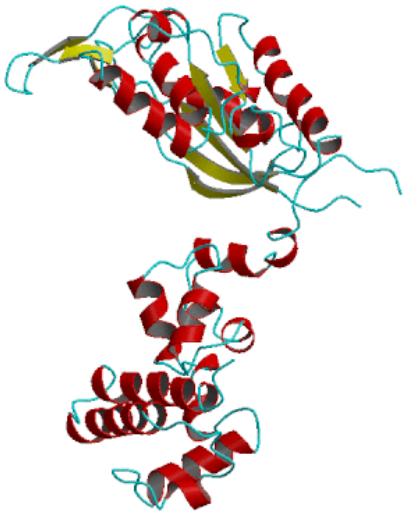
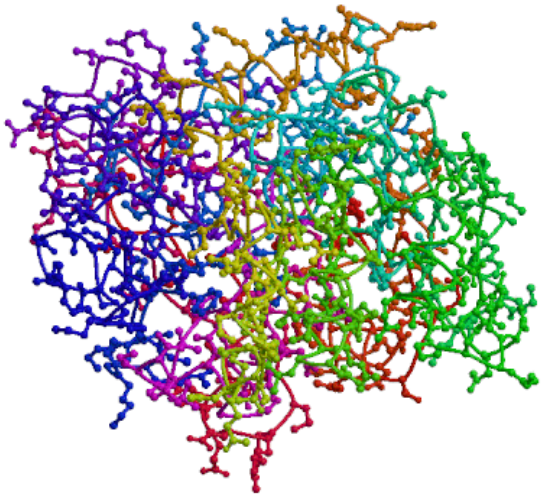
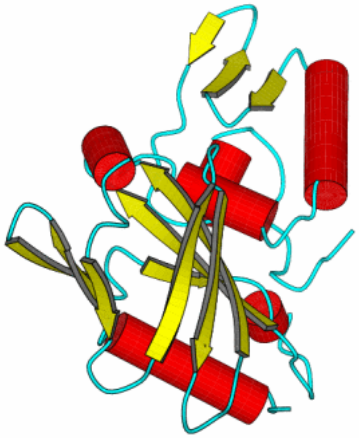
Enzyme Dynamics and Function







http://wishart.biology.ualberta.ca/moviemaker/gallery/index.html

Last Week...

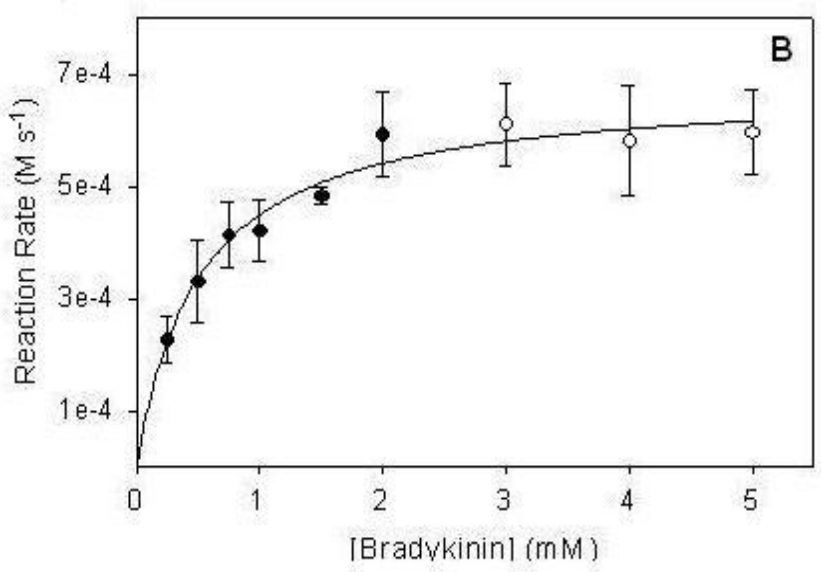
- Last week was all about enzyme kinetics and regulation.

