Self-Assembly

Getting Molecules to Put Themselves Together to Make Nanoscale Devices

Over the past century, chemists have become lords of the covalent bond, the ultra-strong chemical coupling that results when two neighboring atoms share some of their electrons with each other. Having invented hundreds of chemical reactions that form and rearrange covalent bonds, chemists can now build large, complex molecules almost at will, rivaling nature's ability to create substances and materials with a range of useful properties. Indeed, most of today's life-saving drugs owe their existence to chemists' mastery of the covalent bond.

Now, however, chemists are trying to tackle the harder problem of making what are known as supramolecular nanostructures—huge assemblies of molecules that make up nanoparticles and other nanoscale devices for use in detecting and treating cancer. "When we talk about supramolecular chemistry, about making nanoparticles for use in diagnostic and therapeutic applications, then we're really talking about taking advantage of the weaker, non-covalent interactions that nature uses to put together large assemblies of molecules," said Jinming Gao, Ph.D., who recently moved his research team to the Simmons Comprehensive Cancer Center at the University of Texas Southwestern Medical Center in Dallas. Gao also has a joint appointment at the University of Texas at Dallas.

Supramolecular structures are nothing new to nature. A cell is perhaps the ultimate supramolecular structure, comprising many millions of molecules held together by these weaker chemical forces. Other supramolecular structures include mitochondria, where a cell generates its energy; ribosomes, which make proteins; and viruses. Liposomes are an example of a human-made supramolecular structure.

What is perhaps most amazing about these enormously complex structures is that they put themselves together. Chemists call this process "self-assembly," and it's a process that they would like to make better use of in order to put together new types of nanoparticles and other useful nanoscale structures. "It's hard to imagine how we can put together the complex structures we're now trying to build for drug delivery or nanoscale devices unless we take advantage of self-assembly," said Mehmet Sarikaya, Ph.D., of the University of Washington in Seattle.

A second chemical language

If there's a *lingua franca* for self-assembly and supramolecular chemistry, it's a much subtler one than chemists have dealt with in the past. They understand that this new vocabulary is based on what are called hydrogen bonds, van der Waals forces, π–π interactions and other energetic influences that attract atoms to one another without causing them to formally exchange electrons. The grammatical rules for this new language, however, are still in the making, but researchers are gradually learning the rules for how small molecules recognize one another and assemble to make stable, large structures. As they master these rules, they are beginning to use them to assemble large molecular complexes by design. For example, Samuel Stupp, Ph.D., of Northwestern University in Evanston, IL, has purposefully created mushroom-shaped assemblies that resemble cellular receptors and nanometer-wide ribbons and rods using self-assembly. Each of these structures comprises thousands of molecules that come together in an orderly manner to create these stable supramolecular structures, all held in place by non-covalent bonds.

