**Biophysical Chemistry I**

Lecture by Joachim Lätzer

# PHOSPHORYLATION AND CELL SIGNALING

# Overview

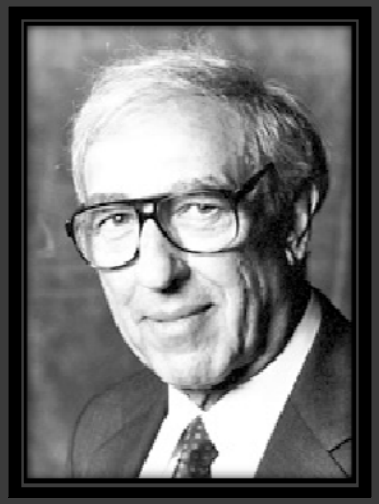
- Introduction to phosphorylation
- Examples of different Signals
- Structural Manifestation
- Biophysical characterization of phosphorylation

# Introduction to phosphorylation

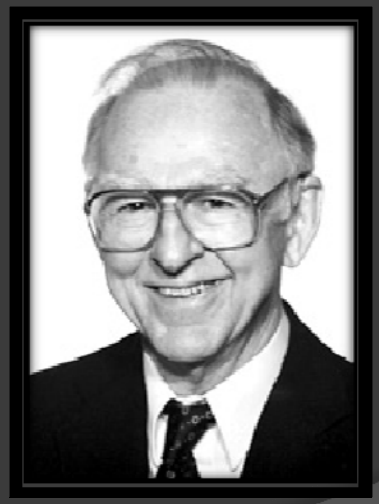
- Fischer and Krebs discovery
- Why phosphorylation is useful

# The Nobel Prize in Physiology or Medicine 1992

"for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism"



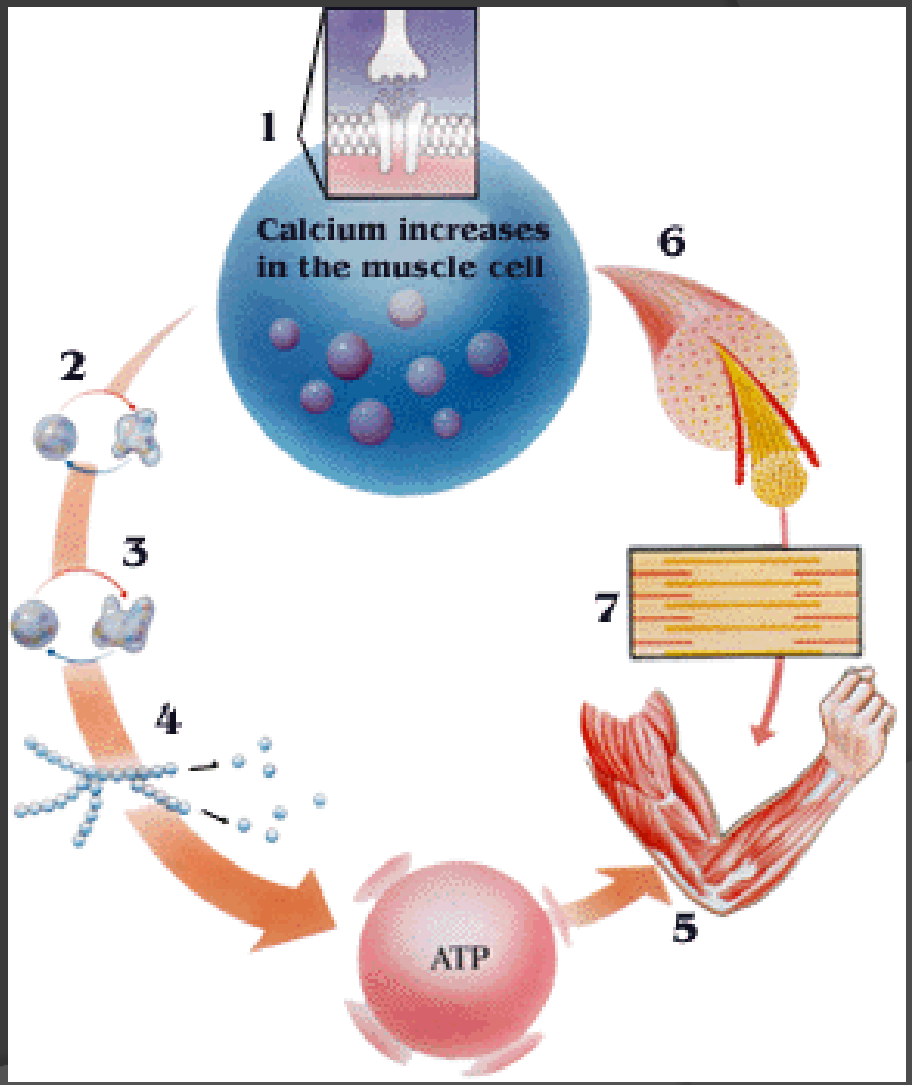
Edmond H. Fischer



Edwin G. Krebs

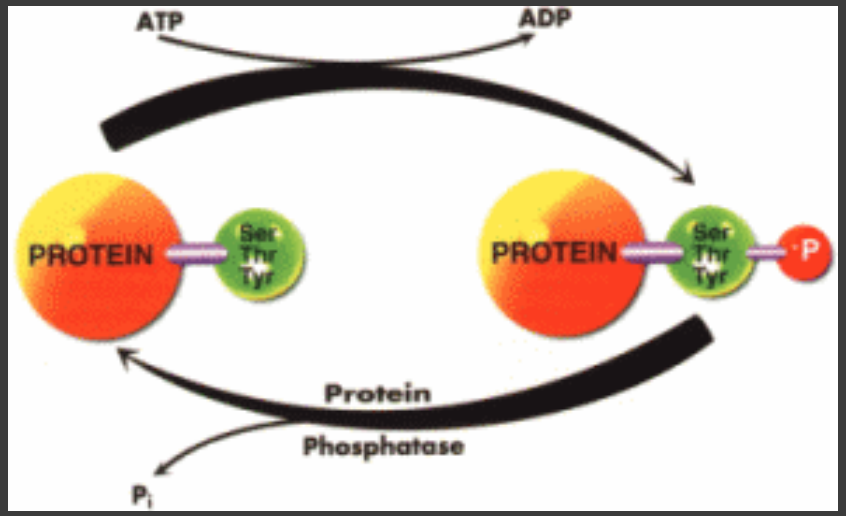
# Breakdown of glycogen to glucose

1. Calcium signal
2. Calcium activates phosphorylase kinase
3. Phosphorylase kinase phosphorylates phosphorylase, which is activated
4. Glycogen is broken to glucose → ATP
5. Muscle work using ATP
6. Muscle contains muscle cells
7. Contraction