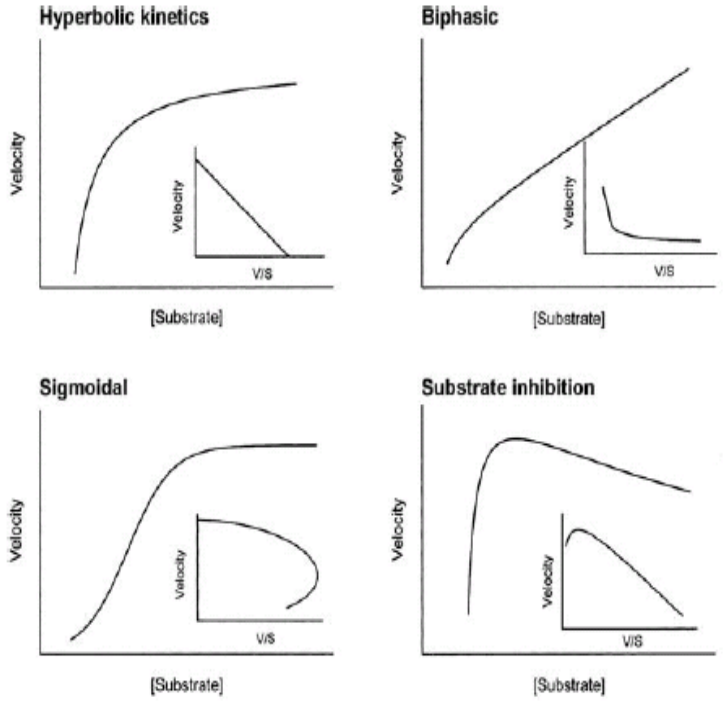

Leonor Michaelis
1875–1949


Maud Menten
1879–1960

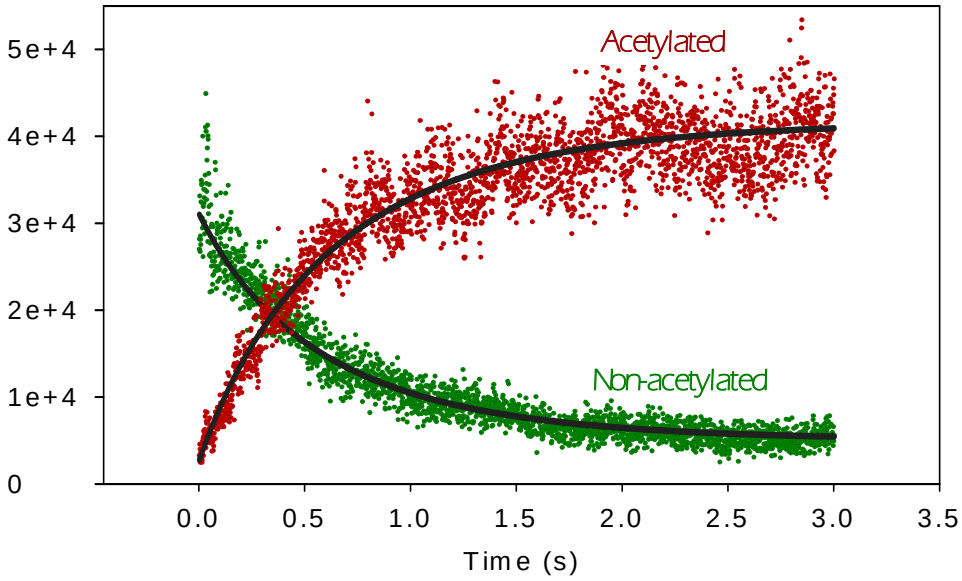
$$K_M$$

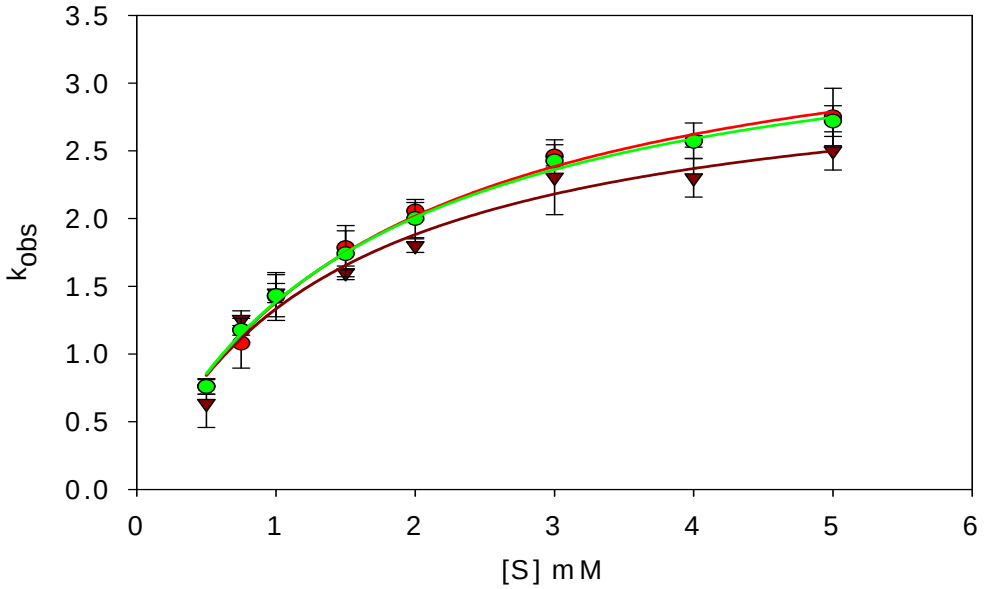
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_{cat}} E + P$$





Last Week...


Acetylated
Non-acetylated
5e+4
4e+4
3e+4
2e+4
1e+4
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Time (s)


k_{obs}
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
[S] mM

Review of Macroscopic Steady State Parameters

- K_M: The equilibrium constant between free enzyme and substrate and *all* enzyme complexes

$$\frac{[E]+[S]}{\sum_{i=1}^{i}[EC]_i}$$

- The substrate concentration at which the initial reaction velocity V is at ½ V_{max}

$$[S] = K_M \longrightarrow V = \frac{V_{max} K_M}{2K_M} = \frac{V_{max}}{2}$$

- A Measure of the '*looseness* of binding' between E and S

Rates associated with breakup of the complex

$$K_M = \frac{k_{-1} + k_{cat}}{k_1}$$

Small K_M = tight binding

Big K_M = loose binding

Intrinsic rate of complex formation

Review of Macroscopic Steady State Parameters

- k_{cat}: A function of all first order rate constants between ES and P. ***Cannot be greater than any of the 'forward' microscopic rates.***

i.e. if $k_{cat} = 5\ s^{-1}$, no forward microscopic rate can be $< 5\ s^{-1}$

- k_{cat} is sometimes called the 'turnover number' because is gives the ***maximum number of 'turnovers' per active site, per unit time.***

TABLE 6–7 Turnover Numbers, k_{cat}, of Some Enzymes

Enzyme	*Substrate*	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Enzyme Efficiencies

- Catalytic efficiency is often given as: $\frac{k_{cat}}{K_M}$

k_{cat} ← How fast? (bigger = better)

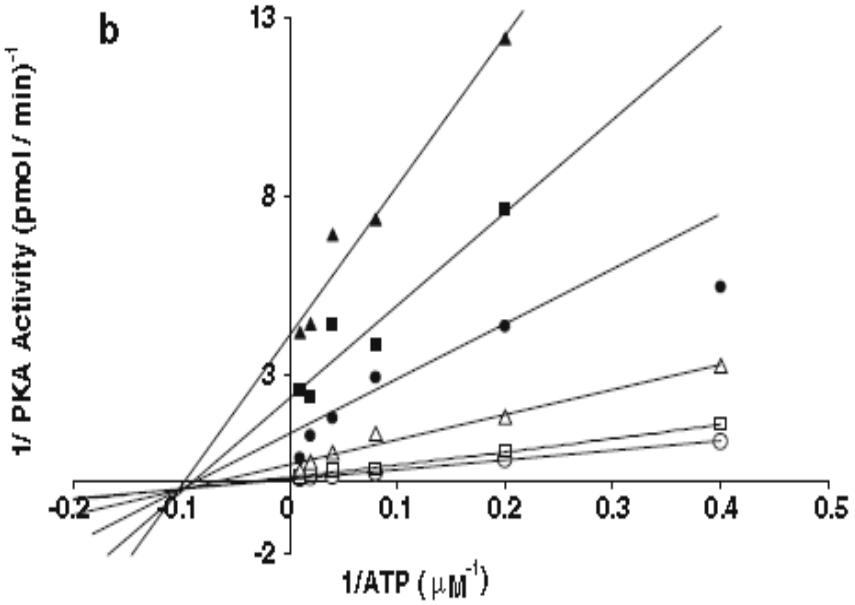
K_M ← How loose? (smaller = better)

