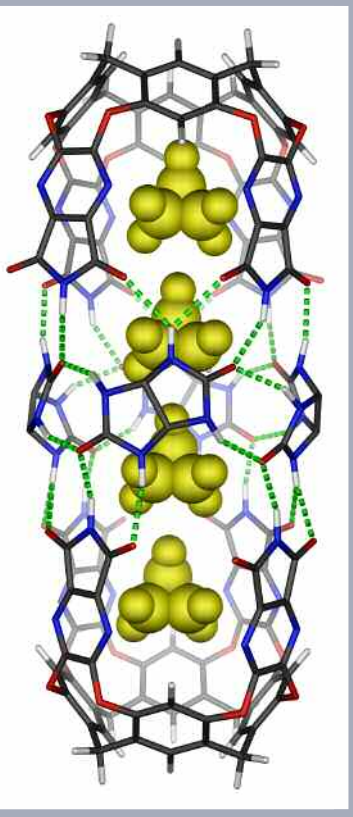
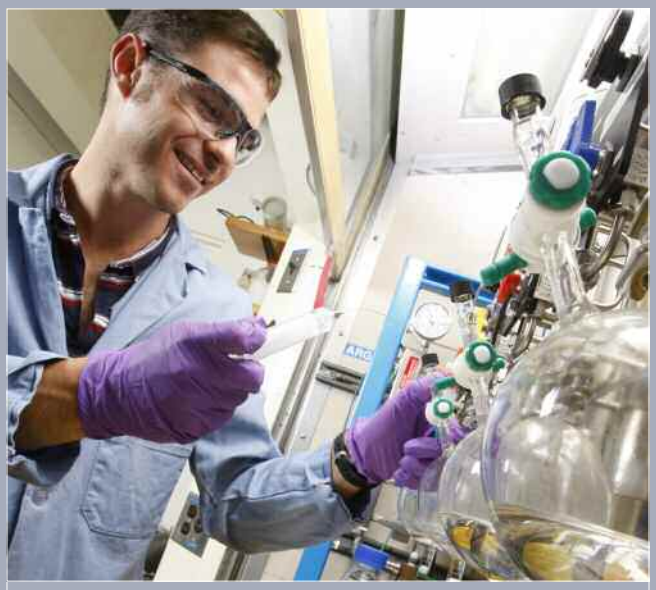
Skaggs Institute for Chemical Biology

Gas encapsulation. Four cyclopropane molecules are reversibly bound in self-assembled, hydrogen-bonded capsules under ambient conditions. The gas pressures in these complexes deviate from ideal gas behavior as a result of CH-π interactions with the capsule wall. A stable arrangement results when approximately 40% of the space is occupied. Work done in the laboratory of Julius Rebek, Jr., Ph.D., professor.





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*** Joint appointment in the Department of Molecular Biology*

**** Joint appointment in the Molecular and Integrative Neurosciences Department*

***** Joint appointment in the Departments of Chemical Physiology and Chemistry*

****** Joint appointment in the Department of Neurobiology*

† Deceased

†† Joint appointments in the Departments of Molecular Biology and Immunology

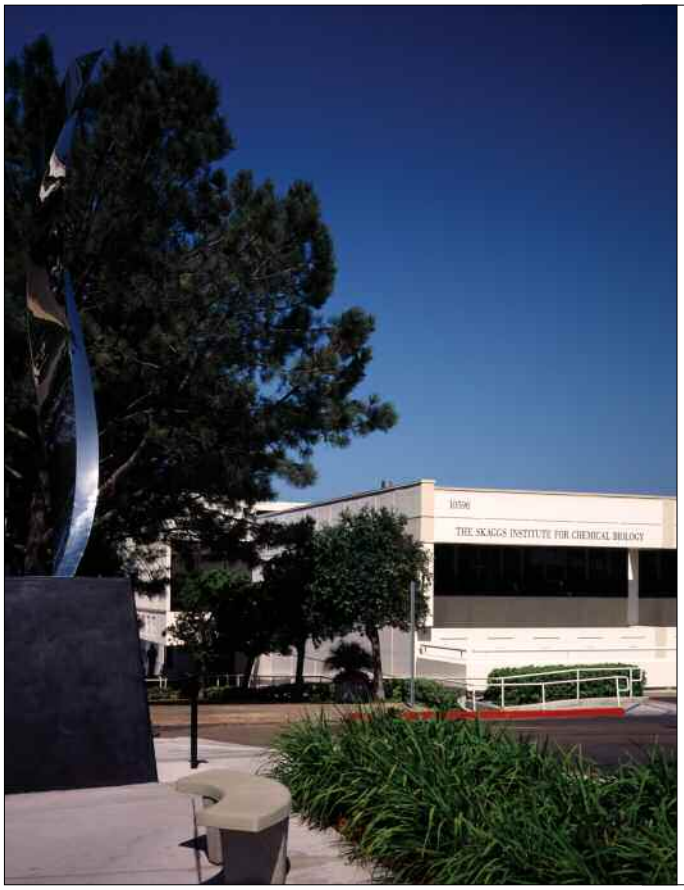
††† Joint appointments in the Departments of Chemistry and Molecular Biology

