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Interfacing Living Cells with Biodetection Platforms

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David G. Whitten Symposium

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Sandia National Laboratories

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Background and Motivation

Living cell-based sensors have proven effective for real-time detection of hazardous agents, near-neighbors, or unknown agents

Cells have been engineered to respond to:

- Stress, toxicity, DNA damage, or death by rapidly producing (or decreasing production) of bioluminescent, fluorescent, or electroactive molecules.†

CANARY Cell Line

- Developed by Lincoln Labs at MIT‡
- Employ B-cell line (lymphocytes) engineered to produce bioluminescent signal via calcium-sensitive bioluminescent protein within seconds of pathogen binding to membrane-bound antibodies
- Require replenishment with new cells every two days
  - Limits use to high priority buildings in developed and stable regions
  - Substantial genetic engineering effort increased CANARY stability to five days§

† Lei, Y. et al. *Anal. Chem.* **2006**, *568*, 200;
Baeumner, A. J. et al. *Anal. Bioanal. Chem.* **2003**, *337*, 434;
Kohler, S. et al. *Fresenius J. Anal. Chem.* **2000**, *366*, 769.

‡ Rider, T. H. et al. *Science* **2003**, *301*, 213.

§ Petrovick, M. S. et al. *Biotechnol. Bioeng.* **2010**, *106*, 474.

Encapsulation of Living Cells

Development of a functional biocompatible interface between immobilized/encapsulated cells and the macro world:

- Allow interaction of cells with external stimuli
  - Target analytes & sensor transducer
- Maintain cell viability
  - Provide access to nutrients, removal of wastes, & limit growth

Cell encapsulation methods†

- Use of synthetic polymers or natural protein or polysaccharide derived hydrogels most widely reported for living cell encapulation
- Most studied system: Alginate
- Other systems: PEG, chitosan, collagen, agarose, etc.

Ideal for tissue and organ replacement or regeneration.
Less ideal for sensing due to: difficulty monitoring and transducing information regarding state of cell, cell growth and replication, need of nutrients

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† Murua, A. et al. *J. Control. Release* **2008**, *132*, 76.

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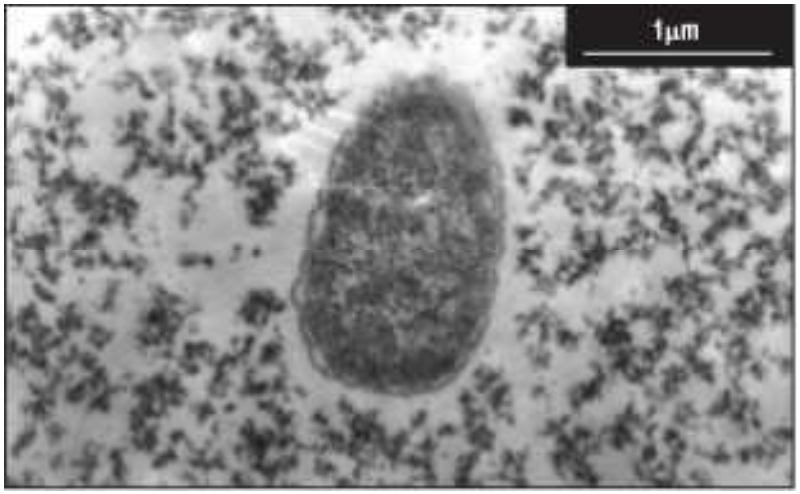
Sol-gel Derived Materials for Entrapping Cells

Encapsulation of whole cells in silica gels has attracted considerable attention as these matrices are biologically inert, easily processed, can be tailored to provide desired material and chemical properties.†

- Two routes typically followed to make solgel more bio-friendly
  - Two step procedure: acid hydrolysis followed by neutral pH encapsulation
  - Acidification of aqueous sodium silicate/colloidal silica solutions

- In both cases solgel synthesis is still too cytotoxic and requires addition of ameliorants:
  - PEG, glycerol, gelatin, alginate, etc.
- Require addition of exogenous buffer or nutrients to maintain viability.‡
- Physically entrapped cells do not influence or actively interact with matrix



TEM of *E. coli* entrapped in glycerol containing solgel, Nassif, N. et al. *Nat. Mater.* **2002**, *1*, 42.

† Avnir, D. et al. *J. Mater. Chem.* **2006**, *16*, 1013.
‡ Nassif, N. et al. *J. Mater. Chem.* **2003**, *13*, 203.

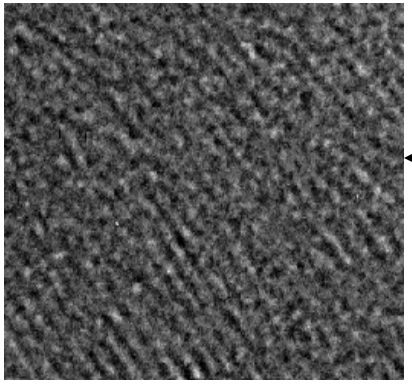
Biocompatible Sol-Gel with Ordered Nanostructure

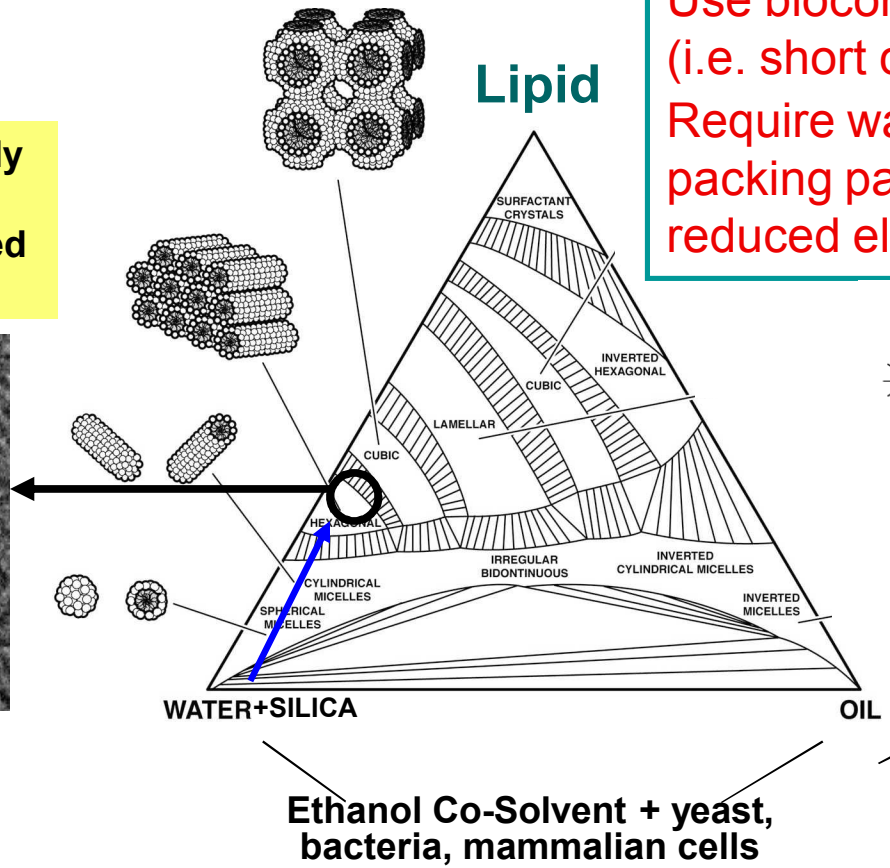
Traditional surfactants toxic:

