Systembiologie 551-1174-00L

Beyond Metabolism

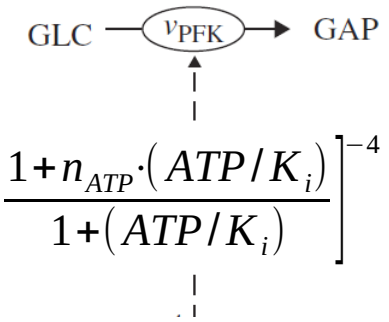
6 April, 2017

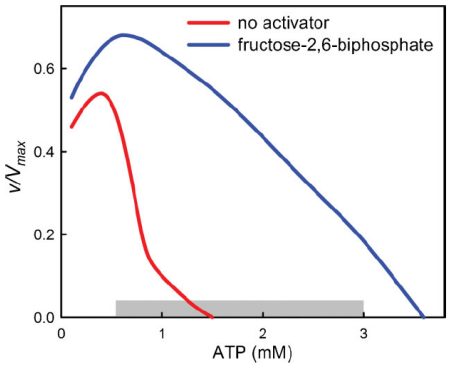
Uwe Sauer, Molecular Systems Biology
Jörg Stelling, D-BSSE

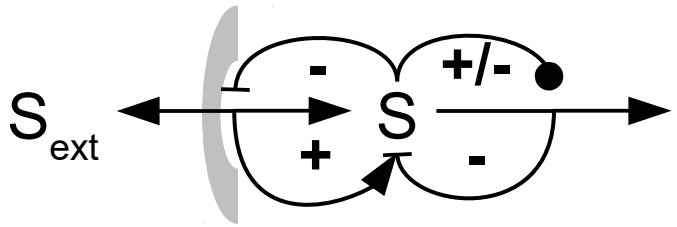
Content:

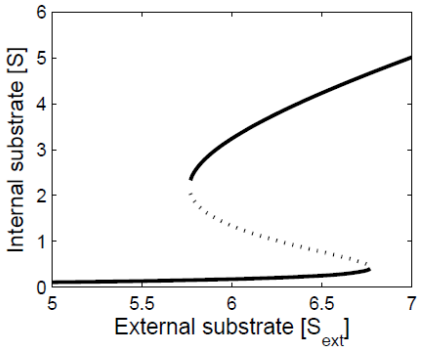
- Signaling pathways - networks (US)
- Dynamic models for cell signaling (JS)
  - Principles: Src kinase
  - Application in drug discovery
- Exams (US/JS)

Revisiting Dynamics: Substrate Inhibition









- Single-enzyme regulation may appear ‘meaningless’ when considered without the network context.
- Wiring and dynamics are critical (negative feedback for homeostasis vs. positive feedback for dynamic memory).

Example (Paradigm) for Signaling: Src Kinase

A SARCOMA OF THE FOWL TRANSMISSIBLE BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.*

BY PEYTON ROUS, M.D.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

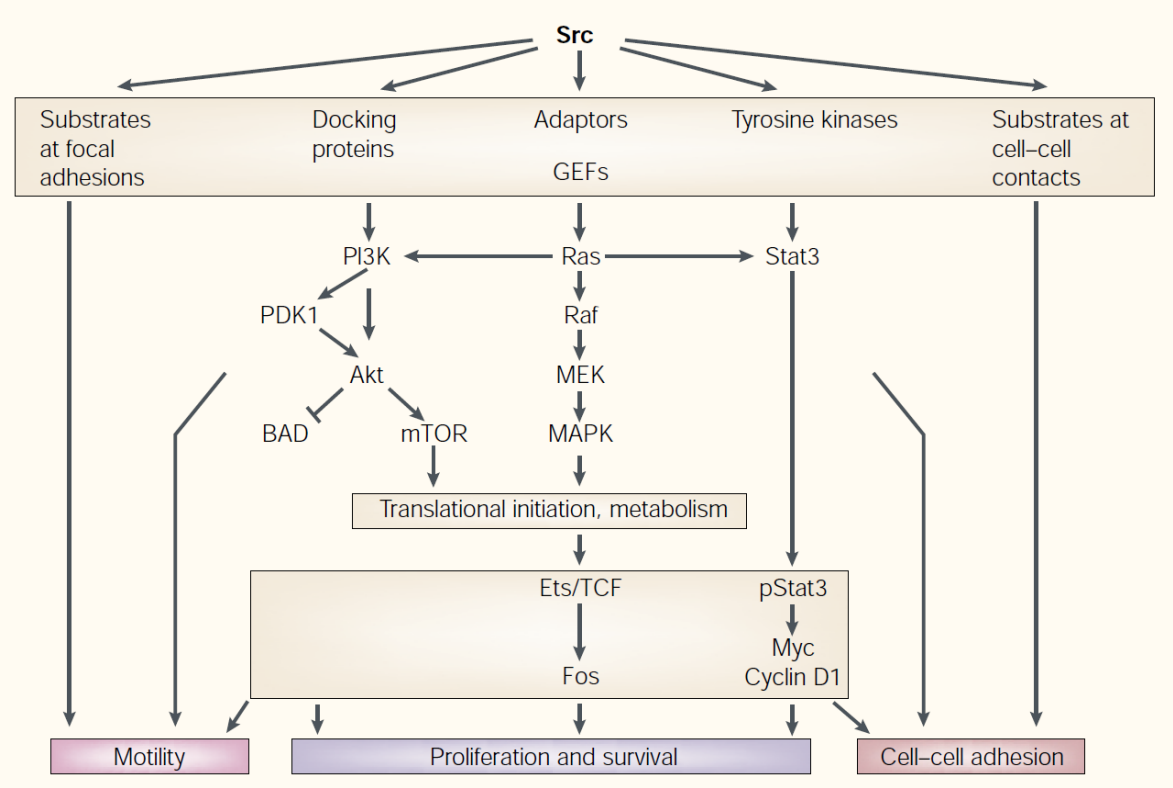
* Received for publication, February 9, 1911.

[1] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 696.

[2] Peyton Rous, *Jour. Am. Med. Assn.*, 1910, lv, 1805; 1911, lvi, 198.

- First demonstration of virus-induced cancer.
- First oncogene (cellular Src protein).
- First tyrosine kinase (non-receptor associated).

Src Kinase: A Key Regulator of Cell Signaling

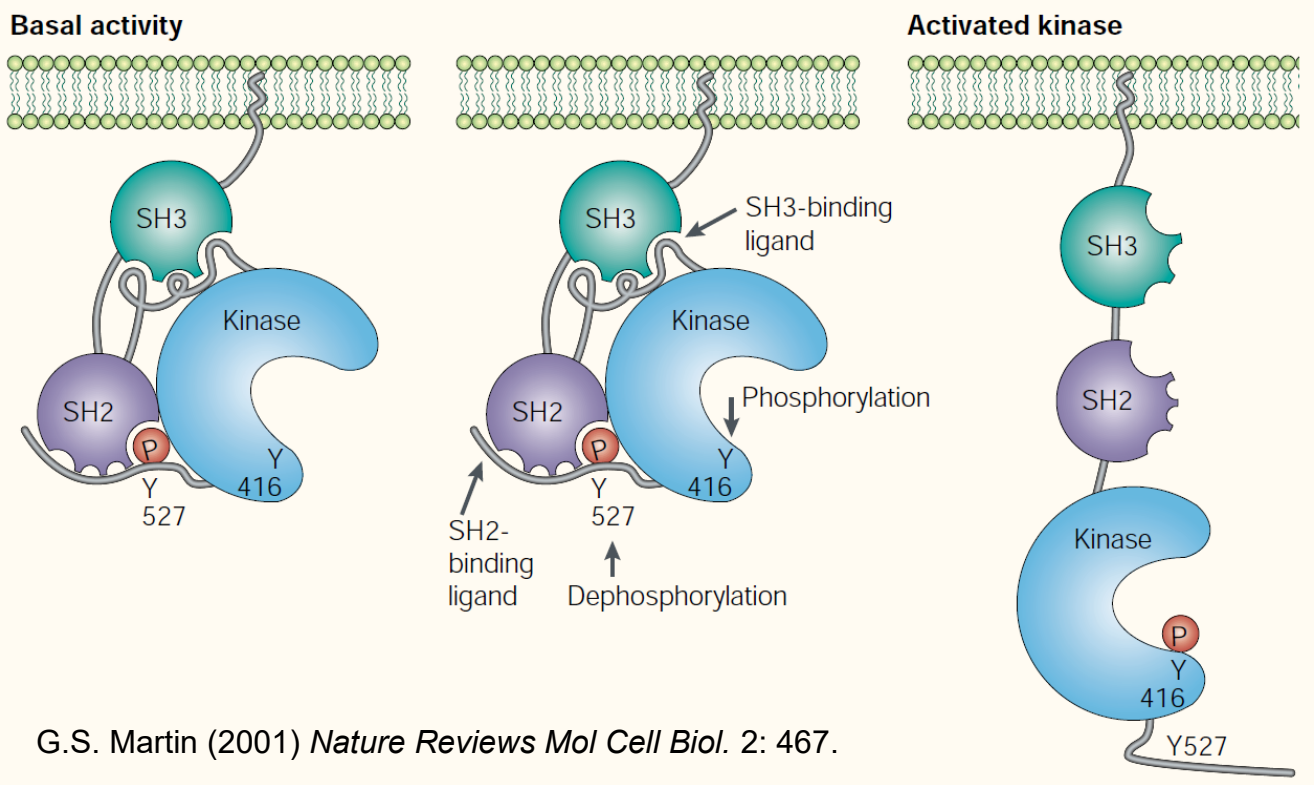


G.S. Martin (2001) *Nature Reviews Mol Cell Biol.* 2: 467.

