

*Part II - Applications of MultiSeq
Evolution of Translation: Dynamics of
Recognition in RNA:Protein Complexes*

*Part III – Towards in silico Cells:
Simulating processes in entire cells*

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Biology

NIH Resource Macromolecular Modeling and Bioinformatics
Workshop 2012

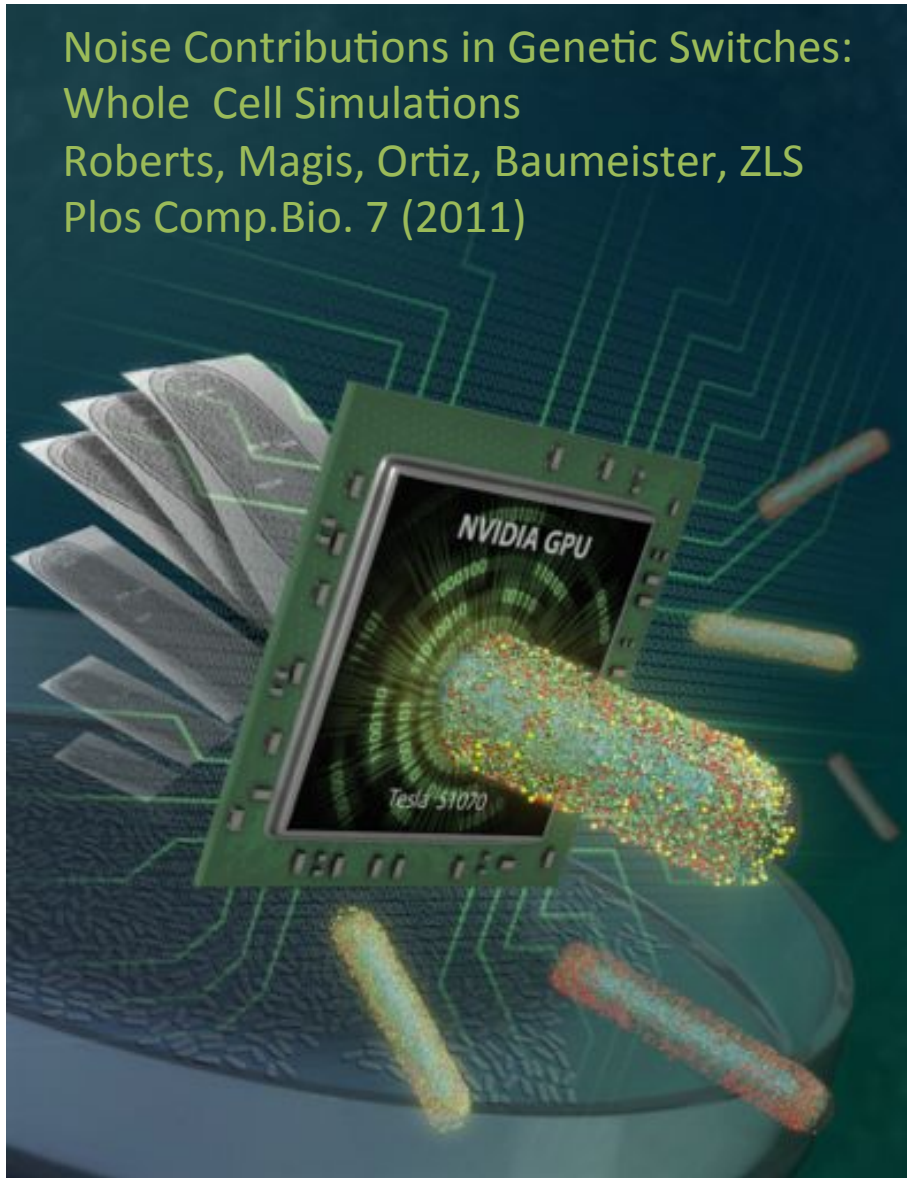


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Cellular Processes in Bacterial Cells

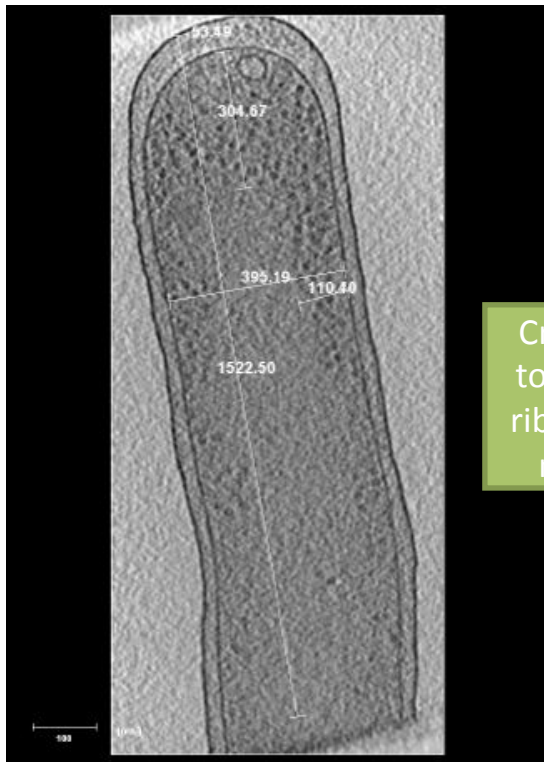
Noise Contributions in Genetic Switches:
Whole Cell Simulations
Roberts, Magis, Ortiz, Baumeister, ZLS
Plos Comp.Bio. 7 (2011)



- Assemble cells for in silico studies with molecular crowding from CET & proteomics data
- Lac Genetic switch in E. coli
- Stochastic gene expression models
- Kinetic parameters from in vitro & SM experiments
- Compare solns with and without spatial heterogeneity
- Reaction-diffusion on a 3D lattice using GPUs for an entire cell cycle

Cellular Models from Single Cells

Slow growing *E. coli* cells
with 3000 ribosomes



Cryo-electron
tomography –
ribosomes and
membrane



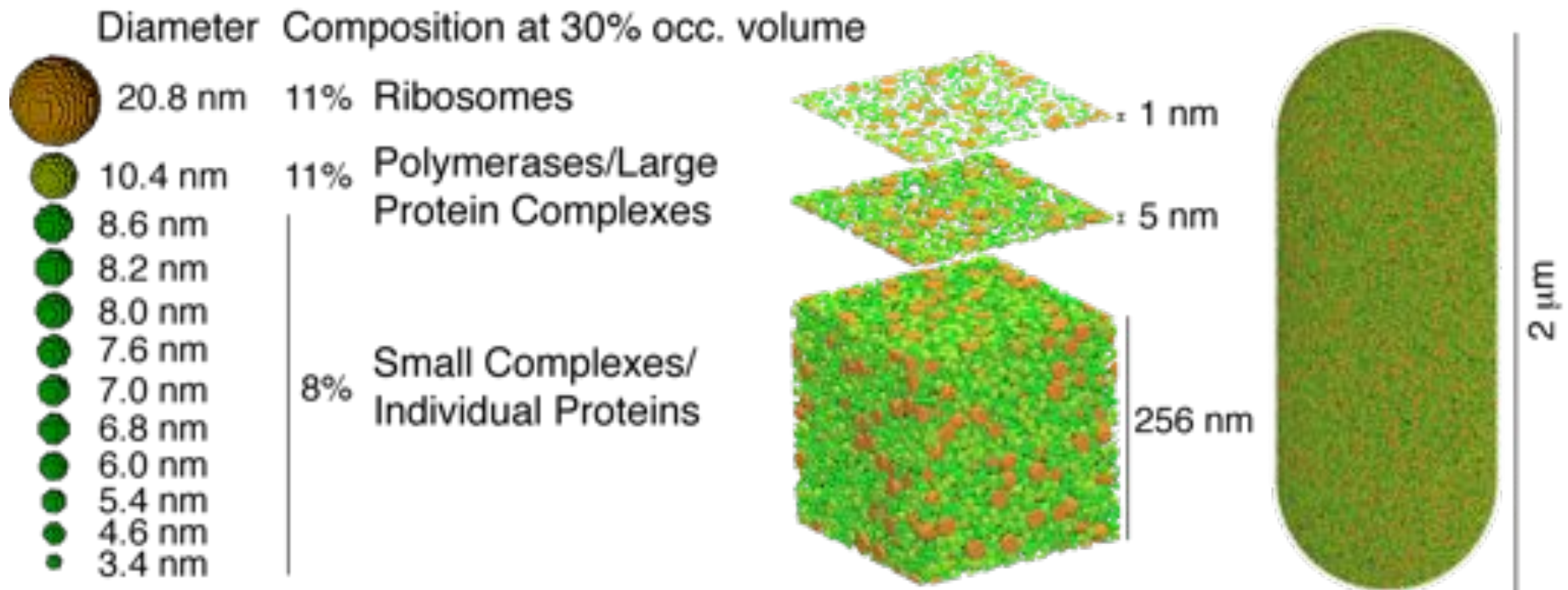
Modeling –
nucleoid and *in vivo*
obstacles



