Redesigning a bacterial two-component system to exhibit desired responses

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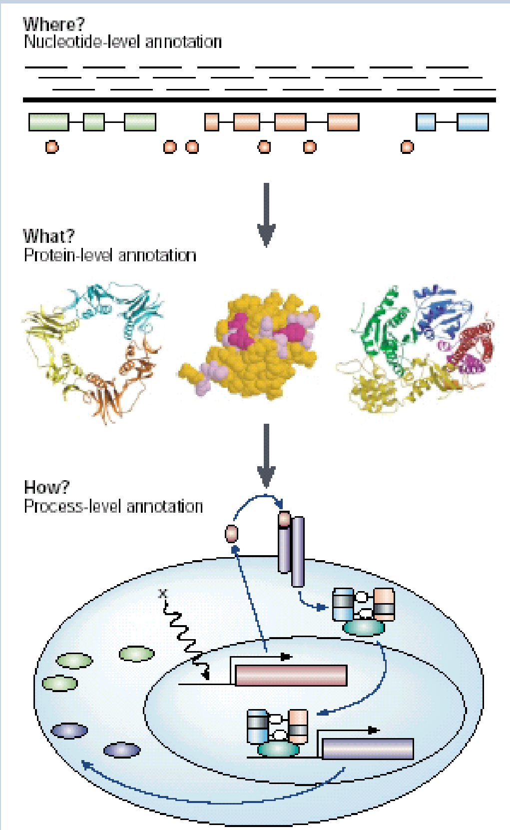
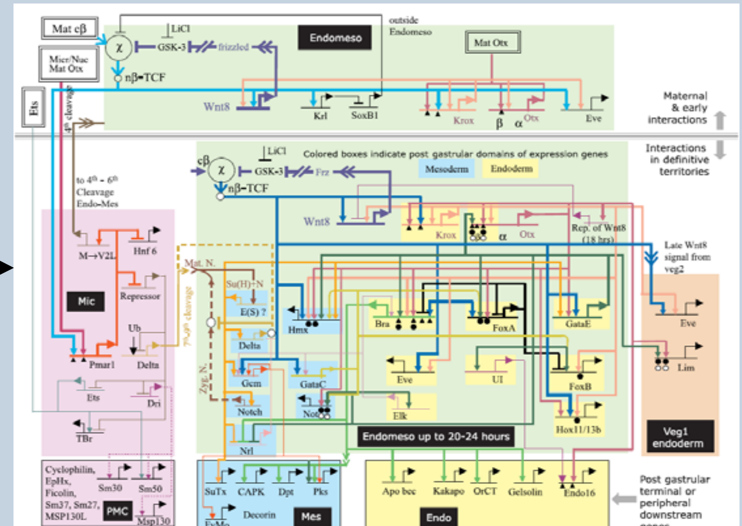
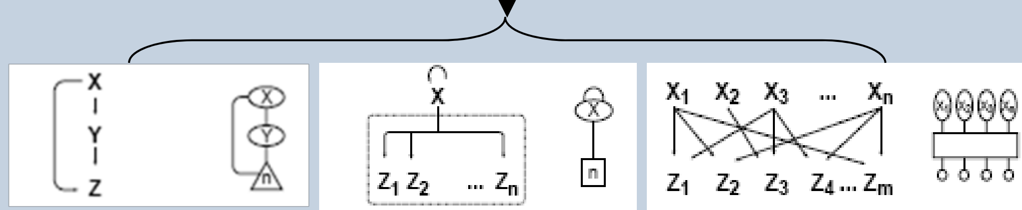
Modern biology: from sequences to networks to modules



Stein L., *Nat Rev Genet.*, 2001



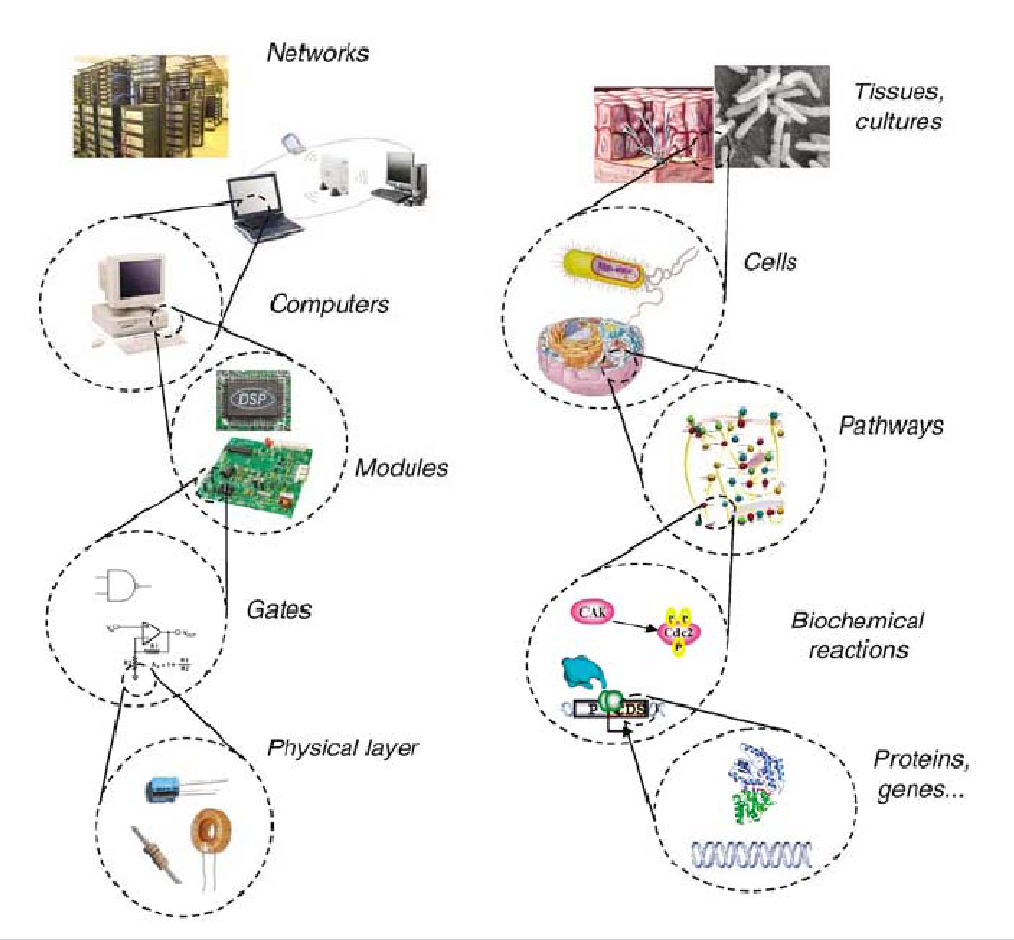
E. H. Davidson et al., *Science*, 2002



R. Milo et al., *Science*, 2002

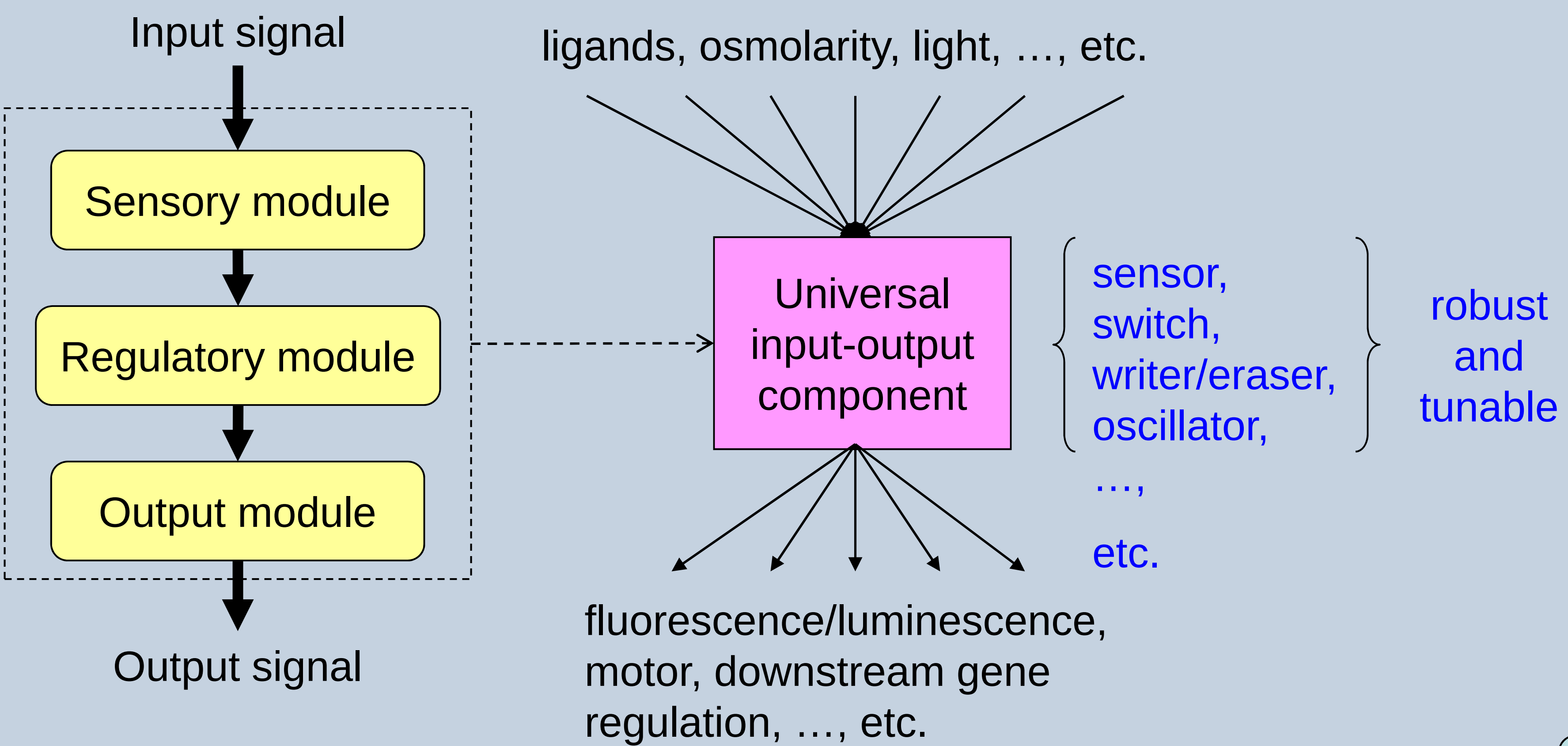
Modular, bottom-up synthetic biology

- Challenge: designing and constructing biological components with robust and desired responses, and scale-up to higher organizational levels.
- Idea: Can we design a universal biological component which consists of simple modular elements, and has potential to exhibit many different responses as desired?

