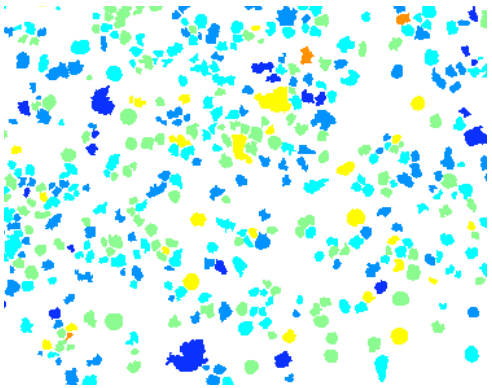
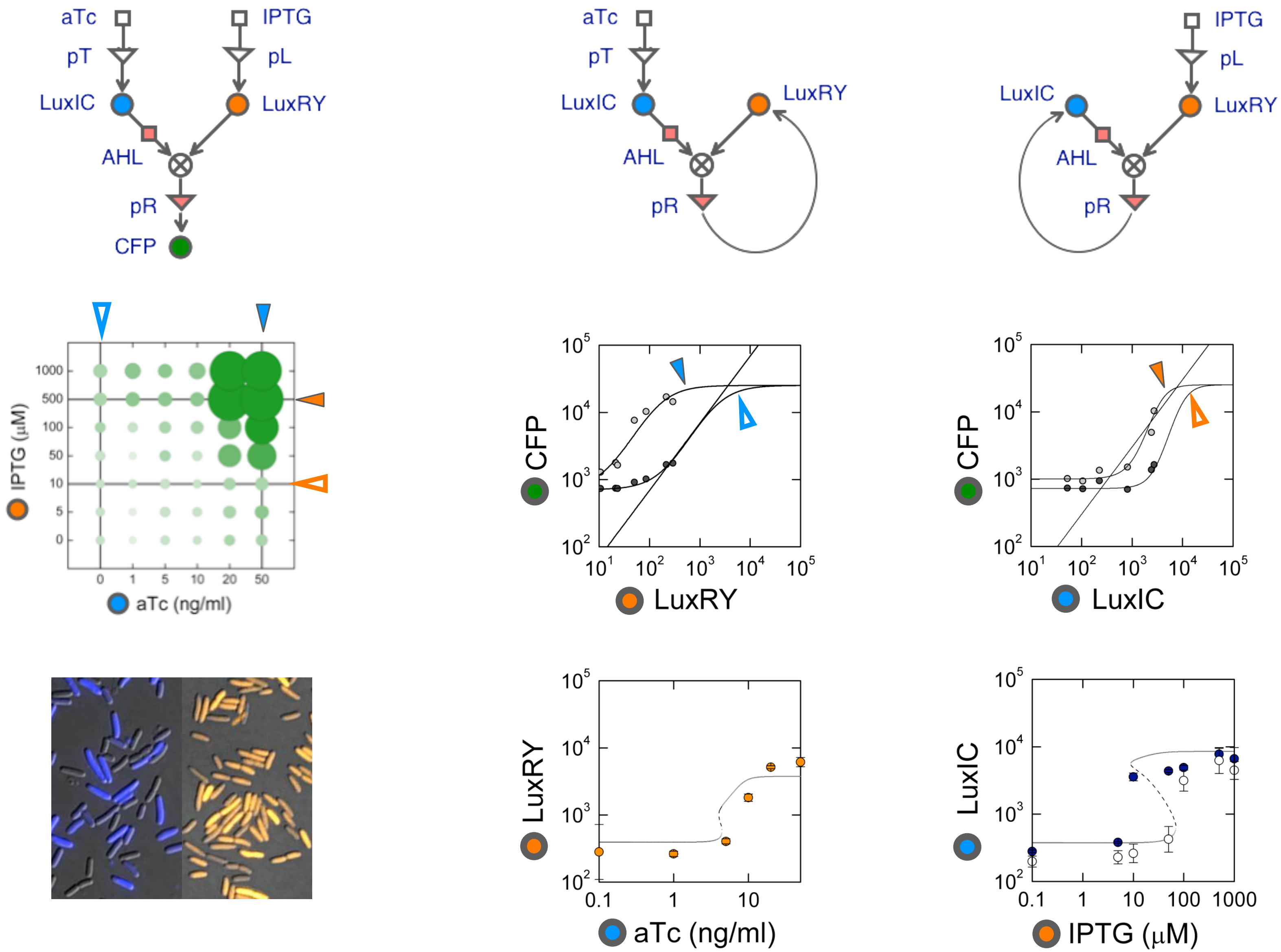
The origins, consequences, and uses of cell-to-cell variability



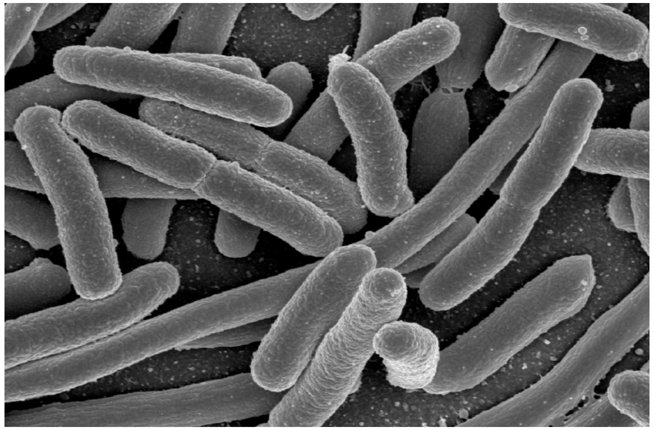
Mukund Thattai
National Centre for Biological Sciences,
Tata Institute of Fundamental Research

ICM Satellite Conference
August 2010

What I'm <u>not</u> going to talk about... open loops and closed loops in bacteria



Variability is a fundamental property of living matter at molecular scales...



