A vesicle bioreactor as a step toward an artificial cell assembly

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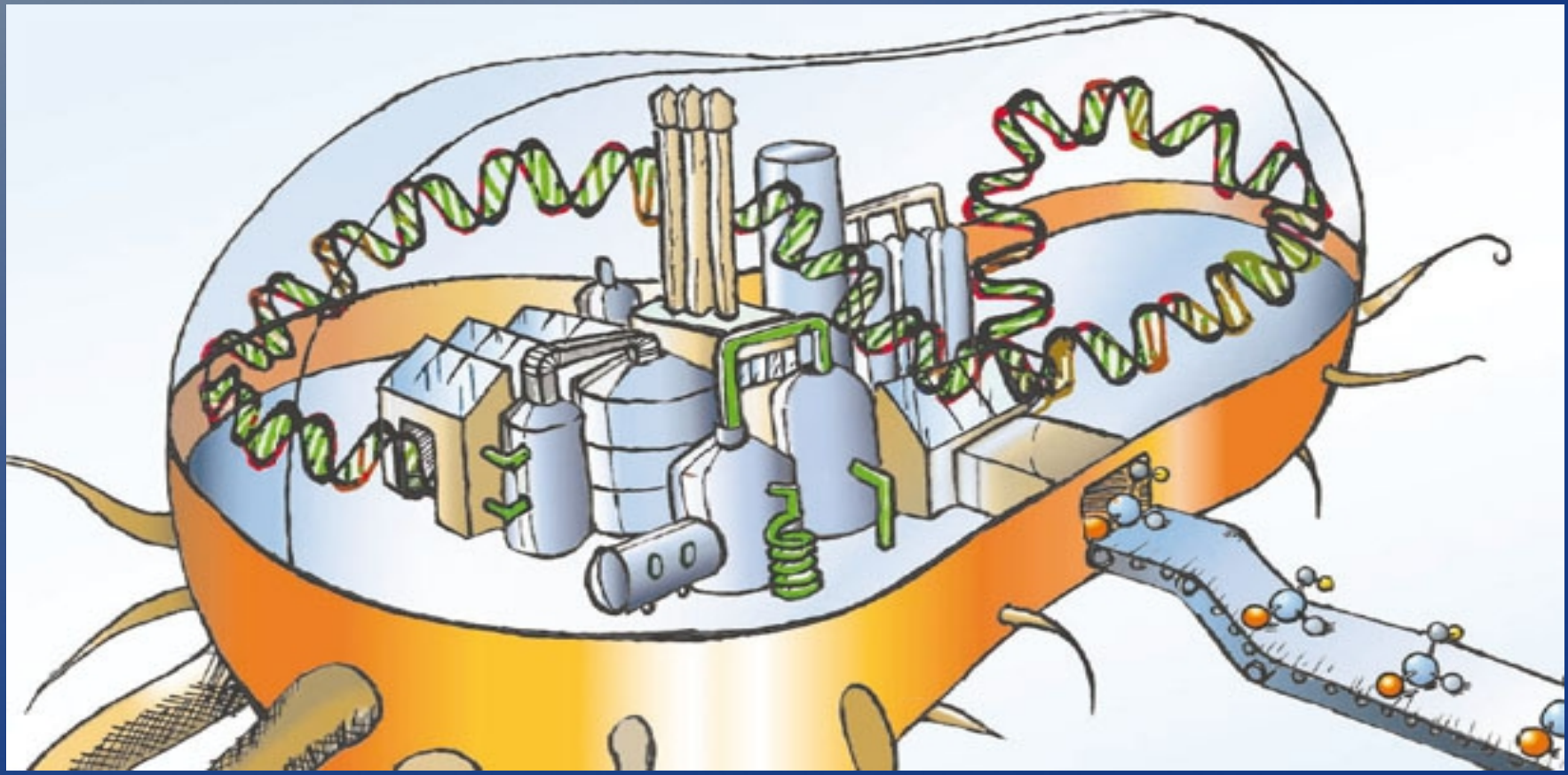
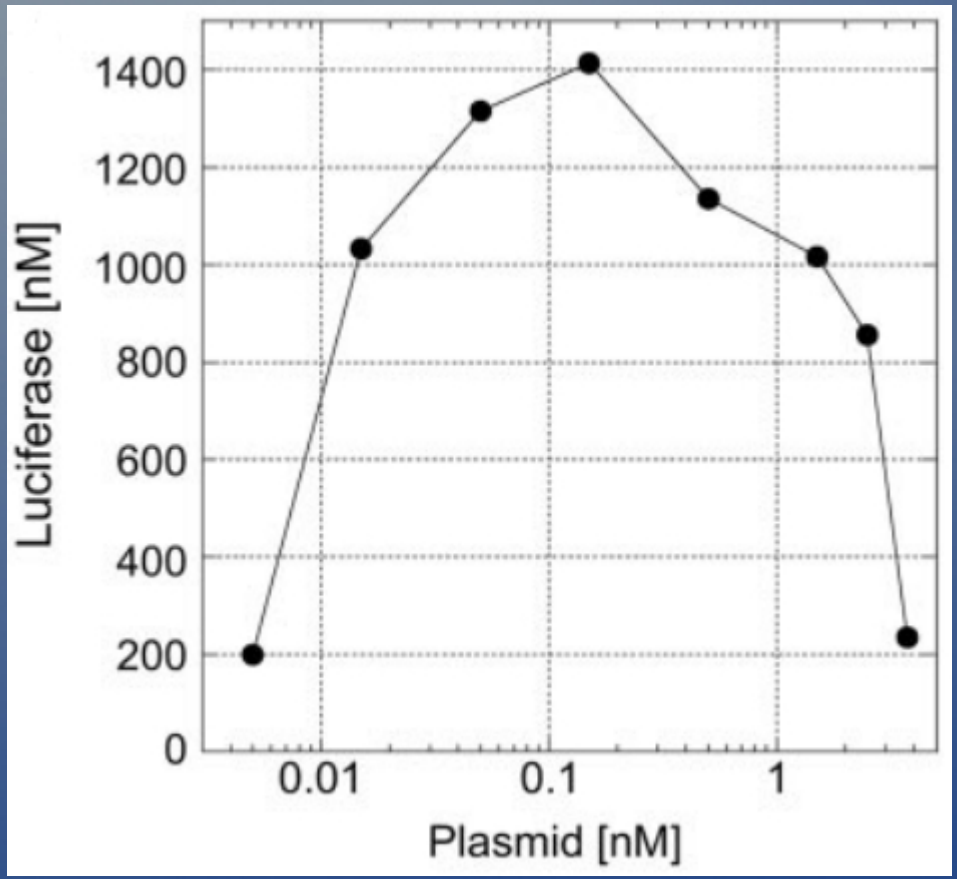