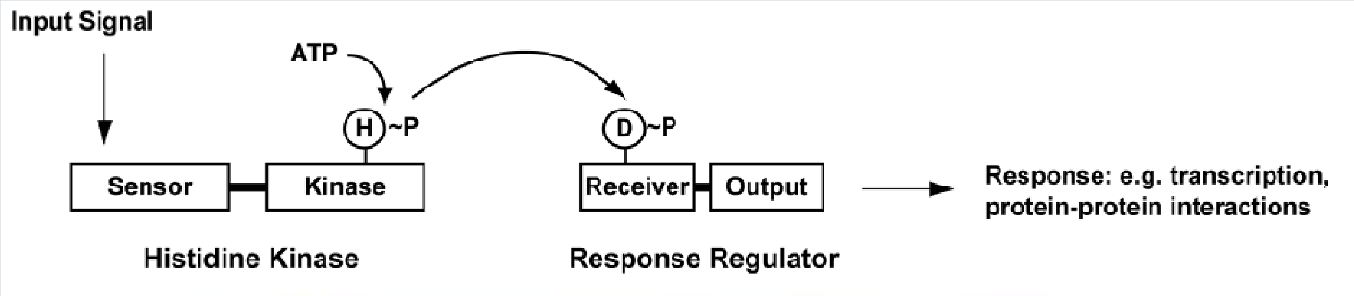


E. Andrianantoandro et al., *Mol. Syst. Biol.*, 2006

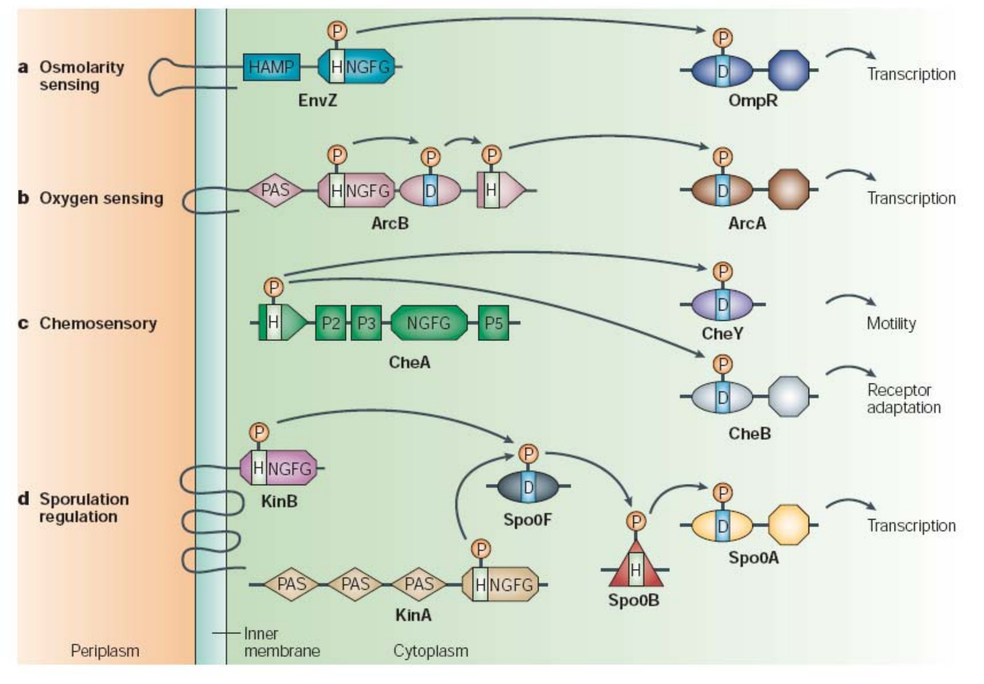
Idea of designing a universal input-output component for synthetic biology



The modular, specific, and speedy bacterial two-component system as a target to redesign

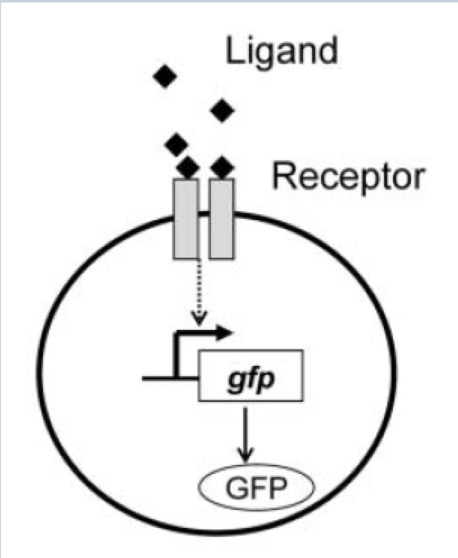


Skerker *et al.*, 2005

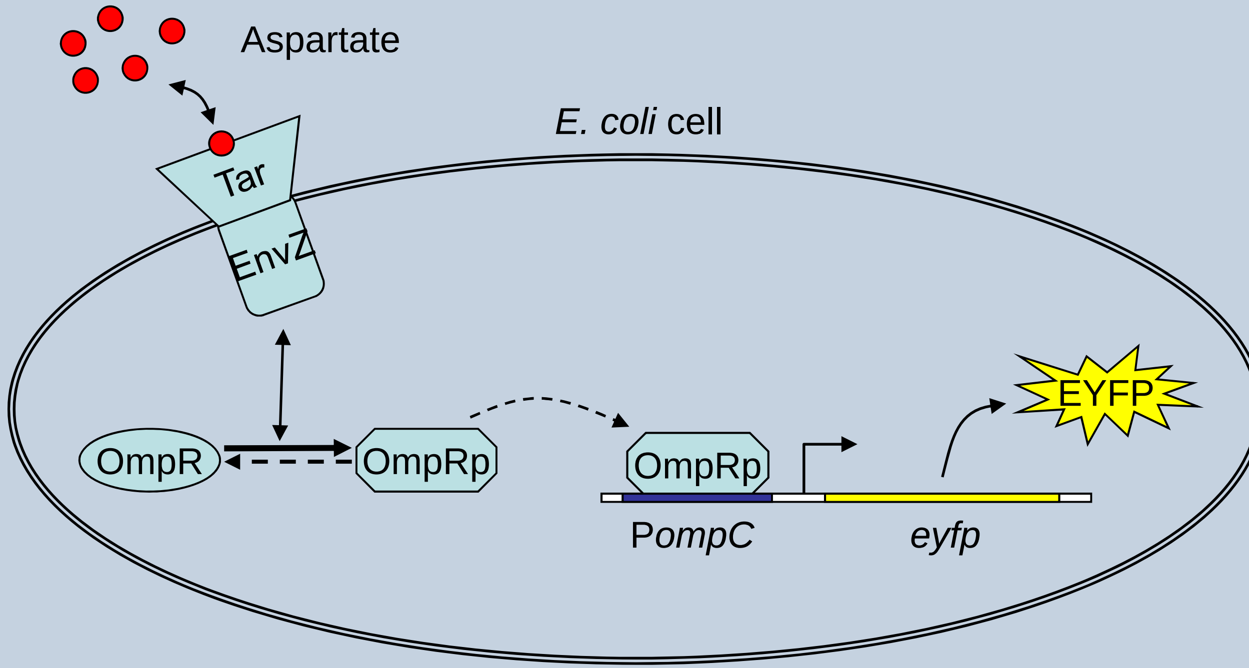


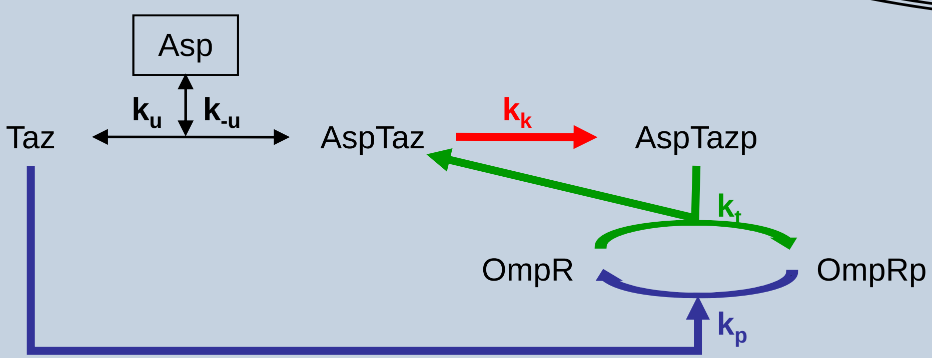
Wadhams and Armitage, 2004

The chimeric Taz-OmpR system



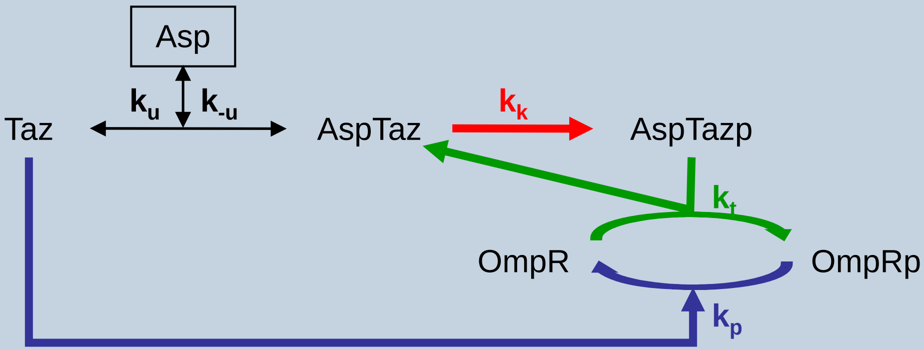
C. Tan et al., *Mol. BioSyst.*, 2007





- k_u/k_{-u}: binding/dissociation of aspartate
- k_k: autophosphorylation of Taz
- k_t: phosphotransfer from Taz to OmpR
- k_p: phosphatase activity of Taz

Part I: The robust, switch-like Taz-OmpR system



Solving two different ODE models at steady-state

without considering ATP/ADP

$$\mathrm{u + T} \underset{k\text{-}u}{\overset{ku}{\rightleftharpoons}} \mathrm{uT} \xrightarrow{kk} \mathrm{uTp}$$

$$\mathrm{uTp + R} \underset{k\text{-}1}{\overset{k1}{\rightleftharpoons}} \mathrm{uTpR} \xrightarrow{kt} \mathrm{uT + Rp}$$

$$\mathrm{T + Rp} \underset{k\text{-}2}{\overset{k2}{\rightleftharpoons}} \mathrm{TRp} \xrightarrow{kp} \mathrm{T + R}$$

Rp = (km2+kp)*kk*ku*u / (kp*k2*kmu) = C_1*u

with considering ATP/ADP

$$\mathrm{u + T} \underset{k\text{-}u}{\overset{ku}{\rightleftharpoons}} \mathrm{uT}$$

$$\mathrm{A + T} \underset{k\text{-}A}{\overset{kA}{\rightleftharpoons}} \mathrm{TA}$$

$$\mathrm{A + uT} \underset{k\text{-}A}{\overset{kA}{\rightleftharpoons}} \mathrm{uTA} \xrightarrow{kk} \mathrm{uTp + D}$$

$$\mathrm{uTp + R} \underset{k\text{-}1}{\overset{k1}{\rightleftharpoons}} \mathrm{uTpR} \xrightarrow{kt} \mathrm{uT + Rp}$$

$$\mathrm{TA + Rp} \underset{k\text{-}2}{\overset{k2}{\rightleftharpoons}} \mathrm{TARp} \xrightarrow{kp} \mathrm{TA + R}$$

u ~ U_{total} when U_{total} >> T_{total}

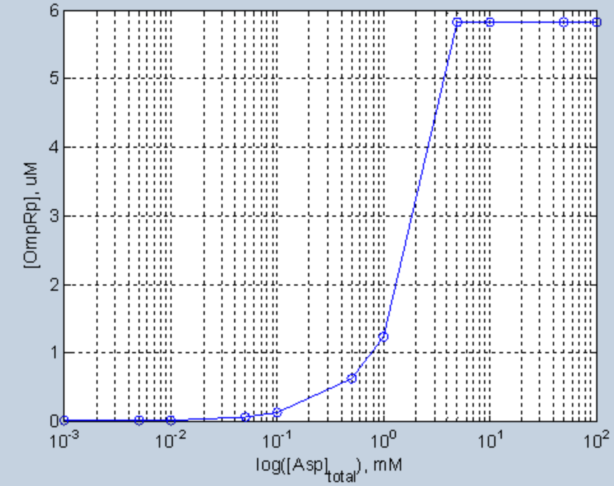
Rp = (km2+kp)*kk*ku*u*kmA / (kp*k2*kmu*(kmA+kk)) = C_2*u

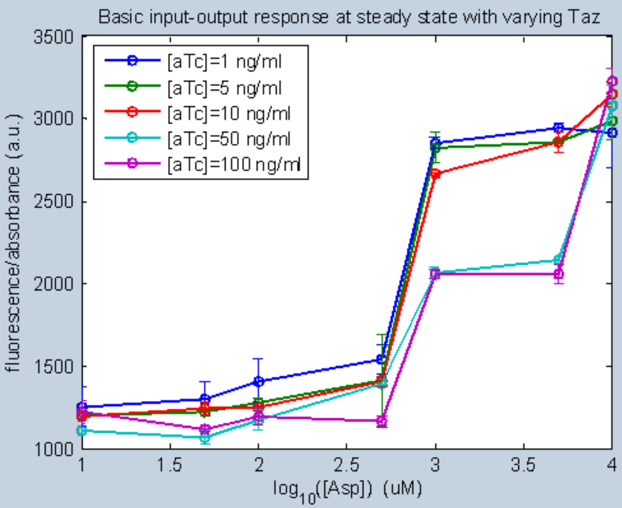
The intrinsic robustness of the Taz-OmpR system also makes it less tunable

