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Supramolecular chemistry at the interface of biology, materials and medicine

Eric V. Anslyn*[1] and Steven C. Zimmerman*[2]

Editorial

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Address:
[1]Department of Chemistry, University of Texas, Austin, TX 78712, United States and [2]Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

Email:
Eric V. Anslyn* - anslyn@austin.utexas.edu; Steven C. Zimmerman* - sczimmer@illinois.edu

* Corresponding author

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Guest Editors: S. C. Zimmerman and E. V. Anslyn

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What do art, auto-mechanics, a rural Australian and Chinese village, two civil wars, and house building have to do with supramolecular chemistry? Unless you are an avid cover-to-cover reader of the Thematic Series of the *Beilstein Journal of Organic Chemistry* and happened upon the issue entitled "Supramolecular chemistry at the interface of biology, materials and medicine", there is no possible way you would see the connection between these disparate items. In fact, these are part of the childhood recollections of several leading practitioners in the field of supramolecular chemistry and the authors of the mini-reviews in this Thematic Series. Before explaining the purpose of these recollections in more detail, an overview of this Thematic Series is needed.

We had two main goals in putting this Thematic Series together: (1) to highlight where the field of supramolecular chemistry is today, how it got there, and where it is going, and (2) to provide personal, autobiographies of leading practitioners of the field. The most important goal was to have a diverse group of experts in the field of supramolecular chemistry give an account of the state-of-the-art from their own unique perspective and subarea. There is an enormous amount still to learn about the fundamental nature of noncovalent interactions and particularly how to design and synthesize molecules that complex other molecules or are able to assemble spontaneously into three-dimensional structures. Thus, a major focus of the mini-reviews in this issue is on developing the supramolecular toolkit and better understanding how individual tools work and how they can be used to construct complex systems non-covalently.

The field of supramolecular chemistry has advanced over the past three decades and that success has allowed for a dramatic expansion in the field. In particular, much more attention is now paid to solving societal problems using supramolecular tools and the principles of supramolecular chemistry. The title of this Thematic Series was chosen to highlight three fields – biology, materials, and medicine – where the interface with chemistry has led to many important applications of supramolecular chemistry. Indeed, the reader will learn about how supramolecular discoveries in the laboratory have been translated into commercial products that can both improve and even save human lives.

It is now cliché to note that the earliest interest in supramolecular chemistry can be found in Emil Fischer's famous lock and key analogy for enzymatic catalysis. If the origins of the field can be traced to that analogy made over a century ago, the event that propelled the field of supramolecular chemistry forward like no other occurred about thirty years ago. Thus, the joint 1987 Nobel Prize to Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen "for their development and use of molecules with structure-specific interactions of high selectivity" [1] is often cited by senior members of the field as an inspirational event that both validated the field and signaled its future potential. The mini-reviews herein illustrate how that potential has been realized in multiple areas across a wide chemical landscape over the past thirty years. The articles also highlight future challenges and opportunities.

The second goal of this Thematic Series is more unusual. Over drinks at a workshop we wondered why our colleagues and we chose to pursue a career in supramolecular chemistry. Was it because as children we all played with Lego building blocks or Tinker Toys? Or was it some other reason? In turn, that got us thinking about the more human aspects of science. What did our parents do and how did they, along with our early life experiences influence our careers? What choices were made along the way and how did our research careers unfold? Rarely do students hear the stories behind the publications and the careers. Thus, the diversity in our authors was intended to have those stories reflect faculty at different stages of their careers, in different countries and with very different backgrounds.

At their core, these reviews are about the science of supramolecular chemistry and where the field is today and where it is going in the future. They also offer an intimate portrait of the people behind the work. We know that our authors greatly enjoyed telling their stories and we hope that the readers, particularly students, will find this perspective interesting and perhaps even helpful and inspirational.

Eric V. Anslyn and Steven C. Zimmerman

Austin, Urbana, May 2016

Reference

1. "The Nobel Prize in Chemistry 1987". *Nobelprize.org.* Nobel Media AB 2014. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1987/ (accessed May 13, 2016).

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supramolecular chemistry: from aromatic foldamers to solution-phase supramolecular organic frameworks

Zhan-Ting Li

Review

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Address:
Department of Chemistry, Fudan University, 220 Handan Road, Shanghai 200433, China

Email:
Zhan-Ting Li - ztli@fudan.edu.cn

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Abstract

This mini-review covers the growth, education, career, and research activities of the author. In particular, the developments of various folded, helical and extended secondary structures from aromatic backbones driven by different noncovalent forces (including hydrogen bonding, donor–acceptor, solvophobicity, and dimerization of conjugated radical cations) and solution-phase supramolecular organic frameworks driven by hydrophobically initiated aromatic stacking in the cavity of cucurbit[8]uril (CB[8]) are highlighted.

Review

Childhood and growing up

I was born on July 23rd, 1966 in the small, remote village of Fang-Liu (a combination of two common Chinese family names), which is located in Shangcai County in the Henan Province of central China. Living in the distant countryside, my childhood was simple and quiet. Later I learned that from 1966 to 1976 China had seriously suffered from the so-called Cultural Revolution. In 1972 I entered primary school. My class had about fifteen students. We were all children from the same village. For the first two years, we had only one teacher, Xi Liu, who taught us Chinese and arithmetic. Once per week he also taught music, mainly singing. Since we did not have a permanent classroom, Mr. Liu frequently moved the class to different empty rooms that he found in the village. As I remember it, I studied in at least four “classrooms”. So every day we all needed to bring a small stool from home to “go to school”, but I did not feel this was a burden. Actually this was possibly the happiest stage of my life because Mr. Liu was a neighbor and a family friend, and my scores in Chinese and arithmetic were always the best. In addition to going to school, I also spent a lot of time reading Chinese novels that I could find after I was able to read. Although there were no opportunities for modern sports such as basketball and football, I liked playing ground chess, a two-person game that was very popular during poor times but has now nearly completely disappeared. I enjoyed the chess

game very much because I could often win even against adults. However, I never dreamed that I would become a chemistry professor in Shanghai many years later.

In 1979, I entered Caigou High School, which was located in Caigou Town one kilometer away from my home village. In 1977, China reinstated the long suspended national college entrance examination, thanks to the comeback of Mr. Xiaoping Deng, the greatest Chinese leader. For young people living in the rural areas, acceptance into college was the only route to change their fate at that time. Therefore, I studied very hard and again got the highest total score in the school in the national college entrance examination in 1981. In autumn of that year, I enrolled in the Department of Chemistry at Zhengzhou University. I chose chemistry as my major only because my chemistry score was 95 (out of 100), which was higher than my math or physics scores. I had no idea on how a university picked its students, but just instinctively believed choosing chemistry meant a better chance for being admitted. After more than 30 years, I still remember the days when I studied in Caigou High School. It was quite good at that time in the county, but it could not attract teachers and students in later years due to its remote location and was closed about ten years ago by the local government.

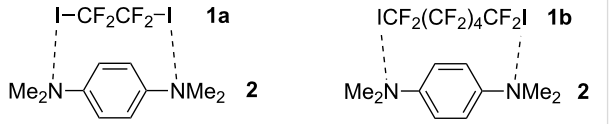
Studying at Zhengzhou University

Zhengzhou is the capital of the Henan Province. Being admitted to the University gave me the opportunity to leave Shangcai County and to take a train for the first time. The Chemistry Department enrolled a total of 120 undergraduates in that year. In the 1980s, the department had only the essential courses of inorganic, organic, physical and structural chemistry and chemical engineering, which were accompanied by a series of fundamental experiments in the same semester. This was the typical course system of the day in China and so all students received the same training. Many years later, China introduced the credit system and both mandatory and optional courses were offered. By the end of the third year, students were required to choose one of the above "secondary degree" disciplines. I chose organic chemistry because I felt it was the easiest course among the others. In particular, I enjoyed one course called "Organic Synthesis Skills" taught by Professor Zhixin Huang, which introduced multistep synthesis. I must say that since 1987 I have benefited greatly from this course as a graduate student at the Shanghai Institute of Organic Chemistry (SIOC). In the spring of 1985, the second semester of the fourth year, I entered Professor Zhendong Chen's lab to perform my dissertation research. I stayed in the lab for about three months and prepared several ferrocene-derived conjugated molecules to study the homologous linearity [1]. I later realized that this was an important research project in physical organic chemistry in China in the 1980s. After graduation from Zhengzhou University, I was assigned to work at Henan Medical University as a teaching assistant. Two years later, I was enrolled as a graduate student at SIOC, the top research institution for organic chemistry in China.

Studying at SIOC: N···I interaction

I joined SIOC in late August of 1987. For many years, graduate students in the first year studied physical organic chemistry, organic synthesis, and organic analytical chemistry. Impressively, all students performed 6–8 multistep synthesis experiments that involved the use of all standard organic synthesis techniques. This was a challenge to many students, but all received systematic training in organic synthesis upon completion of the experiments. In 1988, I entered Professor Ching-Sung Chi's group in the Laboratory of Organic Fluorine Chemistry for a master's degree. The lab is well-known for its long-standing research on reactions of fluorine-containing molecules. Professor Chi left as a visiting scholar at the University of Fribourg, Switzerland shortly after I joined the group and I was actually advised by Professor Yong-Da Lin. I finished the synthesis of several fluorine-containing macrocycles and published my first research paper in the journal Heterocycles [2]. The starting materials for these macrocycles were initially designed for the preparation of biologically active molecules, which was the main project in this laboratory. Using them to prepare new macrocycles became a small independent dissertation project for me. In 1990, I joined Professor Qing-Yun Chen's group as a Ph.D. candidate. Professor Chen is a distinguished, esteemed Chinese chemist in organic fluorine chemistry. His group developed new trifluoromethylation reagents, which found many practical applications [3]. I respect him for his persistent passion for science. Even at the age of 86 in 2015, he still goes to his office and advises his students and remains active in the field of fluorine chemistry. I received my Ph.D. degree in December of 1992. My dissertation research focused on photo-induced reactions of perfluoroalkyl iodides and pentafluoroiodobenzene with arenes, aromatic ethers and amines and heterocycles. These reactions led to the perfluoroalkylation or pentafluorophenylation of the aromatic compounds. One series of reactions involved liquid tetrafluoro-1,2-diiodoethane (**1a**) or dodecafluoro-1,6-diiodohexane (**1b**) and solid *N*,*N*,*N*',*N*'-tetramethylphenylene-1,4-diamine (**2**). We found that when mixing in chloroform, the 1:1 mixtures readily gave a high yield of solid adducts, which melted at 85 and 65 °C, respectively. Elemental analyses supported a 1:1 stoichiometry for the solid adducts, and ^{19}F NMR spectra showed downfield chemical shifting of the ICF_2 signal of **1a** and **1b** [4]. Clearly, an important intermolecular interaction occurred which led to the solidification of the mixtures [5]. However, we did not obtain the crystal structure of the mixtures, although I had been to Beijing

for X-ray diffraction experiments at Peking University. We thus proposed that the two compounds formed 1:1 charge-transfer complexes (Scheme 1). Currently, this N···I interaction is termed as halogen bonding, which is widely used in supramolecular crystal engineering [6,7].

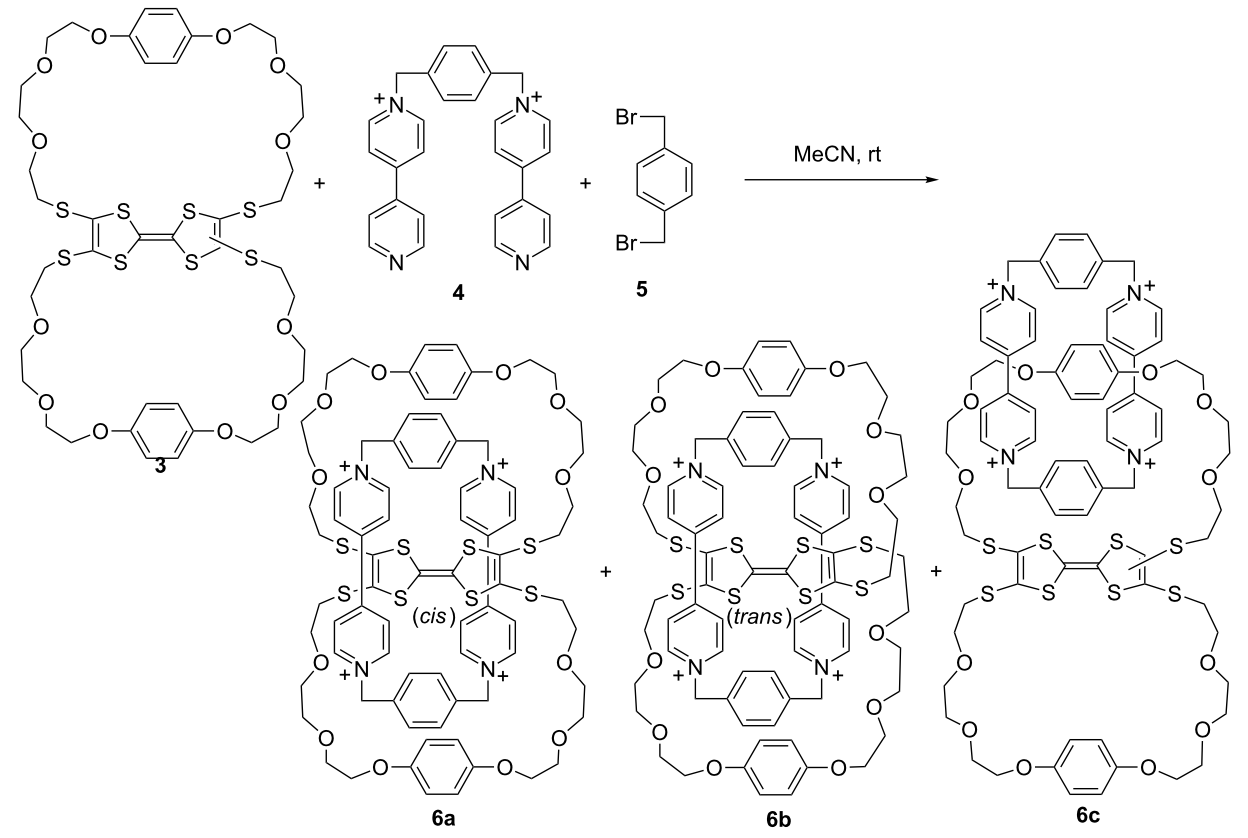


Scheme 1: Proposed structures of complexes between **1a** and **1b** with **2**.

My Ph.D. training in synthetic methodology and fluorine chemistry had an important influence on my research activity. When I initiated a project, the first thing I would think of and discuss with my students is the synthetic route for the target molecules. Fluorine-containing molecules have always been my favorite. When I performed my postdoctoral research in Denmark, I used fluorine-containing precursors to build catenanes [8], and many years later, I utilized fluorine as a hydrogen bonding acceptor to develop aromatic amide and triazole foldamers [9,10].

Postdoctoral research at the University of South Denmark: donor–acceptor interaction-driven catenanes

From October 1994 to December 1995, I performed postdoctoral research with Professor Jan Becher at Odense University (currently University of South Denmark) in Denmark. Through his research career, Professor Becher studied sulfur-containing molecules and since the early 1990s, tetrathiafulvalene (TTF) supramolecular chemistry. Before I went to Odense, the group had developed a very useful method of in situ generation of TTF thiolate anion from cyanoethylated precursors, which greatly simplifies the modification of the TTF core [11]. Using this method, I could prepare bimacrocycle **3** in a short time. This macrocycle was used to template the formation of the so-called tetracationic "blue box" [12,13] from **4** and **5** to give rise to the unique pseudo[3]catenanes **6a** and **6b** (Scheme 2), together with a trace amount of [2]catenane **6c** with the tetracationic cyclophane holding one of the peripheral benzene rings [14]. The TTF unit in **6a** and **6b** adopted a stable *cis* or *trans* configuration, although typically the two configurations easily isomerize into each other in solution. Macrocycle **3** is a brown solid due to the existence of the TTF unit. Catenanes **6a** and **6b** are blue as a result of the charge-transfer complex between the TTF and bipyridinium units, whereas catenane **6c** is orange,



Scheme 2: The formation of catenanes **6a–c**.

which is attributed to the charge-transfer complex between the dioxybenzene and bipyridinium units. Impressively, the three catenanes could be separated from each other using a short silica gel column. Three colorful bands could be observed from the column, which made it easy to collect the respective solutions. Another impressive occurrence was the difference in the solubility of the catenanes with different counterions in water and organic solvents. With hexafluorophosphate as the counterion, the catenanes were well-dissolved in acetonitrile, while with chloride, their solubility was poor.

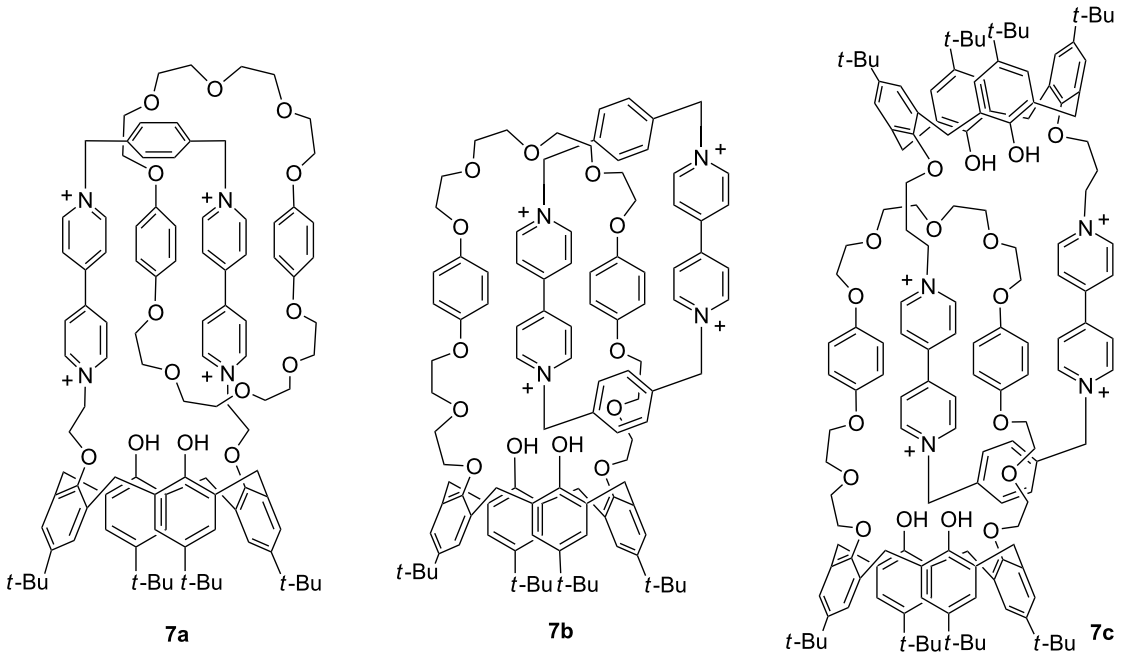
My stay in Odense initiated my research in supramolecular chemistry. For many years, I maintained interest in interlocked systems. TTF is also a favorite. Many years later at Fudan, I initiated a project to study the potential of its radical cation stacking in controlling the folded conformation of linear molecules and two- and three-dimensional supramolecular polymers and frameworks. My life in the small town of Odense was also memorable. Its calm is in sharp contrast to the bustle of Shanghai where I had lived since 1987. Odense is also the hometown of Hans C. Andersen, the great Danish writer of fairy tales. Since I had read his books before, I visited the small Hans Christian Andersen Museum, which is situated in a house in the old town where he was born.

Early research at SIOC

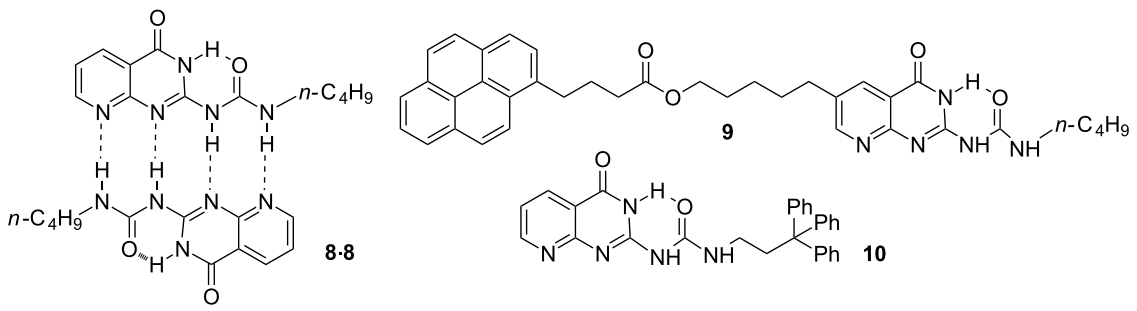
In January 1996, I returned to SIOC. Although I had hoped to continue research in fluorine chemistry, I was assigned to work for a big contract project in the laboratory of physical-organic chemistry. After finishing the contract research, I also did a small project: constructing calix[4]arene-derived catenanes, such as **7a–c** [15] (Scheme 3). I chose this project because I believed that I could make progress in a short time. Most of the work was done by myself, but I also received assistance from several young technicians. We succeeded in investigating the effect of the calix[4]arene moiety on the relative rotation of the two rings using ^{1}H NMR, and from 1998 to 2000, we published four papers from this project. However, this was generally a tough period for me. Due to some nonacademic reasons, I could not build an efficient research group after several years of effort. Therefore, I decided to make a change.

Research at the University of Illinois

In October 2000, I joined Professor Steven C. Zimmerman's group at the University of Illinois at Urbana. The Zimmerman group had been well-known for pioneering works in hydrogen bonding-related achievements. In 1998, the group reported the extremely stable quadruple hydrogen-bonded dimers **8**·**8** [16] (Scheme 4). By using an ^{1}H NMR dilution technique, a lower limit to the dimerization constant K_{dim} (>10^7 M^{-1}) was estimated. Professor Zimmerman hoped to make an accurate determination of the binding stability. Thus, I prepared compound **9**, which bore a pyrene unit [17] (Scheme 4), based on previously published research by Sijbesma and co-workers, who had taken advantage of the excimer signal of the pyrene dimer to evaluate the stability of their famous quadruple hydrogen bonded 2-ureido-4[1*H*]-pyrimidinone dimers [18]. The excimer exhibits an emission band at 500–600 nm, which is separated



Scheme 3: The structures of cantenanes **7a–c**.



Scheme 4: The structures of dimer **8·8** and compounds **9** and **10**.

from that of the monomer. I thus determined the K_{dim} of **9** as 3.0×10^7 M^{-1} in chloroform saturated with water ([water] ≈ 0.45 M) and 8.5×10^7 M^{-1} in freshly opened chloroform ([water] ≈ 17 mM).

Dimer **8·8** had another three tautomers [16]. Thus, I also tried to obtain the single crystal structure of the binding motif. In total I prepared 28 derivatives by introducing different substituents and succeeded in growing the single crystal of compound **10** [17] (Scheme 4). The crystal structure showed the formation of a homodimer of the N(3*H*) protomer that was stabilized by four intermolecular N–H···O hydrogen bonds and the protomer itself was rigidified by an intramolecular six-membered N–H···O hydrogen bond (Figure 1). This motif was the one that had the highest proportion in chloroform.

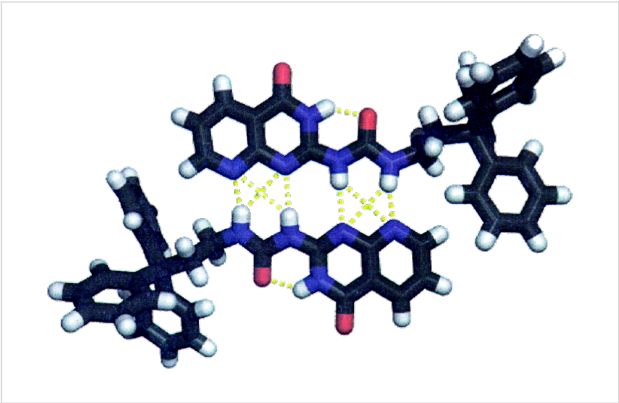
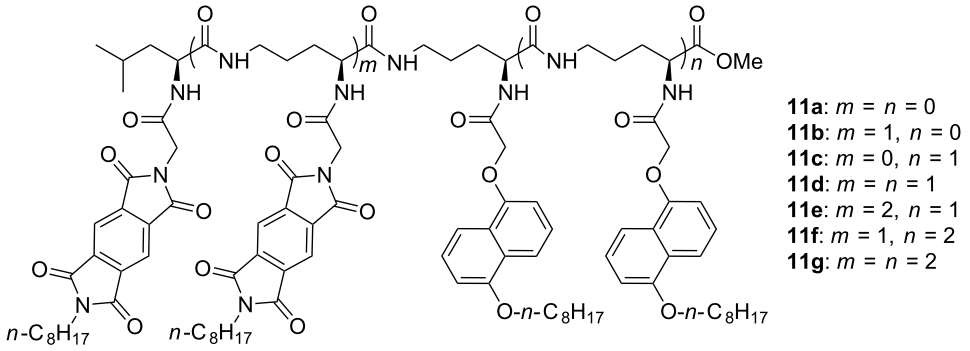


Figure 1: X-ray structure of **10** showing a quadruple hydrogen-bonded dimeric motif [17].

Research on foldamers: applications for molecular recognition and self-assembly

Donor–acceptor interaction and π-stacking for folding. In January 2001, I returned to SIOC again. When I left SIOC for Urbana in 2000, I had built a small research group with two graduates. Thanks to the persistence of Professor Xi-Kui Jiang [19,20], one of the greatest physical organic chemists in China, my small group survived until I returned to the institute. I was so impressed by the work of Professor Zimmerman on the folding of linear urea derivatives driven by intramolecular hydrogen bonding [21], that in 2001, our group started several projects searching for new folded frameworks. All the projects were done by Ph.D. students. Xin Zhao, who is currently a professor at SIOC, finished the first project in 2004. He prepared L-ornithine-derived δ-peptides **11a–g**, which bore one to three electron-deficient pyromellitic diimide (PDI) and electron-rich 1,5-dioxynaphthalene (DAN) units on the two sides of the backbones [22] (Scheme 5). An intramolecular donor–acceptor interaction between the DAN and PDI units,



Scheme 5: The structures of compounds **11a–g**.

which is a well-established noncovalent force [23,24], induced the backbones to fold into zipper-featured foldamers in less polar chloroform and polar DMF (Figure 2). This finding was supported by ^{1}H NMR, UV–vis, and fluorescence quenching studies. As expected, the folding state became more compact for longer sequences, which possess more donor–acceptor interacting sites. UV–vis experiments indicated that the folding state remained, even at 150 °C in DMF.

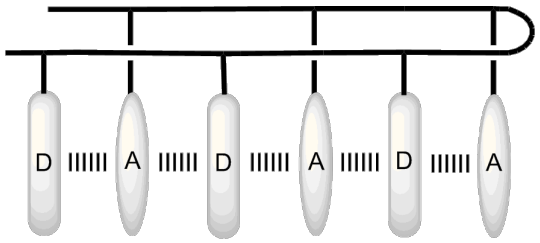
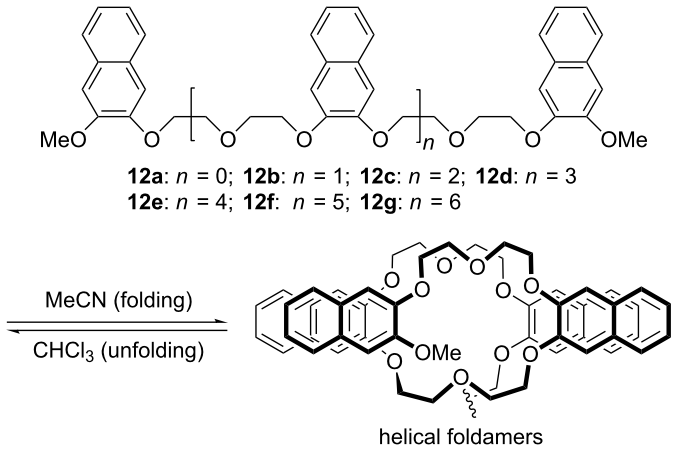


Figure 2: Zipper-featured folding motif of δ-peptides **11a–g** driven by the cooperative donor–acceptor interaction [22].

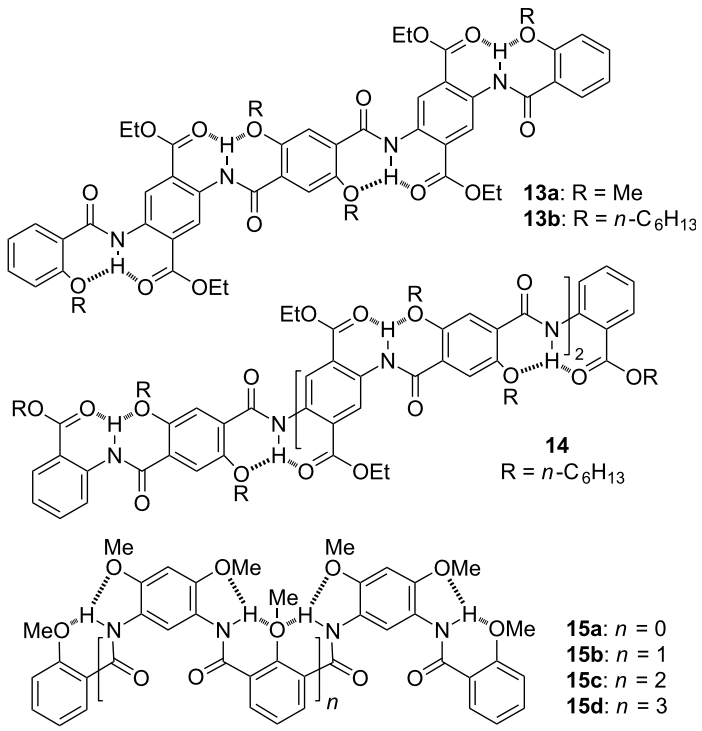
Encouraged by the above work, Junli Hou, who is currently a professor at Fudan University, prepared naphthalene-incorporated oligo(ethylene glycols) **12a–g** [25] (Scheme 6). UV–vis, ^{1}H NMR, and fluorescence experiments in chloroform–acetonitrile binary solvents revealed that the naphthalene units in longer **12f–h** stacked intramolecularly to induce the oligomeric chains to form a helical conformation at high acetonitrile content (Scheme 6). The compact helical conformation gave rise to a cavity similar to that of 18-crown-6 and thus could complex ammonium or ethane-1,2-diaminium in acetonitrile. The stability of the complexes increased with the elongation of the ethylene glycol chains.

Aromatic amide oligomers: extended secondary structures. Our students also tried to make use of hydrogen bonding to control the conformation of aromatic amide backbones. Previously, Hamilton [26], Gong [27], Huc and Lehn [28], and Huc [29] had developed several series of elegant folded frameworks. We thus focused on the creation of extended frameworks, which we expected to be useful for the design of functional materials [30]. In 2004, Zongquan Wu, who is currently a professor at Hefei University of Technology, reported the formation of straight conformations by oligomers **13a,b** and **14**, which was driven by successive intramolecular hydrogen bonding [31] (Scheme 7), whereas Jiang Zhu, who is currently an associate professor at North Sichuan Medical College, described the zigzag conformation of oligomers **15a–d**, which is also stabilized by intramolecular hydrogen bonding [32] (Scheme 7).

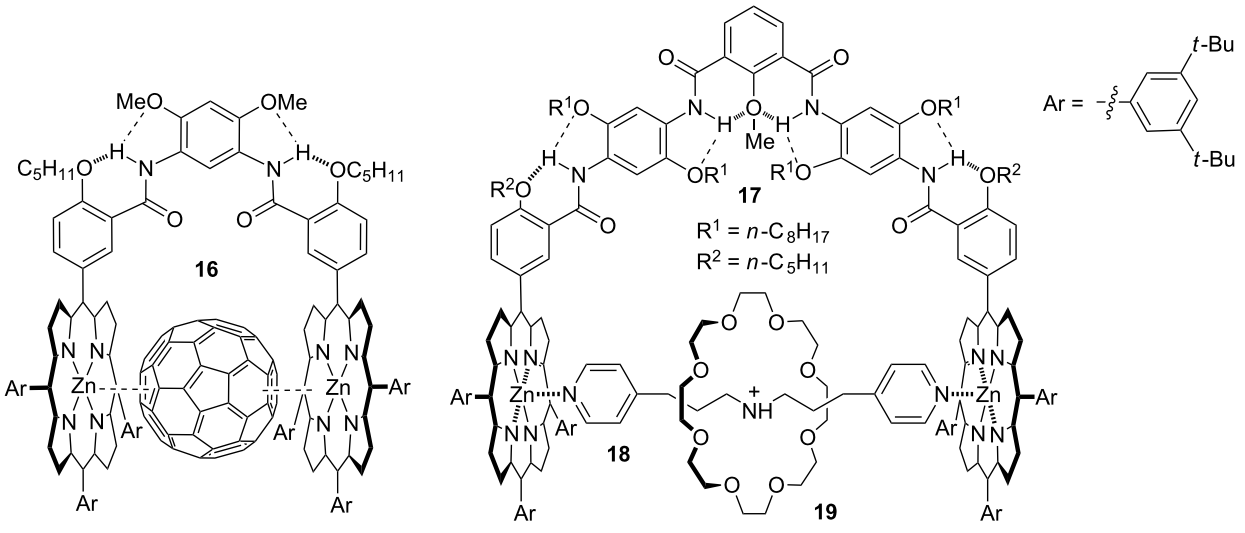
The aromatic units in oligomers **13–15** can be easily combined into one sequence. By changing their number and position, sequences of different length and shape or in a controlled conformation can be designed [33,34]. Thus, oligomers **13–15** may be considered as structural prototypes for creating new modifiable backbones. For example, porphyrin-appended U-shaped molecular tweezers **16** and **17** have been produced (Scheme 8). Compound **16** complexed C_{60} or C_{70} or their derivatives in chloroform or toluene through porphyrin–C_{60} stacking [35], while compound **17** strongly complexed **18** in the chloroform–acetonitrile binary medium [36] (Scheme 8). Because **18** could further form a threaded complex with 24-crown-8 **19** driven by multiple O···H–N hydrogen bonds, the three components self-assembled into a unique dynamic [2]catenane. Under low temperature, this dynamic [2]catenane could be quantitatively generated. The intramolecular hydrogen bonds formed by the aromatic amide linkers remarkably



Scheme 6: The structures of compounds **12a–g** and the formation of the helical conformation by the longer oligomers.



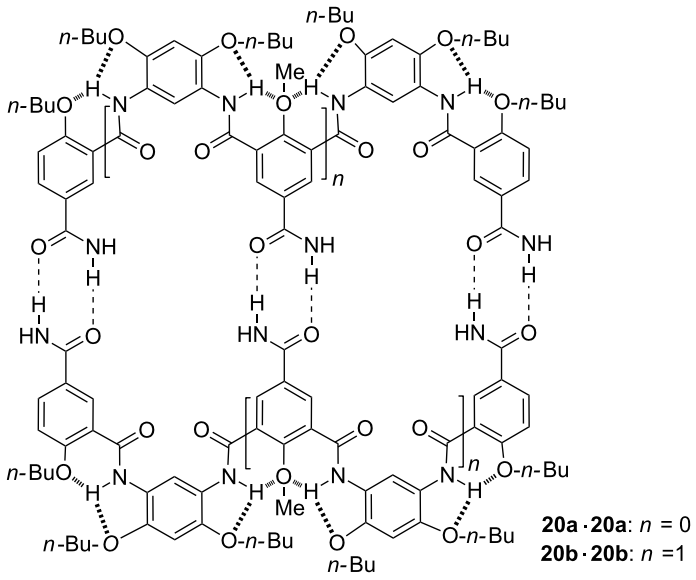
Scheme 7: The structures of compounds **13a,b**, **14**, and **15a–d**.



Scheme 8: The structures of complex C_{60} ⊂ **16** and dynamic [2]catenane formed by compounds **17–19**.

enhanced the complexation of the porphyrin units towards the fullerene and the bipyridine ligand.

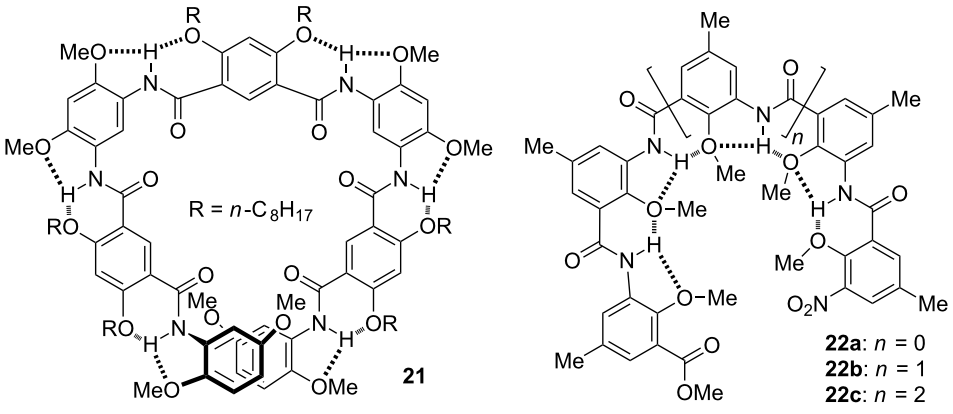
Jiang further introduced amide subunits to the *para*-position of the ether groups of the benzamide rings of oligomers **15a** and **15b** to produce **20a** and **20b** [37], respectively (Scheme 9). ^{1}H NMR dilution experiments in chloroform-*d* revealed that both compounds formed stable homodimers **20a·20a** and **20b·20b**, with K_{dim} being 3.0×10^3 and 2.3×10^5 M^{-1}, respectively. In contrast, even in nonpolar benzene-d_6, the K_{dim} for the dimerization of benzamide was only 40 M^{-1}. The result again shows that the intramolecular hydrogen bonding of the aromatic amide backbones promoted the appended amide subunits to bind in a cooperative manner by preorganizing the backbones.



Scheme 9: The structure of homodimers **20a**·**20a** and **20b**·**20b**.

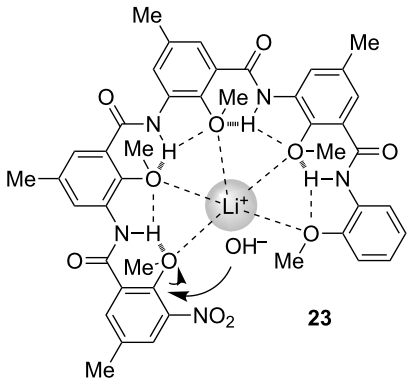
Aromatic amide oligomers: folded and helical secondary structures. In 2005, Huiping Yi finished the synthesis and characterization of aromatic amide oligomers **21** and **22a–c** [38,39] (Scheme 10). These two series of oligomers folded into helical secondary structures driven by intramolecular hydrogen bonding, which is typical for aromatic amide backbones [40-42]. The diamine and diacyl chloride precursors for **21** had been used by Gong and co-workers to prepare the first family of hydrogen bonding-promoted aromatic amide macrocycles [43]. The main aim for designing these folded structures was to explore their potential functions as acyclic receptors. Moore et al. had utilized this approach to investigate the binding of *m*-phenylene ethynylene foldamers for nonpolar organic molecules in polar media [44]. All the C=O oxygen atoms of **21** point into the cavity of the helix, which has a diameter of approximately 0.8 nm. Thus, **21** complexed alkylated saccharide derivatives and a guest with three hydroxyl groups in chloroform. The binding also induced the backbone of **21** to produce helicity bias [38]. The methoxy groups of **22a–c** are all located inwards. These oxygen atoms are potential hydrogen bonding acceptors. ^{1}H NMR and fluorescence experiments in chloroform showed that this series of foldamers complexes primary and secondary alkyl ammonium products [39].

In most cases, we investigated the binding of foldamers for different guests in less polar chloroform. However, during the synthesis of the **22** series, which involved the hydrolysis of the methyl ester at one end with lithium hydroxide in heated dioxane–water solution, Huiping found that the nitro-bearing anisole unit at the other end was also hydrolyzed to afford a



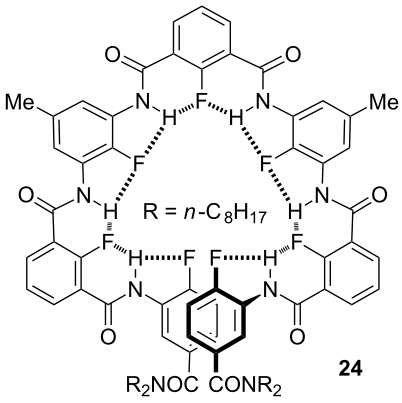
Scheme 10: The structures of foldamers **21** and **22a–c**.

phenol derivative [45]. In contrast, a short control did not exhibit similar reactivity. These observations suggested that the folded conformation, such as in **23**, promoted the hydrolysis of the anisole, which we ascribed to the complexation of the foldamer to Li^+ cation (Scheme 11). We proposed that this complexation increased the efficient concentration of the OH^- anion and also activated the nitro-bearing anisole. This result supports that the intramolecular MeO···H–N hydrogen bonding worked even in a highly polar solvent. The theoretical study by Pophristic and co-workers also shows that this hydrogen bonding exists, to some extent, in polar aqueous environments [46]. The high stability of the folded conformation of the **22** series also make them useful frameworks for creating a variety of complicated supramolecular architectures [42].



Scheme 11: Complexation-promoted hydrolysis of foldamer **23**.

Although alkoxyl groups had been popularly used as acceptors for intramolecular hydrogen bonding [40-42], I was also interested in looking for other substitutes. For this purpose, I envisioned fluorine might be a good candidate, given its highest electronegativity. An investigation by Dunitz and Taylor led to the conclusion that "organic fluorine hardly ever accepts hydrogen bonds, that is, it does so only in the absence of a better acceptor" [47,48]. We conjectured that fluorine might work as an acceptor for intramolecular five- and six-membered F···H–N hydrogen bonding for aromatic amides because of their co-planarity. Chuang Li thus prepared oligomer **24** and shorter analogues [9]. Systematic ^{1}H NMR experiments in chloroform-*d* and crystal structure analysis of model molecules all supported that fluorine was engaged in the expected intramolecular F···H–N hydrogen bonding and **24** formed a helical conformation (Scheme 12). Moreover, Chuang found that intermolecular F···H–N hydrogen bonding could also be formed between **23** and aliphatic ammonium and, at high concentrations in chloroform, a chiral aliphatic ammonium induced **23** to produce helicity bias.



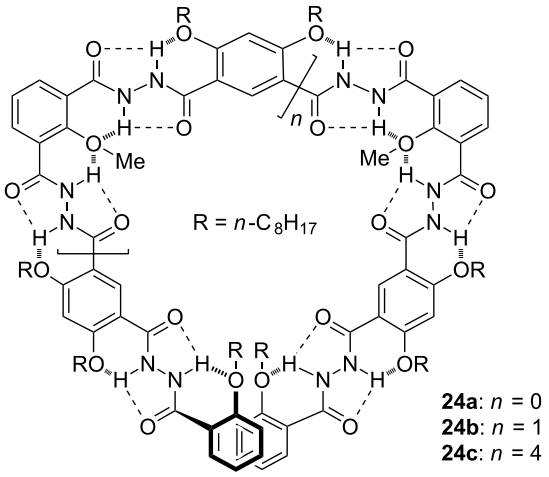
Scheme 12: The structure of foldamer **24**.

One important difference between fluorine and alkoxy groups is that fluorine does not cause a steric effect on aromatic stacking. Zeng and co-workers demonstrated this difference by crystallizing a fluorine-bearing pentagon macrocycle [49-51]. No stacking was observed for the methoxy-derived pentagon macrocycle. In contrast, their fluorine-engaged pentagon macrocycle stacked strongly. Moreover, the macrocycle stacked in the 2D space to give rise to the mathematically predicted, most densely packed lattice for a C_5-symmetric macrocycle [49].

Jiang and co-workers also prepared fluorine-containing quinoline oligoamides, which they found self-assembled into unique double and quadruple helices [52,53]. The fluorine atoms in the oligomers induced the backbone to fold by forming intramolecular F···H–N hydrogen bonding, whereas the small size of fluorine allowed the folded sequences to stack into double and quadruple helices. In an elegant study, they prepared fluorine-containing hybrid sequences with different aromatic subunits, whose helical states could hold one linear molecule to give rise to foldamer-derived dynamic rotaxanes [54].

Aromatic hydrazide foldamers: Aromatic hydrazides have a high propensity towards co-planarity [55,56]. Nowick introduced this unit into peptide backbones to increase the stability of artificial β-sheets [55]. Junli hoped to extend the backbones of hydrogen-bonded foldamers and thus prepared oligomers **24a–c** [57] (Scheme 13). The octoxy groups provided solubility in common solvents such as chloroform. These and the methoxy groups formed successive hydrogen bonds to induce the backbones to form folded conformations, which have a cavity of about 1 nm in diameter. These folded structures could host alkylated saccharides in chloroform, which was stabilized by intermolecular hydrogen bonding formed between the carbonyl oxygen atoms of the foldamers and the hydroxy groups of the saccharides. By changing the position of the alkoxy groups, the shape of the backbones can be readily tuned. The large cavity

tolerates the existence of the methoxy groups for the backbones to stack efficiently. Thus, the backbones can stack to form vesicles or gelate organic solvents depending on the appended side chains [42]. Jiang and co-workers prepared methoxy-free backbones [58]. It is expected that these backbones should exhibit increased conformational flexibility. However, they still displayed quite strong binding capacity to anions and saccharides.

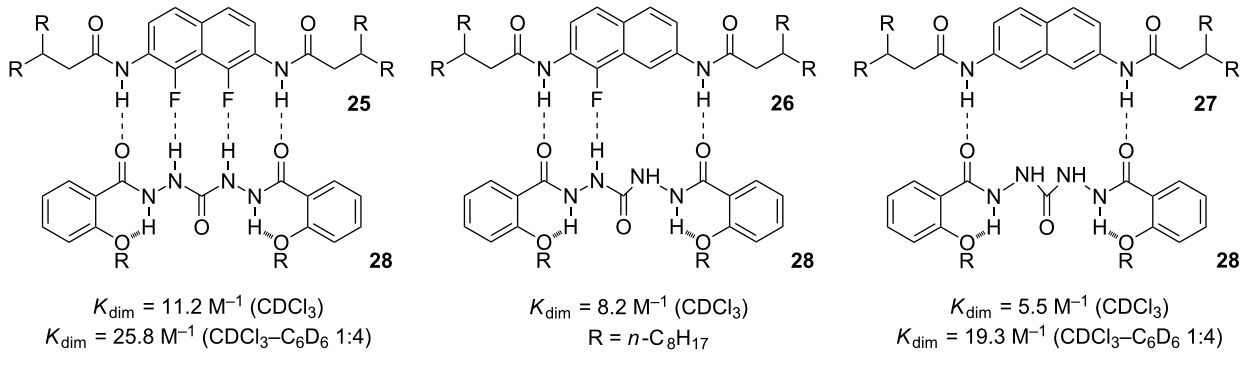


Scheme 13: The structures of foldamers **24a–c**.

Studies on N–H····X (X = F, Cl, Br, I) hydrogen bonding. At SIOC, our group is the only one dedicated to physical-organic chemistry. Thus, we maintained a longstanding interest in the fundamental aspects of noncovalent forces. The establishment of intramolecular N–H····F hydrogen bonds raised the possibility of quantitative assessment of intermolecular N–H····F hydrogen bonds. Since this hydrogen bonding is generally weak, Yanhua Liu prepared compounds **25–27** [59] (Scheme 14), which have the identical backbones, in order to evaluate the stability of the heterodimers formed between them and **28** [56]. In chloroform, K_{dim} for the three complexes was determined to be 11.2, 8.2 and 5.5 M^{-1}, respectively. In less polar binary chloroform–benzene (1:4, v/v), the K_{dim} values of the first and third complexes were increased significantly to 25.8 and 19.3 M^{-1}, respectively. These results indicate that the intermolecular N–H····F hydrogen bond did exist for aromatic derivatives, but is quite weak.

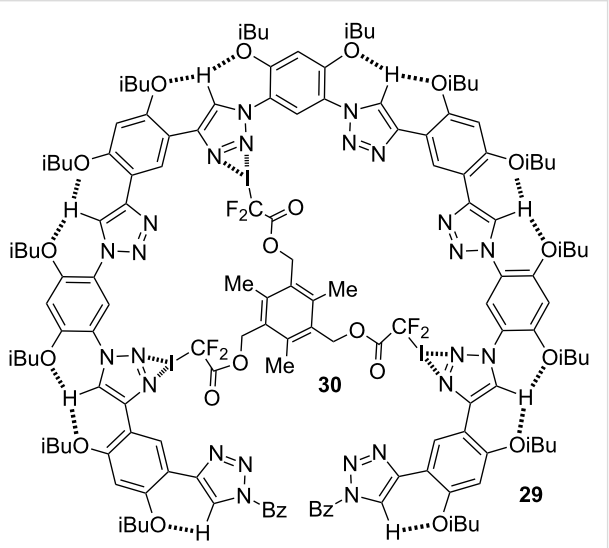
After the establishment of the intramolecular N–H····F hydrogen bond motif, we further prepared various model molecules to exploit the possibility of forming similar N–H····X (X = Cl, Br, I) hydrogen bonding motifs by aromatic amide derivatives [60]. We found that all these halogen atoms are able to form this hydrogen bonding, but the stability decreases successively, which is consistent with the decrease of their electronegativity, but may also reflect the increase of the van der Waals radius. These weak intramolecular hydrogen bonds cannot compete with the strong intermolecular N–H···O=C hydrogen bonds of the amide groups. Thus, for their formation, the latter has to be suppressed. For the same halogen atom, the five-membered N–H····X (X = Cl, Br, I) hydrogen bond is generally easier to form than the six-membered one. One straightforward explanation for this difference is that the formation of the former would confine the rotation of one single bond, while for the latter, it would confine two single bonds. Jiang and Huc and co-workers successfully utilized the five-membered N–H···Cl hydrogen bond to construct quinoline amide-derived double helices [53].

C–H····X (X = OR, F) hydrogen bonding-driven 1,2,3-triazole foldamers. In 2008, several groups independently described that the intermolecular C–H····Cl^- hydrogen bonding could induce benzene-linked 1,2,3-triazole oligomers to form folded or helical conformations [61-63]. Currently, this family of foldamers have found wide applications in anion binding and design of photo-active molecular devices [64-66]. We were interested in developing inherently folded structural patterns for aromatic oligotriazole backbones. In 2009, Yuanyuan Zhu



Scheme 14: Proposed structures of heterodimers **25**·**28**, **26**·**28**, and **27**·**28**.

established that 1,5-diphenyl-1,2,3-triazole formed an intramolecular six-membered C–H···OMe hydrogen bonds [67]. In 2012, Beye Lu further demonstrated that fluorine, chlorine or even bromine could form similar intramolecular C–H···X hydrogen bonds [68]. Using the C–H···O hydrogen bonding motif, Liyan You created a family of oligotriazole foldamers, including **29** (Scheme 15), which had a cavity of approximately 1.8 nm in diameter [69]. At first we expected that this series of triazole foldamers would be good hydrogen bonding acceptors. Intriguingly, the foldamers did not exhibit observable binding affinity to saccharides or amide derivatives even in less polar chloroform. Liyan designed different molecules to evaluate their binding to these triazole foldamers. He finally found that these foldamers were good halogen bonding receptors for tritopic and ditopic guests, including **30** in dichloromethane or its mixture with hydrocarbons.



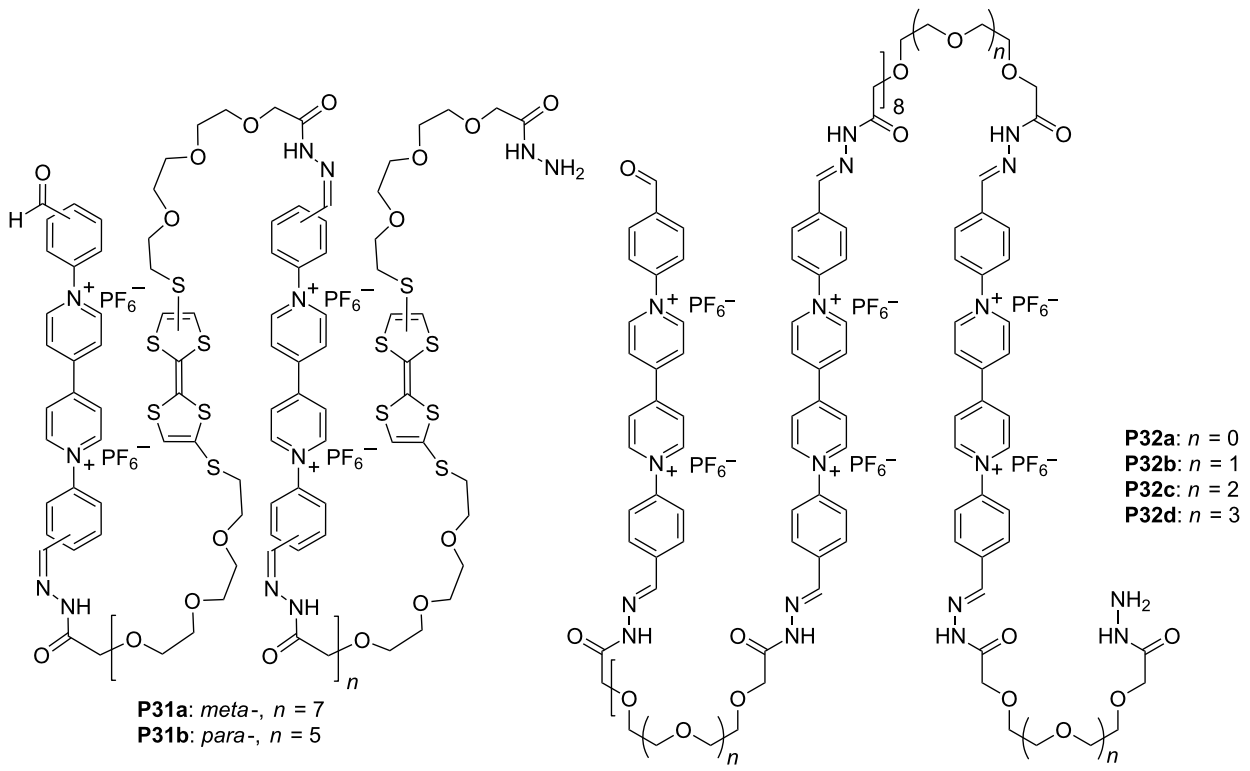
Scheme 15: Proposed structure of complex formed by **29** and **30**.

Yanhua further utilized the C–H···F hydrogen bonding to induce the folding of the same series of benzene/triazole oligomers [10]. Recently, Jiang and co-workers reported that when fluorine or chlorine was introduced to the 2-position of the *meta*-substituted benzene linkers, the corresponding triazole oligomers folded to give new foldamers, which were stabilized by successive intramolecular C–H···F or C–H···Cl hydrogen bonds [70].

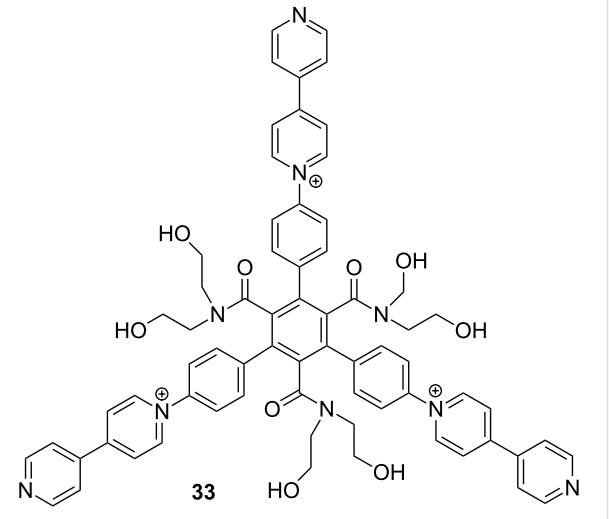
Conjugated radical cation dimerization-driven pleated foldamers. The stacking of the radical cations of viologen or TTF were observed in 1964 and 1979 [71,72]. This stacking is typically weak. Several approaches have been developed to enhance this stacking [73-76]. As a result, strong stacking, which leads to the formation of stable homodimers, has been observed in many elegantly designed molecules and supramolecules. In recent years, this stacking has been utilized as a tunable, noncovalent force to induce the formation of interlocked systems and molecular switches [77,78]. Our long-standing interest in foldamers prompted us to explore the application of this noncovalent force for the generation of new, folded patterns. Thus, Lan Chen prepared polymers **P31a** and **P31b** from the corresponding dialdehyde and di(acylhydrazine) precursors by forming dynamic hydrazone bonds [79] (Scheme 16). This dynamic covalent chemistry approach allowed for quick synthesis of viologen/TTF-alternating polymers. Driven by the intramolecular donor–acceptor interaction between the TTF and viologen units, the polymers folded into pleated conformations in acetonitrile. Upon oxidation of the TTF units to radical cation $TTF^{\bullet+}$, the polymers adopted flexible conformations. When the viologen units were reduced to radical cations, the radical cations stacked intramolecularly to induce the backbone to form another kind of pleated secondary structure. Yunchang Zhang further illustrated that upon reducing the viologen units into radical cations, their intramolecular stacking also induced polymers **P32a–d** to form pleated conformations [80] (Scheme 16). This occurred despite the fact that the folding of **P32a** needed the assistance of alkaline metal ions like Li^+.

Solution-phase supramolecular metal–organic frameworks (MOFs)

Periodicity is the key feature of single crystals in which molecules arrange repeatedly in the three-dimensional space. Porous crystals such as metal–organic frameworks (MOFs) exhibit many unique properties mainly due to their large surface area. However, the achievement of periodicity is a challenge in solution for self-assembled architectures as a result of the impact of the solvent and the weakness of noncovalent forces that hold monomeric components together. In early 2012, I discussed the initiation of a project with Kangda Zhang to explore the possibility of generating honeycomb, supramolecular frameworks in water. In a short time, he prepared one triangular target molecule. However, the molecule was poorly soluble in water. He then prepared compound **33** [81] by introducing three hydrophilic amide chains, which provided good solubility in water (Scheme 17). Shortly after, Kangda revealed that the three phenyl-bipyridine units strongly stacked in the two-dimensional space when mixing with 1.5 equiv of cucurbit[8]uril (CB[8]), which could encapsulate the stacked bipyridine dimers [82,83]. In collaboration with Yi Liu at Lawrence Berkeley National Laboratory in the United States, we conducted solution-phase, small-angle X-ray scattering synchrotron experiments on the mixture of **33** and CB[8] in water (1:1.5, 3.0 mg/mL), which supported the periodicity of the 2D honeycomb SOF structures in solution. Liang Zhang



Scheme 16: The structures of polymers **P31a,b** and **P32a–d**.



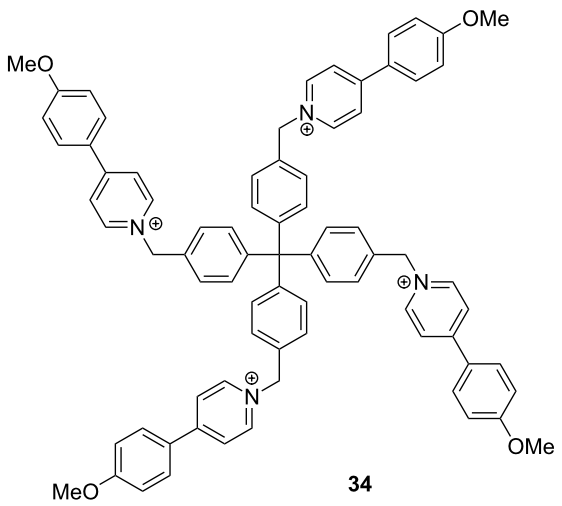
Scheme 17: The structure of compound **33**.

found that the stacking of viologen radical cations could also drive a similar triangular molecule to form 2D SOF, which was further stabilized with CB[8] by encapsulating the stacking radical cation dimer [84]. Very recently, Zhao and co-workers reported that a donor–acceptor interaction could drive the formation of square-shaped 2D MOFs from a porphyrin-derived bipyridinium precursor and 2,6-dioxynaphthalene-derived ditopic precursor [85]. In this case, the 2,6-dioxynaphthalene–dipyridinium donor–acceptor complex was also stabilized by CB[8]. These results showed that generation of 2D SOFs can be induced by discrete noncovalent forces.

In 2014, Jia Tian further extended the concept of solution-phase SOF to 3D space from the self-assembly of tetrahedral **34** and CB[8] [86] (Scheme 18). The 1:2 mixture in water generated a 3D, homogeneous SOF of diamond topology. The pores of the 3D SOF could be observed by high-resolution TEM. As a supramolecular "ion sponge", the framework adsorbed various anionic guests, including drugs, peptides and DNA. These new 2D and 3D homogeneous SOFs all have defined cavities or pores, which are expected to display new, interesting properties [87].

Future perspectives

My research on foldamers was heavily affected by the work of Professor Xi-Kui Jiang on the self-coiling of organic molecules in aqueous media driven by hydrophobicity [19]. At an early stage, we developed quite a number of folding patterns to explore the so-called functions. Finally, I established the hydrogen-bonded aromatic amide and hydrazide sequences as a longstanding research area. With these sequences as a struc-



Scheme 18: The structure of compound **34**.

tural basis, we were able to investigate many interesting phenomena or concepts in supramolecular chemistry such as stimuli responsiveness, "sergeant–soldier effect", and supramolecular devices [88]. It is also noteworthy that the preorganization feature of the amide sequences can remarkably increase the selectivity of macrocyclization [42,51,89]. By making use of the dynamic covalent chemistry approach, we have demonstrated that complicated macrocycles can be obtained in nearly quantitative yields [90].

My research on aromatic foldamers has lasted for more than ten years. In collaboration with Junli, we recently found that the tubular cavity of hydrazide foldamers could mediate the transmembrane transport of K^+ [91]. One ongoing project is to explore the routes for improving the transport selectivity for proton, cations and anions by making use of helical foldamers as channels. Long helical foldamers can theoretically form deep spring-shaped tubes. However, the synthesis and characterization of long foldamer polymers has been a challenge [92-94]. I hope that we can find solutions to address this issue in the future. Particularly, we are interested in obtaining stable, single macromolecular tubes, probably after suitable postmodification. Solution-phase SOFs are new, homogeneous, porous architectures. As ordered supramolecular polymeric electrolytes, this family of self-assembled systems may be developed as useful adsorbing materials. Currently we are investigating new behaviors of molecules or macromolecules adsorbed by SOFs in solution.

Acknowledgements

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Life lessons

Jonathan R. Nitschke

Review

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Address:
Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom

Email:
Jonathan R. Nitschke - jrn34@cam.ac.uk

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Abstract

Reminiscing about his younger self: "I mean I can't very well just 86 [in American slang, to "86" is to eject, remove, or discard someone or something, J.R.N.] this guy from my life. On the other hand, if through some as yet undeveloped technology I were to run into him today, how comfortable would I feel about lending him money, or for that matter even stepping down the street to have a beer and talk over old times?" — Thomas Pynchon, *Slow Learner*

Review

I was raised in Syracuse, New York (USA), and went to a series of state schools of varying quality. Only the faces of the bad teachers stick with me, but I remember the names, too, of a few of the good ones – Karen Curry (High School Biology), Michelle Grosnick (Earth Science). Following my parents' divorce I moved to Gainesville, Florida when I was 16, where I enrolled in the International Baccalaureate program of Eastside High School. I had been drawn to chemistry for a few years by then. My chemistry teacher, Susan Zoltewicz (wife of Professor John Zoltewicsz, University of Florida), recognized my interest, and very kindly arranged for me to do a weekly afterschool apprenticeship with a lab technician named Charlie, whom I helped to set up all manner of chemical demonstrations and experiments at the University of Florida.

I went on to study at Williams College in Massachusetts. I knew chemistry was going to be my major subject, but my interests were broad, leading me to think that a liberal arts education might be a good fit. I remember greatly enjoying courses on art history and 20th century German history, along with a tutorial-style course on heterocyclic chemistry by J. Hodge Markgraf. Two summers stand out in my memory, when I carried out internships at Nanoptics, a company in Gainesville that makes optical fiber and various devices that incorporate it. The company was small enough at the time for me to enjoy considerable interaction with its founder and CEO, Jim Walker, who had been in charge of internally-initiated physics experiments at Fermilab. I had several challenging and very engaging jobs to do, including putting a disassembled fiber-spinning machine

back together, and programming a microcontroller to run a stepper motor. I'm pretty sure that Jim's good word got me in to Berkeley for Ph.D. studies, despite mediocre results for my senior project (with Lee Park at Williams) and during a summer internship at SRI in Menlo Park, California.

I arrived at Berkeley with the impression of having gotten in by the skin of my teeth – that I would have to work twice as hard as anyone else just to scrape through. The first year of the program was a trial by fire, involving coursework – an excellent Physical Organic Chemistry course taught by Bob Bergman stood out – teaching a laboratory section, choosing a supervisor, and racing to get work done for the crucial first-year report. I remember waking up at 4 am during my first year, deeply concerned that I couldn't get a reaction to work consistently, and so heading in to lab to have another go. Although this was an incredibly poor idea from a safety perspective, the underlying attitude served me well. I loved the environment at Berkeley – the place was fizzing with intellectual energy, manifested both as world-class scholarship and as Hunter S. Thompson-style craziness.

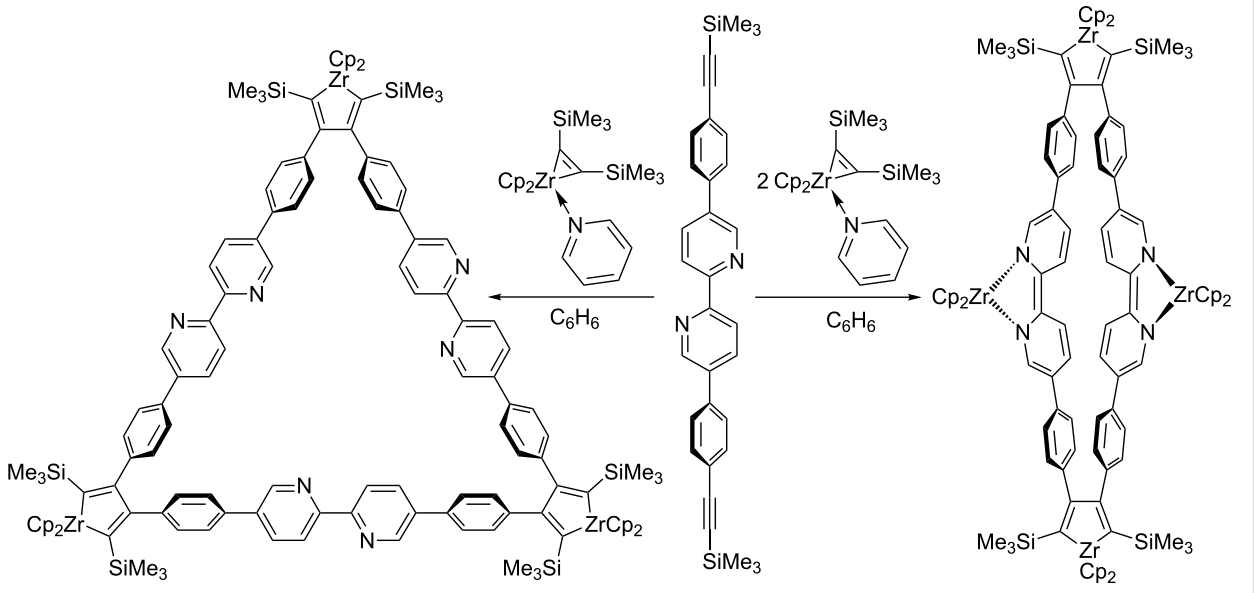
Under T. Don Tilley's supervision, my Ph.D. work involved the development of zirconocene-mediated macrocyclization reactions to make a series of new structures under thermodynamic control, two examples of which are shown in Scheme 1 [1,2].

This Ph.D. work gave me a taste for organic chemistry involving transition metals, and an interest in structures that form under conditions of thermodynamic equilibration.

Towards the end of my Ph.D. work, Jean-Marie Lehn gave a talk at Berkeley, a highlight of which was some of Bernie Hasenknopf's latest work on circular helicates [3,4]. I remember being greatly impressed by the intricacy and beauty of these assemblies, and struck by the relative simplicity of their precursors. I spent considerable time over the following weeks digging through the literature, which involved quite a bit more physical activity in those pre-digitization days, in pulling great volumes down from high shelves, reading the work, photocopying the most interesting papers, and tracing back through the references for other papers, to gain context and depth. I came away convinced that was the kind of chemistry that I wanted to do for a postdoc.

So I wrote to Jean-Marie, and ultimately was offered a place in his labs in Strasbourg. I asked two Berkeley alumni who had recently finished postdocs there about their experiences, and was advised quite strongly against going! It's too difficult to get work done there, they said, and the French don't like Americans. A bit of back-and-forth led me to conclude that I might make it through OK if I polished up my high-school French, and tried to tone down some of the American cultural characteristics that seemed least compatible with the French worldview – the 'in your face' attitude that can sometimes compel action in the US, I reckoned, would likely backfire in France.

After a slow start, I ended up getting some good results in a project related to some new dynamic-covalent grid complexes [5]. This work didn't come to fruition before I went onto the academic job market, however! I thus had few US interviews,



Scheme 1: Zirconocene-coupled dimeric (right) and trimeric (left) macrocycles [1,2].

and only one offer from Case Western by the end of my time at Strasbourg. I had also applied for a 'maître-assistant' position at the University of Geneva in Switzerland, thinking of it mostly as practice for the 'real' US interviews. The interview went well; I was promised full scientific independence and the opportunity to supervise two Ph.D. students. In the end I chose Geneva because everything I needed was there, the teaching load was relatively light, and it seemed that I might stand a better chance of recruiting good students there. The senior members of the Department and School also impressed me; it seemed that advice and mentoring would be available for the asking. These first impressions held up well with time.

The group's first two Ph.D. students were hired from the University of Strasbourg, where there was no shortage of very well qualified M.Sc. students in search of opportunity. I interviewed several and extended offers to the two best, David Schultz and Marie Hutin, who did not let me down. Their intelligence and hard work laid the foundations of the group's work to the present day. Just after concluding the interviews, I was called to Berne, where a senior chemistry professor interviewed me on the Swiss National Science Foundation proposal that I had submitted. I did not make out so well as Marie and David, being informed that the work that I proposed was much too ambitious – it would require the mastery of techniques and concepts to which I had never been exposed. "Write a new proposal," came the advice, "convince us that you can get good work done quickly with limited means."

Although this rejection was devastating, time has told that it was the best advice that I could have gotten at that point in my career, delivered in a context that I could not ignore. My senior colleagues at Geneva came through with bridge funding so that I could still give Marie and David their promised places – a kindness for which I remain grateful – and the next proposal I wrote obtained modest funding.

This proposal, and the research programme that followed, involved the use of old chemistry – Daryle Busch's metal-templated imine-bond forming reaction [6] – in new ways. We called it subcomponent self-assembly to emphasize the use of simple precursors to build complex products. The preparations and crystal structures of three of these products are shown in Figure 1.

