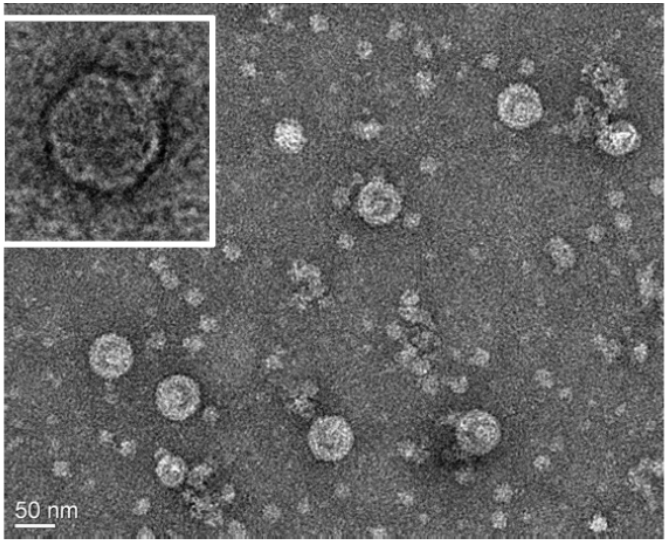
Cell-free TX-TL systems for synthetic biology

Vincent Noireaux, University of Minnesota

Caltech workshop, August 2013

Outline:

- Introduction - Motivations.
- Cell-free TX-TL.
- Synthetic gene circuits and phage synthesis.
- Artificial cell.



Electron microscopy of T7 bacteriophages expressed in a test tube.

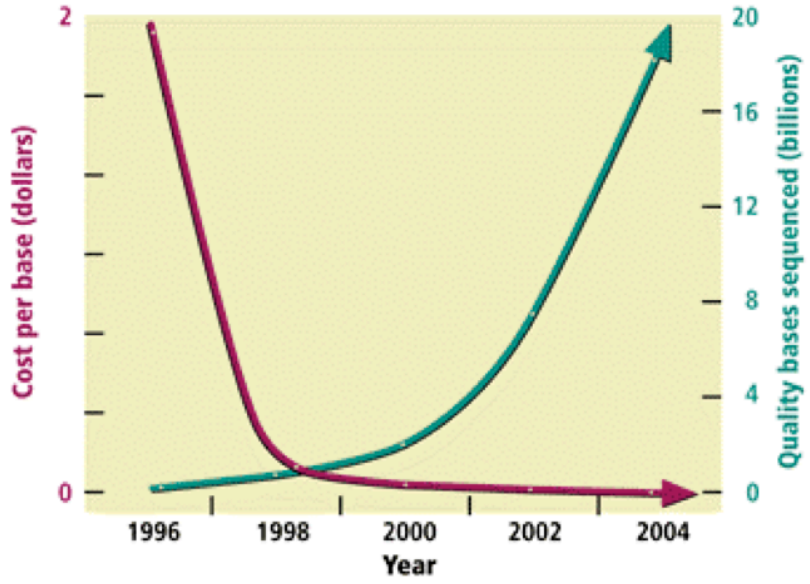
1
Introduction – Motivations

- **Cell-free synthetic biology.**
- **bottom-up construction of biochemical systems.**

Synthetic biology era

The design and fabrication of biological components and systems that do not exist in the natural world:

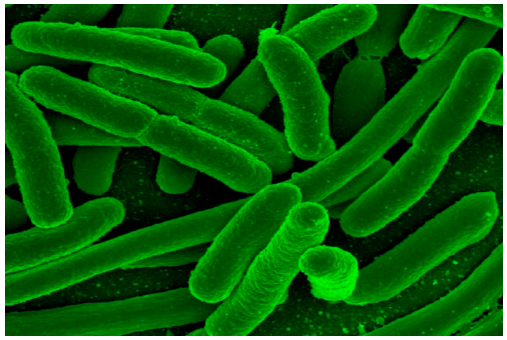
- to understand gene regulation and make simple computations.
- to use them either as molecular-scale factories.
- to create new hybrid materials.



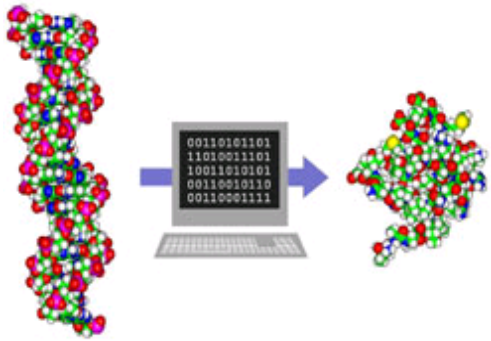
DOE – Human Genome Project

Synthetic biology platforms

in vivo



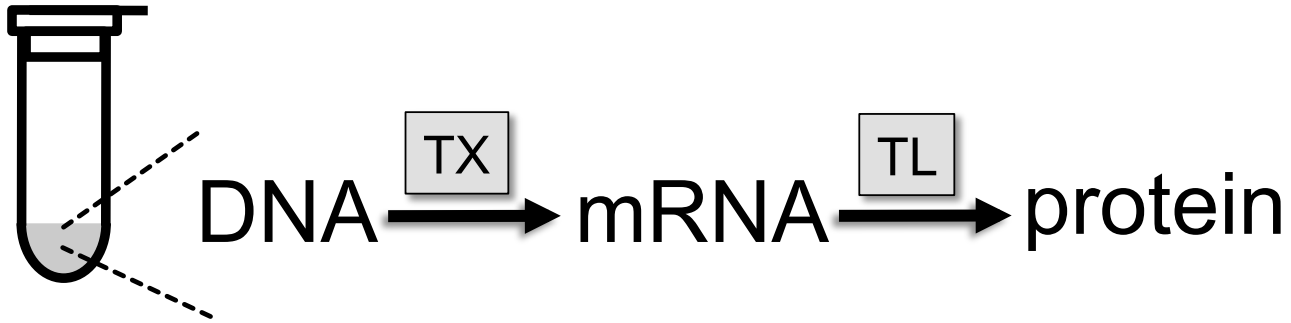
in silico



in vitro

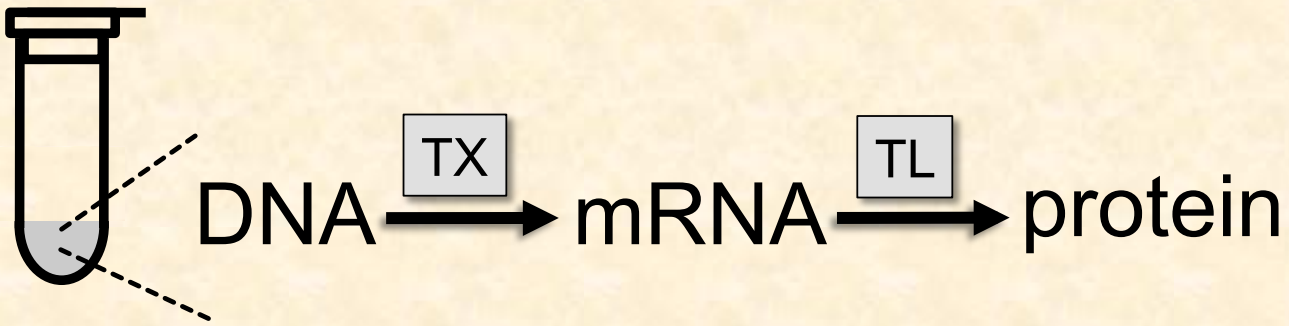
Synthetic biology in a test tube
(cell-free synthetic biology)

Construction of living systems in a test tube from the DNA program.



- bottom-up, reductionist and constructive approach.
- no endogenous information.
- no interference and response from an organism.
- more freedom of control and design compared to in vivo.

2
Cell-free TX-TL



Cell-free TX-TL systems

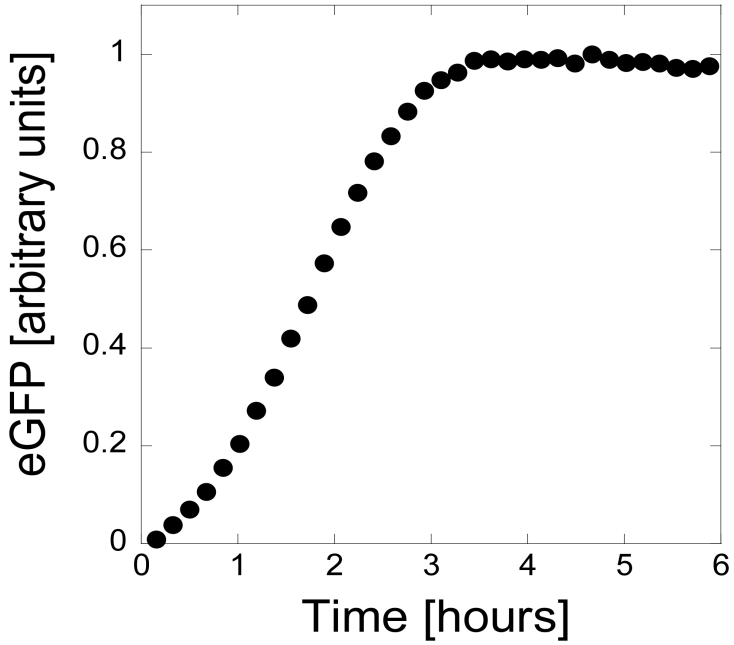
(a brief history)

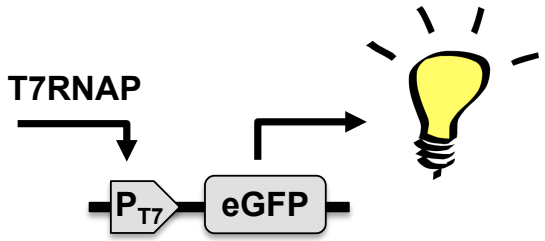
- 1961: first cell-free protein synthesis study.
  (Matthaei and Nirenberg)
- 70s: gene regulation unraveled with cell-free systems.
- 90s: invention of the efficient hybrid cell-free system.
  - large scale protein synthesis.
  - high throughput proteomics.
  - protein evolution.
  - industrial applications.