†††† Joint appointment in the Department of Cell Biology

††††† Rebek laboratory staff. Staff of the other members are listed in their respective departments

‡ Appointment completed

‡‡ Appointment completed; new location shown



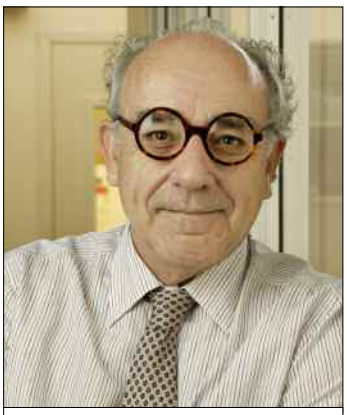
THE SKAGGS INSTITUTE FOR CHEMICAL BIOLOGY

In 1996, The Scripps Research Institute established The Skaggs Institute for Chemical Biology, made possible by a gift of more than $100 million to The Skaggs Institute for Research from Aline W. and L.S. Skaggs.

Scientific members of the Skaggs Institute hold dual appointments in various departments at Scripps Research. These scientists have broad expertise in areas including the structure of biological macromolecules, chemical and antibody catalysis, synthetic and combinatorial chemistry, molecular recognition, and molecular modeling methods. With the achievements of its staff, the Skaggs Institute has assumed its research identity in the United States and throughout the world at the interface of biology and chemistry.

Director's Overview

The Skaggs Institute for Chemical Biology was established in 1996 by a spectacular gift from L.S. "Sam" Skaggs. During the past 12 years, more than 100 million dollars has been awarded in research support for members of the Skaggs Institute. Currently, the funding supports 31 principal investigators, 99 postdoctoral fellows, and 61 graduate students. The mission of the institute is to conduct science that leads to new medicinal agents to relieve suffering. Here I describe some of the progress made by members toward these goals. More details can be found in the individual reports.



Julius Rebek, Jr., Ph.D.

Stephen Mayfield has used his genetically modified algae to produce carbon-neutral liquid biofuels, a splendid result at a time when fossil fuels reserves are dwindling. Algae can produce biomass at a rate higher than terrestrial plants do and can be used to synthesize therapeutic proteins. In short, algae are a versatile and renewable energy source.

M. Reza Ghadiri has developed new cyclic peptide mimetics as scaffolds to present amino acid side chains involved in protein-protein interactions. Using the triazole as a peptide bond surrogate, he has developed useful bioactive probe molecules that imitate the 3-dimensional pharmacophore of naturally occurring tetrapeptides.

M.G. Finn continues to modify the surfaces of intact viral capsids by using, among other methods, click chemistry. These modifications have been used to display carbohydrates on the exterior capsid surface as well as polycations that efficiently inhibit the action of heparin.

Jeff Kelly, the new chairman of the Department of Molecular and Experimental Medicine, is studying the role of amyloidosis in diabetes. Deposits of amylin in the pancreas are related to the compromised function of these secretory cells that characterize the disease.

Jamie Williamson has developed a powerful enzymatic synthesis of nucleotides such as adenosine triphosphate. The process involves 28 enzymes but can be carried out in 60% yield starting from glucose, carbon dioxide, ammonia, and serine. The synthesis is ideal for isotopically labeled products for use in nuclear magnetic resonance analysis of the structure of proteins and nucleic acids.

Ullrich Müller is studying the hair cells of the inner ear that are the principal mechanosensors for the detection of sound and head movement. He is unraveling the molecular composition of the mechanotransduction machinery in these cells by identifying the genes that control their functions.

