Principles of protein phosphorylation

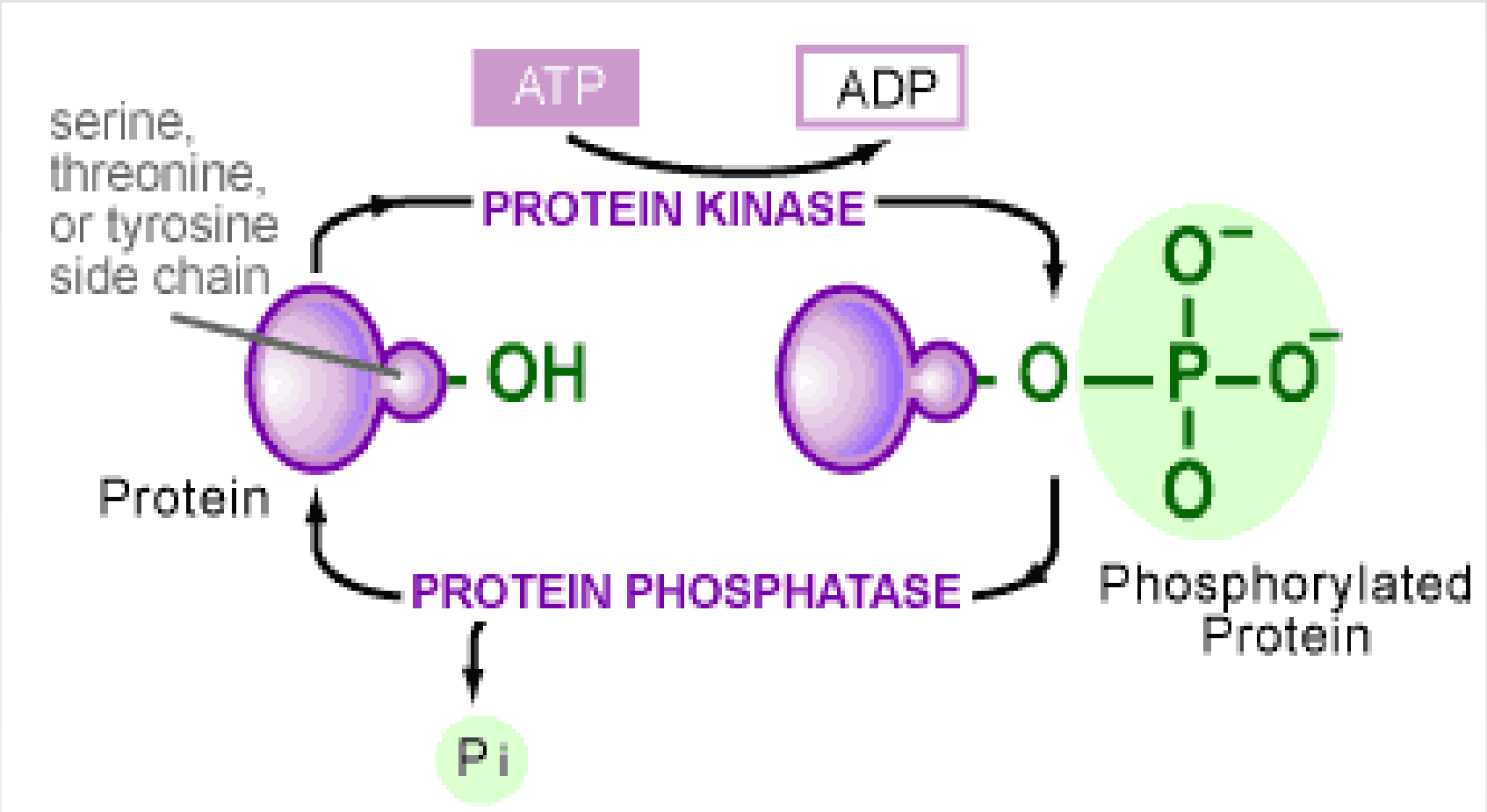
Biophysical Chemistry 1, Fall 2010

Signalling “cascades”

