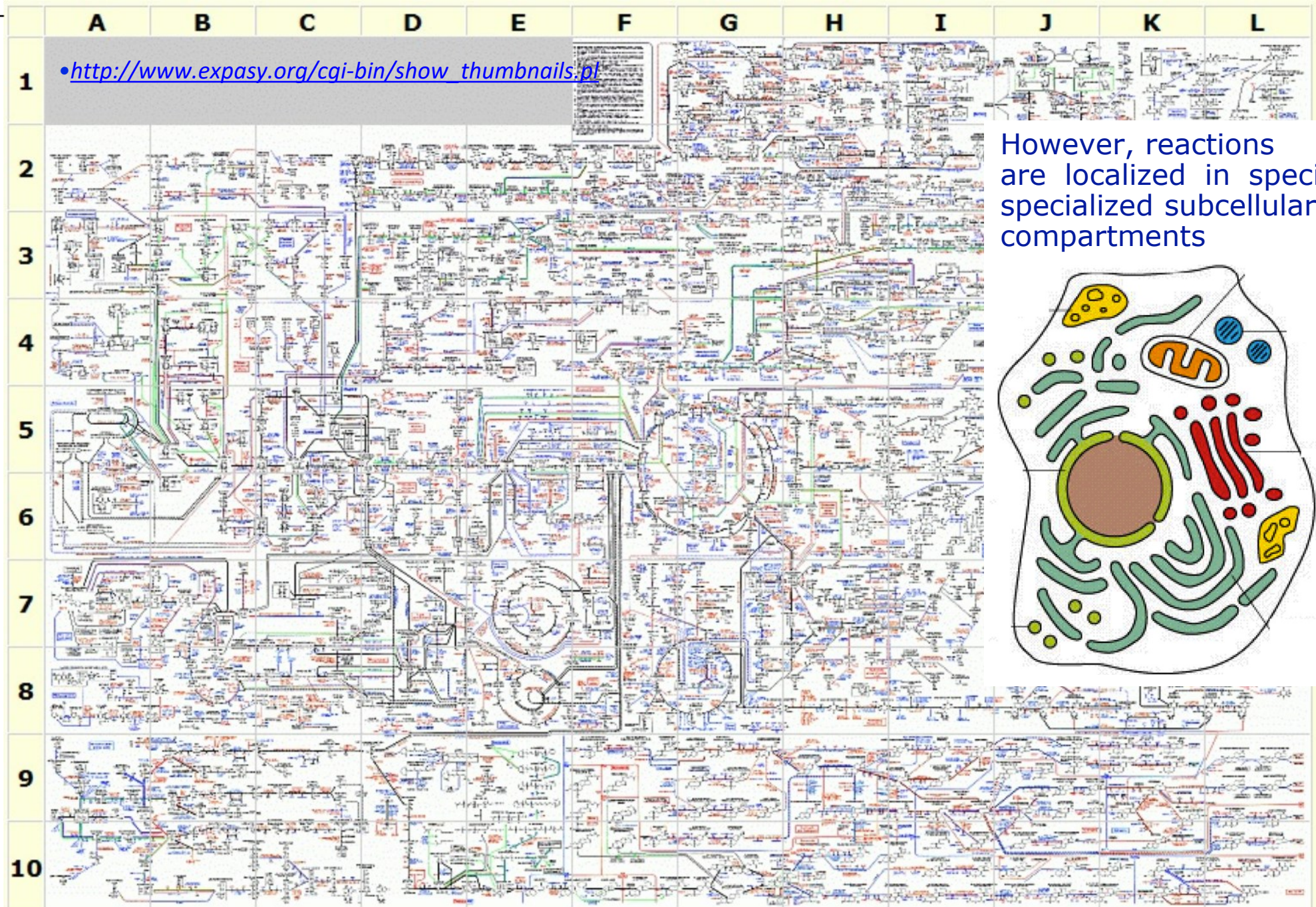
Protein domains mediating interactions

W_03 • Protein - Protein interactions domains
Scaffolds and co-incidence
PPI's and networks

W_04 • Protein - Membrane
Lipid-binding domains
Manipulation of location
Activation

"the right molecules at the right place at the right time"

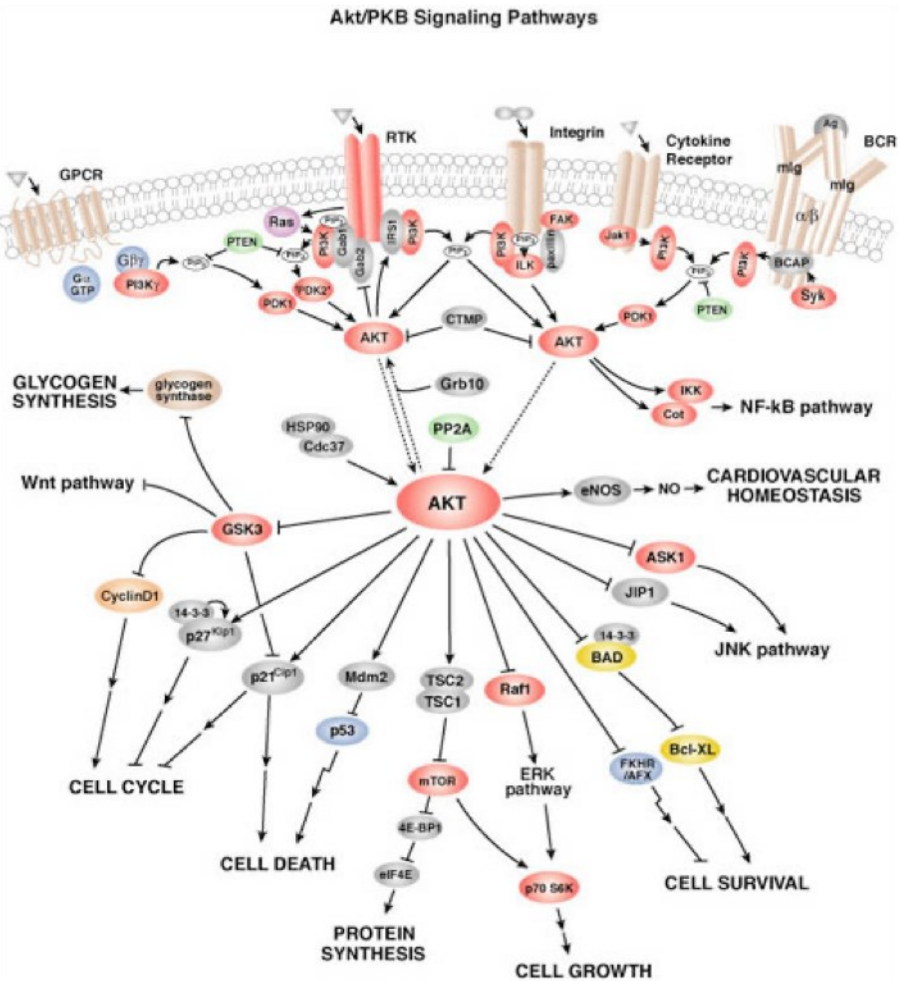
Metabolic pathways: The early days of Biochemistry

