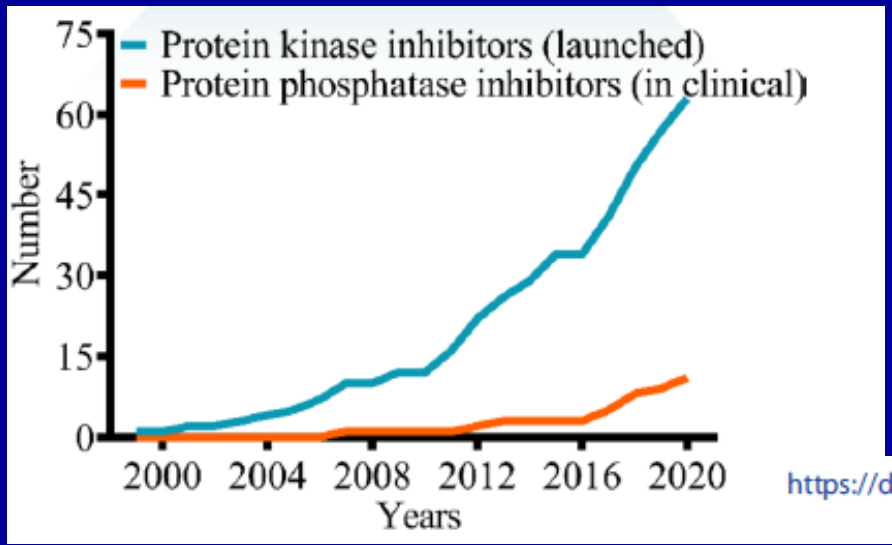
(Protein Kinases)

vs

Protein Phosphatases

-ancient enzymes essential to cell signaling and cellular regulation

-new targets for Pharmaceuticals



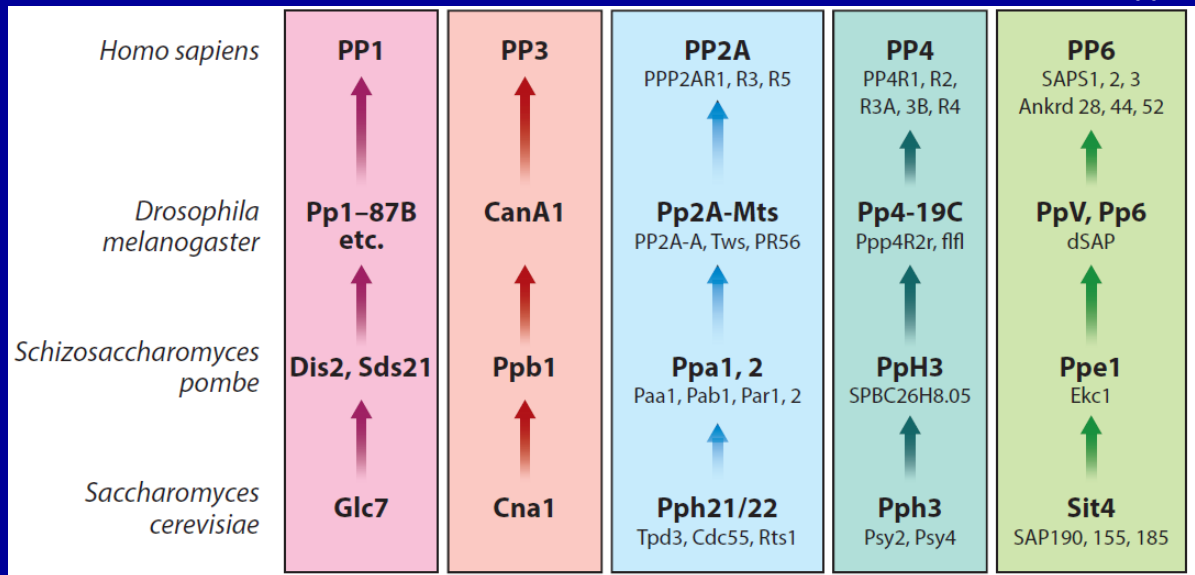
https://doi.org/10.1021/acs.jmedchem.1c00631
J. Med. Chem. 2021, 64, 8916−8938

Protein Phosphatases (PPPs) are highly conserved through evolution among of all enzymes...

Proteins	unit of evolution (millions of yrs for 1% change)
Histone H4	400
Calmodulin	350
Histone H3	330
PP-2Aα	100
PP-1α	88
PP-2β	66
Histone H-2A, H-2B	60
GDH	55
Tubulin	40
PKA	39
Collagen	36
Gαi	32
K+ channel	22

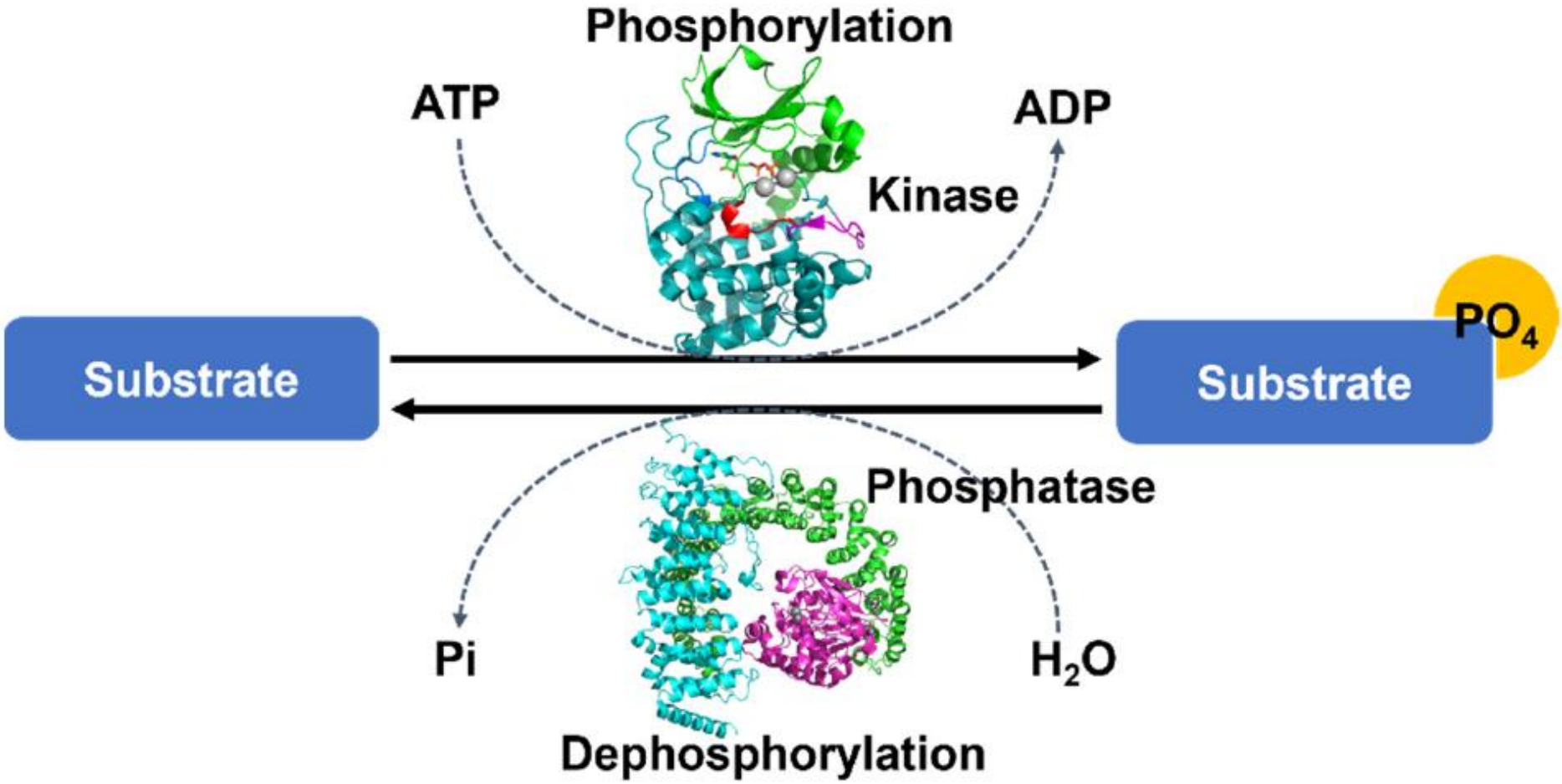
From H.C.-Li 2001

PPPs of all three families are present from yeast to plants and mammals.
And in many cases, also in prokaryotes (bacteria and archaebacteria).



Protein Phosphorylation

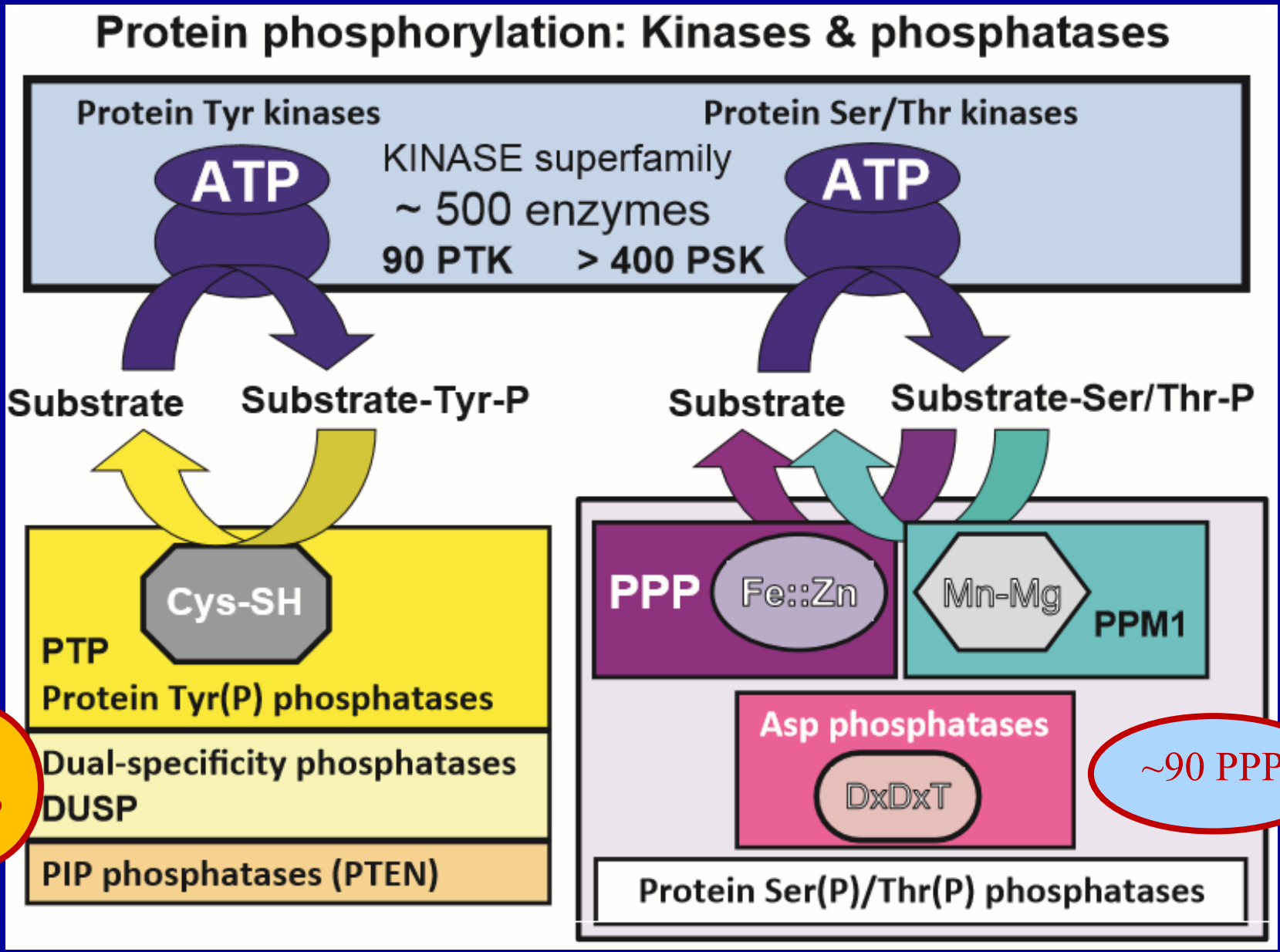
rapid and reversible biochemical reactions



A molecular on/off switching mechanism

"Writer & Eraser"

