CONTRIBUTION OF DETERMINISTIC BISTABILITY AND NOISE TO CELL FATE TRANSITIONS

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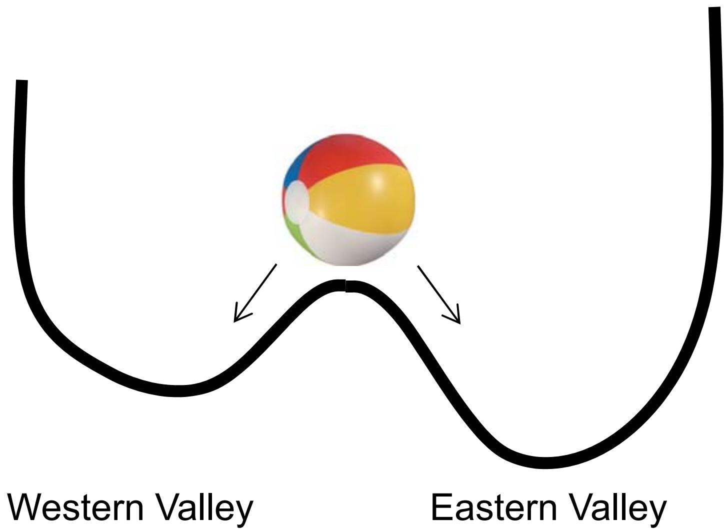
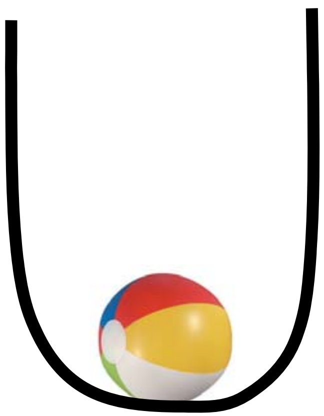
What is bistability?

- Existence of two stable states



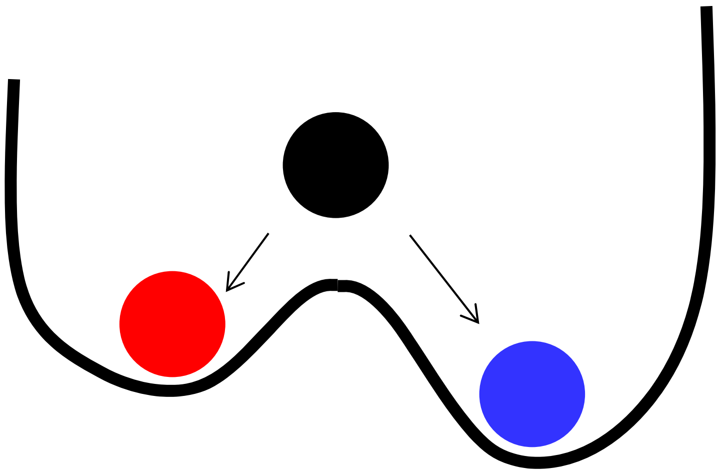
Bistable system



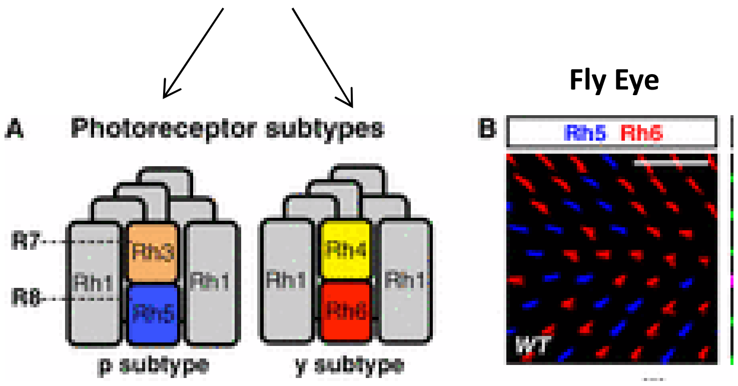
Monostable system

Bistability in biology

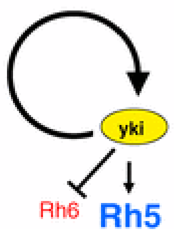
- Alternative cell fates: differentiation
- All cells in the body are result of cell fate decisions / instructions



Alternative cell fates



Jukam et al (2013) Science

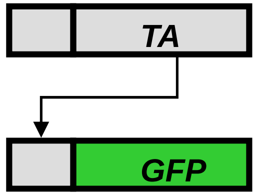


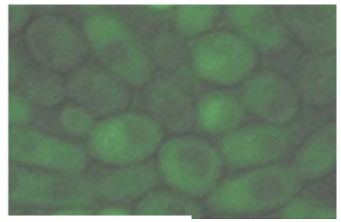
- Alternative cell fates in fly eye: cells with different rhodopsins detect different **wavelengths**
- The Drosophila eye comprises about 800 unit eyes,
- P(ale) and Y(ellow) subtypes are distributed randomly in the retina in a p:y ratio of ~30:70,
- p versus y fate is established by a bistable transcriptional **feedback loop** including yki

- How do feedback loops generate bistability?

Constitutive regulation

- (Absence of feedback loops)
- All cells behave uniformly = their fate is **determined (**Deterministic behavior in gene expression)
  (when grown in homogenous environment)
- Graded expression

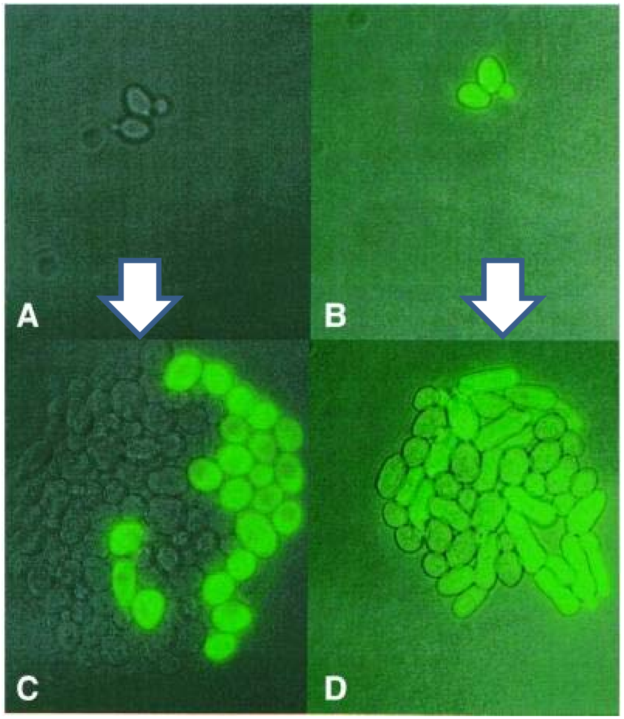




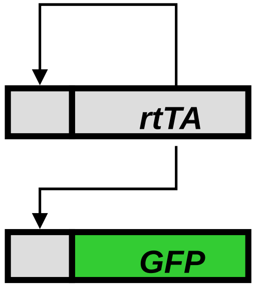
Constitutive regulation

Becskei, Séraphin & Serrano: EMBO J (2001)

Feedback regulation and bistability: two cell fates

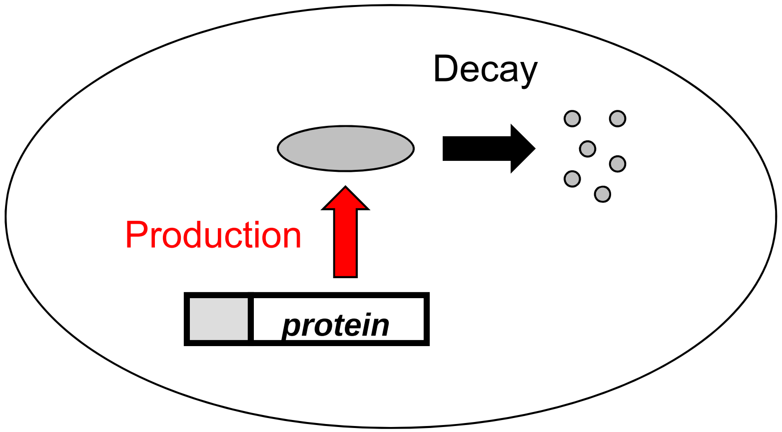


Becskei, Séraphin & Serrano: Positive feedback loop, EMBO J (2001)

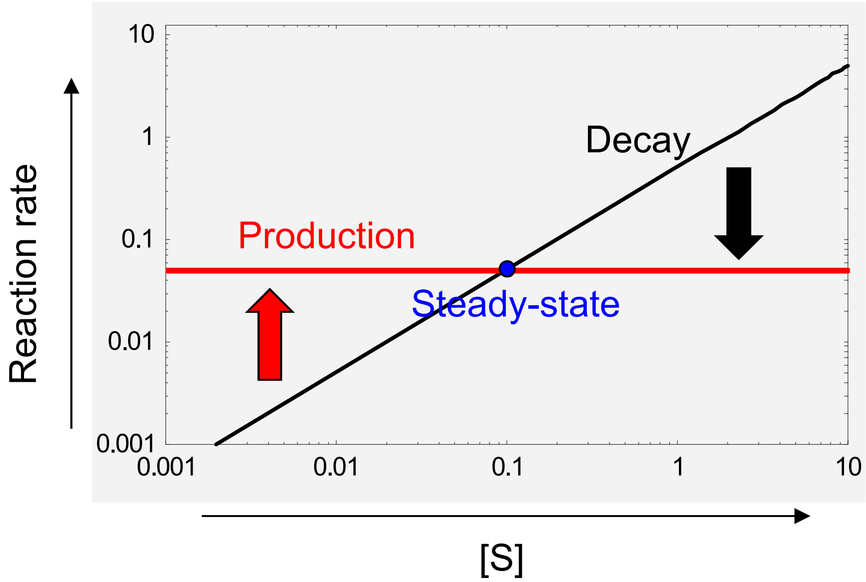


- Positive feedback loop: two cell fates.
  - Counterintuitive: why not only a single high state?
- Stochastic behavior
  - It is impossible to predict exactly which of the cells will switch to the high state
  - But it is possible to make predictions on the distribution of the cells:
  - E.g. 45% of the cells will switch to the ON (green) state

