Bioprocess Intensification at the Intersection of Biology and Advanced Manufacturing

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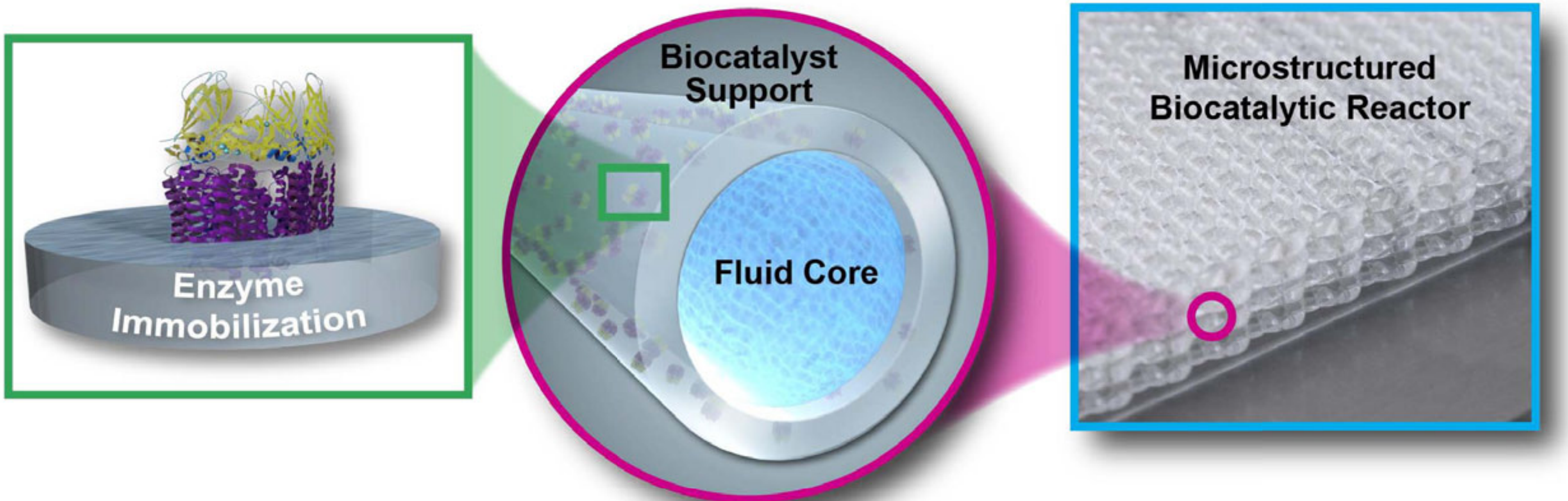
Collaborators: Prof. Amy Rosenzweig **(Northwestern)**; Prof. Alfred Spormann **(Stanford)**



Laboratory Directed Research and Development

BETO Listening Day July 30, 2017

Centralized Feedstocks

Emerging Distributed Feedstocks e.g. biogas, syngas, biosolids, food waste, CO_2 streams

Process/Reactor Needs for Emerging Feedstocks:

- **Efficient at small scales**
  - e.g. modular reactors: surface area dependent (gas phase reactants, electron transfer)

- **Low Capital Investment**
  - Mild operating conditions, high process intensity, reduced downstream processing

Technical Innovations for Bioprocess Intensification:

- **Higher Intensity: Minimize volume/carbon devoted to metabolism (Cell-free)**
- **Higher Stability: More Process Flexibility**
- **Advanced Materials to Enhance Cell-Free Processes and Mass Transfer**

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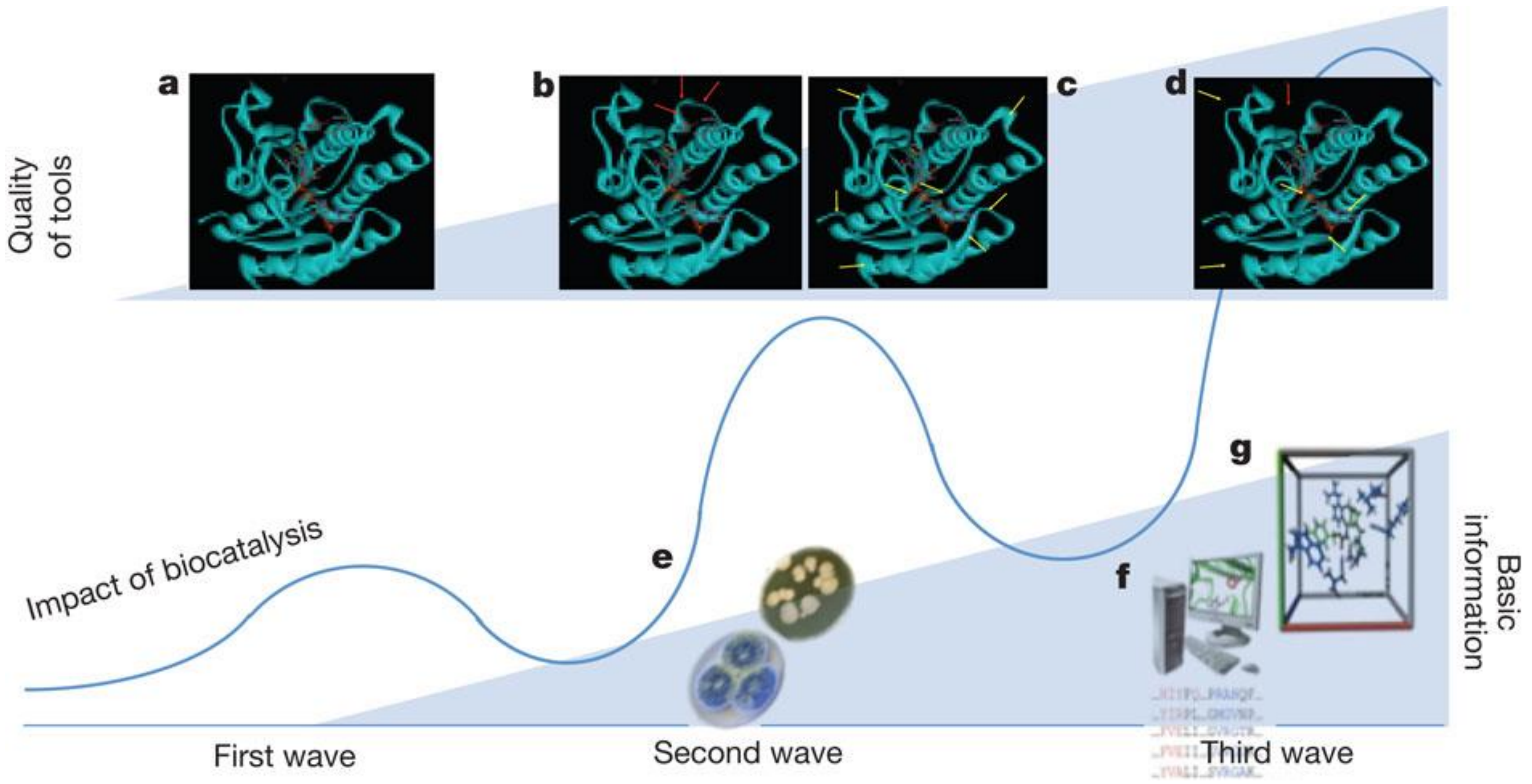
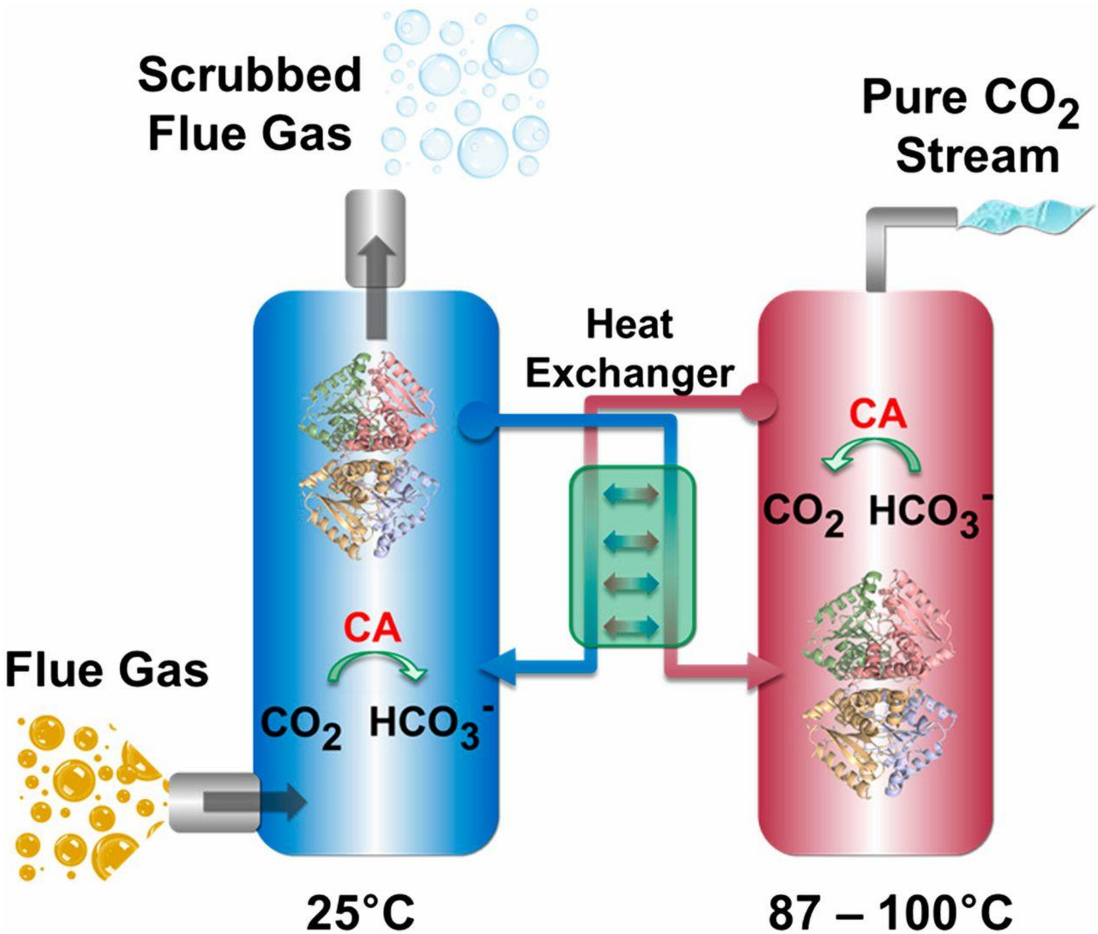
"3rd Wave" of Biocatalysis: Smaller, Smarter Libraries, Rational Design