Protein Phosphorylation: Kinases & Phosphatases

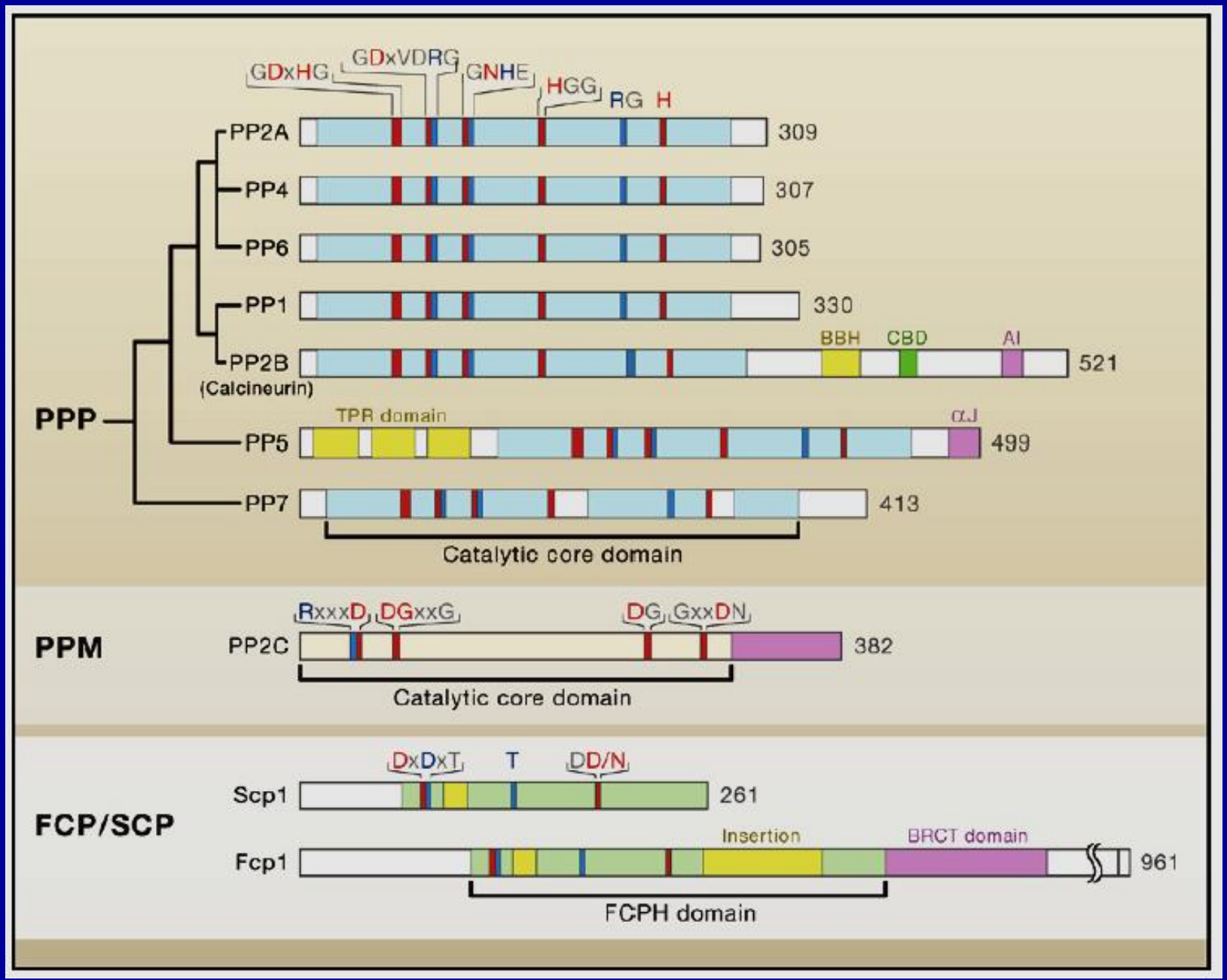


Protein Ser/Thr Phosphatases

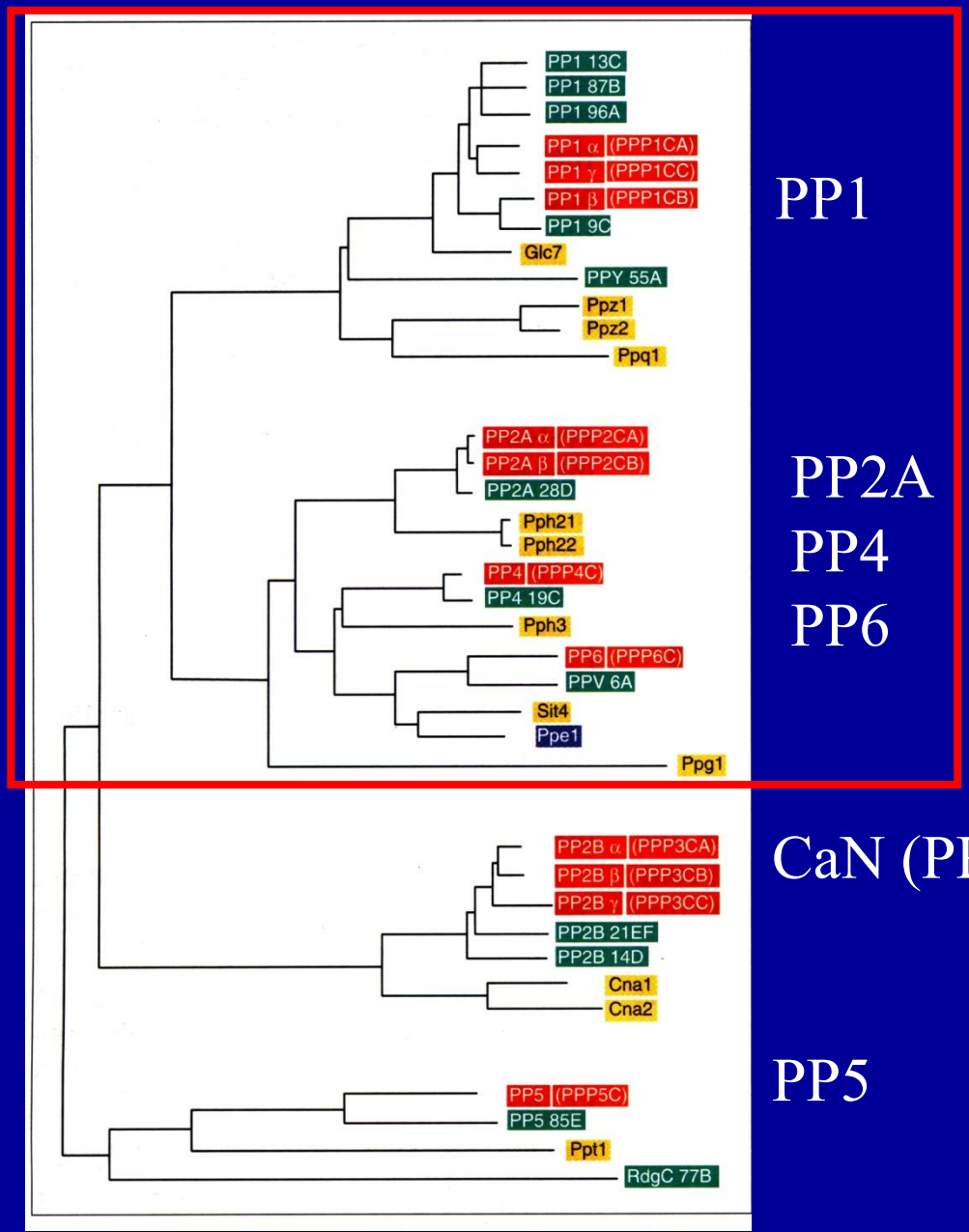
Phosphoprotein phosphatases

(PPP family)

Three families of Ser/Thr Protein Phosphatases



The PPP Family of Protein Ser/Thr Phosphatases



PP1

PP2A

PP4

PP6

CaN (PP2B)

PP5

Toxin-sensitive PPP

Red = human
Green = Drosophila
Yellow = yeast

Inhibiting PPPs

PP-1 and PP-2A are inhibited by **okadaic acid** (shellfish toxin) and **microcystin** (cyclic peptides produced by cyanobacteria which are potent hepatotoxins).

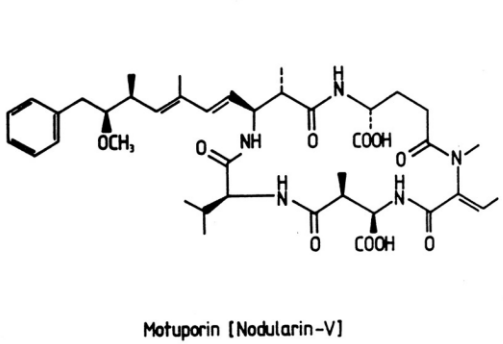
Also PP-4, PP-5 and PP-6 are inhibited, while PP-2B is inhibited by higher (mM) concentrations.

PP-2B is a target of **cyclosporin A** and **FK506** (immunosuppressants)

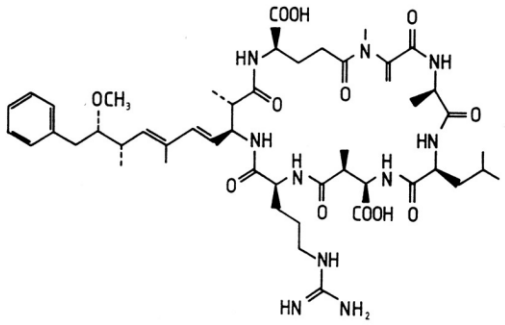
Cyclosporin A (CsA) is a lipid soluble fungal undecapeptide (Mr=1,203) widely used in transplantation for graft rejection; functions as blocker of T cell activation or proliferation. CsA binds cyclophilin (CyPA) and this complex binds B subunit of calcineurin in presence of calcium/calmodulin to inhibit PP activity.

FK506 is a bacterial (Streptomyces) product, a macrocyclic lactone structurally unrelated to cyclophilin that complexes with FKB binding protein (FKBP12 from the TGF-β receptors signaling) to inhibit calcineurin PP activity.

Natural Toxins from Diverse Sources Bind and Inhibit PPP Protein Phosphatases

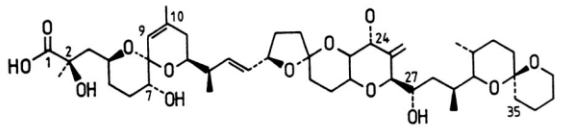


Motuporin [Nodularin-V]



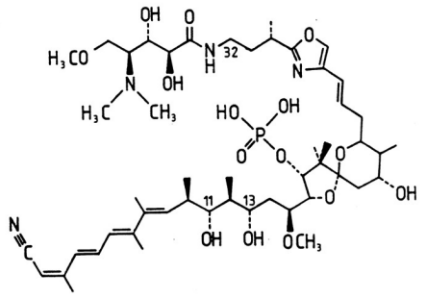
Microcystin-LR

Blue-green Algae



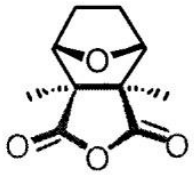
Okadaic acid

Dinoflagellates
Prorocentrum lima

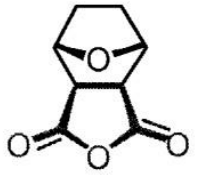


Calyculin A

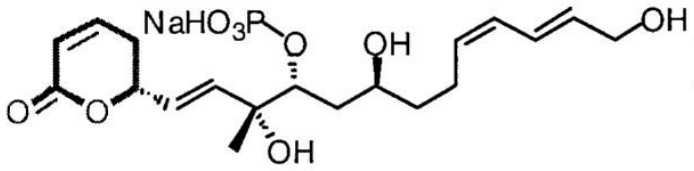
Blister beetle *Coleoptera*



Cantharidin



Norcantharidin

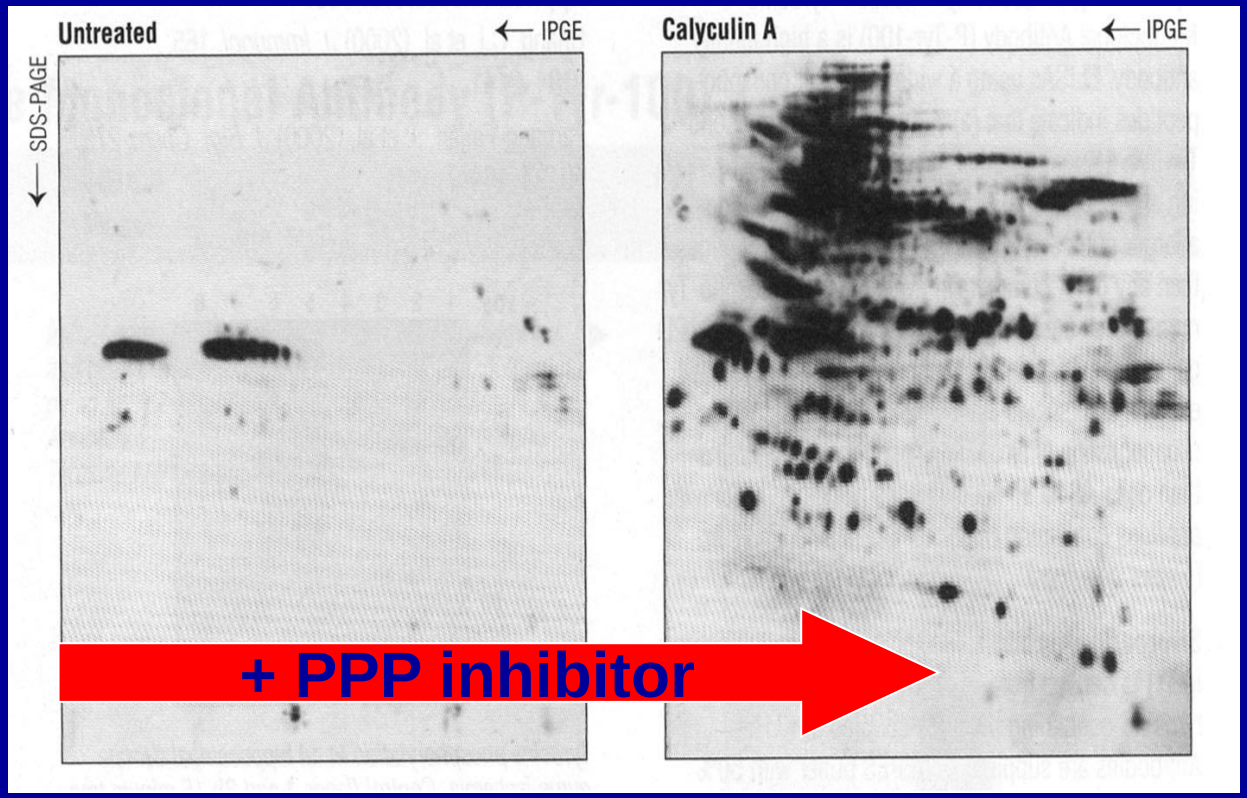


Fostriecin

Streptomyces (fostreus)

