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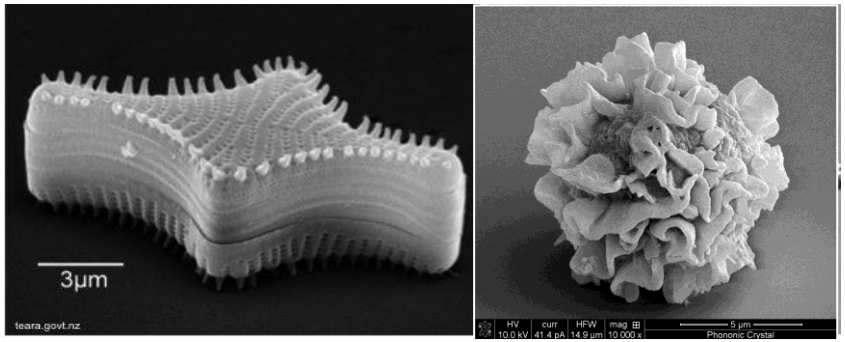
Sandia National Laboratories

Complex Composites: *Directed by Cells*

Gordon Conference on Bio-Interface Science, June 15-20, Lucca, Italy 2014

Bryan Kaehr[1], Jason Townson,[2] Eric Carnes[1], Atul Parikh[3], Jeff Brinker[1,2]



Diatom Frustule Mammalian Cell Frustule

DOE BES, AFOSR, NCI, DTRA, NSF IGERT, SNL LDRD, NIH/EPA CEIN, NSF

[2]University of New Mexico

Darren Dunphy
Helen Baca
Graham Timmins
Maggie W-Washburn
Patrick Johnson
Annikka Jensen
Jennifer Pelowiz
David Padilla
Ying-Bing Jiang
Katie Epler
Robbie Trujillo

[1]Sandia National Labs

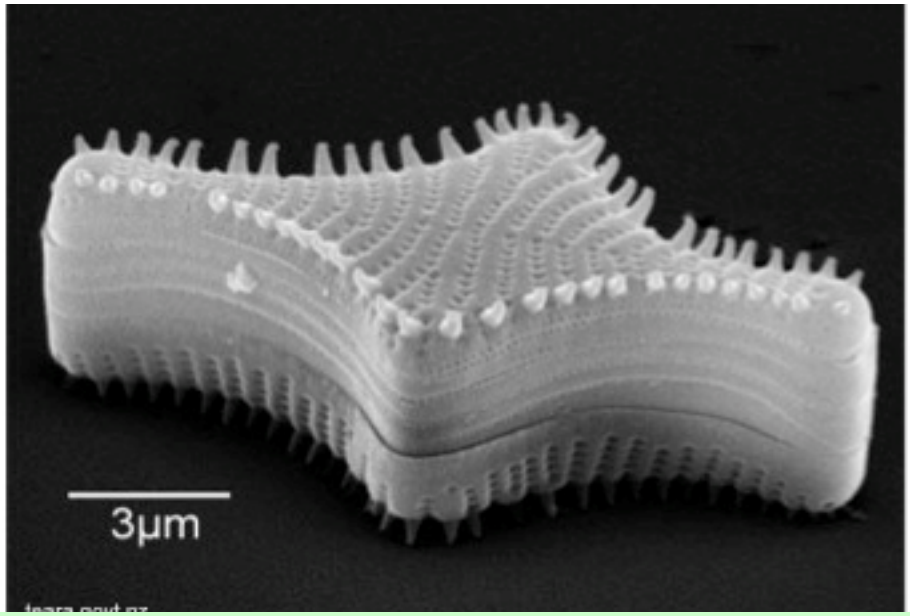
Todd Alam
Jason Harper
Seema Singh
Susan Rempe

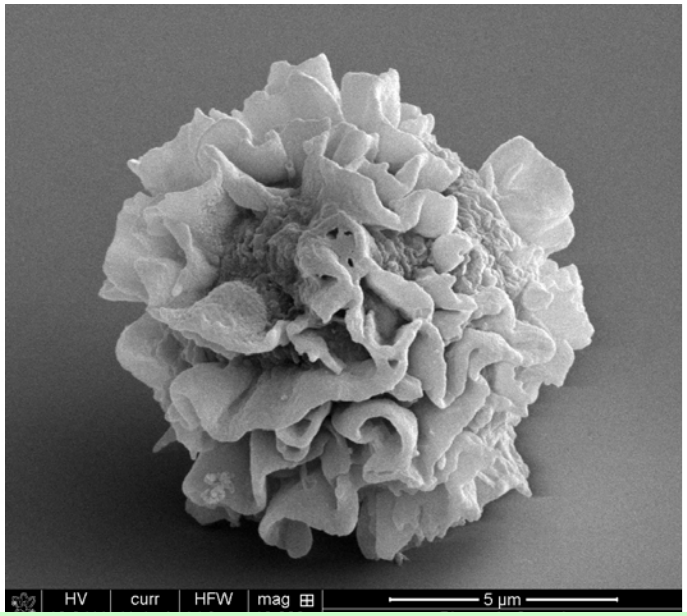
Outside Collaborations

Constantine Khripin, NIST
Jie Sun, UIUC
Eric Kendall, UC Davis
Lucio Ciacchi, Lutz Madler, Bremen
[3]Atul Parikh, UC Davis
Liangfang Zhang, Peter Wang, UCSD

U.S. DEPARTMENT OF ENERGY NNSA National Nuclear Security Administration

Silica @ Cells: A special relationship





- Silica@Surfactant/Amphiphilic Interfaces
  - Sol-Gel chemistry and Evaporation Induced Self-Assembly of 'Mesoporous Silica'
- Silica@Cells
  - Cell-Directed Assembly (CDA) of conformal 3D silica nanostructures
  - Protein-Directed assembly of silica within lithographically-defined 3D-scaffolds
  - Replication of mammalian cells and organisms *in silica* – *'Zombie Cells'*
- Membranes@*Mesoporous* Silica – 'Protocells' and enhanced fluidity and phase separation

Water Replacement Hypothesis: Silicic acid ($Si(OH)_4$) participates in tetrahedrally coordinated hydrogen bonding networks and behaves thermodynamically like water. It can replace water at hydrophilic/biomolecular interfaces – where it can be amphoterically condensed to silica via catalysis by proximal proteins (or other biomolecular components)

Evaporation-induced self assembly (EISA) of silicic acid precursors into periodic silica/surfactant mesophases – predictable from alcohol-water-surfactant phase diagram w/o silica

Sol-gel chemistry - combined with self- and directed assembly

Confinement Induced Quorum Sensing of individual S. aureus
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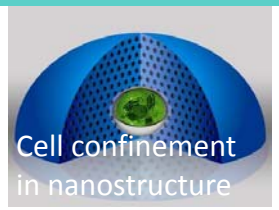

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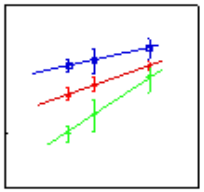

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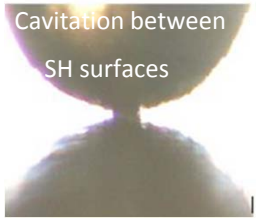

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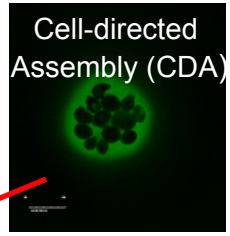

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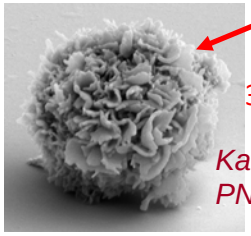

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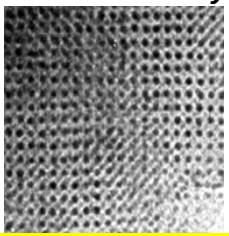

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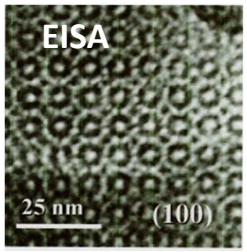

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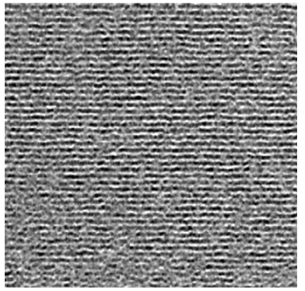

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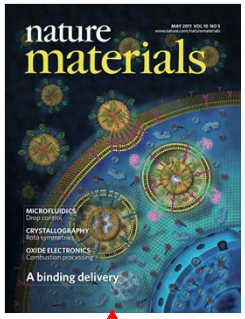

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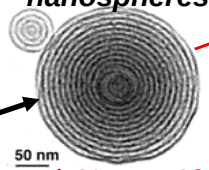

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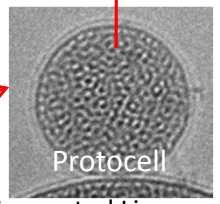

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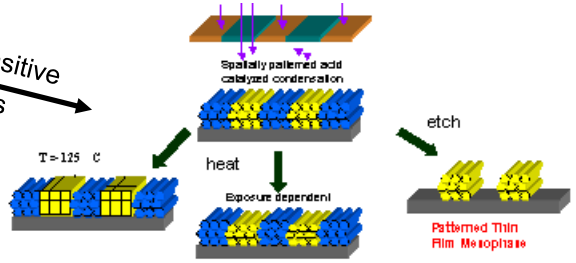

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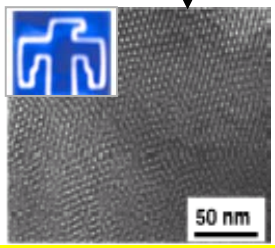

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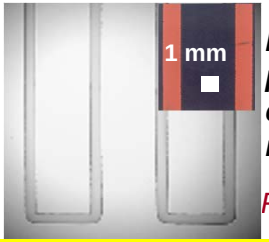

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Physical/Chemical Isolation Of Individual Cells
Aerogels Via Springback
Evaporation Induced self-assembly (EISA)
Multi-component (EISA)
Nano-indentation modeling / spectroscopy
Fractal SH surfaces via springback
Aerosol-assisted Self-Assembly
Cell-Directed-assembly
3D nanocrystal self-assembly
(EISA) using polymerizable surfactant
EISA of photosensitive nanostructures
"smart ink"

During evaporation, (poly)silicic acid replaces water at hydrophilic interfaces

Evaporation-induced self assembly (EISA) of silicic acid precursors into periodic silica/surfactant mesophases – predictable from alcohol-water-surfactant phase diagram w/o silica

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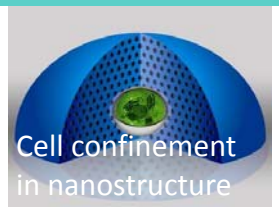

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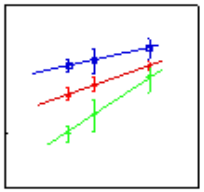

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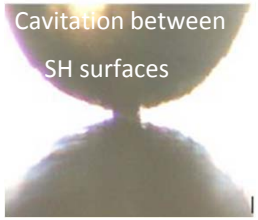

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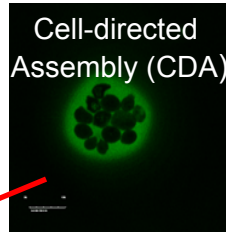

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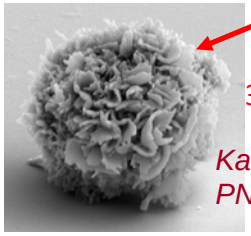

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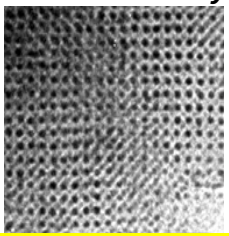

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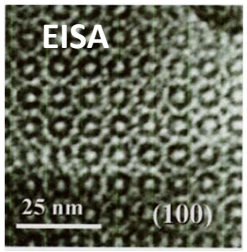

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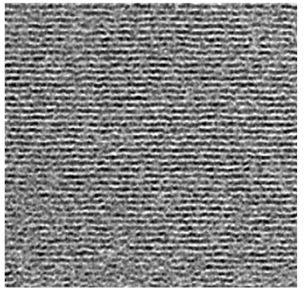

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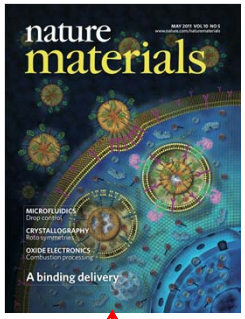

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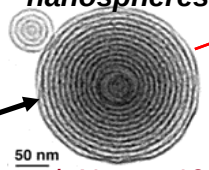

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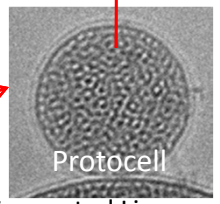

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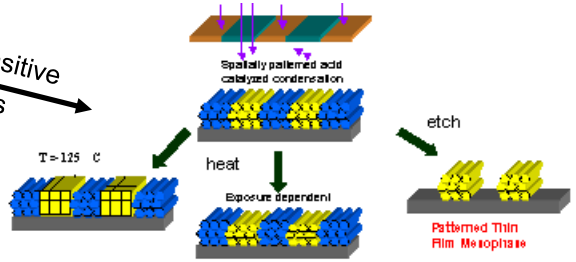

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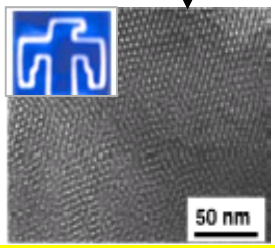

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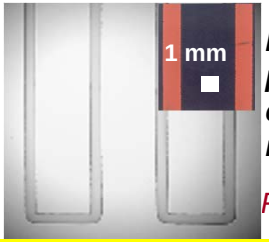

Direct writing of porous & composite nanostructures

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Physical/Chemical Isolation Of Individual Cells

Aerogels Via Springback

Evaporation Induced self-assembly (EISA)

Multi-component (EISA)

Aerosol-assisted Self-Assembly

Nano-indentation modeling / spectroscopy

Fractal SH surfaces via springback

Cell-Directed-assembly

3D nanocrystal self-assembly

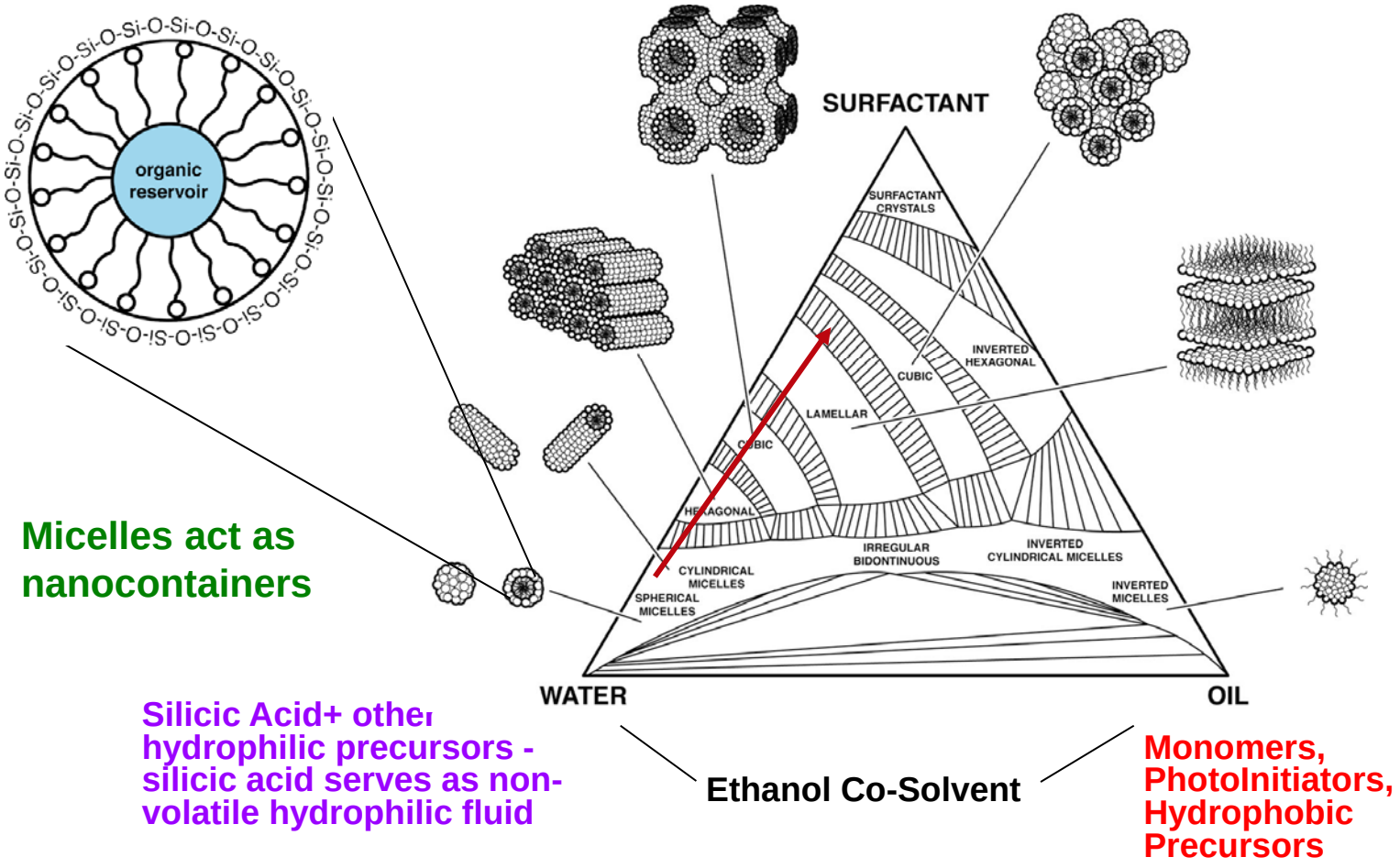
(EISA) using polymerizable surfactant

EISA of photosensitive nanostructures

"smart ink"

Consider cells as energy dissipating catalytic scaffolds to direct the formation of silica

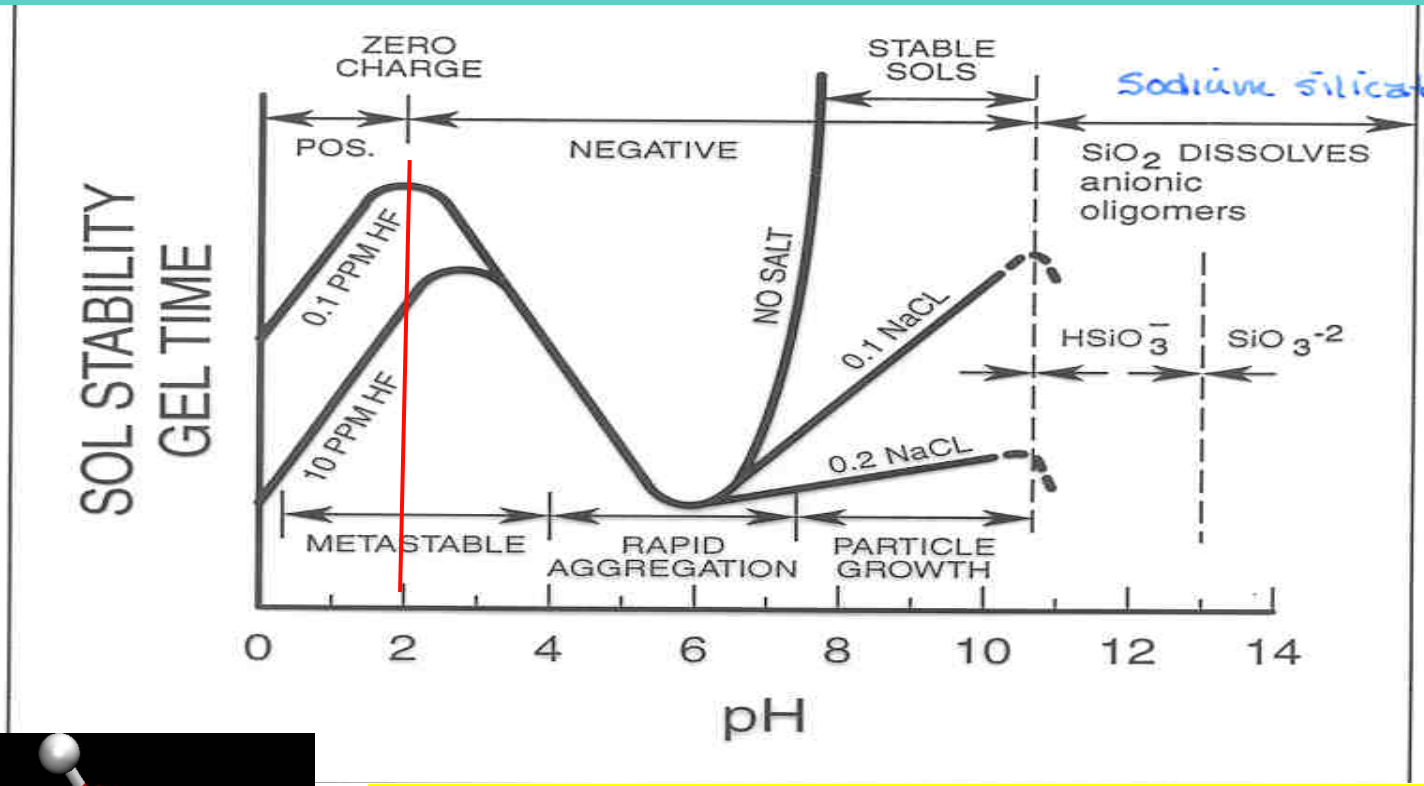
EISA: Use evaporation to drive self-assembly of periodic surfactant mesophases under thermodynamic control – water progressively replaced with silicic acid $Si(OH)_4$ by evaporation

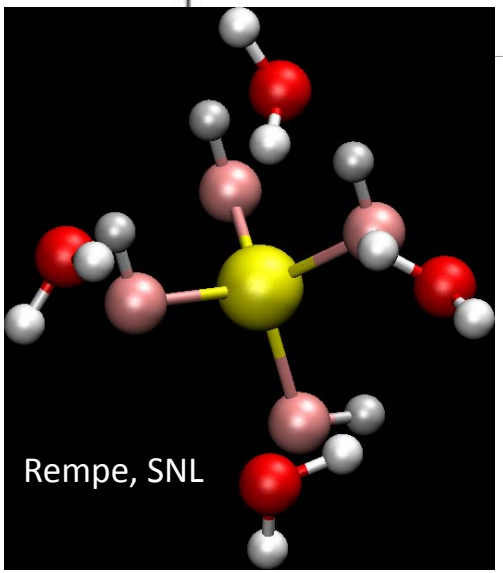


Detergent phase diagram adapted from Scriven and Davis

For further reference, see papers on evaporation induced self-assembly (EISA) of porous (*Lu et al. Nature, 1997*) composite (Sellinger et al. *Nature,* 1998) particulate *(Lu et al.Nature*, 1999) and patterned (*Fan et al. Nature 2000, Doshie et al. Science 2000)* nanostructures

Key: Employ acidic conditions that suppress silica condensation / gelation, enable self-assembly, and allow high fidelity replication of bio-interfaces





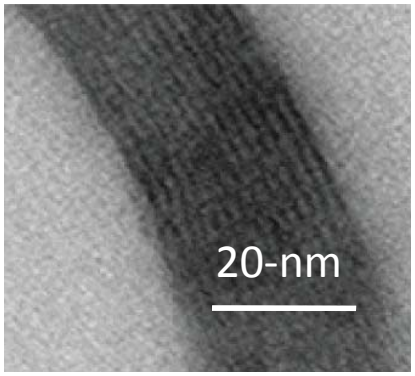
Does the tetrahedrally-coordinated, hydrogen bonded network of water surrounding neutral silicic acid stabilize it against bimolecular nucleophilic substitution?

– other metal hydroxides undergo rapid condensation at their isoelectric points – (Hamaker or Madelung Constants?)

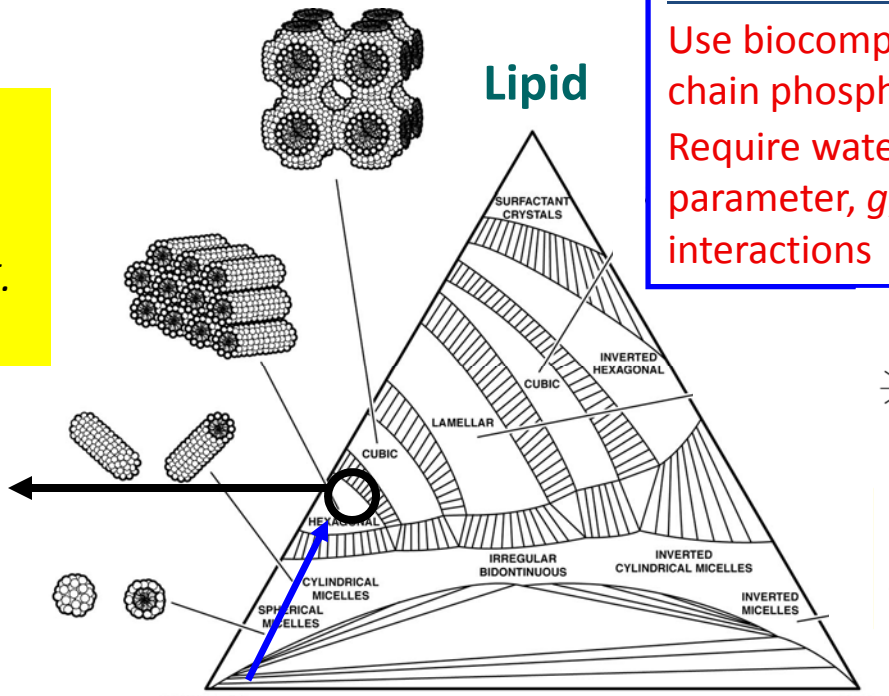
– comparable hydrogen bond strengths (H_2O, ≡Si(OH), H_2O/(OH)Si≡

EISA performed with silicic acid and lipids results in conformal cell/silica interfaces that withstand drying and evacuation and preserve aspects of cell viability

Hexagonally ordered silica/lipid mesophase templated by *di*C_6PC during spray drying of *E. coli*



Stiffer than bone…

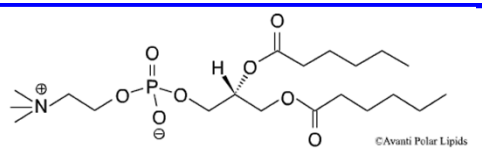


Ethanol Co-Solvent + Yeast, Bacteria, pH ~ 2.2

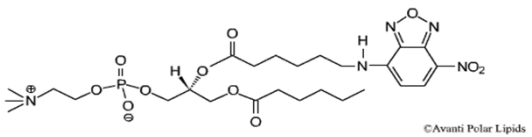
Traditional surfactants toxic:

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

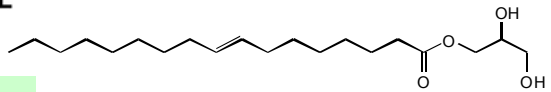
Require water solubility, low packing parameter, *g*, and reduced electrostatic interactions



*di*C6PC



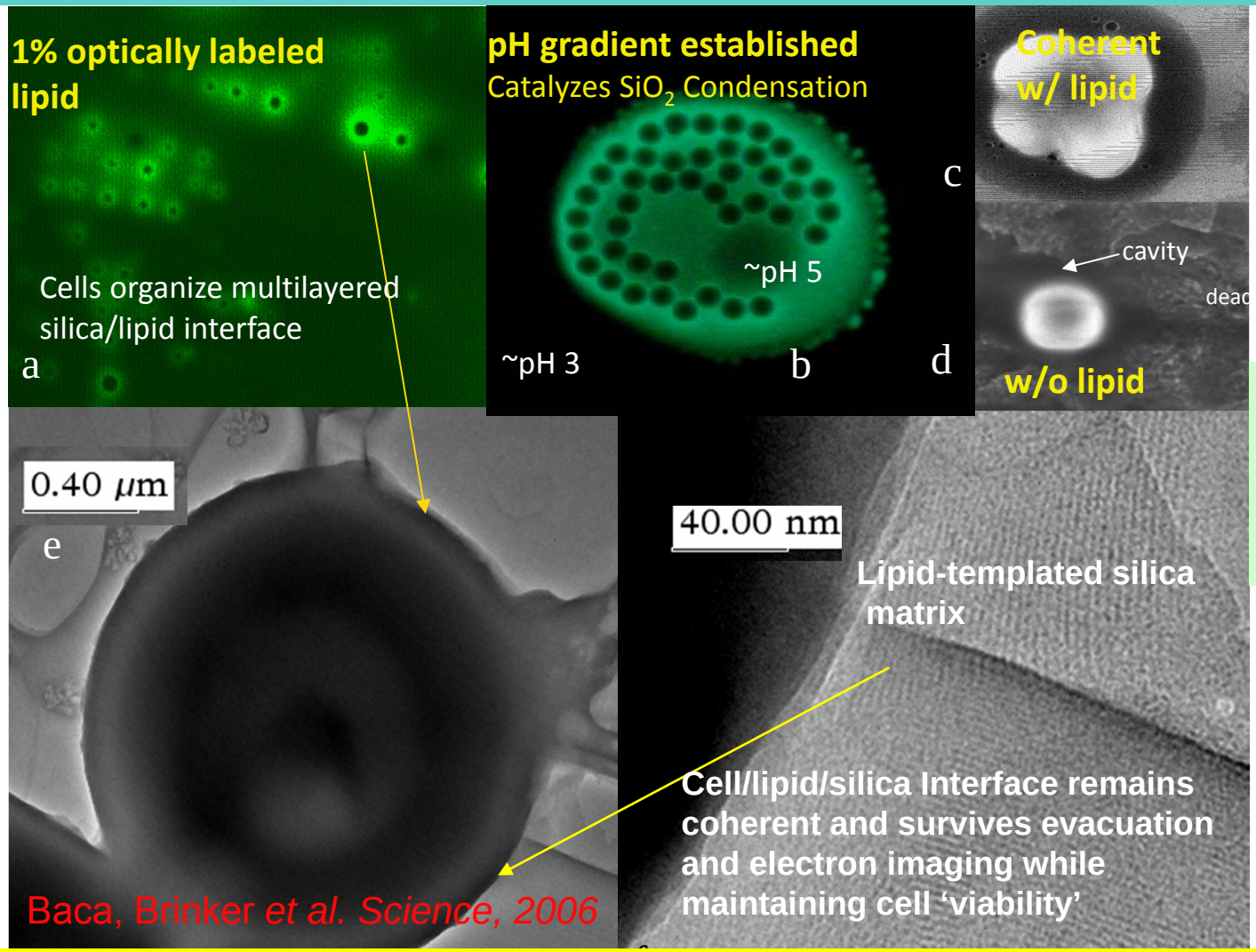
*di*C6PC-NBD



Glycerol monooleate

During EISA of homogeneous EtOH/H_2O/lipid/silicic acid systems, evaporation of EtOH and then water drives self-assembly of lipid/silica mesophase – WATER REPLACED with SILICA – substitution of water with silicic acid does not perturb phase diagram

EISA performed with added yeast results in conformal 3-D lipid-silica-cell interface – It remains coherent with surrounding silica/lipid nanostructure and maintains aspects of viability upon dying and evacuation

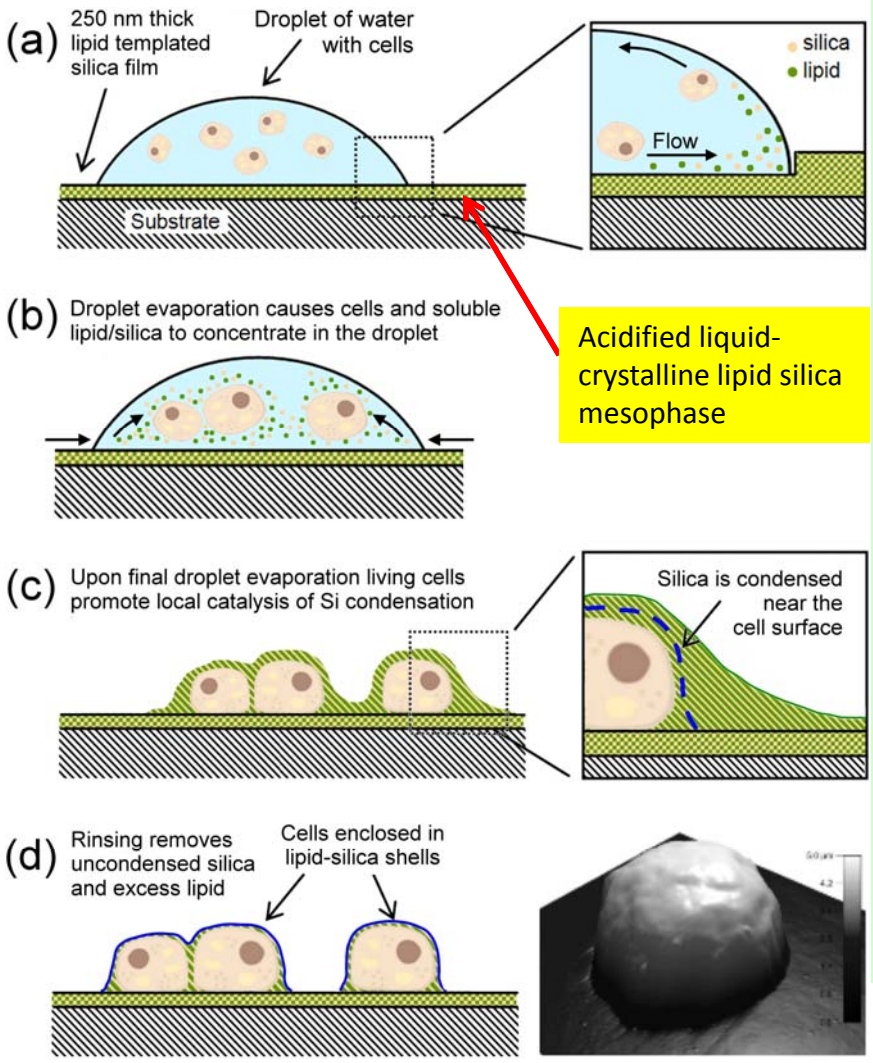


Spin-coating – (here) or spray drying, dip-coating etc.

What underlies the formation of a conformal, coherent interface that survives drying and evacuation with no dimensional change?