Constitutive gene expression: Steady-state solution



$$\frac{ds}{dt} = p - \gamma s$$

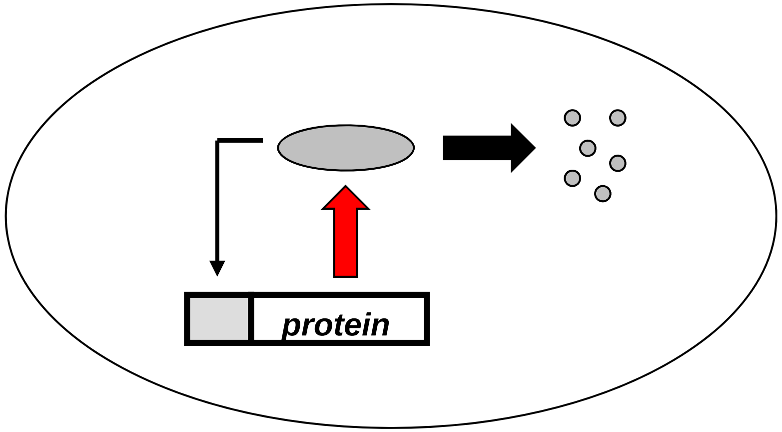


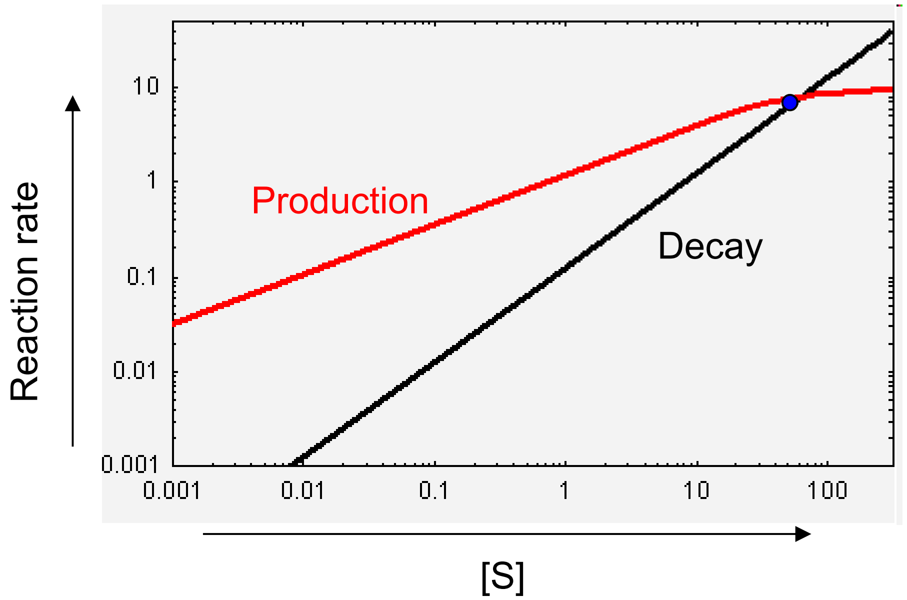
$$\frac{ds}{dt} > 0 \qquad \frac{ds}{dt} = 0 \qquad \frac{ds}{dt} < 0$$

- Protein level can changed e.g. by injecting additional “S” into the cell. After some time, the “S” would decay to the original steady-state.

$$s = \frac{p}{\gamma}$$

Positive-feedback with hyperbolic production-rate





$$\frac{dS}{dt} = p_0 + p_1 \frac{fS}{K + fS} - \gamma S$$

- p0 basal transcription
- p1 transcription induced by a transcriptional activator
- f proportion of active transcriptional factors (e.g. phosphorylated, bound by an inducer)

- Protein binding to DNA is described with the same saturation equation as protein-ligand binding.

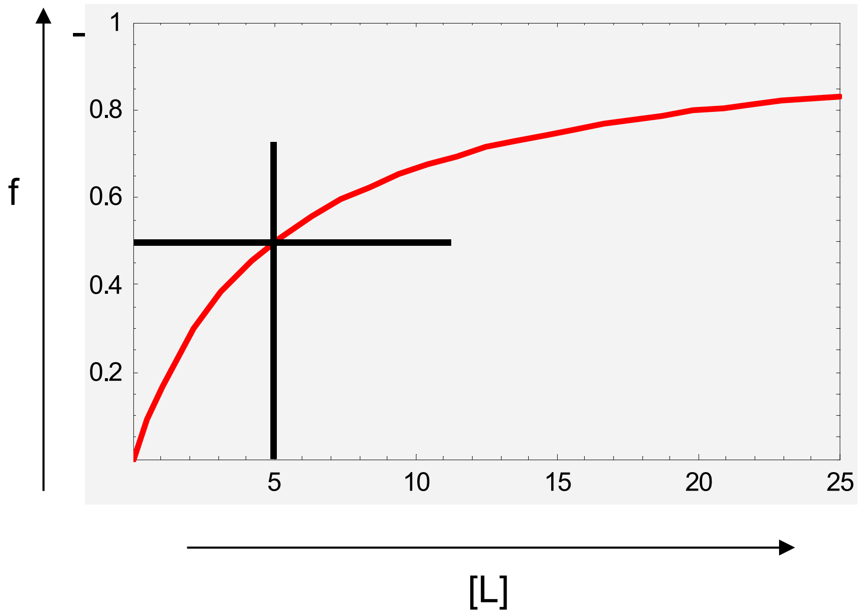
Saturation in protein – ligand binding: Hyperbolic function is a "linear function" for biologists

- Equilibrium binding of ligand (L) to protein (P)
  - Free protein P_f , complexed protein P_c
  - K: equilibrium dissociation constant
  - $L + P_f \leftrightarrow P_c$
  - f: fraction of protein binding sites occupied by ligand
    - Bound protein / total protein

$$K = \frac{L P_f}{P_c}$$

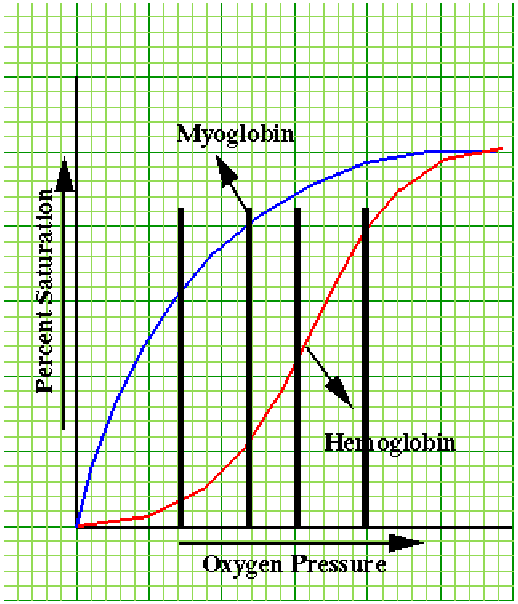
$$f = \frac{P_c}{P_c + P_f} = \frac{\frac{P_f L}{K}}{\frac{P_f L}{K} + P_f} = \frac{L}{K + L}$$

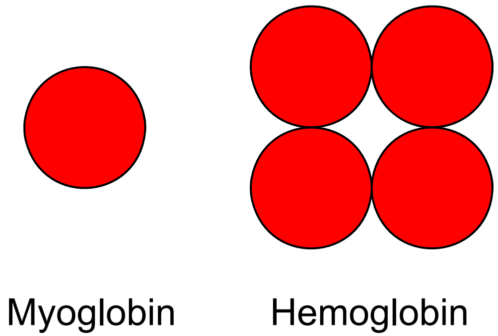
- Equation is analogous to the Michaelis-Menten equation and has a hyperbolic shape
- Rectangular hyperbola is defined by asymptotes at K and f=1.
- 50% saturation when L = K



A prototypical binding reaction: Oxygen transport and hemoglobin

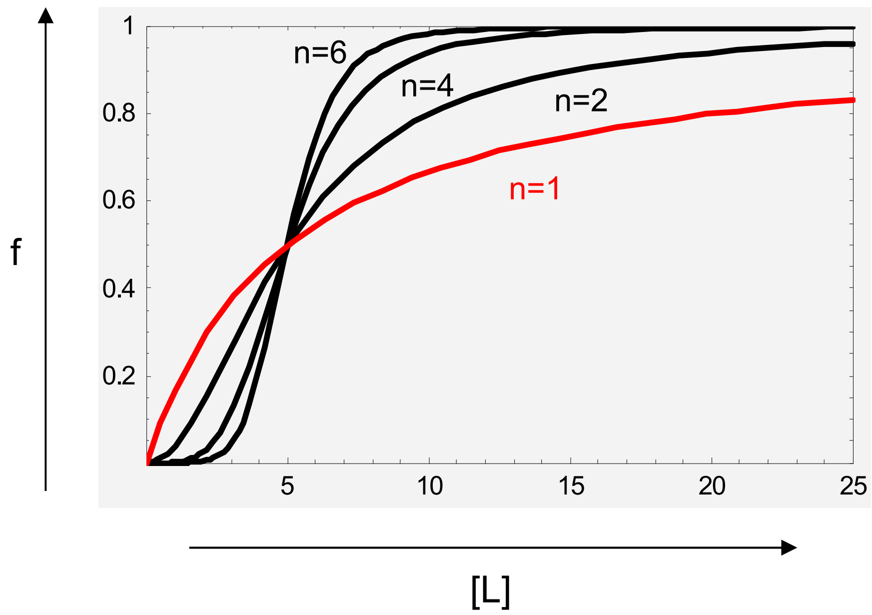
- Oxygen transport is more efficient due to the switch-like (nonlinear) behavior in the oxygen binding of hemoglobin.
- The cooperative action of hemoglobin subunits leads to a higher amount of oxygen released when oxygen pressure decreases.





