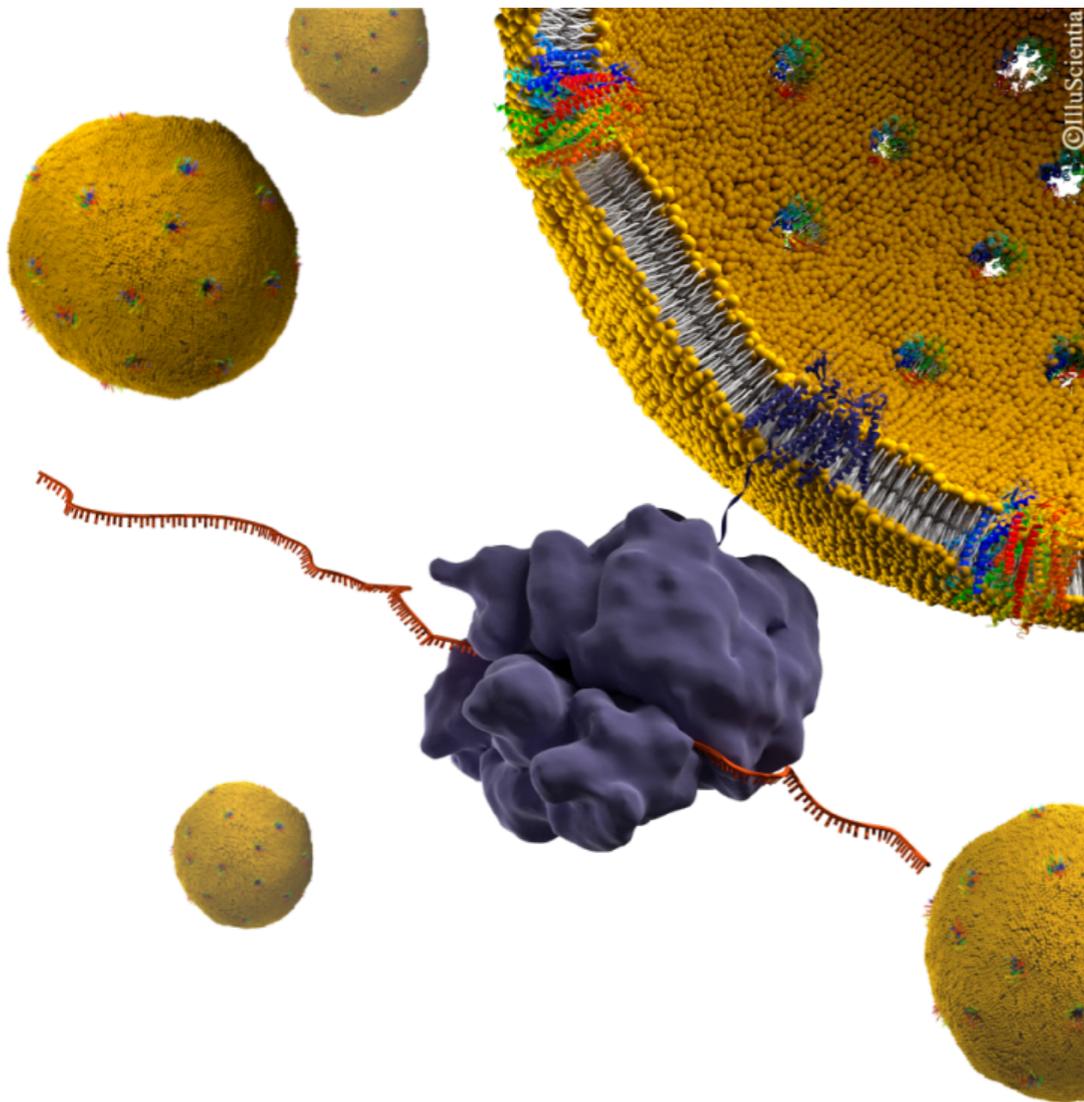
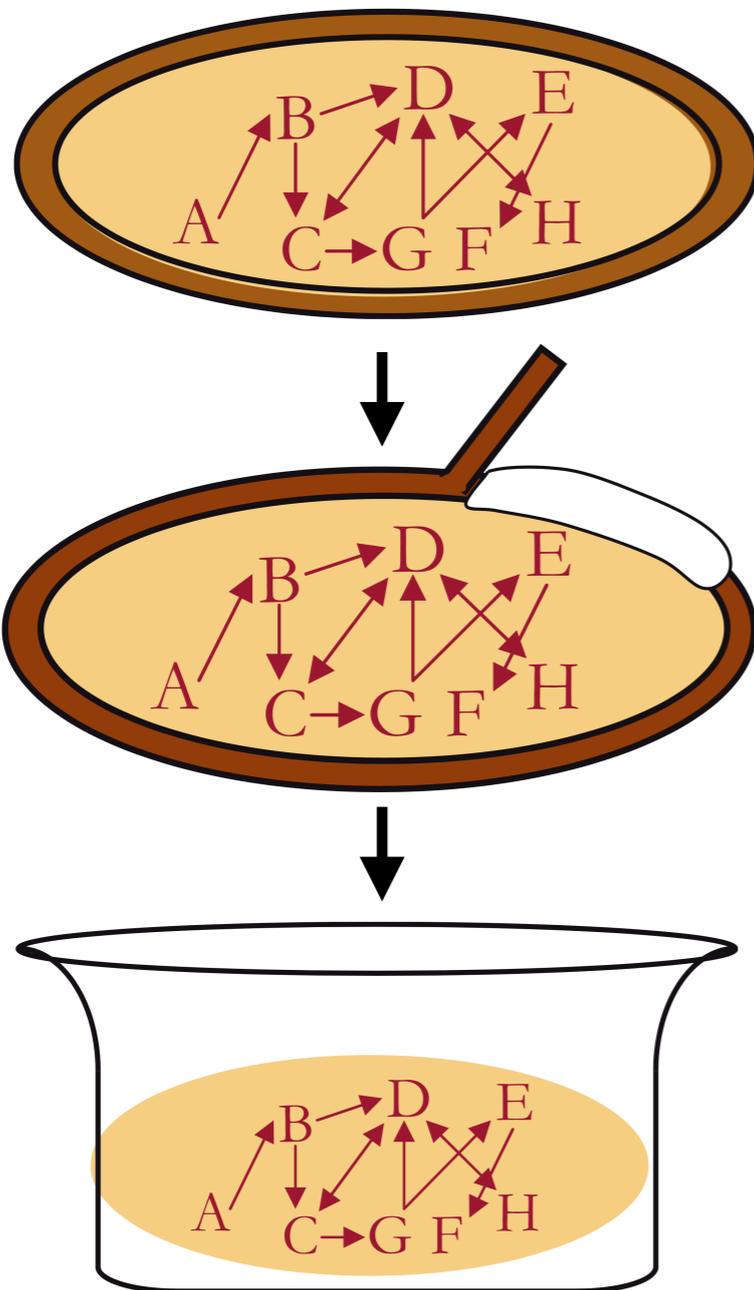


Structure



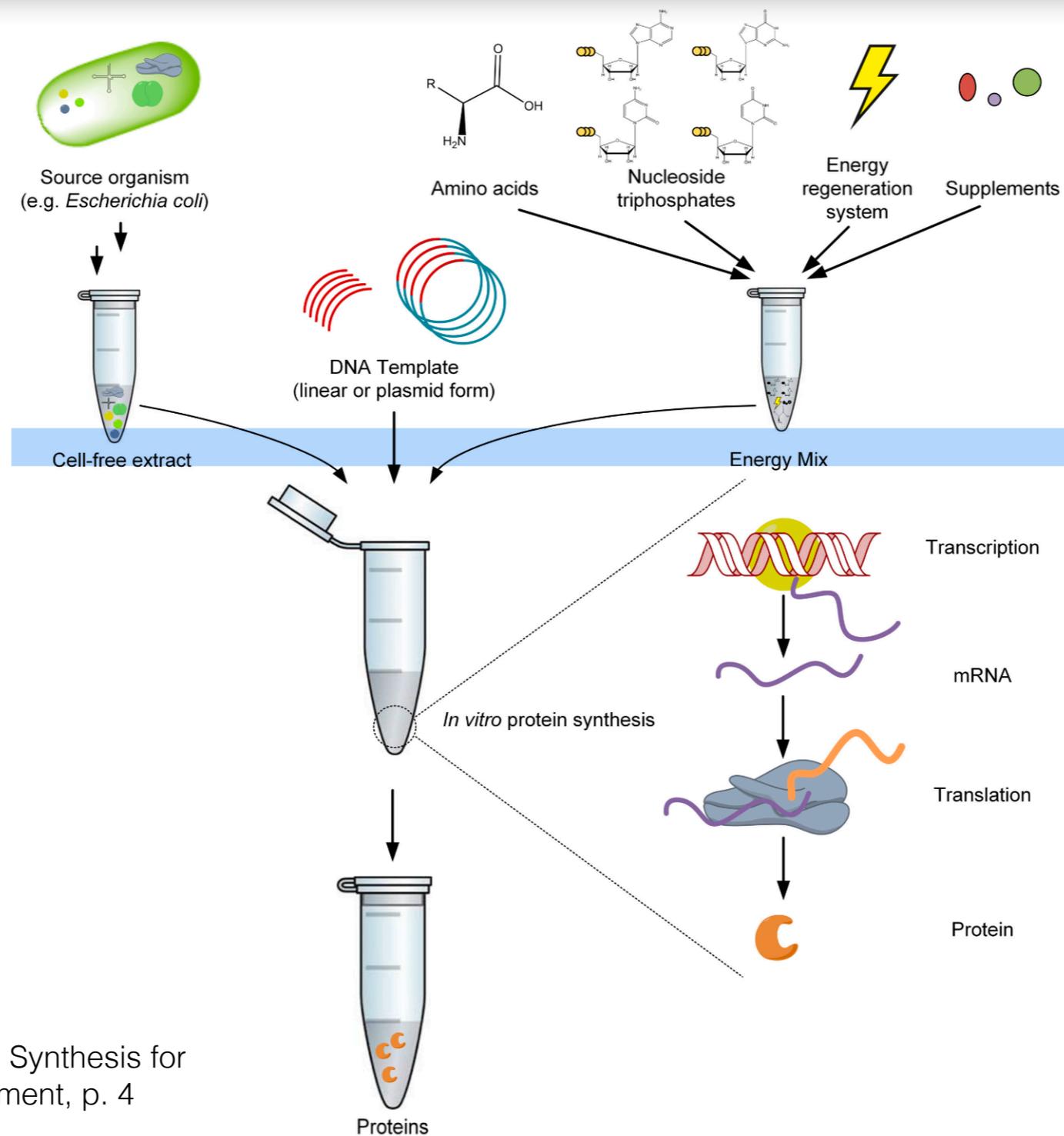
1. Introduction
2. Principles of cell-free genetic circuit assembly
3. A vesicle bioreactor as a step toward an artificial cell assembly
4. Current research