- Src = Phosphotyrosine kinase with multiple functions in cell signaling (proteomics: ~350 potential Src substrates).

How can one protein integrate many signals?

Src Kinase: Mechanism of Activation



G.S. Martin (2001) *Nature Reviews Mol Cell Biol.* 2: 467.

- Regulation by two key phosphorylation events (Y527: intramolecular inhibition; Y416: kinase domain activation).

How can we model Src activation dynamics?

Models for Cell Signaling: Same Concepts

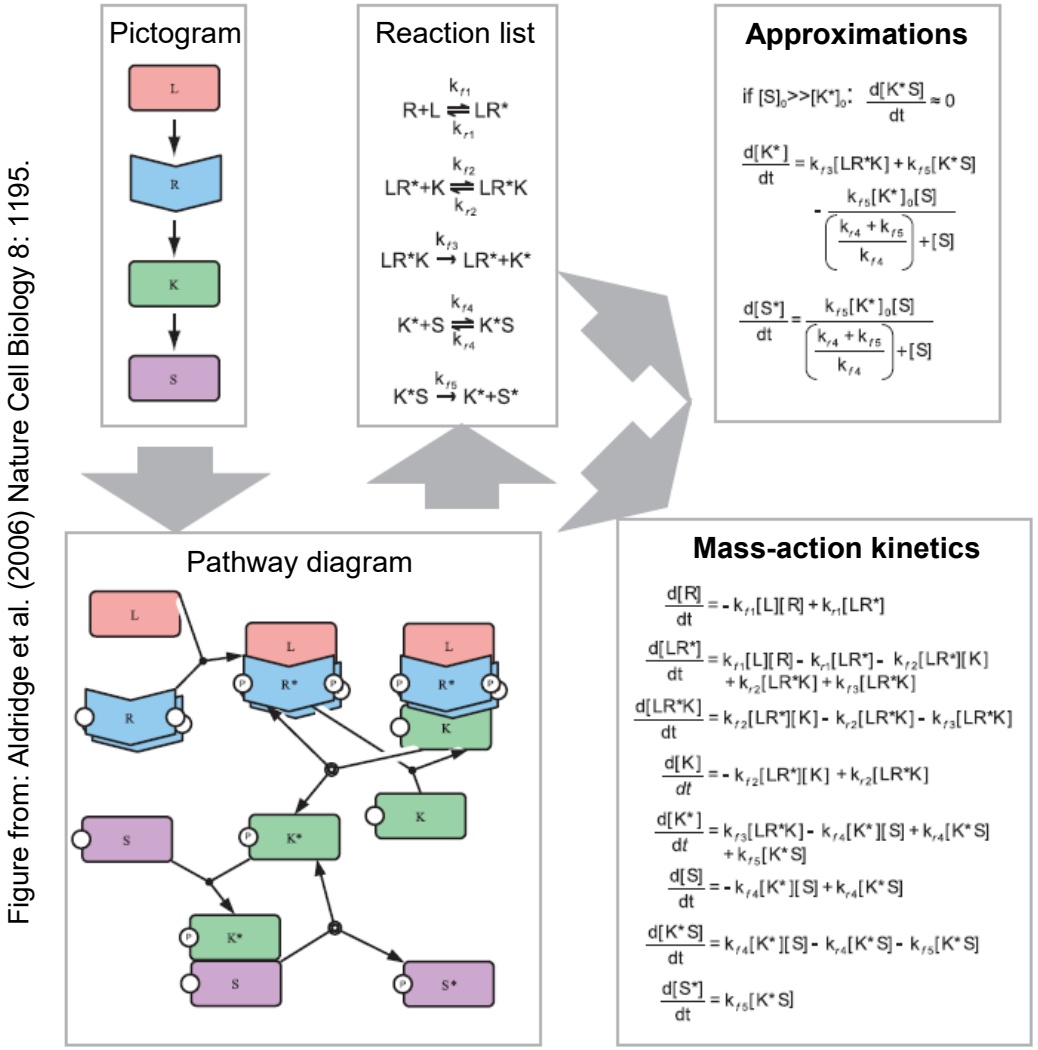
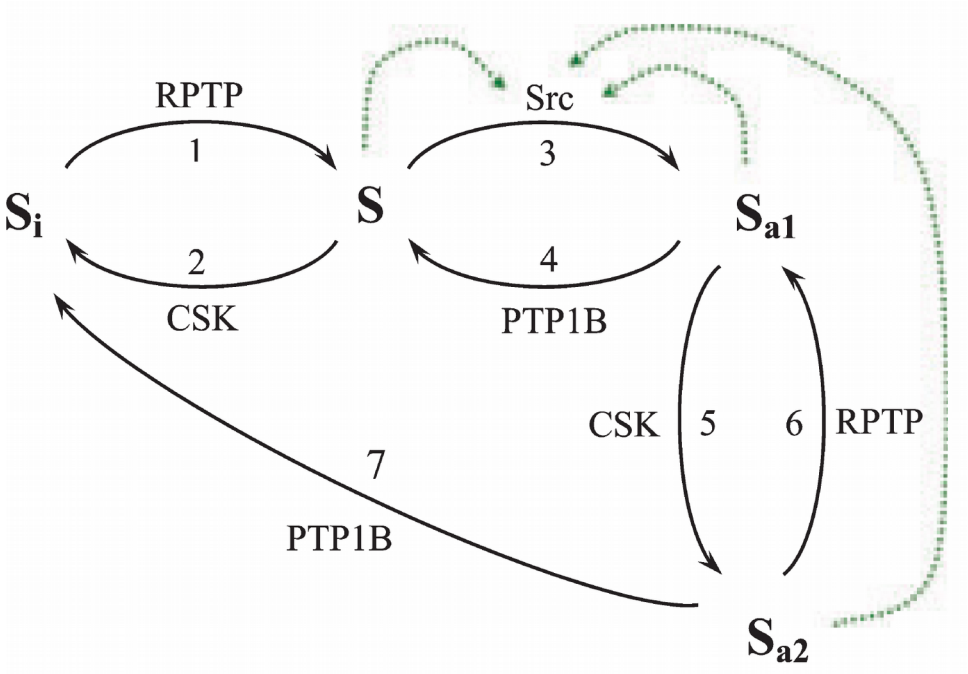


Figure from: Aldridge et al. (2006) Nature Cell Biology 8: 1195.

$$\frac{d\,\mathbf{x}(t)}{dt} = f\left(\mathbf{x}(t), \mathbf{u}(t), \mathbf{p}\right)$$

Example: Model for Src Kinase

- S_i: inhibited (pY527, Y416)
- S: partial act. (Y527, Y416)
- S_{a1}: active (Y527, pY416)
- S_{a2}: active (pY527, pY416)



N. Kaimachnikow & B. Kholodenko (2009) *FEBS J.* 276: 4102.

- Src model with four states, seven reactions, autoactivation.
- States are **not** independent → Not Michaelis-Menten!

Example: Model for Src Kinase

- $\mathbf{S_i}$: inhibited (pY527, Y416)
- $\mathbf{S}$: partial act. (Y527, Y416)
- $\mathbf{S_{a1}}$: active (Y527, pY416)
- $\mathbf{S_{a2}}$: active (pY527, pY416)

- Autocatalytic network:

$$\mathrm{S} + \mathrm{S} \underset{k_{\mathrm{S}}^{\mathrm{r}}}{\overset{k_{\mathrm{S}}^{\mathrm{f}}}{\rightleftharpoons}} \mathrm{S} \cdot \mathrm{S} \xrightarrow{k_{\mathrm{S}}^{\mathrm{cat}}} \mathrm{S} + \mathrm{S_{a1}}$$

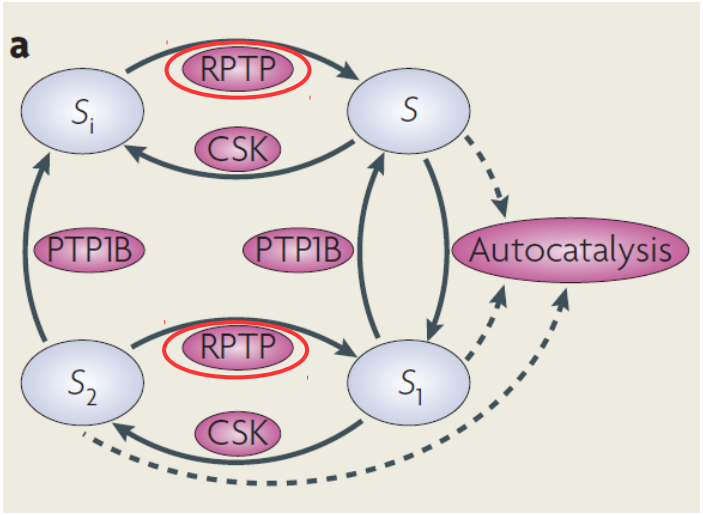
$$\mathrm{S_{a1}} + \mathrm{S} \underset{k_{\mathrm{a1}}^{\mathrm{r}}}{\overset{k_{\mathrm{a1}}^{\mathrm{f}}}{\rightleftharpoons}} \mathrm{S_{a1}} \cdot \mathrm{S} \xrightarrow{k_{\mathrm{a1}}^{\mathrm{cat}}} \mathrm{S_{a1}} + \mathrm{S_{a1}}$$