Use biocompatible surfactants (i.e. short chain phospholipids)

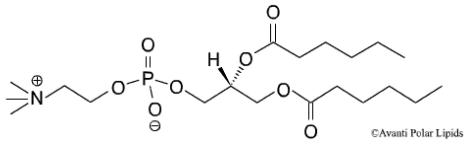
Require water solubility, low packing parameter, *g*, and reduced electrostatic interactions

Uncalcined **Hexagonally ordered silica nanostructure templated by *di*C_6PC**

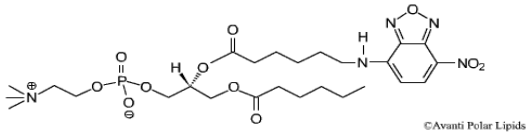




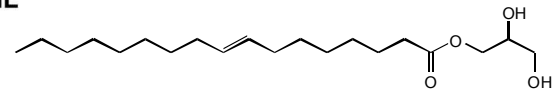
Ethanol Co-Solvent + yeast, bacteria, mammalian cells



*di*C6PC



*di*C6PC-NBD



Glycerol monooleate

Biocompatible surfactant-directed self-assembly of inorganic host matrix creates nanostructured silica network that maintains internal fluidic architecture - uniformly sized nanopores and channels remain water-filled, develop and maintain 3D gradients

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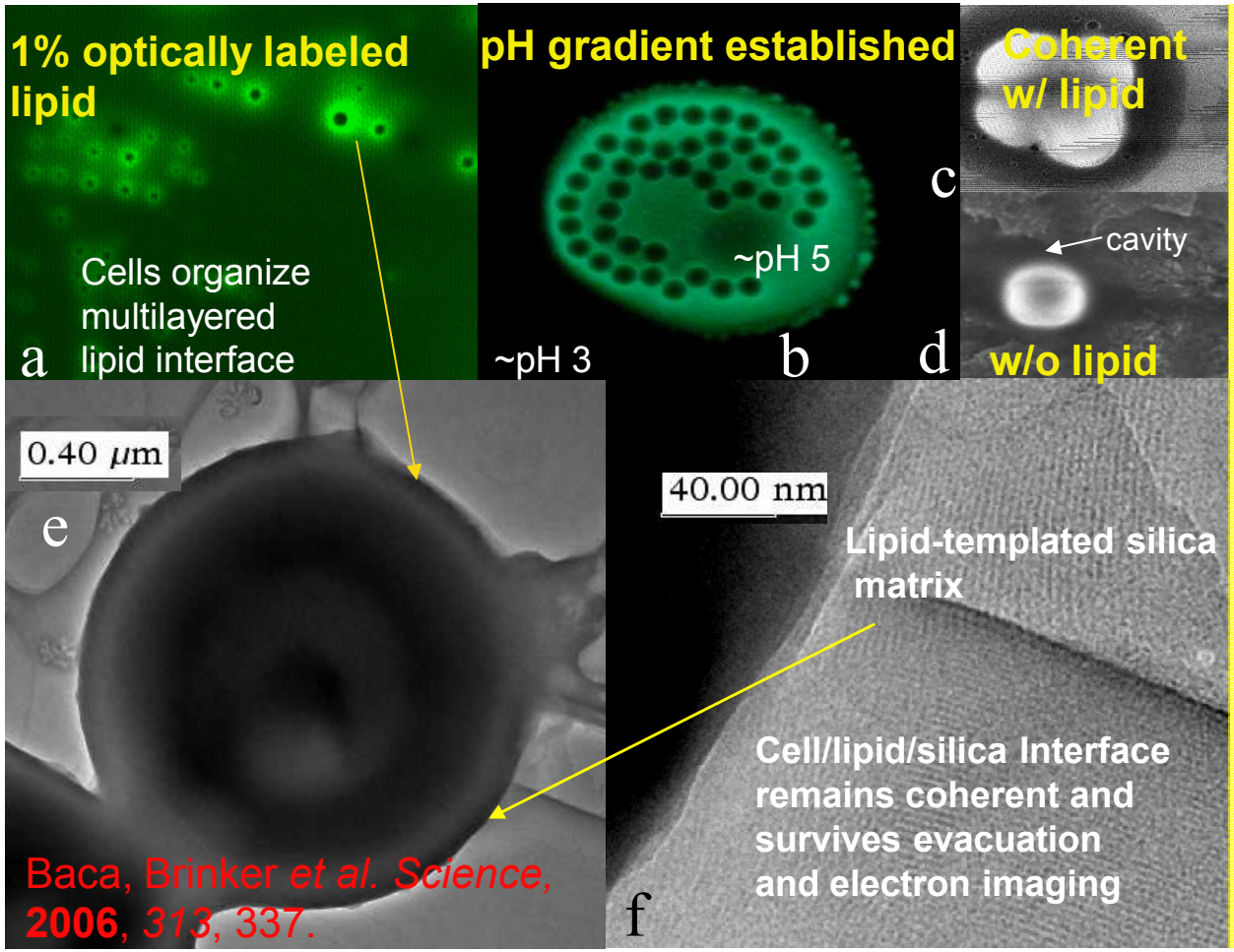
Cell Directed Assembly (CDA)