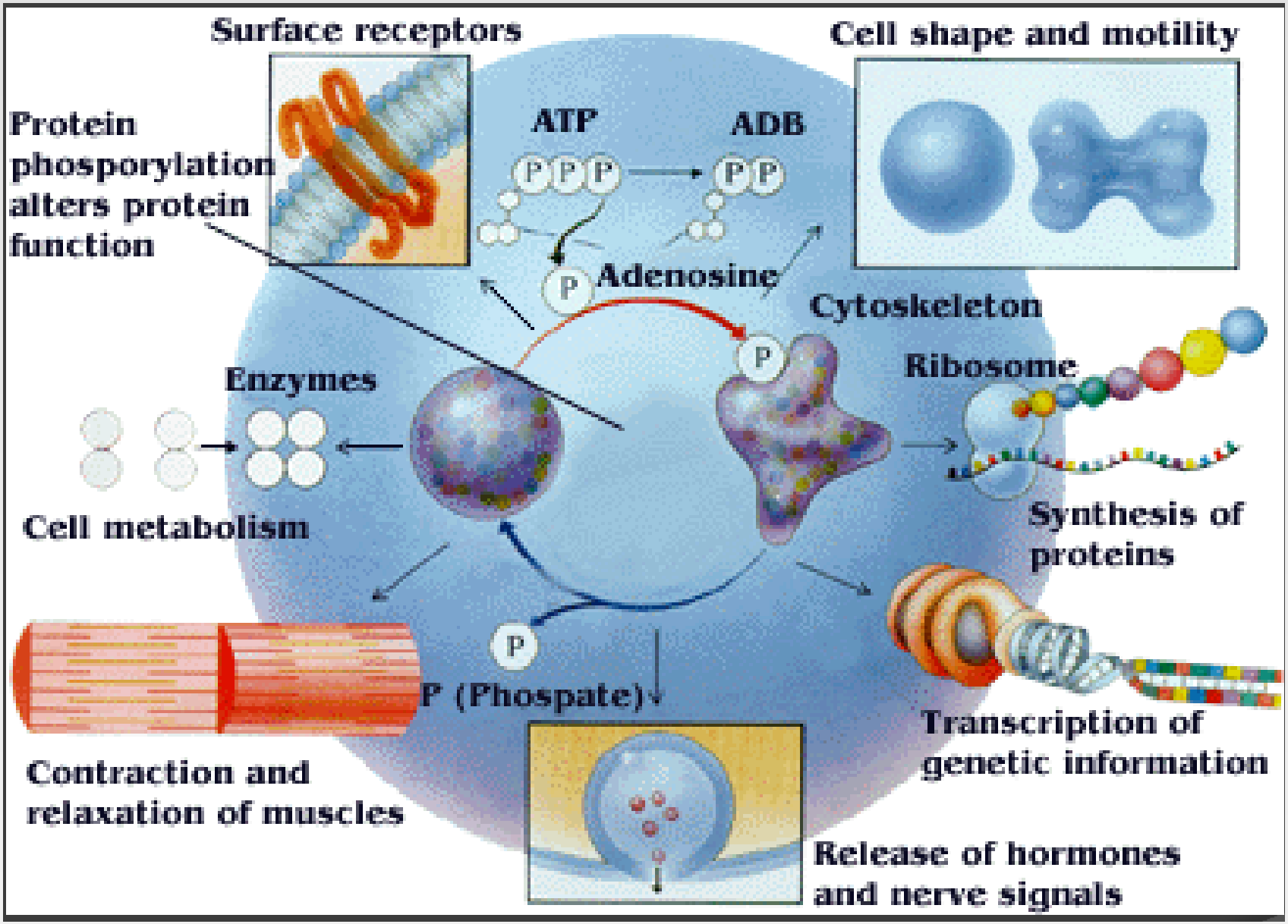
Structural biology of phosphorylation

Web assignment: http://pkr.genomics.purdue.edu

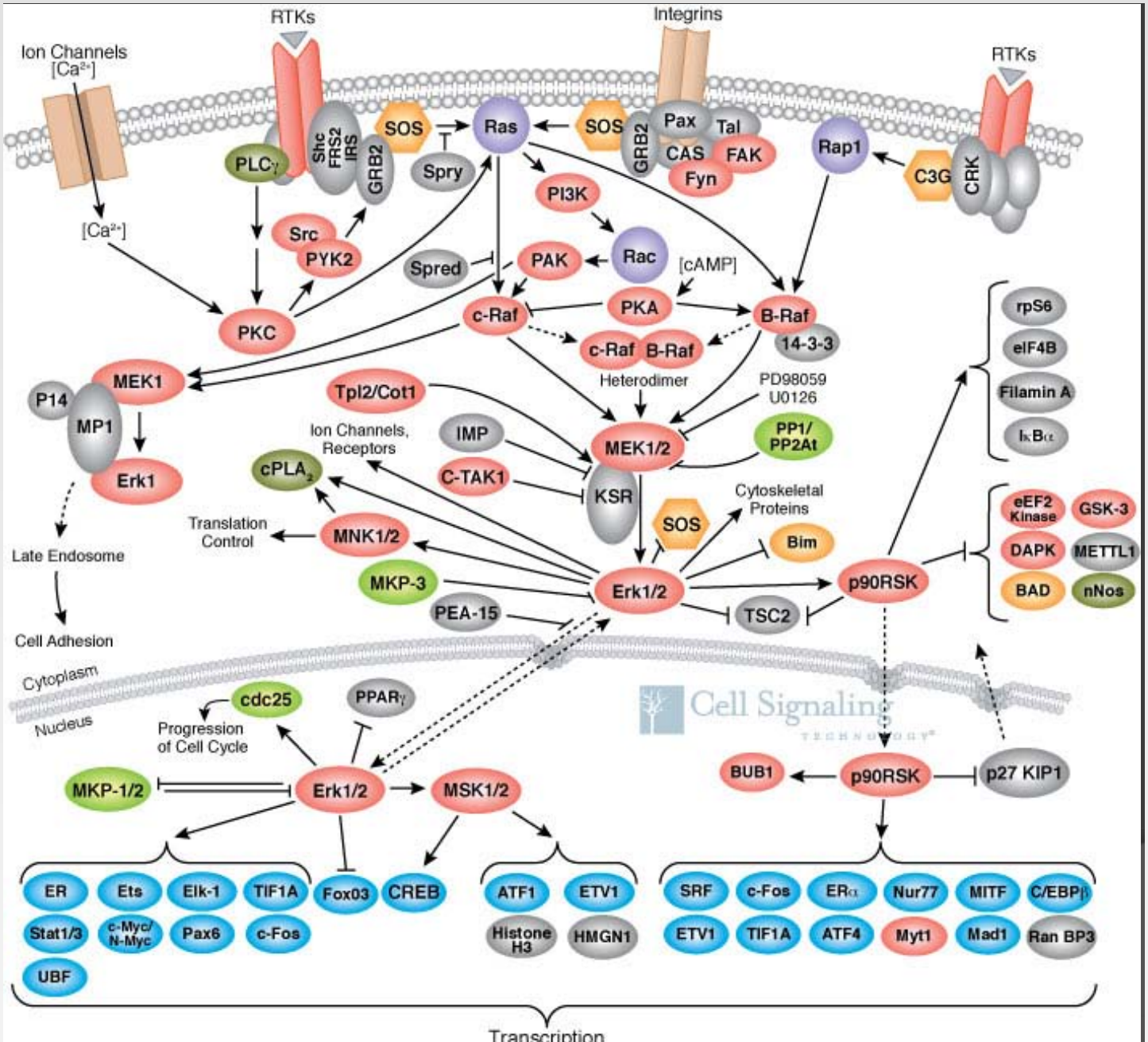
Kinases and phosphatases



Signalling overview



Cells are way too complex!



Rous sarcoma virus (RSV)