http://www.biooekonomie.de/BIOOEKO/Navigation/DE/Foerderung/foerderbeispiele,did=167528.html

1 Extract characterization



→ *plasmid concentration for all experiments: 0.5 nM*

Expression with *E.Coli* extract in cell-free solution:

- *Firefly luciferase & eGFP as reporter proteins*
- *> 1nM: Saturation of translation machinery (probably)*
- *< 0.1 nM: plasmid expression not linear with template concentration*

2 Encapsulation and expression

- Cytoplasmic extract:

100 mg/mL protein

50-100 mM salts

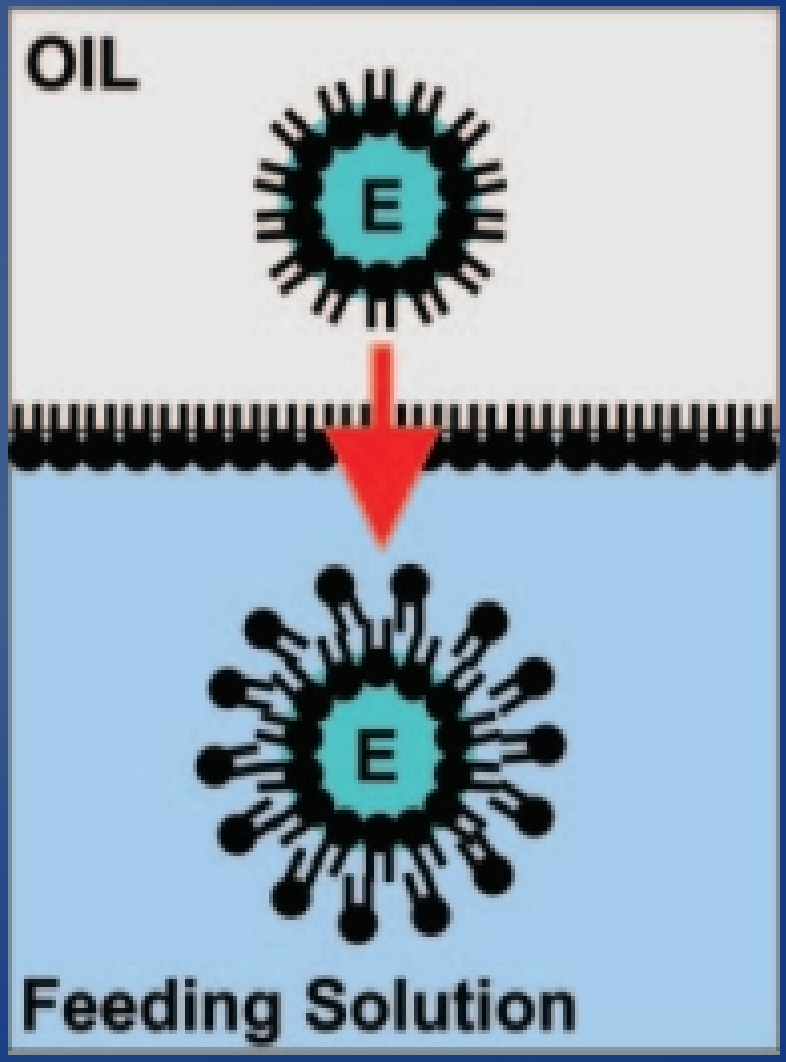
10-20 mM ions

- Feeding solution:

ribonucleotides

amino acids

- Arrow = Centrifugation

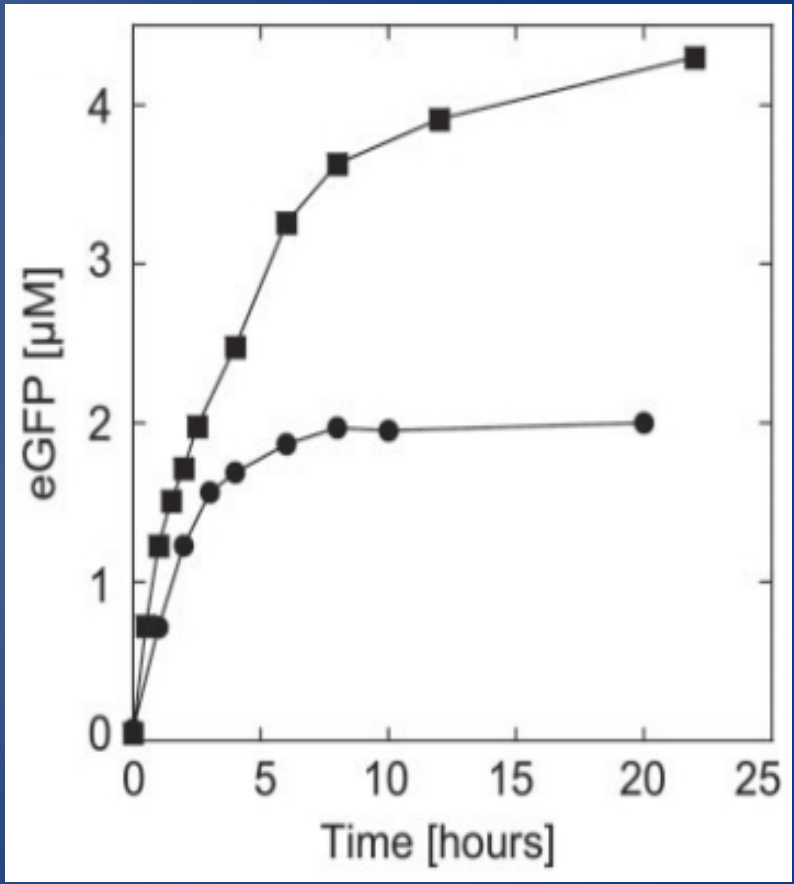


2 Encapsulation and expression

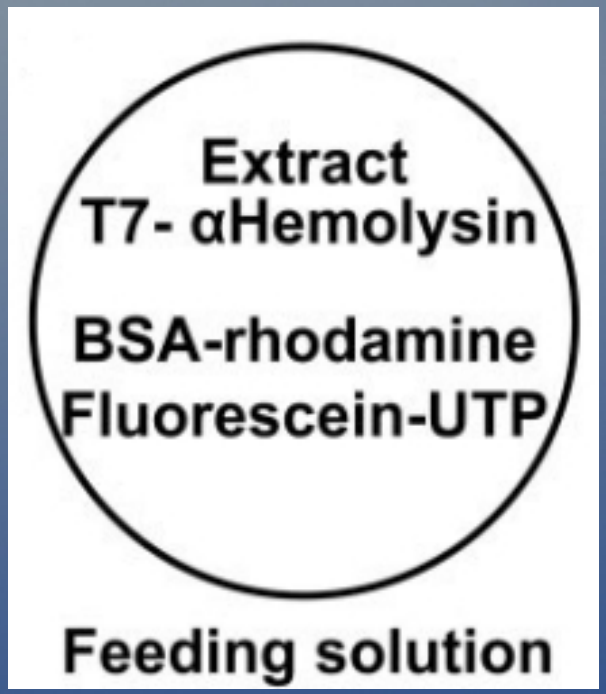
Expression of eGFP inside a vesicle under different osmotic pressure:

- 100% extract encapsulated, 100% feeding solution (squares)
- 50% extract & 50% feeding encapsulated,

feeding supplemented with 4% extract (circles)



3 Membrane permeability

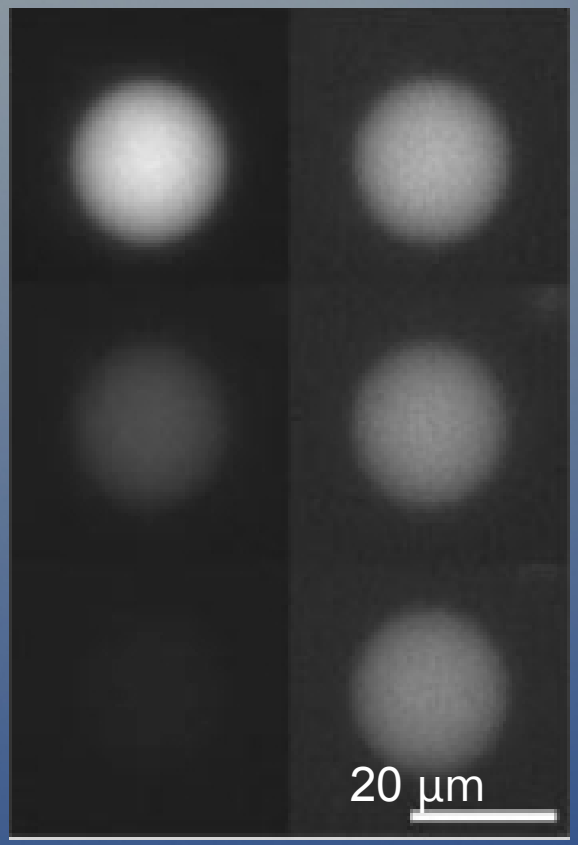


Expression of α-hemolysin toxin inside the vesicle:

- Vesicle contains:
  - α-Hemolysin (0.5 nM)
  - BSA protein (labeled) (6 μM)
  - Ribonucleotide UTP (labeled) (35 μM)

