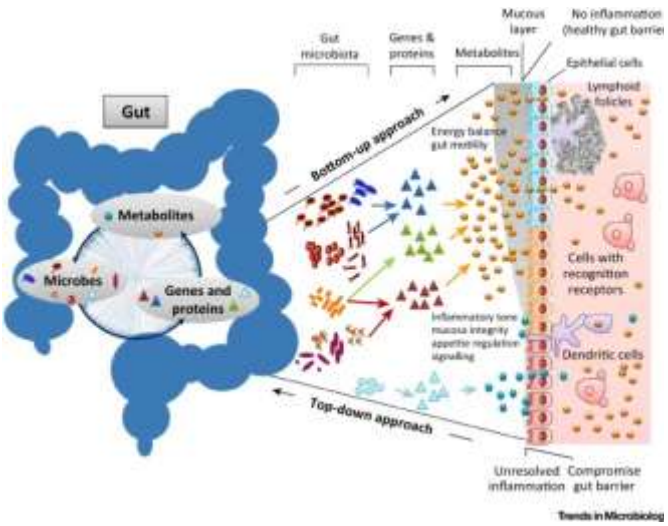
Communicating with and controlling biology via biofabrication, synthetic biology, and microelectronics

William E. Bentley

Robert E. Fischell Institute for Biomedical Devices
Institute for Bioscience and Biotechnology Research
Fischell Department of Bioengineering
Department of Chemical and Biomolecular Engineering

NSF Nanoscale Science and Engineering Grantees Conference , Alexandria, VA, 2018

Grand Challenges – building better medicines & new methods for discovery



1794 Diabetes Volume 64, May 2015

Franklin F. Duan, Joy H. Liu, and John C. March

CrossMark

Engineered Commensal Bacteria Reprogram Intestinal Cells Into Glucose-Responsive Insulin-Secreting Cells for the Treatment of Diabetes

Diabetes 2015;64:1794–1803 | *DOI: 10.2337/db14-0635*

John March
Cornell

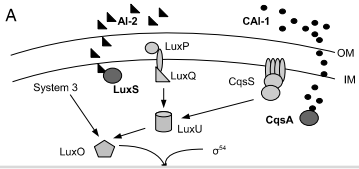
Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model

Faping Duan and John C. March[1]

Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853

Edited by Rita R. Colwell, University of Maryland, College Park, MD, and approved May 13, 2010 (received for review February 1, 2010)

**To investigate the possibility of using commensal bacteria as signal mediators for inhibiting the disease cholera, we stably transformed *Escherichia coli* Nissle 1917 (Nissle) to express the autoinducer molecule cholera autoinducer 1 (CAI-1) (shown previously to prevent virulence when present with another signaling molecule, autoinducer 2, at high concentrations) and determined the effect on *Vibrio cholerae* virulence gene expression and colonization in an infant mouse model. We found that pretreatment of mice for 8 h with Nissle engineered to express CAI-1 (Nissle-cqsA) greatly increased the mice's survival (92%) from ingestion of *V. cholerae*. Pretreatment with Nissle-cqsA for only 4 h increased survival by 77%, whereas ingesting Nissle-cqsA at the same time as *V. cholerae*



Molecular Systems Biology 7; Article number 521; doi:10.1038/msb.2011.55
Citation: *Molecular Systems Biology* 7:521

www.molecularsystemsbiology.com

molecular systems biology

Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen

Nazanin Saeidi[1], Choon Kit Wong[1], Tat-Ming Lo, Hung Xuan Nguyen[2], Hua Ling, Susanna Su Jan Leong, Chueh Loo Poh* and Matthew Wook Chang*

School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore
[1] These authors contributed equally to this work

Matthew Chang
NUS

Molecular Systems Biology 9; Article number 636; doi:10.1038/msb.2012.71
Citation: *Molecular Systems Biology* 9:636
www.molecularsystemsbiology.com

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molecular systems biology

Autonomous bacterial localization and gene expression based on nearby cell receptor density

Hsuan-Chen Wu[1,2], Chen-Yu Tsao[1,2], David N Quan[1,2], Yi Cheng[3], Matthew D Servinsky[4], Karen K Carter[2,5], Kathleen J Jee[1], Jessica L Terrell[1,2], Amin Zargar[2,5], Gary W Rubloff[3], Gregory F Payne[1,2], James J Valdes[6] and William E Bentley[1,2,5,*]

[1] Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA, [2] Institute for Bioscience and Biotechnology Research, University of Maryland, College Park, MD, USA, [3] Department of Material Science and Engineering, University of Maryland, College Park, MD, USA, [4] Sensors and Electron Devices Directorate, US Army Research Laboratory, Adelphi, MD, USA, [5] Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD, USA and [6] US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, USA
* Corresponding author. Institute for Bioscience and Biotechnology Research, University of Maryland, 5115 Plant Sciences Building, College Park, MD 20742, USA. Tel.: +1 301 405 4321; Fax: +1 301 405 9953; E-mail: bentley@umd.edu

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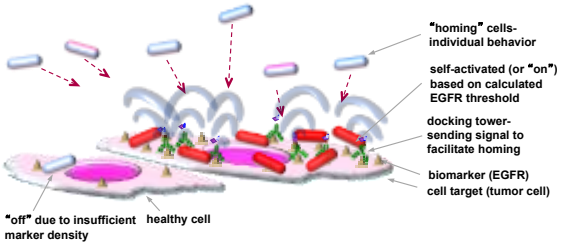
Hsuan-Chen Wu
NTU



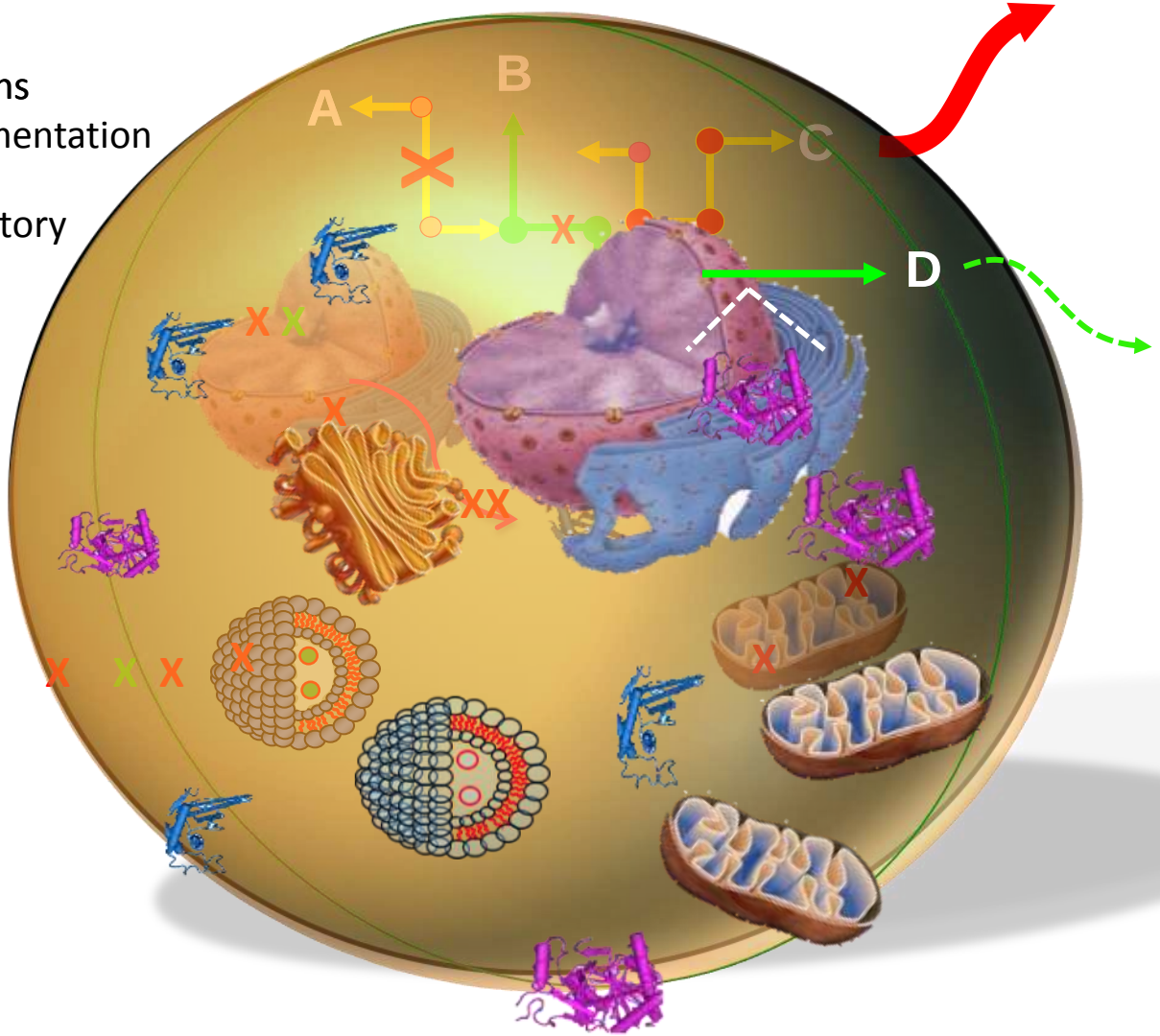
Cell Engineering

Synthetic Biology & Metabolic Engineering

Targeted mutations
& transgene augmentation

Pathway & regulatory
Engineering

Whole genome
engineering



Cell Engineering

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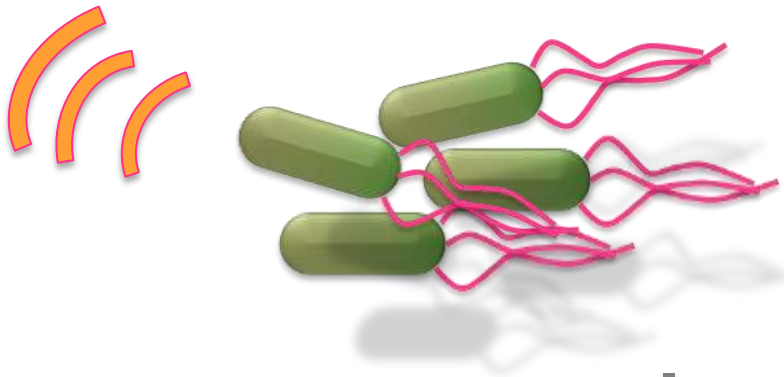
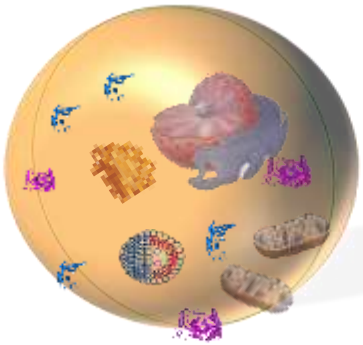
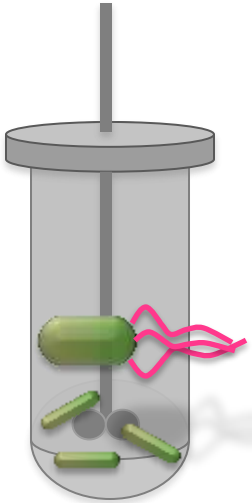