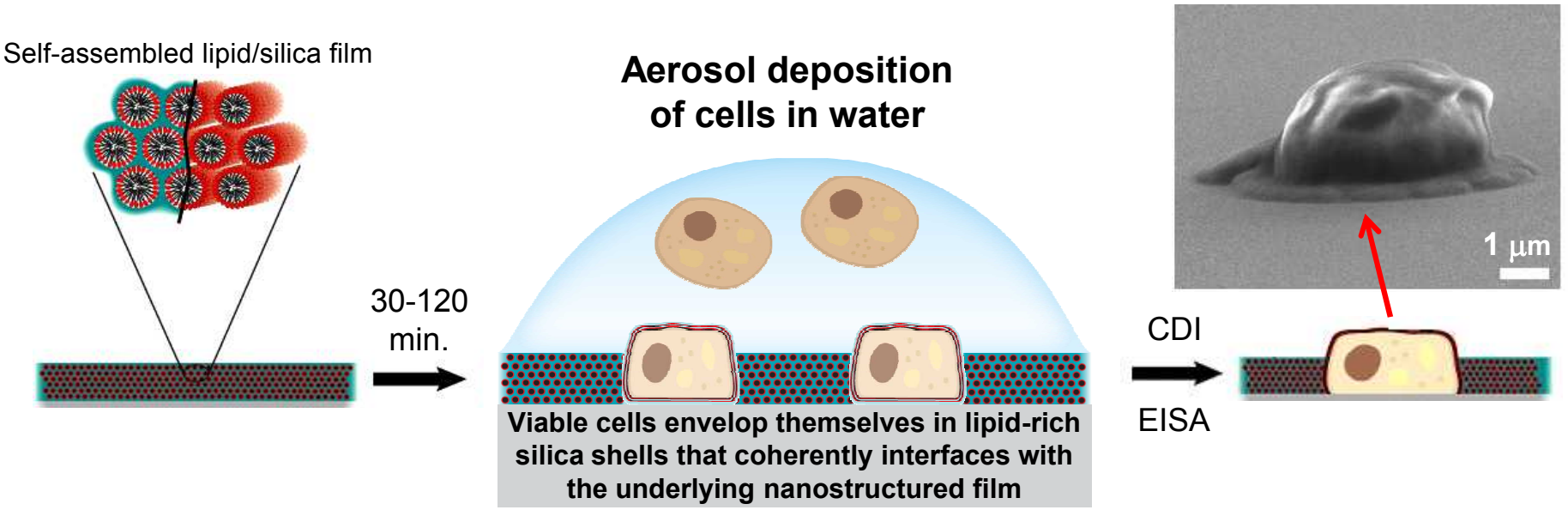
CDA - Yeast cells (*S. cerievisiae)* added directly to lipid-silica precursor solution *actively* intervene during EISA directing the organization of a novel bio/nano interface



- Cells accumulate lipid-rich shell - species specific, accommodates proteins and nanocomponents
- Coherent interface forms between cells and silica nanostructure - forms also by active integration into preformed lipid/silica film
- Cells immobilized in silica avoid biaxial tensile stresses - appears to actively mediate stress
- DTRA program *develops mechanistic understanding and extends to mammalian cells*

Cell Directed Integration (CDI)

CDI - Yeast cells added onto preformed lipid-templated mesoporous silica film *actively* reconstruct the surface encapsulating themselves in a lipid-rich silica shell

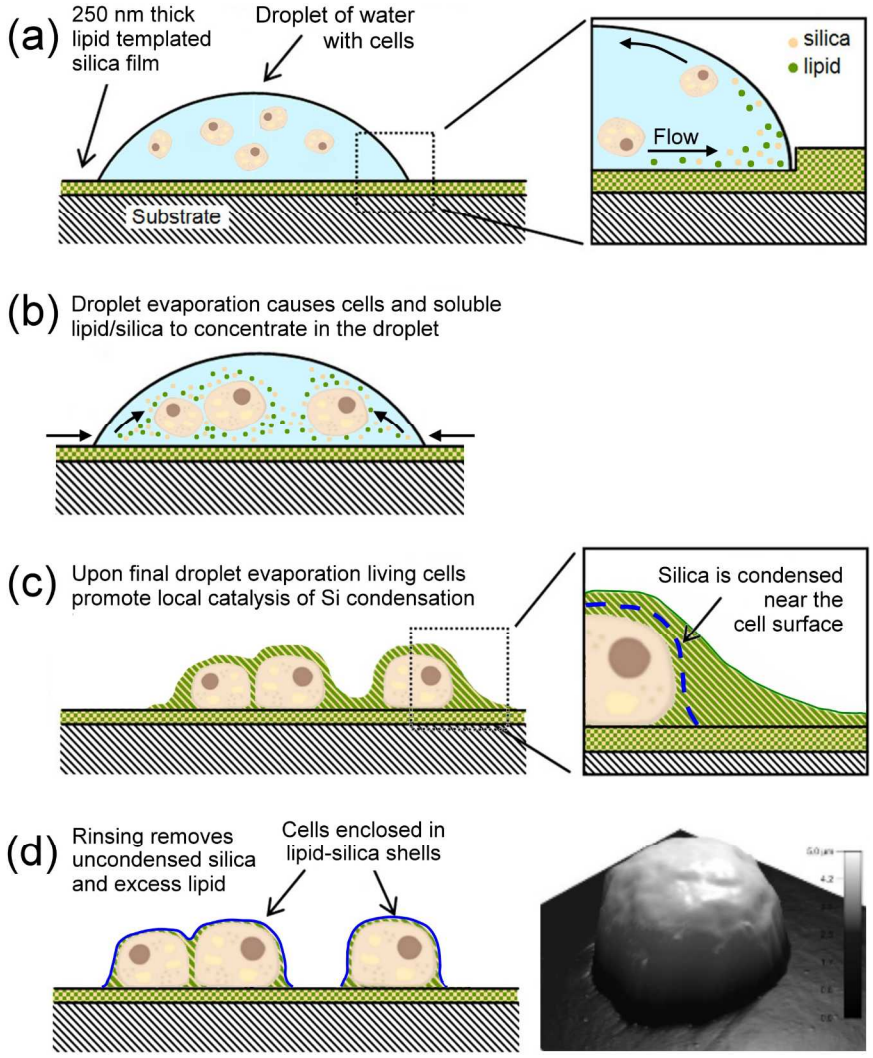


Additional Advantages of CDI

- *Reduces stress exerted on cells during hydrolysis & condensation → Mammalian Cells*
- *Original nanostructure of bulk film is maintained*
- *Improved accessibility to external environment via rapid transport through thin lipid-silica shell*
- *Lithographic patterning of preformed film → Define regions for cell immobilization, fluidic channels*

Mechanism of Cell-Directed Integration

Cell-Directed Integration†



a) Film Solubilization

- Introduction of an aqueous suspension of cells onto slowly condensing lipid/silica film solubilizes components of the film
- Marangoni and capillary flow arises from temperature and viscosity gradients

b) Droplet Evaporation

- Cells, and solubilized lipid and silica progressively concentrate in droplet

c) Viable Cells Facilitate Silica Condensation

- Cells increase local pH in response to evaporation induced osmotic stress
- Membrane proteins/peptides catalyze silica condensation
- Exponential phase cells exhibit higher integration efficiency, while stationary phase cells exhibit higher viability

d) Lipid-Rich Silica Shell

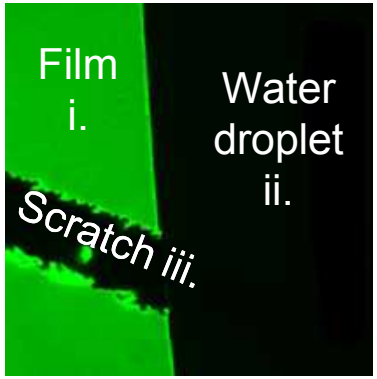
- Rinsing the substrate leaves cells encapsulated in a lipid-rich silica shell