Ortiz, et al. (2010), *J. Cell Biol.* 190:613-21

Roberts, Magis, Ortiz, Baumeister, ZLS *Plos Comp. Biol.* (2011)

In vivo Crowding in *E. coli* – Fast Growing



Immobile obstacle classes

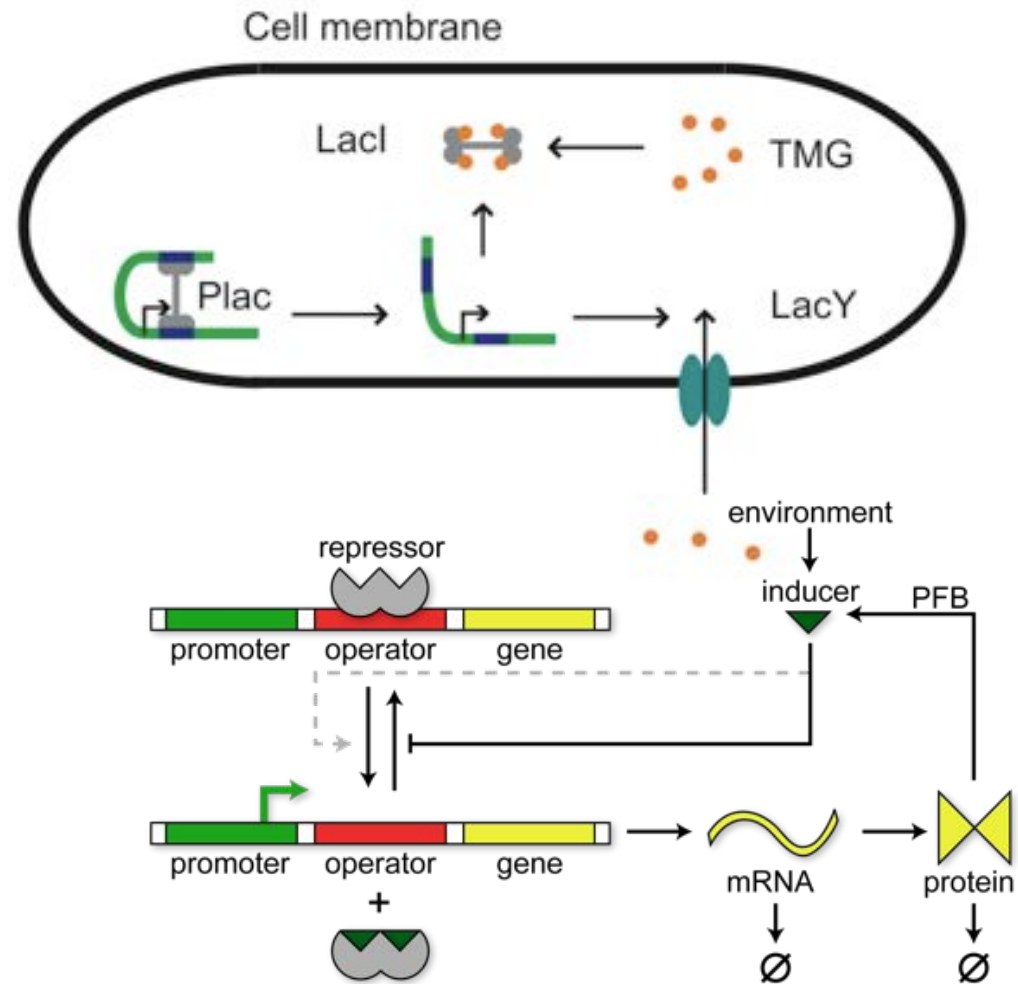
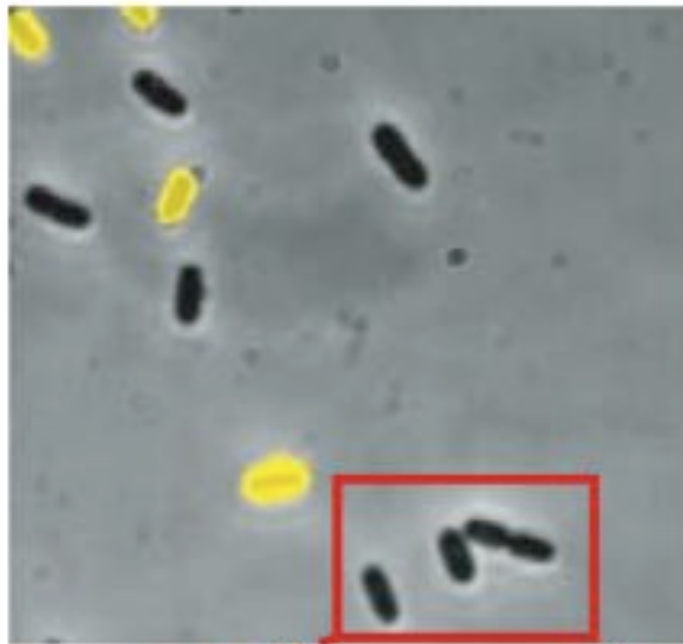
Abundances from proteomics studies

Fast growth phenotype (55 min) – uniformly distributed obstacles

Reaction diffusion master equation simulations on GPU

20-30,000 ribosomes randomly placed

Lac genetic switch in E. coli



- Kinetic model for lac regulatory circuit
- Stochasticity & population heterogeneity?
SM experiments (Xie, Science 2007, 2008):