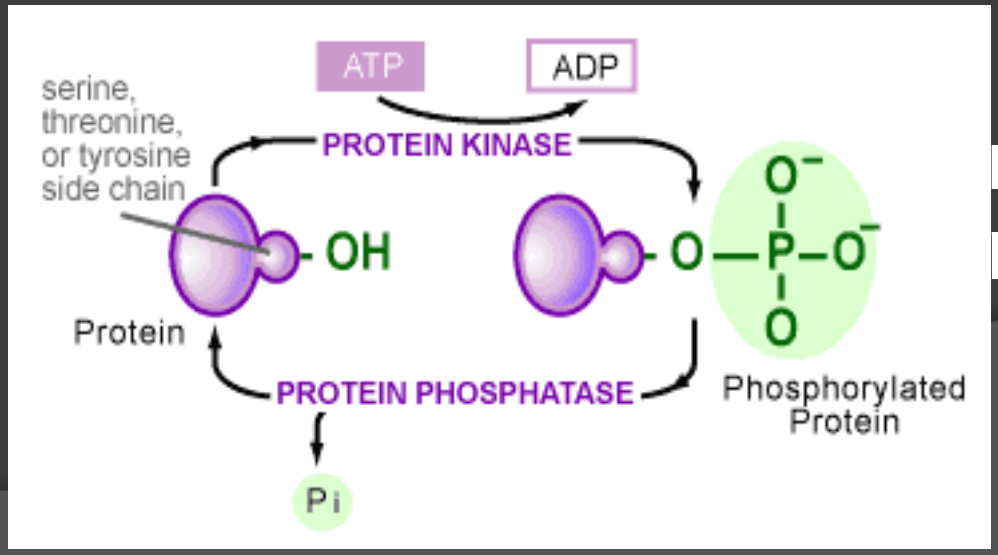
# Reversible protein phosphorylation

**Enzymatic reaction**
**$\Delta G$~12kcal/mol**




**Posttranslational Control**

**Phosphatase dephospho-rylates**

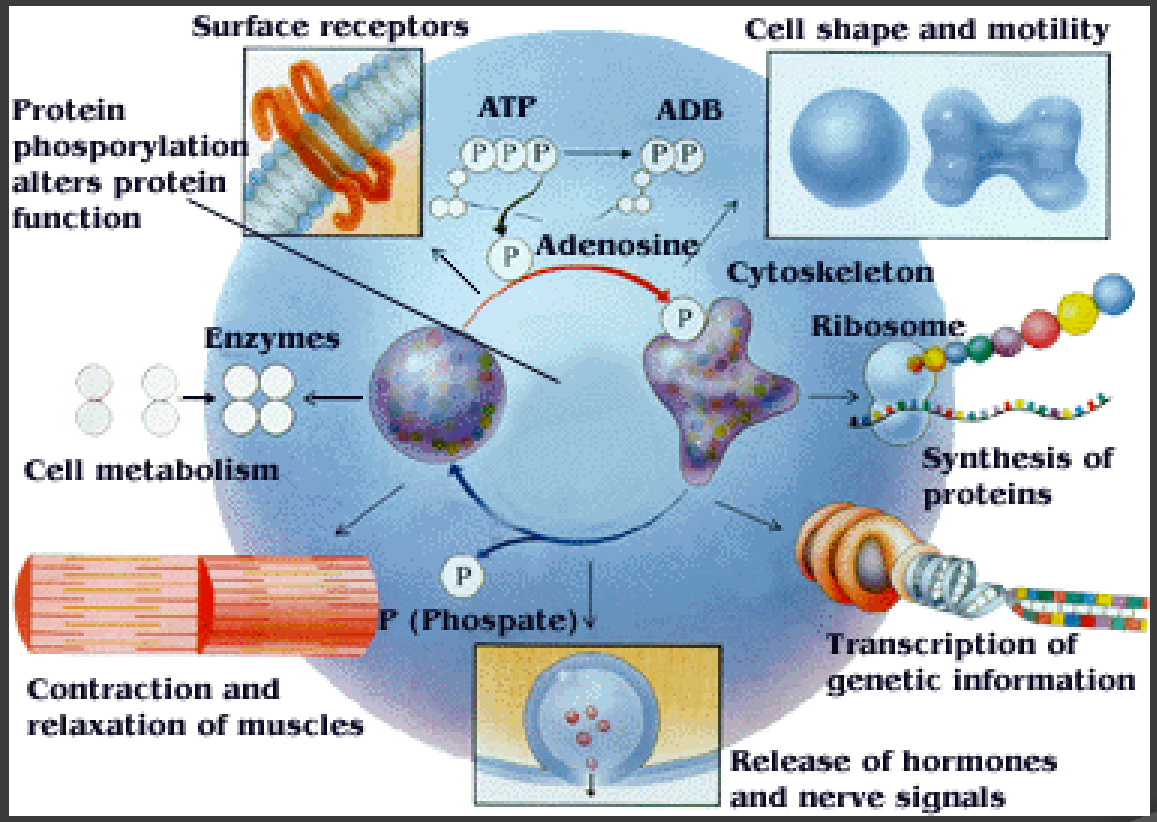



**Kinase phosphorylates**

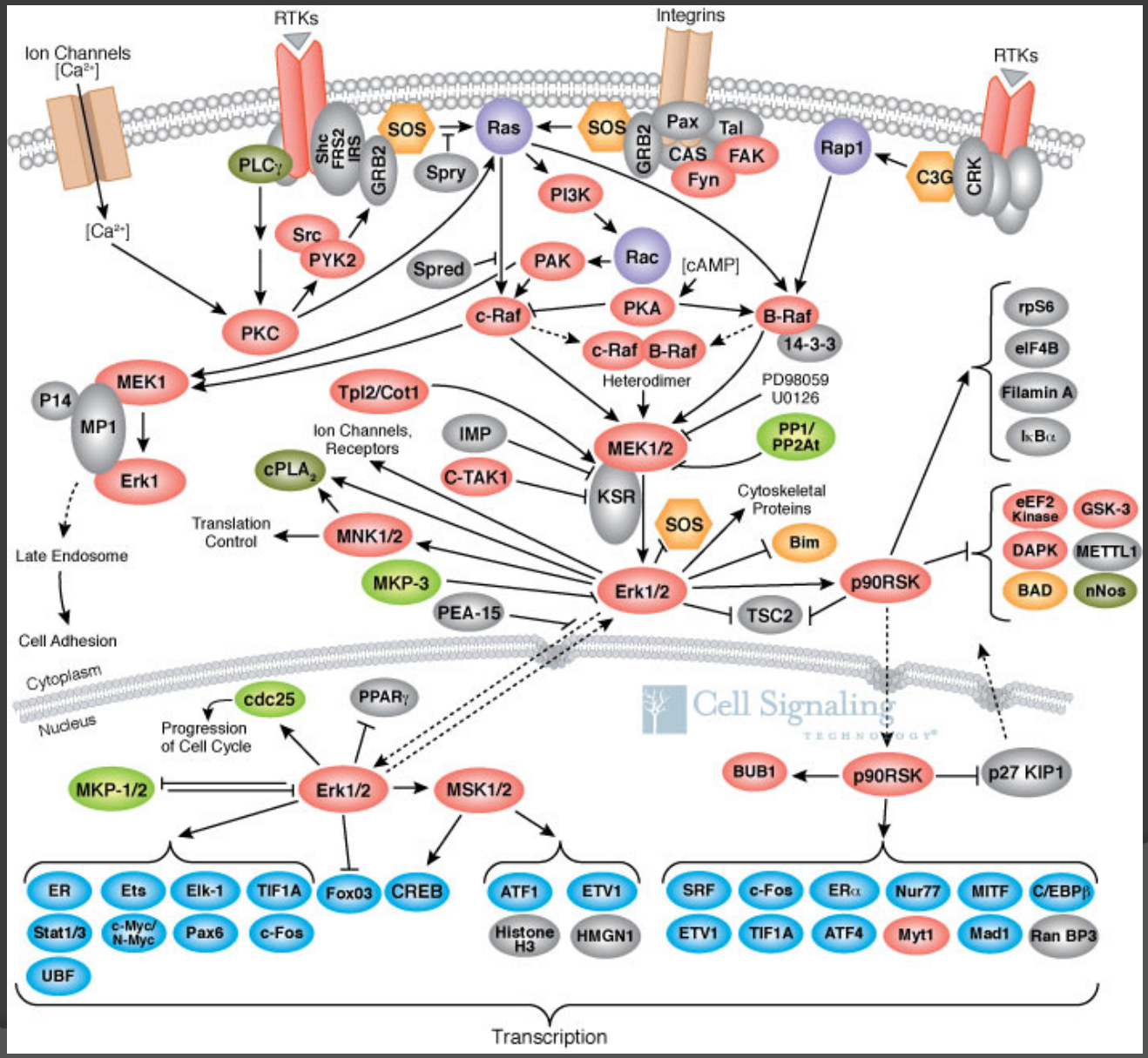
# Examples of different Signals

- General Examples
- MAPK/ERK signaling pathway: cell division
- Oncogene: Cancer and MAPK/ERK
- NF-KB signaling pathway: phosphorylation and inhibition

# General Examples

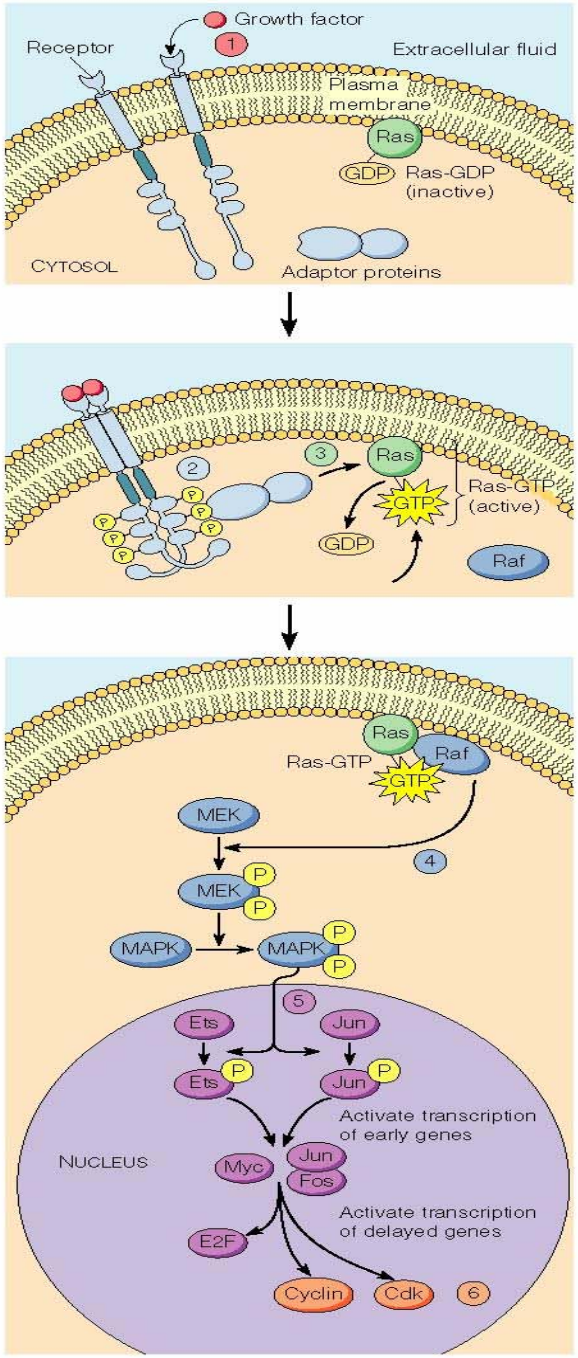


# MAPK/ERK Signaling Pathway



# Simplified view

1. Growth factor binds to a receptor
2. located in the cell membrane- this activates:
3. Grb2
4. Sos
5. Ras
6. Raf
7. MEK
8. Map Kinase



