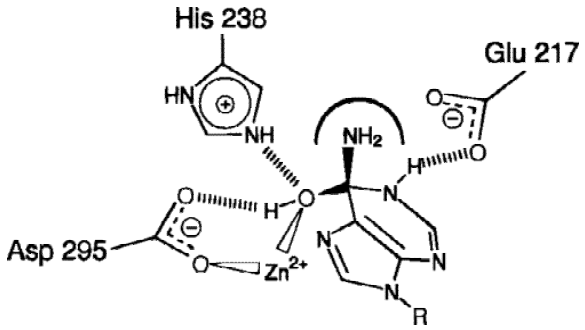
Answers to Problem Set 3.

1. Adenosine deaminase and its mechanism appear at first glance to be very similar to the cytidine deaminase mechanism that you looked at in detail in ps 2. One helpful ingredient in thinking about catalysis is to have a structure of a ground state analog and a transition state analog. Different interactions in the ts, not observed in the gs, could help identify the mechanisms of catalysis. The structure in Figure 1a is thought to be indicative of a ground state conformation, while the structures in Figure 1b and Figure 2 should be indicative of transition state binding. The structure in Figure 1B was a surprise as the crystallization was carried out with the PR (3) and the enzyme catalyzed a stereospecific addition of a zinc activated water or hydroxide to the C6 position of PR. The differences between the the structures in 1A and 1B can potentially give information about catalysis. The data has been taken from two papers by Quiocho and his group published in Biochemistry 32, 1689-1694 (1993 and Biochemistry 37, 8314-24 (1998). First let us look at what the three structures have in common that allows adenosine to bind. All three structures reveal a H bond interaction between N-3 and the amide NH of G184, and unusually between N-7 and a protonated D296. The environment of D296 must be unusual to keep it's side chain protonated. In all three structures the D19 forms H bond interactions with the 2'-hydroxyl of the sugar. A difference between the gs structure and the two ts structures is the interaction with E217. In the gs structure the N in the base is absent. In the two ts analog structures the E is not protonated in the H bonding interaction. These structures suggest that E might be protonated in the gs as suggested by the authors addition of a proton to E217 in Figure 1A. Once again the environment of the protein must cause the pKa of this E to be altered.



A comparison of all three structures suggests that four residues play a very important role in catalysis: E217, H238, D295 and the zinc. The gs structure suggests that zinc, with the help of H238 has activated water for nucleophilic attack on the purine ring. Water could bind to the zinc, its pKa is reduced and histidine picks up the proton. The histidine could function to electrostatically stabilize the attacking hydroxide. The D295 could assist in helping align the hydroxide to attack the base with the appropriate stereo chemistry. The location of the zinc and the aspartic acid dictate the stereochemistry of attack. It should be noted that the role of His as a GBC to assist in water activation is controversial from mutagenesis studies. If the mutagenesis studies are correct, then it could be that E217 initially deprononates the water and is now in the protonated state ready to protonate the N1 of the base. This role would be similar to the one proposed for cytidine deaminase. The E217 appears to be in an excellent position to protonate the N1 of the base. Analysis of the distances in the available structures (not accessible to you) suggest that E217 is too far removed to deprotonate the water. If in fact the E217 is too far, then the protonated E217 must reside in an environment to perturb its pKa.

The ammonia elimination pathway is more difficult to deconvolute from the available structural information. It is possible as in the case of cytidine deaminase that the E217, now deprotonated, could shuttle a proton from the 6-OH to the NH_2 leaving group. Histidine could assist in formation of 6-O^- structure. Note in the case of Figure 1A, E217 is far removed from the 6-HO and the question of flexibility within the active site remains an issue. The structures available do not reveal this required flexibility. An alternative pathway could use D295 in a similar role as that proposed for E217. However, given the structures, and the fact that D295 is coordinated to the zinc, it may be difficult for this residue to move in a fashion to protonate the leaving NH_2 on the opposite face from the 6-O.