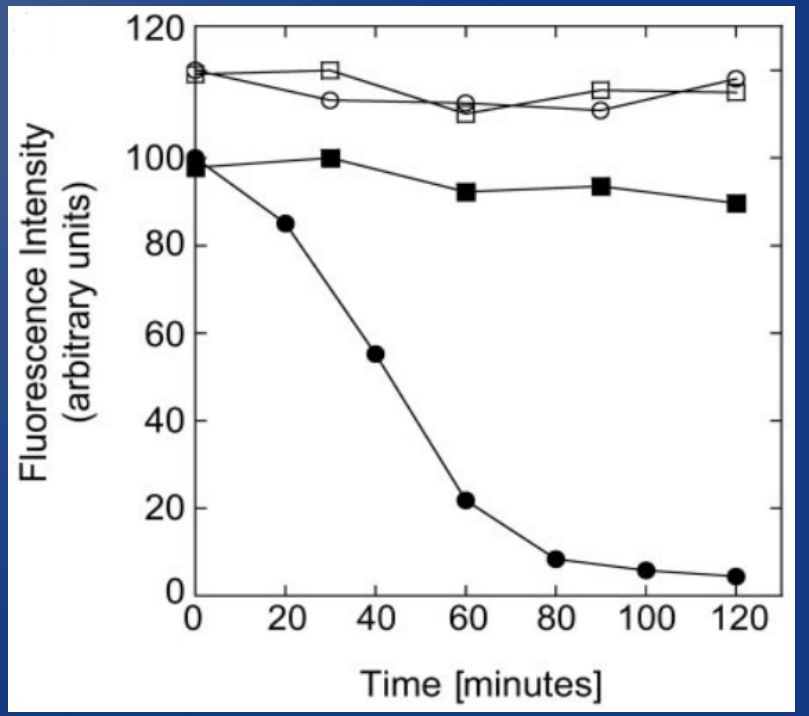
α-Hemolysin creates pores with a specific mass cut-off

3 Membrane permeability



Time sequence of vesicle fluorescence (10, 70, 120 min):

- Left, circles: fluorescein-UTP
- Right, squares: BSA-RITC
- Negative control: Firefly luciferase instead of toxin



→ Synthesized toxin is functional & amino acids and ribonucleotides can diffuse in and out while extract is kept inside.