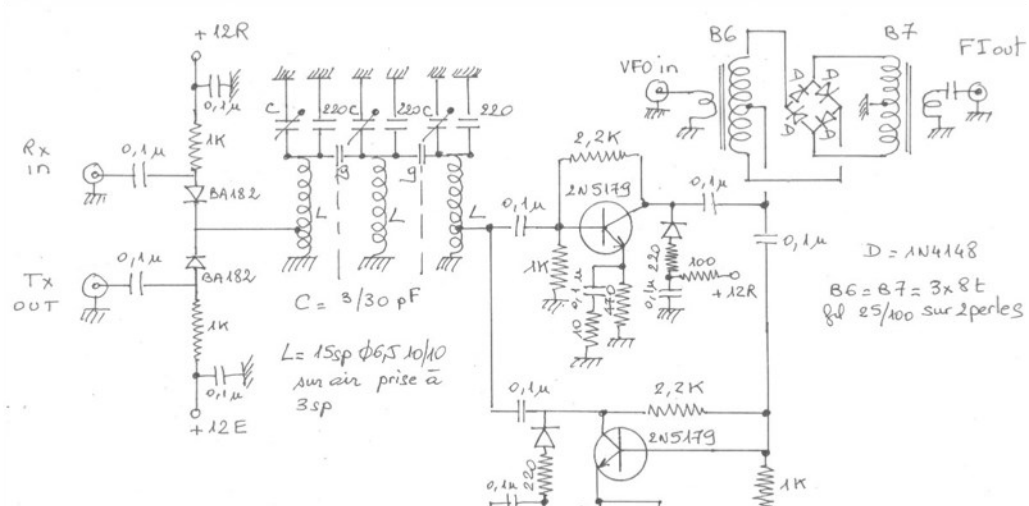


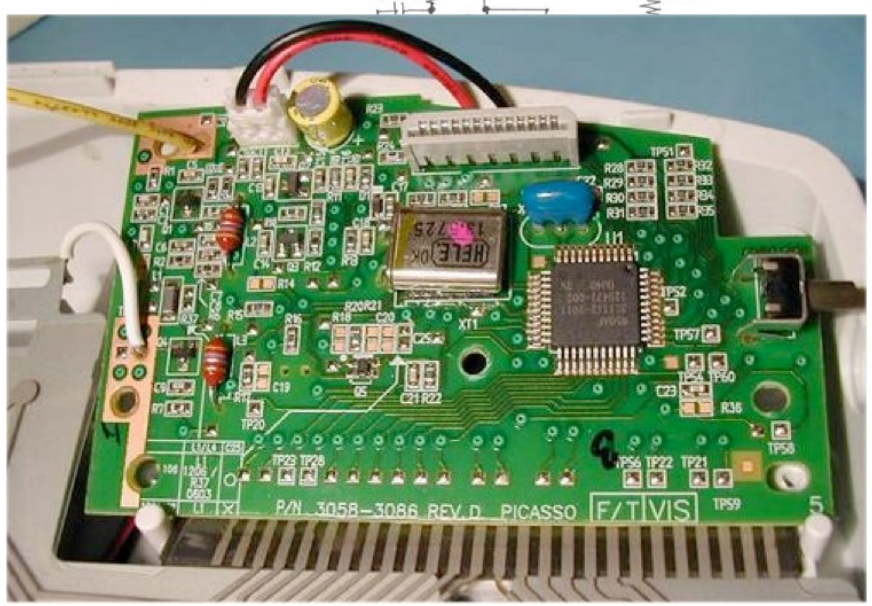
However, reactions are localized in specific specialized subcellular compartments

Cellular signalling - Biological networks

- The cell is extremely complex
  - many intertwined multi-dimensional networks of signalling and metabolism
  - a strong **spatial & temporal organisation** of **molecular interactions**

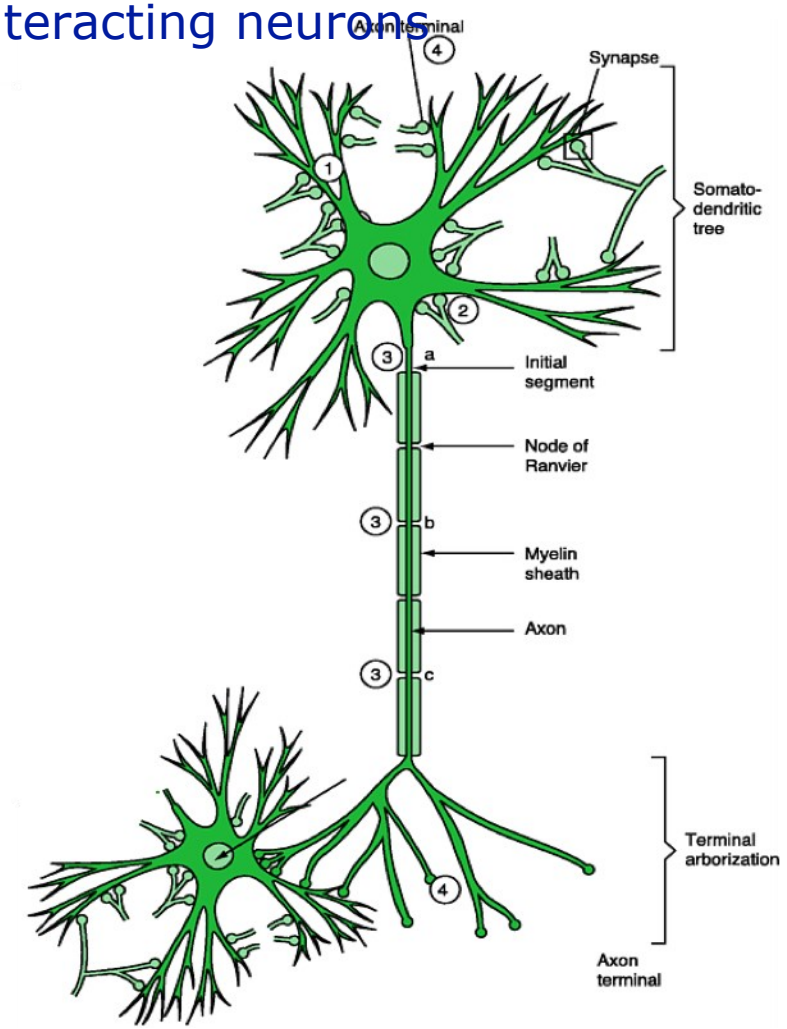




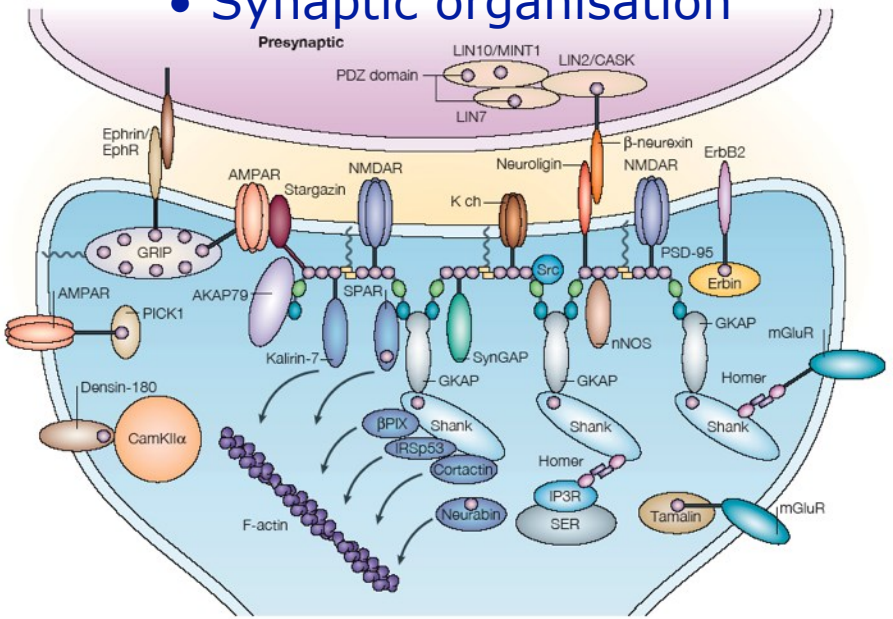


Cellular signalling - Molecular organisation

- Interacting neurons



- Synaptic organisation



Signalling events within a cell
- Right place
- Right time
- Proper arrangement
- Specificity & right partners

through ***reversible*** and **adjustable localised** molecular interactions.

Hammond "Cell & Mol Neurobiology"
Kim, Nat Rev Neurosci 5 (2004) 772

Space & time in signalling: A theoretical example

E.g. for the phosphorylation state of protein M (purple hue indicate [M-Pi].