Not developed and not optimized to program or study biochemical systems in vitro.

Limitations of the conventional hybrid TX-TL CFS:

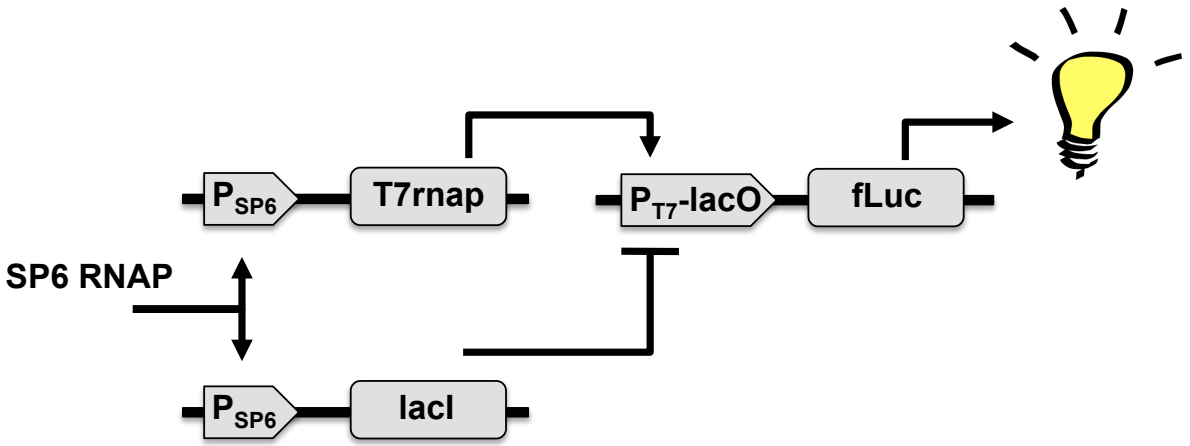
- TX: limited to a few bacteriophage RNA polymerases.
  repertoire of regulatory parts way too small.
- No control of mRNA and protein degradation.
- No control of the expression dynamics.

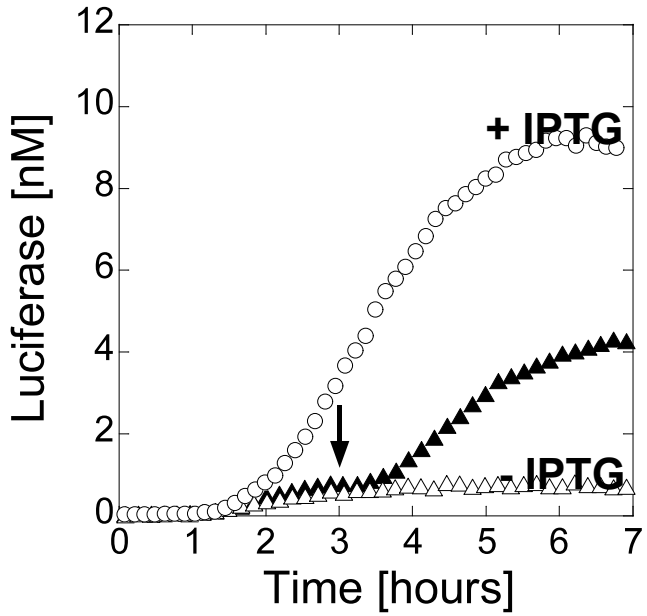




- 3-6 hours of expression.
- 0.5-1mg/ml of protein synthesized (20-35μM for eGFP)
- E. coli: $[Protein]_{ave}$ = 500nM
- 10μl reactions.

Circuits with conventional TX-TL CFS:





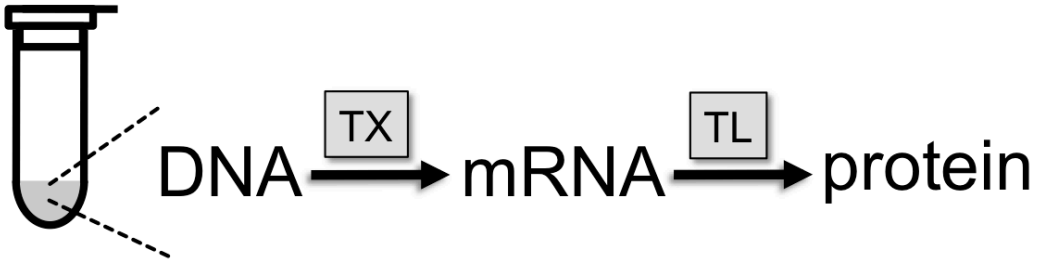
Noireaux et al, PNAS, 2003.

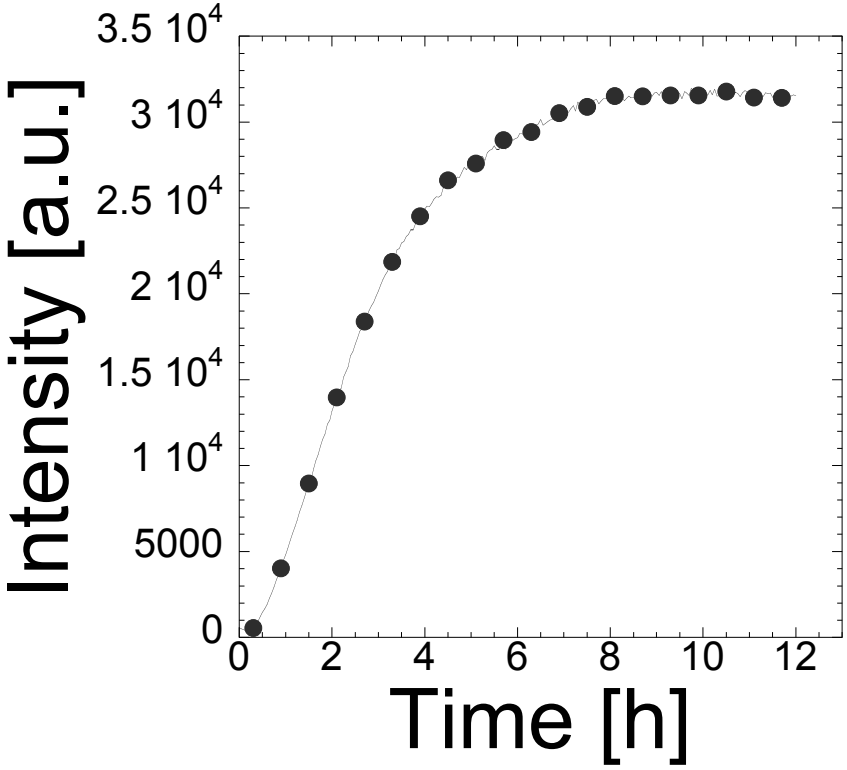
An ‘all’ E. coli cell-free TX-TL system

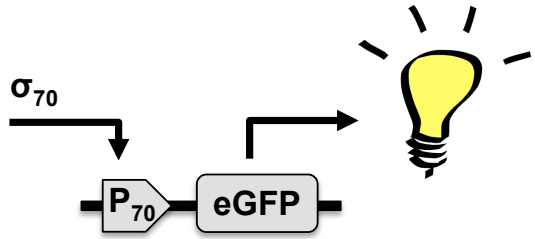
- **crude extract preparation:**
  - E. coli cells (fast, reproducible).
  - cytoplasm is extracted.
  - endogenous DNA and mRNA are degraded.
  - the extract contains:
    - **transcription machinery: σ_{70} and core RNAP.**
    - **translation machinery: ≈ 100 molecules.**
  - dilution factor: 20-30 times compared to in vivo.
    (protein :250-300mg/ml in vivo, 10mg/ml in vitro)

- **reaction (≈ 10µl):**
  - crude extract.
  - buffer: energy, building blocks (nucleotides, AA).
  - plasmid DNA program prepared in the lab.

1-gene characterization







- 3-6 hours of expression.
- 0.5-1mg/ml of protein synthesized (20-35µM for deGFP)
- 10µl reactions.

Shin and Noireaux, JBE, 2010a.

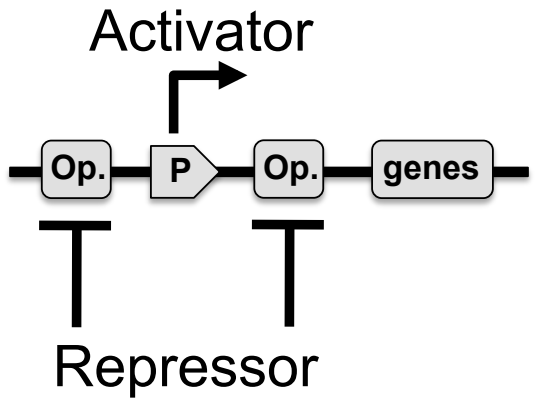
TX repertoire

Transcriptional activation

- Core RNAP + σ_{70}: housekeeping TX.
- 6 others E. coli sigma factors: 19, 24, 28, 32, 38, 54-NtrC.
- 2 bacteriophage RNA polymerases: T7 and T3.
- all the E. coli regulatory parts (promoter/operators) available.