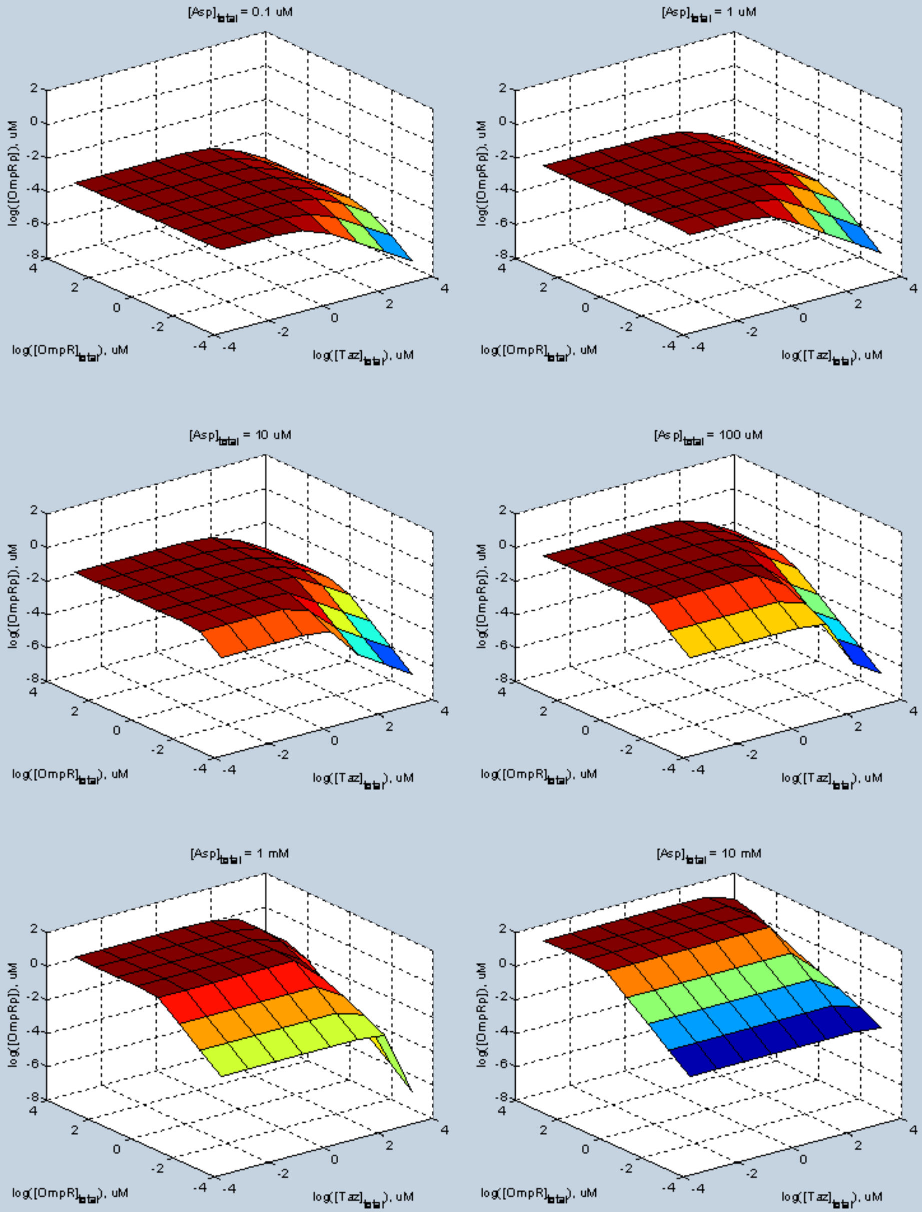
- The original Taz-OmpR system acts as a robust switch. The steady-state output of the system is conditionally robust within the range of normal biological conditions.

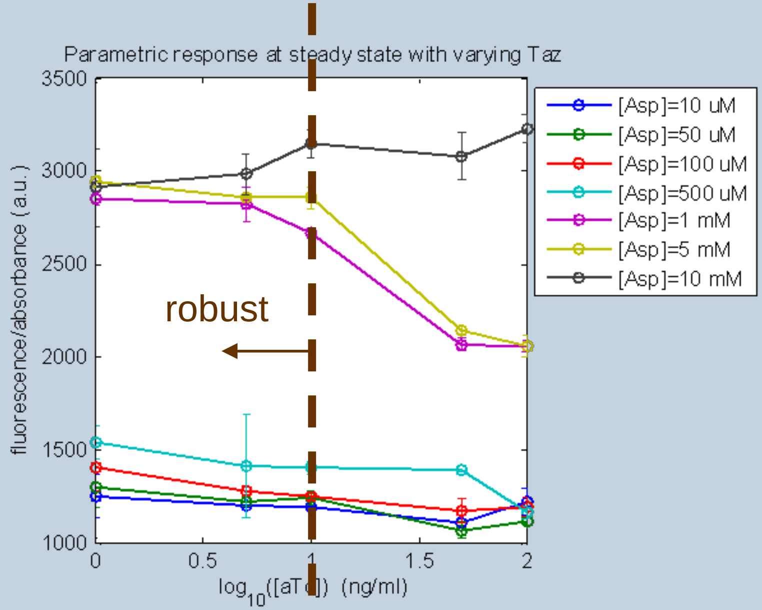
- The output is tunable only at high $[Taz_T]$ low $[OmpR_T]$ with high $[Asp_T]$.

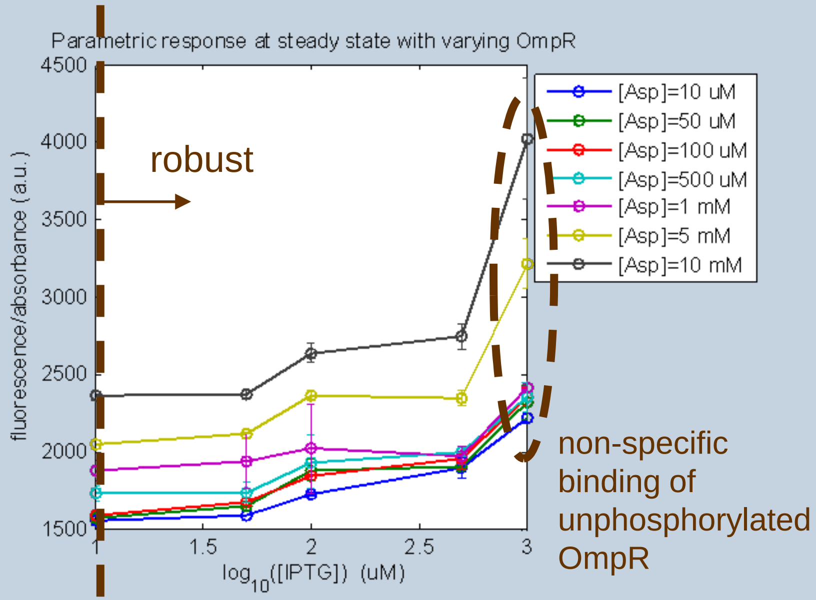
- However, over-expressing membrane protein Taz may cause toxicity to the cell, and deleting the high-level regulator OmpR may mess up the whole regulation network in the cell.



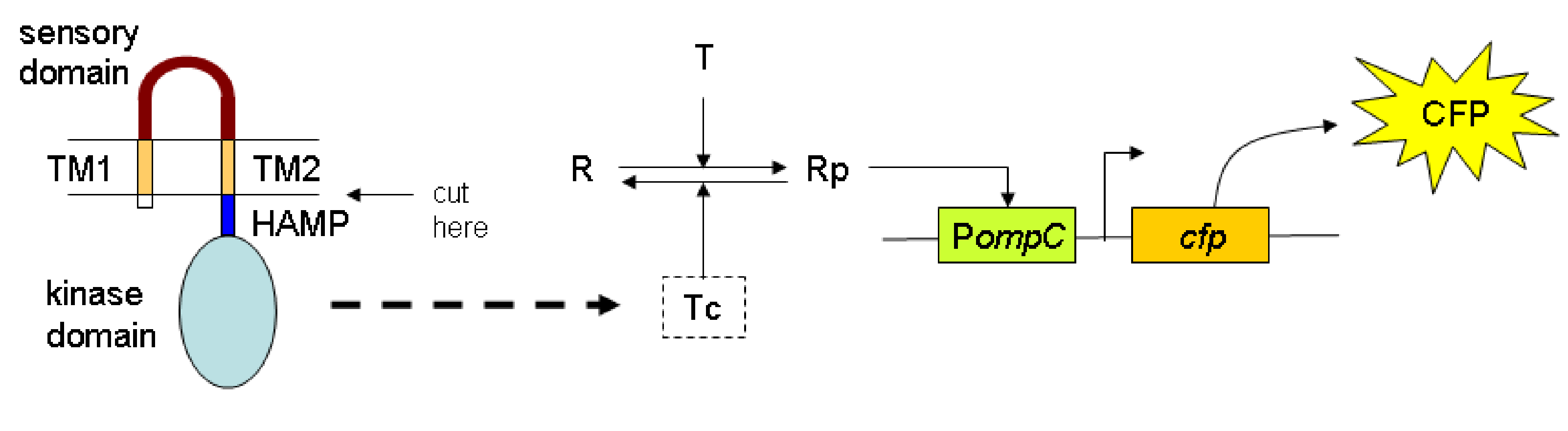


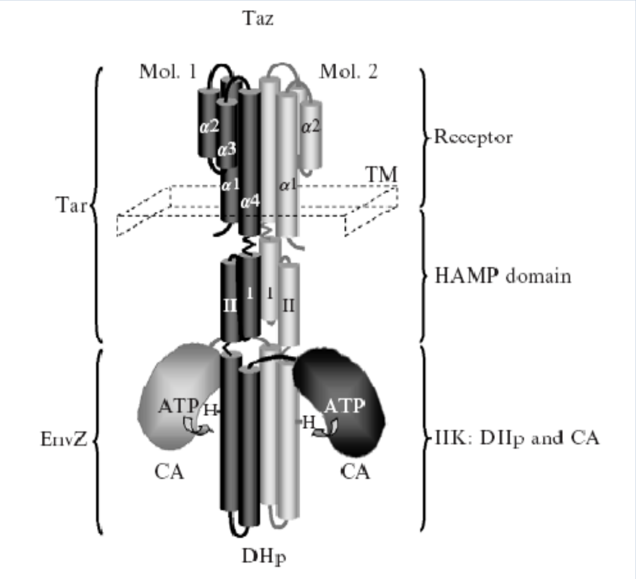

[Asp]total = 0.1 uM
[Asp]total = 1 uM
[Asp]total = 10 uM
[Asp]total = 100 uM
[Asp]total = 1 mM
[Asp]total = 10 mM
log([OmpRp]), uM
log([OmpR]total), uM
log([Taz]total), uM


Parametric response at steady state with varying Taz
fluorescence/absorbance (a.u.)
log10([aTc]) (ng/ml)
[Asp]=10 uM
[Asp]=50 uM
[Asp]=100 uM
[Asp]=500 uM
[Asp]=1 mM
[Asp]=5 mM
[Asp]=10 mM
robust


Parametric response at steady state with varying OmpR
fluorescence/absorbance (a.u.)
log10([IPTG]) (uM)
[Asp]=10 uM
[Asp]=50 uM
[Asp]=100 uM
[Asp]=500 uM
[Asp]=1 mM
[Asp]=5 mM
[Asp]=10 mM
robust
non-specific binding of unphosphorylated OmpR