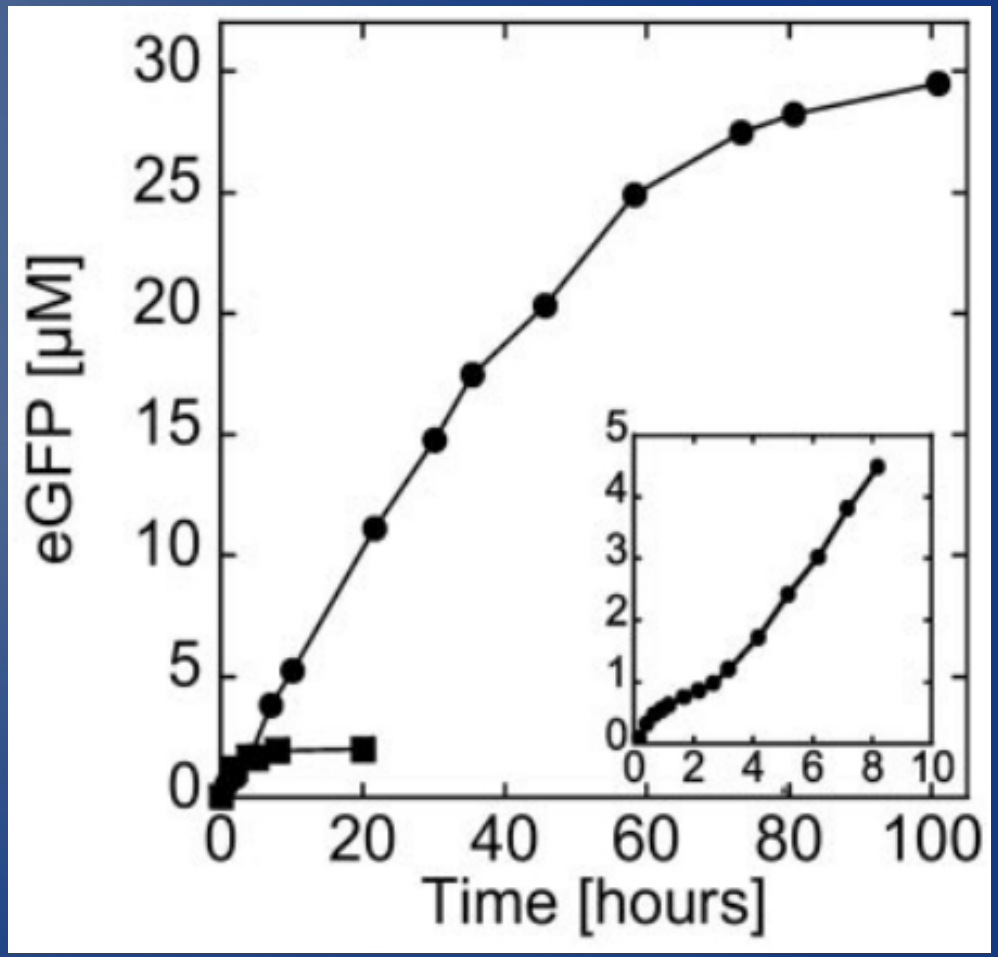
4 Long-Lived Bioreactor

- With α-hemolysin:

Expression observed for >4 days (circles)

- Without α-hemolysin:

Expression observed for 5h (squares)



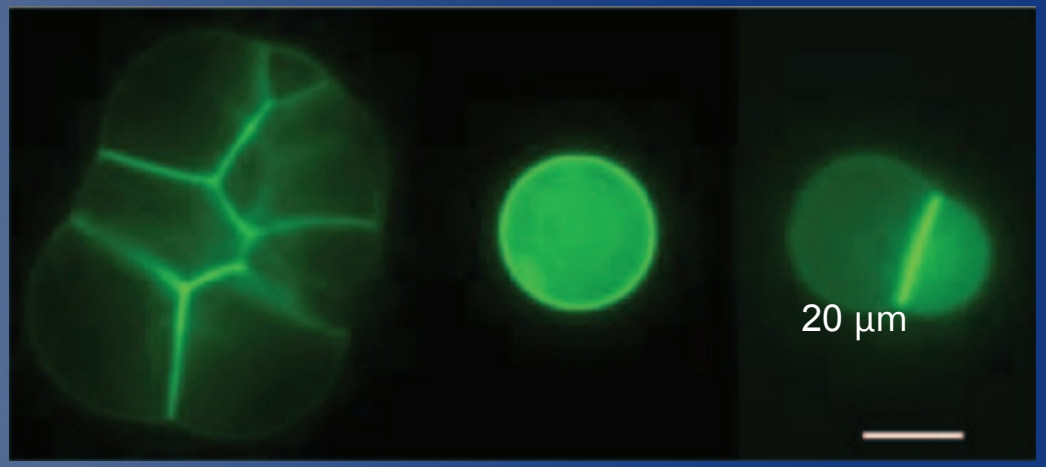