Alternative mechanism of cell-directed integration – Condensation of silicic acid is catalyzed @ cell-silica interface

Cell-Directed Integration†



a) *Film Solubilization*
- Introduction of an aqueous suspension of cells onto weakly condensed lipid/silica film solubilizes film components
- Marangoni and capillary flow arises from temperature and viscosity gradients

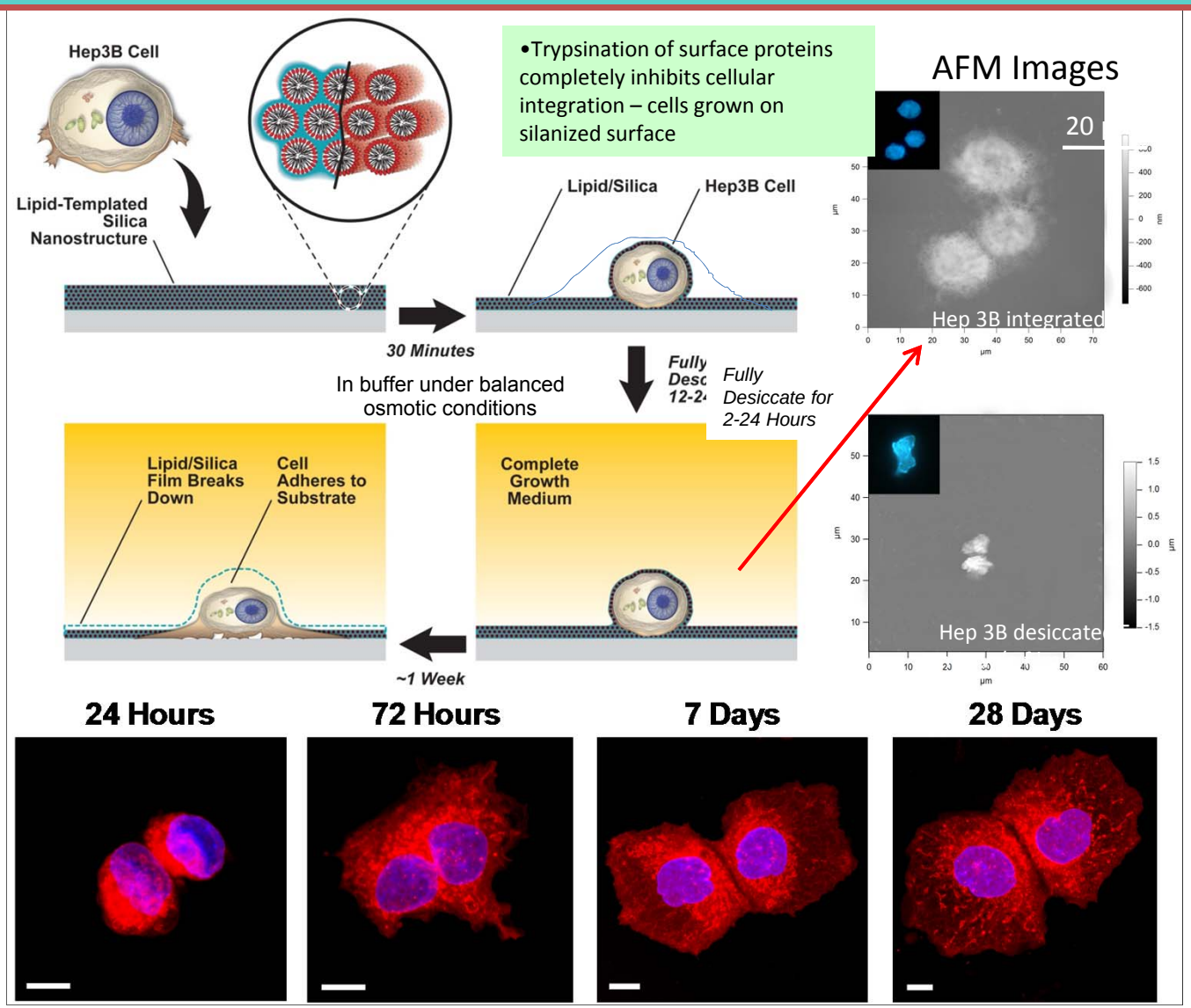
b) *Droplet Evaporation*
- Cells, and solubilized lipid and silica progressively concentrate in droplet

c) ***Cells Direct Silica Deposition***
- Cells increase local (μm scale) pH in response to evaporation induced osmotic stress – catalyzing 3D self-integration
- *W/O Evaporation under balanced osmotic conditions, cell surface components also catalyze silica condensation, allowing integration of mammalian cells

d) *Lipid-Rich Silica Shell*
- Rinsing the substrate leaves cells encapsulated in a conformal ultra-thin
- silica shell

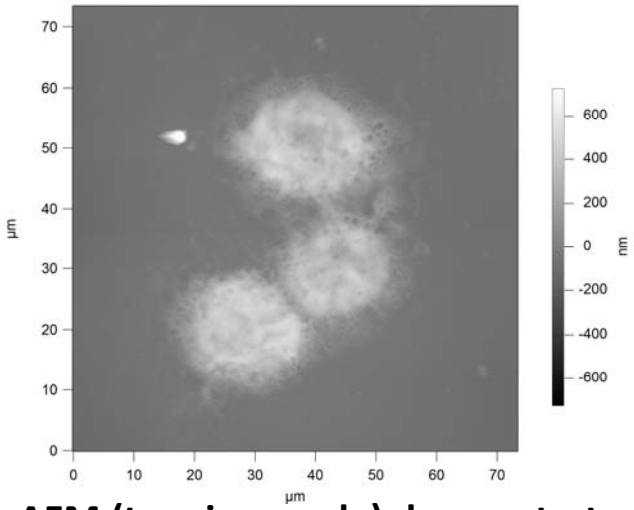
† Harper *et al*. *ACS Nano*, **2010**, *4*, 5539-5550.

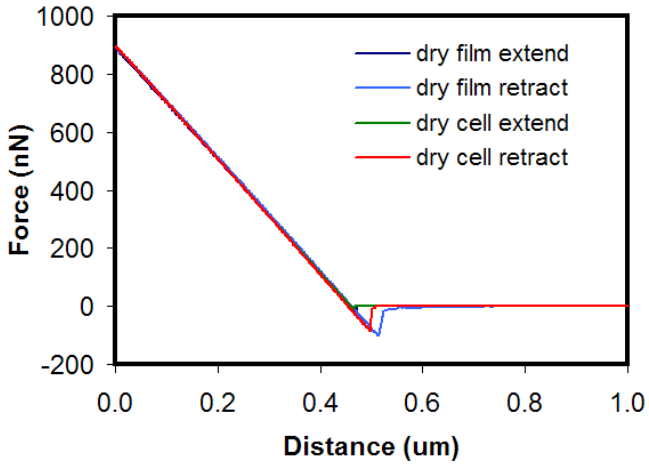
Mammalian Cells (Hep 3B) Integrate into Lipid-Templated Silica Nanostructures via 'Cell-Directed Integration' Without Evaporation



Upon dissolution of the silica matrix in fetal bovine serum, cells spread but do not replicate; they are synchronized and arrest in the cell cycle Ashley et al. *unpublished*

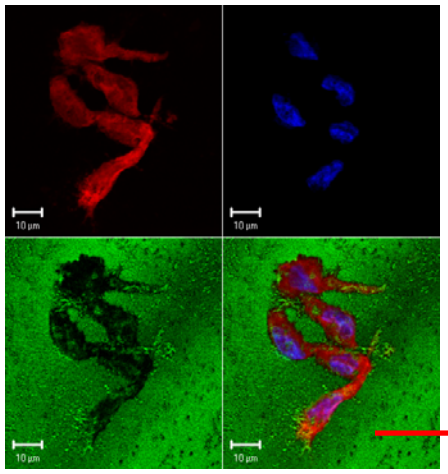
Surrounding 3D silica interface is ultra-thin, conformal, and stiff – it preserves cell integrity and 'viability' upon drying



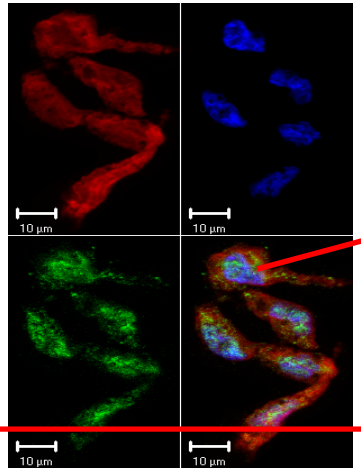


AFM (tapping mode) demonstrates that immobilized Hep3B cells (desiccated for 1 hour) retain their morphology; the regions around the cell are as stiff as the bulk film

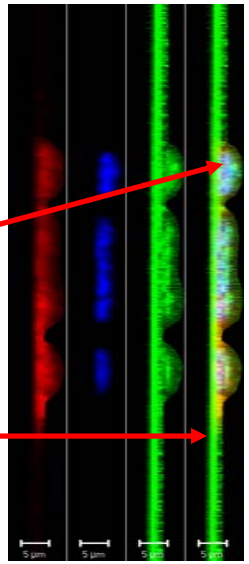
Immobilized Hep3B cells (stained with CellTracker Red and Hoechst 33342) aggregate NBD-labeled lipid (green) when desiccated



NBD-lipid in the bulk film

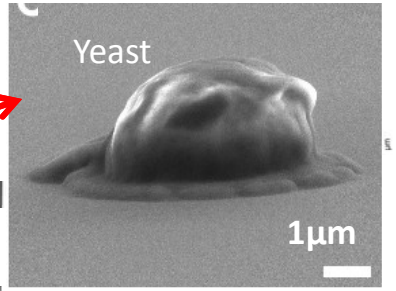


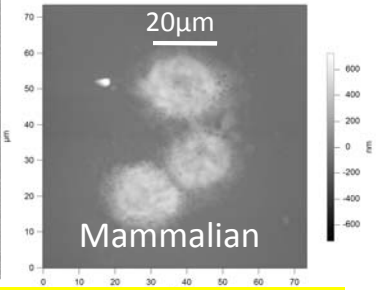
NBD-lipid aggregated around the cell

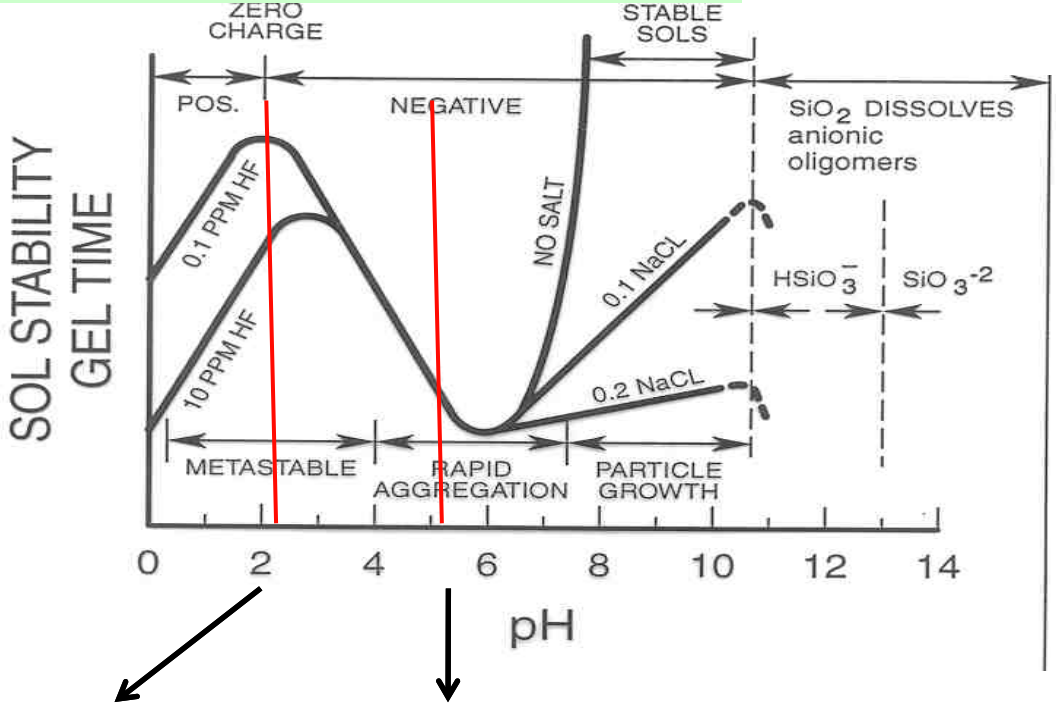


In Cell Directed Integration, what underlies the formation of a coherent, self-limiting, defect-free interface that survives drying and evacuation with no dimensional change – is it generalizable?

Initial Hypothesis: Silica condenses within a µm-scale pH gradient created at cell surface via osmotic stress response







Matrix pH

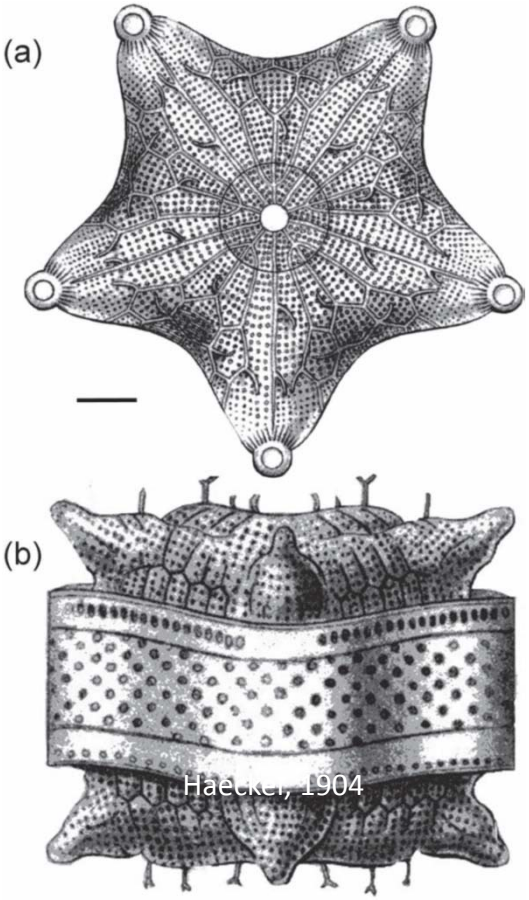
Cell surface pH

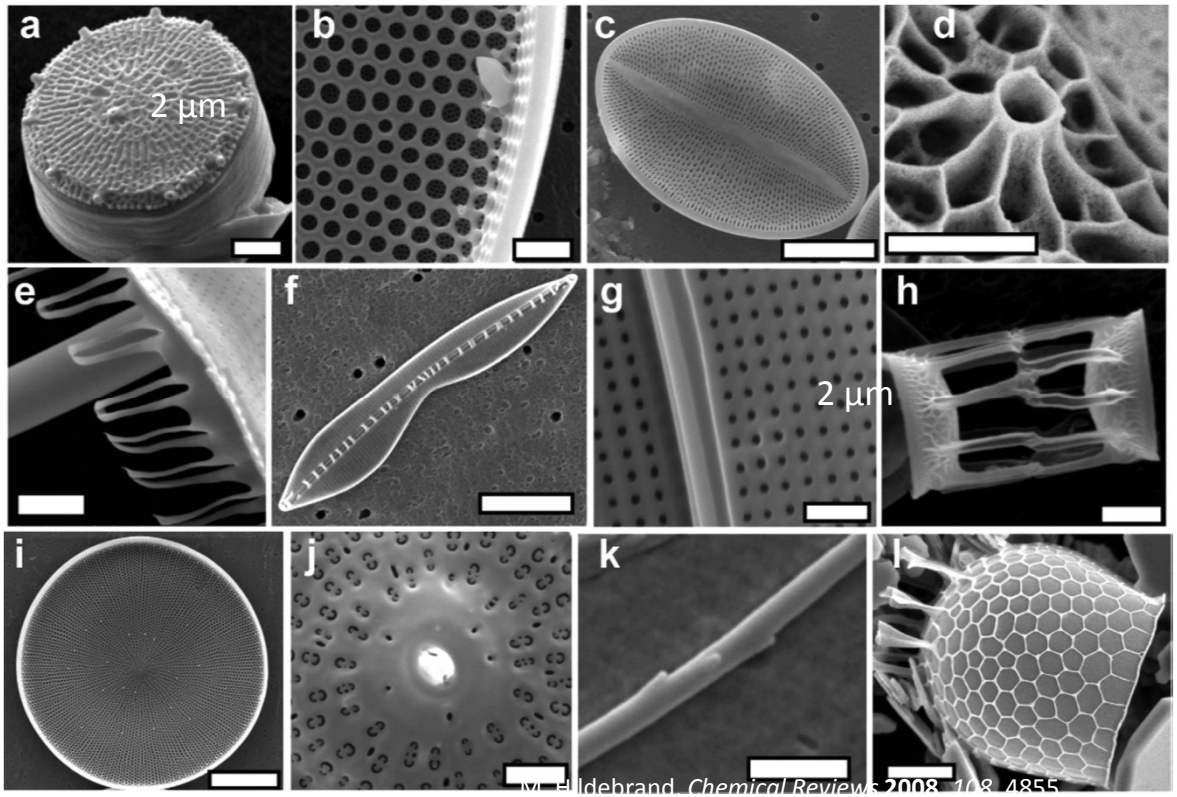
But, Osmotic stress not necessary…

Also, if pH promoted 'gelation', what preserves dimensional features and 'viability' of the cell upon drying, evacuation, etc., where structure should be subjected to tensile, capillary stresses and undergo substantial shrinkage?

Iler, The Chemistry of silica, Wiley, 1979
Brinker and Scherer, Sol-Gel Science , AP, 1990

Consider Cell-Directed silica deposition in natural systems – *diatoms* have been a fascination since the invention of the microscope, but how do they form? *Peptide specific interactions - tested by hydrolysis and condensation of TMOS* by proteins extracted from diatoms etc.





So far…A variety of proteins/enzymes with differing pIs (isoelectric points) have been extracted from diatoms and have been shown to direct silica condensation to produce globular silicates

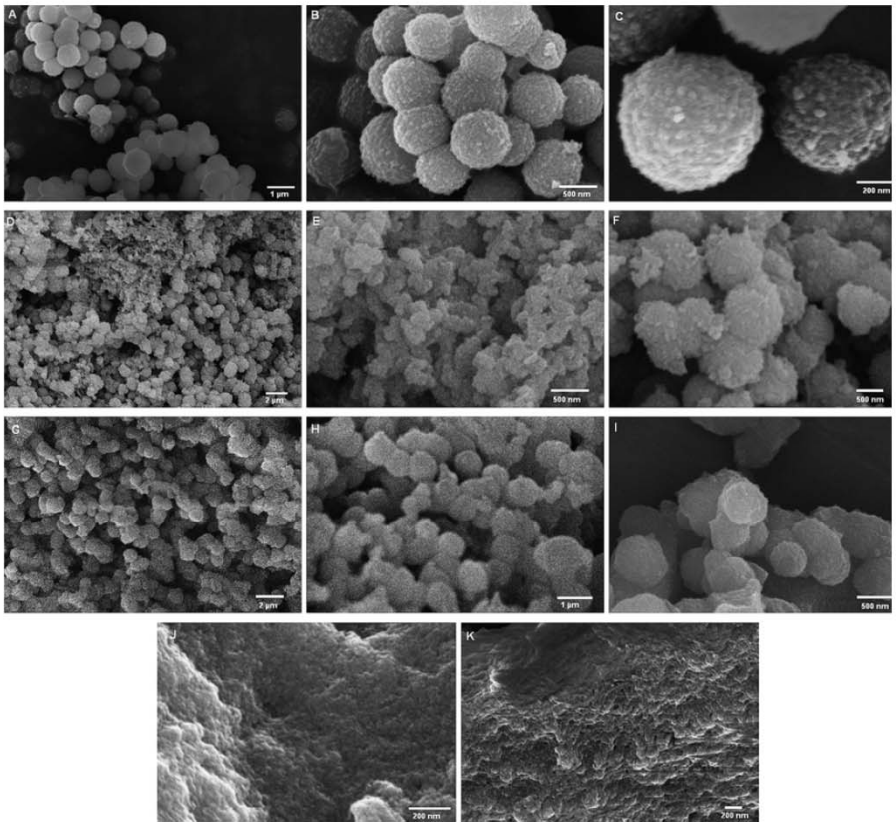


Table 1 Silica precipitating ability of various enzymes

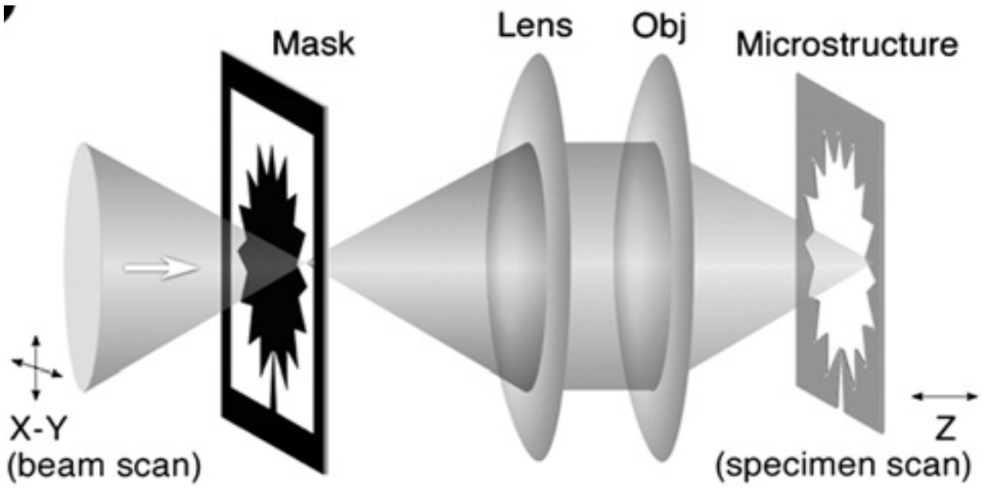
Enzyme	pI	Product	Physical state of solid silica	Time
Negative control	—	Gel		9 hours
Trypsin	10.5	Solid	Bimodal nanoparticles 100–200 nm + 700–950 nm	10 minutes
Papain	8.8–9.6	Solid	Nanoparticles 500–650 nm	15 minutes
Bromelain	9.6	Solid	Monolith	25 minutes
Tritirachium album proteinase K	8.9	Solid	Nanoparticles 450–900 nm	1 hour
Candida antarctica lipase A (CAL)	7.5	Solid	Monolith	15 minutes
Alkaline phosphatase	4.5	Gel		9 hours
Rennin	4.5	Gel		8 hours
Rhizopus oryzae lipase (ROL)	6.9	Gel		7 hours

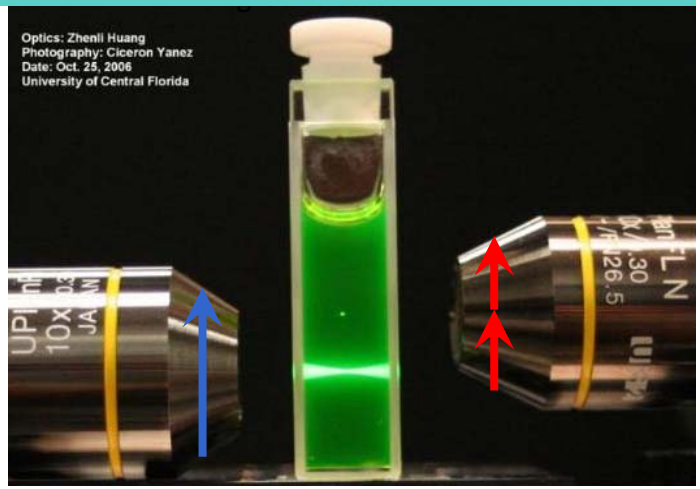
J. Mater. Chem., 2009, 19, 7606–7609

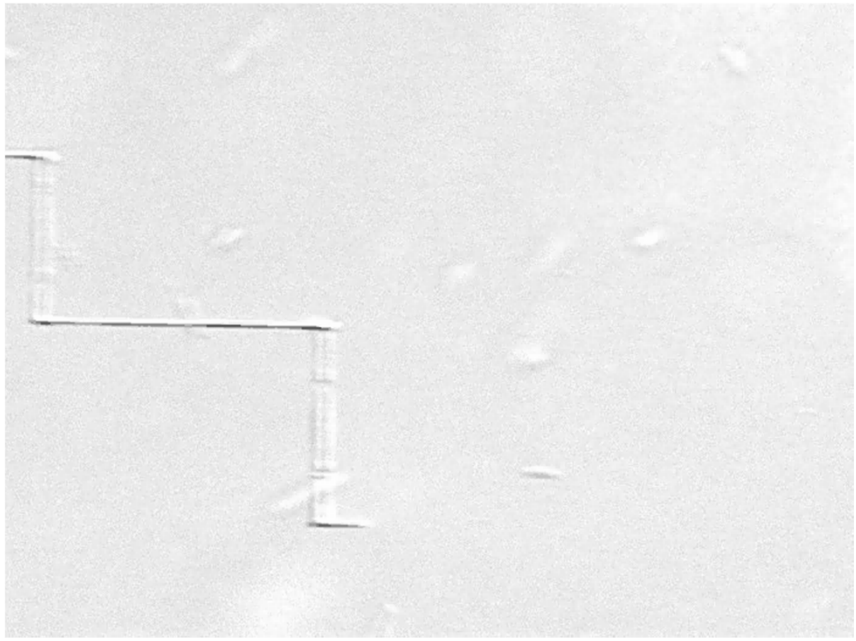
Monosilicic acid $Si(OH)_4$, which occurs in natural habitats in concentrations between 1 and 100 mM is silica source - Polyamines may catalyze the polycondensation of silanol groups

Hypothesis: protein display on 3D scaffolds presents a crowded organizational motif that locally concentrates soluble silica and catalyzes condensation

Bryan Kaehr (SNL)

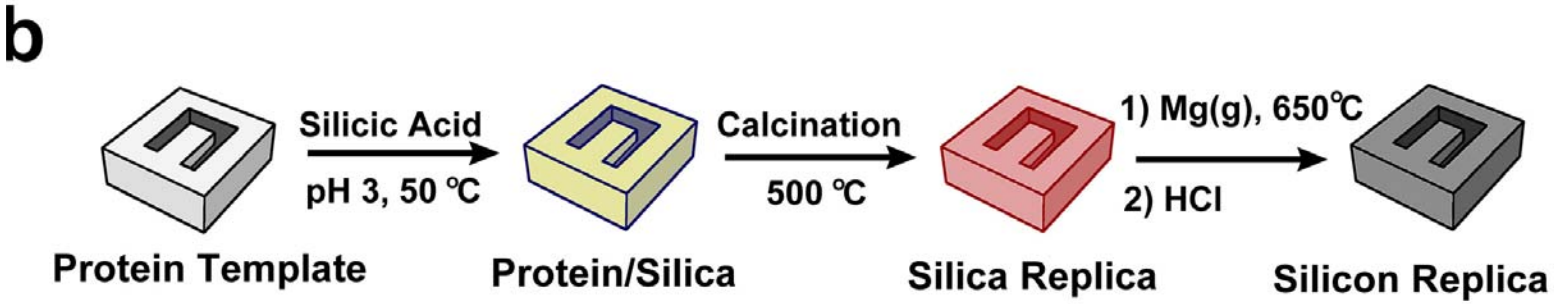


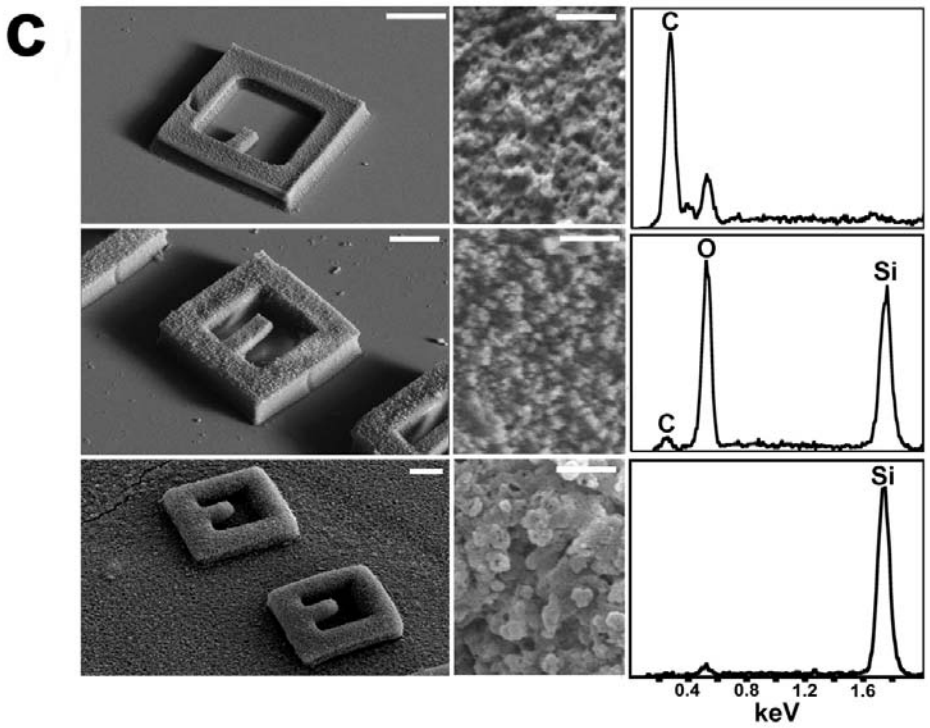


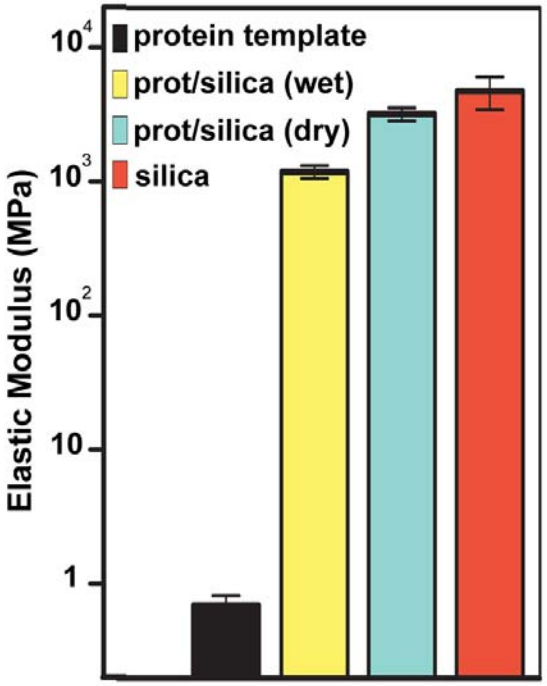


Light from a femtosecond titanium: sapphire laser is sent through a confocal scan box to raster the beam

Protein-directed Materials Deposition Using Volumetric Templating within a User-Defined Protein Scaffold Fabricated by Multi-Photon Lithography







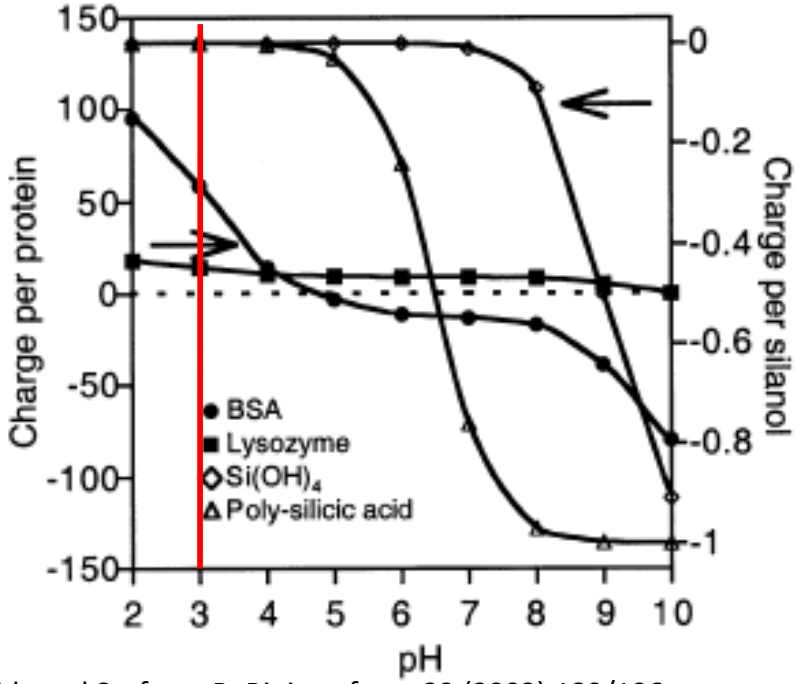
C. Y. Khripin, C. J. Brinker and B. Kaehr*, Soft Matter, 2010, 6, 2842-2848

Silica Deposition Occurs with Moderate to Modest Electrostatic Interactions – *using many different protein templates*

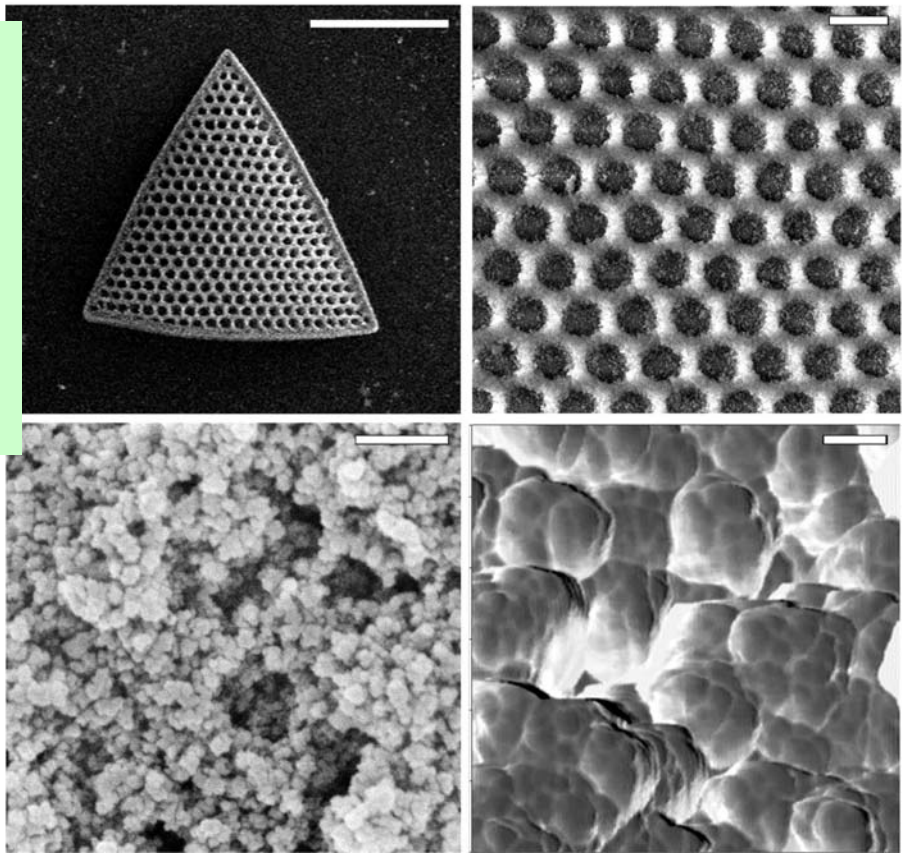
•Incubation of lithographically-defined BSA protein hydrogel with polysilicic acid (100mM) at pH 3 •silica nucleation, growth, and coarsening are templated volumetrically within the 3D scaffold

•Overall/net electrostatic interactions are modest – hydrogen-bonding dominates

• 16-nm primary particles form, aggregate and coarsen within 3D protein scaffold.