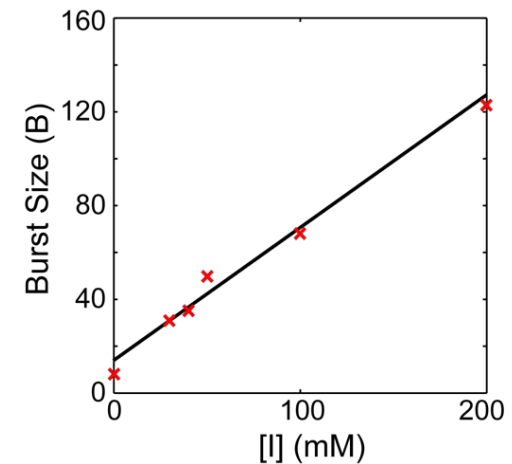
Kinetic Model of *lac* System

Reaction	Param	Stochastic Rate	Units	Source ^o		
Lac operon regulation						
$R_2 + O \rightarrow R_2O$	k_{ron}	2.43e+06	$M^{-1}s^{-1}$	M		
$IR_2 + O \rightarrow IR_2O$	k_{iron}	1.21e+06	$M^{-1}s^{-1}$	M		
$I_2R_2 + O \rightarrow I_2R_2O$	k_{i2ron}	2.43e+04	$M^{-1}s^{-1}$	M		
$R_2O \rightarrow R_2 + O$	k_{roff}	6.30e-04	s^{-1}	S		
$IR_2O \rightarrow IR_2 + O$	k_{iroff}	6.30e-04	s^{-1}	S		
$I_2R_2O \rightarrow I_2R_2 + O$	k_{i2roff}	3.15e-01	s^{-1}	M		
Transcription, translation, and degradation						
$O \rightarrow O + mY$	k_{tr}	1.26e-01	s^{-1}	M		
$mY \rightarrow mY + Y$	k_{tn}	4.44e-02	s^{-1}	S		
$mY \rightarrow \emptyset$	k_{degm}	1.11e-02	s^{-1}	S		
$Y \rightarrow \emptyset$	k_{degp}	2.10e-04	s^{-1}	M		
Lac inducer-repressor interactions						
		TMG	IPTG	TMG	IPTG	
$I + R_2 \rightarrow IR_2$	k_{ion}	2.27e+04	9.71e+04	$M^{-1}s^{-1}$	M	K
$I + IR_2 \rightarrow I_2R_2$	k_{i2on}	1.14e+04	4.85e+04	$M^{-1}s^{-1}$	M	K
$I + R_2O \rightarrow IR_2O$	k_{iopon}	6.67e+02	2.24e+04	$M^{-1}s^{-1}$	M	K
$I + IR_2O \rightarrow I_2R_2O$	k_{i2opon}	3.33e+02	1.12e+04	$M^{-1}s^{-1}$	M	K
$IR_2 \rightarrow I + R_2$	k_{ioff}	2.00e-01		s^{-1}		K
$I_2R_2 \rightarrow I + IR_2$	k_{i2off}	4.00e-01		s^{-1}		K
$IR_2O \rightarrow I + R_2O$	k_{iopoif}	1.00e+00		s^{-1}		K
$I_2R_2O \rightarrow I + IR_2O$	$k_{i2opoif}$	2.00e+00		s^{-1}		K
Inducer transport						
$I_{ex} \rightarrow I$	k_{id}	2.33e-03		s^{-1}		K
$I \rightarrow I_{ex}$	k_{id}	2.33e-03		s^{-1}		K
$Y + I_{ex} \rightarrow YI$	k_{yion}	3.03e+04		$M^{-1}s^{-1}$		K
$YI \rightarrow Y + I_{ex}$	k_{yioff}	1.20e-01		s^{-1}		K
$YI \rightarrow Y + I$	k_{it}	1.20e+01		s^{-1}		K

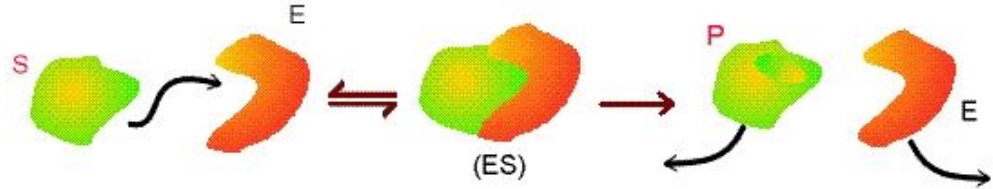
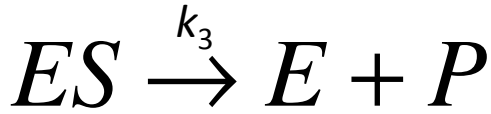
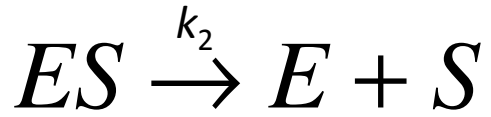
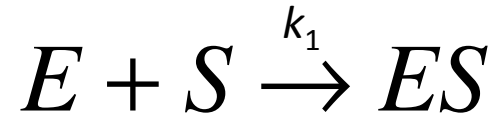
K – in vitro kinetic experiment

S – single molecule experiment

M – model parameter fit to single-molecule distributions



Analysis of biochemical reactions



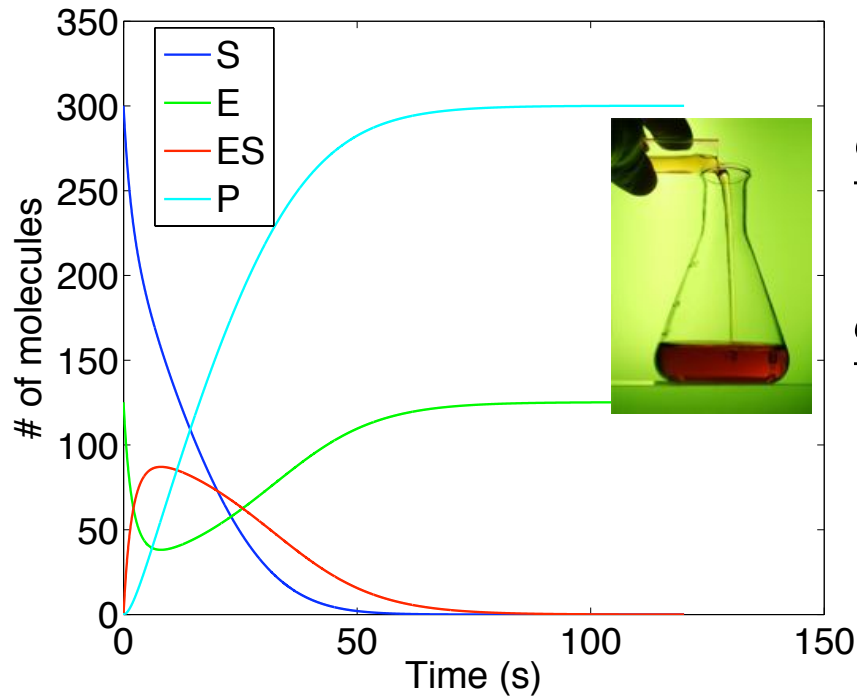
$$\frac{d[S]}{dt} = k_2[ES] - k_1[E][S]$$

$$\frac{d[E]}{dt} = k_2[ES] + k_3[ES] - k_1[E][S]$$

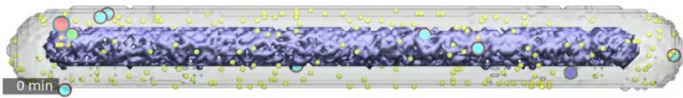
$$\frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES]$$