Each of these products was prepared simply by mixing the precursors shown in water, and each represents a different way to arrange four metal ions in space, going from a one-dimensional linear array (Figure 1a) [7] to a two-dimensional grid (Figure 1b) [8] to a three-dimensional tetrahedron (Figure 1c)

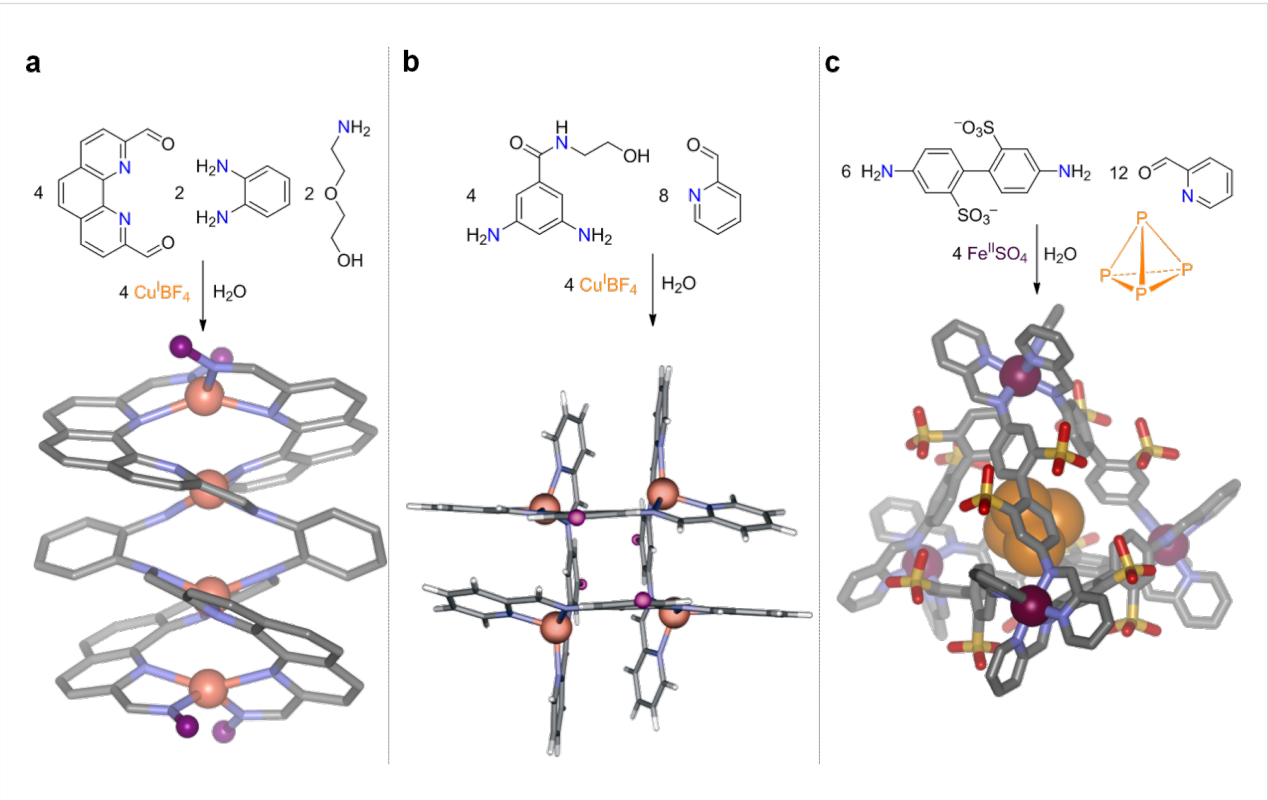


Figure 1: Complex structures prepared from simple subcomponents: a) Cu^{I}_4 helicate [7]; b) Cu^{I}_4 grid [8]; c) Fe^{II}_4 tetrahedral cage [9,10].

[9], which turned out to be capable of binding white phosphorus (P_4) and rendering it air-stable [10]!

The work went well at Geneva – we were publishing in good journals, and I managed to win a Swiss National Science Foundation Assistant Professorship, which would have allowed me to modestly expand the group. My senior colleagues praised my accomplishments, but advised caution: I was not on a tenure track, and no future at Geneva could be guaranteed.

I thus set about looking for new opportunities, and I applied for an open lectureship at Cambridge. I was delighted to get the job, although it meant leaving behind the funding I had just won in Switzerland.

I hadn't anticipated the psychological gulf between the relatively rosy Swiss funding situation and the harsh headwinds of UK academia – much less research funding to go round meant for much sharper competition! Proposal ideas that had been funded with great scores in Switzerland were mercilessly skewered when submitted to the Engineering and Physical Sciences Research Council (EPSRC), our primary UK science funder. Each failed proposal hurt badly, but I made common cause with others – banding together with Richard Layfield and Paul Lusby to protest our first grants' rejection to the head of EPSRC, for example. And I slowly got a sense of what a good grant proposal looked like by UK standards, and how to write one. We kept going in lab, seeking to generate new results of the kind that could underpin a successful UK grant proposal.

The story of P_4 encapsulation [10] garnered substantial positive attention, and an invited *Nature* Q&A piece on systems chemistry [11] probably helped, too. Ultimate success in attracting funding from the European Research Council (ERC) and EPSRC was the sweeter for the many failures that had preceded it. It has also been a delight to see others making use of subcomponent self-assembly to solve new puzzles, citing our development of the technique [12-25].

Over the past few years, we have developed a series of new functional structures. A leitmotiv of my group's work is the integration of a new class of hollow container molecules, invented by my group, into complex and dynamically-responsive systems and materials. Three of these containers are shown below: Figure 2a depicts an $Fe^{II}_8L_6$ cubic cage with walls constructed from porphyrins that binds guests such as fullerenes and coronene [26]; Figure 2b shows a $Co^{II}_{10}L_{15}$ pentagonal prism that embeds five anions, such as PF_6^- (shown) or ClO_4^- in its walls, and a sixth – usually chloride – in its center [27]; Figure 2c illustrates a $Fe^{II}_{12}L_{12}$ *pseudo*-icosahedron with *mer* stereochemistry to its Fe^{II} centers, shown encapsulating $B_{12}F_{12}^{2-}$ [28].

We also have a flourishing line of enquiry into conjugated polymers that are held together by metal-ion templation – the structure shown in Figure 1a was a key precursor to this work. In collaboration with Richard Friend in the Physics Department at Cambridge, we have built these polymers into devices that emit white light [29], or that show blue-shifted emission at higher voltages [30], intriguingly.

Key questions that my group and I hope to address over the next few years include:

1. How can we design a system of chemical assemblies to work together in a network, to accomplish a function collectively?

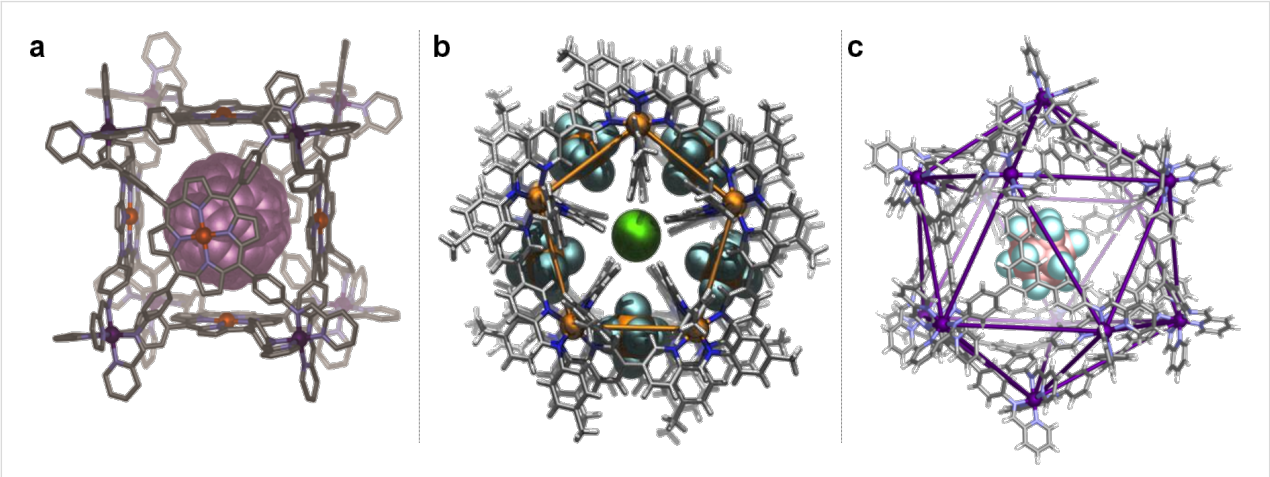


Figure 2: a) A cubic cage [26]; b) a pentagonal prism [27]; c) a *pseudo*-icosahedron [28].

2. Given that increasingly fine-grained control over self-assembled structure is being achieved, how can we design a self-assembling process with a target function in mind, such as light emission or the catalytic transformation of a substrate?

Both of these questions are predicated upon the idea of shifting intellectual effort away from designing and synthesizing complex molecules, and towards understanding and controlling the processes and systems of self-assembly.

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Smart molecules for imaging, sensing and health (SMITH)

Bradley D. Smith

Review

Open Access

Address:
University of Notre Dame, Notre Dame, IN 46530, USA

Email:
Bradley D. Smith - smith.115@nd.edu

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Abstract

This autobiographical review provides a personal account of the author's academic journey in supramolecular chemistry, including brief summaries of research efforts in membrane transport, molecular imaging, ion-pair receptors, rotaxane synthesis, squaraine rotaxanes, and synthtavidin technology. The article concludes with a short perspective of likely future directions in biomedical supramolecular chemistry.

Review

From the land of kangaroos to the home of the Fighting Irish

I grew up in Myrtleford, a small town in the foothills of the Australian alpine region (Australia has mountains?). The town population of ≈2,500 has hardly changed in fifty years, and I return there regularly to visit my parents. I attended the local schools and although I was clearly one of the smarter students, a recent review of written teacher comments reveals a propensity to be the class clown. Most of my teenage years were spent on the local golf course (Figure 1), but I was sufficiently successful in the statewide exams to gain entry to the University of Melbourne as an undergraduate science major. Mediocre performances in maths and physics soon left chemistry as the only viable option for serious advancement, and it was in my third year that I encountered my first research experience under the supervision of Dr. Roger Read (now at the University of New South Wales). The project goal was modest but I enjoyed the personal ownership and also the camaraderie of working in a large open lab. I was sufficiently successful to get accepted into a fourth year of study for B. Sc. Honors, and I joined the group of Dr. David Kelly and worked on an NMR project on the structure of carbocations. I was lucky enough to stumble upon an unusual naphthalenium rearrangement process and I worked hard enough to earn co-authorship of a full paper in the Journal of the American Chemical Society [1]. As the year progressed I began to think about future plans. It was very common for Australian college graduates to head overseas on lengthy world trips, but I greatly enjoyed my research experience, so a reason-

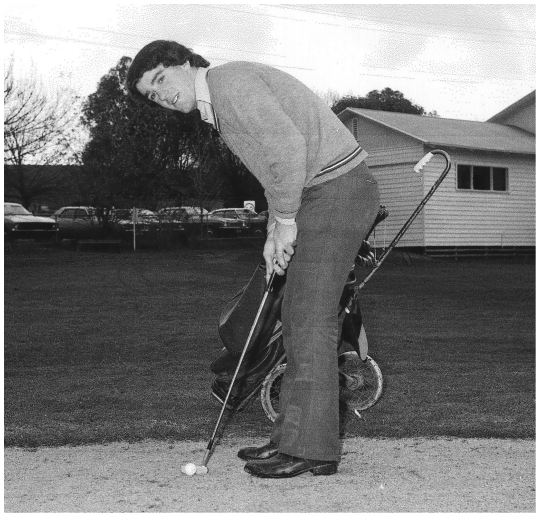


Figure 1: The author as a teenager in his school uniform, but on the nearby Myrtleford golf course.

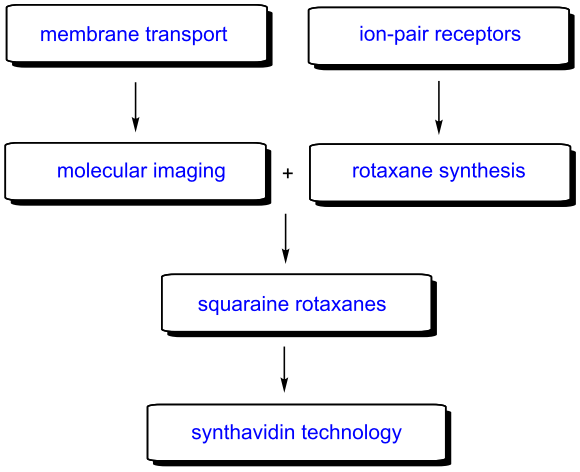
able compromise was graduate studies abroad. I met with Dr. Kelly for suggestions and the outcome was a plan to join the lab of his former mentor, Professor Lloyd Jackman, an Australian who had moved from Melbourne to Penn State University in the United States. Professor Jackman was a pioneer in the use of NMR methods for studies of organic chemistry, and my graduate project was to elucidate the aggregated structures of lithium enolates in a weakly polar solvents using a range of multinuclear NMR methods [2]. The work involved vacuum line preparation of the samples and extensive measurements of NMR parameters. Jackman was a wonderful academic advisor, a passionate scientist who also enjoyed sports and recreation. He was beloved by his students and respected by his colleagues as a scholar and a gentleman. A quote that I sometimes attribute to Jackman but it may in fact predate him is, "A physical organic effect has to produce more than a 1000-fold difference to be truly interesting". Upon graduation I moved to Oxford University in the UK for postdoc studies in the group of legendary Professor Jack Baldwin, at the height of his work on penicillin biosynthesis. The lab was full of very talented people, but the enormous size of the group made it unwieldy. After 15 months and a few quick papers I moved back to the US for a second postdoc with Professor Koji Nakanishi at Columbia University in New York City. Professor Nakanishi was a larger than life character, who formed strong bonds with his students and postdocs. He worked very long hours in an office that was open to the labs and often joined his students for meals or late evening drinks. He was a natural showman and dazzled his lecture audiences with magic tricks and engaging stories. The lab worked on an array of technically challenging projects concerning natural products isolation and their mode of action. Nakanishi worked closely with several pharmaceutical companies and it was inspiring to see the immense resources that large companies could focus on important pharmaceutically projects. So when I started looking for a permanent job, I was initially torn between academics and industry. But my interviews at universities seemed to progress more smoothly and in 1991 I accepted an attractive offer to start a tenure-track position in the Department of Chemistry and Biochemistry at the University of Dame, the home of the Fighting Irish.

My Ph.D. and postdoc training covered a wide range of physical organic and bio-organic topics but none of them could be considered supramolecular chemistry. So it was a little risky to start an independent academic career in a field with no previous experience. Indeed my new lab had to start from the beginning and learn all the rudimentary supramolecular experimental techniques such as measuring binding constants and conducting transport experiments. The lack of background knowledge was an obvious impediment, but at times it was helpful because I was not biased to revisit old supramolecular problems from the previous decade. As I look back at my independent research publications, it seems that the more highly cited papers were often projects that required the lab to learn a new experimental technique or move into a completely new research area. So while the learning curve was steep, the eventual reward was a more impactful outcome.

Major research projects

In Scheme 1 is a flow diagram of major topics that my research group has pursued over the last 25 years. I maintain a group of about ten full-time co-workers, along with a handful of undergraduate researchers, and at any time about two thirds of the co-workers are organic chemists and the rest are biochemists. Most of the projects have started out as structure design problems where the goal was to prepare a set of organic molecules with specific supramolecular functions. Once the molecules were made they had to be tested and in the early days this typically involved simple test-tube studies, but increasingly the testing systems became more biological and more relevant to human health. This required us to collaborate with an expanding number of research specialists. Collaboration can be highly rewarding and without doubt it is the best way to maintain an internationally competitive program in modern supramolecular chemistry. But young investigators must quickly recognize that collaboration is an exercise in human relationships, and it works best when there is obvious benefit to both parties and strong lines of communication.

The following sections expand on Scheme 1 and provide short summaries of the major research projects. The main purpose is to illustrate the chronological flow of thoughts and events that led me to pursue a series of supramolecular ideas that may seem



Scheme 1: Chronological progression of Smith group research projects.

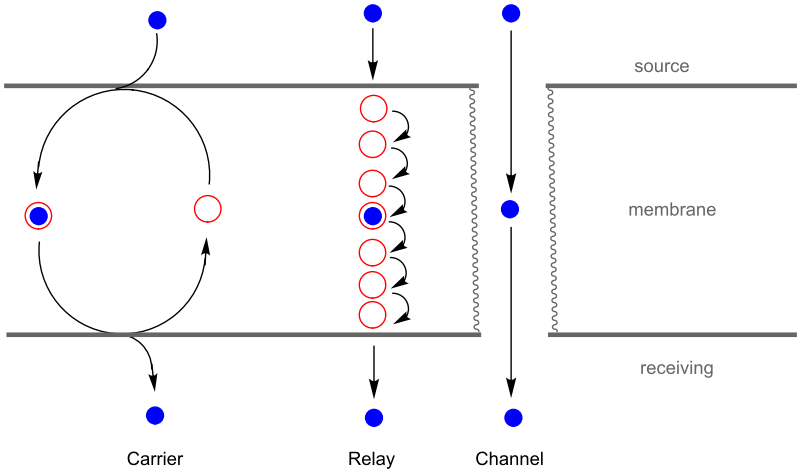
from the outside to be disconnected. Since this article is highly autobiographical, it does not cite all of the seminal and inspirational work done by other research groups, and I apologize for any distortion of credit. To assist the interested reader, each research topic includes references to relevant review articles by leading groups in the field.

Membrane transport

Molecular transporters that alter the concentration gradients of anions and biomolecules across a cell membrane have various biological effects as reagents for cell biology research and as potential pharmaceuticals [3,4]. In the early 1990's, most of the supramolecular research on facilitated membrane transport using carrier molecules focused on metal cation transport. As a young professor, with an inexperienced group, we started out looking at synthetic transport carriers for biomolecules such as sugars, nucleotides, amino acids, and catecholamines [5] (Scheme 2). Later on we started looking at chloride transport and we enjoyed a very productive collaboration with the British group of A. P. Davis to develop a series of mobile carriers for chloride ions across liposome and cell membranes [6]. There continues to be a strong community interest in chloride transporters as they are expected to exhibit interesting biological activity [7]. In addition, my group developed some of the first synthetic molecules to promote flip-flop of phospholipids across cell membranes, and thus alter the membrane structure and function. In particular, we discovered compounds that can scramble the transmembrane distribution of phosphatidylserine, a crucial signaling phospholipid. The synthetic scramblases make phosphatidylserine appear on the surface of cells and induce subsequent secondary biological signaling processes such as blood clotting and cell clearance by macrophages [8].

Molecular imaging

Our work on synthetic phospholipid scramblases required us to develop methods for quantifying phospholipid levels on the surface of biological membranes [9,10]. Biomembrane science is very challenging because the self-assembled bilayer is a soft and dynamic object that is hard to characterize with spectroscopic methods. To paraphrase a former US president, "we pursue biomembrane science not because it is easy but because it is hard" [11]. We realized that most of the targeted fluorescent molecular probes for phospholipids were derivatives of large protein systems and we felt that we could devise small synthetic mimics of the binding pockets. Our primary phospholipid target was phosphatidylserine, which gets exposed on the cell surface during the process of apoptosis or programmed cell death. Thus, phosphatidylserine is an excellent biomarker of



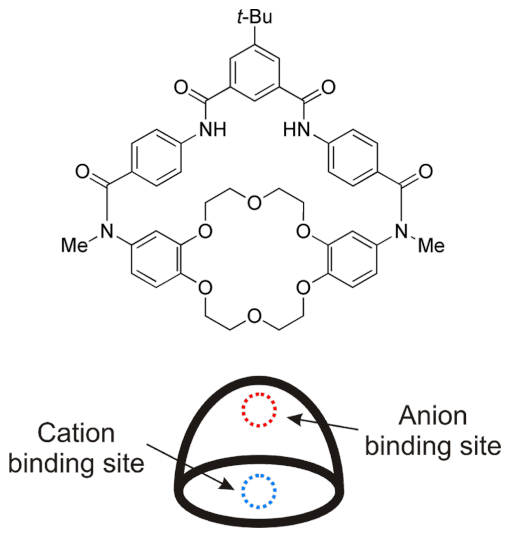
Scheme 2: Molecular transporters promote translocation of ions or hydrophilic biomolecules across a synthetic or biological membrane.

cell death and an attractive target for molecular imaging. We reasoned that synthetic anion receptors such as zinc dipicolylamine (ZnDPA) coordination complexes would selectively recognize the anionic head group of phosphatidylserine [12]. Furthermore, binding strength is amplified by electrostatic attraction of the cationic receptor to the apoptotic cell membrane with a negative surface potential. We developed a family of fluorescent ZnDPA probes for fluorescence microscopy and flow cytometry methods for preclinical research [13-16] (Figure 2). Many of these fluorescent probes are used as imaging reagents to quantify the level of cell death in a range of biomedical samples [17]. We also developed some nuclear isotopic labeled probes for in vivo imaging of living subjects [18]. An eventual goal of this ongoing research project is to invent clinical imaging methods that will improve patient care by rapidly evaluating the efficacy of cancer treatment or the extent of cardiovascular disease.

The capability of these ZnDPA probes to target anionic cell surfaces led us to pursue molecular imaging of microbial infection, an unsolved research problem of high biomedical significance. We demonstrated targeted optical imaging of bacterial infection in animal models and subsequently developed optical probes for photodynamic therapy of bacterial infection [19-22]. The latest discovery is a set of molecular ZnDPA probes that selectively target parasite infections in living subjects such as Leishmaniasis, a lethal disease that afflicts many millions of people around the world [23].

Ion-pair receptors

Our early experience with membrane transport raised awareness of the need for transport carrier molecules that could simultaneously complex both the anion and the cation [24,25]. Starting in 2000 we prepared a number of relatively simple macrobicyclic receptors that could bind salts as either contact ion-pairs or as solvent separated ion-pairs [26-28] (Scheme 3). Many X-ray crystal structures were acquired and they provided excellent atomic scale insight. The liquid extraction and membrane transport properties were evaluated and we demonstrated symport processes that used anion concentration gradients as energy sources to drive cation transport uphill [29].



Scheme 3: Macrocyclic receptor that binds solvent separated ion-pairs.

Rotaxane syntheses

Building on literature ideas about “wheeled nucleophiles [30,31].” we prepared the reactive ion-pair in Scheme 4 and trapped the bound phenolate as an uncharged [2]rotaxane struc-

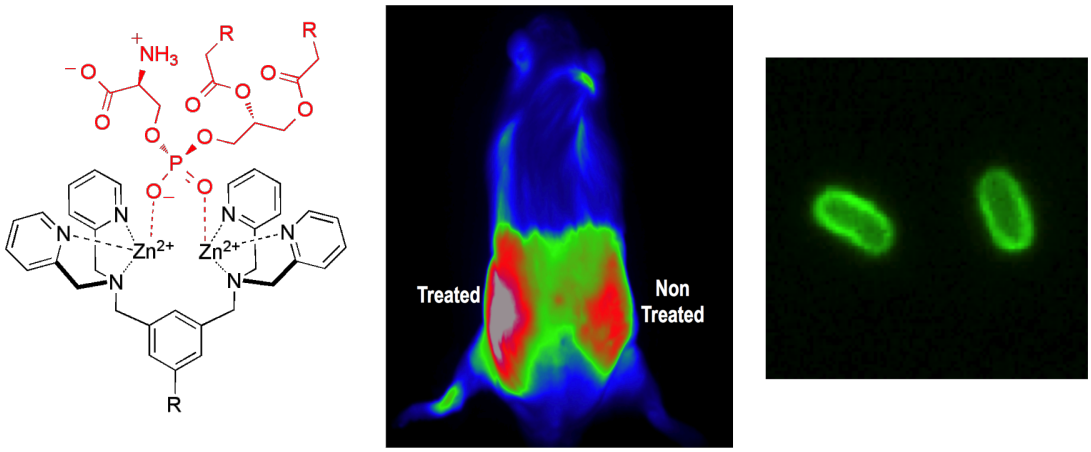
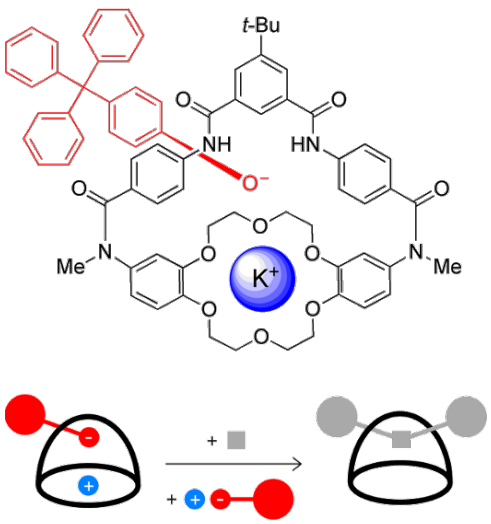


Figure 2: (left) Association of ZnDPA probe with phosphatidylserine head group. (middle) False colored fluorescence image of a living rat bearing two tumors. There is increased accumulation of the cell death probe in the tumor that has been treated by focal beam radiation. (right) Fluorescence image of bacterial cells stained with fluorescent ZnDPA probe.



Scheme 4: Trapping a macrocyclic receptor containing a reactive ion-pair produces an interlocked [2]rotaxane.

ture [32]. The ability of the macrobicyclic receptor to accommodate a solvent separated ion-pair was vital for efficient rotaxane formation. The interlocked molecule retained its salt binding ability and association of the ions modulated the rotaxane structural dynamics [33,34].

Squaraine rotaxanes

The interests in molecular imaging and rotaxane structures merged in 2005 with the discovery and development of squaraine rotaxanes as a novel family of deep-red fluorescent dyes with extremely high brightness and stability [35,36]. A key finding was the importance of the interlocked rotaxane structure for protecting the encapsulated squaraine from chemical attack by water. Squaraine rotaxanes can be conjugated with targeting ligands, and bioimaging studies have shown that they enable fluorescence microscopy and mesoscale imaging of diverse biomedical targets such as tumors, infection, bone, cell death, and brown adipose tissue [37,38] (Figure 3). Several of these molecular probes are commercially available for preclinical research applications including acceleration of the anticancer and obesity drug discovery, facile monitoring of bone growth, and next-generation fluorescence-guided surgery [17]. An off-shoot of this work was discovery of novel chemiluminescent squaraine rotaxane structures [39]. Nanoparticles containing these self-illuminating molecules enable high sensitivity imaging of deep-tissue target sites in living subjects [40].

A recent spin-off from the squaraine rotaxane project uses homologous croconaine dyes to absorb 800 nm laser light and cleanly convert the energy into heat without producing reactive singlet oxygen [41-43]. The dyes enable new types of nanoscale

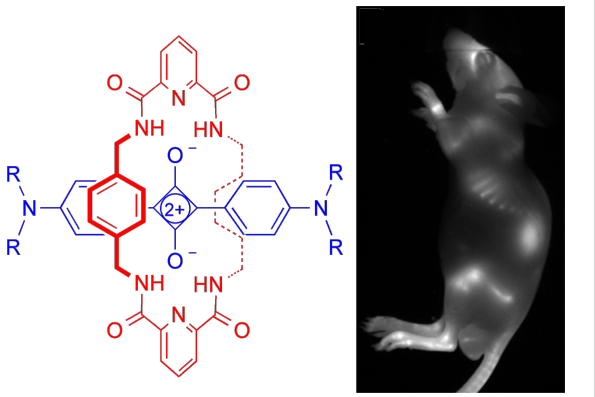
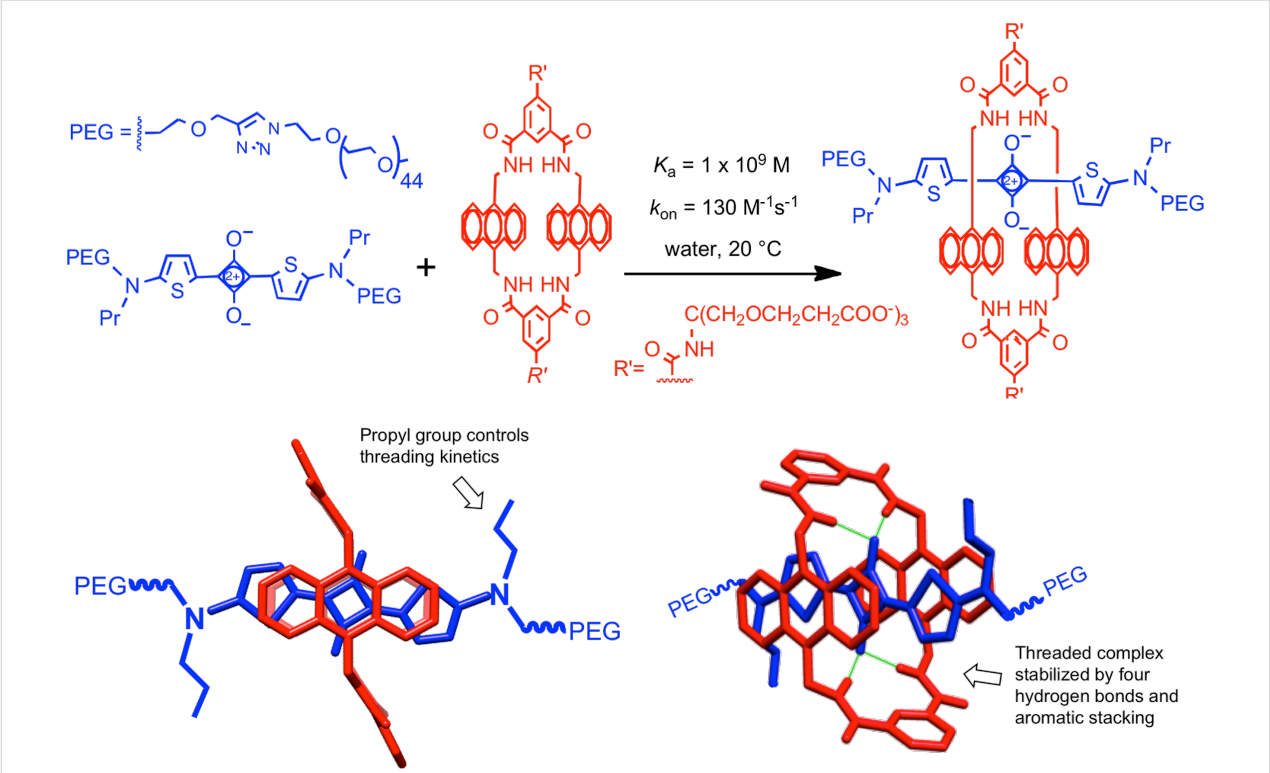


Figure 3: (left) General structure of a squaraine rotaxane dye. (right) Fluorescence image of a living mouse dosed with a squaraine rotaxane probe that selectively targets bone. Reprinted with permission from [37]. Copyright (2013) American Chemical Society.

heating technologies that release sensitive payload such as dyes, drugs, oligonucleotides, or proteins. The dyes can also be loaded into nanoparticles for anticancer photothermal therapy in preclinical animal models. Another use for these dyes is as contrast agents for photoacoustic imaging where short laser pulses are converted into sound waves that are detected using an ultrasound scanner.

Synthavidin technology

While working with the molecular building blocks to make squaraine and croconaine rotaxanes we discovered a remarkable self-assembly process that we call Synthavidin (synthetic avidin). A simple example is illustrated in Scheme 5 [44]. A water soluble tetralactam macrocycle with anthracene sidewalls is threaded by squaraine dyes that are flanked by long PEG chains to give highly stable complexes with nanomolar dissociation constants. The rate of macrocycle threading is insensitive to the length of the appended PEG chains. But the threading kinetics are greatly affected by the steric size of the second *N*-substituent at each end of the squaraine dye, and an *N*-propyl group produces a perfect mixture of kinetic and thermodynamic properties. The nanomolar affinity in water is exceptionally strong for a synthetic cyclophane host molecule, and it is driven by a large favorable change in enthalpy. The two oxygen atoms on the encapsulated squaraine dye form hydrogen bonds to the four macrocycle NH residues and there is coplanar stacking of the squaraine aromatic surfaces with the anthracene sidewalls of the macrocycle. We are beginning to use Synthavidin technology as a self-assembly platform to rapidly fabricate libraries of targeted multivalent probes for fluorescence imaging of over-expressed receptors on the surface of cancer cells. We are hopeful that the probes will be useful for therapeutic applications such as fluorescence-guided surgery and intra-operative photodynamic therapy.



Scheme 5: (top) Basis of Synthavidin technology. A fluorescent squaraine dye that is flanked by PEG chains can rapidly thread a tetralactam macrocycle in water to produce a highly stable complex. (bottom) Two views of the threaded complex highlighting the structural features controlling the threading kinetics and thermodynamics.

Life as an academic supramolecular chemist

This is my 25th year at the University of Notre Dame, which is located in South Bend, Indiana, 90 miles from Chicago. My wife's parents live a few hours away and my two daughters are presently undergraduates at the university. Typically, our family holidays are spent within Indiana or we travel all the way to Australia. Travel is an integral part of academic research, and it is always enjoyable to visit a new part of the world. I have little ability to learn new languages and I am very grateful that English is the universal language of science. I can hardly imagine the challenge of writing papers and presenting lectures in a non-native language. In the summer time, my primary source of recreation is golf or cycling. In the Fall, many weekends are filled with the social aspects of college football. It may hard for non-American readers to imagine tail-gate parties in the campus parking lots with tens of thousands of like-minded fans. But college football is essentially the university's social season and it provides a great backdrop for hosting visitors to the campus.

A trend that often occurs over the span of an academic career is a change in the primary motivation for conducting research. The goal of most young investigators is to achieve a list of well-defined accomplishments (obtain a major grant, publish high impact papers, get promoted, speak at major conferences, etc.) but for senior investigators there is often a growing desire to make a broader contribution that has lasting impact. This contribution could be in any of the three major components of academic life, namely, research, teaching, or administration. It is my observation that chemists often are well-suited for administration, most likely because they have experience managing relatively large research groups. In my own case, I serve my university as Director of the Notre Dame Integrated Imaging Facility, a campus wide research core that houses about a dozen major instruments that are maintained by seven staff members (Figure 4). It has been very rewarding to create this facility and see it grow to help the broader research effort of the university. Of course, the most obvious way to make a significant contribution to academic research is to publish high impact papers, and there is tremendous gratification when other investigators employ ideas or techniques that were first developed in your own lab. A tangible outcome of our lab is production of new fluorescent molecular probes and I am proud that more than dozen of our probes are commercially available and used by researchers around the world [17].

The US is an exciting place to conduct academic research, especially for a young investigator since there is opportunity to

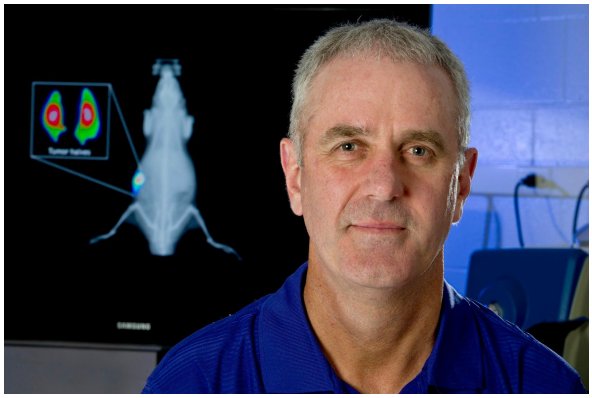


Figure 4: The author as director of the Notre Dame Integrated Imaging Facility.

quickly build a large independent research group. But the US granting system has strong Darwinian elements and there is always pressure to procure research funding. The grant writing process can sometimes be tiresome, but it also can be enlightening. As a reminder to always view the glass as half-full, I have the following quotation posted in my office; "In academics, the situation is often desperate but never very serious". In other words, I am a tenured professor who has the privilege of spending every day with bright young students who have open minds and a desire to learn. My job is to guide them and to channel their optimism into fulfilled potential. There is no doubt that at career-end, my greatest overall contribution will be the outstanding group of young scientists who have trained in my lab and gone on to make important contributions to their families, communities, and broader society.

Future directions in biomedical supramolecular chemistry

Recently, I co-authored a book chapter entitled "Applications of Synthetic Receptors for Biomolecules [45]." The chapter grouped the most common applications into four general groups; Separations, Imaging and Sensing, Catalysis, and Pharmaceutical Activity – important topics that will continue to attract the attention of many supramolecular chemistry labs around the world. In addition, the chapter provided a short summary of four emerging themes in biomolecule recognition that are likely to garner increased attention over the next decade; Logic Devices, Biomolecule Responsive Materials, Drug Delivery, and Biomolecule-Fueled Molecular Machines. While there are presently "proof of concept" studies for each of these research themes [46,47], I feel there is a need to advance beyond prototype systems. If supramolecular chemistry is to maintain its legitimacy as a field worthy of major academic and industrial investment, it has to clearly show a pathway that leads to valuable new technologies.

Effective biomolecule recognition requires both strong affinity and high selectivity, and for investigators who wish to develop association systems that operate effectively in water I offer two words of advice "surface area". In 2003, Houk and coworkers published a survey of all known natural and synthetic host/guest binding systems and concluded that the best predictor of strong affinity is the amount of solvent accessible surface area that is buried upon binding [48]. Typically, this surface area is amphiphilic; that is, it contains a mixture of polar and non-polar functional groups. In water, the non-polar groups drive affinity due to hydrophobic effects, and the polar groups produce selectivity due to directional interactions, such as hydrogen bonding. It is insightful to consider the protein–protein recognition and self-assembly processes that are crucial for cell growth and signaling. These precisely controlled association systems have been refined over billions of years of evolution. The selective recognition is driven by protein–protein interactions that operate at an interface with a surface area that is usually in the range of 1200 to 2000 $Å^2$. This is more than an order of magnitude greater than the surface area of the largest synthetic molecular hosts, and I think there is an important underlying message here – to achieve biologically relevant binding systems with truly useful dynamic properties, the supramolecular community should build much larger synthetic hosts. How can this be done? One approach is to create covalently linked polymers that are programmed to fold up into three dimensional structures that mimic the topologies of proteins and nucleic acids. Perhaps a more facile fabrication strategy is to employ hierarchal self-assembly methods to create robust nanoscale architectures with precisely positioned functional groups. I am attracted to this latter strategy for enhanced biomolecule recognition and indeed our emerging efforts with Synthavidin technology are small steps in the general direction. This vision merges classical supramolecular chemistry with materials self-assembly. The projects will be technically challenging, as they will require nanoscale characterization methods that are not familiar to most small molecule chemists. Thus, collaboration is likely to be a key element for this type of multiscale work. Intellectually, the investigators need to broad minded and willing to learn the language and culture of other research fields. Early success is not guaranteed but the journey will be fascinating.

Acknowledgements

First and foremost, I am extremely grateful to all of the students who have worked in my lab over years, and the names of many are listed in the references. It has been a sincere pleasure to know each one and I have high expectations for their future success. I also wish to acknowledge my administrative assistant, Theresa Bollinger, for her outstanding attention to detail and professional office management. I am also grateful to the University of Notre Dame for providing an excellent research

infrastructure and my colleagues in the Department of Chemistry and Biochemistry for providing a collegial work environment. Most of the projects described in this article were supported with funding from the NSF or the NIH (USA).

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Learning from the unexpected in life and DNA self-assembly

Jennifer M. Heemstra

Review

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Address:
Department of Chemistry and the Center for Cell and Genome Science, University of Utah, 315 S 1400 E, Salt Lake City, Utah 84112, United States

Email:
Jennifer M. Heemstra - heemstra@chem.utah.edu

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Abstract

The greatest lessons in life and science often arise from the unexpected. Thus, rather than viewing these experiences as hindering our progress, they should be embraced and appreciated for their ability to lead to new discoveries. In this perspective, I will discuss the unexpected events that have shaped my career path and the early stages of my independent research program.

Introduction

"Jennifer is not skilled at science." This judgment was delivered upon me at the age of fourteen by my eighth grade science teacher, and was part of a recommendation that I not be placed on the science-intensive track of study as I entered high school. The extent to which this recommendation limited my access to science courses should have effectively ended any chance of my going on to pursue a career in this field. However, this event instead allowed me to view science as a fun hobby instead of a required subject, which led me to discover my love of science and began to pave the road to my future career in chemistry. At every step along this road, I benefited from fantastic mentors who shared with me their enthusiasm for life and learning. I also grew to recognize my love of supramolecular chemistry, and this has been the constant theme driving my research choices throughout my career. Much like a negative judgment about my aptitude for science unexpectedly led me to a career in chemistry, my lab has found that our greatest insights into the molecular recognition and self-assembly properties of DNA have come from unexpected results or failed experiments.

Review

Entering high school, I knew that I enjoyed math, but was unsure of my career goals. Looking for a way to stay entertained after school, my friends suggested that I join them on the Science Olympiad team. My response was that this was obviously a terrible idea, as I was "not skilled at science." They thankfully convinced me to join despite this, and in Science Olympiad, I found my first true mentor, Dr. Marcia Sprang. Dr. Sprang taught the advanced Chemistry and Physics courses at my high school, and also coached the Science Olympiad team. Over the four years that I was involved in Science Olympiad, Dr. Sprang convinced me that you don't need "skill"

to be a scientist, but rather you just need a love of learning and a passion for discovery. During this time, she also served as a model for the type of mentor that I would later hope to become – providing consistent encouragement and wisdom, while teaching students that through hard work, they can achieve things that they never thought possible. Reflecting on this story, I have only recently come to appreciate that had I been labeled "skilled at science," I would have felt tremendous pressure to excel in this area, which likely would have killed my enthusiasm for the subject. However, being told that I had no natural aptitude for the subject removed this pressure, allowing me the freedom to pursue science for the pure enjoyment of learning.

Entering college at the University of California, Irvine, I brought with me the love of science instilled by my experiences in Science Olympiad and the mentoring of Dr. Sprang. However, I was still unsure which area of science I wanted to pursue. I began my college career convinced that I wanted to major in Biology, but quickly realized that this was not the right fit. As I found myself adrift and trying to formulate a new career plan, I decided to dispatch with the required Organic Chemistry courses, which were almost universally dreaded by my fellow Biology majors. I expected these courses to be difficult and frustrating, but instead found that learning about organic molecules and solving the complex puzzle of multi-step synthesis was fun and gratifying. Around this same time, I decided to begin working towards my honors thesis, which required me to find a lab where I could do research. Considering the amount of fun I had in my Organic Chemistry course, I thought that it would be interesting to experience research in a chemistry lab. I read through all of the faculty research descriptions, and while I was still years away from recognizing an underlying love of supramolecular chemistry, I was immediately drawn to the work of Prof. James Nowick, whose lab was focused on constructing and studying artificial β-sheet structures.

I am thankful that James provided me the opportunity to join his lab, as it was this experience that brought me from thinking of chemistry as a "fun course that I took" to something that I might want to spend my life pursuing. Even though I was just an undergraduate student, James (and my postdoctoral mentor, Dr. Mark Wilson) provided me with a significant amount of freedom in the lab. The ability to formulate a hypothesis, design experiments, and then test whether or not my ideas would work afforded me a sense of satisfaction that was entirely new and utterly intoxicating. This experience not only convinced me that I wanted to pursue a Ph.D. in Chemistry, but also continues to influence my mentoring style in my independent career. Specifically, I recognize that what convinced me I wanted a career in research was the freedom and autonomy of asking and answering my own questions, even just in the initial context of troubleshooting a project that had been designed for me. As a result, I not only have a great enthusiasm for mentoring undergraduate students in my own lab, but I place a high value on giving each student their own project, or a distinct piece of a larger project, so that they also can experience this joy of autonomy. As I began to ponder the next stage of my career, I recognized a second, very important lesson that I had learned from James – the importance of working for wonderful people. While the process of discovery was what had made me fall in love with research, having an advisor who was enthusiastic and supportive was what made it fun to come into lab each day. Even more importantly, I recognized that even though I was only an undergraduate researcher, James would be an ally and supporter throughout my career.

By the time that I was choosing a graduate program, I had learned the phrase "supramolecular chemistry" and recognized my excitement for the process of designing, building, and studying functional molecular architectures. In the process of researching graduate programs, I read a series of papers from the lab of Prof. Jeff Moore at the University of Illinois describing their pioneering work on helical phenylene ethynylene foldamers. As with many things in my scientific career, I was drawn to this work because it was "just *so* cool." While I was still an undergrad, James offered to introduce me to Jeff by email, and this started a series of conversations about exciting future project ideas. I also came to realize that Jeff was exactly the type of mentor I hoped to find in graduate school, as he was a genuinely kind person, providing encouragement to the students in the lab, but also providing them the freedom to develop into independent scientists. On my first day in Jeff's lab, I was given the freedom to choose which new idea I wanted to pursue, and every day after that, I was given the freedom to stumble, make mistakes, figure things out for myself, and ultimately "learn how to learn." This process that Jeff fostered in his lab now forms the core of my mentoring philosophy. The part of my job I enjoy most is discussing research (and life in general) with the students and postdocs in my lab, especially since I am fortunate to have a research group that is filled with creative, motivated, and independent-minded scientists. Inspired by my time in the Moore Group, a large fraction of these conversations end with me saying something along the lines of "there must be a way to do this, but I don't know exactly how – you get to go and figure it out." My proudest moments are when one of my group members has taken on one of these challenges, mastered things that I have no idea how to do, and then they spend our lab meetings teaching these new concepts and ideas to me and the rest of the lab. In my opinion, achieving this level of independent learning and thinking is the pinnacle of success in a Ph.D. career.

During my Ph.D. studies, I had tremendous fun designing and studying organic foldamers, but I also began to be attracted to the especially privileged molecular recognition and self-assembly properties of nucleic acids. I think that Jeff actually recognized this before I did, as he chose the additional faculty members on my thesis committee to be Profs. Steve Zimmerman and Scott Silverman, who are both leaders in the field of nucleic acid molecular recognition. As my graduation neared, Jeff, Steve, and Scott all enthusiastically encouraged me to pursue postdoctoral research and then a career in academia. After a brief detour into industry while I waited for my husband to finish his Ph.D., I was fortunate to have the opportunity to join the lab of Prof. David Liu at Harvard University. Considering that I had made an early departure from my Biology major as an undergrad, I now found myself working in a chemical biology lab with almost no formal training in molecular or cellular biology. Fortunately, David provided me the freedom to make mistakes and learn from those around me, which allowed me to grow in my knowledge of this field that was almost entirely new to me. Additionally, David and my other former advisors provided me with critical mentoring as I went through the process of applying, interviewing, and negotiating for an academic position. This experience further reinforced to me the value of having great people on your side as you strike out into the world on your own.

Looking back on my early career, it is clear that gender and work-life balance issues have in many ways shaped the path that I have taken. I have expressed my perspective on some of these issues in depth elsewhere [1], and thus will only mention the topic briefly here. The data show that there is significant progress that still needs to be made to increase diversity and accommodate work-life balance in the sciences [2], and arguably much of this change needs to be wrought at the institutional level. However, I hope that my story demonstrates that every individual can make a tremendous impact in the areas of diversity and equity through the mentorship and advocacy that they provide to others.

As the students in my own lab near graduation and start to consider their next move, I encourage them to choose the place where they know they will thrive, both scientifically and personally. Not surprisingly, choosing to work for a wonderful mentor or at a company with supportive management is a central part of this advice. I know that I would not be where I am without the mentors who continue to support, encourage, and serve as role models for me in my independent career. I am honored to now have the opportunity to pay that debt forward by serving as a mentor to students and postdocs in my own lab, and it is my great hope that as these individuals go out from my lab, they will propagate this legacy of positive mentoring as they themselves move into positions of leadership.

In starting my independent career at the University of Utah, I never intentionally made a decision to work in a specific area of science. Instead, I brainstormed to generate a series of ideas, then narrowed down my list to the few that I was most excited about. This ended up being an interesting process, as I was able to look at the result and gain insight into who I was as a scientist, at least at that moment in my career. All of the project ideas that made the final cut involved using a combination of molecular recognition, self-assembly, and nucleic acids to build functional architectures for applications in biosensing or bioimaging (Figure 1). As my research group translated these (and many of their own) ideas into actual experiments, we began to recognize a paradigm in which our projects are designed with an applica-

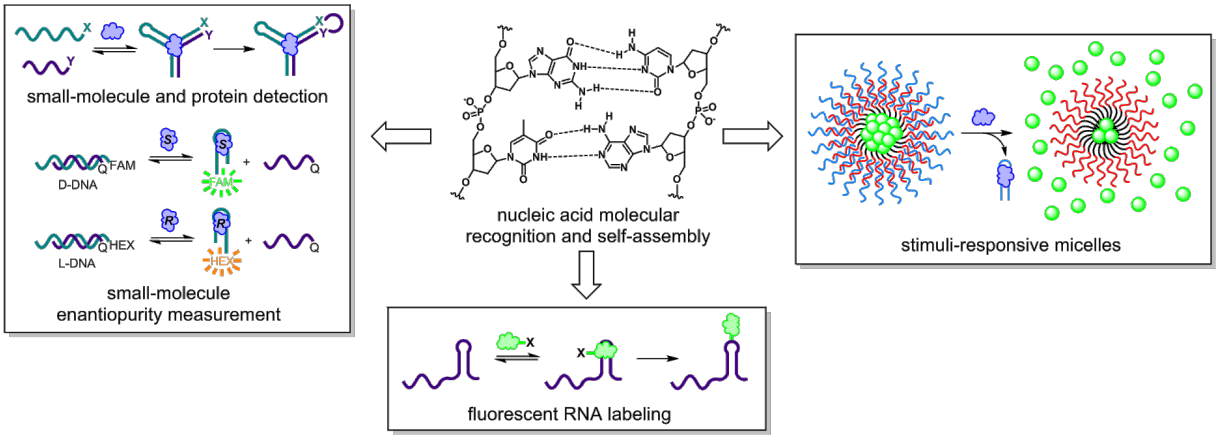

Figure 1: The common thread that is woven throughout our research program is the utilization of nucleic acid molecular recognition and self-assembly to generate functional architectures for biosensing and bioimaging. Adapted with permission from [3]. Copyright (2014) American Chemical Society. Adapted with permission from [4]. Copyright (2015) American Chemical Society.

tion in mind, as we want to make things that will benefit society. However, while we are excited when one of our ideas works as planned, some of our greatest moments are when something doesn't work for an unexpected reason. These "failed" experiments, while initially frustrating, have frequently led us to learn something new about the interactions of nucleic acids with each other or with ligands such as small molecules. These new insights are often the key to making a project successful, and sometimes even inspire the design a new project, but they can tend to get lost among all of the data in a manuscript, or relegated to the supporting information. Thus, in this perspective article, I am excited to have the opportunity to highlight some of these stories of unexpected results and what we learned from them.

During my initial brainstorming of project ideas, I was very drawn to the idea of working with DNA split aptamers. These recognition elements are comprised of two DNA (or RNA) sequences that selectively assemble in the presence of a small-molecule or protein target [5,6]. Thus, they combine my two favorite themes, as they use molecular recognition to drive self-assembly. At the point that we began our research program, all of the reported work on split aptamers had focused on detecting non-covalent assembly for systems at equilibrium [7]. Building upon this work, we were intrigued by the question of whether these DNA assembly events could be covalently trapped, which would allow the initial binding event to be "remembered" even if the target became unbound from the split aptamer. We reasoned that this might improve the sensitivity of split aptamer-based detection assays, and also that by converting the presence of a target molecule into the output of a DNA ligation event, we might be able to use split aptamers in assay formats that were previously inaccessible with these affinity reagents.

In our first attempt to demonstrate this principle of split aptamer ligation, we functionalized one fragment of the cocaine-binding DNA split aptamer [8] with a cyclooctyne and the other fragment with an azide. Although the cyclooctyne and azide are inherently reactive towards one another [9], we hypothesized that in the context of the free split aptamer fragments at low concentrations, the second order reaction would be relatively slow; however, addition of cocaine would drive assembly of the DNA strands, placing the two reactants in close proximity to one another and thus dramatically increasing the reaction rate (Figure 2). In our first experiment, we tested the hypothesis that the untemplated second order reaction would be sufficiently slow to prevent accumulation of the background signal. We were surprised to observe significant ligation between the split aptamer fragments, even in the absence of cocaine. While our initial thought was that this background was the result of a second order reaction between the functional groups on the DNA strands, this was quickly disproven, as we found that the untemplated ligation yield was not dependent upon DNA concentration. This led us to take a step back and think more critically about the fundamental principles behind split aptamer assembly. We quickly recognized that the function of split aptamers relies on a thermodynamic balancing act in which the enthalpic gain of base pairing and base stacking in the assembled state is weighed against the entropic cost of assembly. According to this logic, split aptamers will function best when the enthalpy for hybridization between the DNA strands is tuned such that in the absence of the ligand, this enthalpic gain is not quite sufficient to overcome the entropic cost of assembly. This poises the DNA strands at the brink of assembly, where the small amount of additional enthalpy gained through target binding can dramatically shift the equilibrium to favor the assembly of the split aptamer. To test this hypothesis, we carried out a simple experiment in which we measured the ligation yield for the two split aptamer fragments in the absence of cocaine, but in the presence of varying concentrations of sodium chloride, as increasing ionic strength increases the enthalpic gain for nucleic acid duplex formation [10]. We found that the ligation yield consistently increased with increasing ionic strength, which served as an initial validation of our hypothesis regarding the thermodynamics of split aptamer assembly. In the short term, this allowed us to overcome the challenge of background signal by reducing the ionic strength, and we were delighted to observe dose-dependent ligation of the split aptamer fragments with increasing concentrations of cocaine [11].

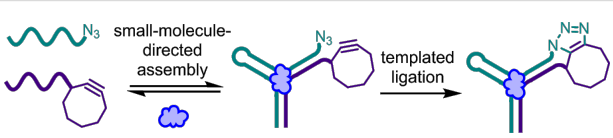

Figure 2: Split aptamers use molecular recognition to drive the assembly of two DNA strands. Placing reactive functional groups at the termini of the split aptamer fragments enables these assembly events to be covalently trapped. Reprinted with permission from [11]. Copyright (2011) American Chemical Society.

While the insight we gained into balancing the thermodynamics of split aptamer assembly seemed fairly straightforward, the lessons we learned from this unexpected experimental result played a critical role in our success with many of the experiments that soon followed. Having recognized that split aptamer assembly could be tuned much like the dial on a radio, we soon became enthralled by our ability to shift the equilibrium for this assembly process in predictable ways. As described above, this was initially achieved by simply changing the ionic strength of the solution. However, when we moved to experiments in biological fluids, where the ionic strength is difficult to change, we

began to explore the tuning of enthalpy through rational mutations to the DNA sequences themselves. We were gratified to find that by adding or taking away base pairs, we could reliably tune the equilibrium for assembly of the split aptamer fragments. This provided us with the power to adapt the cocaine split aptamer to function with optimal signal-to-background in higher ionic strength samples such as human blood serum and artificial urine media [12].

Our initial proof of concept experiments demonstrated that we could use our split aptamer ligation method to measure the concentration of a small-molecule target, but doing so required analysis via gel electrophoresis. Thus, we sought to create an assay that would be capable of the high throughput needed for clinical diagnostics applications, and we were specifically drawn to the enzyme-linked immunosorbent assay (ELISA) that is the current gold standard in this field. As shown in Figure 3, we constructed our enzyme-linked assay by immobilizing one fragment of the split aptamer on the surface of a microplate, then adding the test sample along with a solution containing the other split aptamer fragment functionalized with a biotin. In the first step of the assay, the concentration of the target molecule is transduced into a dose-dependent ligation of the split aptamer, which then allows for pull-down of a streptavidin-functionalized reporter enzyme to provide a colorimetric output [13]. This assay format highlighted the utility of our covalent trapping approach, as enzyme-linked assays typically require multiple washing steps, and loss of target binding during these steps results in loss of signal. In contrast, our method enables target binding events to be "remembered" through covalent trapping of the assembled split aptamer. Thus, the ability to generate target-dependent signal is preserved even if target binding is lost in the washing steps.

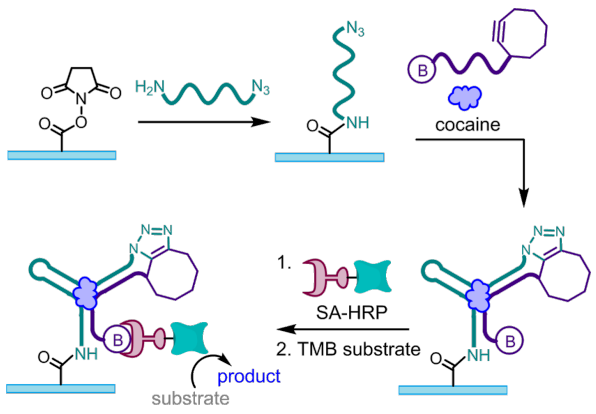

Figure 3: Split aptamer ligation can be used to construct an enzyme-linked assay for small-molecule detection. Conversion of the small molecule signal into a dose-dependent covalent ligation event allows signal to be produced even if target binding is lost. Reprinted with permission from [13]. Copyright (2012) American Chemical Society.

At this point, we were excited by our successes in the development of small-molecule detection assays, but we recognized that making practical use of these assays would require DNA split aptamers for other small-molecule targets. We were initially surprised to find that while there were over 100 DNA aptamers for small-molecule targets [14], at the time, only two of these had been successfully converted to split aptamers [5,6]. We reasoned that this dearth of split aptamers was a result of the fact that many aptamer structures cannot be split without compromising substrate binding. To overcome this challenge, we hypothesized that the three-way junction architecture of the cocaine aptamer could be a privileged structure for the engineering of aptamers into split aptamers, as it offers two putative splitting sites that are distant from the typical target binding site (Figure 4a). Excitingly, Stojanovic and co-workers had recently demonstrated that SELEX could be carried out using a structurally biased library to generate aptamers having the necessary three-way junction architecture [15]. Using our insights regarding the thermodynamics of split aptamer assembly, we developed a method for rapidly and reliably converting these three-way junction aptamers into split aptamers (Figure 4b). We

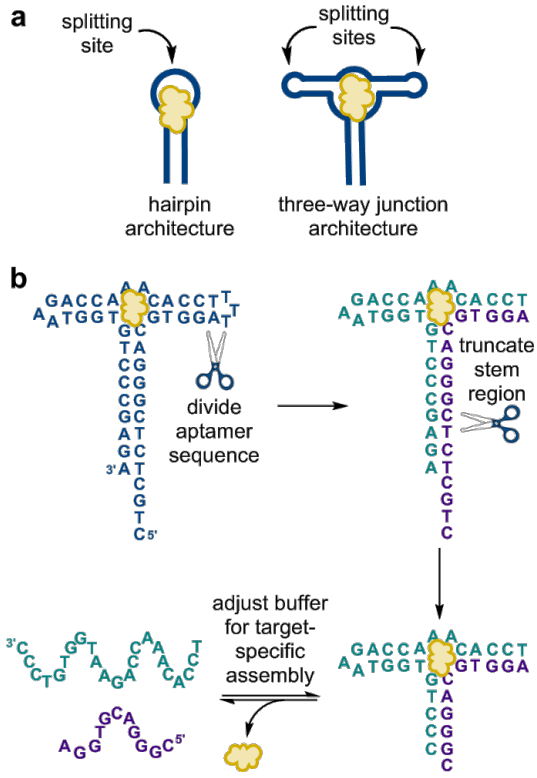

Figure 4: (a) In contrast to the more typical hairpin aptamer structure, we hypothesized that the three-way junction would provide a privileged architecture for generating split aptamers. (b) Split aptamers can be engineered by dividing a three-way junction aptamer, then systematically truncating the stem region to fine tune the thermodynamics of assembly. Adapted with permission from [16]. Copyright (2013) American Chemical Society.

first divided the sequence by removing one of the loop regions, and then systematically truncated the base-pairing stems until we achieved the desired assembly properties. As predicted by our thermodynamic model, we found that split aptamer sequences having fewer base pairs could function in high ionic strength solutions, while sequences having more base pairs functioned better in lower ionic strength solutions [16]. Importantly, our success in this endeavor was again closely tied to the insights we gained from our initial unexpected results in our split aptamer ligation experiments. Looking to the future, we expect that the ability to reliably generate split aptamers for new targets of interest will greatly expand the utility of this class of recognition elements.

In parallel with our efforts aimed at small-molecule detection, my lab was also intrigued by the challenging task of measuring small-molecule enantiopurity, as this is a key factor in the synthesis of pharmaceutical intermediates and other high-value chemicals. Enantiopurity can be measured by chiral chromatographic methods, but this process is limited to a few thousand samples per day [17]. In contrast, fluorescence based methods have potential to provide throughput on the order of 10^5–10^6 samples per day [18]. We envisioned that aptamers could serve as powerful recognition elements for fluorescence-based high-throughput enantiopurity measurement, and the first key element to our approach was the concept of reciprocal chiral substrate selectivity. According to this principle, aptamers having the same sequence, but synthesized from opposite enantiomers of DNA, will bind to opposite enantiomers of a target molecule with identical affinity and selectivity [19]. The second key element to our approach was the ability of DNA structure-switching biosensors to transduce the presence of a target molecule into a dose-dependent fluorescence output [20]. In this sensor format, a short quencher-labeled complementary strand is hybridized to the fluorophore-labeled aptamer, and equilibrium favors duplex formation in the absence of the target. However, addition of the target molecule shifts this equilibrium to favor displacement of the complementary strand, thus generating the dose-dependent signal.

Using the previously reported structure-switching biosensor for L-tyrosinamide (L-Tym) [21], we synthesized both the L- and D-DNA sequences, but labeled these enantiomeric biosensors with orthogonal fluorophores (Figure 5). In our initial experiments, we utilized fluorescein (FAM) and cyanine 3 (Cy3), however, we observed that the difference in fluorophore structure resulted in an approximately two-fold difference in the equilibrium constants for the sensors. This was surprising, as the fluorophores are small molecules attached to the termini of much larger DNA molecules. However, we found this lesson very informative, as it showed that dyes and other functional

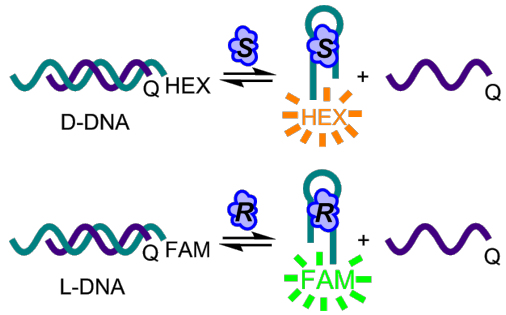

Figure 5: Structure switching biosensors convert the presence of a target into a dose-dependent fluorescence signal, and construction of biosensors from opposite enantiomers of DNA enables rapid measurement of enantiopurity using two-color fluorescence. Adapted with permission from [4]. Copyright (2015) American Chemical Society.

groups that we frequently append to DNA are not as innocuous as we assume them to be. Rather, they can have a dramatic impact on the assembly properties of the DNA sequences. In our experiments, this was particularly noticeable, as the structure-switching sensors are tuned to have an equilibrium near unity, which allows small amounts of the target to trigger displacement. Thus, in these systems, subtle changes to the energetics of DNA assembly can have rather large effects on the position of equilibrium. We were fortunately able to overcome this challenge by replacing the Cy3 with a hexachlorofluorescein (HEX). HEX and FAM are spectrally orthogonal, but have similar chemical structures and electrostatic properties, and we found that they provided enantiomeric sensors having nearly identical equilibrium constants. To test our enantiopurity analysis method, the two biosensors were incubated together with varying ratios of L- and D-Tym to construct calibration curves relating the observed fluorescence output to concentration for each enantiomer. Comparison of our calculated versus actual % L-Tym for these measurements revealed a high level of both accuracy and precision, and we were also able to demonstrate the use of our sensors to accurately monitor yield and enantiopurity in chemical reactions [4]. In the context of this project, our unexpected observation regarding the impact of dyes on DNA assembly was something that we merely needed to overcome. However, this experience provided us with an improved understanding of the function of structure-switching sensors, which has proven critical to our current experiments aimed at rapidly generating these sensors for new small-molecule targets of interest.

While I am extremely passionate about science, I find that I am most creative and happy when I can occasionally escape to pursue other hobbies. Living in Salt Lake City, I joke that my hobbies are the “Utah usual,” which includes rock climbing, road and mountain biking, snowboarding, and hiking. Among

these, rock climbing, and specifically bouldering, is my favorite, as there is something special about the movement and flow of a good boulder problem. And, the intense focus required in bouldering to elucidate these complex sequences of movements is one of the few things that can completely wipe my brain clear of the stresses of deadlines and funding (Figure 6). I also very much enjoy spending time with my husband and two sons, whether we are traveling, enjoying outdoor activities, or just playing with Lego blocks.



Figure 6: The author intensely focused on climbing the boulder problem "The Angler" during a research group outing to Joe's Valley, UT. Photo credit: Zhesen Tan.

Conclusion

Looking toward to the future of aptamer-based sensors, I feel that there is still much to learn about the thermodynamics and kinetics of sensor assembly and target recognition. Inevitably, many of these discoveries will be made much like those I've highlighted above – through experiments that initially did not go as we had planned. However, I am also very thankful for the researchers who are intentionally delving into these questions and uncovering new insights on a regular basis. Understanding these principles is the key to not only designing better sensors, but also to making sure that we are generating the best possible recognition elements when we set out to select for new aptamers. In surveying the landscape of applications for DNA-based sensors, I am most excited about recent progress in the use of aptamers inside of living cells, as there is a wealth of information that can be gained regarding the concentrations and flux of small molecules within these dynamic and complex environments. Additionally, the ability to fluorescently monitor targets such as small molecules inside of living cells could prove to be valuable for synthetic biology applications such as metabolic engineering. Bringing these technologies to the point that they are routine and broadly applicable will clearly involve significant advances in molecular and cellular biology. However, contributions from supramolecular chemistry will also be critical, as these will provide the key to understanding and engineering the complex molecular architectures of aptamer-based sensors.

Acknowledgements

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Bright molecules for sensing, computing and imaging: a tale of two once-troubled cities

A. Prasanna de Silva

Review

Open Access

Address:
School of Chemistry and Chemical Engineering, Queen's University, Belfast BT9 5AG, Northern Ireland

Email:
A. Prasanna de Silva - a.desilva@qub.ac.uk

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Abstract

The circumstances in Colombo, Sri Lanka, and in Belfast, Northern Ireland, which led to a) the generalization of luminescent PET (photoinduced electron transfer) sensing/switching as a design tool, b) the construction of a market-leading blood electrolyte analyzer and c) the invention of molecular logic-based computation as an experimental field, are delineated. Efforts to extend the philosophy of these approaches into issues of small object identification, nanometric mapping, animal visual perception and visual art are also outlined.

Review

Prologue

Colombo, Sri Lanka: A civil war leads to 100,000 needless deaths. Belfast, Northern Ireland: Another civil war and another 3,500 needless deaths. Thankfully, both wars exhausted themselves after 25 years. Colombo returned to calm in 2009, while Belfast did the same in 2005. I call both these places home. I have seen good times, bad times and good times again. Through all these times, a bit of supramolecular photochemistry [1,2] kept happening and this is my story.

My paternal grandfather was the schoolteacher in our Colombo suburb and so, the value of learning was instilled into me from age zero. A sharpening of the interest opened up when my mother bought me a flood-damaged encyclopedia of science and technology. An influential high-school teacher, Errol Fernando, focussed me further towards chemistry. Due to a shortage of chemistry lecturers in the University of Colombo, the British Council sent us a Glaxo alumnus, Vincent Arkley. He not only enthralled us with personal stories of vitamin B_{12} synthesis, but was also instrumental in opening a channel to Ph.D. study at the Department of Chemistry at Queen's University Belfast. His former protégé at Glaxo, Ron Grigg, had risen to be the Chair of Organic Chemistry at Belfast, but recruiting

decent Ph.D. students to Belfast during the troubles had been hard. Ron Grigg was persuaded by Vincent Arkley to take on several Sri Lankans. I was fortunate to work with James Grimshaw who was hugely knowledgeable in electro- and photochemistry, while Ron Grigg also kept an eye out for me. My paternal grandmother's failing health persuaded me to return to Sri Lanka in 1980, following a very happy Ph.D./post-doctoral time in Belfast. She had been one of my carers throughout my childhood and this was my chance to return the favour. Also, I had been greatly influenced by my mother's ability to help and care for others, even to the point of sacrifice and even against opposition. Chemistry took a back seat during the next six years as I took on a carer role and as Sri Lanka descended into a very dark place. The Department of Chemistry at the University of Colombo gave me a job, which I will always be grateful for. These eventful years proved to be the crucible in which my current research directions were forged. Two of our papers from the University of Colombo were accepted by *J. Chem. Soc., Chem. Commun*. [3,4]. When my grandmother passed on, I had an international phone call. It was Ron Grigg. He offered condolences and offered me a chance to return to Queen's. The continuing troubles had made the recruiting of decent lecturers to Belfast difficult as well. Following family conferences, I was able to return to old friendships in Belfast. There I have stayed.

Research beginnings

At the time that I was studying for a Ph.D. in organic photochemistry [5], the field was in transition. The study of single functional groups was nearing completion and attention was shifting to the meeting of two functionalities in a photon field. One of the most influential concepts that emerged from this ferment was photoinduced electron transfer (PET) [6,7], especially following the previous realization of its central role in green plant photosynthesis. It appealed to the physicochemical side of me that PET allows one to think primarily in terms of redox potentials, while atomic details remained secondary. Also, when a lumophore was involved, the emission could be easily observed and measured. Especially in its intramolecular manifestation, a 'lumophore–spacer–receptor' system would behave in a modular fashion [8-10] – an accurate case of molecular engineering with attendant advantages of predictive behaviour which is rare in chemical contexts. Since an electron trying to leave a receptor would be electrostatically held back by a cation held there, it was clear that PET processes could be switched 'off' by an externally impressed chemical command. Since PET and luminescence compete for the deactivation of the same excited state, it was equally clear that a luminescence signal could be switched 'on' by chemical command. Therefore, we were fortunate to be able to introduce a general design tool of luminescent PET sensing/switching [11-15], which even handled anions and neutral species besides cations [12,16].

When the chemical command comes from what is present in the molecular neighbourhood, the three-module supramolecular system takes on the role of a sensor. Since the sensor is of nanometric dimensions, the general problem of detection and measurement of the occupants of very small spaces opens up to solutions. Scientists skilled in cell biology were to take this solution to exquisite levels in the coming years [17,18]. When the chemical command is manipulated by the scientist, the three-module system and its higher versions take on the role of a miniature information processor, e.g., a logic gate [16,19-21]. Indeed, molecular sensors and logic gates are related in several ways [16]. Both of them are rooted in two (or higher) -state equilibria between free and bound forms of a molecule, so that sensors become the simplest logic gates. However, the sensor's ability to smoothly measure small variations in a chemical concentration [22,23], which is an analogue (rather than digital) function, arises from mass action of a large population of molecules.

