Molecular Trees: A New Branch of Chemistry

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If the atomic organization of organic and inorganic matter could be defined precisely on a nanometer scale, then "smart" materials might be fabricated that could revolutionize many technologies. Among the possibilities, nanoscale optical, electronic, and mechanical devices would enable further miniaturization of information processing and energy transduction. Designed materials capable of recognizing molecules and metal ions could be used as molecular filters, selective chemical catalysts, or chemical sensors. Many chemists and materials scientists are trying to tackle the problem of controlling matter in the size range of 1 to 10 nm from opposite ends: The synthetics chemist traditionally builds large structures from smaller subunits, whereas others typically use semiconductor processing techniques that are limited to scales above 50 nm. In this issue, Zimmerman, Zeng, Reichert, and Kolotuchin (*1*) report the self-assembly of synthetic molecular subunits to form nanoscopic particles of precisely defined structure.

Just a few decades ago, it would have been staggeringly difficult to synthesize copies of complicated nanometer-size molecules found in nature. Now, this task can be accomplished by selecting reagents from the vast array assembled mainly during the last 30 years. Step-by-step, bond-by-bond synthesis of large molecules from smaller ones is an effective way to prepare complex natural products and analogs for pharmaceutical testing, but not for controlling molecular architecture on a larger scale. The reproduction of natural products continues to stimulate creative development of synthetic methodology, but the synthesis of large structures designed for specific functions will become a major challenge for synthetics chemists in the next century. Synthesis is the weak link in the design-synthesis-testing feedback loop; progress could be accelerated through synthesis of smaller molecules capable of aggregating to form "supramolecular" structures (*2*).

Practitioners of both "top-down" and "bottom-up" nanoscale materials synthesis have borrowed from nature the concept of molecular self-assembly (*3*), which originates from studies on the spontaneous assembly of infective tobacco mosaic virus particles from 2130 identical protein molecules and a single strand of viral RNA (*4*). The "self" in self-assembly indicates that the subunits are "programmed" with the information necessary for error-free assembly of a specific supramolecular structure (*2, 3*). Self-assembly can be applied in two different ways to the fabrication of nanoscale materials. Spontaneous molecular aggregation occurs during the formation of specific phases, such as films, membranes, vesicles, micelles, liquid crystals, and solids. This is an important area of research, but crystallization has proven very difficult to control, and fluid phases are not homogenous on a molecular level. Specific interactions between surfaces and molecules in films can be exploited to produce a promising class of two-dimensional substances known as self-assembled monolayers. The other general approach is to design molecules having binding sites positioned for specific aggregation to form larger complexes. These complexes can then be crystallized or used to form partially ordered phases. The ultimate level of control over nanoscale organization will be achieved when complexes can be designed to aggregate and form specific "supra-supramolecular" structures.

To design materials based on self-assembling molecules, chemists need a detailed understanding of the factors controlling molecular interactions. Thanks to the pioneering work of Cram (*5*), Lehn (*2, 6*), Pedersen (*7*), and others over the last 25 years, important advances have been made in the design of molecular complexes in which synthetic molecules are used to mimic natural molecular recognition processes. Earlier studies in supramolecular or host-guest chemistry focused on the complexation of alkali metals by crown ethers and cryptands, but in the last decade, effective artificial receptors have been developed for recognition of neutral organic molecules by hydrogen bonding (*8, 9*).

Self-assembled complexes are appearing from the laboratories of chemists in increasing numbers. Over the last two decades, ligands having binding sites for more than one transition metal atom were designed, synthesized, and found to produce self-assembled metal complexes of predictable composition and geometry (*2, 3, 10*). The double-helical and triple-helical "helicates" are an important class of self-assembled metal complexes (*10*). The self-assembly concept has also been used to simplify the synthesis of multiply linked and knotted molecular rings (*11*) and to produce cavities capable of encapsulating small molecules (*12*). The use of hydrogen bonding to control molecular self-assembly has been explored recently by the research groups of Hamilton (*13*), Rebek (*12*), Whitesides (*14*), Zimmerman (*15*), and others.

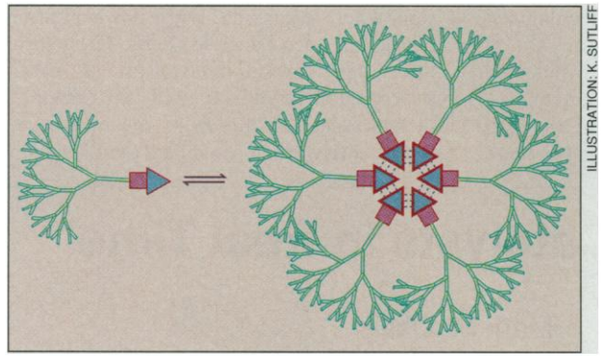

ILLUSTRATION: K. SUTLIFF

Self-planting trees. Equilibrium-controlled self-assembly of six wedge-shaped molecules bearing highly branched side chains. The six pie wedges are held together by hydrogen bonds between carboxylic acid groups. Contact between the branched side chains apparently stabilizes the 9-nm assembly through attractive van der Waals forces.

