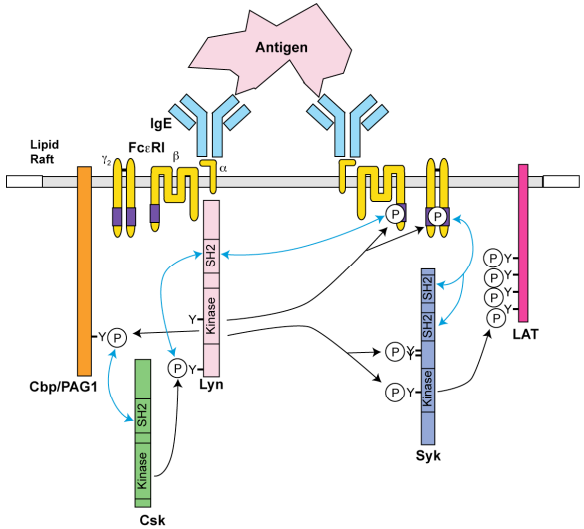
# (Still More) Rule-Based Modeling of Biological Signaling

Simulation Methods, Examples, misc.





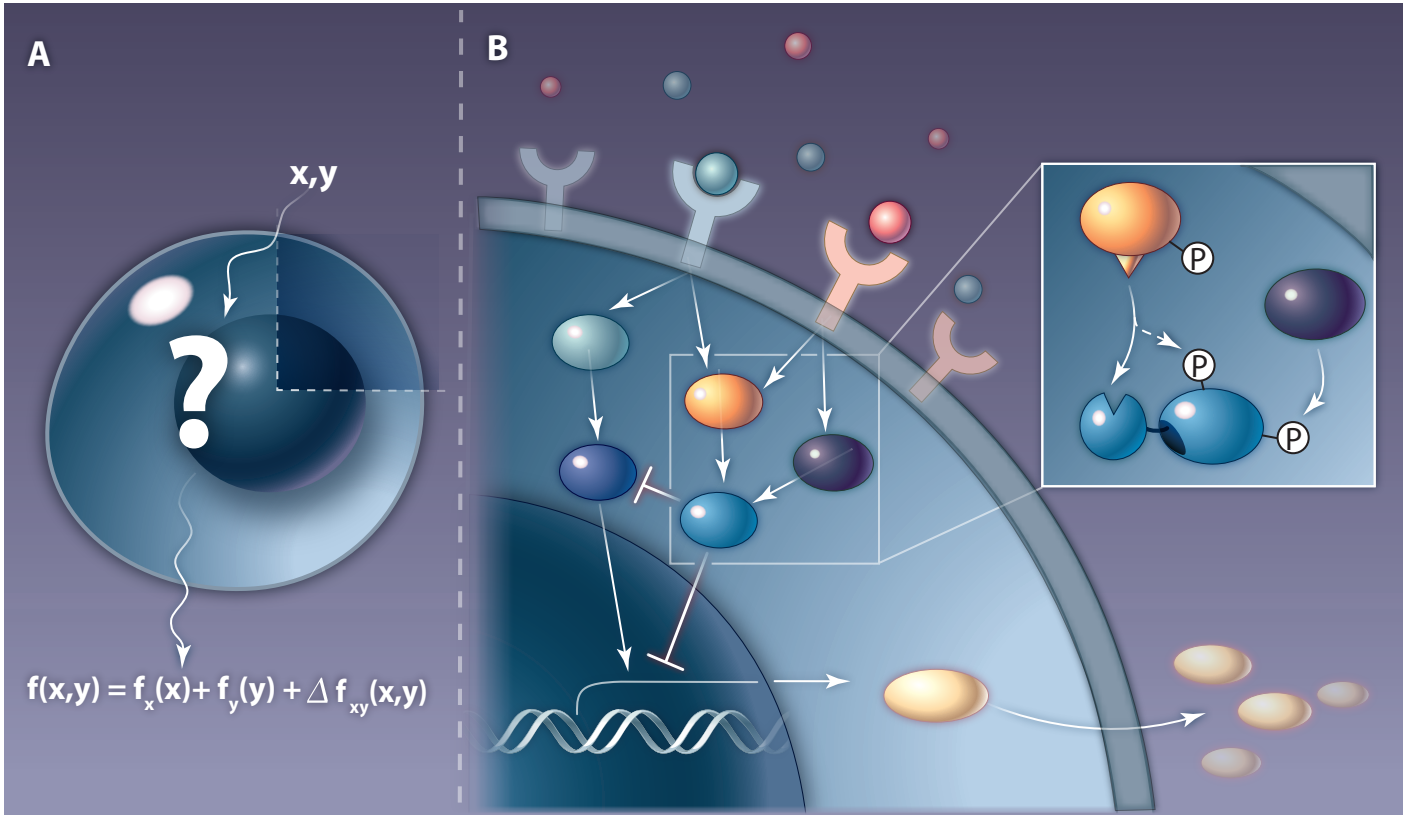
**Jim Faeder**

*Department of Computational Biology*
*University of Pittsburgh School of Medicine*


*3rd q-bio Summer School*

*Los Alamos, New Mexico*
*August 3, 2009*

# Layers of Complexity in Cell Signaling

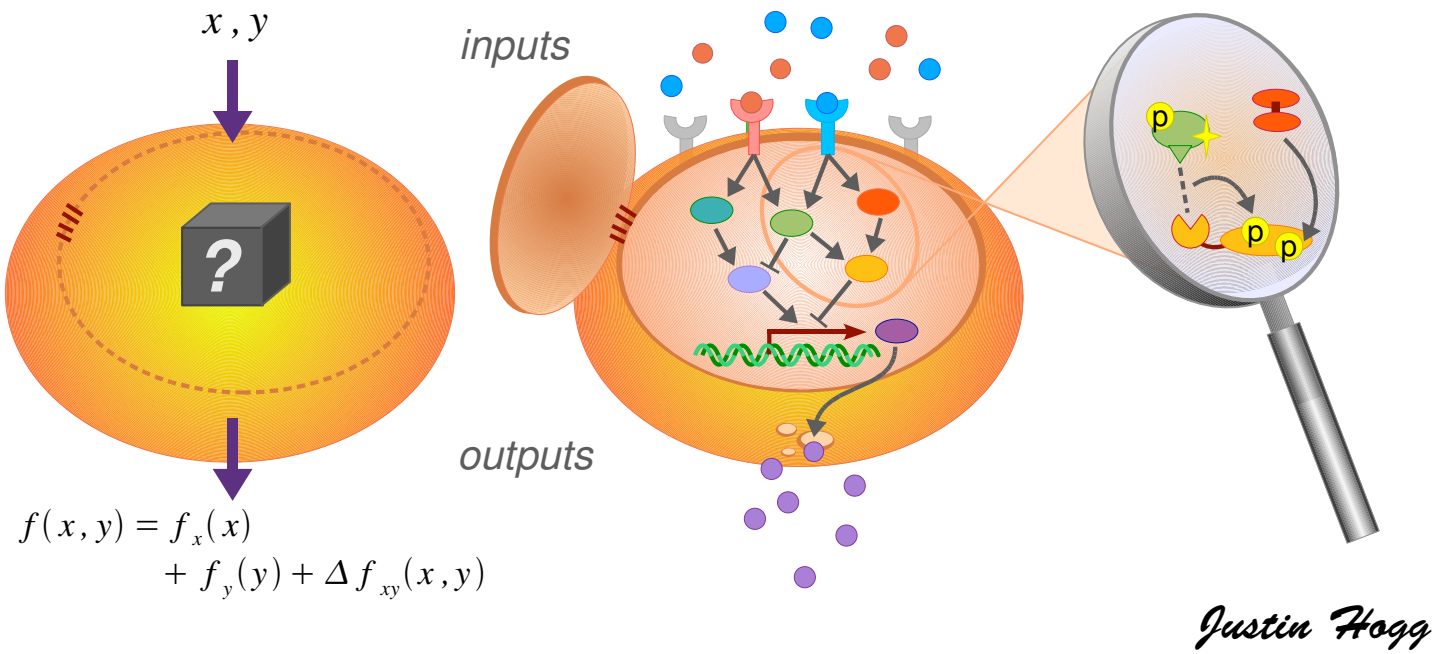



**How does a cell combine multiple inputs?**

**How complex is the machinery of signaling?**

**How can we make sense of the complexity?**

# Layers of Complexity in Cell Signaling



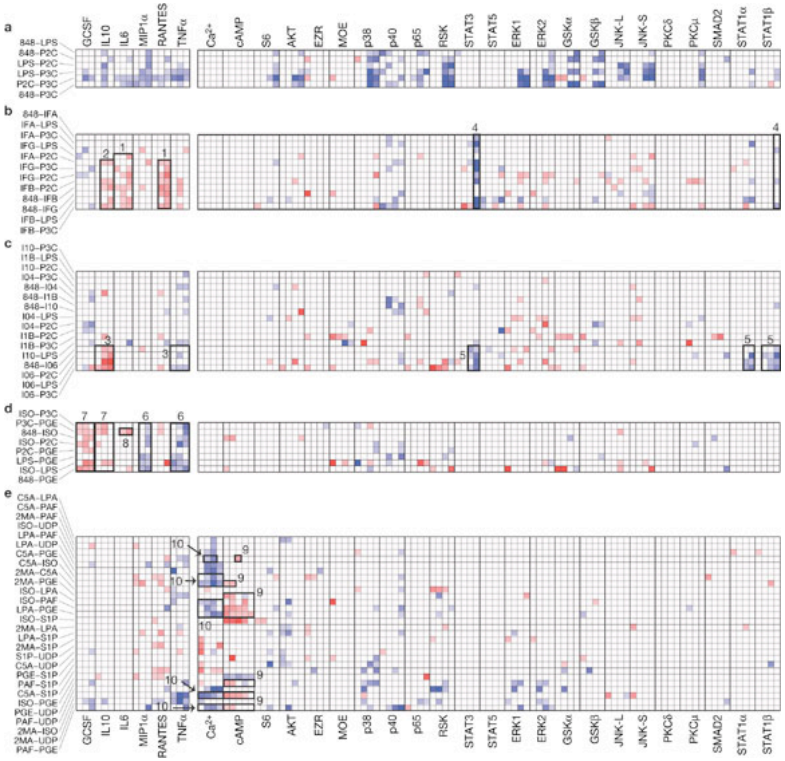

**How does a cell combine multiple inputs?**

**How complex is the machinery of signaling?**

**How can we make sense of the complexity?**

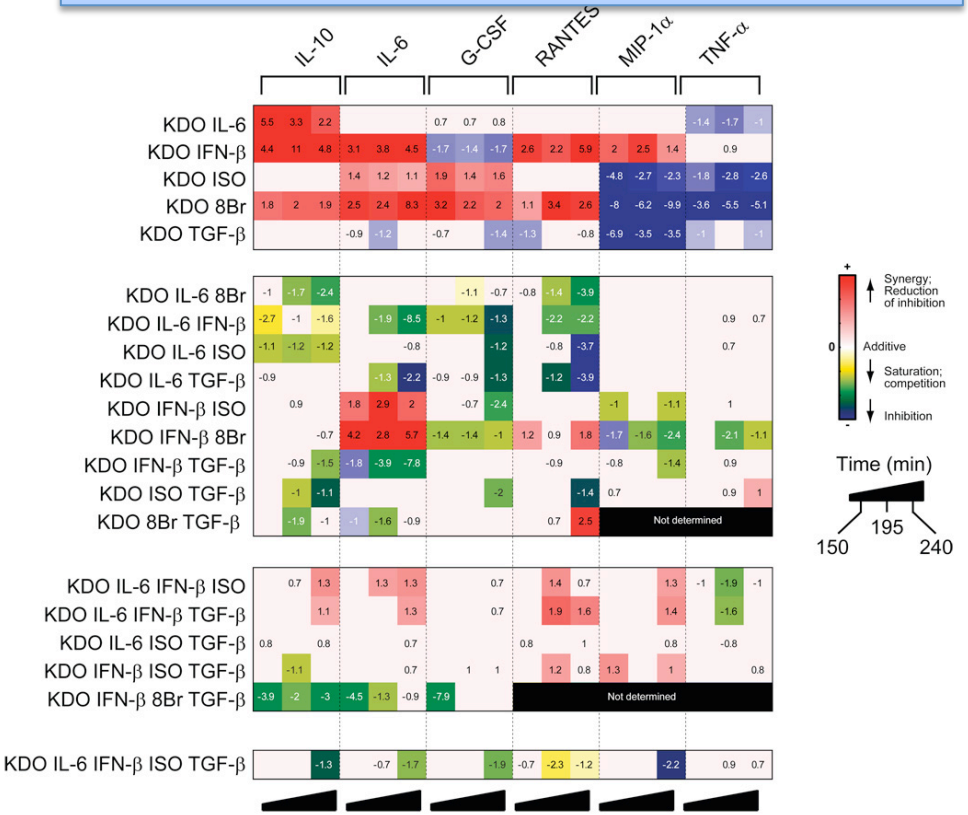
# The Black Box Perspective

22 ligands / 231 combinations / 39 outputs



Natarajan et al. *Nat. Cell Biol.* **8**, 571 (2006).

Responses to selected 3 way and higher combinations




Hsueh et al. *Sci. Signal.* **2**, ra22 (2009).

# The Black Box Perspective

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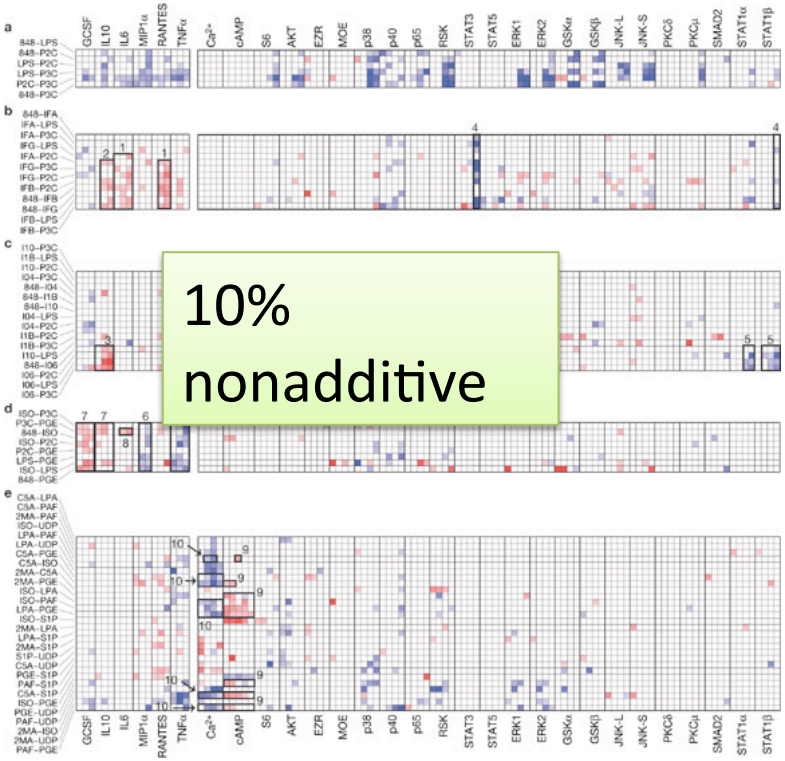


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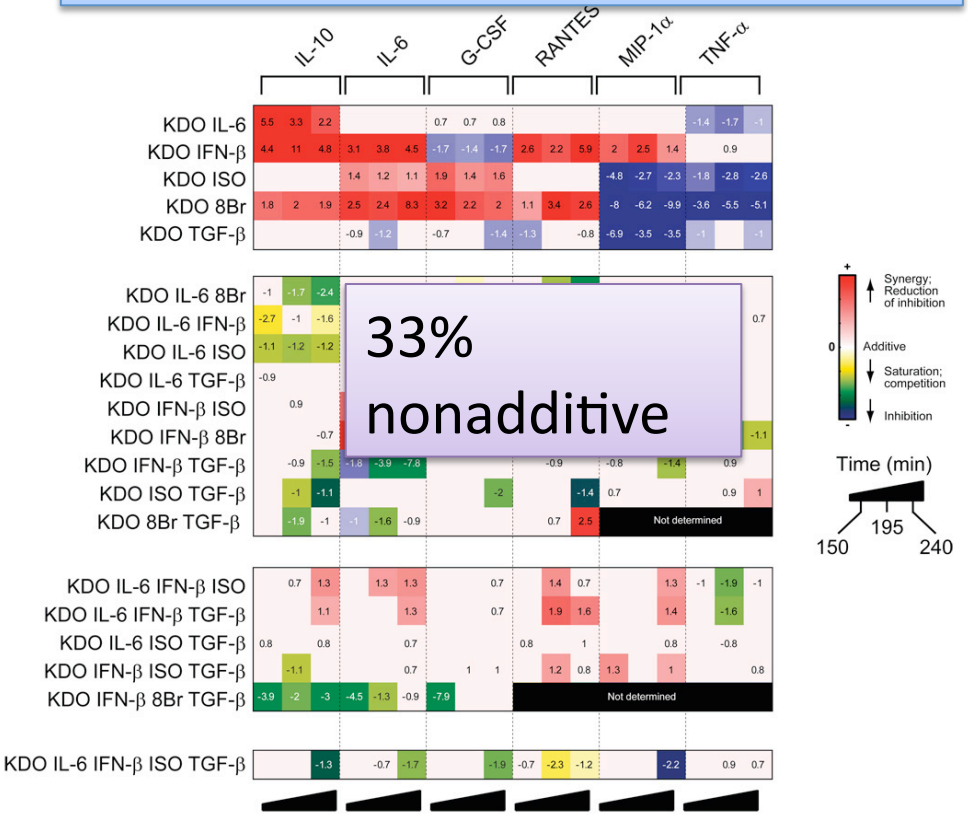



Hsueh et al. *Sci. Signal.* **2**, ra22 (2009).

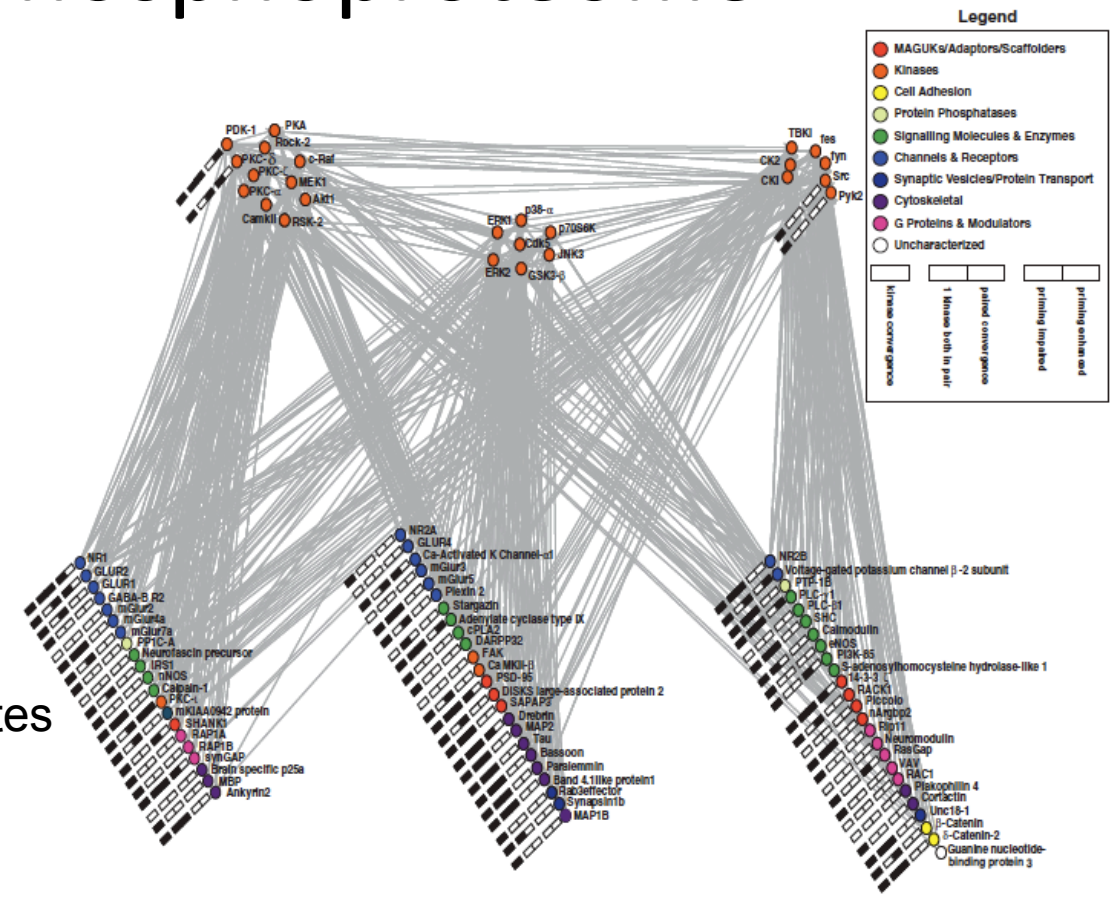
# A Wiring Diagram
# The Phosphoproteome

*Following stimulation by a single ligand (NMDA) of a single receptor (NMDAR)*

- 25 Kinases
- 300 sites / 166 observed

- 29 sites / kinase
- > 2 kinases / site

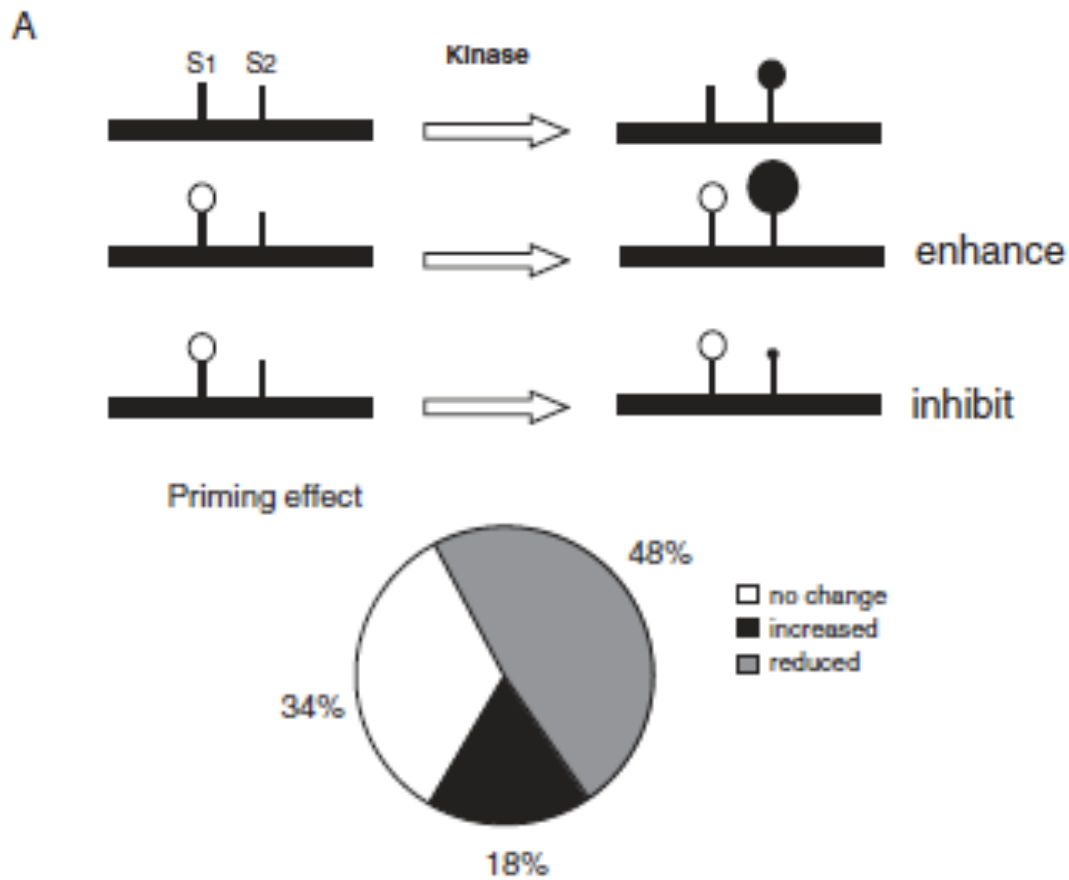
- 65% of proteins contain P-sites in binding domains




M. P. Coba et al. Neurotransmitters drive combinatorial multistate postsynaptic density networks. *Sci. Signal.* **2**, ra19 (2009).

# Cooperativity in Phosphorylation

- 66% of 200 peptides exhibit "priming"

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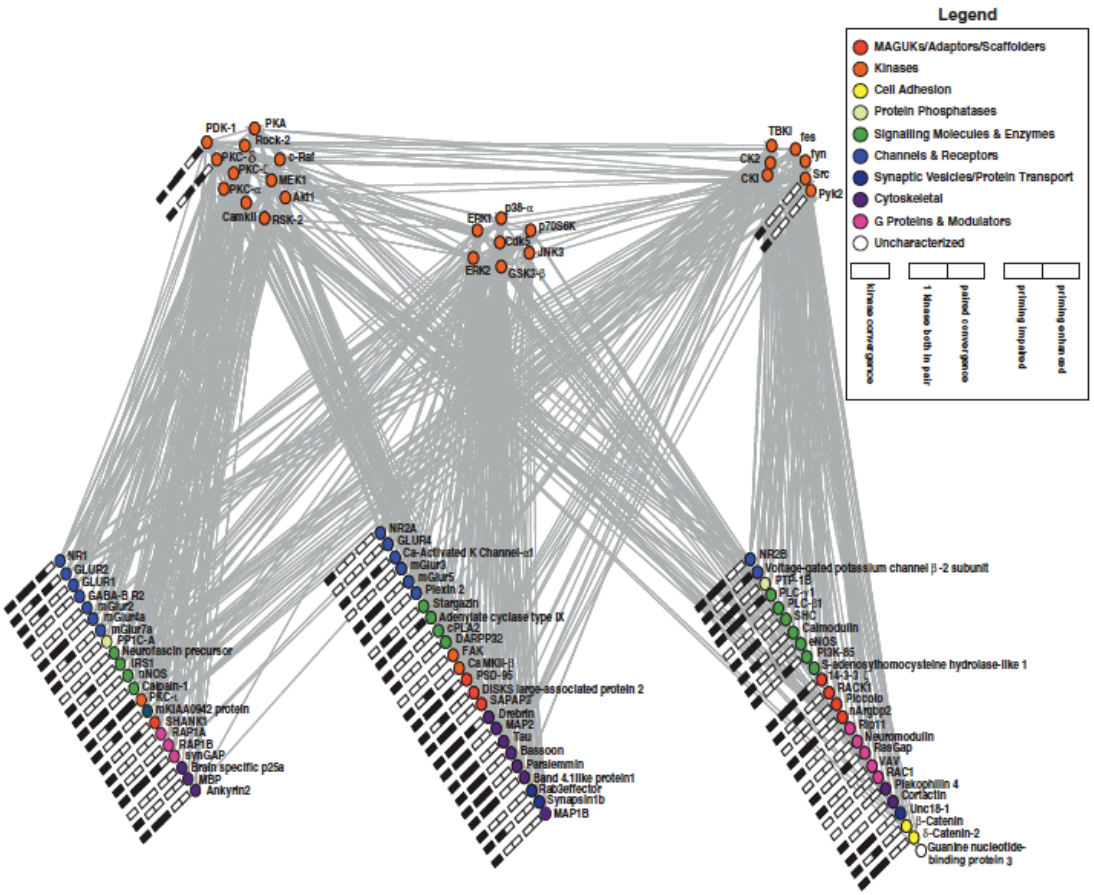
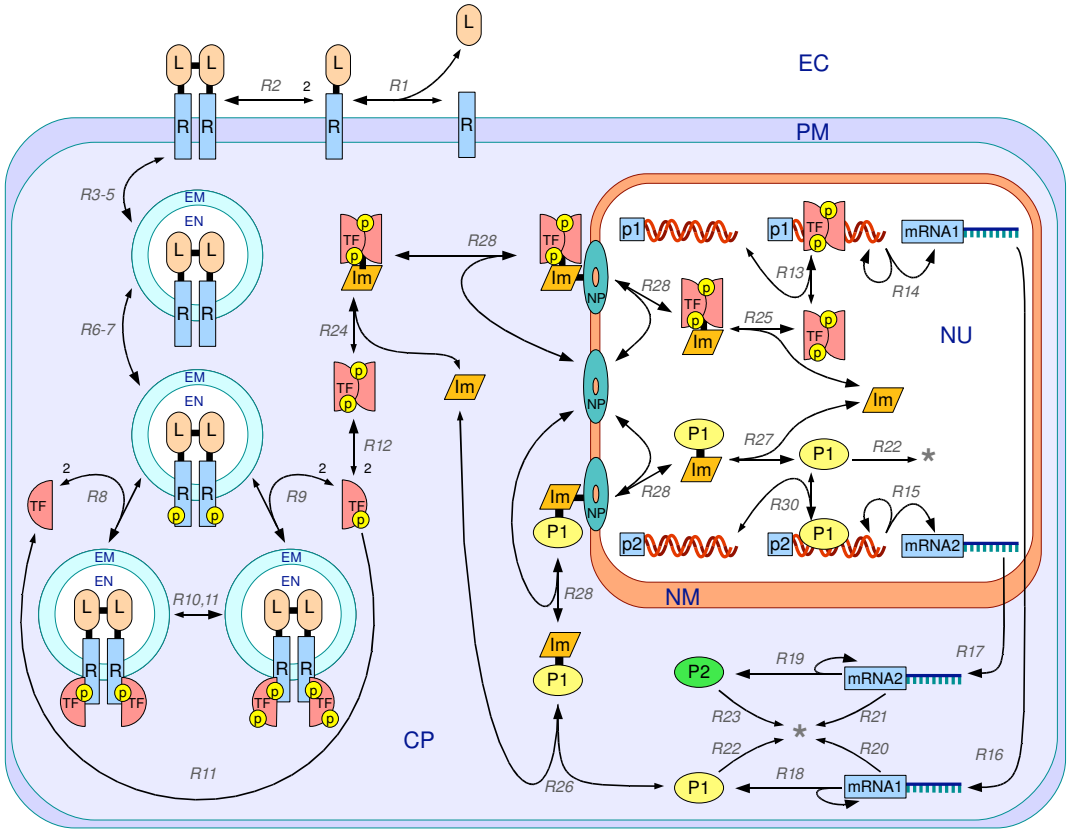



W. S. Hlavacek, J. R. Faeder, The complexity of cell signaling and the need for a new mechanics. *Sci. Signal.* **2**, pe46 (2009).

# Toward whole cell modeling

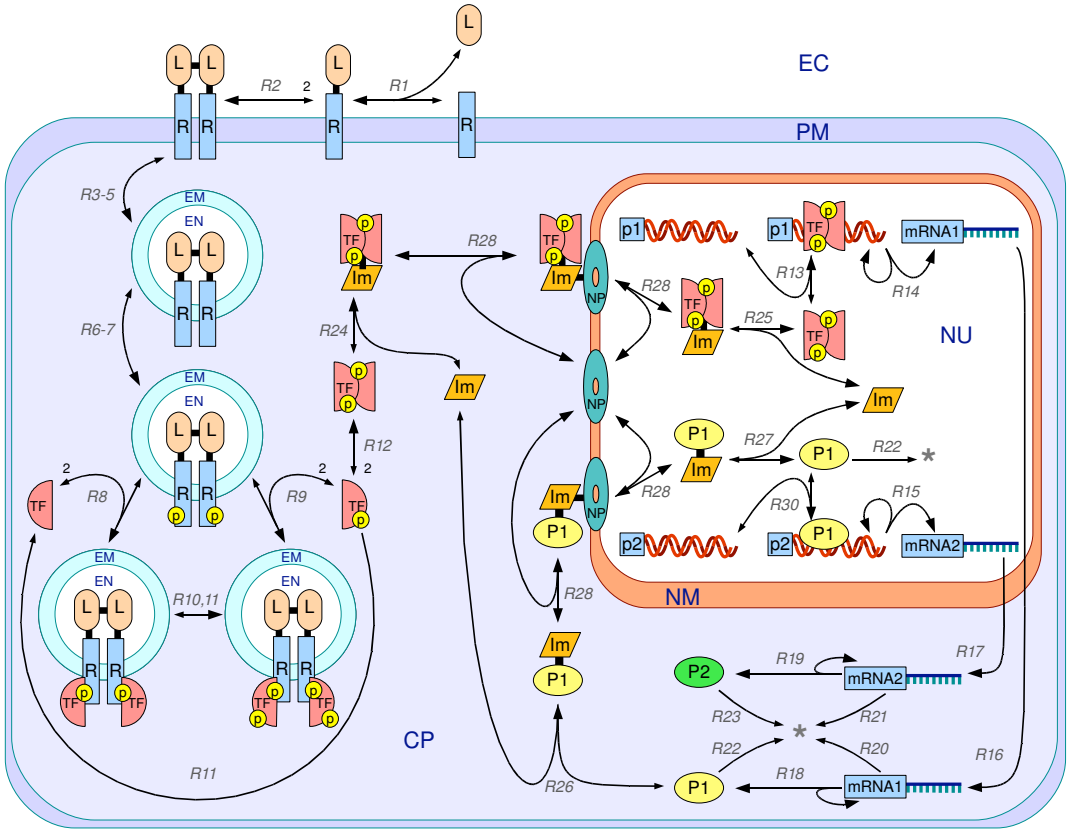



Justin Hogg

Key Questions:

- What parts of cell machinery are critical to the outcome?
- What controls the cell-to-cell variability?
- Can that variability be utilized by the organism to promote survival?

# Toward whole cell modeling

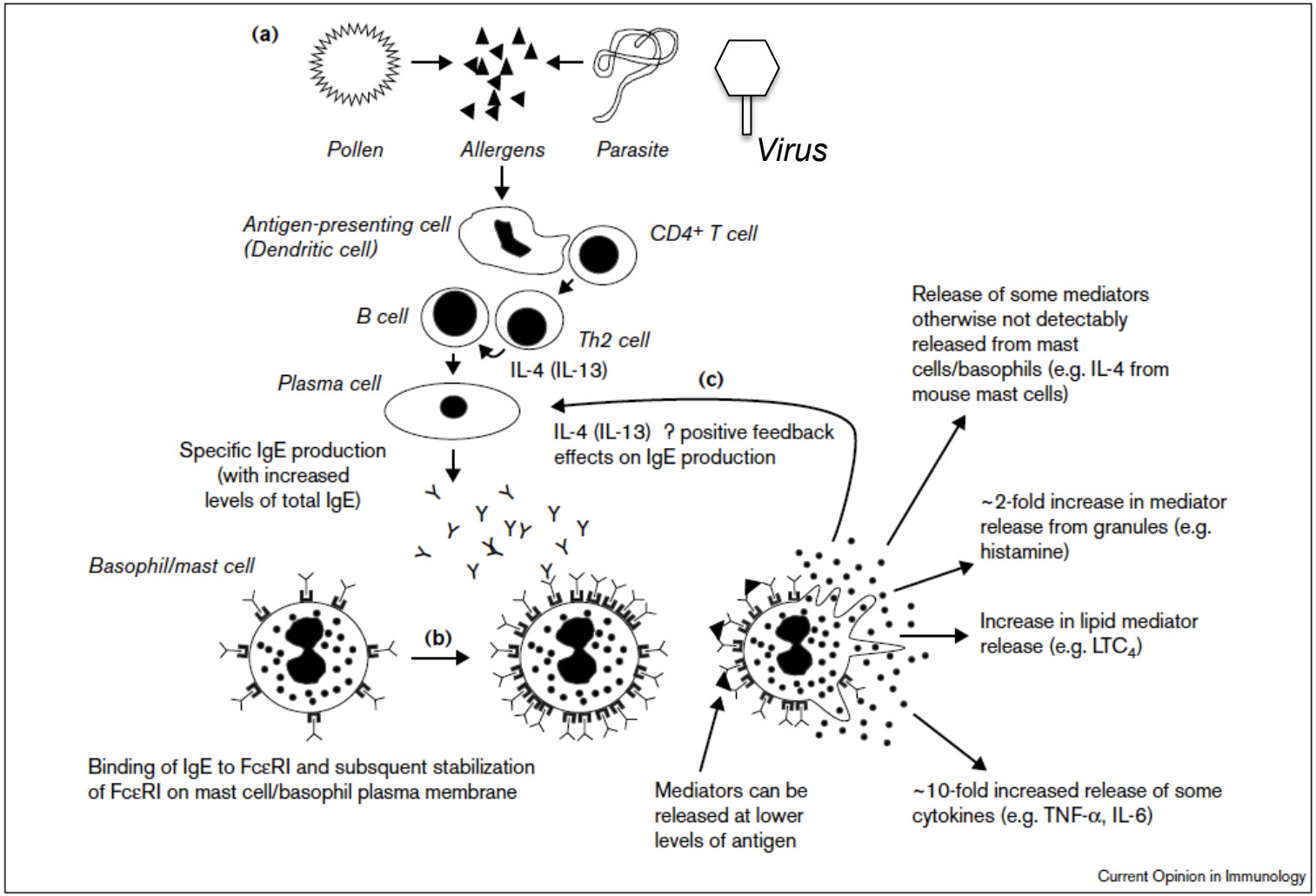



Justin Hogg

Approach:

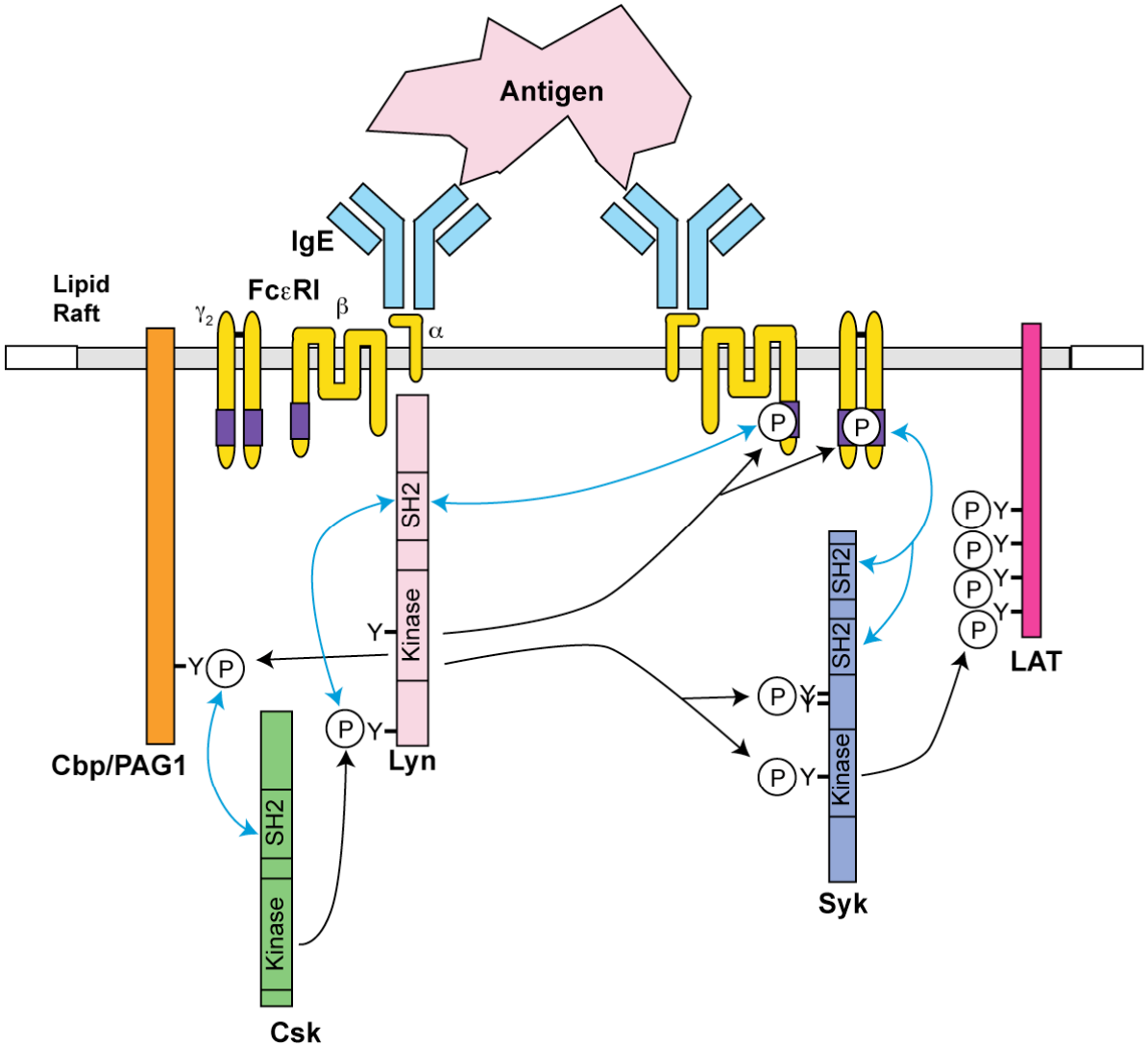
- Rule-based modeling
  - Phosphorylation and site-specific interactions
- Compartmental topology of the cell: *implicit* ➔ *explicit*