We were able to demonstrate that the chemically switchable 'lumophore–spacer–receptor' system naturally harnesses the diversity available in each of the three modules. For instance, the lumophore could be a fluorescent dye [3,24], a room temperature phosphor [25,26], or a lanthanide-based emitter [27,28]. Colleagues showed that even a quantum dot [29] would fit the bill. The receptor could be an amine [3,24,30], an amino acid [18,31], a crown ether [32] or a cryptand [33] and the spacer could be an oligomethylene chain [34] or nothing at all [35,36]. Since such diverse systems allow the addressing of various problems and since the design is usually straightforward, the PET sensor/switch design tool has been taken up by about 330 laboratories (Figure 1, PET maps) so far.

A bit of medical diagnostics

While it was clear from the beginning that fluorescent PET sensors would be useful, we saw no practical path to such development. Serendipity had to smile in the form of the interest and the commercial will of Roche Diagnostics before such a path would open. The molecular engineering capabilities of the fluorescent PET sensor design were initially put to the test to quantitatively plan an 'off-on' sensor for sodium in whole untreated blood. Since the normal Na^+ level is 0.1 M, our receptor needed to have a binding strength (β_{Na}) of 10 M^{-1} in neutral water. An *N*-(2-methoxy)phenylaza-15-crown-5 ether [37] fitted the bill, besides having good selectivity characteristics. PET thermodynamics were matched by the use of a 4-aminonaphthalimide fluorophore [38-40], which, in the presence of blood, also could be conveniently excited by a blue



Figure 1: Approximate world maps of sources of fluorescent PET sensors/switches. Only the names of corresponding authors from the literature are given. Adapted from [15], Copyright (2015) The Royal Society of Chemistry.

light-emitting diode once the red cells were filtered out. **1** [41] and relatives for K^+, Ca^{2+}, H^+ and (indirectly) CO_2 are immobilized within a millimetric channel in the chemistry module of the OPTI point-of-care analyzer (Figure 2) now sold by Optimedical Inc. [42], with sales of over $130 million thus far. An appropriately adjusted version for veterinary use is sold by IDEXX Laboratories [43] with sales of around $400 million. The structural formulae of **1** and other molecules are given in Figure 3.

A particularly touching aspect of this story occurred when Roche executives informed me that OPTI analyzers had been sold to the Sri Lanka ambulance services during the civil war there. Imagine the scene, if you will. A victim of a suicide bombing is lying in the road, fighting for life in the midst of others who have lost that fight. A paramedic identifies the sinking victim and stabilizes him/her. A sample of venous blood is drawn and analyzed in an OPTI cassette within 30 seconds. The gas and electrolyte levels in the victim's blood are telephoned to the hospital so that a blood bag can be prepared to match the salt levels of the victim before the ambulance fights through the Colombo traffic to the Accident and Emergency ward. This precaution prevents salt shock occurring during transfusion. Before the OPTI was available, many victims died

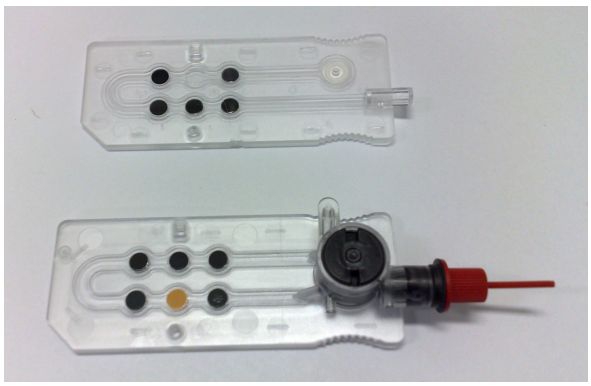

Figure 2: OPTI™ cassettes sold by Optimedical Inc. (http://www.optimedical.com). Photograph is reprinted from [10], Copyright (2008) The Royal Society of Chemistry.

on the operating table due to salt shock even though the surgeons and nurses had followed their procedures assiduously. In other words, some of these Colombo residents (like I was) are now alive thanks to a fluorescent PET sensor.

In addition to this ex vivo application, which had an easier accreditation process through the medical watchdog institutions as compared to an in vivo version, there are emerging instances where fluorescent PET sensors are immobilized on the tips of optic fibres placed in a vein, to allow continuous monitoring of blood. Glucose monitoring [44,45] is one such success [46]. Problems of white cell attack and subsequent covering up of the fibre tip, which frustrated previous commercialization efforts along this path [47], appear to have been beaten by the use of new biocompatible hydrogel coatings. The small size of molecules allows their successful use in these millimetric spaces.

A bit of map-making

Map-making is not the exclusive domain of cartographers and surveyors. There are sub-nanometric environments such as those bounding membranes where concentrations of species like H^+ can be radically different to what is found in the bulk water [48,49]. Since membrane-bounded H^+ forms the heart of the field of bioenergetics [50], these concentrations need to be located as a function of position with respect to the membrane. Such maps can be constructed by using fluorescent PET sensors equipped with extra modules for fine positioning and for reading the position, e.g., **2** [51]. The former task is achieved by using groups of various hydrophobicity to allow the sensor to reach an equilibrium position in a membrane–water interfacial region [52]. The second challenge is addressed with fluorophores which achieve charge-separated excited states [12,53] so that their interaction with the local dipoles of the neighbourhood causes shifts in the emission spectra. Simultaneous monitoring of wavelength and intensity data to obtain position and H^+ concentration information respectively, is another example of two-dimensional fluorescence sensing [54]. Maps of local H^+ concentration versus position show the rapid fall-off of proton

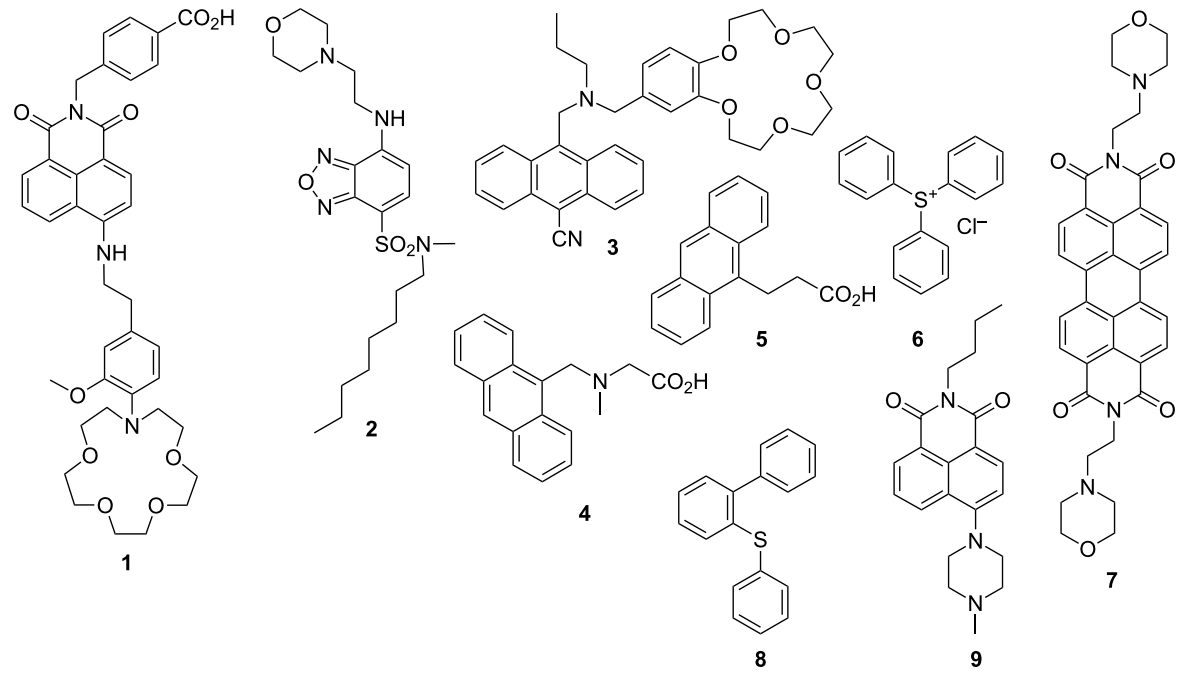

Figure 3: Structural formulae of the molecules discussed in this paper.

levels as non-polar membrane surfaces are approached [51]. So we see that even sub-nanometric spaces can be accessed by fluorescent PET-based molecules in order to shed light on these tiny worlds.

Emulating a bit of digital electronics

I was introduced to digital electronics in undergraduate physics classes but priceless 'hands-on' encounters were arranged by Satish Namasivayam. The devices came off the page of the textbook at that time and the logic gate hardware of computers lost a bit of their mystique. George Boole's disciples had shown how the Master's ideas concerning binary digits in communication [55] could be developed into information processors [19,20]. The modern manifestations of these processors are the logic gates cut from silicon. Of course, stored-program computers (as the name suggests) require software in order to command and coordinate complex computations. I was fortunate to receive programming instruction from Gihan Wickramanayake (University of Colombo) and Albert Smith (Queen's University Belfast) and their colleagues, which gave me an appreciation of how different logic gate arrays inside a computer are called into service at different stages of a run.

It took time for circumstances to change sufficiently to allow molecules to be considered as possible information processors [56]. Then it dawned on me that the 'lumophore–spacer–receptor' system could be elaborated into Boolean logic devices with chemical inputs and luminescence output. The first device, which initiated molecular logic-based computation as an experimental field, arose in the form of a 'lumophore-spacer$_1$–receptor$_1$–spacer$_2$–receptor$_2$' system, **3** [57], which behaved as an AND logic gate driven by Na^+ and H^+ inputs. In other words, luminescence emission was strong only when both Na^+ and H^+ were present at high levels. Each input, when supplied to the device at sufficiently high concentration, knocks out a PET pathway from its corresponding receptor to the lumophore. Even a single PET pathway disables emission. Selective receptors were the key.

It is perhaps worth noting that a simple expansion of the 'lumophore–spacer–receptor' system, with the attachment of an additional spacer and receptor, allowed chemistry to crossover into computer science at least in conceptual terms [16,58,59]. More generally, there was recognition that a chemical reaction (by its very name) involves a humanly noticeable response of the molecular device to some inputs such as reagents and reaction conditions. Or, in other words, chemistry is full of input–output devices based on molecules and materials. This diversity has continued to attract nearly 400 laboratories from various backgrounds and disciplines into the molecular logic field up to now (Figure 4) [16,21,60-64].

Molecular logic-based computation can be put to use in situations which benefit from the small size of fluorescent molecules. For instance, it can be useful in drug discovery. Many of these programs employ sub-millimetric polymer beads as carriers of drug candidates. These beads have to be tagged with some identification so that they can be tracked as they go through the processes of discovery and evaluation. However these beads are too small to be tagged by semiconductor-based radiofrequency identification (RFID) chips [66], which would otherwise have been the obvious choice. Molecular computational identification (MCID) tags come to the rescue [67,68]. Fluorescence colour is a useful identifier [69] but sufficient diversity is not generated in this way. Substantial diversities are created when the emission of the fluorescence colour is made conditional upon various controllable input parameters, such as the chemical nature of the input, the logic type of the response pattern and the threshold of the input concentration which triggers the response. Double tagging leads to large diversities and also gives access to multi-valued logic. The latter has a higher information content than binary versions [16,70-75], and is not error-prone under our experimental conditions of microscopic examination after washing. In contrast, multi-valued logic is very error-prone under normal conditions of computation with stored programs due to error accumulation over many computational steps. Even cases as simple as H^+-driven YES and PASS 1 logic gates (**4** and **5** respectively) are useful as MCID tags either individually or in combination, via the acid–base control of the blue fluorescence. A YES gate produces a strong light output only when the input is present at a high level. On the other hand, a PASS 1 gate produces a strong light output whether the input is present or not. Figure 5 shows the blue fluorescence output of these and other gates on polymer beads in acid and alkaline conditions. These examples and the blood analyzer described previously (which is a Na^+-driven YES gate with green fluorescence output) are proof that even the simplest molecular logic gates have worthwhile uses [16].

Emulating a bit of psychology

The process of visual perception or attention protects us all everyday, by evaluating every approaching object for its threat potential [76,77]. Psychologists have found this is achieved by the retina detecting the edges of the object [78]. The physiological basis is that, within milliseconds, the image received by the rod and cone cells is passed up to the ganglion cells which can use a wiring scheme by which a group of rod and cone cells pass their responses to a single ganglion cell so that an output is eventually fired into the optic nerve only if the central rod/cone cell is illuminated while the surrounding cells are in the dark (or vice versa). The edges so extracted contain far less information than the original image so that it can be sent to the brain for quick comparison with other edges held in easily accessible

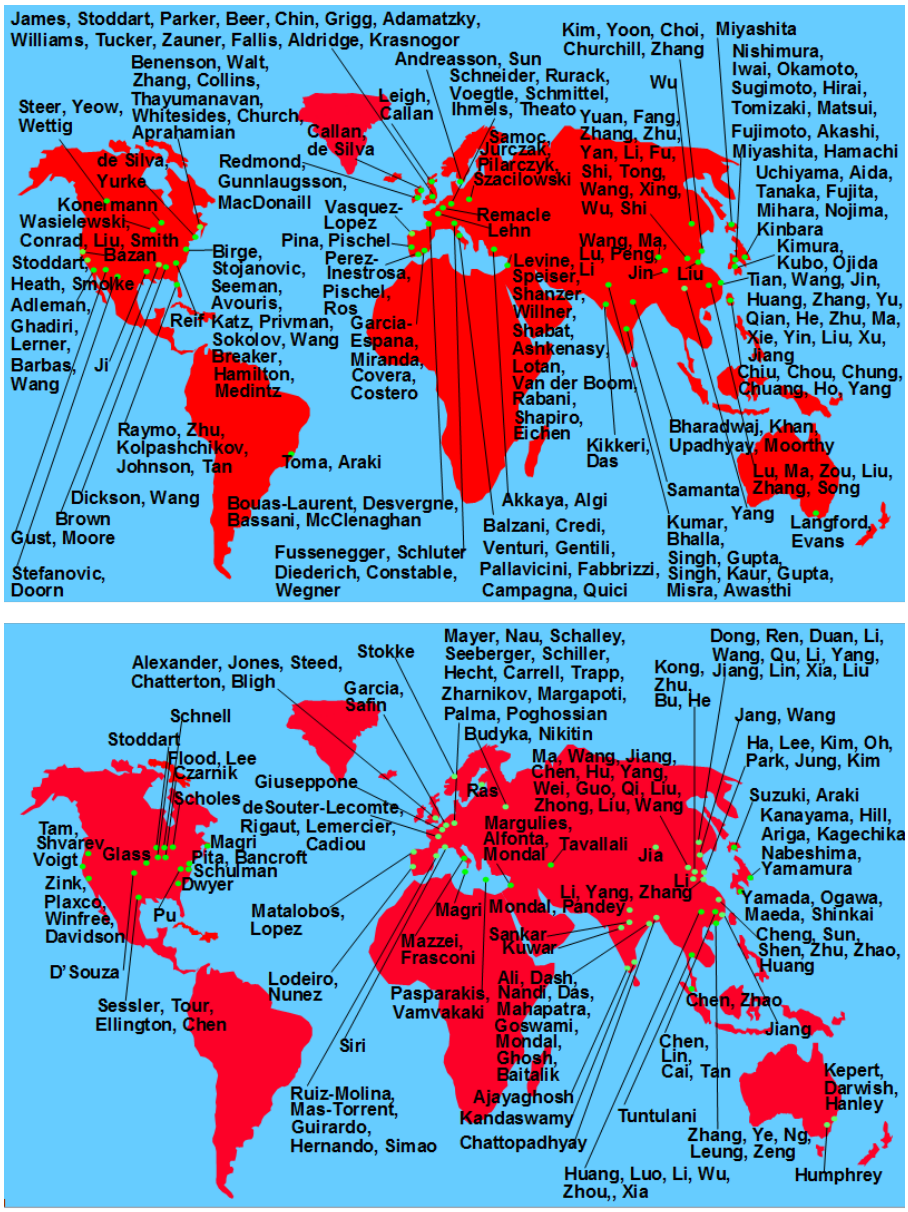

Figure 4: Approximate world maps of the sources of molecular logic devices. Only the names of corresponding authors from the literature are given. The simplest cases of single-input, single-output binary logic devices are not included. Adapted from [65], Copyright (2015) The Royal Society of Chemistry.

memory. Once the object is identified through its edges, appropriate activation of leg muscles will enable the person to flee the scene if necessary.

Edge detection can also be achieved in a semiconductor computing context, but not with a logic gate array alone. A full stored-program computer [19,20] running edge-detection software such as the Canny algorithm [79] is required. This allows different logic arrays to be brought into action as each line in the algorithm is called out, for example. Then, light intensity gradients (or second derivatives) can be detected. Such programs are even available on mobile telephones nowadays. Such a fundamental aspect of information processing in the animal and technology worlds has also been emulated by films of bacteria, after suitable genetic modification [80], and also by reactive networks of rather high molecular-mass oligonucleotides [81], both of which involve high levels of organization in space-time.

The challenge we faced was to emulate this deep-seated animal behaviour with small molecules with no organization other than to spread them out on paper [82]. Such spreading would create a

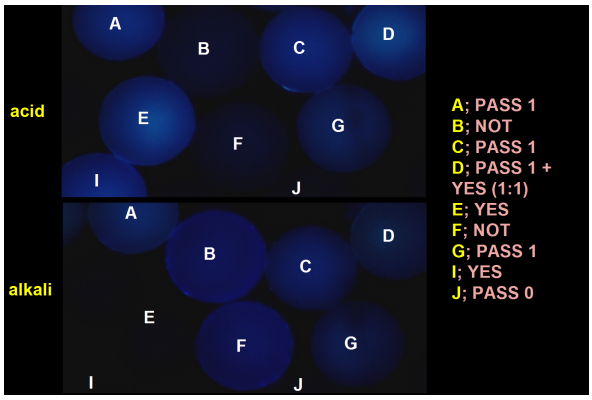

Figure 5: Fluorescence micrographs (excited at 366 nm) of 0.1 mm polymer beads carrying MCID tags. The beads are treated with (a) acid and (b) alkali in aqueous methanol (1:1, v/v). The logic gate type of each bead is given in the legend. Photograph adapted from [67], Copyright (2006) Macmillan Publishers Limited.

graphical user interface somewhat akin to those found in a touch-screen of a mobile telephone or in a mouse-driven screen of a stored-program computer. The treated paper could build an image after receiving a projection of the object. We do this by using a photoacid generator **6** (used to sculpt features in silicon chips) [83] in combination with a H^+-driven 'off-on' fluorescent sensor **7** [84,85], which is also a H^+-driven YES logic gate with fluorescence output. A weak pH buffer (Na_2CO_3) is used to poise the system in an 'off' fluorescent state at the beginning. Costs are kept low by employing a common two-colour ultraviolet lamp for writing (254 nm) and reading (366 nm) the information.

Writing with 254 nm produces protons in the irradiated regions, which quickly overcome the weak buffer so that the fluorescence of **7** is switched 'on'. This occurs by removal of the PET process occurring from the amine side groups to the perylene-tetracarboxydiimide lumophore [84,85]. So, a positive photograph is produced at short irradiation times (Figure 6). However continued irradiation builds up the concentration of the photoproduct **8**, whose electron richness allows it to engage in a PET-based bimolecular quenching process, so that the freshly-created fluorescence is killed off again (Figure 6). This is a fluorescence 'off–on–off' process driven by writing light dose. 'Off–on–off' processes are common [86-94], with enzyme activity as a function of pH and tunnel diode current as a function of voltage being just two disparate examples [90]. 'Off–on–off' processes can also be understood as XOR logic behaviour [16,21] or more generally as a ternary logic function [16].

While the 'off–on–off' fluorescence function eventually returns most of the image to a dark state, the edges of the image remain brightly fluorescent. Diffusion of H^+ down the gradient at the edges allows protons to outrun the lumbering **8** and create a thin region of protonated **7** which escapes the quenching. The thinness of this bright region is determined by the diffusion coefficient of H^+ in the matrix. We dry the paper carefully so that the diffusion coefficient of H^+ is about an order of magnitude lower than that found in bulk water [85]. This procedure results in edges of 1–2 mm thickness during an experiment runtime of about 30 minutes (Figure 6). Too much or too little drying destroys the edge detection capability quite sharply [82]. It is important to note that the edge regions are governed by much more than light dose-driven XOR logic. Indeed, the construction of serially integrated molecular logic gate arrays has required innovative schemes in the hands of several laboratories [26,95-105]. It is also sobering to realize that the parallel processing seen in the current example involves around a quadrillion molecules of the sensors to create an edge of an object about 4 cm square. What's a quadrillion? One way to imagine this is with a bit of economics. A quadrillion dollars is

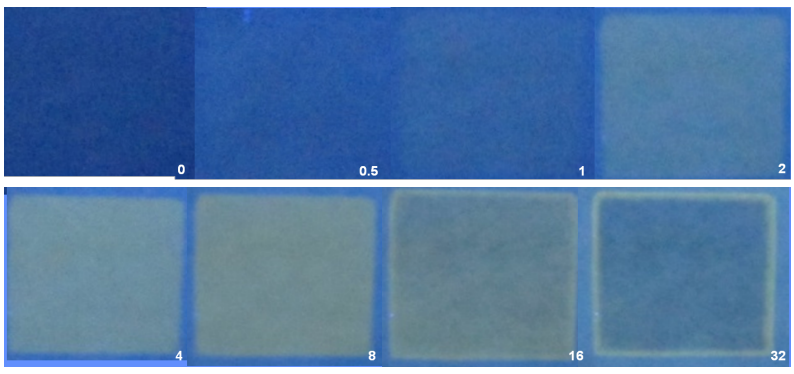

Figure 6: Photographs of fluorescent images (excited at 366 nm) after writing with 254 nm light through a 'square' mask onto the substrate, containing **6**, **7** and Na_2CO_3, for varying cumulative times in minutes as noted in each photograph. Scale bar = 4.0 cm. Photograph reprinted from [82], Copyright (2015) American Chemical Society.

around the total debt plus derivatives traded in the world (2013 figures). In contrast, the total world gross domestic product is only 70 trillion dollars.

Emulating a bit of arts

The achievement of edge detection by small molecules allowed us to apply this idea to visual arts at a rudimentary level [85]. Outline drawing is perhaps the simplest level of sketching. Many paintings begin life as an outline drawing so that we are connecting here with a celebrated part of human culture. It seems that an outline drawing is the accurate representation of the edges detected by the artist while observing the object. Our edge-detecting composition of **9** [106], **6** and pH buffer transforms the shamrock object into a green outline suitable for a St. Patrick's Day celebration (Figure 7). The main difference between the circumstances of Figure 6 and Figure 7 is that now the object includes arbitrarily complex curves and acute angles, as an artist would.

Figure 8 illustrates the different ways in which children, computers and molecules achieve outline drawing. Children follow the outline directly, probably by employing their edge detection ability, from an arbitrary point on the outline until the circuit is completed. Computers, running a version of the Canny algorithm [79], take an image and raster scan it so that the edge pixels emerge horizontal line by horizontal line. Thus the outline arises from a vertical stack of edge pixels. Logical molecules take an initial photographic image, expand it slightly and then erase the original photograph to leave behind the thin expansion region as the outline. This behaviour can be modelled semi-quantitatively using well-known equations of acid-base equilibria and diffusion [85].

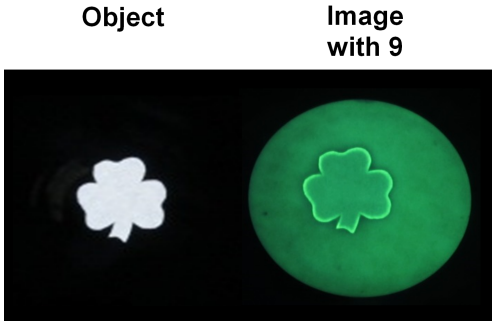

Figure 7: Backlit shamrock object and fluorescence image (excited at 366 nm) on paper containing **6**, **9** and Na_2CO_3, following writing with 254 nm light for 16 min. The filter paper diameter is 11.0 cm. Photograph reprinted from [85], Copyright (2015) The Royal Society of Chemistry.

A bit of arts

The arts offer an excellent balancing influence for scientists. For me, one non-scientific aspect of life needs to be mentioned. Sri Lanka is a drumming nation and Northern Ireland has a very rich music tradition. I have been immensely fortunate to absorb some part of these traditions. Percussion and drumming have been an essential part of me as long as I can remember. I was fortunate to be introduced to an Irish traditional band 17 years ago and we have kept on playing since then.

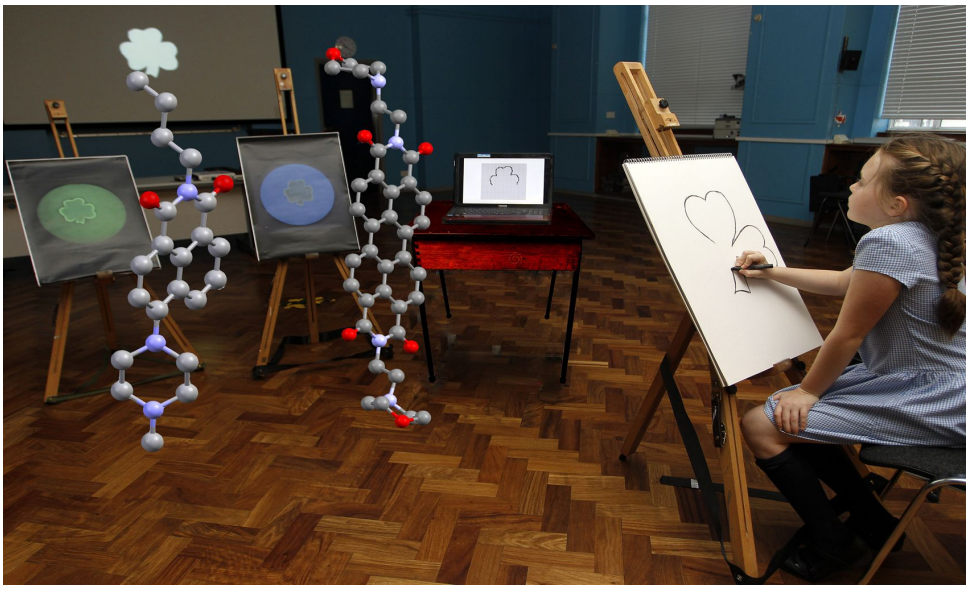

Figure 8: Comparison of how a child, a computer and the molecules **7** and **9** draw the outline of a shamrock object on the wall screen. Photograph used by permission of Karen-Louise Daly, Brian Daly and Aodhan O'Raghallaigh.

Epilogue

Since supramolecular chemistry concerns molecule–molecule interactions, its principles can be adapted to miniaturize people–people interactions or people–object interactions to the molecular level in favourable instances [107]. It is notable that such interactions may involve information transfer of some kind. Enabling molecules with the ability to gather, store, process and transmit information is a very worthwhile enterprise, especially because molecules can access important spaces where no devices but molecules may enter. Additionally, the smallness of molecules allows the mobilization of huge numbers of them in parallel so that large-area problems can be solved. The above sections have provided some examples where bright (super)molecules exploit these capabilities from a variety of contexts. I hope that younger and brighter minds will expand this variety much more. After all, there is a lot of human experience waiting to be miniaturized for useful purposes.

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

A journey in bioinspired supramolecular chemistry: from molecular tweezers to small molecules that target myotonic dystrophy

Steven C. Zimmerman

Review

Open Access

Address:
Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

Email:
Steven C. Zimmerman - sczimmer@illinois.edu

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Abstract

This review summarizes part of the author's research in the area of supramolecular chemistry, beginning with his early life influences and early career efforts in molecular recognition, especially molecular tweezers. Although designed to complex DNA, these hosts proved more applicable to the field of host–guest chemistry. This early experience and interest in intercalation ultimately led to the current efforts to develop small molecule therapeutic agents for myotonic dystrophy using a rational design approach that heavily relies on principles of supramolecular chemistry. How this work was influenced by that of others in the field and the evolution of each area of research is highlighted with selected examples.

Review

Early childhood and overview

I was born on October 8, 1957 in Evanston, Illinois, the second of three boys. Our parents, Howard E. Zimmerman and Jane Zimmerman, née Kirschenheiter, were very much in love and also remarkably different people. My mother was a rebellious, direct-speaking, very liberal, yet religious Christian who never graduated from high school. My father was a soft spoken, politically conservative, nonpracticing Jew, who not only obtained a B.S. and Ph.D. from Yale University, but went on to do postdoctoral work with Robert Burns (R.B.) Woodward at Harvard University. He was the first on either side of the family to get a college degree. With the exception of my father, my family was primarily working class (mailman, construction worker, Navy man, etc.). Family photographs are shown in Figure 1.

The clash of cultures in the family challenged my sense of identity and I grew up feeling like a foreigner, an outsider, in a homogeneous, white, Christian, middle class-neighborhood. My remarkable mother made sure we appreciated our father's



Figure 1: Photographs of Howard E. Zimmerman (July 5, 1926–February 12, 2012) (left) and Jane Zimmerman (née Kirschenheiter) (December 24, 1928–January 21, 1975) (center) as young adults, and the author (right) giving a Breslow group meeting presentation in graduate school (Havemeyer Hall, Columbia University).

heritage. Although we were raised with traditional Christian holidays, in early adulthood I recognized in myself a sense of humor and an outlook on life that was distinctly Jewish. I was drawn to the books of Saul Bellow and Isaac Bashevis Singer, and New York City. What does any of this have to do with chemistry? Chemistry provided a sense of belonging and identity from an early age simply because my father was immersed in an occupation I didn't understand but recognized as being very exciting. His passion for his work was obvious, so as a young boy I told people I also wanted to be a chemist. Furthermore, the long line of remarkably talented chemistry graduate and postdoctoral students that came to my house for Z-group parties were like an extended family. They were all "cool" people and some, for example, John McCall and Laren Tolbert even served as babysitters. This was a family I wanted to join. The occasional visiting faculty member solidified my choice of chemistry as a career. What child wouldn't be excited by Koji Nakanishi cutting ropes in two only to have them magically reconnect!

Undergraduate and graduate studies and an NSF-NATO postdoc

The Department of Chemistry at the University of Wisconsin was an extraordinarily stimulating place in the mid to late 1970s. I was fortunate to do undergraduate research with Professor Hans J. Reich, investigating the mechanism of the singlet oxygen reaction with alkenes and studying the oxidation of selenide/sulfide mixtures using ozone and singlet oxygen [1]. In my senior year, I took three graduate level courses, which was an amazing experience. Professors Charles P. (Chuck) Casey and Harlan L. Goering taught the physical organic course (Chem 641), Professor Barry M. Trost and Edwin Vedejs taught the synthesis course (Chem 841) and Hans Reich a more informal, once-a-week, mechanisms (arrow-pushing) class. For each lecture, Trost or Vedejs passed out several pages describing various methods of synthesizing several natural product substructures with a rather lengthy bibliography. I naively thought that this bibliography was an assigned reading list rather than a list for future reference. It was a wonderful mistake that led to my learning an enormous amount of exciting synthetic chemistry. Indeed, in going to Columbia University for graduate school I had every intention of working for Professor Gilbert Stork or W. Clark Still. But Professor Ronald Breslow's enzyme reaction mechanisms course, my first class ever on anything resembling biology or biochemistry, was so exciting that I decided to join the Breslow group and work on pyridoxal/pyridoxamine enzyme analogs [2-4]. Not only was Ron Breslow a wonderful and inspirational mentor, he had built an extraordinarily stimulating group of coworkers that he himself described as "people that you will hear from in the future, not people who will disappear into the woodwork." Indeed, my labmates during the period from 1979 to 1983 included Jik Chin, Robert Corcoran, Tony Czarnik, Sam Gellman, Don Hilvert, Uday Maitra, Dave Okrongley, Russ Petter, Darryl Rideout (coincidentally a Madison West High School classmate), Alanna Schepartz, Alan Schwabacher, George Trainor, Craig Wilcox, and Jeff Winkler.

Ron Breslow had broad interests, with projects ranging from developing artificial enzymes, to novel anti-aromatic compounds, to remote C–H activation of steroids, to determining hydrocarbon pK_a values using electrochemistry. The lesson learned, and one I tried to put into practice in my independent career (see below), is that it is very much possible to run a research group focused in quite different areas of chemistry. With an NSF-NATO postdoctoral fellowship, I spent just under two years with Sir Alan Battersby at the University of Cambridge where we completed the total synthesis of sirohy-

drochlorin, an intermediate in the biosynthesis of vitamin B12. Then in July 1985, it was off to the University of Illinois at Urbana-Champaign.

Molecular tweezers and a paradigm shift in host–guest chemistry

Developing molecular tweezers was one of the main projects I started in my independent academic career at Illinois. The idea originated at Columbia when I began to teach myself the biochemistry and biology lacking in any of my formal coursework. For example, one summer that process involved taking J. D. Watson's "Molecular Biology of the Gene" [5] on the subway to a Long Island beach on weekends. The beautiful structure of DNA and its intercalation complexes of aromatic dyes were especially intriguing. In broader reading, I sought to understand better the so-called nearest-neighbor exclusion principle (NEP), wherein intercalators at full saturation of a DNA helix bind every other site (i.e., one intercalator per two base-pairs) [6].

Figure 2a schematically shows how insertion of monointercalators at full saturation leads to a DNA helix with intercalation sites only half occupied. Le Pecq and coworkers studied bisacridines such as **1** (Figure 2b) [7]. Consistent with the NEP, **1** formed a very tight bisintercalation complex with its spermine-derived linker chain spanning two base-pairs (Figure 2c). However, with shorter linkers that can only span a single base-pair, a monointercalation complex forms (Figure 2d). In fact, with bisintercalators the situation is considerably more complicated with the apparent width of the intercalator determining whether the nearest neighbor exclusion principle is obeyed. Although the principle remains poorly understood even today, my idea as a graduate student was to make a bisintercalator that was so rigid it could not form the mono-intercalated complex in Figure 2d. The ultimate goal was to develop a small molecule ligand that might intercalate at sites that lack a conventional neighboring intercalation site, for example, the ends of DNA double helices, replication forks, or abasic sites.

My original research proposal was submitted August 16, 1983 and was entitled "Synthesis of a Rigid 'Molecular Tweezer' with Novel DNA Binding Potential." The name "molecular tweezer" was inspired by Howard Whitlock's 1978 report [8] of compound **2** containing two caffeine units linked by a rigid diyne spacer (Figure 2e). Whitlock noted that conventional bisintercalators such as **1** would have their affinity for oligonucleotides significantly reduced as a result of intramolecular π–π aromatic stacking. Whereas the diyne spacer would prevent such stacking, it would not prevent mono-intercalation; therefore, we sought a spacer that would enforce a C-shape on the intercalator units. Compound **3** was one of two highly rigid tweezers proposed (Figure 3). The R-substituent, $-CH_2CH_2NMe_3^+$, along with the phenanthridinium units ("wide" chromophores obeying the NEP), were an obvious attempt to provide water solubility to a highly aromatic structure. In Roger Adams' Laboratory, Craig Vanzyl, Greg Hamilton, and I were able to prepare molecular tweezer **4** and several substituted analogs, but none that were water soluble [9,10].

Fortunately, the excitement surrounding the 1987 Nobel Prize to Cram, Lehn, and Pedersen had generated an enormous

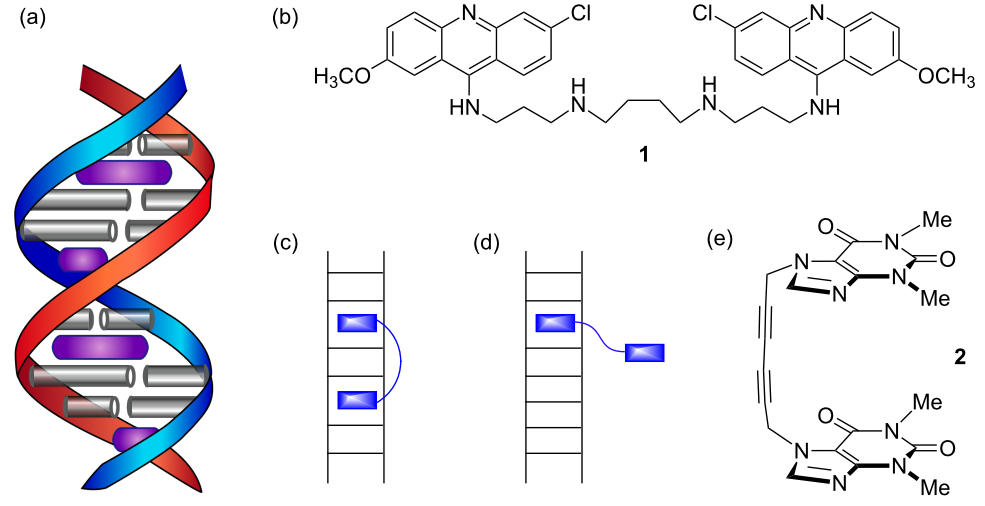

Figure 2: (a) Schematic double helix fully saturated with intercalator (in purple) according to the neighbor exclusion principle (NEP). (b) Bisintercalator with spermine linker. (c,d) Bisintercalator with long linker spanning two base-pairs and short linker preferring mono-intercalation to obey NEP. (e) Whitlock's "rigid" molecular tweezer.

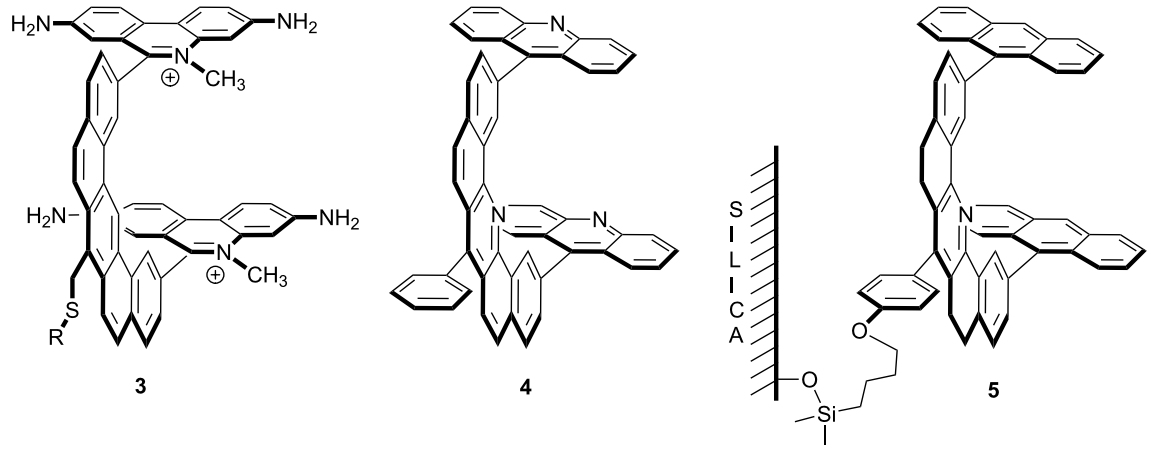

Figure 3: Bismethidium molecular tweezer **3** proposed as a graduate student at Columbia University, which was quite close in structure to **4**, synthesized and studied at Illinois. Chemically bonded stationary phase **5** used for HPLC assay of nitrated aromatics and for quantitative enthalpy determinations.

interest in host–guest chemistry and there was at this time a move to go beyond cyclic crown ethers. In particular, the groups of Rebek [11] and Hamilton [12] and many others were developing hosts capable of complexing more structurally challenging organic guests such as nucleobases. We quickly discovered that in chloroform solution, **4** and its analogs could bind nitrated aromatic compounds, such as 2,4,7-trinitrofluorenone. Nitrated polycyclic aromatics and polynitrated fluorenones were known pollutants so Kurt Saionz covalently linked the molecular tweezers to silica gel (see **5**, Figure 3), making chemically bonded stationary phases which were packed into HPLC columns that selectively retained and separated nitrated aromatics [13]. The HPLC columns proved to be very useful for quickly measuring $\Delta H°$ of complexation and, indeed, multiple guests could be measured at once [14]. The HPLC method of determining complexation $\Delta H°$ values was extended by Vincent Kwan to hydrogen bonding host–guest complexes [15].

Molecular tweezers that complex adenine and analysis of binding interactions

The idea of incorporating hydrogen bonding functionality into the molecular tweezer was appealing because it meant that aromatic stacking and hydrogen bonding might cooperate to give higher binding constants and guest selectivity. However, the preparation of a rigid aromatic spacer with a functional group converging on the binding cleft was not only a significant synthetic challenge, I questioned whether the group might not distort the spacer, thereby altering its dimensions. I was able to prepare small quantities (<100 mg) of **6** and **7** and crystallize both for X-ray analysis (Figure 4) [16]. The solid state structure of **6** revealed significant distortion and, indeed, a highly nonplanar aromatic spacer that was not suitable for the desired molecular tweezer. In contrast, analog **7** with nitrogen atoms in the peri-positions was a much more planar and suitable candidate.

Weiming Wu was able to prepare molecular tweezer **9** and showed it to bind 9-propyladenine in chloroform with a very high association constant of K_{assoc} = 120,000 M^{-1} [17-19]. What role do the hydrogen bonding and aromatic stacking play? As seen in Figure 4, this system provides an excellent example of what Jencks called "complex additivity of binding energies" [20]. The aromatic cleft of molecular tweezer **11** showed no affinity for adenine **8** and the carboxylic acid **10** bound **8** rather weakly ($-\Delta G°$ = 3.3 kcal/mol), yet the cleft and acid group cooperate together to give the very stable complex **8·9**. One simple interpretation in line with Jencks' idea is that the entropy paid by the hydrogen bonding is sufficient to allow the enthalpy of the aromatic stacking to be observed. However, there are other explanations for the high stability observed in the **8·9** complex. The aromatic cleft may serve to desolvate the carboxylic acid, thereby improving its hydrogen bonding capability. However, Monte Carlo simulations by Blake and Jorgensen indicate that the cleft of **9** is actually a good host for chloroform and that the carboxylic acid is solvated by more than one chloroform molecule [21]. Another possibility is that the aromatic cleft might somehow decrease the acidity of the acid group making it a better hydrogen bond donor. However, titrations in a mixed aqueous–organic solvent suggest that the carboxylic acid within the molecular tweezer is actually less acidic and likely a less effective hydrogen bonding unit [22].

Preorganization and cost of freezing single bond rotations

The studies above show the utility of the molecular tweezer approach in complexing large organic guests while at the same

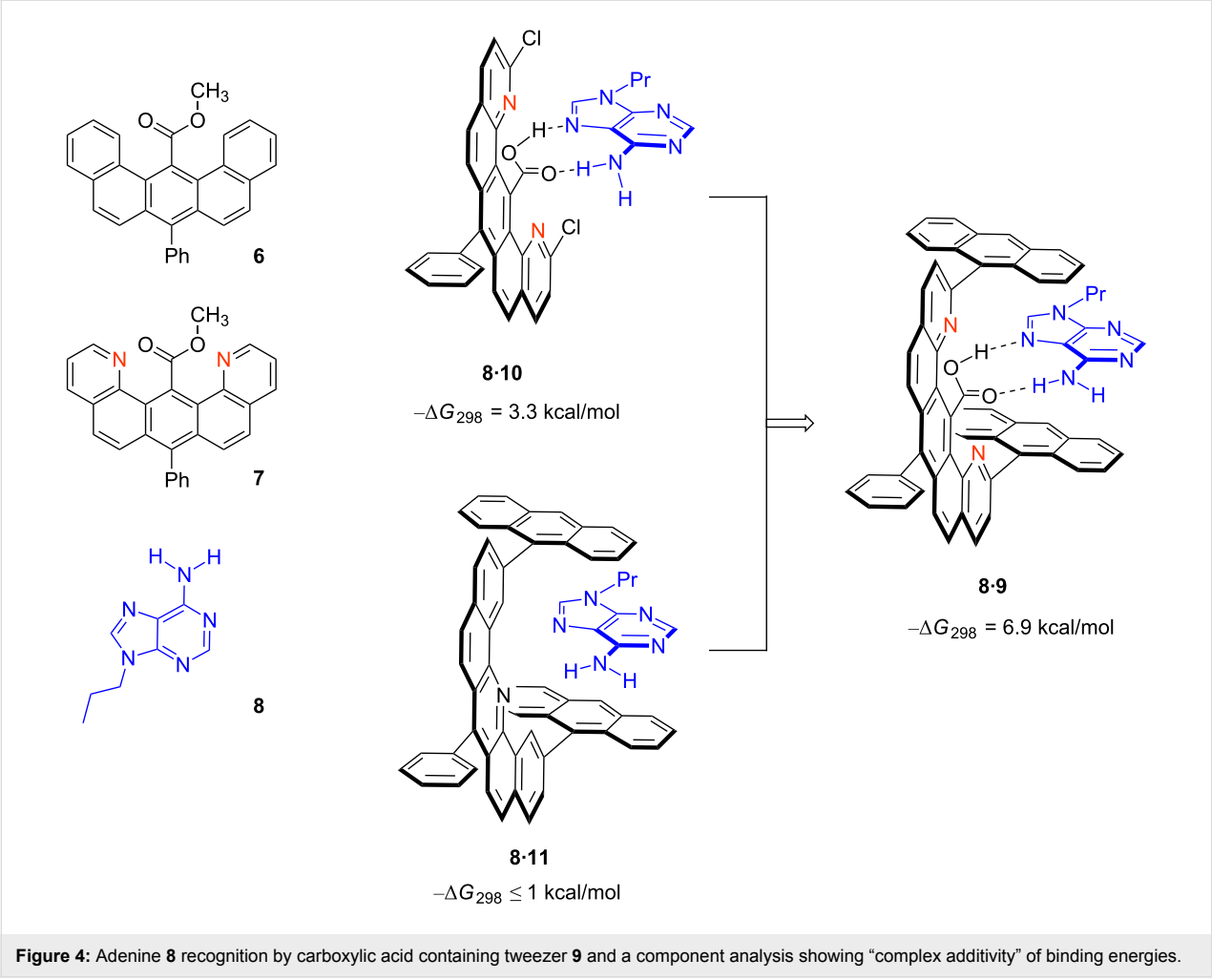

Figure 4: Adenine **8** recognition by carboxylic acid containing tweezer **9** and a component analysis showing "complex additivity" of binding energies.

time uncovering important design criteria in host–guest chemistry. Regarding design criteria, the ability to synthesize structurally analogous molecular tweezers provides an unprecedented opportunity to develop a wide range of important structure–property relationships. For example, a lot had been written about the importance of Cram's preorganization principle in host design, but no one had measured the energy cost of locking a single bond rotation in a host–guest complex. Monica Baloga, Milan Mrksich, and I prepared three new molecular tweezers **12**–**14**, where the spacer units possess zero, one, and two aryl–aryl single bonds (i.e., **14** → **13** → **12**) [23]. Their K_{assoc} values with 2,4,5,7-tetranitrofluorenone (**15**) in chloroform were then measured. As seen in Figure 5, freezing each single bond rotation increases complex stability by about 0.9 kcal/mol. This value is in line with a value of $T\Delta S°$ = 0.6 to 1.2 kcal/mol previously suggested by Jencks and Page as the cost paid to freeze out each single bond rotation in a ring-forming reaction [24]. Later Dudley J. Williams, in the context of analyzing the vancomycin complex with D–Ala–D–Ala containing peptides, suggested the cost of freezing a free rotation to be between 0.4 to 0.9 kcal/mol – a value that is also close to what we had measured [25]. In Williams' case, the value was derived from the entropy of fusion within a homologous series of alkanes, not an analysis of a host–guest system. All these values suggest that freezing out a single bond rotation is not terribly costly but association constants can drop significantly if too much flexibility exists; freezing five single bonds within a complex would lower its stability by as much as 10^4-fold.

Research on molecular tweezers goes mainstream

Since our early studies on molecular tweezers, numerous examples have appeared in the literature. A comprehensive review is not possible, but a few examples from other investigators are presented to illustrate the breadth of structure and function that has been achieved over the past three decades. One of the earliest examples is a molecular tweezer developed contemporaneously with our own efforts. Roeland Nolte and his team first reported the synthesis and X-ray structure of **16** (Figure 6, R = OH) as the basic building block [26]. The now familiar

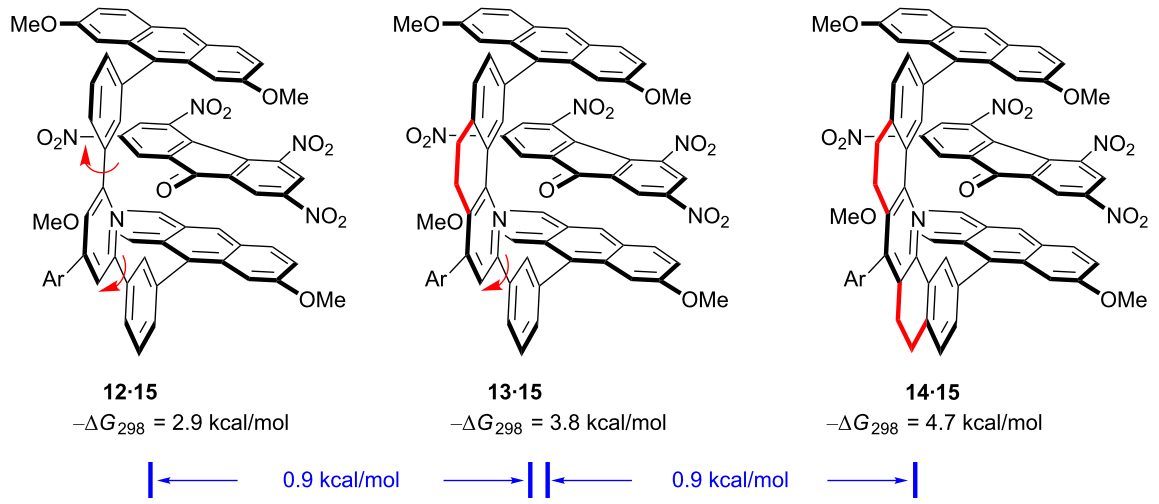

Figure 5: The first determination of the cost of freezing single bond rotations in a host–guest complex.

glycoluril motif of **16** clearly has the rigid C-shaped required, and a subsequent report showed it to be capable of complexing dihydroxybenzenes using a combination of aromatic stacking and hydrogen bonding to the urea carbonyl groups [27]. The aromatic stacking surface can be expanded as in molecular tweezer (clip) **17** (Figure 6), but interestingly, this compound is

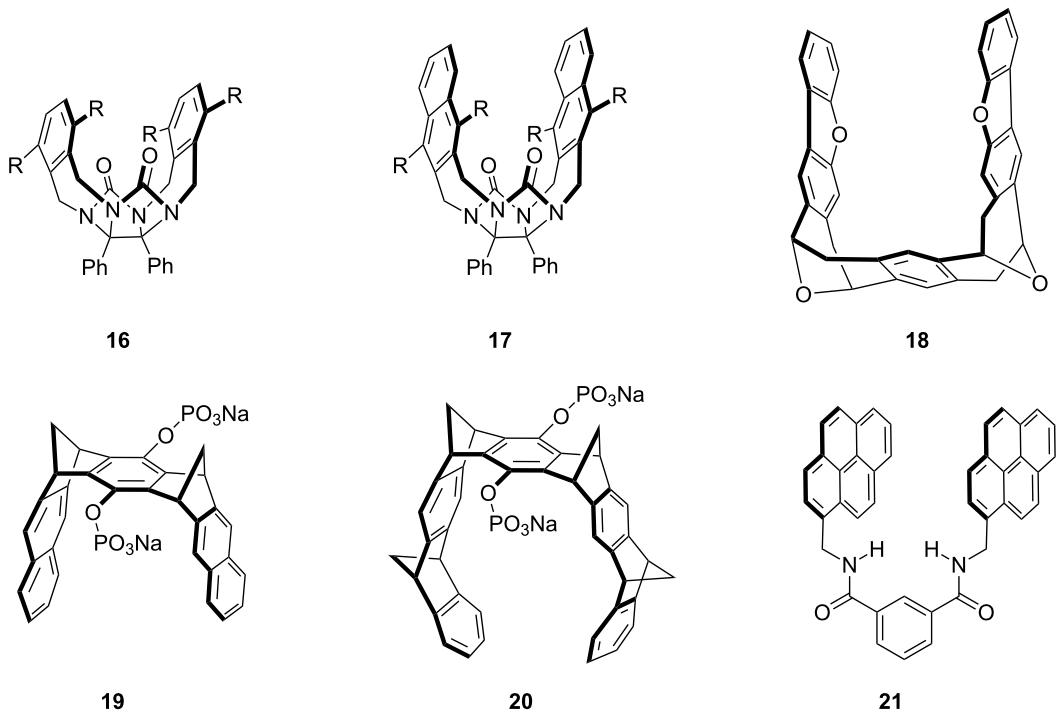

Figure 6: Glycouril-based molecular clip **16** and **17** developed by Nolte, an example of Harmata's chiral Kagan-ether tweezer **18**, two of Klärner's water-soluble tweezers (**19** and **20**), and pyrene tweezer **21** studied by Colquhoun.

not active whereas the 1,8-substituted naphthalene is, providing a clip that works by an induced fit mechanism (structure not shown) [28]. The Nolte group went well beyond the simple molecular clip architecture with a wide range of molecular and supramolecular architectures derived from the simple glycoluril motif [28].

Another early molecular tweezer developed by Harmata was notable because of its chirality. Harmata and his undergraduate student, Tom Murray (who later obtained his Ph.D. in my group), showed how two units of Kagan's ether provide the necessary C-shaped geometry [29]. Then, Harmata himself synthesized tweezer **18**, reporting its solution binding studies and a beautiful solid-state inclusion complex with trinitrobenzene [30]. This series of molecular tweezers was reviewed in 2004 [31].

A related class of achiral molecular tweezers was developed by Klärner et al., with their first report appearing in 1996 [32]. These di-, tri-, and tetramethylene-bridged aromatic systems (e.g., **19** and **20**) were prepared by consecutive Diels–Alder reactions and have been shown to exhibit a remarkably rich host–guest chemistry [33]. Perry Corbin, Steven Dell, and I were pleased to work with Frank Klärner and his group on linking a host analogous to **20** to silica and to study the solvent effects on host–guest complexation chemistry using our HPLC method [34].

Many of the molecular tweezers described above were reported many years ago. Were the water-soluble tweezers we set out to prepare in 1985 ever made? Where does the field stand today? We found that our molecular tweezers are well preorganized for dimerization, limiting their use in water [22], whereas Klärner and colleagues discovered that their tweezers **19** and **20** are water-soluble but tend not to self-associate [33]. This has allowed their use in a range of biomolecular recognition applications, which appear quite promising. For example, tweezer **20**, also called CLR01, has been found to bind the accessible lysine and arginine groups of proteins and thereby: (1) inhibit the toxicity of amyloidogenic proteins in cell culture, (2) modulate Aβ protein oligomerization, and (3) disintegrate preformed Aβ fibrils [35]. CLR01 (**20**) was recently shown capable of antagonizing seminal amyloids involved in HIV infection [36].

Beyond the impressive biomedical application described above, very simple molecular tweezers such as **21** have found use in materials and information storage applications. Howard Colquhoun and coworkers have used **21** to recognize sequence information in polymers [37,38], to form healable, supramolecular nanocomposites [39], and even to form supramolecular inkjet printing inks [40]. It is clear that molecular tweezers have found diverse applications, many of which derive from their cleft-like architecture. For example, the recognition of the polymer by a macrocycle would necessitate the threading of the polymer through the macrocycle and the act of reading the polymer sequence would have to be performed sequentially rather than random access.

Macrocycles make an appearance: macrocyclic bisintercalators and our early efforts to develop DNA probes

As just illustrated, one of the major advantages of the molecular tweezer approach to molecular recognition, and analogously the cleft approach pioneered by Rebek, Hamilton, and others (vide supra), is the ability to recognize large guests, for example, part of the surface of a large macromolecule or biomolecule. Indeed, our original goal of challenging the NEP was guided by the observation that all synthetic bisintercalators of DNA were nonmacrocyclic. DNA bisintercalating natural products with macrocyclic peptide scaffolds (such as echinomycin) were known as early as 1957, but these bound with the macrocycle in a single groove [41]. We wondered whether a macrocyclic bisintercalator that required a linking chain to reside in each groove would be able to bind given that a local melting would be required for one linker to pass to the other side of the double helix. Shown schematically as **22** (Figure 7), such a complex would be an example of what later would be known as a reversible, supramolecular catenane [42,43].

Carol Lamberson synthesized macrocyclic diacridine **23** with spermine and diamide linker chains long enough to allow binding according to the nearest neighbor exclusion principle (e.g., a complex such as **22**). All the data collected showed that this "topologically constrained" bisintercalator was indeed able to insert both acridine rings into double helical DNA; however, we were only able to infer the formation of complex **22** [44]. The data from a combined kinetic, NMR, and modeling study in collaboration with David Wilson were most consistent with complex **24**, although **22** was also consistent with much of the data [45].

Beyond the novelty of the binding mode, we thought these macrocyclic bisintercalators might serve as probes of DNA breathing and further might have particularly slow dissociation kinetics, thereby improving their ability to inhibit enzymatic DNA processing. However, students were not so interested in this area of research, perhaps because I had little training in DNA/small molecule recognition. Whatever the reason, we temporarily suspended this research effort. Fortunately, others were more persevering and pushed the concept forward in important new directions. Earlier work by Jean-Marie Lehn and coworkers showed that bisacridine **25**, which they called cyclo-

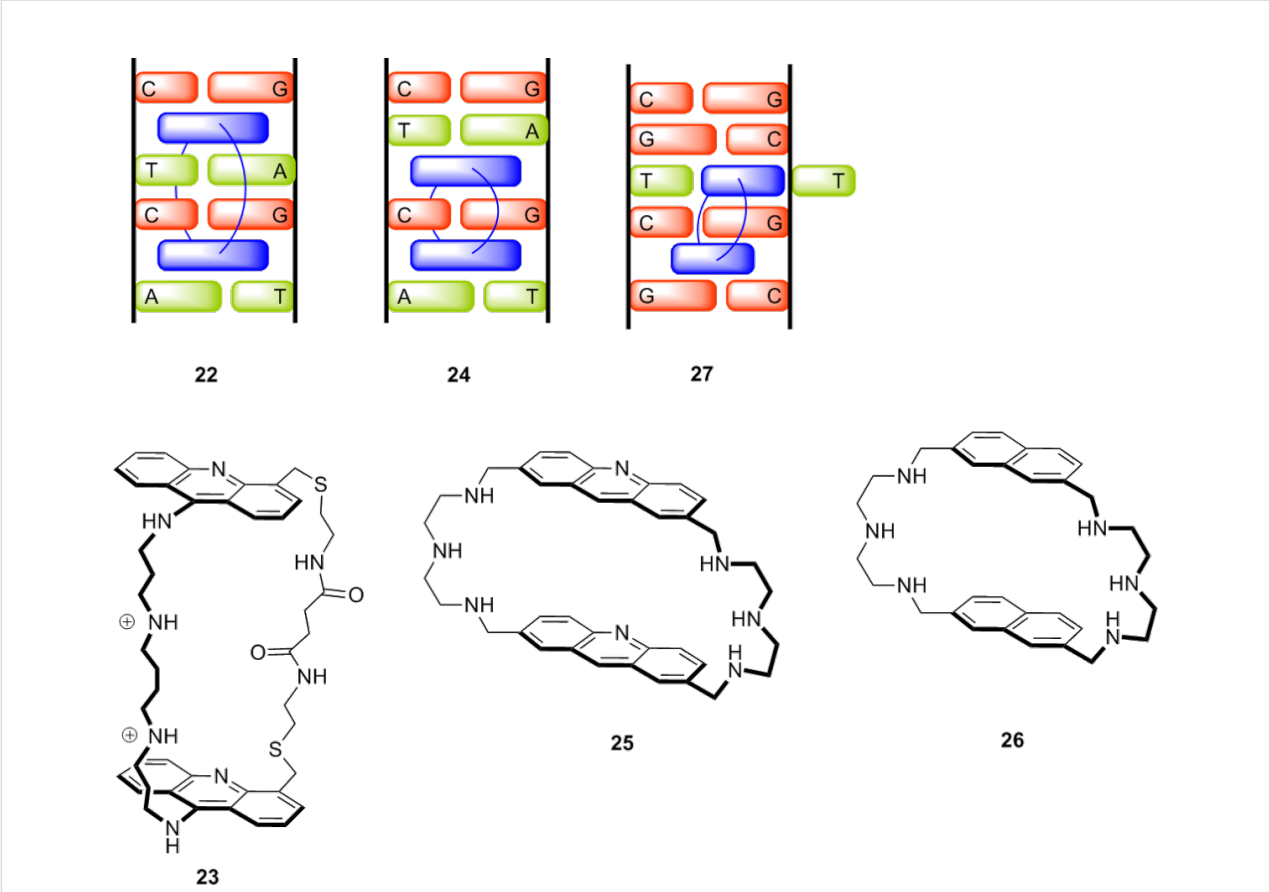

Figure 7: Macrocyclic diacridines and dinaphthalene and the intercalation complexes they may form with one chain in each groove.

bisintercalators, selectively bound DNA hairpins [46]. The same compound was found to selectively photo-cleave abasic sites in DNA [47]. Teulade-Fichou et al. reported that **25** (and especially the naphthalene analog **26**) showed high selectivity in binding pyrimidine mismatches [48]. The affinity of **26** for a TT mismatch in DNA was sufficient to inhibit the binding M. TaqI and, thus, potentially interfere with mismatch repair enzymes that have been implicated in certain diseases [49]. In 2009, Brent Iverson and coworkers reported that a cyclic bisnaphthalenediimide formed a complex with $d(CGGTACCG)_2$ that provided a well-resolved NMR and clear NOE signals between the linking chains and both the major and minor grooves [50]. Additional studies on this class of DNA ligands as part of an excellent review of other small molecules recognizing DNA mismatches has been recently published [51].

Sometimes a break can be productive and refreshing

In the year or so prior to my tenure and promotion, I thought about taking on a new and seemingly more challenging problem. At that time, there was a lot of discussion and some reports on molecular-recognition-driven self-assembly. The grand challenge was to design small subunits that would spontaneously form complex structures noncovalently, ultimately with some particular function. So we began in earnest to develop such systems, focusing primarily on hydrogen bonding. This effort amounted to a nearly 20-year break from DNA intercalators and molecular tweezers. Ed Fenlon, Tom Murray, and Perry Corbin started investigating DNA base-pair analogs and were later joined by Zhanting Li [52], Taiho Park, Jordan Quinn, and Eric Todd [53-56]. The goal was to understand the design principles that lead to high affinity [50,57,58], and then to apply that knowledge to self-assembling systems. Inspired by the work of Lehn [59], Whitesides [60], and Wuest [61], Brook Duerr, Yuguo Ma, Dave Reichert, and Fanwen Zeng developed discrete cyclic assemblies of small molecules [62,63] and dendrimers [64,65] – work that ultimately led Cyrus Anderson, Darrell Kuykendall, Ying Li, Taiho Park, Kwansima Quansah, and Mauricio Suarez to a broader range of supramolecular polymers, including liquid crystals [66], network blends [67], alternating copolymers [68], reversible adhesives [69], and redox-responsive supramolecular blends [70,71].

The circle of life or circling back within a career?

One of my colleagues at Illinois, John Katzenellenbogen, advised me early in my career not to entirely give up work in any area in which I gained experience. His sage advice was that in time those skills and knowledge, combined with general progress in chemistry could ultimately be leveraged into new and interesting ideas. So around 2007, Jonathan Arambula in my group returned to the idea of molecular-tweezer-like compounds and their ability to bind DNA. But unlike my early research, we were interested in linkers or spacers that minimally allowed or even forced the two chromophores to stack on one another.

The DNA target in which we became interested was a repeating sequence $(CTG)_n$ in the *DMPK* gene on chromosome 19. The sequence becomes unstable when $n > 50$, undergoing progressive expansion to give CTG^{exp} [72,73]. This inheritable genetic defect, with a frequency of about 1 in 8000 to 1 in 20,000 worldwide, leads to the incurable neuromuscular disease known as myotonic dystrophy type 1 (DM1) [74]. Many of the disease symptoms were attributed to aberrant sequestration of an alternative splicing regulator, MBNL1, by the expanded CUG transcript (CUG^{exp}) into nuclear foci [75]. We sought a small molecule that would selectively complex CTG or CUG repeats, both of which were known to form hairpin structures (e.g., see Figure 8a). The structure of $r(CUG)_6$, reported by Berglund in 2005 [76], revealed clear opportunities for a rational design approach. The stem-loop structure adopts an overall A-form structure but with little distortion in the backbone that would allow the U–U mismatches to form hydrogen bonds. Our early experience with Janus bases in self-assembly [61,63,77] suggested the use of a melamine (2,4,6-triamino-1,3,5-triazine) unit for formation of a base-triplet, as shown in Figure 8b. Additional reports by Lehn [78] and McLaughlin [79] supported this approach and the melamine unit has more recently been used extensively by Bong [80].

So why did we once again become interested in bisintercalators and the question of rigid vs flexible linkers? The triaminotriazine unit was designed to provide selective recognition of U–U or T–T mismatches, but on its own was viewed as unlikely to provide significant binding affinity because the hydrogen bonding simply involves replacing the hydrogen bonds to water. Coupling the triaminotriazine recognition unit to an intercalator provided the hydrophobic driving force for binding. Of course, nonspecific intercalation would be problematic so here we took advantage of the intramolecular stacking between the intercalator and the recognition unit to reduce off-target binding. Thus, as shown schematically in Figure 8c, the "stacked-intercalator"

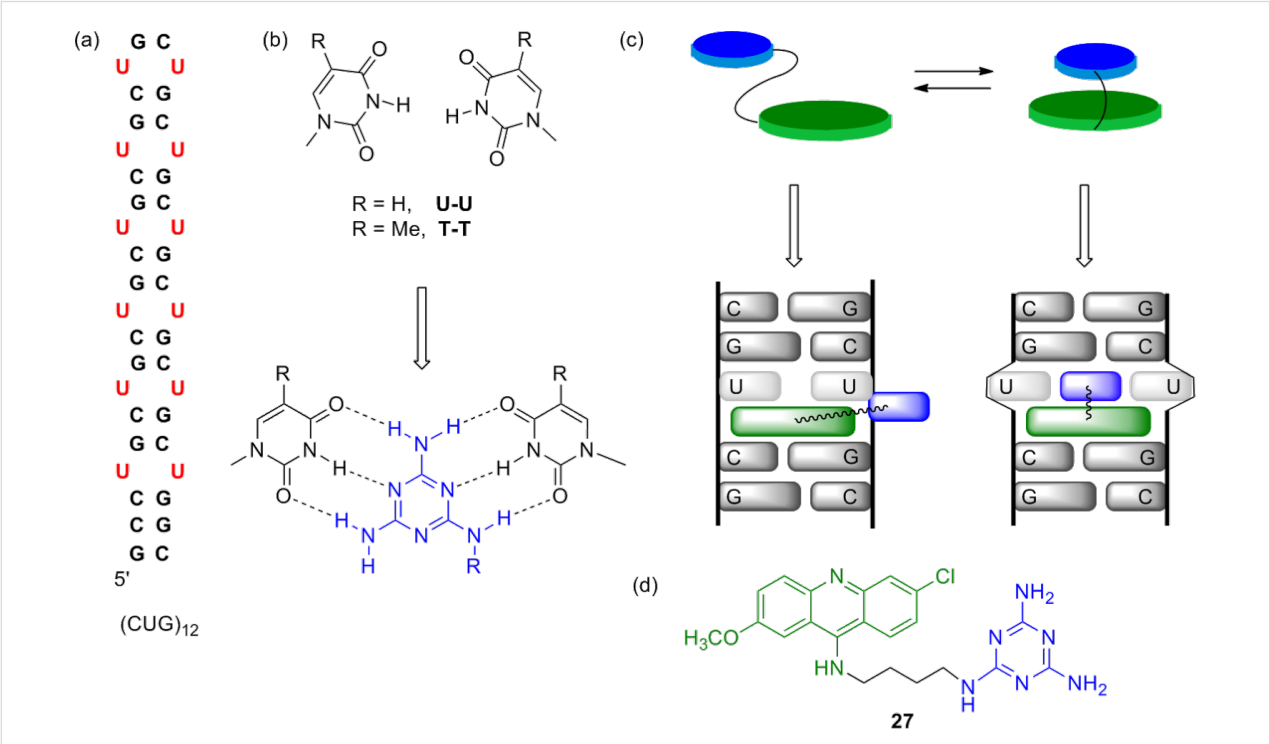

Figure 8: (a) Hairpin structure of CUG^{exp} seen in $(CUG)_{12}$. (b) U–U or T–T mismatches with no inter-strand hydrogen bonds, hypothetically forming base-triplet with 2,4,6-triamino-1,3,5-triazine. (c) Aromatic recognition unit tethered to intercalator in stacked and unstacked conformation. (d) Ligand **27**, an inhibitor of MBNL1N sequestration.

is too thick to insert between base-pairs but could potentially insert at U–U or T–T sites by forming a base-triplet. Lhomme had shown that nucleic bases tethered to 9-aminoacridines by methylene linker chains, as Whitlock had suggested, preferred stacked structures in water [81]. Nonstacked analogs were shown to recognize and even cleave abasic sites [82].

With this rational design in mind, Jonathan Arambula prepared ligand **27** and a number of control compounds and found it to be a dCTG- and rCUG-selective ligand with apparent K_D values of about 300–400 nM [83]. A very fruitful collaboration with Anne Baranger's group was initiated and they developed an electrophoretic mobility shift assay (EMSA) using MBNL1N and r(CUG)$_{12}$ and showed that **27** was indeed an inhibitor of MBNL1N sequestration. However, **27** did not appear to enter cells easily and was poorly soluble and cytotoxic.

Amin Jahromi recognized that acridine-containing compounds had been previously induced to enter cells and cell nuclei by attaching amino groups that take advantage of a polyamine transporting system [84,85]. Thus, compound **28** (Figure 9b) was prepared and found to inhibit formation of the MBNL-CUG^{exp} nuclear foci in DM1 model cells. Indeed, it was possible to follow live cells and watch the foci dissolve in real time using time-lapse confocal microscopy [77]. The logical next step was increasing affinity for CUG^{exp} by dimerizing ligands **27**/**28**. Indeed, dimer **29** exhibited a bivalent effect of 133 and was an extremely potent inhibitor of the MBNL1N-(CUG)$_{12}$ complex [86,87].

