MMBioS Meeting, Feb 2017

Model Checking Techniques for Systems Biology Modeling: A Case Study of Neurotransmission

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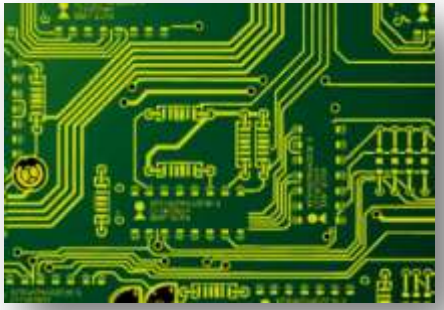
Complex Systems

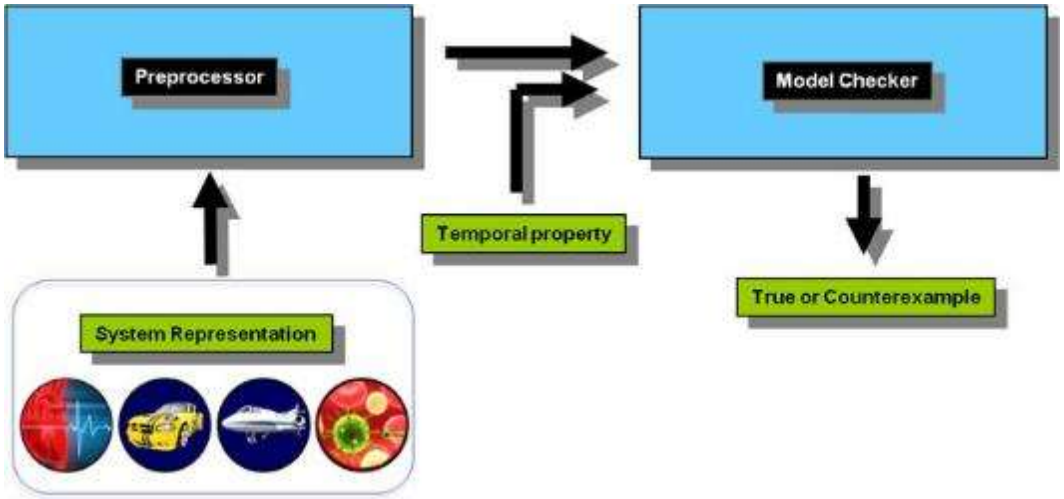
Model Checking

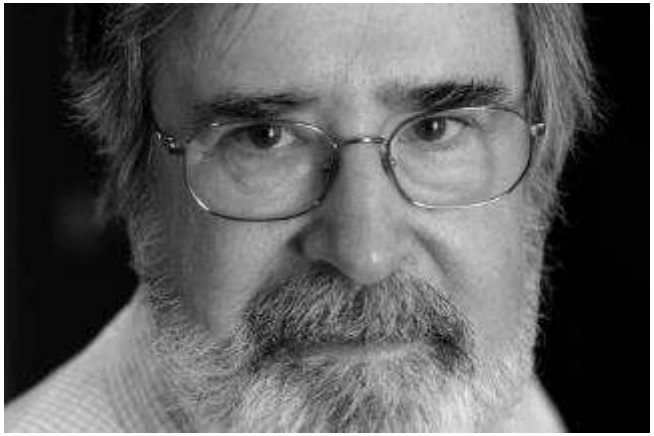
- Detect bugs in a variety of hardware and software applications
  - E.g. microprocessor, railway system, satellite-control software
- Many industrial successes
  - Intel, IBM, Apple, Microsoft, Motorola, Airbus, etc.



Model Checking

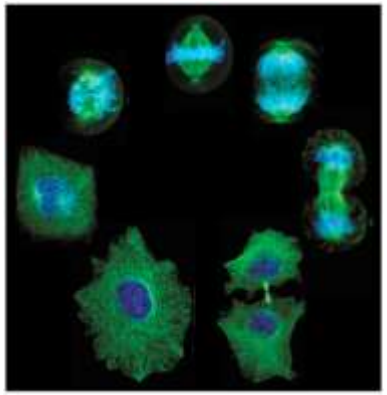
- An automated method to formally verify a system's behavior with respect to a set of properties





Edmund M. Clarke

Biological Machines



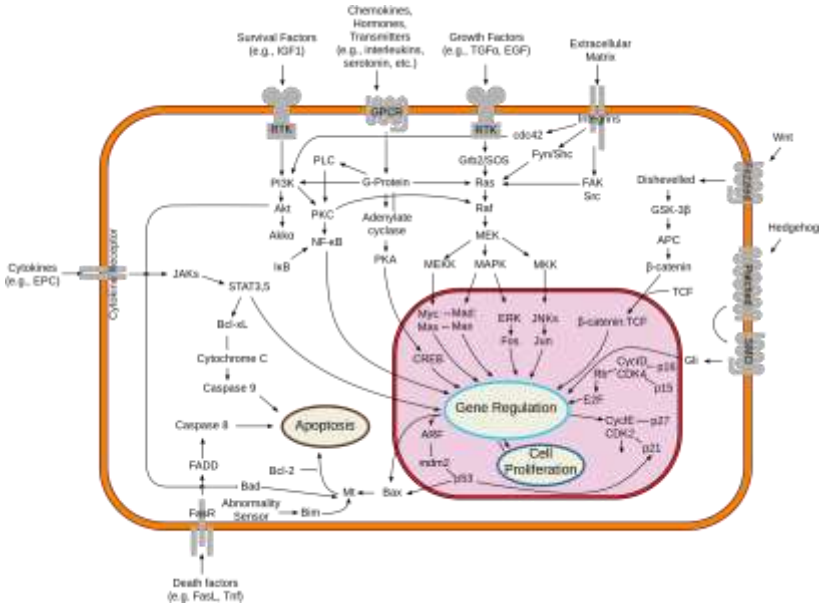
Cell cycle

ATP synthase

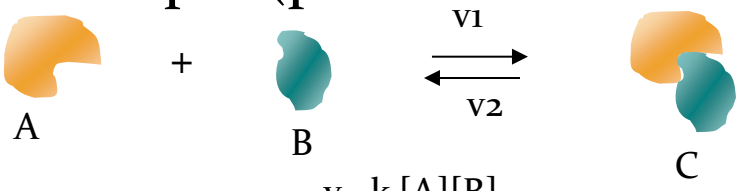
Microtubule assembly, vesicle transport driven by motor proteins, protein synthesis by ribosome, power station mitochondria

Systems Biology Modeling

- Mathematical formalisms
  - Ordinary Differential Equations
  - Petri Nets
  - Hybrid Automata
  - Markov chains (e.g. CTMC)
  - ***BioNetGen language***
  - …



- ODE Example (protein association):



$v_1 = k_1[A][B]$

$v_2 = k_2[C]$

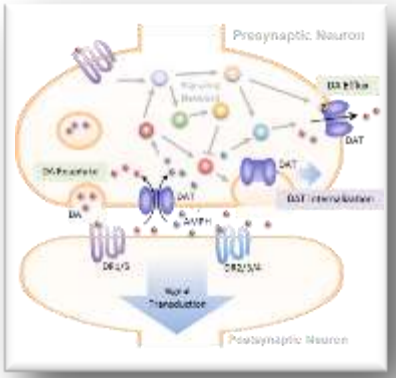
$d[B]/dt = - v_1 + v_2 = k_1[A][B] - k_2[C]$

Mass action law

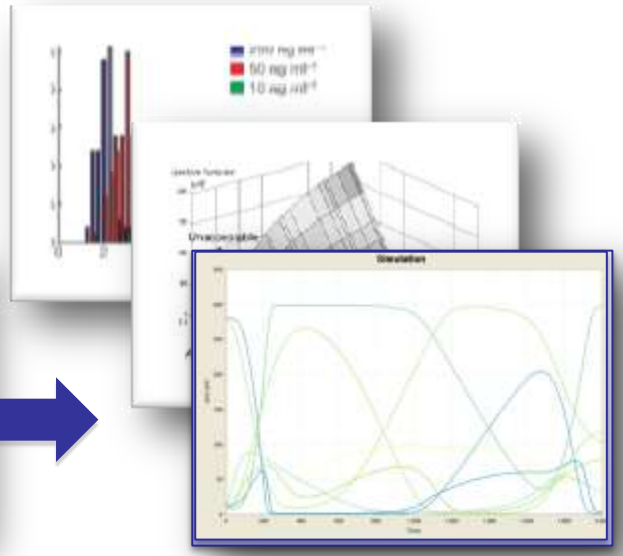
Problems Faced

- Which hypothesis is more plausible?
- **How to estimate unknown model parameters?**
- Which component is critical to the dynamics?
- How to control to system to get a desired behavior?

Model checking can help!

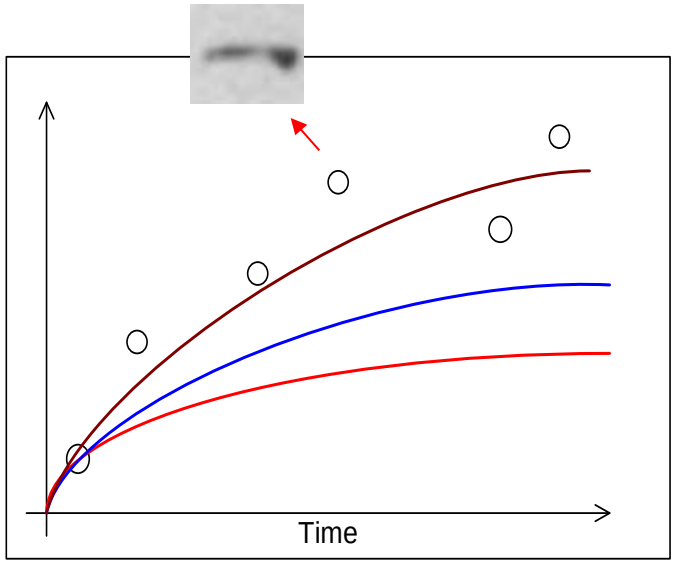



```
begin molecule type
L() R()
end molecule type
begin reaction rules
L(r) + R(l) <-> L(r!1).R(l!1) kp1, km1
end reaction rules
generate_network()
simulate({method=>"ode",t_end=>500,n_s
teps=>500})
```



Parameter Estimation

- Goal:
  - Find values of parameter so that model predictions can match experimental data (e.g. time serials, steady state)



krbNGF = 0.33, KmAkt = 0.16, kpRaf1 = 0.42 … …

target

krbNGF = 0.49, KmAkt = 0.08, kpRaf1 = 0.97 … …

krbNGF = 0.88, KmAkt = 0.21, kpRaf1 = 0.05 … …

Optimization Approach

- Minimize the difference between model prediction and experimental data

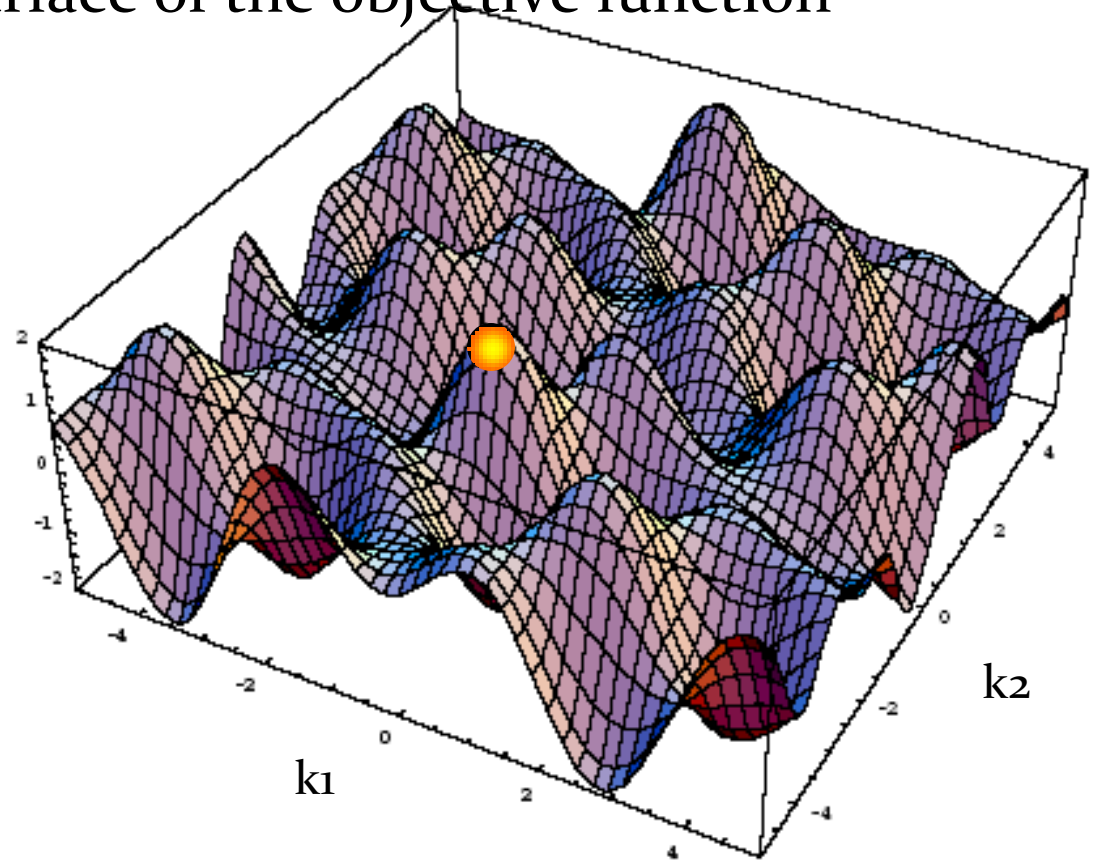
Given data $\tilde{\mathbf{x}}(t_j)$, find $\mathbf{k}$ to

minimize $J(\mathbf{k}) = \sum_j ||\mathbf{x}(t_j; \mathbf{k}) - \tilde{\mathbf{x}}(t_j)||^2$