# Mast Cells / IgE Signaling in Host Defense

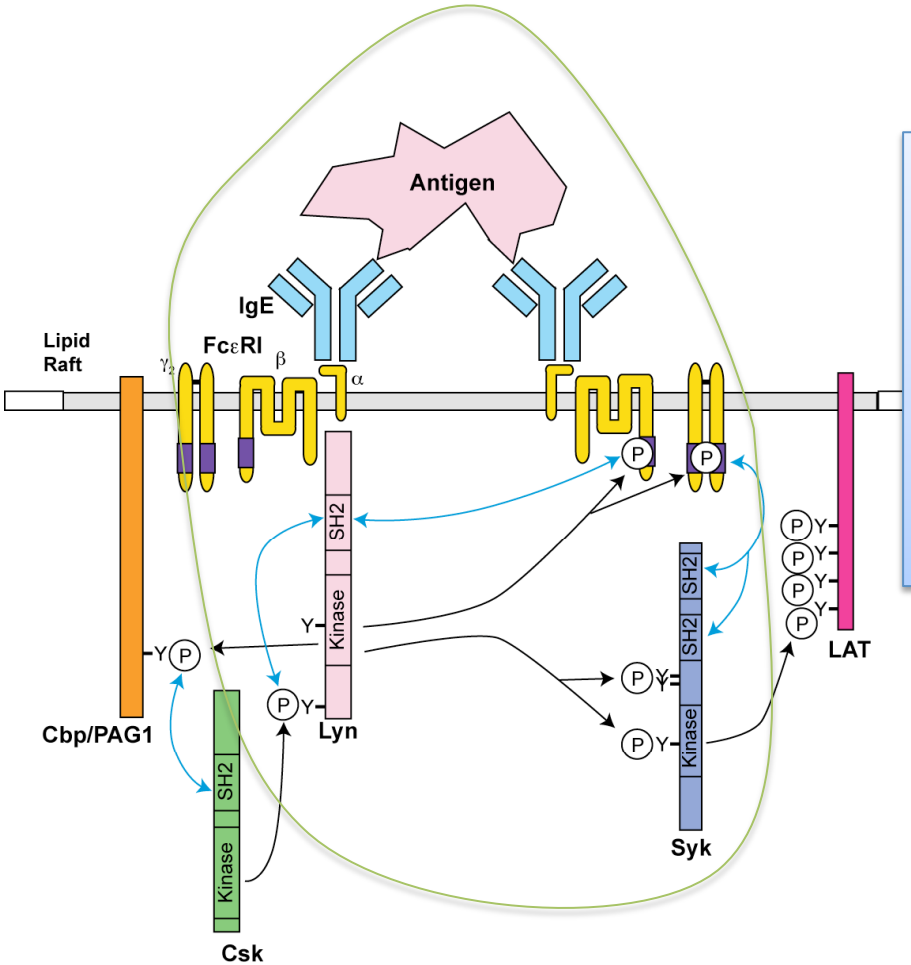



Wedemeyer et al., 2000

# Early events in FcεRI signaling



# Syk activation model



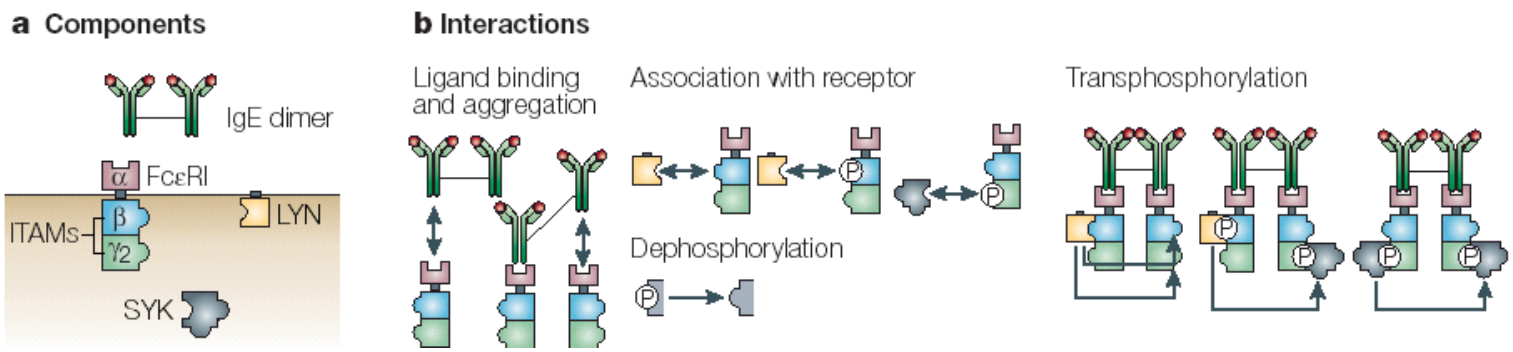

## Key variables

- ligand properties
- protein expression levels
- multiple Lyn-FceRI interactions
- transphosphorylation

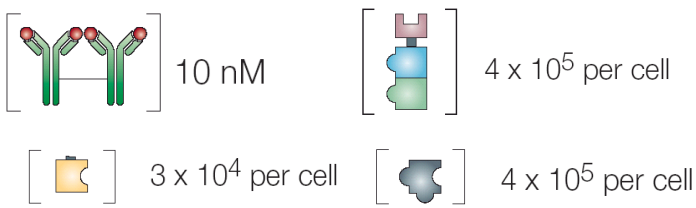
*Mol. Immunol.,*2002
*J. Immunol., 2003*

# Standard modeling protocol

1. Identify components and interactions.




2. Determine **concentrations** and **rate constants**




3. Write and solve model equations.

$$\dot{\mathbf{x}} = \mathbf{S} \cdot \mathbf{v}(\mathbf{x})$$

# Combinatorial complexity

**States of the Model**

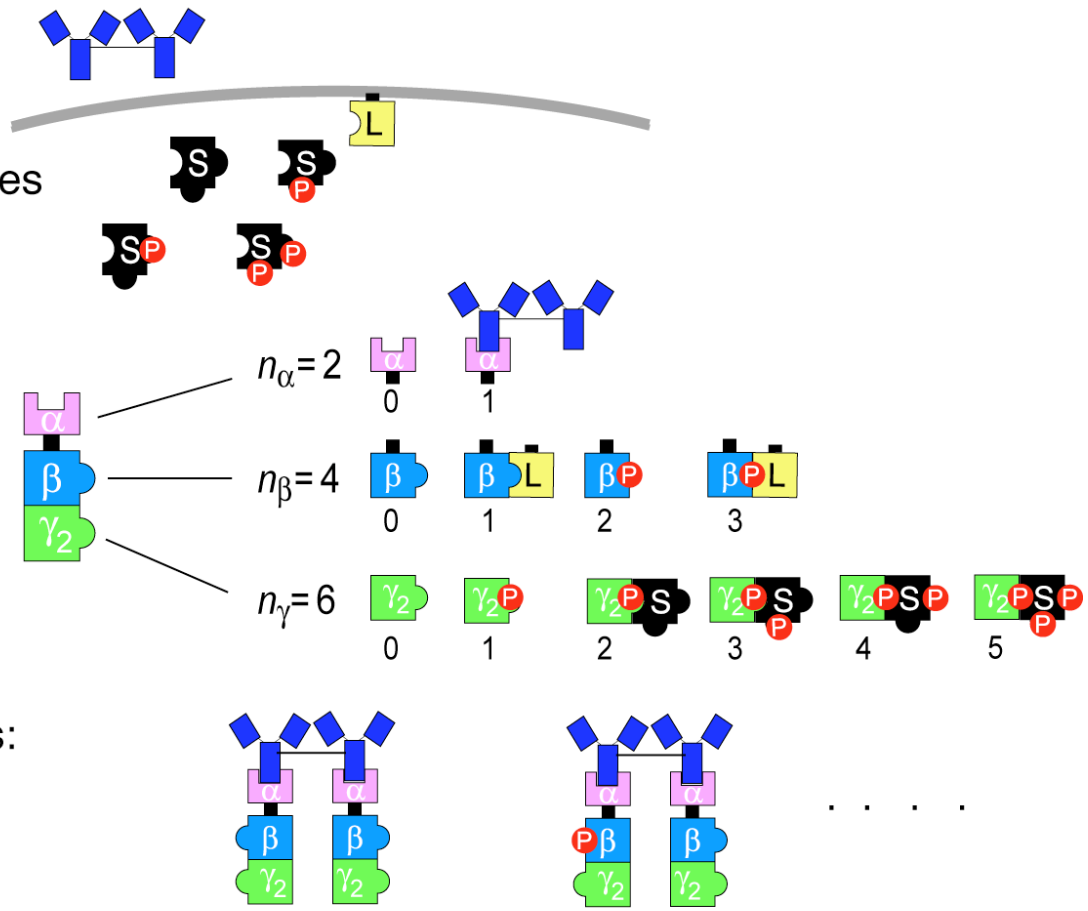
6 free nonreceptor states

48 monomer states:

$n_\alpha n_\beta n_\gamma$

300 dimer states:

$n_\beta n_\gamma (n_\beta n_\gamma + 1)/2$




**The model has 354 states**

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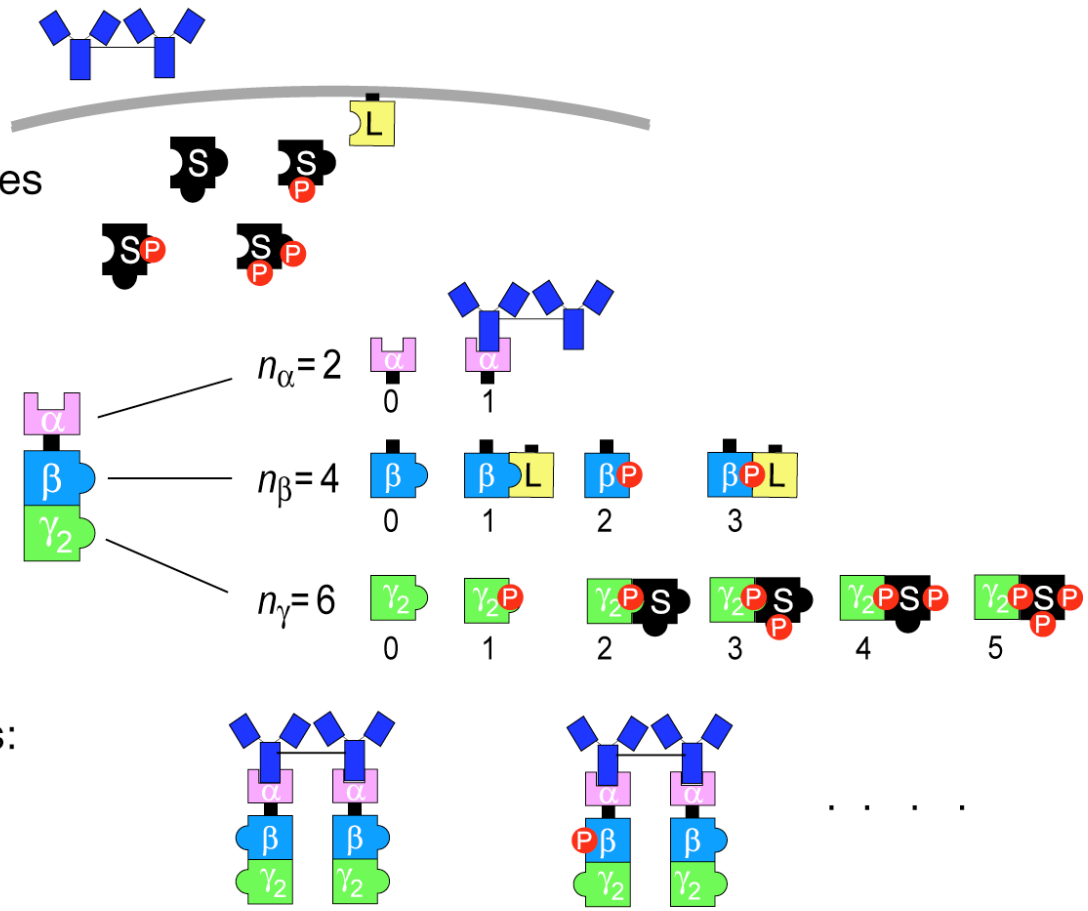
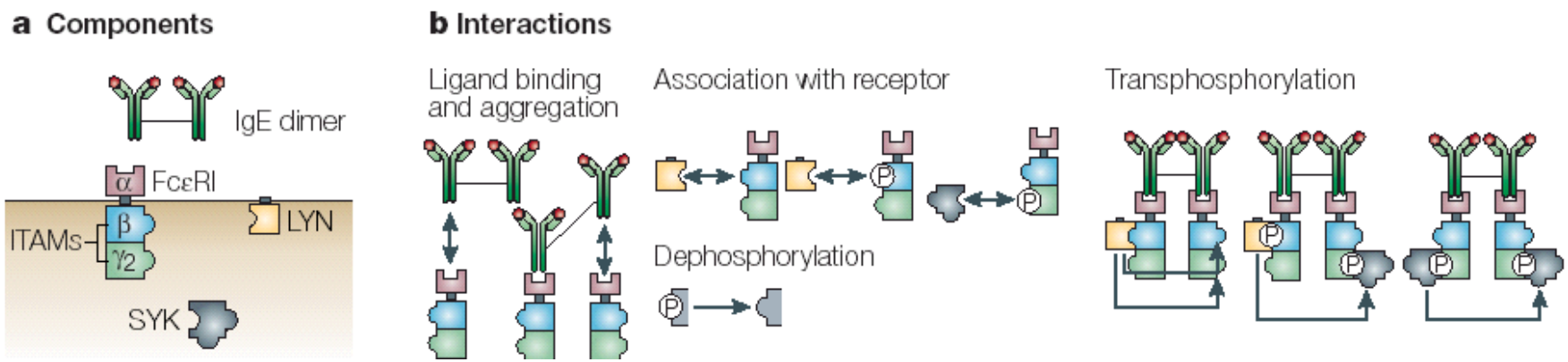



**The model has 354 states (2954 if the ligand was a trimer)**

# Addressing combinatorial complexity

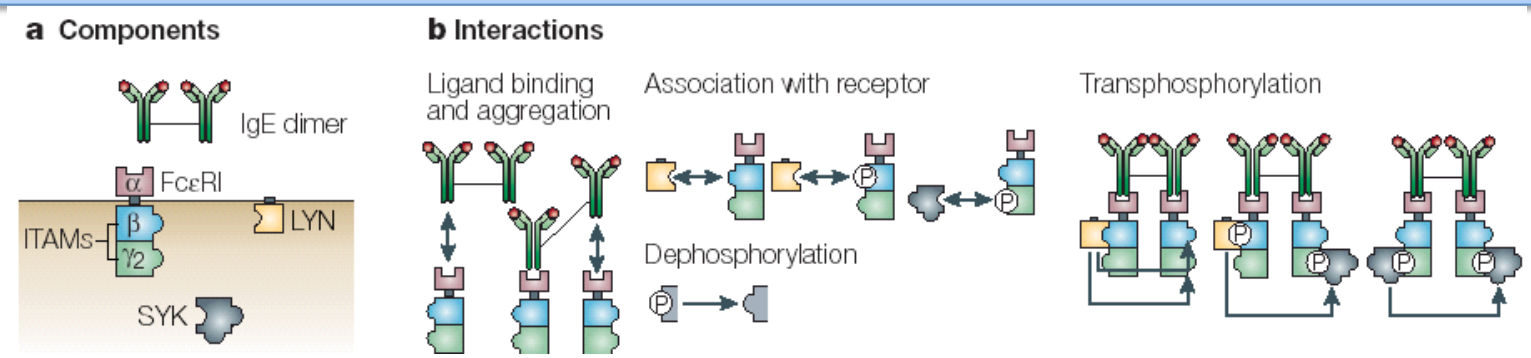



**354 species / 3680 reactions**

- **Standard approach** – writing equations by hand – won't work!
- **New approach**
  - Write model by describing interactions.
  - Automatically generate the equations.

# Rule-based modeling protocol

1. Define components as *structured objects* and interactions as *rules*.

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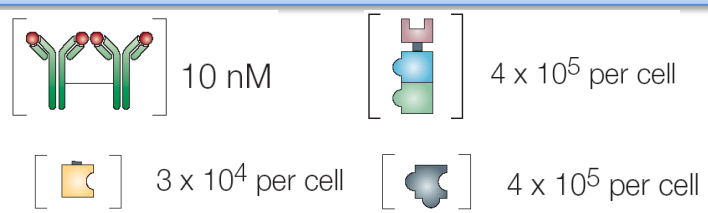
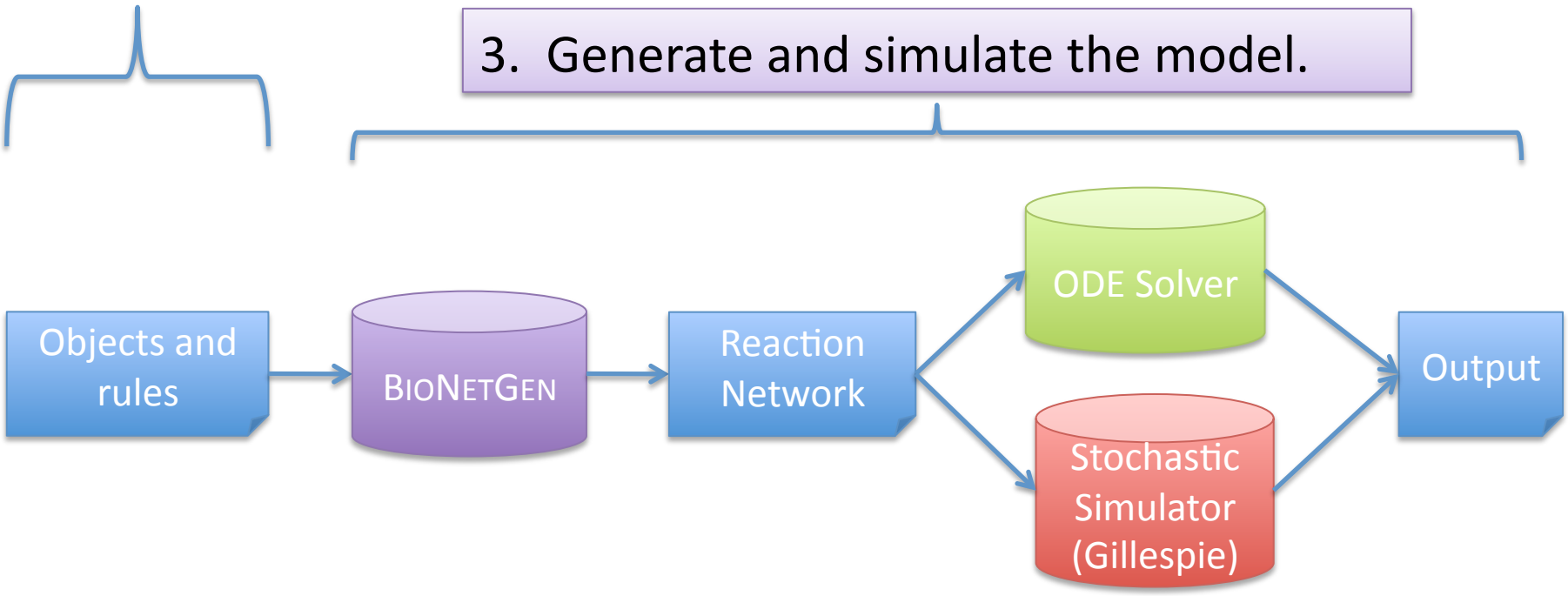



3. Generate and simulate the model.

# Rule-based modeling protocol

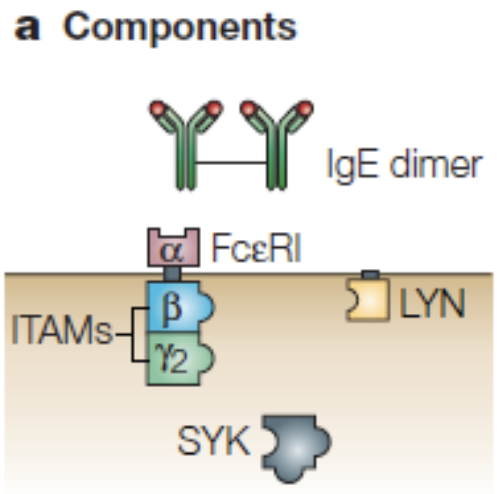
1. Define components as *structured objects* and interactions as *rules*.
2. Determine **concentrations** and **rate constants**
3. Generate and simulate the model.




http://bionetgen.org

Faeder, Blinov, and Hlavacek, *Methods Mol. Biol.* (2009)

# Defining Molecules

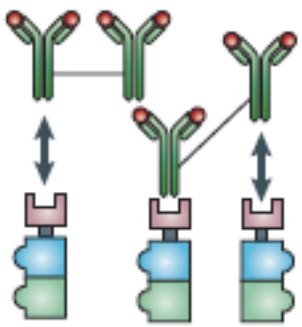



<u>BioNetGen Language</u>

```
IgE(a,a)
FceRI(a,b~U~P,g2~U~P)
Lyn(U,SH2)
Syk(tSH2,lY~U~P,aY~U~P)
```

# Defining Interaction Rules

Ligand binding and aggregation

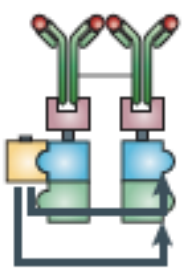


BioNetGen Language

```
IgE(a,a)+ FceRI(a)<-> IgE(a,a!1).FceRI(a!1)
…
```

binding and dissociation

Transphosphorylation



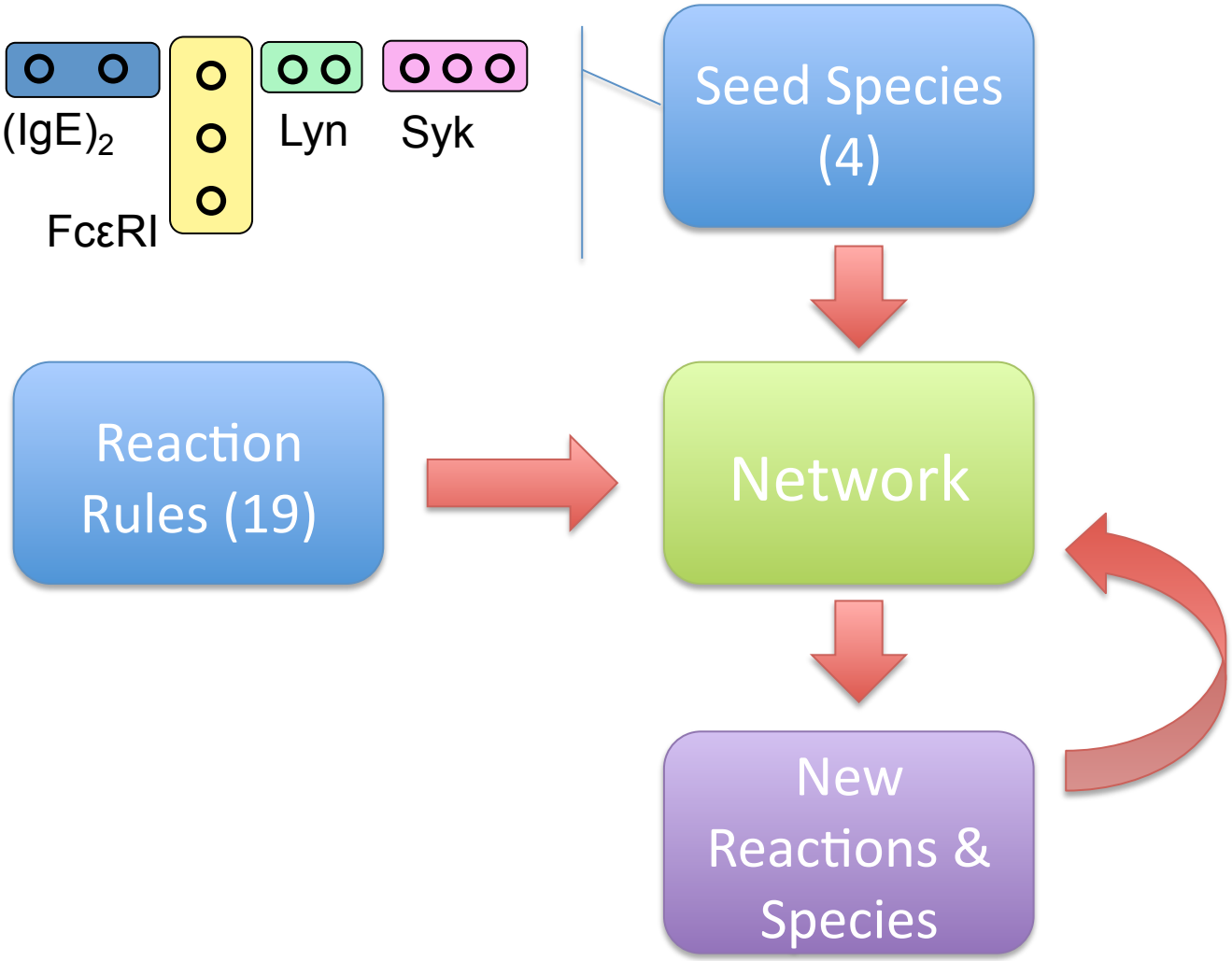
```
Lyn(U!1).FceRI(b!1).FceRI(b~U)-> \
Lyn(U!1).FceRI(b!1).FceRI(b~P)
```

component state change

# Automatic Network Generation

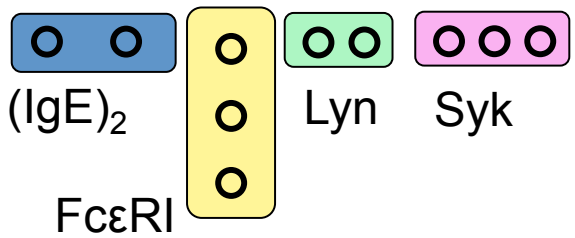
FcεRI Model



# Automatic Network Generation

FcεRI Model



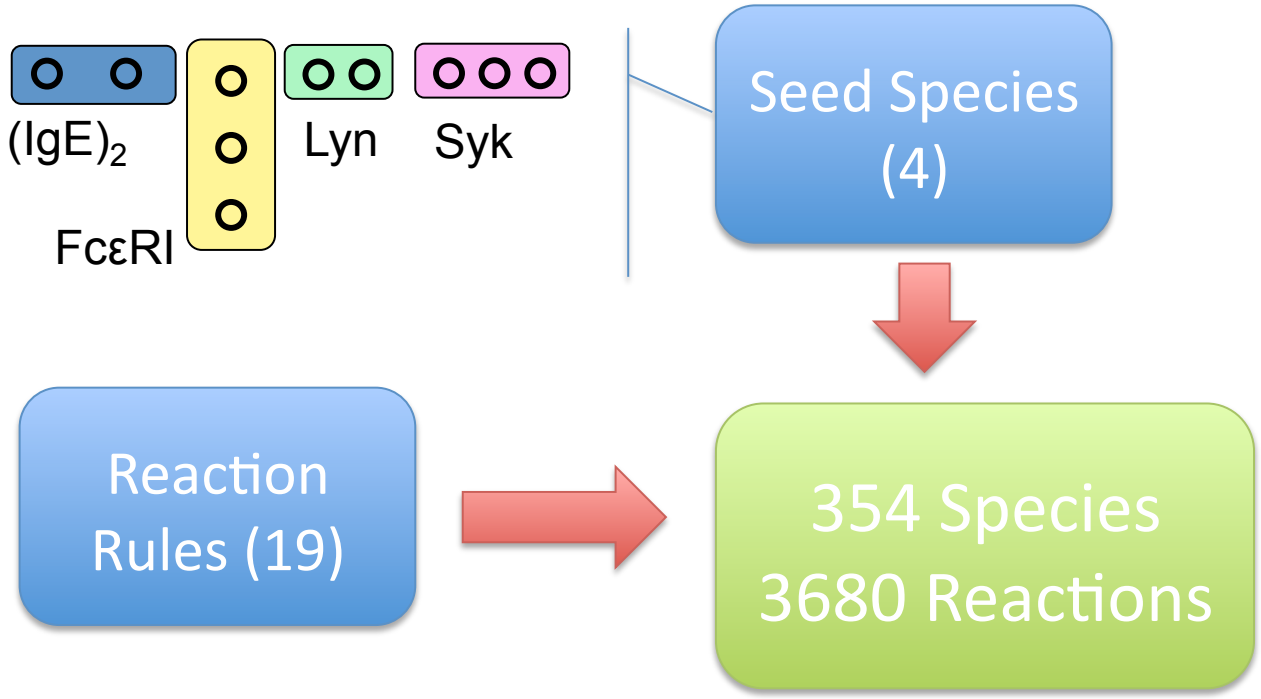

Seed Species (4)

Reaction Rules (19)

354 Species
3680 Reactions

# Automatic Network Generation

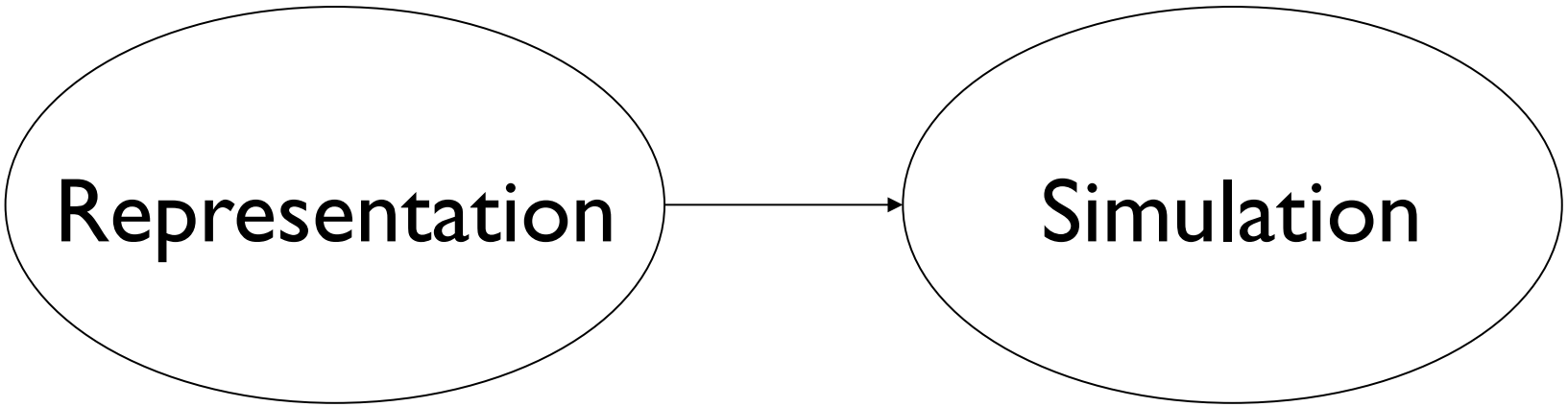
FcεRI Model




$$N_{\text{parameters}} \propto (N_{\text{rules}} + N_{\text{seed species}}) << N_{\text{reactions}}$$

# Modeling cell signaling

**AIM:** Model the biochemical machinery by which cells process information (and respond to it).




BIONETGEN Language
kappa
etc.

ODE, PDE
Stochastic Simulation Algorithm
Kinetic Monte Carlo
Brownian dynamics

# Advantages of Formal Representations

- Precise interaction-based language for biochemistry – knowledge representation
- Concise representation of combinatorially complex systems
- Documentation and model readability
- Modularity and reusability
- Accuracy and rigor

Hlavacek et al. (2006) *Sci. STKE*, **2006**, re6.

# Related Work

- StochSim
- Moleculizer
- Simmune
- κ-calculus / κ-factory
- little b
- Stochastic Simulation Compiler
- meredys
- …

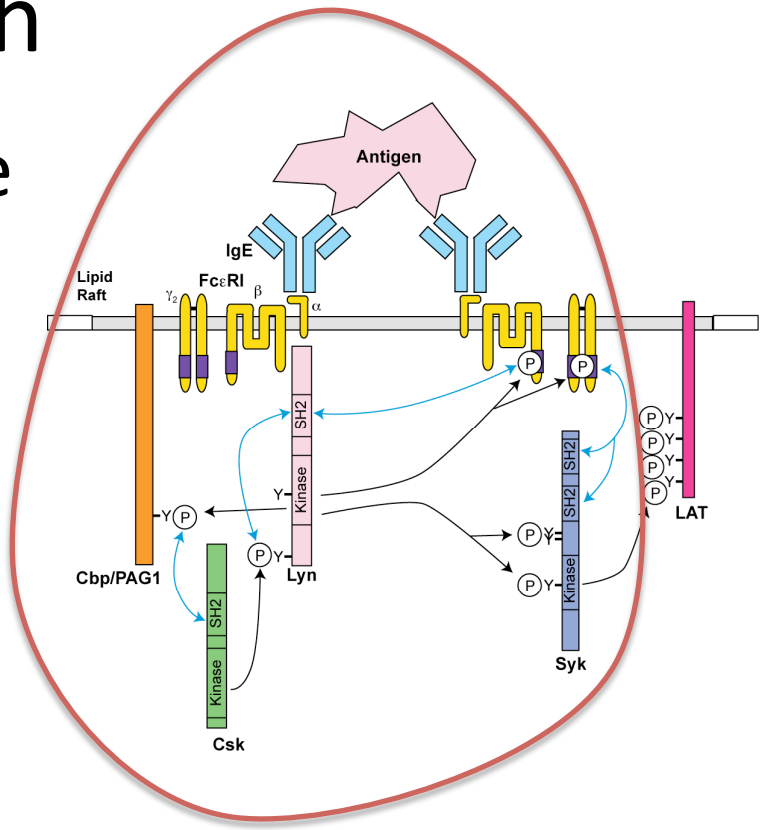
# Systems Modeled

- IgE Receptor (FcεRI)
  - Faeder et al. *J. Immunol.* (2003)
  - Goldstein et al. *Nat. Rev. Immunol.* (2004)
  - Torigoe et al., *J. Immunol.* (2007)
  - Nag et al., *Biophys. J.*, (2009) [LAT]
- Receptor aggregation
  - Yang et al., *Phys. Rev. E* (2008)
- Growth Factor Receptors, other
  - Blinov et al. *Biosyst.* (2006) [EGFR]
  - Barua et al. *Biophys. J.* (2006) [Shp2]
  - Barua et al. *J. Biol. Chem.* (2008) [PI3K]
  - Barua et al., *PLoS Comp. Biol* (2009). [GH / SH2B]
- Carbon Fate Maps
  - Mu et al., *Bioinformatics* (2007)
- TCR (Lipniacki, *J. Theor. Biol.*, 2008)
- TLR4 (An & Faeder, *Math. Biosci.*, 2009)

See http://bionetgen.org for complete list.

# Limits of the network generation approach

- Extending model to include Lyn regulation results in >20,000 states.



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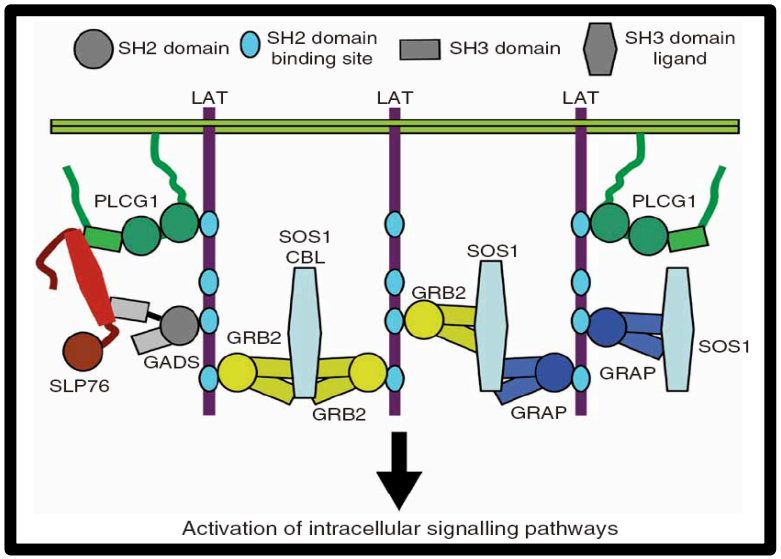
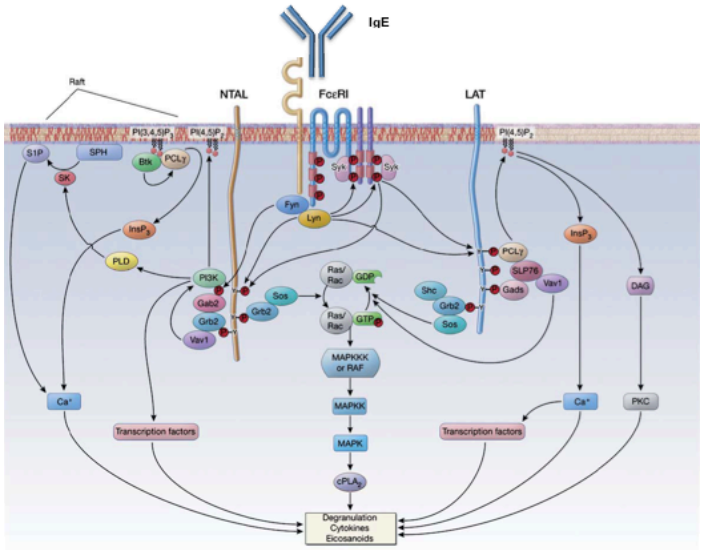
- LAT may form large oligomers under physiological conditions.




Houtman et al., *Nat. Struct. Mol. Biol.* (2006)
Nag et al., *Biophys. J.* (2009)

# Limits of the network generation approach

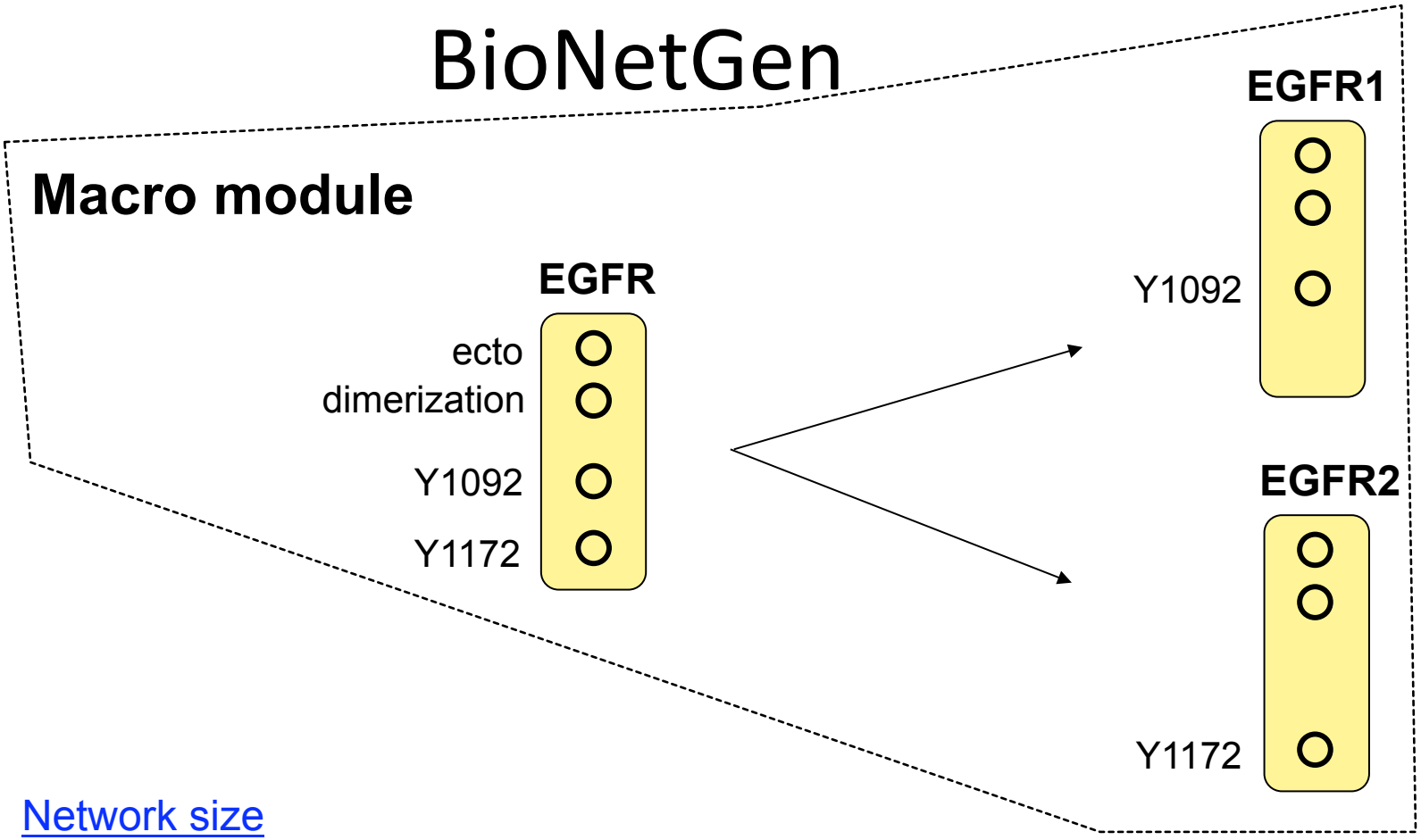
- Extending model to include Lyn regulation results in >20,000 states.
- LAT may form large oligomers under physiological conditions.
- Many more components are still missing. Networks can easily reach “Avogadro limit”



# Handling the combinatorial explosion

- **Model truncation**
  - Arbitrary
  - Loss of accuracy / convergence check
- **Model reduction**
  - Applies when sites act independently (multiplicative ➔additive)
  - Limited by cooperativity (FcεRI model is not reducible)
  - See Feret et al. *PNAS* (2009); Conzelmann et al. *BMC Syst. Biol.* (2008); Borisov et al., *IET Syst. Biol.* (2008)

# Automated model reduction in BioNetGen



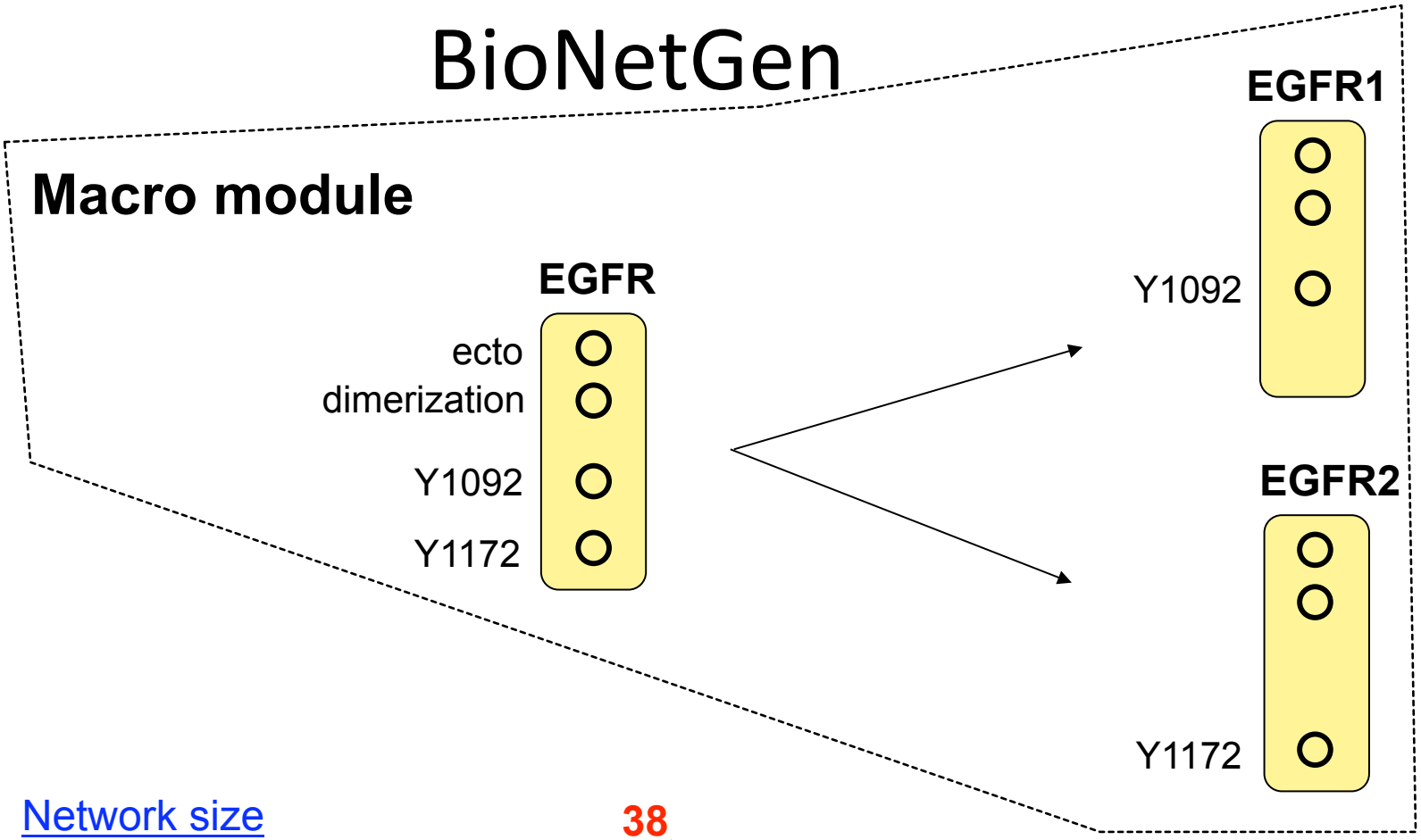

Network size

**356** species ➔ **59** species

**3749** reactions ➔ **277** reactions

Borisov et al., *IET Syst. Biol.* (2008)

# Automated model reduction in BioNetGen



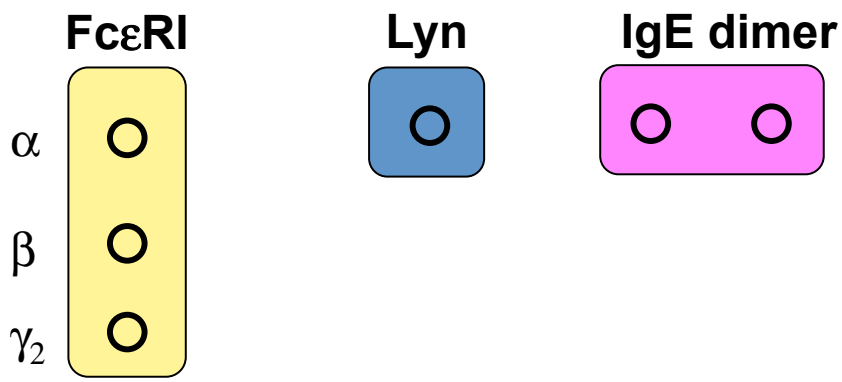

Network size

**356** species ➔ ~~**59**~~ **38** species

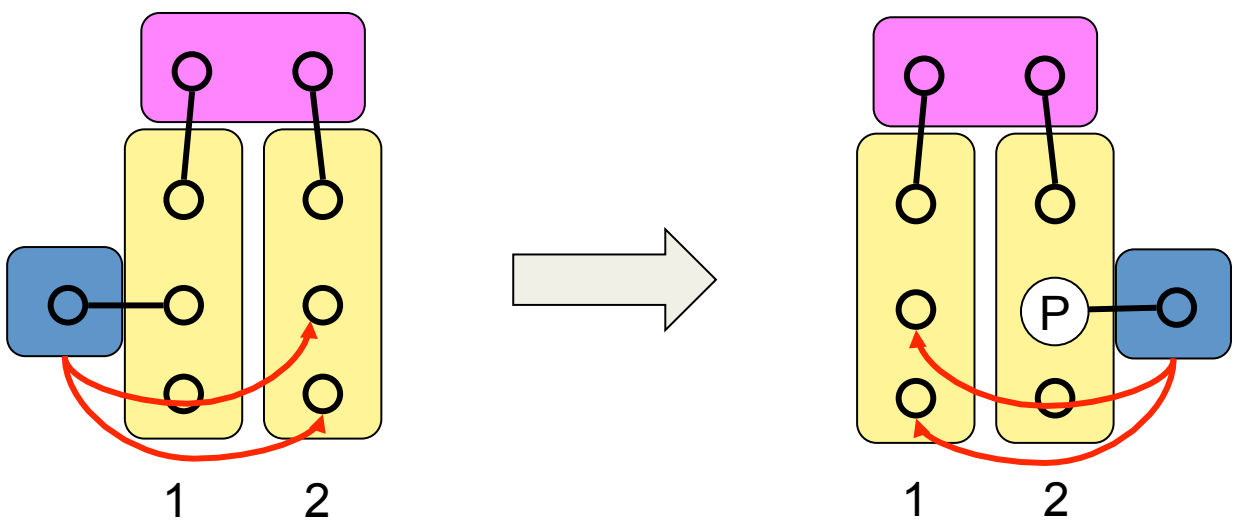
**3749** reactions ➔ ~~**277**~~ **123** reactions

Conzlemann, Faeder, and Hogg, unpublished.