The possibility of off-target activity by the acridine ligands resulting from the unstacked conformation (Figure 8c) led Chun-Ho Wong to search for an alternative scaffold to drive the recognition [88]. He was attracted to the bisamidinium ion in **30** for three reasons: (1) it is a nuclear localizing agent analogous to nuclear fluorescent stains like DAPI, (2) Butcher reported the NMR structure of DB213, an analogous bisamidinium ligand, bound in the groove of the HIV-1 frameshift site (FS), and (3) the (CUG)$_6$ X-ray and HIV-1 FS NMR structures were both

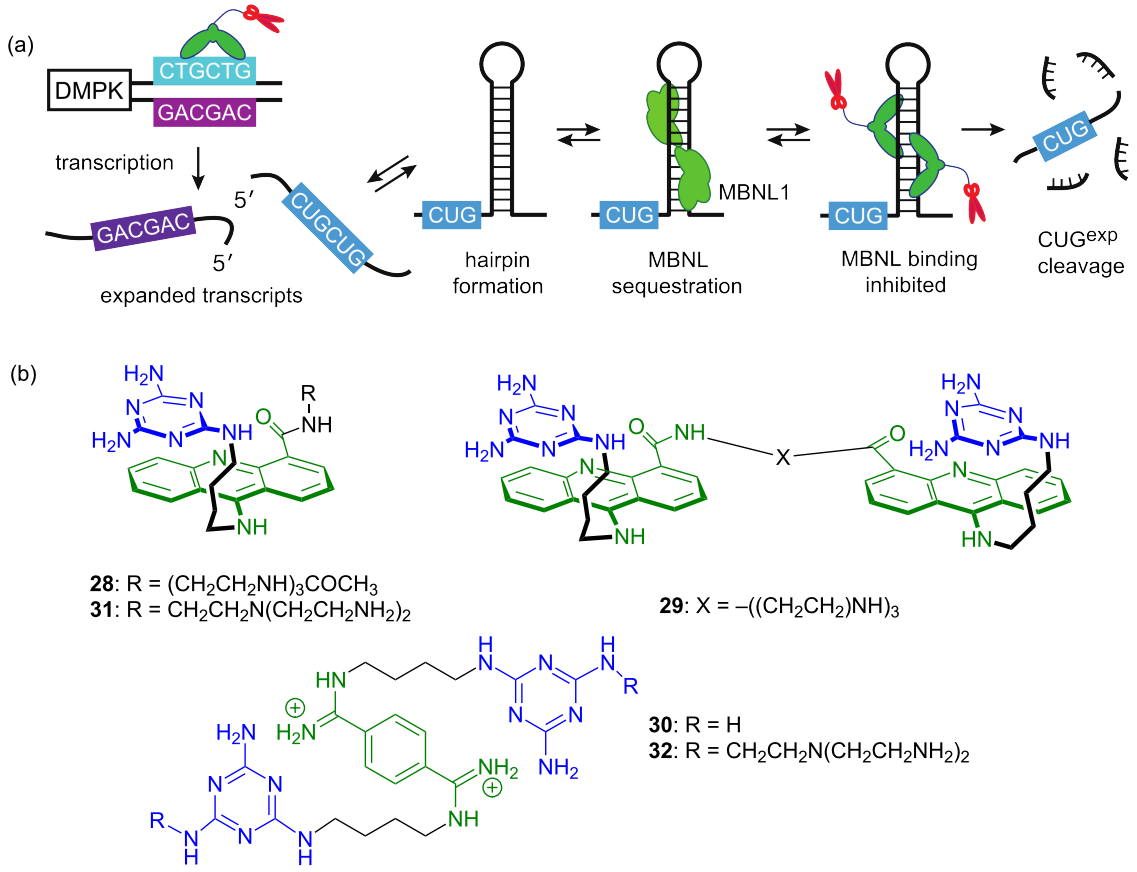

Figure 9: (a) CTG trinucleotide repeat expansion in *DMPK* gene produces expanded transcripts, one which CUG^{exp} sequesters splicing regulator MBNL1. Three modes of disease intervention: binding to CTG^{exp} to inhibit transcription, binding to CUG^{exp} to inhibit MBNL1 binding, and RNase-like cleavage of CUG^{exp}. (b) Ligands **28**–**32**.

A-form and similar suggesting replacement of the ammonium ions in DB213 with triaminotriazine units. Ligand **30** was studied in collaboration with both Anne Baranger and Paul Hergenrother and it was found to have low cytotoxicity, enter DM1 model cells, dissolved the MBNL1 foci and partially corrected the missplicing of two key pre-mRNAs, *cTNT* and *IR*. A terrific collaboration with Professor Edwin Chan's group at the Chinese University of Hong Kong allowed the compounds to be tested in vivo using a DM1 *Drosophila* model that looked at the rough eye phenotype with i$(CUG)_{480}$ flies. Ligand **30** showed significant and dose-dependent improvement in the rough eye phenotype, whereas the negative control, DB213 showed much weaker activity. Much less effort has been devoted to developing small molecules to treat DM2, which originates in a CCUG expansion, but Lien Nguyen, Chun-Ho Wong, and JuYeon Lee used similar rational design approaches and found lead agents that are selective for this RNA as well [89].

Although the gain of function mechanism that has CUG^{exp} sequestering MBNL1 is well supported, it is clear that the disease pathobiology is more complex. For example, Ranum recently reported [90] that both the CUG^{exp} and CAG^{exp} undergo repeat-associated non-ATG (RAN) translation to produce up to nine homopeptides, some of which are known to be toxic and involved in other disease [91]. Lien Nguyen and Long Luu considered the possibility of rationally designing ligands that could operate on multiple targets in the DM1 pathobiology. They designed and studied ligands such as **31** and **32**. These ligands were shown to bind the DNA that causes DM1, interacting with CTG^{exp} to inhibit transcription to CUG^{exp}, also binding CUG^{exp} that slips through inhibiting its sequestration of MBNL1, and, with the catalytic amino/ammonium/imidazole groups, slowly cleaving the CUG-RNA to prevent RAN translation [92]. Edwin Chan and his group again studied the in vivo activity of our compounds, showing that **32** reversed two separate CUG^{exp}-induced phenotypes in transgenic DM1 *Drosophila*, specifically the rough eye phenotype and larvae crawling mobility.

Conclusion

Detours, perspectives, and future studies

I was Department Head or Interim Department Head for a total of eight years (1999–2000 and 2005–2012). It was an honor to serve our outstanding faculty, staff, and students and to follow luminaries such as William A. Noyes, Roger Adams, Herb Carter, Larry Faulkner, and Gary Schuster. Being department head was without a doubt the most difficult thing I did during my career. It was a period of extraordinary personal growth, having learned how to work with a wide range of people and manage a complex organization. The expressions "herding cats" and "drinking from a fire hose" are apropos descriptors as it was more than a full time job and extremely demanding in other ways. Although it was hard to take time away from research and teaching, the department head job was interesting, challenging, and highly rewarding in seeing the department move forward, especially with the help of our loyal alumni.

We entitled the thematic issue containing this contribution "Supramolecular chemistry at the interface of biology, materials and medicine." Some of the examples presented herein illustrate the potential of the supramolecular approach to lead to advanced therapeutic agents. In particular the Klärner molecular tweezers that complex lysine-containing peptides may lead to agents that dissolve Alzheimers plaques or inhibit their formation. Our own efforts to create small molecules to target the toxic RNA involved in myotonic dystrophy have expanded to include multitarget drug-discovery approaches where supramolecular design principles led to DNA and RNA-selective small molecules that function even in the complex organisms (i.e., *Drosophila*).

Do we know so much about supramolecular interactions that all of the focus now should be on applications in biology, materials and medicine? The answer to this question is emphatically "No!" At a recent NSF workshop comprised of physical chemists and supramolecular chemists focused on water, the supramolecular chemists mostly agreed that water was not a special solvent, it just occupied an extreme, with low polarizability and a high cohesive nature. In stark contrast, the physical chemists showed plots indicating that water was unlike any other liquid and was clearly special. There also remain debates about whether the π-cation or face-edge aromatic interactions are unusual or even important. Therefore, model studies that shed light on the strength and nature of supramolecular contacts continue to be critically important.

One important emerging area I would like to highlight is the development of complex supramolecular systems. So much of supramolecular chemistry is inspired by biology, it is only natural that the complexity of biological systems be modeled in supramolecular systems. Thus, future developments will lead to multicomponent supramolecular structures/systems that evolve over time or communicate or respond to external or internal stimuli. Dynamic covalent chemistry has moved in this direction [93], and some initial efforts in this area using supramolecular chemistry have also appeared. For example, Andy Wilson's group reported a sequence of supramolecular recognition events that proceed in a controlled and defined manner, the specific pathway guided by what is present in solution [94]. This primitive model of a signaling cascade points to what may be possible as this area develops.

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

My maize and blue brick road to physical organic chemistry in materials

Anne J. McNeil

Review

Open Access

Address:
Department of Chemistry and Macromolecular Science and Engineering Program, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109-1055, United States

Email:
Anne J. McNeil - ajmcneil@umich.edu

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Abstract

Similar to Dorothy's journey along the yellow brick road in *The Wizard of Oz*, this perspective carves out the path I took from my early childhood fascinations with science through my independent career at the University of Michigan (maize and blue). The influential research projects and mentors are highlighted, including some fortuitous experimental results that drew me into the field of supramolecular chemistry, specifically, and organic materials, broadly. My research group's efforts toward designing new sensors based on small molecule gelators are described. In particular, I highlight how our design strategy has evolved as we learn more about molecular gelators. This perspective concludes with some predictions about where molecular gels, as well as my personal and professional life, are headed.

Review

One of my earliest memories involves pouring water down our driveway and watching the newly formed river break into small streams that later rejoined. Like most children, I was incredibly curious about the natural world around me. I set up terrariums and aquariums, used microscopes to examine leaves and insects, and kept spiders, snakes and turtles as pets. Although I sometimes dabbled with my brother's chemistry set, I often found the simple experiments boring. At 15, I started working at the local library because I had an insatiable appetite for learning. Bringing home new reading material each night was worth the monotony of re-shelving books each day. It was here that I began exploring potential careers in astronomy, chemistry, ecology, and geology. At the time, there was no clear favorite and my uninspiring high school classes did little to tilt the balance. As I began considering college, I realized I was most interested in the chemical phenomena within each field. Learning that light emitted from stars comes from hydrogen fusing to form helium, or that a pond's carbonate concentration affects buffering capacity and health, or that traces of iron in quartz lead to the purple color of amethyst, was all fascinating

to me. It is cliché, but I recognized the centrality of chemistry in the natural sciences and, as a result, I wanted to learn more.

At the College of William and Mary (W&M) my appreciation for chemistry grew as I took classes from engaging professors, including Dr. Gary Rice and Dr. Trevor Hill. Dr. Rice wore shorts and t-shirts to class, cracked jokes, amazed us with fire-laden and/or explosive demos, and made balancing equations interesting. Dr. Hill had the opposite persona; the (untrue) rumor was that he invented Teflon while working at DuPont, was incredibly wealthy, and taught purely for the love of chemistry. The aura that surrounded him was palpable and he did not disappoint. Because of him I began to see organic chemistry in the world around me, and I was hooked. My father still reminisces about the time I spent our three-hour ride home describing the molecular basis behind the stretchiness of rubber bands, among other things. I could not stop talking and we both knew that I had found my passion.

With encouragement from my faculty advisor, Dr. Debbie Bebout, and second-semester organic chemistry professor, Dr. Rob Hinkle, I began doing independent research. I was fortunate that Rob agreed to take me on for what turned out to be a three-year research experience. My initial goal was to identify six isomeric fragmentation products that were first observed by former student Dave Thomas (Figure 1). Once identified, I was tasked with elucidating the mechanism(s) that led to those products. What drew me into the lab was applying what I learned in class (e.g., substituent effects) to this unknown research question. I was driven by the desire to collect new data, and provide new information about the reactivity of these compounds [1,2]. Rob was a great mentor and role model; he had high expectations for himself and worked hard to achieve them. I was fortunate to have another great, albeit unofficial, mentor at W&M, Dr. Carey Bagdassarian. He was creative and passionate, and he encouraged, supported, and pushed me to be a better person and scientist. I left W&M feeling prepared for graduate work, and excited about the opportunity to gain even more breadth and depth in organic chemistry.

Entering graduate school at Cornell, I wanted to continue using physical organic chemistry principles to solve chemical mysteries. Although Cornell had a great selection of professors doing both fundamental and applied physical organic chemistry, I was most interested in working with Professor Dave Collum. He was known for unraveling complex mechanisms using a seemingly simple combination of kinetic, spectroscopic, and computational studies. I was fortunate, and will be forever grateful, that Dave accepted me into his group. We embarked on a project aimed at understanding why a lithium enolate alkylation stalled at 70% conversion during a key step in the preparative scale synthesis of a factor Xa inhibitor at Aventis. At the time, identifying solution structures of lithium enolates by NMR spectroscopy was challenging owing to the absence of Li–O coupling and the high symmetry of most common aggregates. Dave suggested we examine nonracemic mixtures to break symmetry (e.g., R_2S_2 versus R_3S_1 tetramers). At low temperatures, however, we saw just two major signals: one peak for the 100% R (and 100% S) aggregate and another peak for a heterochiral 50:50 R/S aggregate. We suspected a dimeric aggregate because our rate studies revealed a first-order dependence on enolate concentration. A major breakthrough occurred when Dave saw spectroscopic data wherein the "baseline junk" emerged as two additional resonances on warming; he excitedly declared that the "junk" was instead the complexity of higher aggregates. Sure enough, the "junk" showed a depen-

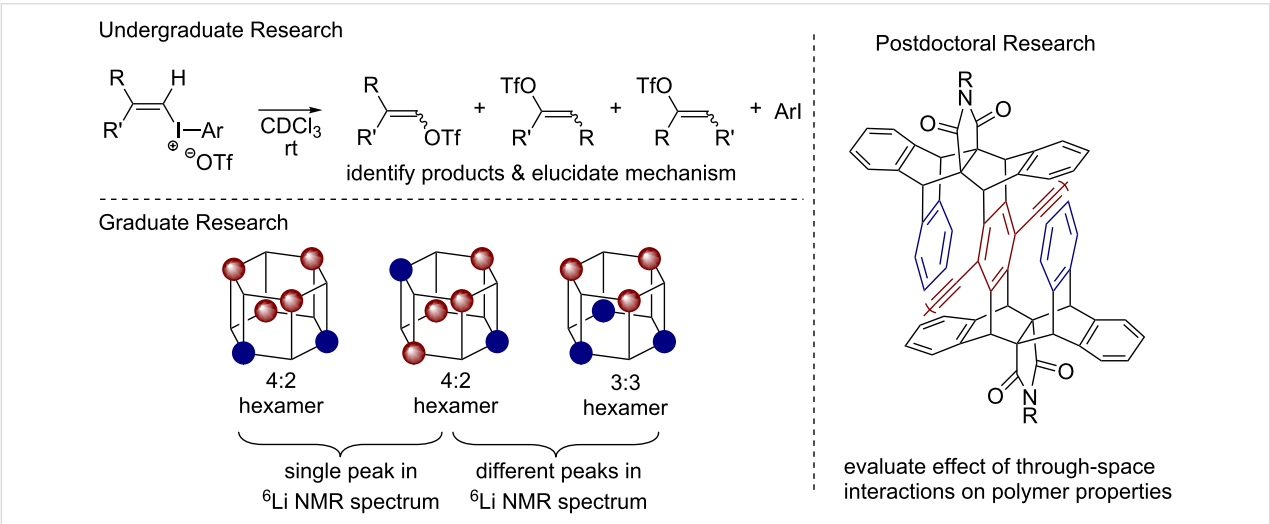


Figure 1: Summary of research experiences prior to independent career.

dence on optical purity consistent with hexameric enolates (Figure 1). We recruited physics graduate student Gil Toombes to help us fit the data using an iterative parametric method and what emerged was a beautiful story [3-5] (and method) that is still being used to determine the aggregation state of lithium enolates [6-9] and alkoxides [10-13]. From Dave, I learned a tremendous amount about being a scientist. One of the most important take-home messages was that all data, whether intentionally collected or not, is part of the story. Dave taught me how to be an efficient experimentalist, how to write papers and grants, and to have a critical eye for everything that you read in the literature. Dave also showed me how to balance an academic career with having a family. He was a great mentor who knew exactly when to push, when to provide assistance, and when to disappear and let me figure it out on my own.

A fortuitous and unusual observation during my graduate work led me into the field of organic materials: I observed an enolate alkylation wherein the rate correlated with how fast it stirred. Because I was measuring rates by quenching independent (but supposedly identical) reactions at various times, I first suspected that the stir-rate effect was due to the initial mixing of reagents depending on each vial's position in a grid on my stir plate. Once I measured the rate in a single round-bottom flask I realized that the stir-rate effect was real. I eliminated obvious culprits, including heterogeneity and high viscosity. Puzzled, I talked about this oddity to whomever would listen and tried every experiment suggested. One day I let a reaction sit unstirred for an hour while I attended a seminar; when I returned, the reaction mixture had formed a gel. In the end, we hypothesized that faster stirring disrupted more supramolecular aggregates, providing additional reactive sites for the alkylation, thereby accelerating the rate.

As I read more about gels and other organic materials, I began to see the field of physical organic chemistry more broadly. For my postdoctoral studies, I wanted to learn more about how (macro)molecular structure influences a material's solution and solid-state properties. I was drawn to Professor Tim Swager's research based on his creativity in (macro)molecular design and applied work with conjugated polymers. Tim pitched ideas for dozens of projects and let me decide where to focus. It took a few months and a few failed projects before I identified a clear research direction. In fact, one of those failed projects led to a new idea: to evaluate the effect of through-space (rather than through-bond) interactions on a conjugated polymer's properties. During this project I synthesized some of the most beautiful molecules I have ever seen, with one and two cofacial arenes surrounding the central arene of the monomer (Figure 1) [14]. The synthetic chemistry was elegant and simple. In the end, we saw substantial through-space effects on the monomer's properties, which were diminished in the polymer. Nevertheless, the cofacial arenes provided a physical, protective barrier from oxygen, and as a result, we observed reduced photobleaching, which is problematic for solid-state applications of conjugated polymers. Working with Tim I learned that new materials are interesting if they offer new properties, a lesson that still influences my research today. I learned how to manage my efforts, including when to stop working on unsuccessful projects. Tim's biggest impact, however, was on my presentation style. Tim critiqued my 10 minute "best poster" Gordon Research Conference talk for over an hour. Every little pixel, color, and bond was discussed. It was an eye-opening experience that has had a long-lasting impact. I am forever grateful for his time and advice.

While on the academic interview circuit, I was fortunate to have Steven Wheeler (a postdoctoral researcher with Professor Ken Houk at the time) in the audience at one interview. He was developing computational methods to evaluate π-stacking interactions, and was intrigued by our surprising substituent effects on the Diels–Alder regioselectivity in our monomer synthesis. Together we designed a collaborative project to further evaluate these effects. These studies led us to conclude that the π system is relatively unimportant and that substituent effects can instead be explained by through-space interactions [15]. Steven, who is now an associate professor at Texas A&M University, is changing the way we understand π stacking, XH/π, and ion/π interactions in organic systems [16-20].

During my postdoctoral studies, I remained fascinated with gels and was inspired by the creative work of many researchers in the field at the time [21-24]. When it came time to assemble a set of job proposals, it seemed natural for one focused on molecular gelation. Specifically, I proposed to develop sensors wherein a chemical stimulus (analyte) reacts with a "latent gelator" and induces gelation (Figure 2). Gel-based sensors were appealing because they provide an unambiguous visual change in the material's physical properties with no interference from colored or opaque samples. Moreover, no instrumentation or training is necessary to interpret the results, thereby providing a portable and potentially inexpensive method for sensing. Considering how naïve my understanding of gelation was at the time, I am still surprised that my proposal idea worked almost exactly as described.

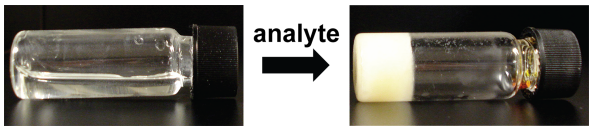


Figure 2: Sensing via analyte-triggered gelation.

Molecular gels form through the self-assembly of small molecules into supramolecular structures, such as ribbons, fibers, and sheets. This self-aggregation is driven by noncovalent interactions, including hydrogen bonding, π stacking, van der Waals interactions, and halogen bonding. Physical interactions amongst these larger structures lead to gel formation. Because noncovalent interactions are involved, gel formation is reversible and can respond to environmental changes. Understanding which molecules will form gels and under what conditions remains a significant challenge. As a consequence, new gelators are often "discovered" by modifying known gelator scaffolds. Although successful, this approach is limited to existing scaffolds and specific solvents, which may not be suitable for every application.

Our work with molecular gels began with two research questions: (1) How can we accurately predict which molecules will form gels? (2) How can we develop sensors where an analyte triggers gel formation? One of my first graduate students, Jing Chen, evaluated the use of an oxidation reaction to convert a nonplanar molecule into a planar one [25]. We hypothesized that this conformational change, combined with an increase in conjugation length, would facilitate self-assembly and gelation via π stacking. This hypothesis was based on Hanabusa's suggestion that unidirectional (1D) intermolecular interactions are necessary for gelation [26]. Excitingly, we found our first gelator (**1a**) after synthesizing just three molecules (Figure 3). Witnessing our first gel form remains one of my career highlights. Further characterization revealed that the π-stacking direction was coincident with the long axis of the fiber, providing support for the 1D interaction hypothesis. The original goal was to use nitric oxide (NO) as the oxidant, because a high concentration of NO in exhaled breath correlates with many diseases [27]. The NO-triggered oxidation and gelation worked, but there were a few limitations. Because NO was largely insoluble in the gelling solvent, we had to sequentially add NO and then additional solvent. When catalytic quantities of NO were used, the reaction rates were too low for sensing in real time. Increased NO concentrations led to gels within a minute, but these concentrations were outside the useful range for breath analysis. Nevertheless, these initial studies laid the foundation for our next effort, which was focused on developing a more sophisticated approach to gelator design.

We hypothesized that the Cambridge Structural Database (CSD), which contains over 700,000 organic and inorganic crystal structures, could be used to identify molecules that exhibit 1D interactions in the solid state. The rationale was that these molecules, or closely related derivatives, might be gelators. We searched the CSD for molecules containing a mercury atom (Hg^{2+}) that was involved in an intermolecular cation–π interaction [28]. We identified molecule **2a**, which exhibited 1D π–cation–π interactions in the solid state (Figure 3). Graduate student Kelsey (King) Carter synthesized just three compounds before gelator **2b** was discovered. Single crystals of **2b** revealed a surprising 1D π-stacking interaction, rather than the expected cation–π interaction. Nevertheless, powder X-ray diffraction (PXRD) analysis revealed that the packing within the single crystal was not representative of the packing within the gel. As a consequence, the gel structure remains unknown. Instantaneous gelation is observed when adding Hg-contaminated water to a solution containing the ligand, albeit at high concentrations

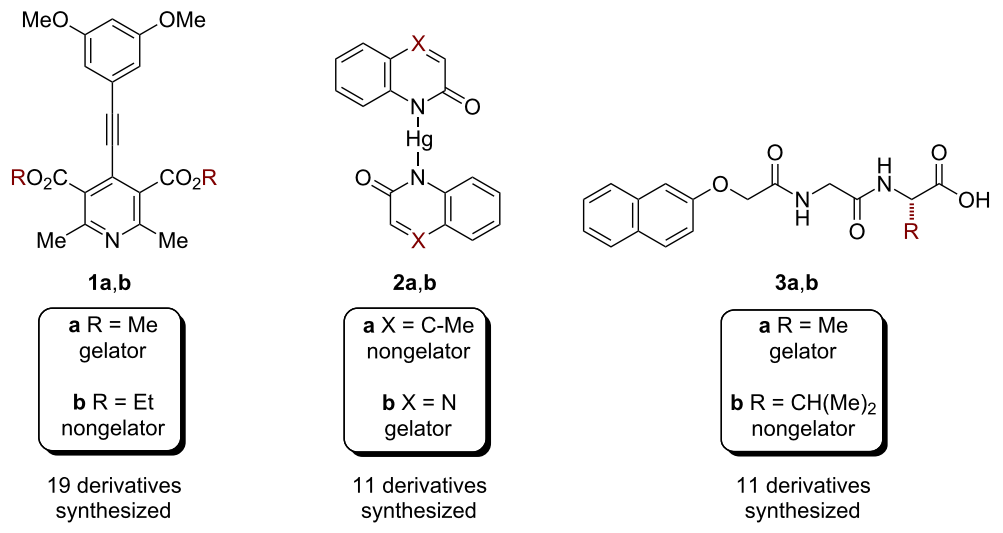


Figure 3: Examples of structurally similar gelators and nongelators examined in our studies.

of Hg^{2+}. We further demonstrated that gelation removes >98% of the Hg^{2+} from the contaminated water, leading to potential applications in environmental remediation [24].

In both studies we observed that seemingly minor changes in structure have a surprisingly strong effect on gelation ability. For example, converting a methyl ester (**1a**) to an ethyl ester (**1b**), which was not expected to affect the 1D intermolecular interactions, made the difference between a gelator and nongelator (Figure 3). Intrigued by the role of structure in gel formation, we asked ourselves, "What is special about the gelators compared to the structurally quite similar nongelators?" To address this question, we synthesized a large group of gelators and nongelators, with the long-term goal of elucidating their unique properties.

In our first effort, we synthesized 19 pyridine-based compounds, wherein 8 were gelators and 11 were nongelators [29]. One hypothesis from the literature was that gelators are "not too soluble or too insoluble" [30,31]. In contrast, we found gelators at both the low and high ends of the solubility spectrum (0.001 to 1 mg/mL), alongside the nongelators. We then probed the hypothesis by Hanabusa regarding the importance of 1D intermolecular interactions [22]. We were able to obtain single crystals of six gelators and five nongelators. Of the six gelator crystal structures, only three had PXRD patterns that matched the gel. Within this limited data set we found 1D intermolecular interactions being present and absent amongst both gelators and nongelators, providing no clear distinction based on molecular packing. Next, we performed a Hirshfeld surface area analysis to quantitatively evaluate the intermolecular interactions within the crystal structures [32]. This analysis provides information about the nature and extent of intermolecular interactions in the solid state. Surprisingly, three nongelators exhibited Hirshfeld surface areas similar to the gelators, suggesting the types of intermolecular interactions (e.g., van der Waals, H-bonding, π-stacking) were similar amongst the gelators and nongelators. Because this analysis involves counting interactions without weighting them according to their influence on the solid-state structure, we began investigating alternative measures of intermolecular interactions.

We hypothesized that gelation might instead depend on the strength of intermolecular interactions in the solid state. To test this hypothesis, graduate student Jing Chen measured dissolution enthalpies (ΔH_{diss}) of both the gelators and nongelators by determining their solubility at various temperatures [25]. The dissolution enthalpy reflects both the solid-state gelator/gelator interactions as well as the solution-state gelator/solvent interactions (Figure 4). Examining all 19 compounds revealed that gelators had higher dissolution enthalpies than the nongelators (on average), suggesting that the gelators had stronger solid-state gelator/gelator interactions and/or weaker gelator/solvent interactions. When comparing the same compounds in a different solvent system, similar trends were observed. To determine whether these results were general, postdoctoral researcher Maria Muro-Small synthesized and measured dissolution enthalpies for 11 dipeptide-based compounds (6 gelators, 5 nongelators) [33]. Peptides were chosen because they represent the largest and most widely investigated class of molecular gelators and their gelation ability is highly dependent on their sequence (e.g., **3a** versus **3b**, Figure 3) [34]. We again observed the trend that gelators exhibit higher dissolution enthalpies. During these studies, we discovered that several dipeptides underwent solid–solid transformations during the solubility measurements. Graduate student Kelsey (King) Carter later observed similar solid–solid transformations with our Hg complexes, precluding further analysis [35]. Ultimately, our take-home message was that strong intermolecular interactions and weak solvent interactions are important in gelation.

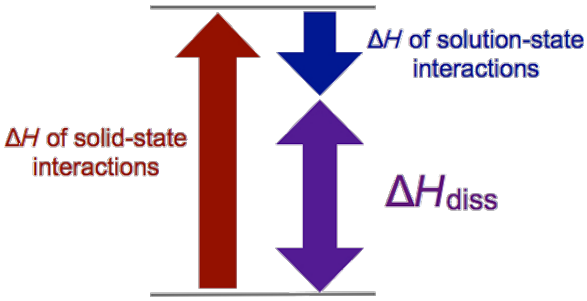


Figure 4: Relationship between dissolution enthalpies and intermolecular interactions. Gelators exhibit (on average) larger ΔH_{diss} than nongelators, suggesting stronger solid-state interactions and weaker solvent interactions.

At this point, we wanted to develop a method that could predict solid-state dissolution enthalpies, rationalizing that such an approach could be useful for identifying new gelators. Graduate student Cheryl Moy ambitiously learned molecular mechanics simulations with mentorship from my colleague Professor Charles L. Brooks III [36]. Our goal was to model the solid-state interactions as well as the solvent interactions. We wanted to avoid starting the simulation with a crystal structure, knowing that this criterion would ultimately limit the structural diversity, so we modeled the solid-state as a liquid. Unfortunately, starting from a random, liquid orientation resulted in gelators and nongelators being enthalpicly indistinguishable. Using the crystal lattice as input provided better estimates of the dissolution enthalpies, but the gelators and nongelators within this limited data set remained indistinguishable.

We then returned to our original CSD approach and modified it to incorporate our new understanding about the importance of strong intermolecular interactions (Figure 5) [37]. Specifically, we hypothesized that the driving forces for anisotropic growth in crystals and gel fibers might be similar. Because needle-shaped crystals form when the intermolecular interactions in one dimension are significantly stronger than the others, we predicted that molecules with needle-shaped morphologies (or their closely related derivatives) might be gelators. To test this hypothesis, we used crystal morphology prediction tools to identify needle-shaped crystals in the CSD. Graduate student Kelsey (King) Carter and undergraduate student Sarah Cox predicted the morphologies of 186 Pb(II)-containing crystals. Focusing on the top 5% and selecting stable and easily synthesized compounds (e.g., **4a** and **5a**), we discovered two new gelators (**4b** and **5b**, Figure 6). Postdoctoral researcher Gesine Veits synthesized nine additional derivatives and found both gelators and nongelators. These exciting results suggested that the driving forces for forming high-aspect-ratio crystals and gel fibers may be similar and guided by strong, directional intermolecular interactions. Overall, this approach for identifying new gelators should be generalizable, and therefore useful for developing new molecular gel-based applications.

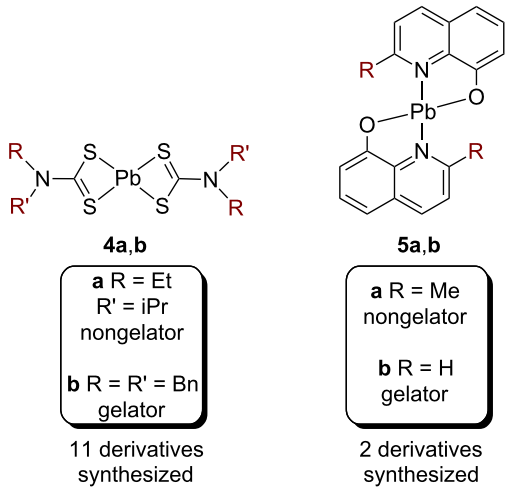


Figure 6: New gelator scaffolds identified by predicting crystal morphologies.

Alongside these fundamental studies, we were interested in applying our new gelators in sensing platforms. Because analyte-mediated sensors rely on a chemical transformation to take place prior to gel formation, the reaction rate should be fast, or ideally instantaneous. While most of our sensors were designed with this criterion in mind, we were sometimes surprised to find slower and/or lower yielding reactions than reported. In these cases, we evaluated the stability of the reaction intermediates [38], measured reaction rates for both the desired and undesired products [39] and optimized the conditions to accelerate the desired transformations [34,35]. We learned from our early efforts that generating highly sensitive sensors required analytes that were efficient catalysts. With postdoctoral researcher Steven Bremmer and collaborator Professor Matt Soellner, we targeted gelation-based sensors using enzymes as the analytes [40,41]. Enzymes were attractive analytes because many diseases are correlated with their overactivity and/or overexpression. Specifically, we selected proteases, which play important roles in many biological processes, including blood clotting, apoptosis, and pathogenesis. Prior to our work in this area, there were several examples of enzyme-triggered gelation [42-44]; however, most of these systems were not responsive to physiological enzyme concentrations and not generalizable. We hypothesized that an enzyme-triggered cleavage that separates a recognition sequence from a gelator would represent a general and modular strategy for detecting proteases (Figure 7) [36]. The key advantage of this system is that simply swapping out the recognition sequence can lead to gelation-based sensors for other proteases. We applied this method to three different proteases and demonstrated that gelation could occur under physiological enzyme concentrations. We later generalized the

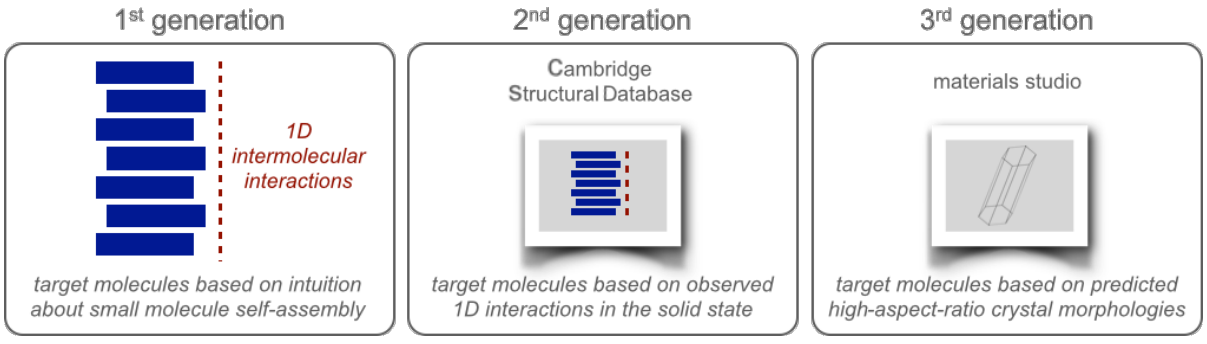


Figure 5: Evolution of our design strategy for identifying new gelators.

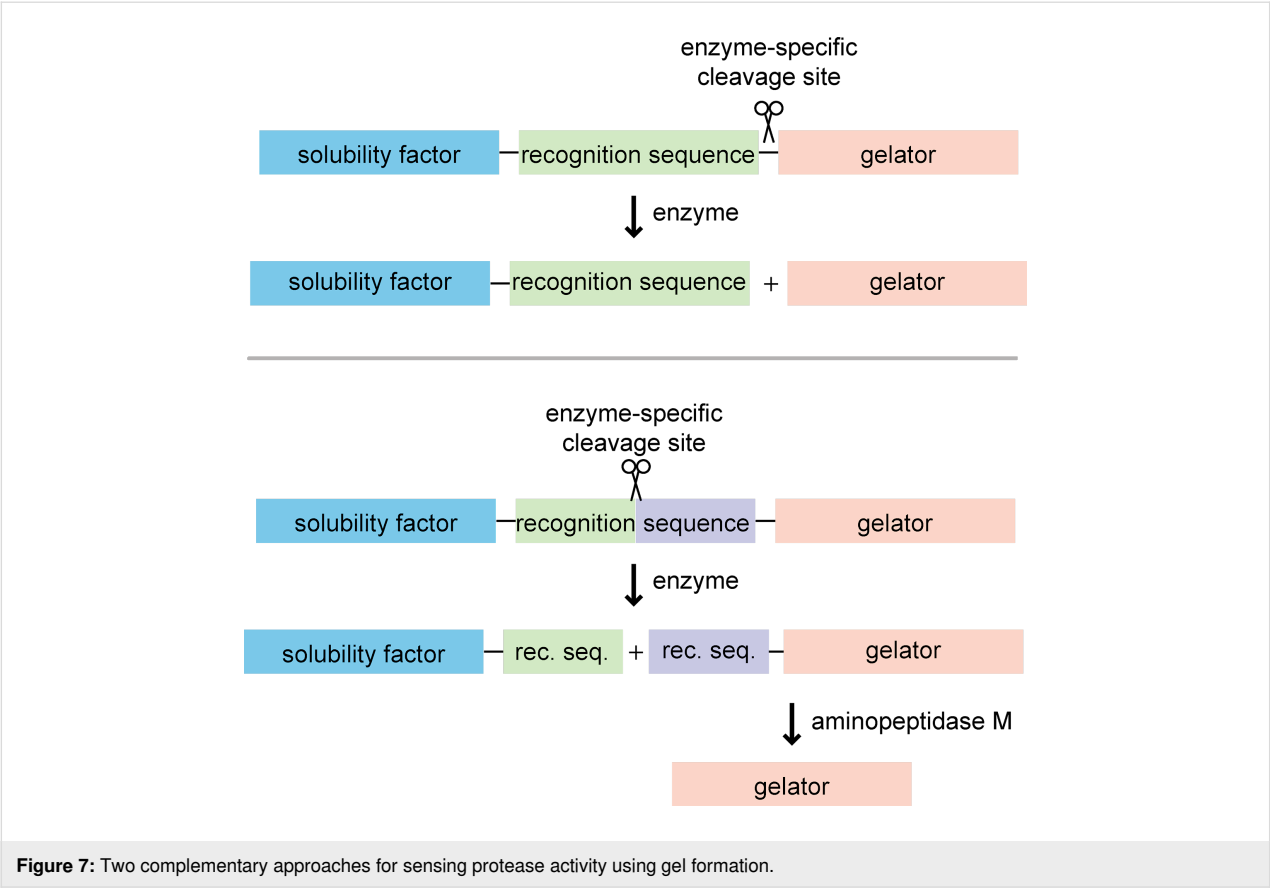
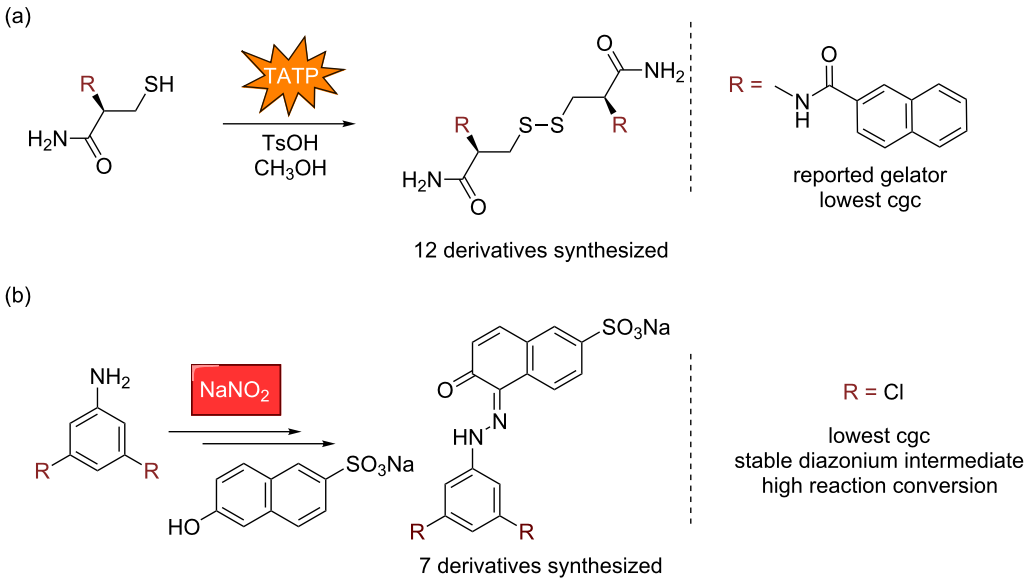


Figure 7: Two complementary approaches for sensing protease activity using gel formation.

approach even further to include proteases with internal cleavage sites (Figure 7) [37].

Additionally, we began investigating alternative ways to improve the sensitivity of gelation-based sensors for noncatalytic analytes. Graduate students Cheryl Moy and Danielle Zurcher evaluated "disassembling" polymers wherein an analyte-mediated end-group cleavage triggers depolymerization, releasing many gelators for each analyte [32,45]. We investigated three different polymeric scaffolds but have not yet successfully polymerized any monomer that forms a gel when released. We briefly investigated several methods of increasing sensitivity by lowering the critical gel concentration (cgc) using external (nongelator) modifications. For example, we decreased the cgc by lowering the solvent volume and/or reducing the vial diameter [35]. In a different example, we hypothesized that polymeric additives, commonly utilized to alter crystallization processes, might be useful for lowering the cgc in gel formation. Graduate student Yash Adhia, working with undergraduates Tracy Schloemer and Maria Perez, screened a series of commercially available polymers and discovered that adding poly(acrylic acid) (PAA) led to an incredible 90% reduction in the cgc of **1a** [46]. We determined that PAA was adsorbing onto the gel fibers during growth, which decreased the growth rate, leading to thinner fibers that were either longer or greater in number. These morphological changes reduced the cgc likely through additional physical entanglements from the longer and/or more prevalent fibers. Combined, these results suggested that additives may be a simple method to modify gel formation without having to alter the molecular structure.

We have also explored the more conventional approach of modifying a known gelator to identify a new gelator with a lower cgc [34,35]. Graduate student Jing Chen, working with undergraduate Weiwei Wu, developed a sensor to detect milligram quantities of the explosive triacetone triperoxide (TATP) [35]. We synthesized 12 derivatives and found just three additional gelators; however, none of them exhibited lower cgcs than the original, reported gelator (Scheme 1a). Graduate student Danielle Zurcher, working with undergraduate Julian Díaz Romero, developed a sensor for nitrite contamination in water sources [34]. In this case, five additional compounds were synthesized, all of which were gelators, with some exhibiting lower cgcs than the known gelators (Scheme 1b). Given the mixed results of this approach, we believe that our CSD-morpholgy prediction method represents a better strategy for identifying new gelators for specific applications moving forward.



Scheme 1: Sensors based on modifying known gelator scaffolds.

Although I started my independent career just eight years ago, so much has changed in my life, both personally and professionally. I met my wonderful husband (Professor Matt Soellner), got married and now have two awesome children (Evie and Emily). I am happiest when we are outside, exploring our world together (Figure 8). Professionally, I have taken on new leadership roles at the University of Michigan and elsewhere. My lifetime love of learning has led me down a path to better understand how other people learn. This path involved starting a funded research program in chemistry education. I am grateful that I can continue exploring new research areas as a professor. I have also realized that, like the library of my youth, there is no better place for me than the University of Michigan. I am surrounded by an amazing group of colleagues within my department and on campus. I am especially grateful for my mentors here at the University of Michigan: Professors Brian Coppola, Carol Fierke, Adam Matzger, Tim McKay, Melanie Sanford, and John Wolfe, who each have played a significant role in my professional development. I am looking forward to seeing what additional changes will occur in my family and career in the future.



Figure 8: Enjoying the outdoors with my family, especially when it involves mud! Photo credit: Donald A. McNeil.

Conclusion

Overall, we have developed a strategy for discovering new gelators that focuses on molecules exhibiting strong, 1D intermolecular interactions in the solid state. This approach is simple to execute and expected to be generalizable. We anticipate that this new design strategy will transform gelator discovery from its current random screening to a more rational approach. With this streamlined approach to identifying new gelators, both the number and utility of molecular gel-based applications should increase. In addition, we have performed both fundamental and applied studies toward gelation-based sensors for a diverse set of analytes. Many of the strategies described herein can be used to improve other gel-based applications. Moving forward, we plan to capitalize on our experiences by embarking on a new (to us) research direction: exploring gels as templates for synthesizing materials with high surface areas.

The future of molecular gels remains bright. Two areas of growing importance include: (i) applying advanced solid-state

characterization methods to gain insight into the molecular packing within gel fibers, and (ii) efforts towards correlating gelation ability with solvent properties (e.g., Hansen solubility parameters). These studies can provide further insight into gelator/gelator interactions as well as solvent/gelator interactions. Analogous to our efforts, these studies need to move from a post-experiment rationalization to a predictive model to be useful for gelator design. It is here where computational methods can and should play a significant role.

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First and foremost, I want to thank the hard-working undergraduate, graduate, and postdoctoral researchers with whom I have had the pleasure to work with over the last eight years on this research direction as well as others. I also want to acknowledge the many insightful discussions I have had with my co-workers and colleagues at the University of Michigan. I want to thank 3M, the Arnold and Mabel Beckman Foundation, the Office of Naval Research (N000140910848 and N000141210604), and the University of Michigan for supporting this work. I also want to thank the Alfred P. Sloan Foundation and the Camille and Henry Dreyfus Foundation for research and teaching fellowships.

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Art, auto-mechanics, and supramolecular chemistry. A merging of hobbies and career

Eric V. Anslyn

Review

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Address:
Department of Chemistry, University of Texas, Austin, TX 78712, USA

Email:
Eric V. Anslyn - anslyn@austin.utexas.edu

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Abstract

While the strict definition of supramolecular chemistry is “chemistry beyond the molecule”, meaning having a focus on non-covalent interactions, the field is primarily associated with the creation of synthetic receptors and self-assembly. For synthetic ease, the receptors and assemblies routinely possess a high degree of symmetry, which lends them an aspect of aesthetic beauty. Pictures of electron orbitals similarly can be seen as akin to works of art. This similarity was an early draw for me to the fields of supramolecular chemistry and molecular orbital theory, because I grew up in a household filled with art. In addition to art, my childhood was filled with repairing and constructing mechanical entities, such as internal combustion motors, where many components work together to achieve a function. Analogously, the field of supramolecular chemistry creates systems of high complexity that achieve functions or perform tasks. Therefore, in retrospect a career in supramolecular chemistry appears to be simply an extension of childhood hobbies involving art and auto-mechanics.

Review

Introduction

The field of supramolecular chemistry abounds with beautiful and aesthetically pleasing molecules. From Stoddart’s rotaxanes [1,2], Sauvage’s knots [3,4], Rebek’s capsules [5], Fujita’s 3-D MOFs [6,7], to Atwood’s clusters [8,9], our field is associated with creating complex structures, often of very high symmetry. This makes ChemDraw structures, space-filling models, or ball and stick renderings very akin to objects found in modern art [10]. Can one look at an Atwood cluster without thinking of Geometric Abstract Art? Maybe one can, but the similarly is striking (Figure 1).

Besides having an aspect of beauty, supramolecular structures are created to achieve a chemical function or task. These functions range from imparting mechanical changes [11-13], to altering material properties [14,15], to manipulating biological ramifications [16]. Thus, not only are the assembled chemical

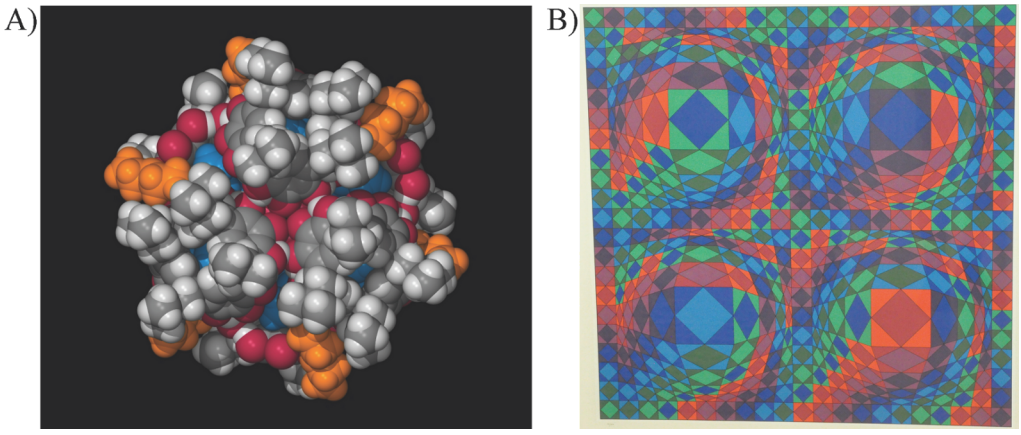

Figure 1: A) An Atwood Cluster, picture donated from Jerry Atwood. B) Vasarely serograph, personal photograph from EVA.

entities visually striking, they also have real-life practical applications. This combination of art and function undoubtedly had a large influence on why my career transitioned into the field of supramolecular chemistry.

Earliest inspirations

My father, Samuel Anslyn Jr., was an industrial artist. In World War II and later he worked as an artist rendering exquisitely detailed charcoal sketches, and airbrush mock-ups of airplane and ram-jet parts. Before becoming an art and drafting teacher at a Glendale Community College, he was the art director at Marquardt Corporation [17]. My house and garage are filled with the most wonderful renderings of airplane parts (Figure 2A), as well as large oil paintings of other industrial and mechanical structures, such a locomotives (Figure 2B). As he aged, his need to express creativity converted to being an auto mechanic, restoring old Jaguars and Porsches to the level of award winning Concours D'Elegance vehicles.

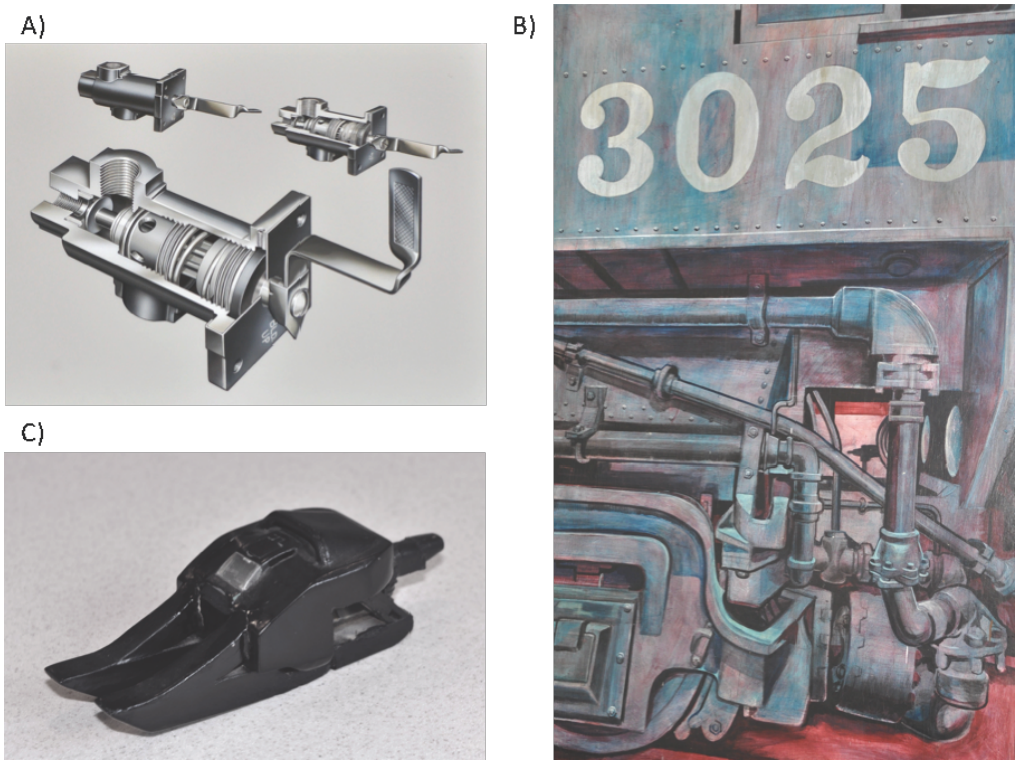

Figure 2: A) An airplane part air-brush rendering (S. S. Anslyn, 1950's). B) A mural of a locomotive engine (S. S. Anslyn, 1971). C) A "destructo" created by Brian Heidsiek in approximately 1973. All graphics are personal photographs by EVA, who has the copyright to every photo used herein.

Growing up in a household with a father that was both an artist and a mechanic, it became natural to build plastic and balsawood models as a hobby and to work on motorized vehicles. My neighbor from age 7, Brian Heidsiek, became an industrial designer himself. As children we raced and worked on go-karts. We also created numerous models from scratch, culminating in what were known as a series of "destructos", i.e., vehicles that were indestructible and saved the world from disasters (Figure 2C), inspired by the cartoon show "The Thunderbirds" [18]. Even to this day, my hobbies still involve go-kart racing and restoring old cars.

Thus, after 40 years of hindsight – considering my childhood with an artist/mechanic for a father, and a best friend with whom I built functional models, it is not surprising that my career has focused on the creation of new molecular and supramolecular structures designed to execute a particular function or achieve a certain task. Further, while we all know "beauty is in the eye of the beholder", many of our group's chemical structures are exquisite, at least in my own somewhat biased opinion. In fact, even after 27 years as a Professor, I experience a thrill when we get a crystal structure because they invoke an aesthetic response (Figure 3). The combination of art and function is fully analogous to both my father's and neighbor's designs, except that the "art" of my group is visualized on the nanoscopic scale rather than on a macroscopic scale of my dad and friend. Thus, my childhood exposure to the combination of art and function has clearly led me to the field of supramolecular chemistry.

Origin of a love of organic chemistry, orbitals, and complexity

All organic chemists, and in particular supramolecular chemists, must share an enjoyment in creating new chemical entities of our own inspiration. My passion for organic and organometallic synthesis was first developed when performing undergraduate research at the California State University Northridge (CSUN) under the tutelage of Dr. Edward Rosenberg. At this undergraduate institution my major was pre-med, with all the associated drive and motivation to do well, accompanied with the annoying behavior of such students. For example, my major was chemistry solely because a larger fraction of B.S. chemistry majors were accepted to medical school than other majors. I had never taken a chemistry class in high school, yet it was my declared major. Further, because the counselors advised that undergraduate research was a good exercise to build a curriculum vitae for entrance into medical school, research was one of my pursuits from freshman year throughout my under-

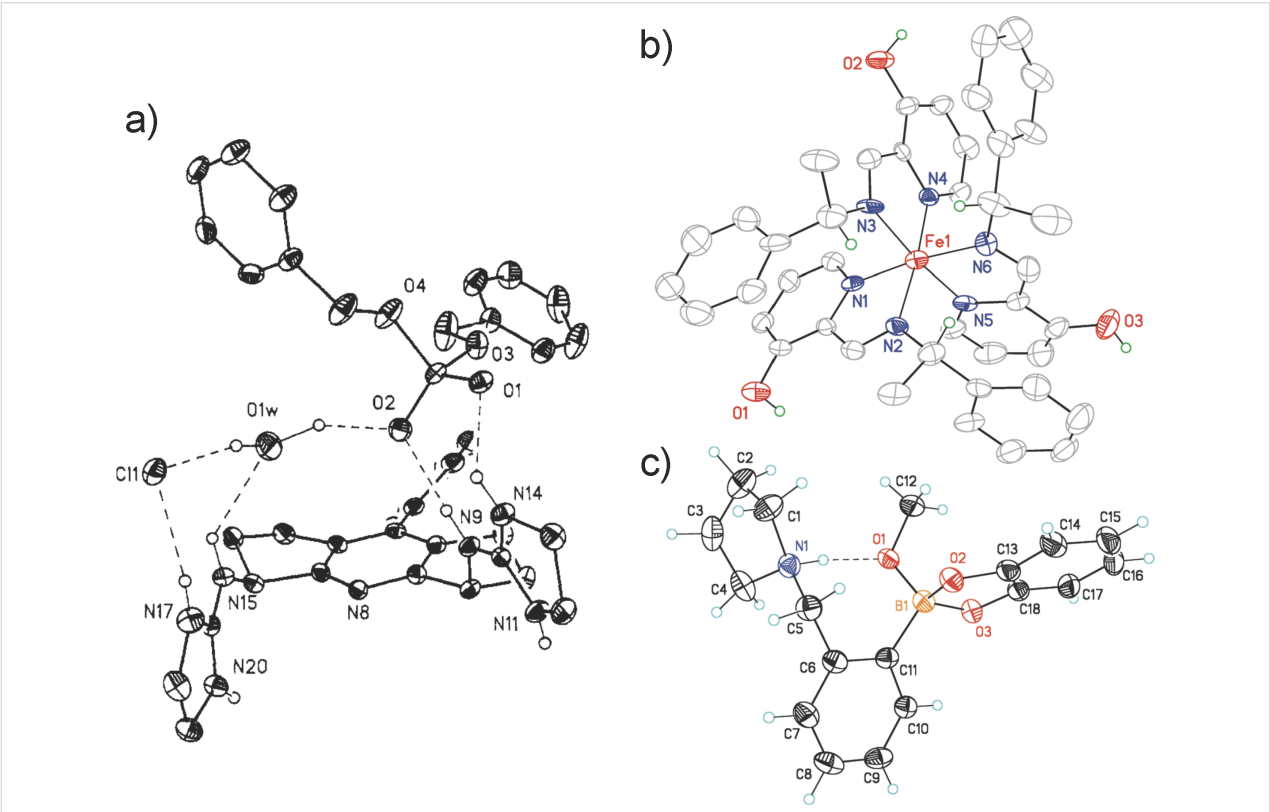

Figure 3: Representative crystal structures of various complexes we have created over the years, that in my own opinion are particularly beautiful. Collage reproduced with permission from renderings in reference [19-21]. Copyright 1993, 2009, and 2012 The American Chemical Society.

graduate career. The project entailed the use of variable-temperature NMR to measure the dynamics of ligand migration in trimetallic osmium clusters [22,23]. Dr. Rosenberg was an inspirational figure, and his pursuit for scientific knowledge was infectious. In particular, he imparted a love of deciphering mechanistic puzzles.

To me, mechanistic puzzles are similar to deciphering how to fix an internal combustion engine; both involving diagnosis of the problem within a large "black box" and fixing it with tools appropriate for the job: valve spring compressors, feeler gauges, socket wrenches, etc. As organic chemists, we propose hypotheses explaining how Mother Nature works, and we have particular experimental tools to test our theories: kinetics, isotope effects, solvent effects, etc. [24].

Reactions with multiple components all working in concert to achieve a function beyond that of the individual parts (known recently as "emergent properties" [25,26]) is likewise analogous to an internal combustion motor (Figure 4). Numerous

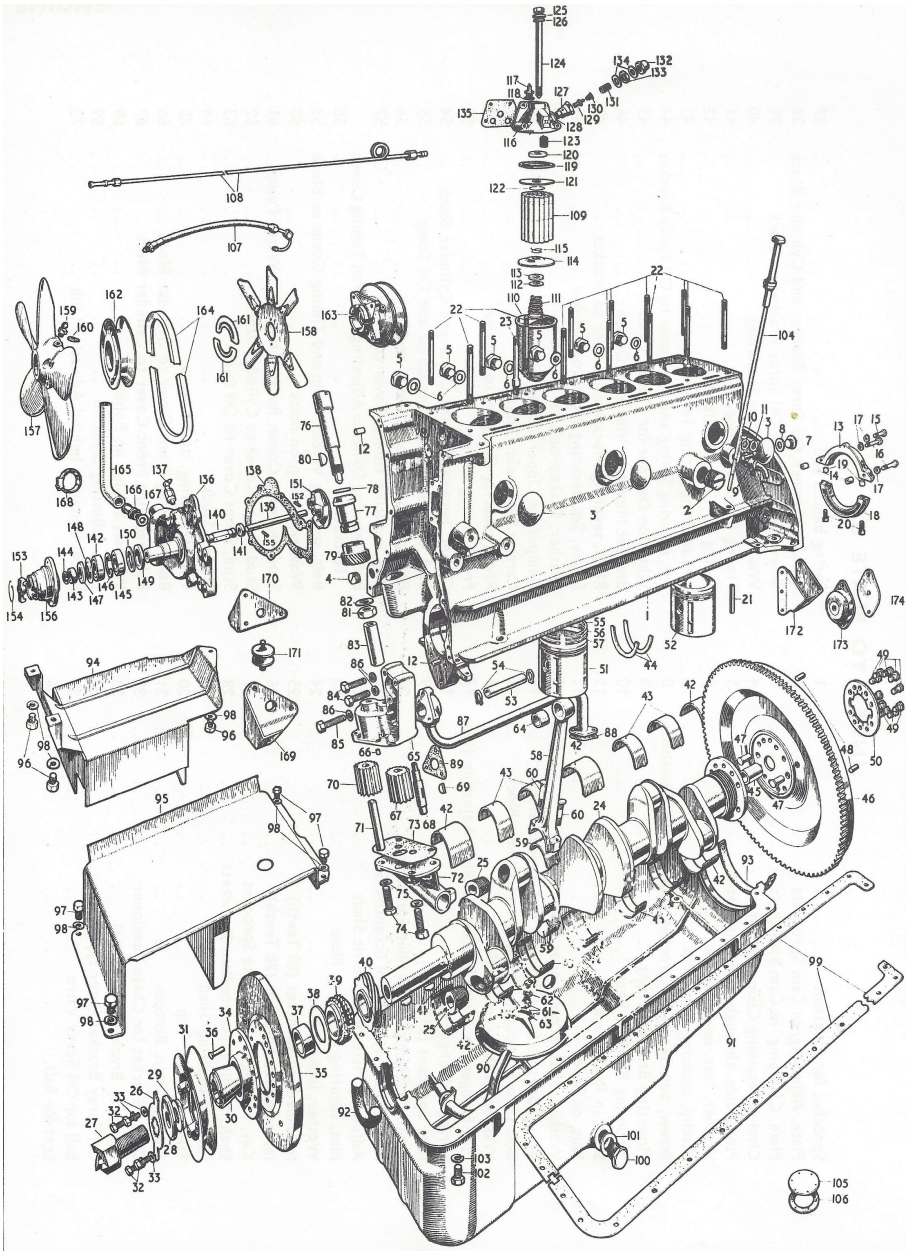

Figure 4: Exploded view of a 1953 Mk VII Jaguar in-line six internal combustion motor (bottom end), overhauled by EVA in 1976. Personal photograph by EVA.

parts: pistons, values, crankshafts, cams, etc., all combine together to create a force that propels the automobile. The expanded version of a 1953 in-line six-cylinder bottom-end from an Mk VII Jaguar owner's manual is a powerful image that accentuates the idea of emergent properties.

As described below, the field of differential sensing, in which our group works extensively, takes the responses from a suite of receptors and creates patterns for diagnostic purposes that are beyond what can be achieved by the separate components alone. In fact, we are currently working to push this even further creating multicomponent cascades of reactions yielding a final result that the individual reactions themselves cannot achieve. With the hindsight described in this article, it is clear that my inspiration to pursue such research is driven by similar hobbies from my childhood.

From my first introductory organic chemistry class I have had a fascination with electron orbitals. The artistic similarity and aesthetic reaction to molecular orbital theory is obvious. Even if beauty is in the eye of the beholder – can anyone really question that HOMOs and LUMOs (Figure 5) are beautiful representations? When considered in this manner, electronic structure theory takes on a completely different aspect, that Kandinsky would have appreciated [27].

Graduate school and Post-Doc

After receiving a B.S. in chemistry from CSUN, medical school at the University of Southern California (USC) was the next destination. But, this lasted only about two weeks. In the evenings, my inorganic and organic chemistry textbooks [28,29] were calling to me rather than required physiology and anatomy books. Thus, after withdrawing from USC, the next year was spent continuing research with Ed Rosenberg and creating signs. With my friend Andy Trapani, we started "Eric's Signs". This company made Styrofoam lettering for sides of buildings modeled off of a company's business card and logo. It was quite successful and could have blossomed into a business career in industrial art.

After a one-year break from schooling, Caltech was my destination, where Dr. Robert Grubbs accepted me into his group. This

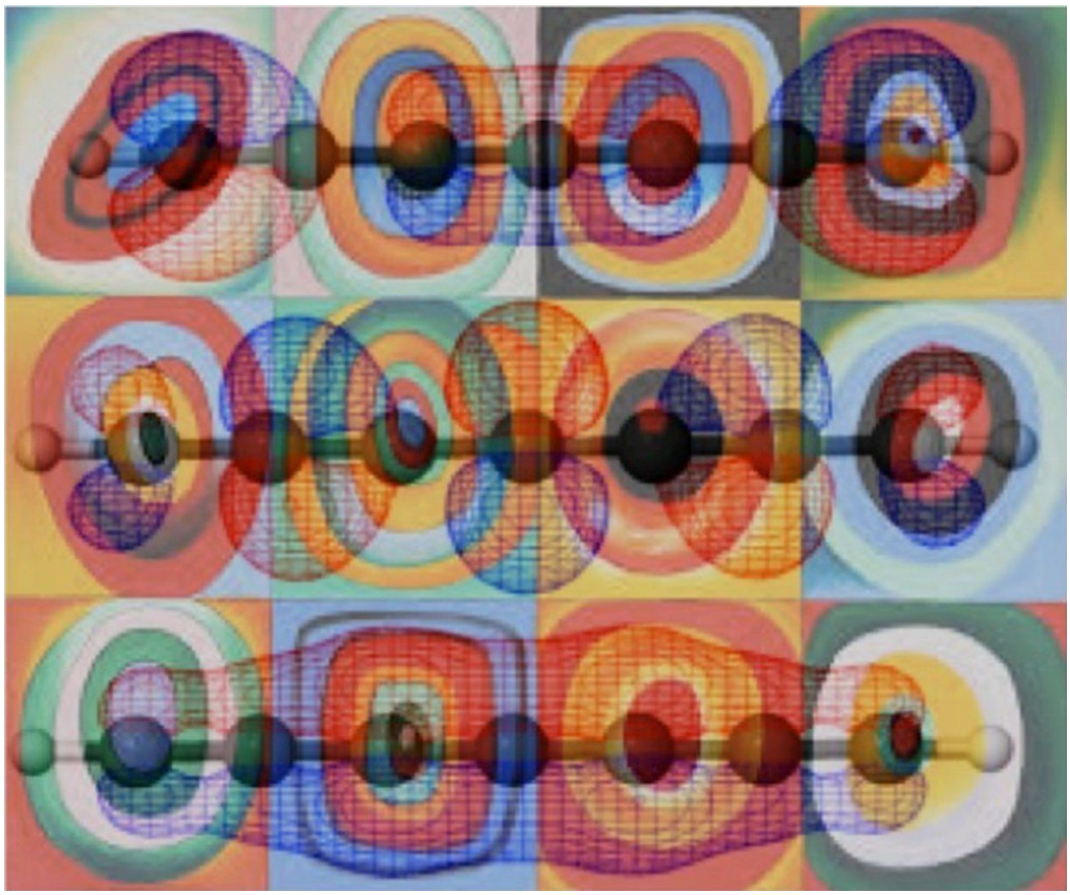

Figure 5: Kandinsky's Concentric Circles. (http://amazinglittleartiststves.weebly.com/student-artwork/category/kandinsky) , with orbitals computed by John Stanton (personal communication). Collage created by EVA.

was one of the most important and impactful decisions of my life. Dr. Grubbs was another inspirational individual with a love of science that permeated through his group. He has an innate instinct of when chemistry will and will not work. Part of my Ph.D. thesis was computational under the direction of Dr. William Goddard, involving molecular orbital theory and the rendering of orbitals. Both Professors taught me various aspects of the art of physical organic chemistry. In addition, Dr. Dennis Dougherty welcomed me to his group meetings, where various topics of supramolecular chemistry were common. Little did either of us suspect we'd co-author a physical organic textbook together about 15 years later [24].

Using the combined experience from Grubbs, Goddard, and Dougherty, physical organic chemistry as applied to biological problems was a main interest, which became the topic of my post-doctoral work with Dr. Ronald Breslow at Columbia University. Breslow has the quickest mind of anyone I've ever met and his enthusiasm for his group's work knows no bounds. When he would enter a laboratory to hear the latest news, it was an explosion of energy. He wanted to hear about everything, even the latest TLC conditions. The atmosphere of his group inspires all members to go as far as possible in academia.

Early academia

The question everyone has to confront when starting in academia is "what to do?". Initially I took an easy route. My post-doctoral work with Ronald Breslow was focused on enzyme mimics for the hydrolysis of RNA [30,31]. Thus, continuing in this vein but using a different approach, that of guanidinium groups in preorganized scaffolds that created clefts, was the route my group pursued [19,32,33]. This was the era in supramolecular chemistry of Rebek's Kemp-triacid clefts (**1**) [34,35] and Zimmerman's tweezers (**2**) [36-38]. My own molecular designs were also reminiscent of these precedents (**3**) (Figure 6).

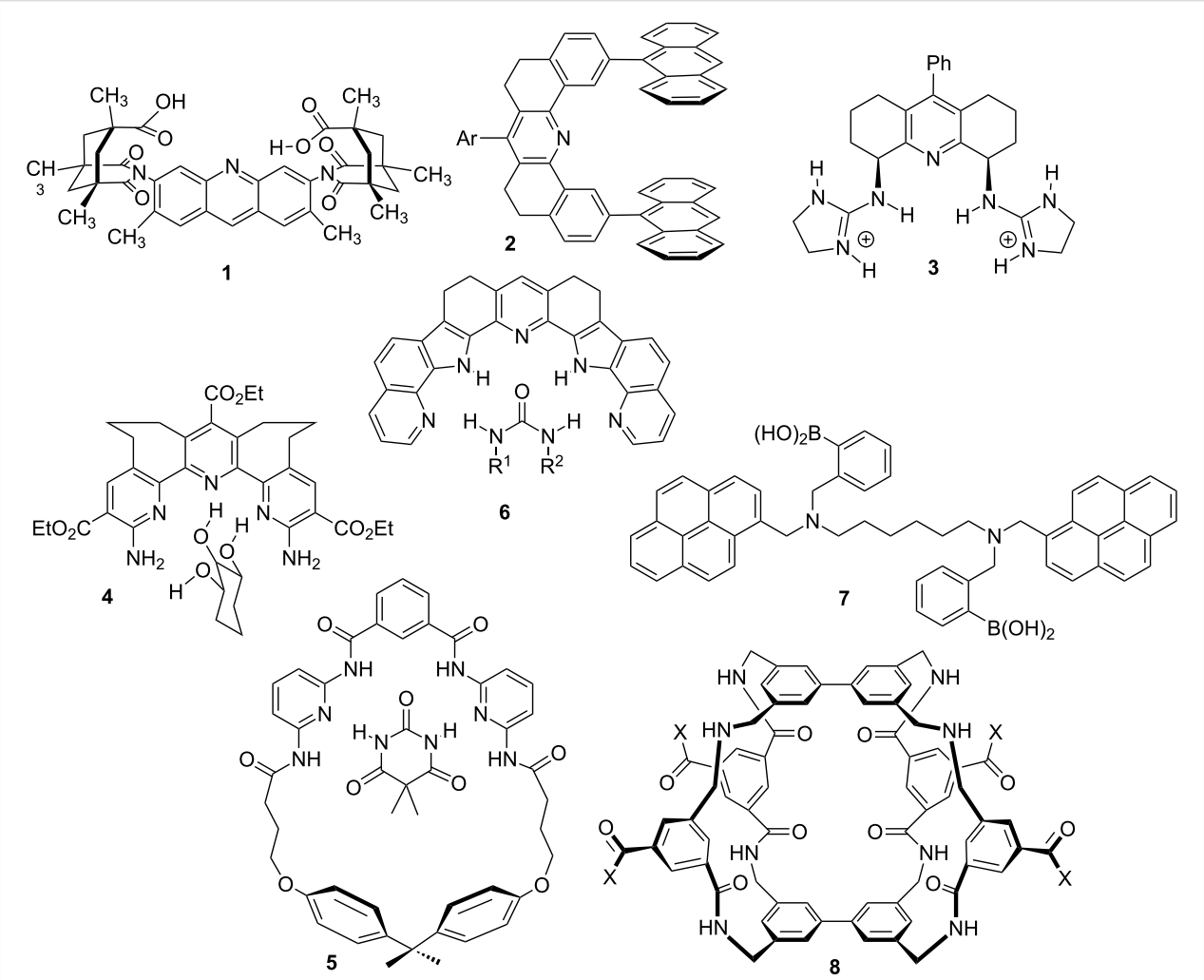

Figure 6: A potpourri of chemical receptor designs that influenced our group's work **1**, **2**, **5**, **6**, **7**, **8**), along with a few of our own (**3** and **4**) [34-45].