Courtesy: Jinming Gao, Ph.D., University of Texas

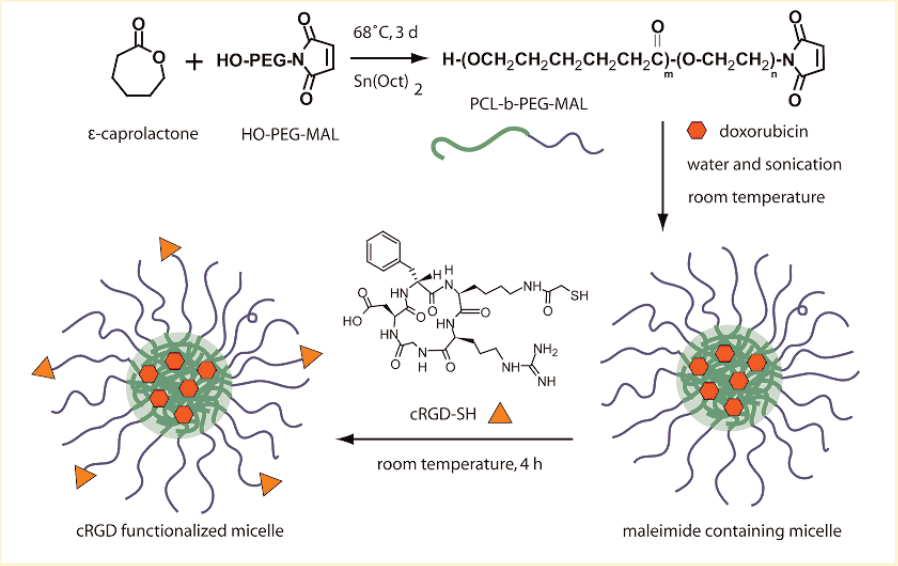

Figure 1.
Synthesis of MAL-PEG-PCL copolymer and preparation of cRGD-functionalized, DOXO-loaded micelles.

Nature has inspired most of these advances, and for good reason. From relatively simple supramolecular complexes that bring together a few protein subunits to create something functional, such as hemoglobin, to more complex entities, such as the ribosome, cell membrane, and bone, biological systems make heavy use of non-covalent bonds, particularly hydrogen bonds and hydrophobic interactions. Hydrogen bonds form when a hydrogen atom is shared between two electron-rich atoms, such as oxygen or nitrogen, while hydrophobic interactions cause hydrocarbon-rich portions of molecules to pack closely together to minimize their exposure to water. These two forces combine, for example, to stabilize the double helix of DNA: hydrogen bonds pull the two strands together, while the hydrophobic rings of the nucleic acid bases stack upon one another to make the strands more rigid. This stacking is further stabilized by the interactions between π–electron clouds that circle above and below these rings, a result of the special electronic structure of the rings that are found in nucleic acid bases and many other biomolecules.

Hydrophobic interactions are key to the nanoparticles that Gao and his colleagues are creating via self-assembly. These particular nanoparticles are known as micelles, and they are made using block co-polymers that are part water-loving (hydrophilic) and part water-hating, or hydrophobic. When mixed together in water, the hydrophobic stretches of the linear polymer molecules come together in order to minimize their exposure to the watery environment. This self-assembly process yields a rugged nanoparticle with a solid core composed of the hydrophobic portions of the polymer and a shell comprising the hydrophilic segment of the same polymer chains. When other hydrophobic molecules, such as the anticancer drug doxorubicin, are added to the initial mixture of polymer molecules, the resulting micelle encapsulates that molecule in its hydrophobic core (see Figure 1).

The hydrophilic surface of these micelles provides ready anchoring points for targeting molecules. One example of such a targeting molecule is a synthetic peptide known as cRGD. This molecule comprises five amino acids linked together in a ring, and it binds to a protein known as $\alpha_v\beta_3$, which is over-expressed on the surface of the newly formed blood vessels that nourish growing tumors. Gao and his colleagues have developed a way of attaching cRGD to an already-formed, doxorubicin-loaded micelle that does not affect the integrity of the micelle structure. Atomic force microscopy images of the targeted micelle particles showed them to be spherical and fairly uniform in size, with an average size of 43 nanometers (see Figure 2). *In vitro* experiments showed 30-fold more of the targeted micelles were readily taken up by cells that over-express $\alpha_v\beta_3$ compared to the uptake of untargeted micelles.

Courtesy: Jinming Gao, Ph.D., University of Texas

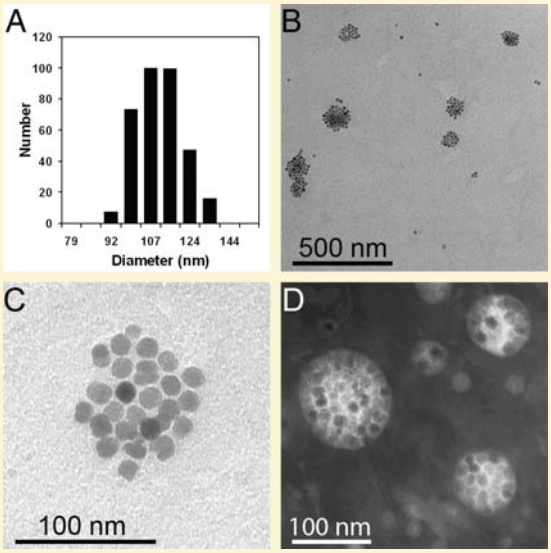

Figure 2.
(A) Dynamic light scattering histogram showing the size distribution of SPIO (16 nm)-loaded polymeric micelles based on PCL5k-b-PEG5k copolymer (mean diameter: 110 nm). (B) TEM of these micelles at low magnification. Isolated clusters of SPIO particles were observed. (C) A high magnification TEM image of the micelle (D) TEM of the same micelle sample after negative staining by 2% PTA. SPIO cluster was found to localize inside the "bright" hydrophobic cores of micelles.