# Cooperativity leads to irreducibility

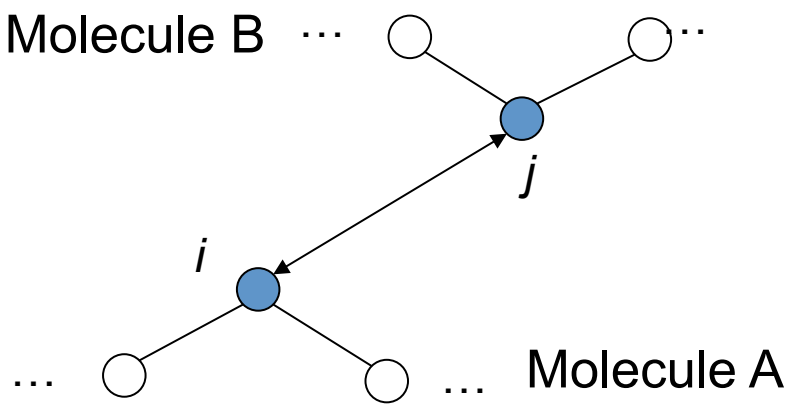



**Transphosphorylation by Lyn couples sites**



# Interaction-based formulation of chemical kinetics

Molecular Dynamics (MD)




Dynamics depend on force between *i* and *j* (Newton)

# Interaction-based formulation of chemical kinetics

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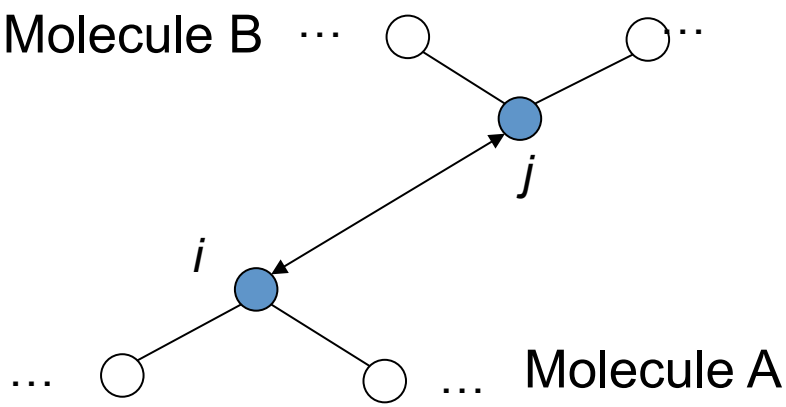


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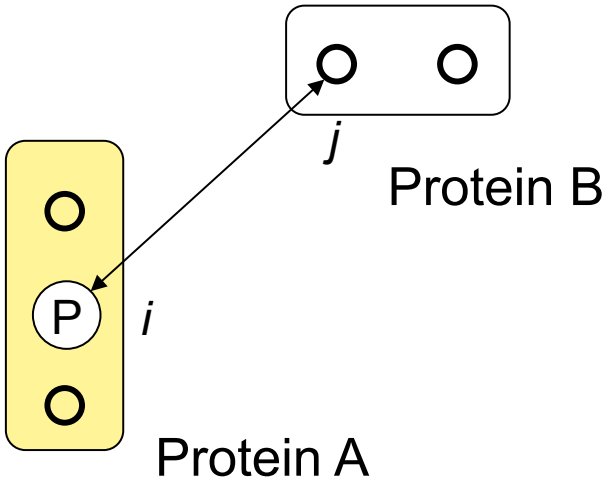
Chemical Kinetics




Dynamics depend on reaction propensity between *i* and *j* (Gillespie)

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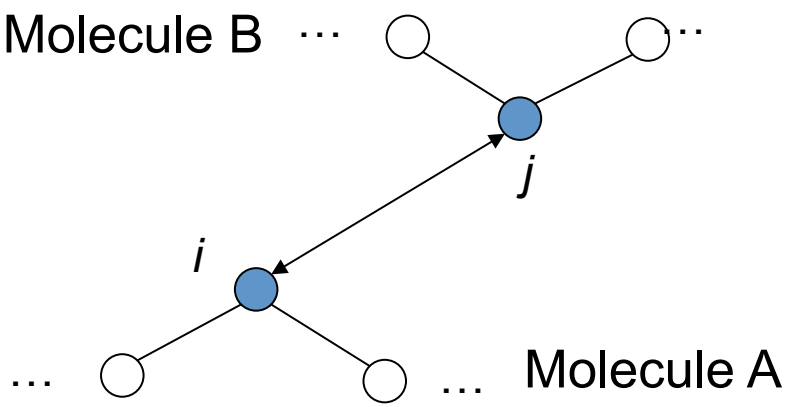


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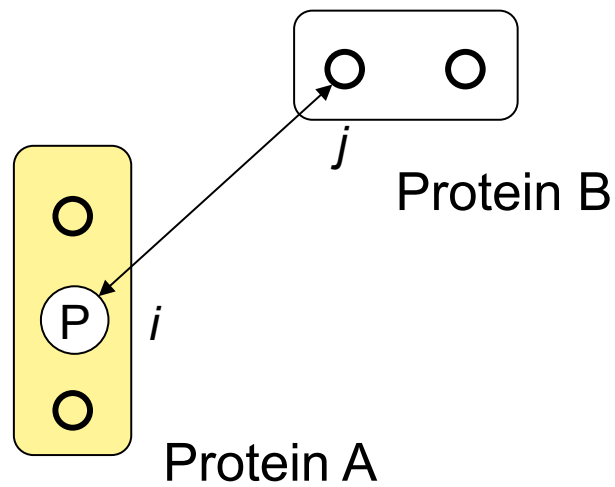


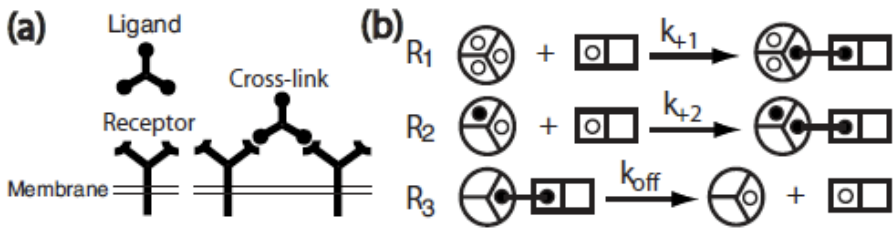

Dynamics depend on reaction propensity between *i* and *j* (Gillespie)

**Key point**: Dynamics depend on time-varying quantities that may be computed on-the-fly. *Don't have to enumerate possibilities in advance.*

PHYSICAL REVIEW E **78**, 031910 (2008)

# Kinetic Monte Carlo method for rule-based modeling of biochemical networks

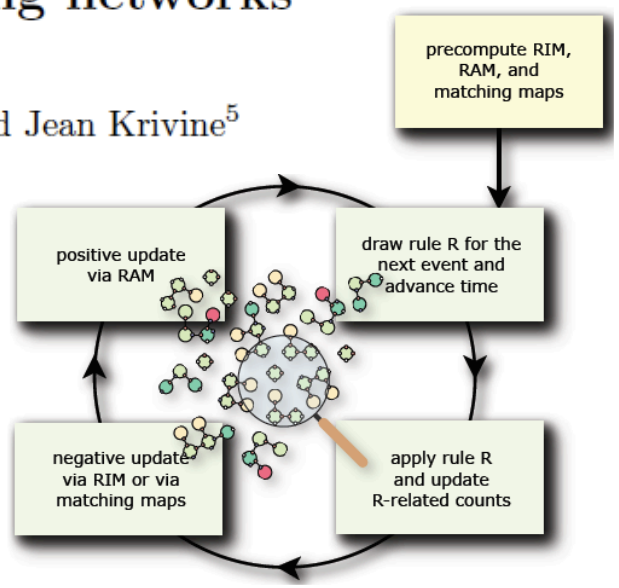
Jin Yang,[1,*] Michael I. Monine,[2] James R. Faeder,[3,†] and William S. Hlavacek[2,‡]




# Scalable simulation of cellular signaling networks

Vincent Danos[1,4⋆], Jérôme Feret[3], Walter Fontana[1,2], and Jean Krivine[5]

*APLAS '07 (invited paper)*



# NFsim

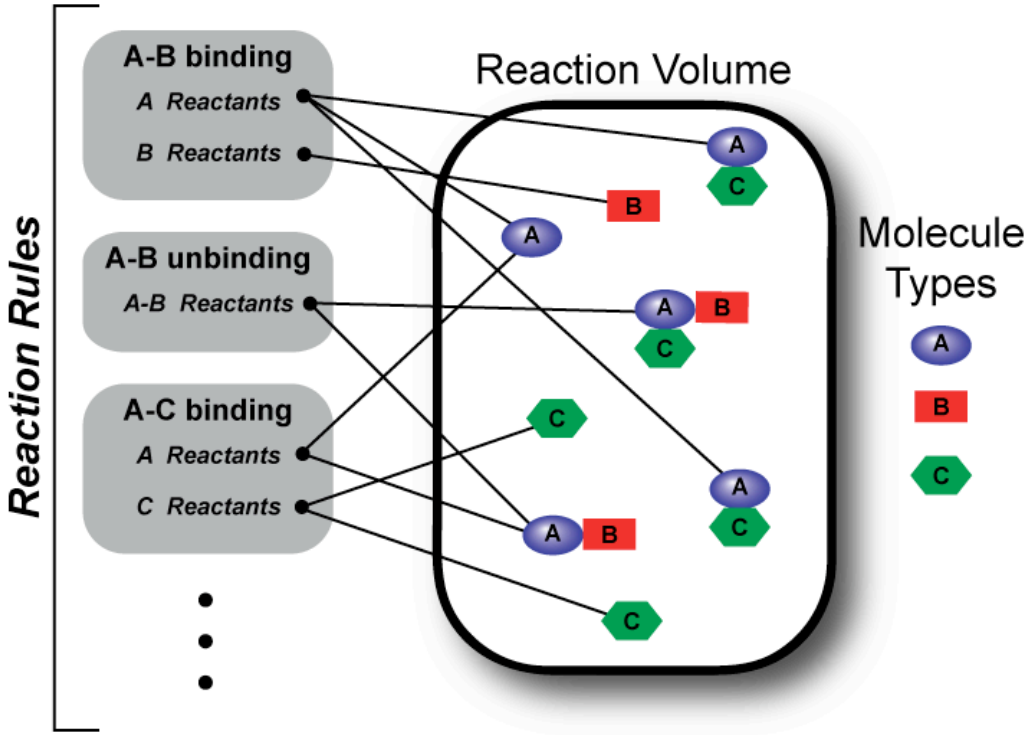
## The Network-Free Stochastic Simulator

Michael Sneddon
Thierry Emonet
*Yale University*

1) Treat molecules as Agents that can be connected together.

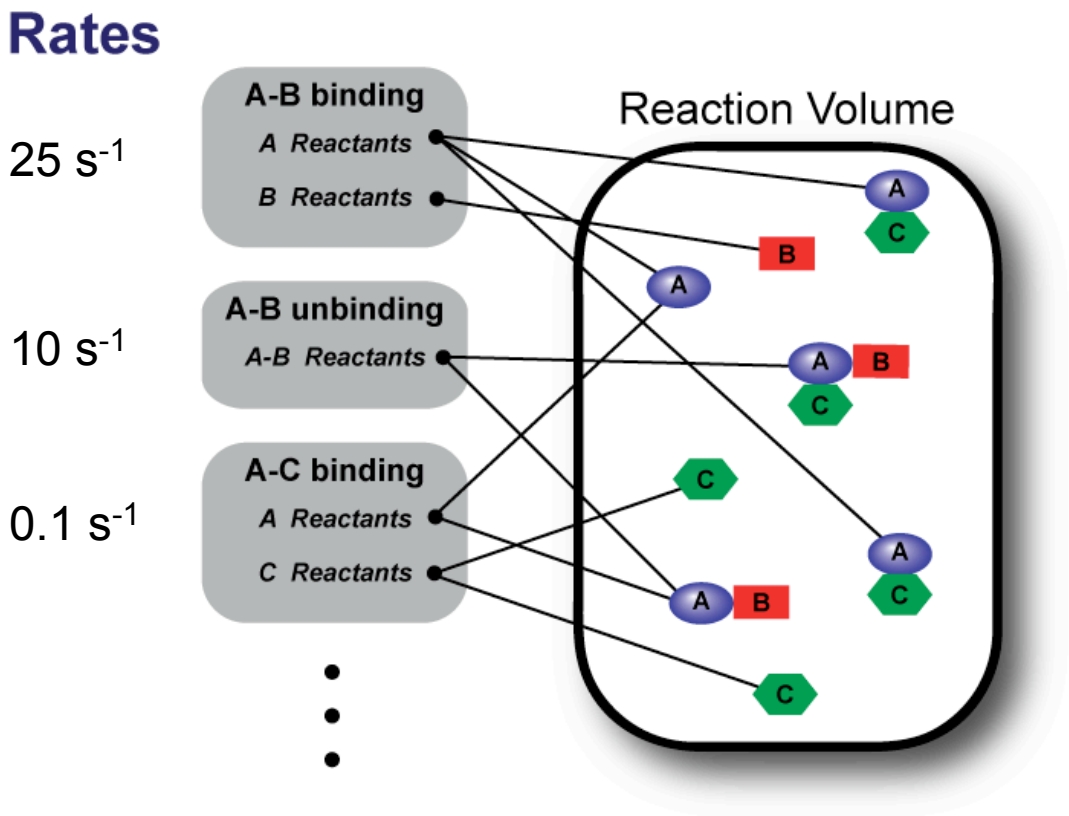
2) Use reaction rules to define interactions.

3) Simulate with an Agent-based extension to the Gillespie Algorithm



# The Event Scheduler

## An Agent-based Extension to the Gillespie Algorithm




Pointers to possible reactants stored on Rule-Reactant lists.

Sneddon, Faeder, and Emonet, in preparation.

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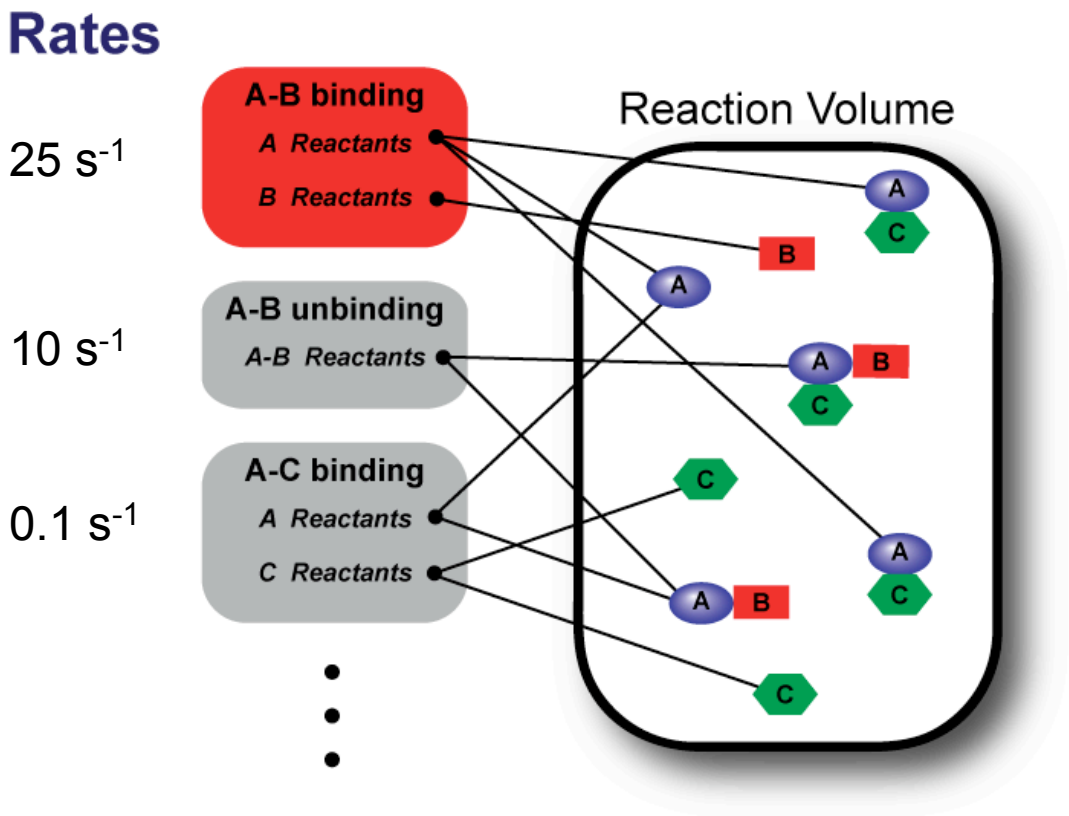


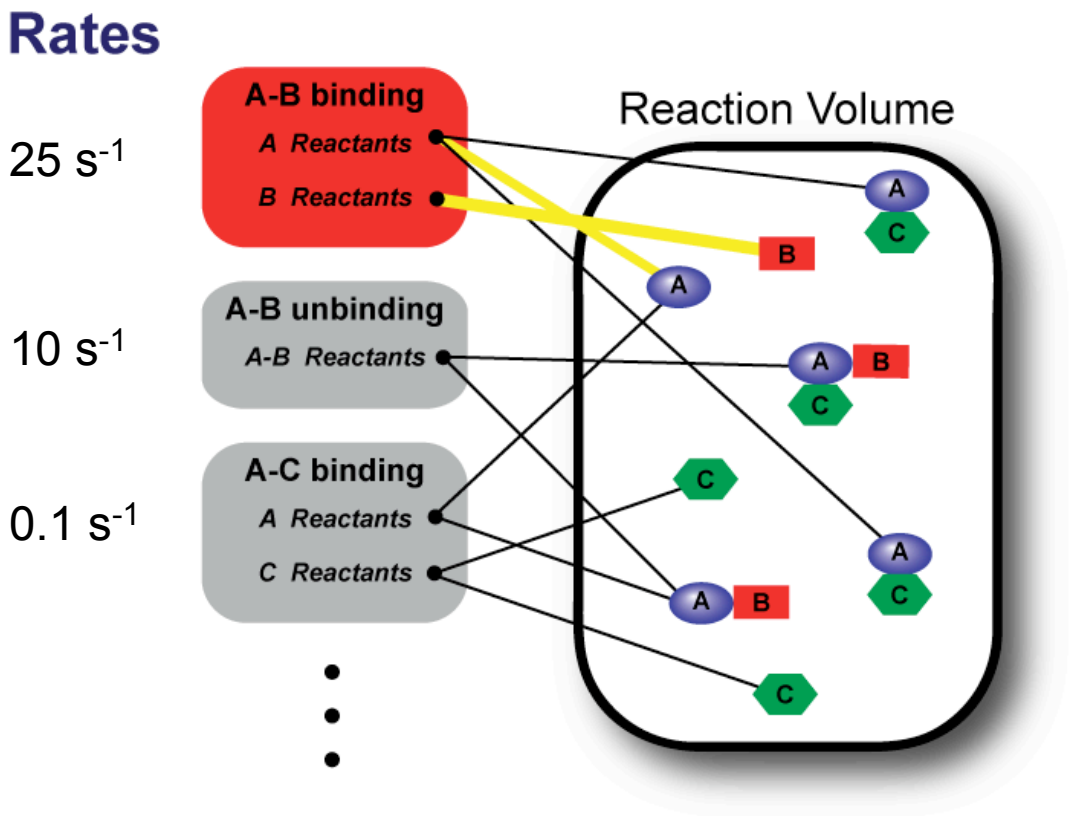

As in Gillespie, the waiting time to the next event is sampled.

Here, the next RULE is chosen stochastically (not the next reaction).

Sneddon, Faeder, and Emonet, in preparation.

# The Event Scheduler

## An Agent-based Extension to the Gillespie Algorithm




The molecule Agents that will react are randomly selected.

Sneddon, Faeder, and Emonet, in preparation.

# The Event Scheduler

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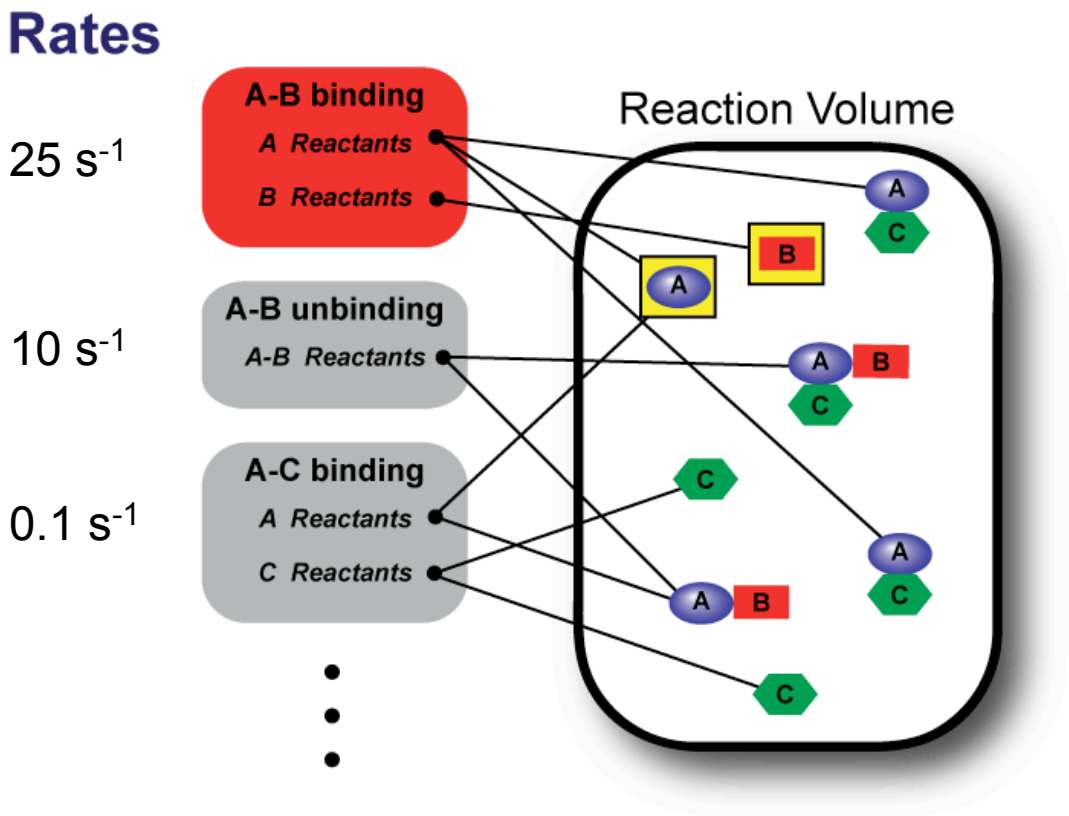


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Sneddon, Faeder, and Emonet, in preparation.

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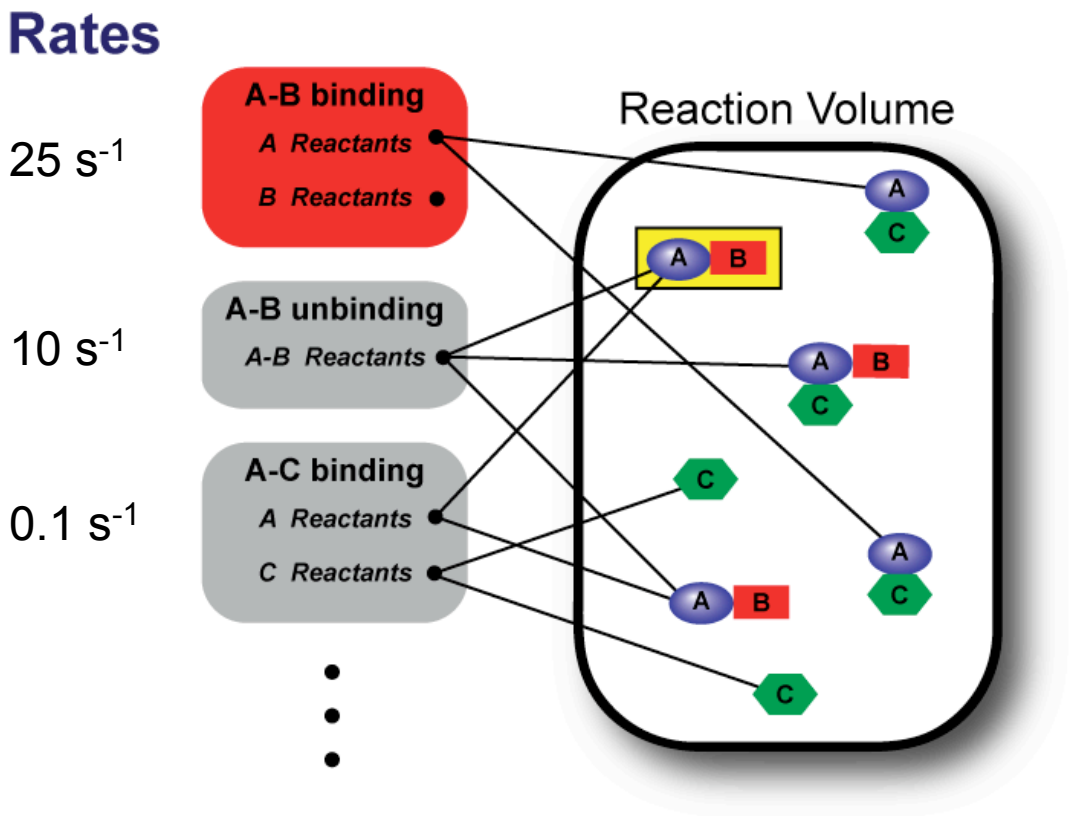
## An Agent-based Extension to the Gillespie Algorithm

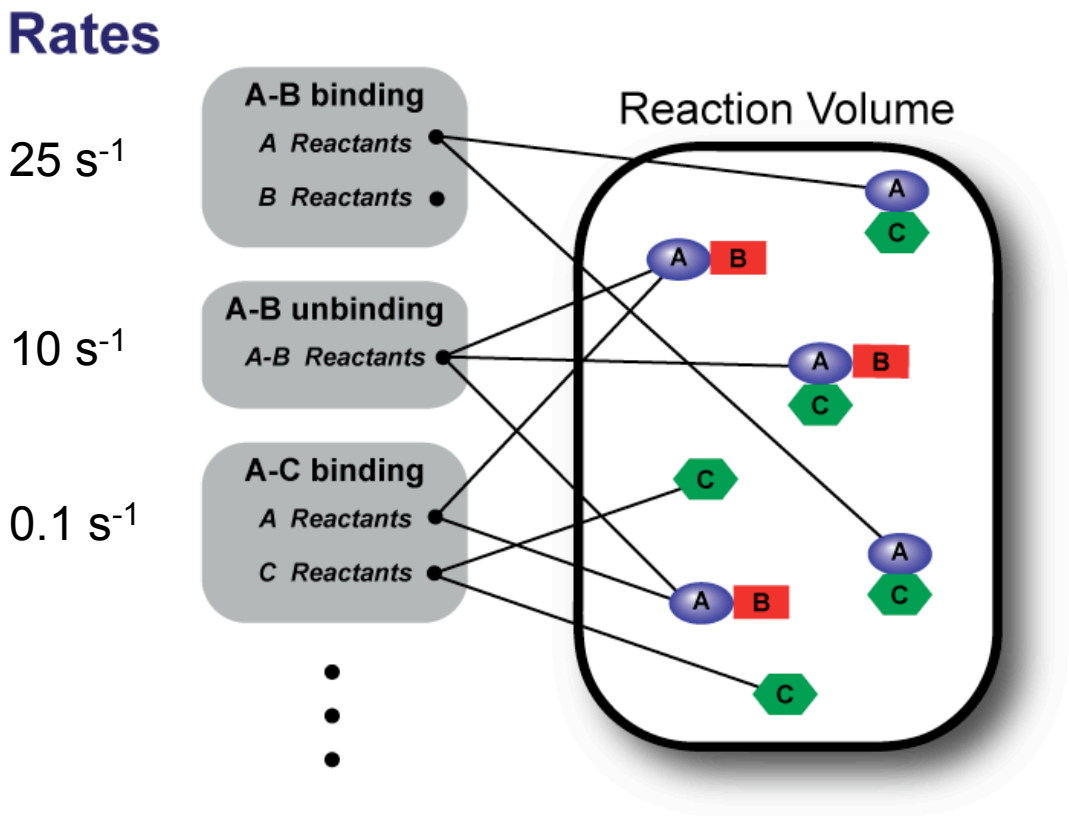



The molecule Agents update themselves and "reschedule" themselves by updating their Rule-Reactant Pointers.

Sneddon, Faeder, and Emonet, in preparation.

# The Event Scheduler

## An Agent-based Extension to the Gillespie Algorithm




The system is advanced by the sampled time.

Sneddon, Faeder, and Emonet, in preparation.

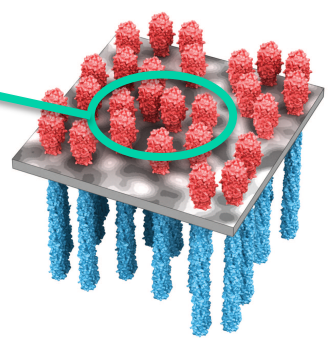
# NFsim Core Simulator Features

1) Modular C++ code base and highly efficient implementation

2) Operates seamlessly with BIONETGEN

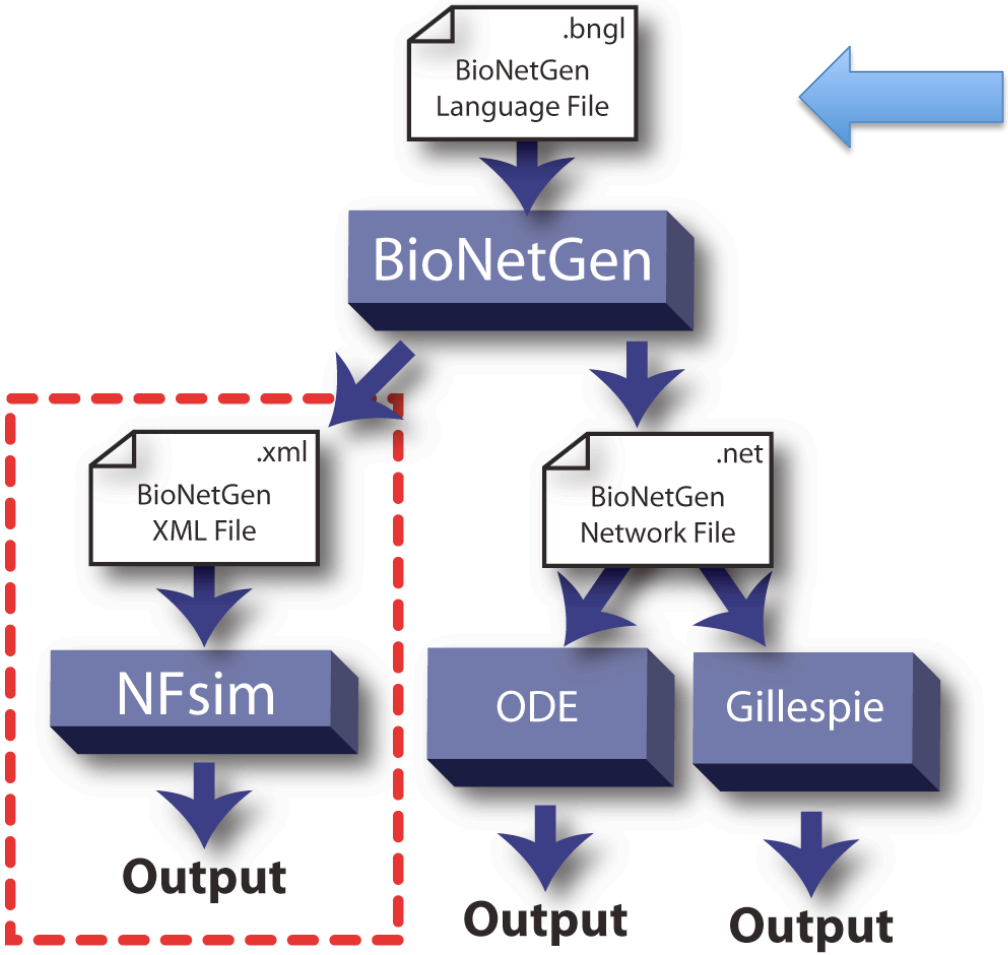
3) Extended BioNetGen Language handles
   1) Spatial compartments
   2) System variables in rate law expressions

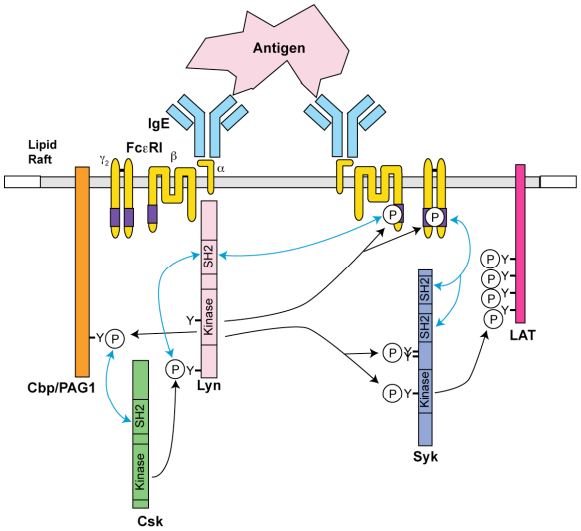
cooperative receptor interactions



```
MethLevel(x) = 1*R1(x)+2*R2(x)+
     3*R3(x)+4*R4(x)+5*R5(x)+6*R6(x)+
     7*R7(x)+8*R8(x)
```

# Integration with BioNetGen



# DYNSTOC: An Alternative Network-Free Approach Based on Null Event Sampling

*Systems biology*

**Bioinformatics**

**Simulation of large-scale rule-based models**

Joshua Colvin[1], Michael I. Monine[2], James R. Faeder[3], William S. Hlavacek[2,4], Daniel D. Von Hoff[5], and Richard G. Posner[1,6*]

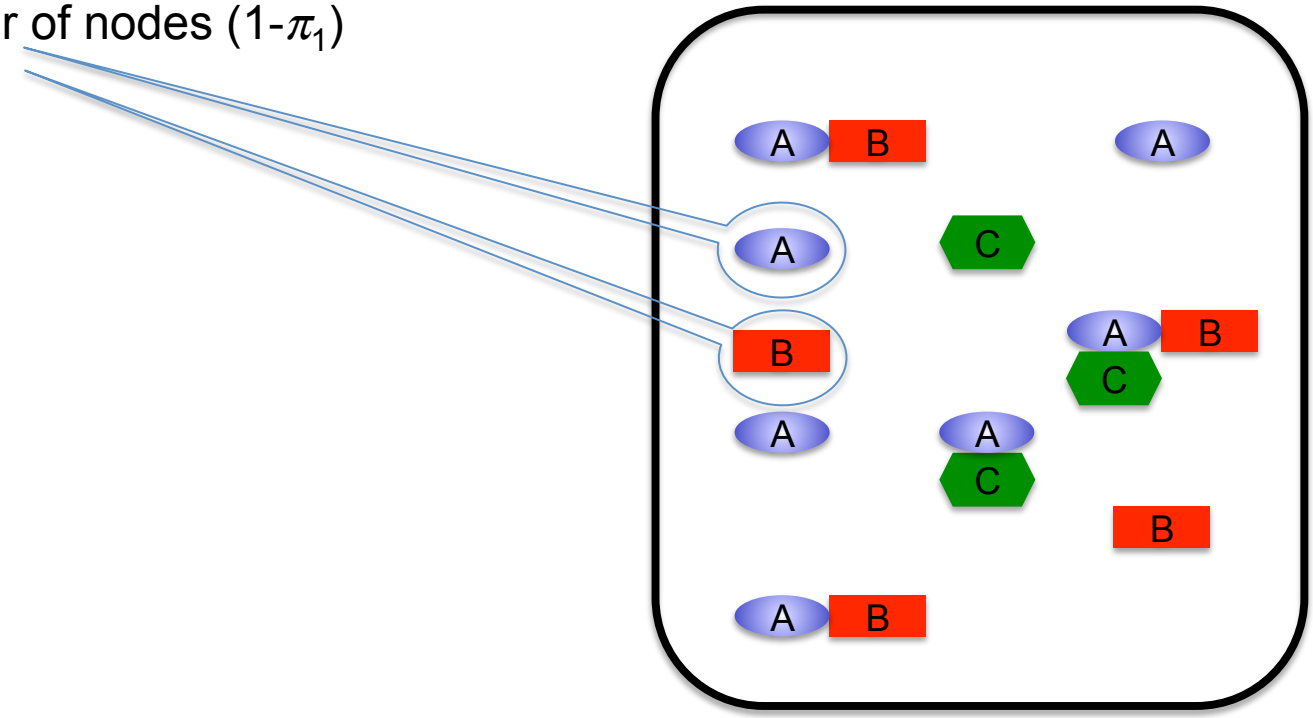
**NFsim:** Event-driven, slower updates

**StochSim:** Fixed time step, fast updates

(Morton-Firth and Bray, 1998)

# DYNSTOC Algorithm

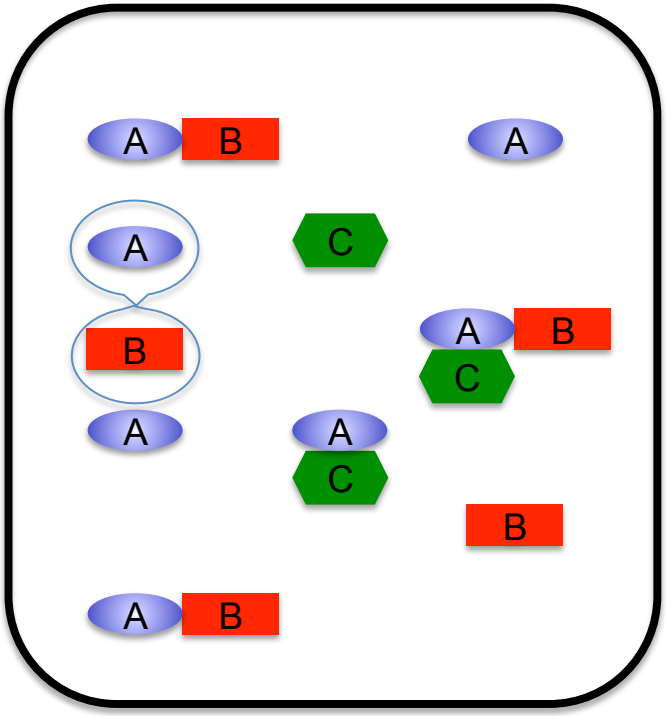
1. Pick a node ($\pi_1$) or pair of nodes (1-$\pi_1$)



# DYNSTOC Algorithm

1. Pick a node ($\pi_1$) or pair of nodes ($1-\pi_1$)

2. Compute reaction probabilities ($p_i$) for $\Delta t$.



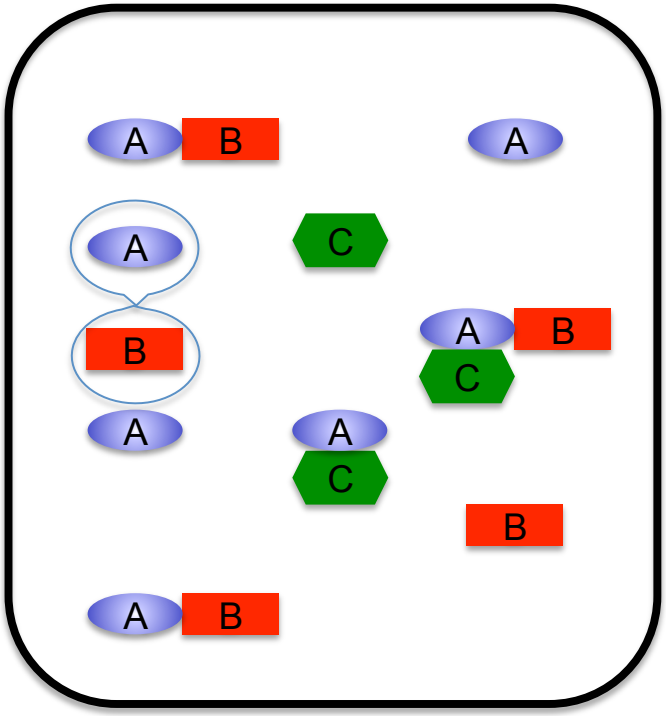
# DYNSTOC Algorithm

1. Pick a node ($\pi_1$) or pair of nodes (1-$\pi_1$)

2. Compute reaction probabilities ($p_i$) for $\Delta t$.

3. Determine if a reaction occurs.

$$\text{smallest } R \text{ s.t. } \sum_{i=1}^{R} p_i \geq \rho \in (0,1)$$



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1. Pick a node ($\pi_1$) or pair of nodes ($1-\pi_1$)

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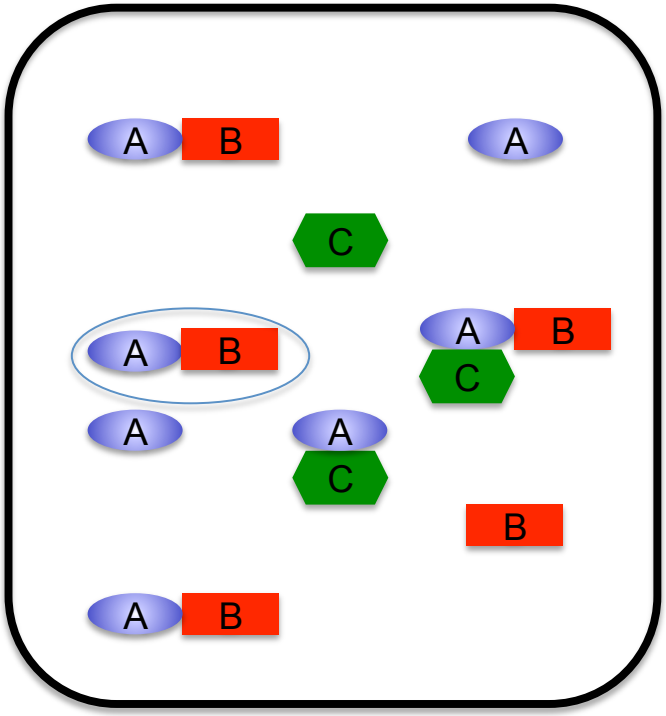
4. If yes, fire reaction; otherwise, *null* event.