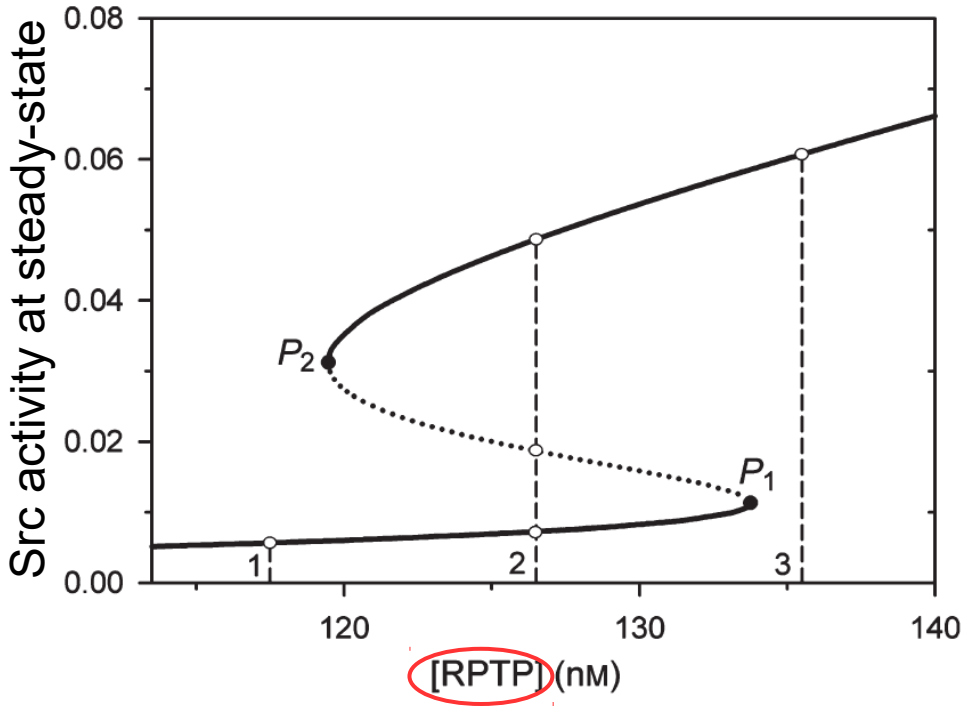
$$\mathrm{S_{a2}} + \mathrm{S} \underset{k_{\mathrm{a2}}^{\mathrm{r}}}{\overset{k_{\mathrm{a2}}^{\mathrm{f}}}{\rightleftharpoons}} \mathrm{S_{a2}} \cdot \mathrm{S} \xrightarrow{k_{\mathrm{a2}}^{\mathrm{cat}}} \mathrm{S_{a2}} + \mathrm{S_{a1}}$$

N. Kaimachnikow & B. Kholodenko (2009) *FEBS J.* 276: 4102.

- Src model with four states, using mass-action kinetics.
- States are **not** independent: $$v_3 = \left(\frac{k_{\mathrm{S}}^{\mathrm{cat}}}{K_{\mathrm{S}}} [\mathrm{S}] + \frac{k_{\mathrm{a1}}^{\mathrm{cat}}}{K_{\mathrm{a1}}} [\mathrm{S_{a1}}] + \frac{k_{\mathrm{a2}}^{\mathrm{cat}}}{K_{\mathrm{a2}}} [\mathrm{S_{a2}}] \right) [\mathrm{S}]$$

Principle: Dynamic Memory

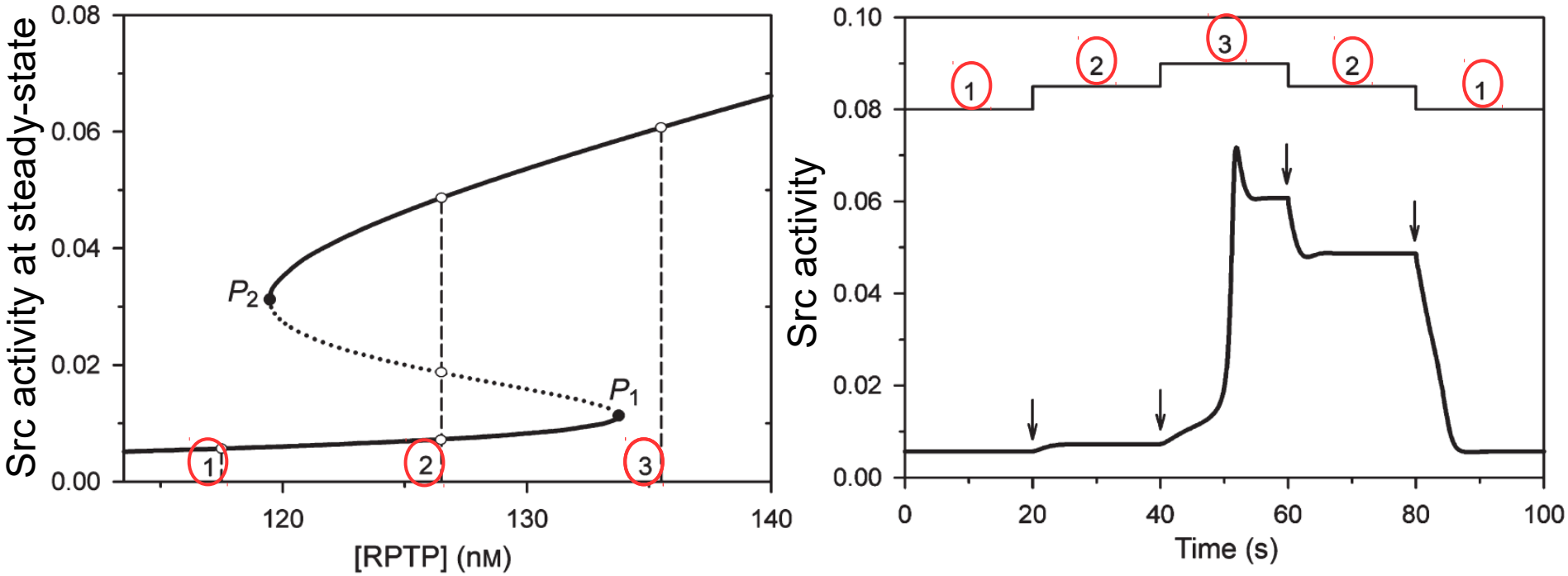




N. Kaimachnikow & B. Kholodenko (2009) *FEBS J.* 276: 4102.

- Src autocatalytic activation implies positive feedback → Dynamic memory is possible (depending on context).

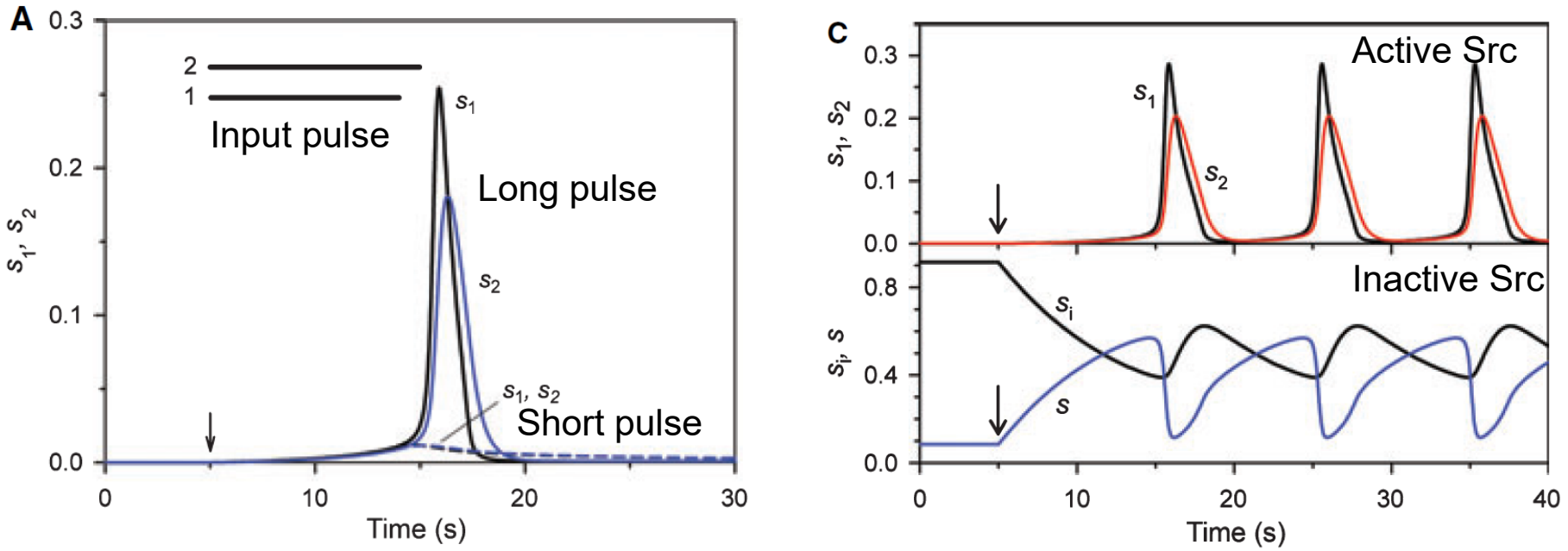
Principle: Dynamic Memory



N. Kaimachnikow & B. Kholodenko (2009) *FEBS J.* 276: 4102.

- Src autocatalytic activation implies positive feedback → Dynamic memory is possible (depending on context).