Transcriptional repression

- a set of 5 repressors: lac, tet, ara, CI, Cro.



mRNA and protein degradation

- mRNA mean lifetime ≈ 12-13 min for deGFP
  Can be shortened down to 0 min with MazF toxin.

- Protein mean lifetime ≈ ∞.
  Can be shortened with endogenous AAA+ proteases.

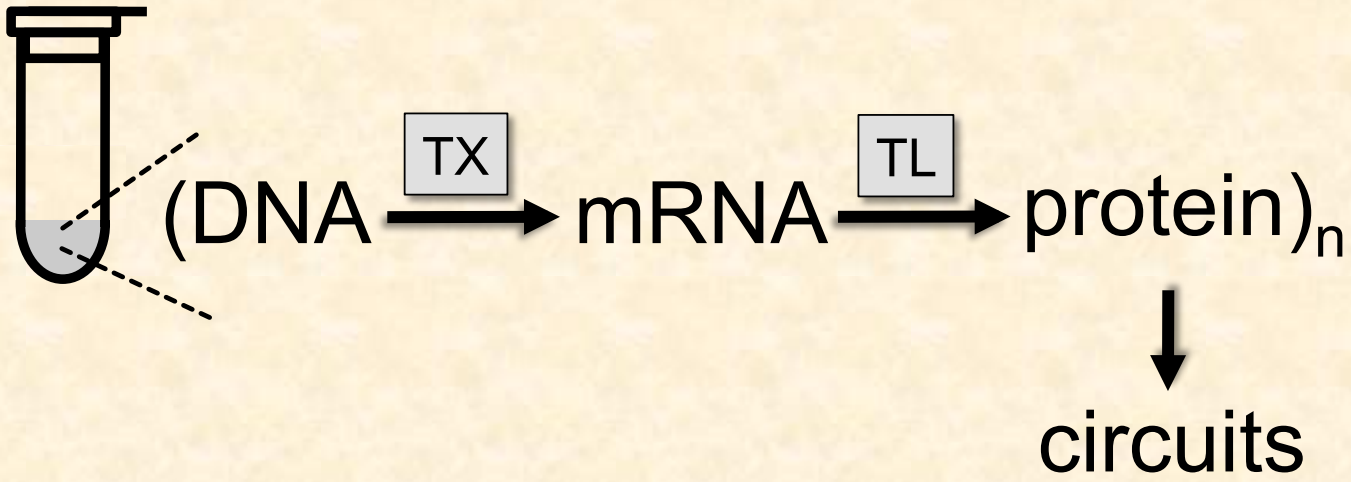
Shin and Noireaux, JBE, 2010b.

Conclusions:

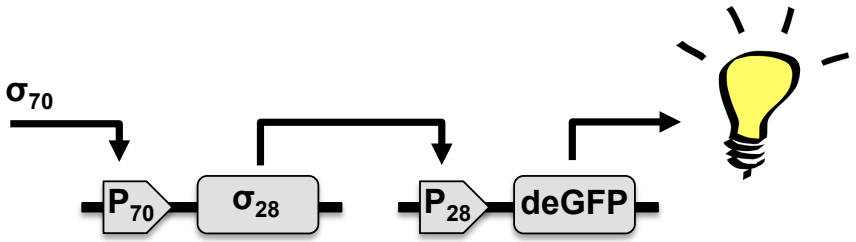
- all E. coli cell-free TX-TL toolbox.
  - repertoire of 14 TX regulatory parts.
  - control of synthesis and degradation rates.
- coarse-grained model of TX/TL processes.
- next steps: better cell-free TX-TL (currently optimizing).
- collaboration: Roy Bar-Ziv (Weizmann).

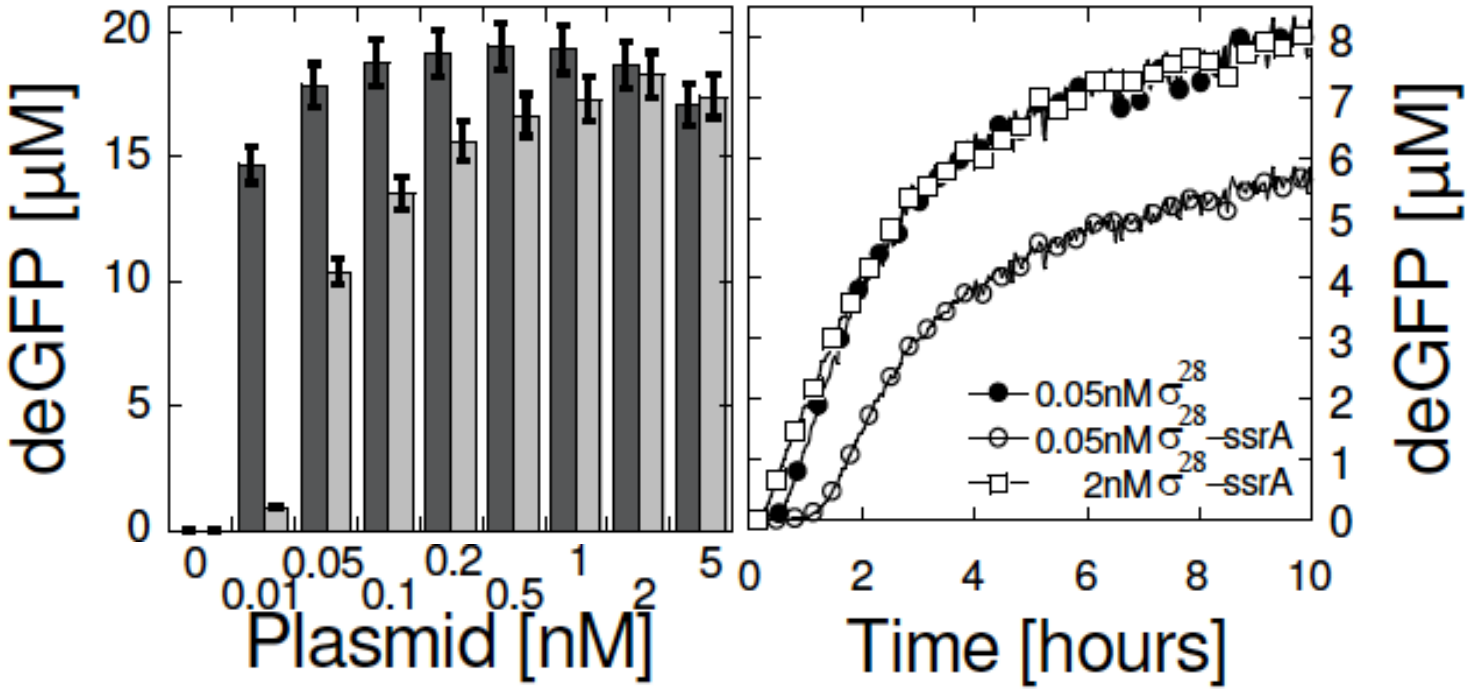
- Noireaux et al. PNAS 2003.
- Shin and Noireaux. J. Biol. Eng. 2010a
- Shin and Noireaux. J. Biol. Eng. 2010b.
- Karzbrun et al. PRL 2011.
- Shin and Noireaux. ACS Synthetic Biology 2012.