# What does map kinase (ERK) do?

- **Map kinase activates CDK1 which turns on cell division**
- **Map kinase enters the nucleus and activates transcription factors**
- **Transcription factors then bind to DNA to turn on genes that lead to cell division**

# Question: What happens if the signal pathway is modified ?

- Usually cancer
  - Caused by viruses
  - Caused by mutations

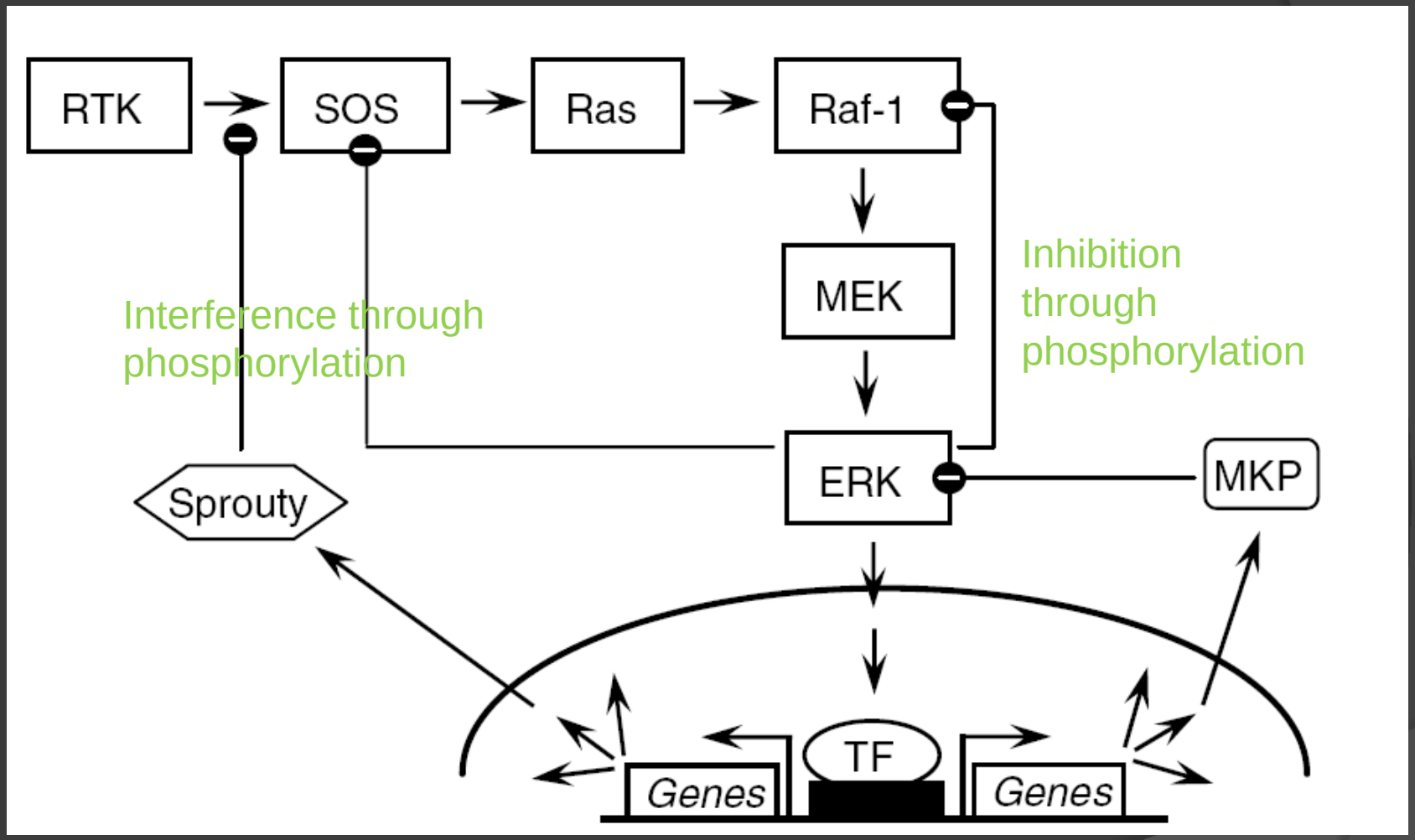
# Example: Rous sarcoma virus (RSV)

- 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

# Example: Rous sarcoma virus (RSV)

- 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

# MAPK/ERK and Cancer




Modified cell communication ➡ CANCER

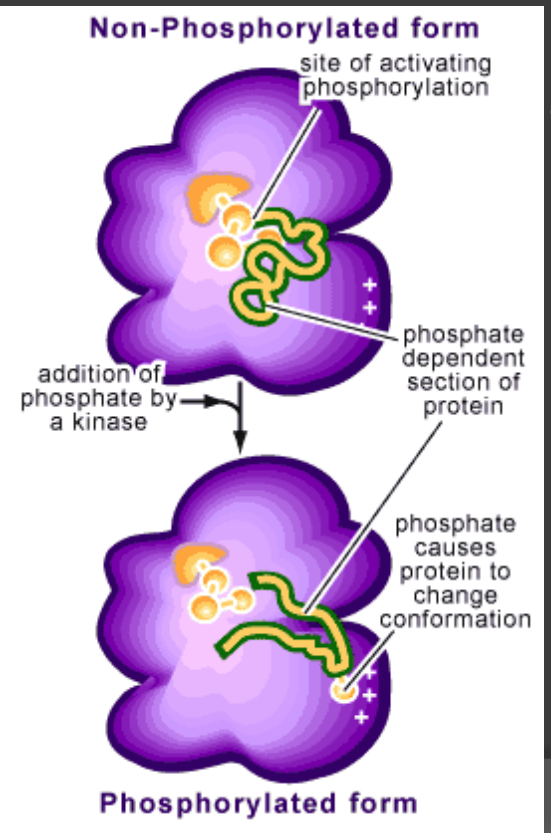
**"MAP kinase signalling pathways in cancer"** *Oncogene* (2007) **26,** 3279–3290

# Structural Manifestation

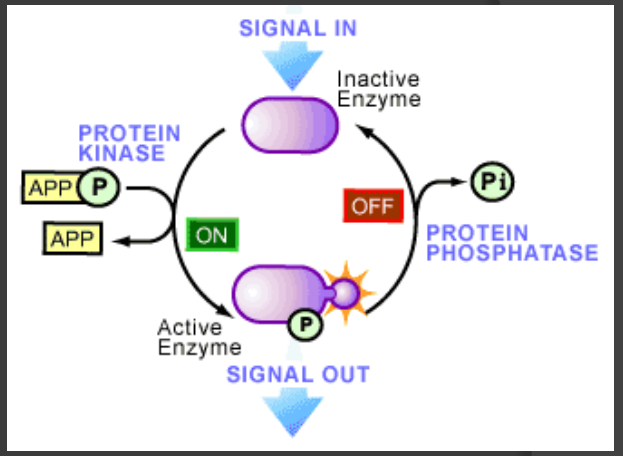
- Conformational change
- Reorientation
- Ordering
- Disordering
- Alternate Binding

