Molecular communications in four dimensions

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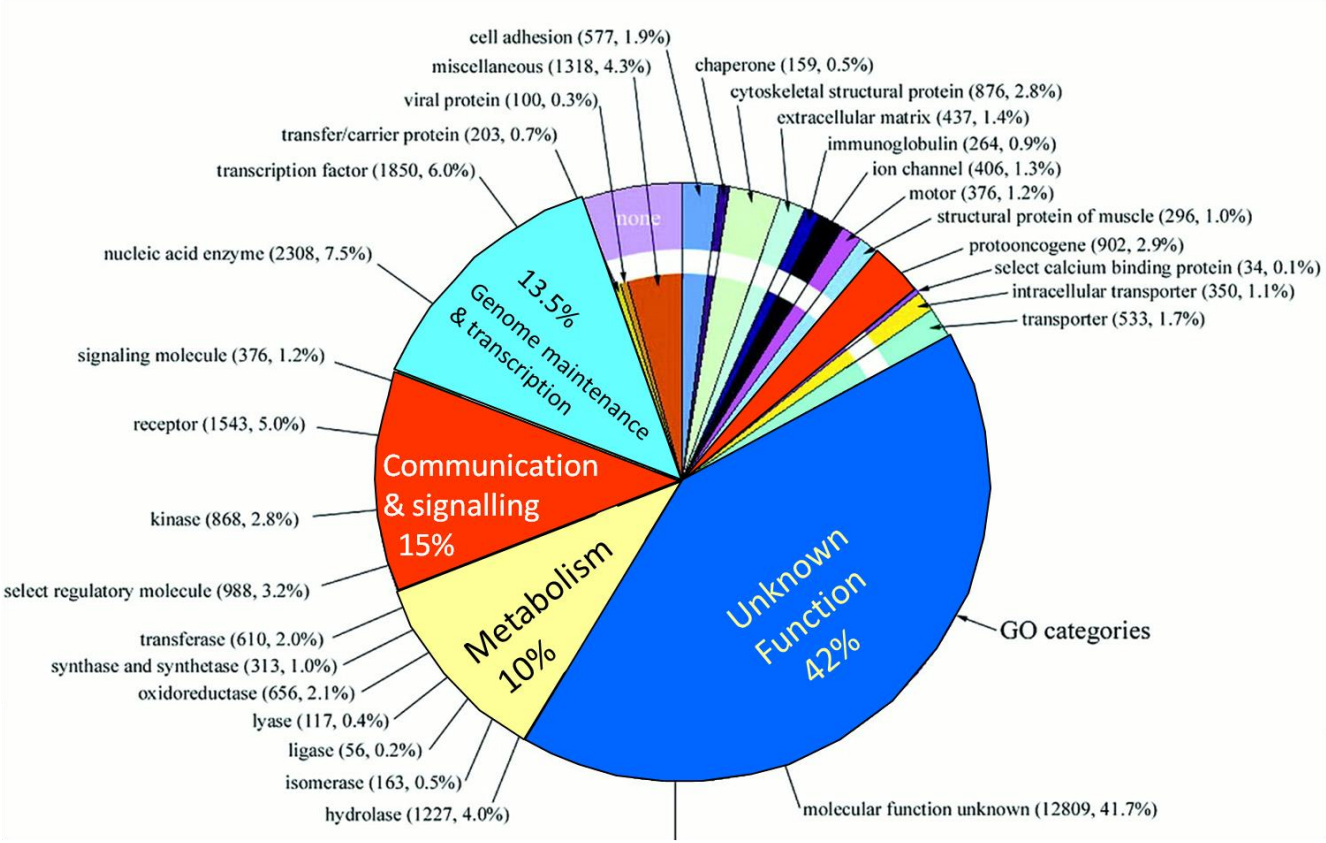
SBI – Who we are

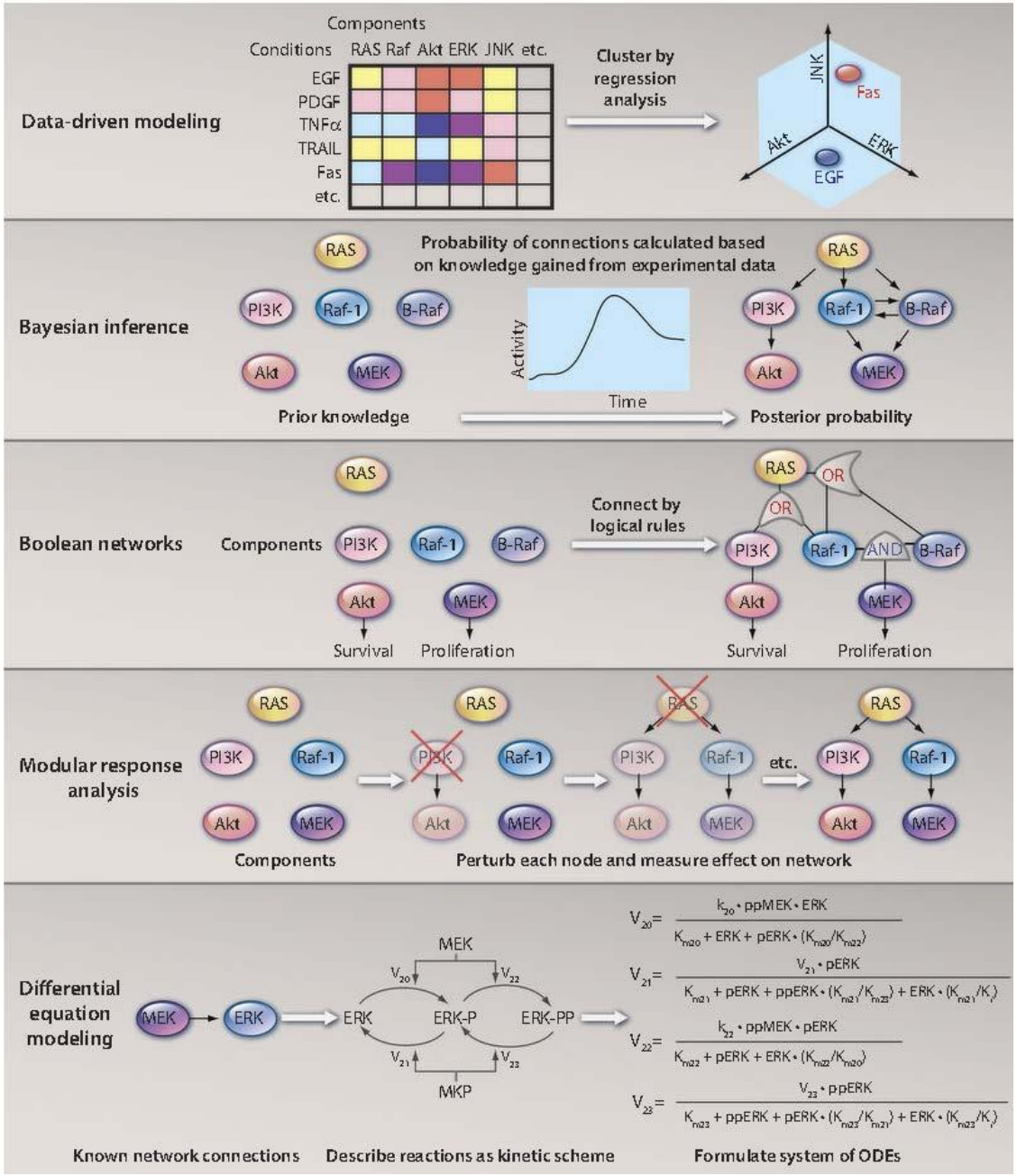
- Founded 2009 by a CSET award from Science Foundation Ireland
- ~60 staff from >20 different nations
- wet and dry under one roof
  - highly integrated research capacity
  - attractive for clinical collaborations

Why focus on signal transduction networks?

Signal transduction networks provide the **communication** within and between cells. **15% of our genes** are dedicated to **communication.**



Approaches to model cellular signalling networks



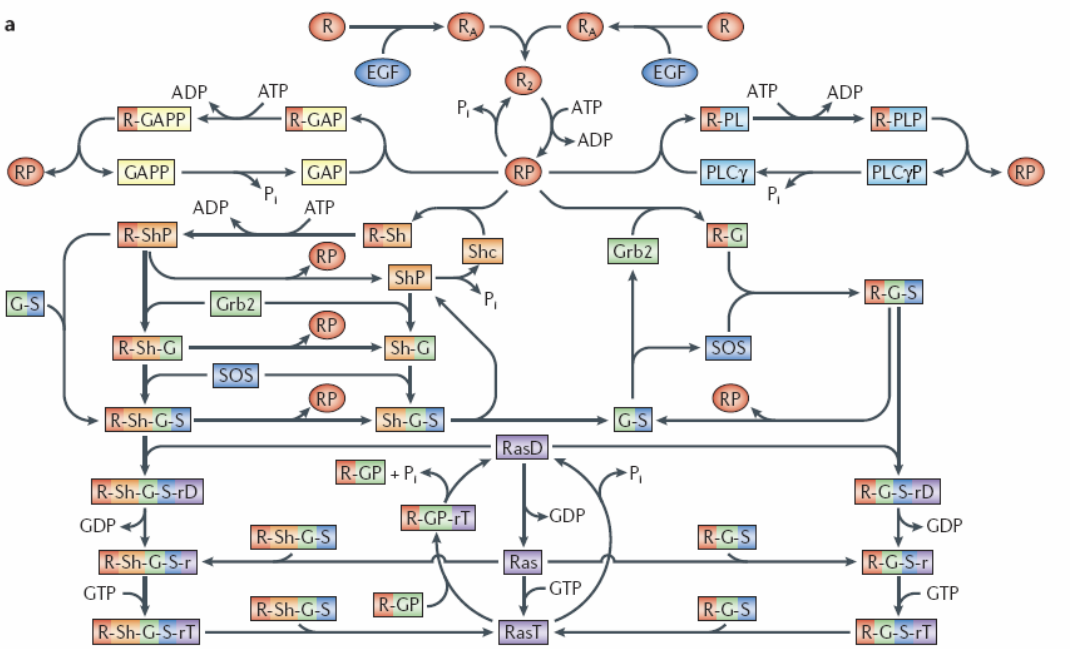
Abstract approaches

More mechanistic approaches based on physico-chemical kinetics

Kholodenko et al, Science Signaling, 5 re1 (2012)

Challenges of the Bottom-up Simulation Approach: Network circuitry and kinetic parameters

A dynamic system: $dx/dt = f(x, p).\ x = x_1,\ldots, x_n,\ p = p_1,\ldots, p_m$

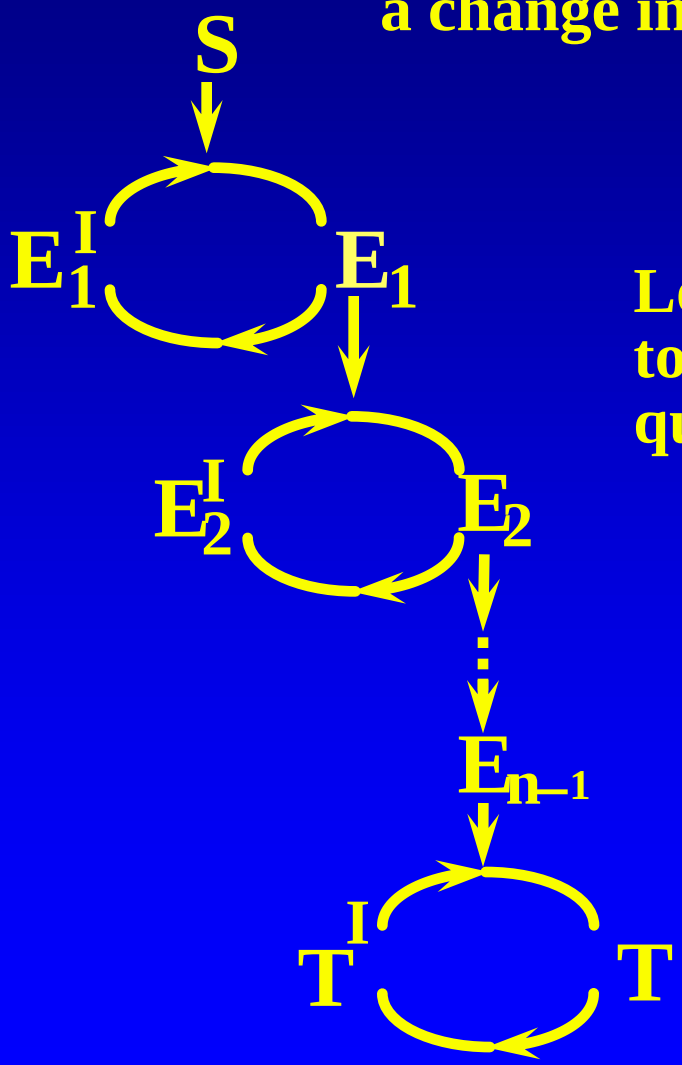


The challenge is to determine the network circuitry and kinetic parameters.

Models are trained on the kinetic data that describe the trajectories $x_i(t)$ for selected network nodes/phase variables.

Kholodenko, B.N. (2006) Nat. Rev. Mol. Cell Biol.
Kholodenko *et al* (1999) J Biol Chem

How to Quantify Global Responses to Input Signal?



Global Response: the sensitivity (*R*) of the target *T* to a change in the signal *S* (can be directly measured)

$$R_S^T = d\ln T / d\ln S\big|_{steadystate}$$

Local Response: the sensitivity (*r*) of level *i* to preceding level *i*-1. The coefficients r_i quantify local connections in the network

$$r_i = \partial\ln E_i / \partial\ln E_{i-1}\big|_{Level\ i\ steadystate}$$

System response equals the PRODUCT of local responses (connection coefficients) for a linear cascade:

$$R_S^T = r_1 \cdot r_2 \cdot \ldots \cdot r_{n-1} \cdot r_n = \prod(path)$$

Kholodenko *et al* (1997) FEBS Lett. 414: 430

Modular Response Analysis (MRA): Untangling the Signaling Wires

BOX 1 QUANTIFICATION AND UNRAVELLING OF NETWORK INTERACTIONS

How is the network wired?

Mathematical description

$dx_i/dt = f(x_1,\ldots, x_n,p_i)$.
p_i – perturbation parameters (siRNA)

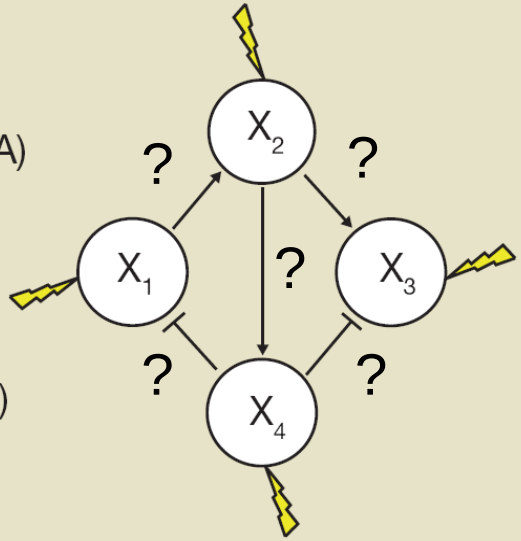
Jacobian matrix $(\partial f_i/\partial x_j)$

Relative strength of connection from node *j* to node *i* is given by

$r_{ij} = \partial \ln x_i/\partial \ln x_j = - (\partial f_i/\partial \ln x_j)/(\partial f_i/\partial \ln x_i)$

Global response of a node *j* to aperturbation p_k is given by

$R_{jk} = \partial \ln x_j/\partial p_k$



Experimental procedure

- Make perturbation
- Measure global responses
- Repeat steps above until all nodes have been perturbed
- Calculate connection coefficients r_{ij} using measured global responses R_{jk}

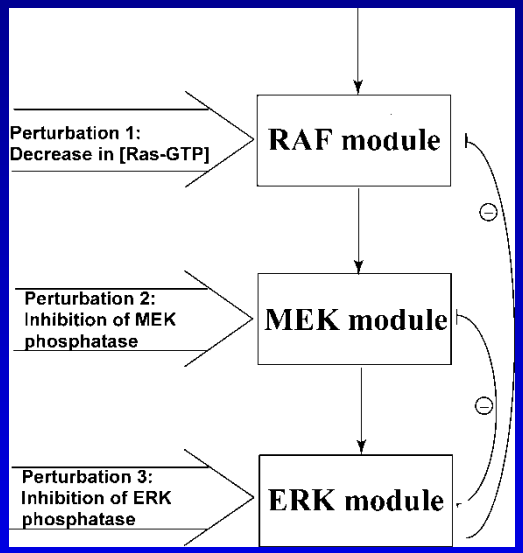
$$\sum_j r_{ij} R_{jk} = 0$$

Kholodenko B.N. Nature Cell Biol (2007) 9: 247

Kholodenko et al (2002) PNAS 99: 12841

Step 1: Determining Global Ras/MAPK Cascade Responses to Three Independent Perturbations

a) Measurement of the differences in steady-state variables following perturbations:



$$\Delta \ln X \approx 2(X^{(1)} - X^{(0)})/(X^{(1)} + X^{(0)})$$

1

$$\begin{pmatrix} \Delta_1 \ln(Raf\text{–}P) \\ \Delta_1 \ln(MEK\text{–}PP) \\ \Delta_1 \ln(ERK\text{–}PP) \end{pmatrix}$$

2

$$\begin{pmatrix} \Delta_2 \ln(Raf\text{–}P) \\ \Delta_2 \ln(MEK\text{–}PP) \\ \Delta_2 \ln(ERK\text{–}PP) \end{pmatrix}$$

3

$$\begin{pmatrix} \Delta_3 \ln(Raf\text{–}P) \\ \Delta_3 \ln(MEK\text{–}PP) \\ \Delta_3 \ln(ERK\text{–}PP) \end{pmatrix}$$

b) Generation of the system response matrix

–7.4	6.9	3.7
–6.2	–3.1	8.9
–12.7	–6.3	–3.4

10% change in parameters

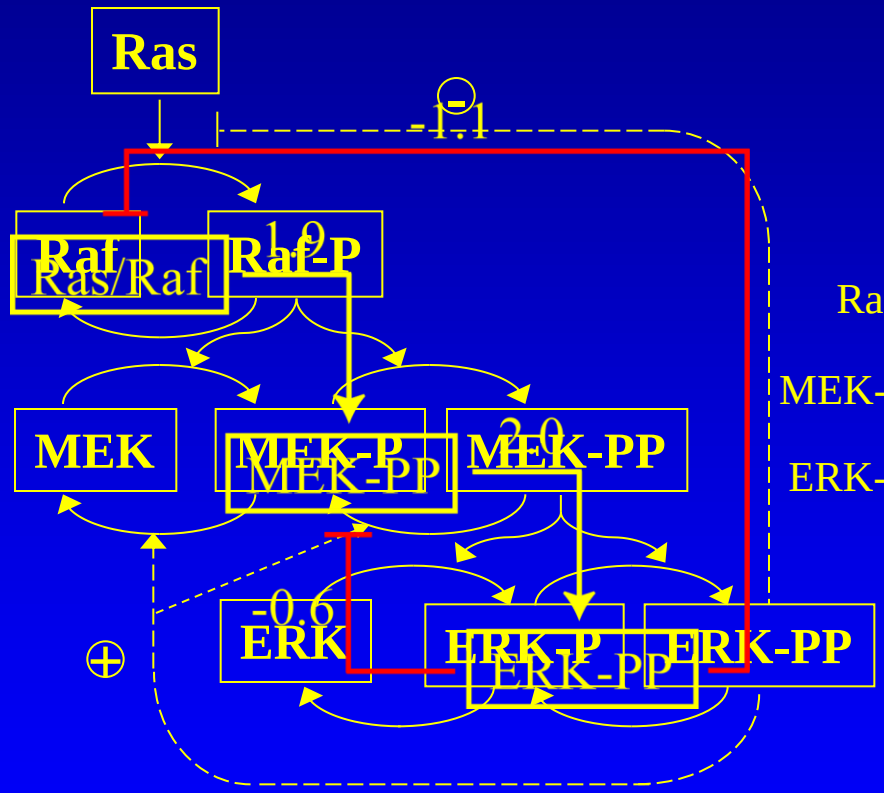
–55.5	–46.3	–25.0
–44.8	20.3	–56.8
–85.7	39.4	21.8

50% change in parameters

Kholodenko et al (2002) PNAS 99: 12841

Step 2: Calculating the Ras/MAPK Cascade Interaction Map from System Responses

$$\mathbf{r} = -\left(dg(\mathbf{R}^{-1})\right)^{-1}\cdot\mathbf{R}^{-1}$$



Two interaction maps (local response matrices) retrieved from two different system response matrices

	Raf-P	MEK-PP	ERK-PP
Raf-P	−1	0.0	−1.1
MEK-PP	1.9	−1	−0.6
ERK-PP	0.0	2.0	−1

	Raf-P	MEK-PP	ERK-PP
Raf-P	−1	0.0	−1.2
MEK-PP	1.8	−1	−0.6
ERK-PP	0.0	2.0	−1

Known Interaction Map

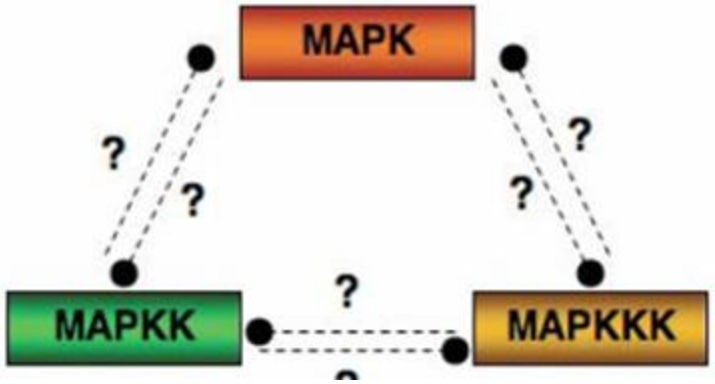
	Raf-P	MEK-PP	ERK-PP
Raf-P	−1	0.0	−1.1
MEK-PP	1.9	−1	−0.6
ERK-PP	0.0	2.0	−1

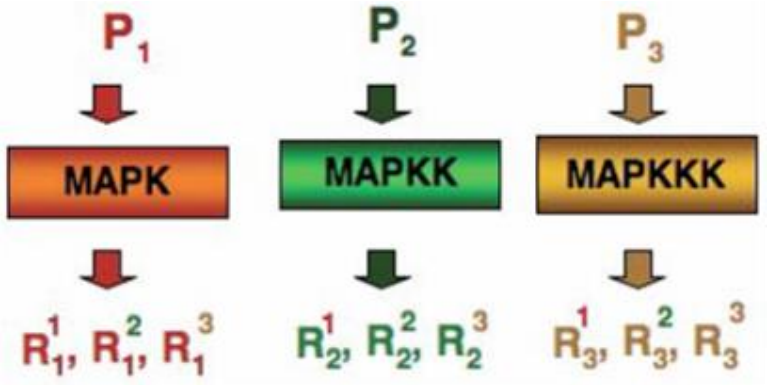
Kholodenko et al (2002) PNAS 99: 12841

Experimental Application of the Unraveling Method

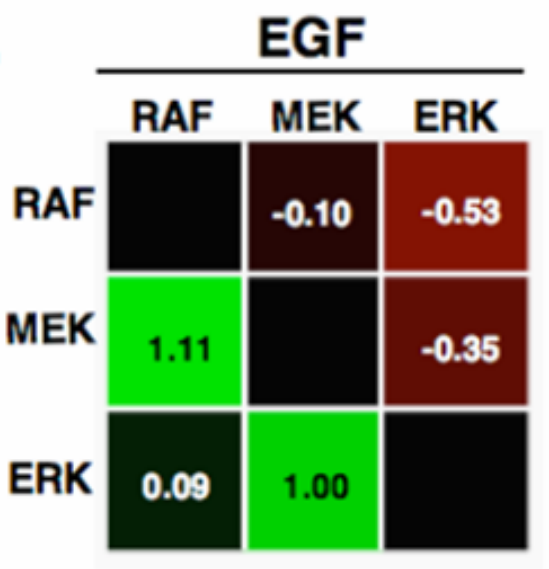
Santos S.D.M., Verveer P.J. & Bastiaens P.I.H. Nature Cell Biol. 2007

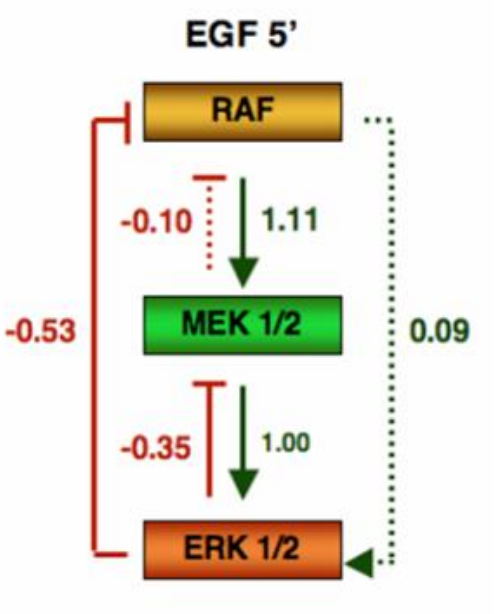
How is the MAPK Network Wired?

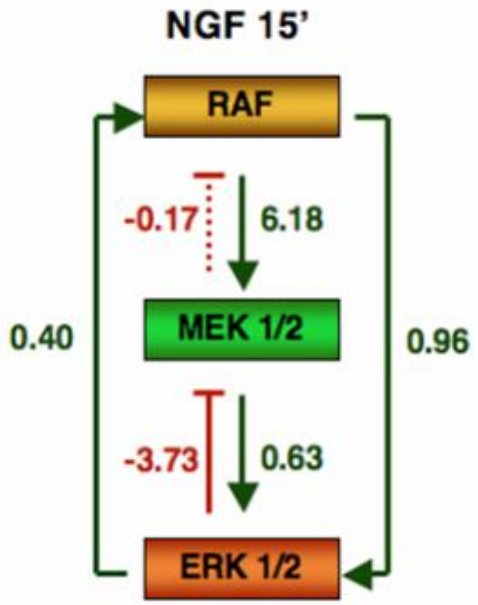




Growth factor induced MAPK network topology determines PC-12 cell fate







Modular Response Analysis (MRA)

Advantages
- Gives direction of connections
- Quantifies strengths of connections

Disadvantages
- Every network component needs to be perturbed
- Sensitive to measurement & biological noise

Bayesian Modular Response Analysis (BMRA)

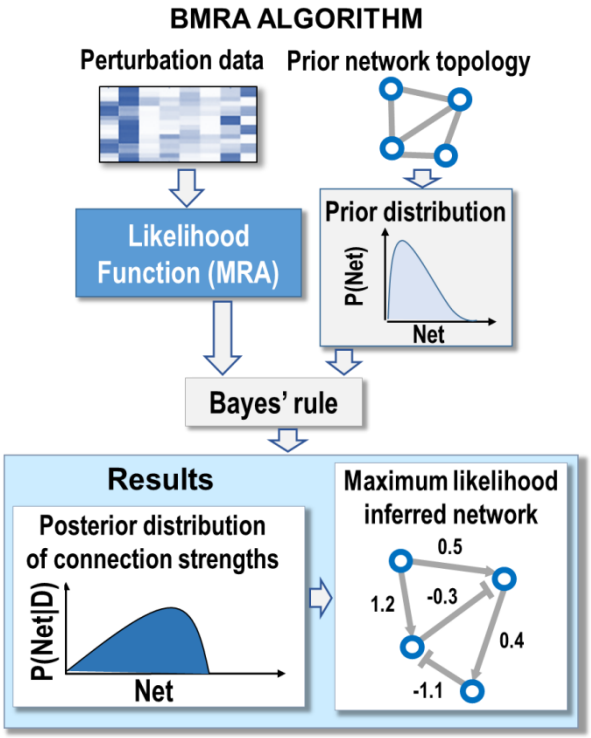
$$\sum_{j=1,j\neq i}^{n} r_{ij}R_{jk} = R_{ik}; i \neq k; i,k = 1\ldots n$$

$$\sum_{j=1,j\neq i}^{n} A_{ij}r_{ij}R_{jk} + \epsilon_{ik} = R_{ik}; i = 1\ldots n, k = 1\ldots n_p^i, i \neq k$$

Binary Variable:
Aij=0 if rij =0
Aij=1 if rij≠0

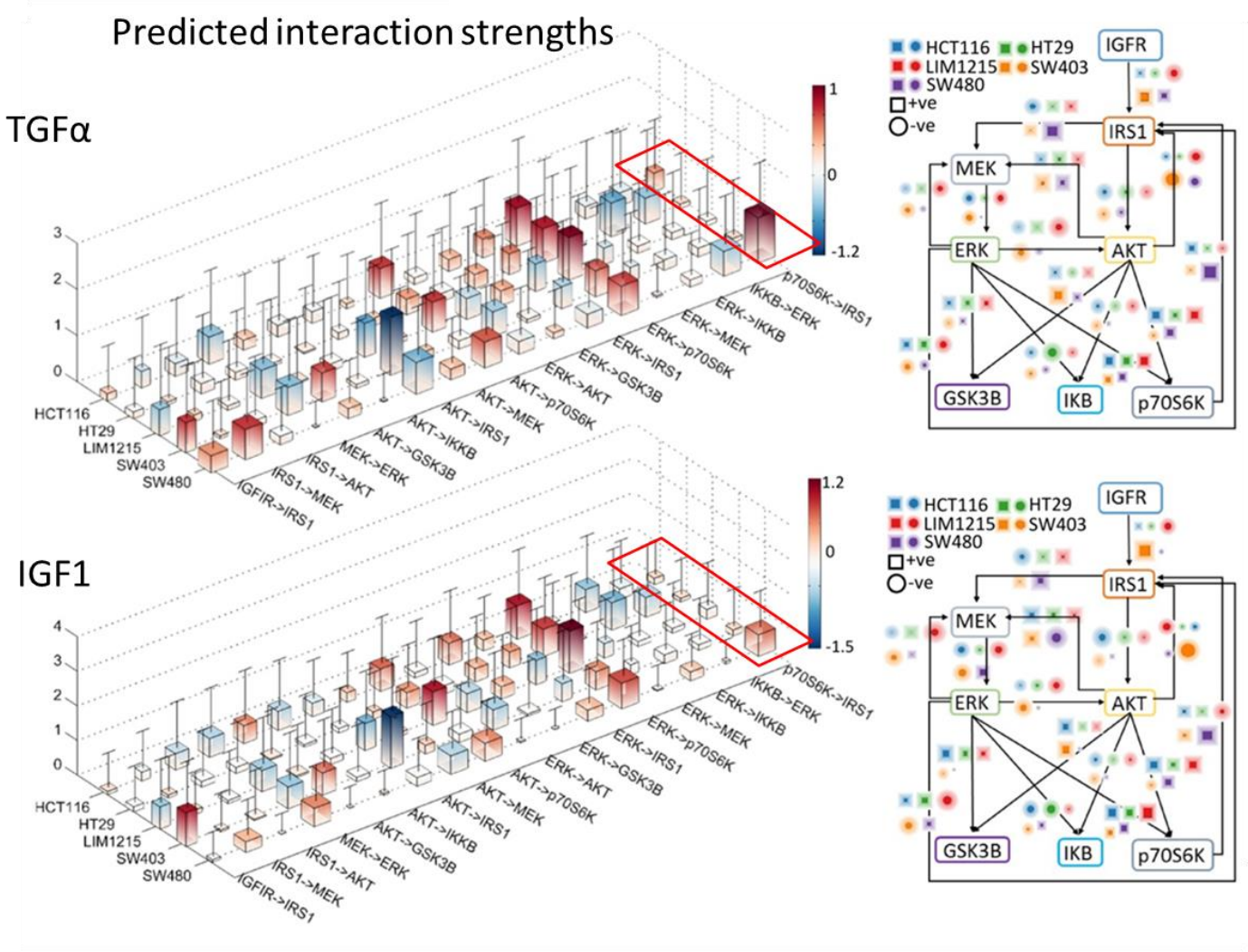
Stochastic variable that accounts for the error caused by noise

Determined by Bayesian Variable Selection Algorithm (BVSA), which is a MCMC approximation to estimate whether a connection exists or not



Santra et al (2013). BMC Syst Biol 7: 57; Halasz et al (2016) Sci Signal. 9, ra114.

Colorectal cancer: Cell-line specific connections inferred

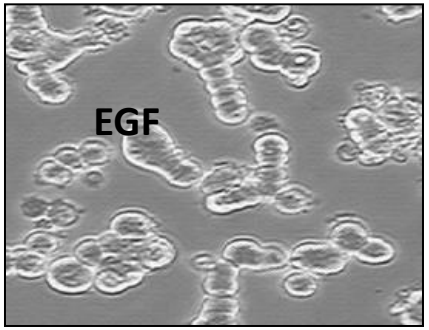


p70 S6K phosphorylation feedback to IRS1 is dramatically different across five CRC cell lines. Breaking the p70 S6K-mediated feedback sensitizes mutant KRAS cells to EGFR inhibitors.

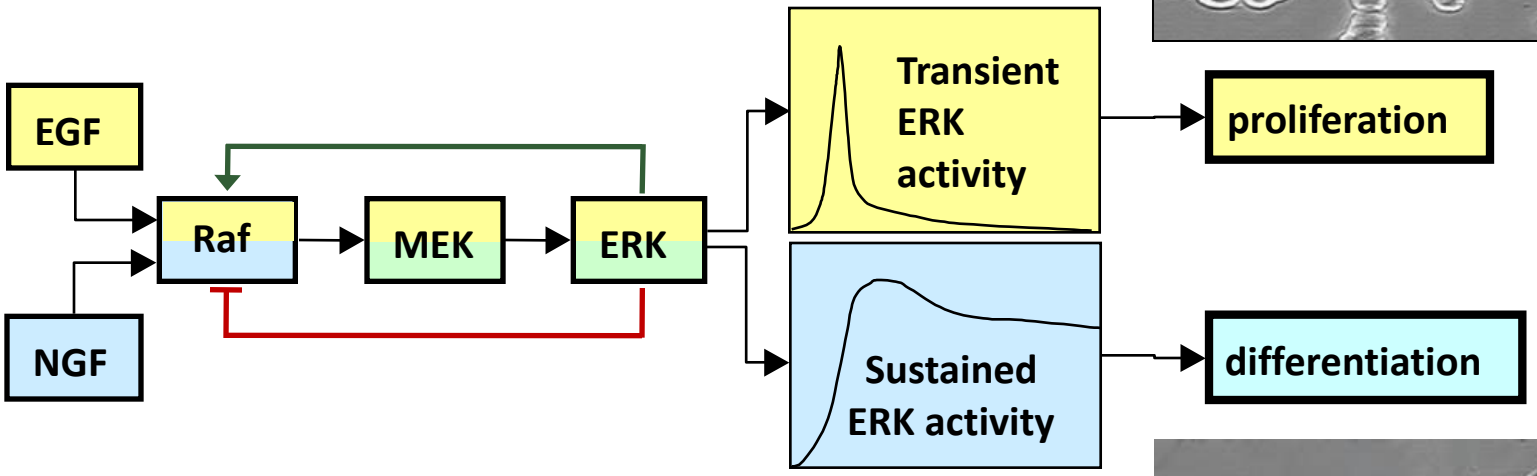
Halasz et al (2016) Sci Signal. 9, ra114.

Challenge: How Can External Cues Lead to Unique Cellular Responses if Signaling Pathways are Shared?

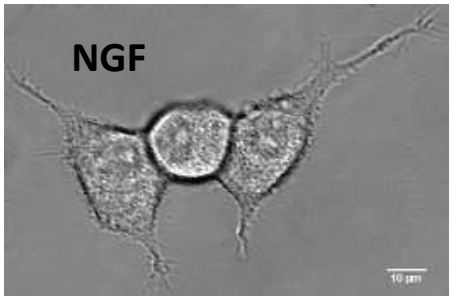
PC12 cell model: Epidermal growth factor (**EGF**) supports proliferation, whereas Nerve Growth Factor (**NGF**) causes irreversible differentiation.



Activation kinetics of ERK determines cell fate:

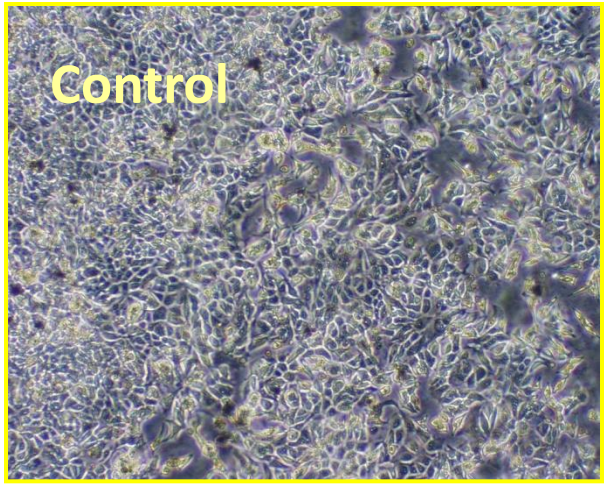


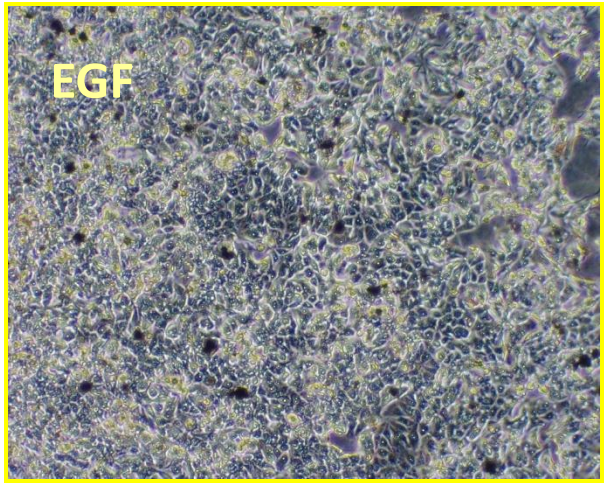
Another cell model with ligand-specific ppERK responses is MCF cancer cells stimulated with EGF or HRG

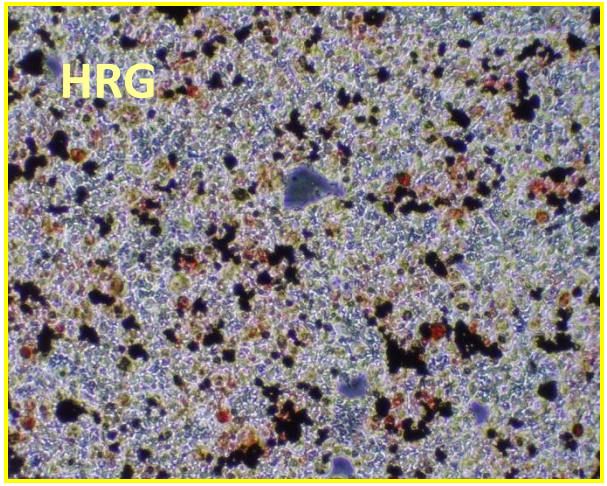


Ligand-Specific Cell-Fate Control in MCF-7 Cells

Epidermal Growth Factor (EGF) has high affinity for ErbB1, induces transient ERK signalling and causes proliferation





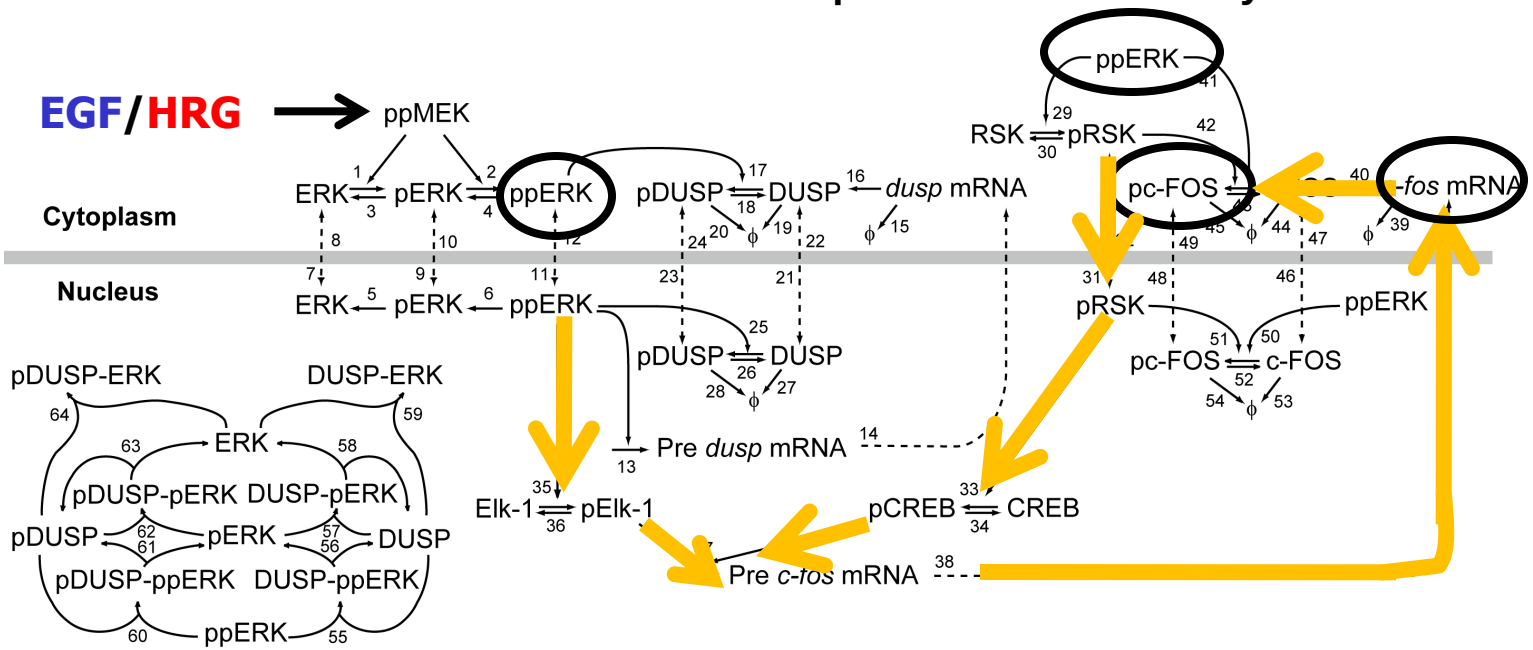


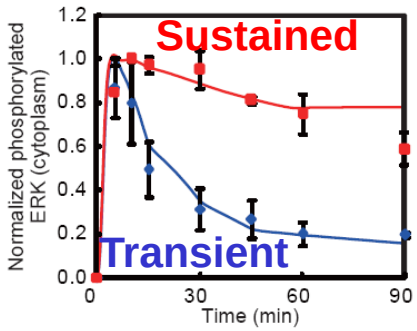
Heregulin (HRG) has high affinity for ErbB3/ErbB4, induces sustained ERK signalling and causes differentiation

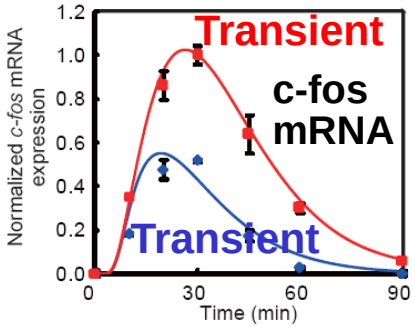
Differentiated cells produce lipid and beta-lactoalbumin

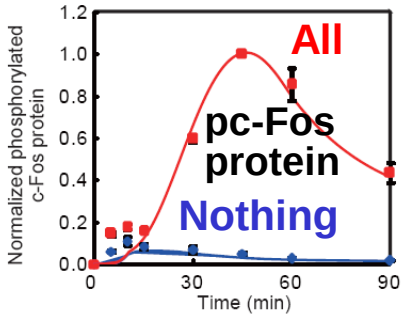
Modeling Signal Specificity of c-Fos Expression

Key events: ERK activates RSK, and both ERK and RSK control *c-fos* transcription through activation of TFs Elk-1 and CREB. Nascent c-Fos protein is stabilized by ERK and RSK



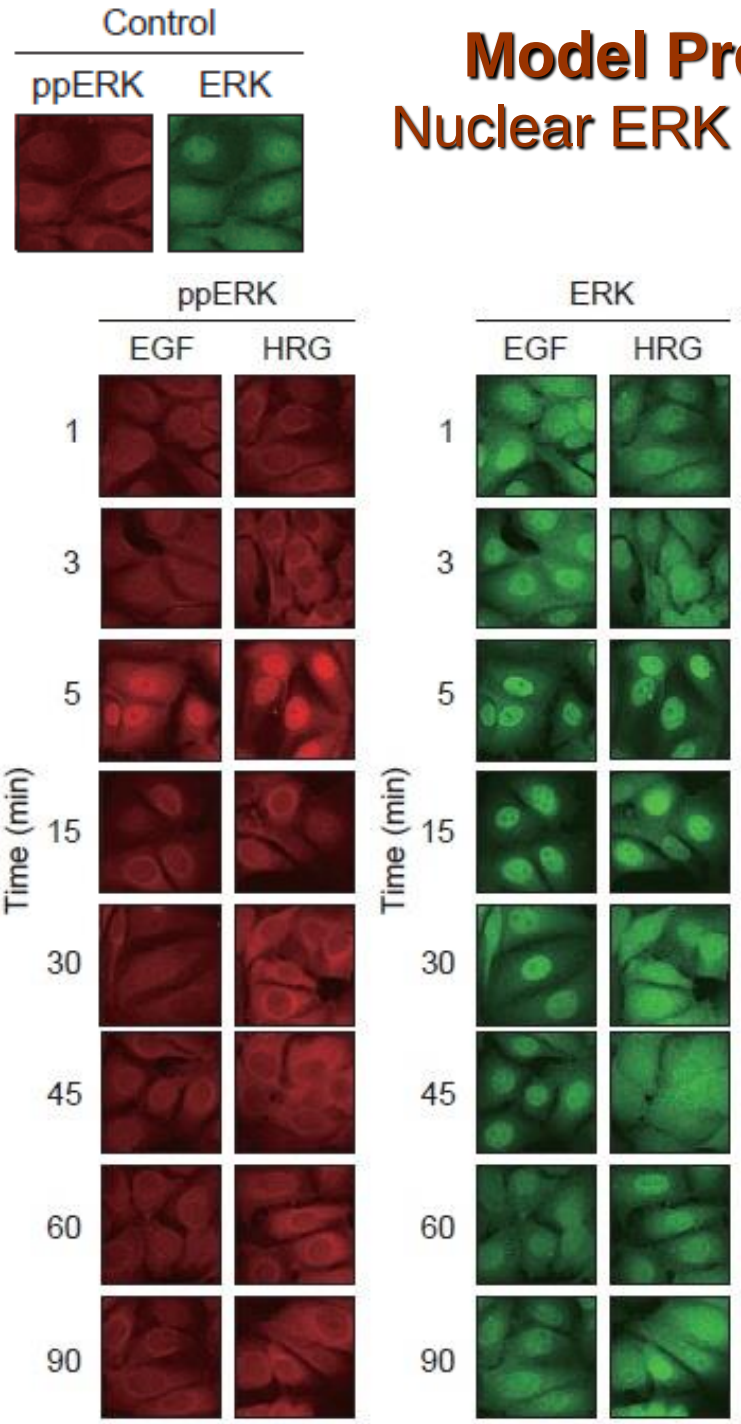


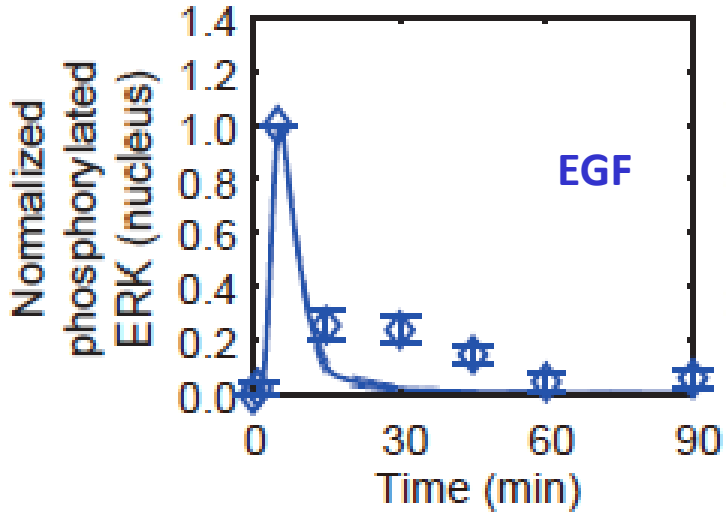


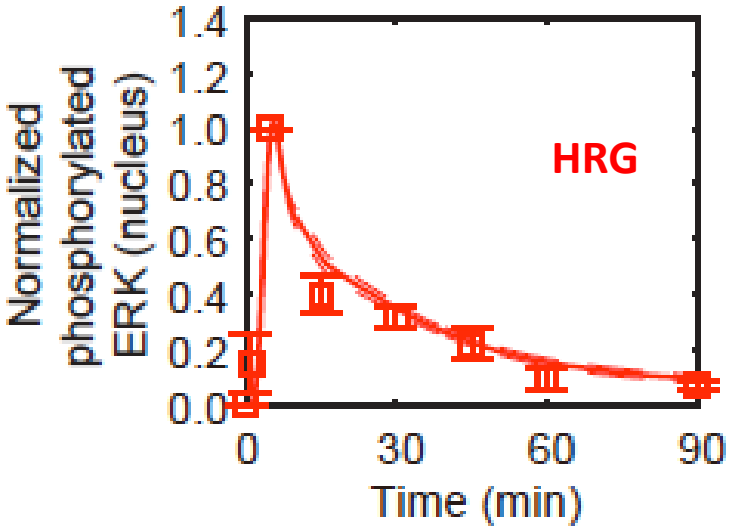


What Controls These Ligand-Dependent Responses in MCF7 cells?

Model Prediction and *In vivo* Validation:
Nuclear ERK Signaling is Transient for EGF & HRG



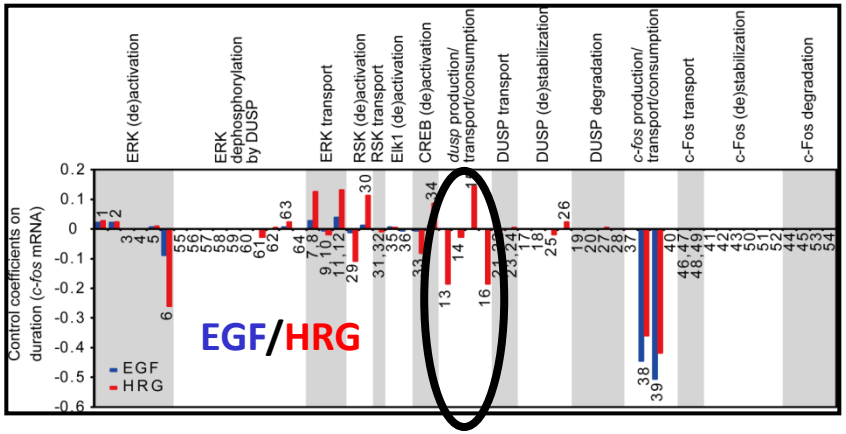




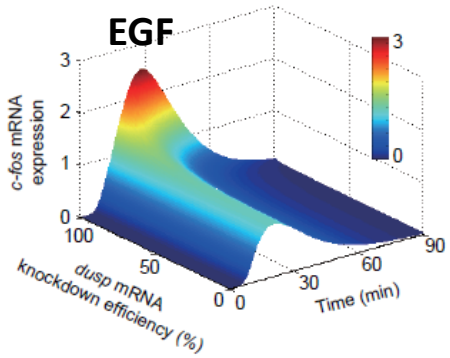
Nakakuki et al, Cell, 141: 884 (2010)

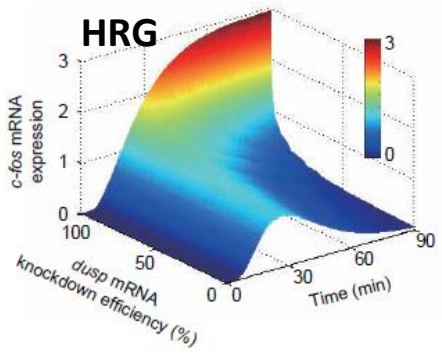
Model Suggests Critical Role of Dual Specificity Phosphatases (DUSP)

Sensitivity Analysis of *c-fos* mRNA Duration

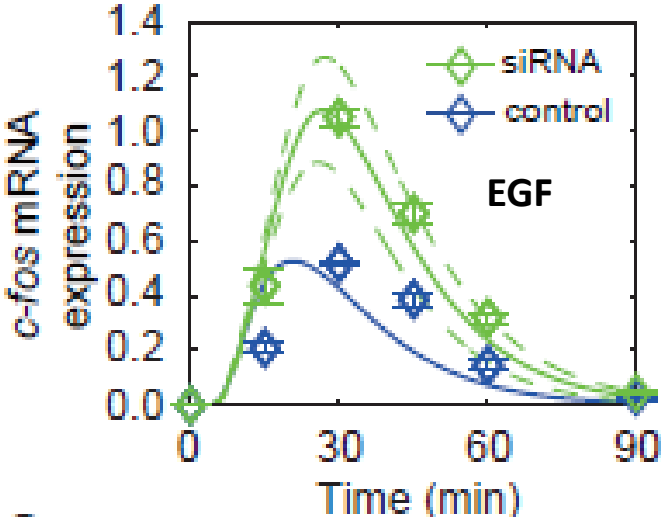


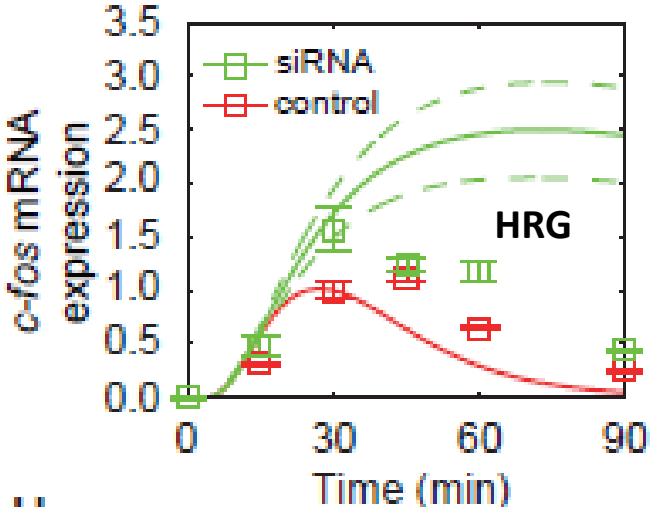
***In silico* effects of *dusp* knockdown**





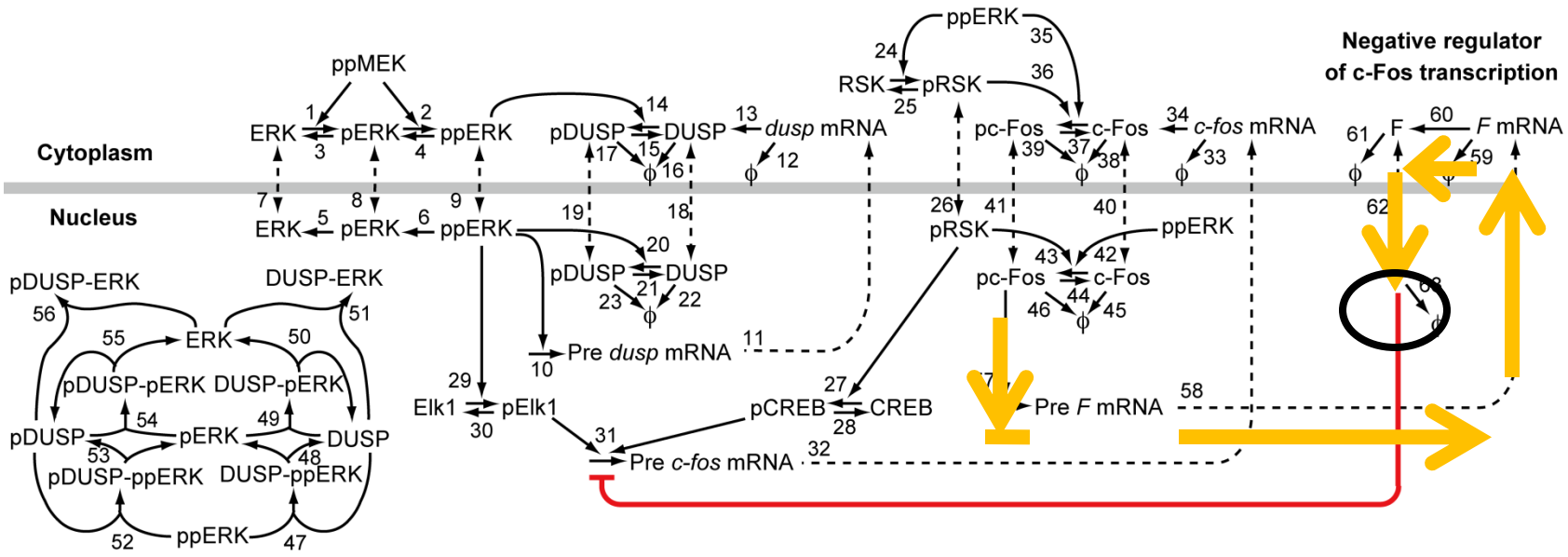
Experimental Data Only Partially Confirm the Model

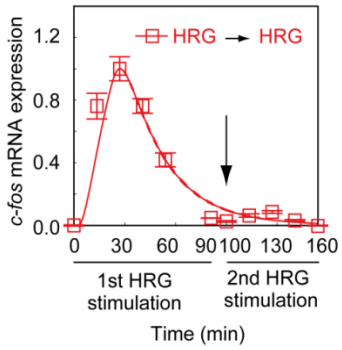


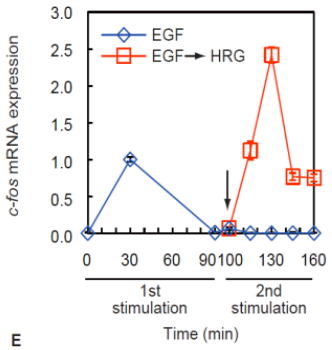


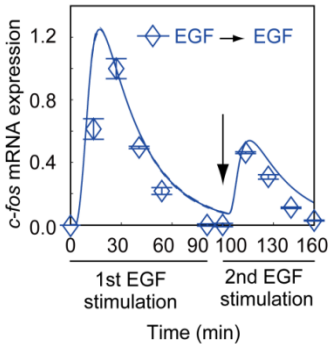
DUSP knockdown increased *c-fos* mRNA expression for EGF, as predicted, but not by HRG, in contrast with the model predictions

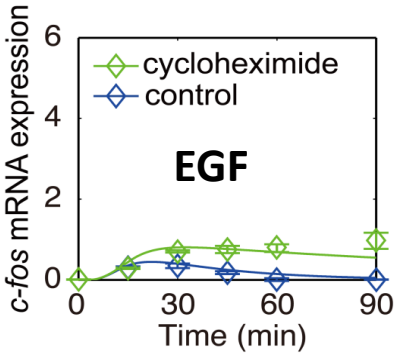
Pivotal Role of Transcriptional Negative Feedback Loops

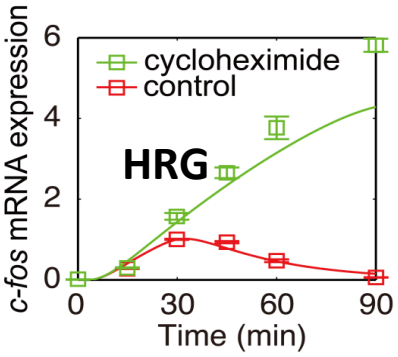










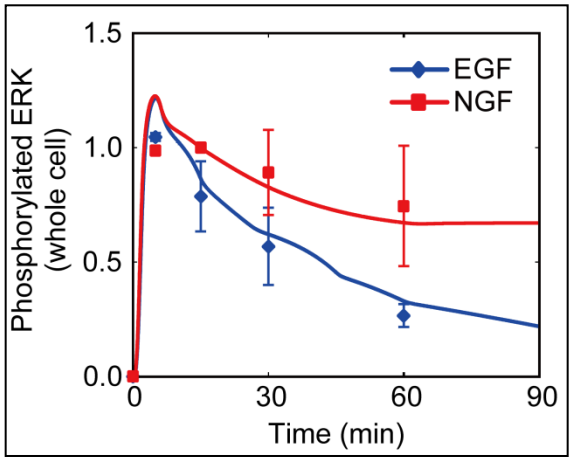


Double-pulse and cycloheximide experiments demonstrate that there is a novel transcriptional repressor of *c-fos* expression that is induced by HRG, but not by EGF.

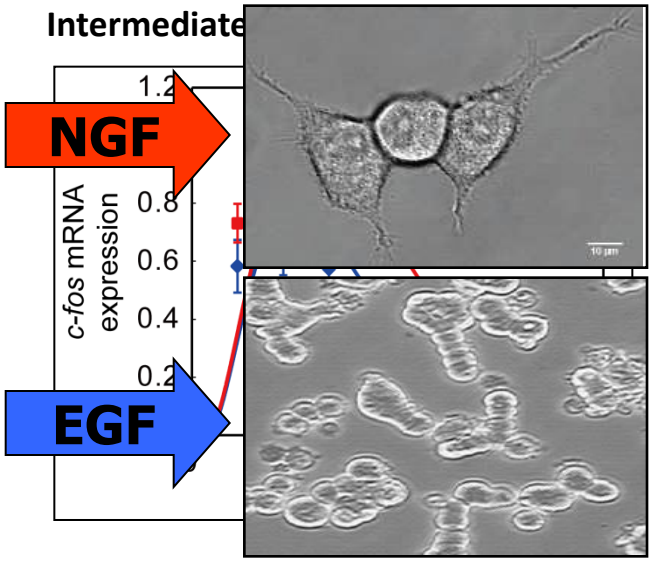
Nakakuki et al, Cell 141: 884 (2010)

Ubiquitous Control Mechanisms of c-Fos Expression: PC12 System Revisited

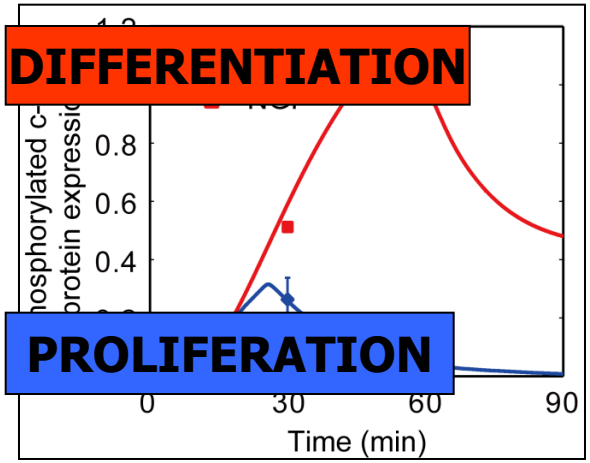
Input signal – ppERK



Intermediate

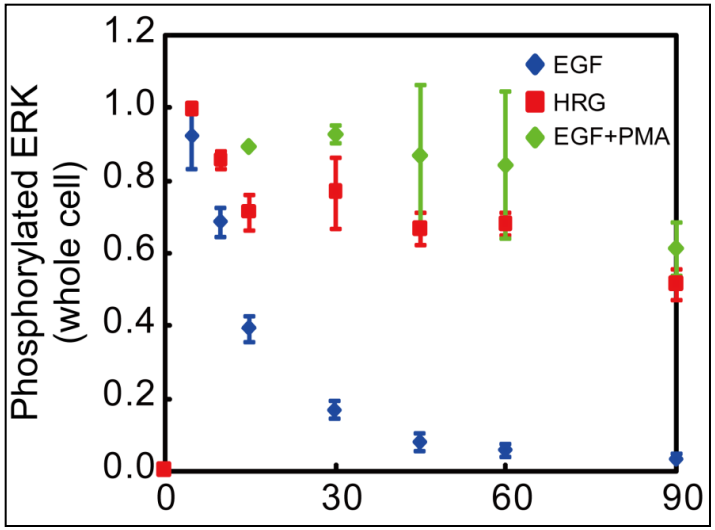


Output – phosphorylated c-Fos

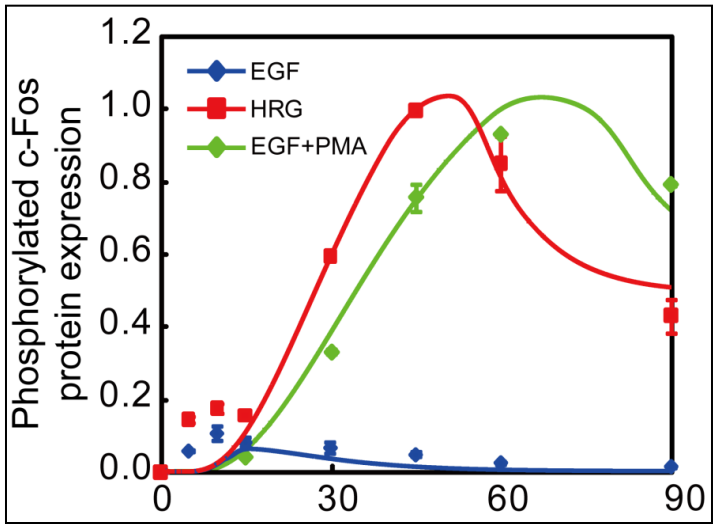


Rewiring cell-fate decisions in MCF-7 cells

PMA converts the transient EGF-induced input signal into sustained ppERK input

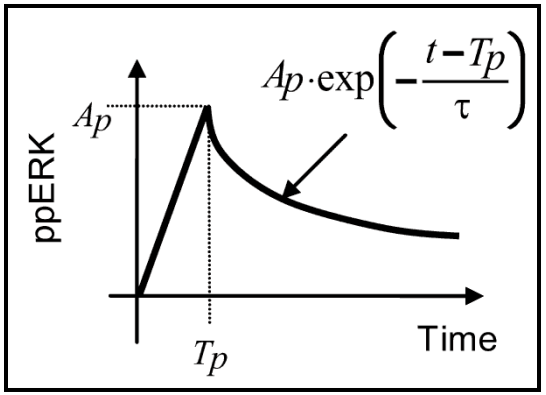


Spatio-temporal regulation of the c-Fos expression cascade transforms MCF-7 cell decisions



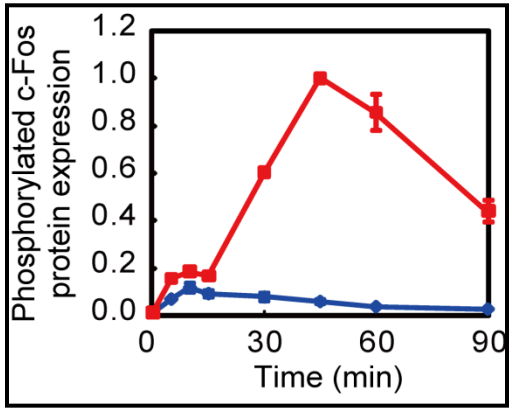
Conclusion: Control Principles of the c-Fos Expression System

Input - ppERK Dynamics (simplified description)

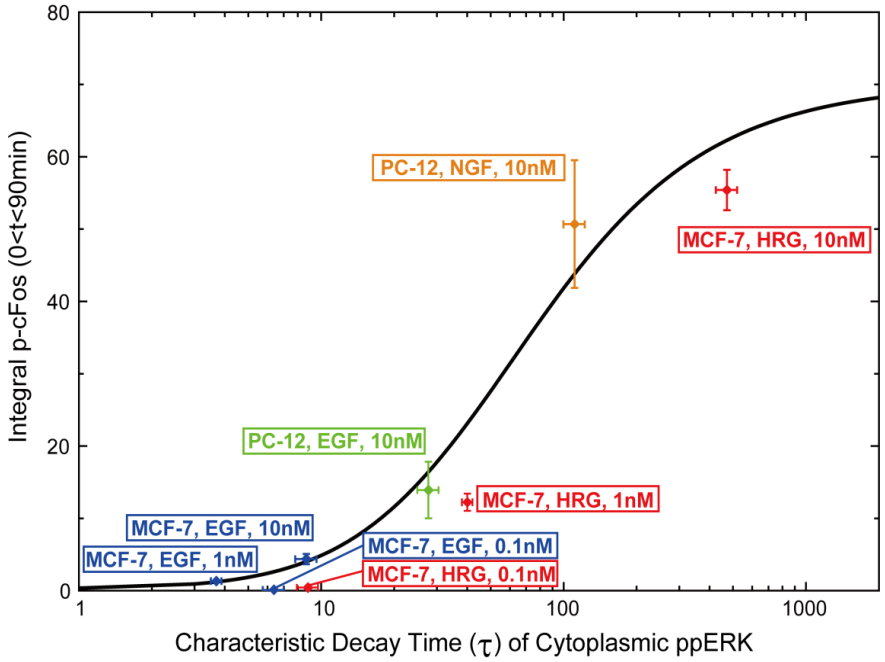


c-Fos Expression System

Output - pc-Fos Dynamics (responses integrated over time)



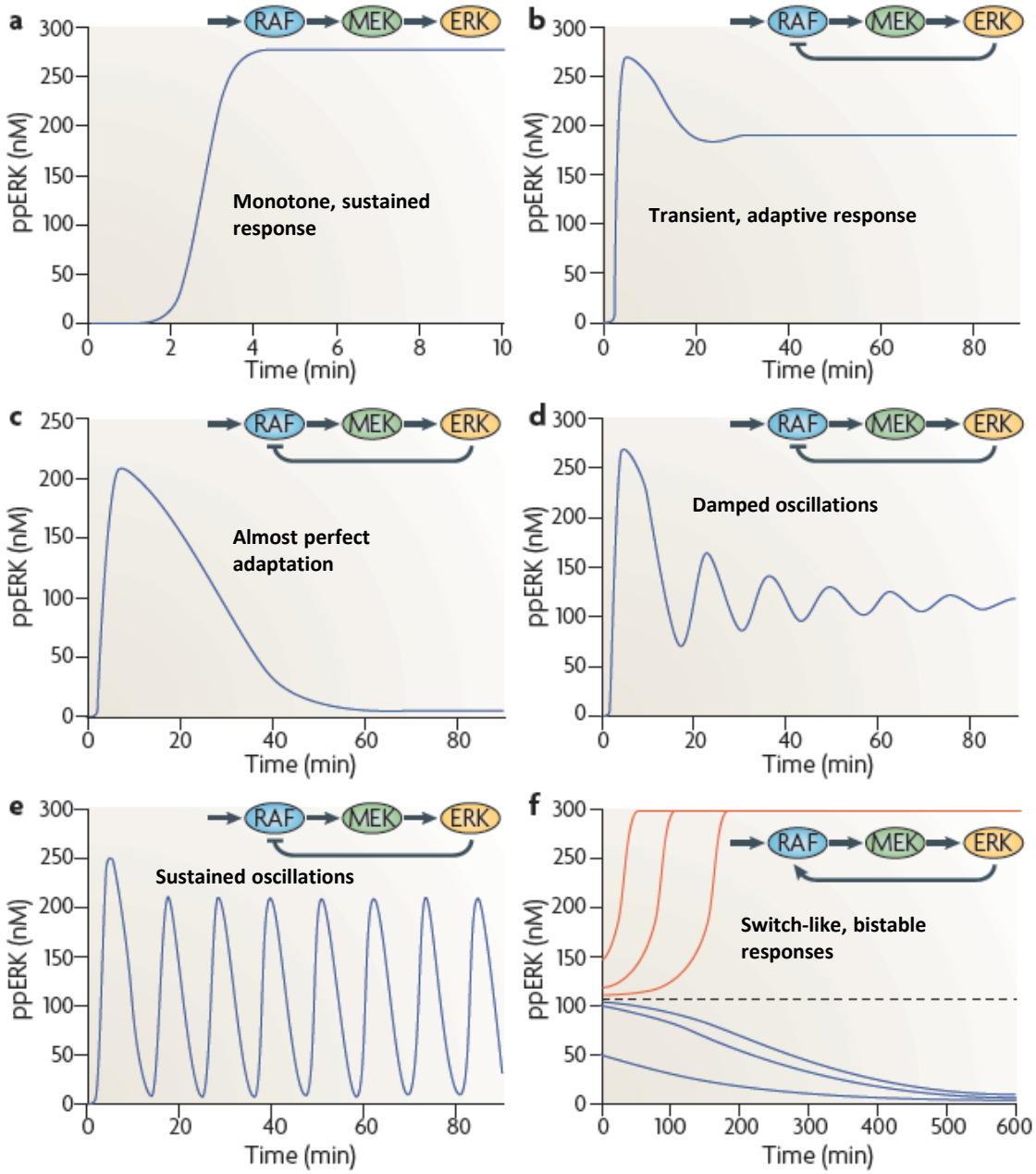
Unified Relation Between ppERK Input Decay and pc-Fos Output Integrated Response



Nakakuki et al, Cell 141: 884 (2010)

Catching 4-D Dynamics Surfing the Net

How do MAPK dynamic responses change with feedback?

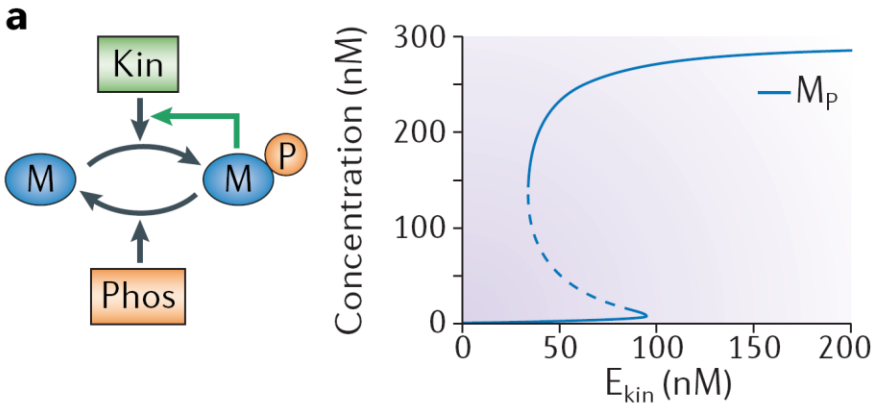


MAPK cascades display remarkably different temporal responses to a stimulus depending on feedback.

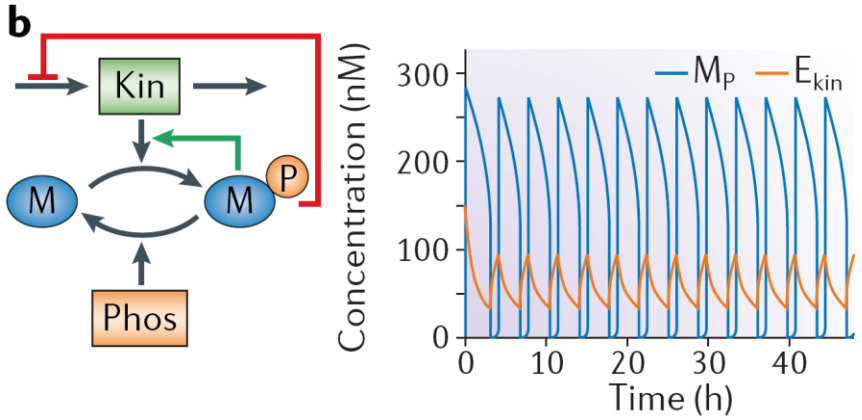
Identifying MAPK feedback circuitry is not a trivial task

Kholodenko et al, Nature Rev Mol Cell Biol (2010)

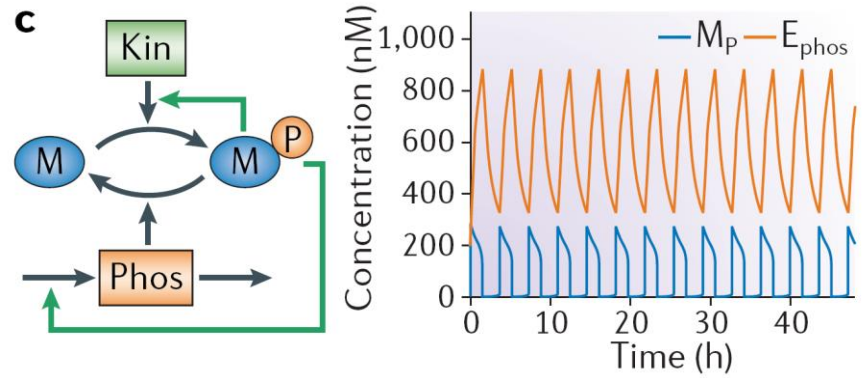
Simple motifs displaying complex dynamics



Bistability and hysteresis arise from product activation (destabilizing positive feedback)

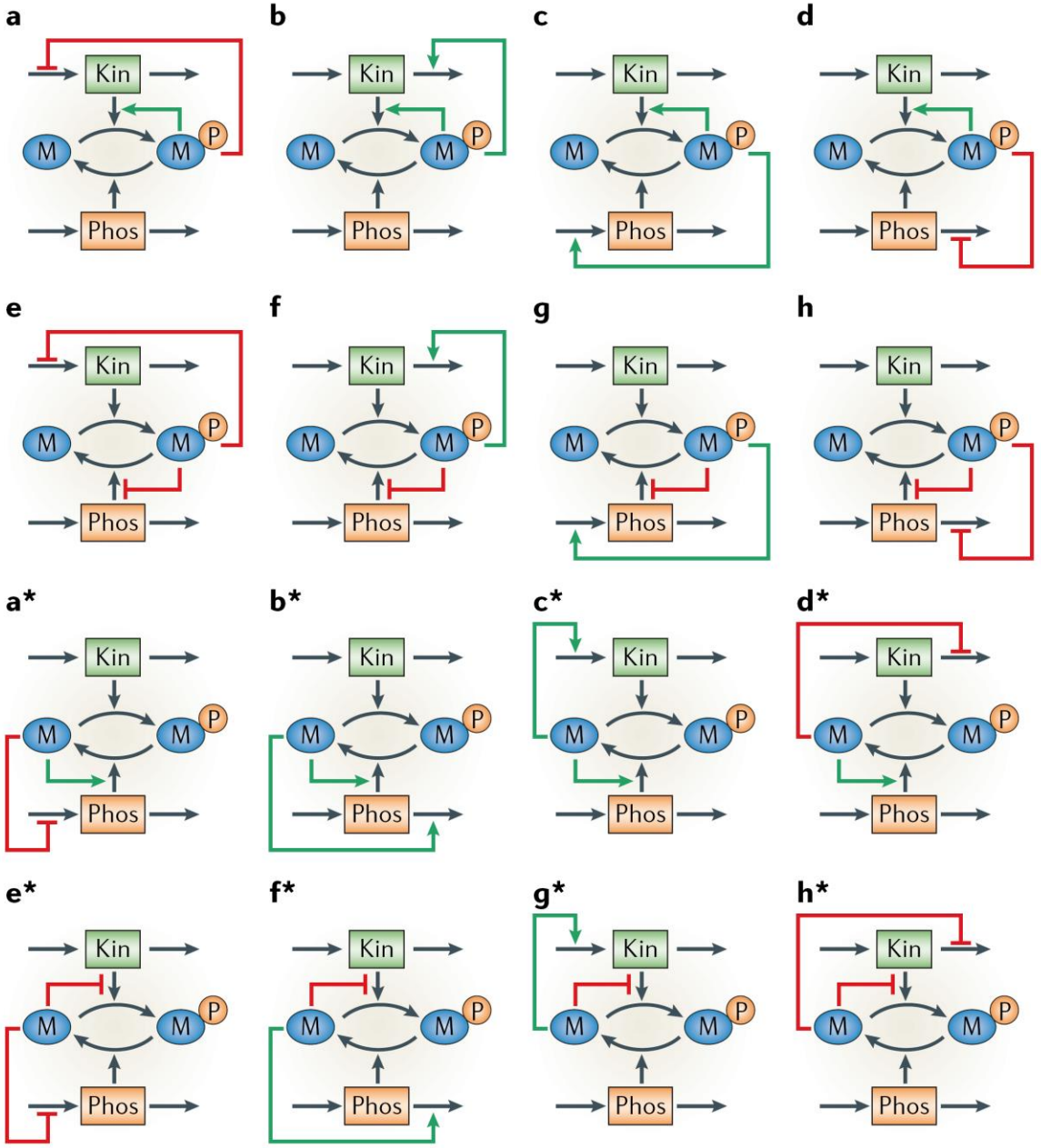


Relaxation oscillator is brought about by positive feedback plus negative feedback



Kholodenko, B.N. (2006)
Nat. Rev. Mol. Cell Biol.

Simple motifs displaying complex dynamics



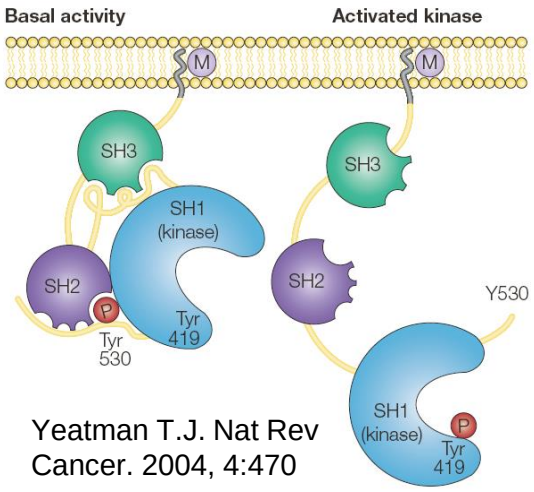
32 feedback designs that turn a universal protein modification motif into a bistable switch and a relaxation oscillator.

Complex dynamics is a robust design property.

All rates and feedback loops obey simple Michaelis-Menten type kinetics.

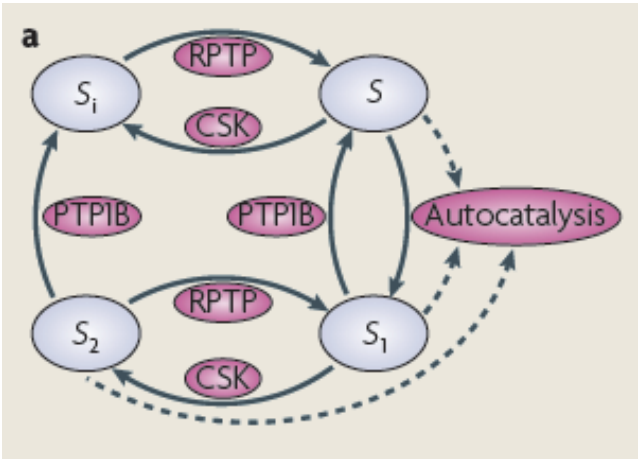
Kholodenko, B.N. (2006) Nat. Rev. Mol. Cell Biol.

Src Kinase Exhibits Complex Dynamics



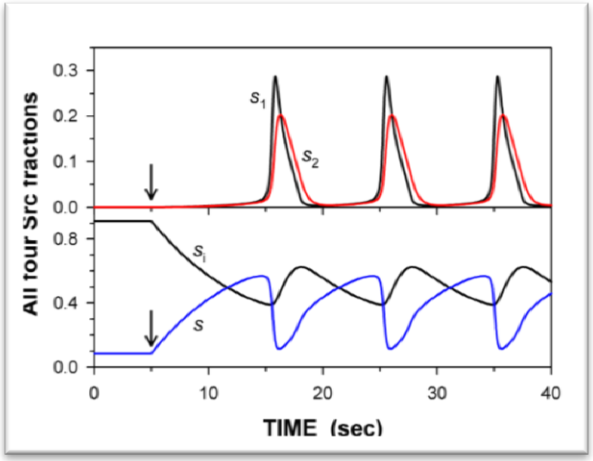
Yeatman T.J. Nat Rev Cancer. 2004, 4:470

Kinetic diagram: Transitions between inactive (S_i) and active (S_1 and S_2) forms of Src

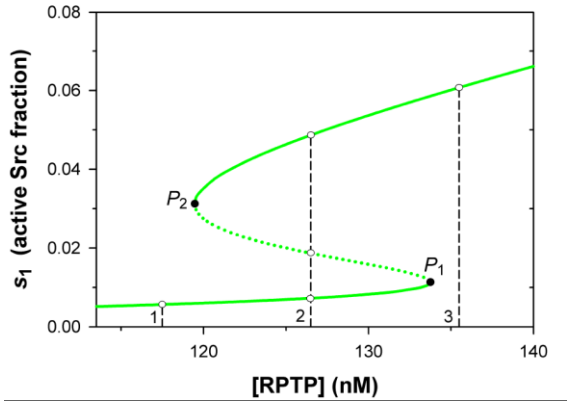


Kholodenko et al, Nature Rev Mol Cell Biol (2010)

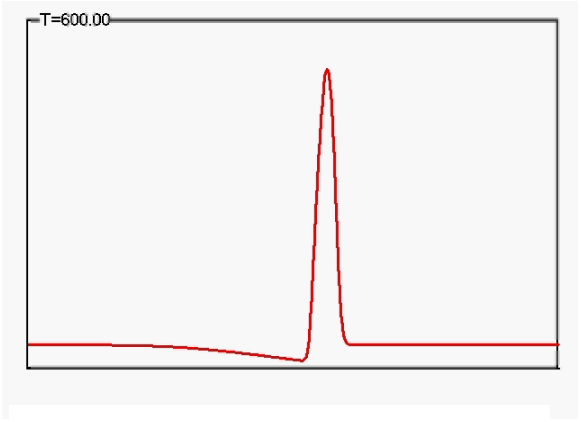
Oscillations



Bistable behaviour and hysteresis

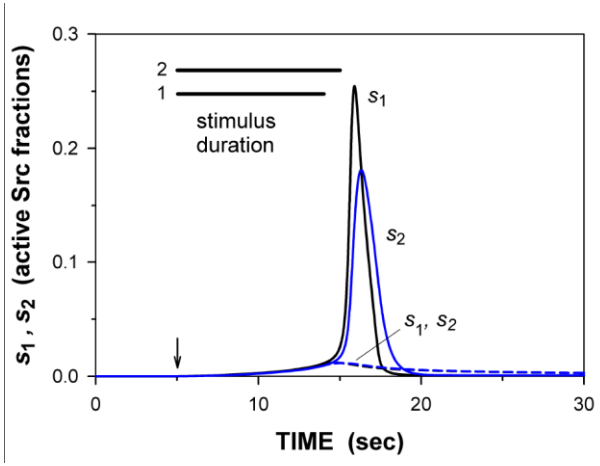


Spatial propagation of excitable pulse

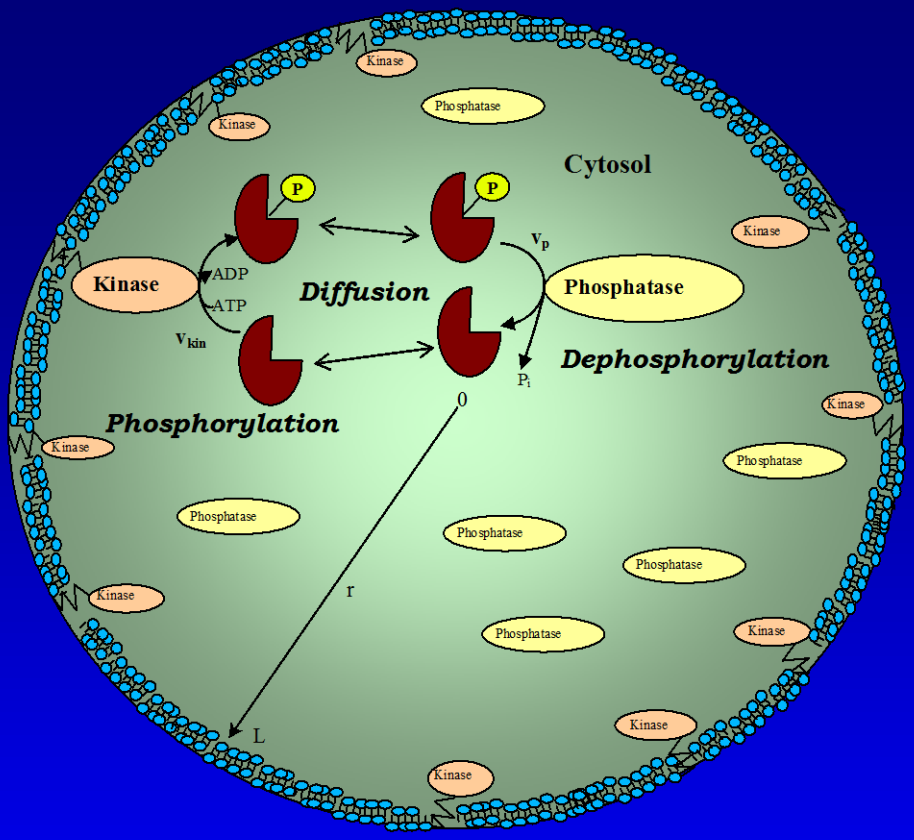


Distance from the local stimulus

Excitable behavior



Spatial gradients of phospho-proteins



$$\frac{\partial c_p}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial c_p}{\partial r}\right) - k_p c_p$$

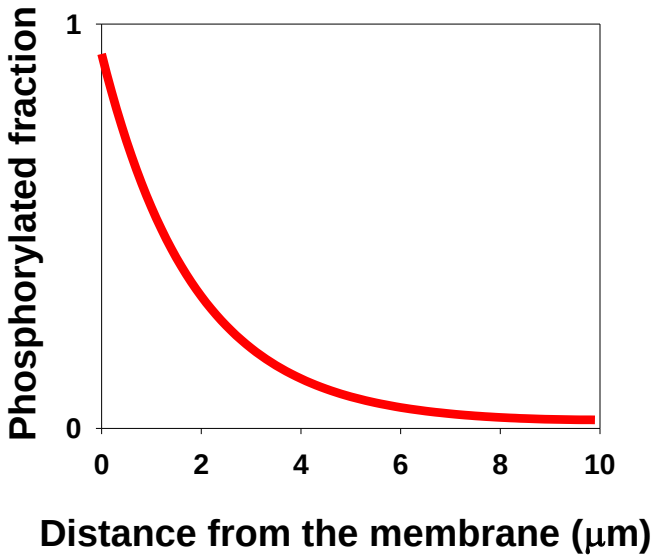
$$\left.\frac{\partial c_p}{\partial r}\right|_{r=L} = \frac{L}{3D} v_{kin} \cdot \quad \left.\frac{\partial c_p}{\partial r}\right|_{r=0} = 0.$$

c_p - the concentration of the phospho-protein; $k_p c_p$ - the phosphatase rate; D is the diffusion coefficient .

Steady-state solution

$$c_p(r) = const \cdot (e^{\alpha r} - e^{-ar})/r; \;\; \alpha = \sqrt{k_p / D}$$

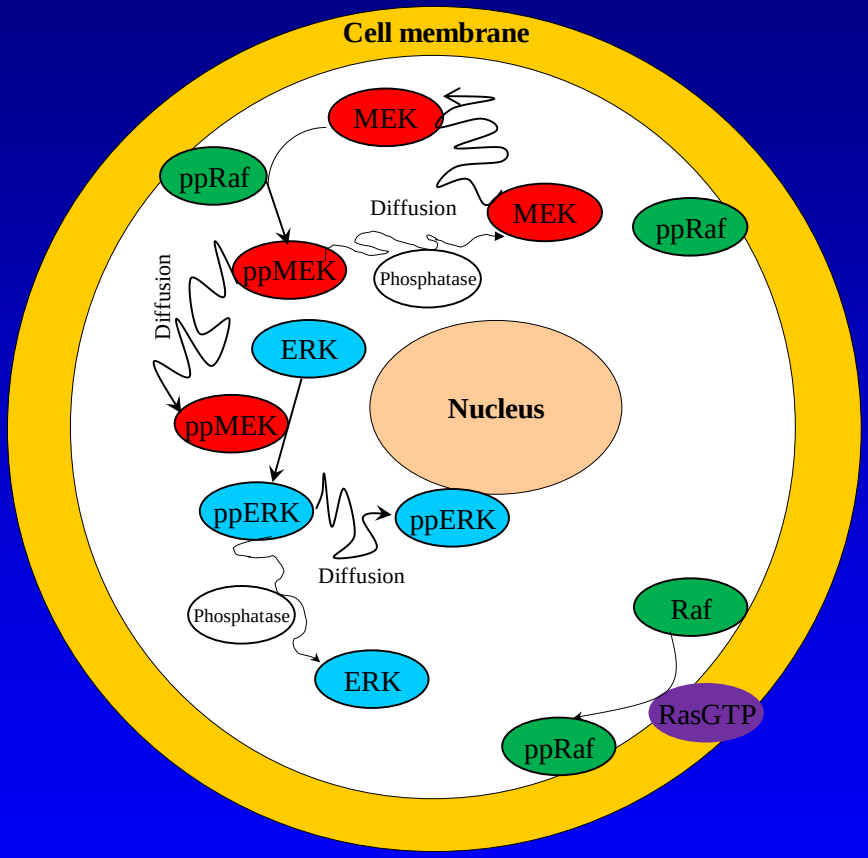
Steady-state gradient is exponential with the characteristic length $L_{gradient} = \sqrt{D / k_p}$

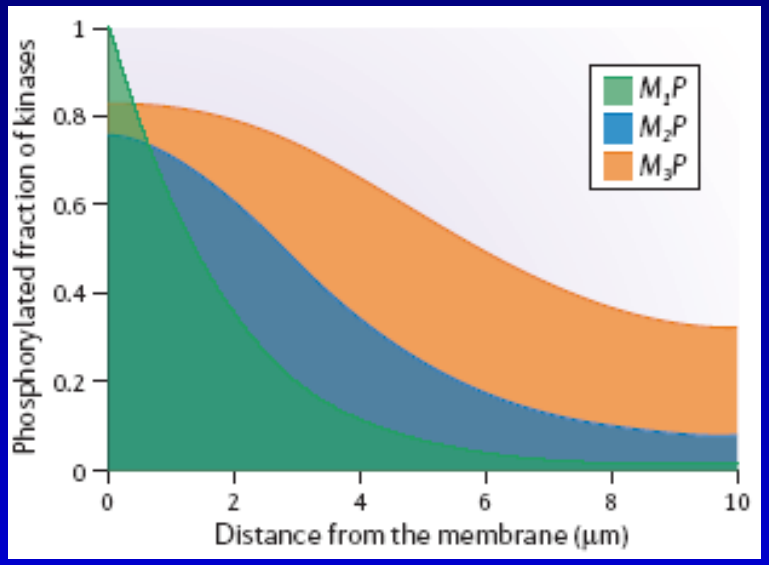


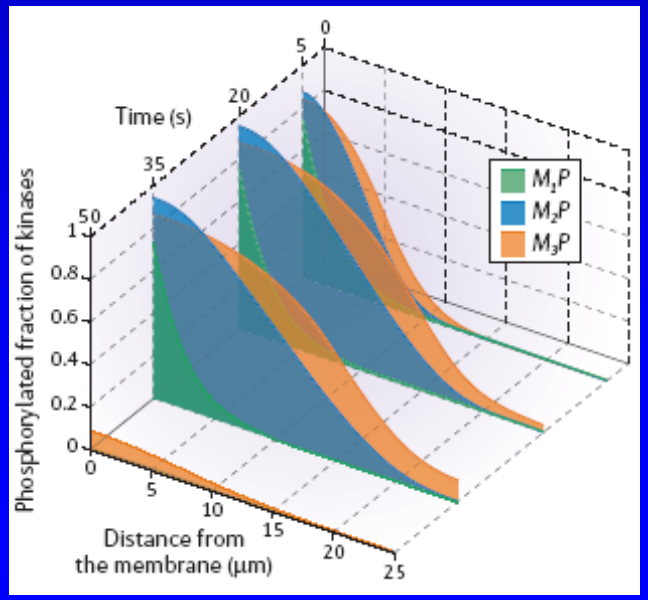
Brown & Kholodenko (1999) FEBS Lett. 457: 452.

Spatial propagation of phosphorylation signals

Existence of several levels in a cascade facilitates signal transfer

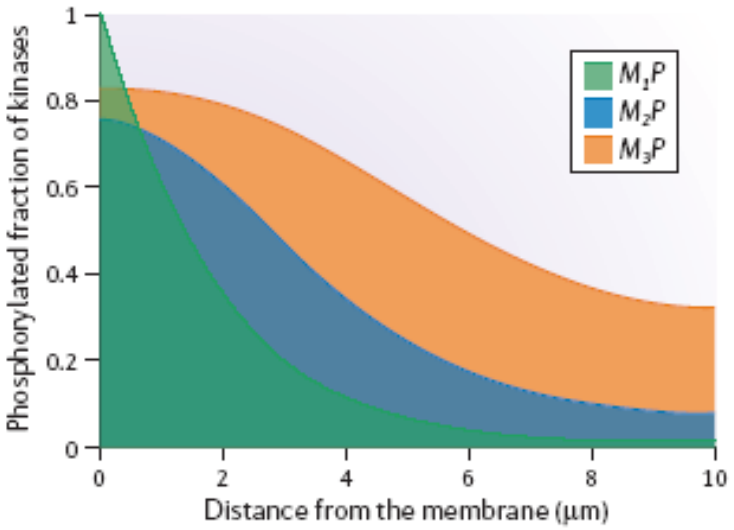


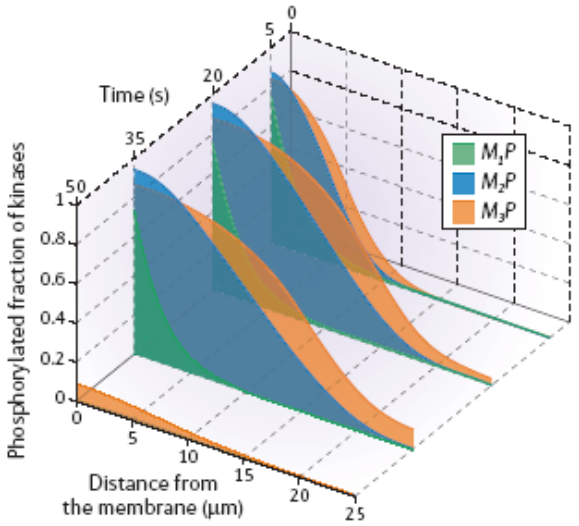




Brown & Kholodenko (1999) FEBS Lett. 457: 452.
Kholodenko, B.N. (2006) Nat. Rev. Mol. Cell Biol.

Spatial propagation of phosphorylation signals

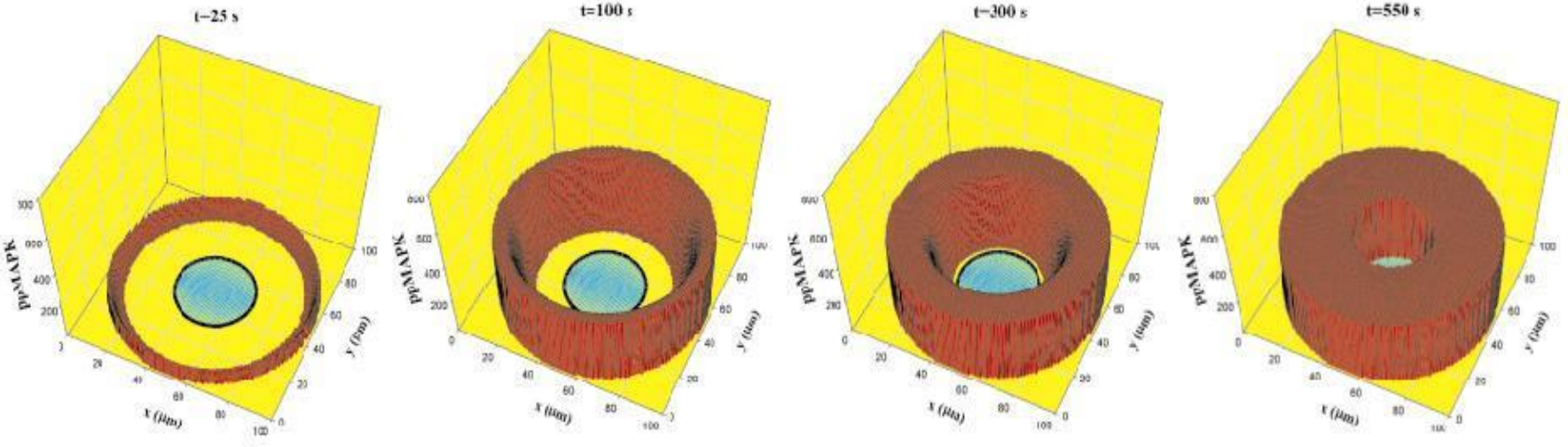




Existence of several levels in a cascade facilitates signal transfer

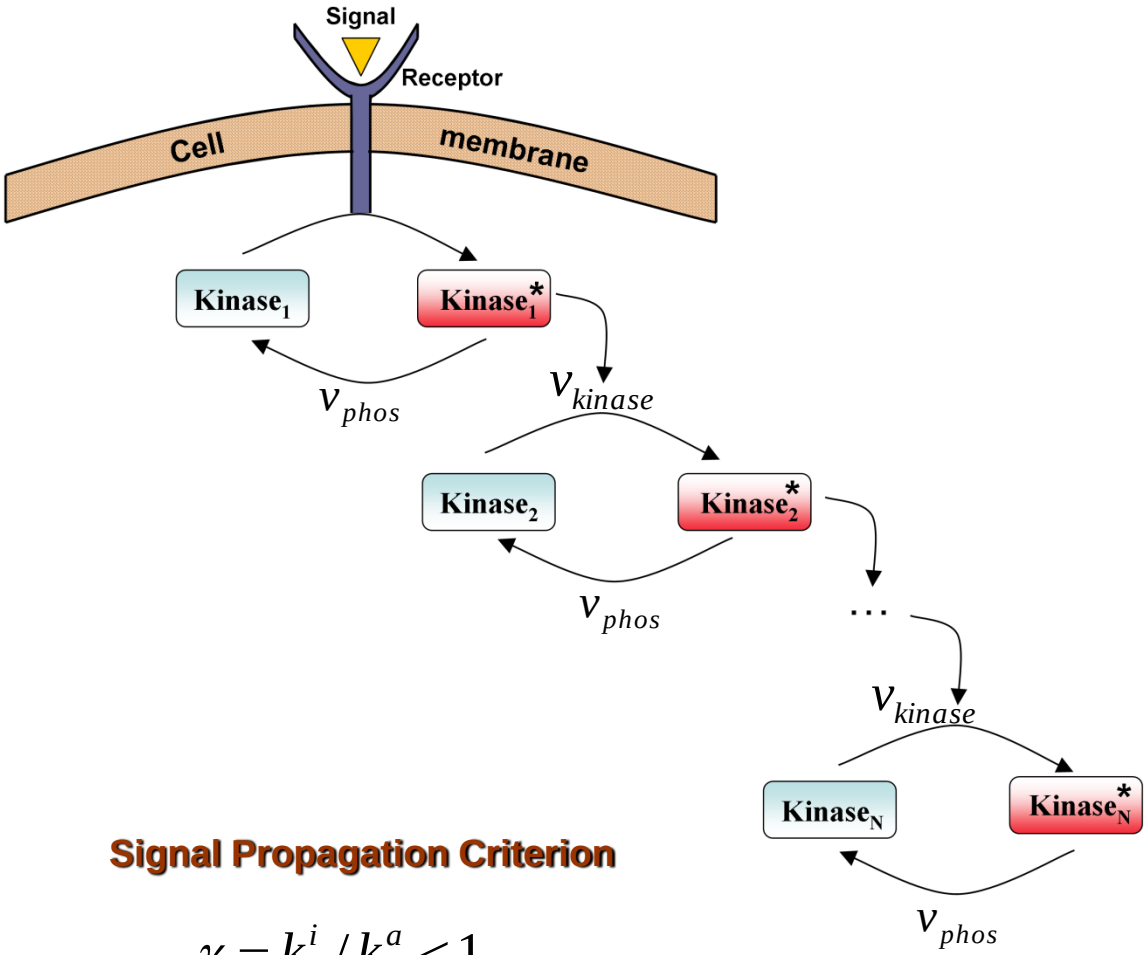
Kholodenko, B.N. (2006) Nat. Rev. Mol. Cell Biol.

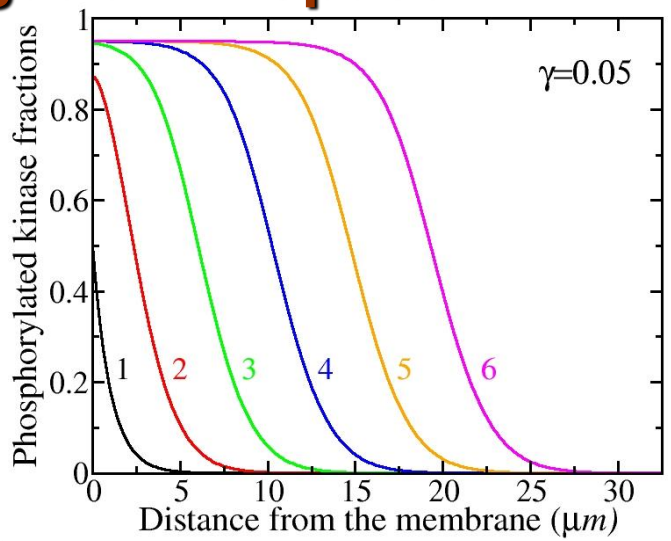
Phosphoprotein Waves Arising from Bistability in Kinase Cascades Propagate Signals over Spatially Extended Areas

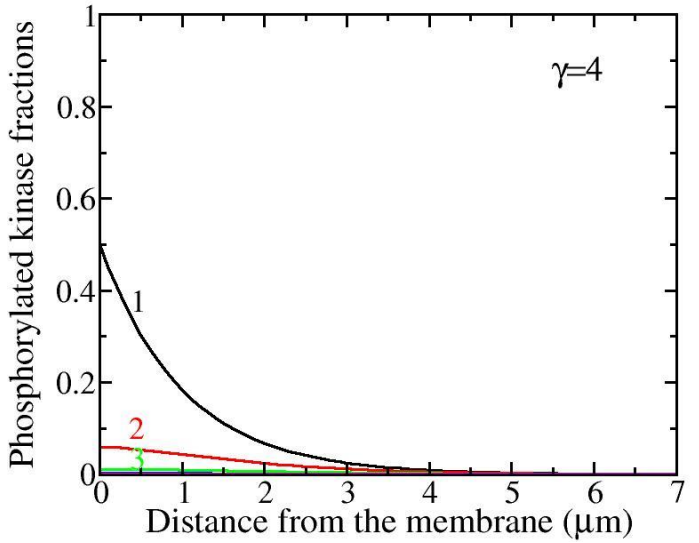


Markevich et al (2006) Mol Sys Biol 2:61

Conditions for Signal Propagation in Space







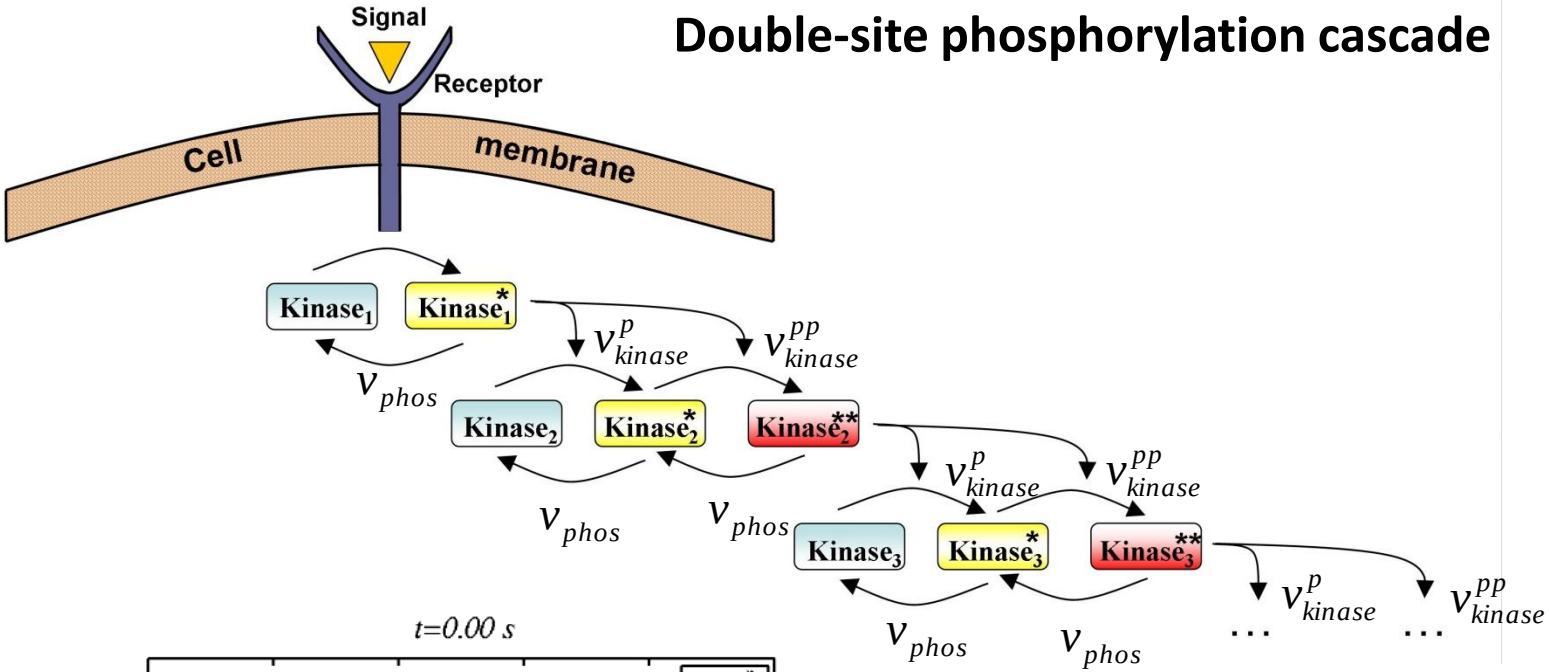
Signal Propagation Criterion

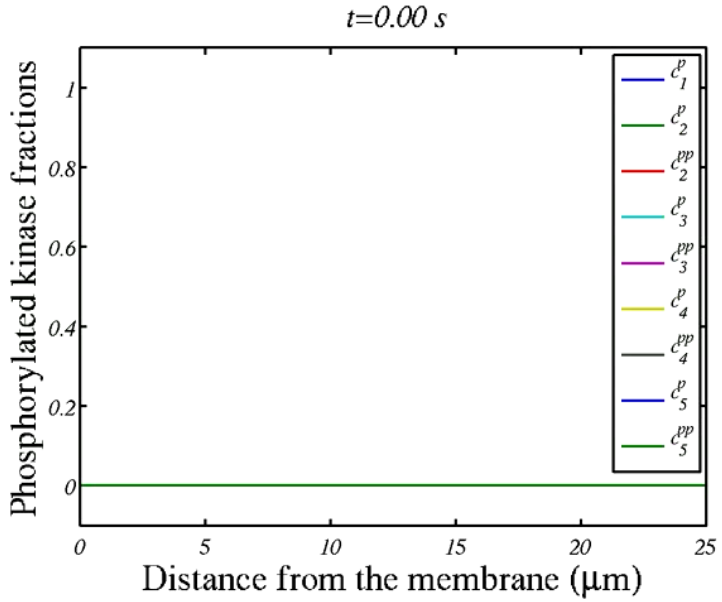
$$\gamma = k_n^i / k_n^a < 1$$

$$k_n^a = \frac{V_{\max}^{kinase_n}}{K_M^{kinase_n}}, \quad k_n^i = \frac{V_{\max}^{phos_n}}{K_M^{phos_n}}$$

Munoz-Garcia J., Neufeld Z. & Kholodenko B. N. (2009) PLoS Comput Biol. 5, e1000330

Conditions for Signal Propagation in Space





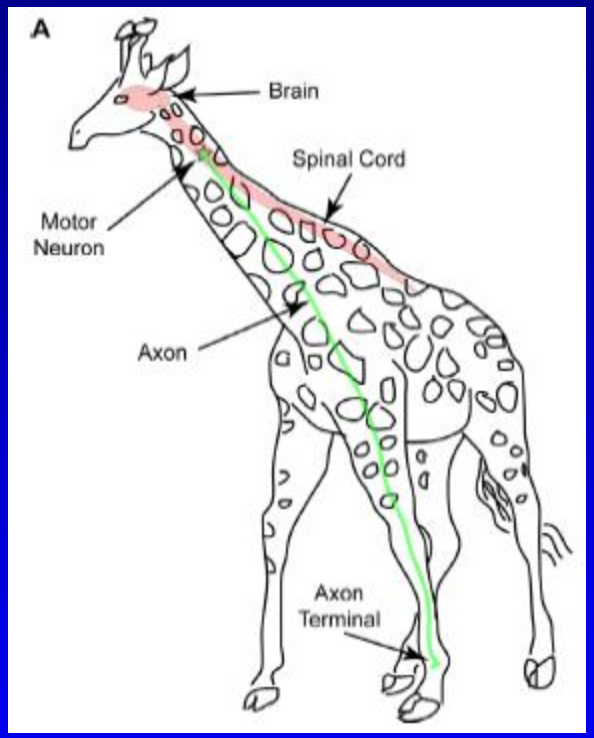
Signal propagation criterion

$$\gamma^{p} = k_i^{p} / k_a^{p}, \gamma^{pp} = k_i^{pp} / k_a^{pp}$$

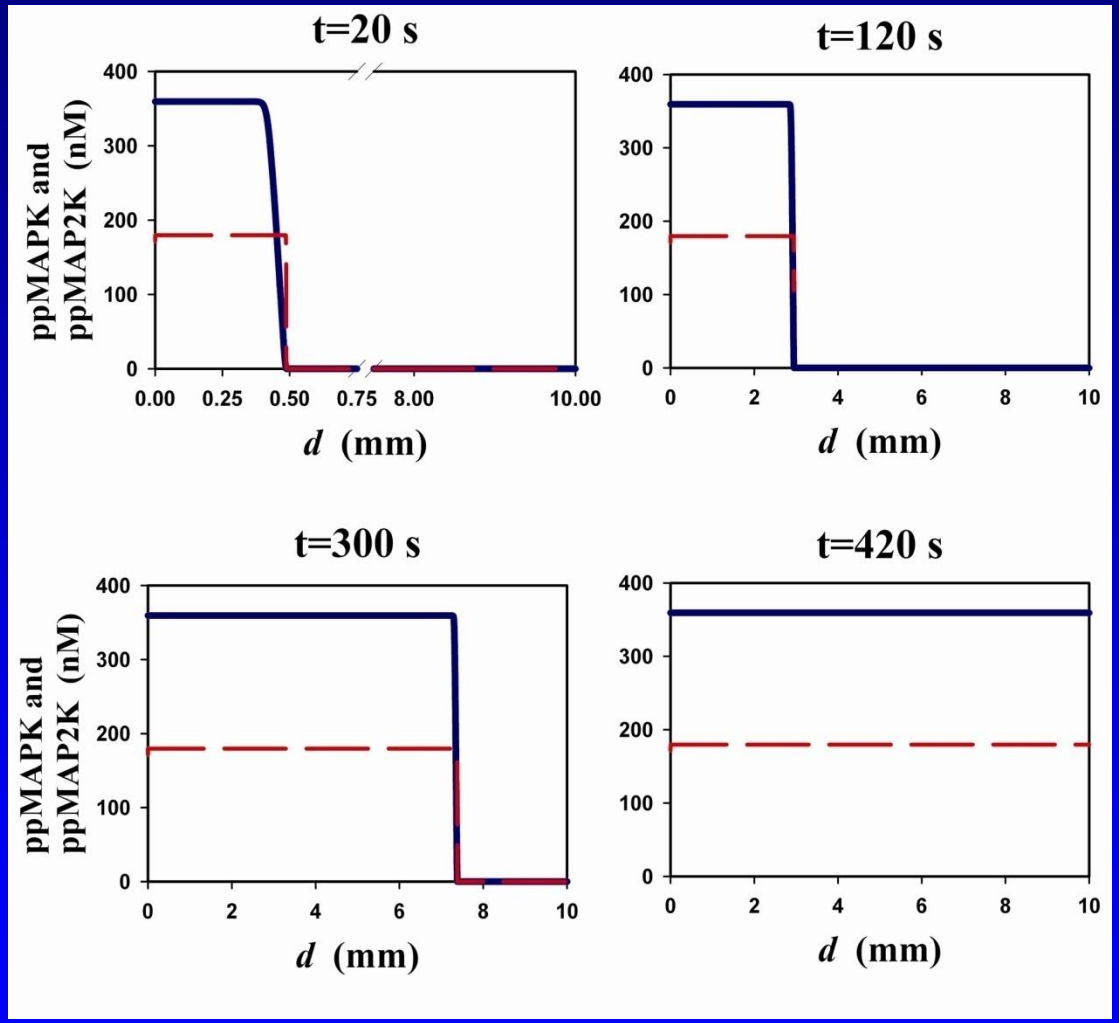
$$\gamma^{p}\gamma^{pp} < 1/4$$

Muñoz-García *et al* *Biophys. J* (2010)

Traveling wave carrying a binary phosphorylation signal over exceedingly long distances



Molecular-motor driven retrograde transport takes more than 72 hrs



Solid black lines - ppMAPK Dashed red lines – ppMAP2K

Markevich et al (2006) Mol.Syst.Biol. 2:61

RhoGTPase activity cycles and control of cell migration

- RhoGTPases cycle between a GDP-bound, inactive form and a GTP-bound, active form.
- The cycle is regulated by GEFs and GAPs (and GDIs).

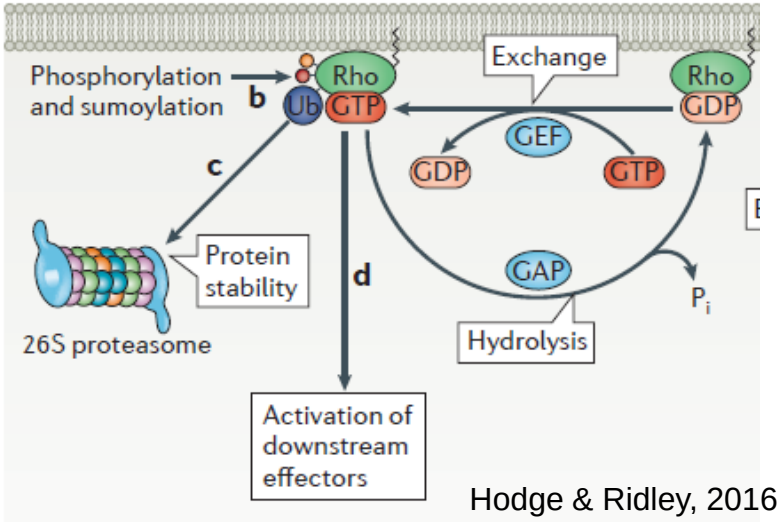
- ***How RhoGTPases regulate migration:***

o Rac1 RAC–GTP activates WAVE and hence ARP2/3 complex, leading to lamellipodia formation at the leading edge.

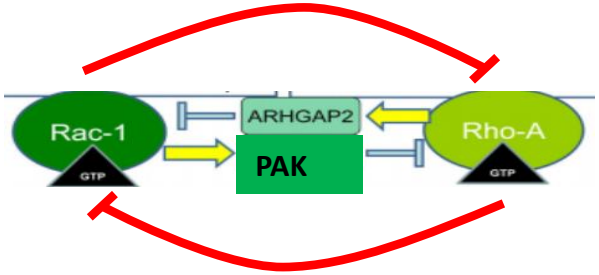
o RhoA promotes the rear retraction (through ROCK-mediated MLC phosphorylation) and the formation of actin stress fibres.

o Cdc4 recruits VASP to promote filopodium formation at the leading edge.

o RhoGTPases promote the assembly of integrin-based, matrix adhesion complex.

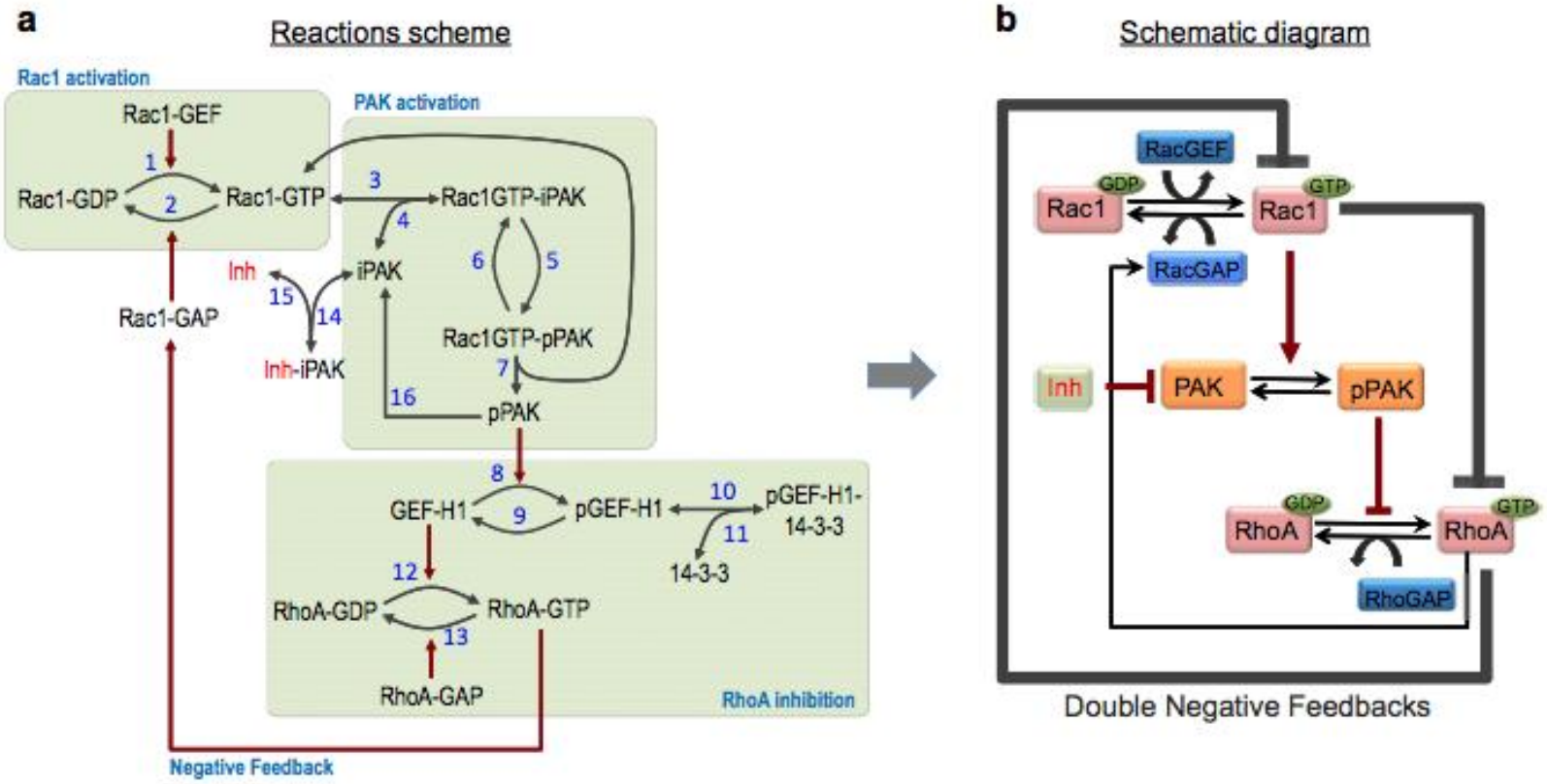


Hodge & Ridley, 2016



Rac1 and RhoA mutually inhibit each other (Guilluy et al, 2011)

Modelling the Rac1-RhoA interaction network

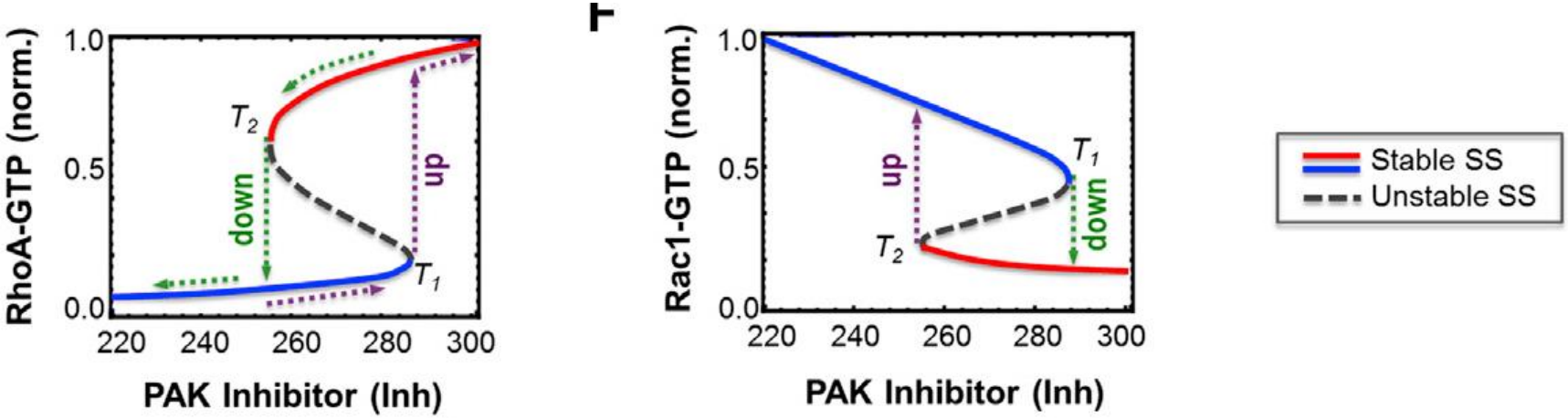


- In MDA-MB-231 mesenchymal breast cancer cells, Rac1 activates its effector kinase PAK, which phosphorylates and inhibits GEF-H1, a major GEF for RhoA in these cells.
- RhoA activates its effector kinase ROCK, which in turn phosphorylates and activates FilGAP and ArhGAP22, which are Rac1-specific GAPs, thereby inhibiting Rac1.

How will the Rac-Rho network respond to perturbations of negative feedback from Rac1 to RhoA by an inhibitor (IPA-3) of PAK?

Byrne et al. Cell Systems (2016)

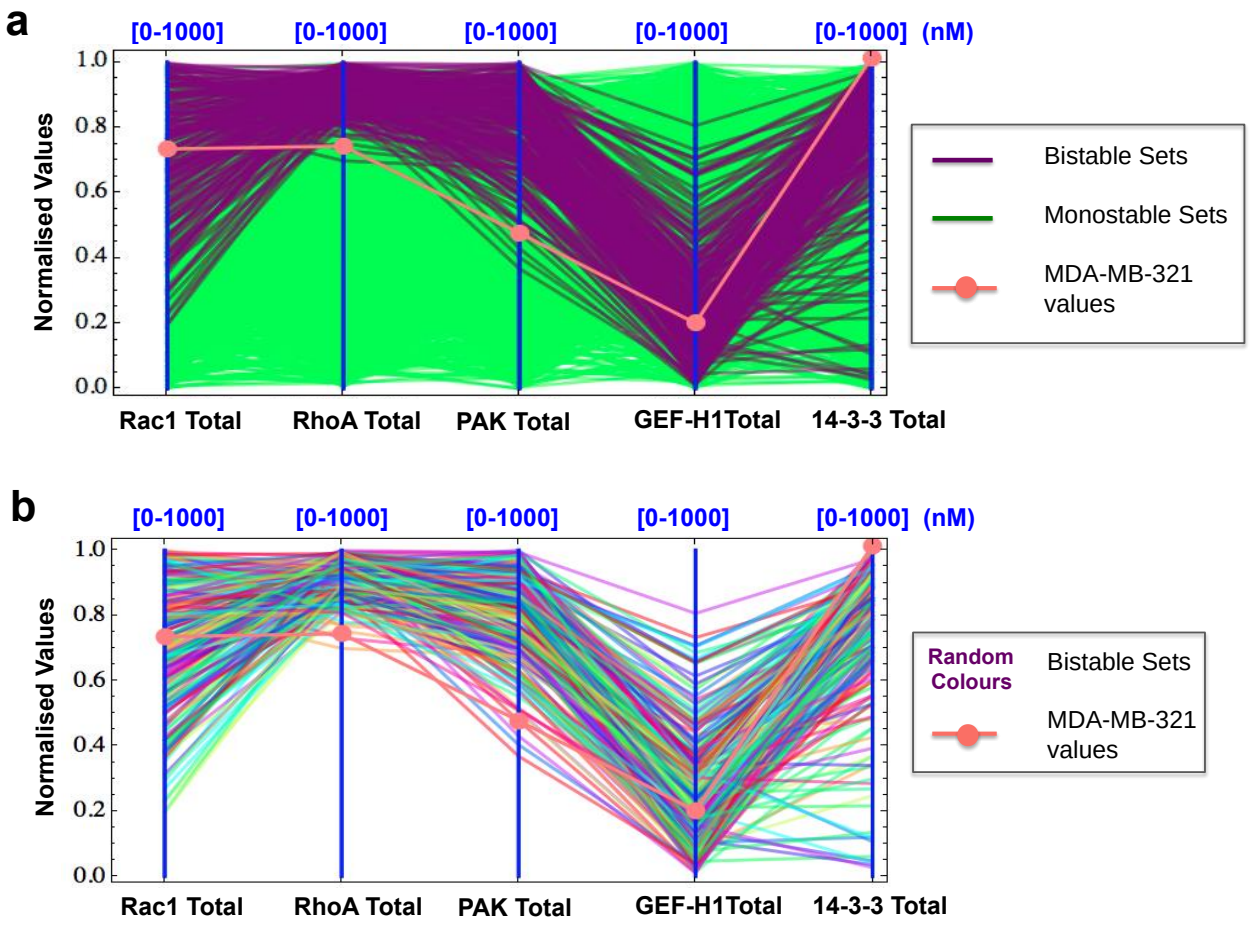
Model predicts bistability in MDA-MB-231 cells

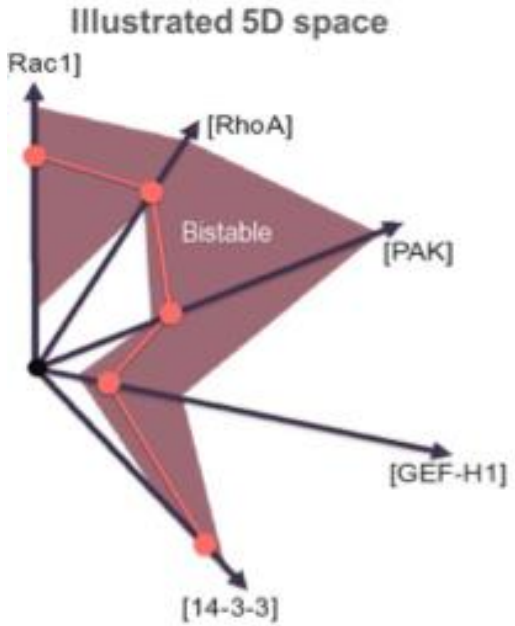


Bistability: Two stable steady states (the 3rd is unstable) coexist at the same parameter values.

Hysteresis: Switch-Up and Switch-Down occur at different parameter values. At a given parameter value the state of a system depends on the history (initial state)

Applying DYVIPAC to the Rac1-RhoA model

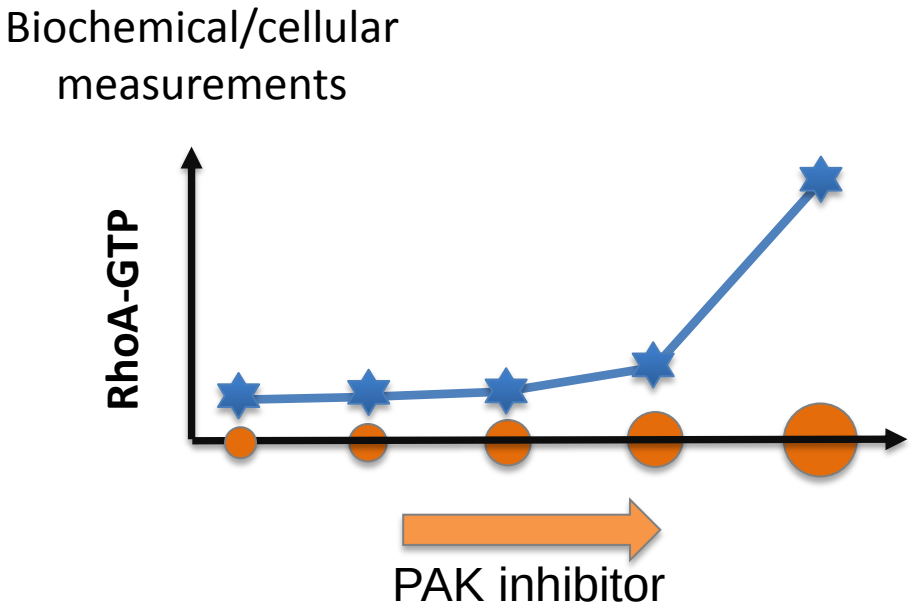




The measured parameters in MDA-MB-231 cells (protein abundance of GTPases, PAK, GEF-H1, and 14-3-3) belong to the bistability regime

Byrne et al. Cell Systems (2016)

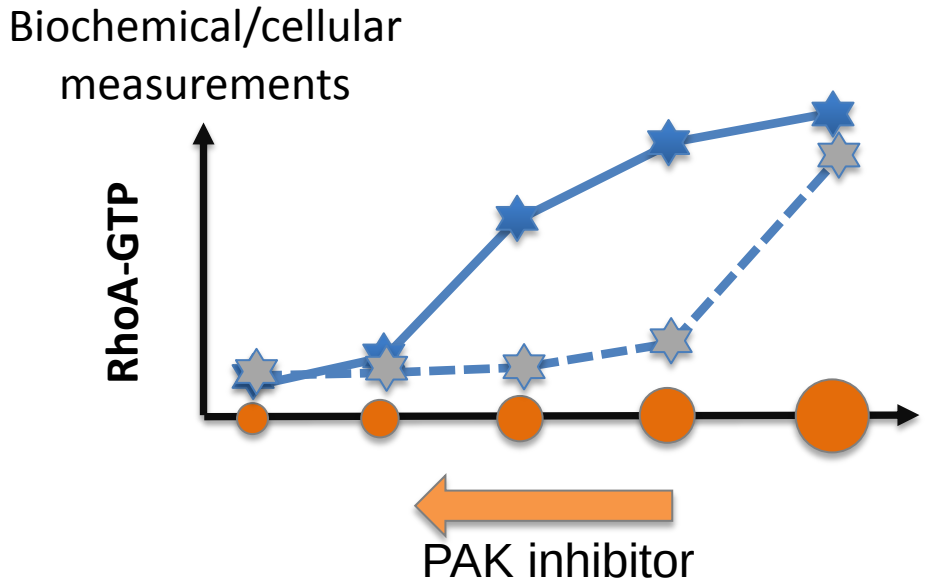
Testing bistability of RhoA-Rac1 network



Detecting Hysteresis

Experimental setup:

1. Use gradually increasing doses of a small molecule inhibitor and measure new steady-state levels of the protein activity of interest.

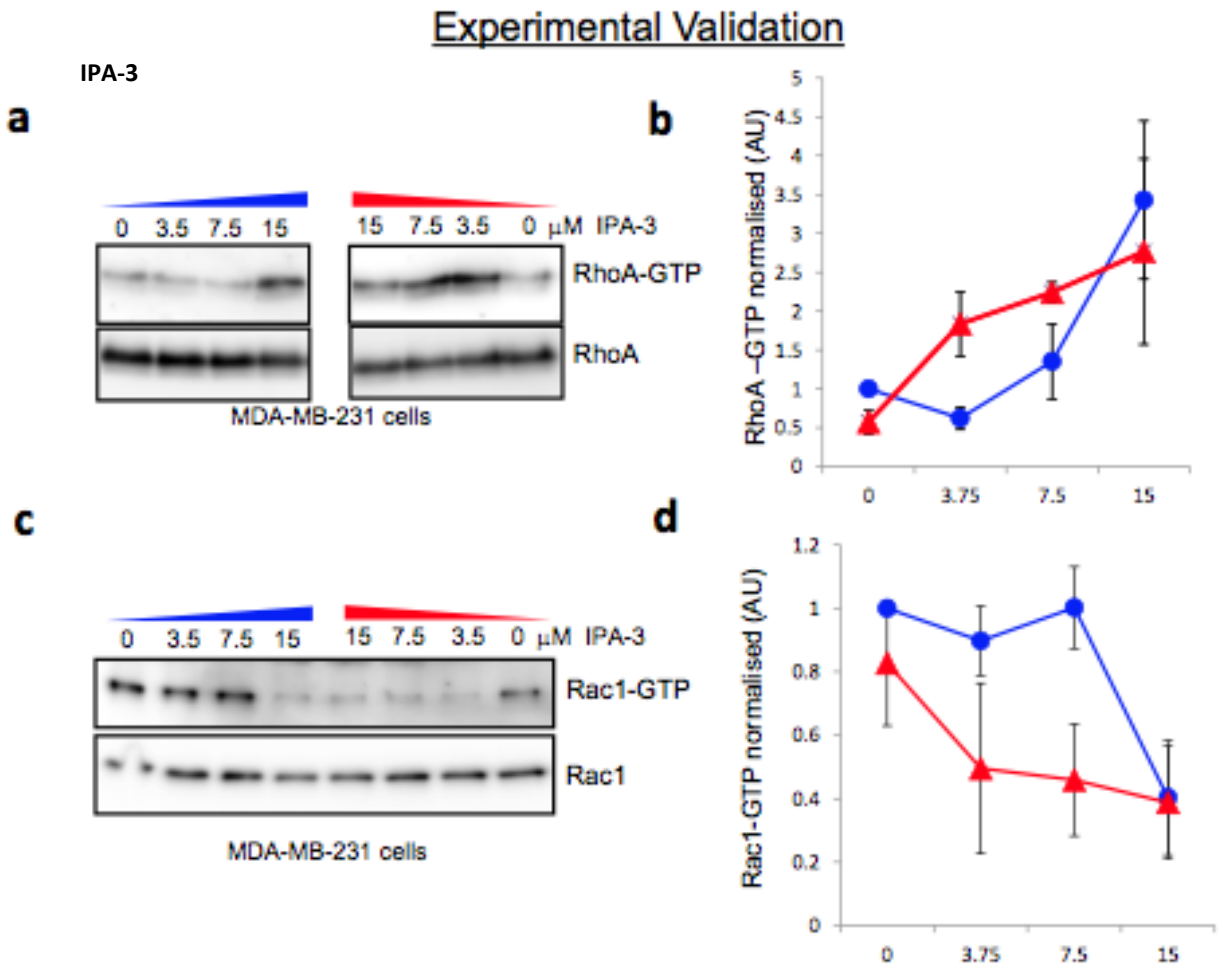


Detecting Hysteresis

Experimental setup:

2. Lock the system in the state with the highest inhibitor dose. After washing inhibitor out, gradually reduce inhibitor dose and measure new steady-state activity levels

Experimental validation in MDA-MB-231 cells



BLUE: PAK inhibitor, IPA-3, was given for 40 min at gradually increasing doses

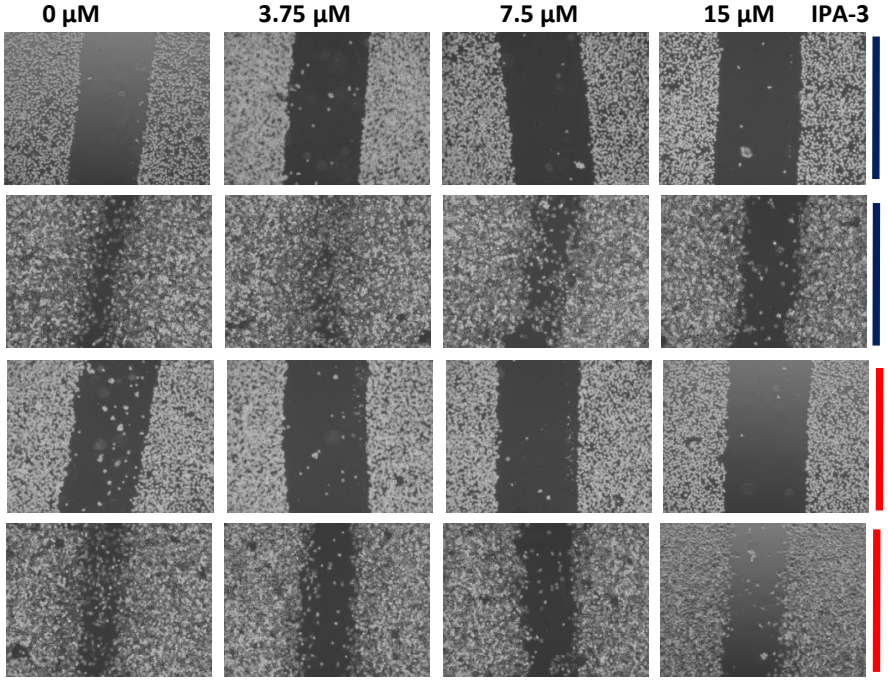
RED: After incubating cells with 15 uM IPA-3 for 20 min, the inhibitor was washed out (half-life is ca. 2 min) and cells were incubated for additional 20 mins with IPA-3 at gradually decreasing doses

Rac1/RhoA activity levels exhibit hysteresis and bistability

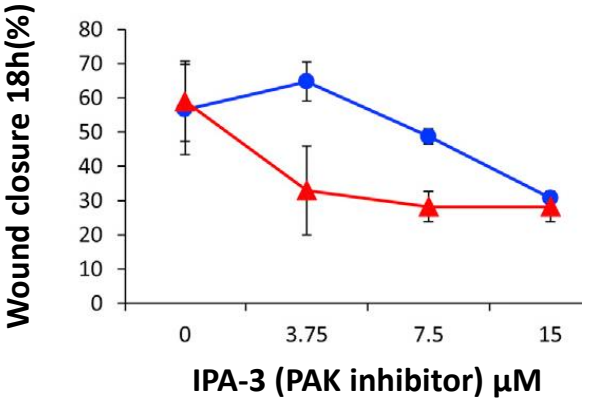
Byrne et al. Cell Systems (2016)

Biological responses: Bistable behavior of cell migration

Directed cell migration: wound healing assay

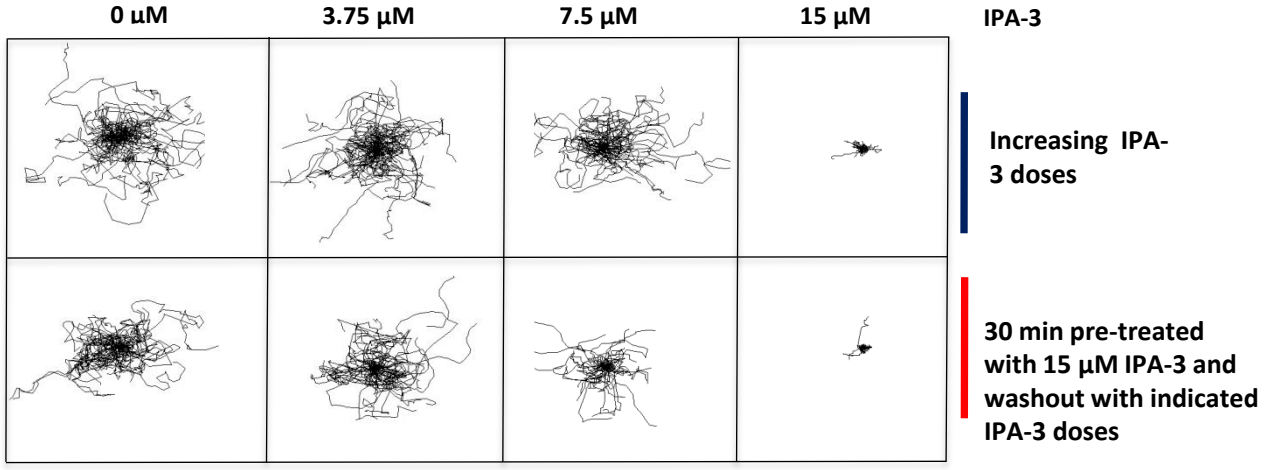


Hysteresis of wound closure for increasing vs decreasing (pre-treated cells) IPA-3 doses

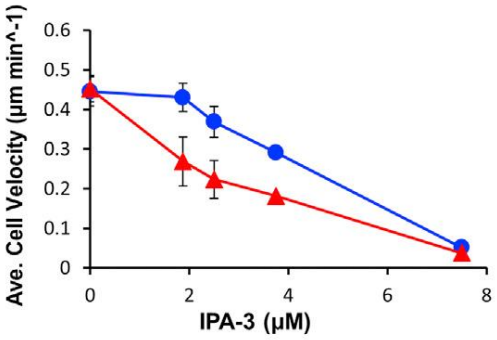


Random Cell Migration

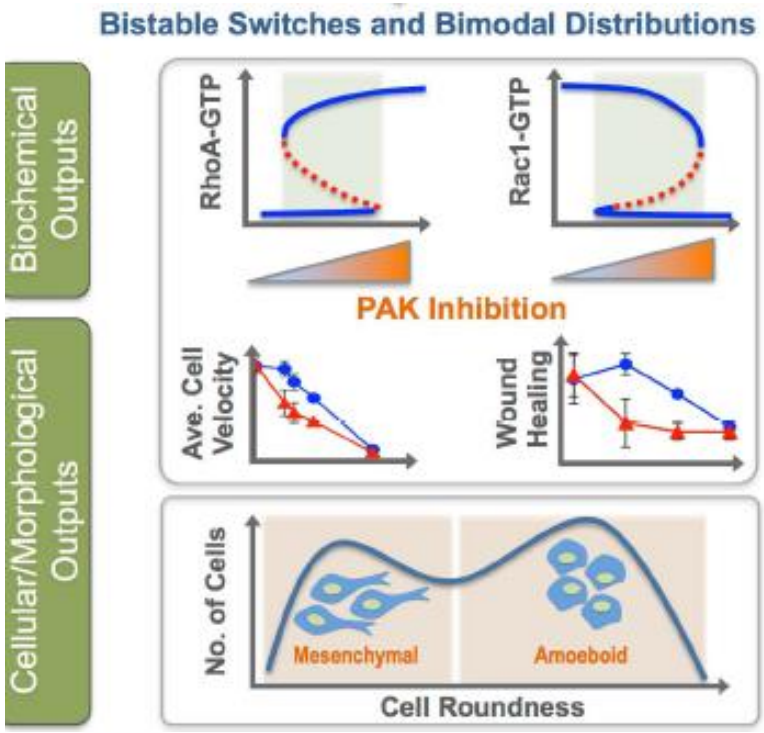
Images are taken every 20min.
Migration path is tracked for 12 hr



Hysteresis of the average cell speed vs IPA-3 doses

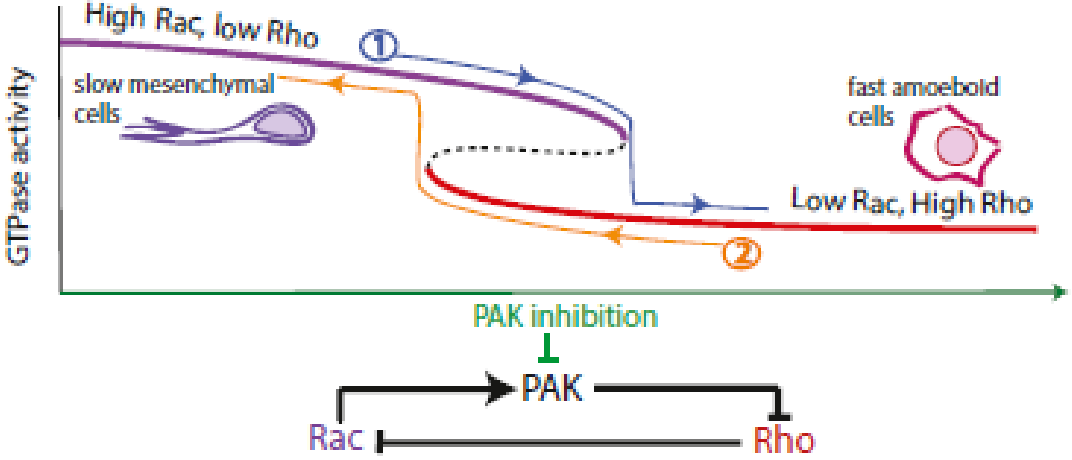


Conclusions



Bistability in the biochemical Rac-RhoA networks leads to bistability in downstream cell's phenotype ranging from cell shape and motility to multi-cellular wound healing.

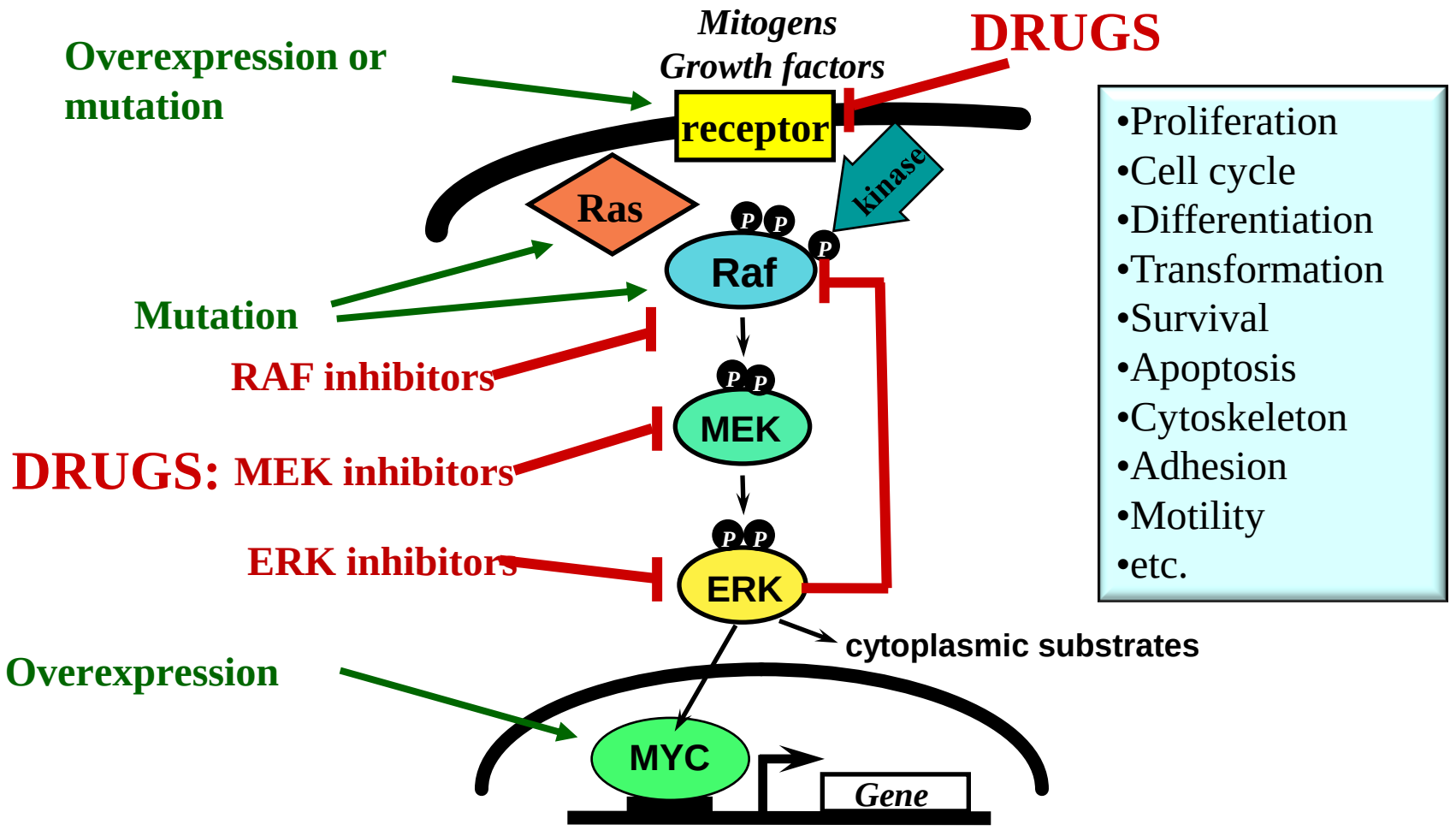
Byrne et al. Cell Systems (2016)



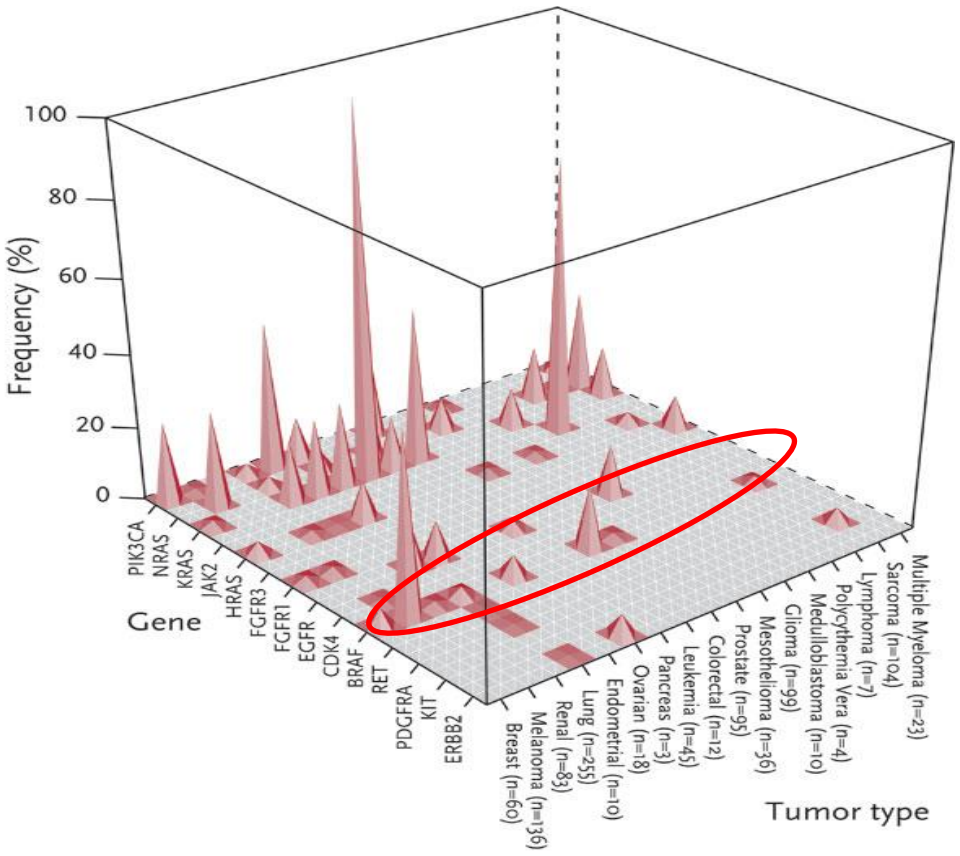
Starting from mesenhymal, high Rac & low RhoA state, PAK inhibition led to a switch to high Rho & low Rac amoeboid behavior. Starting from high Rho & low Rac amoeboid cells and reducing PAK inhibition results in a distinct trajectory. It flips to Rac dominated, mesenchymal behavior at a much lower PAK inhibition.

Edelstein-Keshet L. Cell Systems (2016)

MAPK/ERK signalling pathway is a hot drug target, but resistance inevitably occurs



BRAF mutations, RAF dimerization & drug resistance



Thomas *et al*, Nat Gen, 2007

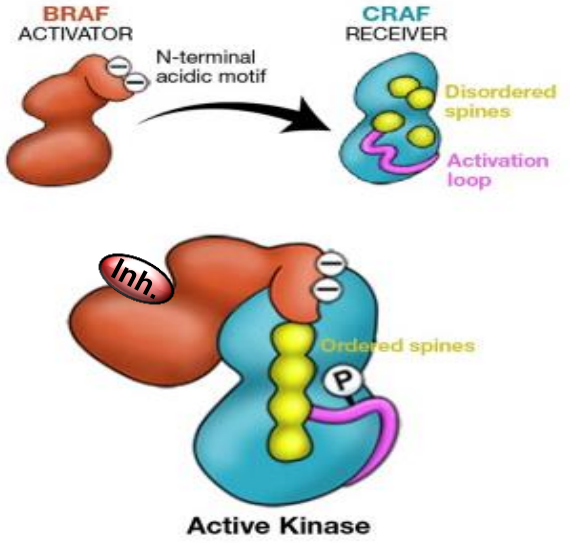
- BRAF activation mutations (most frequent BRAF V600E) are found in many cancers. These cancers are often resistant to specific BRAF inhibitors.
- Side effects: treating melanoma with BRAF inhibitors causes skin tumours (squamous cell carcinomas, keratoacanthomas) due to paradoxical activation of ERK-signalling in cells with wild-type BRAF (Hall-Jackson et al., 1999).
- This ERK activation is related to BRAF homo/heterodimerization (Poulikakos et al., 2010, Hatzivassiliou et al., 2010; Heidorn et al., 2010).
- Why does BRAF kinase dimerization conveys drug resistance?

Why does BRAF-CRAF heterodimerization convey drug resistance?

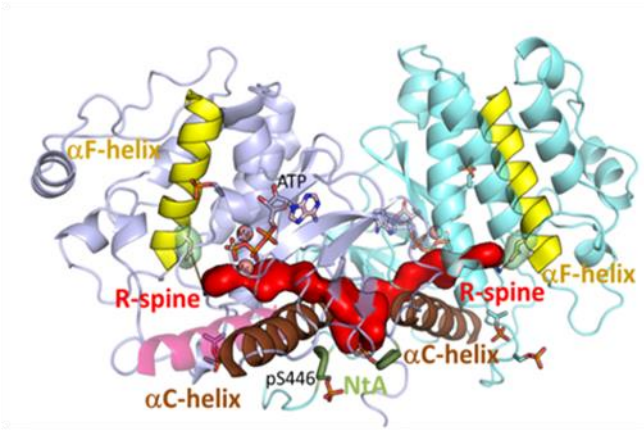
- **Dimerization is part of physiological RAF activation. RAF heterodimerization increases the total RAF kinase activity > 10 – 20-fold** (Rushworth et al. Mol Cell Biol 2006)

- **RAF inhibitors facilitate RAF homo/heterodiimerization** (Poulikakos et al. Nature 2010)

- **Drug-bound RAF protomer allosterically activates the other free protomer (spine assembly). Therefore, a dimer that has bound only one inhibitor molecule is still active.**

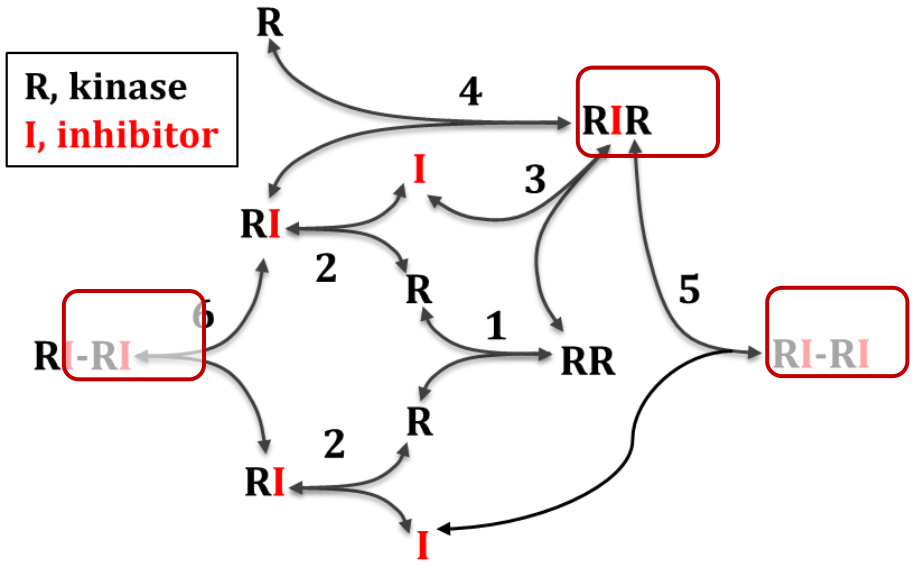


Hu et al. Cell 2013 154:1036.



Why is the 2nd inhibitor molecule unable to effectively bind and fully inhibit the dimer?

Kinase dimers & drug resistance – a thermodynamic view



Dimerisation & drug binding is a cycle.
In a cyclic process the thermodynamic free energy fluxes = 0 at equilibrium

Two thermodynamic factors F and G describe allosteric inhibitor effects:
Factor F < 1 determines the facilitation of dimerisation by inhibitor:

$$D1 \propto F*M$$

M and D1 are the Kd's of drug binding to monomer R and free dimer RR.

Factor G is determined by the structural changes in the other RAF protomer:

$$D2 \propto (G/F)*D1$$

D2 is the Kd of the 2nd drug molecule binding to semi-inhibited dimer RIR.

These thermodynamic constraints imply:

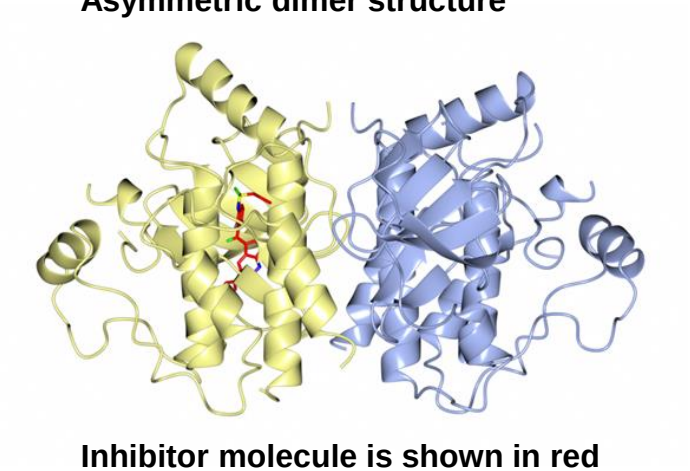
1) **Inhibitors that induce dimerization will preferentially induce accumulation of RIR dimers with only one protomer inhibited. These dimers are active.**

2) **The RIR to RI-RI transition is disfavoured as the binding constant for the second inhibitor molecule drops (Yao et al. Cancer Cell. 2015).**

Kholodenko B.N. Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects. *Cell Reports,* 12, 1939-1949 (2015).

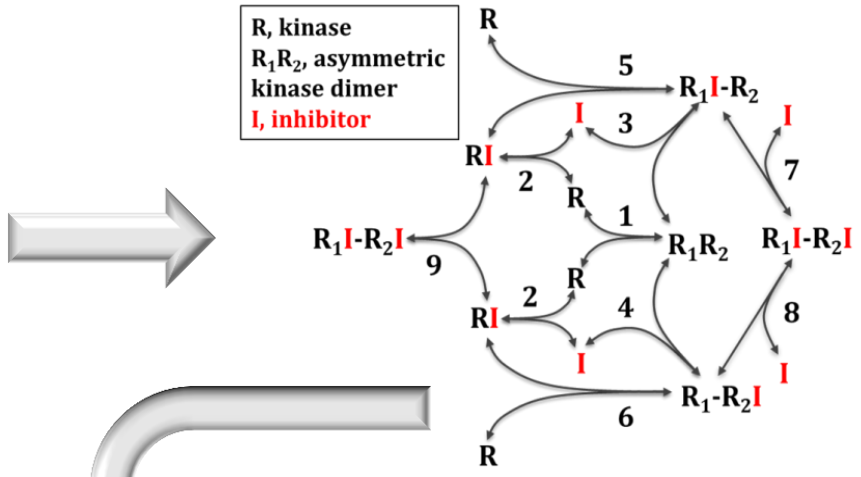
BRAF-CRAF heterodimerisation – thermodynamic view

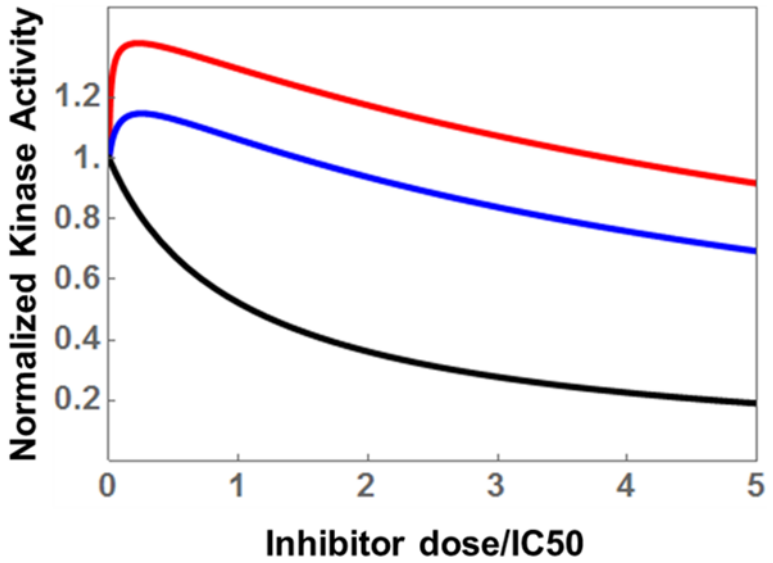
Asymmetric dimer structure



Inhibitor molecule is shown in red

Asymmetric dimer - inhibitor interaction model





Inhibitor binding & dimerization are highly cooperative

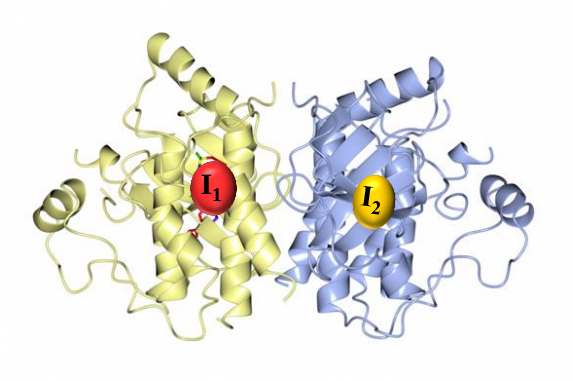
Inhibitor binding & dimerization are cooperative

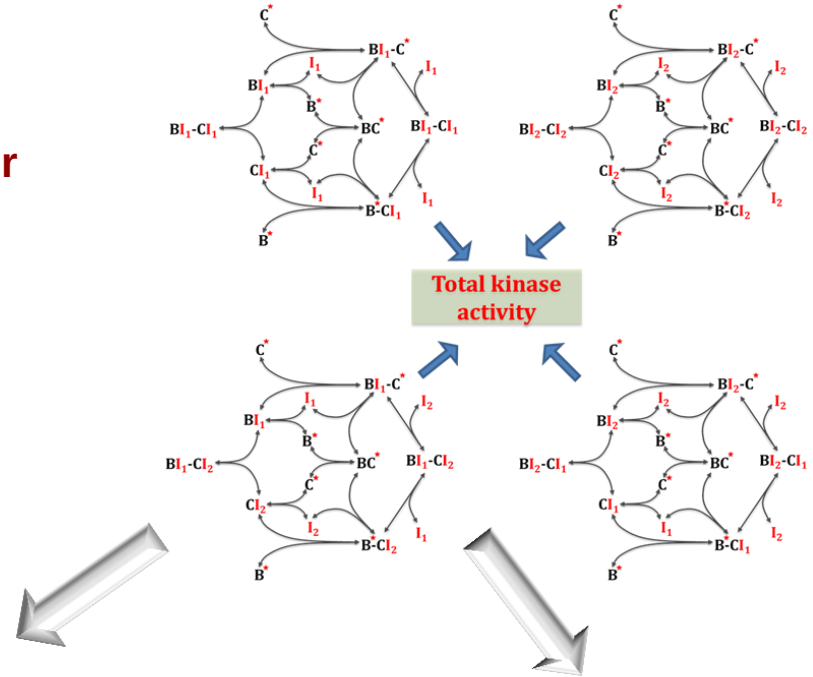
Inhibitor binding & dimerization are independent events

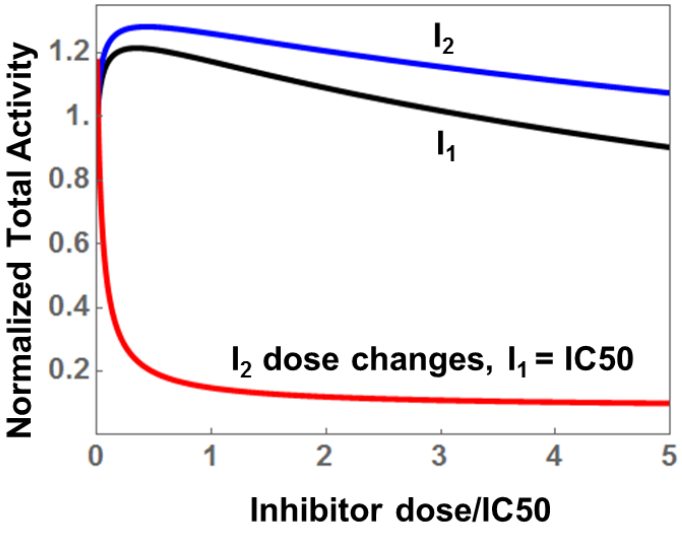
Kholodenko B.N. Cell Reports, 12:1939 (2015).

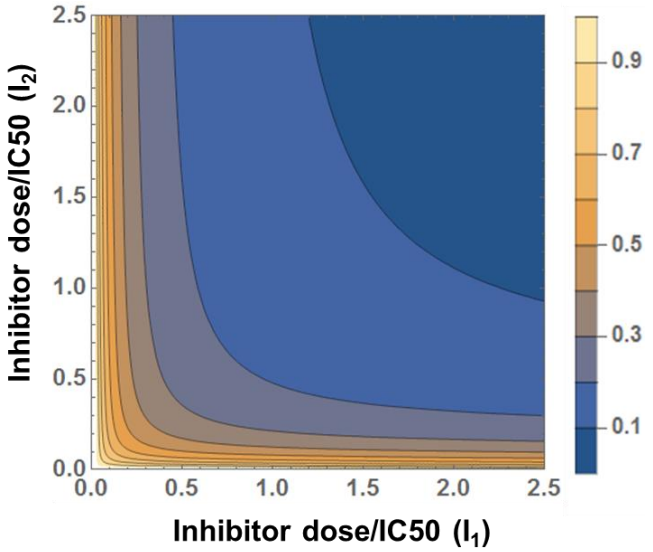
Breaking dimerisation-induced drug resistance

Consider thermodynamics of RAF inhibitors that bind to different RAF conformations in the asymmetric dimer

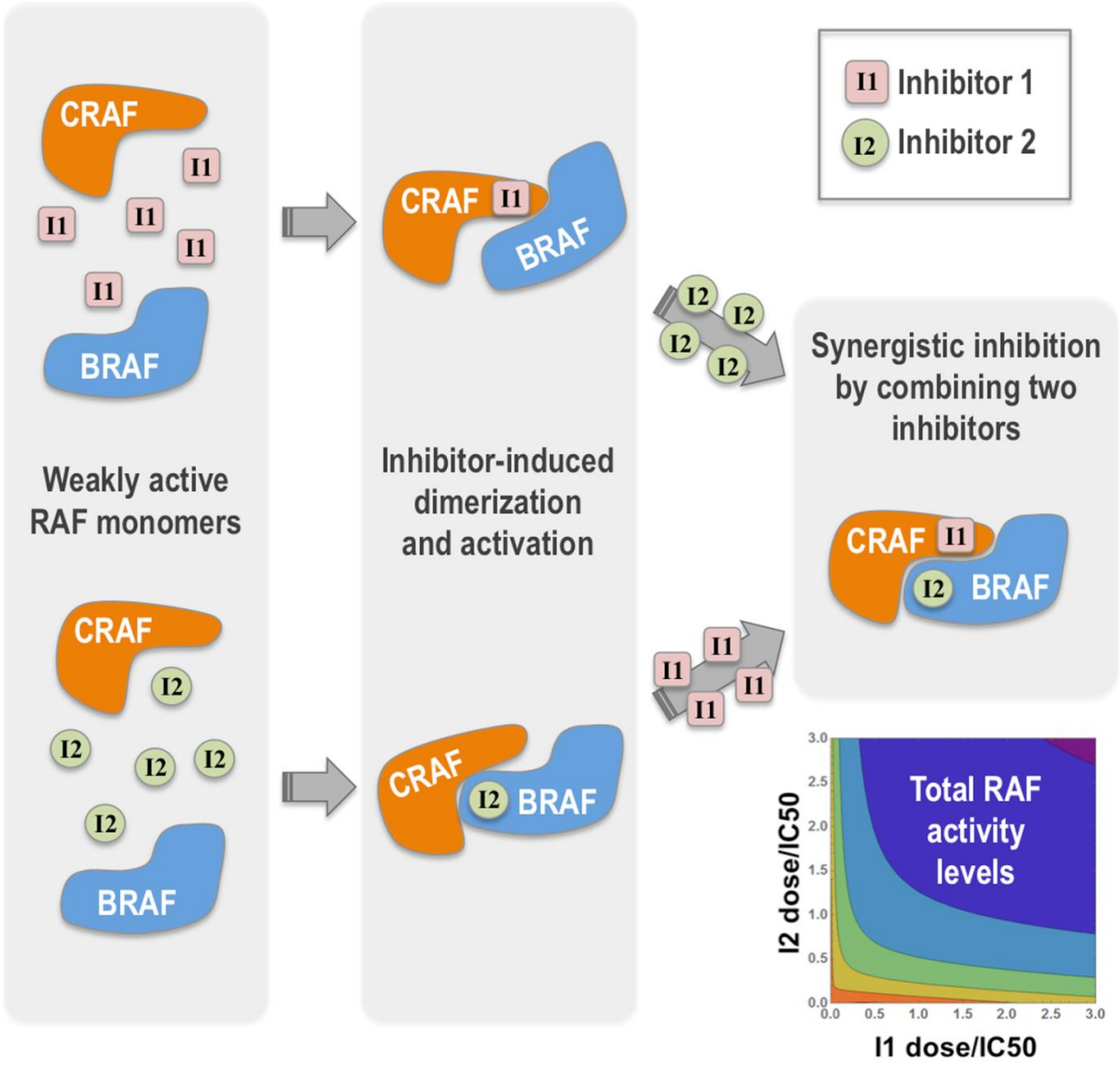








Breaking dimerisation-induced drug resistance



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