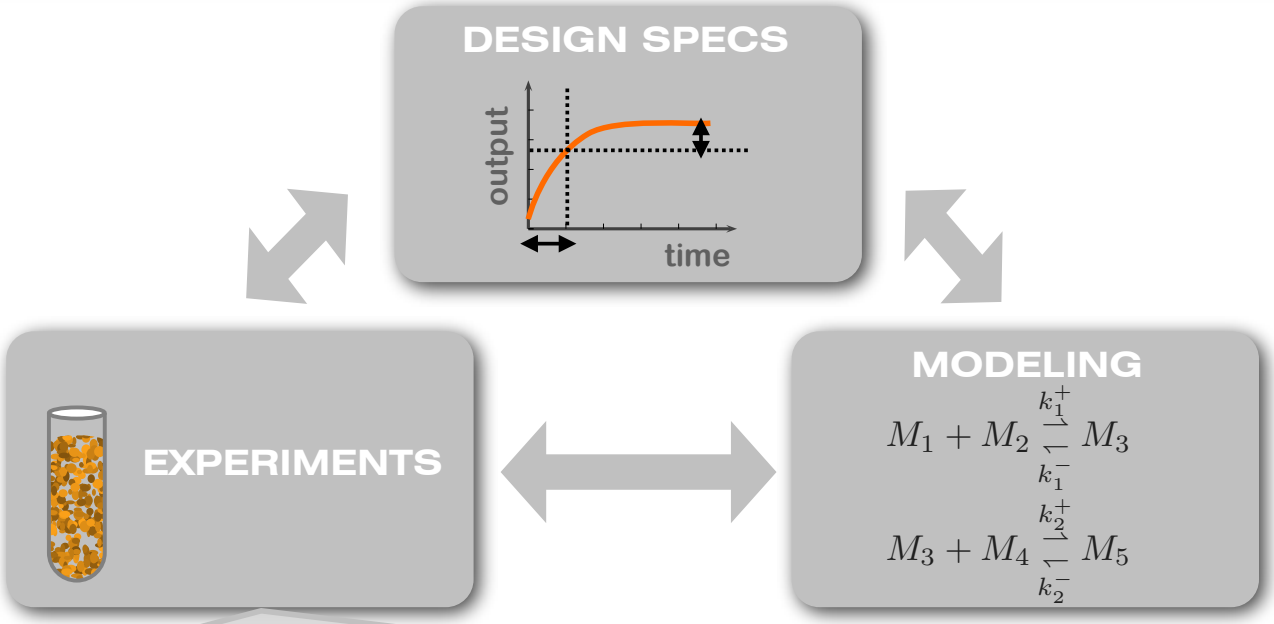
Programming and screening dynamic molecular networks in vitro

Elisa Franco, UC Riverside

Engineering dynamic molecular systems

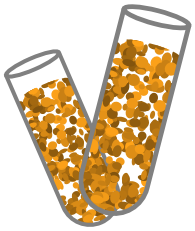
Molecular programming project (NSF expedition)



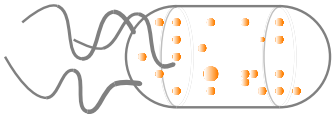
	in vivo:	in vitro:
Pros	- Test devices in actual working environment - Self-replication (cells = microfactories)	- Simple - Easier to quantify - Explore new chemistries
Cons	- Complex! - Unknown, unmeasurable interactions	- Not the “real thing” - Closed, non renewable system

Scope of research in vitro

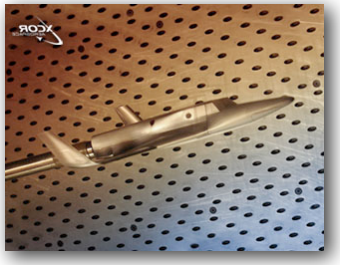
Biological "wind tunnel":

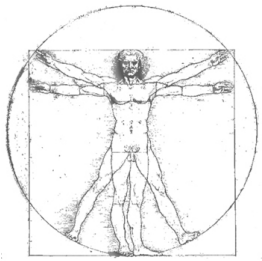


in vitro



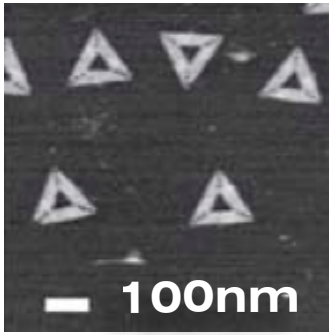
in vivo



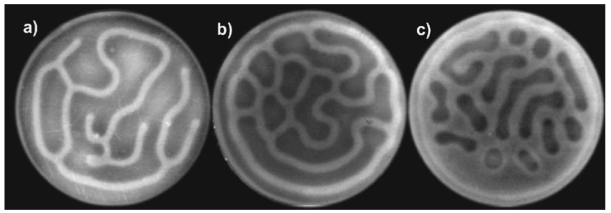


animal/
human
testing

Generate new devices, exploit molecular machinery in non strictly biological contexts, study toxic pathways...

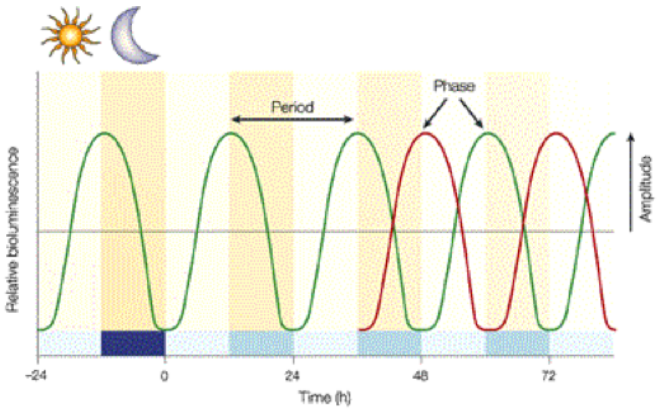


P. Rothemund, Nature 2006



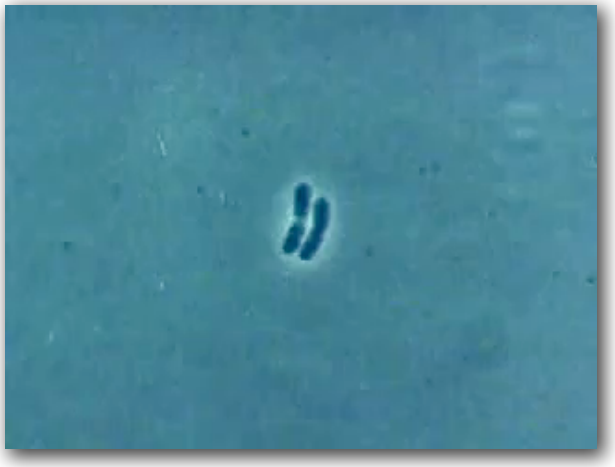
Szalai and De Kepper, 2008

Case study: Oscillators



In vivo oscillators are complex!!
(more in Frank's talk)

Can we create simple molecular clocks from the bottom up?



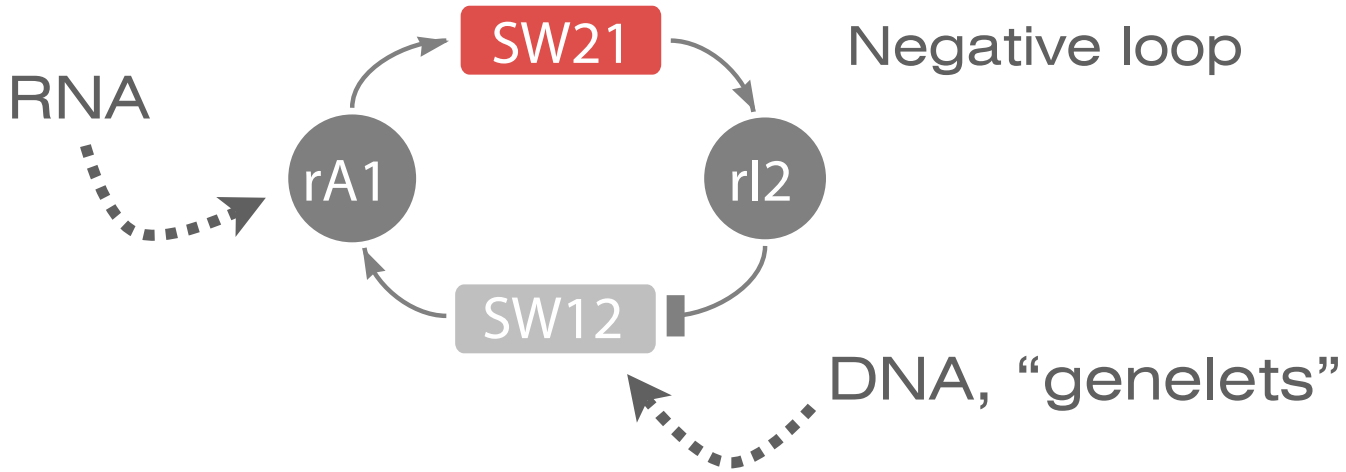
Why?

- Design principles
- Self-sustained signal generators
- Timers
- Patterning...