# Structural Effect of Phosphorylation

Phosphorylation is an important regulatory mechanism




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

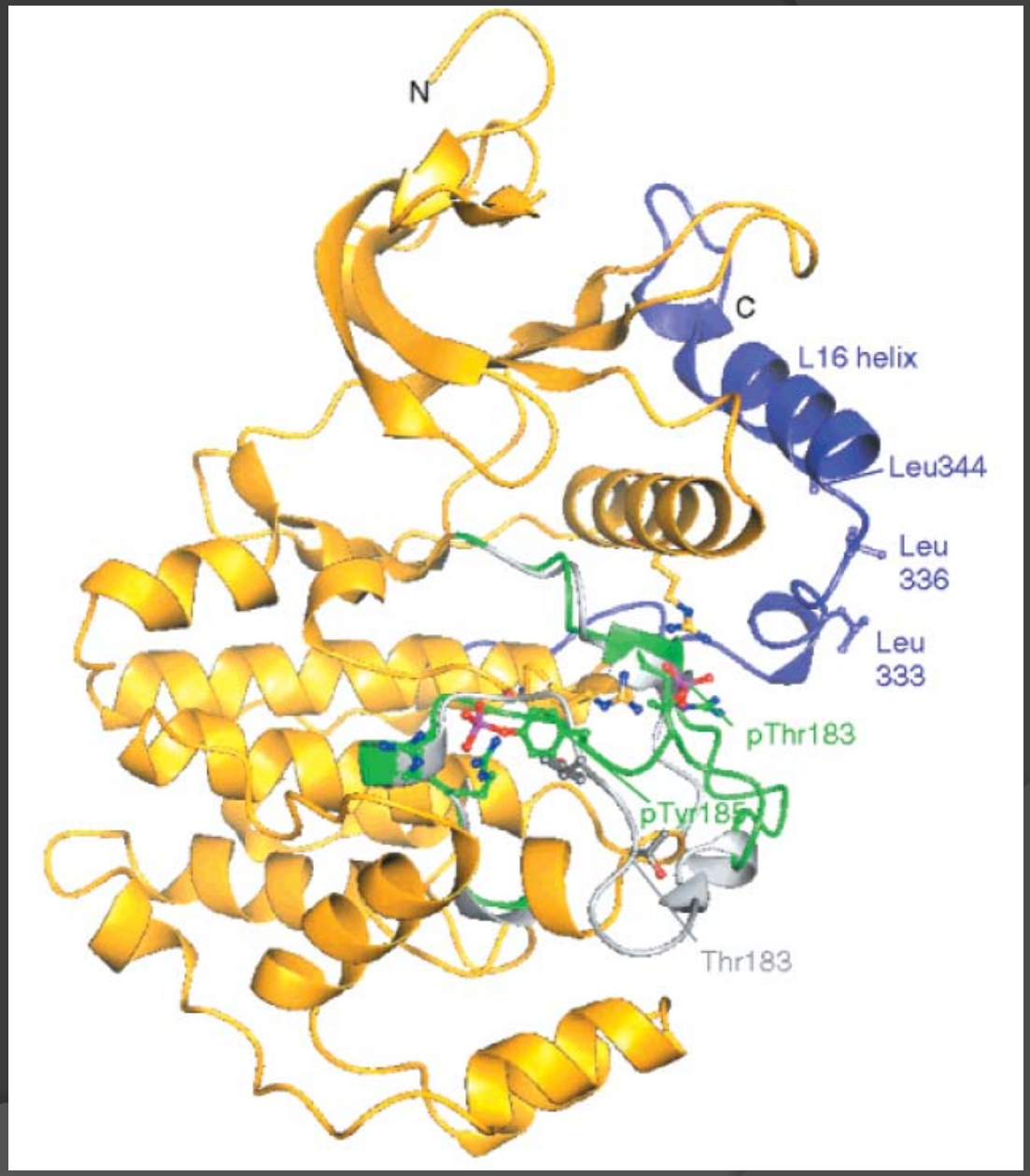



Effect of phosphorylation is manifold

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

# Conformational Change in ERK2

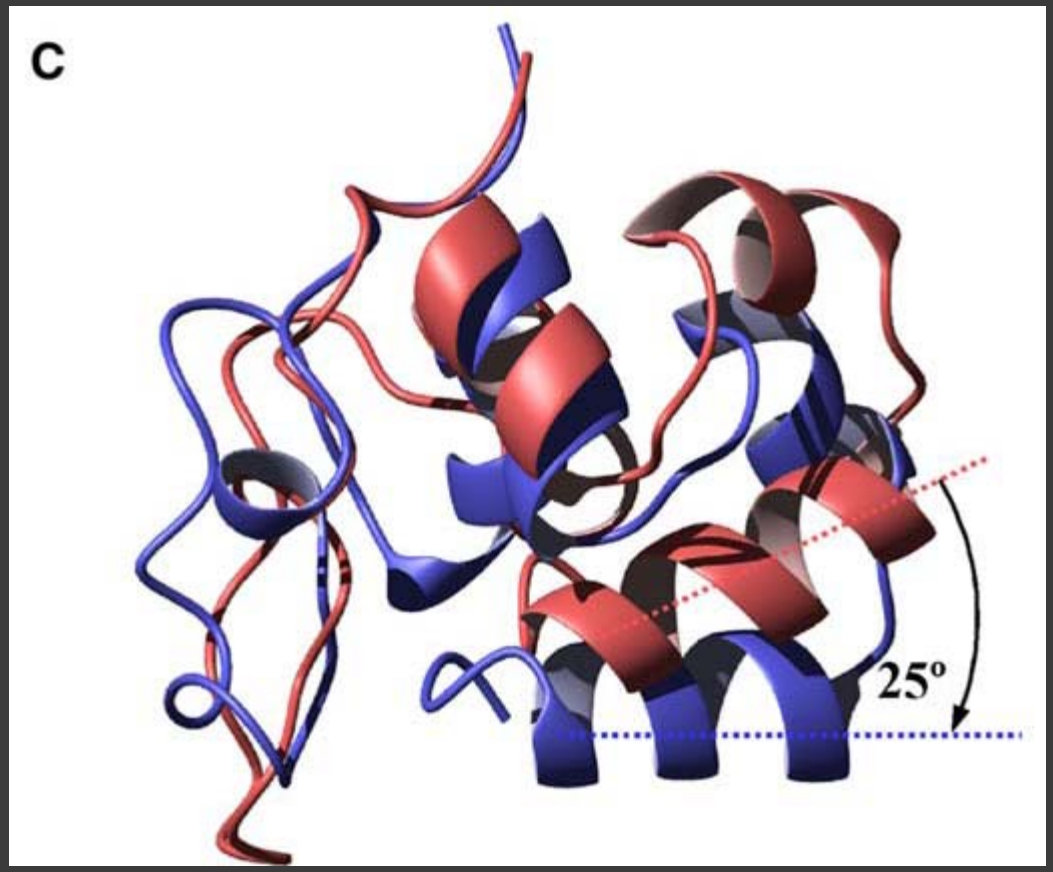
- Grey closed form
  - pTyr185 blocks binding site
- Green open and active form




Johnson and Lewis, Chemical Reviews, 2001, Vol. 101, No. 8

# Reorientation: A conformational switch

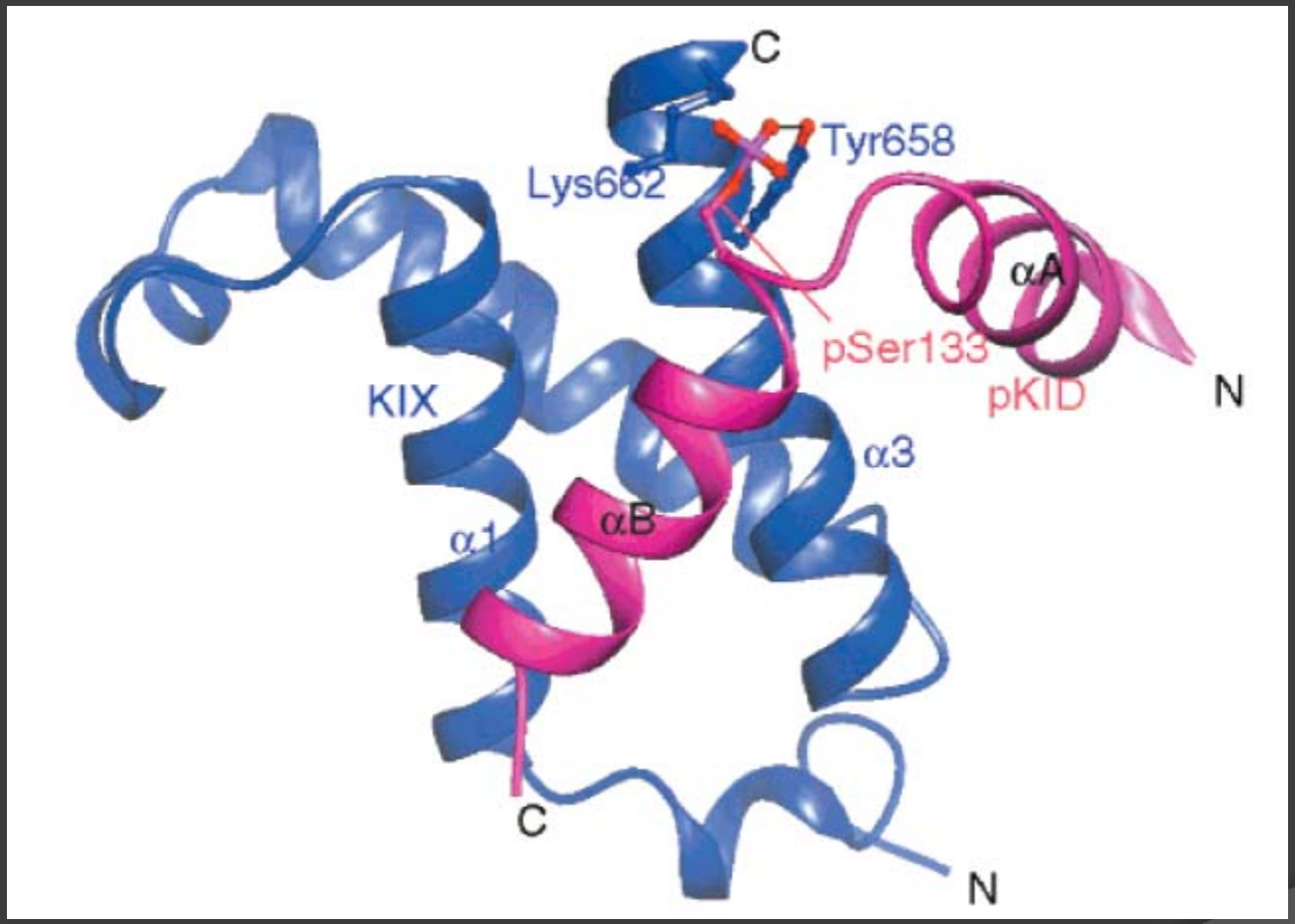
DHP (red) and
DHPs74e (blue)