UT Bornscheuer *et al.* *Nature* **485**, 185-194 (2012)

Example: Directed Evolution of Carbonic Anhydrase



Oscar Alvizo et al. PNAS 2014;111:16436-16441

The Stability of Carbonic Anhydrase was Improved ~5 Million Fold

Technical Innovations for Bioprocess Intensification:

- Higher Intensity: Minimize volume/carbon devoted to metabolism (Cell-free)

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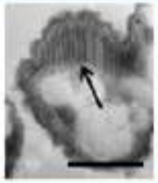
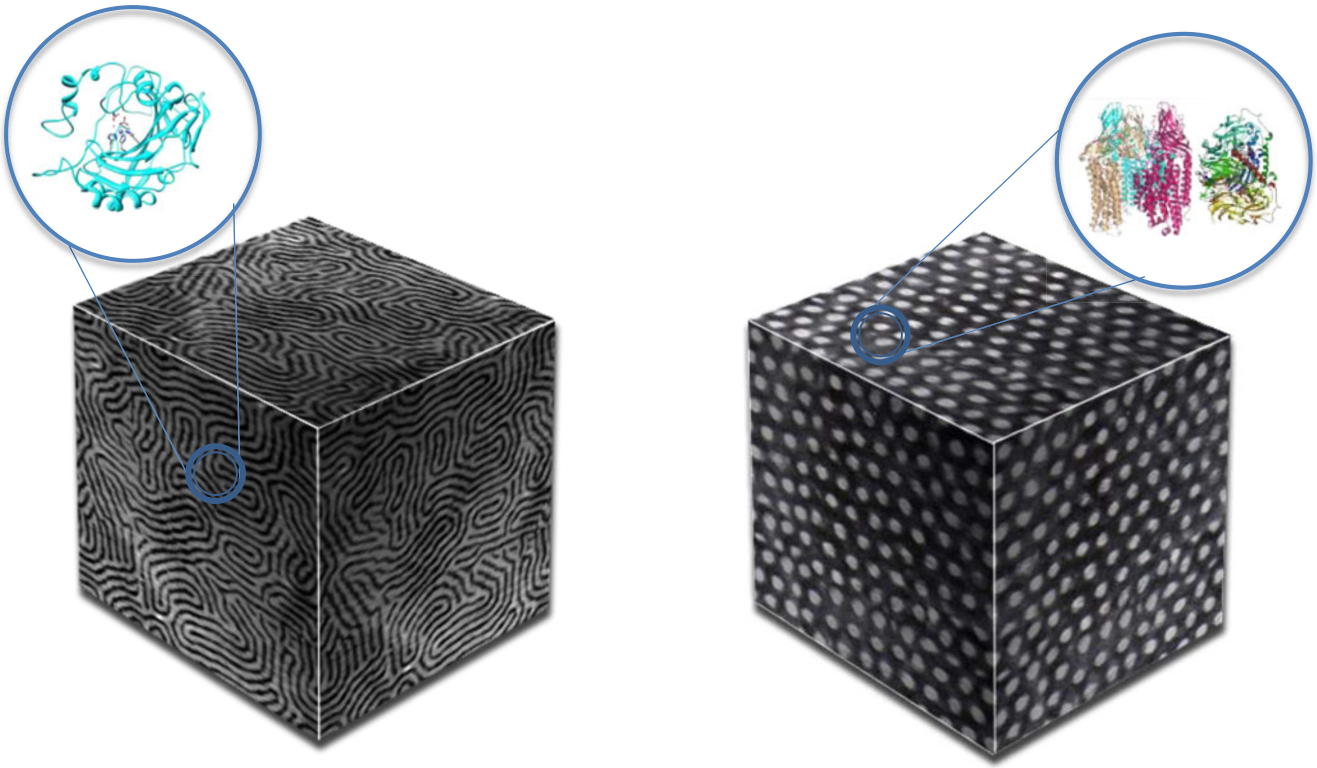
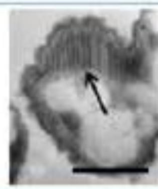
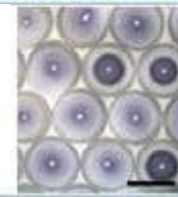
Biology uses materials to make enzymes work better:

Biology's Design Strategies	Biological Structures
Enzyme-substrate co-localization	
Cellular compart-ments with selective membrane barriers	
Positioning enzymes at interfaces	

Embedding Enzymes in Functional Materials

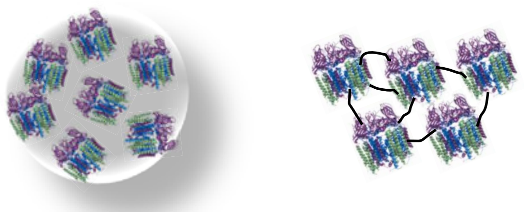


We can now mimic biology's design strategies using advanced manufacturing

Biology's Design Strategies	Biological Structures	**Proposed** Artificial Design Strategies	**Proposed** Synthetic Architectures	**Function** of Architectures
Enzyme-substrate co-localization		Functional copolymer network structures		Increase substrate concentration to increase catalytic rates
Cellular compart-ments with selective membrane barriers		Permeable polymer microcapsules embed-ded in bulk materials		Protect enzymes from competing reactions and prevent cofactor diffusion
Positioning enzymes at interfaces		Printing of enzyme-embedded material gradients		Enable most efficient use of enzymes

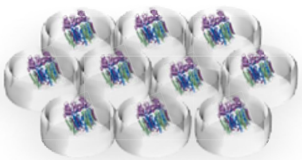
How Can Materials Meet the Potential of Engineered Enzymes?

1st Gen: immobilization — Enzyme re-use



Adsorption, crosslinking

2nd Gen: stabilization — Re-use + extended lifetime/ Organic solvents



Encapsulation (sol gel, mesoporous)

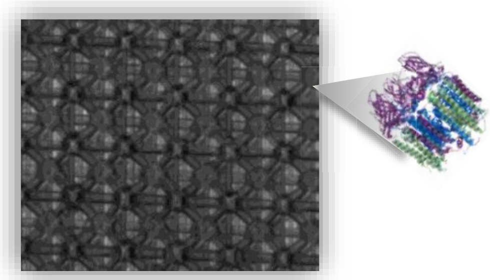
3rd Gen: directed assembly

Synergy with Materials:

Enhanced Mass Transfer

Permeable compartments

Enhanced Electron Transfer



Enzyme embedded materials with tunable architectures

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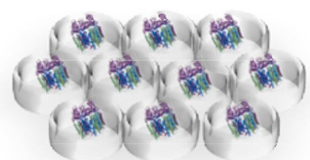

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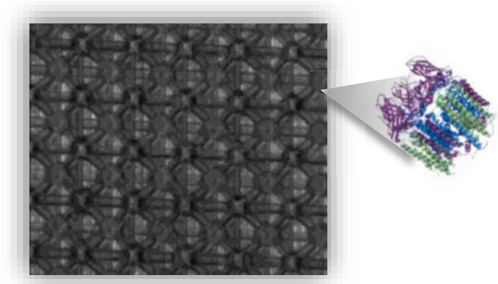


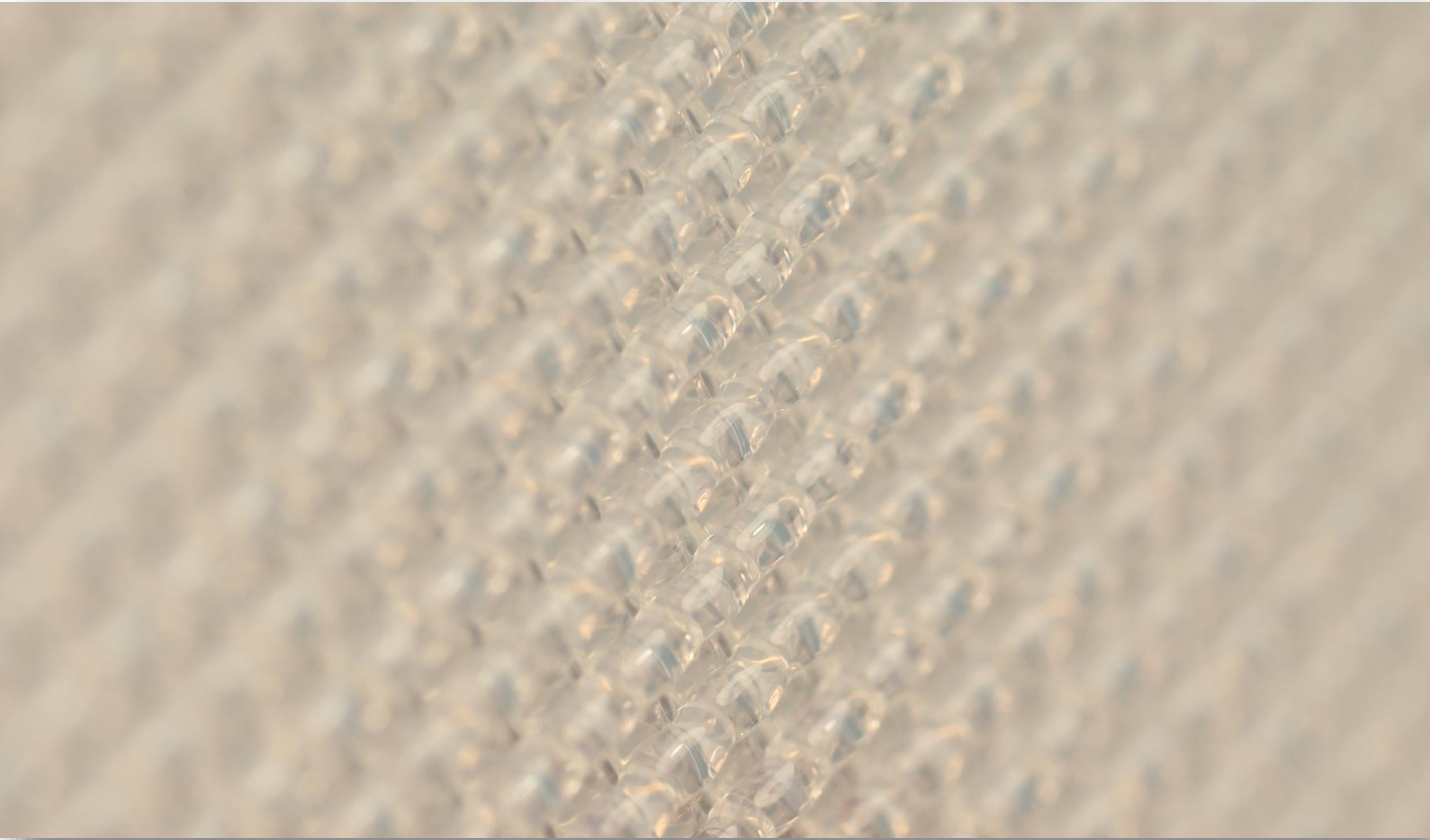
Enzyme embedded materials with tunable architectures