- the phosphatase **Phos** is homogeneously distributed in the cytosol, but
- the kinase **Kin** is bound to

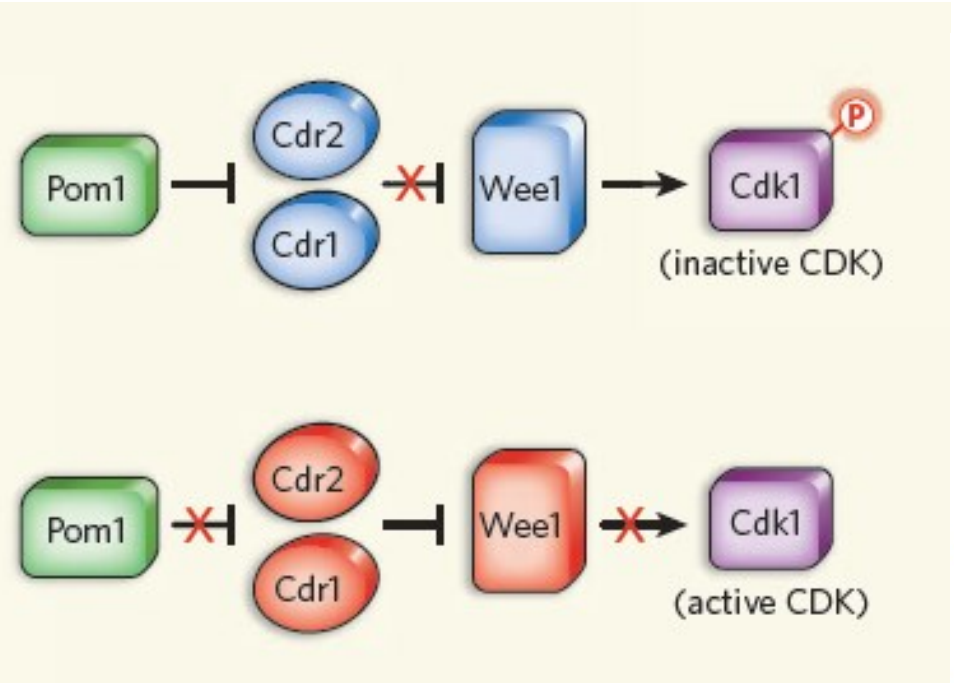
a central structure or the plasma membrane

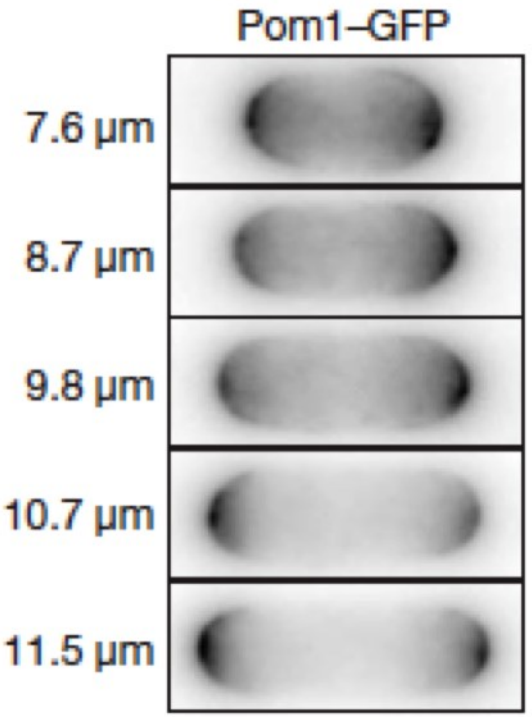
Kholodenko, Nat Rev Mol Cell Biol 2006

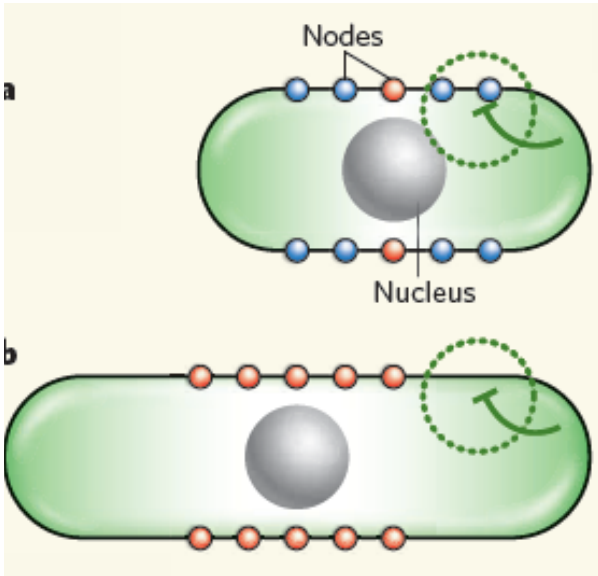
Space & time in signalling: A cellular mechanism

Regulation of cell division of yeast.

Observations:
- Only big cells divide
- All components involved seem to be present continuously
- When nuclear Cdk1 is active => cell division
- Pom1 (green) inhibits Cdk1 via a cascade







Moseley, Nature 2009

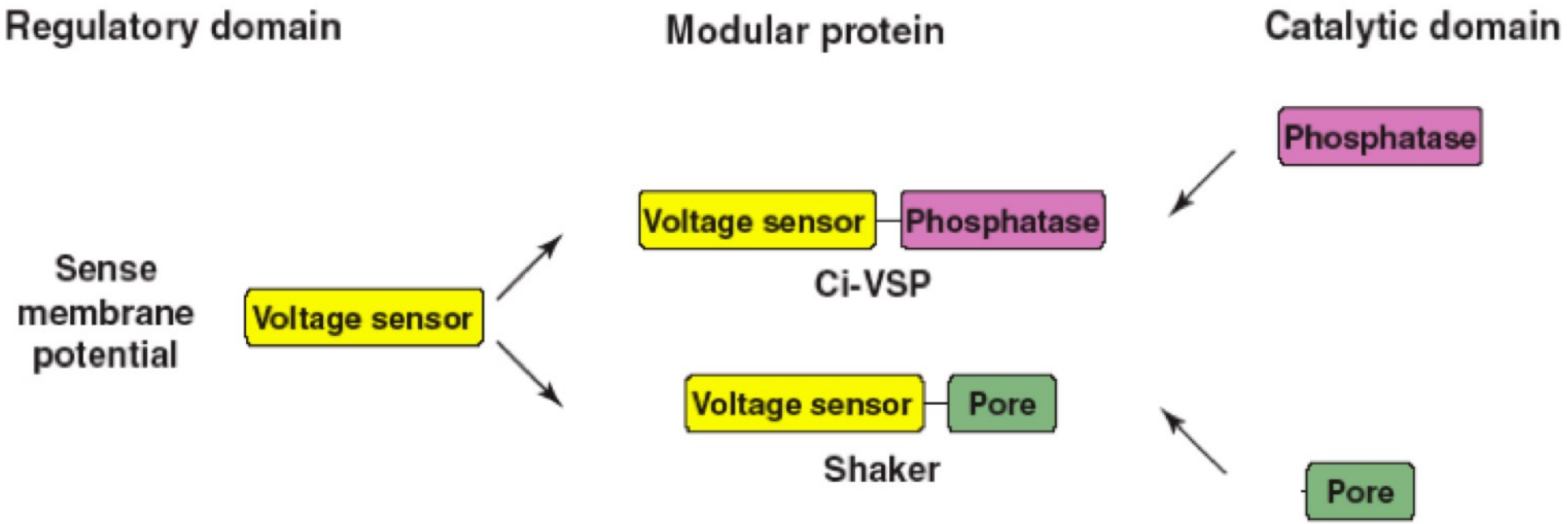
Proteins : Modular assembly of functional domains

- Domains: - 50-150 residues that fold autonomously
  - specific functionalities
  - > 1’000 types of domains in genome

- Eukaryotic proteins comprise often several domains
  - a combinatorial approach yields shear infinite possibilities

