The Chemistry of the Antibody Molecule

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Immunochemistry has traditionally focused on understanding the molecular basis of antibody binding affinity and specificity. In the last 20 years a major shift has occurred in the field. We are now asking how we can exploit the remarkable properties of the antibody molecule and the immune response in chemistry—from testing basic theories of enzymatic catalysis to the synthesis of new catalysts. Moreover, the concept of molecular diversity, which underlies these experiments, is now being applied to a large number of other problems—from libraries of biopolymers to libraries of solid-state materials. Importantly, theses studies are providing fundamental molecular insights into many biological, chemical, and physical phenomena, as well as changing the way we approach the synthesis of new molecular function.

1. Introduction

1.1. Synthesis at the Interface of Chemistry and Biology

Synthesis has been and will continue to remain a cornerstone of chemistry. It provides access to new molecular structures with novel chemical, biological, and physical properties, ranging from antibiotics and genes to conducting polymers and superconductors. Synthesis also provides a unique opportunity to test fundamental principles of chemistry and gain new insights into chemical phenomena. For example, our understanding of reactive intermediates has come in large part from iterative synthetic and mechanistic studies.

As the field of synthesis continues to evolve there will be an increasing shift in focus from the synthesis of structure to the synthesis of molecular function. Unfortunately, we as chemists are not nearly as sophisticated in our ability to rationally design and synthesize molecules with defined chemical, biological, or materials properties, as we are in our ability to synthesize complex molecular structures. How then do we develop synthetic strategies that enable us to create molecules with novel functions efficiently? One answer is to look to biology for guidance. After all, living organisms are unparalleled in their ability to generate molecular function—from molecular recognition by antibodies to energy conversion by the photosynthetic complex. Unfortunately, the synthetic machinery of the cell is not as easily adapted to new targets as are traditional synthetic methods. However, if we combine the "synthetic strategies" and processes of nature with the methods and principles of classical synthetic chemistry, it should be possible to generate new molecular function that is not accessible by either approach alone.

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1.2. The Synthesis of Biological Catalysts

To illustrate this chemical–biological approach to synthesis, consider the generation of enzymatic catalysts. Enzymes catalyze biological transformations with exquisite specificity and efficiency using a limited number of functional groups—they do not have organolithium reagents or metal hydrides available. Given their remarkable catalytic function, considerable effort has gone into understanding the molecular basis of enzymatic catalysis using an array of spectroscopic and mechanistic tools. Nevertheless, the best test of our understanding of the principles of biological catalysis is in the synthesis of new enzymes. But how does one synthesize something as complex as an enzyme when we do not even yet understand the rules that govern the folding of amino acid sequences?

The answer comes from our understanding of the evolutionary process Nature uses to create enzymes. This process, at its most fundamental level, involves genetic recombination and mutation to generate a large diverse library of molecular scaffolds. This library is then subjected to natural selection in order to identify those molecules with the requisite catalytic function. Further rounds of mutation and selection lead to improved function.[1] This approach is very different from that of traditional synthetic chemistry, in which molecules are designed and synthesized one at a time, and optimized in an iterative fashion by structural refinement. Unfortunately, in contrast to laboratory synthesis, natural evolution occurs on

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an evolutionary timescale of up to billions of years. The challenge then in the synthesis of new biological catalysts is to recreate the basic features of enzyme evolution on the laboratory timescale.

If one accepts the basic principle that catalytic function results from the selective use of binding energy to stabilize transition states or to destabilize ground states preferentially, then the problem is simplified to one of synthesizing highly selective molecular receptors.[2] While this remains a major challenge for synthetic chemistry, there does exist a biological solution to the problem of molecular recognition. It is a well-known fact in immunochemistry that the immune response can generate an antibody that is complementary to virtually any foreign molecular structure presented to it. The process whereby these selective, high-affinity receptors are generated resembles in many ways the natural evolution of enzymes[3–5] (Table 1).

Table 1. A comparison of the evolution of enzymes and antibodies.

Enzymes	Antibodies
exon shuffling	V-D-J rearrangement
gene duplication	batteries of V, D, and J gene elements
accumulation of point mutations	somatic hypermutation
natural selection	clonal selection
timescale: $10^1 - 10^8$ years	timescale: weeks

The immune system "synthesizes" on the order of 10^8 different germline antibodies as initial solutions to the recognition problem by assembling the full-length antibody gene from the different V, D, J, and C gene segments (V = variable, J = joining, D = diversity, and C = constant segments). If each gene segment can be derived from any one of multiple genes, and insertion or deletion can occur during recombination of the segments, large numbers of antibody sequences can be generated (Figure 1). This particular "synthetic" strategy focuses molecular diversity in six hypervariable loops that make up the ligand-combining site on a core eight-stranded antiparallel β-sheet scaffold.[6] Once a germline antibody is selected from the pool based on its affinity for an antigen, additional structural diversity is generated by a process of affinity maturation in which somatic mutations are introduced throughout the variable region. Iteration of this process leads to a high-affinity antibody.

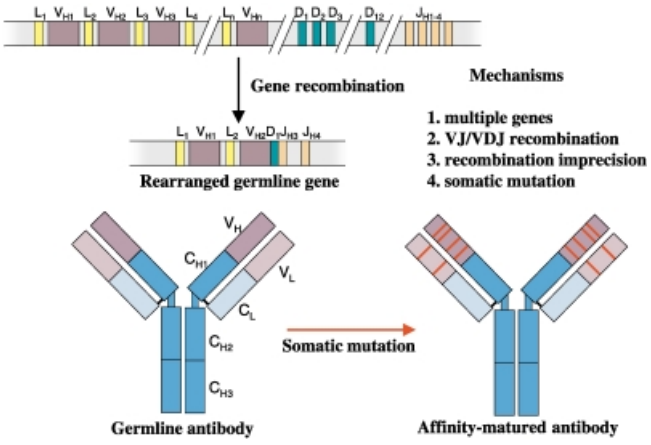

Figure 1. The generation of immunological diversity by genetic recombination and somatic mutation.

The key "synthetic" strategy used by the immune response, the generation of molecular diversity, is the same as that used in natural selection. The fundamental difference is in the timescales, the latter process occurs over millions of years; in contrast, the immune response occurs over a period of weeks. Consequently, if one can direct the immunological evolution of an antibody down the same path as the natural evolution of an enzyme, one should, in principle, generate enzyme-like catalysts from antibodies. This can be done by programming the immune response with mechanistic information about the chemical transformation of interest, in much the same way that mechanistic insight drives the development of classical synthesis and synthetic methodologies. But in the former approach, the principles and tools of chemistry are used *together* with the powerful biological notion of molecular diversity to synthesize new function. Herein we describe a number of experiments in antibody catalysis that illustrate this new synthetic paradigm.

