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# **In silico model-based inference: applications to anti-tumor immunity**

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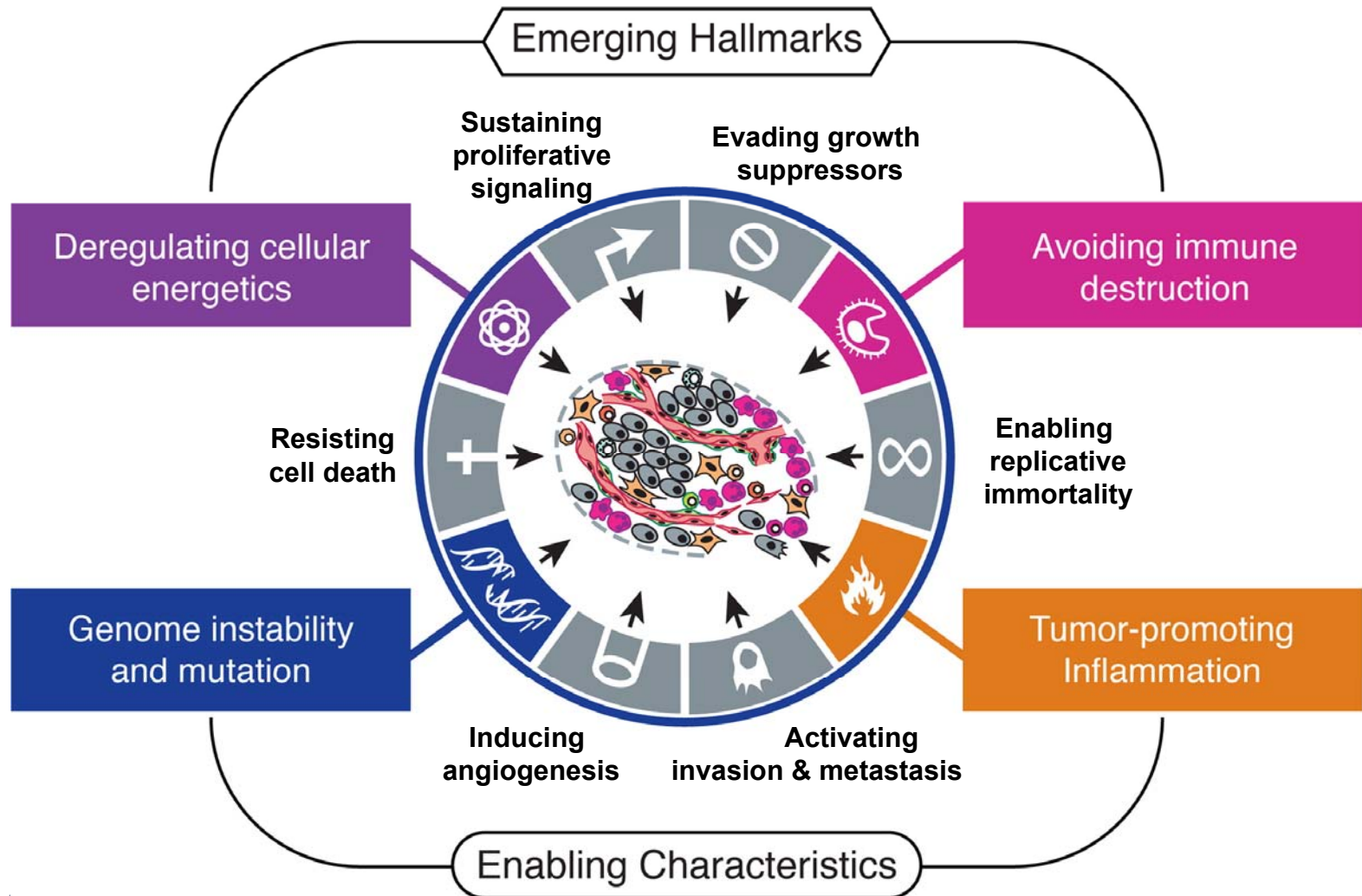
# Summary

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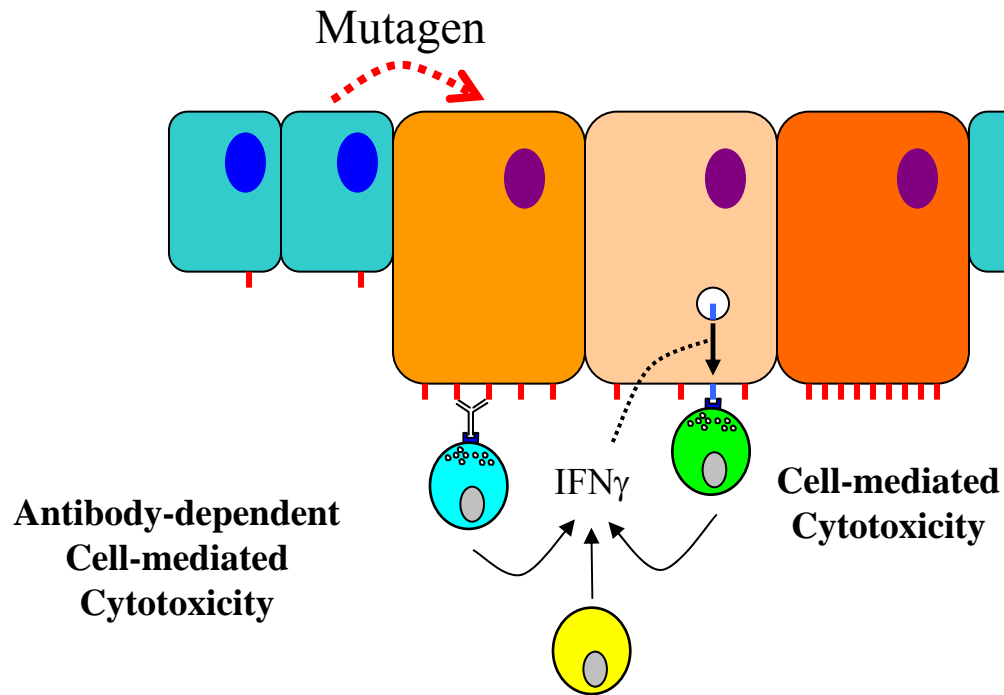
- Anti-tumor immunity
- *In silico* model-based inference
- Examples
  - Simple enzyme kinetics
  - Cell signaling
  - Tumor immunology



# Immunescape recognized as an emerging hallmark of cancer






# The right cytokine response shapes cell-mediated anti-tumor immunity



- **Local delivery of IL-12 enhances anti-tumor immunity** (Kerkar et al. Cancer Res 2010)
- **Cancer is an evolutionary process** (Klinke *submitted* 2012)

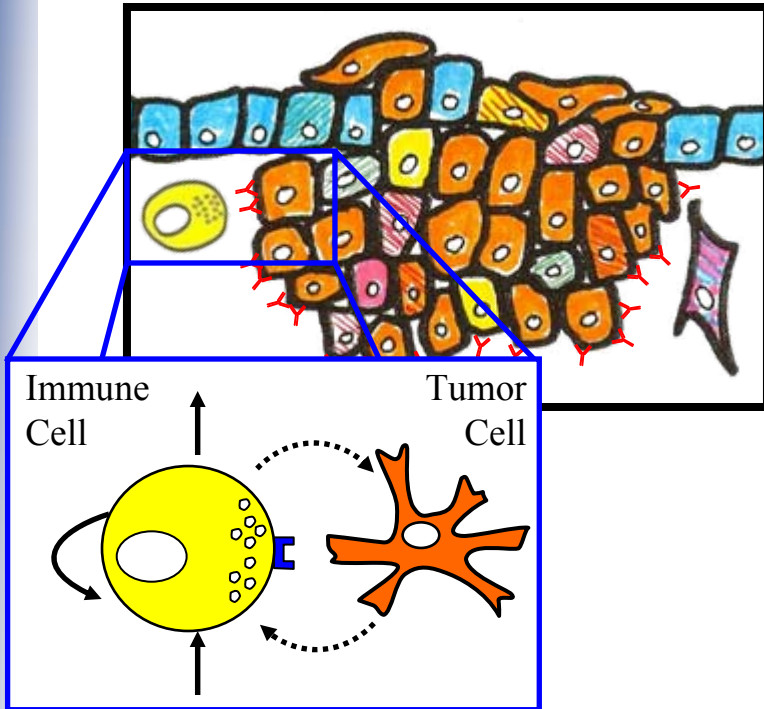
**Do tumor cells alter immune fitness landscape by interfering with endogenous Interleukin-12?**



<u>IL-12</u>	<u>Key Cell Types</u>
Activates NK cells	 Natural Killer Cell
Promotes Th1 differentiation	 T Helper Cell
Promotes effector differentiation	 Cytotoxic T Lymphocyte

# Biological problem poses constraints on plan of attack

Klinke *Mol Cancer* (2010) 9:242.



- Create minimal experimental system where immune cells exhibit well-characterized response
- Dynamical system spans minutes to days
  - Multiscale – need to deconvolute cell fate from signaling events.
  - Slaving – response of system governed by slow events
- Rich prior knowledge regarding causal relationships – competing hypotheses
- Kinetic importance of nodes/edges is unclear
- Evaluate competing causal hypotheses using available data



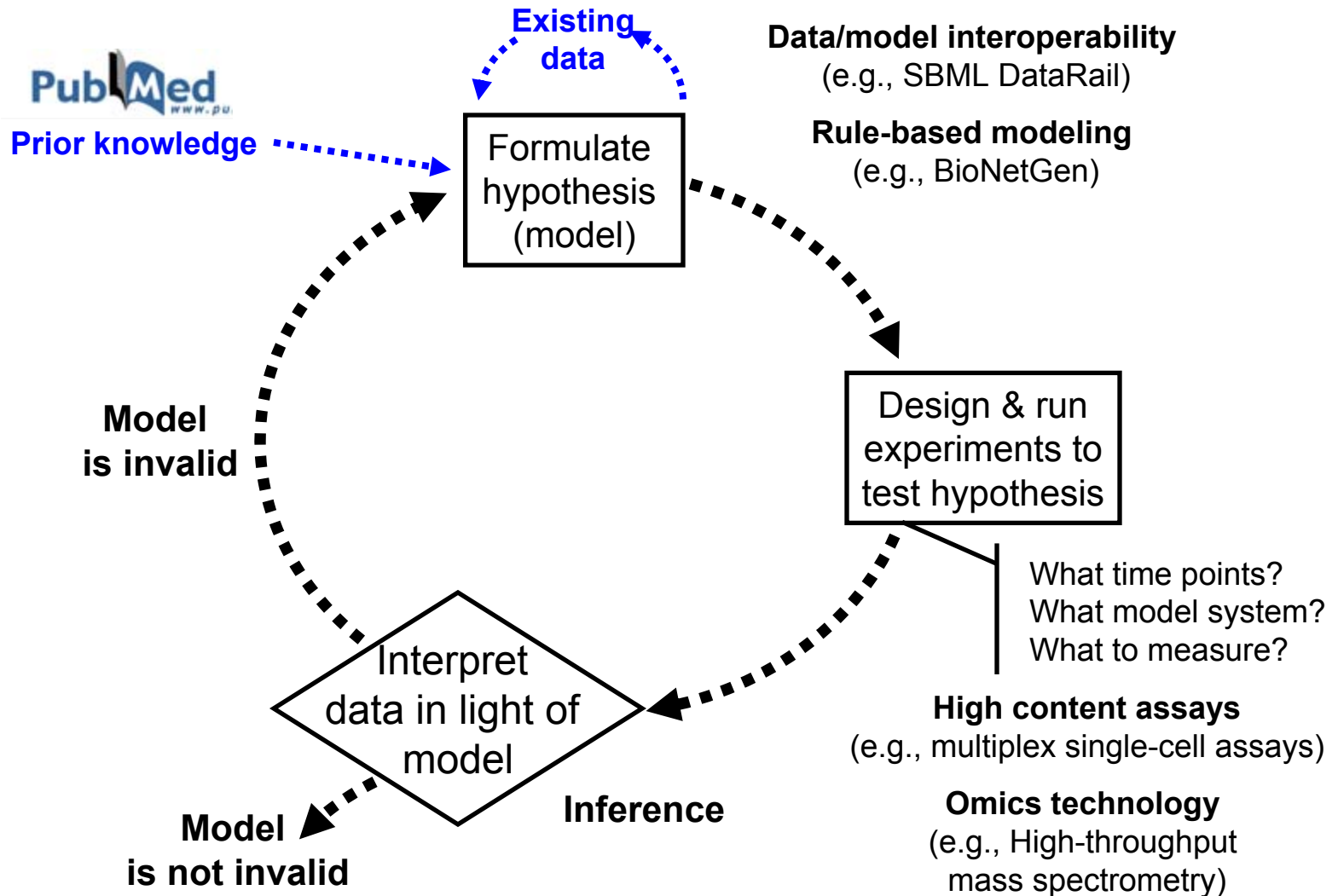
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# The scientific method is a structured activity used to improve understanding of systems



# Inference is the logical reasoning about our understanding of a system using observations

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- Our understanding of the system can be expressed in terms of a model (M)
- Logic can be extended using probability
- Probability is conditioned
  - $P(Y|M)$  : observing an event (Y) is conditioned on a cause (M)