J: objective function

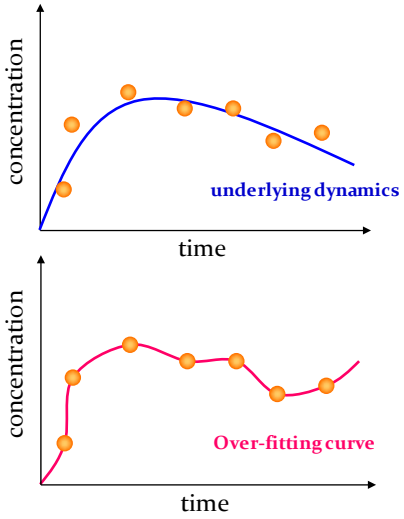
Example: Steepest Decent

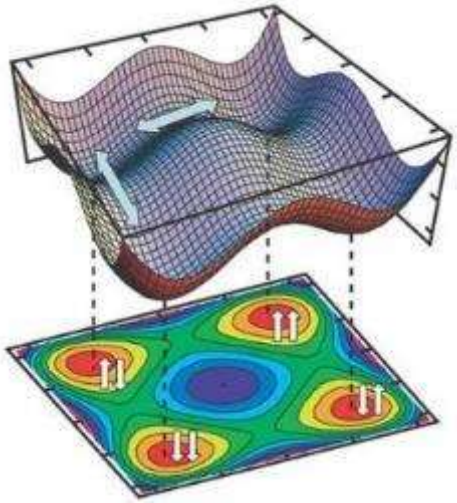
- Update following the direction of steepest descent on the hyper-surface of the objective function



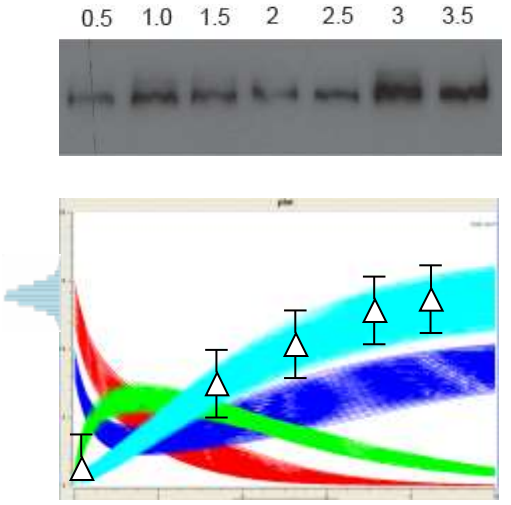
Many Challenges

- The curse of dimensionality
- Over-fitting
- Non-identifiable models
- Inherent uncertainty of data



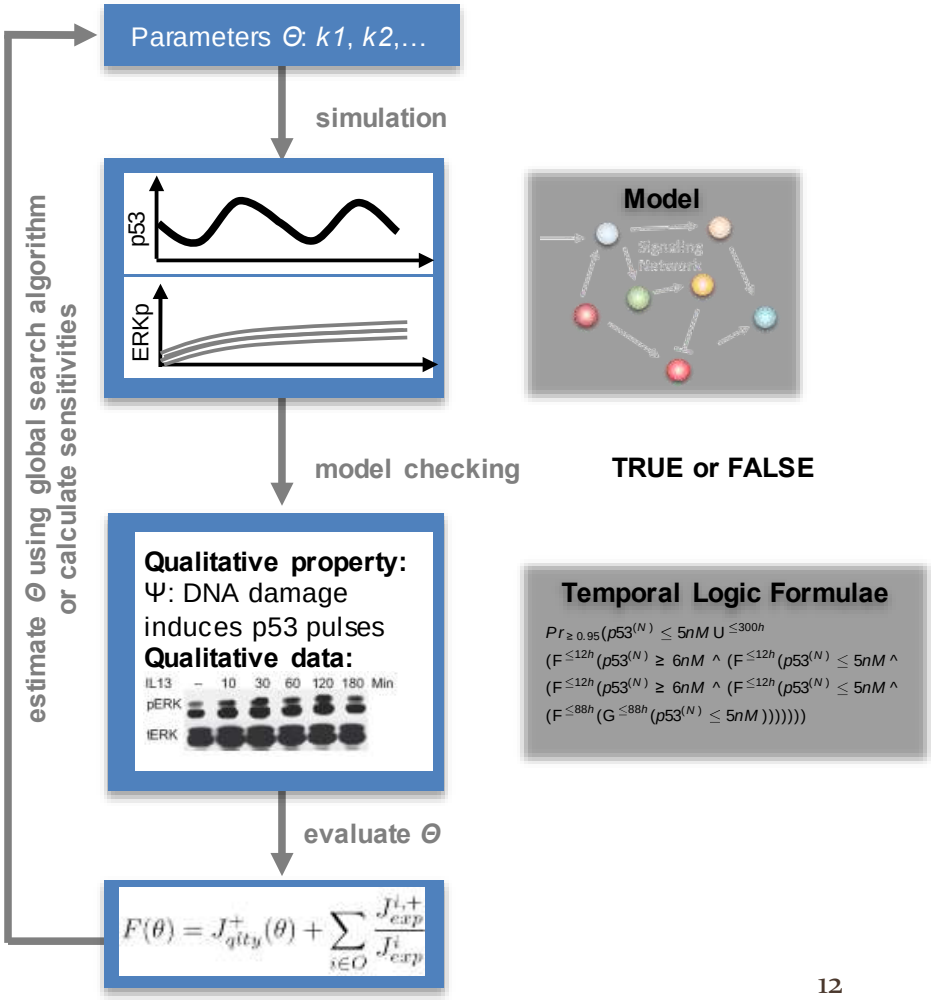


Kim et al. 2007



Our Solution

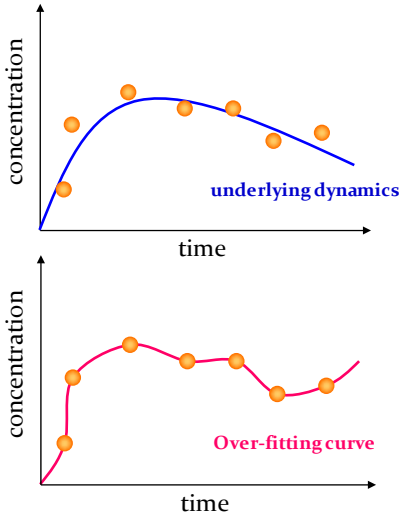
- A statistical model checking (SMC) based approach
  - Encode training data as a ***bounded linear temporal logic (BLTL)*** formula
  - Evaluate candidate parameters using SMC
  - Perform global optimization (e.g. stochastic ranking evolutional strategy, SRES)

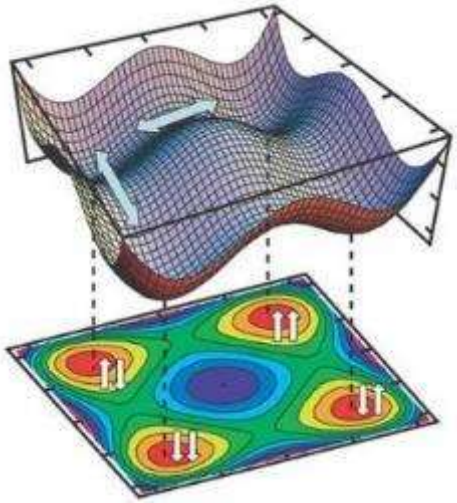


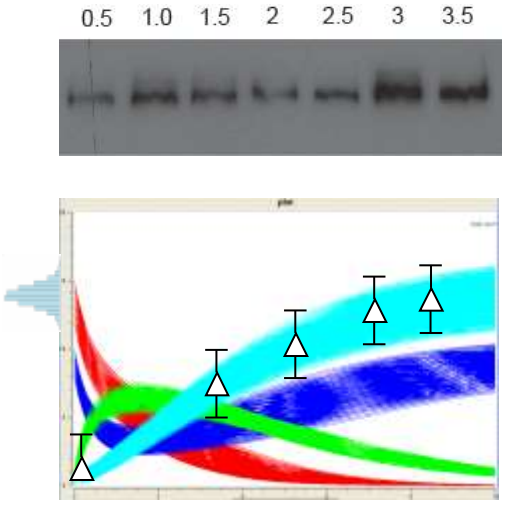
Palaniappan et al, CMSB, 2013, Liu & Faeder, BIBM, 2016

Our Solution

- Advantages
  - Utilize both ***quantitative*** and ***qualitative*** knowledge
  - Deal with uncertainty of the biological system/data
  - Good scalability due to the power of statistical testing







Kim et al. 2007

How to encode knowledge?

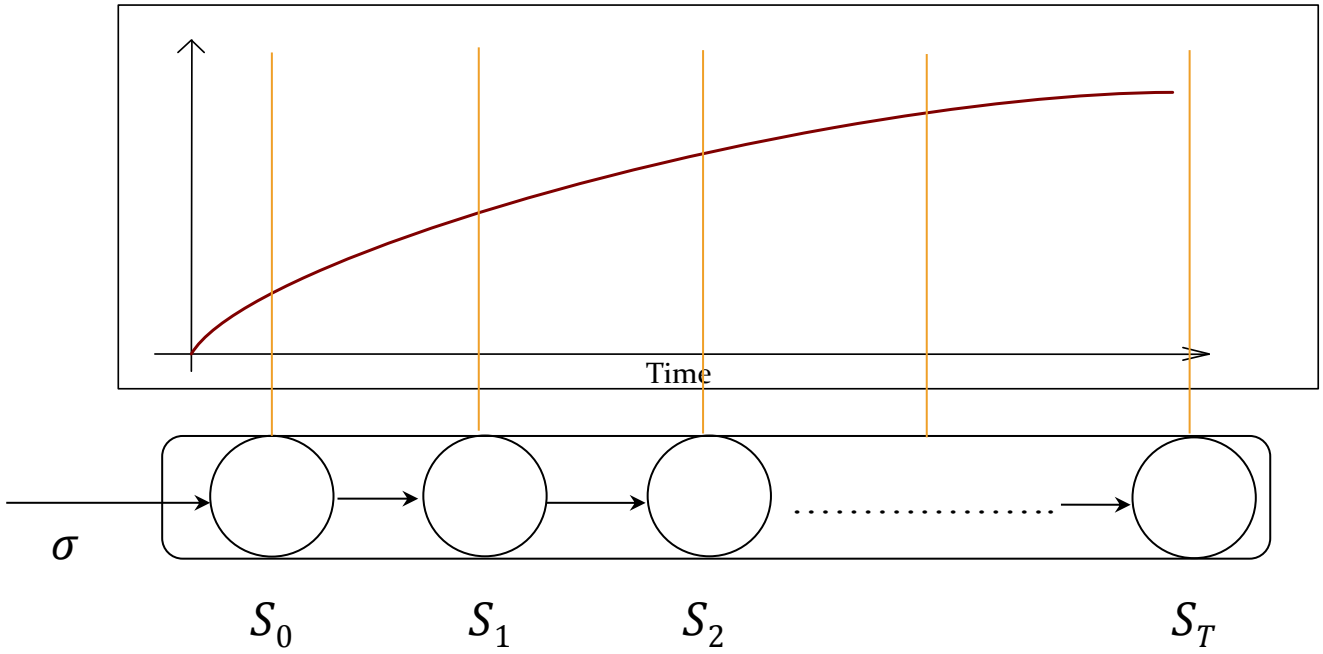
- E.g.
  - "ERKp level is between 10nM and 20nM"
  - "*Caspase-3 level sustains once it reaches threshold 30nM*"
- Temporal logic
  - A bounded linear temporal logic for biological properties (CSMB'13)

$$C3 \le 1nM \mathbf{U}^{10h} (\mathbf{F}^{\le 56h} (C3 \ge 30nM \wedge \mathbf{G}^{\le 44h} (C3 \ge 30nM)))$$

Amir Pnueli

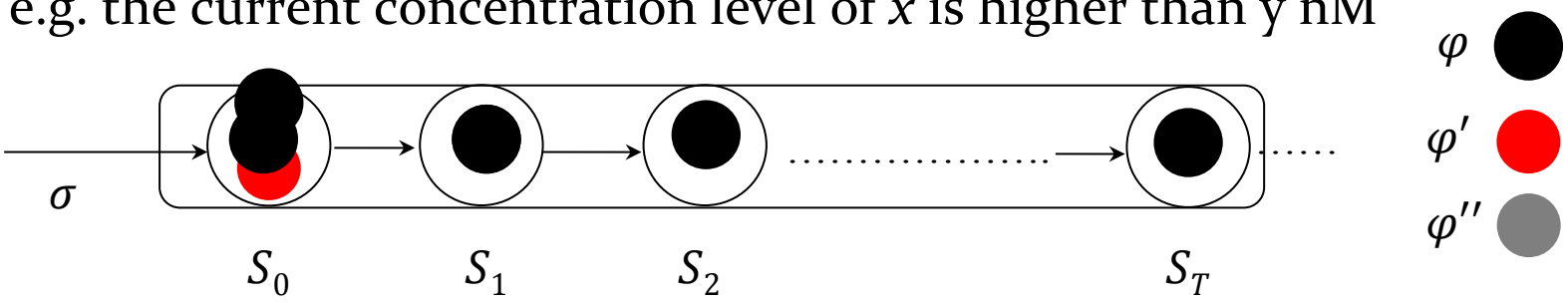
BLTL

- A finite set of time points: $\mathbf{T} = \{0,1,\ldots,T\}$
- A trajectory is represented by
  $\sigma = (s_o, t_o), (s_1, t_1), \ldots (s_T, t_T) \ldots$