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

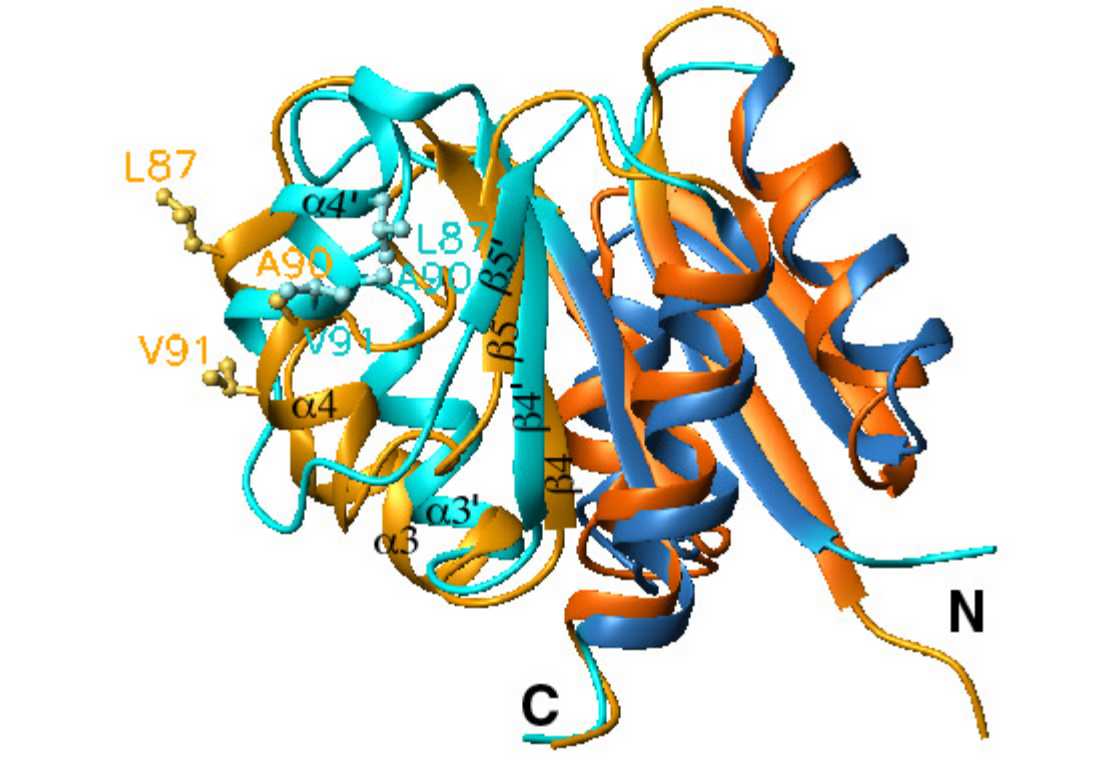
Title: A phosphorylation-induced conformation change in dematin headpiece
Author(s): Jiang ZHG, McKnight CJ
Source: STRUCTURE Volume: 14 Issue: 2 Pages: 379-387 Published: FEB 2006

# Ordering events: KIX and pKID




KIX and phosphorylated pKID

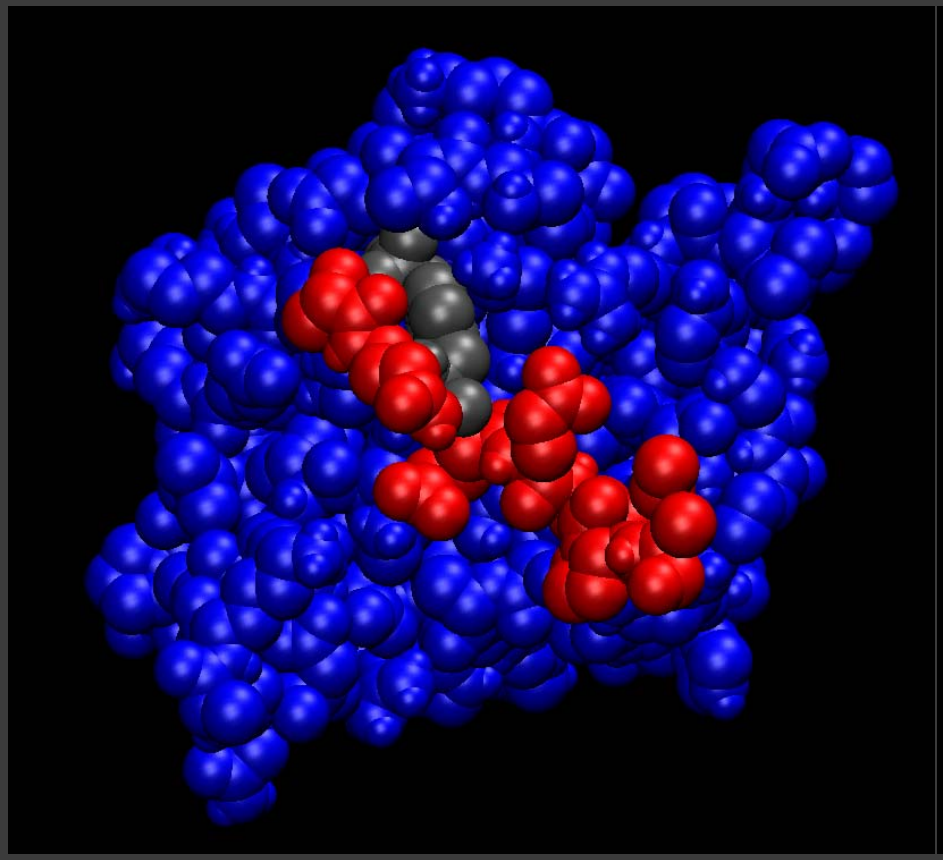
# Disordering: NtrC, a molecular switch upon phosphorylation