Cause (M): Happy

Unhappy

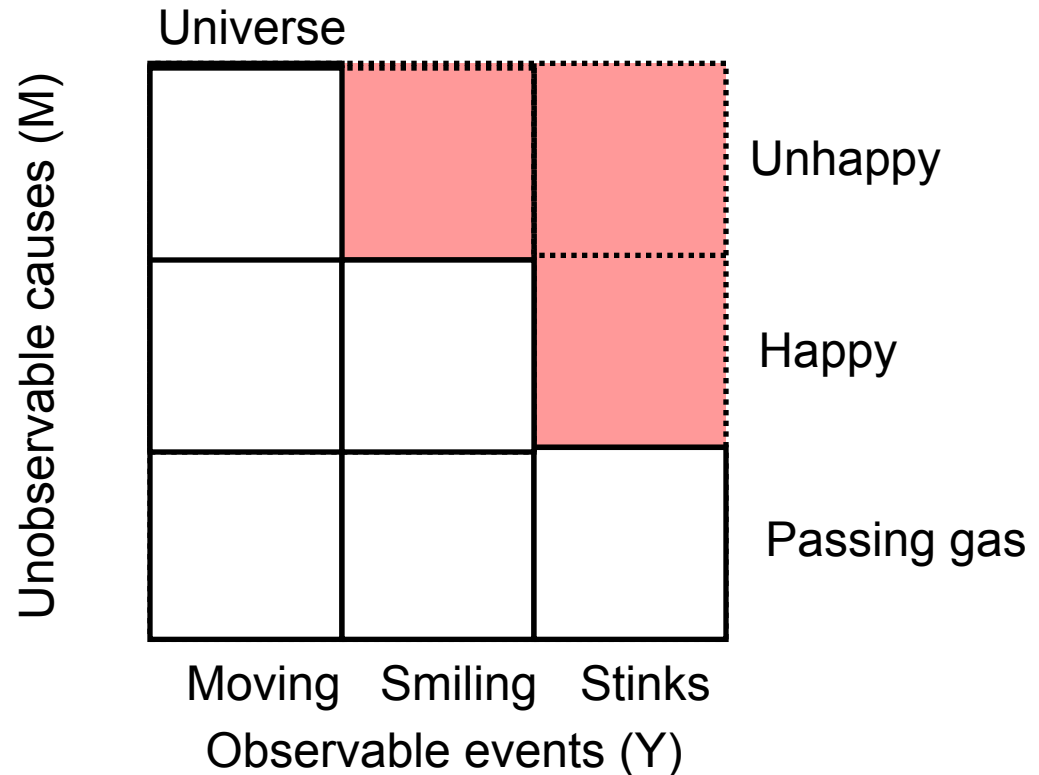
Event (Y):





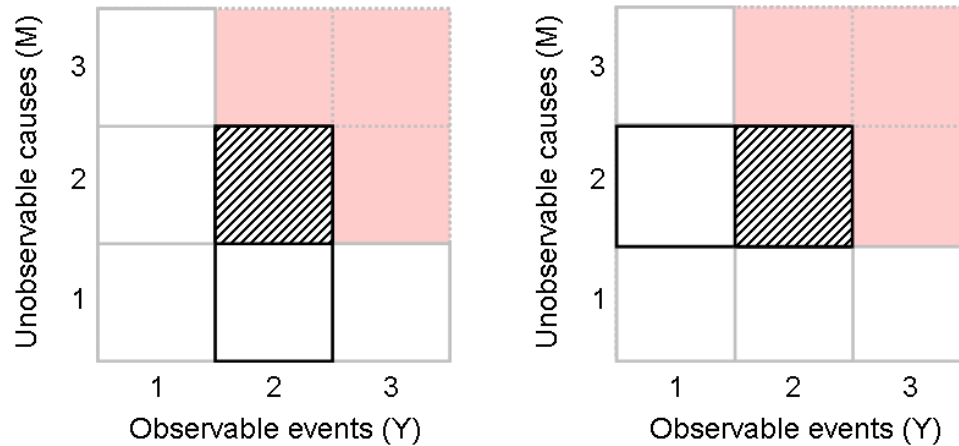
# Logical reasoning in the context of cell signaling primarily involves inductive inference

- Increase understanding of system by reasoning backwards from uncertain observations to a cause:  
 $P(M|Y)$



But how do we relate  $P(M|Y)$  to  $P(Y|M)$ ?

# Conditional probability is the same irrespective of whether one conditions on observation or cause



$$P(M_i | Y) \cdot P(Y) = P(Y | M_i) \cdot P(M_i)$$

Generally:  $P(M | Y) = \frac{P(Y | M) \cdot P(M)}{P(Y)}$

Prior belief in model

Likelihood:

Evidence:

$$P(Y | M) \propto \frac{1}{\sum_{i=1}^n (y_i - y_{Mi})^2}$$

$$P(Y) = P(Y | M) \cdot P(M) + P(Y | \bar{M}) \cdot P(\bar{M})$$



If error ( $\varepsilon$ ) =  $(y_i - y_{Mi}) \approx N(0, \sigma^2)$

# Empirical Bayesian approach is a contemporary alternative for model-based inference

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- Mathematical models are an expression of our belief in how information flows within cells.
- The level of confidence in our beliefs must account for the uncertainty associated with the parameters and the data used in calibrating the model.

$$P(\hat{Y} | M) = \iint P(\hat{Y} | \Theta, M) \cdot \underbrace{P(\Theta | M, Y)}_{\text{posterior}} \cdot P(Y) d\Theta dY$$

$$\text{Bayes Theorem: } P(\Theta | Y, M) \cdot P(Y) = \underbrace{P(Y | \Theta, M)}_{\text{likelihood}} \cdot \underbrace{P(\Theta | M)}_{\text{prior}}$$

$$P(\hat{Y} | M) = \iint P(\hat{Y} | \Theta, M) \cdot \underbrace{P(Y | \Theta, M)}_{\text{likelihood}} \cdot \underbrace{P(\Theta | M)}_{\text{prior}} d\Theta dY$$

- High performance computing is used to compute these integrals.



# $P(Y|\Theta, M)$ can be viewed from two different perspectives

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## Frequentist viewpoint:

- Random observations,  $Y$ , but  $\Theta$  and  $M$  are discrete fixed or “true” values:  
 $P(Y|\Theta_F, M_F)$
- A priori identifiability / Maximum likelihood
- Regress equations to data
- Apply MCMC convergence to parameters

## Bayesian viewpoint:

- $Y$ ,  $\Theta$ , and  $M$  exhibit uncertainty (randomness):  
 $P(Y|\Theta_R, M_R)$
- Available data limit ability to determine parameter values (practical identifiability)
- Can we distinguish among competing causal hypotheses given data?
- Apply MCMC convergence to predictions



# Integration with respect to observed data ( $\mathbf{Y}$ ) is a sum

- $\mathbf{Y}$  is a collection of different types of experimental data:  $\mathbf{Y} = \{Y_1, Y_2, \dots, Y_n\}$

$$P(\mathbf{Y} | \Theta, M) = P(Y_1 | \Theta, M) \cdot P(Y_2 | \Theta, M) \cdots P(Y_n | \Theta, M) = \prod_{j=1}^n P(Y_j | \Theta, M)$$

- Each data set may also have multiple measures:  $Y_j = \{y_1, y_2, \dots, y_{m_j}\}$

$$P(Y_j | \theta_i, M) \propto \left[ \frac{\text{Max}(Y_j)^2}{\sum_{k=1}^{m_j} (y_k - \hat{y}_k(\theta_i | M))^2} \right]^{\frac{m_j}{2}}$$

- Likelihood is related to the normalized sum of squared error.

$$P(\hat{Y} | M) = \int_{\text{all } \Theta} P(\hat{Y} | \Theta, M) \cdot \prod_{j=1}^n \left[ \frac{\text{Max}(Y_j)^2}{(Y_j - \hat{Y}_j(\theta_i | M))^T \cdot (Y_j - \hat{Y}_j(\theta_i | M))} \right]^{\frac{m_j}{2}} \cdot \underbrace{P(\Theta | M)}_{\substack{\text{proposal} \\ \text{distribution} \\ \text{(prior)}}} d\Theta$$

- Integration with respect to parameters is difficult.

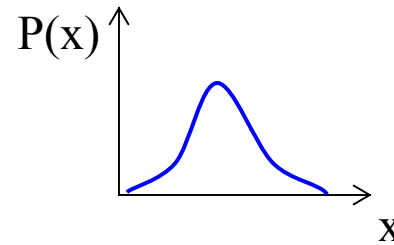


# An adaptive Markov chain Monte Carlo algorithm is used for integration

- Monte Carlo integration:
  - Numerical method used for integrating complex integrals using a random sample. Samples are weighted by probability.

$$\bar{x} = \int_{\text{all } x} x \cdot P(x) dx$$

$$\bar{x} = \frac{1}{N} \cdot \sum_{i=1}^N x_i \cdot P(x_i)$$



Randomly sample  $x \rightarrow$   
calculate  $p(x)$

Convergence: used to assess how many samples are needed to provide a good estimate of integrand

- Markov Chain Monte Carlo integration:
  - Better suited to high dimensional problems where high likelihood regions are highly structured
  - Random samples are obtained using a random walk, including a new step in walk is based upon likelihood.
  - Proposal distribution reflects prior information as to parameter (in)dependence that can be conditioned on data (an Adaptive MCMC)



# Summary

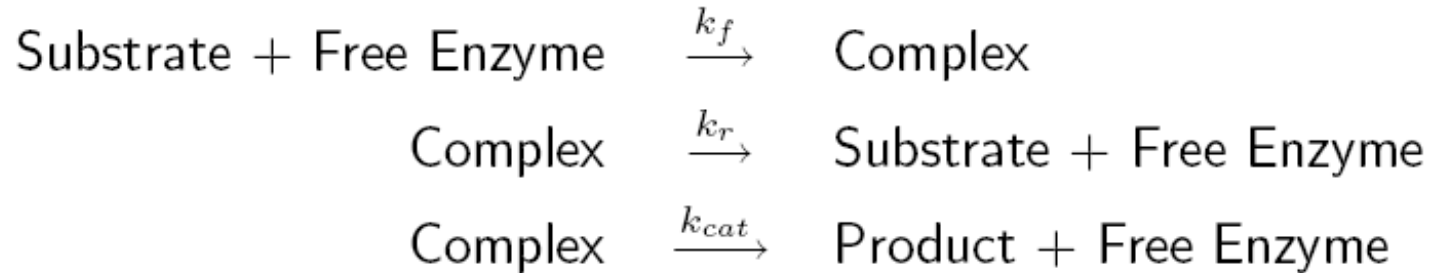
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# A simple enzyme kinetics example using adaptive Markov chain Monte Carlo integration

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Dynamical System:

$$\frac{dS}{dt} = -k_f \cdot E \cdot S + k_r \cdot C$$

$$\frac{dC}{dt} = k_f \cdot E \cdot S - (k_r + k_{cat}) \cdot C$$

$$E_{Tot} = E + C$$

Known:

$E_{Tot}$  - Total enzyme concentration

Unknown:

Rate constants { $k_f$ ,  $k_r$ ,  $k_{cat}$ }  
 2 different initial substrate concentrations.

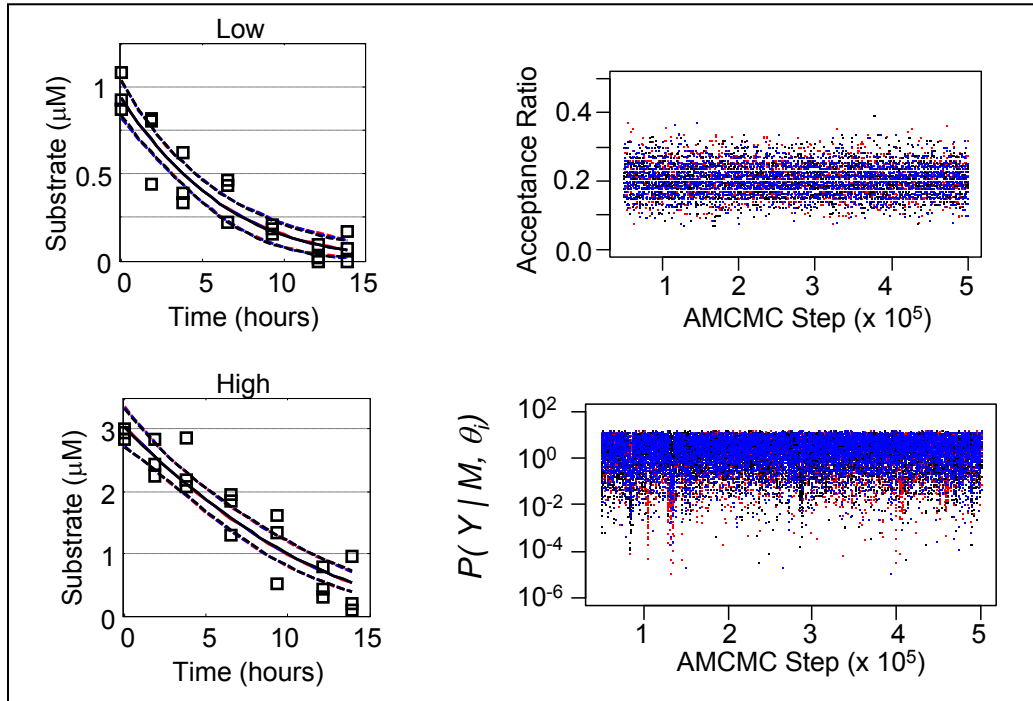
Measurement error:

10% RMS

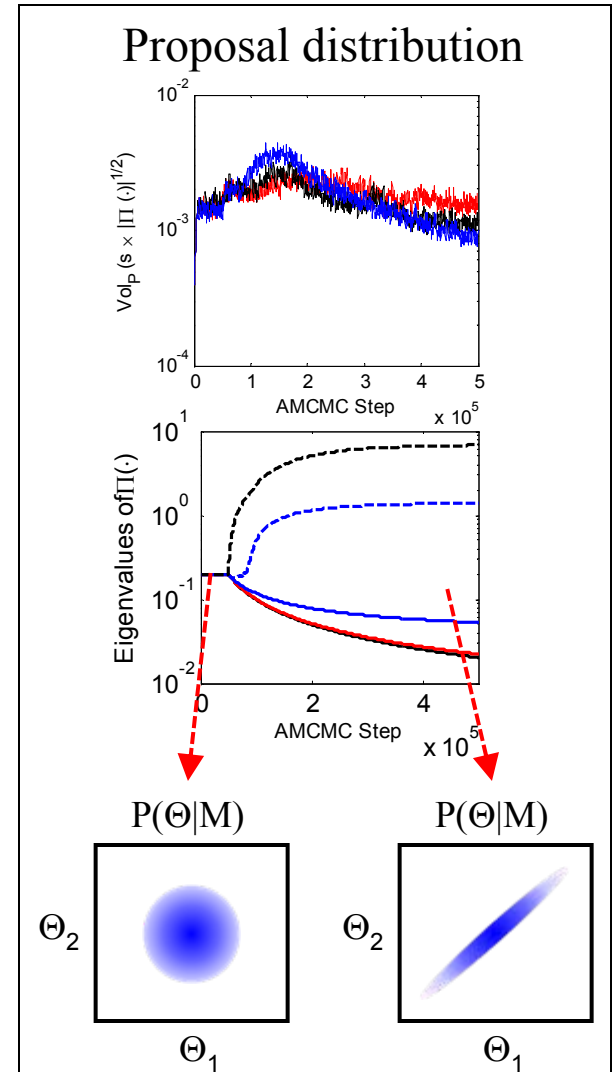




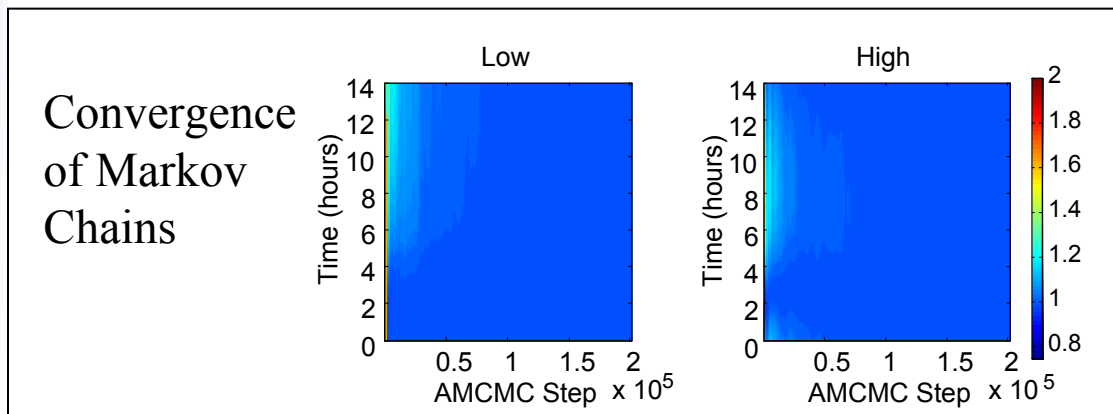
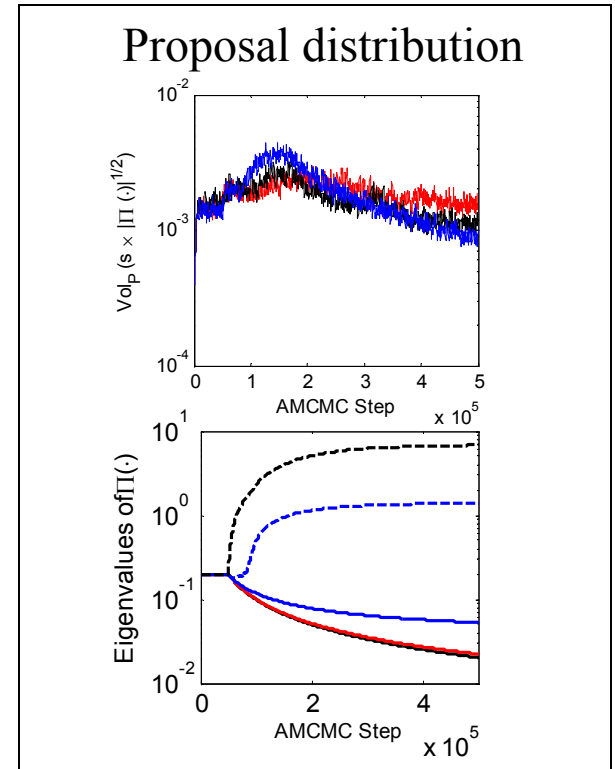
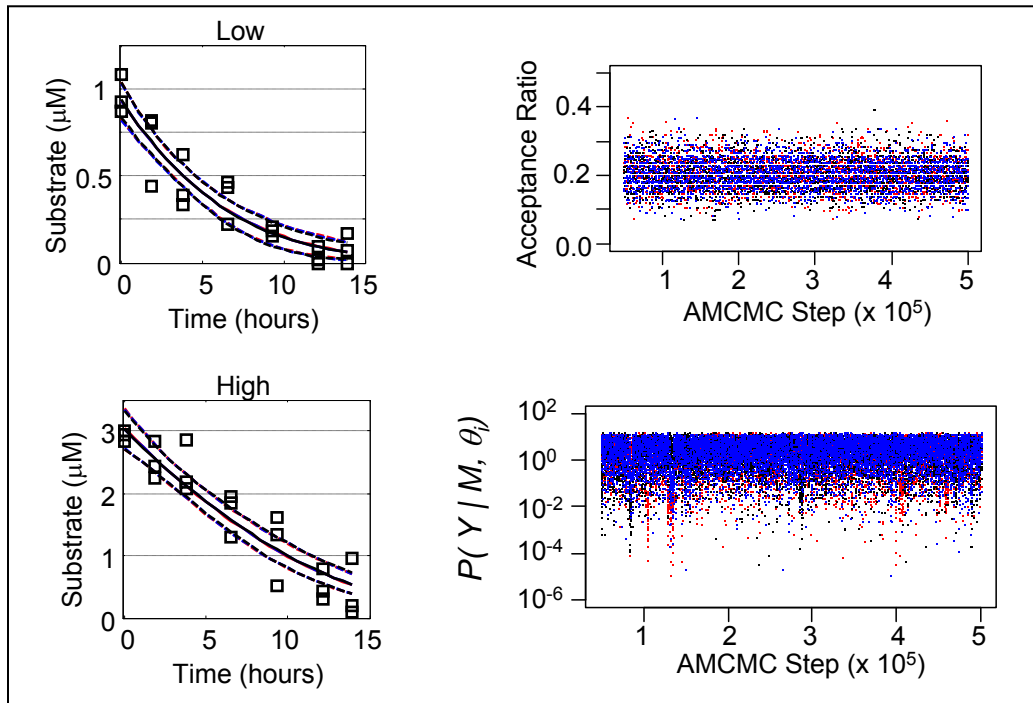
# A simple enzyme kinetics example using adaptive Markov chain Monte Carlo integration



Proposal distribution exhibits diminishing adaptation

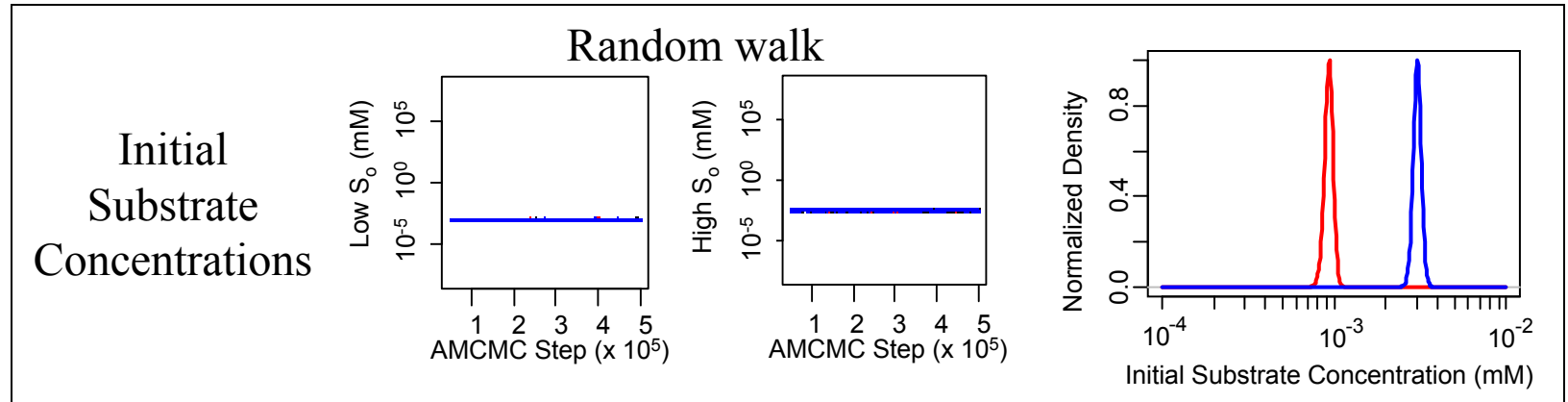


# A simple enzyme kinetics example using adaptive Markov chain Monte Carlo integration

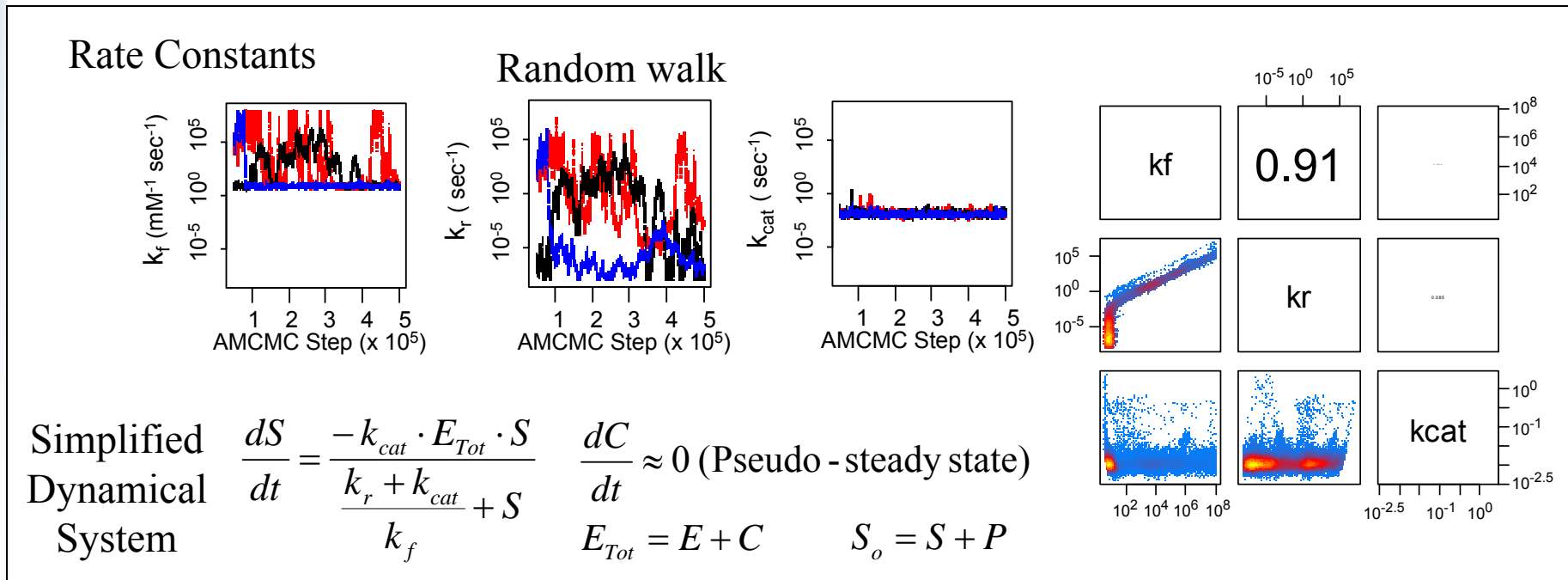
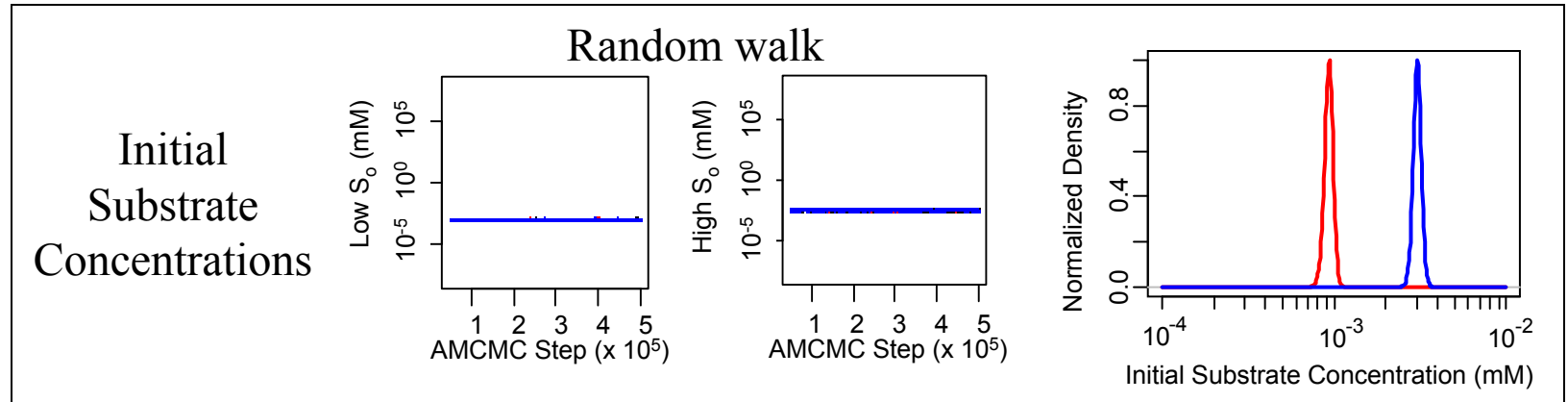