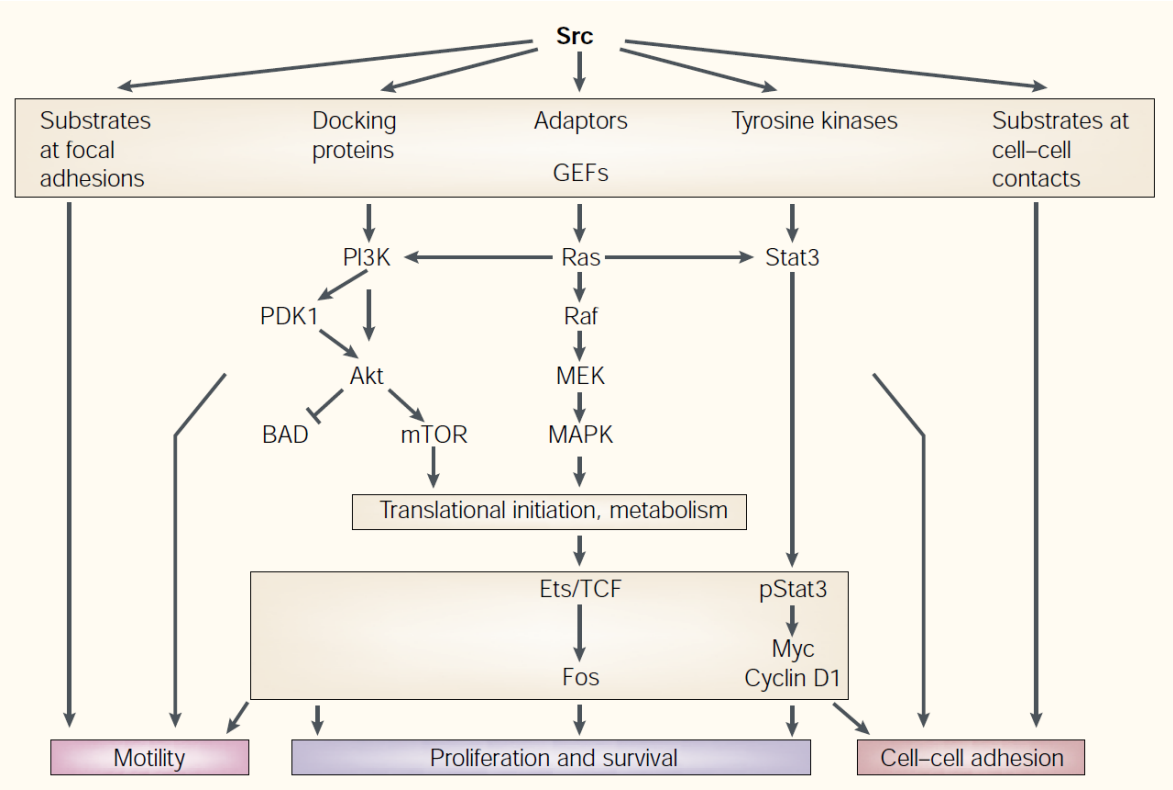
Src Kinase: Versatile Dynamic Behaviors



N. Kaimachnikow & B. Kholodenko (2009) *FEBS J.* 276: 4102.

- Excitability (left): Src responds with pulse of activity to signals above (2) but not below (1) threshold duration.
- Oscillations (right): In the right context, kinase activity oscillates autonomously (for constant inputs).

Principle: Integrated Cell Signaling



G.S. Martin (2001) *Nature Reviews Mol Cell Biol.* 2: 467.

- Conclusion: Such pictures can be misleading because signals are (dynamically) separated in time (and space).

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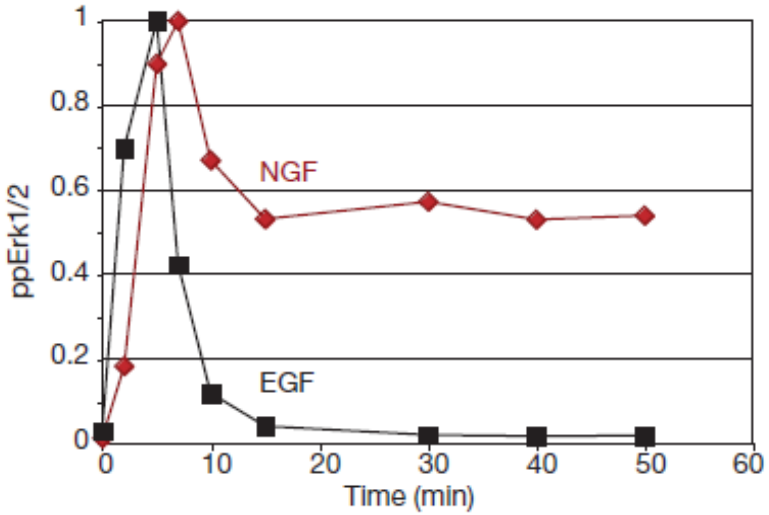
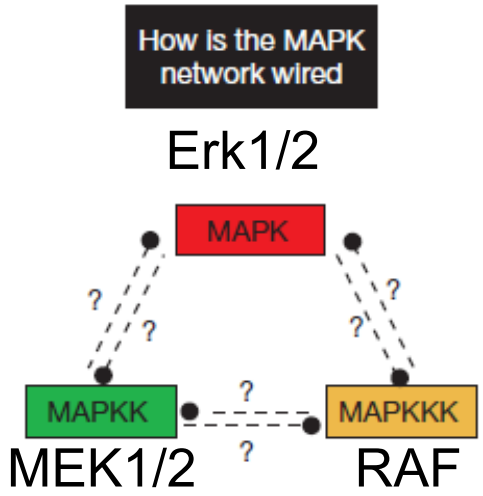
Example: MAPK Signaling in Human Cells

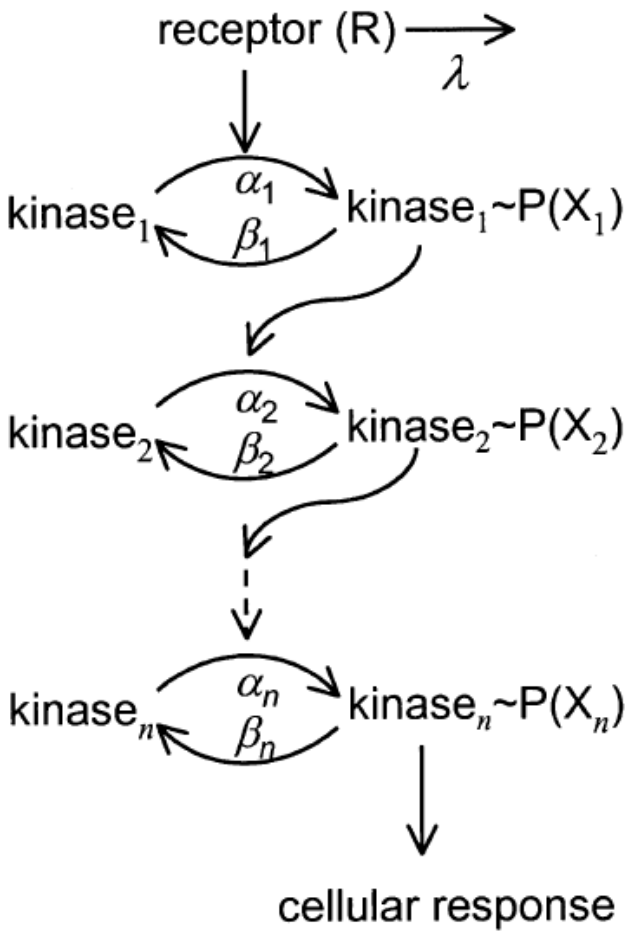




S. Santos et al. (2007) *Nature Cell Biol.* 9: 324-330.

- Qualitatively different output of MAPK cascade signaling for different growth factor stimulations: NGF induces differentiation, EGF induces proliferation.

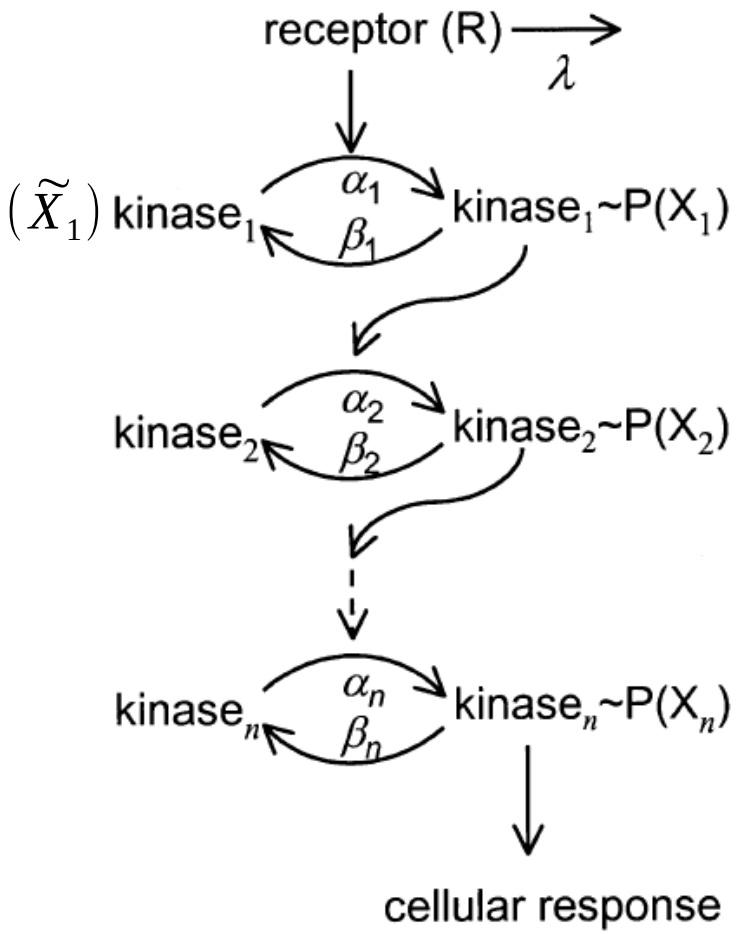
MAPK Cascade Signaling: General Questions



- How does a common pathway structure generate distinct dynamic behaviors (e.g., transient vs. sustained signaling)?
- Why do MAPK cascades have usually three levels?
- Which components influence signaling most?

