Systems Biology: A Personal View
XIII. Intra-cellular systems IV: Signal-transduction and networks

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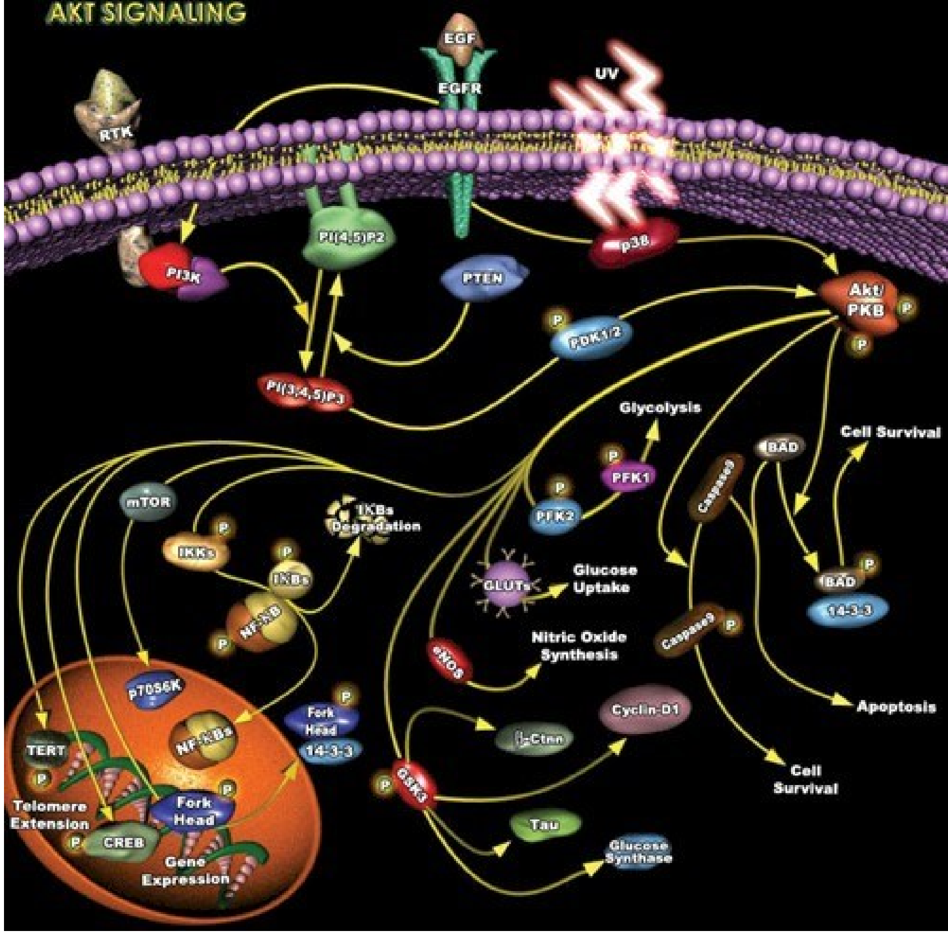
Intra-cellular biochemical networks

- ☐ Metabolic networks
  Nodes: metabolites (substrates & products of metabolism)
  Links: chemical reactions (directed)
- ☐ Genetic regulatory networks
  Nodes: Genes & Proteins
  Links: regulatory interactions (directed)
- ☐ Protein-Protein interaction network
  Nodes: Proteins
  Links: physical binding and formation of protein complex (undirected)
- ☐ **Signaling network**
  Nodes: Signaling molecules e.g., kinase, cyclicAMP, Ca
  Links: chemical reactions (directed)

Intra-cellular Signaling Network

The mechanism:

a sequence of linked biochemical reactions inside the cell, carried out by enzymes (e.g., kinases/phosphatases that catalyzes transfer of phosphate groups from/to a substrate)



The system:

A network whose nodes are enzymes, and links are reactions

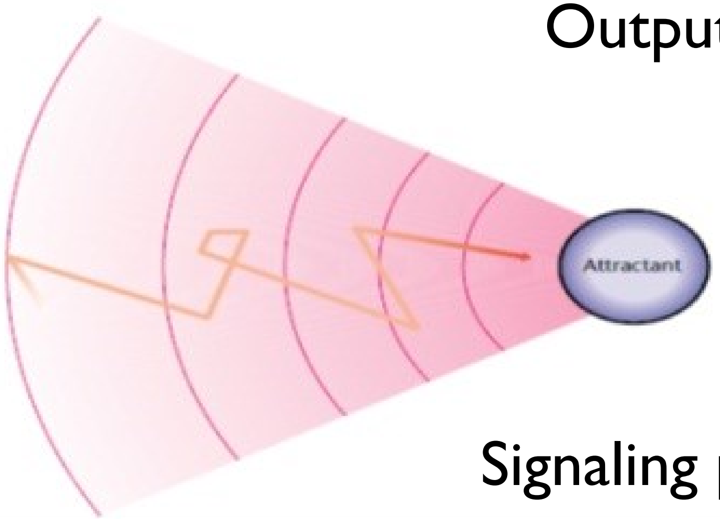
Emergence:

Interactions among reactions → signal-transduction by which cell converts signal/stimulus to specific response

“Nervous system” for the cell

Example: Chemotaxis in *E coli*

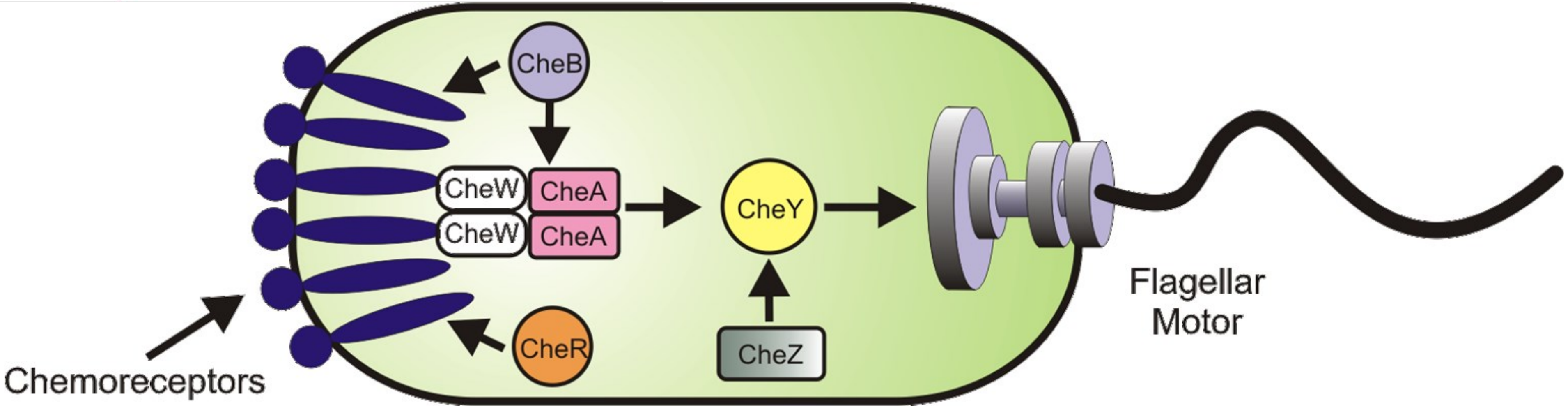
http://2011.igem.org/

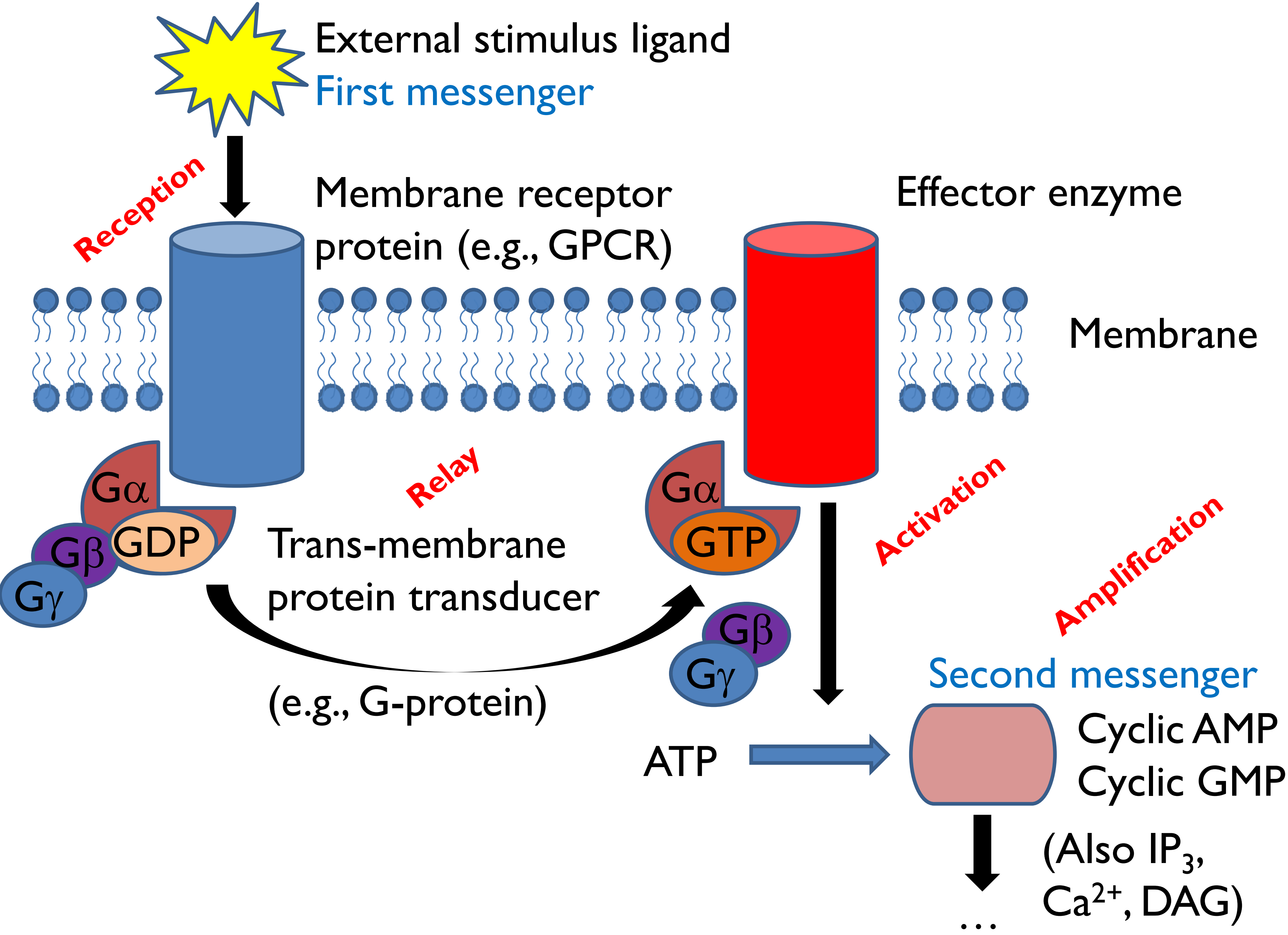


Input: chemical substances (e.g., nutrient)
Output: physical movements (bacterial motion)