To find right cells, we employ methods
that survey increasingly larger populations and
ultimately select based on one or two traits:



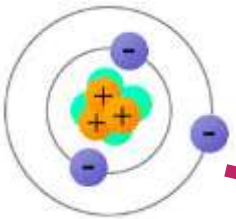
e.g., productivity &
tolerance



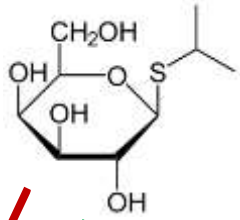
We bound phenotypic
“noise” that stems from
the heterogeneous cues

Cell Engineering

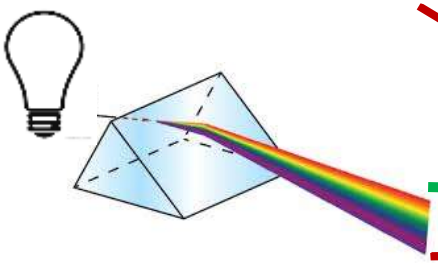
Synthetic Biology & Metabolic Engineering

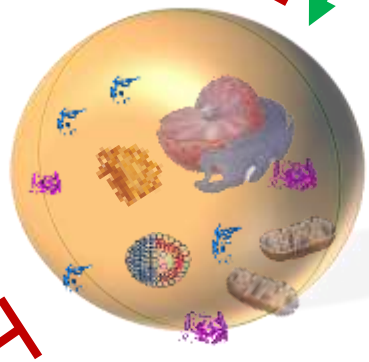


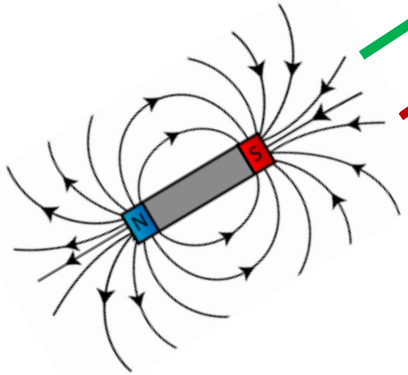
Spatiotemporally control stimulation to engineered (permissive) cells



e^-







…and while orthogonality is attractive, circuits & systems converge

Biofabrication

The use of biological components, biological means, or mimics of biological processes for synthesis or assembly.

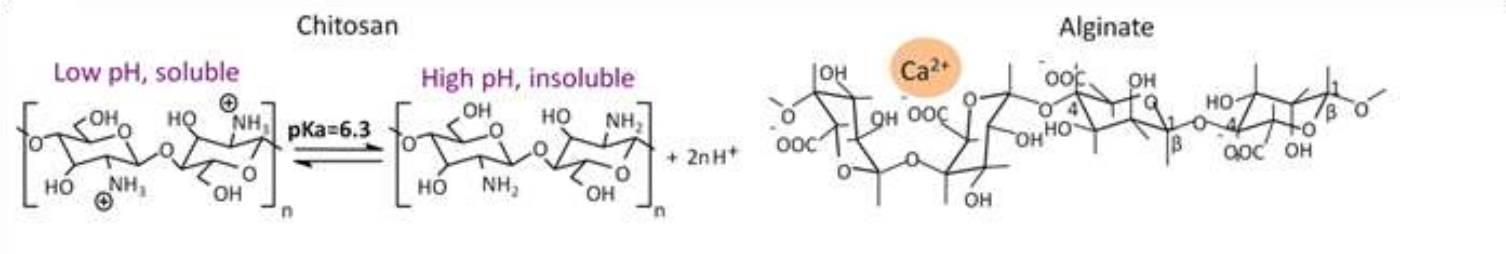
Stimuli-respor
assembly

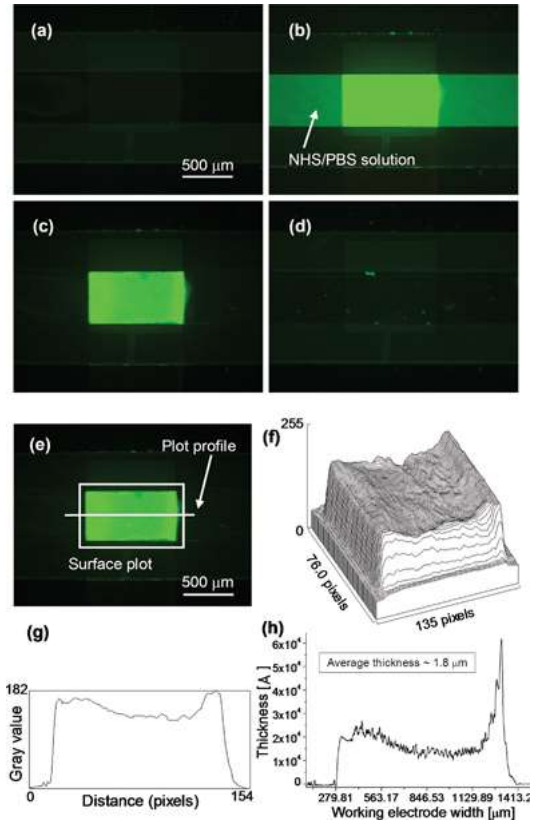
Enzymatic as

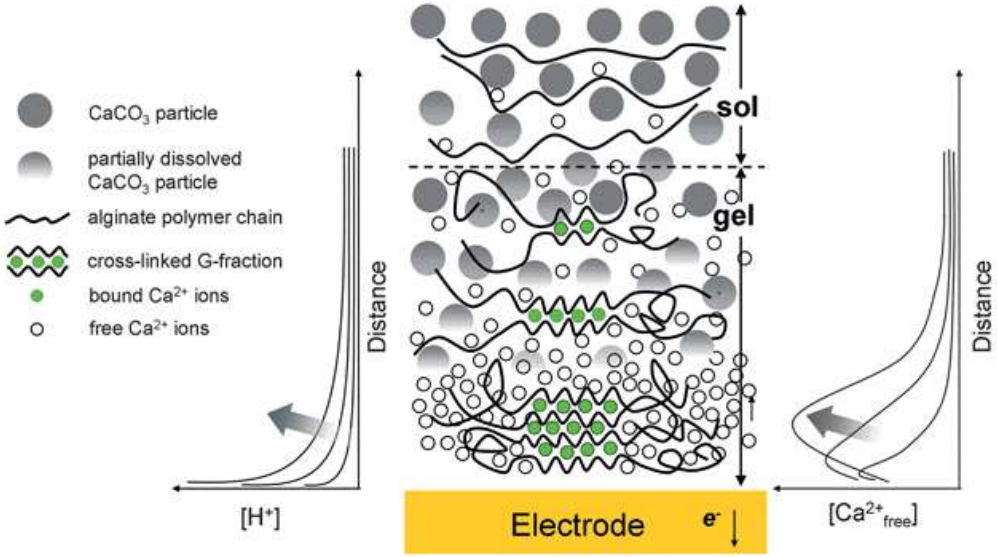
Self assembly

Stimuli-responsive Assembly

pH, temperature, pressure, light, etc.