- So, an efficient enzyme will have a high k_{cat} (fast) and a low K_M (tight substrate binder)

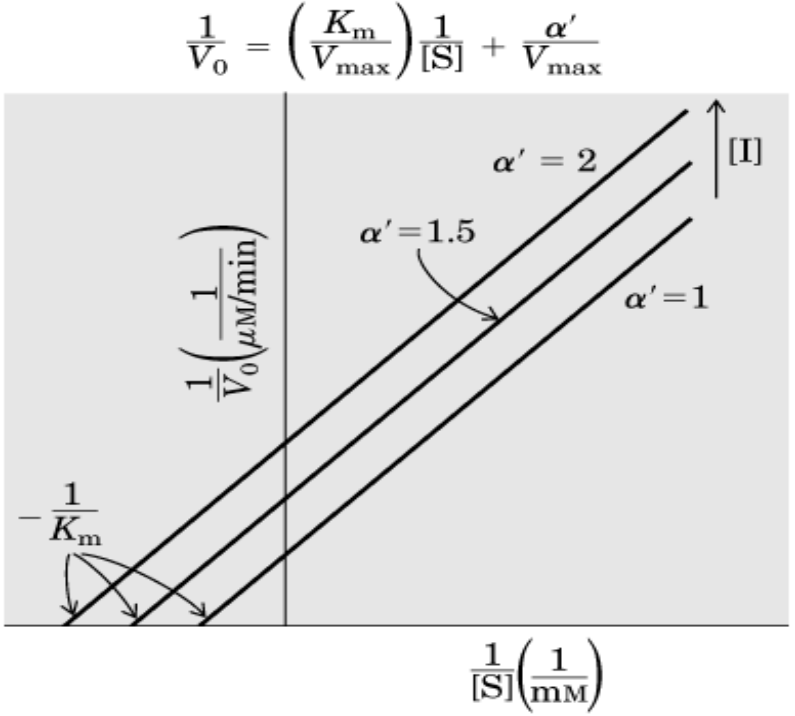
Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Superoxide dismutase	Superoxide ion ($O_2^{-\cdot}$)	3.6×10^{-4}	1.0×10^{6}	2.8×10^{9}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Errata! Uncompetitive Inhibition vs. Mixed Inhibition

- Mixed:

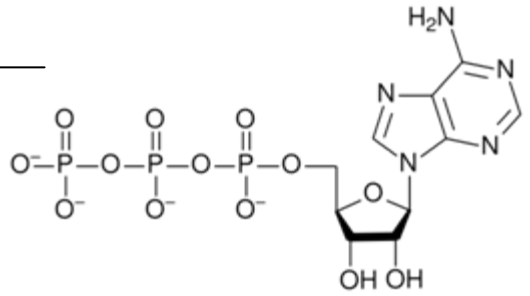


- Pure Uncompetitive:

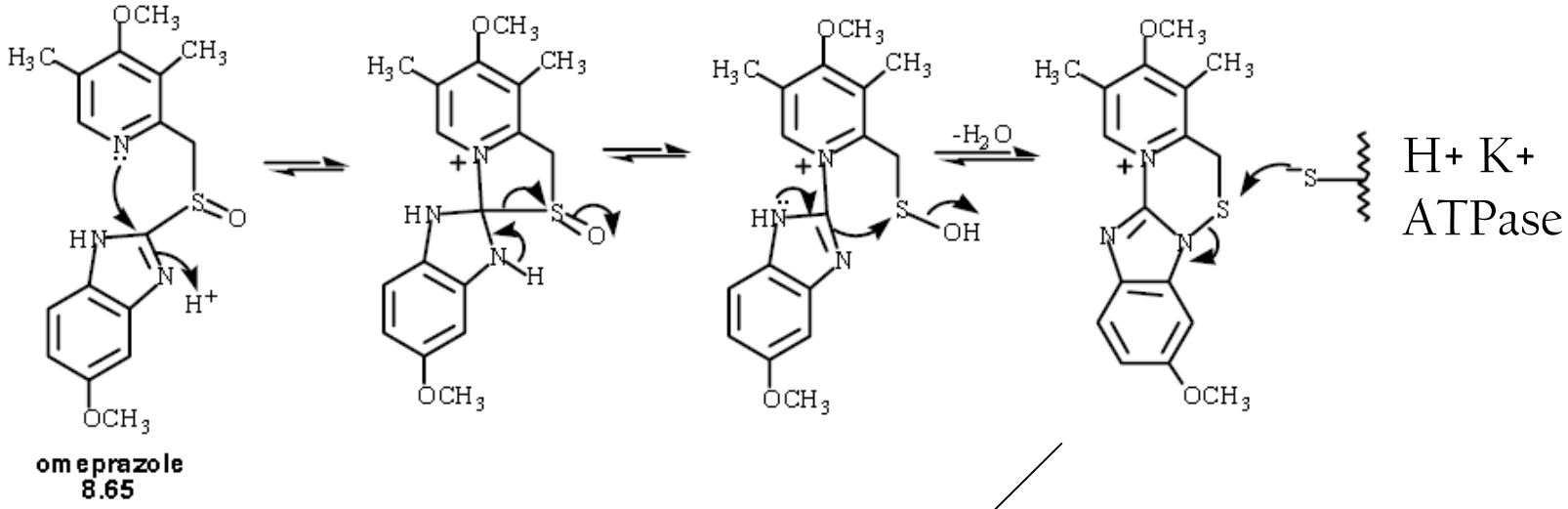


K_M/V_{max} = unchanged!!