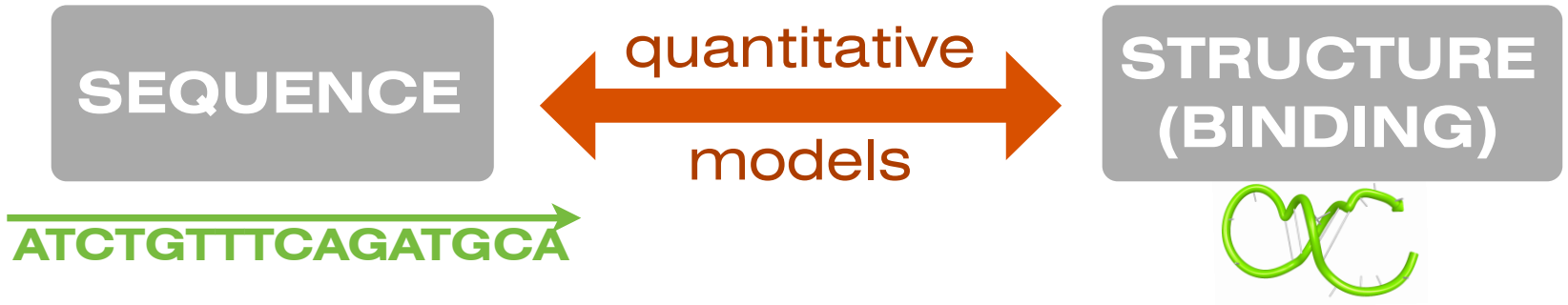
An in vitro molecular oscillator

Kim & Winfree, MSB 2011 Franco et al. PNAS 2011

Nucleic acids + off the shelf proteins



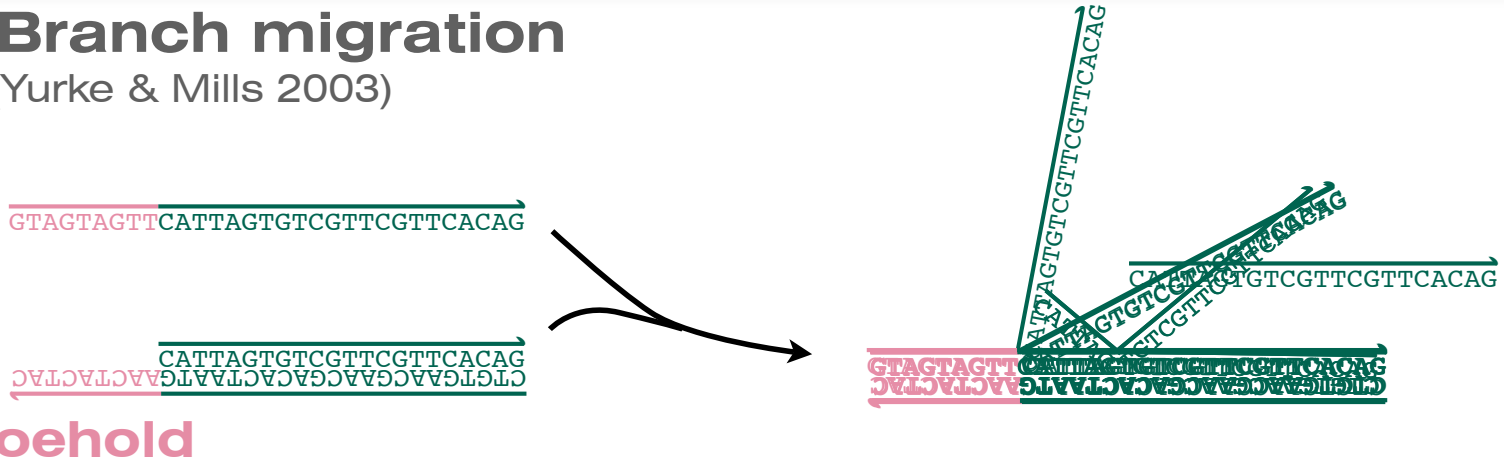
Why nucleic acids?



Key ideas behind design of DNA dynamical reaction networks

1) Branch migration

(Yurke & Mills 2003)



We can implement Arbitrary Biochemical Networks

Soloveichick et al. 2010

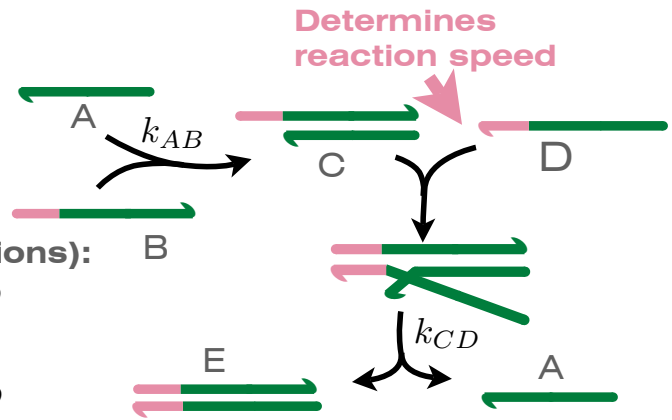
Reactions:

$$A + B \overset{k_{AB}}{\rightharpoonup} C$$

$$C + D \overset{k_{CD}}{\rightharpoonup} E + A$$

ODEs (concentrations):

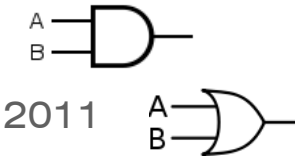
$$\dot{A} = -k_{AB}AB + k_{CD}CD$$
$$\dot{B} = -k_{AB}AB$$
$$\dot{C} = +k_{AB}AB - k_{CD}CD$$
$$\dot{D} = -k_{CD}CD$$
$$\dot{E} = +k_{CD}CD$$



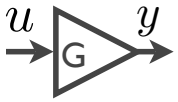
Toehold-mediated branch migration

DEVICES AVAILABLE

- **Logic circuits,**
  Seelig et al. 2006
  Qian and Winfree, 2011



- **Catalysts/Amplifiers,**
  Zhang et al. 2007

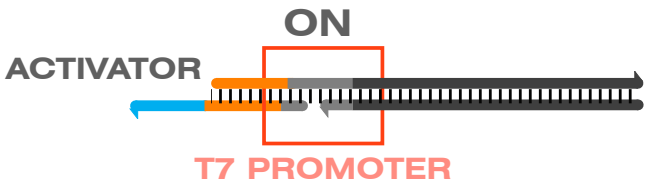


- **Oscillators, toggle switches…**
  Kim et al. 2006
  Franco et al. 2011

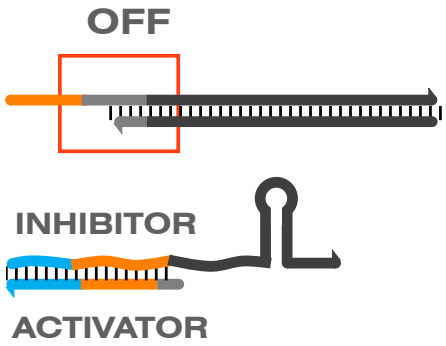
Key ideas behind design of DNA dynamical reaction networks

2) Promoter displacement

(Kim et al. 2006)

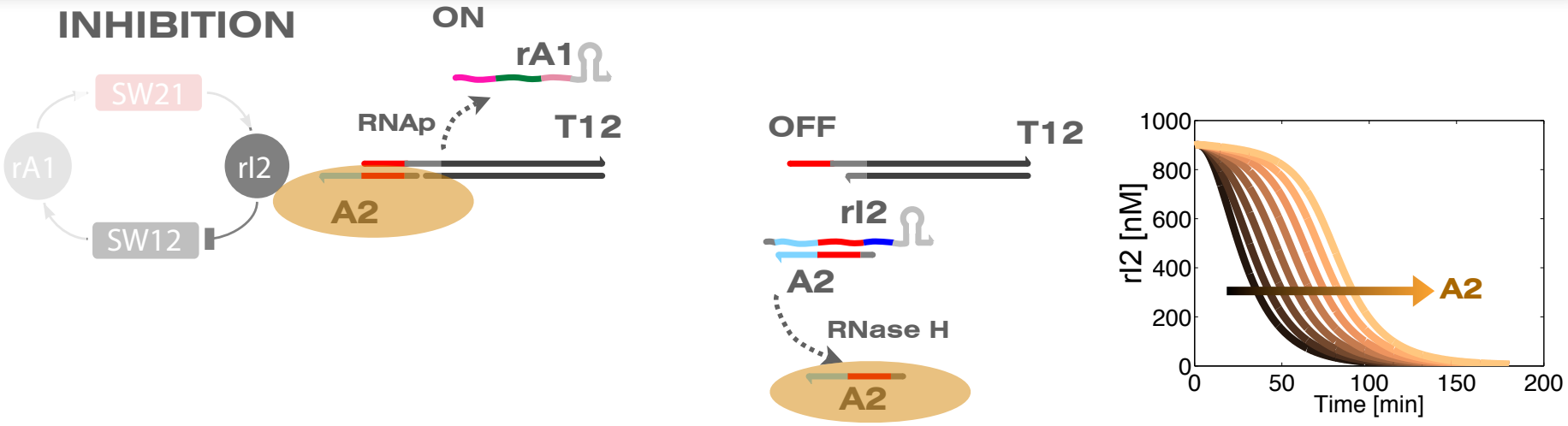


short synthetic genes
(genelets)
70-100 base pairs

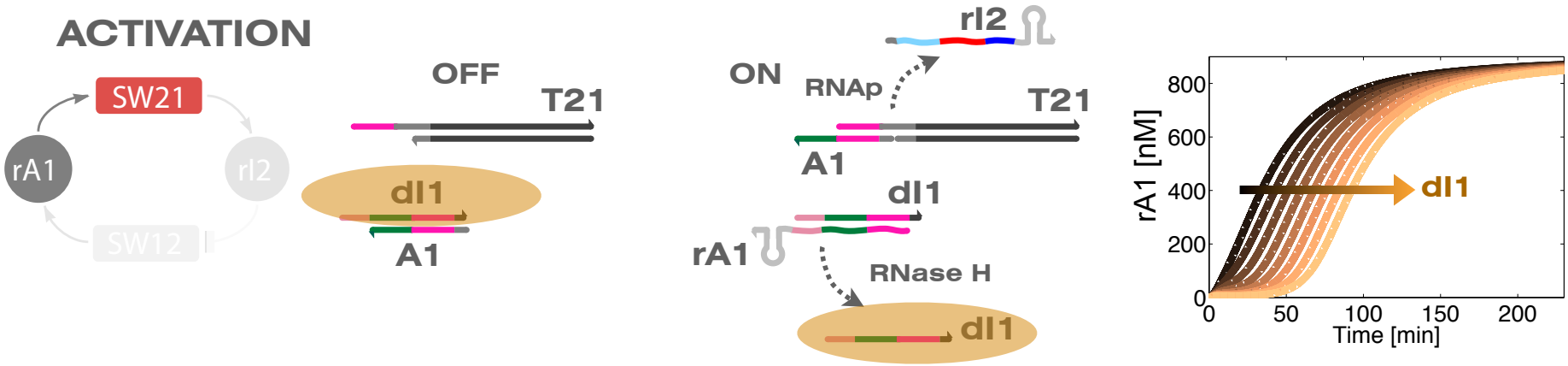


Branch migration and promoter displacement can be combined to create synthetic gene networks