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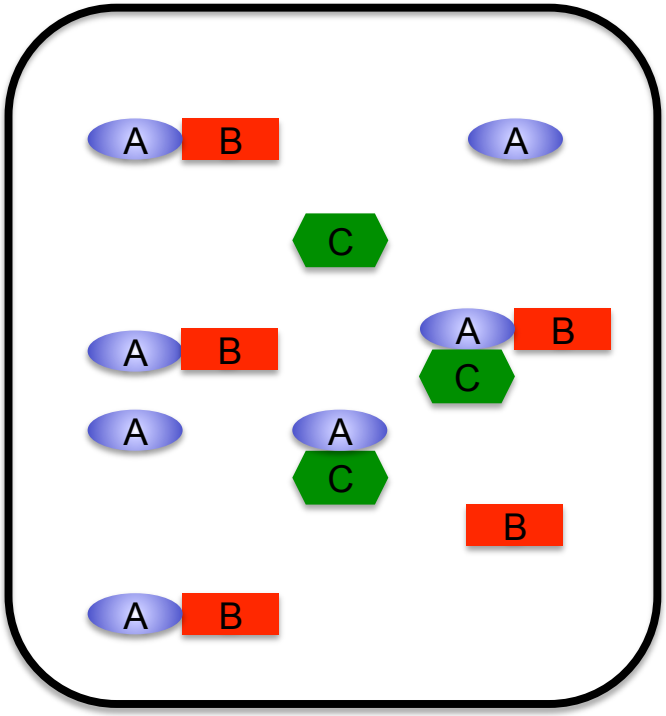
3. Determine if a reaction occurs.

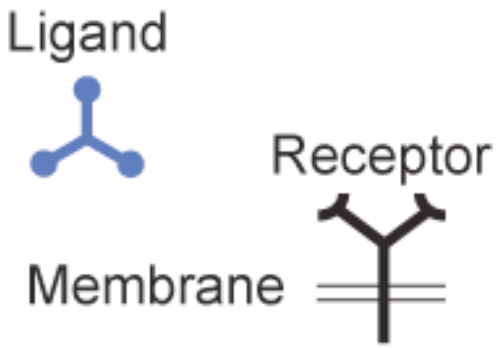
4. If yes, fire reaction; otherwise, *null* event.

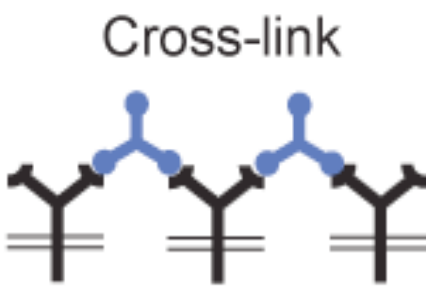
5. Increment time:
   $t = t + \Delta t$



# Trivalent Ligand Bivalent Receptor (TLBR)

**Goldstein and Perelson (1984)**



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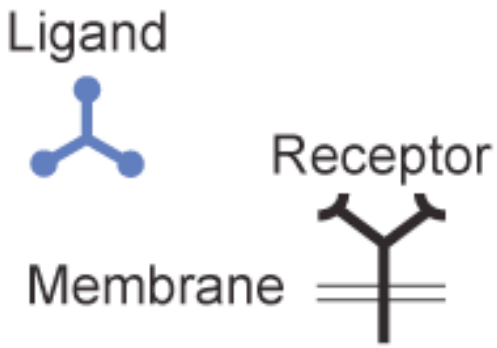
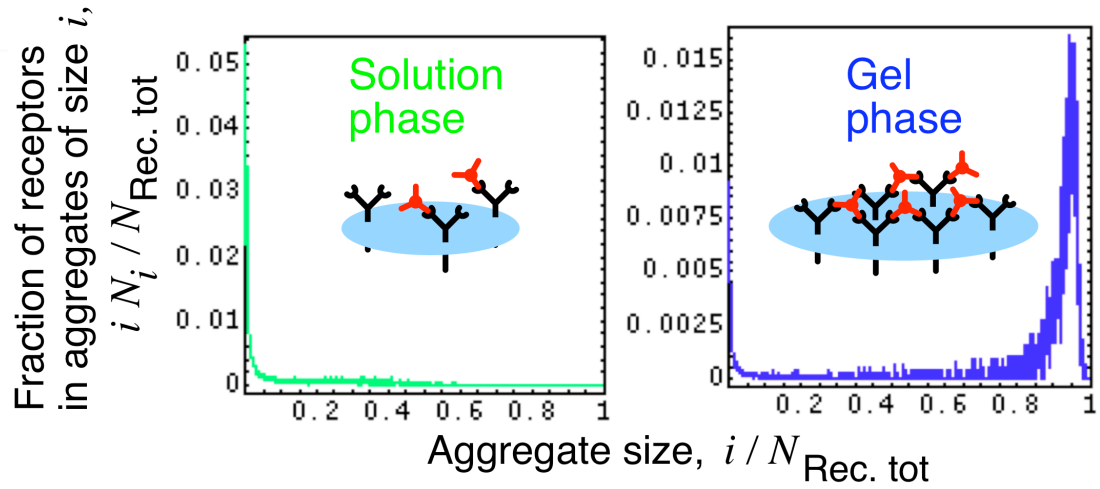
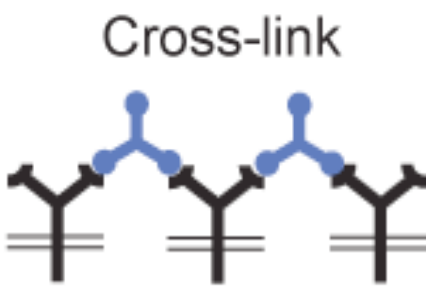
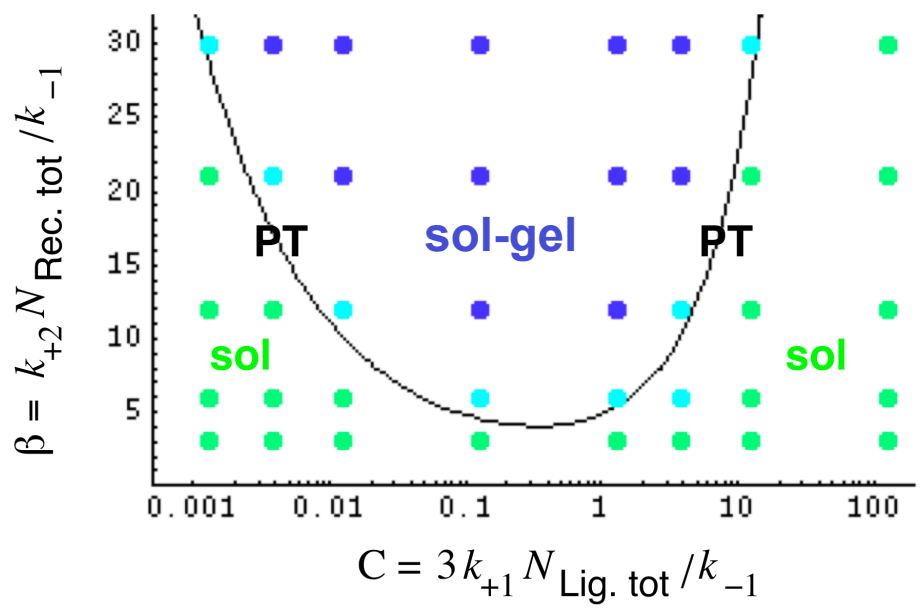


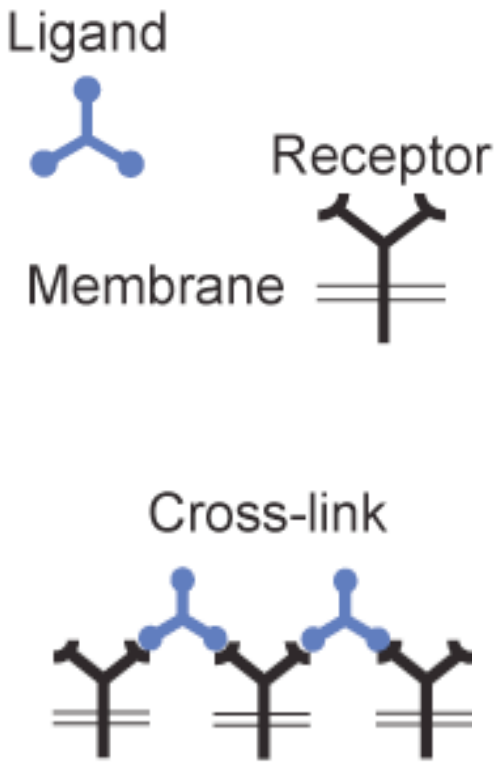



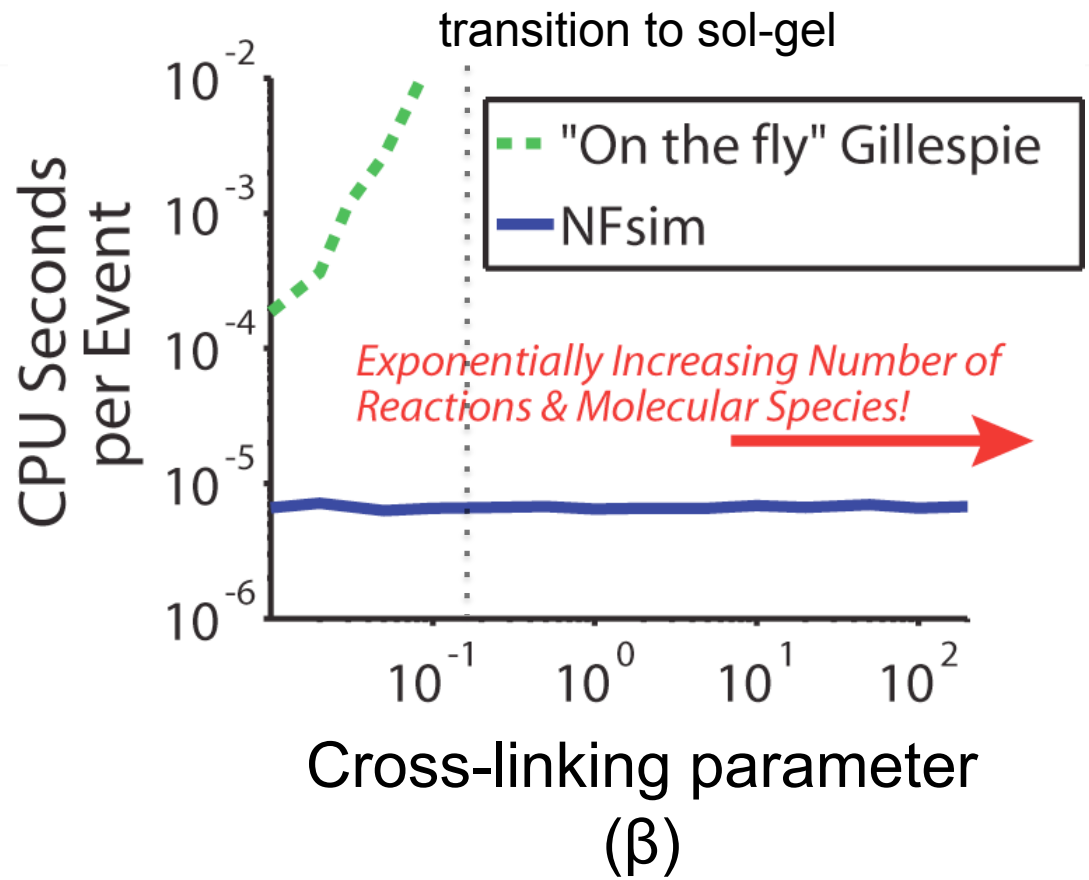



# NFsim's Performance

**Trivalent Ligand, Bivalent Receptor (TLBR) System**




3000 Receptors, 10,000 Ligands

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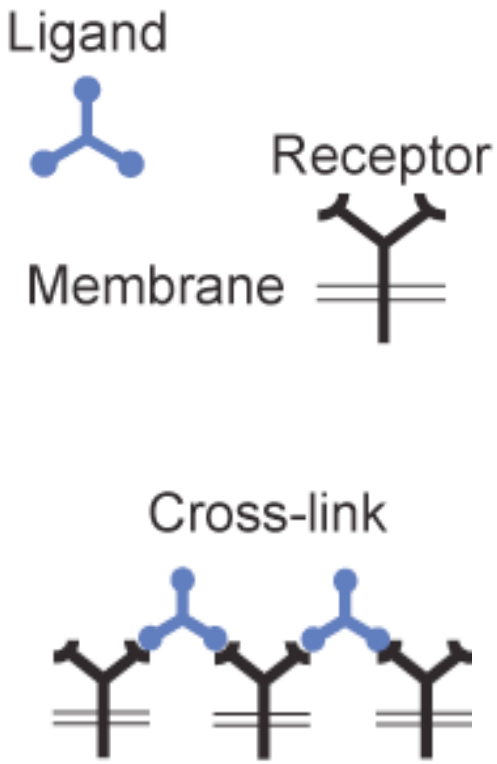
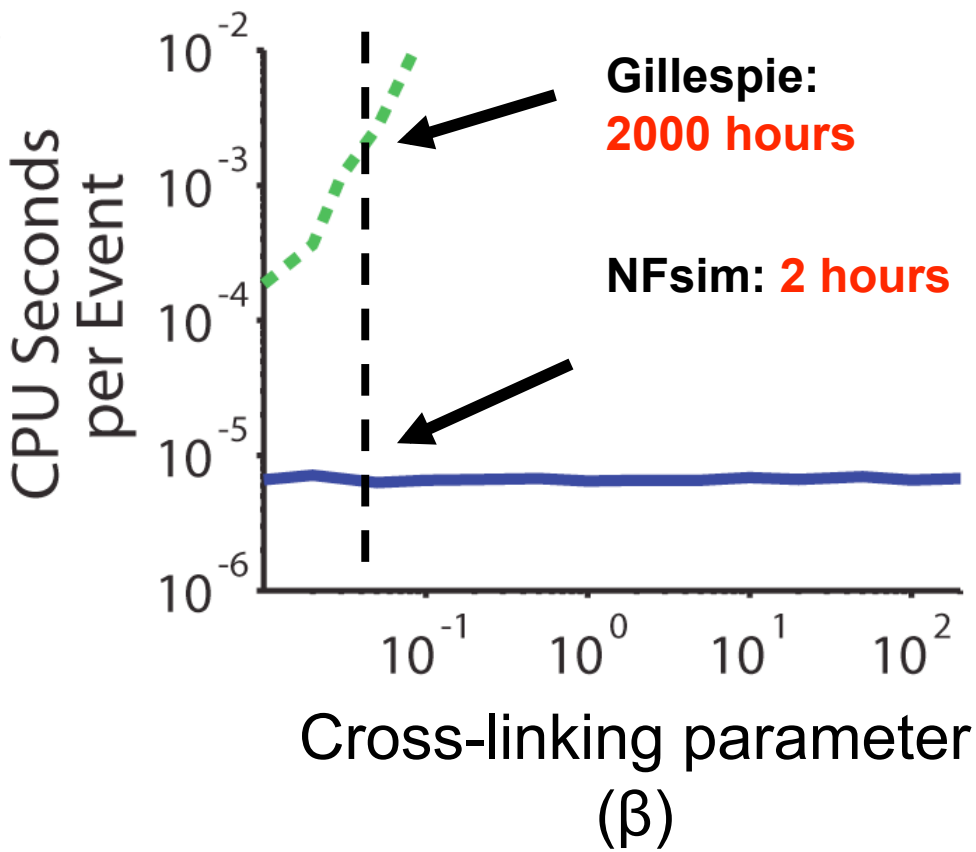
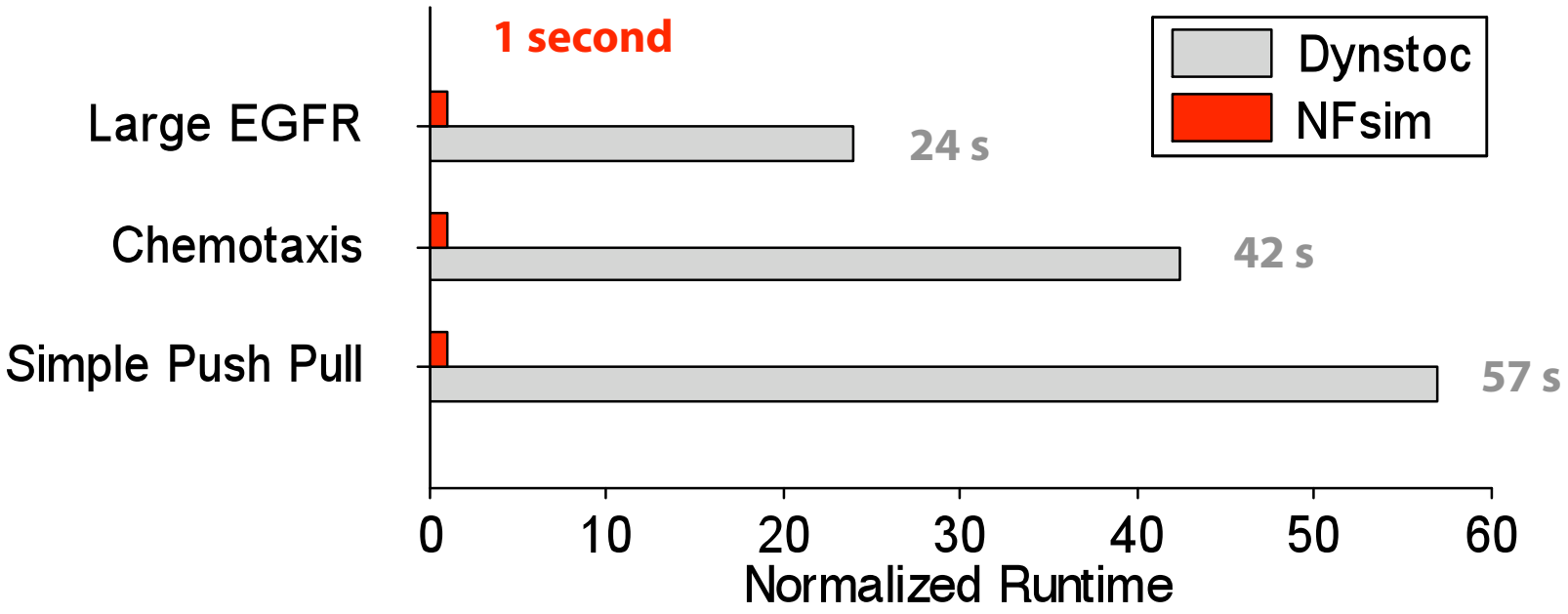


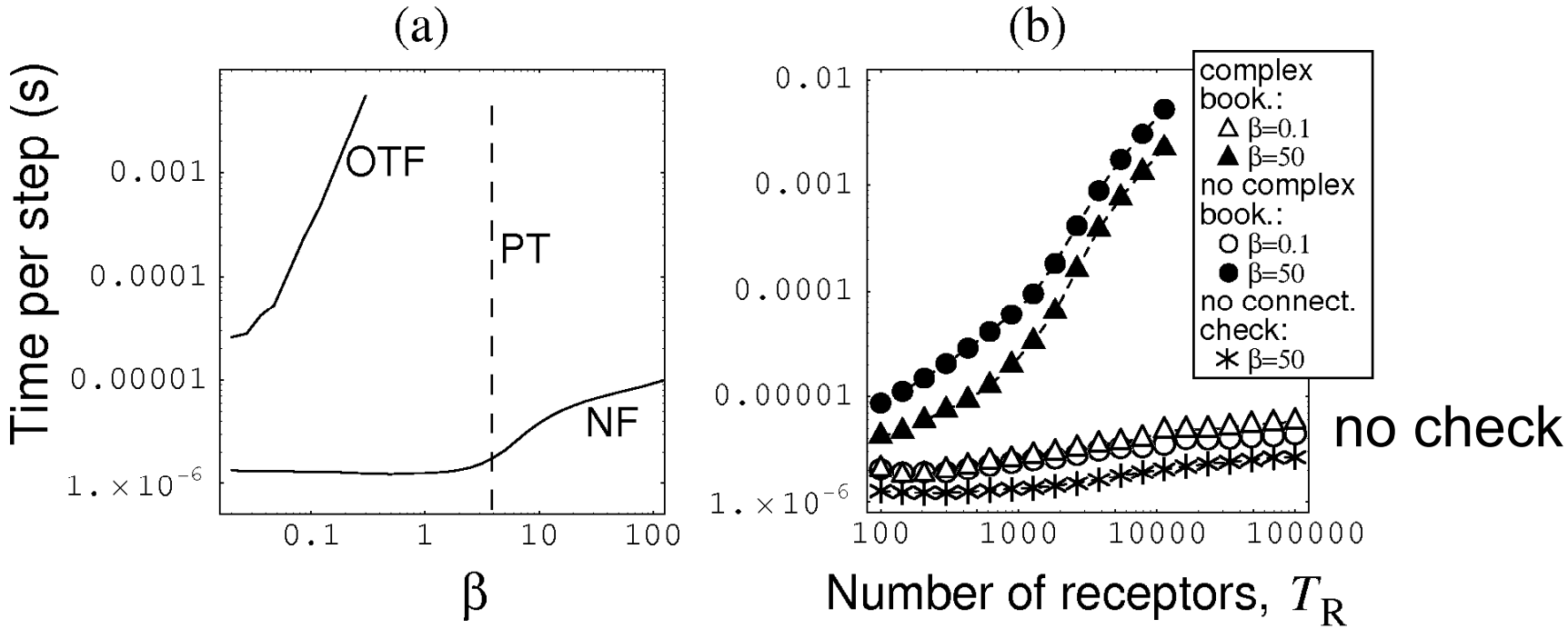

3000 Receptors, 10,000 Ligands

# NFsim's Performance

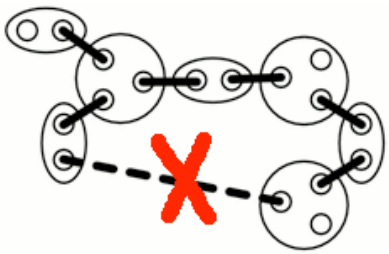
## Comparison to the StochSim / Dynstoc Approach



# Performance

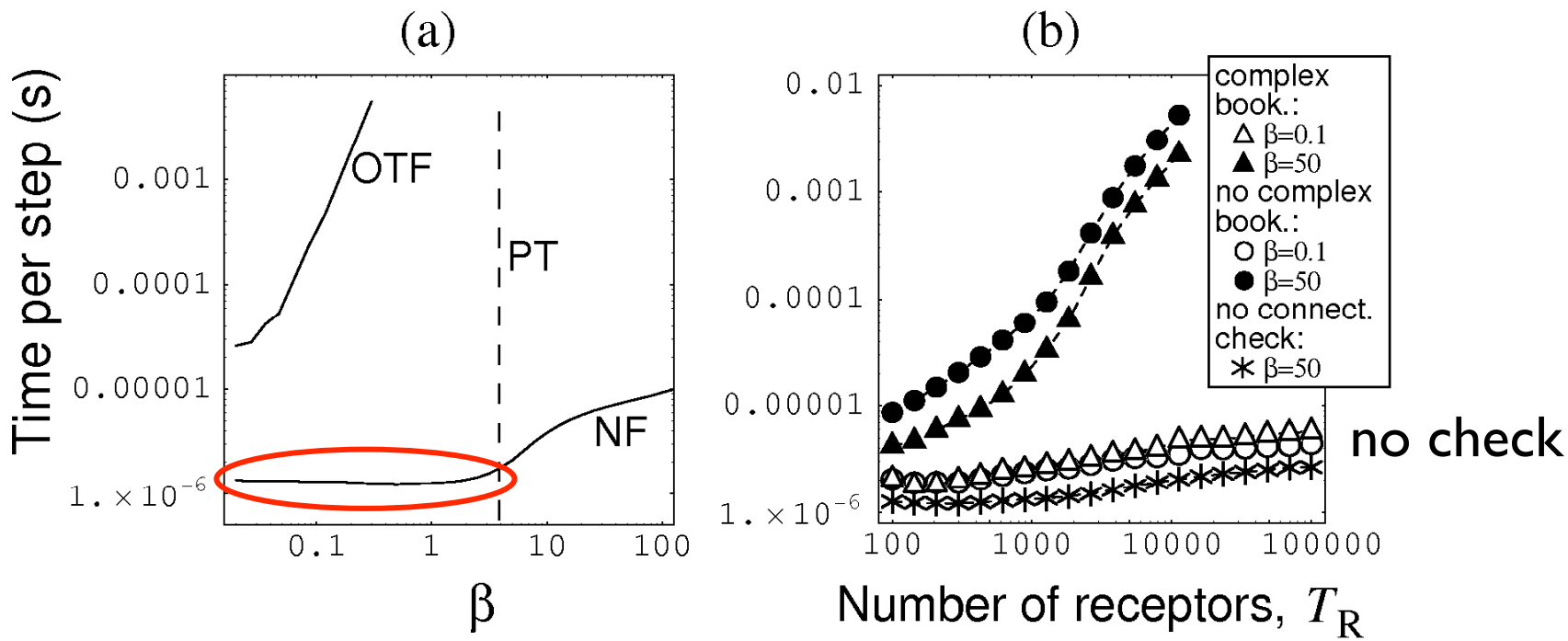



Modest increase in cost at gel phase boundary due to connectivity check



Michael Monine

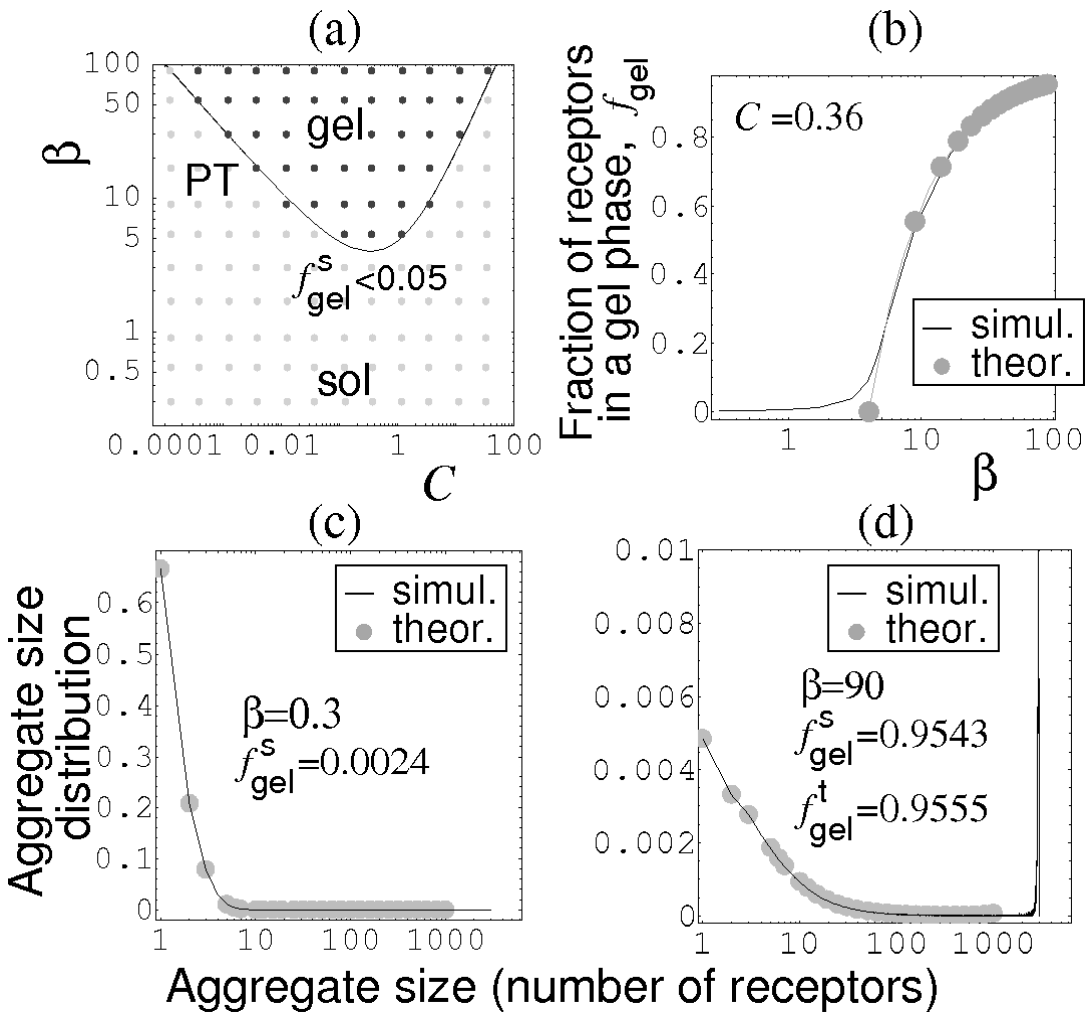
# Performance




Network size increases dramatically, but cost is constant

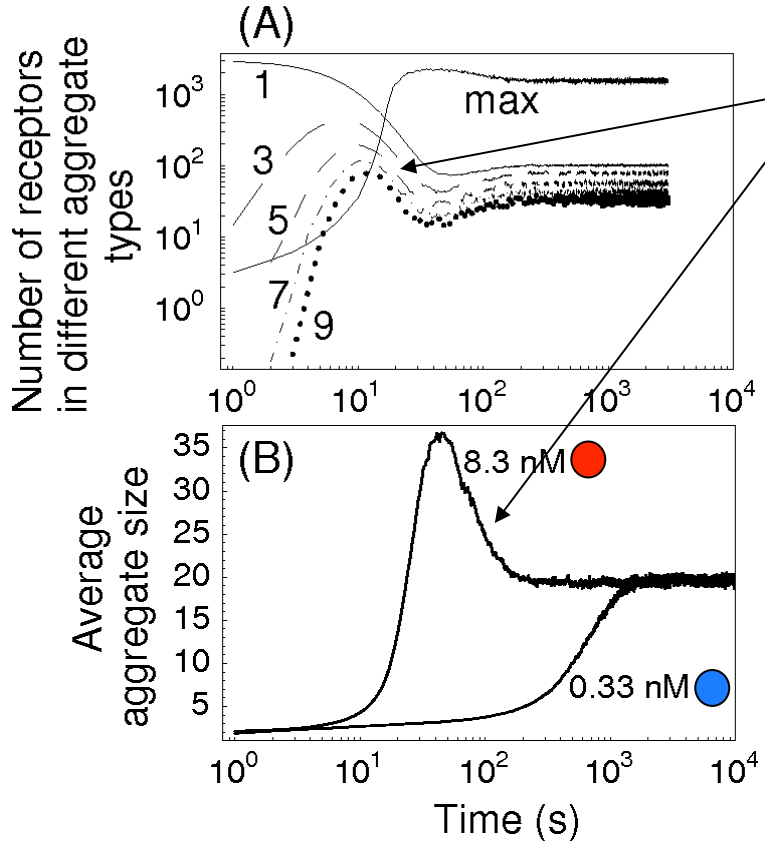
Yang, Monine, Faeder & Hlavacek, *Phys. Rev. E* (2008)

# Validation based on comparison with Goldstein-Perelson Theory



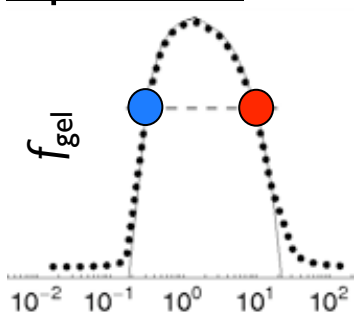

Monine *et al.*, submitted

# Kinetics of Aggregate Formation




At high ligand concentration, intermediate aggregates form transiently

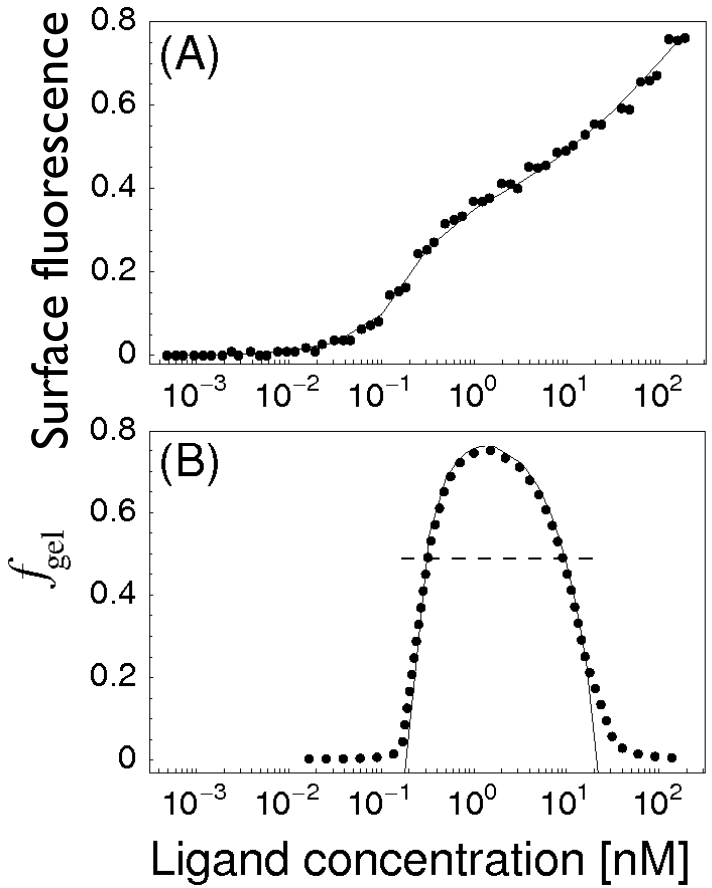
Equilibrium




Ligand concentration

Monine *et al.*, submitted

# Trivalent DNP ligand binding to IgE-FcεRI



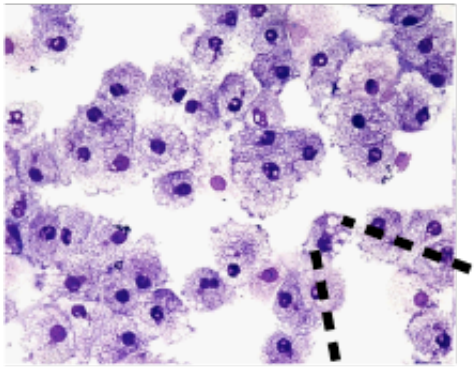

Flow cytometry data from R. Posner (NAU/TGen) for fluorescein-labeled trivalent DNP ligand.