Introduction



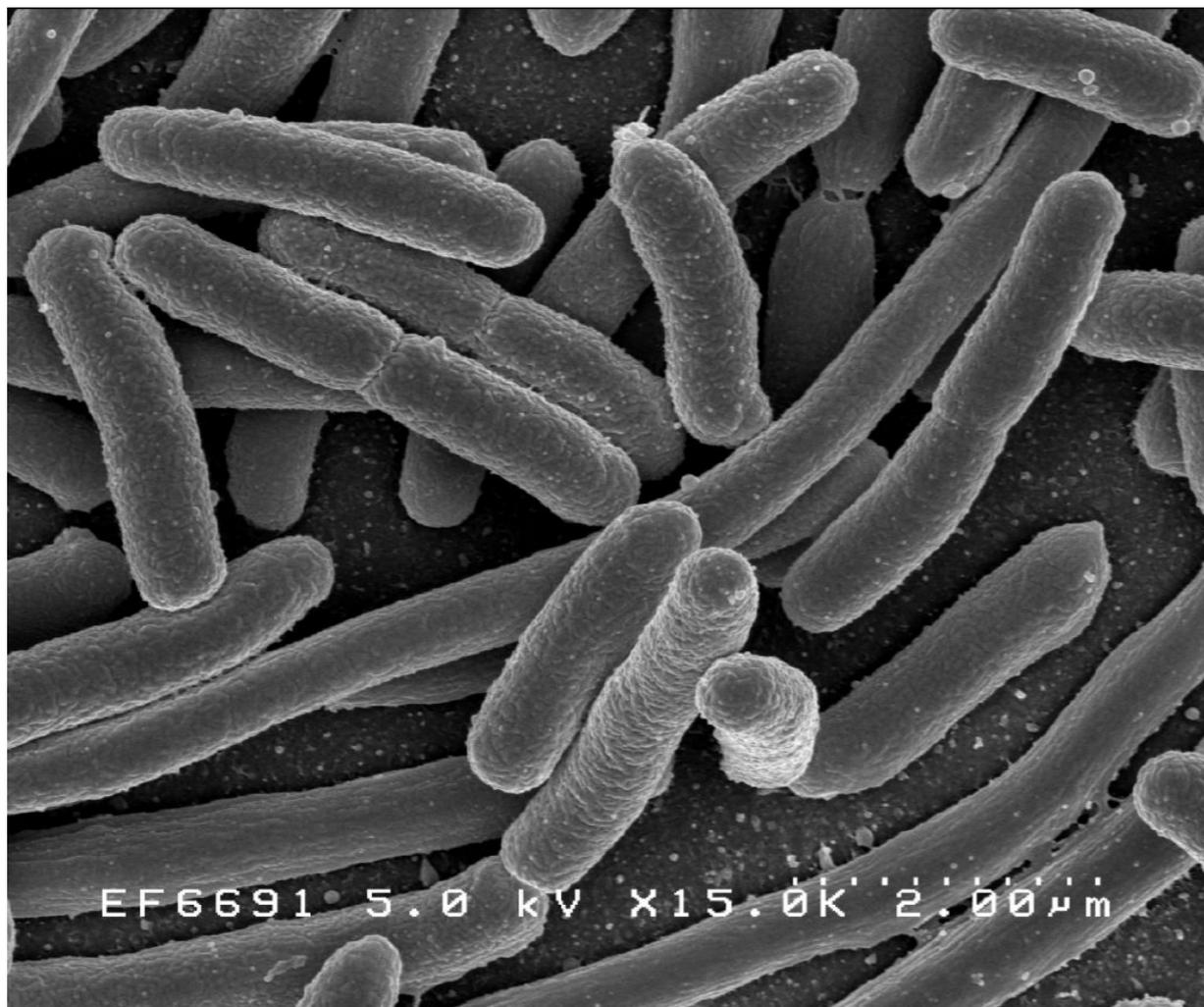
- Defined as “the reproduction, study, and exploitation of complex biological processes **WITHOUT** intact cells”
- Focus on basic biochemical reactions
- Ability to activate and control protein production

Basics of Cell-free Protein Synthesis



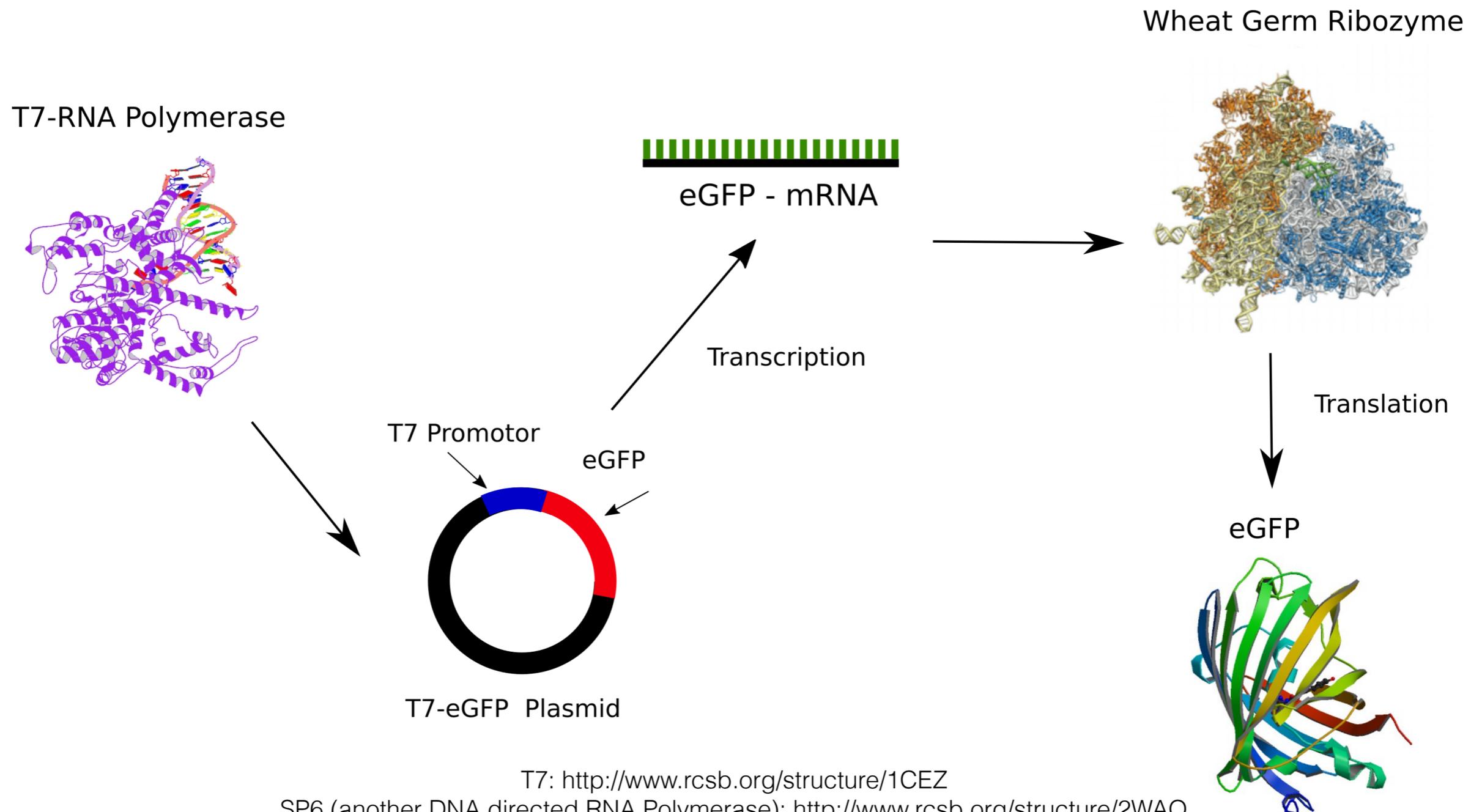
Cell-free Genetic Circuit Assembly

Introduction



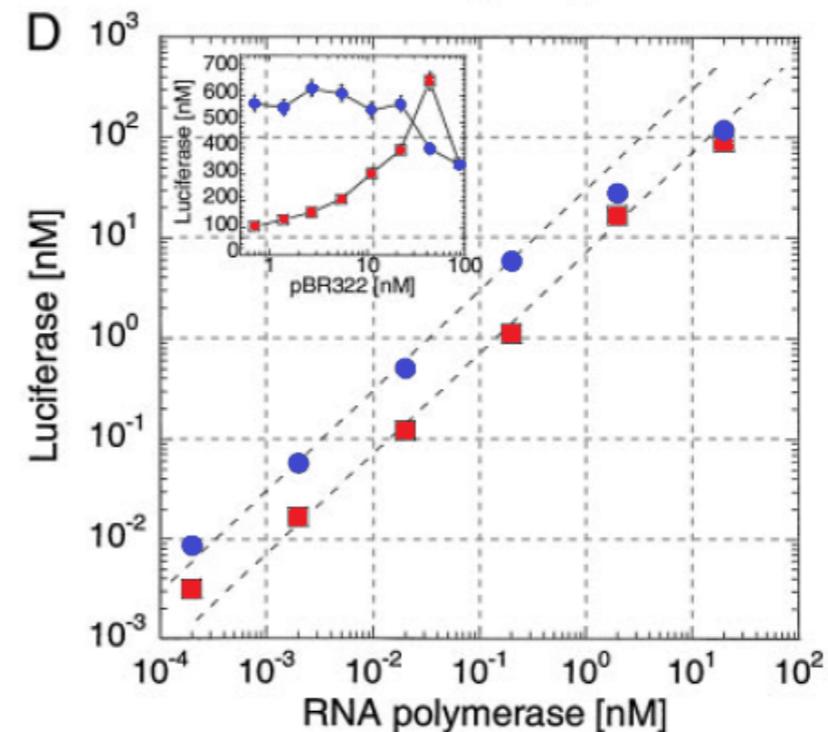
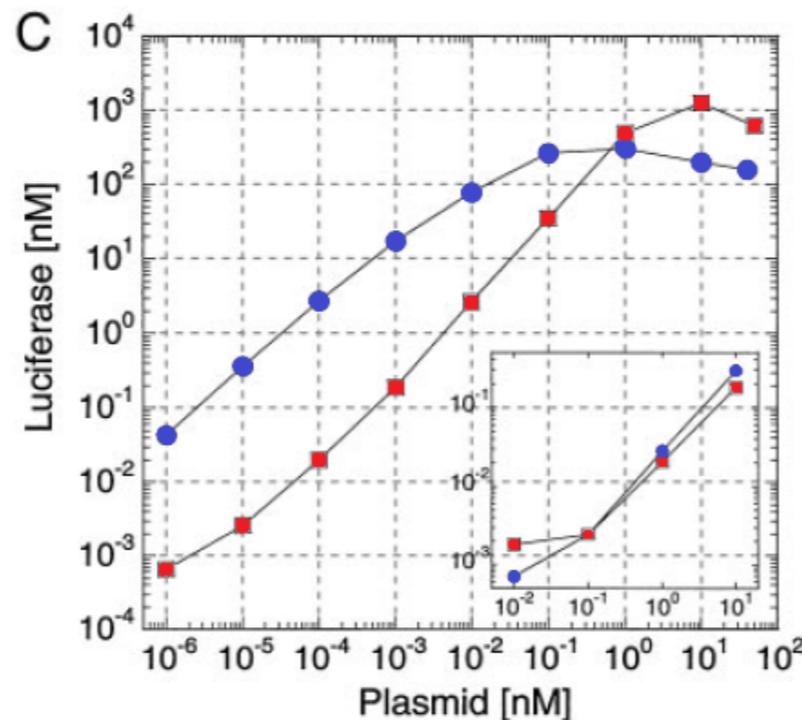
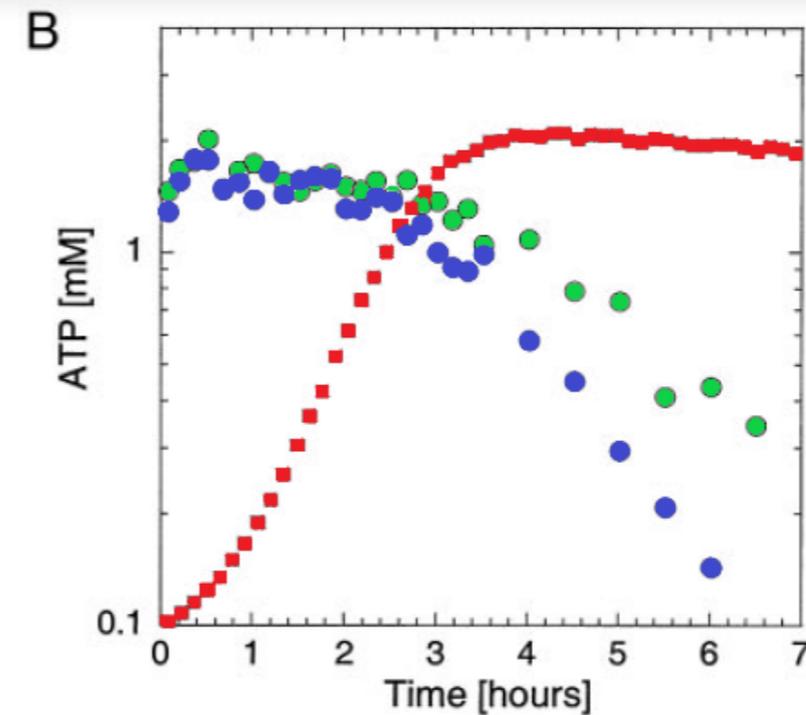
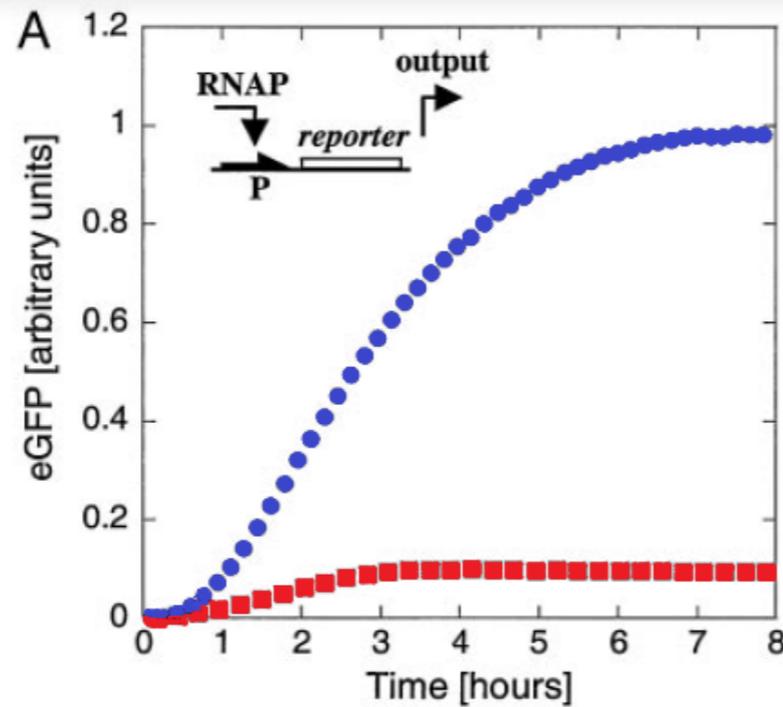
- Construction of synthetic genetic circuits
- Two and three step cascades of protein translation
- Introduction of regulatory elements