BLTL

- Atomic (elementary) proposition: $x\#\, y, \# \in \{>, <, =, \leq, \geq\}$
  - e.g. the current concentration level of *x* is higher than y nM



- The formulas are built over operators $\wedge, \vee, \neg, \mathbf{O}, \mathbf{G}^{\leq t}, \mathbf{G}^{t}, \mathbf{F}^{\leq t}, \mathbf{F}^{t}, \mathbf{U}^{\leq t}, \mathbf{U}^{t}$

$\sigma(o) \vDash \varphi \vee \varphi'$, $\sigma(o) \vDash \varphi \wedge \varphi'$, $\sigma(o) \vDash \varphi$, $\sigma(o) \vDash \sim\varphi''$

$\sigma(o) \vDash O(\varphi)$, φ is true in the next state

$\sigma(o) \vDash \varphi U^{\leq t} \varphi'$, φ will be true until φ' is true

$\sigma(o) \vDash F^{\leq t}(\varphi')$, φ' will be true some time in the future

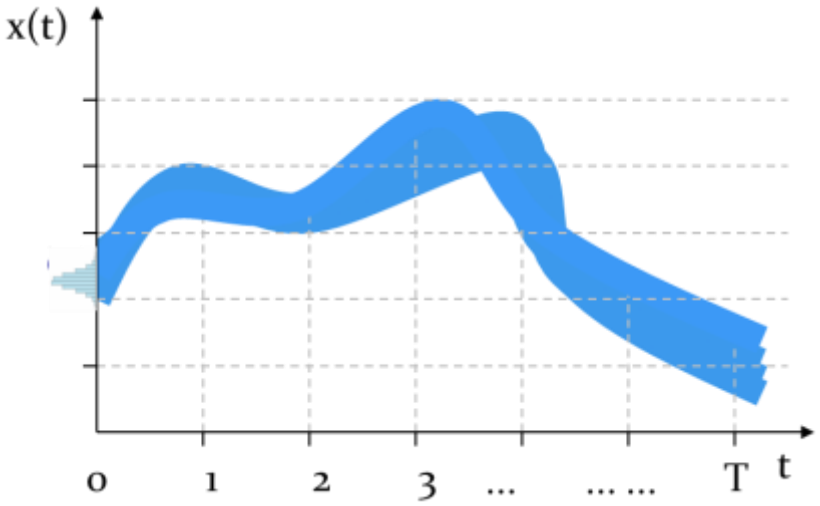
$\sigma(o) \vDash G^{\leq t}(\varphi)$, φ will be globally true in the future

$\sigma(o) \vDash F^{t}(\varphi)$, φ is true at time point t

Probabilistic BLTL

- Example:
  - *Caspase-3 level sustains once it reaches threshold 30nM with a probability at least 0.95*

$$\Pr_{\geq 0.95}(C3 \leq 1nM \mathbf{U}^{10h}(\mathbf{F}^{\leq 56h}(C3 \geq 30nM \wedge \mathbf{G}^{\leq 44h}(C3 \geq 30nM))))$$



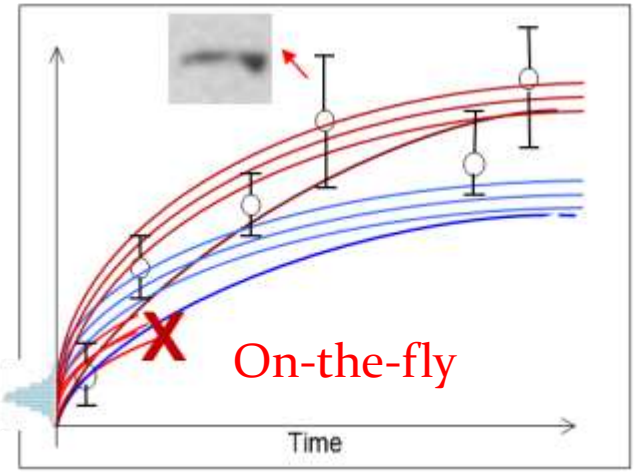
SMC of PBLTL formulas

- Check $M \models \Pr_{\geq r}(\psi)$ using a sequential hypothesis test between

$$\text{H0}: p \geq r + \delta \text{ and } \text{H1}: p \leq r - \delta$$

- Generate a sequence of sample trajectories: $\sigma_1, \sigma_2, \ldots$
- Verify each trajectory and determine whether accept Ho or H1 based on Type I/II error bounds (α, β):

$$q_m = \frac{[r-\delta]^{(\sum_{i=1}^{m} y_i)}[1-[r-\delta]]^{(m-\sum_{i=1}^{m} y_i)}}{[r+\delta]^{(\sum_{i=1}^{m} y_i)}[1-[r+\delta]]^{(m-\sum_{i=1}^{m} y_i)}}$$



Knowledge Encoding

- Quantitative experimental data

$$\psi_i^t = \mathbf{F}^t(l_i^t \le x_i \wedge x_i \le u_i^t)$$

$$\psi_{\exp} = \wedge_{i \in O}(\wedge_{t \in T_i} \psi_i^t)$$

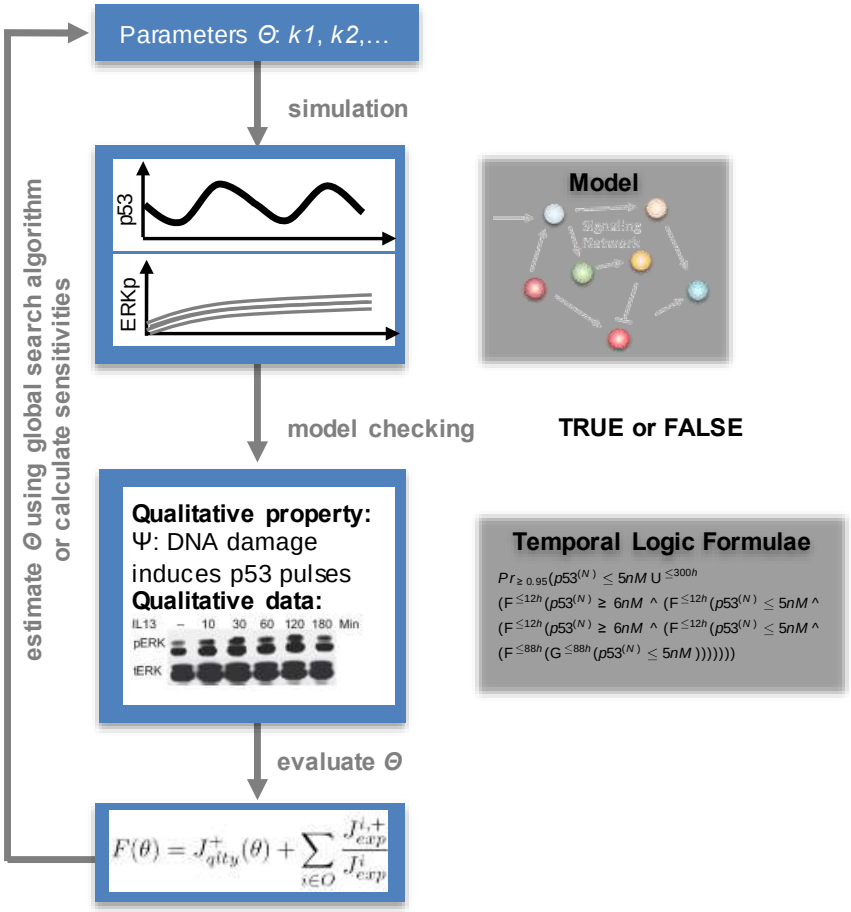
- Qualitative properties of the dynamics
  - E.g. transient/sustained activation, oscillatory behavior, bistable, …
  - *'trend'* formulas: ψ_{qlty}

- PBLTL formula: $\Pr_{\ge r}(\psi_{\exp} \wedge \psi_{qlty})$

SMC based Parameter Estimation

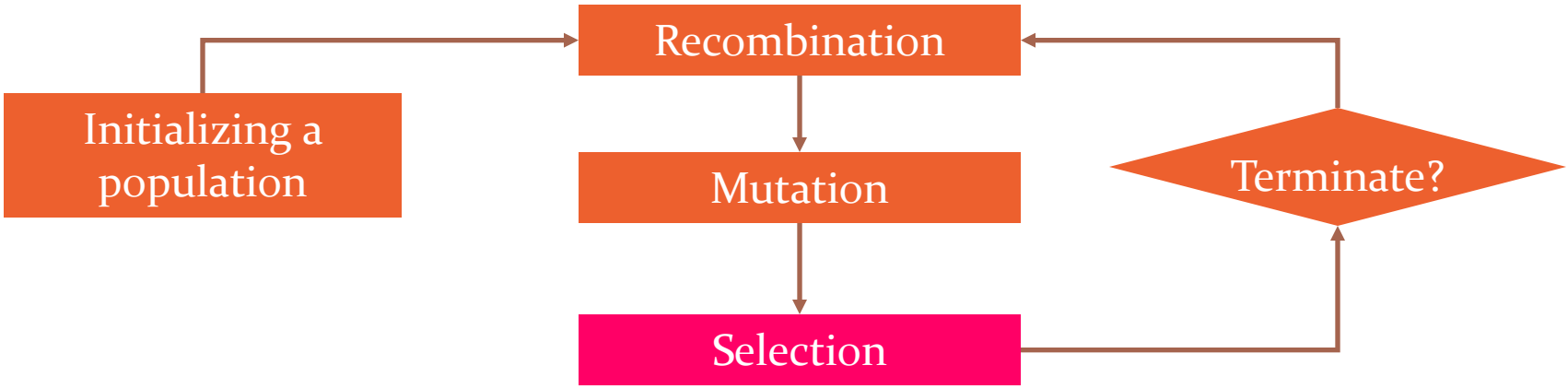
1. Guess θ_l
2. Verify $\psi_{\text{exp}} \wedge \psi_{qlty}$ with the chosen strength
3. Compute $F(\theta_l)$
4. Terminate or make a new guess (based on search strategy e.g. SRES) and repeat step 1

$$F(\theta) = J^{+}_{qlty}(\theta) + \sum_{i \in O} \frac{J^{i,+}_{exp}}{J^{i}_{exp}}$$



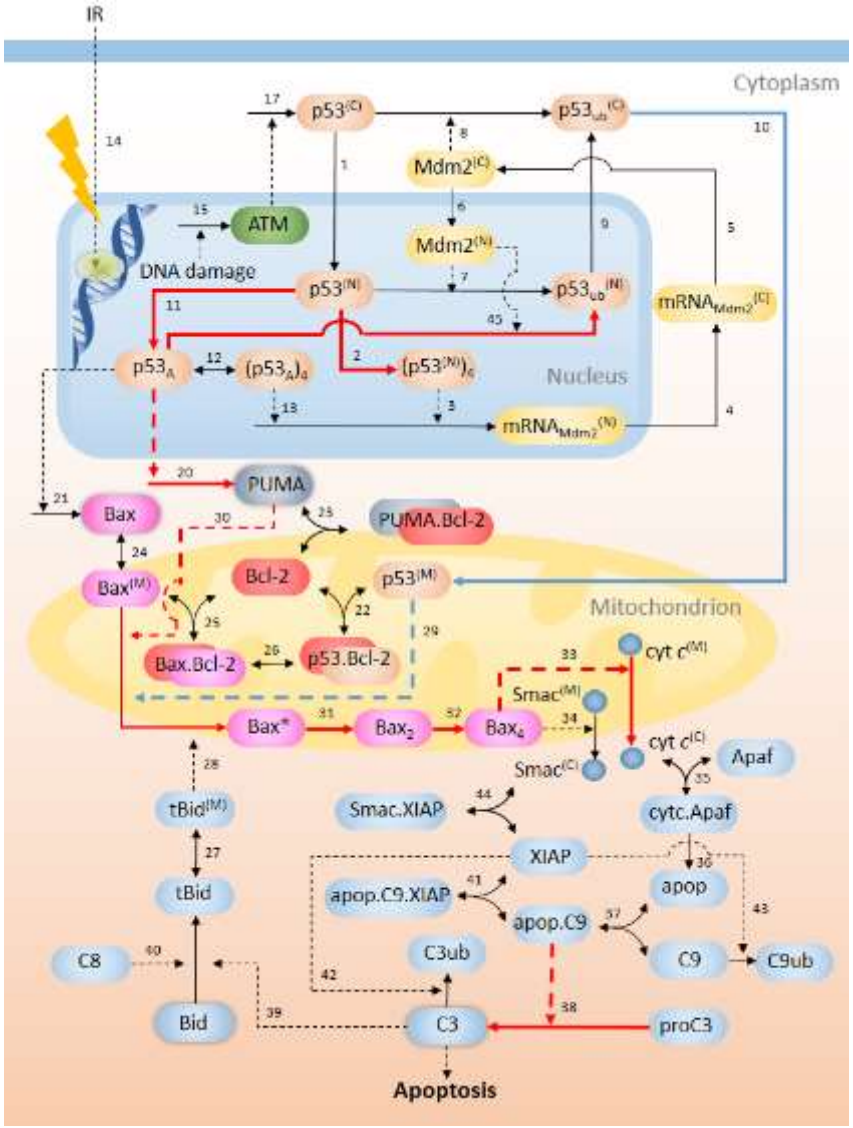
Stochastic Ranking Evolutionary Strategy