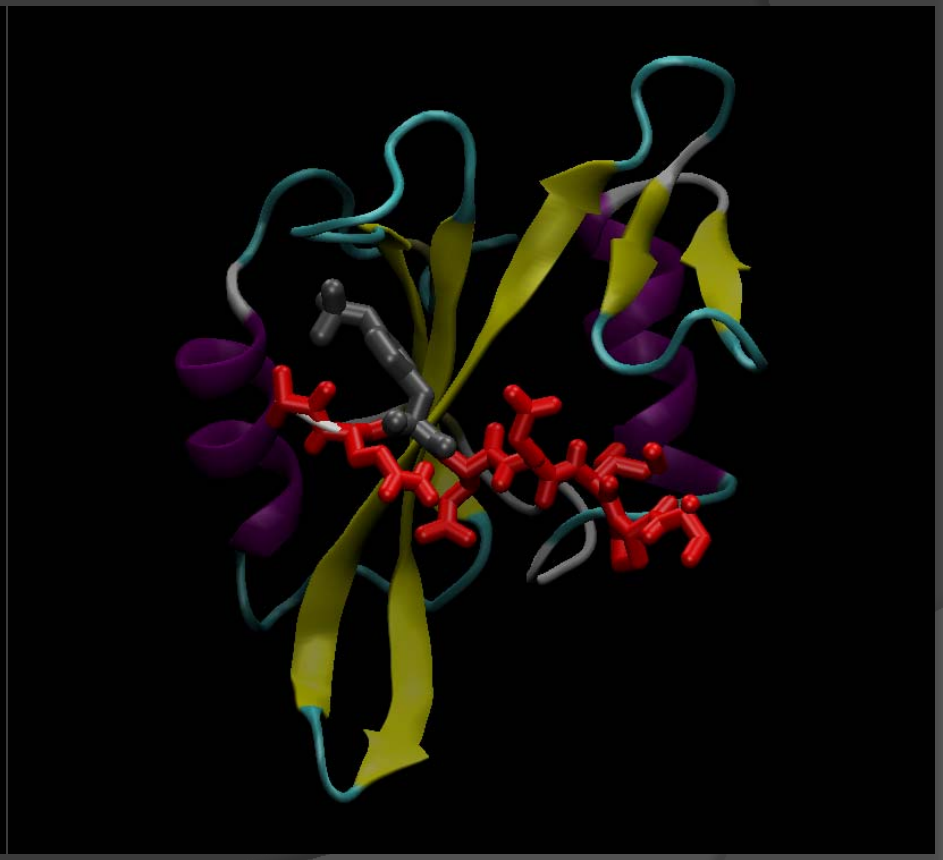



Orange-yellow: unphosphorylated NtrC
blue-cyan: phosphorylated NtrC

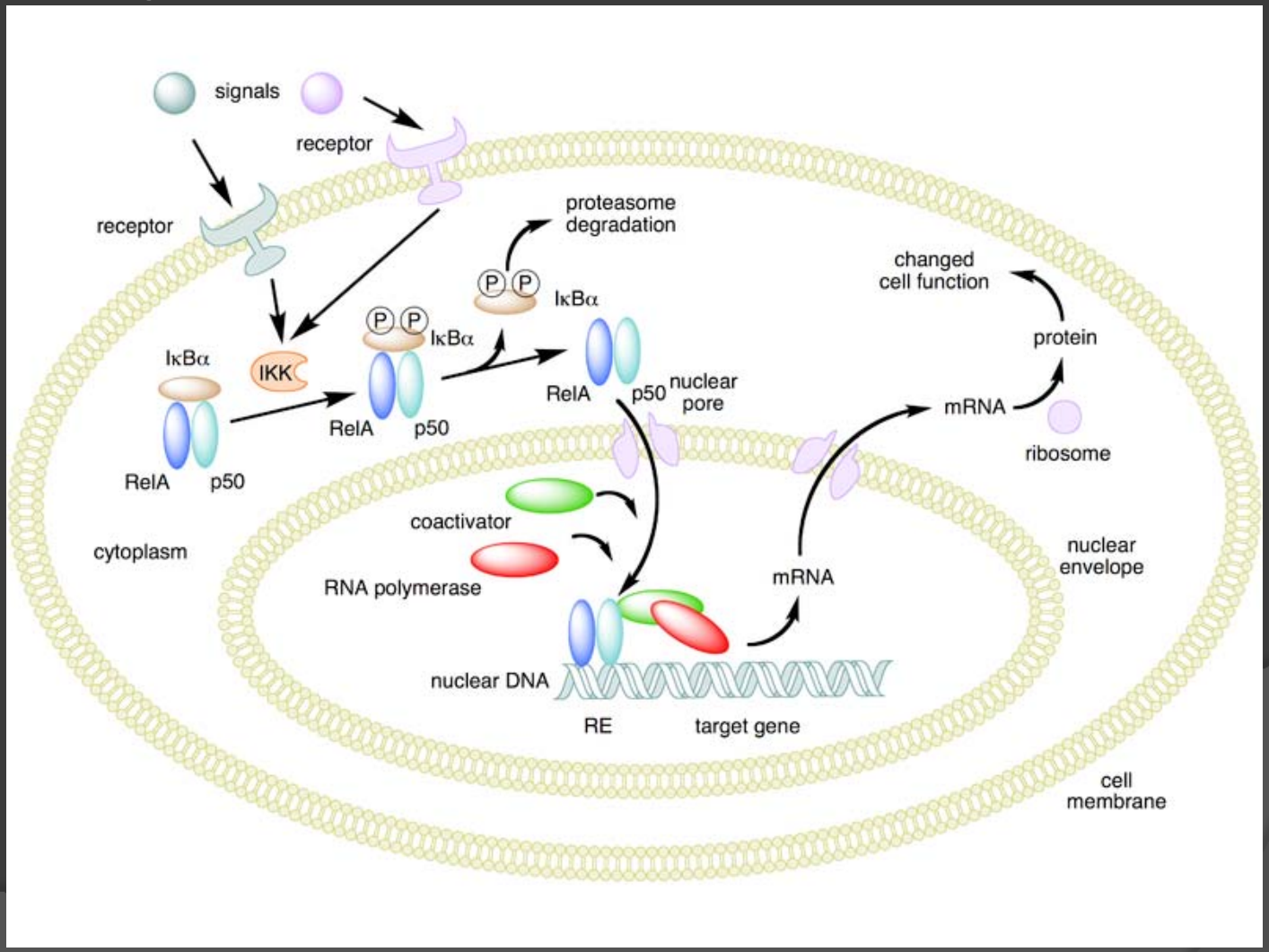
*Volkman et al., Science 2001, 291, 2429-33*

# Alternate Binding: SRC SH2 domain binding



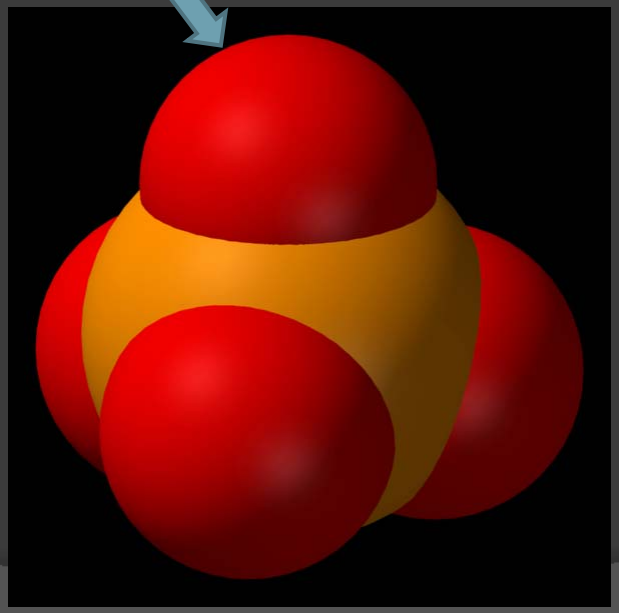


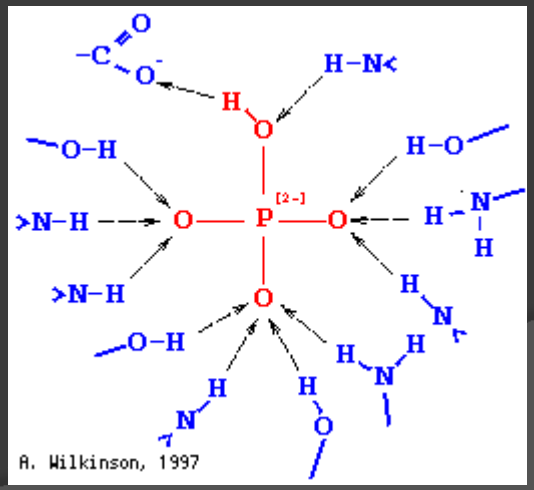
# Phosphorylation leads to unbinding in the NF-KB signaling pathway



# Can we understand (predict) the effect of phosphorylation

- Electrostatics
- Hydrogen bonding
- Size





# Biophysical Methods

- Crystallography
- NMR methods
- Cryoelectronic imaging
- Kinetic and thermodynamic measurements
- Modeling methods

# Early Wisdom on use of computations

*"There is a more general lesson to be drawn from the example of the genetic code. This is that, in biology, some problems are not suitable or not ripe for a theoretical attack for two broad reasons. The first I have already sketched--the current mechanisms may be partly the result of a historic accident. The other is that the "computations" involved may be exceedingly complicated.*

*" . . . These difficulties do not mean we should not look for the broad principles involved (for example….), but it does mean that it may be better to try to go around such problems and not try to tackle them head on at too early a stage."*

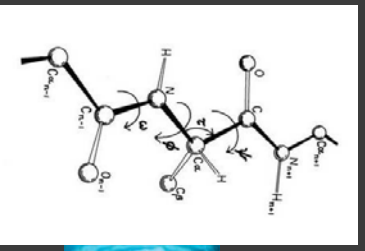
Francis Crick

*"What Mad Pursuit: A Personal View of Scientific Discovery"*

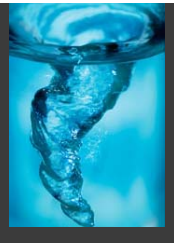
Basic Books, 1988

# Prediction of Phosphorylation Effects

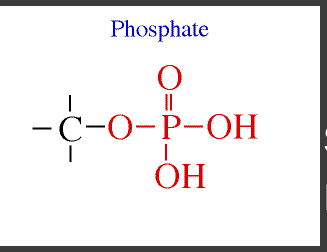
$$H_{AMW} = H_{BB} + H_{AM} + H_{RG} + H_{contact} + H_{water} + H_{burial}$$



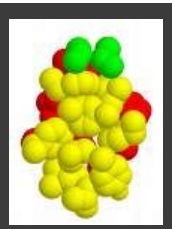
Assure correct polypeptide chemistry



Direct and water-mediated contacts, optimized with strategy based on maximizing $T_F/T_g$ (max Energy gap, min excess ruggedness )