Ehud Keinan has proposed a general synthetic strategy of using a simple pentagonal core to produce chemical capsids that are approximately the size of spherical viruses. He has modeled the assembly and dissociation of these systems under controlled environmental conditions and has made progress in synthesizing the molecules that have the proper shapes and recognition surfaces.

Dale Boger and his group work on inhibiting enzymes that control natural painkillers such as anandamide. They have developed synthetic molecules that are more efficient than ibuprofen and are similar to morphine in potency as analgesics in neuropathic pain.

Carlos Barbas used a reductionist approach on catalytic antibodies to identify the key features of their catalytic abilities. He has shown that simple chiral amines can be nearly as effective in asymmetric catalysis for many reactions that make complex carbon-carbon bond arrays.

Geoffrey Chang has developed x-ray crystallography to characterize molecules involved in multidrug resistance. These molecules transport small drug molecules from inside the cell to outside and are involved in the efflux of antibiotic compounds. The goal is to develop inhibitors of the process that can be used in the treatment of infections.

Gerald Joyce, dean of the Scripps Research faculty, has developed "evolution on a chip." This method combines a large population of RNA molecules and computer controlled microfluidic chips that allow adaptation to occur through hundreds of cycles in a few days. He has also developed small molecules that can trigger RNA enzymes to catalyze their own formation: molecular replication.

Kim Janda is working to manipulate the chemical biology of cell-to-cell signaling known as quorum sensing. His findings have applications in controlling virulence and infectivity of bacterial and other microbial agents.

Peter Schultz continues to add more amino acids to the repertoire of synthetic biology. Proteins made from amino acids with an expanded genetic code can confer an evolutionary advantage and improved pharmacologic properties. These proteins are directed to applications in biomedical technology.

Ian Wilson continues to study those few potent but broadly neutralizing antibodies that recognize HIV type 1. The elusive goal is still to develop the structural information in these complexes for use in a vaccine.

Lisa Stowers studies neural circuits that underlie innate behavior. She uses olfactory stimulus in rodents to identify the neurons involved. Her studies suggest that maternal-infant behavior in rodents is also triggered by olfactory mechanisms.

In prebiotic chemistry, a debate continues on the relative importance of replication vs metabolism in the origins of life. Albert Eschenmoser is making progress on both of these fronts. He and his group make use of ever-simplified backbones derived from glyceric acid for replication and explore the chemistry of glyoxylate for metabolism.

Chi-Huey Wong has invented a new method for the ligation of peptides in which attached sugars are used as delivery vehicles. The intent is to optimize the methods to achieve the total synthesis of therapeutic glycoproteins as single isomers.

In my own research group, we continue to explore the behavior of molecules in small spaces. These arrangements, known as encapsulation complexes, isolate molecules from the medium and expose unusual behaviors, shapes, and reaction intermediates that cannot be seen in solution.

Among the honors bestowed on the Skaggs investigators, 2 were particularly noteworthy. Peter Wright, chairman of the Department of Molecular Biology, was elected to the National Academy of Science, and Tamas Bartfai, Chairman of the Molecular and Integrative Neurosciences Department, was elected to the Swedish Academy of Sciences. Members of the Skaggs Institute won numerous national and international prizes and earned many honorary degrees in the past year.

My colleagues and I are grateful for the continued support of the Skaggs Institute for Research. They provide generous funding for basic science at the interface of chemistry and biology.

Investigator's Report

Reversible Encapsulation: Molecules at Close Range

J. Rebek, Jr., D. Ajami, M. Ams, E. Barrett, T.J. Dale, H. Dube, R.J. Hooley, J.-L. Hou, T. Iwasawa, S. Kamioka, A. Lledo Ponsati, L. Moisan, F.P. Restorp, M. Schramm, S. Shenoy, C. Turner, H. Van Anda, S. Xiao

MOLECULAR ENCAPSULATION

Molecules with appropriate curvature and hydrogen-bonding capabilities can self-assemble into "host" container structures when suitable "guests" are present. In Figure 1, 2 units of the module **1** assemble into the cylindrical capsule **1.1** in the presence of smaller molecules that fill the space inside properly. The coencapsulation of 2 guests within a host must fulfill certain requirements: the congruence of molecular shapes, the compatibility of lengths, the conformity with volumes, and the complementarity of chemical surfaces. The examples shown in Figure 1 reveal that these criteria can be met by many combinations of guests.