- A variation of evolutionary strategy
- Select best λ solutions according to a probabilistic formula
- One of the best performing global method in parameter estimation (*Moles et al, Genome Res 2003*)



Benchmarking

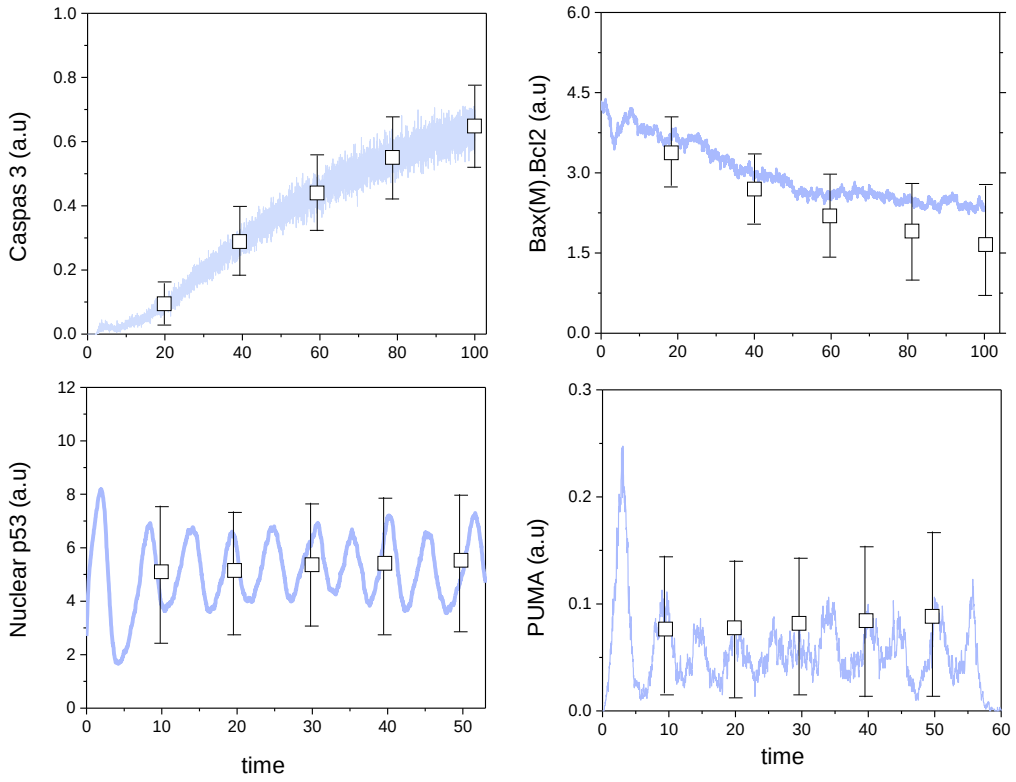
- p53-induced apoptosis
  - 86 rules
  - 160 parameters (10 unknown)
- Synthetic training data
  - 4 species at 5 time points
  - 3 qualitative properties:
    - Mmd2 reaches its peak before p53
    - Sustained caspase-3 once its level reaches certain threshold
    - p53 pulses induce oscillatory behaviors of target genes



Liu et al, Sci Rep, 2014

Benchmarking

- Running time: 4.2 hours
- Reproduce quantitative data



Benchmarking

- Reproduce qualitative behaviors
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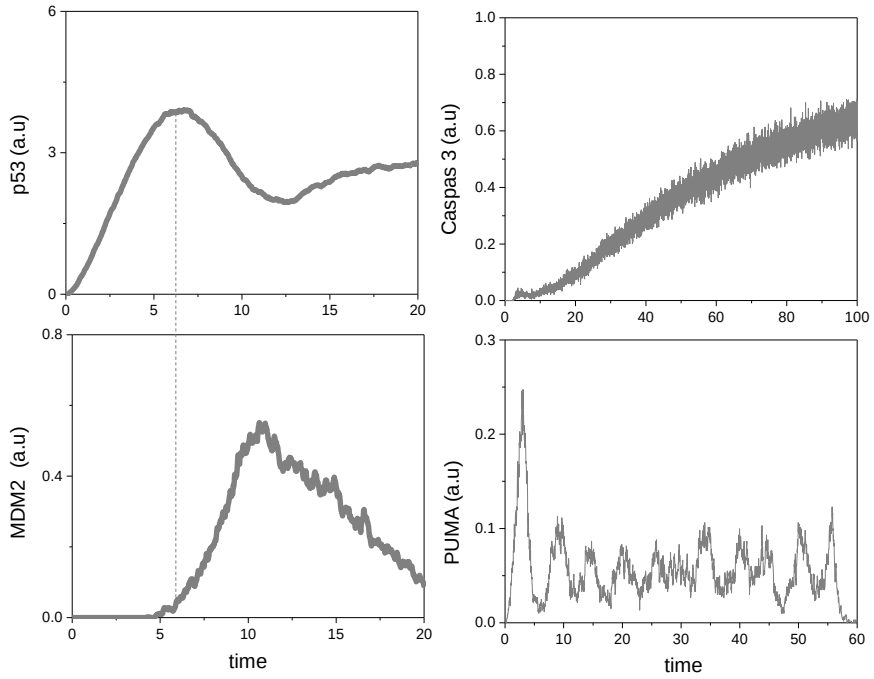
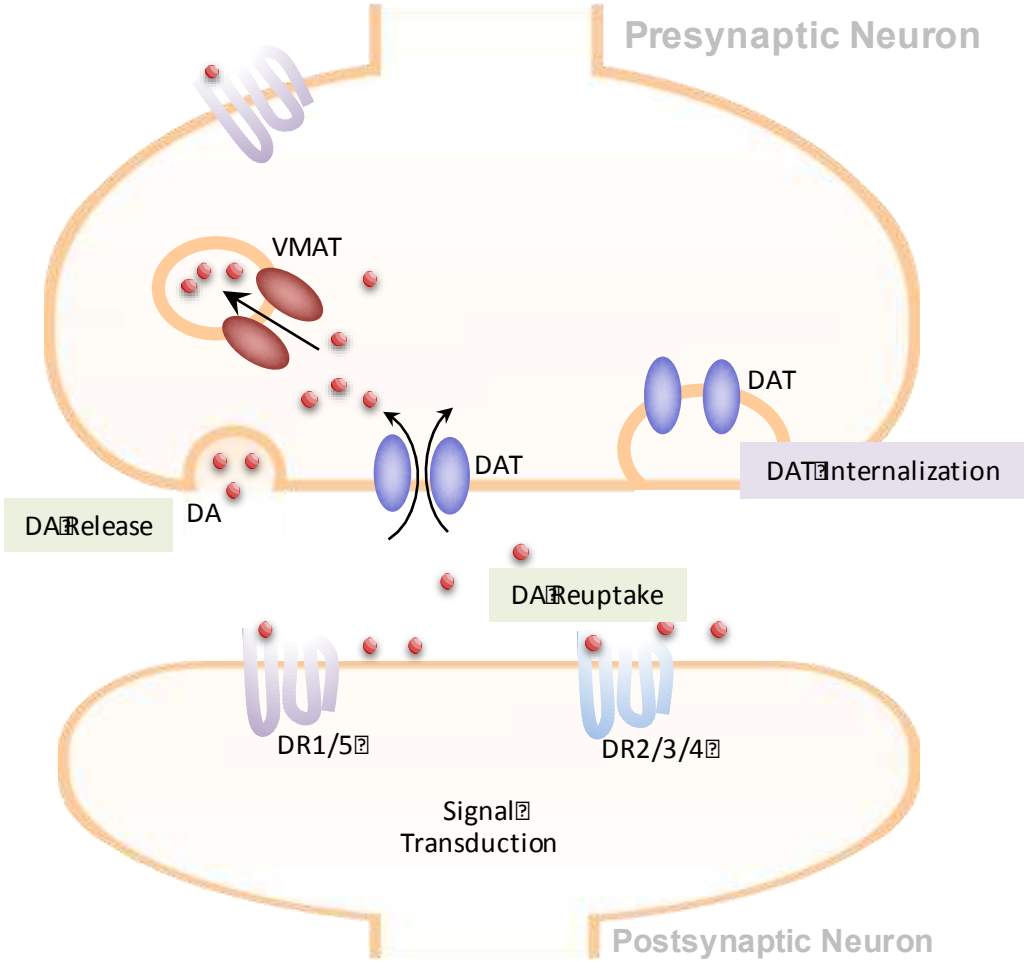


Amphetamine (AMPH)

- Induce euphoria and hyperactivity by increasing extracellular dopamine
- AMPH enters DA neurons via DAT
- 'Block' VMAT2
- Enhance DA efflux
  - via PKC, CaMKII, G-protein pathways
- Stimulate DAT internalization
  - via Rho pathway



Susan Amara, DBP1

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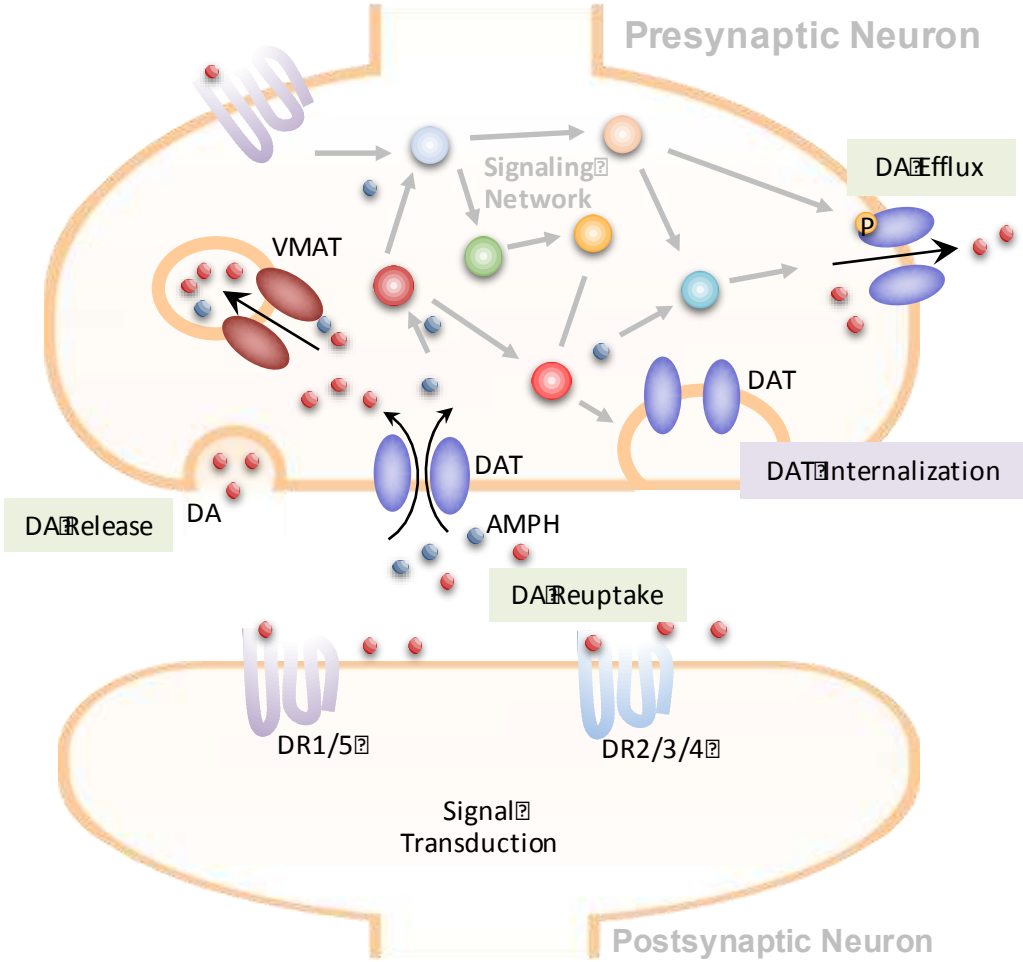
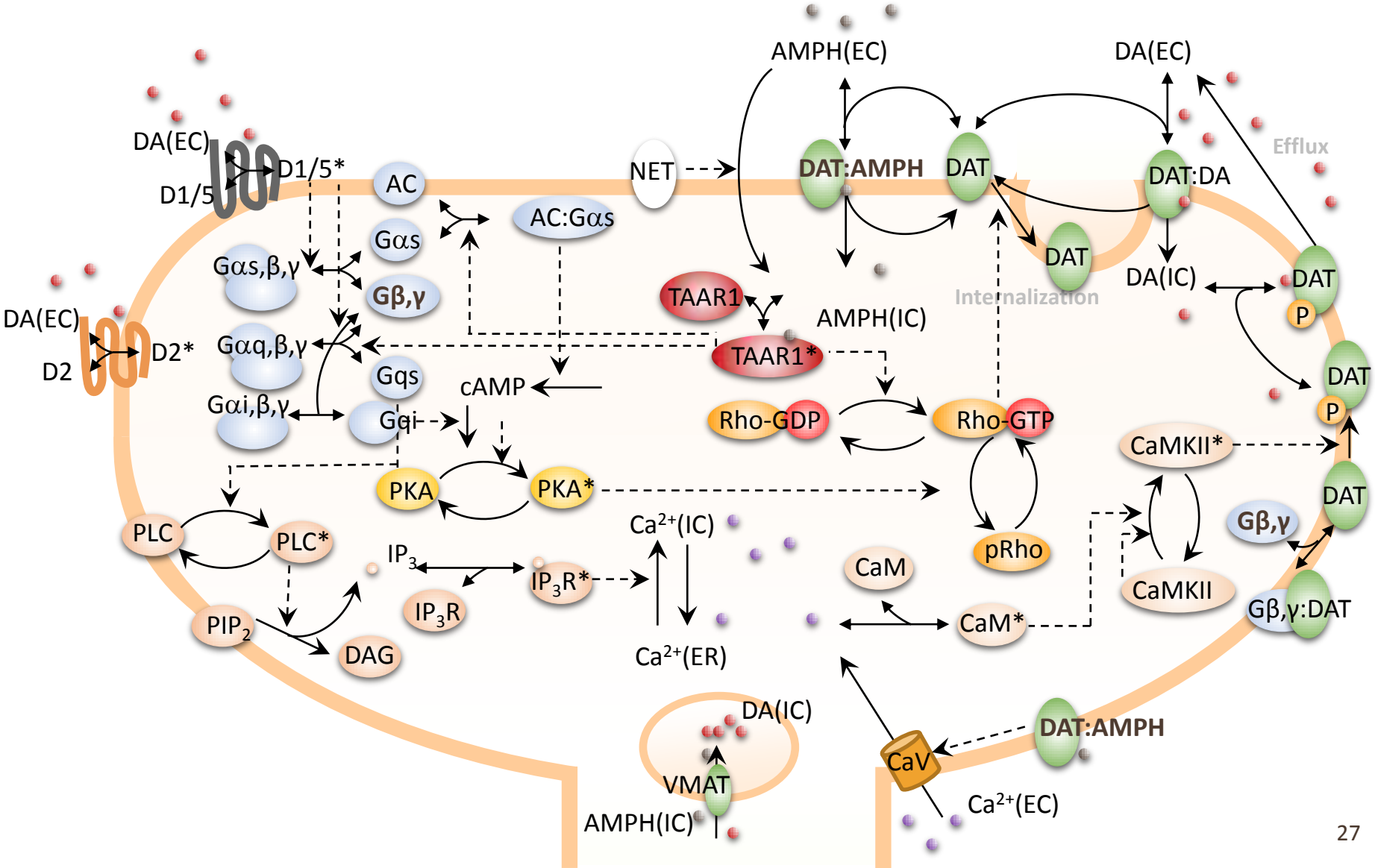
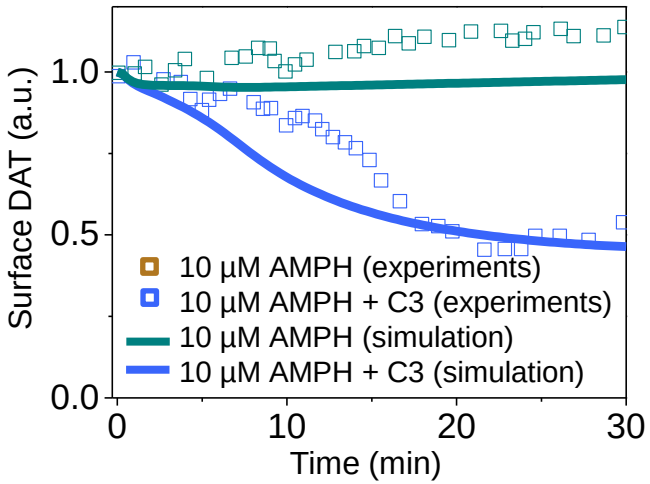