3
Elementary cell-free circuits

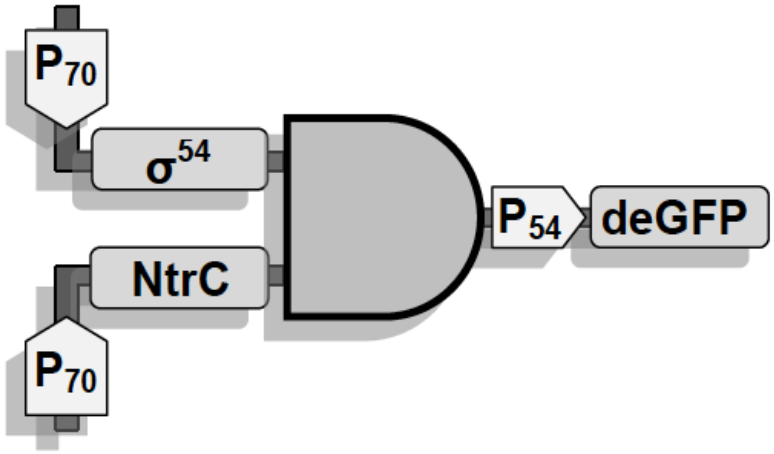


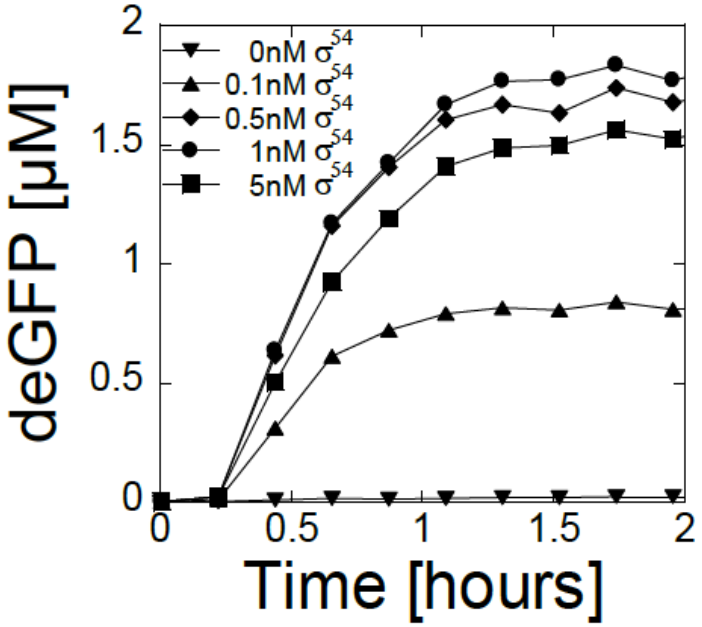
TX activation cascade



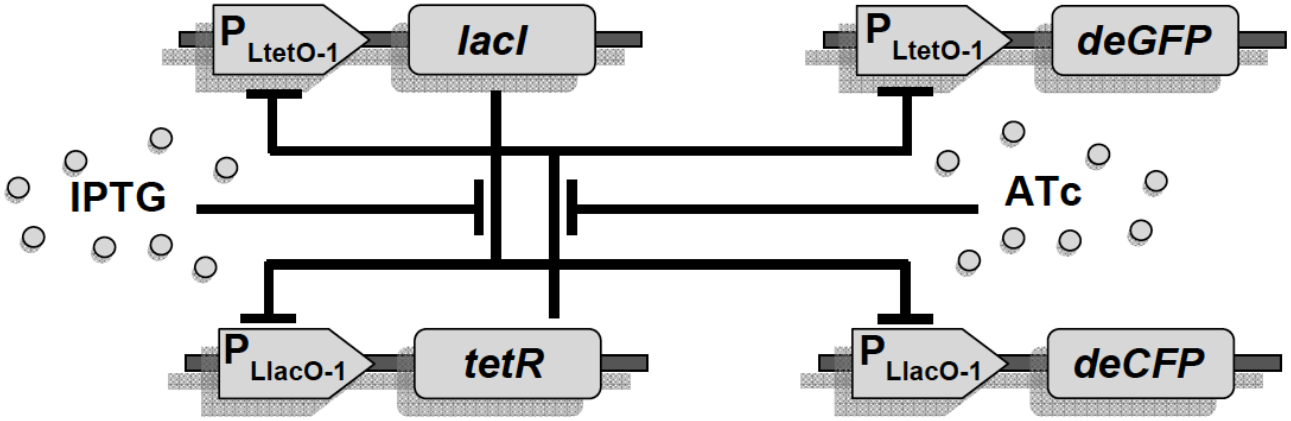


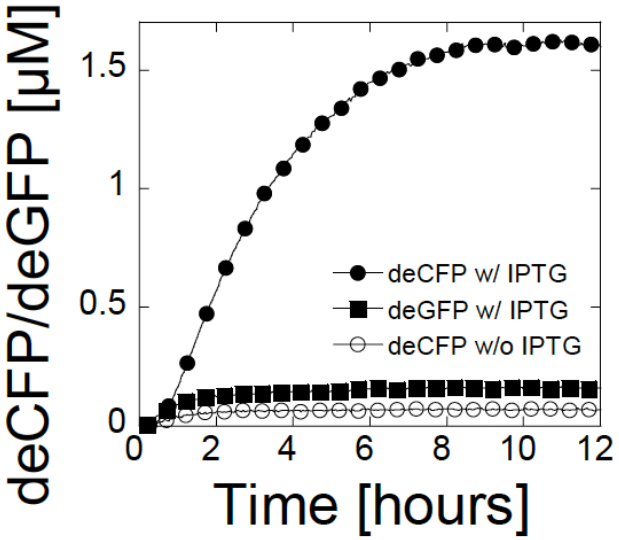
AND gate S54-NtrC

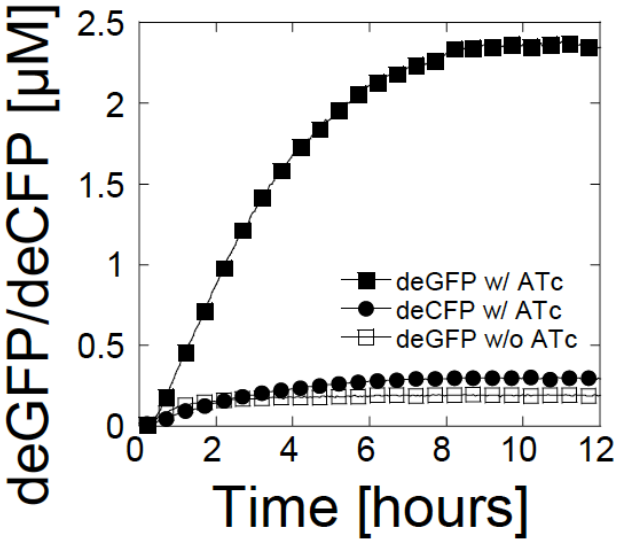




4 outputs circuit





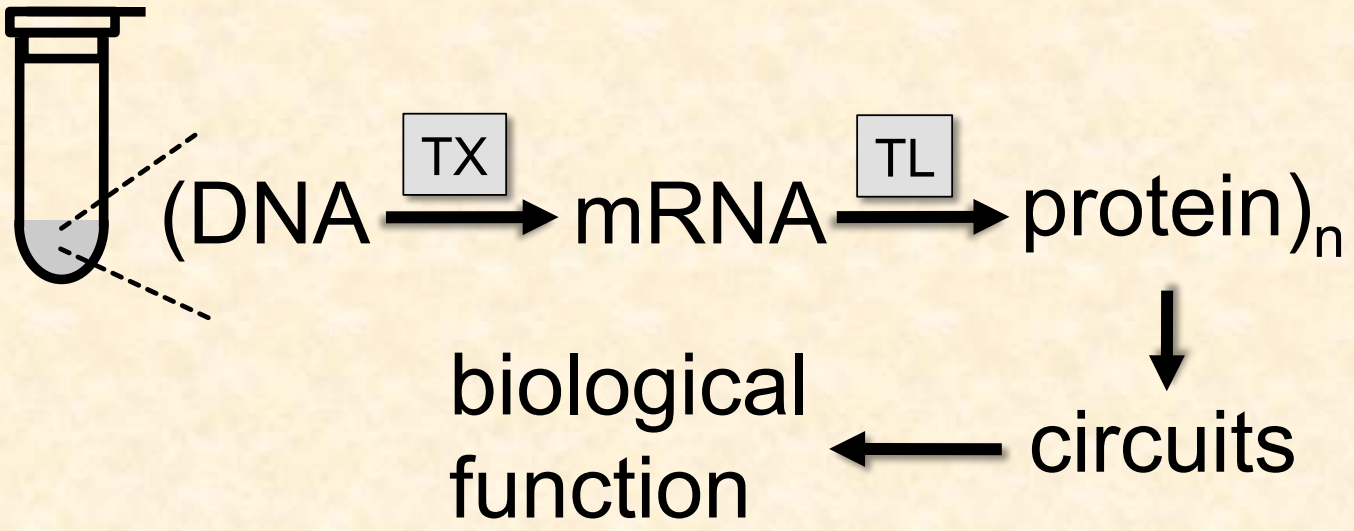


Conclusion:

- constructed and characterized small cell-free circuits.
- next steps: change cloning technique, test larger circuits.
- collaboration: Richard Murray (Caltech)
  Chris Voigt (MIT)

- Shin and Noireaux. ACS Synthetic Biology 2012.

4
Genome-sized circuits
Bacteriophage synthesis



Genome scale circuits

(information and self-organization)

- What is the real capacity of the system to construct circuits and living systems?

CFR batch mode: [Protein] = 25-30μM
E. coli: $[Protein]_{ave}$ = 500nM

} **50-60 genes**

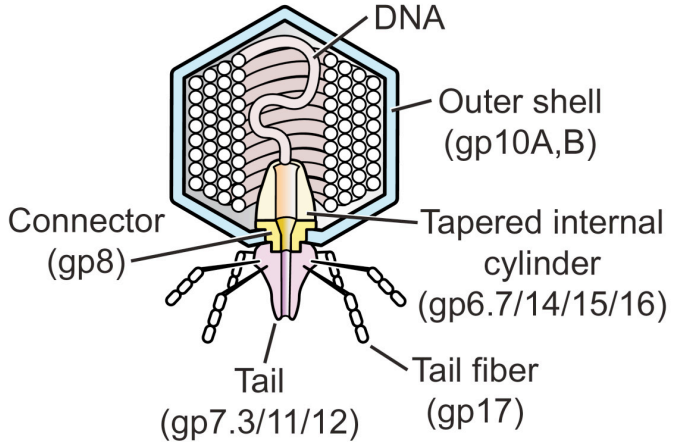
- Test the system with genome-sized information.