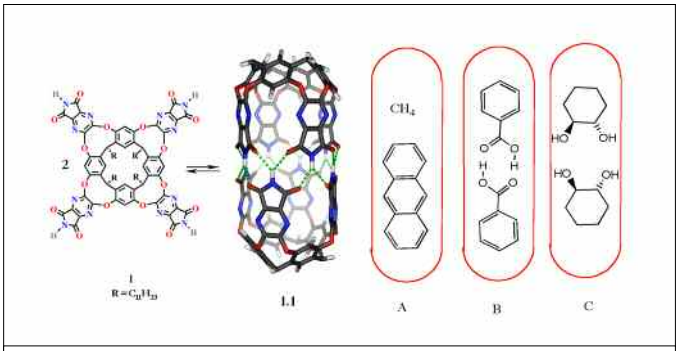


Fig. 1. Formula of the module **1** and its dimeric capsule **1.1**. The capsule is shown without peripheral alkyl groups, and the cartoon used elsewhere in this report is shown with a large and a small guest (A), 2 identical guests (B), and a guest and its mirror image (C).

REMOTE INFLUENCES

Molecular encounters in solution last typically a billionth of a second, and the collisions take place randomly. But molecules in capsules can be detained for hours, and their collisions are guided by their arrangement inside. The long contact times of coencapsulated molecules and their fixed orientation in the space allow observation of very subtle differences. For example, coencapsulation of isopropanol with isomers of the diol shown in Figures 2A and 2B leads to 2 different complexes.

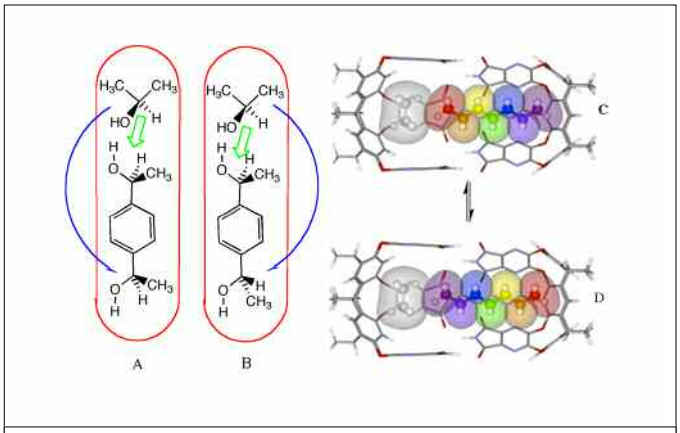


Fig. 2. A and B, Coencapsulation amplifies magnetic effects of remote asymmetric centers. The NMR signals for the isopropyl alcohol (top) are influenced by the nearby chiral center (green arrow) as well as the distant center (blue arrow). C and D, Coencapsulation of ethane (gray) and heptane (colored spheres). NMR experiments show that heptane tumbles inside the capsule; the ethane makes contact with atoms at either end of heptane but not with the atoms in the middle.

The asymmetric center near the isopropanol is the same in both, but the distant centers are of opposite chirality or handedness. The nuclear magnetic resonance (NMR) signals are different for the 2 complexes. Such influences cannot be seen in bulk solution because of the rapid exchange of partners and the free rotational motions that average the effects.

The coencapsulation of ethane and heptane is shown in Figures 2C and 2D. NMR experiments revealed that ethane makes contact with both ends of the heptane. This situation is caused by the end-over-end tumbling of heptane in the capsule. It is thought that heptane coils into a more compact shape during the tumbling motion.

REACTIVITY

The chemical reactions that take place only slowly in bulk solution can be dramatically enhanced inside capsules. One reason is the long contact times of the 2 coencapsulated reactants. Another reason is a concentration effect; each molecule inside has a concentration of 4 M, even if the concentrations outside are 1000-fold lower. A third reason is the limited motion of the capsule itself; the capsule acts as a solvent cage already organized for the reaction. The reaction of an isonitrile with a carboxylic acid can be accelerated by confining both components to a capsule. The elusive initial addition product has been directly observed by using NMR methods. The capsule not only accelerates the reaction but also acts as a catalyst. The product is released, and the capsule can be refilled with starting reagents (Figs. 3A and 3B).