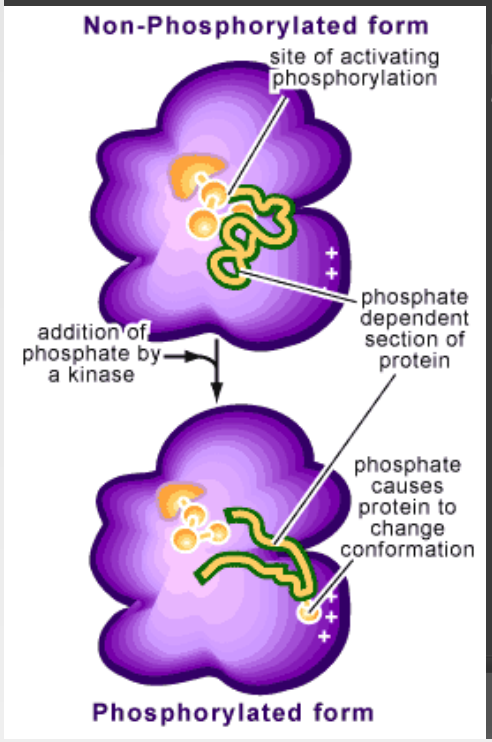
- gag - encodes capsid proteins
- pol - encodes reverse transcriptase
- env - encodes envelope proteins
- src - encodes a tyrosine kinase that attaches phosphate groups to the amino acid tyrosine in host cell proteins

