Tracking Protein Allostery in Evolution

- Glycogen phosphorylase frees sugars to provide energy
- GP orthologs diverged 600,000,000 years can respond to transcription controls, metabolite concentrations and post translational modifications

Metabolites are Glucose, AMP, ATP and PTMS are Ser or Thr Phosphorylation
Antique Phosphorylase

- non allosteric; transcriptional control
- binding site for maltodextrin magenta
- PLP cofactor- vitamin B6, (yellow) - side chain of Lys-680
- The active site between the N-terminal and C-terminal domains
- maltose -purple
Yeast GP has controls

N-terminal residues 1-22, corresponding to the N-terminus of muscle phosphorylase, ribbon in white. The unique N-terminal extension of yeast phosphorylase (residues -1 through -39) is drawn as a ribbon in pink.
Yeast GP has controls

Unphosphorylated enzyme: N-terminal extension blocks the active site.
Phosphorylation moves the N-terminal extension to subunit interface to displace the inhibitor, glucose 6-phosphate (orange).
Regulated GPs have two states, inactive and active

How is the switch between conformations made?

Triggers can vary, the response must settle the chemistry of catalysis

Yeast and human GPs use the same mechanism to stabilize the active conformation - a cluster of hydrophobic side chains form, but from different starting configurations
Orange hydrophobic residues cluster in active Yeast GP

Clustered

Distributed
Given the built-in conformational change

It is easy to engineer a new allosteric trigger
Engineering an allosteric switch

**Fig. 1** Activation sites in native and engineered phosphorylases. Stereo view of the activation domain (dimer interface) of three phosphorylase structures represented by α-carbon ribbon diagrams. The phosphorylated enzyme \(^2\) residues 1–80 is shown in blue, side chain atoms of phosphoserine (the phosphate atom is pink) and the two liganding arginines (43 and 69) are also shown. The AMP bound structure \(^1\) residues 10–80 is green; side chain atoms for residues 42, 72 and 75 are forming bonding interactions with AMP. The nickel bound structure of V45H+Y75H residues 13–80 is red; Ni\(^{2+}\) is shown as a white sphere with the liganding histidine side chains (45 and 75).
Ser Pi can activate, AMP can activate, in mutant only Ni ion activates
A baroque assembly of chaperone and client
**SptP** binds unfolded to **3 SicP** chaperones.
How is a universal protease inhibitor made?
Multiple interfaces of ecotin
Ecotin-domain swapped tetramer
A chain is as strong as its weakest link
A net is stronger than its strongest link

• Chain
• Tetramer net
A single side chain can configure a functional protein interface
Ecotin bound thrombin molecule
Analytical Ultracentrifuge and Gel chromatography are simplest ways to measure $M_r$ in solution
AUC and GEL Chromatography
Caffeine
Mw 194
Binds to 100,000 protein
SPR measures binding of small molecules to protein
Sensitivity 100 D relative to 100,000 D
Information in a Sensorogram
Measuring added mass by light
The Octet

- BioLayer Interferometry (BLI) monitors the binding of proteins and other biomolecules to their partners directly in real time.
- Binding interaction continuously monitored by measuring the change in thickness of the protein layer on the biosensor tip.
The Octet has Crude Sample Compatibility

- Only molecules that bind to or dissociate from the biosensor surface produce a signal change.
- Proteins can be assayed in crude mixtures (cell lysates) or in DMSO (up to 10%), and glycerol, reducing sample preparation.
Octet Biosensor measures Ligand induced protein to protein disociation

Concept: inhibitor compound changes protein structure to release protein from binding partner
Free energy calculations for the three complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Insulin dimer</th>
<th>Trypsin-PTI</th>
<th>Haemoglobin αβ dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissociation constant ( K_D ) (molar)†</td>
<td>(10^{-5})</td>
<td>(10^{-13})</td>
<td>(&lt;10^{-8})</td>
</tr>
<tr>
<td>Free energy of dissociation ( \Delta G_D ) (kcalorie mol(^{-1}))†</td>
<td>7</td>
<td>18</td>
<td>&gt; 11</td>
</tr>
<tr>
<td>Translational/rotational free energy( \Delta G'_D ) (kcalorie mol(^{-1}))</td>
<td>23</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Free energy required for association ( \Delta G''_D ) (kcalorie mol(^{-1}))§</td>
<td>30</td>
<td>45</td>
<td>&gt; 38</td>
</tr>
<tr>
<td>Surface area buried in the complex (Å(^2))</td>
<td>1,130</td>
<td>1,390</td>
<td>1,720</td>
</tr>
<tr>
<td>Hydrophobic free energy (kcalorie mol(^{-1}))¶</td>
<td>28</td>
<td>35</td>
<td>43</td>
</tr>
</tbody>
</table>

*Data at neutral pH near 2° C from refs 13-14.
†\( \Delta G_D = -kT \ln K_D \).
‡Calculated from the translational/rotational partition functions.
§\( \Delta G''_D = \Delta G'_D + \Delta G_D \).
¶25 calorie mol\(^{-1}\) per Å\(^2\) of buried surface area.
Fig. 1. This association pathway for a homodimer is a simplified version of the general pathway. Only one intermediate (a) occurs, rather than the dimeric and monomeric intermediates a’, b’, and (ab’). The individually solvated molecules, a’, must first change conformation, a, and then overcome a diffusional barrier.
Two States in Association

Fig. 2. Solvated proteins associate through hydrophobic interactions in step 1. Each molecule loses overall rotational and translational degrees of freedom relative to the other due to binding. Specific contacts such as electrostatic pairings and hydrogen bonds occur between subunits in step 2. Adapted from Ross and Subramanian (1981).
Fits of Buried Surface to Experimental Free Energy

hydrophobic atoms alone correlate poorly with the free energy of association

Counting the polar and nonpolar buried surface, and the weighting factors, the correlation is 96%.
Conclusions

• buried polar atoms contribute favorably if paired, unfavorably in unpaired and hydrophobic atoms contribute favorably.

• entropy lost on complex formation is not well explained- it was made an adjustable parameter in these studies: one of a total of 3 parameters for 15 observable.

• H bond average is -0.24 kcal/mole. Range is 0 to -.71: 44 of the bonds are charged and contribute -0.8 to -1.5.
Structural and thermodynamic characterization of free and complexed ACTR and CBP
Solution structure of the ACTR–CBP complex—ACTR is pink and CBP blue
Sequences Compared
Interface interactions