Synergy or Transport in Enzyme Assembly

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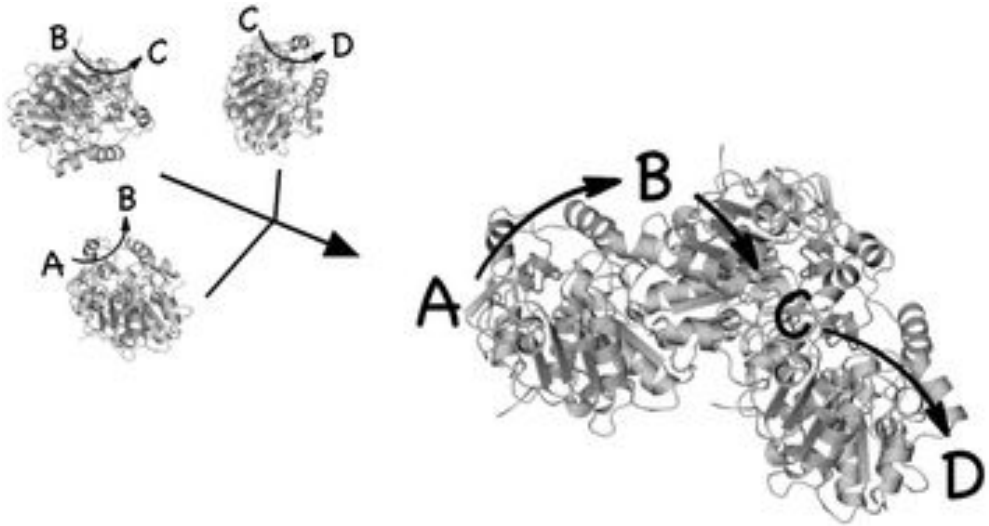
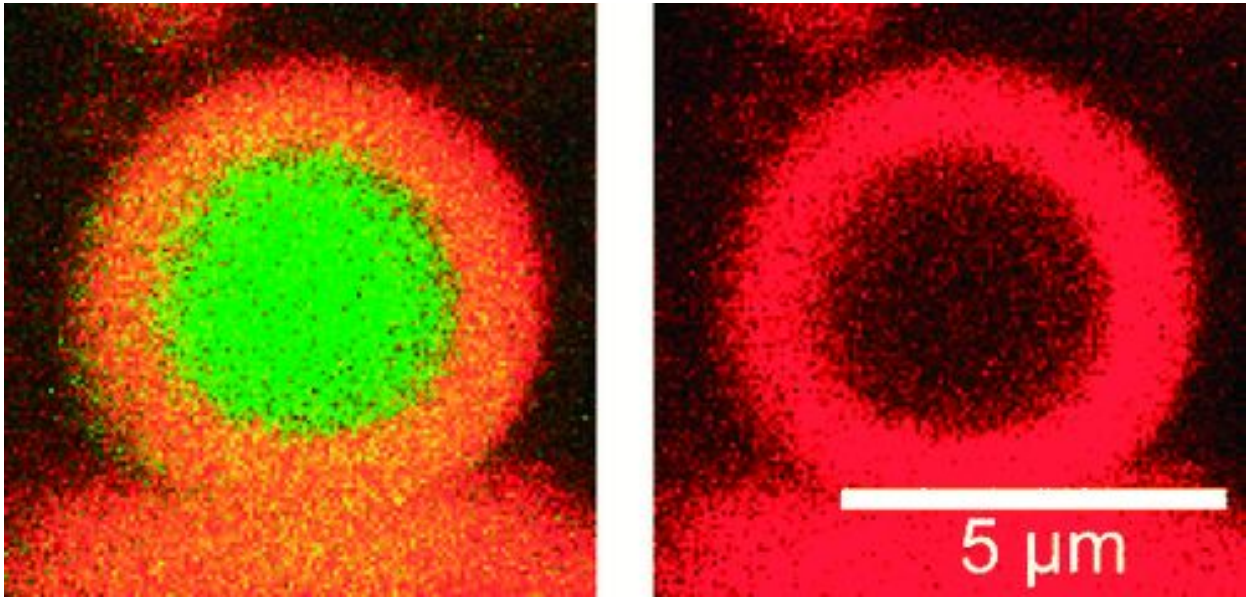
Multi-Step Enzymatic Reactions Catalyze Metabolic Processes