Convergence  
of Markov  
Chains

# A simple enzyme kinetics example using adaptive Markov chain Monte Carlo integration



# A simple enzyme kinetics example using adaptive Markov chain Monte Carlo integration



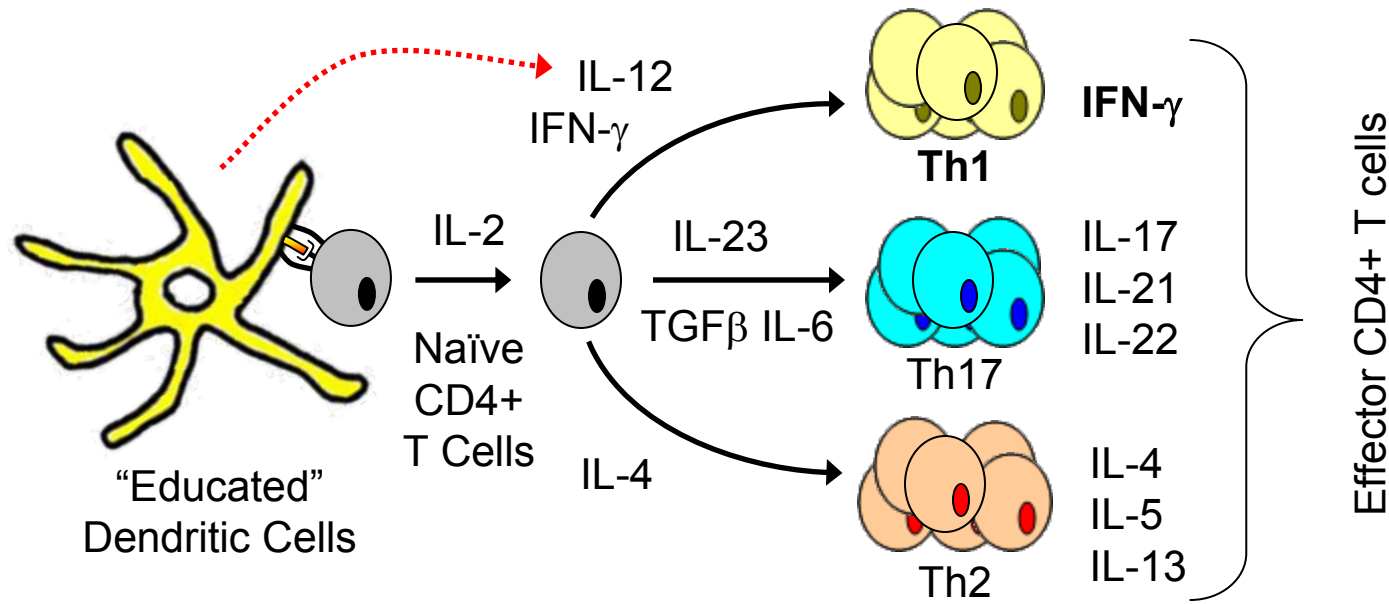
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# Cytokines direct T helper cell polarization



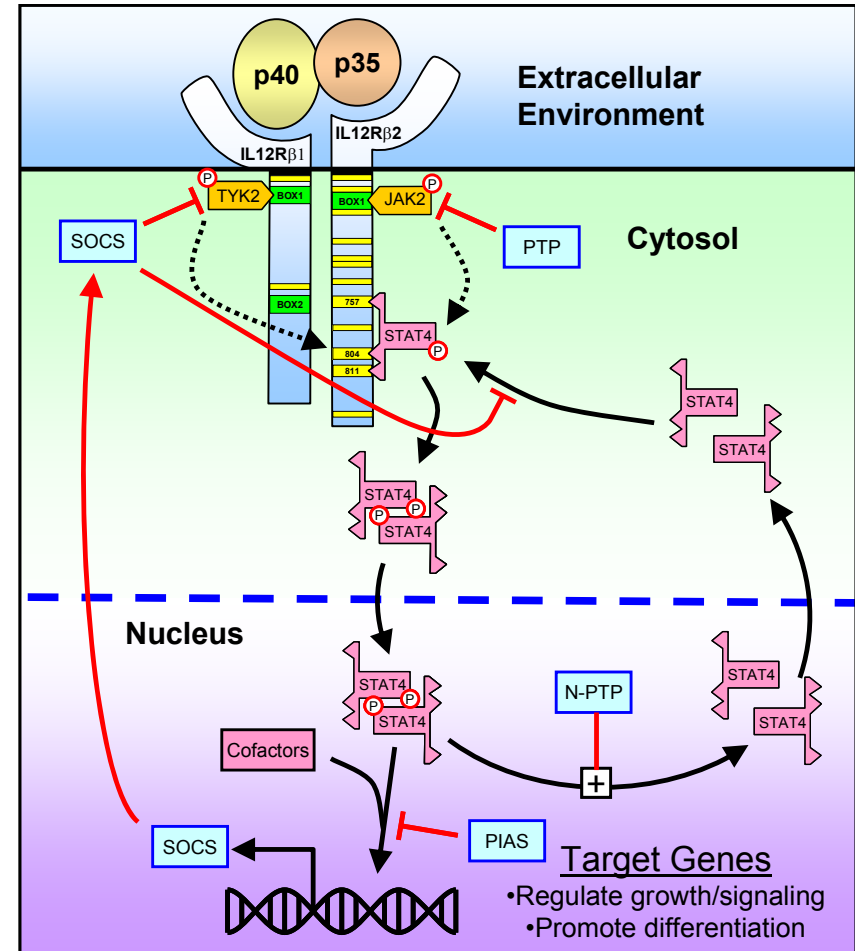
T helper cells:

- Recognize antigens
- Presented in the right context
- Produce cytokines that are influenced by autocrine and paracrine biochemical cues



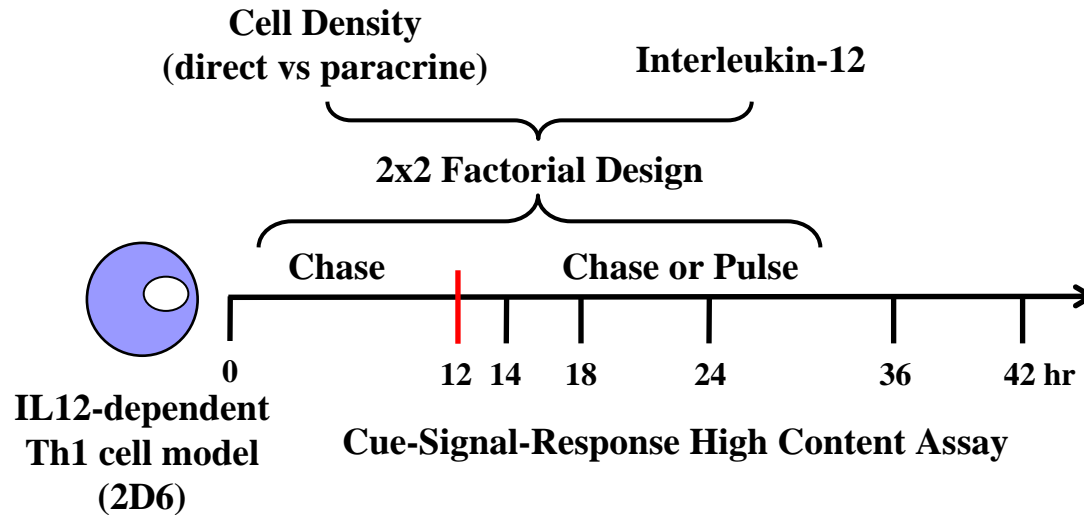
# Interleukin-12 promotes Th1 polarization

- The IL12 signaling pathway is a member of *JAK/STAT* family of signaling networks
  - Signal strength regulated via positive and negative feedback
  - Role of feedback in normal physiology unknown (Murray *J Immunol* 2007)



Can model-based inference be used to evaluate competing hypotheses regarding how  $T_H1$  cells interpret IL-12?

# We acquired a high content, quantitative cue-signal-response data set to test competing hypotheses

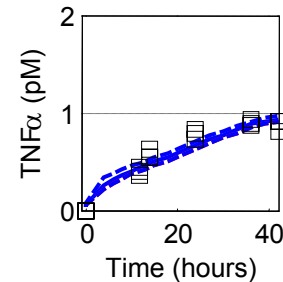
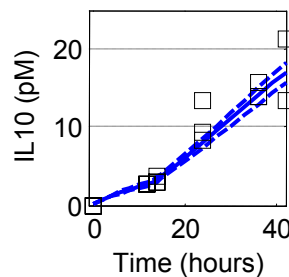
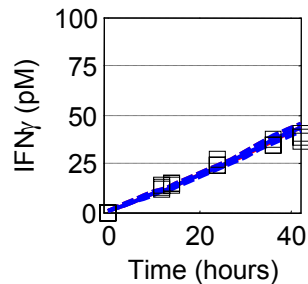
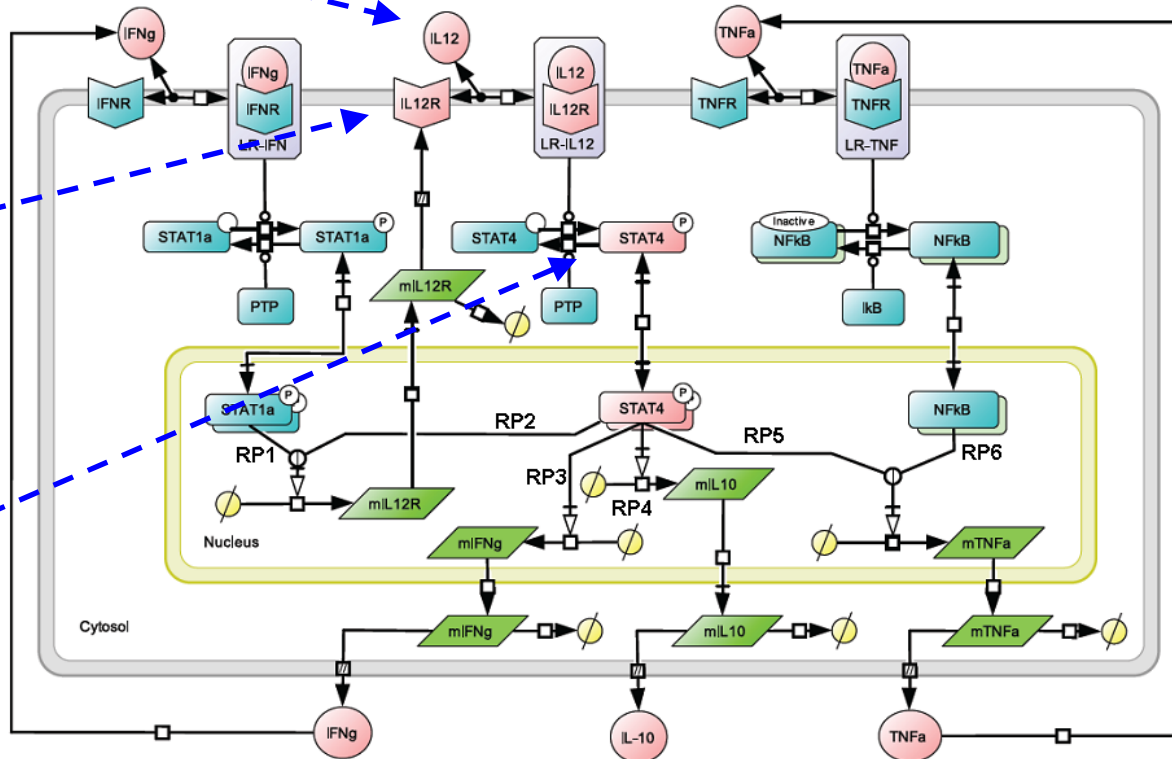
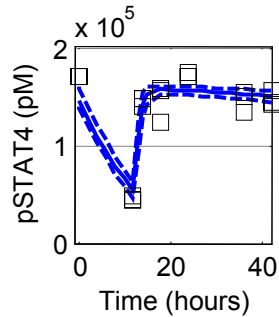
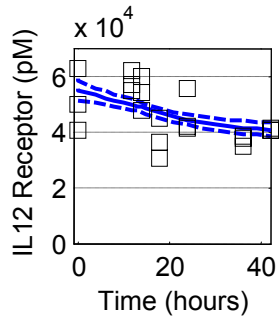
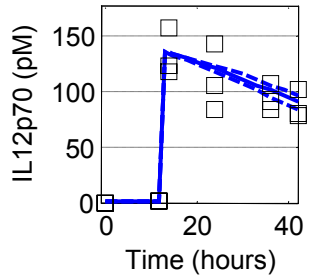


