

Chemistry 5.07 Biological Chemistry
Fall Semester, 2013

Lecture 5 and 6 Enzymes and Catalysis. Without enzymes there would be no metabolism.

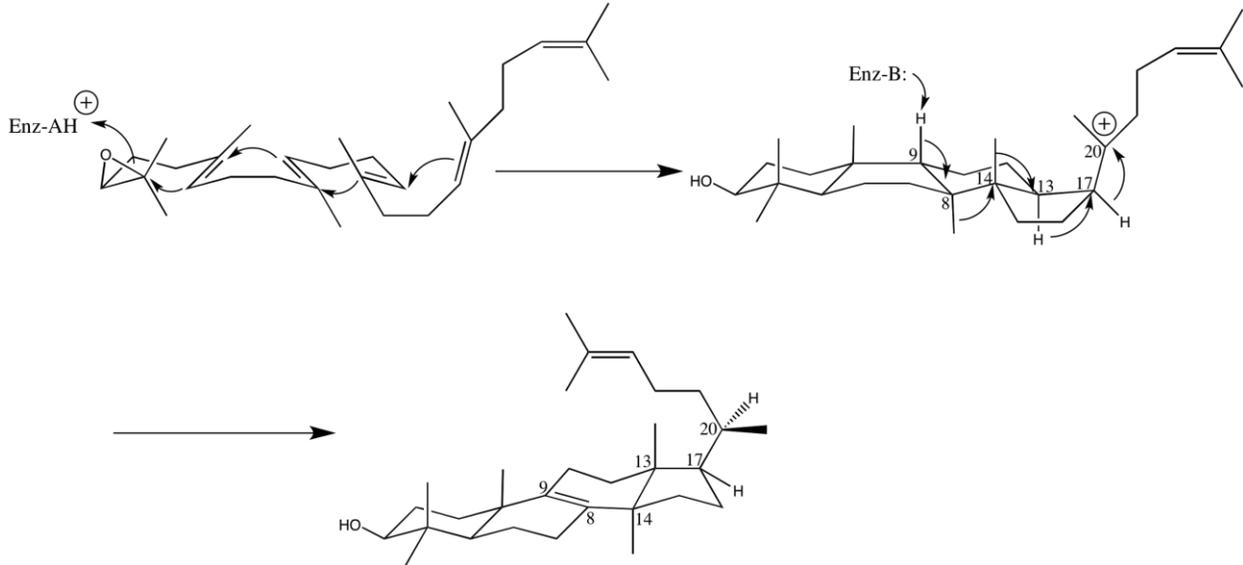


Figure by O'Reilly Science Art for MIT OpenCourseWare.

Adapted from Figure 1 of Poralla, Karl. "Profound insights into squalene cyclization." *Chemistry & biology* 11, no. 1 (2004): 12-14.

Figure 1. Cyclization of oxidosqualene: Lanosterol is the precursor to cholesterol and steroids. Cyclization of the (3S)-2,3-oxidosqualene to produce lanosterol is catalyzed by lanosterol cyclase. This single enzyme creates 7 asymmetric centers stereospecifically at 37°C, at pH 7!!

The awesome power of enzymes:

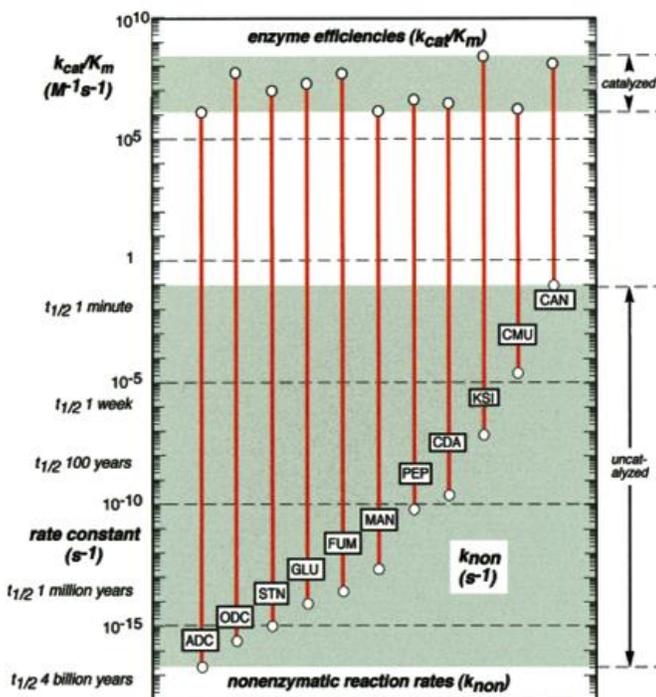
I. Definition of a catalyst: an entity (organic, inorganic, organometallic, protein or RNA) that increases the rate of a reaction without itself being changed in the overall reaction. A catalyst has NO effect on the solution equilibrium of a reaction, it increases the rate of approach to equilibrium.