$$\frac{d[P]}{dt} = k_3[ES]$$

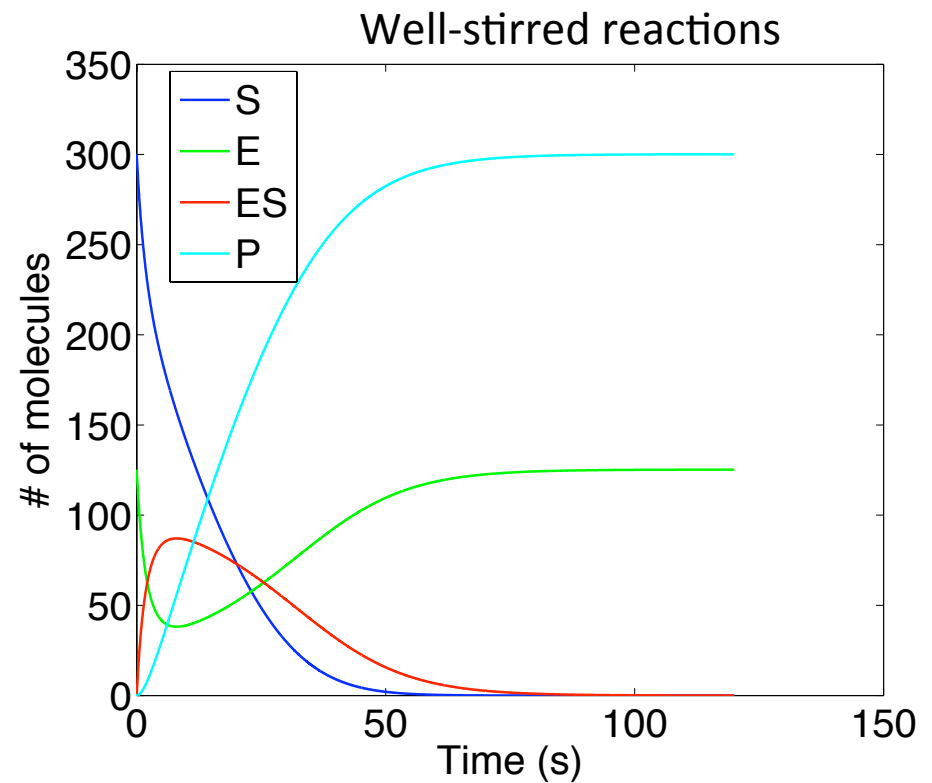
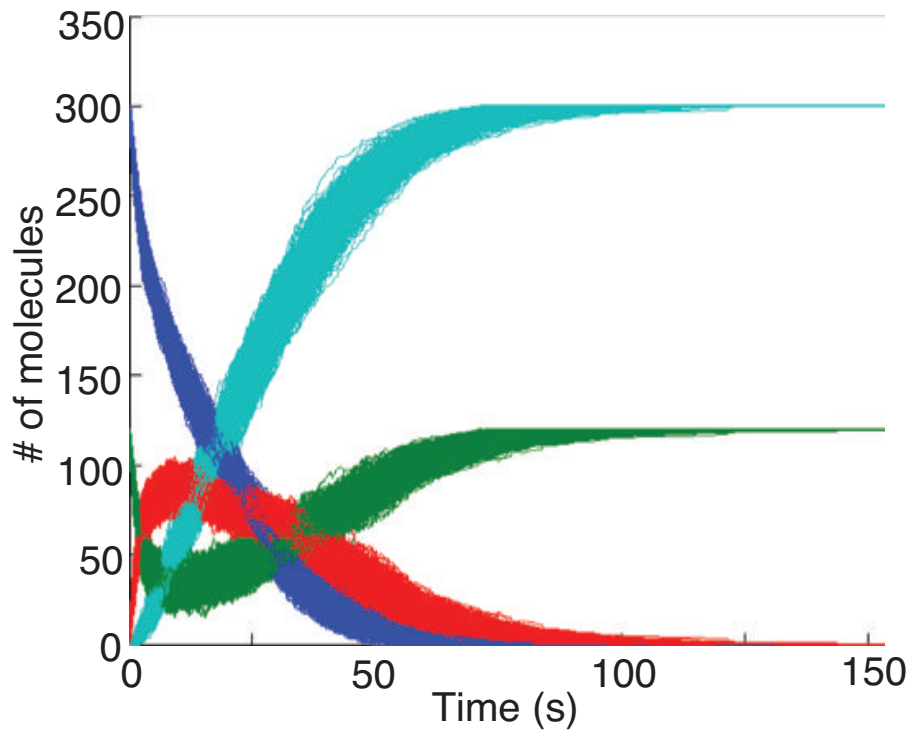
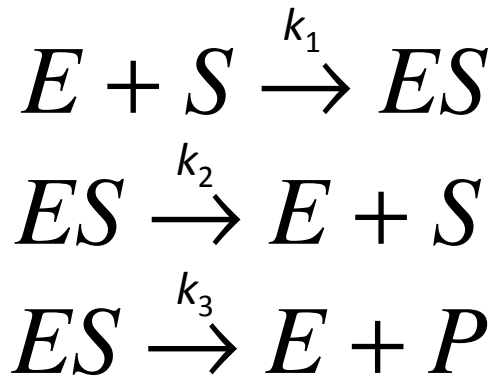
In cells hundreds of such reactions exist, some involving only a few molecules (9 repressors, 1DNA, ...) others have thousands of reactants.



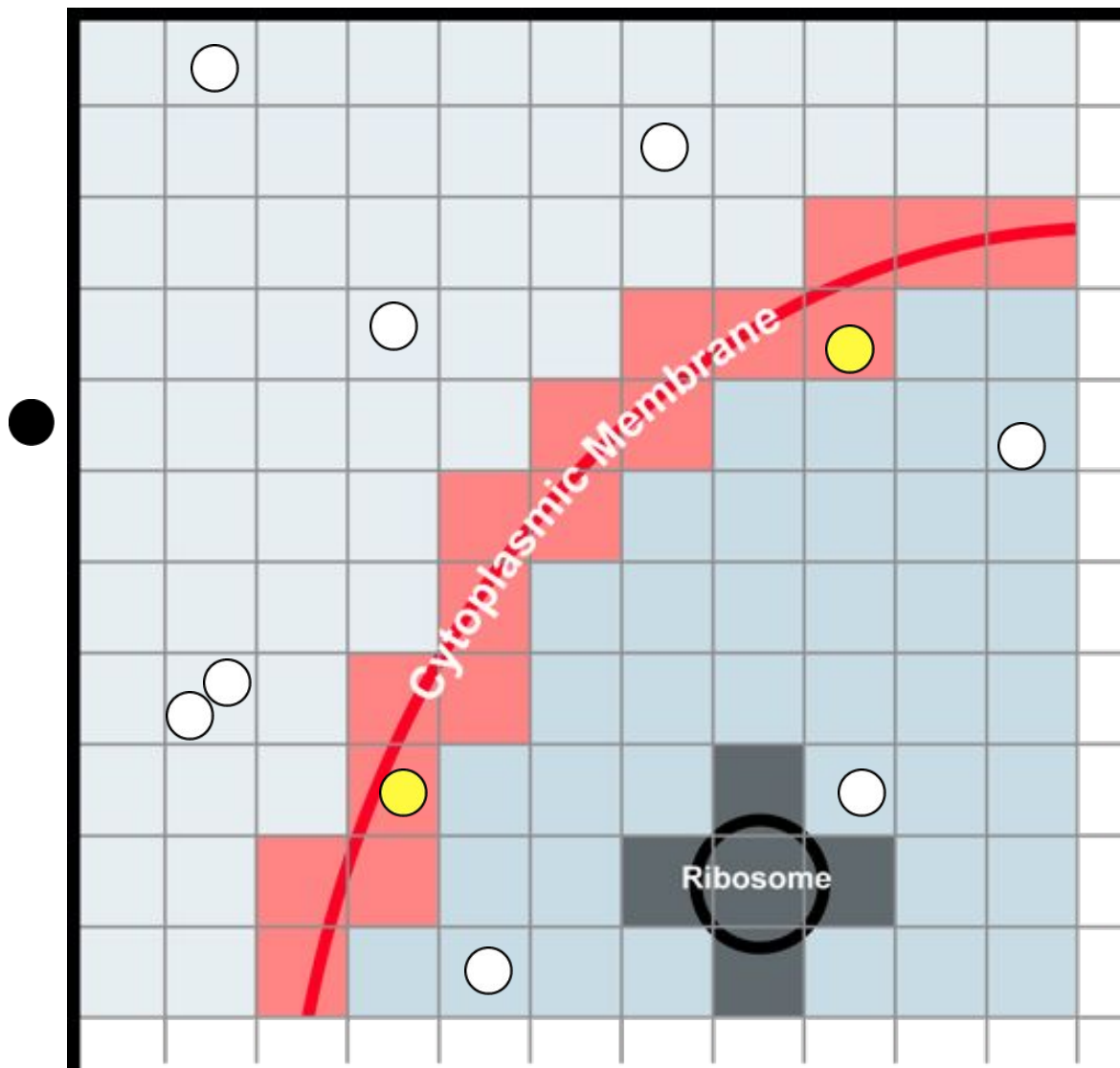
Stochastic vs. Deterministic Solutions



Cell is shaken, but not well-stirred
Gillespie's SSA on GPU
(2000 threads)