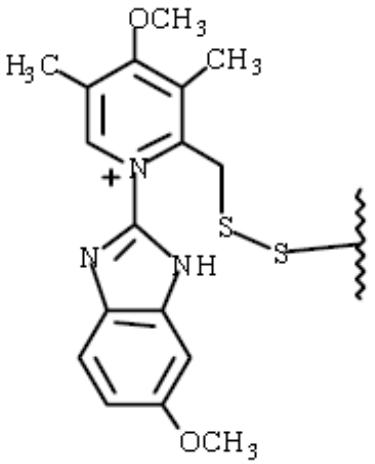
Suicide (Irreversable) Inhibition



- Omeprazole: Best drug ever!



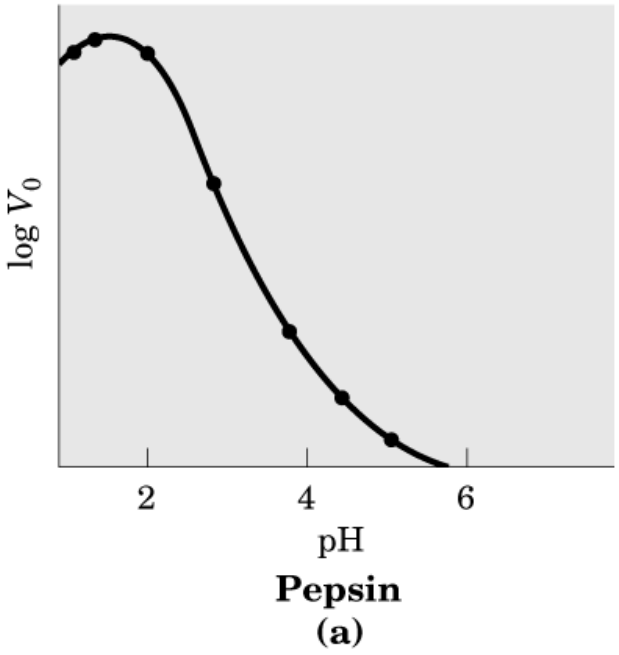
omeprazole
8.65



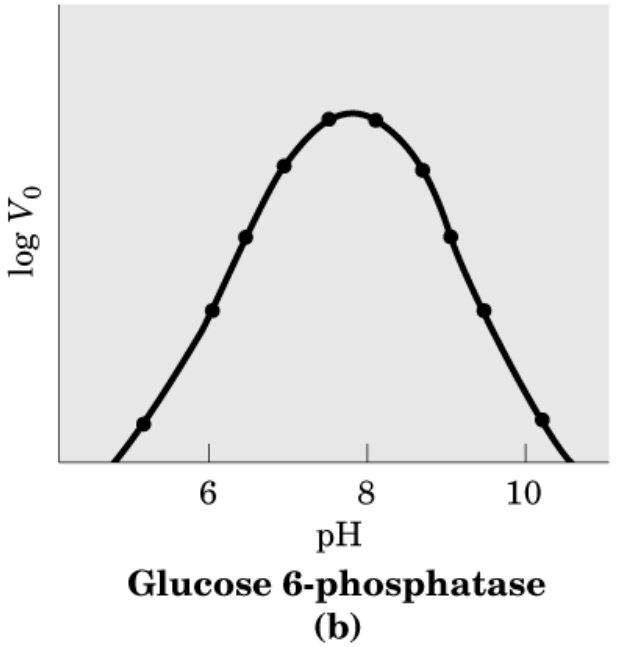
- Stuck!

Enzyme Control by pH

- All enzymes have a pH optimum which is usually associated with the ionization state of a critical (usually catalytic) residue

