Formal Analysis Provides Parameters for Guiding Hyperoxidation in Bacteria using Phototoxic Proteins

Qinsi Wang

Carnegie Mellon University

Joint work with Natasa Miskov-Zivanov, Cheryl Telmer, Edmund M. Clarke

GLSVLSI 2015 Pittsburgh, USA

Bacteria-Killing

- ~ at least 5 nonillion ($5x10^{30}$) bacteria
- much of Earth's biomass is made up of bacteria
  - friendly bacteria
  - harmful bacteria

Bacteria-Killing

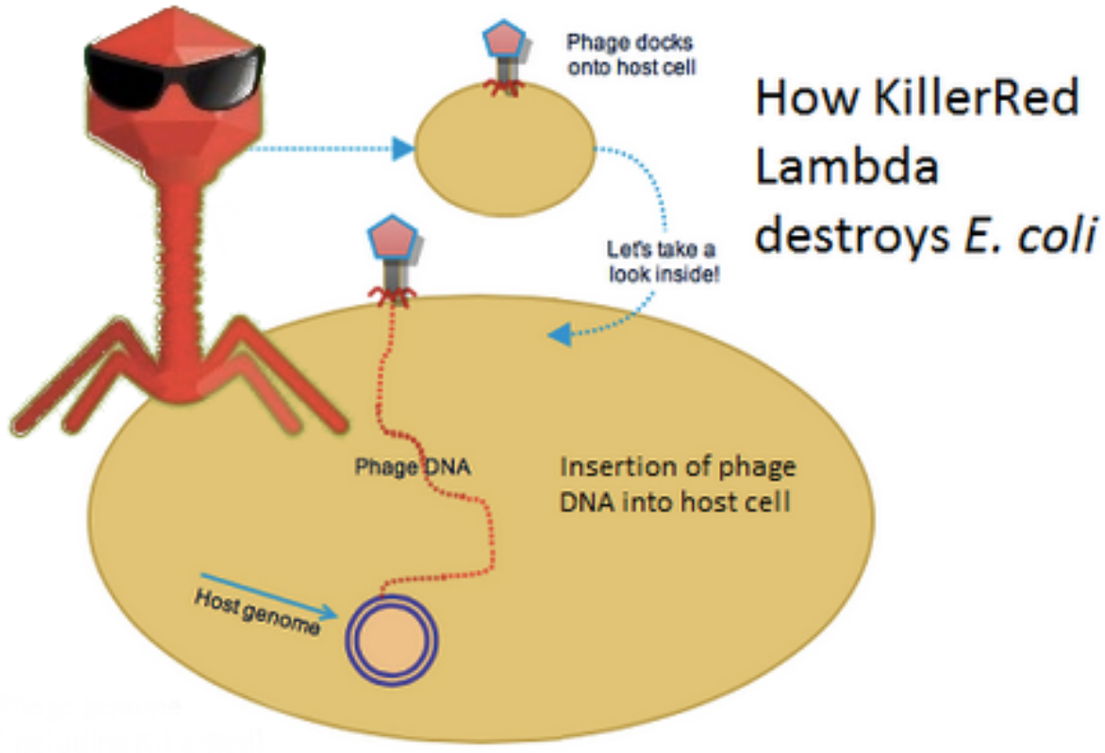
- ~ at least 5 nonillion ($5x10^{30}$) bacteria
- much of Earth's biomass is made up of bacteria
  - friendly bacteria
  - harmful bacteria

The Downside of Antibiotics

- discovery of antibiotics has been quickly followed by the development of antibiotic resistance.
- use of antibiotics often kills both good and bad microorganisms
- threats with overuse and misuse of antibiotics

Phage Therapy for Bacteria-Killing

- Phages are viruses that infect bacteria
- Evolve to manipulate the bacteria cells and genome, making resistance to phages difficult to achieve

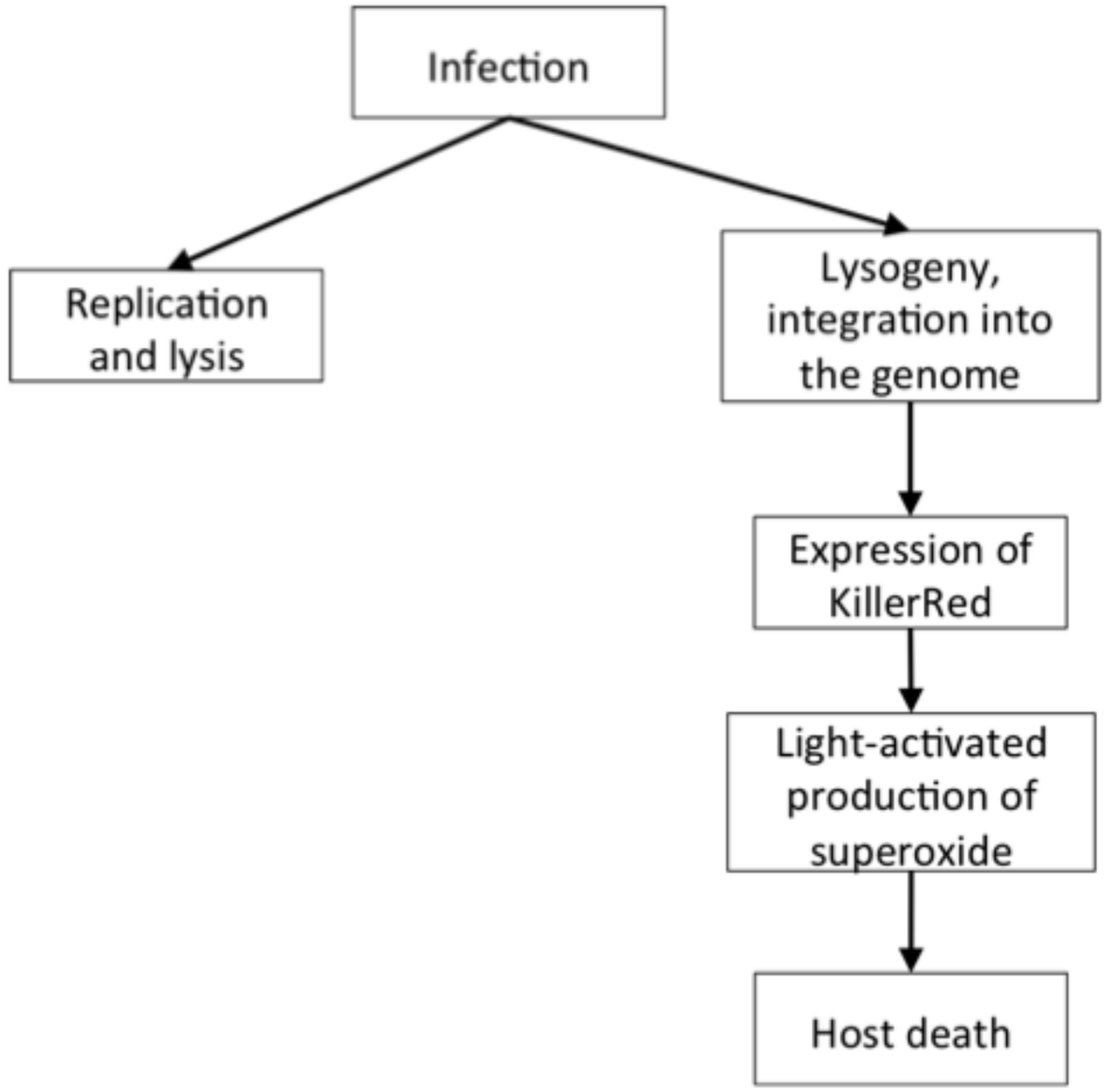


How KillerRed Lambda destroys *E. coli*

- Phages are complex and utilize many host pathways such that they cannot be inactivated or bypassed.
- Phages infect only specific hosts and can kill the host by cytolysis.