- Bacteriophages:

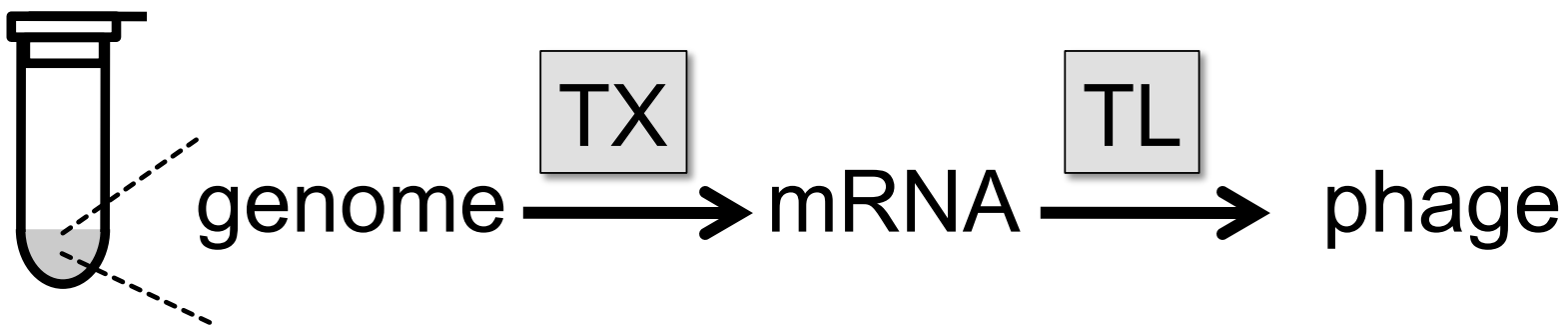
- search for genomes composed of ≤ 60 genes.
- with molecular biology technically accessible.
- condition/bottleneck: complexity of the interaction with the host (beyond TX-TL).

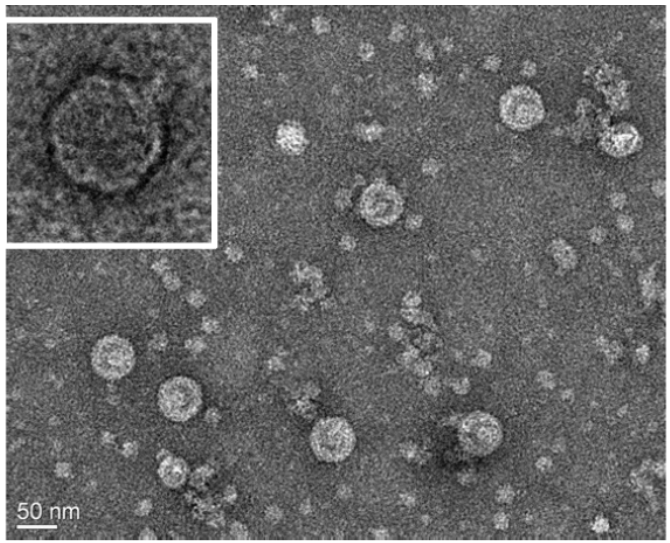
Phage T7

- lytic coliphage.
- 40 kbp, 60 genes (35 with known functions).
- almost host independent (2 host proteins required).
- has its own RNA polymerase.
- has its own DNA polymerase.



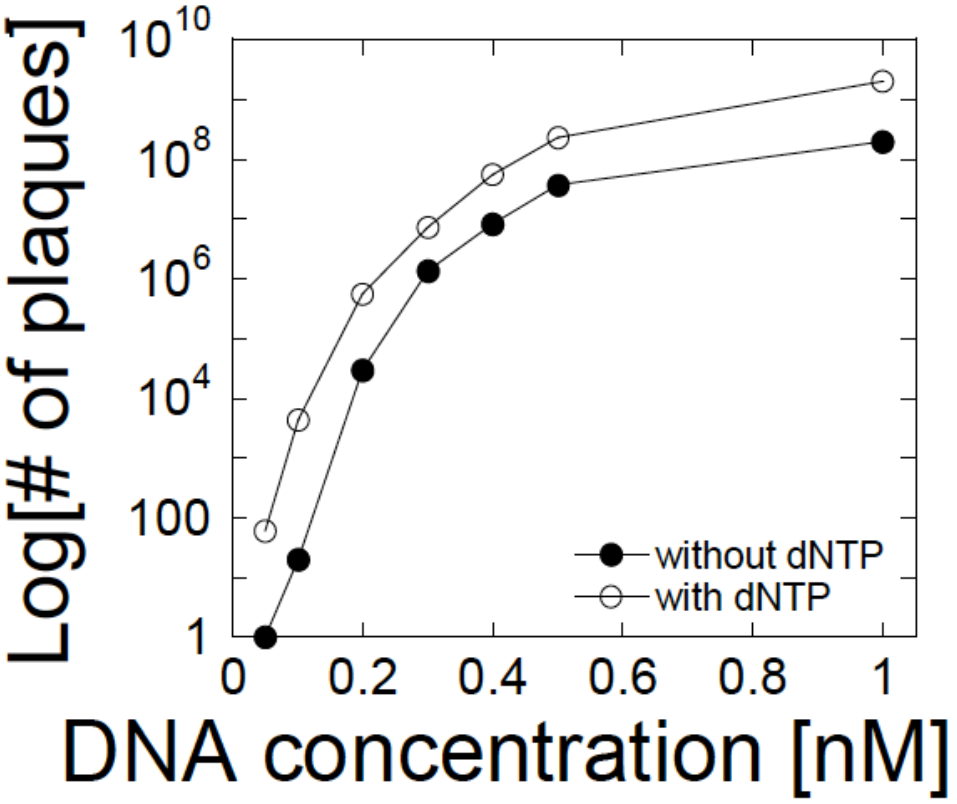
Phage T7 synthesis in a test tube





- TEM image
- 5-6 hours of incubation
- batch mode reaction

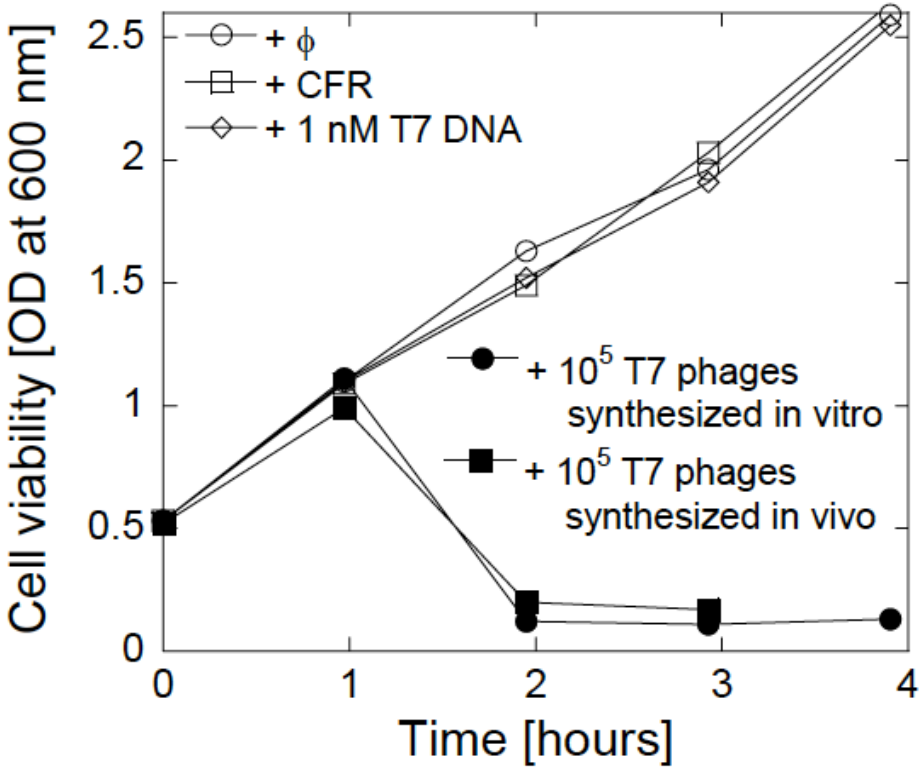
T7 Genome replication



- up to 200 times greater with dNTPs.
- a few billion of functional phages per milliliter synthesized after 5-6 hours of incubation in batch mode.