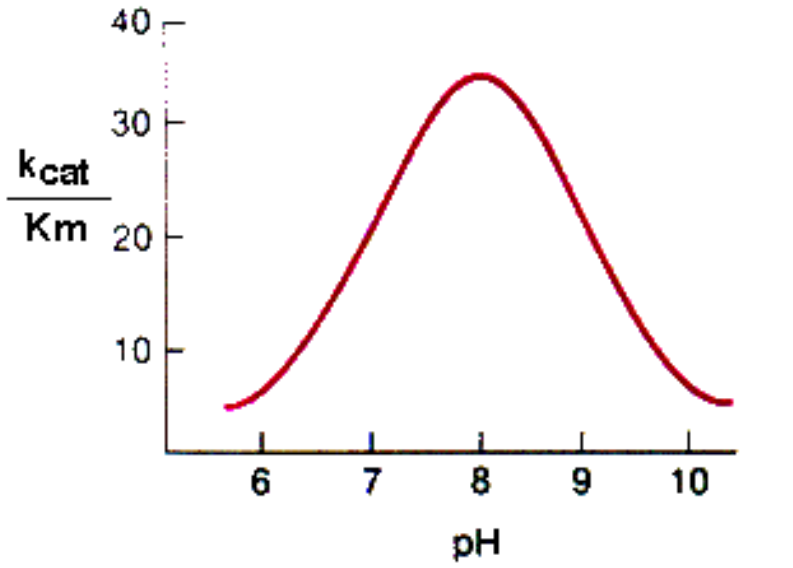


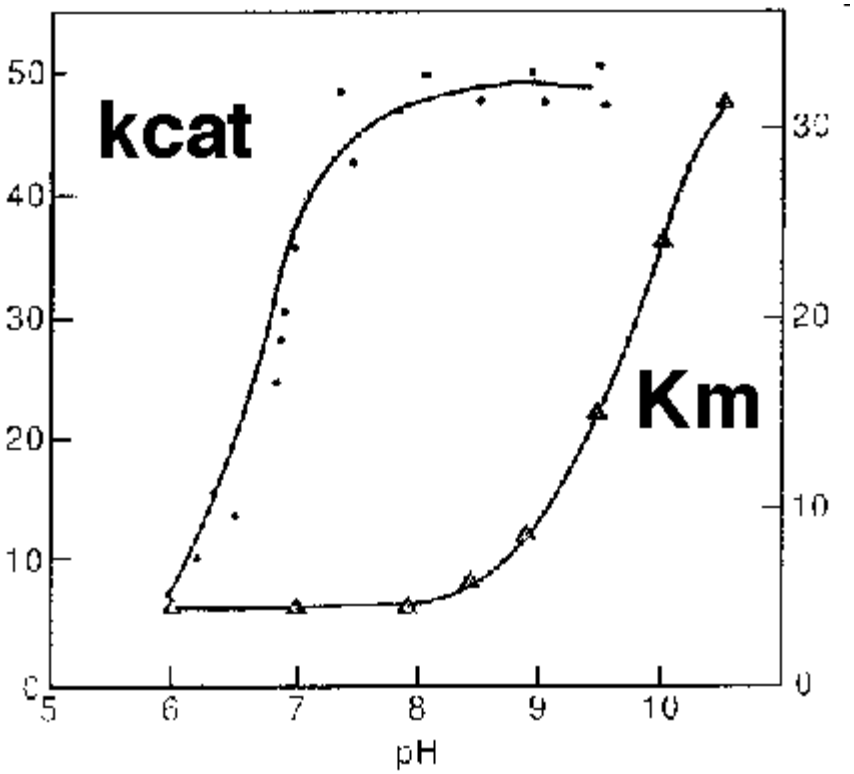
Catalytic Residue: Aspartate



Catalytic Residue: His (2° amine)

pH and Chymotrypsin





Catalytic Residue: Serine... ...?

pKa Ser = ~13!

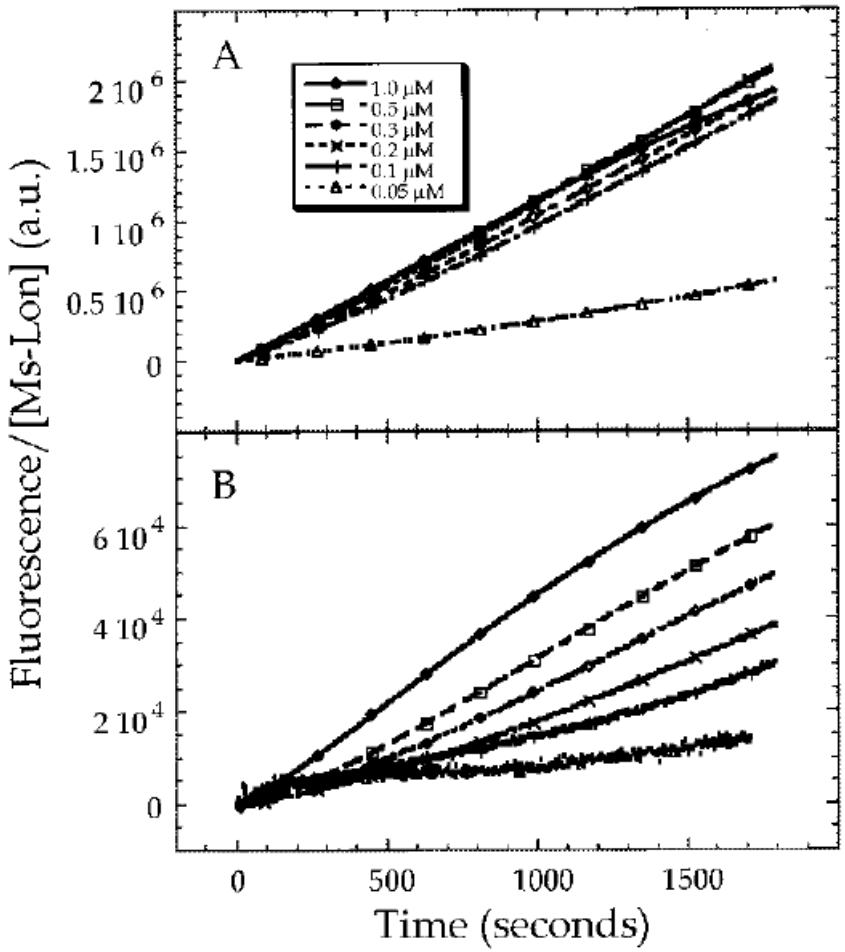
- Mechanism is reliant on Histidine!

Conformational changes increase K_M

http://www.bio.mtu.edu/campbell/401lec16ap2.html

Enzymatic Control by Oligomerization

- Protease Activity of Lon (a multimeric ATP dependent protease)



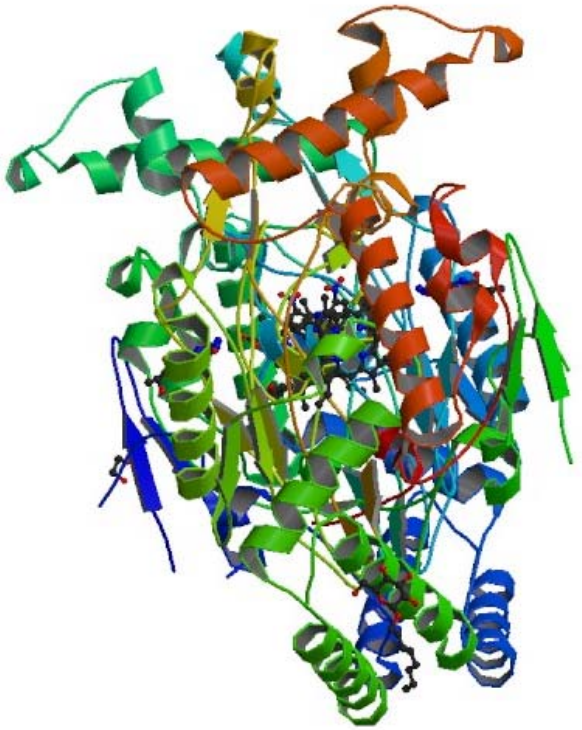
Oligomeric form favored
($Mg^{2+}\uparrow$)

Monomeric form favored
(Mg2+↓)

Biochemistry **2001,** *40,* 9317-9323

Enzyme Dynamics and Function Intro

- When you get a crystal structure of a protein, it looks like this:



- It was originally thought that we could just figure out where the small molecules will bind, in what orientation and we've have a good idea of how the enzyme works. And sometimes this works!