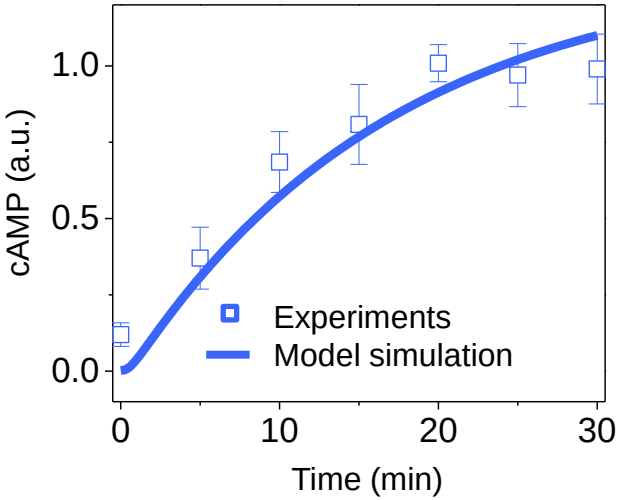
Susan Amara, DBP1

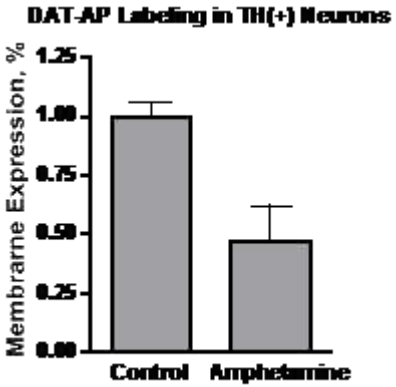
A Kinetic Model

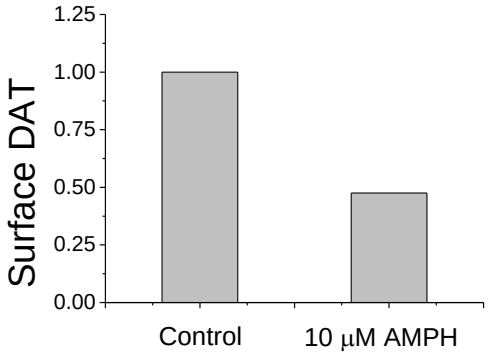


Training data





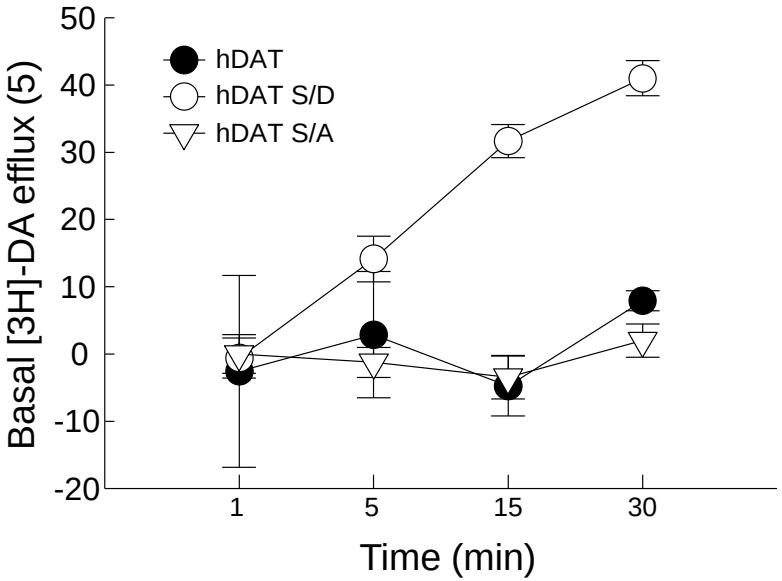


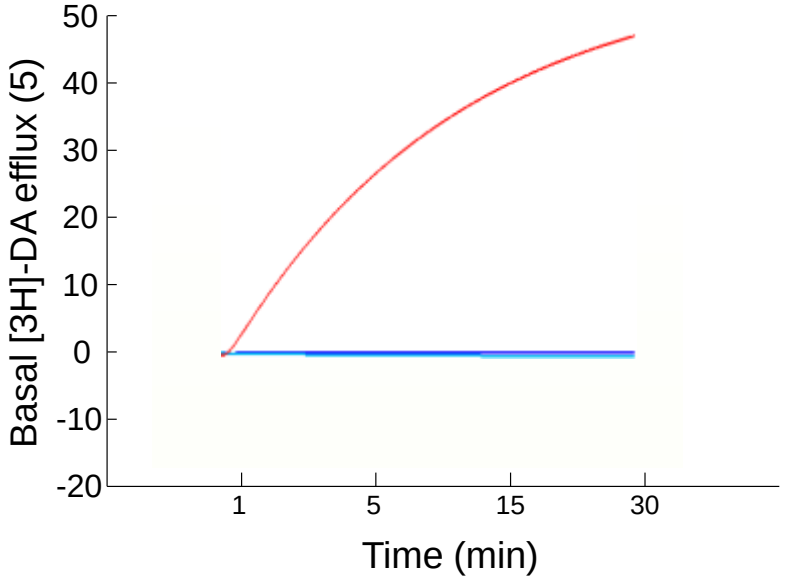


Wheeler et al, PNAS 2015

Training Data

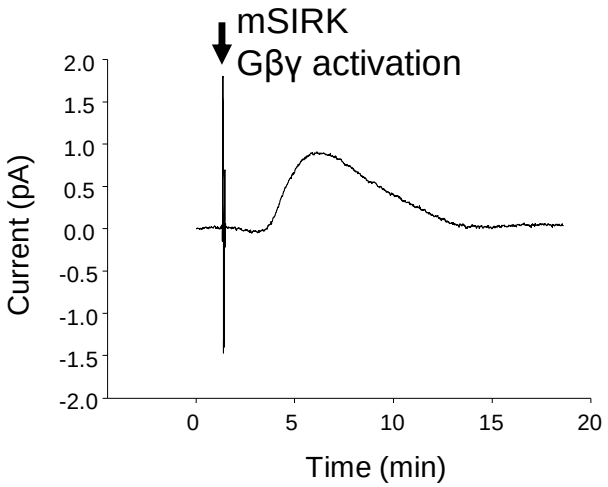
- Effect of N-terminal Serines Phosphorylation on DA efflux

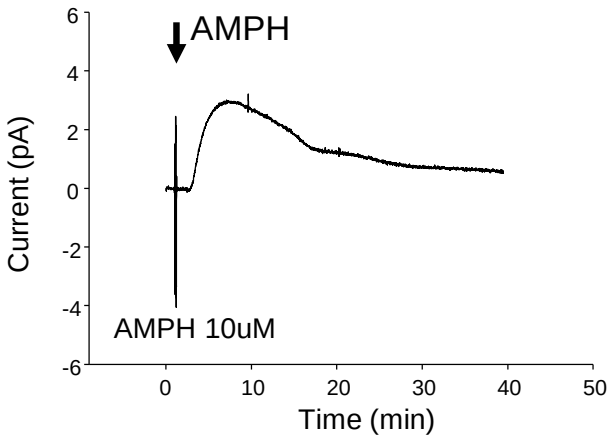


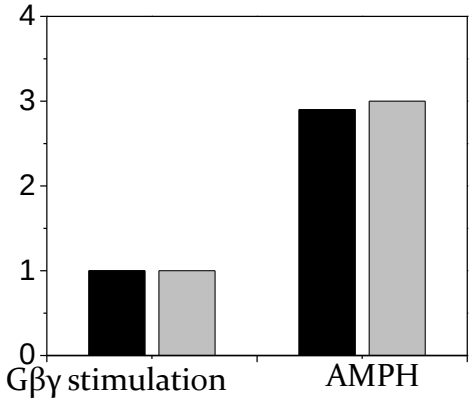


Training Data

- Amperometric recordings for DA efflux after Gβγ stimulation in CHO cells expressing DAT