2. Strategies for the Synthesis of Catalytic Antibodies

2.1. Transition-State Stabilization

One of the earliest theories put forth to explain enzymatic catalysis was Pauling's notion of transition-state stabilization: "The assumption that the enzyme has a configuration complementary to the activated complex, and accordingly has the strongest power of attraction for the activated complex, means that the activation energy for the reaction is less in the presence of the enzyme than in its absence, and accordingly that the reaction would be speeded up by the enzyme."[7, 8] One should be able to test this notion experimentally by chemically programming the immune response with a stable molecule which mimics the presumed structural and electronic features of the rate-limiting transition state for a particular reaction. The result should be the evolution of an antibody binding site with maximum complementarity to the transition state versus substrate or product. This antibody should catalyze the reaction by selectively stabilizing the bound transition state and thereby lowering the free energy of activation of the reaction.

To test whether one can synthesize enzymatic function using this approach, we attempted to generate antibodies that catalyze acyl transfer reactions. The transesterification reaction illustrated in Figure 2a is thought to proceed through a tetrahedral transition state generated by addition of alcohol **1** to ester **2** to produce a tetrahedral intermediate **3**, which breaks down to give product **4**. Antibody 13D6.1 generated to the phosphonate diester **5**, which mimics the charge distribution and geometry in the transition state, was found to catalyze the reaction with an effective molarity (EM; the concentration of substrate required in the absence of catalyst to achieve the same rate) of $\approx 10^4 - 10^5$ M.[9] This value begins to approach the theoretical maximum of 10^8 M calculated by

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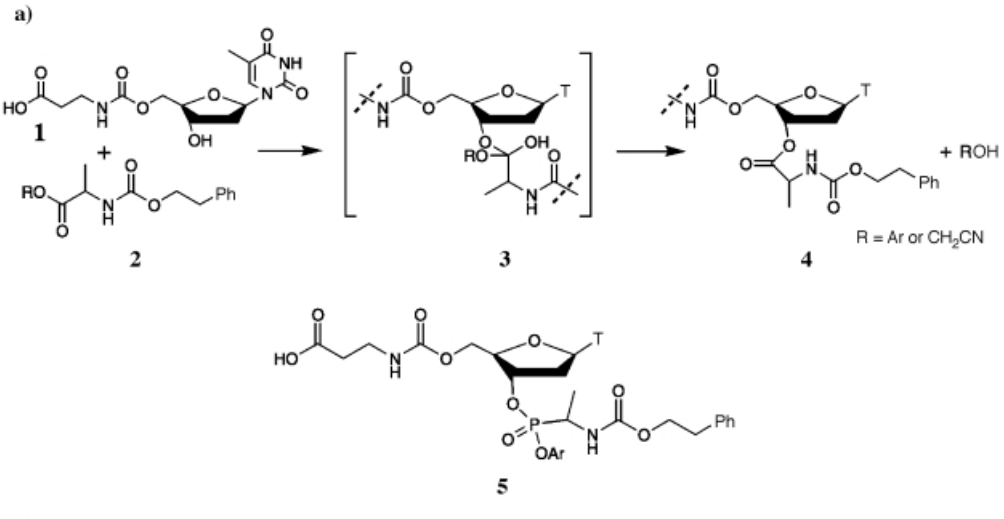

Figure 2. a) Acyl transfer from the ester **2** to the alcohol **1** catalyzed by antibody 13D6.1, which was generated against the phosphonate diester **5**; b) NMR structure of the Michaelis complex, with **1** shown in blue and **2** in orange.

Page and Jencks for a bimolecular reaction in which all translational and rotational modes along the reaction coordinate are frozen out in an enzyme active site.[10] NMR spectroscopic studies of the Michaelis complex (Figure 2b) confirmed that the antibody binds the substrate **1** in an optimal geometry for attack on the ester carbonyl group of **2**.[11] This antibody-catalyzed reaction is also characterized by remarkable selectivity—the antibody does not catalyze acyl transfer to water despite its presence at 55 M. Such selectivity is similar to that seen in enzymes such as triosephosphate isomerase, which is able to sequester bound intermediates from water.

Not only is it possible to create an enzyme-like catalyst by this approach, one can also recreate other mechanisms by which enzymes have evolved to catalyze similar acyl-group transfer reactions. For example, an antibody which was generated against the phosphonate monoester **9** (Figure 3a) is also a highly selective and efficient acyl transfer catalyst (EM = 10^5 – 10^6 M).[12] However, in this case the reaction was found to proceed through the formation of an acyl-antibody intermediate. Based on X-ray crystallographic and mutagenesis studies, the mechanism likely involves attack of the active site Ser 98^H residue on the bound ester[12, 13] (Figure 3b). In this example, immunological evolution has converged on a mechanism similar to that used by serine proteases and esterases to catalyze acyl-transfer reactions.

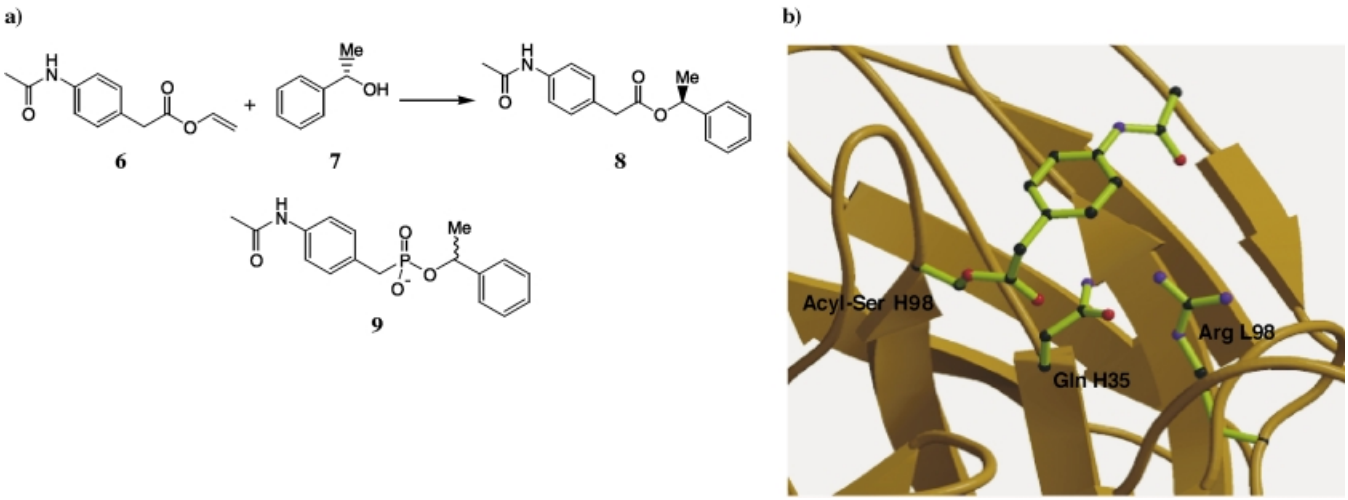

Figure 3. a) Acyl transfer from the ester **6** to the alcohol **7**, catalyzed by antibody 21H3, which was generated against the hapten **9**; b) modeled structure of the acyl–antibody intermediate based on the X-ray crystal structure of the antibody–hapten **9** complex.