Protein Ser/Thr Phosphatases Are Dominant over Protein Kinases

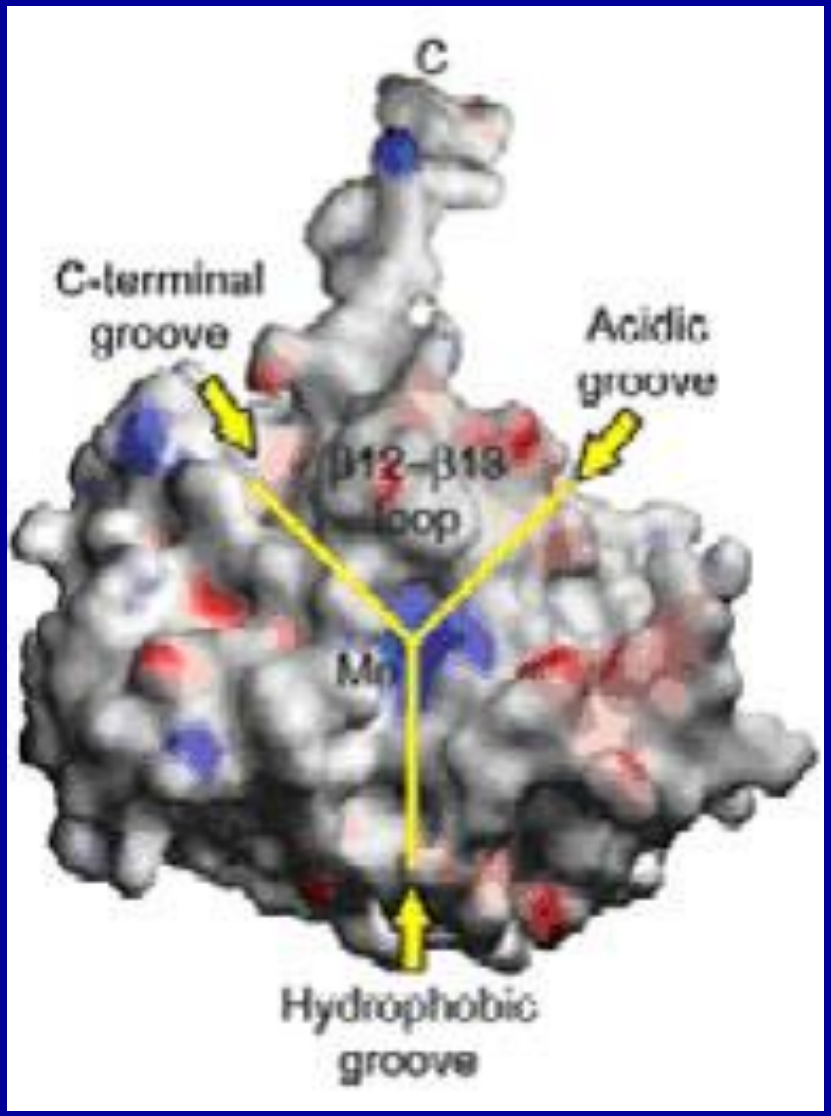
(*most proteins are **maintained** in a dephosphorylated state!*)

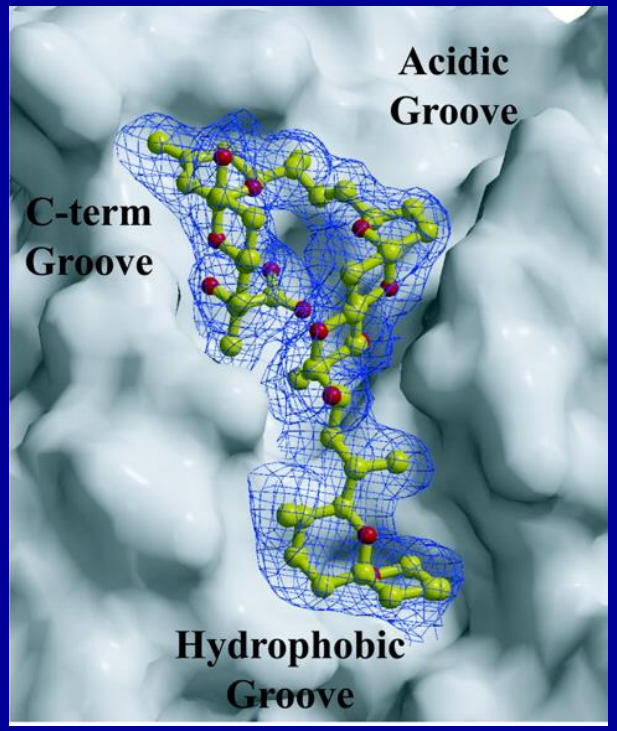


Cell Signaling Technology 2002 catalogue (pg. 15): Western blot analysis of whole cell lysates of Jurkat cells,.untreated with 0.1μM calyculin A for 20 minutes prior to lysis, using Phospho-Thr antibody.

Catalytic Subunit of Protein Phosphatase-1 (PP1)

PP1, the most widely expressed protein Ser/Thr phosphatase that is responsible formore than 50% of all dephosphorylation reactions in humans…

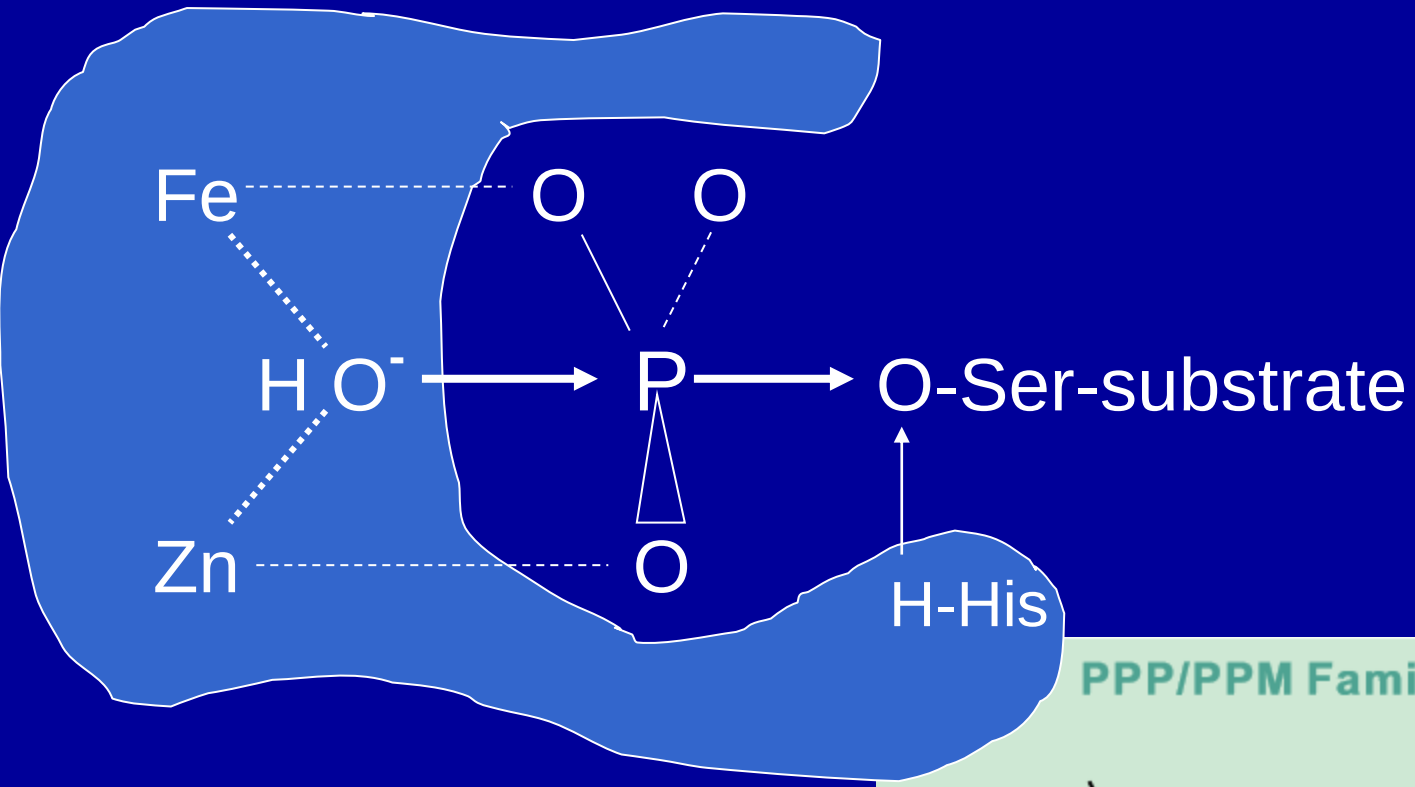


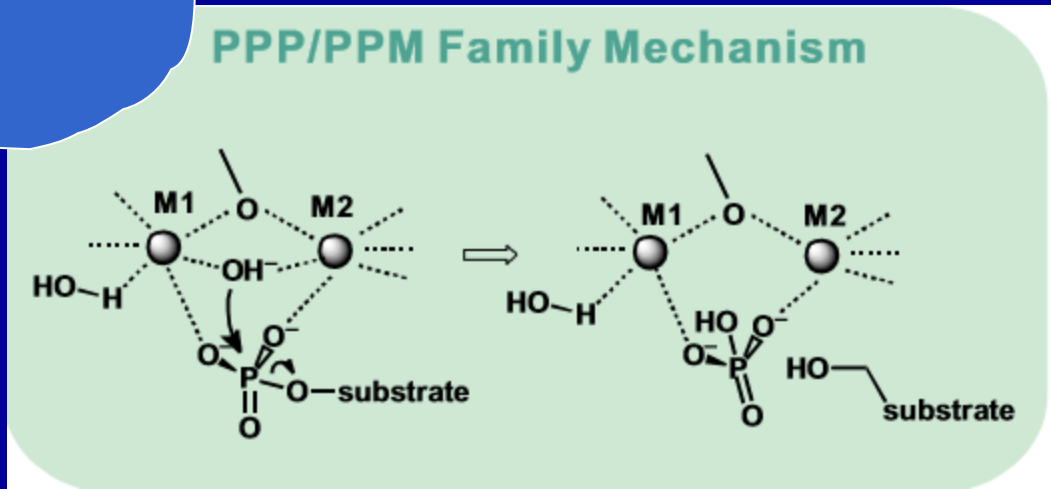


Okadaic Acid Binds at the Active Site of PPP Protein Phosphatases

Mechanism of Phospho-Ester Hydrolysis by PPP Phosphatases:

in-line attack of metal-activated hydroxide, with trigonal bipyramid intermediate and inversion of stereochemistry protonation of the leaving alcoholic group by the His of the active site.





Type-1 Protein Phosphatase (PP1)

1. Bi-metallic active site with Fe and Zn

2. 3D structure - beta sheet and alpha helix clusters

3. Isoforms α, $\gamma 1$, $\gamma 2$, δ
   differences mostly in C terminal, allow specific antibodies

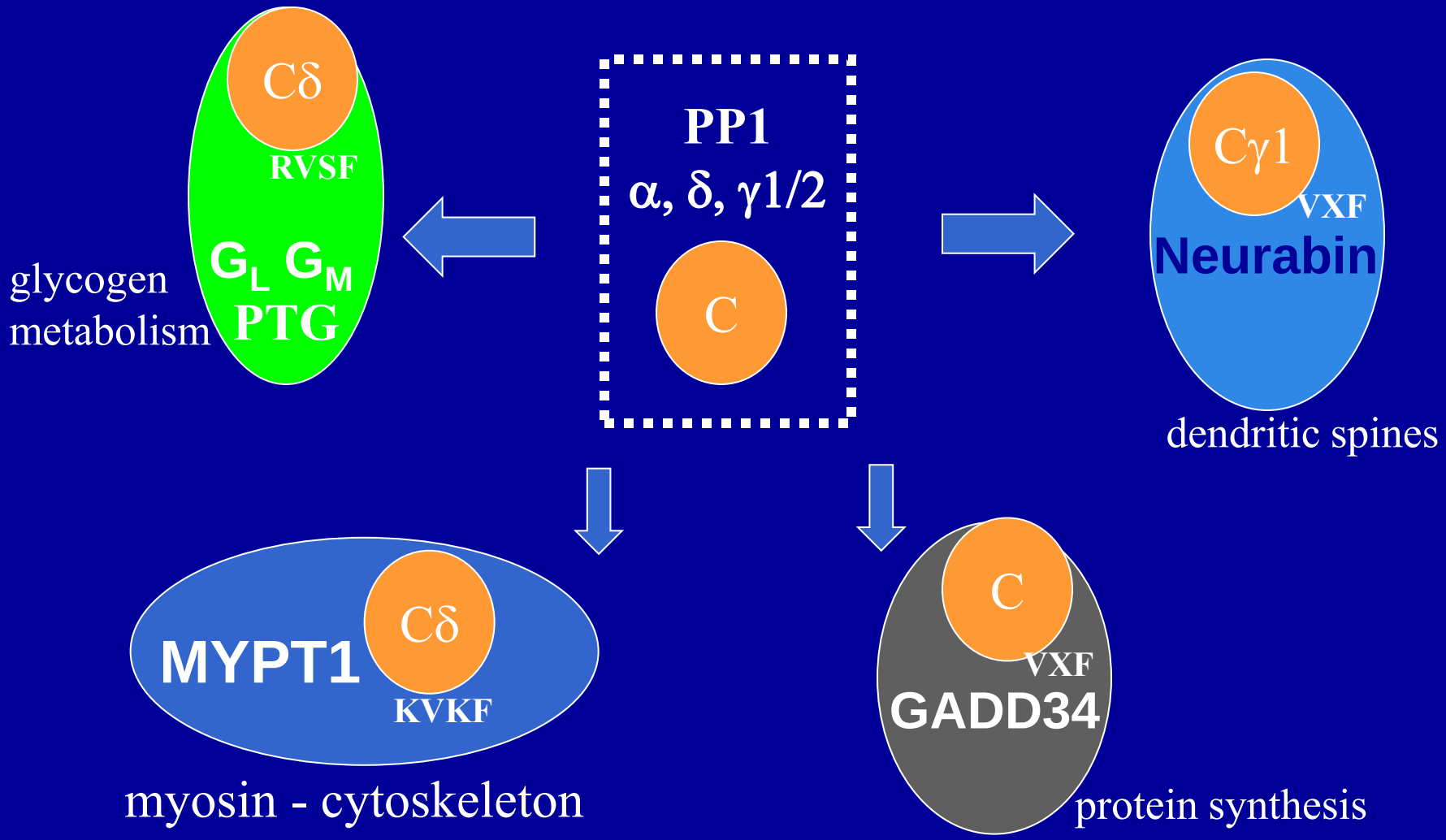
alpha	NPGG**RPITPP**RN--SAKAKK
gamma	--AT**RPVTPPR**GMITKQAKK
delta	NSG-**RPVTPPR**TANPPK-KR

4. > 200 regulatory subunits

5. Toxins - microcystin, okadaic acid, calyculin A bind at active site (3D structures)

Protein Ser(P)/Thr(P) Phosphatase - PP1

many different regulatory-targeting subunits
complex with common catalytic subunit



Type-2A Protein phosphatase (PP2A)

Catalytic subunit

1. Bimetal center Fe::Zn and catalytic mechanism same as PP1

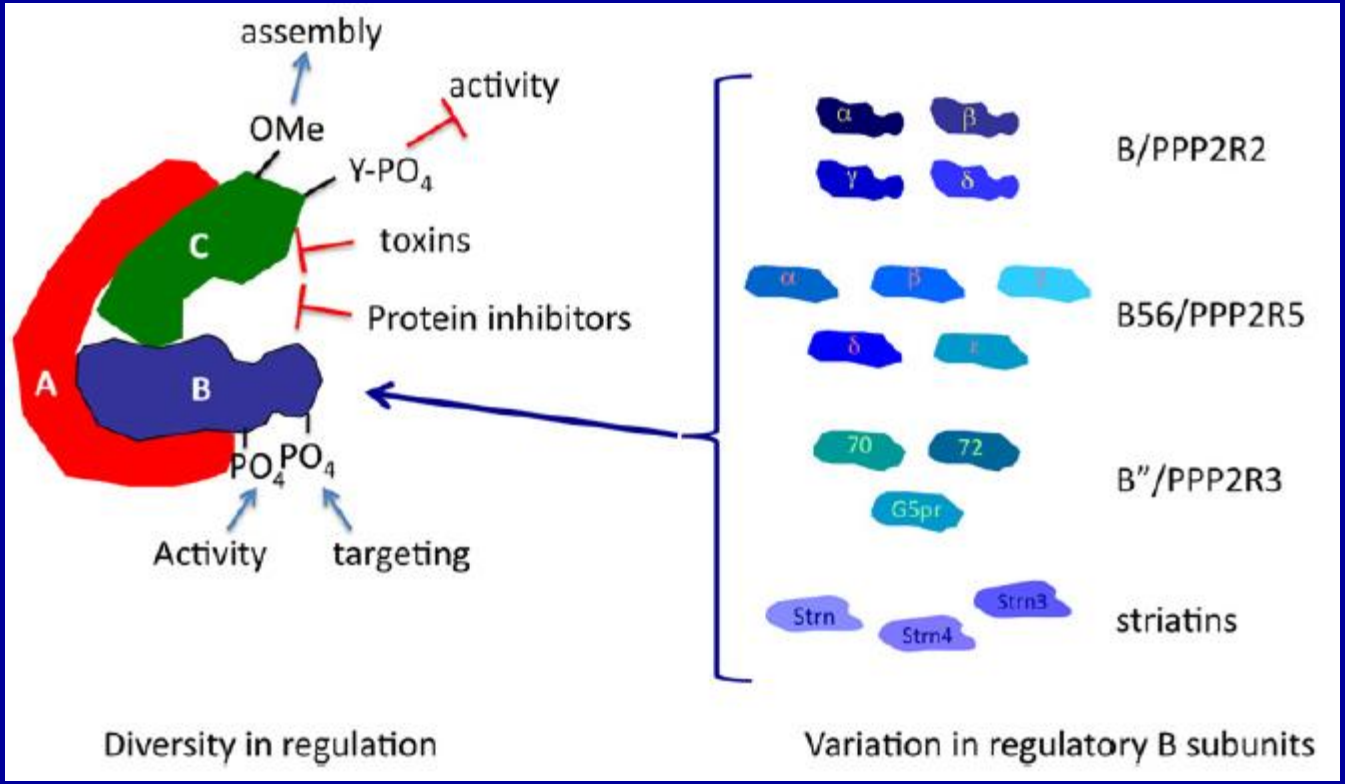
2. 3D structure…known in complex with A and in ABC

3. Isoforms α, β 10:1 ratio, essential for development

4. **DYFL**$_{COOH}$ motif at C terminus conserved
   phosphorylation - PTKs, eg. Src, JAK
   methylation - PMT and PME, alters subunit association

5. Toxins – MCLR and OA bind at active site.
   Differences between PP1 and PP2A in β12-β13 loop
   =>differences in inhibitory doses

Protein phosphatase 2A describes a panoply of phosphatases



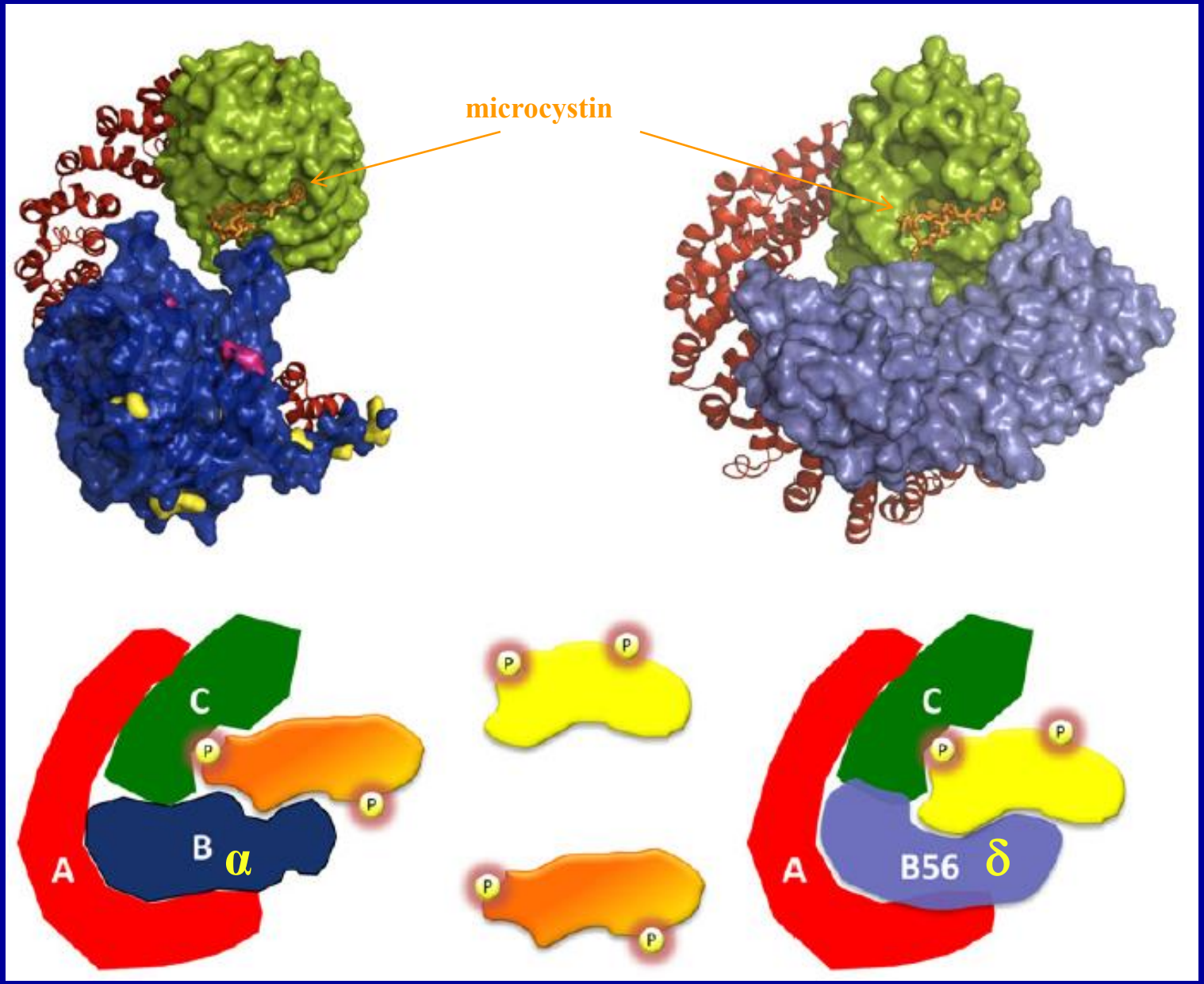
The common heterotrimeric form of PP2A contains a pair of the catalytic subunit (C), the structural A/PR65 subunit and a regulatory/targeting B subunit (at least 15 distinct B subunits are known).

Various cellular and viral proteins interact with PP2A components as indicated…

Virshup, DM (2000) Current Opinion in Cell Biol 12:180-185

PP2A can be > 80 different "enzymes"

The Structural Basis of Substrate Recognition by PP2A



PP2C=Mn^{2+}/Mg^{2+}-dependent PPase (PPM)

A. Catalytic subunit
   1. unrelated to PPP but bimetallic Mg:Mn active center
   2. isoforms α, $\beta 1$, $\beta 2$, etc.
   3. many new family members in plants genome

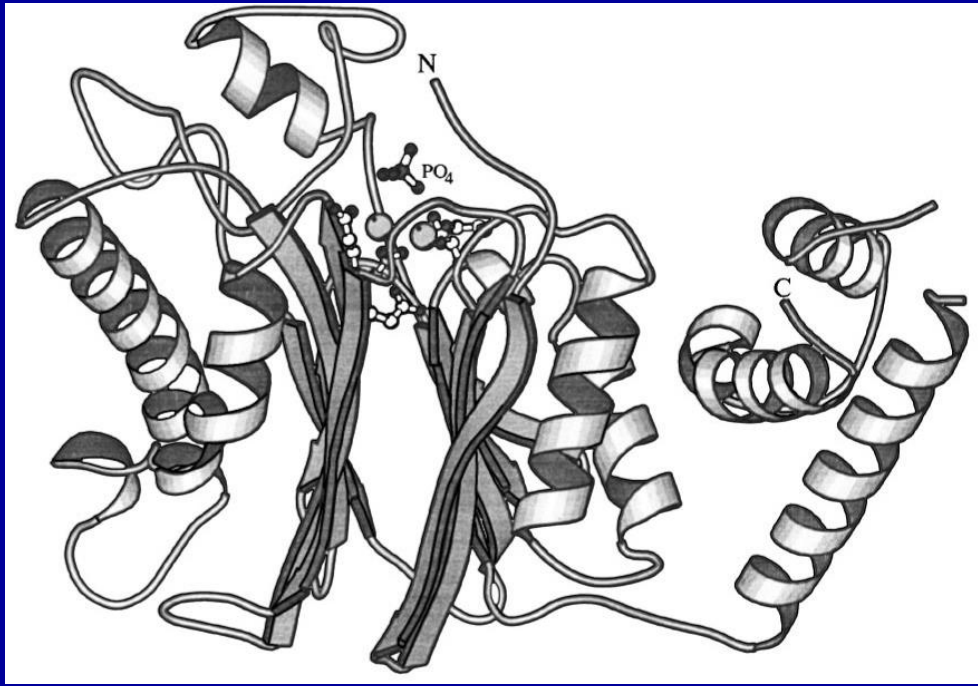
B. Regulatory subunits - none?

C. Inhibitor Proteins - none?

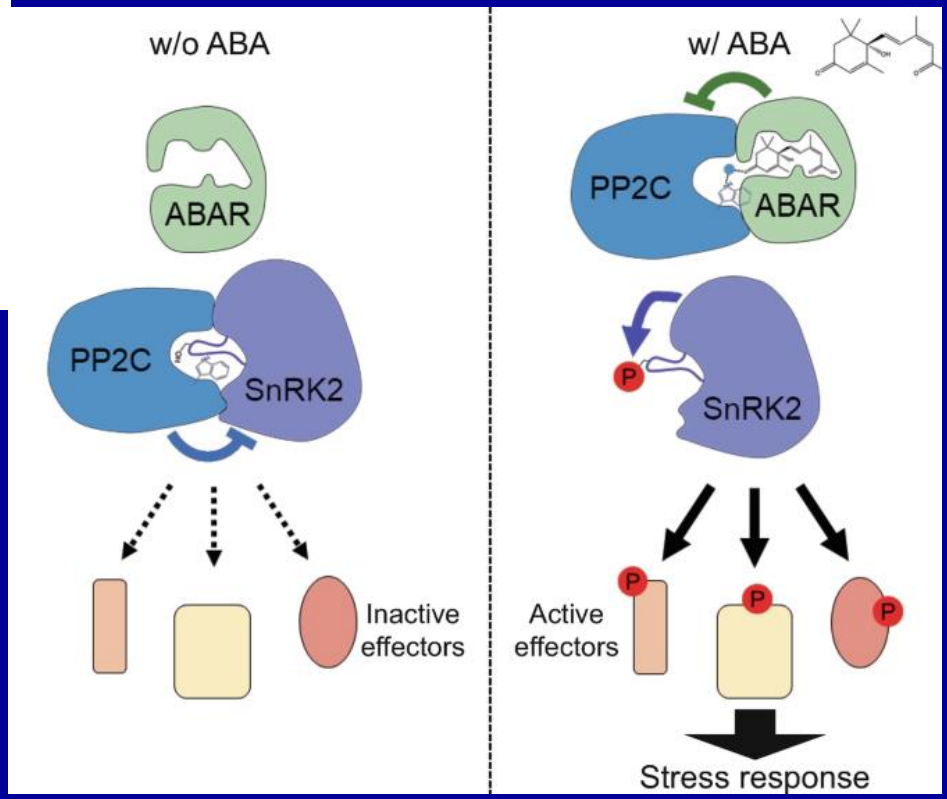
D. Substrates
   1. CDKs
   2. the kinase activation loop
   3. PI3K
   4. Glycogen synthase

   …others

Human Protein Phosphatase 2C
Metal (Mn^{2+})-dependent Phosphatase (MPP)

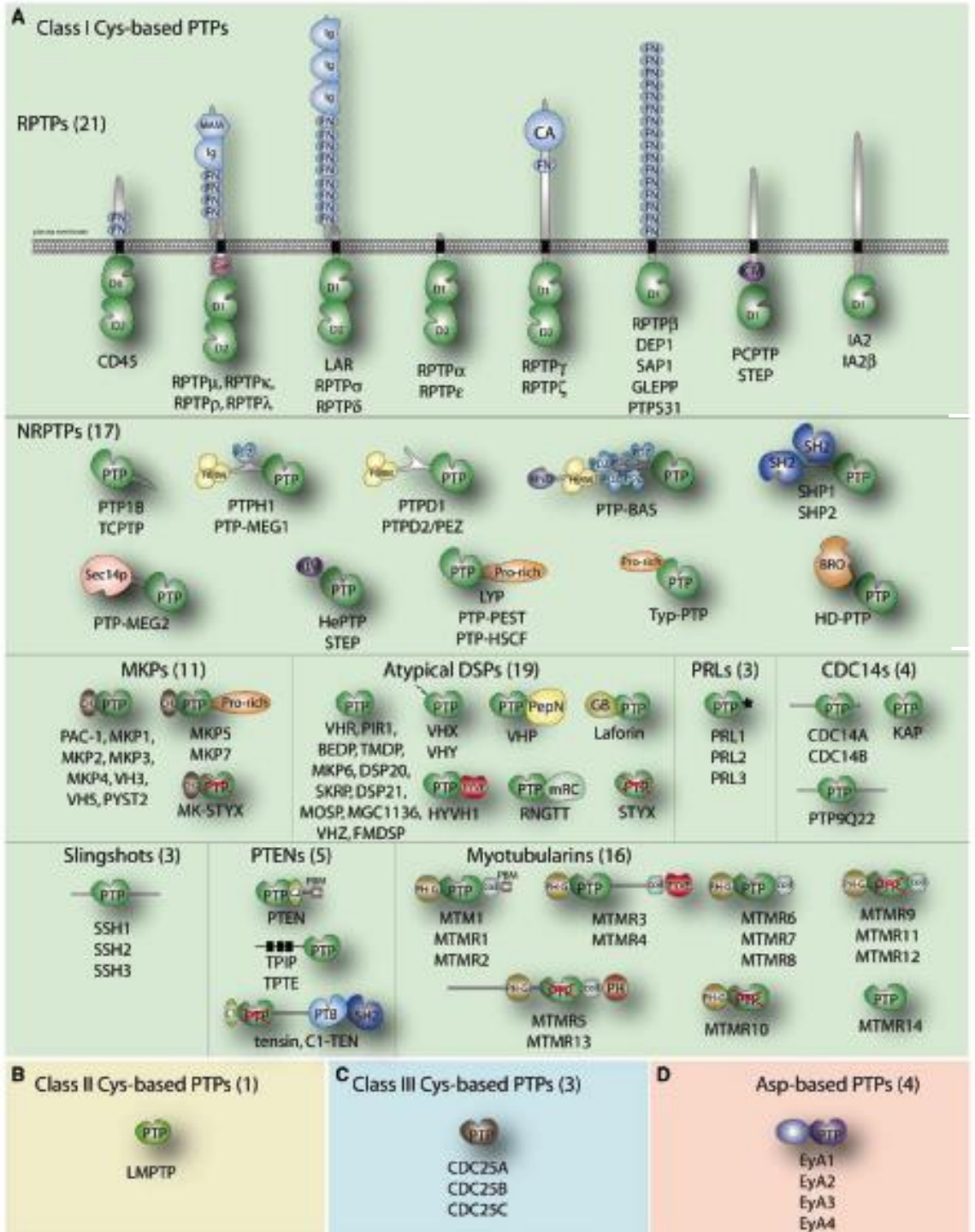


Abscisic Acid–Mediated Plant Stress Responses



Protein Tyrosine Phosphatases (PTPs)

PTP(-like) Phosphatases in The Human Genome (107 genes): ~90 active enzymes



transmembrane

cytoplasmic

assorted others

Cell (2004) 117:699

Crystal structures of six PTP domains show a **conserved fold and Cα-backbone**

Superimposition of PTP1B (magenta), RPTPα (gray), RPTPμ (red), LAR (blue), SHP1 (green) and SHP2 (yellow).