Mutations, viruses and cancer

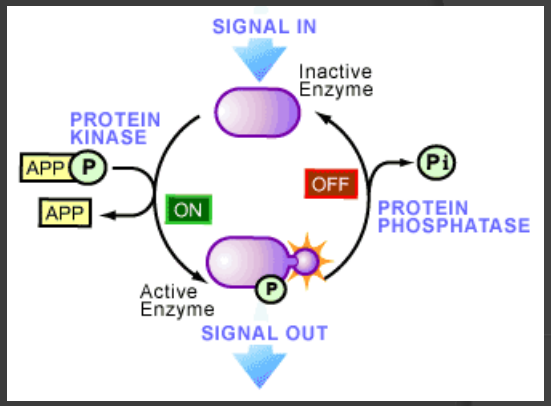
- v-src lacks the C-terminal inhibitory phosphorylation site (tyrosine-527), and is therefore constitutively active as opposed to normal src (c-src)
- Continuous cell profileration ⟹ tumor

Biophysics of signalling

Phosphorylation is an important regulatory mechanism



Can reversible attach/detach a phosphate and therefore switch "on"/"off" the function

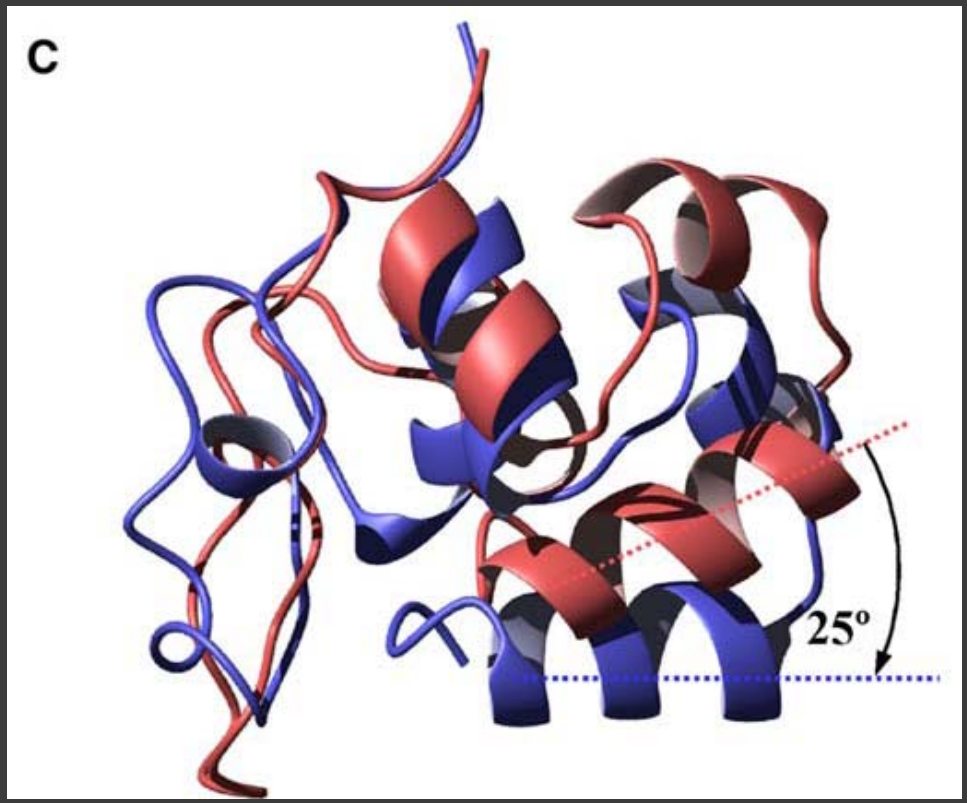


Effect of phosphorylation is manifold

- Conformational change
- Ordering/disordering
- Electrostatic effects
- Alternate binding behavior