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One interpretation of this phenomenon is that because of the limited "reagent pool" available to proteins, there are only a limited number of ways to accomplish particular chemical transformations.

2.2. Strain

Another classical theory of enzymatic catalysis is the notion of substrate strain put forth by Haldane in the 1930s.[14] Rather than selectively stabilize a transition state, Haldane argued that enzymes (with β-glucosidase as an example) use binding energy to strain or distort the bound substrate: "The enzyme would thus tend to pull the salicin molecule apart…(or) push the molecules of glucose and saligenin together. The same hypothesis might clearly be applied to other hydrolytic enzymes. Using Fisher's lock and key simile, the key does not fit the lock quite perfectly but exercises a certain strain on it" (Figure 4). This theory has been quite difficult to prove, despite considerable mechanistic effort. Once again synthesis offers an opportunity to test this notion by using it as the basis for the immunological evolution of a catalytic antibody.

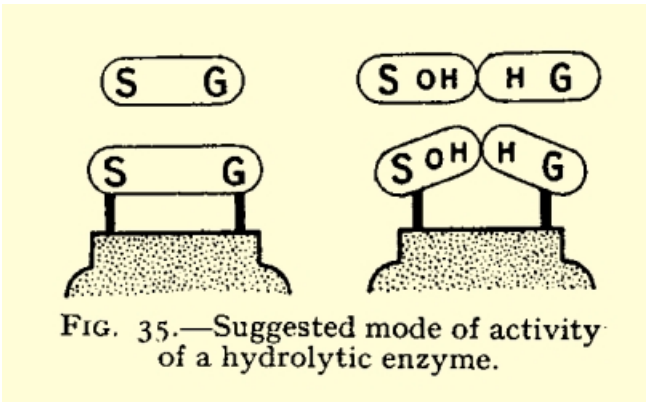

Figure 4. Haldane's strain theory of enzyme catalysis with β-glucosidase as an example (from reference[14]).

The enzyme ferrochelatase catalyzes the last step in heme biosynthesis—metal-cation insertion into the porphyrin ring. The enzyme is thought to catalyze the reaction by distorting the porphyrin ring out of planarity, which facilitates the chelation of the metal ion by the lone pairs of the pyrrole nitrogen atom.[15] If this is indeed the case, then one should be able to synthesize a ferrochelatase by generating antibodies against a distorted porphyrin. To test this concept, antibodies were generated against *N*-methylmesoporphyrin (**12**), a mimic of the strained substrate **10**, synthesized by alkylating an internal nitrogen atom of the mesoporphyrin (Figure 5a). The antibody 7G12 was able to catalyze the metalation of mesoporphyrin (**10**) with Zn^{2+} ions, with rates comparable to the natural biosynthetic enzyme.[16] Resonance Raman studies have shown that the porphyrin ring is distorted out of planarity in an alternating up–down mode.[17] The X-ray crystal structures of both the antibody–*N*-methylmesoporphyrin and antibody–mesoporphyrin complexes provide direct structural evidence for the strain hypothesis—in both

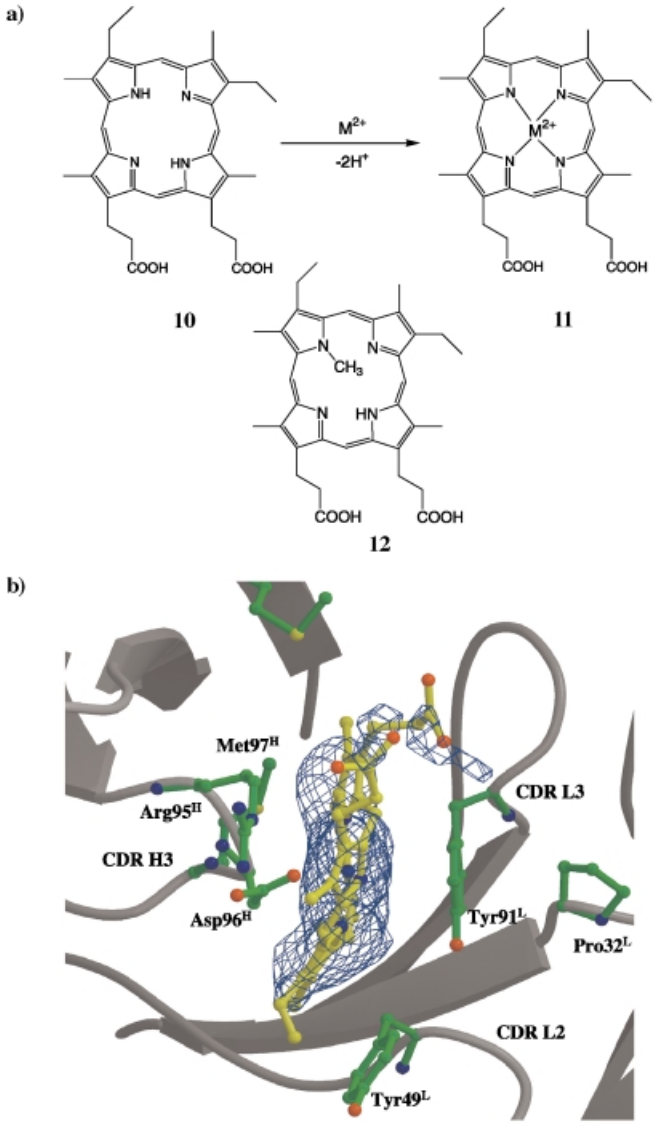

Figure 5. a) Metalation reaction of mesoporphyrin (**10**) catalyzed by antibody 7G12, which was generated against *N*-methylmesoporphyrin (**12**); b) the X-ray crystal structure of the antibody 7G12–**10** Michaelis complex with the *Fo*–*Fc* electron density contoured at 2.0σ. The structure of **10** is shown in yellow, residues of 7G12 that make critical packing interactions with **10** are shown in green.

cases the ring system is clearly distorted (Figure 5b).[18, 19] This distortion is mediated by packing interactions between the pyrrole rings and the Tyr 49^{L} and Tyr 91^{L} residues in the active site. Thus the synthesis of catalytic antibodies not only yields new biological catalysts, but also tests and validates fundamental principles of enzyme catalysis.