Colloids and Surfaces B: Biointerfaces 29 (2003) 189/196

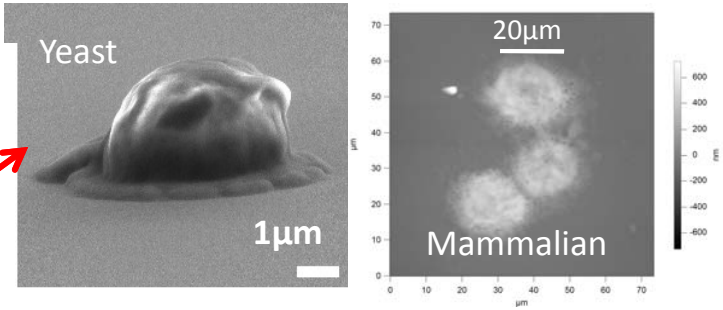


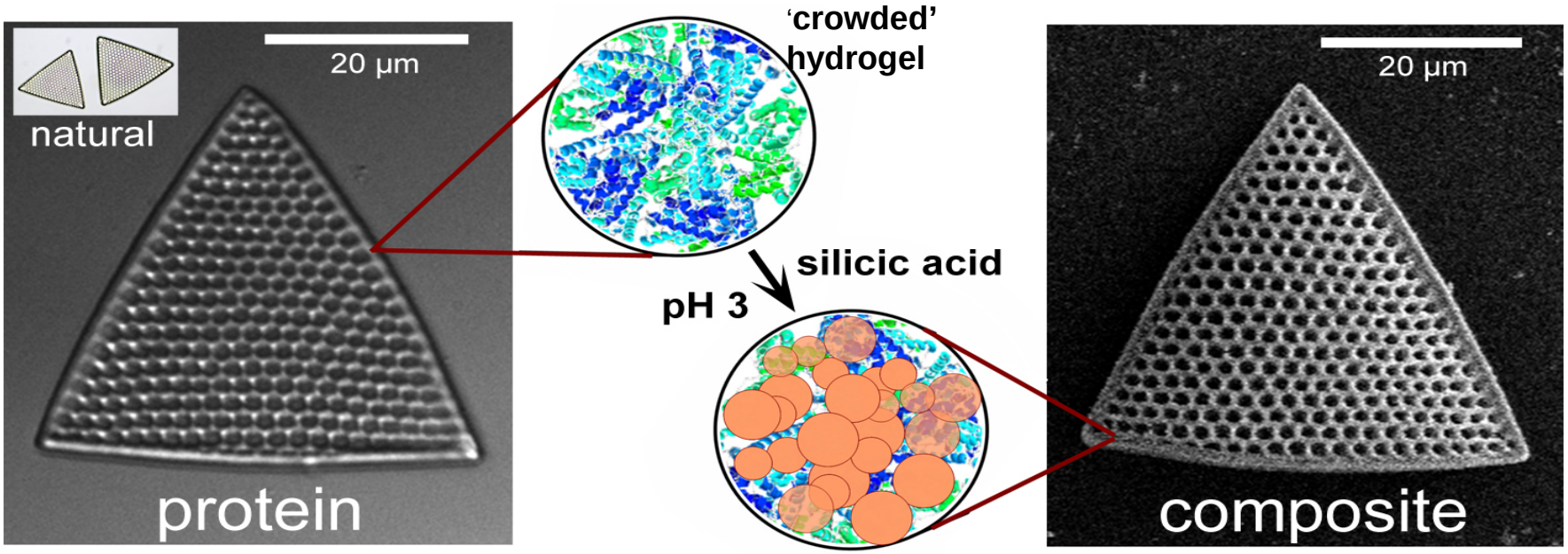
Scale bars are (top) 20 µm, 2 µm, (bottom) 200nm, 50nm

Protein directed silica deposition occurs non-selectively for proteins with different pIs – multivalent patterns of hydrogen bonding within crowded scaffold

Scaffolded MPL defined 3D protein architectures direct the formation of arbitrary user-defined silica materials

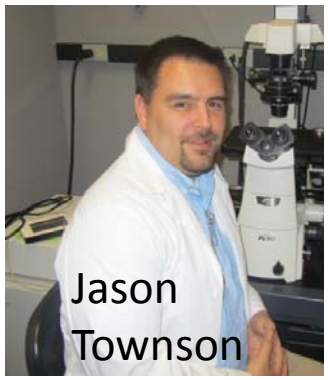
cell surface proteins/components may similarly direct conformal dimensionally stable silica deposition in cell-directed assembly

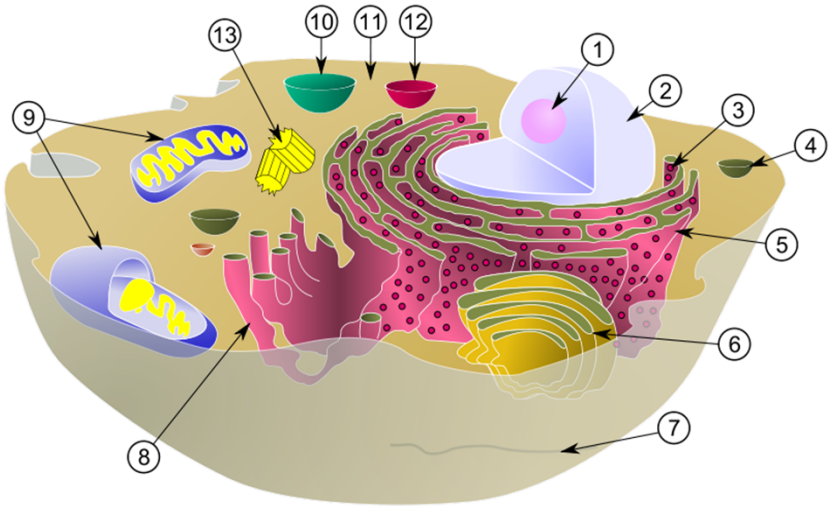


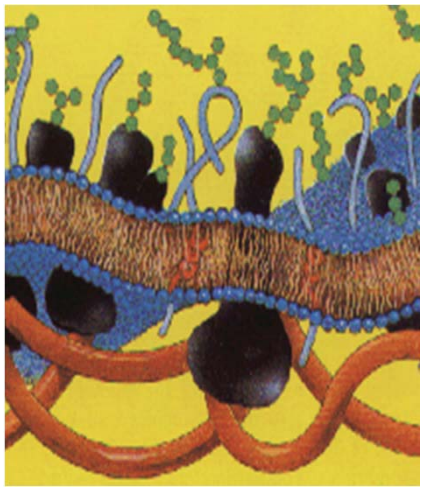


Khripin, C.Y., Pristinski, D., Dunphy, D.R., Brinker C. J., Kaehr, B.* (2011) ACS Nano, 5, 1401-1409.

Hypothesis: the highly crowded cellular microenvironment can serve as a 3D bio-molecular scaffold of catalysts with which to direct conformal, dimensionally stable silica deposition

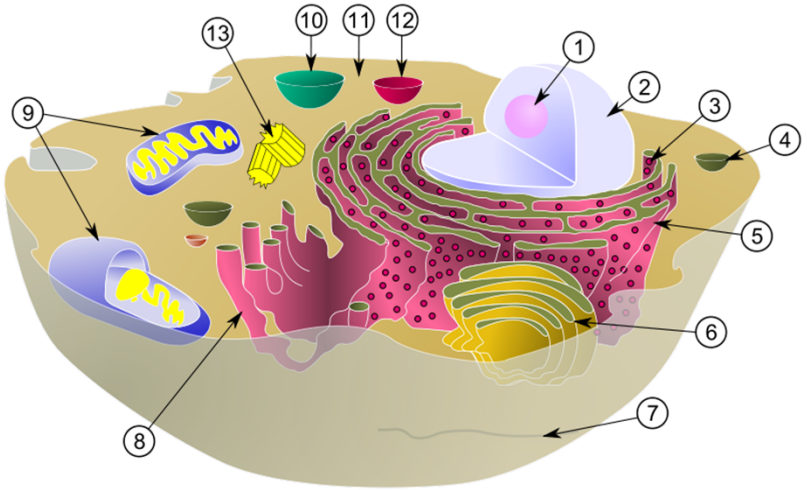






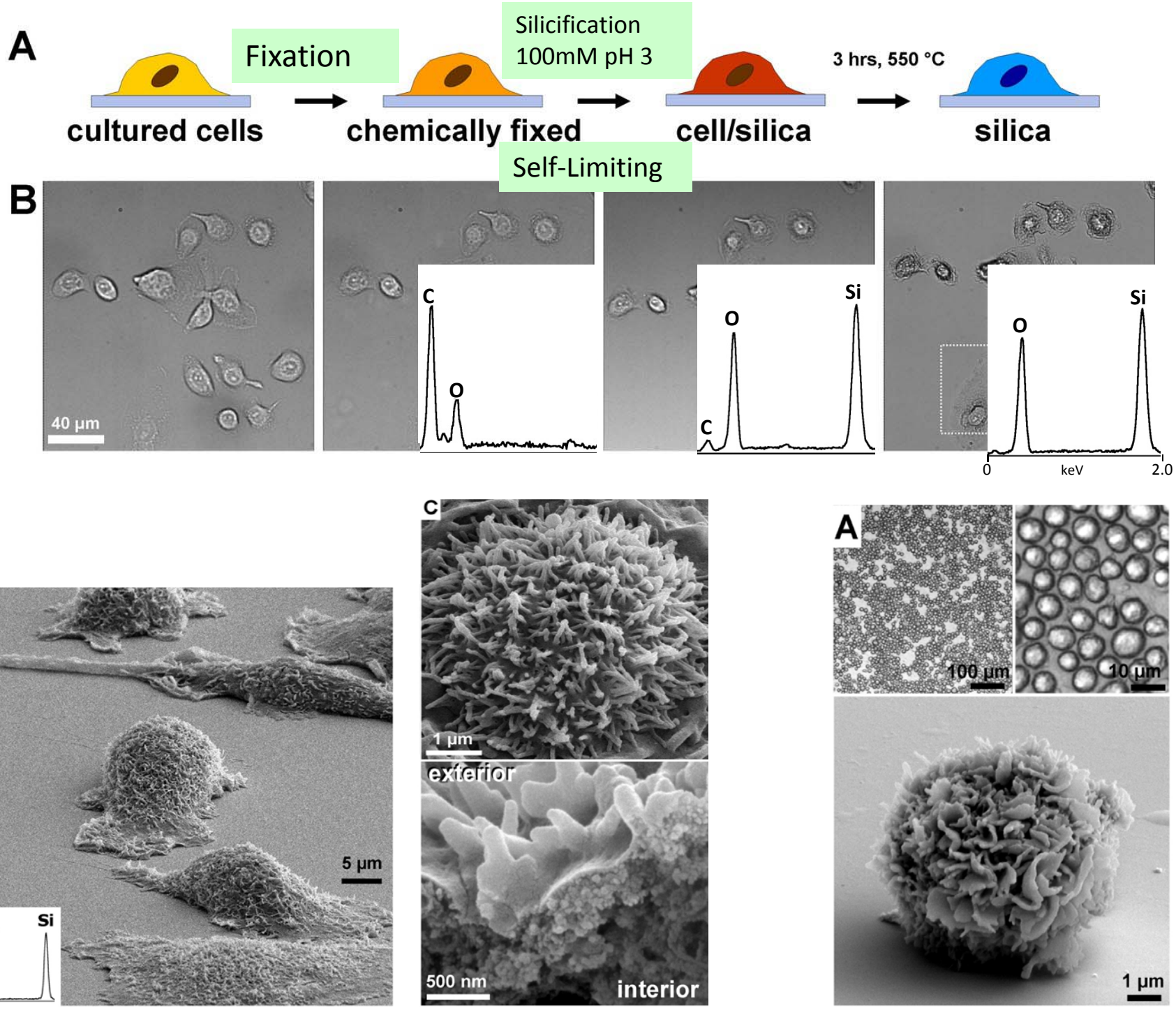
Schematic of typical animal cell, showing subcellular components. Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

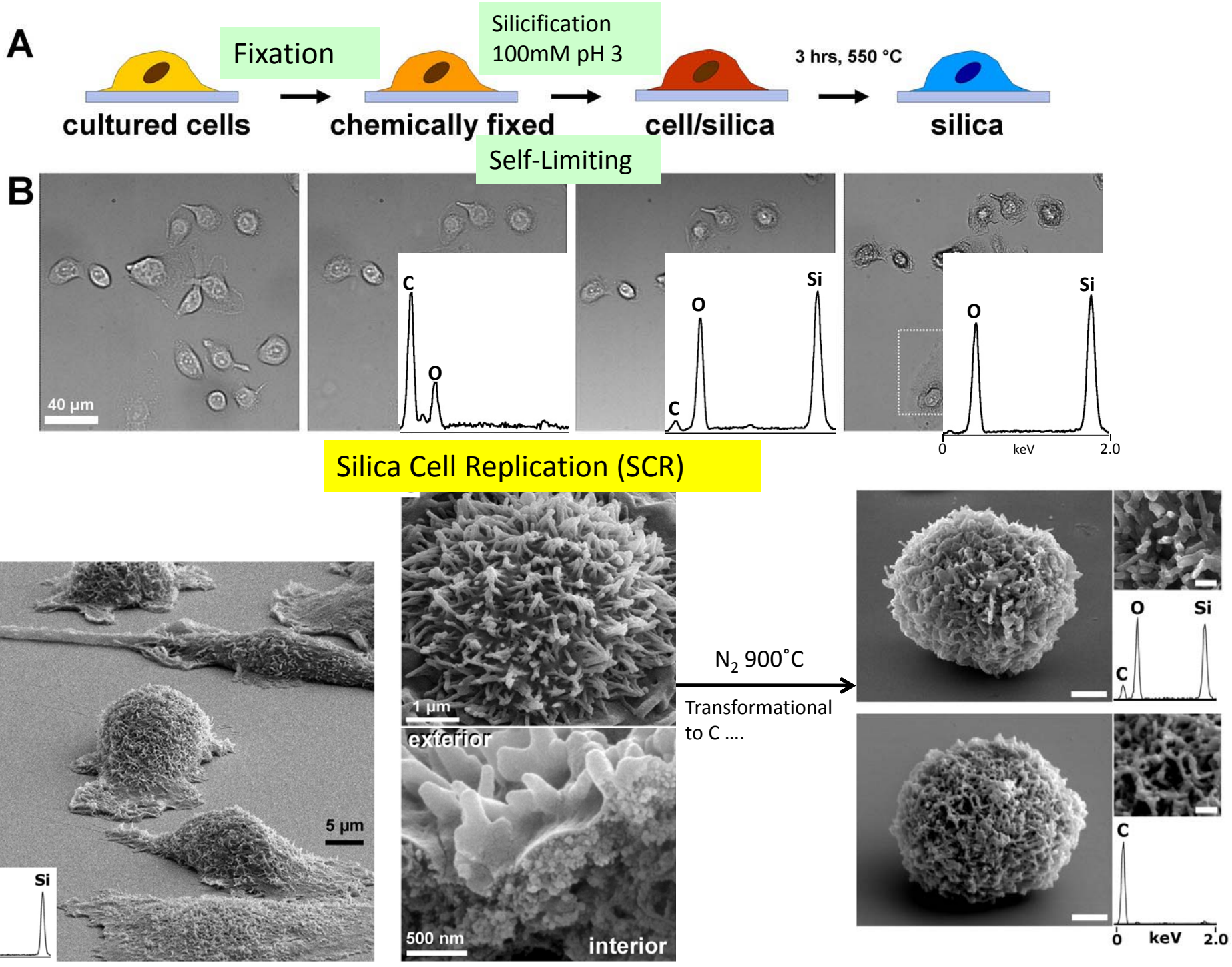
Hypothesis: the highly crowded cellular microenvironment can serve as a 3D bio-molecular scaffold of catalysts with which to direct conformal, dimensionally stable silica deposition?



Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

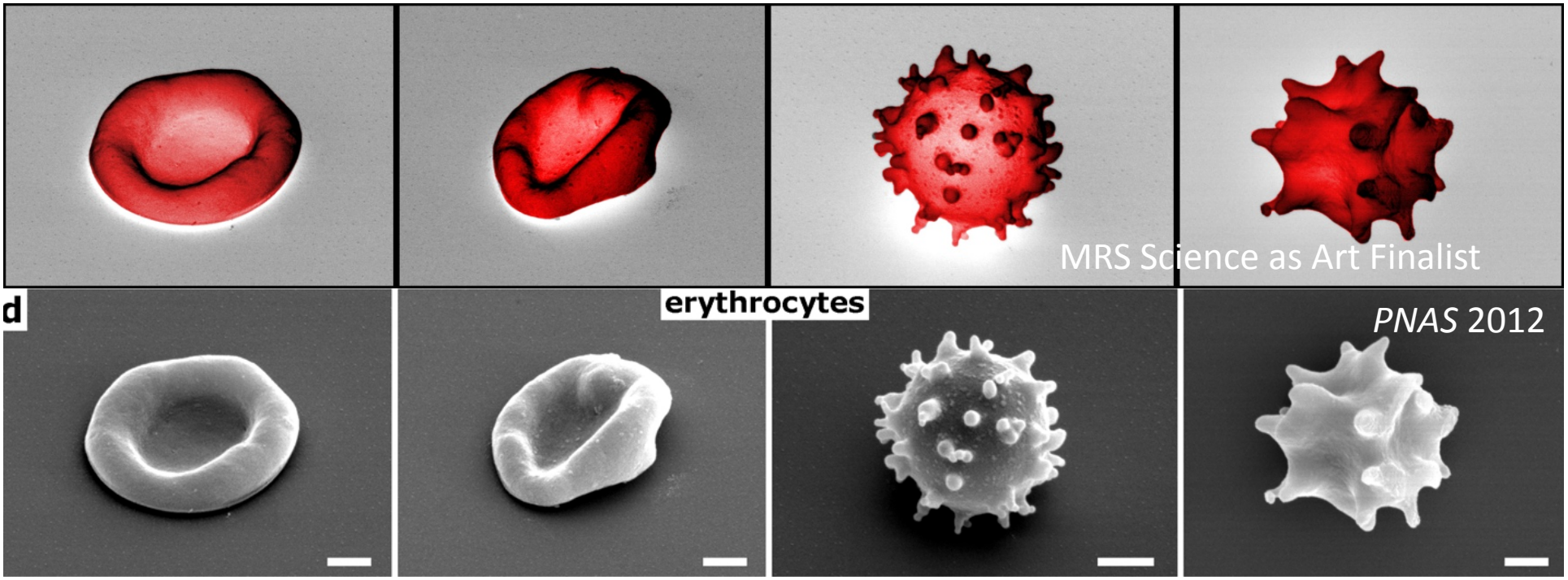
Cryo-TEM Tomography - beta cells preserved *in situ* in pancreatic islet tissue isolated from mice March et al. PNAS 2004; 101: 5565-5570


A
Fixation
Silicification
100mM pH 3
3 hrs, 550 °C
cultured cells
chemically fixed
cell/silica
silica
Self-Limiting
B
40 µm
C
O
Si
0
keV
2.0
B
5 µm
O
Si
C
1 µm
exterior
500 nm
interior
A
100 µm
10 µm
1 µm


A
Fixation
Silicification
100mM pH 3
3 hrs, 550 °C
cultured cells
chemically fixed
cell/silica
silica
Self-Limiting
B
40 µm
C
O
O
Si
C
O
Si
0
keV
2.0
Silica Cell Replication (SCR)
B
5 µm
O
Si
1 µm
exterior
500 nm
interior
N2 900˚C
Transformational
to C ….
O
Si
C
C
0
keV
2.0

Use exquisite sensitivity of cells to environmental factors to program cell shape, which is faithfully replicated *in silica*

Blood cells and their varying morphologies induced by osmotic stress are replicated with high fidelity – *dissipative materials assembly?*

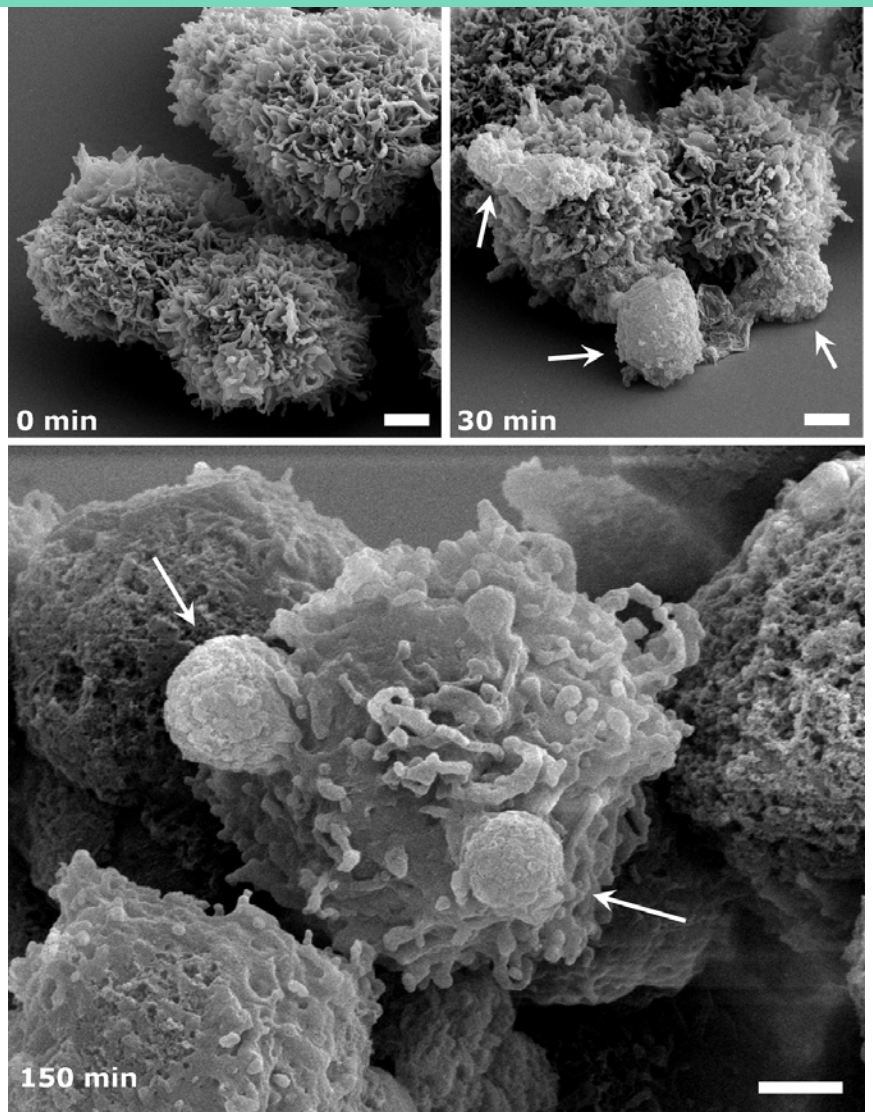


Scale bars = 1µm

❿Increasingly abnormal/crenate morphology resulting from increasing levels of osmotic stress – energy consumed/transduced to alter cell shape and protein expression, which are protected and preserved within silica and transformable to other chemistries

- Cells can be decorated with NPs etc. prior to shape change

Direct phenotypic changes and capture the dynamics of these changes on the time scale of minutes

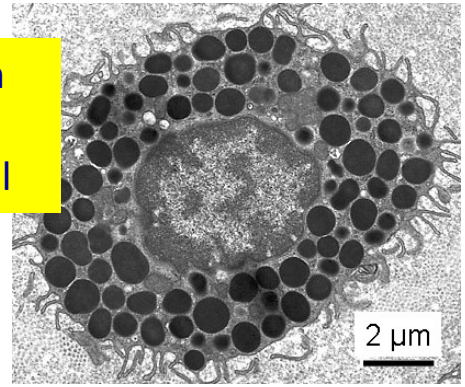


Clusters of calcined (500 °C, 3 h) silica replicas templated from 4T1 cells incubated in 5 **µM doxorubicin to induce apoptosis. Arrows denote apoptotic** blebs. **(Scale bars, 2 µm.)**

Stimulate and Replicate Cellular Dynamics: Inflammatory Response of Rat Basophilic Leukemia (RBL) Cells

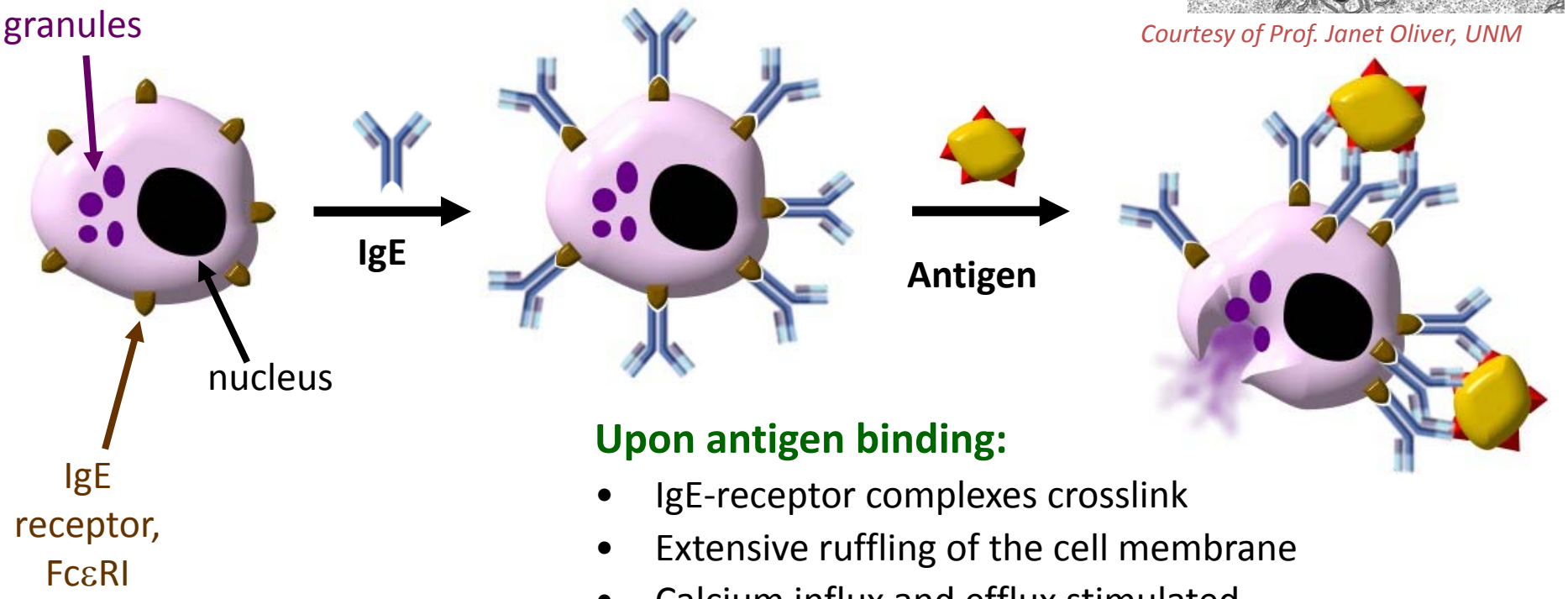
Basophiles are a class of white blood cells chiefly responsible for inflammatory and allergic response – antigen binding initiates cascade of signaling and phenotypic events

TEM cross-section image of antigen-stimulated RBL cell



Courtesy of Prof. Janet Oliver, UNM

Mechanism of Response†

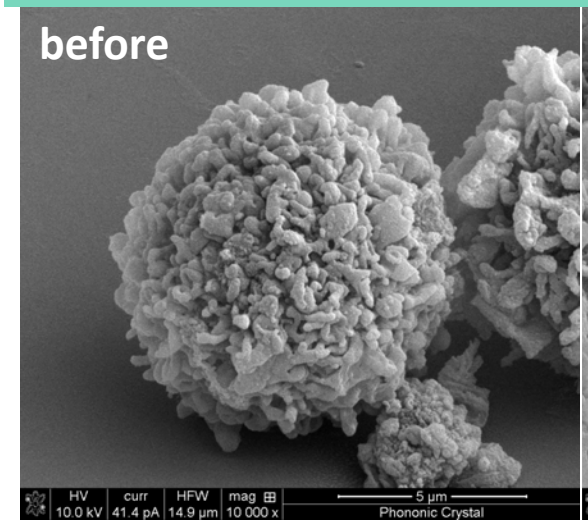


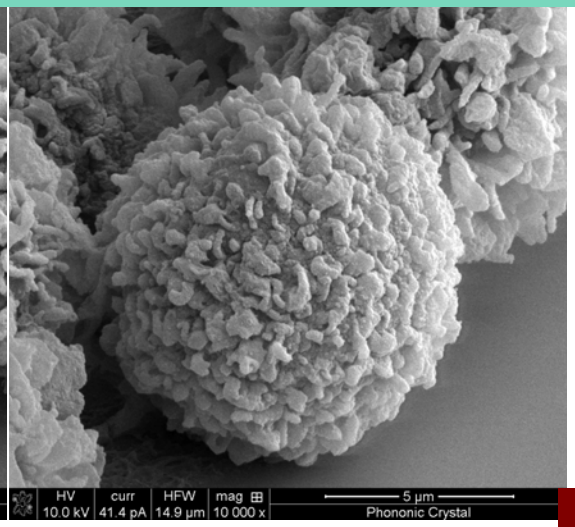
Upon antigen binding:

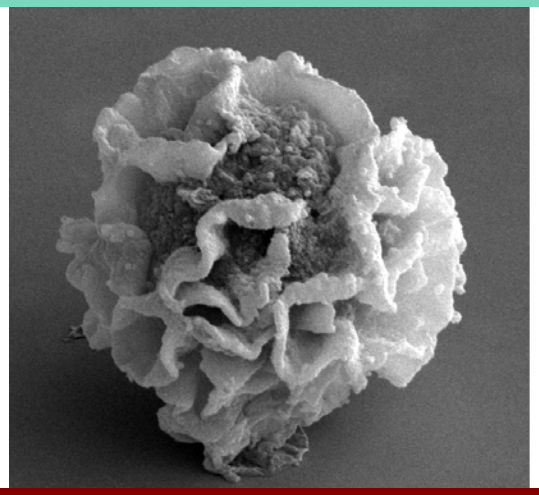
- IgE-receptor complexes crosslink
- Extensive ruffling of the cell membrane
- Calcium influx and efflux stimulated
- Contents of granules (i.e. histamine) expelled

† Lee *et al. Cytometry* **1992**, *13*, 127.

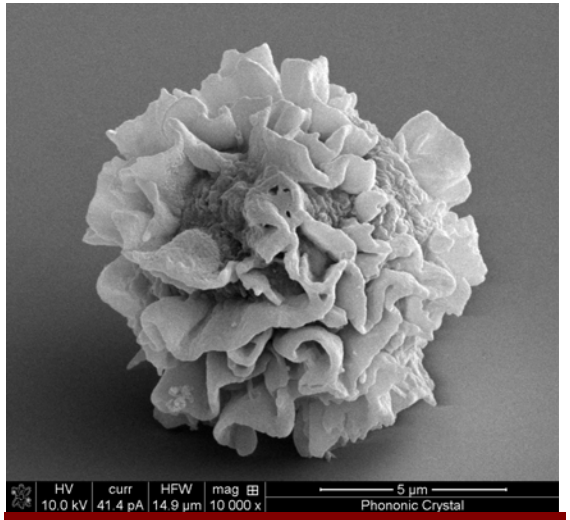
Silica replicas of RBL-2H3 cells before and after FcεRI cross-linking in response to antigen lipopolysaccharide



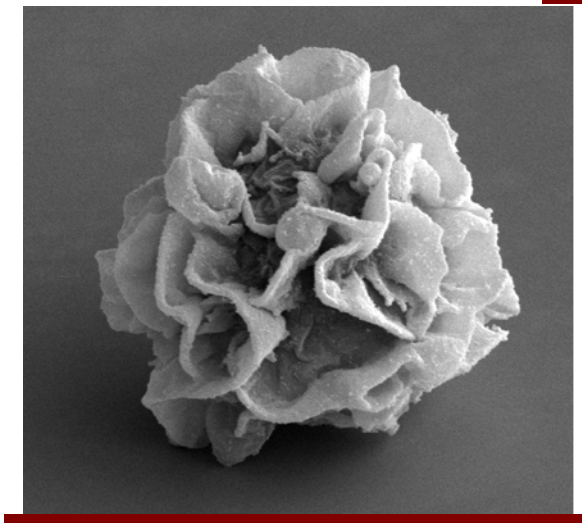




After LPS + 15 minutes



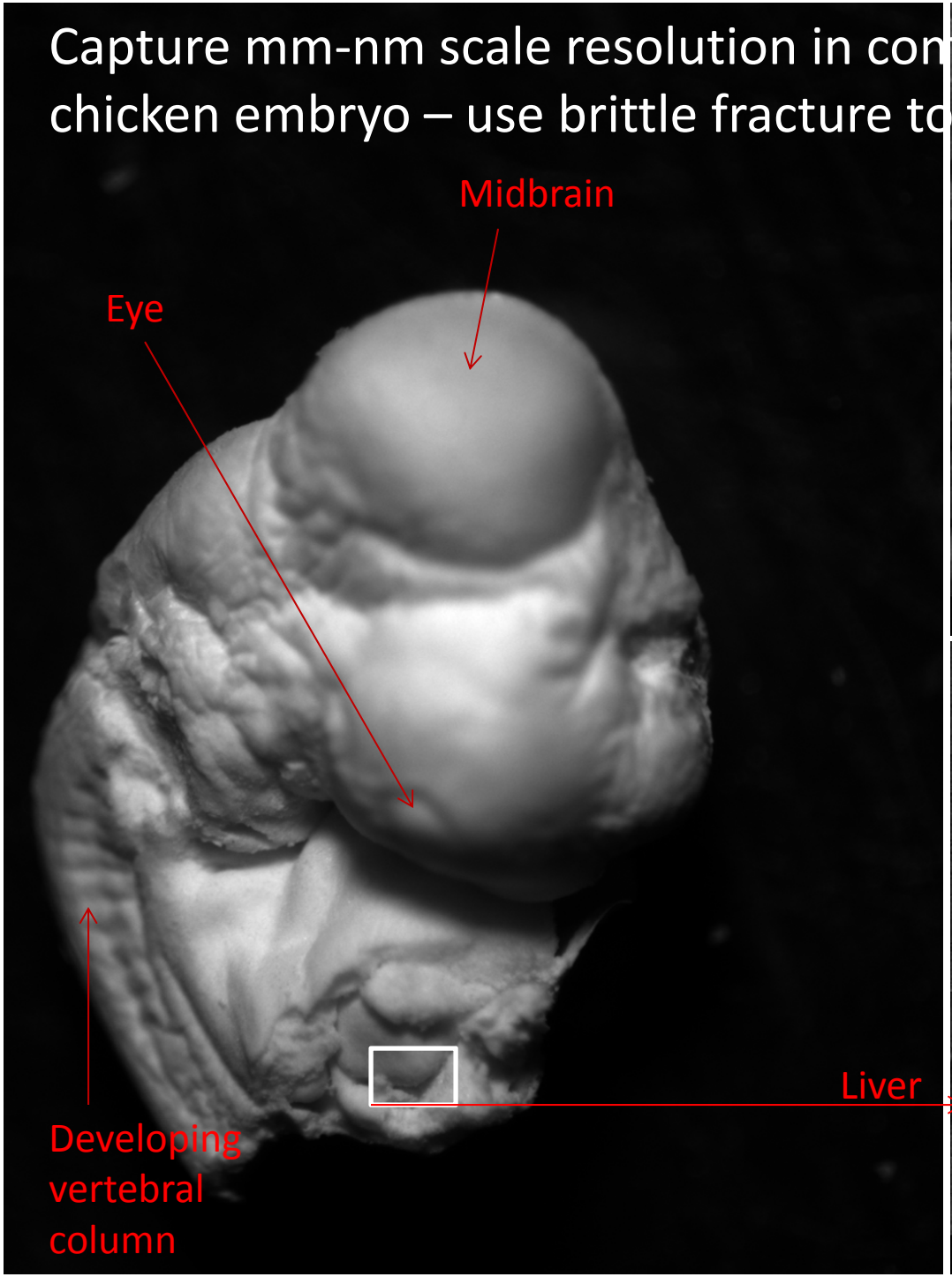
After LPS + 30 minutes

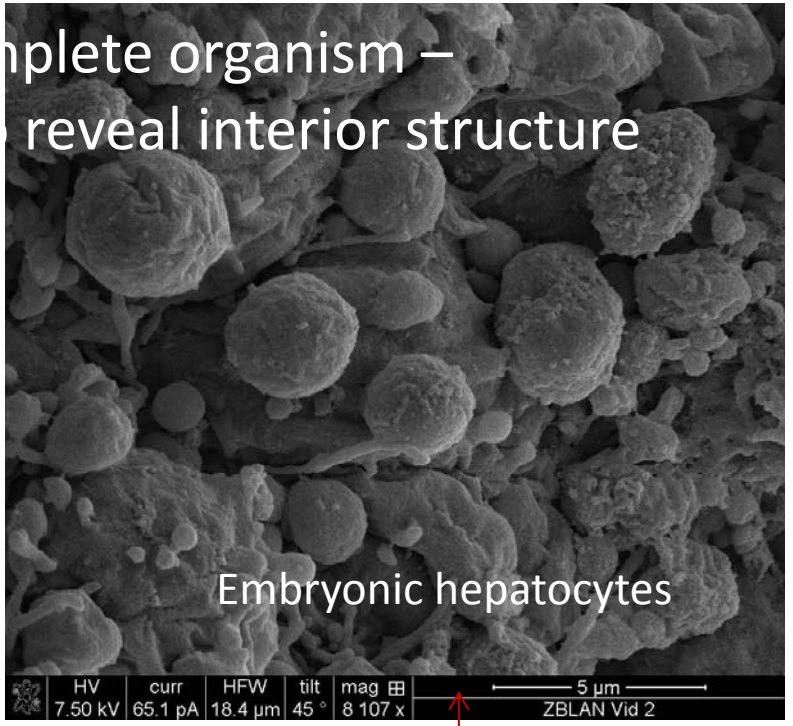


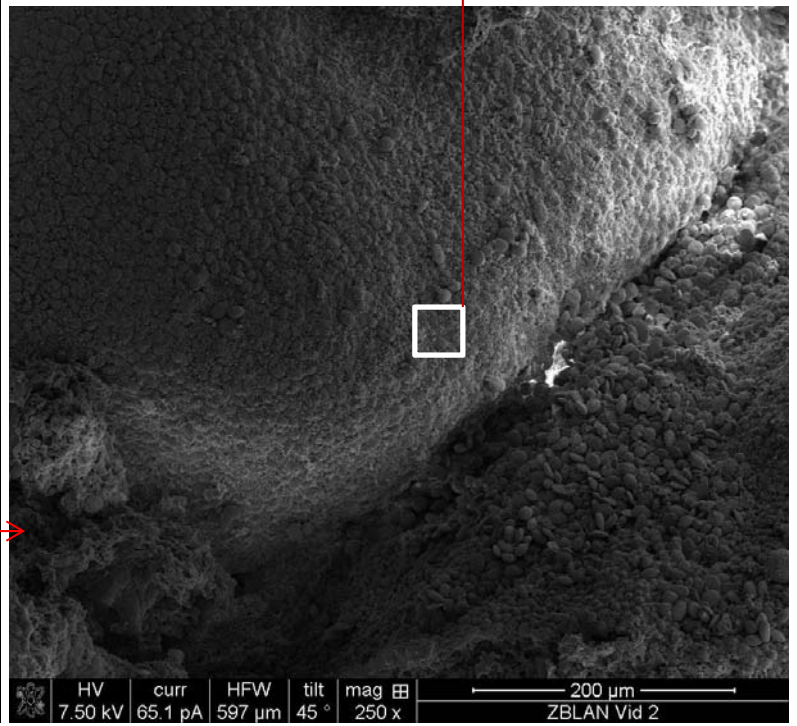
After Calcination 550˚C

Dynamic malleability of cell used to program cell morphology, which is preserved with dimensional fidelity in robust silica composites and replicas – extend with cellular processing of molecular and NP cargos and transformational chemistry, e.g. Sandhage Ga Tech