† Harper *et al. ACS Nano*, in review

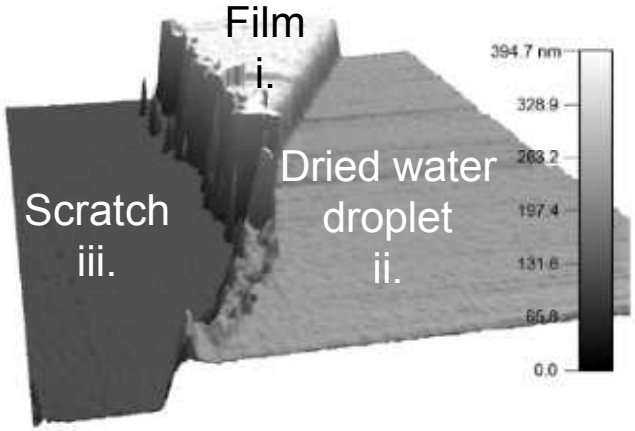
Lipid Templated Silica Film Solubilization

Lipid and silica are solubilized from the slowly condensing lipid-templated silica film upon introduction of an aqeuous droplet

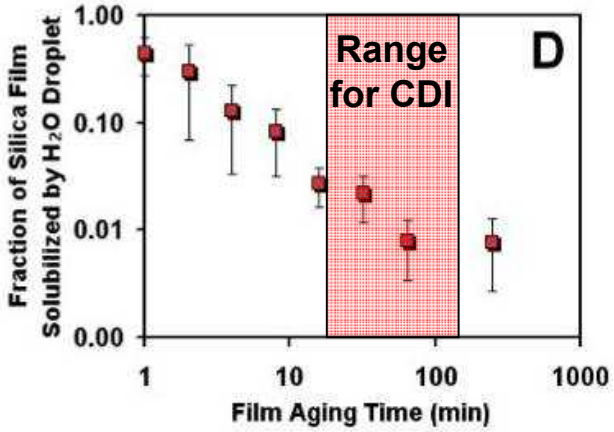
Fluorescence Image
(NBD tagged lipid)



AFM Measured Topography



Faction of Si Solubilized



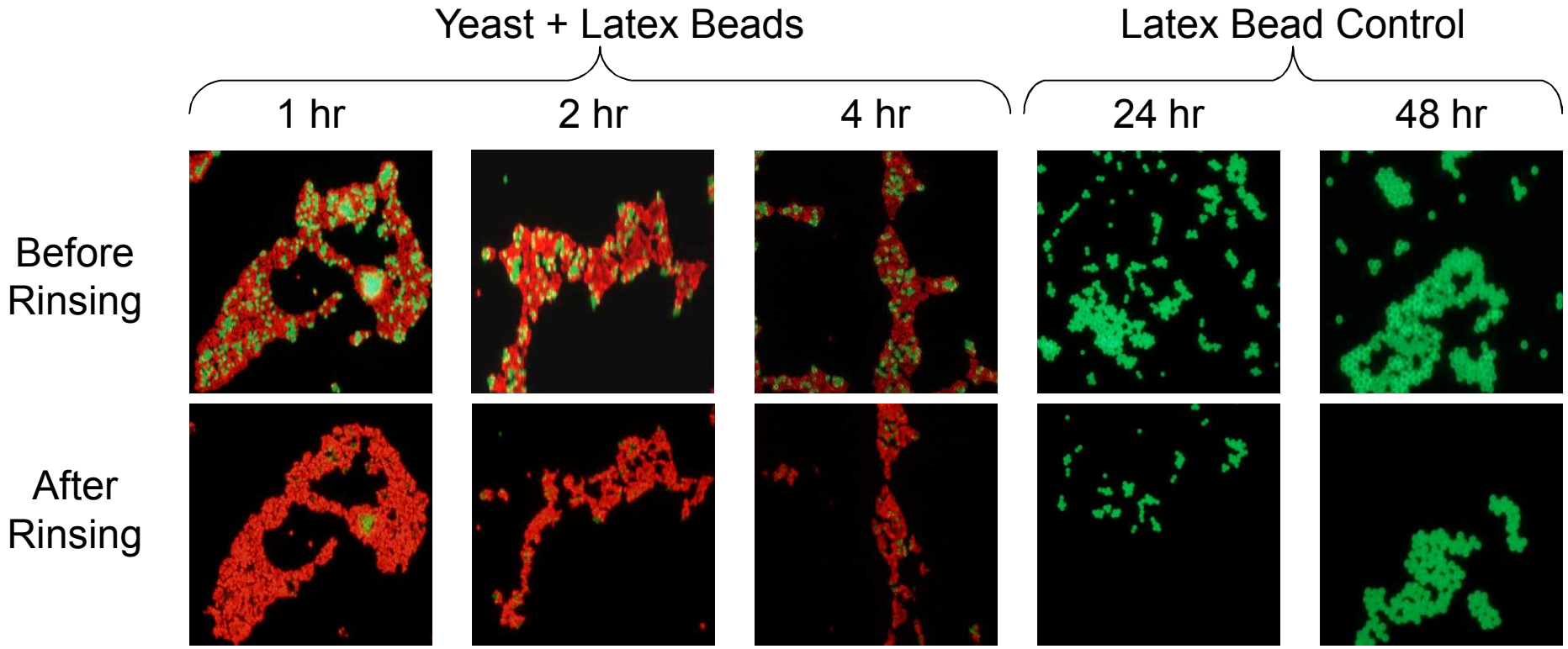
i. **Pristine lipid/silica matrix film**
ii. **Film exposed to water droplet left to evaporate**
iii. **Film removed exposing underlying coverslip**

Film thickness decreases from ~230nm to ~110nm, a 50% loss

Silica dissolution depends strongly on the ageing time of the film, i.e. its extent of condensation prior to introduction of droplet. To ensure stability of film structure, while allow sufficient Si to be solubilized to encapsulate cells, films were aged 30-120 min prior to introducing cell solution.

Viable Cells Actively Facilitate Silica Condensation

Yeast cells are not removed from the film by rinsing, while beads are easily washed away under timescales shorter than that required for silica condensation



RED = Yeast stained with Syto 64

GREEN = 5 μm fluorescent latex beads

A combination of a 1:1 ratio of beads:yeast were allowed in integrate into a 30 minute old lipid-templated silica matrix. At each time point (1, 2, 4, 24 and 48 hours) films were rinsed to remove non-integrated material.