In vivo RDME Sampling



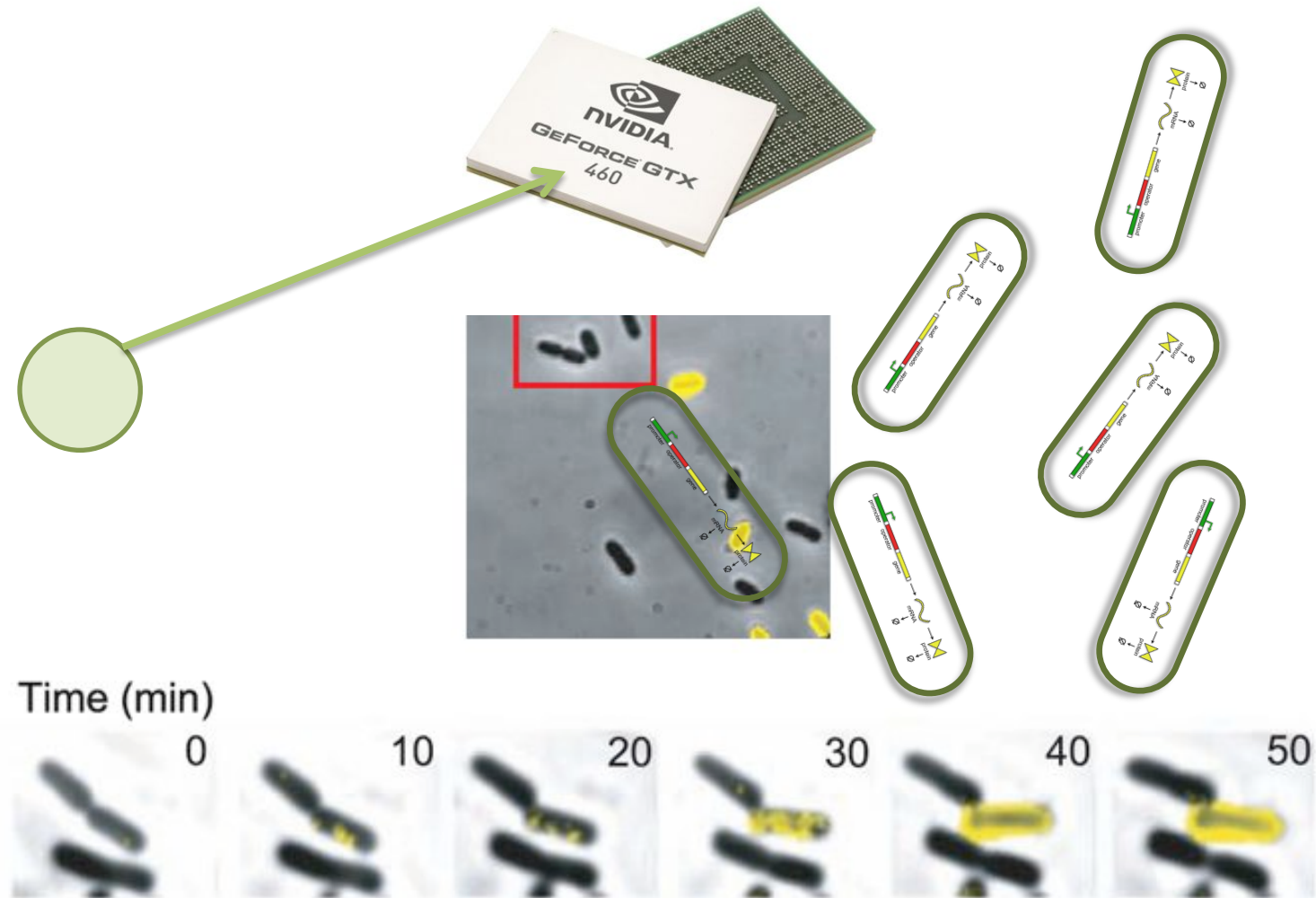
Divide space into equally sized subvolumes which can each contain multiple reactive species (particles)

Assume subvolumes are well-stirred

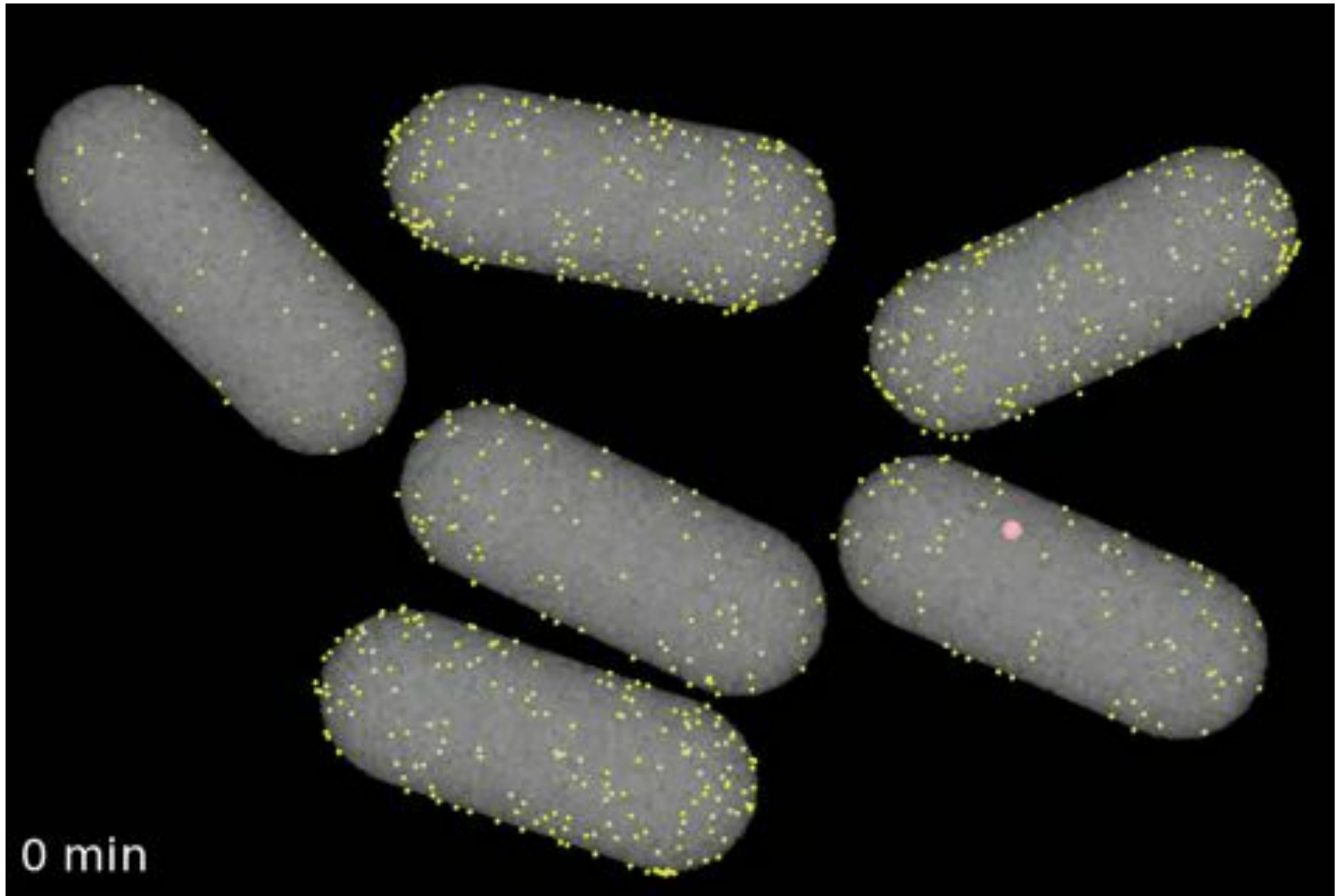
During each time step particles randomly react with other particles in the subvolume according to well-stirred chemical kinetics (Gillespie)

After each time step particles randomly move to neighboring subvolumes according to transition probabilities that depend on the particle's diffusion coefficient as well as the type of both subvolumes