Phage Therapy for Bacteria-Killing w. A Phototoxic Protein

- Many phages are temperate
  - enter a lysogenic phase
  - cannot lyse and kill the host bacteria
- A lysogenic phage + A phototoxic protein
  - KillerRed protein (phototoxic)



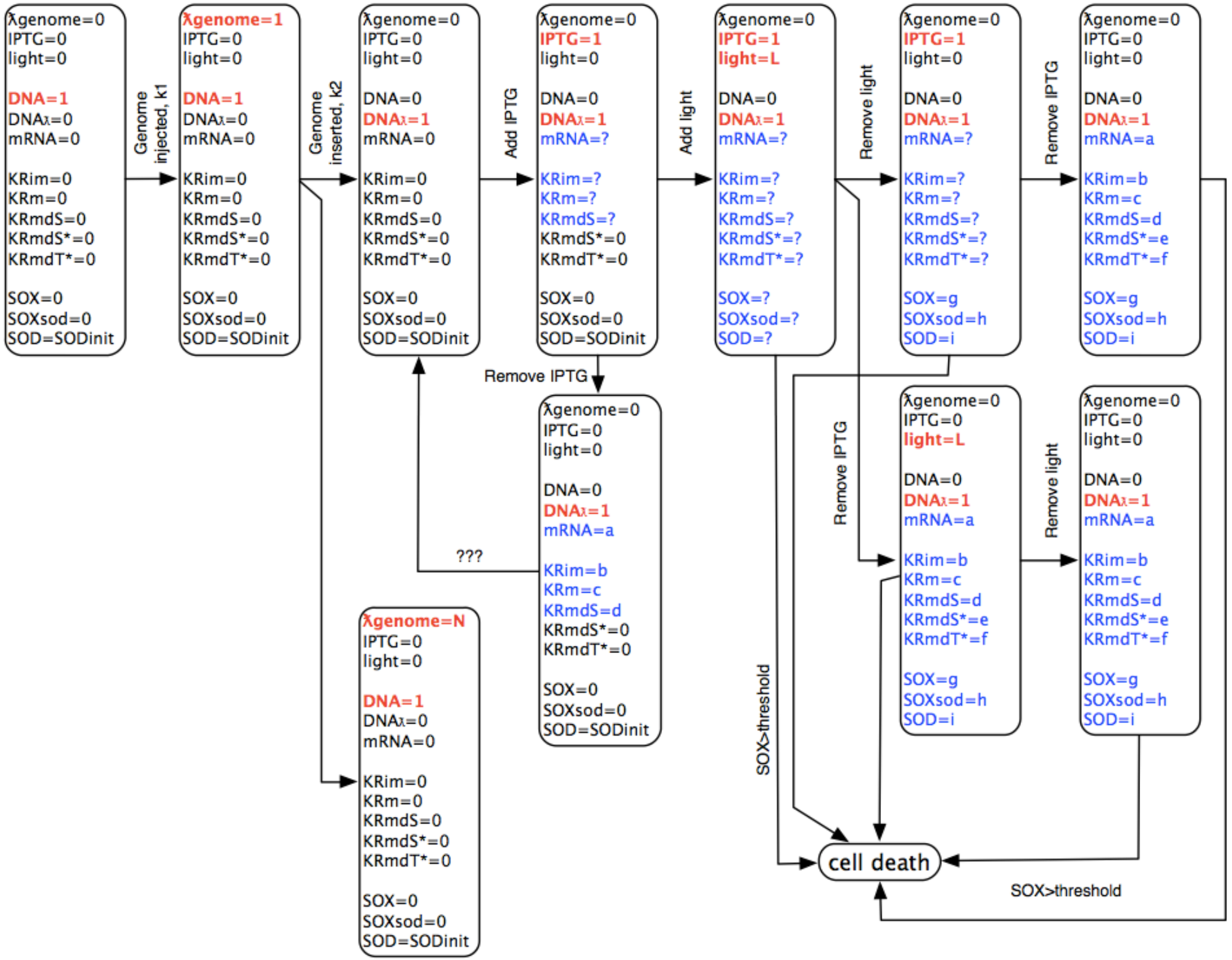
Our KillerRed Model

- synthesis and action of the KillerRed protein over three phases:
  - induction at 37 degrees;
  - storage at 4 degrees to allow for the protein maturation; and
  - photobleaching at the room temperature
- several stages of interest:
  - mRNA synthesis and degradation;
  - KillerRed synthesis, maturation, and degradation;
  - four distinct KillerRed states;
  - Superoxide production; and
  - Superoxide elimination