Since completing that experiment, Gao's group has also prepared targeted micelles containing a novel hydrophobic formulation of iron oxide nanoparticles. By varying the size of the iron oxide nanoparticles, the researchers succeeded in preparing micelles that could be detected by magnetic resonance imaging (MRI) at concentrations as low as 5.2 nanomolar. At that level of sensitivity, these micelles could provide a way of detecting sparse cancer-related surface markers such as $\alpha_v\beta_3$. In fact, Gao's group is in the process of preparing cRGD-targeted, iron oxide-loaded micelles for doing just that, and he looks forward to moving these micelles toward human clinical trials. "That's the big reason I moved my laboratory—to take advantage of the opportunities for clinical development that UT-Southwestern has set up in oncology."

Hydrophobic bonding is also what holds together the core-shell nanoparticles that Gregory Lanza, M.D., and Samuel Wickline, M.D., both at the Washington University School of Medicine in St. Louis, created and that biotechnology company Kereos, also based in St. Louis, is developing for targeted imaging and therapeutic delivery. These nanoparticles, some of which are now in human clinical trials for tumor imaging, have a biodegradable perfluorocarbon core surrounded by a lipid shell that stabilizes the particle. Hydrophilic drugs, imaging agents or targeting molecules can be attached to the exposed portion of the lipid, while hydrophobic drugs or imaging agents will embed themselves in the heart of the lipid layer. One unusual property of these nanoparticles is that when they contact a targeted cell, the lipid shell of the nanoparticle and the lipid bilayer of the cell exchange components. Through this contact-mediated process, drug molecules in the shell get passed to the targeted cell. This interchange could not happen if the outer shell were covalently bonded to the core.

Courtesy: Mehmet Sarikaya, Ph.D., University of Washington, Seattle

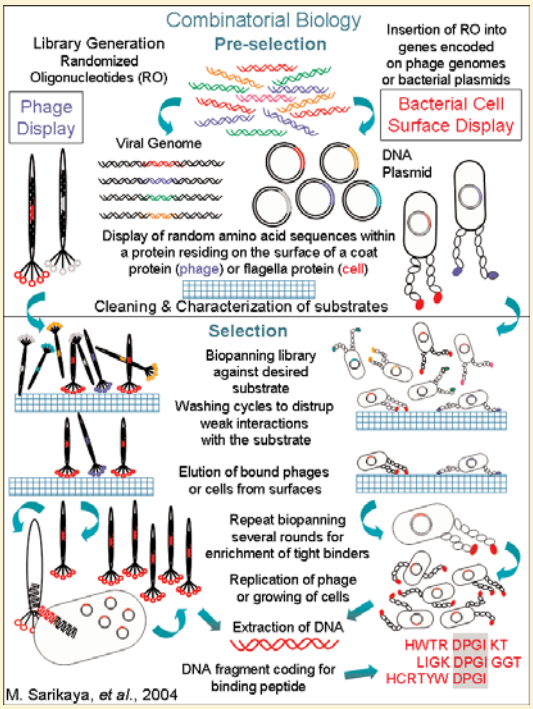

Figure 3.
The technology for creating and screening millions of peptides on the surface of a virus can rapidly lead researchers to molecules capable of binding to specific materials or inorganic molecules.