- But sometimes it doesn't! Why? ...WHY!!!...

- Because proteins have to MOVE to carry out their function!

Why Study Motions?

- Catalysis is inherently dynamic... if nothing else, substrate binding induces repositioning of catalytic residues

- It has been proposed that protein motions in the Michaelis complex are directly linked to the catalytic reaction coodinate.

- Dynamics is crucial to ligand binding

- And allostery

- Proteins have therefore not only had to evolve based on their structural properties, but also on their available dynamics modes.

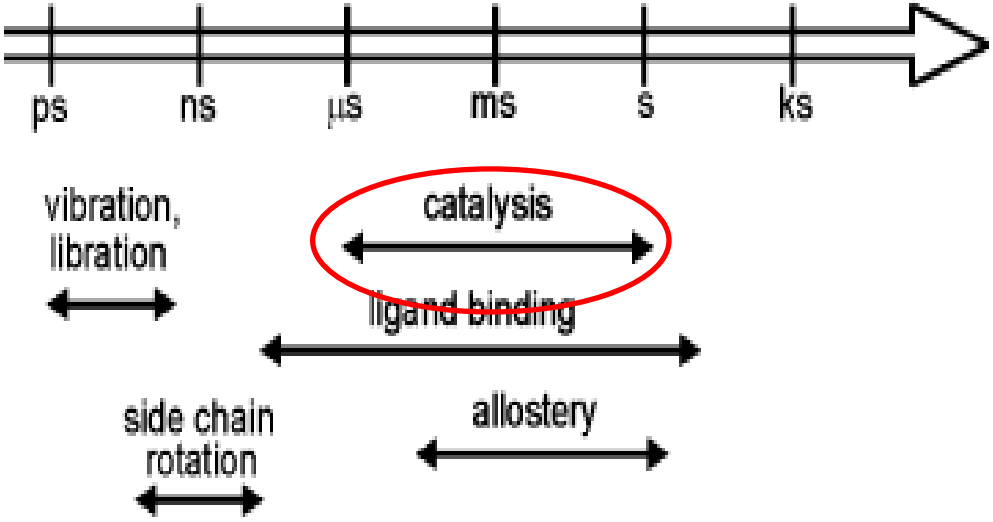
DNA Polymerase

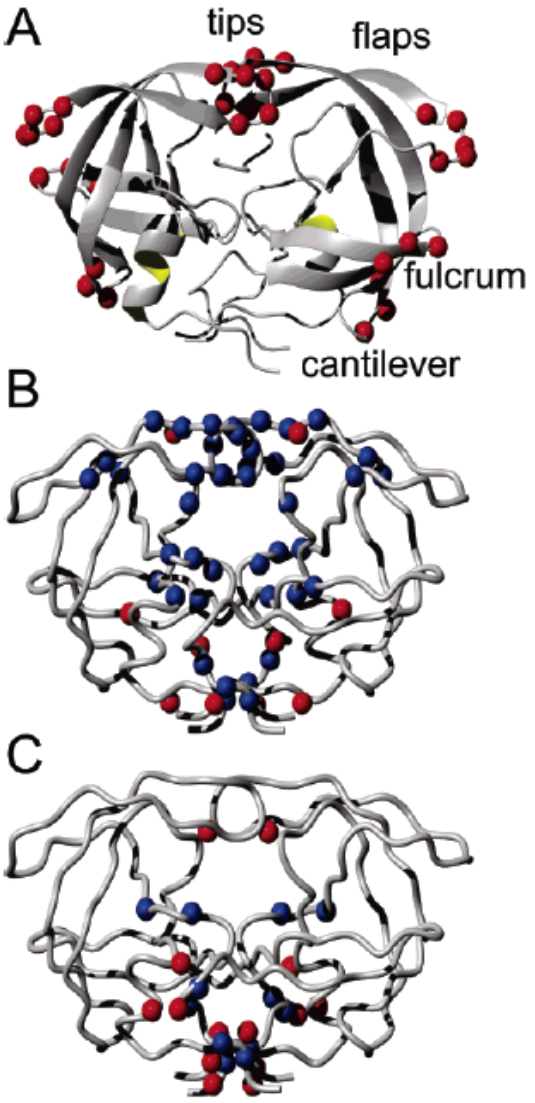
http://wishart.biology.ualberta.ca/moviemaker/gallery/index.html

Motions and Time-scales

- Polypeptide chains are intrinsically capable of motion – backbone phi/psi, rotation of side chains...

- These motions occur over a wide range of time-scales:





ps

µs

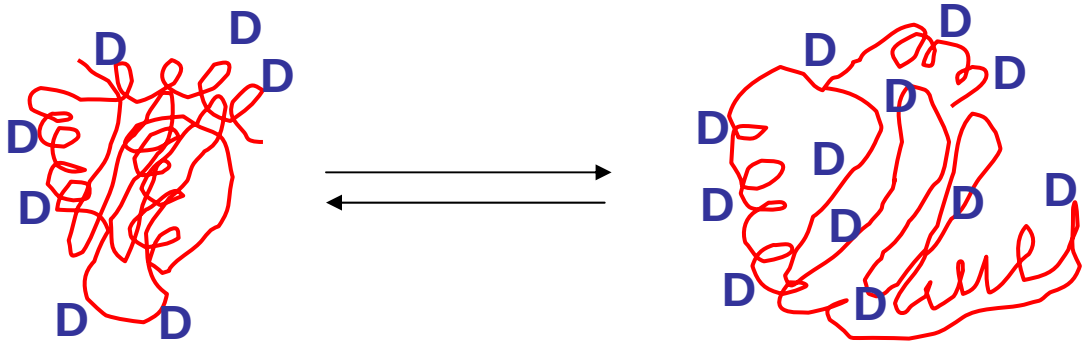
ms

HIV Protease

Enzyme Dynamics

- Sounds good in theory, but how do we prove it? Can we find any examples?

- How can we see these motions?



Ground State (low energy, common)

Excited state (high energy, rare)

- Hydrogen/deuterium exchange: Put protein in D_2O – the backbone protons (NH from peptide bonded N) will exchange with solvent D.