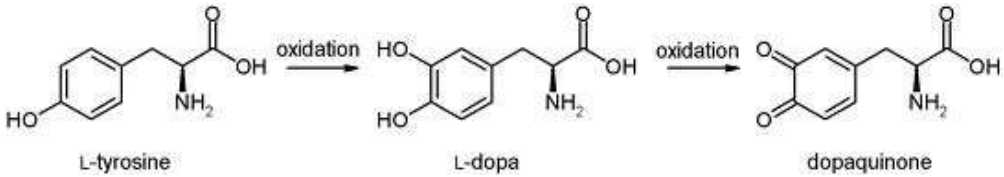


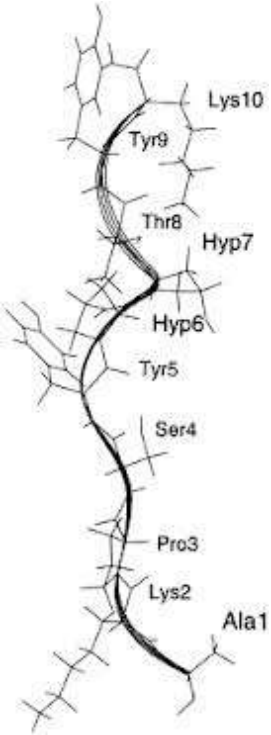


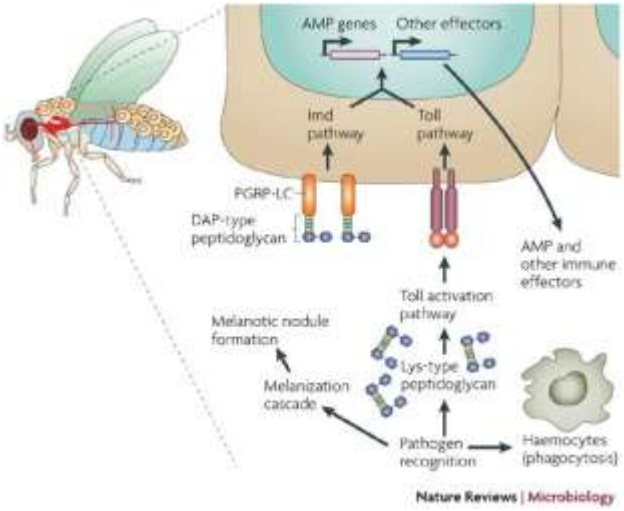


Biofabrication Enzymatic Assembly

phenoloxidase, tyrosinase

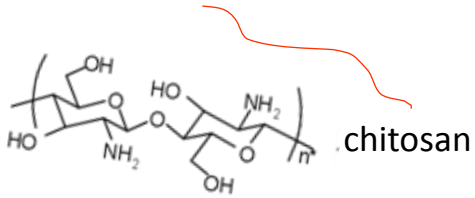


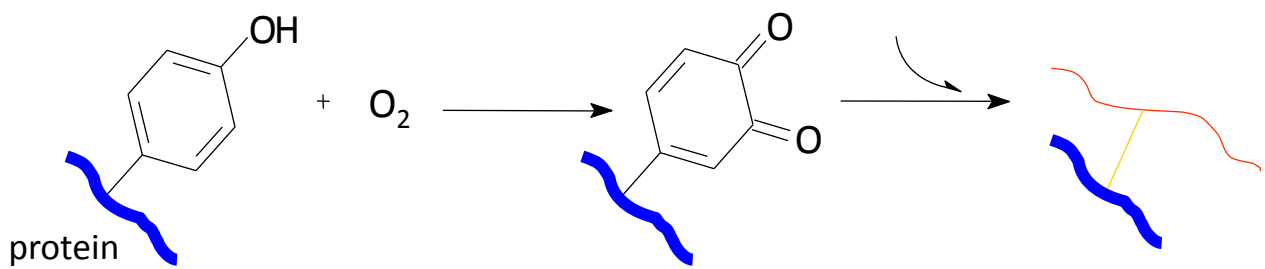




Enzymatic Assembly - tyrosinase to construct protein-chitosan conjugates

"Pro-tag" w/ 5 tyrosyl (Y_5) residues





"activated" protein

protein-chitosan conjugate

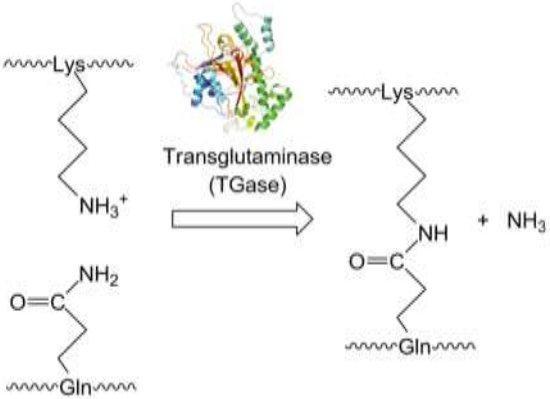
green fluorescent protein – chitosan conjugate

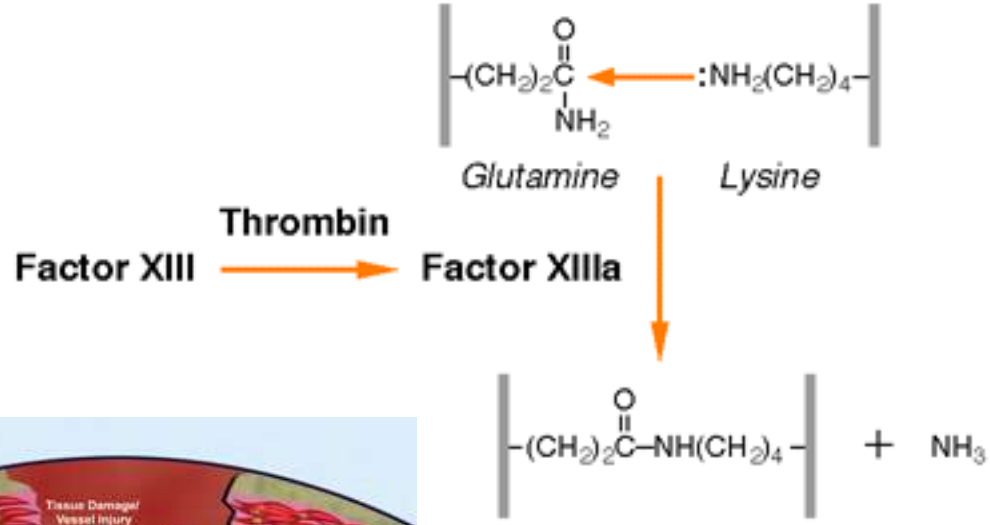
***Biopolymers* 64: 292 (2002); *Biomaterials* 24: 2831 (2003); *Biotech. Bioeng*. 93:1207. (2006)**

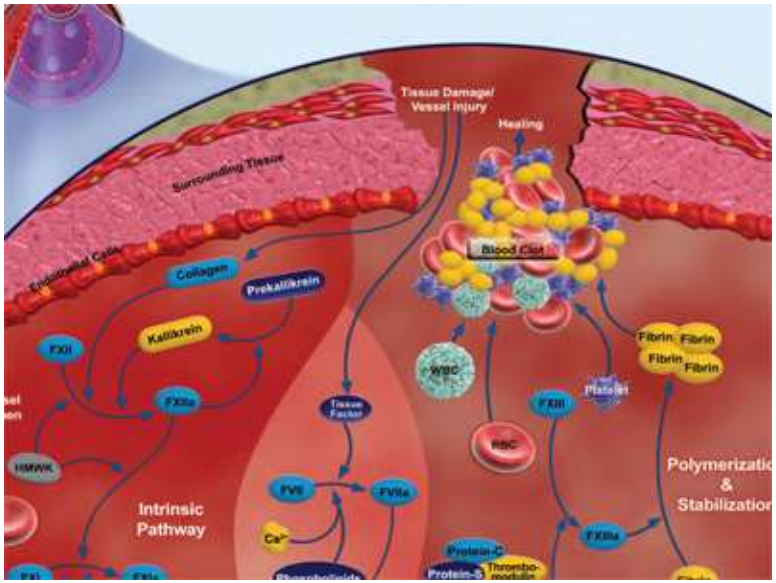
Biofabrication

Enzymatic Assembly

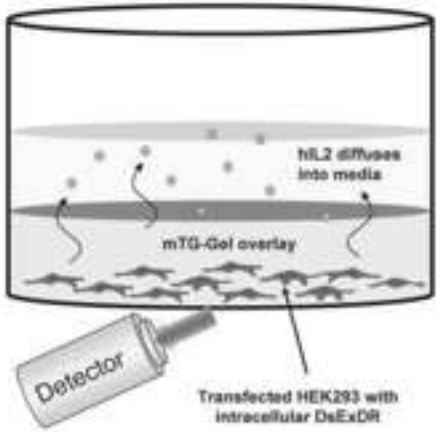
transglutaminase

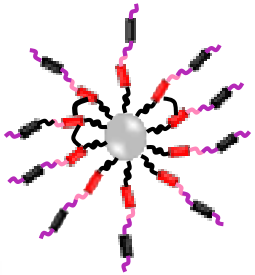


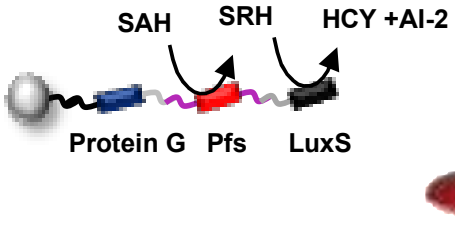


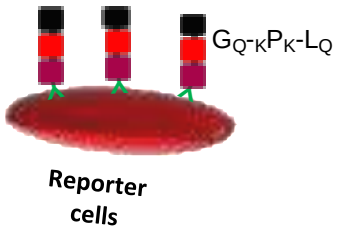


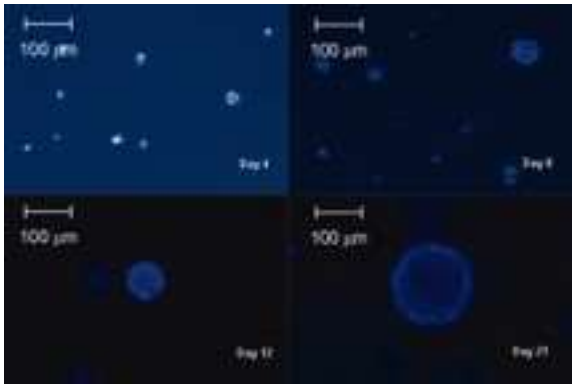
Transglutaminase to construct tissue engineering scaffolds & protein-peptide structures







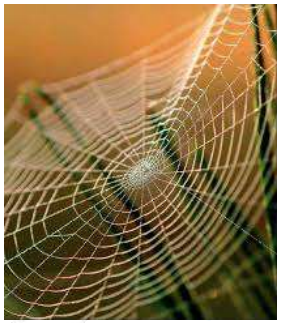




Yung, C.W., *et al.* , *J. Biomed. Mat. Res. A.* (2007)
Yung, C.W., *et al*., *J. Biomed. Mat. Res. A.* (2010)
Yung, C.W., *et al*., *Met. Eng*. (2006)
Yung, C.W., *et al*., *Biotech. Bioeng*. (2006)