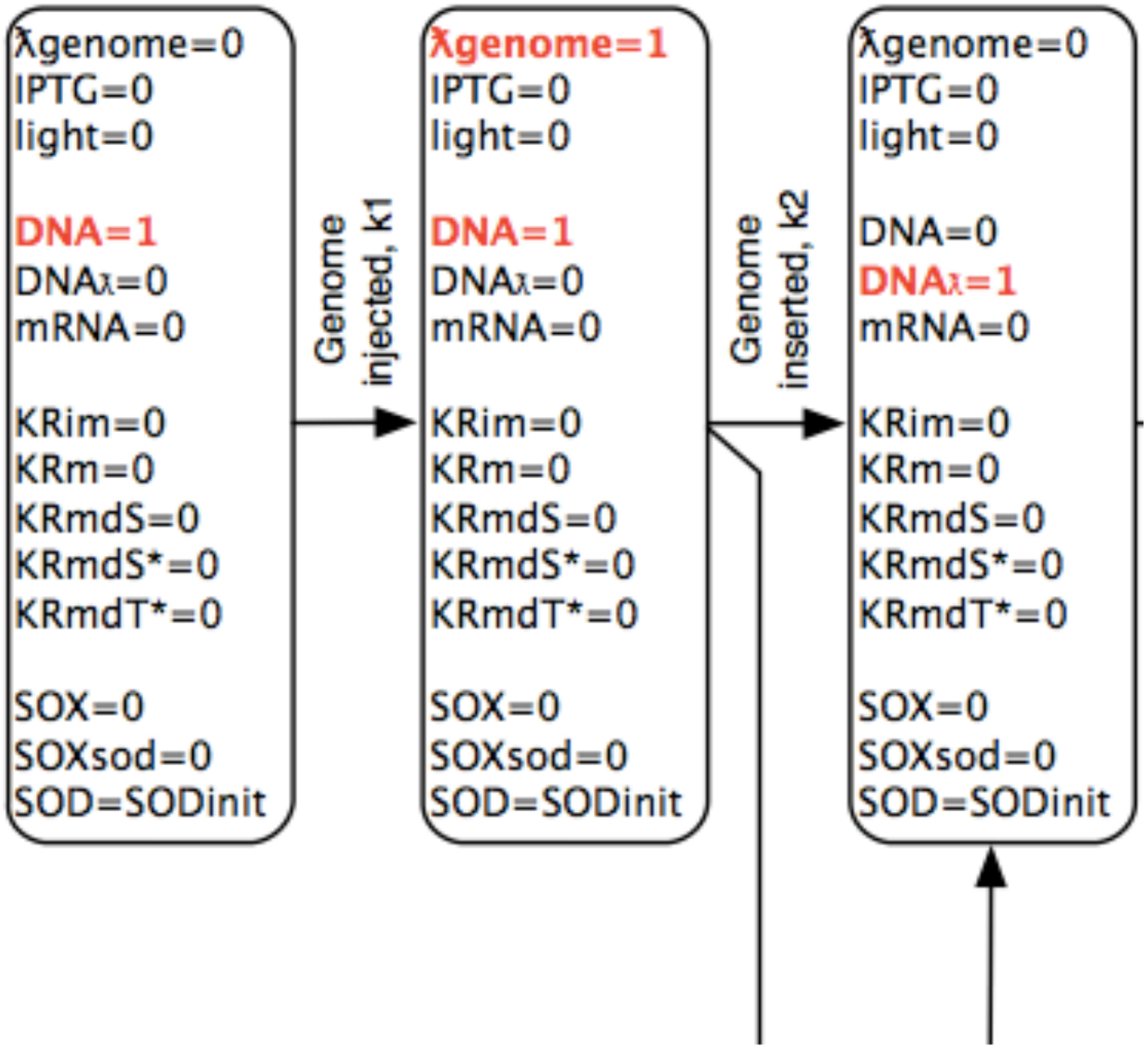
Our KillerRed Model – System States

State	State description	Input
S_0	Initial system state, bacteria cell, without phage	n/a
S_1	Phage genome injected	λ-phage genome
S_2	Phage genome replication (lytic cycle)	Genome replication
S_3	Phage genome within bacterial DNA (lysogenic cycle)	Genome insertion
S_4	Gene transcription, translation	Addition of IPTG
S_5	Gene transcription decrease	Removal of IPTG
S_6	Activation of KillerRed	Light turned ON
S_7	Mixture of KillerRed forms, no activation	Light turned OFF
S_8	Mixture of KillerRed forms, transcription decrease	Removal of IPTG
S_9	Mixture of KillerRed forms, no activation, transcription decrease	Removal of IPTG
S_{10}	Mixture of KillerRed forms, transcription decrease, no activation	Light turned OFF
S_{11}	Cell death	SOX>threshold

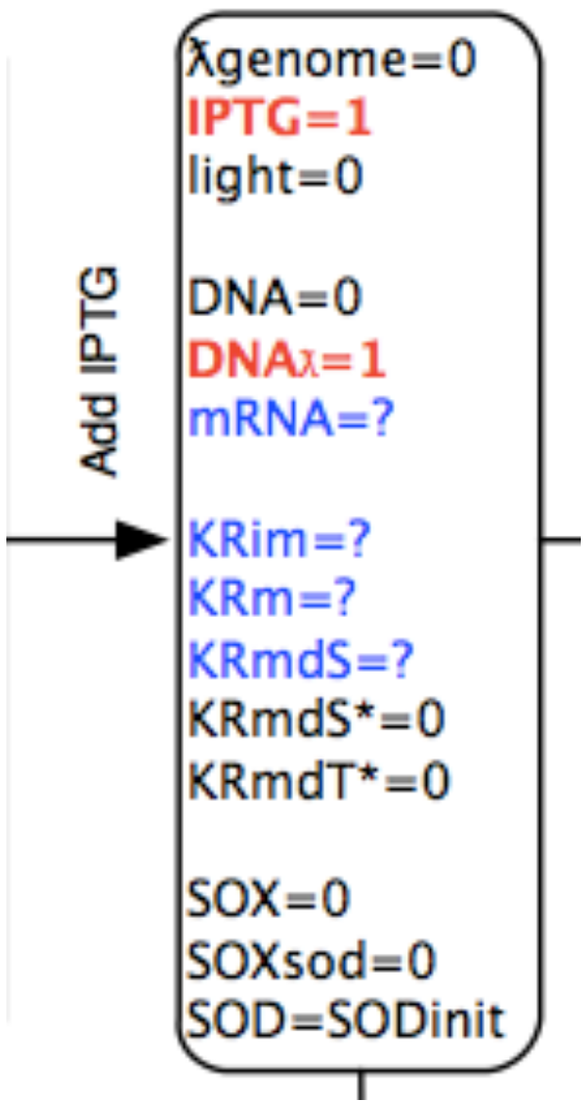
Our KillerRed Model – Hybrid Model



Injection of λ–phage genome



Addition of IPTG



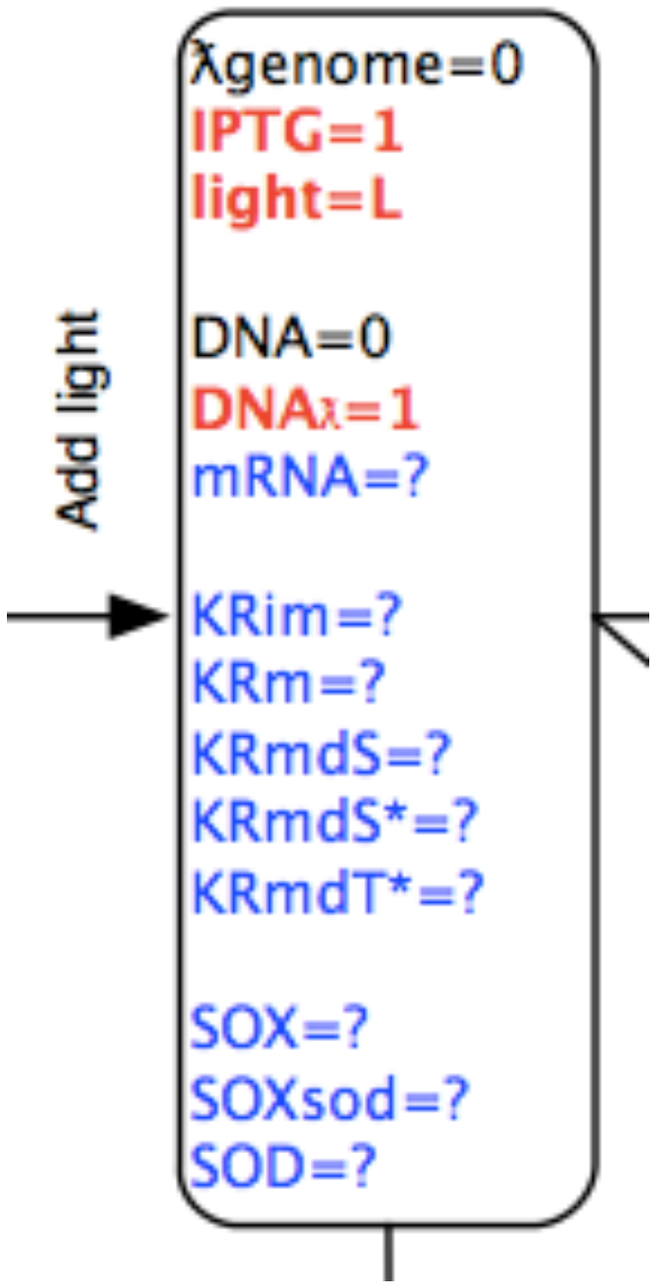
- When IPTG is added to the system, the transcription starts.
- mRNA transforms into immature KillerRed molecules.
- Immature synthesized KillerRed (KRim) -> mature KillerRed form (KRm) -> a dimer (KRmd)

$$\frac{\mathrm{d}[mRNA]}{\mathrm{d}t} = k_{RNAsyn} \cdot [DNA] - k_{RNAdeg} \cdot [mRNA]$$

$$\frac{\mathrm{d}[KR_{im}]}{\mathrm{d}t} = k_{KR_{im}syn} \cdot [mRNA] - (k_{KR_m} + k_{KR_{im}deg}) \cdot [KR_{im}]$$

$$\frac{\mathrm{d}[KR_{mdS}]}{\mathrm{d}t} = k_{KR_m} \cdot [KR_{im}] - k_{KR_{mdS}deg} \cdot [KR_{mdS}]$$