Chemotaxis: Bacteria move along chemical gradient, towards food and away from noxious substances

Signaling pathway components for *E coli* chemotaxis





External stimulus ligand

First messenger

Reception

Membrane receptor protein (e.g., GPCR)

Effector enzyme

Membrane

Relay

Trans-membrane protein transducer

(e.g., G-protein)

Activation

Amplification

Second messenger

ATP

Cyclic AMP

Cyclic GMP

(Also IP_3, Ca^{2+}, DAG)

…

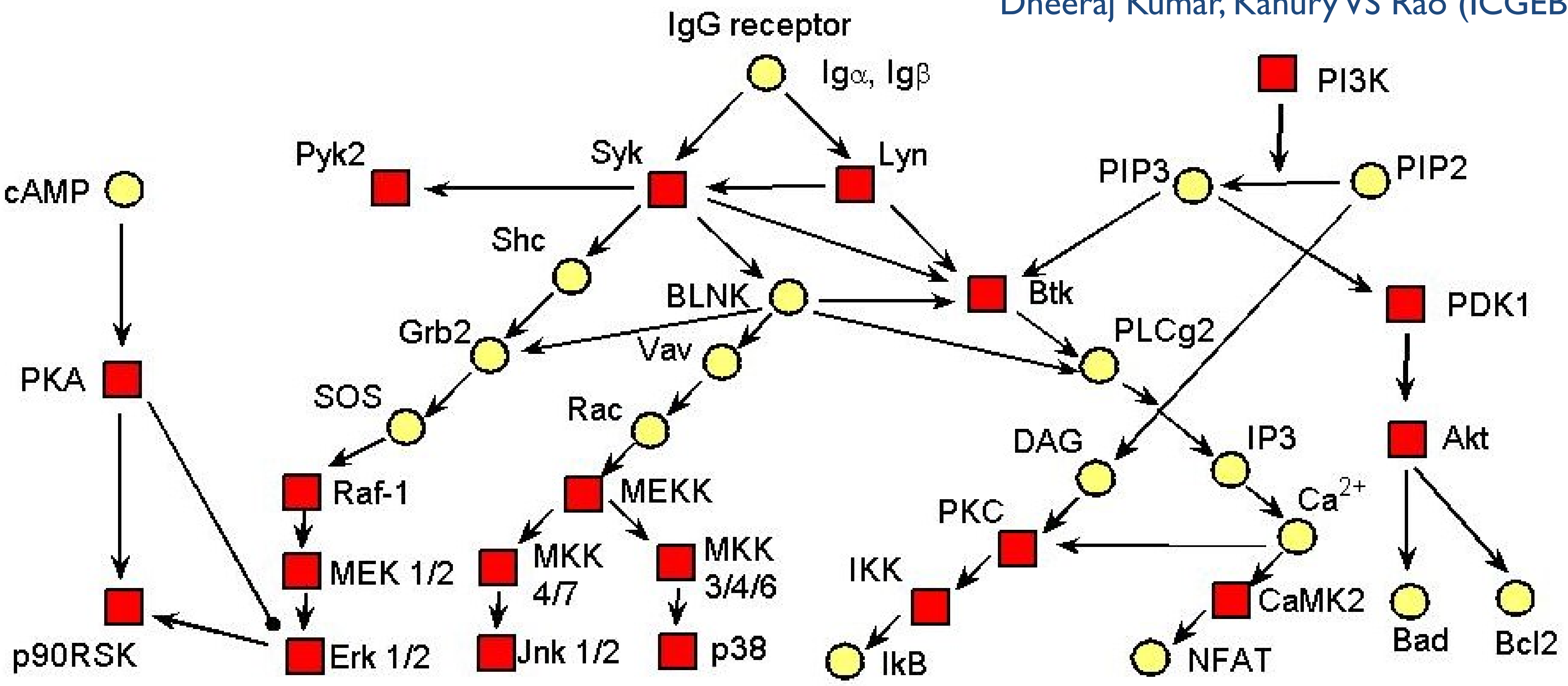
How does the signaling network allow the cell response

☐ to be sensitive to various different stimuli, and,

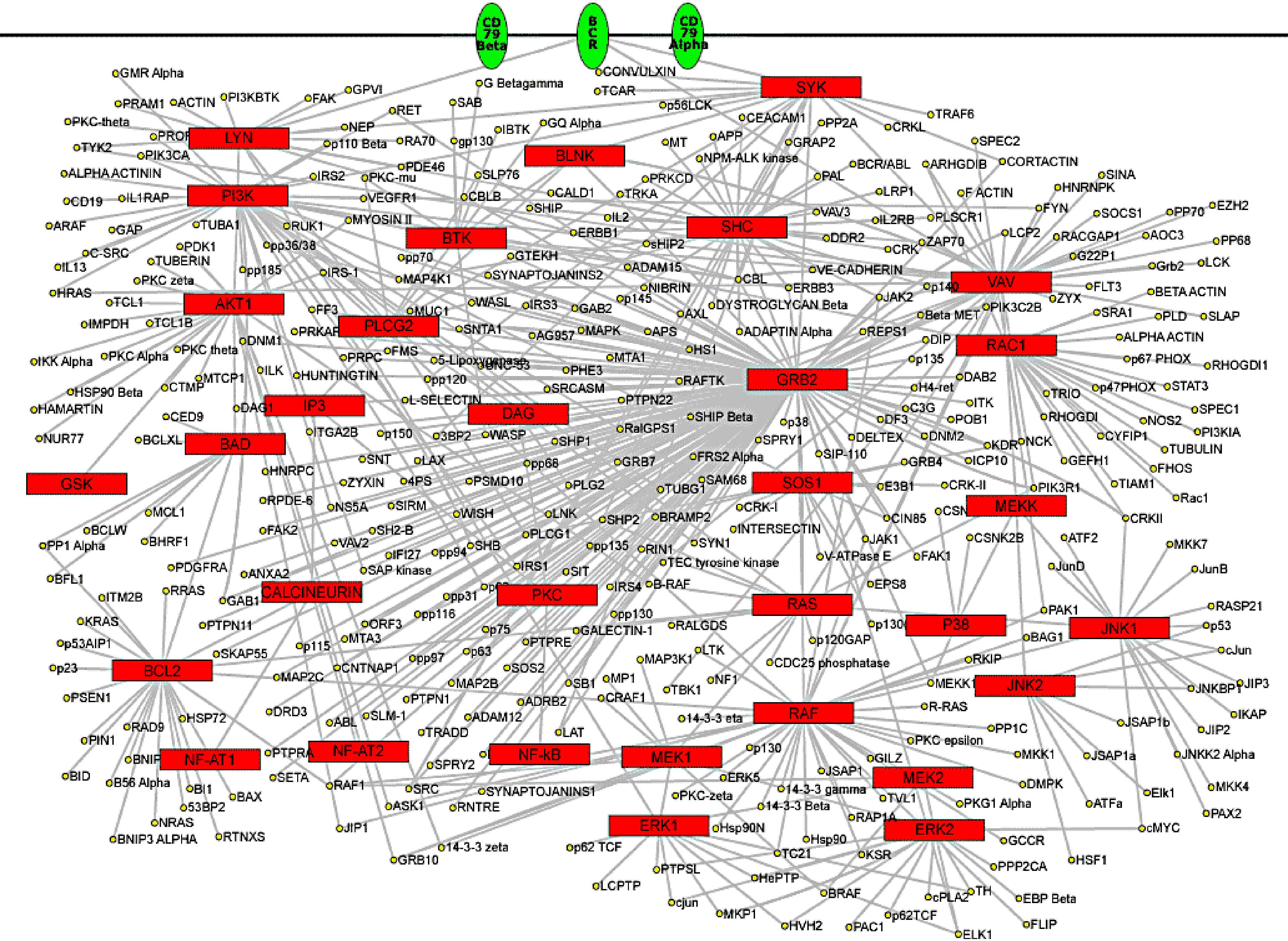
☐ yet robust enough to withstand noise ?