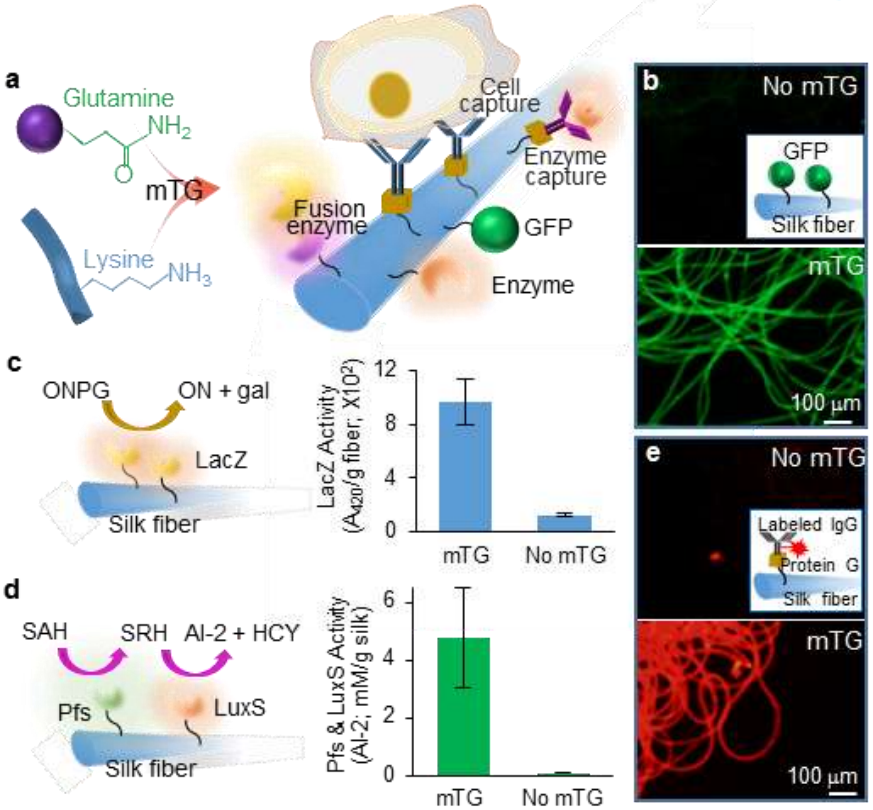
Bhokisham, N., *et al., Met. Eng.* (2016)
Bhokisham, N., *et al., Cell Mol. Bioeng*. (2017)

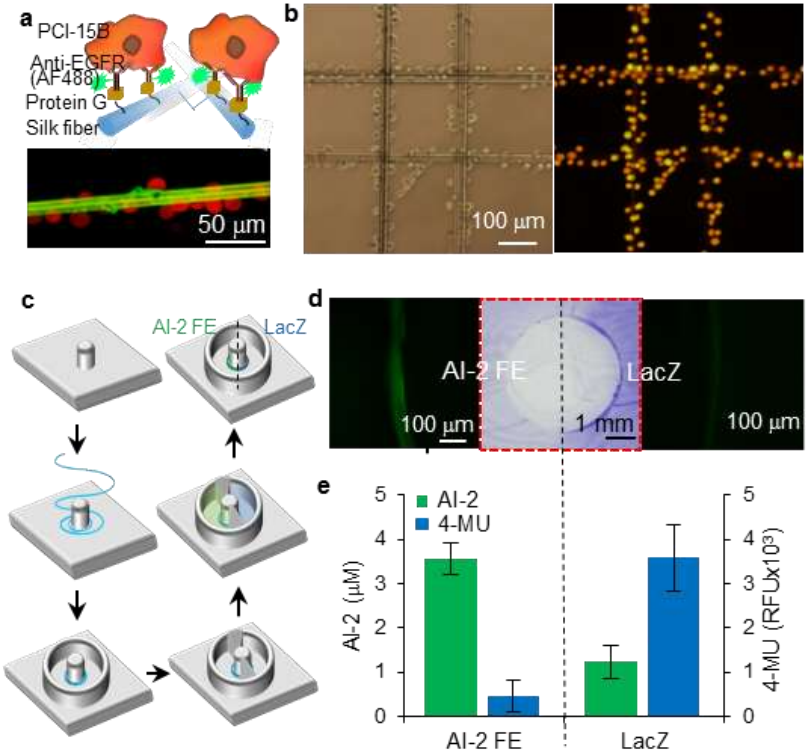
Facile assembly of cells and proteins …



spider silk

tyrosinase and transglutaminase

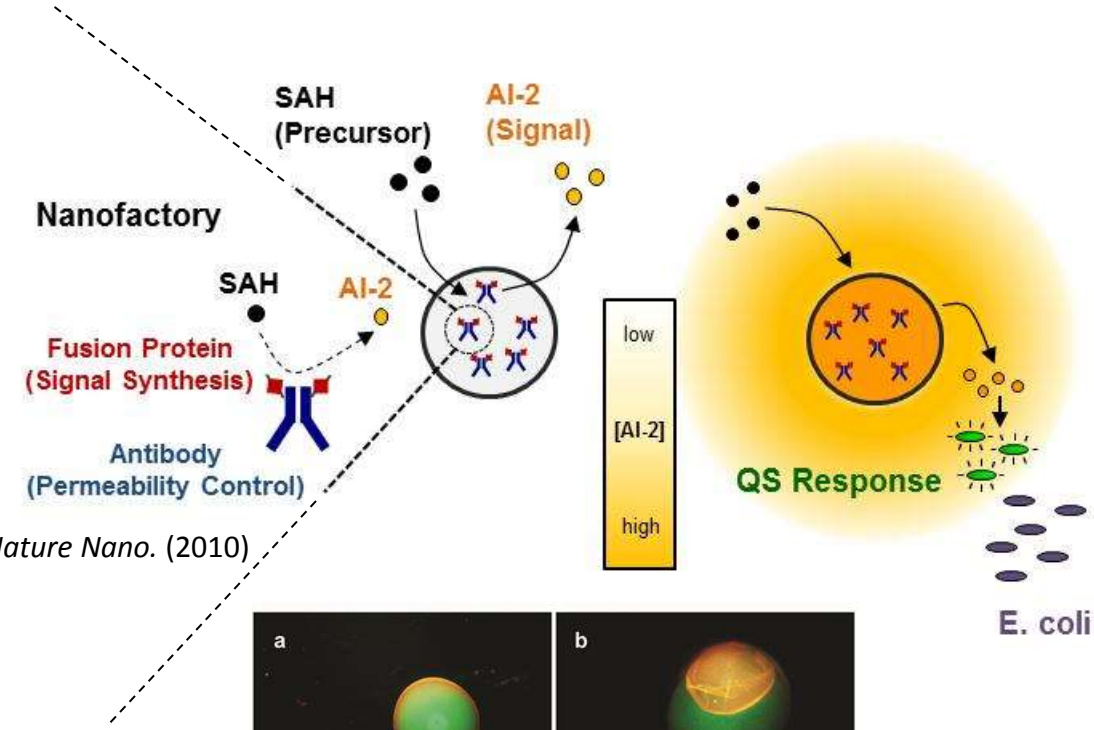




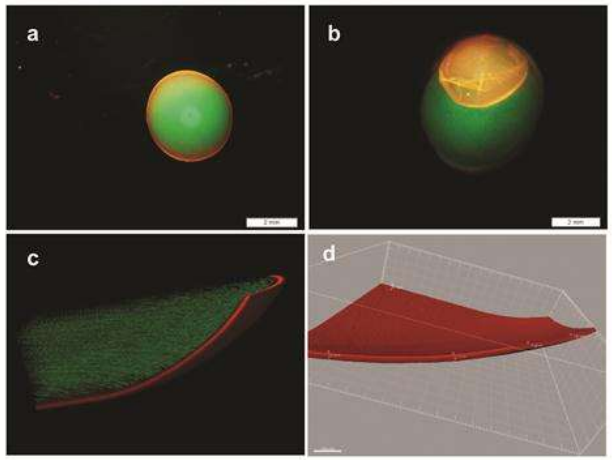
Wu et al., Biotech. Bioeng., 2017; Bhokisham et al., Met. Eng., 2016

Creating "artificial cells" for guiding bacteria …

Apoorv Gupta



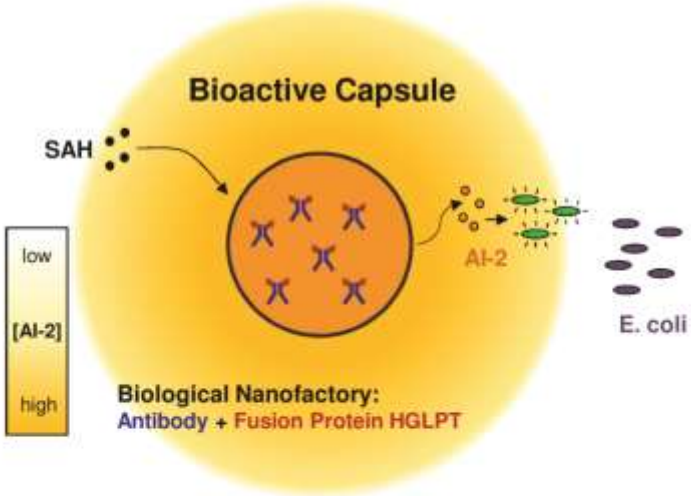
Fernandes, R., *et al., Nature Nano.* (2010)

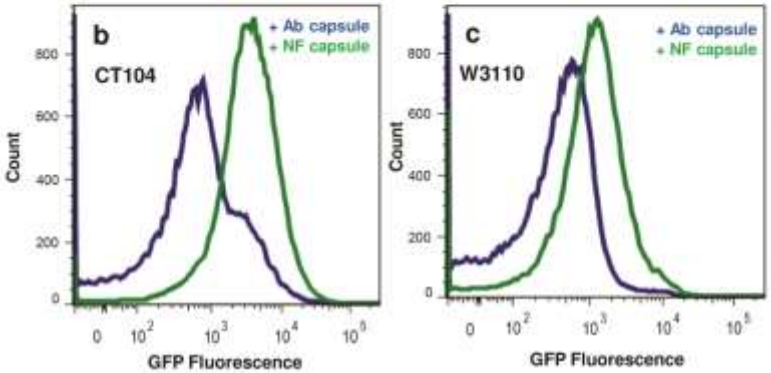


Apoorv Gupta

Gupta, A., et al., *Biotech. Bioeng*. 2012

Can "artificial cells" modulate the microbiome?