Fit model parameters lie within gel formation region

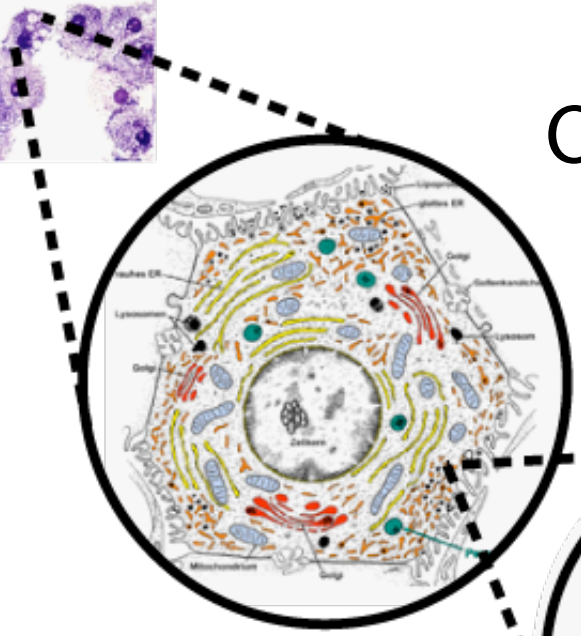
Finding is robust to inclusion of steric effects and ring formation in model

Monine *et al.*, submitted

# Agents at all Scales of Biology



Cells as Agents



Organelles as Agents

Molecules as Agents

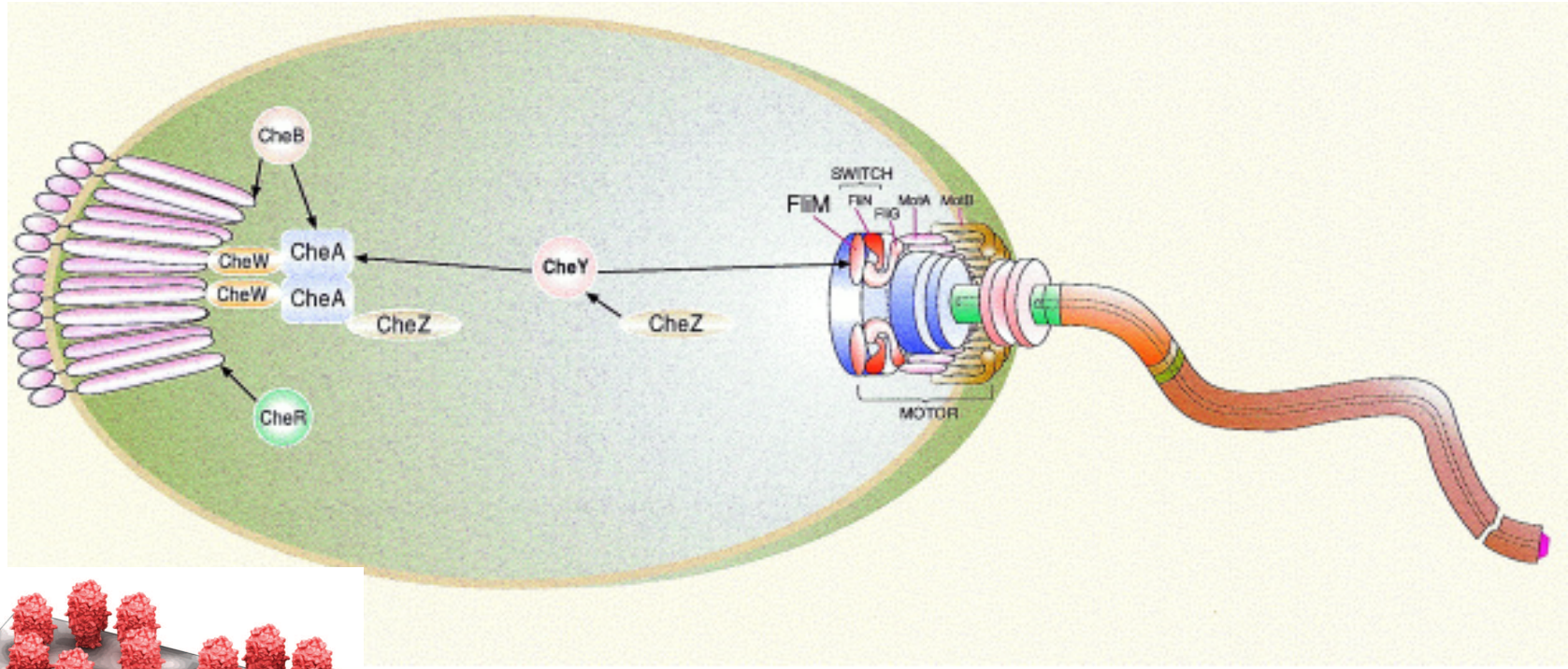
**Goal:** Multiscale Agent-based, simulation of biological systems, building up from the stochastic molecular level

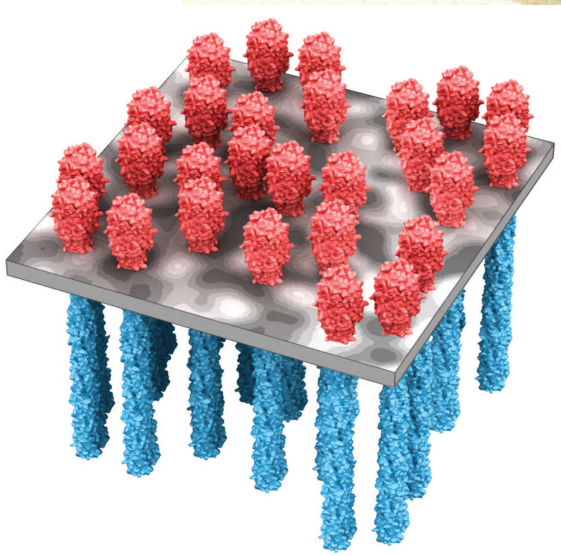
Cell and Population Level Behavior

↑

Molecular Level Interactions

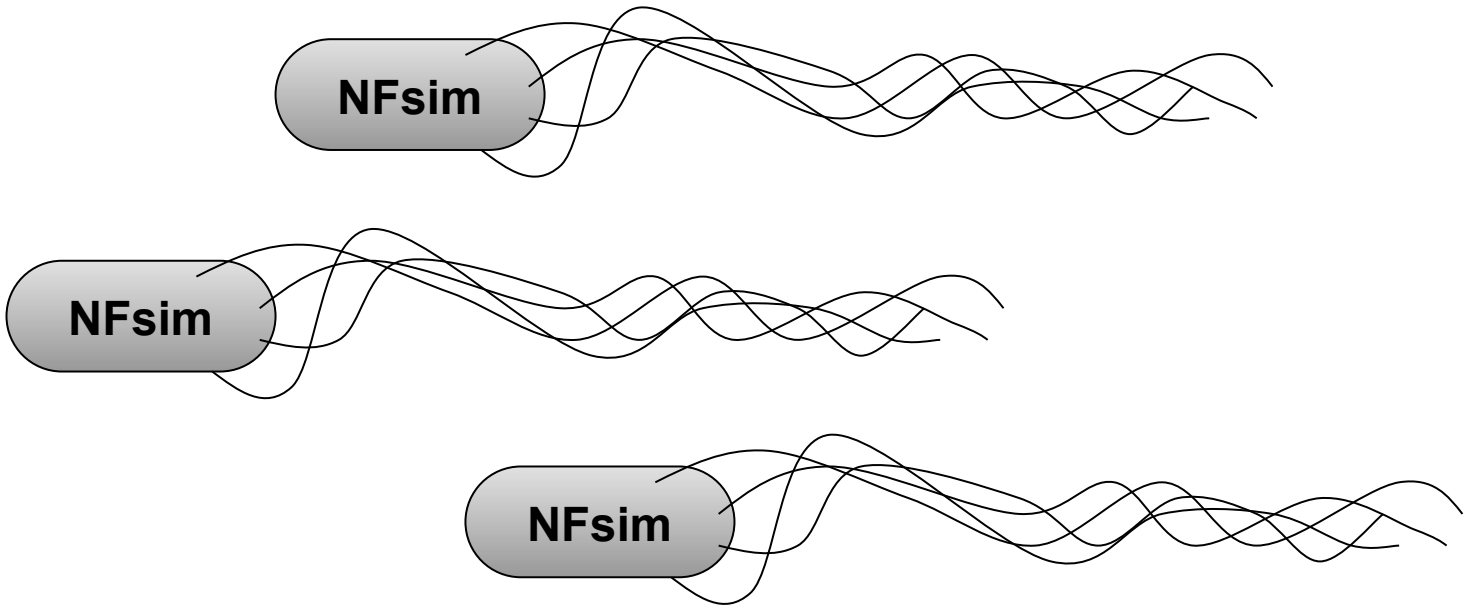
# Complexity in Chemotaxis Signaling



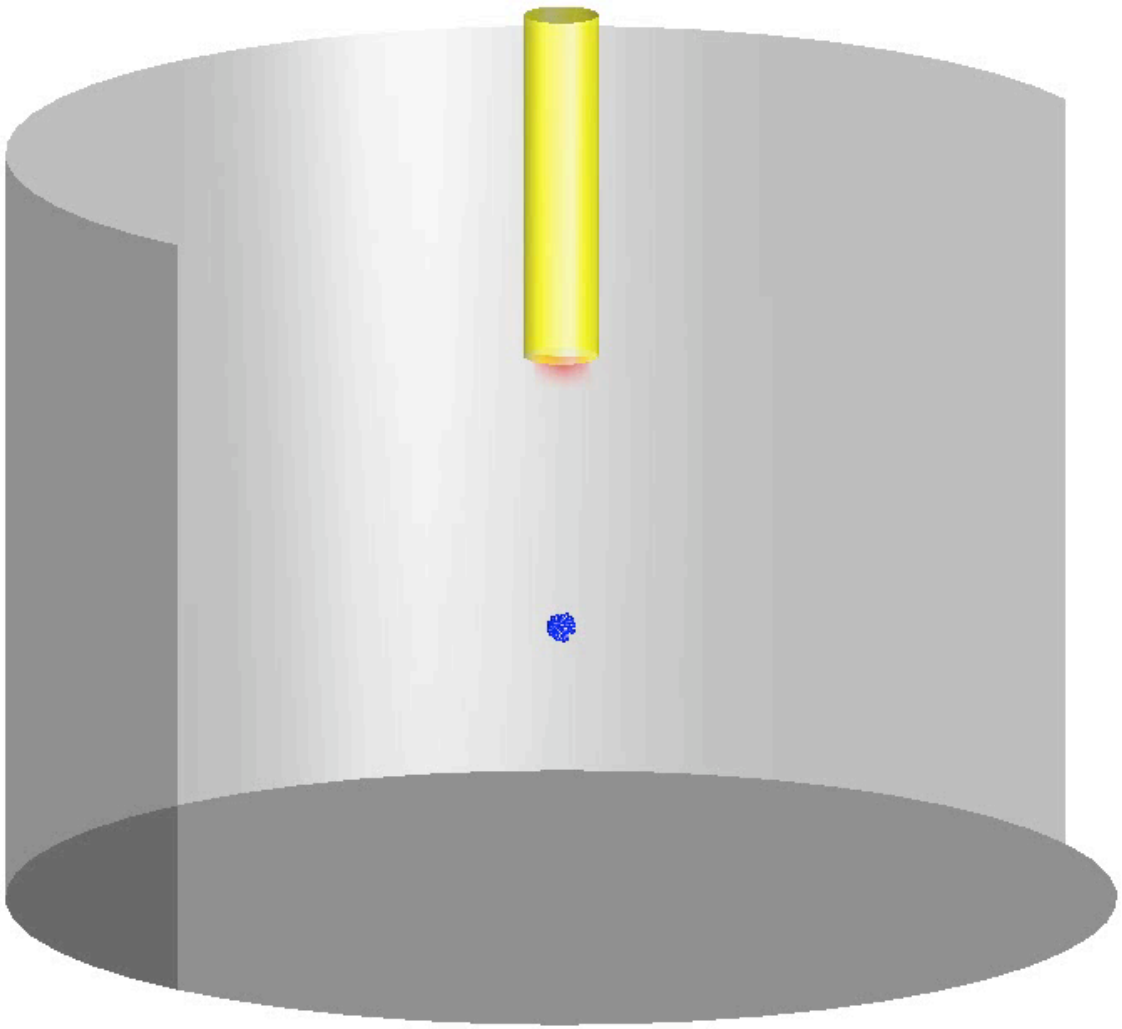



**Receptor aggregation makes simulation difficult**

# NFsim can be embedded into other higher level agents



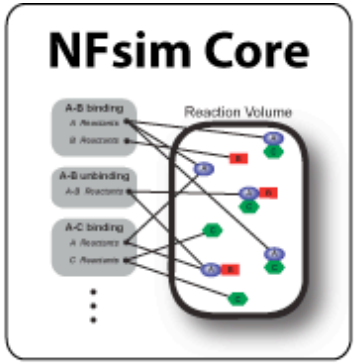
# Digital Chemotaxis Experiments



Elapsed Time: 0 minutes

**200 *E. coli* Cells**
**2mm from Capillary**
**10mM Attractant**

**40 min simulation**




Emonet Lab
Yale University

**BioNetGen Interface, Simulation Methods**
Faeder Lab
University of Pittsburgh

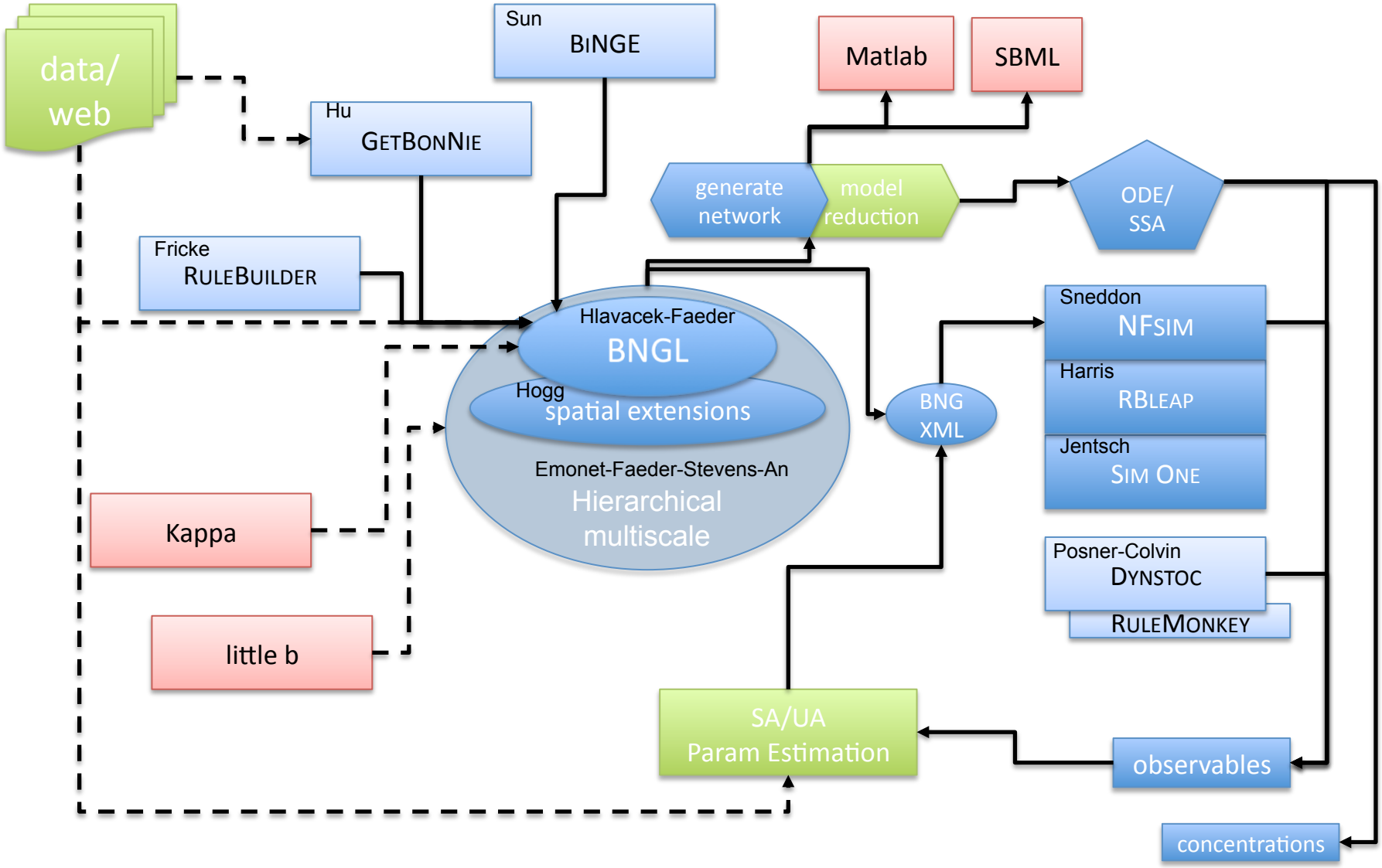
**Parallel Computing**
Rick Stevens Group
Argonne National Lab

**Applications to Medicine, Knowledge Representation**
Gary An Lab
Northwestern University

# Conclusions

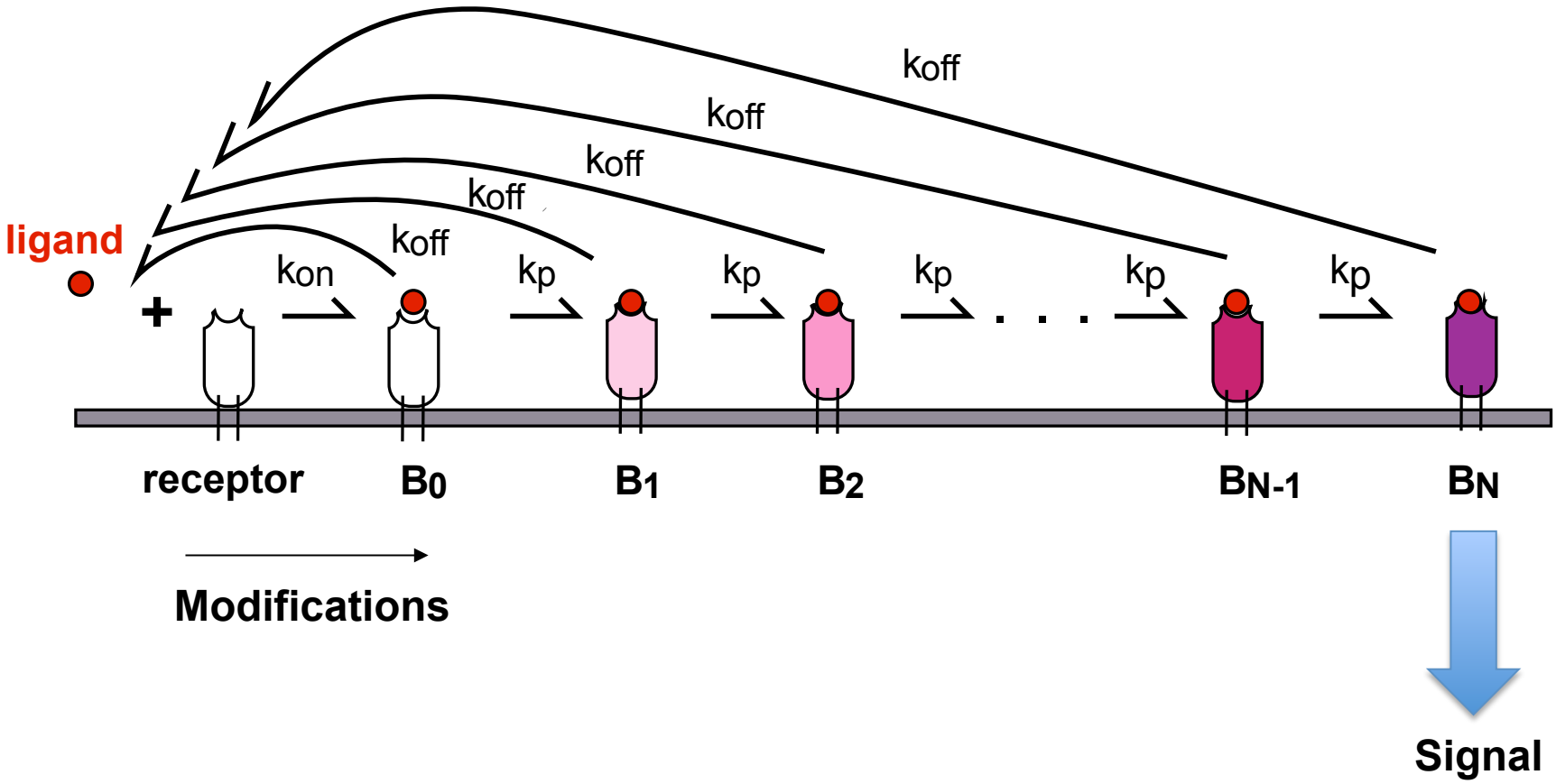
- Kinetics and stoichiometry of complex formation can have a profound effect in signal transduction.
- Modeling these effects requires a new approach to modeling that addresses the issue of *combinatorial complexity*.
- Rule-based (or *interaction-based*) modeling is such an approach.
- *Network-free* simulation is a powerful technique that circumvents combinatorial complexity.
- *Network-free* enables simulation of arbitrarily complex intracellular networks and allows embedding in multi-scale models.

# BioNetGen Universe (circa Summer, 2009)



# Kinetic Proofreading in Receptor Signaling

- Ligand dissociation rate can determine ligand efficacy




T. W. McKeithan, *PNAS*, **92**, 5042-5046 (1995).

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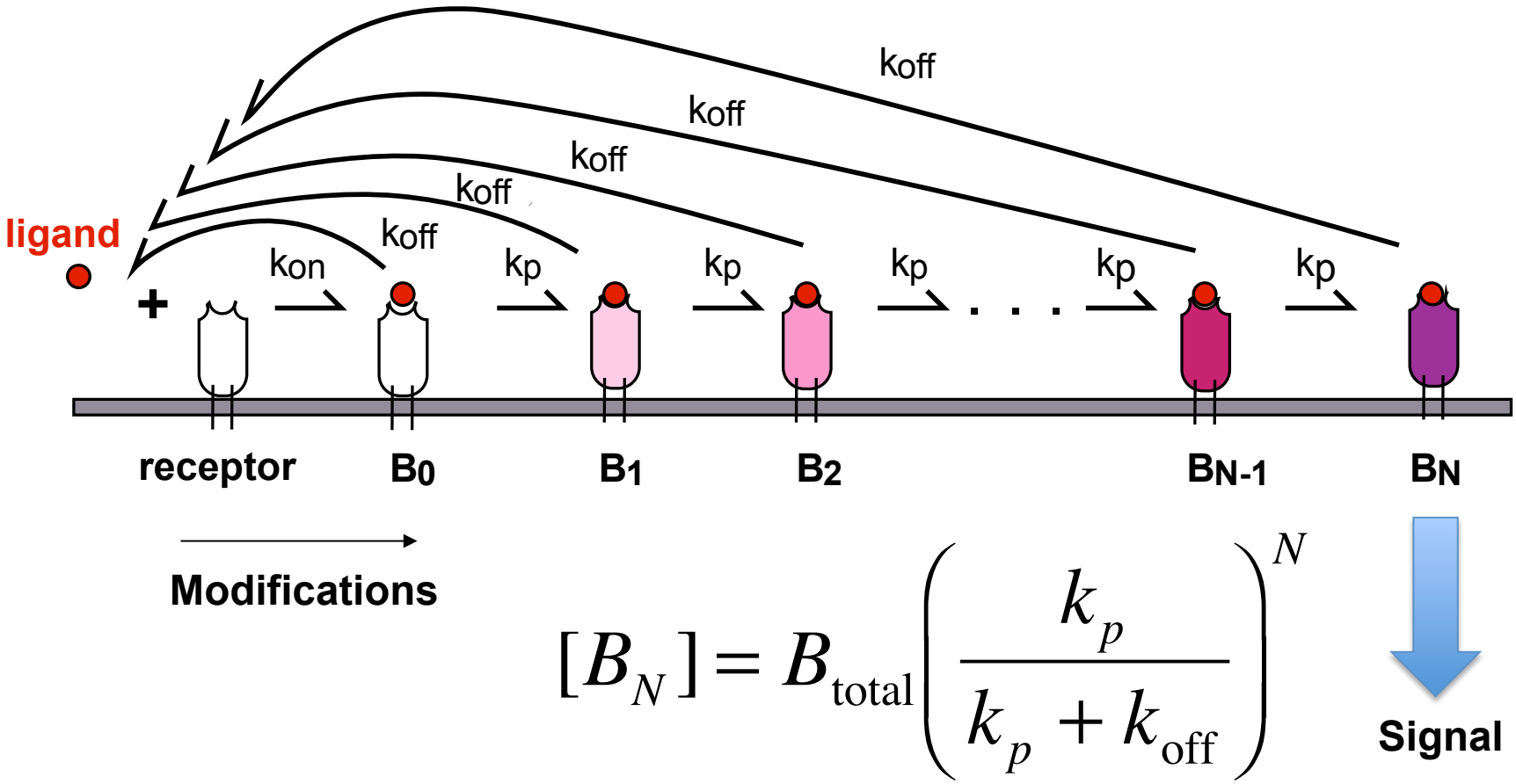


$$[B_N] = B_{\text{total}} \left( \frac{k_p}{k_p + k_{\text{off}}} \right)^N$$

T. W. McKeithan, *PNAS*, **92**, 5042-5046 (1995).

# Kinetic Proofreading in Receptor Signaling

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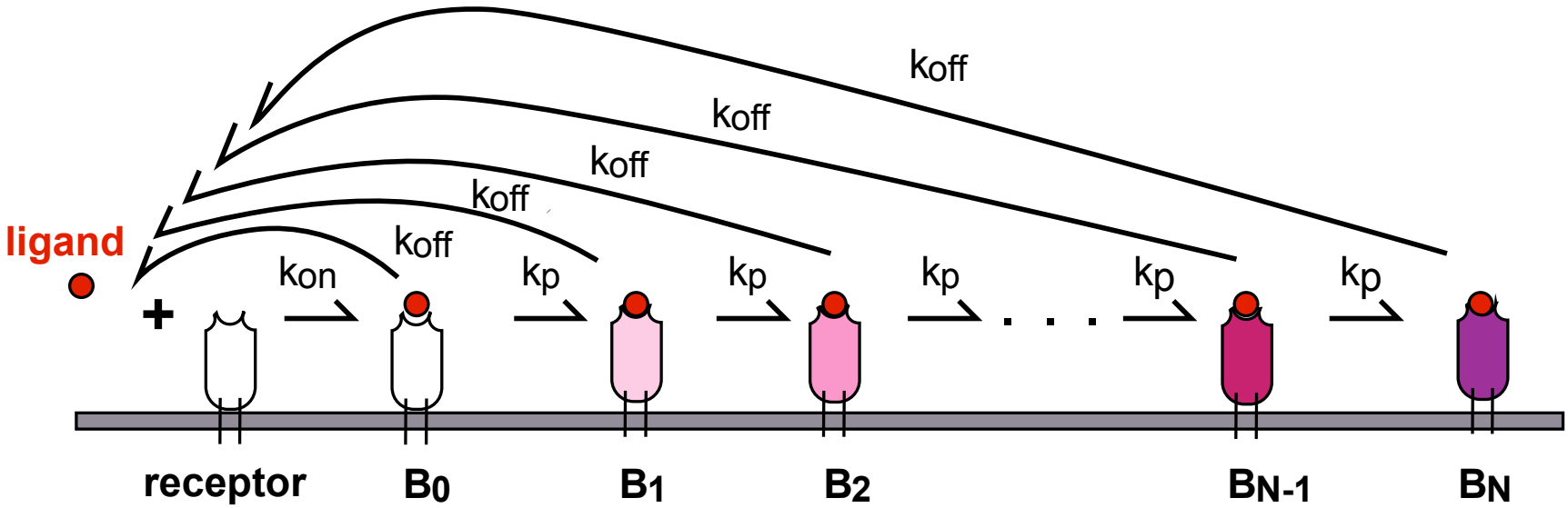



**Modifications**

**Enhancement ratio**

$$[B_N]/[B'_N] = \left(\frac{k_p + k'_{\text{off}}}{k_p + k_{\text{off}}}\right)^N$$

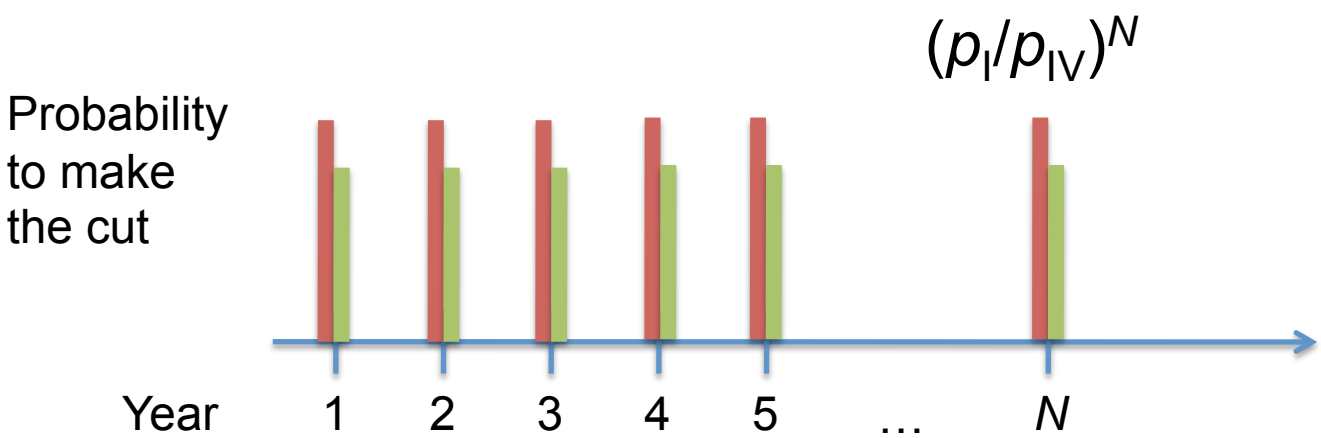
**Signal**

T. W. McKeithan, *PNAS*, **92**, 5042-5046 (1995).

# Kinetic Proofreading in Sports

Malcolm Gladwell, *Outliers*.

- Many sports (and education systems) have cutoff dates to establish eligibility
- Having a birthdate close to the cutoff date confers a small but tangible advantage



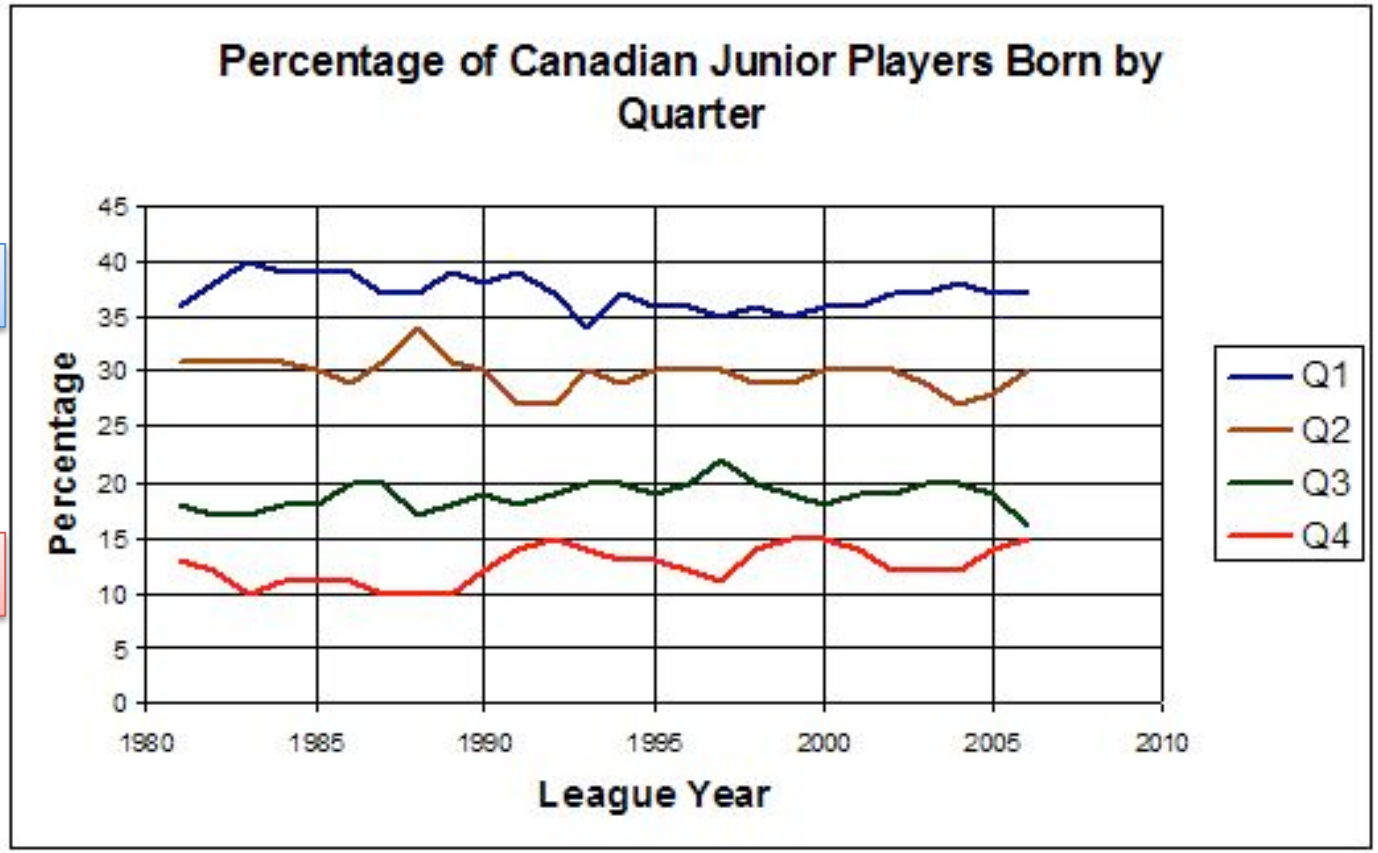
# Kinetic Proofreading in Sports

Malcolm Gladwell, *Outliers*.

Born Jan-Mar

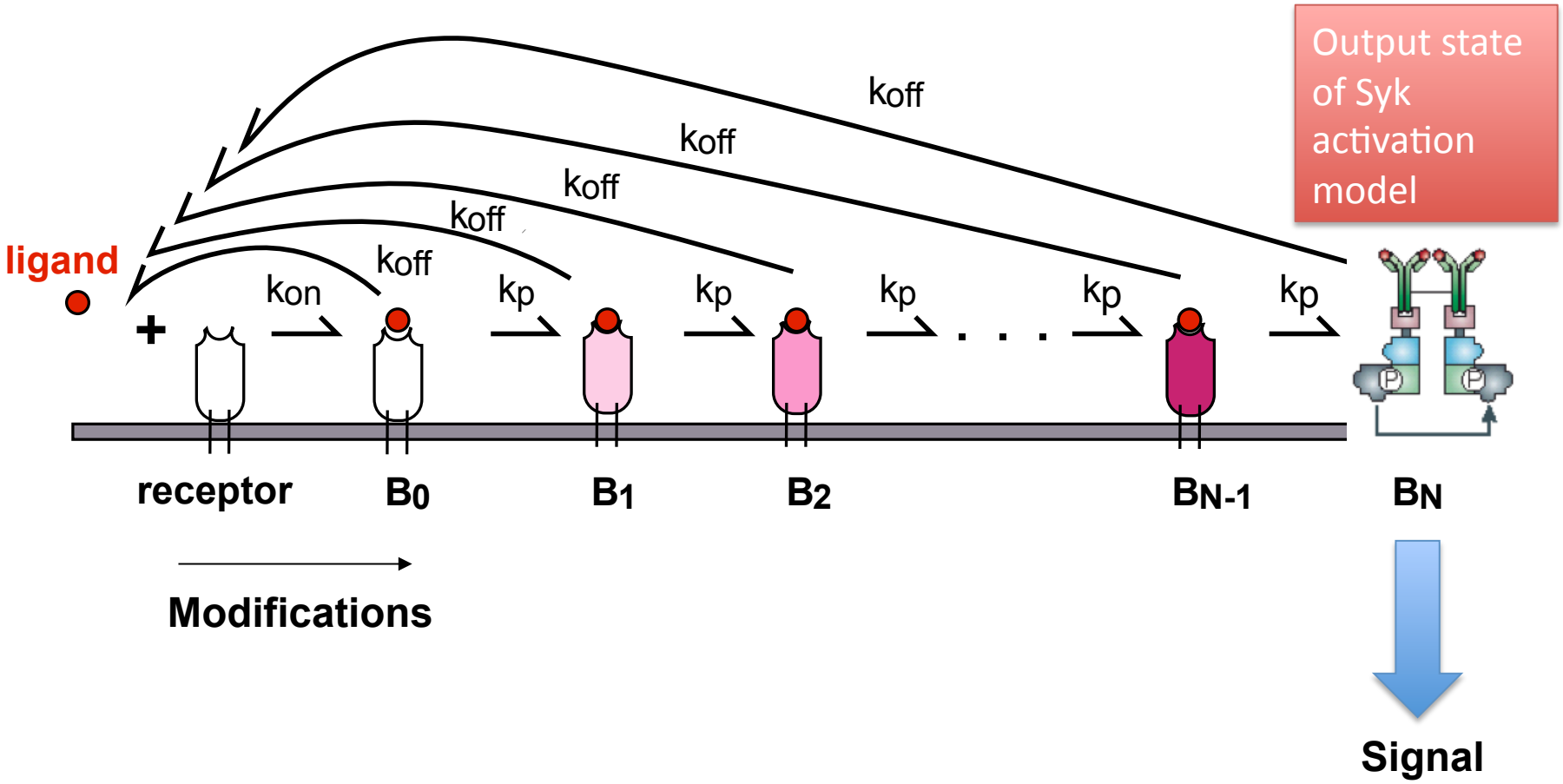
2.5-4 fold!

Born Oct-Dec



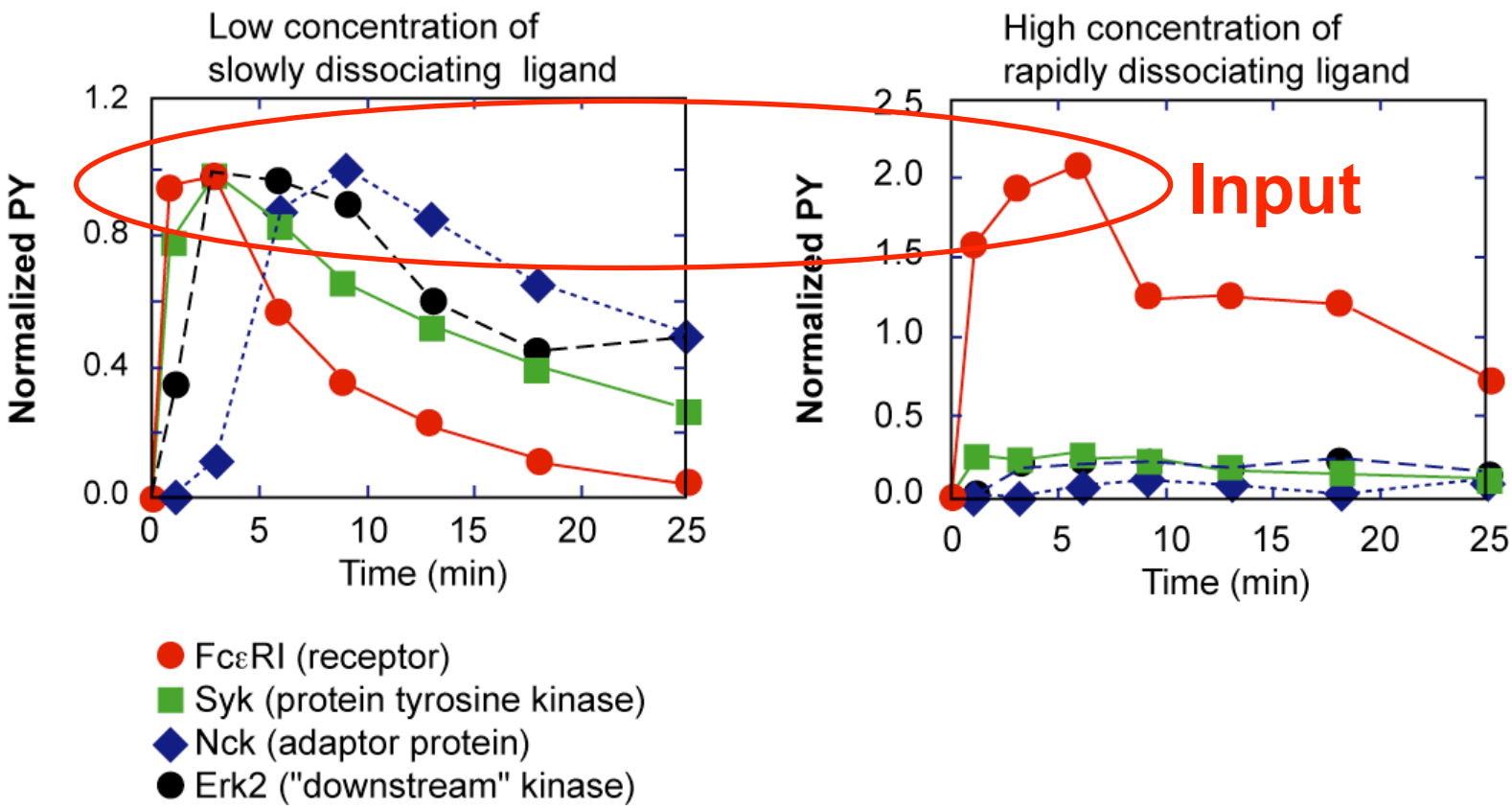
# Kinetic Proofreading in Receptor Signaling

- Ligand dissociation rate can determine ligand efficacy



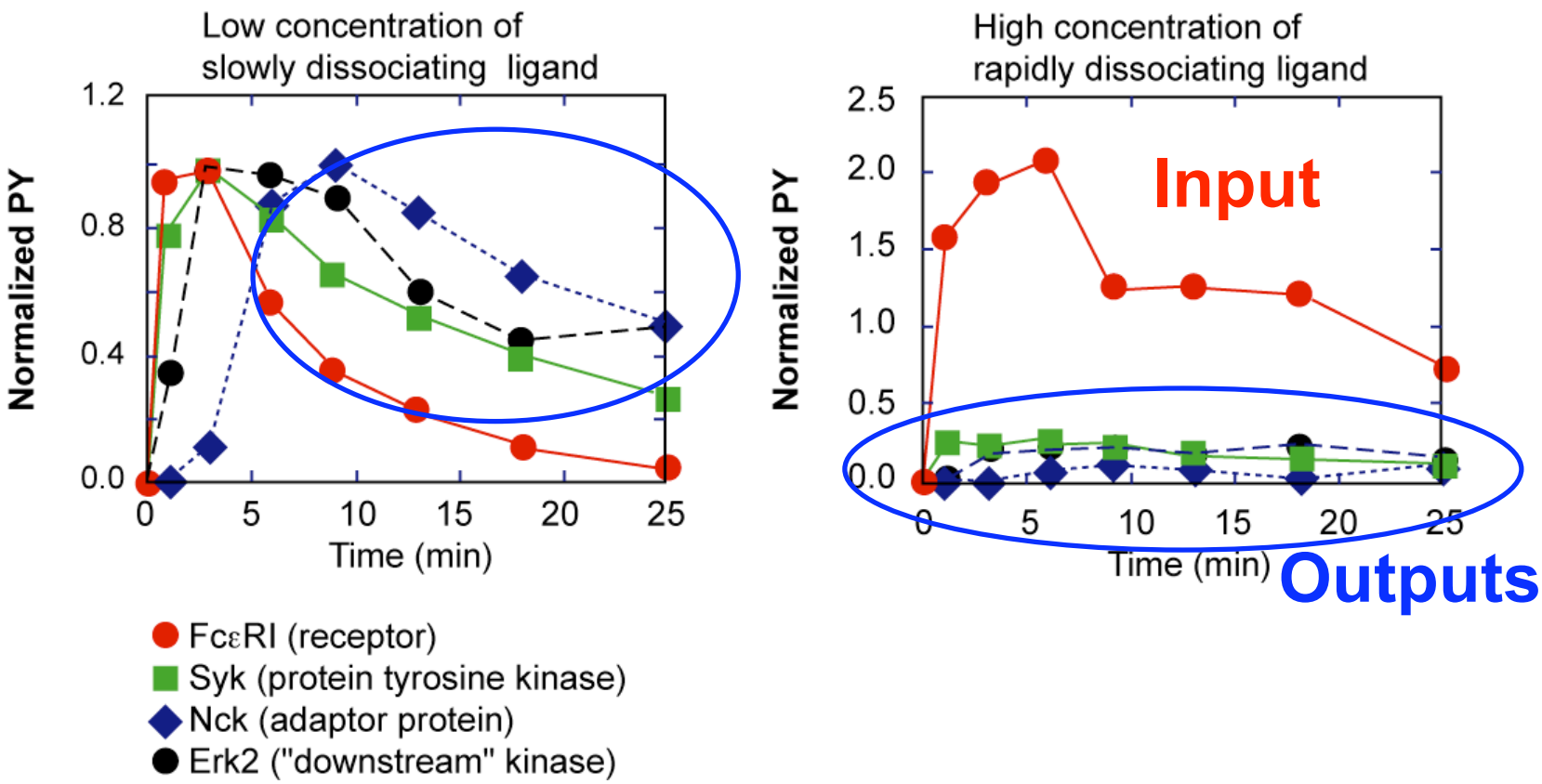

T. W. McKeithan, *PNAS*, **92**, 5042-5046 (1995).

# Evidence for Kinetic Proofreading in Mast Cell Responses to Two Ligands



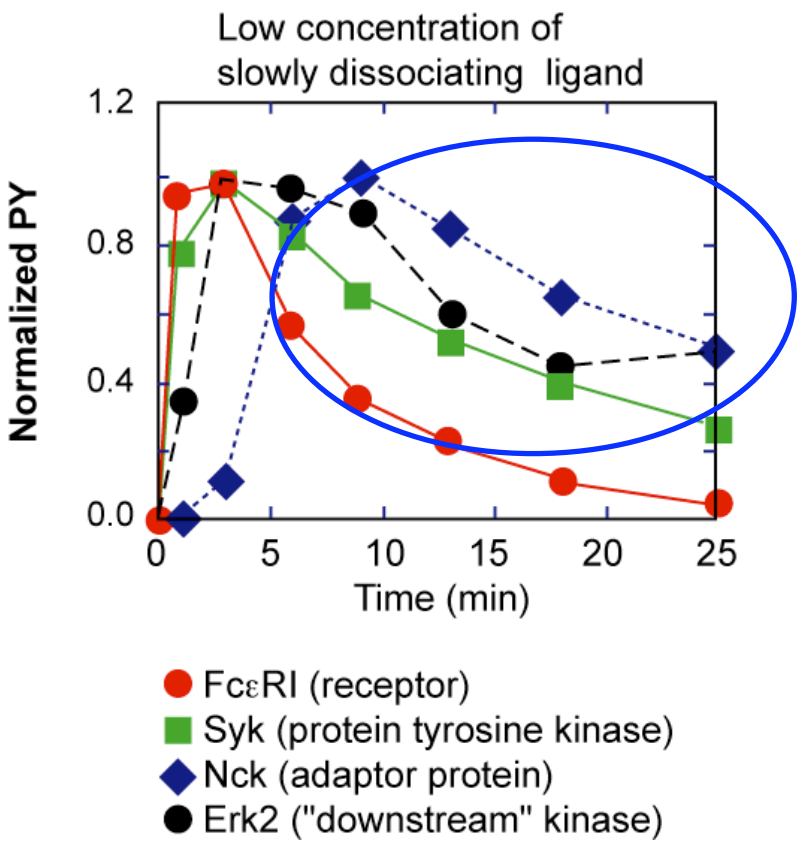

Torigoe, Inman & Metzger, *Science*, **281**, 568 (1998)