Cell-free Genetic Circuit Assembly



T7: <http://www.rcsb.org/structure/1CEZ>
 SP6 (another DNA directed RNA Polymerase): <http://www.rcsb.org/structure/2WAQ>
 Firefly luciferase: https://en.wikipedia.org/wiki/Firefly_luciferase

Cell-free Genetic Circuit Assembly

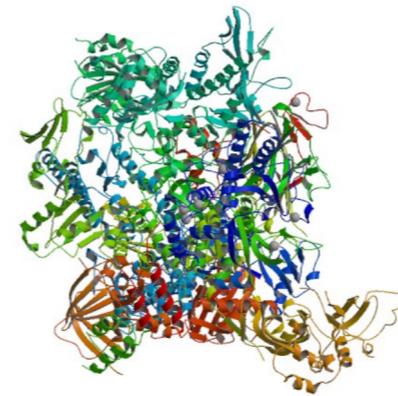


Cell-free Genetic Circuit Assembly

T7-RNA Polymerase

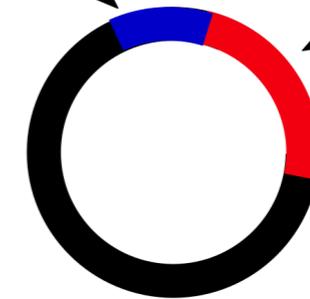


SP6-RNA Polymerase



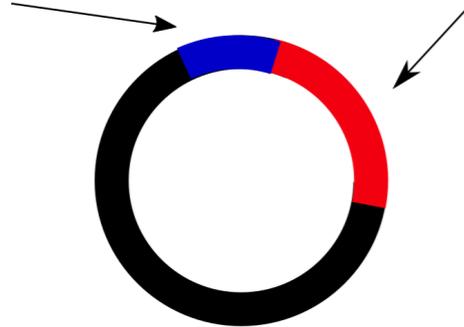
SP6 Promotor

Firefly Luciferase



T7-Promotor

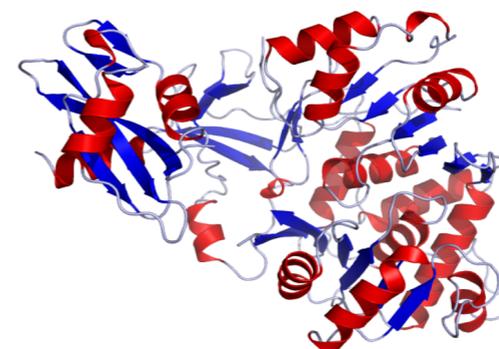
SP6-RNA Polymerase



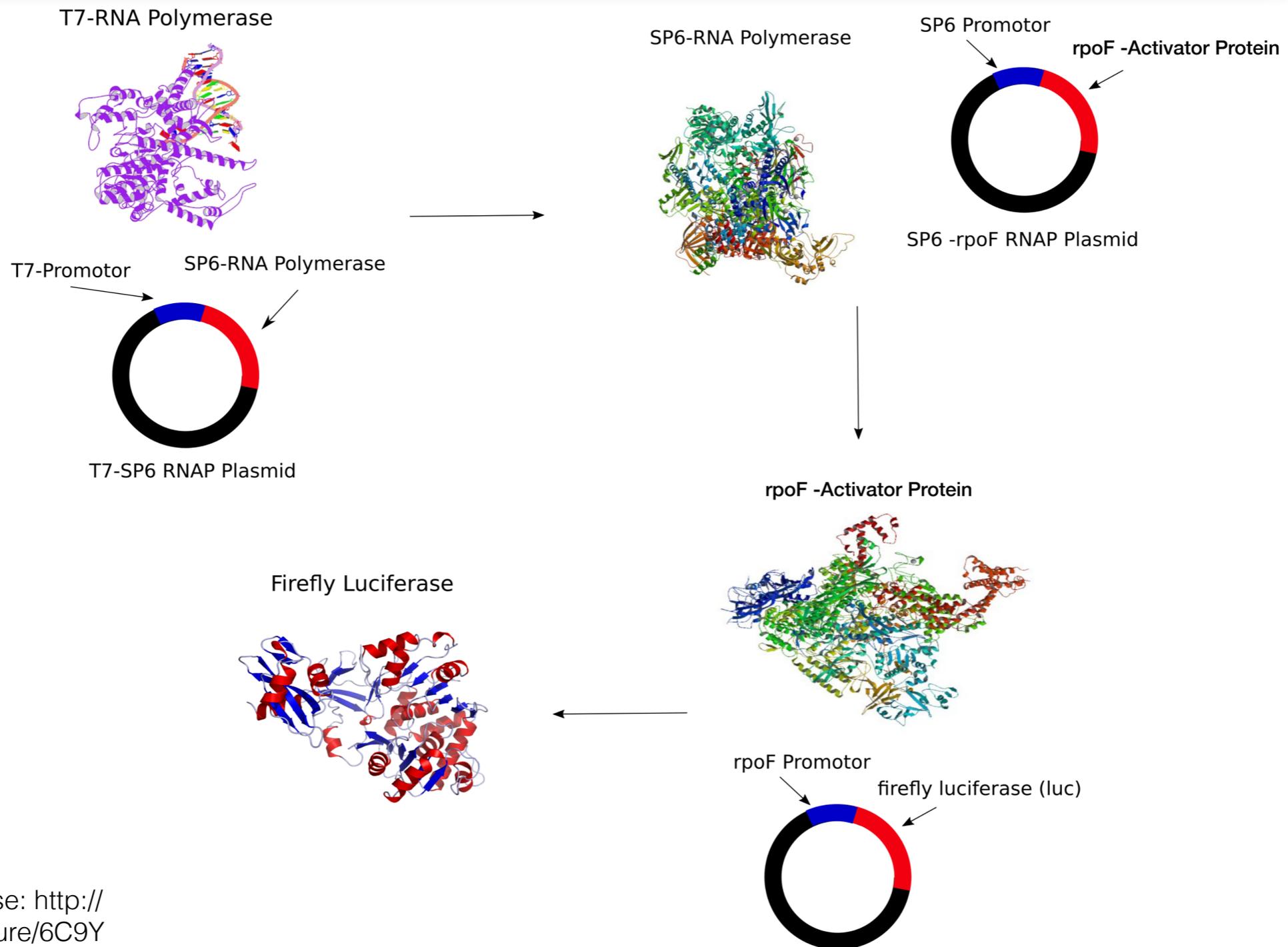
T7-SP6 RNAP Plasmid

SP6 -firefly luciferase Plasmid

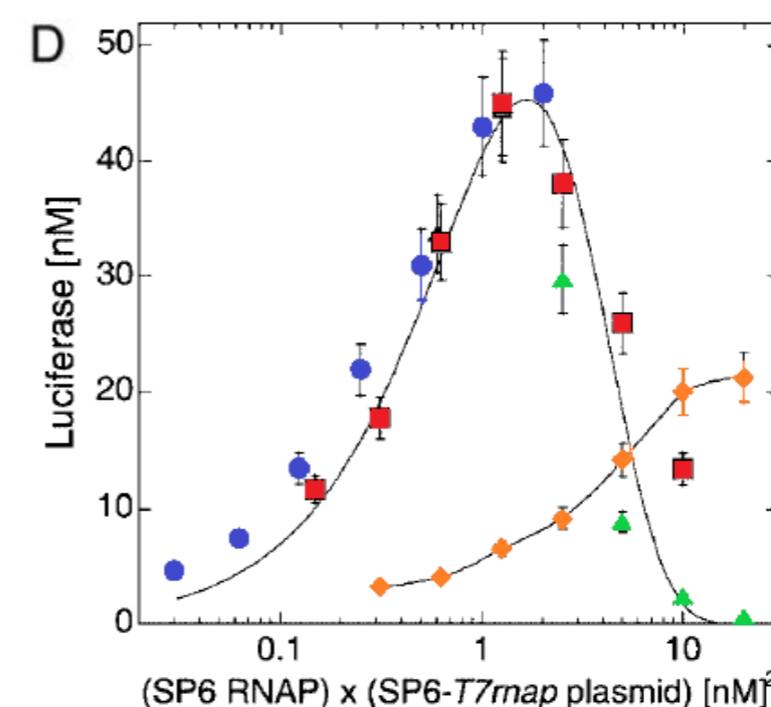
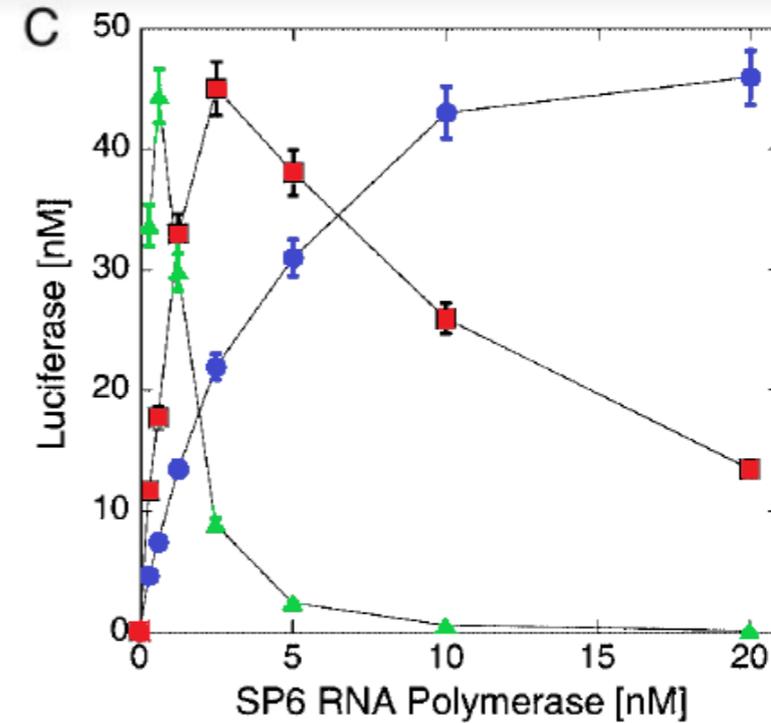
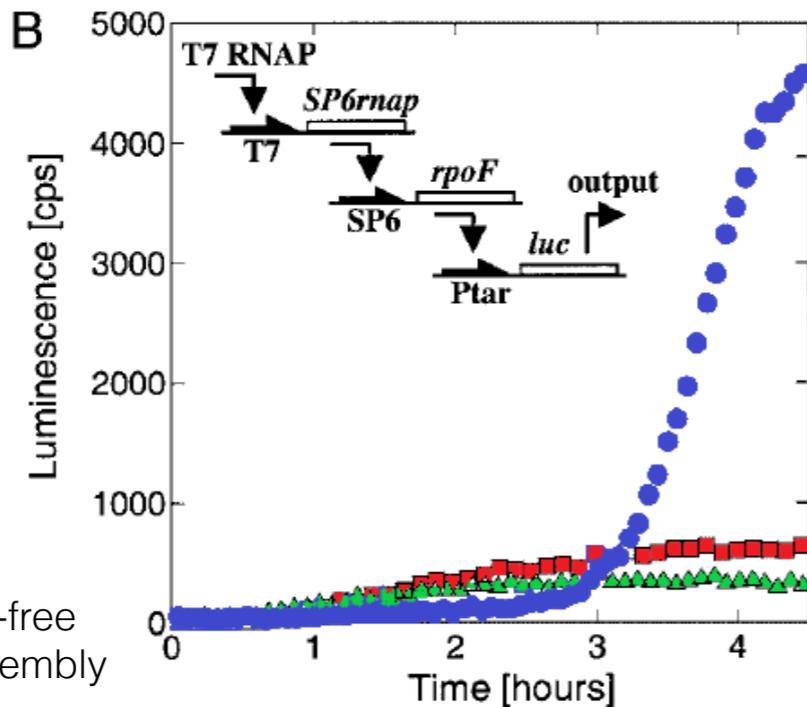
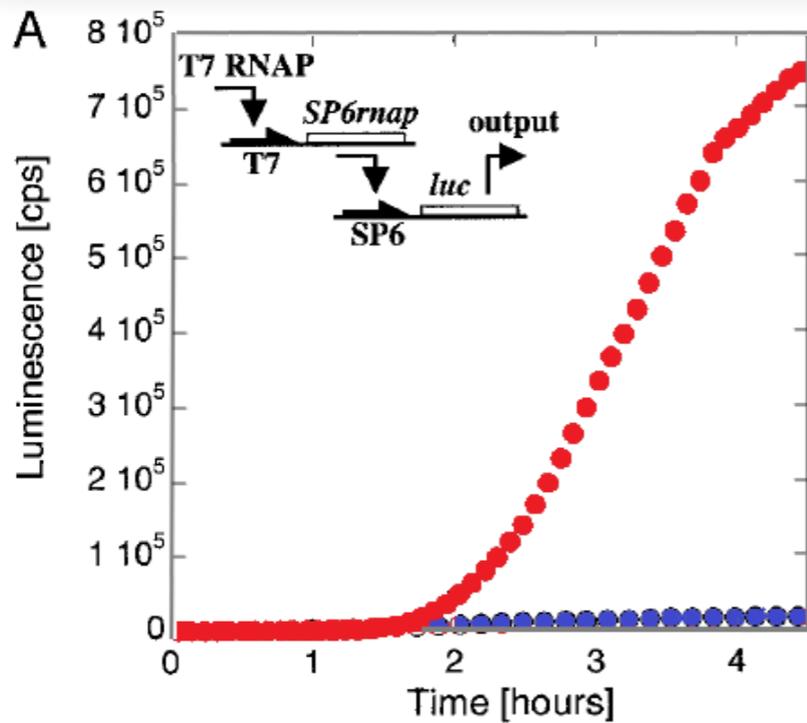
Firefly Luciferase