Addition of Light



$$\frac{\mathrm{d}[mRNA]}{\mathrm{d}t} = k_{RNAsyn} \cdot [DNA] - k_{RNAdeg} \cdot [mRNA]$$

$$\frac{\mathrm{d}[KR_{im}]}{\mathrm{d}t} = k_{KR_{im}syn} \cdot [mRNA] - (k_{KR_m} + k_{KR_{im}deg}) \cdot [KR_{im}]$$

$$\frac{\mathrm{d}[KR_{mdS}]}{\mathrm{d}t} = k_{KR_m} \cdot [KR_{im}] - k_{KR_{mdS}deg} \cdot [KR_{mdS}]$$

$$\frac{\mathrm{d}[KR_{mdS}]}{\mathrm{d}t} = k_{KR_m} \cdot [KR_{im}] + k_{KR_f} \cdot [KR_{mdS^*}] + k_{KR_{ic}} \cdot [KR_{mdS^*}] + k_{KR_{nrd}} \cdot [KR_{mdT^*}] + k_{KR_{SOXd1}} \cdot [KR_{mdT^*}] - k_{KR_{ex}} \cdot [KR_{mdS}] - k_{KR_{mdS}deg} \cdot [KR_{mdS}]$$

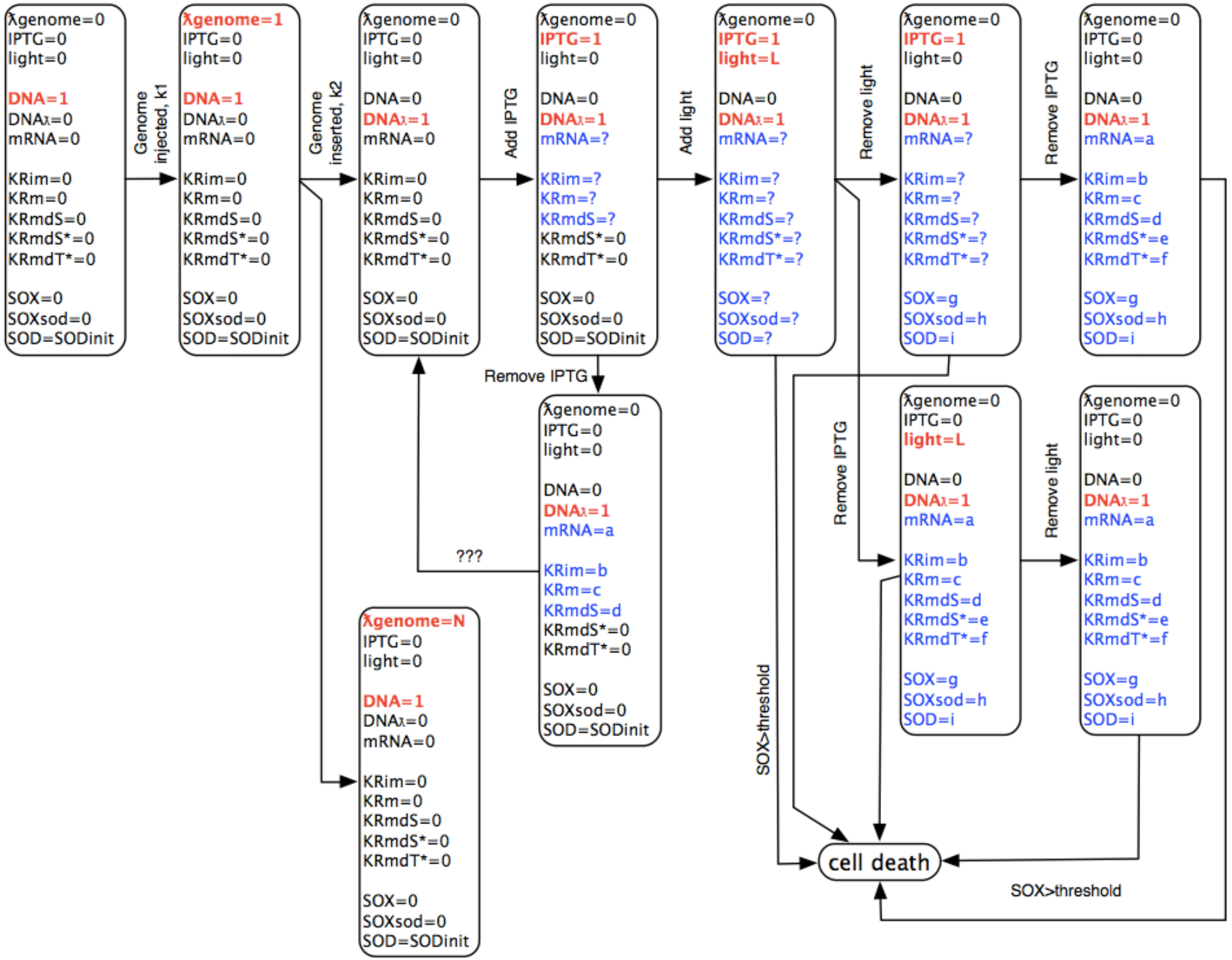
$$\frac{\mathrm{d}[KR_{mdS^*}]}{\mathrm{d}t} = k_{KR_{ex}} \cdot [KR_{mdS}] - k_{KR_f} \cdot [KR_{mdS^*}] - k_{KR_{ic}} \cdot [KR_{mdS^*}] - k_{KR_{isc}} \cdot [KR_{mdS^*}] - k_{KR_{mdS^*}deg} \cdot [KR_{mdS^*}]$$

$$\frac{\mathrm{d}[KR_{mdT^*}]}{\mathrm{d}t} = k_{KR_{isc}} \cdot [KR_{mdS^*}] - k_{KR_{nrd}} \cdot [KR_{mdT^*}] - k_{KR_{SOXd1}} \cdot [KR_{mdT^*}] - k_{KR_{SOXd2}} \cdot [KR_{mdT^*}] - k_{KR_{mdT^*}deg} \cdot [KR_{mdT^*}]$$

$$\frac{\mathrm{d}[SOX]}{\mathrm{d}t} = k_{KR_{SOXd1}} \cdot [KR_{mdT^*}] + k_{KR_{SOXd2}} \cdot [KR_{mdT^*}] - \frac{\mathrm{d}[SOX_{sod}]}{\mathrm{d}t}$$

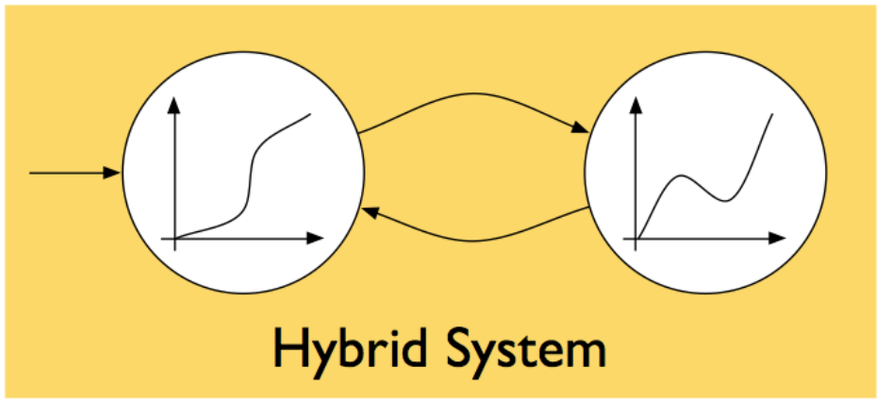
$$\frac{\mathrm{d}[SOX_{sod}]}{\mathrm{d}t} = k_{SOD} \cdot V_{maxSOD} \cdot \frac{[SOX]}{K_m + [SOX]}$$