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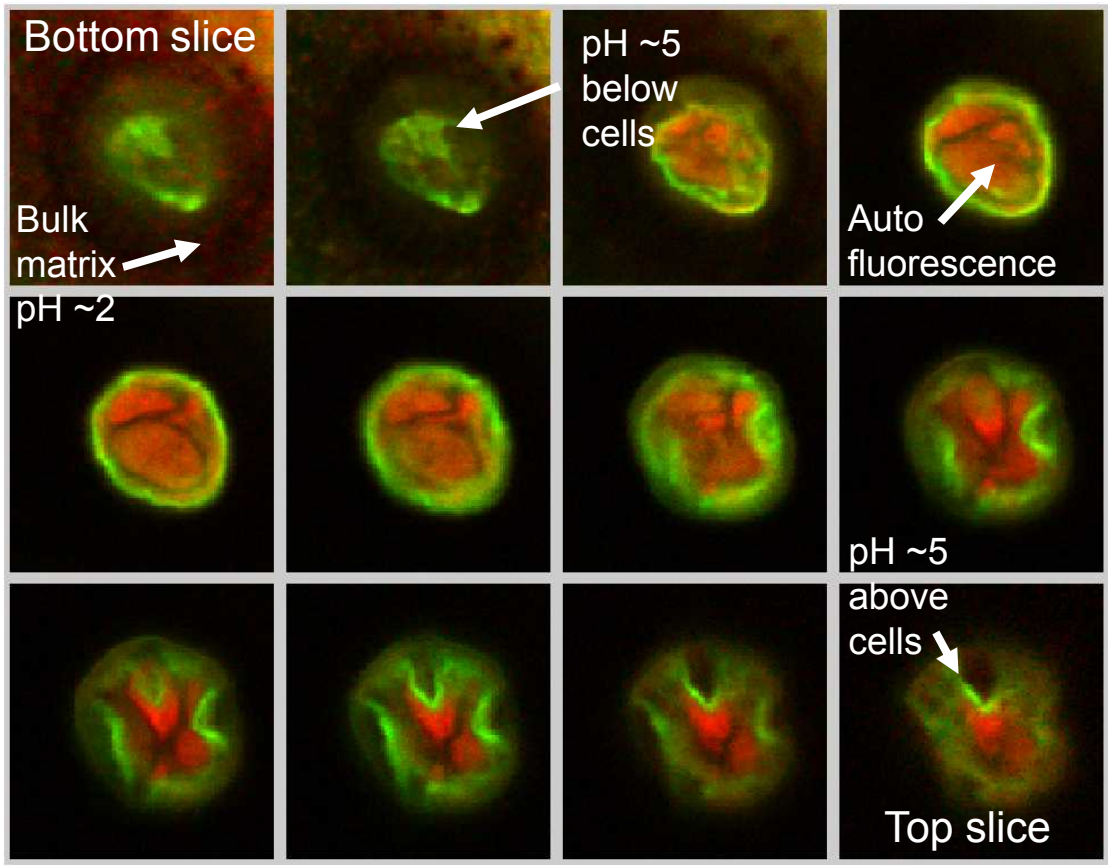
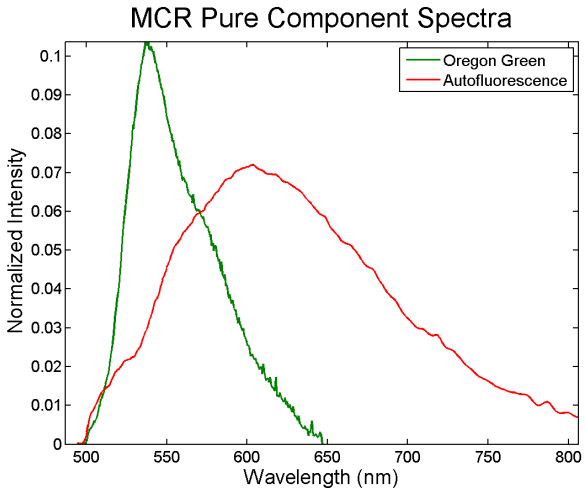
Active Cells Develop a Local 3D Gradient in pH

CDI Encapsulated Yeast Cells Develop Fully 3D pH Concentration Gradients at Interface with the Lipid-Silica Matrix

Hyperspectral Confocal Slices of a Three Yeast Cell Cluster





GREEN = Pixels with Oregon green pH sensitive dye spectra

RED = Pixels with yeast auto-fluorescence spectra

Gradient in pH exists both above and below cells

In collaboration with Howland Jones & Ryan Davis, SNL

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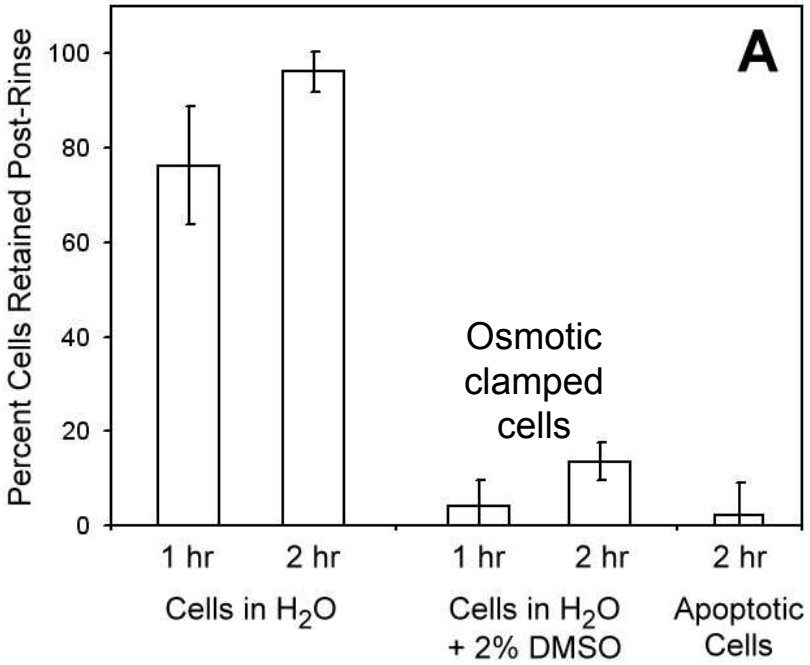
Turning Off Cell-Directed Integration