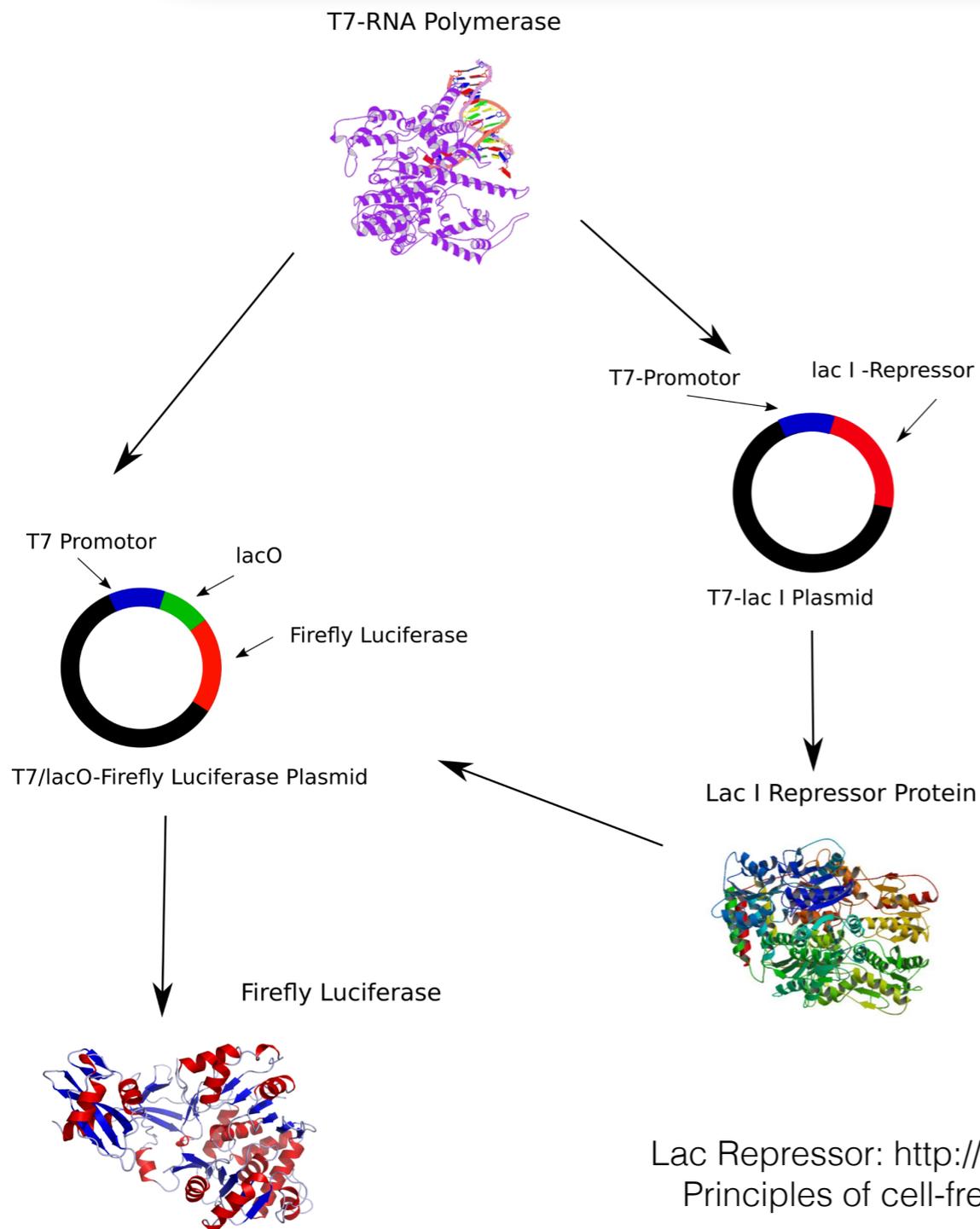
Cell-free Genetic Circuit Assembly



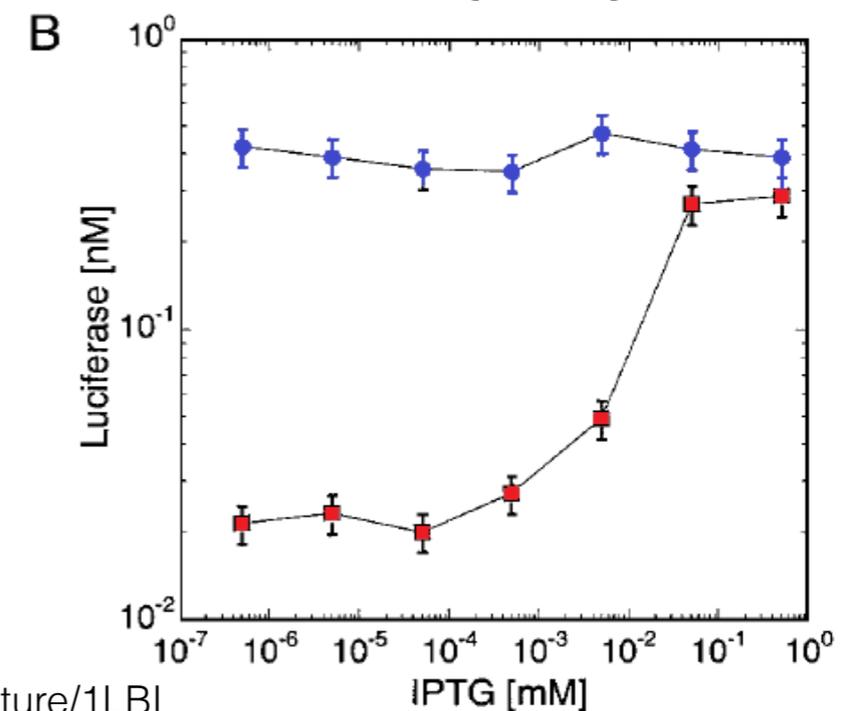
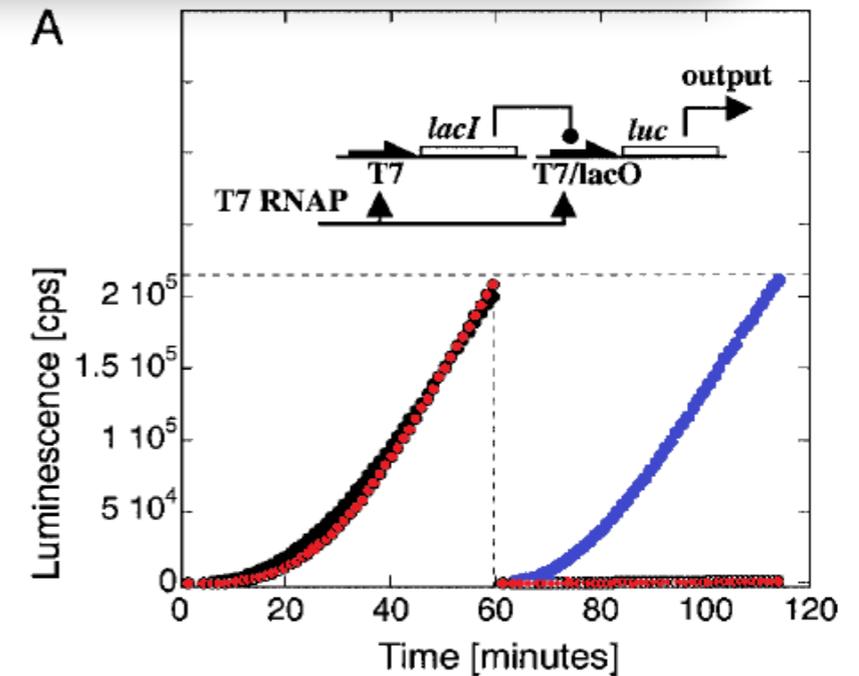
Cell-free Genetic Circuit Assembly