Example: B-Cell Response signaling network

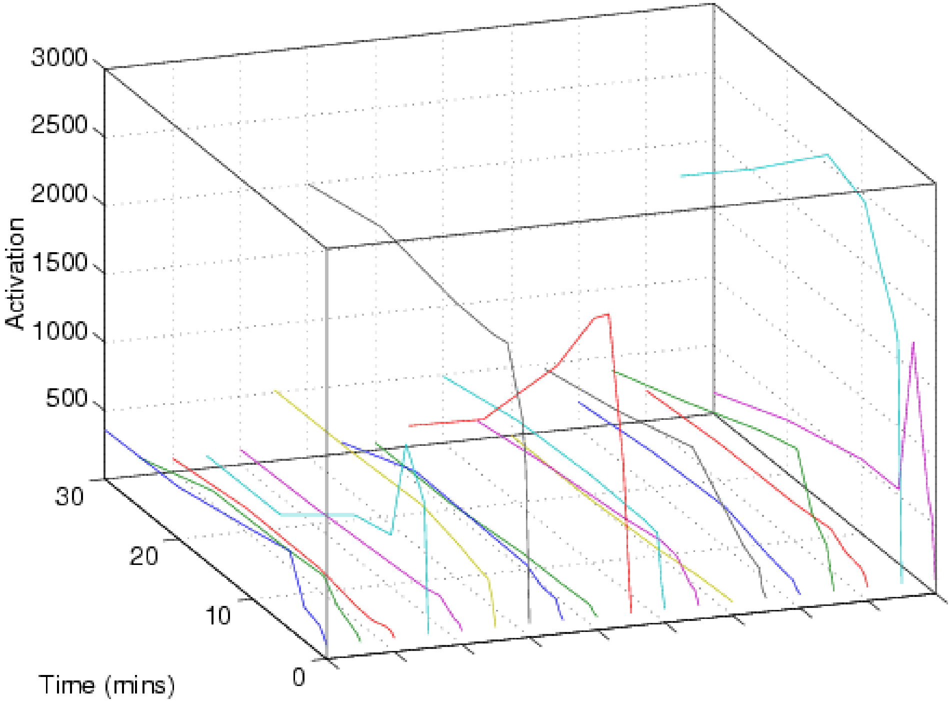
Dheeraj Kumar, Kanury VS Rao (ICGEB)



- Breakdown of communication → disease.
- Hijacked by intracellular infectious agents for proliferating.


CD 79 Beta
BCR
CD 79 Alpha
GMR Alpha
CONVULXIN
G Betagamma
SYK
GPVI
TCAR
PI3KBTK
FAK
PRAM1
ACTIN
SAB
p56LCK
RET
TRAF6
CEACAM1
PKC-theta
PP2A
GQ Alpha
NEP
CRKL
IBTK
PROF
LYN
APP
RA70
SPEC2
gp130
MT
GRAP2
p110 Beta
TYK2
PIK3CA
BLNK
NPM-ALK kinase
BCR/ABL
ARHGDIB
CORTACTIN
PDE46
ALPHA ACTININ
IRS2
PKC-mu
SLP76
PAL
SINA
PRKCD
HNRNPK
LRP1
CALD1
F ACTIN
TRKA
PI3K
IL1RAP
VEGFR1
CBLB
CD19
EZH2
SHIP
FYN
VAV3
PP70
SOCS1
IL2
PLSCR1
MYOSIN II
IL2RB
ARAF
TUBA1
RUK1
SHC
GAP
ERBB1
LCP2
AOC3
RACGAP1
BTK
DDR2
PP68
ZAP70
sHIP2
pp36/38
PDK1
CRK
C-SRC
GTEKH
G22P1
pp70
TUBERIN
LCK
Grb2
IL13
ADAM15
pp185
VE-CADHERIN
IRS-1
SYNAPTOJANINS2
MAP4K1
CBL
PKC zeta
VAV
p140
FLT3
NIBRIN
ERBB3
BETA ACTIN
HRAS
JAK2
ZYX
p145
TCL1
WASL
AKT1
IRS3
DYSTROGLYCAN Beta
PIK3C2B
GAB2
FF3
MUC1
SRA1
AXL
Beta MET
PLD
SLAP
IMPDH
TCL1B
PLCG2
SNTA1
MAPK
APS
ADAPTIN Alpha
REPS1
PRKAR
AG957
ALPHA ACTININ
DIP
DNM1
RAC1
PKC theta
HS1
FMS
PKC Alpha
PRPC
MTA1
p67 PHOX
p135
5-Lipoxygenase
IKK Alpha
RHOGDI1
PHE3
ILK
HUNTINGTIN
DAB2
MTCP1
pp120
RAFTK
GRB2
STAT3
p47PHOX
CTMP
H4-ret
SRCASM
HSP90 Beta
TRIO
L-SELECTIN
PTPN22
ITK
IP3
DAG1
C3G
HAMARTIN
SPEC1
DAG
CED9
SHIP Beta
RHOGDI
POB1
DF3
NOS2
p38
RalGPS1
ITGA2B
PI3KIA
p150
3BP2
WASP
CYFIP1
DNM2
DELTEX
NUR77
BCLXL
SHP1
SPRY1
BAD
KDR
NCK
TUBULIN
SIP-110
FRS2 Alpha
SNT
LAX
GEFH1
ICP10
GRB7
GRB4
pp68
FHOS
HNRPC
SAM68
4PS
PSMD10
ZYXIN
SOS1
TIAM1
PLG2
E3B1
CRK-II
GSK
PIK3R1
TUBG1
Rac1
RPDE-6
SIRM
MEKK
CSN
NS5A
CRK-I
MCL1
WISH
LNK
BRAMP2
CIN85
CRKII
SHP2
INTERSECTIN
BCLW
SH2-B
FAK2
PLCG1
CSNK2B
ATF2
JAK1
BHRF1
VAV2
SYN1
MKK7
SHB
PP1 Alpha
pp135
RIN1
pp94
IFI27
FAK1
V-ATPase E
TEC tyrosine kinase
IRS1
JunD
PDGFRA
JunB
SAP kinase
SIT
ANXA2
BFL1
EPS8
B-RAF
IRS4
RRAS
CALCINEURIN
PKC
pp31
ITM2B
GAB1
RAS
RASP21
PAK1
pp116
pp130
KRAS
ORF3
PTPN11
RALGDS
P38
p130
p53
JNK1
p75
GALECTIN-1
BAG1
MTA3
p120GAP
PTPRE
p53AIP1
p115
LTK
cJun
p63
SKAP55
pp97
MAP3K1
RKIP
CDC25 phosphatase
CNTNAP1
SOS2
p23
BCL2
MAP2C
MP1
NF1
SB1
MAP2B
MEKK1
JIP3
JNK2
JNKBP1
TBK1
PSEN1
CRAF1
PTPN1
ADRB2
R-RAS
DRD3
RAF
IKAP
SLM-1
ADAM12
HSP72
14-3-3 eta
ABL
JSAP1b
RAD9
PP1C
JIP2
TRADD
LAT
PIN1
PKC epsilon
p130
NF-AT2
PTPRA
MKK1
JNKK2 Alpha
JSAP1a
NF-kB
MEK1
GILZ
BNIP
NF-AT1
SPRY2
JSAP1
BID
SETA
ERK5
MEK2
B56 Alpha
RAF1
DMPK
MKK4
BI1
SRC
14-3-3 gamma
SYNAPTOJANINS1
Elk1
BAX
PKC-zeta
TVL1
PKG1 Alpha
ATFa
ASK1
RNTRE
14-3-3 Beta
53BP2
PAX2
RAP1A
NRAS
ERK1
JIP1
Hsp90N
cMYC
ERK2
RTNXS
BNIP3 ALPHA
Hsp90
GCCR
14-3-3 zeta
p62 TCF
TC21
KSR
GRB10
HSF1
PPP2CA
PTPSL
HePTP
LCPTP
BRAF
TH
cPLA2
EBP Beta
cjun
MKP1
p62TCF
FLIP
HVH2
PAC1
ELK1

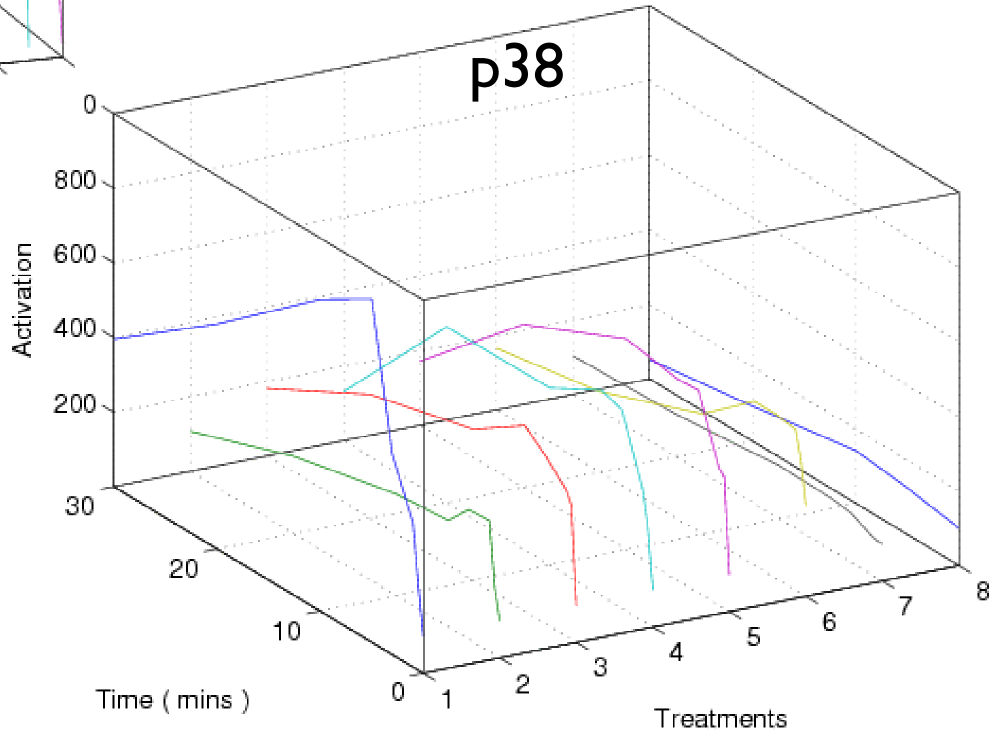
Reconstructing the complete set of interactions



Under normal condition measure activation

Let’s focus on a specific kinase

How does it respond when activation of particular nodes in the network are blocked ?

