

Lecture 6: Allostery

I. Introduction

We have investigated three major topics related to protein dynamics:

1. **Theoretical.** The main themes in this topic are (a) Boltzmann ensembles and the theory that all possible states in the distribution are sampled but at different frequencies; (b) Calculations using the heat capacities of proteins demonstrate that even under native-state conditions a protein samples very high energy, unfolded states.
2. **Computational.** MC and MD simulations show the conformational diversity sampled by a protein; probability density maps can be used to describe “average” structures but much conformational variability underlies a typical average protein structure.
3. **Experimental.** Hydrogen exchange experiments provide strong evidence that proteins partially unfold even under native conditions and that different segments unfold with different frequencies. This experimental data is consistent with the theory and computational predictions.

Now we come to a fourth topic related to protein dynamics, that of protein **function**. In the lecture and lab for this block, we will explore how thinking about protein dynamics can help us understand protein function.

A. Allosterism. Proteins function as small machines and many proteins have both an active site (for substrate binding and/or catalysis) and a regulatory site (for binding modulators or effectors). Many proteins have a regulatory site far removed both in amino acid sequence and in 3D distance from the active site. *How does a distant regulatory site communicate with the active site?*

A similar question applies to the case of proteins with multiple ligand binding sites in which binding at one site affects the binding to other distant sites. As an example, consider the cooperative binding of oxygen by hemoglobin. Hemoglobin binds four molecules of oxygen, one for each heme in the molecule. A plot of oxygen binding *versus* oxygen concentration (Figure 1, below) shows that the hemoglobin curve is sigmoidal (green curve) in contrast to the curve for another oxygen binding protein, myoglobin, that is hyperbolic (black curve). Myoglobin binds only one oxygen molecule per protein and the hyperbolic curve indicates an uncomplicated single site binding isotherm. The sigmoidal behavior for hemoglobin indicates that oxygen binding to hemoglobin is positively **cooperative**, i.e. the binding affinity at any oxygen concentration is dependent on how many oxygens are previously bound – the affinity of later binding oxygen molecules is greater than early binding oxygen molecules. Therefore, there must be communication between the oxygen binding sites. But the model of hemoglobin structure on the right shows that the heme groups (red spheres) are not in contact with each other.

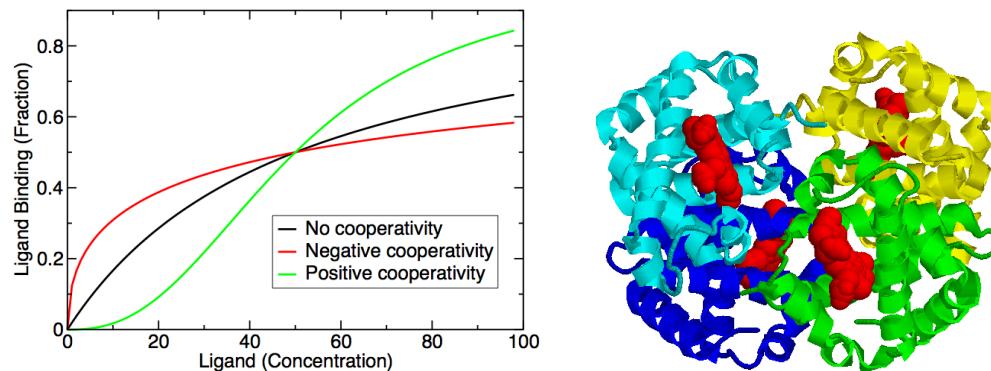


Figure 1. Left, Binding isotherms can demonstrate no cooperativity, positive cooperativity or negative cooperativity. Right, the heme groups in hemoglobin are not in contact with each other yet the binding of oxygen to each heme is communicated to the other heme groups.

Note that it is also possible to have *negative cooperativity* (Figure 1, red curve) where early binding ligands are bound with greater affinity than subsequent binding ligands. For all these cases where distant binding sites affect each other the term **allostery** was introduced by Monod, Changeux and Jacob in 1963 (JMB 6[4] 306). Many proteins are **allosteric** and this concept is a topic in first year biochemistry courses.

The following are historically important characteristics of allosteric proteins:

- Separate binding sites on a protein affect each other.
- Binding sites may be distant from each other on the protein.
- Requires protein flexibility - protein exists in at least two states.
- Effectors may inhibit or activate binding of the primary ligand.
- Display cooperative binding isotherms.

Most examples of allostery in proteins have benefited from the experimental observation of two different conformations, active and inactive (or high affinity and low affinity binding). In these cases, two different models may be used to explain allosteric behavior: (1) **Induced fit**: The first binding event induces a new protein conformation which binds a subsequent ligand with different affinity, or (2) **Conformational selection**: The first binding event shifts a pre-existing ensemble of protein conformations and changes the average binding affinity of the population. Historically, the idea that multi-domain proteins such as hemoglobin could exist in different conformations (or states) and that a ligand stabilized specific states has been the standard, but for single domain proteins the idea of “induced fit” has been preferred as a model. More recently theoretical simulations have shown that both mechanisms are possible in all proteins and that the distinguishing parameters are the rate of conformational transition and rate of diffusional collision by effector.

A confounding example of cooperative binding has long been observed in some enzymes that exhibit apparent allosteric behavior but are single subunit, single binding site proteins. Furthermore, recent experimental examples have been found in which the conformation of the active and inactive forms of a protein are *identical* within the error of

X-ray diffraction, i.e., there is no apparent conformational change. How can allostery be explained in these cases?

In this lecture, we will see how a consideration of the conformational dynamics of proteins can explain cooperative binding behavior for all the above examples.

II. Types of conformational dynamics in allostery

A. With static flexibility. The classic example of cooperative allosteric behavior accompanied by observable conformational change is hemoglobin as mentioned above. There are two main models that have been used for many years in attempts to explain the action of hemoglobin and they are illustrated in the following figure.