Cell Death

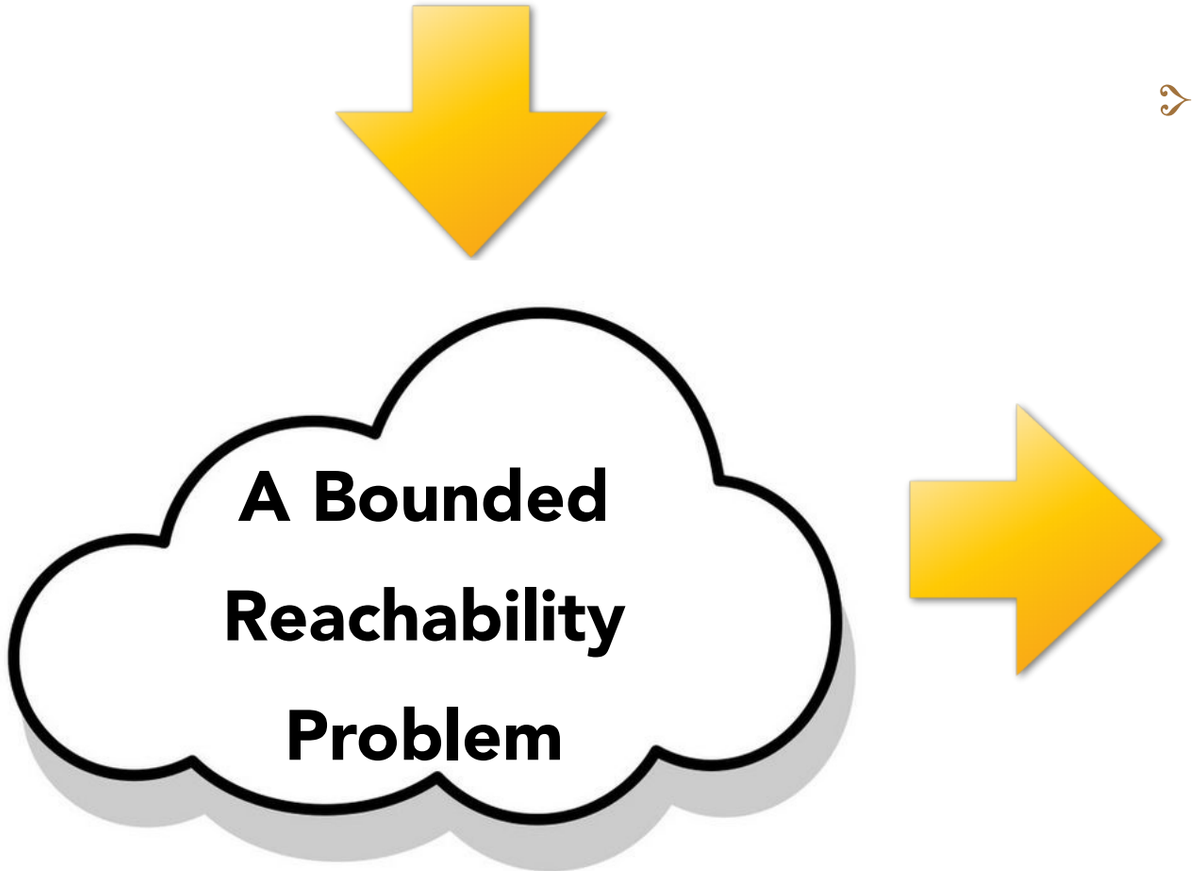

λgenome=0
IPTG=0
light=0
DNA=1
DNAλ=0
mRNA=0
KRim=0
KRm=0
KRmdS=0
KRmdS*=0
KRmdT*=0
SOX=0
SOXsod=0
SOD=SODinit
Genome injected, k1
λgenome=1
Genome inserted, k2
Add IPTG
DNAλ=1
mRNA=?
KRim=?
KRm=?
KRmdS=?
KRmdS*=0
KRmdT*=0
Add light
IPTG=1
light=L
KRmdS*=?
KRmdT*=?
SOX=?
SOXsod=?
SOD=?
Remove light
SOX=g
SOXsod=h
SOD=i
Remove IPTG
mRNA=a
KRim=b
KRm=c
KRmdS=d
KRmdS*=e
KRmdT*=f
Remove IPTG
???
λgenome=N
SOX>threshold
cell death
SOX>threshold

What we want to know?

- Model Falsification: whether consistent with existing experimental observations.



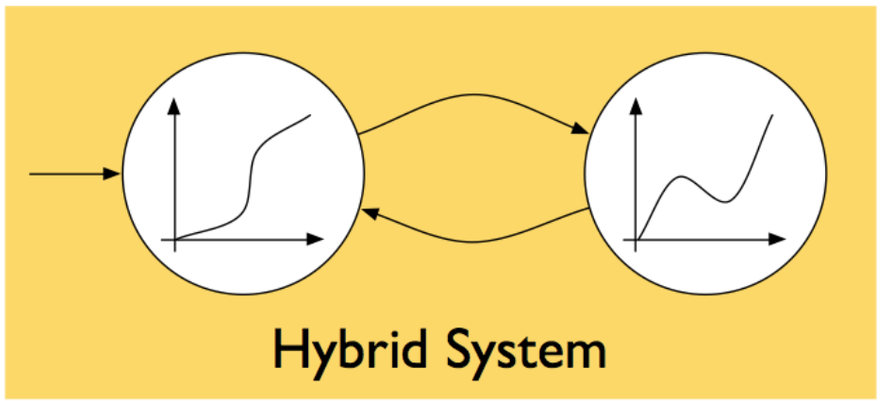
Discrete Control + Continuous Dynamics



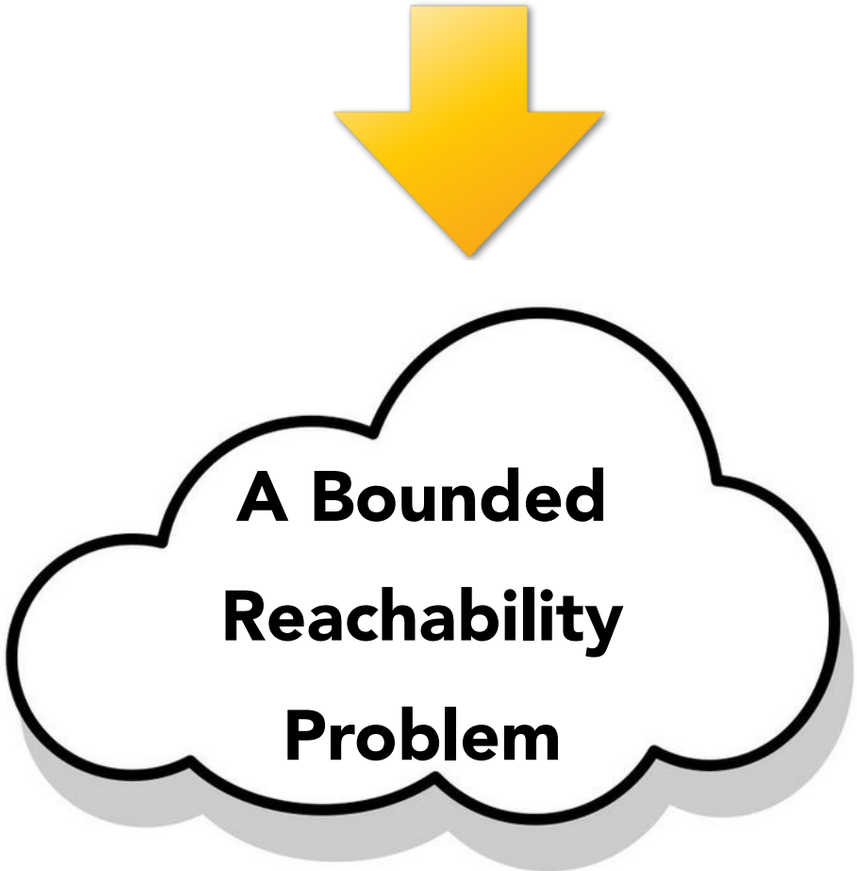
- Expressing each experimental observation as a goal region, is there any number of steps k in which the model reaches the goal region?
  - If none exists, the model is incorrect regarding the given observation.
  - If, for each observation, a witness is returned, we can conclude that the model is correct with regard to a given set of experimental results.