This antibody also catalyzes the H_2O_2-dependent oxidation of organic substrates. We have shown that one can further evolve the catalytic efficiency of this antibody in vitro. A library of active-site mutants was displayed on phage and selected based on the ability to oxidize the substrate tyramine linked to biotin. Oxidation of the tyramine moiety results in covalent attachment of biotin to the peroxidase antibody displayed on phage, which can then be captured by streptavidin beads. A single Tyr 49^{L}Trp mutant selected by this method led to a 20-fold increase in rate.[19] Active mutants are

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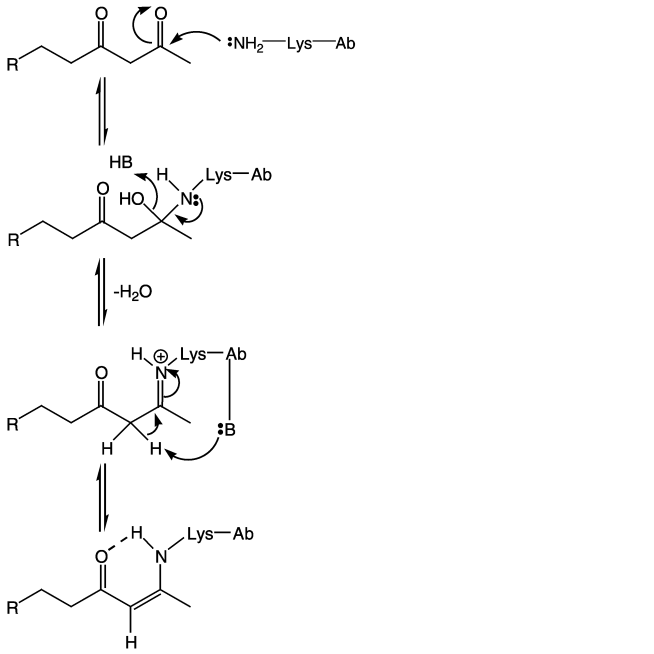
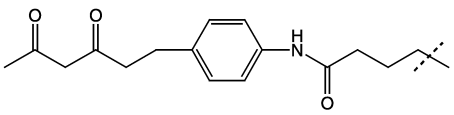
currently being subjected to DNA shuffling to enhance catalytic efficiency further.

2.3. Covalent Catalysis

Another important principle of enzymatic catalysis is covalent catalysis—the use of covalent intermediates to lower the free energy of activation for a reaction.[20–22] How much can the formation of these intermediates contribute to the catalytic efficiency of enzymes? To answer this question, an effort was made to generate an antibody that catalyzes the aldol reaction—the key carbon–carbon bond-forming reaction in organic synthesis. This reaction is catalyzed in glycolysis by an aldolase through the initial formation of a Schiff base intermediate with an active-site lysine.[23] By using the β-diketone **13** as an immunogen[24, 25] (Scheme 1), the evolution of an antibody with a reactive lysine in the active site was chemically programmed. Antibodies containing an





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Scheme 1. Generation of an aldolase antibody by reactive immunization with the 2-diketone hapten **13**.

appropriately positioned lysine residue were selected based on their ability to form a stable Schiff base (a process termed reactive immunization) with β-diketone protein conjugate.

The result of this experiment was an antibody (38C2) that catalyzes aldol reactions with exquisite specificity and rates close to the natural biosynthetic enzyme. In fact, this catalytic antibody has a broad substrate scope and can catalyze a wide array of aldol reactions with very high enantioselectivities (Figure 6a; >98% *ee*).[26–28] Antibody 38C2 has also been used to carry out the chiral resolution of β-hydroxyketones on gram scale,[29] as well as the selective activation of prodrugs. Moreover, catalytic antibodies (93F3 and 84G3) have been generated that catalyze aldol reactions with the opposite enantioselectivities, again with high *ee* values.[30]

Solution of the X-ray crystal structures of these antibodies reveals a deep hydrophobic pocket containing a buried lysine residue[26] (Figure 6b). This structure is quite similar in many respects to the corresponding enzyme despite the very different frameworks used by these proteins—another example of convergent evolution. These experiments again show that, through a combination of immunological diversity and basic chemical principles, efficient catalysts that rival enzymes can be created.

2.4. Selective Transition-State Stabilization in Disfavored Reactions

Not only can one "synthesize" antibodies that stabilize a rate-limiting transition state, one can generate antibodies that differentially stabilize two competing transition states to afford the kinetically disfavored reaction product which corresponds to the higher energy transition state. Once such example is an antibody-catalyzed elimination reaction in which the antibody 1D4 preferentially stabilizes the disfavored transition that leads to the *syn* elimination of HF from the β-fluoroketone **14** (Scheme 2)[31]. This transition state is calculated to be roughly 5 kcal mol^{-1} higher in energy than that for the favored *anti* elimination reaction. In fact, the antibody catalyzes exclusive formation of the disfavored product. The X-ray crystal structure of the hapten **15**–antibody complex reveals the structural basis for the *syn* binding mode of the substrate and periplanar base-catalyzed elimination.[32] A number of other disfavored reactions have also been catalyzed by antibodies, including epoxide ring opening,[33] Diels-Alder[34, 35] and cationic rearrangement reactions.[36–38] Thus, with the help of antibody binding energy, one can control transition-state energies in ways that might be difficult with classical synthesis.

2.5. Antibody Binding Energy and the Excited-State Reaction Manifold

The previous examples have focused on the use of antibody binding energy to affect the relative energies of various species along a thermally populated reaction coordinate. However, there have also been experiments carried out in which antibody binding energy has been used to control the photophysics and photochemistry of excited-state energy surfaces.[39] For example, the *trans*-stilbene hapten **16** was used to elicit a panel of monoclonal antibodies that display a range of fluorescent spectral behavior when bound to **16** (Figure 7a). The antibody 19G2 afforded a blue fluorescence indicative of an excited-state complex or "exciplex" between *trans*-stilbene and antibody with a fluorescence quantum yield (ϕ_f) of $\approx$0.80 and a radiative lifetime of >30 ns. The behavior of the blue antibody is reminiscent of what occurs as solutions