Part II: The graded, tunable writer-eraser design

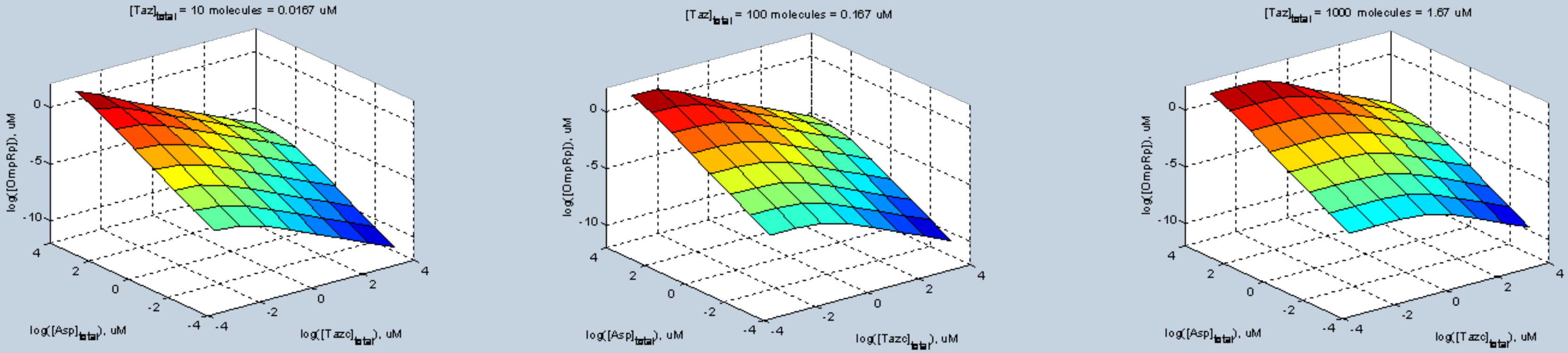


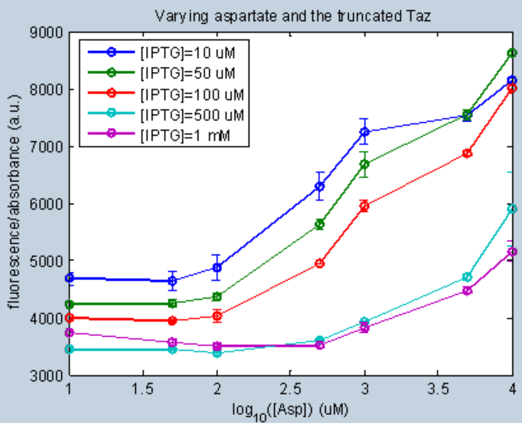


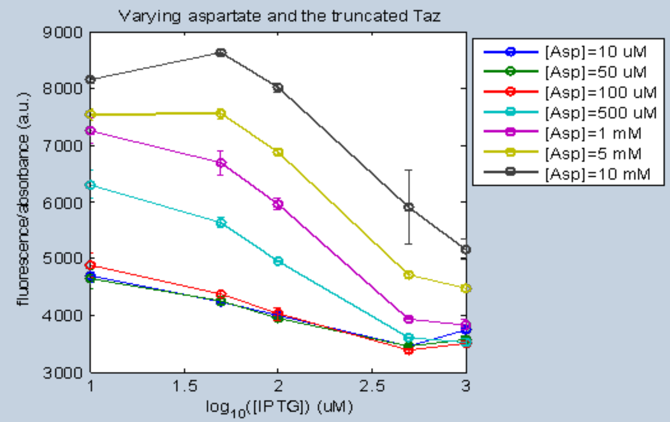
Yoshida *et al.*, 2007

- For the original Taz-OmpR system, it is difficult to tune the steady-state level of output OmpRp in biological conditions.
- The truncated version of Taz, Tazc, consisting of only the HAMP domain and the kinase domain, can be an additional phosphatase for the redesigned system.

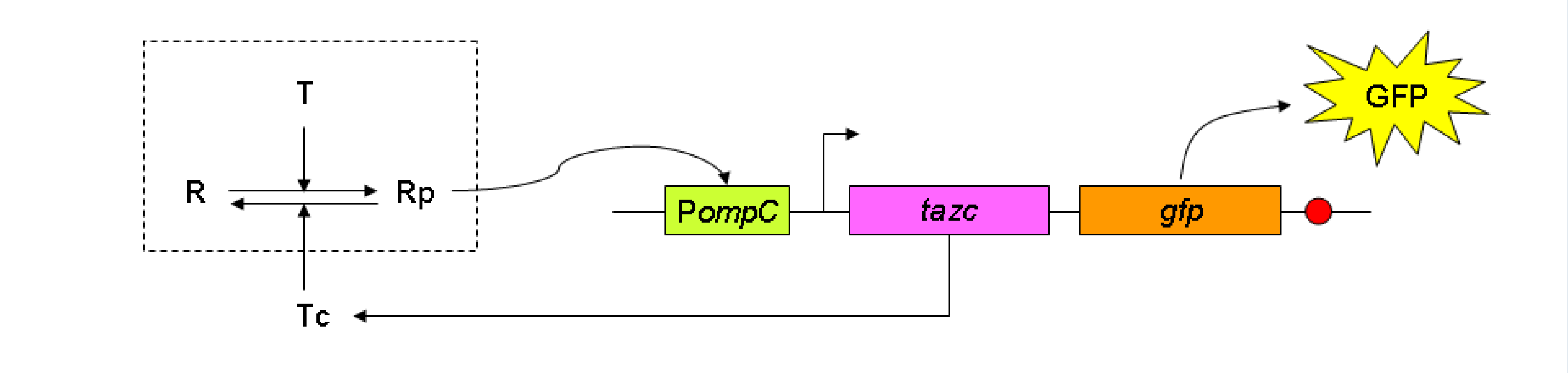
- The steady-state level of OmpRp can be tuned in a wide range, and can still respond to the level of input aspartate.
- The response becomes more graded, i.e., the linear region is much larger than the original system.
- In short, the system is redesigned from a robust switch to a tunable sensor.





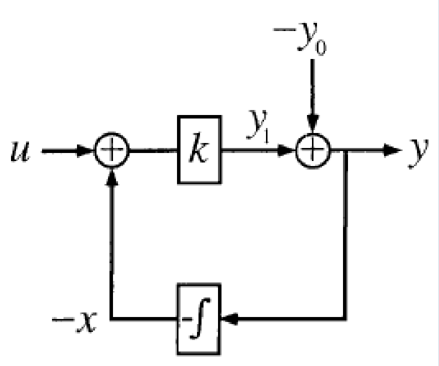


Part III: The adaptive feedback design

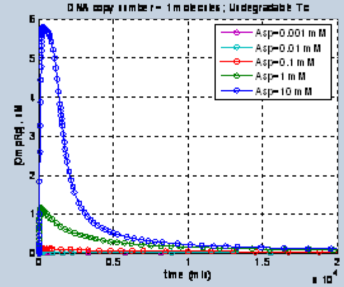


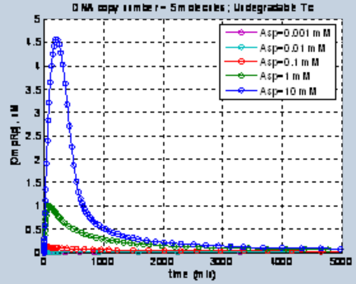
- Using P*ompC* to control the expression of the truncated Taz in order to make a negative feedback for the Taz-OmpR system.

- In theory, adaptation can happen within some parameter regions. However in experiment only two parameters are able to be tuned:
  - The copy number of plasmid carrying Tazc
  - The degradation rate of Tazc.

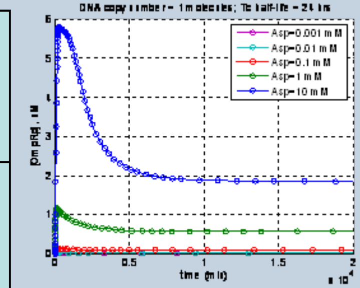


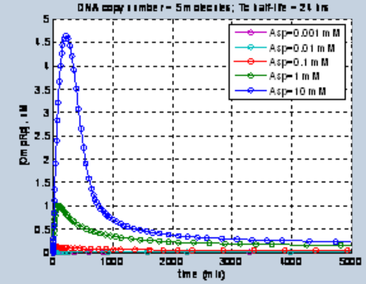
T.-M. Yi et al., *PNAS*, 2000

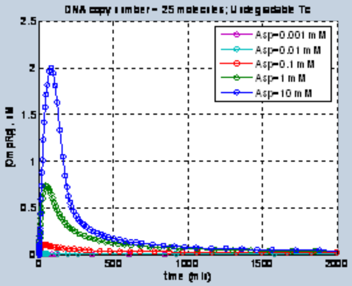


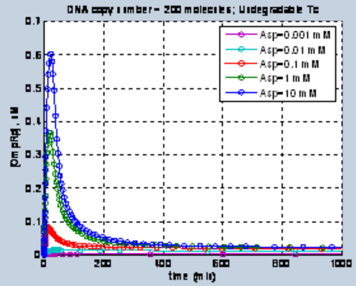


1 copy (genome)	5 copy (pSC101)
25 copy (p15A)	**200 copy (pUC19)**







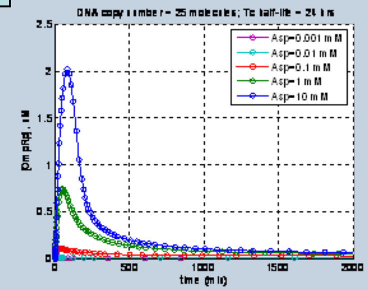


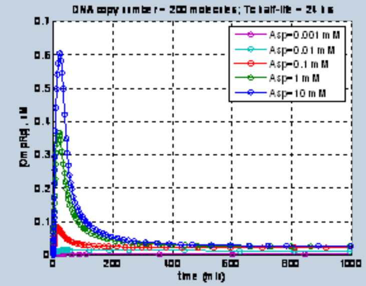
not degrade

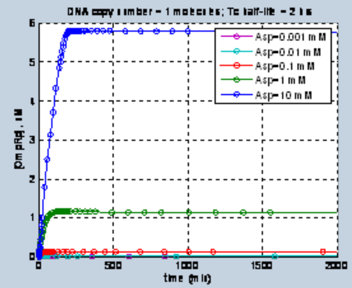
(hypo.)

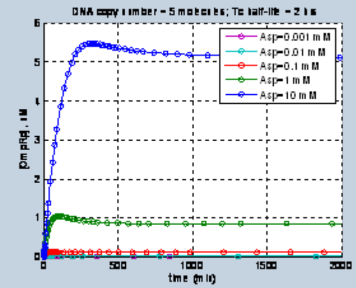
half-life 24 hrs

(hypo.)







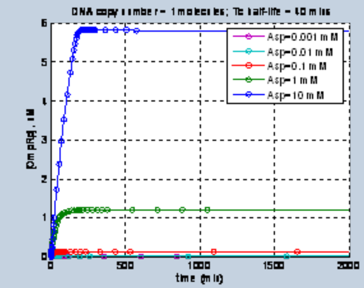


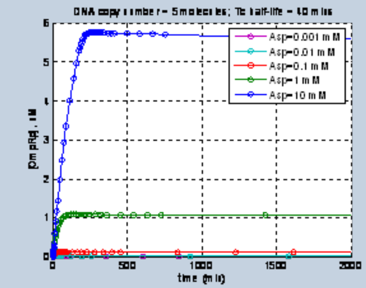
half-life 2 hrs

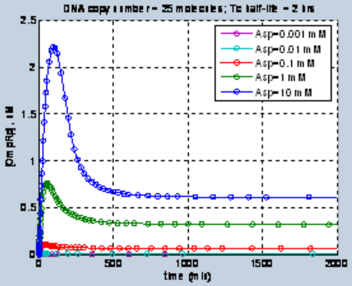
(ASV tag)

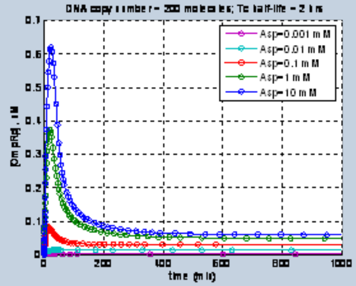
half-life 40 mins

(LVA tag)