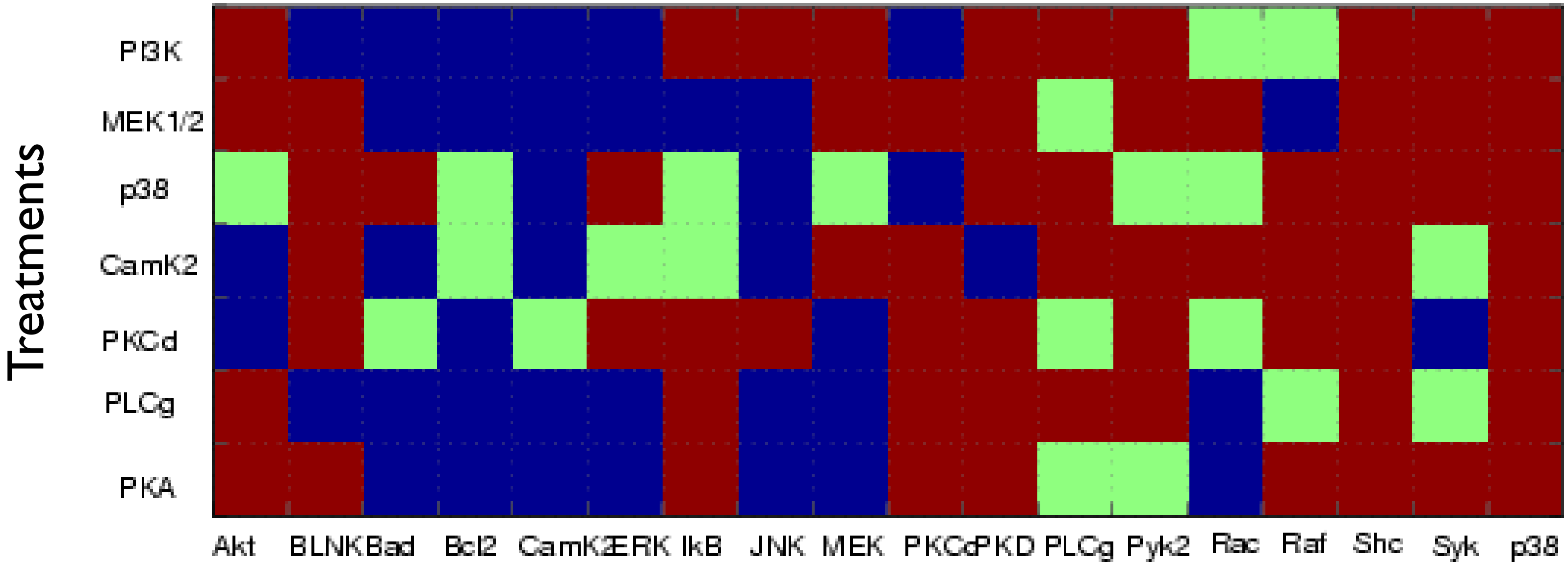


Correlation analysis of activity

Dheeraj Kumar, Kanury VS Rao (ICGEB)

Which nodes influence which other nodes ?

Block activation of a node, and find out how other nodes behave in its absence



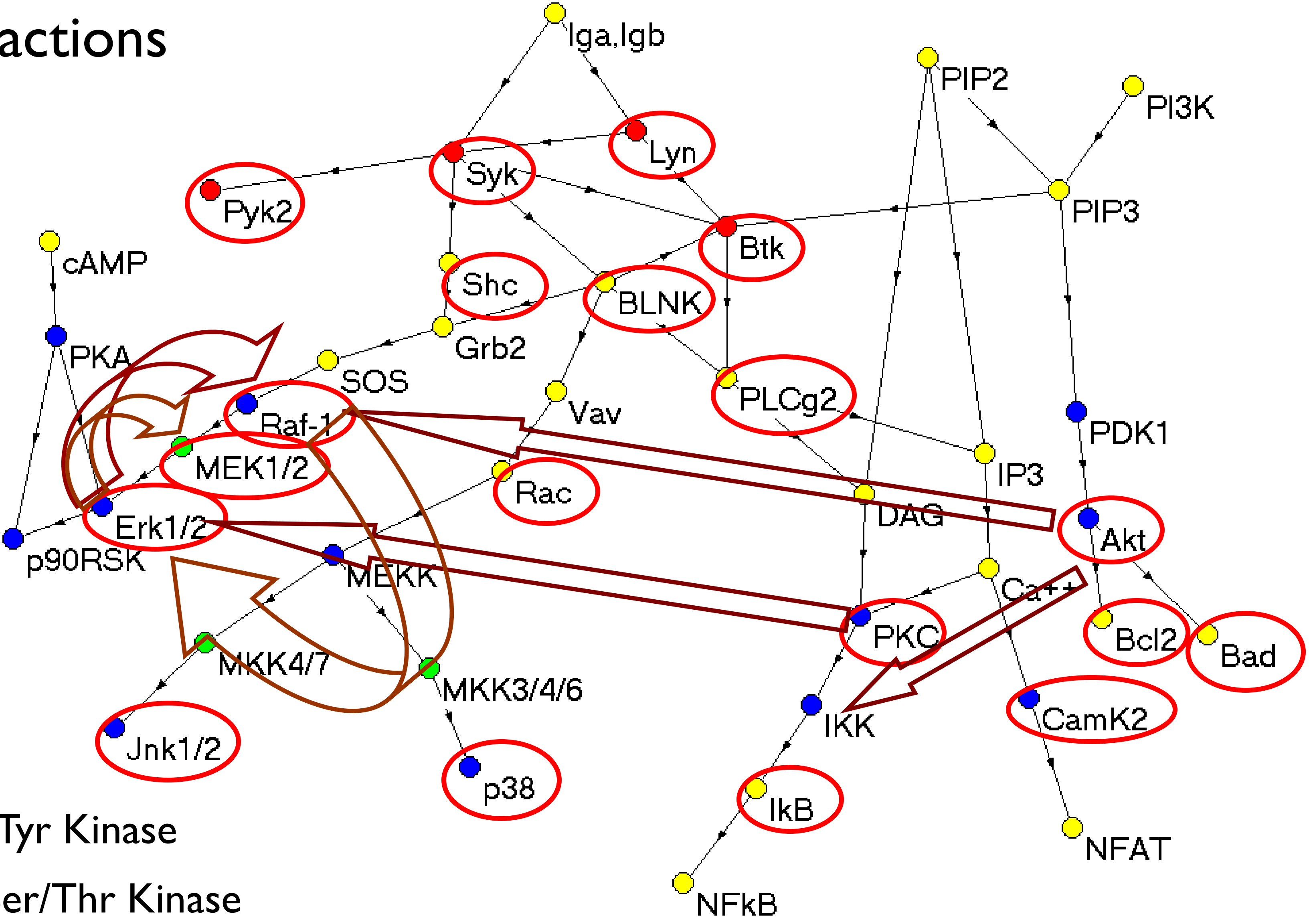
Treatments	Akt	BLNK	Bad	Bcl2	CamK2	ERK	IkB	JNK	MEK	PKCd	PKD	PLCg	Pyk2	Rac	Raf	Shc	Syk	p38
PI3K	🟥	🟦	🟦	🟦	🟦	🟦	🟥	🟥	🟥	🟦	🟥	🟥	🟥	🟩	🟩	🟥	🟥	🟥
MEK1/2	🟥	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟥	🟥	🟥	🟩	🟥	🟥	🟦	🟥	🟥	🟥
p38	🟩	🟥	🟥	🟩	🟦	🟥	🟩	🟦	🟩	🟦	🟥	🟥	🟩	🟩	🟥	🟥	🟥	🟥
CamK2	🟦	🟥	🟦	🟩	🟦	🟩	🟩	🟦	🟥	🟥	🟦	🟥	🟥	🟥	🟥	🟥	🟩	🟥
PKCd	🟦	🟥	🟩	🟦	🟩	🟥	🟥	🟥	🟦	🟥	🟥	🟩	🟥	🟩	🟥	🟥	🟦	🟥
PLCg	🟥	🟦	🟦	🟦	🟦	🟦	🟥	🟦	🟦	🟥	🟥	🟥	🟥	🟦	🟩	🟥	🟩	🟥
PKA	🟥	🟥	🟦	🟦	🟦	🟦	🟥	🟦	🟦	🟥	🟥	🟩	🟩	🟦	🟥	🟥	🟥	🟥

🟥 positive effect 🟩 no effect 🟦 negative effect

Surprise: e.g., p38 affects and is affected by many other nodes !

Why ?