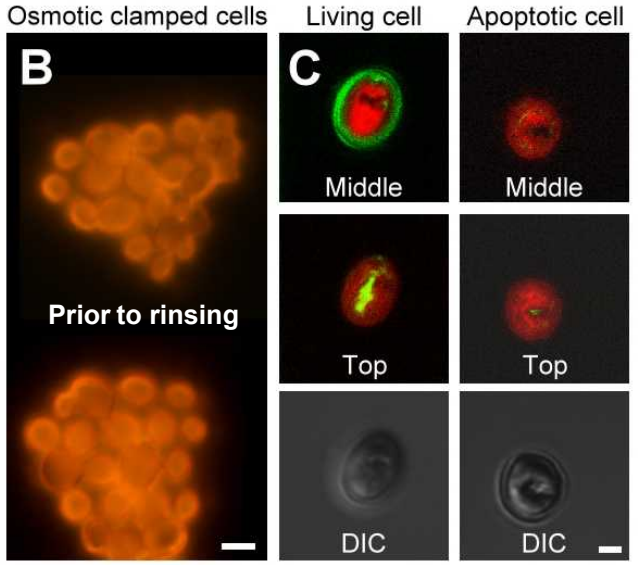
The majority of yeast cells introduced to the lipid-silica matrix in water integrate into the film and form localized pH gradients.

Apoptotic yeast, or yeast cells under osmotic-clamp,† do not integrate into the film and do not form gradients in pH.

Integration Efficiency



With Oregon Green pH probe



RED = Yeast stained with SYTO 64

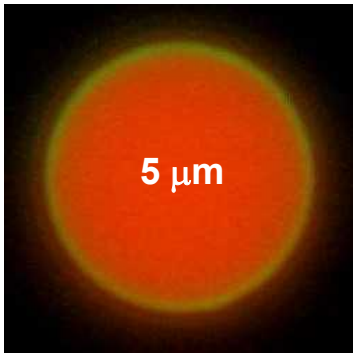
- Development of pH gradient is required for integration.
- Si condensation rate increases significantly in higher pH region resulting in lipid-rich silica shells at cell-matrix interface.

† Yang *et al. Biochim. Biophys. Acta* **1991**, *1080*, 138.

Cell Surface Proteins/Peptides Assist Integration

Synthetic and natural polycationic macromolecules are known to catalyze silica condensation.†

Lysozyme Coated Bead

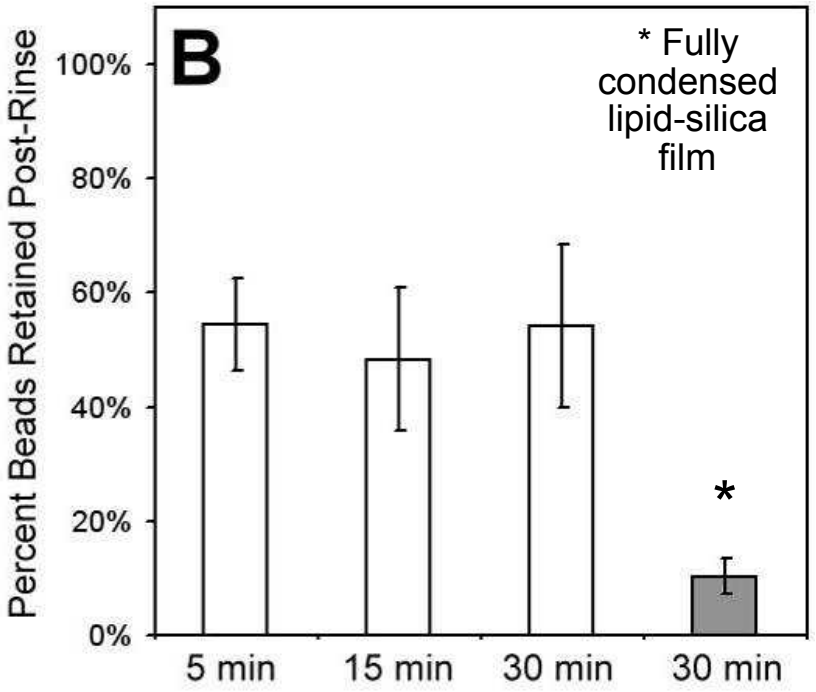


Lysozyme protein (pI ~ 10) was crosslinked to carboxyl functionalized orange fluorescent beads via carbodiimide chemistry

Protein conjugation verified by treatment of beads with NHS-Alexa Fluor 488

† Patwardhan *et al. Chem. Commun.* **2005**, 1113; Lopez *et al. Curr. Nanosci.* **2005**, *1*, 73.

Integration Efficiency



Neutrally charged latex beads show only ~5% integration under identical conditions.

Polycationic regions on a cell may contribute to integration. However, this is a minor mechanism of integration for bacterial and yeast cells.

Towards Mammalian Cell Encapsulation

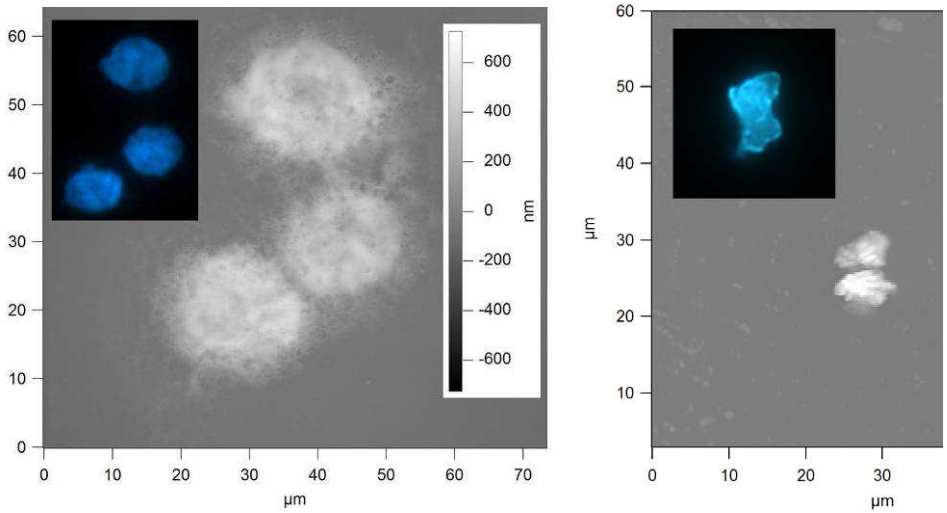
Governing Mechanisms of CDI

- Osmotic stress induced development of local pH gradient
  - → **Exerts severe stress on larger and more fragile mammalian cells**
- Polycation induced silica condensation
  - → **Appears to be a significant mode of Si deposition over longer times with cells in solution**

Mammalian Cell Encapsulation - Path Forward

- CDI with wet cells over longer times
- Poly-ion polymer layering to protect cells and enhance Si deposition
- Incorporate ameliorants and media components into sol-gel
- Vapor phase Si deposition

Encapsulated Mammalian Hep3b Cells



Hepatoma cancer cells (Hep3b) integrated into the silica-lipid film (left) retain their morphology when dried, whereas unintegrated cells (right) shrivel.