An example: Protein Kinase C

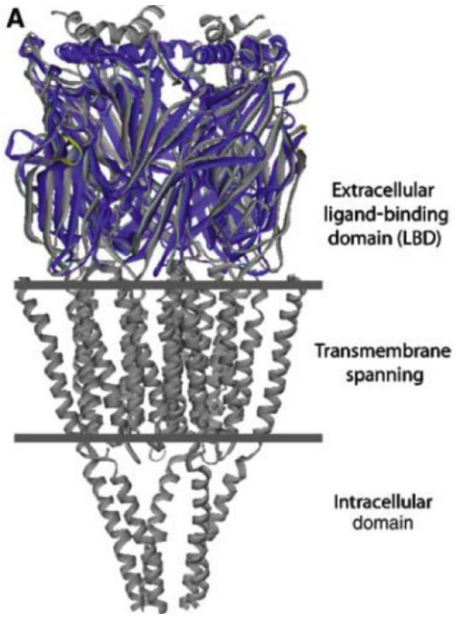
Combination of domains with different functions



Ligand-gated ion channels: binding domain ->

channel domain ->

intracellular domain ->



Bhattacharyya Ann Rev Bc 2006

Many domains have been identified

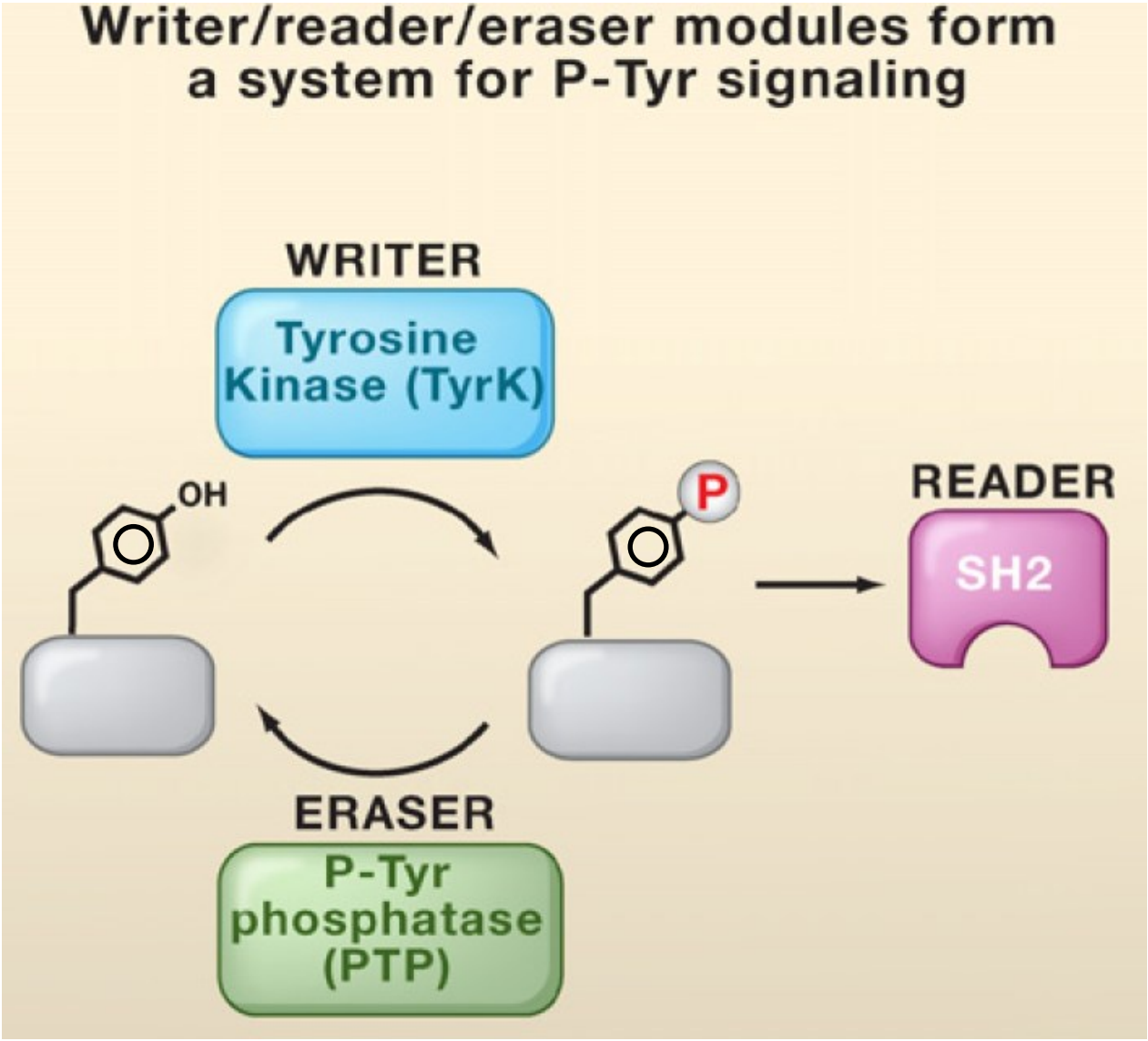
Examples of protein domains involved in molecular recognition:

Domain	**Generally recognised sequence**	**Abundance in human genome**
SH2	-**p**Y-x-x-hydrophobic-	352
PTB	-hydrophobic-x-N-P-x-**p**Y-	141
SH3	-P-x-x-P-x	894
WW	-P-P-x-Y-	307
14-3-3	-R-S-x-**p**S-x-P-	19
PDZ	-E-S/T-D/V- C-terminus	918
PH	phospholipids	±250
C2	phospholipids	641

NB: human genome encodes approximately 3.10^4 proteins

M-M Zhou, Bhattacharyya Ann Rev Bc 2006

Phosphorylation : A common signalling motif



Writers:

- Kinases :

  activity is

  context-dependent

Erasers:

- Phosphatases :

  activity is often

  constitutive

Readers :