Inserting "missing" connections from database of protein interactions



- 🔴 Tyr Kinase
- 🔵 Ser/Thr Kinase
- 🟢 Dual specificity Kinase
- 🟡 Other

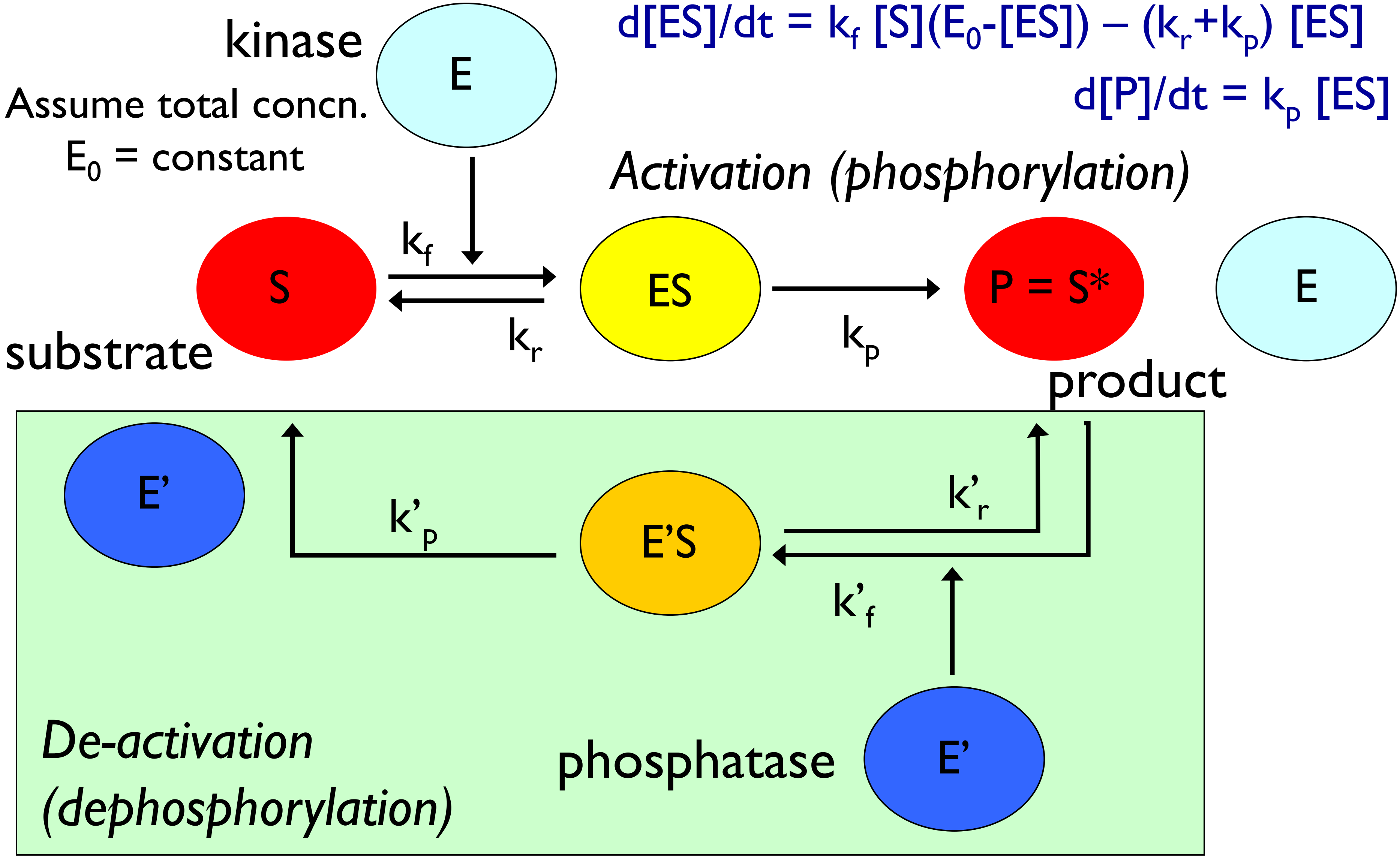
Does not always explain everything

Dynamics of Kinase Activation

$$d[S]/dt = -k_f [S][E] + k_r [ES] = -k_f [S](E_0 - [ES]) + k_r [ES]$$

$$d[ES]/dt = k_f [S](E_0 - [ES]) - (k_r + k_p) [ES]$$

$$d[P]/dt = k_p [ES]$$



kinase

Assume total concn.
E_0 = constant

Activation (phosphorylation)

substrate

product

De-activation (dephosphorylation)

phosphatase

Michaelis-Menten equation

Steady-state assumption: $d[ES]/dt = 0$

Maud Menten
(1879-1960)

Leonor Michaelis
(1875-1949)

acsundergrad.wordpress.com

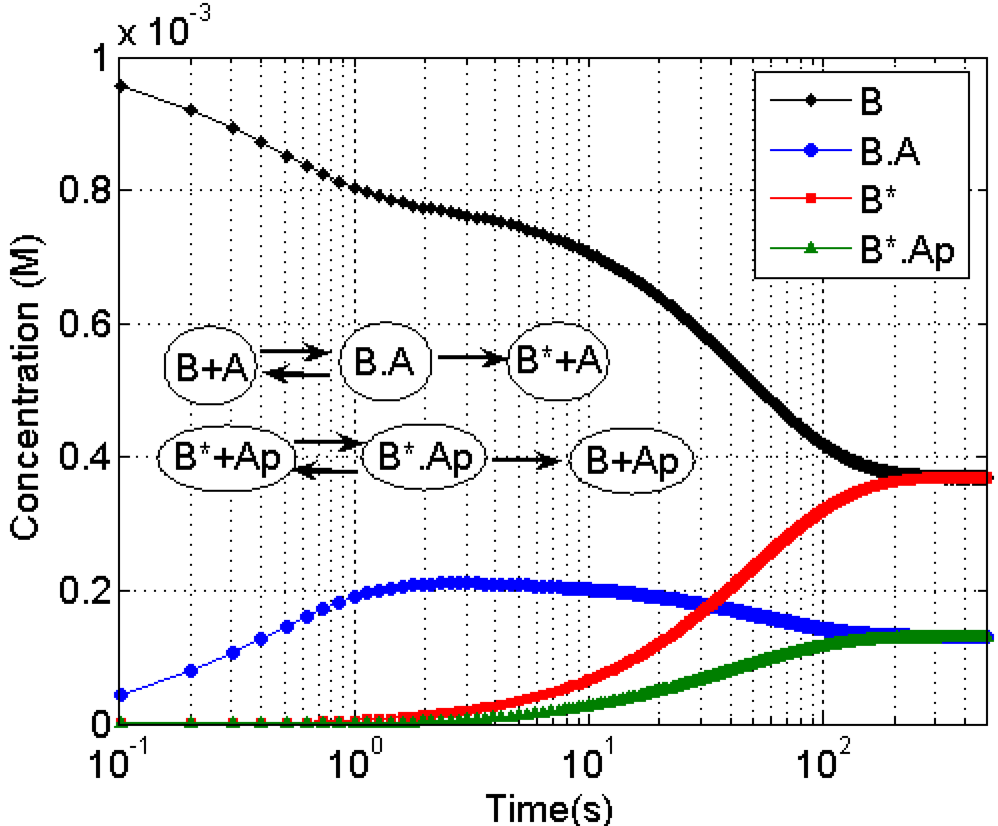
$$\Rightarrow \quad [ES] = E_0\,[S] \,/\, (\,[S] + K_m\,)$$

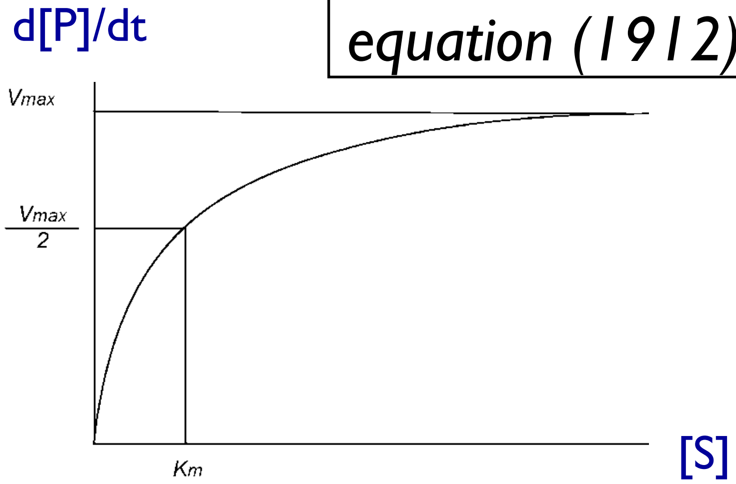
with $K_m=(k_r+k_p)/k_f$

$$\Rightarrow \quad d[P]/dt = k_p\,E_0\,[S] \,/\, (\,[S] + K_m\,)$$

But is the quasi-steady-state hypothesis valid ?

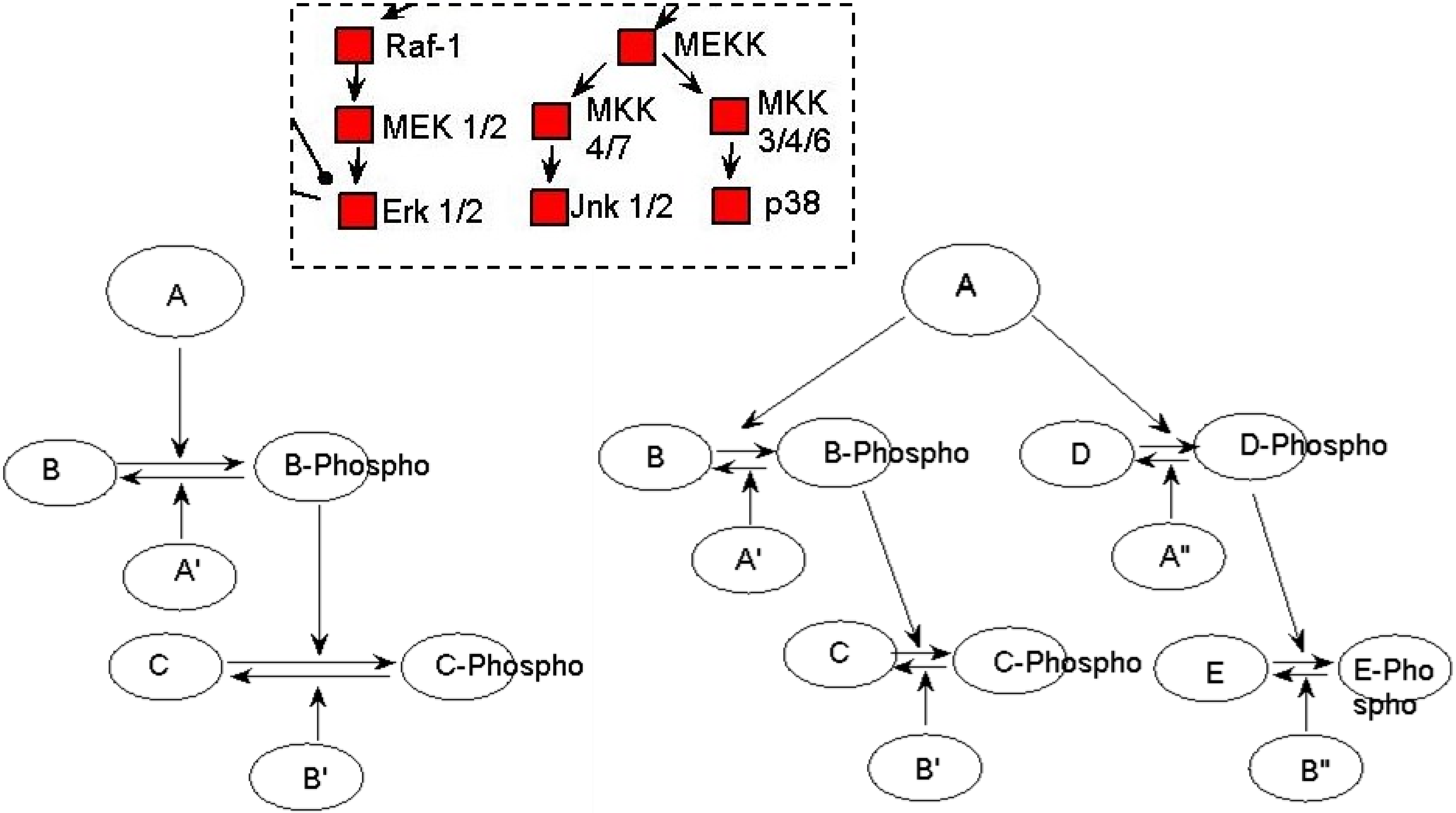
Michaelis-Menten equation (1912)





The steady-state assumption can give misleading results, especially in multi-step cascades !

