Noise in bacterial gene expression: Experiments and models

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My history

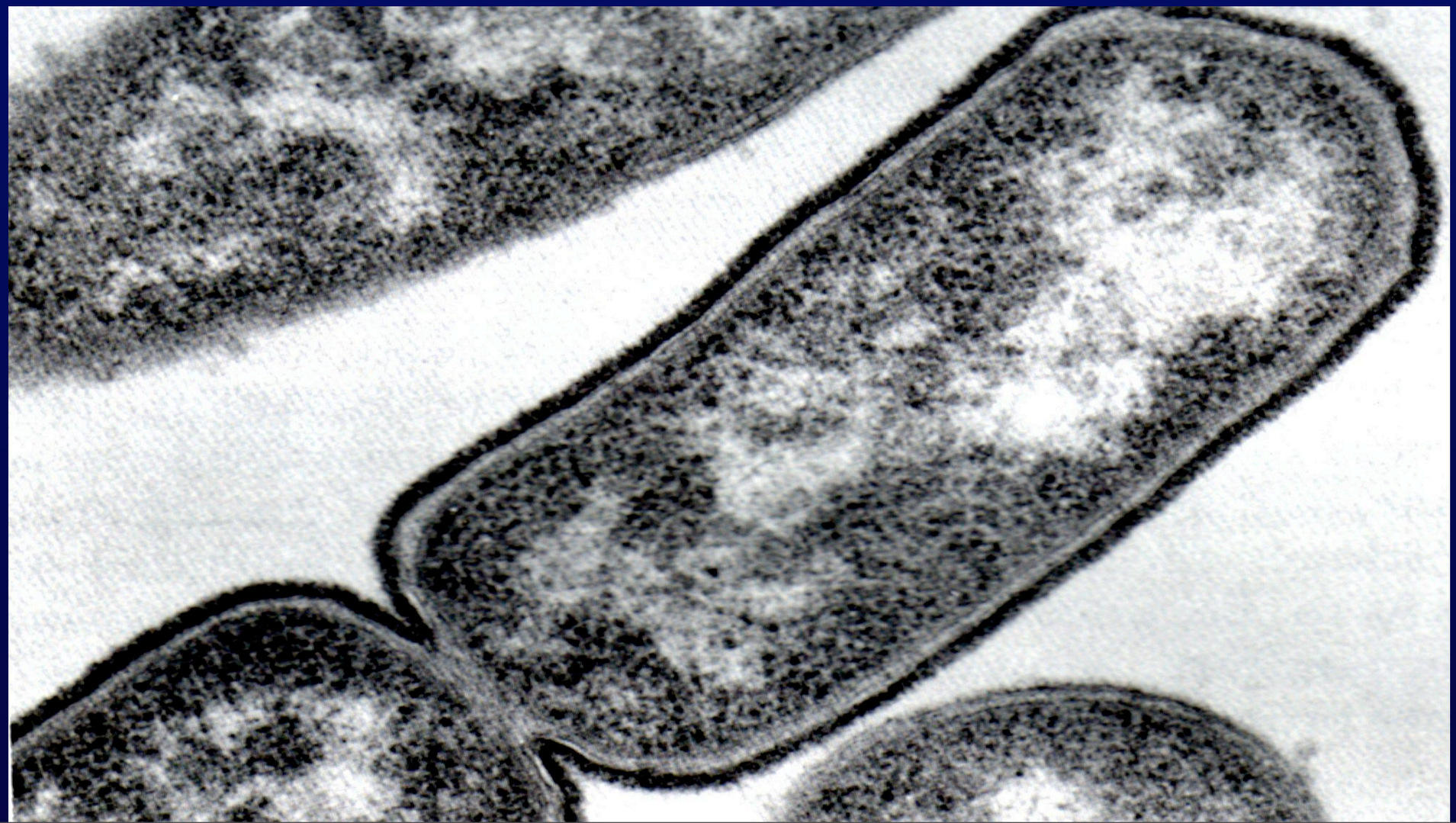
- PhD: University of Toronto engineering
  - Gabe D'Eleuterio, Space Robotics group (aerospace)
  - Models of coupled neural oscillators to control a walking robot
- Postdoc: Center for BioDynamics, Boston University
  - Neural/genetic oscillator synchronization with Nancy Kopell
  - Gene modelling with Jim Collins
- Faculty: Chemistry/Physics,Toronto (since July 2003)

People

People

The cell

- A little package of goo, in a wrapper
- Key molecules:
  - Proteins: do most of the work
  - DNA: codes for proteins



Genome sequencing projects

Genome sequencing projects

Secret of Life Solved!

Cells fully understood!

Molecular biology finished!

- Why is this not true?
- Having the pieces doesn’t mean we know how they function and fit together (dynamics, network behaviour)

Questions

- Big Question #1: How do we predict cellular behaviour?
  - Sequence data gives us the components, now how do we understand the system?
- Big Question #2: How can we *control* cellular behaviour?
  - Diseases, pathogenic invasions: involve alterations of natural dynamics
  - Can we reestablish normal function?

Dynamics of the cell

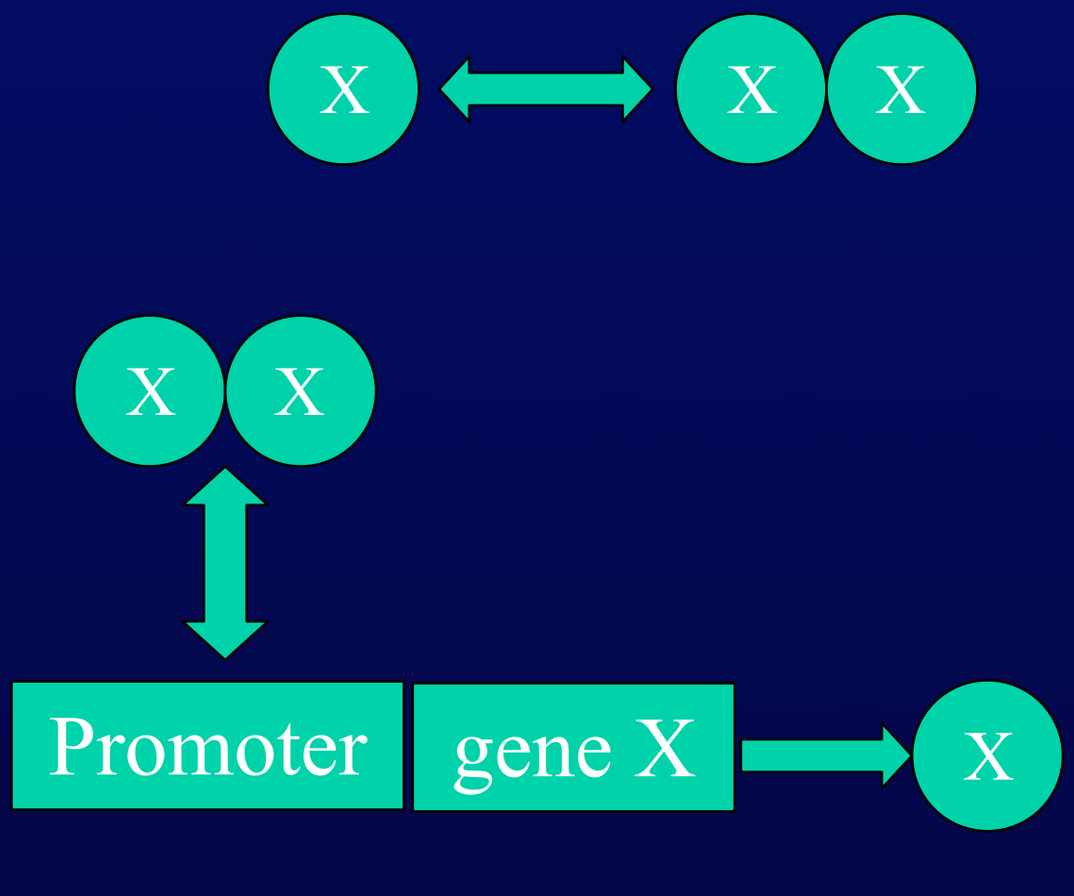
- Dynamics: How a system's state evolves over time
- State of a cell:
  - Numbers of biomolecules/complexes
  - Proteins, mRNA, DNA-protein complexes ...
- Time evolution:
  - Driven by biochemical reactions
  - Transcription, translation, binding ...

If cells were beakers ...

1. Use standard kinetics to form models
2. Identify the set of relevant biochemical reactions
   - List all species of interest
   - Include all reactions that affect those species
3. Determine rate constants
   - Production, degradation, binding
4. Derive dynamics from the chemical kinetics
5. Main problem: sheer scale

Formulating models

- Represent production, binding, degradation as a set of reactions
- Use chemical kinetics to turn reactions into a mathematical model



$$D + X_2 \xleftrightarrow{k_1 / k_{-1}} D_X$$

$$X + X \xleftrightarrow{k_2 / k_{-2}} X_2$$

$$D \xrightarrow{k_T} D + X$$

$$D_X \xrightarrow{\alpha\, k_T} D_X + X$$

$$X \xrightarrow{k_x}$$

Formulating models

- Translate reactions into rate equations for each species

$$D + X_2 \xleftrightarrow{k_1 / k_{-1}} D_X$$

$$X + X \xleftrightarrow{k_2 / k_{-2}} X_2$$

$$D \xrightarrow{k_T} D + X$$

$$D_X \xrightarrow{\alpha\, k_T} D_X + X$$

$$X \xrightarrow{k_x}$$

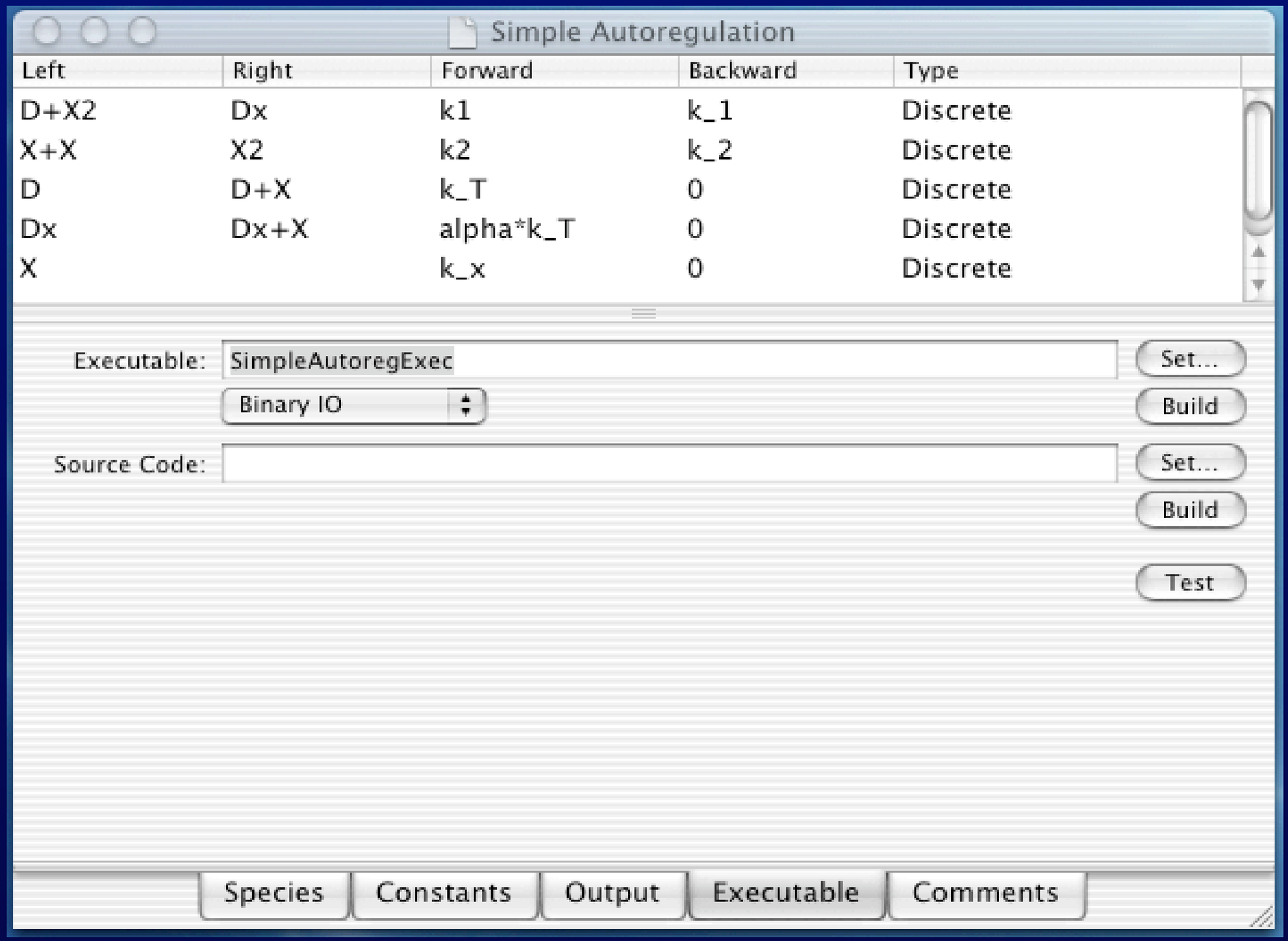
$$\dot{x} = -2k_2x^2 + 2k_{-2}x_2$$

$$+ k_T d$$

$$+ \alpha\, k_T d_x$$

$$- k_x x$$

Autoregulation in BioNetS



Adalsteinsson, McMillen, and Elston. BMC Bioinformatics 5:24 (2004).

But cells differ from beakers ...

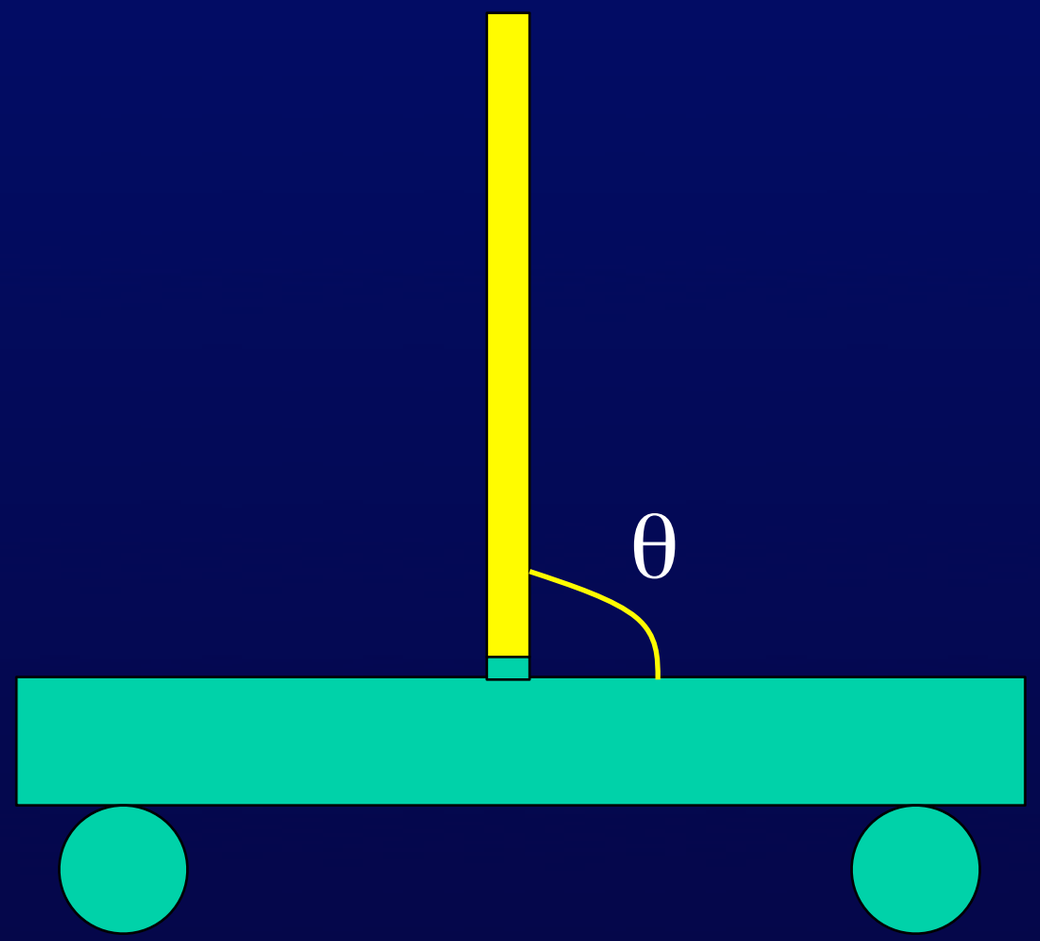
1. Hard to reach into and control
   - Design of synthetic network controllers
2. Small size / small numbers
3. Growth and division
   - Cells double, cut in half
4. Individual histories and identities
   - Cell-to-cell variation
5. High complexity
   - Model reduction methods are valuable
6. Crowded molecular environment
   - How do crowded kinetics change?