Capture mm-nm scale resolution in complete organism – chicken embryo – use brittle fracture to reveal interior structure



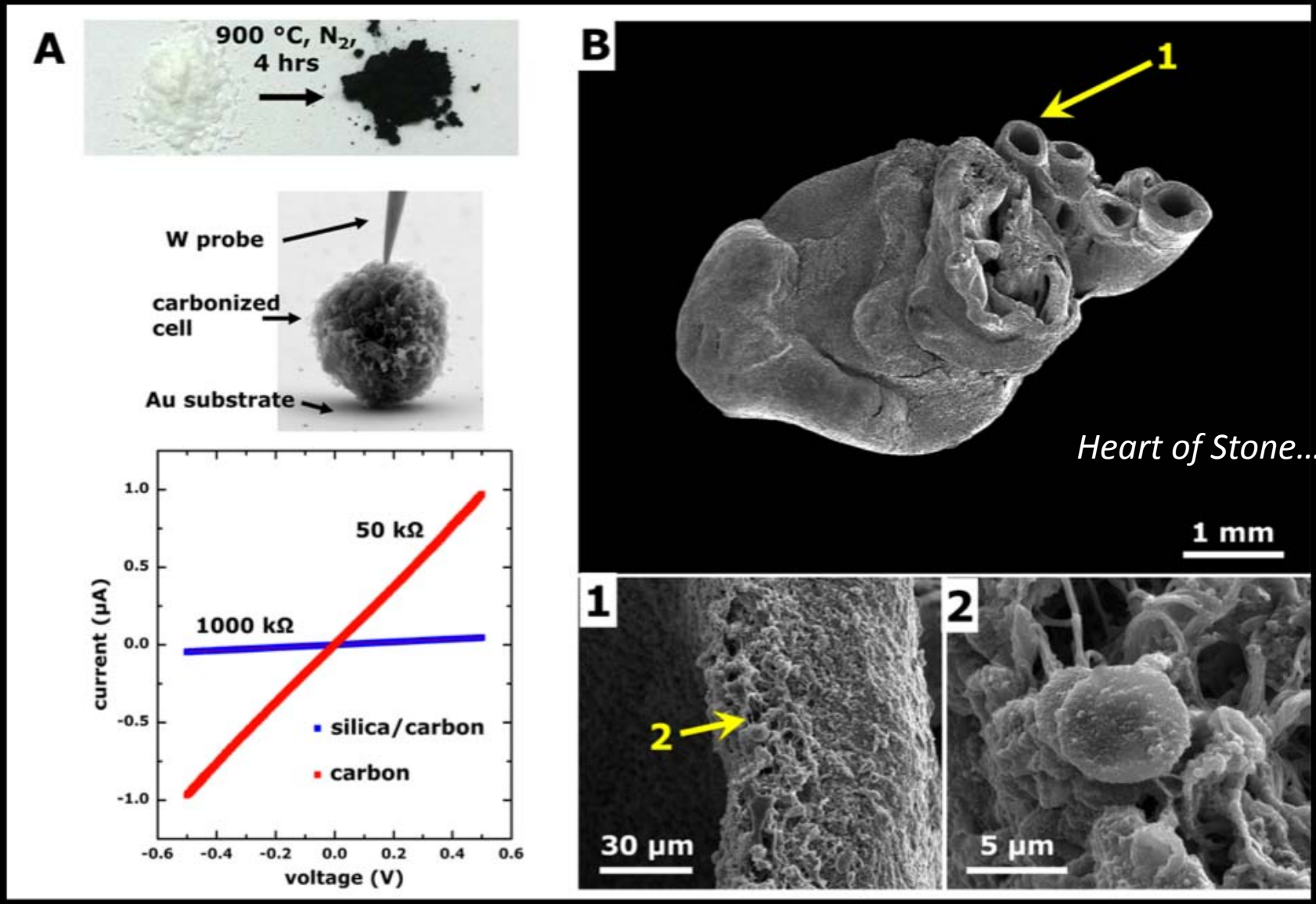




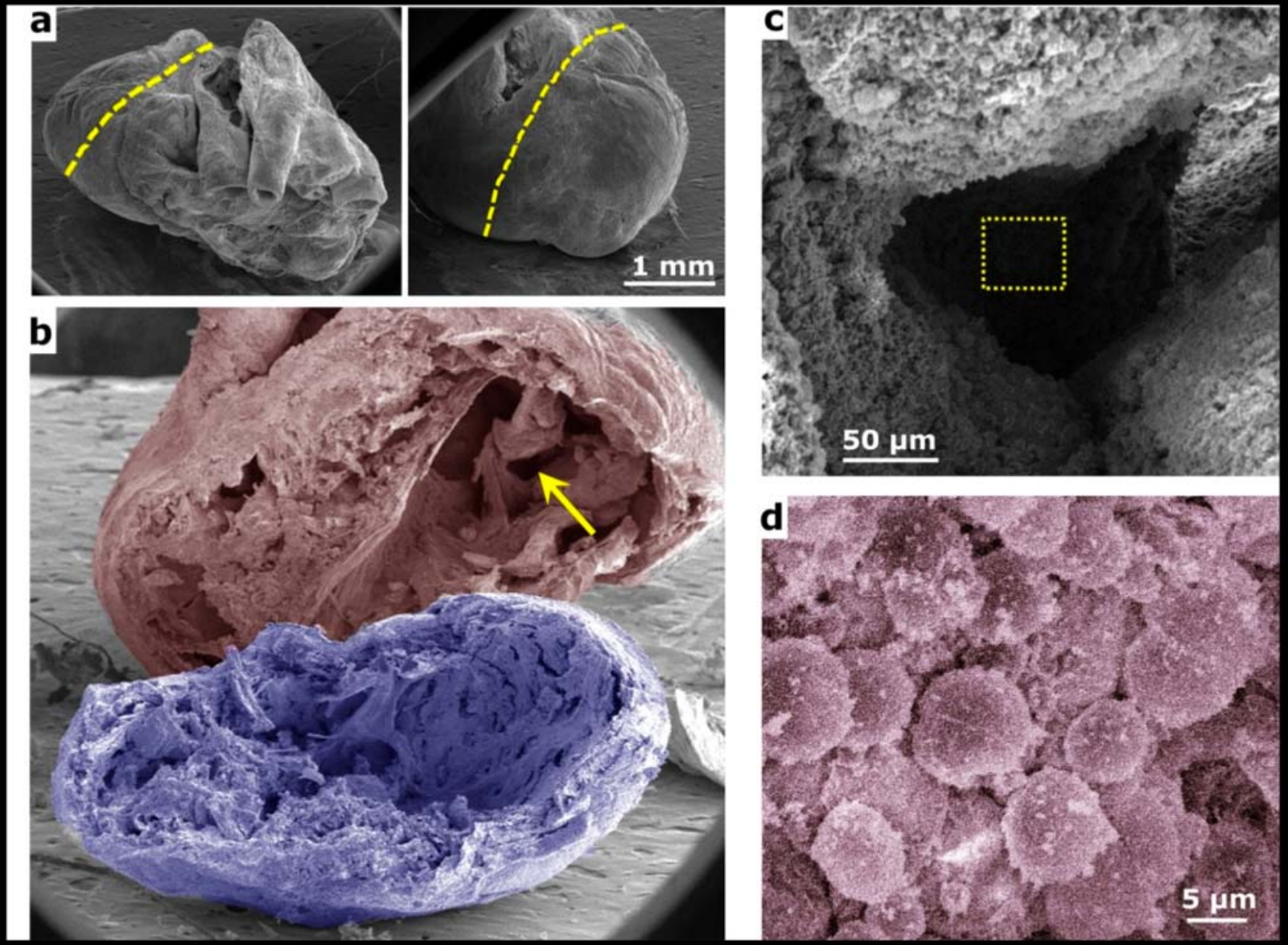
‘Brittle’ Fracture of the Organism: Allows us to capture with high fidelity selected regions within the interior the organism – here red and white blood cells within the venous system of the chick embryo liver

carbonization

1000 C, H5N, 12 hrs



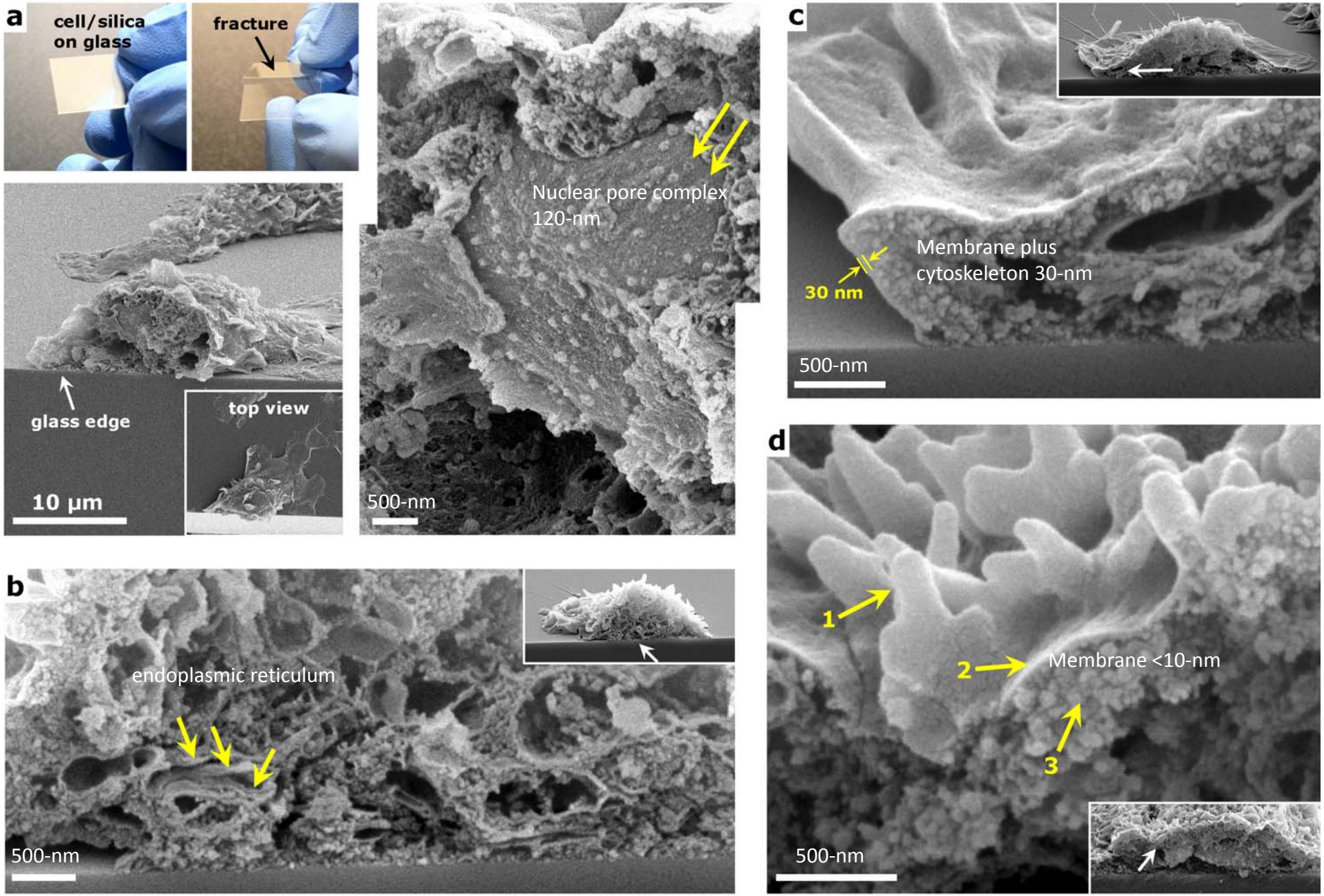
Sectioning and imaging without conductive coating



30 kV, ~nA

Jason Townson, chx embryo in vivo model

What is the ultimate resolution/precision of the replication process?



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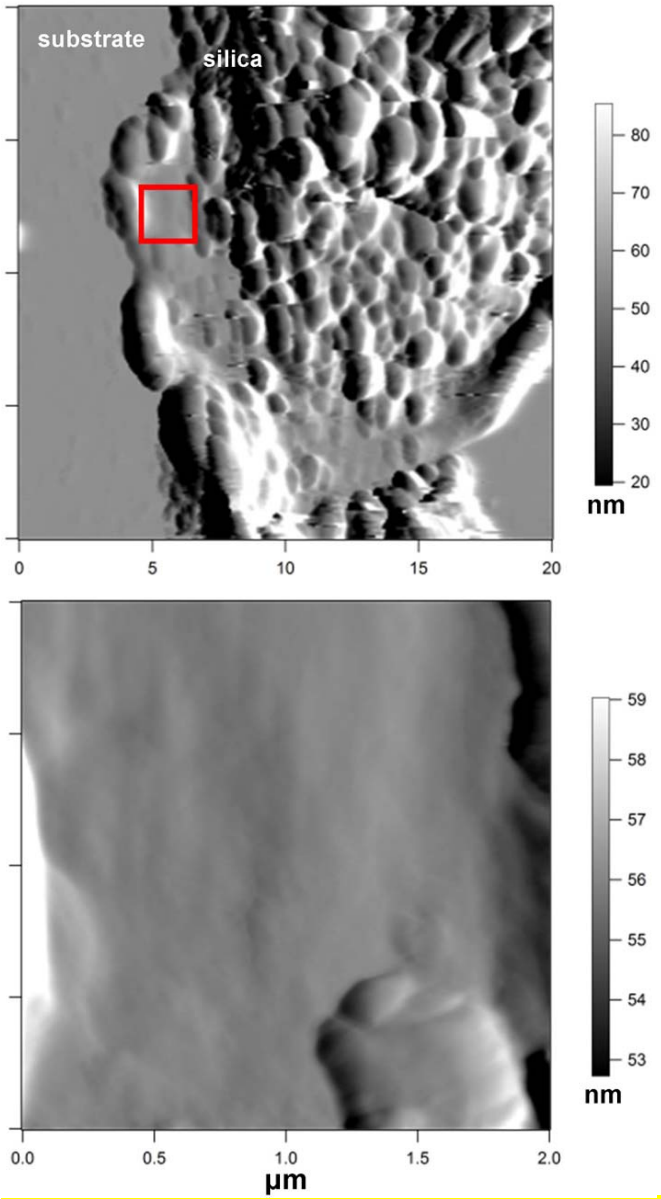
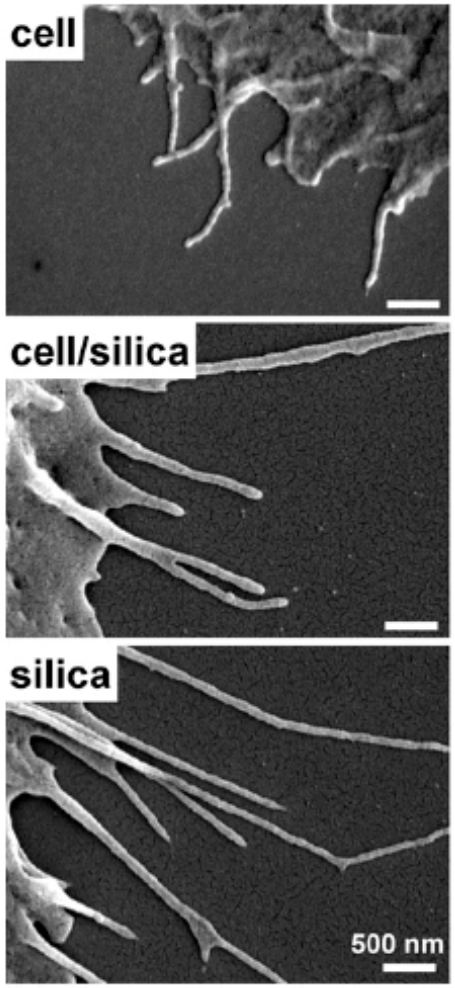
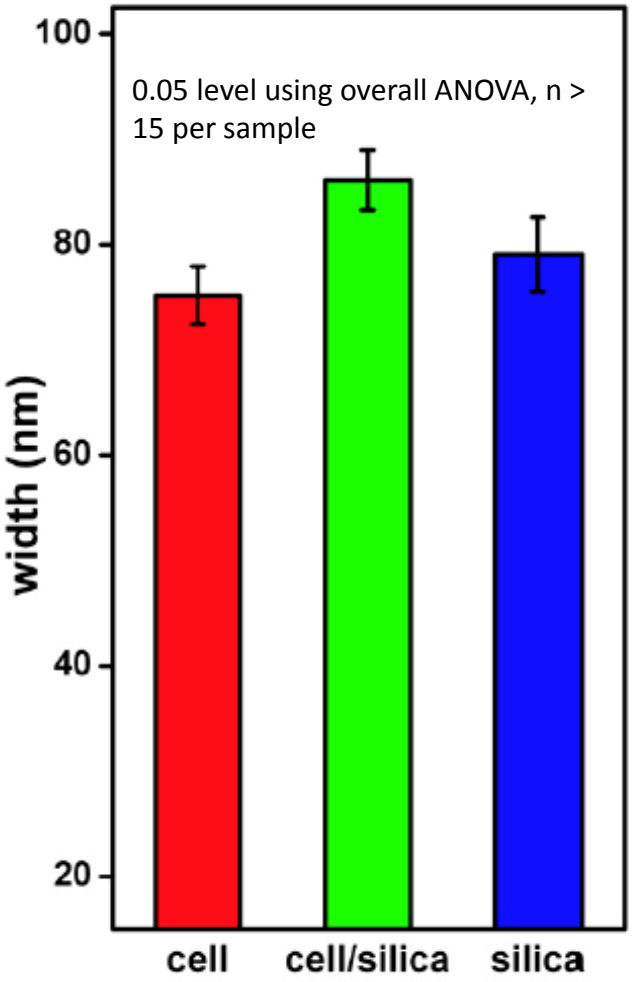




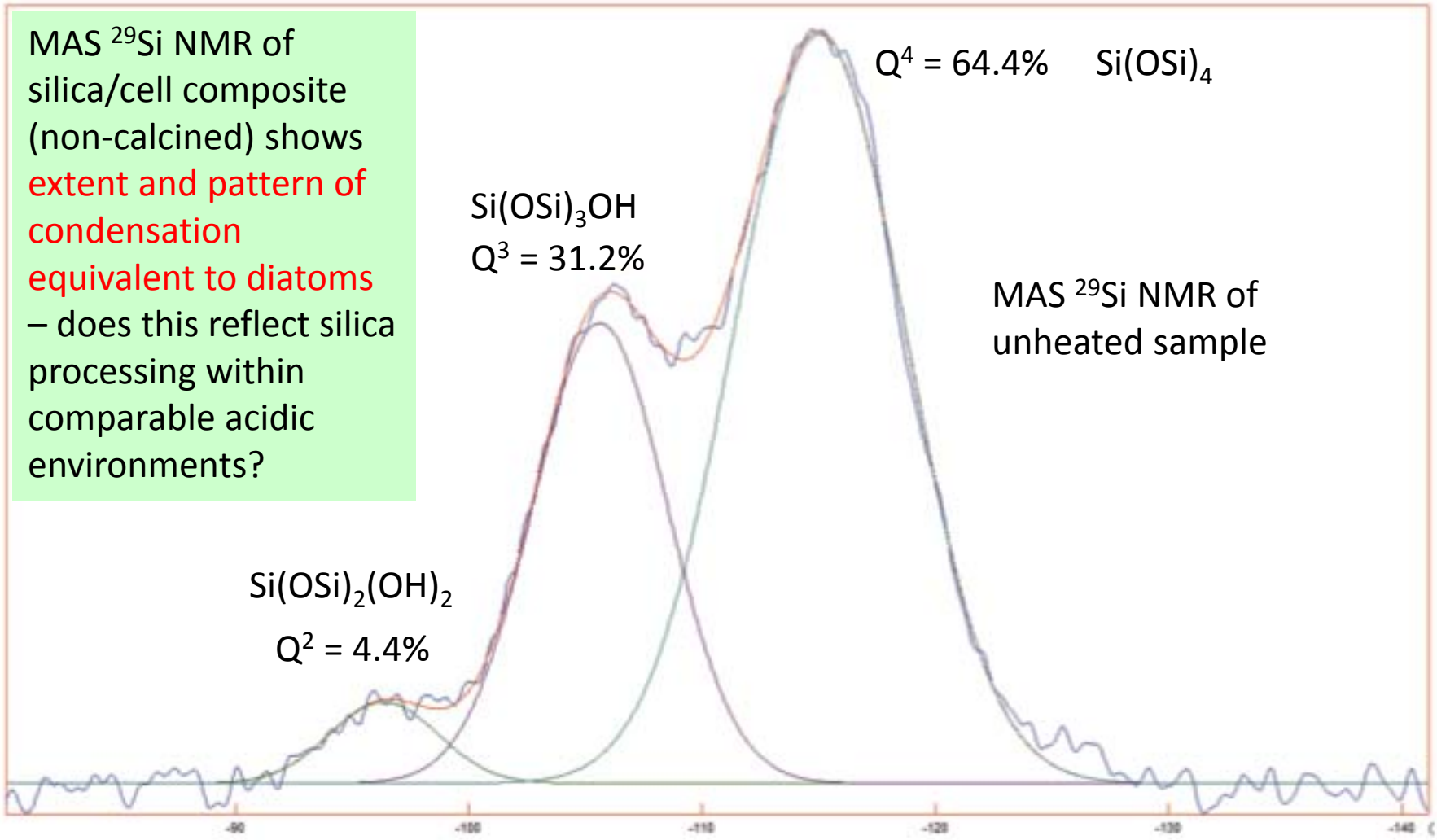


Feature dimension suggests silica deposition thickness limited to < 6-nm; calcination results in cell replica with ~2-nm precision, AFM featureless at 2-nm scale

SEM analysis of filopodia: mean width of fixed cells (75 nm), cell/silica composites (86 nm), and silica (79 nm) derived from substrate-bound differentiated AsPC-1

How do we understand silica cell replica chemistry/mechanism and how does it compare with that of diatoms?

MAS ^{29}Si NMR of silica/cell composite (non-calcined) shows extent and pattern of condensation equivalent to diatoms – does this reflect silica processing within comparable acidic environments?



Silica cell replicas have an extent of silica condensation and Q^n distribution nearly identical to that observed and conserved in marine diatoms

Table 1 Integral areas of the Q^2, Q^3, and Q^4 moieties in % (estimated error ±2) and Q^4/Q^3 ratios calculated from the quantitative (single-pulse) solid-state ^{29}Si NMR spectra of the biosilica deposits of freeze-dried complete cells and extracted cell walls of the diatoms *Chaetoceros debilis*, *Chaetoceros didymum*, *Cylindrotheca fusiformis*, and *Nitzschia angularis*, as well as synthetic silica gel

Species (nature of sample)	Area (%) $Si(OSi\equiv)_4$ [Q^4]	Area (%) $Si(OSi\equiv)_3(OH)$ [Q^3]	Area (%) $Si(OSi\equiv)_2(OH)_2$ [Q^2]	Q^4/Q^3
Chaetoceros debilis (complete cells)	64	34	2	1.9
Chaetoceros didymum (complete cells)	63	33	4	1.9
Cylindrotheca fusiformis (complete cells)	63	35	2	1.8
Nitzschia angularis (complete cells)	64	33	3	1.9
Chaetoceros debilis (extracted cell walls)	69	28	3	2.5
Chaetoceros didymum (extracted cell walls)	69	26	5	2.7
Chaetoceros didymum (cell walls extracted at room temperature)	70	27	3	2.6
Chaetoceros didymum (extracted cell walls, after storage for 12 months)	70	27	3	2.6
Cylindrotheca fusiformis (extracted cell walls)	72	26	2	2.8
Nitzschia angularis (extracted cell walls)	69	27	4	2.6
Synthetic silica gel	74	24	2	3.1

Anal Bioanal Chem (2003) 375 : 630–634
DOI 10.1007/s00216-003-1769-5

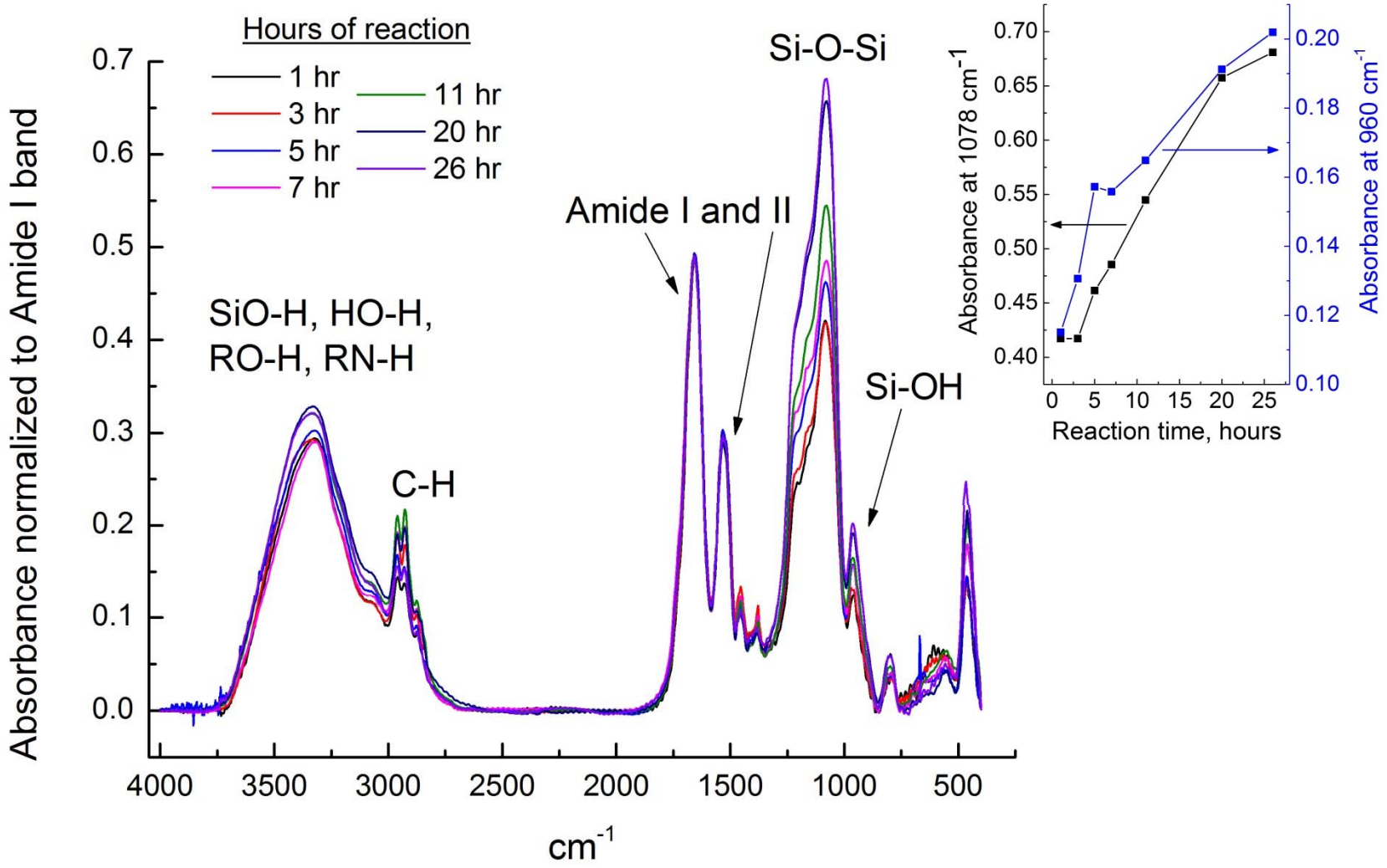
ORIGINAL PAPER

R. Bertermann · N. Kröger · R. Tacke

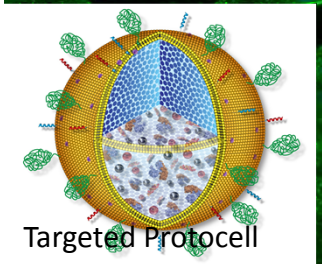
Solid-state ^{29}Si MAS NMR studies of diatoms: structural characterization of biosilica deposits

Coincidental? Perhaps but it suggests a similar condensation chemistry environment.