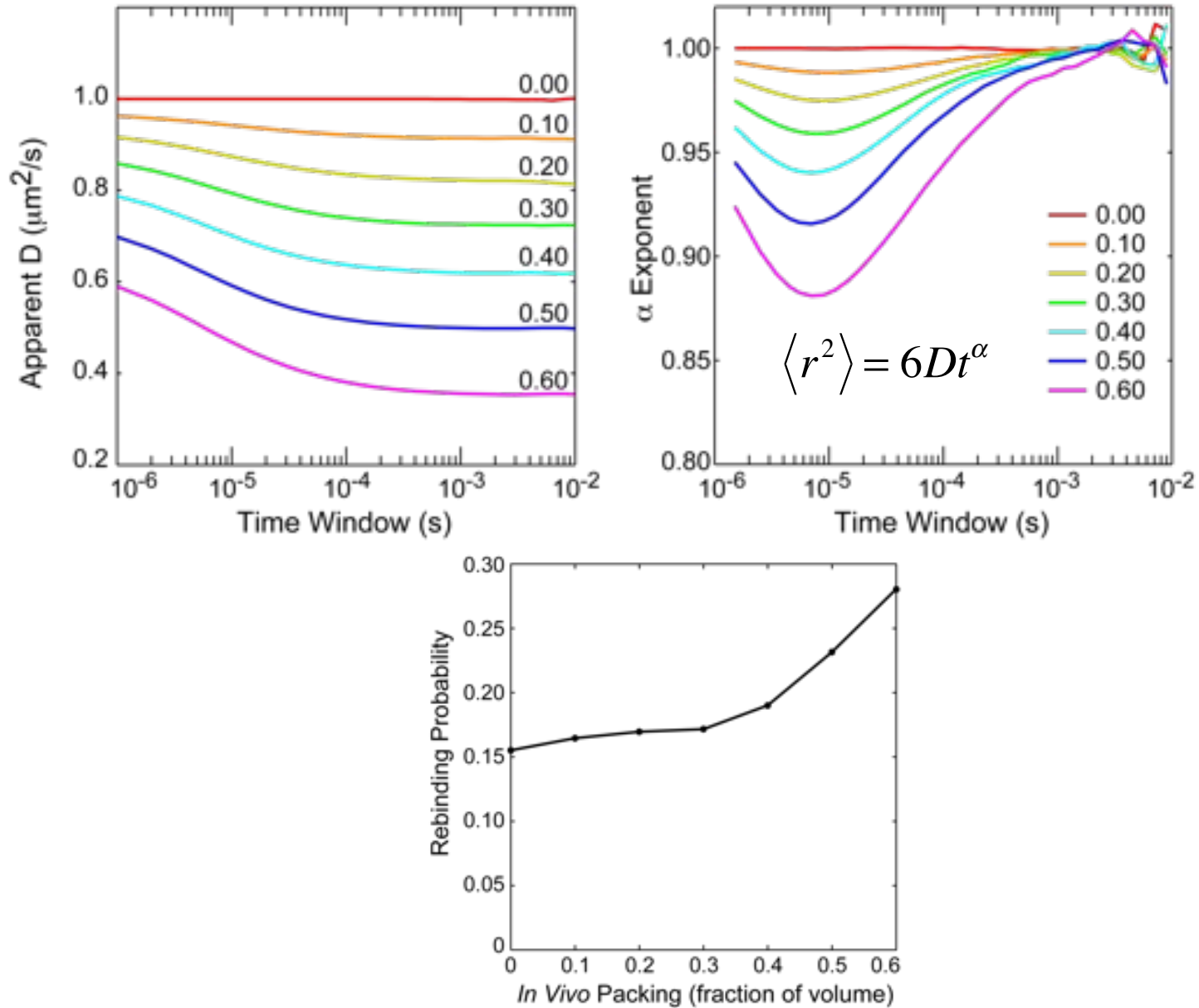
Motivation



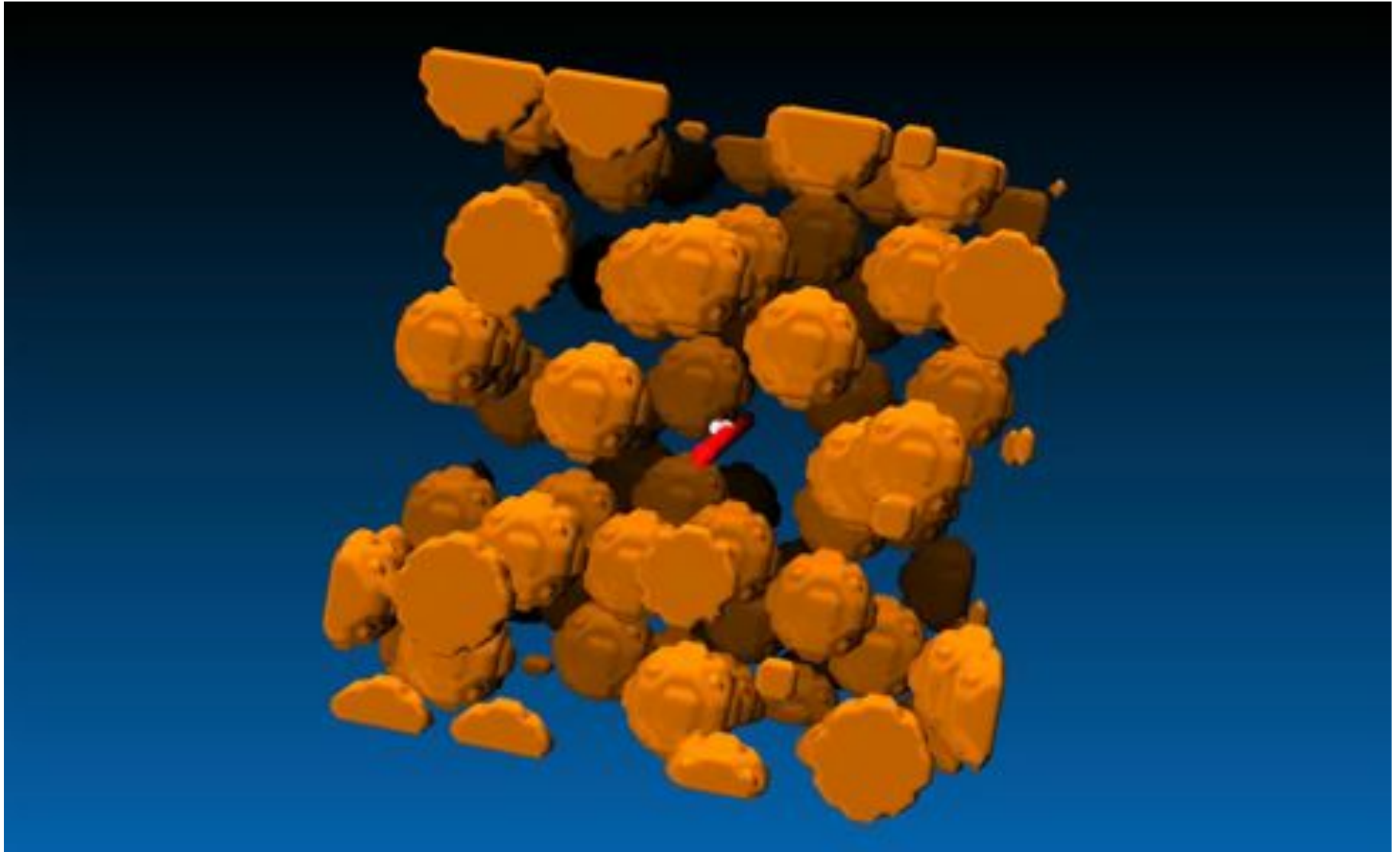
Switching in Fast Growing *E. coli* Cells – Bursting of mRNA