1. Most catalysts in biology are proteins. The exception is the ribosome (the translation factory)!

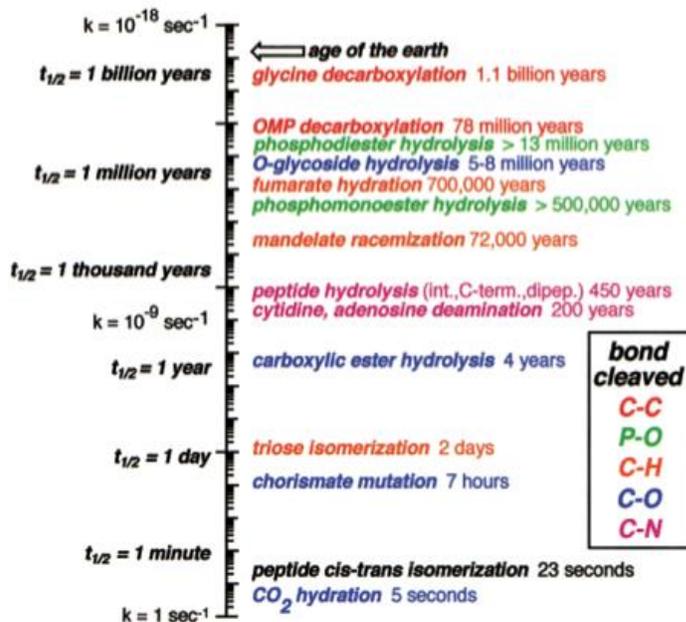
2. Enzymes accelerate the rates of reactions relative to non-enzyme catalyzed reactions by factors of 10^6 to 10^{15} (Figure 2A). Figure 2B shows non-enzymatic reaction rate constants of reactions found in the cell. From Figure 2B, you can see for example, that peptide bond hydrolysis at neutral pH occurs with a rate constant of 10^{-9} s^{-1} , yet typically enzymes that catalyze this reaction have turnover numbers (k_{cat}) of 50 s^{-1} . The catalytic **efficiency** (**proficiency, specificity**) of an enzyme (or any catalyst) is given by the kinetic parameter $k_{\text{cat}}/K_{\text{m}}$. We will learn about k_{cat} and $k_{\text{cat}}/K_{\text{m}}$ and their usefulness in thinking about catalysis in the next Lecture. Figure 2A shows that $k_{\text{cat}}/K_{\text{m}}$ for enzyme catalyzed reactions all occur within a small range (10^6 to $10^9 \text{ M}^{-1}\text{s}^{-1}$) and that the non-enzyme catalyzed reactions have reaction rate constants that span many more orders of magnitude. Enzyme catalysts have been optimized (We will see that this range of numbers represents diffusion control of an enzyme (E) finding its substrate (S) in solution). [aside: You might think about how one would measure the rate of a reaction with a half-life of a billion years as in the non-catalyzed reactions shown in Figure 2B!]

3. Enzymes catalyze their reactions stereospecifically and are highly specific for their substrates. However, studies in the test tube (in vitro) with synthesized substrate analogs never encountered in vivo, suggest that enzymes are not as specific as we once thought. They are specific in vivo!