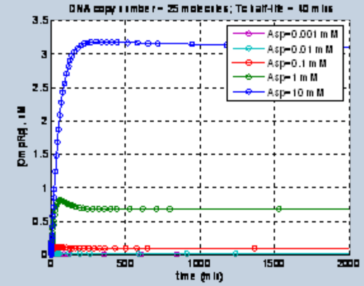


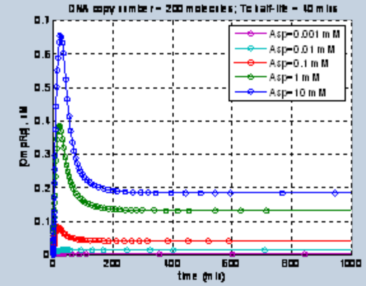






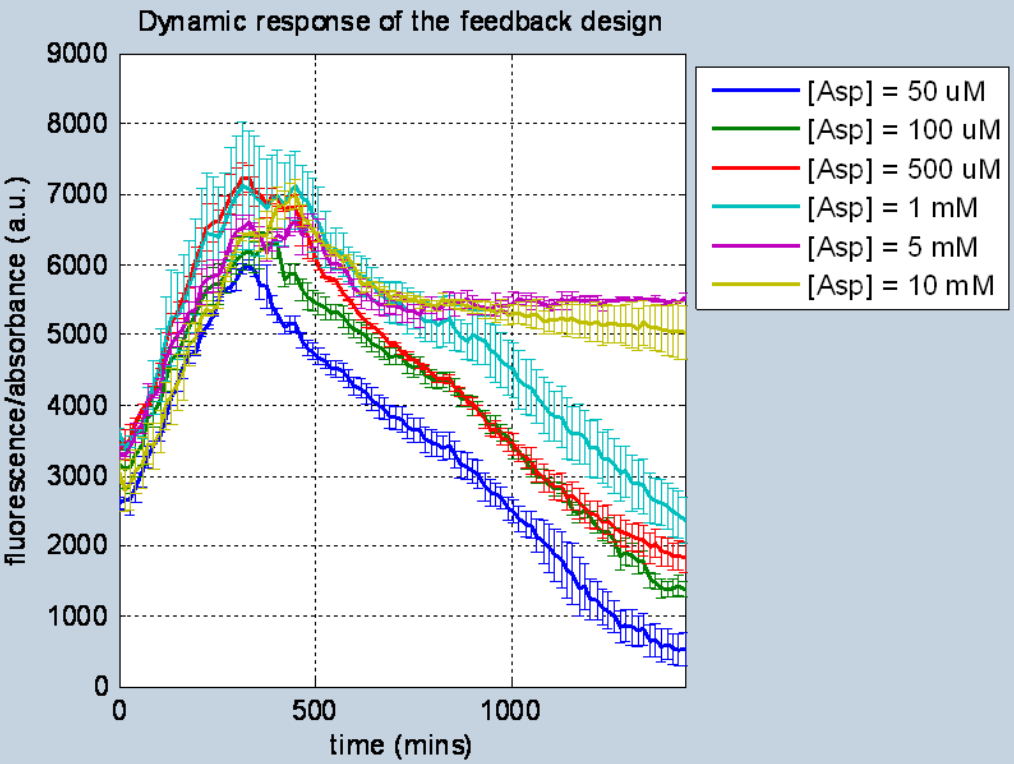
- Best guess for experiment: high-copy plasmid & untagged Tazc



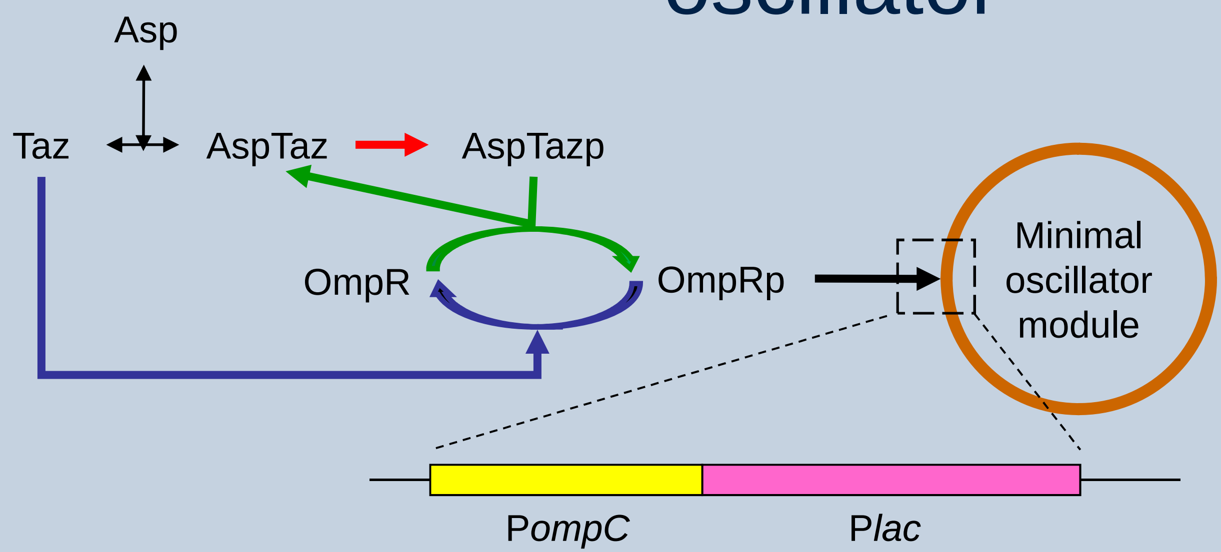


Experiments for the adaptive feedback design

- The redesigned system exhibits adaption in experiment.
- The remaining output level at steady state is tunable by the level of input aspartate.



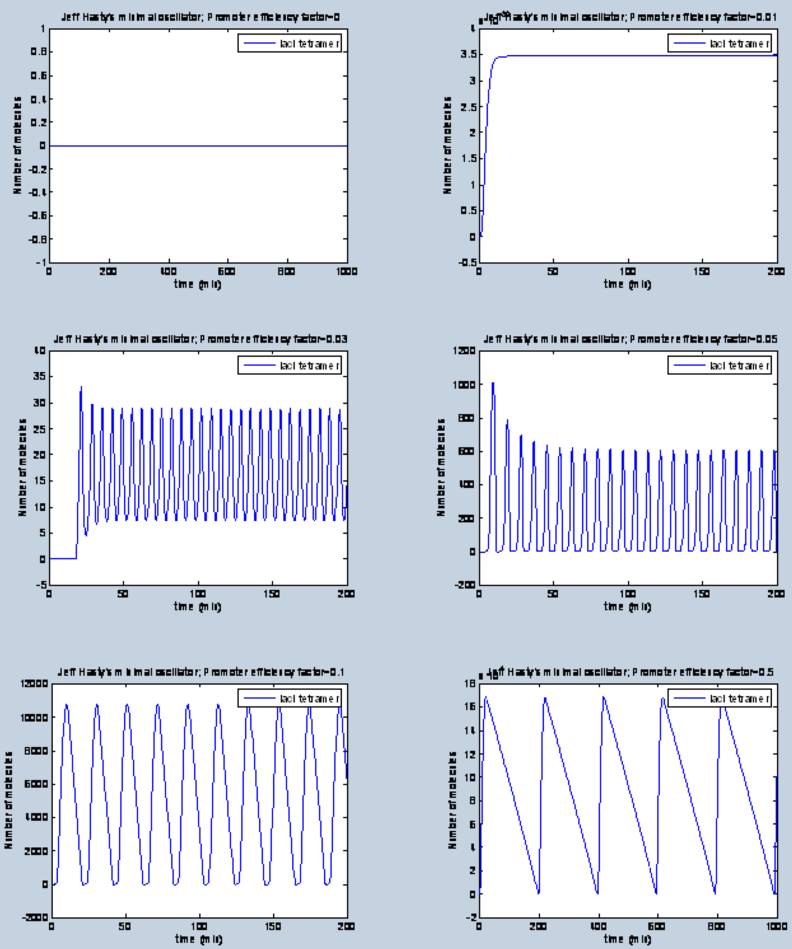
Part IV: The design of an input-driven oscillator

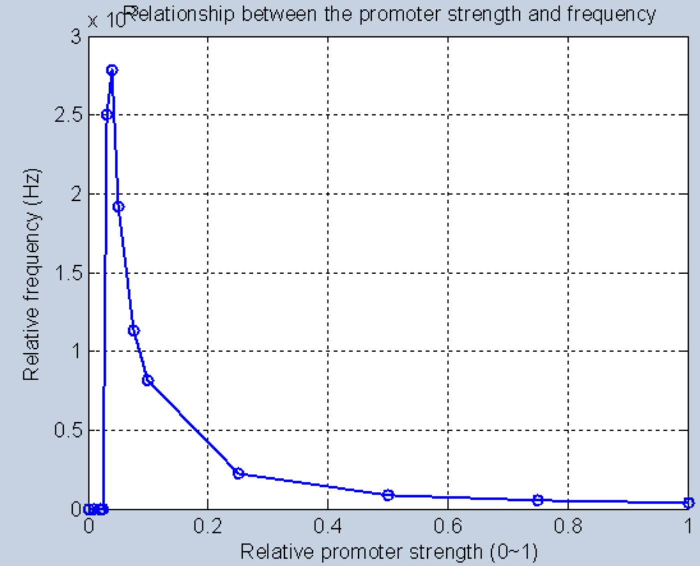


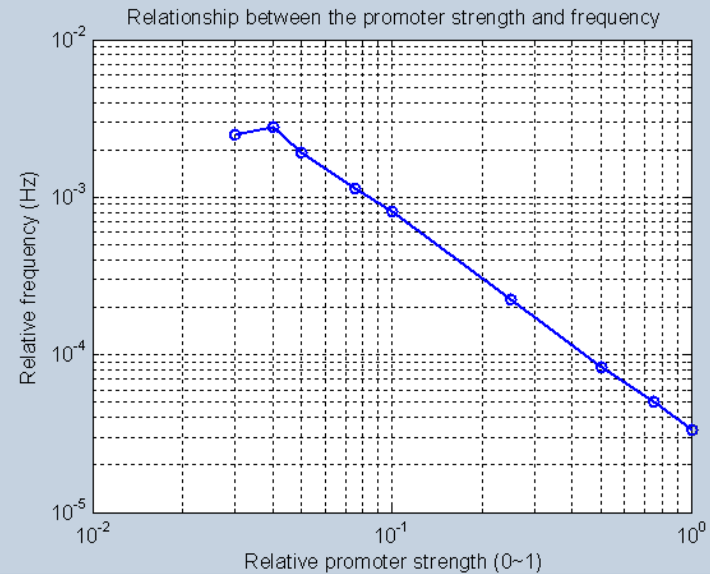
function generator

- A combinational promoter which consists of P*ompC* and part of P*lac* is designed to connect the Taz-OmpR system with a minimal oscillatior module.
- The oscillation is driven by the input aspartate, and the frequency is based on the level of input aspartate, like a biological function generator.

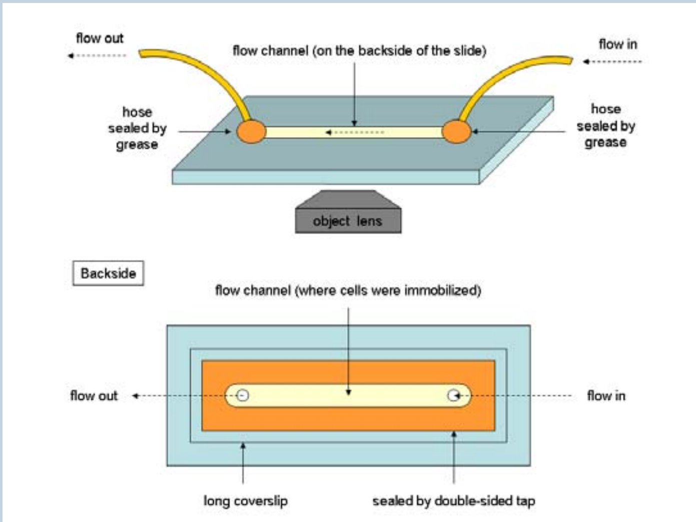
Simulations for the input-driven oscillator