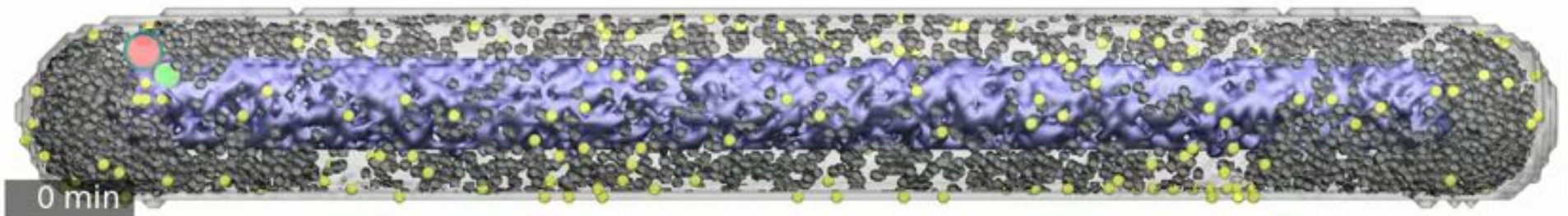
Anomalous Repressor Rebinding



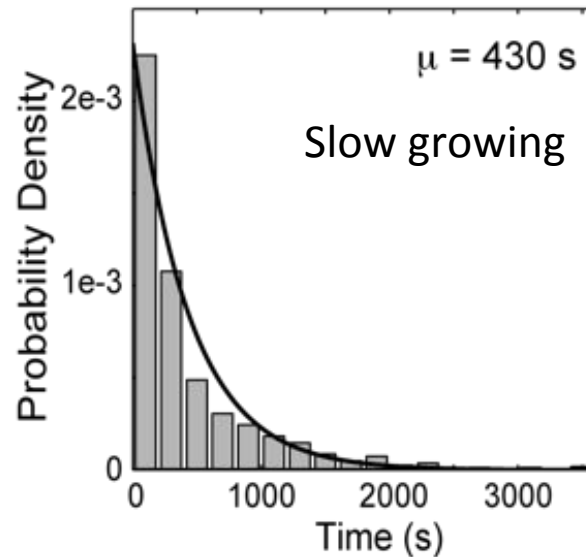
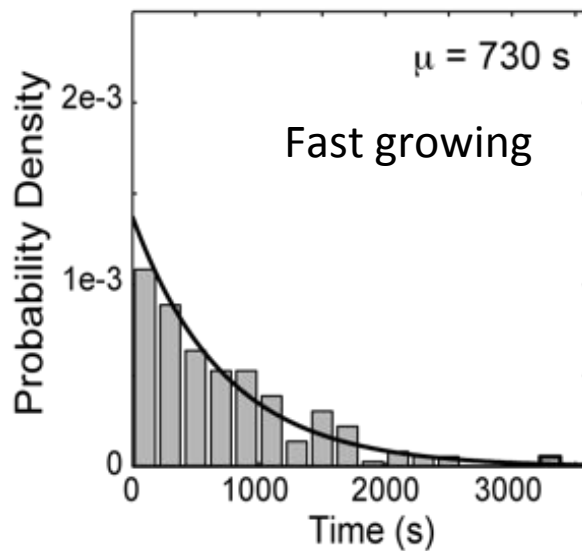
Effect of *in vivo* crowding on repressor re-binding (uniform distribution of ribosomes)



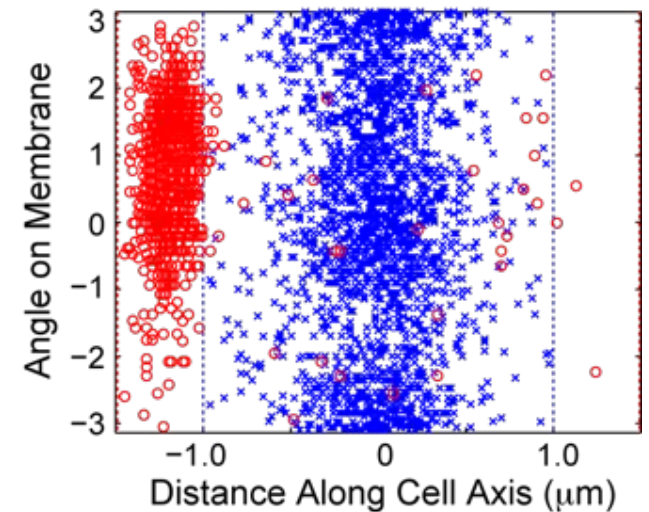
In vivo – Slow Growing Cells



Lifetimes Repressor-Operator Complexes

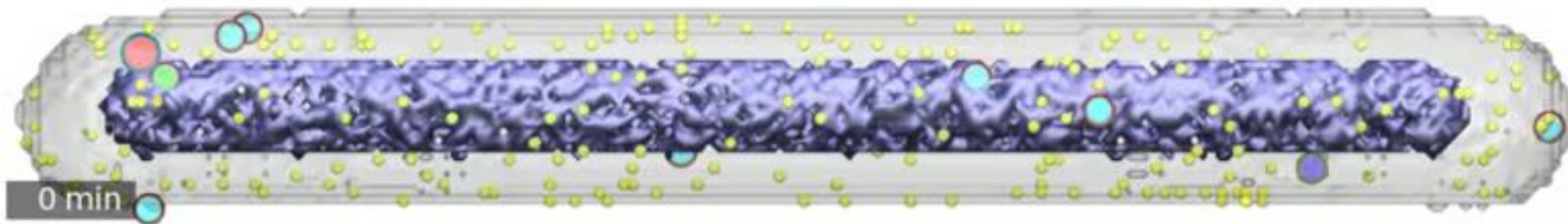


Position mRNA on membrane



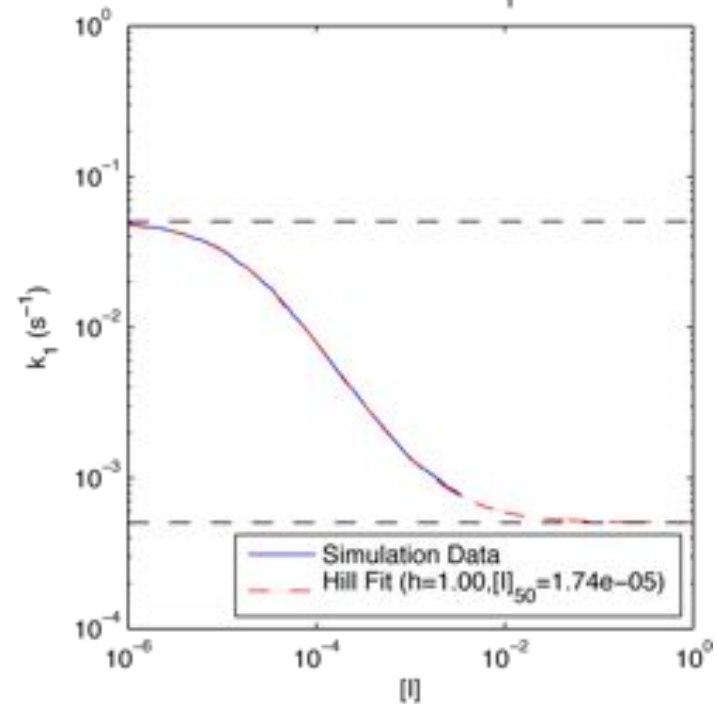
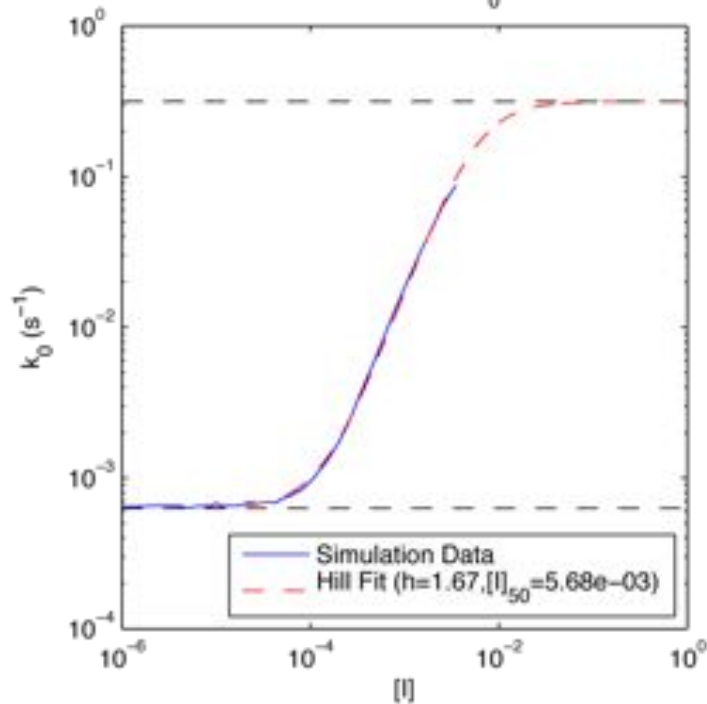
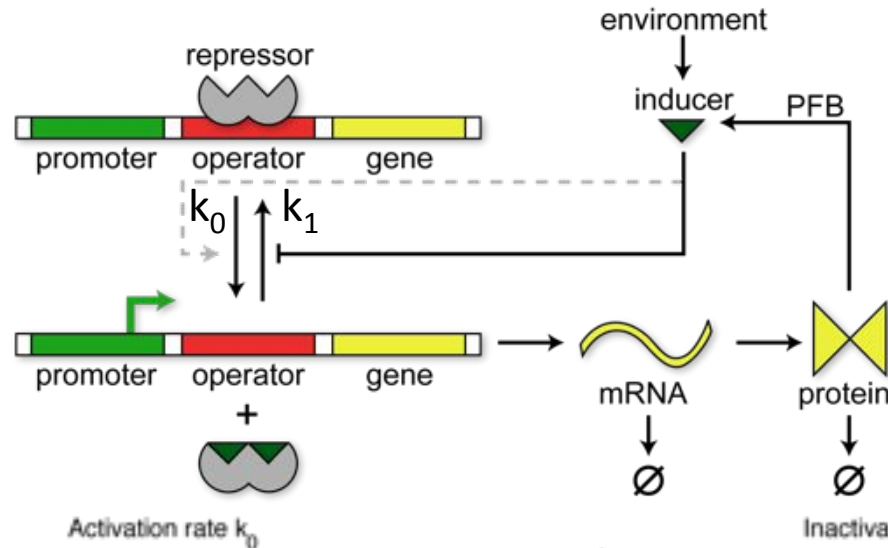
Repressor dynamics – *in vivo* model of slow growing cells