A.



B.



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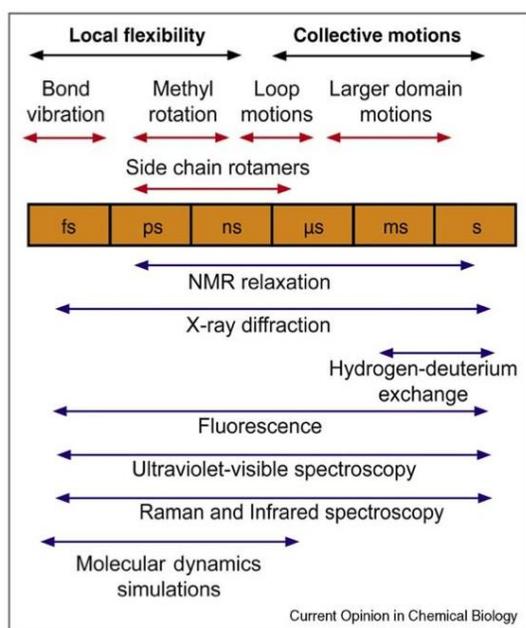
Source: Wolfenden, Richard, and Mark J. Snider. "The depth of chemical time and the power of enzymes as catalysts." *Accounts of chemical research* 34, no. 12 (2001): 938-945.

B. Source: Lad, Chetan, Nicholas H. Williams, and Richard Wolfenden. "The rate of hydrolysis of phosphomonoester dianions and the exceptional catalytic proficiencies of protein and inositol phosphatases." *Proceedings of the National Academy of Sciences* 100, no. 10 (2003): 5607-5610. Copyright © 2003 National Academy of Sciences, U.S.A.

Figure 2. Enzyme power. A. Logarithmic scale of k_{cat}/K_m and k_{non} values for some representative reactions at 25°C. The length of the vertical bar represents transition-state affinity or catalytic proficiency. B. Half-times of biological reactions proceeding spontaneously in water at 25°C in the absence of a catalyst.

4. Enzymes catalyze a limited repertoire of reactions compared to the number of reactions that chemists have created using the periodic table. Nature is considerably more effective at catalyst design than a chemist. However, her catalysts are much more complex with molecular weights of 10 kDa to MegaDas, while chemists create catalysts of molecular weights ranging from 500 Da to a H^+ . The side chains of the amino acids of proteins, as noted in Lecture 2 and 3, play the central role in catalyzing these reactions.

5. The region of the protein where the chemical reaction occurs is called the active site and there may be as few as 10 amino acids surrounding (enclosing) this site (recall the Enolase superfamily (TIM barrel) and the residues required to bind the metal). However, it is now clear that amino acids very far removed from the active site often play an essential role in catalysis by affecting protein side chain alignments via conformational change(s) of the protein and consequently its activity. [The role of dynamics in these changes and hence in catalysis is actively being debated, although more recent reports suggest that a consensus is being reached. See *Biochemistry* 52, 2068-77 (2013) and *Biochem Soc Trans* 40, 515-21 (2012).] Figure 3 indicates the time scales of the motions of proteins and their methods of detection.



Courtesy of Elsevier, Inc., <http://www.sciencedirect.com>. Used with permission. Source: Nash ne, Vishal C., Sharon Hammes-Schiffer, and Stephen J. Benkovic. "Coupled motions in enzyme catalysis." *Current opinion in chemical biology* 14, no. 5 (2010): 644-651.

Figure 3. Timescales of molecular motions and their means of detection.

6. Nature has also made additional use of coenzymes (small organic and inorganic clusters) bound in the active site to expand the catalytic diversity of reactions that she can access (Figure 4; also see Lexicon of reactions). These coenzymes will be discussed in Lectures 7-10 and

throughout our discussion of metabolism. Many of these cofactors are derivatives of vitamins or decorated metal centers.