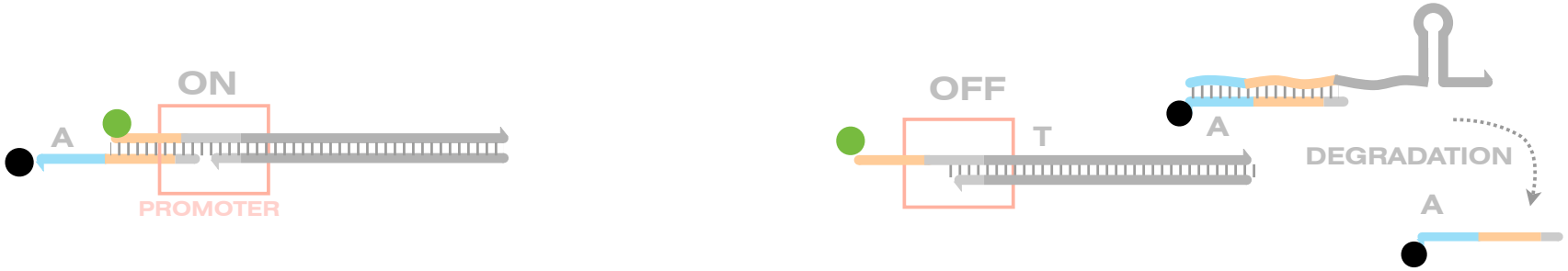
Repression: promoter displacement
Activation: promoter “restoration”



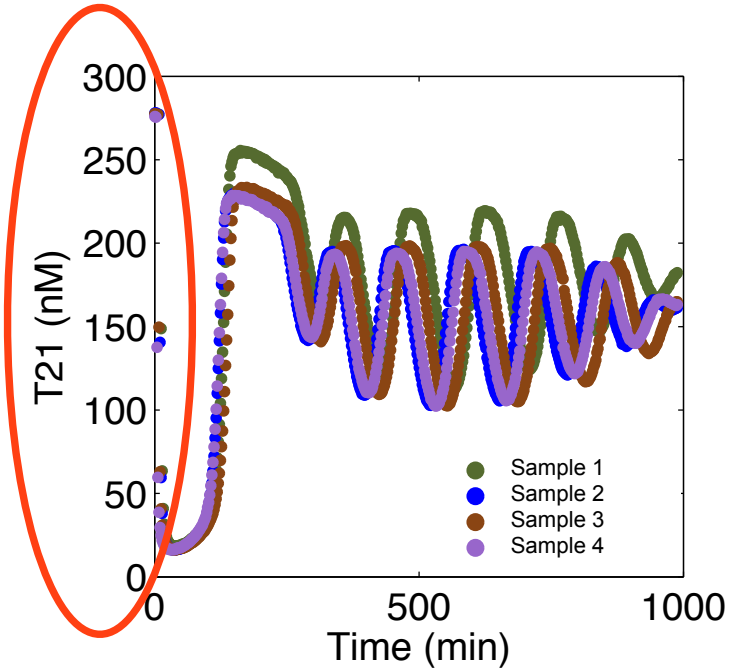
Reaction speeds are programmable (toeholds)!

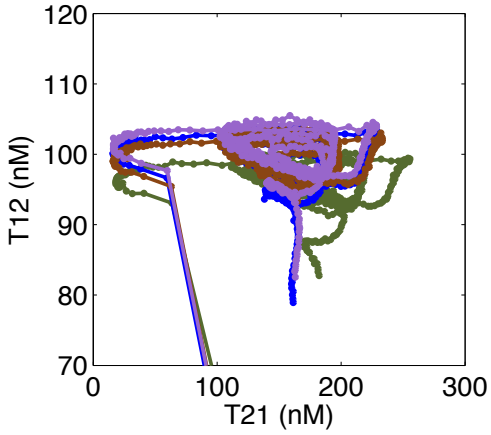


What are we measuring?



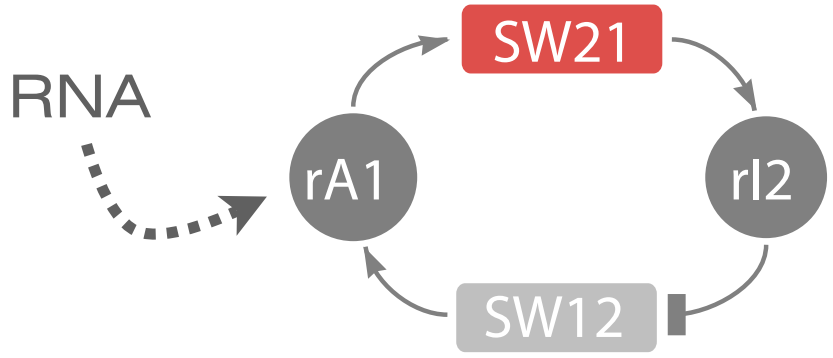
FLUOROPHORE-QUENCHER PAIRS MONITOR ON/OFF STATE OF GENELETS





A simple model

Franco et al. PNAS 2011



DNA, “genelets” + thresholds

$$\frac{d[rA1]}{dt} = k_p[SW12] - k_d[rA1]$$

$$\tau\frac{d[SW21]}{dt} = [SW21^{tot}]\frac{\frac{[rA1]^m}{KA^m}}{1+\frac{[rA1]^m}{KA^m}} - [SW21]$$

$$\frac{d[rI2]}{dt} = k_p[SW21] - k_d[rI2]$$

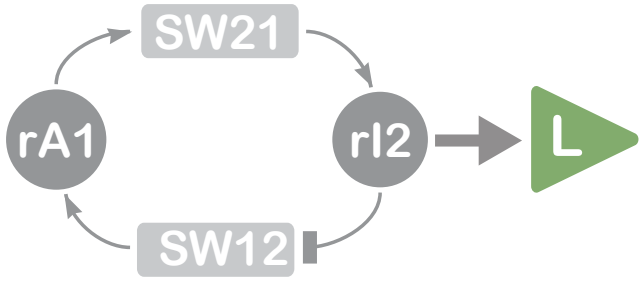
$$\tau\frac{d[SW12]}{dt} = [SW12^{tot}]\frac{1}{1+\frac{[rI2]^n}{KI^n}} - [SW12]$$

INTERCONNECTION OF TWO MONOTONE SYSTEMS (Angeli & Sontag 2008)

- RNA PRODUCTION/DEGRADATION: **Loop Gain**
- DNA THRESHOLDS dI1, A2: **Delay**

Molecular programming challenges we explored

1. Interconnection of modules / signal transmission



Suppose we want to use the oscillator as a signal generator.
Can we transmit the signal effectively to downstream devices (loads)?
(Domitilla's talk)

2. High throughput screening of circuit behavior

Suppose we have several candidate circuits in vitro, how can we assess their dynamic behavior experimentally with few, high throughput experiments?

1. Transmission of oscillations to a molecular "LOAD", DNA Tweezers

Yurke et al. Nature 2000

Example target: dl1

GTGTGTAGTAGTAGTTCATTAGTGTCGTTCGTTCACAG

dl1

GTGTGTAGTAGTAGTTCATTAGTGTCGTTCGTTCACAG

OPEN

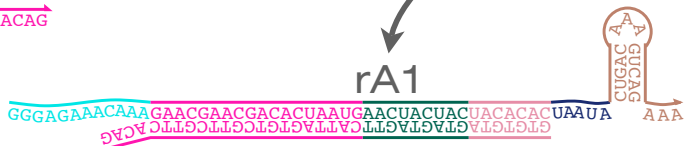
CLOSED

rA1

dl1

GTGTGTAGTAGTAGTTCATTAGTGTCGTTCGTTCACAG

RNase H



rA1

dl1

A large molecular load perturbs the oscillator

Kim & Winfree, MSB 2011 Franco et al. PNAS 2011

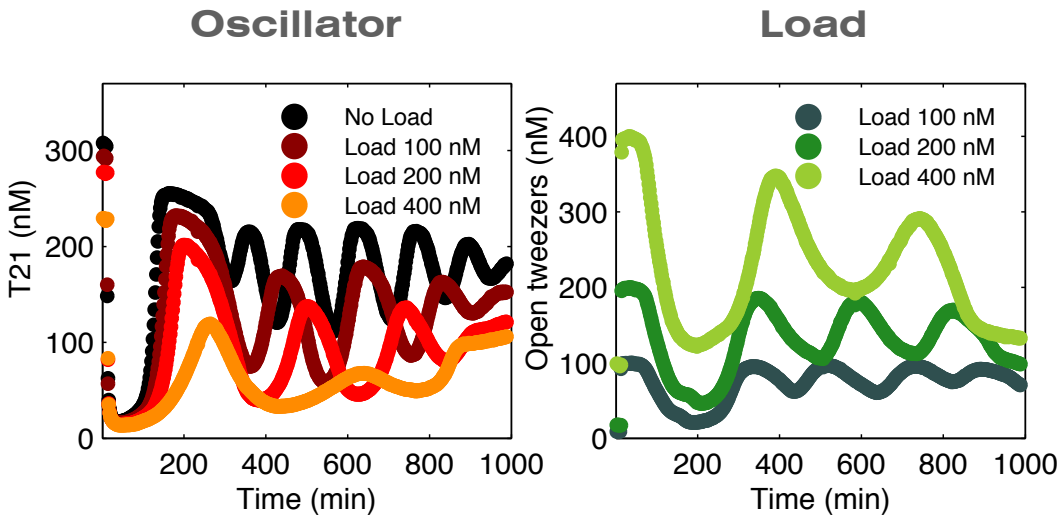
Oscillator model:

$$\frac{d[rA1]}{dt} = k_p[SW12] - k_d[rA1]$$

$$\tau\frac{d[SW21]}{dt} = [SW21^{tot}]\frac{\frac{[rA1]^m}{KA^m}}{1+\frac{[rA1]^m}{KA^m}} - [SW21]$$

$$\frac{d[rI2]}{dt} = k_p[SW21] - k_d[rI2]$$

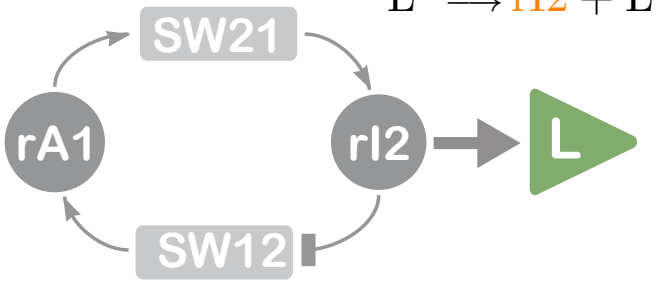
$$\tau\frac{d[SW12]}{dt} = [SW12^{tot}]\frac{1}{1+\frac{[rI2]^n}{KI^n}} - [SW12]$$



Load model:

$$rI2 + L \xrightarrow{k_f} L^a$$

$$L^a \xrightarrow{k_r} rI2 + L$$



Consumptive coupling

Quasi steady-state approximation:

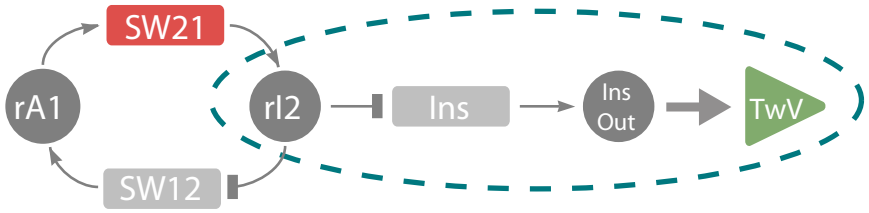
$$\frac{d[rI2]}{dt} = k_p \cdot [SW21] - k_d \cdot [rI2] + \overbrace{k_r \cdot [L^{tot}]\frac{k_f[rI2]}{k_r + k_f[rI2]}}^{\text{consumptive}}$$

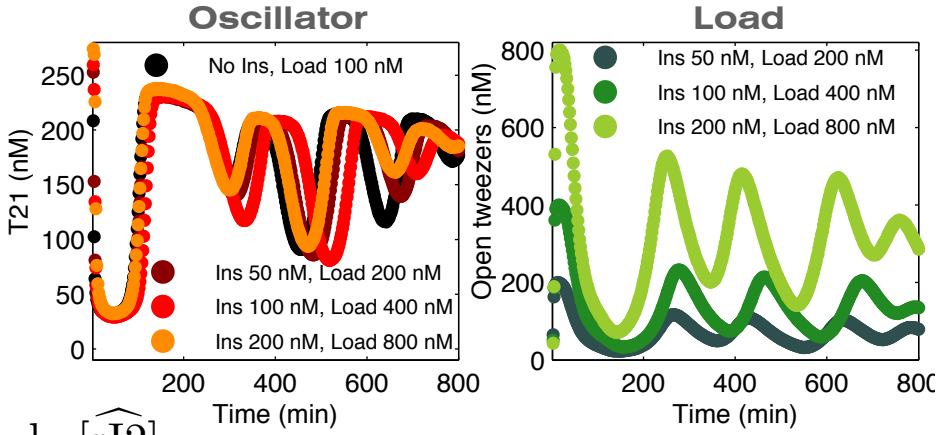
$$\frac{d[rI2]}{dt} = k_p \cdot [SW21] - k_d \cdot [rI2] - k_r \cdot [L^{tot}]\frac{k_f[rI2]}{k_r + k_f[rI2]}$$

$$[\widehat{L^a}](t) = [L^{tot}]\left(1 - \frac{k_r}{k_r + k_f[rI2]}\right)$$

Insulation: robustness to load

Franco et al. PNAS 2011
Del Vecchio et al. MSB 2008



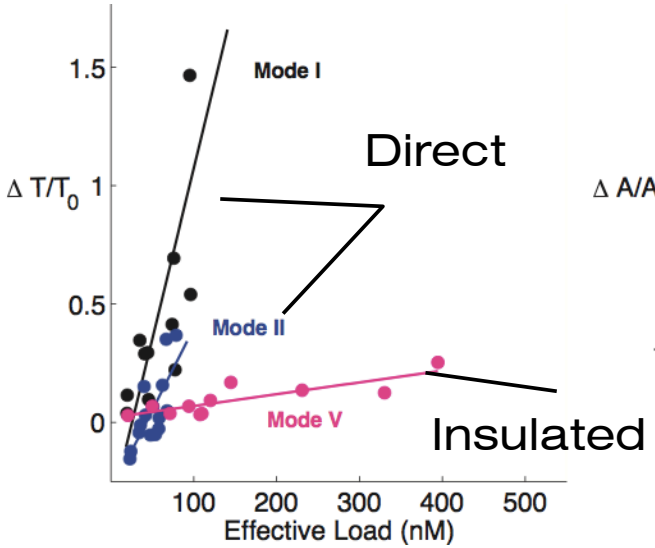


$$\frac{d[\widehat{rI2}]}{dt} = k_p \cdot [SW21] - k_d \cdot [\widehat{rI2}] - k_r \cdot [Ins^{tot}] \frac{k_f [\widehat{rI2}]}{k_r + k_f [\widehat{rI2}]}$$

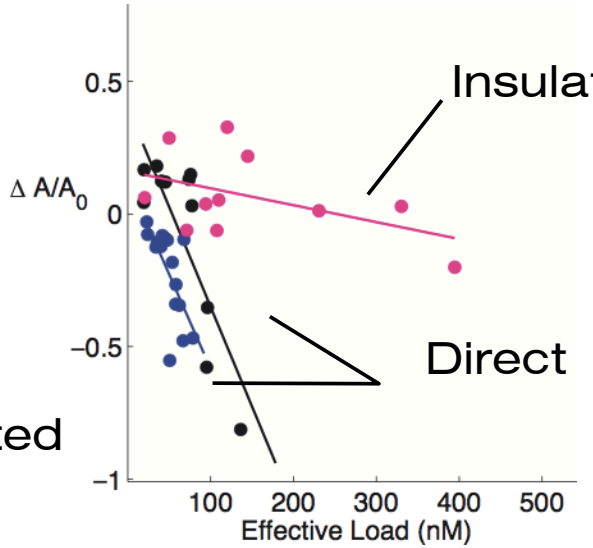
$$[\widehat{L^a}](t) = [L^{tot}] \left(1 - \frac{k_r}{k_r + k_f [InsOut]}\right)$$

- Small amount of threshold A2 is used to activate Ins genelet
- Ins genelet amplifies the signal

Period perturbation



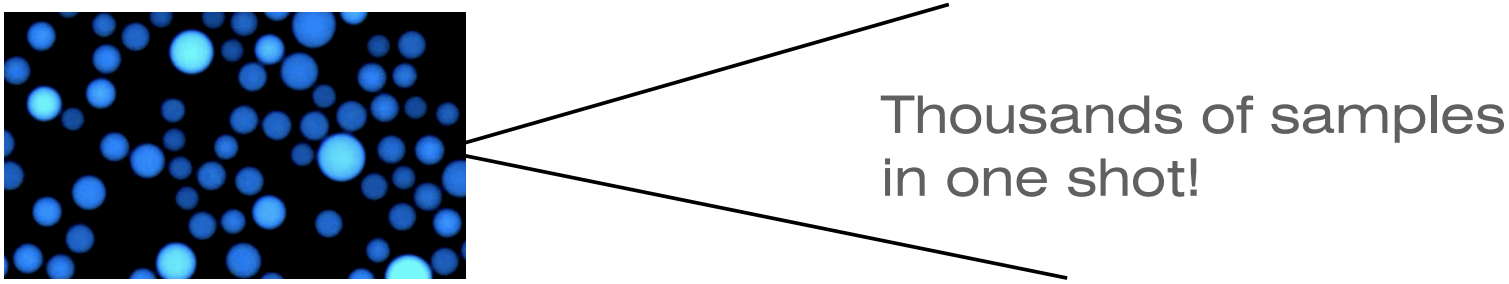
Amplitude perturbation



2. High throughput screening in microscale compartments.

Weitz et al.
Nature Chemistry 2014

Run the reactions in water-in-oil droplets

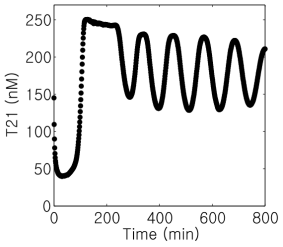


- High throughput screening, reproducibility

- Stochastic effects?