Mass Transfer Example: Cell-Free methane conversion

Efficient GTL process for small and remote methane streams Needed: Suggests Biological Process

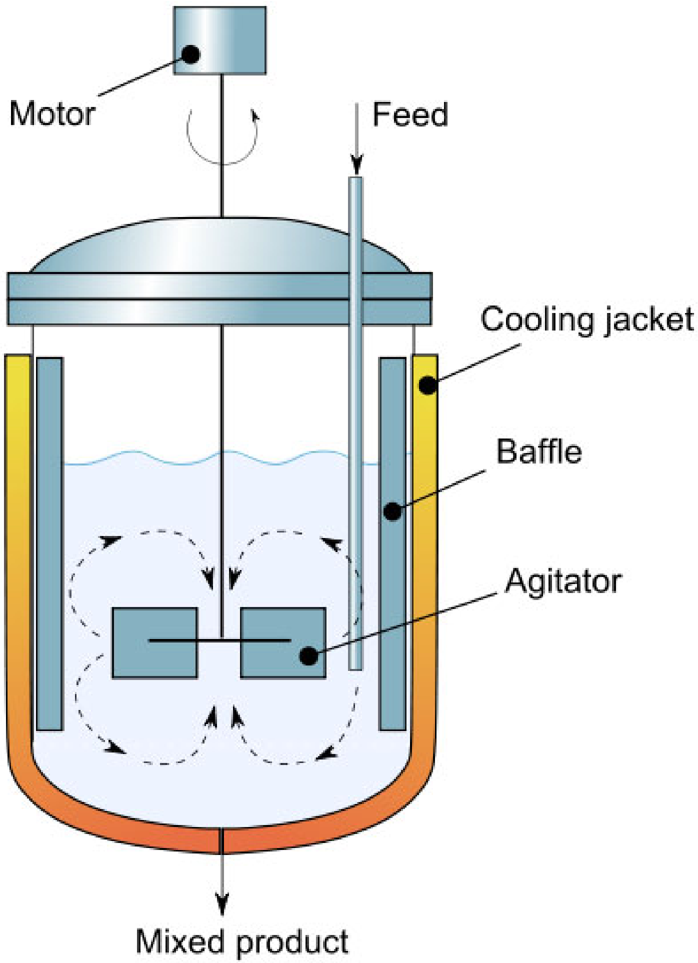
*Fei, Q., et. al., 2014. Bioconversion of natural gas to liquid fuel: Opportunities and challenges. *Biotechnology Advances* 32, 596–614. 1.; Haynes, C. A. & Gonzalez, R. Rethinking biological activation of methane and conversion to liquid fuels. *Nature Chemical Biology* **10,** 331–339 (2014).

Approach: Printed Bioreactor



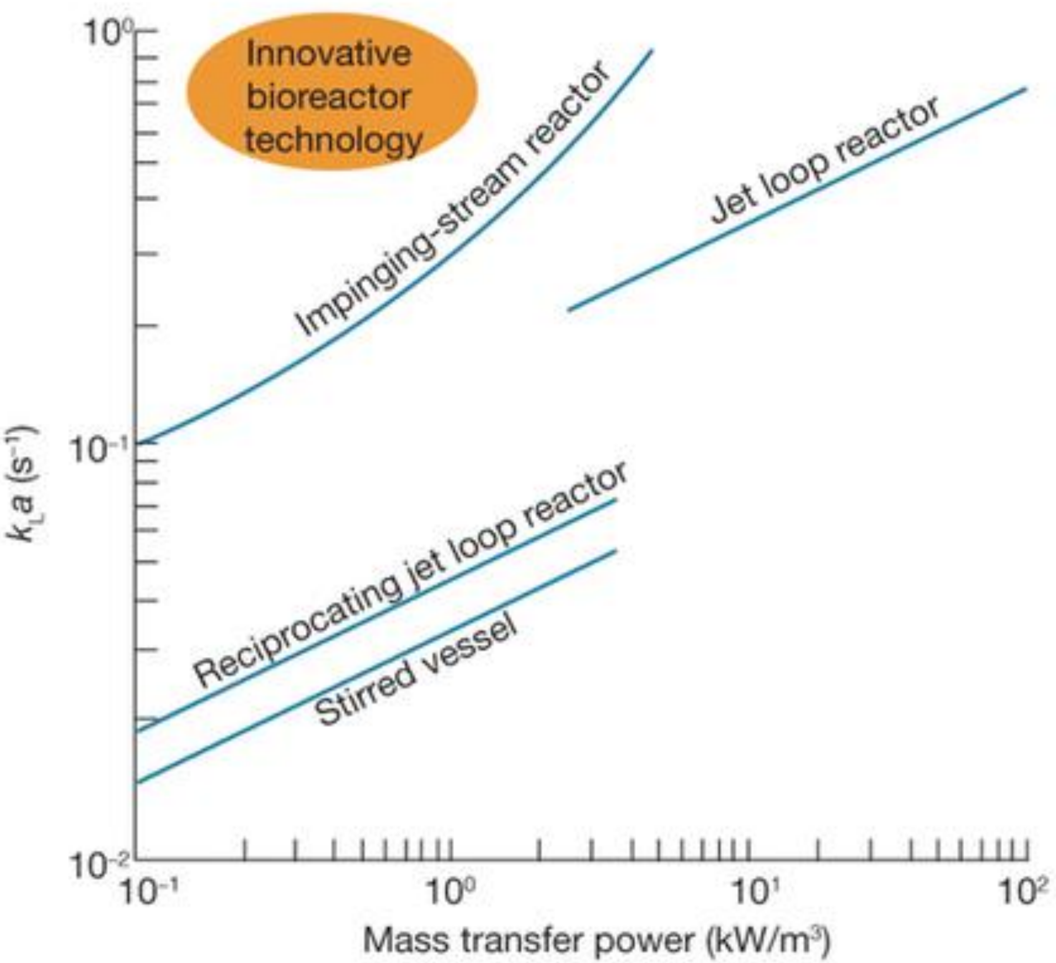
Why would we want to do that?

The stirred-tank reactor is slow and inefficient for gas phase reactants (e.g. CH_4, O_2, CO, H_2, CO_2)



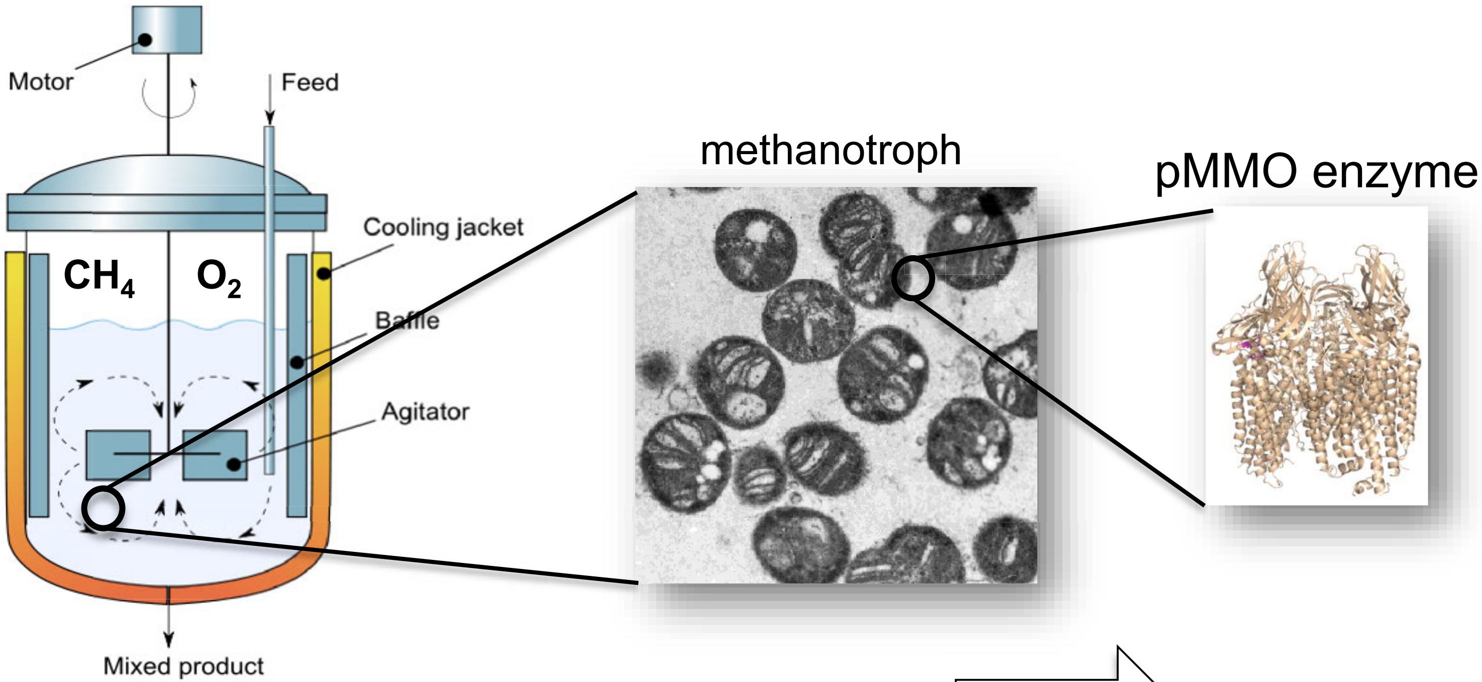
- Poor Mass Transfer
- Low Volumetric Productivity