- Flow cytometry-based high content observations
  - Cell density – AccuCount calibration beads
  - Cell viability – Caspase 3 cleavage
  - Quantitative cellular signals (MFI → Copy number)
    - Phosphorylated STAT4
    - IL12 receptor  $\beta 2$  and IL12 receptor  $\beta 1$
  - Biochemical cues / Cell Response – Cytometric bead array
    - IL12p70 TNF- $\alpha$  IL-10 IL-6 IFN- $\gamma$  MCP-1
- 924 data points – 7 time points, in triplicate

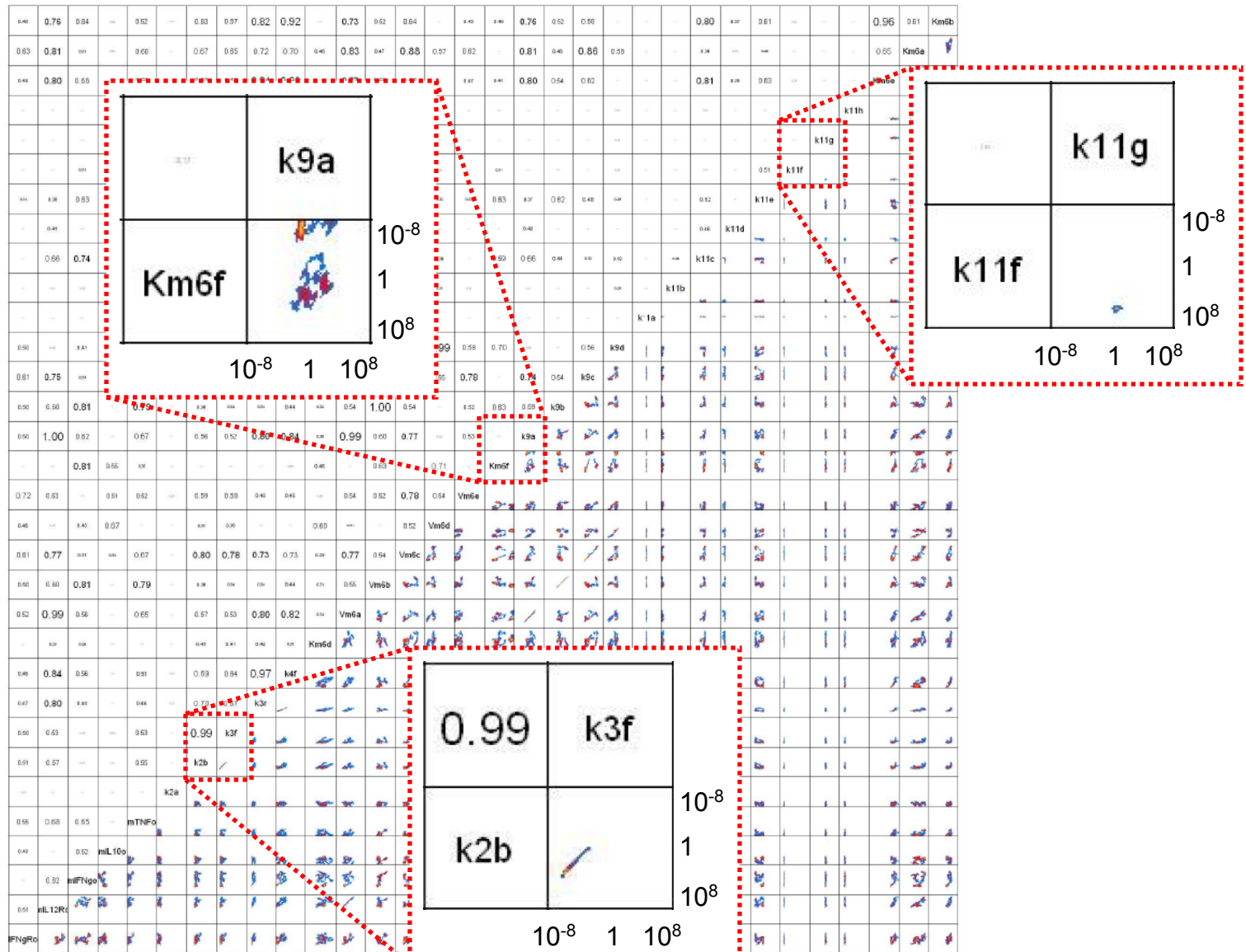




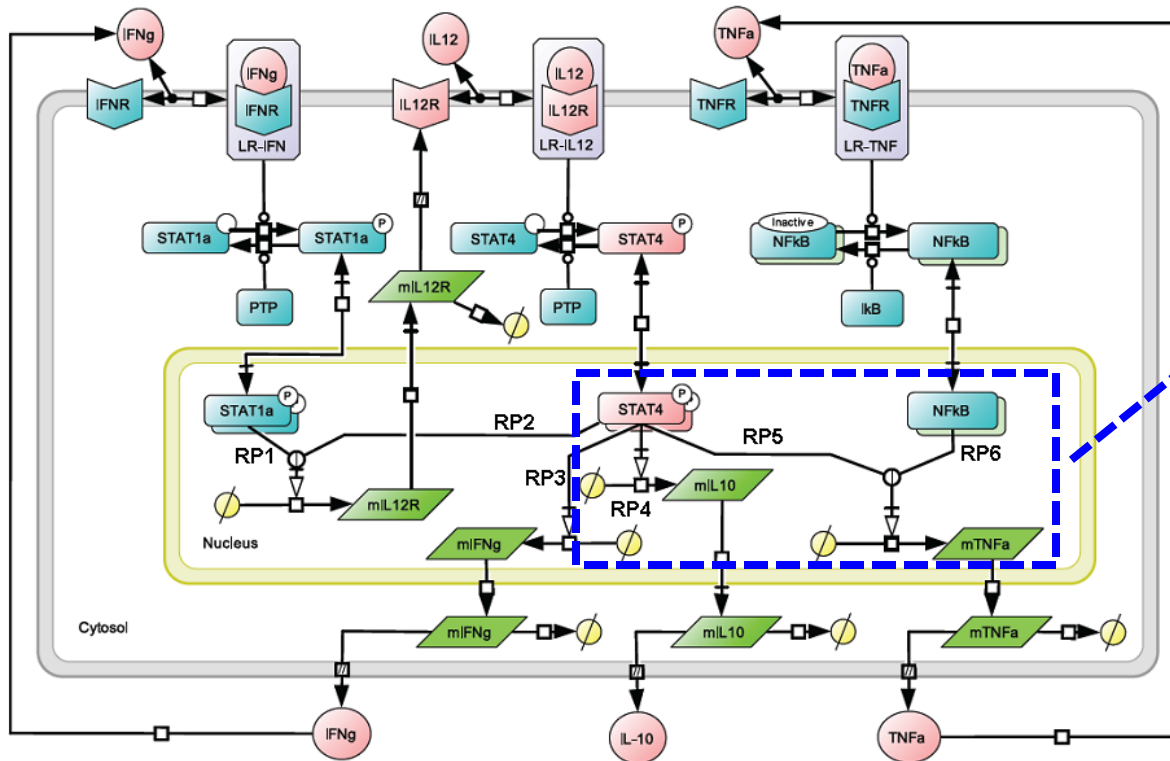
# Model encodes competing hypotheses and was calibrated to data



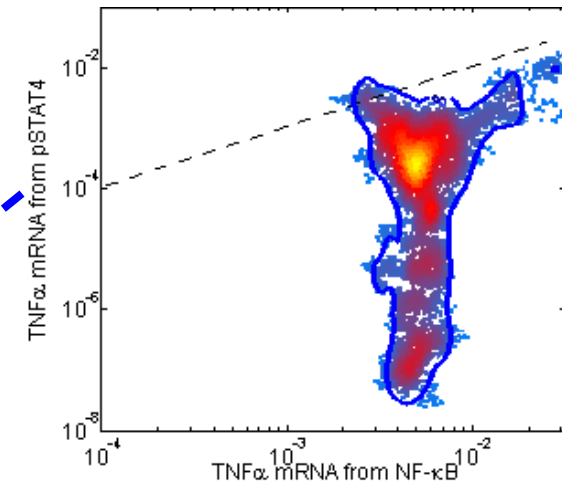
# Parameters are selectively informed by data and exhibit correlation



# Posterior distribution suggests that 2D6 cells produce $\text{TNF}\alpha$ via an autocrine feedback loop



RP5

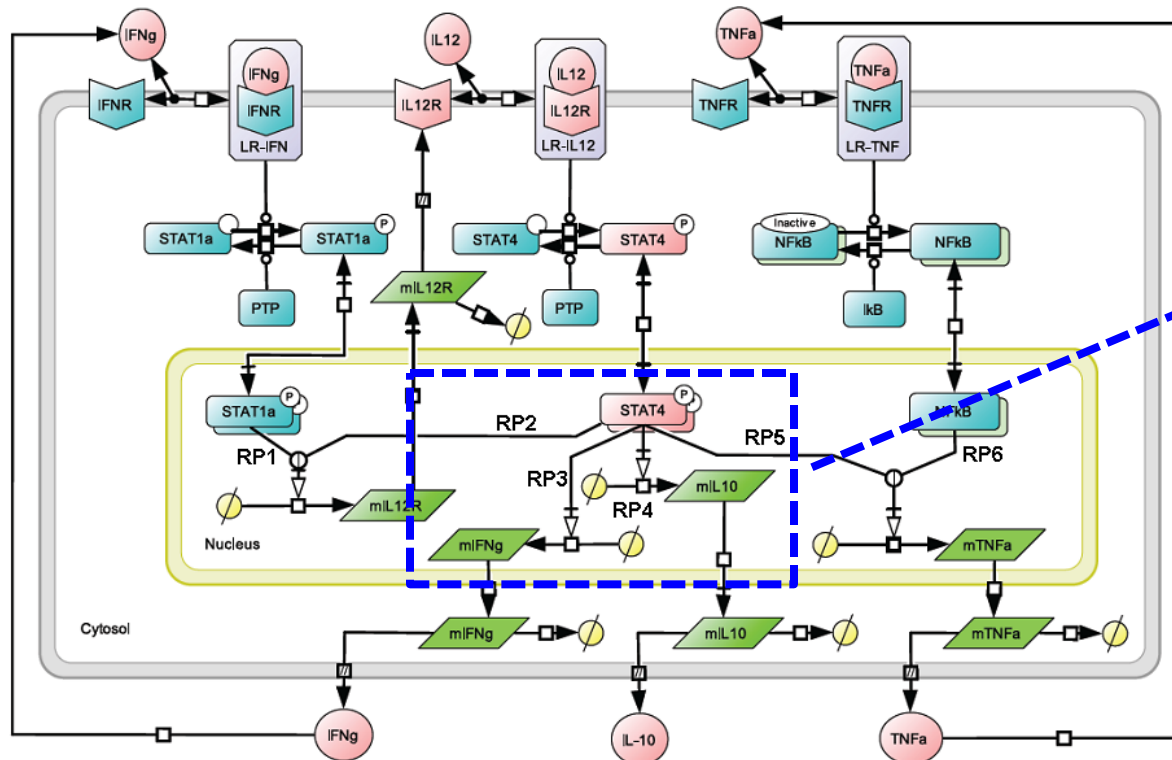


RP6

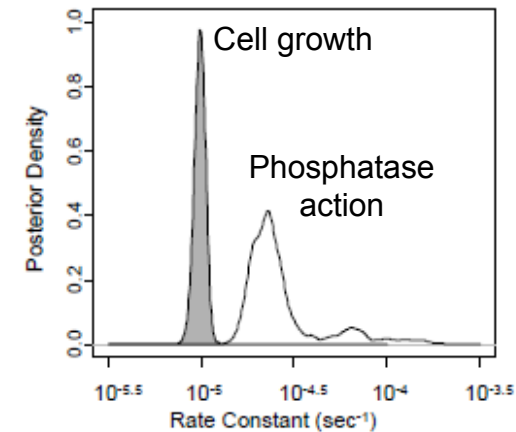
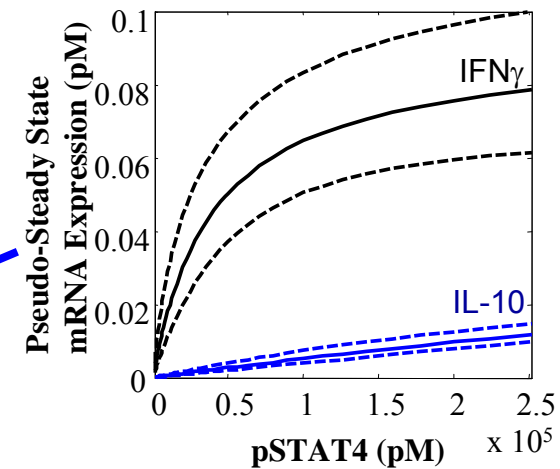
- Autocrine feedback loop regulates  $\text{TNF}\alpha$  production in 2D6 cells.
- $\Pr(\text{RP6} > \text{RP5} | Y, M) > 96.7\%$
- Experimentally validated prediction.