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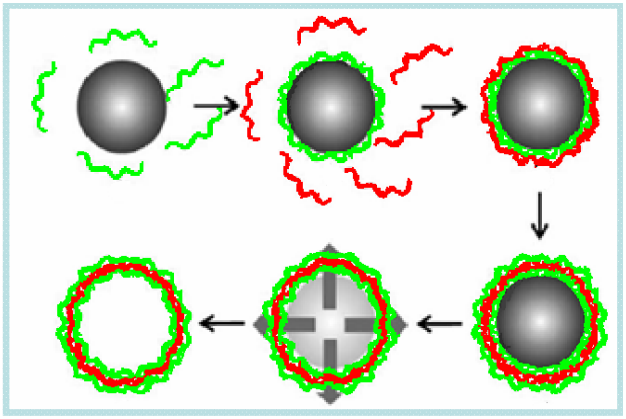
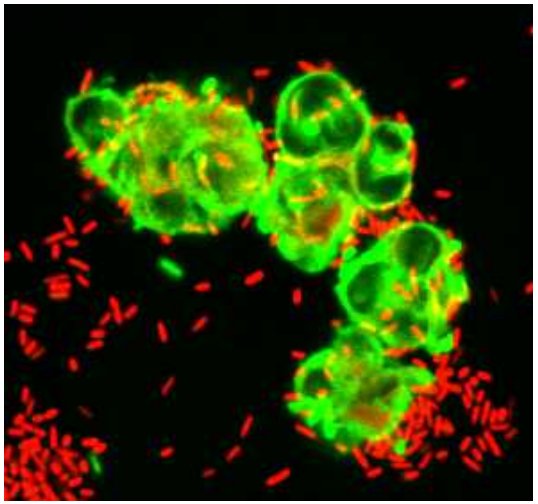
Polyion Layer-by Layer Deposition onto Cells

Conjugated Polyelectrolyte Capsules†





"μRoach Motels"

- Layer-by-layer deposition of polyelectrolytes onto 5 mm spherical supports
- Polyelectrolyte capsules bind strongly to bacterial & mammalian membranes
- White light activates biocidal activity
  - → Mammalian cells exhibit significantly reduced toxicity

Collaboration with Whitten Lab

→ *Use mammalian cells as support for LbL with polyelectrolytes*

→ *Polycationic layers will serve as catalysts for Si deposition forming Si shell encapsulated cells*

† Corbitt, Whitten *et al. ACS Appl. Mater. Interfaces* **2009**, *1*, 48.

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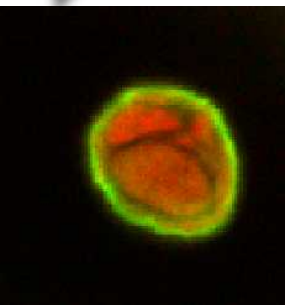
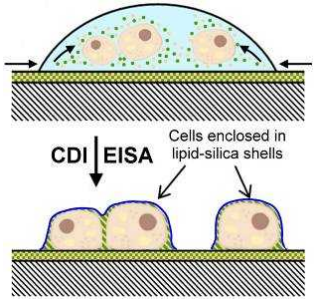
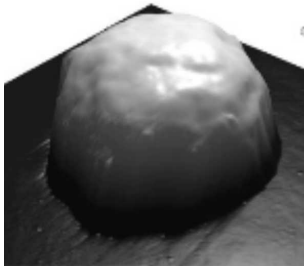
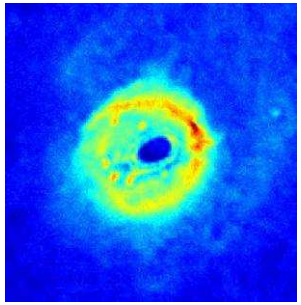
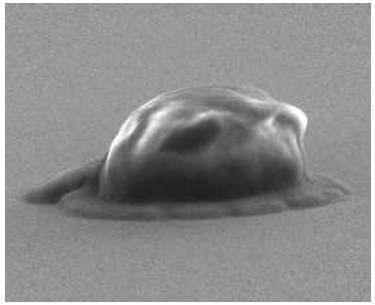
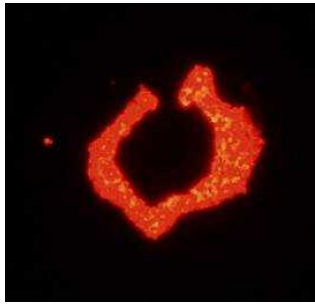
Acknowledgements

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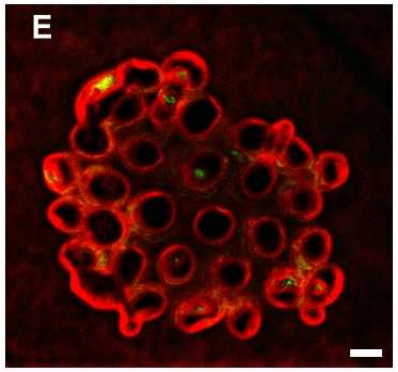
Engineered 3D Bio-Nano Interfaces

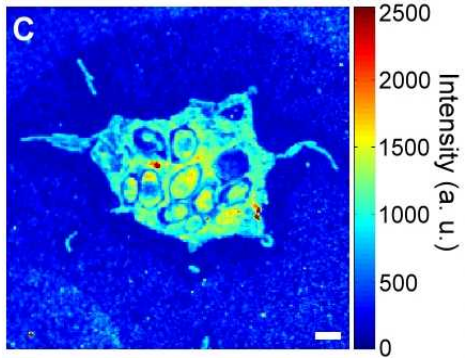
Auxiliary nanocomponents (liposome lipids, quantum dots, exogenous proteins) are localized at the cell-matrix interface allowing development of complex active and accessible bio/nano interfaces not achievable by other synthetic methods.

Localization of Liposome Lipid‡



POPC liposome lipid (red) added with cells during CDI has higher priority for cell surface. –No such affinity observed in original solution with cells, or with other liposomes.