From 1989–1993 achieving tenure was a major goal, and I believed pursuing novel biologically relevant targets using supramolecular chemistry would make a unique and new contribution. Thus, our group created some of the earliest reported monosaccharide receptors (**4**) that exploited hydrogen bonding based recognition in chloroform [39,40]. The receptor designs from Hamilton (**5**) [41] and Thummel (**6**) [42] at around the same period of time clearly influenced my own designs. This was approximately 1992, and looking back at the polyol receptors (such as **3**) shows how far this field progressed. The use of boronic acids of Shinkai/James (**7**) [43-45] and the large cavities reported by Davis (**8**) [46] for binding saccharides has advanced the field far beyond our primitive designs (Figure 6).

The synthetic receptors we created were designed to answer basic science questions about enzyme mechanisms [19,47], rate enhancements from ion-pairing and general acid-catalysis [48], as well as reveal the strengths of hydrogen bonding. Interspersed among this work using synthetic receptors there was a continued fascination with approaching mechanistic problems using more classical physical organic methods, and we published a series of papers on the mechanisms of glycoside [49,50] and phosphoester hydrolysis [51].

Why textbooks?

After achieving tenure, it is natural to reflect "Whew, what now?" In answering such questions, one dominant thought continued to recur – the most influential people on my perception and knowledge of chemistry were Grubbs, Breslow, Rosenberg, Lowry and Richardson, as well as Morrison and Boyd. These later two set of individuals were the authors of my graduate [52] and undergraduate [29] textbooks on organic chemistry, respectively. This thought brought the realization that a textbook has a far broader and extended effect on influencing how students think about chemistry when compared to what our research was ever likely to achieve. Thus, through a series of fortunate events the graduate-level textbook "Modern Physical Organic Chemistry", co-authored with Dennis Dougherty, was the result [24]. Similarly, due to a long friendship with Brent Iverson, dating back to graduate school, the undergraduate book "Organic Chemistry" was produced [53]. Writing the graduate level textbook was the most educational thing I've done, and ranks among the most gratifying experiences of my career.

After tenure

Much of our group's work prior to tenure was addressing mechanistic aspects on the timing of proton transfers in hydrolysis reactions [51,54-56]. While the approaches we used to answer these questions where mechanistically interesting, there was a question – "How many people really care about such subtle details?" Thus, while mechanistic pursuits remain a constant in our group's work, we switched from using synthetic receptors as mechanistic probes to using such receptors for sensing purposes. The impetus for doing so was driven by an attempt to have a higher impact with our work, but, admittedly, was also due to serendipity.

Several events moved our group's research toward sensing applications. One was having a synthesis to create **9** as an RNA hydrolysis catalyst. Structure **10** was building up in a vial as a byproduct of synthesizing **9**, and we had no idea what to do with it. That was until one day sitting at my desk drinking a Fresca soda and reading the ingredients, the first of which was sodium citrate, the light bulb went off - Bingo! Compound **10** should definitely bind citrate quite strongly, even in a highly competitive media, due to the fact that all the hydrogen bonding will be strengthened from the additional ion-pairing (**10**). This hypothesis proved to be correct [57]. Hence, the idea was to use **10** as an optical sensor for citrate. But, we needed a signaling protocol, and neither **10** nor citrate possess a chromophore. The usual approach would have been to covalently attach a chromophore to the receptor, but we wanted a more general approach, one that would not require additional synthesis. Upon remembering that the Breslow group would follow the displacement of fluorophores bound in the cavity of cyclodextrins to measure K_{eq} values, our idea was to instead exploit the displacement as the sensing modality. Thus, the idea of an indicator-displacement assay (IDA) was born [58,59]. As with so many "new" ideas in chemistry, the approach had actually been used before, by Inouye and Shinkai [60,61]. IDAs are now one of a handful of standard approaches to creating optical sensors [62].

Our group optimized the citrate receptor design by incorporating a single boronic acid (**11**) [63] and measured citrate in soda pops [64], vodkas [65], and most recently showed that such receptors can be used in dialysis clinics to monitor citrate anticoagulation therapy [66]. The optimization procedure for the citrate receptor followed a classic "lock and key" design strategy (Figure 7). The receptor was preorganized by the hexasubstituted benzene [67] to present two guanidinium groups and the boronic acid in a spatial manner to best complement the citrate "key" to the receptor "lock" (**11**). In other studies, using a lock and key design approach led to very selective and high affinity receptors for heparin [68,69] and 2,3-bisphosphoglycerate [70].

Although reading the label of a Fresca soda indeed sparked the idea of pursuing a citrate sensor, the idea of working on sensing had been percolating in my mind for a while. A. P. De Silva was pioneering the use of PET (photoinduced electron transfer) signaling [71], Seiji Shinkai (and his post-doctoral associate

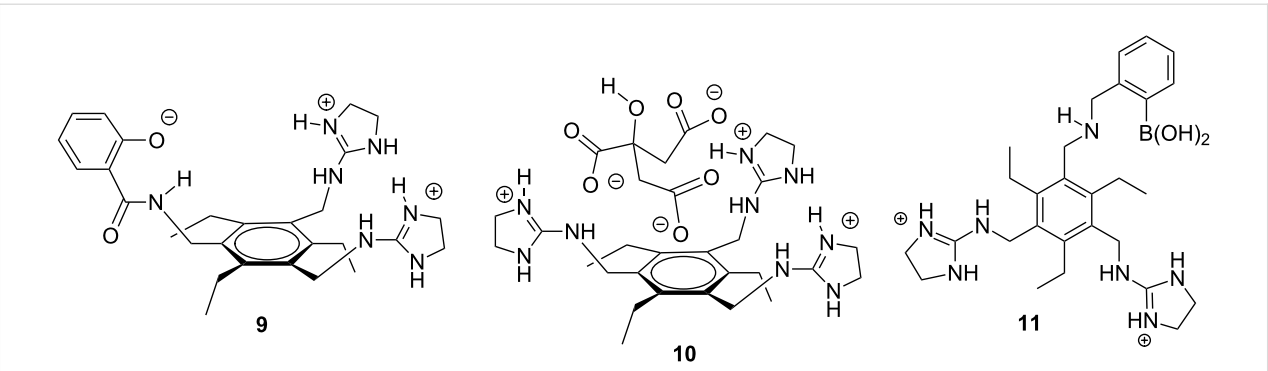

Figure 7: Evolution of design of our citrate receptor [63-67].

Tony James) were creating sugar sensors [43-45], and Anthony Czarnik had published his landmark treatise "Desperately Seeking Sensors" [72]. These three individuals are the true fathers of "Supramolecular Analytical Chemistry", even if our group later introduced this terminology [73].

Sensing a paradigm shift

Whereas selectivity has been a goal for many studies using synthetic receptors, in part because Clark Still once noted that synthetic receptors could be as selective as antibodies [74], a chance lunch led me to consider moving in an entirely different direction toward the end of the 20th century. My ex-colleague, Dr. John McDevitt (now at NYU) told me over lunch about devices known as "electronic noses" [75,76], which are analytical devices that have arrays of cross-reactive entities whose signals (most commonly electrochemical) can be deciphered by chemometric routines to create patterns (also called "fingerprints") for the composition of gases/vapors. In collaboration with McDevitt, as well as Drs. Jason Shear and Dean Neikirk, we created one of the earliest "electronic tongues", which simply meant we were analyzing solution compositions rather than gases [77]. Our device emulated design principles emanating at that time from David Walt [78,79] and Ken Suslick [80,81], who were pursuing similar goals.

The field of electronic noses and tongues has a biomimetic origin. Having worked with Breslow as a post-doctoral fellow, who coined the word biomimetic [82], the idea of mimicking the mammalian senses of taste and smell as an approach to chemical sensing seemed obvious. Mammalian chemical sensors do not use highly selective, lock and key-like, receptors, but instead rely on a series of low-selectivity but cross-reactive receptors that create a pattern [83]. These patterns act as fingerprints, to recognize and diagnose future foods and beverages. Whereas the field of electronic noses in the late 1990s was very sophisticated, in general the analytical chemistry community did not incorporate principles of supramolecular chemistry into their designs, and furthermore were primarily limited to vapor analysis.

We did not have an immediate epiphany that the supramolecular community could have a large impact in this field, but instead this realization came gradually, as studies from the group led to the conclusion that a lack of selectivity could be powerful. Furthermore, it was a reviewer of one of my early grants in this area that made me realize what I had not already recognized; we were, and still are, "making lemonade out of lemons". In essence, we were taking advantage of the fact that synthetic receptors lack a high level of selectivity.

One of the earliest studies from our group that revealed how a lack of selectivity could be useful involved the age of scotch whisky [84]. We found that the same receptor we had optimized for citrate (**11**) would indiscriminately bind tannic acids, which are species that leach from oak barrels as the whisky ages. Using this one receptor to signal all tannic acids, we could create an IDA that correlated with the age of the whisky. In a second similar study, we took advantage of our early fledgling interest in chemometrics (see more below), and showed how an artificial neural network (ANN) could be used to analyze mixtures of cross-reactive receptors with indicators to accurately quantitate concentrations of the very similar analytes malate and tartrate [85]. Subsequently, the Severin group has also nicely exploited both mixtures of receptors and indicators [86,87], as well as spatially arrayed versions, to diagnose other very subtle differences in analytes [88].

To distinguish the idea of using selective receptors from that of using cross-reactive arrays we coined the term "differential sensing" [89]. The idea was to highlight the most important factor in this biomimetic approach – that the receptors all acted differently from one another. The responses from all the receptors would need to be interpreted by a chemometric protocol [90], such as principal component analysis (PCA), linear

discriminate analysis (LDA), hierarchical cluster theory (HCT), or an ANN. A course at Georgia Tech University, held in approximately the year 2000, was my basic training in the methods. Admittedly, the extensive linear algebra discussed was above my comprehension, but much of what was taught ultimately resulted in a manuscript that we hope helps the supramolecular chemistry community to use these methods [91].

Among the earliest work from our group using the electronic tongue and chemometrics were methods to differentiate ATP, GTP and AMP [92], phosphorylated peptides [93], as well as a technique to identify sweeteners in coffee and tea [94]. Each of these studies used combinatorial libraries of peptides around a preorganized scaffold or with a known targeting agent; these include **12**, **13**, and **14**, respectively (Figure 8). Such achievements would have been difficult for supramolecular chemistry groups using the standard lock and key approach to create synthetic receptors due to the difficulties in creating receptors with the appropriate specificity.

In the beginning, the supramolecular chemistry community was not receptive to this kind of work. Quite well known chemists, and close friends, had comments such as "you're going to put us all out of business", "this is not science", or "you've lost your way".

The electronic tongue created with McDevitt possessed beads placed in micromachined divots on a silicon chip (Figure 9A), and solvents and samples were introduced to the system via an external HPLC [69]. While the miniature nature of the system was intriguing, such a device was going to be difficult for supramolecular chemists to adopt. More user-friendly variants for spatially arraying receptors for solution analysis were thus created by others, such as Pavel Anzenbacher's sol–gel approach [95,96]. Yet, the absolute easiest way to array receptors

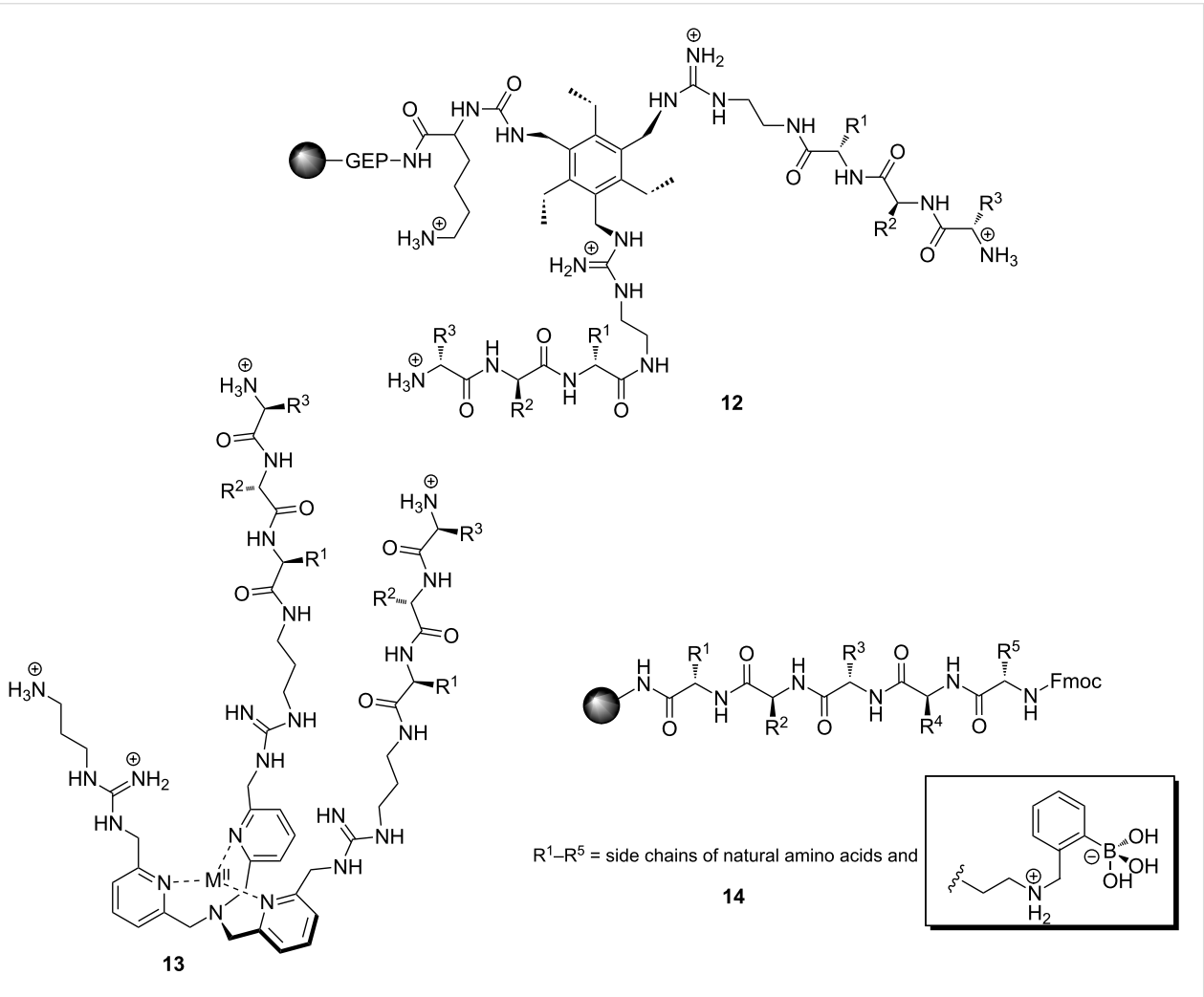

Figure 8: Combinatorial peptide library designs used for differential sensing purposes [92-94].



Figure 9: Concept behind the electronic tongue, with micromachined divets that hold beads placed in an array. While not micromachined, a much simpler analog that accomplishes much of the same concept is just a simple 96-well plate.

is to use commercially available plates, such as plastic 96-well plates, because the receptors can be dissolved and then dispensed by common 8- and 12-channel pipettes. To our knowledge, one of the earliest reports of using such a plate with supramolecular chemistry and chemometrics sensing techniques was from Lavigne [97-99]. His work inspired our own group to move to 96-well plates, and from that point forward the field of differential sensing has exploded (Figure 9B) [100]. But, for my own tastes the work from Vince Rotello on biological applications [101,102] should inspire supramolecular chemists to move the field toward pathology.

In our own laboratories the most recent uses of differential sensing have focused upon trying to push the limits of the technique. Three studies were driven by attempting to see how far the idea of using cross-reactive arrays could be pushed for solution-based analysis of complex, and/or subtly different, analytes. Probably the most well-known set of studies from our group are on wines. As with our earlier work, the approach uses a suite of combinatorial peptides as differential receptors. The peptides are biased with a large fraction of the amino acid histidine, are metallated with Cu, Ni, and Zn, and bind indicators to create a series of IDAs that can classify wine varietals, hang time, correlate with the human taste response of astringency (Figure 10A), and identify percentages in blends [103-105]. While this work was started simply as a means of seeing if we could create assays that would parallel human taste responses, we have since found that wine fraud may be a real-life application for the method.

As has Rotello, we are taking our differential sensing work to the biological arena. Kinases are enzymes that are involved in cellular signaling and regulation. Monitoring their activity has commonly involved the creation of highly selective peptides that respond to only one kinase [108,109]. This can be viewed as a lock and key approach, while as described herein, many groups have shown that the differential sensing approach may be more applicable for certain applications. Thus, we took a suite of peptides containing the SOX fluorophore, and analyzed their ability to classify MAP kinase identity, concentrations, and inhibitors thereof [106,110]. The chemometric analysis of the data (Figure 10B) revealed that most of the peptides were phosphorylated by each kinase, and that unexpected activity was found for inhibitors.

Among the most challenging guests that we could envision for supramolecular chemistry to tackle are glycerides. Glycerides often differ only by numbers of methylene groups, positions of double bonds, and stereochemistry of the olefins. It seems impossibly difficult to create a synthetic receptor that could bind selectively trielaidin over trielroselaidin (differing only by double bond position in each fatty acid chain, Figure 11) and a second synthetic receptor that did the opposite. Thus, if successful, the demonstration of a differential sensing approach that could classify glycerides, determine their structural features, and quantitate concentrations, would be a large validation of the method. To accomplish this we used serum albumins as the cross-reactive receptors, paired with a series of hydrophobic indicators [107]. The method worked extremely well (Figure 10C), and we are currently pursuing the analysis of adipocyte extracts in collaboration with Sanofi–Aventis for diabetes studies.

What's next?

Where should the field of supramolecular analytical chemistry be moving, and therefore what inspirations are there for our group? Undoubtedly, because it is my first love, my group will continue to study mechanisms of organic reactions, molecular recognition, and photophysical techniques. In each study, we'll be driven to create imaginative new approaches and complex

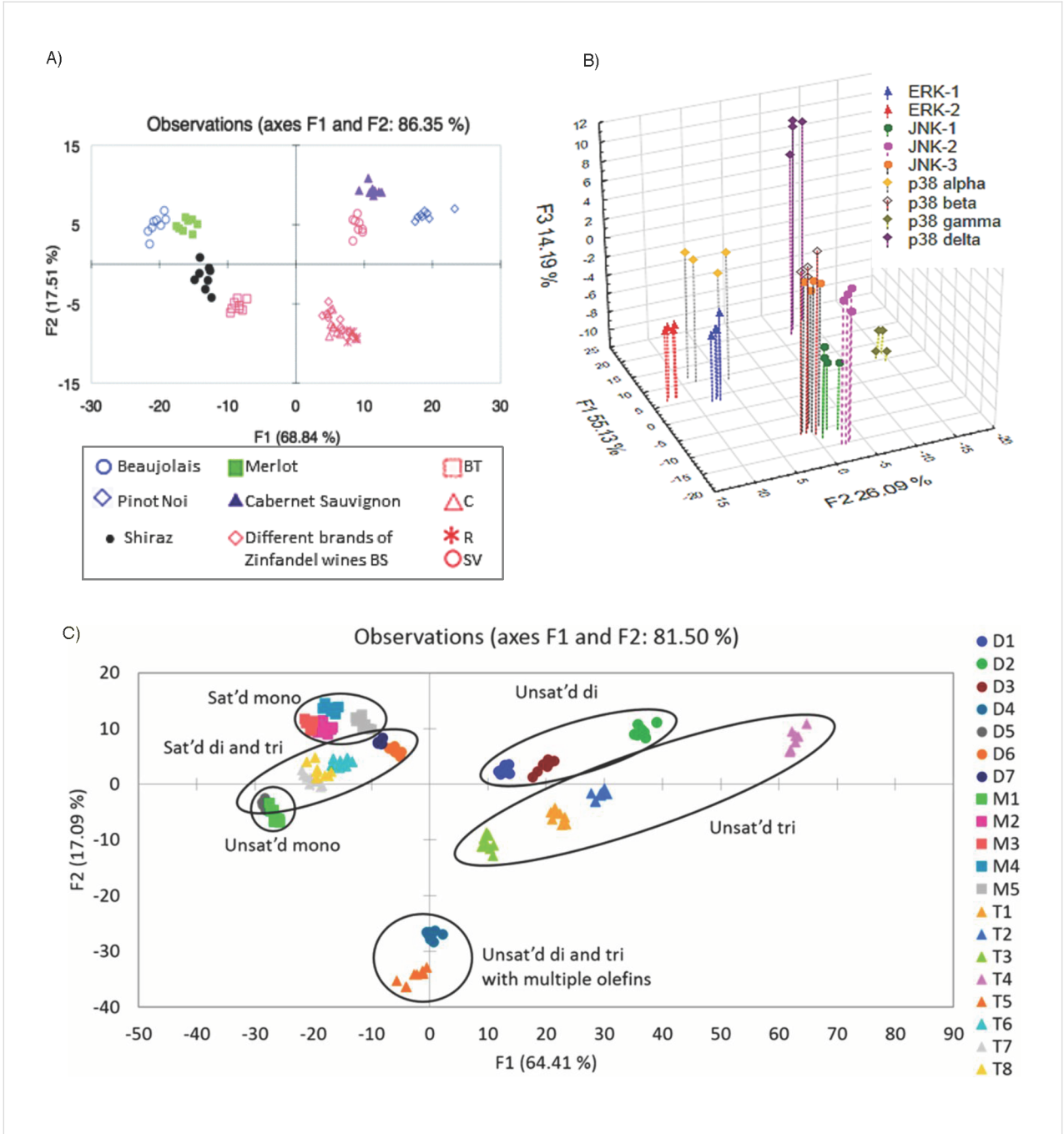

Figure 10: a) LDA plot of the response from different wine varietals with array Z [103]. b) Three-dimensional LDA plot of the response from the SOX-peptides showing in vitro differentiation of nine MAP kinases [106]. c) LDA plot of data collected from 96-well plates [107]. The array components consisted of BSA and HSA (100 μM), glyceride (90 μM), DNSA (60 μM), ANS (60 μM), NBD-FA (60 μM), metathesized glyceride (90 μM), AF (100 μM), and DNSA (60 μM) in phosphate buffer with <5% (v/v) THF. Cross-validation: 98%.

physical entities of beauty that perform functions and tasks. As discussed above, we'll continue to create differential sensing arrays for new, ever-expanding applications. However, after the analysis of glycerides, we feel there is no longer a need to see how challenging a class of analytes can be tackled. Instead, it is now necessary to make the methods truly practical for real-life applications.

But, far more important than my own group's work, to survive and thrive, supramolecular analytical chemistry must create results that are widely recognized by the chemical community. Our field has to have broad impact, not only advancing the basic science of molecular recognition and chemical reactivity, but also using this information to influence how other scientists perform their own studies. In this regard, differential sensing

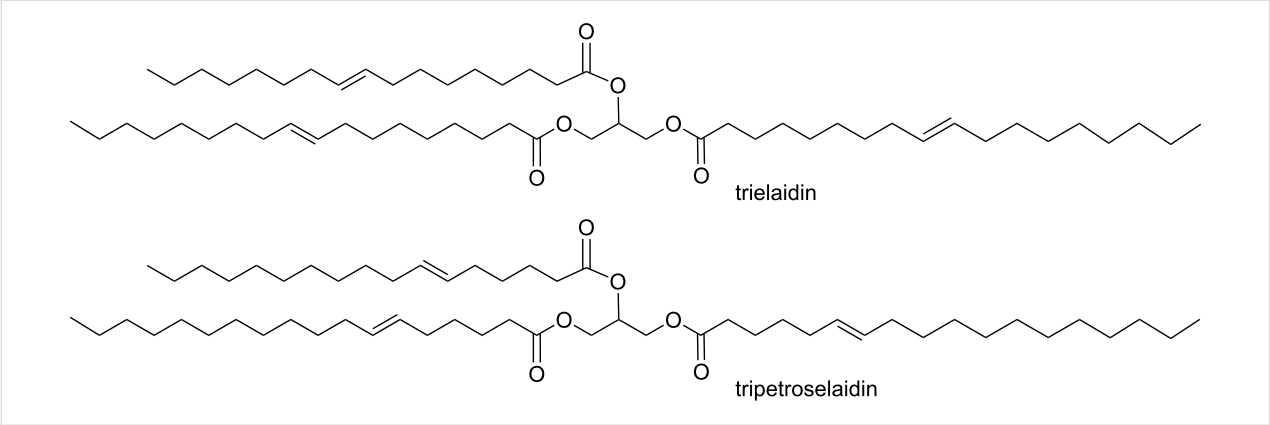

Figure 11: Two seemingly impossible targets to make highly selective receptors for.

must find a "Grand Challenge" that it, and only it, can solve. The first artificial nose companies, Aromascan and Cyrano, have fallen by the wayside, primarily for lack of a real market application. It is true that solution-based differential sensing is likewise struggling for a solid foothold in the economy. In contrast, imaging agents are clearly a frontier. These are the kinds of sensors that Tony Czarnik originally envisioned [72], and commercial success has been achieved with the company Life Technologies, formally Molecular Probes.

The directions that Vincent Rotello is taking the field of differential sensing, toward that of biological applications, is one clear future. We are similarly moving to biological applications with kinase and lipid analysis, and cellular classification. But, even beverage analysis, complex mixture authentication, and drug metabolism, are still important areas for differential sensing applications.

In addition, the work of Scott Phillips [111-113] and Doron Shabat [114-116] are currently inspirational for our group's research efforts. They are both using auto-inductive and cascade reactions, for signal amplification purposes. Given their advances, we are currently using our own physical organic chemistry insights to amplify the responses of molecular recognition in single analyte or array sensing. This is an area where supramolecular and physical organic chemists can create ensembles with many components that create properties that emerge which are greater than the individual parts alone.

Conclusion

In summary, it is clear after a 27-year career in supramolecular chemistry that my group's work is just a continuation of my childhood. This childhood was driven to emulate my father and have fun with my neighbor Brian. Just as I did during these formative years, my current group strives to make complex systems, with numerous moving parts, to achieve a function. This is similar to creating and fixing internal combustion motors on cars and go-karts, as well as designing and constructing from scratch balsa wood models. My current weekend hobbies are not any different, leading my wife to often observe "you're replicating your childhood". Further, while not necessarily designed to be objects of art, the compounds our group creates, and those that the field of supramolecular chemistry generally creates, are indeed beautiful. Even molecular orbital theory creates objects of worthy of artistic notice. This is what initially drew me to the field, and the aesthetic feelings evoked to the complexity of chemical assemblies are still my driving force for the creation of imaginative and novel systems chemistry.

If there is a lesson here, it is that one should take advantage of their strengths. Our hobbies as children, and as adults, don't necessarily need to be significantly different than our careers. They can meld together, and thus work and recreation become one and the same.

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Self and directed assembly: people and molecules

Tony D. James

Review

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Address:
Department of Chemistry, University of Bath, Bath, BA2 7AY UK

Email:
Tony D. James - t.d.james@bath.ac.uk

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Abstract

Self-assembly and directed-assembly are two very important aspects of supramolecular chemistry. As a young postgraduate student working in Canada with Tom Fyles my introduction to Supramolecular Chemistry was through the self-assembly of phospholipid membranes to form vesicles for which we were developing unimolecular and self-assembling transporter molecules. The next stage of my development as a scientist was in Japan with Seiji Shinkai where in a "Eureka" moment, the boronic acid templating unit (directed-assembly) of Wulff was combined with photoinduced electron transfer systems pioneered by De Silva. The result was a turn-on fluorescence sensor for saccharides; this simple result has continued to fuel my research to the present day. Throughout my career as well as assembling molecules, I have enjoyed bringing together researchers in order to develop collaborative networks. This is where molecules meet people resulting in assemblies worth more than the individual "molecule" or "researcher". My role in developing networks with Japan was rewarded by the award of a Daiwa-Adrian Prize in 2013 and I was recently rewarded for developing networks with China with an Inaugural CASE Prize in 2015.

Review

Beginnings

When at school I became distracted very easily and while this may not be a good thing for getting on in education it is the one aspect of my personality I have found to help me as a scientist. Since being easily bored can drive you to look for something more interesting – in science the something more interesting is a new discovery. Therefore, while getting side-tracked is not a very good thing in everyday life, for me it has allowed me to flourish as a scientist, since being "side-tracked" often opens up new and exciting areas of research not possible if you just follow the flock.

At school and as teenager in the early 1980's I needed to decide what to do as a career. Luckily, around this time of decision in my life I happened to watch a programme by the Nobel Prize Physicist Richard Feynman on Horizon in 1981 [1] "The Pleasure of Finding Things Out". His eloquence and ability

to convey science to everyone amazed me and inspired me to want to experience the same joy in science that he had in abundance.

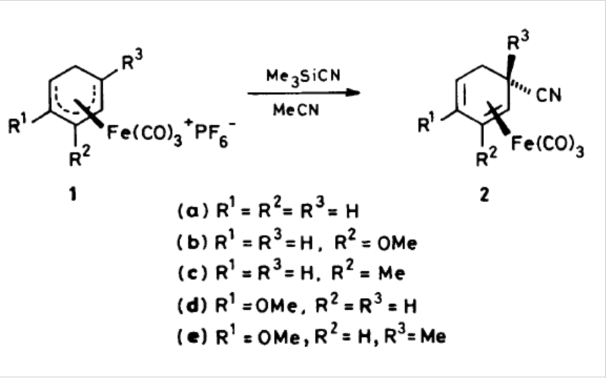
Richard P. Feynman (1918–1988) explains how he has an artist friend who describes a flower he is holding *"I as an artist can see how beautiful this is but you as a scientist take this all apart and it becomes a dull thing,"* Richard P. Feymann does not agree, since as a scientist he can observe the beautiful inner workings and structure of the flower which adds new layers and dimension to the observation of the flower.

Interestingly at this time I was probably a better artist than scientist, and so this comment by Feynman struck a very strong cord with me. Therefore, in 1984 after leaving the Abraham Darby School in Telford, I chose to go to The University of East Anglia (UEA) to study chemistry. So why chemistry? To be truthful this is a combination of factors. Firstly, I found chemistry to be the most interesting and exciting science in school. Secondly, the chemistry industry in the UK was very strong, thus, on finishing my degree I anticipated that a suitable job would be available (an important factor given my working class roots). Thirdly, the chemistry course at UEA offered a one year placement in the USA [2]. While these types of courses are now very common at the time this was a novel concept.

Therefore, I spent a year (1984-1985) as a visiting student at the University of Massachusetts in Amherst. The year I spent at UMass (ZooMass) contributed to my love of American recreational culture and travelling in general. The year in Amherst started the chain reaction of events leading to my current career. This starts with my move in 1986 to Canada and the University of Victoria for a PhD with Tom Fyles and is followed by a move in 1991 to Kurume, Japan to work with Seiji Shinkai as a Postdoctoral Research Fellow. Finally, in 1996 after 10 years outside the UK, I returned home to the University of Birmingham as a Royal Society University Research Fellow.

Research

My first true research was at UEA and as a final year project student with G. Richard Stephenson [3]. This project opened my eyes to "research" as something you did rather than just read about. The project involved evaluation of the alkylation reaction of trimethylsilyl cyanide with tricarbonyl(*η*5-cyclohexadienyl)iron(1+) salts (Scheme 1). The reaction was shown to involve the isocyanide isomer, produced in rate-limiting preequilibration. The rate of reaction was proportional to the concentration of MeSiCN, with reactive metal complexes, but was independent of the concentration of the dienyl salt. With deactivated 2-methoxy substituted dienyl salts, a change in the rate-limiting step was observed.



Scheme 1: Reaction of trimethylsilyl cyanide with tricarbonyl (*η*5-cyclohexadienyl)iron(1+) salts. Reproduced with permission from [3]. Copyright 1987 Royal Society of Chemistry.

Coupling my new found love of North America and research, I decided to look for a PhD and as it happens the University of Victoria had an advert in chemistry in Britain (Now Chemistry World) for PhD Fellowships in chemistry. The rest as they say is history, I applied was successful and started my research career in Victoria with Professor Thomas M. Fyles [4-10]. The choice of Tom as PhD mentor was a really great one for me. Tom is an outstanding scientist but more importantly for me he was a wonderful mentor. The project I worked on with Tom had many ups and downs and involved some quite tricky synthetic chemistry (which is not my forte – this will be attested to by all those who have worked with me and seen the pile of broken glassware in the wake of my synthetic efforts). However, throughout the project Tom was always very positive and full of new ideas when things did not quite work as we had hoped. It is through working with Tom that I realised that to do great science and make great discoveries was a roller coaster ride and he taught me a very important lesson which was that what we consider as "bad" results were just as important as the "good" ones. From working with Tom I learnt a lot and in particular how to be a good supervisor capable of nurturing (or at least knowing how to) good research – summing up in Tom's own words this was the "take home message from my PhD."

During my PhD we developed both self-assembling supramolecular pore formers as well as unimolecular ion channels for the transport of metal ions across biological membranes. The project required both the synthesis and evaluation of a number of transporter molecules (Figure 1).

Post-doctoral research

For many the PhD is enough so why did I want more? I think this is because Tom had shown me how to appreciate the bad results or research failures. I was also inspired by the tactile lecture of Donald J. Cram at Pacifichem (1987), where he sent a CPK model of a carcerand around the audience, and the 1990

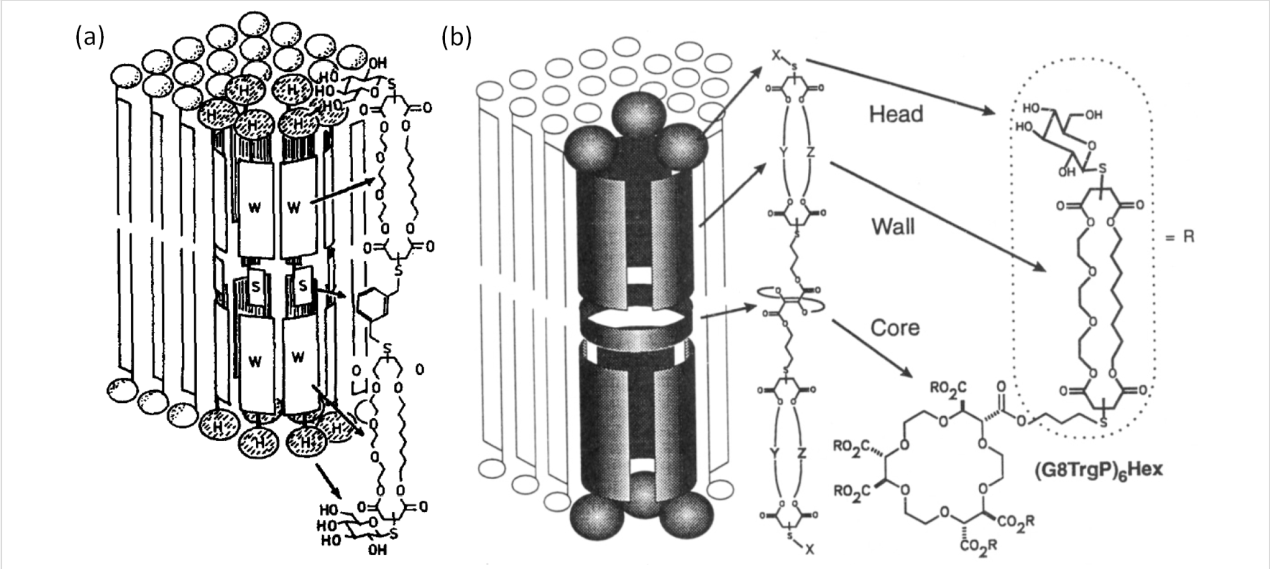


Figure 1: (a) Supramolecular pore formers. Reproduced with permission from [6]. Copyright 1990 Elsevier. (b) Unimolecular ion channel. Reproduced with permission from [9]. Copyright 1993 The American Chemical Society.

lecture by Sir J. Fraser Stoddart in Halifax (Canada) where he used beautiful language and amazing colours to convey difficult concepts. (Note that at this time most lectures were colourless). Last but not least, I still had a strong desire to travel to a new research position in Japan as a research fellow for Seiji Shinkai as part of his newly formed ERATO (JST) Chemirecognics Project. This was quite a leap because other than for the fact that I was studying Karate at the University of Victoria, I knew very little about Japan and almost-nothing about the Japanese Language. (Karate had taught me to count from 1 to 10).

Having just finished a project with a lot of multistep synthesis, I was immediately drawn to projects as part of the boronic acid research group. The boronic acid group were developing carbohydrate receptors from what looked like simple molecules requiring just one or two steps to synthesize.

Therefore, while working with the late Takaaki Harada, we developed a simple colorimetric system able to "read-out" the chirality of sugars. The system we developed used the colour of liquid crystals to determine chirality and was quickly prepared in just two steps. The addition of our D-glucose boronate complex to a liquid crystal system the colour changed from green to red, however for the L-glucose system the colour changed from green to blue [11,12] (Figure 2 depicting the same colour changes for D- and L-fucose). On observing these amazing colour changes of the "green" liquid crystal system [13], we realised that the obvious place to publish the work was "*Chem. Commun.*" [14] (given that the green was a similar colour of the cover [15] of the Journal at that time).

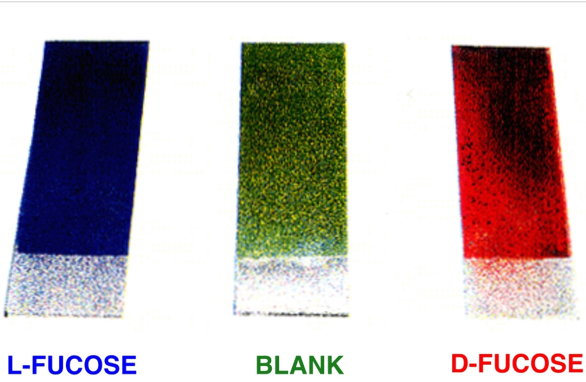
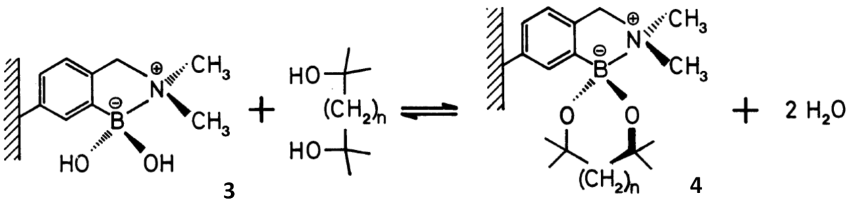


Figure 2: An intelligent liquid crystal to read out saccharide structure as a color-change. Picture provided by Seiji Shinkai, Director of the ERATO Chemirecognics Project (1990–95) [13].

Following a truly inspiring talk by A. P. De Silva who visited the Chemirecognics project in Kurume and on a long flight back from a conference in the Netherlands (XVIII ISMC 1993, Enschede The Netherlands), I had the "good idea" to combine receptor units used by Gunter Wulff (Scheme 2) [16] and the fluorescent photoinduced electron transfer (PET) pH sensors developed by A. P. De Silva (Figure 3) [17] in order to develop a fluorescence sensor for saccharides [18]. Thus creating a system where the neighbouring nitrogen lowered the working pH of the boronic acid and provided a fluorescence signalling mechanism to report saccharide binding (Figure 4).

Sensei (Seiji Shinkai) then gave me my next and most important academic lesson – if you discover "gold" in one area of research you should keep on digging in that area – the hope is



Scheme 2: Polymeric boronic acid receptor units developed by Wulff. Reproduced from [16]. Copyright 1982 International Union of Pure and Applied Chemistry.

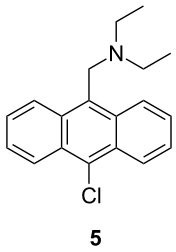


Figure 3: Fluorescence photoinduced electron transfer (PET) pH sensor developed by A. P. De Silva.

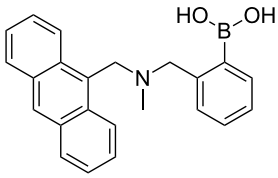


Figure 4: Fluorescence PET sensor for saccharides.

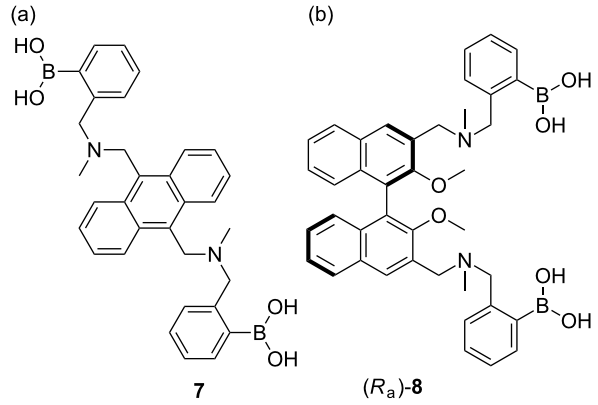


Figure 5: (a) Glucose selective PET system. (b) Chiral discriminating PET system.

that you will discover a "seam of gold" leading to even more research achievements. This result, as far as my career goes was a Eureka moment, for which I count myself very lucky, this very simple discovery was the start of a very big "seam of gold" that has continued to fuel my research to the present day [19-27]. After, we discovered this simple system we very quickly went on develop a glucose selective system [28,29] (Figure 5a). It is very rewarding to see that the basic framework of this glucose selective system is still be used by Takeuchi for the development of implantable sensors [30-32].

Some moments during your research career can be very memorable – one such moment was being confronted by Shinkai Sensei with a bottle of champagne to celebrate that our chiral discriminating system had just been accepted for publication in Nature [33] (Figure 5b).

At the 20th International Symposium on Macrocyclic Chemistry [34] in 1995, it was my great pleasure to meet and chat with Prof. J. Fraser Stoddart who had inspired me as PhD student to do research in the first place. He had recently moved to the University of Birmingham and suggested that I apply for a Royal Society University Research Fellowship to move and start my independent academic career in Birmingham.

I was very excited with the prospect of this opportunity and after a simple application procedure was very lucky to be awarded a Fellowship and so in 1996 I moved to Birmingham start my independent career. Before, I left Japan Sensei (Seiji Shinkai) gave me some excellent research advice by reminding me that – "even monkeys fall from trees" – in other words to remember as I moved to the next stage of my academic career, that everyone makes mistakes [35]. The time I spent in Japan was a very important time in my career and also life. Since, I left Japan with not only some excellent research results, more importantly I left with my Fiancée – Eriko Furukawa (we were married at St Michael's Church in Madeley on 3rd May 1996).

The excellent research links I nurtured during my time at the Shinkai Chemirecognics project (1991–1995) have continued to flourish and in recognition of these collaborative research projects with Japan, myself and Seiji Shinkai as team leaders were awarded a Daiwa-Adrian Prize in 2013 [36] for "Chemonostics: Using chemical receptors in the development of simple diag-

nostic devices for age-related diseases" with a team including Steven Bull (University of Bath), John Fossey (University of Birmingham), Kazuo Sakurai [37-41] (University of Kitakyushu) and Yuji Kubo [42-47] (Tokyo Metropolitan University).

Independent research – Birmingham

Once again I was inspired by De Silva who gave a wonderful talk in Birmingham where he discussed fluorescence based logic systems [48]. During his talk I realised that you could easily combine the De Silva cation sensor [49] with the simple boronic acid sensor [18] to prepare a new logic based system. Therefore, with my first PhD student Chris Cooper [50] we combined these two receptor elements and developed an AND logic system for "cations" and "diols" (Figure 6) [51,52].

In 1998 Michael Bell from Beckman Coulter approached me about working together on a fluorescent glucose sensor system. Therefore, in July and few weeks after the birth of my son Joseph "Joey" Hiro Furukawa James, I visited Beckman-Coulter Inc., Advanced Technology Center in Brea, Orange County, California to discuss a project directed towards the development of fluorescent glucose sensors. Research towards modular systems was started in Birmingham with Susumu Arimori an excellent PDRF from the Shinkai group [53].

My time at the University of Birmingham had been rewarding, however, with a young family I felt that the security offered by a more permanent position was needed. As chance would have it at that time the University of Bath was looking for an Organic Chemistry Lecturer and in 2000 I moved to take a position in Bath. This was a great move for me from a personal as well as career perspective, since Bath was a very supportive and friendly department. I was also lucky to be appointed at the same time as Toby Jenkins and Steven Bull who have been very good friends and collaborators over the last 15 years.

Independent research – Bath

One of the first things I did at Bath was to expand and develop the modular saccharide selective fluorescent sensors which was part of the project with Beckmann-coulter started as a Royal Society University Research Fellow at the University of Birmingham [54,55]. My family also expanded on arriving in Bath with the birth of my daughter Elinor "Ellie" Yoko Furukawa James.

As with many industrial projects Beckman-Coulter Inc. has subsequently moved on to other areas of research. However, as luck would happen Glysure Ltd. [56] in the form of Barry Crane approached me in 2006 about our fluorescent glucose selective systems, after reading our paper in *Perkin Transactions 1* [55], therefore, together we set about improving these systems in order to develop a practical sensor system for use in intensive care units (ICU) [57] (Figure 8). The collaboration with Glysure Ltd. continues to flourish and over the years has been funded by many routes including a TSB project [58] on "A Calibration Free Continuous Invasive Sensor Targeted at Glycaemic Control in the ICU". This project is worthy of mention since it started a long standing association between myself and John S. Fossey who was the researcher co-investigator on the project.

The GlySure Continuous Intravascular Glucose Monitoring (CIGM) depicted in Figure 8 has recently secured a CE Mark as the world's first and only Continuous Intravascular Glucose Monitoring System (CIGMS). The award of this CE Mark allows GlySure to market their device in Europe. They are currently conducting a UK-based trial to facilitate use of the device across all adult Intensive Care patients.

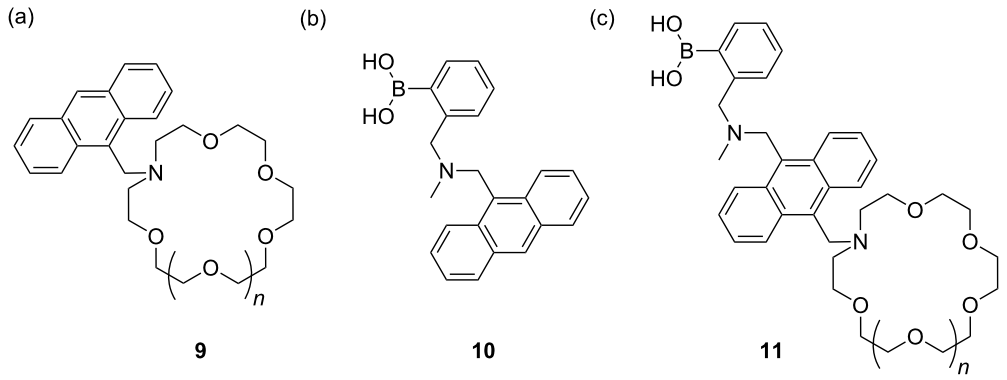


Figure 6: (a) Fluorescence photoinduced electron transfer (PET) cation sensors developed by A. P. De Silva. (b) Fluorescence photoinduced electron transfer (PET) saccharide sensor. (c) Fluorescence AND logic sensors for D-glucosamine hydrochloride.

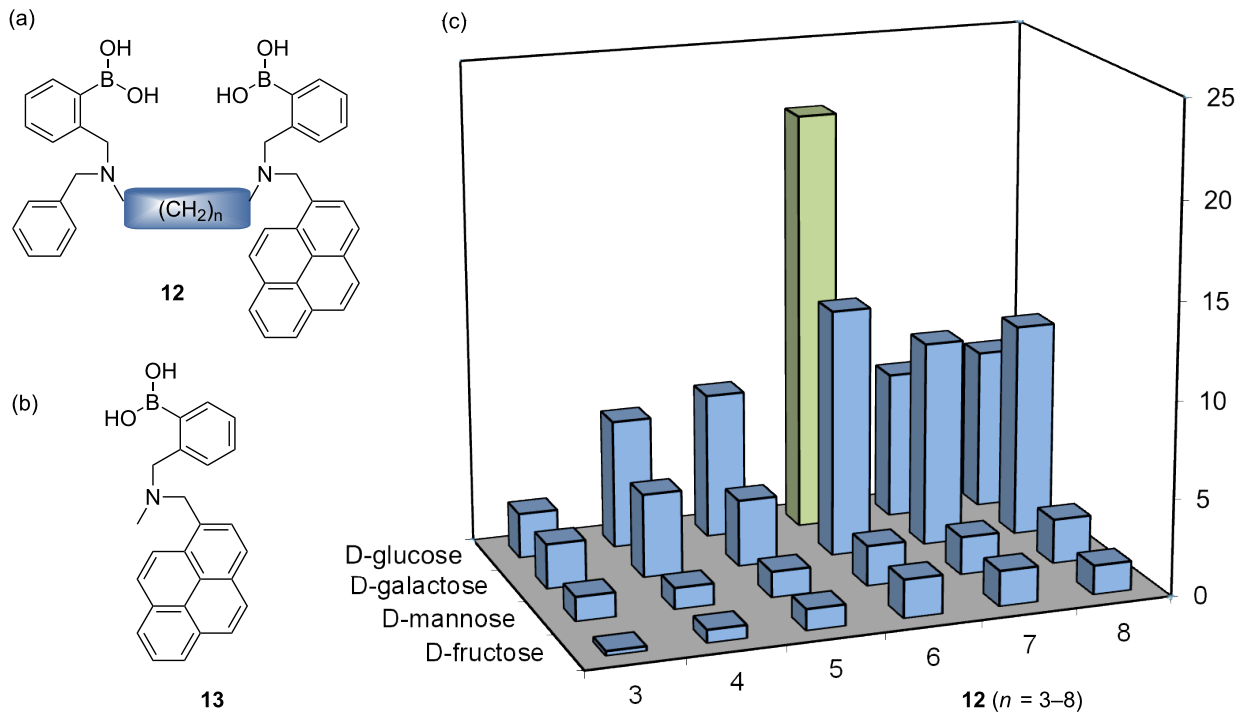


Figure 7: (a) Pyrene diboronic acids (n = 3–8). (b) Pyrene monoboronic acid. (c) Block chart showing the relative stability K_{rel} of saccharide complexes. Obtained from the observed stability constants (K_{obs}) for D-glucose, D-galactose, D-fructose and D-mannose with pyrene diboronic acids (n = 3–8) divided by the observed stability constants (K_{obs}) with pyrene monoboronic acid, to yield relative values with saccharides.

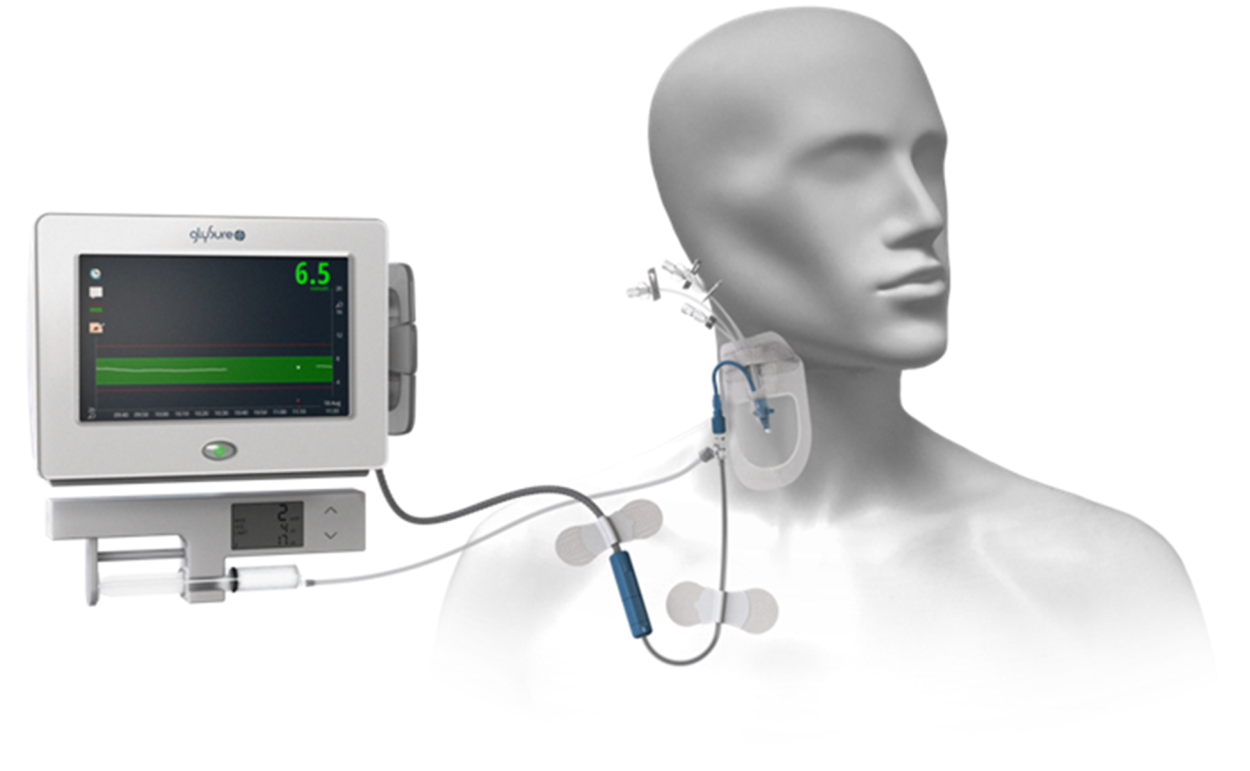
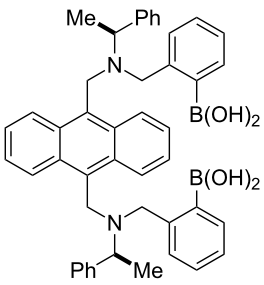


Figure 8: Glysure Continuous Intravascular Glucose Monitoring (CIGM) System. Image provided by Nicholas P. Barwell Glysure Ltd. [56].

Westheimer's Discovery [59] – that "A couple of months in the laboratory can frequently save a couple of hours in the library" makes you realise how important it is to read and keep up to date with the literature. Taking that one step further, it is also very important to keep abreast of papers that cite your own work. This is clearly illustrated using a paper by Todd A. Houston, who discovered that the racemic form of our chiral binol boronic acid **8** formed very strongly complexes with tartaric acid [60]. This paper made us realise that our chiral binol boronic acid **8** should be able to discriminate the enantiomers of tartaric acid and also bind strongly with other sugar acids. Therefore, with Jianzhang Zhao an outstanding Postdoctoral Research Fellow in my group and currently a Professor at Dalian University of Technology, we decided to investigate the properties of the enantiomerically pure forms of the receptor with chiral tartaric acid and other sugar acids. It turned out that the chiral system **8** (*R* or *S*) was very good at differentiating the enantiomers of tartaric acid and also bound bind strongly with other sugar acids [61] (Figure 9).

While, we were very happy with these results, we realised that the system could be improved by changing the fluorophore from binol, to a much better fluorophore such as anthracene. Therefore, we combined the structural design of the glucose selective sensor **7** with chiral building blocks to prepare two sensor (*S*,*S*)-**14** and (*R*,*R*)-**14** (Figure 10).



(*S*,*S*)-**14** and (*R*,*R*)-**14**

Figure 10: Chiral discriminating sensor (relative stereochemistry shown) constructed using a good fluorophore (anthracene).

After finishing as a Postdoctoral Research Fellow at Bath in 2005 Jianzhang Zhao took up an academic position at Dalian University of Technology. We have continued to collaborate and in particular we worked together to improve the chiral discriminating systems. In order to improve the chiral systems we designed sensors using a d-PET rather than the normal a-PET fluorescence sensing mechanism. With d-PET systems the fluorophore is the electron donor and the protonated amine/boronic acid moiety as the acceptor (the reverse is true for normal a-PET systems). Therefore, d-PET systems have a significant advantage over the a-PET systems at acidic pH, since

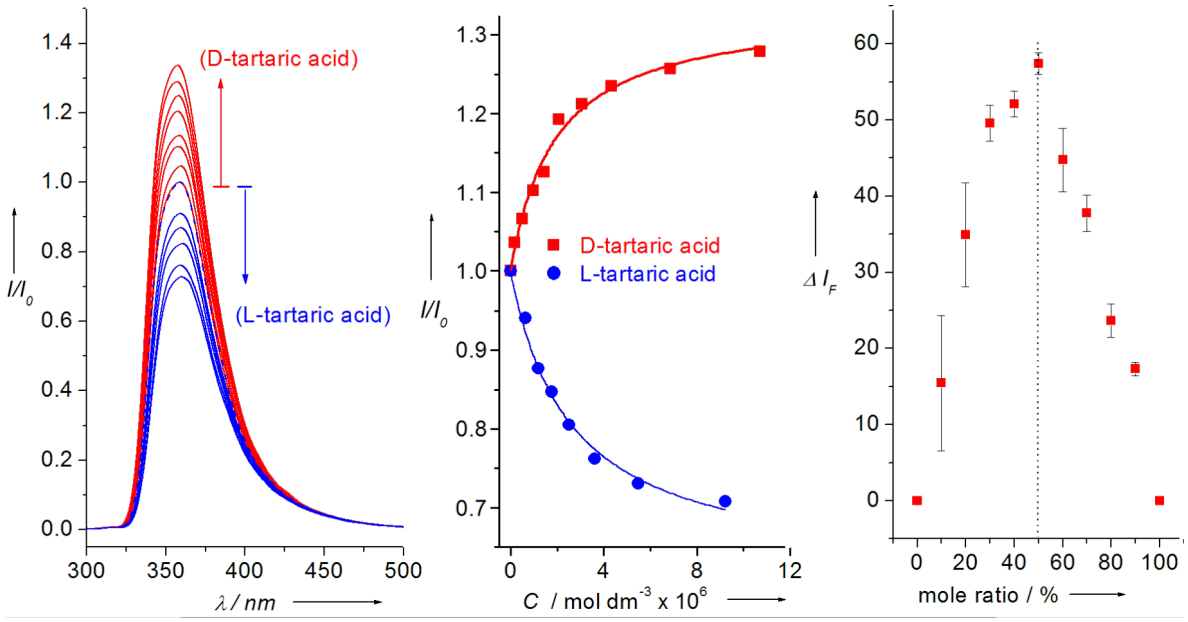


Figure 9: Chiral discrimination of D- and L-tartaric acid by (*R*)-**8** at pH 5.6. [(*R*)-**8**] = 5.0 × 10^{-6} mol dm^{-3}, at pH 5.6 in 0.05 mol dm^{-3} NaCl (52.1% methanol in water), λ_{ex} at 289 nm, 22 °C. The pH was kept at 5.6 with NaOH/HCl. (Left) Emission spectra. (Center) Normalized emission intensity) as a function of added tartaric acid concentration. Lines are fit to 1:1 binding isotherm (Right) Job plot of (*R*)-**8** with D-tartaric acid at a constant total concentration [D-tartaric acid] + [*R*] = 5.0 × 10^{-6} mol dm^{-3}; λ_{em} at 358 nm. Reproduced with permission from [61]. Copyright 2004 John Wiley and Sons.

background emission for these sensors is much lower. This is particularly important given that we were developing receptors for acidic guests such as tartaric acid and sugar acids [62-65] (Figure 11).

The best chiral discriminating d-PET system was constructed using a phenothiazine fluorophore **17** and **18** [66]. The phenothiazine fluorophore was chosen because it is a very strong electron donor. These sensors resulted in an eight fold contrast ratio, which was much better than the two fold obtained for the carbazole-based d-PET fluorescent sensors. Using sensor **18** with the largest spacing between the boronic acid receptors resulted in excellent enantioselective discrimination of D- and L-tartaric acid (Figure 12).

The Japan World Cup in 2002 was responsible for starting a number of enduring collaborations. Matthew Davidson, Steven Bull and myself spent approximately two weeks from May to June travelling around Japan presenting research talks at 13 research institutes and universities [67]. Two of the collaborations established during this trip are worthy of note since they became part of the team awarded the 2013 Daiwa-Adrian Prize. Yuji Kubo (TMU) who was at that time based at Saitama University, who I had first met during the XVIII ISMC in 1993 was one and the other was Kazuo Sakurai (Kitakyushu University) who as it happens had employed Susumu Arimori after he left my group at the end of 2001 as a Post-Doctoral Research Fellow. The two weeks on the road also allowed Steve and me to discuss research. Steve is interested in chiral catalysis and I am interested in chiral sensing so a collaborative project using boron as a chiral catalyst was a good idea. We quickly came up with a research plan over a beer or two (Figure 13). Then on our return to the UK we quickly put these ideas into practice with the investigation of a “chiral boron reagent” formed between

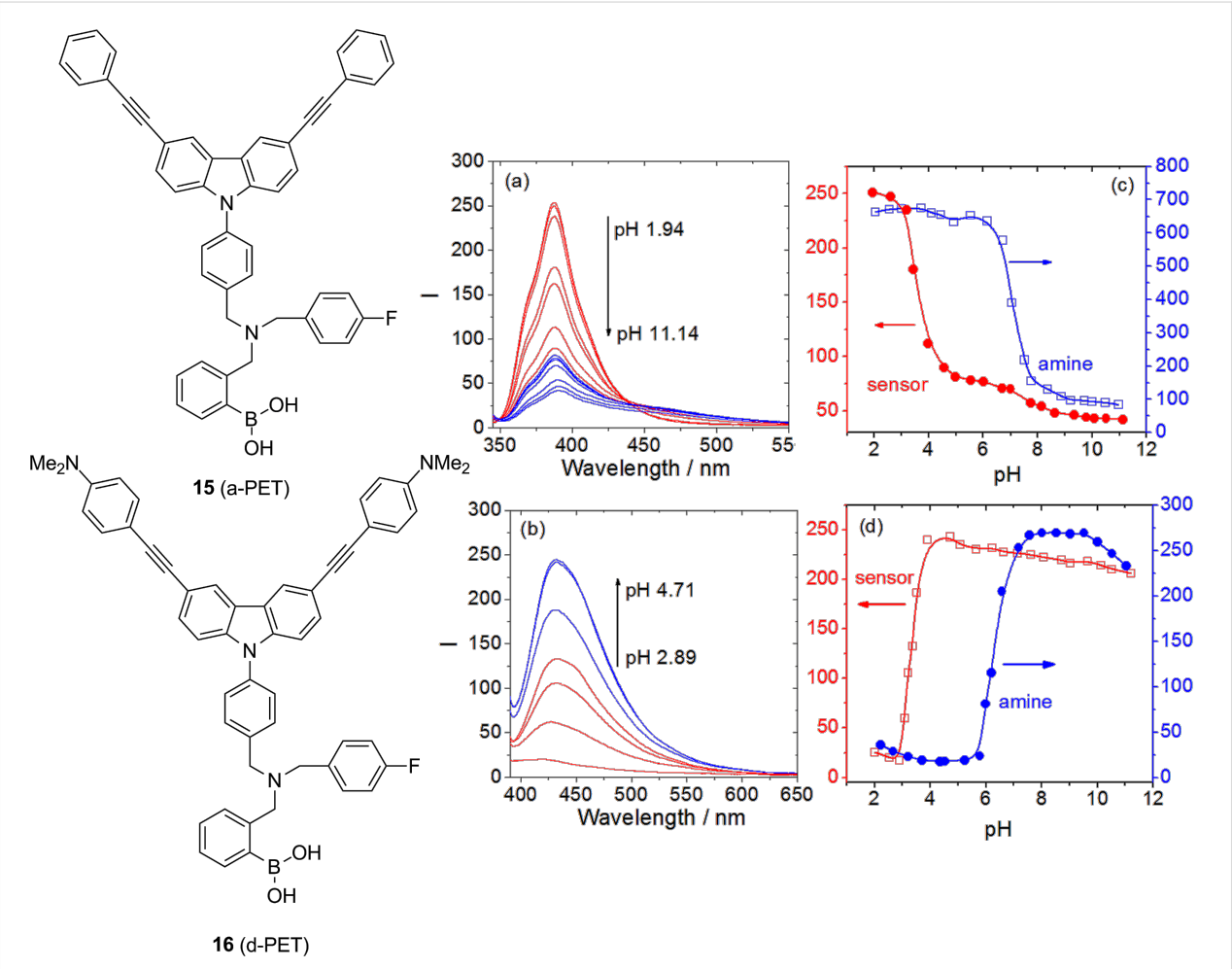


Figure 11: Fluorescence emission intensity-pH profile of: (a) Sensor **15**: 1.0×10^{-6} mol dm^{-3} (λ_{ex} 370 nm, λ_{em} 435 nm. (b) Sensor **16**: 1.0×10^{-6} mol dm^{-3} (λ_{ex} 335 nm, λ_{em} 390 nm). In 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). Emission spectra of the sensors (c) **15** (1.0×10^{-6} mol dm^{-3}), λ_{ex} 370 nm; (d) **16** (1.0×10^{-6} mol dm^{-3}), λ_{ex} 335 nm. Sensors in 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water, w/w), 25 °C. Reproduced with permission from [64]. Copyright 2010 American Chemical Society.

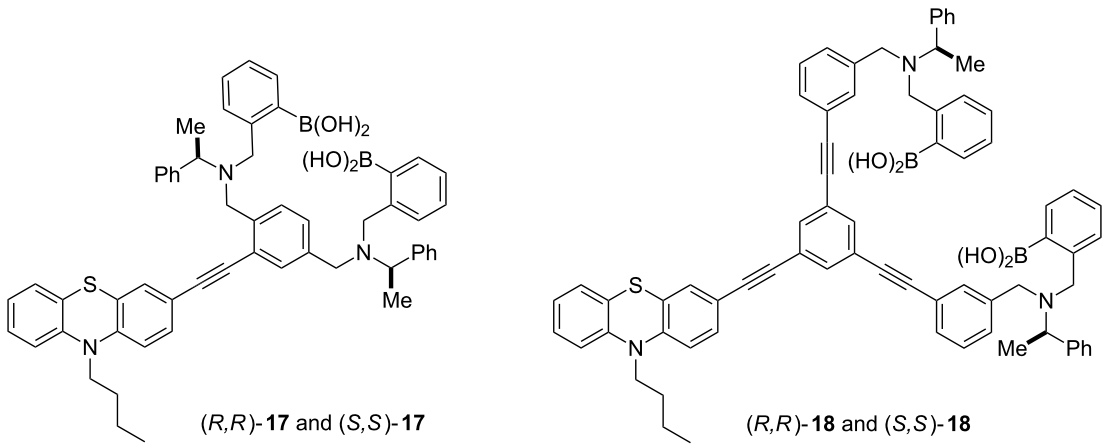


Figure 12: Modular chiral discriminating d-PET systems (relative stereochemistry shown).

binol and trimethoxy borate for the Lewis acid catalyst of diastereoselective aza-Diels–Alder reactions [68] (Figure 14). While, the structure of the "chiral boron reagent" still remains unknown during our investigation of analogues we discovered a very interesting three-component self-assembly.

Chiral binol, a chiral amine and 2-formylbenzeneboronic acid spontaneously self-assemble to quantitatively form a stable complex. Interestingly, when the chirality of the binol or amine was fixed the ^{1}H NMR of the complexes formed using a scalemic mixture of the other chiral partner (diol or amine) results in a ^{1}H NMR where signals due to both diastereomeric complexes are clearly separated. The diastereomeric imine signals are particularly useful since they are in a region of the spectra clear of interference from signals due to the binol (diol) or amine. Integration of the pair of imine signals provides a diastereomeric ratio which can be related to the enantiomeric excess of the original scalemic mixture of binol (diol) or amine (Figure 15).

The three-component system was very versatile and we could use the complexes to determine the enantiomeric excess (ee) of



Figure 13: With Matthew Davidson and Steven Bull during "World Cup" lecture tour of Japan in 2002. (Left) Private photo taken in Osaka. (Right) Photo taken in Tokyo by Katsuhiko Ariga.

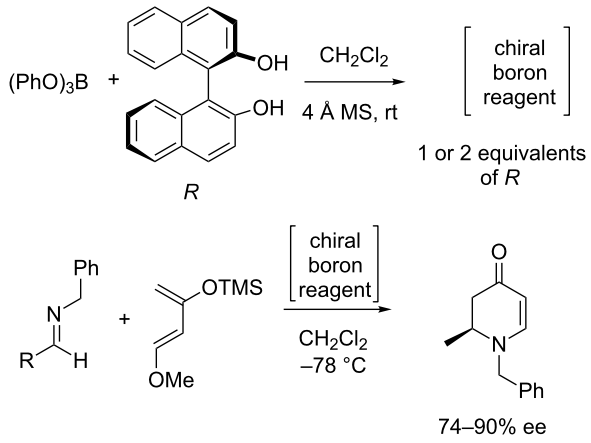


Figure 14: Preparation of chiral boron reagent and use as catalyst for aza-Diels–Alder reactions.

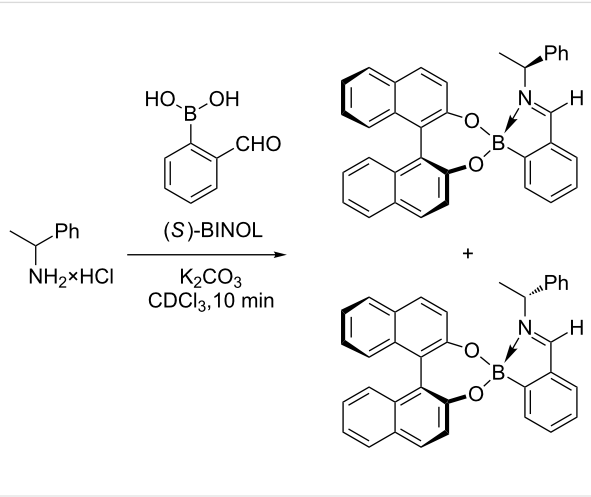


Figure 15: Chiral three component self-assembling system.

amines [69,70], diamines [71], amino alcohols [72], hydroxylamines [73] and diols [74,75] using predominantly ^{1}H NMR but we also demonstrated that by using 4-fluoro-2-formylbenzeneboronic acids then ^{19}F NMR could also be used [76]. We have also used the three component systems in collaboration with Jim Tucker (University of Birmingham) to measure the ee of binol via electrochemistry [77]. The system has also been used with Eric Anslyn (University of Texas at Austin) to determine the ee of amines using circular dichroism (CD) spectroscopy [78]. The system was more recently used to measure the ee of amines using fluorescence [79].

The UK Office of Science and Innovation (OSI) signed a bilateral agreement with the Chinese Ministry of Education in April 2007, making funding available for the scheme to develop enduring collaborations between UK and Chinese scientists. The UK and China had clearly recognised the need to bring together scientists to develop new networks. The programme was looked after by the Royal Society in the UK and and the China Scholarship Council (CSC) in China.

This was great timing given my good links with Jianzhang Zhao and having just returned from East China University of Science and Technology (ECUST) in Shanghai where I presented at the International Conference on Molecular Machines and Sensor (ICMMS). Therefore, we Jianzhang Zhao and Tony James (with Steven Bull and John Fossey) applied for funding to host a Thematic Workshop during 2008 on "Catalysis and Sensing for our Environment" (CASE) in Bath. We were very lucky in obtaining the funding [80] and the CASE Network was established [81-83].