Studying progressively more complex kinase cascades

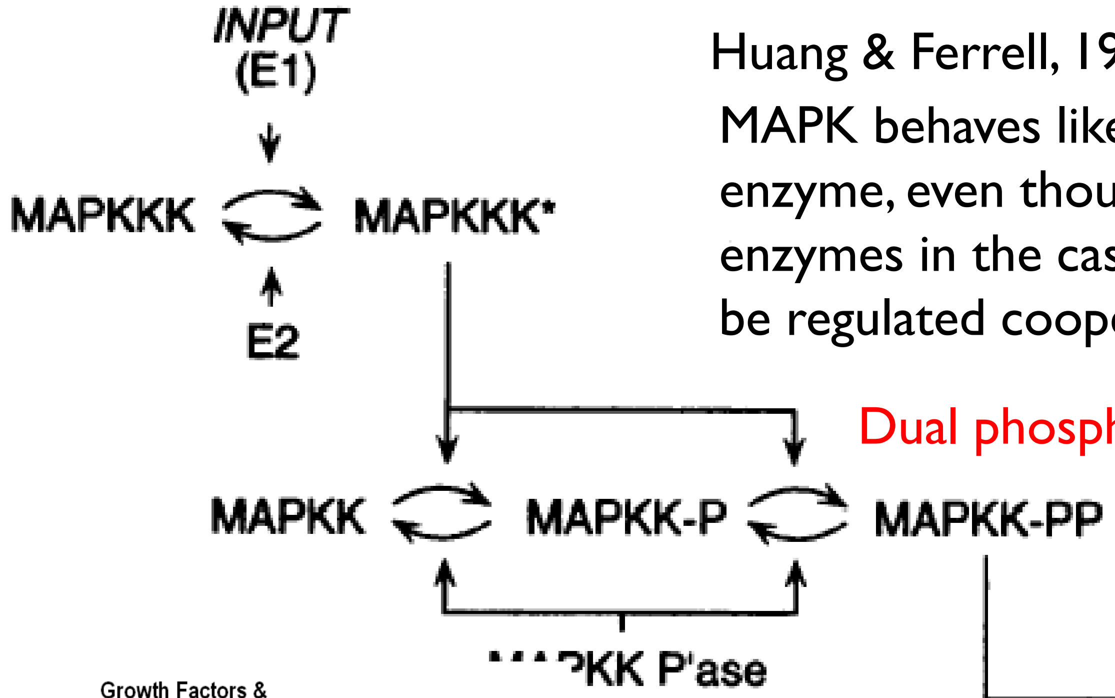


The MAP-Kinase cascade

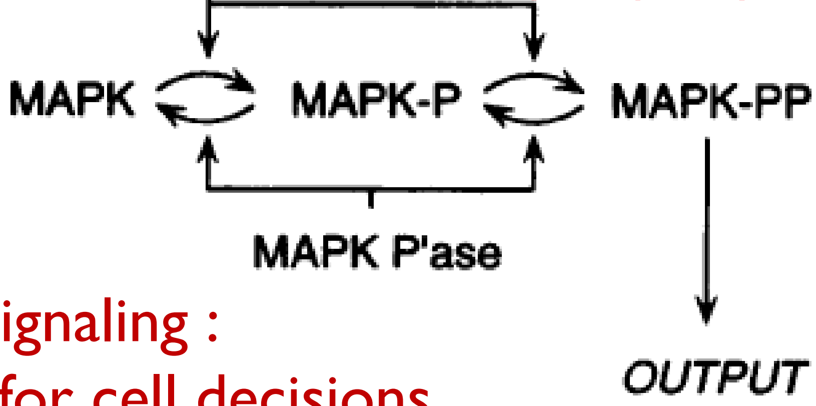
Present in all eukaryotic cells

Huang & Ferrell, 1996

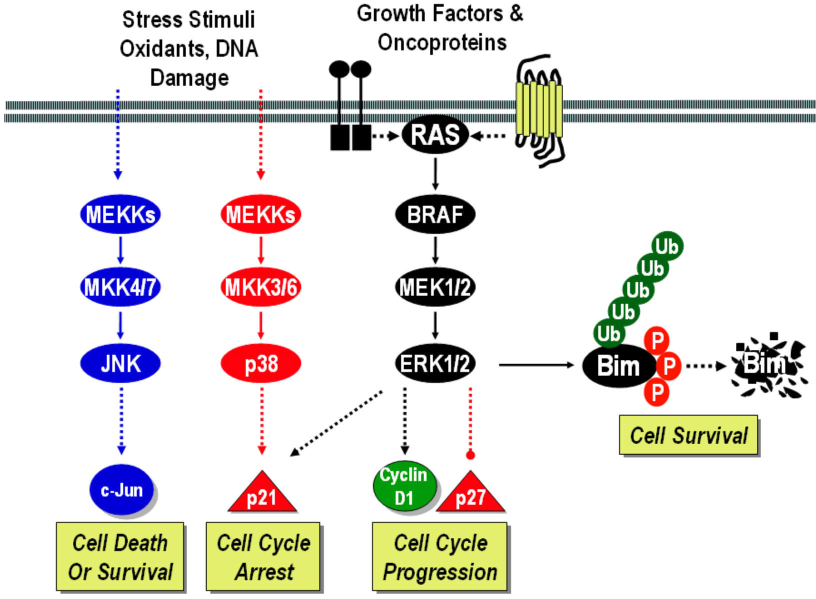
MAPK behaves like a highly cooperative enzyme, even though none of the enzymes in the cascade are assumed to be regulated cooperatively.



Dual phosphorylation



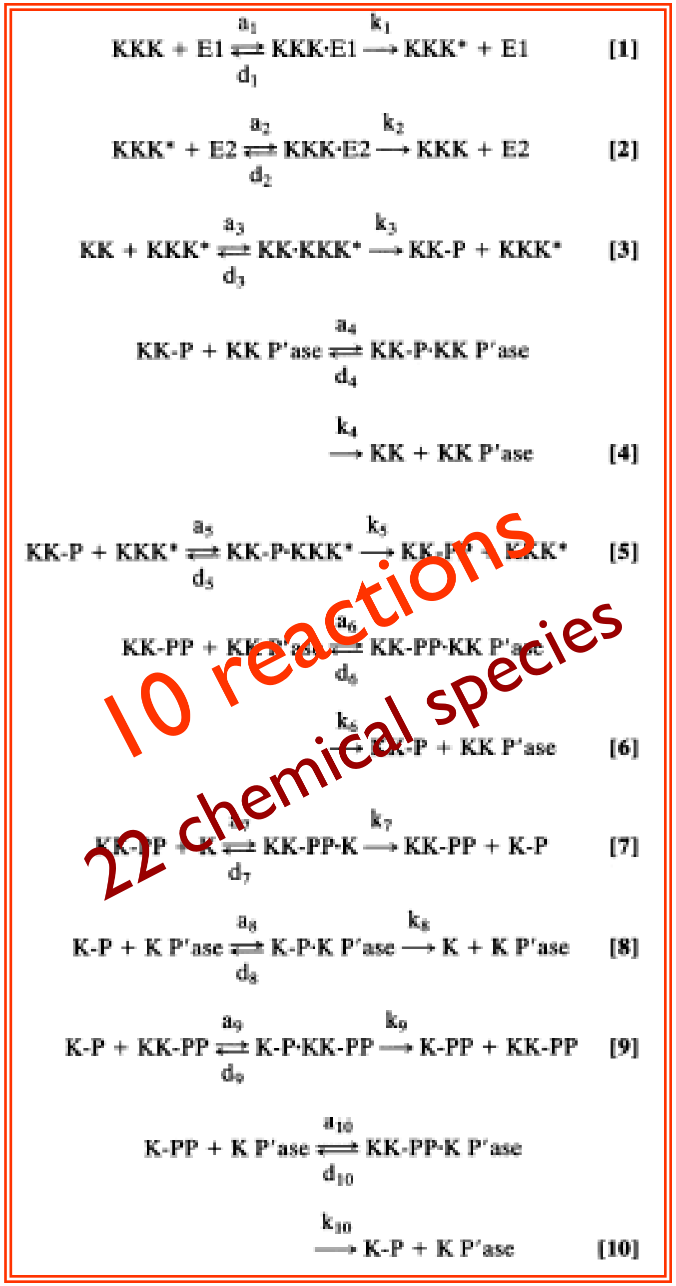
Dual phosphorylation



MAPK signaling :
Critical for cell decisions
on proliferation,
differentiaton & apoptosis

The Huang-Ferrell model (1996)

Connects MAPK cascade structure to its dynamics



$$\mathrm{KKK} + \mathrm{E1} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{KKK{\cdot}E1} \xrightarrow{k_1} \mathrm{KKK^*} + \mathrm{E1} \qquad [1]$$

$$\mathrm{KKK^*} + \mathrm{E2} \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{KKK{\cdot}E2} \xrightarrow{k_2} \mathrm{KKK} + \mathrm{E2} \qquad [2]$$

$$\mathrm{KK} + \mathrm{KKK^*} \underset{d_3}{\overset{a_3}{\rightleftharpoons}} \mathrm{KK{\cdot}KKK^*} \xrightarrow{k_3} \mathrm{KK\text{-}P} + \mathrm{KKK^*} \qquad [3]$$

$$\mathrm{KK\text{-}P} + \mathrm{KK\ P'ase} \underset{d_4}{\overset{a_4}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KK\ P'ase}$$

$$\xrightarrow{k_4} \mathrm{KK} + \mathrm{KK\ P'ase} \qquad [4]$$

$$\mathrm{KK\text{-}P} + \mathrm{KKK^*} \underset{d_5}{\overset{a_5}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KKK^*} \xrightarrow{k_5} \mathrm{KK\text{-}PP} + \mathrm{KKK^*} \qquad [5]$$

$$\mathrm{KK\text{-}PP} + \mathrm{KK\ P'ase} \underset{d_6}{\overset{a_6}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}KK\ P'ase}$$

$$\xrightarrow{k_6} \mathrm{KK\text{-}P} + \mathrm{KK\ P'ase} \qquad [6]$$

$$\mathrm{KK\text{-}PP} + \mathrm{K} \underset{d_7}{\overset{a_7}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}K} \xrightarrow{k_7} \mathrm{KK\text{-}PP} + \mathrm{K\text{-}P} \qquad [7]$$

$$\mathrm{K\text{-}P} + \mathrm{K\ P'ase} \underset{d_8}{\overset{a_8}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}K\ P'ase} \xrightarrow{k_8} \mathrm{K} + \mathrm{K\ P'ase} \qquad [8]$$