"...many crucial cellular functions such as biosynthesis and cellular signaling are controlled by multi-step enzymatic reactions that take place simultaneously with unsurpassed efficiency and specificity"[1]



1. https://doi.org/10.1371/journal.pone.0097103
2. https://doi.org/10.1039/C1SM06452E

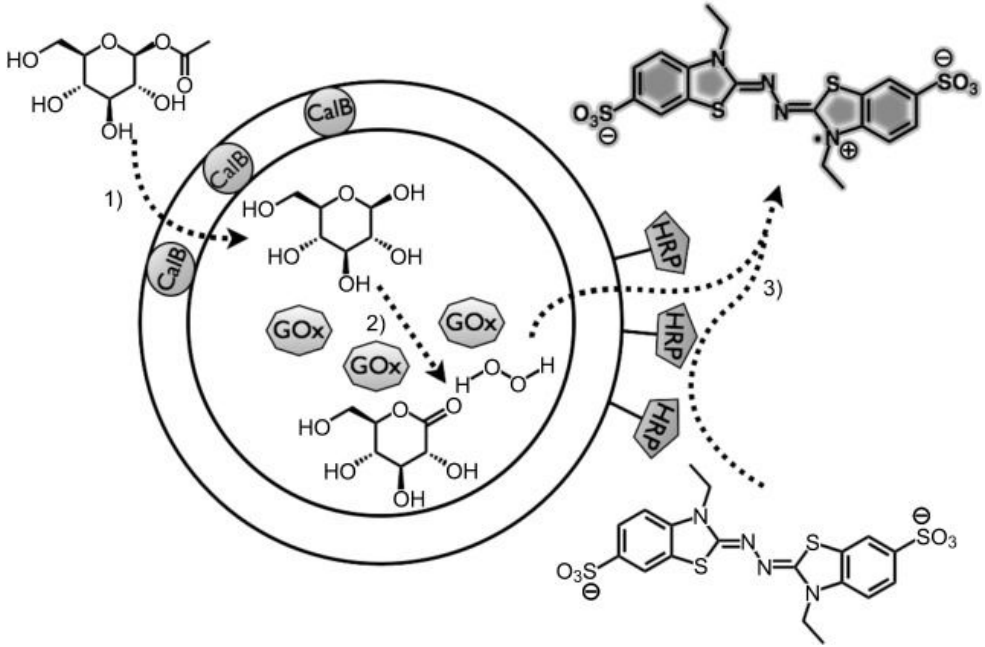
Use of Vesicles to Mimic Complex Enzyme Systems



Cross-sectional images of the giant vesicle observed by confocal laser scanning microscopy

https://doi.org/10.1002/cbic.200300630

Advances in the Design of Complex Enzymatic Systems



Schematic representation of the multistep reaction. 1) Monoacetylated Glucose 2) Oxidation of glucose to gluconolactone by GOx in the inner aqueous compartment,providing a molecule of hydrogen peroxide. 3) Hydrogen peroxide is used by HRP to convert ABTS to ABTS.+. HRP is tethered to the polymersome surface.

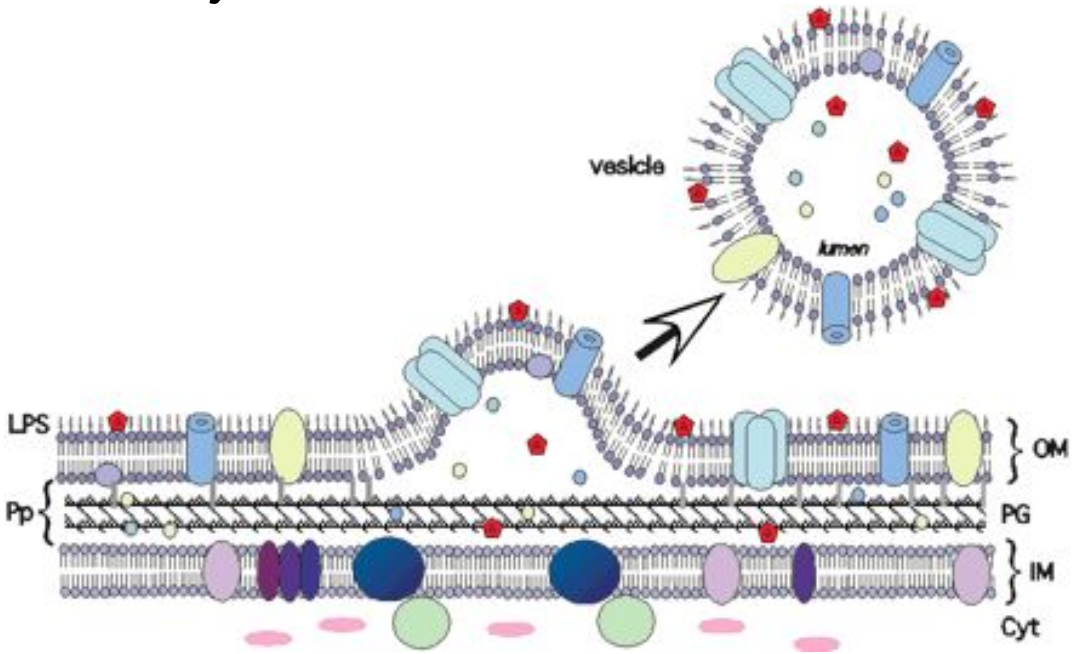
https://doi.org/10.1002/chem.200802114

Limitations

- Thermodynamic and mechanical instability of liposomes
- Low permeability of polymersomes
- Tedious and multiple-step syntheses and conjugations of polymersomes

Park et al. Approach

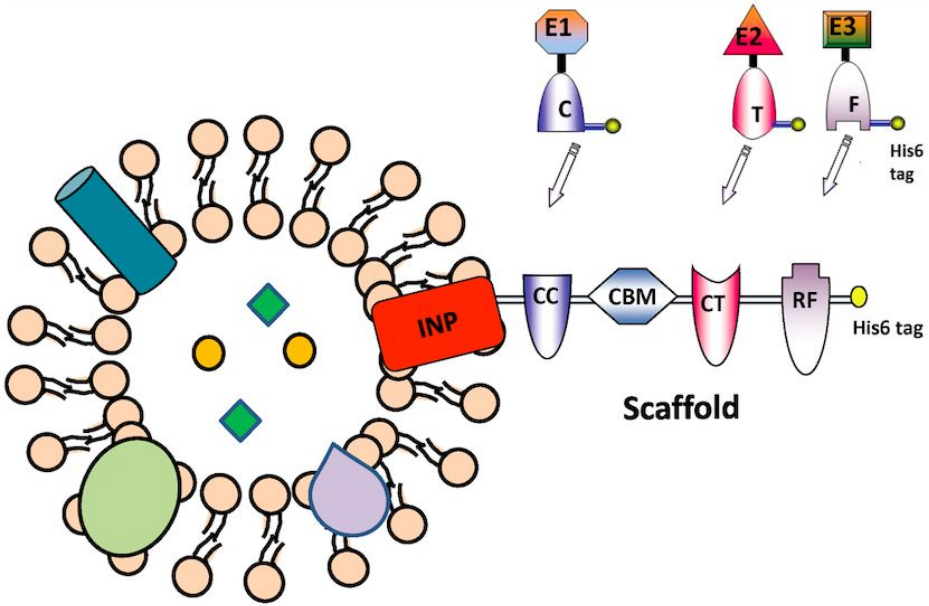
OMVs as synthetic nanoreactors



Model of vesicle biogenesis. OM vesicles are proteoliposomes consisting of OM phospholipids and LPS, a subset of OM proteins, and periplasmic (luminal) proteins. (LPS) Lipopolysaccharide; (Pp) periplasm; (OM) outer membrane; (PG) peptidoglycan; (IM) inner membrane; (Cyt) cytosol.

10.1101/gad.1299905

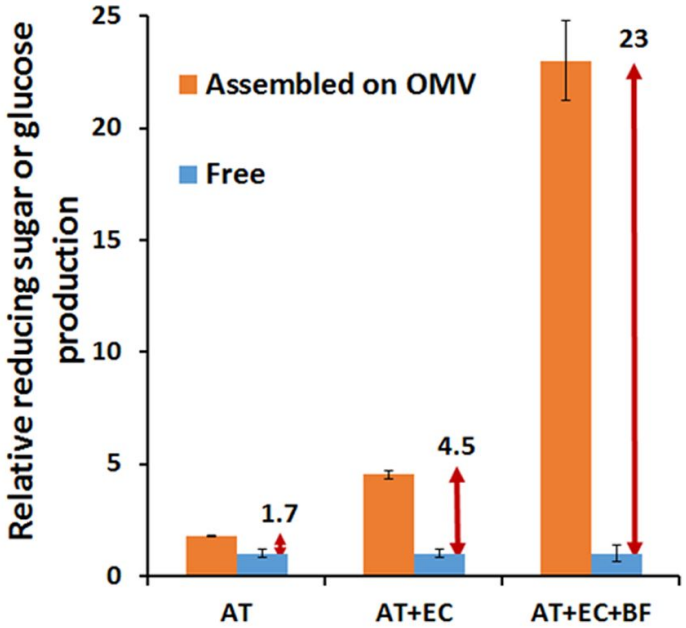
Synergistic assembly of multiple enzymes



Functional assembly of multiple enzymes on engineered bacterial outer membrane vesicles (OMVs). A trivalent scaffold containing three orthogonal cohesin domains, DocC (from *C. cellulolyticum*), DocT (from *C. thermocellum*) and DocF (from *R. flavefaciens*), and one cellulose-binding module, is displayed onto OMVs using the ice nucleation protein (INP) anchor. The specific interaction between each cohesin-dockerin pair enables the sequential assembly of three dockerin-tagged cellulases (E1, E2 and E3) onto the OMVs at their corresponding position (C, T or F).

https://doi.org/10.1371/journal.pone.0097103

Key Results and Conclusions from Paper



Production of reducing sugars (AT and AT+EC) or glucose (AT+BC+RF) from PASC by enzyme-assembled OMVs or the same amount of free enzymes.

- 23-fold increase in conversion of cellulose to glucose
- Increase in conversion is due to the synergy developed between the three enzymes

https://doi.org/10.1371/journal.pone.0097103

Our Model

Aim of this Study

To determine the possibility of enzyme spatial organization being the sole cause of enhanced production of glucose

Methodology and Governing Equations

Diffusion Control: Random Walk

- Diffusion time >> reaction time
- Reaction rates only depend on physical movements of the molecules

Kinetic Control: Tau Leaping

- Conversion is instantaneous
- Reaction rates ignored as diffusion effects are negligible

$$k = \frac{1}{average\ reaction\ time} = \frac{average\ steps\ to\ the\ reaction}{time\ per\ step}$$

$$rate = \frac{V_{max} \cdot [S]}{K_M + [S]}$$

Assumptions

1. No side reactions occur.

2. Enzymes can be react with another substrate outside of its intended one and be deactivated.

Random Walk for Diffusion Control

Success v. Failure:

- Success: producing C within defined number of random steps
- Failure:
  - Max steps were taken without producing C
  - A interacted with E3 before E2, deactivating E3

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Walk  1  completed in  636441  steps, A reached E3 before E2. Simulation failed :(

Successful! A+E2 -> B
Successful! B+E3 -> C
Walk  2  completed in  668645  steps.

Walk  3 completed in  1000000  steps, no reaction

Walk  4  completed in  355551  steps, A reached E3 before E2. Simulation failed :(

Walk  5 completed in  1000000  steps, no reaction
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Next Steps for Diffusion Control

- Run simulation more to determine rate constants
- Plot relationship between overall reaction and varying r_a and r_b

$$k = \frac{1}{average\ reaction\ time} = \frac{average\ steps\ to\ the\ reaction}{time\ per\ step}$$

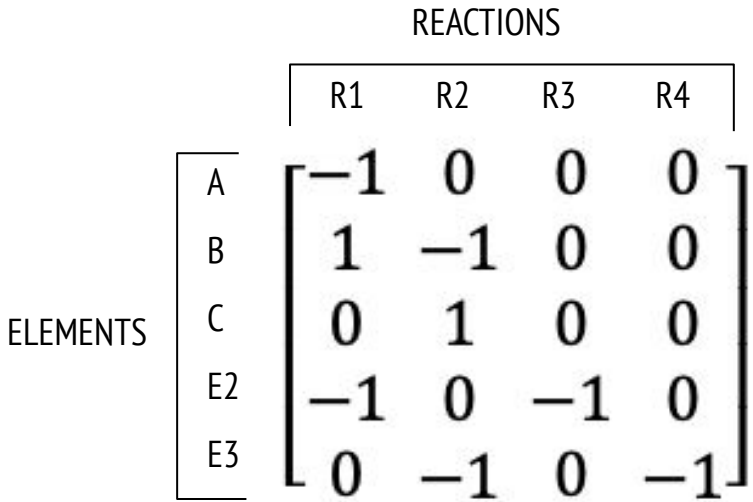
Tau Leaping for Kinetic Control

R1: A + E2 -> B

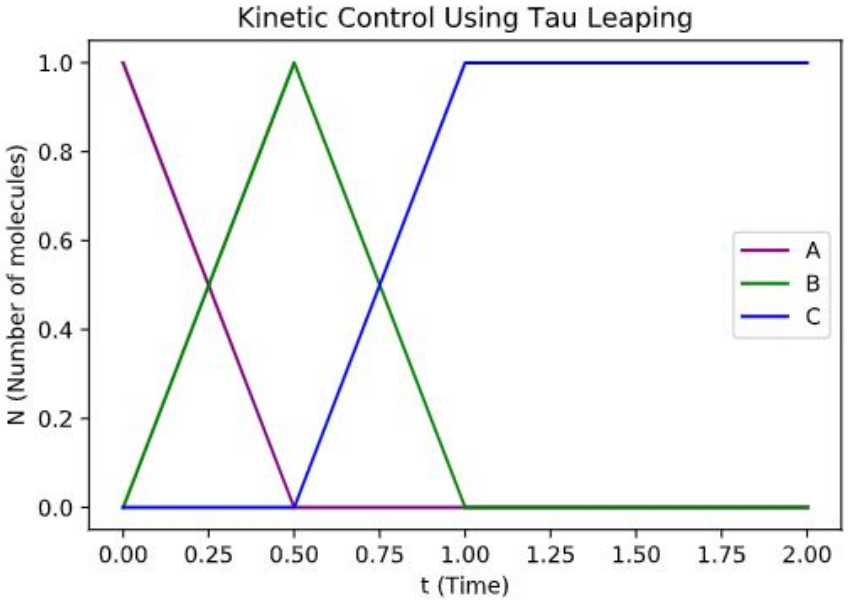
R2: B + E3 -> C

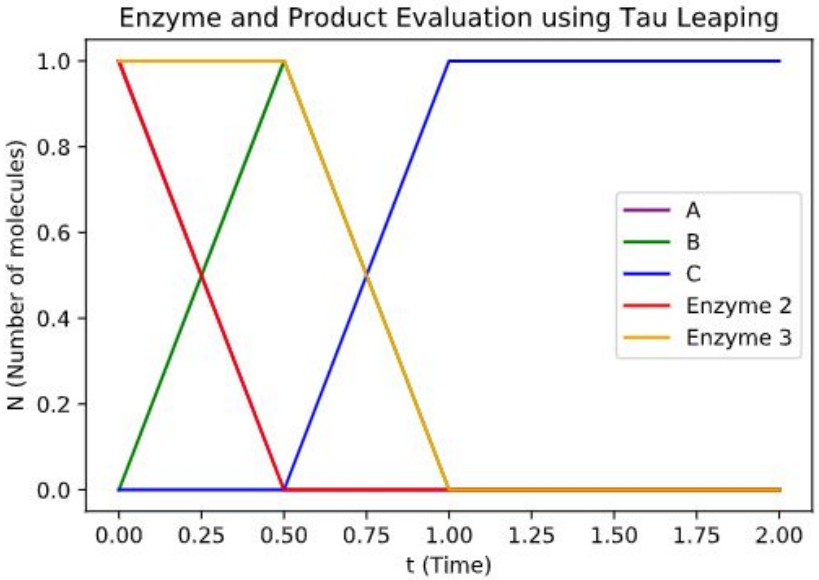
R3: E2 degrades

R4: E3 degrades



Preliminary Results





Project Next Steps

- Finalize Diffusion Controlled System
- Run combined Python Script for a Diffusion and Kinetic System
- Analyze results to determine if spatial organization is a viable possibility for the concentration increase received by Park et al.

Questions?

References

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