1. Cells are stubborn

- By “stubborn,” we mean: hard to control (fiendishly uncooperative!)
- Want to be able to exert control over cells from the inside
  - “Killer app”: the in-cell cancer detector that kicks cell into apoptosis
- Hope is to learn to design synthetic regulatory networks capable of this
  - We’re working on a system that cures disease - but only in bacteria

Control systems

- The "game" in control systems is to alter a system's dynamics so that it has desired properties



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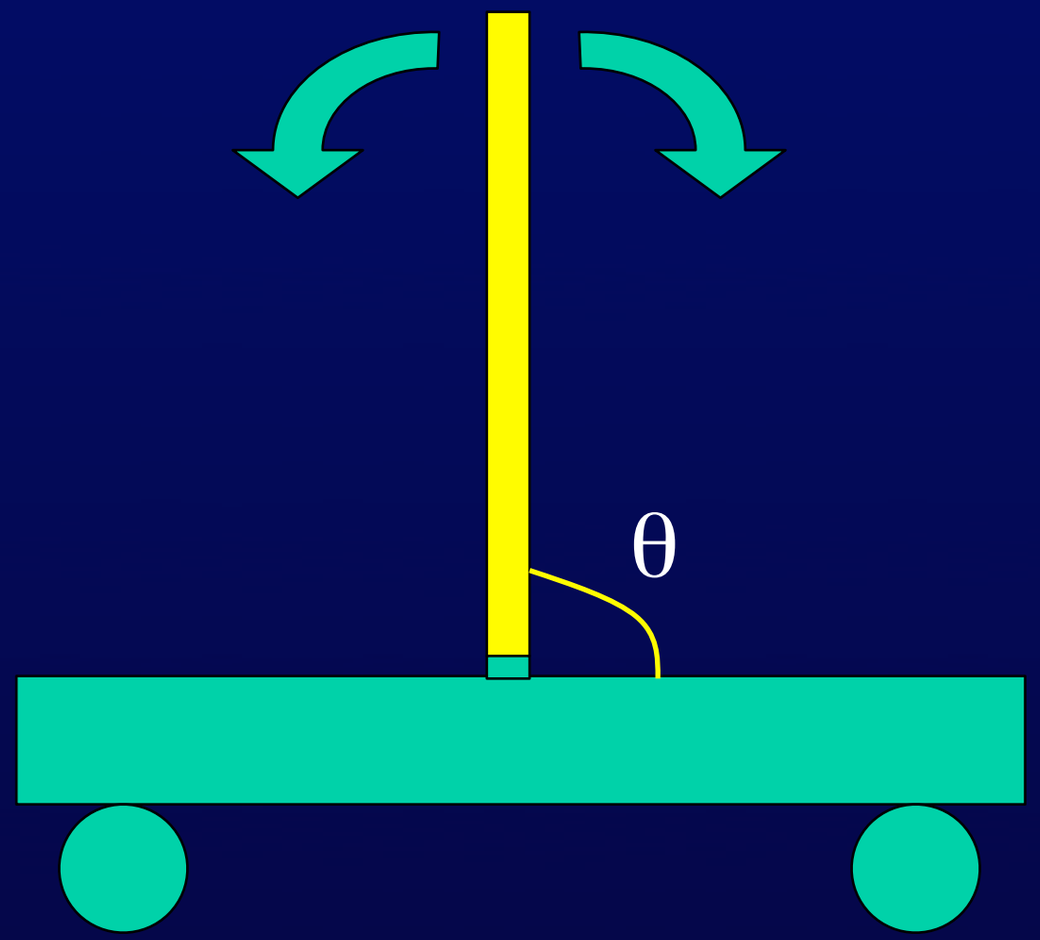

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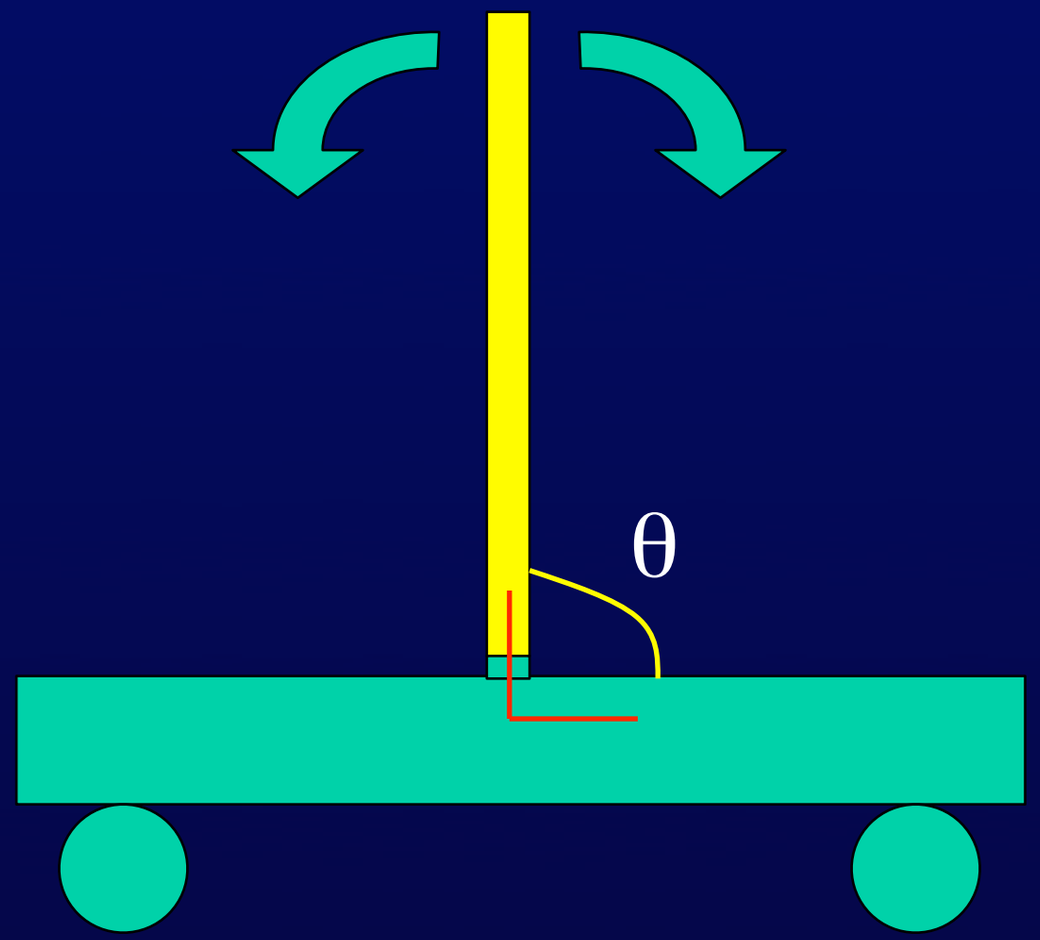

Control systems

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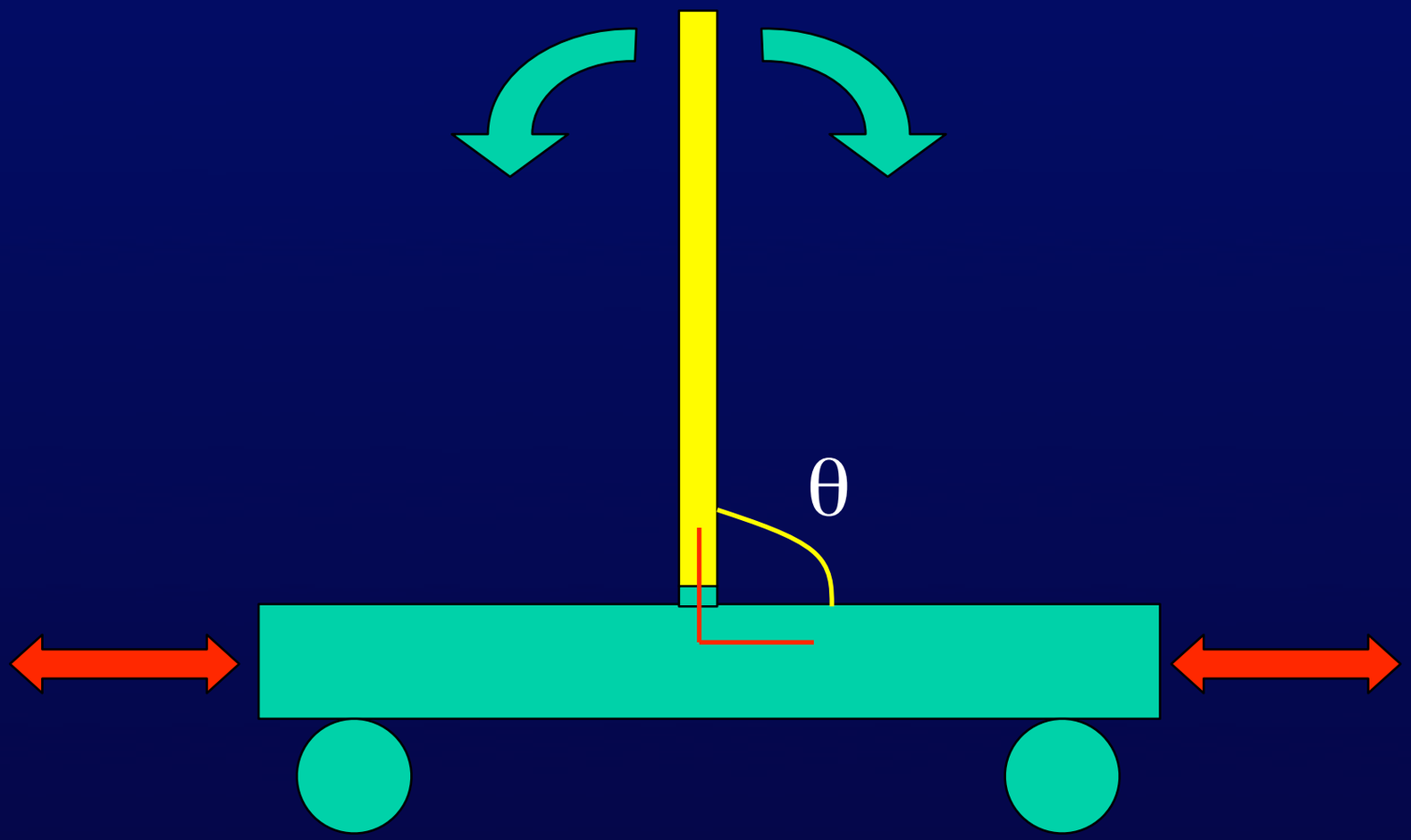
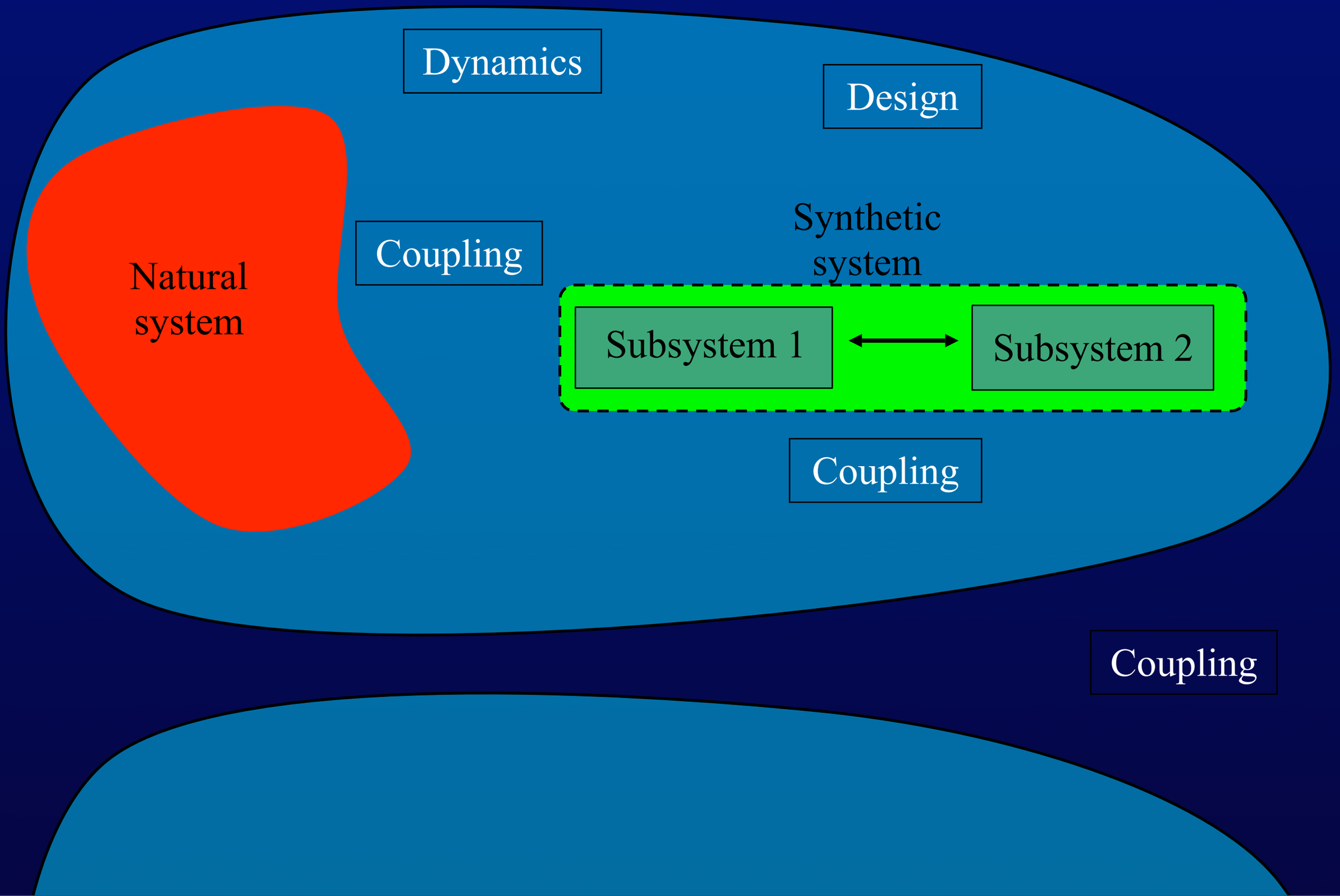
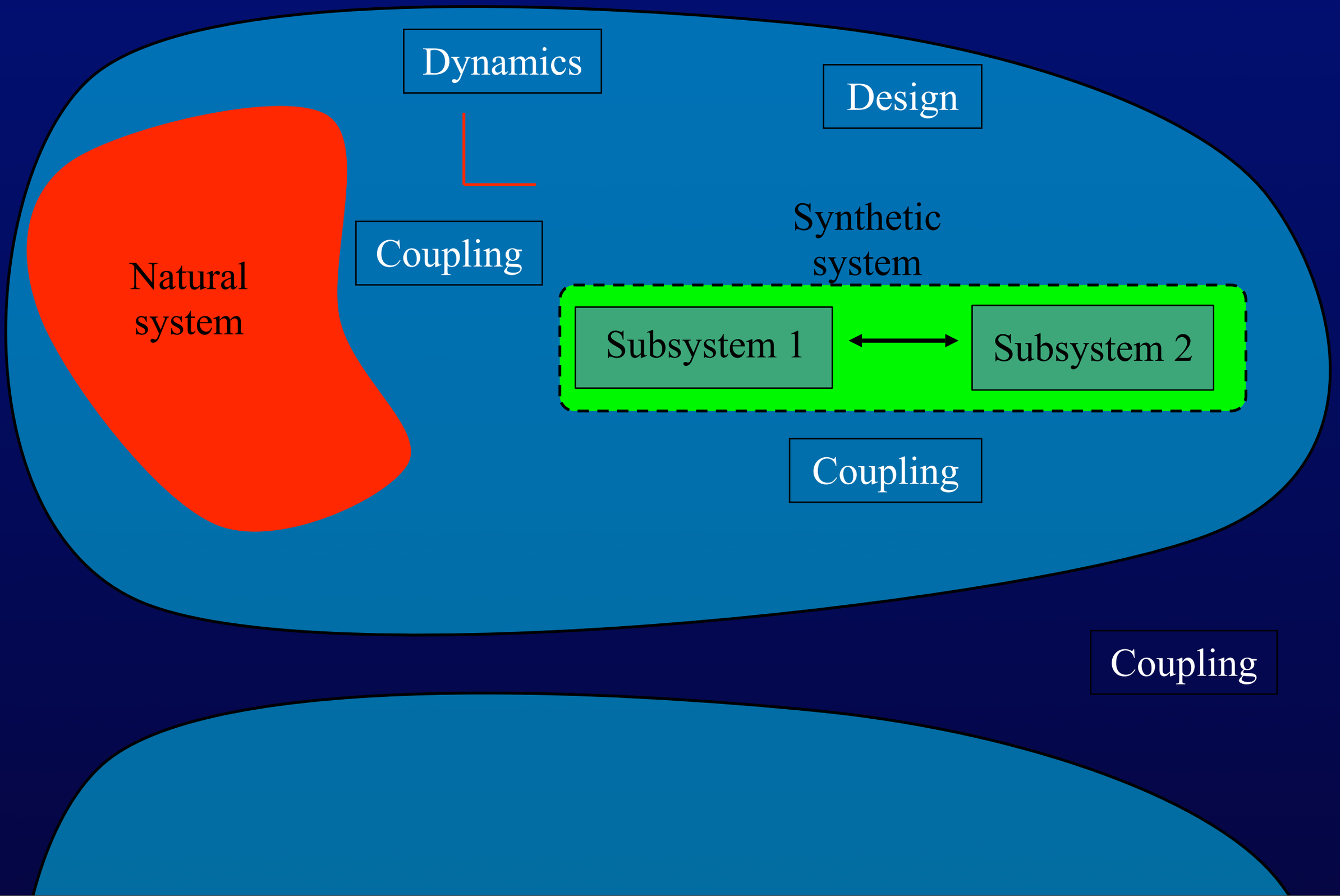