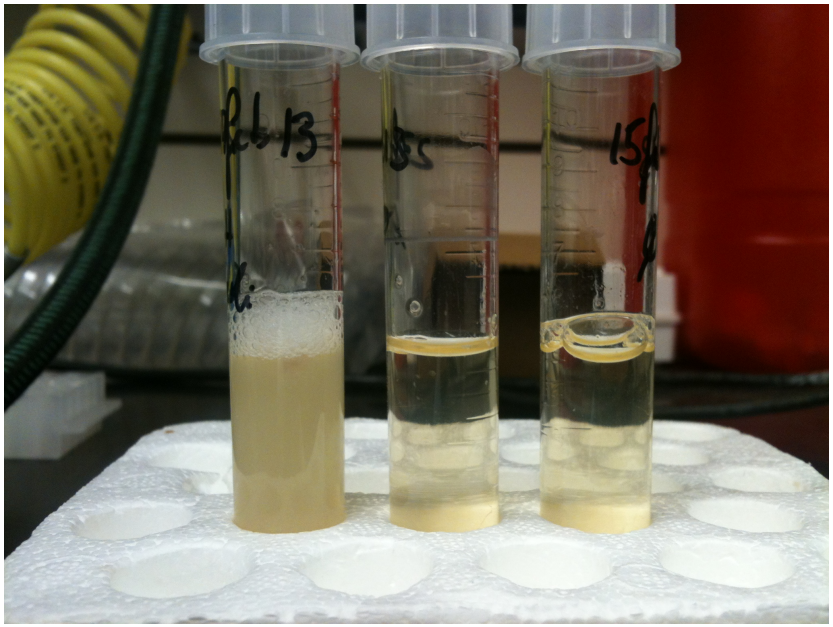
T7 - E. coli Infection test

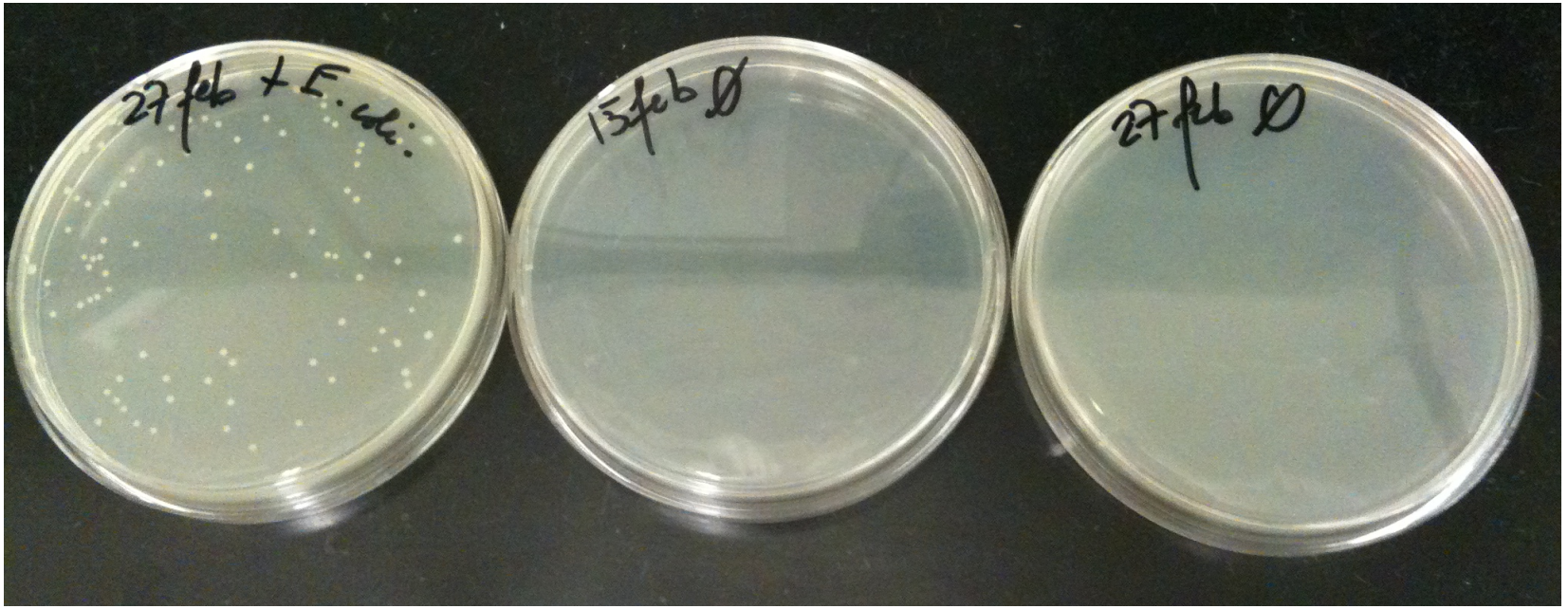
No difference observed between *in vivo* and *in vitro* synthesized phages.



- phages per cell ≈ 100.
- phage cycle ≈ 25 min.
- E. coli division ≈ 30 min.

Extract
Quality control

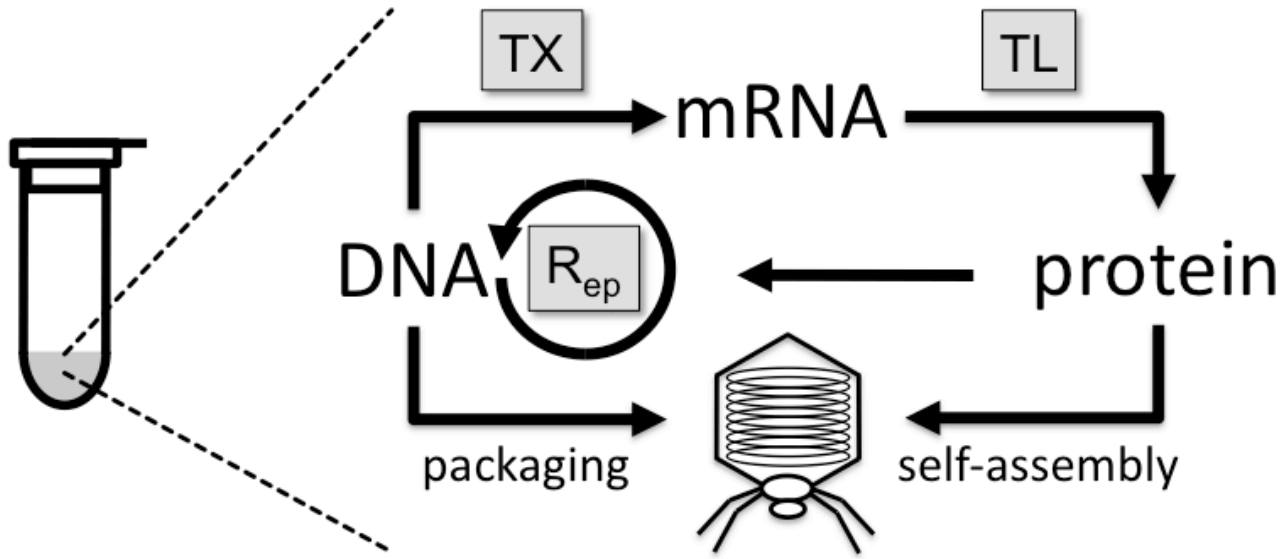




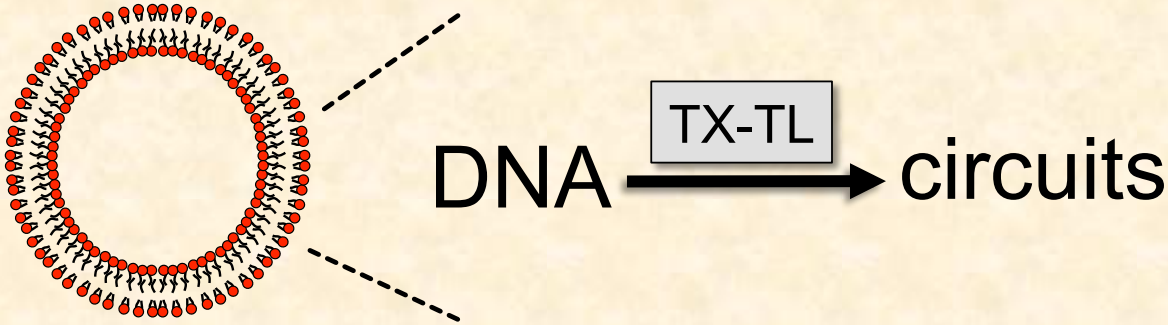
Conclusion:

- DNA replication and viral self-assembly in a test tube.
- semi-continuous TX-TL.
- next steps: better cell-free TX-TL.
  simplified DNA replication.

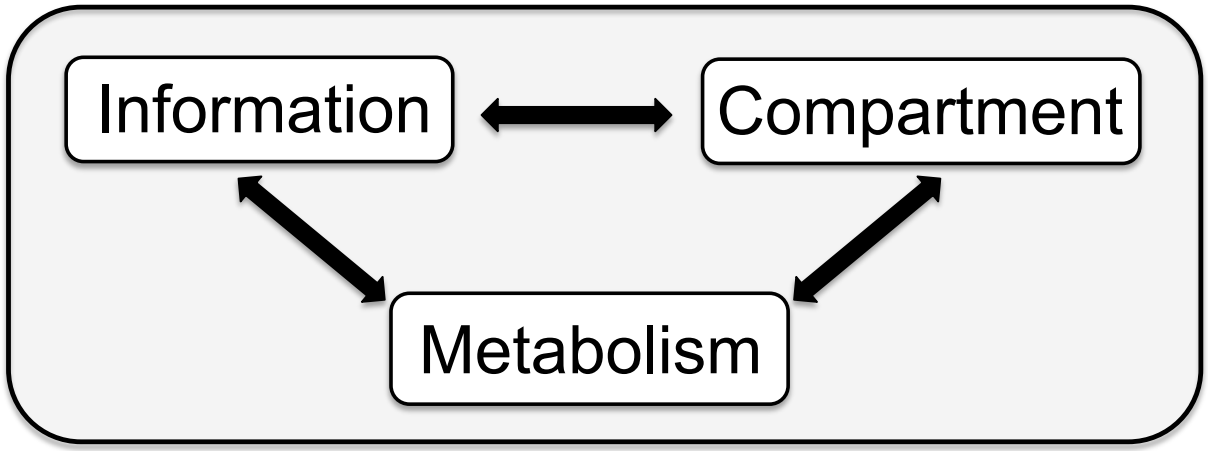
- Shin, Jardine and Noireaux. ACS Synthetic Biology 2012.