All life is made of cells

Cells are small

Small means noisy

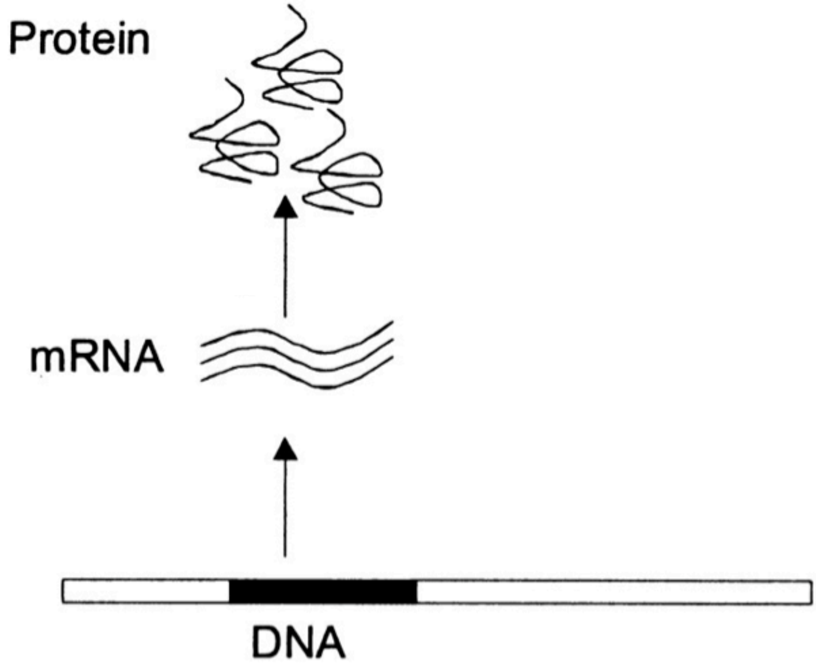
... but macroscopic outcomes are precise

Cells contain low numbers of macromolecules

~ 10 - 1000

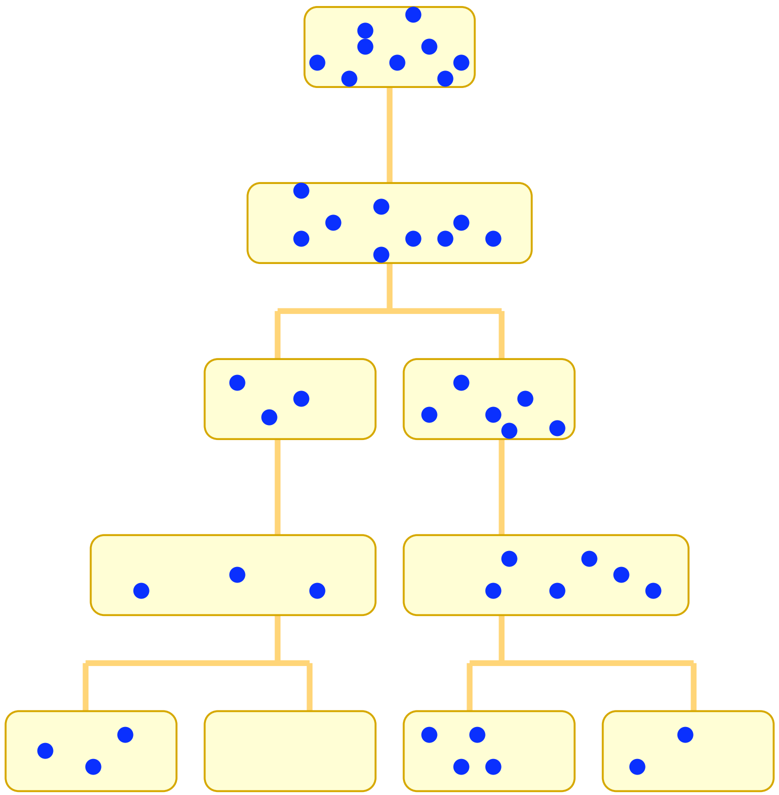
~ 10 - 100

~ 1

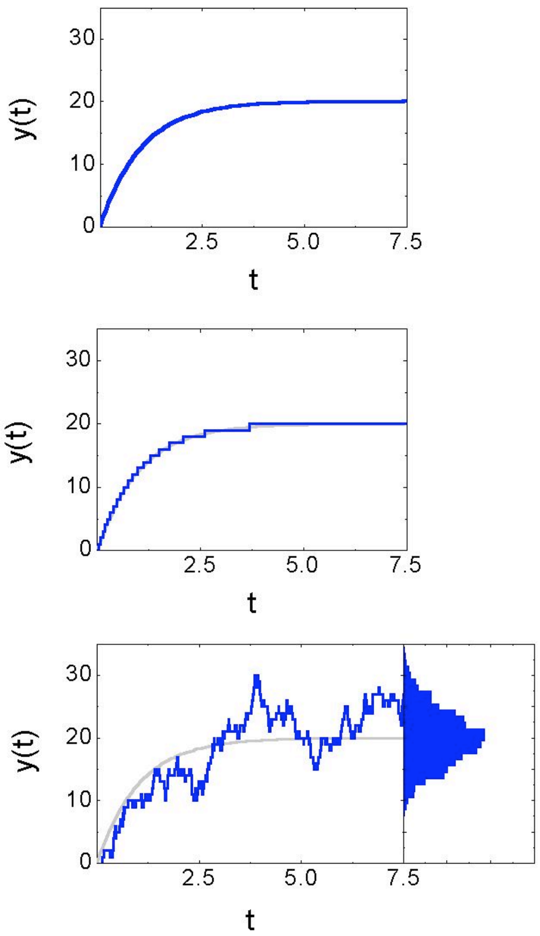


“Noise” arises from the discreteness of molecules and reactions

dilution



synthesis

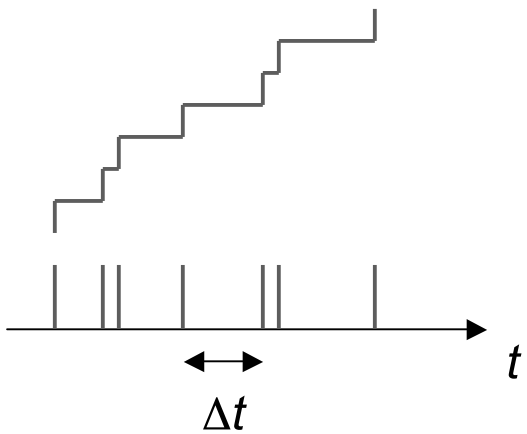


continuous

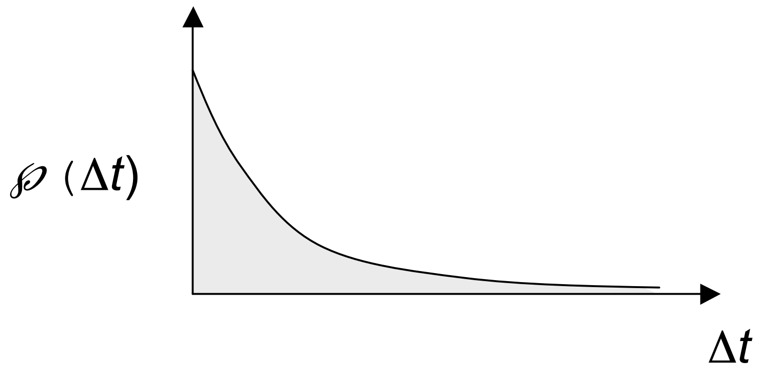
discrete, metronome

discrete, ‘popcorn’