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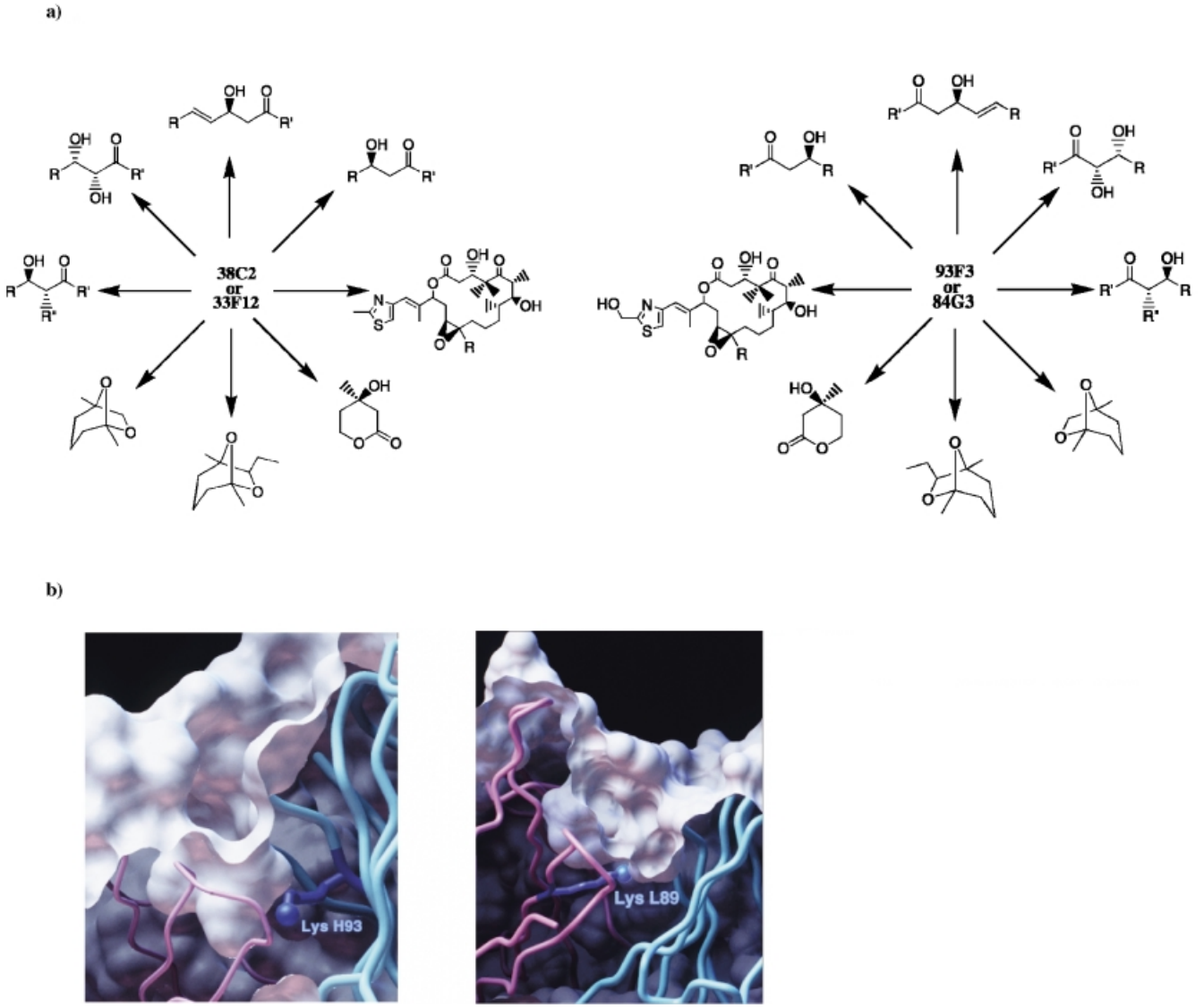
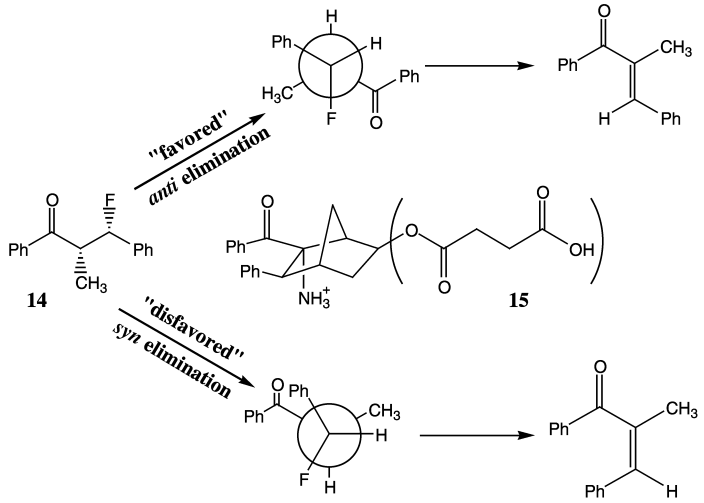


Figure 6. a) Broad substrate scope of antibody-catalyzed aldol reactions. The two antibodies have antipodal activities; b) substrate binding pockets for the antibodies 33F12 (left) and 93F3 (right). The light chain is shown in pink and the heavy chain in blue. The active-site lysine residue is also shown.



Scheme 2. Disfavored *syn*-elimination reaction catalyzed by the antibody 1D4, which was generated against hapten **15**.

of *trans*-stilbene are cooled to very low temperatures, which restricts the isomerization pathway in the excited state and therefore increases the fluorescence quantum yield. Yet, unexpectedly, cooling of a blue-fluorescent antibody complex below a critical temperature (250 K) caused disappearance of the blue emission and produces a purple complex. Thus, antibody 19G2 not only controls the isomerization coordinate of *trans*-stilbene, but also dynamically couples this manifold with active-site residues (Figure 7b). These experiments, and others involving excited-state photocleavage reactions,[40] provide a unique experimental approach for controlling the energetics and interconversion of short-lived excited-state species.

2.6. Endogenous Antibody Catalysis

Given the demonstrated catalytic potential of the antibody molecule, is it possible that endogenous catalytic antibodies

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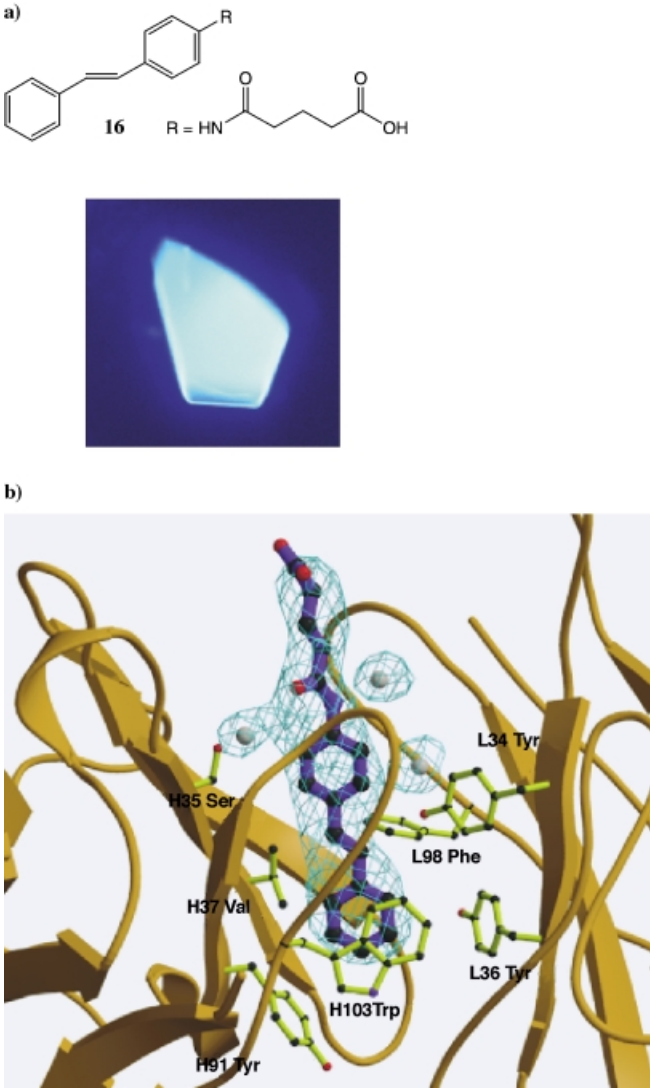

Figure 7. a) Structure of the *trans*-stilbene **16** and a crystal of the Fab 19G2–**16** complex under UV irradiation; b) X-ray crystal structure of Fab 19G2–**16** complex with hapten **16** in blue. The *Fo*–*Fc* electron-density map was contoured at 2.0σ.