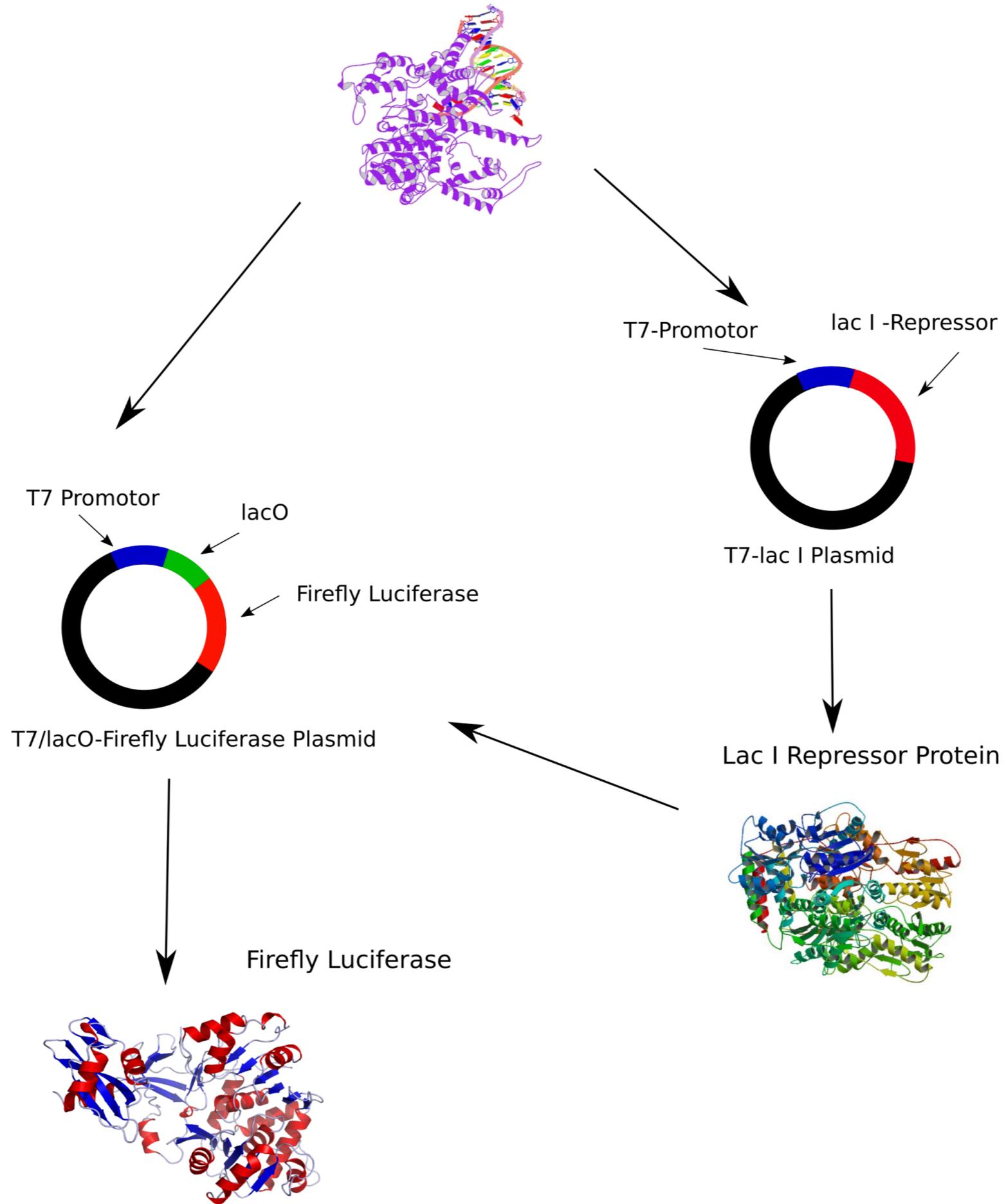
Cell-free Genetic Circuit Assembly



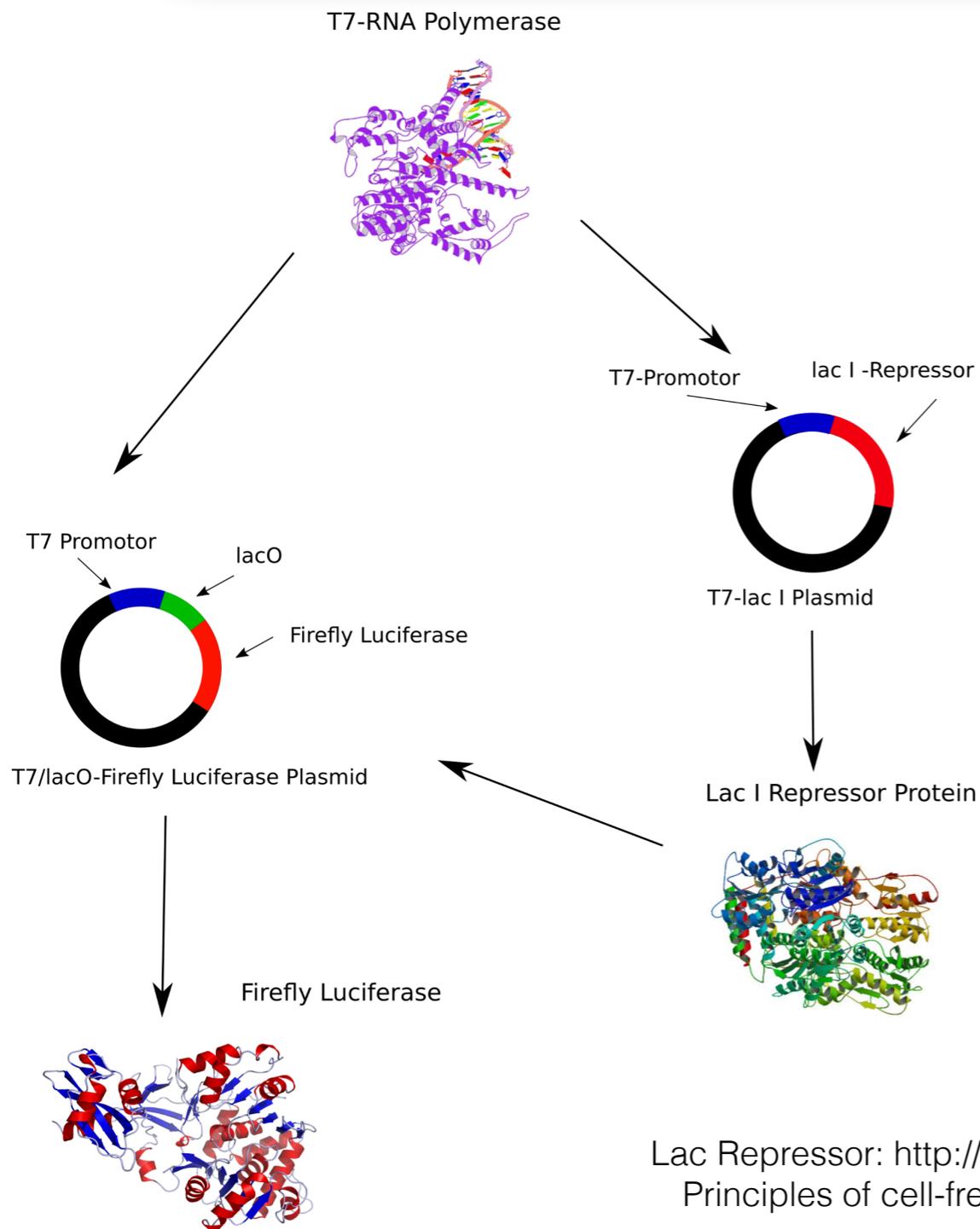
Lac Repressor: <http://www.rcsb.org/structure/1LBI>
Principles of cell-free genetic circuit assembly



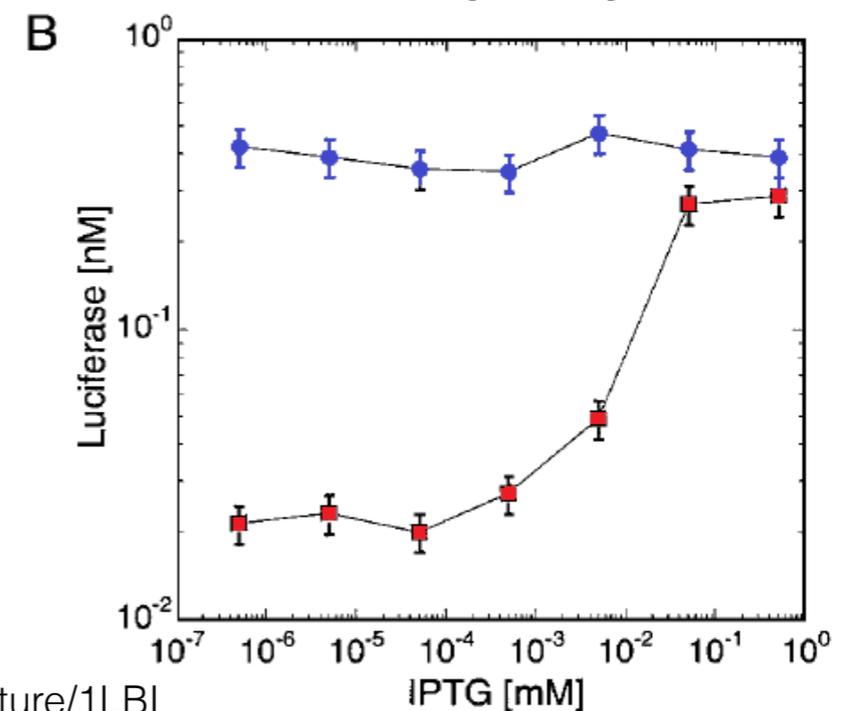
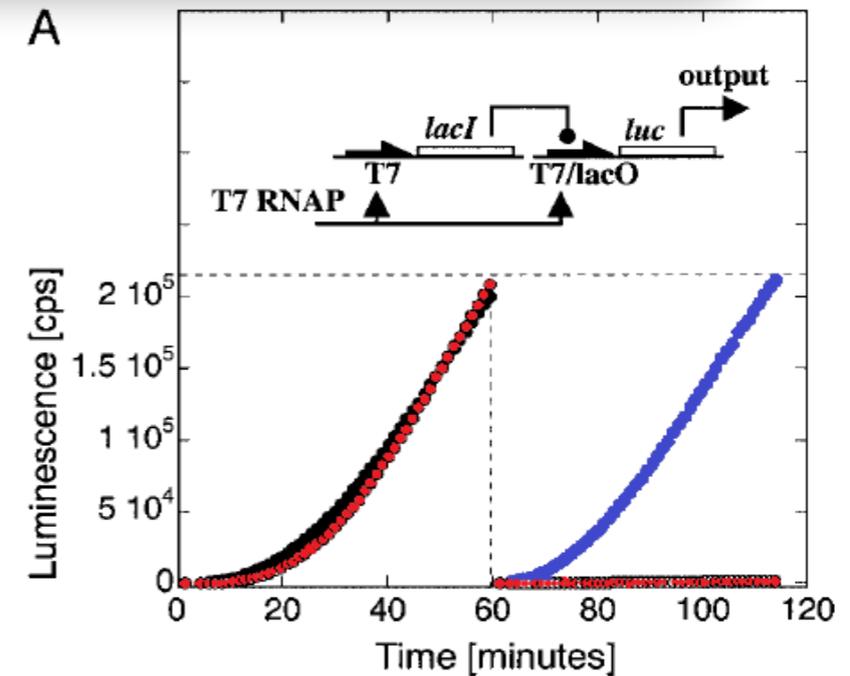
T7-RNA Polymerase