New Bioreactor Technology Needed



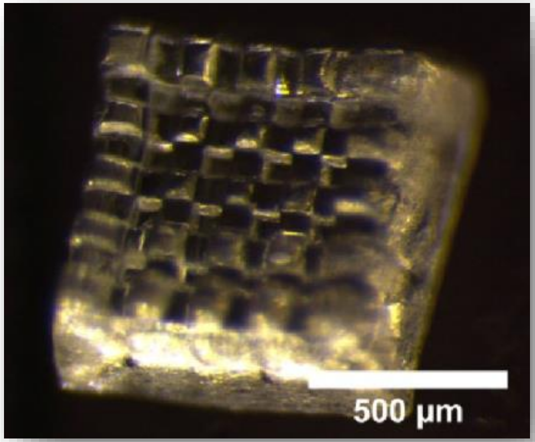
Haynes and Gonzalez, *Nature Chemical Biology* 10, 331–339 2014

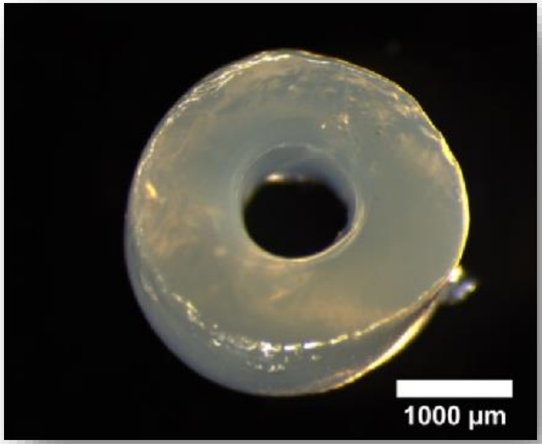
Printed pMMO Bioreactor to Intensify the Process



$$CH_4 + O_2 + 2H^+ + 2e^- \xrightarrow{\text{pMMO}} CH_3OH + H_2O$$

Direct printing of pMMO: control of surface area

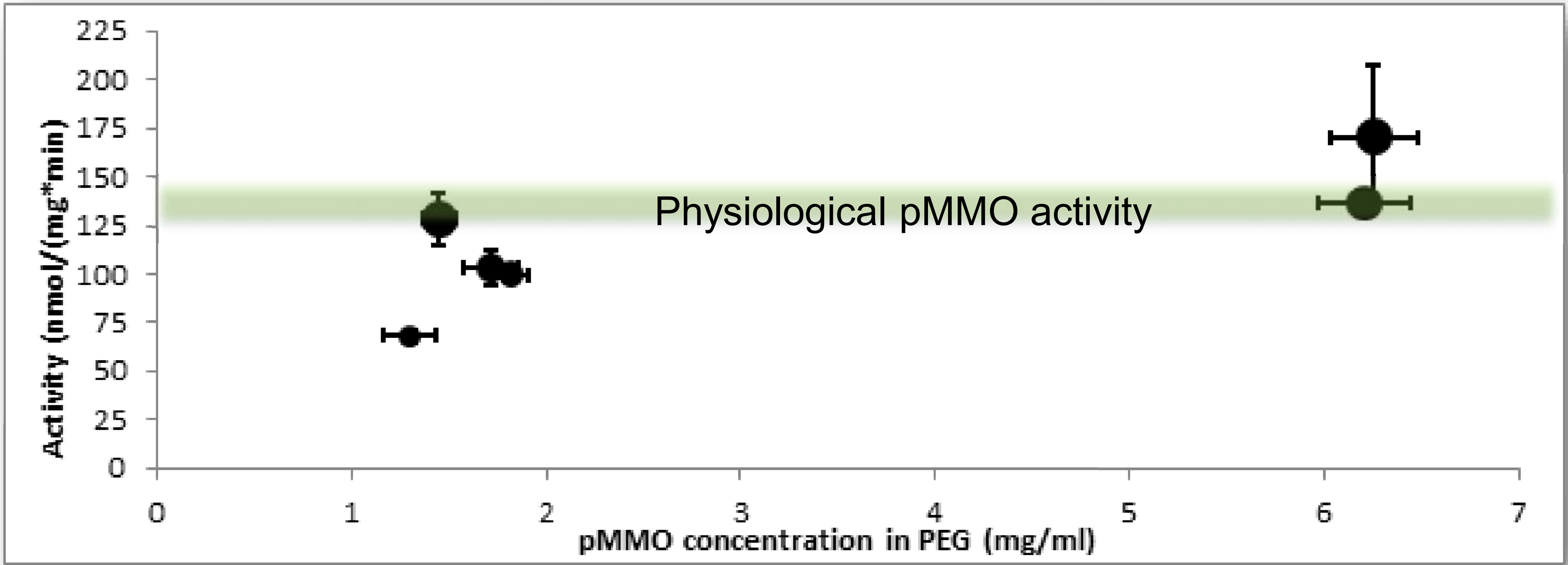




Zheng *et al.*, Science 344 (6190): 1373-1377 (2014)

Printed pMMO: increased protein concentration and activity

Printed pMMO

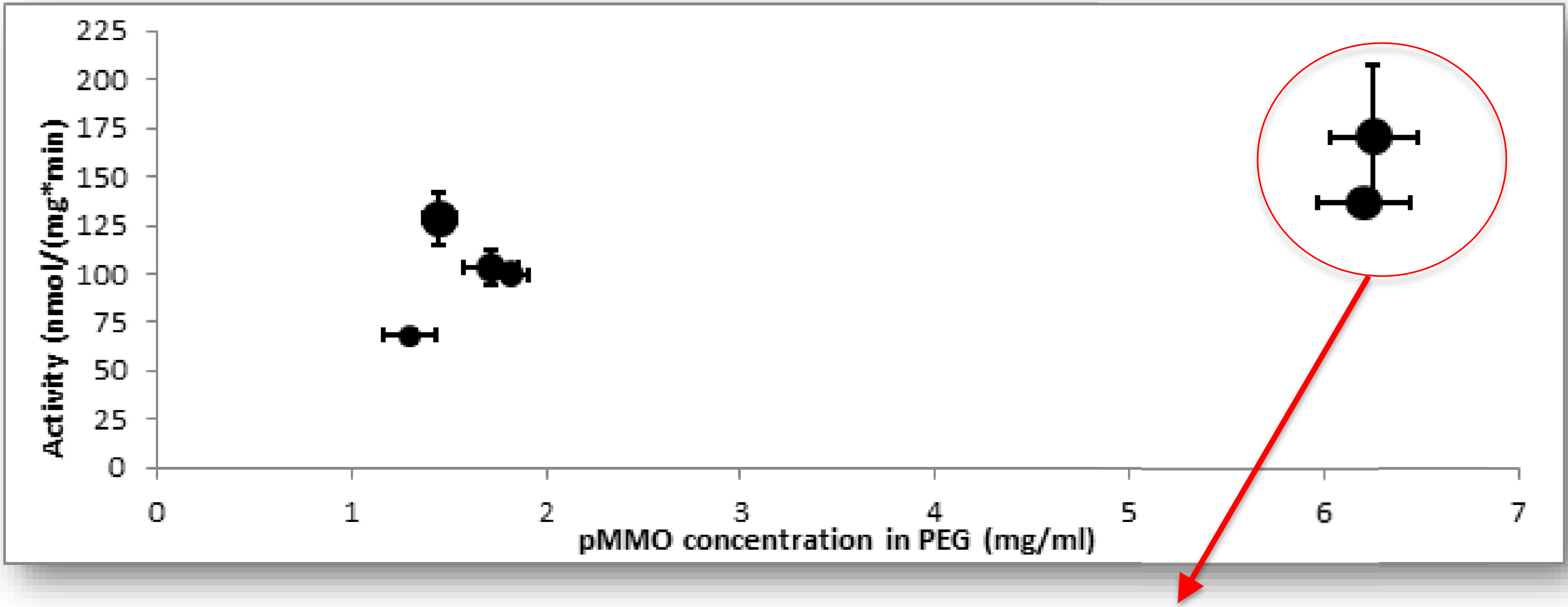


Physiological activity of pMMO achieved in a printed material

Blanchette, C. D. *et al. Nature Communications* **7,** 11900 (2016).