Vitamin	Coenzyme	Human Deficiency Disease
B ₁ (Thiamine)	Thiamine pyrophosphate (TPP)	Beriberi
B ₂ (Riboflavin)	Flavin coenzymes (e.g., FAD)	Deficiency in humans rare
B ₃ (Pantothenate)	Coenzyme A (CoA)	Deficiency in humans rare
B ₆ (Pyridoxine)	Pyridoxal phosphate (PLP)	Deficiency in humans rare
B ₁₂ (Cobalamin)	Cobalamin coenzymes (e.g., 5-deoxyadenosyl cobalamin)	Pernicious anemia
Biotin	Biocytin	Deficiency in humans rare
Folic Acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide cofactors	Pellagra

Figure 4. Coenzymes expand the catalytic diversity of enzymes from the side chains we discussed in Lecture 2 (see Lexicon).

II. Conceptualization of Catalysis using Transition State Theory

Transition state theory has given us an intellectual framework to think about barriers to chemical reactions and enzymatic reactions and as a consequence, how these reactions might be catalyzed. Linus Pauling (yes the same Pauling of 2^o structure fame) stated in the 1940s, that enzymes are excellent catalysts because they bind the transition states (ts) of the reaction they catalyze better than substrates and hence lower the activation barrier for the reaction. The transition state of a reaction is the point of highest free energy (life-time of 10⁻¹⁵ s or fs) on a reaction coordinate,

that is, the point where bonds are being made and broken (Figure 5A). Many reactions have intermediates between the S (substrate) and P (product) and hence multiple transition states. The intermediate (I) has all of its bonds fully formed, although it still might be chemically very unstable and challenging to characterize (Figure 5B).

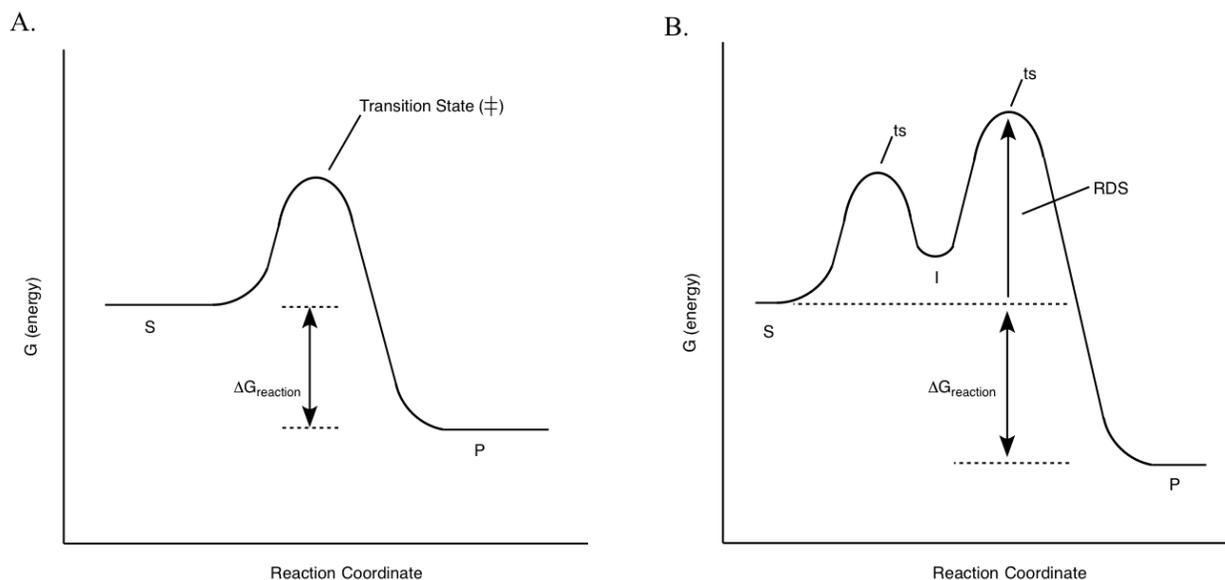


Figure 5. Enzyme catalysis and reaction profiles for two idealized enzyme-catalyzed reactions, one with a single transition state (left, A) and another with two transition states and an intermediate (I) (right, B).