Localization of Film Residing Qdots‡

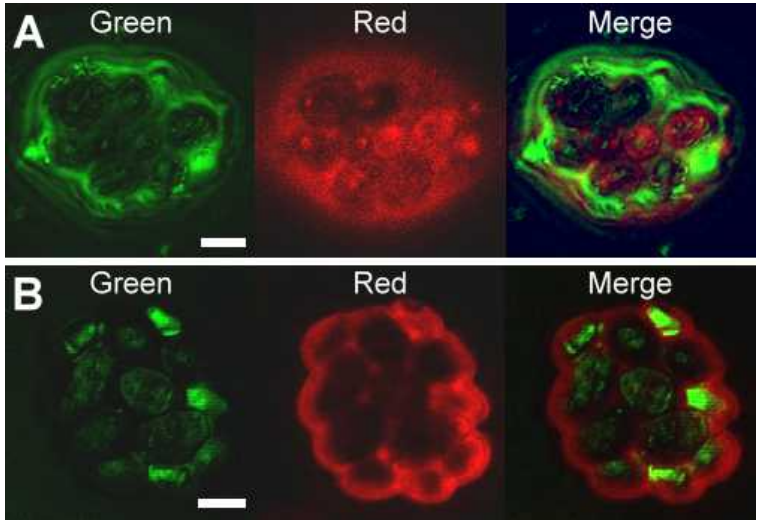


655nm emitting Qdots in lipid-silica matrix are localized near cell surfaces during CDI. Some Qdots endocytosed by cells.

Localization and Orientation of Active Bacteriorhodopsin (bR) Transmembrane Protein†

bR protein (red) added with short-chain *di*C_6PC lipid (green)

bR protein (red) added in DMPC liposomes (green) with using *di*C6PC



We observe more conformal localization of bR introduced in liposomes - DMPC is selectively localized at the cellular surface with preference over *di*C_6PC. Liposome introduced bR was observed to modulate pH gradients demonstrating retained functionality and preferential orientation.

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‡ Harper *et al. ACS Nano*, **2010**, in review

† Carnes *et al. J. Amer. Chem. Soc.* **2009**, *131*, 14255.

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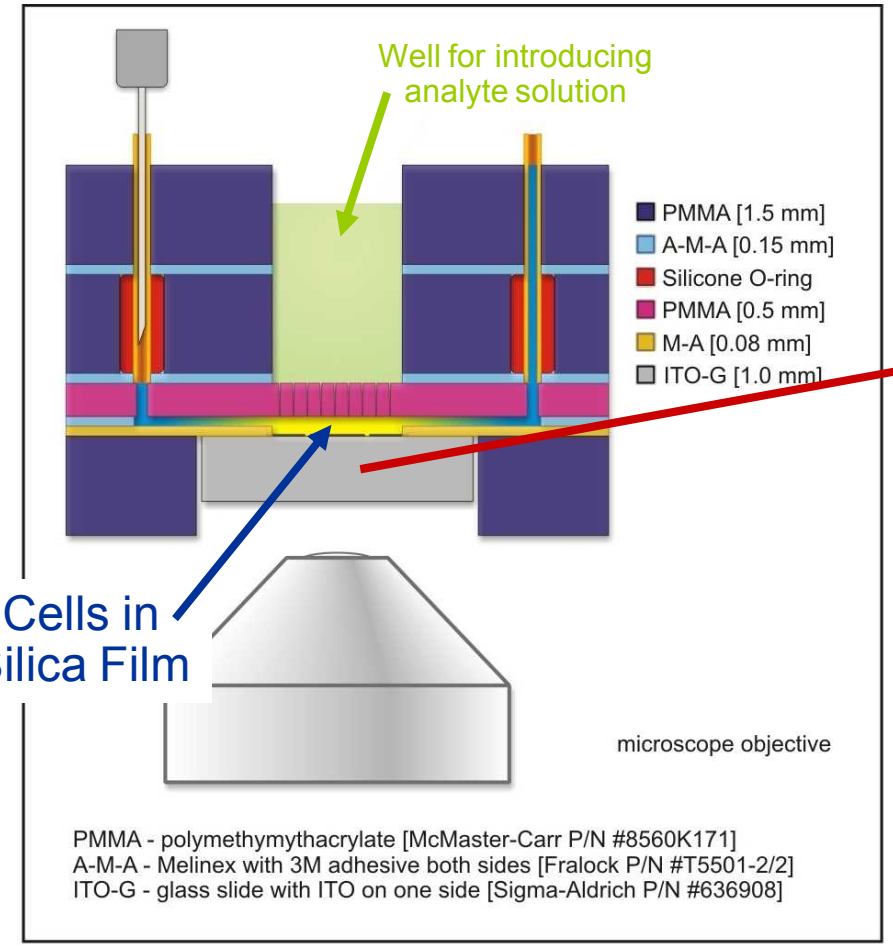
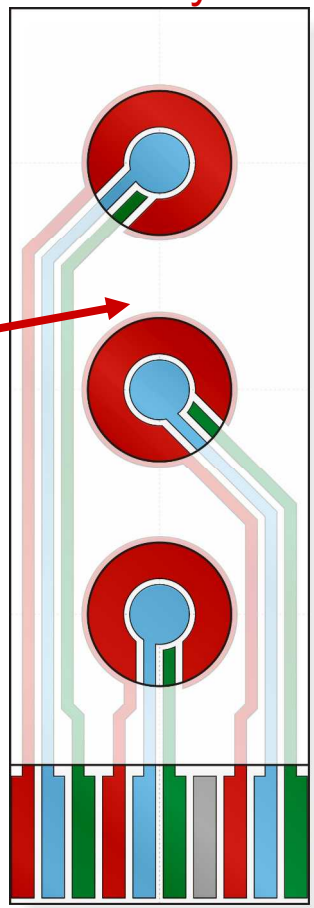
Whole Cell-Base Orthogonal Biosensor

Cartridge with Optical/Fluorescent & Electrochemical Detection Modes

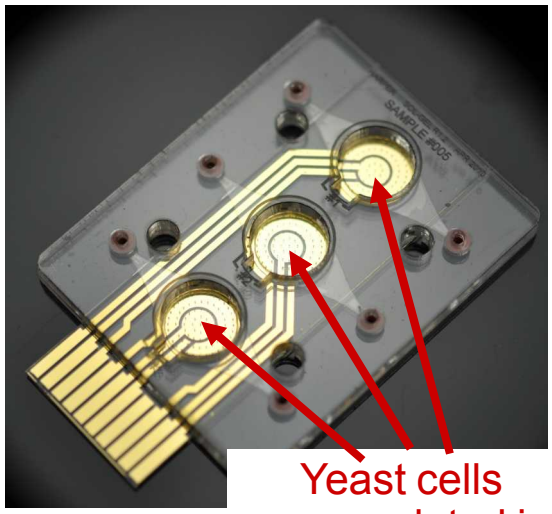
Cartridge Cross-Section



ITO Electrode Array



Assembled Cartridge



- Microfluidic cartridge fabricated from inexpensive plastics allowing fast prototyping
- Cartridge allows formation of silica encapsulated yeast thin film over transparent (ITO) electrodes

In collaboration with Thayne Edwards, SNL