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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NIH Resource Macromolecular Modeling and Bioinformatics
Atlanta Workshop 2011

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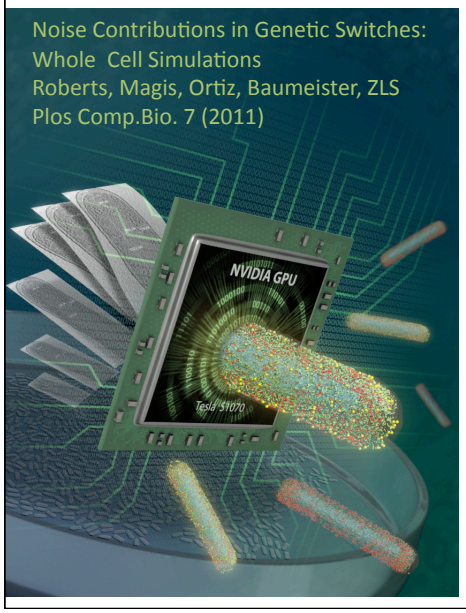
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Cellular Processes in Bacterial Cells



- 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

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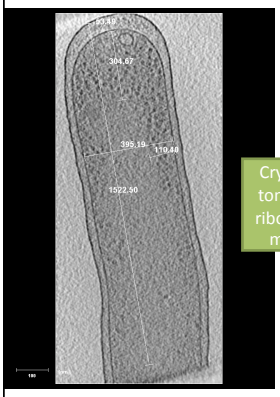
Cellular Models from Single Cells

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)

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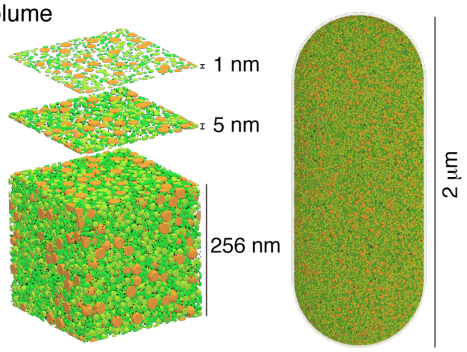
Ridgway et al., (2008) *Biophys J*
Roberts, Stone, Sepulveda, Hwu, Luthey-Schulten (2009) *IEEE Proc. HPCB - Italy*

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

Diameter	Composition at 30% occ. volume	
20.8 nm	11%	Ribosomes
10.4 nm	11%	Polymerases/Large Protein Complexes
8.6 nm	8%	Small Complexes/ Individual Proteins
8.2 nm		
8.0 nm		
7.6 nm		
7.0 nm		
6.8 nm		
6.0 nm		
5.4 nm		
4.6 nm		
3.4 nm		



20-30,000 ribosomes randomly placed

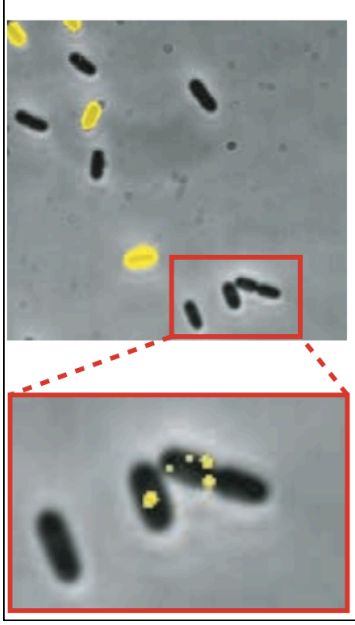
Immobile obstacle classes

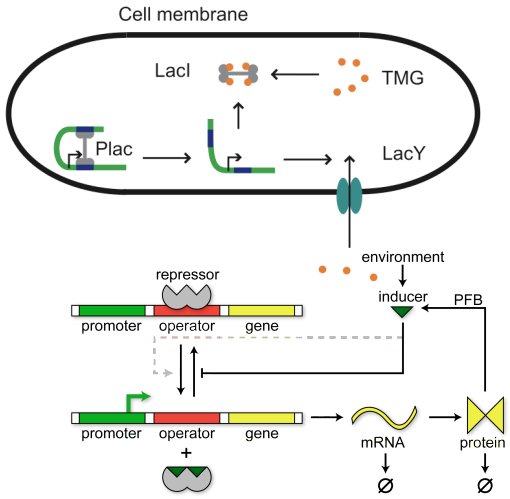
Abundances from proteomics studies

Fast growth phenotype (55 min) – uniformly distributed obstacles

Reaction diffusion master equation simulations on GPU

Lac genetic switch in E. coli





- Kinetic model for lac regulatory circuit
- Stochasticity & population heterogeneity?