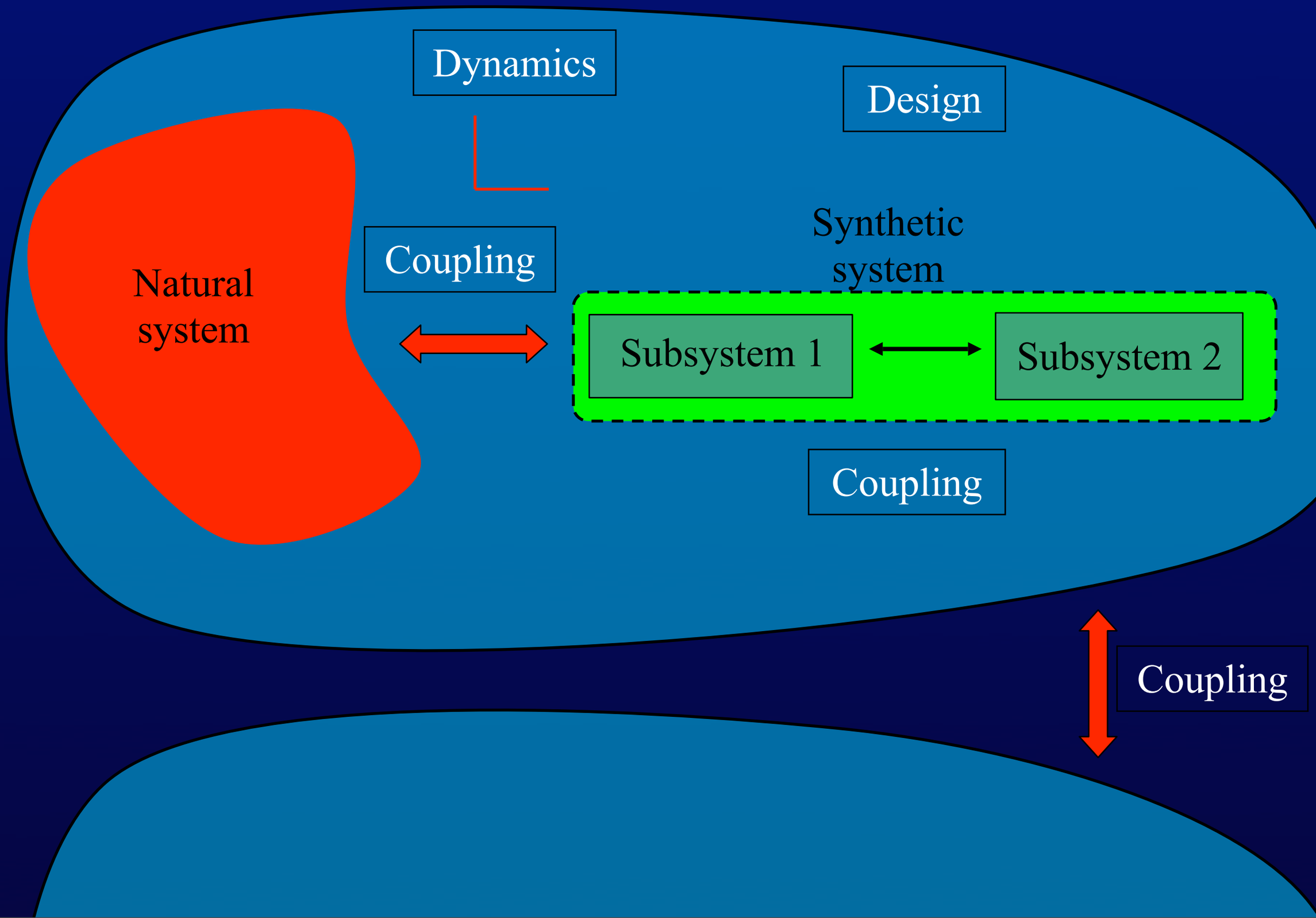
Cellular control - requirements



Cellular control - requirements

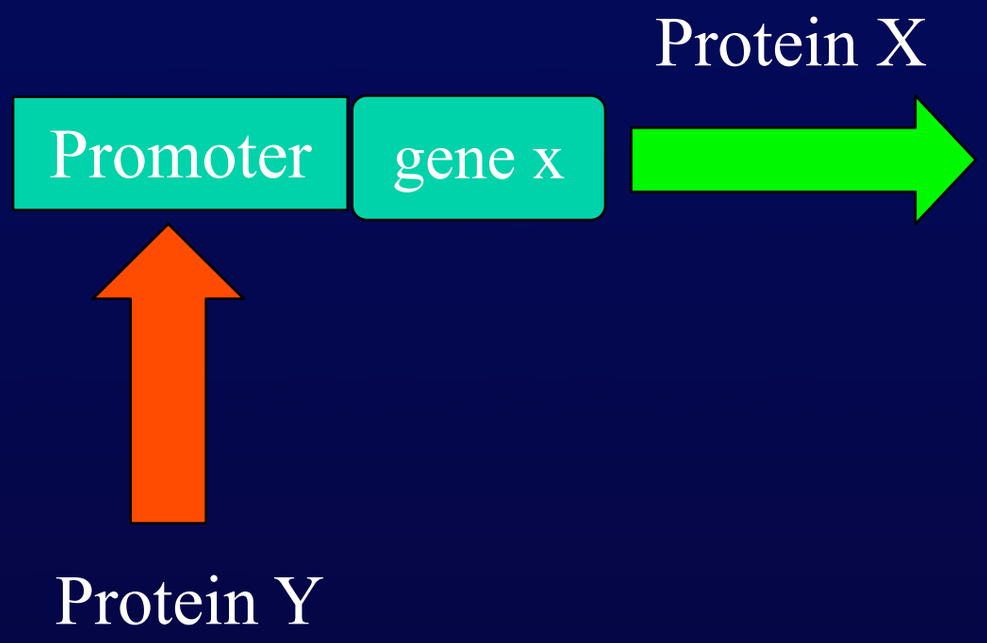


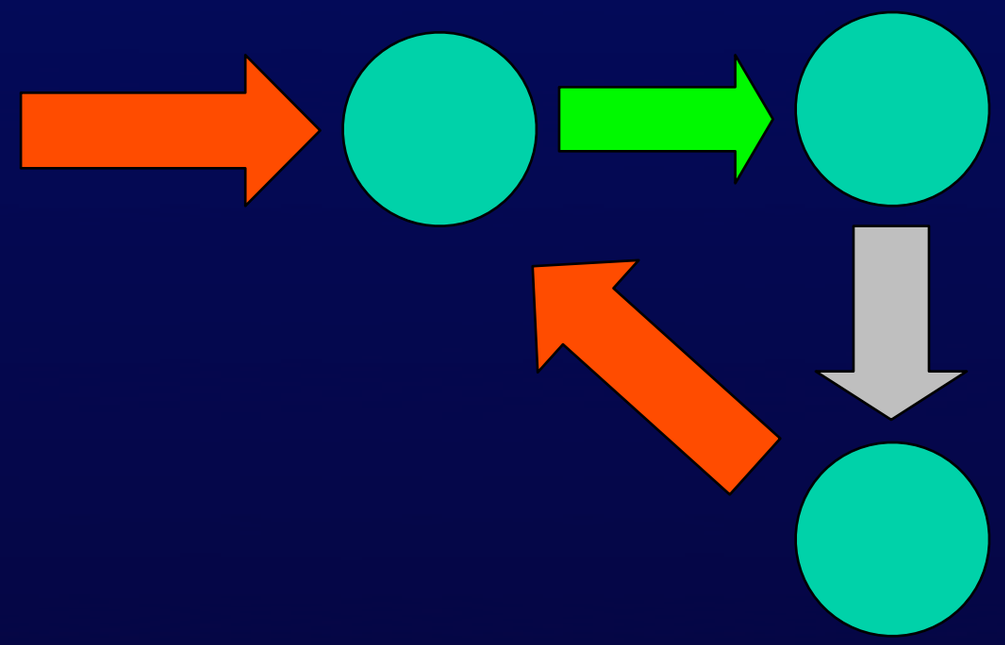
Cellular control - requirements



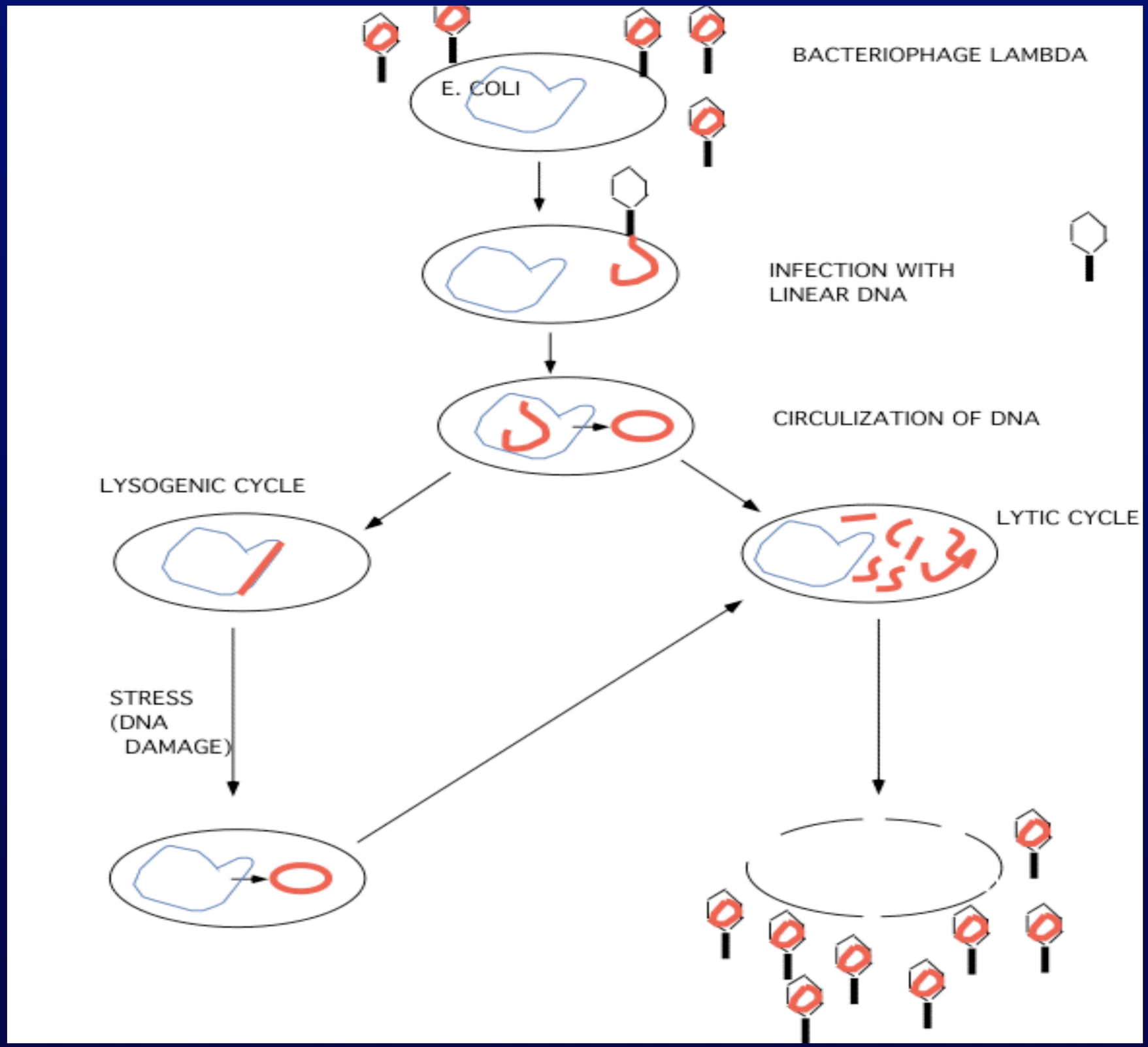
Designing synthetic networks

- Node in the network = a promoter/gene pair
- Choice of promoter = choice of input(s)
  - Sets which proteins affect the node
- Choice of gene = choice of output(s)
  - Sets which genes are affected by the node





Bacteriophage lambda

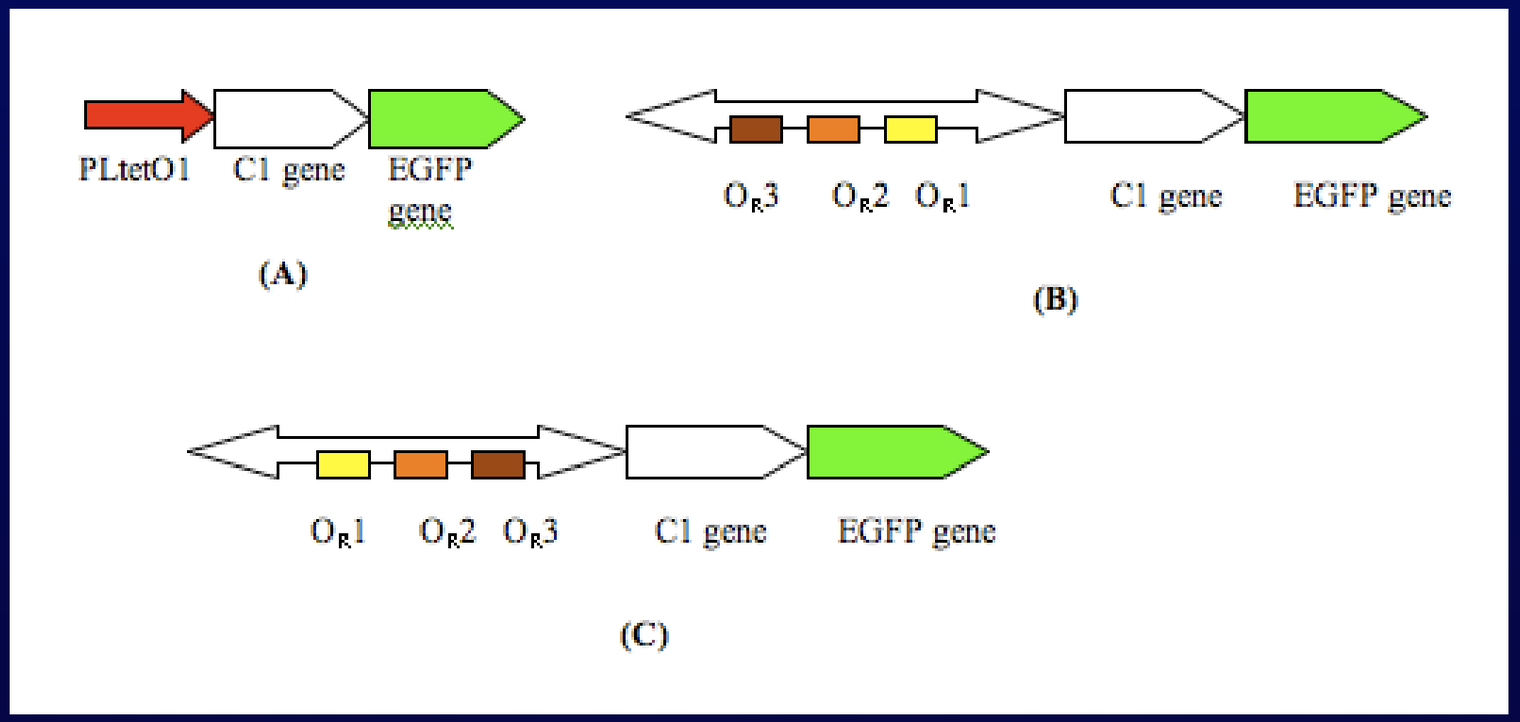


Lysis prevention

- Use a cell-resident network to prevent a fatal disease
  - Currently in bacteria, but looking to the future, and human medical applications
- Prevent lysis in E. coli infected by λ
  - Protein CI: maintains lysogeny, prevents lysis; SOS response causes RecA to cleave CI monomers, CI drops
  - Protein Cro: expression leads to lysogeny

Lysis prevention

- Sense onset of lytic pathway using a CI-repressed promoter
- When CI level drops, (lysis coming!), produce extra CI to maintain lysogeny
- Status: Working towards it



Sangram Bagh

2. Cells are small

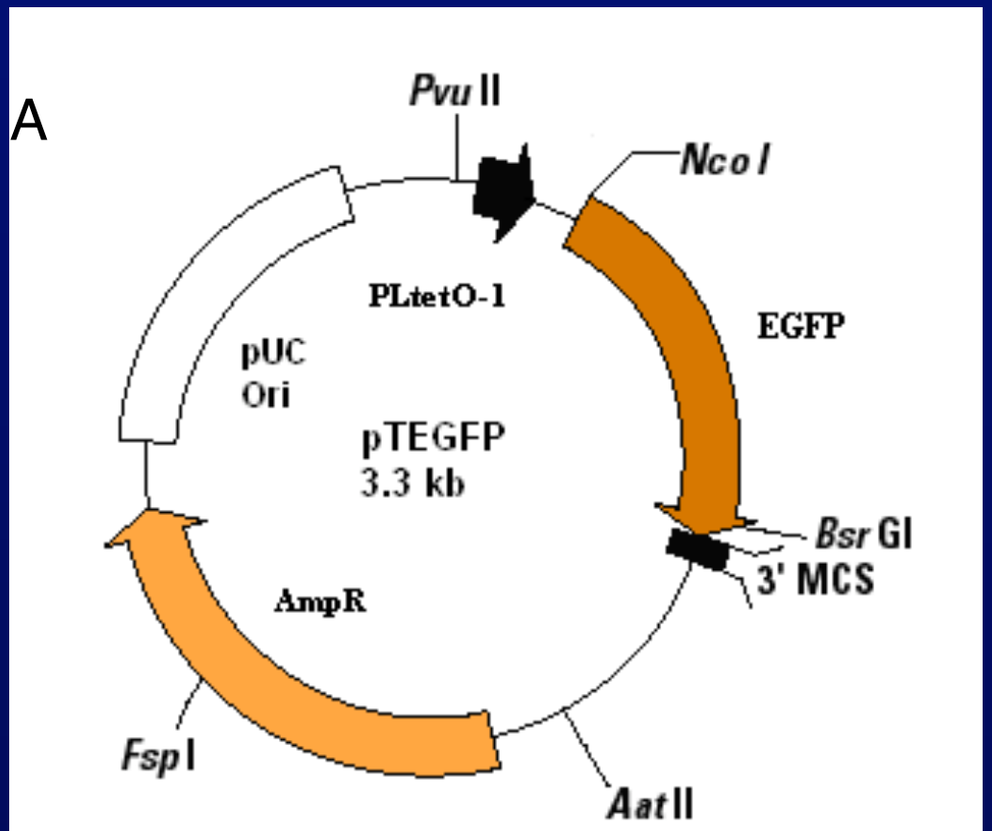
- Chemical kinetics:
  - Random interactions of molecules
  - For moles of particles, rates effectively deterministic
- But for small numbers of molecules, fluctuations become significant
- Inside a cell:
  - Small numbers of molecules, not moles
- Question: What are the actual numbers?
  - Recent work: Swain and Elowitz (binomial division); Ghaemmaghami (blotting); Barkai (fluorescence)

Unregulated system

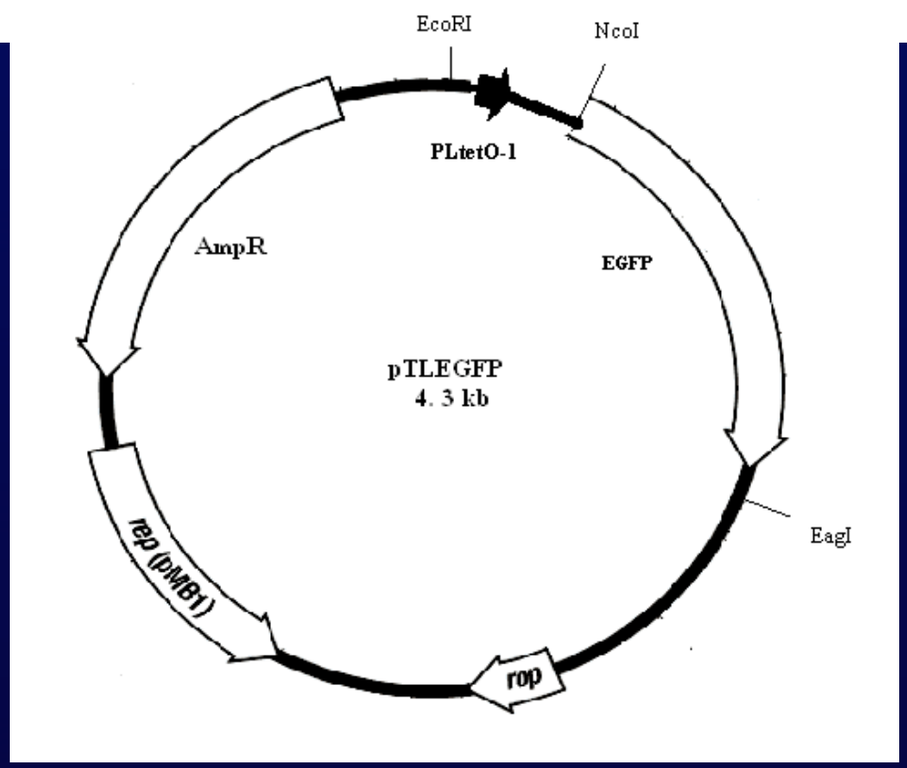
- Constructed a simplified system:
  - Promoter with no (known) regulatory feedback present, $P_L$tetO1
  - Expressing EGFP directly
- Inserted into *E. coli* cells on plasmids with two different replication patterns:
  - High copy number plasmid: replicates as rapidly as it can, circa 400 copies/cell
  - Medium copy number plasmid: feedback reduces replication, circa 40 copies/cell
- Examined four strains of bacteria