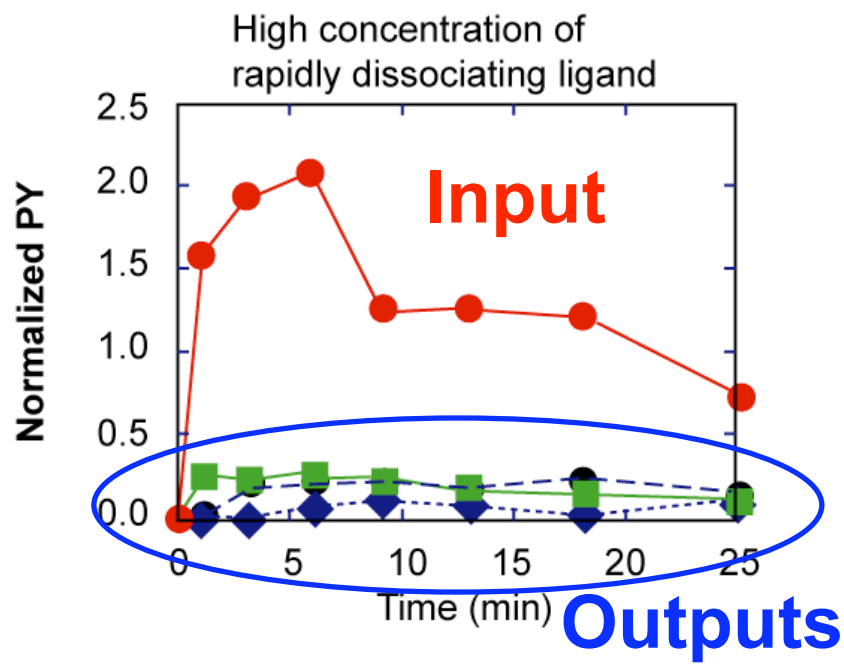
# Evidence for Kinetic Proofreading in Mast Cell Responses to Two Ligands




Torigoe, Inman & Metzger, *Science*, **281**, 568 (1998)

# Evidence for Kinetic Proofreading in Mast Cell Responses to Two Ligands

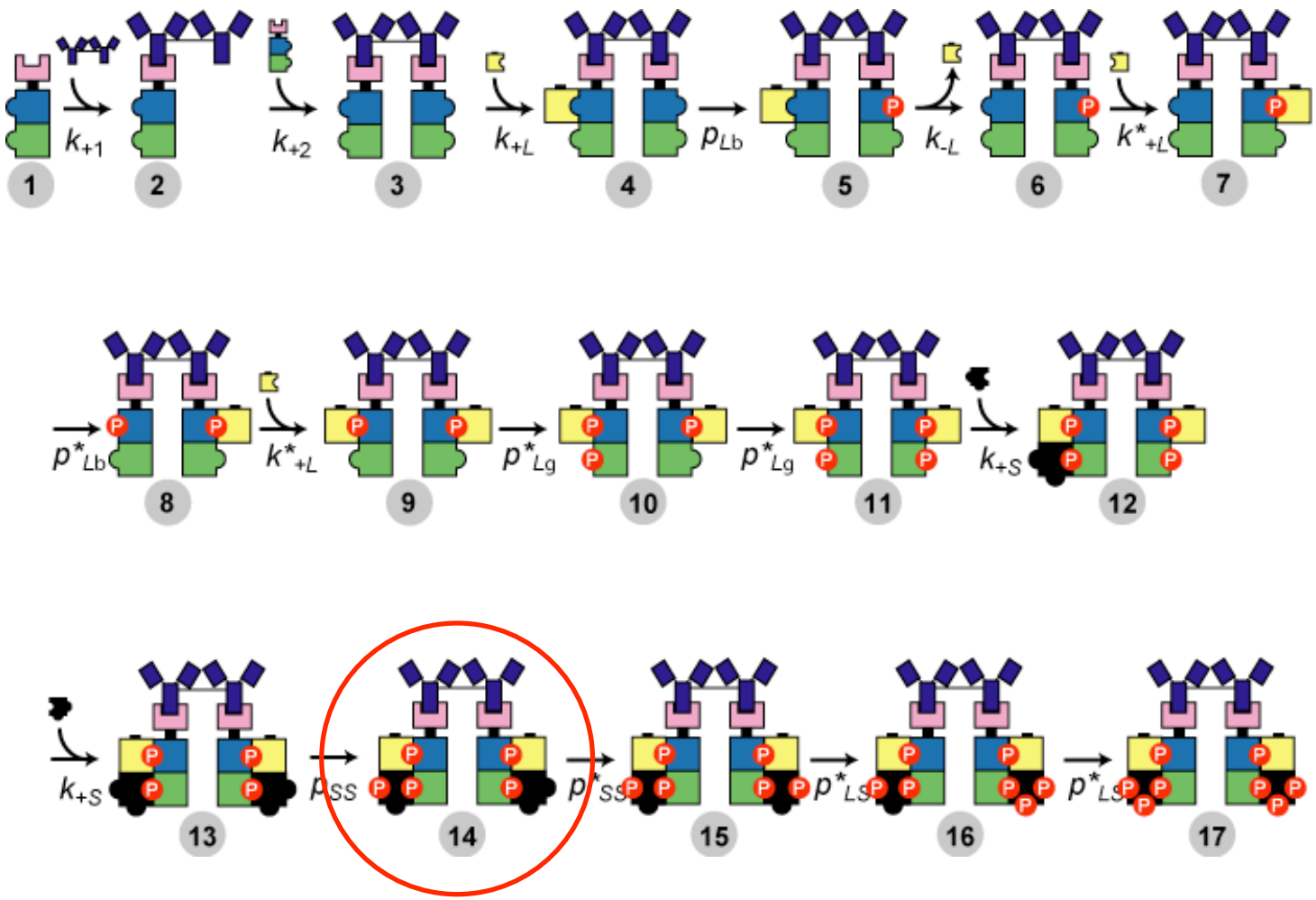


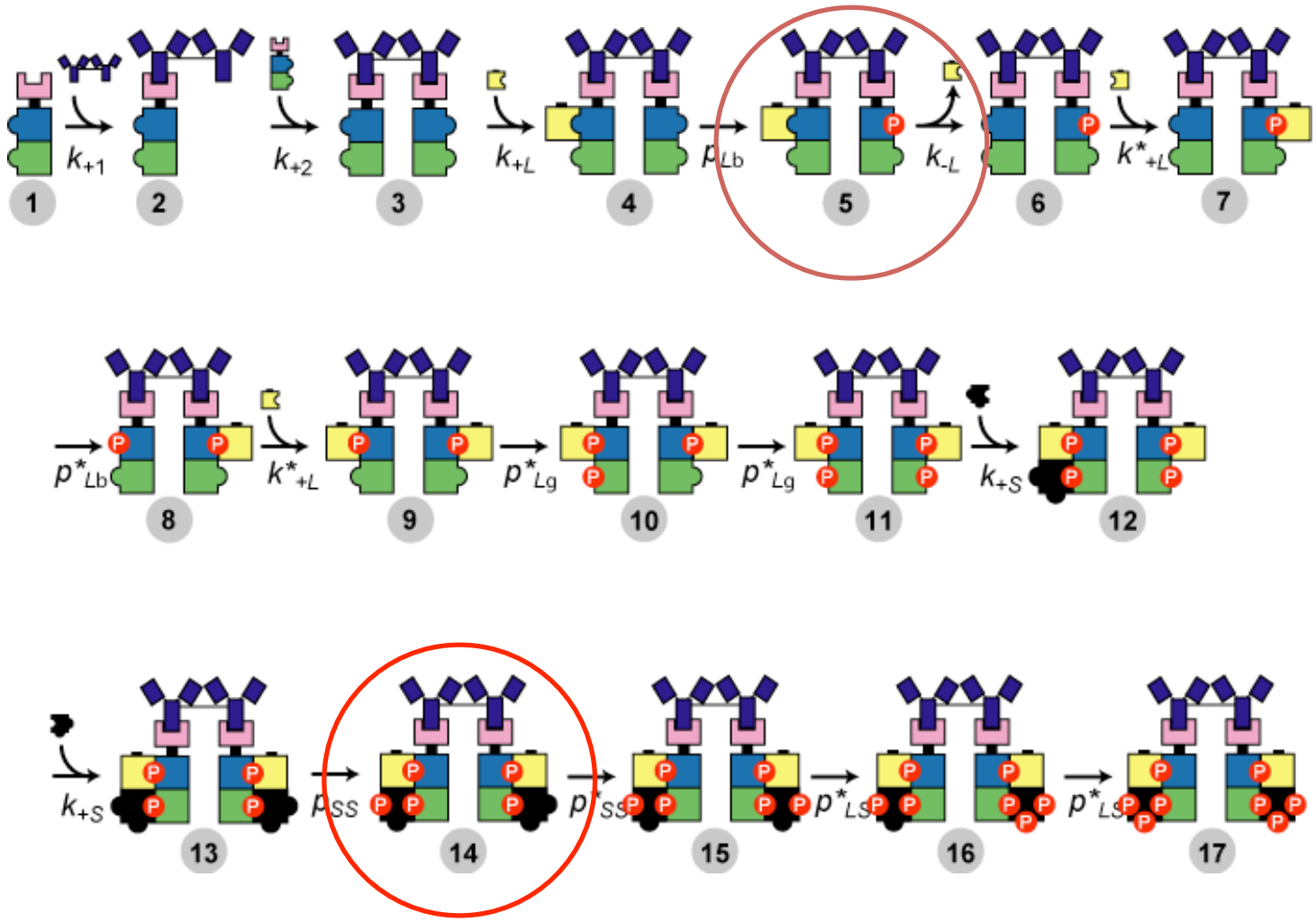

Ligand with shorter dwell time gives low Syk phosphorylation

Torigoe, Inman & Metzger, *Science*, **281**, 568 (1998)

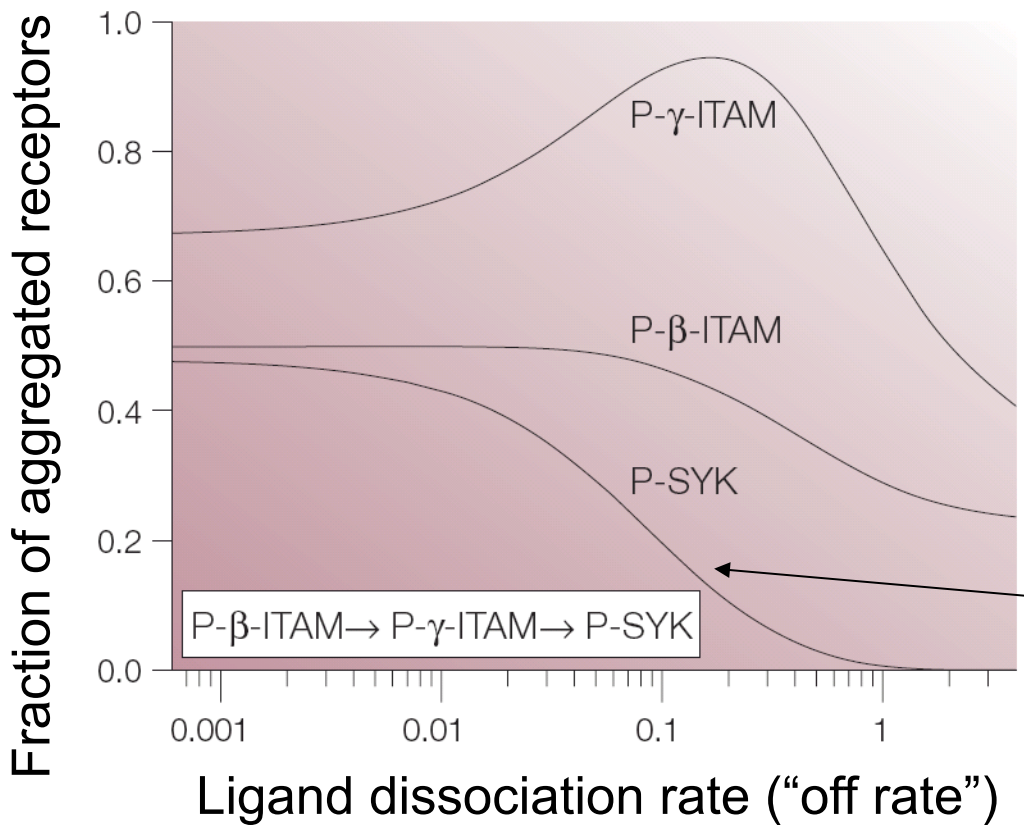
# Large number of reaction events required for Syk activation



# Small number of reaction events required for receptor phosphorylation



# Kinetic proofreading of Syk activation but not receptor phosphorylation




Rapid fall in efficiency of Syk phosphorylation

Goldstein *et al.* (2004) *Nat. Rev. Immunol*. **4**, 445-456.

# Bimodal dose-response curves

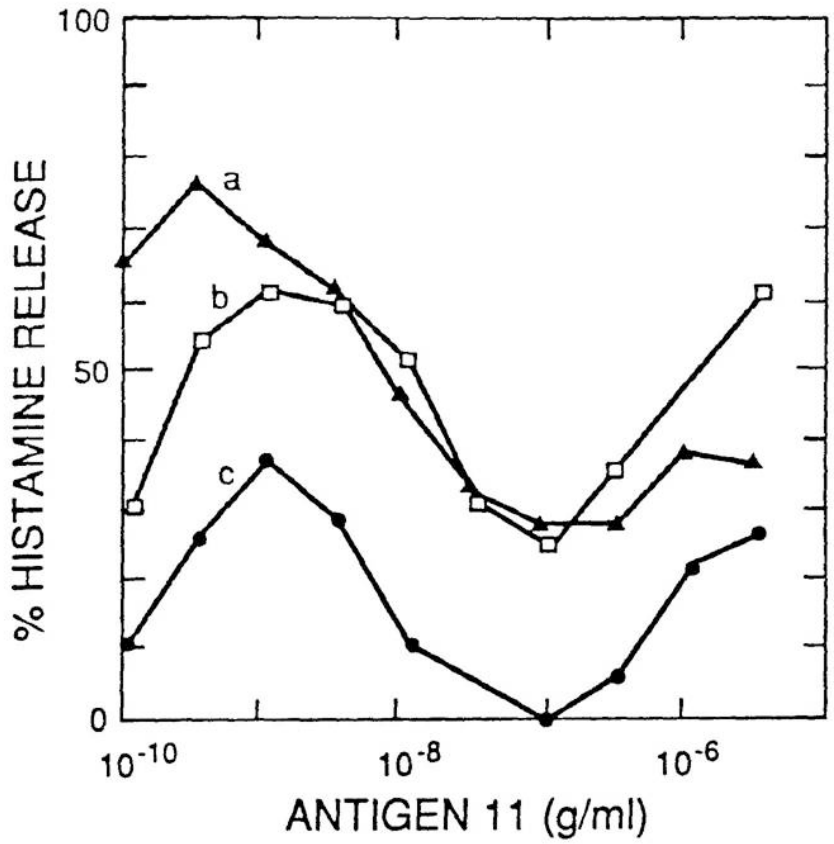



FIGURE 12 Histamine release curves from basophils from three donors who were allergic to the dust mite *Dermatophagoides farinae* (Weyer et al., 1982). Antigen 11 is a highly purified component of *D. farinae*. DeLisi and Siraganian (1979) observed similar double-humped histamine release curves with rabbit basophils.

B. Goldstein, in *Theoretical Immunology, Part One*, Ed. A. S. Perelson

# Bimodal dose-response curves

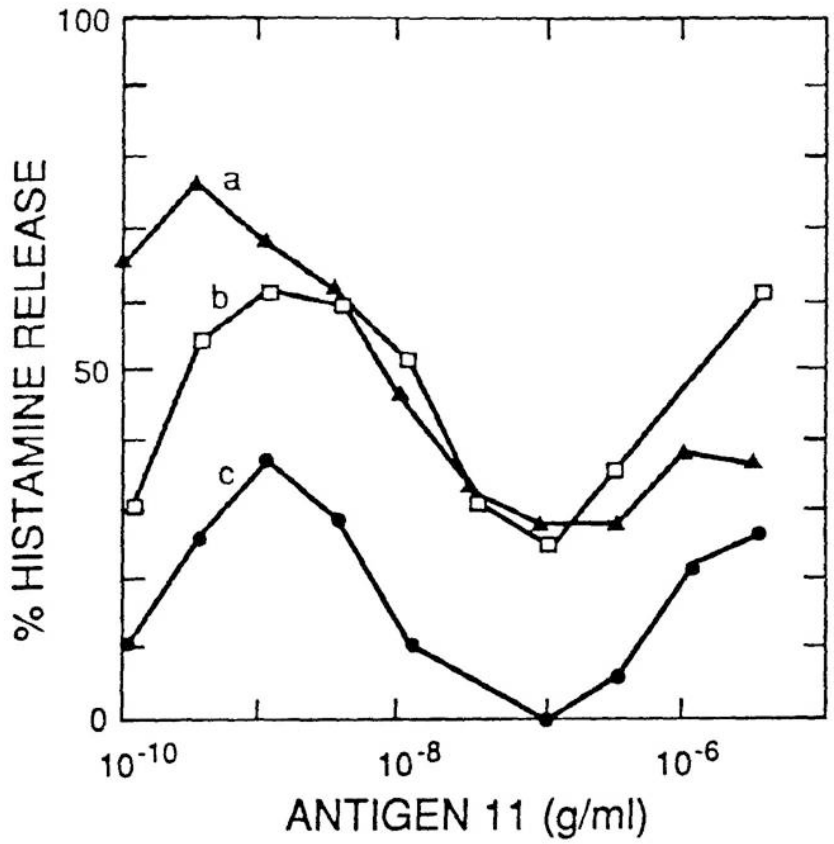


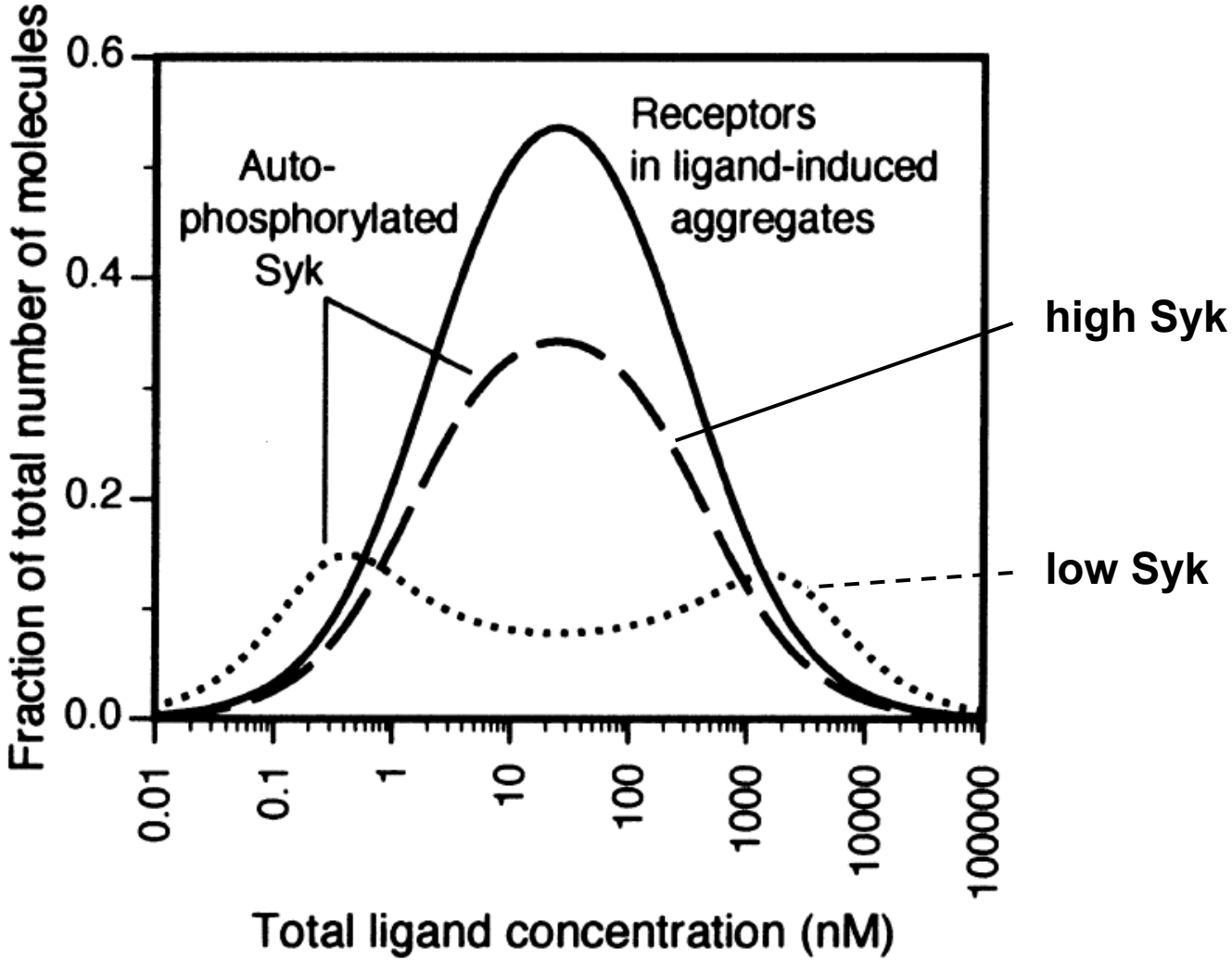

FIGURE 12 Histamine release curves from basophils from three donors who were allergic to the dust mite *Dermatophagoides farinae* (Weyer et al., 1982). Antigen 11 is a highly purified component of *D. farinae*. DeLisi and Siraganian (1979) observed similar double-humped histamine release curves with rabbit basophils.

Syk expression is highly variable in human basophils (5,000-60,000 copies per cell)

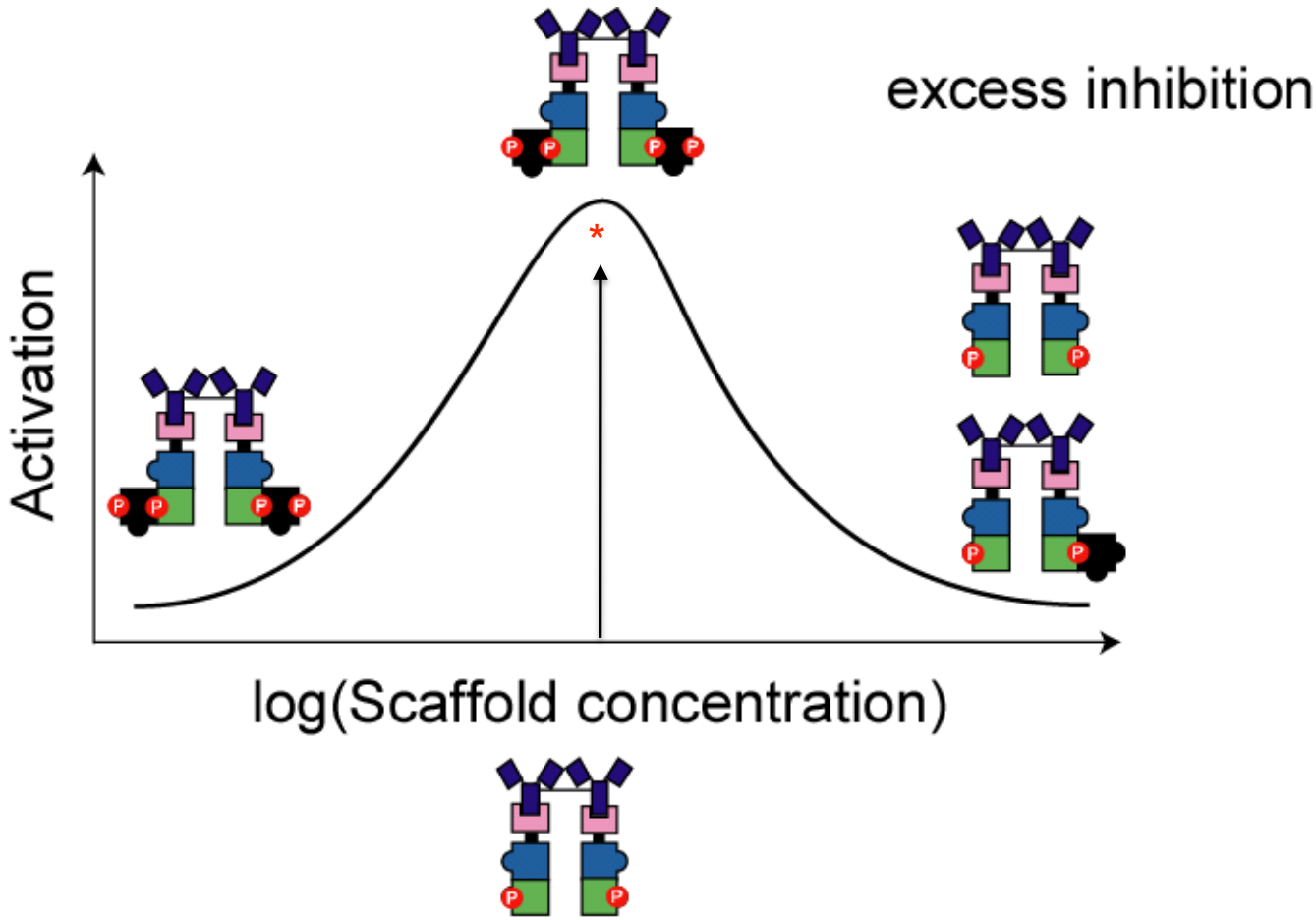
MacGlashan (2007)

B. Goldstein, in *Theoretical Immunology, Part One*, Ed. A. S. Perelson

# Dose-response curves for reversibly binding ligand

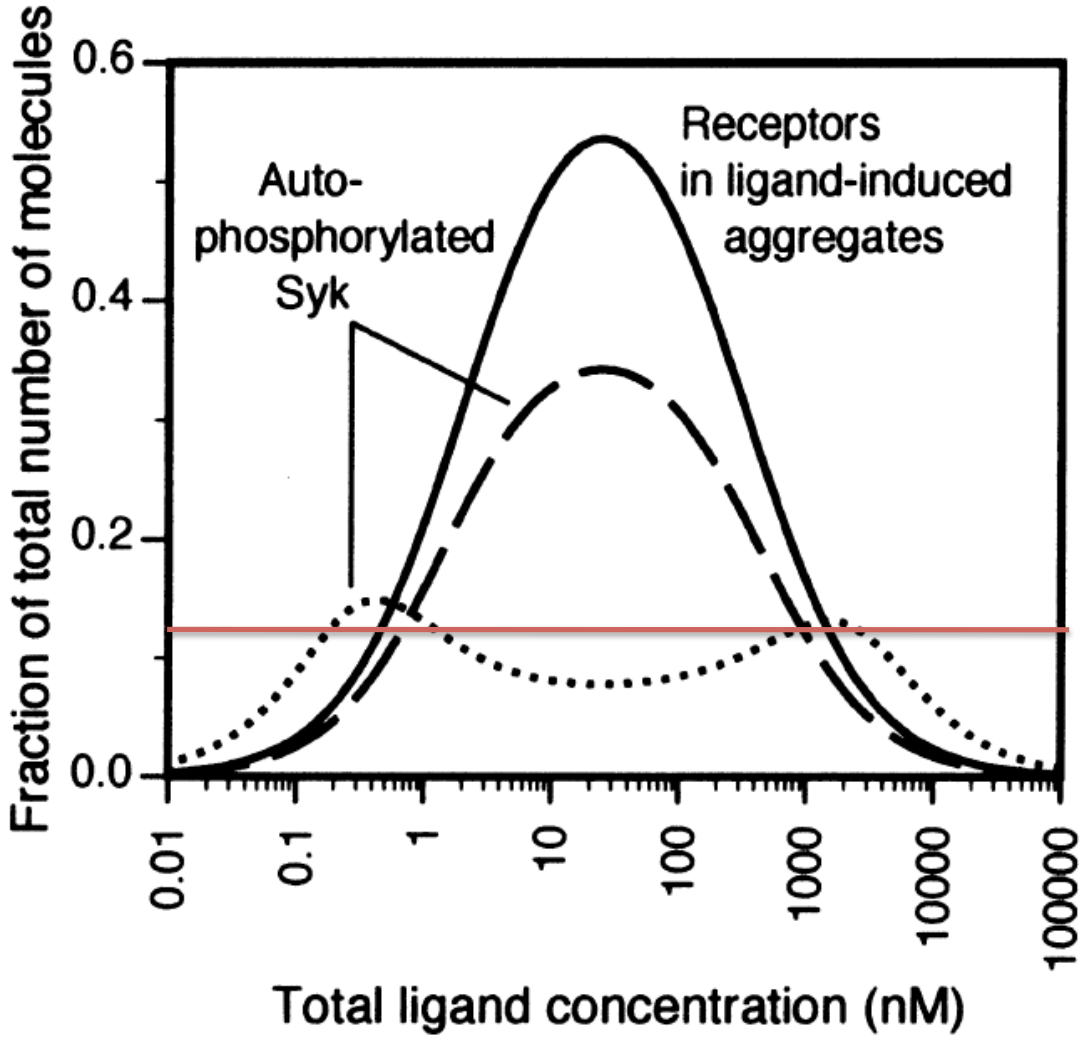


# The multivalent scaffold effect




*Syk and scaffold concentrations are equal

Bimodal response occurs when Syk concentration below maximal number of aggregated receptors



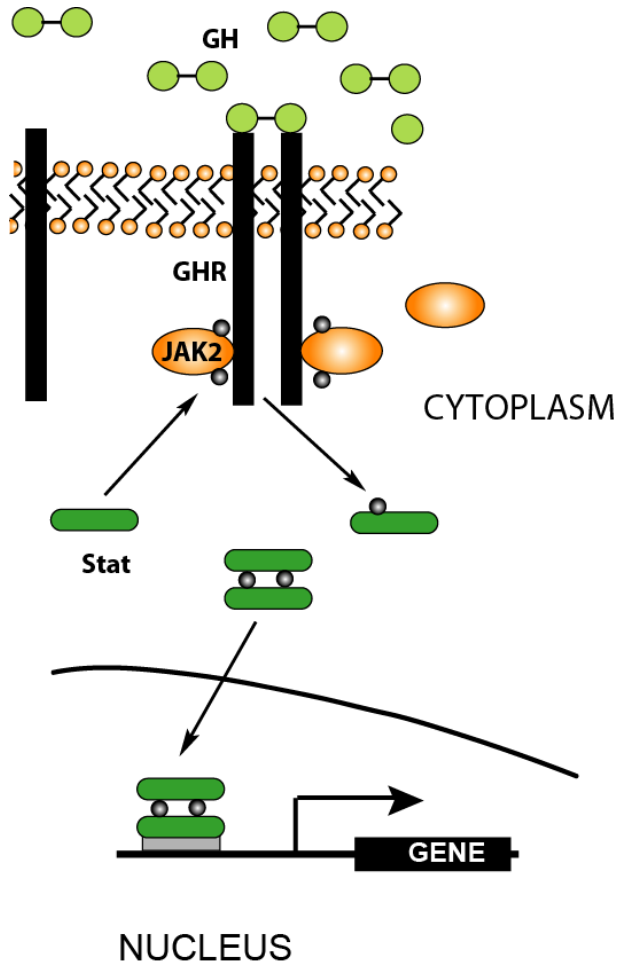

$R_{agg} = Syk_{tot}$

# Growth Hormone / Jak2 Signaling

Dipak Barua

Jason Haugh

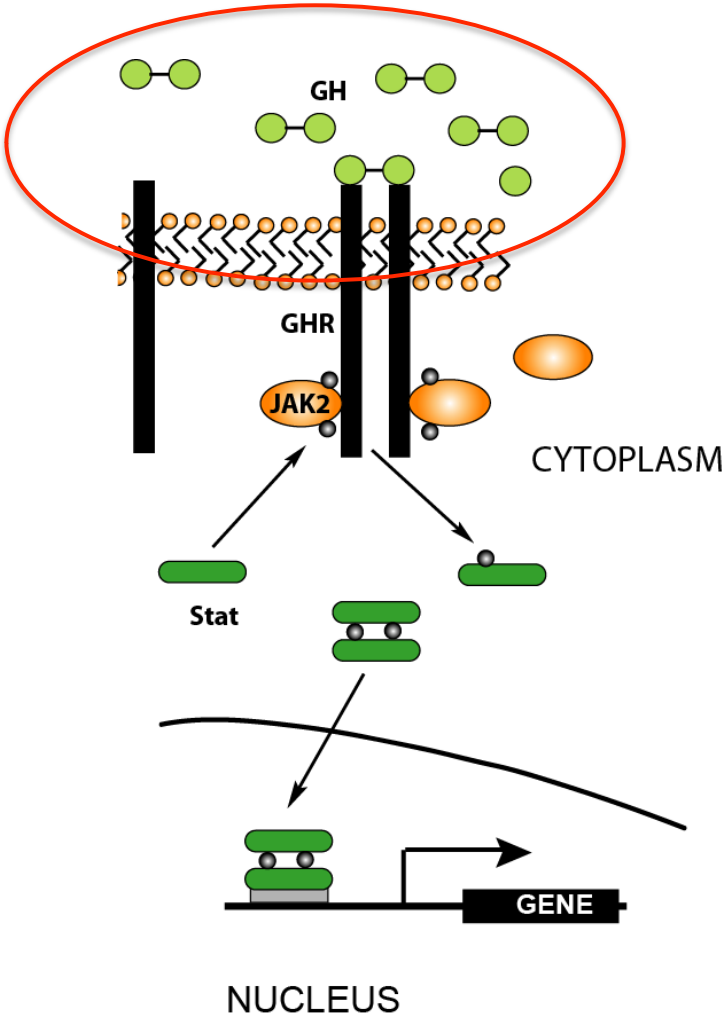
**NC State University**




Barua et al., *PLoS Comp. Biol.* (2009)

# Growth Hormone / Jak2 Signaling

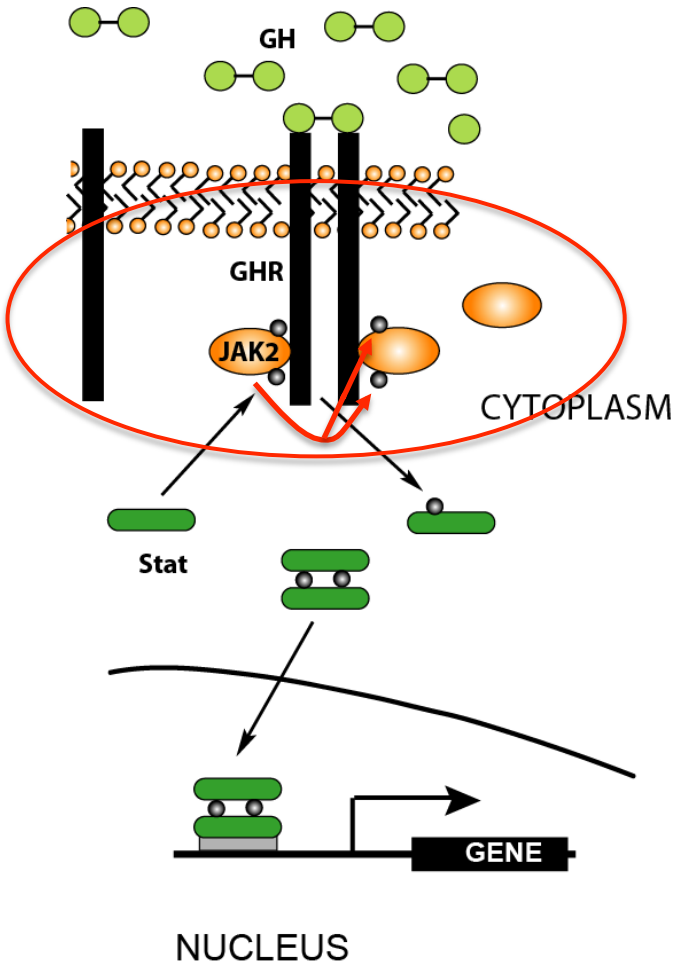
Ligand binding and receptor aggregation



# Growth Hormone / Jak2 Signaling

Ligand binding and receptor aggregation

Jak2 recruitment and transautophosphorylation

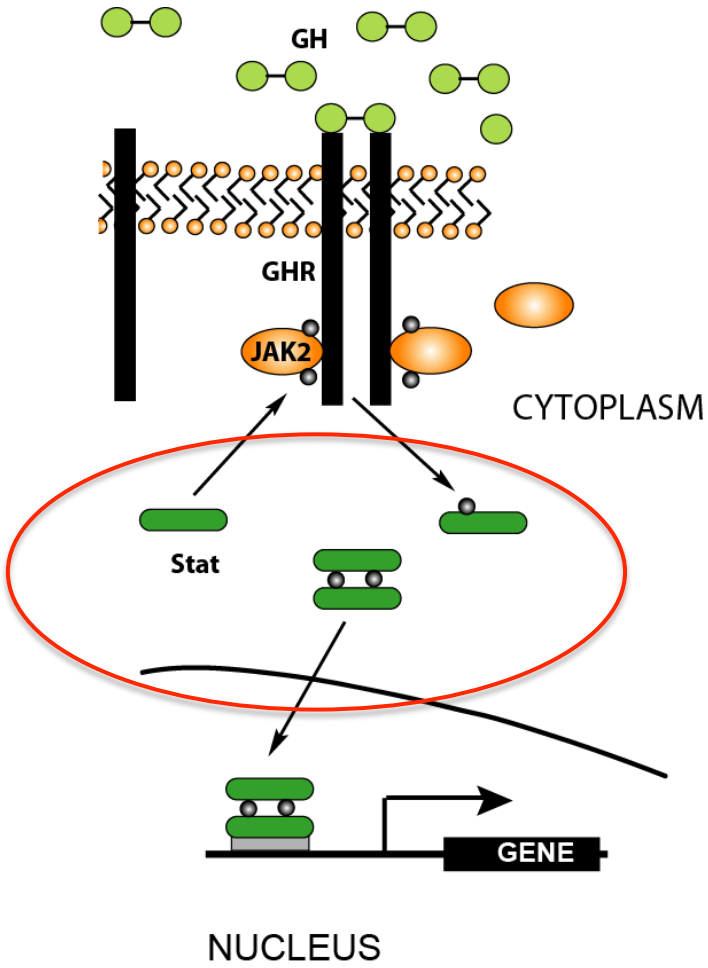


# Growth Hormone / Jak2 Signaling

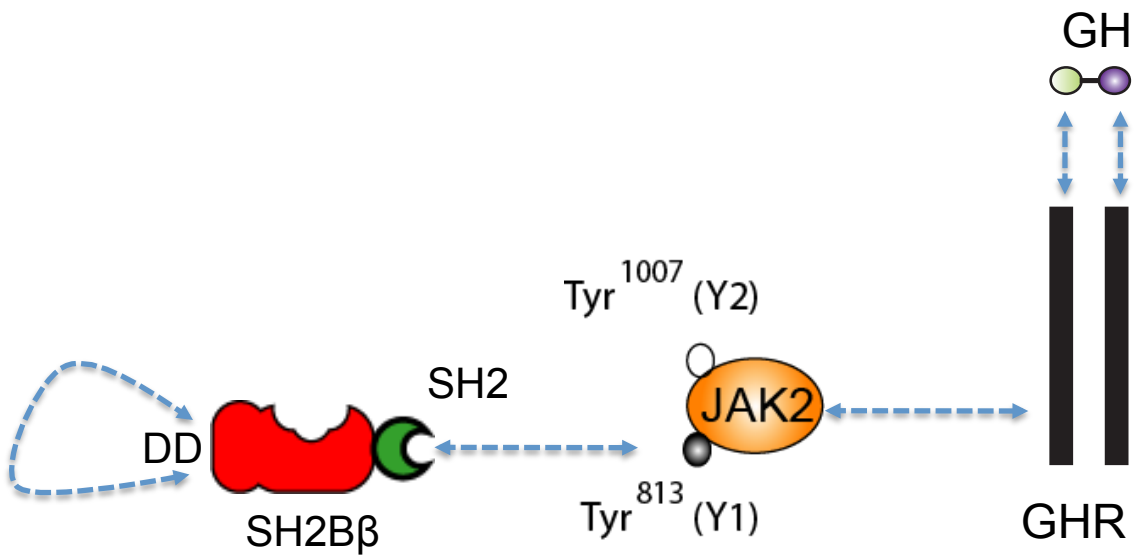
Ligand binding and receptor aggregation

Jak2 recruitment and transautophosphorylation

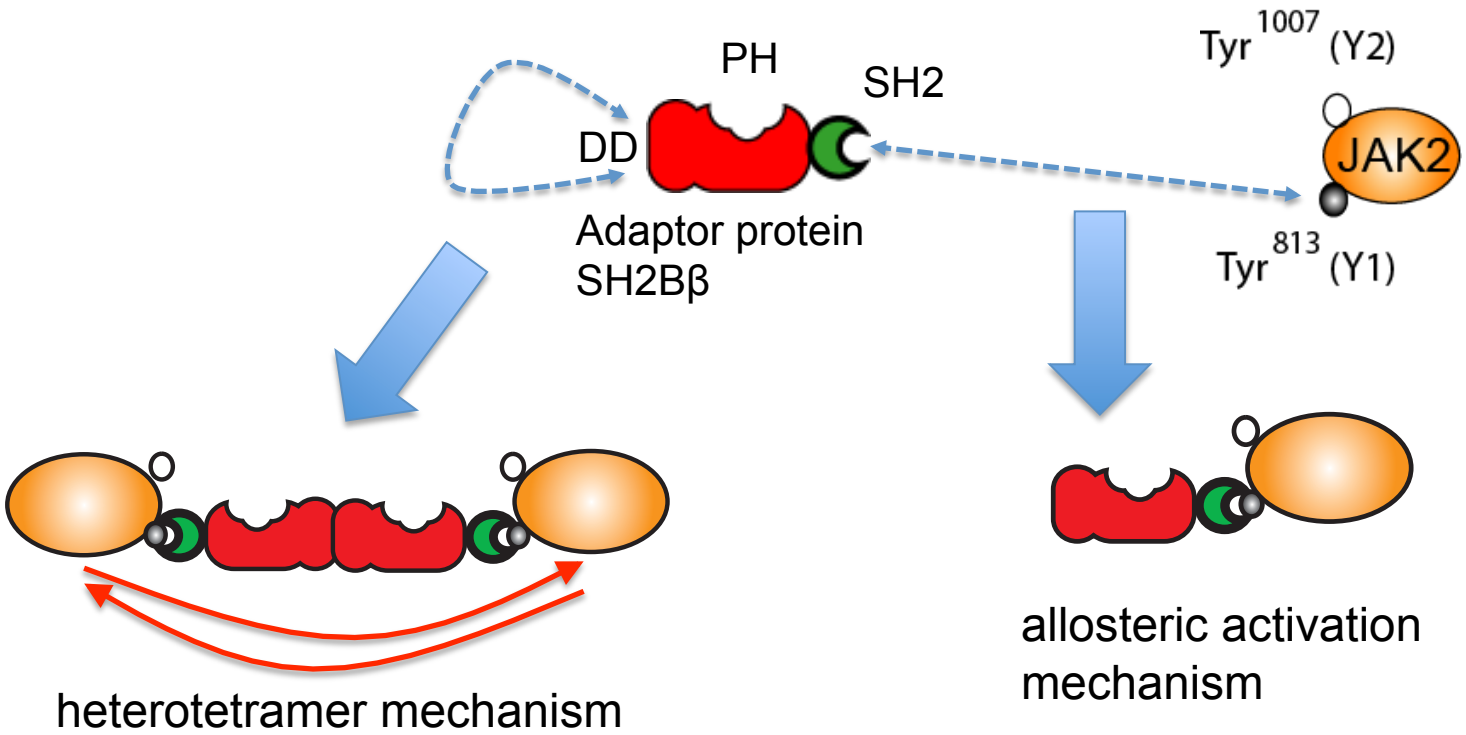
Stat3/5b phosphorylation, dimerization, and translocation