Cooperativity - Hill function

- Each subunit in a n-subunit protein binds to a ligand
- $nL + P_0 \leftrightarrow P_n$
- Concerted binding:
  - We assume that some interaction among the subunits forces all the binding sites to be either simultaneously occupied or simultaneously empty.
  - Protein with empty binding sites P_0
  - Protein with all sites occupied P_n
  - The Hill function has a sigmoidal shape when n > 1, a sign of cooperativity.



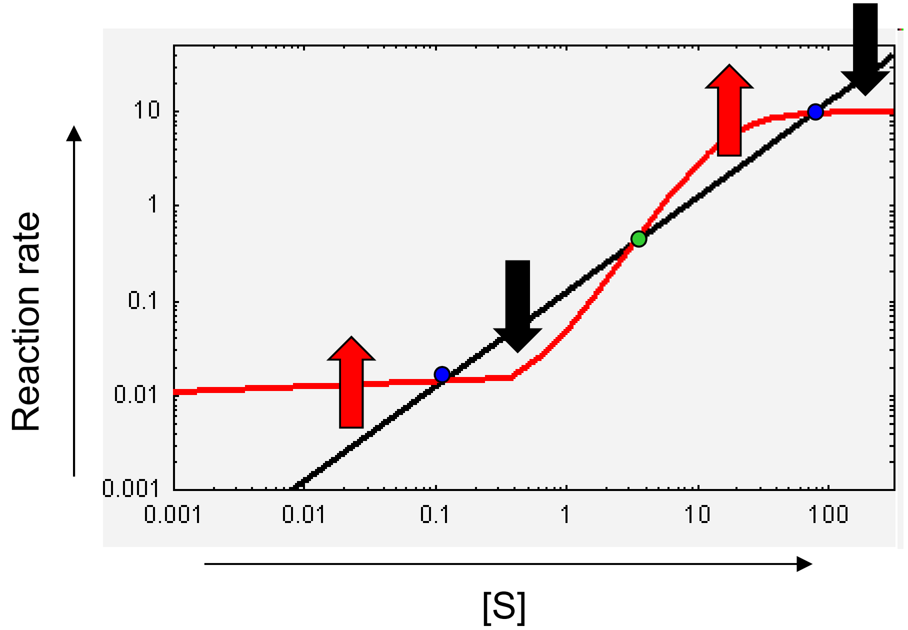
$$K = \frac{L^n P_0}{P_n}$$

$$K_n = 5^n$$

$$f = \frac{P_n}{P_n + P_0} = \frac{\frac{P_0 L^n}{K}}{\frac{P_0 L^n}{K} + P_0} = \frac{L^n}{K + L^n}$$

Positive feedback with sigmoidal production rate

$$\frac{dS}{dt} = p_0 + p_1 \frac{(fS)^2}{K + (fS)^2} - \gamma S$$



- Nonlinear sigmoidal production rate
  - The TF binds to 2 sites in the promoter
- The steady-state equation is a cubic expression with the possibility of three real roots.
- In this case, there are two stable and one unstable fixed points.
- The two stable fixed points correspond to two expression levels.
- Parameters:
  - f=0.04, p0=0.01, p1=10, K=1

Logarithmic sensitivity (derivative) characterizes biological nonlinearities

Logarithmic sensitivity (S)

$$S = \frac{\partial \ln f(S)}{\partial \ln S}$$

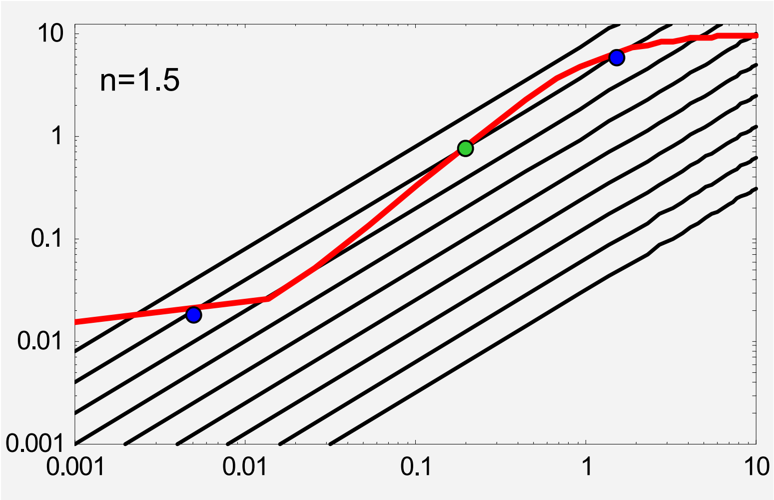
- If S > 1, the system is ultrasensitive
- S for the Hill function
  - And in the limit of low S

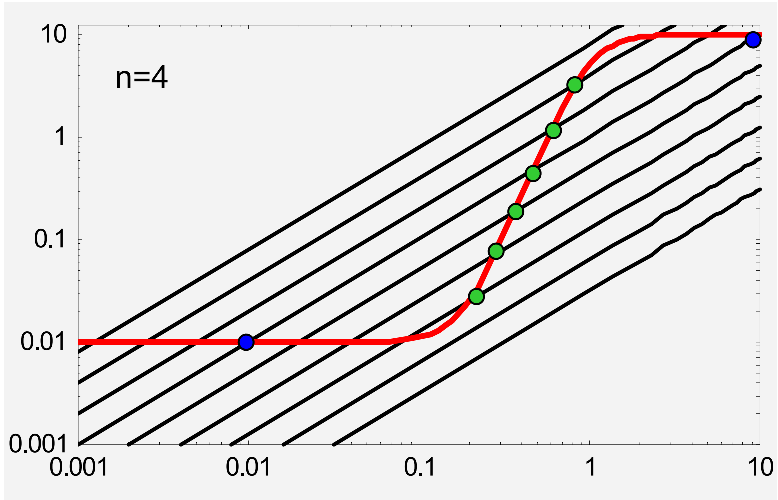
$$\frac{\partial \ln \dfrac{S^n}{K^n + S^n}}{\partial \ln S} = \frac{\partial \ln S^n}{\partial \ln S} - \frac{\partial \ln(K^n + S^n)}{\partial \ln S} = \frac{S}{S^n}\frac{nS^{n-1}}{1} - \frac{S}{1}\frac{nS^{n-1}}{K^n + S^n} = n - n\frac{S^n}{K^n + S^n} = n\frac{K^n}{K^n + S^n}$$

$$\lim_{S \to 0} n\frac{K^n}{K^n + S^n} = n$$

At low concentration the sensitivity is equal to the Hill number

Robustness of bistable behavior





- Larger the cooperativity (sigmoidal nonlinearity) the more robust the bistability
- Curve with small Hill number (n=1.5) has only one triple intersection while n=4 entails six triple intersections.
- Number of subunit only defines the maximal **attainable Hill number** but this is usually not reached.
- Realistic Hill numbers: Hemoglobin with 4 subunits has *n* = 2.8. For few proteins n=3 to 4.
- Most of the protein complexes are display no or little cooperativity (present survey of data).
- Production rate curves span 3 orders of magnitude (0.01 to 10). Basal activity is usually set by incomplete control.

Maximal bistable range of a parameter is related to Sensitivity

- Conditions of bifurcation (C):

$$C^{(1)}(\omega;\alpha) = f(\omega;\alpha) - \omega = 0,$$

$$C^{(2)}(\omega;\alpha) = \frac{\partial f(\omega;\alpha)}{\partial \omega} - 1 = 0,$$

$$C^{(3)}(\omega;\alpha) = \frac{\partial^2 f(\omega;\alpha)}{\partial \omega^2} = 0.$$

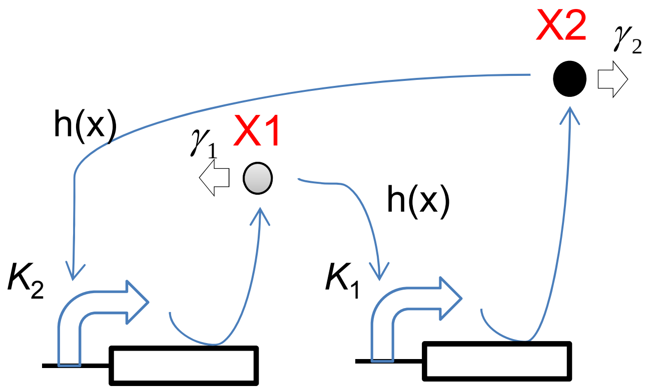
$$\frac{d\alpha_i}{d\alpha_k} = -J^{-1}_{\omega ij}\frac{dC}{d\alpha_k} = -\frac{\det J_{\omega kj}}{\det J_{\omega ij}}.$$

- General result: the maximum of with respect to two reaction parameters defines the extremum of the bistable range of a third parameter.

$$\frac{\partial S^f_\omega}{\partial \alpha_C} = 0 \text{ and } \frac{\partial S^f_\omega}{\partial \alpha_k} = 0.$$