What chemistry actually looks like



The rate *k* is the probability of a single event per unit time



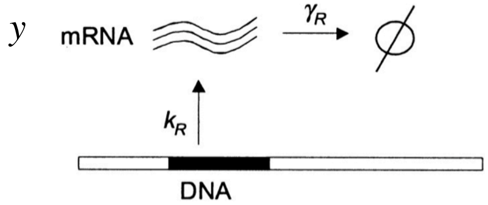
inter-event times are exponentially distributed...

$$\wp(\Delta t) = k e^{-k\Delta t}$$

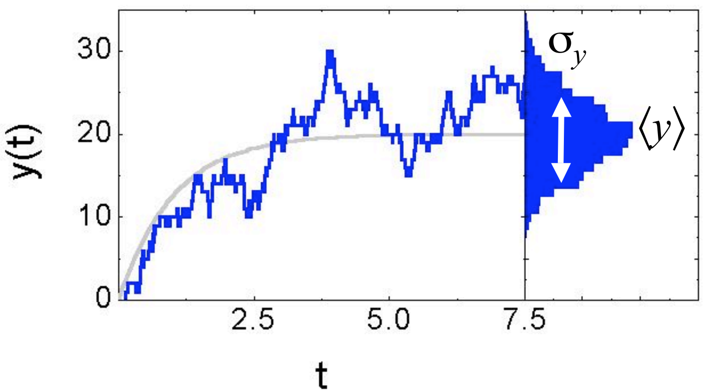
...and can be simulated using a uniform random variable

$$\Delta t = \frac{1}{k}\ln\left(\frac{1}{u}\right)$$

mRNA: the Poisson distribution



$$\wp_y = \frac{\bar{y}^y}{y!} e^{-\bar{y}} \qquad \frac{\sigma_y^2}{\langle y \rangle^2} = \frac{1}{\langle y \rangle}$$

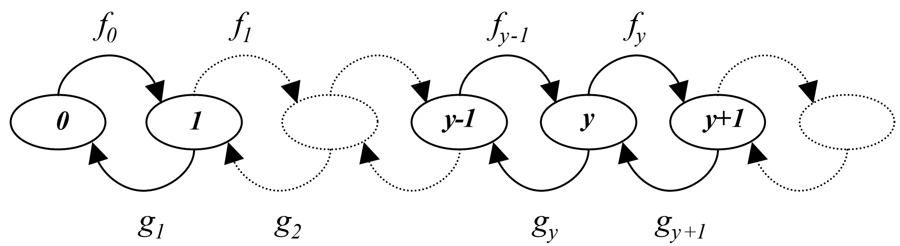


Deterministic

$$\frac{dy}{dt} = k_R - \gamma_R y \equiv f_y - g_y$$

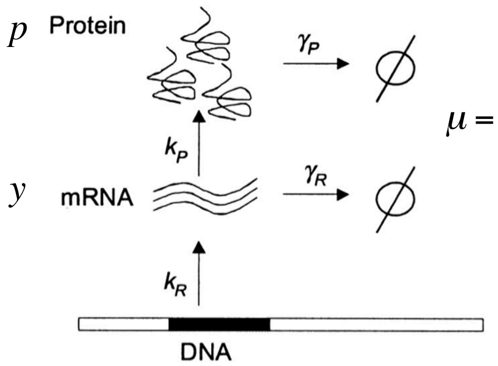
Probabilistic: the Master Equation

$$\frac{\partial}{\partial t} \wp_y = -(f_y + g_y)\wp_y + f_{y-1}\wp_{y-1} + g_{y+1}\wp_{y+1}$$



Thattai & van Oudenaarden, PNAS (2001)

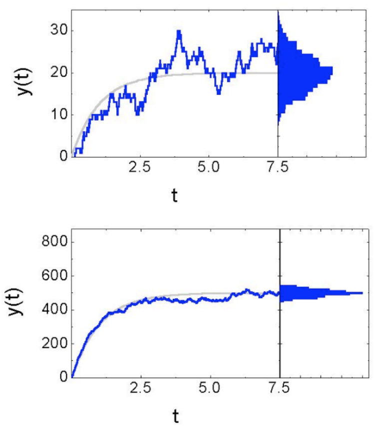
Gene expression: three ways to reduce noise



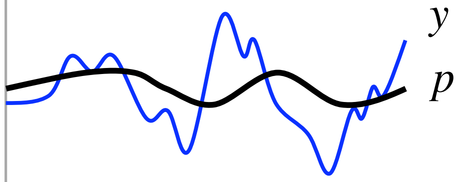
$\mu = \gamma_P / \gamma_R$

$$\frac{\sigma_p^2}{\langle p\rangle^2} = \frac{1}{\langle p\rangle} + \frac{1}{\langle y\rangle}\frac{\mu}{1+\mu}\left|\frac{\partial \ln\langle p\rangle}{\partial \ln\langle y\rangle}\right|^2$$