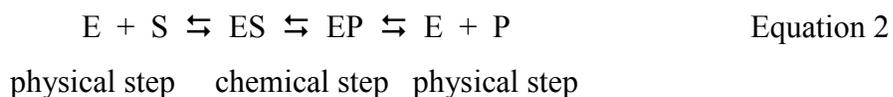
TS theory assumes that the ground state (gs) of the reaction is in equilibrium with the transition state of the reaction and allows one to use thermodynamics to describe rates. One can derive the following expression for the measurement of rate constant (k_{obs}) that you have seen in either Freshman Chemistry or Chemistry 5.60. k_{obs} is an experimentally measurable rate constant for product (P) formation from substrate (S), T is temperature in $^{\circ}\text{K}$, k_{B} is the Boltzmann constant, κ is the transmission coefficient which is the probability that the breakdown of the activated complex will be in the direction of product, not starting material, ΔG^{\ddagger} is the free energy of activation and R is the ideal gas constant.

$$k_{\text{obs}} = (\kappa k_{\text{B}} T / h) e^{-\Delta G^{\ddagger} / RT} \quad \text{Equation 1}$$

Equation 1 tells us that **the rate of the reaction decreases as the energy of activation increases**. Thus to accelerate the rate of the reaction one needs to find some way to reduce this barrier. Most reactions are multistep (Figure 5B). The rate of the overall reaction can only be as

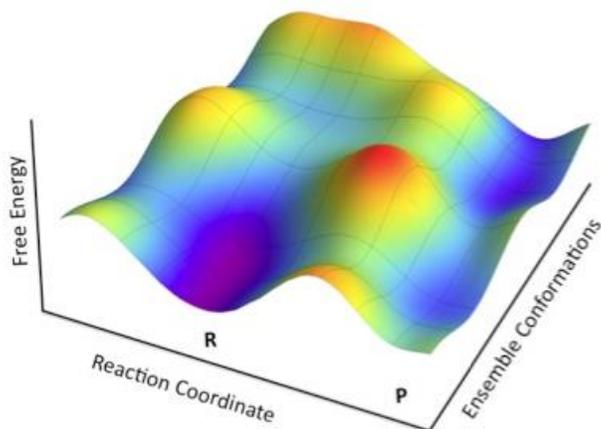
fast as the slowest elementary step. The rate-determining step (RDS) is the bottle-neck of the reaction. Thus lowering the barrier of a non rate-limiting step has little effect on the rate of the overall reaction.

TS theory is used to think about enzymes, the catalysts for all of the steps in metabolic pathways:



Through billions of years of evolution, enzymes have evolved in many cases to a state where they are **rate-limited by a physical step or steps and not a chemical step**. [The physical steps could be substrate binding or product release or conformational changes]. The enzymatic reactions are often diffusion controlled, that is the slow step is the E finding its S in solution. Diffusion control in enzymatic systems range from 10^6 to $10^9 \text{ M}^{-1}\text{s}^{-1}$ and is described by k_{cat}/K_m . See Figure 2A. You will see that k_{cat}/K_m is a bimolecular rate constant. If physical steps are rate limiting, then studying the chemistry is challenging as it is masked by the physical steps. **As with all mechanistic studies, one must perturb the system to see the chemistry.**

A recent view of catalysis, which will replace the current text-book descriptions (Figure 6), is that the reaction coordinate is multidimensional:



Courtesy of Annual Reviews, Inc. Source: Klinman, Judith P., and Amnon Kohen. "Hydrogen tunneling links protein dynamics to enzyme catalysis." *Annual review of biochemistry* 82 (2013): 471.

Figure 6. Free energy landscape for an enzyme-catalyzed reaction with protein motions emphasized. The reaction coordinate axis describes the local environment that facilitates the chemical reaction and the ensemble coordinate axis represents sampling of multiple states that occur (Reactants, R, and Products, P) at all stages along the reaction coordinate. R and P valleys are ground states before and after the reaction and the peaks represent the transition ready states (TS).

