

Sex to order

A BIRTH announcement with a difference is made by D. G. Cran and colleagues in the latest issue of *The Veterinary Record* (**132**, 40–41; 1993) — the production of three female and three male calves from eggs fertilized by spermatozoa preselected for sex. Behind the news, which in the long run will be good news for beef and dairy farmers, is the refinement of a flow cytometry technique for sorting sperm according to whether they contain X or Y chromosomes. Use of a fluorescent dye and laser detection means that the 4 per cent or so difference in DNA content between X- and Y-bearing sperm can be distinguished with reasonably high efficiency. The birth (after embryo transfer into heifers) of the calves with sexes as predicted was the test that the sperm remain viable after their buffeting, but it will be some time before sexed embryos become available commercially. The necessary large-scale trials have yet to be completed.

Exclusion principle

TRACKING the motion of iridium atoms over the surface of an iridium crystal, S. Wang and G. Ehrlich find a curious *cordon sanitaire* just three atoms wide around a surface defect (*Phys. Rev. Lett.* **70**, 41–44; 1993). The defect, deliberately introduced, is a small cluster of iridium atoms which mimics the step that forms on a surface as a crystal grows. Alternately freezing the crystal to locate the atoms with field-emission microscopy and then warming it to allow them to wander, the authors find that although the atoms might move anywhere just beyond the exclusion zone, they are never seen inside it. Eventually, the lone atoms can suddenly join the central cluster. What keeps them out of the exclusion zone is not clear — tests seem to rule out the presence of an energetic barrier.

Myosin multiplicity

Acanthamoeba contains three forms of myosin I, the tailless monomeric myosin that forms no filaments. I. C. Baines *et al.* (*J. Cell Biol.* **119**, 1193–1203; 1992) have now determined their distribution in the cell and inferred something of their functions. From its location it seems that myosin IA acts in nipping off the small plasma membrane vesicles and moving them between the membrane and cytoplasm; IB turns up in the fraction of large vacuoles and phagocytic vesicles that derive from the active membrane regions, and thus most probably drives the extension of the membrane; and IC is the only form seen in the contractile vacuole and so is probably involved in its function. It will be especially interesting to discover how these myosins recognize their chosen sites.

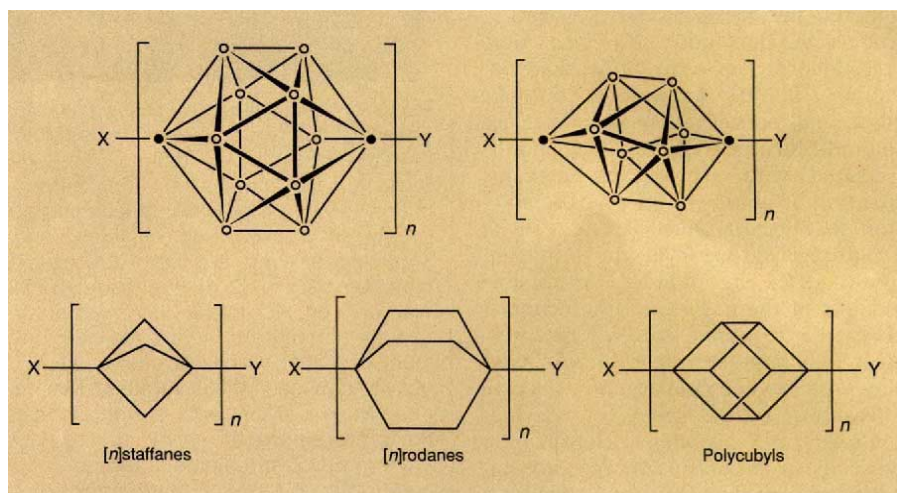
Carborod molecular scaffolding

Jeffrey S. Moore

As miniaturization of electronic, photonic and mechanical devices approaches the molecular scale, chemists are recognizing the possibility of building the smallest possible objects from the atom up. This creates the need for the molecular construction kit, which would probably include an assortment of molecular sticks of different lengths, connectors, and a mechanism to bring the pieces together. But even the simplest component of this kit, the non-collapsible rod,

carborane cage means that the carborods uniformly fill space around their longitudinal axis. Thus, of all the so-called 'rigid-rod' molecules, the carborods most closely resemble cylindrical pillars. They are available in lengths that are integral multiples of about 4.5 Å, the average repeat distance of the carborane monomer.