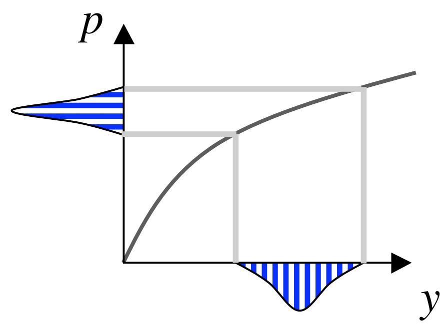
large numbers



time averaging

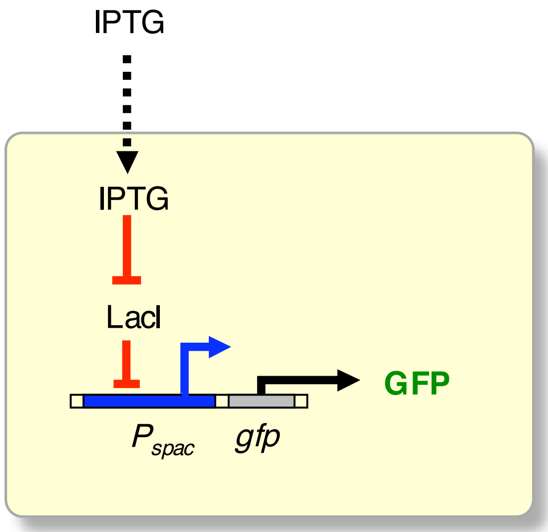


logarithmic gain

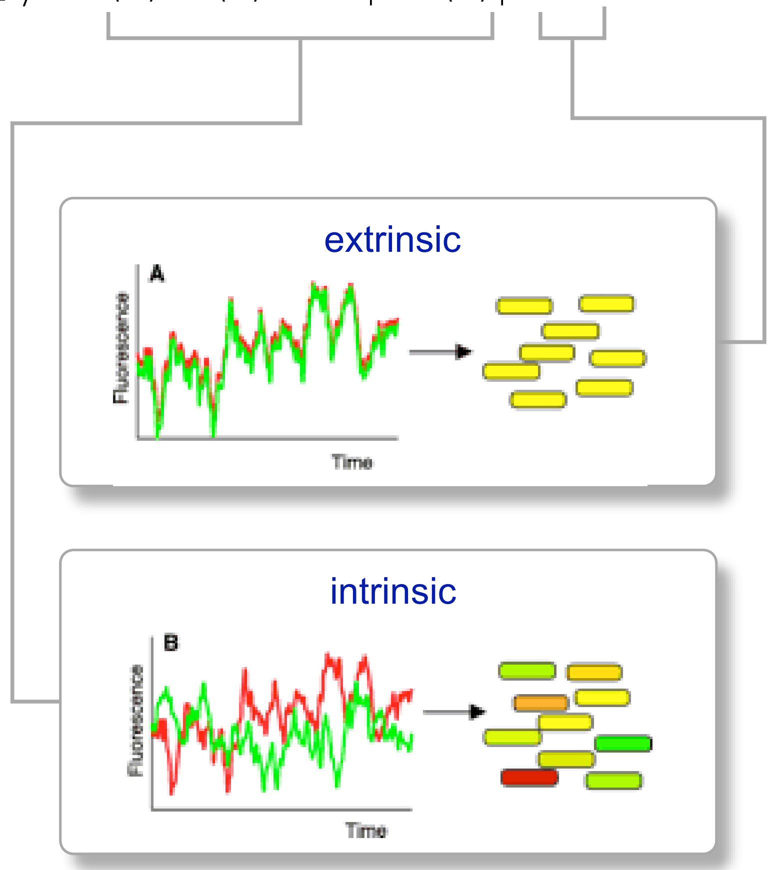


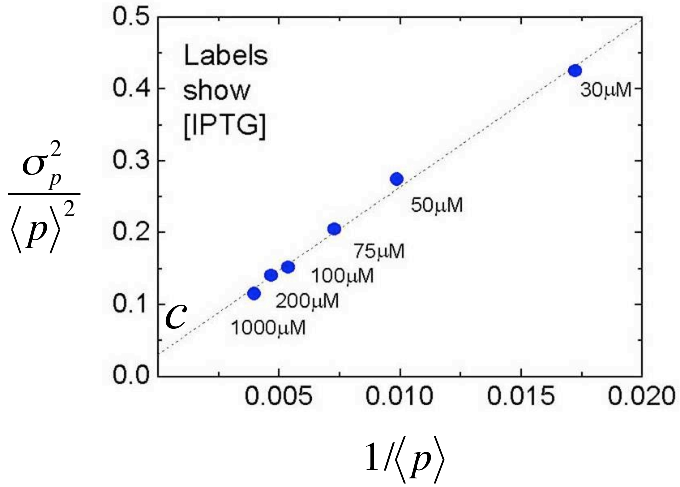
Thattai & van Oudenaarden, PNAS (2001)

Intrinsic and extrinsic noise



$$\frac{\sigma_p^2}{\langle p\rangle^2} = \frac{1}{\langle p\rangle} + \frac{1}{\langle y\rangle}\frac{\mu}{1+\mu}\left|\frac{\partial \ln\langle p\rangle}{\partial \ln\langle y\rangle}\right|^2 + c$$





Ozbudak et al., Nature Genet., 2002; Elowitz et al., Science, 2002

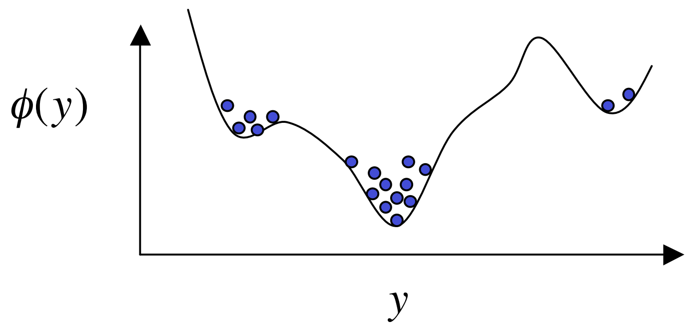
Molecule numbers obey a diffusion equation

Fokker-Planck

$$\frac{\partial}{\partial t}\wp_y \approx -\frac{\partial}{\partial y}(f_y - g_y)\wp_y + \frac{1}{2}\frac{\partial^2}{\partial y^2}(f_y + g_y)\wp_y$$

$$\frac{\partial}{\partial t}\wp_y \approx \quad + \frac{\partial}{\partial y}\phi'(y)\wp_y \quad + \quad \frac{1}{2}\frac{\partial^2}{\partial y^2} D_{eff}\wp_y$$

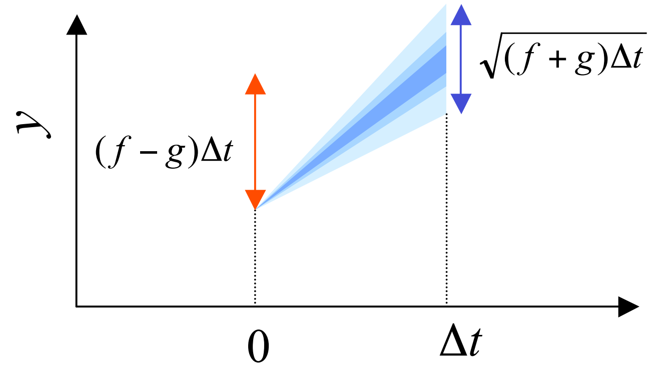
drift diffusion



Langevin

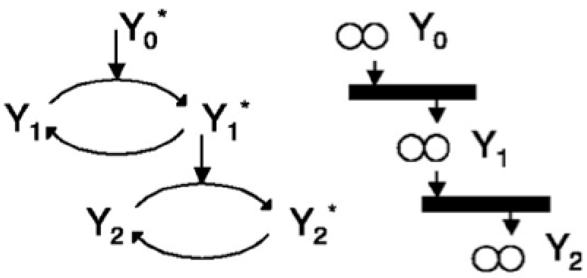
$$\frac{dy}{dt} = f(y) - g(y) + \eta(t)$$