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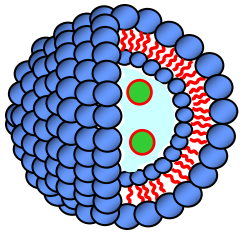
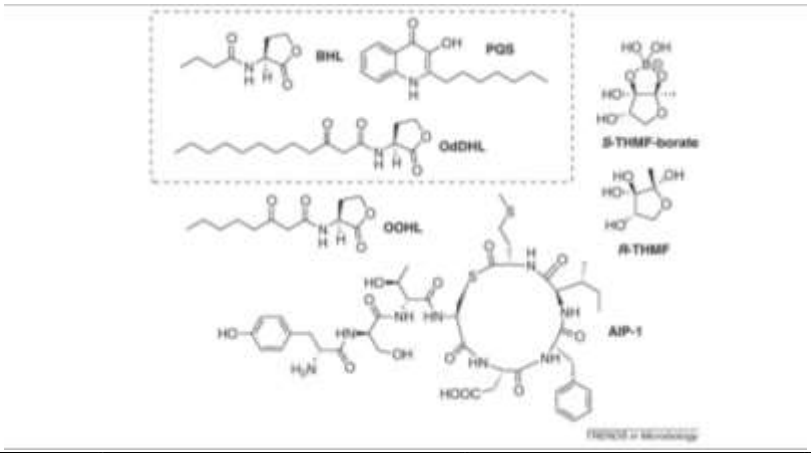
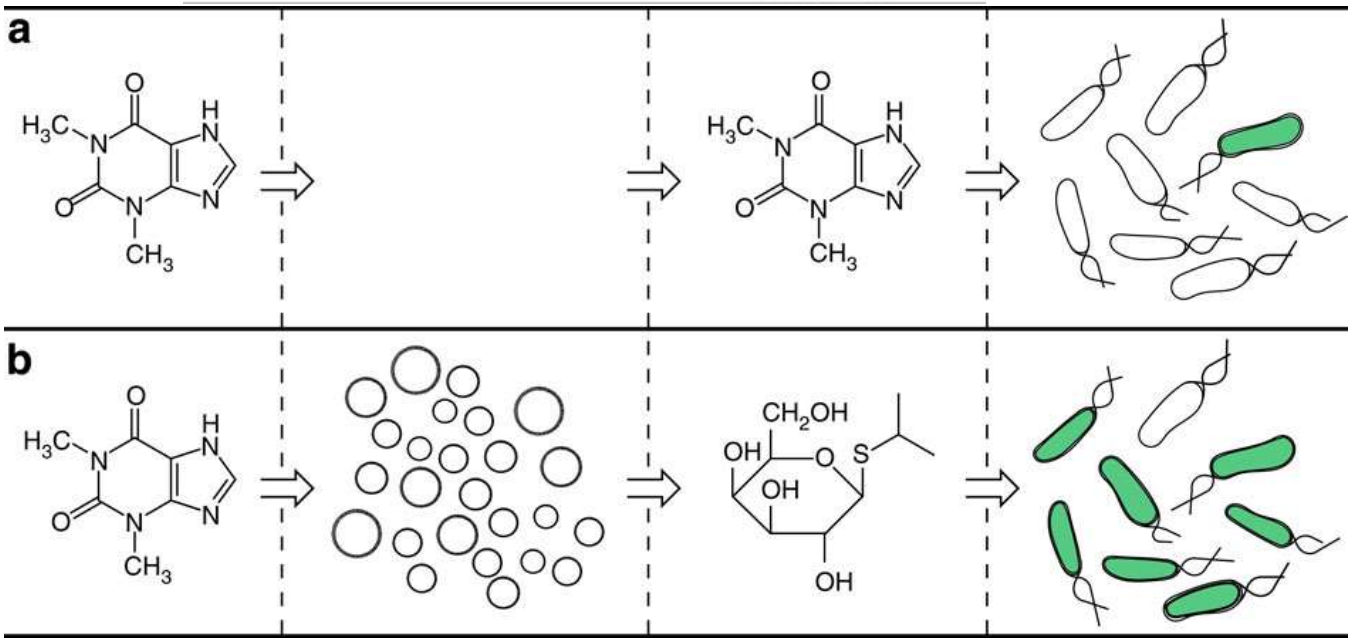
Enzyme loaded capsules

Gupta, A., et al., *Biotech. Bioeng*. 2012

“Artificial” cells with more capability….

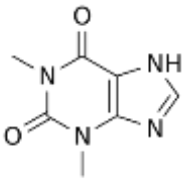


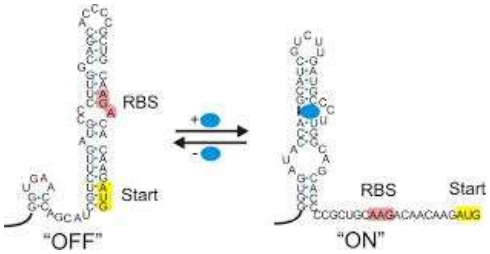


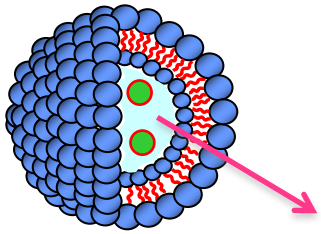


Lentini et al. (2014) *Nature Communications*

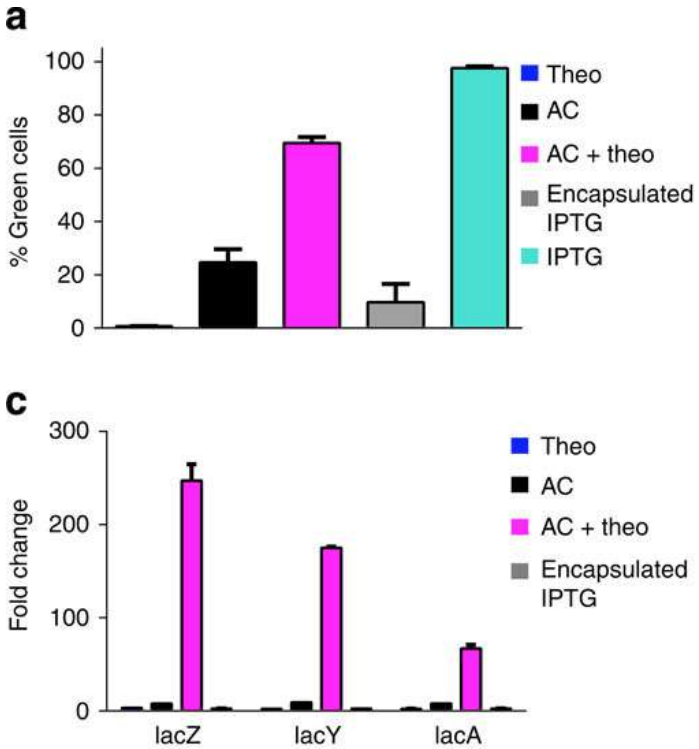
“Artificial” cells with more capability….

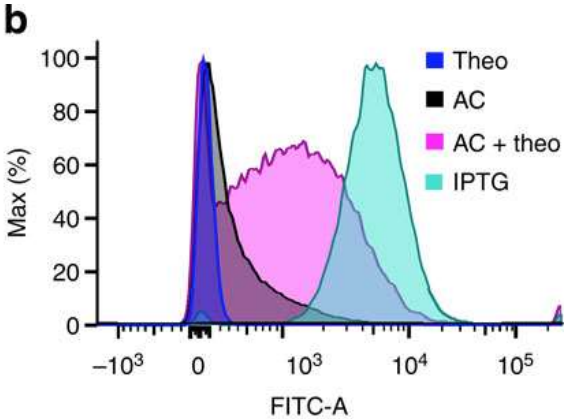


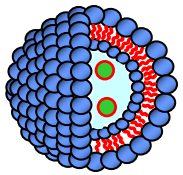




1. Expression of αHL
2. Haemolysis
3. Release of IPTG



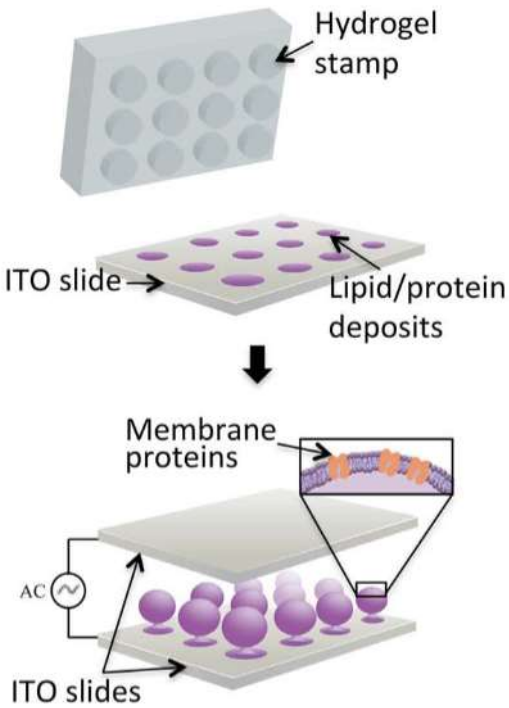




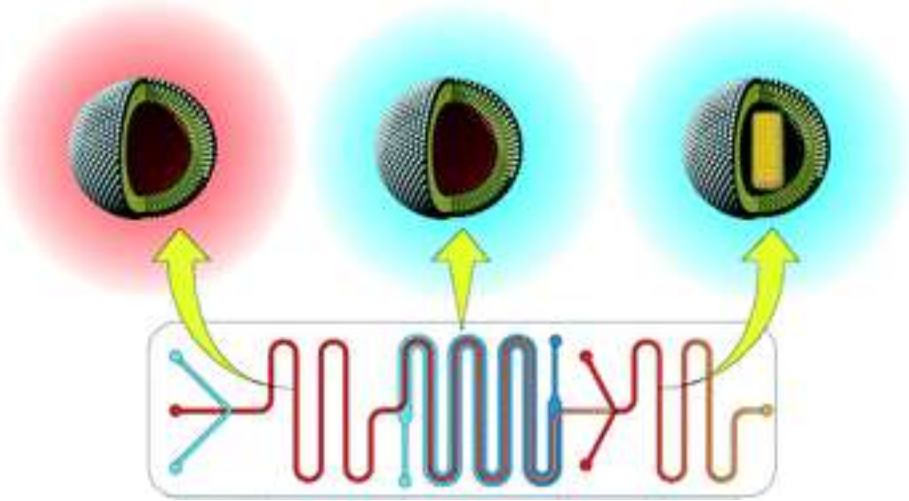
Roberta Lentini **Jessica Terrell**

Lentini et al. (2014) *Nature Communications*

Organizing Structure - Biofabrication and Assembly….