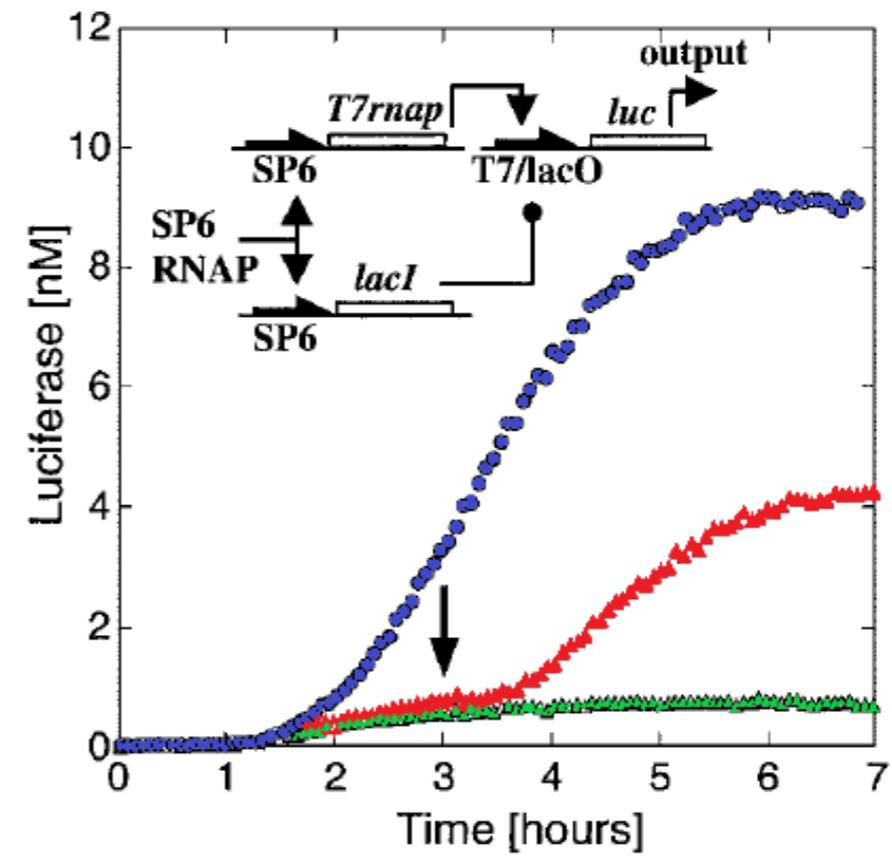
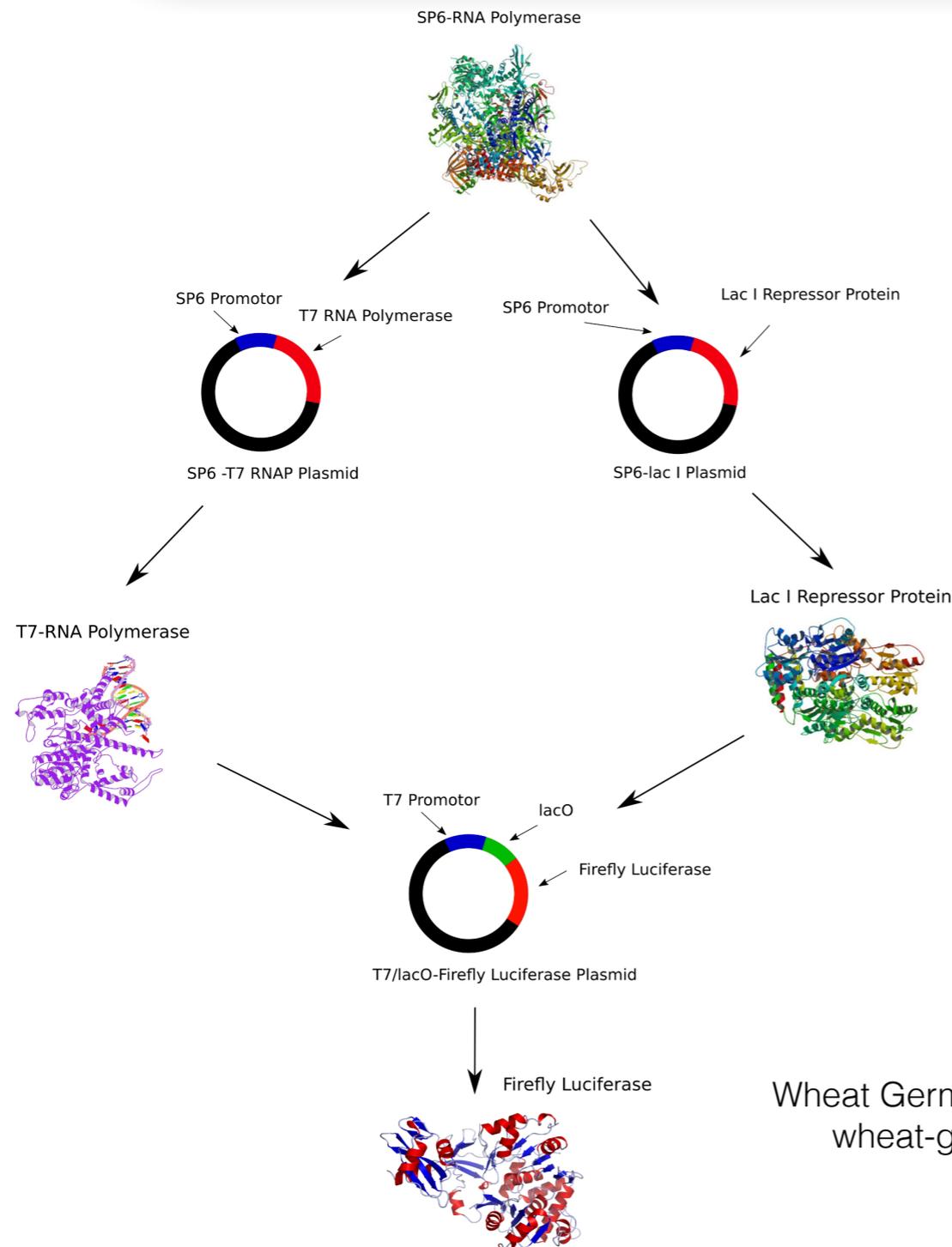
Cell-free Genetic Circuit Assembly



Lac Repressor: <http://www.rcsb.org/structure/1LBI>
 Principles of cell-free genetic circuit assembly



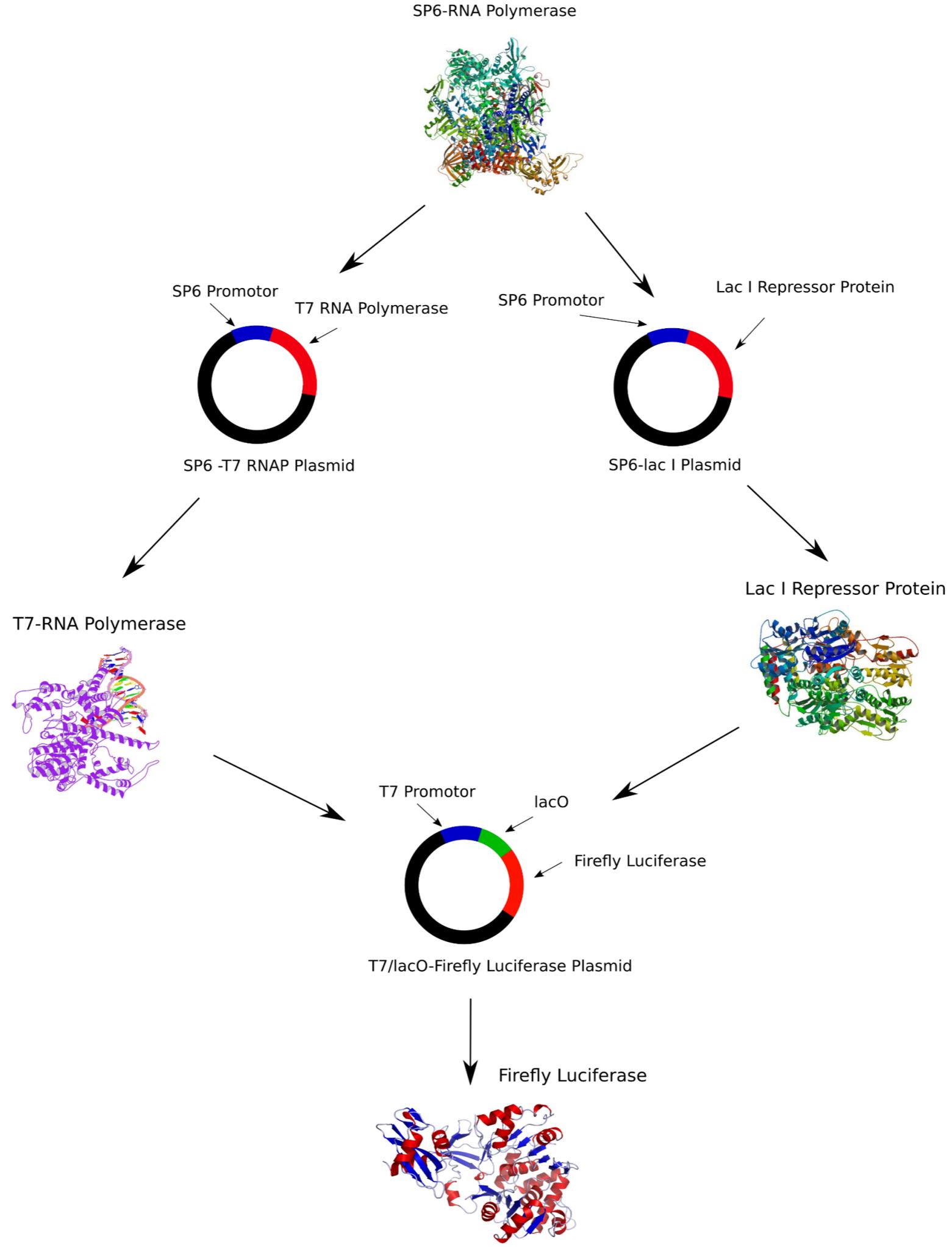
Cell-free Genetic Circuit Assembly



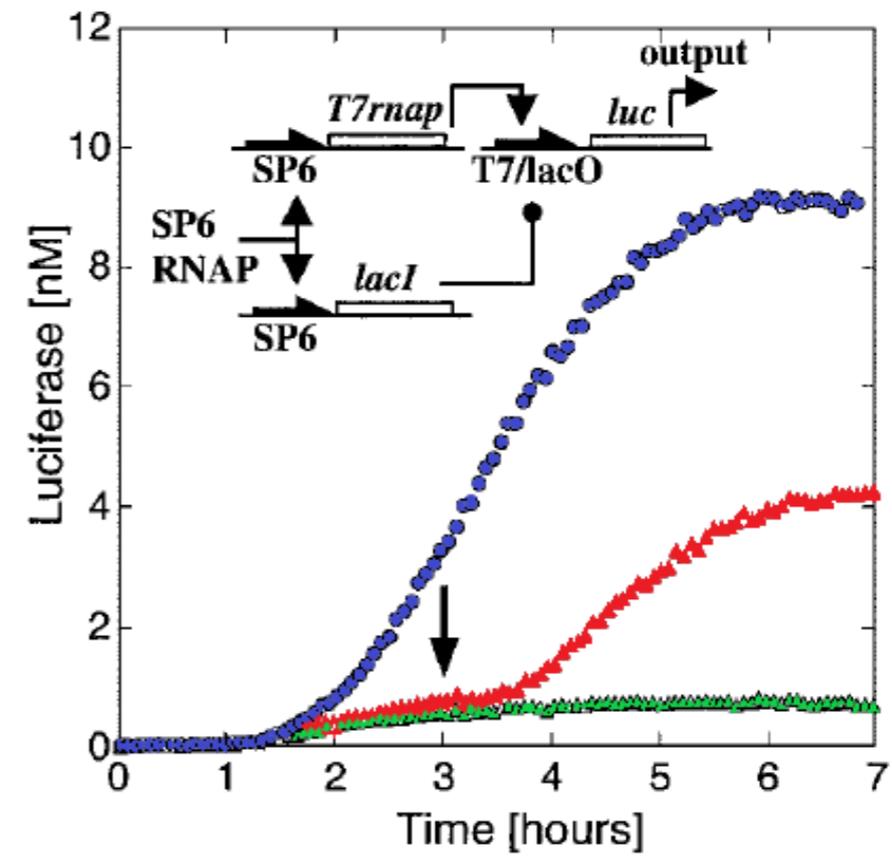
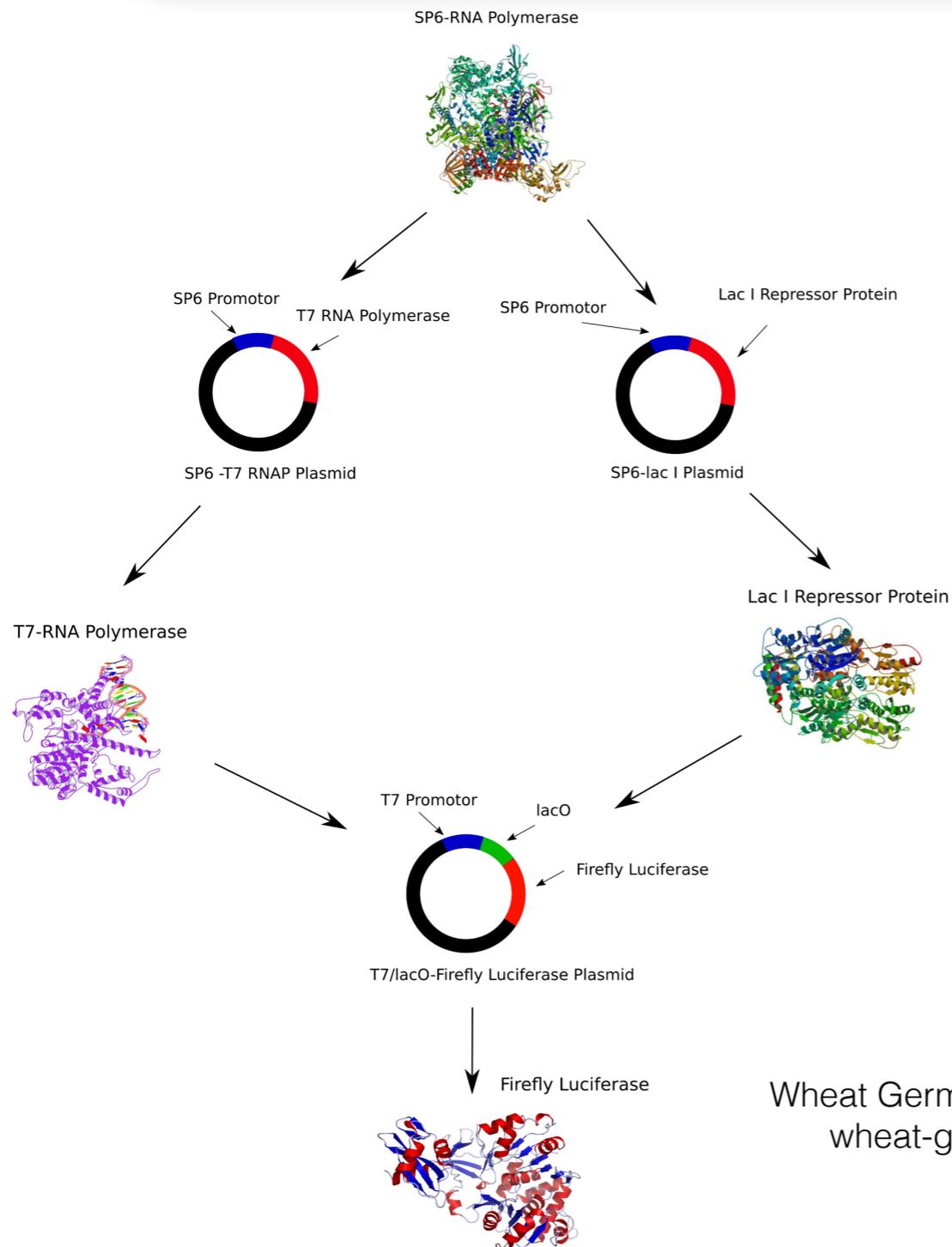
eGFP: <http://www.rcsb.org/structure/2Y0G>