"A scientist has to work very hard to get to the point where he can be lucky." – Robert B. Woodward (1917–1979)

The 7th Catalysis and Sensing for our Environment Symposium was held in Dublin on 9–10 July 2015 and jointly hosted in Ireland by Thorfinnur Gunnlaugsson (Trinity College Dublin), Donal O'Shea (Royal College of Surgeons in Ireland) and Robert Elmes (Maynooth University) [84]. It was my great honour to be presented an Inaugural CASE prize by Eric Anslyn at this meeting for my contributions to sensing and helping establish the CASE Network [85].

Thanks to the CASE Network enduring collaborations have been established in China with outstanding academics including Yun-Bao Jiang [86-93], Xuhong Qian [94-96], Yitao Long [97-106], Weiping Deng [107] ,Weihong Zhu [108-113] and Xiao-Peng He (Franck) [114-118]. In addition excellent, links and collaborations with other countries have also developed as a direct result of these networking meetings. Including the recent collaboration on chiral discrimination with Pavel Anzenbacher, Jr. (Bowling Green State University) and Tsuyoshi Minami (Yamagata University) [79,119] that was originated during discussion over several Margarita's at CASE2013, which was hosted by Eric Anslyn and Jonathan Sessler in the University of Texas at Austin.

The ethos of the CASE meetings since the onset has been to bring together world leading researchers and junior scientists on an equal footing and to provide an environment where research ideas can be openly discussed in order to establish new collaborations and develop new ideas.

The future

"Prediction is very difficult, especially if it's about the future." – Niels Bohr (1885–1962)

The problem with predicting the future of research is that as scientists we are very literal and specific, but, it is much easier to be correct if your predictions are broad based. Consider the book "*Les Propheties*" by Nostradamus, while his predictions may be seen as vague, this makes them open to different interpretations and as such they are as topical and up-to-date as when they were written.

"Study the past, if you would divine the future." – Confucius (551–479 BC)

This quote is close to my heart – you will see from this perspective that many of the advances in research that I have been involved with have to some extent required an understanding of the past. This is probably true for all scientists since the past is the published literature and we rely on these publications to build the future.

The outlook for boronic acid based receptors is very bright with many new areas of research on the horizon and many results near the cusp for translation into practical systems or devices in the near future. In particular Glysure Ltd. has demonstrated that boronic acid based receptors can be used to continuously monitor glucose concentrations in the ICU. The clear success of this system, points to the potential future use of this type of fluorescent boronic acid based glucose sensor for home use and the potential to incorporate the sensor into devices for closed loop treatment of diabetic patients.

One important practical application for boronic acid based receptors that is currently emerging is in the measurement of protein glycation. Glycated proteins are potentially important biomarkers for sugar-related non-communicable disease states, such as atherosclerosis, autoimmune diseases, cancer, and Alzheimer's disease (AD). Some researchers have even coined the phrase Type 3 diabetes for AD. While it remains to be determined whether the formation of AGEs in AD, and other age-related diseases, is a primary or secondary event, detection of specific glycated proteins may provide a very useful tool for diagnosis, monitoring, and treatment of the increasingly wide range of disease states that are associated with uncontrolled levels of glucose in the bloodstream.

With Jean van den Elsen (University of Bath) we have developed a simple and cost-effective analytical technique for the detection of glycated proteins in a variety of biological samples, including plasma and brain homogenates. This electrophoresis-based analytical method evolved from our work with John Fossey [120] and relies on the reversible covalent binding of a fluorescent boronic acid to glycated proteins that enables them to be detected [121-123]. This fluorescent phenylboronic acid gel electrophoresis method (Flu-PAGE), is currently being used to analyse the glycated protein profiles of brain homogenates from an AD mouse model, human cortex homogenates and plasma and serum samples from diabetes sufferers, with the aim of identifying disease-specific glycated proteins.

The ultimate aim of this work is to develop early stage diagnostic tests allowing for faster intervention and treatment; in the future it may be possible to cure a condition before the patient is even aware of symptoms. Obviously these developments will also facilitate the evaluation of particular intervention and ultimately improve in the efficiency of treatment strategies.

The two examples given above, one for the home and closed loop treatment of diabetes and the other for a simple diagnostic test for Alzheimer's disease (AD), clearly demonstrate that the near future of boronic acid based receptor research is assured. What about over the longer term? Rather than give specific predictions, I prefer to be more like Nostradamus and provide a prophecy, in which I predict that in the future the most important applications for boronic acid based receptors will be in the area of disease theranostics. In part these applications will develop as a direct result of the increased importance of animal, tissue and cellular imaging techniques using boronic acid receptors [124].

"*Education is the passport to the future, for tomorrow belongs to those who prepare for it today.*" – Malcolm X (1925–1965)

Author biography



Tony D. James is a Professor at the University of Bath, he obtained his BSC from UEA (1986), PhD from the University of Victoria (1991) and was a PDRF with Seiji Shinkai in Japan

(1991–1995). He was a Royal Society Research Fellow at the University of Birmingham (1995–2000), and University of Bath (2000–2003). He has been a visiting professor at Tsukuba, Osaka and Kyushu Universities, an AMADEus invited professor at the University of Bordeaux and is a guest Professor at East China University of Science and Technology, Xiamen University, Shandong Normal University, Nanjing University and is a Hai-Tian (Sea-Sky) Scholar at Dalian University of Technology. In 2013 he was awarded a Daiwa-Adrian Prize and in 2015 received the Inaugural Catalysis and Sensing for our Environment (CASE) Prize. His research interests include boronic acid based receptors for the fluorescence sensing of saccharides.

Acknowledgements

This is a personal account describing research carried out by myself and my group over the last 30 years. Throughout, the account I have acknowledged the people whose friendship and collaboration have made all of this possible. I am particularly indebted to the efforts of my many collaborators, postdoctoral researchers, PhD students, visiting students, and undergraduate students, without who this would have been a very short read. The following organisations and institutions are thanked for both past and present support: the University of East Anglia, the University of Victoria, the ERATO (JST) Chemirecognics Project, the University of Birmingham, the University of Bath, the Great Britain Sasakawa Foundation, the Daiwa Anglo-Japanese Foundation, the Engineering and Physical Sciences Research Council (EPSRC), the Japan Society for the Promotion of Science (JSPS), the Leverhulme Trust, the China Scholarship Council, The British Council, Defence Science and Technology Laboratory (DSTL), the Nuffield Foundation, Prostate Cancer UK, Dunhill Medical Trust, Alzheimer's Research UK (ARUK), Beckman-Coulter Inc, Zeneca Group PLC, Unipath Ltd, Smart Holograms, Glysure Ltd, Quotient Diagnostics, the Royal Society and the Royal Society of Chemistry. I would particularly like to thank The Catalysis and Sensing for our Environment (CASE) network [83] for providing excellent networking opportunities, since many of the results contained in this personal perspective are a direct result of the network.

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Creating molecular macrocycles for anion recognition

Amar H. Flood

Review

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Address:
Department of Chemistry, Indiana University, Bloomington, IN 47405, USA

Email:
Amar H. Flood - aflood@indiana.edu

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Abstract

The creation and functionality of new classes of macrocycles that are shape persistent and can bind anions is described. The genesis of triazolophane macrocycles emerges out of activity surrounding 1,2,3-triazoles made using click chemistry; and the same triazoles are responsible for anion capture. Mistakes made and lessons learnt in anion recognition provide deeper understanding that, together with theory, now provides for computer-aided receptor design. The lessons are acted upon in the creation of two new macrocycles. First, cyanostars are larger and like to capture large anions. Second is tricarb, which also favors large anions but shows a propensity to self-assemble in an orderly and stable manner, laying a foundation for future designs of hierarchical nanostructures.

Review

"Well, maybe it started that way. As a dream, but doesn't everything. Those buildings. These lights. This whole city. Somebody had to dream about it first. And maybe that is what I did. I dreamed about coming here, but then I did it."
Roald Dahl, *James and the Giant Peach*

Early childhood influences

I was born and raised in Napier, a small town in New Zealand best known as a vacation destination. It was an idyllic place to be brought up where, as kids, we had the freedom to dream. Part of that freedom was born of formative experiences by my father's side. My father was, and still is, a builder and an outdoorsman. When I was with him, we were either following plans to build a house or making plans to have an adventure. I now recognize that these same skills are used every day in science. In the first case, I learned the satisfaction of making something new (Figure 1), be it a new house or a new molecule. In the second, I learned how to forge a path into unknown territory [1] using only a simple set of core skills; I may have replaced map reading with the scientific method but it involves the same spirit of exploration. I also learned that if I could see the mountaintops on the horizon, I had a fair chance of being able to climb them one day. So it was with my dreams of becoming a scientist.

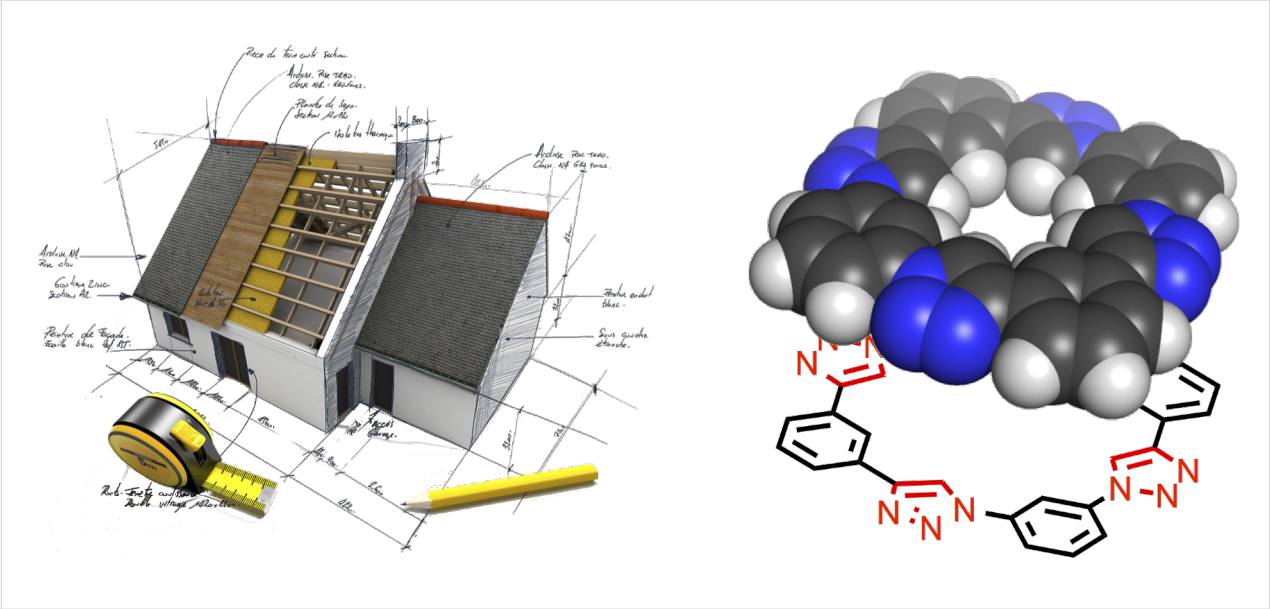

Figure 1: The design and building of a house is just as satisfying as that of a new molecule and often takes the same amount of time (left: Franck Boston copyright 123RF.com).

Mentors and inspirations

I pursued a bachelor of science at the University of Otago in the city of Dunedin where scholarly life was central to almost everything. I selected chemistry for my major as it provided the most explanatory power of the world around me. I did not start research until my honors year. At the time, I was excited by the research of Keith Gordon and requested to join his group. I worked with Keith on the creation and understanding of metal polypyridyl dyes [2] designed for use in solar cells [3]. I loved being in the laboratory doing research and I continued this work through my Ph.D.

From Keith I learned the importance of designing function into molecules and exploiting them in a homologous series [4] to better extract meaning from their measured properties. I came to appreciate a healthy mix of computation, synthesis and characterization, and I endeavor to use that approach every day. Yet, in spite of all the careful planning, I also learned the importance of simply trying it, or as Keith would say, “suck it and see”.

During my Ph.D., I learned how to be an independent scientist, but I was dreaming of something more, something bigger, and beyond New Zealand’s place at the other end of the world. Looking outward, Fraser Stoddart’s research, in particular, the molecules he made [5] that can be programmed to move and change shape (rotaxanes and catenanes) had hooked my attention. Quite apart from his gift for creating new molecules that are also functional, the number of people who shared co-authorship with him intrigued me.

Joining Professor Stoddart’s laboratory in 2002 as a postdoctoral researcher was an important step in my professional life. I ended up working closely with him, which ultimately provided me with immense perspective. I will admit that it took a long and hard climb from New Zealand into the UCLA group; it was all action and every step took me closer to my mountaintop. I learned everything I could from Fraser: how to run big and small research projects, write scientific papers, and give engaging scientific presentations at conferences. I gained priceless experience. He opened the doors to the world of science that I had only dreamed about during my years at Otago. Being given the chance of independence at Indiana University as an assistant professor was a welcome next step to fulfilling the dream.

Career accomplishments and highlights

Macrocycle discoveries

Macrocycles have been key to the group’s research findings. The timeline of macrocycle structures (Figure 2) illustrates the depth in triazolophanes [6] and the breadth in the cyanostars [7] and tricarb [8]. Their creative design and their roles in scientific learning will be described in the accounts to follow.

The efforts on macrocycles are particularly rewarding. They are aesthetically and geometrically appealing to work with. In our stables we have triangles, squares and pentagons represented by triacarb, triazolophane and cyanostar macrocycles. The analogy to donut shapes is obvious and the hole in their middles can be both filled with anions and observed using real-space scanning tunneling microscopy (STM) imaging. Their shape persistence

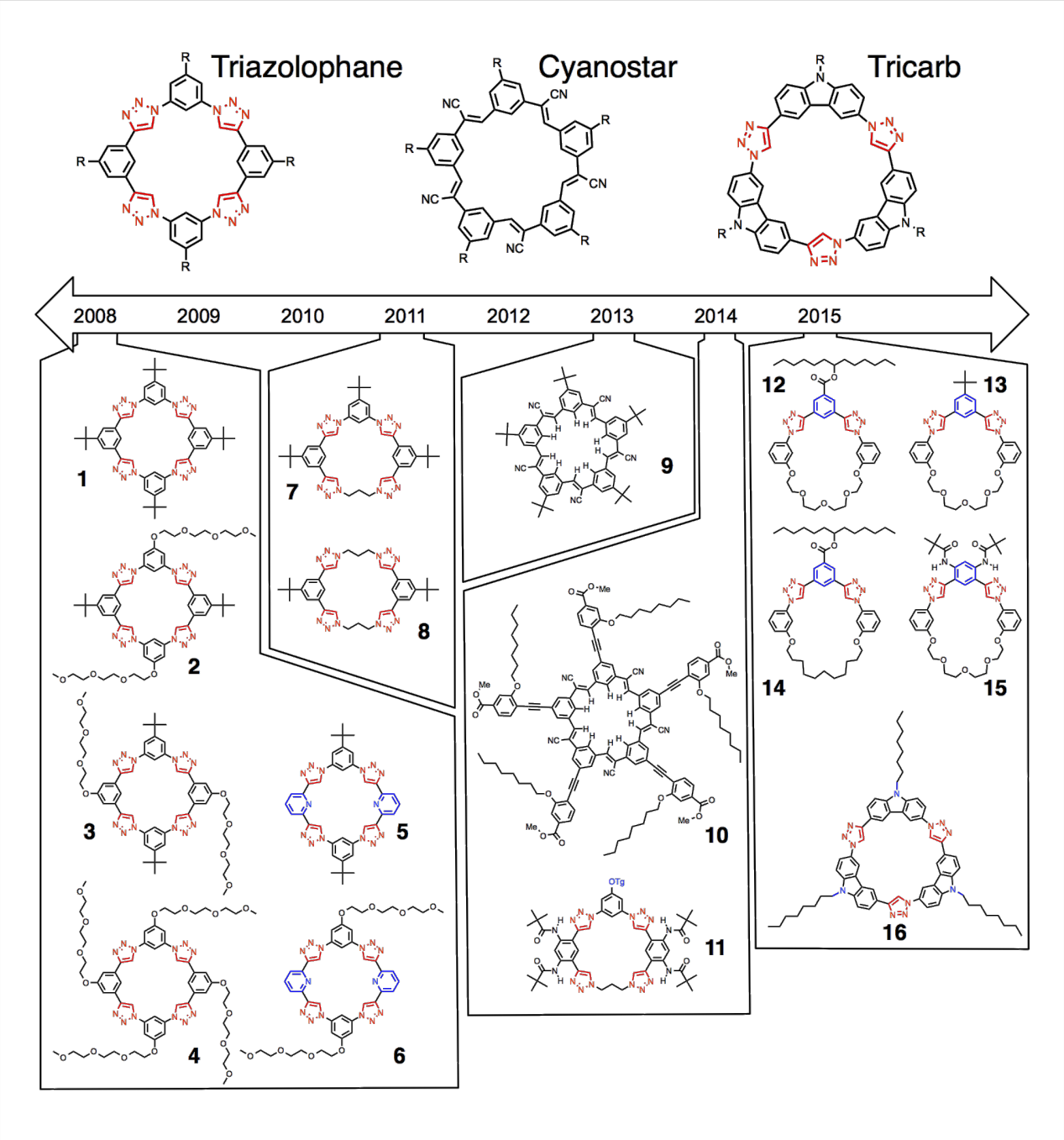

Figure 2: Timeline of anion-binding macrocycles.

makes them ideal for the study of structure–property relationships to enable deep understanding of anion recognition phenomena.

The identification of 1,2,3-triazoles as linkers, ligands and building blocks. The synthetic creation of macrocycles sets the scene for the group's initial and ongoing activities in anion recognition. Triazolophanes [6] (Figure 1) were the first of these macrocycles and the origin of their design warrants description because it represents the first time we made something new.

Triazolophanes were inspired by an overarching philosophy for synthetic design that was derived from Roald Hoffmann's idea of chemistry being "the same and not the same" [9]. At around this time (2006), click chemistry [10] (Figure 3) had caught my attention for its prevalence but I did not know why it was being used so much. All I could venture was that it was an old reac-

tion, the Huisgen cycloaddition, made good (regioselective) with the aid of copper catalysis. Taking that idea on face value, I reasoned that click chemistry was a new and extremely effective way to make 1,2,3-triazoles. It is not often that either new or newly refined reactions emerge but when they do, they represent plenty of scope for creative chemical design.

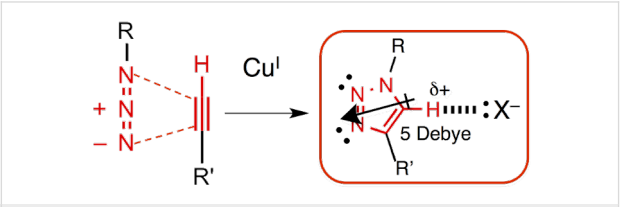

Figure 3: Click chemistry's copper-catalyzed azide–alkyne cycloaddition (CuAAC) forms 1,2,3-triazoles that stabilize anions by CH hydrogen bonding and ion–dipole interactions.

At the time, I observed that the triazole linkages were used to bring together two modules, be they a fluorescent tag "clicked" onto a protein or redox-active group "clicked" together with a polymer. But I also thought there must be some latent functionality deriving from the intrinsic structure of the triazole that remained unexplored. It was clear later that Stefan Hecht and Steve Craig had similar thoughts.

Invoking Hoffmann's dictum [9], I originally wondered if the triazoles were the same as pyridines for the purpose of transition-metal coordination, an area in which I received my Ph.D. training with Keith Gordon. I asked my postdoctoral co-worker at the time, Yongjun Li, to assist with testing the idea. We prepared a series of analogs to the common terpyridine ligand (Figure 4a,b). Once made, they indeed bound metals, for example, Fe(II), Ru(II) and Eu(III) [11]. It was at this point that I lifted my head and asked the next critical questions: how are triazoles not the same as pyridines and what does the coordination chemistry teach us about how these 1,2,3-triazoles like to behave?

All our observations indicated that triazoles are sterically small. In the iron complex, we saw the ferrous ion's preference for triazole change to a water molecule when oxidized up to the harder ferric ion. This process did not occur with the terpyridine control complex. Thus, we reasoned water could easily slip past the triazole nitrogens but not past the pyridine with the steric protection provided by a CH group (see the red arrows in Figure 4c for the overlay of the two ligands). The ruthenium complex showed intermolecular π stacking of ligands on neighboring complexes in the solid state; yet terpyridine analogs did not. Again, having a nitrogen atom in the triazole heterocycle instead of a CH provides less steric interference. Finally, the Eu(III) complex was more hydrolytically stable. With the aid of

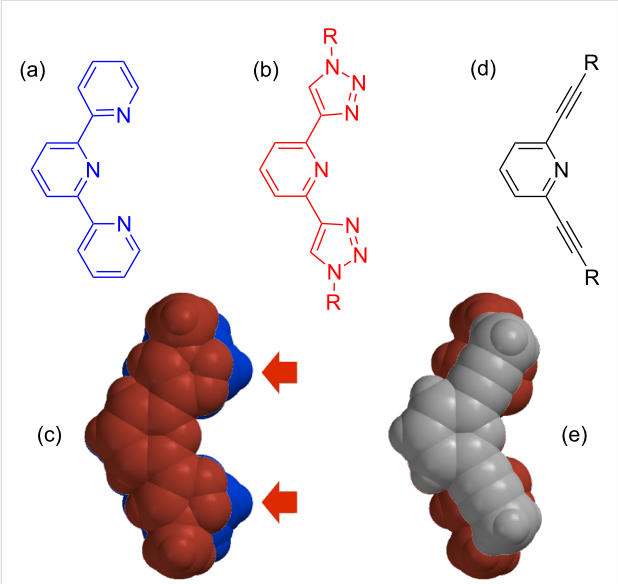

Figure 4: These molecular compounds are the same and not the same.

a crystal structure, we saw the smaller size of the triazoles again circumvented the steric destabilization seen in the CH groups in pyridine that poke uncomfortably into neighboring ligands.

The coordination chemistry taught us that triazoles are sterically small, perhaps small enough to be as innocent as ethynyl units (e.g., Figure 4d and overlay in Figure 4e). If that idea was true, then triazoles became prime candidates for making coplanar structures same as Moore's [12]. With this realization in mind and a pen in hand, the creation of triazolophanes emerged.

The creation of triazolophanes. Sitting in a lecture hall listening to a seminar was the perfect place for doodling molecular designs with triazoles. I started by drawing a triazole and then drew one benzene on either side (Figure 5a). At the time, I was fixated on linear molecules but that was not how this benzene–triazole–benzene triad looked. I recall being bothered that the pentagon shape of the triazole forced me to bend away from linearity; clearly not the same as carbon–carbon triple bonds. But, when I took a calming breath and looked again, I embraced the curvature and just kept on drawing (Figure 5b). Before I really knew what was happening, the alternating benzenes and triazoles quickly and quietly generated a circle resembling a new macrocycle (Figure 5c). I recall being amazed at how well the two ends came together and I thought it was a little too convenient. So, with plenty of time left in the lecture, I carefully redrew the molecular design as a sequence of hexagons and pentagons. I used as much precision as possible and made use of the lines on the notepad for guidance; a hangover

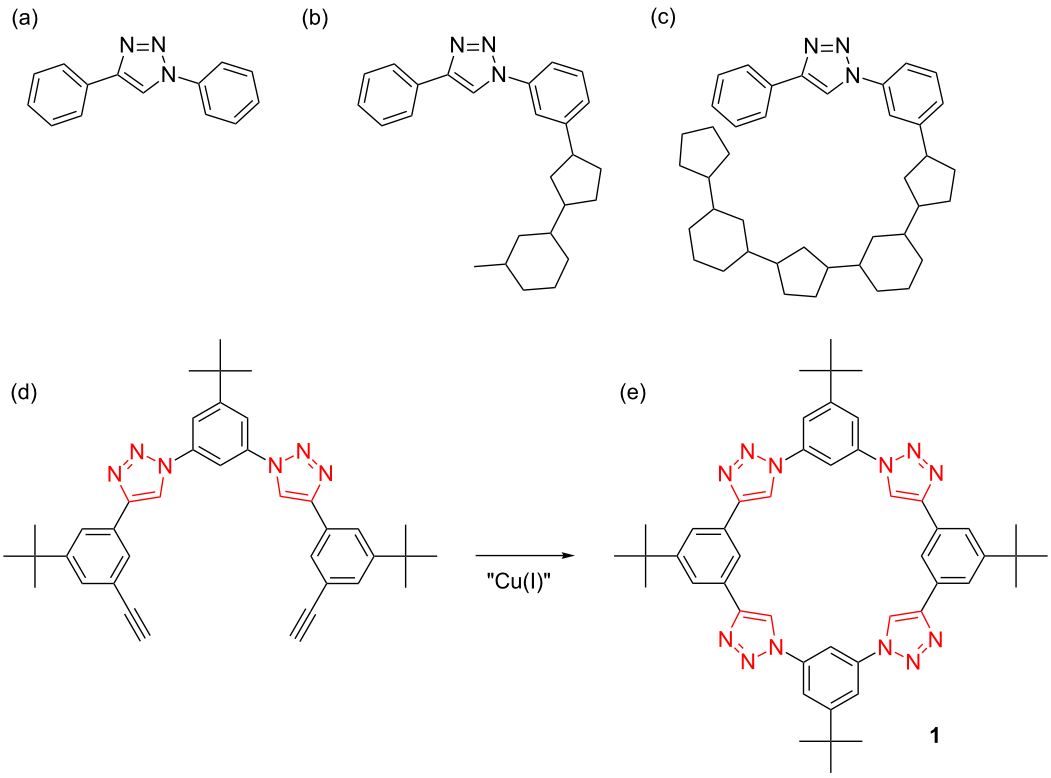

Figure 5: (a, b, c) Sequence of chemical sketches leading to triazolophanes. (d) The precursor that led, by CuAAC, to the (e) macrocycle.

from my father's influence as a builder. If anything, the design got sharper upon redrawing.

A few more things happened that same day to benefit the realization of the macrocycle. I tested the idea using simple molecular mechanics to recapitulate the hand-drawn image with a more realistic model. Later in the day, I asked Yongjun Li, my postdoctoral co-worker at the time, if he thought he could make it, and he said yes. Yongjun and I were fortunate to have built up a working relationship at the time where I would share my latest idea with him and vice versa, then one of us would proceed to knock the idea down. But this time around, the idea fully captured both of our imaginations.

As always, the synthetic pathway leading to a new compound needs to be forged by the traveler making the journey for the first time. While I was happy to see the first few legs pass uneventfully, Yongjun had to pause a long while at the precursor to the macrocycle (Figure 5d). We even considered a few consolation prizes. It is a testament to the perseverance and skills of Yongjun Li that he was able to navigate all the possible dead ends to bring about the first preparation of the macrocycle (Figure 5e). To this day, the stepwise procedure [6] we currently use to make triazolophanes still largely follows the path he set for it.

Anion binding to triazolophane macrocycles. At the time when the triazolophane was drawn on the notepad, the central cavity clearly beckoned a guest; after all, nature abhors a vacuum [13]. With hydrogens running around the cavity, it appeared to be predisposed towards anions. However, no one would have anticipated the chloride affinity would be as high as it was.

Triazolophanes bind chloride with an affinity that surpasses many other macrocycles. The affinity that we have come to accept as final for equilibrium **1** + Cl^- ⇌ **1**·Cl^- is K_1 = 4,700,000 M^{-1} in dichloromethane [14]. This affinity is all the more special and unusual for coming from a receptor that only bears the so-called "weak hydrogen bond" deriving from CH donors [15].

We contend that the triazole CH donors are not weak: the cluster of three nitrogen atoms act together to polarize the CH bond far beyond the intrinsic electronegativity difference of carbon and hydrogen. Added to these four triazoles in the triazolo-

phane are the four benzenes, which also deliver CH donors. Yet, the question of how all these donors perform so well inside the triazolophane defined a research agenda that continues to this day. Ideas such as the macrocycle's rigid shape-persistence must also play a role.

Testing structure–property relationships for triazolophanes: electronics, halide selectivity, π-stacked sandwich formation, and flexibility. If the phenylene CH groups were playing a role, we reasoned from Benjamin Hay's work [16] that electronics would alter the hydrogen bonding strength. We made, characterized, and compared the chloride affinity to a series [17] of triazolophanes with substituents spanning from electron donating *tert*-butyl to more electron donating alkoxys (**1**–**4**) (Figure 2). As expected, the chloride affinity decreased across the series, for example, reducing by a factor of four for **1**·Cl$^-$ versus **4**·Cl$^-$ (Figure 6). Interestingly, this series allowed us to show that stronger binding occurs in pockets with triazoles linked to the phenylenes through the nitrogen atoms located north and south. We attribute this effect to the electron-withdrawing nitrogen and better angular alignment (Figure 3) of the triazole's dipoles.

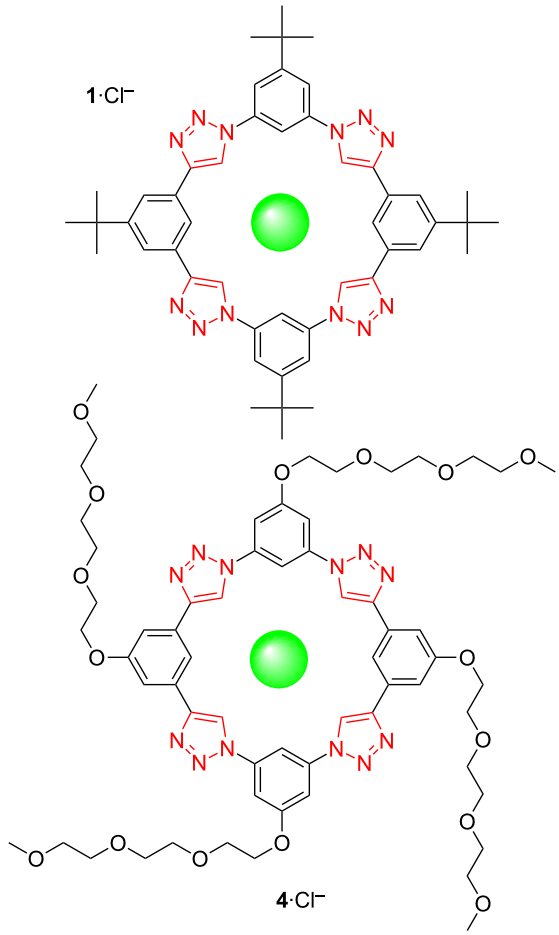

Figure 6: Variation in phenylene substituents weakens chloride affinity from **1** to **4**.

In response to Michael Haley's question during the first presentation of the triazolophanes in 2007 at Yoshito Tobe's International Symposium on Novel Aromatics (ISNA-12) on Awaji Island, Japan, we undertook a study of the size selectivity [17]. We found triazolophanes are size-matched to chloride and bromide ($K \approx 10^6$ M^{-1}) with fluoride being too small ($K \approx 10^5$ M^{-1}) and iodide ($K \approx 10^4$ M^{-1}) too large. The binding constants reflect this order. As suggested by Jonathan Sessler in conversations, the bromide is better size-matched but its charge density is just a little less than chloride such that the affinity for chloride is a little higher in dichloromethane. The biggest surprise (and clearest vindication for the rigidity of the triazolophane macrocycle) is the weak fluoride affinity, being an order of magnitude lower than chloride. Typically, charge-dense fluo-

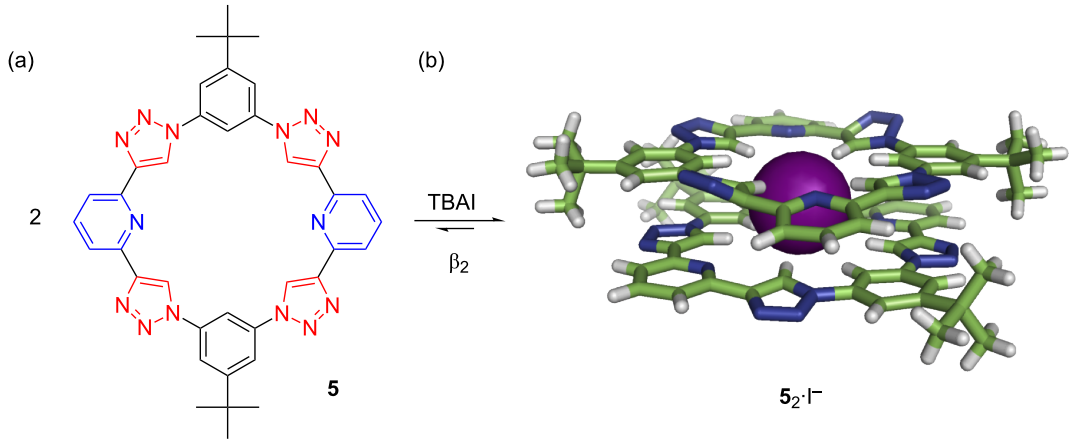

Figure 7: (a) Pyridyl triazolophane and (b) its high-fidelity sandwich around iodide (crystal). Adapted with permission from [20], copyright 2010 Royal Society of Chemistry.

ride produces the largest affinity when the receptor can conform to the fluoride's smaller size to produce shorter, stronger contacts. Calix[4]pyrroles are a case in point [18]. This conformational rearrangement cannot occur within the triazolophane and so, while the fluoride may have shorter hydrogen bonds, it has fewer contacts than chloride.

For iodide, it is simply too big for a single triazolophane; so it was a fortuitous and pleasant discovery that, with pyridyl rings [19], 2:1 sandwich complexes form around iodide (Figure 7). In fact, we saw such extreme positive cooperativity that we could not clearly distinguish the 1:1 affinity and reported instead just a fix on the 2:1 stability constant of $\beta_2 = 8.6 \times 10^{10}$ M^{-2}. Interestingly, we observed a rotational offset between the two π-stacked macrocycles that we attributed to the emergence of favorable *anti*-parallel pairing of local dipoles between the two macrocycles.

The size selectivity of the macrocycles attested both to the triazolophane's rigidity and that preorganization may be crucial for the large binding affinity. To test this idea, we examined the macrocyclic effect using an oligomer (Figure 8a) that folds up around chloride [17]. The affinity decreased by four orders of magnitude to ≈100 M^{-1}, perfectly consistent with expectations. Then, we reduced the rigidity of the macrocycle by replacing two phenylenes with two propylenes (Figure 8b), which decreased the affinity to ≈1,000 M^{-1} [21].

The affinities we got wrong and our steps to right the wrongs

Side-by-side comparisons of different structures and different anions rely upon the correct estimate of the binding affinity. We originally missed a few features that impacted the accuracy of the binding constants. While these issues are quite common, their impact can be huge: our affinities had to be modified twice

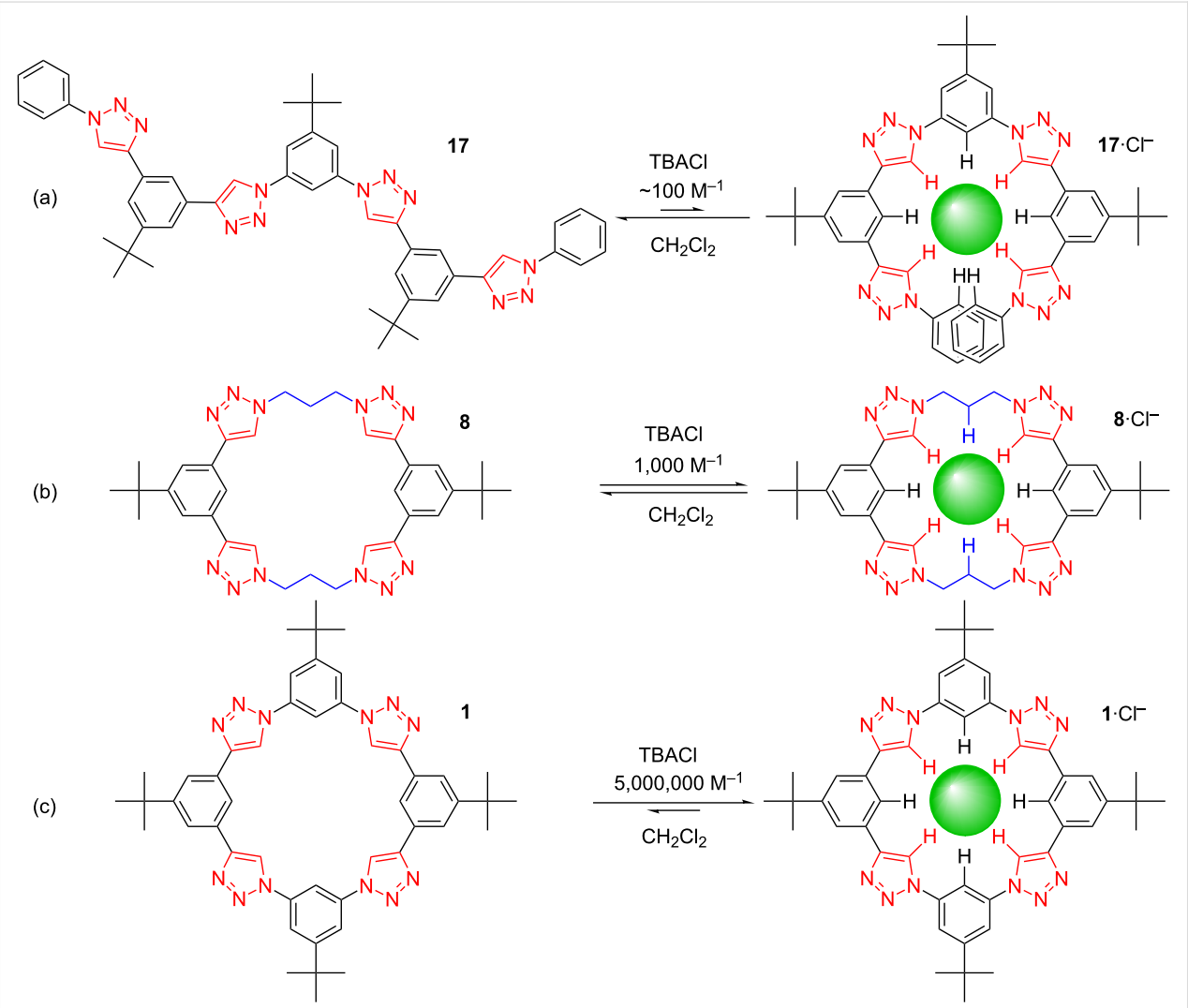

Figure 8: Testing the (a) macrocyclic effect, and (b) effect of rigidity against (c) the parent triazolophane.

from K_a = 130,000 M^{-1} to 11,000,000 M^{-1} to 4,700,000 M^{-1} [6,14,17]. That is, differences of 100 and then two; factors that have an impact when trying to compare two sets of results.

We had three errors. First, we failed to conduct our titration experiments at concentrations close to the initially determined dissociation concentration, K_d = 8 μM (= $1/K_a$). Our first titrations were conducted at such high concentrations (100 μM) that the moment we added any chloride, it directly converted reactants (macrocycle) into products (1:1 complex). With little to none of the empty macrocycle present after equilibrium was established, the ratio of products to reactants was poorly represented by the observed UV–vis absorption data. We also failed to appreciate that the 2:1 sandwich complexes could also form in solution, a finding we only considered after seeing the 2:1 sandwiches around iodide [19]. What we needed to do was include an additional equilibrium to account for this species.

The third problem with ionic titrations is ion pairing [22], particularly in solvents with dielectric constants (ε) less than 20. Every salt (e.g., tetrabutylammonium chloride (TBACl)) is different and its propensity to be paired in solution should always be evaluated for the concentrations at which the titrations are being conducted. If it is paired, then the receptor has to outcompete the anion from the counter cation in order to be complexed. While ion pairing may be just a little healthy competition, it is too often overlooked because its spectroscopic signature is often hard to detect. Furthermore, once a 1:1 or 2:1 complex has formed, that species is just one big greasy anion and it too can engage with the counter cation. Like the excellent series of papers by Sessler, Gale and Schmidtchen [23,24], we learned all of this the hard way. In their case, when they changed the counter cations, their binding constants were not constant! In our case, when we changed concentrations, the binding constants for the 1:1 triazolophane–chloride complexes were not constant.

We ended up taming our menagerie of ion pairing and complexation equilibria in a tour de force thermodynamics study [14]. Here I present the full set of four equilibria (Figure 9a) we settled on for the reaction between macrocycle, anion and the TBA^+ counter cation. Thus, we have the 1:1 species (K_1), formation of the 2:1 sandwich (K_2, $\beta_2 = K_1 \times K_2$), formation of the ion pair complex (K_{ipc}), and competition from ion pairs (K_{ip}).

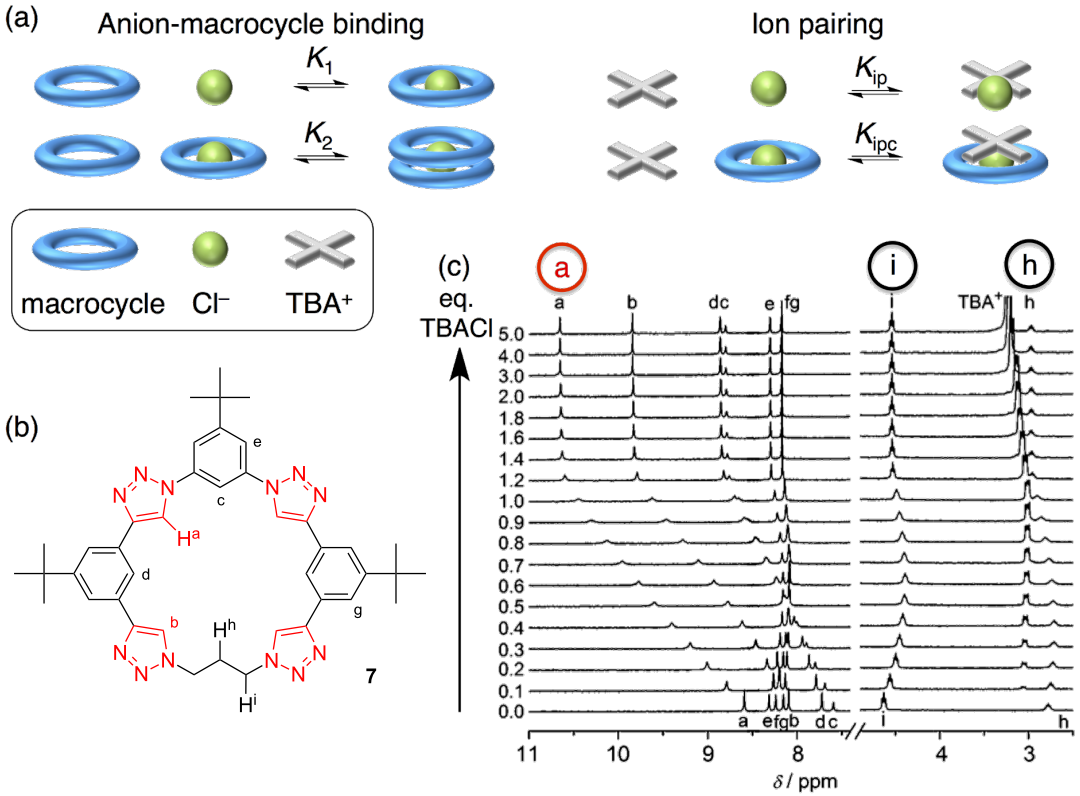

Figure 9: (a) Representations of the four equilibria that dominate in dichloromethane for which the (b) propylene triazolophane's (c) ^{1}H NMR titration data is particularly reflective of the four-equilibrium model. (c) Adapted with permission from [14], copyright 2011 Wiley.

As a tribute to the four equilibria and the four species that are formed, the ^{1}H NMR titration data for triazolophane **7** is exemplary (Figures 9b,c). Proton H^a reflects the 1:1 species by stopping its movements after 1 equiv of chloride is added. Protons H^h and H^i have inflection points after 0.5 equiv of chloride are added, which is the equivalence point for the 2:1 complex. We observe the ion pairing of the TBA$^+$ with the 1:1 complex in the cation's α proton (≈3 ppm), which has an inflection point at 1 equiv. Consistent with its complexation as a 1:1:1 species, the diffusion NMR signature for the TBA$^+$ cation's α proton showed a lower diffusion coefficient at 1 equiv when it forms the larger ion pair complex MC·Cl$^-$·TBA$^+$.

Ultimately, any accurate assessment of an equilibrium constant requires inclusion of all the equilibria. There are a few tricks [25]. Some of the less stable species can be diluted out and ion pairing can be avoided by using more polar solvents. Nevertheless, the equilibria operating in solution still need to be assessed. Fortunately, the minor ones can often be omitted. Either their inclusion has a negligible impact on the fitting or they contribute less than 5% to the overall distribution of species in solution such that they are poorly represented in the titration data.

These days, we undertake such detailed multiequilibria analyses about once every graduate student so that they learn how to analyze data accurately. We also make use of more polar solvents that avoid ion pairing. But, in those cases, the triazolophanes then start to self-associate [17,19]. The story of macrocycle self-association is still unfolding in our hands.

None of the multiequilibria fitting would be possible without the use of appropriate software. This allows me to highlight a rewarding collaboration with Douglas Vander Griend who wrote and updates software called SIVVU [26,27], which is the spelling of UVVIS backwards to reflect the idea of the deconvolution of absorbance data into equilibria. In addition, we have benefitted from the use of HypNMR, allowing for the fitting of ^{1}H NMR titration data. Both software have their limitations but their usage as tools to unravel complex equilibria is without parallel.

Cooperativity of ion–pair complexation

The most recent undertaking of ion pairing is to make it a feature and to examine, in glorious detail, how ion pairs can be bound cooperatively inside designed macrocycles [28]. When positive cooperativity emerges, novel selectivity can be engineered [22]. Despite this possibility, we recognized that a deep understanding of ion pairing is still in its infancy. Ultimately, we quantified the cooperativity involved in the salt binding to an aryl–triazole–glycol macrocycle (Figure 2, **12**–**15**, and Figure 10) and examined the cooperativity using theory. We investigated $NaClO_4$ and NaI (Figure 10b) experimentally and NaI, NaBr, NaCl theoretically. Theory is able to do things that are impossible in experiment (Figure 10c). As a result, and for the first time, we quantitatively determined that allostery contributed to ≈30% of the cooperativity. The remaining 70% came from Coulombic cooperativity, which dominated the size-dependent trend such that NaCl proved to have greater cooperativity than NaI.

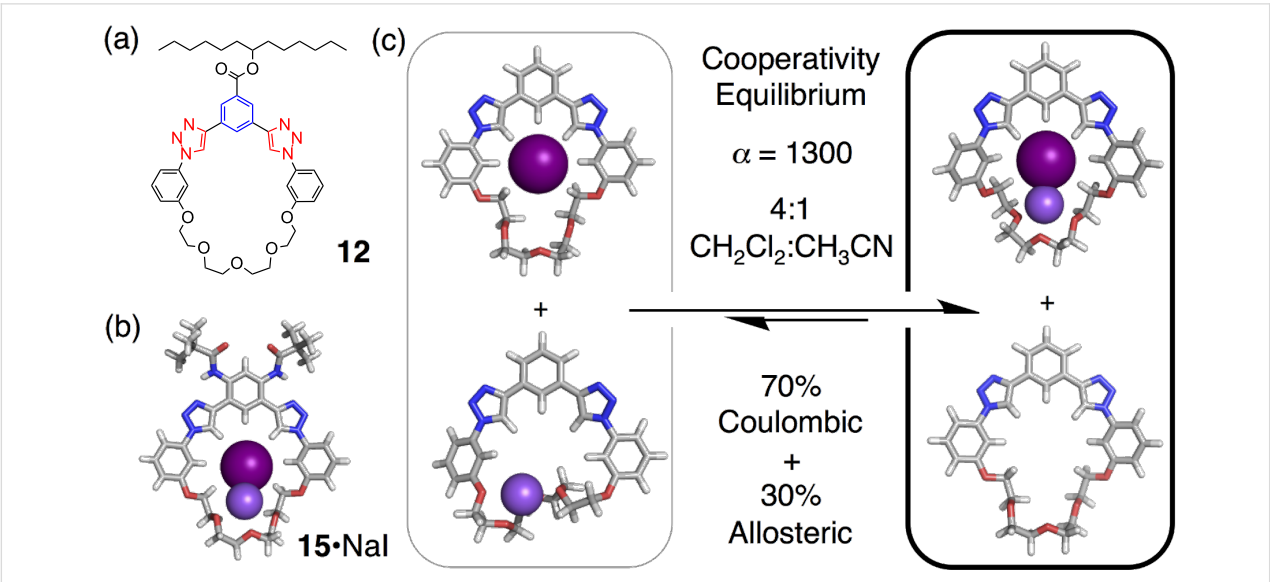

Figure 10: Representations of (a) aryl–triazole–ether macrocycle **12** and (b) the ion-pair crystal structure of **15** with NaI. (c) Cooperativity of ion pairing was revealed using a theoretical assessment of all species. Geometry-optimized structures are shown; note the NaI complex matches the crystal.

Using theory for understanding

With accurate values for the 1:1 stabilities of various anion complexes deconvoluted from a veritable stew of other equilibria, quantum chemistry can be used to get an accurate picture of binding. First, we learn how to bring experiment and theory into agreement, then delve deeper into the origins of binding, which also provides a basis for computer-aided receptor design. The fact that triazolophanes are shape-persistent helps immensely for finding agreement between experiment and theory.

Working in collaboration with Krishnan Raghavachari, we investigated some of the early ideas about how the triazolophane performs. We confirmed it to be highly preorganized [21,29], that the triazoles are responsible for the majority of the stabilization, and that benzenes offer CH hydrogen bonds at 40% the strength of triazoles. We have examined anions of increasing complexity, ranging across the simple halides, to diatomic cyanide, and triatomic bifluoride. The halides fluoride, chloride and bromide largely follow experiment [29]. However, the cyanide study [30] produced some surprising results. We found in gas-phase calculations and solution-phase experiments that cyanide binds as well as chloride (Figure 11a). Theory helped explain these findings wherein the cyanide rotates in-plane to behave as a pseudospherical anion. In addition, we found nitrogen forms shorter hydrogen bonds [31] even though carbon is the site of covalent bond formation.

Computer-aided receptor design

We used our collective knowledge to execute our first computer-aided receptor design project [28]. As with the cyanide study, we used the binding of chloride as a yardstick against which to compare and enable understanding of the binding of the triatomic anion bifluoride, $[F–H–F]^-$. Unlike cyanide binding, we saw that while chloride and bifluoride had the same affinity in gas-phase calculations, they differed experimentally in solution (Figure 11b). Judging from the calculated geometry, we wagered that the bifluoride anion did not fit very well and that solvent was pulling it out of the binding pocket. We went through a few cycles of computer-aided receptor design to optimize the geometry of the complex. Out targets were to equalize chloride and bifluoride affinities in the gas phase, and to optimize geometries with the anions located in-plane with the macrocycle in order to avoid solvation effects. The hypothesis was that the affinities should also be equal in solution, and after synthesizing the new design, we confirmed the hypothesis (Figure 11c).

Cyanostar macrocycles

Putting all the lessons from the triazolophanes to the test, co-worker Semin Lee designed a wholly new macrocycle, called cyanostar [7] (Figure 12). The motivation for this new work was to generalize the idea of activating CH hydrogen bond donors using electron-withdrawing groups. In cyanostilbene, Lee recognized the cyano group could polarize the CH

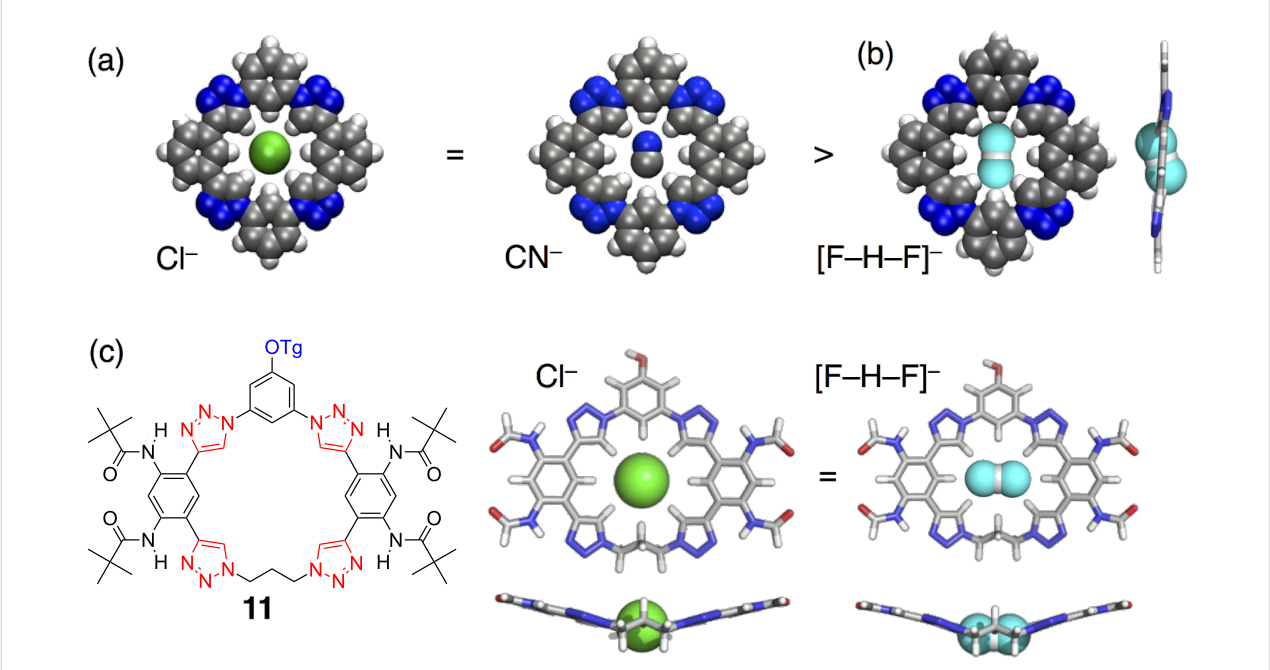

Figure 11: Chloride is used as a comparator for (a) cyanide and (b) biflouride. (c) Computer-aided receptor design was used to optimize the structure to make the chloride's affinity equal to that of bifluoride. Part (a) adapted with permission from [30], copyright 2011 Wiley. Part (c) adapted with permission from [32], copyright 2014 American Chemical Society.

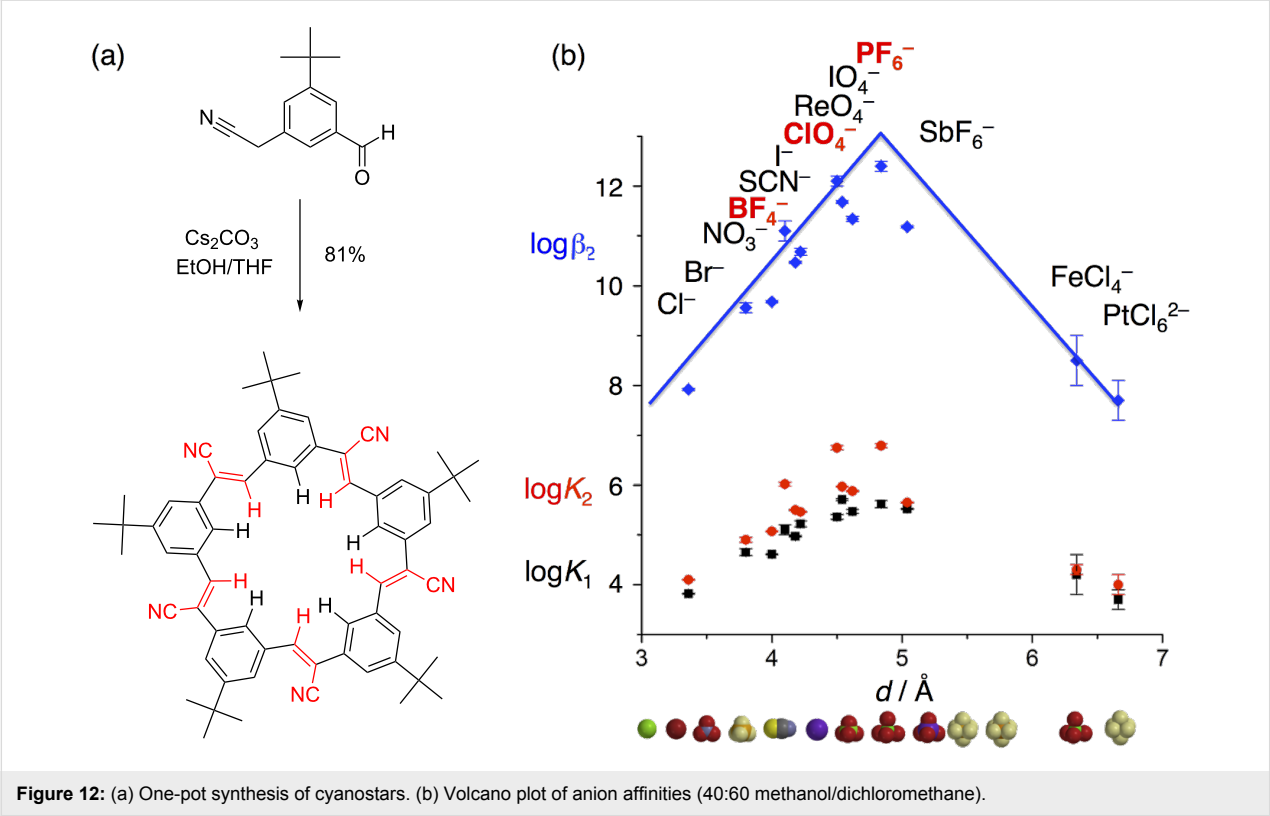

Figure 12: (a) One-pot synthesis of cyanostars. (b) Volcano plot of anion affinities (40:60 methanol/dichloromethane).

bond. Following some molecular modeling, he settled on a five-fold symmetric target that demanded a difunctional building block for the Knoevenagel condensation that forms cyanostilbenes. Treating the material to carbonate base-catalyzed conditions, he acquired the product in high yields, now optimized to 80% and scaling nicely up to 10 g quantities.

The effectiveness of the central binding pocket for stabilizing anions was unexpected. Larger than triazolophanes by a whole angstrom, the cyanostar's cavity was expected to offer weak binding: its cavity was much less electropositive than triazolophanes and its 4.5 Å size was only complementary to anions known to be weakly coordinating [33]. Despite these perceived shortcomings, the affinity towards anions like PF_6^- and ClO_4^- was some 6–9 orders of magnitude higher than most others that had been seen prior [34]. Fortunately, Sindelar's bambusuril [35] has now been shown to offer similar affinity towards these anions.

The performance of the cyanostar macrocycles is still being explored. The large binding pockets and the C_5 symmetry provide a basis for a lot of new chemistry. This includes use of phosphodiesters as templates for the synthesis of [3]rotaxanes [7] (Figure 13). The other aspect of these macrocycles arising

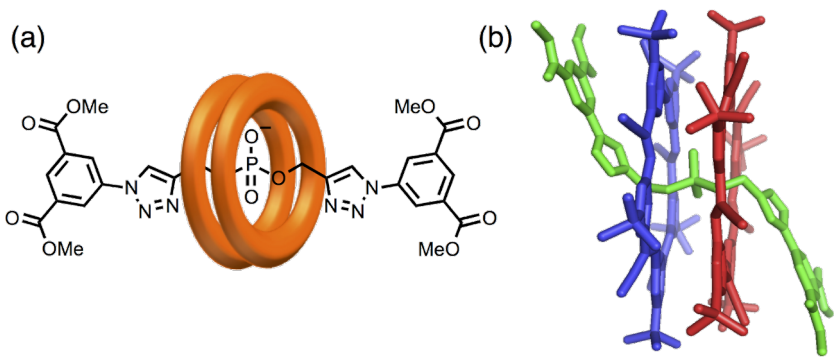

Figure 13: (a) Representation and (b) crystal structure of cyanostar-based [3]rotaxane.

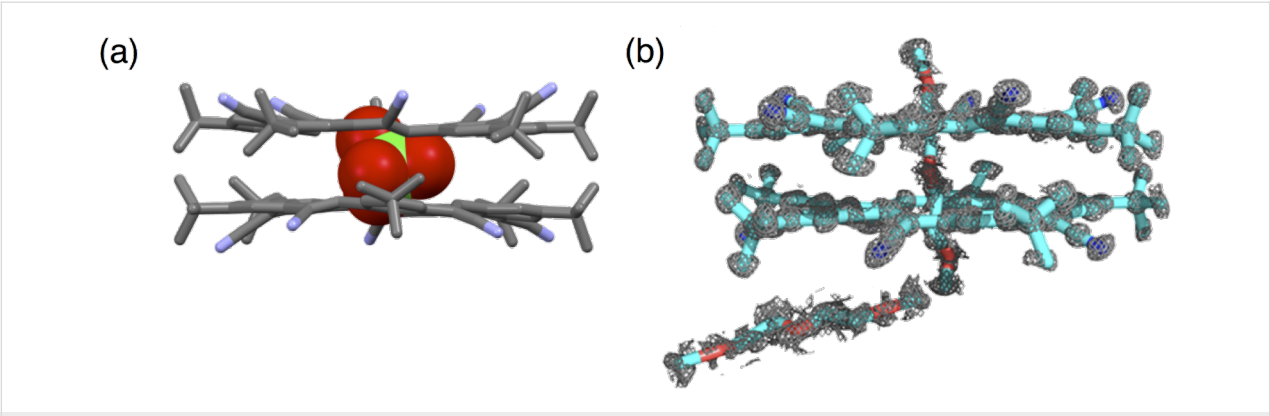

Figure 14: Crystal structures of cyanostar sandwich around (a) perchlorate and (b) diglyme (molecules shown with stick models and representative electron-density contours). Part (a) reproduced with permission from [36], Copyright 2014 Royal Society of Chemistry; Part (b) adapted with permission from [37], copyright 2015 American Chemical Society.

from their π surface is their propensity to stack. This can be seen in the crystalline phase with dimer formation either around anionic guests like perchlorate [36] (Figure 14a) or, in the absence of an anion, around adventitious solvents of crystallization, like diglyme (Figure 14b) [37] .

The π-surface was enhanced by extending the cyanostar's extremities (Figure 15a) in a bid to program the molecule's self-assembly into a 2D array on graphite [36]. This work was undertaken in collaboration with Steve Tait who is able to resolve molecules using STM. It was gratifying to see the molecules assemble and even more so to observe (Figure 15b) the macrocycle's star shape.

Tricarbazolo triazolophane macrocycles

Perhaps co-worker Semin Lee had acquired a taste for discovery because he created another class of macrocycle. The tricarbazolo triazolophanes [8] (tricarb for short, Figure 16a)

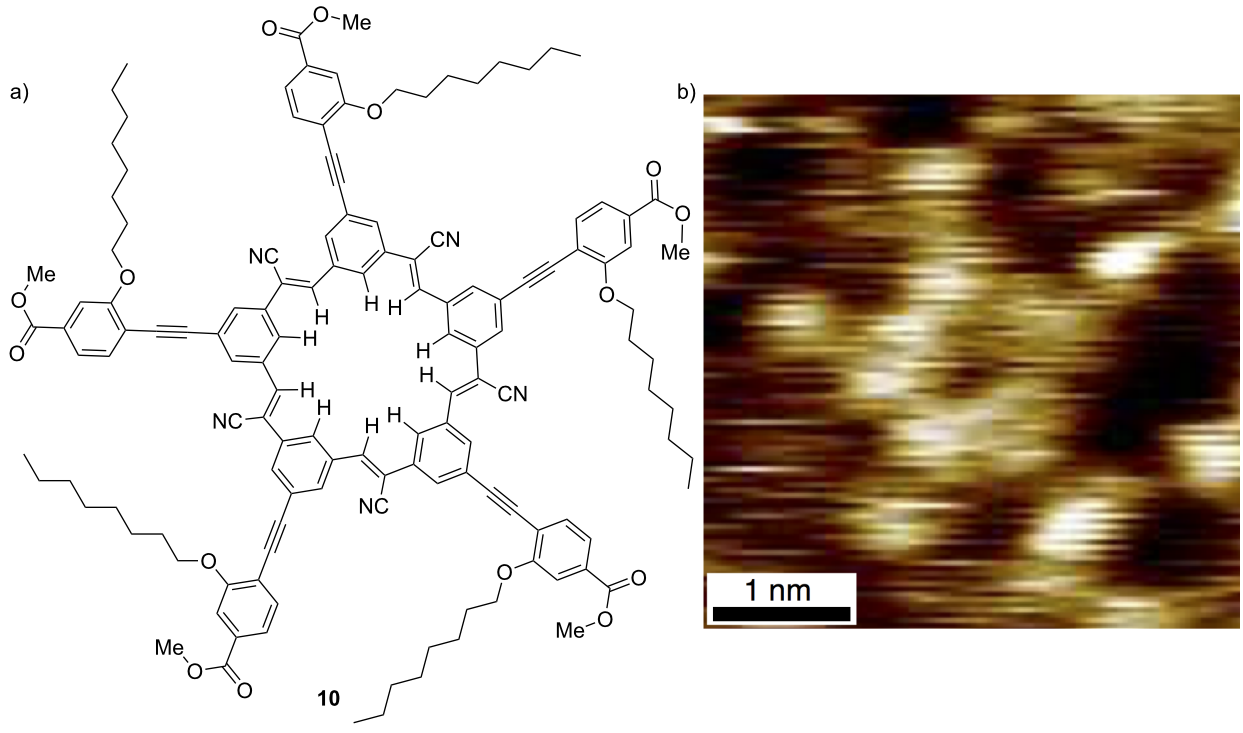

Figure 15: (a) Star-extended cyanostar and an (b) STM image cropped from a 2D lamellar lattice. Part (b) adapted with permission from [36], copyright 2014 Royal Society of Chemistry.

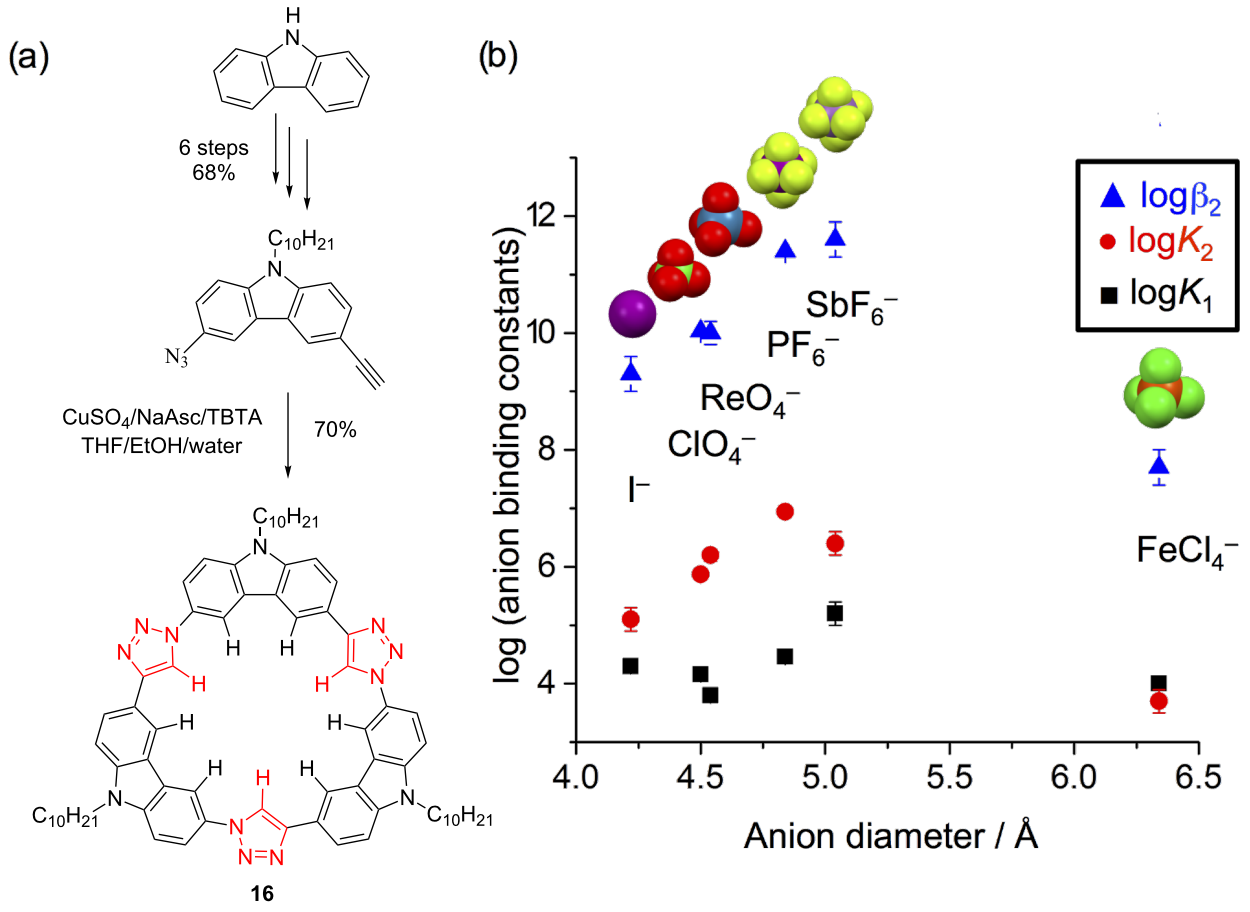

Figure 16: (a) Synthesis and one-pot macrocyclization of the tricarb macrocycle. (b) Volcano plot of anion affinities (20:80 methanol/chloroform). Part (b) adapted with permission from [8], copyright 2016 Wiley.

were designed to be made in one pot using click chemistry and to prepare another potent binding pocket for anions composed solely of CH hydrogen bond donors. Now just a little larger than cyanostars at 4.8 Å, their affinities showed peak affinity for slightly larger anions (Figure 16b). The tricarb structure is highly planar and this is where its properties begin to depart from cyanostar's. This planarity leads to extremely high self-association behavior. While a crystal structure has so far been elusive, the pattern of molecular packing has been examined from studies of surface assembly.

We inspected the tricarb macrocycle's surface self-assembly and anion binding (Figure 17a) using STM and were surprised by what we saw. Clear in the imaging (Figure 17b) were their shapes, looking like lumpy donuts; a shape similar to the one offered by Japan's Mister Donut. Interestingly, these macrocycles displayed a reliable propensity for clean self-association into 2D patterns of fused rosettes (Figure 17c). This pattern allowed us to readily discern binding of iodide anions as bright features constituting a pattern with the same unit-cell dimensions as the parent honeycomb. These macrocycles had another characteristic that was unexpected; they showed concentration-driven stacking directly on top of one other into uneven multi-layered ultra-thin films (Figure 17d) about three to four layers thick. This layering is unusual for such self-assemblies and, thus, this is a fruitful area for us to investigate further by considering how to program the outsides of macrocycles to direct 3D packing.

Crescent receptors

Crescent receptors, while not as elegant as macrocycles, are succinct models for testing ideas. Over the years (Figure 18), we have tested intramolecular hydrogen bonding for preorganization (**19**) [38], new CH hydrogen bonding from naphthalimides (**20**) [39], and investigated the transfer of function to polymeric constructs (**21**) [40]. The most recent crescents (**22**) provided a basis to investigate surface self-assembly [41], anion binding and switching at interfaces [42]. They also provide a base for foldamers; a series of crescents linked together into oligomers with interesting dynamic shapes.

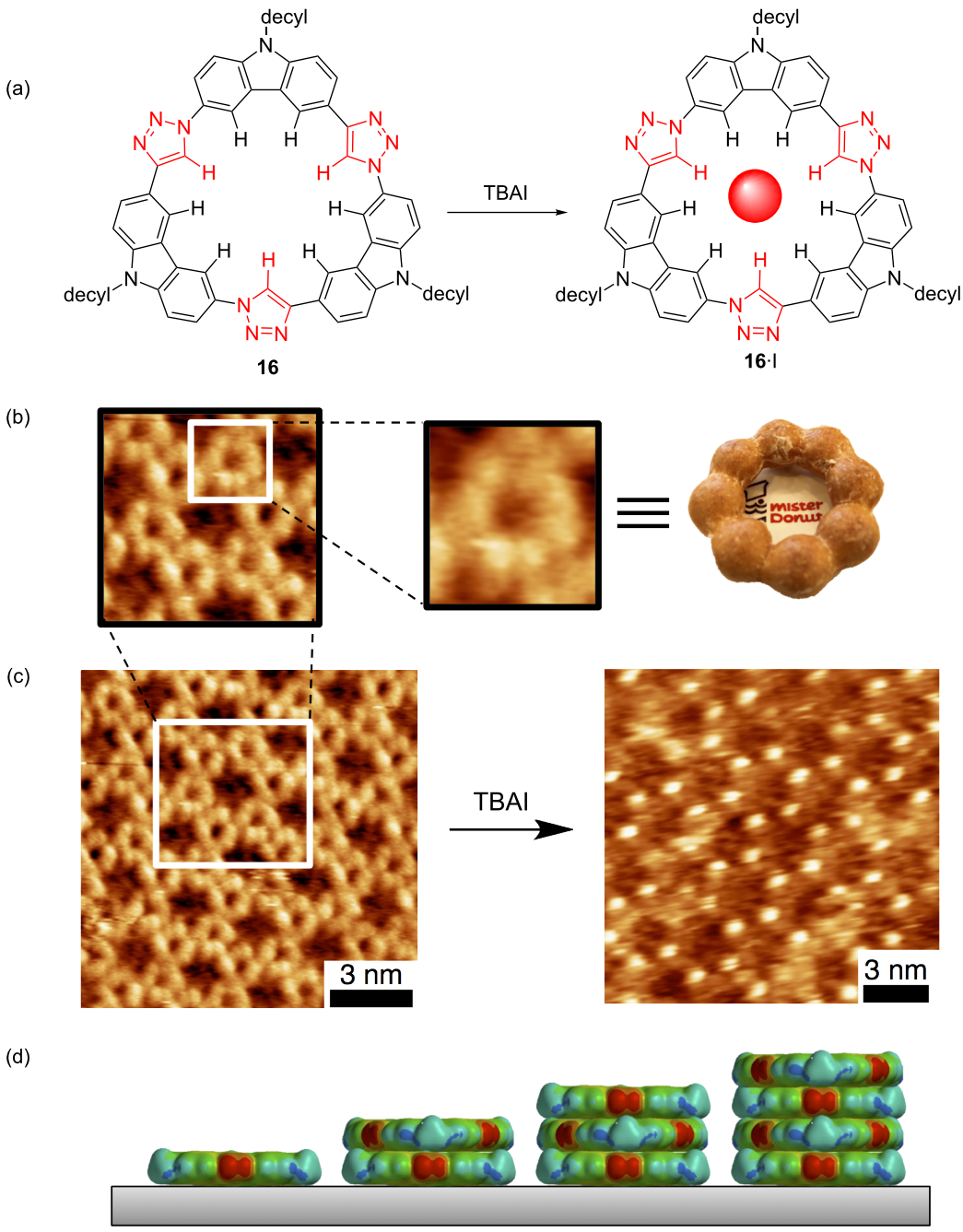

Figure 17: (a) Tricarb binds iodide. (b) Tricarb's single-molecule STM image resembles a donut. (c) Honeycomb surface patterning of tricarb before and after binding iodide. (d) Representations of how tricarb stacks on graphite. Parts (b) and (c) adapted with permission from [8], copyright 2016 Wiley.

Foldameric anion receptors

Complementing our creation and study of macrocycles is work on foldamers (Figure 19). With their flexibility, foldamers are excellent "character foils" to rigid macrocycles. As a class of compounds, foldamers [43] are highly modular and thus benefit greatly from the prevalence of azido and alkynyl building blocks that serve as precursors to click chemistry. Our research program is bioinspired [44] for the control of anions for separation purposes. We have utilized them for regulating chloride [45]. Starting with an on–off binding and release ratio of about 10 with foldamer **23** [46], and, by using secondary contacts often seen in biology, we have reached up to a ratio of 84 with

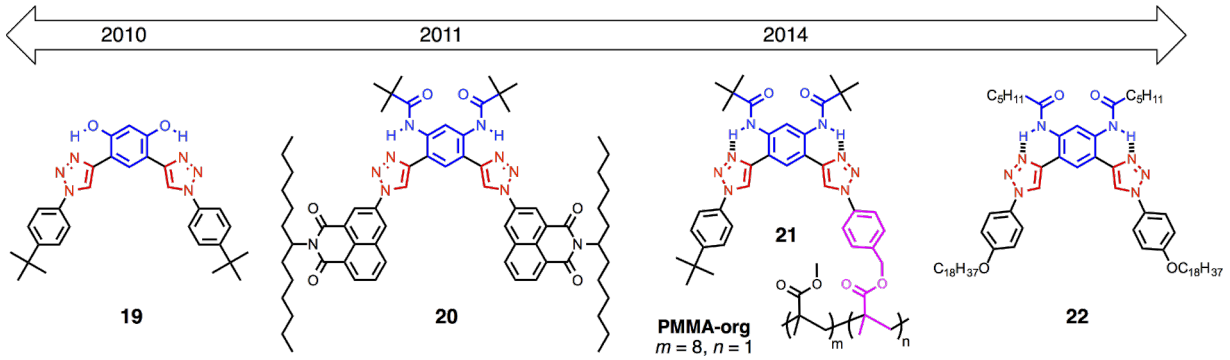

Figure 18: Timeline of crescent-shaped anion receptors.

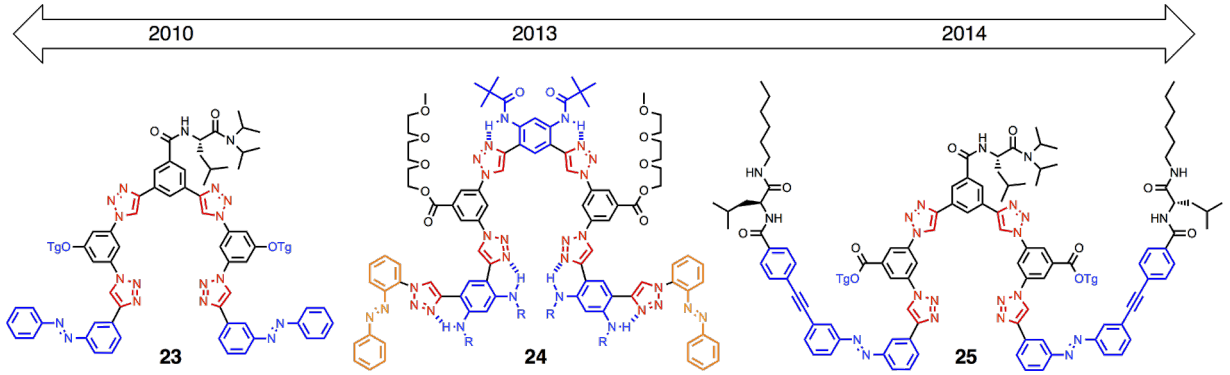

Figure 19: Timeline of anion-binding foldamers.

25 [47]. We also saw protein-like driving forces elicit unprecedented Cl^- binding in semi-aqueous solutions (50:50 water/acetonitrile). The aryl-triazole foldamer **24** formed a double helix with overall stability of $\beta_2 = 10^{12}$ M^{-2} [48]. Importantly, while we observed clear penalties to the binding affinity when we added extra water, we also saw that extra water increases the stability of the duplex. The analogy to hydrophobic collapse in proteins is clear.

Other passions, interests, and activities

Outside of research, my other passions are family, mountain biking, and visits to New Zealand and Japan. The family is young and the mountain biking is new, so finding the right balance with research is critical and rewarding.

My group and I are also enjoying 3D printing of molecules (Figure 20), an activity that Ognjen Miljanic and I recently described [49]. Having early access to a full-color 3D printer in Indiana University's School of Fine Arts helped my early adoption of holding and seeing molecules. Now we use it for "image training" (thanks to Makoto Fujita for the turn of phrase) as much as to show off the shape and function of molecules.



Figure 20: Family portrait of 3D-printed molecular receptors.

Future perspective

There are a few grand challenges inspiring us going forward. Underpinning each of them is a central philosophy: that the chemical nature of all matter motivates us to consider how we might design molecules to have an impact on the world of human experience. The anion recognition work has over-

arching implications across a broad swath of modern life, from human biology, to the environment, to industry. The opportunity for the use of molecules in separations and environmental remediation is of continuing importance to us. The ongoing effort to plumb the depths of anion recognition synergizes with this goal, and it also brings computer-aided receptor design into focus as a very real prospective for the future [32].

We have recently been inspired to program the surfaces of molecules to direct their hierarchical assembly into molecular materials. The discovery of tricarb's 3D assembly at interfaces [8] is igniting this work. Our inclination to collaborate with theorists also opens up the chance to design those hierarchical assemblies using computer-aided design. The long-term ramifications include the ability to create semiconductor structures by simple self-assembly. Thus, the goal is to contribute scientific understanding on how to program the nanostructures of advanced technology by employing bottom-up molecular design.

Acknowledgements

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From steroids to aqueous supramolecular chemistry: an autobiographical career review

Bruce C. Gibb

Review

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Address:
Department of Chemistry, Tulane University, New Orleans, LA 70118, USA

Email:
Bruce C. Gibb - bgibb@tulane.edu

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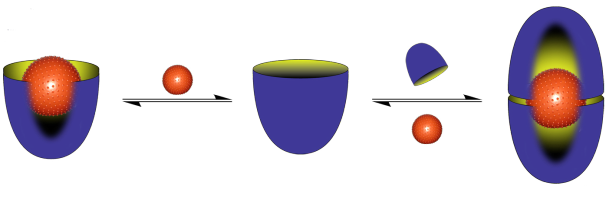
Abstract

The focus of my group's research is aqueous supramolecular chemistry; we try to understand how chemical entities interact with water and consequently how they interact with each other. This personal history recounts my career experiences that led to his involvement with this fascinating area of science.

Review

I recall a conversation with J. Fraser Stoddart some years ago that now seems pertinent to this review. He asked a simple question, "what did your parents do for a living?" For the record, my father and dear departed mother worked together in their own watch-makers and jewelers. My father built and repaired timepieces, whilst my mother worked on the jewelry side of things. In contrast, my interests center round something more mundane, but devilishly more complicated: water. More specifically, my research group is interested in the interactions between water and species dissolved in it. In short: aqueous supramolecular chemistry. We investigate supramolecular processes in water in order to identify new and interesting phenomenons, and to contribute to Science's understanding of the properties of aqueous solutions. As discussed below, the latter can be bifurcated into two general topics: the hydrophobic effect and the Hofmeister effect. However, specific molecular-level understandings of these phenomena are far from complete. We'll return to this in due course, but a summary of a large part of our research – the formation of 1:1 complexes and 2:1 capsular complexes in aqueous solution – is shown in Scheme 1 [1].

Why did Fraser Stoddart care about my parent's occupation? The premise of the question, as Fraser said after I responded, was that he thought that supramolecular chemists had an artistic, visual-spatial bent. That's certainly true in my case, when not delving into research my hobbies are photography and cooking; the former is very visual, the product of the latter is too, and both are artistic in their own right. Indeed, art is in my



Scheme 1: The formation of a 1:1 complex and a 2:1 supramolecular nano-capsule complex from bowl-shaped "cavitand" molecules.

family; my father didn't only enjoy the artistry of building and repairing watches and clocks, but he is also a keen watercolor artist. Moreover, my (elder) brother is a trained architect. It was my artistic side that initially drove me to consider going to university and studying architecture, but my brother beat me to designing buildings and urban landscapes. So a combination of my ego and my scientific persona drove me ultimately to the physical sciences; I attended Robert Gordon's Institute of Technology in Aberdeen (Scotland) to study a degree in Physical Sciences, and in my third year I opted to major in Chemistry. If I wasn't going to be designing buildings, I'd design molecules instead! The alternatives of physics or biochemistry also seemed like fun, but I was attracted to chemistry by the intrinsic beauty of organic chemistry. There is an ineffable artistry to a reaction scheme that, combined with the chess-like options of organic synthesis that have been built up over the centuries, can be as beautiful as it can be beguiling. And so, in my honors year it was time to work on a research project under the guidance of one of the graduate students in the department. Naturally, I opted for an organic project: The synthesis and reactivity of 7-amino-2-phenyl-6-azaindolizine (Scheme 2). Fortuitously, this project was to be guided "on the ground" by Derek McHattie, a third year Ph.D. student possessing a keen mind and a healthy disdain for conformity. As is often the case with undergraduate research, The Boss, in my case Dr. Martin Fraser, was "up at 35,000 feet" and was a lot less accessible than Derek. And so it was Derek, whom I'm fortunate enough to still consider a good friend, who really introduced me to organic chemistry research. Derek sent me to work on the first step, the unoptimized desulfurization of 2-thio-6-methyluracil using Raney-Nickel to form 6-methyl-4-hydroxypyrimidine (Scheme 2). It turned out I was a wiz at this organic synthesis lark (as Derek liked to say). I took the yield from 73% to quantitative by doing what organic chemists do, changing one parameter at a time whilst keeping an eye on the yield and the practicality of the reaction and workup. And so my first foray into organic synthesis was a success; I had gone mano-a-mano with Mother Nature and won. I was hooked! Of course, Nature was to put me in my place on plenty of other occasions; she truly does guard her secrets well. But I had the bug, and loved the never-ending competition. Those universal constants and physical laws are totally reliable and allow you to know your opponent to an acceptable (but far from complete!) degree. Picking a battle with Mother Nature is just a sheer delight.

Subsequently I went on to study my Ph.D. degree. I opted for the same university, but switched to the Pharmacy Department to work on the synthesis of steroids under the tutelage of Dr. Philip J. Cox and Dr. Steve M. MacManus. Phil, an X-ray crystallographer, was my director of studies and he needed new steroids synthesized and structurally characterized [2,3]. So he brought on-board Steve – a natural products chemist – to guide me through the synthesis of the different targets. The overall goal of my work was the development of new steroidal contraceptives, and the key structural theme of the different androstane targets was the presence of a cyclopropane moiety in the A-ring. Figure 1 shows two of my favorite molecules synthesized in my work.

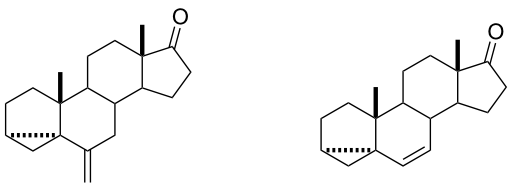
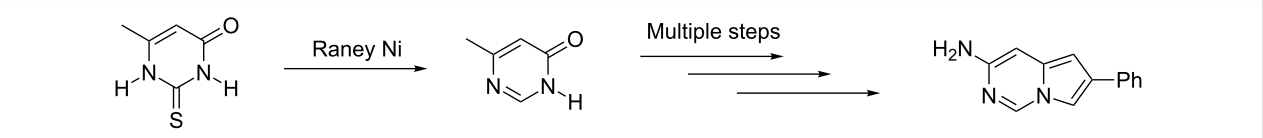


Figure 1: My two favorite compounds for my Ph.D. dissertation, "The Synthesis and Structural Examination of 3α,5-cyclo-5α-androstane Steroids".

What fascinated me about these molecules – and I now realize that this was a sign of my physical-organic chemistry leanings – was the spectroscopic difference between these molecules and how these compared to analogous steroids whereby the cyclopropane group in the A-ring is replaced with a conjugating C=C double bond. To cut a long story short, vinyl cyclopropanes are conjugated, albeit to a lesser degree than dienes, and NMR, IR and UV–vis analysis showed up some fascinating differences



Scheme 2: Abbreviated synthesis of 7-amino-2-phenyl-6-azaindolizine.

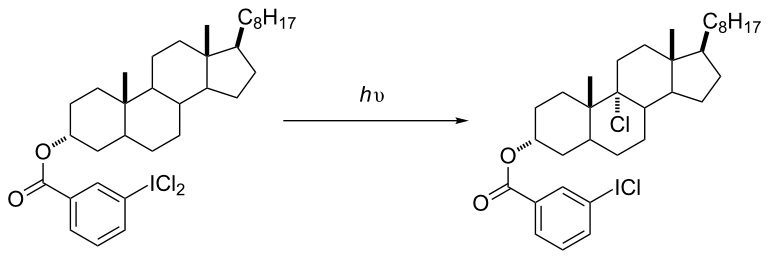
between these steroids that could be related to their structures derived from molecular mechanics.

This topic was not however the greatest, lasting memory from my Ph.D. studies. Neither was manually dredging the Chemical Abstracts for each literature search (if only the students of today could understand!). No, my greatest, lasting memory from my years as a Ph.D. student was an old paper I found from the labs of Ronald Breslow [4]. In particular, what caught my eye was the conversion of 3α-cholestanyl *m*-iodobenzoate dichloride to the corresponding 9α-chlorosteroid via internal hydrogen abstraction (Scheme 3).

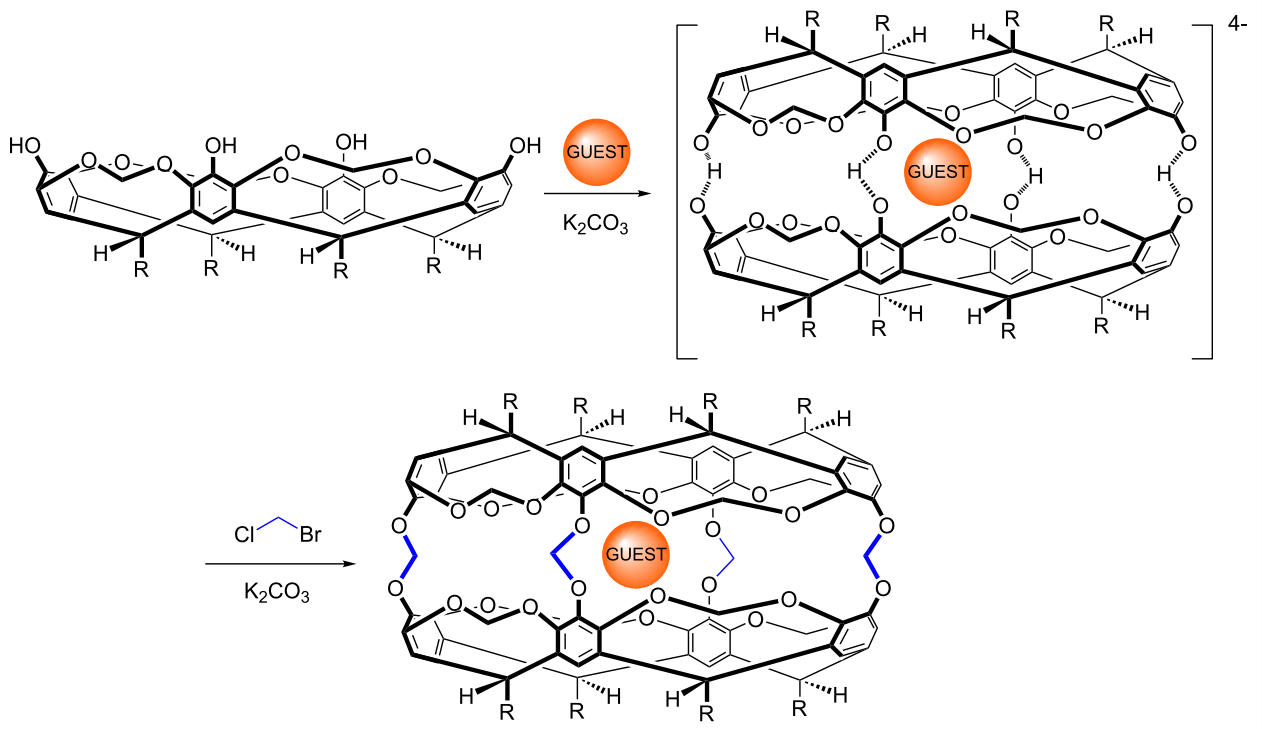
It was not that I was trying to make 9-halogeneated derivatives, or indeed do anything remotely analogous to what Breslow was accomplishing. It was simply that having worked with steroids for a couple of years I appreciated that they really are just a, "slab of hydrocarbon". Hence this example of selective, remote functionalization of the steroid framework was captivating. This captivation was itself pivotal to my career path, and it is a constant reminder to me (and hopefully young practitioners I've told this story to) how important it is to keep one's eye on the literature at all times. Not by having an unknown algorithm carry out an automated keyword search each week, but by actually sitting down with a cup of your favorite drink and perusing individual journal issues. The simple fact is that you can't catch important, interesting research peripheral to your current studies with SciFinder. Life is so much more than either your brain or an algorithm, and if you expose yourself to other chemistries going on around you might be very surprised what pops up.

In my case, the Breslow paper pointed me towards the cytochrome P450 family of enzymes, or more specifically, the subset of this class of enzymes that play an important role in steroidogenesis. How does the body synthesize all those steroid hormones? How does Nature carry out remote functionalization on these slabs of hydrocarbon? The answer of course involves a heme-cofactor and an exquisitely shaped hydrophobic pocket/active site. Ronald Breslow was asking similar questions and addressing it with cyclodextrin derivatives as enzyme mimics, but as I neared the end of my Ph.D. degree I confess I knew nothing of this facet of supramolecular chemistry.

In 1991, as lab work started to be replaced by thesis writing I found myself with two concerns: 1) was I going to regret pioneering thesis writing at my department using Wordstar 5.0? 2) What was I going to do next? On the first topic, I think it was ultimately a good idea not to write my thesis by hand and then get it typed up, but I will never forget all the (physical) cutting and pasting of printed Chemdraw 2.0 images into gaps in my thesis text; what a drag! As for the more important task of the next step in my career, I was not happy with the sort of job interviews I was getting in the UK, and as a result, I decided to upend everything and move out to Vancouver, British Columbia (BC). Now this was not entirely on a whim, 18 months earlier my ultimately to be wife, Corinne, had moved there to carry out polymer research at the University of British Columbia (UBC) with Donald Brooks, so I figured a few months exploring options in the west coast of Canada would be at least fun and potentially fruitful. Upon arriving I gathered up all the department brochures I could get my hands on (yes, there was no internet) and quickly discovered a whole range of potential labs to work in. There were many programs of research at UBC, Simon Fraser University, and the University of Victoria that appealed to me, but of all the people I talked to, the work of John Sherman at UBC appealed the most. John had carried out his Ph.D. with Don Cram at UCLA right at the (Nobel Prize winning) pinnacle of Don's career, and like so many people in the group at the time had worked with cavitands. In fact, John was – to cut a long story short – instrumental to Don's publications on the synthesis and properties of soluble carceplexes formed by the dimerization and covalent linking of tetrol-cavitands (Scheme 4). When I first arrived on the scene one of John's projects was to understand this templated reaction. I had discovered supramolecular chemistry! Alas, this very project was being driven by Bob Chapman; an outstanding chemist who's now a Natural Health Products Program Leader at the National Research Council in Canada [5-7].



Scheme 3: An inspiring chlorination from the group of Ronald Breslow.



Scheme 4: The carceplex reaction.

So the good news was that I had "discovered" molecular concavity and host–guest chemistry, research that I instantly related to the action of the Cytochrome P-450s and other enzymes. Unfortunately however, there was the small problem of financial support. From John's perspective I had literally appeared out of the blue, so as there was no possibility of support I simply volunteered to take a gratis appointment. So I got to work; sustained by Corinne's stipend that – thanks to my cooking skills – fed the two of us much more successfully than did Corinne's cooking for one (pasta with fresh air anyone?).

I had three related projects in John's labs: one was to see if noble gases such as xenon could template the above reaction (short answer, not in my hands); one was to synthesize new cavitands with functionality at their feet (the R groups in Scheme 4) [8]; and the third was to devise a way in which four α-helical peptides could be coupled to the rim of a cavitand scaffold to form mimics of four-helix bundles or caviteins (Figure 2) [9,10]. These latter two projects worked well, although I ended up moving on before the cavitein work could be brought to full fruition; it was more a case of me "clearing the brush" and setting things up for new graduate student Adam Meso to really start to bring out the best in the project [11]. But I'm jumping ahead of myself here. After a few months with John, and an unsuccessful RSC post-doc fellowship application to try and make my position more concrete, things improved considerably when John managed to find a way to support me.

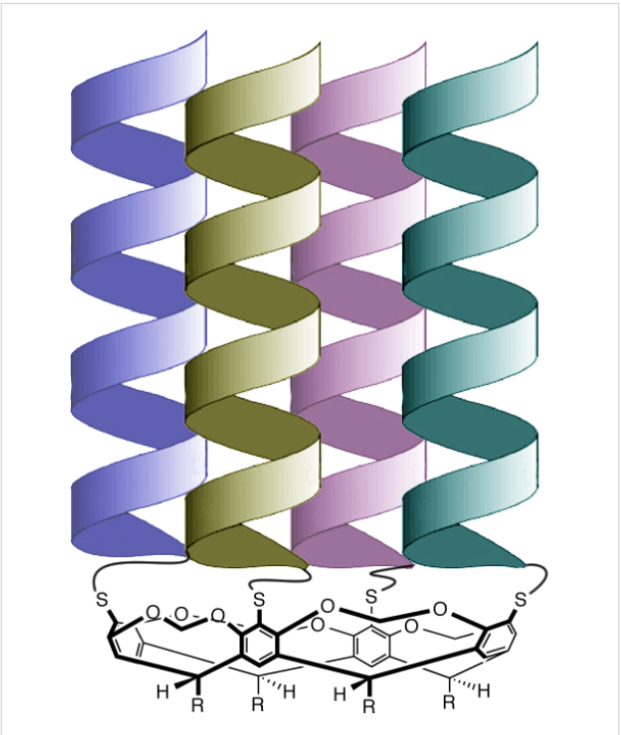


Figure 2: Schematic of a cavitein.

Now I was actually being paid to do what I wanted to do. I still don't know where that money came from, but it was pivotal. Thanks John!

I made lots of friends and memories in beautiful BC, but career wise I recall two moments that had the greatest effect on my career. The first was when another faculty member whom I had initially spoken with whilst looking for a position, turned up in John's lab to try to hire me away. I guess John had mentioned to his colleague that I was able to synthesize molecules and I had therefore suddenly become an attractive prospect. I turned my suitor down; he was a big-time player, but it was John who had shown me faith. That stated, the offer was a nice confidence-booster. Here I was at a department that was – at least in terms of number of researchers – literally fifty times larger than where I had completed my Ph.D.; but I was apparently still quite good at this "synthesis lark." That calibration help me no end. The second BC-moment was a career chat with John. It was a simple conversation about what I was to do next, but it suddenly made me realize the obvious: that there was the option of staying in North America to continue my career. And so that is what I opted for; in large part because the prospect was so exciting, but also because having completed my studies in Scotland at a small school I felt a career in the UK would be hampered by the country's predilection to put too much stock in pedigree. Looking into my options, John and I narrowed things down to either a post-doc with Donald Cram at UCLA, or a position at NYU with James Canary. The possibility of working with Don was provocative, but this was right at the end of his career and in all likelihood I would probably end up making carceplexes, and after working with John I wanted something new. And so I opted to swap the wilds of BC for the wilds of New York City. After a wonderfully exciting road trip across the continent, Corinne, and I arrived in Manhattan on a beautiful Sunday evening in October. The images of Bleecker Street and Greenwich Village life that evening will always be with me.

Living in Manhattan and working with Jim proved to be a real eye-opener. What fun! Jim and I turned out to be almost exact opposites; in a good way. My project was to synthesize some tris(2-pyridylmethyl)amine (TPA)-based ligands and study their zinc complexes (Figure 3) as mimics of the enzyme Carbonic Anhydrase. The ligands for these complexes are notoriously difficult to work with chromatographically, but I managed to synthesize a few derivatives that not only replicated the zinc binding site, but also some of the functionality that 'dangles' above the zinc-bound water in the crystal structure of the enzyme. In addition to synthesis, the project also involved a good deal of potentiometry, and this began to teach me that synthesizing enzyme mimics was a difficult challenge of balancing the need to create functionality for the active site, whilst maintaining suitable water solubility. Whilst I was busy doing all of this with Jim, Corinne was working in the labs of Neville Kallenbach, then Chair of the Chemistry Department. Corinne and Neville got on like a house on fire, and so I got to know Neville very well.

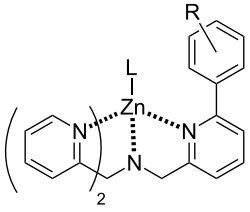


Figure 3: General structure of zinc-TPA complexes.

Jim also had a big influence on my career, and I can recall two key moments where his wisdom helped point me in the right direction. The first came as we wrote a review on metallo-enzyme mimics [12]. Near the end of the whole process I mentioned to Jim that it was kind of disappointing that when synthesizing an enzyme mimic nobody had really, truly integrated a hydrophobic pocket with the catalytic machinery required for whichever catalytic conversion was targeted. People such as Breslow had coupled these two facets of a shape-selective catalyst together, but no one had integrated these two aspects of an enzyme to the level Nature does. Arch critic that I am, I turned on my own research; "how can we ever expect to get the zinc-bound water in our complexes to be the strong acid it is in Carbonic Anhydrase if we're not fully taking into account context?" Jim gave me his bemused/slightly-exasperated/are you kidding me kind of look – the one that can put anybody in their place – and said, "why don't you go off and synthesize your own synthetic hydrophobic pocket and then you can add all the catalytic machinery you want." Twenty years later, all I can say is that I'm still trying. Stay tuned Jim!

The other key conversation I had with Jim was very much career-related. I was leaning towards an academic job, but what sealed it for me was when Jim said words to the effect of, "Do you want to be a cog in a wheel, or do you want to be your own boss and take your own independent thoughts and turn them into something?" That kindled the independent streak within, and I thought of my mother and father and all my ancestors that had had their own businesses. Why not do the same in an academic setting and see what comes forth?

And so I applied for every academic job that came up that year: 54 there were, 2 in Canada, 2 in the UK, and 50 in the US. Considering my pedigree, some of the institutions were quite frankly out of my depth. In fact, to be honest, all of them arguably were. I just didn't have the CV bulging with big important papers backed up by heavy-hitters in the field. Still, you've got to try don't you? To cut a long story short, after many late nights of writing three proposals – with the invalu-

able help of Corinne and our good friend Niels Van der Veen running around the library digging up papers – everything cerebral was ready. Then I turned to our neighbor Julie Kaplan – the events and outreach guru at NYU Chemistry – to give myself and my stack of applications that certain *je ne sais quoi*. After spending all I could on everything from a respectable outfit for me to posh paper for my CV, I was ready.

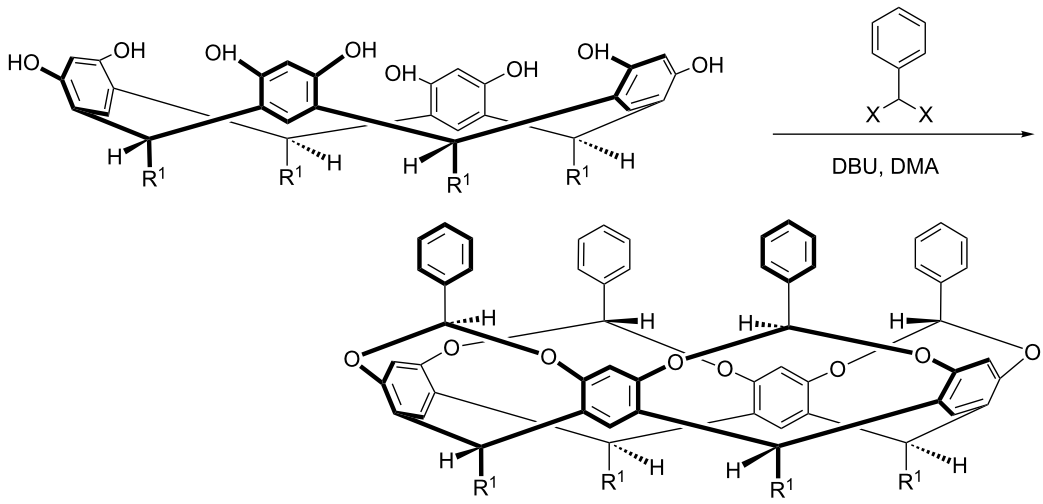
There was then that quiet period between sticking dozens of applications in the mailbox and the first rejections; the one where your boss is happy because you have your focus back. By the time everything was done and dusted I had one university in the US wanting to take me down for an interview, and an offer of a post-doctoral position with David Reinhoudt in the Netherlands (plan B). Knowing now how departments in the US carry out faculty searches, I now appreciate that I had struck gold. I never saw the letters that John, Jim and Neville wrote, but they must have said some nice things. And so I went through the interview process at the University of New Orleans and did well enough to get an offer. I had my foot in the door! In a life-changing, bifurcated fork moment, I declined the post-doc in Europe with David, and Corrine and myself said farewell to New Your City and drove south. You don't need a map to find New Orleans; you just follow the steep gradient in relative humidity until it reaches a maximum.

Like New Orleans itself, UNO Chemistry was a bit of a conundrum. Here was a relatively small university, recognized more locally for being a commuter campus than known nationally as a hive of research. But the Chemistry Department was an aberration on campus. It had a full complement of young, enthusiastic faculty, was receiving more federal research dollars than it had at any time in its history, and was publishing more high impact publications than ever before. Moreover, with 18–20 faculty and 60–70 graduate students there was a small but thriving research community; and one that was anticipating a move into a brand-new building.

We started setting up the lab in August 1996, the new building was not going to be ready for another 10 months, so the space we were given was, how to put this, an afterthought at the end of a long tiring meeting that occurred sometime before I arrived. Still, you could do chemistry in the space, and that's exactly what we set about doing. I say we, because we'd arrived in New Orleans without securing a position for Corinne, and so as a prelude to seeking gainful employment she decided to help out setting up the lab and initiating some of the chemistry. And so before a student could join at the end of the semester, Corinne and myself had a small academic enterprise purring away in little more than a small, sweaty cupboard. It turned out that Corinne and myself worked very well together, and so slightly by accident this turned into a permanent working relationship. Corinne is the yin to my yang (and vice-versa), and our working relationship has taught me many things, including that the culture in the biosciences of having a permanent technician/lab manager(ess), as part of a research group is an excellent idea. Having a permanent pair of feet on the ground whilst you hover over the lab at 35,000 feet is good for everyone. Moreover, a permanent manageress helps ensure that the on-the-ground lab culture and history is maintained; an especially important point if you only have two or three graduate students in the group. I don't think we'd have it any other way now, but since it is unusual in the US chemistry community to have a lab manageress – especially one your married to – it is a constant struggle to find funding.

Of the three proposals I'd written we decided to initially work on two: what was nicknamed the "chiral canyons project" and another project on the synthesis of deep-cavity cavitands. The first was inspired by Murray Goodman's work [13] on the covalent templation of small, stable collagen-like triple-helices, and involved positioning a metal-ion coordinating template into a collagen structure such that the "active site" was situated in one of the chiral grooves of the triple-helix. In contrast, the second project on cavitands was focused on making a wholly synthetic hydrophobic pocket rather than one in a proteinaceous hybrid. We needed a peptide synthesizer for the canyons project and a round bottom flask for the cavitand work, and as we had to buy the former via a long, drawn-out purchasing process, and the latter could be picked up from the department store, the cavitand work was the first thing we did. The first new reaction carried out in our chemistry cupboard was to deepen the cavity of resorcinarenes (Scheme 5).

To attempt this stereoselective "bridging" of resorcinarenes we ordered both benzal chloride and benzal bromide; because both were in the Aldrich catalogue, and because, as I'm fond of telling students, you should never assume you know what Nature will do and should always maximize your chances when investigating a reaction. Anyway, both benzal halides were cheap (e.g., $35.15 for 25 g of benzal bromide; as the first compound we ordered I still have the (framed) order card on my wall). The idea was to quickly deepen the resorcinarene bowl, but the reaction involved making eight C–O bonds and creating four stereogenic centers in the shown, phenyl ring "up" configuration. If one ring pointed "in", then the cavity was no more. I had my doubters with this reaction, and it failed miserably with benzal chloride (we fished out <5% of the desired cavitand). However, if short R groups such as methyl were avoided and benzal bromide was used instead, yields were near 60% in selected polar-aprotic solvents, i.e., ~93% yield per bond and ~88% diastereoselectivity [14-16]. Unfortunately a reviewer at



Scheme 5: Stereoselective bridging of a resorcinarene with benzal halides.

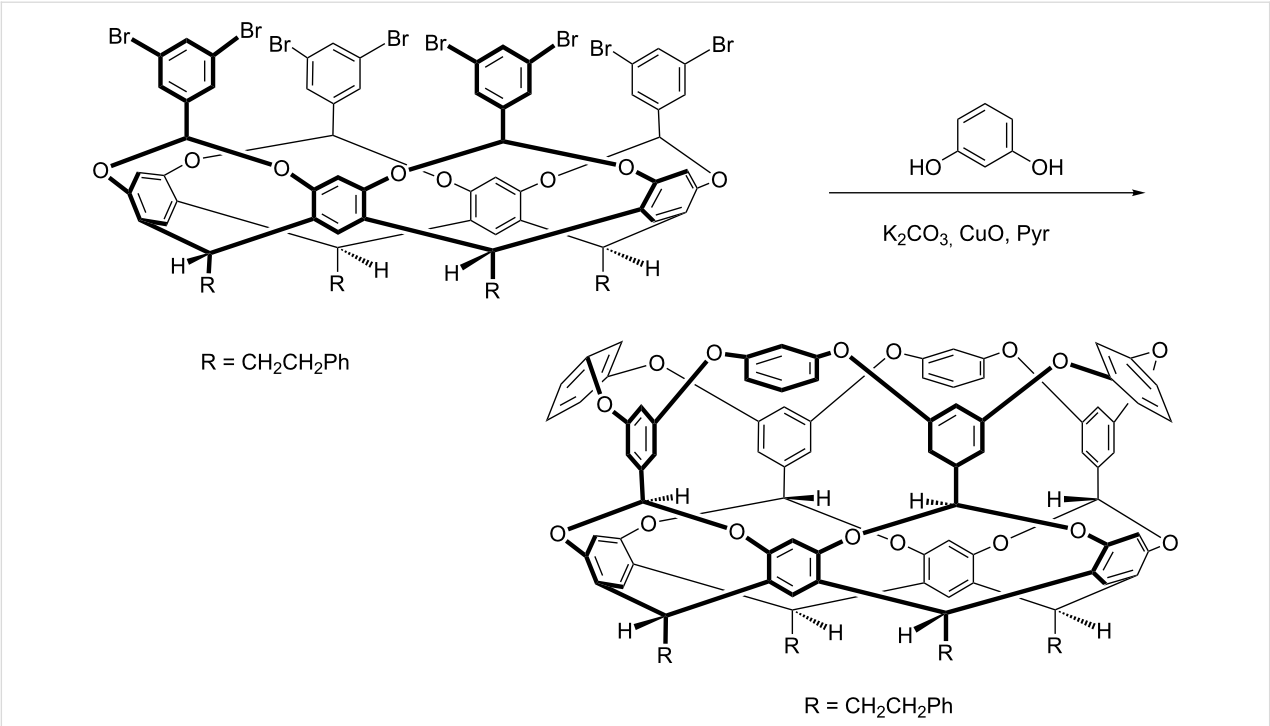
the journal that we first submitted our manuscript to was also a doubter, even after we sent all the spectra, crystal structure information, and whatever other piece of data we thought might be useful to back up the text and supporting information. Alas, it was to no avail and the journal rejected the manuscript. It turned out that no information would have been sufficient, as the reviewer confided many years later, they just didn't believe our yields. I guess that was kind of a compliment.

As well as being our first successful reaction in the lab, this benzal bridging was also a gateway to speaking at my first international meeting. I had no idea at the time, but one of the organizers of the meeting had unsuccessfully tried the same reaction a year or two earlier… using unfortunately benzal chloride. They never tried using the bromide, so when they saw my abstract they were naturally intrigued, and before long, I was invited to give a flash presentation at the meeting.

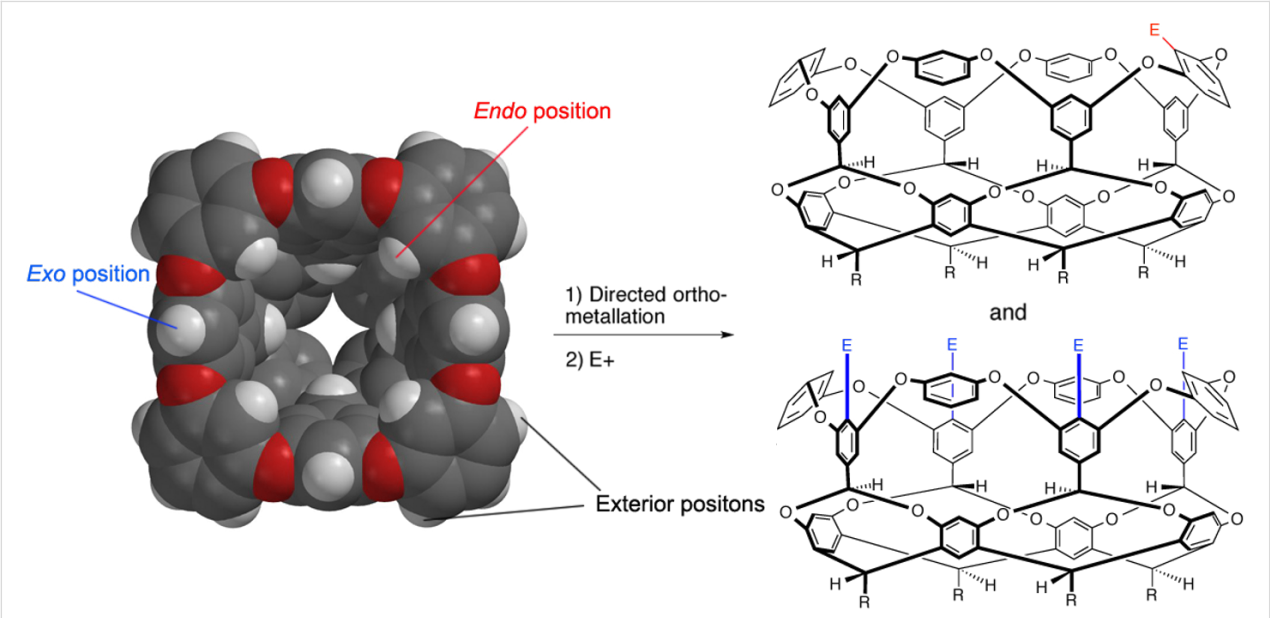
As we anticipated, the benzal-bridged cavitand shown in Scheme 5 was not a particularly good host; the free rotation of the aromatic rings ensures a poorly defined cavity. Moreover, although we could insert a range of functional groups into this second row of aromatic rims simply by using substituted benzal bromides [15,16], generally these cavitands were very acid sensitive. And so, using whatever means possible to avoid stray protons, we set out to rigidify the structure of the cavity. Many procedures were conceived, but the one most unlikely to succeed on paper – an eight-fold Ullmann ether reaction – was the one that ultimately worked best. Thus, treatment of the shown octa-bromide (Scheme 6) with resorcinol led to the insertion of a third row of aromatic rings and the corresponding deep-cavity cavitand [17,18]. This "weaving process" can be carried out with many resorcinol derivatives, but the necessity of using variations on the classical Ullman ether reaction (none of the milder modern-day variations have worked in our hands) does limit the product range somewhat.

These highly preorganized deep-cavity cavitands proved to be much better hosts, and the biggest determinant for guest binding beyond size compatibility with the pocket is the crown of four inward-pointing benzal C–H groups near the base of the pocket. Linked to two oxygen atoms, each C–H group is relatively electron deficient and therefore – at least on the scale of C–H hydrogen bonding – a reasonable donor. Synergistically however, four groups can readily control guest selection and orientation within the pocket. For example guests with halogen atoms bind relatively strongly by placing their halogen atom down into this crown [17,18]. Moreover, functional groups introduced into the entrance/rim of the host during the weaving step can also force guests to adopt specific orientations [19-21]. Beyond these structural features, the biggest determinant in guest binding was the solvent. Solvents such as DMSO gave the strongest complexations, whilst halogenated solvents gave the weakest. Apparently the former does not solvate the cavity well.

One of the remarkable features of these cavitands is that although structurally quite complex, because of their well-defined structure they can be readily functionalized [22,23]. For example, directed *ortho*-metallation and quenching with electrophiles could at first blush theoretically lead to a plethora of products because there are sixteen aromatic carbons (labeled *endo*-, *exo*- and *exterior*- in Scheme 7) that are doubly activated by O-substituents around the rim of the pocket. It tran-



Scheme 6: An eight-fold Ullman ether "weaving" reaction.



Scheme 7: Directed *ortho*-metallation of the deep-cavity cavitands, showing the mono-*endo* substituted to tetra-*exo* substituted products.

spires that lithiation does not occur at the exterior positions of these hosts, so there are only (!) eight positions where reaction occurs. However, by control of stoichiometry and the nature of the organo-lithium only a grand total of ten different products of the possible sixty-nine can be formed. Hence by careful control of the conditions, compounds ranging from the mono-*endo* substituted to tetra-*exo*-substituted (Scheme 7) can be isolated in useful yields. The highly defined nature of the intermediate lithiate anions is important to the control of these reactions. Thus, much wider ranges of products are observed if direct electrophilic substitution is used as an alternative strategy of host functionalization [24].

As well as proving to be useful hosts, these deep-cavity cavitands also demonstrated that resorcinarenes could be used as covalent templates to form macrocycles. For example, the deep-cavity cavitand shown in Scheme 8 can be treated with excess BBr_3 to cleave the four, benzal-bridges and liberate a new family of aromatic macrocycle [25]. Interestingly, as these hosts are tetra-acetals we initially tried removing the template under acidic conditions. However, even reflux in 1:1 $EtOH/H_2SO_4$ for a week failed to affect the starting material. Evidently, under reversible conditions, a broken acetal can easily reform before the other three are themselves cleaved. An irreversible acetal cleavage mechanism is therefore key to success.

Meanwhile, because our chiral canyons project required some new, tripodal zinc-binding ligands for templating a collagen helix, the whole endeavor had itself become very syntheses oriented. We designed two general families of tris-pyridyl hosts to bind zinc ions within our proposed chiral canyons (Figure 4) [26-28]. The shown macrocycle turned out not to be a strong binder of zinc, but did demonstrate enantioselective binding of chiral amino acids that was dependent on the nature of the counter-anion [27,28]. On the other hand the shown tris-pyridylmethane ligand did bind zinc quite well, with its propensity to form the desired 1:1 host–zinc complex rather than a potentiometrically inert 2:1 complex controlled by the nature of the pyridyl substituents [26]. However, with the increasing momentum of the cavitand project it was proving harder to get students to work on this project, and with only a small group possible at UNO, taking these tris-pyridylmethane ligands into chiral canyons never materialized.

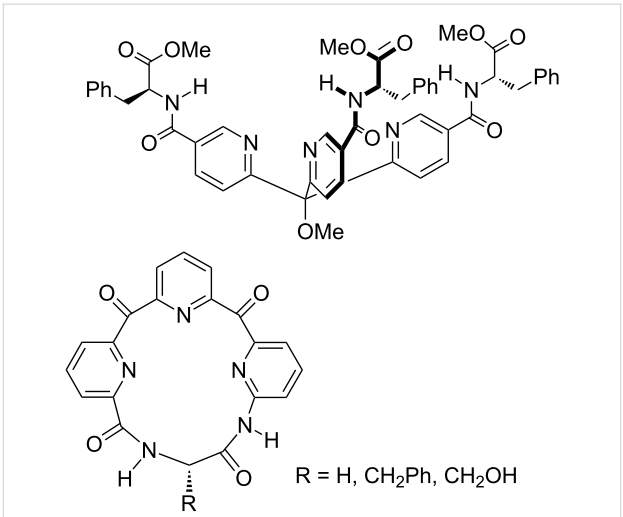
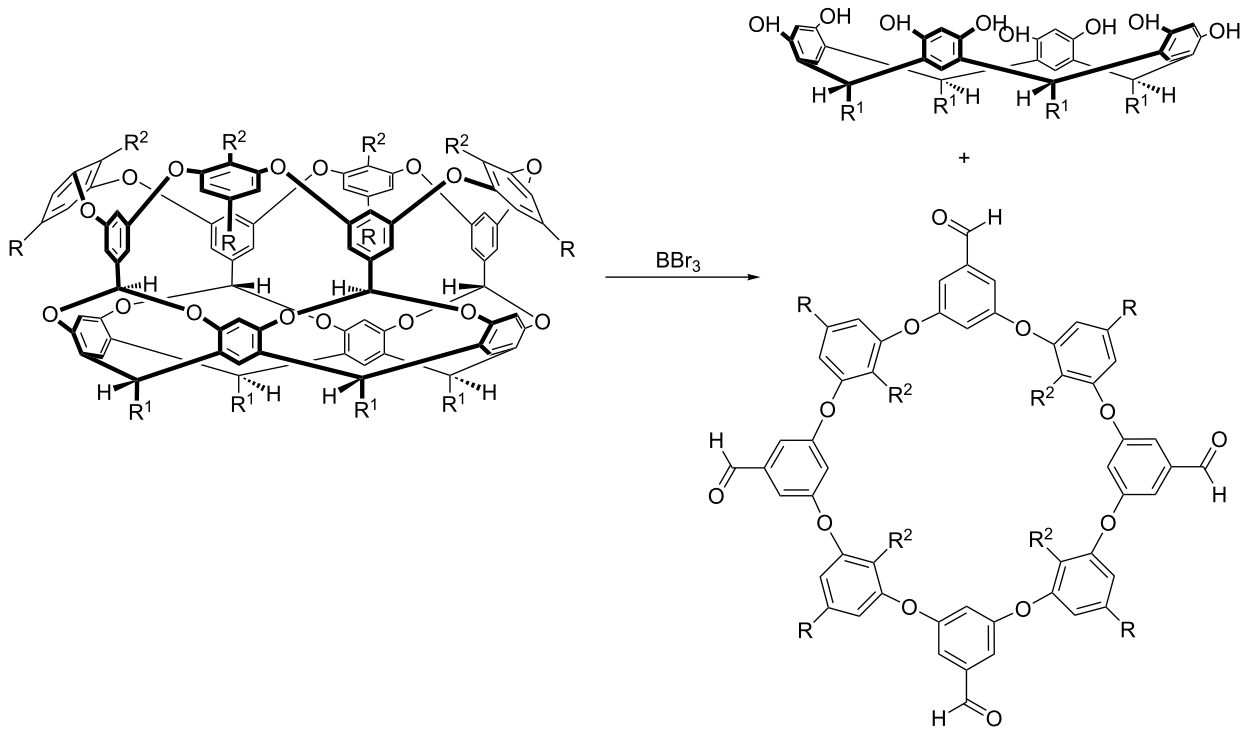


Figure 4: Tris-pyridyl hosts.

There was however a serious problem with our cavitand work: we were working in organic solvents, not water. Throughout my career, water had been just under the surface of all my research



Scheme 8: Macrocycle synthesis via resorcinarene covalent templates.

endeavors: I had been working towards water-soluble cavitands and making four-helix bundles that assembled via the hydrophobic effect at UBC, I had been working with water-soluble zinc complexes at NYU; even non-water-soluble steroids rely in part on the hydrophobic effect to bind. The common denominator was water, "the solvent of life". However, although we understand water quite well, we are far from fully understanding aqueous solutions [29,30], and to be frank, it's hard to contribute to this understanding if you are working in chloroform. Something had to be done.

We examined several approaches to making deep-cavity cavitands water-soluble, but the one that worked best led to what has been commonly called octa-acid (Figure 5) [31,32]. Now we had a host that: 1) was evenly "coated" with water-solubilizing carboxylic acids/carboxylates to bestow good water solubility under basic conditions, and; 2) possessed a rim of hydrophobicity around the entrance to its interior hydrophobic pocket. We envisioned that driven by the hydrophobic effect this host would readily form 1:1 complexes in water. More importantly, because the pocket and rim of the host would be poorly solvated, in the presence of an apolar guest we envisioned the host would dimerize around it. In other words, we hoped that the hydrophobic effect, would drive capsule assembly. To our knowledge this represented an entirely different approach to the assembly of container molecules; up until that point strategies such as hydrogen bonding or metal coordination were the focus of attention.

Our first investigations with octa-acid looked at assembly around steroids [31]. There was obviously a personal reason for this, but more importantly we wanted guests that were very hydrophobic and rigid. Our concern was – and this proved to be completely unfounded – that two octa-acids in a capsule would slip and slide all over each other. In other words, with a lack of what are traditionally viewed as functional groups that can direct assemblies, the structural definition and kinetic stability of 2:1 host–guest complexes might be low. We therefore wanted to introduce a bias by using very rigid guests. We were therefore grateful to discover that a range of steroids, from as small as estradiol to as large as cholesterol formed exceedingly stable capsular complexes (Figure 6).

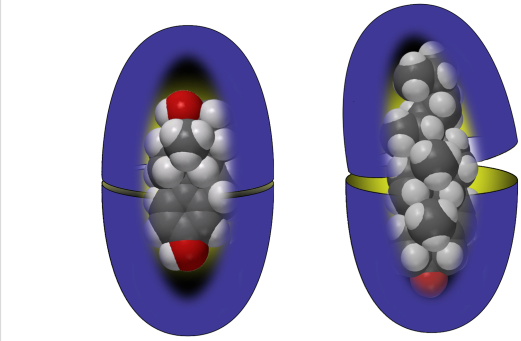


Figure 6: Cartoons of the 2:1 host–guest complexes of estradiol (left) and cholesterol (right).

Regarding their stability, even at 5 μM, NMR showed complete encapsulation of estradiol; giving a minimum apparent binding constant for the empty capsule (the host is actually monomeric in the absence of guests) of 1×10^8 M^{-1}. When the steroid was very large, e.g., cholesterol, NMR showed that the capsular complex was near its carrying capacity, but the complex was still stable on the NMR timescale.

It transpires that any molecule that would energetically rather not be in water and is small enough to fit, forms a capsular complex with octa-acid. There are two caveats to this statement. First, very small guests such as methane form 1:1 complexes; the cut-off for simple alkanes is between ethane and propane; the former forms a 1:1 complex whilst the latter forms a kinetically stable 2:2 capsular complex. Second, if guest binding generates a rather hydrophilic portal region, then dimerization of the host will not occur. For example the binding of charged

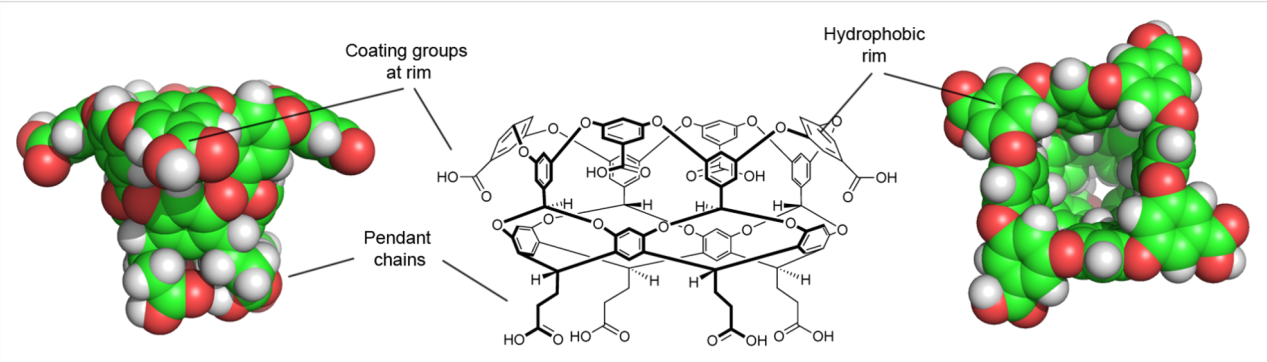


Figure 5: (Center) Chemical structure of the octa-acid host. (Left and right) Respective space-filling representations of octa-acid showing side and plan views.

amphiphiles such as *n*-octanoate lead to 1:1 complexes [33,34]. With these two points noted, a wide range of guests trigger dimerization to form the capsule with ostensibly sixteen negative charges on its exterior and a dry, water-free nano-space for harboring a guest or guests. The range of guests that form 2:1(2) host–guest complexes is illustrated by the examples in Figure 7. As expected, if a co-solvent is added then these complexes are broken down. Thus, screening of eight different co-solvents for their ability to break capsular complexes revealed that the most powerful solvent for doing so is acetonitrile, whilst solvents such as DMSO and MeOH require three times as much to do likewise [35].

Water solubility can be bestowed in many ways, and although eight negatively charged carboxylates have proven to be one of the most reliable approaches to date, it is not the only approach. For example, working with Scott Grayson across town at Tulane University we successfully formed the dendrimer–coated host shown in Figure 8 [36], as well as wide series of polymer coated derivatives coupled using "Click" chemistry [37].

Forming stable complexes for a broad range of guests, the capsules formed by octa-acid are ideal nano-scale (yocto-liter) reaction vessels. Our first foray into this topic relied on collaboration with Vaidhyanathan Ramamurthy, and examined a wide range of photophysics, and photochemical conversions [38-41]. One particularly enjoyable example of a reaction in the capsule formed by octa-acid came through collaboration between our group, Ramamurthy's, and the group of the late Nicholas Turro (Figure 9) [42,43]. If a solution of a capsular complex of the sensitizer dimethylbenzil is irradiated, the encapsulated sensitizer is excited and can transfer its energy to an oxygen molecule adventitiously entering the capsule. (Although guest exchange is slow on the NMR timescale, there is a much faster dynamic breathing of the capsule that allows small molecule entry and egress [44].) The resulting singlet oxygen can escape the capsule, and if there is a second complex containing a substrate then the $^1O_2$ can enter that cavity and react with the encapsulated substrate. In these experiments the substrate was a 1-methylcycloalkene, two copies of which bind to the capsule in specific, "methyl down" orientations. The first step of the reaction between singlet oxygen and the alkene is allylic hydrogen atom abstraction, and although there are three possible H-atoms for reaction, because of the specific guest-binding motif only an H-atom at the 3-position is removed. The result is therefore a 90% yield of the 1-peroxide rather than the 2- or 5-peroxide. In essence therefore the octa-acid not only facilitates reaction by dissolution in aqueous solution, it also engenders highly selective photochemical conversion via the transfer of chemical information – in the form of $^1O_2$ – from one capsule to another.

A second interesting example of a reaction in the capsule formed by octa-acid illustrates the effect of guest packing upon reactivity [45]. Eight *n*-alkyl dibenzyl ketones (R = CH_3 through *n*-C_8H_{17}) were found to form 2:1 capsular complexes capable of undergoing Norrish-type I and/or type II photoreactions. NMR analysis revealed that, depending on the size of the R group, the guests adopted one of three principle packing motifs (Figure 10a). In essence, the host acts as an external template to promote the formation of distinct guest conformations. As a result of their packing motif, the methyl, ethyl, and *n*-propyl dibenzyl ketones yielded large amounts of the decarbonylated product as well as sizable amounts of a rearranged product (Figure 10b). In contrast, the different packing motif of the *n*-butyl and *n*-pentyl guests yielded equimolar amounts of two rearrangement products. Finally, in further contrast the *n*-hexyl, *n*-heptyl, and *n*-octyl ketones yielded large amounts of Norrish-type II products. These results highlight the difference between macro-scale and nano-scale reactors: within yocto-liter

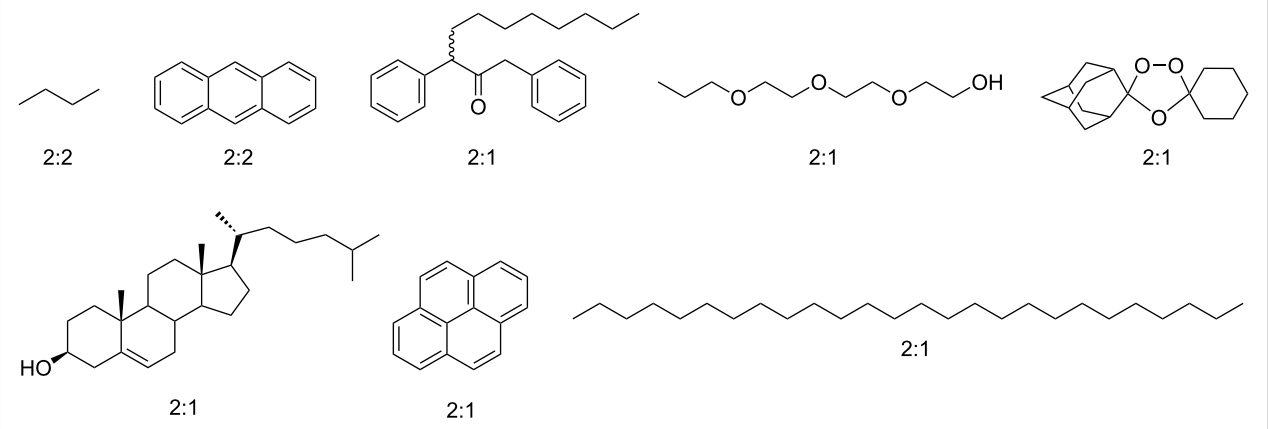


Figure 7: Representative guests for the capsular complexes formed by octa-acid (stoichiometry shown in parenthesis).

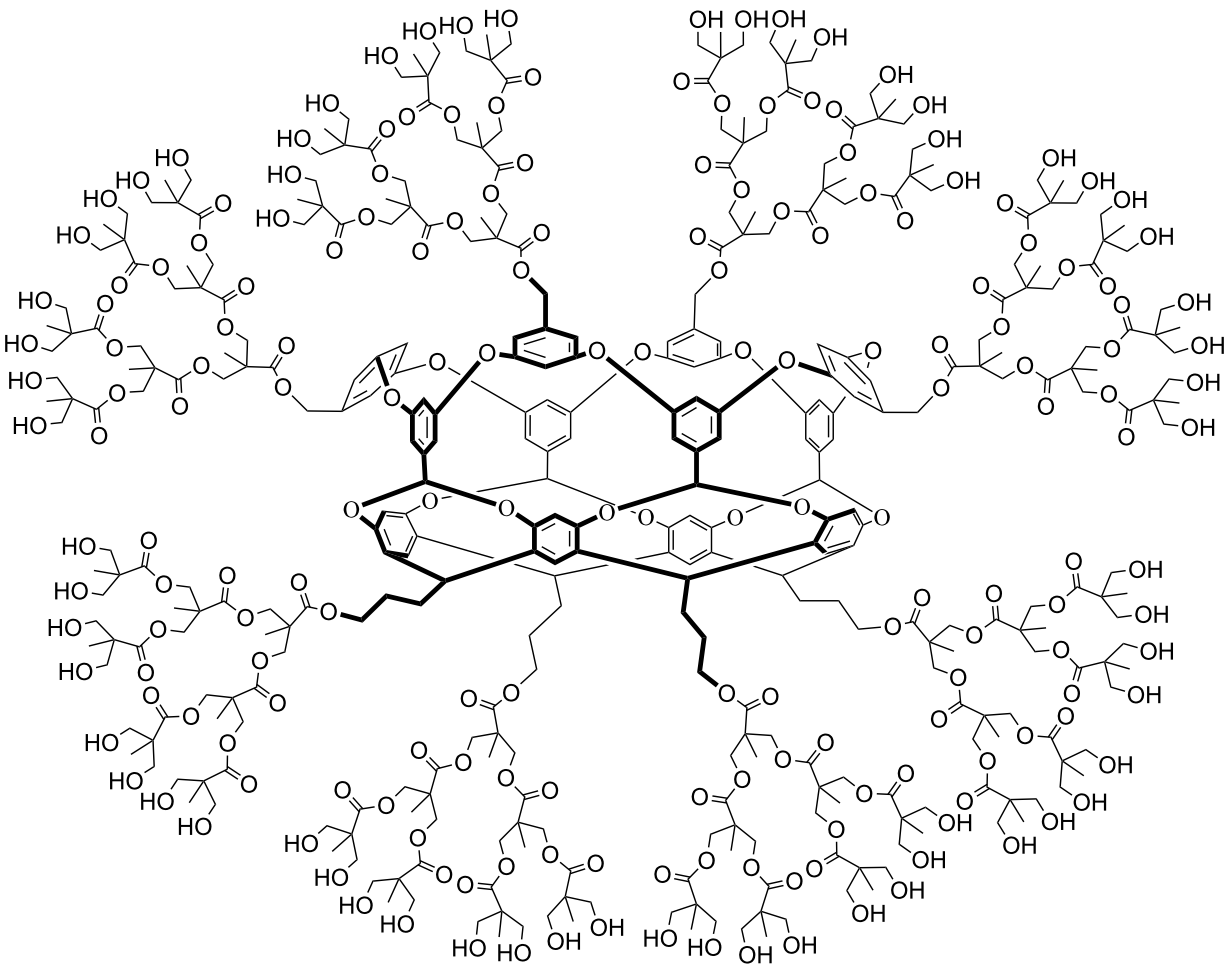


Figure 8: A dendrimer-coated cavitand.

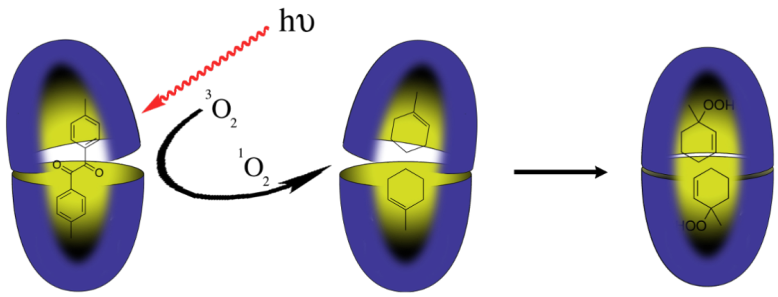


Figure 9: Selective oxidation of olefins by singlet oxygen.

vessels there are multiple opportunities for intimate contact between substrate and nano-reactor. The result is exceptionally high cage effects in which the two radicals that form within the capsule can only react with themselves. Moreover, these intimate contacts open up entirely new product landscapes by engendering rearrangement reactions not seen in solution.

The flip-side of capsules as yocto-liter reaction flasks are capsules as a means of molecular protection, and whilst the former has continued to gain prominence, the latter was (and still is) poorly developed. To explore this idea we carried out the kinetic resolution of pairs of constitutionally isomeric esters in the presence of octa-acid [46]. The concept is straightfor-

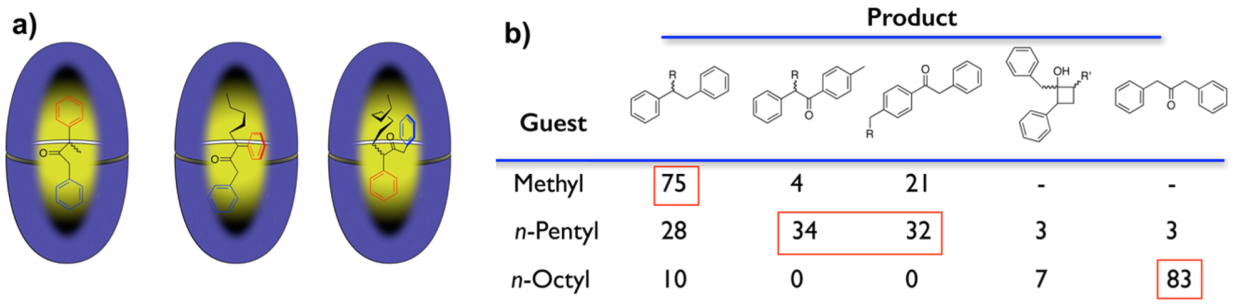


Figure 10: a) Preferred packing motifs of methyl, pentyl and octyl guests. b) Product distribution observed for the complexes of the same three guests.

ward. The chosen esters (Figure 11) are so similar that it is difficult to separate them chromatographically, and in basic solution they cannot be kinetically resolved because they undergo hydrolysis to the corresponding inseparable acids at approximately the same rate. How would the capsule formed by octa-acid affect this situation? To keep things manageable we focused on pairs of esters. In the presence of octa-acid one of the esters preferentially binds to the capsule and is kept "safe" in the confines of the container, whilst the weaker binding competitor is primarily left in solution and undergoes hydrolysis. Ideally therefore, none of the stored ester undergoes hydrolysis, before all of the second reacts. We discovered that the esters all formed kinetically stable 2:1 host–guest complexes and bound with the following relative affinity to the host: methyl > *n*-pentyl > *n*-butyl > ethyl > *n*-propyl. Furthermore, each of these complexes was exceptionally stable at room temperature (<5% reaction after 1 month). However, at 100 °C the hydrolysis rates were reasonable, and when pairs of esters were pitted in competition the kinetic resolutions shown in Figure 11 (table) were obtained. To account for this data we built a Michaelis–Menten model that showed how the kinetics of hydrolysis in the presence of octa-acid is dependent on both the equilibria between the free and bound species and the relative rate with which the free esters undergo reaction.