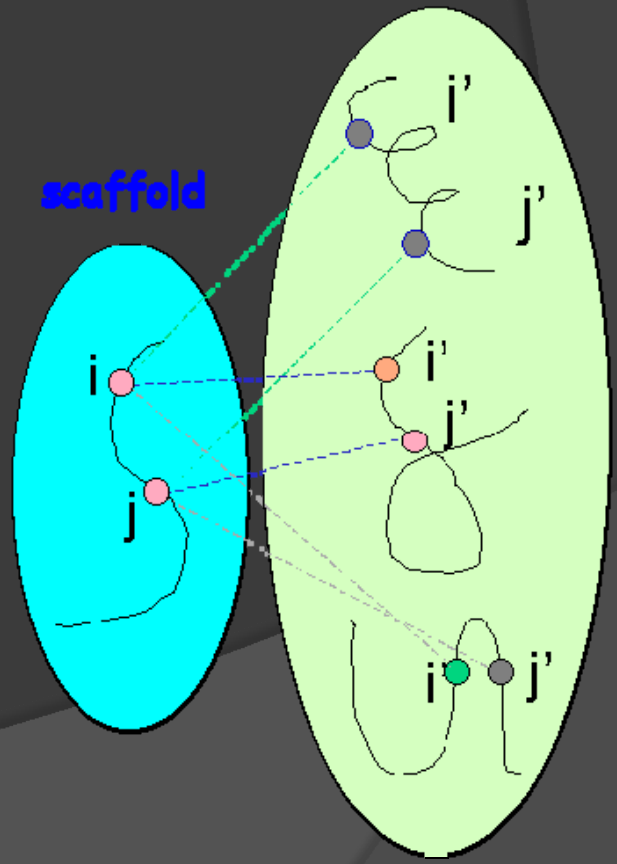



Introduce 21st charge: supercharged glutamic acid to mimic phosphoresidue



$H_{burial}$ gives density preferences for each amino acid

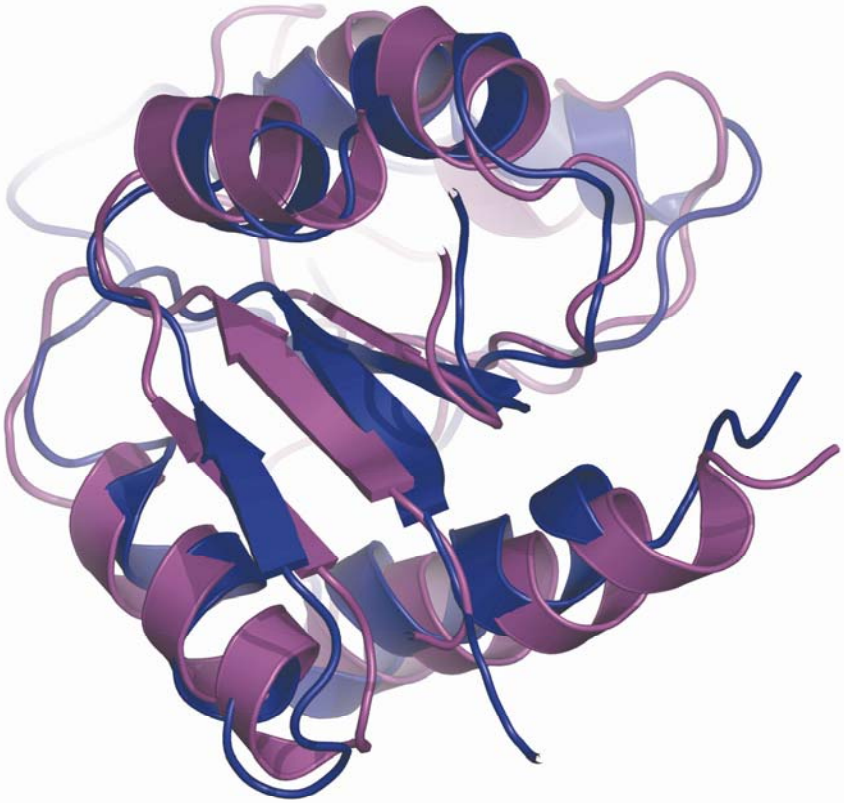
For $H_{AM}$ aligned to X-ray memory proteins




AMW: Papoian et al., PNAS , 2004 ,101(10):3352-7.

# Prediction of $^{p}$NtrC with the Phospho AMH

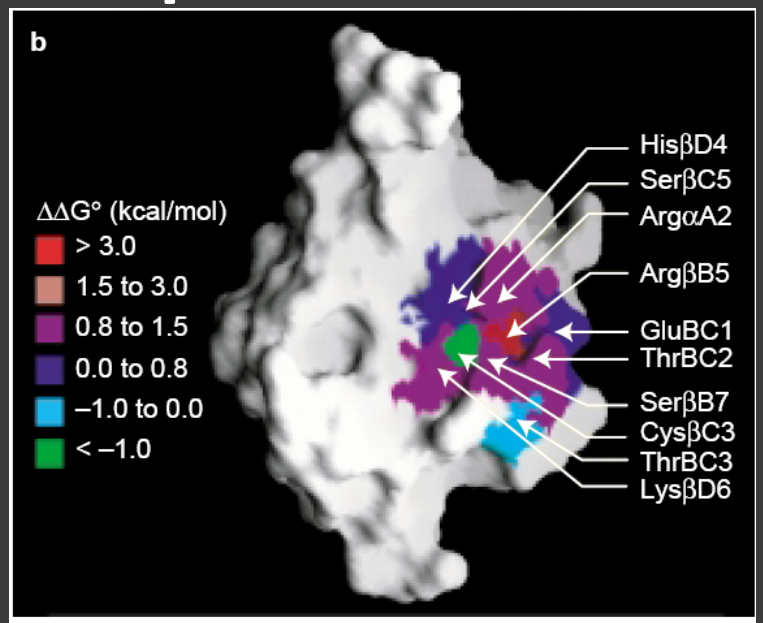
**Rmsd = 2.3Å**
**CE Z-Score = 6.0**

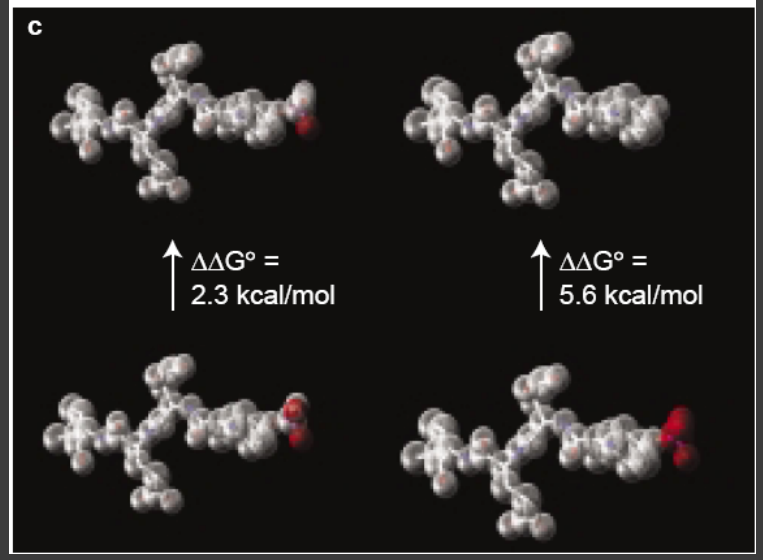


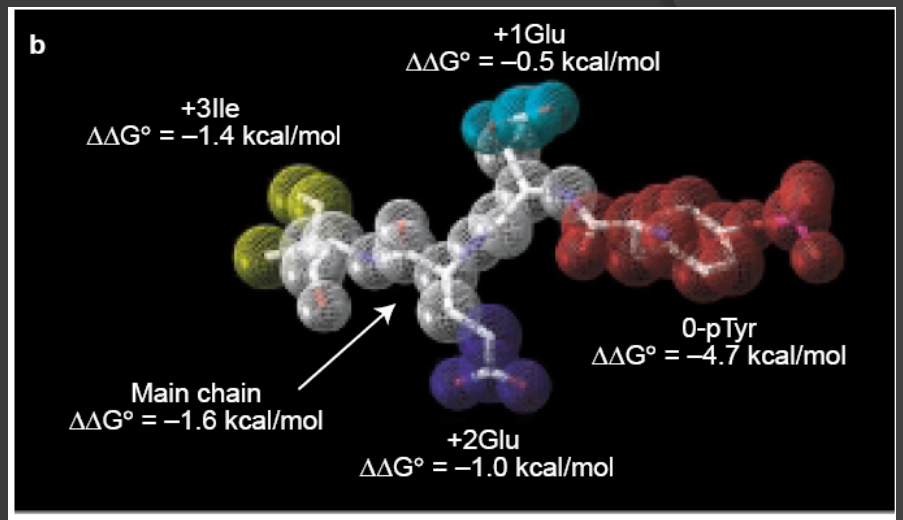
# Example: SH2 domains as drug targets for diseases

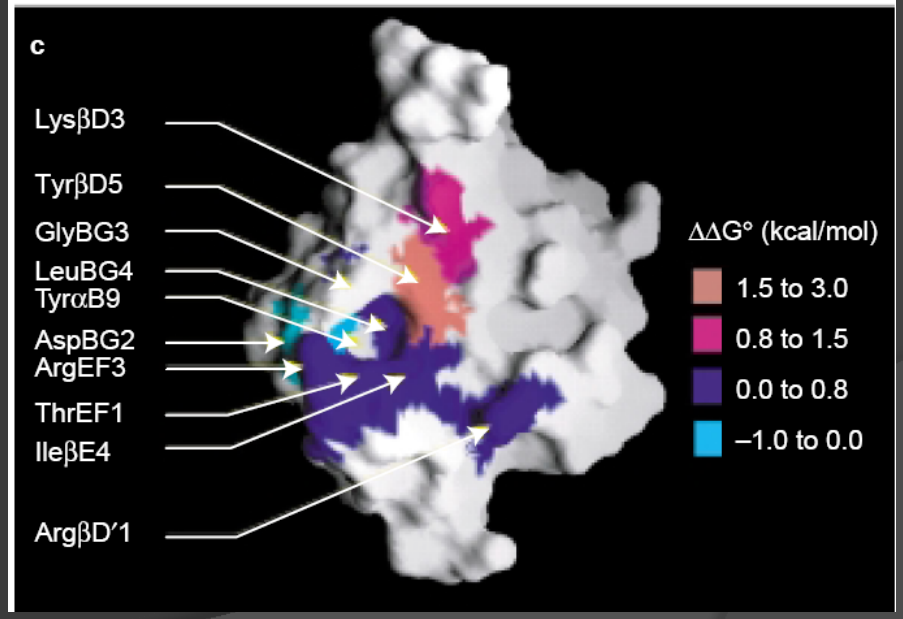
| Disease | SH2 domain-containing target |
|---|---|
| AIDS | Lck, Hck |
| Allergy and asthma | Syk, Lyn |
| Anaemia | SHP-1 |
| Autoimmune disease | ZAP-70 |
| Breast cancer | Grb2, Grb7, Src |
| Cancer | p85, Shc, Grb2, GAP |
| CML and ALL | Grb2, Crkl |
| Erythroleukaemia | Shc |
| Inflammatory disease | STATs |
| Pre-B-cell leukaemia | Btk |
| Myelodysplastic syndrome | Tec |
| Osteoporosis | Src |