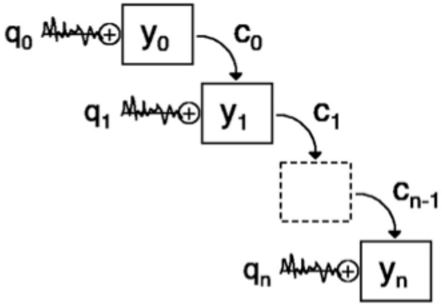
$$\langle \eta(t) \rangle = 0, \quad \langle \eta(t)\eta(t+\tau) \rangle = (f+g)\delta(\tau)$$



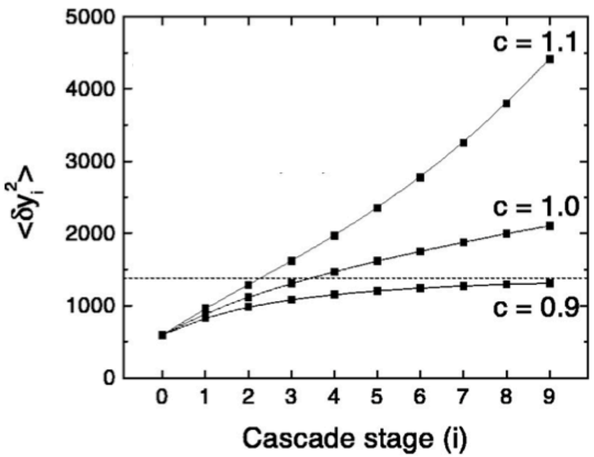
📖 Thattai & van Oudenaarden, Biophys. J., 2002

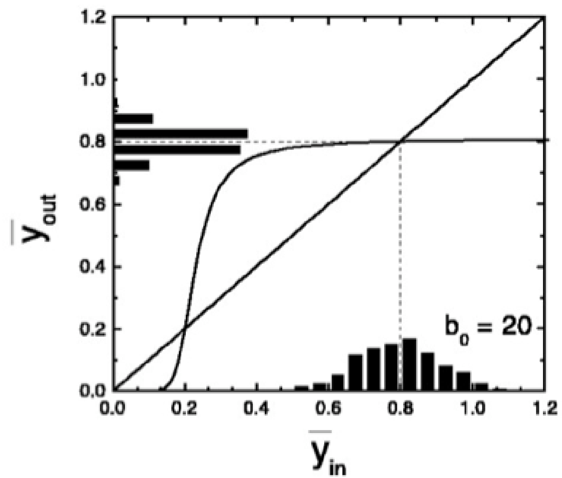
Noise can propagate through a cascade





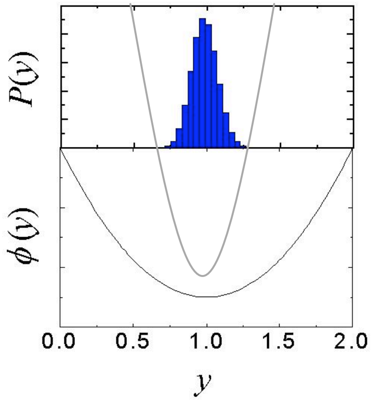
$$\left\langle \delta y_i^2(\omega) \right\rangle = \frac{q_i + c_{i-1}^2 \left\langle \delta y_{i-1}^2(\omega) \right\rangle}{1+\omega^2}$$

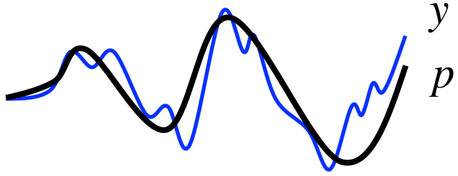




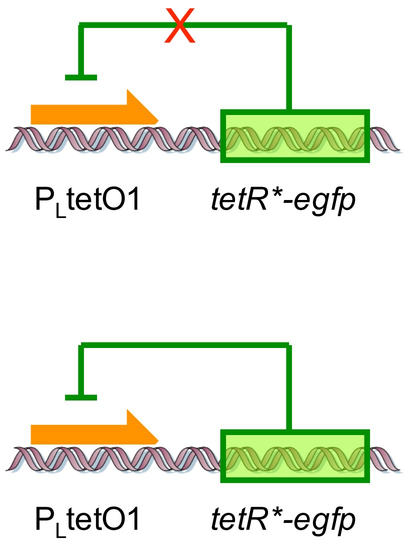
Thattai & van Oudenaarden, Biophysj, 2002

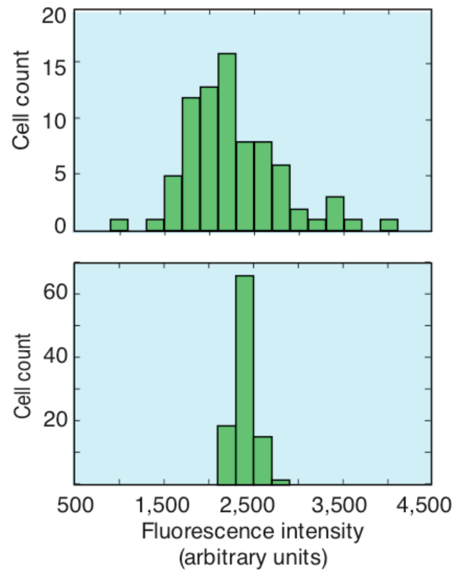
Negative feedback can reduce fluctuations





But care needed: rapid response can sometimes increase noise

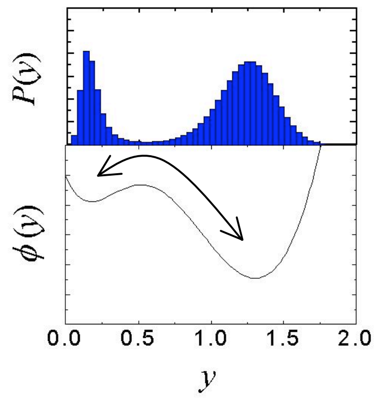


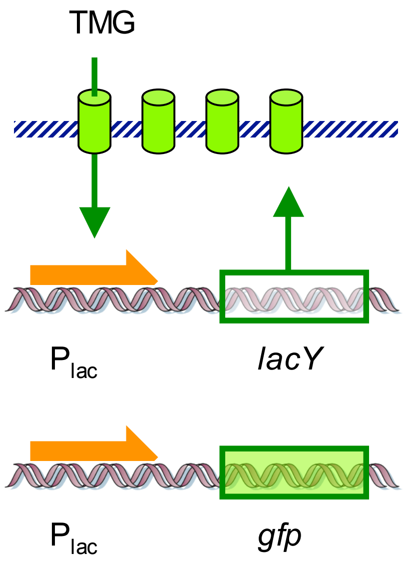


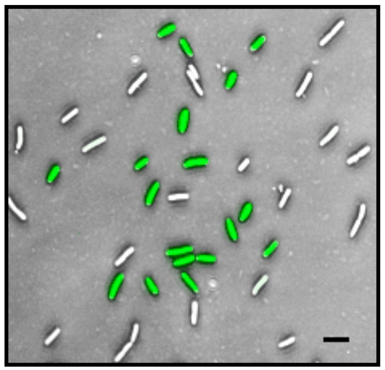
Figures adapted from Hasty et al, 2002

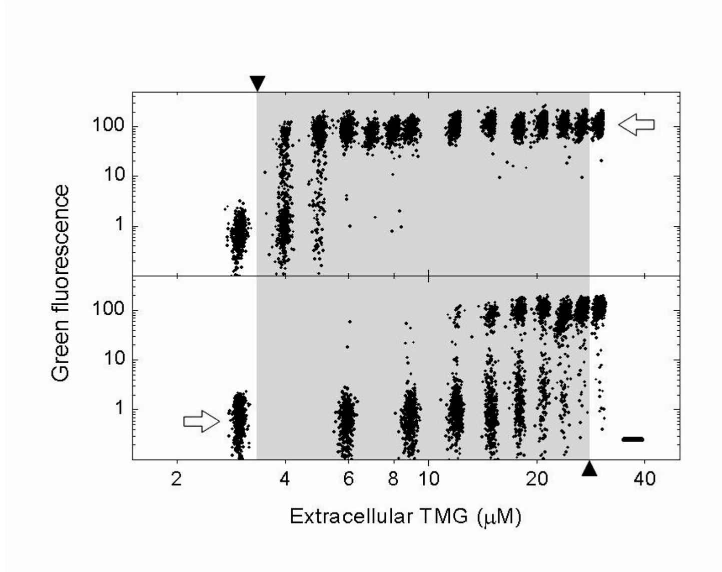
Becskei & Serrano, Nature, 2000

Noise can flip a bistable switch





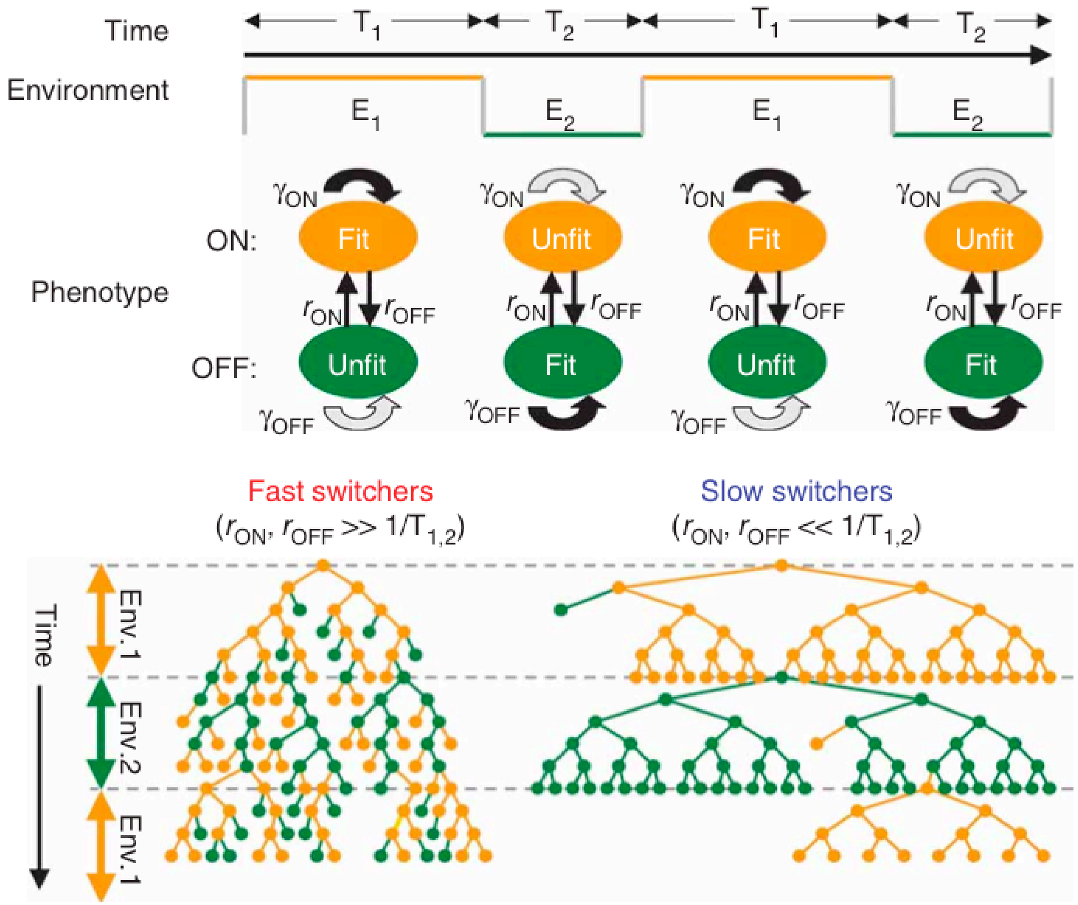




Ozbudak & Thattai et al., Nature, 2004

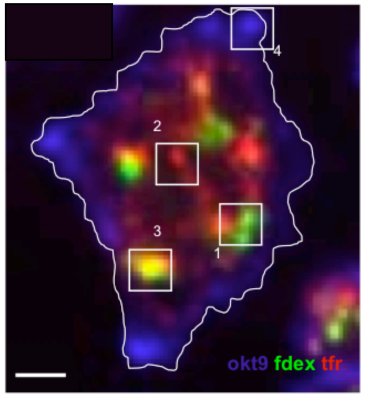
Single cells can use noise to hedge bets

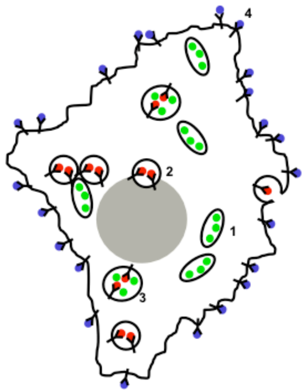
Cells switch between two states, each optimized to a different environment



Thattai & van Oudenaarden, Genetics, 2004; Acar, Mettetal & van Oudenaarden, Nature Genet., 2008; Kussell et al., 2005