Signalling by reorientation

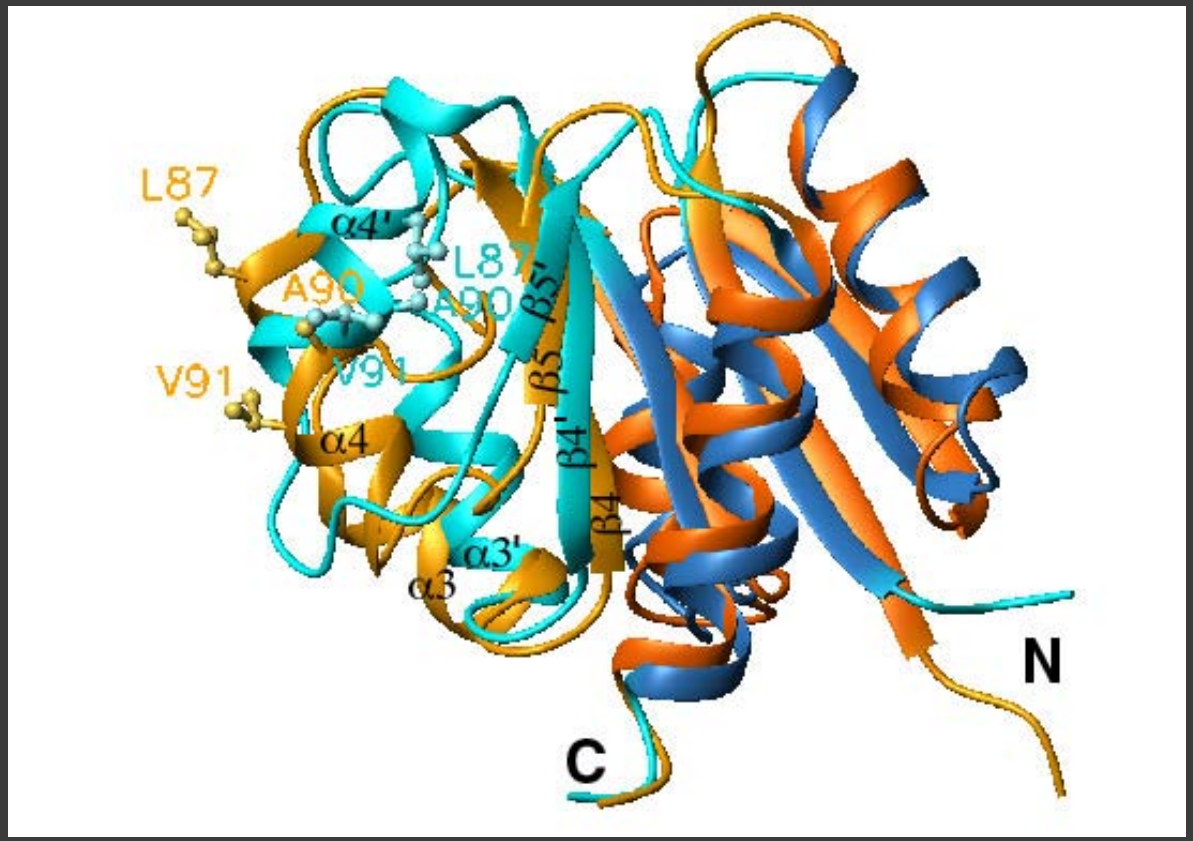
DHP (red) and
DHPs74e (blue)



Rmsd = 2.5Å
Z-Score = 4.6
(>3.6 same fold)

Title: A phosphorylation-induced conformation change in dematin headpiece
Author(s): Jiang ZHG, McKnight CJ
Source: STRUCTURE Volume: 14 Issue: 2 Pages: 379-387 Published: FEB 2006