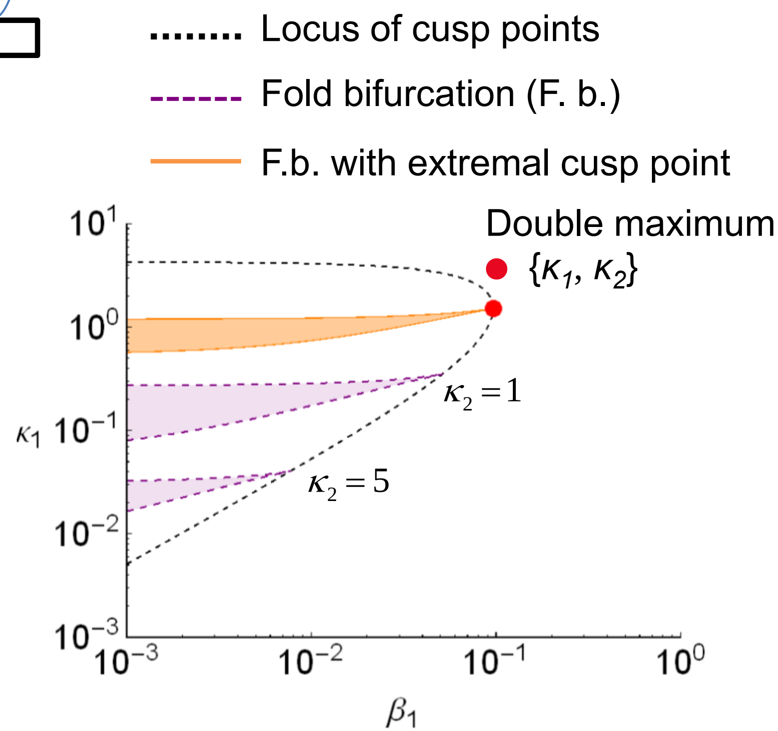
Majer et al (2015) Physical Biology

An example: feedback by two activators



- The broadest bistable range in *beta* (basal transcription rate) is attained when the affinities of activator binding to DNA (*kappa*) are equal.

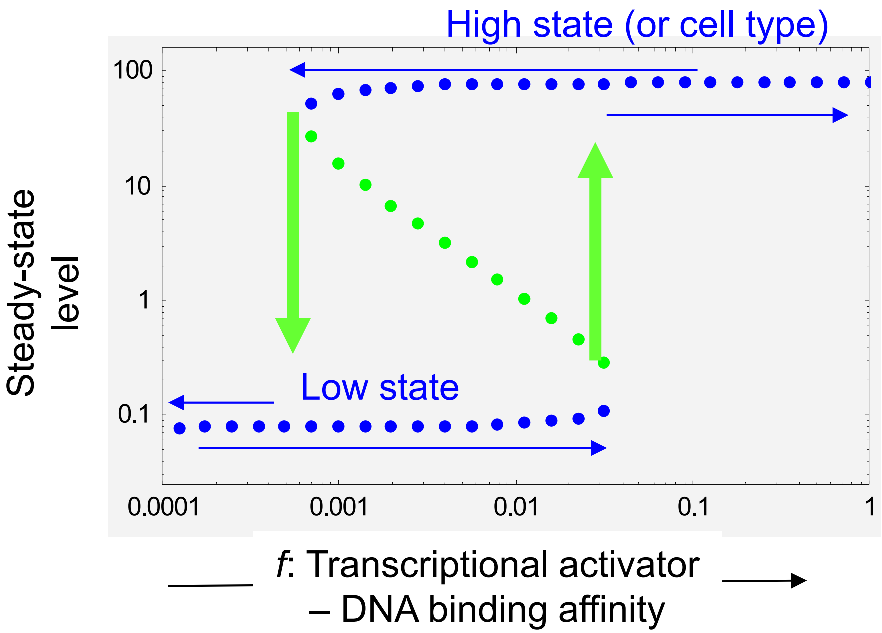
$$\kappa_1 = \kappa_2 = \frac{\left(\frac{n-1}{n+1}\right)^{-1/n}\left(n^2-1\right)}{4n}.$$



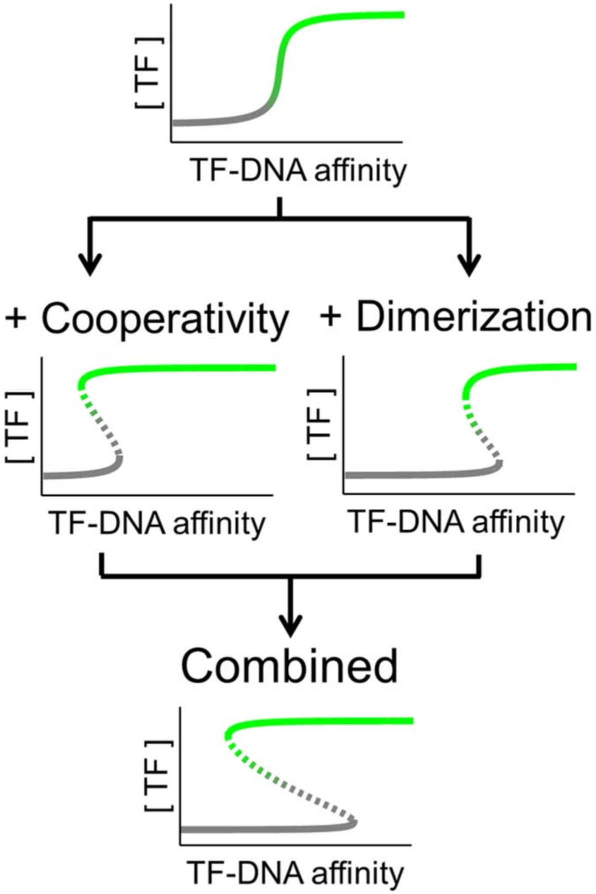
Majer et al (2015) Physical Biology

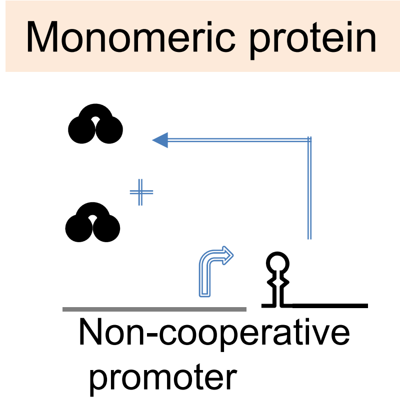
Experimental detection of bistability

- Dependence of the steady-state behavior on the initial condition
  - cellular memory
- Varying a system parameter (e.g. affinity) reveals if **hysteresis**, a hallmark of bistability, exists.

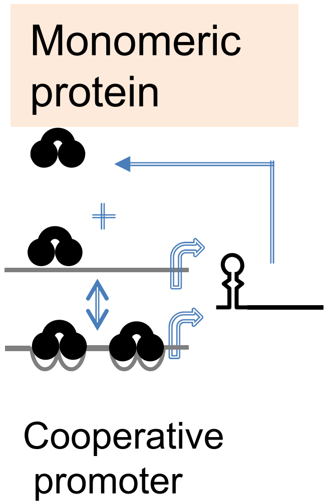


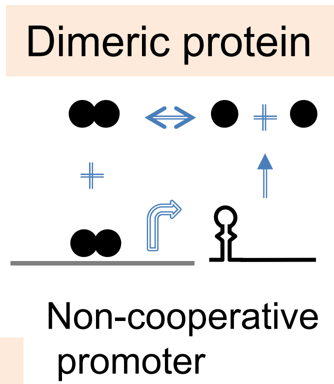
Systematic variation of reactions with sigmoidal dose-response