SM experiments (Xie, Science 2007, 2008):

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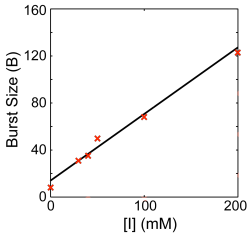
Kinetic Model of *lac* System

Reaction	Param	Stochastic Rate		Units	Source[a]	
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 degredation						
$O \rightarrow O + mY$	k_{tr}	1.26e-01		s^{-1}	M	
$mY \rightarrow mY + Y$	k_{tn}	4.44e-02		s^{-1}	S	
$mY \rightarrow \varnothing$	k_{degm}	1.11e-02		s^{-1}	S	
$Y \rightarrow \varnothing$	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_{iopoff}	1.00e+00		s^{-1}	K	
$I_2R_2O \rightarrow I + IR_2O$	$k_{i2opoff}$	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



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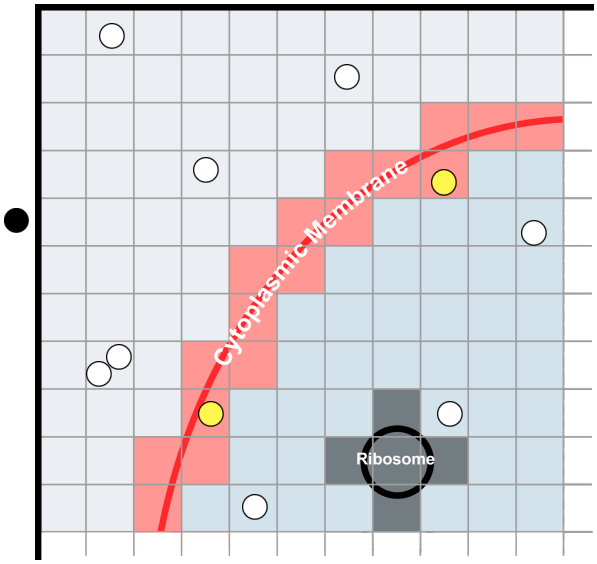
In vivo RDME Sampling



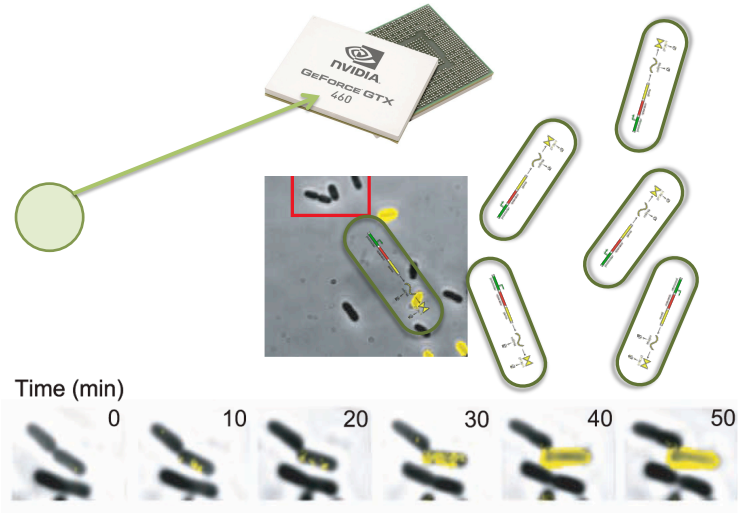
Divide space into equally sized subvolumes which can each contain multiple reactive species (particles)