may exist in humans? Paul and co-workers have reported that antigen-specific autocatalytic antibodies can be found in the serum of patients with autoimmune disease.[41] In particular, antibodies have been found that efficiently hydrolyze vasoactive intestinal peptide (VIP). The mechanism for the formation of these endogenous catalytic antibodies is to date unknown. It may involve the formation of antiidiotypic antibodies, an approach that has been used in the laboratory to generate antibody esterases and phosphodiesterases.[42] Alternatively, it may be that a selection based solely on binding affinity can in some cases lead to catalysis. Indeed, monoclonal antibodies independently generated to a VIP-protein carrier conjugate were shown to hydrolyze VIP with a catalytic efficiency (k_{cat}/K_m) of $6 \times 10^5\,M^{-1}\,min^{-1}$.[41] Molecular modeling and mutagenesis studies suggest that these antibodies have a catalytic dyad (Ser–His) in the active site. A similar catalytic dyad has been identified crystallographically in a phosphonate-specific esterolytic antibody.[43]

3. The Evolution of Binding Energy and Catalysis

Not only does antibody catalysis give us an opportunity to test fundamental notions of enzymatic catalysis by "synthesis", it also gives us a unique opportunity to characterize the evolution of binding energy and catalytic function in proteins. As we have seen, the combinatorial and mutational processes of the immune system are similar in many respects to those that occur during the natural evolution of enzymes (Table 1 and Figure 1). However, because the immune response occurs in real time, one has the opportunity to analyze the entire immunological evolution of a catalytic antibody—from the germline precursor to the affinity-matured catalyst, as well as putative intermediates along the pathway. Such an analysis has been carried out for five catalytic antibodies to date, and a number of fundamental insights have emerged.

Consider, for example, the evolution of the ferrochelatase catalytic antibody 7G12. As described above, this antibody adopts a similar catalytic mechanism to the naturally occurring enzyme. The germline antibody accumulates five somatic mutations (two in the light chain and three in the heavy chain) as it undergoes affinity maturation.[18] These mutations lead to a 100-fold increase in binding affinity with a concomitant increase in catalytic efficiency. The X-ray crystal structures of the germline (hapten bound and free) and affinity-matured (hapten bound and free) antibodies have been determined[18, 19] (Figure 8). The affinity-matured antibody binds the *N*-methylmesoporphyrin in a Fischer "lock and key" fit binding mode[44] as expected for a high-affinity binding site in which the active site conformation is preorganized. In

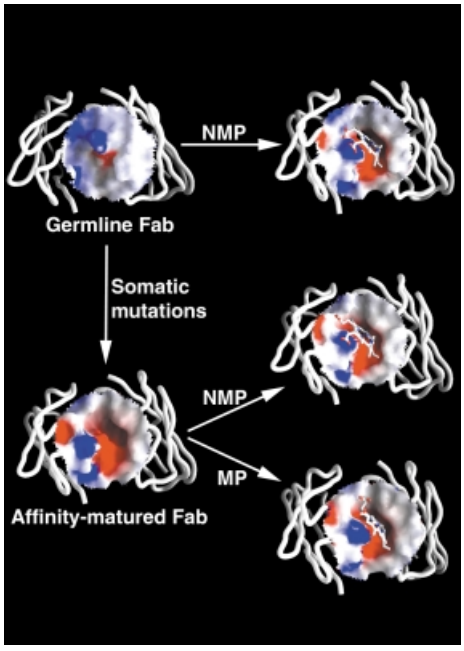

Figure 8. The difference in electrostatic surface potential of the antibody-combining site in the germline Fab and the affinity-matured Fab of antibody 7G12 and their different responses to the binding of hapten *N*-methylmesoporphyrin (NMP) **12** and substrate mesoporphyrin (MP) **10**. The red and blue colors correspond to negative and positive surface potential, respectively.

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contrast, the germline antibody undergoes a significant conformational change upon binding of the hapten, especially in the CDR H3 loop. This induced-fit binding conformation is locked by somatic mutation, in particular through a $Ser\,97^{H}$-Met mutation, which introduces a kink into the backbone of the CDR H3 loop. In addition, there is an $Ala\,32^{L}$Pro somatic mutation, which reinforces the packing interaction with $Tyr\,91^{L}$ that appears to distort the bound porphyrin ring.[18]

The conformational flexibility seen in the ferrochelatase germline ligand-free and -bound structures is also evident in three of the other four germline antibodies that have been structurally characterized: a hydrolytic antibody (48G7),[45, 46] an oxy-Cope catalyst (AZ28),[47, 48] and a redox-active antibody (28B4).[49, 50] In each case significant side-chain reorganization of the germline active site occurs upon ligand binding, whereas there are no significant structural differences in the ligand-bound and -free forms of the affinity-matured antibody. Somatic mutations play a key role in fixing the optimal active site conformation.[18, 46, 48, 50, 51] Only the germline antibody for a Diels–Alder catalyst does not show this behavior; in this case the germline light chain was shown to be polyspecific.[52]

Over half a century ago there was considerable debate over the mechanisms by which the immune system is able to evolve selective, high-affinity receptors for a multitude of ligands. Once it was established that the immune system can produce a large number of antibodies with different sequences through recombination and somatic mutation, this sequence diversity was widely accepted as the basis for the tremendous binding potential of antibody repertoire.[3–5] However, Haurowitz, Breitl, and Pauling argued that conformational diversity could also account for the virtually infinite binding potential of the antibody molecule.[53, 54] Just as a human hand can bind and adapt its shape to a large number of structures, so could an antibody active site change its shape to complement a virtually infinite number of ligands. This theory was termed the chemical-instruction theory (Figure 9).

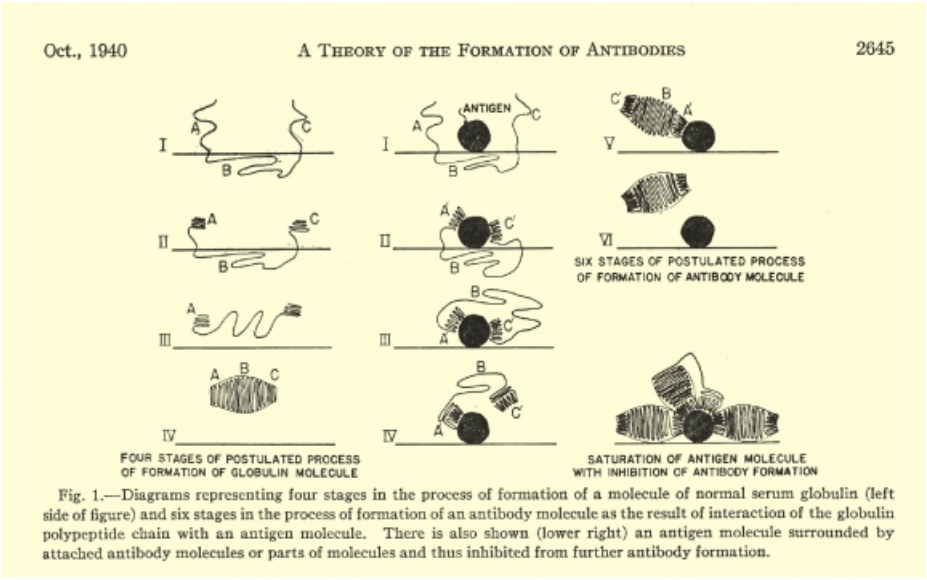

Fig. 1.—Diagrams representing four stages in the process of formation of a molecule of normal serum globulin (left side of figure) and six stages in the process of formation of an antibody molecule as the result of interaction of the globulin polypeptide chain with an antigen molecule. There is also shown (lower right) an antigen molecule surrounded by attached antibody molecules or parts of molecules and thus inhibited from further antibody formation.