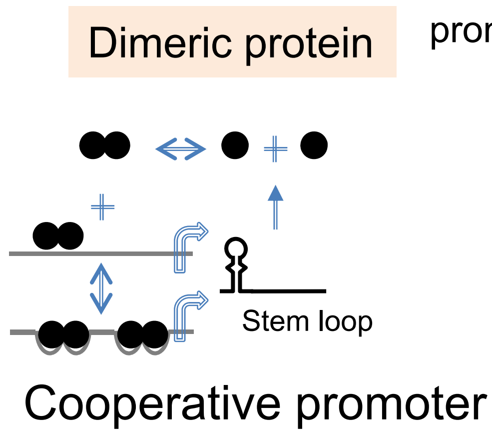




- Dimerization can also generate sigmoidal response

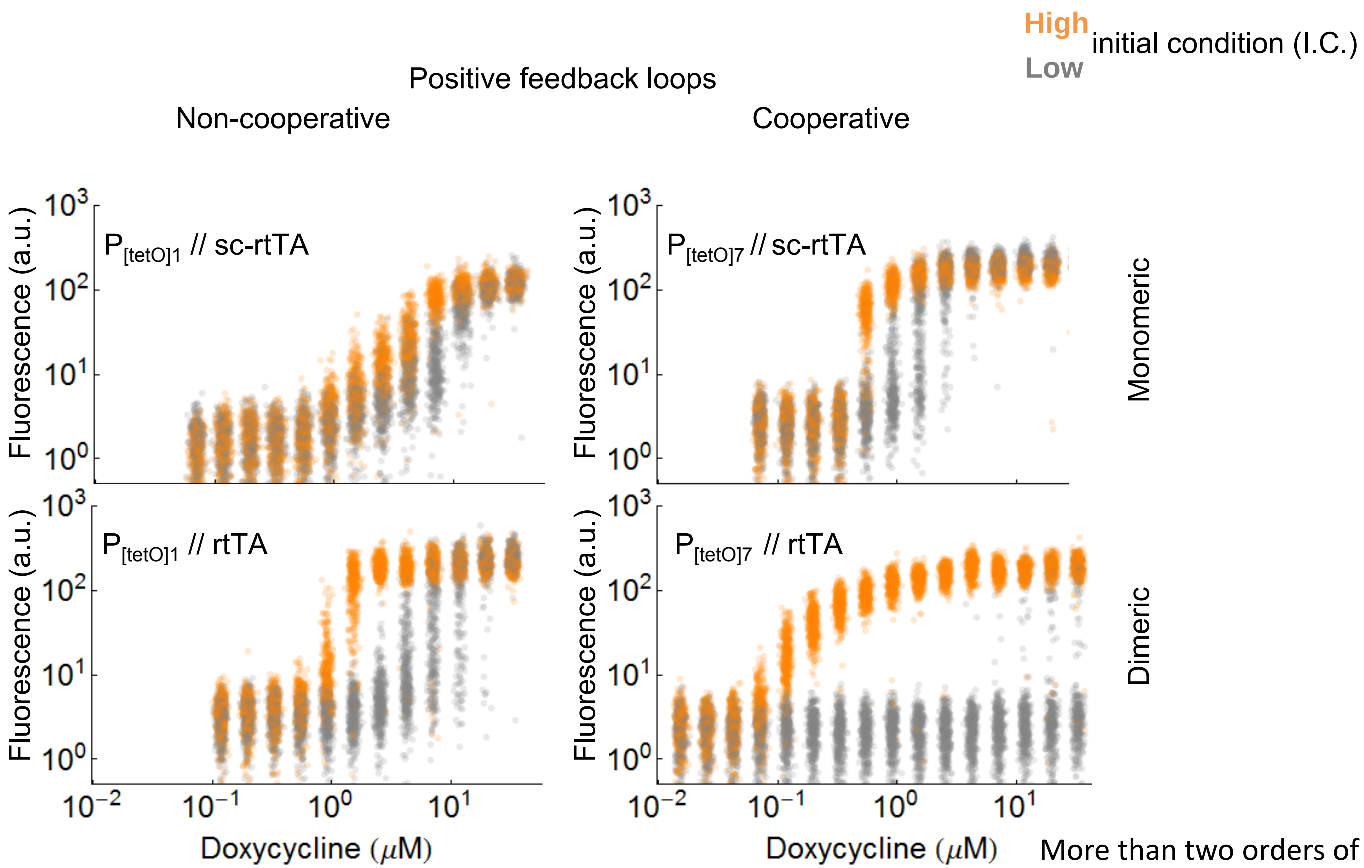






Robust bistability (cellular memory) by the joint effect of protein dimerization and cooperativity

Hysteresis experiments



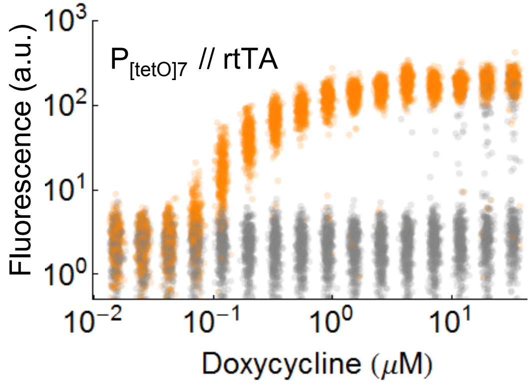
More than two orders of magnitude of parameter values with bistability

Hsu et al (2016) *Cell Reports*

Negative feedback loop weakens bistability

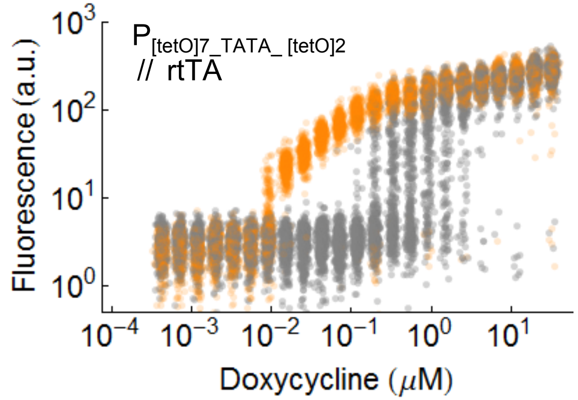
Cooperative, dimeric

High / Low initial condition (I.C.)

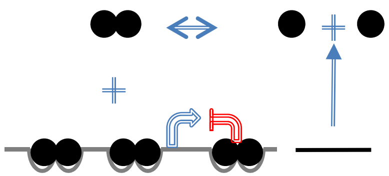


Positive feedback

C



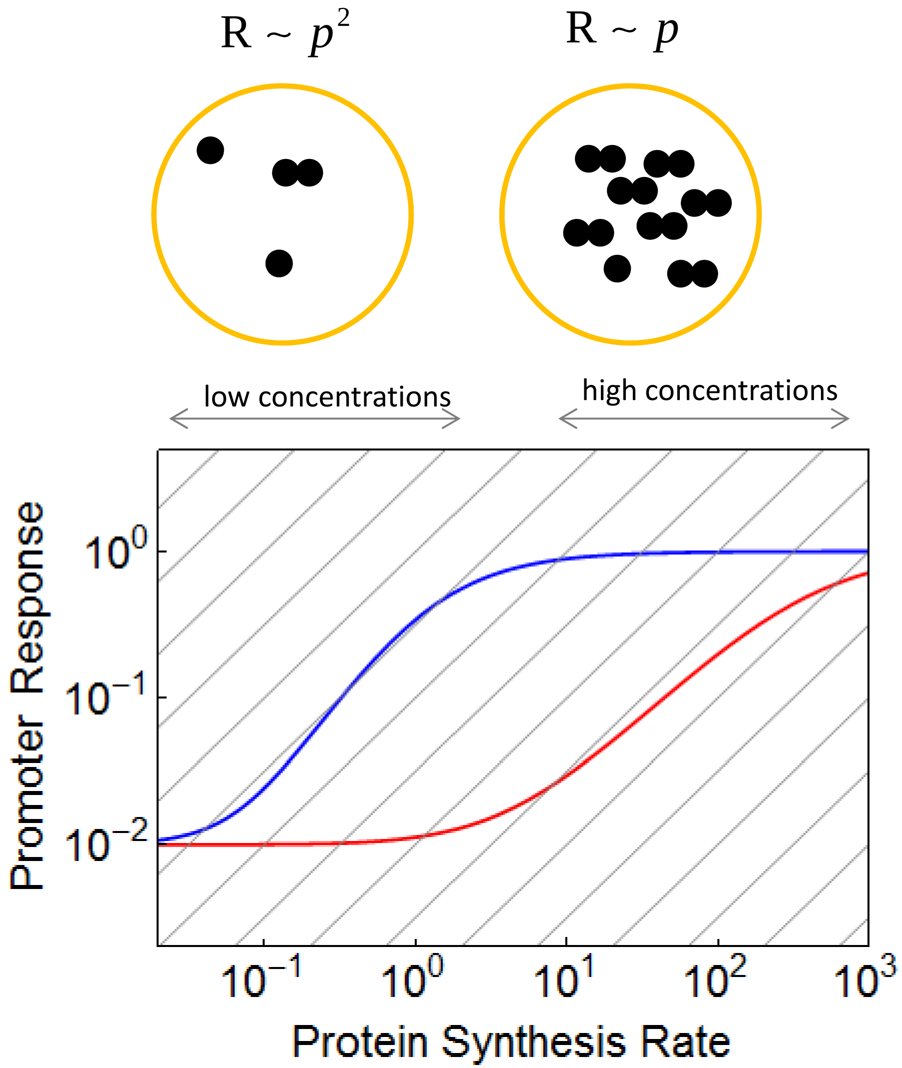
Positive & negative feedback loop



Positive feedback extended with negative feedback: $P_{[tetO]7\_TATA\_ [tetO]2}$ // rtTA

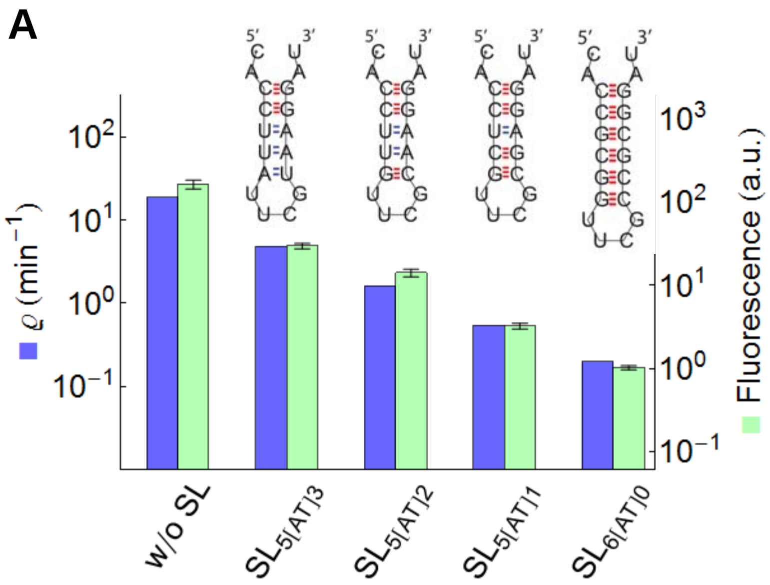
Sigmoidal effect due to dimerization arises only at low concentration