Silicification proceeds largely with little perturbation of the hydrogen bonded hydroxyl network – and with preservation of protein associated vibrational features – *evidence for self-limitation*



Surface antigens (epidermal growth factor receptors) remain accessible during initial stages of silicification



Targeted Protocell

A549, 0 h

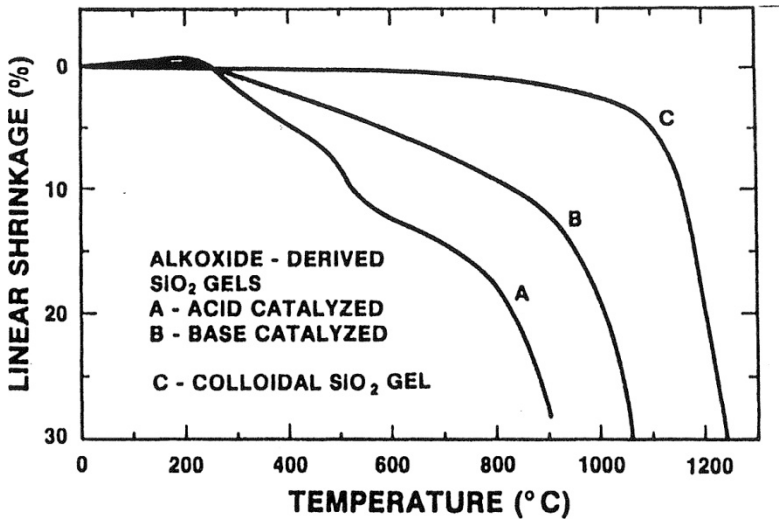
A549, 2 h

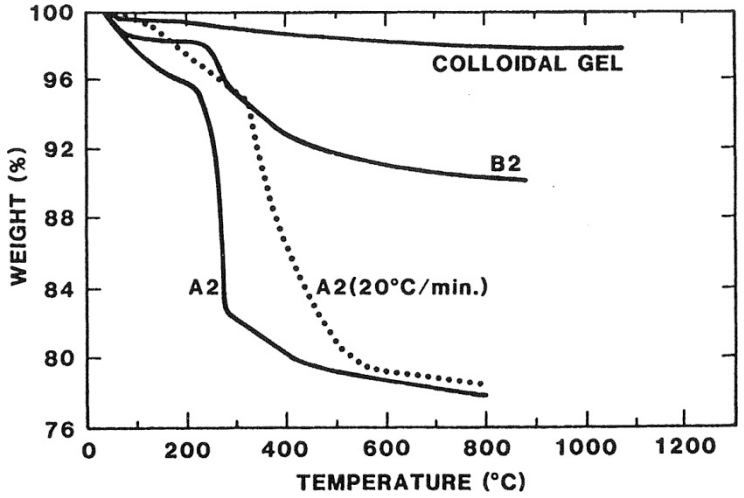
A549, 4.5 h

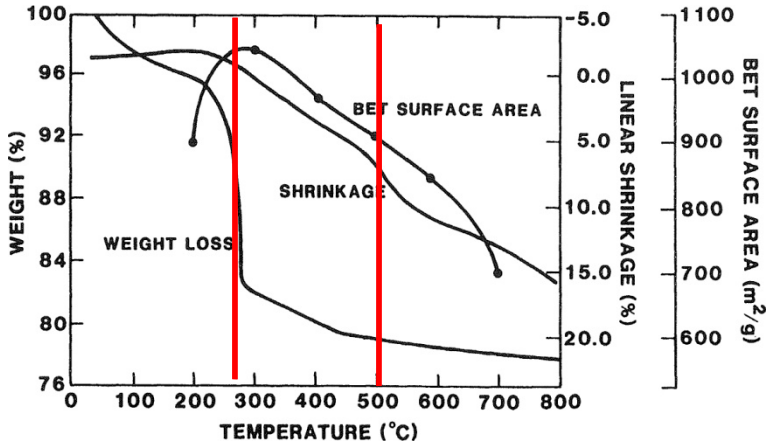
A549, 6 h

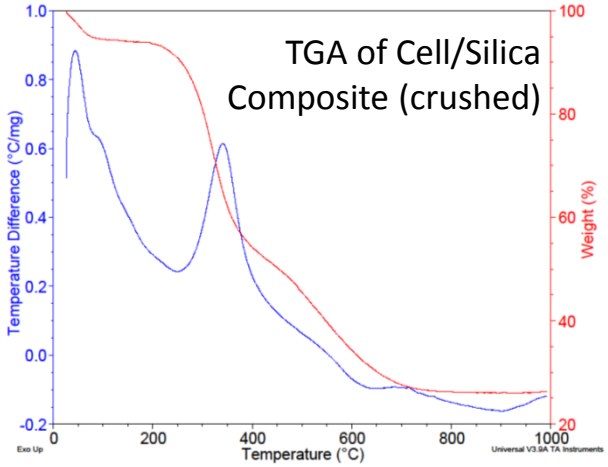
20 µm

Dimensional stability to > 500˚C is completely unexpected based on previous sintering studies of acid-catalyzed silica xerogels (CJB and Scherer, Sol-Gel Science AP 1990)



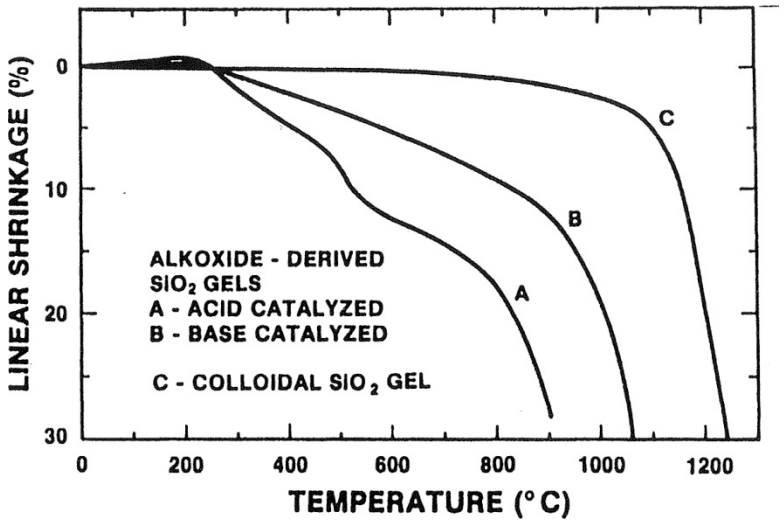


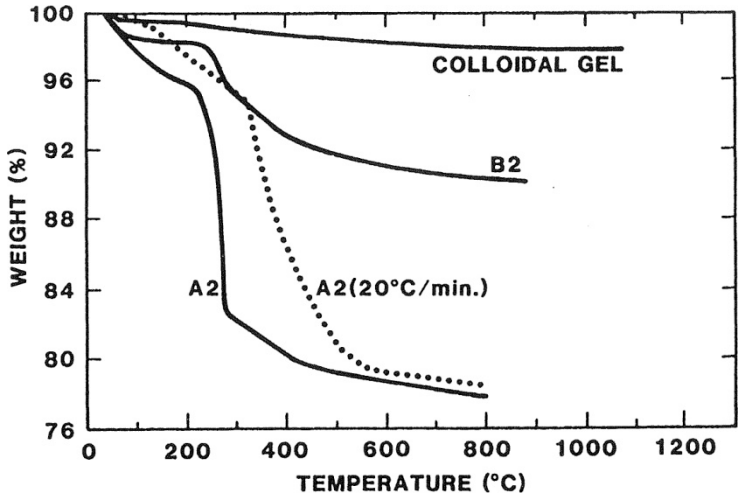


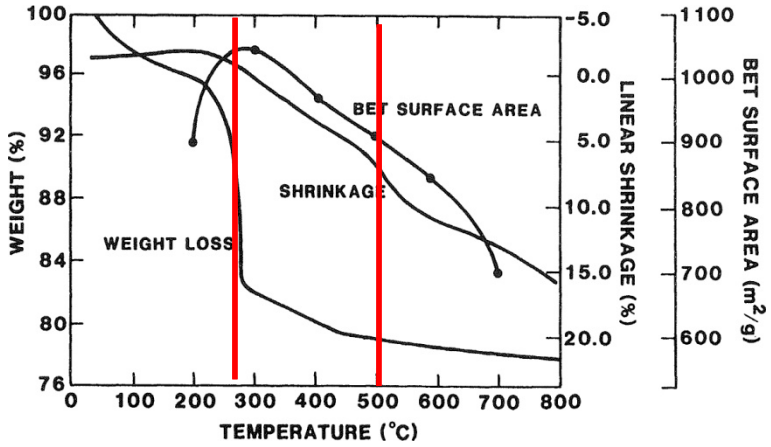


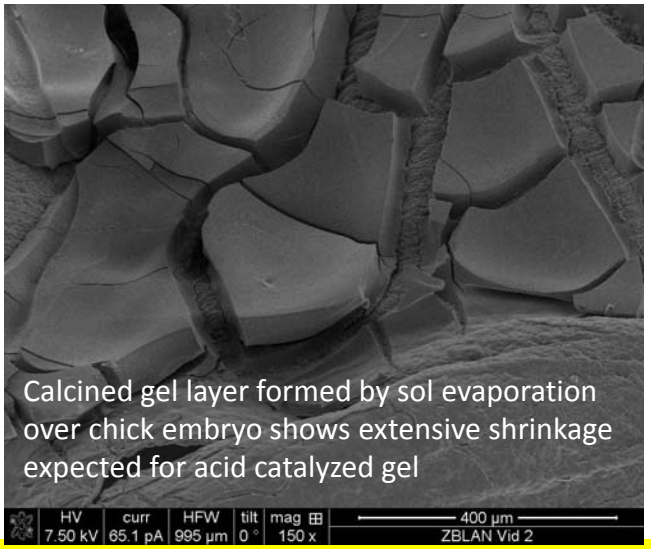
Continued silica condensation reactions are apparent from weight loss – absence of shrinkage suggests an adherent ultra-thin 3D layered network that accommodates shrinkage in thickness direction

Dimensional stability to > 500˚C is completely unexpected based on previous sintering studies of acid-catalyzed silica xerogels (CJB and Scherer, Sol-Gel Science AP 1990)









Continued silica condensation reactions are apparent from weight loss – absence of shrinkage suggests an adherent ultra-thin 3D layered network that accommodates shrinkage in thickness direction enabling dimensional preservation

Key Features of Silica Cell Replication

- pH 3 silicic acid (100mM) does not self-condense – pH 3 near the isoelectric point of monosilicic acid
- Silica deposition occurs uniformly inside and outside the cell and is *self-limiting* – 3D scaffolded catalytic surface – complementary to LbL surface sol-gel process (Sandhage)
  - what is diffusivity and condensation mechanism?
  - are membranes in tact and does diffusion occur through Na^+ channels?

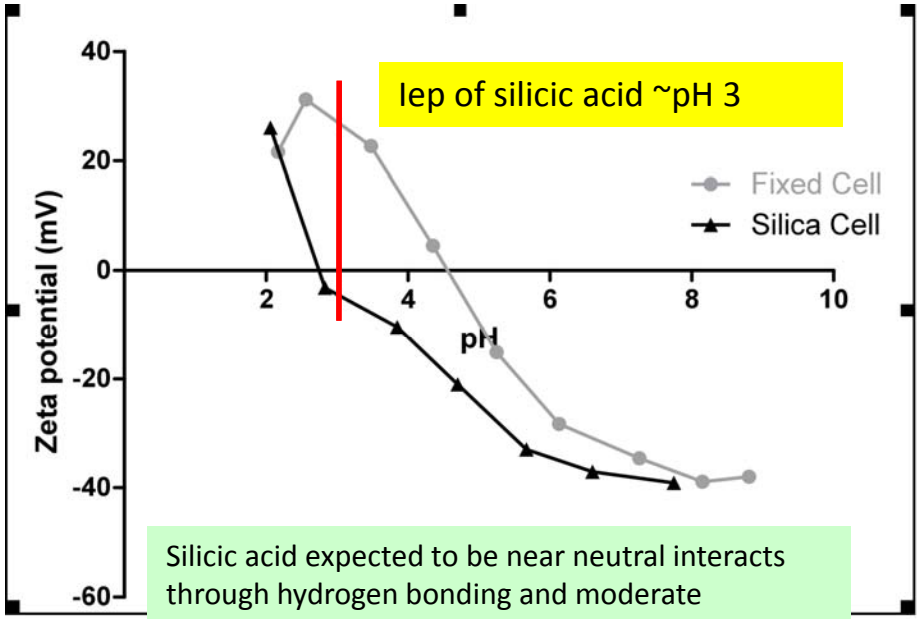
- From the standpoint of 'sol-gel processing' cell-silicified structures are remarkably resistant to drying and calcination
  - Mechanically completely connected and robust (modulus/density scaling?)
  - Absence of high curvature structures that would result in drying and sintering stress
  - Ultra-thin silica layer allows condensation shrinkage to be accommodated in thickness direction

- Ultimate nanostructure can be featureless and defect-free ~ 2-nm precision

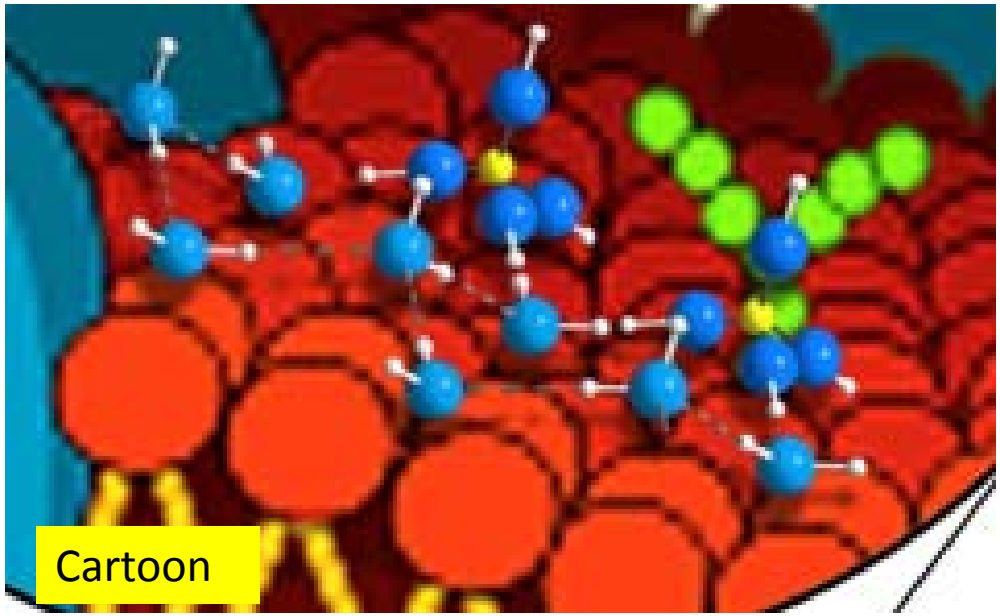
- Preserved biomolecular structure (FTIR) and functionality? (with caveat of fixation)
  - Does de-silicification reveal silica occluded structure and re-generate biofunctionality? if yes dried structure could be stored and re-activated
  - Can we avoid/reverse fixation?

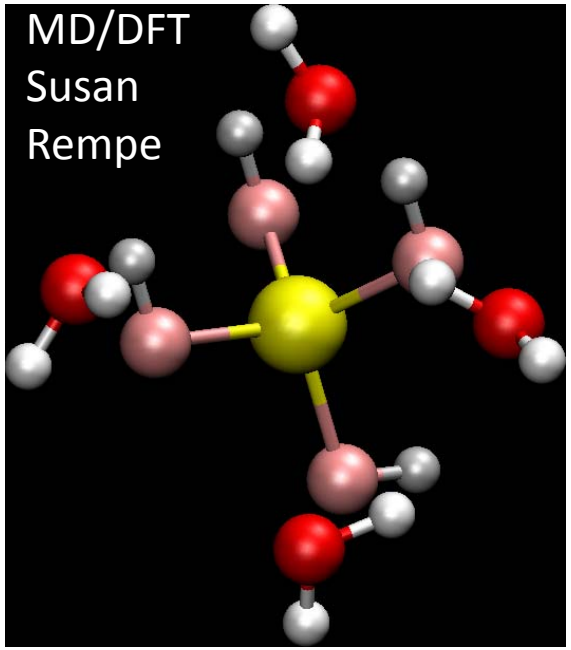
- Self-Consistent Mechanism?

Water Replacement Hypothesis



Silicic acid expected to be near neutral interacts through hydrogen bonding and moderate electrostatic interactions

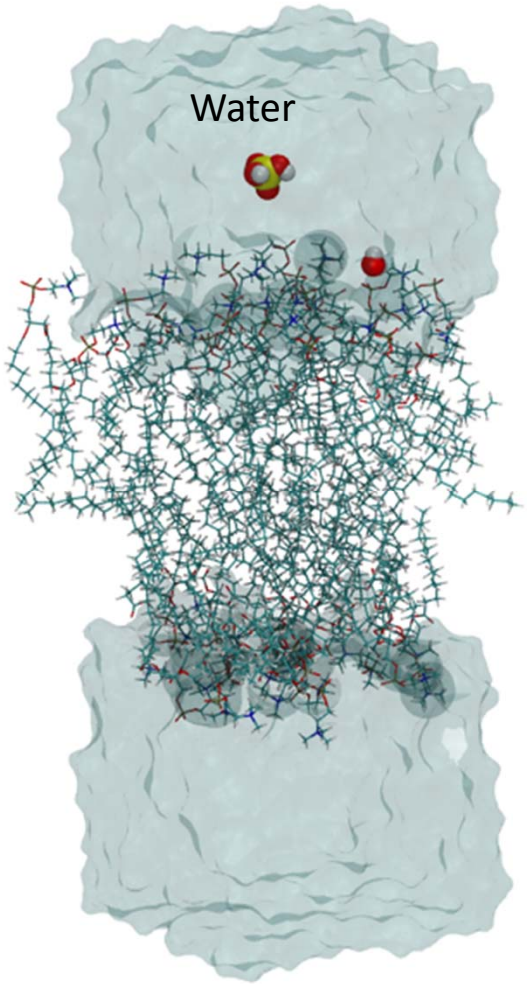


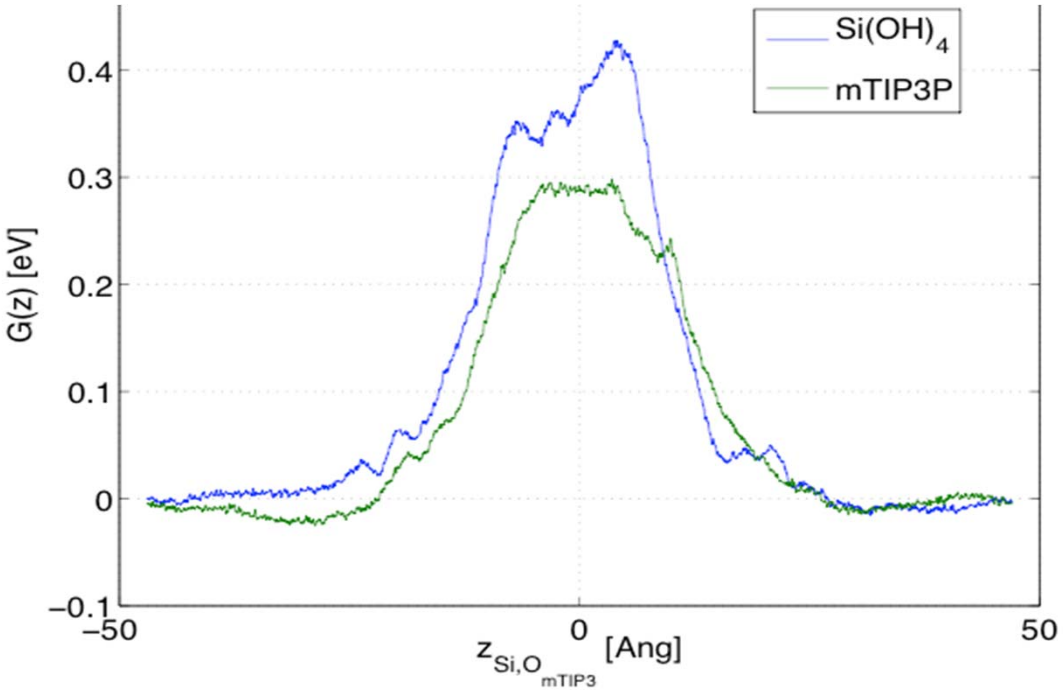


Water Replacement Hypothesis – Can silicic acid diffuse throughout cell, replace/displace hydrogen-bonded interfacial water at cellular/biomolecular interfaces and be concentrated and catalyzed by proximal membrane associated proteins, carbohydrates (other components) to form stable interfacial silica network in self-limited process?

Do water associated surfaces represent an optimized mechanically connected network that if replaced with silica would result in 3D dimensional stability?

Isomorphism of silica and water- both form structures composed of tetrahedral units sharing vertices to form four connected infinite networks – can silicic acid replace/displace water at a biological interface?





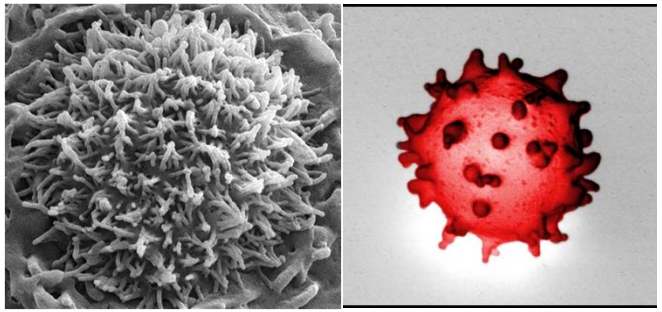
- $Si(OH)_4$ force field created
- RDF verified against quantum mechanical (DFT) calculation
- Atomistic model of POPC bilayer
- Metadynamics/REMD/REST simulations of $Si(OH)_4$ and H_2O diffusion through bilayer

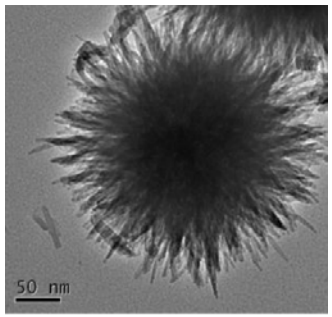
Lucio Ciacchi *et al.*, *JACS* 134 4 2407-2413 (2012) – work above unpublished

For monomer no energy minimum is observed at lipid headgroup interface…dimers

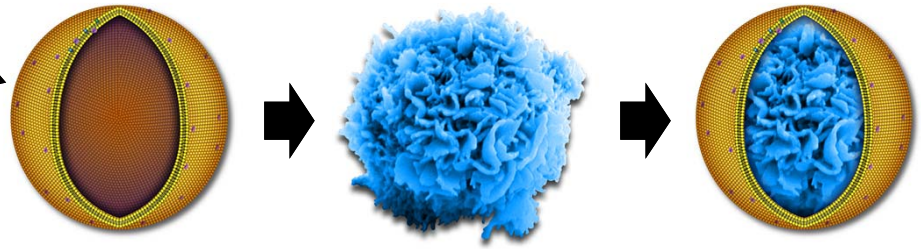
Summary/Directions

- Alternative to Freeze-Fracture
- Optimize procedures to maximize functional activity - de-silicification to re-reveal all functional components
- Program shape and gene expression using genetic engineering, differentiation, environmental exposure.
  - Upregulate enzymes/phenotypes of interest – preserve enzymes in their native crowded states in powders that withstand desiccation
- Use Cellular Processing of NPs
- Protocellular construct amenable to synthetic biology and transformation chemistry (C, Si, TiO_2…)
  - Incorporate native and non-native proteins within and external membrane bilayers/receptors, etc
- Preserve biomolecular components and function without Cryo –preservation
- Direct cell behavior with silica 'decoys'



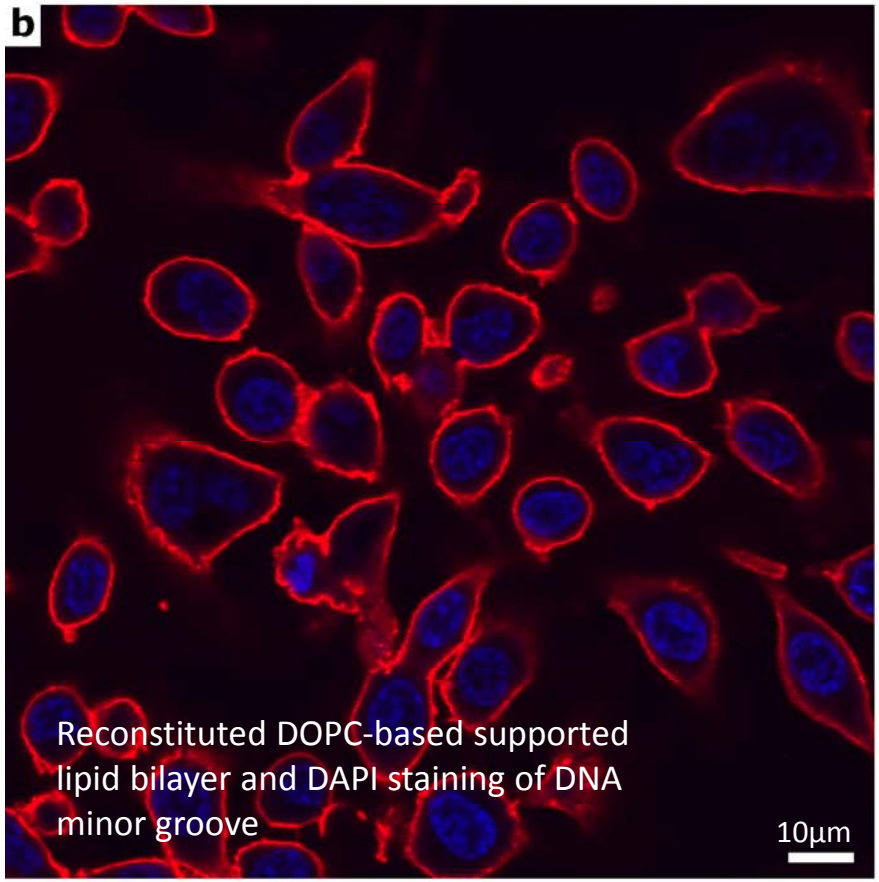


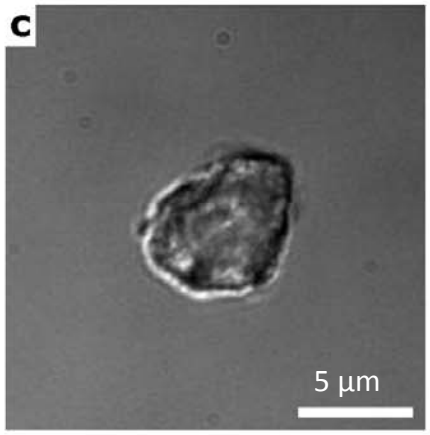
$LnPO_4$ created by processing of ReO in macropinosome, Nel et al. UCLA

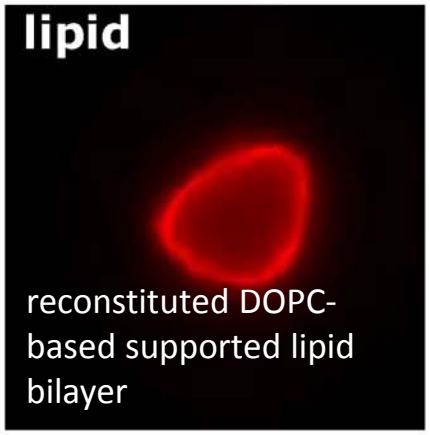


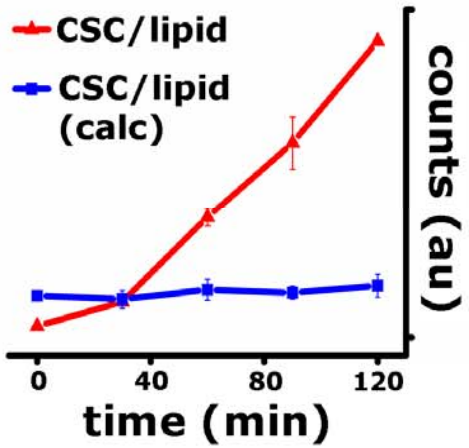
Protocellular platform for synthetic biology

Reconstitution of Cellular Function in 'Protocellular' Replica- Supported Lipid Bilayer Construct Tested with Viability Probe

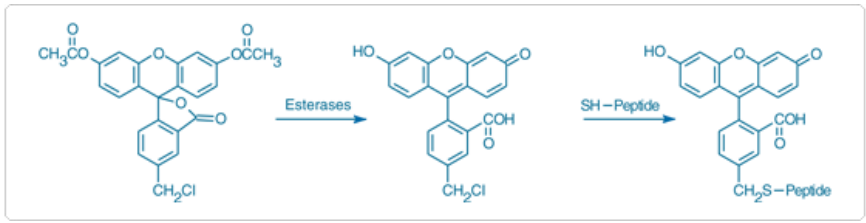








Liposomes fuse spontaneously (and selectively) to cell replicas non-calcined or calcined – what are its biophysical characteristics?



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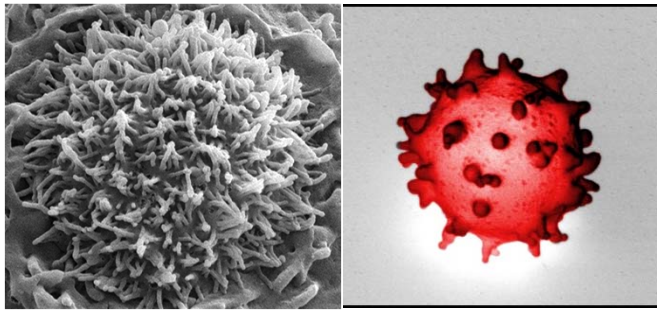

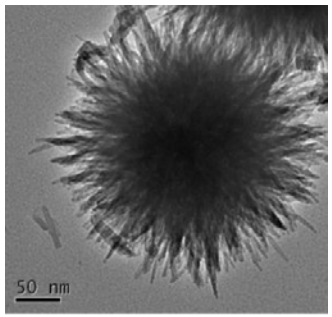

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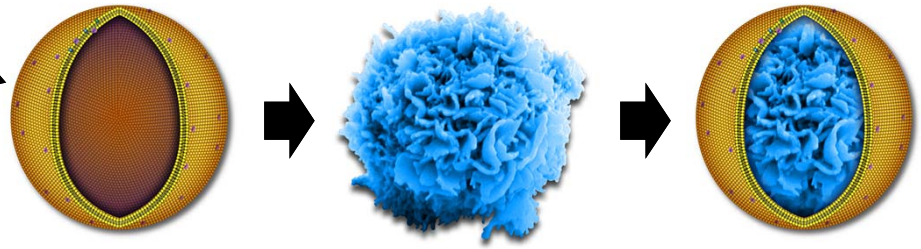
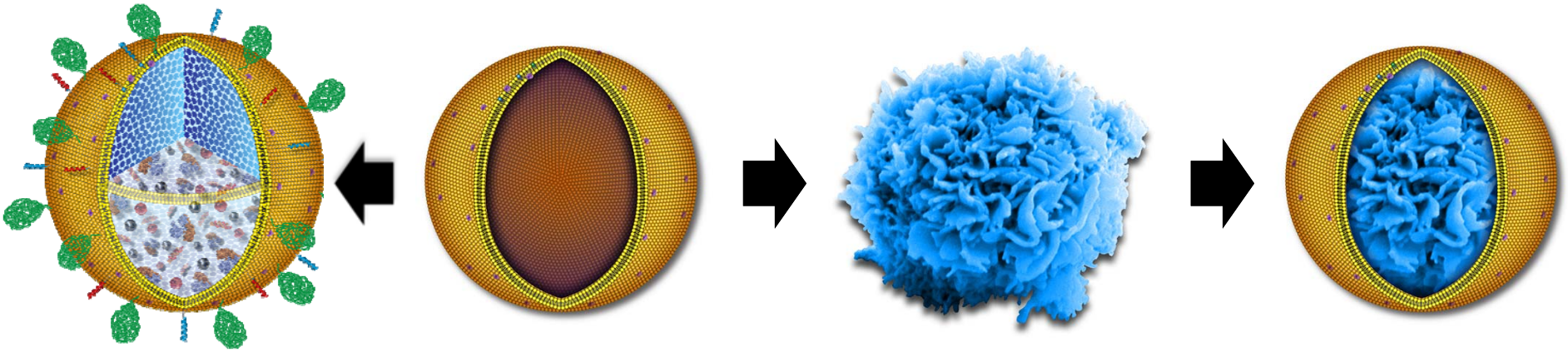
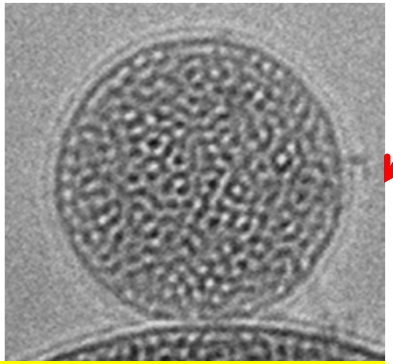


Protocellular platform for synthetic biology – dissipative assembly

Future: What are the biophysical properties of mesoporous and replica-supported lipid bilayers? – Natural cell membranes are supported on porous scaffolds



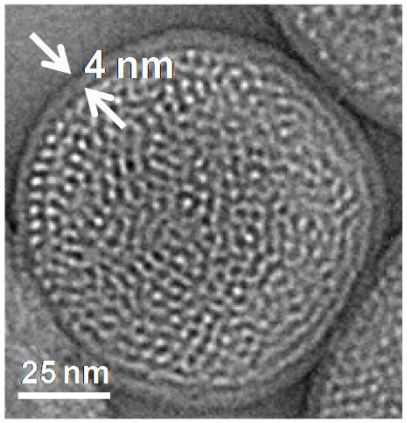


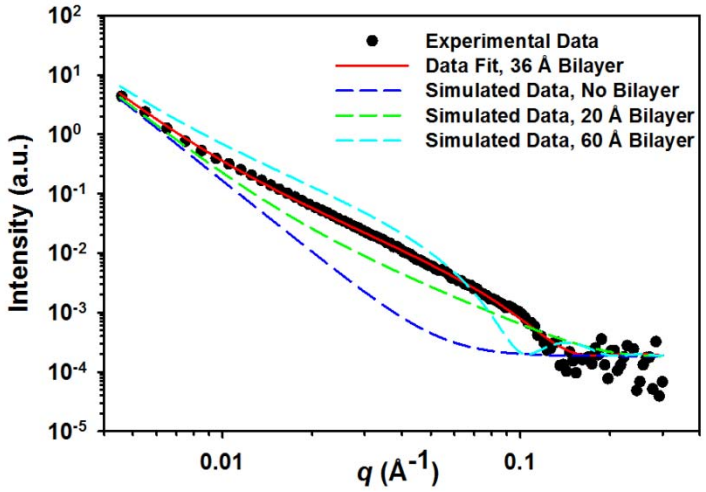
Nanoporous particle-supported lipid bilayer – *Nature Mater. 2011*

- Nanoporous support suppresses lipid melting transition temperature (FRAP)

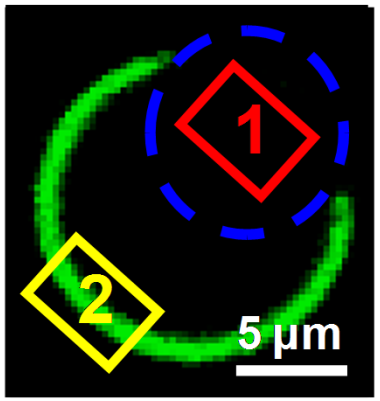
Silica cell replica supported DOPC bilayers stained with Texas red – *PNAS 2012*

Liposome Fusion Creates a Supported Lipid Bilayer (SLB) that has enhanced lateral fluidity compared to solid supported SLB

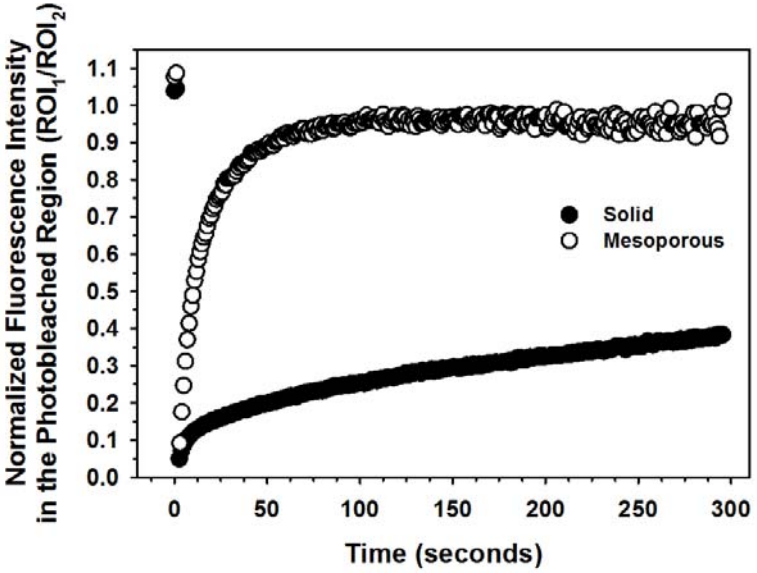


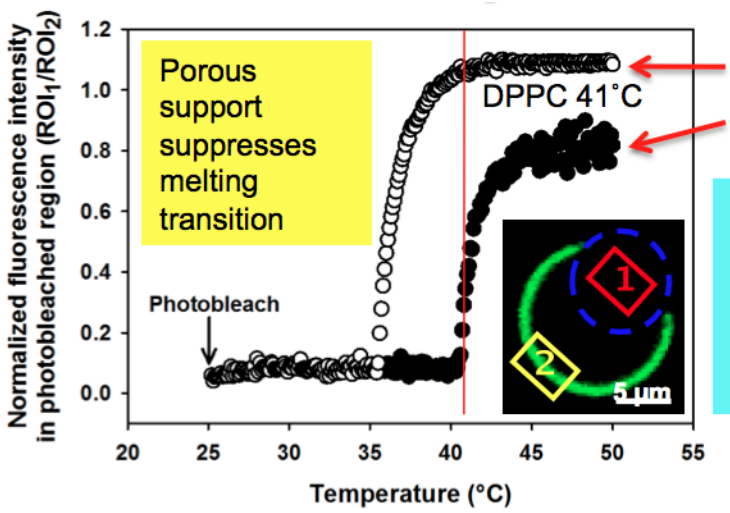


TEM and SANS demonstrate that SLBs are 3.6-nm thick



FRAP demonstrates that SLBs are fluid



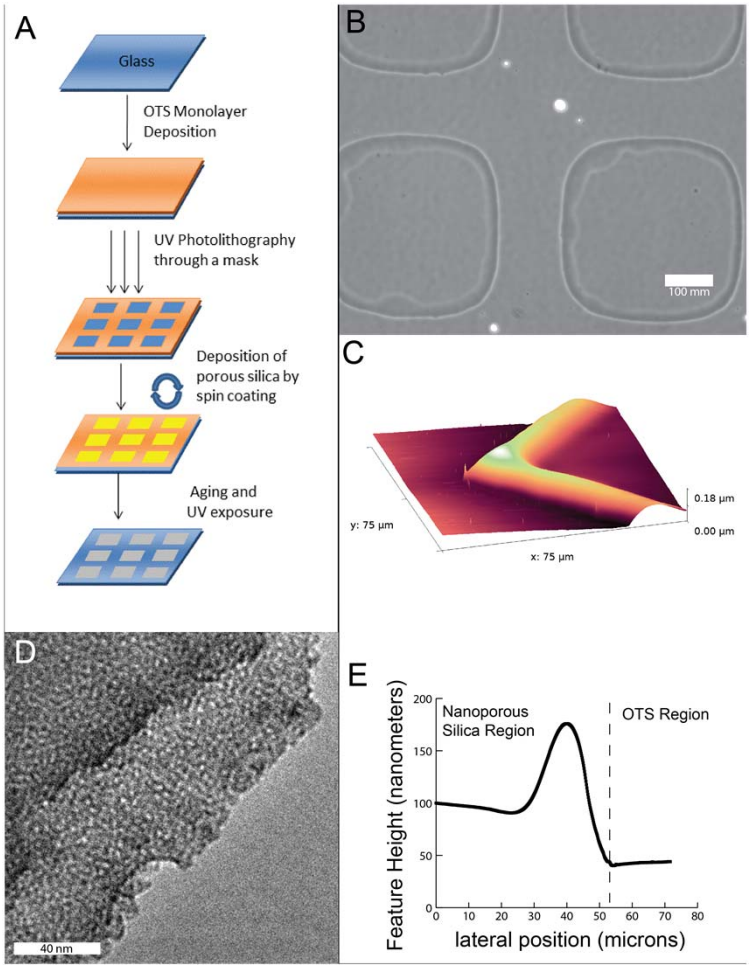


Enhanced SLB fluidity enables peptide recruitment to the HCC surface

Ashley et al. *Nature Mater. 2011*

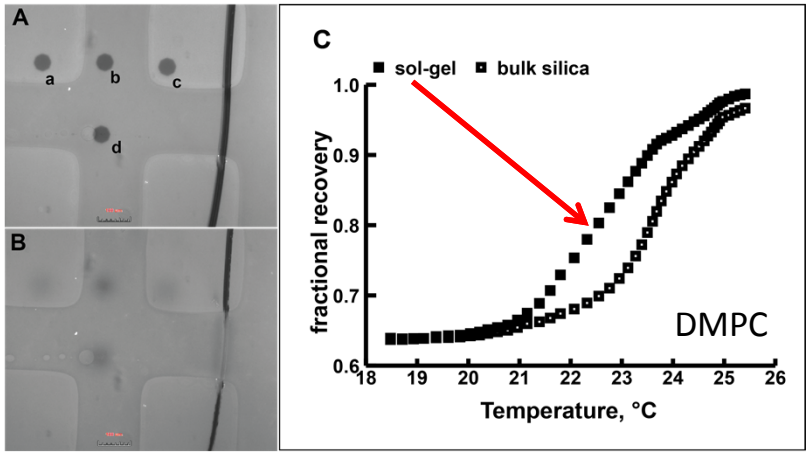
Lithographically-patterned mesoporous substrate spatially modulates fluidity and phase separation of supported membranes