R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

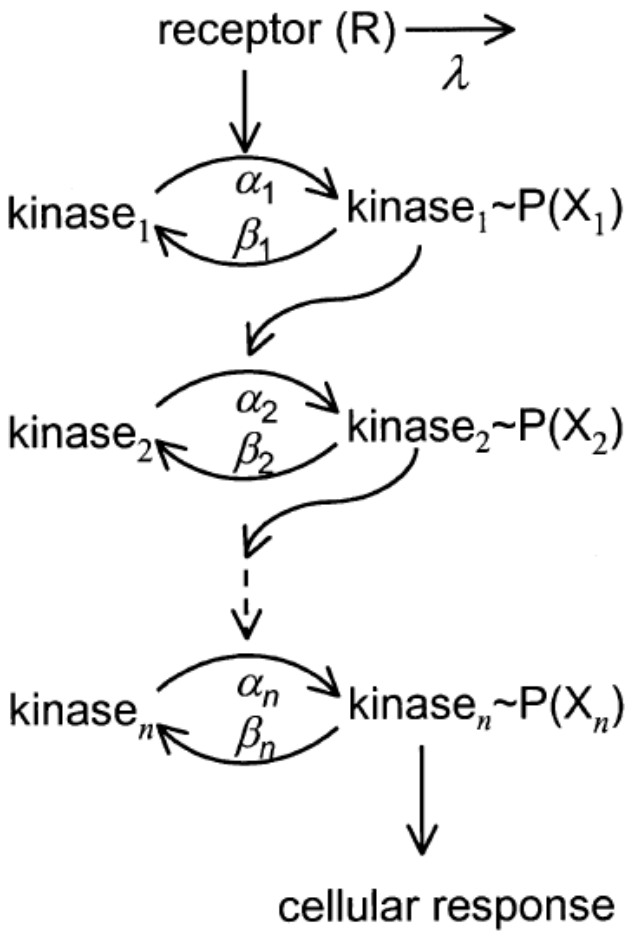
An Abstract Model of MAPK Cascade Signaling



R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

- Kinase cascade with $i=1...n$ levels (kinases).
- States are concentrations of:
  - Active, phosphorylated kinase X_i
  - Inactive kinase $\widetilde{X}_i$
- Parameters for kinase (α_i) and phosphatase (β_i).
- Input: receptor signal $R(t)$.

An Abstract Model of MAPK Cascade Signaling



Figures from: R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

- Kinase activity ($i > 1$):

$$\frac{dX_i}{dt} = v_{p,i} - v_{d,i} = \tilde{\alpha}_i X_{i-1} \tilde{X}_i - \beta_i X_i$$

- With total concentration C_i :

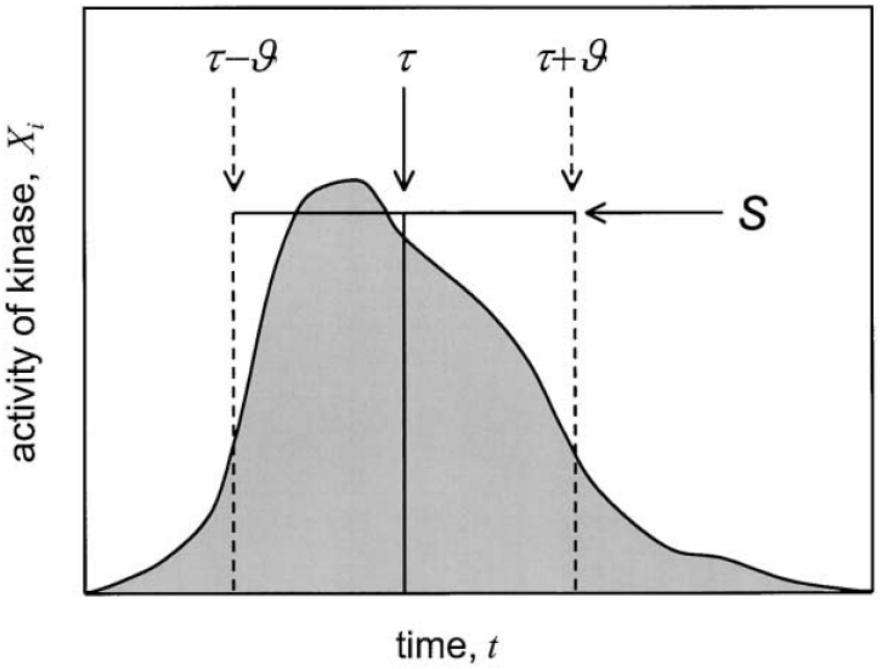
$$\frac{dX_i}{dt} = \alpha_i X_{i-1}\left(1 - \frac{X_i}{C_i}\right) - \beta_i X_i$$

- Activation by $R(t) = \exp(-\lambda t)$:

$$\frac{dX_1}{dt} = \alpha_1 R(t)\left(1 - \frac{X_1}{C_1}\right) - \beta_1 X_1$$

How to quantify signaling performance?

Signaling Cascades: Performance Criteria



R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

- Signaling time τ: Expected time of arrival of the signal.
- Signal duration υ: Variance of the expected time.
- Signal amplitude S: Average amplitude during signaling.

Signaling Cascades: Weak Activation

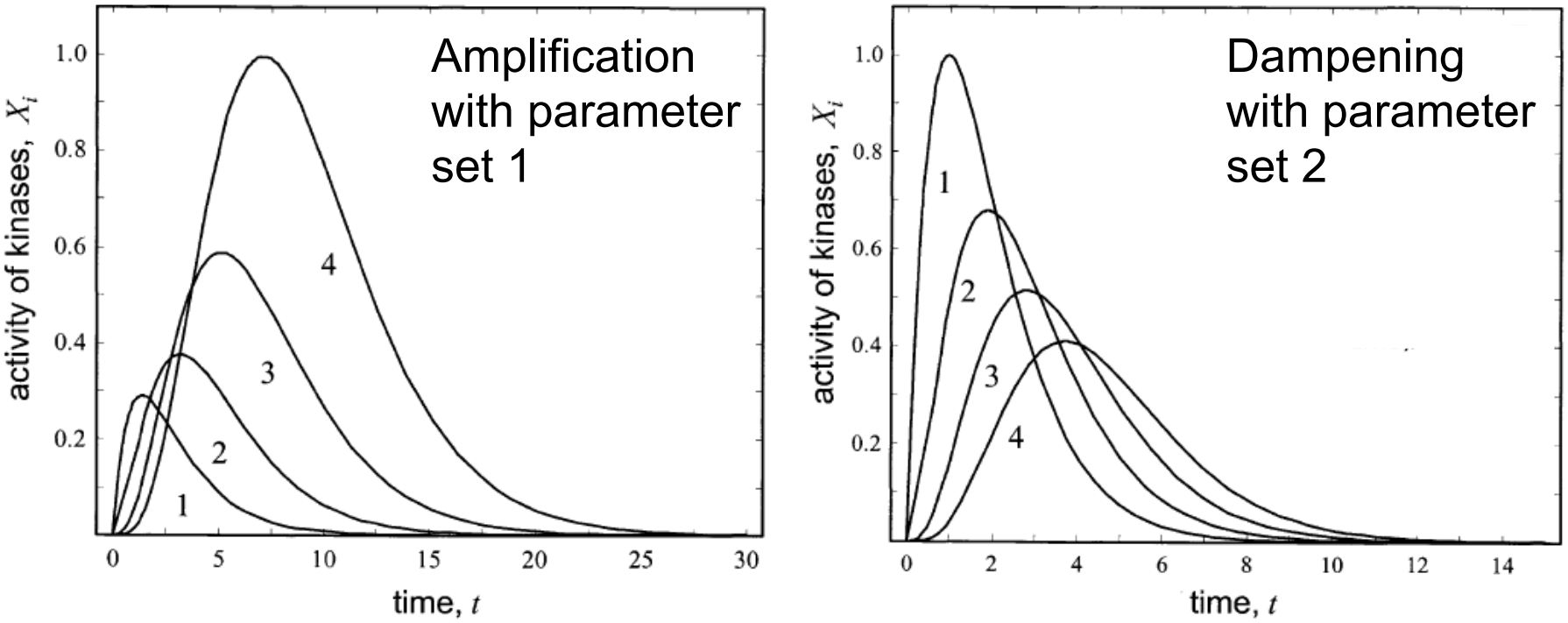
- Assume 'weak activation' of all kinases: $X_i << C_i$

$$\frac{dX_i}{dt} = \alpha_i X_{i-1}\left(1 - \frac{X_i}{C_i}\right) - \beta_i X_i \longrightarrow \frac{dX_i}{dt} = \alpha_i X_{i-1} - \beta_i X_i$$

- Analytic solutions for performance of the cascade:
  - Signaling time: $\tau = \frac{1}{\lambda} + \sum_{j=1}^{n} \frac{1}{\beta_j}$
  - Signal duration: $\vartheta = \sqrt{\frac{1}{\lambda^2} + \sum_{j=1}^{n} \frac{1}{\beta_j^2}}$
  - Signal amplitude: $S = \frac{S_0 \prod_{k=1}^{n} \frac{\alpha_k}{\beta_k}}{\sqrt{1 + \lambda^2 \sum_{j=1}^{n} \frac{1}{\beta_j^2}}}$

Kinase-independent (no α)!