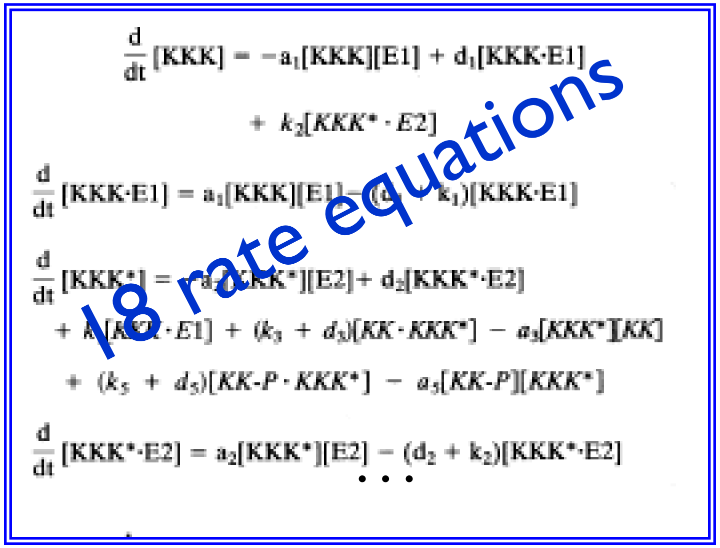
$$\mathrm{K\text{-}P} + \mathrm{KK\text{-}PP} \underset{d_9}{\overset{a_9}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}KK\text{-}PP} \xrightarrow{k_9} \mathrm{K\text{-}PP} + \mathrm{KK\text{-}PP} \qquad [9]$$

$$\mathrm{K\text{-}PP} + \mathrm{K\ P'ase} \underset{d_{10}}{\overset{a_{10}}{\rightleftharpoons}} \mathrm{K\text{-}PP{\cdot}K\ P'ase}$$

$$\xrightarrow{k_{10}} \mathrm{K\text{-}P} + \mathrm{K\ P'ase} \qquad [10]$$

10 reactions

22 chemical species



$$\frac{d}{dt}[\mathrm{KKK}] = -a_1[\mathrm{KKK}][\mathrm{E1}] + d_1[\mathrm{KKK{\cdot}E1}] + k_2[KKK^* \cdot E2]$$

$$\frac{d}{dt}[\mathrm{KKK{\cdot}E1}] = a_1[\mathrm{KKK}][\mathrm{E1}] - (d_1 + k_1)[\mathrm{KKK{\cdot}E1}]$$

$$\frac{d}{dt}[\mathrm{KKK^*}] = -a_2[\mathrm{KKK^*}][\mathrm{E2}] + d_2[\mathrm{KKK^*{\cdot}E2}] + k_1[KKK \cdot E1] + (k_3 + d_3)[KK \cdot KKK^*] - a_3[KKK^*][KK] + (k_5 + d_5)[KK\text{-}P \cdot KKK^*] - a_5[KK\text{-}P][KKK^*]$$

$$\frac{d}{dt}[\mathrm{KKK^*{\cdot}E2}] = a_2[\mathrm{KKK^*}][\mathrm{E2}] - (d_2 + k_2)[\mathrm{KKK^*{\cdot}E2}]$$

. . .

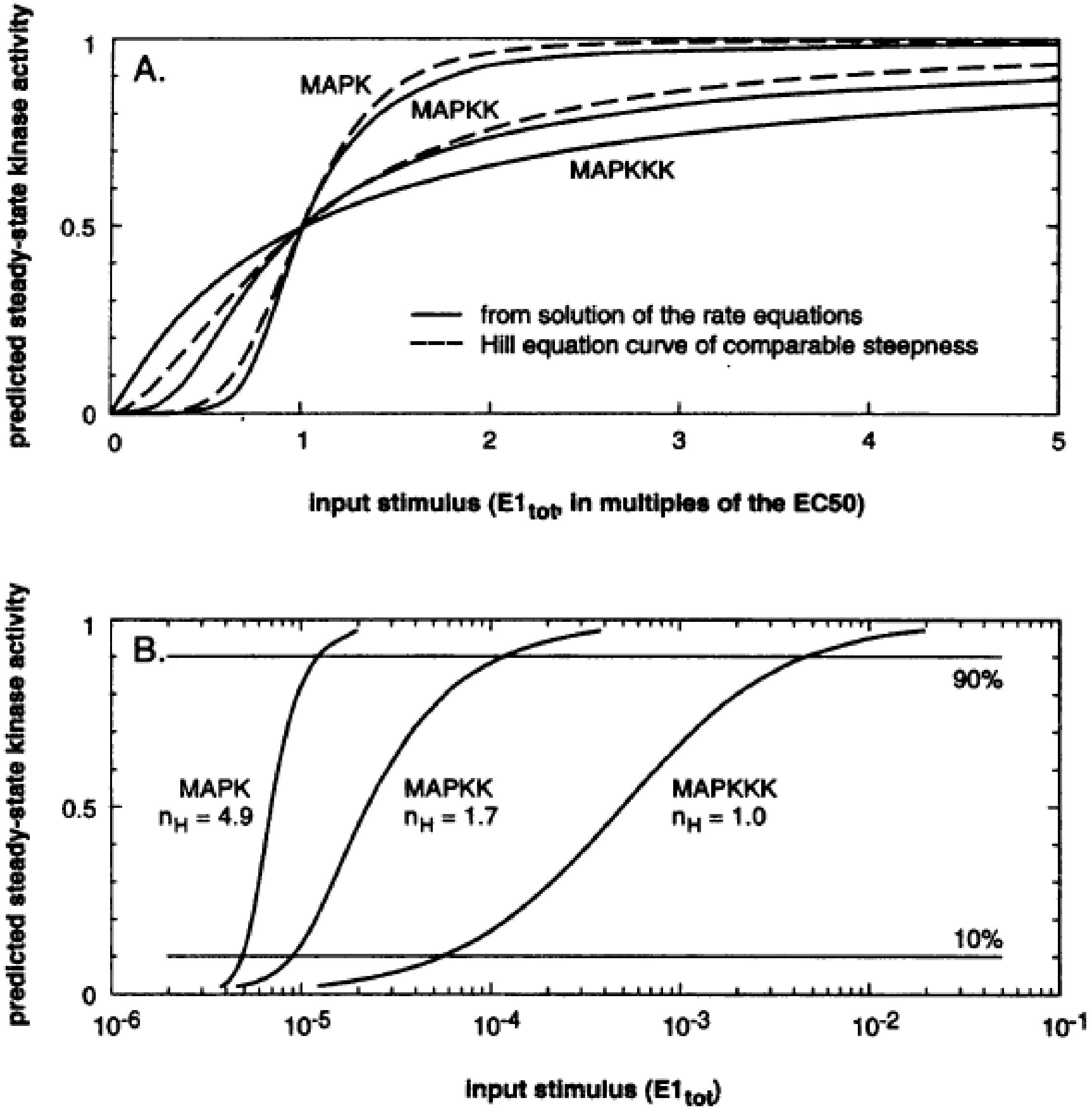
18 rate equations

[KKK]=3nM
[KK]=1.2 μM
[K]=1.2 μM
[E2] = 0.3 nM
[KK P'ase] = 0.3 nM
[K P'ase] = 120 nM

….

36 parameters + signal strength

Ultrasensitivity in stimulus-response of MAPK cascade by dual phosphorylation



Huang & Ferrell, PNAS (1996)

First phosphorylation of MAPKK driven by linearly increasing input stimulus (MAPKKK*) ⇒ rate & equilibrium level of phosphorylation of the substrate increase **linearly** with input.
Second phosphorylation driven by a linearly increasing input stimulus (MAPKKK*) **and** a linearly increasing substrate concentration (MAPKK*) ⇒ rate & equilibrium level increase as the **square** of the input stimulus.

Fit to Hill equation:

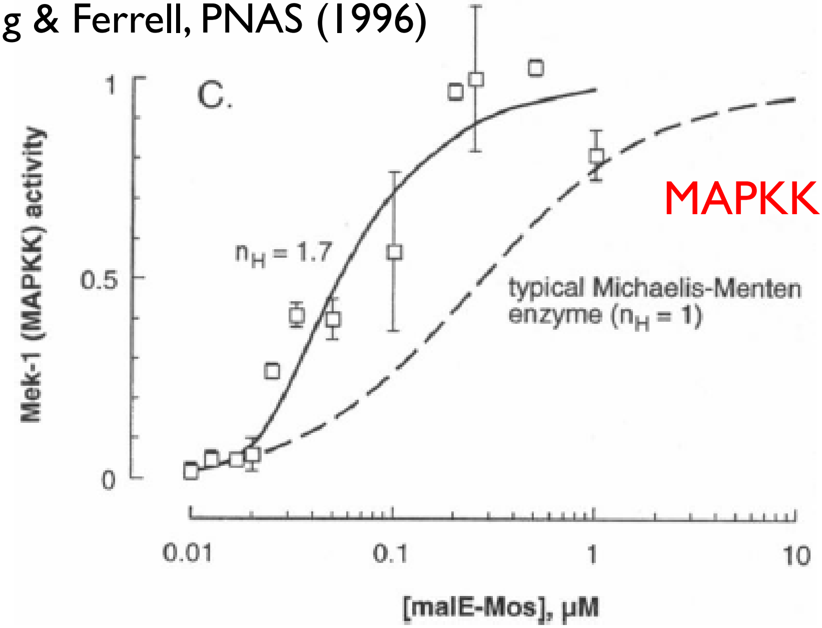
$$y = x^{nH}/(K+x^{nH})$$

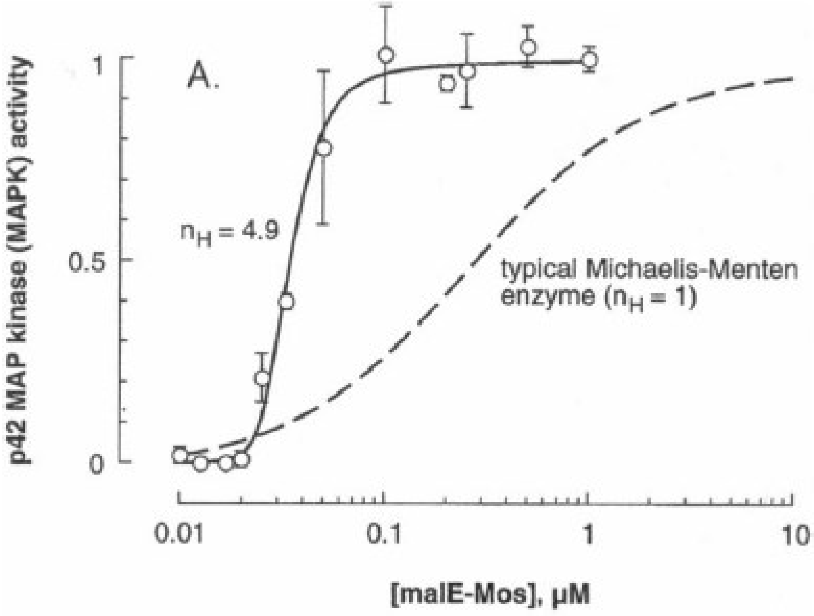
nH: Hill coefficient represents degree of cooperativity in ligand binding to enzyme or receptor (=1 for independent binding, >1 implies cooperative effect)

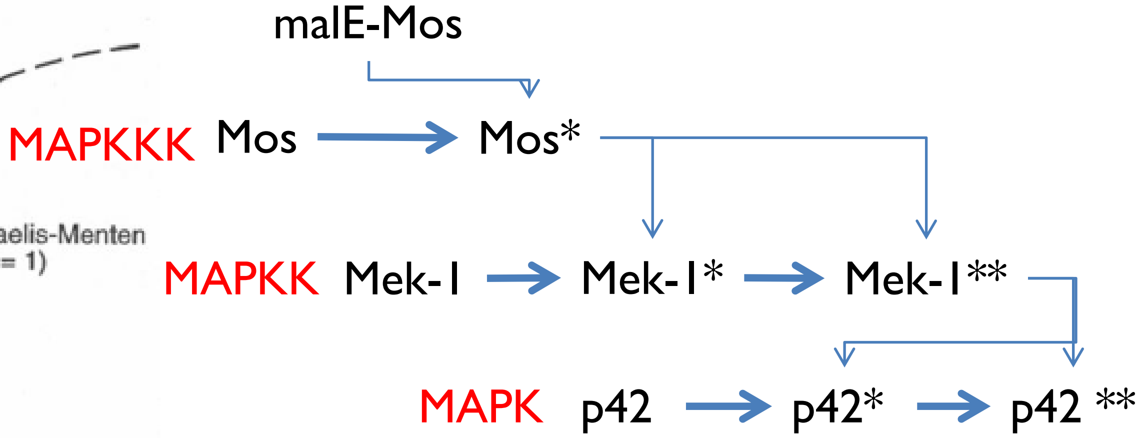
A V Hill
(1886-1977)

Experimental validation in *Xenopus* oocyte extract

Huang & Ferrell, PNAS (1996)







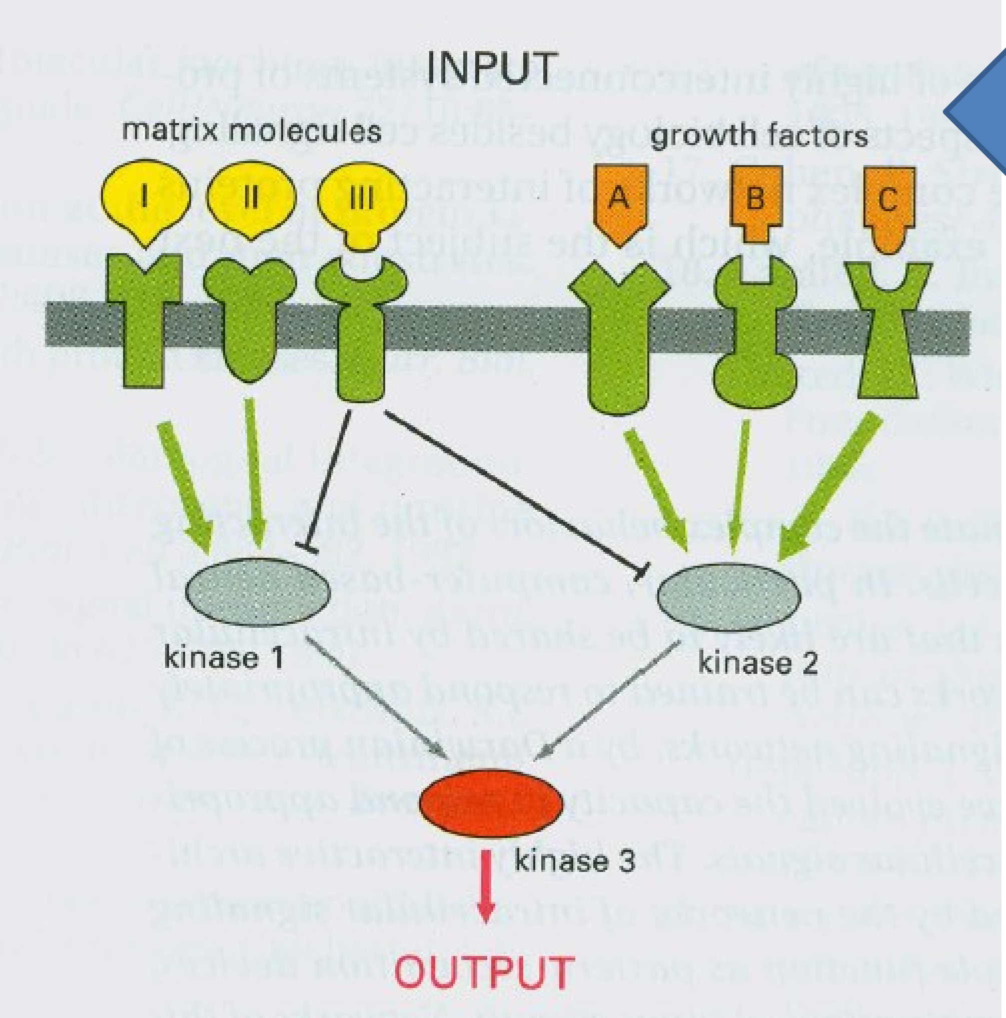
Various concentrations of bacterially-expressed malE-Mos (signal E1) added to prepared Xenopus oocyte extracts

Reactions incubated at room temperature for 100 min – length of time was sufficient to allow Mek-1 (MAPKK) and p42 (MAPK) to reach steady-state activity levels

Agreement with model predictions

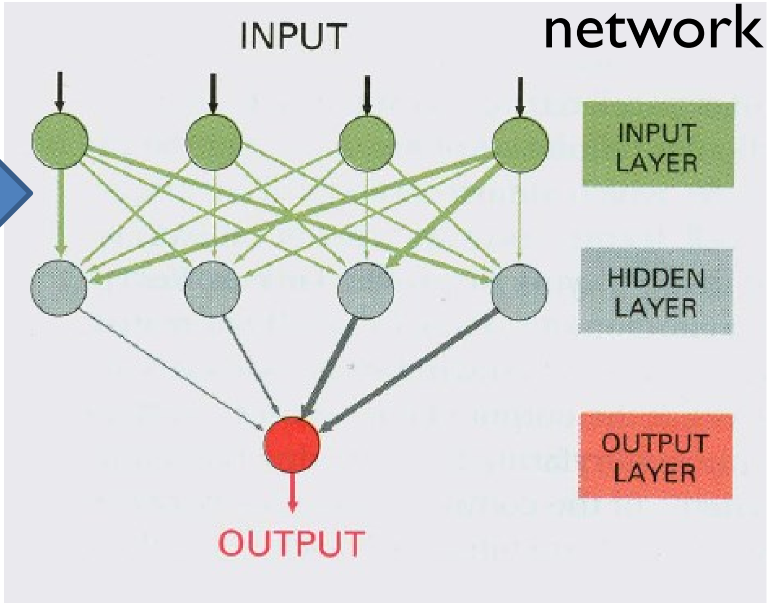
Intra-cellular signaling networks: "Neural" networks trained by evolution

simple hypothetical signaling network

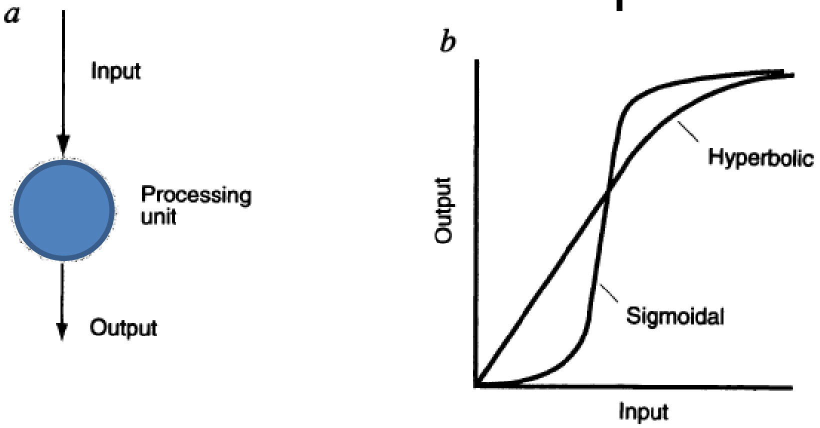


Alberts et al, Molecular Biology of the Living Cell, 3rd ed

Multi-layer feed-forward neural network



The elements of computation



D Bray, Nature (1995)