# Experimental data




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

# Analogues that bind SH2 domains

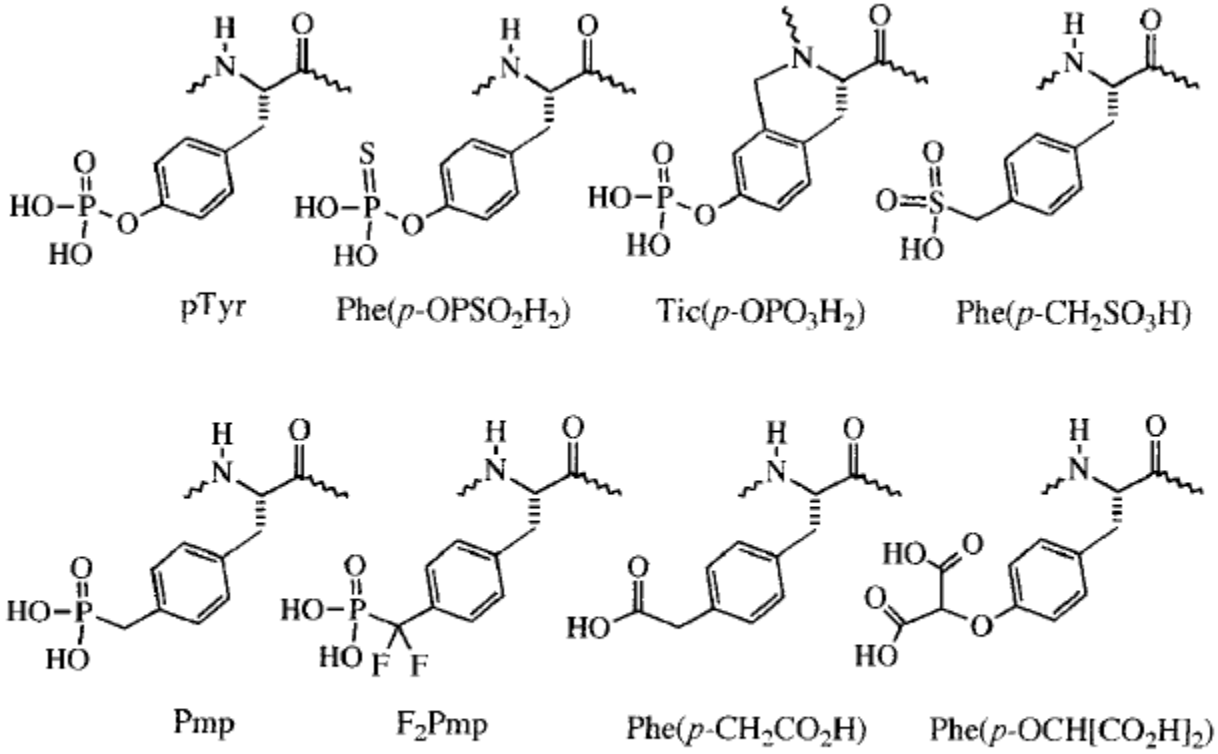



**FIGURE 6** Chemical structures of pTyr and various analogues including phosphatase-resistant bioisosteres of the phosphate moiety, such as phosphonates, sulfonates, and carboxylates.

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

# Binding Data

**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<br>pY pY+1 pY+2 pY+3 | 1.0<br>($IC_{50}$ = 0.7 $\mu M$) |
| **3** | Ac-Tyr-Glu-Glu-Ile-Glu | <0.001 |
| **4** | Ac-Pmp-Glu-Glu-Ile-Glu | 0.025 |
| **5** | Ac-$F_2$Pmp-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<br>($IC_{50}$ = 22 $\mu M$) |

# Prediction with an AA force field

## The AMBER force field

$$V(r^N) = \sum_{\text{bonds}} \frac{1}{2} k_b (l - l_0)^2 + \sum_{\text{angles}} k_a (\theta - \theta_0)^2 + \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \left\{ \epsilon_{i,j} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - 2 \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} \right\} + \sum_{\text{torsions}} \frac{1}{2} V_n [1 + \cos(n\omega - \gamma)]$$

## A sequence with unknown structure

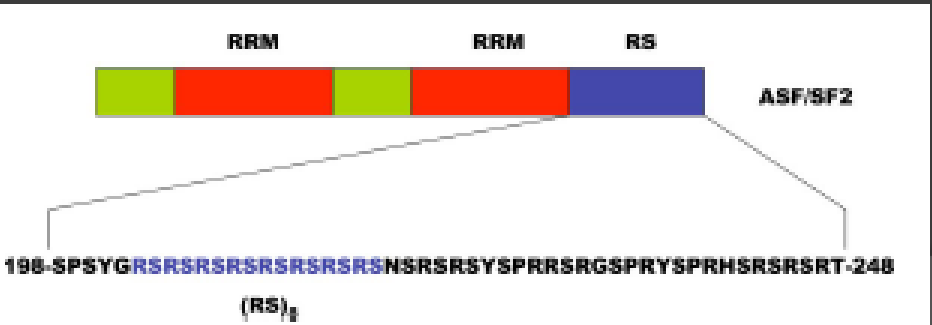
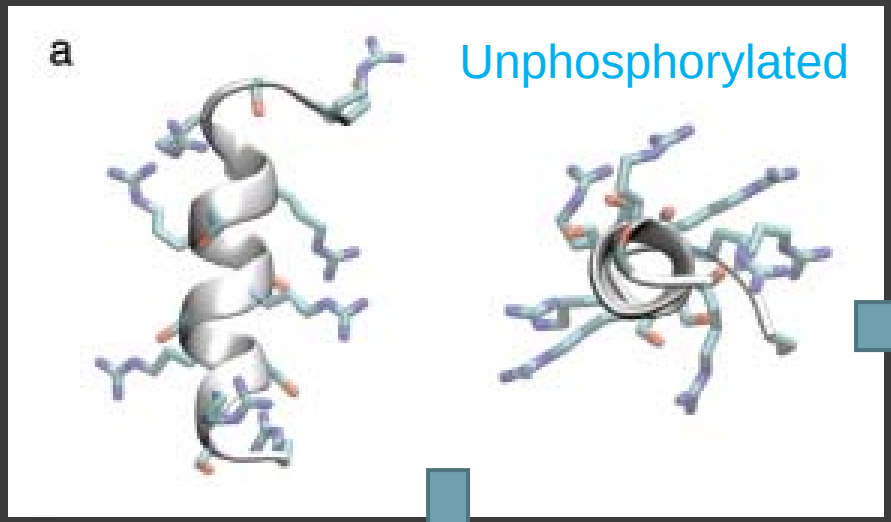
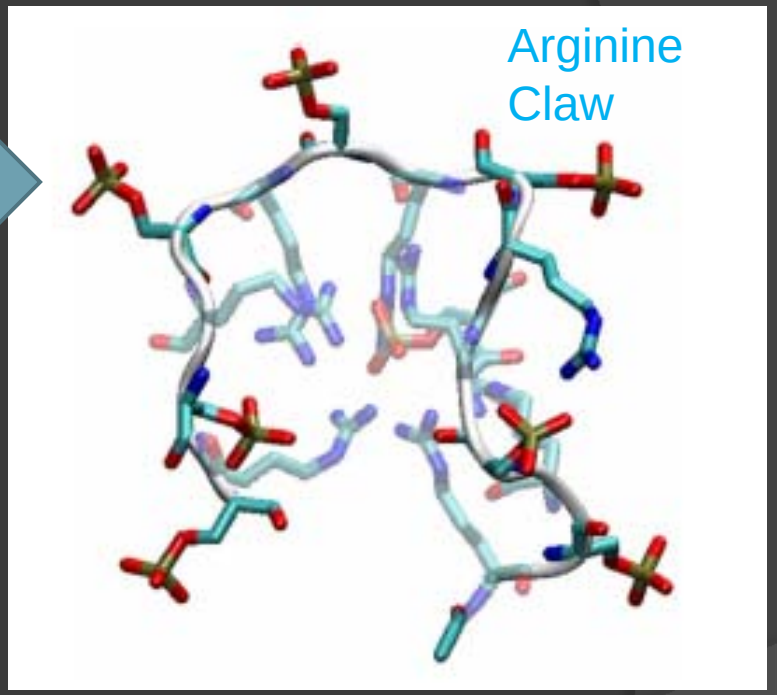
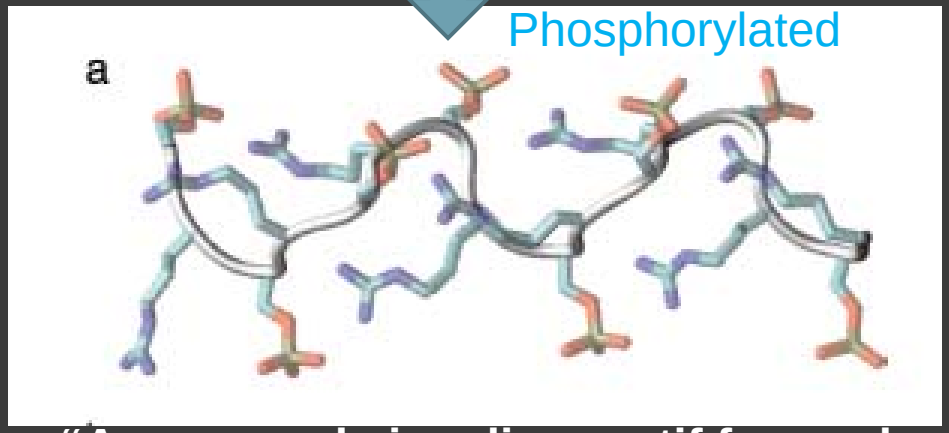



Fig. 1. A cartoon view of the sequence of ASF/SF2 with the RS domain shown in detail.

# Results from MD simulations




**"A proposed signaling motif for nuclear import in mRNA processing via the formation of arginine claw", Hamelberg et al, PNAS 2007, 104:14947-14951**