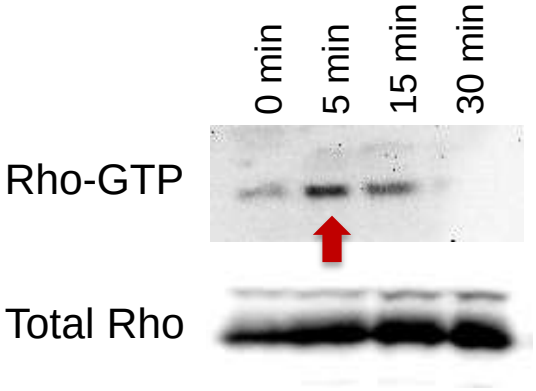


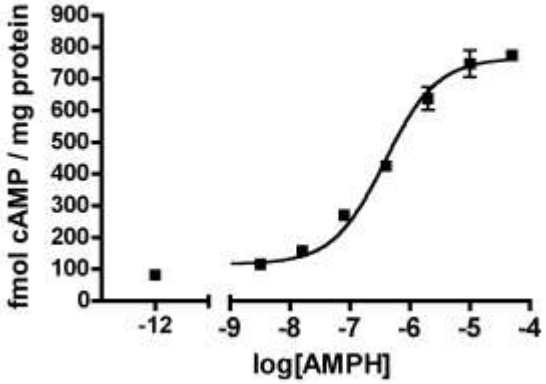


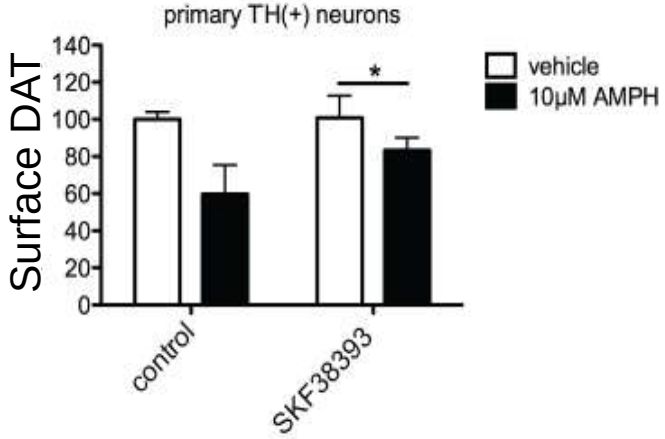


Model reproduces test data

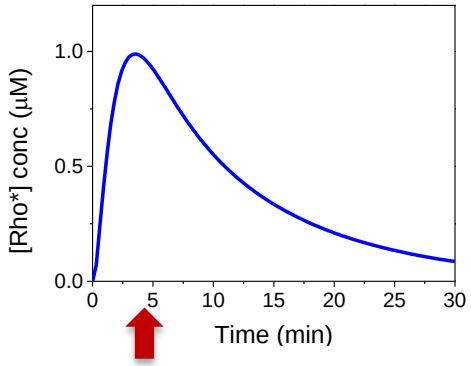
Experimental data

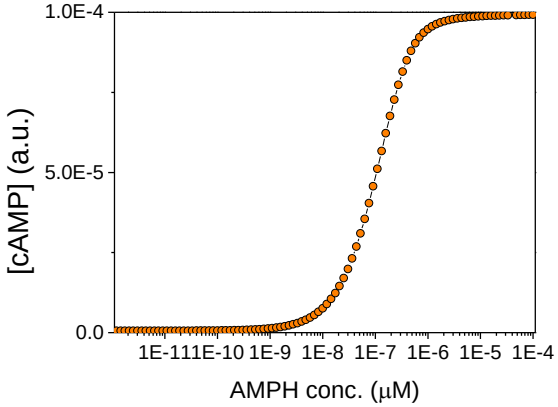


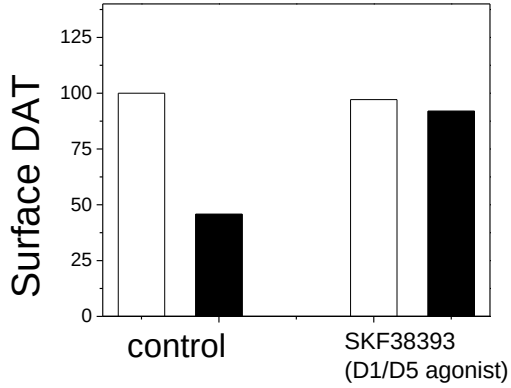




Model prediction



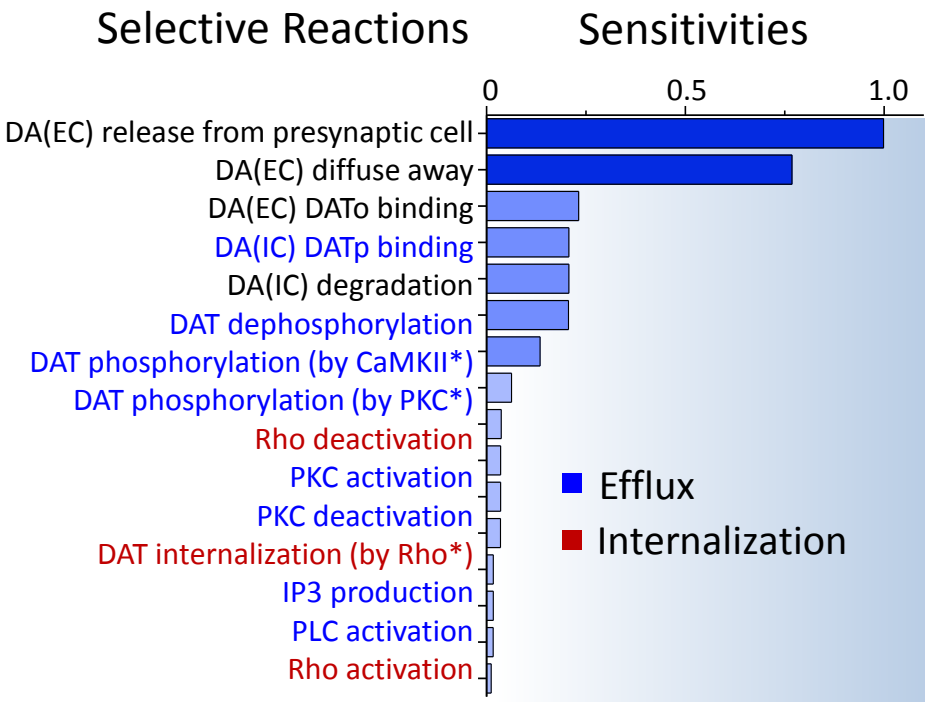


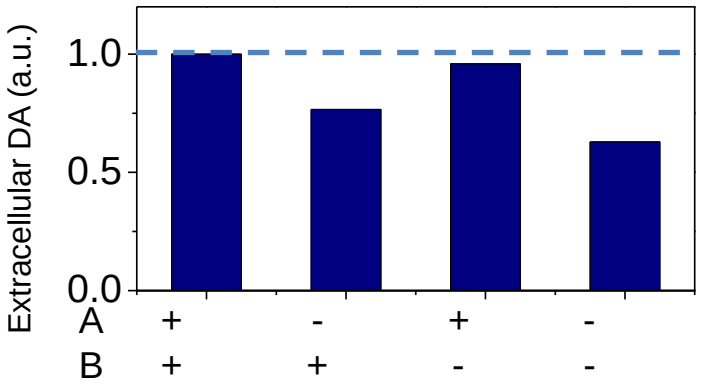


Wheeler et al, PNAS 2015

Model Predictions

- Sensitivity analysis suggests that AMPH modulates DA(EC) level mainly through the DA efflux pathways, than DAT internalization
- Simultaneously block DAT internalization and DA efflux pathways synergistically enhance DA reuptake

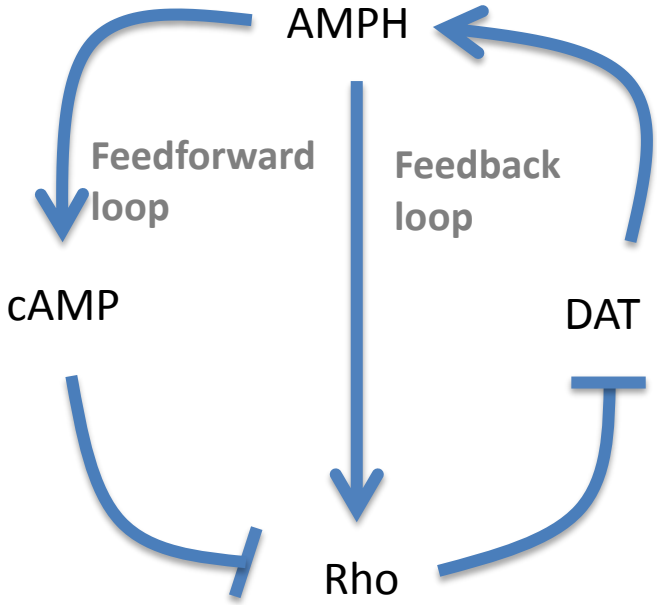


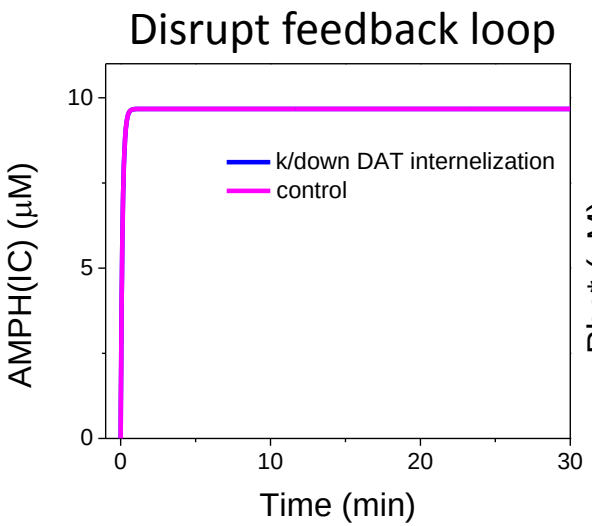


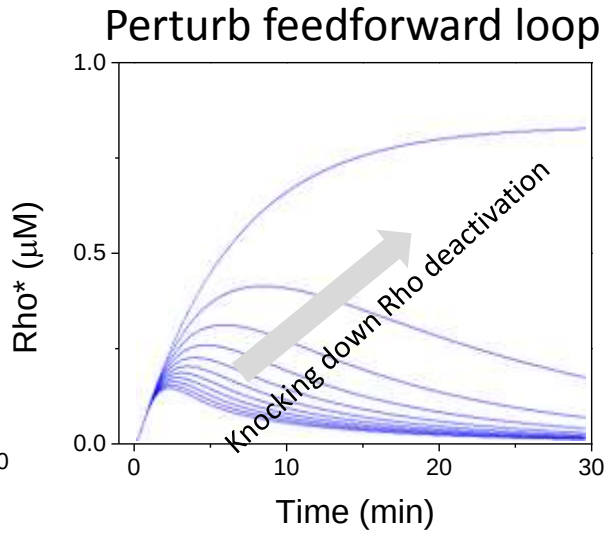
A: DAT phosphorylation by CaMKKII*
B: Rho activation

Model Predictions

- How Rho mediated feedforward/back loops fine-tune AMPH induced DAT internalization
  - The role of the feedback loop is insignificant
  - The feedforward loop governs the time window of Rho activation







Conclusion

- A SMC based approach for the parameter estimation of rule-based models
- Utilize both quantitative and qualitative knowledge
- Deal with uncertainty of biological systems/data
- Good performance due to the power of statistical testing and online model checking

Our MC-based Techniques

Core Technology

System Representation
- DBN (***Bioinformatic, 2012***)
- ODEs (***CMSB'13***)
- Stochastic models (***Sci Rep, 2014***)
- Hybrid Automata (***CMSB'14, HSCC'15, HSB'15***)
- Boolean Network (***CMSB'16***)
- Rule-based models (***BIBM'16***)

Model Checking
- Statistical model checking
- Probabilistic model checking
- δ-decision model checking
- Symbolic model checking

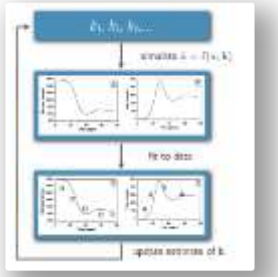
SAT

UNSAT

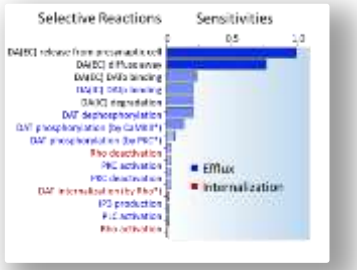
Temporal Property
- Bounded Linear Temporal Logic
- Quantitative property
- Qualitative behaviors

Analysis Methods

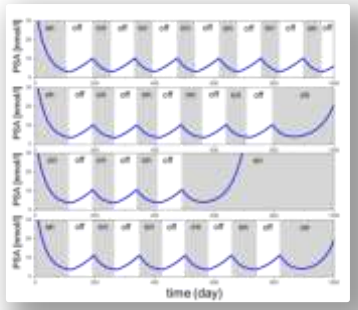
Parameter estimation

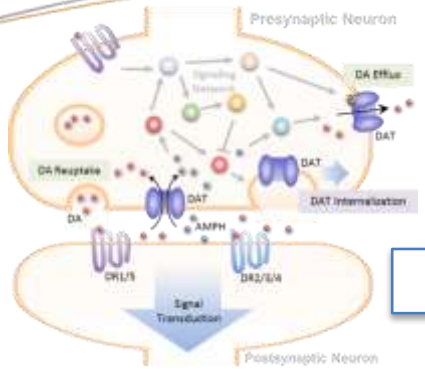


Sensitivity analysis



Predict therapeutic strategies

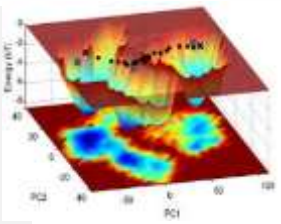




Model Construction

BioNetGen Modeling & Analysis

```
begin reaction rules
L(r) + R(l) <->
L(r!1).R(l!1) kp1, km1
end reaction rules
generate_network()
simulate({method=>"ode",t_e
nd=>500,n_steps=>500})
```

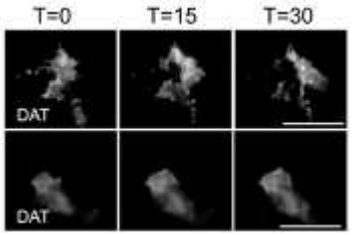


Energy Landscape

Kinetic Modeling

Biological System & Data

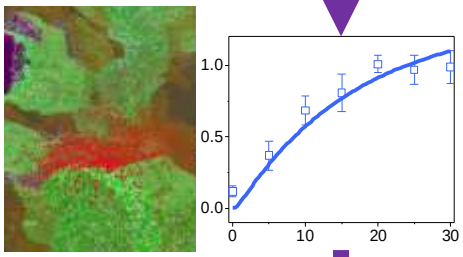
Parameter Estimation & Model Validation

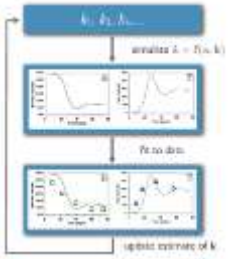


MCell Simulations

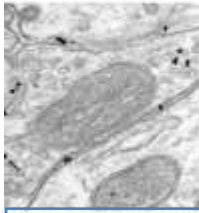
Image analysis

Model Checking Techniques



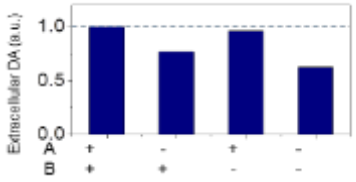


Model Analysis

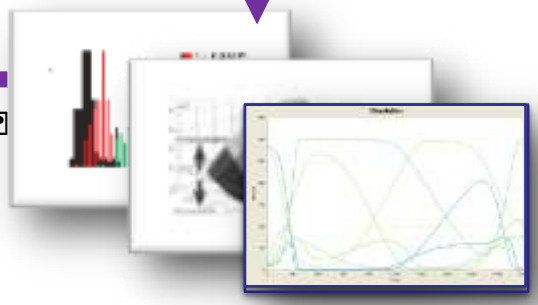


EM Data

Model Refinement



New Insights & Hypotheses



Discovery of new therapeutic strategy

Acknowledgements

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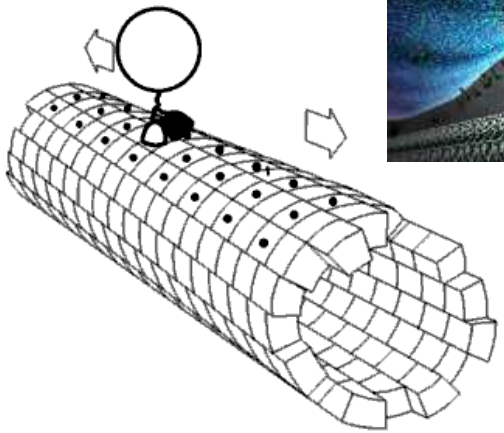
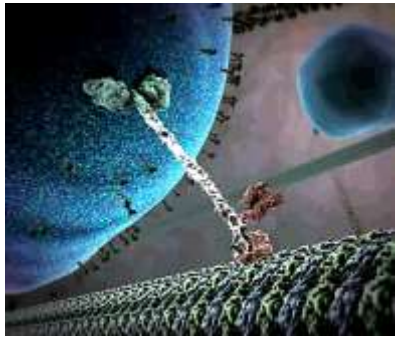
Edmund M. Clarke's Lab

National University of Singapore
P.S. Thiagarajan's Lab

Questions?