Printed pMMO: ARPA-e REMOTE targets reached

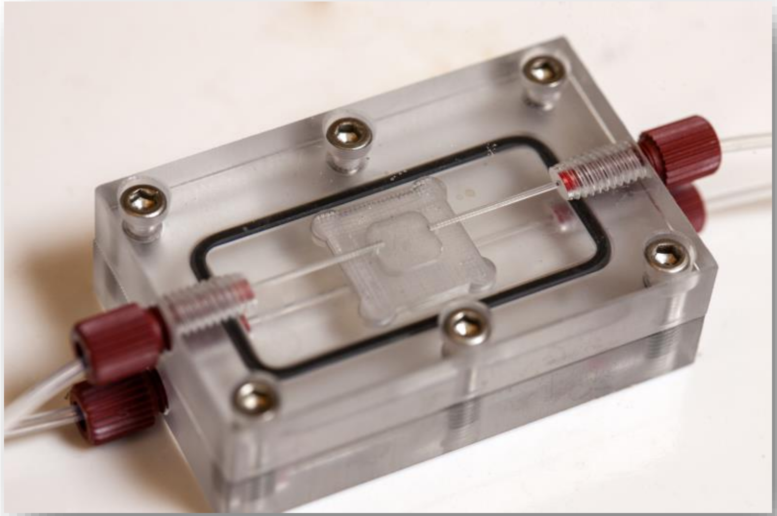
Printed pMMO

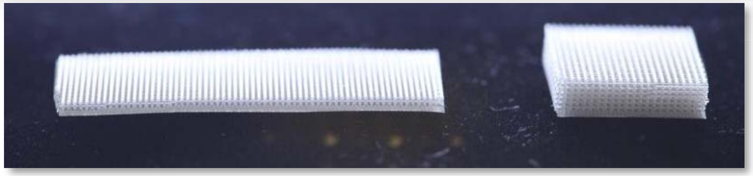


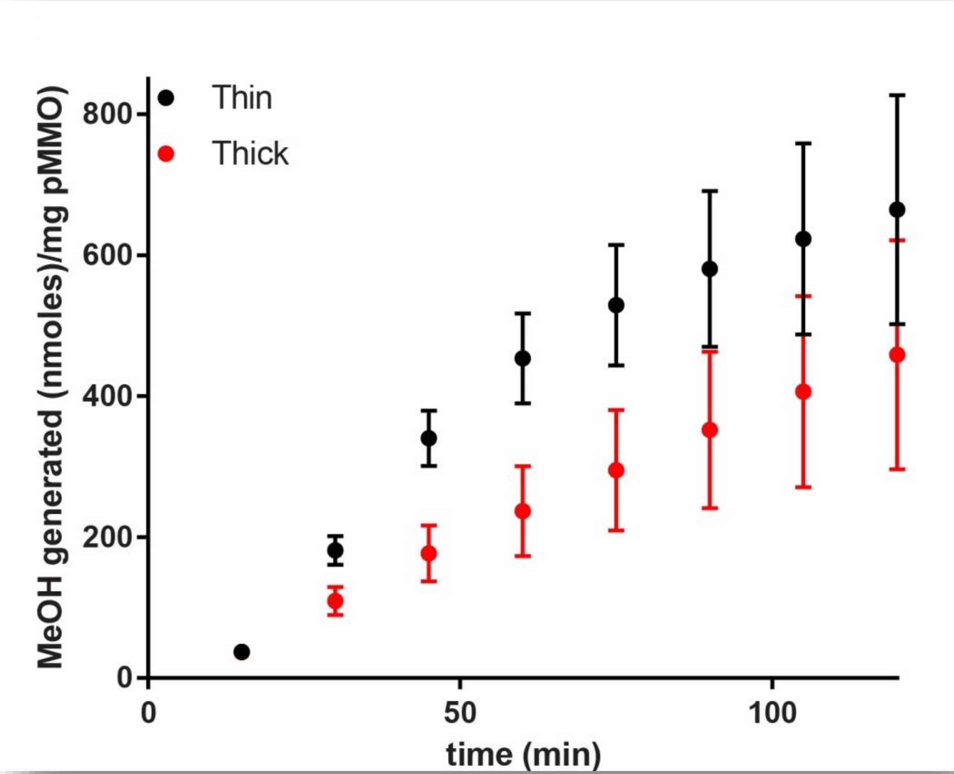
Corresponds to >2g MeOH/L/hr (with unoptimized structures)

Haynes and Gonzalez. Rethinking biological activation of methane and conversion to liquid fuels. *Nature Chemical Biology* **10,** 331–339 (2014).

Printed pMMO membranes enabled continuous methanol production at gas- liquid interface



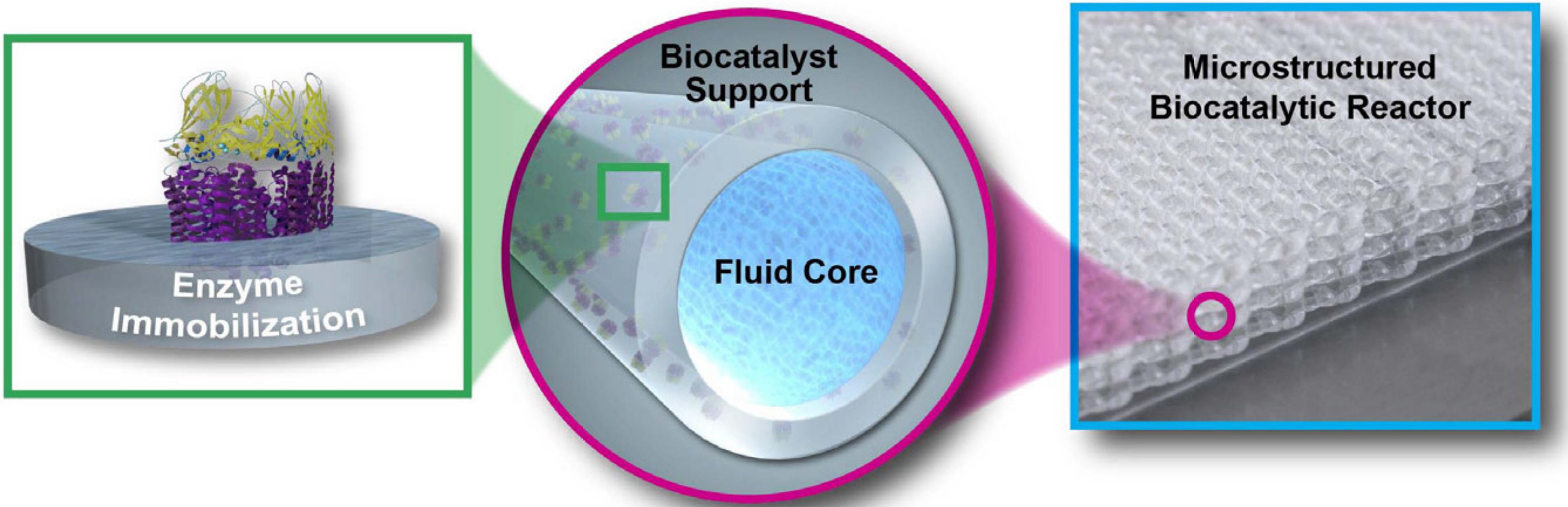




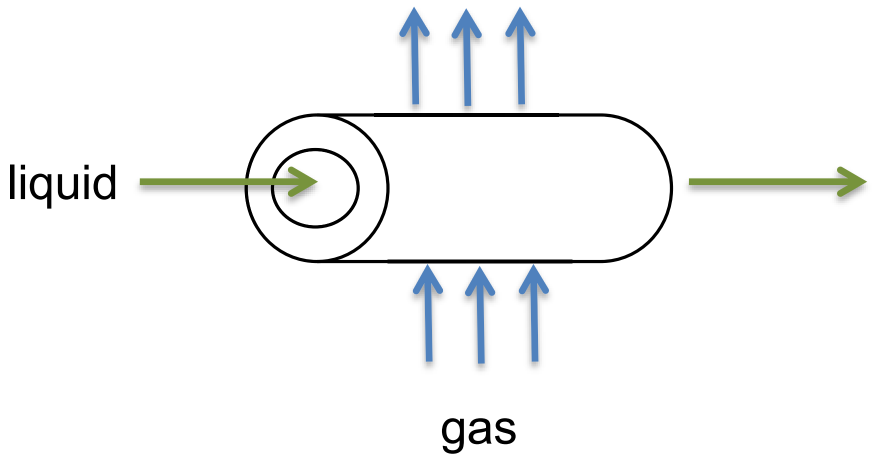
- **Thin pMMO lattice → higher activity**

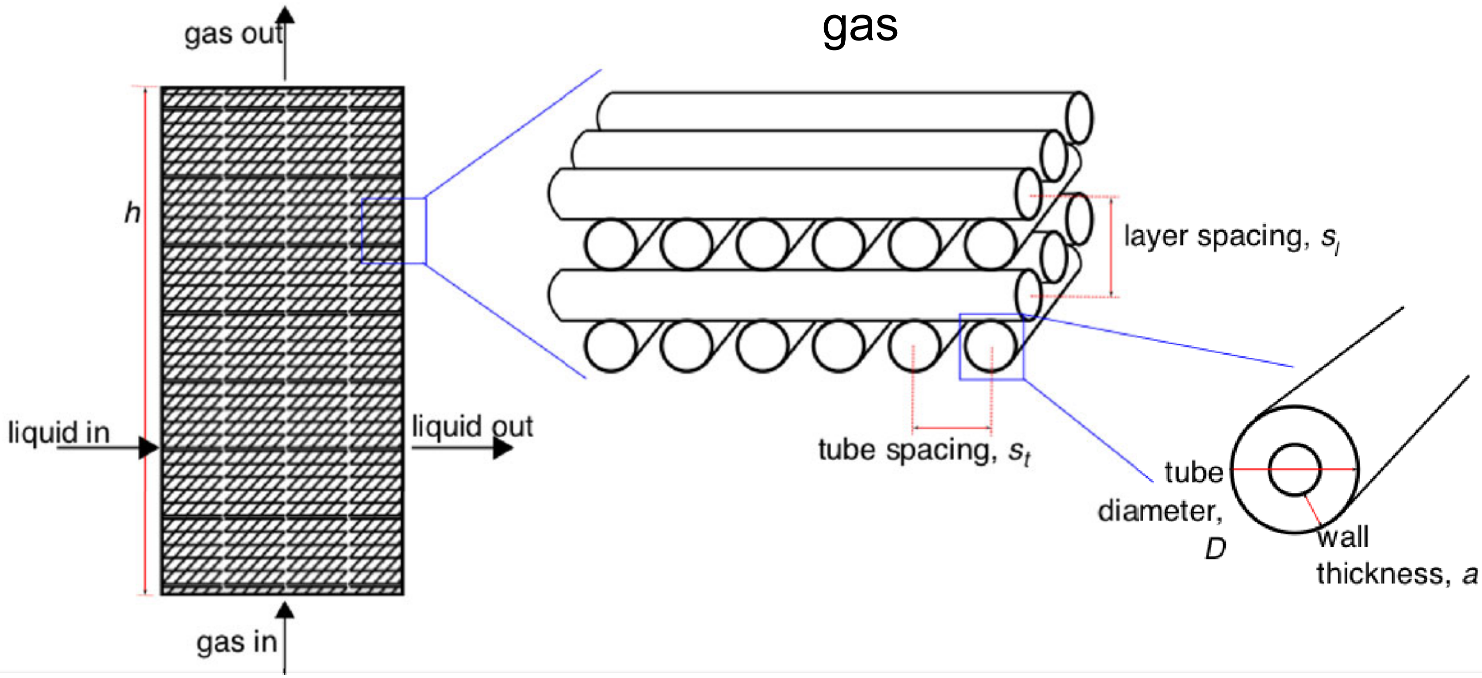
- **Membrane is Progress, But Can We Do Better?**

Potential Reactor Design: “Printed Tube Reactor”