Figure 9. The "chemical-instruction theory" of Pauling (from reference [54]).

We now see with these first structural studies of germline antibodies that conformational diversity does indeed play a key role in germline-binding potential, and allows the germline antibody to adapt many different structures (and ligand-binding modes).[18, 46, 48, 50] This conformational diversity dramatically increases the number of possible germline-combining-site structures. The active-site conformation of the affinity-matured antibody (which has optimal complementarity to the bound ligand) is locked not by folding of the rest of the antibody molecule (as proposed by Pauling), but rather by somatic mutations. Importantly, these studies have also shown that somatic mutation can be either in the active site, or significantly removed in distance, and affect ligand binding through coupled secondary-sphere interactions.

Not only do these experiments provide fundamental new insights into the molecular basis of the immune response, they are relevant to the evolution of the binding and/or catalytic function of other proteins as well. For example, recently it has been shown that many receptor surfaces for protein ligands are also conformationally amorphous, which allows them to bind a large number of different protein and small molecule ligands using the same molecular surface.[55] Moreover, mutational studies of enzymes are beginning to show that one can significantly affect the binding and catalytic properties of enzymes through mutations outside the active site, in much the same way that somatic mutations throughout the antibody variable region affect the maturation of binding affinity.[56] This realization is significantly changing current mutagenesis strategies for the in vitro evolution of enzymes to reflect those used by the immune system.[57]

Similar studies of the immunological evolution of the antibody AZ28, which catalyzes an oxy-Cope rearrangement, suggest that the conformational diversity of the germline repertoire can also play a dynamic role in catalysis. It was thought that an antibody generated to the chairlike hapten **19** might catalyze the related oxy-Cope rearrangement (Scheme 3) by binding the acyclic substrate **17** in a cyclic conformation with favorable π-orbital alignment.[58] Such stereoelectronic effects are thought to play a key role in enzymatic catalysis.[59, 60] Surprisingly, the germline antibody accelerated the reaction by 3×10^5 fold (within a factor of three of the corresponding enzyme chorismate mutase), while the affinity-matured antibody of AZ28 was roughly 100 fold less active. Structural studies of the affinity-matured antibody again showed that the hapten is bound in a lock and key conformation with packing interactions between the antibody and 2,5-aryl substituents locking the substrate into the desired cyclic conformation (consistent with NMR spectroscopic studies of the Michaelis complex).[47] An anionic substituent effect also appears to

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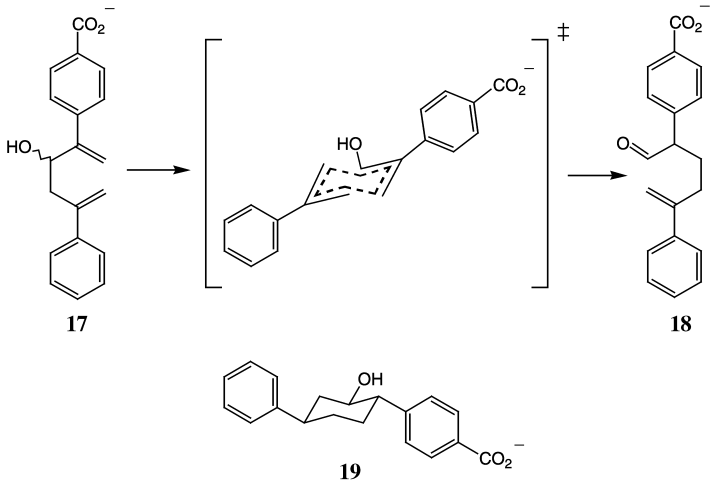

Scheme 3. Transition-state analogue **19** and the oxy-Cope rearrangement catalyzed by antibody AZ28.

contribute to the rate enhancement. However, the 2-aryl ring of bound **19** is orthogonal to the six electron π system, which results in poor orbital overlap in the transition state.[47] The structures of the ligand-bound and -free forms of the germline antibody provide an explanation for the increased rate of this antibody despite its lower affinity for the hapten **19** (Figure 10). Again, there appears to be conformational flexibility in the germline antibody, which in this case allows the 2-aryl ring to rotate into planarity, which increases π overlap and as a result lowers the activation energy.[47, 48, 61] This is of course a static picture and must be confirmed by NMR spectroscopy, but it strongly suggests that the conformational diversity of the germline repertoire can also play a dynamic role in catalysis, much as it is now being realized that side-chain dynamics play a key role in enzymatic catalysis. It may also be possible that the induced-fit mechanisms of enzymes may be vestiges of earlier conformational flexibility that was retained through evolution based on a catalytic, selective advantage.

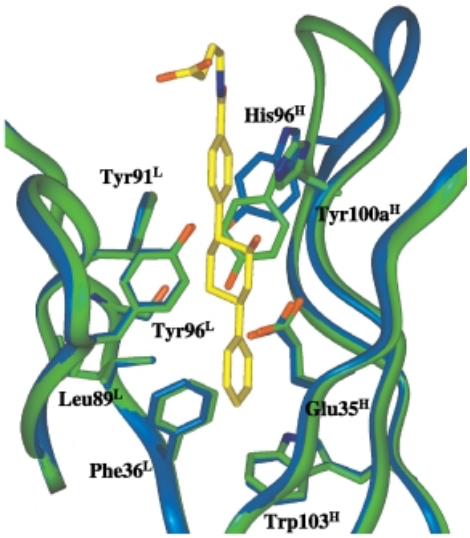

Figure 10. Overlay of the active sites for the germline antibody structures of AZ28 with the hapten **19** (blue) and without hapten (green). The hapten is shown in yellow.