What we want to know?

- Parameter Estimation: How to control the system to reach good states (cell death)?



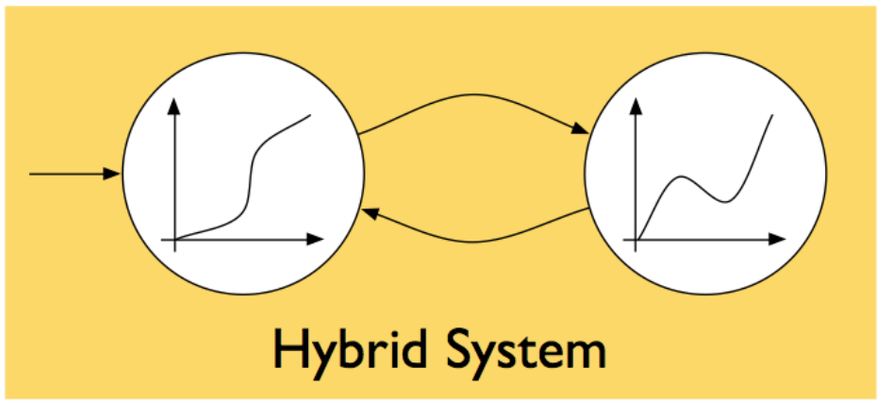
Discrete Control + Continuous Dynamics



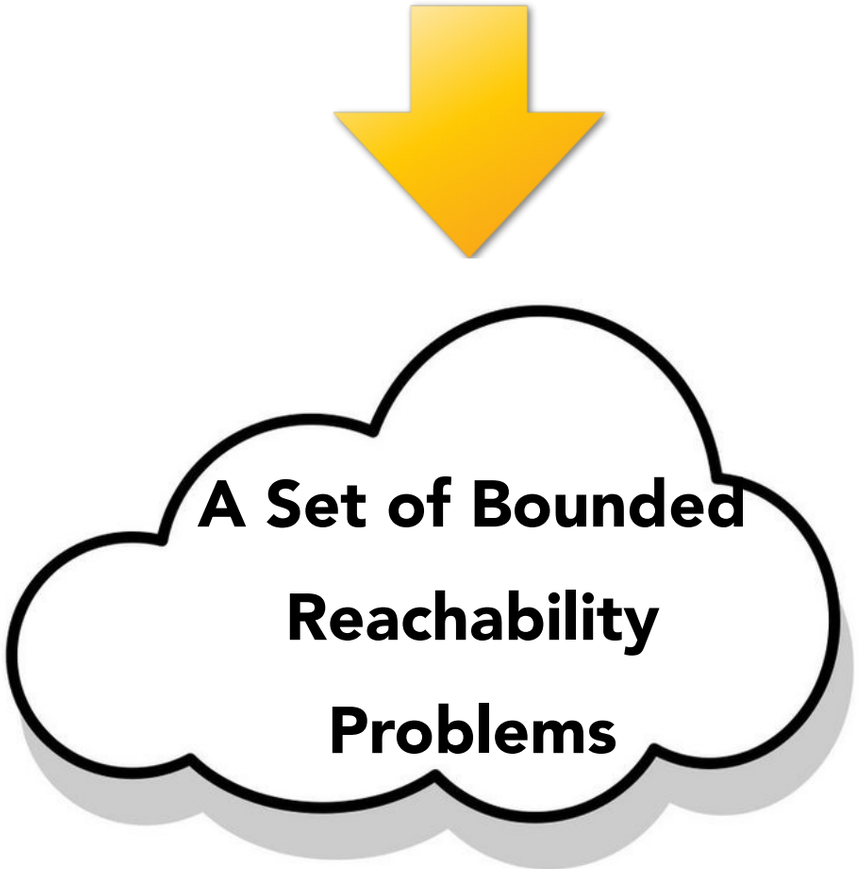
- Does it exist a parameter combination for which the model reaches the given goal region in bounded steps?
- Considering an assignment of a certain set of system parameters, if a witness is returned, this assignment is potentially a good estimation for those parameters.
- The goal here is to find an assignment with which all the given goal regions can be reached in bounded steps.

What we want to know?

- Parametric Sensitivity Analysis: Testing the robustness of the model, Understand the relationships between parameters and the model, etc.



