# AB. ANTIBODY CATALYSIS

By 1947 it had become clear that proteins are responsible for carrying out the majority of biological functions of interest. Two common modes of action by proteins were known. Receptors bind to ligands in an equilibrium fashion, and enzymes catalyze the conversion of **substrates** (reactants in biochem speak) to products. However, there were no structures available and the alpha-helix had yet to be properly modeled by Linus Pauling. However, Linus saw well into the future and predicted that receptor-ligand binding could be linked to enzyme-mediated catalysis by a common theme. "I believe that an enzyme has a structure closely similar to that found for antibodies but with one important difference, namely, that the surface configuration of the enzyme is not so closely contemporary to its specific substrate as that of an unstable molecules… namely the 'activated complex' for the reaction…".

He was right – but it took 40 years to prove it. These notes are intended to cover the ground that links antibodies to enzymes.

## Background on Antibodies

Antibodies are immune system proteins that function by binding to foreign proteins in the body, and then targeting the for disposal. The ligand for an antibody is termed an **antigen**.[1] The challenge for the immune system is that there are a virtually infinite number of foreign proteins that could enter the body and not an infinite number of antibodies. Actually, there are usually $10^7$ different antibody (**Ab**) proteins circulating in the blood at any given time, but the potential for $10^9$ different Ab's exists. How are they produced?

*White Blood Cells and so on*

There are two classes of white blood cells (lymphocytes) that interest us at the moment – those that mature in bone marrow (B cells) and those that mature in the thymus (T cells). B cells are the lymphocytes that produced antibodies. Through a complicated genetic mechanism that is worth your attention[2], any given B cell is responsible for producing one Ab protein (as defined by its primary structure). There are $2.5 \times 10^8$ B cells in the body at any given time producing $10^7$ different Abs, so it is possible to have populations of one kind of B cell producing the same Ab – they are all related to a common ancestor B cell.

B cells wear their antibodies on their surface and parade around the body looking for a foreign protein to bind (Figure Ab.1). That might be a long wait, and it may be that it never finds a protein for which it has high affinity. But in a population of $10^7$ *different* kinds of B cells, one will eventually hit paydirt. That successful B cell will then endocytose (bring into the cell) the foreign protein that it recognizes and chop it to bits. A fragment will then be displayed (bound to) on a protein called the major histocompatability complex (MHC) on the surface of the B cell. A T-cell will see that the B

[1] See Glossary of terms in the Appendix

[2] http://jeeves.mmg.uci.edu/immunology/CoreNotes/Chap08.pdf

cell has hit paydirt and will provide a chemical signal that causes the B cell to be activated and to proliferate. At that point it generates a large number of antibodies that will effectively be the first line of defense against that one particular invader.