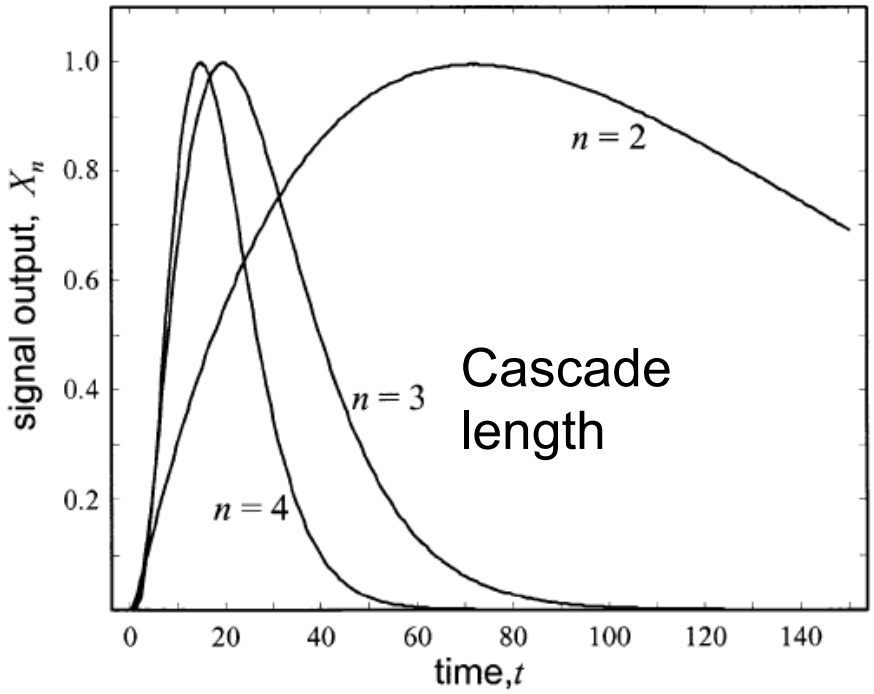
Signaling Cascades: Performance



R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

- Qualitative dynamics: Amplification or dampening of signal traversing the cascade → Parameter-dependent.

Signaling Cascades: Performance



R. Heinrich et al., *Molecular Cell* 9: 957, 2002.

- Counter-intuitive finding: Cascades with more levels can propagate signals faster.

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A 'Real' Model of MAPK Cascade Signaling

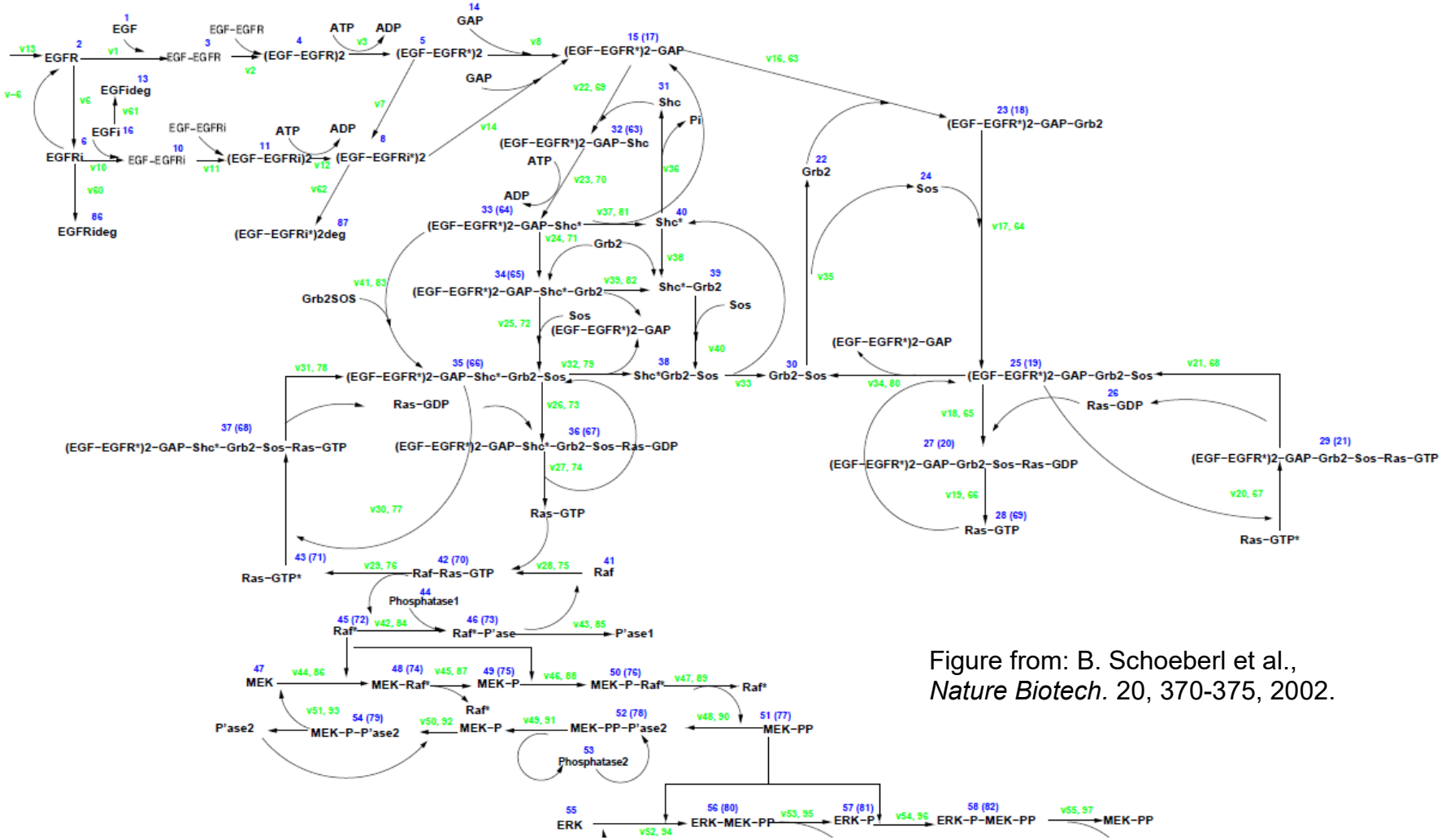


Figure from: B. Schoeberl et al., *Nature Biotech.* 20, 370-375, 2002.

- ODE model for Epidermal Growth Factor (EGF) signaling in humans → Target discovery for anti-cancer drugs.

MAPK Cascade Signaling Network

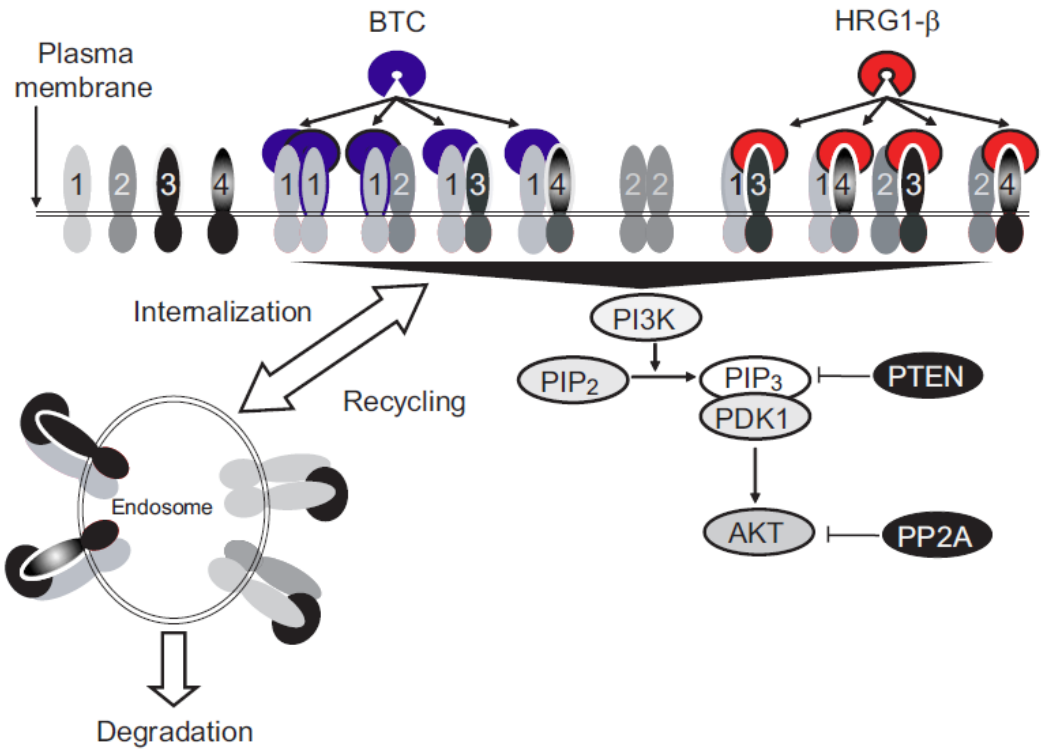


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- Signaling network with four receptors (ErbB1-4) and two ligands (BTC, Hrg1-β) → Growth signaling in cancers.