4. Expanding Upon the Concept of Biological Diversity

The combinatorial strategies embodied by the immune response, and well-known to immunochemists, are now being applied by chemists to a huge number of synthetic challenges.[62] This approach is applicable to any molecular structure that can be assembled either in a stepwise or concerted fashion from a set of molecular precursors, and where a screen or selection for a desired function exists. For example, synthetic libraries of protein and nucleic acids are being screened for novel catalytic and binding properties. Synthetic small molecules can also be assembled in a combinatorial fashion from building blocks to create chemical diversity that can be screened for selective binding to biological receptors (the inverse of the immune response). Combinatorial methods have even been used to screen the entire periodic table successfully for combinations of elements that lead to new materials with novel electronic, catalytic, structural, magnetic, and optical properties (Figure 11).[63, 64] Characterization of

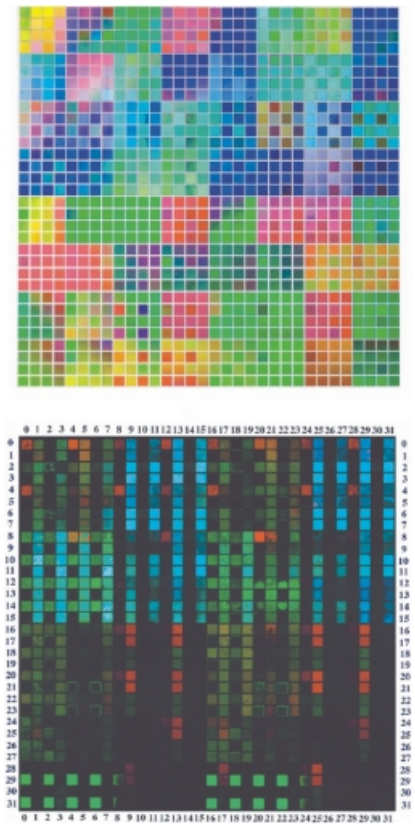

Figure 11. A library of metal oxides that contains novel fluorescent materials under ambient light (above) and UV irradiation (below).

the properties of these new molecules affords new physical, chemical, and biological insights that lead to an improved theoretical understanding of the relationship between molecular structure and function. This mechanistic insight leads in turn to an improved ability to synthesize molecular function.

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5. All Antibodies Are Catalytic—A New Paradigm

The above examples of antibody catalysis underscore the tremendous chemical potential of natural molecular diversity to produce selective catalysts, when programmed by chemists with the appropriate chemical instructions. Over one hundred reactions have been catalyzed to date, including carbon–carbon bond-forming reactions (e.g. Diels–Alder and aldol reactions), redox reactions (e.g. heteroatom, carbon atom, and H_2O oxidation), rearrangement reactions (e.g. sigmatropic rearrangements and cationic cyclization reactions) and hydrolytic reactions (e.g. ester, amide, phosphodiester, and glycosidic-bond hydrolysis). The broad scope of antibody catalysis raises the inevitable question of whether natural antibody catalysis is an intrinsic component of the immune response, which either converts toxic products into harmless materials, or actually generates toxic materials to kill infectious agents. The demonstration of such activity would represent a major shift in the general belief that antibodies simply use their binding to target antigens, which then initiate destruction by using effector systems which are not part of the antibody molecule.

Remarkably, it now appears that all antibodies, regardless of their source or antigenic specificity, are themselves catalysts that have the potential to generate a remarkable array of highly toxic oxidants with the capacity to destroy antigens.[65–68] Some of these oxidants are similar to those known to be generated by phagocytes, whereas others have not been encountered in biology. These findings may demonstrate a shift in the way the humoral component of the immune system is thought to function.

Work carried out over the last century has led to a consensus on how the humoral component of the immune system is organized (Figure 12). The central idea is that, unlike enzymes, antibodies do not have sophisticated chemical functions—they simply function as signaling molecules which activate effector systems upon binding with an antigen. These effector systems, which include the complement cascade and phagocytic cells, are in contrast to antibodies considered to be highly sophisticated chemical cascades that generate reactive proteins and chemical oxidants, respectively, that may be capable of killing infectious agents. But if the antibody itself is a catalyst for the generation of oxidants, then recognition and killing become linked within the antibody molecule itself and the role of the antibody in defense goes far beyond simple signaling. In addition to changing our concepts about the role of antibodies in immune defense, we may gain new insights into the role of antibodies in disease, in that oxidants generated by them may play a previously unrecognized role in the pathology that occurs when this defense system is not perfectly controlled.

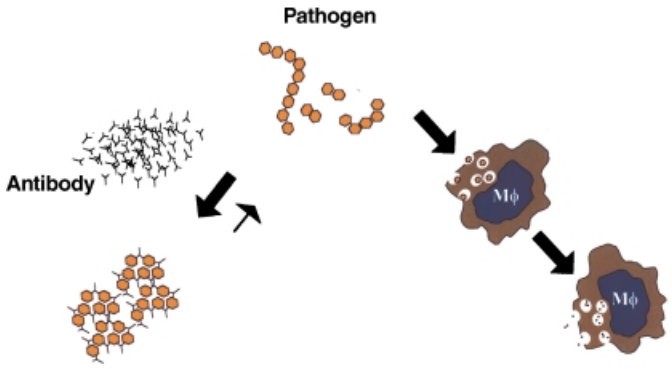

Figure 12. The conventional view of the roles of antibodies in the humoral system: antibodies bind whereas phagocytes destroy pathogens.

Studies of the blue fluorescent antibody[39] mentioned above led to the observation that this antibody and all others characterized to date can produce H_2O_2 from $^1O_2$ without damage to the protein catalyst. Metals or Cl^- ions have been excluded as the source of electrons. On the basis of isotope-incorporation experiments and kinetic data, it has been proposed that antibodies use H_2O as an electron source, which facilitates its addition to $^1O_2$ to form H_2O_3 as an intermediate: [Eq. (1)]

$$^1O_2 + H_2O \rightleftarrows [H_2O_3] \rightarrow H_2O_2 + O_2 \quad (1)$$

X-ray crystallographic studies with xenon indicate putative conserved oxygen-binding sites within the antibody fold, where this chemistry could be initiated. The exact nature of the oxidants produced by the antibody-catalyzed water-oxidation cascade is still under study, but Goddard and coworkers have calculated a number of energetically reasonable pathways by which H_2O_3 can collapse to form H_2O_2.[67, 68] Many of these pathways can be expected to produce highly reactive oxidants not previously observed in biology.

One question often asked concerns the source of the substrate singlet oxygen that antibodies need to initiate the oxidative cascade. Interestingly, when antibody that is bound to the infectious agent signals activation of the cellular effector systems in white blood cells, they generate $^1O_2$ from the reaction of H_2O_2 with HOCl to produce HO_2Cl, which subsequently reacts to afford $^1O_2$. Thus, signaling by the antibody causes its own substrate to be generated such that the very molecule that started the process and is still attached to the infectious agent can generate its own oxidants. While this cascade might seem complicated, it should be remembered that the function of the system is to generate highly toxic substances and their production must be tightly controlled to avoid collateral damage to the host. Because the antibody molecule is the most proximal to the infectious agent, one might expect the oxidant that it generates to be the most effective, while at the same time minimizing collateral damage. This is especially important when the oxidants are short-lived. What is perhaps most surprising is that the field of antibody catalysis, of which the aim was to exploit the selective binding energy of antibody molecules for catalysis, ultimately led to the discovery that these proteins were enzymes all along.

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