During the last decade, there has been an explosive growth in studies of a new type of polymer: the dendrimer (*16–18*). These treelike molecules branch out from a central core to form globular particles. The patterns of chemical bonds in the multiple layers or "generations" radiating from the core resemble fractals. The beauty of dendrimers is that their size and architecture can be specifically controlled in their synthesis. They have been hailed as promising building blocks for fabrication of designed materials, as molecular scaffolds for chemical catalysts, and as potential vehicles for delivery of drugs and immunogens (*18*). Several creative approaches to the synthesis of the dendrimers have been reported, but their usefulness is limited by the tedious nature of stepwise synthesis. A "self-condensing" polymerization approach to dendrimers was reported recently (*17*), but polymerization gives a statistical distribution of products, so molecular structure and properties cannot be controlled accurately.

Zimmerman, Zeng, Reichert, and Kolotuchin (*1*) have applied the principle of self-assembly to dendrimer synthesis.

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They have shown that, when appropriately designed, six wedge-shaped subunits bearing highly branched tails can self-assemble to form cylindrical rosettes of nanoscopic dimensions (see figure). Thus, the organic chemist needs only to design and synthesize one-sixth of the desired structure. The structure of Zimmerman's pie wedge is based on those of his "molecular tweezers" that bind aromatic guest molecules by intercalation between aromatic rings spaced 0.7 nm apart (*8*). These spaces commute with a larger internal void in the supramolecular assembly, and it would be interesting to find out what guests molecules could be bound or trapped in this cavity.

The benzene rings in both layers of each pie wedge bear two carboxylic acid groups oriented at 60° angles with respect to each other. These 60° angles, combined with the linear geometry of the carboxylic acid dimer, dictate that six of the tetracarboxylic acid wedges will form the most stable assembly. Chemical systems seek the lowest energy situation, and the numerous studies on equilibrium-controlled molecular self-assembly point to one conclusion: the favored aggregate is the lowest molecular weight structure that satisfies the number of binding sites and their geometric constraints. Although larger aggregates might also satisfy the hydrogen bonding preferences of the subunits, entropy favors the largest number of particles and, therefore, the smallest particles. Zimmerman's six-wedge model is supported by the observation that discrete assembly of six subunits is most favorable when the branched tails are in good contact. The van der Waals interactions between the surfaces of these globular tails apparently stabilize the supramolecular assembly. Smaller or more flexible tails do not give stable six-wedge complexes; instead, open-ended polymer chains are formed.

The self-assembling dendrimers resemble Whitesides' soluble rosettes (*14*), but their 9 nm by 2 nm dimensions place them in the realm of nanostructures. There is also a superficial resemblance to the ion-mediated assembly of discotic liquid crystals from taper-shaped molecules (*19*), but the structures of dendrimers can be precisely controlled, at least in the vicinity of the core. This self-assembly approach also allows for much better control of molecular architecture when compared with polymerization methods for dendrimer synthesis. The logical next step toward fabrication of nanostructured materials is to apply the principles of molecular recognition at the next level: self-assembly of self-assembled dendrimers.

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Evolving Catalysts in Real Time

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Antibodies that catalyze chemical reactions can be produced at will by immunizing animals with stable analogs of specific transition states (*1*). These antibodies are potentially enormously useful for catalysis of standard reactions in novel formats and, especially, for generation of entirely new catalysts. The field has been hindered by the relative inefficiency of most of the antibodies produced to date, so there have been intense efforts to improve the catalytic ability of these molecules. The reports from Patten *et al.* (*2*), presented in this issue, and from others (*3*, *4*) of x-ray crystal structures of catalytic antibodies bound to transition state analogs are therefore important in laying the groundwork for rational design of better catalysts.

Patten *et al.* (*2*) do more than this, however. They also take a look backward and explore the immunological evolution of their catalytic antibody. They derive the sequence of the original protein that existed before the action of somatic hypermutation, the specific type of mutagenesis that produces higher affinity antibodies over the course of an immune response. The 14,000-fold improvement in affinity that they see between the mutated antibody and the original shows the power of somatic hypermutation. This large improvement in affinity also brings about a 100-fold increase in the catalytic ability of the antibody, suggesting that further gains in affinity might produce even better catalysts.

The antibody studied by Patten and coworkers was generated by immunization with a nitrophenyl phosphonate transition-state analog and catalyzes the hydrolysis of an ester or the related carbonate derivatives. The structure shows that the hapten is bound in a 10 Å–deep pocket surrounded by six tyrosines, with the aromatic nitro group at the bottom. This pocket structure exists in the antibody binding sites for most haptens, including another phosphate analog (*4*) and arsonate (*5*). The identity of the residues surrounding the hapten suggests possible mechanisms of catalysis; mutagenesis implicates some of these residues as critical, particularly a histidine at position 35 of the variable region of the immunoglobulin heavy chain (V_H).

These new data are the most striking illustration yet of how dramatic the effects of somatic hypermutation can be on the affinity of an antibody and even yield some clues as to how this is achieved. B lymphocytes usually first encounter antigens in the lymph nodes, and at this stage they express nonmutated (germline) immunoglobulin M (IgM) antibodies on their cell surface (see figure). After encountering antigen and receiving "help" from T cells (in the form of cytokines), the B lymphocyte can differentiate along several pathways. One of these induces, by an unknown mechanism, somatic hypermutation of the two variable regions V_H and V_L (L indicates the light chain) but not the constant regions (C_H or C_L). Subsequent encounters with antigen select higher affinity variants to advance to the plasma cell stage, which secretes immunoglobulin. This is usually (but not always) accompanied by a rearrangement that brings the V_H exon adjacent to a new C_H, resulting in the secretion of a new immunoglobulin type (such as IgG or IgA) (*6*).

The authors used the heavy and light chain immunoglobulin sequences that they had isolated to find the original germline sequences. They then reconstructed this anti-

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