Inducer binding to repressors causes shorter repressor-operator lifetimes
(ribosomes omitted for clarity)

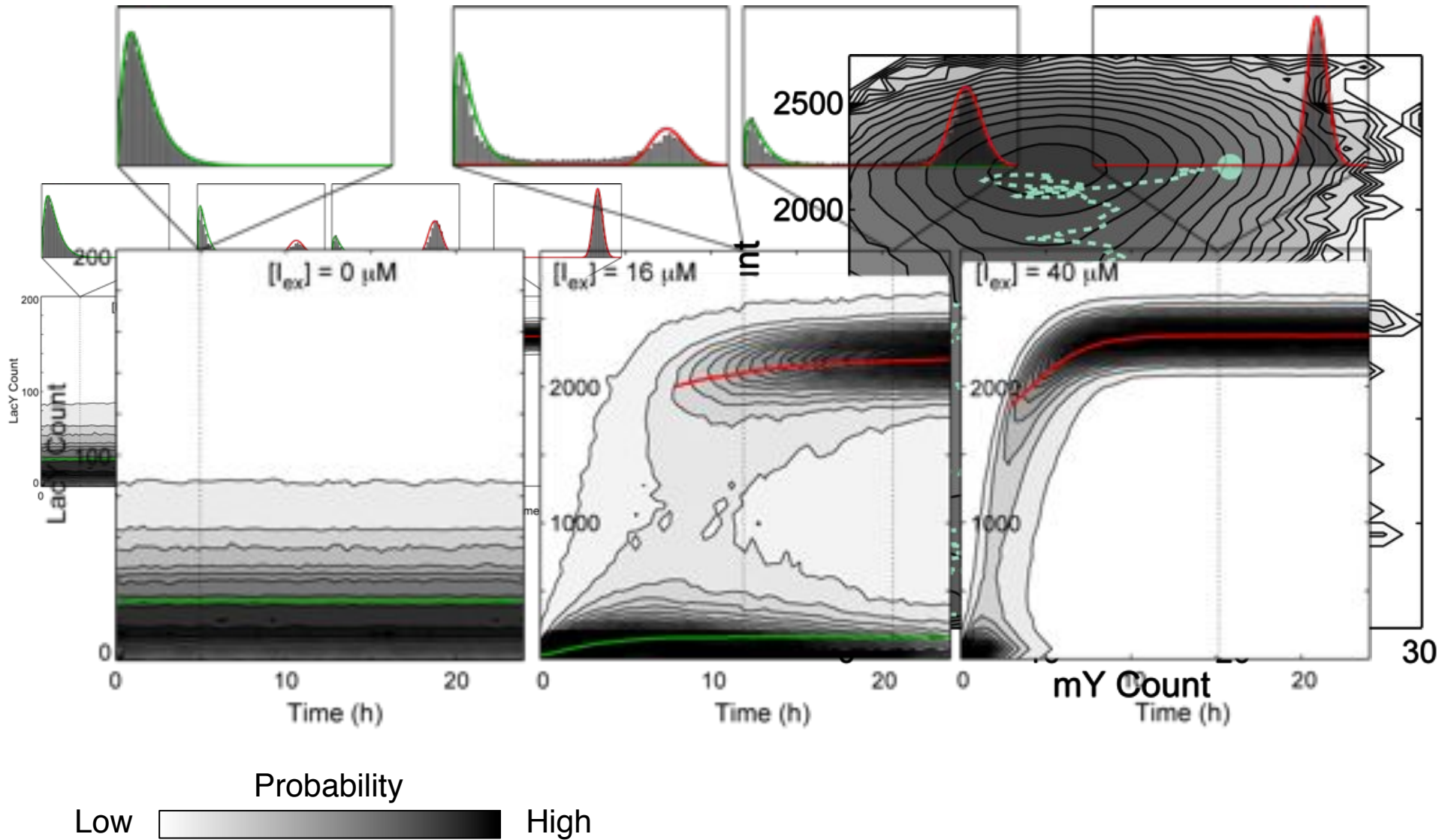


Red – mRNA bursting Yellow – Lac Y protein
Green/white – LacY gene bound/free
Light Blue – Repressor + I₂
Dark Blue -- Repressor + I

Predicting the rates of switching



Switching Behavior



Lattice Microbe Method

Reaction-diffusion master equation (RDME) for diffusion & reactions

- Spatial heterogeneity in sublattices
- Crowded cytoplasmic conditions



Massive parallelization on the GPU permits studying time evolution of cellular systems for the duration of the cell cycle

Lattice Size	Spacing (nm)	Time Step (ms)	Simulation Time per Day
64x64x128	16	50	400 s
128x128x256	8	1.3	20 s
256x256x512	4	0.32	0.5 s

Carl Woese
Bill Metcalf



John Eargle,



Ke Chen,
Jonathan Lai
Elijah Roberts, M.
Ada Yonath Assaf, T. Ernest, J. Cole

Wolfgang
Baumeister



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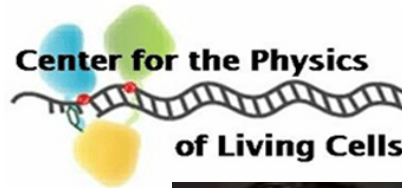
Nathan Price



Piyush Labhsetwar
John Cole



Wen-mei
Hwu (ECE)
Woodson
J. Hopkins



Beckman Institute, NIH Center
VMD, NAMD



GPU



Li Li



Susan Martinis

Taekjip Ha, H. Kim
NCSA & NSF Supercomputers
& Nvidia Center of Excellence



Mike Hallock, Corey Fry, Y. Tang



John Stone

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