# Model of GHR-Jak2-SH2-Bβ Interactions



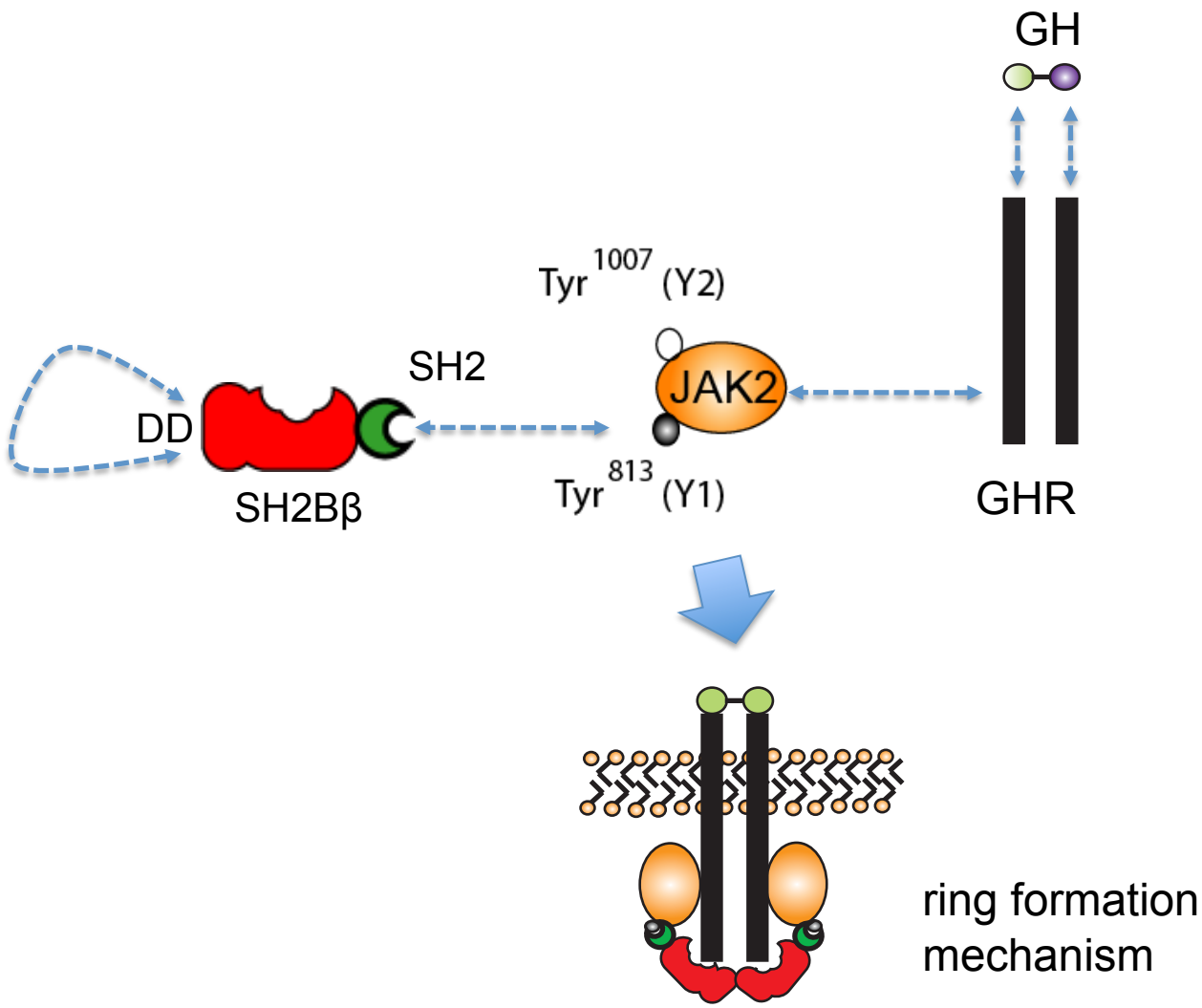
# SH2-Bβ: An Adaptor that Modulates Jak2 Activation by GHR



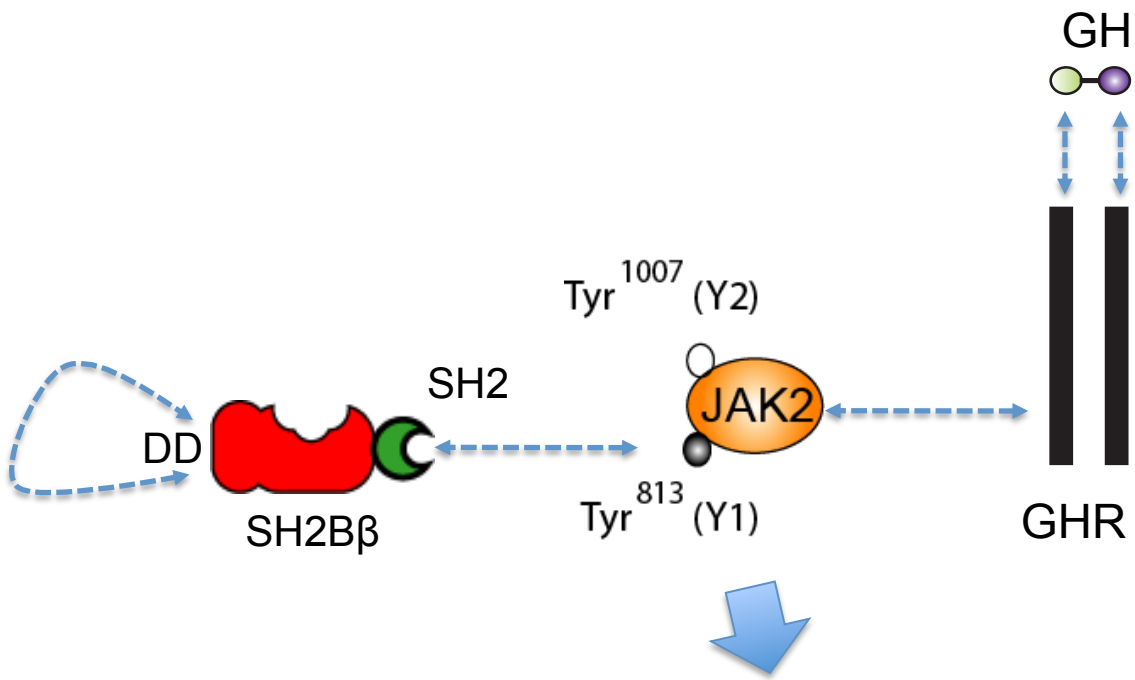

Nishi et al. (2005) *Mol Cell Biol* **25**, 2607-2621.

Rui et al. (2000) *Mol Cell Biol* **20**, 3168-3177

# Model of GHR-Jak2-SH2-Bβ Interactions - Third Mechanism

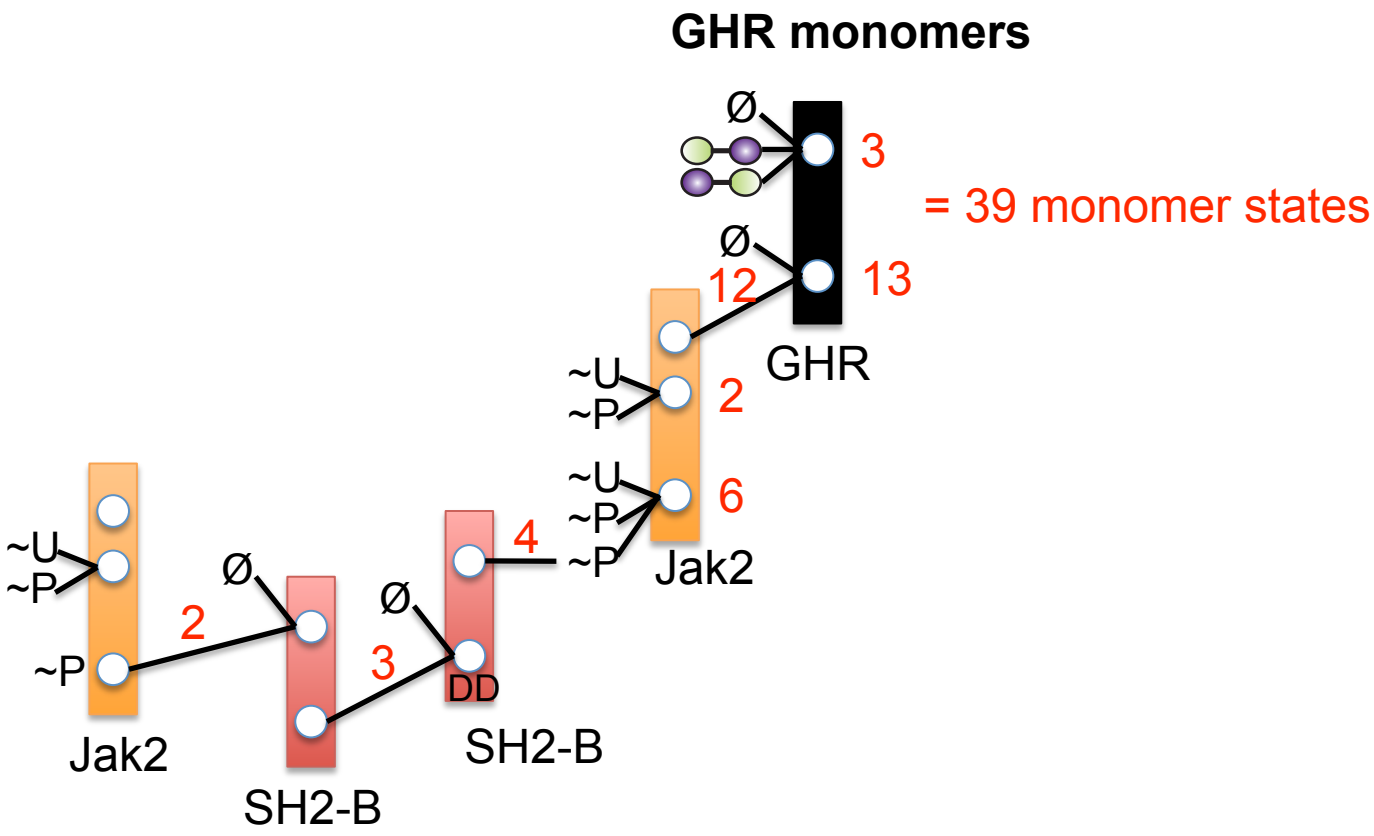


# Model of GHR-Jak2-SH2-Bβ Interactions - Complexity

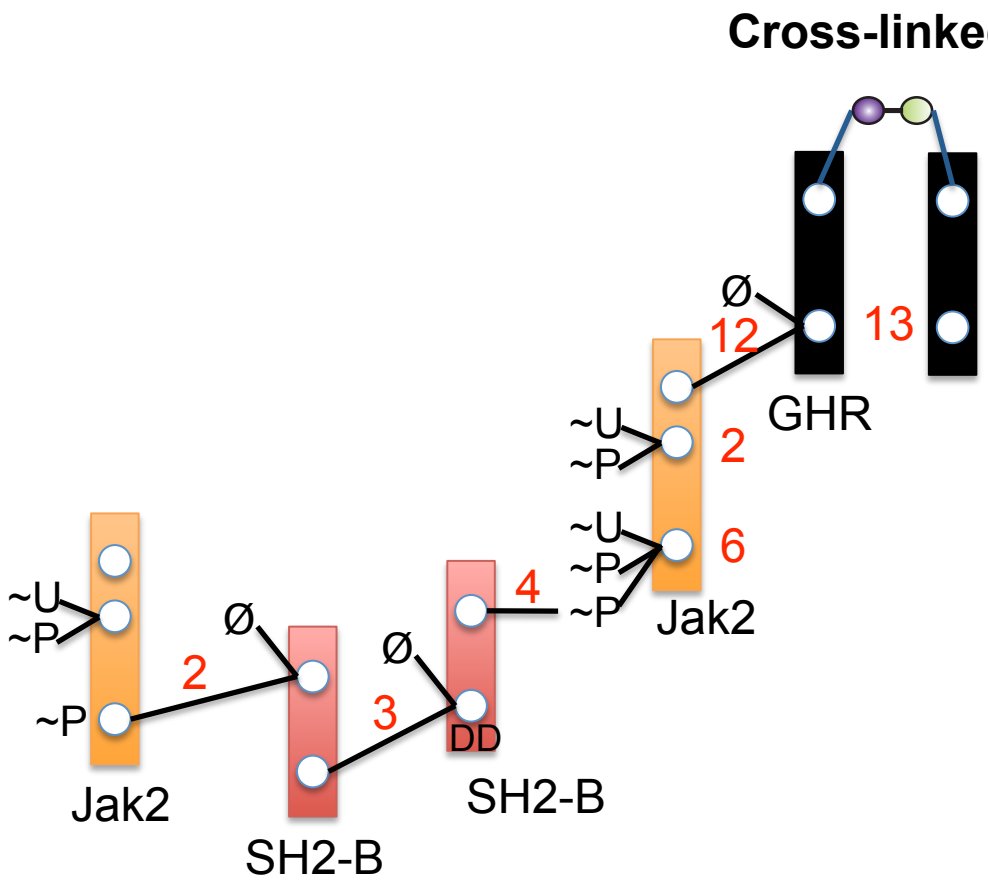



**741 Species / 5,033 Reactions**

# Combinatorial Complexity

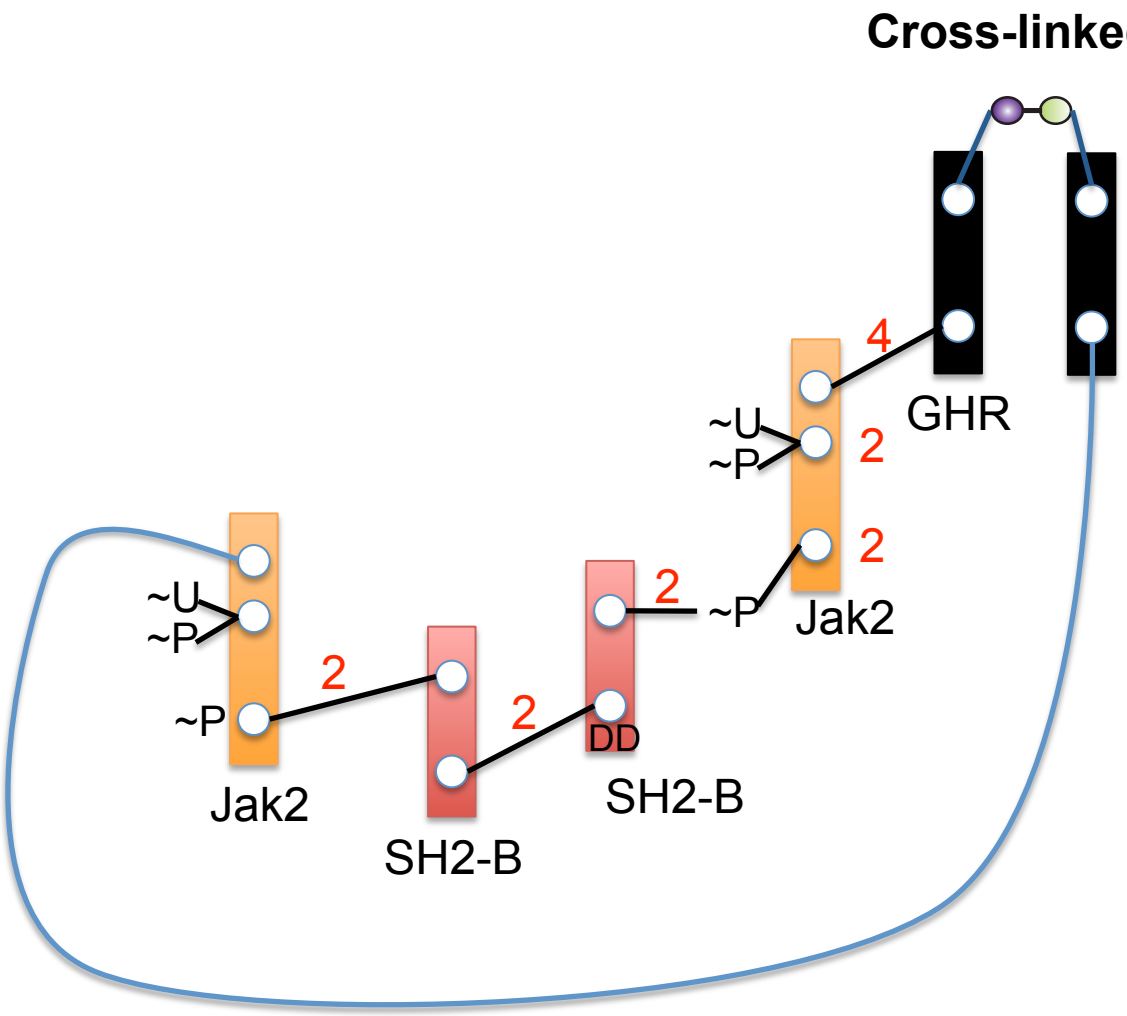


# Combinatorial Complexity



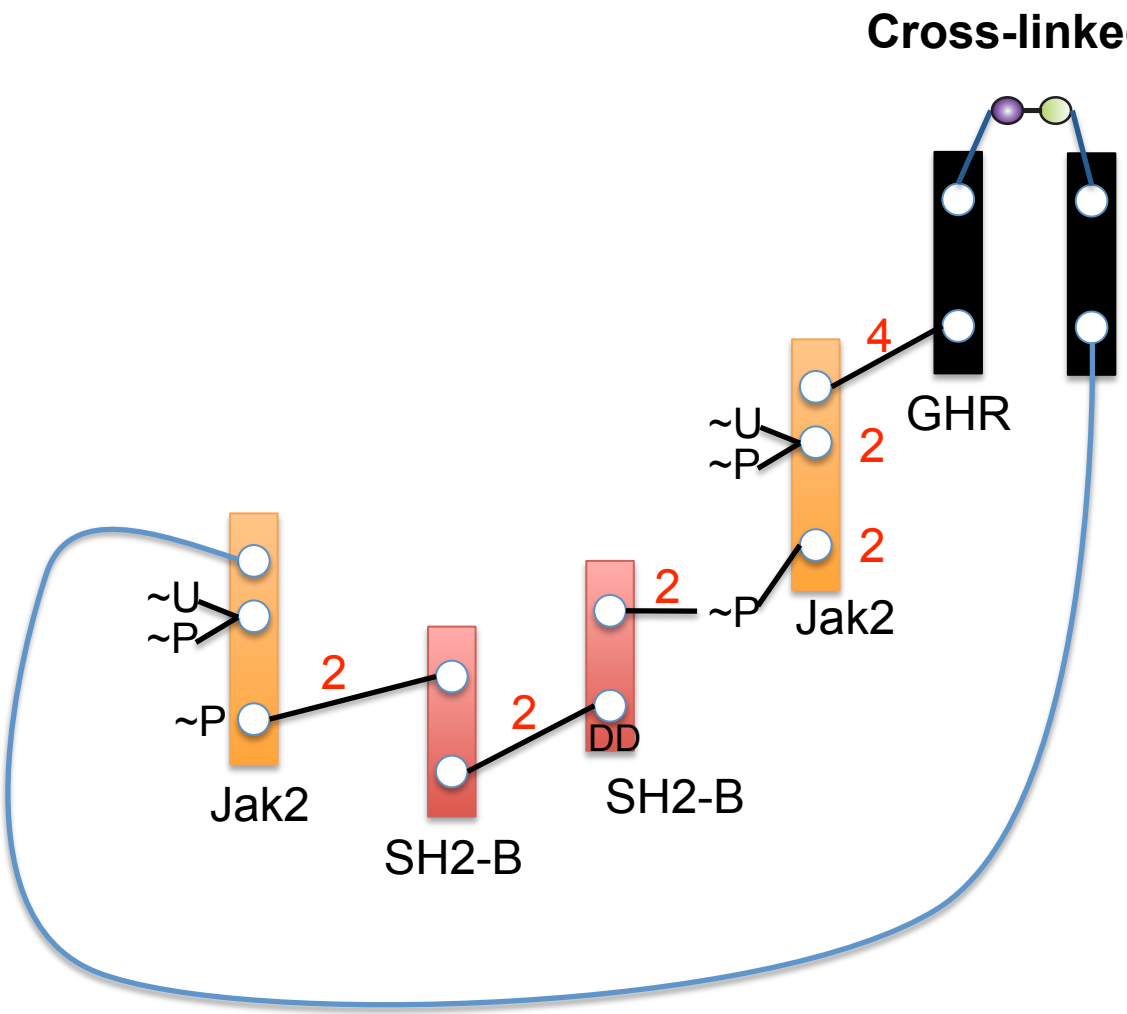

$(13)^2$ dimer (no ring) states

# Combinatorial Complexity




$(13)^2$ dimer (no ring) states
+ 4 dimer rings states
= 173 cross-linked dimers

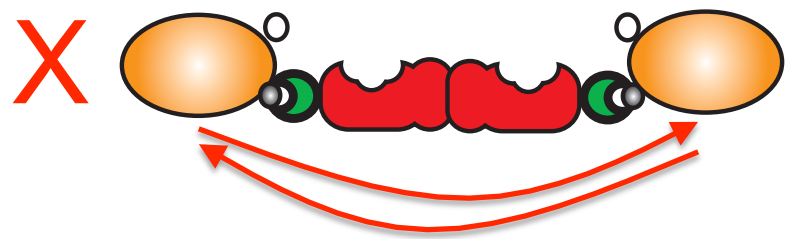
# Combinatorial Complexity




$(13)^2$ dimer (no ring) states
<u>+ 4 dimer rings states</u>
= 173 cross-linked dimers

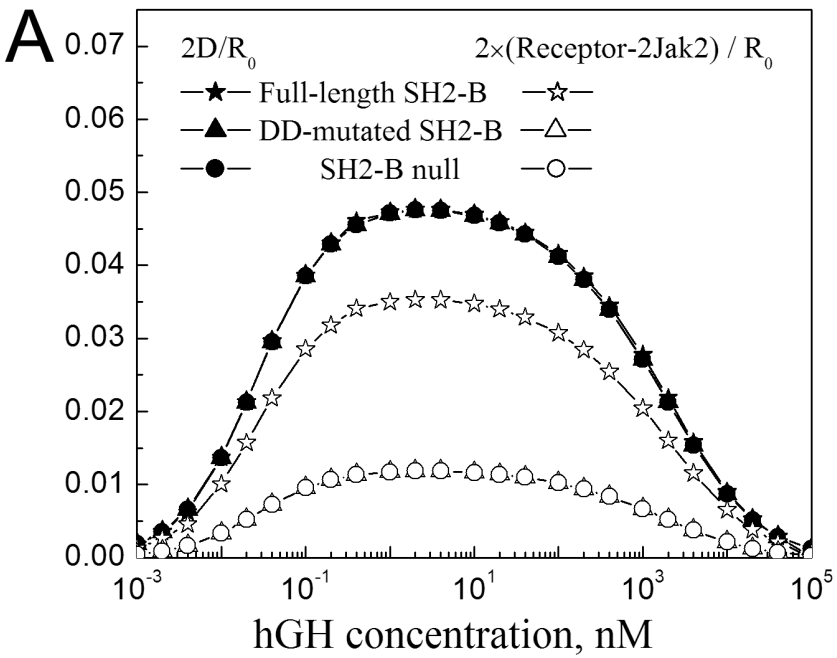
For a more formal analysis, see Danos et al., “Abstract Interpretation of Cellular Signaling Networks”, 2007.

# Model does not support the heterotetramer mechanism

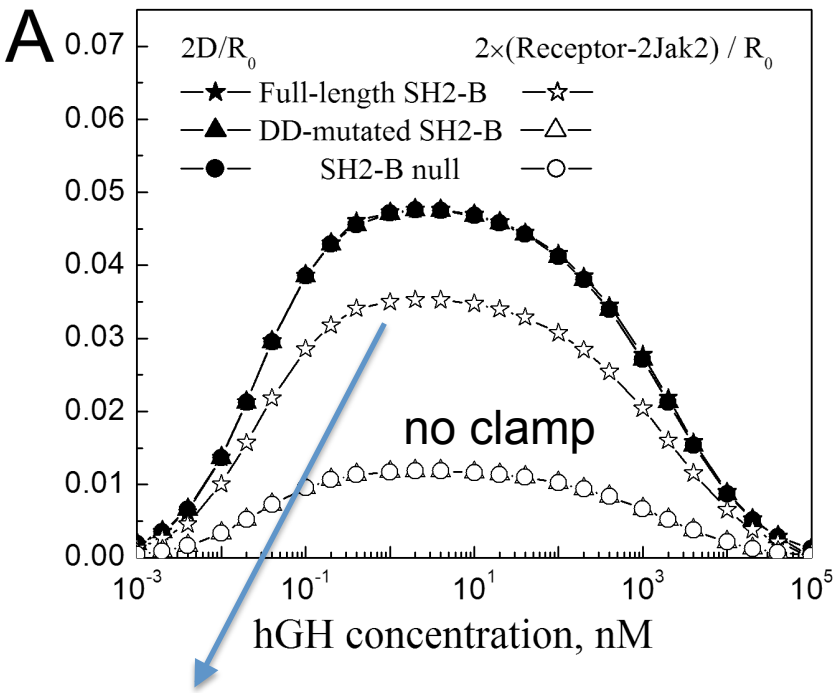


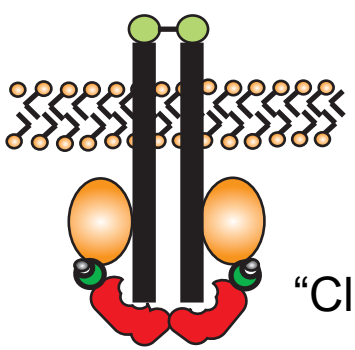

- Complex is short-lived and does not reach substantial concentration
- Observed phosphorylation is probably below detection limit, even when association rate increased
- Model may be an overestimate because it assumes Y813 is initially phosphorylated in the experiment

# In Vivo Results: Basic Cellular Model



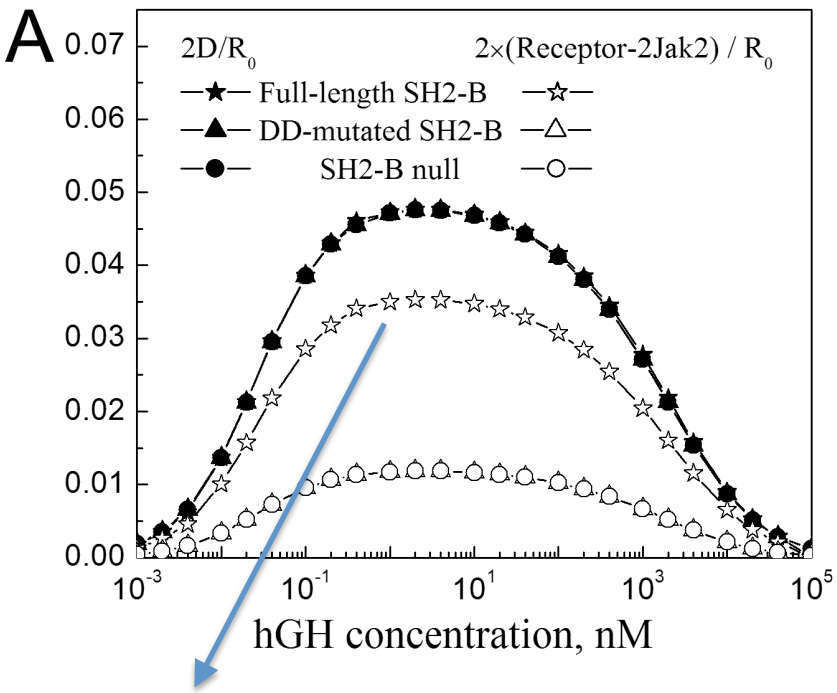
# In Vivo Results: Basic Cellular Model

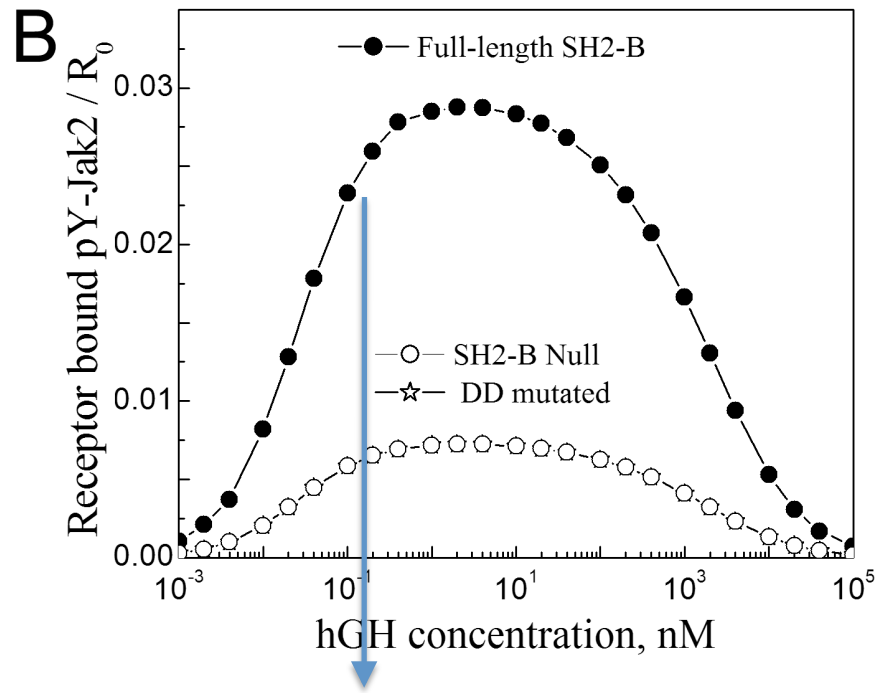


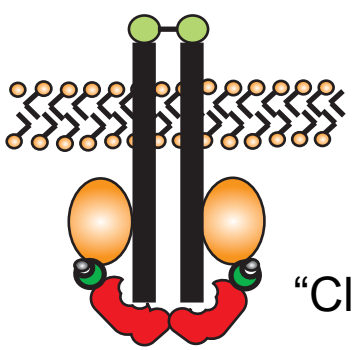



"Clamping"

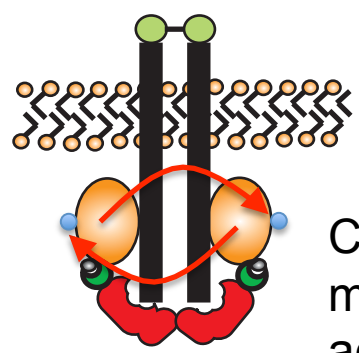
# In Vivo Results: Basic Cellular Model



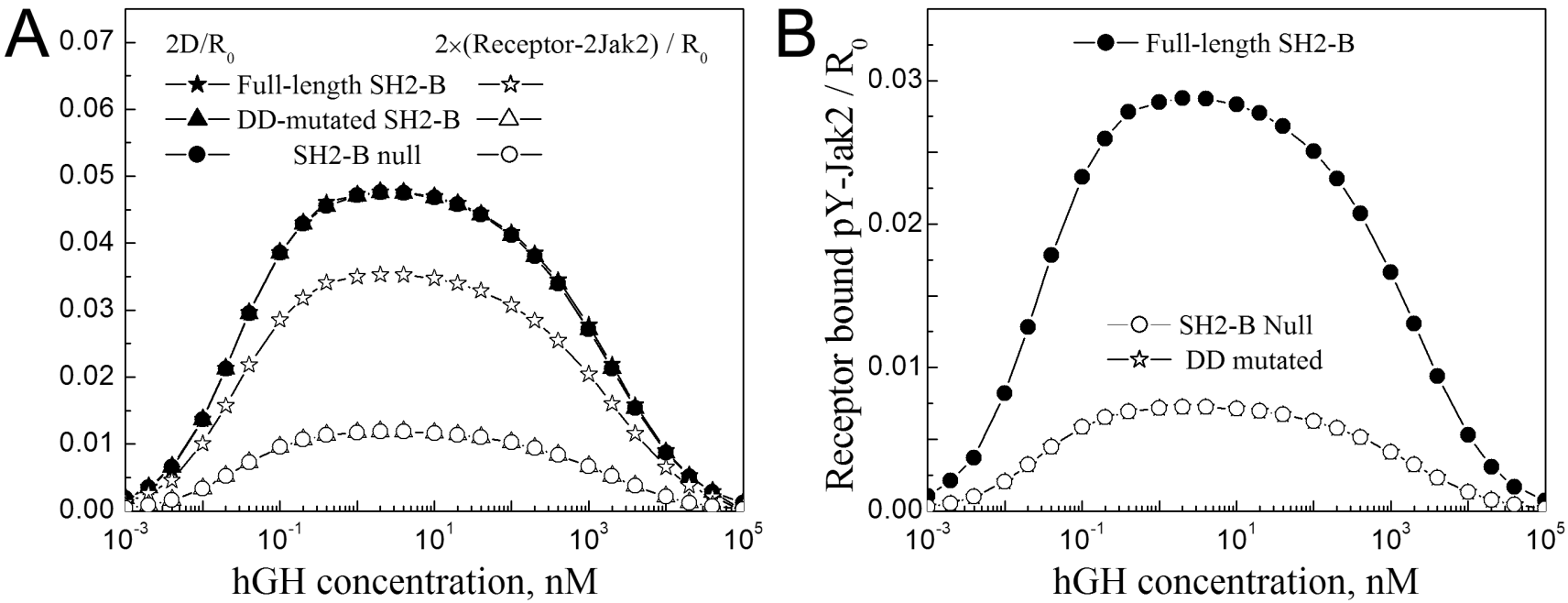



"Clamping"



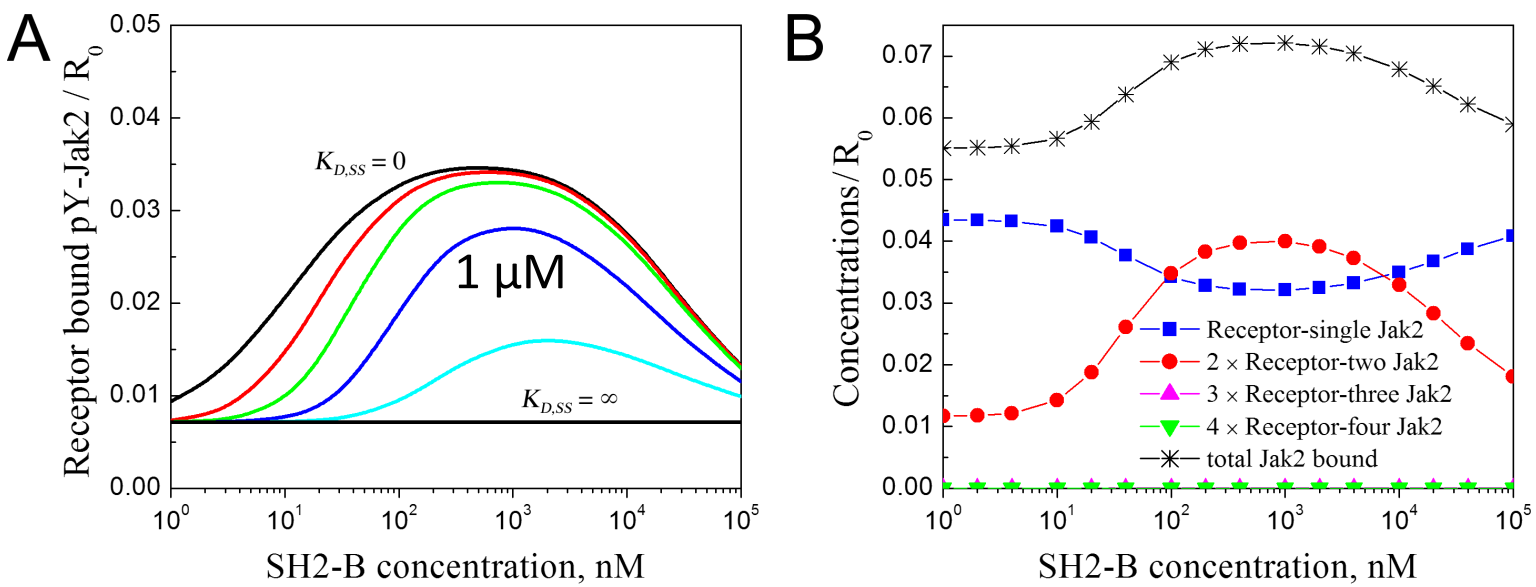
Clamping maintains activation

# In Vivo Results: Basic Cellular Model

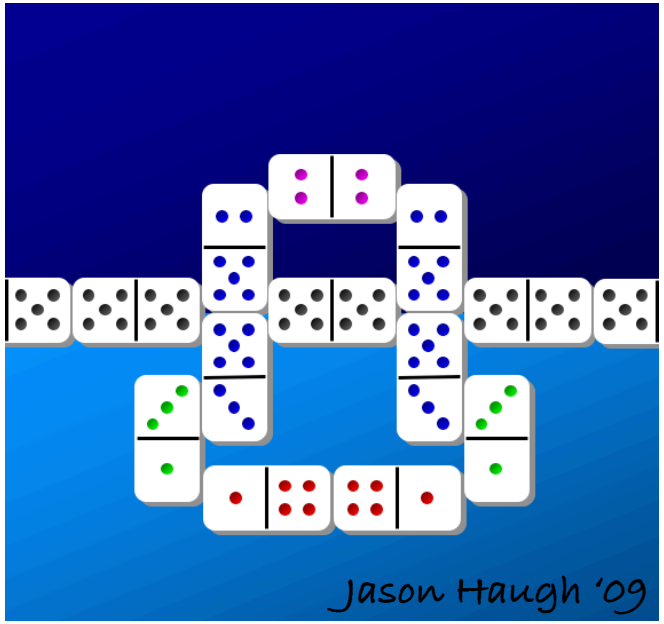



- Receptor dimerization is not affected
- Receptor-Jak2 tetramer is stabilized
- SH2-B gives about 4-fold increase in Jak2 activation
- SH2-B is effective only with intact dimerization domain