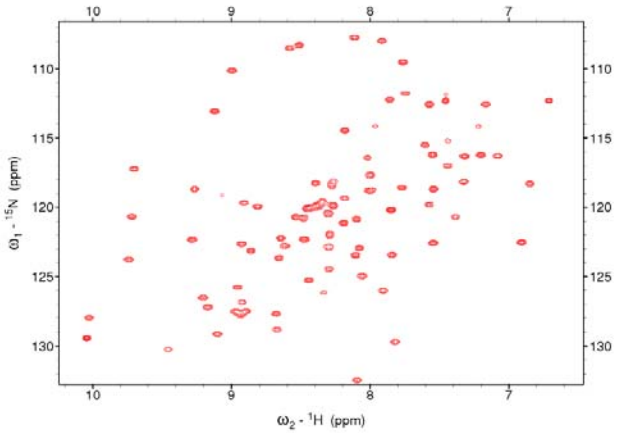
Hydrogen/Deuturium Exchange

- This will make the protein **heavier**.

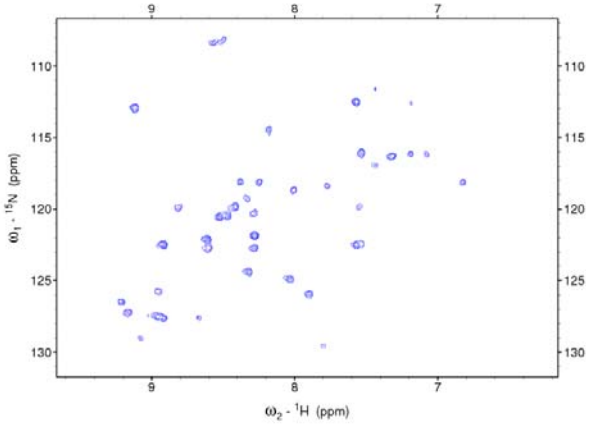
Mass Spectrometry!!

- Sites where exchange has occured (i.e. where the NH has exchanged to ND) will become NMR silent!

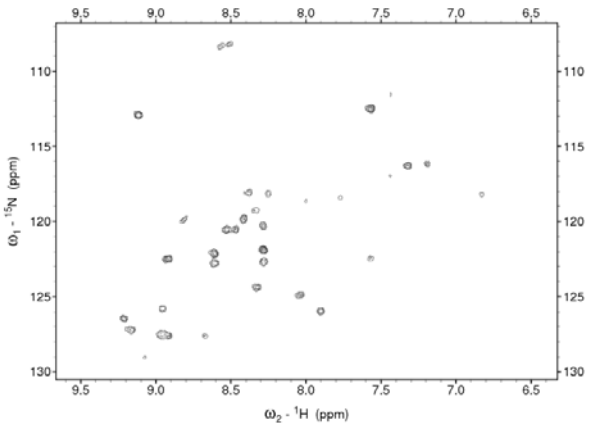
Site specific 'exchange profile'



t = 0



t = 5min

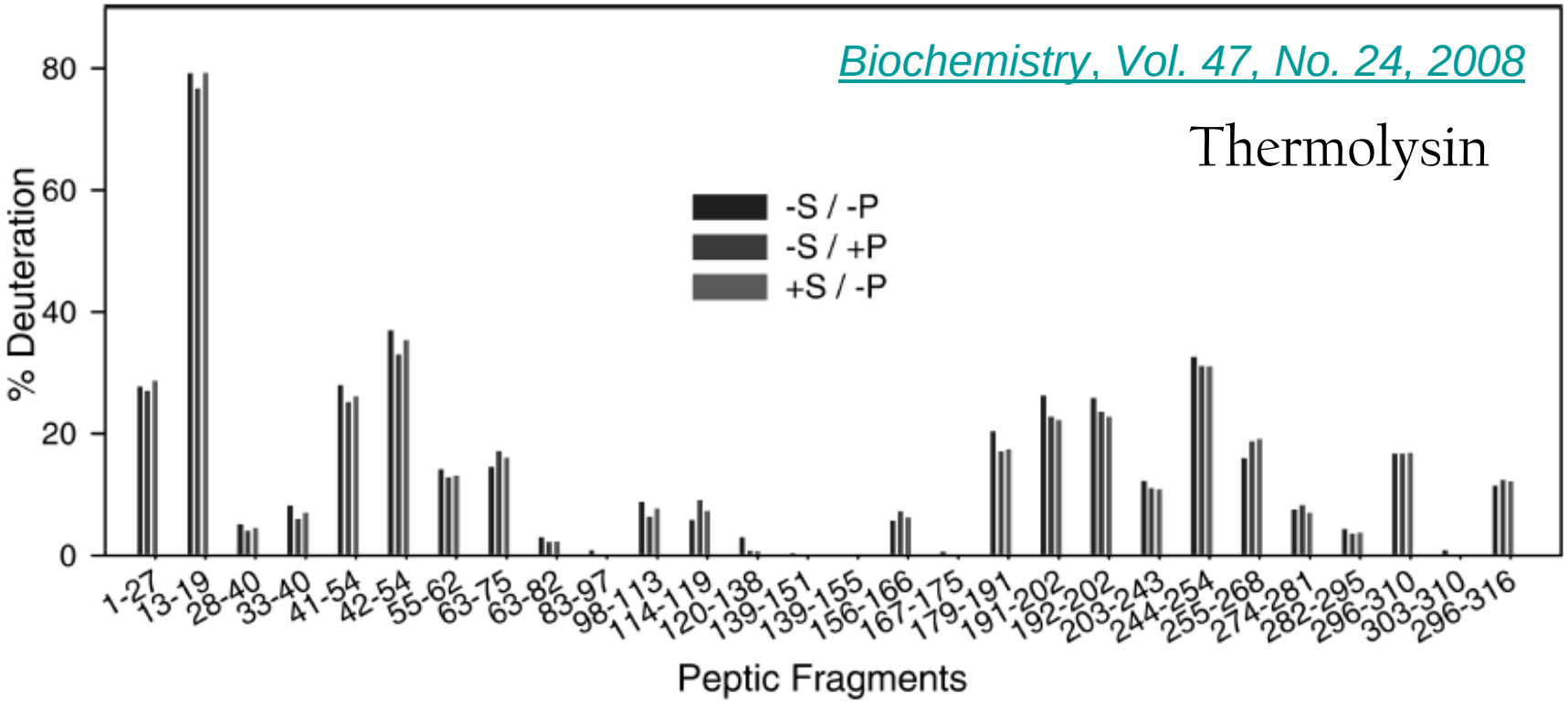


t = 1 hr

Back to Our Story…

- How do we prove that motion in enzymes is important?