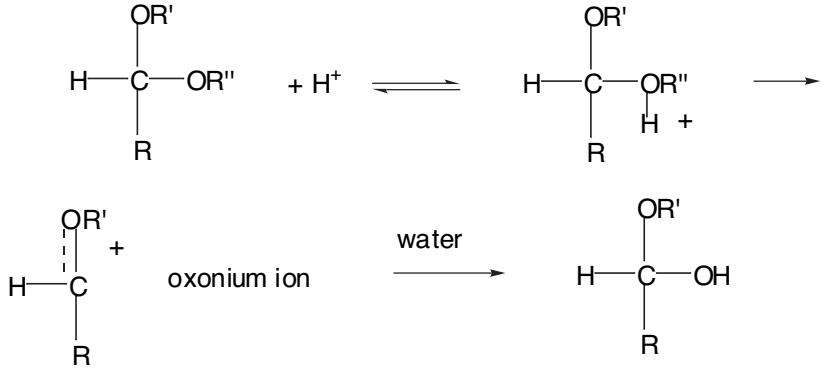
One needs to point out that one cannot carry the analogy between cytidine deaminase and adenosine deaminase too far. The structures are completely different as are the environments within the active site. They share in common the use of zinc and a carboxylate as playing an important role in catalysis.

b. The structures of DCF and HDPR (1A) are remarkably similar. The key interaction that provides tight binding is the coordination of the hydroxyl grooup to the zinc. This interaction is not accessible in the gs structure. The 6-OH of DCF is also H bonded to

the D295 and H238 in identical fashion to hydrated PR (1B). All of the other interactions, as noted above, are similar as well. This HO is the key to the tight binding of the ts relative to the gs through the zinc, H and D. The S isomer does not possess the appropriate stereochemistry to bind and still retain the pentacoordinate environment around the zinc. Hence one has much weaker binding.

c. The mechanistic options are described above in part a. The role of E217, D295 and H238 in the acid and base chemistry is still open to debate and a number of possible mechanisms exist given the available information.

2. Lysozyme has long been used as an example of ground state destabilization and its importance in catalysis. The details of the proposed mechanism and the controversy are outlined in detail in Voet and Voet (p. 385). The details of how the binding site for 6 sugars was identified are discussed in Voet as well. Lysozyme catalyzes hydrolysis of a glycosidic bond and involves the conversion of an acetal to a hemiacetal. The mechanism you learned in organic chemistry involves protonation of leaving group, followed by generation of a resonance stabilized oxonium ion intermediate. These types of intermediates are planar. You really did not need to know this information to solve the problem, you just needed to realize that the lactone (4) is planar and you were given that the D site bound its sugar in a non-chair unusual planar configuration.



In the active site of lysozyme to acidic amino acids play an essential role (probably general acid/base and electrostatic stabilization) in catalysis. This is the Phillips model that suggested the importance of strain in catalysis. The data in Tables 1 and 2 provided the evidence for this original model. Measurements of binding equilibria (Table 2) of various oligosaccharides to lysozyme indicated that all the saccharide residues except the one binding to the D subsite contributed favorable energetics

towards the binding of the substrate to lysozyme. From the Table binding of NAM to the D site required a free energy imput of 12 kcal. The Phillips model used this data to suggest that the energy imput was used to strain the substrate to look more the planar ts. The chair form was strained to a half chair, planar form. This notion of using binding energy for catalysis was supported by the rate accelerations observed in Table 1 when sugar oligomers extending to the fifth and sixth positions in the active site were occupied. The lactone (4) binds much tighter than the normal substrate suggesting that it may be a ts analog for the reaction. The binding energy is used to bind as there can be no reaction. The lactone is planar as is proposed for the sugar resdue binding in the D site. An xray structure has supported this model and the lactone of the tetrasaccharide binds in the D site and is in a half chair conformation. For those of you who remembered the mechanism of acetal hydrolysis, oxonium ions are also planar and therefore tight binding of the lactone has been used to support this mechanism. This is the answer I was looking for. The description in Voet and Voet tells you about additional experiments and theory that suggest that this type of destabilization is insufficient to account for all the rate acceleration. This is not surprising from what we have learned in class. In the case of lysozyme, the role of acid base catalysis, electrostatic stabilization of the ts and the role of covalent catalysis are still being debated.