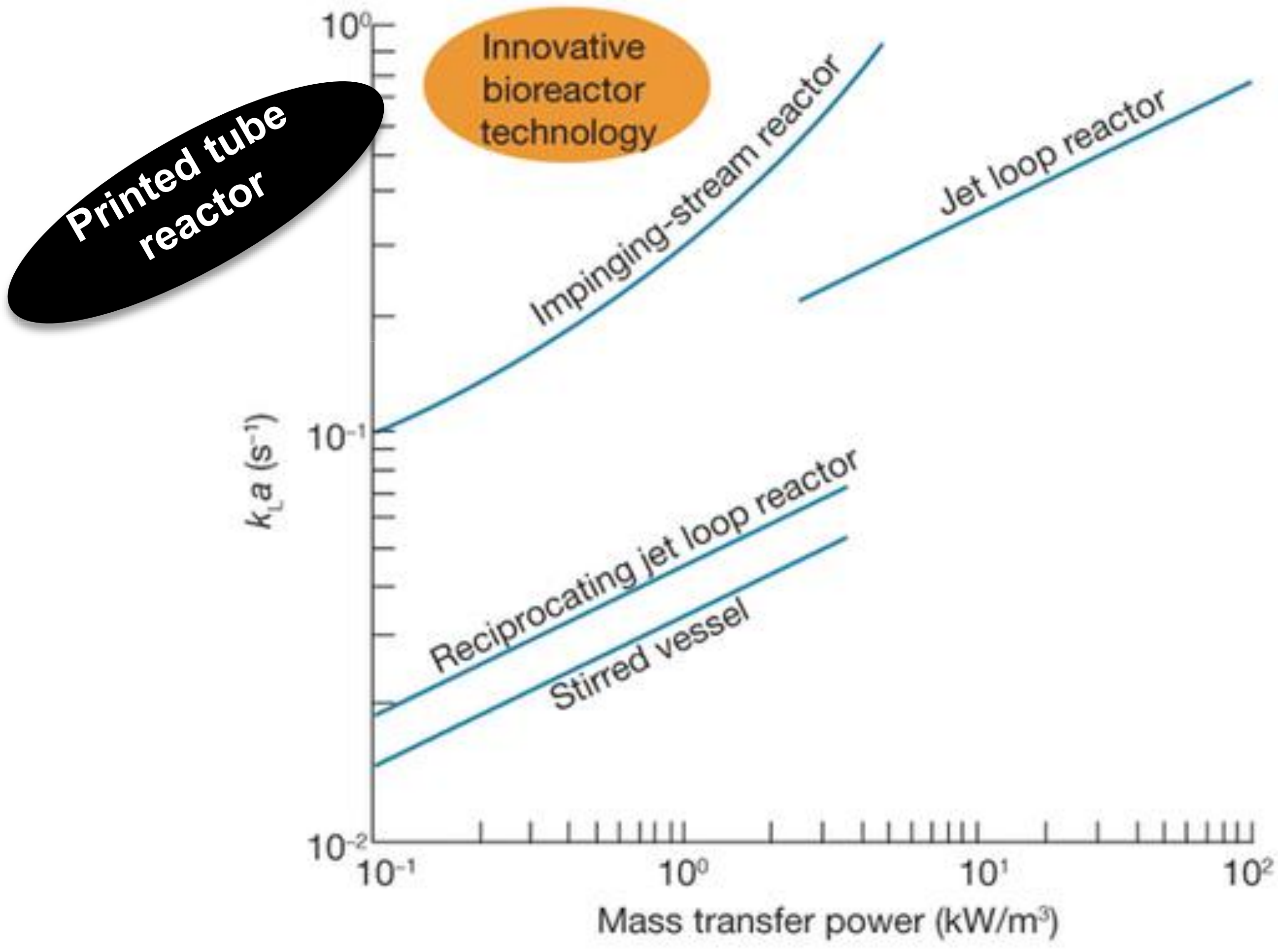


Printed Tube Reactor: Surface Area Created By Structure & Independent of Pressure Drop



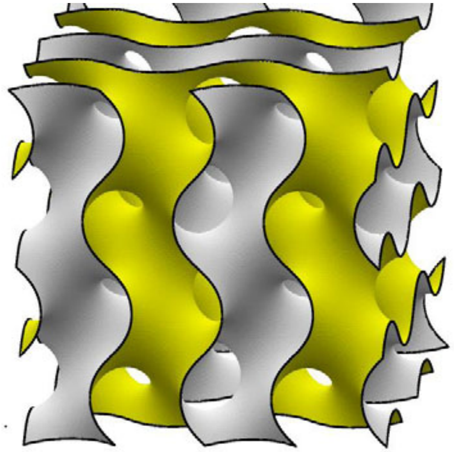


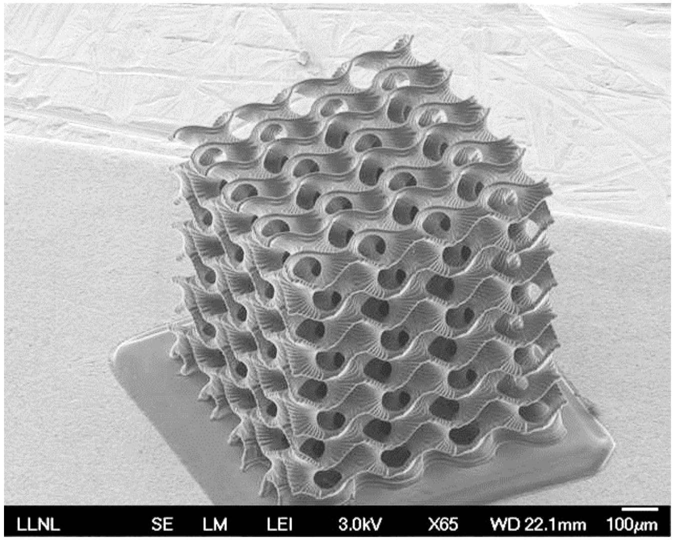
Printed tube reactor: High mass transfer rate + energy efficiency



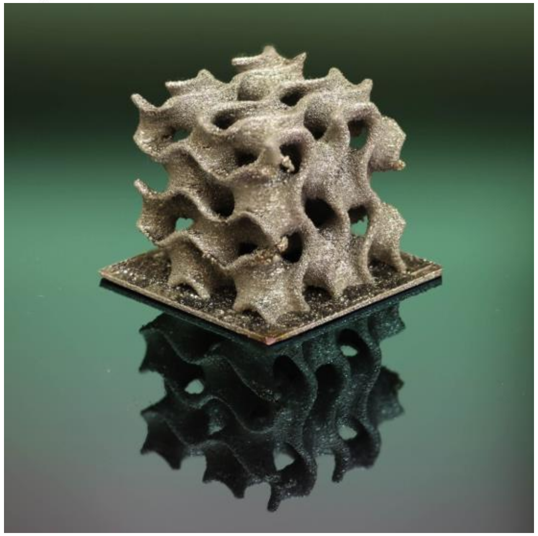
Haynes and Gonzalez, *Nature Chemical Biology* 10, 331–339 2014

Gyroid reactors: only possible with additive manufacturing



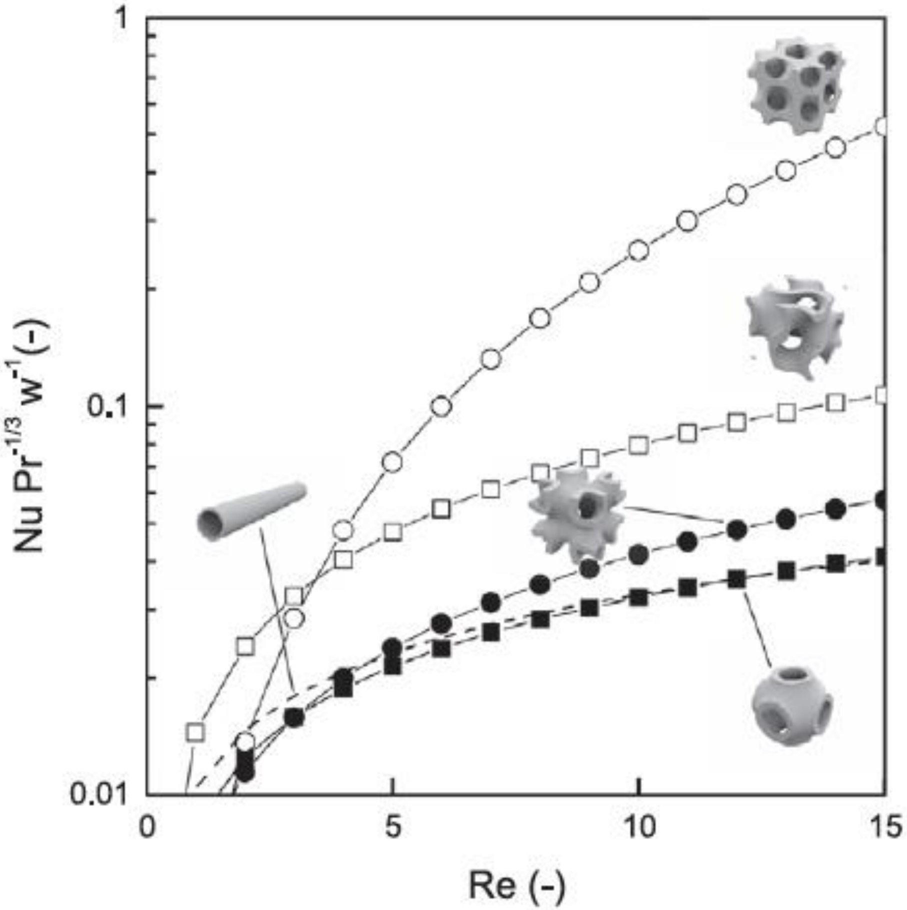


Polymer Gyroid Reactor (LLNL)



Stainless Steel Gyroid Reactor (LLNL)