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

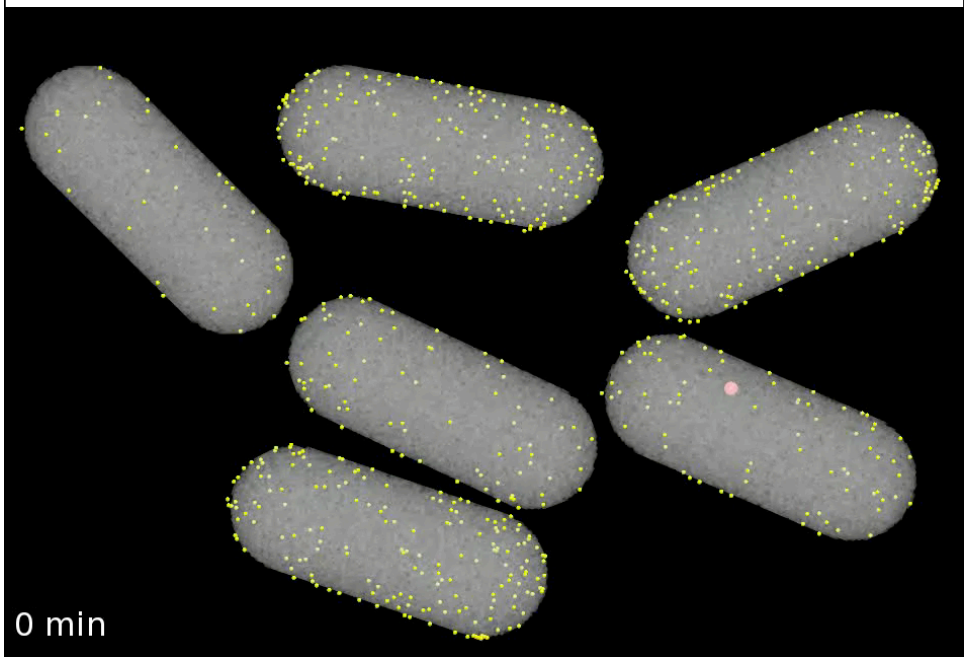
Motivation



Choi, Cai, Frieda, Xie (2008) *Science*

Noise contributions in genetic switches:Bacterial cell simulations, Roberts, Magis, Ortiz, Baumeister, ZLS (2010) submitted

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



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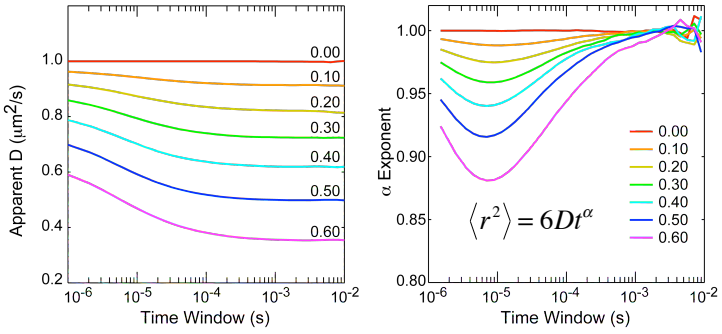
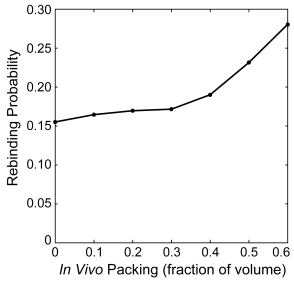
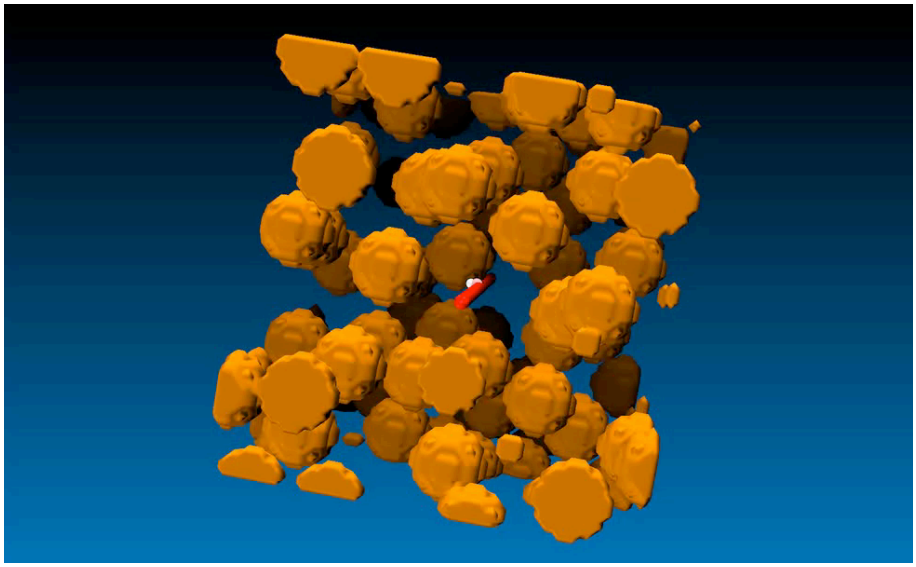
Anomalous Repressor Rebinding





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



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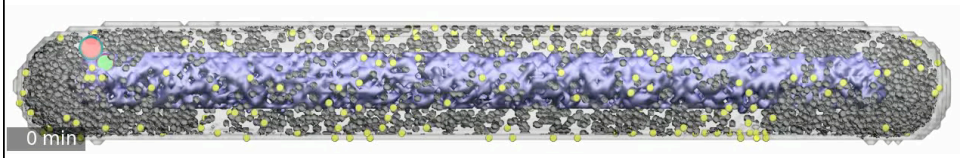
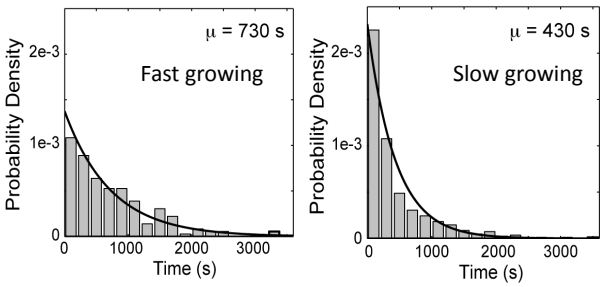
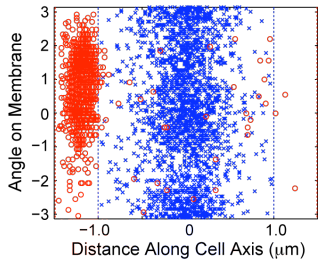
In vivo – Slow Growing Cells