MAPK Cascade Signaling Network

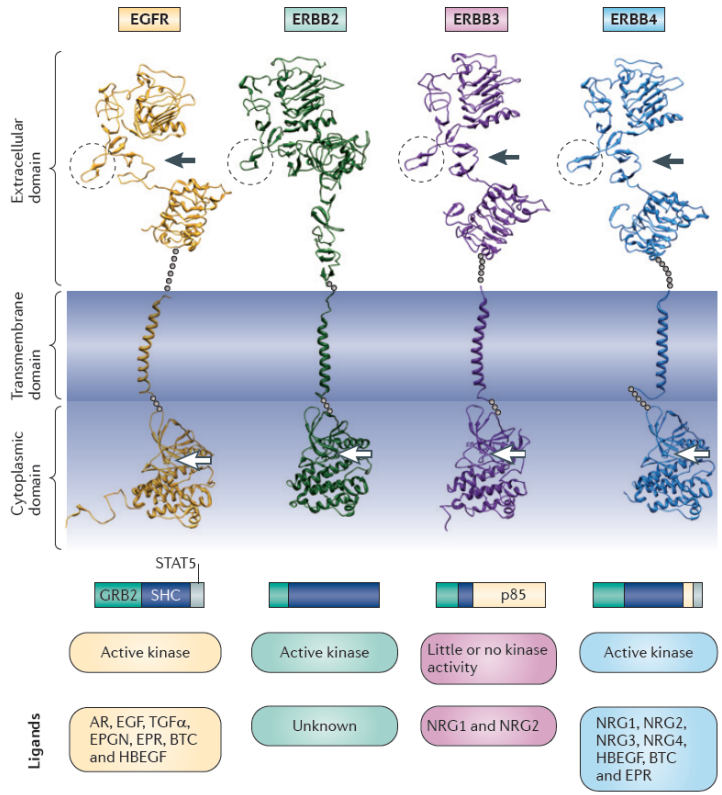


Figure from: Y. Yarden & P. Gines, *Nat. Rev. Cancer* 12, 553 (2012).

On notation:

- NRG1 = HRG1
- EGFR = ErbB1

- Signaling network with four receptors (ErbB1-4) and two ligands (BTC, Hrg1-β) → Growth signaling in cancers.
- Special case ErbB3: receptor without kinase activity.

Model Development for ErbB Signaling

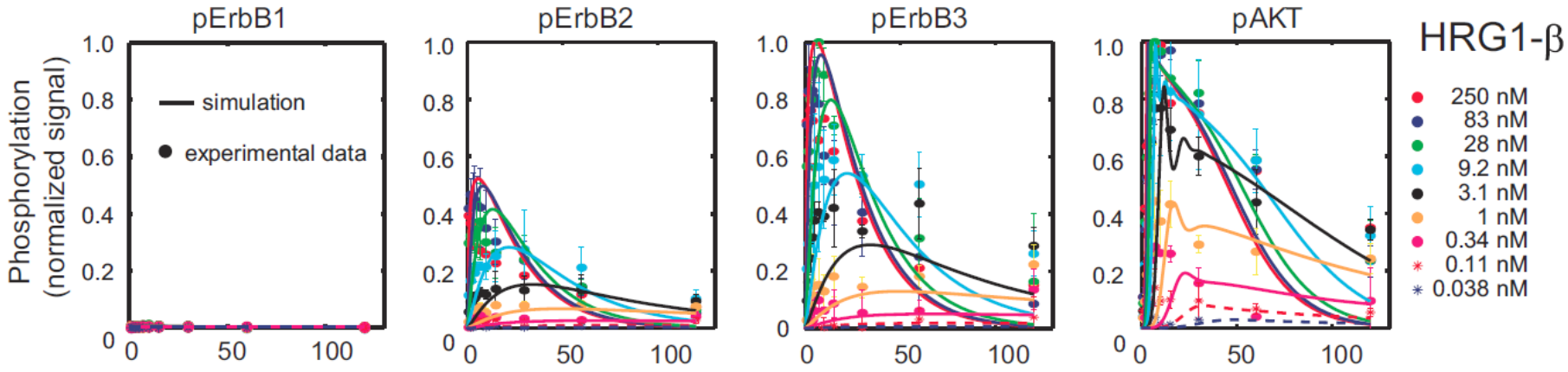


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- ODE model with ~500 states and ~230 parameters.
- Challenge: Calibrating parameters with experiments.
- Needs: Dynamic data, different ligand doses, ...

What are problems associated with many parameters?

Model Development for ErbB Signaling

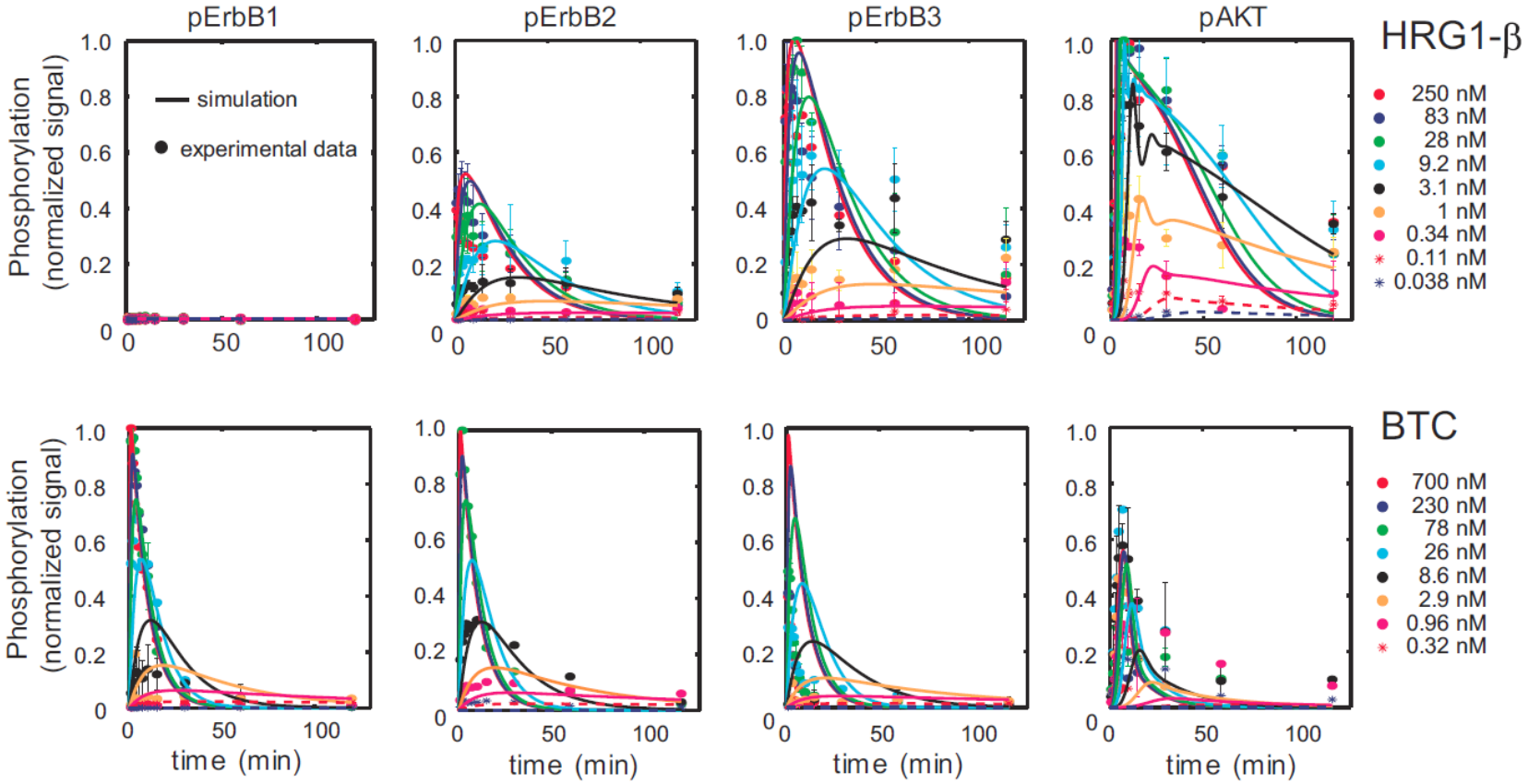
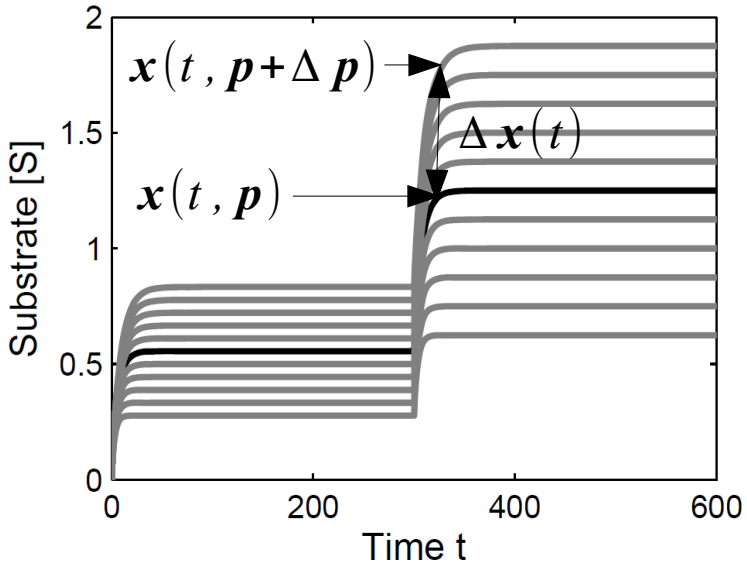


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- Calibrated model describes experiments reasonably.

How to use the model to identify potential drug targets?

Concept: Parameter Sensitivities



$$S(t) \approx \frac{\Delta x(t)}{\Delta p}$$

- **Parameter sensitivities $S(t)$:** Changes of system states in response to (small) changes in model parameters around a reference parameter set.