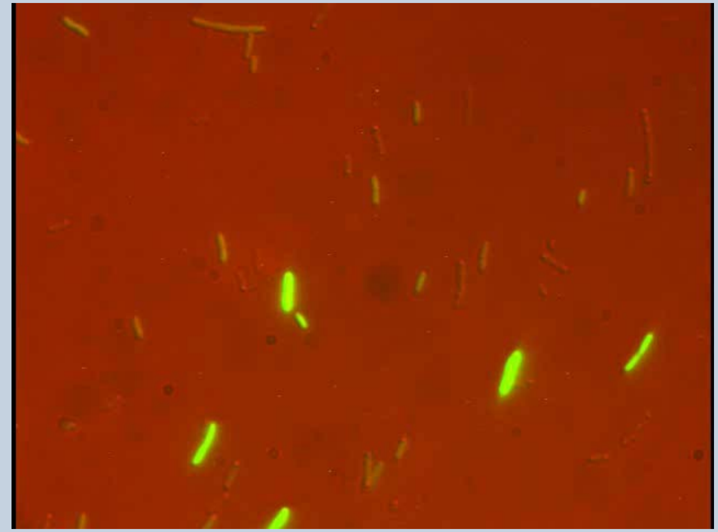


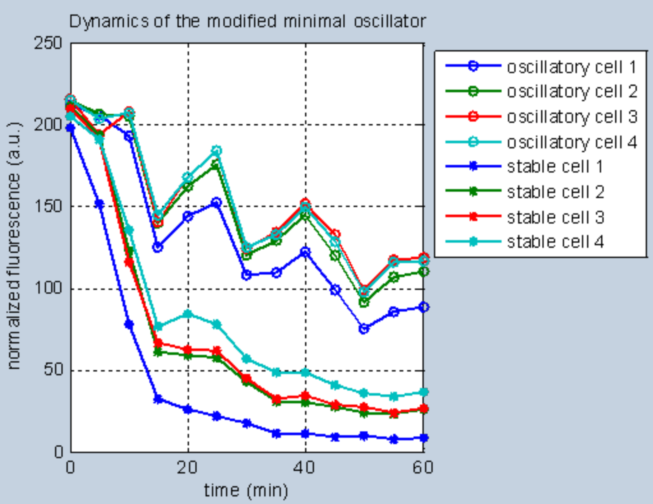


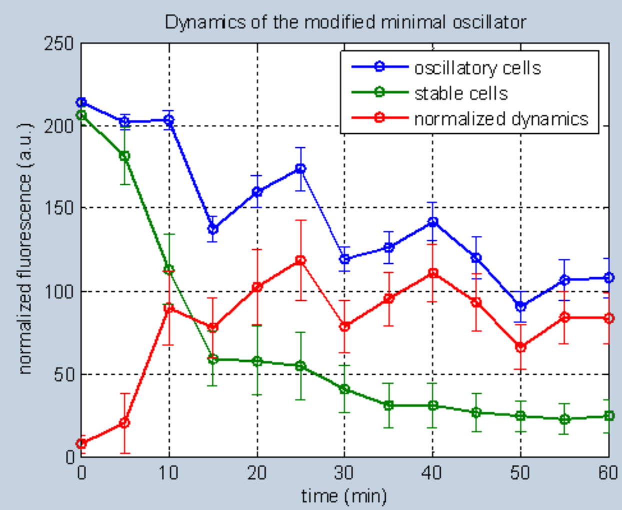


Experiments for the input-driven oscillator



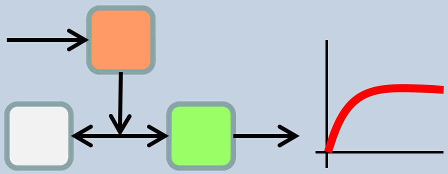




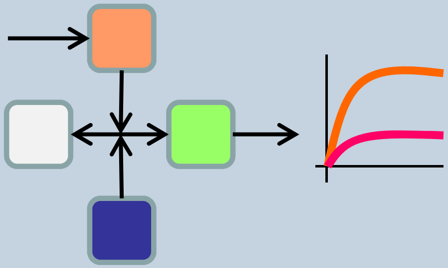


Summary: Redesigning a bacterial two-component system to exhibit desired responses

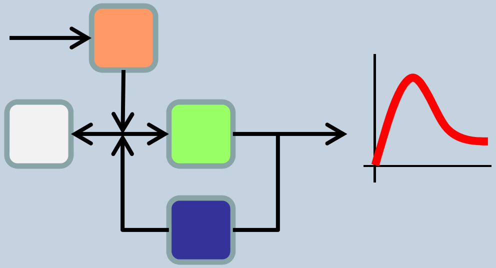
(1) Robust switch



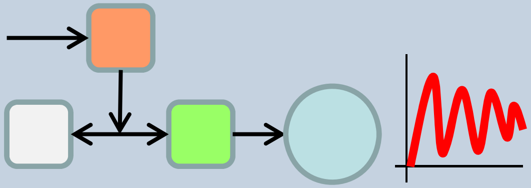
(2) Tunable sensor



(3) Adaptive feedback network

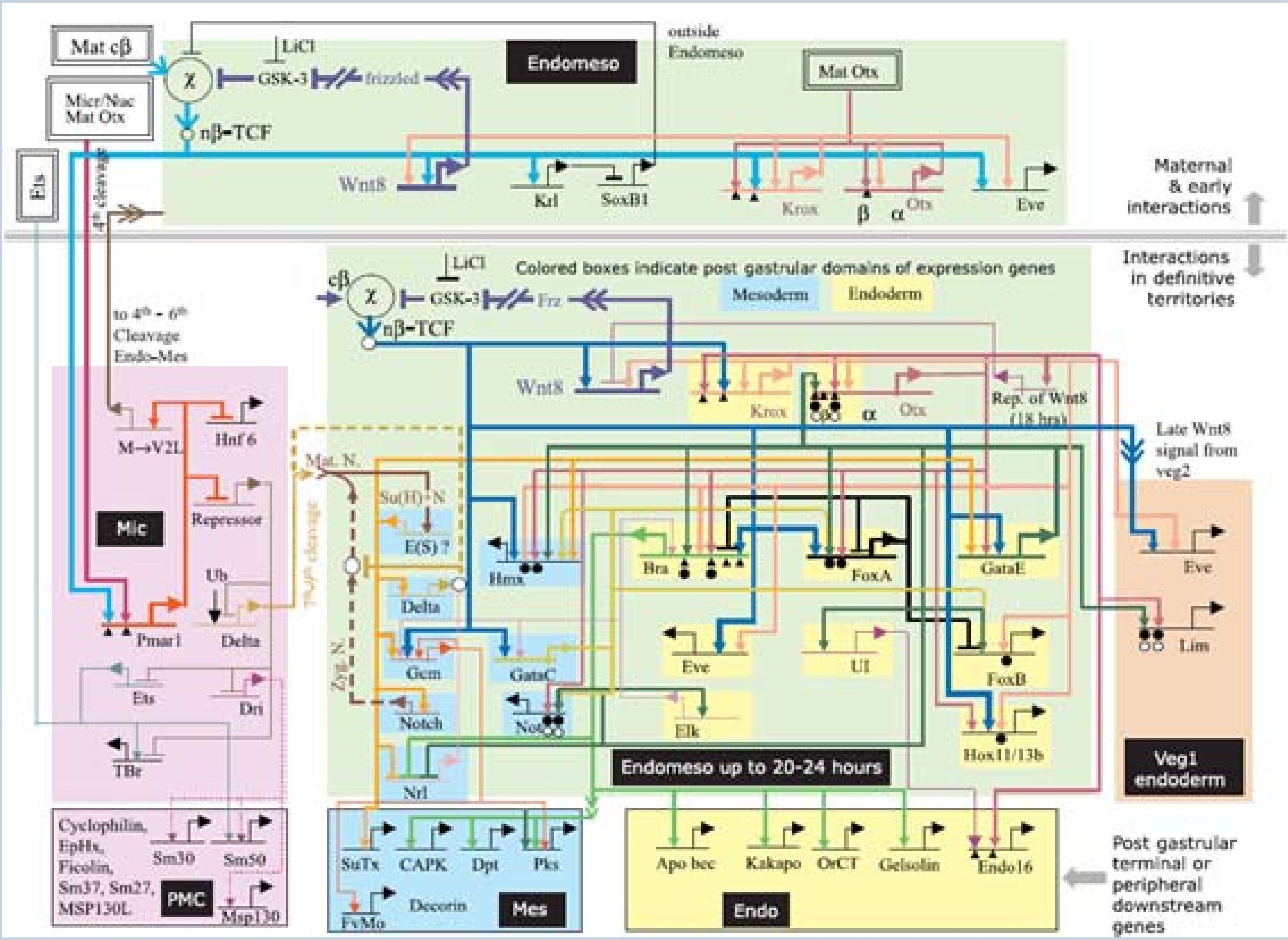


(4) Input-driven oscillator



The sensory, regulatory and output modules are all switchable now!

How to deal with large biological networks?



Binding/dissociation

$$
\begin{aligned}
\frac{d[mRNA(DnaK)]}{dt} &= Ktr_1.[\sigma^{32}: RNAP : ph] - \alpha_{mRNA}.[mRNA(DnaK)] \\
\frac{d[DnaK_t]}{dt} &= K_{TL}.[mRNA(DnaK)] - \alpha_{prot}.[DnaK_t] \\
\frac{d[mRNA(FtsH)]}{dt} &= Ktr_2.[\sigma^{32}: RNAP : ph] - \alpha_{mRNA}.[mRNA(FtsH)] \\
\frac{d[FtsH_t]}{dt} &= K_{TL}.[mRNA(FtsH)] - \alpha_{prot}.[FtsH_t] \\
\frac{d[mRNA(protease)]}{dt} &= Ktr_3.[\sigma^{32}: RNAP : ph] - \alpha_{mRNA}.[mRNA(protease)] \\
\frac{d[protease_t]}{dt} &= K_{TL}.[mRNA(protease)] - \alpha_{prot}.[protease_t] \\
\frac{d[mRNA(HslVU)]}{dt} &= Ktr_4.[\sigma^{32}: RNAP : ph] - \alpha_{mRNA}.[mRNA(HslVU)] \\
\frac{d[HslVU_t]}{dt} &= K_{TL}.[mRNA(HslVU)] - \alpha_{prot}.[HslVU_t] \\
\frac{d[mRNA(\sigma^{32})]}{dt} &= Ktr_5.[\sigma^{70}: RNAP : pg] - \alpha_{mRNA}.[mRNA(\sigma^{32})] \\
\frac{d[\sigma^{32}_t]}{dt} &= \eta(T) \cdot K_{TL} \cdot [mRNA(\sigma^{32})] - r\alpha_{prot}.[\sigma^{32}_f] - \alpha_{FtsH}.[\sigma^{32}: DnaK : FtsH] \\
&\quad - \alpha_{protease}(T).[\sigma^{32}:DnaK:protease] - \alpha_{HslVU}(T).[\sigma^{32} : HslVU] \\
\frac{d[Pfolded]}{dt} &= K_{fold}.[Punfolded : DnaK] - K(T).[Pfolded]
\end{aligned}
$$

Gene expressions

$$
\begin{aligned}
[\sigma^{70}: RNAP] &= K_1.[\sigma^{70}_f].[RNAP_f] \\
[\sigma^{32}: RNAP] &= K_2.[\sigma^{32}_f].[RNAP_f] \\
[RNAP : D] &= K_3.[RNAP_f].[D_t] \\
[\sigma^{32}: DnaK : FtsH] &= K_4.[\sigma^{32}: DnaK].[FtsH_f] \\
[\sigma^{32}: DnaK] &= K_5.[\sigma^{32}_f].[DnaK_f] \\
[\sigma^{32} :DnaK : protease] &= K_6.[\sigma^{32} : DnaK].[protease_f] \\
[\sigma^{32} : HslVU] &= K_7.[\sigma^{32}_f].[HslVU_f] \\
[Punfolded : DnaK] &= K_8.[Punfolded].[DnaK_f] \\
[\sigma^{32}: RNAP : ph] &= K_9.[\sigma^{32}: RNAP]([ph_t] - [\sigma^{32}: RNAP : ph]) \\
[\sigma^{70}: RNAP : pg] &= K_{10}.[\sigma^{70}: RNAP]([pg_t] - [\sigma^{70}: RNAP: pg]) \\
[\sigma^{70}: RNAP : D] &= K_{11}.[\sigma^{70}: RNAP].[D_t] \\
[\sigma^{32}: RNAP : D] &= K_{12}.[\sigma^{32}: RNAP].[D_t]
\end{aligned}
$$

(manual process, though)