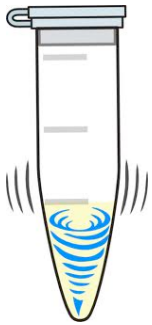
Circuit components

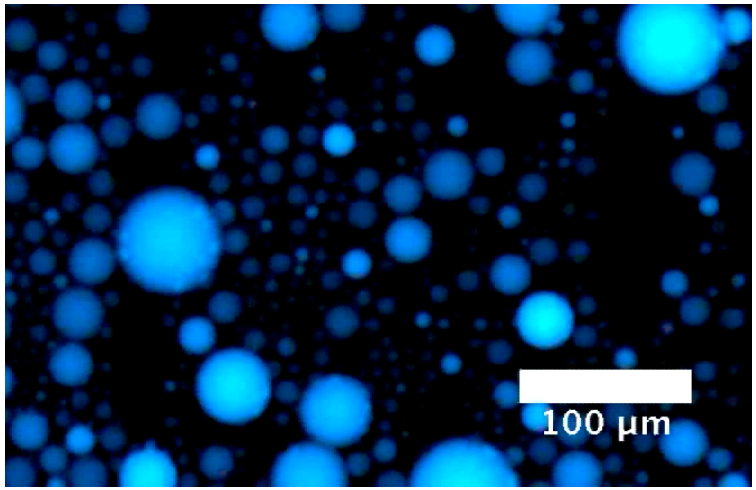
BULK oscillator



“Shaken not Stirred”

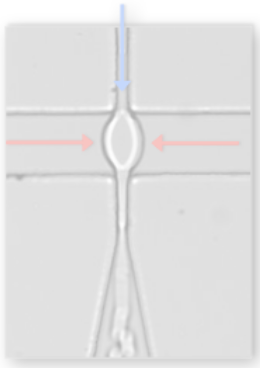
(Water in oil emulsion)



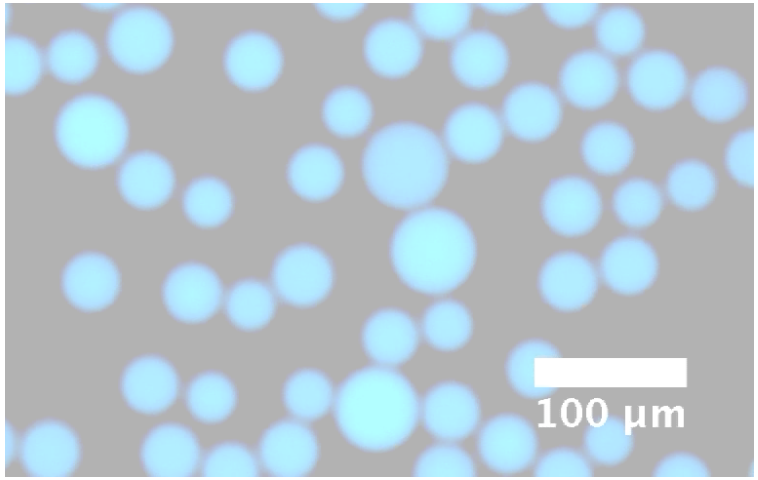


Microfluidics

aqueous solution

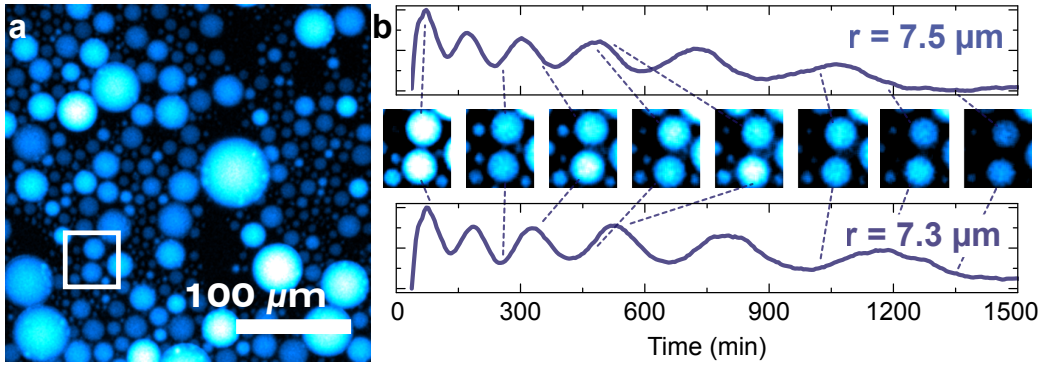


oil phase

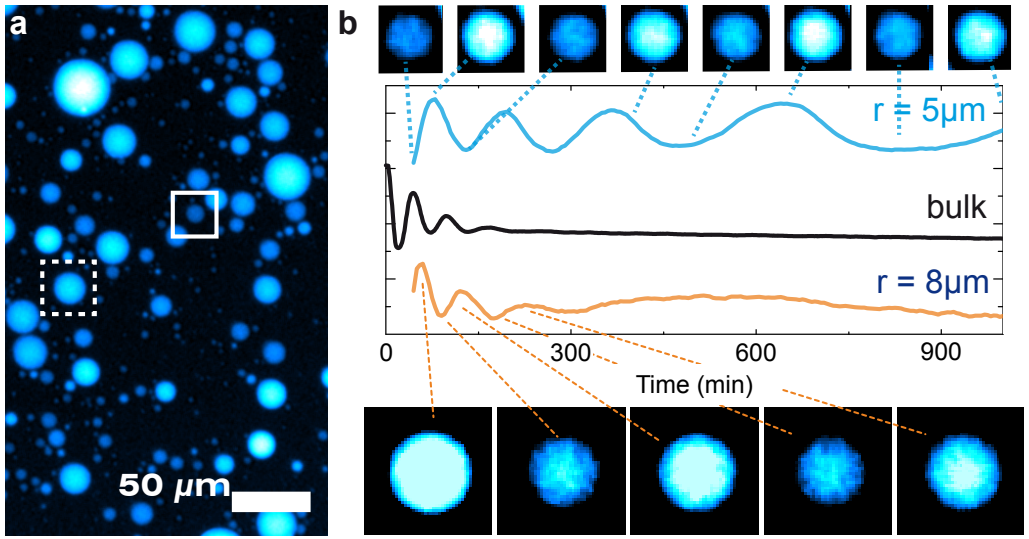


Emulsions:
variability in the population of oscillators

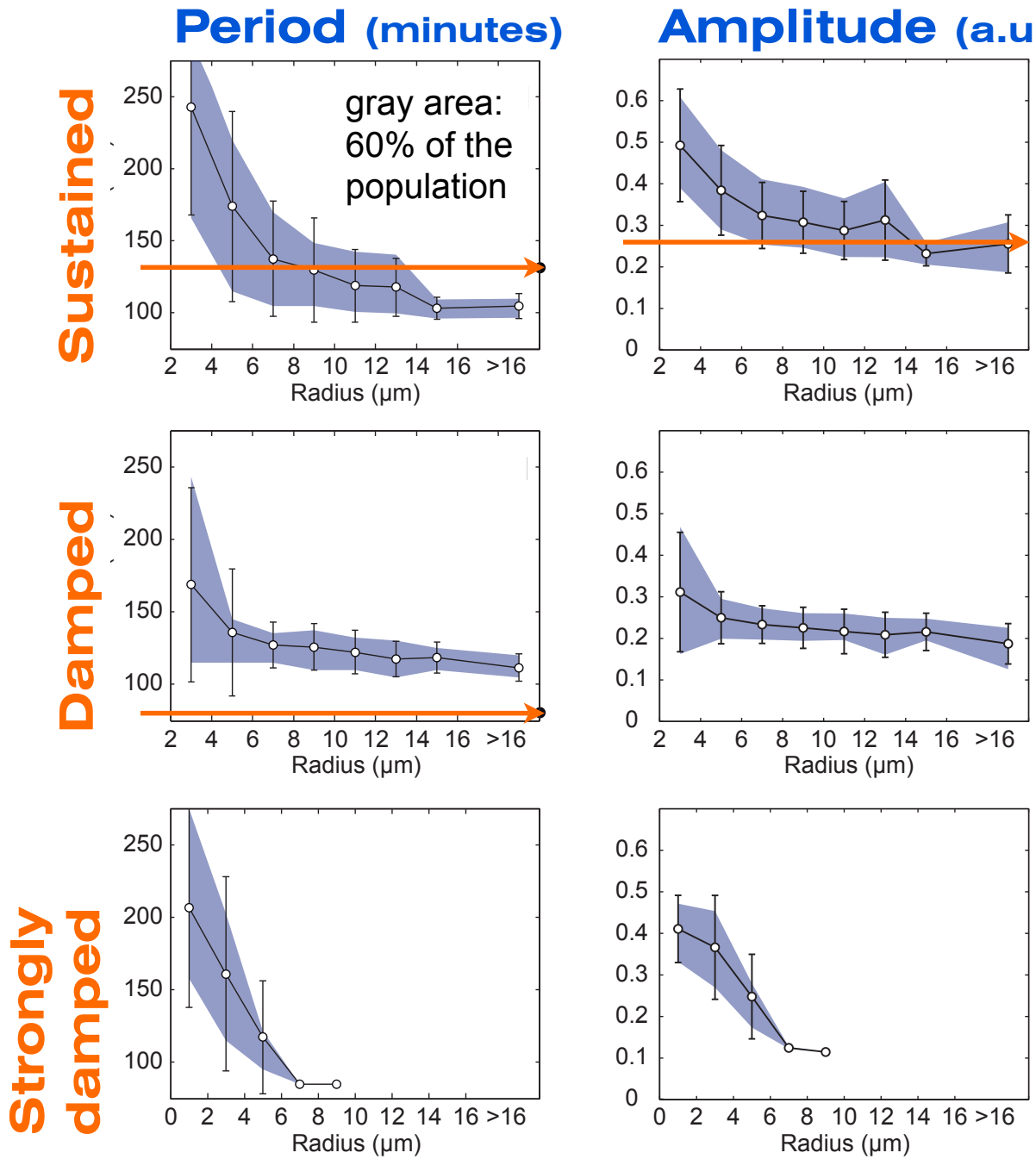
BULK: Sustained oscillations



BULK: Damped oscillations



Different operating points of bulk solution



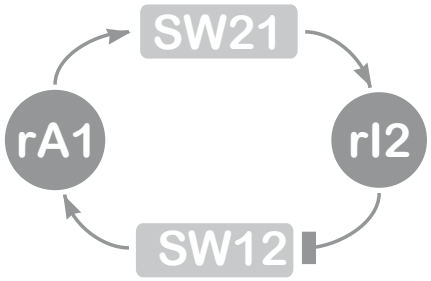
SMALL RADIUS

MORE VARIABILITY

Not surprising.