The capsule formed by octa-acid is also capable of bringing about physical separations. One facile way to examine this is to consider the absorption of gases from the gas phase into a solution phase. We therefore examined the uptake of butane into an aqueous solution of octa-acid to form the corresponding 2:2 host–guest complex. In doing so we determined that it was possible to form methane solutions that were 200 times higher in concentration than the maximal solubility of the gas in water. The concentration of butane within the capsule itself was esti-

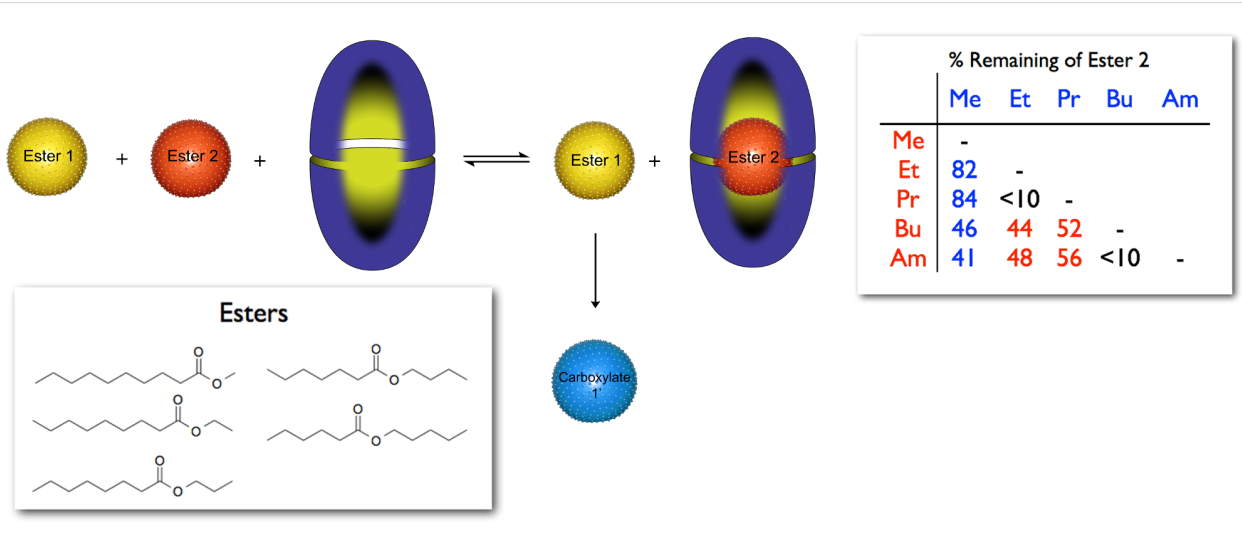


Figure 11: Schematic of the competition of two esters for the capsule formed by octa-acid. The ester that binds weakest to the host (arbitrarily Ester 1) remains in solution and is hydrolyzed to the corresponding carboxylate. The table shows the percentage of Ester 2 remaining after all of Ester 1 has been hydrolyzed. The color of the font of listed values indicates which ester of the pair predominated (red listed vertically, blue horizontally).

mated to be ~0.5 M, i.e., akin to its supercritical state. If (for fun) this guest is treated as an ideal gas, then the pressure within the capsule can be calculated to be roughly 100 atmospheres. Other hydrocarbon gases could be encapsulated. For example, methane and ethane formed 1:1 host–guest complexes, and propane formed a 2:2 complex. Interestingly, the extra methylene group of butane meant that it bound to the host twelve times more strongly than propane, and this allowed selective butane complex formation from a 1:1 propane–butane mixture (Figure 12). In short, aqueous solutions can be used to separate gases; to paraphrase Cram, the synthesis of the host prepays the energy requirements for gas separation.

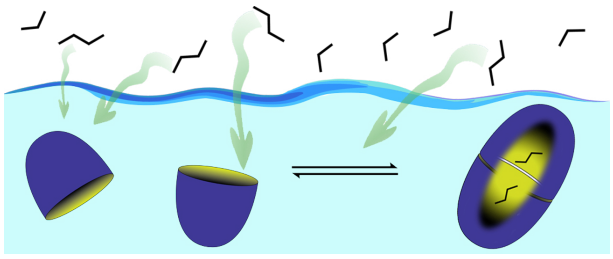


Figure 12: Schematic of the inter-phase separation of propane and butane; the latter binds more strongly to the capsule formed by octa-acid and so is selectively sequestered into aqueous solution.

As discussed, the precise binding motif of an encapsulated guest – how it packs within the inner space of the capsule – is a significant factor in determining how guests undergo reaction. More generally, how a guest packs will dictate its overall affinity for the capsule and hence will affect any application that these types of complex are applied to. One straightforward approach to examining how flexible guests pack within these capsules is to consider the *n*-alkanes, the homologous series of which is available in pure form all the way up to $C_{50}H_{102}$. In considering this we have found that for the octa-acid dimer the boundary between two or one guest encapsulation is C_8H_{18} [47], but that guests longer than $C_{12}H_{26}$ must compress in some manner in order to be accommodated. There are three principle ways that such large guests undergo compression [48]. For alkanes such as $C_{14}H_{30}$ the guest adopts a helical form; one that is of high energy in solution because of the gauche interactions down the length of the chain, but within the capsule maximizes non-covalent contacts between host and guest. With larger species such as $C_{18}H_{38}$ the guest must adopt a J- or U-shape within the cavity; a motif that is sometimes seen when fatty acids bind to fatty-acid transport proteins. Finally, the third and highest energy guest motif within the capsule can be observed with guests such as $C_{26}H_{54}$. This guest packs with each methyl terminus in separate poles of each hemisphere, but the bulk, central region of the guest forms a flattened disc that lies in the equator of the capsule prizing the two hemispheres apart. Thus in profile the bound guest resembles a spinning-top. This same study [48] also examined the consequences of covalently linking together two octa-acid molecules. For this "dimer" host, guests smaller than *n*-heptadecane ($C_{17}H_{36}$) bind to the host in a similar manner as they do to the assembled capsule of octa-acid. However, guests larger than $C_{18}H_{38}$ do not adopt a U-shaped packing inside a 1:1 complex, but instead induce the formation of a more capacious D_{2d} dimeric assembly that allows the accommodation of two copies of the guest.

A related deep-cavity cavitand that we have worked with is tetra-*endo*-methyl octa-acid (TEMOA, Figure 13). The four methyl groups at the rim of this host project inwards and upwards so as to subtly change the shape of the pocket by reducing the width of the portal but increasing its depth. The result is a host with approximately the same carrying capacity as octa-acid, but very different binding and assembly properties. For example, the binding profile of octa-acid using the metric of *n*-alkane complexation is as expected. The smallest of guests form 1:1 complexes, whereas with guests larger than ethane a

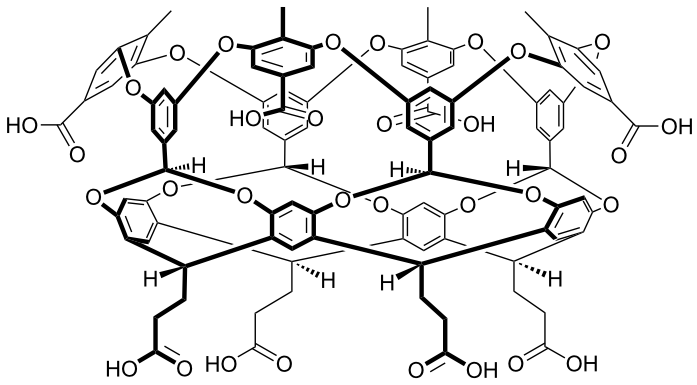


Figure 13: Structure of tetra-*endo*-methyl octa-acid (TEMOA).

2:2 or 2:1 capsular complex is formed. In contrast the binding profile of TEMOA is non-monotonic: very small and medium sized guests form 1:1 complexes, whereas small and large guests form dimeric capsular assemblies [49]. This unique property comes about because the four methyl groups reduce the relative stability of the dimer capsule so that guests such as C_5H_{12} promote the formation of a 2:2 capsule, but near the transition point from two to one internalized guest, C_7H_{16} destabilizes the capsular form and leads instead to a 1:1 host–guest complex. Only with C_9H_{20} – one copy of which can adequately fill the capsule – does the host revert to an assembled form. This unusual non-monotonic profile carries over when the formation of hetero-host complexes formed between octa-acid and TEMAO and *n*-alkane guests are examined [50].

This reduced propensity of TEMOA to dimerize means that when the guest is as large as $C_{17}H_{36}$, rather than form a dimer the cavitand switches to a tetrameric, pseudo-tetrahedral, 4:2 host–guest complex (Figure 14) [51]. This is possible because the methyl groups that sterically hinder dimerization interfere less with an assembly as the angle between the two interfacing surfaces of a pair of cavitands increases from 0° (in a dimer) to ~70.5° (in a regular tetrahedron). This senary assembly many be higher in free energy, but because this container has a volume of four hosts *plus* that of a roughly tetrahedral volume in the center of the assembly, the guests are afforded more space: ~1400 Å^3 in total. The assembly properties of TEMOA do not end there. With even larger guests such as $C_{24}H_{50}$ the host forms the corresponding octahedral 6:3

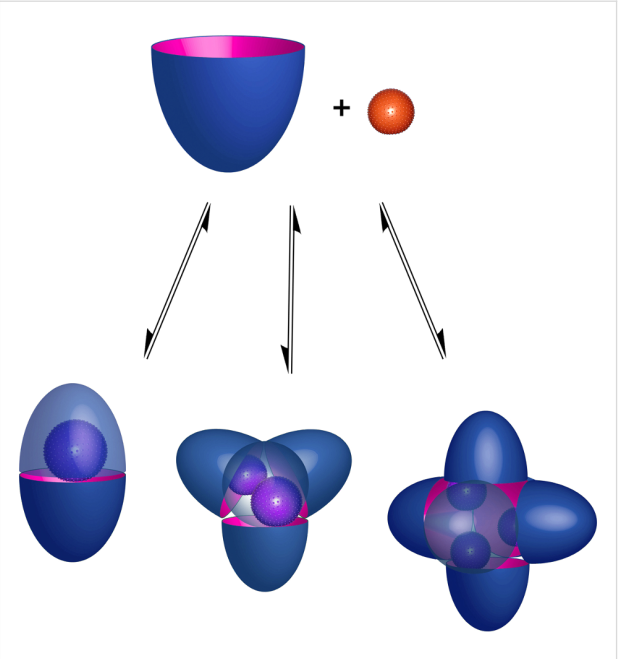


Figure 14: Assembly properties of TEMOA.

nonary complex with an internal volume of ~3400 Å^3, i.e., the volume of the six cavitands plus the roughly cubic central void at the center of the inner space. This represents one of the largest container systems synthesized to date, and certainly the largest synthesized with the help of the hydrophobic effect.

To summarize, the combination of octa-acid, TEMOA and the hydrophobic effect have allowed us to form mono-dispersed 1:1, 2:1, 2:2, 4:2 and 6:3 complexes with molecule weights up to ~11.5 kD. Furthermore, these entities demonstrate that orchestration of the hydrophobic effect can be used to bring about a range of unique phenomena, including novel chemical conversions, and chemical or physical separations.

Concomitant to using the Hydrophobic effect to engender unusual phenomena such as those outlined above, we have also been involved with improving Science's understanding of both the Hydrophobic effect and the Hofmeister effect [29]. Contributing to understanding these phenomena is truly an interdisciplinary affair, and in particular, over the last few years we have collaborated with computational chemists whose unique skills allow us to "visualize" what water is doing within the solvation shell of solutes. One exciting facet of these collaborations is the SAMPL exercise in which we and other supramolecular chemists a priori determine the thermodynamics of complexation for a series of processes, and keep the results under wraps whilst computational groups around the world attempt to predict the data [52]. This competition gives the computationalists a unique opportunity to try and hone their skills, which in return provides feedback to those interested in the a priori prediction of the thermodynamics of binding. For example, being able to predict ahead of time which ligands binds best to a protein binding-site has the potential to save the pharmaceutical industry billions of dollars in drug development.

Collaboration with my former colleague Steven Rick has provided considerable insight into the solvation of octa-acid. Briefly, this work showed that between 0–7 (~4.5 on average) water molecules can be found within the hydrophobic pocket [53]. These water molecules are relatively high in energy, in so much as they cannot form their regular complement of hydrogen bonds seen in the bulk (~3.6) but instead must form weaker interactions with the concavity; and the deeper the water molecule the higher its energy and the lower its translational diffusion constant, but the higher its rotational diffusion constant. Overall however, the pocket would rather be solvated: ΔG_{hyd} ΔH_{hyd} and $T\Delta S_{hyd}$ were determined to be approximately, –5, –20, and 15 kcal mol^{-1} for 4 or 5 water molecules.

The addition of salts to water or aqueous solutions lead to a plethora of phenomenon all classified under the banner of "the

Hofmeister effect". The classical manifestation of the Hofmeister effect is the ability to increase or decrease the apparent solubility of organic solutes dissolved in water. Thus for example, sodium sulfate will decrease the solubility of a protein causing it to precipitate from solution. In contrast, salts such as sodium thiocyanate cause proteins to become more soluble, and in doing so break apart their tertiary structure. For a long time it has been appreciated that it is the anion that plays the more significant role in this Hofmeister effect; the question is how [54]?

We first started to investigate the Hofmeister effect in 2005 when we were on a forced group sabbatical at the University of Texas, Austin. The principle driving force for our near year long relocation was hurricane Katrina that devastated New Orleans, south east Louisiana, and southern Mississippi in the fall of 2005. One of my best memories of this difficult period arose from the twelve offers we received from colleagues around the country (and indeed beyond!) to temporarily move and set up shop in University X. We chose UT Austin, both because it was possible to get to New Orleans with an eight hours drive (important for rebuilding our house and helping rebuild the department), and because of the Department's supramolecular strengths. Indeed, the coordinated and gracious charity of Jonathan Sessler (provider of research funds whilst we sought emergency support from the likes of the National Science Foundation and Department of Energy) and Eric Anslyn (who provided us with research space) was too attractive to turn down; especially since Jonathan's neighbors, Syd & Arnie Popinsky, were offering us a room for the first week or two until we settled in.

Although a much larger department than UNO, UT Austin had a poorer ratio of NMR instruments to students, and so we focused mostly on isothermal titration calorimetry (ITC) based research. There was the small matter of sneaking into a closed city and slipping under the radar of the National Guard to "steal" our own hosts, reagents, and ITC instruments; but that story is perhaps for another time. Back in Austin with everything we needed, we got down to some research… and of course filling out countless forms for insurance claims and requests for financial aid from the Federal Emergency Management Administration (FEMA).

Our first results that came out from this period demonstrated that the thermodynamics of guest binding to octa-acid were extremely perturbed by the presence of different salts [55]. As expected, the Hofmeister effect was indeed in operation: salting-out sodium sulfate increased guest association, whilst salting-in sodium perchlorate weakened binding considerably. The surprise was that the root cause of the latter was competitive binding of the anions to the hydrophobic pocket of our anionic host. A follow-on full paper expanded in these results considerably and provided a detailed thermodynamic picture of how the concentration and nature of salts influence hydrophobic solute association [56].

But why did some salts cause binding to increase? We answered this question back in New Orleans, not at UNO, but rather at Tulane University. Being a private institution Tulane had weather Katrina relatively well. Moreover, it had also weathered the withering Louisiana politics of the last decade that has resulted in the largest cuts to higher education in the nation. One metric of the effects of this political and economic upheaval was that between Katrina and the present day, the UNO chemistry faculty decreased from 18–20 to 5; with a concomitant decrease in the graduate student and post-doc population. Sometime before the Department numbers bottomed-out, the opportunity to move across town to Tulane arose and I accepted. It was a hard decision leaving UNO in such a state; things had been going so well before the storm. But if you think doing science is hard, try doing it in a dispiriting environment where no institutional decision was ever favorable (or even neutral). To take but one example, despite the best efforts of our then Chair (Matt Tarr), when I left the department it had functioned for two years with no secretary whatsoever.

Back to the science. Why did some salts cause guest binding to strengthen? An answer came when we studied the binding of a much weaker guest to octa-acid; namely perchlorate [57]. Just as we observed with the binding of hydrophobic guests, perchlorate association was influenced by the presence of other salts: sodium chloride and fluoride caused the affinity to increase whereas sodium thiocyanate caused its affinity to decrease (Figure 15). The data from this NMR study allowed us to build a model for how K_a changed as a function of salt concentration. Briefly, two factors accounted for >90% of the observed changes in perchlorate affinity as a function of co-salt ionic strength: where present, anion binding to the hydrophobic pocket caused perchlorate affinity to decrease, but in all cases sodium ion condensation to the exterior carboxylates of the host reduced its negative charge and hence increased anion affinity.

Cation binding to groups such as carboxylates is well documented, but why would an anion bind to a hydrophobic pocket? Elsewhere it has relatively recently been demonstrated that such salting-in anions have an affinity for the air–water interface, and furthermore it has been suspected for some time that the same anions have a weak affinity for the macromolecule–water interface (see references in references [29] and [54]), at least in part because such ions have relatively low free energies of hydration. Still, these energies are high, and it is frequently

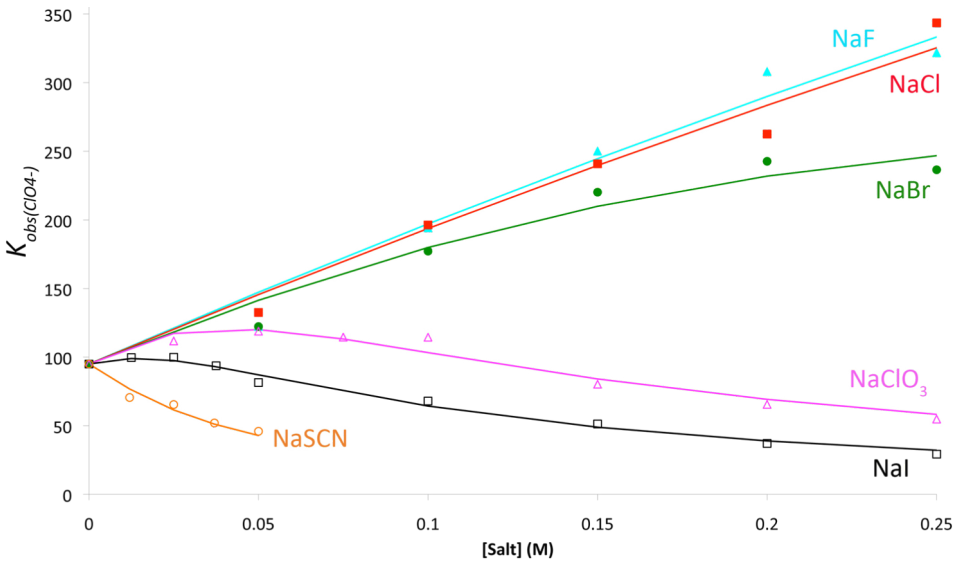


Figure 15: How salts influence the association constant (K_a) for the binding of ClO_4^- to octa-acid (Figure 4). The indicated points correspond to NMR-derived data, whilst the lines correspond to the model devised (see text).

commented on in supramolecular chemistry how difficult ion recognition in water is precisely because this solvation shell has to be removed. But does it? Our latest Hofmeister-related paper revealed detailed thermodynamic data for a wide range of anions binding to octa-acid, and suggests that complexation only involves partial ion-desolvation [58]. These results builds on our earlier in silico studies [53]; anion binding to the pocket appears to involve simply replacing one or two high-energy water molecules with the anion. The details of why this should be are yet to be determined.

Conclusion

So that's roughly where we are regarding our exploration of the hydrophobic effect, the Hofmeister effect, and what can be done with them. Our research over the last two decades or so has revealed that it is possible to harness the properties of water to engender molecular containment and a range of new phenomena. More importantly perhaps, in trying to understand why we see what we see we are revealing new information about the hydrophobic and Hofmeister effects that dovetails with many aspects of recent water research carried out within other fields. Gratifyingly however, there is still a lot to learn about the interactions between water, salt and solute. Here's hoping the next two decades will be at least equally revealing!

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BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Organic chemistry meets polymers, nanoscience, therapeutics and diagnostics

Vincent M. Rotello

Review

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Address:
Department of Chemistry, University of Massachusetts-Amherst, 710 North Pleasant Street, Amherst, Massachusetts 01003, USA

Email:
Vincent M. Rotello - rotello@chem.umass.edu

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Abstract

The atom-by-atom control provided by synthetic organic chemistry presents a means of generating new functional nanomaterials with great precision. Bringing together these two very disparate skill sets is, however, quite uncommon. This autobiographical review provides some insight into how my program evolved, as well as giving some idea of where we are going.

Review

My roots – synthetic organic

My interest in chemistry built on my incessant tinkering. When I was young, I was the sort that would take things apart to see how they worked. I also put them back together, occasionally with no extra parts… Eventually, the ability to create overcame the desire to dissect, and I started pursuing photography. My efforts in this domain progressed until I started considering art as a career. Alongside this artistic pathway, however, I developed an interest in chemistry. What intrigued me at the start was connectivity – how atoms could be strung together. I explored both art and photography at Illinois Institute of Technology, but science and scientists ended up deciding me. It started with Chem. 237, Organic Chemistry. This course was taught by Pete Johnson, who introduced me to retrosynthetic analysis, and in the process showed me how I could achieve the connectivity I had doodled when younger. This classroom work was followed up soon thereafter by practical training at the hands of Phil Garner. Phil was an old school 'guts and glory' synthetic type, and I learned from him everything I needed to about the practical side of synthetic organic. This work culminated in the first paper I co-authored [1], where I learned painful lessons with the Fieser triangle at the twilight of the pen and ink era.

With my belly full of synthetic fire, I sought out a place to pursue my trade. In the mid-80s Yale University was hotbed for synthesis, and picking out an advisor was a difficult choice. I ultimately chose Harry Wasserman, based on the freedom he gave his group in choosing and attacking synthetic and physical challenges. During this period I developed and finished a variety of syntheses. My break came, however, when looking in the back pages of the journal *Heterocycles* in the "New Natural Products" section. I noticed a rather interesting new molecule known as rapamycin that had the vicinal tricarbonyl motif in vogue in the Wasserman group. When I suggested we go after this molecule, Harry demurred, pointing out its complexity and that "nobody would be interested". I kept my eye on the literature, however, and when Dave Williams published the "Central America" fragment of the related macrolide FK-506, I urged Harry to see if he could get some intermediate that I could use to demonstrate our methodology for tricarbonyl synthesis. Dave generously obliged by sending ~200 mg of an advanced intermediate. With hands shaking, I cracked the vial and started on the synthesis. Working with small-scale reactions (<1 mg), I eventually worked out a high-yielding synthesis of the Williams fragment [2]. This synthesis generated some buzz, catching the eye of folks like Sam Danishefsky and facilitating my next move.

Moving to Legoland

Sorting out what I wanted to do after grad school was a bit of a challenge. In those days I knew I wanted to be an academic, but what I wanted to do scientifically was an open question. I started thinking about proposals for postdoctoral fellowships, but the synthetic ideas I generated didn't fire me up like I thought they should. I really enjoyed the power of organic synthesis, but I wanted to do something with the molecules that I laboriously fashioned. Once again my love of connectivity kicked in, this time with supramolecular chemistry. I started thinking of molecules instead of atoms as building blocks. I looked around for professors with a like mind, and applied to Julius Rebek at Pittsburgh. Between when I applied and when I joined as an NSF postdoctoral fellow Julius had moved to MIT for his brief stay in the Boston area before heading off to Scripps.

While in the Rebek group I used my synthetic abilities while gaining the insight into physical organic chemistry that has informed the rest of my career. I started out working on self-replicating systems [3], developing new systems that had novel capabilities, including external regulation [4]. I also took on a brutal project, focused on the synthesis of water-soluble analogs of Kemp's triacid. This project was a massive effort, with a huge number of reactions required to optimize the initial steps. We did, however, obtain the desired receptors and observe some interesting binding processes in water [5]. During this part of my time in the group, Julius offered that if I stayed an additional year I could work on projects of my own. During this time I discovered my inner mentor. Much to the annoyance of my labmates, I built a veritable army of undergraduates, pursuing supramolecular chemistry, along with a project in fullerenes [6,7].

After finishing my postdoctoral work, I moved to the University of Massachusetts. I chose UMass based on its quality and longstanding reputation for collaborative research. Upon arrival, I collected a fired up group of graduate students and went to it. I maintained my fullerenes project [8], and initiated a set of three other supramolecular projects. Of these projects, our work on flavoenzyme models was the one that really took off. Using electrochemistry, we were able to gain a real understanding of how forces such as hydrogen bonding [9], aromatic stacking (Figure 1) [10] and donor atom–π interactions [11] influence the energetics of redox processes. We also used integrated experimental and computational techniques to actually establish what hydrogen bonding does to the electrons in hydrogen-bonded complexes [12]. Finally, we put this all together to generate molecular switches and devices [13]. This work has continued through our long productive collaboration with Graeme Cooke, now at University of Glasgow, moving from redox-active systems [14,15] to photovoltaics (along with Ifor Samuel at St. Andrew's) [16].

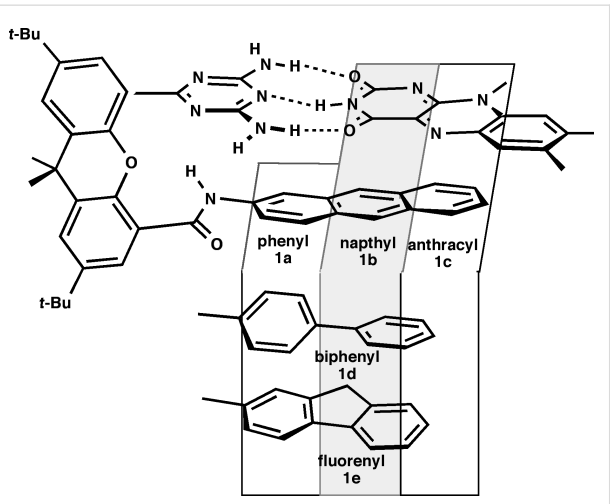

Figure 1: Flavoenzyme model system for determining the role of aromatic stacking in flavin redox processes. Reprinted with permission from [10]. Copyright (1997) American Chemical Society.

Go big, go nano

After four or so years of work on redox-modulated recognition two things happened. First, I realized that we could predict what would happen with the systems before we made them. This took

much of the fun out of the work. Simultaneously, I received tenure, and tried to sort out what I wanted to do for my next career phase. Our next move was into polymers, where we started the conceptual journey we are still taking. The key question we asked is "we know what happens when you have one host–guest dyad, but what happens when you have 10, 50, 100 on a polymer?" On a straightforward level, we were able to demonstrate that we could use non-covalent sidechain modification between multivalent polymers and monovalent guests to generate "plug and play polymers" (Figure 2) [17]. We also showed that we could self-assemble a polymer around an electroactive guest, effectively encapsulating and isolating it [18].

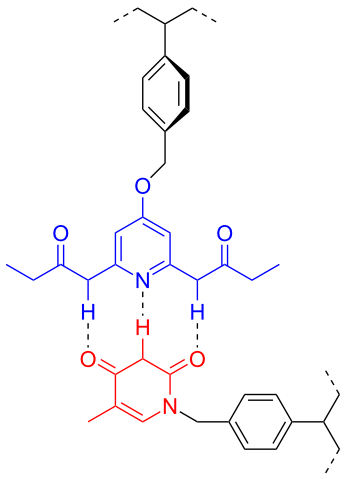

Figure 2: Recognition element-functionalized polymers for 'plug and play' modification and self-assembly.

The research really became interesting when we started mixing multivalent complementary polymers together. When we mixed together diaminopyridine and uracil polymers together in chloroform we generated a turbid solution. Under the microscope we found that the turbidity surprisingly arose from vesicular structures [19]. Through quite a bit of experimentation we determined that the unprecedented self-assembly process was driven by self-sorting of the polymer chains to provide vesicle walls with denser recognition elements in the middle than at the outside [20].

While we were working with polymers, we were just starting to move into nanoparticles. As with the polymers, we started off studying the interactions of recognition element-functionalized nanoparticles with monovalent guests – in this case our old friend flavin where we showed modulation of the flavin redox potentials [21]. Taking this research one step further, we created a nanoparticle with a mixed monolayer consisting of hydrogen bonding and aromatic staking sidechains. When we incubated this NP with flavin we observed an increase in binding over time, i.e., we were able to template the particle to the guest [22]. We have since demonstrated this templation with peptides [23] and are (still!) trying to definitively show templation to proteins.

As I mentioned above, I am an incessant tinkerer, a trait that has rubbed off on the group. When students mixed complementary versions of the polymers and nanoparticles described above, we were quite surprised to find that we generated regular spherical and network structures (Figure 3) [24]. This "bricks and mortar" assembly process provides a modular system where structure and stoichiometry of the components drives structure formation. These assemblies set us on a path of generating nanocomposite materials, including regular structures using diblock copolymers [25,26] and nanoparticle–protein [27,28] and nanoparticle–nucleic acid composites [29].

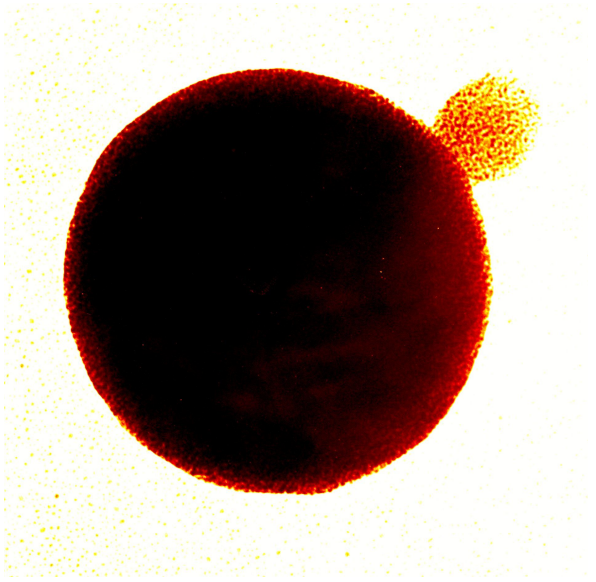

Figure 3: Recognition-mediated assembly of nanoparticle–polymer constructs. Reproduced from [24].

Concurrently with our 3D self-assembly, we pursued the use of nanoparticles for surface modification. This research has focused on the use of these particles to efficiently impart functional properties to surfaces [30]. including anti-fouling properties [31]. When combined with nanoimprint lithography [32] this process gives us access to nano-textured nanopatterned surfaces [33,34]. We have recently employed this strategy to control cell growth on surfaces [35], including using the surface properties of the nanoparticle to dictate cell selectivity [36].

Nanoparticles meet biology

Our move into nanocomposites coincided with our efforts to interface materials and biology – the current core focus of the lab. We started off looking at nucleic acids, where we showed that cationic nanoparticles could bind to anionic DNA and inhibit transcription [37]. We also developed a number of strategies for delivery of small molecules, including glutathione-mediated release of covalently attached thiols [38], as the effective release of drugs adsorbed into the cationic monolayers of nanoparticles [39], and even photoactivatable drug [40] and DNA release [41]. When we started looking at how particles interact with proteins, however, very little was known about how these materials would play together. We found out pretty quickly that the answer was "not very well". Binding of the nanoparticle induced protein denaturation, with loss of bioactivity [42]. We hypothesized that this denaturation arose from interaction of the protein with the hydrophobic elements of the simple ligands we were using. This hypothesis led us to create what we call the "tabula rasa" ligand [43], namely a ligand that features a hydrophobic interior for self-assembly, and a short tetra(ethylene glycol) layer to block interactions of the hydrophobic interiors with proteins [44]. These particles were indeed "blank" (but not as blank as our later zwitterionic “corona-free’ particles [45]), and behaved like high molecular weight poly(ethylene glycol) [46]. Once we appended simple anionic and cationic recognition elements to the surface these system bound proteins with high affinity [47] and some degree of selectivity [48]. What was surprising is that not only did the particle–protein binding process not denature the proteins, it actually stabilizes them [46]! This result stills surprises researchers who follow the dogma that proteins must denature at interfaces. One of the other observations we made using isothermal titration calorimetry is that nanoparticle–protein interactions using the tabula rasa-based particles mimicked the thermodynamics of protein–protein interactions quite well [49].

Once we were able to have proteins and nanoparticles work in harmony [50] we started working on designing systems with emergent behavior, i.e., where the particle–protein complex behaves differently than either of the two components. A prime example of this synergy is when we showed that nanoparticle–enzyme complexes showed altered substrate selectivity [51], with the particle dictating the enzyme kinetics by acting as a "filter" for substrate and product [47]. Another area where we demonstrated synergy is in the area of Pickering emulsions. These emulsions are made by interfacial assembly of particles at oil–water interfaces. We showed that nanoparticles and proteins could be self-assembled at this interface [52], retaining their activity. When the oil core was crosslinked, these systems worked even better, enhancing enzyme activity [53], even under extreme chemical and thermal conditions [54].

As we were learning the rules for nanoparticle-biological interactions we started looking into applications for these self-assembled materials. Our first real success came when we demonstrated very efficient DNA transfection (i.e., gene delivery) using gold nanoparticles [55]. In later work we improved on our gene delivery vehicles [56], demonstrated the delivery of siRNA [57] and enzymes [58] using nanoparticle assemblies. All of these systems (as well as essentially all of the other examples of nanomaterial delivery vehicles in the literature) occurred via endosomal uptake. The problem with this route is that what goes into the endosome tends to stay in the endosome, and eventually be degraded. Since most of the interesting things in cells require access to the cytosol (including materials destined to the nucleus), this entrapment is a major limitation [59].

One of the beauties of supramolecular chemistry is its modularity. We started looking into the use of the Pickering emulsions described above for delivery applications. There was a challenge: nobody (including us) could generate emulsions with diameters small enough (<200 nm) for use as in vivo delivery vehicles [60]. Once again, supramolecular chemistry came to the rescue. Anslyn showed that guanidinium groups bound strongly to carboxylates [61]. This led to the surmise that this interaction could be used to "pin" arginine-capped nanoparticles to oil droplets comprised of fatty acids. This trick worked remarkably well, providing ~150 nm nanocapsules. These capsules were unstable in serum however. Reaching back to our nanocomposite work, we were able to use bricks and mortar assembly of anionic proteins (transferrin) and cationic nanoparticles to create stable capsules [62]. These capsules delivered hydrophobic drugs and dyes to cells very effectively. A puzzling question arose however: dyes were delivered into cells much faster than the particles on the outside of the capsule. Clearly, endosomal uptake would result in identical rates of uptake, leading us to surmise that uptake occurred through a membrane fusion process.

Driven by the desire to deliver biological payloads directly to the cytosol, we tested our system for the very challenging goal of protein delivery using green fluorescent protein (GFP). It worked even better than we hoped, with complete cytosolar distribution of the GFP observed (Figure 4) [63]. This ability to "dump" proteins into cells is unprecedented, allowing us to deliver proteins capable of intracellular localization – the next frontier of targeting [64]. We also made use of the oil interior of the capsule to provide dual protein (caspase 3) and therapeutic (paclitaxel) delivery where the two payloads worked synergistically for chemotherapy [65]. Being supramolecular types, we figured we could swap out the anionic proteins used above for anionic siRNA [66]. In this case we were right – we

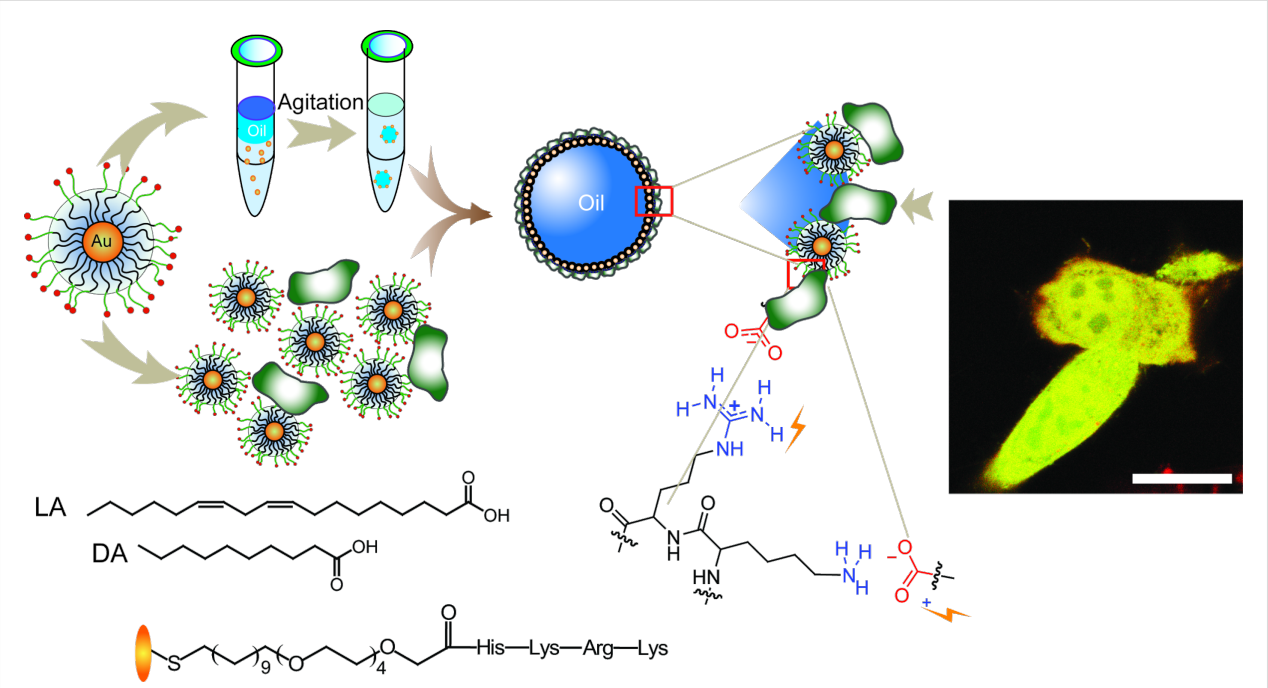

Figure 4: Cytosolic delivery of GFP to cells using nanoparticle-stabilized nanocapsules. Adapted with permission from [63]. Copyright (2013) American Chemical Society.

can deliver siRNA directly into the cytosol with great efficiency [67].

Concurrent with our delivery efforts, we were working on ways to track nanoparticles in living systems. Back in 2008 I had a student working jointly with Richard Vachet, and I suggested the rather crazy notion of taking some cells, putting in nanoparticles, and then throwing them in the matrix-assisted laser desorption ionization (MALDI) mass spectrometer without matrix. Quite surprisingly, all the student could see was the ligand on the particle – with essentially no signal from all of the rest of the stuff found in cells [68]. This was the start of a collaboration that continues to this day, where we have used laser desorption ionization (LDI) to characterize ligands on particles [69,70], supramolecular chemistry in cells [71], track NPs in fish [72] and plants [73], and to image NPs in animal organs – after shooting them into mice [74]. More recently, we bought an inductively coupled plasma (ICP) mass spectrometer that allows us to quantify elements. We have used ICP together with LDI to determine the stability of quantum dots [75] and gold nanoparticles in cells [76]. Once again, a tribute to doing the occasional eccentric experiment.

Building nanonoses

As we were developing our delivery vehicle platforms, we were starting to think about sensing applications. One of the things that we observed in our protein binding work was that our nanoparticles had varying affinities for different proteins [77]. This differential affinity made our particles potential sensor elements for employing the "chemical nose" strategy to proteins. The challenge was that particles bound to proteins look quite similar to particles that aren't. Clearly, we needed a means of transducing protein binding. The breakthrough occurred at the Fall 2006 ACS meeting where the ever-charming Uwe Bunz and I had the simultaneous inspiration of using Uwe's highly fluorescent poly(phenylene ethynelene) "molecular wire" polymers as transducers. These polymers would be quenched by the gold core of the nanoparticles while bound, regaining fluorescence after being displaced by protein analytes. This strategy worked quite well, allowing us to discriminate proteins at considerably lower concentration than prior studies [78]. Using the supramolecular chemist's ability to oversimplify, we soon extended these studies to bacterial [79] and mammalian cells [80] under the pretext that cell surfaces are "complex solutions" confined to surfaces [81].

While Uwe's polymers were incredibly useful, they had two limitations. The first limitation is that they tended to aggregate and self-quench in complex media, wiping out the signal. This aggregation became an issue when we attempted to sense changes in serum profiles. We overcame this issue using Nature's fluorescent polymers, namely fluorescent proteins. Using gold nanoparticles and green fluorescent protein we were able to rapidly detect very small changes in serum protein

profiles in undiluted serum [82] a project that we are currently testing in human studies. I should point out that we have managed to marry the strenths of the polymer and fluorescent protein systems [83] using polymer–protein FRET to provide modulated output [84].

You'll remember that I said that conjugated polymers had two limitations. The second issue we had was lack of choices in emission color – blue and green are easy, yellow is challenging, and getting a red conjugated polymer with a respectable quantum yield is nigh impossible. We had an idea, however, that we could use multi-channel sensing to increase our sensor capabilities, with the goal of creating "one well" chemical nose sensors. To this end we expressed ourselves some red, green and blue fluorescent proteins and set to work on cell surface sensing. Rather than going after something easy, we decided to see if we could discriminate mechanisms of chemotherapeutics. This project worked better than we could have hoped, with complete discrimination of eight different mechanisms, all in the matter of minutes (Figure 5) [85]. The sensor was tested against therapeutics and was able to identify the mechanisms of new drugs in the training set, and equally importantly identify if the therapeutic acted by a mechanism novel to the training set. The capabilities of this sensor are quite impressive, but you may ask "what is it on the cell surface that the sensor is responding too?" We have an excellent clue to that question: changes in glycosylation (through mutation or glycolosis) generate very strong sensor responses, implicating that our sensor responds to changes in glycosylation [86] which is probably also the mechanism in play for our successful determination of biofilms using this platform [87].

Whither next?

You can probably tell from the above ramble that predicting where I am going next is an incredibly challenging question for me. We have a number of areas we are currently exploring, including the creation of nanoparticle-based antimicrobials [88,89] building on our studies showing that nanoparticles are effective against antibiotic-resistant bacteria such as MRSA [90] and nanocapsule systems work against the biofilms [91] made by these nasties [92]. We have also made a foray into bioorthogonal chemistry, using nanoparticles to encapsulate transition metal catalysts, generating “nanozymes” that can catalyze processes in cells that even the cells can’t do [93]. We have even been doing some immunology [94], including turning on the innate immune system [95] and figuring out what macrophages like to eat [96].

We have plenty to work on just taking our systems where they need to go, be it in vivo or in the clinic [97,98]. Broader picture, what I really want to see is the integration of the synthetic and biological worlds to generate new systems that can do things that neither can do alone [99,100]. This vision of "cyborg" constructs with emergent behavior is motivating both my own research and my editorial efforts with *Bioconjugate Chemistry*. I feel that this is an area where chemistry can really impact the human condition, of course keeping in mind the cautionary tales provided by many Sci-Fi novels and movies.

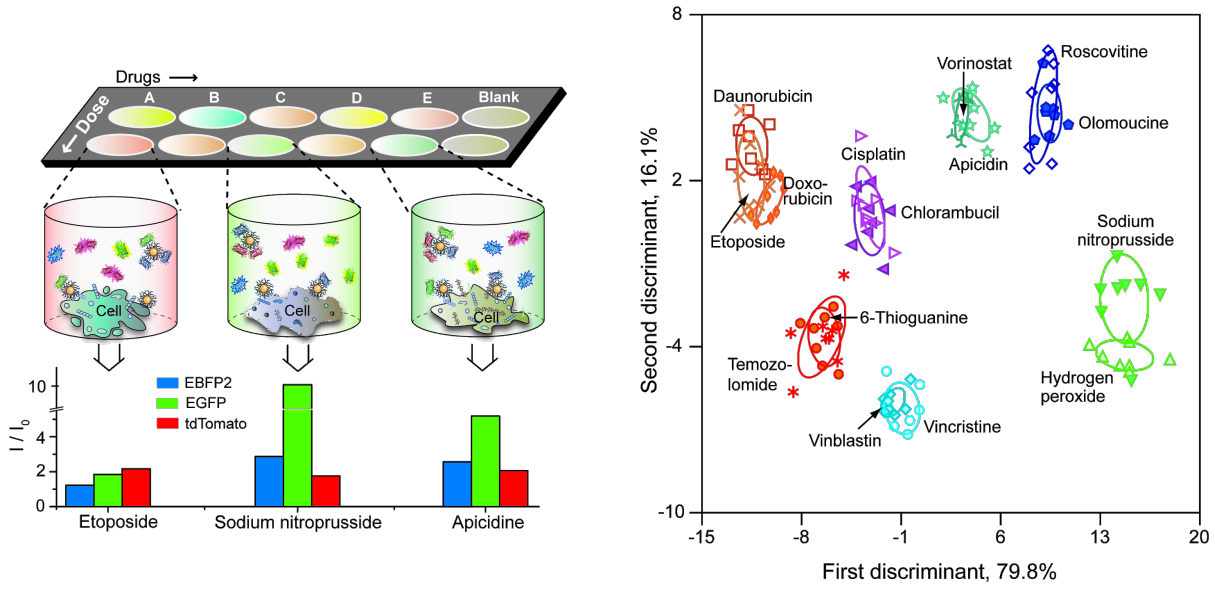

Figure 5: Rapid determination of therapeutic mechanisms using three-channel nanoparticle fluorescent protein nanosensors. Adapted from [85].

Something about the author

Like anyone with a job, I live two lives. As you can probably surmise, science moves me at work. As I travel, I also enjoy the comradeship of scientists around the world, and can become quite evangelical about the roles the scientific community can serve to bridge gulfs between nations and cultures. To be honest, I also enjoy seeing the world and its many wonders, reveling in both the "big" sights and running at dawn in a new town. And I have been accused of traveling on my stomach – I do have a fondness for food .

At home, I am a family man (if not a Family Guy…). Coming from the school that food equals love, my love of cooking is likewise a major factor in my life. The skills I developed as a synthetic chemist give me an understanding of ingredients and techniques that allows me to cook cuisines from around the world. Fortunately my wife and I are inveterate exercisers, and my son's metabolism is still rapid enough to avoid (excessive) weight gain. This exercise is typically done with our rather neurotic Weimaraner Trudy, allowing us to get double duty from our toil.

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From supramolecular chemistry to the nucleosome: studies in biomolecular recognition

Marcey L. Waters

Review

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Address:
Department of Chemistry, CB 3290, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Email:
Marcey L. Waters - mlwaters@email.unc.edu

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Abstract

This review highlights the author's indirect path to research at the interface of supramolecular chemistry and chemical biology.

Review

Childhood influences

When thinking about how to start writing this review, it crossed my mind that the fact that I grew up with a dog named Frodo (Figure 1), named by my dad, says a lot about the environment in which I grew up (I read the Lord of the Rings at age 12 to learn why my dog was named that). Since I have already brought my dad into this, I will begin by saying a bit more about him and his influence on me. My dad is an intensely curious man who loves all things science. He started out as a geologist, but life took him in other directions, and he ended up as a Navy pilot and later an engineer. Nonetheless, my dad never lost interest in his first love, and so I learned a lot about rocks as a kid! I am the middle of three sisters, and we joke that my dad didn't care if he had a son, as long as he had a scientist. My mom is an artist but followed a career path that was available to women at the time: she was a teacher before she had a family. Important for my story, I often overheard her commenting that women could do anything that men could do and that it was an outrage that women got paid less than men for the same work (and still is). Even at 5 or 6 years old, I remember getting angry about this myself and thinking, effectively, "I'll show them". So, between my own inherent interest in math and science (as is common among us, I asked for microscopes and chemistry sets for Christmas), strong encouragement from my dad, and a certain drive to prove something to the world (!) instilled by my mom, I set out on the trajectory that led me to where I am today (with a little help from some influential people along the way).

The winding path to chemistry

Even so, it wasn't a straight path to chemistry professor. In addition to my interest in science, I also loved to build and to draw and paint (my parents got me a real tool set when I was 6 or 7

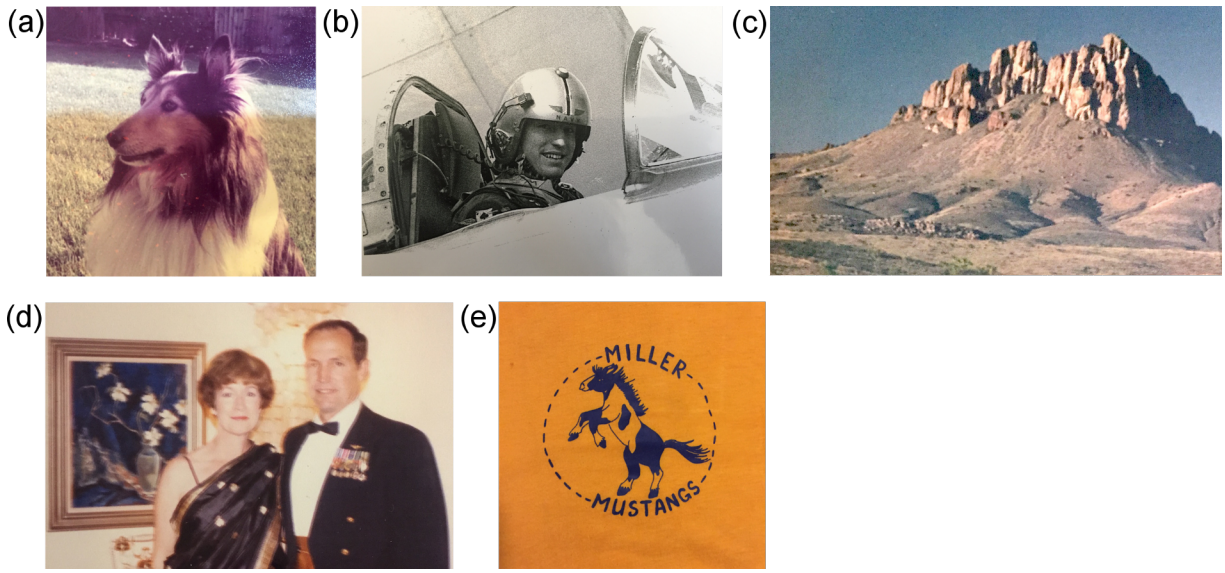

Figure 1: (a) Frodo the dog (copyright to MLW). (b) Ron Waters in an A6 in 1961 at the age of 26 (reproduced with permission from RLW). (c) A picture of Steeple Rock, near Duncan, AZ where we spent many mornings collecting geodes when visiting my grandparents (reproduced with permission from RLW). (d) Sue and Ron Waters in front of a painting by Sue Waters in 1983 (reproduced with permission from ASW and RLW). (e) My first successful artwork – the school logo I designed in elementary school (reproduced with permission from Miller Elementary School).

and I promptly sawed into the picnic table in the backyard). In fact, my high school art teacher encouraged me to pursue art as a career, and for a time I considered architecture. And while early on chemistry was my favorite field of science, I was not inspired by it in High School or in general chemistry in college at UCSD (here's how not to teach genchem: my textbook listed all compounds by their molecular formula, so, for example, acetic acid was $C_2H_4O_2$. Thus, the fact that molecular structure has anything to do with reactivity was completely left out). I actually started out as a bioengineering major in college. However, after getting accepted to the impacted major (one in which only a subset of students is admitted through an application process), I realized I did not have a passion for it. I thought that genetics was interesting, so registered for both genetics and organic chemistry with the plan of being a biochemistry major. I had heard all of the dreadful stories about organic chemistry and actually went into the class with a bit of a sick curiosity (I had figured out in high school that I often like subjects that others dreaded, so I was not deterred by the dorm-room rumors of O-Chem). It turned out that, as is true with many organic chemists I know, I fell in love with the logic of organic chemistry (I cannot understate the influence of Professor Charles Perrin, who taught organic chemistry from a mechanistic perspective with beautiful clarity). I also suspect that the visual nature of the material appealed to me, as I inherited some degree of artistic aptitude from my mother and had always excelled at spatial relations like my dad. Thus, visualization of concepts like stereochemistry came easily to me, unlike many of my peers. I did pursue one semester of research in genetics, but at that point I found biology too vague for me; molecular level detail was what satisfied my curiosity. Indeed, it was only later once I felt I had a strong molecular understanding of molecular recognition principles that underpinned all of the cartoons of protein complexes that I turned back toward biology.

On to graduate school in organometallic chemistry

Once I "found" organic chemistry, the path to graduate school was relatively direct. My TA, Rich Engler, encouraged me to pursue research, and I did so, joining the group of Professor Perrin, who had engaged me in organic chemistry in the first place. I had a penchant for physical organic chemistry (I wanted to know how things worked), so this was a good fit for me. I also participated in a summer NSF-REU program at Columbia University in Professor Ged Parkin's group and got a taste of inorganic chemistry and all the fun of Schlenk line and glovebox techniques. I quickly decided that I wanted to attend graduate school and also very early on decided I wanted to be a professor. This was largely because I knew my research interests leaned toward the fundamental, but recognizing that there were few women faculty in the sciences in the late 1980's to early 1990's, there may have been a small part of me with something to prove, just like the 5-year old overhearing her mother's conversations!

One interesting aside was the reaction of my parents (both of whom were the first in their families to go to college and

whose childhoods bring up memories of rationing during World War II) when I told them I wanted to get a Ph.D. in chemistry. My mom said, "I don't think we can afford that." I explained that I could get paid to get a Ph.D. and she told me that I better check on that because that couldn't be right. Every time I teach a big lecture class I make sure to tell my students about research opportunities, grad school, and getting paid to get a Ph.D., because I know there are still students out there just like me who didn't come from a family of Ph.D.s and don't know how the system works, and that you can still get paid to get an education!

With my research experiences in physical organic chemistry and inorganic chemistry, and with the boom in organometallic research at that time, I chose to pursue mechanistic organometallic chemistry for my Ph.D. at the University of Chicago in the group of Bill Wulff. Research in the group spanned organometallic methodology, asymmetric catalysis, total synthesis, and mechanistic studies. I opted for the latter and spent my Ph.D. studying the mechanism of the Wulff–Dötz reaction [1], while at the same time gaining a broad background in methodology and synthesis (Figure 2). I had a fantastic time in graduate school, with an advisor who loved to stand at the chalkboard and talk science for hours (one of my fondest memories). He was just the right mix of hands-on and hands-off for me, and knew how to motivate students through enthusiasm instead of pressure. As an example, we had group meetings on Friday mornings but no schedule. On Thursday afternoons, Bill would walk through the lab and talk to everyone about their latest results. Then on Friday morning, he would call on people to present their work. It didn't take long to realize that he called on people with exciting new results, so everyone wanted to present at group meeting. Unlike many of my peers in graduate school, who left with a Ph.D. but no longer with a love of science, I made it through more enthusiastic than ever due to the positive mentorship I received. Reflecting on my own experience versus those of my peers in graduate school has had a significant impact on how I run my own group.

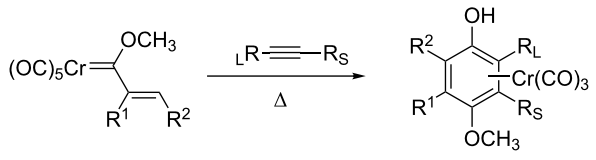

Figure 2: The Wulff–Dötz reaction.

A turn to bioorganic chemistry

An interesting thing happened while I was in graduate school. I found myself reading papers in a relatively young field: supramolecular chemistry. This interest did not simply spring forth on its own, however; the seeds were planted when I was an undergraduate. I took a graduate physical organic chemistry class from Jay Siegel, who was an assistant professor at the time. In his class, in addition to presenting the usual material, he covered recent published literature on molecular recognition that caught my attention, such as Dennis Dougherty's work on cation–π interactions (Figure 3a) [2]. Thus, while at the time I was intent on studying organometallic chemistry, my interest in supramolecular chemistry increased the more I read through graduate school, and particularly molecular recognition in aqueous solution, which I viewed as the most challenging and most important medium for molecular recognition. This led to my decision to postdoc for Ron Breslow at Columbia University, who is known for biomimetic chemistry, but at the heart of his cyclodextrin-based enzyme mimics is molecular recognition in water. Breslow's style was very different than Wulff's, but he was also a very supportive, positive advisor. While I never had a female mentor, I never felt the need for one in these research groups (both departments had one woman on their faculty during my time in those departments).

Starting out on my independent career – combining peptide chemistry and supramolecular chemistry

I learned a great deal of things during my postdoc and it was a great experience for me. However, one thing I learned was that I did not want to start out my independent career trying to design and synthesize a functional molecule (receptor, enzyme mimic, etc), only to find out after several months of synthesis that it did not function as planned! I wanted to utilize versatile chemistry that allowed me to synthesize and evaluate the compound of interest quickly and modify it rapidly for further mechanistic studies. This led me to become a peptide chemist! This was a risky move as an assistant professor to venture into a new field in which I had no established record. But I have always been one to follow my interests, and it worked out for me in the end.

I continued to be interested in aromatic interactions and their potential role in biology as a postdoc. Seminal work probing the electrostatic component of π–π stacking and edge-face aromatic interactions as well as cation–π interactions was being published at the time, as well as tantalizing suggestions about their relevance in biological structure and function. In particular, I was inspired by work of Dennis Dougherty [2], Francois Diederich [3], Sam Gellman [6], Eric Kool [5] and Jeremy Sanders [4] to name a few working in the area at the time (Figure 3). I was particularly interested in addressing whether aromatic interactions provided a degree of selectivity that is not possible with classic aliphatic, hydrophobic interactions, based on the electrostatic component of aromatic interactions.

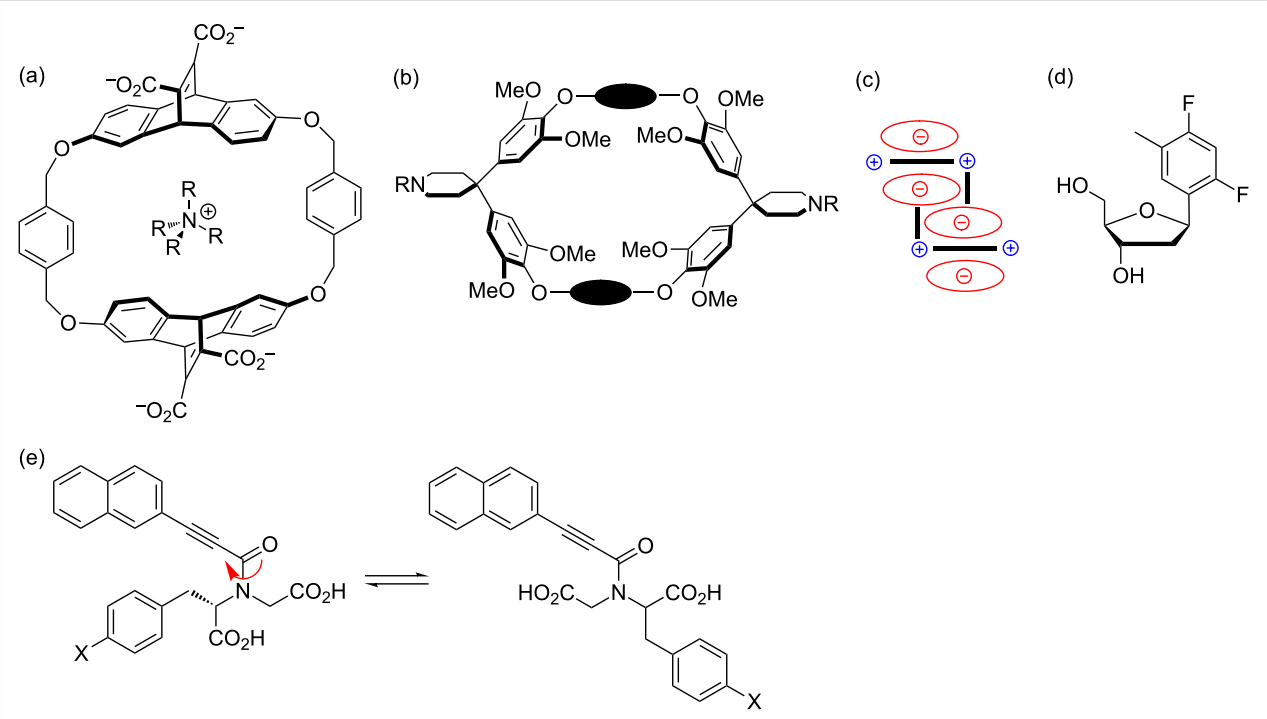

Figure 3: Work by others that inspired my interests. (a) Cyclophane receptors from Dennis Dougherty's group in the late '80's and early '90's that demonstrated cation–π interactions [2]. (b) Cyclophane receptors from the Diederich group in the late '80's and early '90's that demonstrated the "nonclassical hydrophobic effect" [3]. (c) The Hunter-Sanders Model for π–π stacking from 1990 [4]. (d) Kool's nonpolar isostere of thymidine from 1995 [5].(e) Gellman's model for π–π stacking in aqueous solution [6].

In the late 1980's and early 1990's, much work was also done defining the factors that stabilize monomeric α-helices, including the role of noncovalent interactions such as salt bridges, as exemplified by the pioneering work by Baldwin [7,8] and Kallenbach [9]. Thus, when I started at UNC in 1999, I decided to investigate the use of α-helical scaffolds to investigate aromatic interactions, including π–π and cation–π interactions in aqueous solution. The goal was to develop biologically relevant model systems to study these interactions in aqueous solution and to gain insight into the nature of these interactions, their biological relevance, and also see if we could use them to influence structure and function. Peptides were very appealing because of the ease of synthesis and the ease of systematic variation and we published several papers using α-helical scaffolds [10,11]. However, one limitation of α-helices is that their folding is not two state, thus requiring indirect methods to measure the influence of a noncovalent interaction on folding.

About that time, several papers had been published reporting the first monomeric, modestly folded, non-aggregating β-hairpins in aqueous solution [12-15]. My first student, Chad Tatko, read a paper by Gellman [15] on one of these early β-hairpins and suggested that we use it as a scaffold for exploring aromatic interactions. This was an attractive scaffold because a two-state approximation for folding was reasonable in most cases and the β-hairpin is far more amenable to NMR analysis than α-helices, which were usually characterized by Circular Dichroism (CD). Additionally, because the sidechains in β-hairpins interdigitate, they provide relatively isolated positions for evaluating noncovalent interactions, making them a superb model system. Chad and I set out on this course, which led to the publication of more than a dozen papers on a wide range of aromatic interactions in aqueous solution (Figure 4) [16-32]. At the same time, our model systems provided significant insight into the features that contribute to folding of β-hairpin peptides and β-sheets, an area that lagged decades behind the general understanding of α-helices. Beyond using β-hairpins as scaffolds for physical organic chemistry, we also developed some of the first functional β-hairpins that bound nucleotides and ssDNA, mimicking a class of β-sheet proteins, thus expanding on the sequence-structure-function paradigm with minimalist structures (Figure 5) [33-36]. More recently this work has been extended into catalytic β-hairpins that serendipitously utilize aromatic interactions to maximize catalysis [37,38]. Along the way, we had some fun naming the hairpins that had the most interesting properties, including Chadtide – our first model system (after Chad Tatko) [16], Saratide – which binds ATP (after Sara Butterfield) [33], Sarah–Zachtide – which investigates a carbohydrate–π interaction (after Sarah Kiehna and Zachary Laughrey) [27,28], Bobtide – which contains a cation–π interac-

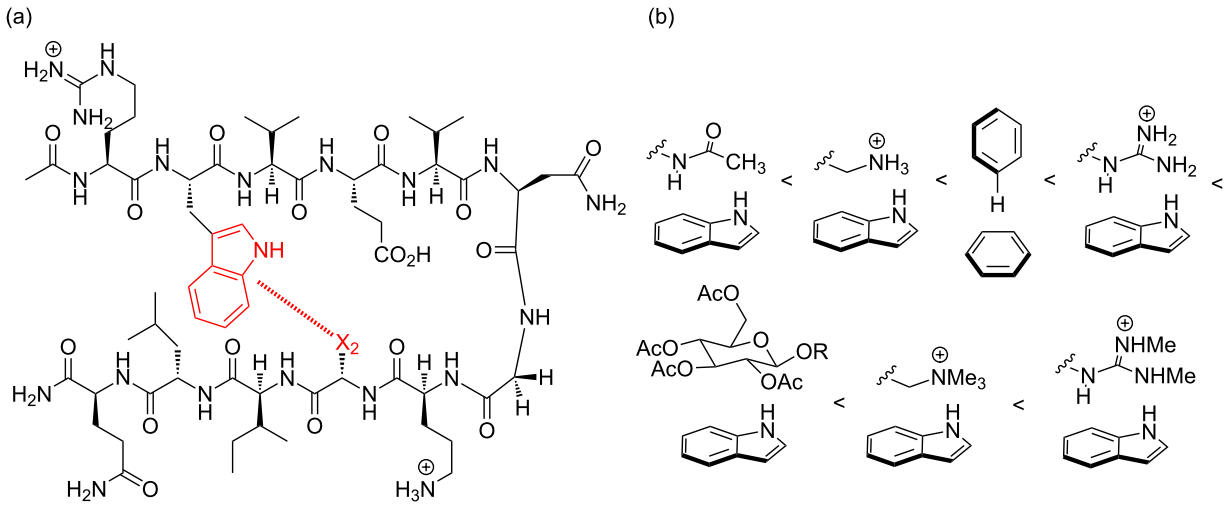

Figure 4: (a) Model β-hairpin for investigation of aromatic interactions. (b) Examples of noncovalent interactions studied, from weakest to strongest [16-32].

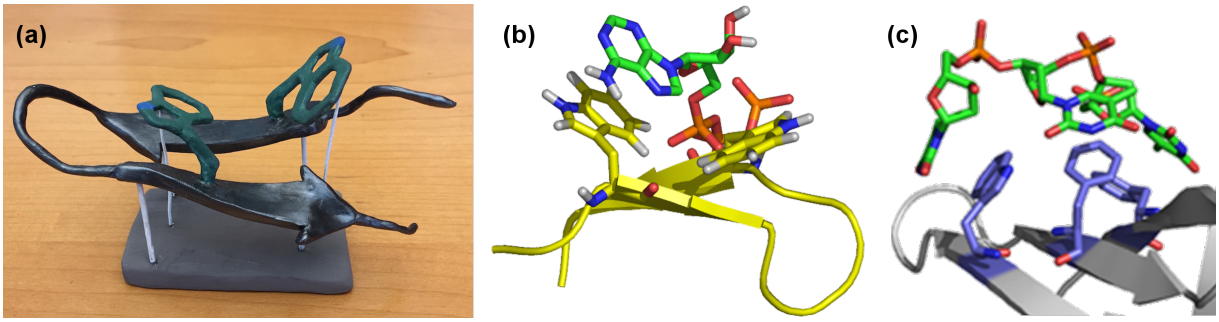

Figure 5: (a) A clay model of our WKWK peptide (aka "Saratide") made by Jes Park, a former graduate student in the group (reproduced with permission from Jessica Park). (b) Computational model of Saratide bound to ATP. (c) Inspiration from Nature: an OB fold bound to ssDNA.

tion with KMe3 (trimethyllysine) and was the most stable β-hairpin reported at the time (after Robert Hughes) [22,23], and Alextide – for which folding can be turned on or off with post-translational modifications (after Alex Riemen) [32].

Biological significance and a shift in focus

While studying aromatic interactions in β-hairpins in the early 2000's, an important biological discovery was made: a crystal structure of a protein that binds to trimethyllysine (KMe3), an important post-translational modification involved in controlling gene expression, shows that it recognizes the trimethylammonium group via an aromatic cage (Figure 6a) [39]. This suggests that the binding is driven by cation–π interactions. We thus studied the influence of lysine methylation and the significance of the positive charge in our β-hairpin model systems [23,24], and then moved into studying the actual protein–peptide interaction as well, providing the first definitive evidence that cation–π interactions provided the dominant component to binding in this important class of interactions [26].

This work led to several important formal and informal collaborations with others doing research in the area of chromatin remodeling, and thus paved the way for a new direction in our research. I had been fascinated by the groundbreaking work of Jeremy Sanders and co-workers on dynamic combinatorial chemistry (DCC) while being a graduate student and postdoc (Figure 7) [42,43]. Like folded peptides that self-assemble into their functional state, DCC allows molecules to self-assemble in the presence of a template. Moreover, DCC is highly amenable to structure–function studies, since only a new monomer must be synthesized, rather than an entirely new receptor. With my

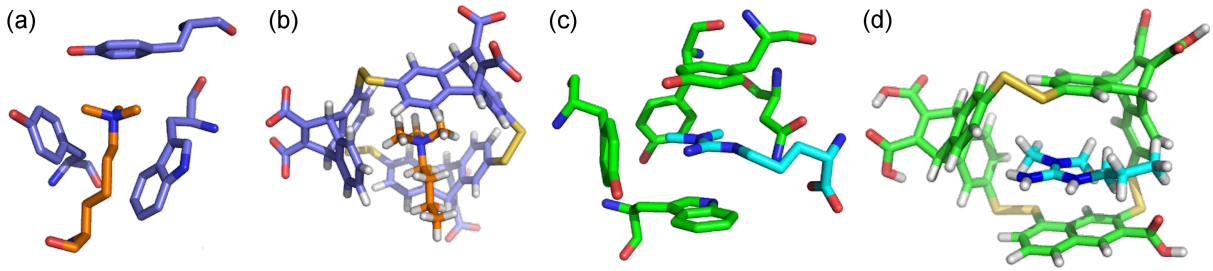

Figure 6: (a) Binding pocket of the *Drosophila* HP1 chromodomain (blue) bound to trimethyllysine (orange), PDB code: 1KNE [39]. (b) Computational model of a synthetic receptor, **A_2N** (blue), bound to KMe3 (orange) [40] (c) Binding pocket of the SMN tudor domain (green) bound to asymmetric dimethylarginine (aDMA, cyan). (d) Computational model of a synthetic receptor, **A_2D** (green), bound to aDMA (cyan) [41].

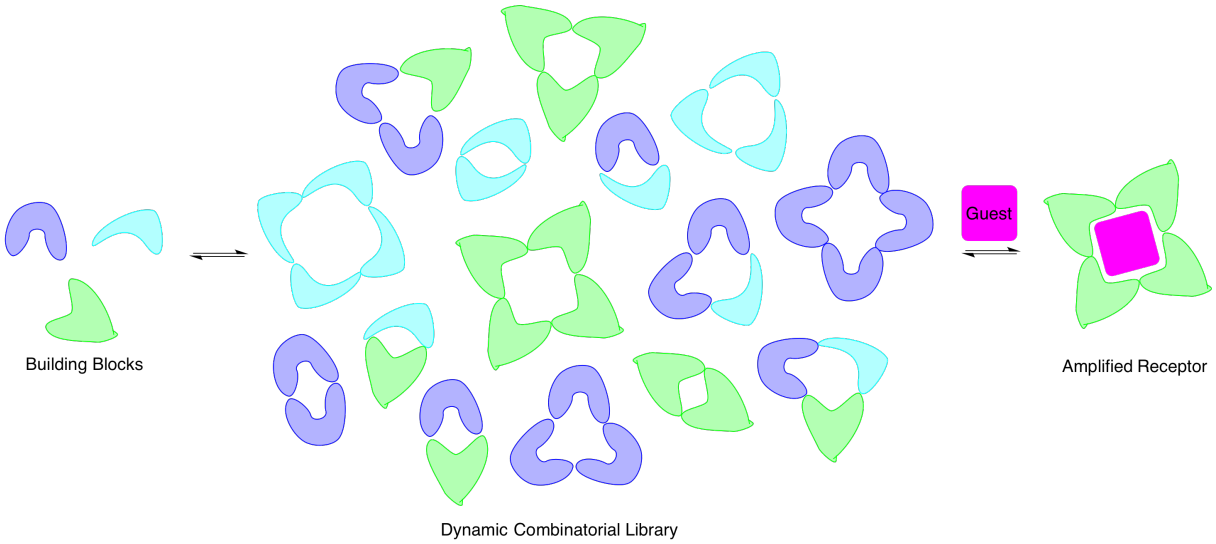

Figure 7: Dynamic combinatorial chemistry [41,42].

interest in trimethyllysine provided a significant problem in which DCC seemed to be a promising solution. It turns out that the main tool for sensing protein post-translational modifications such as trimethyllysine are antibodies, but antibodies have significant limitations in this context, as they are too sequence specific. Thus, we aimed to develop synthetic receptors that would mimic the binding pockets of proteins to recognize trimethyllysine, but not the surrounding sequence. This turned out to be an ideal problem to address using DCC, and we have now developed a number of synthetic receptors for methylated lysine and arginine that have applications as sensors for these modifications (Figure 6).

Lessons learned

As a child, my parents said that I "marched to my own drummer". In my career I have continued to follow my interests wherever they have led me, which at points has meant effectively changing fields. This means that I always have new things to learn, which always keeps me interested. I look forward to seeing what is around the corner in the years to come.

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