Kendall, Parikh et al

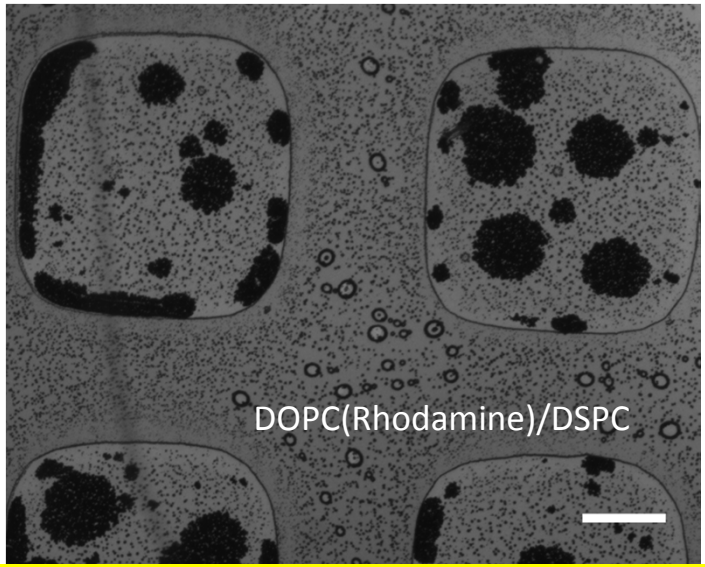


Lithographically-defined Mesoporous patterns

Locally enhanced fluidity

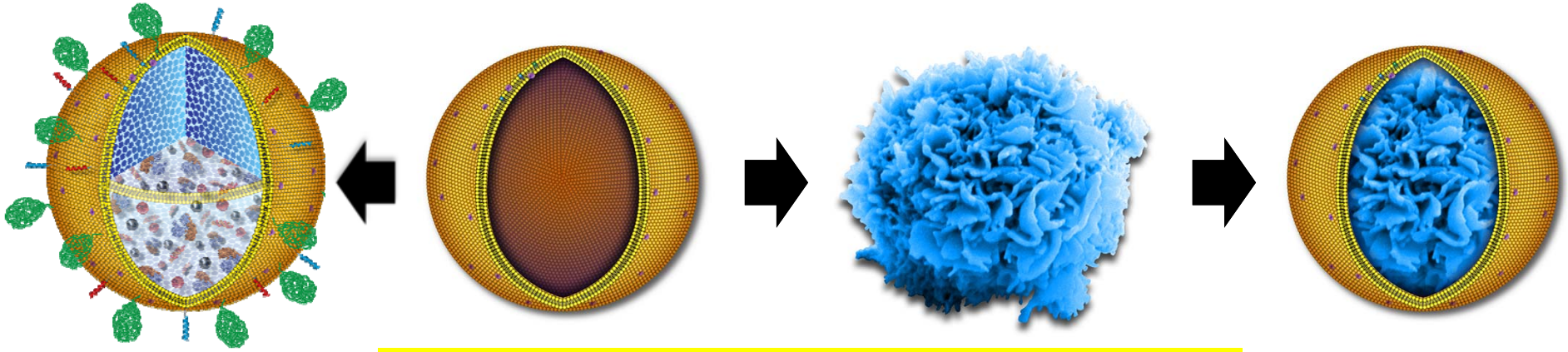


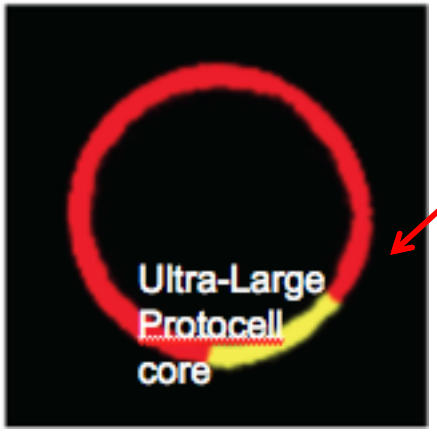
Larger domains – Disproportionation



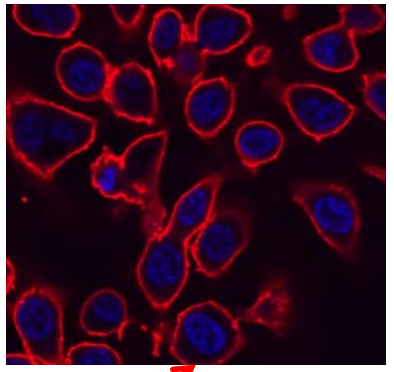
Topographic 'Diode' – relevant to raft formation?

Future: What are the biophysical properties of nanoporous/replica supported lipid bilayers? – Can we reconstitute cellular function in a reductionist system?





- Nanoporous support suppresses lipid melting transition temperature (FRAP)
- Observe global phase separation of raft forming lipid mixtures, which is kinetically limited on solid supports (Sasaki)
- Does porous scaffold enable/mediate protein-binding induced curvature
- Cytoskeletal mimic may result in new biophysical characteristics
- Cell replica scaffold enables reconstitution of molecularly crowded cellular environment – platform for synthetic biology – dissipative assembly



Nonintercalating Nanosubstrates Create Asymmetry between Bilayer Leaflets

Sameer Varma,*[,†,§] Michael Teng,[‡] and H. Larry Scott[†]

[†]Department of Biological, Chemical and Physical Sciences, Center of Molecular Study of Soft Condensed Matter, Illinois Institute of Technology, Chicago, Illinois 60616, United States

[‡]Illinois Mathematics and Science Academy, Chicago, Illinois 60506, United States

Asymmetry in the diffusivity of the upper and lower leaflets and overall reduction in apparent diffusivity

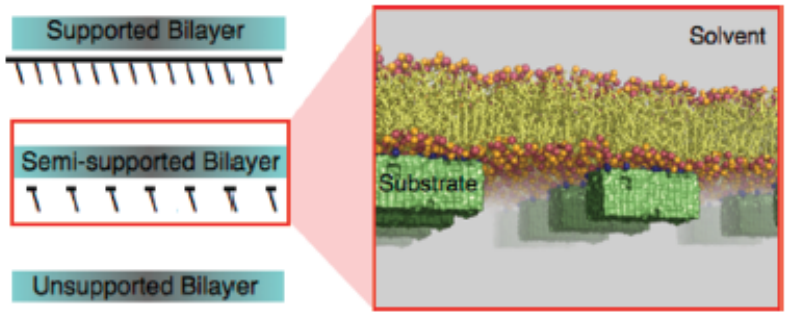


Figure 1. Cartoon of a semisupported POPC bilayer. The phosphate and choline lipid groups are illustrated as pink and orange spheres. The substrates are shown as green solids, and the functional groups on the substrate surface are drawn as blue spheres. The atomic coordinates were taken from the 0.53 μs snapshot of a molecular dynamics simulation of a POPC bilayer supported by a substrate carrying 20% hydroxyl surface coverage.

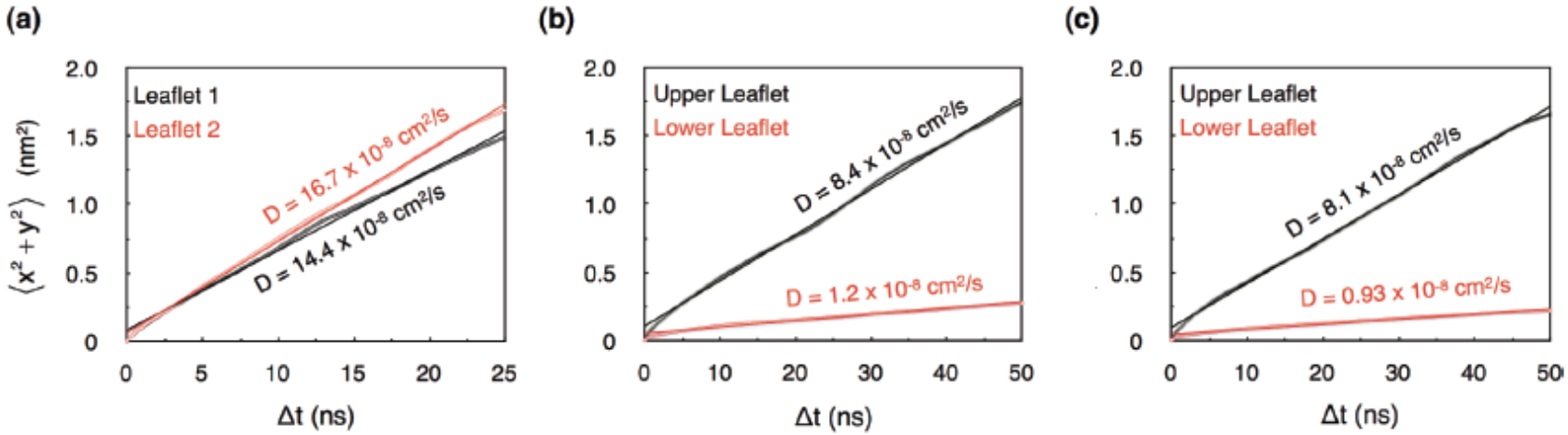
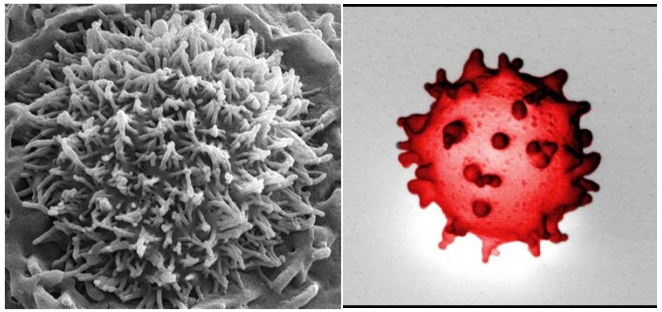
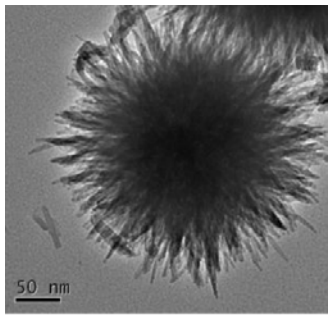


Figure 6. Lateral mean-square displacement of lipids: (a) unsupported bilayers, (b) bilayers supported on substrates with 10% hydroxyl surface coverage, and (c) bilayers supported on substrates with 20% hydroxyl surface coverage. The straight lines are least-squares fits, which yield diffusion constants through the Einstein relationship. The attractive electrostatic force between the substrate hydroxyls and the lipid dipoles slows down lipid diffusion, although differently in two leaflets. Lipids in the lower leaflet diffuse at rates almost an order of magnitude slower than those in the upper leaflet.

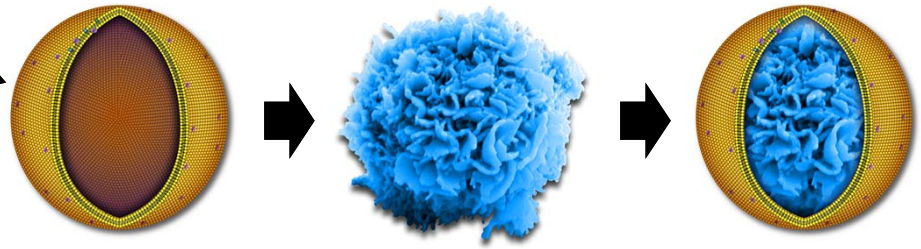
Summary/Directions

- Alternative to Freeze-Fracture
- Optimize procedures to maximize functional activity - de-silicification to re-reveal all functional components
- Program shape and gene expression using genetic engineering, differentiation, environmental exposure.
  - Upregulate enzymes/phenotypes of interest – preserve enzymes in their native crowded states in powders that withstand desiccation
- Use Cellular Processing of NPs
- Protocellular construct amenable to synthetic biology and transformation chemistry (C, Si, TiO_2…)
  - Incorporate native and non-native proteins within and external membrane bilayers/receptors, etc
- Preserve biomolecular components and function without Cryo –preservation
- Direct cell behavior with silica 'decoys'



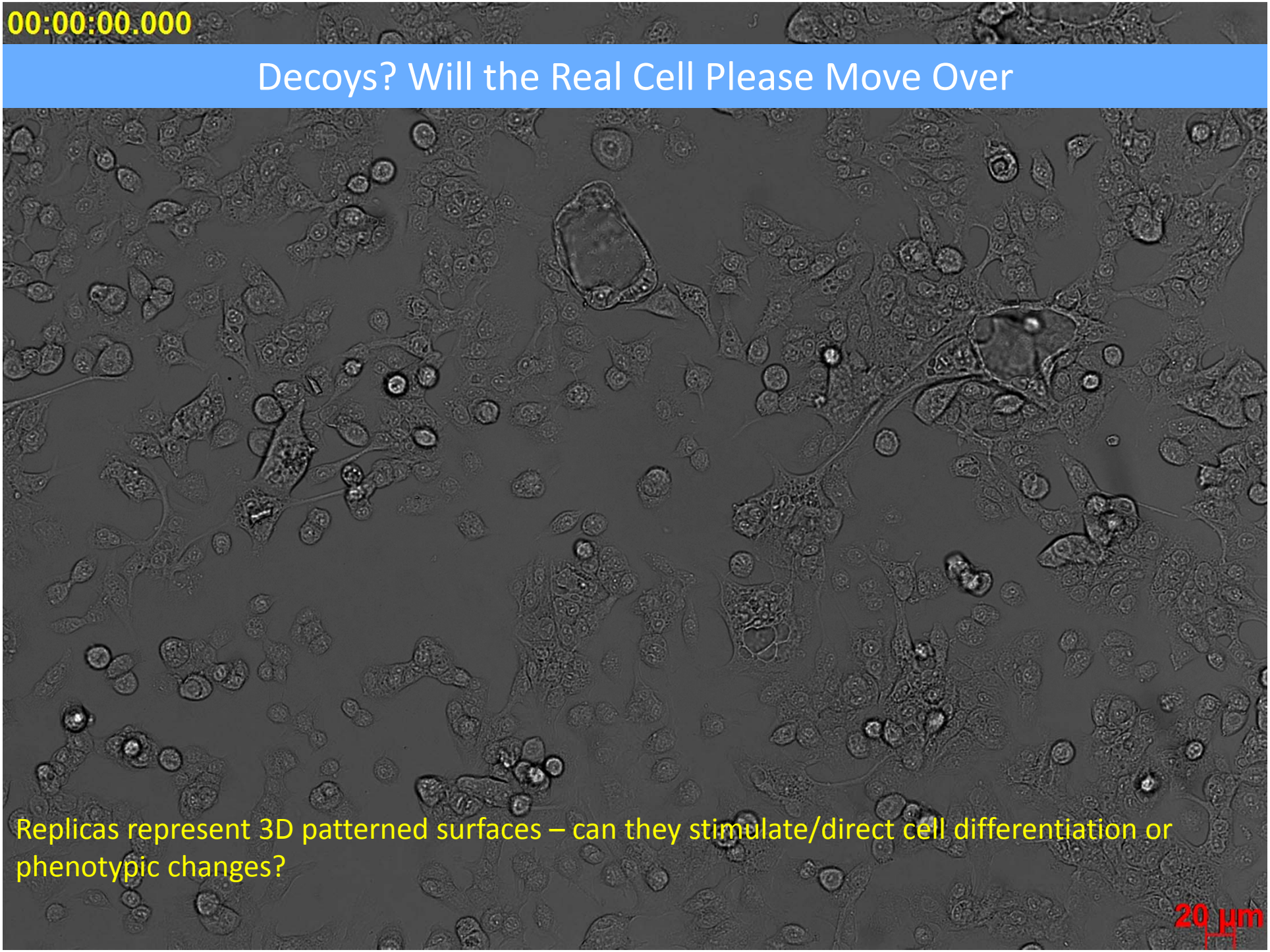


$LnPO_4$ created by processing of ReO in macropinosome, Nel et al. UCLA

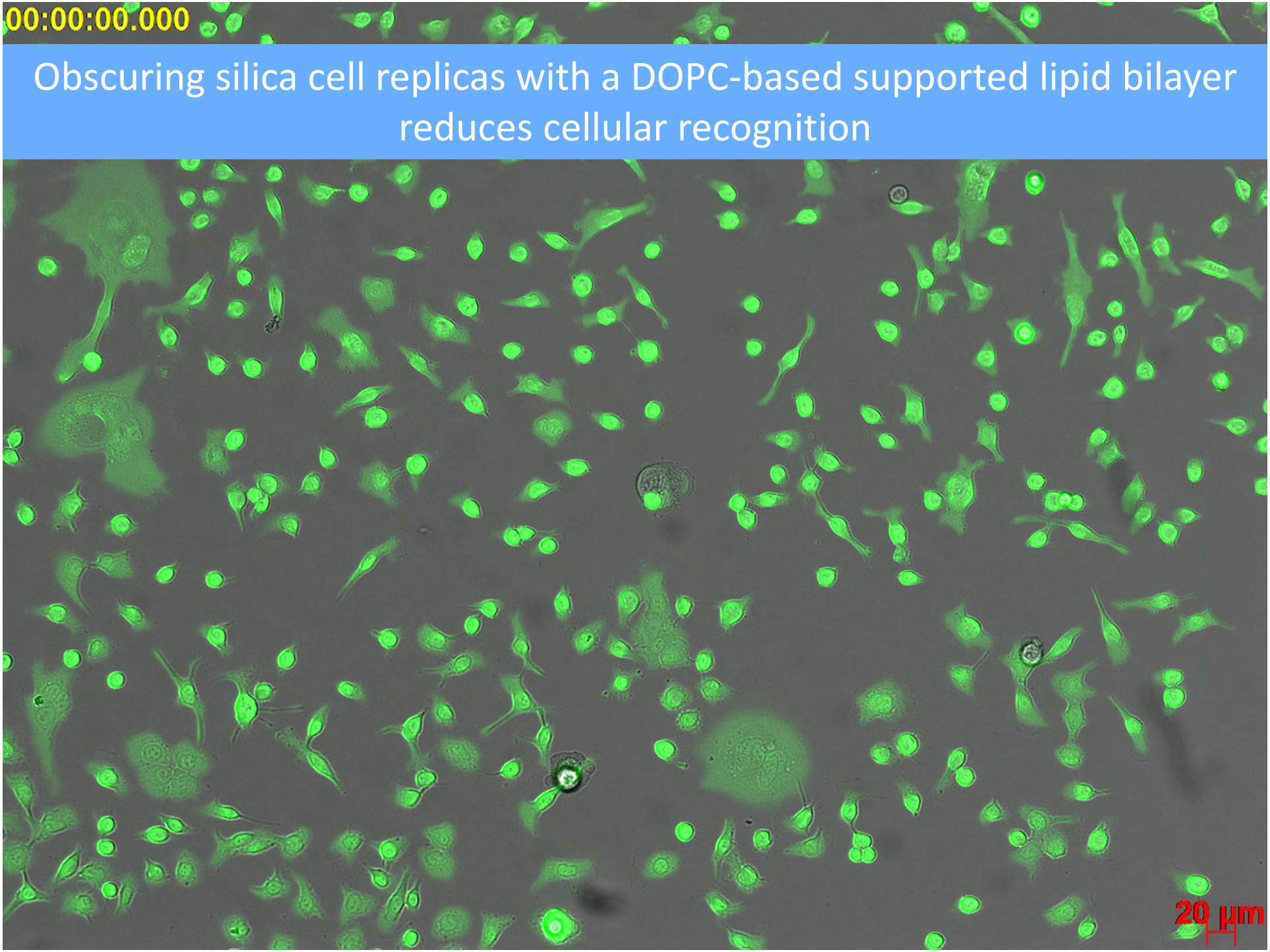


Protocellular platform for synthetic biology – dissipative assembly

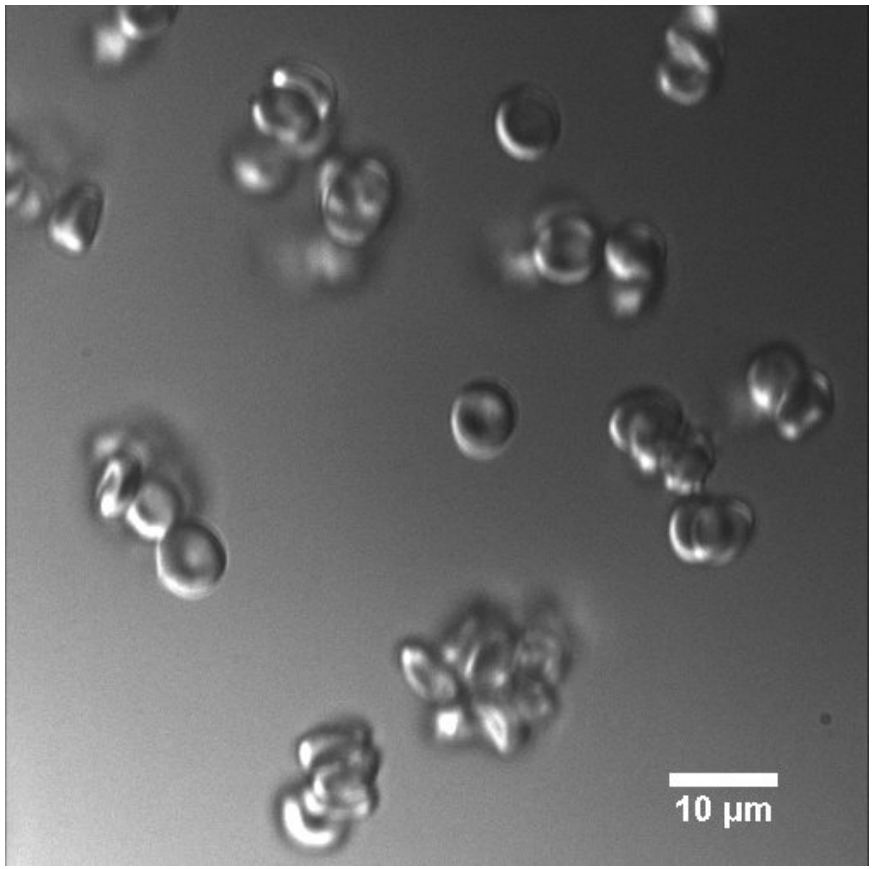
Decoys? Will the Real Cell Please Move Over

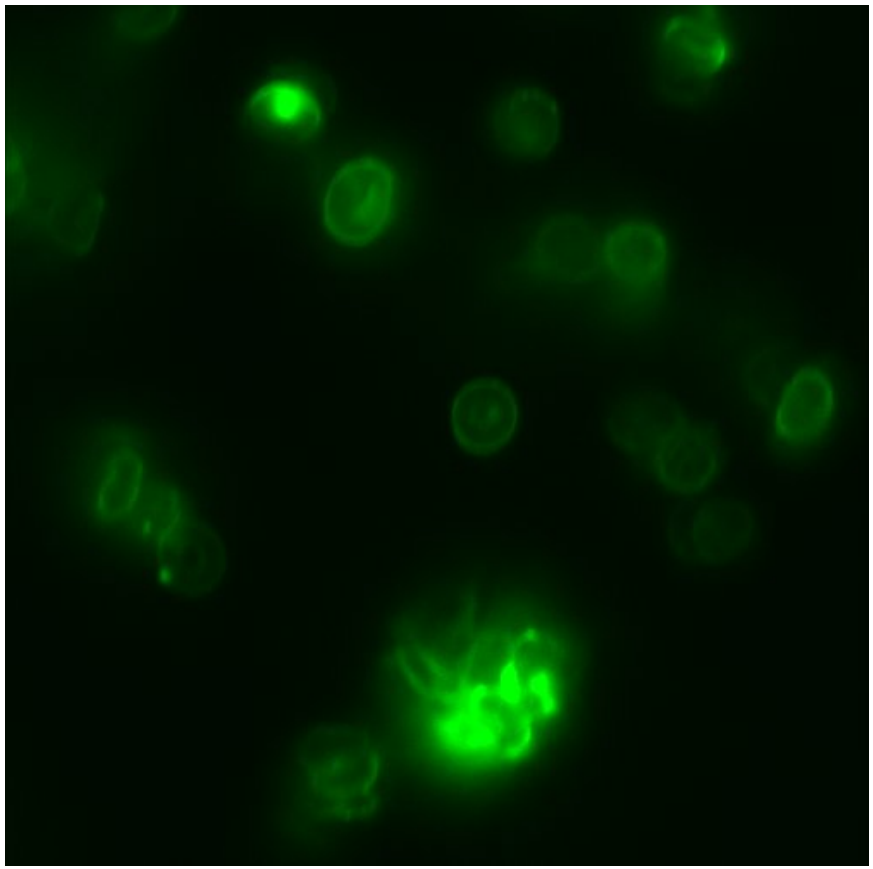


Obscuring silica cell replicas with a DOPC-based supported lipid bilayer reduces cellular recognition



RBC silica-cell with fused with Human RBC membrane (FITC-DOPC label) – How is it recognized *in vitro* and *in vivo?*

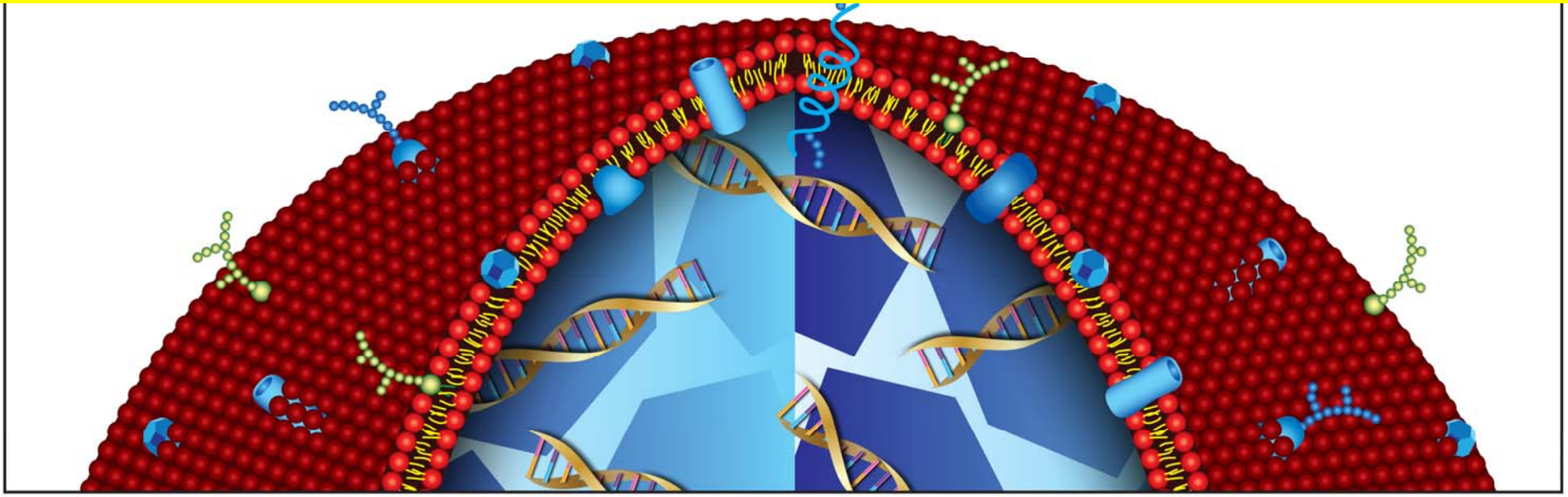




P.S. Scaffold for reconstituting cellular function in a compartmentalized object that can achieve and maintain concentration/energy gradients

Can recognition of ‘self’ or ‘non-self’ be used to mediate macrophage uptake, circulation, and bio-distribution?

MSNP Enveloped with Native Red Blood Cells –display self-recognition peptides….



= Cholesterol

= Glycolipid

= Channel Protein

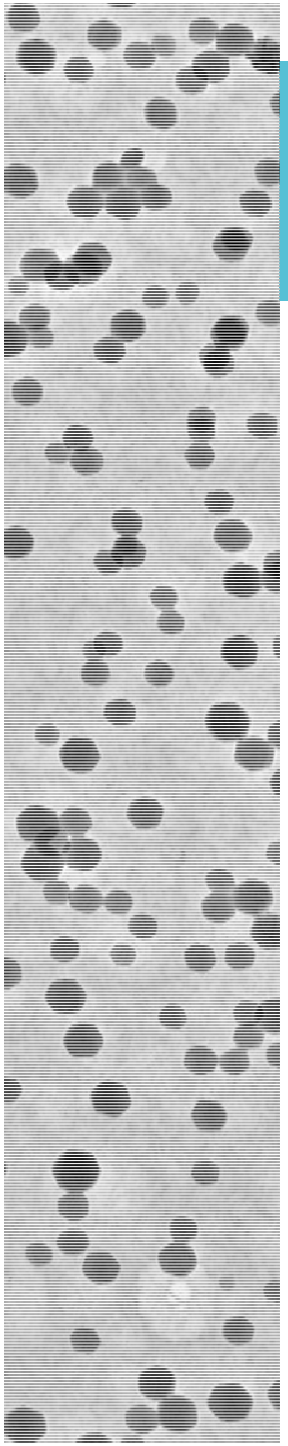
= Glycoprotein with carbohydrate attached

= Peripheral Protein

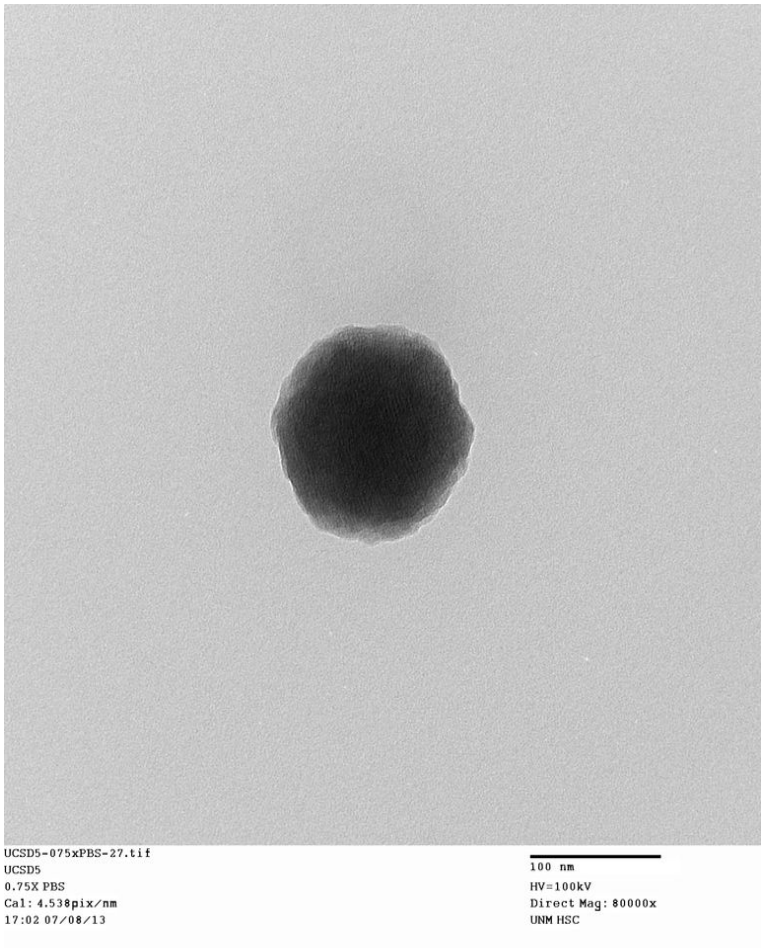
= Integral Protein

= Alpha Helix

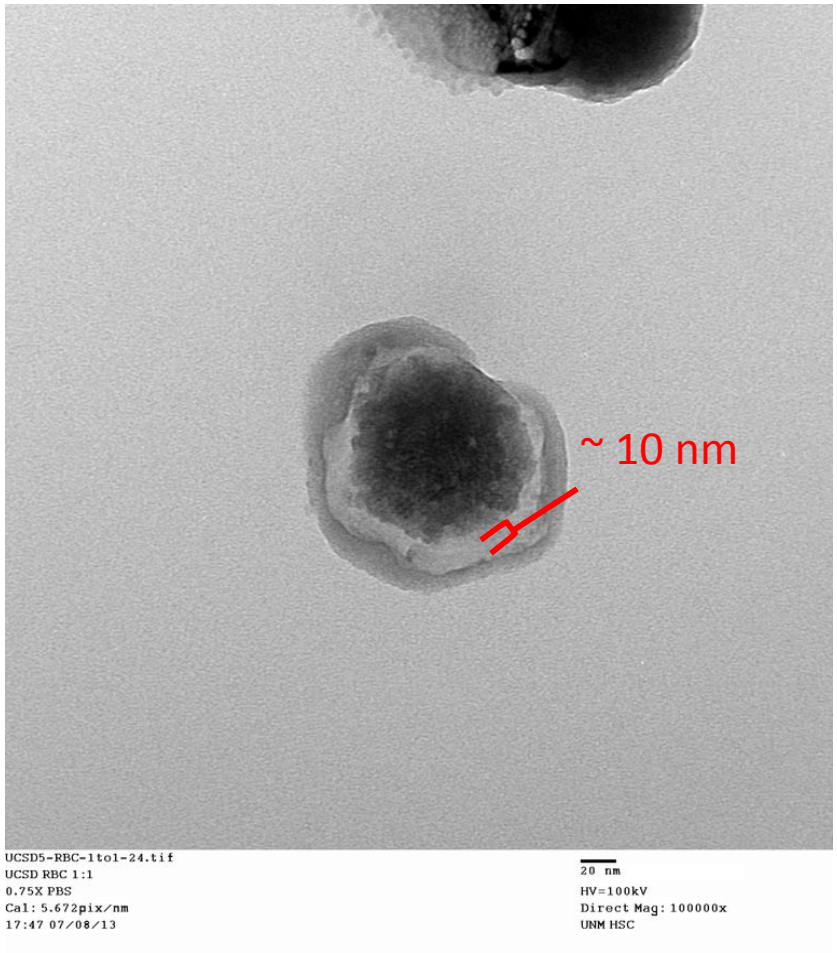
Collaboration with Liangfang Zhang Group UCSD