5
Artificial cell
Cell-free TX-TL in cell-sized compartments



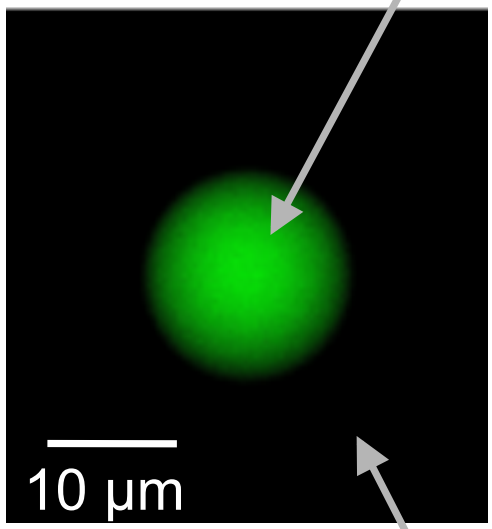
Bottom-up artificial cell

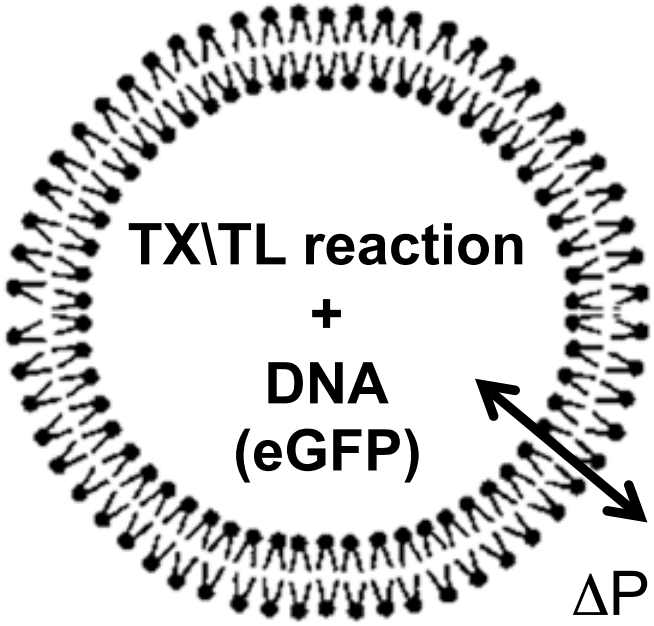


- Unique property: self-reproduction
  - each part is essential.
  - each part is made of molecular machineries.

- Far objective:
  - construct a ‘predictable’ artificial cell from scratch.
  - capture the cooperative link between the 3 parts.

Encapsulation inside lipid vesicles

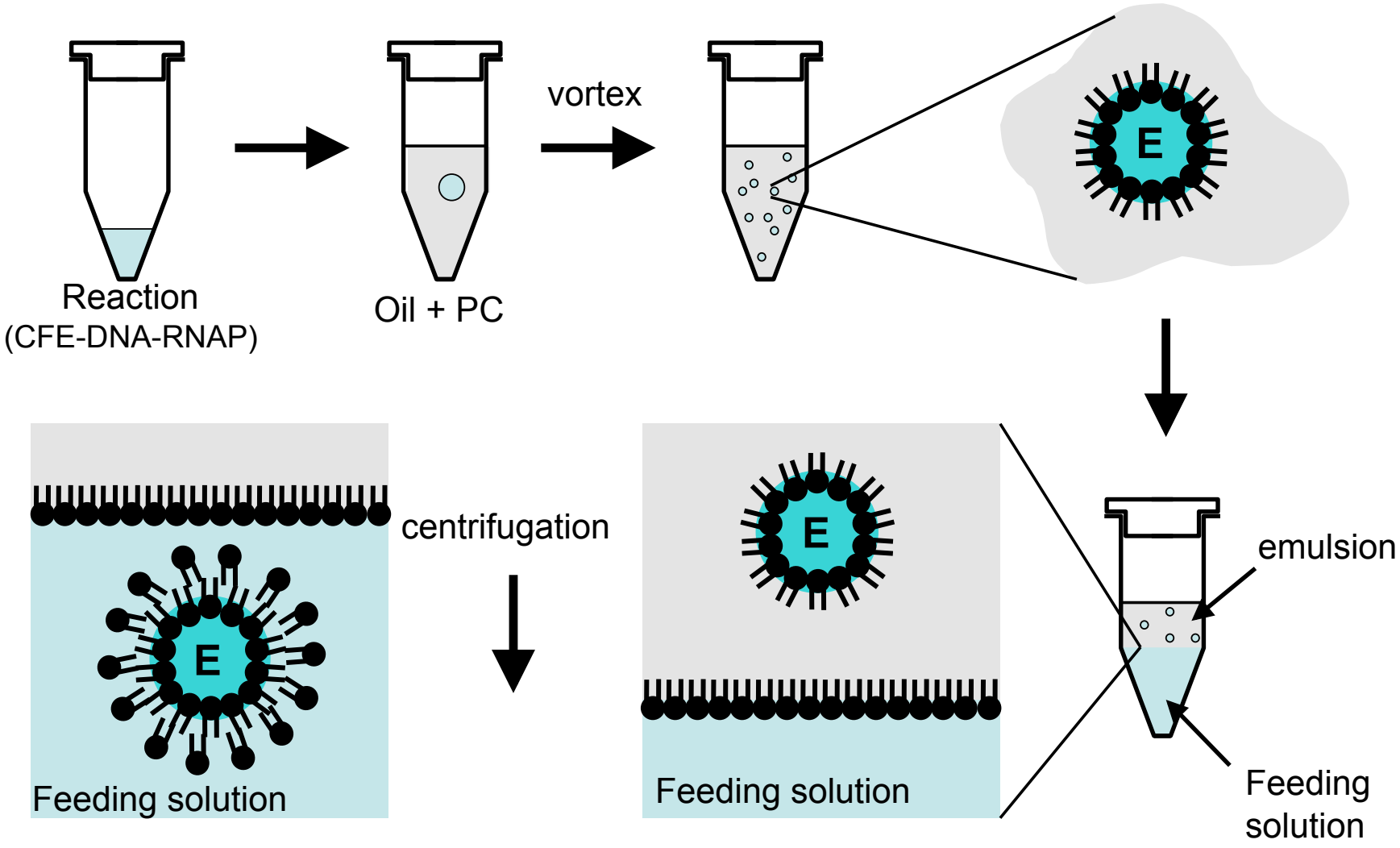




$$\Delta P = c\,k_B T = \frac{2\gamma}{R}$$

Feeding solution
(ATP, GTP, building blocks, buffer)

Encapsulation method

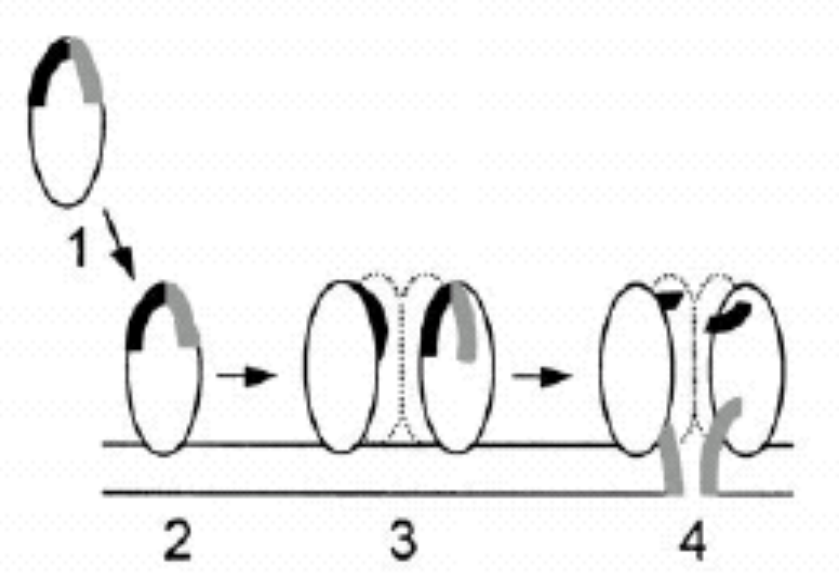


Pautot et al., 2004.