Another promising aspect of self-assembly is the ability to marry organic and inorganic materials, something that materials scientists have had difficulty accomplishing. "If you don't have to rely on covalent bonds, then there are many avenues to bring organic and inorganic molecules together in a structurally durable manner," said Buddy Ratner, Ph.D., director of the Center for Engineered Biomaterials at the University of Washington, Seattle. Ratner, together with Thomas Boland, Ph.D., who is now at Clemson, showed in 1995 just how easy this melding could occur. "We made the accidental discovery that if you dipped a piece of gold into a dilute solution of nucleic acid in ethanol that, boom, they self-assemble into ordered 2-dimensional films that were bound tightly to the gold. It was that simple."

Since then, chemists have developed a wide range of techniques for marrying the organic world of proteins, carbohydrates and nucleic acids to the inorganic world of metals and semiconductor materials, such as silicon and glass. Ratner's group, for example, has developed several systems that self-assemble into large supramolecules capable of coating the surfaces of implantable medical devices. One such system is designed to respond to ultrasound and could be used to create drug delivery devices that would only release their payload when irradiated with focused ultrasonic energy at the site of a tumor. Ratner characterized recent results using this material as "very promising."

Many of the methods chemists are developing for self-assembling organic and inorganic materials take their cue from biology. "Nature has evolved a wide range of proteins whose role is to bind inorganic materials such as [the mineral component of] bone," explained Sarikaya who is engineering peptides to bind specifically to different types of inorganic materials without the need for covalent bonds. "After all, proteins are the ultimate tool for self-assembly because it's part of their function to recognize specific molecules and materials and when they find the molecules to stick to them, they do with a great deal of strength."

Sarikaya and his colleagues use combinatorial biology techniques such as phage display and cell-surface display, which harness a virus' or a bacterium's protein-making mechanisms to generate random peptide sequences on the virus' outer coat or cell's membrane, respectively (see Figure 3). The millions of resulting peptides can then be screened rapidly to determine which ones bind specifically to a desired molecule or material. The goal of this work is to create a toolbox of peptides that can be used to marry biomolecules to metals and other inorganic materials, which could prove useful for developing biocompatible *in vivo* biosensors and drug delivery devices.

Over a hundred years ago, Hermann Emil Fischer of Germany invented the field of organic chemistry through his work developing chemical reactions for making covalent bonds. Besides winning himself the second Nobel Prize in Chemistry ever awarded, Fischer's accomplishments set off a chemical revolution that has given us a wealth of life-saving drugs. Nearly twenty years ago, chemists Donald Cram, Jean-Marie Lehn, and Charles Pederson conducted pioneering work in self-assembly that also won a Nobel Prize in Chemistry. Though it's still too early to predict the ultimate impact of their work, it is safe to say that without self-assembly, the field of nanotechnology would hold far less promise for changing the way cancer is detected and treated.

— Joe Alper

References

Goncalves IC, Martins MC, Barbosa MA, Ratner BD. Protein adsorption on 18-alkyl chains immobilized on hydroxyl-terminated self-assembled monolayers. *Biomaterials*. 2005. 26(18):3891-99.

Kwok CS, Mourad PD, Crum LA, Ratner BD. Self-assembled molecular structures as ultrasonically-responsive barrier membranes for pulsatile drug delivery. *J Biomed Mater Res.* 2001 Nov;57(2):151-64.

Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, Boothman DA, Gao J. cRGD-functionalized polymer micelles for targeted doxorubicin delivery. *Angew Chem Int Ed Engl.* 2004. 43(46):6323-7.

Oren EE, Tamerler C, Sarikaya M. Metal recognition of septapeptides via polypod molecular architecture. *Nano Lett.* 2005. 5(3):415-9.

Sarikaya M, Tamerler C, Jen AK, Schulten K, Baneyx F. Molecular biomimetics: nanotechnology through biology. *Nat Mater.* 2003 2(9):577-85.

Schmieder AH, Winter PM, Caruthers SD, Harris TD, Williams TA, Allen JS, Lacy EK, Zhang H, Scott MJ, Hu G, Robertson JD, Wickline SA, Lanza GM. Molecular MR imaging of melanoma angiogenesis with alphanubeta3-targeted paramagnetic nanoparticles. *Magn Reson Med.* 2005.53(3):621-7.