TEM reveals that MSNP are enveloped with ~10-nm thick membrane

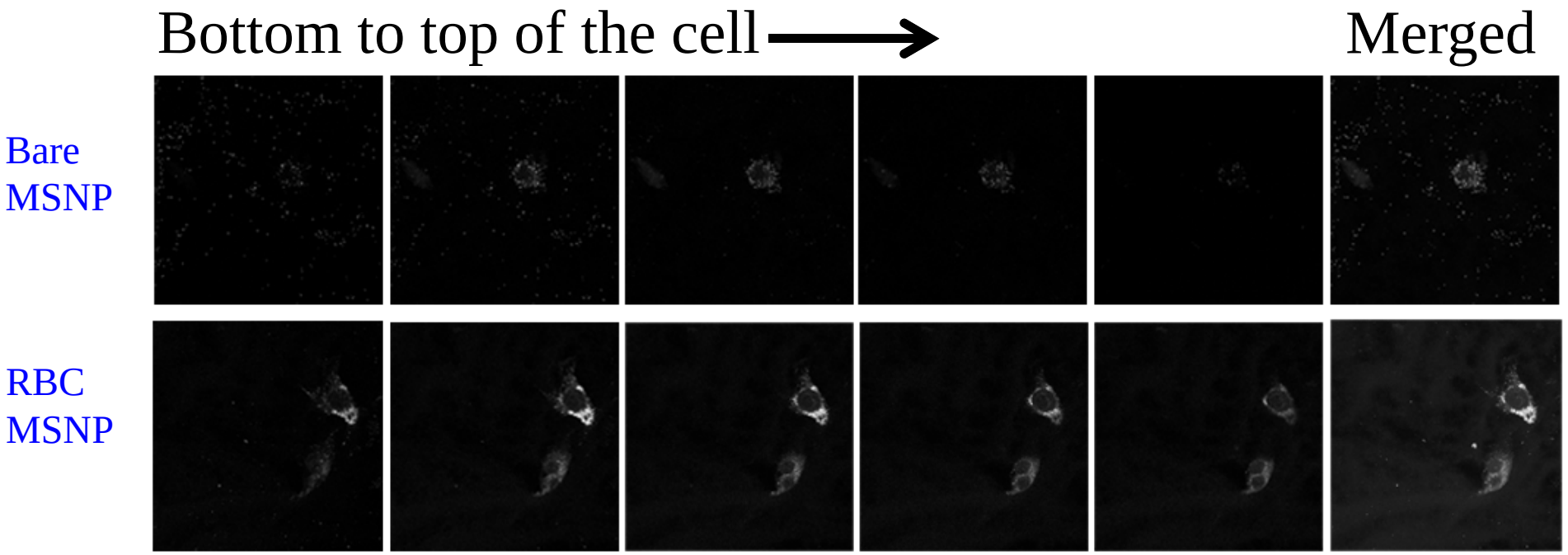


Uncoated Si-COOH MSNP



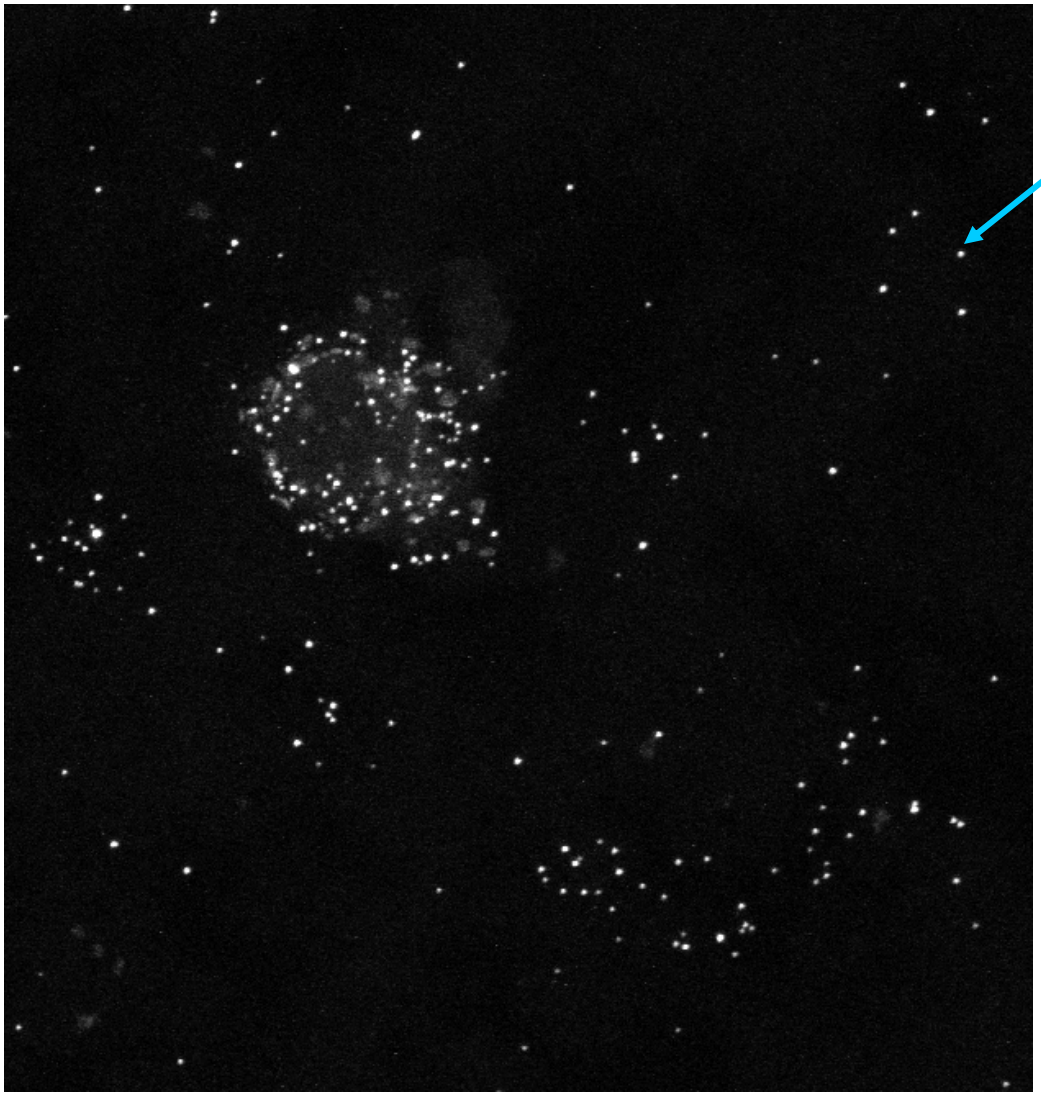
RBC membrane enveloped Si-COOH MSNP

Human RBC ghosts fused on MSNPs enhance specific uptake by mouse Raw264.7 macrophage cells



Macrophages activated with Lipopolysaccharide (LPS)

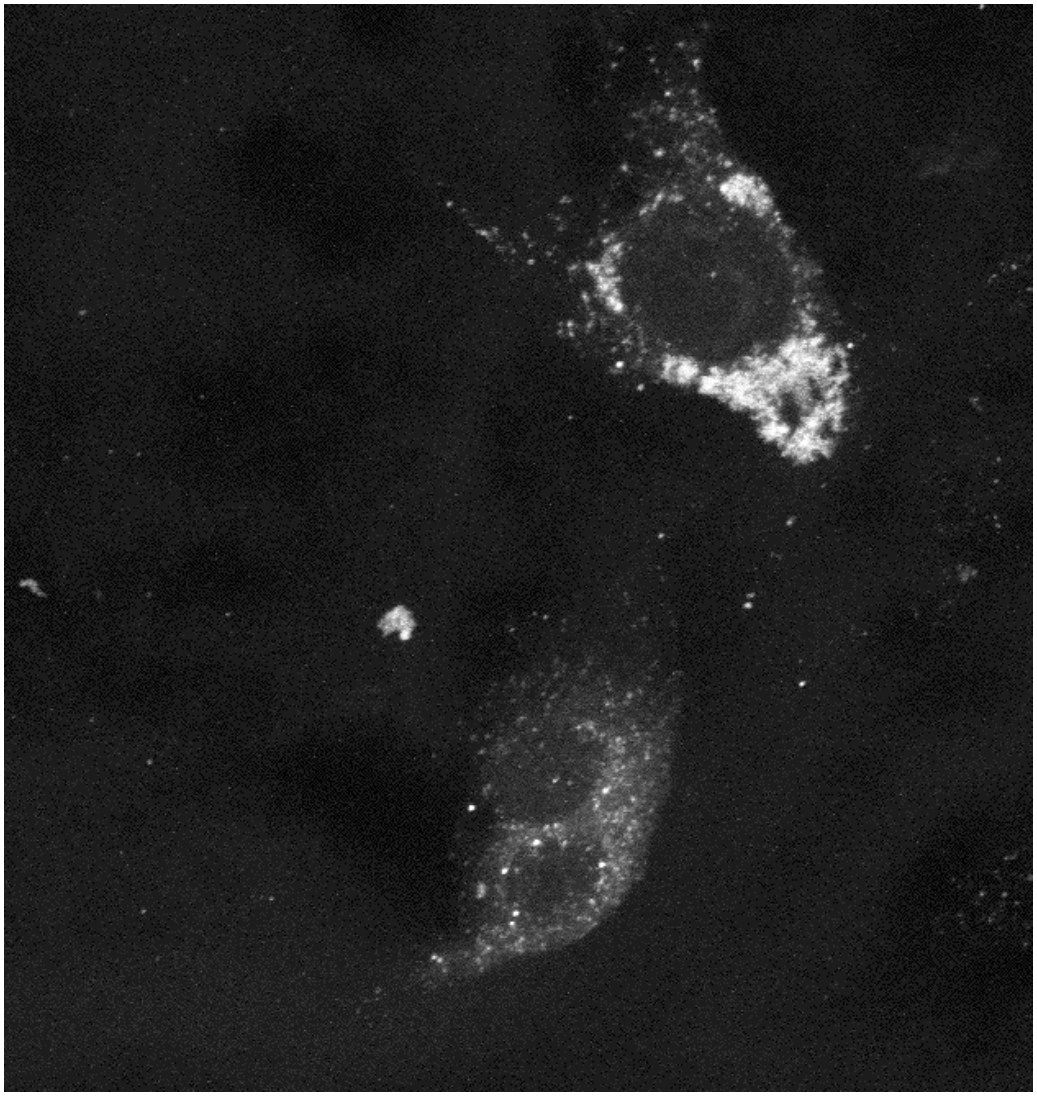
Bare MSNP bind but are weaky internalized by mouse macrophage cells



Non specifically bound Bare Si NP

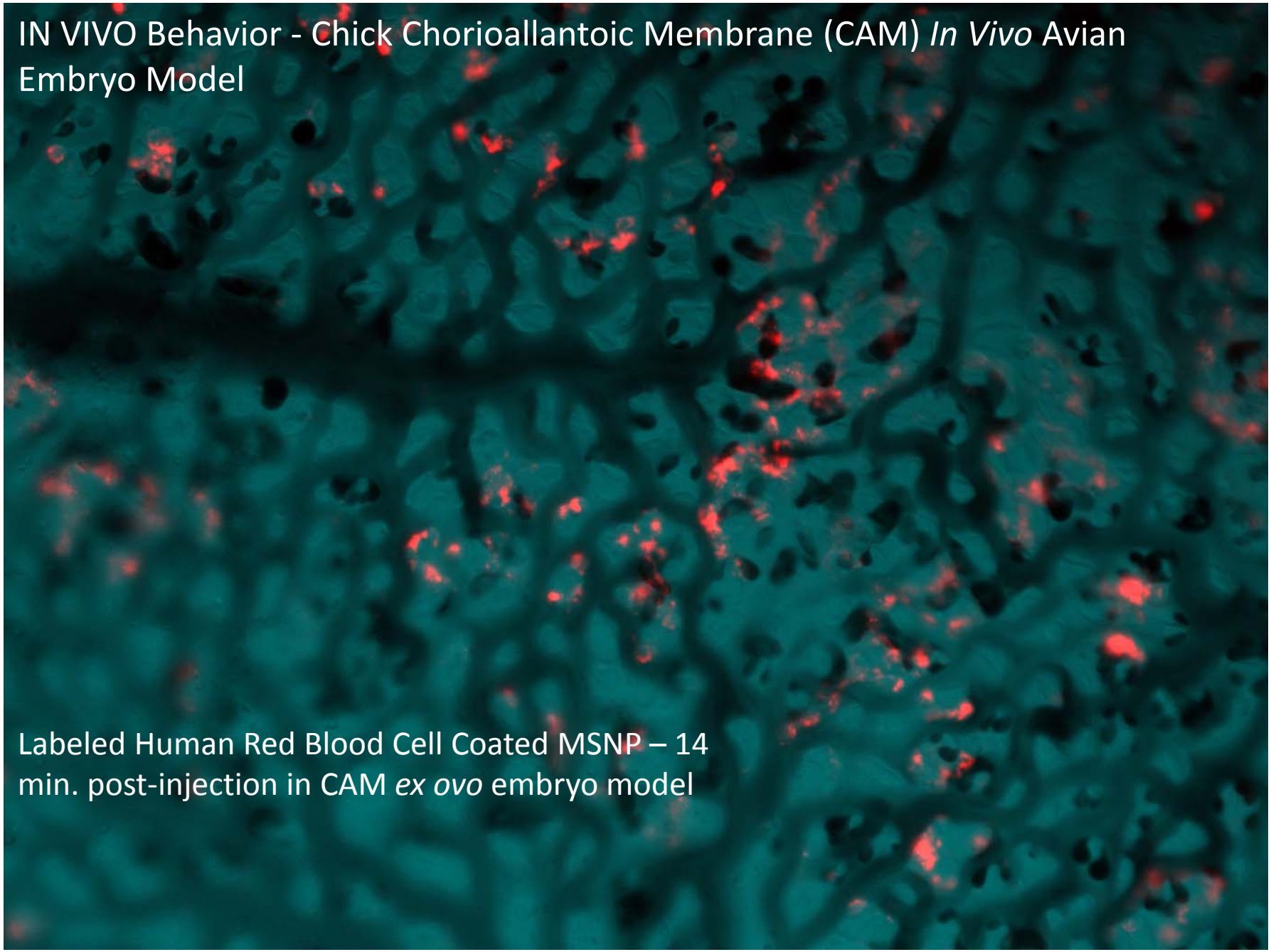
- Significant non-specific binding with **bare** Si NPs
- Besides non-specific binding, internalized bare Si NPs seem to be in the cytosol

MSNPs fused with human RBC ghosts are specifically taken in by mouse macrophage cells

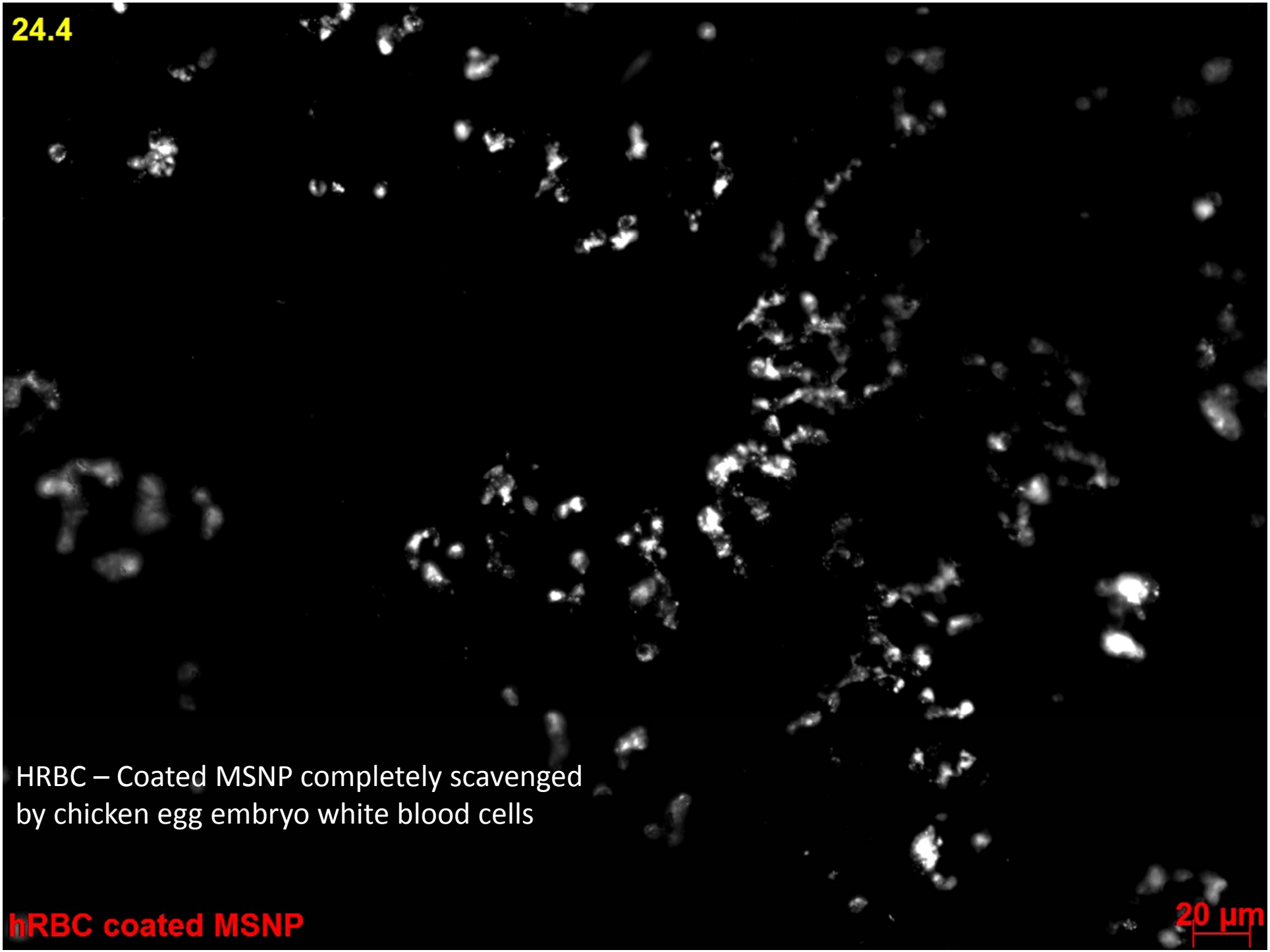


- No significant non-specific binding
- Functionalization of MSNP w/ human RBC ghosts enhances **specific** up take by mouse macrophage cells
- Particles significantly outline **perinuclear** region with some in the cytosol

IN VIVO Behavior - Chick Chorioallantoic Membrane (CAM) *In Vivo* Avian Embryo Model

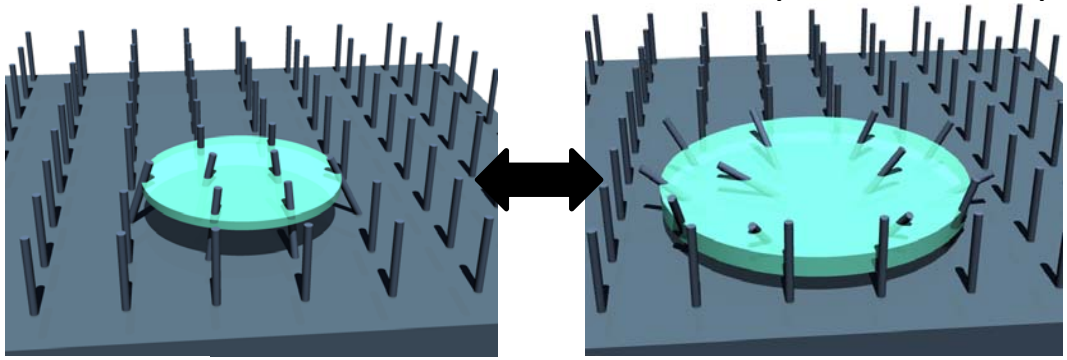


Labeled Human Red Blood Cell Coated MSNP – 14 min. post-injection in CAM *ex ovo* embryo model

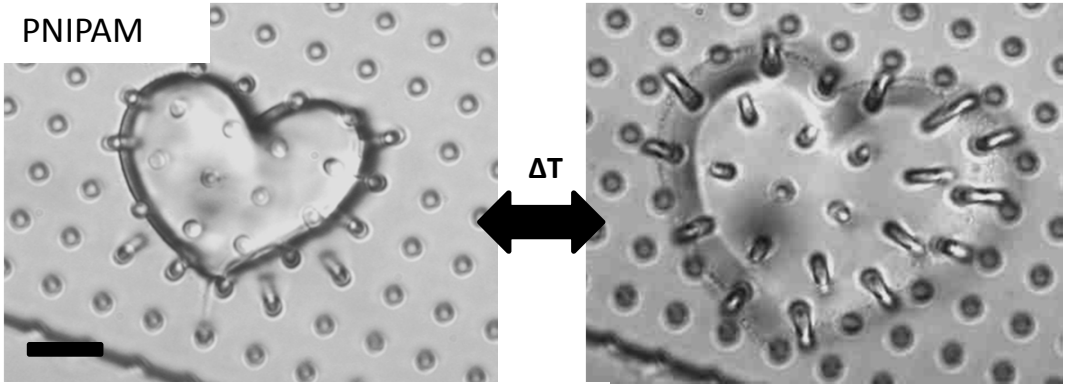

24.4
HRBC – Coated MSNP completely scavenged
by chicken egg embryo white blood cells
hRBC coated MSNP
20 µm

Architectures for Dissipative Assembly – Lithographically patterned hydrogel membranes suspended in wire arrays

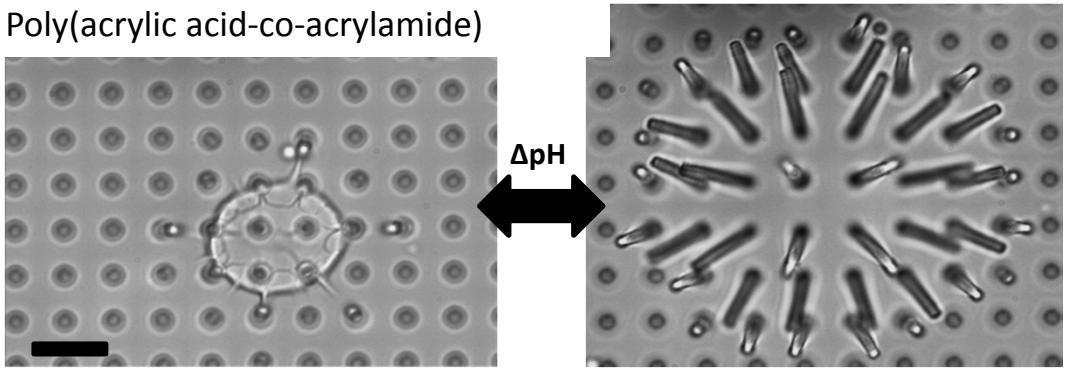
Actuation Response to Temp/ pH



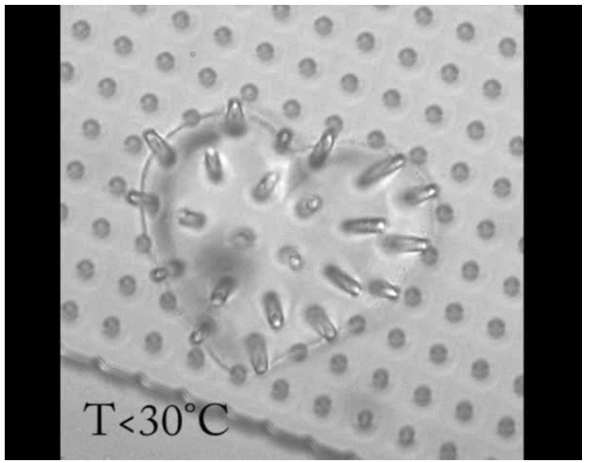
PNIPAM

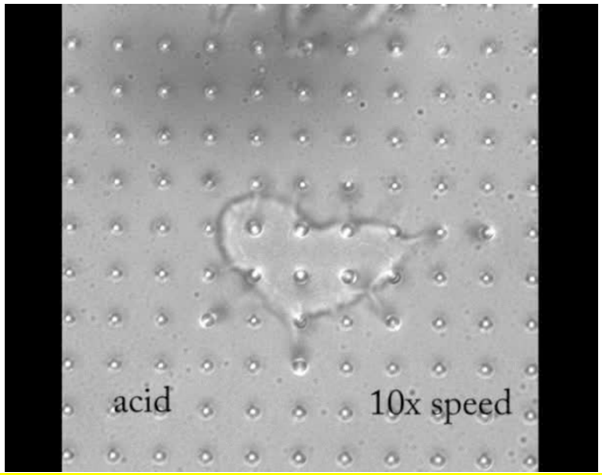


Poly(acrylic acid-co-acrylamide)



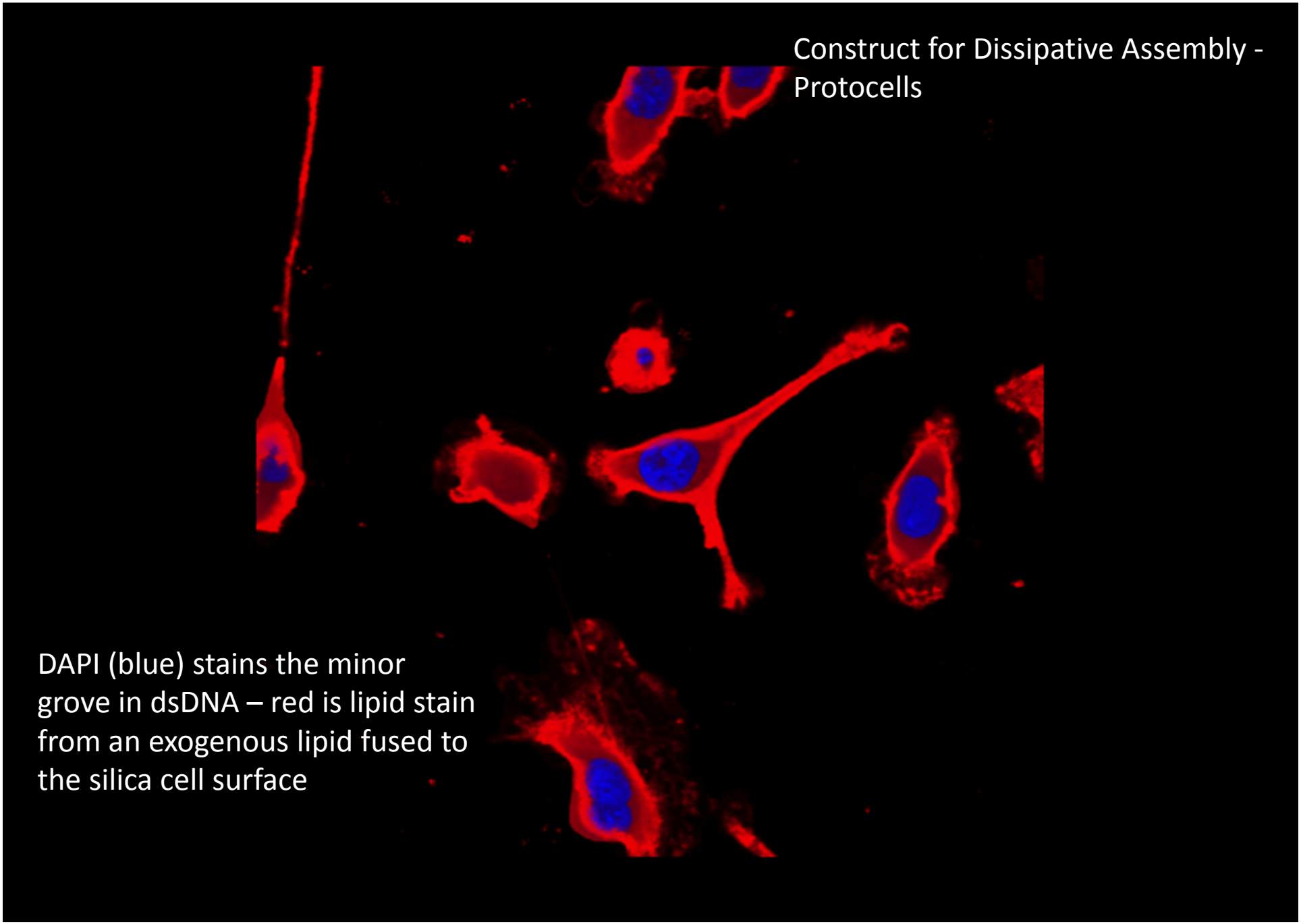
Scale bars = 10 µm



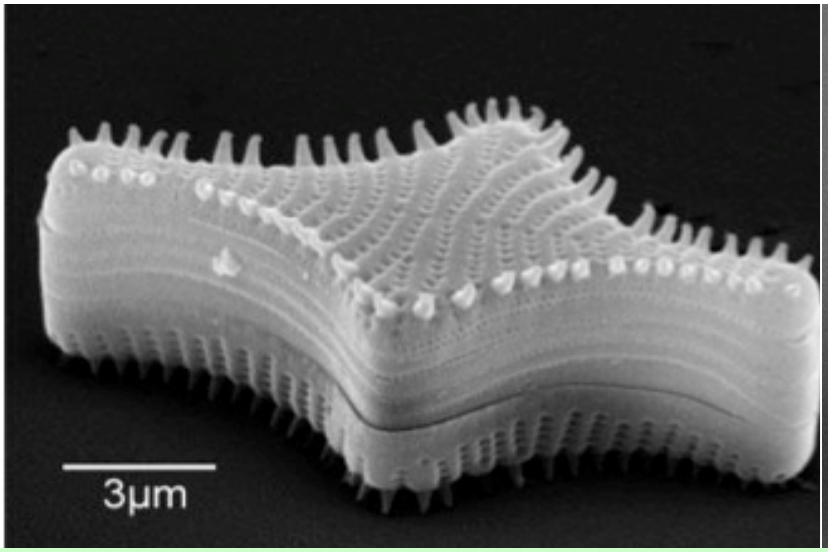


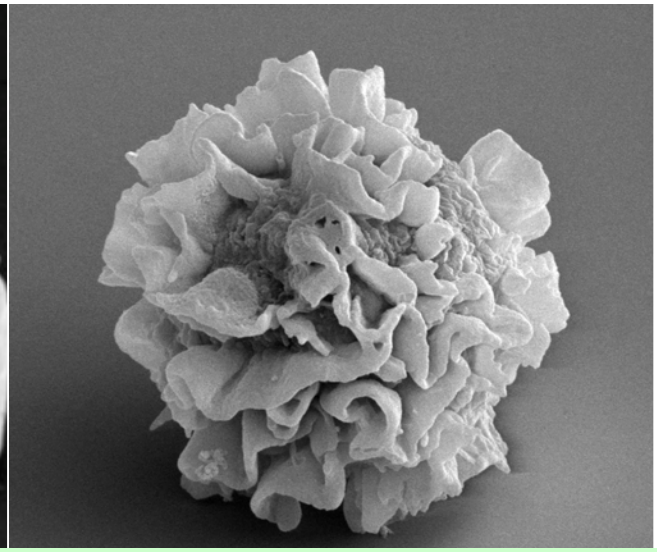
Patterning enables complex patterns of actuation

Angew Chemie 2011 (with JoAnna Aizenberg and Lauren Zarzar, Harvard)


Construct for Dissipative Assembly - Protocells
DAPI (blue) stains the minor grove in dsDNA – red is lipid stain from an exogenous lipid fused to the silica cell surface

Silica @ Cells: A special relationship





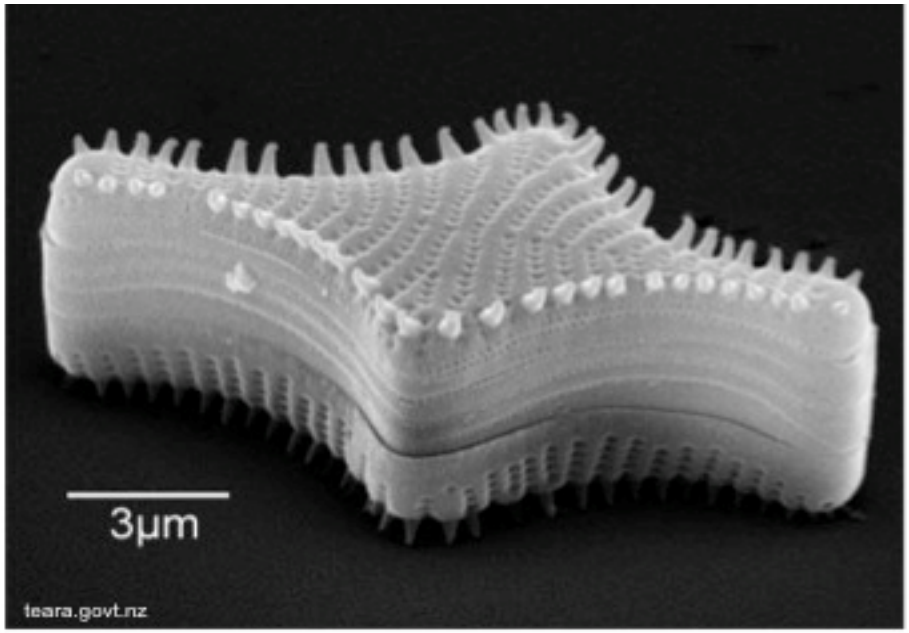
Bryan Kaehr[1], Jason Townson,[2], Eric C. Carnes[1], Yu-Shen Lin[2] and C.E. Ashley[1]

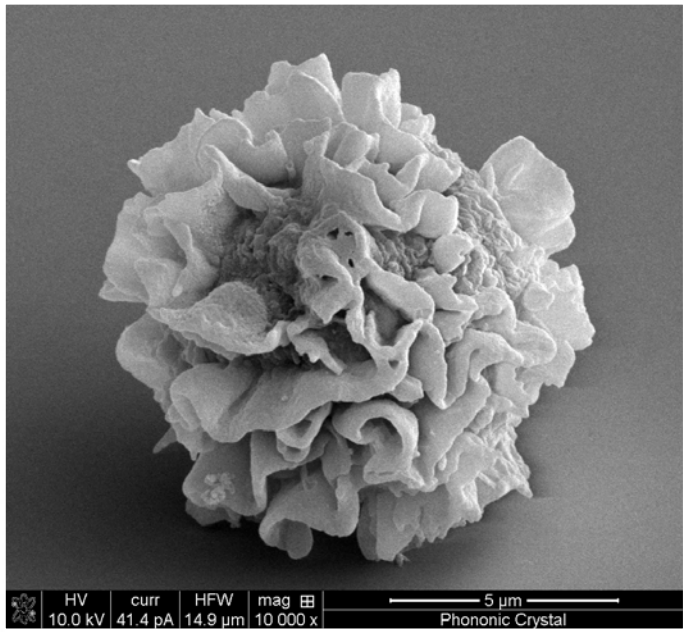
BES Biomolecular Materials Program

Water Replacement Hypothesis: Silicic acid $Si(OH)_4$ behaves thermodynamically like water and can replace water at biomolecular interfaces – silica is 'recognized' and organized by cells - through hydrogen bonding to surface silanols - and is processed by cells in a variety of manners

Interface between cells – water – and ‘silica’ is special and requires further investigation

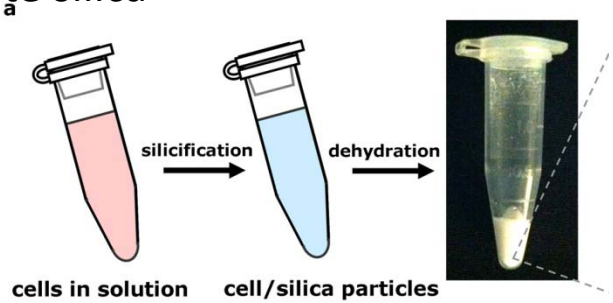
- What thermodynamic relationships exist between water and silicic acid – especially at a hydrophilic interface? Acts like trehalose or glycerol
- Life evolved from aqueous systems supersaturated in silica – do cells sense silica and/or process it?
- Can silicification serve as an alternative means of ‘cryo-preservation’ i.e. freezing in silica?
- Future: self-silicification…..