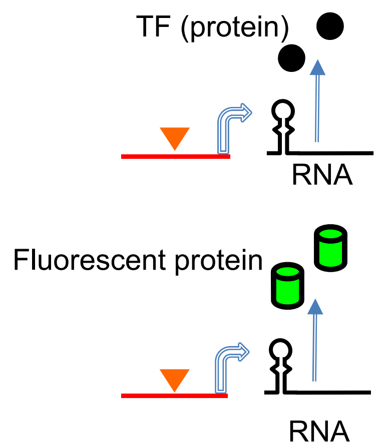
- Quadratic (nonlinear) relation at low concentrations
- Linear relation at high concentrations



Reduction of protein synthesis (translation) by RNA stem-loops

- We weakened the stem structure by shortening the stem length and by increasing the proportion of A-T base pairs.
- We obtained a variety of stem-loops that can tune the translation rate over two orders of magnitude

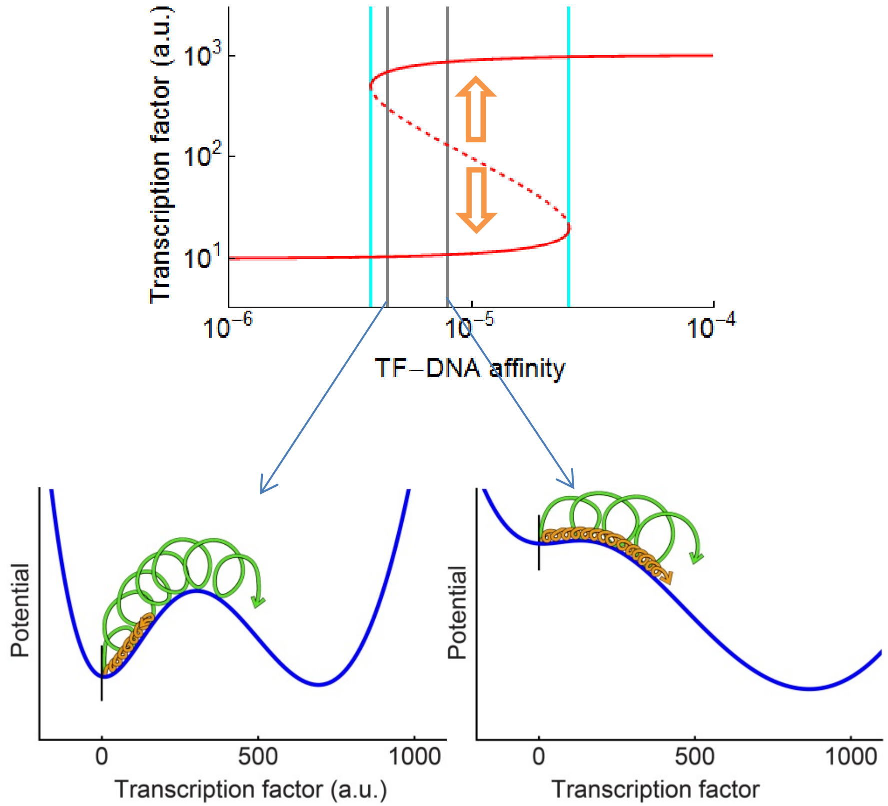


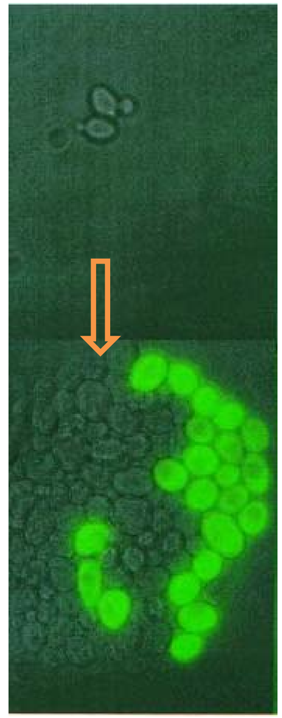


Hsu et al (2016) *Cell Reports*

Bistability is a deterministic concept.

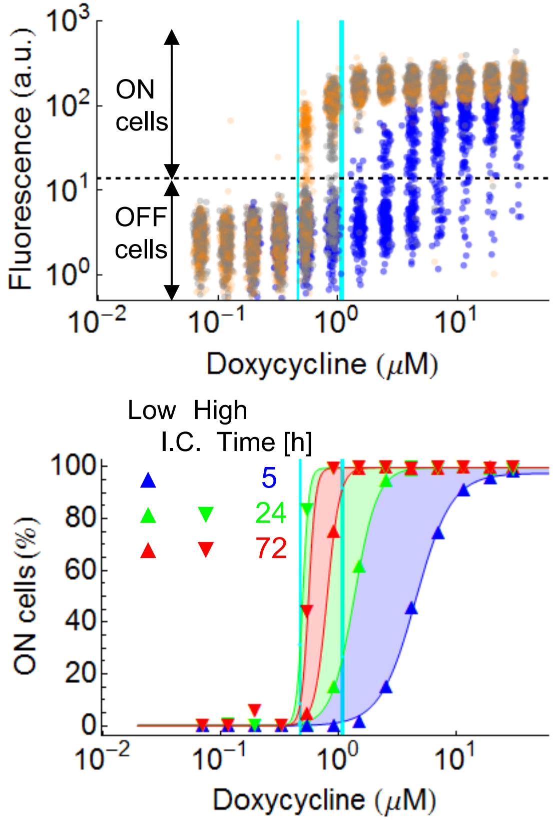
- Initial condition uniquely determines which equilibrium state is reached
  - Deterministic description
- BUT: Concentrations fluctuate in cells
  - Noise makes the system switch from one state to the other. There is no true equilibrium (in the deterministic sense).
- Transition rate depends on
  - Depth of potential well (deterministic)
  - Noise intensity



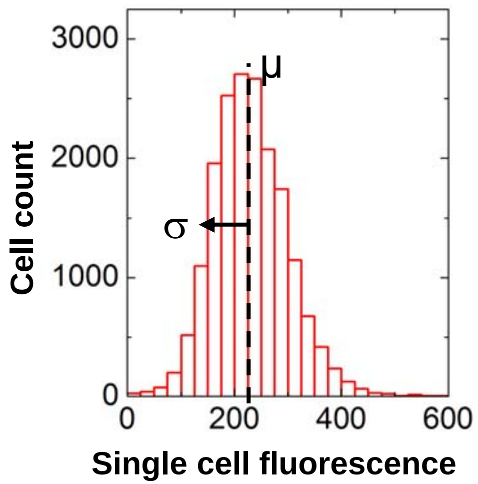


There are stochastic transitions between the two states

- Hysteresis shrinks over time

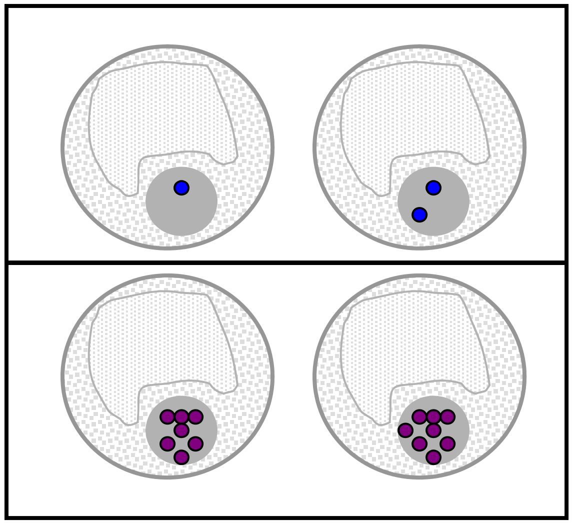


Where is noise coming from?
Small number effect



Noise

$$\eta = \frac{\sigma}{\mu}$$



Poisson's law

$$\eta_1 = \frac{1}{\sqrt{n}}$$

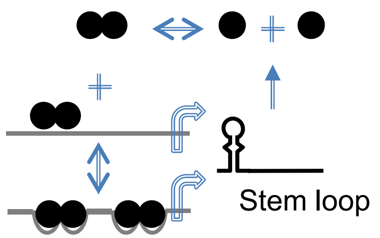
Noise — Number of molecules

- Poisson's law of small numbers (1837): fluctuations are large when few molecules are present in a cell (few events occur).
- mRNAs are the least abundant molecular species:
  - 75% of yeast mRNAs have less than 1 copy / cell, particularly mRNAs of transcriptional factors.

Noise (stochastic process) versus unknown
How to predict noise induced transitions?

- Biology is the kingdom of missing parameters
  - Even if they are available they scatter broadly (especially, the binding constants)
  - Hidden reactions

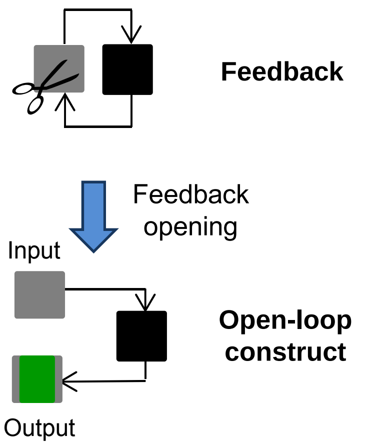
Dimeric protein



Cooperative promoter

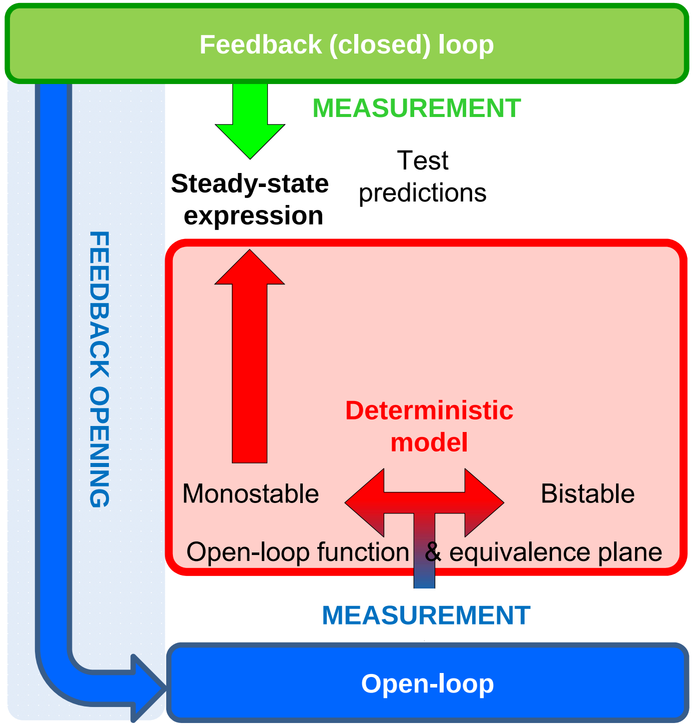
How to identify the deterministic bistability / potential wells?