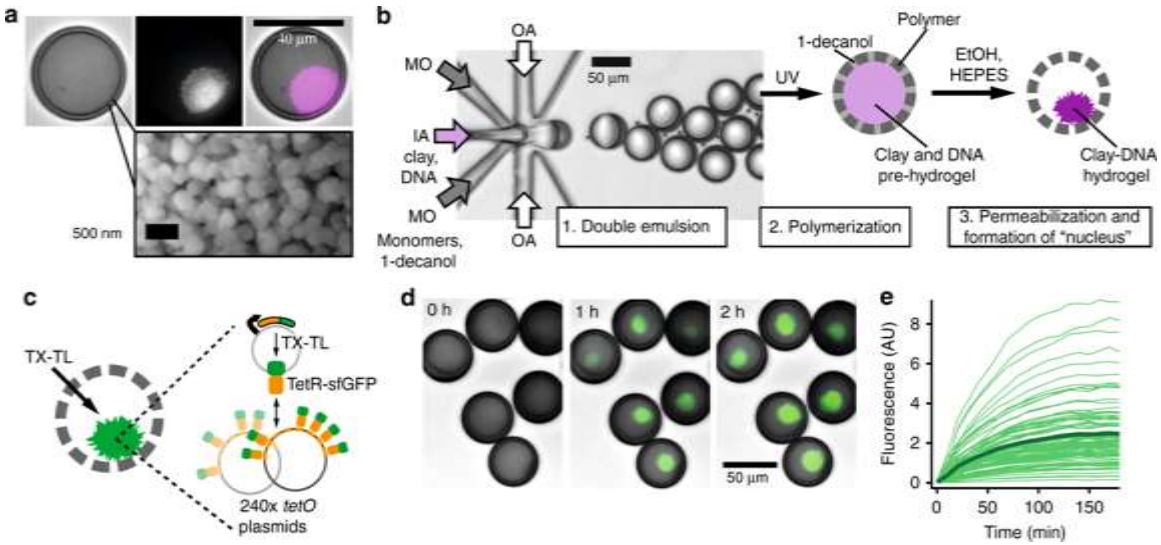


Kang, Wostein, Majid, Adv. Mat., 2013.



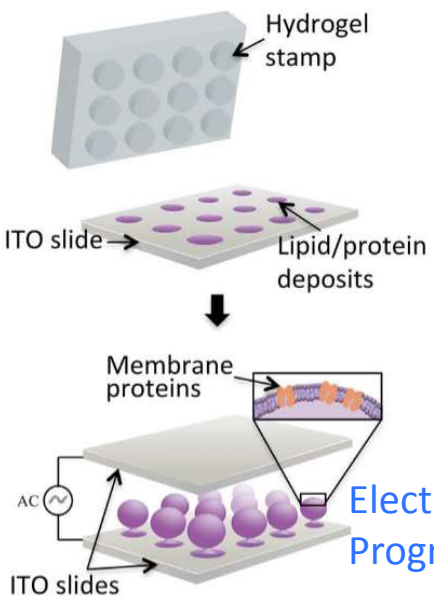
Hood, Vreeland, & Devoe, Lab Chip, 2014

Organizing Structure - Biofabrication and Assembly….



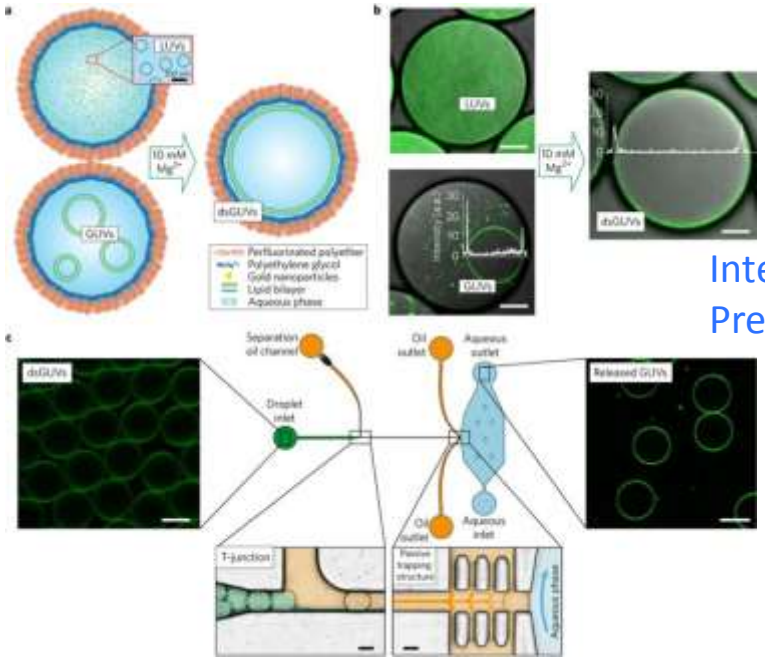
Clay core: Pseudo-nucleus – enables protein display and signaling

Guan et al., eLIFE, 2018



Electroassembly: Programmable

Kang, Wostein, Majid, Adv. Mat., 2013.

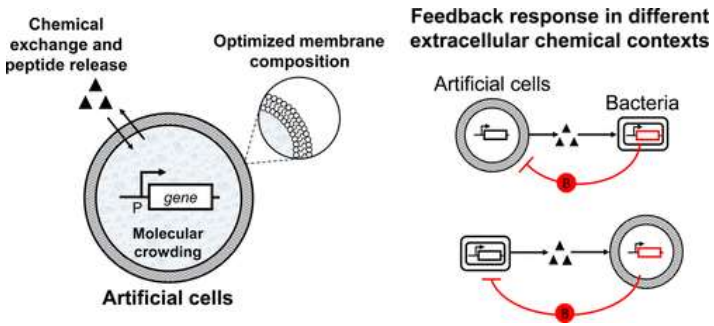


Integrated Hydrogel: Preserves bilayers

Weiss et al., Nat. Mat., 2017

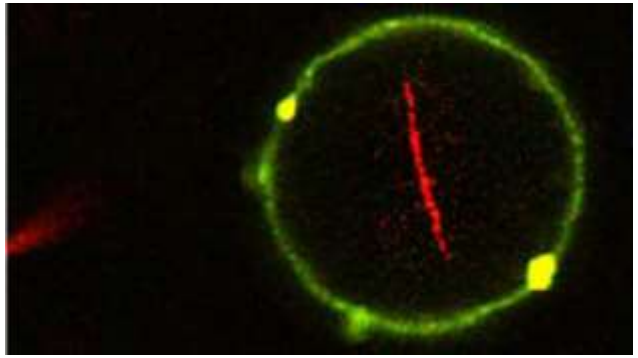
Organizing Structure – Enabling New Function….

Relieving context



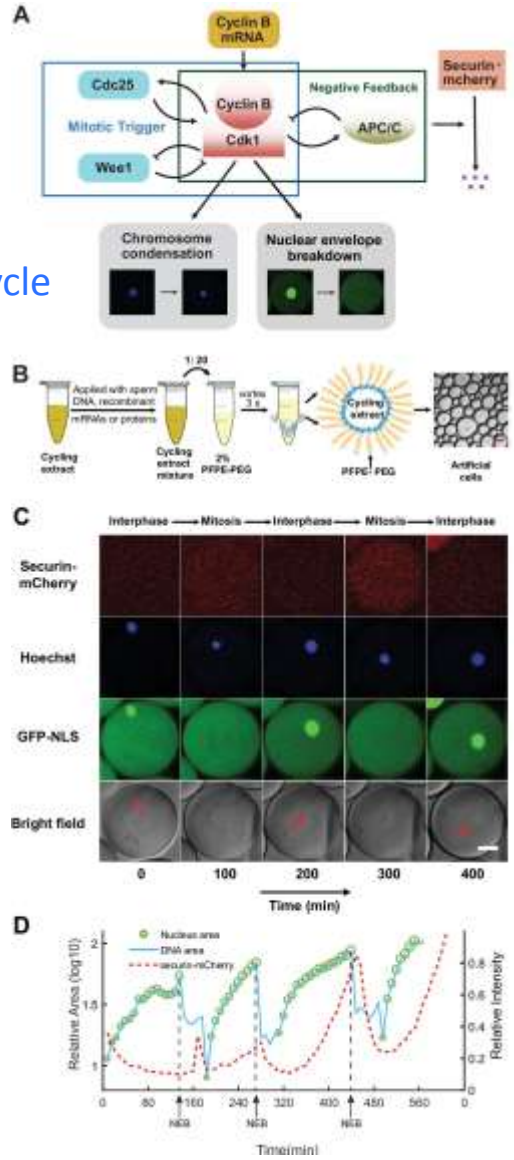
Ding, Tan, et a.., ACS Appl. Mater. & Interfaces, 2018