Besides their geometry, carborane monomers have other characteristics that make them ideal constituents



Molecular scaffolding: top, the two kinds of carborod just synthesized; below, three rigid rods previously synthesized. Solid circles, carbon; open circles, boron (B—H).

has proved pretty elusive. To help fill this gap, independent groups in the laboratories of M. Frederick Hawthorne and Josef Michl have developed a new family of stiff molecular scaffolding, named carborods (X. Yang *et al.*, *J. Müller et al. J. Am. chem. Soc.* **114**, 9719–9721, 9721–9722; 1992).

These new rods are oligomers of *para*-carborane monomers. Carboranes are inorganic cage molecules with boron and carbon vertices. In the *para* isomers, the carbon atoms occupy the apical positions of high-symmetry polyhedra (see figure). Both laboratories report carborods using a 12-vertex carborane monomer having icosahedral symmetry. Michl's group also described a carborod based on a 10-vertex carborane monomer that has a bicapped square antiprism geometry. The carbon atom sites in both of these polyhedra are ideally situated for linear extension through polymerization.

Carborods are thick compared to known rigid-rod molecules (such as so-called $[n]$ staffanes, $[n]$ rodanes and polycubyls, see figure). Their van der Waals diameter is estimated to be more than 7 Å. Moreover, the high symmetry of the

for molecular scaffolding. They are exceedingly robust, having exceptional thermal and chemical stability. They are transparent to ultraviolet and visible radiation, a feature that will be most advantageous in some applications. And perhaps most importantly, carborane chemistry provides a means to prepare rods of discrete length and with specified end-group functionality. This is possible because of the acidity of the terminal CH groups, which offers site-selective reactivity in the growing, multi-vertex carborods.

A practical molecular construction kit will require components with precisely defined structure. Along with uniform length, controlled end-group functionality will be needed to link the rod with connector pieces, through covalent or non-covalent bonding. In making rods of uniform length, one must be able to control the oligomerization reactions. Many of the molecular rods that have been previously described do not allow a means to do this. The methods reported by Hawthorne's and Michl's groups allow carborods to be obtained with modest control. They succeeded in preparing and characterizing end-

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functionalized rods uniquely possessing one, two, three and four carborane repeat units. A single-crystal X-ray structure from Hawthorne's group shows the tetramer to be 17.21 Å in length and almost perfectly linear. This is apparently the longest rigid-rod molecule to be structurally characterized.

The components of a practical molecular construction kit will need to be soluble in common solvents. This might seem like a trivial issue, but chemists as molecular constructors perform their building operations in solution, and rigid rods are notoriously insoluble. Problems of solubility were apparent with the new carborods as well. Nonetheless, Hawthorne's group shows that the poor solubility of the parent tetramer can be overcome through proper choice of end-groups. They hope to use these tactics in combination with an efficient, repetitive synthetic scheme to construct even larger rods.

It is popular to draw analogies between a child's tinkertoys and molecular construction kits, but this overlooks one important difference. Whereas the child can physically handle the components to construct an object piece by piece, to attempt the same in building a molecular object of even moderate size would be cumbersome. The components of a practical molecular construction kit will have to be smart enough to assemble themselves automatically into the desired arrangement. Herein lies the greatest challenge — how to encode the pieces of scaffolding appropriately. This might involve adding extra groups to the pieces, to bias their orientation and position. The chemistry of the non-covalent bond will surely play an important part in this.

Michl's group's effort to produce molecular tinkertoys has already begun to confront the challenge of automatic assembly. Their study included the preparation of Langmuir–Blodgett films — monolayers on a water surface — using carborod building blocks. The carborods were encoded with a carboxylic acid group at one end, a constraint intended to define the orientation of the carborod at the water–air interface. The other end contained a reactive iodide group. The area per molecule of the films was consistent with a monolayer in which the rods packed end-on at the interface. If all goes according to plan, this should leave a layer of exposed iodine atoms. The next step will be to activate the iodine atoms photochemically to allow continued molecular construction on this surface. Chemists will then truly become molecular builders. □

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Evolution *ex vivo*

Jack W. Szostak

BIOLOGICAL evolution is a slow process. But populations of replicating macromolecules can undergo evolution *in vitro* over a timescale more suited to human observers. On page 182 of this issue¹, Lehman and Joyce apply techniques of directed *in vitro* evolution² to modify the catalytic requirements of that much-studied ribozyme — the *Tetrahymena* ribozyme — with some striking results.