# Dynamics of IL-12-induced cytokine production are influenced by deactivation and dilution

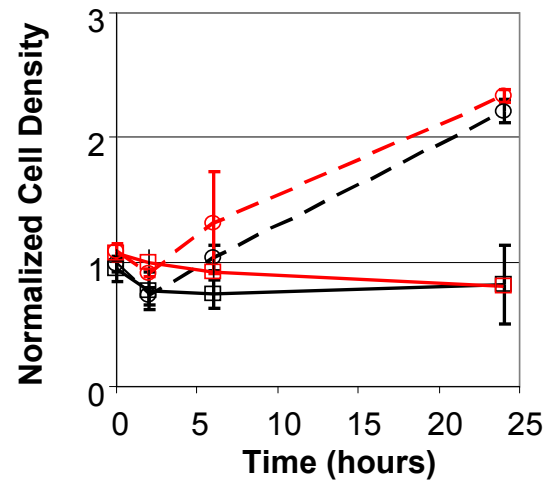


- Activated STAT4 saturates *ifng* but not *il10* promoter.
- Dilution contributes 30% to decay in pSTAT4



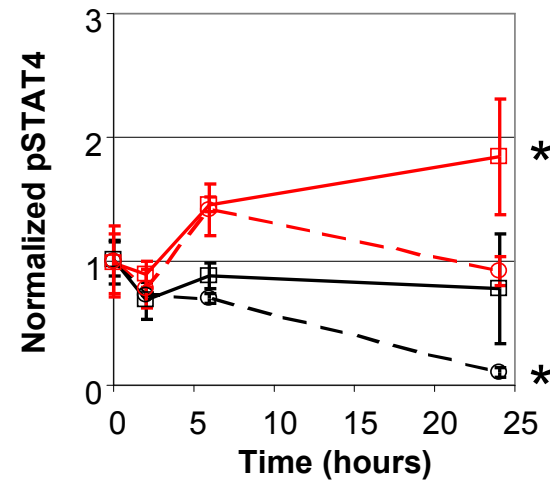
# Inhibiting cell proliferation stops decline in pSTAT4

- Block cell proliferation using Mitomycin C and stimulate with IL-12
- Quantify cell density and pSTAT4 response



Mitomycin C ———

Neg Control - - - -

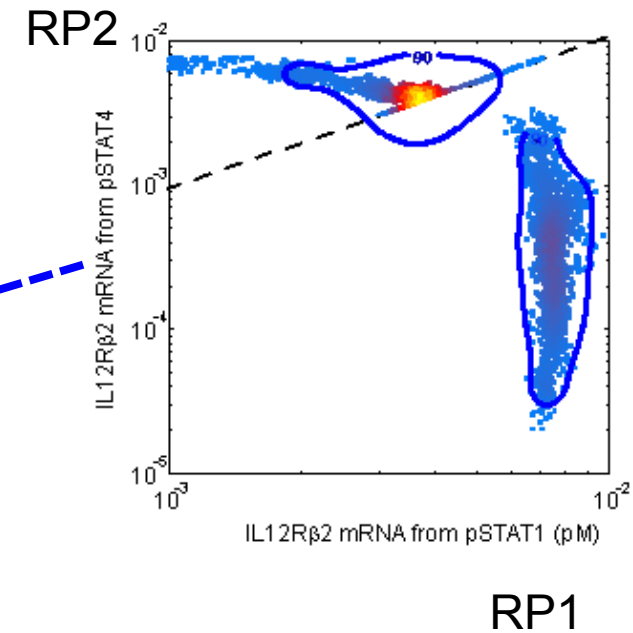
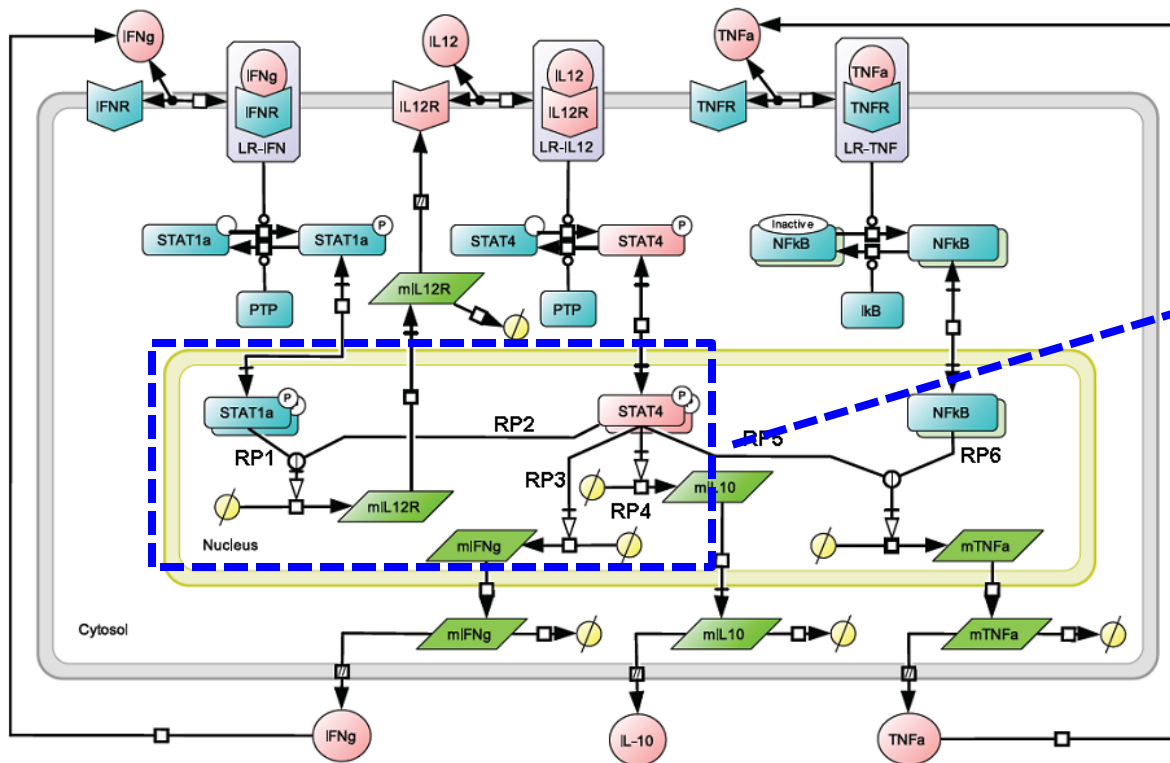


IL-12 ———

Neg Control ———



# Data are insufficient to discriminate between competing hypotheses to control IL12R $\beta$ 2 expression

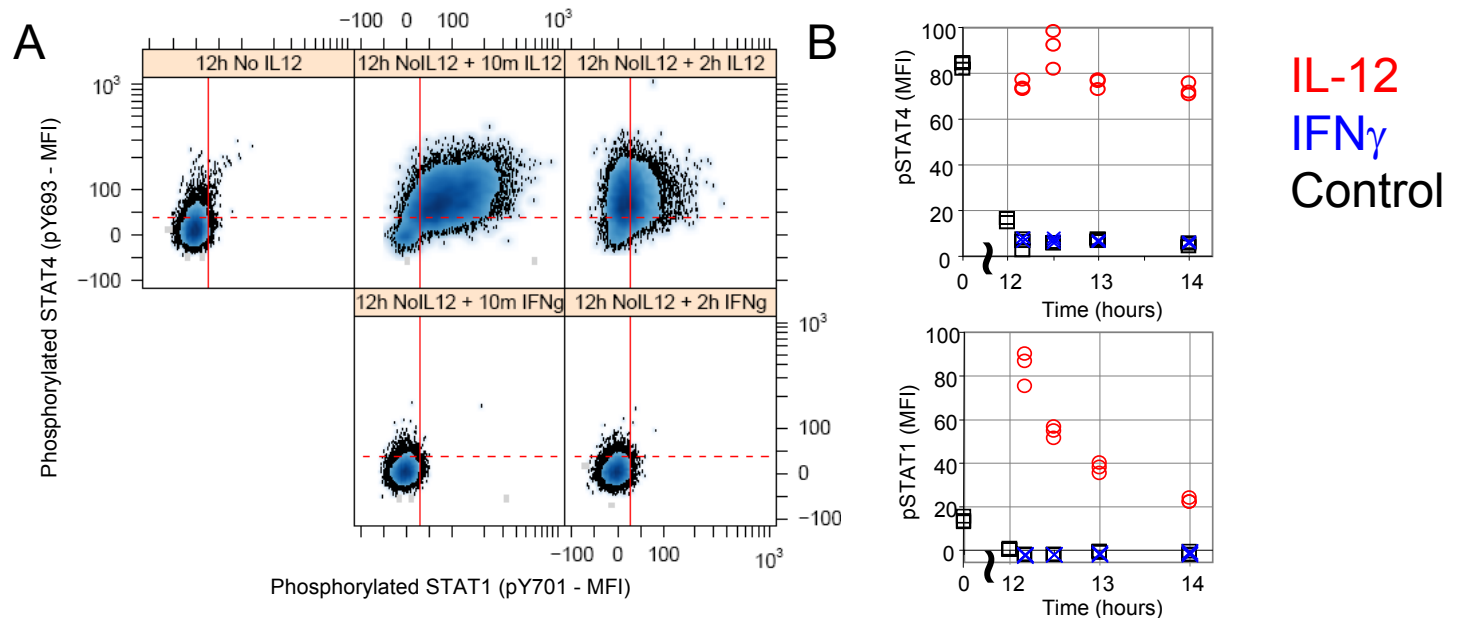


Posterior distributions in the flux through competing pathways is inconclusive.



# Data suggest a lurking mechanism for regulating IL-12 receptor $\beta 2$

- Th1 cells do not typically respond to IFN- $\gamma$ , while naïve CD4<sup>+</sup> T cells do.
- STAT1 activation in response to IFN- $\gamma$  is typically reported at 30' post stimulation – are dynamics important?
- Stimulate 2D6 cells with IFN- $\gamma$  or IL-12, observe rapid time course for STAT1 and STAT4 activation.

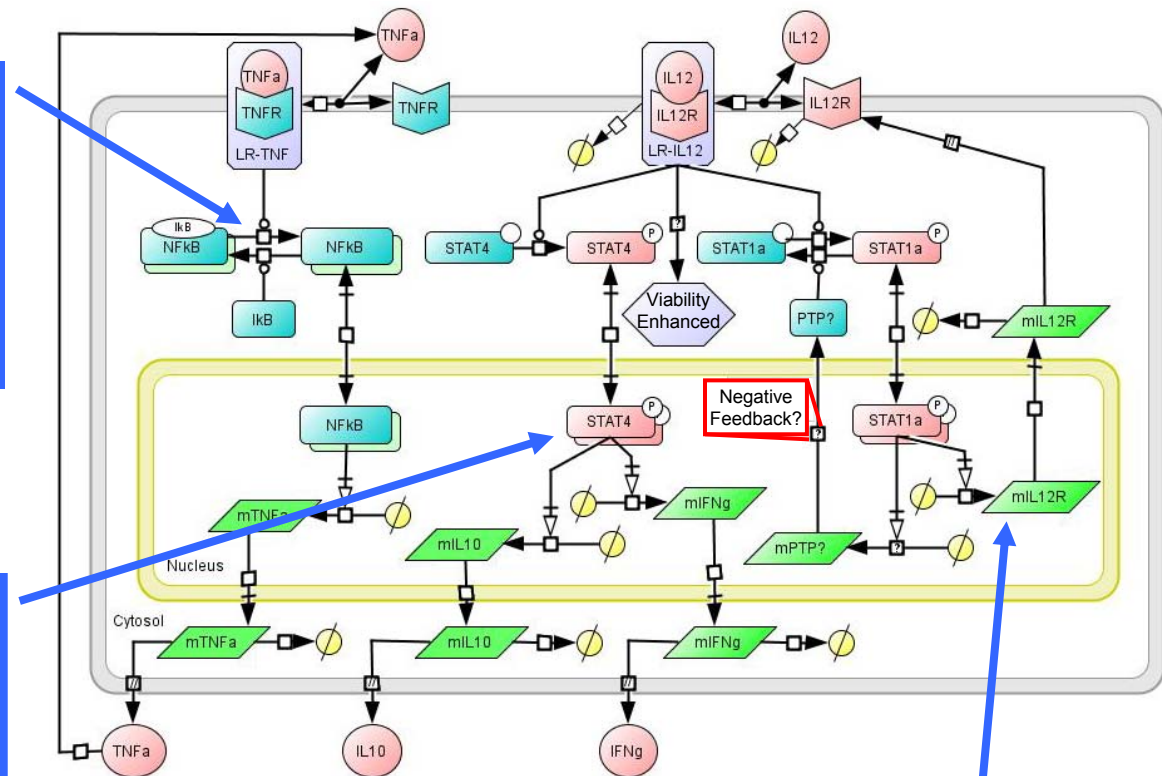


- STAT1 and STAT4 are differentially regulated in response to IL-12

# Cue-signal-response model is revised to reflect new biology

TNF $\alpha$  production is regulated via an autocrine feedback loop – an integral feedback control mechanism to regulate homeostatic proliferation?

The cytokine response of 2D6 cells to IL-12 exhibits memory – temporal component of plasticity in T helper cell fate.



IL-12R $\beta$ 2 is regulated in naïve CD4<sup>+</sup> T cells via an integral feedback control mechanism but shifts to a cell-autonomous mechanism in Th1 effector cells.