ODE Dynamics

We assume that :

$x_i(t) \in [L_i, U_i]$, where $0 < L_i < U_i$

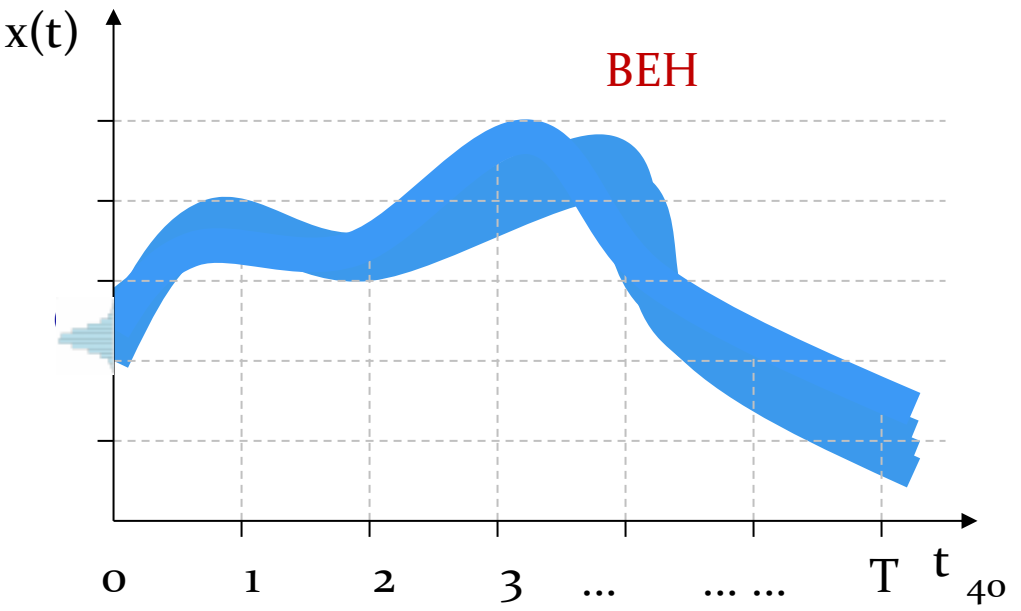
State space : $\mathbf{V} = [L_1, U_1] \times [L_2, U_2] \times ... [L_n, U_n] \subseteq \mathbf{R}_+^n$

$INIT = [L_1^{init}, U_1^{init}] \times [L_2^{init}, L_2^{init}] \times ... [L_n^{init}, L_n^{init}]$

$f_i \in C^1$ for each i, hence $F : \mathbf{V} \to \mathbf{V} \in C^1$

As a result, for each $\mathbf{v} \in \mathbf{V}$, the ODE system will have a unique solution $\mathbf{X}_\mathbf{v}(t)$

$$\frac{d\mathbf{x}}{dt} = F(\mathbf{x}), \mathbf{x} = \{x_1, x_2, ..., x_n\}, \text{and } F(\mathbf{x}(i)) = f_i$$



We define :

flow $\Phi : \mathbf{R}_+ \times \mathbf{V} \to \mathbf{V}$ for arbitrary initial vector $\mathbf{v}$

$\Phi(t, \mathbf{v}) = \mathbf{X}_\mathbf{v}(t)$

Then $\Phi(t, \cdot) \in C^0$

Fixing a maximal time point T, we define a *trajectory* strating from $\mathbf{v} \in \mathbf{V}$ denoted $\sigma_\mathbf{v}$

$\sigma_\mathbf{v} : [0, T] \to \mathbf{V}, \sigma_\mathbf{v}(t) = \Phi(t, \mathbf{v})$

Behavior of our dynamical system is the set of trajectories :

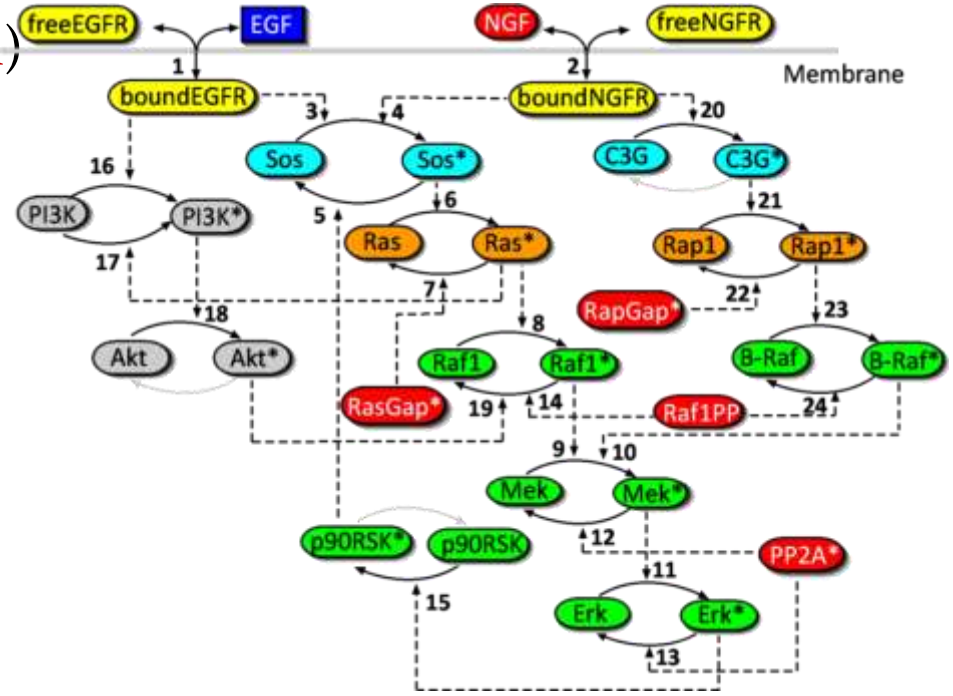
$BEH = \{\sigma_\mathbf{v} \mid \mathbf{v} \in INIT\}$

Case Studies

- Pathway models taken from BioModels database
- Nominal parameters
- Synthetic experimental data
- Qualitative trend

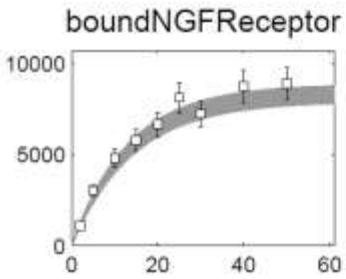
EGF-NGF Pathway

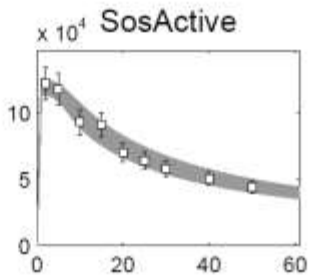
- ODE model (*Brown et al. 2004*)
  - 32 species
  - 48 parameters (20 unknown)
- Training data
  - 7 species, 9 time points
- Test data
  - 2 species, 9 time points

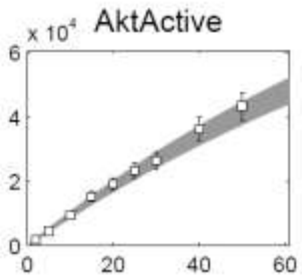


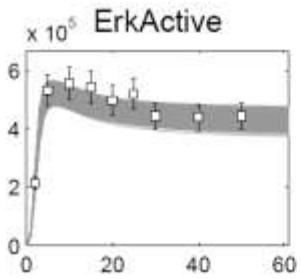
EGF-NGF Pathway

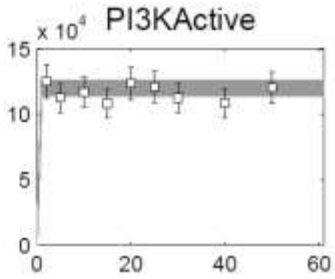
- Running time: 2.23 hours









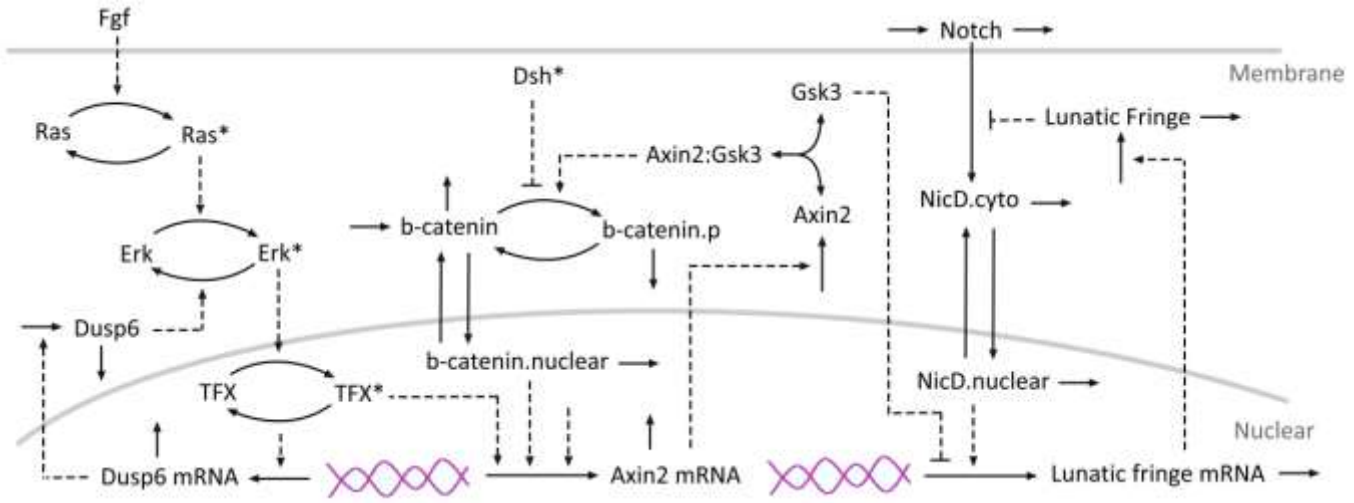


Training data

Test data

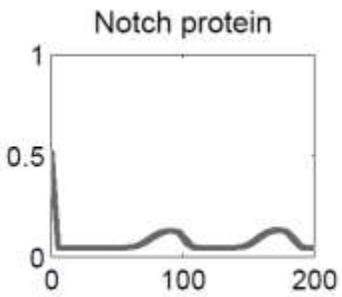
Segmentation Clock Network

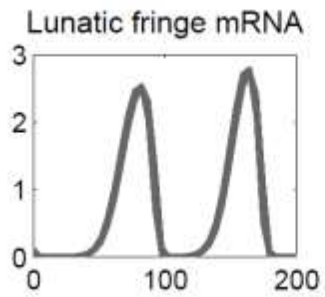
- ODE model (*Goldbeter et al. 2008*)
  - 22 species, 75 parameters (40 unknown)
- Training data
  - Time serials: Axin2 mRNA, 14 time points
  - Qualitative trend: 5 species, oscillatory behavior
    - E.g. $(([LmRNA \leq 0.4] \wedge (\mathbf{F}([LmRNA \geq 2.2] \wedge \mathbf{F}([LmRNA \leq 0.4] \wedge (\mathbf{F}([LmRNA \geq 2.2] \wedge \mathbf{F}([LmRNA \leq 0.4]))))))$
- Test data: Dusp6 protein, qualitative trend