- Take H/D exchange measurements in the presence and absence of substrate! Right?



- Well, actually… no. The amount of H/D exchange (the therefore the dynamics) appear to be the same!!

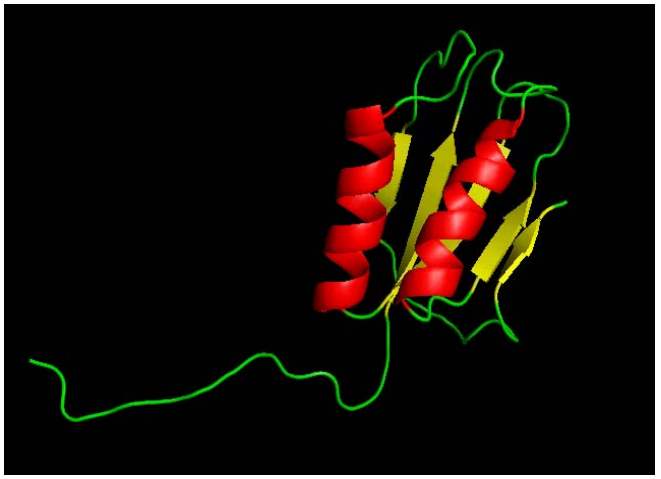
Thermophiles

- So our idea that motion is critical to enzyme function is wrong… or IS IT!!?

- Enter enzymes from Hyperthermophiles:

- These enzymes operate normally at 80°C+, but hardly at all at room temp!

Acylphosphatase from
Sulfolobus solfataricus



- Why not? Are they properly folded? YES

- Right pH? Right Oligomeric state? YES

- Not enough thermal energy for catalytically required dynamics.

Why no difference in H/D exchange?

In 2005, this lady:

Dorothee Kern

And this guy:

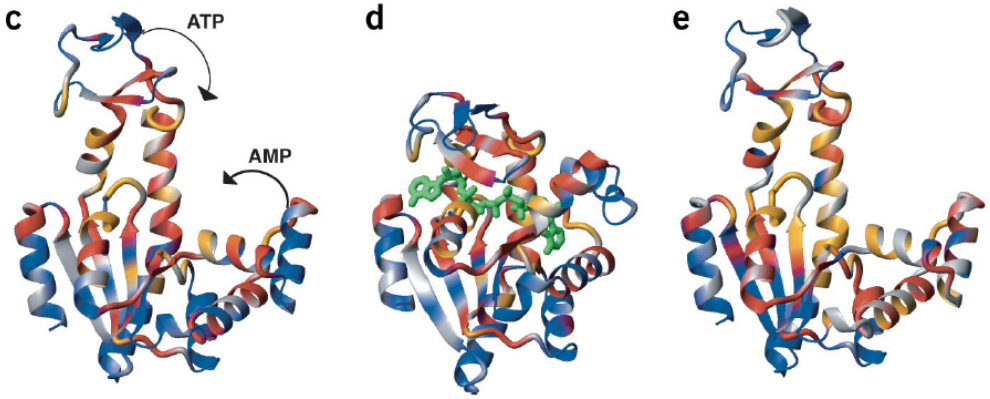
Lewis Kay

provided an answer:

'*We propose that the pre-existence of collective dynamics in enzymes before catalysis is a common feature of biocatalysts and that proteins have evolved under synergistic pressure between structure and dynamics*'

Nature|Vol 438|3 November 2005

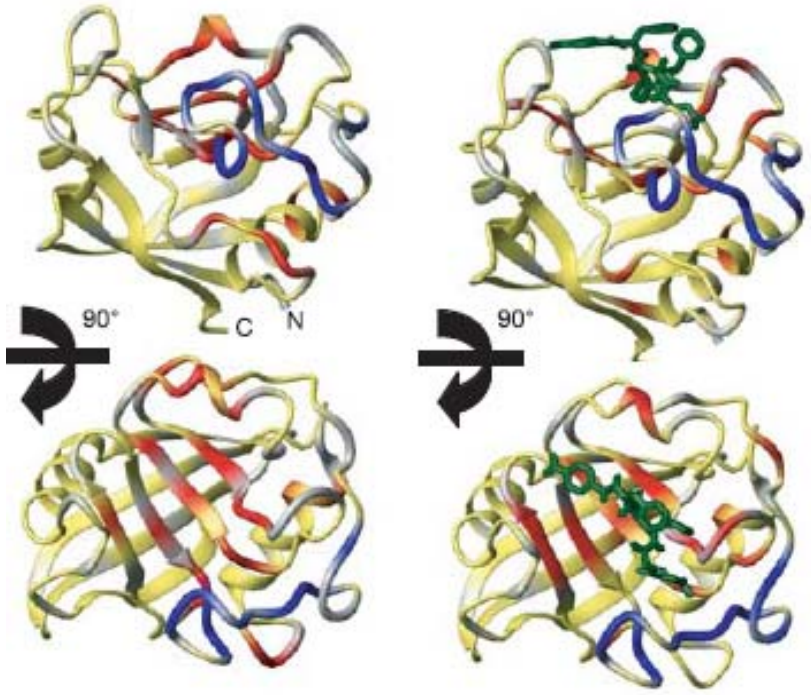
Dynamics are Same with and Without Substrate



Nat. Struct. Biol.|Vol 11|10 2004

Adenylate Kinase

Thermophillic/Mesophillic Enzyme Pair



Nature|Vol 438|3 November 2005

Cyclophillin A (prolyl isomerase)

Dynamics 'hot spots' are the same!

Linearized Michaelis-Menten Kinetics

Linearized Michaelis-Menten Kinetics