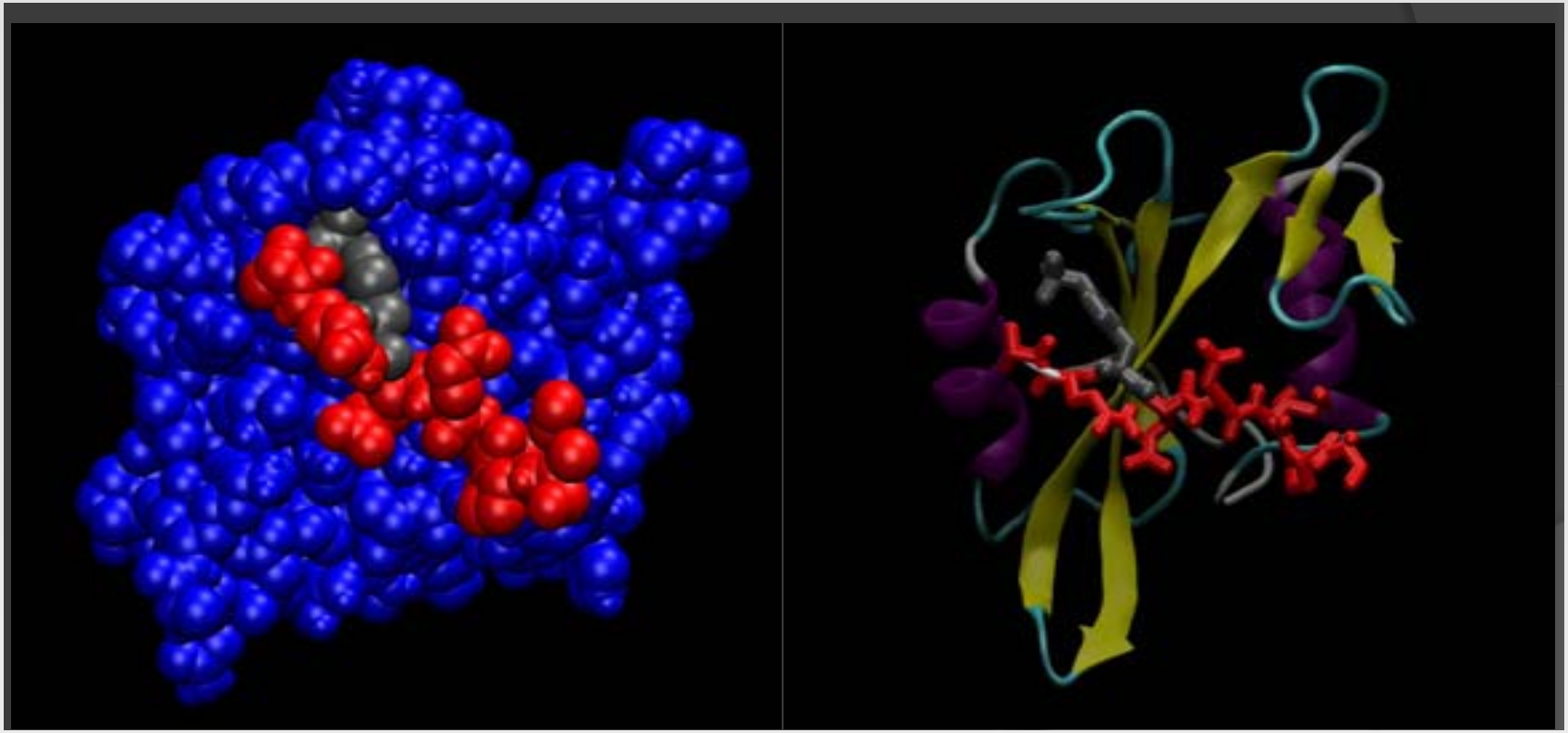
Order/disorder transitions



Orange-yellow: unphosphorylated NtrC
blue-cyan: phosphorylated NtrC

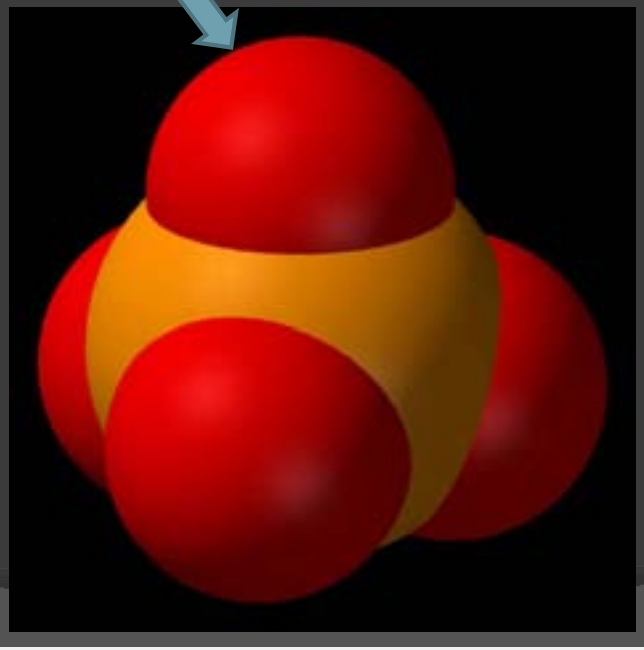
Volkman et al., Science 2001, 291, 2429-33

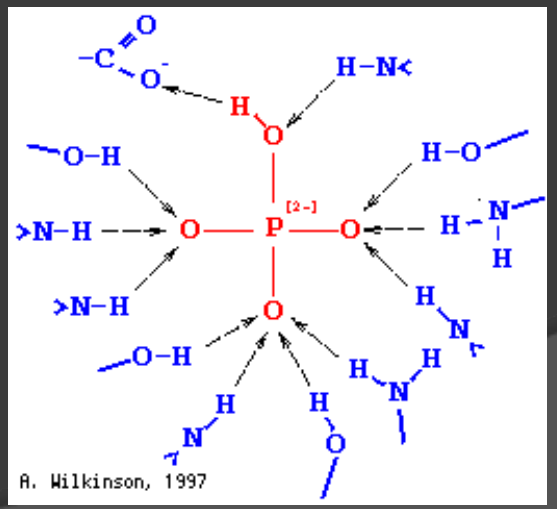
Src/SH2 interactions: binding vs release