Mass balances

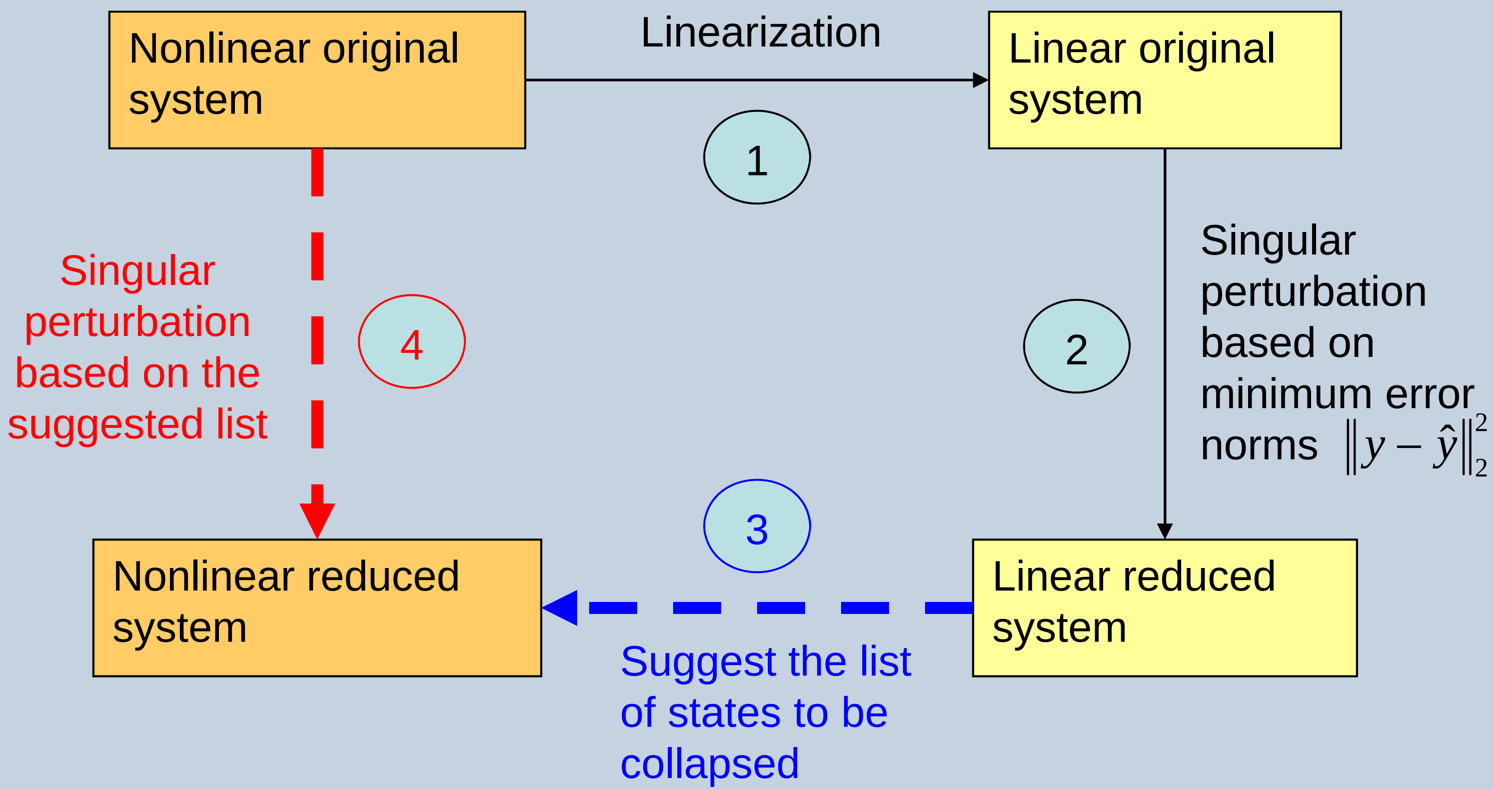
$$
\begin{aligned}
[RNAP_t] &= [RNAP_f] + [\sigma^{70}: RNAP] + [\sigma^{32}: RNAP] + [RNAP : D] + [\sigma^{70}: RNAP : D] \\
&+ [\sigma^{32}: RNAP : D] + [\sigma^{70}: RNAP : pg] + [\sigma^{32}: RNAP : ph] \\
[\sigma^{32}_t] &= [\sigma^{32}_f] + [\sigma^{32}: DnaK : protease] + [\sigma^{32}: RNAP] + [\sigma^{32}: RNAP : D] \\
&+ [\sigma^{32}: DnaK : FtsH] + [\sigma^{32}: DnaK] + [\sigma^{32}: RNAP:ph] + [\sigma^{32} : HslVU] \\
[DnaK_t] &= [DnaK_f] + [\sigma^{32}: DnaK : FtsH] + [\sigma^{32}: DnaK] + [Punfolded : DnaK] \\
&+ [\sigma^{32} : DnaK : protease] \\
[\sigma^{70}_t] &= [\sigma^{70}_f] + [\sigma^{70}: RNAP] + [\sigma^{70}: RNAP : D] + [\sigma^{70}: RNAP : pg] \\
[FtsH_t] &= [FtsH_f] + [\sigma^{32}: DnaK : FtsH] \\
[HslVU_t] &= [HslVU_f] + [\sigma^{32}: HslVU] \\
[protease_t] &= [protease_f] + [\sigma^{32}: DnaK : protease] \\
[Protein_t] &= [Punfolded] + [Punfolded : DnaK] + [Pfolded]
\end{aligned}
$$

Reduced, analyzable form

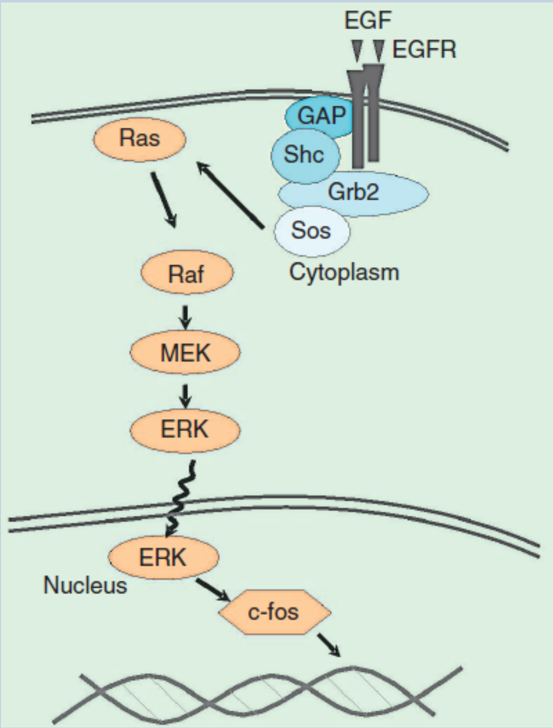
$$
\begin{aligned}
\frac{dS_t}{dt} &= \eta(T) - \alpha_0 S_t - \alpha_s S : D : F \\
\frac{dD_t}{dt} &= K_d S_f - \alpha_p D_t \\
\frac{dU_f}{dt} &= K(T) P_{folded} - K_{fold} U : D
\end{aligned}
$$