Plasmids

A


Pvu II
Nco I
PLtetO-1
EGFP
pUC
Ori
pTEGFP
3.3 kb
Bsr GI
3' MCS
AmpR
Fsp I
Aat II

B


EcoRI
NcoI
PLtetO-1
AmpR
EGFP
pTLEGFP
4. 3 kb
EagI
rop

Strains

Strain	Characteristics
DH5a	F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG 80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17 (r_K^- m_K^+), λ–
Top10	F^- mcrA (mrr-hsdRMS-mcrBC) 80dlacZM15 lacX74 recA1 ara139 (ara-leu)7697 gal/U gal/k rpsL (str^R) endA1 nupG
B/r	F26 his thy
BL21*	(r_k^-, m_k^+) phoA supE44 $^-$ thi-1 gyrA96 relA1

Measurement/calibration

- Measure the "output" (protein expression) by quantifying fluorescence, through flow cytometry and microscopy
- Calculate mean number of EGFP per cell using bulk fluorimetry:
  - Calibrate against known numbers of EGFP in PBS solution (matches cellular pH): yields equivalent # of EGFP in cell culture
  - Use optical absorbance to get cells/ml
  - Divide one by the other to get <EGFP>/cell

Protein numbers

Cell strain	Plasmid	<EGFP>/cell	Div time (min)	Proteins /min
DH5a	High	156,000	36.8	2900
	Medium	23,400	30.5	530
Top10	High	119,000	29.5	2800
	Medium	17,400	29.8	400
B/r	High	144,000	27.9	3600
	Medium	11,000	33.1	230
BL21*	High	46,400	49.9	640
	Medium	5,800	31.6	130

- Order of magnitude: tens to hundreds of thousands of proteins/cell
- Substantial differences across strains

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3. Cells grow and divide

- Unlike beakers, our bacteria double in size every 20-40 min, then cut themselves in half
- Implications for kinetics
  - May need to work in number space rather than concentration space
  - Rates are volume-dependent
  - Differential equations --> maps (maybe?)
- Range of cell sizes complicates matters if you can’t reliably scale away cell size (as in flow cytometry)

“Sawtooth” gene expression

- For high rates of expression, the process of gene expression becomes near-deterministic
  - (In simple-ish models)
- Growth and division induces a sawtooth pattern of protein vs time
- If you see only total fluorescence intensity rather than intensity/size, there’s a contribution to “variability” from the size range

Very simple model

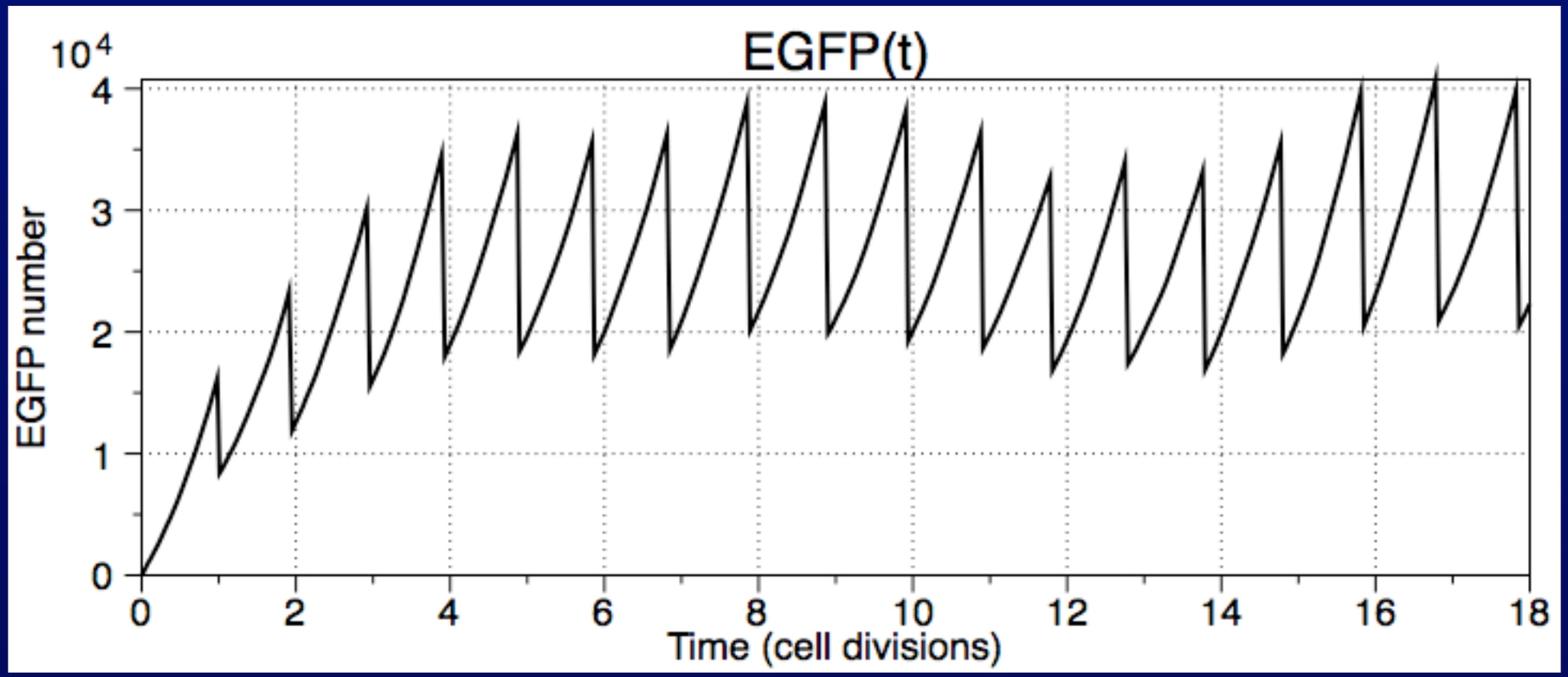
Sawtooth.bnet

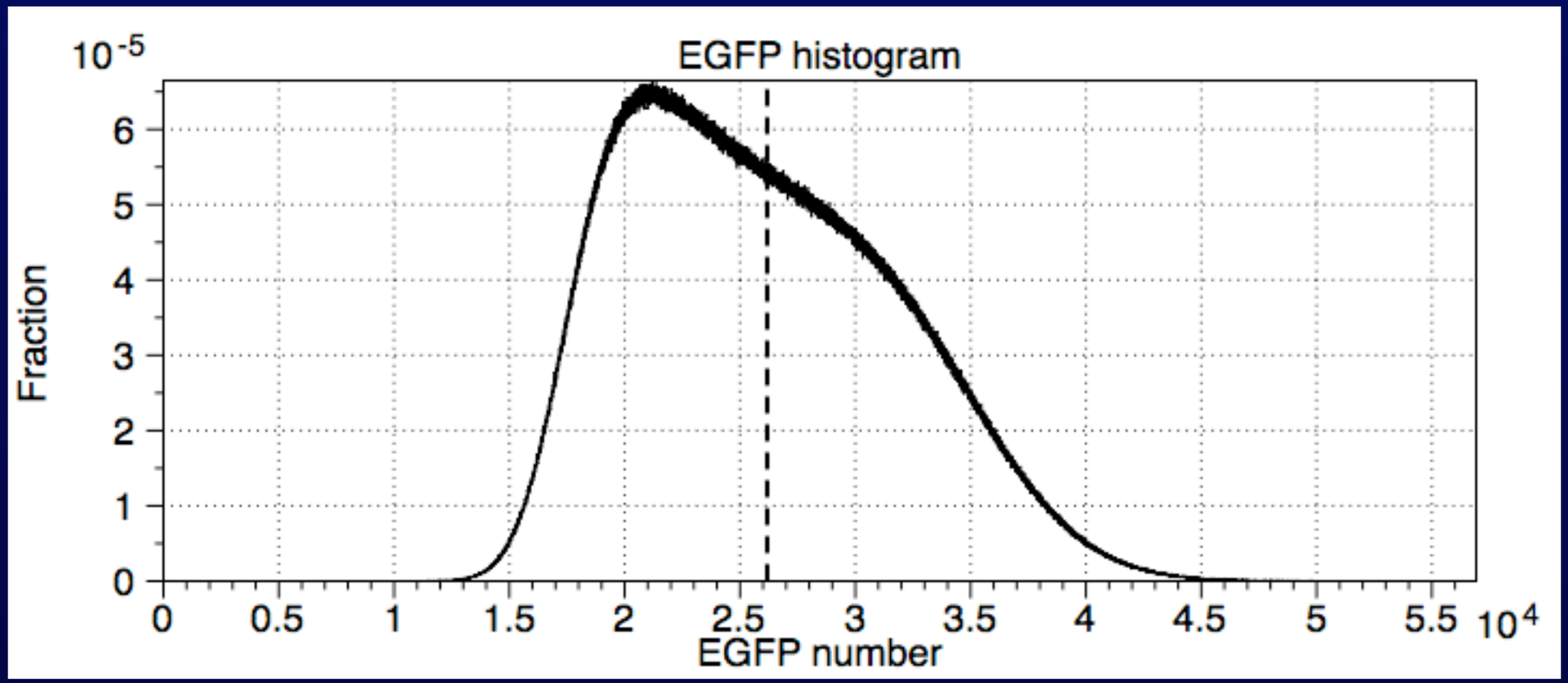
Left	Right	Forward	Backward	Type
	D	k1		Discrete
D	D+G	k2		Discrete
G		k3		Discrete
V	2V	k_v		Discrete

Name	In #	Type	Save	Out #	Hist	Range	Divide
D	1	☑ Discrete	☑ Save	2	☑ Hist.		☑ Divide
G	2	☑ Discrete	☑ Save	3	☑ Hist.		☑ Divide
V	3	☑ Discrete	☑ Save	4	☑ Hist.	200	☑ Divide

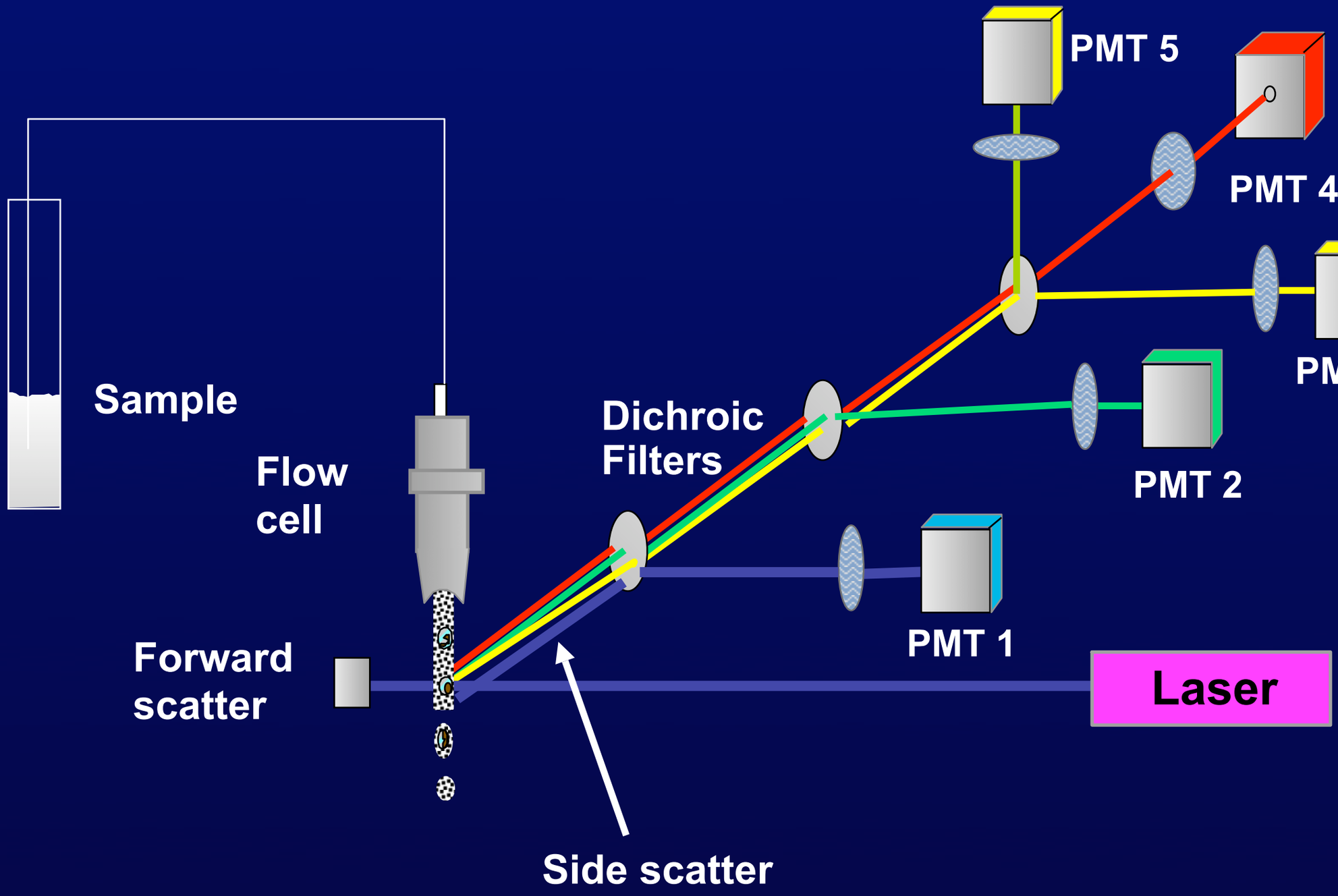
Species | Constants | Output | Expert | Executable | Comments

Simple model outputs



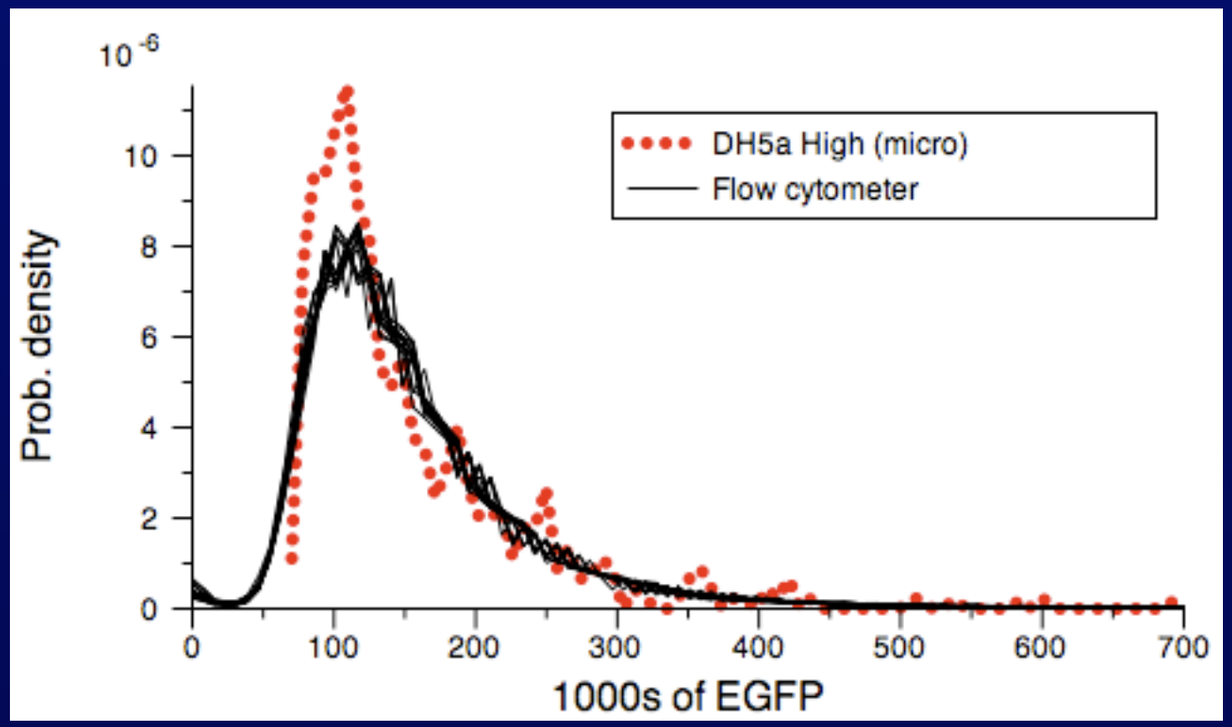


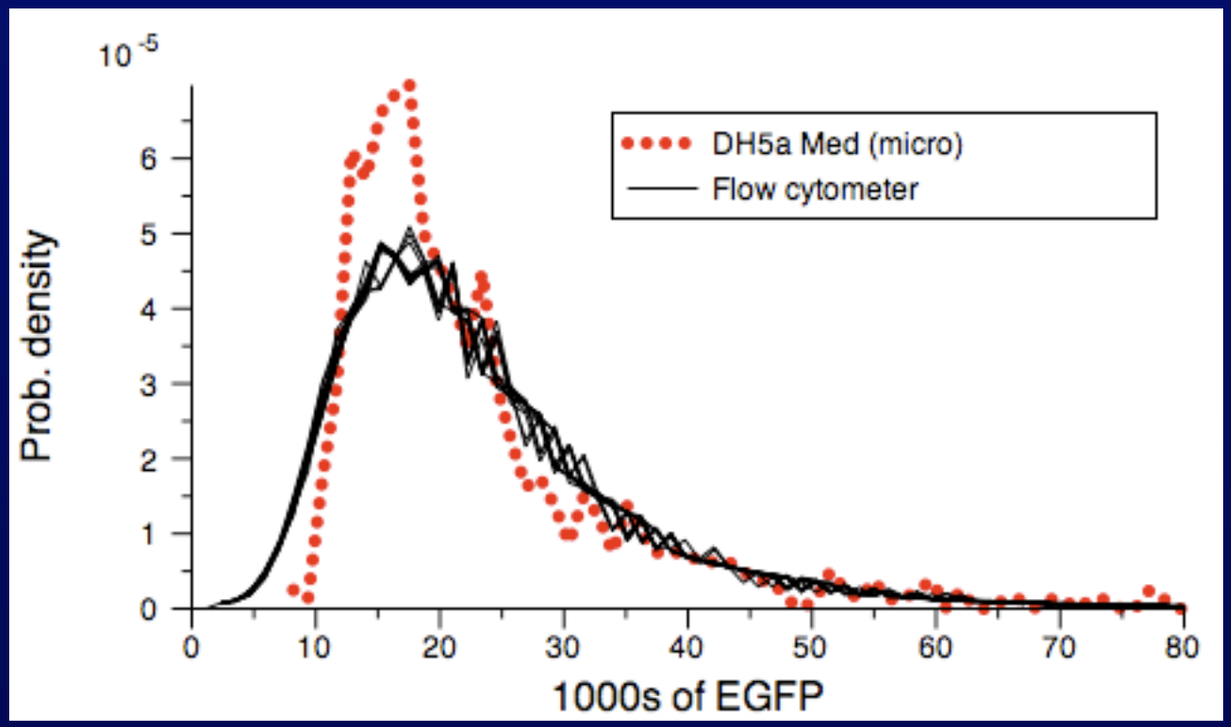
Experiments: Flow cytometry



Protein distributions

- By flow cytometry and microscopy:





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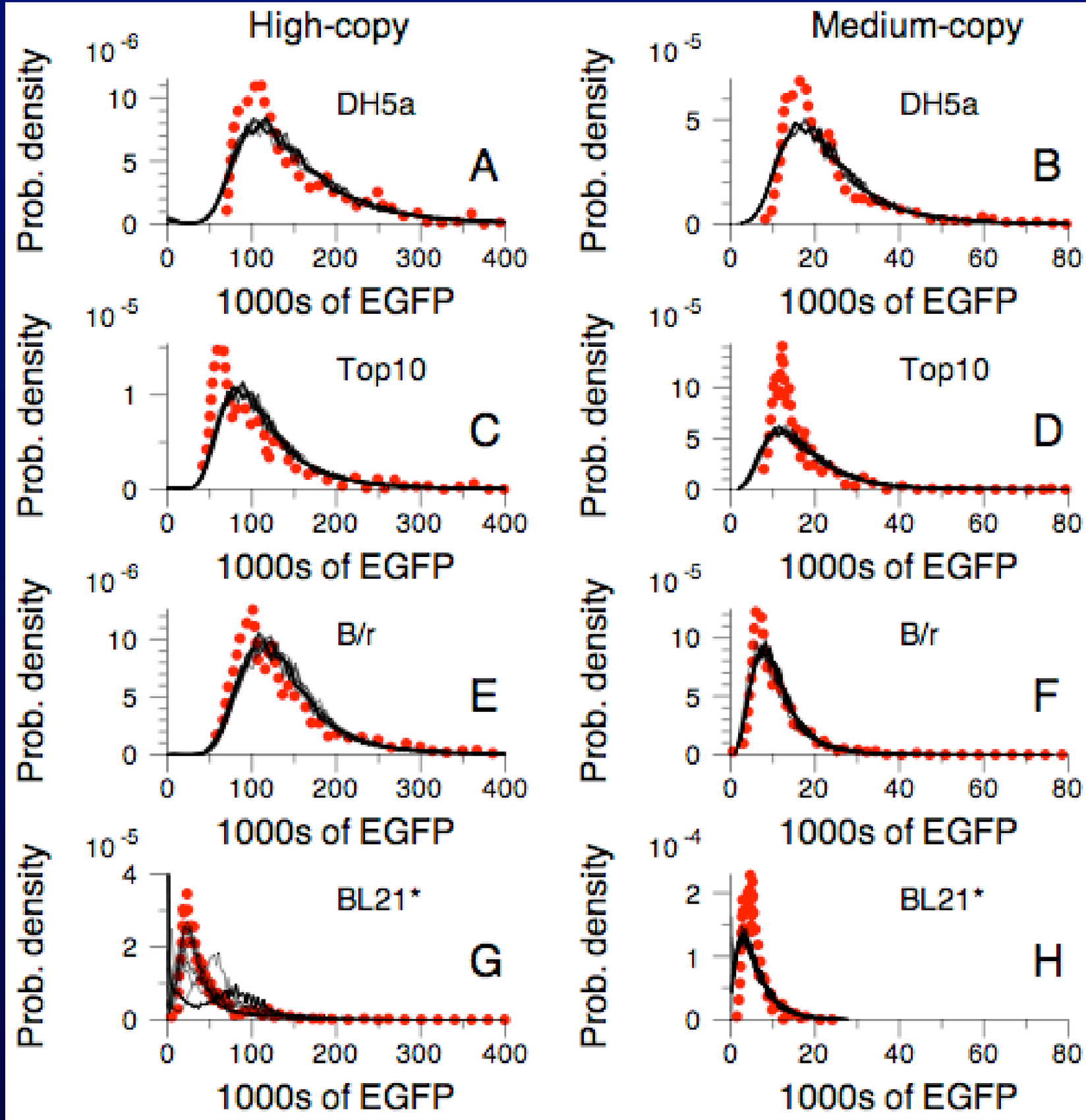
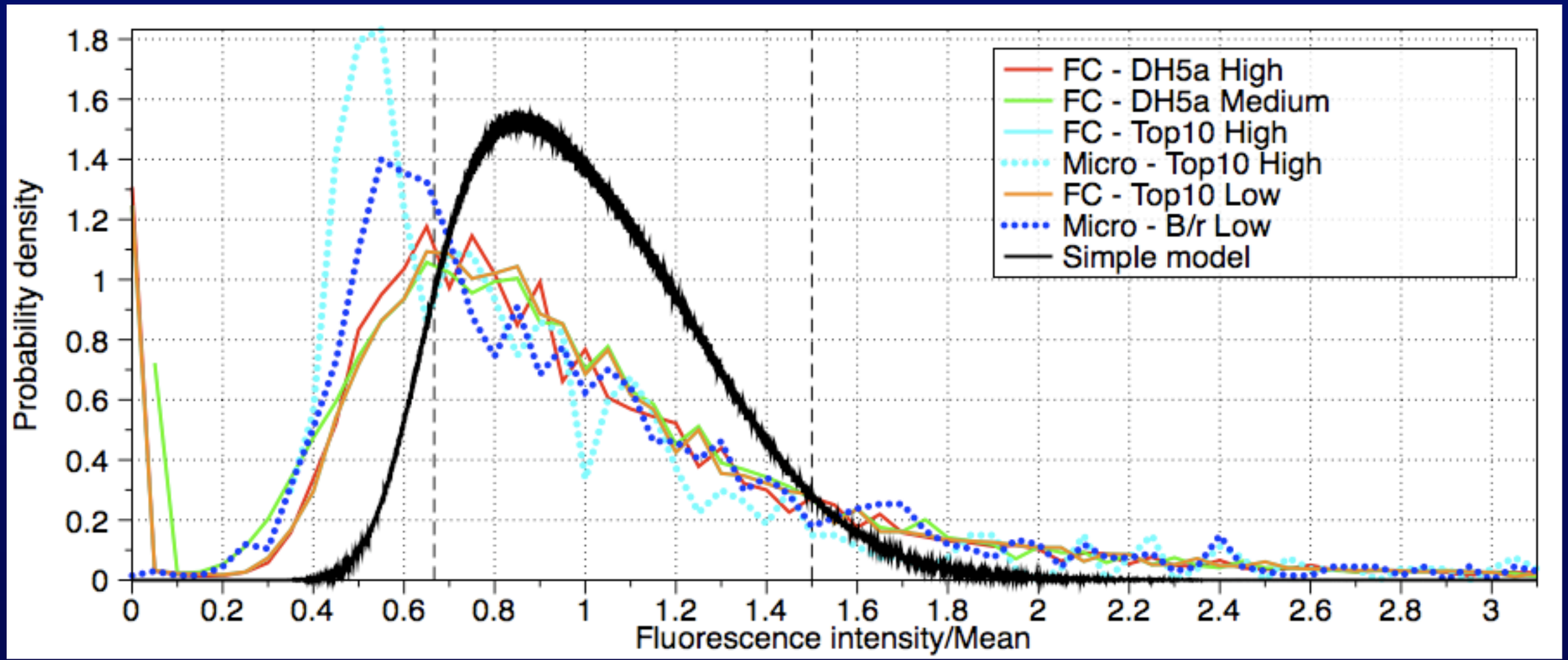
Distributions: all strains

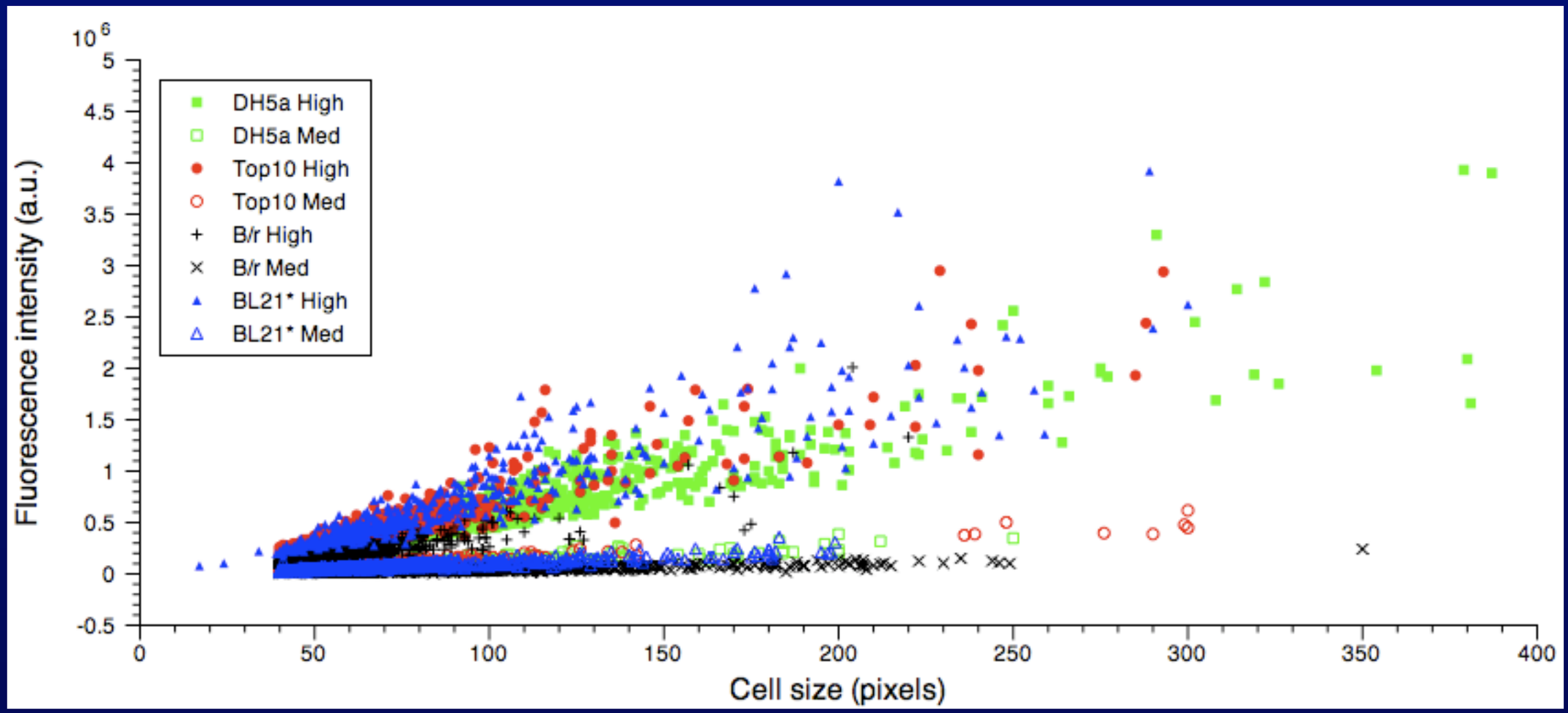


Comparing to model



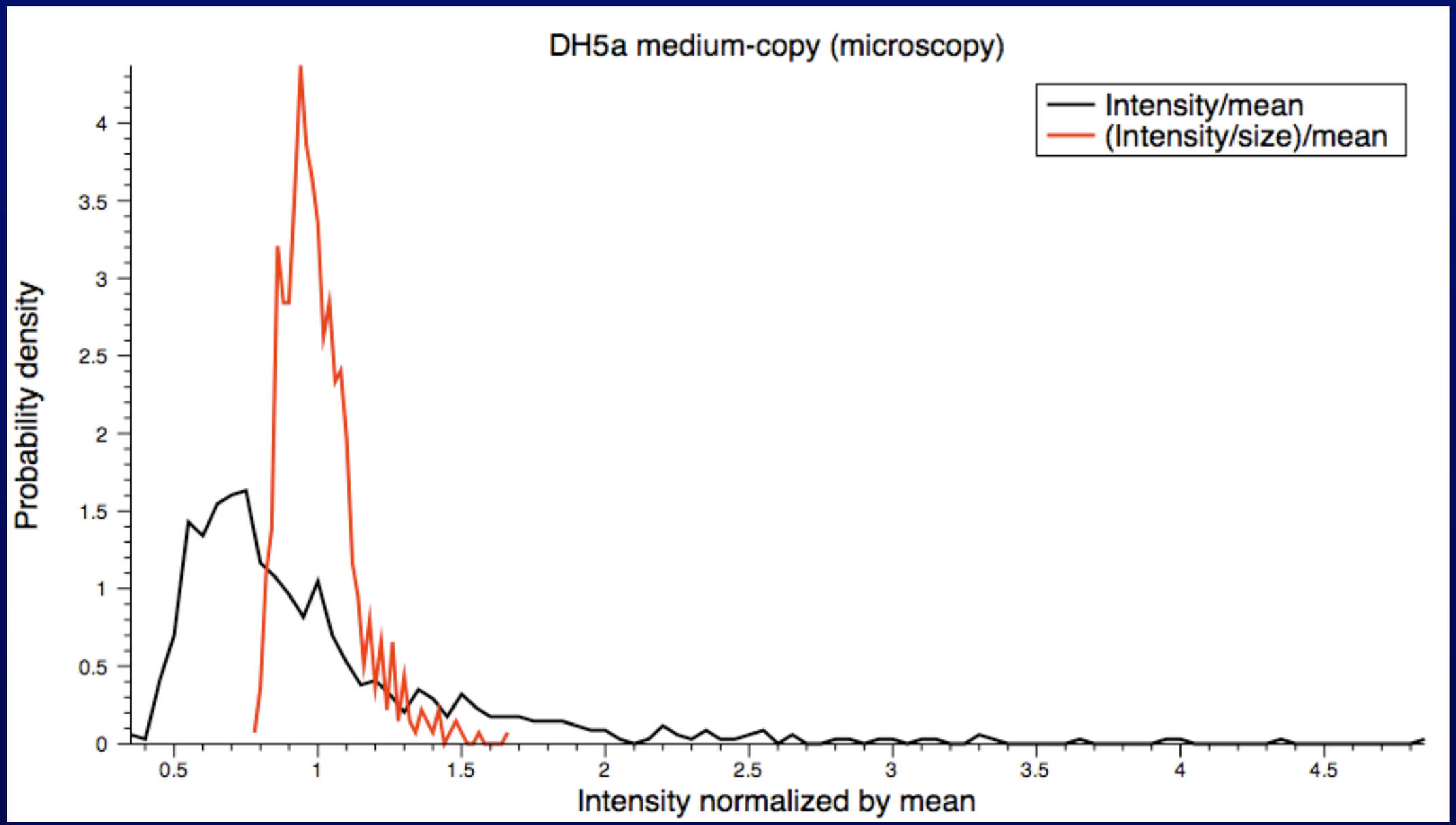
- Simple cell growth picture (V->2V) doesn’t suffice to reproduce actual cell size distributions

Fluorescence vs size



- Strong correlation
- Stronger for medium-copy than high-
- Effect of cell division

Size-scaled histogram



Variability of gene expression

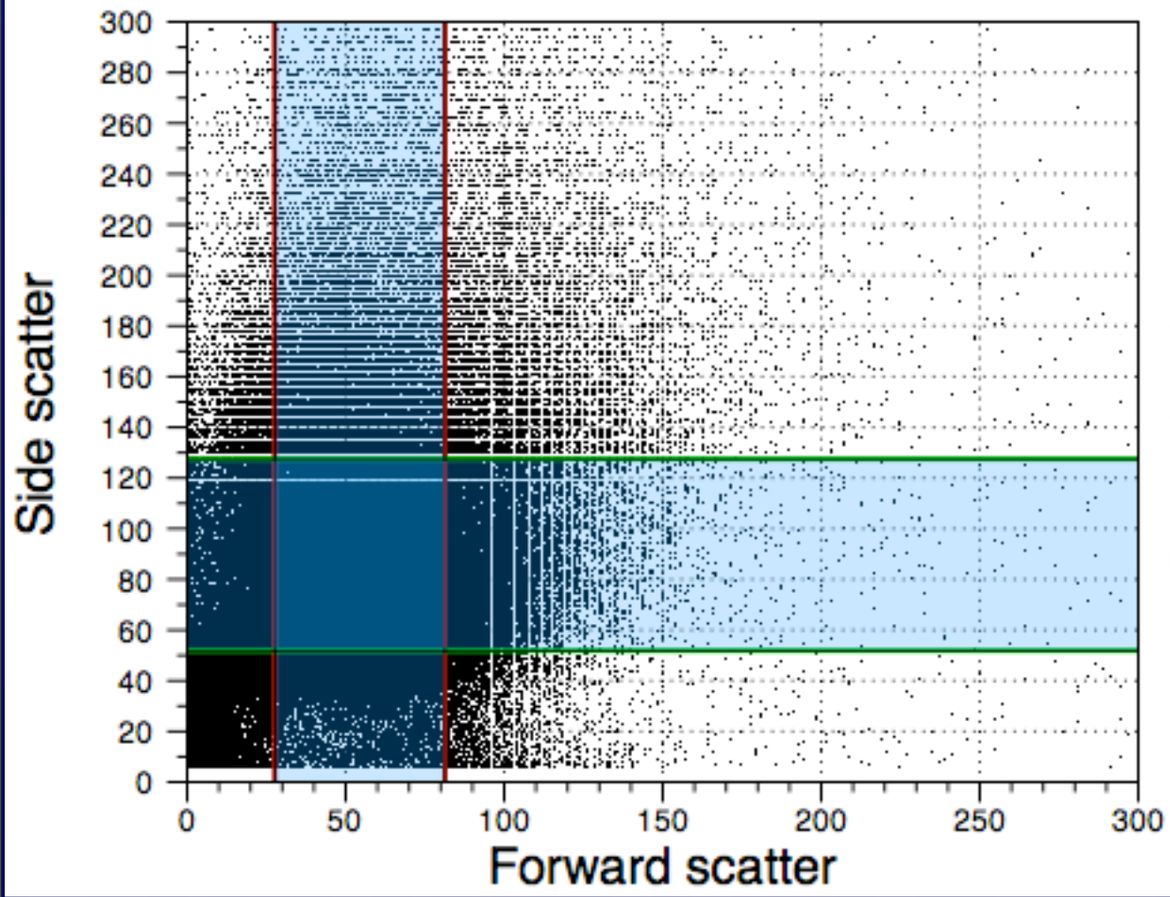
Cell strain	Plasmid	%CV (cytom.)	%CV (micro.)	%CV (micro.) (size scaled)
DH5a	High	55.0	58.4	20.2
	Medium	52.9	56.0	12.6
Top10	High	55.7	76.4	25.0
	Medium	57.7	74.1	12.8
B/r	High	51.5	66.8	24.2
	Medium	58.5	61.0	12.4
BL21*	High	86.0	78.4	29.0
	Medium	75.2	51.7	12.1

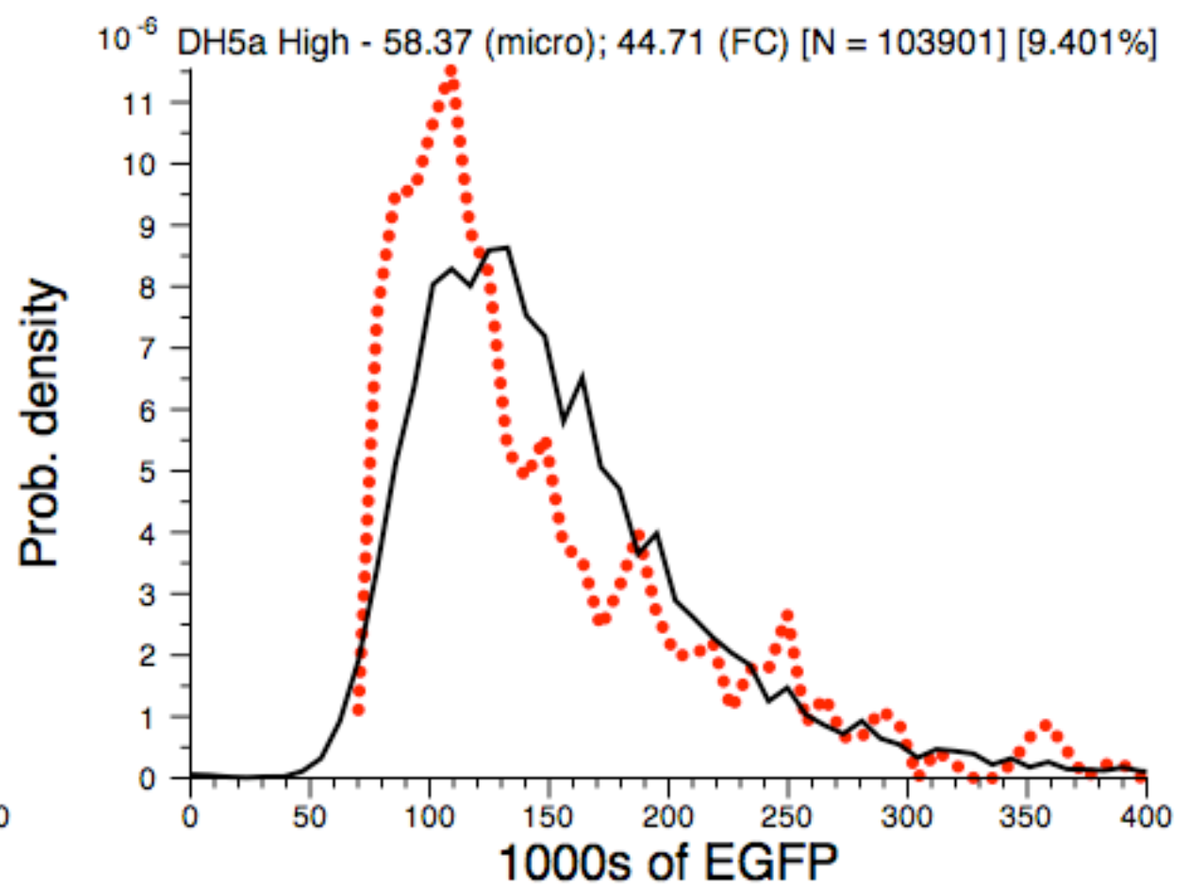
- Substantial drop in CV (std dev/mean) when size is scaled away; note also the consistently lower CVs for the medium plasmid (fewer proteins, but less variable!)

