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.

References

1. S. C. Zimmerman, F. Zeng, D. E. C. Reichert, S. V. Kolotuchin, *Science* **271**, 1095 (1996).
2. J.-M. Lehn, *ibid.* **260**, 1762 (1993).
3. J. S. Lindsey, *N. J. Chem.* **15**, 153 (1991).
4. A. Klug, *Angew. Chem. Int. Ed. Engl.* **22**, 565 (1982).
5. D. J. Cram, *Science* **240**, 760 (1988).
6. J.-M. Lehn, *Angew. Chem. Int. Ed. Engl.* **27**, 89 (1988).
7. C. J. Pedersen, *ibid.*, p. 1021.
8. S. C. Zimmerman, *Top. Curr. Chem.* **165**, 71 (1993); A. D. Hamilton, *Adv. Supramol. Chem.* **1**, 1 (1991).
9. T. W. Bell *et al.*, *Science* **269**, 671 (1995).
10. For alkali metal helicates and references to transition metal helicates, see T. W. Bell and H. Jousselin, *Nature* **367**, 441 (1994).
11. J.-P. Sauvage, *Acc. Chem. Res.* **23**, 319 (1990); D. Philip and J. F. Stoddart, *Synlett* **1991**, 445 (1991).
12. N. Branda, R. Wyler, J. Rebek Jr., *Science* **263**, 1267 (1994); R. S. Meissner, J. Rebek Jr., J. de Mendoza, *ibid.* **270**, 1485 (1995).
13. J. Yang *et al.*, in *Supramolecular Stereochemistry*, J. S. Siegel, Ed. (Kluwer, Dordrecht, 1995), pp. 141–146.
14. G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **254**, 1312 (1991); E. E. Simanek *et al.*, *Tetrahedron* **51**, 607 (1995).
15. S. C. Zimmerman, *J. Am. Chem. Soc.* **57**, 2215 (1992); S. V. Kolotuchin *et al.*, *Angew. Chem. Int. Ed. Engl.* **34**, 2654 (1995).
16. D. A. Tomalia *et al.*, *Angew. Chem. Int. Ed. Engl.* **29**, 138 (1990); G. R. Newkome, C. N. Moorefield, G. R. Baker, *Aldrichimica Acta* **25**, 31 (1992); X. Xu and J. S. Moore, *Angew. Chem. Int. Ed. Engl.* **32**, 246 (1993); *ibid.*, p. 1354; J. M. J. Fréchet, *Science* **263**, 1710 (1994).
17. J. M. J. Fréchet *et al.*, *Science* **269**, 1080 (1995).
18. N. Ardoin and D. Astruc, *Bull. Soc. Chim. Fr.* **132**, 875 (1995).
19. V. Percec, J. A. Heck, D. Tomazos, G. Ungar, *J. Chem. Soc. Perkin Trans. 2* **1993**, 2381 (1993).

Evolving Catalysts in Real Time

Mark M. Davis

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.

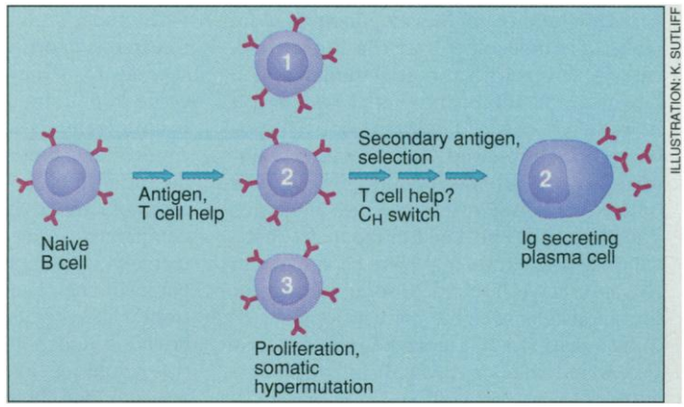
The antibody studied by Patten and coworkers was generated by immunization with a nitrophenyl phosphonate transition-state analog and catalyzes the hydrolysis of an ester or the related carbonate derivatives. The structure shows that the hapten is bound in a 10 Å–deep pocket surrounded by six tyrosines, 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-

The author is in the Howard Hughes Medical Institute, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305-5428, USA. E-mail: mdavis@cmgm.stanford.edu

body minus the nine mutations that were introduced by somatic mutation, expressed the protein in *Escherichia coli*, and compared the kinetics of hapten binding with those of the original. The 14,000-fold difference in affinity between the two resulted mostly from a slower dissociation rate of the mutated antibody. This is by far the largest difference attributable to somatic mutation yet seen in a specific antibody, although it has long been clear that substantial gains in affinity of at least 100-fold accompany this progression in the immune system (*7*). This range of affinities is also likely necessary because of the different environments in which cell surface and secreted antibodies operate. The range of affinities for a number of cell surface receptors that bind other cell surface molecules is quite low, from ~10^{-4} M to 10^{-7} M for adhesion molecules and T cell receptors and their ligands (*8*, *9*). Although they are low affinity in absolute terms, these interactions are quite specific and have an effectively higher affinity because of the massive polyvalency and limited (two-dimensional) diffusion characteristic of cell surface interactions. It is reasonable that the germline version of the antibody investigated by Patten *et al.* is lower in affinity, because it should have first encountered the hapten as a multimeric aggregate or on the surface of some auxiliary cell (*10*). Later, as a secreted antibody, the affinity required is generally much higher (in the nanomolar range) and is achieved by somatic mutation. This affinity is typical of many proteins that bind to soluble ligands.