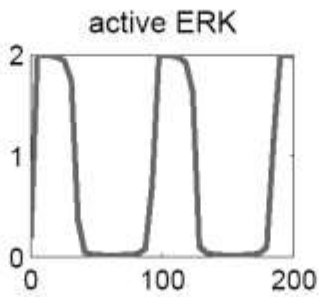


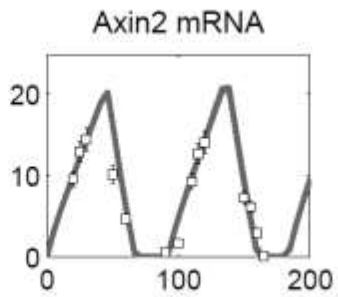
Segmentation Clock Network

- Running time: 2.2 hours

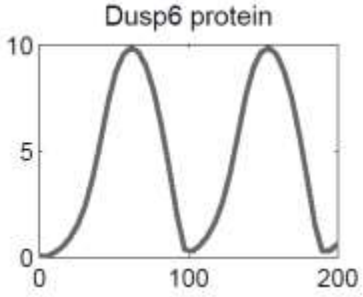








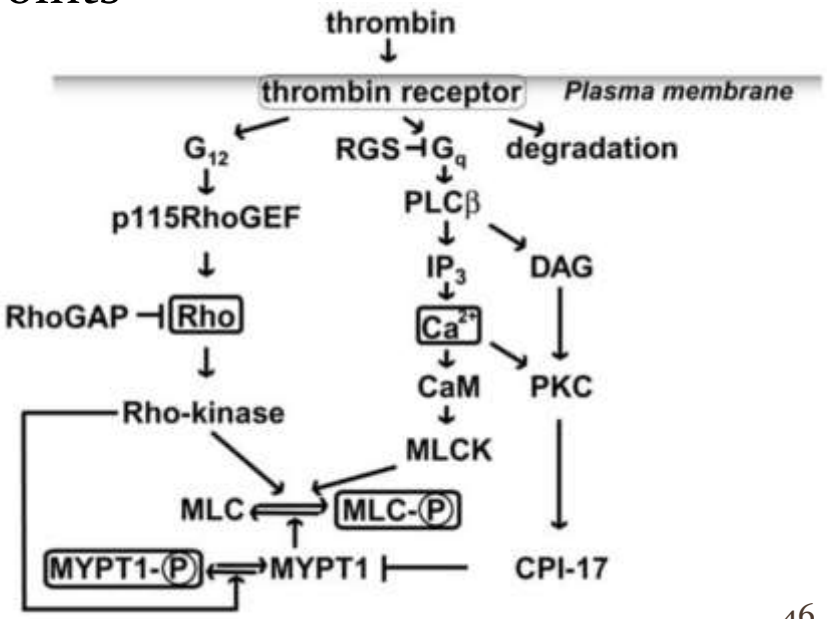
Training data



Test data

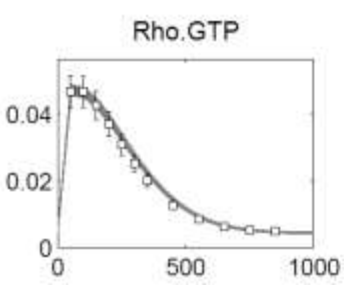
MLC Phosphorylation Pathway

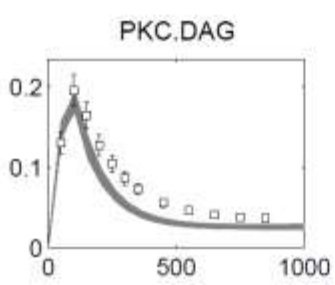
- Regulates the contraction of endothelia cells
- ODE model (*Maeda et al 2006*)
  - **105** species, 197 parameters (**100** unknown parameters)
- Training data
  - Time serials: 8 species, 12 time points
  - Qualitative trend: 2 species
- Test data
  - 2 species, 12 time points

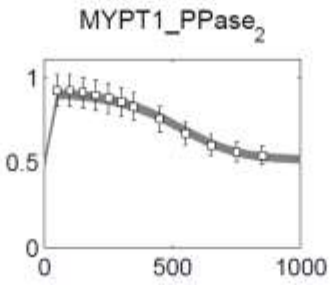


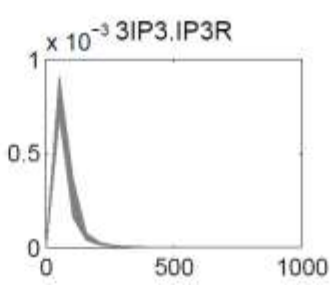
MLC Phosphorylation Pathway

- Running time: 50.67 hours

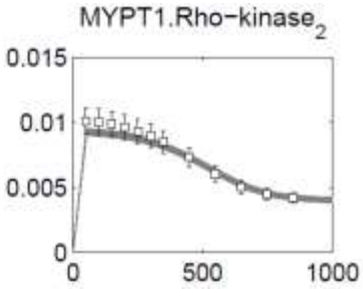








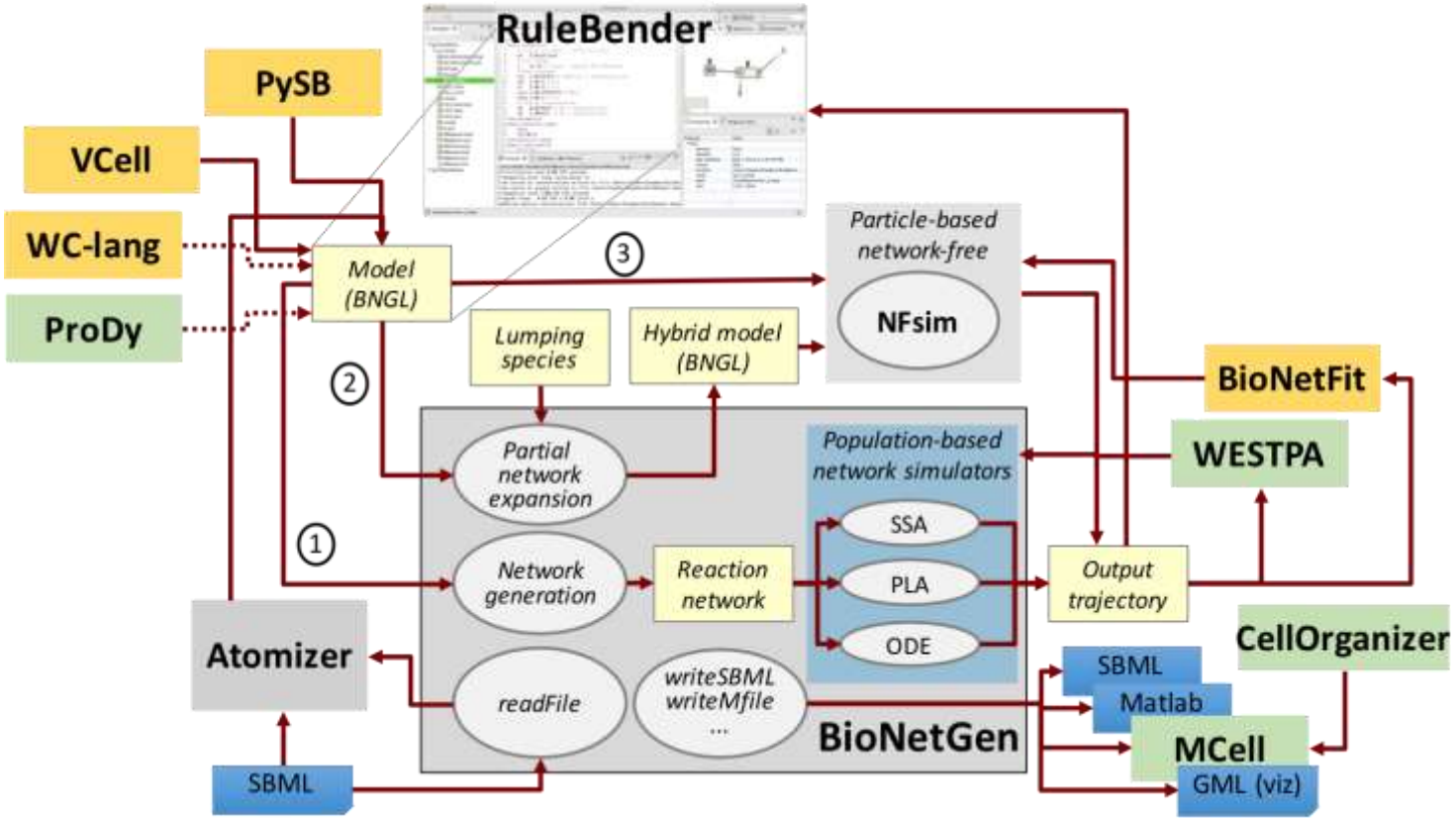
Training data



Test data

Parameter Estimation for BioNetGen

- Current solutions: ptempest, BioNetFit, SBML tools



JR Faeder, unpublished

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UNSAT

Temporal Property
- Bounded Linear Temporal Logic

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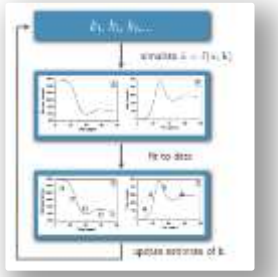
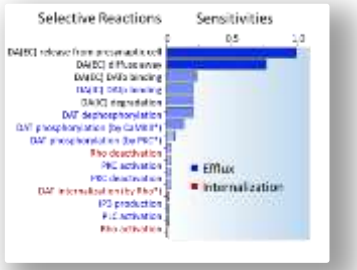
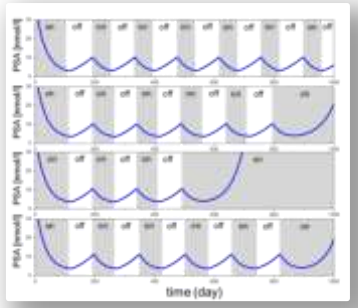
Parameter estimation



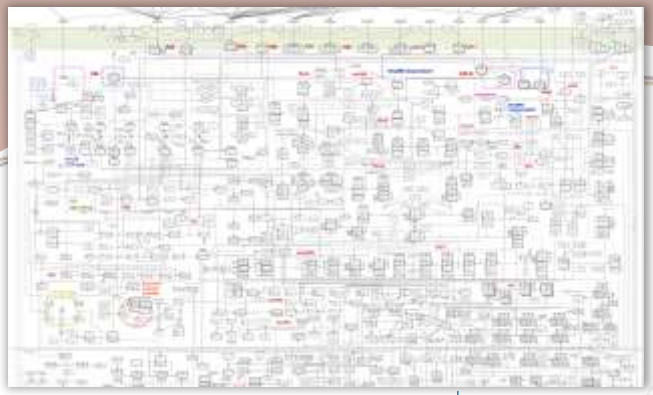
Sensitivity analysis



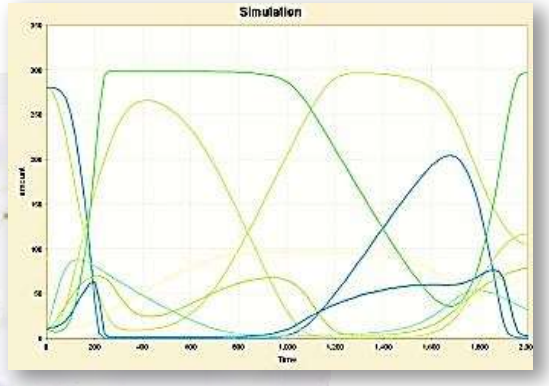
Predict therapeutic strategies



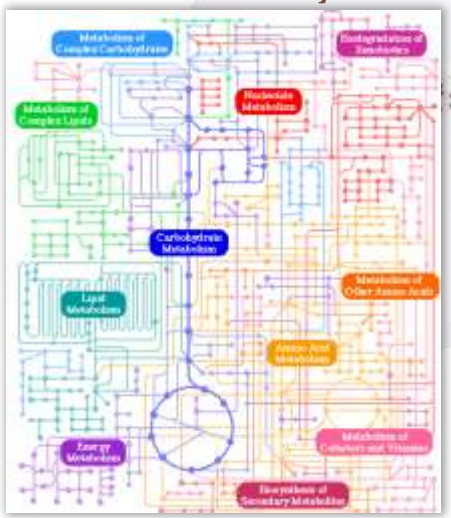
Biological Networks



Signaling Pathways
- Cell death
- Cell differentiation
- Cell proliferation
- Cell migration
- … …

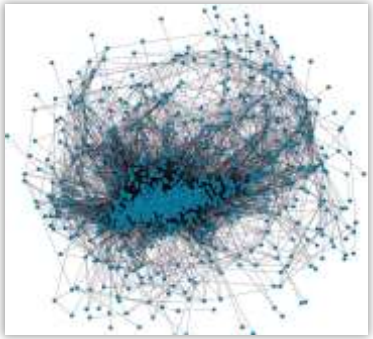


Metabolic Pathways



Macrophage

Gene Regulatory Network



Model Parameters

- Two types of model parameters
  - Initial conditions
  - Rate constants
- Experimental measurements
  - Expensive
  - Not possible to measure all parameters
  - *In vitro* measurements may not reflect the actual physiological conditions in the cell (*Minton, J Biol Chem, 2001*)
  - Cell population-based measurements are not very accurate (*Kim & Price, Phys Rev Lett, 2010*)

On-the-fly Model Checking

- Model checking and generation of trace are coupled i.e. simulate as much as you need.
- Algorithm
  - At each time point we maintain the minimum subset of formulas that need to be true at the state.
  - Based on the simulation, we check the validity of the elements in this set to verify the property
  - Simulation is stopped once the formula has been asserted true/false by the model checking algorithm.
  - We repeat the process of generating simulations and verification until we run enough simulations to satisfy the Wald's statistical test.