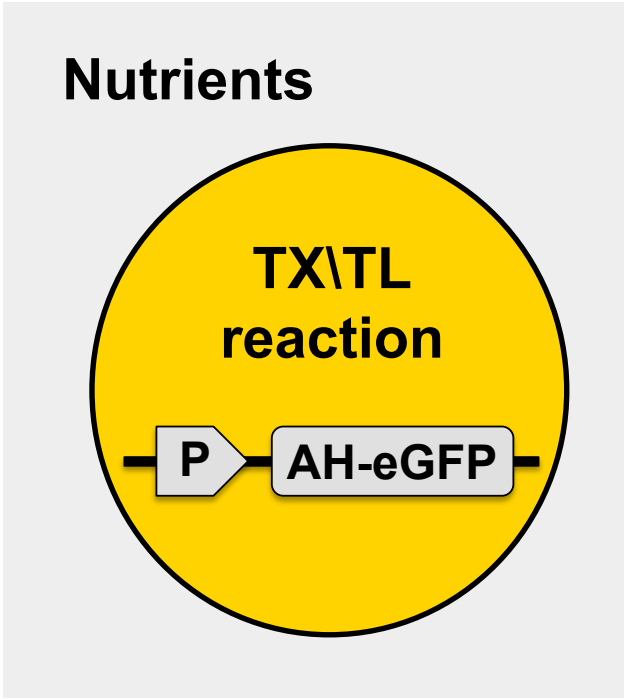
Continuous exchanges

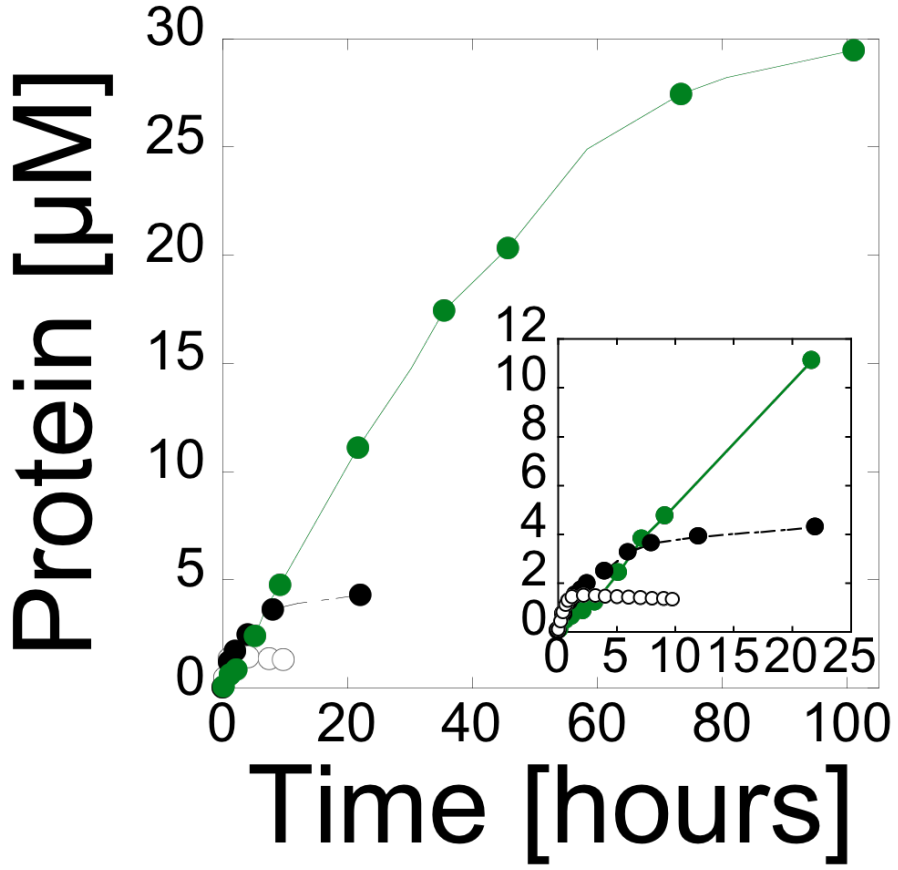
Alpha Hemolysin

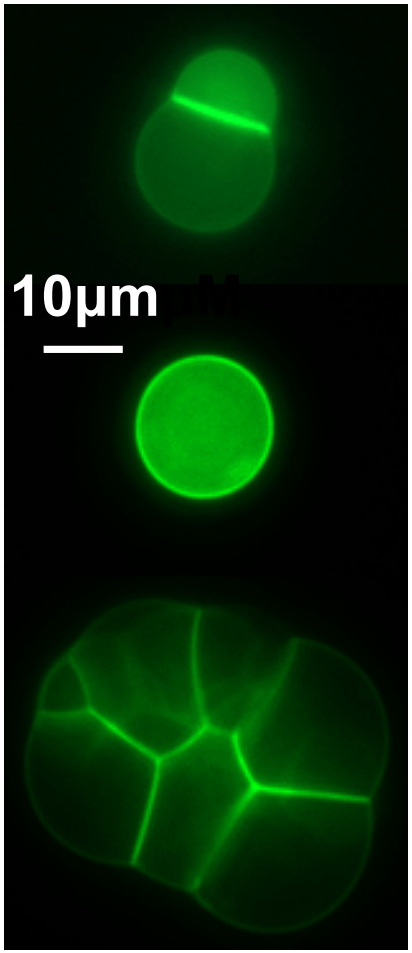
- toxin Staph. Aureus
- soluble monomer
- membrane heptamer
- channel of 1.4nm: 2-3kD



Song et al - 1997







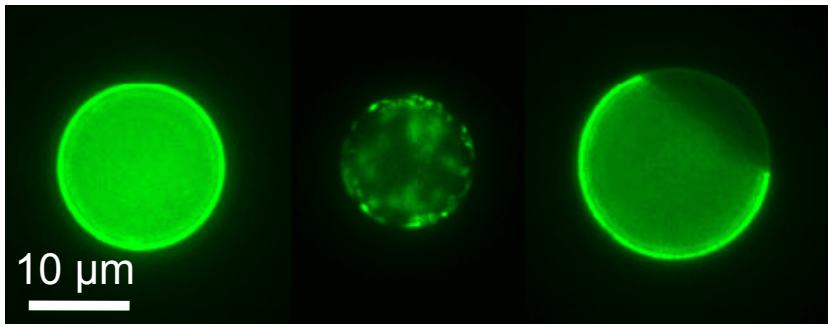
Noireaux and Libchaber PNAS 2004

Protein S-A at the membrane

The membrane is:

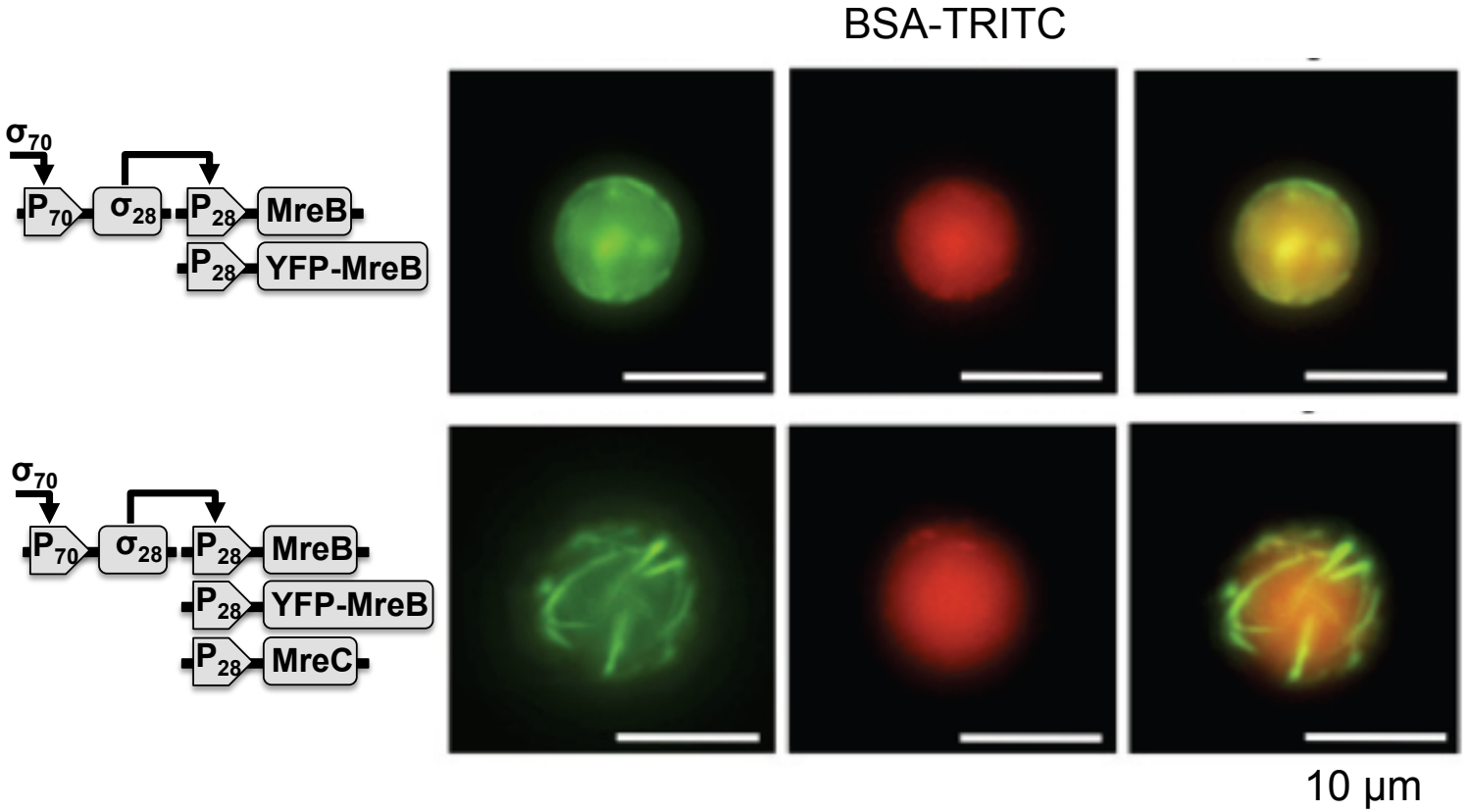
- the physical boundary of the cell.
- a template for self-assembly of proteins.
- essential for spatial organization (symmetry breaking).

Alpha-Hemolysin-eGFP, 25ºC



100%PC	90% PC 10% SM	90% PC 10% DHPC

Cytoskeleton at the membrane



Residual integral membrane protein insertion in cell-free TX-TL system. YidC? Sec system (SecA)?

Work done by:

Jonghyeon Shin (postdoc at MIT, Voigt lab)
Paul Jardine (PI, UMN, virology)

Work sponsored by: