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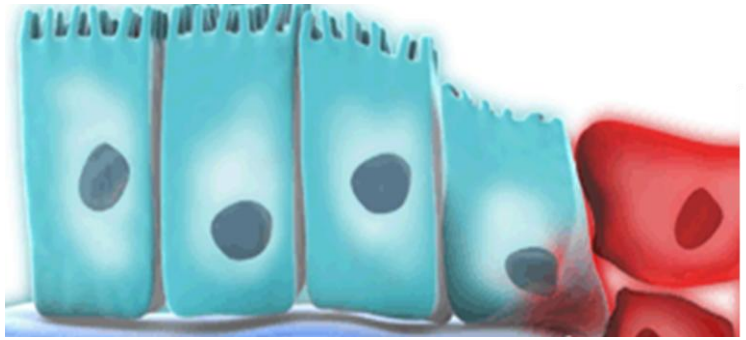
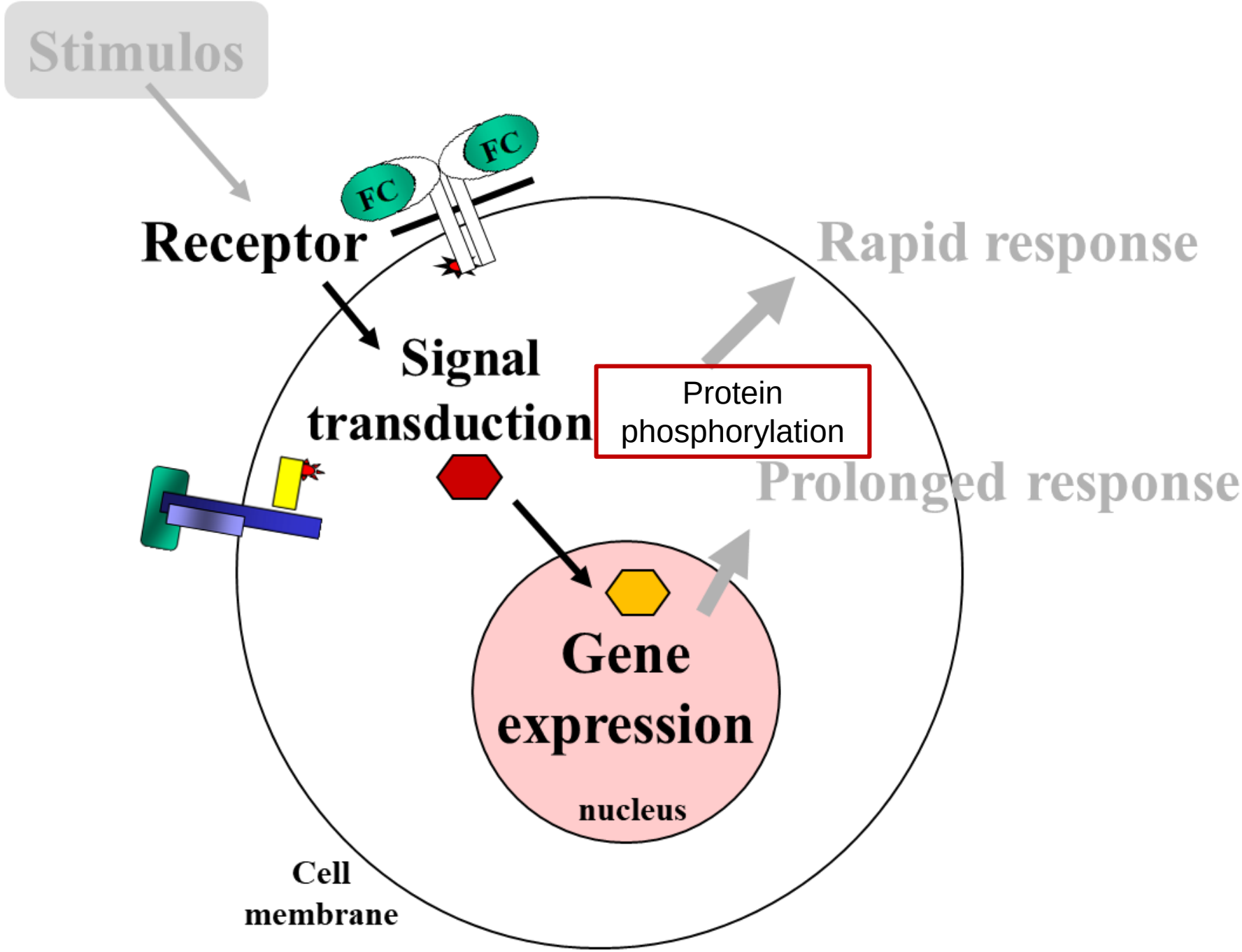
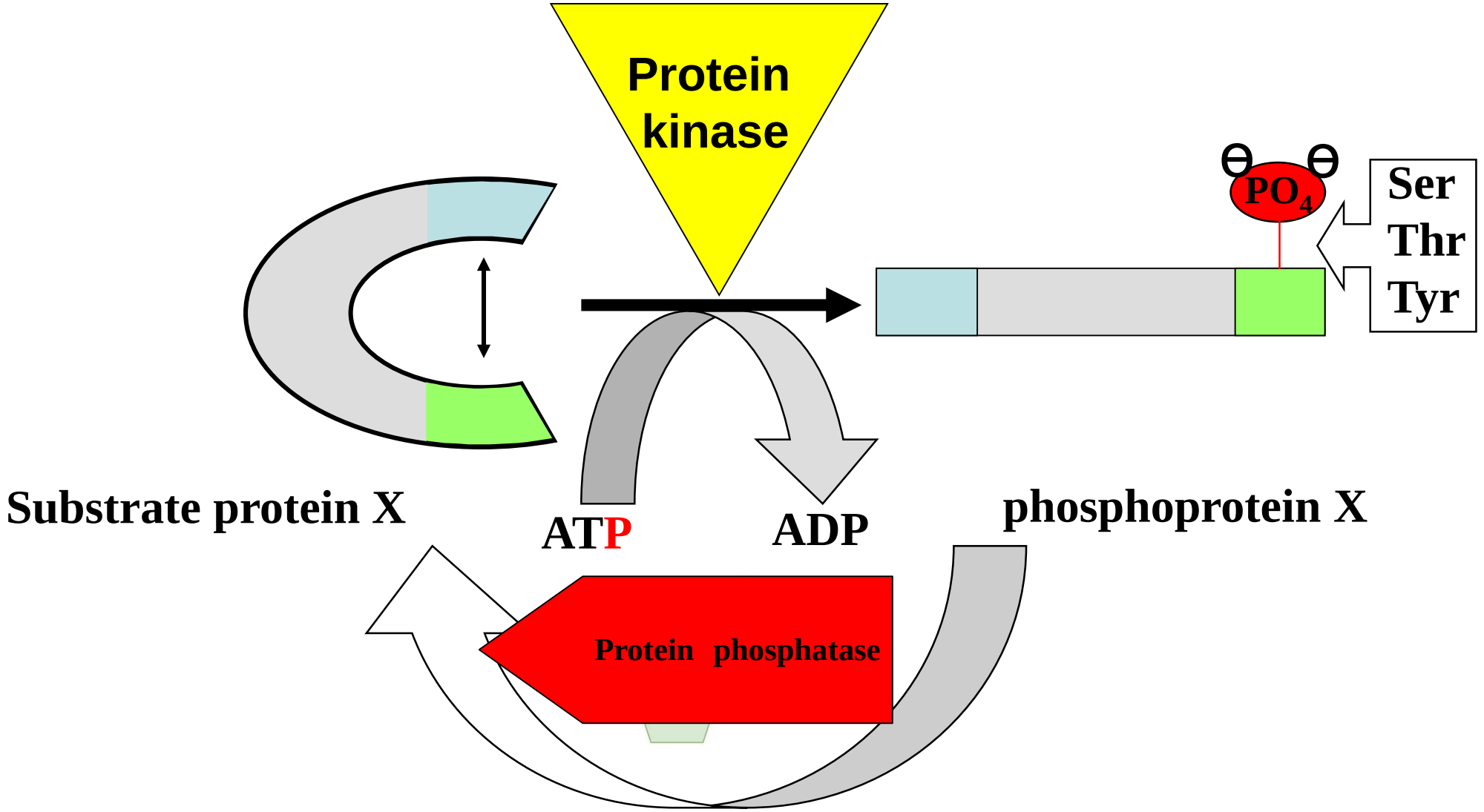


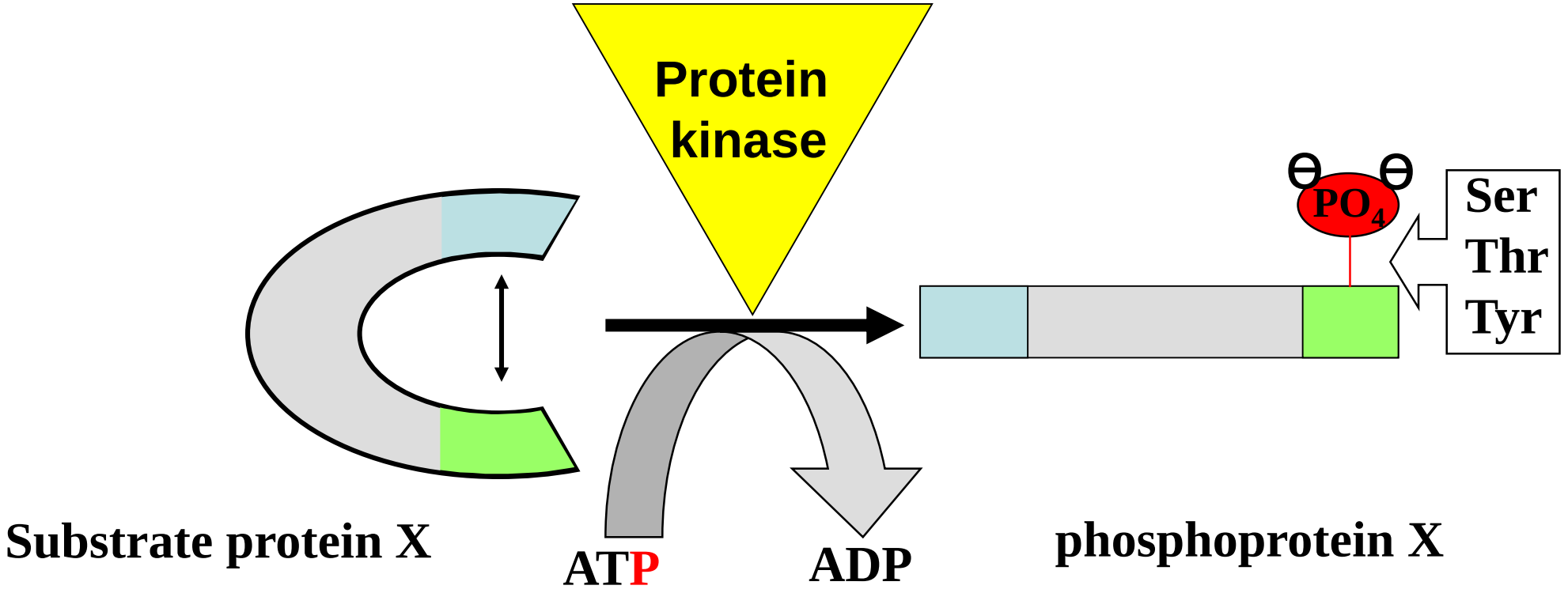
Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

The protein kinase superfamily


Stimulos
FC
FC
Receptor
Rapid response
Signal
transduction
Protein
phosphorylation
Prolonged response
Gene
expression
nucleus
Cell
membrane


Protein kinase
PO4
Ser
Thr
Tyr
Substrate protein X
ATP
ADP
phosphoprotein X
Protein phosphatase



phosphorylation:

- can alter 3D conformation and activity of substrate protein;

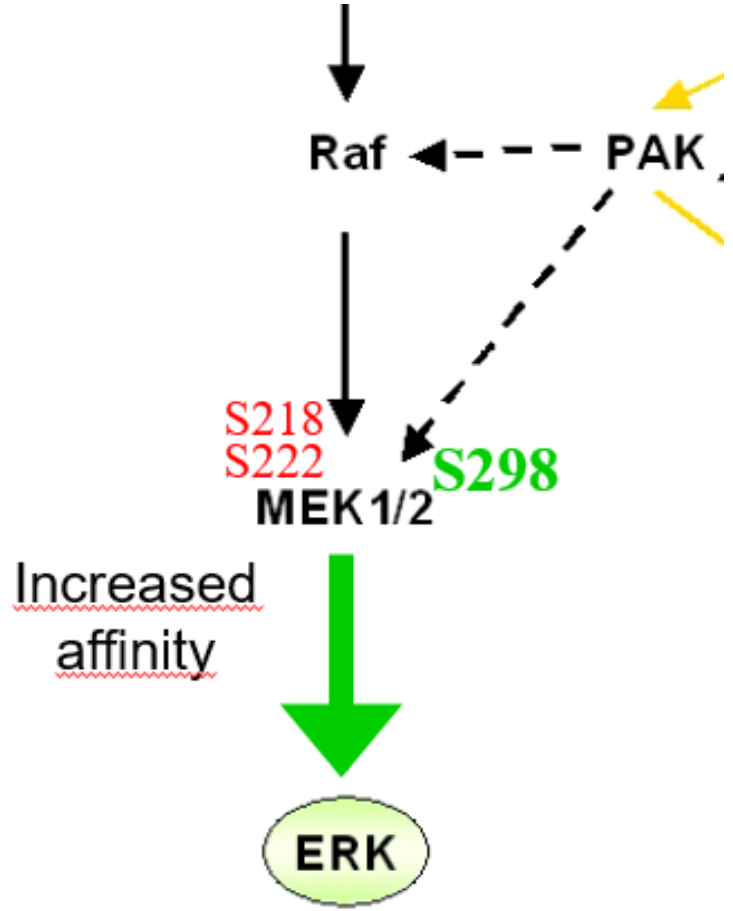
- can create or mask protein interaction motifs in the substrate

inactive ‘Molecular switch’ **active**

Some examples

- Can alter 3D conformation and activity o

- Can create or mask protein interaction m

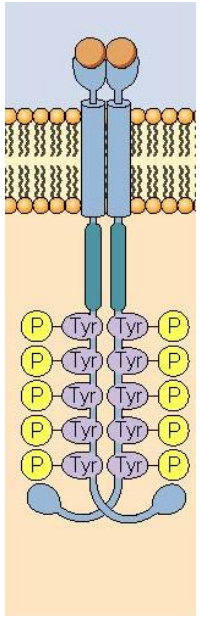


The activity of protein kinases themselves is regulated by their phosphorylation

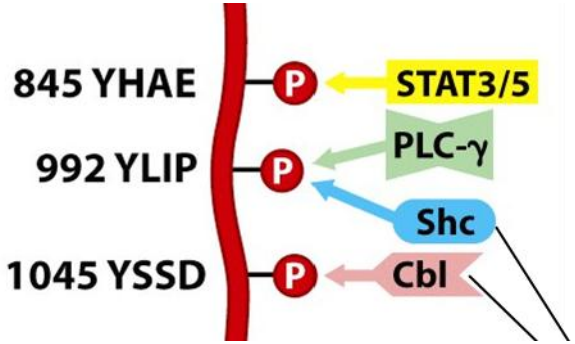
Some examples

- Can alter 3D conformation and activity of substrate protein;

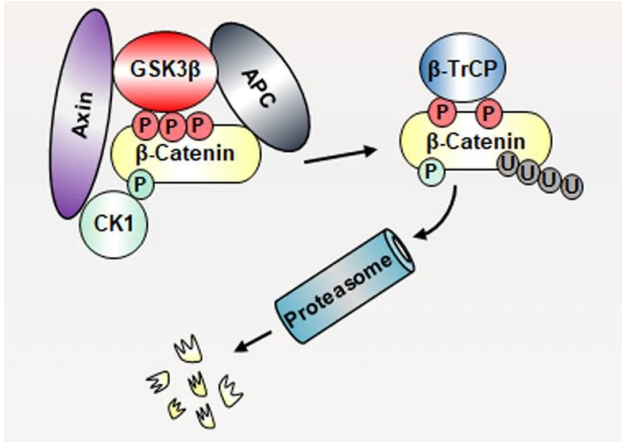
- Can create or mask protein interaction motifs in the substrate



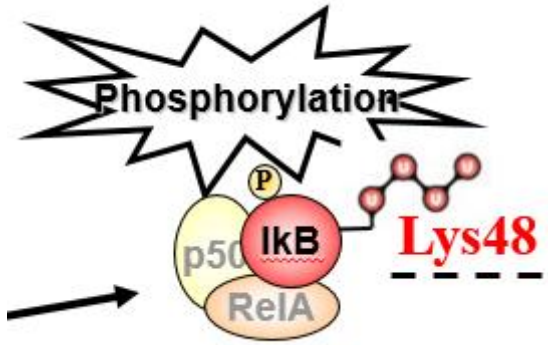
SH2 domain for pTyr



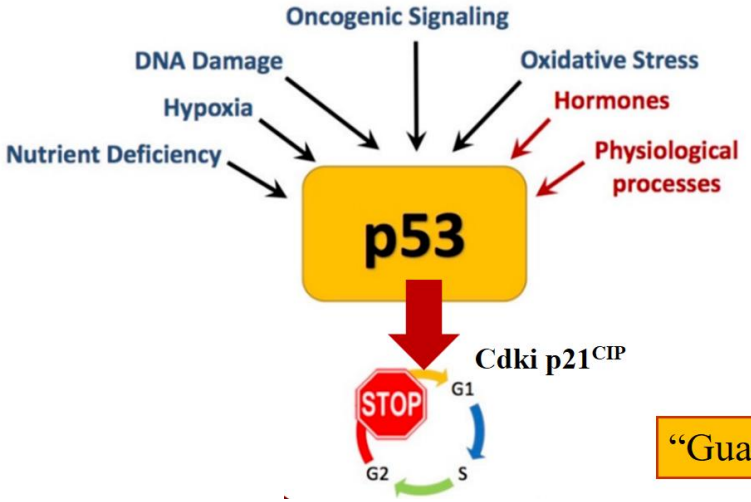
Growth factor receptor



β-catenin ubiquitylation



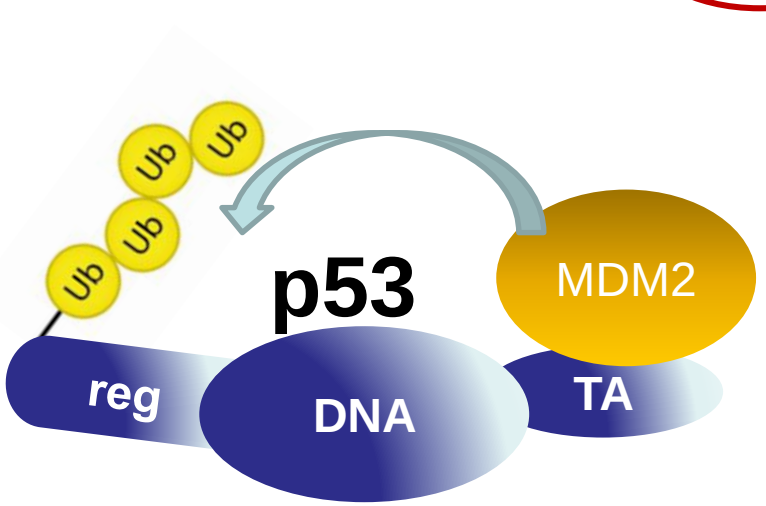
NF-κB ubiquitylation



"Guardian of the genome"

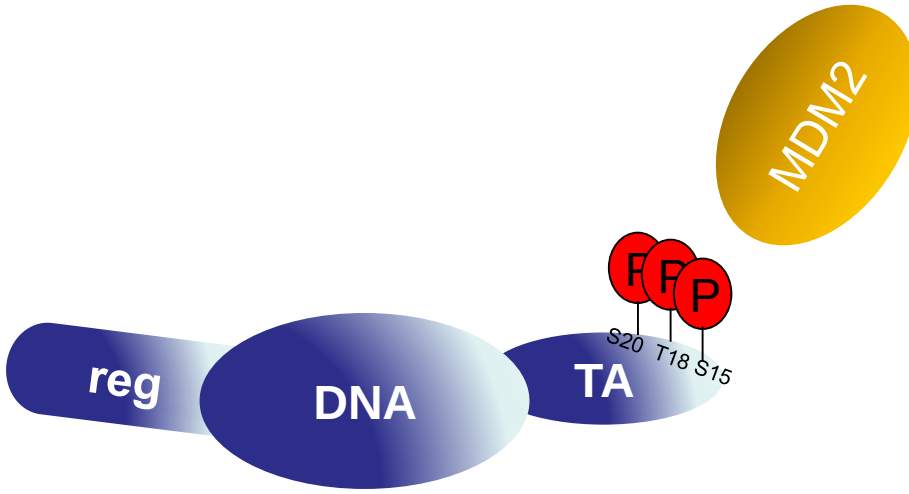
and activity of substrate protein;

- Can create or mask protein interaction motifs in the substrate



DNA damage-activated kinases

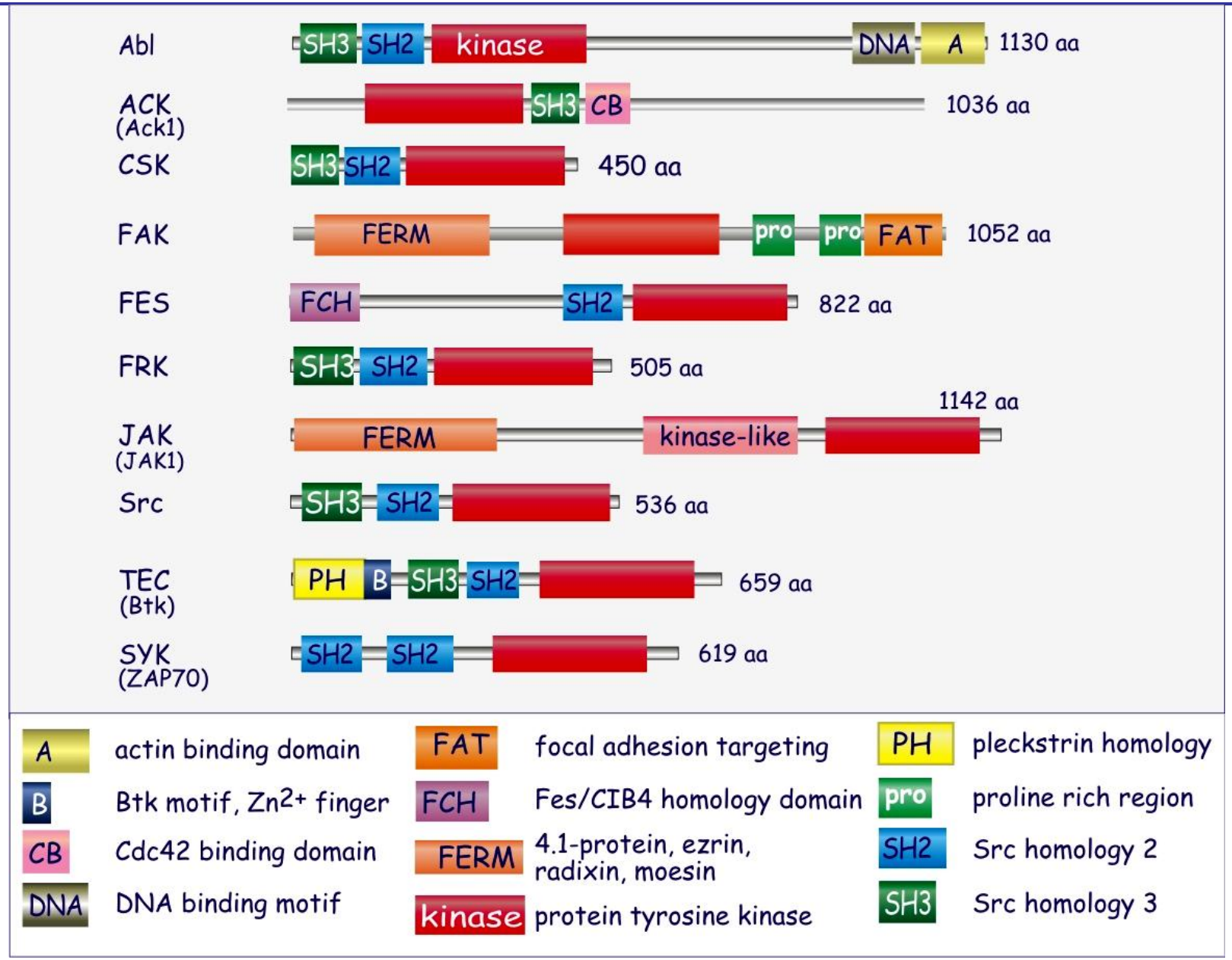
(ATM/ATR, Chk1/Chk2, DNA-PK)



MDM2 binds p53 to promote degradation and inhibit binding to target gene promoters

p53 stabilization and activation of target genes

Protein kinase= catalytic domain + remaining protein

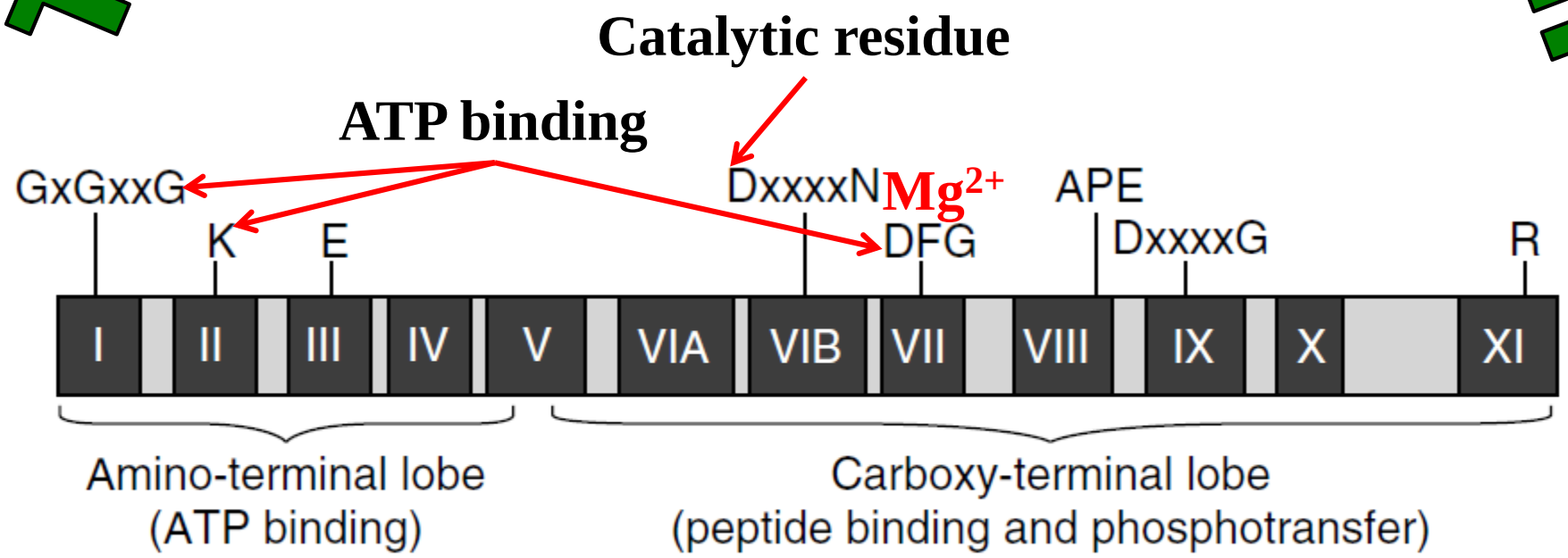


Regulation
Recruitment
Localization

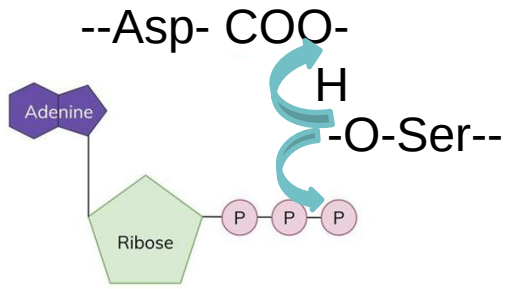
kinase

The catalytic domain

~250 aa

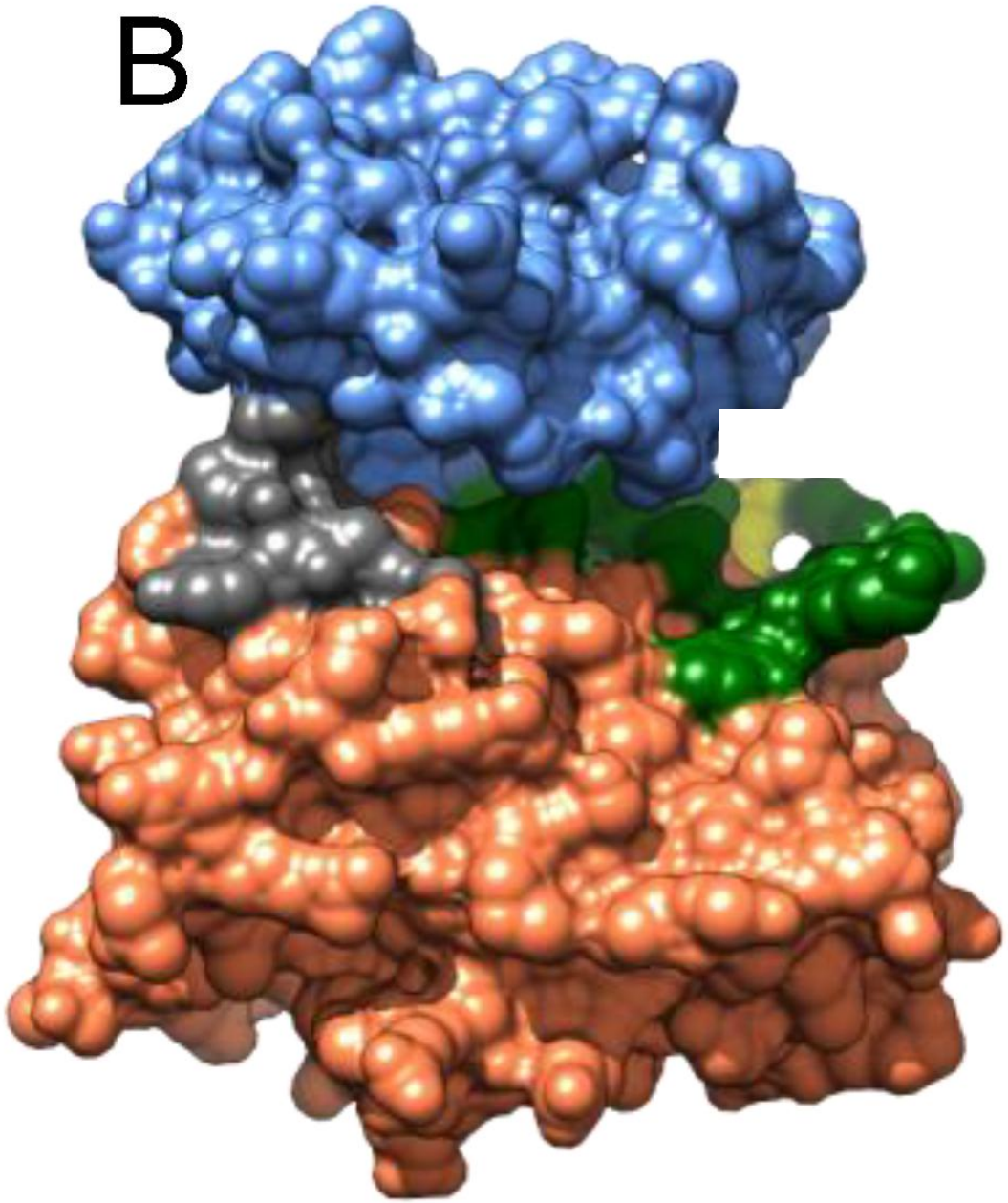


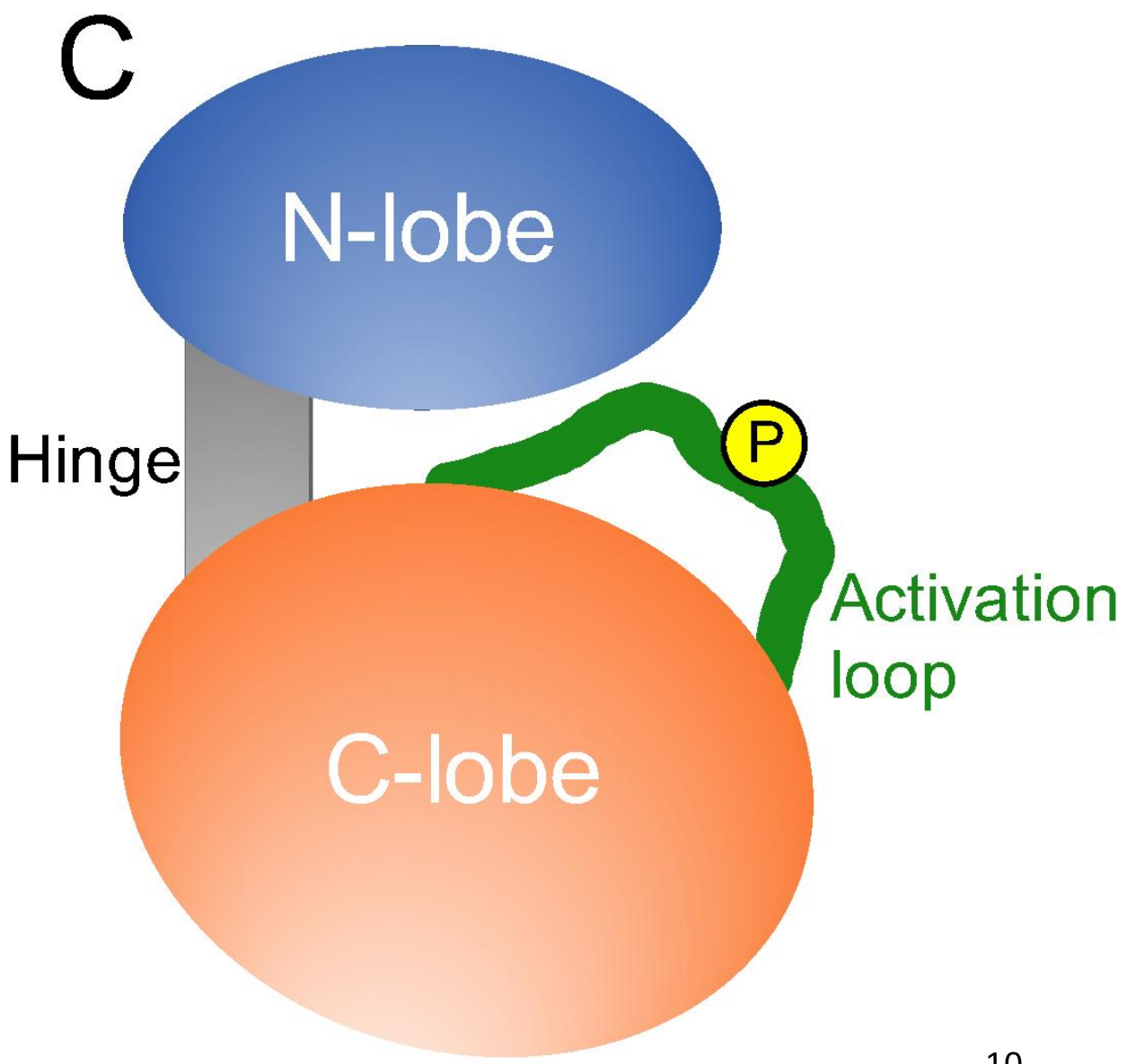
The catalytic Asp=D polarizes with its negative charge the hydroxyl oxygen of a Ser, Thr or Tyr of the protein substrate.
Then, the deprotonated oxygen residue performs a nucleophilic attack on the terminal (γ) phosphoryl group (PO_3^{2-}) of ATP.



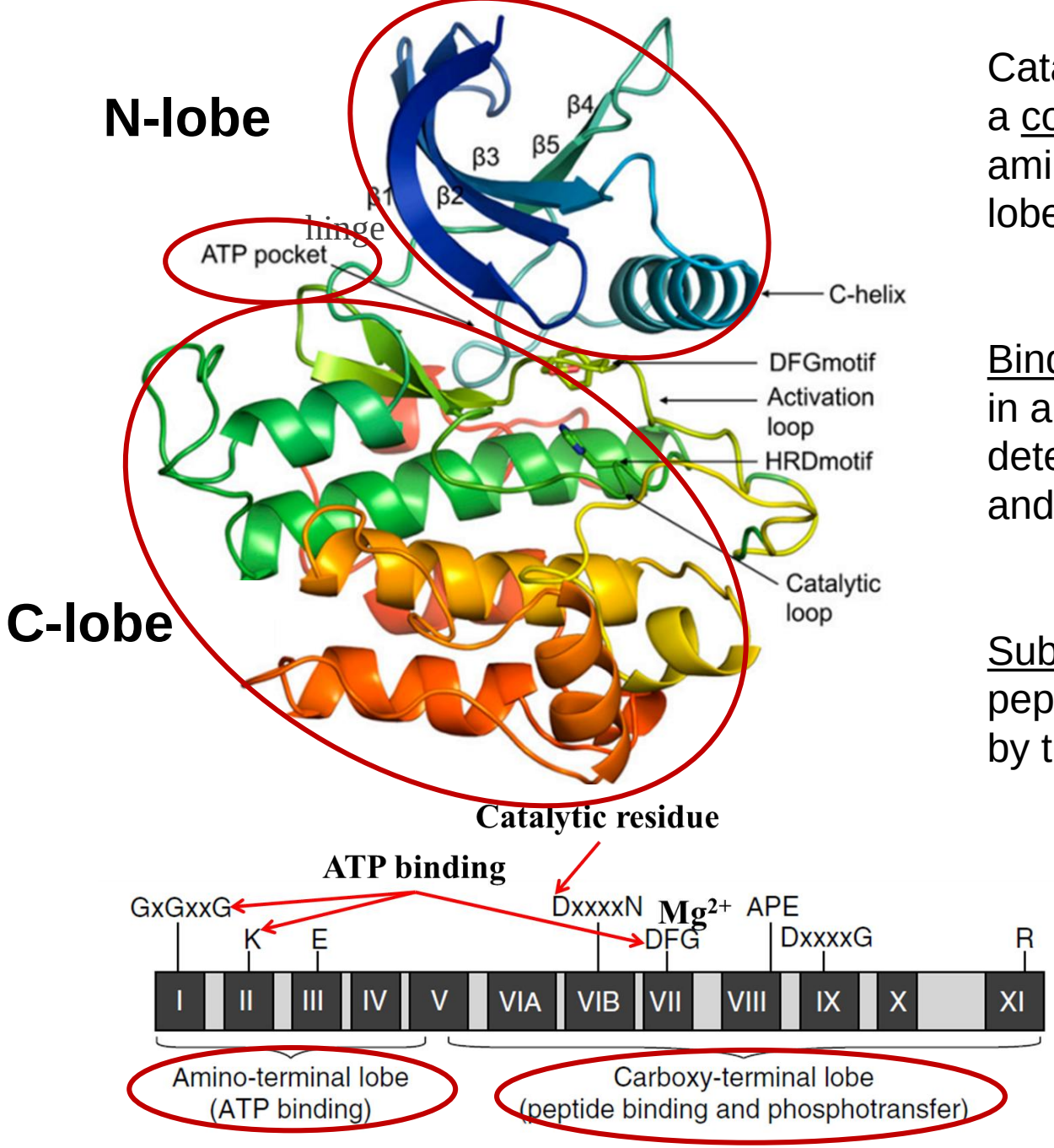
Hanks SK (2003) Genome Biology 4 (5), Article 111

Protein kinase catalytic domain





https://doi.org/10.3390/molecules26030651

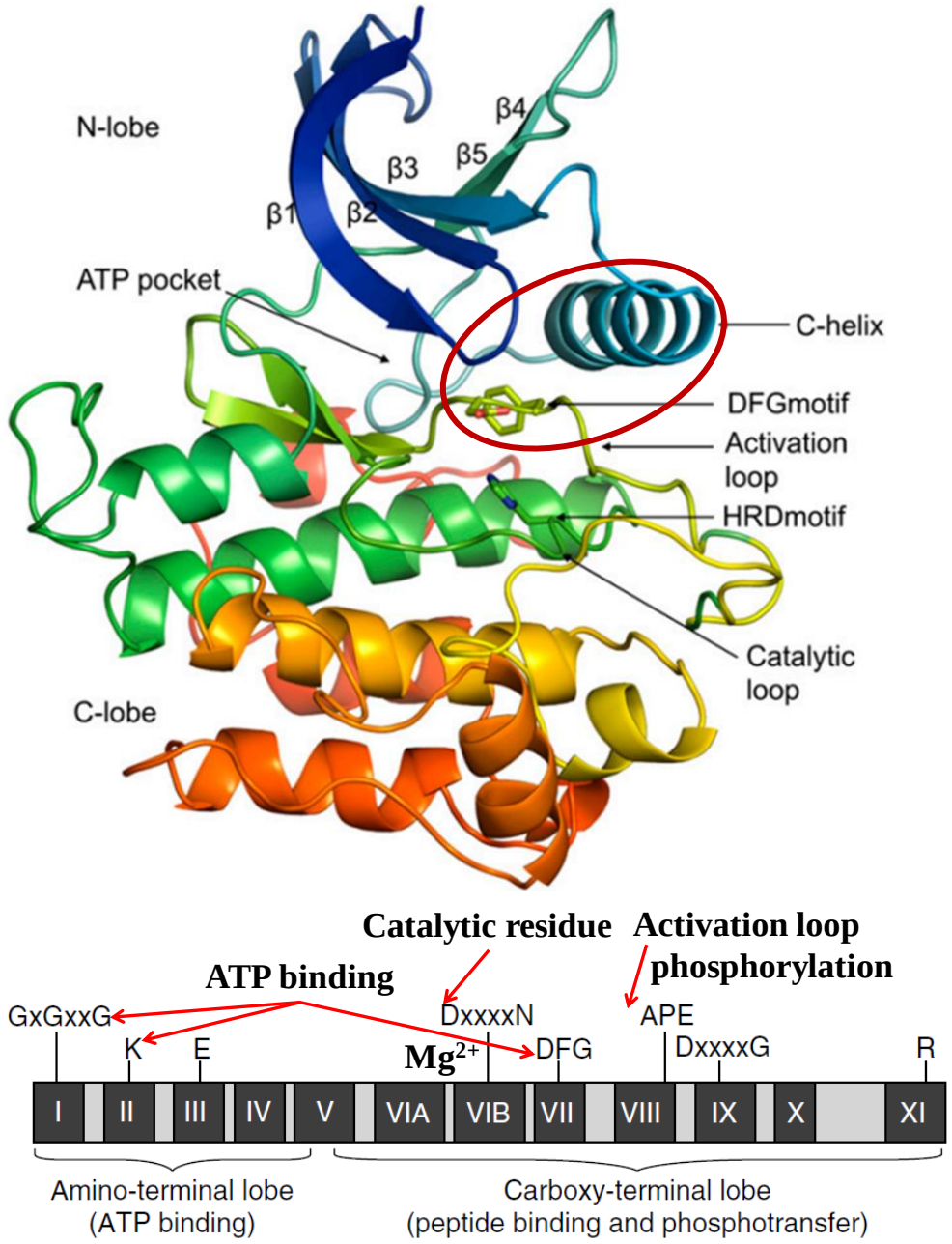


Hanks SK (2003) Genome Biology 4 (5), Article 111

Catalytic PK domain:
a common folding consisting of an amino-terminal and carboxy-terminal lobe

Binding of Mg-ATP:
in a cleft between the two lobes, determined by the amino-terminal lobe and hinge region

Substrate binding:
peptide-substrate binding is mediated by the carboxy-terminal lobe.



Hanks SK (2003) Genome Biology 4 (5), Article 111

Activation of catalytic activity:

the C-helix and the activation loop with the DFG motif need to approach;

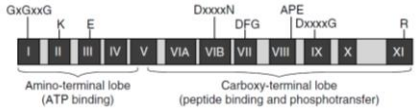
Both peptide regions are flexible between 2 conformational positions: C-helix out/in; DFG out/in;

(Auto)phosphorylation in the activation loop further stabilizes the catalytically active 'in' conformational positions and leads to full kinase activation

➡ phosphorylation:
- can alter 3D conformation and activity of substrate protein

The human kinome

518
protein kinase genes



478 ePKs
conserved eucaryotic domain

40 aPKs
atypical catalytic domain

50 Pseudo-ePK kinases lacking conserved residues

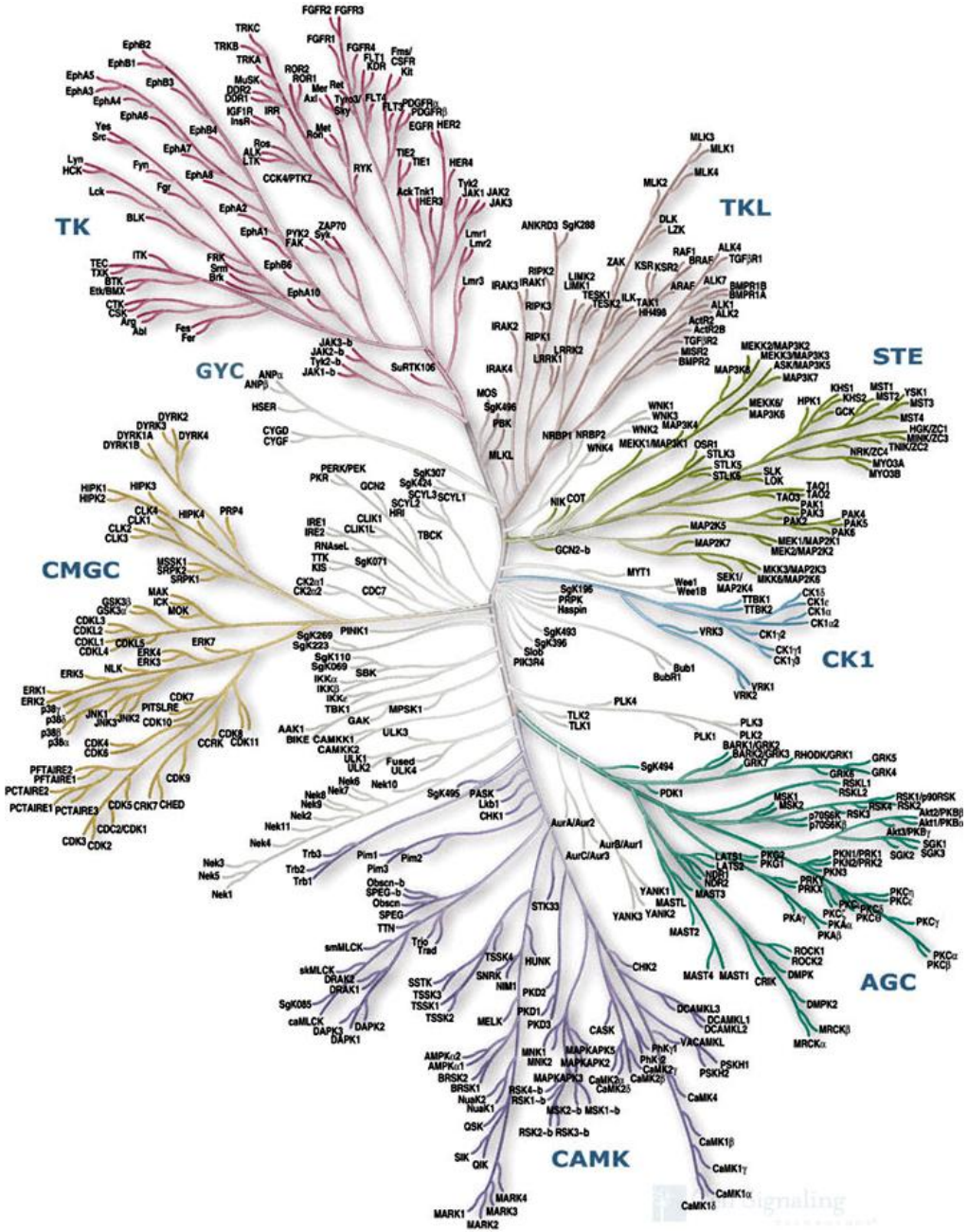
428 ePKs with known or likely kinase activty

8 subgroups:
TK- 84; CAMK- 66; AGC- 61; CMGC- 61;
STE- 45; TKL- 37; CK1- 11; Other- 63

Hanks SK (2003) Genome Biology 4 (5), Article 111

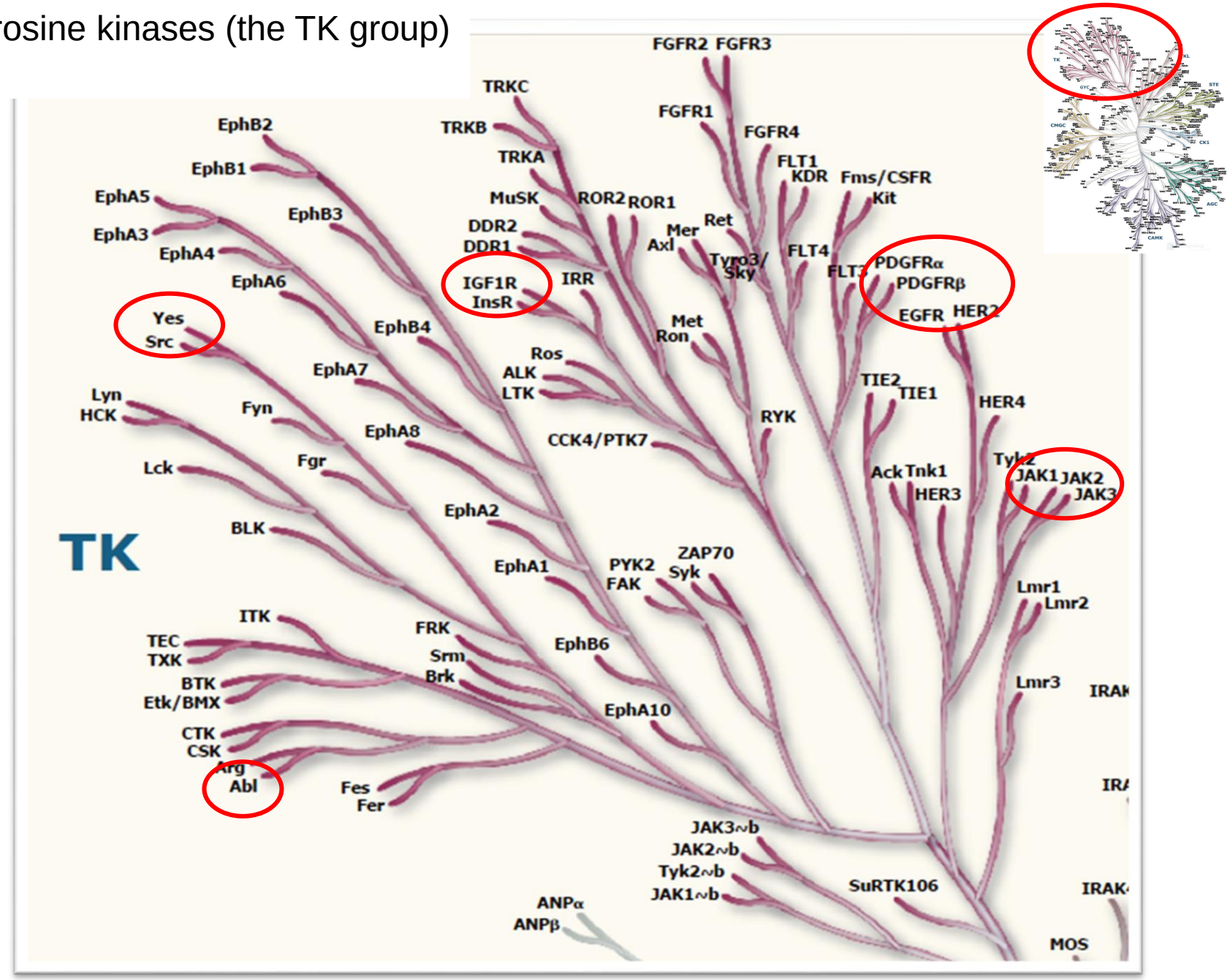
The human kinome tree:

clustering by sequence similarity in the kinase domain led to identification of different subfamilies (incl. 60 receptor kinases)



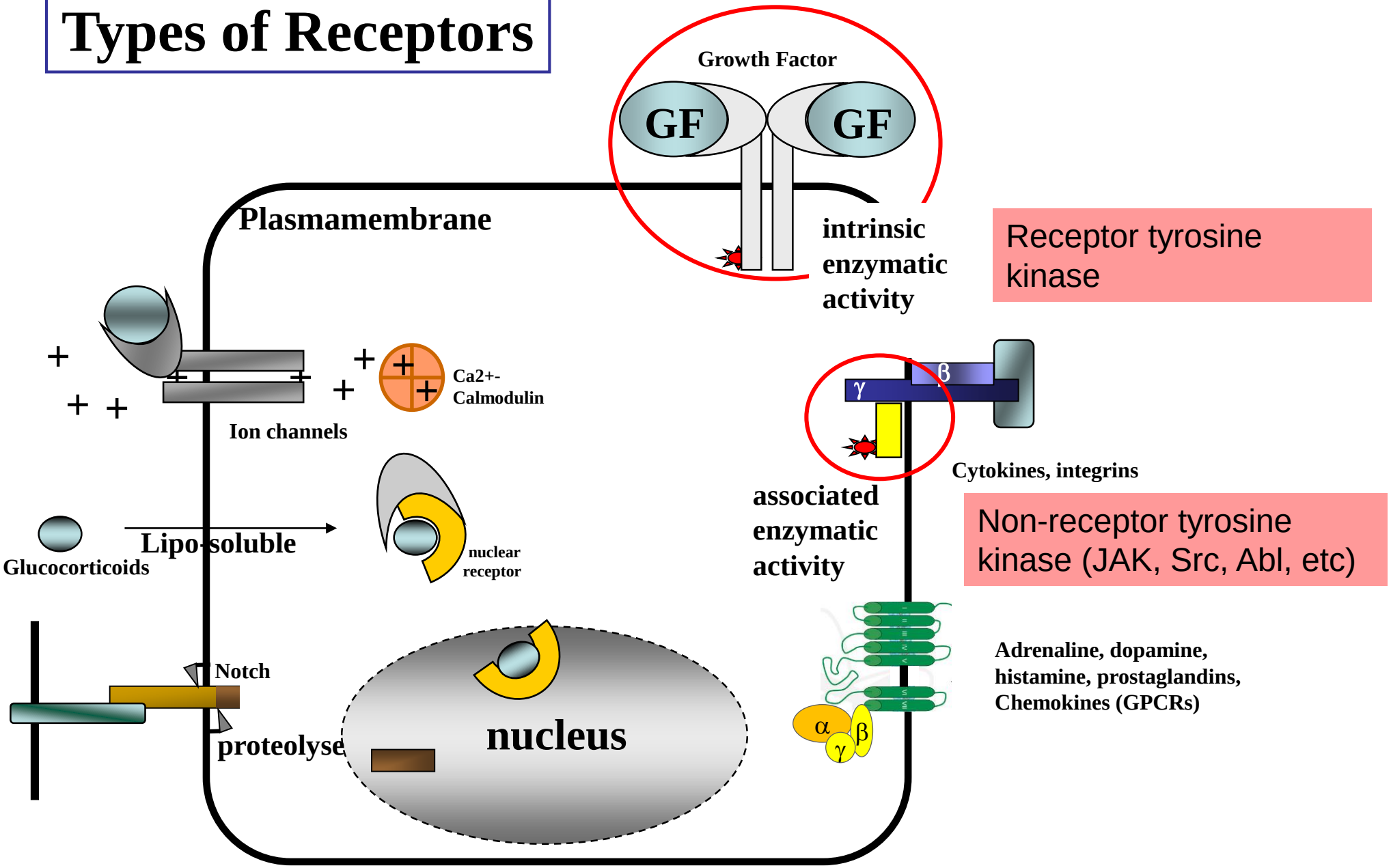
Manning et al. (2002) Science 298, 1912-1934; Hanks (2003) Genome Biol 4: 111

tyrosine kinases (the TK group)



Both, receptor- and non-receptor tyrosine kinase (e.g. EGFR and Abl)

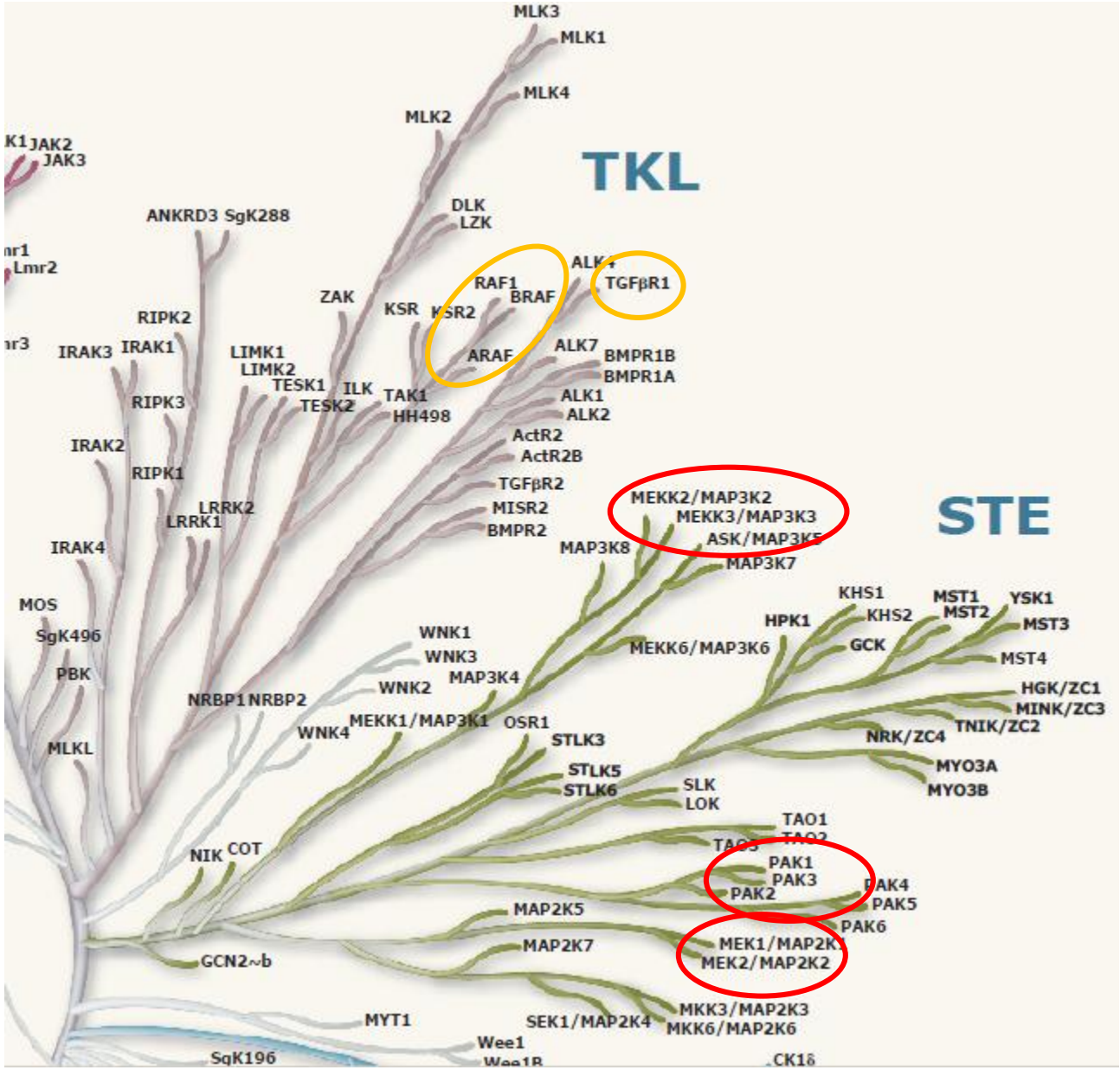
Types of Receptors



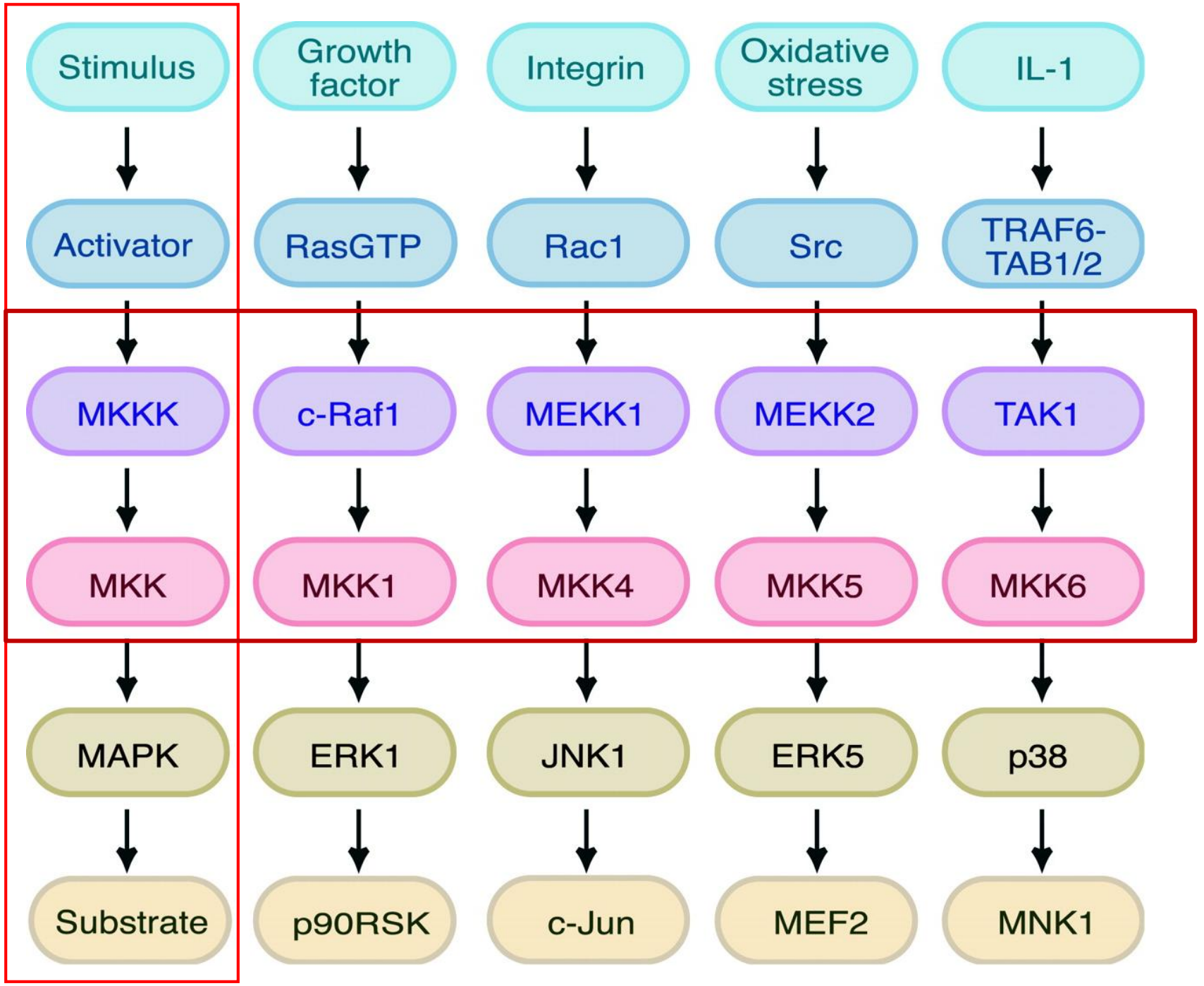
Receptor tyrosine kinase

Non-receptor tyrosine kinase (JAK, Src, Abl, etc)

STE group (yeast **STE**rile mutants-related kinases) and TKL ('tyrosine-kinase like') group



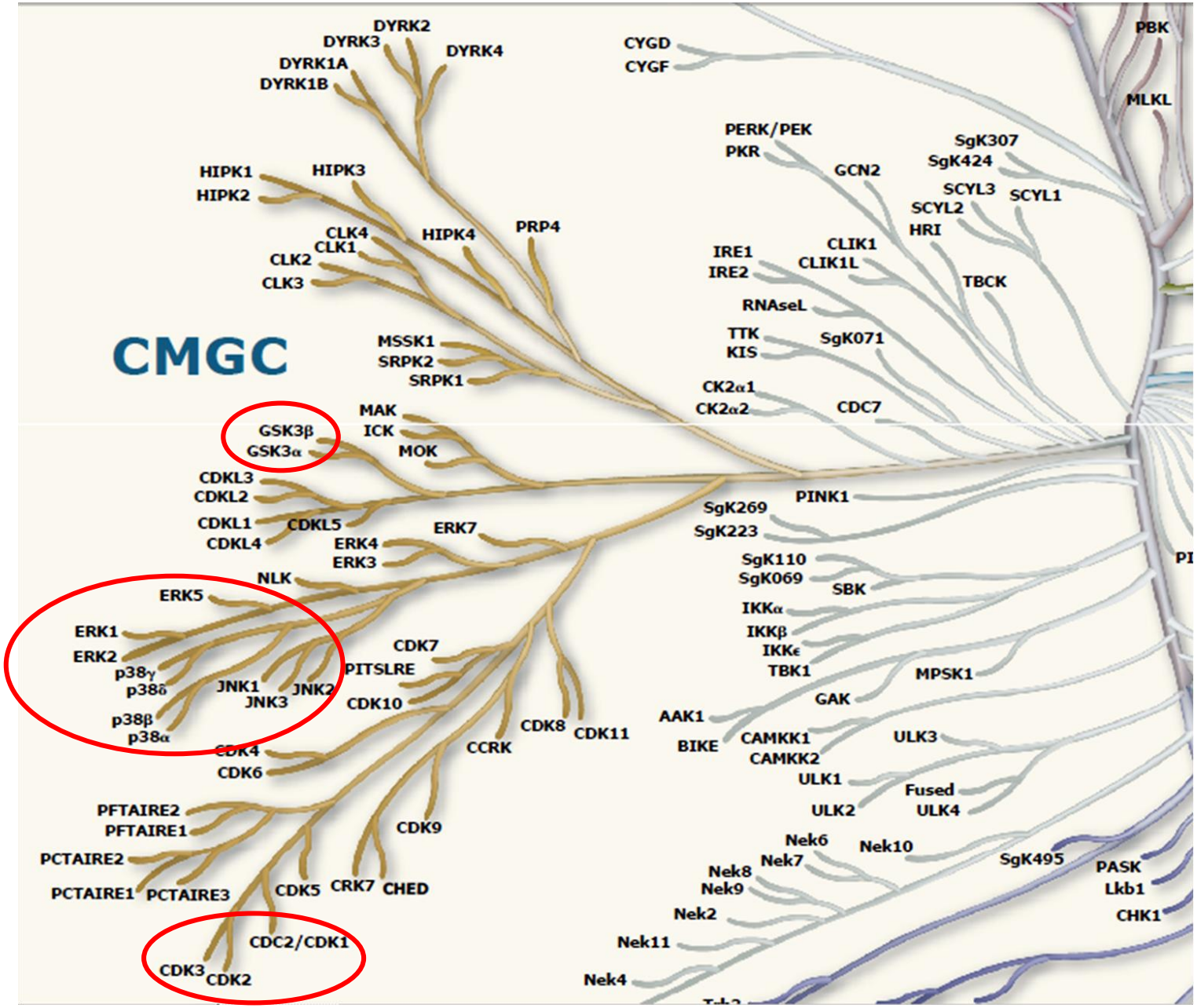
MAPK group members



Four MAP kinase cascades exist

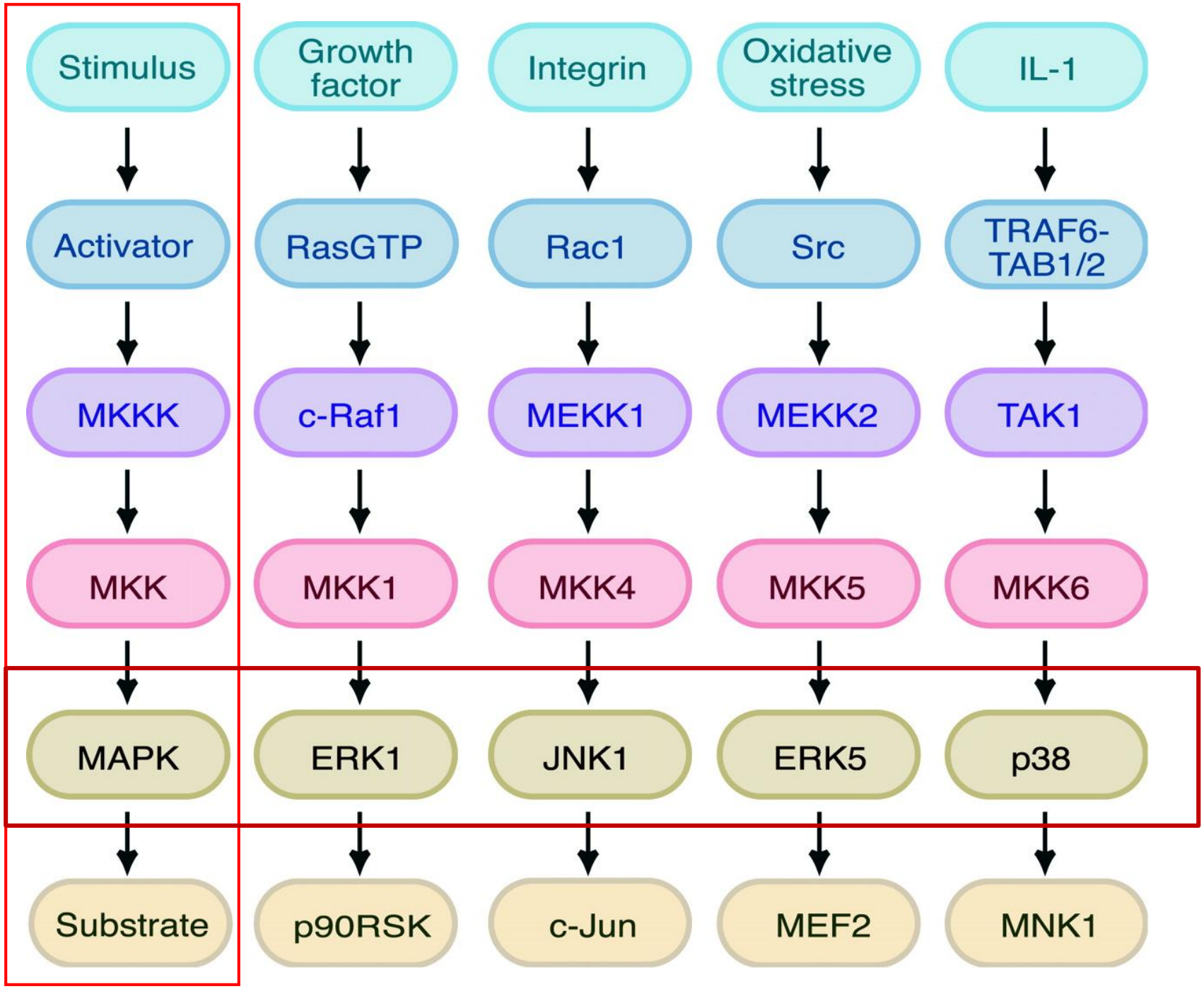
STE group

CMGC group, including the CDK, MAPK, GSK3



Cyclin-dependent kinases

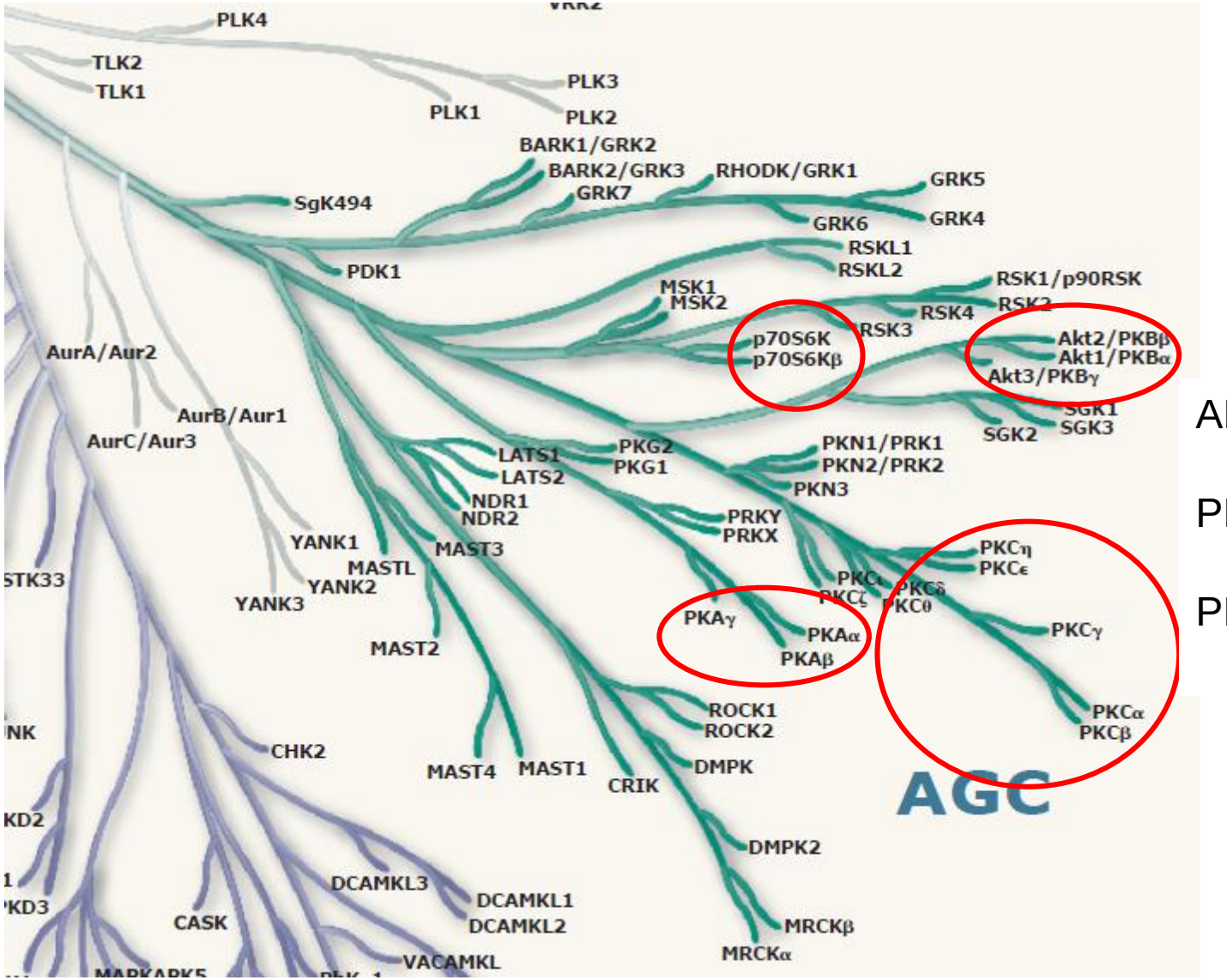
ERK



Four MAP kinase cascades exist

CMGC group

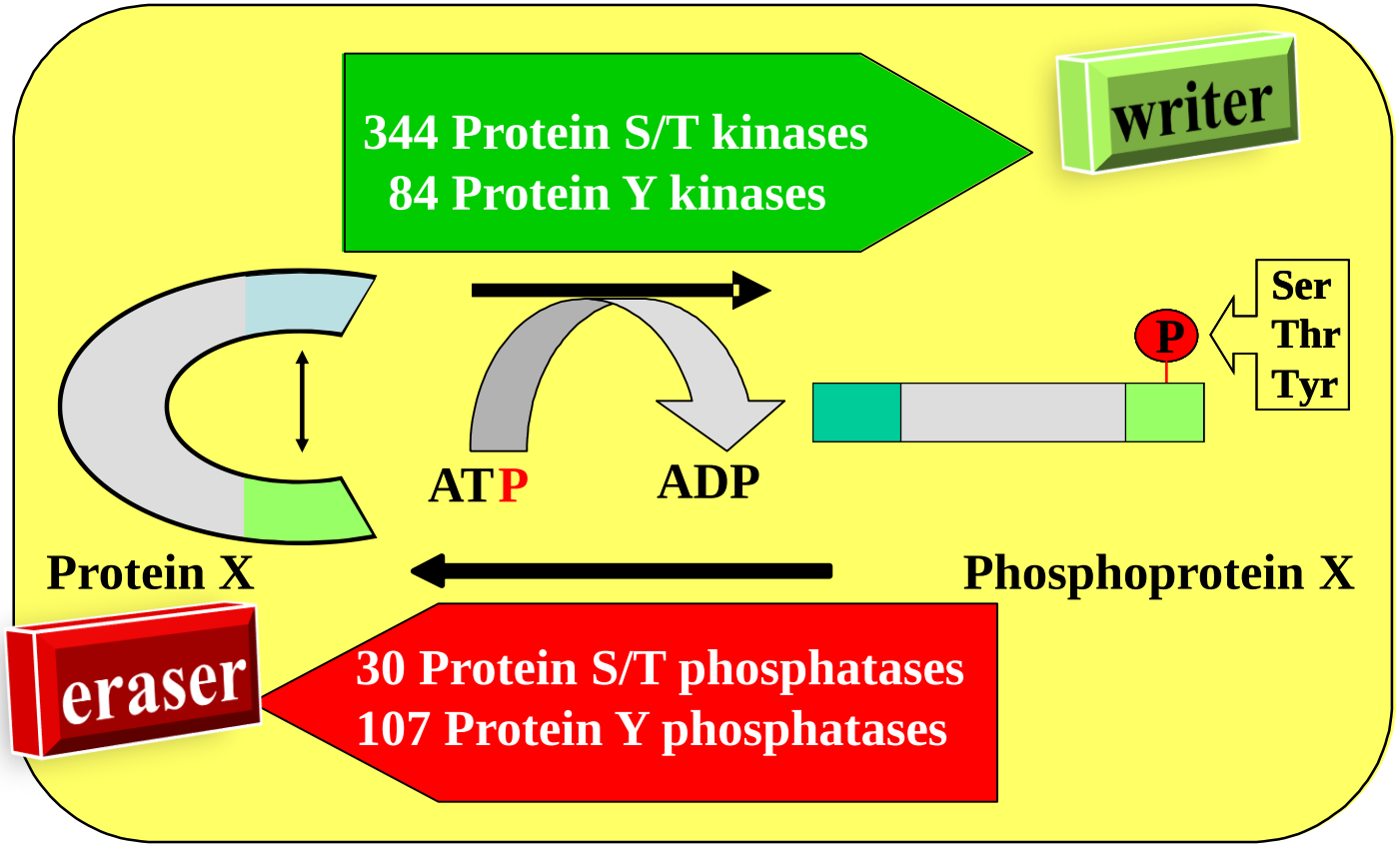
AGC group, cyclic nucleotide- and phospholipid-dependent kinases



AKT- PI3K pathway

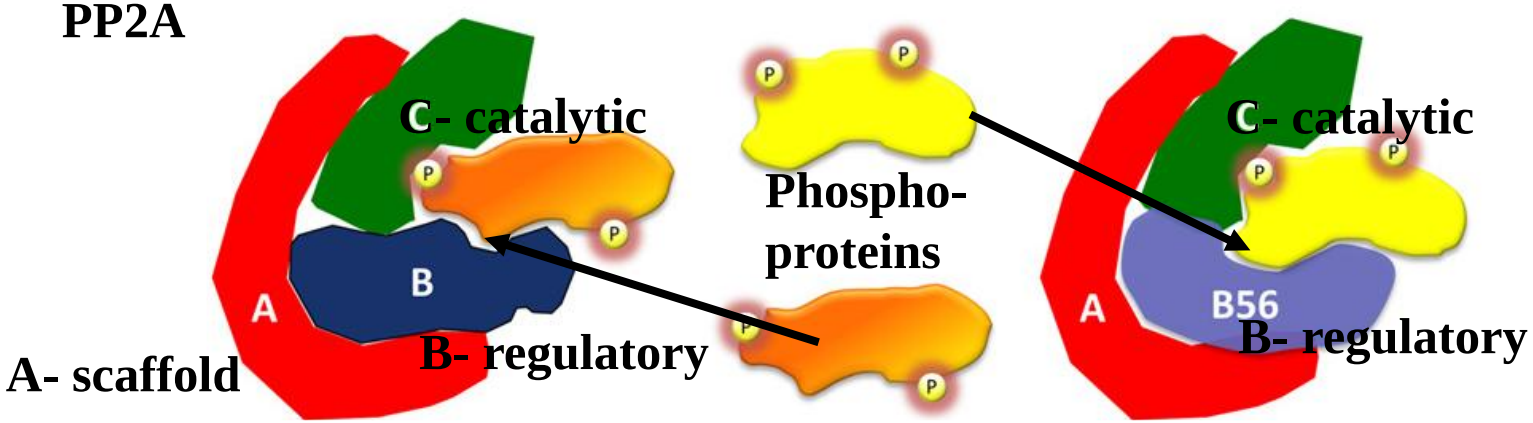
PKA- cAMP-regulated

PKC- DAG-regulated



PTPs have high specificity for their phospho-protein.

PSTPs exhibit low intrinsic sequence specificity. Specific substrate recognition mediated by regulatory subunits

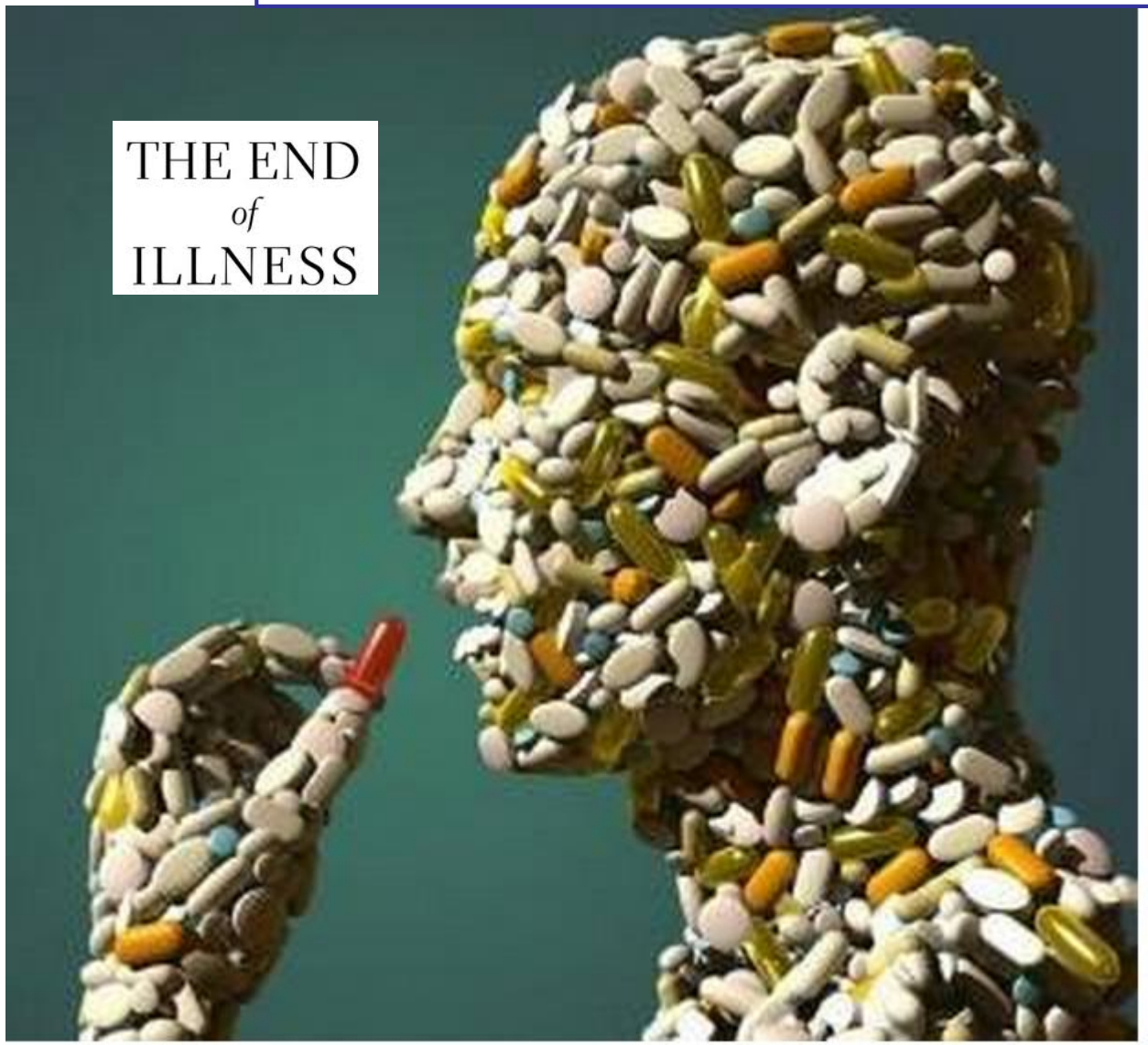


S/T phosphatases are hetero-oligomers of 2 subunits:

- one catalytic (few genes)
- one substrate-specific regulatory (many genes)

Signal Transduction Therapy

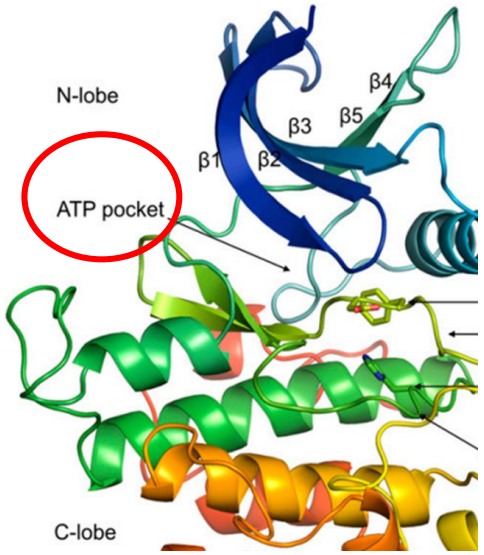
Protein kinase inhibition for disease therapy

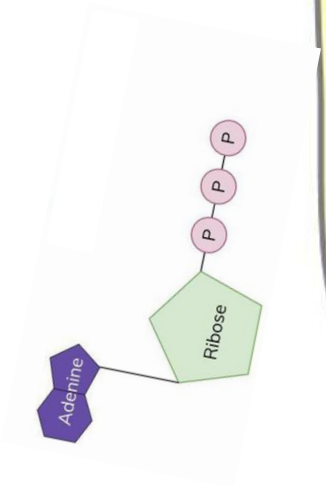


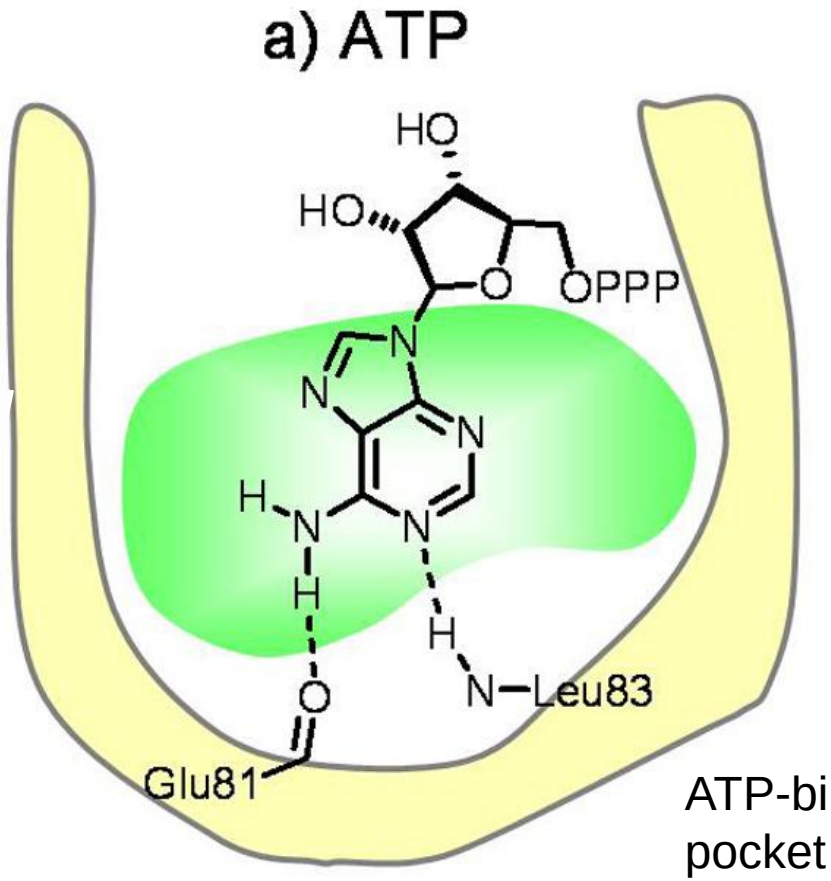
Fast growing list of inhibitors

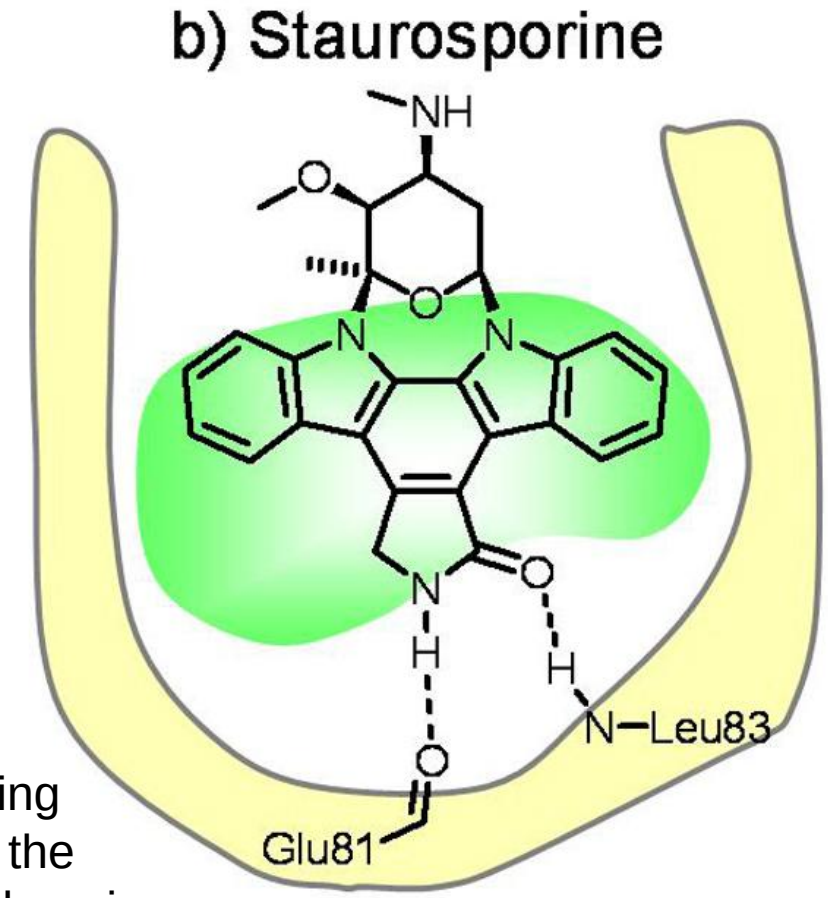
- ~80 FDA-approved in 2024:
- ~180 in clinical trials;
- approximately 30% of current R&D budget spend in pharmaceutical companies;
- only about 10% of kinases have been studied in detail

General protein kinase inhibition by ATP-competitive compounds



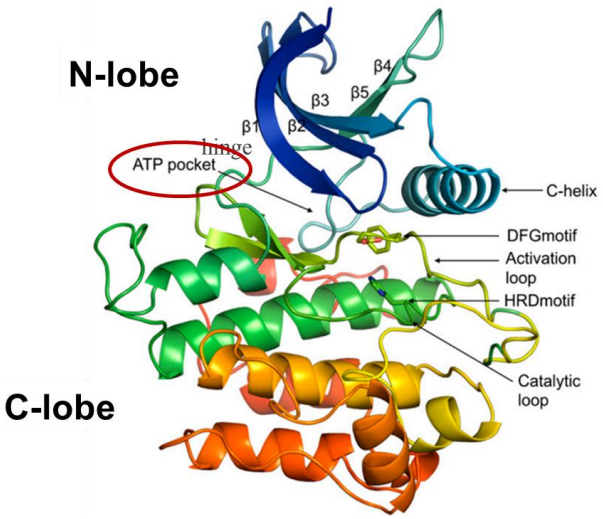




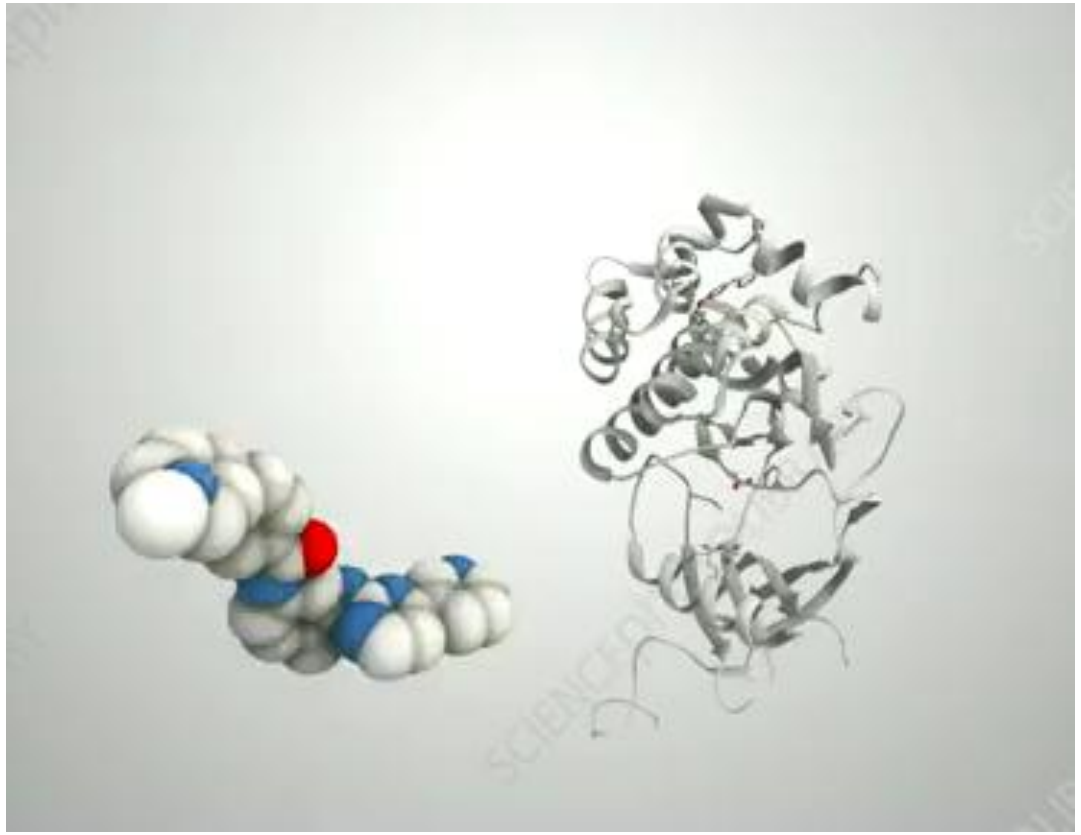


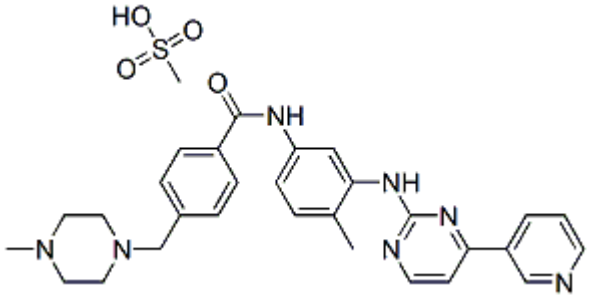
ATP-binding pocket of the catalytic domain

Therapies based on signaling inhibitors



Inhibitor binds to the ATP binding site



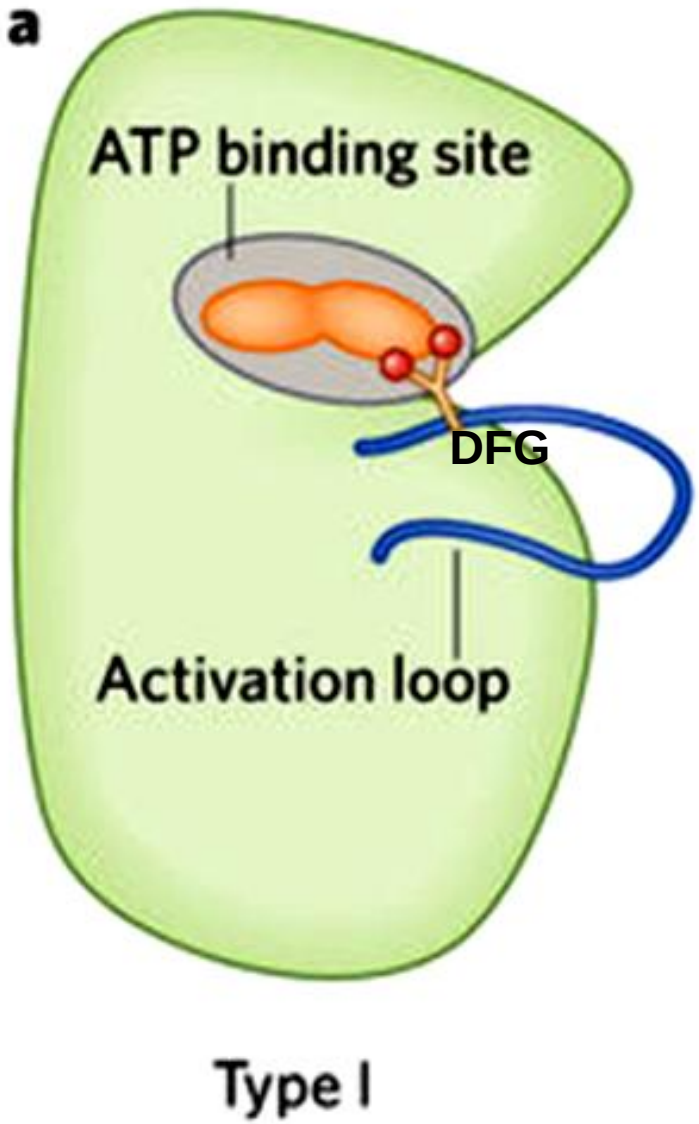


Imatinib or STI-571 or Gleevec

Imatinib
Published in 1996
Clinical trials 1998
FDA approval in 2001.

for the treatment of Chronic myeloid leukemia with constitutively active ABL (due to BCR-ABL fusion gene resulting from translocation t(9;22)(q34;q11))

http://jkweb.mcb.berkeley.edu/external/research-in-progress/5-3/signaling/abl_kinase.html



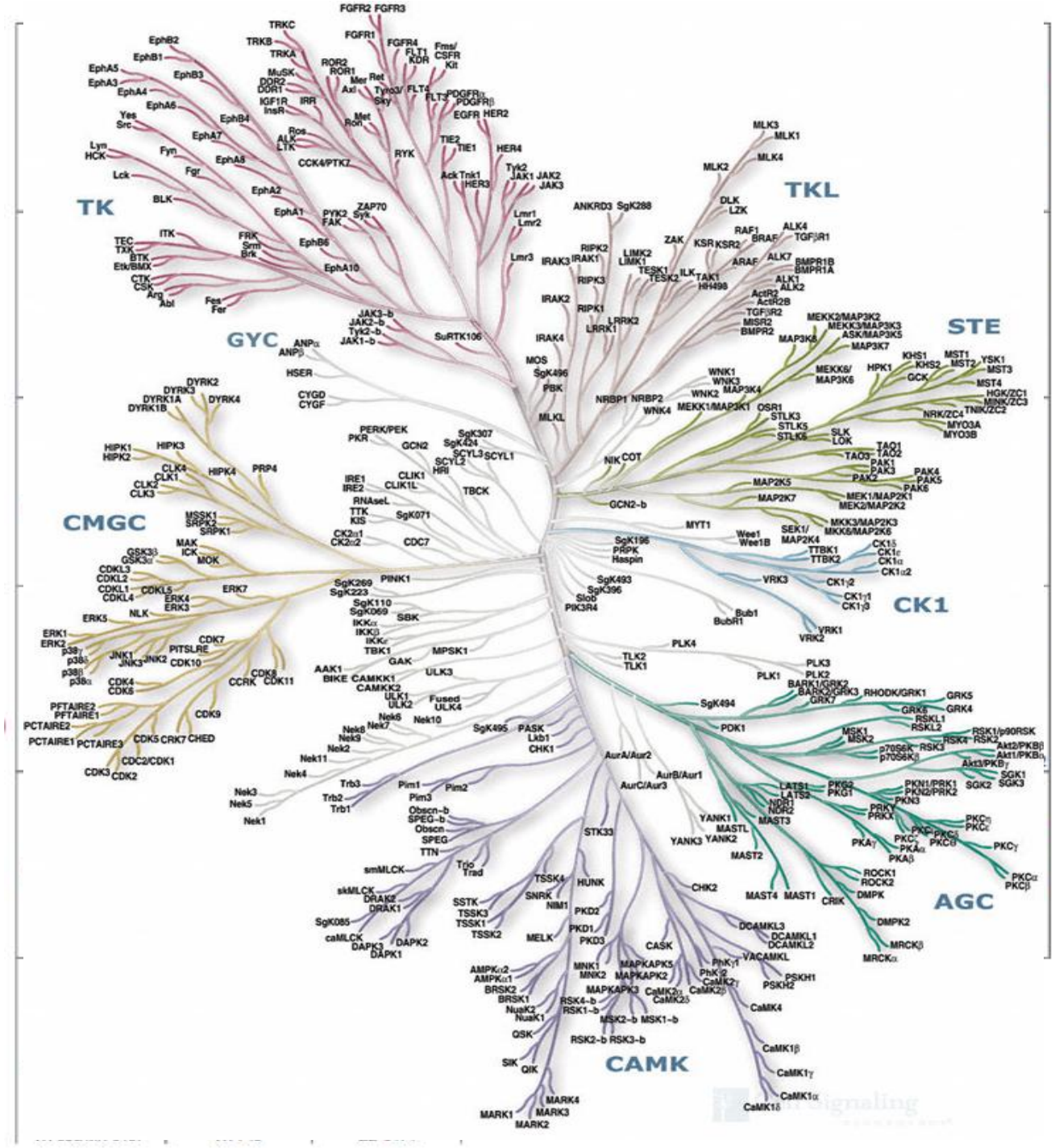
(a) Type I inhibitors (orange)

bind the ATP binding site (gray) of the protein kinase domain (green).

'DFG in' conformation:
(aspartate side chain in the conserved DFG motif at the beginning of the activation loop (blue) faces into the active site).

At present, the majority of clinical and preclinical kinase inhibitors are ATP-competitive

Most ATP binding-site inhibitors have issues ….



Selectivity of protein kinase inhibitors

NATURE BIOTECH 26 (2008)
Measured in *in vitro* protein kinase assays

Selectivity of protein kinase inhibitors

NATURE BIOTECH 26 (2008)
Measured in *in vitro* protein kinase assays

In rare cases, lack of selectivity may be of clinical advantage if

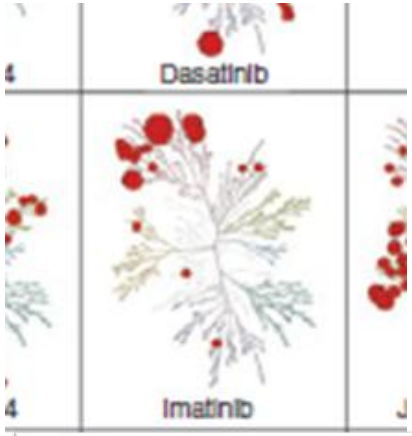
Example 1: the compound may be effective in more than one cancer type

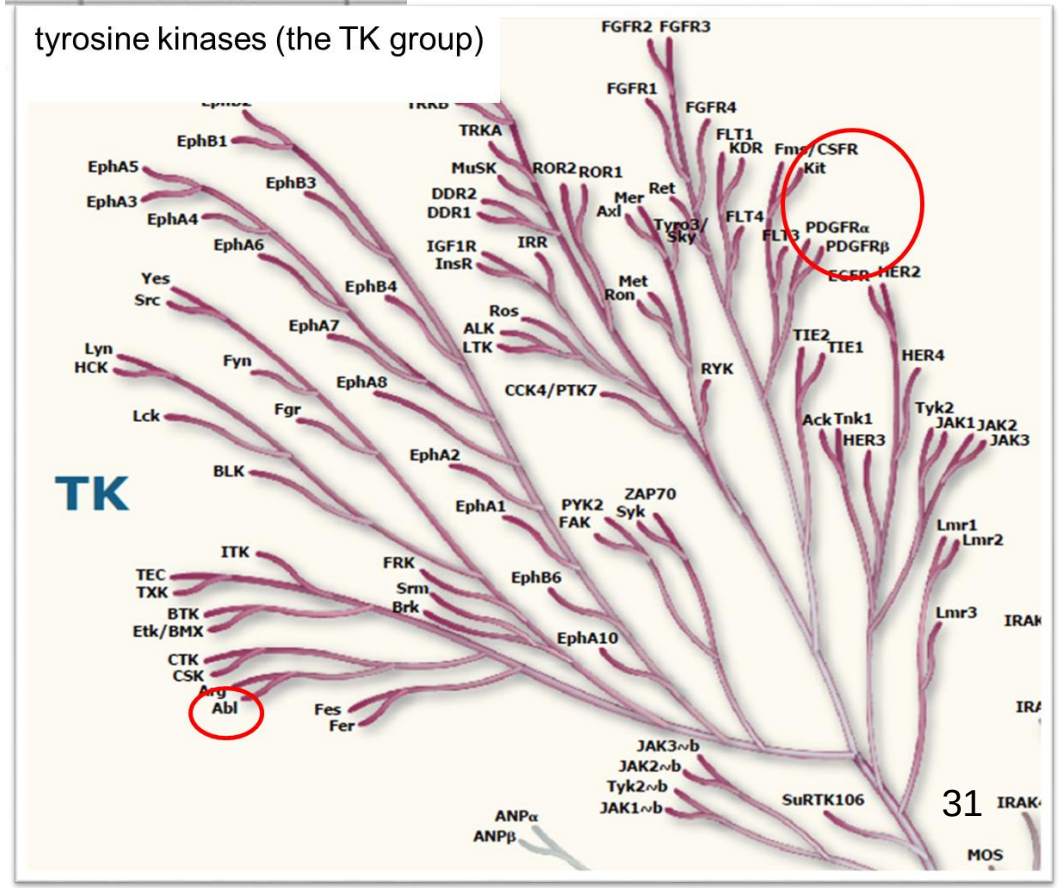
Example 1: Imatinib

Developed to bind to the ATP-binding cleft of ABL non-receptor tyrosine kinase

CML:
Imatinib inhibits BCR–ABL and has transformed CML from a fatal disease into a manageable condition.

Gastrointestinal tumours (GIST):
Imatinib also binds to the receptor tyrosine kinases KIT and PDGF-receptor, which are overexpressed or mutated in GIST





In rare cases, lack of selectivity may be of clinical advantage if

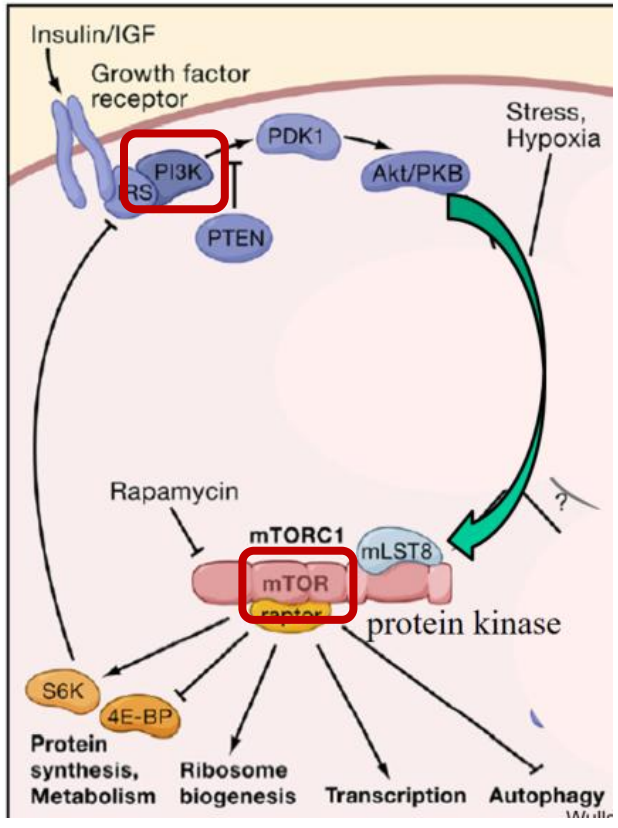
Example 1: the compound may be effective in more than one cancer type

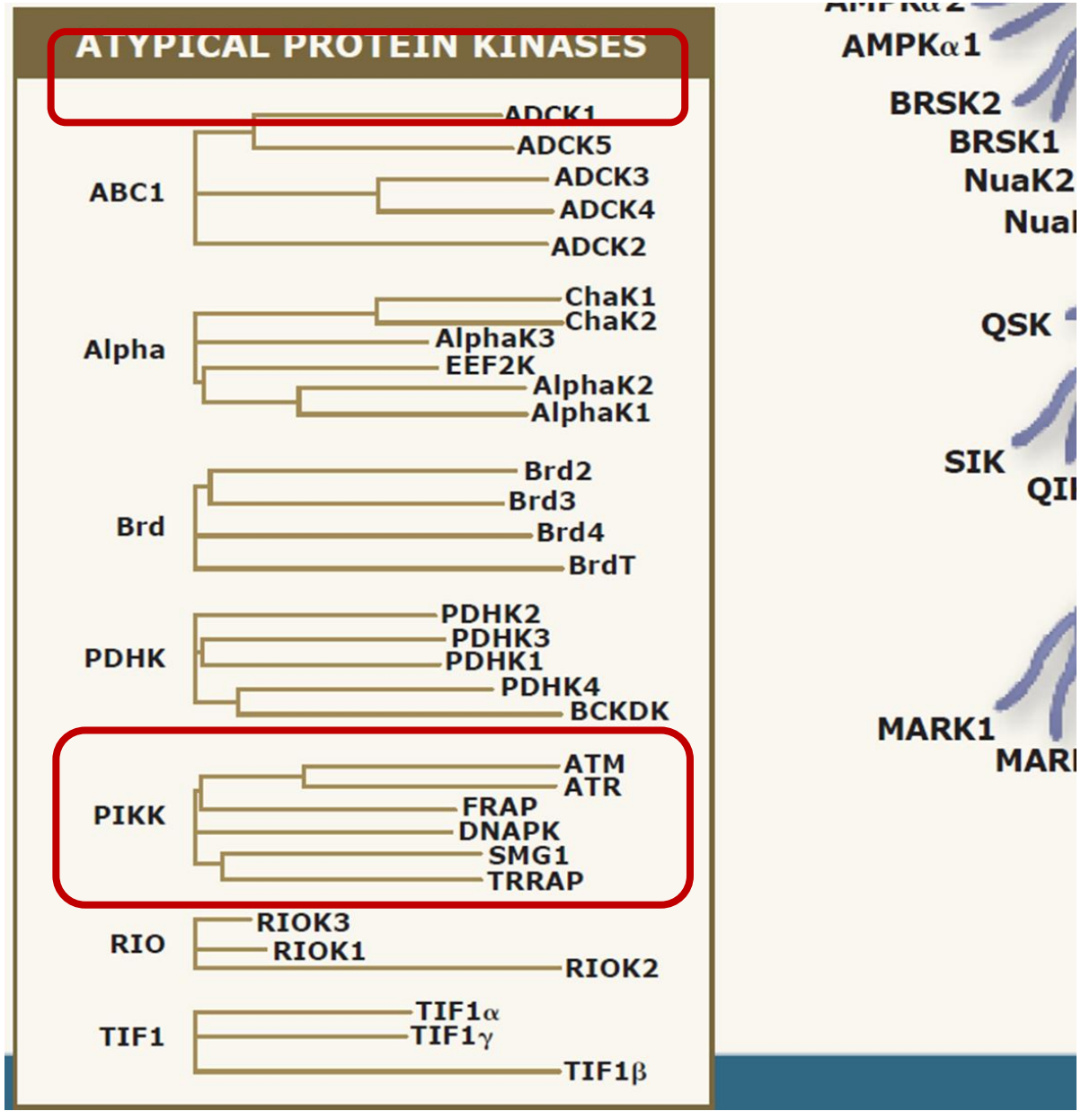
Example 2: the compound inhibits several different kinases in the same signalling cascade (reduces likelihood to develop therapy resistance)

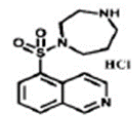
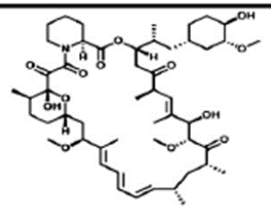
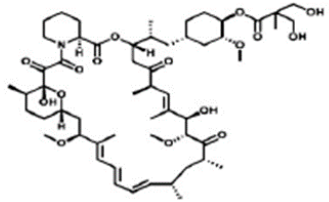
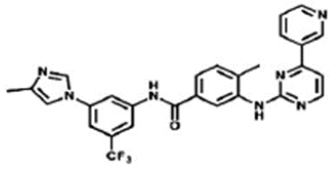
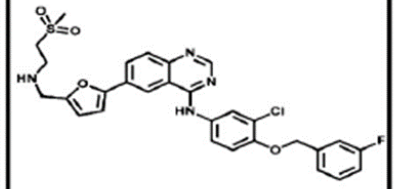
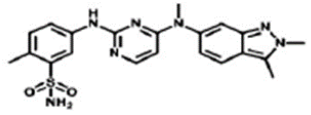
Example 2:
dual PI3K/mTOR inhibitors

(bind to the ATP-binding cleft of PI3K and mTOR kinase)

mTOR (FRAP) shares high sequence similarity in its catalytic domain with PI3K and belongs to a family of PI3K-like kinases (PIKK)

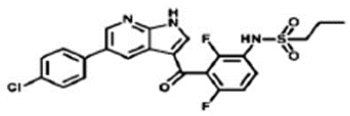
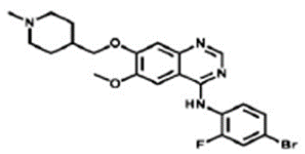
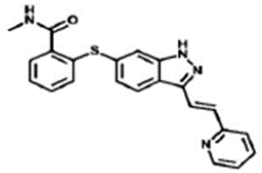
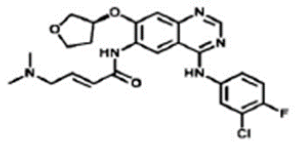
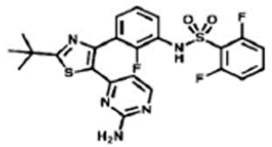
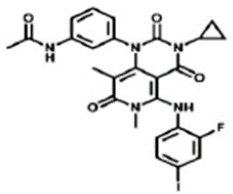




Name	Structure	Reported target	Company	Approved for clinical use
Eril		ROCK	Eisai	1995 cerebral vasospasm (Japan)
Rapamune		mTOR	Wyeth Pfizer	2000 kidney transplantation
Temsirolimus		mTOR	Wyeth Pfizer	2007 advanced renal cell carcinoma
Nilotinib		Bcr-Abl	Novartis	2007 chronic myelogenous leukaemia
Lapatinib		Her2 EGFR	GlaxoSmith Kline	2009 renal cancer
Pazopanib		VEGFR2 PDGFR c-KIT	GlaxoSmith Kline	2009 renal cancer

Some structural examples of approved PK inhibitors

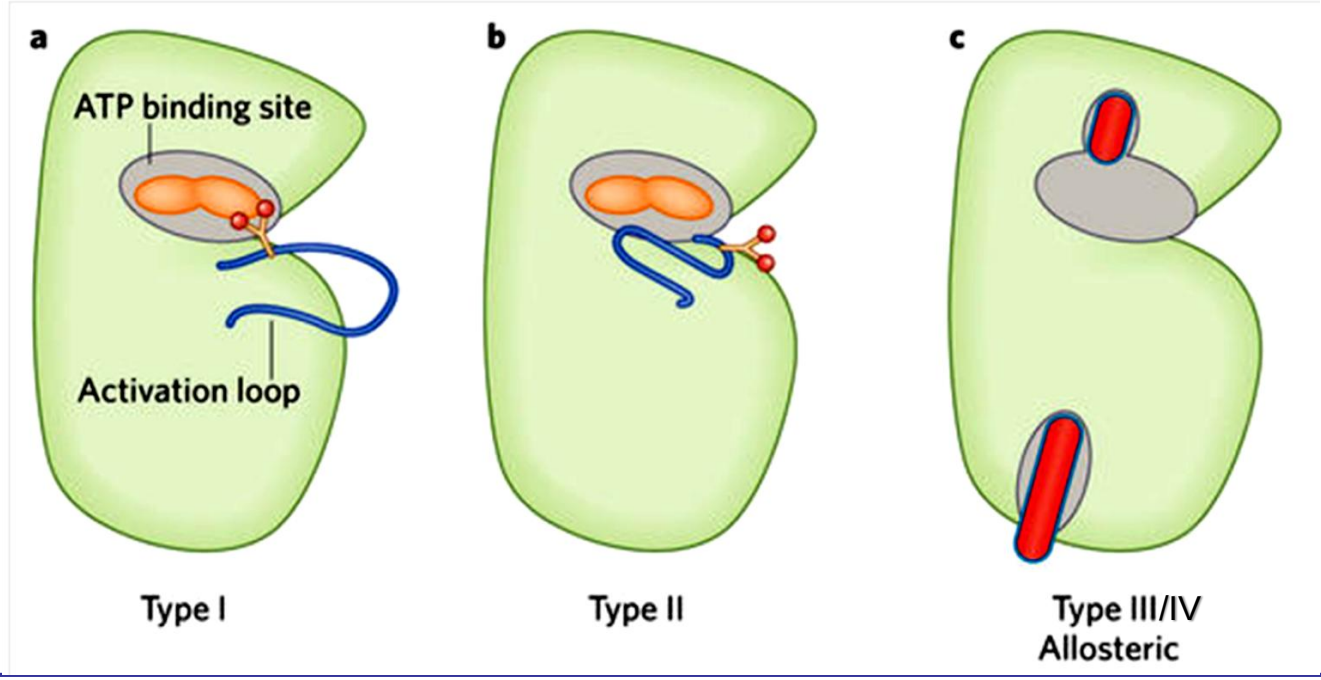
doi.org/10.1021/cb300610s,
ACS Chem. Biol. 2013, 8, 96−104

Name	Structure	Reported target	Company	Approved for clinical use
Vemurafenib		BRAF	Roche	2011 melanoma
Vadetinib		Multiple Tyrosine kinases targeted	Caprelsa IPR Pharms	2012 thyroid cancer
Axitinib		VEGFRs PDGFRB c-KIT	Pfizer	2012 renal cell carcinoma
Afatinib		Her2 EGFR	Boehringer Ingelheim	Not yet NSCLC
Dabrafenib		BRaf	GlaxoSmith Kline	Not yet metastatic melanoma
Trametinib		MEK1/2	GlaxoSmith Kline	Not yet metastatic melanoma

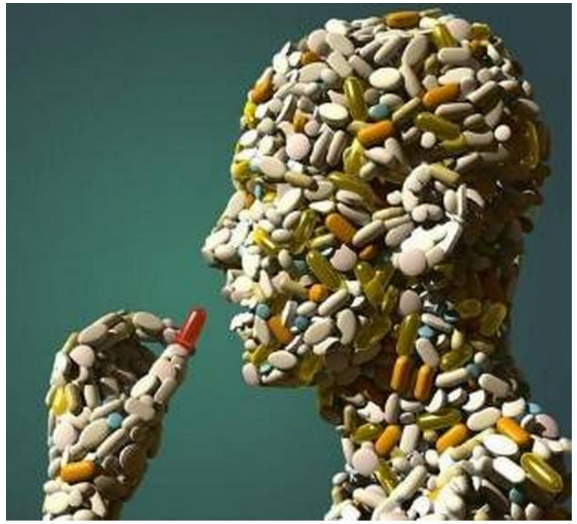
doi.org/10.1021/cb300610s,
ACS Chem. Biol. 2013, 8, 96−104

Nature Chemical Biology **10**, 796–797 (2014) | doi:10.1038/nchembio.1630

More recent review: https://doi.org/10.1016/j.jbc.2022.102247



(**a**) Type I inhibitors (orange) bind the ATP binding site (gray) of the protein kinase domain (green). The aspartate side chain in the conserved DFG motif at the beginning of the activation loop (blue) faces into the active site ('DFG in').
(**b**) Type II inhibitors stabilize a flipped inactive conformation of the DFG motif in which the aspartate side chain faces outwards ('DFG out').
(**c**) Allosteric ligands (red) bind to binding pockets that do not overlap with the active site of the kinase. These binding pockets can be adjacent to or distant from the active site



THE END
of
ILLNESS
??

Therapeutic obstacles

- **Thorough characterization of the molecular pathways is required**

- **Side-effects due to a requirement of the targeted kinase or signalling molecule in other physiologically important functions**

- **Off-target effects of inhibitors (on other PKs or ATP-binding proteins) due to lack of specificity**

- **Emergence of resistance (e.g. through mutations that allow the protein to escape the inhibitor action)**

How to study and measure protein kinase activity?

In vitro protein kinase assays for inhibitor development

Screen chemical compounds in an *in vitro* assay measuring the activity of a specifc PK

Profiling of an identified compound against ~200 other PKs in in vitro assays

Selectivity of protein kinase inhibitors

NATURE BIOTECH 26 (2008)
Measured in *in vitro* protein kinase assays

1. In vitro protein kinase assays to measure activity

Assay component	comment
Buffer	HEPES or TRIS-based, pH 7.5 (why no phosphate buffer ??)
Co-factors	10 mM $MgCl_2$ (or $MnCl_2$)
ATP	**0.1 -0.5 mM**
Substrate	Recombinant protein or synthetic peptide
Kinase	Recombinant or immunoprecipitated
Radioactive ATP*	**2.5-5 mCi**

Incubation: 30-50 μL volume, 30-37ºC, 30 min

* Nowadays detection kits based on colorimetric enzyme assay for ATP consumption

Stop by adding 2x SDS sample buffer; analyze e.g. by SDS-PAGE ± Western blot

Controls in the protein kinase assays

The problems:

1: Recombinant substrate proteins and protein kinases are usually purified from cell or bacterial lysates,
thus, they may not be pure but contain residual amounts of other proteins (kinases or substrates)

2: Some cellular proteins contain ATP binding pockets
that will retain radioactive ATP (eg BSA, Hsp90, ABC transporters)

The solutions:

-- Control tube containing a kinase-dead version of the kinase to be tested.

-- Control tube containing only: substrate protein, reaction buffer and radioactive ATP but not the kinase