Andersen et al Mol. Cell. Biol. 2001

The PTP1B active site

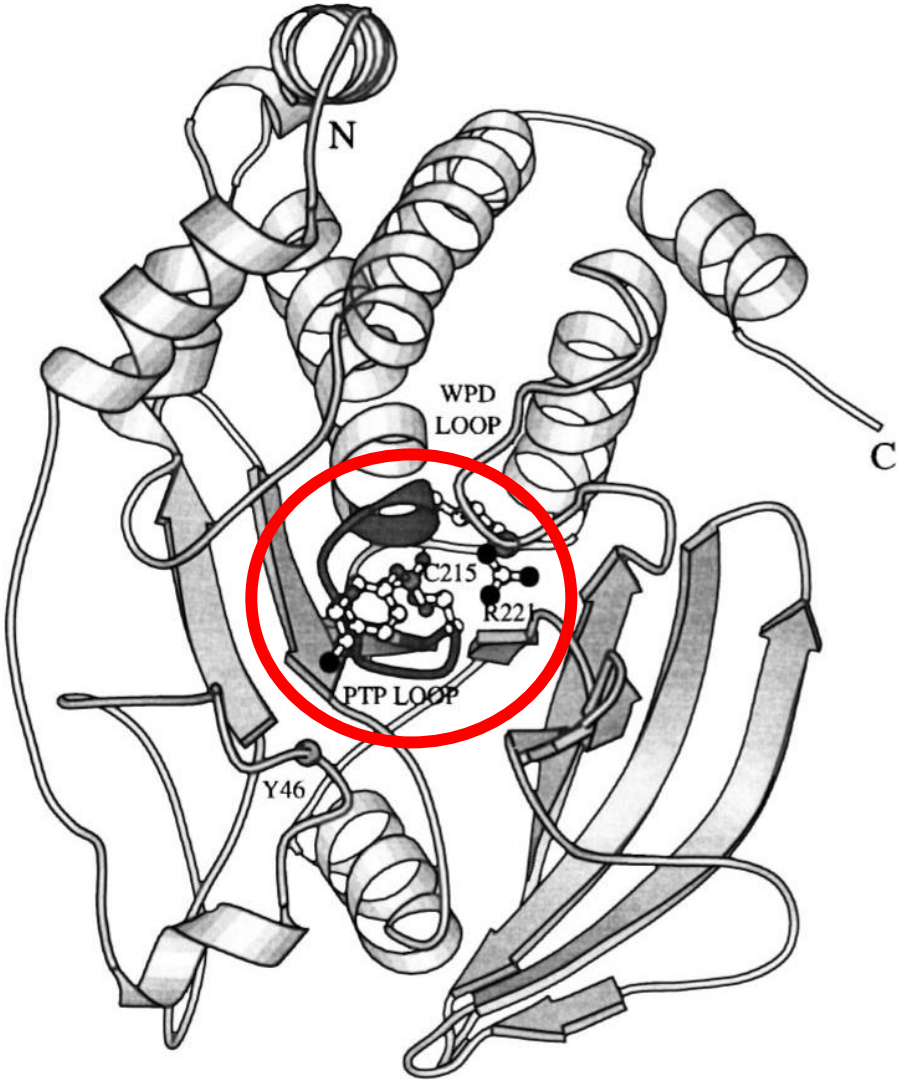
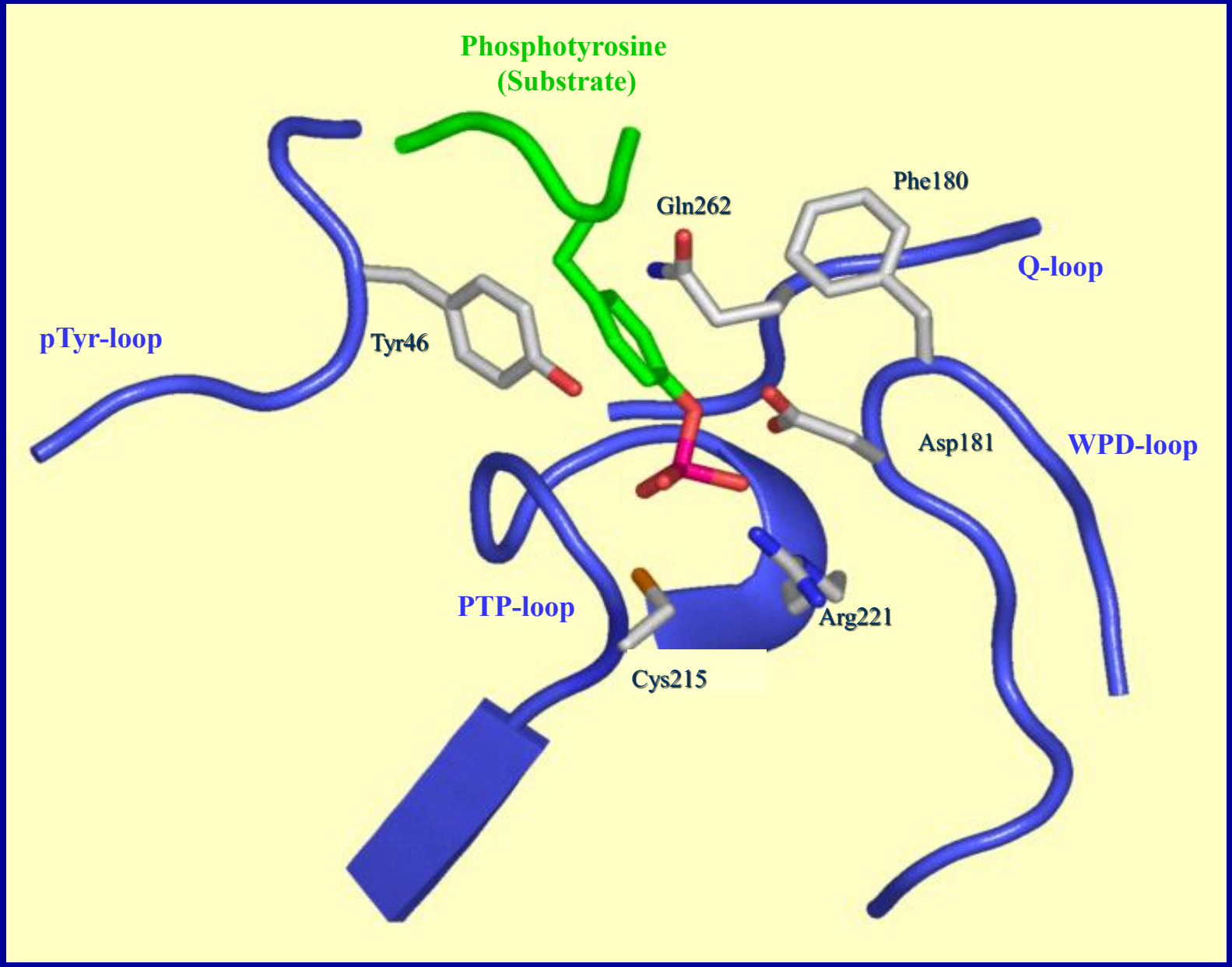
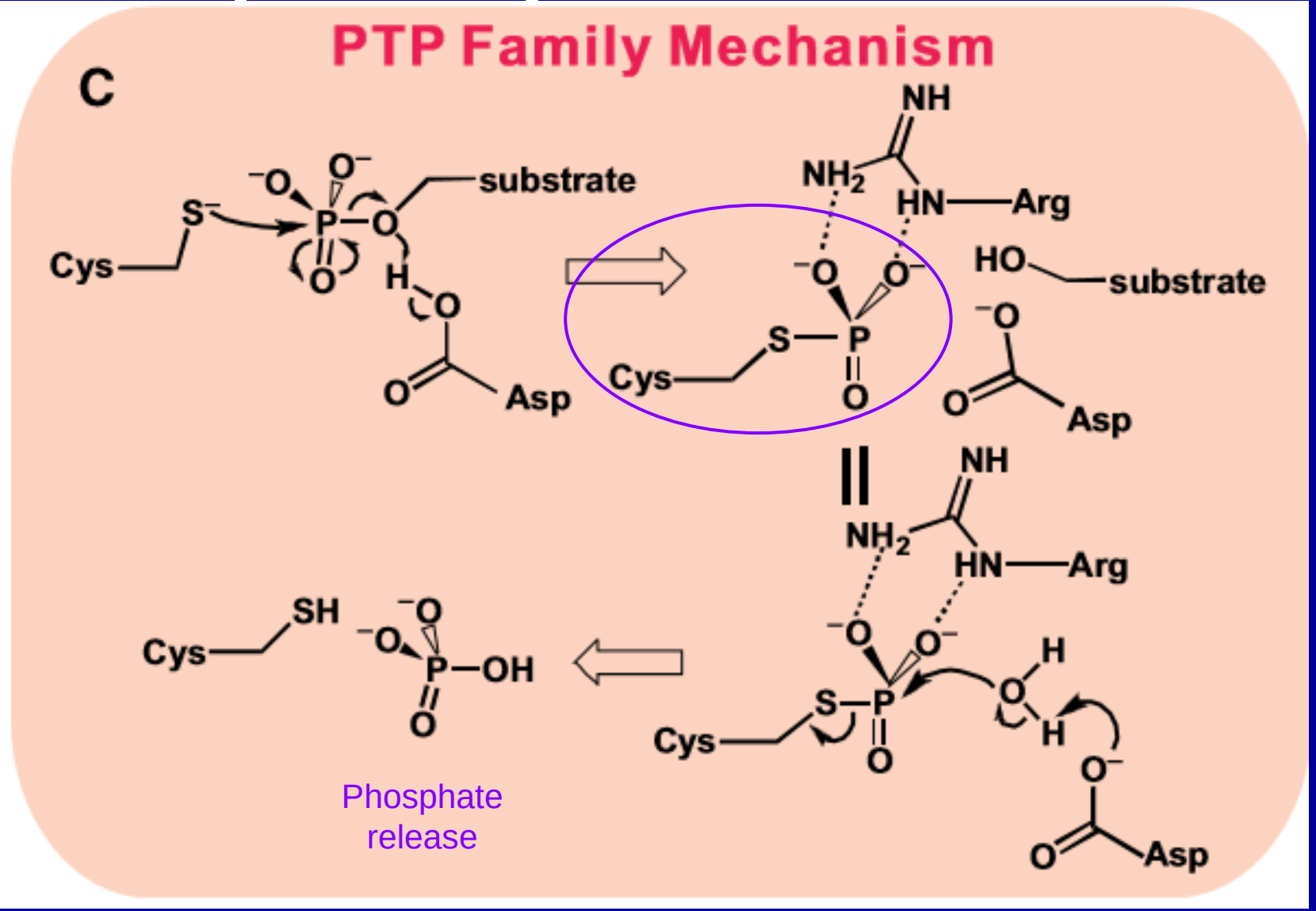


Figure 4 Structure of protein tyrosine phosphatase 1B. The PTP loop (*dark shading*) and WPD loop are indicated, as is Cys 215 and Arg 221 of the PTP loop and the position of the Cα-atom of Tyr 46 of the phosphotyrosine recognition loop.

The PTP1B active site



Catalytic 2-step Mechanism of PTPs



Sub-Families of Tyr Phosphatases (PTPs)

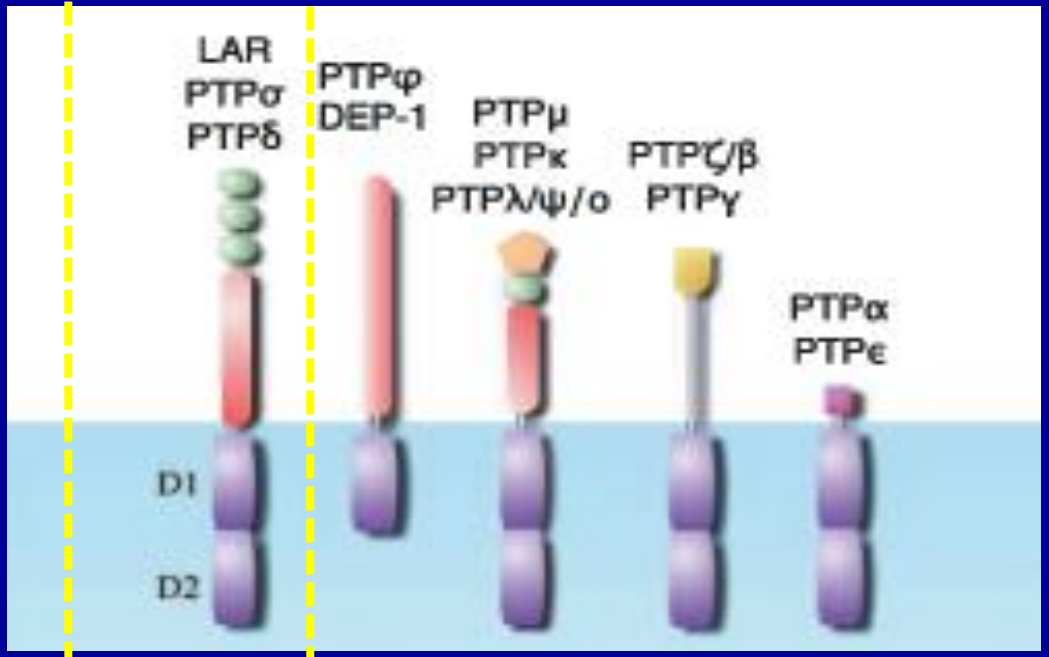
1. Transmembrane PTPs - *the prototype CD-45*

a. common features (most)

1. single TM helix to span membrane
2. double PTP domain, with activity in N terminal (D1) domain
3. large extracellular domains, related to cell-cell adhesion
4. inhibited by dimerization? Oxidation?
5. activators of src kinases by Tyr527 dephosphorylation

b. differences

1. tissue and developmental expression
2. substrate specificity, but few targets known knockouts and trapping mutants
3. inhibitors of active sites as pharmaceuticals



Receptor-like PTPs

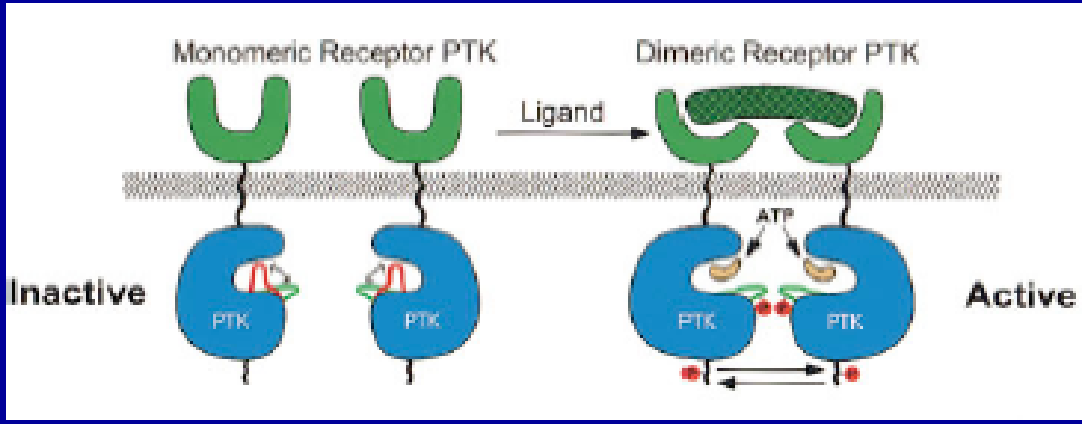
Transmembrane Proteins

Differential expression

E14-16 LAR PTP-sigma PTP-delta

Receptor PTPs catalytic activity is regulated by dimerization:

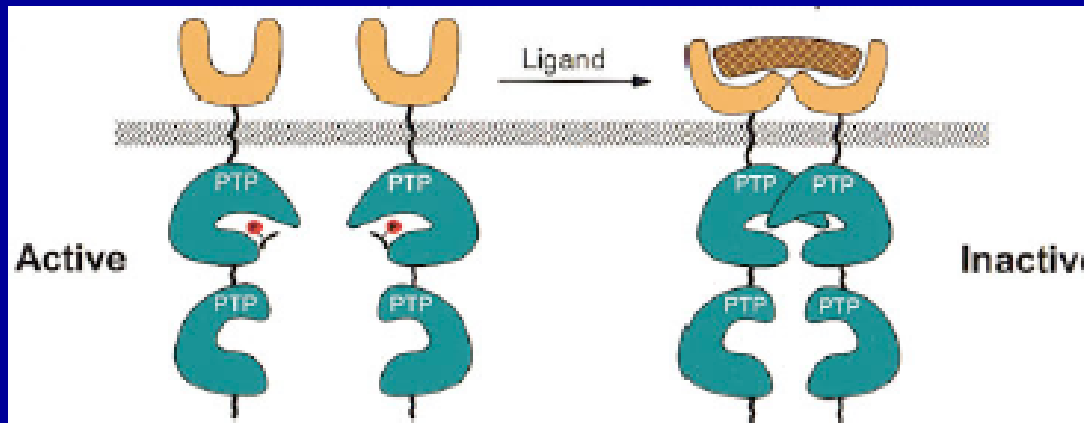
-Monomeric RTKs exhibit weak basal activity. Ligand binding of RTKs leads to dimerization, trans-autophosphorylation, and activation.