Embedding functions



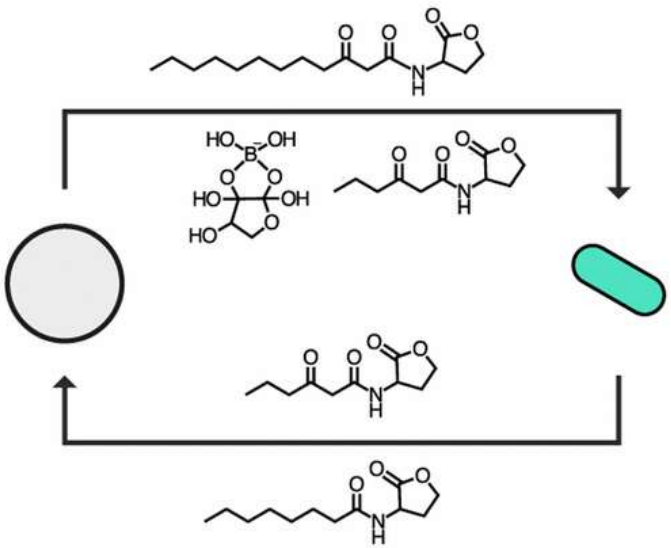
Weiss et al., Nat. Mat., 2017

Adding cell cycle



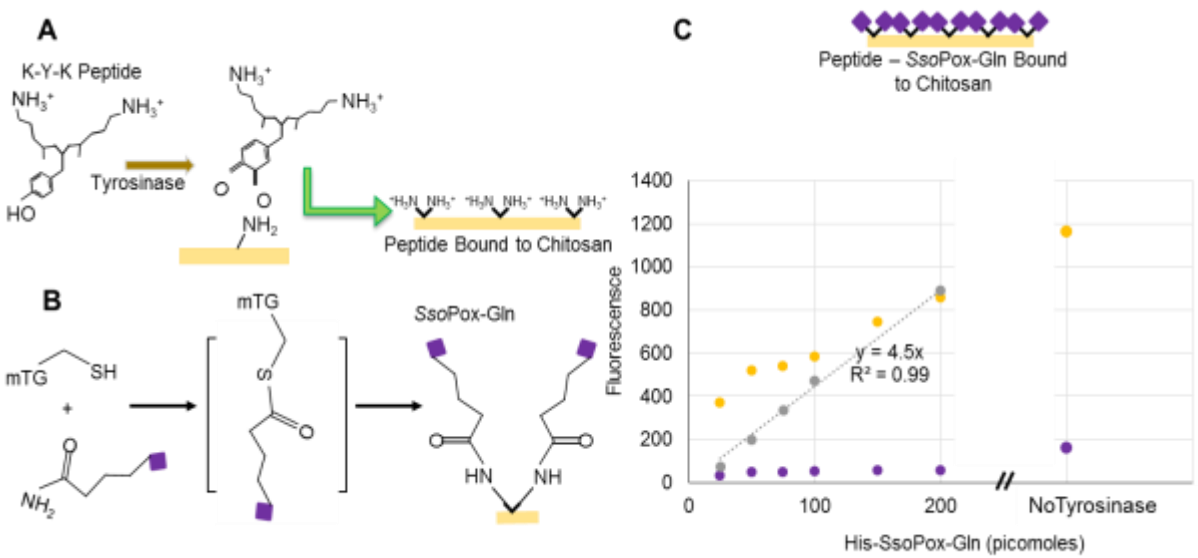
Guan et al., eLIFE, 2018

Organizing Structure – Enabling New Function….



Enabling Crosstalk:
Connecting cells, Turing test

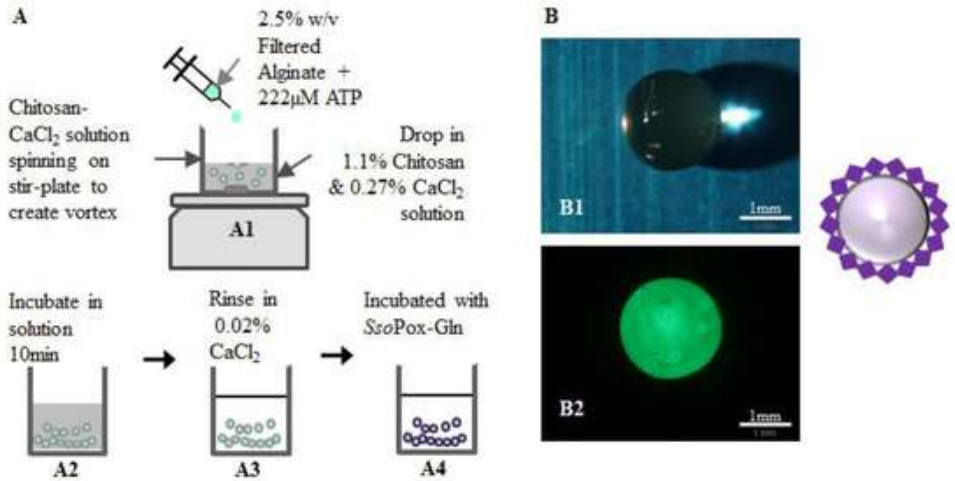
Lentini, Mansy, etal., Central Science

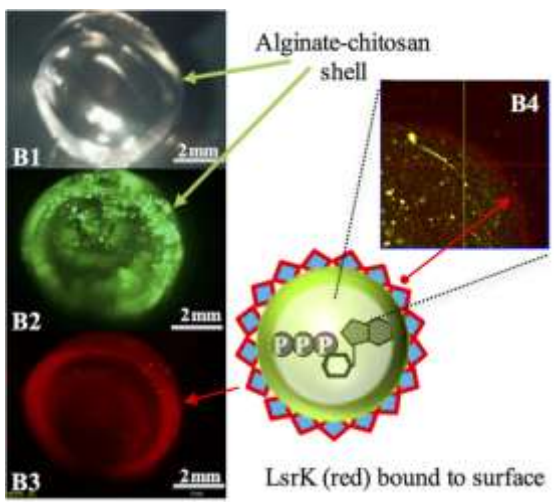


Grafting Enzymes:
Quorum Quenching

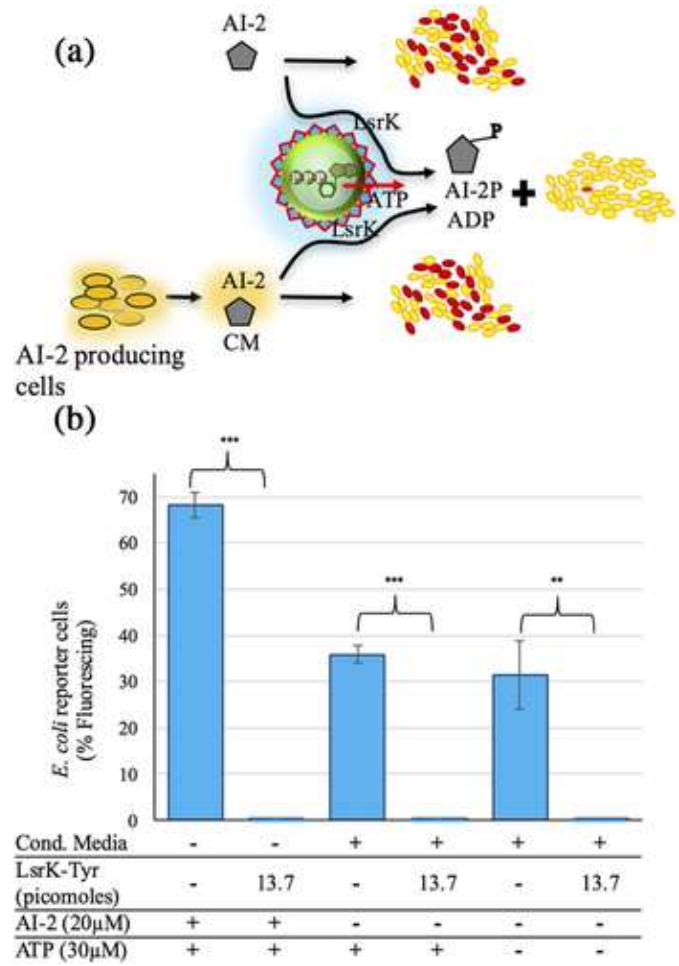
Rhoads, Bentley, et. al., Biot. Bioeng., 2018
Rhoads, Bentley, et. al.., Molecules, 2018

Organizing Structure – Enabling New Function….