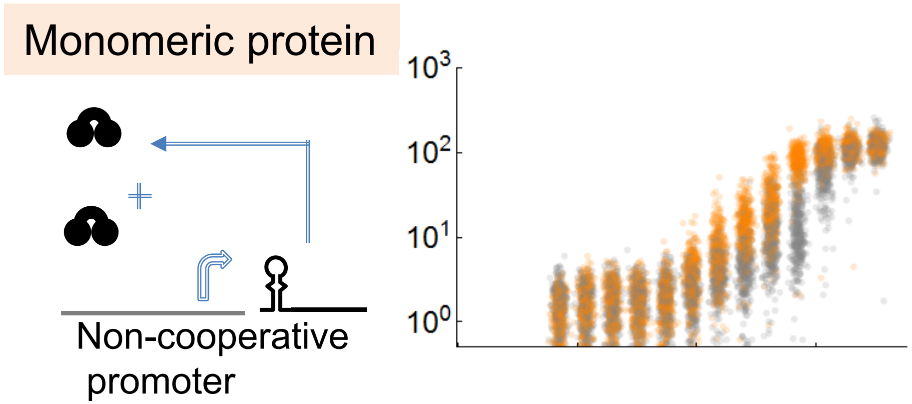
Open-loop function is the **total** response of all the reaction steps in the loop, without the need to resolve **any** of them individually.

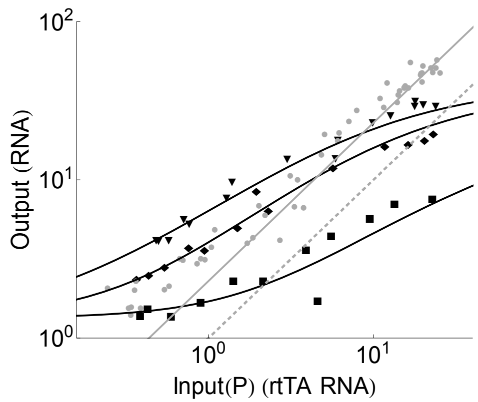


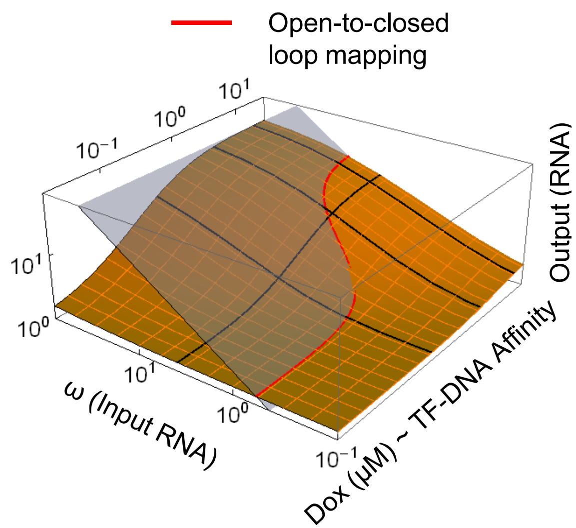
When input = output, then the feedback has a steady-state