Weiss, A and Schlessinger, J (1998) Cell 94:277

Receptor PTPs catalytic activity is regulated by dimerization:

-Monomeric RPTPs exhibit enhanced catalytic activity. Ligand binding of RPTPs leads to dimerization of membrane-proximal PTP domains. 'Inhibitory wedge' sequences from each phosphatase domain interact with the other catalytic domain, preventing substrate binding.



Weiss, A and Schlessinger, J (1998) Cell 94:277

2. Cytosolic PTPs, *the prototype PTP1B*

a. common features

1. single PTP domain, plus targeting sequences
2. specificity for P-Tyr vs P-Ser
3. Phospho-Cys-enzyme intermediate
4. Substrate trap by conformational movement
5. Oxidation-reduction control mechanism

b. differences

1. tissue expression
2. specificity for substrates
3. Inhibition by small molecules

InsR Signaling

Tyrosine Phosphorylation of Insulin Receptor

Muscle

Muscle

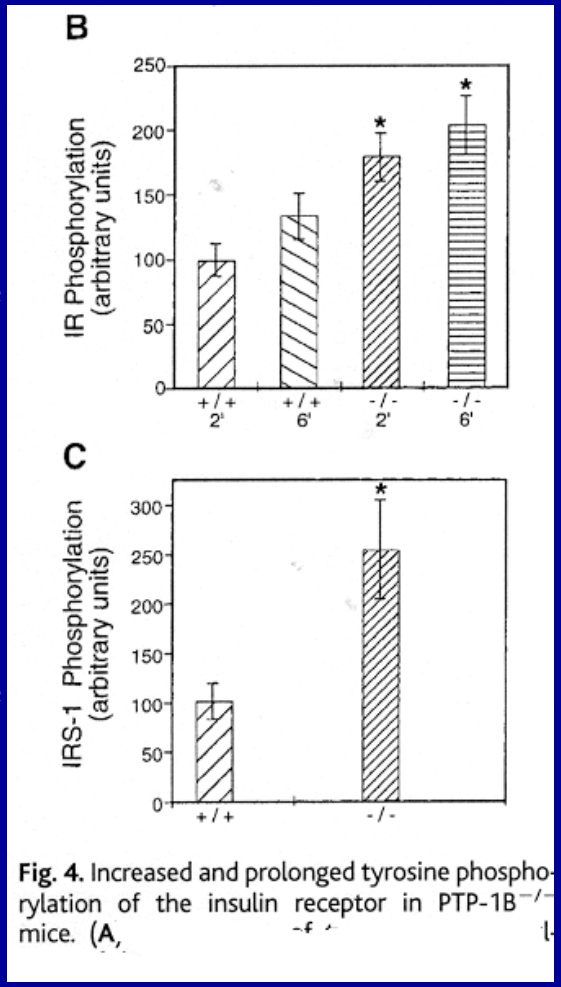
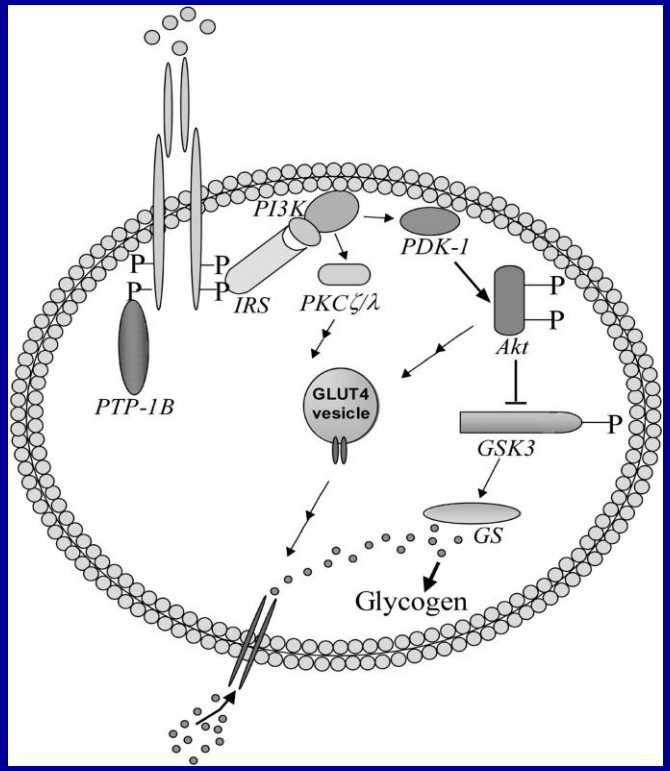


Fig. 4. Increased and prolonged tyrosine phosphorylation of the insulin receptor in PTP-1B$^{-/-}$ mice. (A,



Resistance to High Fat Die

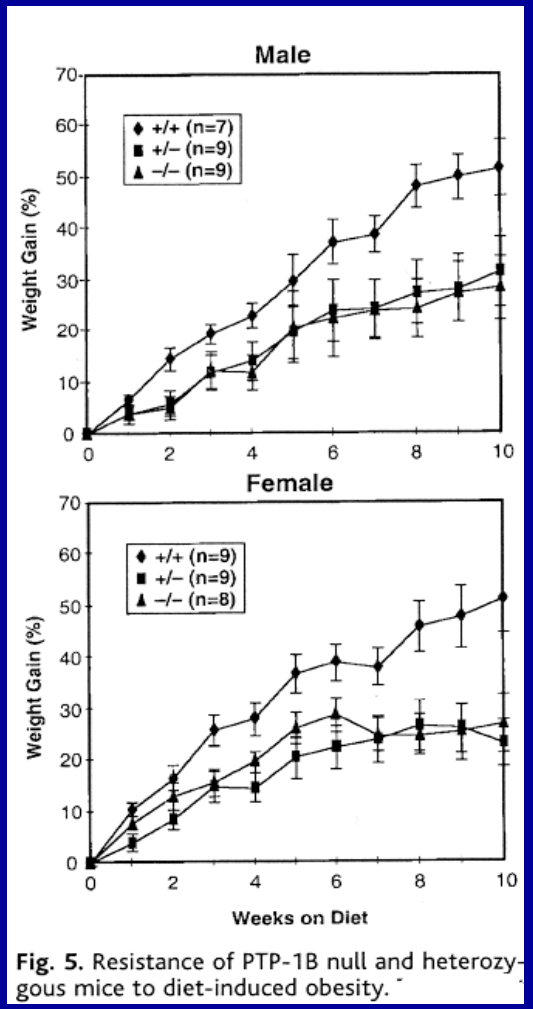
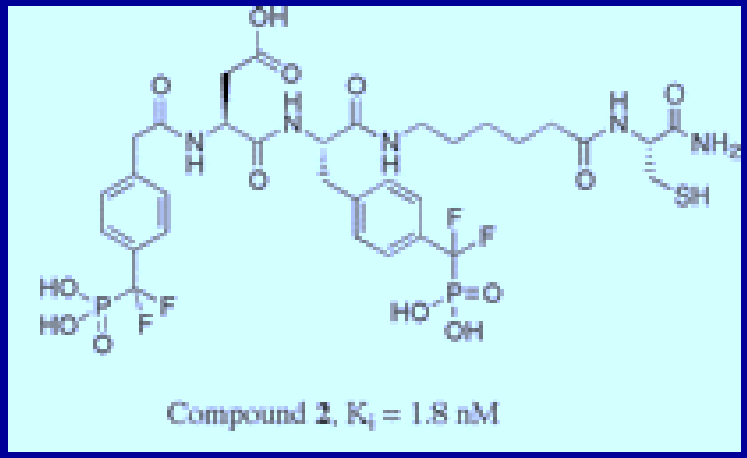


Fig. 5. Resistance of PTP-1B null and heterozygous mice to diet-induced obesity.

Co-Crystal of PTP1B with Chemical Inhibitor - Cmpd2



Compound **2**, K_i = 1.8 nM

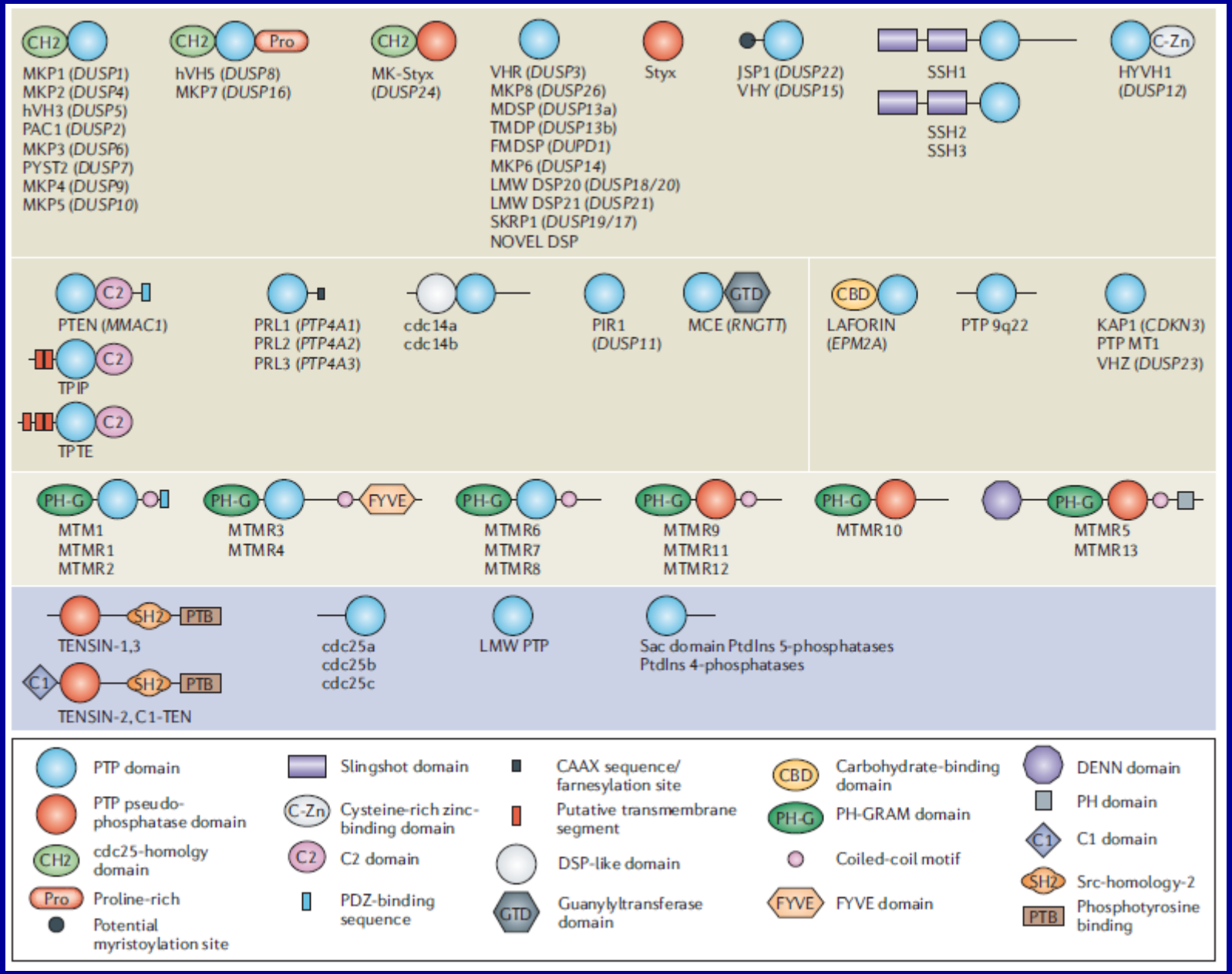
3. Dual Specificity Phosphatases, *the prototype VH1*

a. mechanism common with PTPs, i.e. Cys-based catalytic site,
which is shallow enough to accommodate p-Ser/p-Thr substrates

b. the MKPs (DUSPs), **MAP kinase phosphatases**
binds to MAPK at site using N-terminal domain through CH2 motif, and this activates the MKP terminal catalytic domain
several members : CL100, MKP1, 2, 3, 5, 7

c. the Cdc25 family of **CDK phosphatases**
low activity phosphatase with extreme specificity
large inhibitory domain, activated by phosphorylation
not really a family member- it's like Rhodanese (Thiosulphate transferase: converts CN- in SCN-)