The challenge is daunting; how can we obtain ribozymes with specified properties in the absence of knowledge and understanding of RNA structure sufficient to permit rational design? The answer, in principle, is simple: make all possible changes in a given ribozyme (or at any rate as many changes as are practical, which turns out to be a great many), and then select for the particular changes that alter the properties of the ribozyme in the desired manner. Darwinian evolution — the preferential survival and reproduction of the fittest variants in a population — applies just as much to these replicating populations of informational macromolecules *in vitro* as it does to living organisms on a large scale over long periods of time.

This approach was first applied by Beaudry and Joyce³ to the evolution of a variant form of the *Tetrahymena* ribozyme with altered substrate specificity. Starting with the wild-type *Tetrahymena* ribozyme, which prefers RNA as a substrate, they were able to select for a variant ribozyme with a 100-fold increase in activity on a DNA substrate. This was accomplished by developing methods of mutagenesis, amplification, and especially selection, for the molecules with the desired properties (Fig. 1). The cycle of mutagenesis, selection and amplification was repeated for 10 'generations', at the end of which individual molecules were analysed to determine the mutations responsible for the increase in activity.

In the new work of Lehman and Joyce¹, the same methods have been applied to a new task — changing the metal ion specificity of the ribozyme. The ribozyme requires divalent cations for two distinct roles, folding and catalysis. Although either Ca^{2+} or Mg^{2+} will work at most sites, there is at least one, probably the active site, at which Ca^{2+} will not work⁴. Piccirilli *et al.* have recently shown⁵ that a Mg^{2+} ion interacts directly with the 3' oxygen of the phosphodiester bond that is attacked during the catalytic reaction. This Mg^{2+}

is presumed to stabilize the developing negative charge on the 3' oxygen during chain cleavage. The implication is that an important part of the mechanism of ribozyme catalysis involves binding this Mg^{2+} and directing it towards the appropriate 3' oxygen. The ribozyme– Mg^{2+} interactions involved are not known.

Lehman and Joyce started with a large pool of mutant ribozymes, 2.4×10^{13} by their reckoning, generated by a 5 per

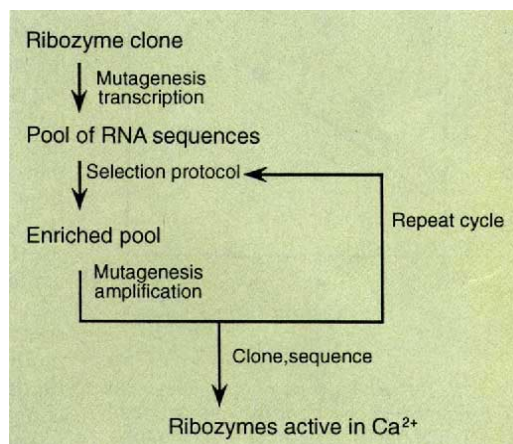


FIG. 1 Cycle of ribozyme mutagenesis, selection and amplification.

cent mutagenesis over 140 nucleotides of the catalytic core of the ribozyme. Such a pool is sufficient to include all five-base changes in the enzyme sequence, and about half of the possible six-base changes. They then selected for mutant ribozymes that could function in the presence of Ca^{2+} with no added Mg^{2+} , using the same selection procedure as in their previous experiment, so that only catalytically active molecules could be replicated. Competition in the Joycean jungle is severe — no more than 0.1–1 per cent of the population survives the selection phase and is allowed to reproduce to form the next generation. Each survivor, however, generates 100–1,000 progeny. Additional mutations were introduced during the amplification phase of each generation by the use of a mutagenic polymerase chain reaction.

One of the exciting aspects of *in vitro* evolution is the ability to assess the changing nature of the population through time. Lehman and Joyce sequenced 50 clones from generations 2, 4, 6 and 8, to see the results of their selection protocol. The molecules in the initial pool contain an average of seven mutations each, as a consequence of the initial 5 per cent mutagenesis over 140 nucleotides. After two generations of