But... source of variability?

Where is variability coming from? What do our models say?



Simple model:

$$\frac{d[rA1]}{dt} = k_p[SW12] - k_d[rA1]$$

$$\tau\frac{d[SW21]}{dt} = [SW21^{tot}]\frac{\frac{[rA1]^m}{KA^m}}{1+\frac{[rA1]^m}{KA^m}} - [SW21]$$

$$\frac{d[rI2]}{dt} = k_p[SW21] - k_d[rI2]$$

$$\tau\frac{d[SW12]}{dt} = [SW12^{tot}]\frac{1}{1+\frac{[rI2]^n}{KI^n}} - [SW12]$$

cannot "make the elephant wiggle its trunk"...

Predictive, detailed model:

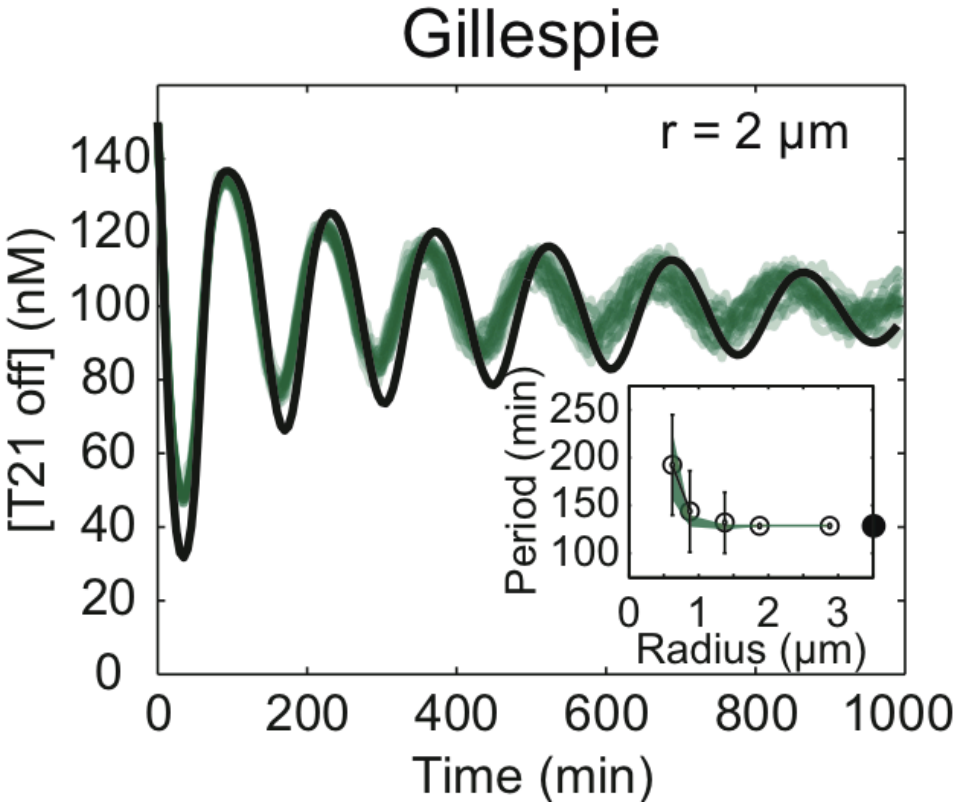
$$\begin{aligned}
T12 + A2 &\xrightarrow{k_{TA,12}} T12 \cdot A2 && \text{(Activation)}\\
T21 + A1 &\xrightarrow{k_{TA,21}} T21 \cdot A1 && \text{(Activation)}\\
A1 + dI1 &\xrightarrow{k_{AI,1}} A1 \cdot dI1 && \text{(Annihilation)}\\
rA1 + dI1 &\xrightarrow{k_{rAI,1}} rA1 \cdot dI1 && \text{(Annihilation)}\\
A2 + rI2 &\xrightarrow{k_{AI,2}} A2 \cdot rI2 && \text{(Annihilation)}\\
T12 \cdot A2 + rI2 &\xrightarrow{k_{TAI,12}} T12 + A2 \cdot rI2 && \text{(Inhibition)}\\
T21 \cdot A1 + dI1 &\xrightarrow{k_{TAI,21}} T21 + A1 \cdot dI1 && \text{(Inhibition)}\\
rA1 + A1 \cdot dI1 &\xrightarrow{k_{AIrA,1}} rA1 \cdot dI1 + A1 && \text{(Release)}
\end{aligned}$$

$$\begin{aligned}
RNAP + T12 \cdot A2 &\underset{k_{-,ON,12}}{\overset{k_+}{\rightleftharpoons}} RNAP \cdot T12 \cdot A2 \xrightarrow{k_{cat,ON,12}} RNAP + T12 \cdot A2 + rA1\\
RNAP + T21 \cdot A1 &\underset{k_{-,ON,21}}{\overset{k_+}{\rightleftharpoons}} RNAP \cdot T21 \cdot A1 \xrightarrow{k_{cat,ON,21}} RNAP + T21 \cdot A1 + rI2\\
RNAP + T12 &\underset{k_{-,OFF,12}}{\overset{k_+}{\rightleftharpoons}} RNAP \cdot T12 \xrightarrow{k_{cat,OFF,12}} RNAP + T12 + rA1\\
RNAP + T21 &\underset{k_{-,OFF,21}}{\overset{k_+}{\rightleftharpoons}} RNAP \cdot T21 \xrightarrow{k_{cat,OFF,21}} RNAP + T21 + rI2\\
RNaseH + rA1 \cdot dI1 &\underset{}{\overset{k_{+,H}}{\rightleftharpoons}} RNaseH \cdot rA1 \cdot dI1 \xrightarrow{k_{cat,H,1}} RNaseH + dI1
\end{aligned}$$

... and the list goes on...
17 ODEs, 24 parameters

Where is variability coming from? What do our models say?

2μm radius-> 33fL
E coli ~1fL

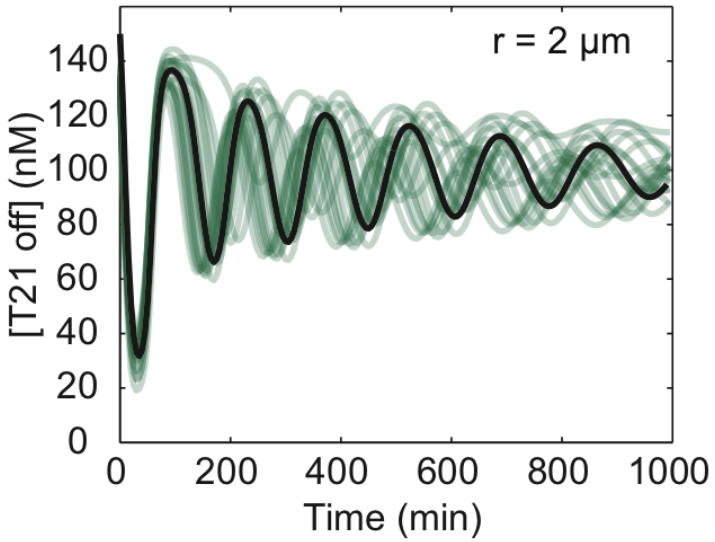


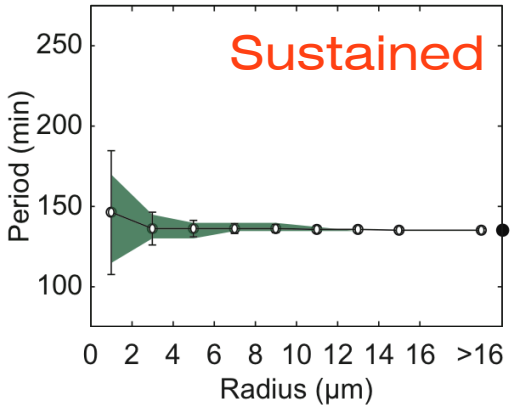
Does not reproduce our measurements...

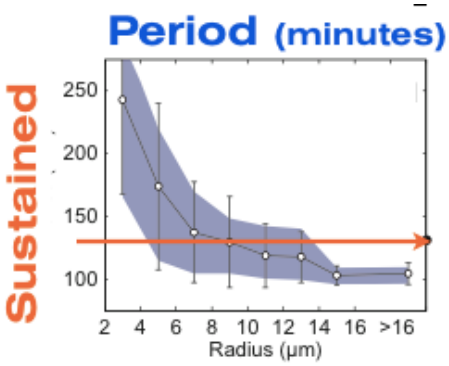
Could the variability be due to partitioning noise?