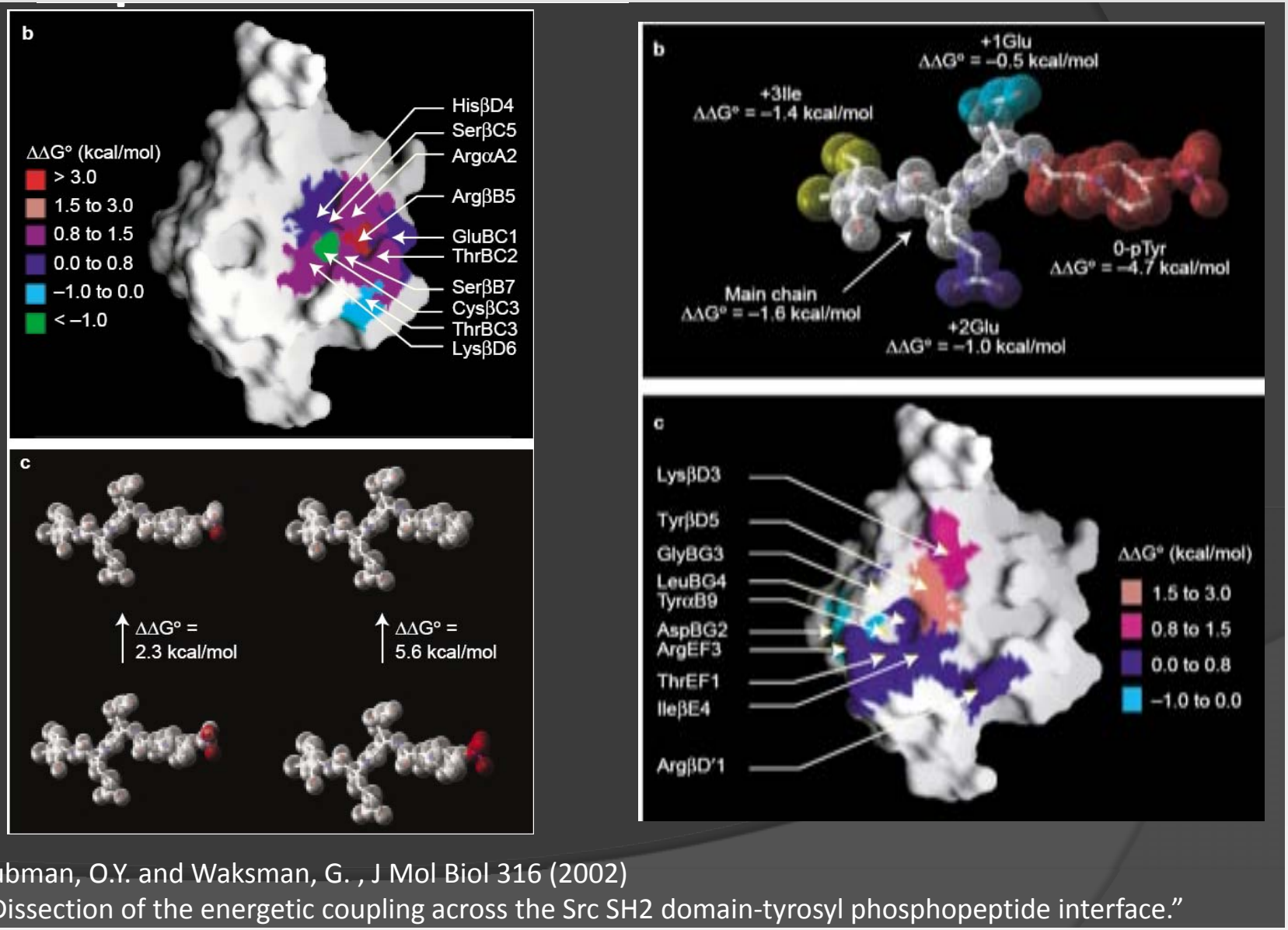
Expected conformational effects

- Electrostatics
- Hydrogen bonding
- Size





Mutational analysis



Lubman, O.Y. and Waksman, G. , J Mol Biol 316 (2002)
"Dissection of the energetic coupling across the Src SH2 domain-tyrosyl phosphopeptide interface."

Looking for analogues

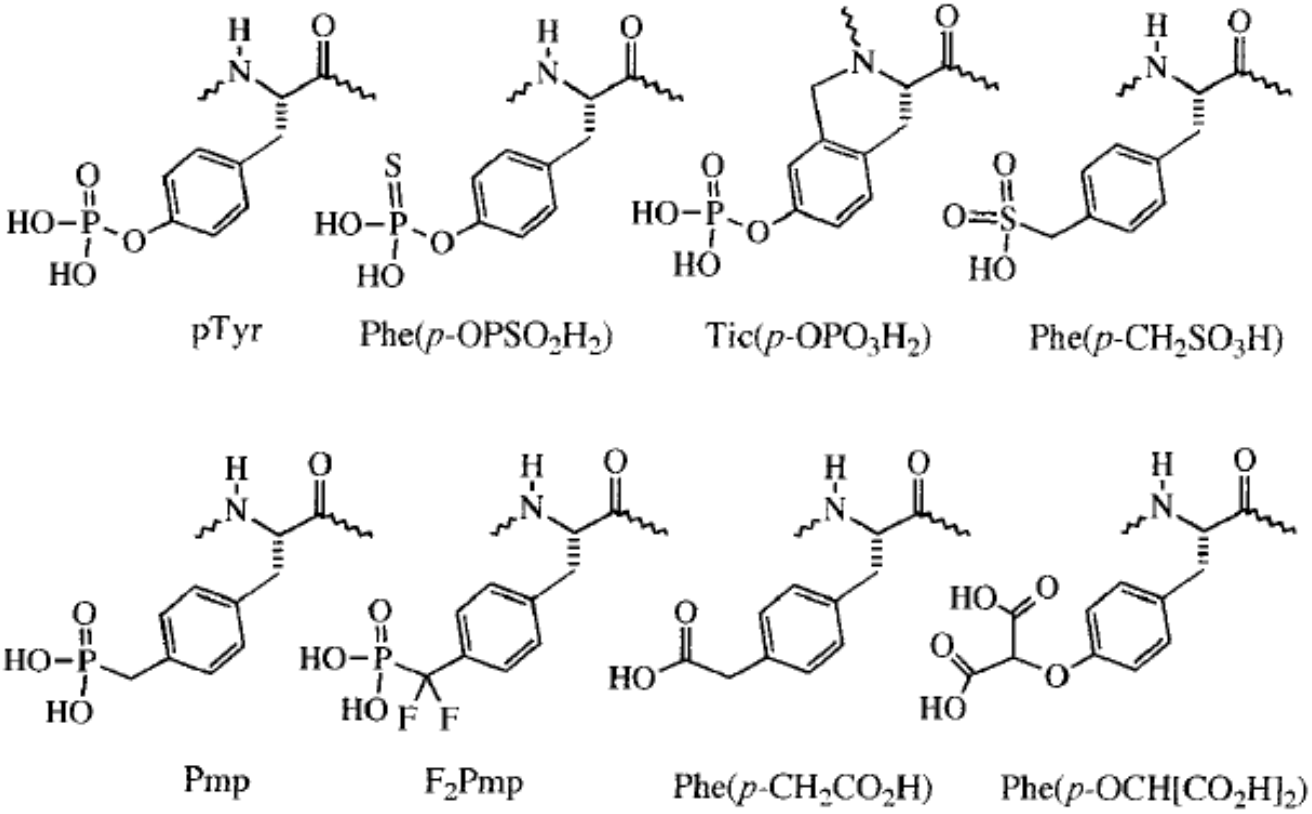


FIGURE 6 Chemical structures of pTyr and various analogues including phosphatase-resistant bioisosteres of the phosphate moiety, such as phosphonates, sulfonates, and carboxylates.

Expected conformational effects

Table IV Structure–Activity Relationships of Phosphopeptide Inhibitors of Src SH2 Binding[10]

Compound	Peptide Structure	Src SH2 Binding Relative Potency
2	Ac-pTyr-Glu-Glu-Ile-Glu pY pY+1 pY+2 pY+3	1.0 (IC_{50} = 0.7 μM)
3	Ac-Tyr-Glu-Glu-Ile-Glu	<0.001
4	Ac-Pmp-Glu-Glu-Ile-Glu	0.025
5	Ac-F_2Pmp-Glu-Glu-Ile-Glu	0.3
6	Ac-Phe(*p*-$OPSO_2H_2$)-Glu-Glu-Ile-Glu	0.6
7	Ac-Tic(*p*-OPO_3H_2)-Glu-Glu-Ile-Glu	0.25
8	Ac-Phe(*p*-CH_2SO_3H)-Glu-Glu-Ile-Glu	0.003
9	Ac-D/L-Phe(*p*-CH_2CO_2H)-Glu-Glu-Ile-Glu	0.001
10	Ac-Gln-Phe(*p*-$OCH[CO_2H]_2$)-Glu-Glu-Ile-Pro-NH_2	~0.03 (IC_{50} = 22 μM)

"Src Homology-2 Domains: Structure, Mechanisms, and Drug Discovery", Sawyer, *Biopolymers*, **47,** 243-63 (1998)