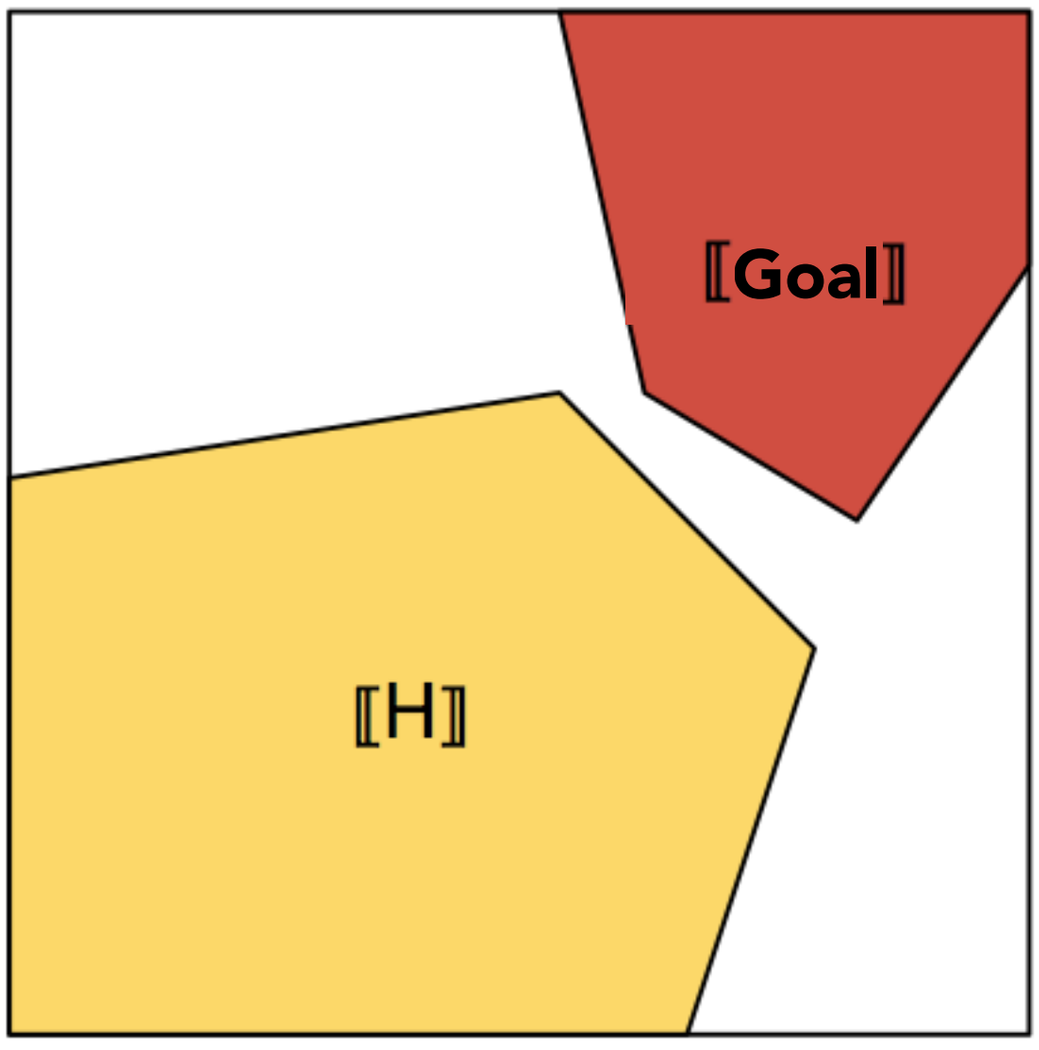
Discrete Control + Continuous Dynamics



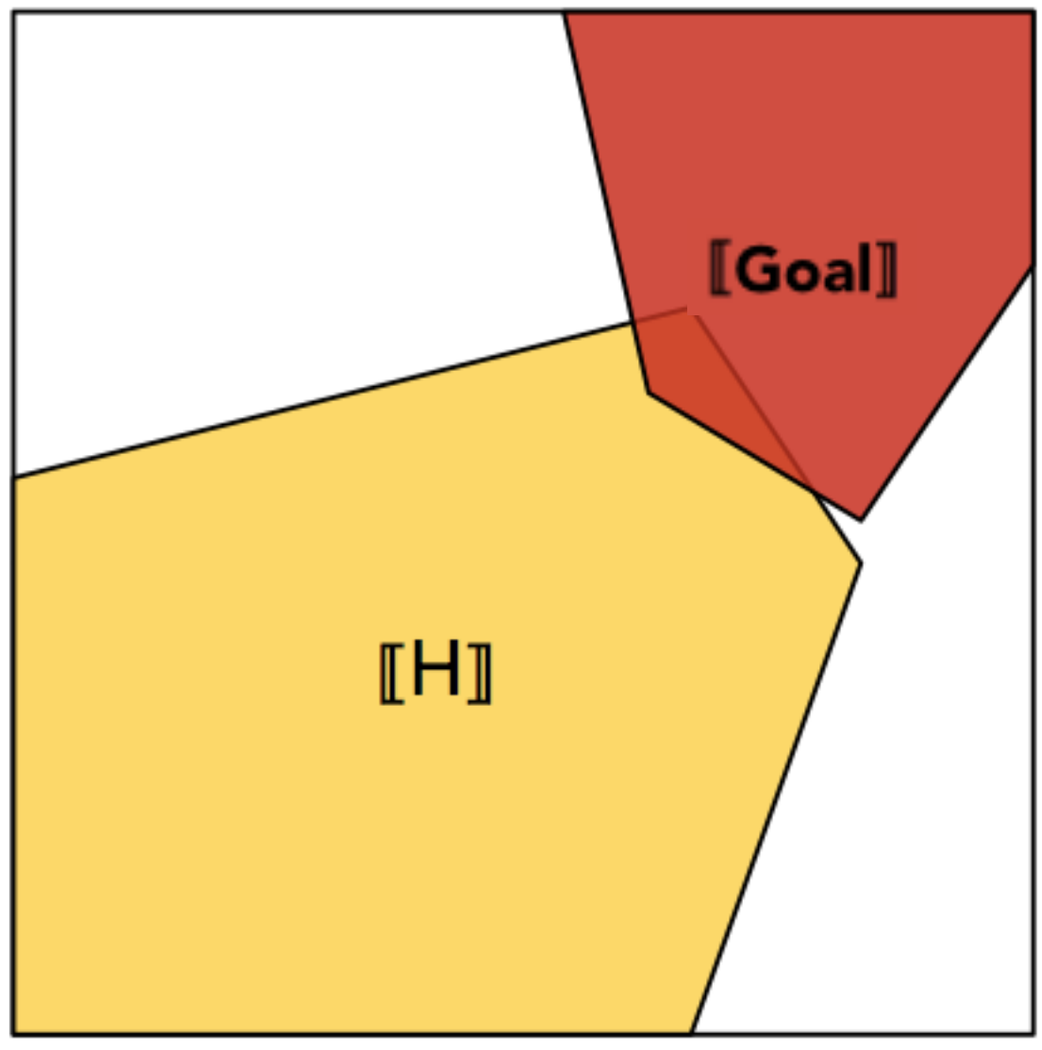
- For different possible values of a certain system parameter, are the results of reachability analysis the same?
  - If so, the model is insensitive to this parameter with regard to the given experimental observations.

Reachability Analysis of Hybrid Systems

- Can a hybrid system H run into a **goal** region of its state space?