Model Analysis: Parameter Sensitivities

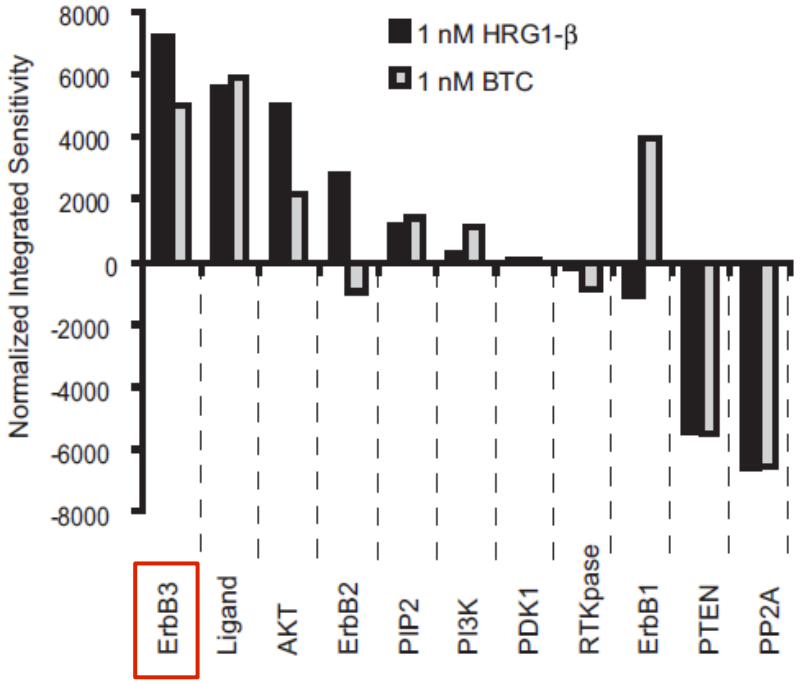


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- **Question:** Which perturbations (e.g. by drugs) are most effective for abolishing growth signaling?
- Surprising result: Perturbing the 'non-active' receptor ErbB3 is predicted to be most effective.

Model Application: Virtual Drug Design

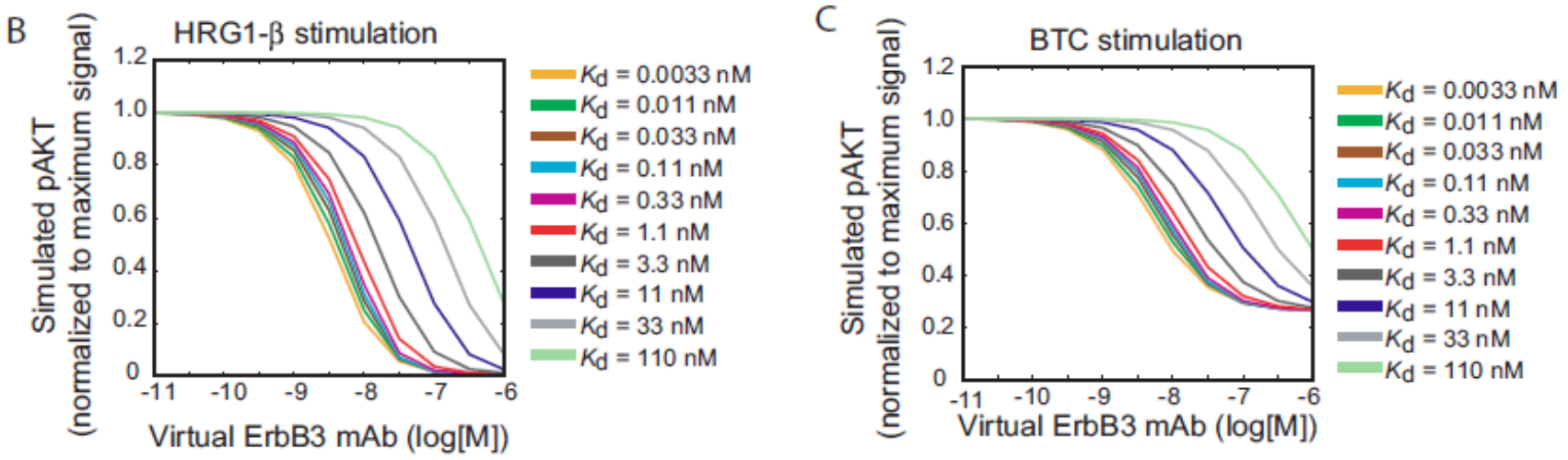


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- **Question:** Can one design a monoclonal antibody (mAb) against ErbB3 that prevents signaling?
- Required mAb affinities are feasible → Produce mAb.

Application in the 'Real' World

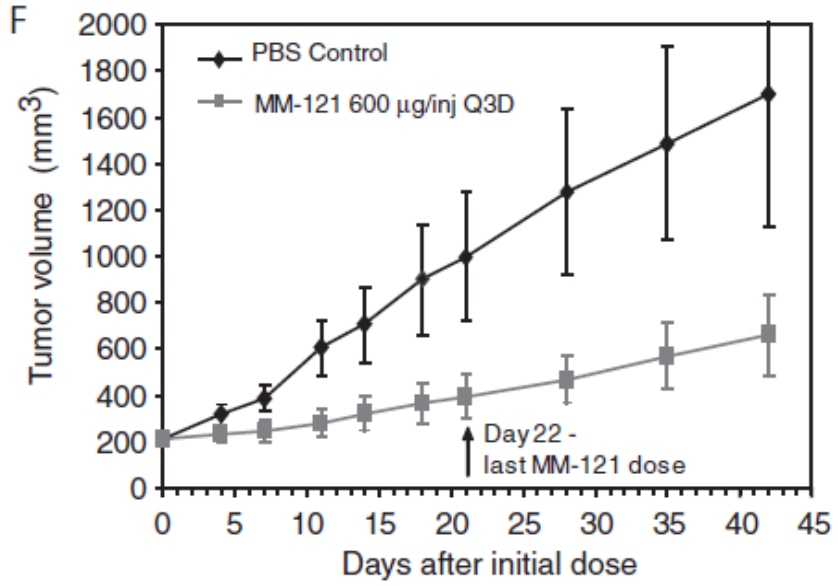
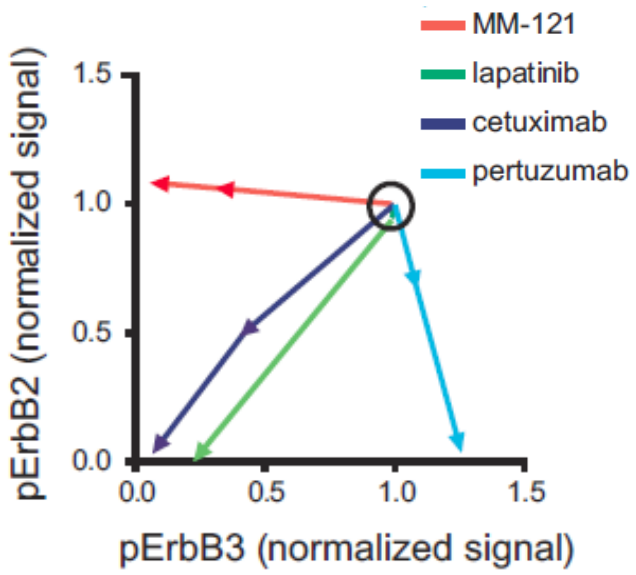


Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).

- **Validation:** In vivo (mouse) experiments, monitor tumor growth with and without mAb (MM-121) treatment → Reduced tumor growth with mAb.

Why Is the Target ErbB3 Relevant?

Figure from: B. Schoeberl et al., *Sci. Signal.* 2, ra31 (2009).



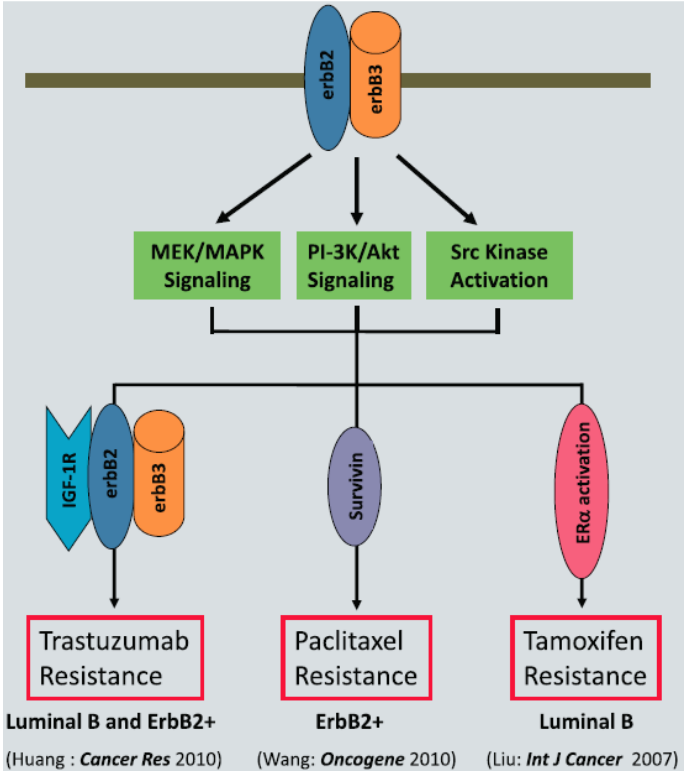


Figure from: J. Ma et al., *Molecular Cancer* 13: 105 (2014).

- Inhibiting ErbB3 has different effects than those of established cancer drugs → One could overcome cancer resistance to drug treatments.

Summary: Teaching Goals II-IV

- Cell signalling follows the same principles as discussed for metabolism: importance of dynamics and feedback.
- Dynamic mathematical modeling generalizes to cell signalling, but assumptions need to be critically evaluated (e.g., Src kinase does not follow Michaelis-Menten).
- Large-scale dynamic models can be applied to identify potential drug targets by analyzing (parameter) sensitivities in cell signaling networks.

"All models are wrong but some models are useful."

George Box (1979)

Robustness in the strategy of scientific model building.