

Bottom-up Synthetic Cells Using CFE

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Mirror Image Biology: Pushing the
Envelope in Designing Biological Systems
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Bottom-up Synthetic Cells Terminology

Coacervate and Protocell: synthesis of cell-sized compartments using prebiotic molecules (peptide, RNA, fatty acids).

Minimal Cell: synthesis of cell-sized compartments using natural molecules (DNA, CFE, lipids, carbs).

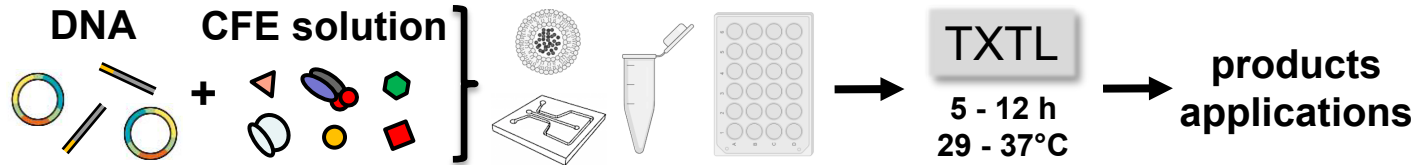
Artificial Cell: synthesis of cell-sized compartments using natural and/or artificial molecules.

CFE: Cell-Free Gene Expression

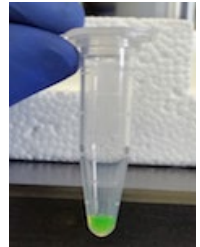
A CFE reaction is a physiological aqueous solution that enables expressing genes outside cells. A CFE reaction is composed of:

- a cell lysate (mostly from bacteria like *E. coli*):
 - contains the transcription machinery (RNA polymerase).
 - contains the translation machinery (ribosomes, tRNAs).
 - does not contain endogenous DNA and mRNA.
- an ATP regeneration system to sustain CFE.
- building blocks and solutes: amino acids, salts.
- preparation of CFE systems is well documented, commercial kits are available.

CFE Workflow

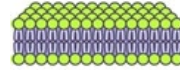
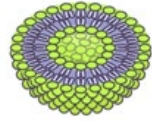


CFE of eGFP

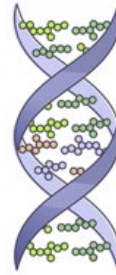


Bottom-Up CFE Synthetic Cells

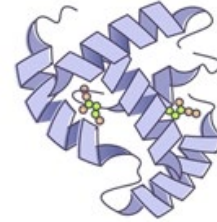
Goal: synthesize cell-sized compartments using natural molecules with natural chirality.