The Dual Specificity Phosphatases



Tonks et al, Nat Rev, 2006

Dual-Specificity Phosphatase: DUSPs

MAPK Phosphatase: Catalytic domain reacts with pTyr-X-pThr

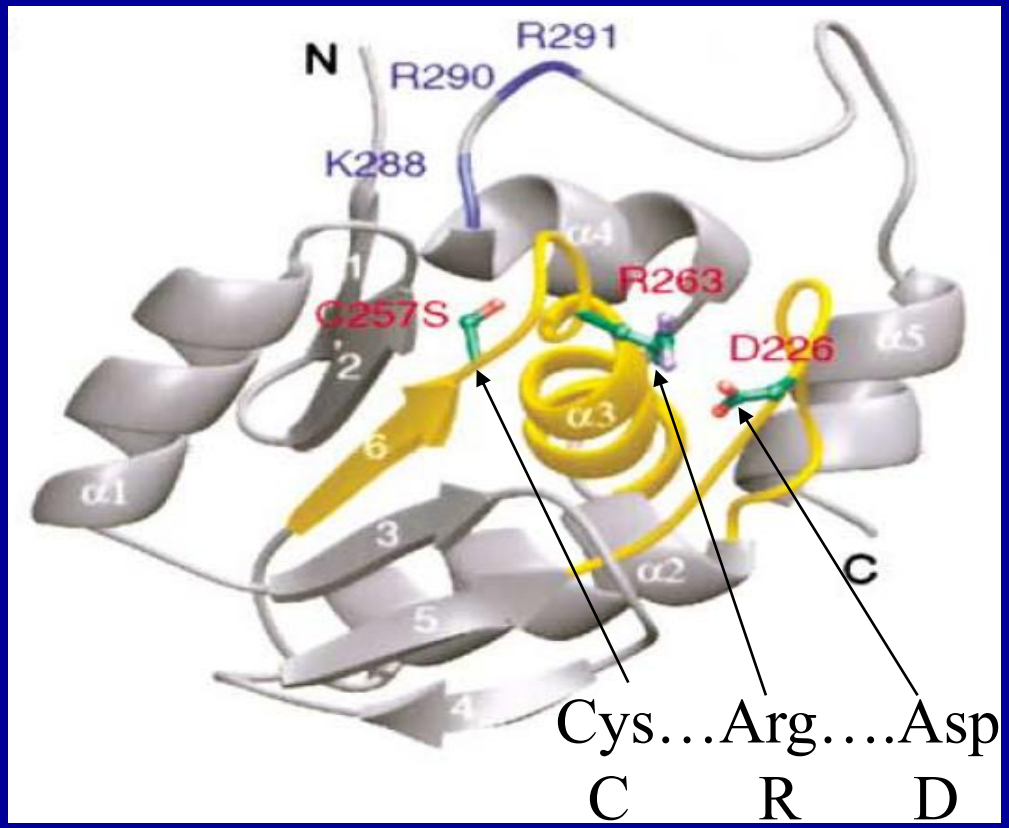
MKP signature sequence is HCXXXXXR:

-Nucleophilic attack of cysteine thiolate anion on MAPK P-Tyr

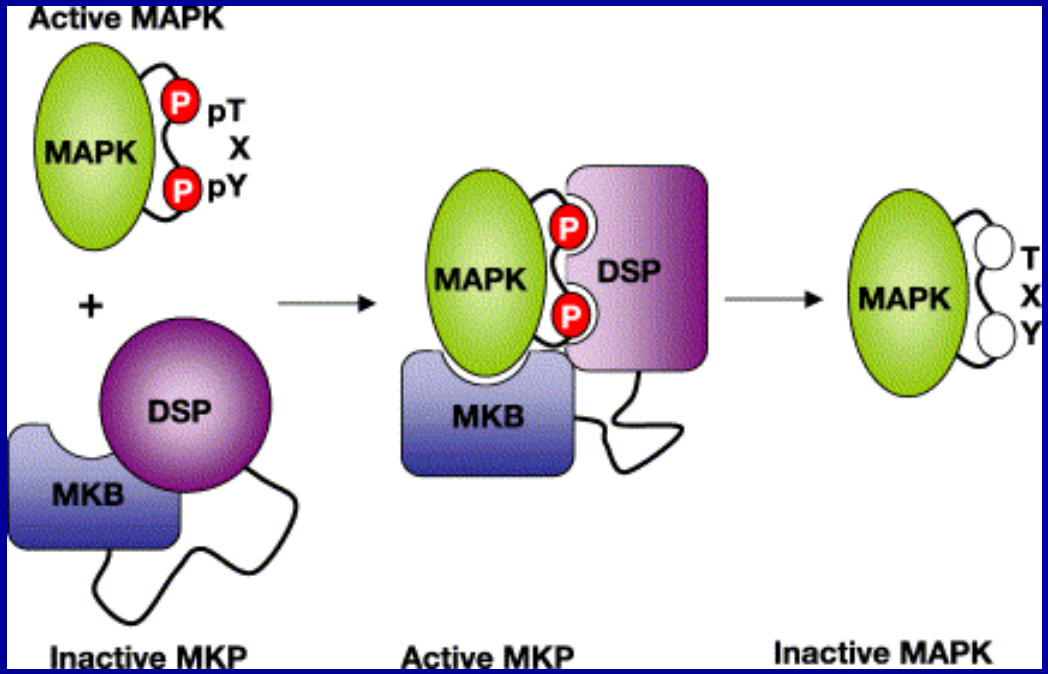
-Aspartate in acid loop donates proton

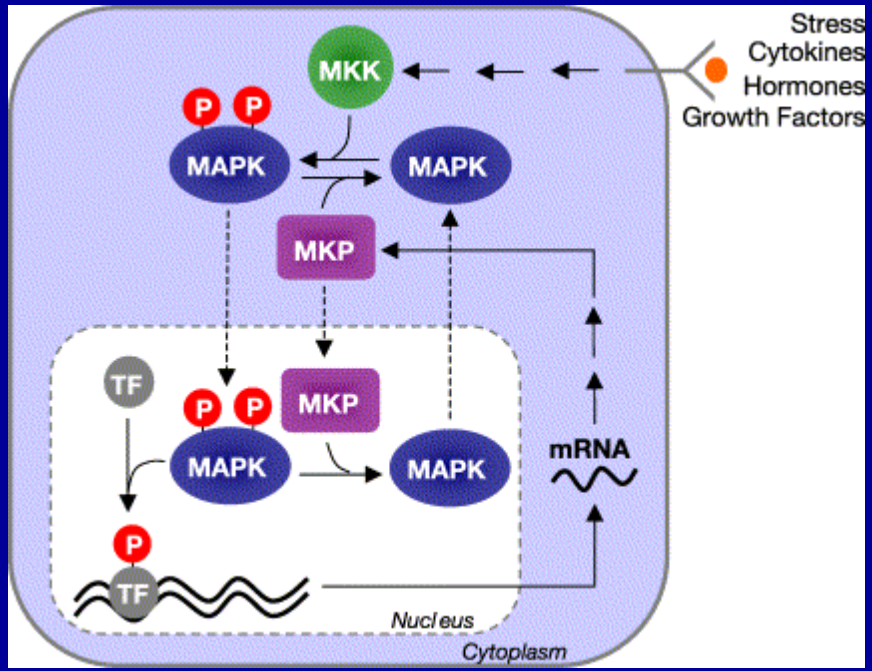
-Arginine coordinates phosphate group of P-Tyr or P-Thr

-Histidine decreases pKa of cysteine so it exists as anion



MAPK Phosphatases : Use of Docking + Catalysis





PTP Inhibitors

PTP Inhibitor Design (e.g. vs. PTP1B for diabetes and Cdc25 for cancer)

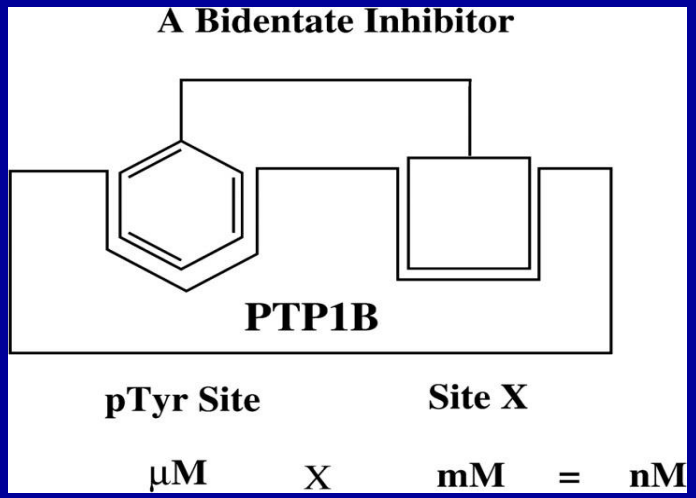
pTyr alone insufficient for high affinity binding to PTPs--adjacent residues contribute to specificity

By analogy, kinase inhibitor specificity determined by binding to region outside ATP binding pocket--for PTPase, pTyr binding domain is smaller than kinase ATP pocket (pTyr takes up ~50% of binding pocket)

So small molecule inhibitors:

--Need to bind PTP catalytic domain and another adjacent region unique to a specific PTP simultaneously to confer specificity (based on structure PTP1B and inh. BPPM)

--Need to penetrate cell membranes



A strategy for creating selective and high-affinity PTP1B inhibitors. Based on the principle of additivity of free energy of binding, high-affinity ligands can be obtained by linking two functional groups that bind to the active site (pTyr binding site) and a peripheral site X. Specificity arises from the fact that site X is not conserved and from the fact that the tethered ligand has to bind both sites simultaneously. Zhang ZY (2002) Annu Rev Pharmacol Toxicol. 42:209-34

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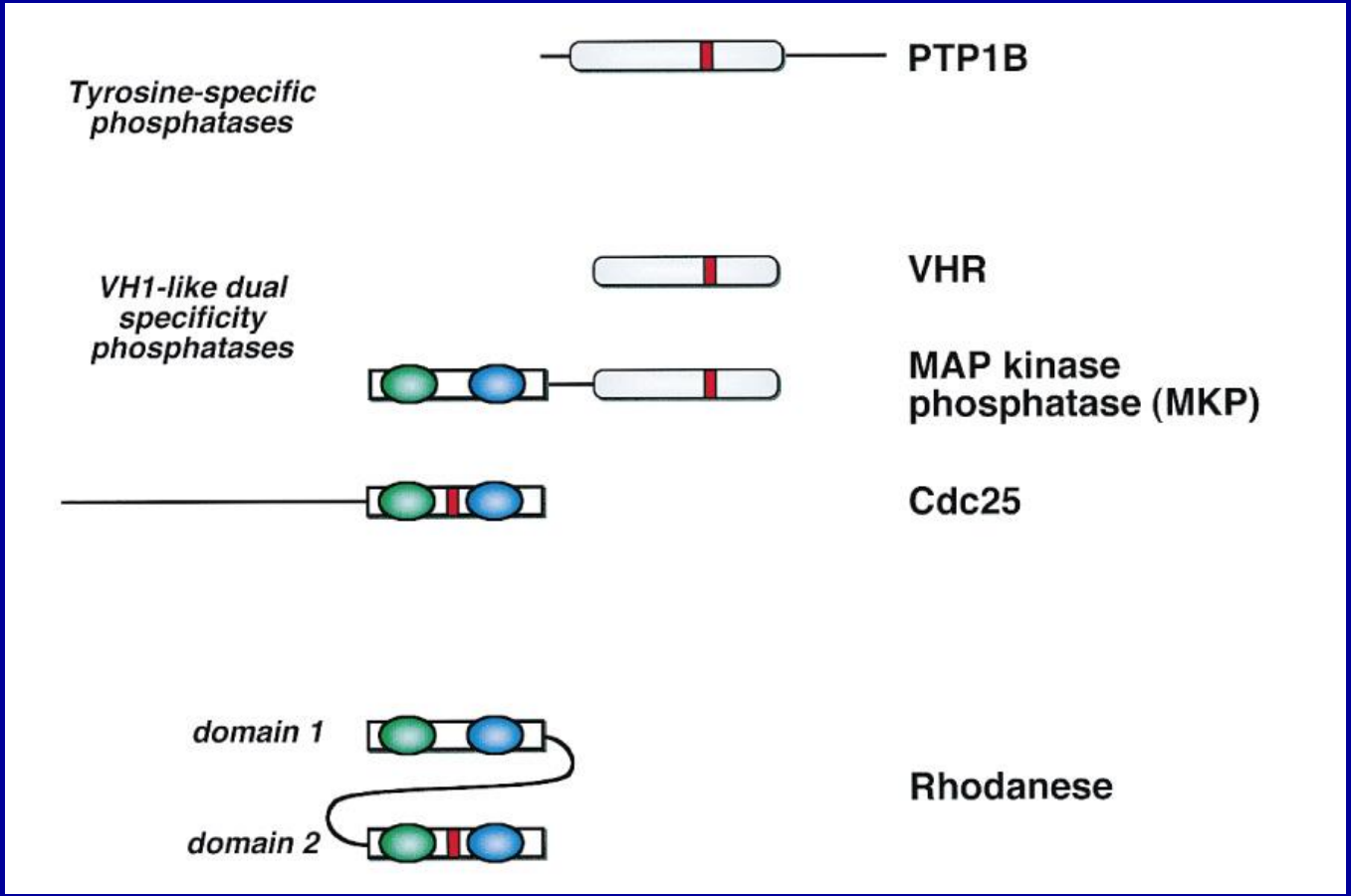
Cdc25 - not really related to other PTPs



Cell cycle. CDK1 (CDC2 or p34cdc2) is inactive in G1 due to phosphorylation on Thr14, Tyr15 and Thr161. Critical threshold concentration of CDK1 at G2/M transition results in increased dephosphorylation of Thr14, Tyr15 by Cdc25, a dual specificity phosphatase, leading to CDK1 activation.