Monostable feedback loop



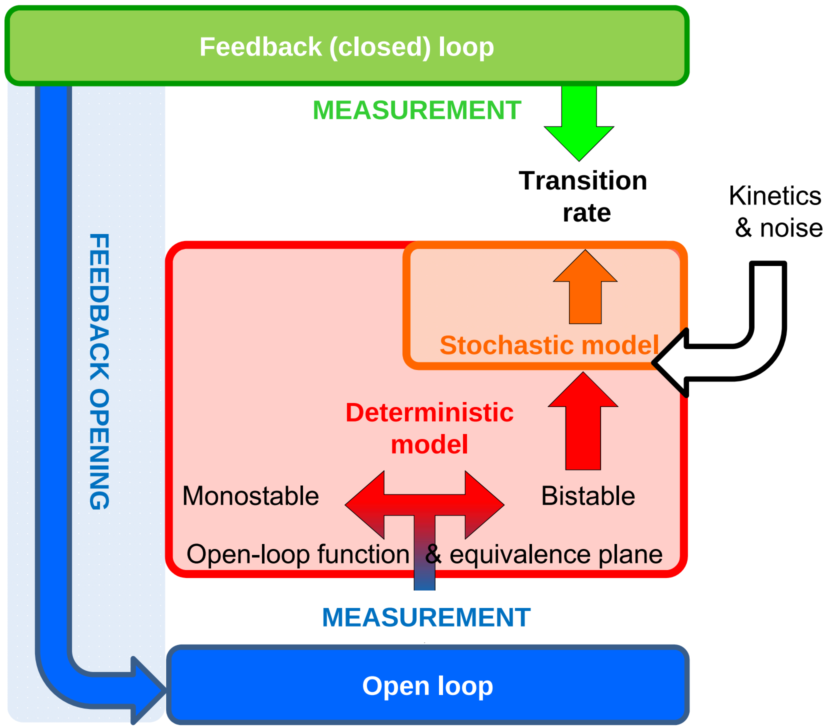


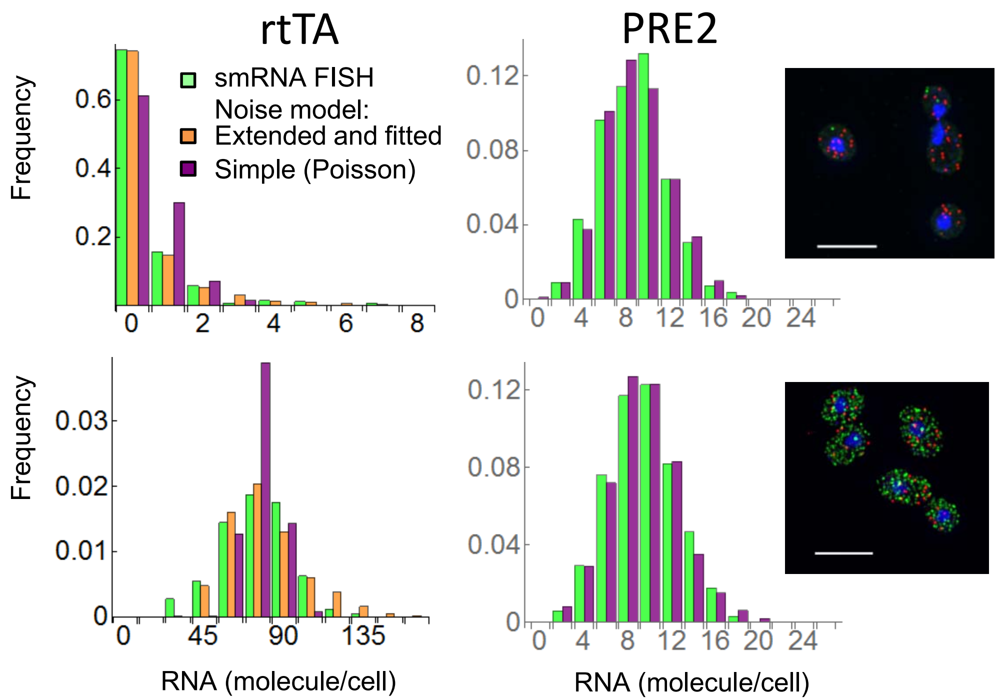


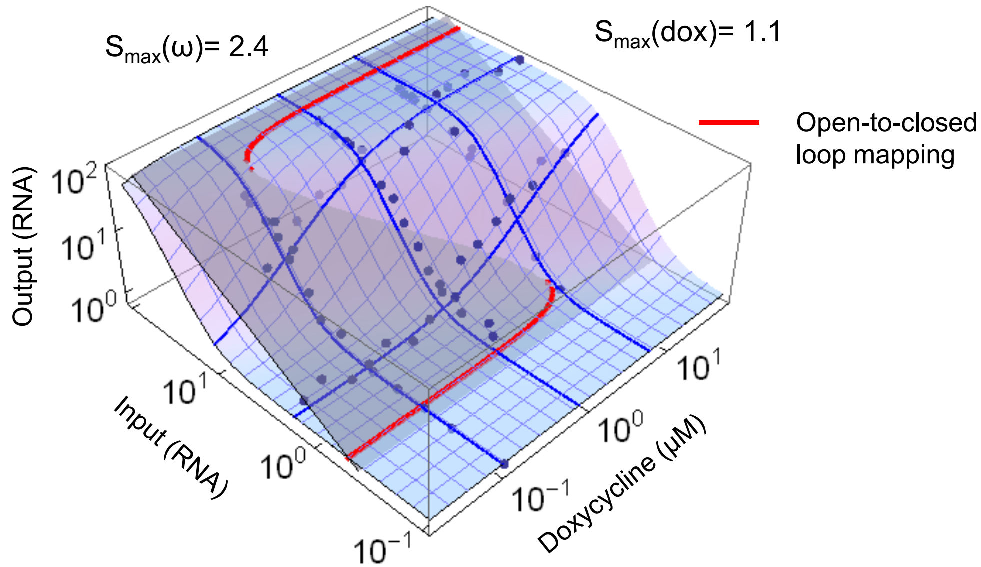


Prediction of transitions by combining deterministic stability and noise

Cooperative – dimeric circuit

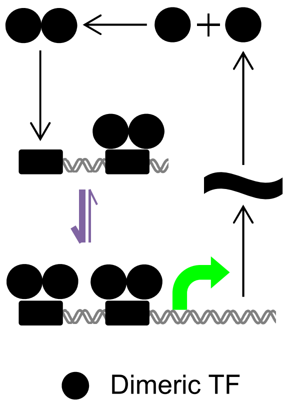


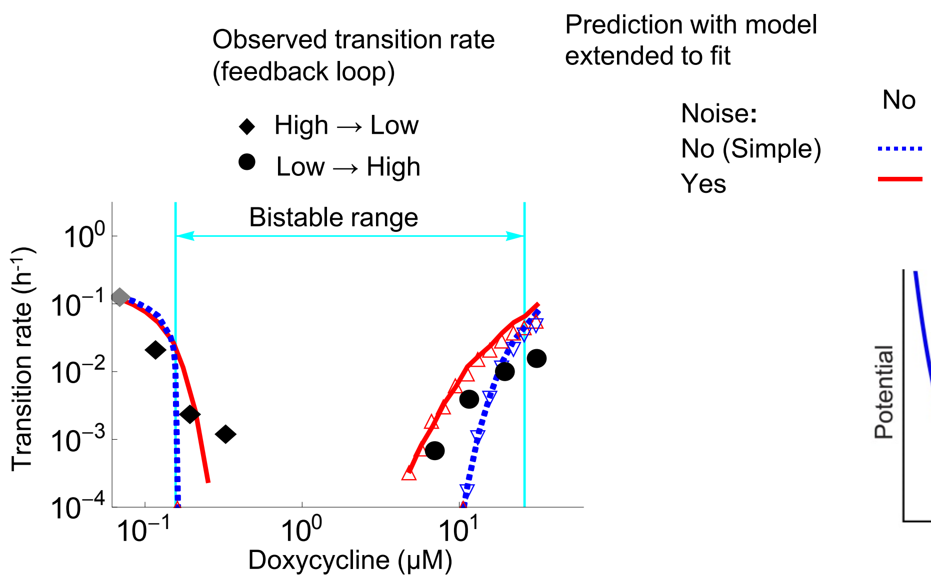


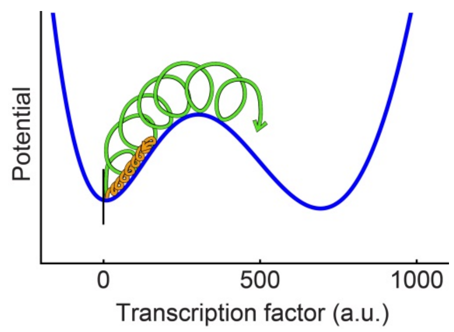


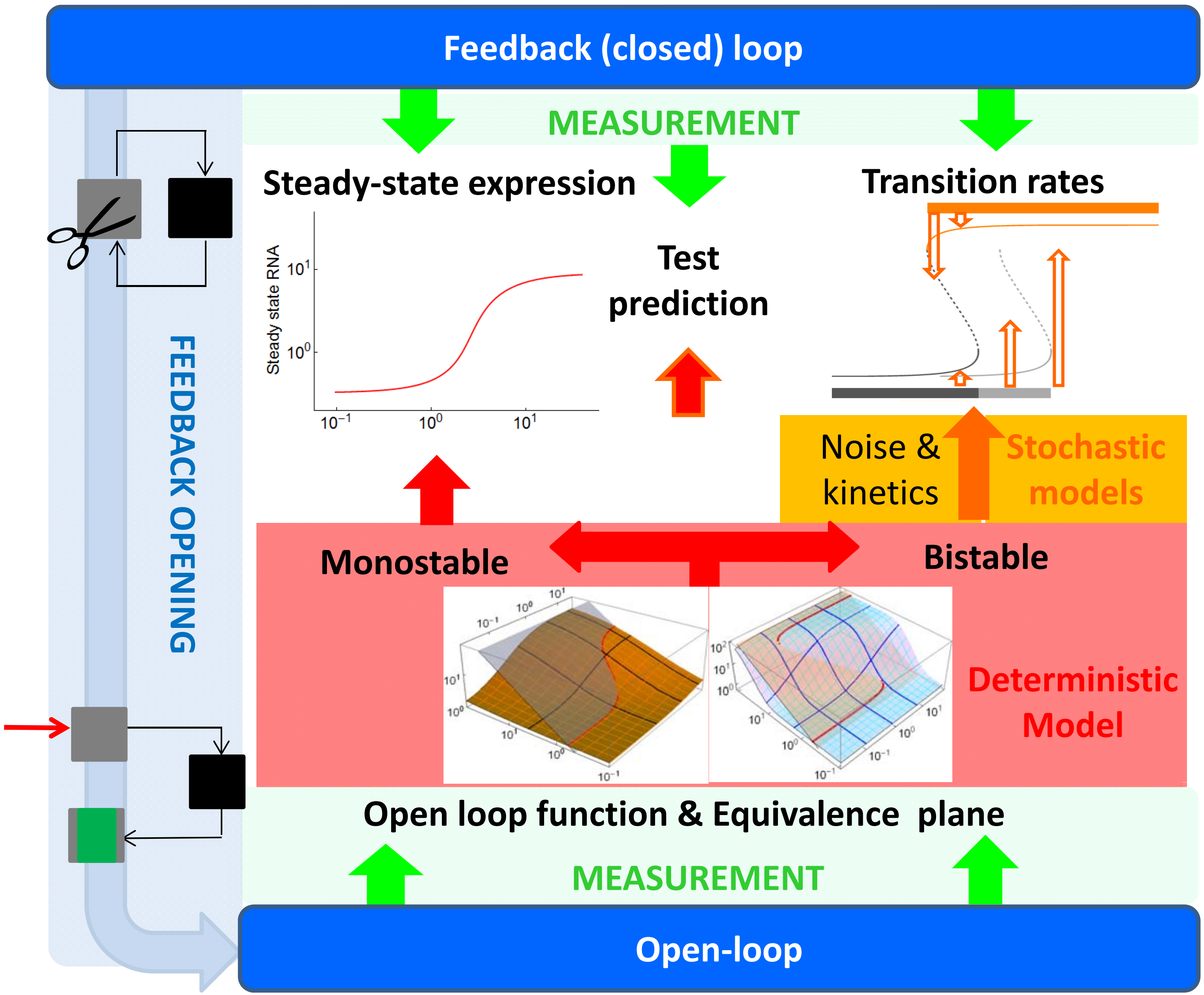
Comparison of predicted and measured transition rates

- Measured (stronger than Poisson) noise explains faster transitions
- Good agreement of measured and predicted noise








Feedback (closed) loop
MEASUREMENT
Steady-state expression
Transition rates
Steady state RNA
Test prediction
FEEDBACK OPENING
Noise & kinetics
Stochastic models
Monostable
Bistable
Deterministic Model
Open loop function & Equivalence plane
MEASUREMENT
Open-loop

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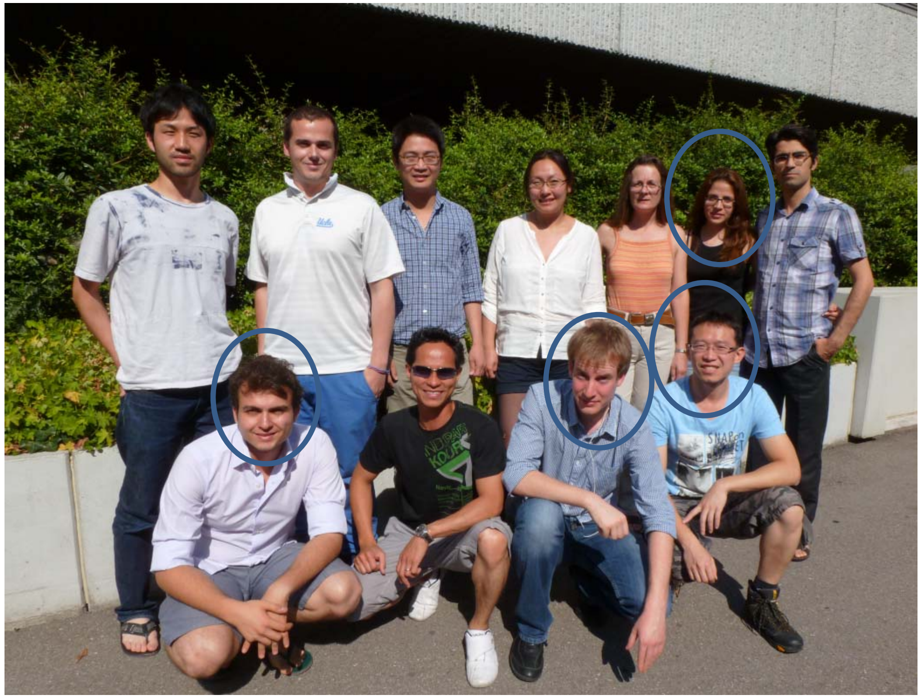

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