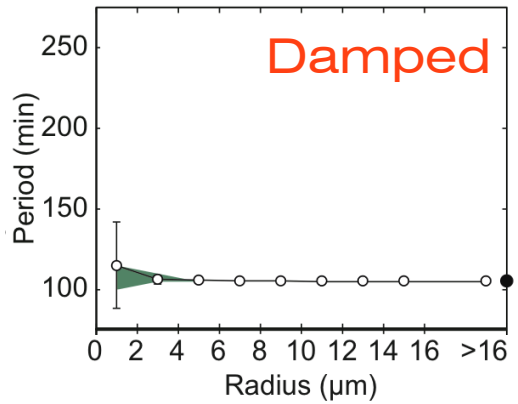
$$p(N|V) = \frac{e^{-\lambda}\lambda^N}{N!} \qquad \lambda = C_0 N_A V$$

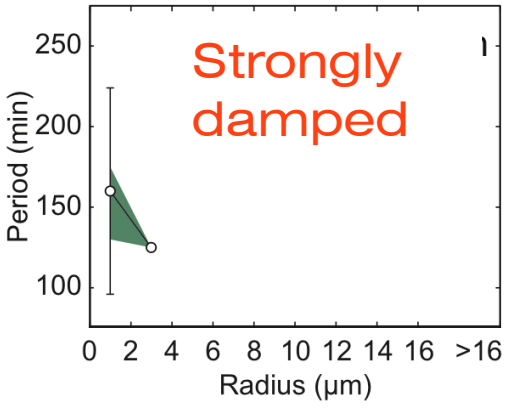
- Stochastic partitioning of reagents
- Deterministic ODEs







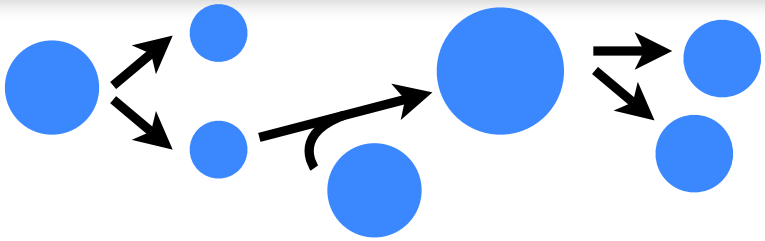




Additional partitioning effects

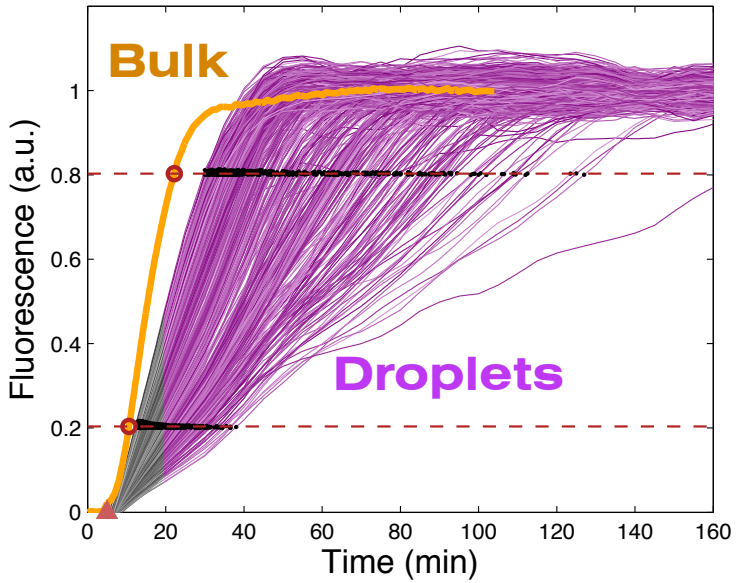
1) Scission/coalescence

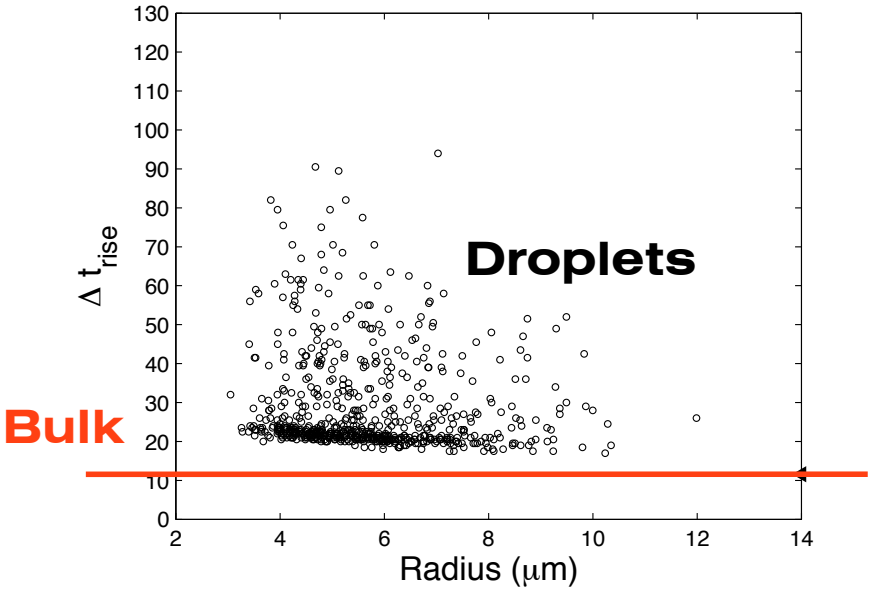
Multiplicative noise



2) Denaturation/loss of enzymes

Rise time in encapsulated **transcription reaction**





3) Aggregation of components

Protein/DNA, DNA/DNA etc

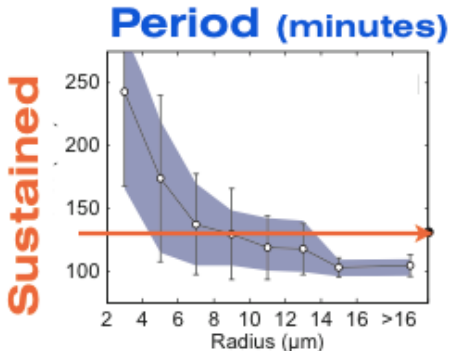
Phenomenological partitioning distribution

Weitz et al. Nature Chemistry 2014

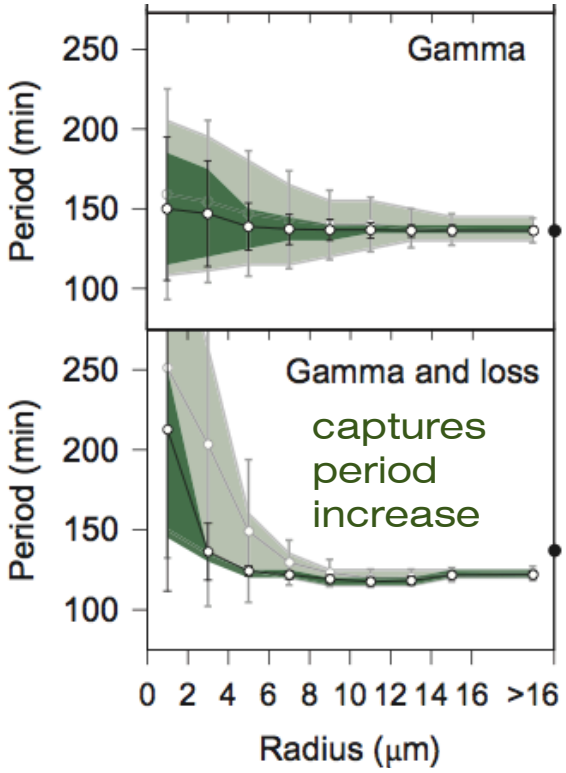
Gamma:

$$p(N|V) = \left(\beta^{\alpha}\Gamma(\alpha)\right)^{-1} V^{\alpha-1} e^{-V/\beta}$$

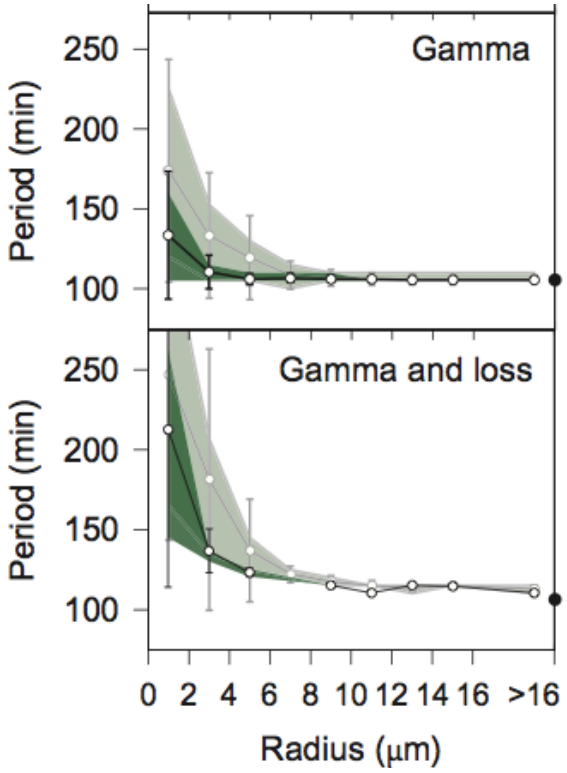
Recall: mean=αβ, var=β*mean. For α=λ , β=1 we recover Poisson