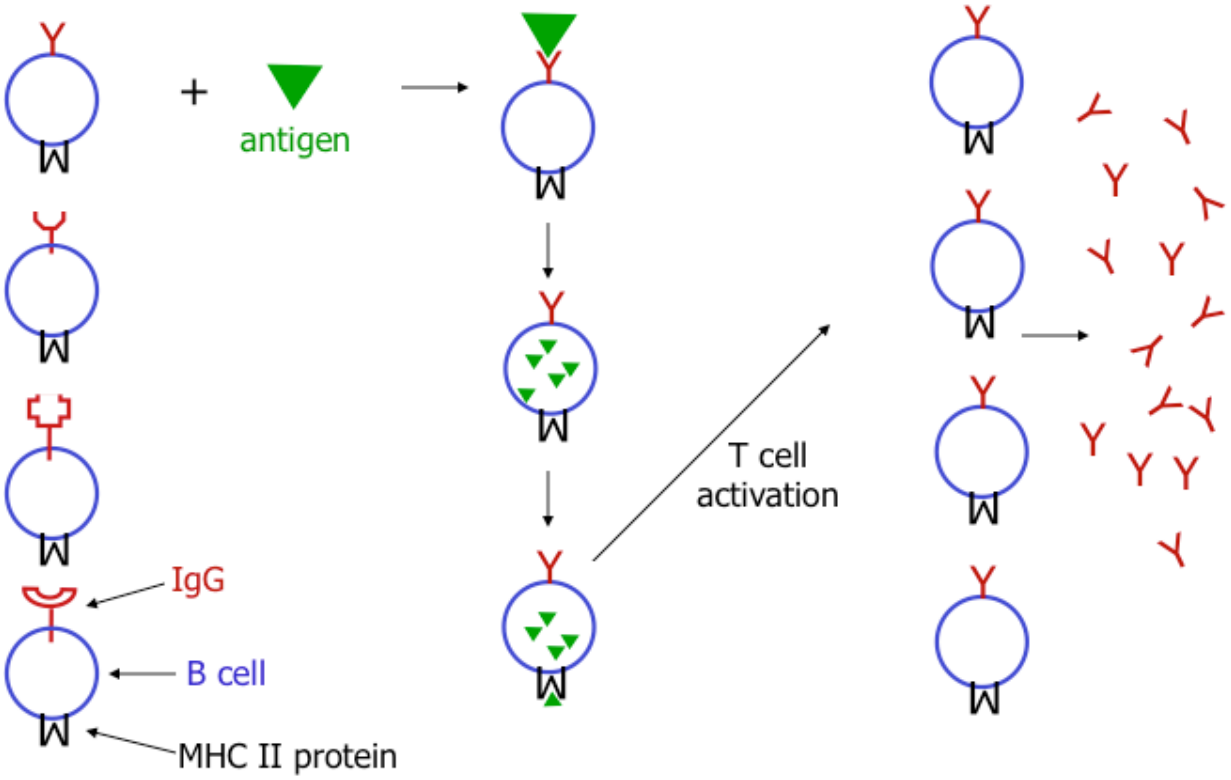



**Figure Ab.1** The activation of one B cell (the one with the "Y" shaped antibody) by antigen binding.

It should be noted that there is a second round of B cell selection. As the primary responder replicates, mutations are made to the gene encoding the antibody. Some of the progeny B cells will be superior to the parent and they will be selected for further reproduction and activation. This process is **affinity maturation** and permits the production of even more effective antibodies than the original one to bind the antigen.

*Structure of the IgG class of Antibodies*

Molecular immunology is a complex field, but I'm going to restrict the discussion here to a limited scope related to a class of antibodies known as IgG (immunoglobulin G). These are the antibodies that circulate in the bloodstream and bind to foreign particles, known as **antigens**. The IgG is an $\alpha_2\beta_2$ tetramer composed of two "heavy" chains and two "light" chains that form a Y-like structure (Figure Ab.2A). The heavy chains are about 440 residues long and built from four discrete, β-sheet rich domains of ~110 residues (the immunoglobulin fold) while the light chain is about 220 residues long and is composed to two immunoglobulin domains (Figure Ab.1B). Each domain is named according to the

A. B.

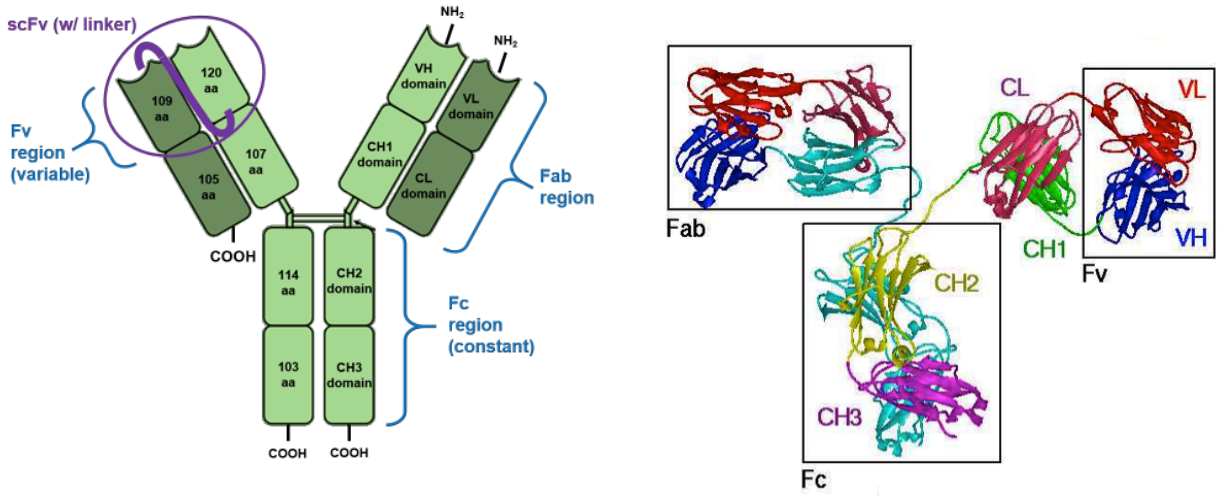



**Figure Ab.2 (A)** Simple diagram of IgG structure. (B) Three-dimensional structure of IgG.

The domains of each chain have a specified label. From the N-terminus to the C-terminus of the heavy chain there are the VH (variable heavy), CH1 (constant heavy 1), CH2 and CH3 domains. For the light chain they are the VL (variable light) and CL (constant light) domains. As the names imply the variable domains possess most of the structural diversity of antibodies and are the domains that bind directly to antigens. There are also named fragments to the IgG. The Fv fragment possesses only the variable domains of each chain (an sFv fragment has those two domains linked together to form a single peptide chain), while the Fab includes the CL and CH1 domains in addition to the variable domains. The Fc fragment is composed only of CH2 and CH3 domains (Figure Ab.2).

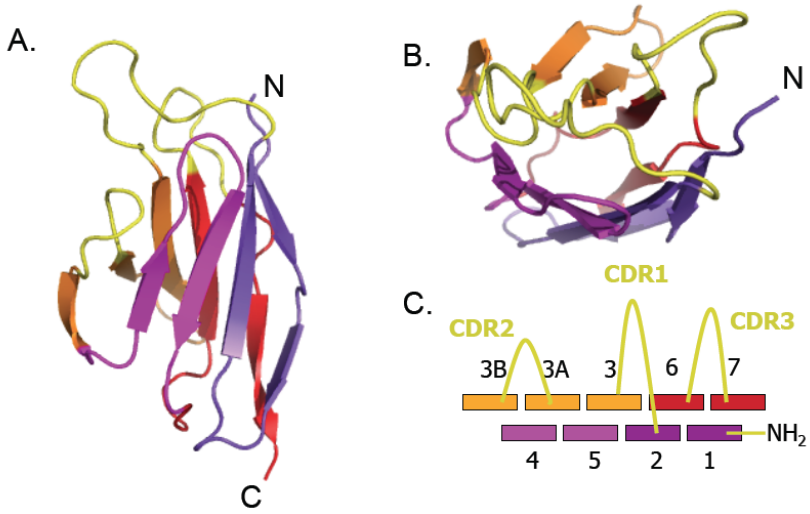



**Figure Ab.3** The variable light domain (A) side on view, showing positions of strands (B) End on view, showing positions of the CDRs. (C) schematic, colored as in A and B labeling strands and CDRs.

