# Completing the circle

## Antibody catalysis

*The use of immunological diversity to generate selective catalysts has come full circle, to the realization that antibodies have an intrinsic catalytic ability to destroy antigens.*

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Historically, immunochemistry has focused on understanding and exploiting the remarkable binding affinity and specificity of antibody molecules. With the advent of antibody catalysis, chemists could ask for the first time whether this sophisticated system of molecular diversity can be used to create new chemical tools — efficient, selective catalysts. From these experiments have arisen a host of antibody catalysts, in some cases with specificities and activities that rival those of enzymes.

Moreover, characterization of the mechanisms and immunological evolution of catalytic antibodies has provided fundamental insights into the evolution of binding and catalytic functions in nature. The field has also had a broader influence on chemistry — chemists are increasingly incorporating the biological idea of molecular diversity into their efforts to synthesize molecules with new functions. Finally, the study of antibody catalysis has led to the realization that all antibodies are oxidative catalysts, suggesting a previously unrecognized role for the antibody molecule in the immune response.

The classic early theories of enzyme catalysis by Linus Pauling and J. B. S. Haldane focused on proteins' ability to use selective binding energy to stabilize rate-limiting transition states or to destabilize substrates in order to lower the free energy of activation of a reaction. The realization that one can similarly direct the binding energy of an antibody molecule provided an experimental approach to test these theories, much as chemists have synthesized small molecules to test mechanistic hypotheses.

Consider, for example, the generation of an antibody that catalyses the metallation of porphyrins. It is thought that the enzyme ferrochelatase, which catalyses this reaction in haem biosynthesis, does so by straining or distorting the planar porphyrin substrate. By generating antibodies against a synthetic mimic of a bent porphyrin, an antibody was evolved with catalytic properties similar to those of the natural enzyme. The X-ray crystal structure of the Michaelis complex provided the first direct structural support for the strain hypothesis. Similar studies have made it possible to analyse the energetic contributions of transition-state stabilization, covalent and general base catalysis, and proximity effects to biological catalysis. In some cases, such as antibodies that catalyse efficient acyl-transfer reactions, the immune system converges on the same mechanisms that are used by enzymes for a reaction, underscoring the remarkable similarity between these two evolutionary systems.

As the chemical strategies for exploiting immunological diversity have improved, so has our ability to generate efficient chemical catalysts. For example, the use of covalent immunization with a β-diketone, in which the hapten selects for an antibody with an appropriately positioned active-site lysine, gave rise to antibody aldolases with selectivities and rates that rival natural aldolases. In contrast to the corresponding enzymes, the antibodies catalyse a broad array of aldol reactions with very high enantiomeric excess.

The natural evolution of enzymes and the immunological evolution of antibodies share many features. Both involve recombination, point mutation and selection. Because the last of these can occur on a laboratory timescale, it provides an opportunity to examine the evolution of binding energy and catalysis in nature. For example, structural and biophysical studies of the immunological evolution of four catalytic antibodies (catalysing acyl transfer, oxidative, pericyclic and metallation reactions) showed that high-affinity catalytic antibodies bind to their ligands in accordance with Fischer's 'lock and key' model. In contrast, the active sites of low-affinity germline precursors undergo significant conformational changes upon ligand binding, resulting in increased antibody–ligand complementarity.

These structural studies of the affinity-maturation process have provided fundamental insights into the molecular origins of antibody affinity and selectivity. In particular, the early hypothesis of Felix Haurowitz and Pauling that germline antibody-combining sites can adapt to many shapes, much like a human hand, seems now to be correct. This conformational diversity, together with sequence diversity, explains the huge binding potential of the germline antibody repertoire.

These studies have also underscored the role of mutations located far from the active site in affecting protein binding and catalysis — an important lesson for protein engineering. Moreover, conformational flexibility has been shown to play a dynamic role in catalysis.

In chemistry, we are seeing an increased focus on the synthesis of molecules with defined biological, chemical or material properties. Unfortunately, our ability to rationally design and synthesize molecules with specific functions is not as sophisticated as our ability to synthesize specific molecular structures. The demonstration that one can use chemical principles and tools (such as transition-state theory and stable transition-state analogues) to exploit immunological diversity to generate selective chemical catalysts has introduced a new strategy into synthesis. Large combinatorial libraries of molecules can now be rationally searched for a particular molecule with the desired catalytic properties. Small-molecule libraries have now been synthesized from synthetic building blocks (rather than DNA), and screened for binding affinity. This approach has even been used to search through the entire periodic table for new combinations of elements with interesting properties.

More than 100 different reactions have been catalysed by antibodies. But do endogenous catalytic antibodies exist? All antibodies have the intrinsic ability to catalyse the oxidation of water by singlet oxygen to generate hydrogen peroxide and other reactive oxygen species, endowing the antibody itself with the ability to destroy pathogens oxidatively. Thus the field of antibody catalysis has come full circle, starting with the realization that immunological diversity can be programmed to generate new chemical functions, and ending with the understanding that antibodies all along have had a remarkable catalytic role. ■

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**Like hands, antibodies can adopt many shapes.**

**FURTHER READING**

Haldane, J. B. S. *Enzymes* (Longmans Green, London, 1930).
Schultz, P., Yin, J. & Lerner, R. *Angew. Chem. Int. Engl. Edn* (in the press).