Wheat Germ Ribozym: https://www.researchgate.net/figure/Structures-of-wheat-germ-and-yeast-eukaryotic-80S-ribosomes-A-and-B-Near-complete_fig2_47544348

Principles of cell-free genetic circuit assembly



Cell-free Genetic Circuit Assembly



eGFP: <http://www.rcsb.org/structure/2Y0G>

Wheat Germ Ribozym: https://www.researchgate.net/figure/Structures-of-wheat-germ-and-yeast-eukaryotic-80S-ribosomes-A-and-B-Near-complete_fig2_47544348

Principles of cell-free genetic circuit assembly

Cell-free Genetic Circuit Assembly

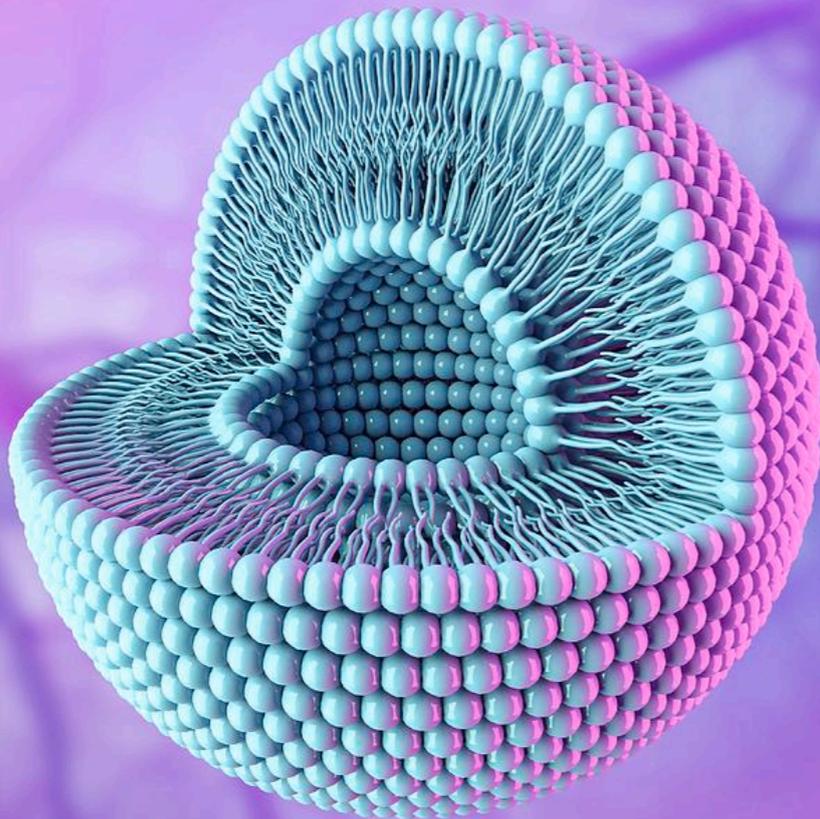
Summary



- Complex genetic circuits work in optimized conditions
- The protein expression can be regulated with simple methods
- Saturation of the translation machinery as limiting factor

A Vesicle Bioreactor for Artificial Cells

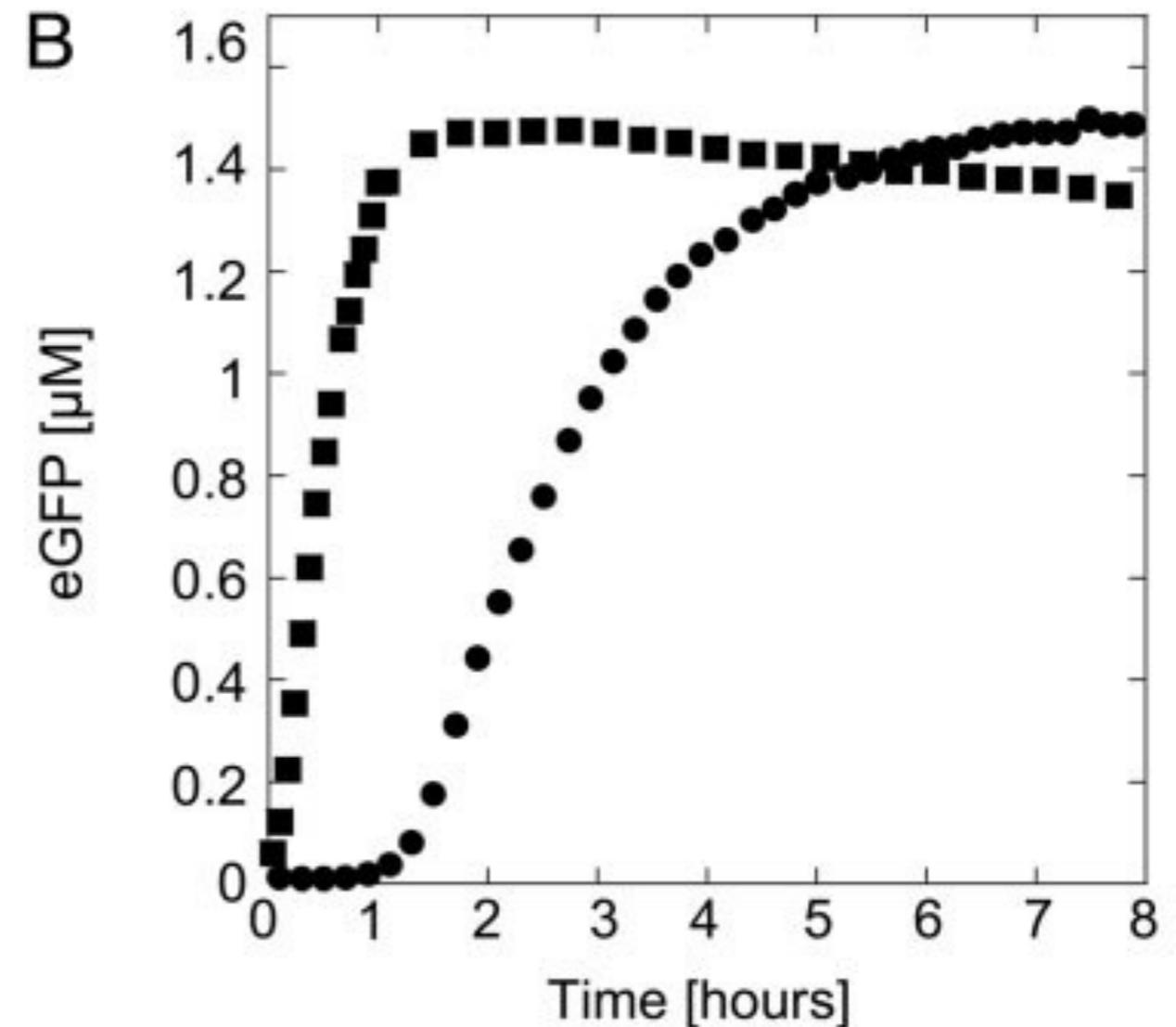
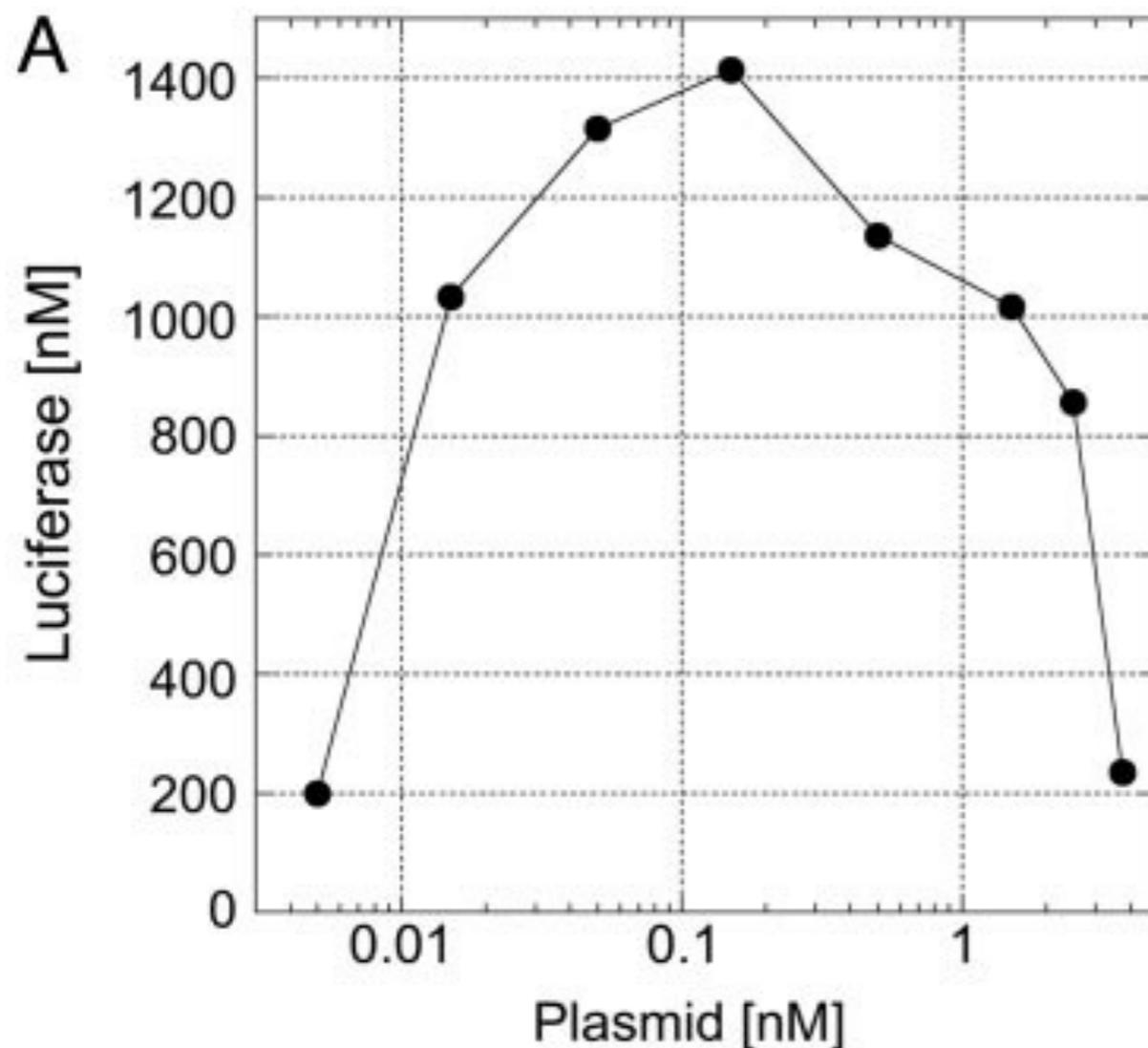
Overview



- Building a cell-like bioreactor with a phospholipid vesicle
- Oxygen diffusion and osmotic pressure as critical parameters
- Encapsulation of active ingredients into the bilayer