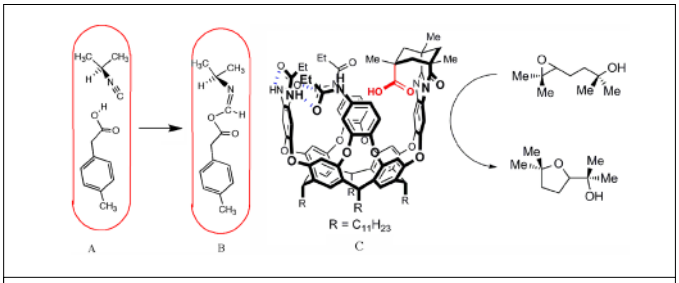


Fig. 3. Trapping a reactive intermediate. The coencapsulation of an acid and isonitrile (A) positions their functional groups for an addition reaction. The intermediate (B) is stabilized by hydrogen bonding with the nearby imides of the capsule, and the rigid cage prevents rearrangements. This intermediate could not be observed in free solution but has a lifetime of nearly an hour in the capsule. The cavitand (C) features an acid catalyst that is trained on any guests inside. The confined space influences the pathway of chemical reactions such as the cyclization shown on the right.

Catalysis also occurs with the cavitand (Fig. 3C). The inwardly directed carboxylic acid can contact any guest that is inside the cavity. The limited space inside favors reactions with compact transition states. The reaction of the epoxide shown is accelerated more than 50-fold and gives exclusively the 5-membered ring, whereas the reaction outside in bulk solution gives both 5- and 6-membered ring products.

PHASE AND PRESSURE IN CAPSULES

What "phase" do molecules experience inside a capsule? When a single or only a few molecules are involved, it is inappropriate to refer to solids, liquids, or gases, yet my colleagues and I have found that molecules in these different phases occupy different fractions of the space inside. The packing coefficients are about 70% for solids, 55% for liquids, and around 40% for gas molecules inside the capsules.

Even if only a single or a few molecules of the gas are present, the pressure inside a capsule can be calculated. When **1.1** is dissolved in cyclopropane-saturated solvent, 3 cyclopropane guests can be detected inside the capsule. The space inside can be calculated by using modeling software; the volume of **1.1** in Figure 4 is 425 Å^3. At 1 atm, a molecule of an ideal gas has a space of approximately 37,000 Å^3, nearly 90 times the space in the capsule **1.1**. Accordingly, 3 ideal gas molecules at ambient temperature in the capsule are at a pressure of approximately 270 atm, yet the system is at equilibrium at room temperature with cyclopropane in solvent at ambient pressure.

The pressures are, of course, unrealistic because these systems are not ideal gases; the gases take up space and their collisions with the walls are not elastic.

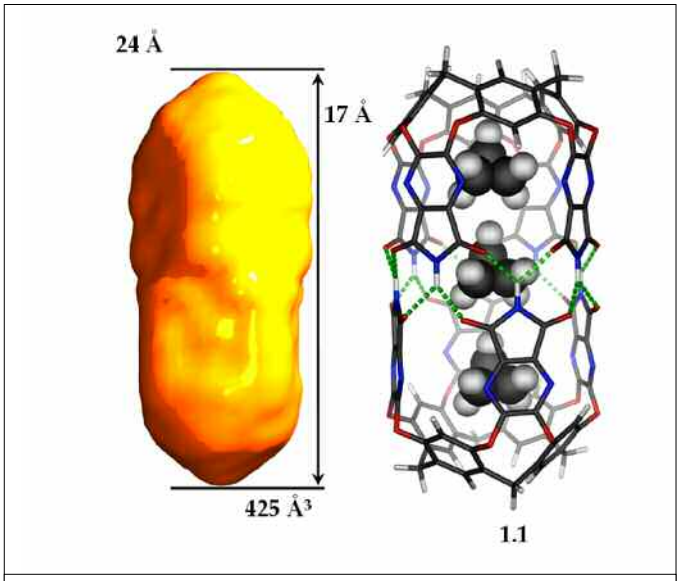


Fig. 4. Left, The shape of the space inside the capsule. The tapered ends can accommodate only the narrowest of functional groups. Right, A total of 3 cyclopropane gas molecules occupy the capsule; the calculated pressure is several hundred atmospheres, but attraction between the surfaces of the gas and the capsule lowers the energy and pressure inside.

The capsule has 16 aromatic panels, and attractions exist between the gas guest and the aromatic inner surface of the host. This binding of carbon-hydrogen bonds to π surfaces lowers the potential energy and the pressure.

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