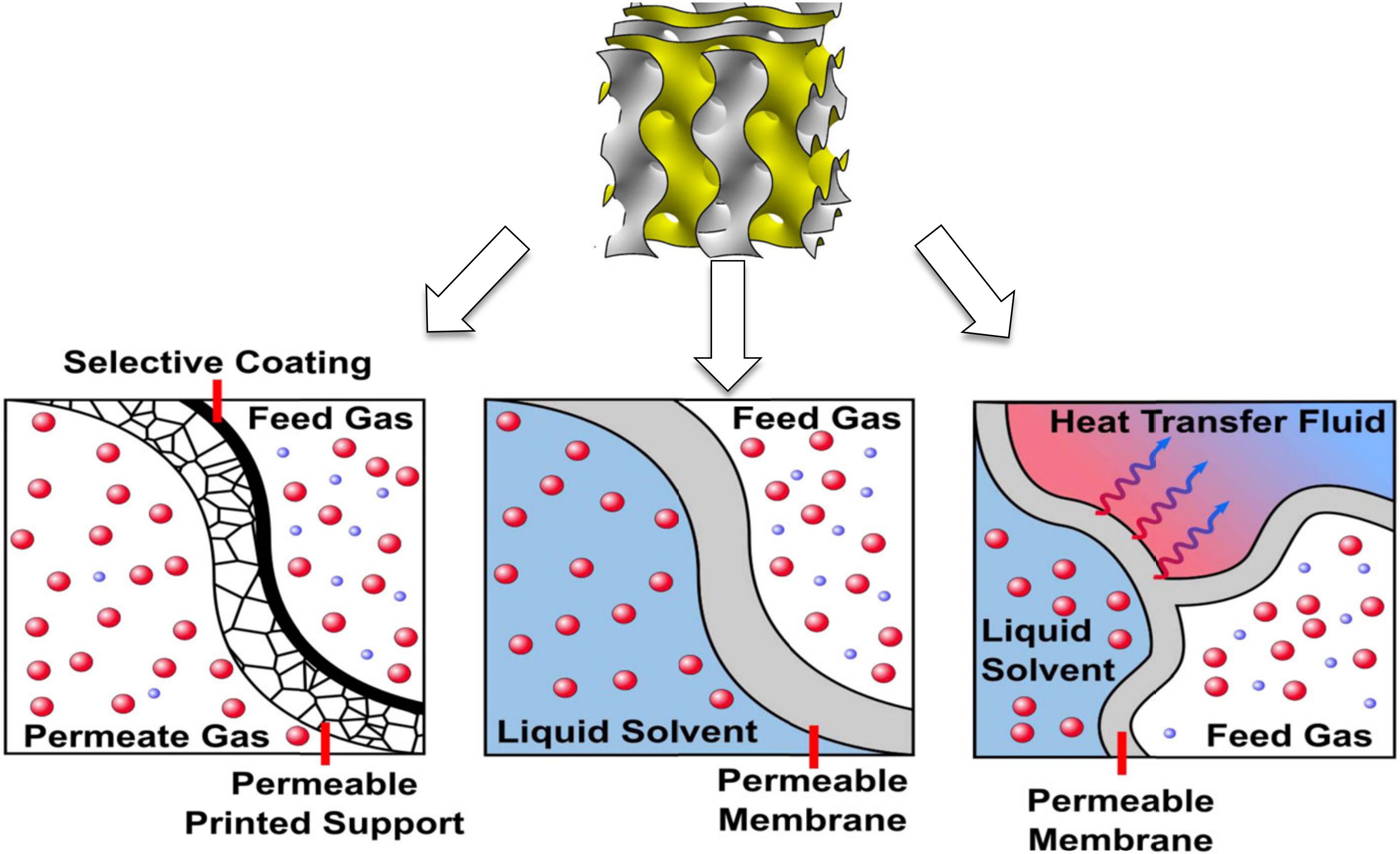
Order-of-magnitude improvement in heat transfer performance over tubes and flat plates.



Heat transfer per unit surface area

T. Femmer et al. *Chemical Engineering Journal* 273 (2015) 438–445.

Possible Reactor Configurations



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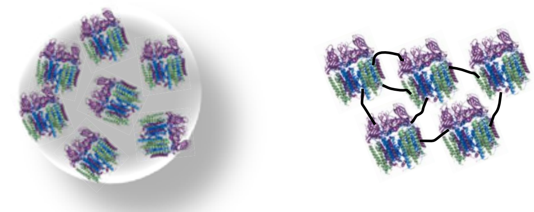

Adsorption, crosslinking

2^{nd} Gen: stabilization — Re-use + extended lifetime/ Organic solvents

Encapsulation (sol gel, mesoporous)

3^{rd} Gen: directed assembly

Synergy with Materials:

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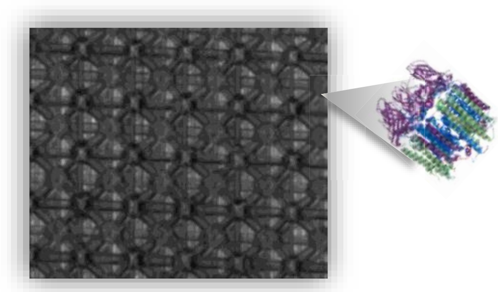
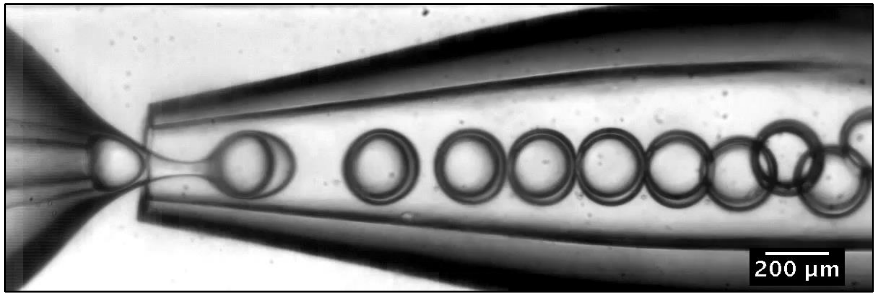
Permeable compartments

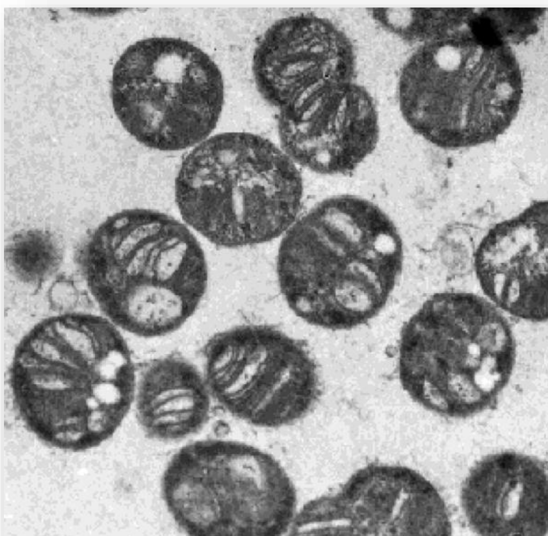
Enhanced Electron Transfer



Enzyme embedded materials with tunable architectures

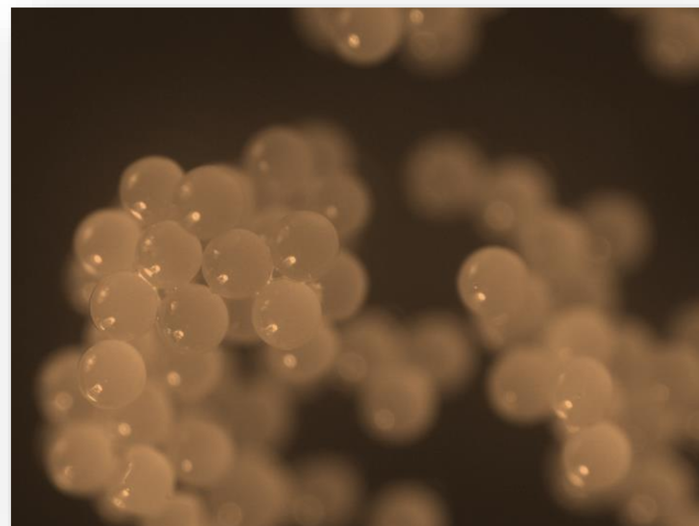
Microencapsulation: Regeneration, Flexible Reactor Configurations, Relevant Length Scales





Lyophilized

Encapsulated



encapsulated *M. capsulatus* proteome

Lyophilized encapsulated whole *M. capsulatus* catalytically active for propylene oxidation

Cells Provided By Calysta

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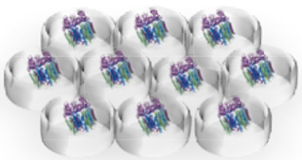

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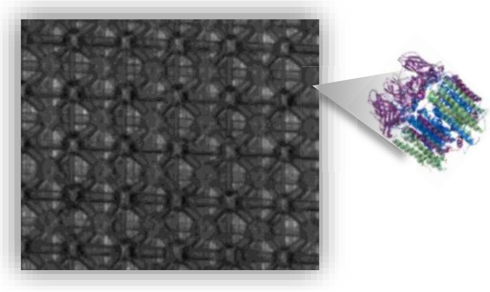
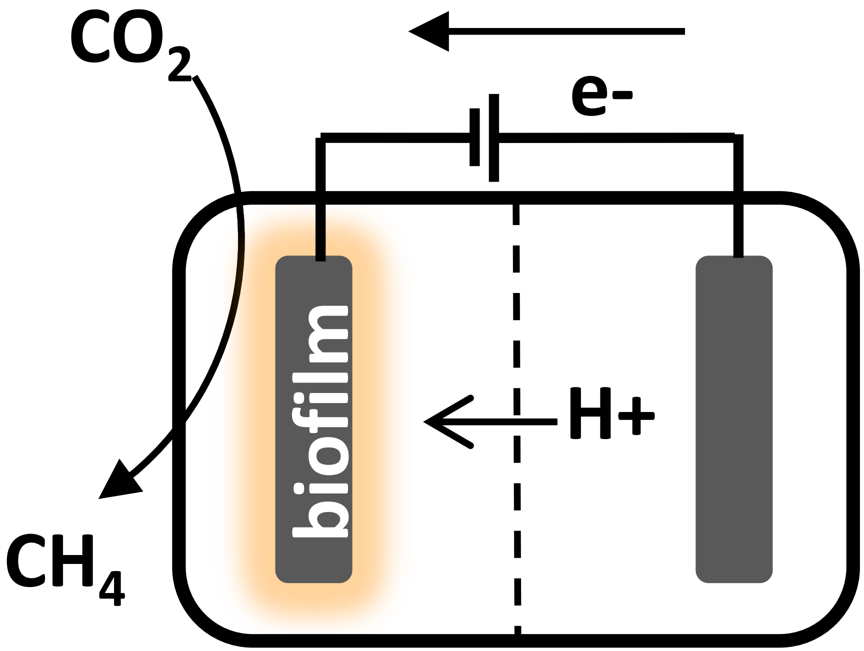


Enzyme embedded materials with tunable architectures

Microbial Electrosynthesis: Reactor Productivity Depends on Current Density (Amps/m^2)