Each domain contains a single β-sandwich fold (also called the immunoglobulin fold). Each domains is composed of two antiparallel β-sheets that are like two slices of bread lying against each other. The constant domains possess one sheet of three strands and a second sheet of four strands. The variable domains are composed of two sheets as well, one with four strands and one with five (Figure Ab.3). The extra strands in the variable domains are labeled 3A and 3B, lying between the positions of strands 3 and 4 found in the constant domains (Figure Ab.3). While some sequence variability exists within the strands, the bulk of the structural diversity exists in three loops at one end of the sandwich that connect strands 2 and 3, 3A and 3B and 6 and 7. The loops are refered to as complementarity determining regions (CDRs) 1-3. It's a big name to say that these are the loops that bind to antigens.

*Antibody-Antigen Interactions*

As noted above, the CDRs of an antibody complex with the surface of an antigen. In general, antigens are proteins. The process of activating B cells, as mediated by T cells, requires that the antigen as a protein component. We won't go into that. Instead, suffice to say that you can inject a mouse with any foreign protein and it will generate antibodies to that protein. Monoclonal antibodies are those generated by a single strain of B cells and have defined sequence and structure. Once upon a time, it was of interest to see what would happen if you injected mice with hen egg white lysozyme – a common protein that poses no threat to any mouse. Several different monoclonal antibodies were recovered that all bind to specific sites (**epitopes**) on the surface of the lysozyme protein and generate high affinity complexes (Table Ab.1, Figure Ab. 4)

**Table Ab.1**. Three antibodies elicited to bind lysozyme. Each binds a different epitope on lysozyme with distinctive surface characteristics.

| **Antibody** | **Contact** | **vdW** | **H-Bond** | **Ion-Ion** | **$K_d$ (M)** |
|---|---|---|---|---|---|
| D1.3 | 685 Å$^2$ | 75 | 15 | 0 | $2 \times 10^{-8}$ |
| HyHEL-5 | 750 Å$^2$ | 74 | 10 | 3 | $5 \times 10^{-11}$ |
| HyHEL-10 | 750 Å$^2$ | 111 | 14 | 1 | $2 \times 10^{-11}$ |

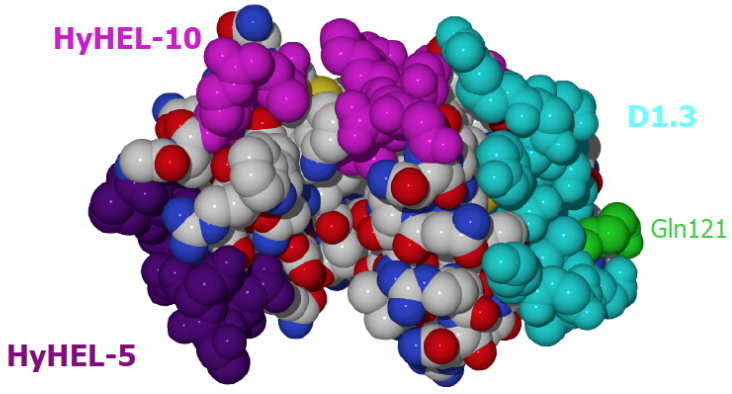



**Figure Ab.4.** Epitopes on the surface of lysozyme. The three different antibodies, HyHEL-5, HyHEL-10 and D1.3, bind to the epitopes color coded on the protein.

One interesting feature that arises from this study is the generality of antibody-antigen recognition. The three epitopes on the surface of lysozyme are non-overlapping and distinct. It's as though *any part of the protein surface* can act as an antibody recognition site. Specificity is general, oddly enough. Any part of the protein's surface appears to be as unique as any other. Also notable is that relatively few H-bonds are involved in specificity (Table Ab.1). Rather, vdW contacts make up the majority of contacts between the antibody and antigen.

A study of D1.3, simply the name of a monoclonal antibody that binds lysozyme, shows that the complex forms exothermically ($\Delta H$ = -21.5 kcal/mol) and with a loss of entropy (-33.7 cal/mol•K). As with protein folding, there is probably a greater entropic cost to complex formation, but that is masked to some degree by an increase in solvent entropy as non-polar surface area is buried on complex formation. While the $\Delta H$ term involves many contacts, specificity for one antigen over another can relate to a single interaction. The chicken lysozyme has glutamine at position 121, while histidine is present in the quail and turkey proteins. Those proteins bind poorly to D1.3 ($K_d > 10^{-5}$ M). However, bobwhite lysozyme – which has a glutamine at position 121, binds just as well as the chicken protein. The rationale for that can be seen in the structure of the D1.3•lysozyme complex (Figure Ab.5), in which Gln121 of lysozyme forms a pair of H-bonds to the backbone of the antibody.

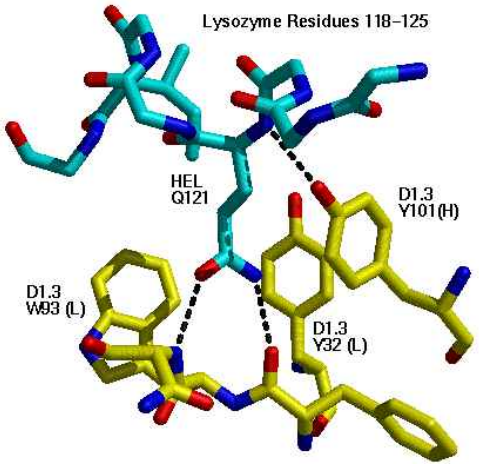



**Figure Ab.5**. Interaction of Gln 121 of lysozyme with the D1.3 antigen-binding region.

## *Generating Antibodies to Small Molecules*

Antibodies are generally elicited in response to protein antigens. However, one can obtain an antibody to a small molecule antigen (called a "hapten") through trickery. By covalently attaching a small molecule to the surface of a protein and injecting into a mouse or rabbit, you will generate an immune response to that hapten-decorated protein. Antibodies that bind a decorated protein will often bind to an epitope that includes the "decoration". Antibodies don't know amino acids from any other small molecule, so a protein surface attached to a hapten is just a protein surface to the antibody. If that surface contains a hapten though, it is often the case that the antibody will also bind the hapten in the absence of the rest of the protein. In that way, one generates a receptor (antibody) selective for a novel small molecule ligand (hapten).

An interesting example of this draws from work in the Wilson lab.[3] I think this was a straw man experiment, but the goal was to develop an antibody that binds cocaine and might thereby be used to detoxify individuals who have overdosed on cocaine by binding up the drug in the blood stream. To do this, a protein has to be decorated with cocaine molecules. That can be achieved by taking an analog of cocaine that possesses a linker that can be used to attach it to the surface of a protein.

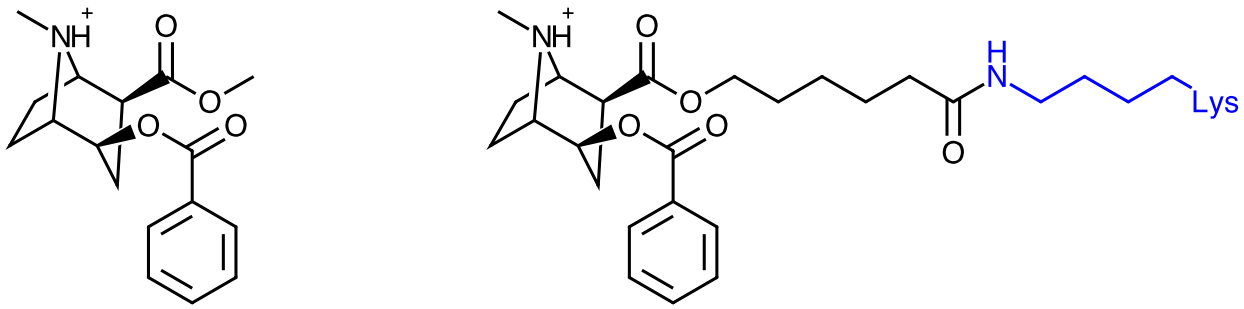
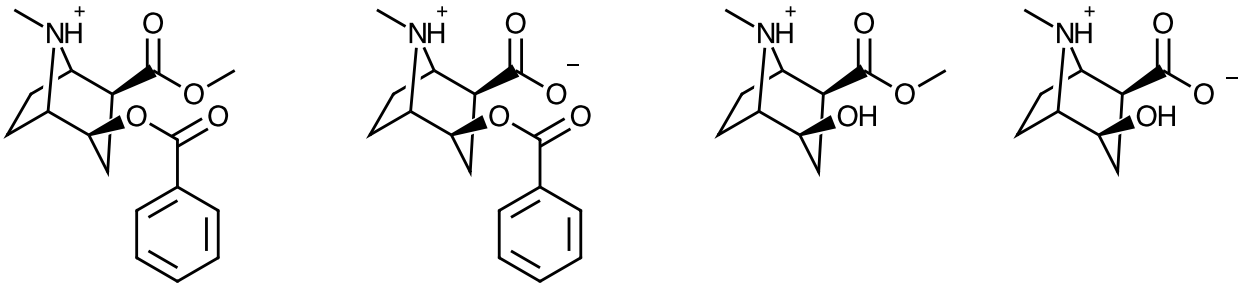



**Figure Ab.6.** At left, structure of cocaine. At right, a cocaine analog in which the methyl ester of cocaine is replaced with a six-carbon linker that can conjugate the analog to a lysine on the surface of a protein (keyhole limpet hemocyanin) thus creating a hapten version of cocaine.

Happily, the strategy worked. An antibody was elicited to a protein, keyhole limpet hemocyanin (KLH; hemocyanin is the oxygen transport protein of the keyhole limpet, a seashell kind of thing), that had been coated with the cocaine analog. The antibody, uselessly named GCN92H2, shows high affinity binding to cocaine ($K_d$ = 100 nM) and much lower affinity to cocaine metabolites (Figure Ab.7). The structure of the complex was solved and the basis for specificity largely lies in shape selective vdW contacts.




$K_d$ = 0.1 μM  $K_d$ = 10 μM  $K_d$ ≈ 1 mM  $K_d$ >> 1 mM

**Figure Ab.7.** At left, structure of cocaine. At right, a cocaine analog in which the methyl ester of cocaine is replaced with a six-carbon linker that can conjugate the analog to a lysine on the surface of a protein (keyhole limpet hemocyanin) thus creating a hapten version of cocaine.

Only one water-mediated H-bond is found in the complex, between the carbonyl oxygen of Ser97 and bridging ammonium group. The cocaine molecule is 95% buried (278 out of 291 Å$^2$) with 75

[3] Larsen et al. (2001) Crystal structure of a cocaine-binding antibody. *J. Mol. Biol.* **311**, 9-15.

vdW contacts between the antibody and hapten. Interestingly, when the benzoyl ester is removed, the $K_d$ increases by about 100-fold, indicating a 2.8 kcal/mol affinity generated by the 33 vdW contacts made to that group, covering about 80 $Å^2$.

So, the idea works, but there's a problem. The lethal dose of cocaine is about 1 g (based on a quick Google – please don't test this!). To bind 1 g (0.003 mol) of cocaine, you would need 0.003 mol of Fab (about 40,000 g/mol), so roughly 120 g of protein. That's why I think this was a silly experiment. It sets up a bad idea to be superceded by a good idea… Let's make an antibody capable of hydrolyzing cocaine. You don't need one mole of catalyst per mole of reactant – you can get by with much, much less.

# Creating a Catalytic Antibody

Back to the topic at hand. The entire purpose of discussing antibody structure was to understand Pauling's principle that enzymes function by binding selectively to transition states. Antibodies bind selectively, but not to transition states. The question was how one might make that happen.

*Generating Antibodies to Transition States*

To create a catalytic antibody, you need one that binds a transition state. Sadly, as noted above, transition states are, as the name suggests, transitory – they live and die within $10^{-12}$ s. Thus one must imagine a small molecule analog of the transition state. The transition state analog (TSA) possesses the same shape and charge distribution as the transition state itself, but is a stable molecule. By decorating a protein with a TSA, one can obtain an antibody that will selectively bind the TSA (and maybe the transition state itself) more tightly that the substrate (the biochemical term for a reactant).

The logic of this process is illustrated in Figure Ab.8. Ideally your antibody catalyst (E), binds the TSA or transition state ($X^{‡}$) more tightly that the substrate (S). That's indicated in Figure Ab.8(A) by the greater negative change in free energy associated for formation of the E•TSA complex than for the E•S complex. If the antibody binds the transition state as tightly as the TSA, the scenario will be as if Figure Ab.8(B). The stabilization of the transition state will be greater than the substrate, leading to a decrease in $\Delta G^{‡}$ for the catalyzed reaction vs. the uncatalyzed reaction.

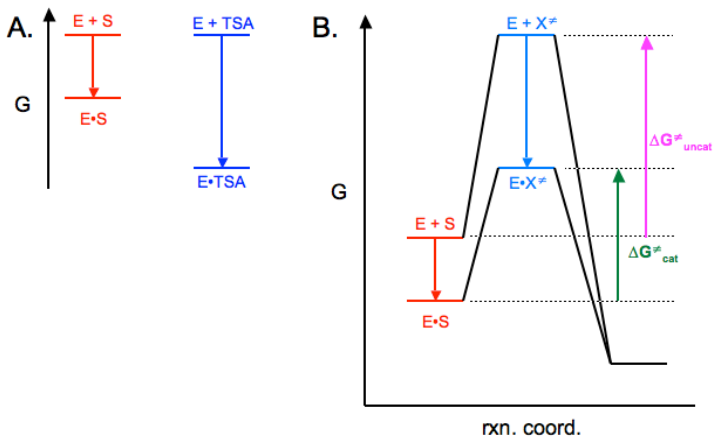



**Figure Ab.8.** (A) An antibody catalyst (E) should bind the TSA more tightly than the substrate, S. (B) If all goes well, it also binds the transition state, $X^{\ddagger}$, more tightly and one will create a scenario with a reduced free energy of activation.

## *An Early Catalytic Antibody*

Consider the base-catalyzed hydrolysis of a carbonate, such as that shown in figure Ab.9. The reaction passes through a tetrahedral intermediate and two transition states. Since the intermediate is presumed to be much higher in energy than reactants or products, we can use Hammond's postulate to argue that the structures of the transition states are close to that of the intermediate. The trick is to devise a molecule that possesses the same geometry and charge characteristics as the presumed transition state. As it turns out, a phosphate group does a good job mimicking the tetrahedral intermediate in carbonate hydrolysis (Figure Ab.9).

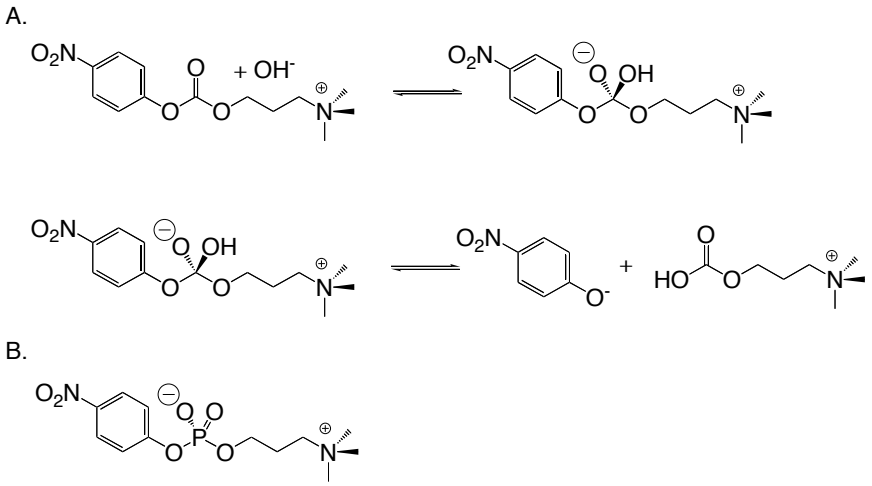



**Figure Ab.9.** (A) Base-catalyzed hydrolysis of a carbonate proceeds in two steps through a tetrahedral intermediate. (B) p-nitrophenylphosphoryl choline is a transition state analog that shares shape (and some electrostatic) similarities to the presumed transition state.

Peter Schultz recognized this and used that structural analogy to use a monoclonal antibody that binds the phosphate shown in Figure Ab.9(B).[4] It is one of the first catalytic antibodies, presumably because the free energy of the transition state is lowered more than the free energy of the substrate. As expected the carbonate substrate does bind less tightly than the phosphodiester TSA:

$E \bullet S \Leftrightarrow E + S \qquad K_d = 200\ \mu M$

$E \bullet TSA \Leftrightarrow E + TSA \qquad K_d = 0.7\ \mu M$

That represents a nearly 300:1 difference in affinity in favor of the transition state analog, or 3.3 kcal/mol (the difference in the arrows in Figure Ab.8(A)). In principle, if the transition state analog mimics the true transition state accurately, the rate acceleration will also be by about 300:1 (Figure Ab.8(B)). Interestingly, the acceleration observed with the antibody over solution rates is observed to be 770 to 1.

$v = k_{uncat}[S] \qquad k_{uncat} = 5.2 \times 10^{-4}\ min^{-1}$ at pH 7

$v = k_{cat}[E \bullet S] \qquad k_{cat} = 0.4\ min^{-1}$ at pH 7

Note that we must specify the pH because hydroxide participates in both reactions.

This is a remarkable result. Several controls were performed to verify the participation of the antibody in catalysis. *See papers for now – I'll add later.*

## *Hydrolyzing Cocaine*

The hydrolysis of a carbonate is hardly noteworthy, and even less impressive is the hydrolysis of a carbonate that has p-nitrophenolate as a leaving group. More interesting have been efforts to achieve catalysis of more difficult reactions of some biological significance. While ester hydrolysis is only one small step up from carbonate hydrolysis, if you can hydrolyze a worthy molecule, then you might have a saleable product. Let's hydrolyze cocaine.

A couple of groups have worked on this problem, but a particularly nice paper came out in 2006 from Ian Wilson's group (see the set up from the earlier study), which couples kinetics to a complete crystallographic exploration of catalysis.[5] As in carbonate hydrolysis, the hydrolytic cleavage of the benzoyl group of cocaine may be achieved by attack of a solvent molecule leading to a tetrahedral intermediate (misleadingly shown as a transition state in Figure Ab.10.) That tetrahedral intermediate may be mimicked by a phosphonate compound resembling cocaine (compound **6** in Figure Ab.10). Even better, if one couples compound **5** from to keyhole limpet hemocyanin, one

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[4] S. J. Pollack, J. W. Jacobs and P. G. Schultz (1986) Selective Chemical Catalysis by an Antibody. *Science* **234,** 1570-1573. Note that Schultz shares priority with Richard Lerner's group, which published in the same issue of Science. A. Tramontano, K. D. Janda and R. A. Lerner (1986) Catalytic Antibodies. *Science* **234**, 1566-1570.

[5] X. Zhu, T. J. Dickerson, C. J. Rogers, G. F. Kaufman, J. M. Mee, K. M. McKenzie, K. D. Janda and I. A. Wilson (2006) Complete Reaction Cycle of a Cocaine Catalytic Antibody at Atomic Resolution. *Structure* **14**, 205-216.

can generate an immune response in which some antibodies will bind strongly to the phosphonate compound **6.**

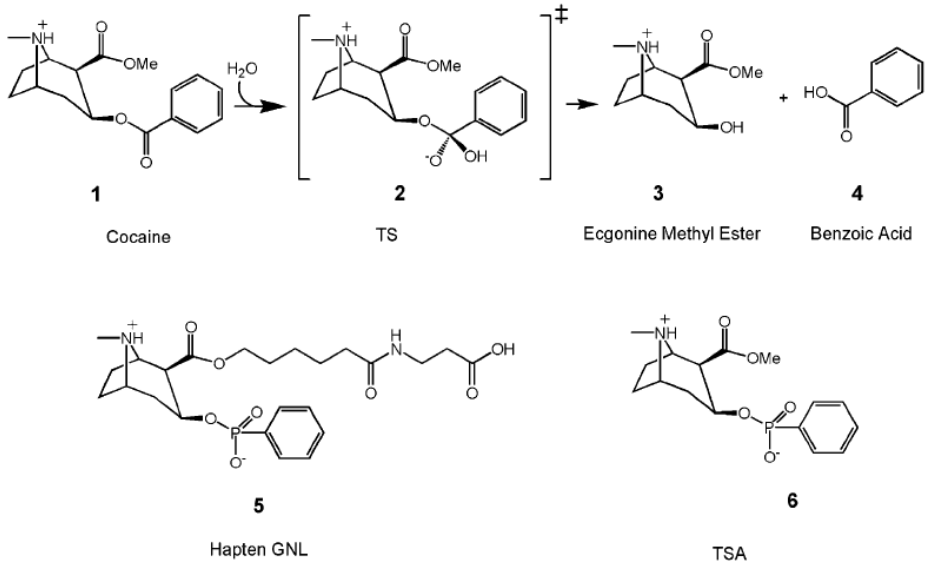


**Figure Ab.10.** Strategy for generating a cocaine hydrolyzing antibody. Compounds 1-4 lie along the reaction coordinate for the hydrolysis of cocaine. Compound **5** may be used as a hapten, linked to KLH, to elicit an immune response. Compound **6** is the transition state analog. (Figure taken from ref. 4.)

The antibody that was selected for characterization does its job. The uncatalyzed rate constant ($k_{uncat}$) for cocaine hydrolysis is 1 x $10^{-4}$ $min^{-1}$, while $k_{cat}$ is 2.3 $min^{-1}$, reflecting a 23000-fold enhancement (5.9 kcal/mol stabilization of the transition state relative to stabilization of the substrate). This can likewise be seen in the $K_d$ for cocaine, the substrate, which is 220 μM (weak binding) and the phosphonate TS analog, which is 0.009 μM (strong binding). The phosphonate binds 24,400 times more tightly than the substrate for the reaction – a difference of 6 kcal/mol. The crystal structure of the antibody complexed to the phosphonate analog reveals the basis of selective transition state analog binding. Arg52 and Tyr50 from the heavy chain do not interact with cocaine (Figure Ab.10A) but form strong H-bonds to the phosphonate group of the TS analog (Figure Ab.10B). Those two H-bonds contribute significantly to the >20,000-fold preference of the antibody for the transition state (and its analog) relative to the substrate and promote catalysis of the cocaine molecule.

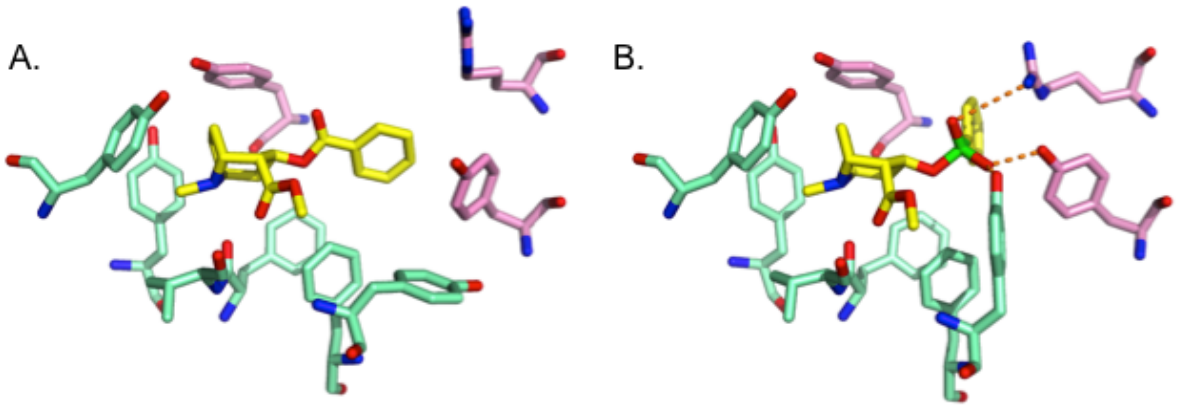


**Figure Ab.11.** (A) Structure of cocaine bound to the catalytic antibody. (B) Structure of the phosphonate transition state analog to the same antibody. Note the arginine and tyrosine residues shift position in the presence of the analog to form H-bonds.

*Antibody that Catalyzed the Chorismate Mutase Reaction*

There has been interest in seeing if one could create a catalytic antibody that can perform a biological reaction as well as a comparable enzyme, natively evolved to perform that reaction. Early investigations probed the chorismate mutase reaction, which proceeds through a Claissen rearrangement (Figures Ab. 12, 13). The biological reaction converts chorismate to prephenate, an essential step in the biosynthesis of aromatic amino acids. As a unimolecular rearrangement it is an ideal target for antibody catalysis since the chief barrier to the reaction is the loss of entropy required for the substrate to attain the conformation required for electrocyclic ring rearrangement.

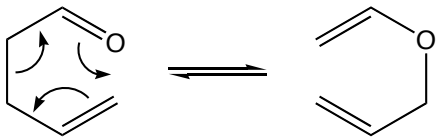


**Figure Ab.10**. The Claisen rearrangement

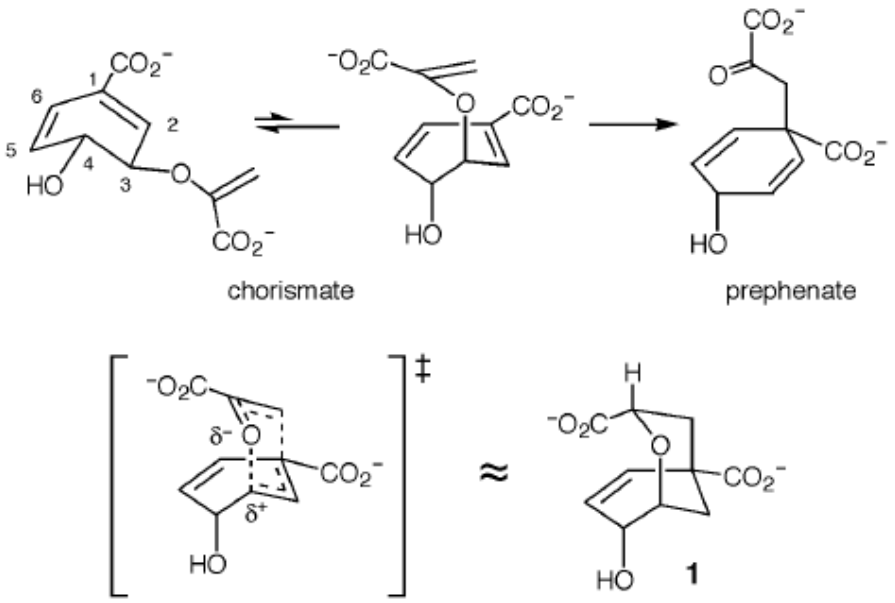


**Figure Ab.13** The Claissen rearrangement of a simple unsaturated aldehyde is shown above. In the middle reaction scheme, the rearrangement of chorismate (at left in two competing conformations) proceeds to give prephenate. At the bottom the presumed transition state is shown along with a transition state analog, **1.**

Use of TSA **1** (Figure Ab.13) as a hapten lead to the isolation of a catalytic antibody capable of promoting the chorismate mutate reaction. As expected, it binds the TSA more tightly than the substrate, by a factor of 100 (Table Ab.2). As expected, it also catalyzes the chorismate mutase reaction roughly 200-fold over its uncatalyzed rate.

**Table Ab.2.** Kinetic and equilibrium data for a catalytic antibody and a natural enzyme catalyzing the chorismate mutase reaction.

| | $k_{uncat}$ or $k_{cat}$ ($s^{-1}$) | $\Delta\Delta G^{\pm}$ (kcal/mol) | $K_d$ for chorismate | $K_d$ for TSA |
|---|---|---|---|---|
| uncatalyzed | $6 \times 10^{-6}$ | N/A | N/A | N/A |
| Catalytic antibody | $1.2 \times 10^{-3}$ | -3.1 | 51 μM | 0.6 μM |
| Natural enzyme | 46 | -9.4 | 67 μM | 3 μM |

Despite that success, the competency of the natural enzyme dwarfs that of the antibody. It binds the TSA more strongly than the substrate, though only a 20-fold difference and it does not bind the TSA as strongly as the antibody. However, the natural enzyme accelerates the reaction by about $10^7$ fold – 100,000 times better than the antibody. How is the enzyme so much more effective? One possibility is that the transition state analog is a poor mimic of the true transition state, so the antibody has been primed to recognize a molecule *similar* to the transition state, but it has not been optimized to the true transition state. Another issue against the antibody catalyst arose from thermodynamic analysis (Table Ab.3). It is noteworthy that both reduce the enthalpy of activation by about 5 kcal/mol over the uncatalyzed reaction. However the entropy of activation is actually less favorable in the antibody than in the uncatalyzed process! That is unlikely due to substrate

effects. The antibody should effectively reduce the entropy of the substrate to a single conformation just as the enzyme would. Instead, Hilvert has argued that the antibody itself must lose entropy as the reaction proceeds to the transition state. Perhaps the antibody retains a great deal of flexibility in its loops while the substrate is bound, but as the reaction progresses, the antibody organizes itself around the substrate and in the process hinders the reaction. That little subtlety is one that nature has undoubtedly countered in the past and is but one of the many virtues that make true enzymes more effective catalysts than antibodies generated to transition state analogs.

Table Ab.3. Thermodynamic parameters for the natural and antibody catalysts.

| | $\Delta H^{\neq}$ (kcal/mol) | $\Delta S^{\neq}$ (cal/molK) |
|---|---|---|
| Uncatalyzed | +20 | -13 |
| Catalytic antibody | +15 | -22 |
| Natural enzyme | +15 | -0.8 |

## *Parting Words*

While no catalytic antibody has yet achieved the catalytic prowess of a natural enzyme, they have been able to generate chemistries that are not catalyzed in nature. The core hypothesis behind catalytic antibodies is that a protein that binds a transition state more tightly than the reactant will accelerate a reaction is clearly supported by the evidence. In fact, as we'll see, some of the best drugs are designed as transition state analogs to reactions catalyzed by enzymes implicated in various diseases.

# Appendix – Glossary of Terms

**Antibody (Ab)** - immune system receptor

**Antigen** - ligand for an antibody (usually a protein)

**B cell** - white blood cell that produces antibodies

**CDR** - complementarity defining region; loop that binds antigen

**Epitope** - part of antigen that binds antibody

**Fab** - Fragment of Ab containing full light chain/half heavy chain

**Hapten** - small molecule that acts as ligand for antibody

**Idiotope** - surface of Ab that interacts with antigen

**Immunoglobulin (Ig)** - class of proteins to which antibodies belong

**Monoclonal Ab** - Ab of defined sequence/structure (as opposed to a polyclonal antibody sample in which many antibodies share a common antigen.

**Variable region** - N-terminal domains of light & heavy chains