Unreachable



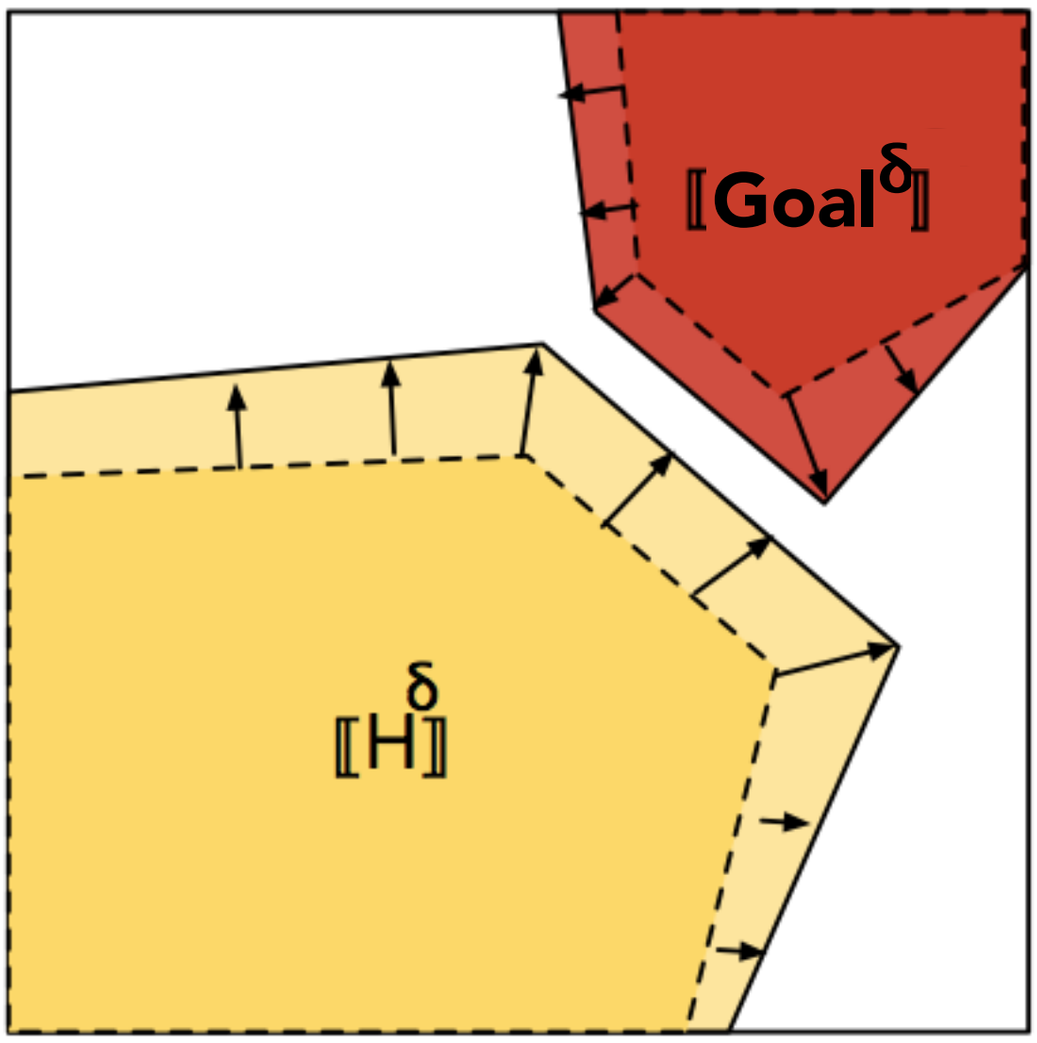
Reachable

Bounded Reachability Analysis of Hybrid Systems

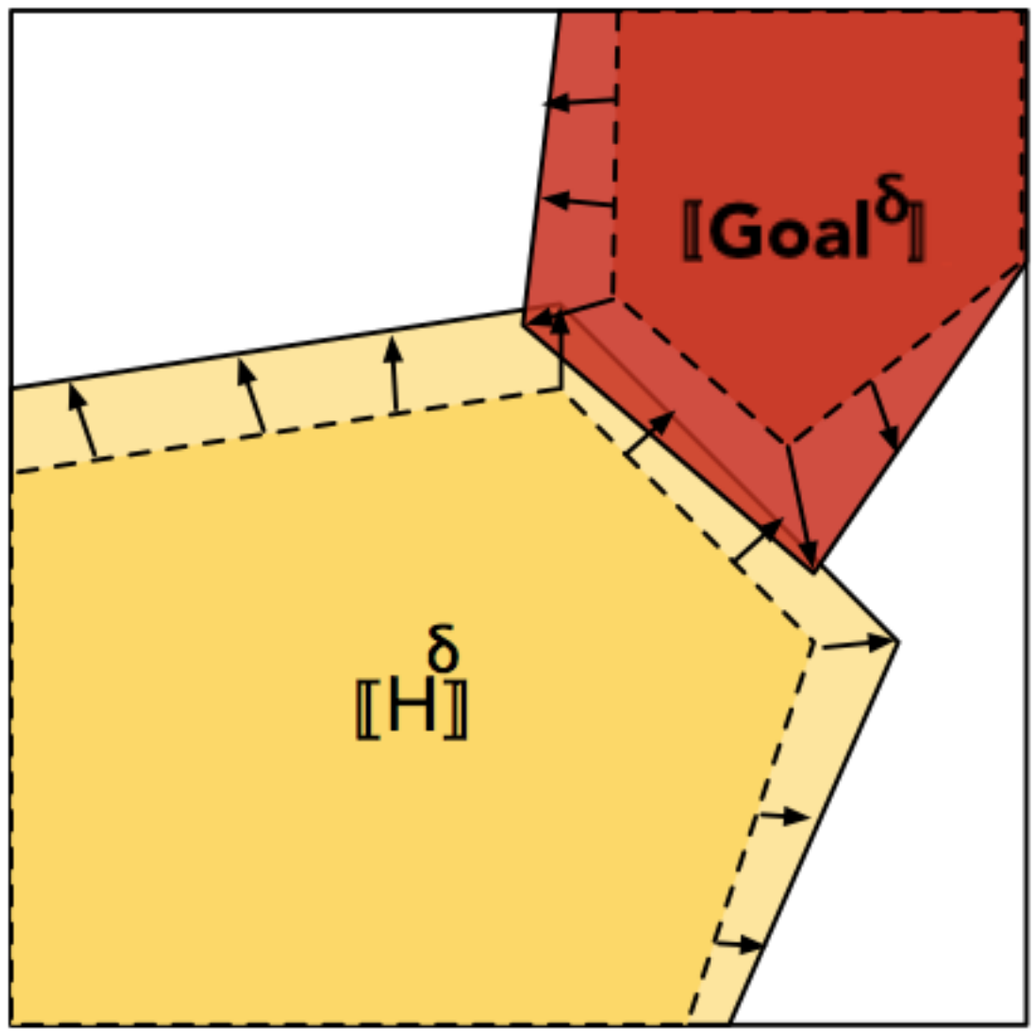
- The standard bounded reachability problems for simple hybrid systems are **undecidable**.
- 1. Give up
- 2. Don't give Up
  - A. Find a decidable fragment and solve it
  - B. Use approximation

δ-Reachability Analysis of Hybrid Systems

- Given $\delta \in \mathbb{Q}^+$, $[\![H^\delta]\!]$ and $[\![Goal^\delta]\!]$ over-approximate $[\![H]\!]$ and $[\![Goal]\!]$ respectively.
- So, the δ-reachability problem asks



Unreachable



δ-reachable

δ-Reachability Analysis of Hybrid Systems

- **Decidable** for a wide range of nonlinear hybrid systems: polynomials, log, exp, trigonometric functions, ODEs …
- Reasonable complexity bound (PSPACE-complete)
- When it says
  - Unreachable – the answer is **sound**
  - δ-Reachable – may lead to an infeasible counterexample,
    you may try a smaller δ and possibly get rid of it

Effect of Delay in Turning Light ON

- Relation between the time to turn ON the light after adding IPTG ($t_{lightON}$), and the total time needed until the bacteria cells being killed (t_{total})

$t_{lightON}$ (t.u.)	1	2	3	4	5	6	7	8	9	10
t_{total} (t.u.)	16	17.2	18.5	20	21.3	22.7	23.5	24.1	25	30

- The earlier we turn on the light after adding IPTG, the quicker the bacteria cells will be killed.

Lower Bound for Duration of Exposure to Light

- Impact of the time duration that the cells are exposed to light ($t_{lightOFF1}$) on the system
- An appropriate range for $t_{lightOFF1}$ which leads to the successful killing of bacteria cells

$t_{lightOFF_1}$ (t.u.)	1	2	3	4	5	6	7	8	9	10
killed bacteria cells	failed	failed	failed	succ	succ	succ	succ	succ	succ	succ

- Keep the light ON for at least 4 time units
- Bacteria cells can be killed within 100 time units when light is ON for 4 time units

Time to Remove IPTG as An Insensitive Role

- The sensitivity of the time difference between removing the light and removing IPTG ($t_{rmIPTG3}$) w.r.t. the successful killing of bacteria cells.

t_{rmIPTG_3} (t.u.)	1	2	3	4	5	6	7	8	9	10
killed bacteria cells	succ	succ	succ	succ	succ	succ	succ	succ	succ	succ

- We have noticed that $t_{rmIPTG3}$ has insignificant impact on the cell killing outcome
- This is in accordance with our understanding of this system, since any additional KillerRed that will be synthesized will not be activated in the absence of light.

Necessary Level of Superoxide

- Check the correctness of our hybrid model by considering various values of SOX_{thres} within the suggested (by Biologists) range - [100uM, 1mM].

SOX_{thres} (M)	1e-4	2e-4	3e-4	4e-4	5e-4	6e-4	7e-4	8e-4	9e-4	1e-3
t_{total} (t.u.)	5.1	5.2	5.4	17	19	48	61	71	36	42

- The bacteria cells can be killed in reasonable time for all 10 point values of SOX_{thres}, which were uniformly chosen from [100uM, 1mM].

- We have also found a broader range for SOX_{thres} - (0M, 0.6667M], with which bacteria cells can be killed.

Conclusion

- A new method of killing bacteria using phage-based therapy with the help of the KillerRed protein
- A hybrid model expressing both continuous and discrete dynamics
- Use a formal technique to investigate some important and interesting properties involving the timing effects of light and IPTG
- Offer new clues on the time to keep the light on and the necessary concentration of superoxide to kill bacteria cells

Thank you!

Any Questions?