Lifetimes Repressor-Operator Complexes

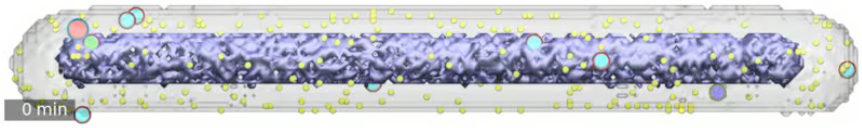


Position mRNA on membrane



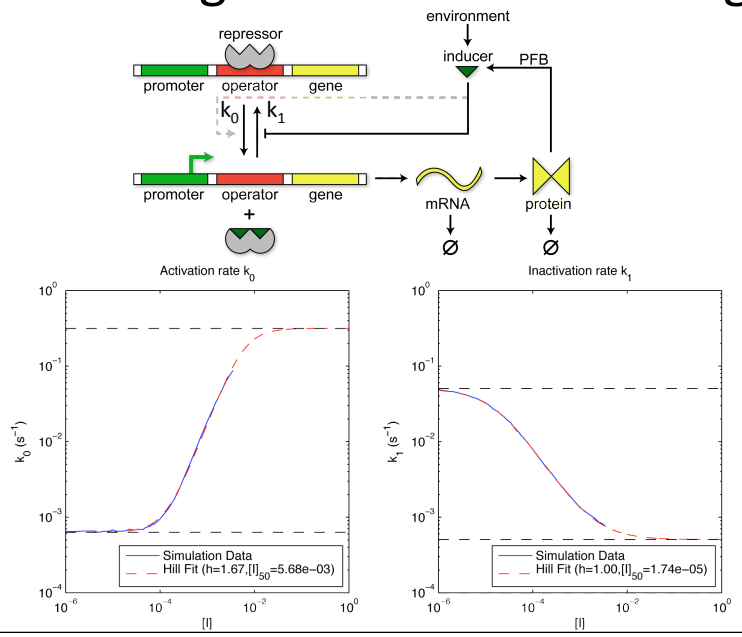
Repressor dynamics – *in vivo* model of slow growing cells

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 + I_2
Dark Blue -- Repressor + I

Predicting the rates of switching



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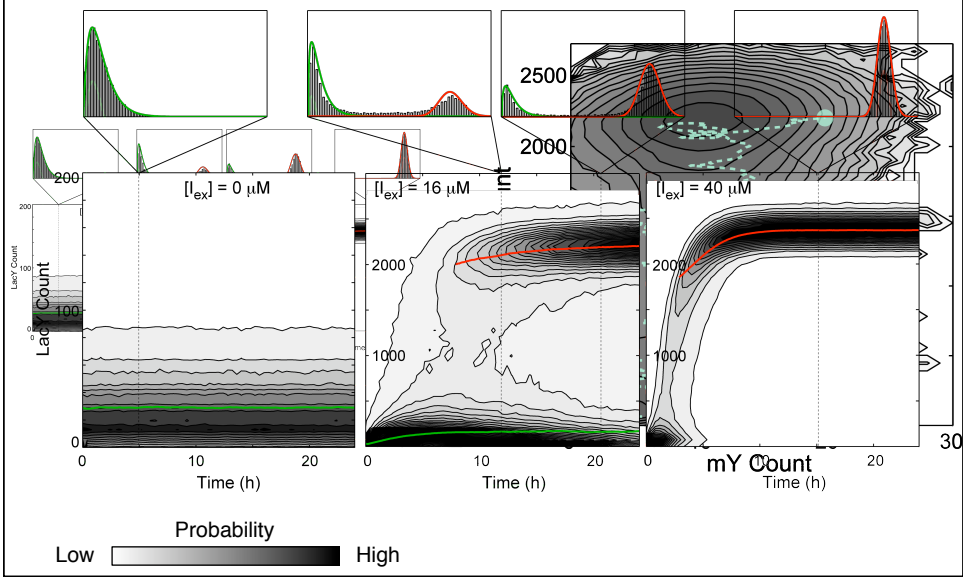
Switching Behavior



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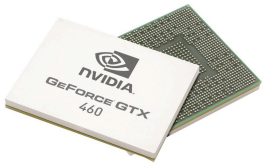
Lattice Microbe Method

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