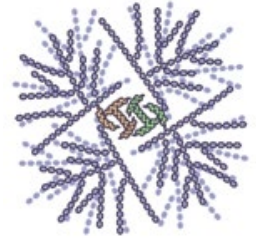
lipids



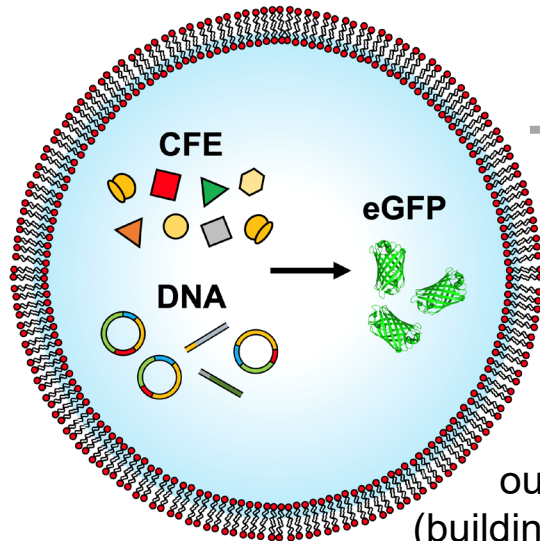
DNA



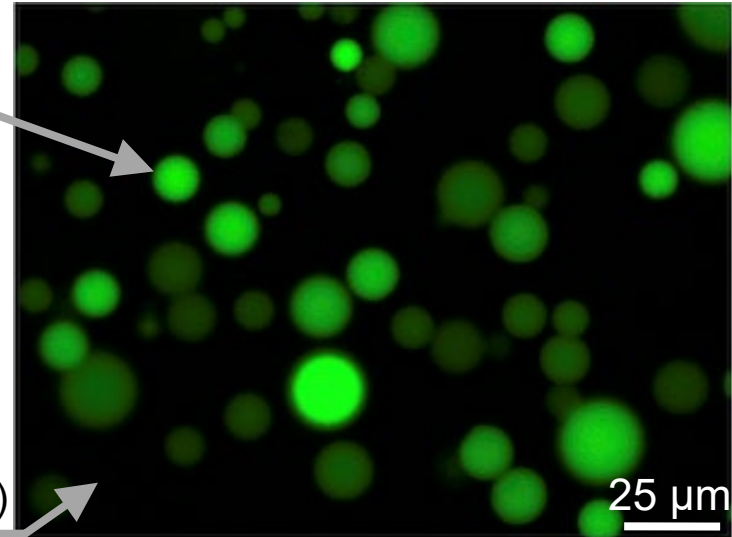
proteins



carbs



outer solution
(building blocks, buffer)

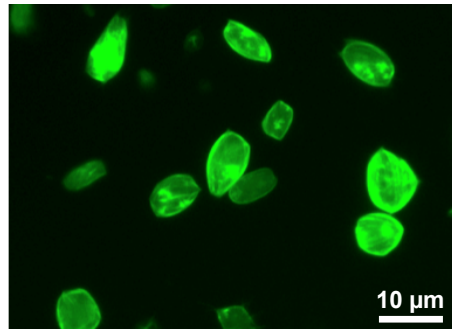
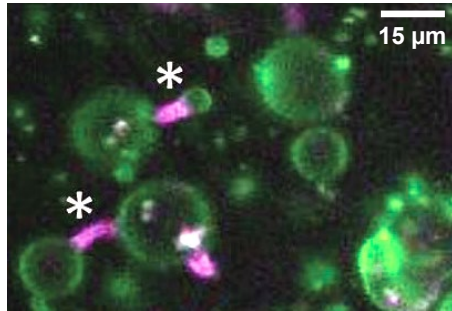


Bottom-Up CFE Synthetic Cells: 3 examples

cytoskeleton

liposome division

liposome shape

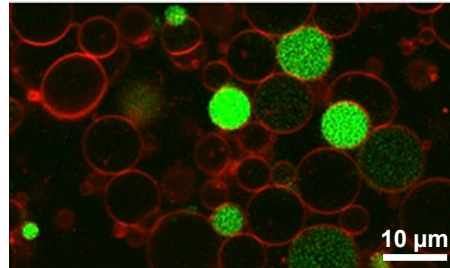
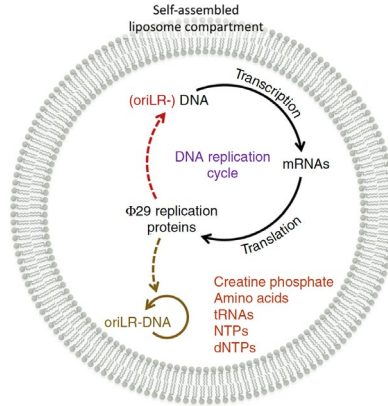


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DNA replication

DNA replication in CFE

synthetic cells

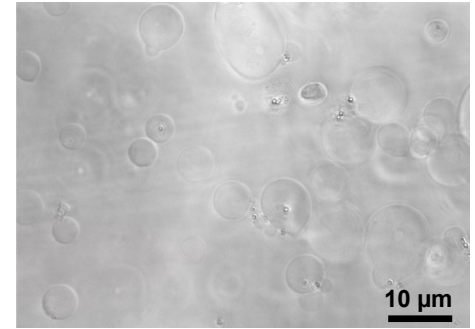
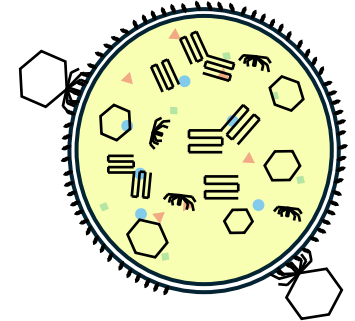


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bacteriophages

Infection of CFE

synthetic cells



10.1101/2025.06.14.659709

Other CFE facts:

- **largest DNA processed in CFE:** phages T4 and T6, 169 kbp, 289 genes. The minimal genome for a self-reproducing cell is estimated to be around 300-400 genes.
- **ribosomes have been partially synthesized in CFE** (assembly of active ribosomes from transcribed).

Other CFE synthetic cells facts:

- **E. coli genome has been encapsulated into cell-sized liposomes.** No cell rebooting observed so far.
- **rebooting the JCVI minimal mycoplasma genomes with a CFE system is being done.**

Build-A-Cell Network: <https://www.buildacell.org/>