Beyond gene expression:
cell-to-cell variability in cellular activity and morphology

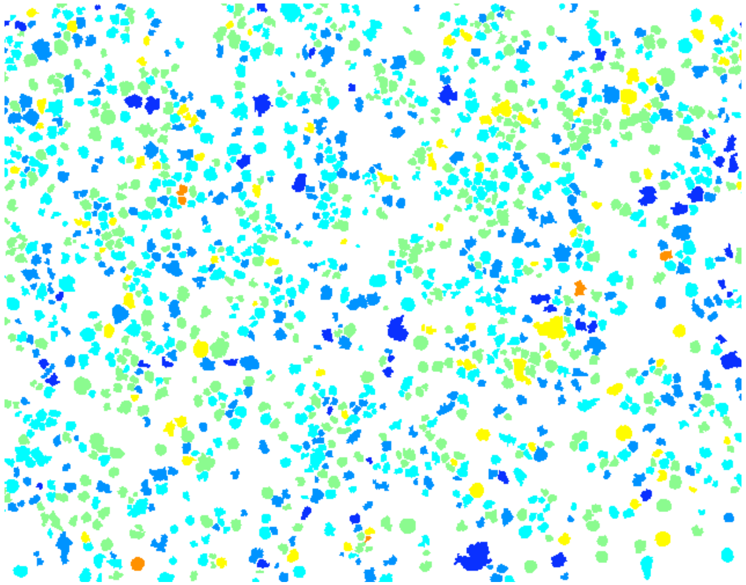




GEEC ("Fld") Pathway: FITC-Dextran 

Clathirin ("Tfr") Pathway: Alexa568-Transferrin 

	FDex	Tfr	Coloc	Okt9	Cell size	Nuclear
Intensity	I1,I2,I3 I4	I5,I6,I7, I8,I9,I10I 11		I12		
Geometry	G1,G2 G3,G4	G6,G7 G8,G9	G5,G10		G11	G12,G13 G14,G15



Drosophila SR+ cells.

Colour-code indicates mean intensity of internalized FITC-Dextran

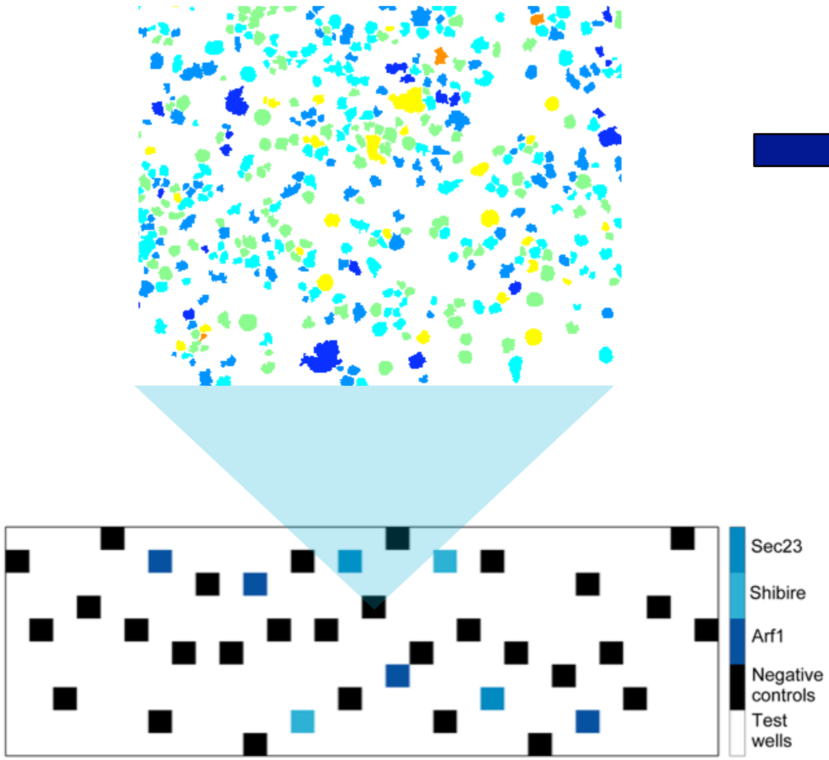
[Data from Jitu Mayor's lab]

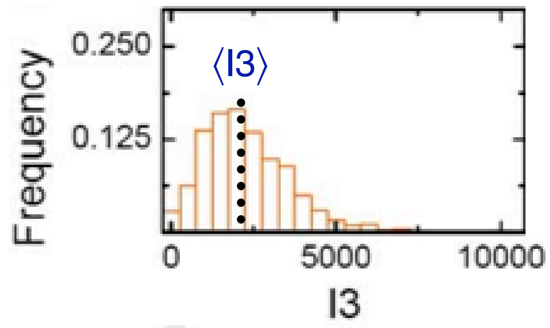
Dey et al., in preparation

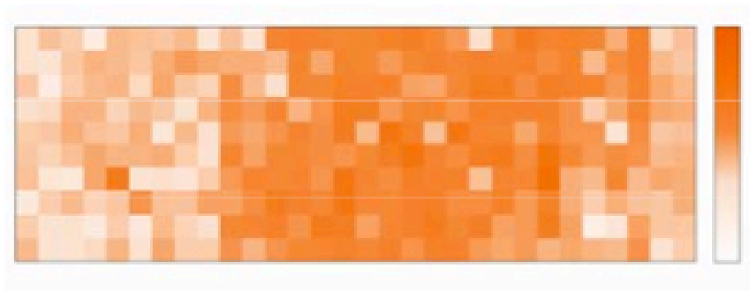
Screening for components of the endocytic machinery

Single-cell images

Population distribution







Slide-format RNAi screen for endocytosis in a Drosophila cell line

Heat map of mean parameter value shows systematic variation

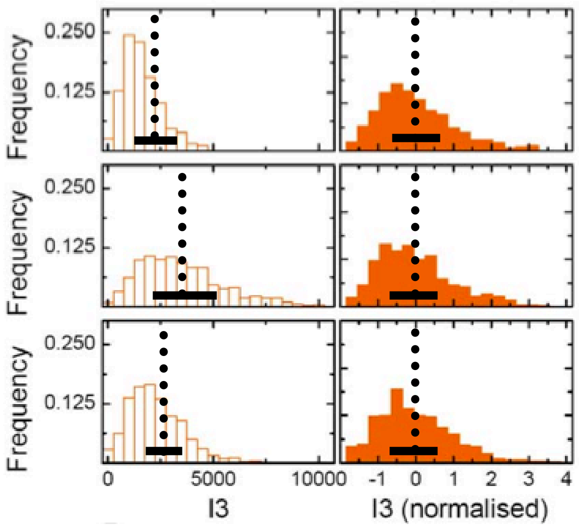
📖 Dey et al., in preparation

Population distributions come to the rescue

Rescale by subtracting the mean and dividing by the stddev

Normalization generates reproducible distributions...