# Summary

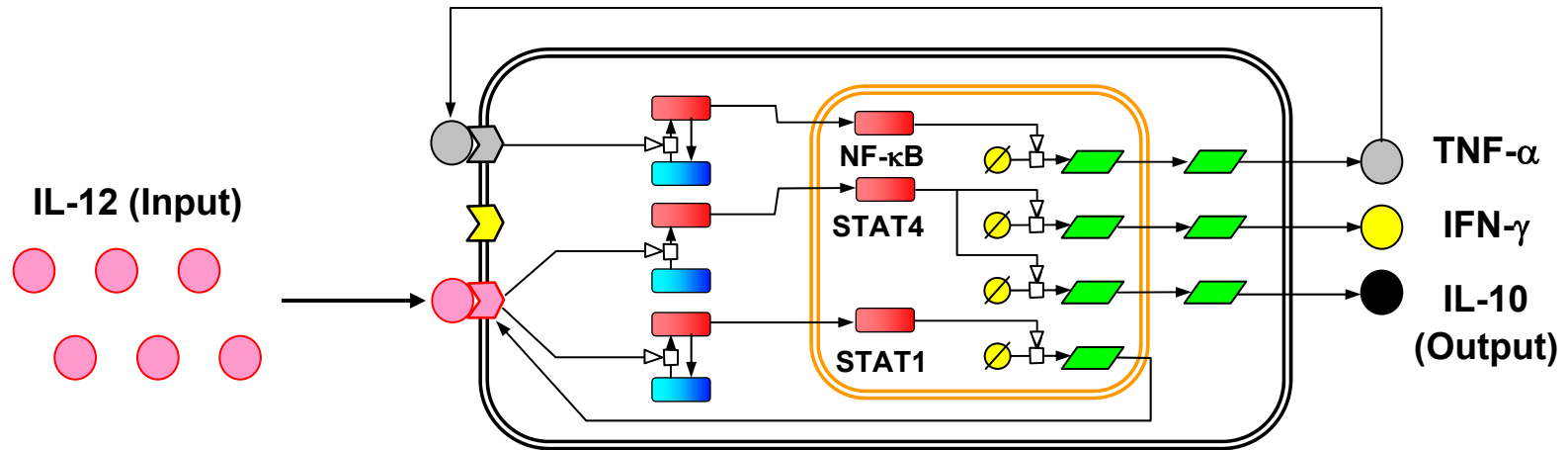
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# Do tumor cells bias the immunoselection landscape through paracrine regulation of Interleukin-12?

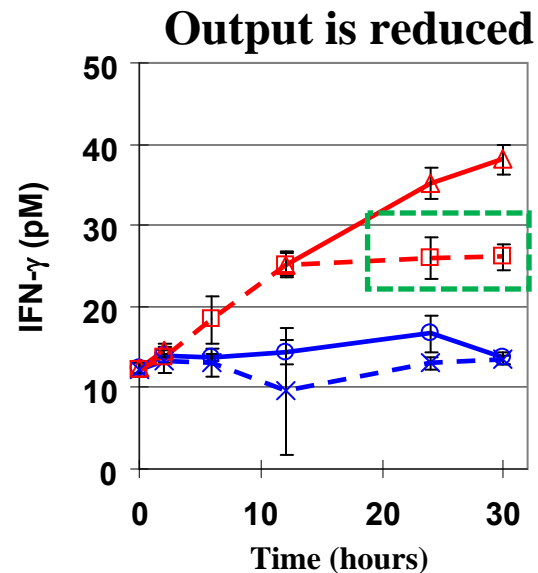
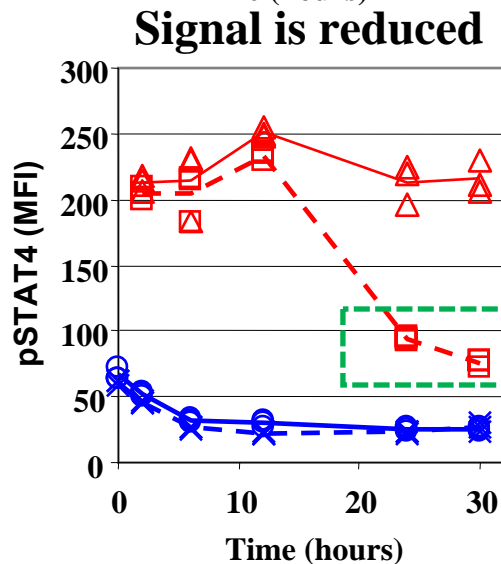
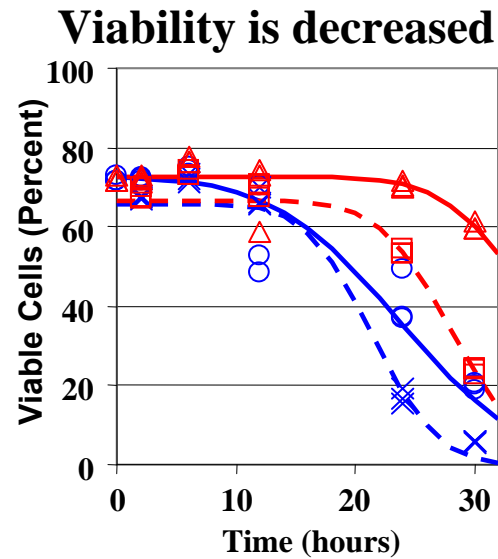
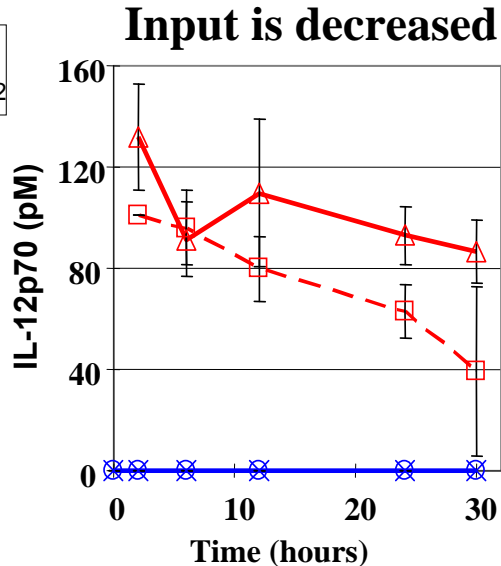
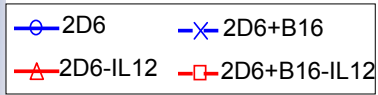
- Create minimal system that recreates local immunosuppression (i.e., a closed-loop system)
- Use an immune cell model for which input-output relationships are well-characterized (Klinke et al. *Sci Signal* 2012.)



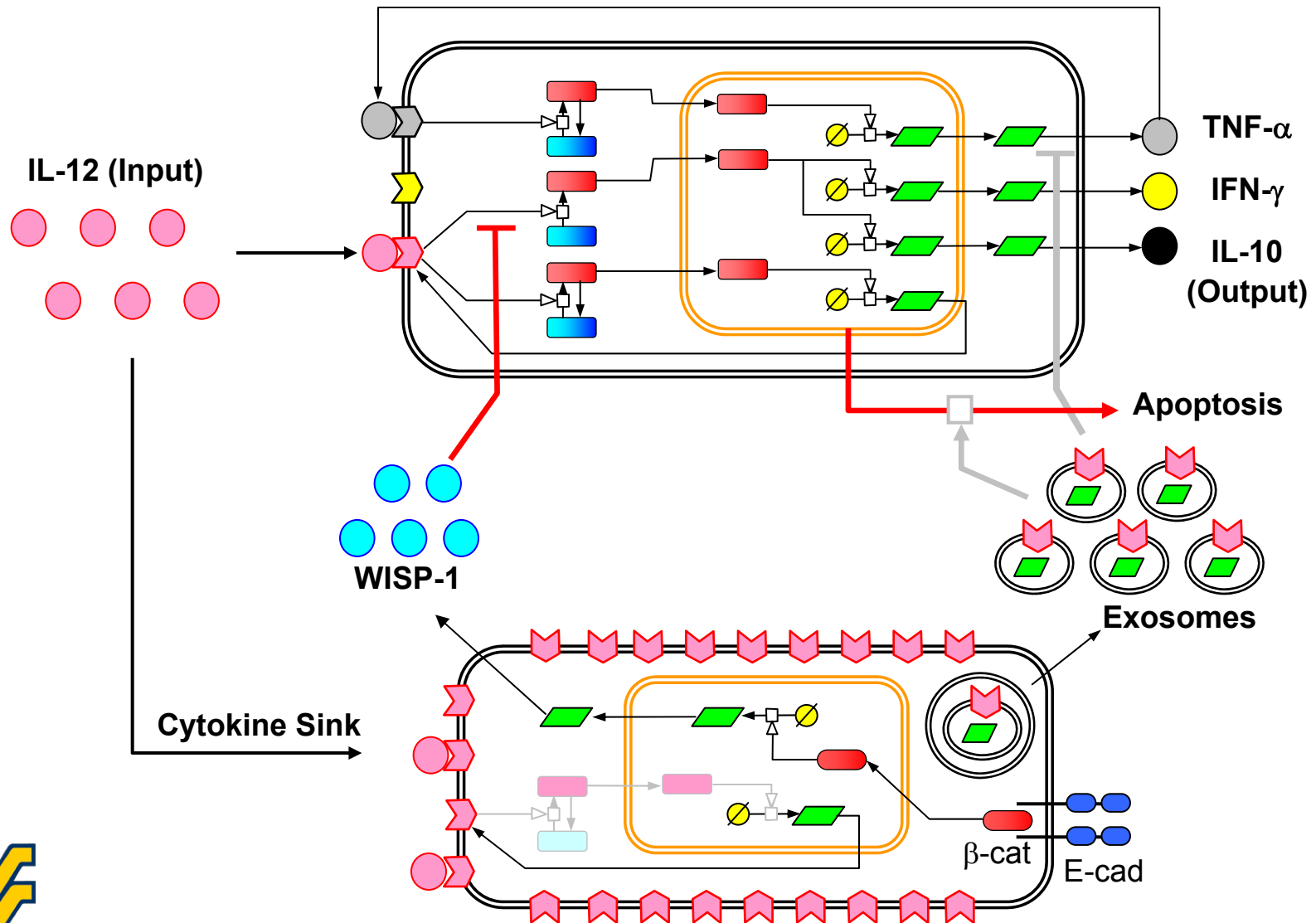
- STAT4 is phosphorylated irreversibly => encodes short-term memory that is limited by cell proliferation and reinforced by low endogenous IL-12
- Employ less biased protein identification methods => 2D-GE/MALDI-TOF MS.



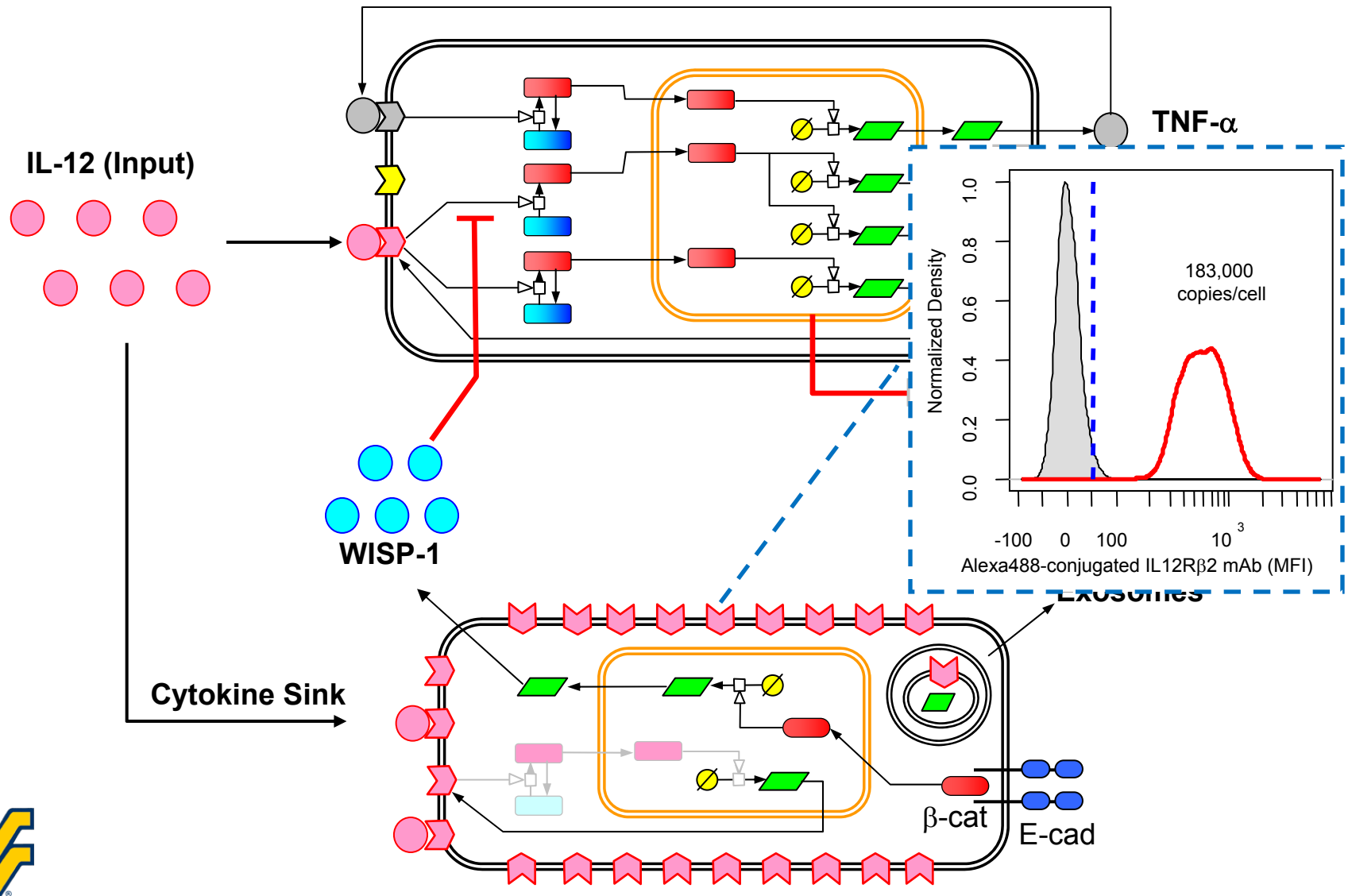
# B16 co-culture with 2D6 cells exhibits closed-loop behavior