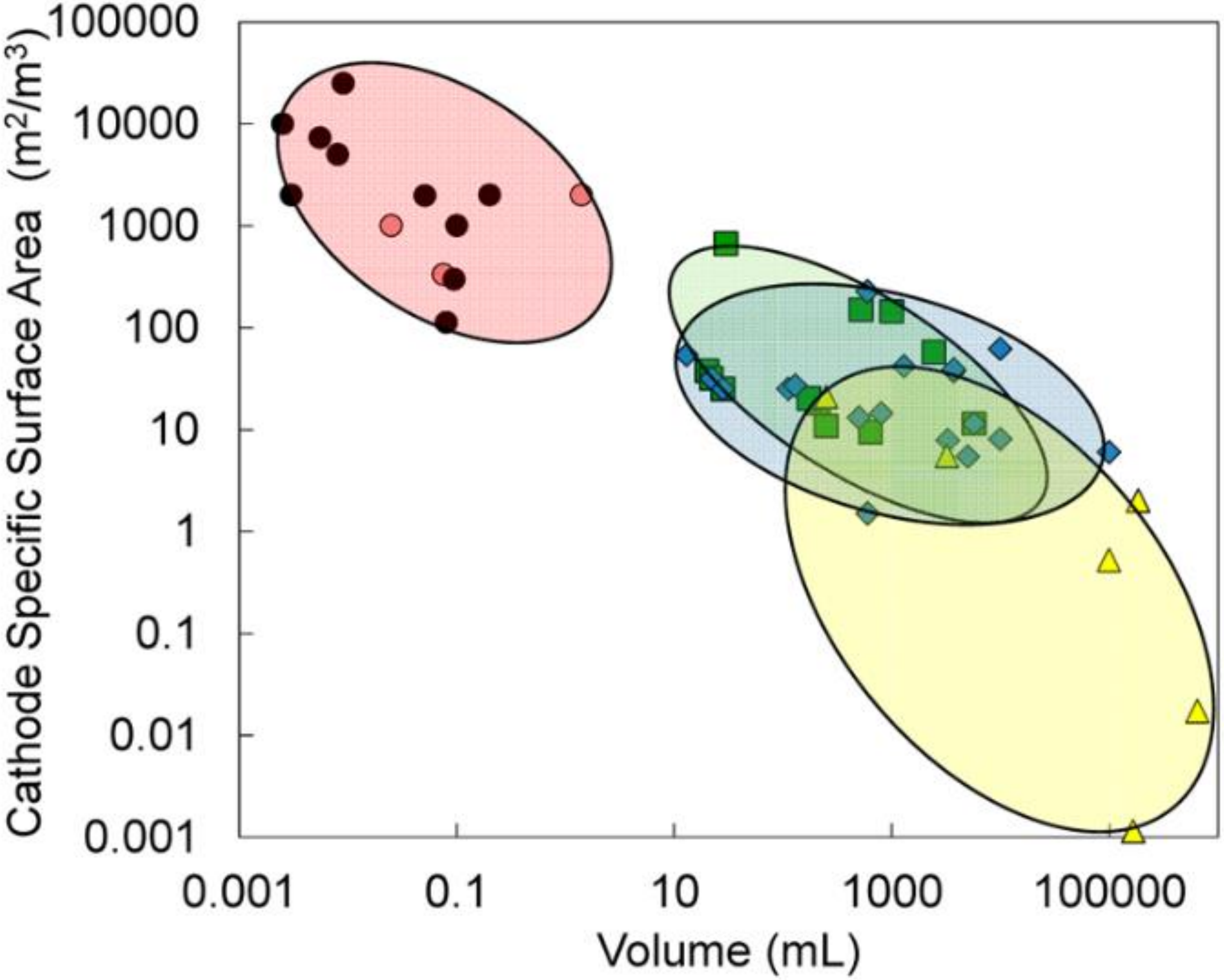


Standard ME cell

Current Density Requires High Accessible Electrode Surface Area

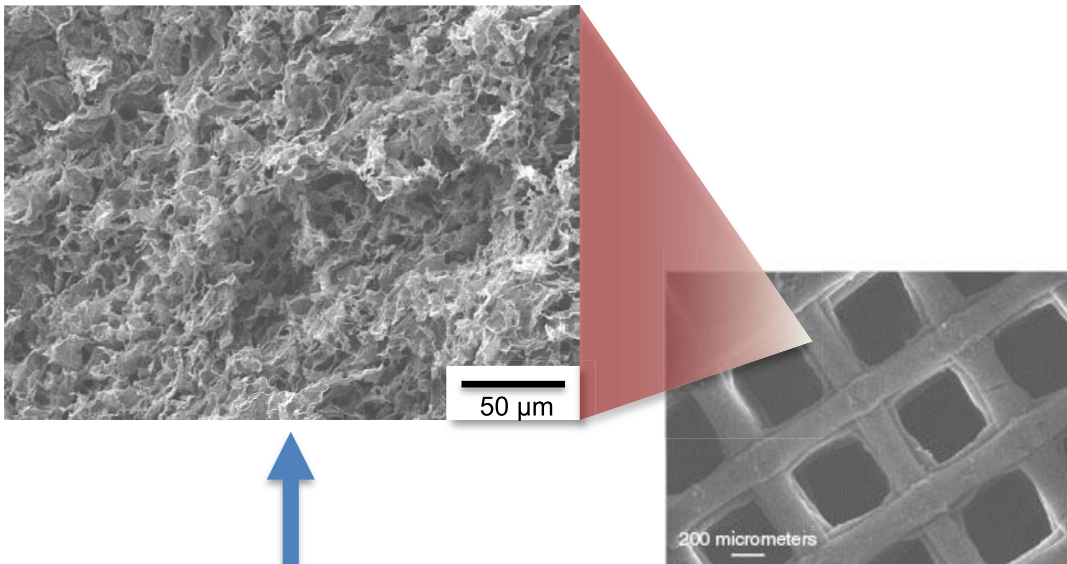
Logan, B. et al. Environ. Sci. Technol. Lett., 2015, 2 (8), pp 206–214

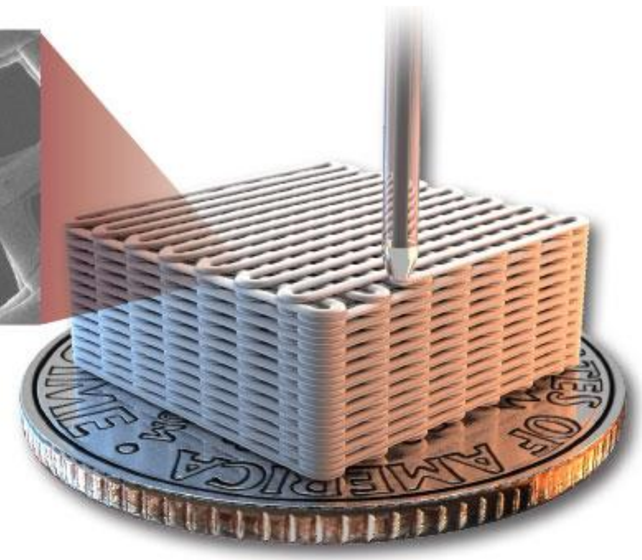
Standard Electrode Materials Difficult to Scale while Maintaining Surface Area



Logan, B. et al. Environ. Sci. Technol. Lett., 2015, 2 (8), pp 206–214

Opportunity: Printed aerogels have hierarchical, scalable surface area; Enzymes can be used for charge transfer



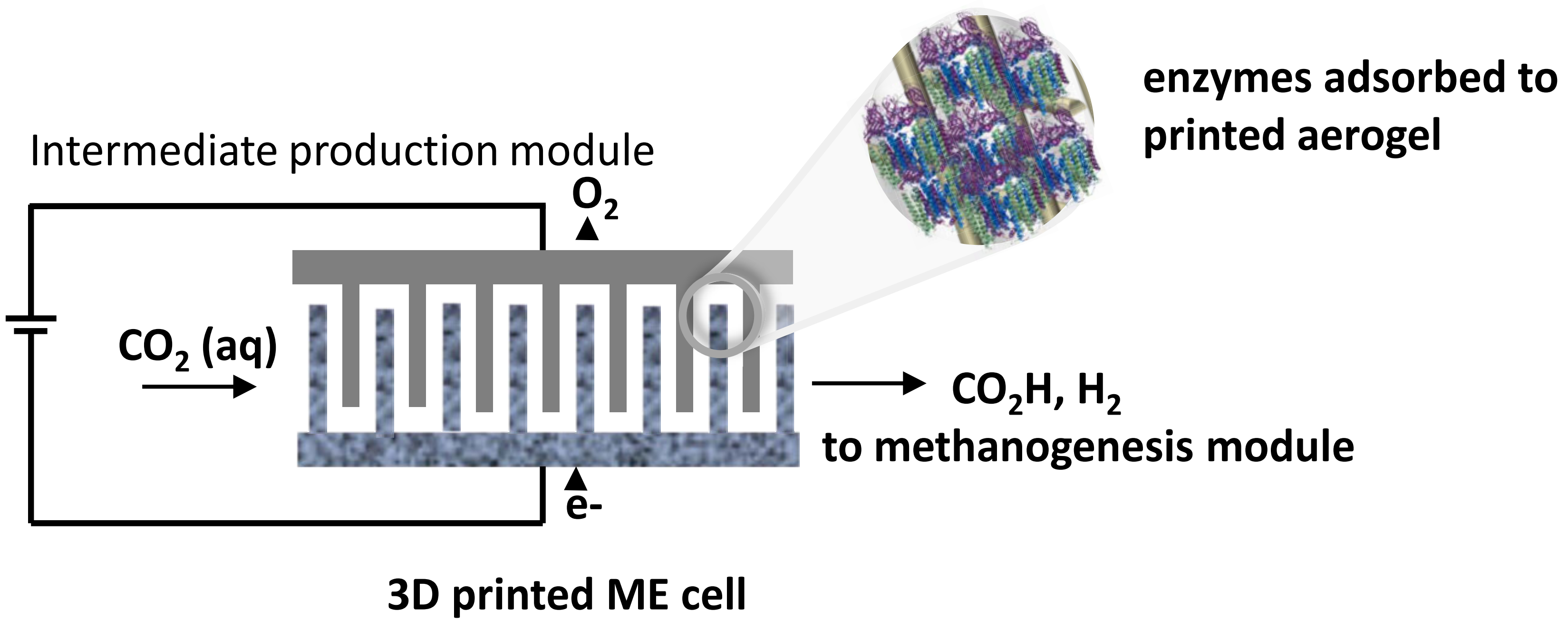


Small pores for enzyme absorption for electron transfer

Larger pores for whole microbes and/or nutrient transfer/mixing

Spormann, A. M. et al. Extracellular Enzymes Facilitate Electron Uptake in Biocorrosion and Bioelectrosynthesis. *mBio* **6,** e00496–15 (2015).

Unique Cell Designs are Available Which Increase Current Density and Decrease Diffusion Distances



Research Needs:

- **Economics, Modeling & Scaling: What is the price of the surface area?**
- **Highly Stable Enzymes (months of operation)**
- **Reducing Equivalents/Cofactors (Elimination/recycling/cheaper alternatives)**
- **Deep understanding of enzyme kinetics and material permeability**

Unprecedented Control in Enzyme Engineering and Materials Synthesis → Rational Design of Biocatalytic Materials and Reactors

- **Small Scale, Modular, Higher Process Intensity**