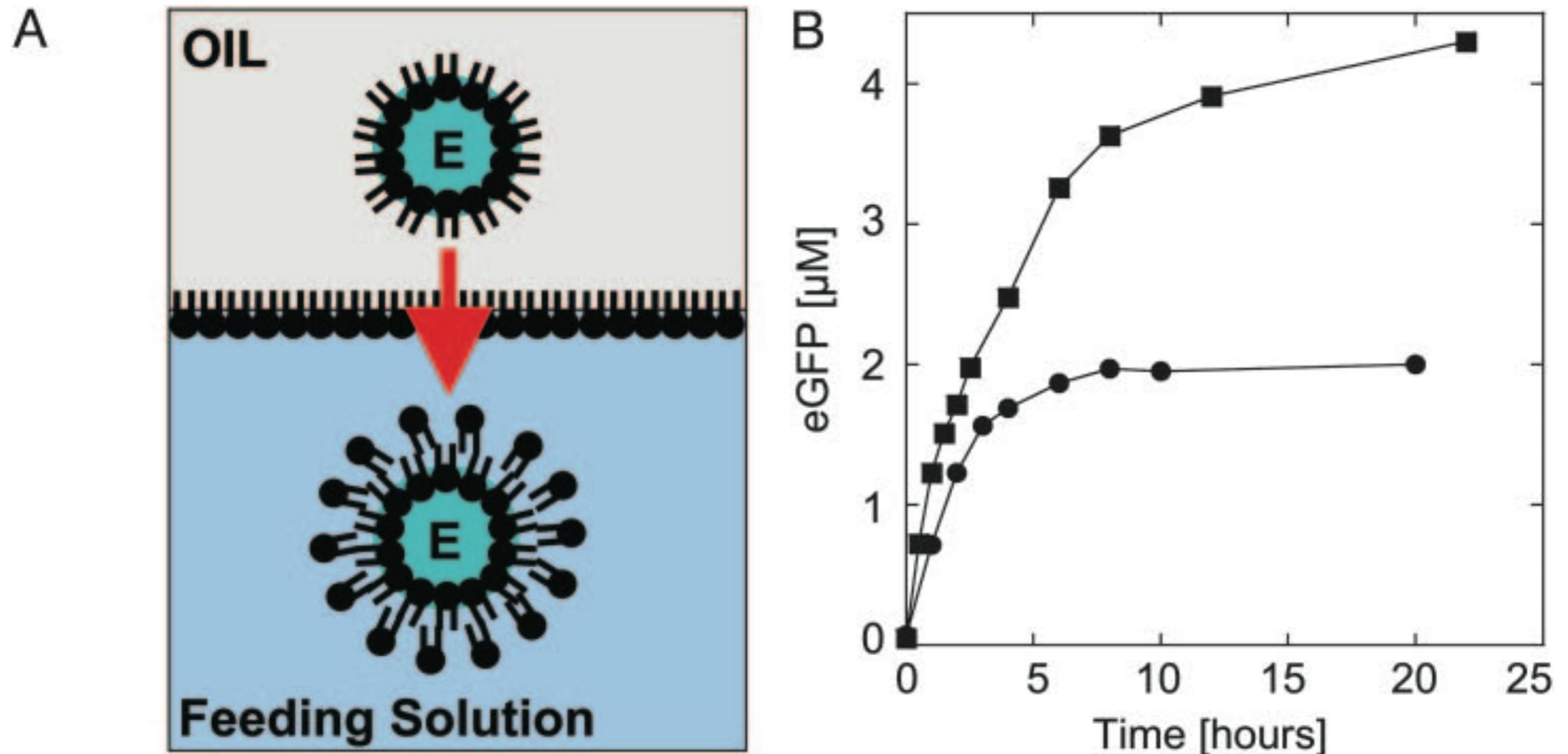
A Vesicle Bioreactor for Artificial Cells

Characterization of the Extract



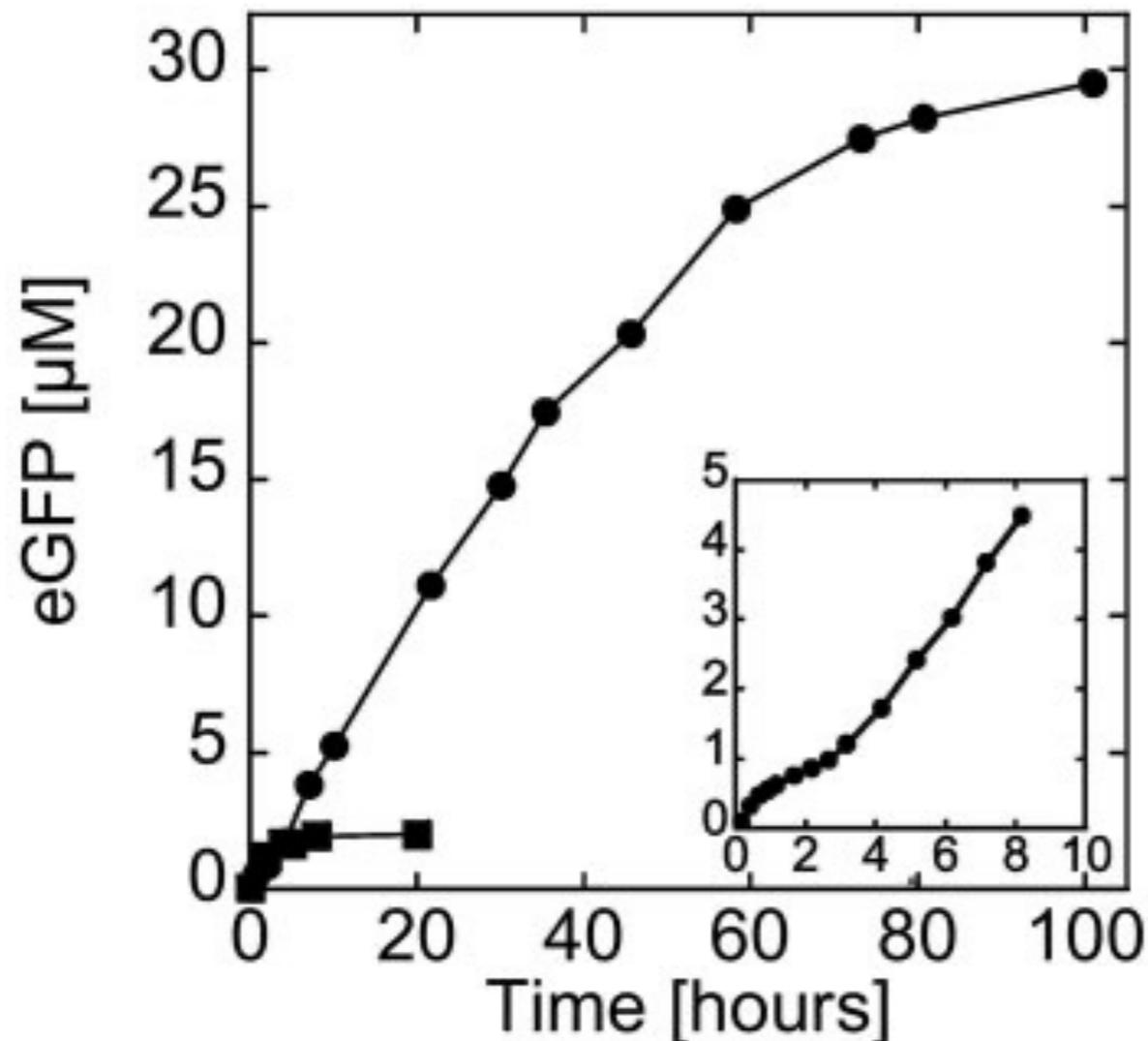
A Vesicle Bioreactor for Artificial Cells

Encapsulation in a Vesicle



A Vesicle Bioreactor for Artificial Cells

Vesicles with α -Hemolysin



- Feeding from surrounding solution through pore
- Ideal candidate: α -Hemolysin protein from bacterium *S. Aureus*
- Expression observed for >4 days with protein production of $\sim 35 \mu\text{M}$

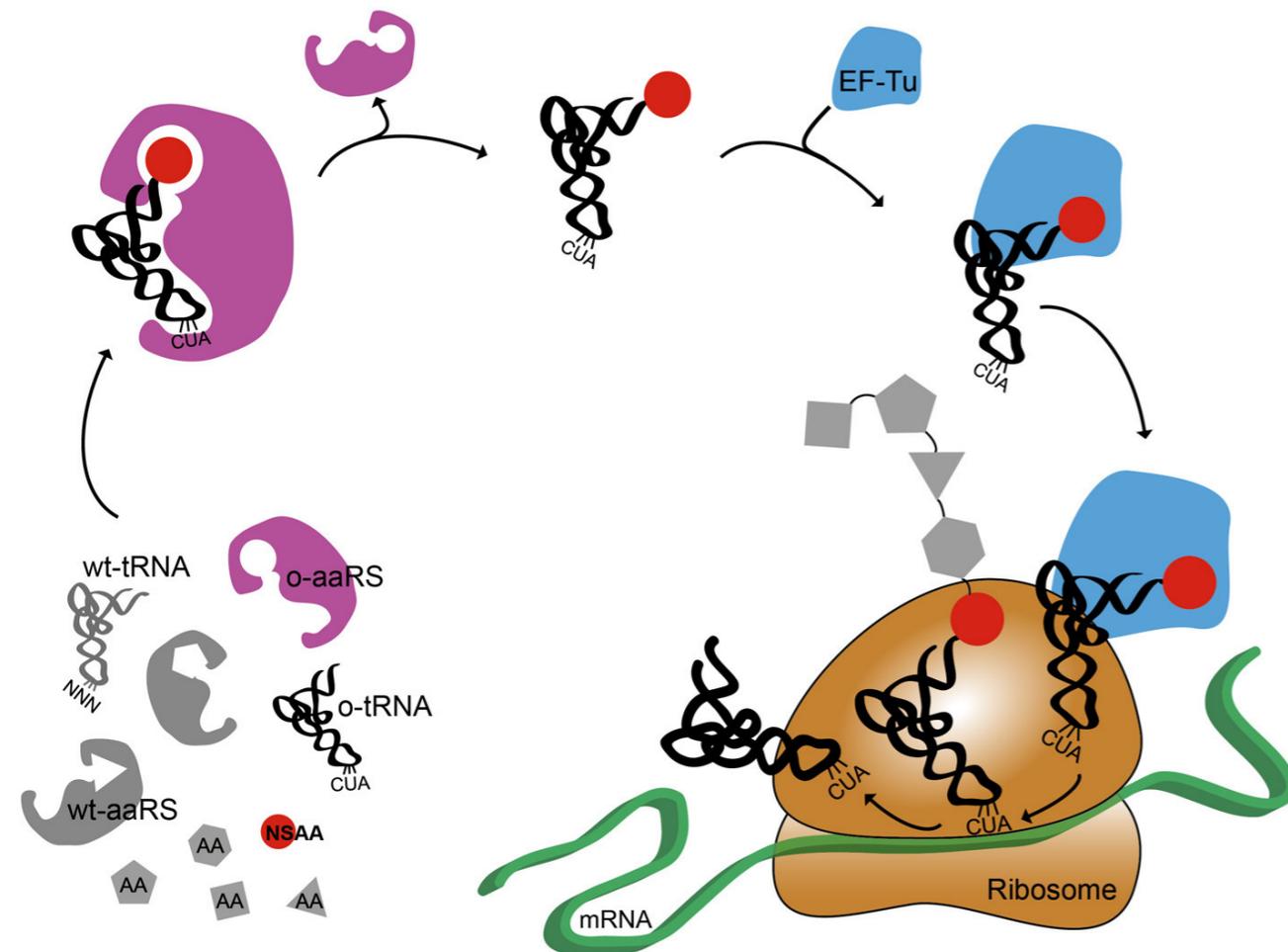
A Vesicle Bioreactor for Artificial Cells

Conclusion



- Transcription and Translation at the scale of a cell
- Limitations of energy and nutrients solved
- Artificial Cells for pharmacology and medical diagnostics possible

Current Research



- Incorporation of unnatural amino acids
- Production of toxic proteins

- Jim Swartz: Developing cell-free biology for industrial applications, Society for Industrial Microbiology 2006
- Jascha Rolf, Katrin Rosenthal and Stephan Lütz: Application of Cell-Free Protein Synthesis for Faster Biocatalyst Development, TU Dortmund University
- Vincent Noireaux, Roy Bar-Ziv, and Albert Libchaber: Principles of cell-free genetic circuit assembly
- Vincent Noireaux and Albert Libchaber: A vesicle bioreactor as a step toward an artificial cell assembly
- Seok Hoon Hong, Yong-Chan Kwon and Michael C. Jewett: Non-standard amino acid incorporation into proteins using *Escherichia coli* cell-free protein synthesis, Frontiers in Chemistry

Questions?



Questions?