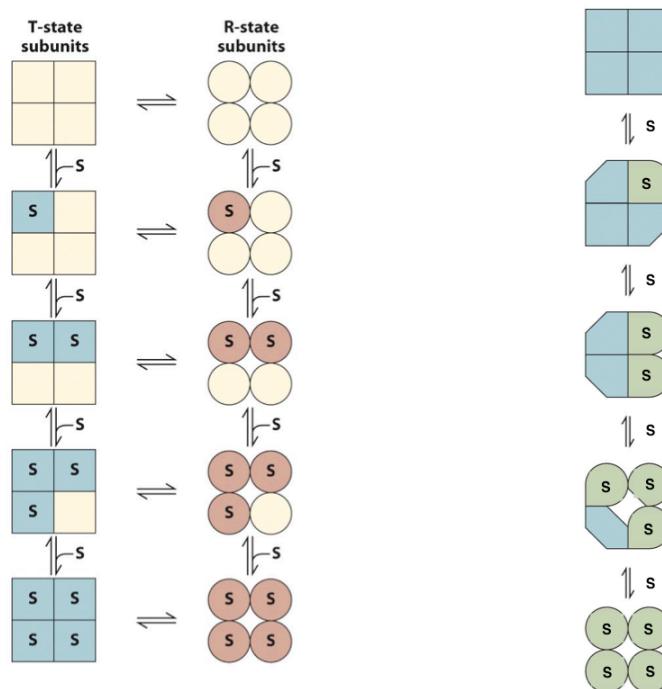


Figure 2. Left, the symmetry model of hemoglobin binding; right, the sequential model of hemoglobin binding.

In the symmetry model, proposed by Monod, Wyman, Changeux (MWC) in 1965 hemoglobin exists in two states; the T-state that has low affinity for oxygen, and the R-state that has high affinity for oxygen. The equilibrium between these two states exists in the absence of ligand (oxygen) but the binding of ligand stabilizes the high affinity state (*circles*). Therefore, the higher the concentration of ligand, the more high affinity state of hemoglobin, i.e. cooperative binding. A hallmark of the symmetry model is that hemoglobin exists in *either* the R- or T-states, there are no hybrid states.

In the sequential model, proposed by Koshland, Nemethy and Filmer (KNF) in 1966 the low affinity T-state is progressively switched to the high affinity R-state by ligand binding. Hybrid states exist because the *coupling energy* between subunits is not sufficient to cause wholesale switching of all subunits when only one subunit changes.

conformation. With very large coupling energies the KNF model becomes identical to the MWC model. Both models explain overall cooperativity but each model predicts differences in the details. A big conceptual difference between the MWC and KNF models is that the MWC illustrates "conformational selection" and the KNF model illustrated "induced fit". Many years of experiments have not settled the question of which is more appropriate, and as we will see, this question is probably now moot.

The difference between conformational selection of a state, and induced fit of a state, can be described as a continuum that depends on the relative rates of conformational change and ligand binding. Consider the figure below.

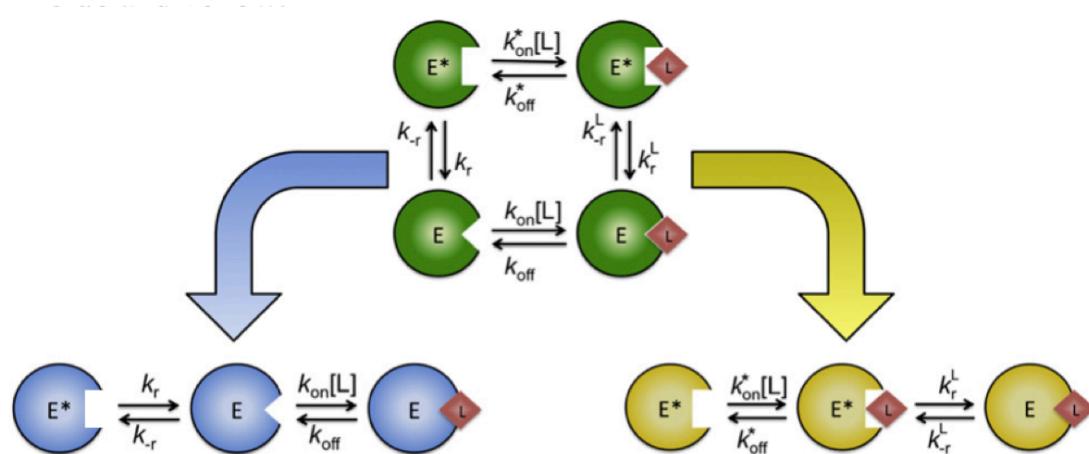


Figure 3. Linkage scheme for protein conformational change and ligand binding. There are two conformational states illustrated by the shape of the binding site on the circular protein labeled E. The ligand is diamond shaped and labeled L.

If the rate of conformational change is greater than ligand binding (left, blue), the binding will result in conformational selection; if the rate of binding is greater than the rate of conformational change (right, gold), the binding can be characterized as induced fit. Indeed, exhaustive sampling of the parameter space for the above rate constants has shown the following continuum (Figure 4).

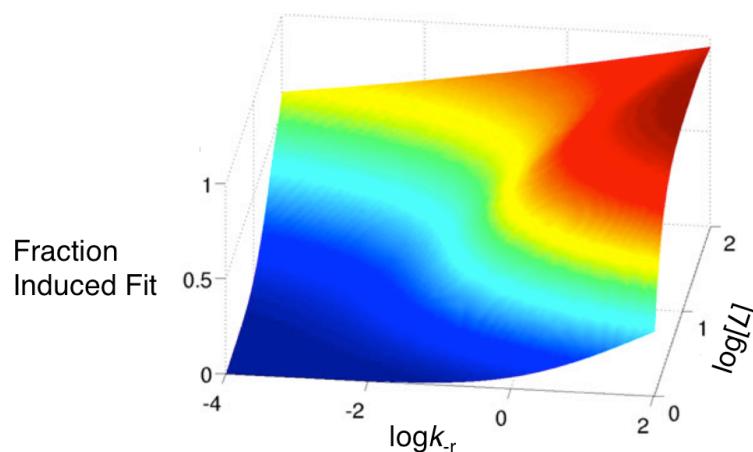


Figure 4. Surface plot of the fraction of induced fit binding compared to conformational selection binding as a function of both the rate of conformational change (k_r) and ligand concentration in arbitrary units. The rate constant, k_r , is defined above in Figure 3.

Note from Figure 4 that *either* an increased rate (k_r) of conformational to the low affinity state *or* increased ligand concentration ($[L]$) can increase the proportion of binding characterized as induced fit (red) instead of conformational selection (blue).

B. Allostery with dynamic flexibility. Catabolite activator protein (CAP) is a transcription factor that controls DNA transcription in bacteria. Cyclic AMP (cAMP) binds to CAP and increases the protein's affinity for DNA. The DNA-bound CAP then recruits RNA polymerase to the site promoting transcription (Figure 5 below).

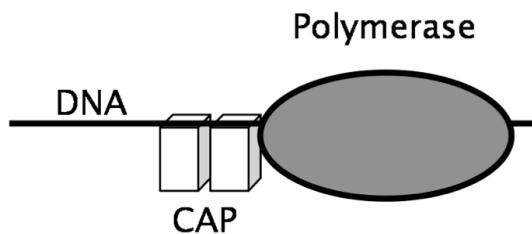


Figure 5. CAP-dependent promoter activation: CAP dimer interacting with DNA and RNA polymerase.

CAP exists as a homodimer of two polypeptide chains and binds two molecules of cAMP. The two cAMP molecules bind with **negative cooperativity**, i.e. the first molecule binds with greater affinity than the second. Yet the distance between the two protein bound cAMP molecules is approximately 20 Å (see Figure 6 below). The following images and experimental results for CAP are from Popovych et al. *Nature Struc. Molec. Biol.* 13:831, 2006.

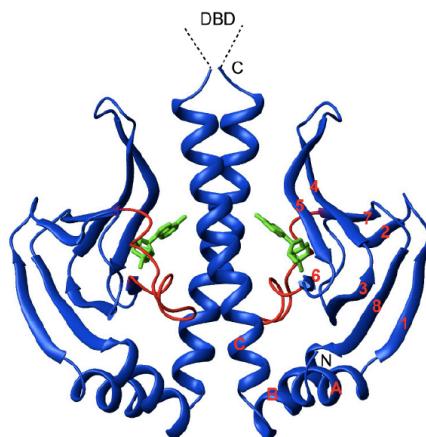


Figure 6. Structure of CAP. The crystal structure of CAP (1G6N) is shown as a ribbon drawing with two bound cAMP molecules in green. The regions of the protein that bind cAMP are shown in red. DBD refers to the DNA binding domain that is not included in the figure.

The issue we want to address is how binding of cAMP to one chain of the dimer is communicated to the other chain so that its affinity for the second cAMP is reduced. Does binding of one cAMP result in conformational changes in both subunits? How do conformational dynamics play a role in this allostery?

1. *Binding of cAMP causes conformational change primarily in one subunit.*
NMR chemical shift experiments measure local environments of a

polypeptide; when the conformation changes the environment of each residue changes. The change in chemical shift ($\Delta\delta$) upon binding a ligand shows which residues change their *conformations*. As seen in Figure 7 the binding of cAMP to the left chain of the dimer results in conformational changes in both the cAMP binding region and throughout the chain. However, the cAMP binding region of the other chain in the dimer (on the right) does not change its conformation. So allostery is not communicated by a shift in the *average* conformational ensemble of the second chain.

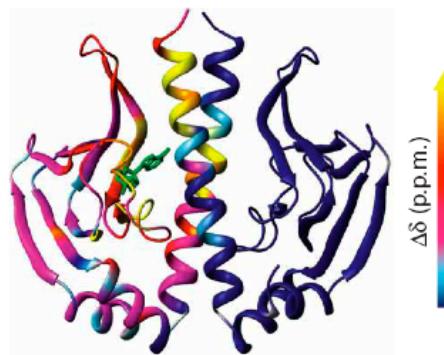


Figure 7. cAMP binding results in conformational change. The NMR chemical shift values for residues in CAP are indicated by a color scale (right). The bound cAMP is green.

2. *Binding of cAMP causes increased conformational fluctuation in both subunits.* In contrast to chemical shift experiments, NMR exchange experiments indicate conformational exchange on the μ s-ms timescale between two different chemical environments; these experiments measure dynamics. The NMR exchange rates (R_{ex}) for CAP in three different states of ligand binding are shown below in Figure 8. Both the unliganded dimer and the liganded dimer with two cAMP molecules bound have low flexibility (right). In contrast (middle) the CAP dimer with one cAMP bound has increased flexibility in *both* chains. The cAMP binding region of the right chain in the middle figure has increased flexibility despite the results above which show that it does not change its *average* conformation! In fact, increases in flexibility are seen as much as 35 Å away from the bound cAMP. **This increased flexibility in the right side subunit is what decreases its binding to cAMP causing negative allostery.**

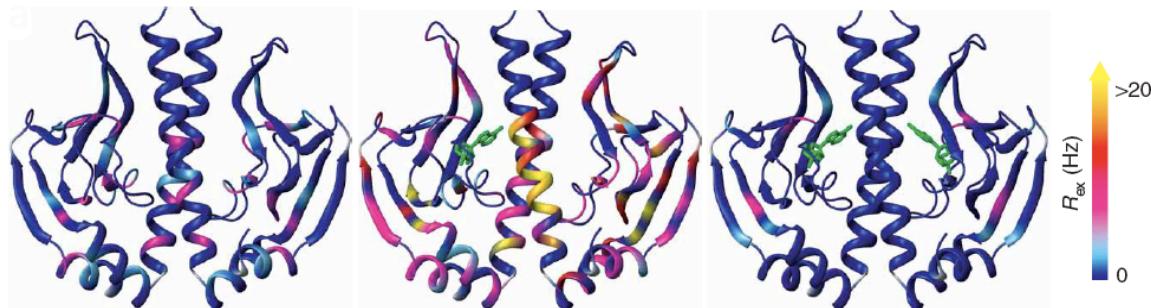


Figure 8. cAMP binding causes changes in flexibility. The NMR R_{ex} values for residues in CAP are indicated by a color scale (right). The bound cAMP is green. Left, no cAMP bound; middle, one cAMP bound; right, two cAMP bound.

3. *Summary.* Binding of cAMP to one subunit of CAP results in a change in the *average conformation* of that subunit but not the other subunit in the dimer. However, changes in the flexibility of both subunits are seen upon one cAMP binding. This result indicates that the allosteric communication between subunits is due entirely to changes in dynamics. *Flexibility* can change, but the *average structure* does not necessarily need to change!

These changes in dynamics are due to a shift in the population not in terms of average conformation but rather in terms of either frequencies or amplitudes of conformational fluctuations.

III. Studying allostery with simulations

How could one investigate long range communication through proteins using molecular simulations? Let us assume that allostery means that a change in conformation or dynamics in one segment occurs at the same time as a change in conformation or dynamics in a distant segment. This means we need a method to measure the *correlation* in changes at different positions along the polypeptide chain during a simulation.

One change we can measure is the partial unfolding of residues and segments along the chain. *Segments that unfold with the same change in free energy are likely to occur with the same frequency and therefore would more likely occur simultaneously.* (Notice the thought process here – we are considering correlated frequencies of separate events, not cause and effect of separate events.)

To explore the above idea we first require an ensemble with many high energy states that have partial unfolding in segments. Think back to the hydrogen exchange experiments where denaturant was added to obtain a population with significant unfolding so the deuterium signal would be increased enough to be measured. One way to obtain a partially unfolded simulation ensemble would be to carry out the simulation at high temperature but there are other methods that we haven't covered in class. For this discussion assume that one has such a simulation ensemble with significant partial unfolding. The exact method used to obtain this ensemble is not important here.

The example we will use comes from a research paper, Pan et al. *Binding sites in *Escherichia coli* dihydrofolate reductase communicate by modulating the conformation ensemble*. PNAS 97;12020, 2000 and the molecular graphics images below are of the crystal structure of the enzyme dihydrofolate reductase (DHFR). This enzyme is necessary for nucleotide biosynthesis and it has two substrates, NADPH and dihydrofolate (DHF). Dihydrofolate reductase is an allosteric enzyme in the sense that binding of NADPH promotes binding of DHF and *vice versa*. The protein residues that bind NADPH are different from the residues that bind DHF so this is an example of true allosteric communication.

We want to calculate the *probabilities* of unfolding for different segments of a protein. To achieve this goal, we need the *free energies* of unfolding so we begin by introducing a method to calculate free energies of conformations in an ensemble.

1. *Free energy calculations.* The probability of each partially unfolded state of a protein is proportional to its free energy change from the native state.

$$P = \frac{g_i e^{-\Delta G_i / RT}}{Z}$$

The Gibbs *free energy* of unfolding for each residue is calculated in the paper by Pan et al. by a very approximate empirical scoring function, or force field. One of the important terms in calculation of changes in free energy when a protein unfolds is the change in interaction of protein with water, termed **solvation free energy**.

It turns out that one can estimate the change in solvation free energy upon unfolding by calculating the amount of new atomic surface area exposed to water by the unfolded segment of the protein. The force field in this example uses solvent **accessible surface area** as an energy parameter to calculate the free energy of unfolding. Calculation of accessible surface area (ASA) is a common procedure in structural biology. A Google search will give you an idea of how important it is. The basic idea is that a “water molecule” (sphere of radius $\sim 1.4 \text{ \AA}$) is rolled around the surface of the molecular structure. The accessible surface area is that surface swept out by the *center* of the rolling sphere while in contact with the van der Waals surface of the atoms. Figure 9 below illustrates the concept of accessible surface area in two dimensions. Red and blue circles represent oxygen and nitrogen atoms, respectively; grey circles represent carbon atoms.

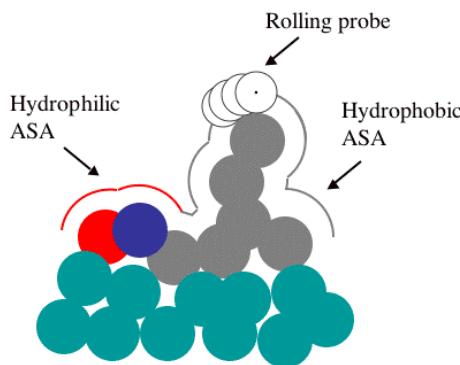


Figure 9. Calculation of accessible surface area.

Atoms which are buried have low or zero ASA while atoms exposed on the surface have greater ASA. **The amount of accessible surface area for each atom is a measure of the interaction of that atom with water.** In the calculation of free energy described above, ASA from polar atoms is energetically more favorable than ASA from hydrophobic atoms. This difference reflects the fact that the energy of interaction between water and polar atoms is energetically favorable (hydrogen bonds and complementary partial charges) while the energy of interaction between water and neutral

carbon atoms gives rise to the hydrophobic effect. In summary:

Polar ASA \propto Favorable solvation free energy

Hydrophobic ASA \propto Unfavorable solvation free energy

ASA measures *free* energies because it is taking into account both potential energy of water/protein interaction and the entropy change of water when it solvates a protein group. We can add a term for the change in **configurational entropy** of the protein when it unfolds (e.g. how many more bond rotations are allowed upon unfolding). Both of these changes can be combined together to make a force field as illustrated in Figure 10 below.

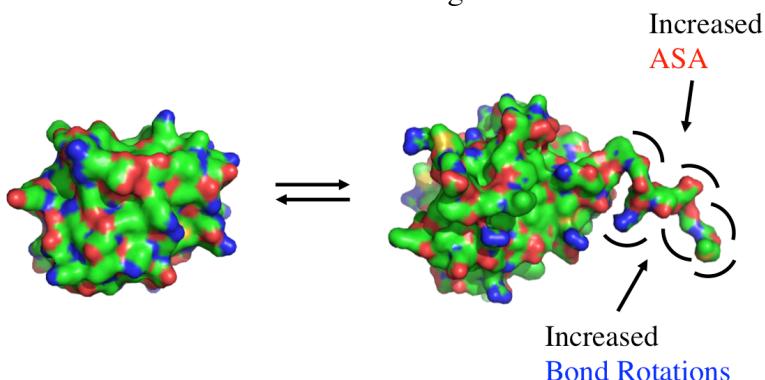


Figure 10. Partial unfolding of a protein segment increases both accessible surface area and degrees of freedom for bond rotations.

The Δ ASA and Δ configurational entropy together constitute a force field for estimating free energy of unfolding.

By summing the Polar and Hydrophobic Δ ASA values and Δ configurational entropy for each partially unfolded conformation of a protein one can estimate the stabilities of the different conformations. If the unfolding of a specific residue or segment causes a large unfavorable change in free energy that residue or segment is considered unlikely to unfold and *vice versa*. Compared to a “complete” force field this is very simplified; but remember how we were able to answer specific questions about helix stability using a very simplified force field composed only of a hydrogen bond scoring function.

2. *Residue stabilities.* So now from the simulation ensemble of dihydrofolate reductase with partially unfolded segments, and a force field to calculate the free energy of each conformation in the ensemble, we can calculate a list of the probabilities of finding a conformation with specific residues unfolded. From this list we can determine the relative stability of each residue. The image in figure 11 below is a static molecular graphics model colored to indicate the residue stabilities. Residues in red are more stable, residues in yellow are the least stable. Notice the helix **end fraying** in helices 1, 2 and 4. Notice also that there appears to be end fraying for the strands making up the beta-sheet on the bottom part of the structure.

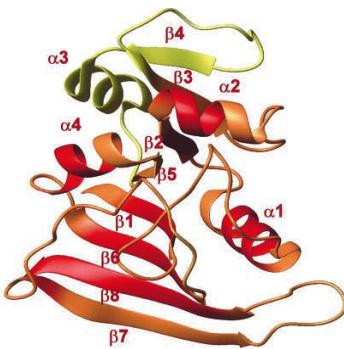


Figure 11. Residue stabilities of dihydrofolate reductase indicated by color. Red = more stable, yellow = less stable. Helices are indicated by α , strands are indicated by β .

3. *Cooperative residue unfolding.* One can use the residue stability data to determine which residues or segments have apparent cooperative conformational changes. If two residues, j and k , are *both* folded, or *both* unfolded, in highly probable protein energy states, the respective residue stabilities will be the same for both residues. Thus any perturbation (such as ligand binding) which changes the probability of protein energy states and affects residue j will also affect residue k , i.e. they will be correlated. This is illustrated in Figure 12 below. On the left the correlation between each pair of residues is plotted in color, red indicates positive cooperativity, blue is zero cooperativity and purple is negative cooperativity (see color bar). Positive cooperativity is defined as two residues having a high probability to be unfolded in the same conformation. Negative cooperativity is defined as one residue likely to be unfolded and another residue folded in the same conformation.

Residues *within* each of the regions labeled A, B and C behave cooperatively and this is not too surprising because they are relatively close in the sequence (they are on the diagonal). Residues within regions C and A also behave cooperatively together as indicated by the region labeled D (off the diagonal) even though these residue pairs are distant in amino acid sequence. The figure on the right shows the structural model of DHFR with the regions color coded: A = red, B = blue, C = green.

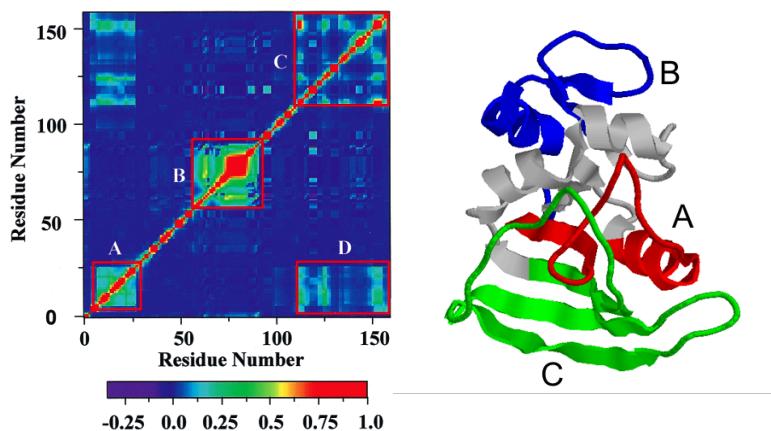


Figure 12. Far left, correlated residue stabilities. Red corresponds to a large positive cooperativity, blue to smaller cooperativity, and purple to negative cooperativity.

Right, protein ribbon segments corresponding to labeled boxes on left; red = A, blue = B, green = C.

4. *Ligand binding induced conformational cooperativity.* The residues that change conformation upon binding of folate are correlated with conformational changes of residues that are distant from the folate binding site. The structural model on the left in figure 13 below shows the cooperative changes in residue stabilities that occur with changes at the folate binding site. Red, yellow and green are positive cooperativity, blue is zero cooperativity and purple is negative cooperativity (within the box). Two protein segments that bind NADPH are positively correlated with folate binding. These two regions are better illustrated in the figure on the right. Here the protein is depicted as a ribbon, the NADPH as magenta sticks and the folate as red spheres. NADPH binding regions (green) that show positive cooperativity with folate binding are distant from the folate (red spheres).

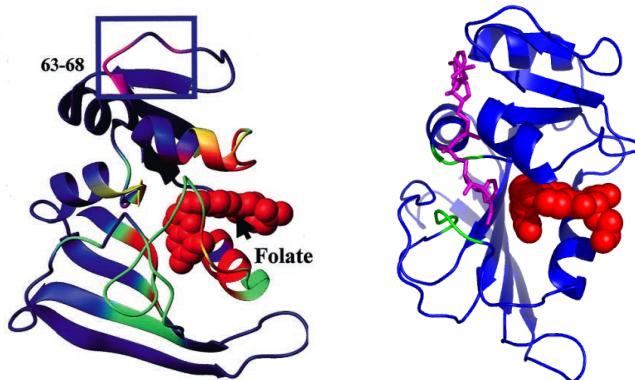


Figure 13. Residue stabilities correlated with folate binding site. Two orientations of DHFR are shown as ribbon structures. Red spheres represent folate. Left, red protein ribbon corresponds to a large positive cooperativity, green to smaller cooperativity, and purple to negative cooperativity. Right, green protein loops correspond to segments of the NADPH binding site. The NADPH is magenta. The green loops on the right (NADPH site) is also green on the left even though they are not contacted by folate.

5. *Summary.* This cooperativity allows us to understand how apparent communication between distant sites can occur without invoking a structural pathway between the sites. The relative stability of distant regions is correlated because these regions are *simultaneously* partially unfolded (or fully folded) in the same conformations found in the ensemble. Again, notice the thought process. We are not looking at cause and effect mechanical linkage. We are looking at correlations of events. If the Boltzmann probabilities are similar the respective events *appear* to be linked.

IV. A unifying statistical mechanical model of allostery.

When it became apparent that the range of conformational states of allosteric proteins included such disordered states as found in intrinsically disordered proteins a new model of allostery based on a statistical mechanical description was put forward by Hilser and Thompson in 2007. This model extends the above ideas on correlated stabilities of protein segments. Figure 14 below shows some examples of the range of allosteric conformationally dynamic states found in proteins.

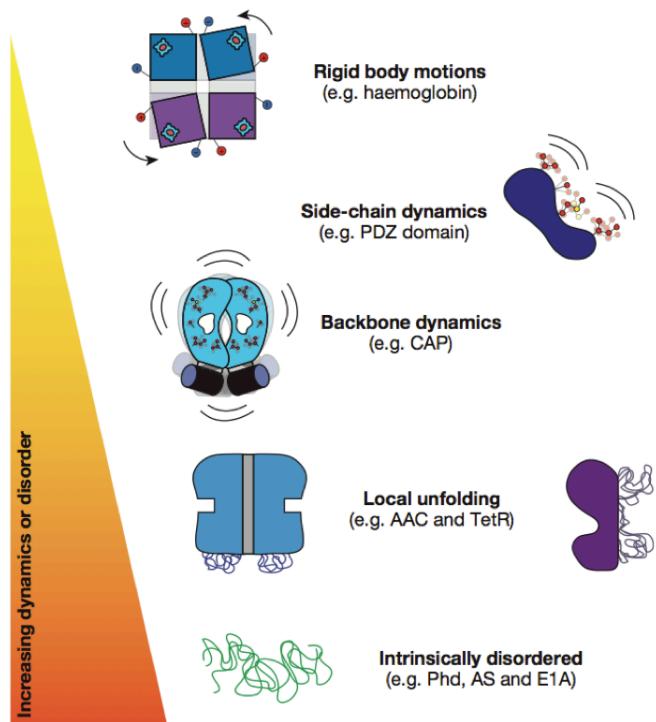


Figure 14. The dynamic continuum of allosteric phenomena. Increasing dynamics, disorder or fluctuations are represented on the vertical axis in the downward direction.

A model that encompasses this range of states without the need to explicitly describe the nature of the states or the pathways of intra-protein structural communication is called the Ensemble Allosteric Model (EAM). Figure 15 below illustrates the EAM for a two-state example.

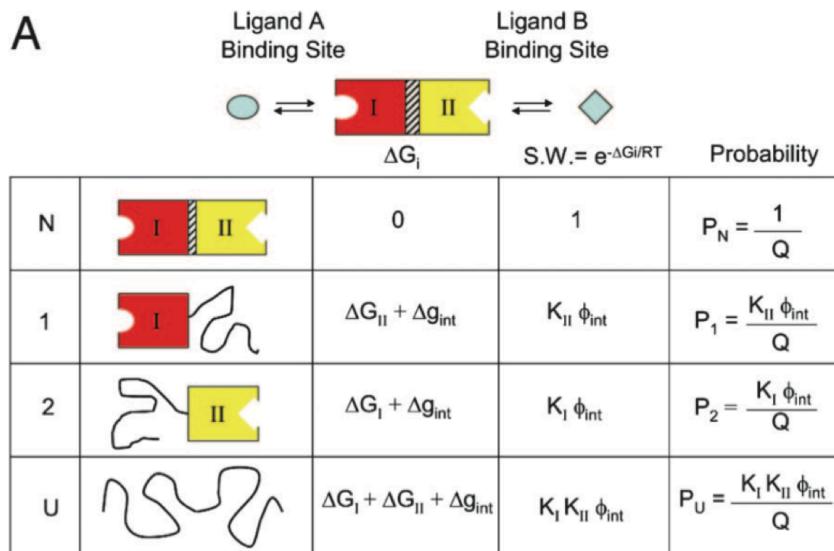


Figure 15. The two-state EAM for allosteric coupling. A hypothetical two-domain protein can bind two different ligands (A and B), one in each domain. Each domain can be folded or unfolded, resulting in four possible states (i.e., N, 1, 2, and U). The hatched area indicates an interaction interface with associated energy of interaction (coupling energy), g_{int} .

This model can be rigorously described using a statistical mechanical formalism as indicated to the right of the protein state cartoons. The partition function Q equals $1 + K_{II}\phi_{int} + K_I\phi_{int} + K_IK_{II}\phi_{int}$, where $K_{II} = \exp(-\Delta G_{II}/RT)$, $K_I = \exp(-\Delta G_I/RT)$, and $\phi_{int} = \exp(-\Delta g_{int}/RT)$. Figure 16 shows an example of how this model explains allostery.

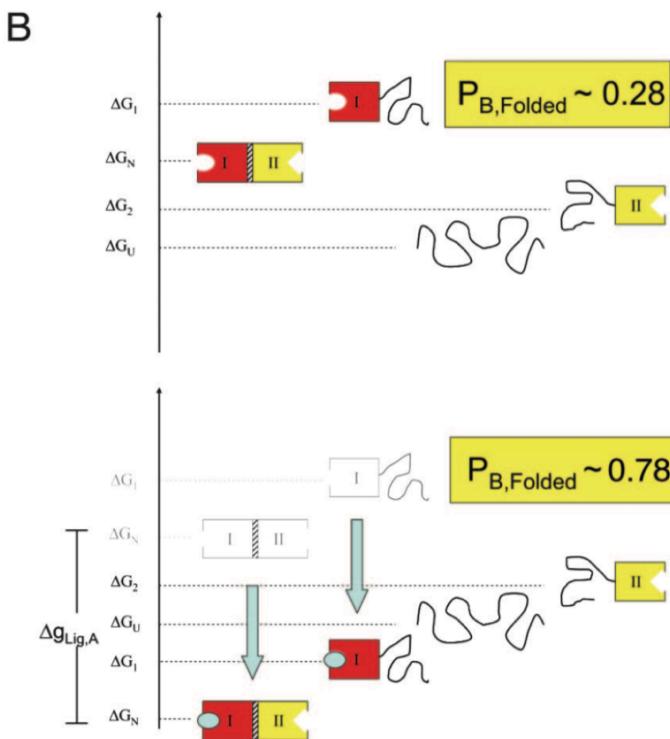


Figure 16. The stabilization of subunit I by ligand A in the above model can increase the fraction of binding-competent subunit II.

In the top panel the state of the ensemble before addition of ligand A is shown. Subunit I is the least stable with a larger ΔG of folding.

At the bottom the binding of ligand A to subunit I redistributes the ensemble probabilities showing a positive allosteric effect. The binding competent state of subunit II increases from a probability of 0.28 to 0.78.

In this example the following reasonable parameters were used, $\Delta G_I = -2.3$ kcal/mol, $\Delta G_{II} = -0.7$ kcal/mol, $\Delta g_{int} = 1.6$ cal/mol, and $\Delta g_{Lig,A} = -3.0$ kcal/mol = the free energy of stabilization of subunit I by ligand A binding.

From the classic allosteric models described above one would expect that the states of different subunits are coupled through a network of interactions and that site-to-site coupling would be maximized when a well-defined pathway through a structure connected the two sites. Instead, a surprising result of an exhaustive search of combinations of ΔG_I , ΔG_{II} , and ϕ_{int} using the EAM was that an inverse relationship between allosteric coupling and the stability within the molecule was observed. This means that exact protein structure is not important - only the relative state energies and interaction energies. This conclusion also is in contrast to a view we will discuss later that emphasizes the importance of specific pathways of communication through the interior of a protein.

Summary. Figure 17 below compares the MWC, KNF and EAM models of allostery for a two state system. The dashed boxes indicate the allowed microstates in each model. An advantage of the EAM is that all possible energy state combinations are allowed and the concept of an interaction energy is included. The nature of this interaction energy is still to be worked out.

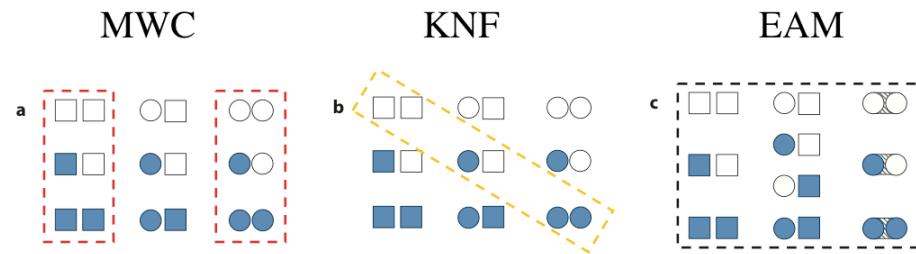


Figure 17. Schematic models of protein allostery. Each subunit of the protein can adopt two conformations, tensed (squares) or relaxed (circles) and two states of ligand binding, unbound (open shapes) or bound (filled shapes). Dashed boxes indicate microstates allowed in each model.

V. Conformational traps and combination therapy.

You should now be convinced that allostery can be explained by both dynamic and static flexibility. So what? What can we use this information for?

Consider the search for pharmaceuticals. Most drugs bind to the active site of an enzyme or receptor and inhibit the binding of the natural ligand to the target protein. There are a limited number of amino acid residues in the active site and viruses and bacteria can mutate these few residues to frequently come up with a variant protein that does not bind the drug but will still bind the natural ligand. In other words, they develop drug resistance.

One way to overcome drug resistance is to administer a cocktail of different drugs against the same protein. The bug may not be able to mutate to a variant protein that binds the natural ligand and *none* of the drugs in the combination. But finding multiple drugs to bind to the same active site is difficult.

The idea of conformational selection by allosteric inhibitors can be used to develop a different class of drugs that would allow a more powerful combination therapy. Below in figure 18 is a scheme illustrating conformational selection. This figure is from Lee and Craik, Science 324: 313 (2009).

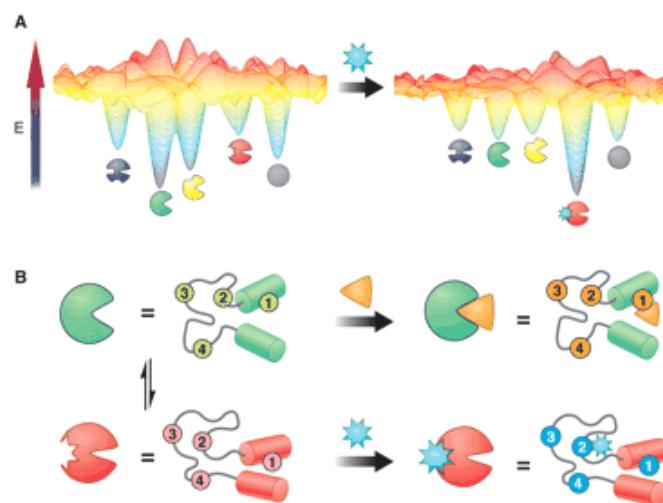


Figure 18. Protein conformational trapping upon ligand binding.

Part (A) of fig. 18 shows shallow wells and transitional barriers of a dynamic free-energy landscape that allow a protein to sample multiple preexisting conformational states, corresponding to the active (green) and inactive (blue, red, yellow, and gray) forms. In panel (A) the global energy minimum shifts to favor the inactive conformation (red) when an inhibitor (cyan star) binds to an allosteric site.

In panel (B) of fig. 18 the active (green) and inactive (red) conformations schematically represent a small enzyme, consisting of a flexible loop connecting two helices. Both conformations are in equilibrium while the enzyme is in the unbound (apo) state. A native substrate (orange triangle) binds to the active site located between the two helices. Alternatively, a small molecule (cyan star) binds to a region with the flexible loop of the inactive conformer, stabilizing that conformer and allosterically inhibiting the enzyme.

The idea for this different drug discovery paradigm is that other inactive forms of the target protein (blue, red, yellow, and gray above) have different allosteric binding sites and ligands that bind to these sites would effectively trap more of the inactive form of the protein. One can search for ligands that bind to other inactive conformational states and these different allosteric ligands may be used in combination. The virus or bacteria would have a harder time finding multiple site mutations that were resistant to all allosteric inhibitors. Expanding the search for inhibitors that bind beyond the active site offers more opportunities to find effective drugs.

The paper by Lee and Craik shows how NMR can be used in a relatively high throughput screening method to find such allosteric inhibitors that bind to different segments of a protein. The drugs Gleevec, Sprycel and Tasigna all bind allosterically to a tyrosine kinase associated with chronic myelogenous leukemia and are examples of drugs in this class.

VI. Allokairy.

It has been known for a long time that single subunit/single substrate enzymes sometimes display typical allosteric (sigmoidal) kinetics. The origin of this behavior could not be due to the coupling of different binding sites, rather these enzymes appear to have a slow conformational relaxation after product release. In other words, they exhibit a **hysteretic effect**. This behavior has recently been called **allokairy** (*allos* = other, *kairos* = time/event). On the left in Figure 19 below a mechanism for kinetically determined cooperative behavior is illustrated. The enzyme is in the high affinity state (H, green) immediately after product (red) release and relaxes back to a low affinity state (L, light blue) with time. High substrate (dark blue) concentrations can keep more of the enzyme in high affinity states thereby enhancing enzyme activity compared to low substrate concentrations.

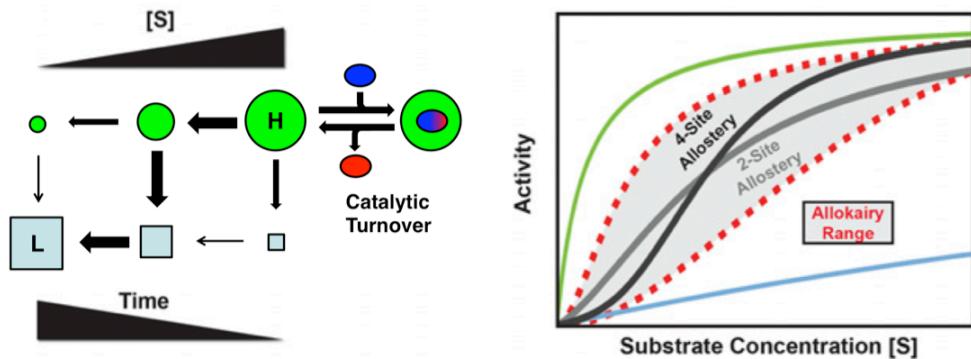


Figure 19. Conformational hysteresis can cause apparent allosteric cooperative enzyme kinetics. Left, the relative fractions of low affinity (squares) and high affinity (circles) states of the enzyme are indicated. These relative fractions are determined by substrate concentration [S] and time after product release. Substrate (dark blue) and product (red) are indicated by ovals. Right, sigmoidal enzyme kinetics are exhibited by allokairic enzymes.

The kinetic plot on the right in Figure 19 demonstrates that allokairic enzymes are more tunable in their cooperativity compared to allosteric enzymes with specified degrees of oligomerization.

VII. Entropy as a protein stabilizing factor.

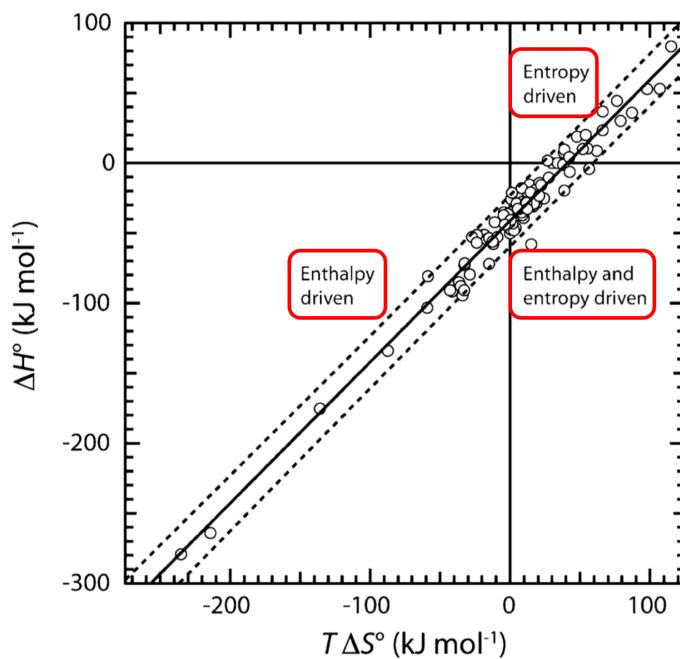
Previously in the course we have focussed on conformational fluctuations of proteins as deviations from the average native state. We first calculated the root mean square internal energy fluctuation for an individual protein molecule and concluded that it was large enough that some molecules in an ensemble were completely unfolded. In our discussion of hydrogen exchange experiments on proteins we saw how different segments of a protein experience different degrees of unfolding even under native conditions (zero denaturant). In both cases the inference was that partial unfolding or increased fluctuation was the *less* favored state.

One paradigm that was commonly accepted for a time is that the binding of a ligand to a protein results in a *decrease* in the fluctuations of the protein. This decrease in fluctuation was thought to stabilize the protein and provide balancing favorable enthalpy to the binding event. Now, with the experimental results on CAP we have the suggestion that increased fluctuation may be a stabilizing factor instead of a destabilizing factor! The increased flexibility seen after binding of one cAMP must contribute to the binding energy of that cAMP or binding would not be favored. Similarly, in DHFR as shown above in figure 13 one segment (residues 63-68, blue box, left figure) displays negative cooperativity with the folate binding site. In other words, this segment shows *increased* fluctuations when the folate site has decreased fluctuations during folate binding. How can this be? Why would Nature include apparent partial unfolding (increased fluctuation) to the binding energy of a ligand?

In a manner analogous to our separation of fluctuations into different *time* resolved categories, dynamic and static, we can also separate fluctuations into different *amplitude* categories. Increasing large amplitude fluctuations (as seen with hydrogen exchange) will

increase the entropy of the protein system but will also probably unfold the protein. This kind of entropy increase therefore will not stabilize the protein. But an increase of small amplitude fluctuations such as increased side chain rotation or increase flexibility in the backbone without changing the average distribution of conformations may increase entropy without conformationally “unfolding” the majority of the protein. In this way increased entropy may actually contribute to the increased stability of a protein-ligand complex through the $-T\Delta S$ term of the Gibbs free energy expression.

The favorable free energy change upon a protein binding a ligand may be due to enthalpy, entropy, or both. Below is a plot of enthalpy change versus entropy change for 100 different protein-protein or protein-peptide interactions. The solid line represents the best fit of a straight line to the data; the dashed lines indicate the 95% prediction intervals. Note the red boxed categories. This dual phenomenon is called **entropy-enthalpy compensation**.



Keywords. You should be able to define or explain each of these terms with a short phrase, mathematical expression or sentence.

Accessible surface area (ASA)
Allokairy
Allosterism
Configurational entropy
Conformational selection
Conformational selection by allosteric inhibitors
Ensemble Allosteric Model (EAM)
Entropy-enthalpy compensation
Hysteretic effect
Induced fit
KNF model of hemoglobin allostery
MWC model of hemoglobin allostery
Negative cooperativity
Positive cooperativity
Solvation free energy