Negative control wells



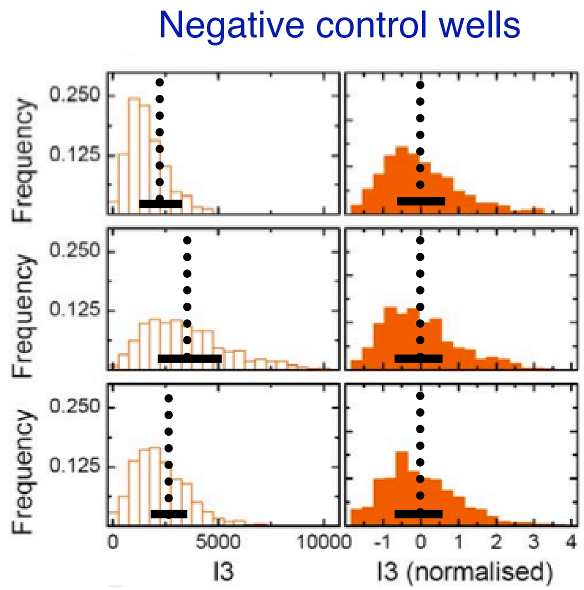
Dey et al., in preparation

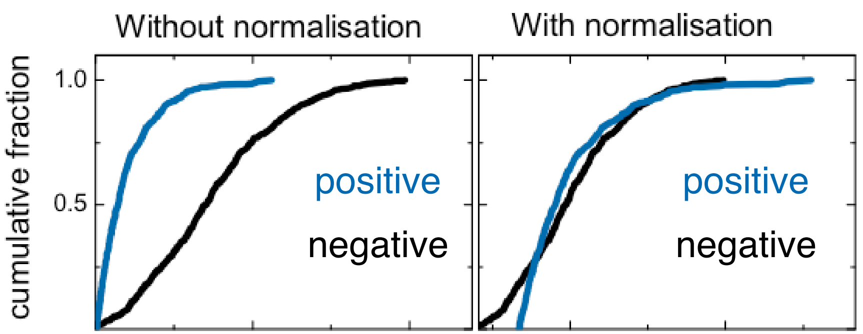
Population distributions come to the rescue

Rescale by subtracting the mean and dividing by the stddev

Normalization generates reproducible distributions...

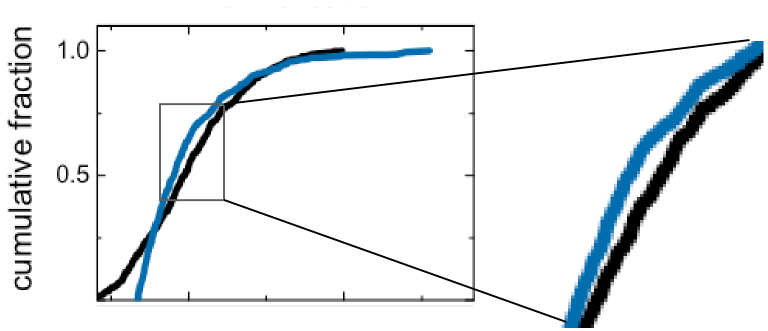
... while preserving the distinction between negative and positive controls



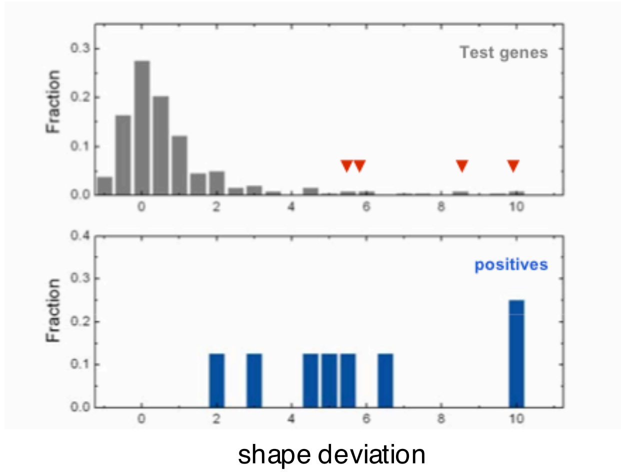


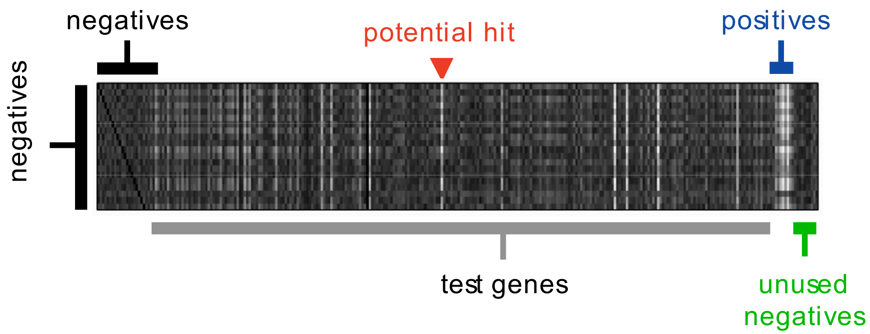
Dey et al., in preparation

We can use variability to find molecular players



A KS-like statistic





GO ID (Description)	GO Term	p-value (corrected)
GO:0005665 (transcription)	DNA-directed RNA pol II, core	3.4e-4 (0.015)
GO:0005686 (mRNA processing)	U2 snRNP	5.7e-5 (<0.01)
GO:0030532 (mRNA processing)	snRNP complex	1.1e-4 (<0.01)
GO:0008541 (proteolysis)	proteasome reg. particle, lid	2.0e-4 (<0.01)
GO:0008540 (proteolysis)	proteasome reg. particle, base	2.2e-5 (<0.01)
GO:0045298 (cytoskeleton)	tubulin complex	1.1e-3 (0.06)
GO:0030126 (vesicular traffic)	COPI vesicle coat	5.8e-5 (<0.01)

Dey et al., in preparation

Origins and consequences of cell-to-cell variability

With: Alexander van Oudenaarden, Ertugrul Ozbudak, Murat Acar (MIT) & Boris Shraiman (KITP)

Exploiting cell-to-cell variability to probe cellular processes

With: Jitu Mayor, Gagan Gupta, Gautam Dey (NCBS)

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