- Proteins with

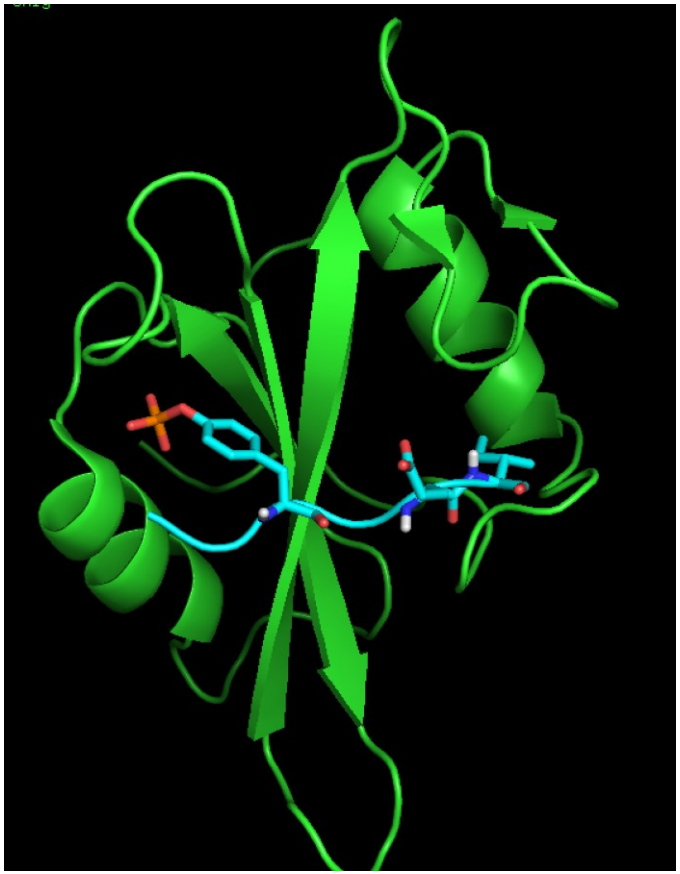
  specific domains

Lin (2010) Cell 142, 661

Example: SH2 domains recognising pTyr

Recognized sequence:
-**Y^{P}**-x-(x)-**hydrophobic**-

- Deep binding site for Y^{P} containing 1 or 2 R's
  +
  multiple H-bonds



Binding "site" for subsequent residues

==> Affinity of SH2-domains for pTyr ranges from 10^{6} to 10^{9} M^{-1}

==> Affinity of non-phosphorylated is ~1'000-fold lower

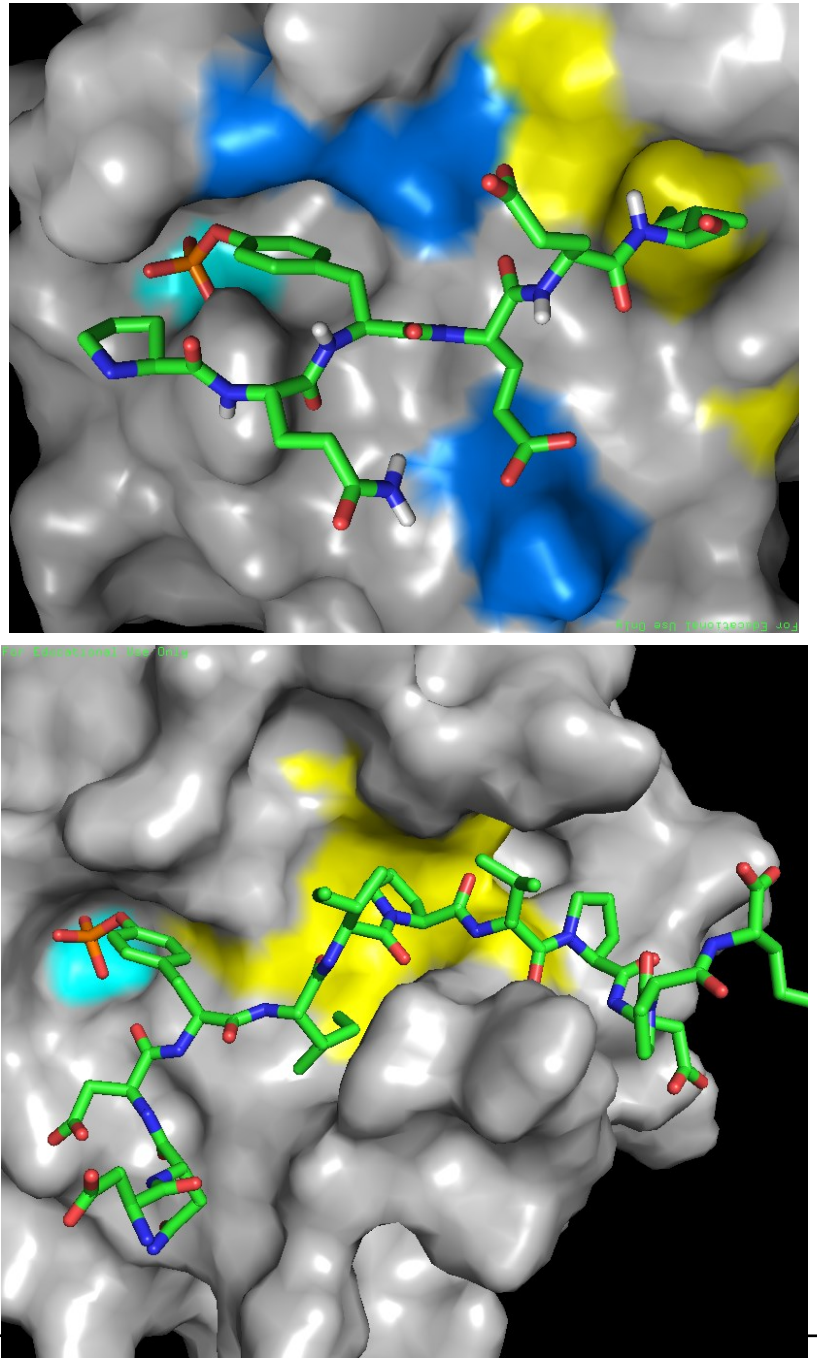
Example: SH2 domains recognising pTyr

Recognized sequence:
-**Y^{P}**-x-(x)-**hydrophobic**-

- Conserved overall structure
- Peptides positioned similarly
- Interactions
  - Y^{P}—-R
  - polar
  - hydrophobic

top:
Src SH2 domain 1SPS.pdb :
PQY^{P}EEI

bottom:
PLCγ SH2 domain 2PLD.pdb :
DNY^{P}IIPLPDPK



How to modulate enzyme activity ?

Enzyme catalytic site

- Regulation of activity - Only activity under certain conditions

Bhattacharyya Ann Rev Bc 2006

Scaffolds - regulation and integration

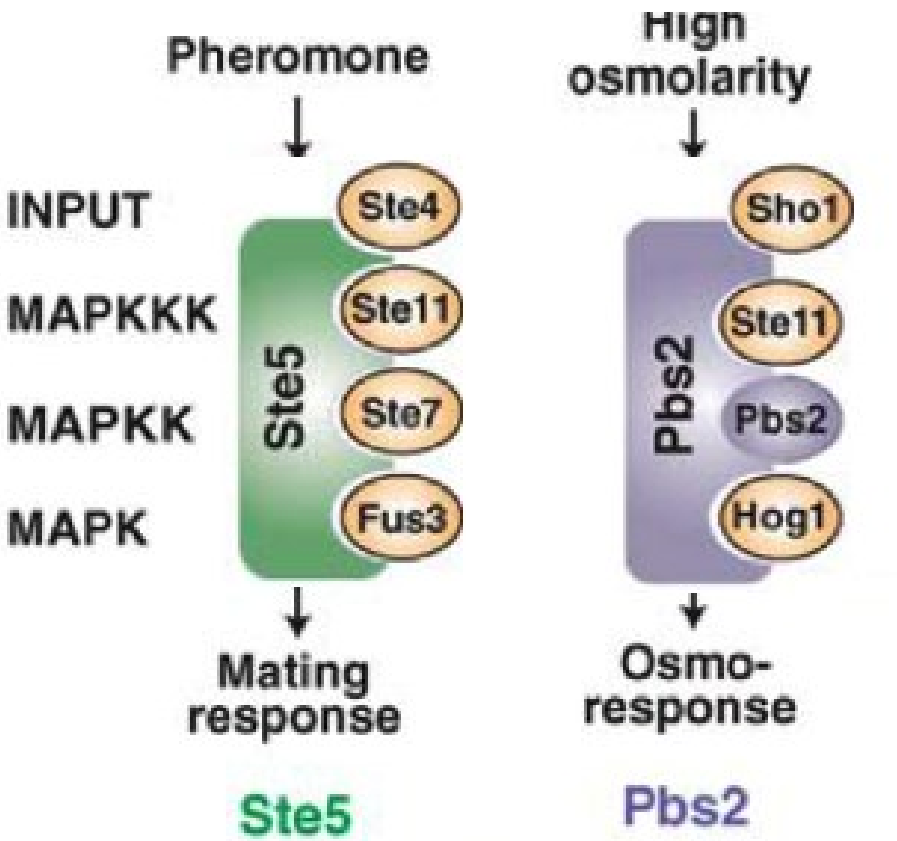
Adaptors & scaffolds: allow certain proteins to get together in a specific orientation and order at a specific site in the cell

ning

Zeke TiCB 2009

Scaffolds - regulation and integration

Comparison of pheromone and osmo-sensing in yeast
- organisation by different scaffolds
- different cellular response

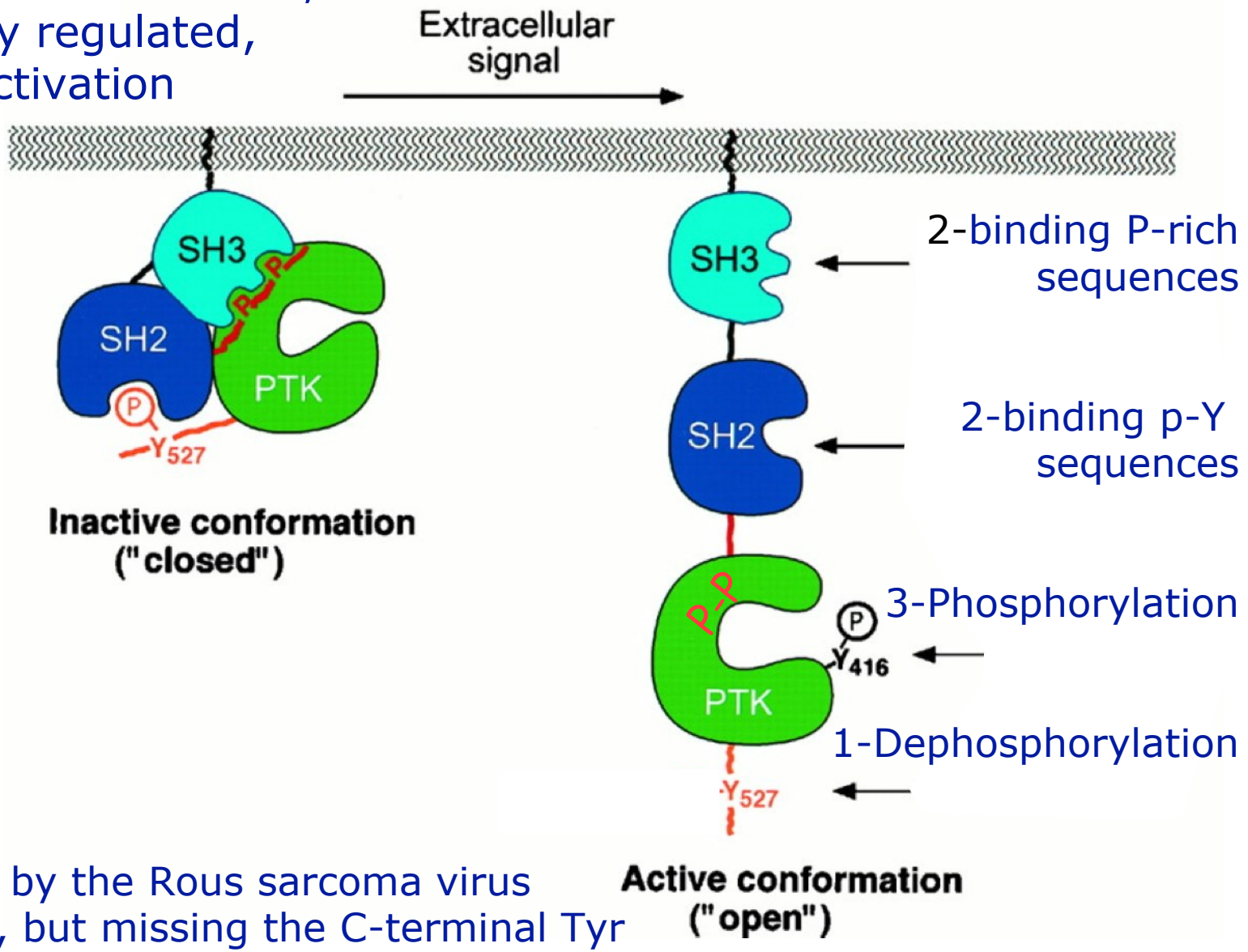


Bhattacharyya Ann Rev Bc 2006

Intra-molecular co-incidence : Regulation of Src kinase

Src: Involved in cell differentiation;
- activity is tightly regulated,
- 4-component activation

Domain	Target
SH3	Pro-rich
SH2	**p**Y
PTK	pY-kinase domain



v-Src: protein encoded by the Rous sarcoma virus
identical to Src, but missing the C-terminal Tyr
=> Active => cancer

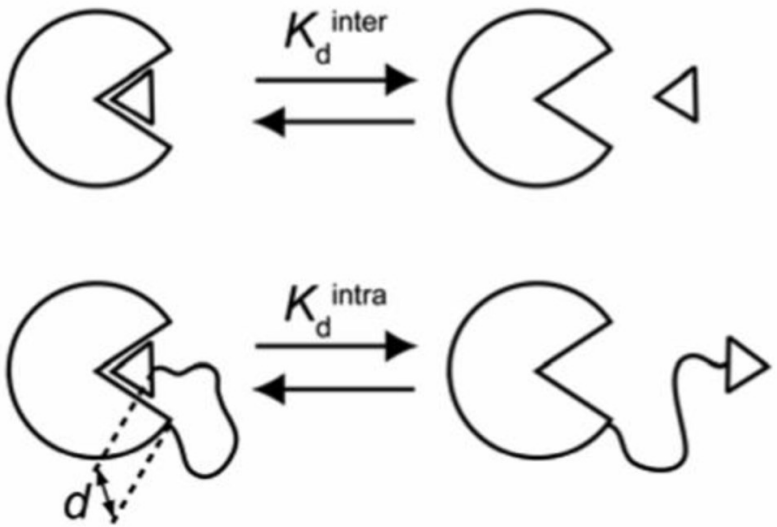
M-M Zhou

Effective molarity

A protein has a binding site to interact with ligand ◁ with K_d^{inter}.

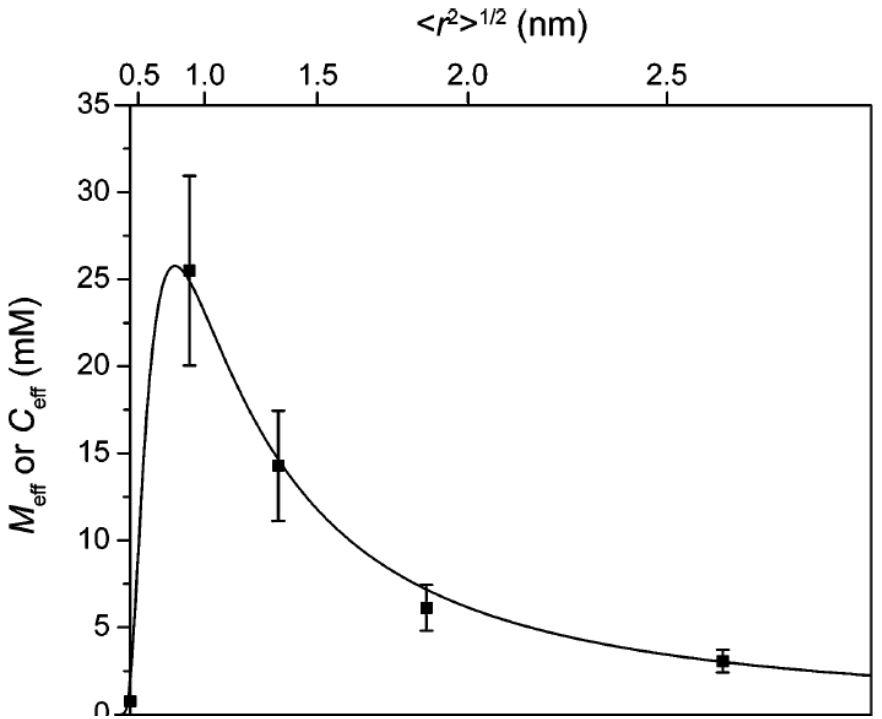
How would tethering the ligand to the protein affect binding to K_d^{intra} ?

Or, what is the effective concentration M_{eff} of the intermolecular ligand ?



$$M_{\text{eff}} = K_{\text{d}}^{\text{inter}} / K_{\text{d}}^{\text{intra}}$$

Effect of linker length



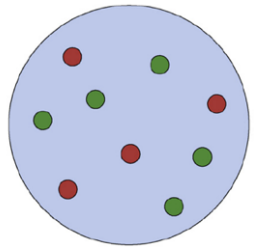
J. AM. CHEM. SOC. 2007, *129*, 1312−1320

Effective molarity - Enabling scaffolds

Scaffold: allows certain proteins to get together in a specific place

- Freely diffusing
  1'000 molecules of Ste_11 and Ste_7 in a yeast cell of 40 μm^3

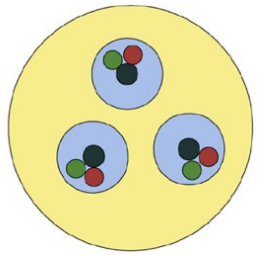
  => **Bulk** concentration = 42 nM



- Bound to scaffolds
  Introduce 1'000 scaffolds Ste_5
  Each scaffold binds 1 pair of Ste_11 and Ste_7

  Assume the diameter of the scaffold to be 100 Å
  => Total Scaffold Volume is 0.014 μm^3

  => **Local** concentration = 120 µM

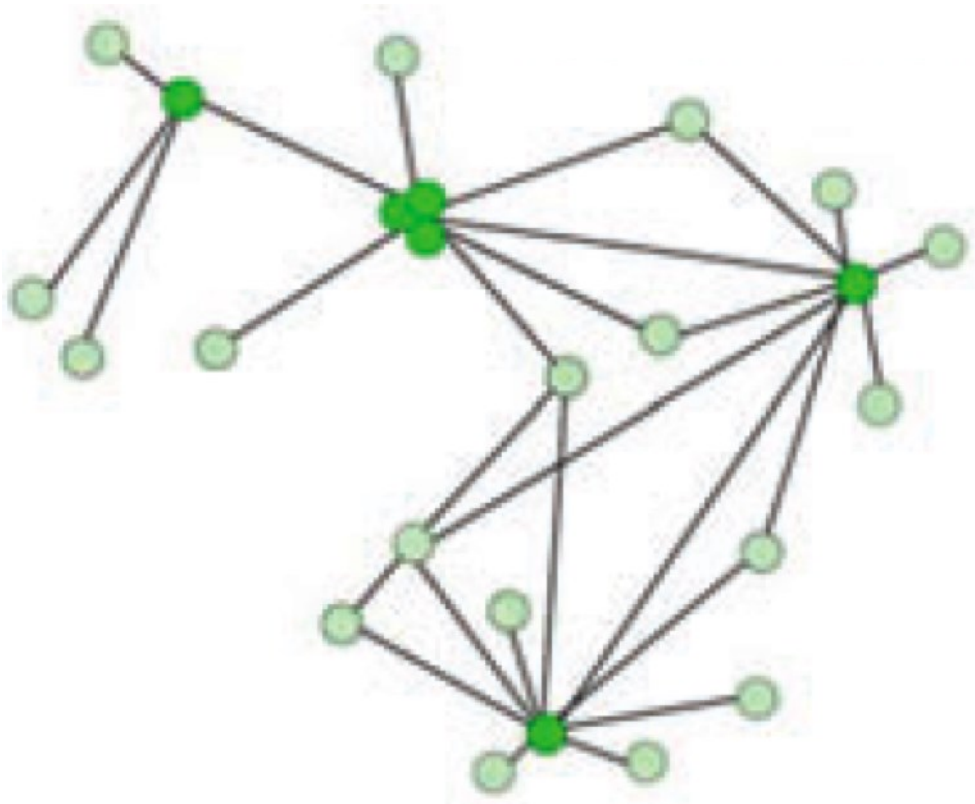


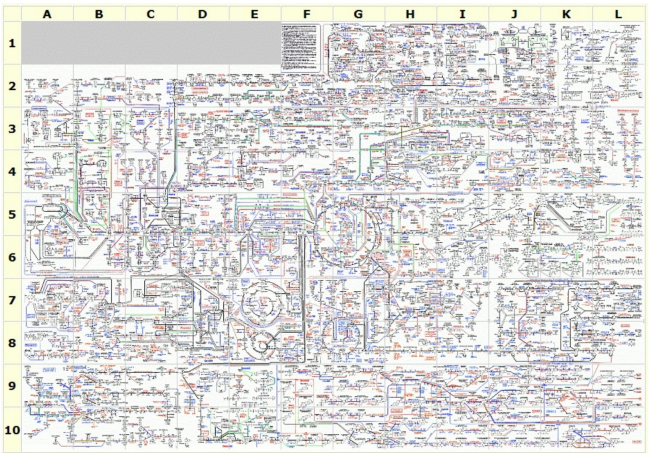
Protein-protein interaction networks

Protein-protein interactions : Genome-wide approaches

- Classical biochemical methods to determine protein-protein interactions: 2-hybrid, GST-pull down, phage display....

  => protein interaction maps, where dots represent proteins
  lines indicate interactions





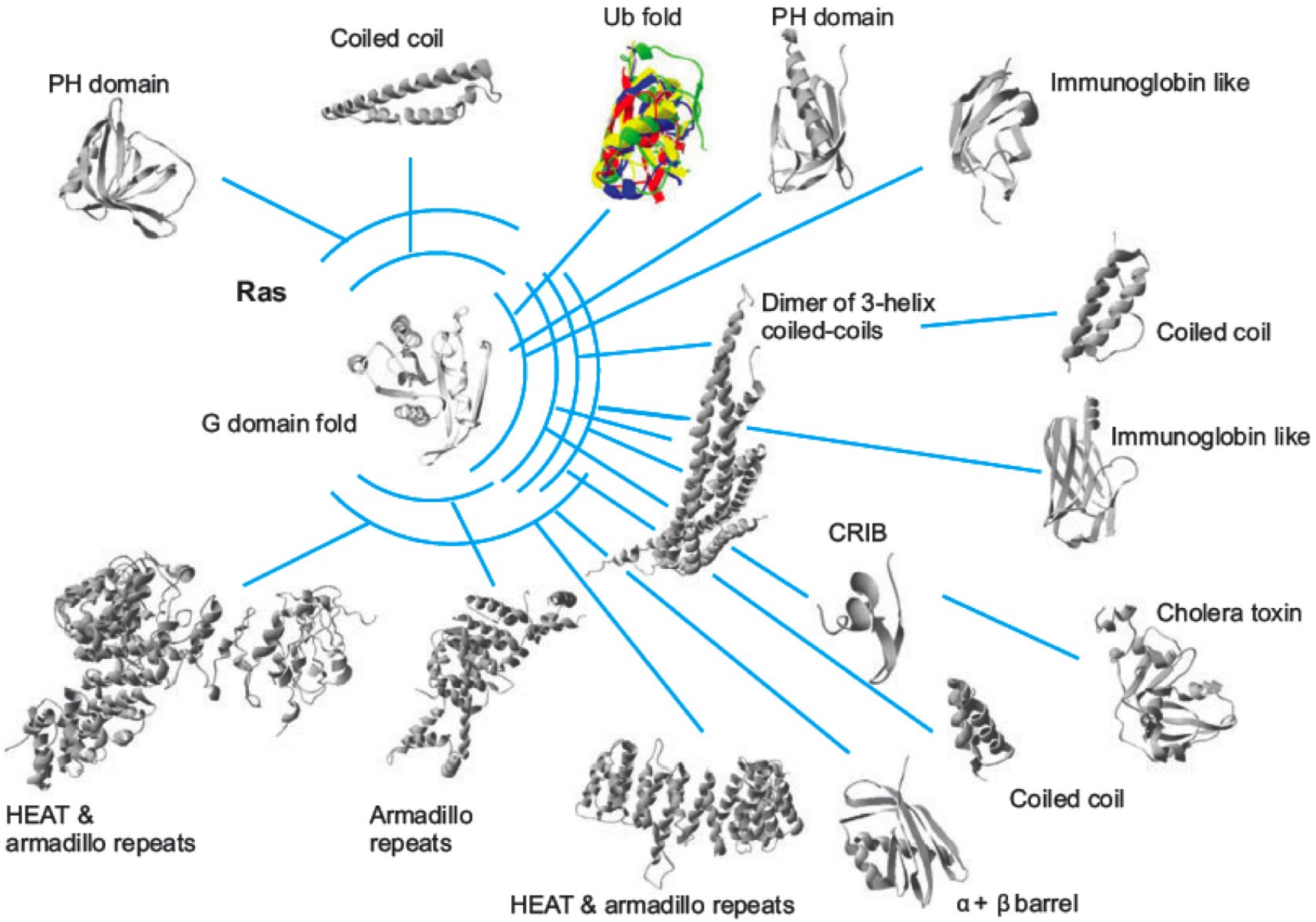
=> Very "simplistic" view of proteins

Protein-protein interactions : Genome-wide approaches

- Domains: - ~1’000 types of domains in genome
  ÷ for > 750, one or more structures are known

  - ~10’000 types of domain - domain interactions are predicted
    ÷ 20% have been structurally characterised

- An example for the Ras-protein:



Linear medication:Disaese => Gene => Pill

”A linear concept” in pharmacy and medicine:

1 disease <=> 1 gene <=> 1 medication

- Identification of a disease:

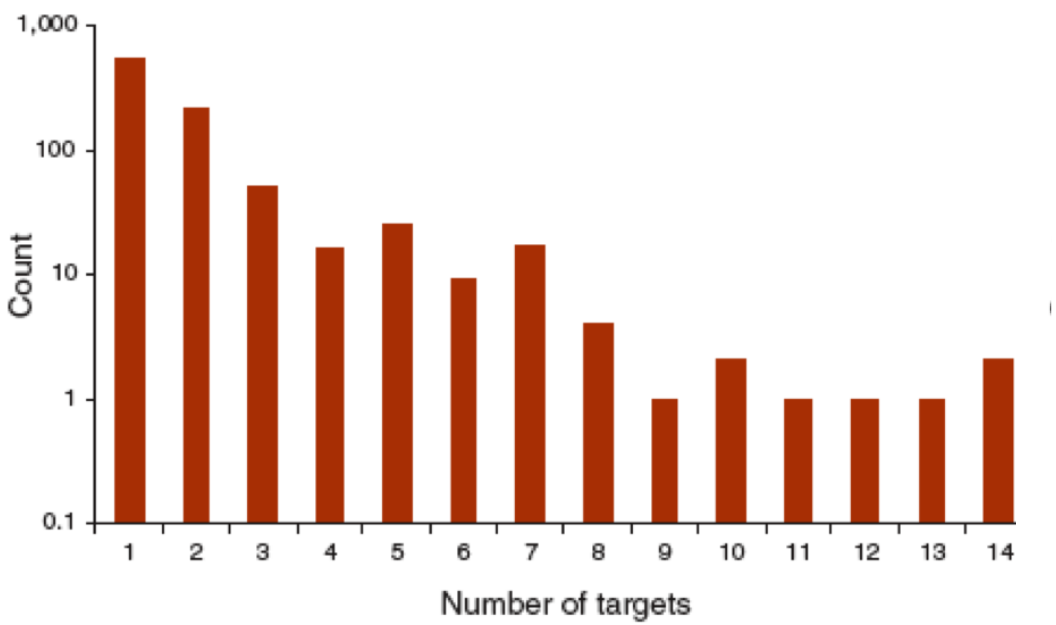
i• Symptoms	Diagnosis or effect
ii• Pharmacology	Well-defined ligand
iii• Signal transduction or Metabolic route	Precisely described mechanism
iv• Gene	Ultimate identification

1 Disease - 1 Gene - 1 Medication ?

- What does statistics say?

Network analysis of all FDA-approved drugs (890)
and their known targets (394)

How many targets per drug?



M.A.. Yildirim, Nat Biotech 25 (2007) p.1119

1 Disease - 1 Gene - 1 Medication ?

Next step:
- integration of drug-target relations into a network
- size of symbol corresponds to number of interactions

890 drugs in total:

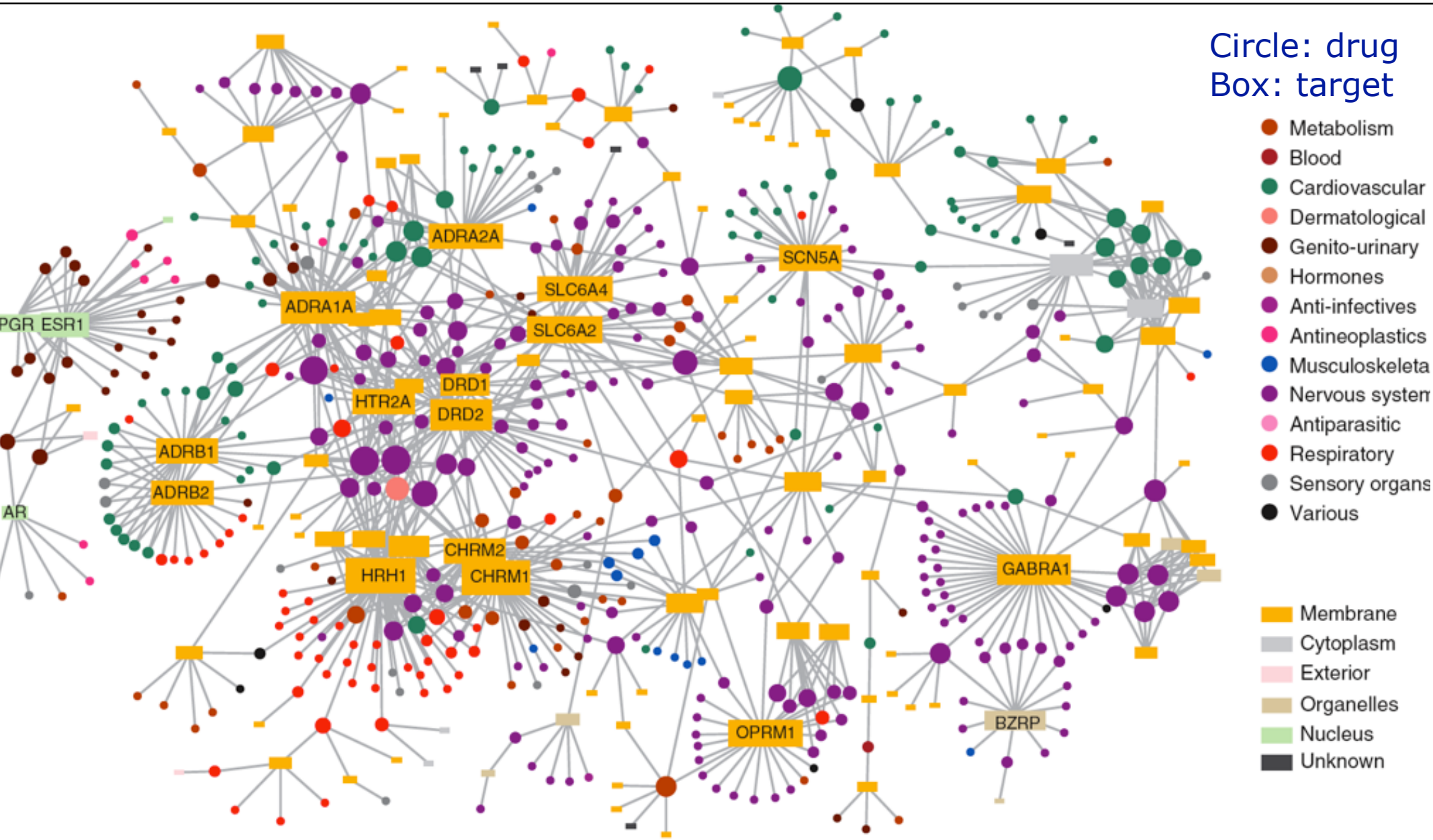
- 102 are linked to only one target: 1 drug 1 target

- 788 are linked to multiple targets, e.g.: 1 drug 3 targets or small networks

- the biggest network contains 476 drugs !!

M.A.. Yildirim, Nat Biotech 25 (2007) p.1119

Drug - target network



Drug - target network

The linear concept *was*:

1 disease <=> 1 gene <=> 1 medication

==> search for selective compounds to avoid unwanted targets and side effects.

Protein - protein interactions & signalling

÷ Reversible interactions with a limited life-time

÷ Domains with specificity for certain sequences

÷ Combination of domains to create a specific function

÷ Interactions affected by signalling

÷ Scaffolds and adaptors dictate order of events

÷ Signalling cascades localised in space and time

÷ Co-incidence and integration

Specific, reversible and adjustable interactions

=> organisation

=> localisation

=> integration

=> enabling

Further reading:

=> Good:"Scaffold Proteins: "Hubs for controlling the flow of cellular information Science 2011

=> Seet: "Reading protein modifications with interaction domains" Nat Rev Cell Biol 2006

=> Zeke: "Scaffolds: interaction platforms for cellular signalling circuits" TiCB 2009

=> Kiel: "Analyzing protein interaction networks using structural information" Ann Rev Biochem 2008