Getting stronger: The progression from cell surface to secreted forms of immunoglobulin. Initially, immunoglobulin genes are rearranged and expressed on the surface of B lymphocytes in the IgM form. If they encounter an appropriate antigen and receive T cell help in the form of cytokines, they may somatically hypermutate their rearranged V region genes, and higher affinity variants are selected to secrete antibodies (*6*).

How does somatic mutation generate this higher affinity? Patten and co-workers find that none of the nine somatic mutants are in direct contact with the antigen. Instead, they affect either residues internal to the binding loops [complementarity determining regions (CDRs)] or, in a few cases, are in neighboring residues. This parallels earlier work by Strong *et al.* (*5*) who saw that a 200-fold increase in affinity between somatic mutants of an antibody to arsonate and its germline predecessor were achieved entirely by mutations in CDR that were not in direct contact with the hapten. Instead, as seen by Patten *et al.*, the most important mutations are involved in inter- or intra-CDR loop interactions. The authors suggest that a unifying mechanism behind this increased affinity could be the stabilization of an optimal binding surface for a particular ligand. [The complex kinetics of some antibody-hapten interactions has suggested that there are multiple binding surface conformations of a given antibody (*11*).]

One variant of this explanation is to consider that affinity is a function of free energy (ΔG) and that free energy is the gain in enthalpy minus the loss in entropy. In this case, mutations that make the antibody binding surface more rigid would decrease the loss of entropy brought about by binding to the antigen and thus increase ΔG and increase the affinity. Alternatively, as discussed by the authors, the changes brought about by the mutations could just be creating a "better" binding site for the hapten. If this were true, however, one would expect at least an occasional mutation in a contact residue, and, although the sample size is small, thus far this has not happened (*2*, *5*).

We now know a little more about both catalytic antibodies and the effects of somatic mutation on antibodies. At the very least, the results of Patten and co-workers suggest that selecting for even higher affinity catalytic antibodies could result in even better catalysts—by further reliance on the immune system during a more lengthy period of immunization or by the more directed approach of mutagenesis with natural or unnatural amino acids.

References

1. P. G. Schultz and R. A. Lerner, *Science* **269**, 1835 (1995).
2. P. A. Patten *et al.*, *ibid.* **271**, 1086 (1996).
3. M. R. Haynes, E. A. Stura, D. Hilvert, I. A. Wilson, *ibid.* **263**, 646 (1994).
4. G. W. Zhou, J. Guo, W. Huang, R. J. Fletterick, T. S. Scanlan, *ibid.* **265**, 1059 (1994).
5. R. K. Strong, G. A. Petsko, J. Sharon, M. N. Margolies, *Biochemistry* **30**, 3749 (1991).
6. A. K. Abbas, in *Cellular and Molecular Immunology*, A. K. Abbas, A. H. Lichtman, J. Pober, Eds. (Saunders, Philadelphia, 1994), pp. 89–95.
7. H. N. Eisen and G. W. Siskind, *Biochemistry* **3**, 996 (1964); C. Berek and C. Milstein, *Immunol. Rev.* **96**, 23 (1987).
8. P. A. van der Merwe and A. N. Barclay, *Trends Biochem. Sci.* **19**, 354 (1994).
9. K. Matsui *et al.*, *Science* **254**, 1788 (1991); S. Weber, A. Traunecker, F. Oliveri, W. Gerhard, K. Karjalainen, *Nature* **356**, 793 (1992); Y. Sykulev *et al.*, *Immunity* **1**, 15 (1994); Y. Sykulev *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12862 (1994); M. Corr *et al.*, *Science* **265**, 946 (1994).
10. H. M. Dintzis, R. Z. Dintzis, B. Vogelstein, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3671 (1976).
11. J. Foote and C. Milstein, *ibid.* **91**, 10370 (1994).

Delivery of Molecular Medicine to Solid Tumors

Rakesh K. Jain

Approximately one-fourth of all deaths in the United States are due to malignant tumors. More than 85% of these are solid tumors, and approximately half of the patients with these tumors die of their disease. The cause of death is usually metastatic disease distant from the original tumor, although uncontrolled primary (or regional) tumors can also be fatal. The distant metastases are treated systemically with chemical and biological agents, but these attempts are often unsuccessful (*1*).

There is widespread expectation that new strategies, collectively referred to as "molecular medicine," have the potential to be dramatically more effective. The new strategies are a product of the remarkable creativity and energy that has been devoted to molecular biology and biotechnology. The resulting agents include monoclonal antibodies, cytokines, antisense oligonucleotides, gene-targeting vectors, and genetically engineered cells. Because of their potent effect on cancer cells in vitro and in some in vivo tumor systems, these agents have been heralded as breakthrough drugs

The author is in the Edwin L. Steele Laboratory, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA. E-mail: jain@steele.mgh.harvard.edu
More details on this topic can be found on the World Wide Web at <http://steele.mgh.harvard.edu>.