Negative feedback in plasmid copy number

- High-copy plasmid (pUC ORI) replicates as fast as it can, constrained only by resources
- Medium-copy (ColE1 ORI) plasmid incorporates negative feedback: interferes with its own replication
- Lower size-scaled CVs for the medium-copy plasmid suggest that the negative feedback keeps copy number less variable, and that shows up in protein CV

Size from cytometry?





- Restricting forward/side scatter region does not have same size-scaling effect as in microscopy

Movie done in DataTank (Visual Data Tools)

“Dark proteins” I

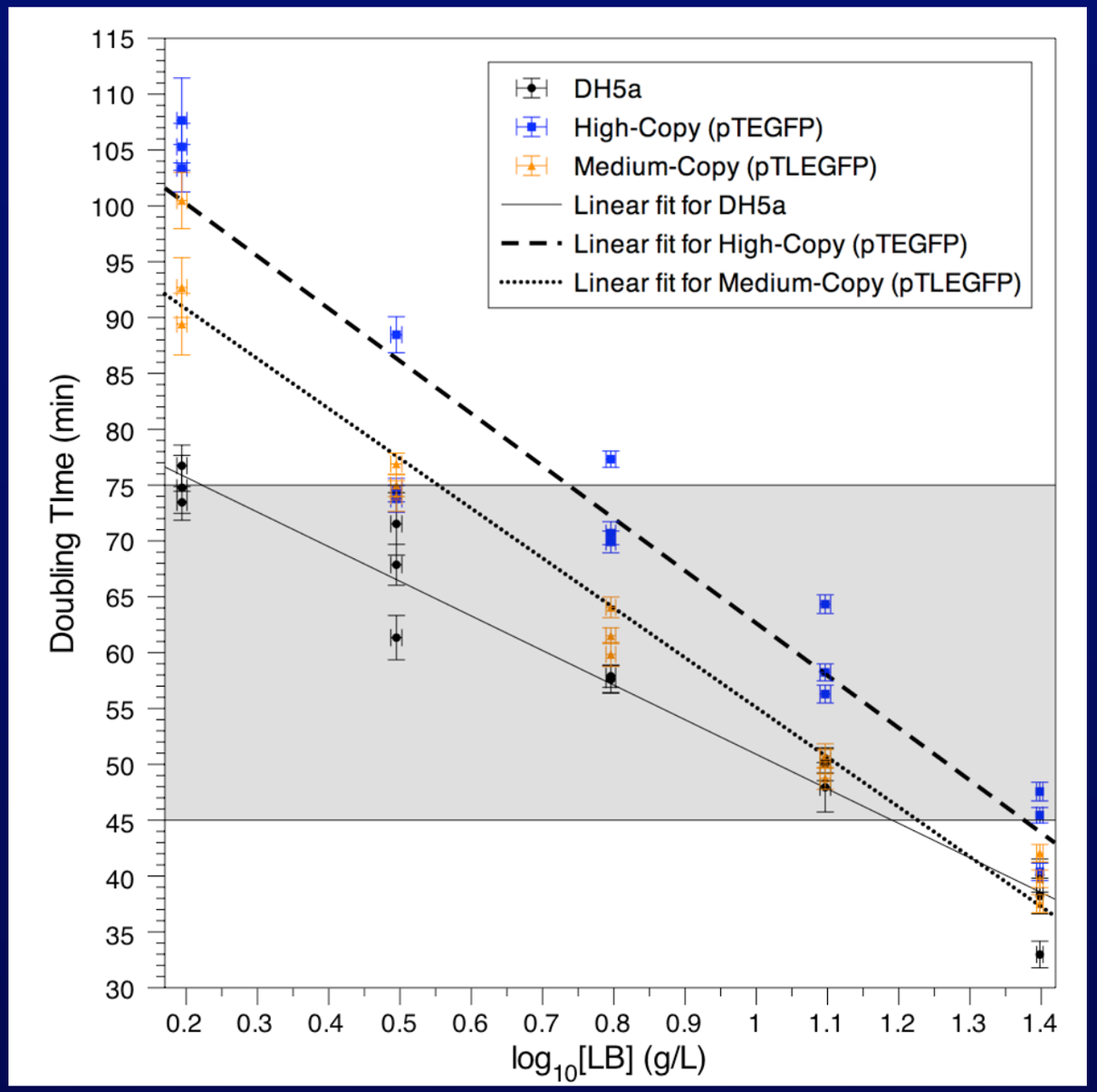
- A problem affecting all studies using fluorescence as a measure of gene expression:
  - You can (of course) only see the proteins that have become fluorescent
- Proteins may be invisible (“dark”):
  - If they are misfolded
  - If they have yet to mature

Inclusion body formation

- Many studies (including ours) use plasmid-based expression
  - Useful as a means of inserting tailored gene networks, to study gene dynamics or as control mechanisms
- High expression levels from plasmids can lead to formation of inclusion bodies
  - Insoluble aggregates of misfolded, non-functional proteins
  - Fluorescent proteins caught in these bodies will not fluoresce

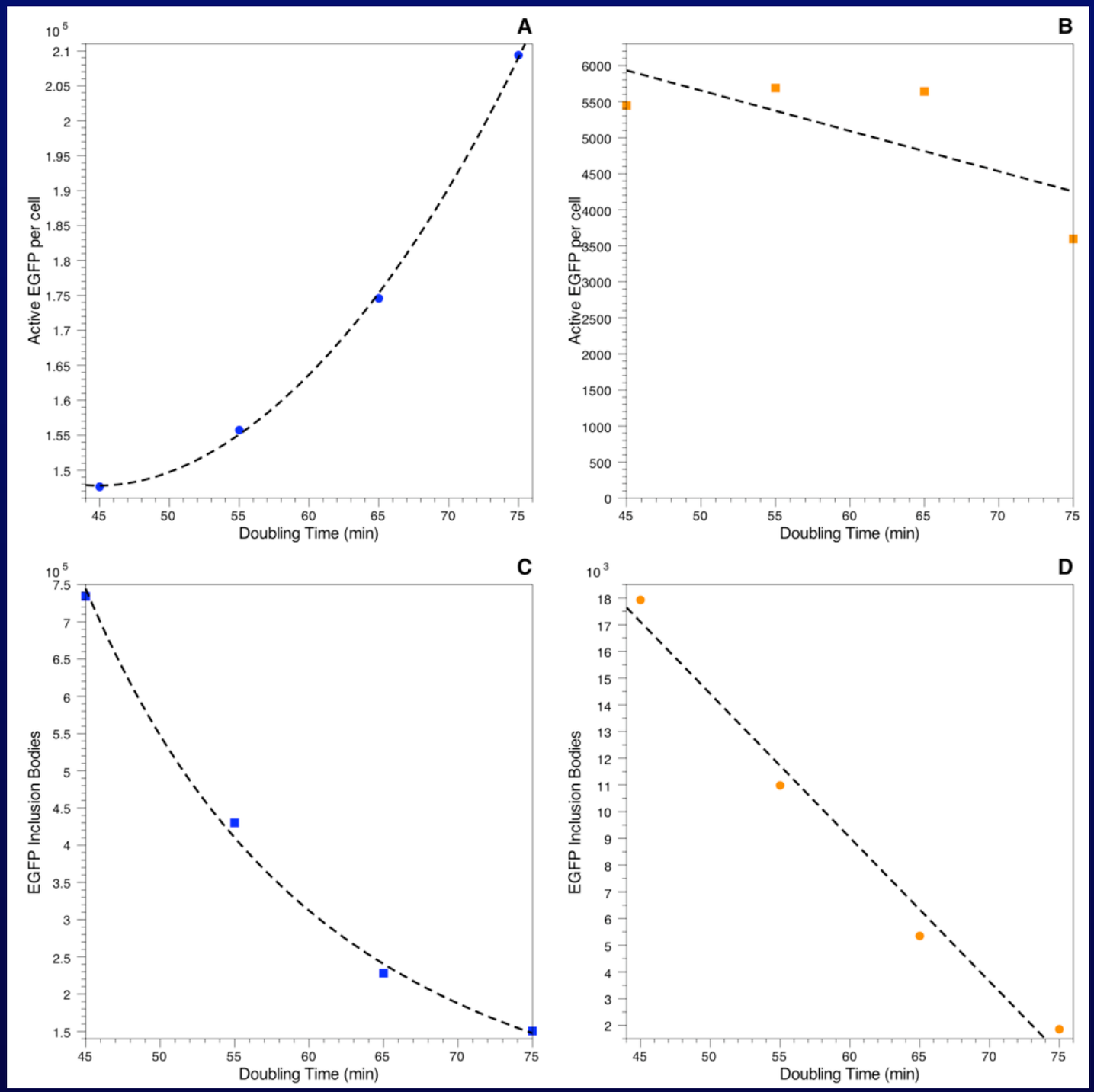
Marco Iafolla

Varying growth rates



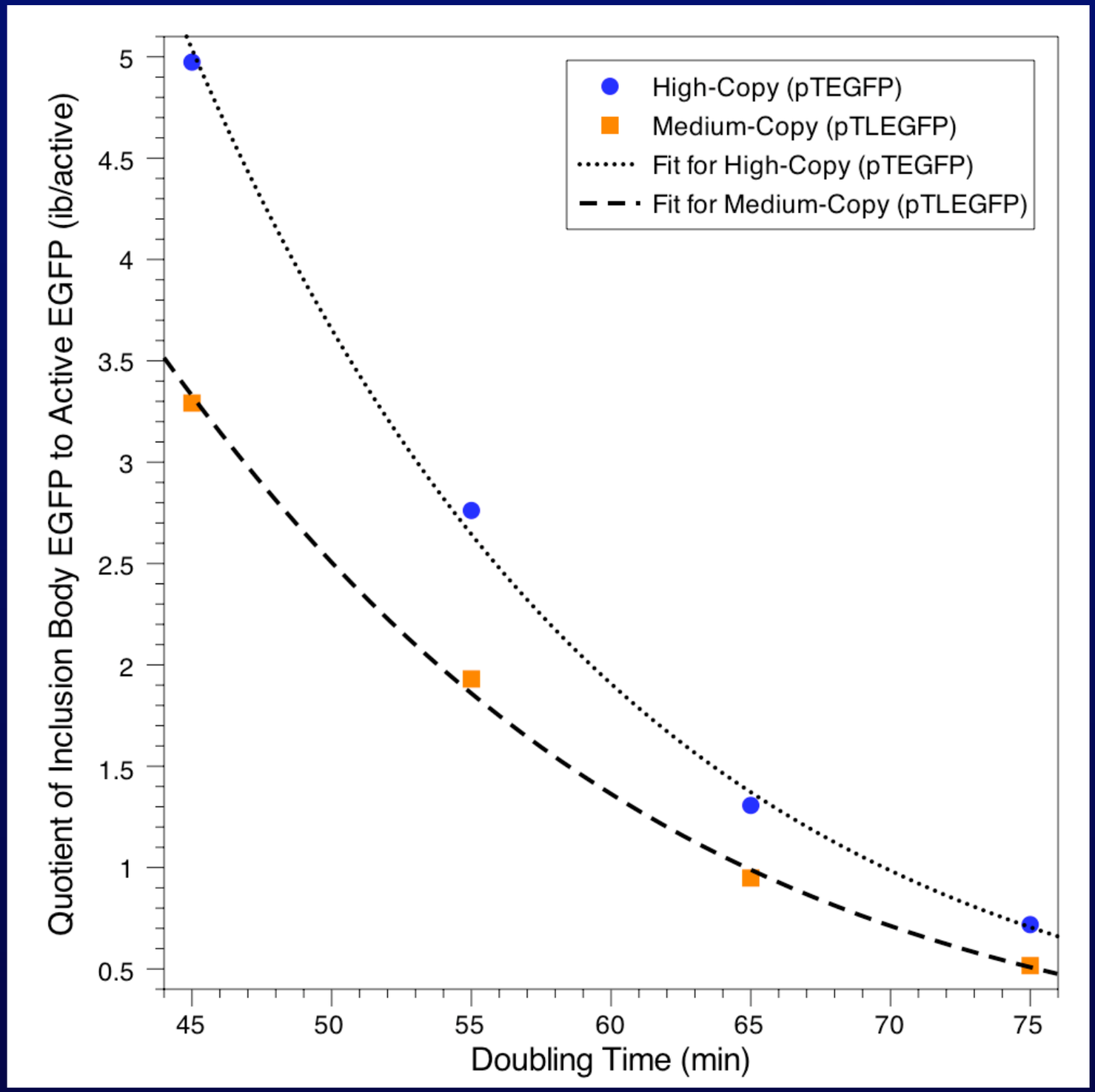
- Bremer and Dennis (1996): a very handy paper with many cellular parameters - all vary with growth rate

Extraction/quantification of inclusion bodies



Marco Iafolla

Ratios of IB to active EGFP

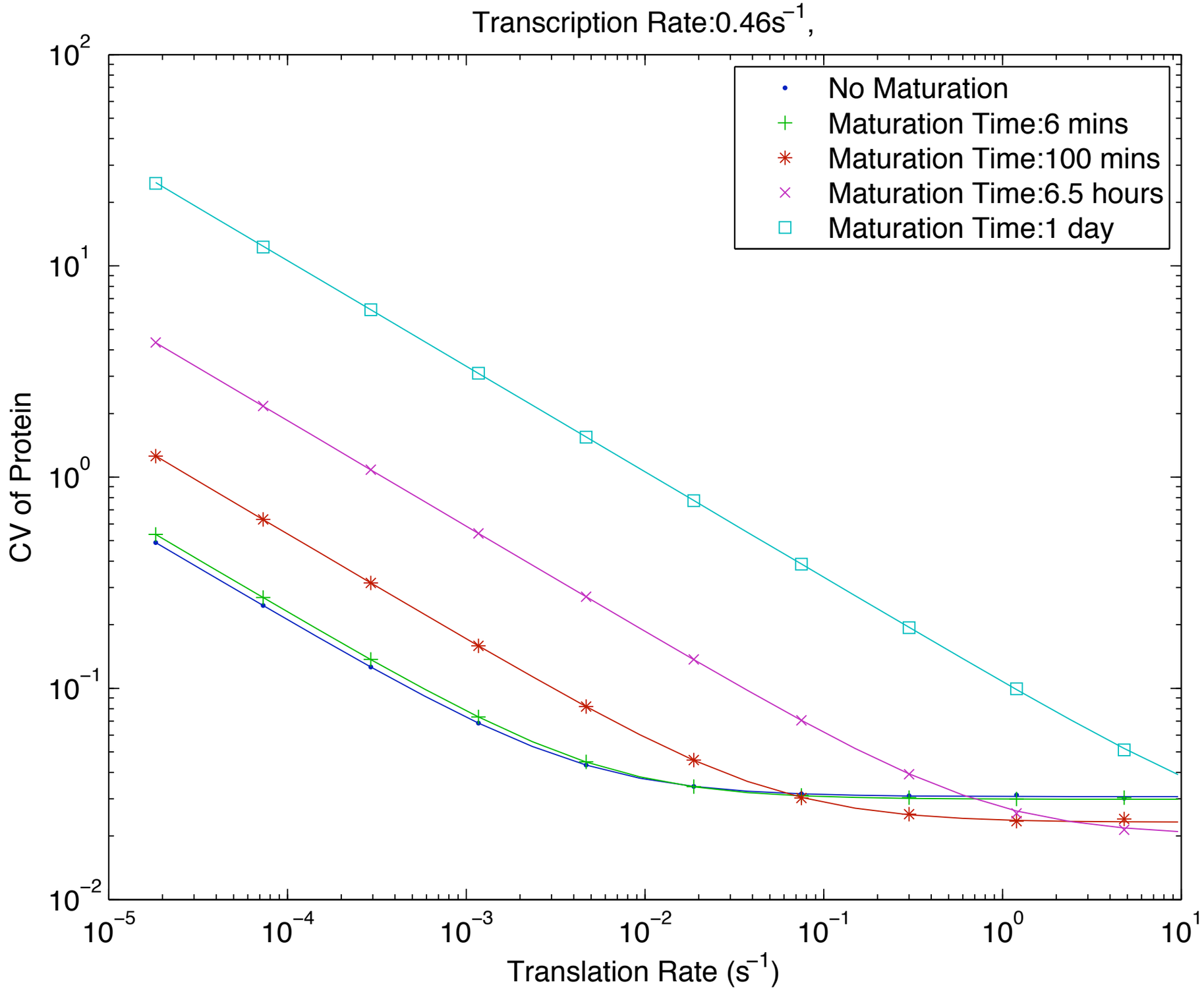


“Dark proteins” II: The Return

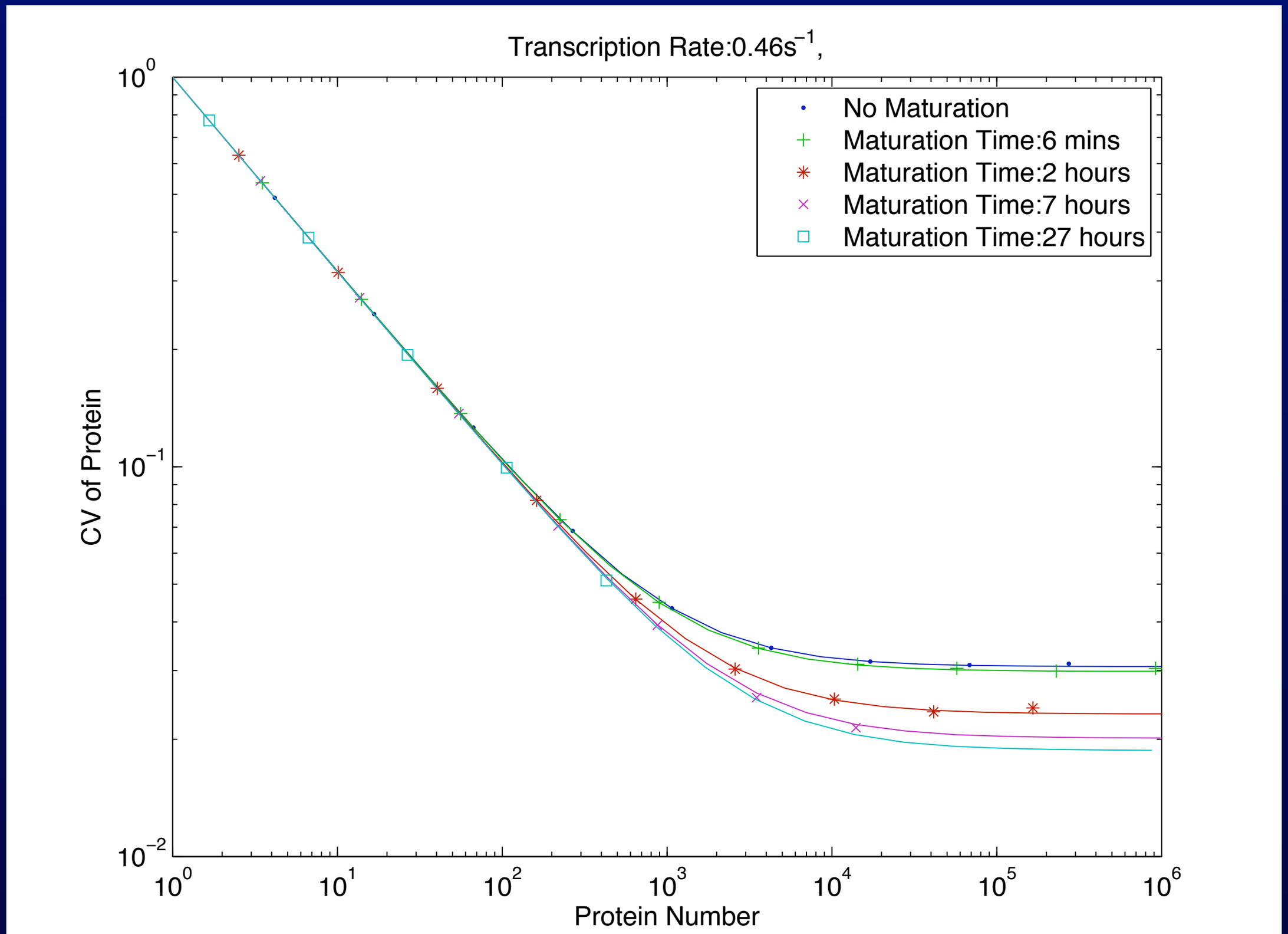
- Proteins are also “dark” in the transient period before they mature and start fluorescing
  - Folding, cyclization, oxidation
  - Maturation rates vary from minutes to days
- Modelling work:
  - At small numbers of proteins expressed, maturation effect increases observed variability (just by reducing numbers)
  - At large numbers, maturation can actually decrease observed variability

C. Guangqiang Dong

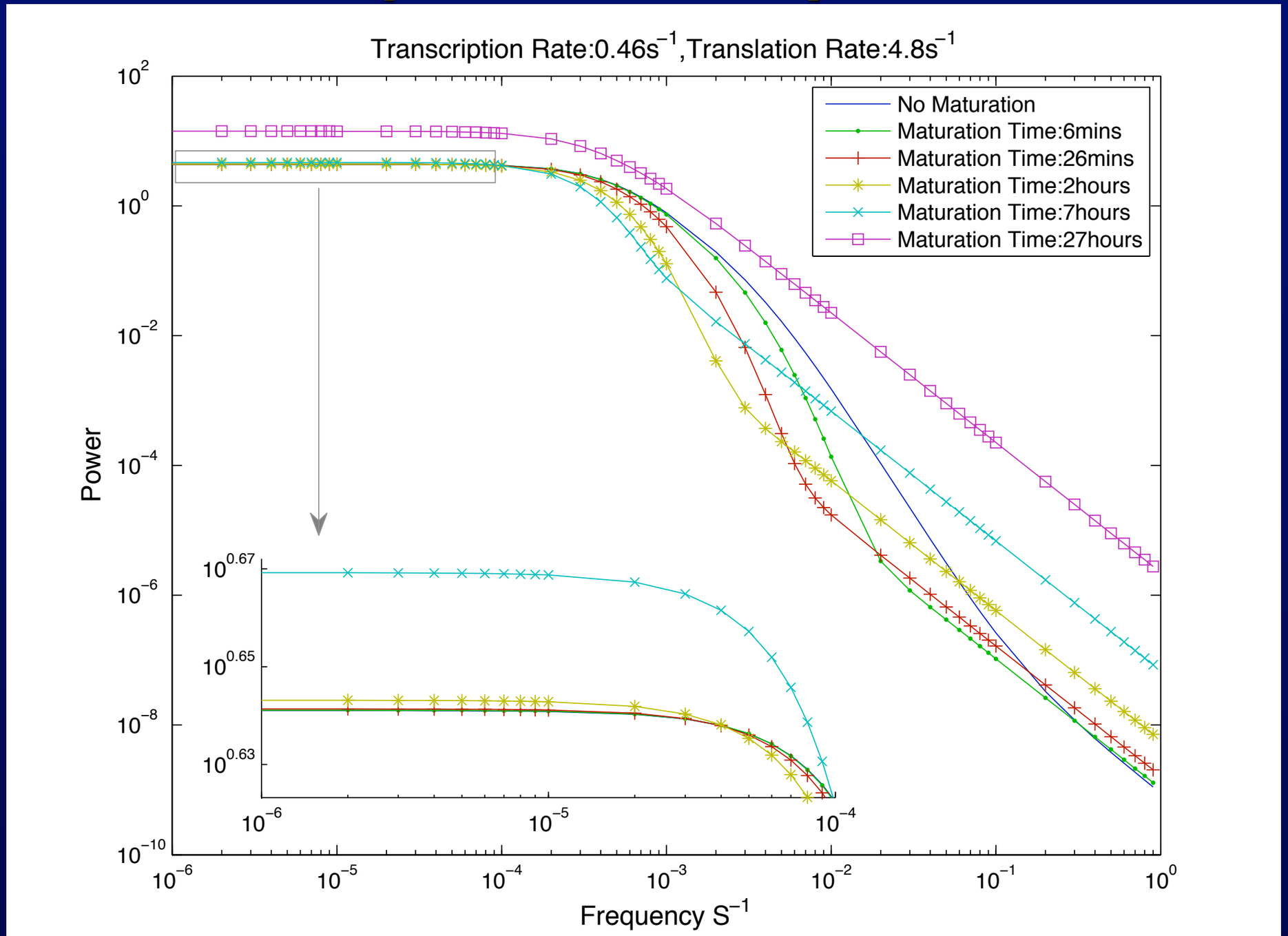
Protein maturation



CV vs <number>


Transcription Rate:0.46s^{-1},
No Maturation
Maturation Time:6 mins
Maturation Time:2 hours
Maturation Time:7 hours
Maturation Time:27 hours
CV of Protein
Protein Number
10^{0}
10^{-1}
10^{-2}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}

Noise power spectrum



- Coming soon: experiments! Vary: maturation rate (proteins), transcription rate (promoters), translation rate (RBS) (Sangram Bagh, C. Guangqiang Dong)

4. Cells are “individuals”

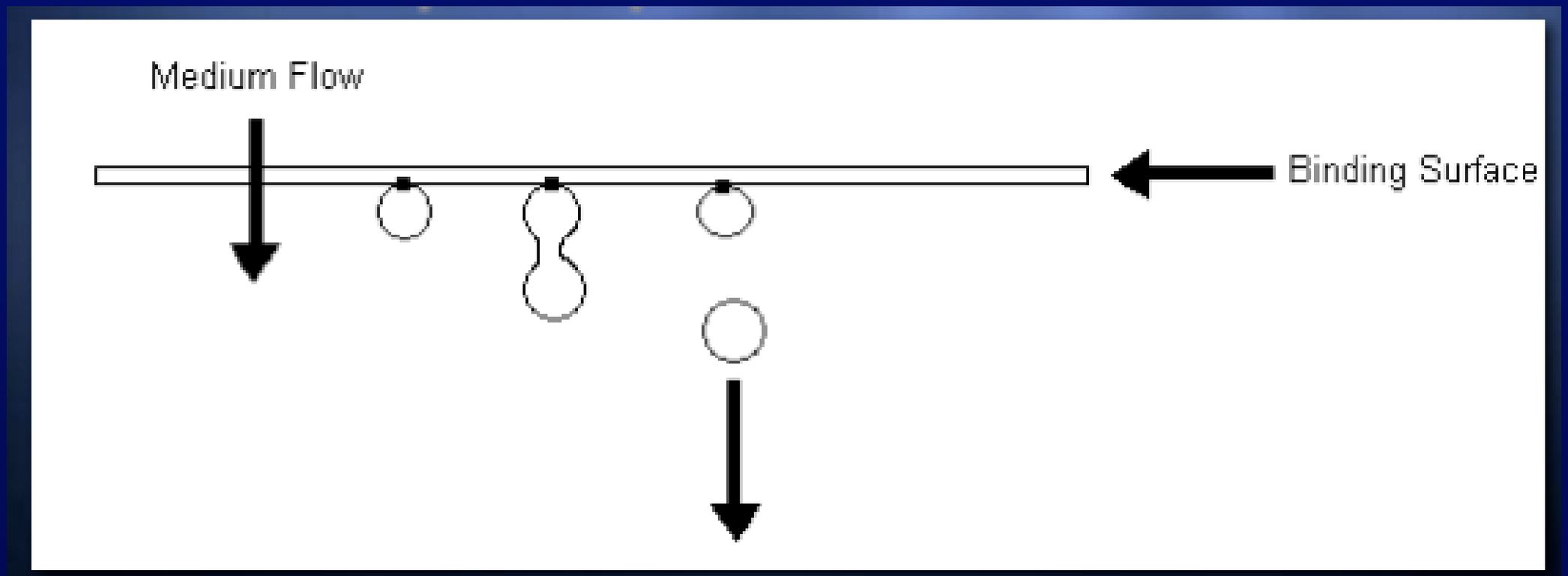
- Cells even in an apparently homogeneous population can have varying individual histories (growth/ division events, nutrient exposure, cumulative effects of fluctuations)
  - Different histories lead to different states
  - Plasmid copy number and cell size: can be viewed as extrinsic or intrinsic noise sources
- We’re interested in the effects of various perturbations on cells’ behaviour

Changing growth conditions

- Using a "baby machine" to examine synchronized cells
  - Early results: synchronized cells (all in nearly same phase of "cell cycle") are less variable than asynchronous cells
  - More than just a size effect: reduction is in the size-scaled variability levels
- Investigating effect of varying nutrient levels by growing cells in a chemostat
  - Early results: chemostat ("continuous culture") cells are more variable than exponentially growing cultures

Luke Jakobowski

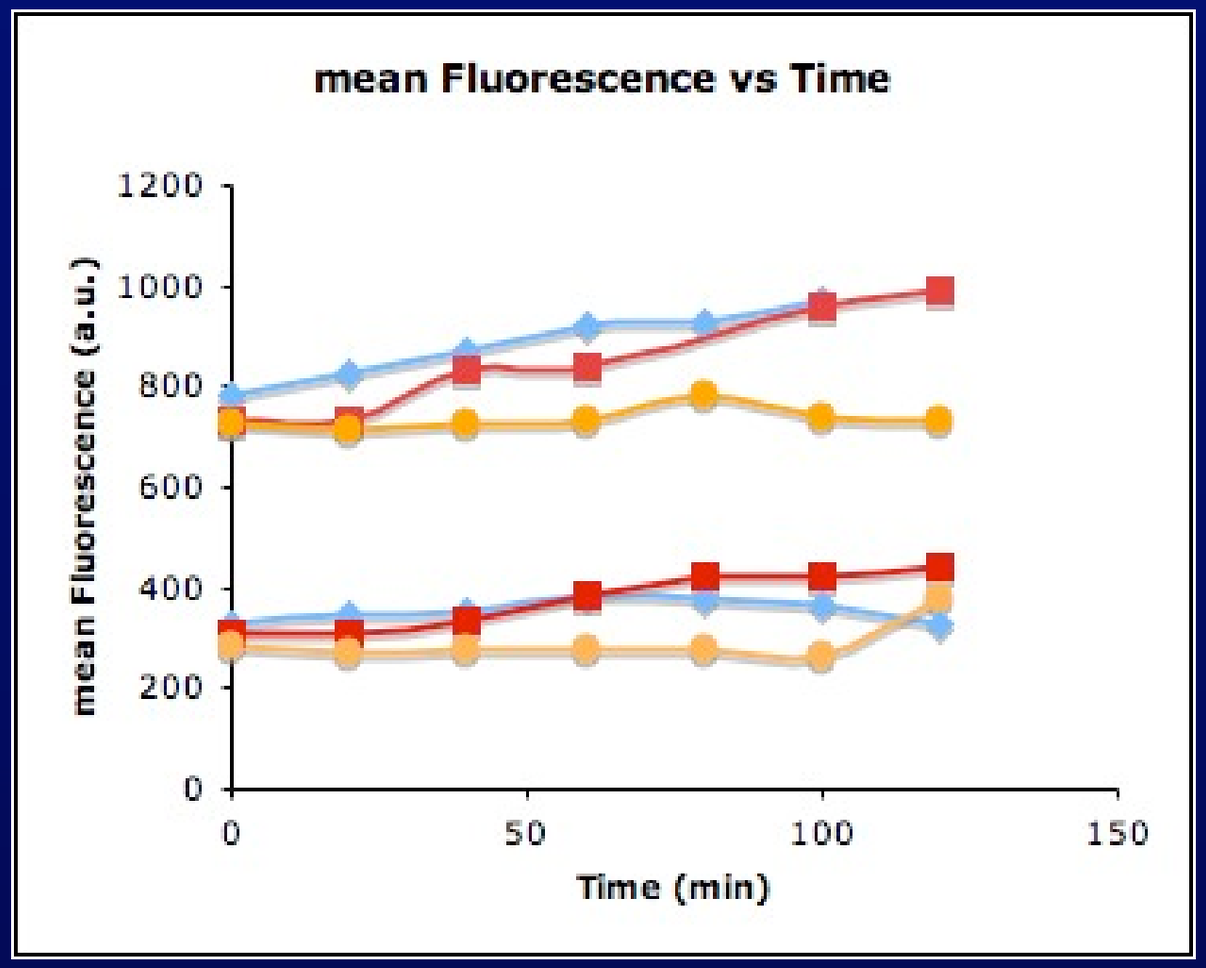
The baby machine



Perturbation by cell sorting

- Cell sorting: can use the flow cytometer to select cells based on any optical output, divert desired cells into a tube
- We’re using this to sort out subpopulations based on brightness (related to EGFP expression level)
  - Observe the distribution of the perturbed population over time, see how (or if) it relaxes back to original distribution

Early cell-sorting results



- Sorted top 10% brightest cells
- Population stays bright over 3 hours
- Grow overnight: back to original dist.(?)

Tharsan Velauthapillai, Mostafizur Mazumder

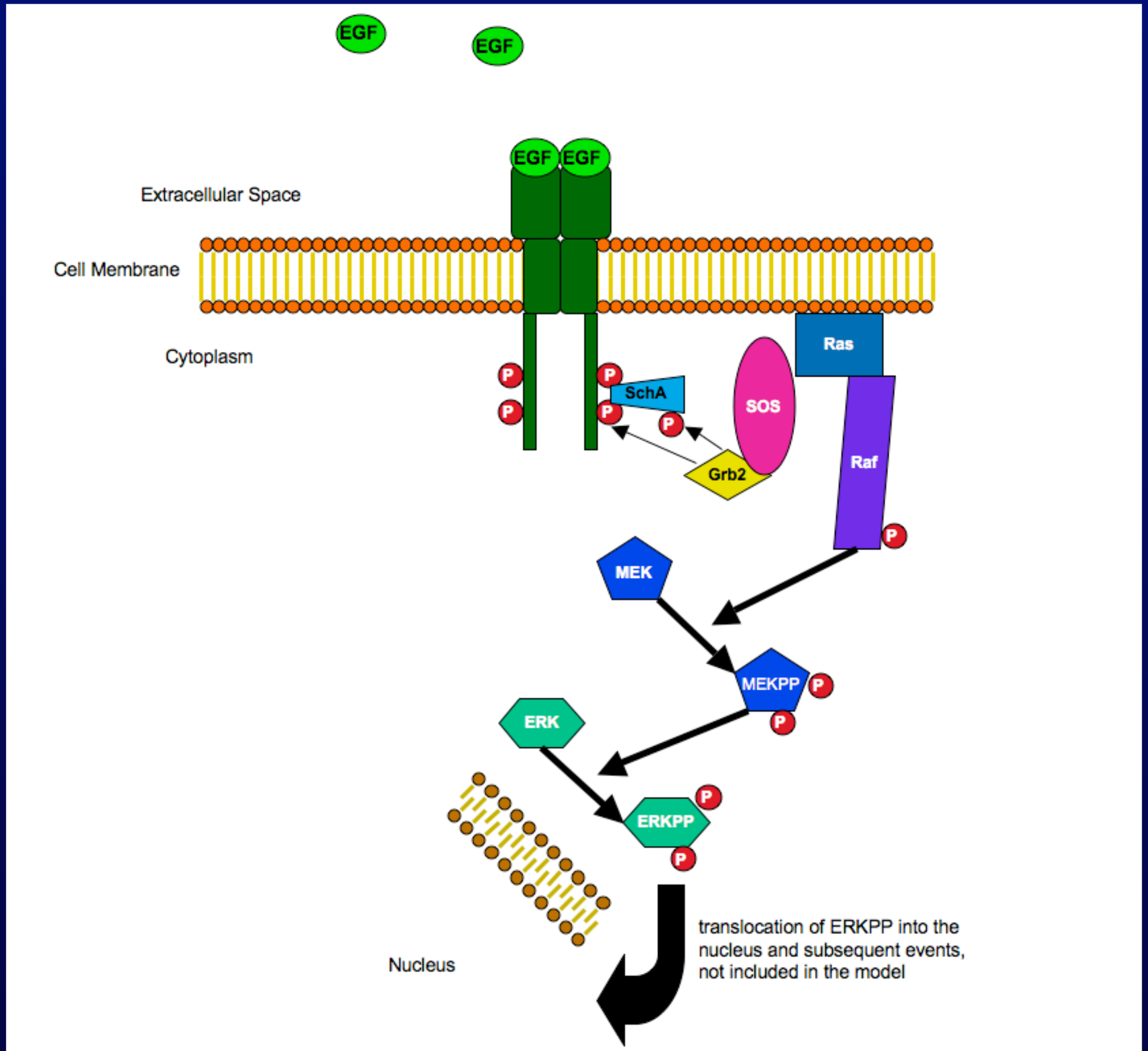
5. Cells are complicated

- Many species and reactions are involved in even a moderately complete description of any biological system
  - Complete model may require dozens (or literally millions!)
- Model reduction methods have been explored for deterministic ODEs
  - Based on time scales, quasi-steady states...
- We're working on methods that can be applied to stochastic systems as well

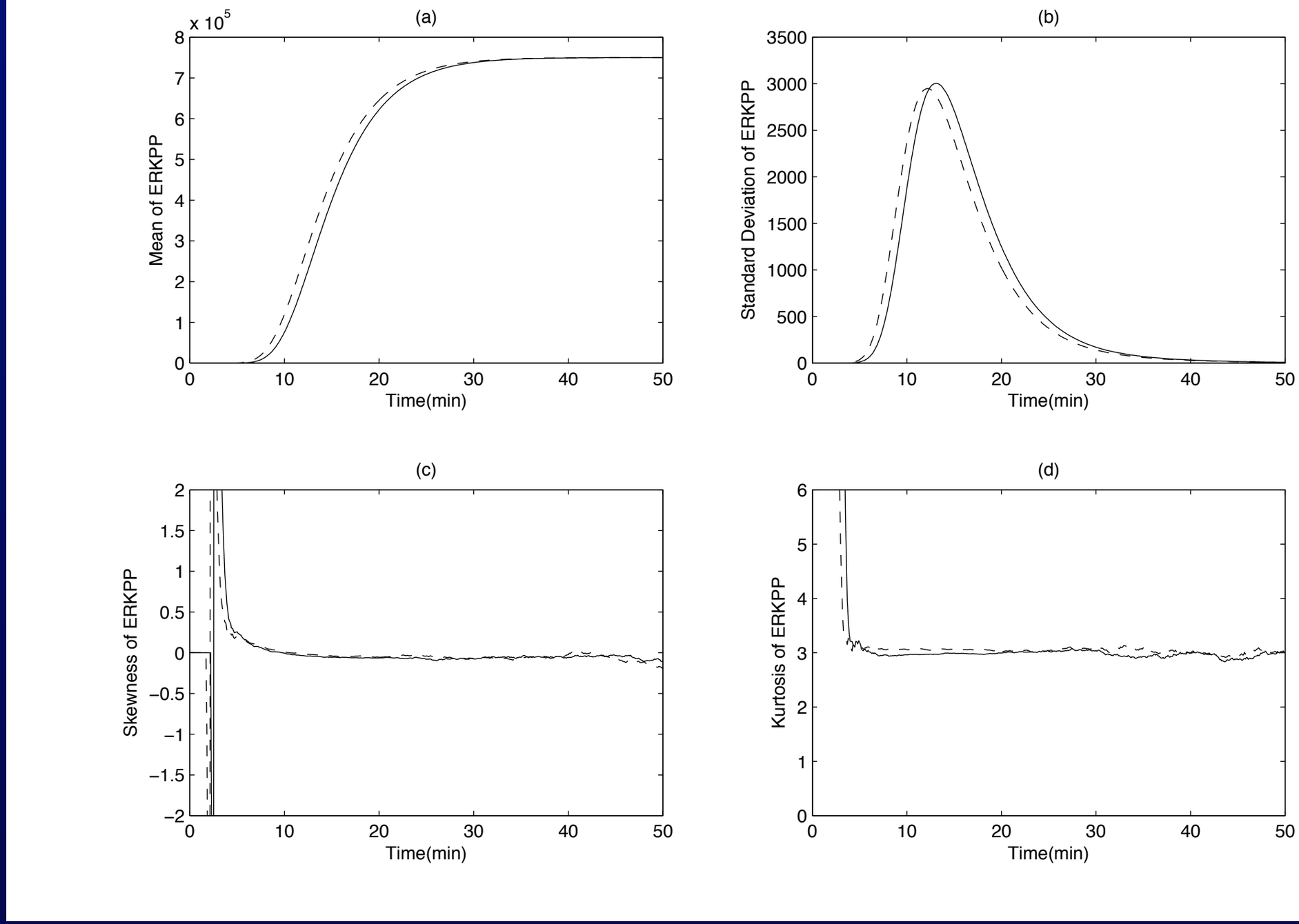
Stochastic model reduction

- Use a known deterministic ODE reduction method:
  - Partition reactions into fast/slow
  - Form reduced system by approximating fast steps as near-instantaneous
- Translate reduced system back into a reaction scheme
- Use stochastic simulator to run it, generate fluctuations
- Result: highly reduced systems still match statistical behaviour of original

Signal cascade model



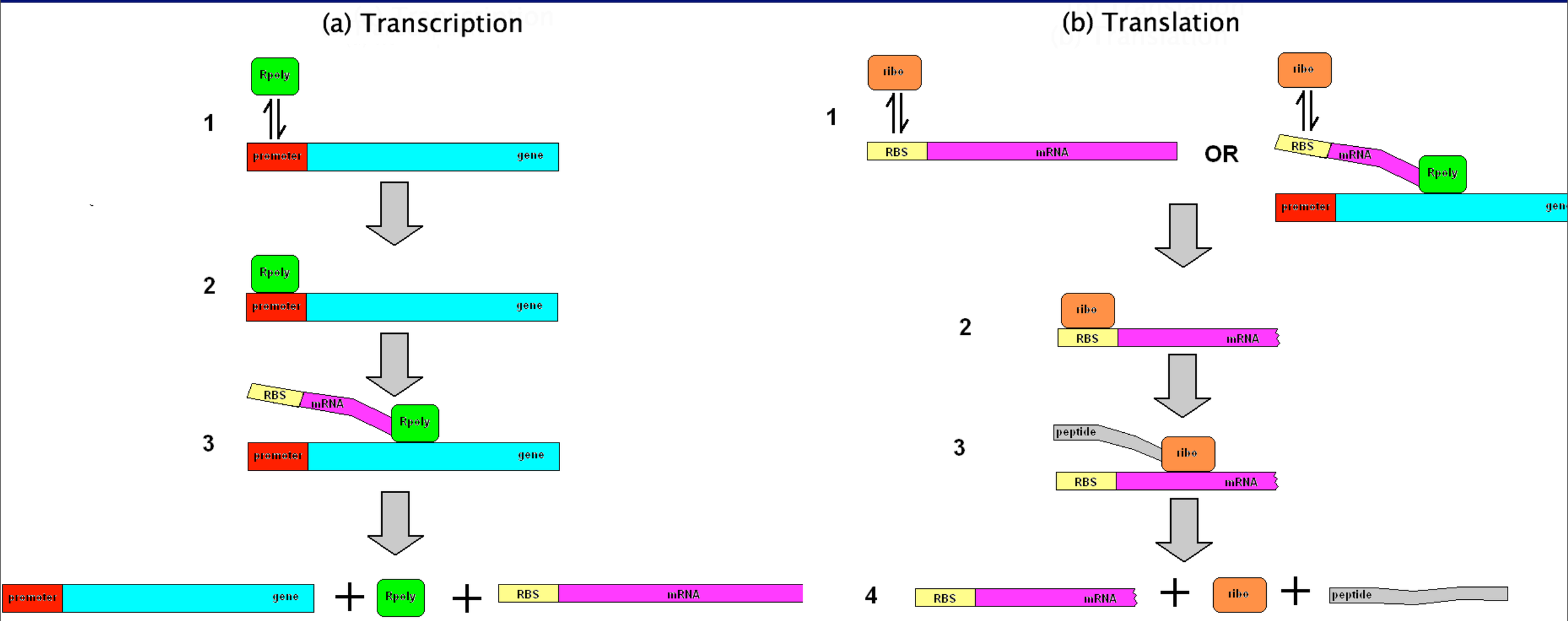
Reduced vs original



- Original: 63 reactions, 41 species
- Reduced: 34 reactions, 27 species
- Dong et al, *J Biol Phys* **32**: 173 (2006)

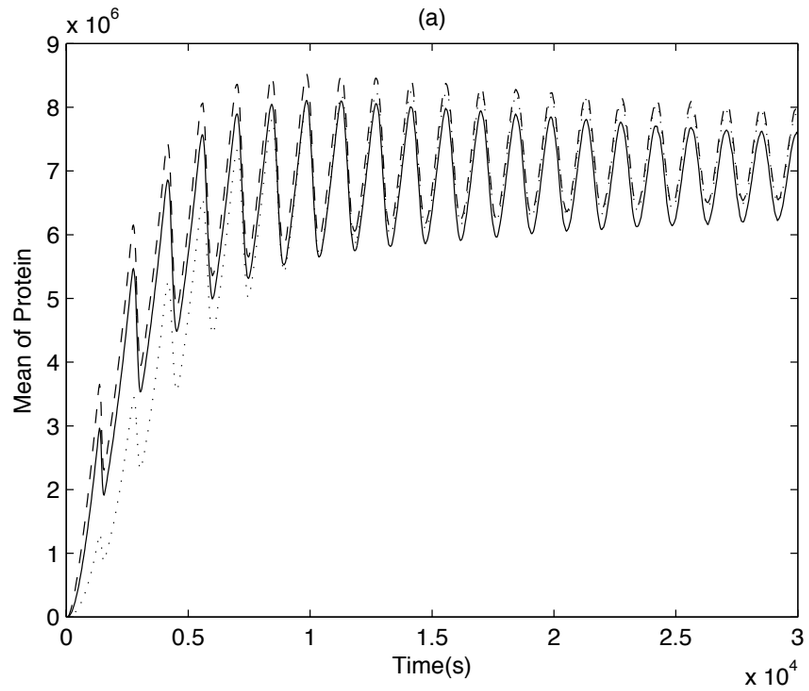
C. Guangqiang Dong

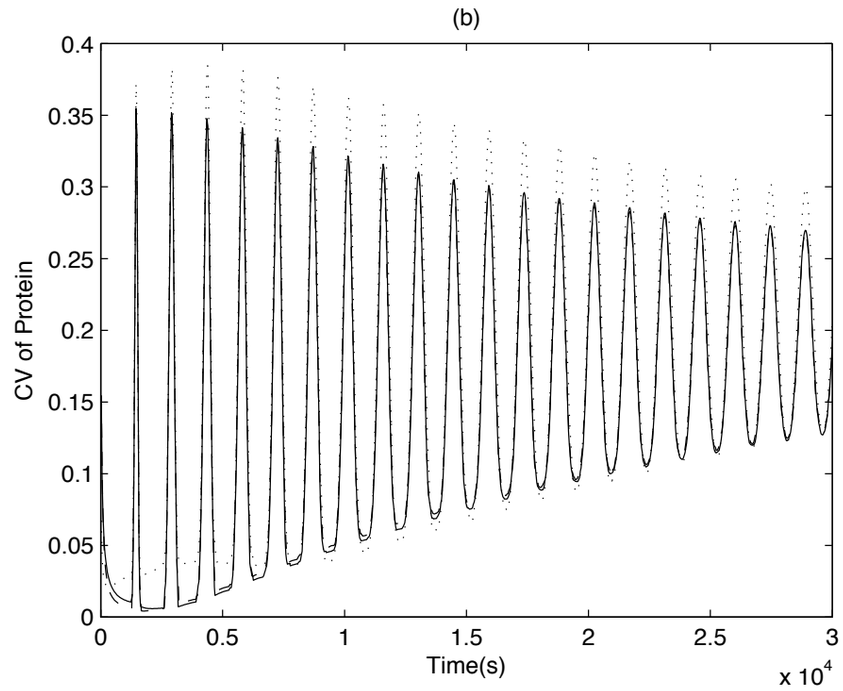
Gene expression model

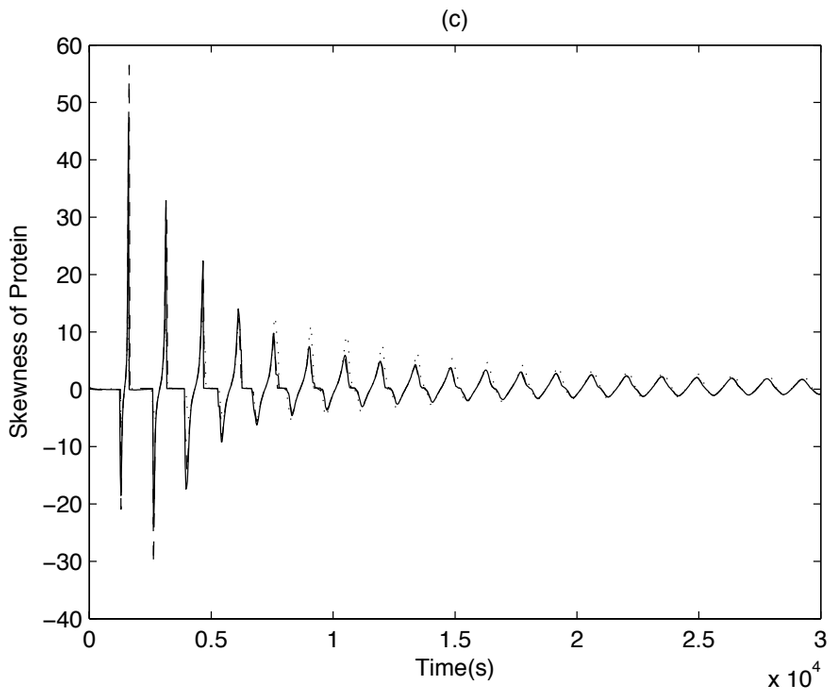


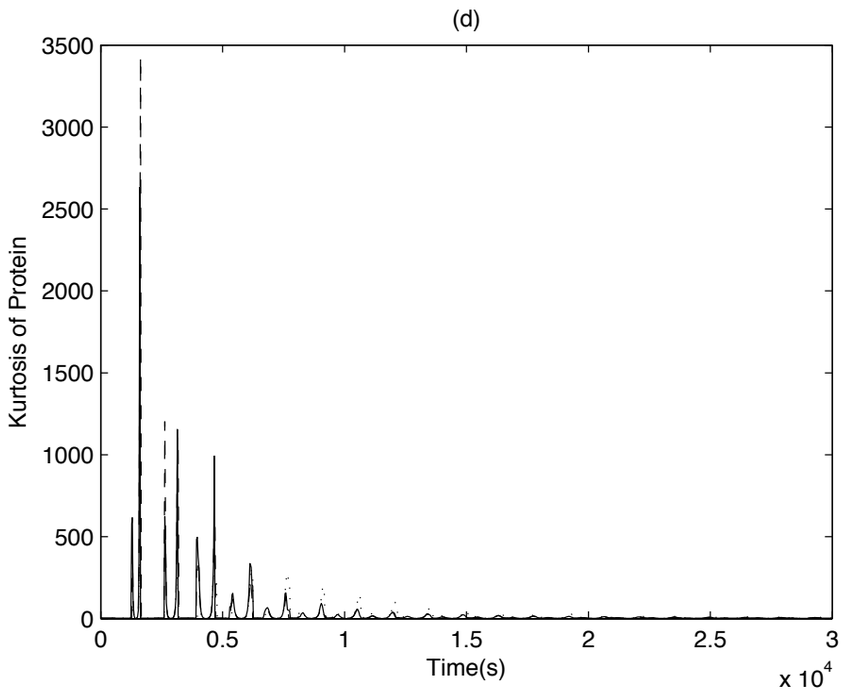
- Original: 71 reactions, 47 species [Iafolla and McMillen, *J Phys Chem-B* **110**: 22019 (2006)]
- Reduced to two different levels:
  - 28 reactions, 29 species
  - 10 reactions, 10 species

Reduced vs original









Caveats

- Works near-perfectly for cascades (feed-forward)
- Still works well for "moderate" feedback
  - But as you crank up feedback strength enough, will eventually break down
- Method can fail with an non-invertible matrix in one of the steps
  - So far, we can't precisely identify the conditions for this to occur
- May need to adjust parameters to achieve an optimal fit

6. Cells are crowded

- Interior of a cell is far from being a dilute solution; more like a paste/gel
- Standard modelling methods tend to assume a well-mixed system
  - Don't keep track of locations of individual molecules, just total concentration or #
  - Crowded environment can have impact on kinetics, and on variability
- We're working on experiments in vitro, to vary crowding level and observe effects
  - Collaboration with Ray Kapral (elegant, fast method of simulating spatial behaviour)

Issues with biochemical kinetic models

- Sometimes I ponder the following two facts:
- Fact #1: Biochemical kinetic models yield useful results
  - Predictive abilities have been demonstrated
- Fact #2: Biochemical kinetics seemingly cannot be *right*, as usually written

Conclusions

- I have no Conclusions
- But some things to note:
  - Biology is interesting, even for physical scientists and mathematicians
  - It's a huge challenge: everything in biology is the least-tractable example of its class
    - Nonlinear, stochastic, far from equilibrium, nonideal, messy, actively uncooperative
  - Fortunately, we tend to love a challenge
  - There's something to be said for working closely with living cells: the daily weirdness tends to promote humility

Acknowledgements

- Funding:
  - NSERC Discovery grant
  - CFI New Opportunities grant (shiny new cell-sorting flow cytometer, baby!)
- People:
  - Christopher McCulloch and Wilson Lee, UofT Dentistry (initial sorter access)
  - The Quantitative Biology Lab students, who do all the real work

Turn back! Extra slides past this point

Expression noise

- Gene expression noise is parcelled into two sources:
  - Intrinsic: from small-number effects on the gene itself
  - Extrinsic: everything else (cell-to-cell variation in components outside gene, e.g. numbers of the enzymes that drive transcription/translation)
- Extrinsic noise: individual cellular environments cause different behaviour
  - Different histories lead to different states
  - Plasmid copy number and cell size: can be viewed as extrinsic or intrinsic noise sources