Two important issues need to be considered:

1. The importance of the **protein conformational landscape** in ligand binding, allostery, catalysis and rate enhancement. Many mutant structures are identical in 3-dimensional models determined by crystallographic methods, but have very different rate properties. Structure is not subtle enough to define function.
2. Catalysis arises from a subset of total protein conformations that achieve simultaneously the specific interactions that in aggregate allow rapid chemistry.

Another important point to remember is that just because a reaction is thermodynamically favored, the reaction can still have a significant kinetic barrier and not proceed.

An example of a thermodynamically favorable reaction is oxidation of glucose to CO₂, one of the major fuels used by our bodies.



However, you all know that your sugar can sit on the table in your house for years.

III. How has nature evolved enzymes to lower the activation barrier?

The huge rate accelerations observed in the conversion of substrates to products in the presence of enzymes are mainly associated with three general mechanisms. Many enzymes use all three mechanisms.

- A. The most important mechanism of catalysis (the one with the biggest effect) is the use of **binding energy**. Binding energy is defined as the free energy released when a substrate interacts with an enzyme. The binding energy is used in part to bind the substrate, but more importantly to lower the activation barrier by some combination of the following:
 - i.) to preferentially bind the transition state of the reaction over the ground state (unique electrostatic or H bonding interactions found in the former state). We will see and calculate the importance of weak non-covalent interactions in TS stabilization. How much rate acceleration can a single H bond unique to the transition state provide toward the observed overall rate acceleration?
 - ii.) to destabilize the ground state
 - iii.) to overcome unfavorable entropic effects (the substrates must be in the correct orientation relative to one another to react, the substrates must be desolvated)
- B. **General acid (GAC) and general base catalysis (GBC)**. General means use of the amino acid side chains in catalysis and not H^+ or HO^- (which are designated specific acid and base catalysts). At pH 7 the concentration of H^+ and HO^- are very low. The side chains of the amino acids play a key role in general acid and base catalysis: the protonated state in GAC and the deprotonated state in GBC. You need to also remember that the environment of the enzyme can perturb the pKa by up to five or six orders of magnitude.
- C. **Covalent catalysis**. Again, the side chains of the amino acids in the active site (S, E, D, K, Y, H, C) can form covalent bonds with substrate in the active site of the enzyme. You will see many examples of this type of catalysis in metabolism.

Both **GAC/GBC** and **covalent catalysis** are prevalent in chemical reactions whose mechanisms have been studied in the 1960s, 70s, and 80s and their contributions to rate acceleration have been measured. Thus the basis for our understanding of the magnitudes of rate accelerations accessible to enzymatic systems is our understanding of the chemical reactions in solution.

Binding energy however, is unique to proteins. You will see over the course of the rest of the semester during our studies of metabolic pathways, many examples of all three mechanisms.

Magnitudes of Rate accelerations of up to 10^8 , 10^2 - 10^3 and 10^2 - 10^3 can be obtained from binding energy, GAC/GBC and covalent catalysis, respectively. To obtain accelerations of 10^{12} , multiple mechanisms are required.

Contributions of weak non-covalent interactions to catalysis: Now let us try to understand how weak non-covalent forces play a role in rate acceleration. As noted above, these weak non-covalent forces (free energy released when a substrate binds to an enzyme) are not used for “tight” binding of the substrate, but for catalysis. Use of a reaction coordinate diagram helps to make this idea clear. Use of Equation 1 above helps us to think about how **unique**, weak non-covalent interactions can be translated into rate acceleration. Think about how many unique interactions are required for rate accelerations of 10^6 to 10^{15} .