- Important:

Pore production must be large enough while extract can run independently of supplementation (inset, first 2h)

4 Long-Lived Bioreactor

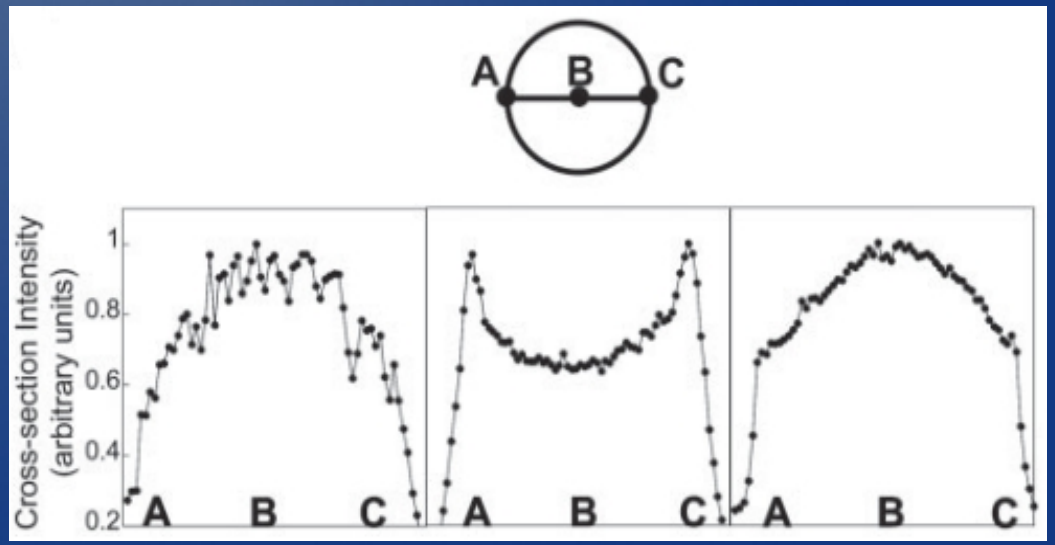
- Accumulation:

α-hemolysin fused to eGFP after 20 – 40 h inside aggregation of vesicles (left), single cell (center), doublet (right)



- Cross section:

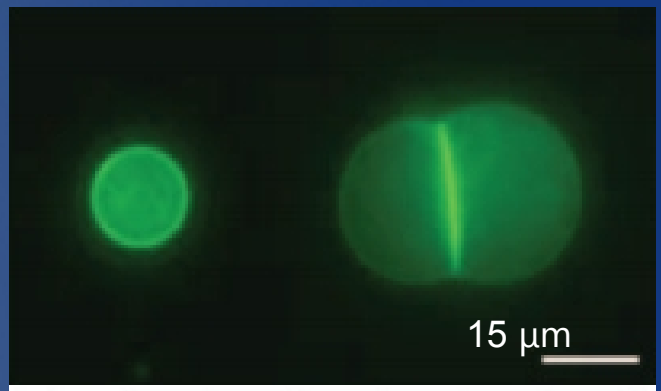
Fluorescence intensity of a single vesicle after 70 min (left), 550 min (center), 4400 min (right)

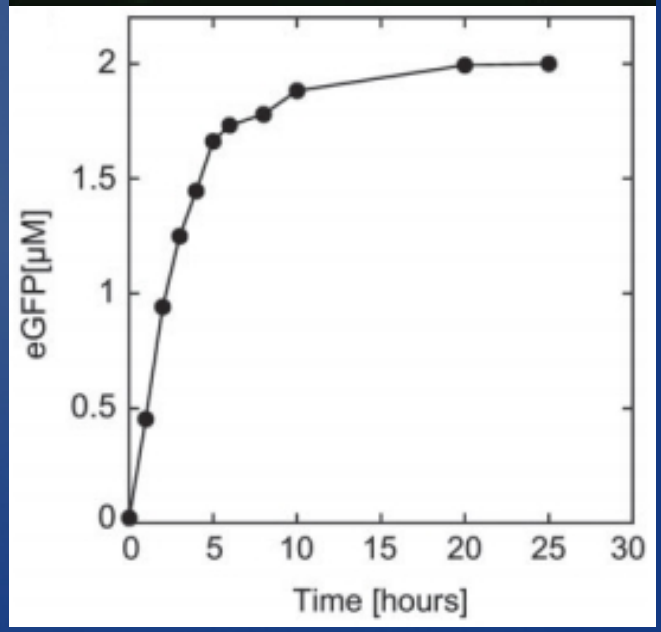


5 Anchoring of proteins

- Accumulation of the artificial peptide 18L (α-helix with a balance of hydrophilic-hydrophobic residues, spontaneously inserts itself into the membrane)
- 18L tagged with eGFP after 5h (top) and kinetics of the expression (bottom)

→ Protein successfully anchored!





6 Conclusions

- Transcription & Translation have been brought at the scale of a cell.
- Energy & nutrient limitations have been solved through the internal expression of a membrane pore and by exchange with environment.
- Anchoring of biopolymers and motor proteins to the membrane by mechanical perturbation.
- Small and enclosed laboratories.

→ Future work:

Improvement of system capacity

Engineering of elementary genome (minimal cell)

7 References

A vesicle bioreactor as a step toward an artificial cell assembly,
Vincent Noireaux and Albert Libchaber, Center for Studies in Physics and Biology, The Rockefeller Univeristy, New York 2004