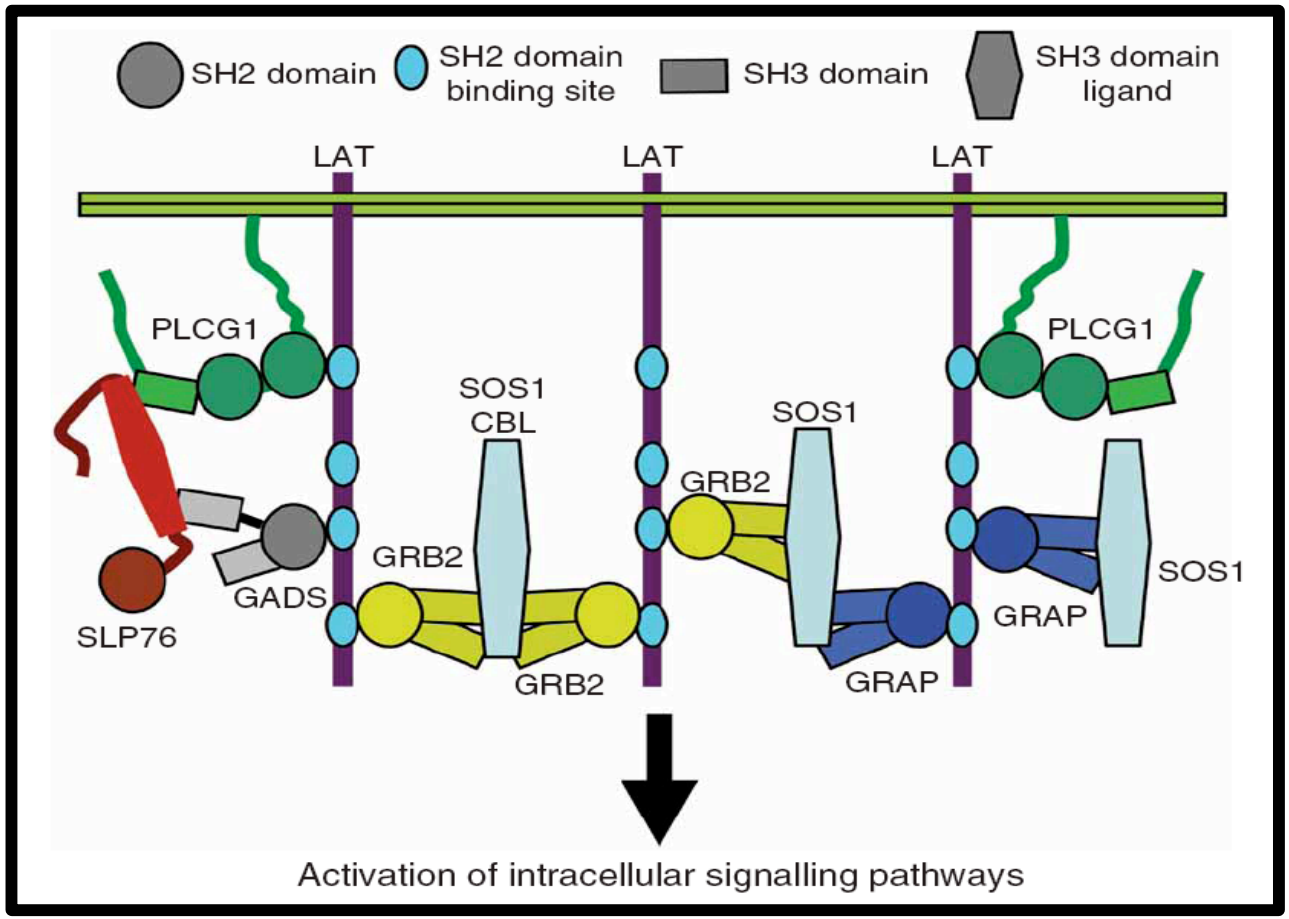
# Analysis of Ring Formation




- *Affinity for dimerization can be weak*
- SH2-B only modestly affects overall Jak2 recruitment
- Complexes with 2 Jak2 are preferentially stabilized
- Recruitment of more than 2 Jak2 is rare
- cross-linking of receptor complexes is negligible


Jason Haugh '09

# LAT oligomerization mediated by GRB2 and SOS1




Houtman et al., *Nat. Struct. Mol. Biol.*, 13, 798 (2006)

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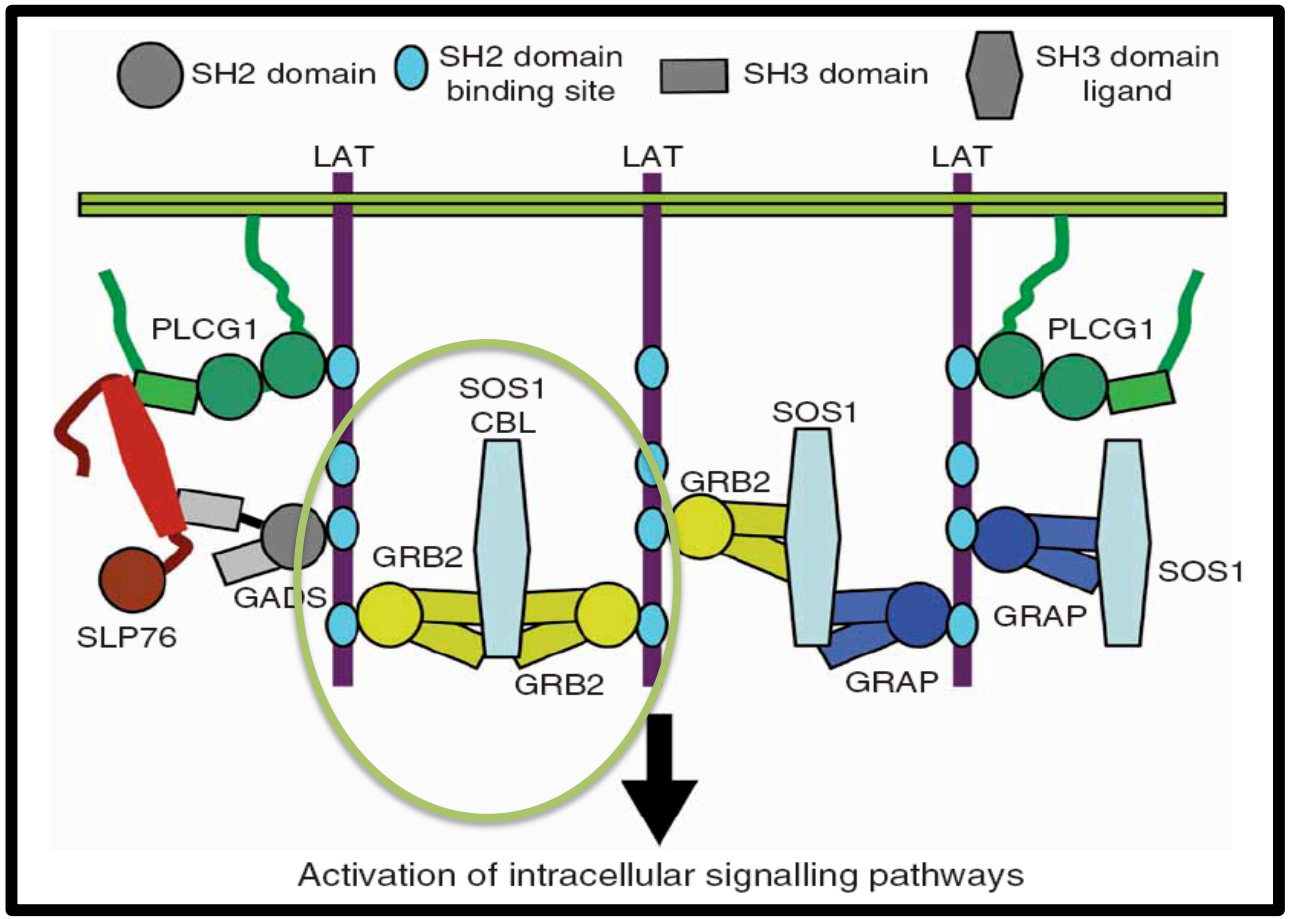



Houtman et al., *Nat. Struct. Mol. Biol.*, 13, 798 (2006)

# LAT oligomerization model

Formation of the 2:1 Grb2:Sos1 complex

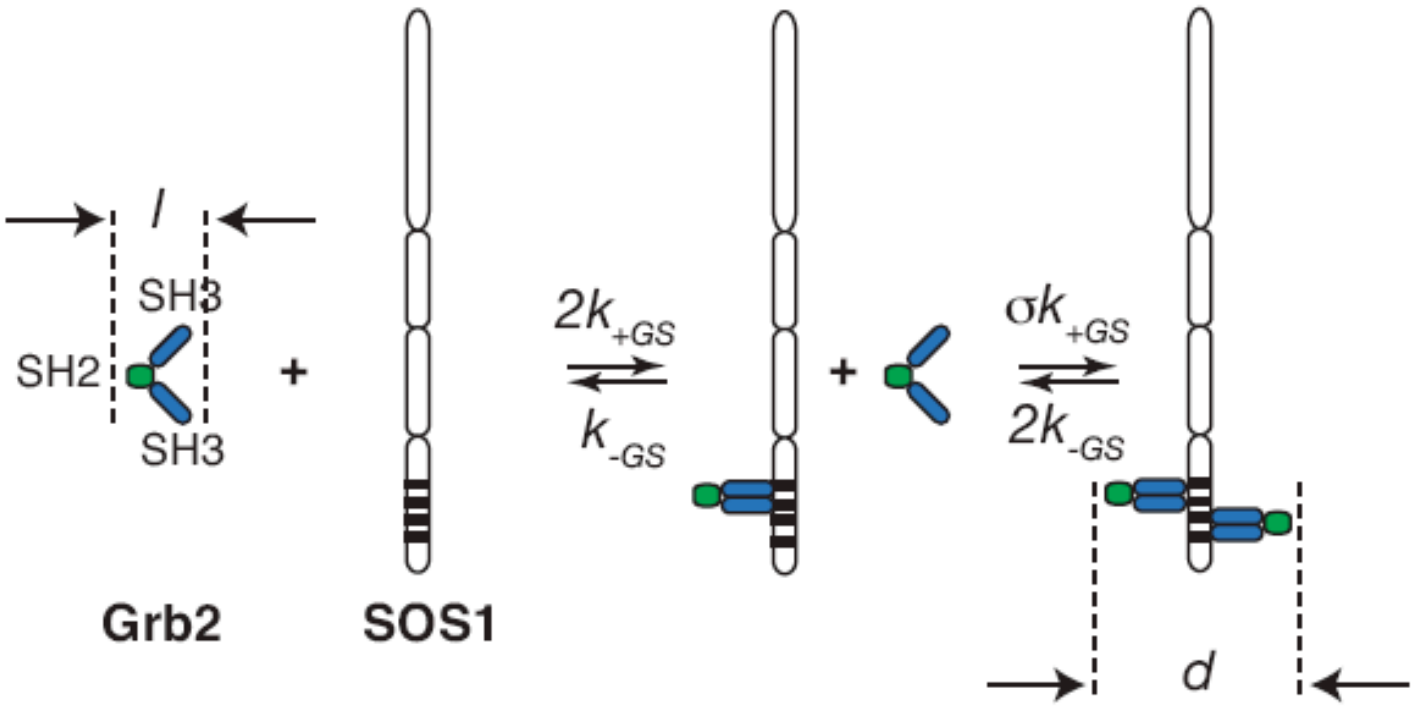
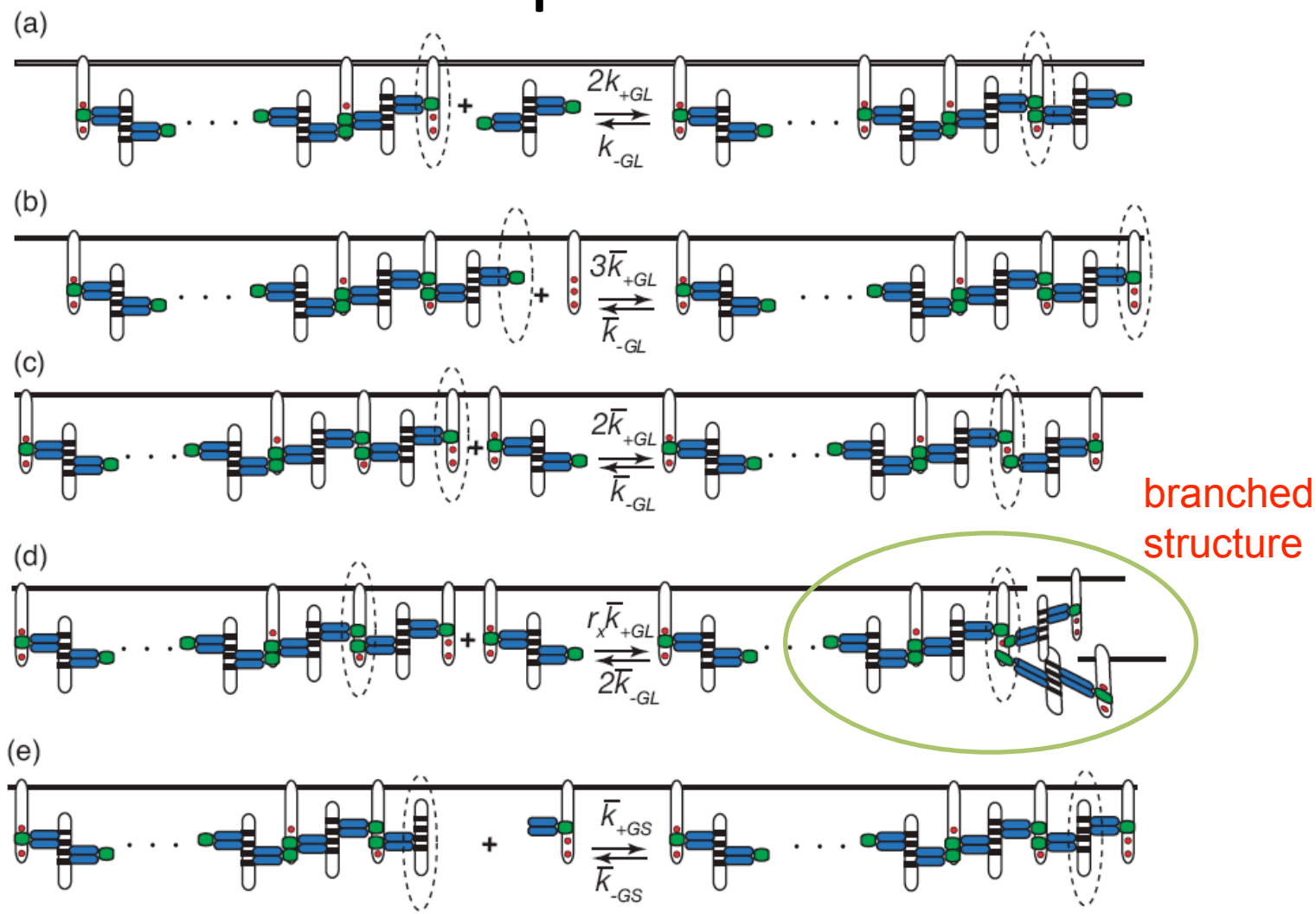



**Figure 1.**

Nag, Monine, Faeder, and Goldstein, *Biophys. J.* (2009)

# Binding and cross-linking via the 2:1 complex




Nag, Monine, Faeder, and Goldstein, *Biophys. J.* (2009)

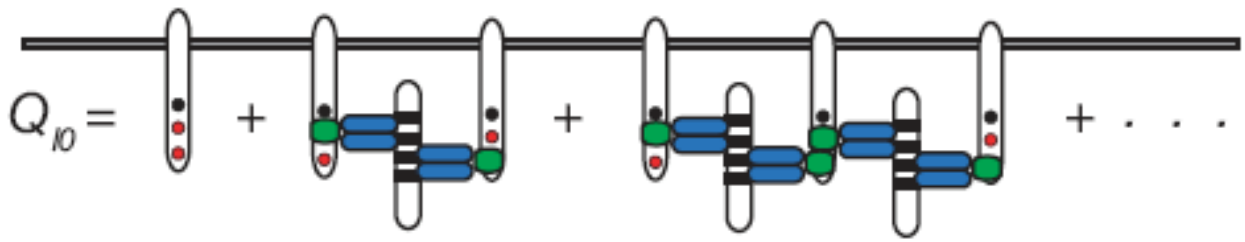
# Parameters of the model

| Parameter | Value | Reference |
|---|---|---|
| $V$ | $3.6 \times 10^{-10}$ cm$^3$ | (42) |
| $A$ | $8.0 \times 10^{-6}$cm$^2$ | (42) |
| $\sigma$ | 0.5 | (15) |
| $K_{GL}$ | $3.4 \times 10^6$ M$^{-1}$ | (15, 21, 23) |
| $k_{-GL}$ | 0.31 s$^{-1}$ | (43) |
| $k_{+GL} = k_{-GL}K_{GL}$ | $1.1 \times 10^6$ M$^{-1}$ s$^{-1}$ | |
| $K_{GS}$ | $2.7 \times 10^6$M$^{-1}$ | (15) |
| $k_{+GS}$ | $9.5 \times 10^4$ M$^{-1}$ | (27) |
| $k_{-GS} = k_{+GS}/K_{GS}$ | 0.03 s$^{-1}$ | |
| $\bar{K}_{GL}$ | $1.7 \times 10^{15}$ mole$^{-1}$ cm$^2$ | see caption[1] |
| $\bar{k}_{-GL}$ | 0.31 s$^{-1}$ | |
| $\bar{k}_{+GL} = \bar{k}_{-GL}\bar{K}_{GL}$ | $5.3 \times 10^{14}$ mole$^{-1}$ cm$^2$ s$^{-1}$ | |

Nag, Monine, Faeder, and Goldstein, *Biophys. J.* (2009)

# Equilibrium theory

Partition function = sum of aggregate concentrations




$$q = w + \frac{1}{\gamma q_0}\left[1 - \frac{1-(1-4\gamma q_0^2)^{\frac{3}{2}}}{6\gamma q_0^2}\right]. \tag{24}$$

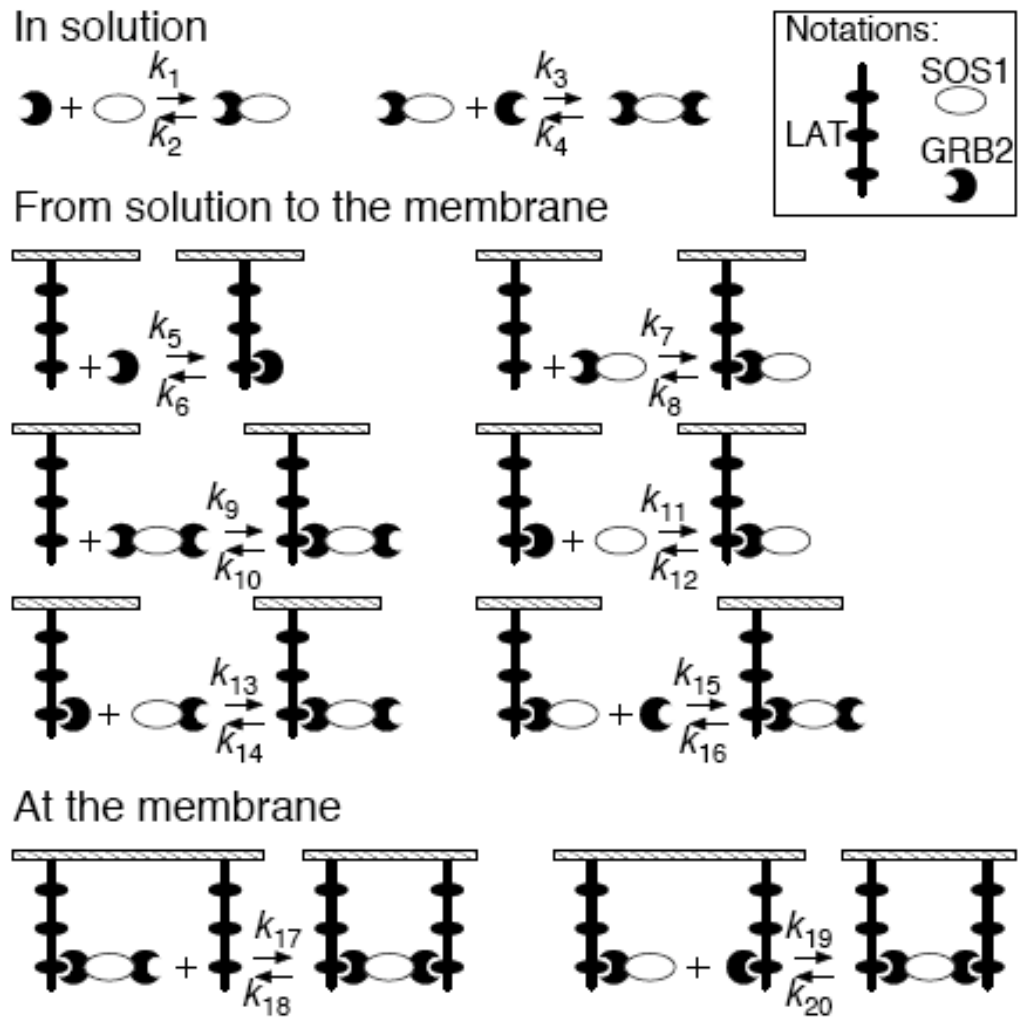
Conservation laws:

$$g + \mu g s + \sigma \mu \theta g^2 s + \left(\frac{AL_T}{VG_T}\right) g \left(\frac{\partial q_l}{\partial g}\right) = 1.$$

$$s + 2\theta g s + \sigma \theta^2 g^2 s + \left(\frac{AL_T}{VS_T}\right) s \left(\frac{\partial q_l}{\partial s}\right) = 1.$$

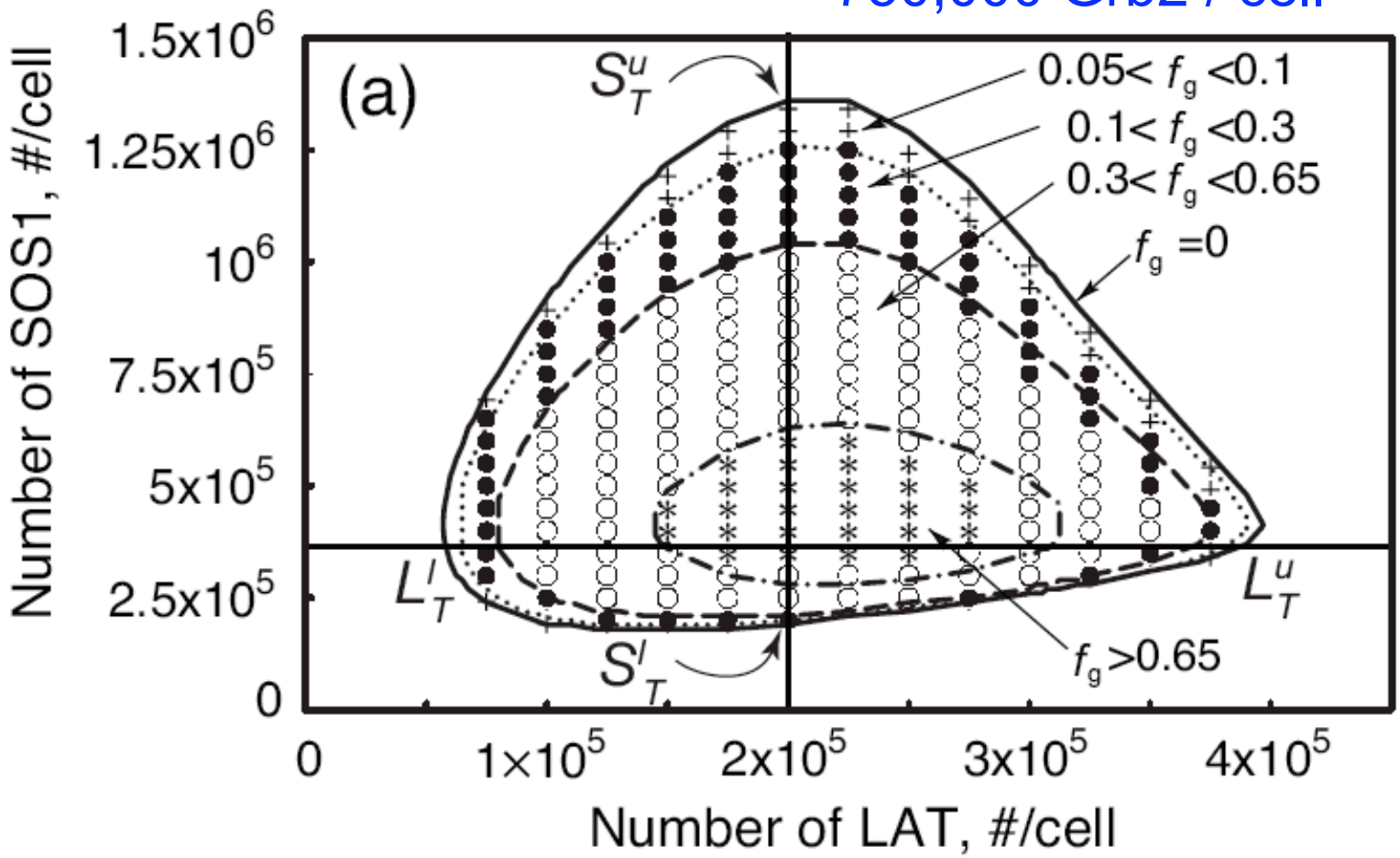
$$l \left(\frac{\partial q_l}{\partial l}\right) = w \left(\frac{\partial q_l}{\partial w}\right) = 1.$$

# Rules for the kinetic model

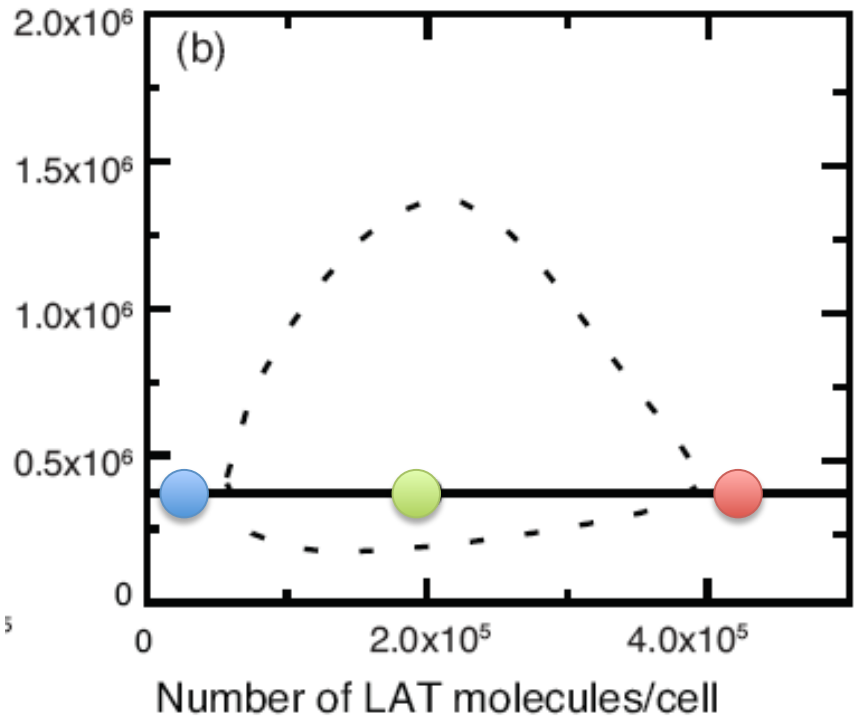


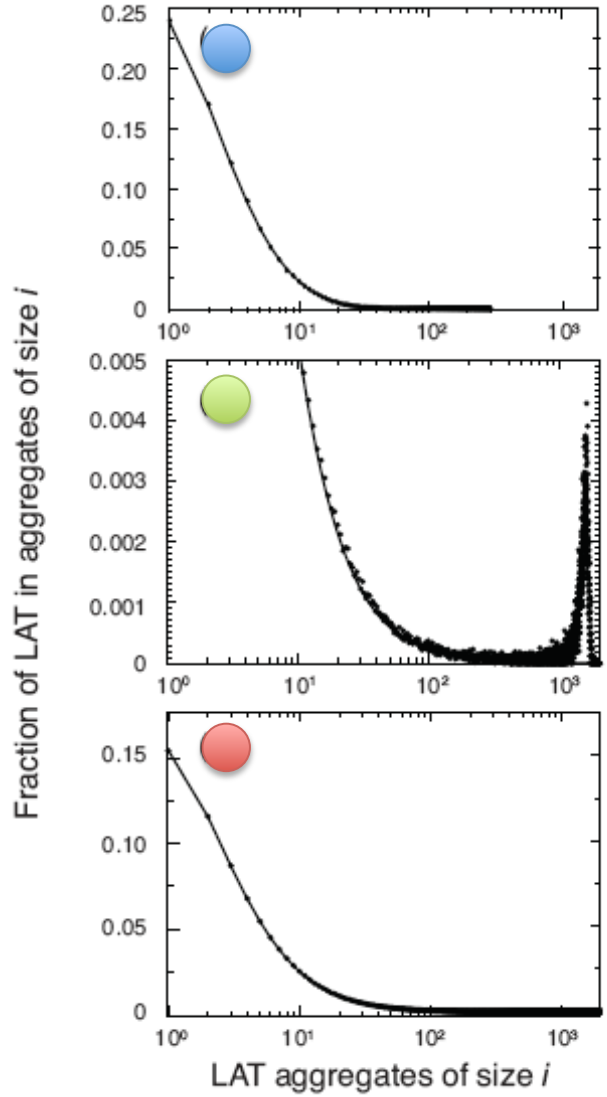
# Phase diagram for sol-gel coexistence

750,000 Grb2 / cell

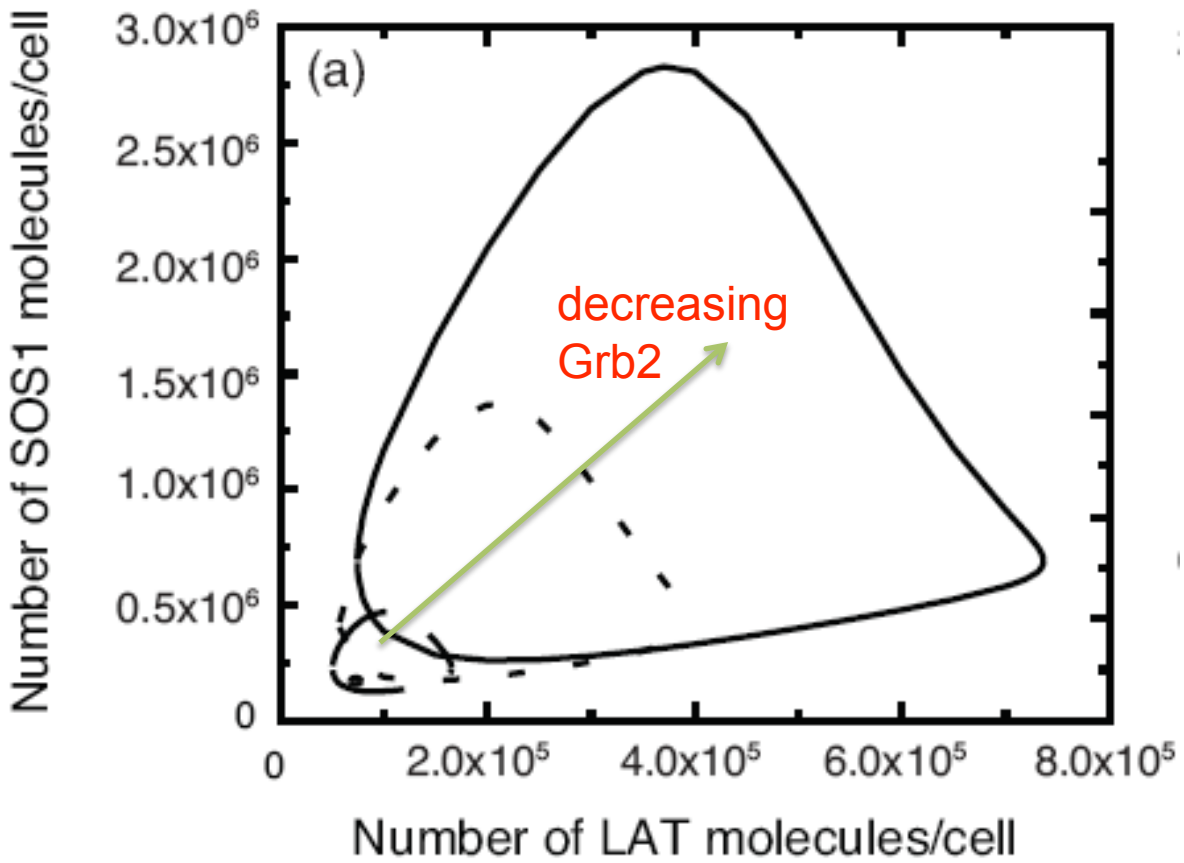


# Distribution of LAT aggregates



# Area of gel phase is sensitive to Grb2 concentration




Do cells embrace or avoid this phenomenon?

# Experimental observation of LAT aggregation

JCB Article

## High resolution mapping of mast cell membranes reveals primary and secondary domains of FcεRI and LAT

**Bridget S. Wilson, Janet R. Pfeiffer, Zurab Surviladze, Elizabeth A. Gaudet, and Janet M. Oliver**

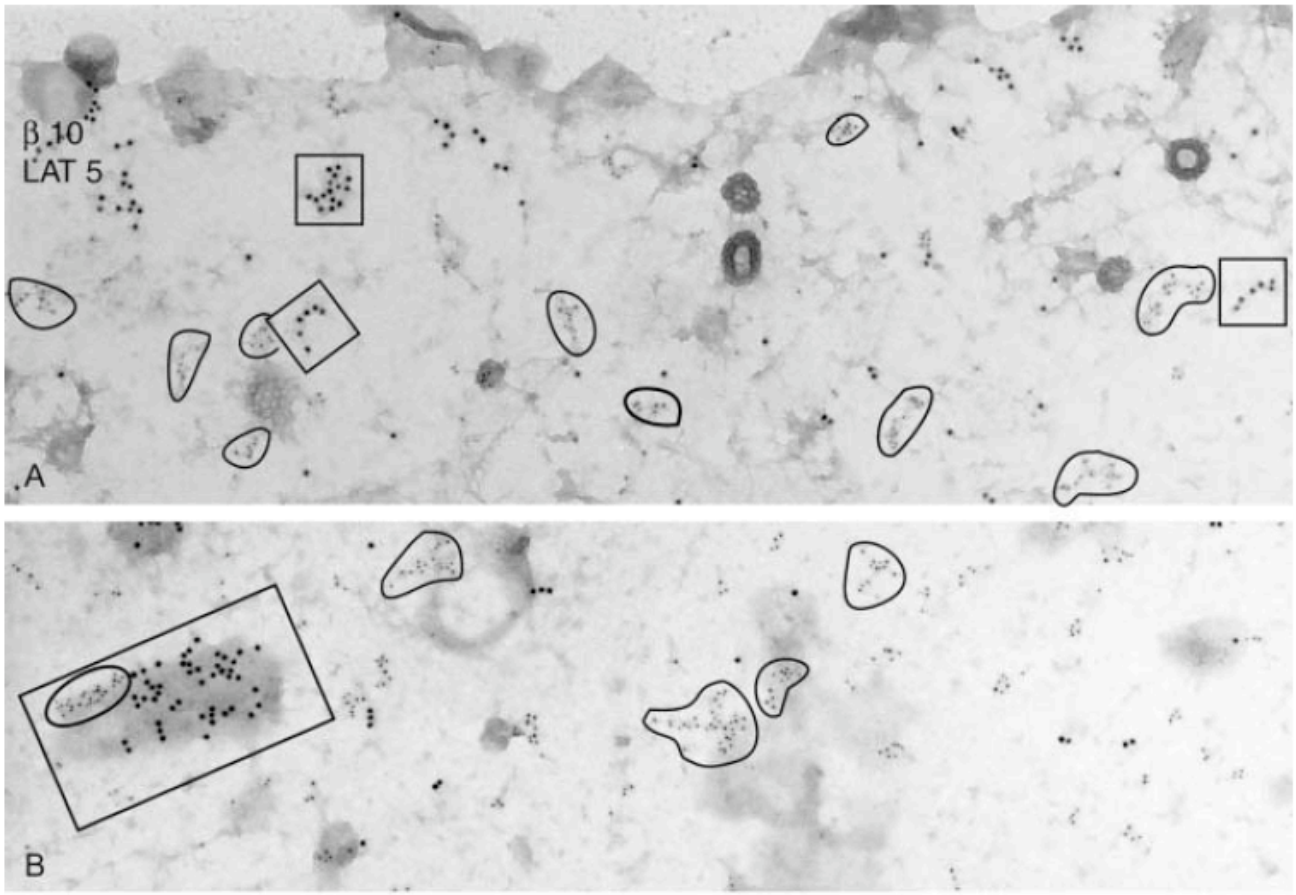
Department of Pathology and Cancer Research and Treatment Center, University of New Mexico Health Sciences Center, Albuquerque, NM 87131

Wilson et al., *J. Cell Biol.* **154**, 645 (2001)

# Experimental observation of LAT aggregation

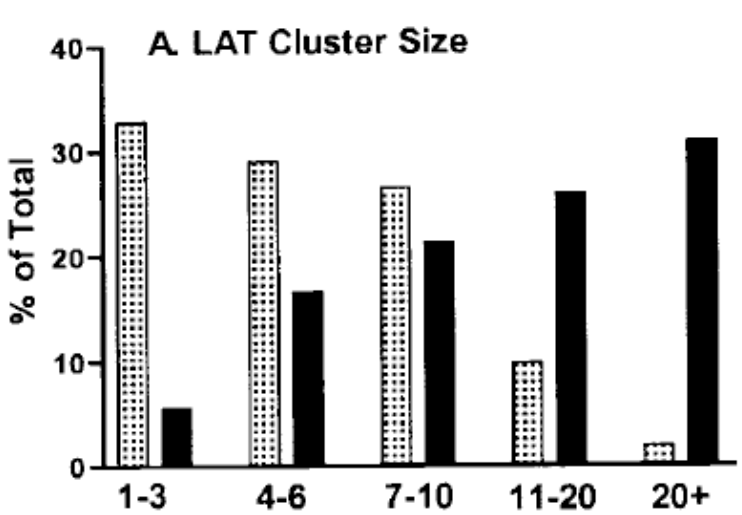
Before FcεRI cross-linking

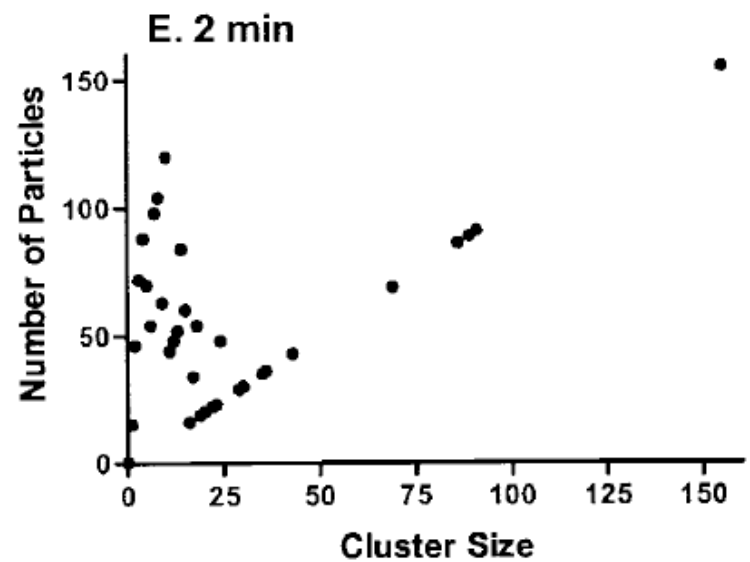
After FcεRI cross-linking




Wilson et al., *J. Cell Biol.* **154**, 645 (2001)

# Experimental observation of LAT aggregation



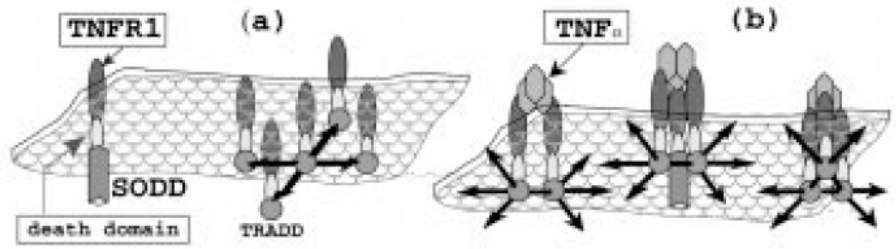




Wilson et al., *J. Cell Biol.* **154**, 645 (2001)

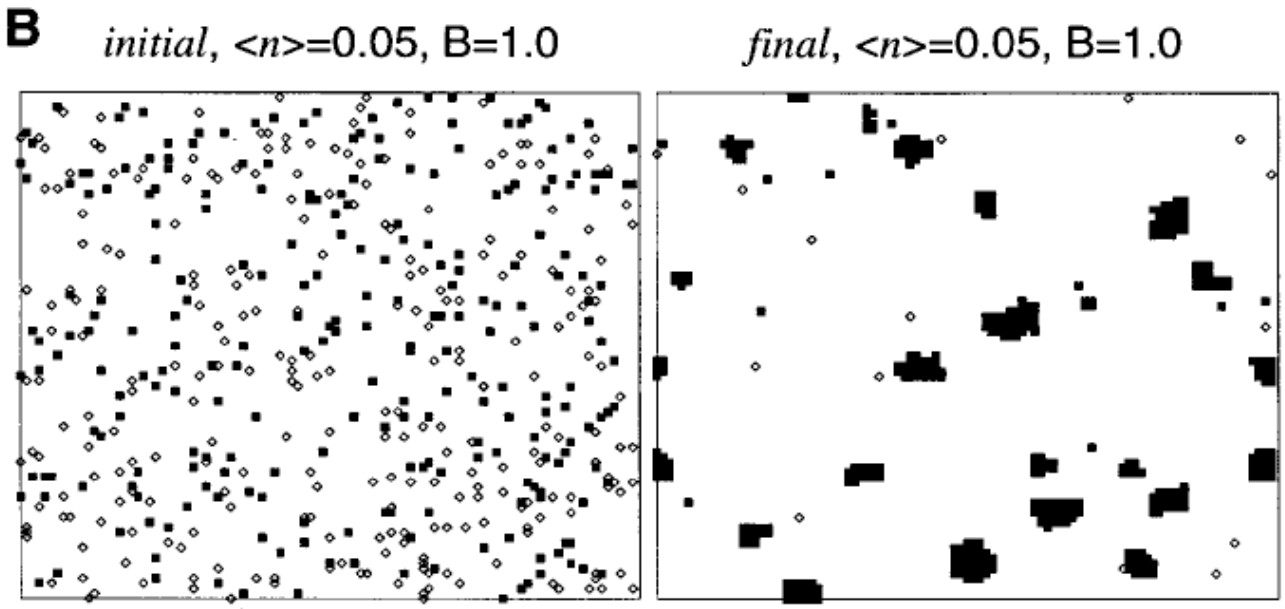
# Open Problem

- Spatial simulations of LAT (and aggregation of other signaling proteins at the membrane
- Possible approaches include
  - Brownian Dynamics simulations
  - Lattice-based (stat. mech.) simulations

# Statistical Mechanics Model for Receptor Clustering




$$H_0(\{n,t\}) = -\sum \mu(t_i) n_i \equiv -\sum \mu\left((R_k L_l S_m T_n)_i\right) n_i$$




Guo and Levine, *J. Biol. Phys.* **26**, 219-234 (2000)