El-Samad *et al.*, 2005

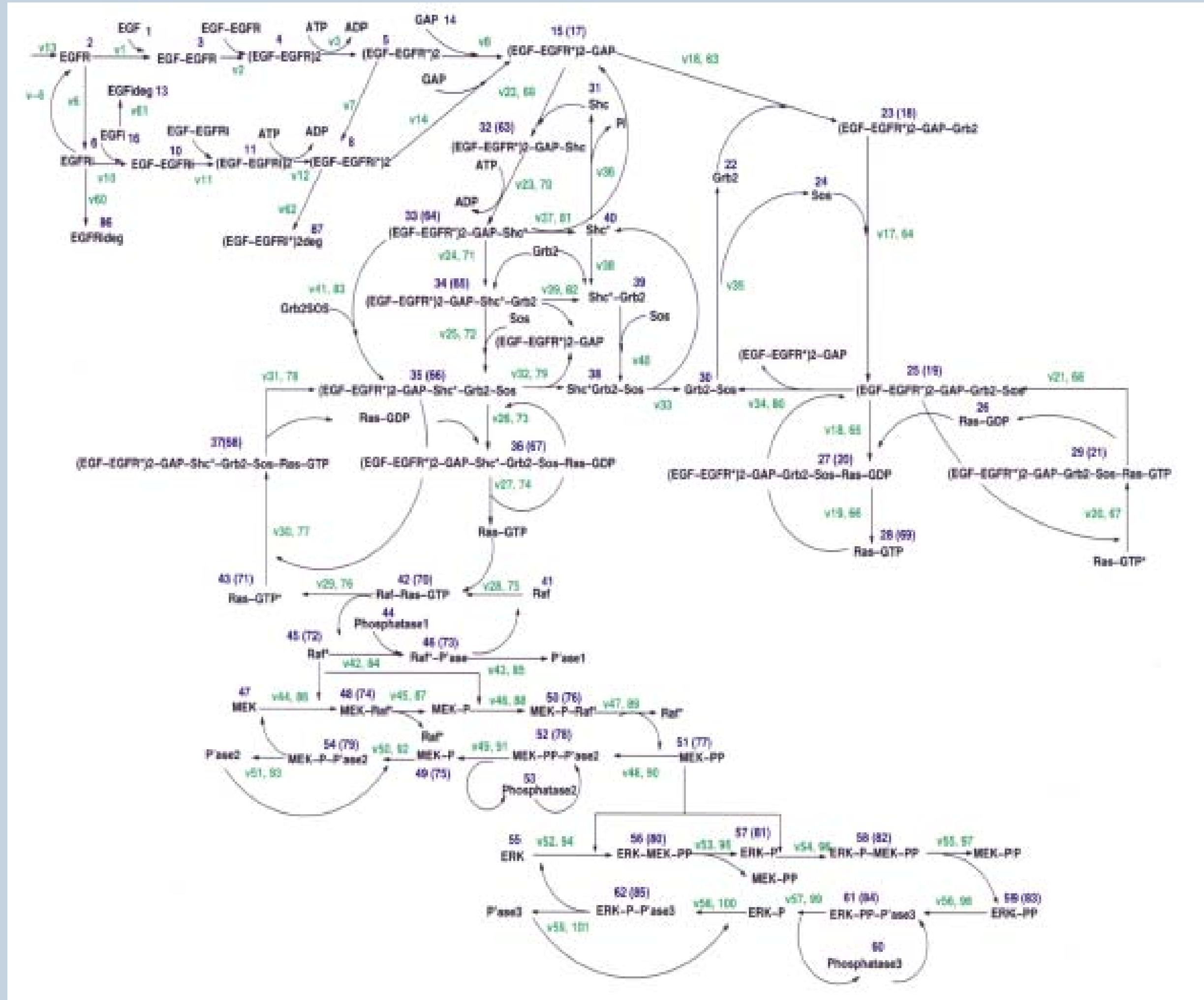
Automated model reduction



Original model of the EGFR-MAPK pathway

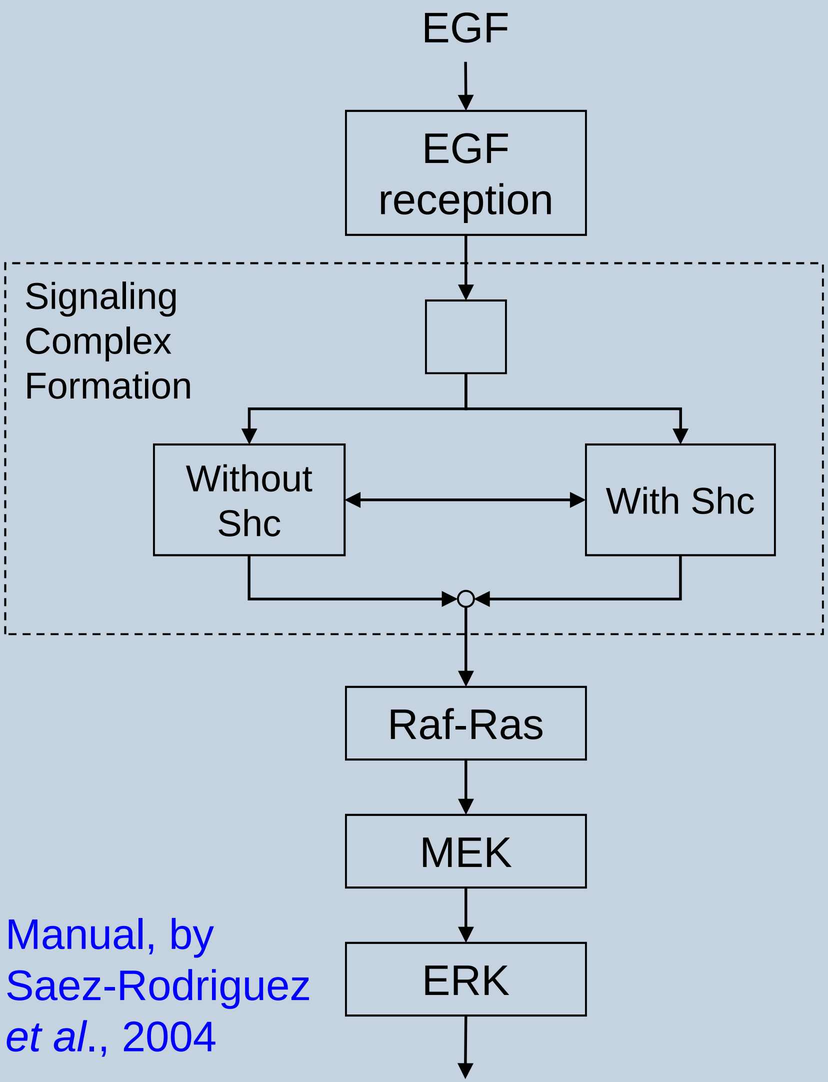


Saez-Rodriguez *et al*., 2004

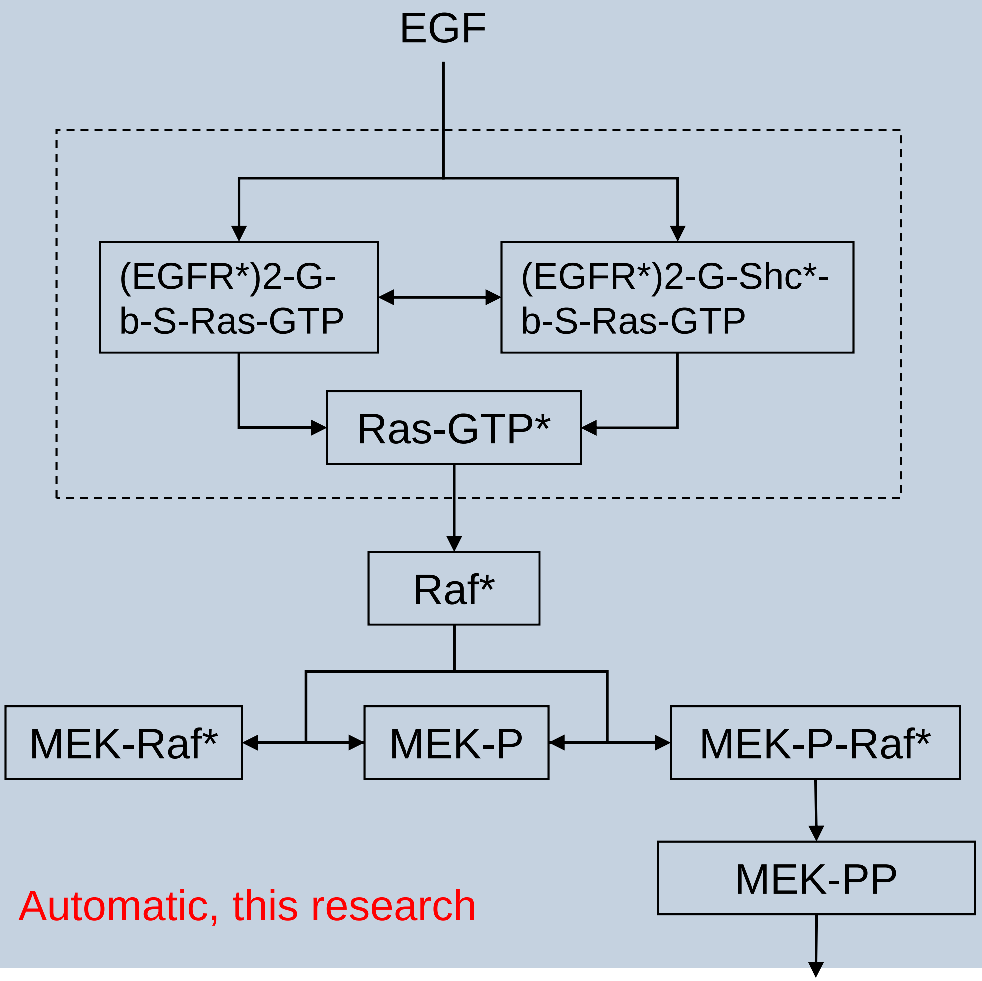


Schoeberl *et al*., 2002

Reduced model of the EGFR-MAPK pathway

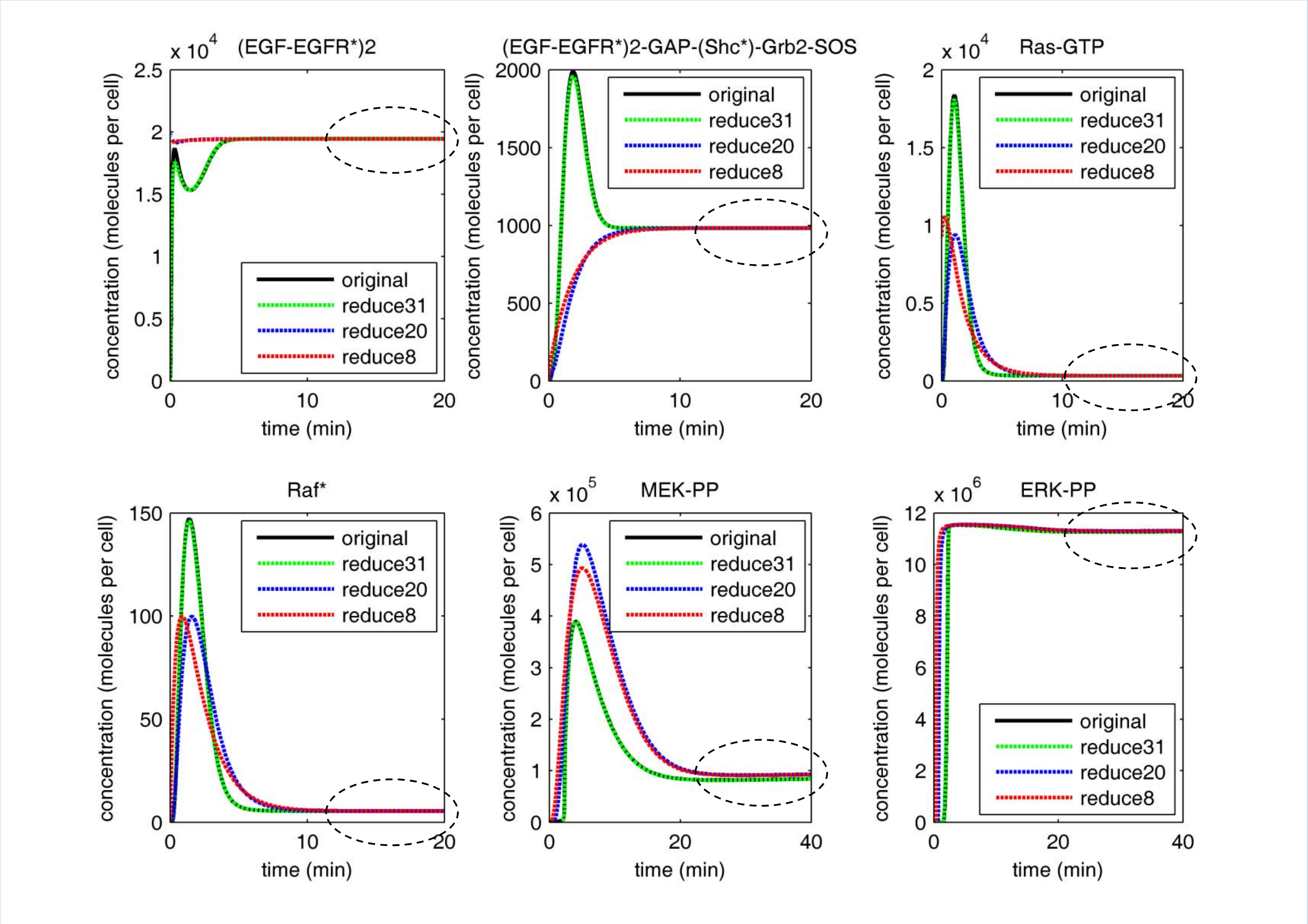


Manual, by Saez-Rodriguez *et al.*, 2004



Automatic, this research

Nonlinear system output comparison



Conclusion: the overall process of redesigning a synthetic biological component

Increase of the organisational complexity →

"Low Level" process

Analyzing the behavior of the original Taz-OmpR system

- Model construction
- Analytical solutions and simulations
- Parametric performance/ robustness analysis

"Medium Level" process

Modifying the original Taz-OmpR system for the desired behaviors

- Writer-eraser design
- Adaptive feedback design

"Higher Level" process

Connecting the Taz-OmpR module with other synthetic modules for new functions

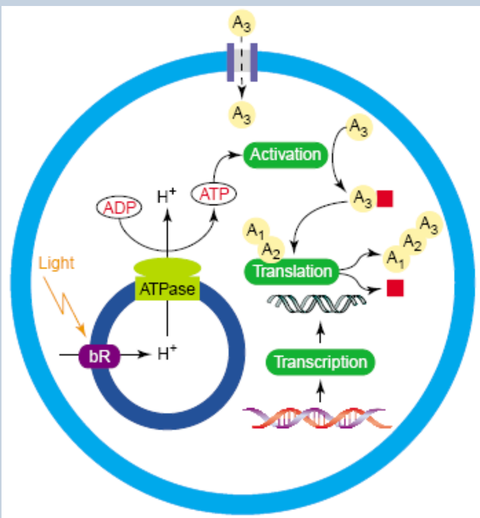
- Input-driven oscillator

Common Technical Bases

- Repository of standardised parts for experimental tests
- Algorithm for linear model reduction analysis

Future aims: Scalable multi-cellular / in-vitro synthetic biological components

- Attempt to make a sense-and-respond artificial cell, better self-sustaining



Pohorille A. and Deamer D., *Trends Biotech.*, 2002

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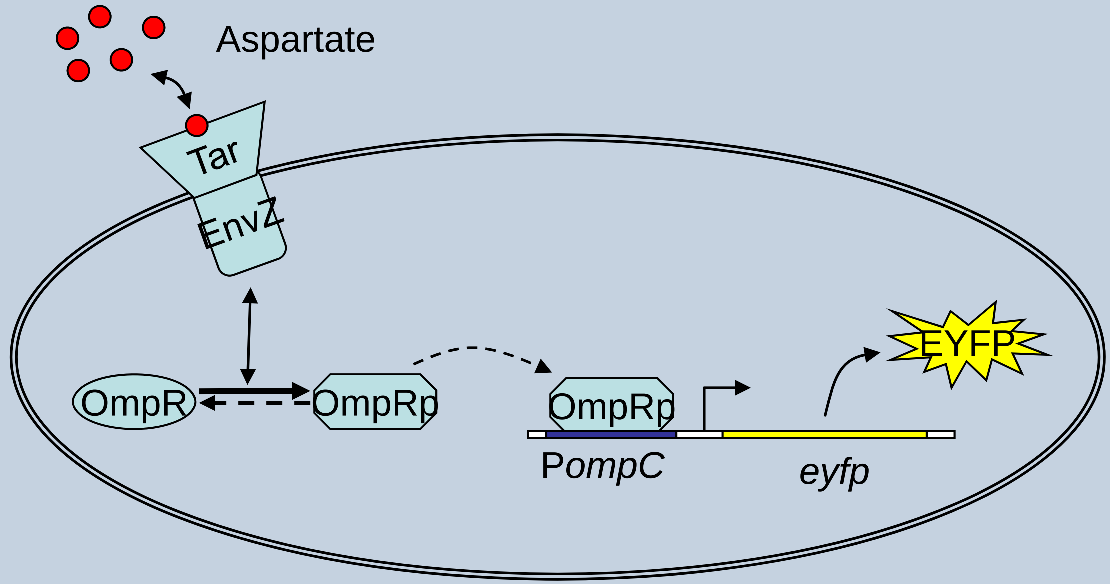
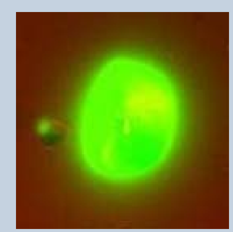


Preliminary test in 2006



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