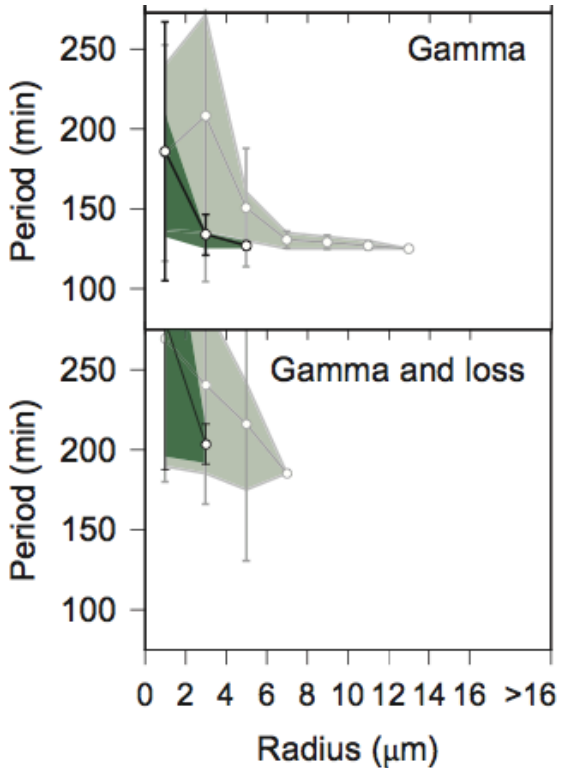
Sustained



Damped



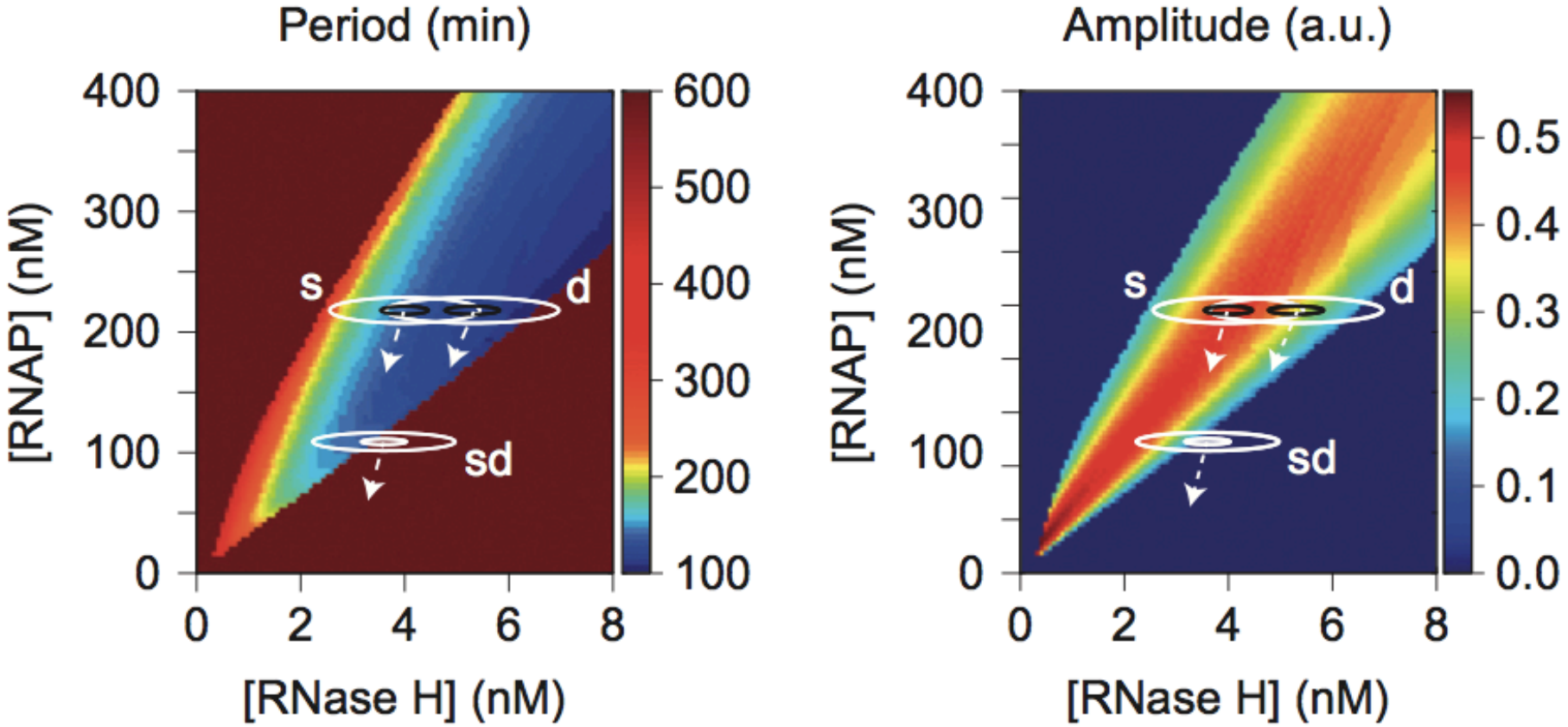
Strongly damped



Biologist: Wait a minute... this is an extremely complex system. Aren't you going to run control experiments to characterize all these sources of partitioning noise? How else are you going to formulate a meaningful model?

EF: Well, we could but it would take us additional years of experiments. Engineers are often happy with minimal models that answer specific questions, neglecting some of the (less important) details.
In this case, we include in our model some additional dynamics that globally capture all the mentioned partitioning phenomena. We do not need to know their mechanistic details.

Phase space “scenario” (numerical)
(RNAP/RNase H drive production/degradation)



Partitioning noise + nonlinearities in the system: possibly large perturbations of the dynamics

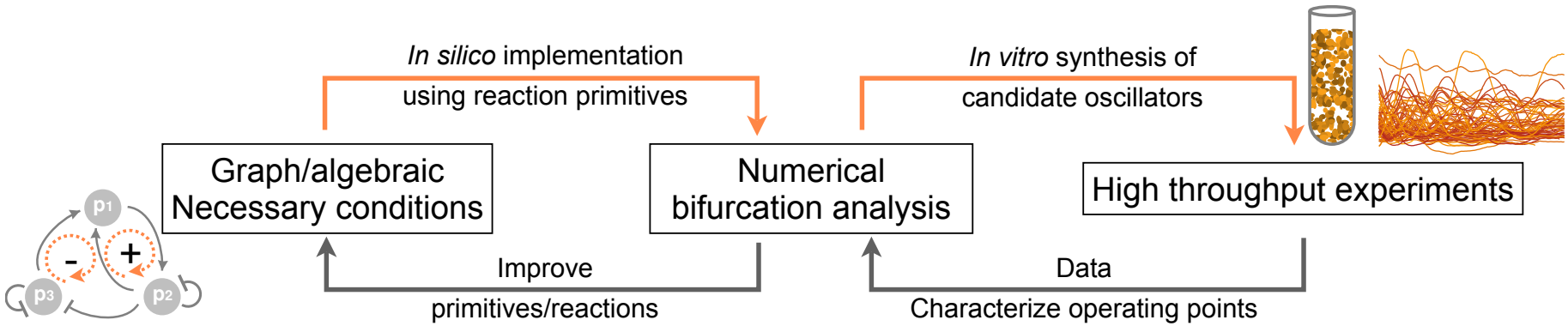
Biologist: This is a nice study but don't we just learn about the behavior of a bunch of DNA reactions inside droplets? I see no general message here...

EF: See next slide

Outlook: screening circuit behaviors

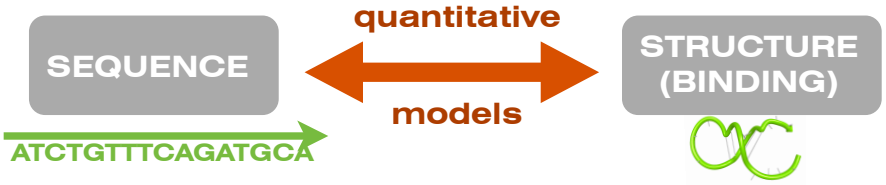
Partitioning noise + nonlinearities in the system: possibly large perturbations of the dynamics

Partitioning noise may be useful!
Provides randomized "initial conditions" to screen sensitivity/robustness of complex circuits around operating point.

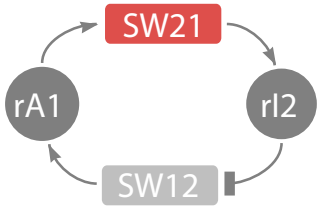


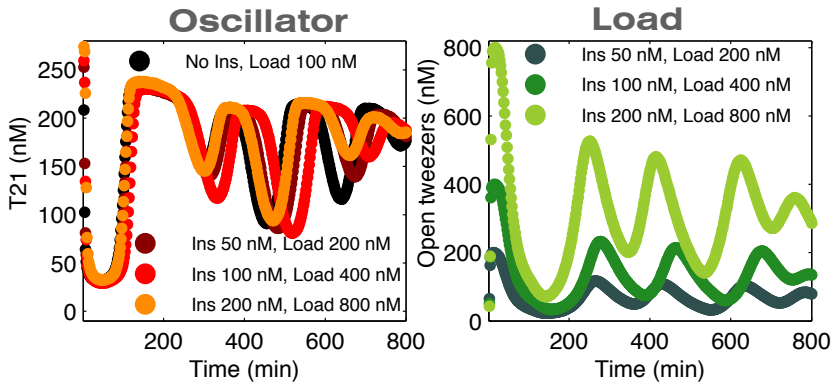
Summary

- Nucleic acids: ideal building material for programming dynamic molecular networks



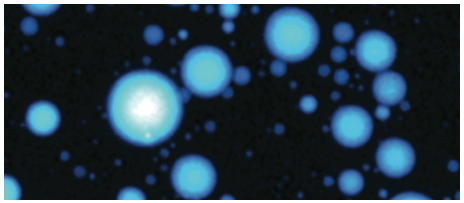
- Case study: oscillator

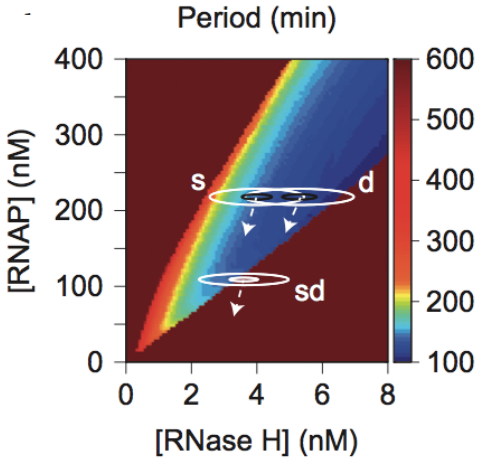




- Signal transmission, insulation

- High throughput screening with droplets, dynamic diversity





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