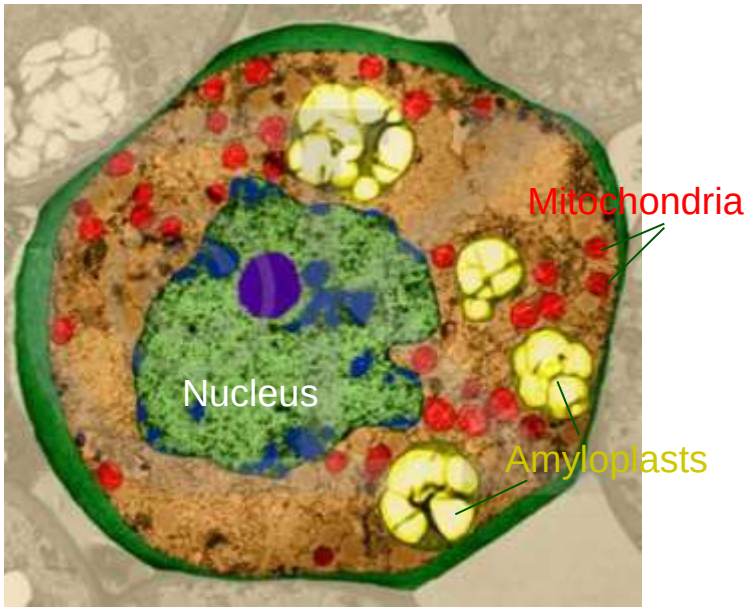
Grafting Enzymes & Embedding Substrates: Quorum Quenching



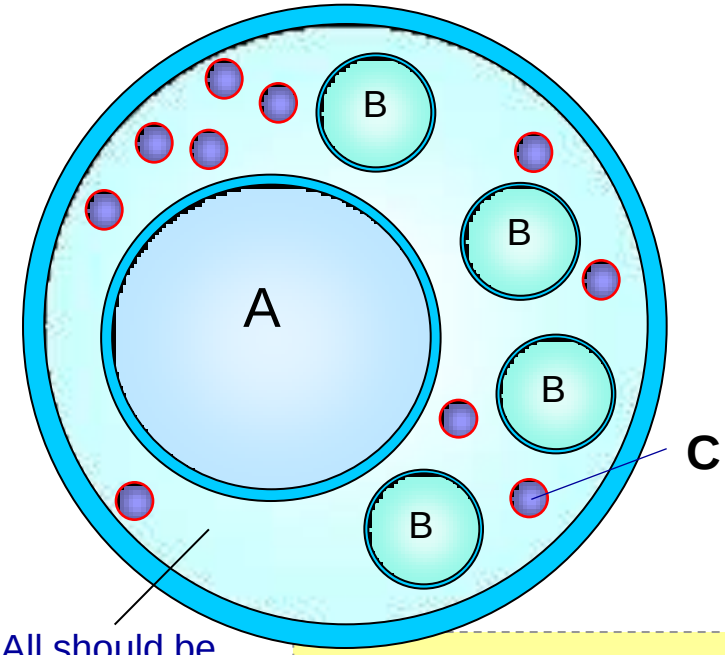
Rhoads, Bentley, etal., Biot. Bioeng., 2018
Rhoads, Bentley, et.a.., Molecules, 2018

Organizing Structure – Functional compartments?

Eukaryotic cells have internal compartments



10 to 100 μm

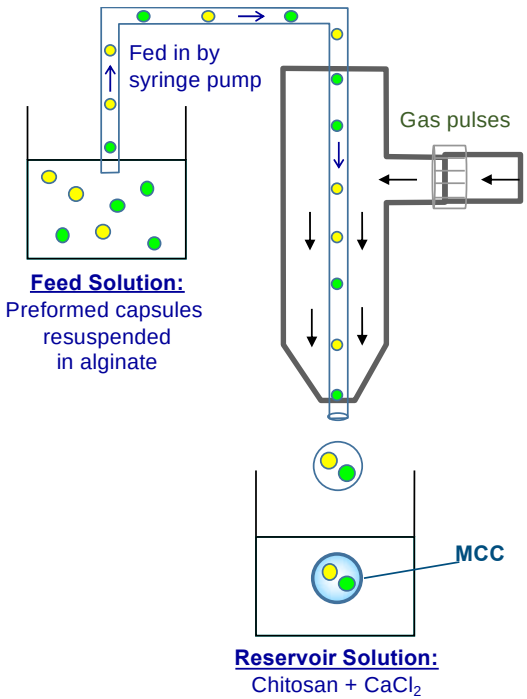


All should be aqueous at normal pH, ionic strength

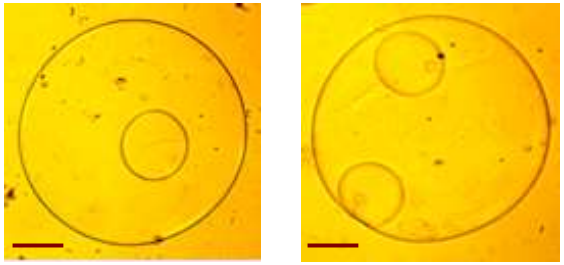
Should be able to control the contents of each type of compartment

Organizing Structure – Functional compartments?

Lysosome?
Vacuoles?
Mitochondria?

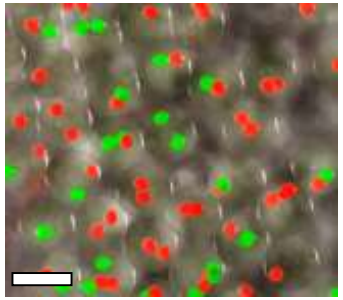


MCCs with different numbers of compartments

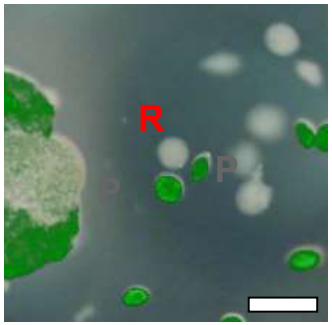


1 compartment 2 compartments

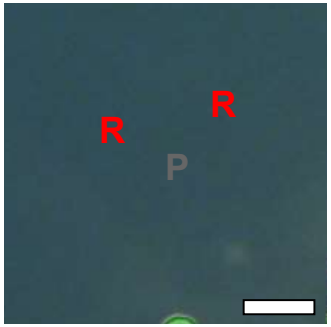
MCCs with distinct contents in each compartment



2 Producers, 1 Reporter



1 Producer, 2 Reporters



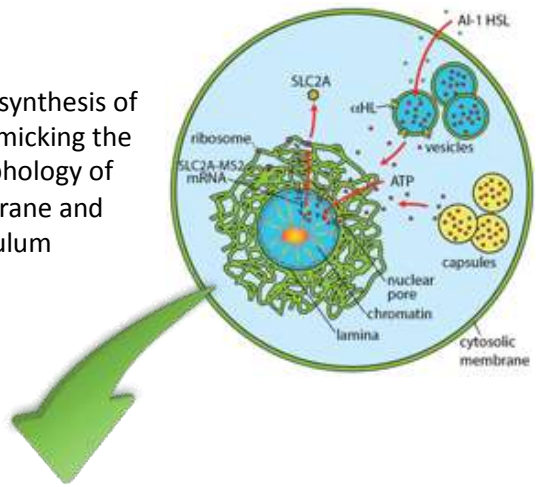
Lu, Oh, Terrell, Bentley, Raghavan*, "A new design for an artificial cell." *Chemical Science*, 2017, *8*, 6893

Organizing Structure – Functional compartments?

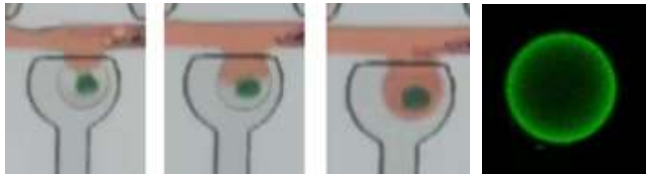
Nuclear Membrane?
Endoplasmic Reticulum?

Cell Biogenesis?

Goal: controllable synthesis of lipid structures mimicking the function and morphology of the nuclear membrane and endoplasmic reticulum



Precise GUV formation in a microfluidic trap

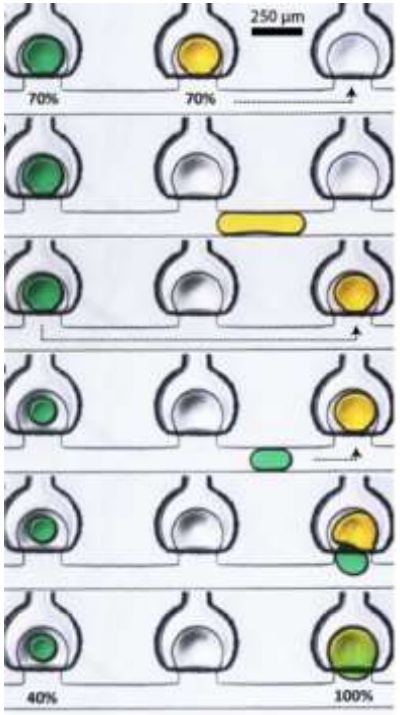


Modification of GUV morphology by chelation with charge-interacting lipids

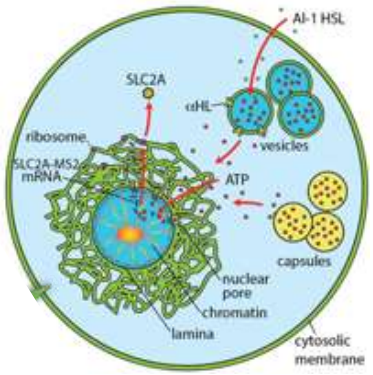
DMPC:PI 9:1

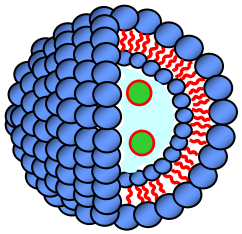
+EDTA (0 min)

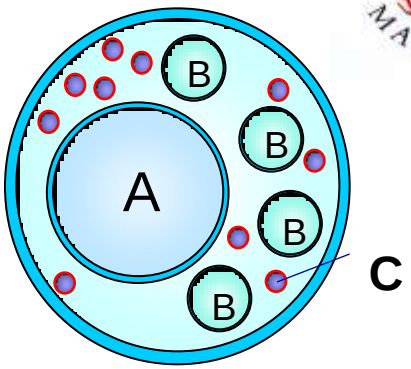
+EDTA (10 min)



Conclusions







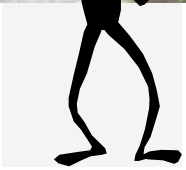
We would like to recognize that signaling & perception can be context-dependent.

Biofabrication provides a means to create context that, in turn, facilitates meaningful communication.

Biofabrication, synthetic biology, and external stimuli can enable hierarchical assembly of biological components and in organized assemblies for advanced function.

We are developing means for organizing advanced functions such as energy generation, signaling, and replication.

Thanks for your attention!!
Questions?



Thanks to our sponsors: NSF, DTRA, EPA, ONR, & RW Deutsch Foundation