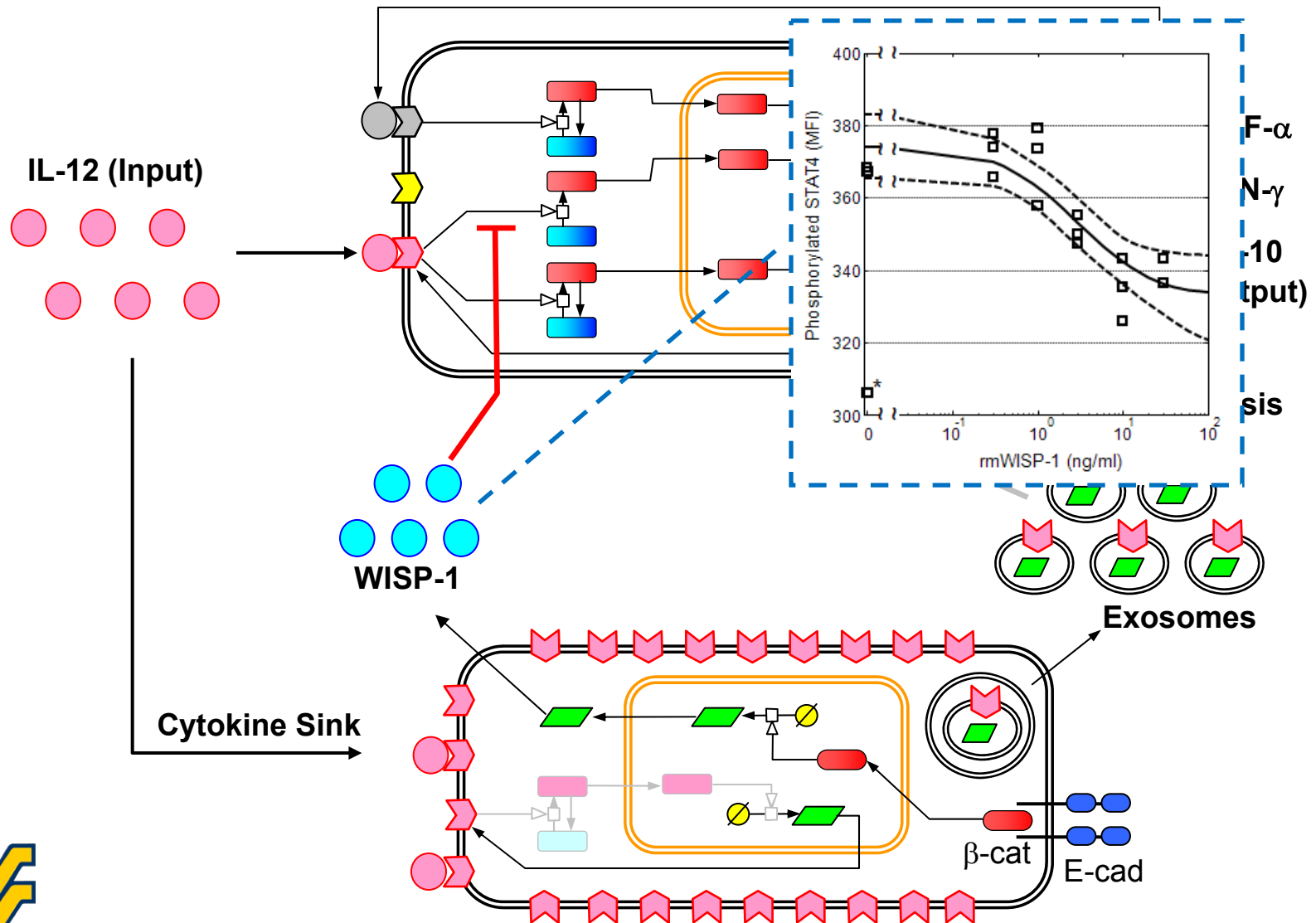
# B16 inhibits cellular response to IL-12 via multiple mechanisms including paracrine action of Wnt-inducible signaling protein-1



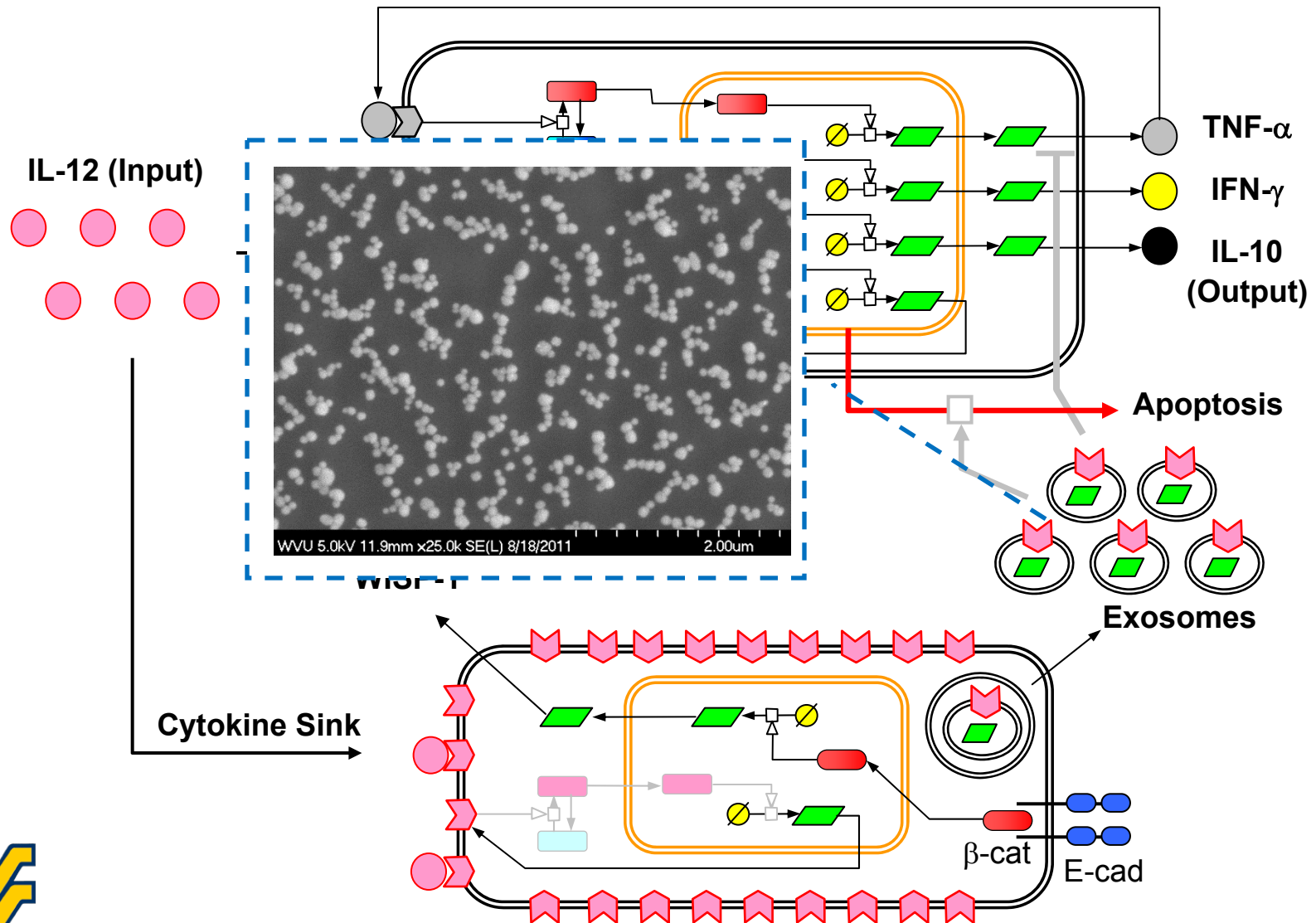
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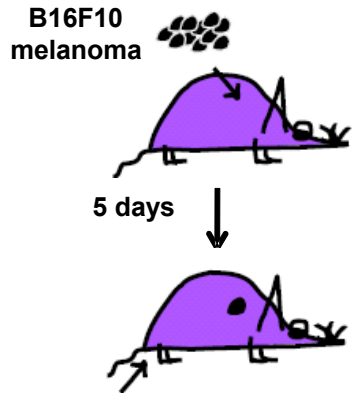
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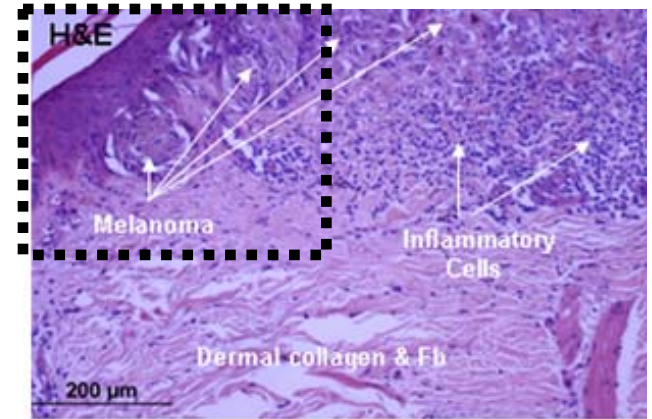
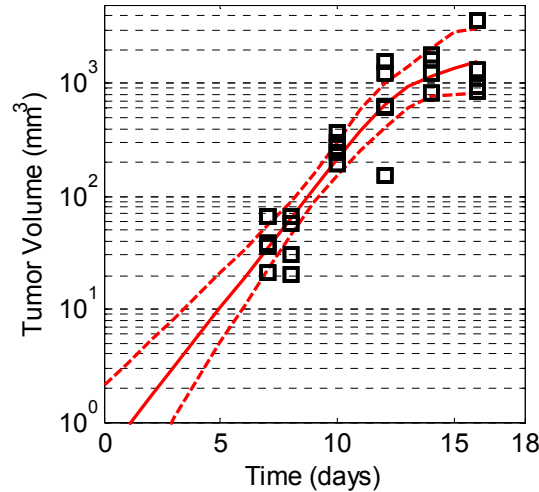
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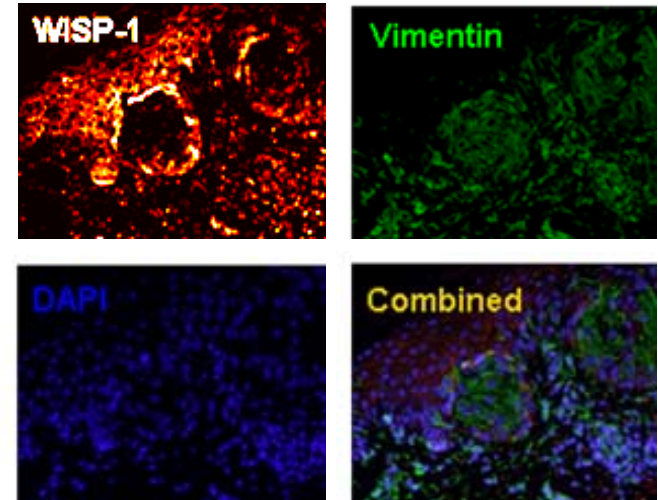
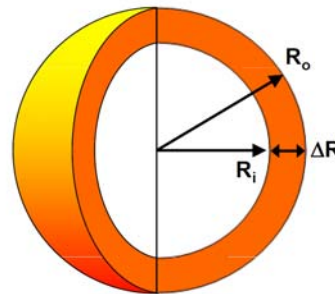
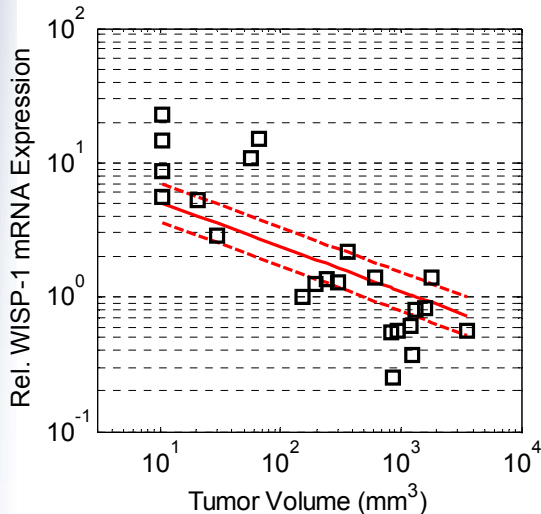
# WISP-1 is expressed at the invasive front of human melanoma tumors



Monitor tumor growth      Quantify WISP-1 and GAPDH mRNA in B16F10 homogenate



Shao et al. Oncogene 2011



Model-based inference used to suggest that WISP-1 is expressed in outer shell of tumor ( $p > 0.95$ )



# Parting Thoughts

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*The most exciting phrase to hear in science, the one that heralds the most discoveries, is not "Eureka!" (I found it!) but "That's funny..."*

– Isaac Asimov

- Lurking mechanisms exist in biological research.
  - IL-12 receptor activates both STAT1 and STAT4
  - Irreversibility of STAT4 phosphorylation
  - Tumors exert paracrine action on immune cells through Wisp1
- *In silico* model-based inference is a contemporary tool for hypothesis testing in dynamical systems.



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