Let us compare the conversion of $S \rightarrow P$ catalyzed by an enzyme and its non-enzyme catalyzed counterpart.

$$k_{\text{enz}} = (\kappa k_B T/h) e^{-\Delta G_{\text{enz}}^\ddagger/RT}$$

and

$$k_{\text{nonenz}} = (\kappa k_B T/h) e^{-\Delta G_{\text{nonenz}}^\ddagger/RT}$$

Let us assume that the rate difference in the reactions under the same conditions is a factor of 10 and the $T = 298^\circ \text{K}$, then

$$k_{\text{enz}} / k_{\text{nonenz}} = 10 = e^{(\Delta G_{\text{nonenz}}^\ddagger - \Delta G_{\text{enz}}^\ddagger)}$$

and

$$\Delta\Delta G^\ddagger = 1.38 \text{ kcal/mol (5.8 kJ/mol)}$$

How much is a H bond worth? The numbers are hard to measure but a typical H bond is worth 2-7 kcal/mol. Thus a unique H bond in the transition state of a reaction, not present in the ground state of the reaction, can give you a rate acceleration of 10^2 to 10^3 .

Summary: mechanisms of catalysis

Binding energy can be used to stabilize the ts, destabilize the gs, desolvate starting materials, orient two substrates relative to one another for chemical reaction. All of these methods require energy that can be supplied in part by the free energy released when the enzyme interacts with the substrate(s).

General acid and general base catalysis using amino acid side chains.

Covalent catalysis using amino acid side chains or cofactors (derivatives of vitamins).

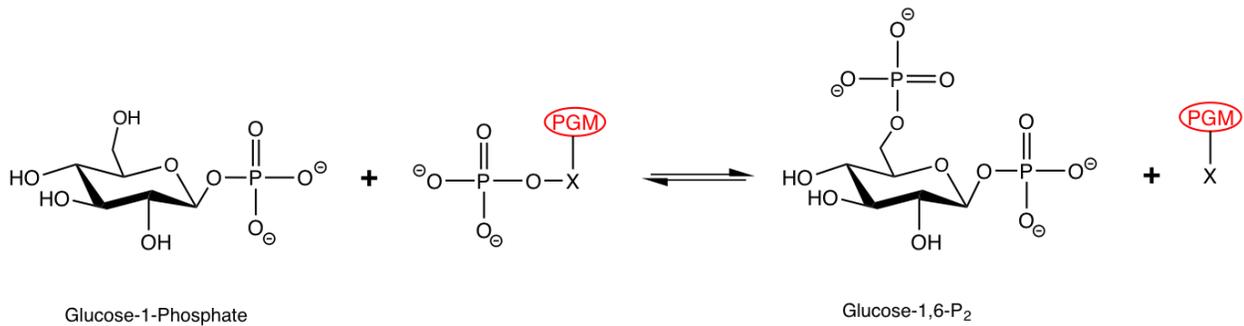
IV. The SECOND important property of enzymes is their SPECIFICITY.

We will define specificity mathematically, but let me give you an amazing example of specificity from a chemist's perspective. While enzymes use binding energy for catalysis, the substrate also **must bind** to the enzyme in a specific fashion. Enzymes in general are highly specific for a single substrate (at least with respect to the metabolites they encounter inside the cell).

However, the K_d (dissociation constant) of enzymes for their substrates is relatively weak and varies from mM to μ M. For catalysis, you must be able to release the product to get multiple turnovers and not bind substrate so tightly that catalysis cannot occur. **A subtle balance is required.**

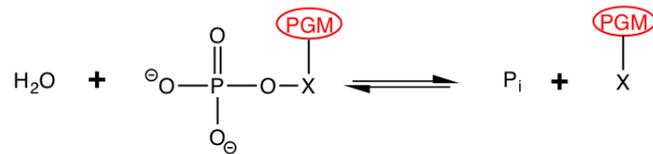
A typical example of the amazing specificity of enzymes is given by phosphoglucomutase (an enzyme in the glycolysis pathway) that catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate. This enzyme involves covalent catalysis with a serine in the active site of the enzyme (PGM-X-OPO₃²⁻ below).

Phosphoglucomutase (PGM)



Equation 2

Phosphoglucomutase can also transfer the phosphate to water in the absence of glucose-1-P.



Equation 3

The relative rates of the reactions shown in Equations 2 and Equation 3 have been measured and found to be $3 \times 10^{10}:1!!$ This is an amazing observation as water and the hydroxyl of glucose have the same chemical reactivity in solution (both have pKas of 16) and water is smaller than G-1-P so can easily fit into the enzyme's active site.

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