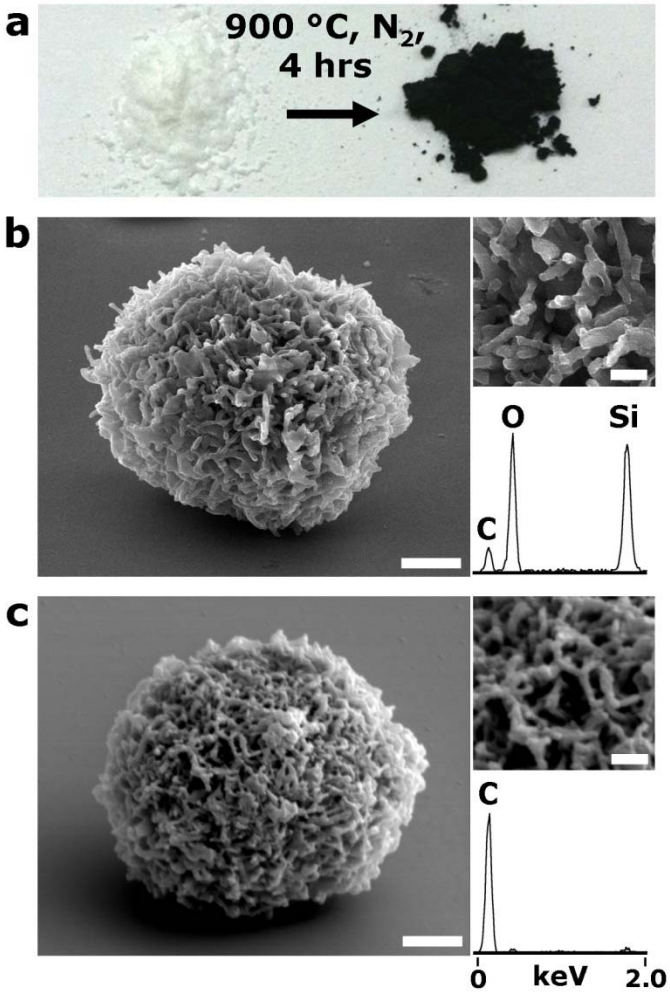


Transformable to Carbon with preserved dimensionality and high conductivity…Does Silica serve to nucleate graphite?

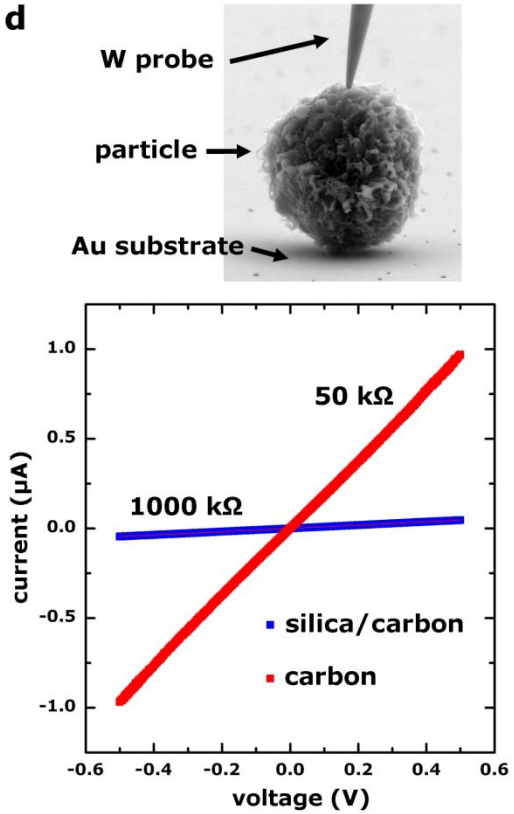
1) Template silica



2) Pyrolysis and etching



3) Electrical characterization



So similar are the basic building blocks of these two materials that it is even possible to form heteronetwork silica/water materials, where the two substances form a continuous framework in which silicate oligomers form part of the water hydrate lattice.

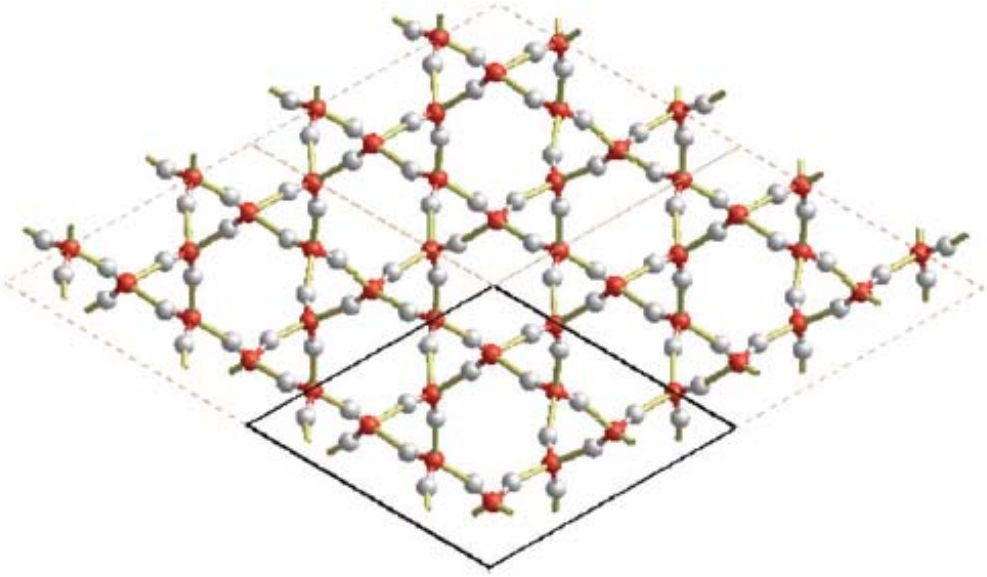


Fig. 4 The hypothetical quartz phase of ice.

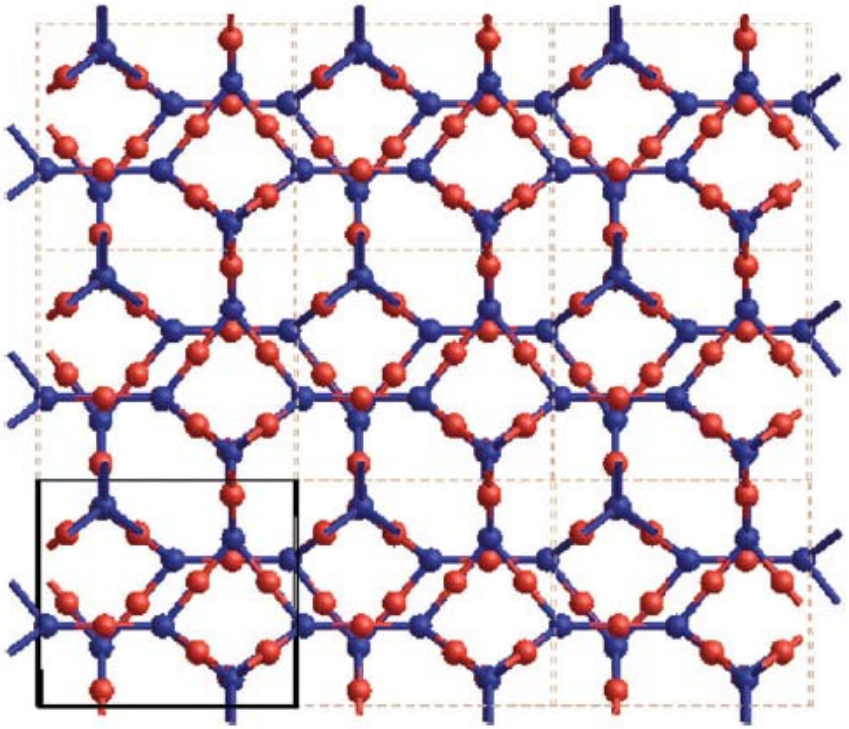
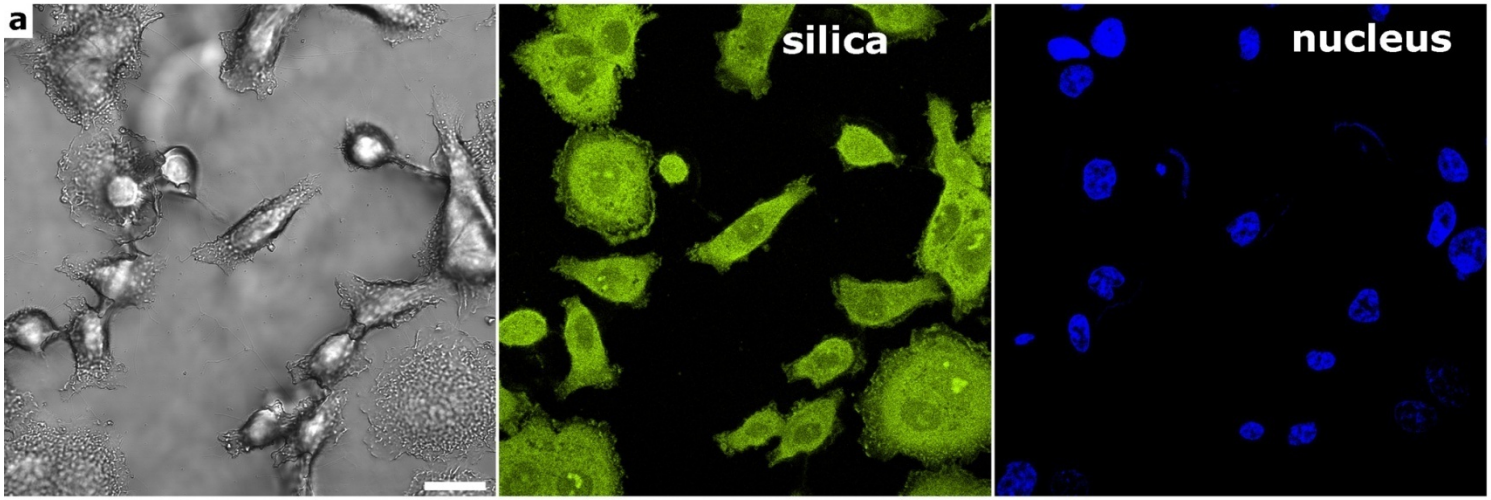
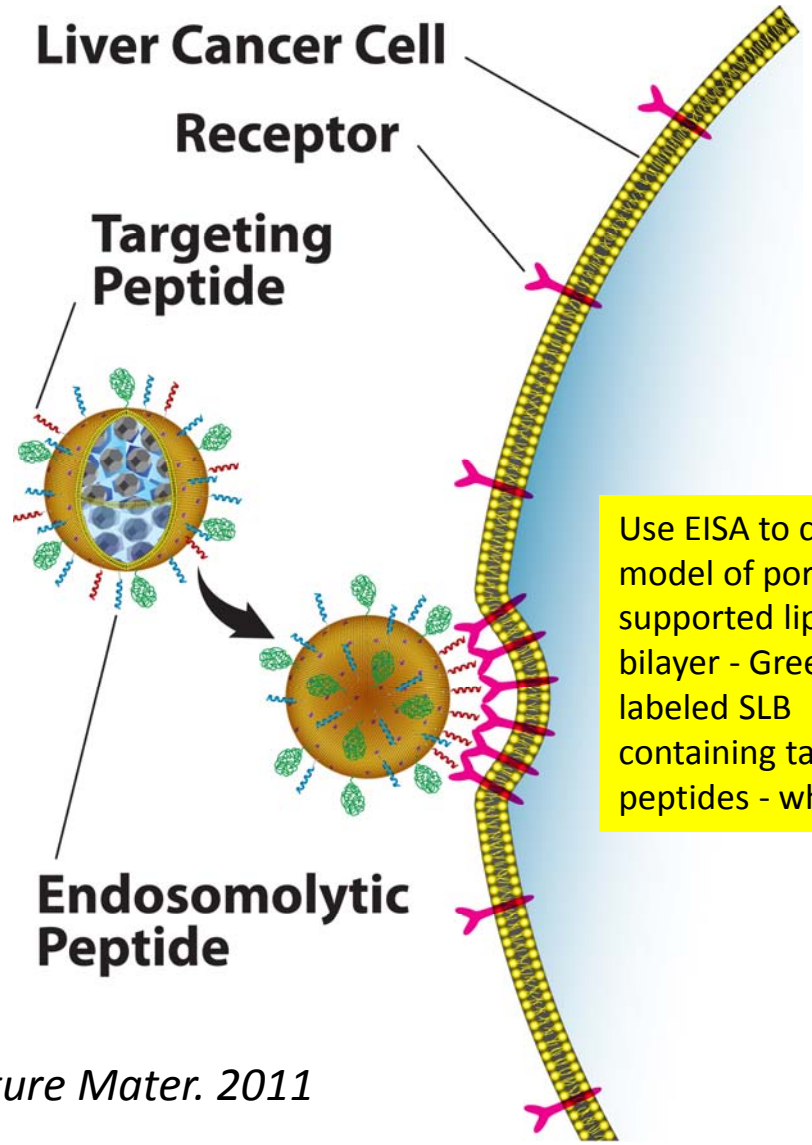


Fig. 3 The hypothetical interpenetrating structure of silica based upon the ice VI structure.

Stabilization of function – ‘Viability’ probe confirms esterase activity

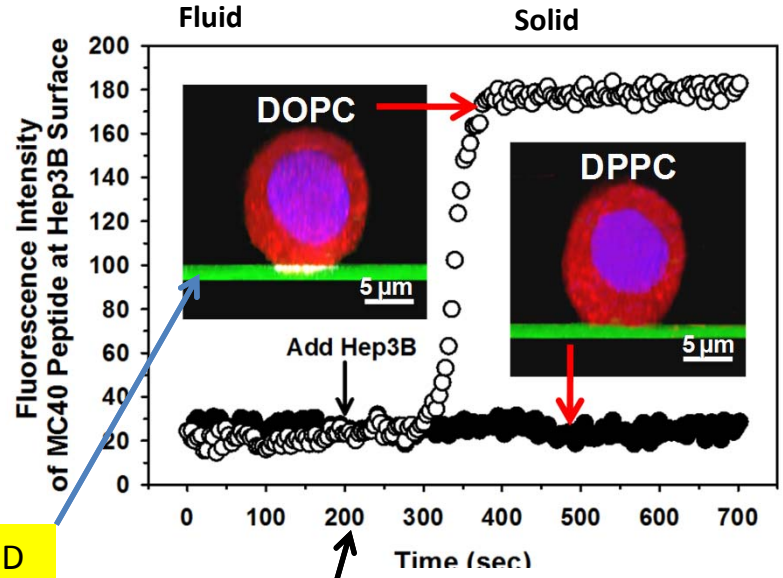


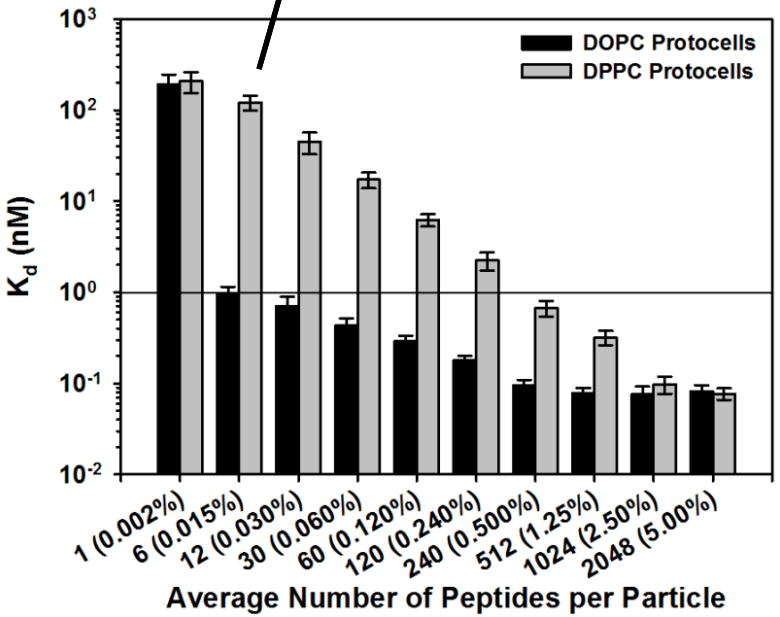
Fluidity of SLB enables targeting peptide recruitment resulting in high affinity binding with low average peptide density



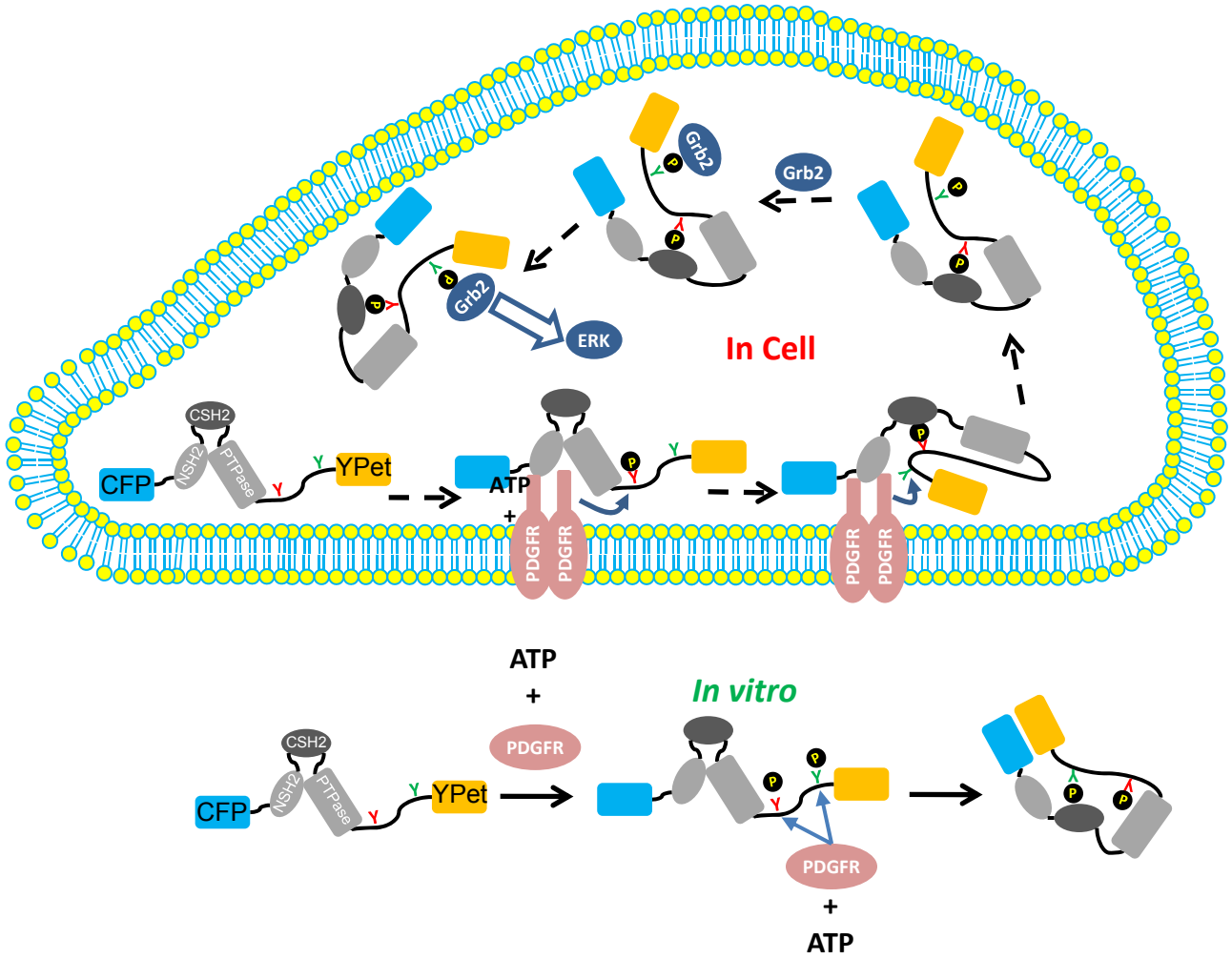
Nature Mater. 2011

Use EISA to create 2D model of porous silica supported lipid bilayer - Green labeled SLB containing targeting peptides - white





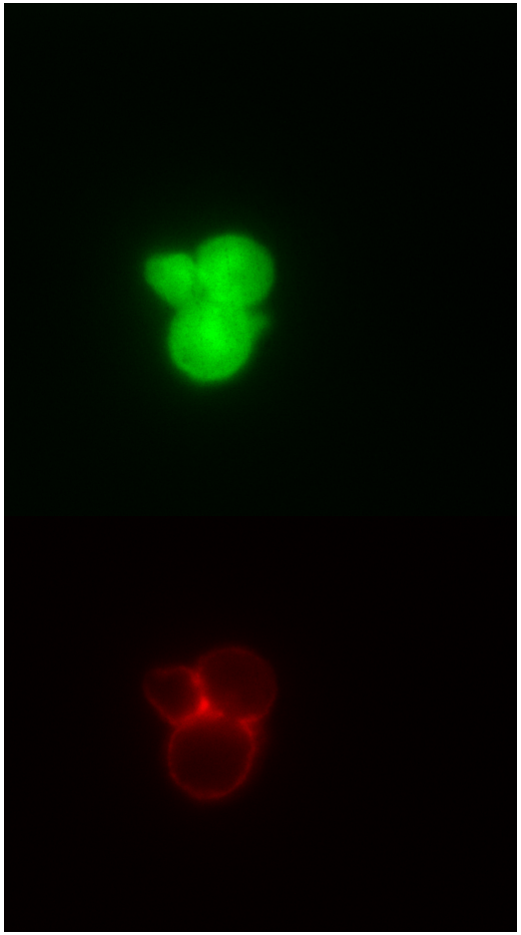
Differential Shp2 (SH2 domain containing tyrosine phosphatase 2) **biosensor reactions in cell and *in vitro***

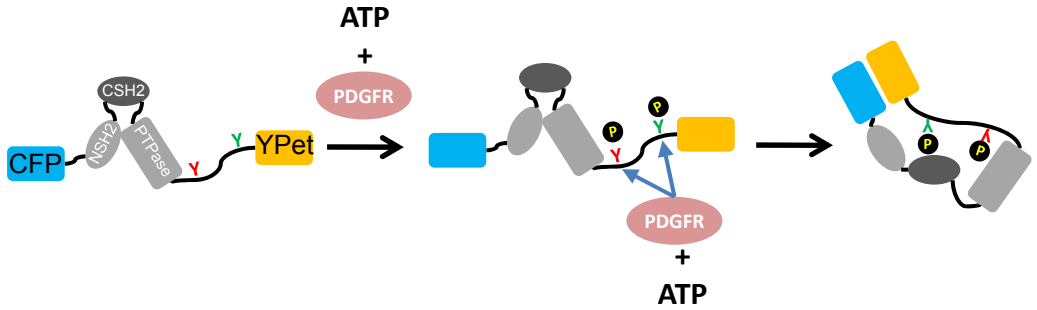


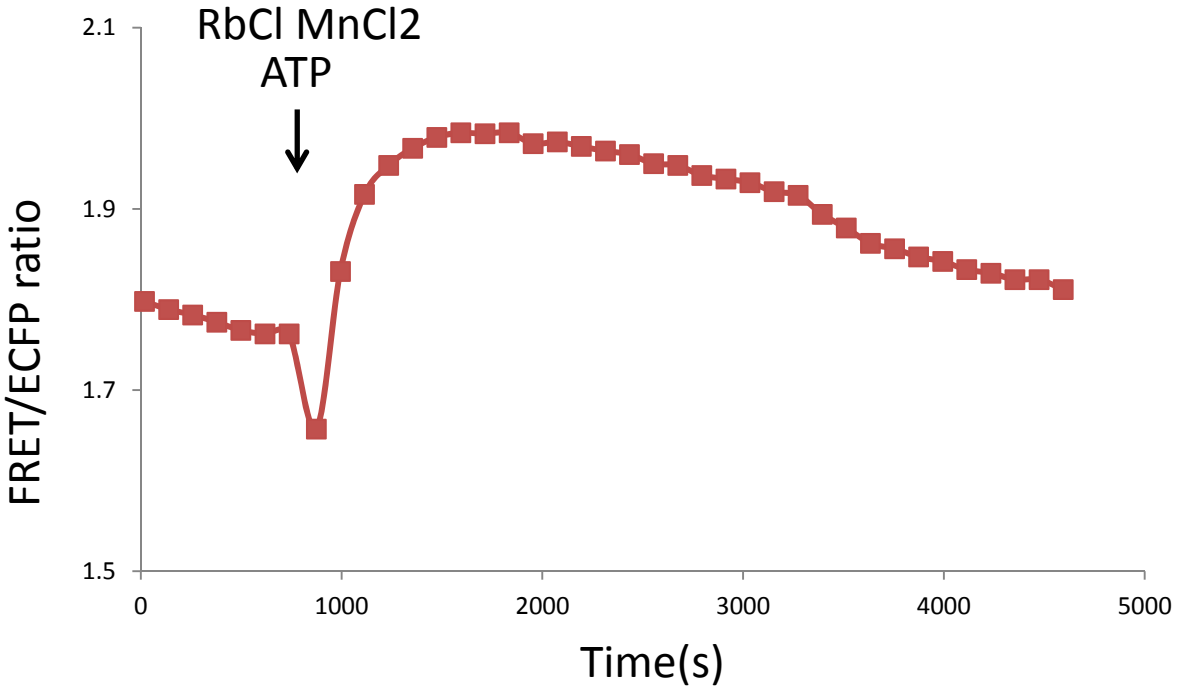
Can we reconstitute biochemical reactions within ‘protocell’ construct and

Silica cell replica core, loaded with Shp2 biosensor with PDGFR, coated with DOPC Texas Red membrane.

Then add ATP, MnCl2 and RbCl together, which permeates membrane to let ATP pass into the core.

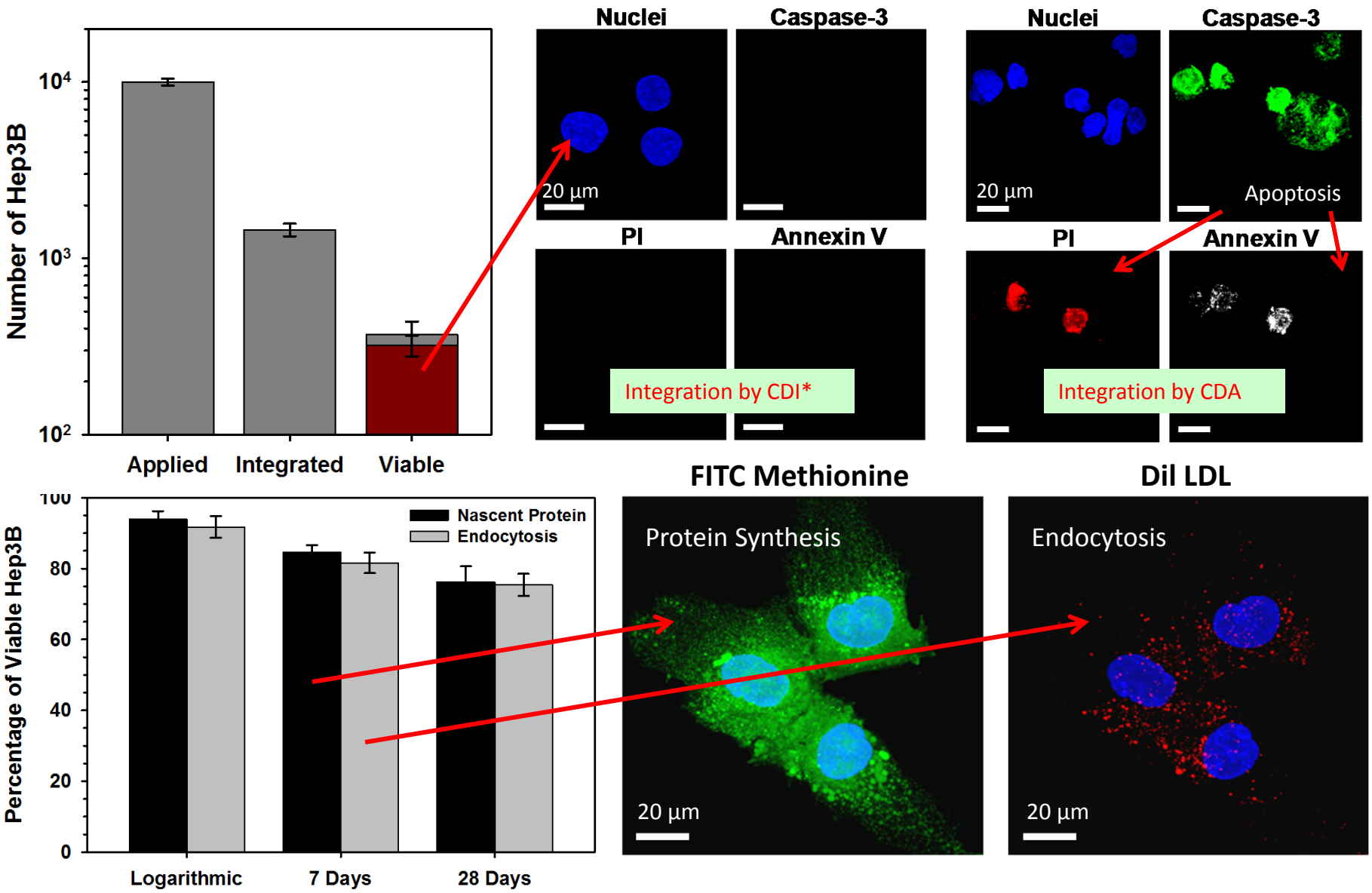




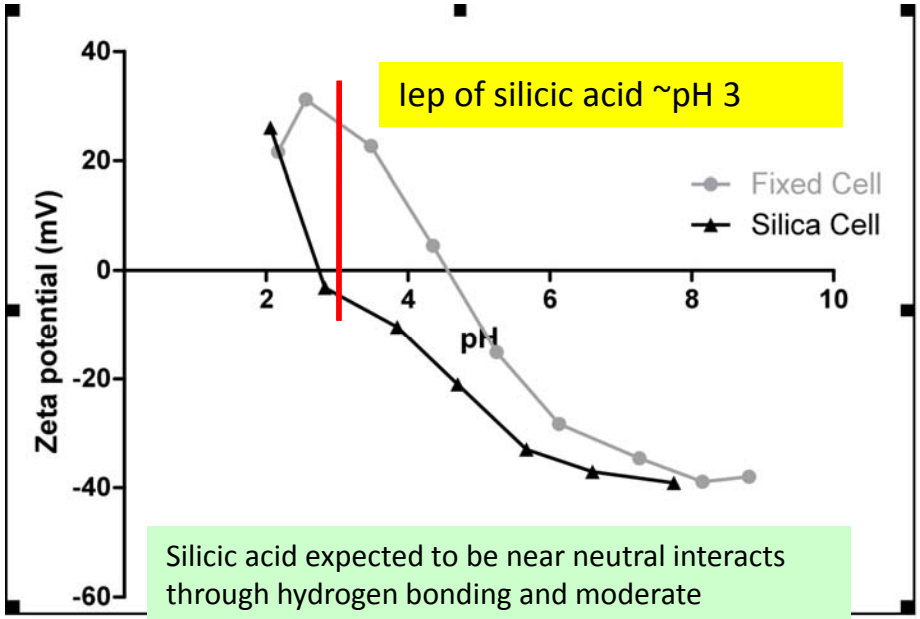


Chemical reaction can happen inside silica cell replica core with membrane boundaries when ATP is supplied.

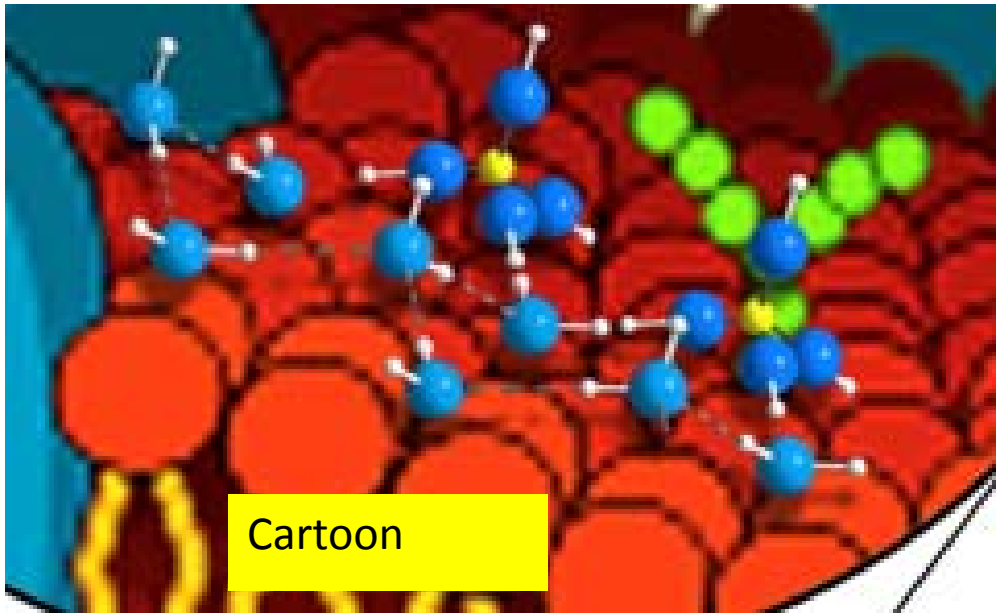
Integrated Cell Remain Viable for > One Month and Are Capable of Synthesizing Proteins and Endocytosing Nutrients – Does integration select for sub population or induce new behavior?

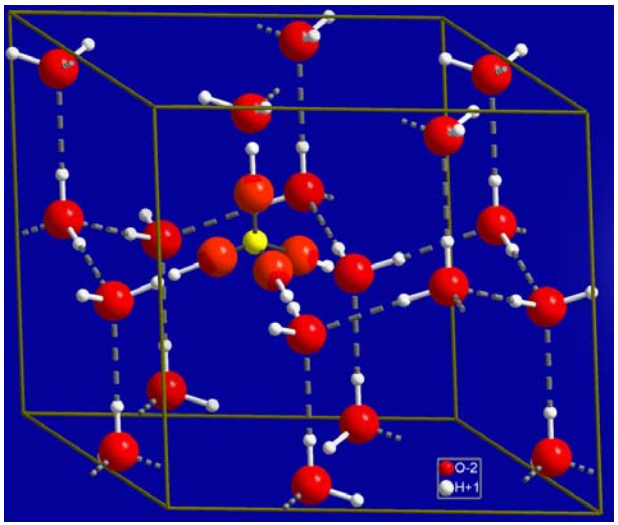


Water Replacement Hypothesis



Silicic acid expected to be near neutral interacts through hydrogen bonding and moderate electrostatic interactions





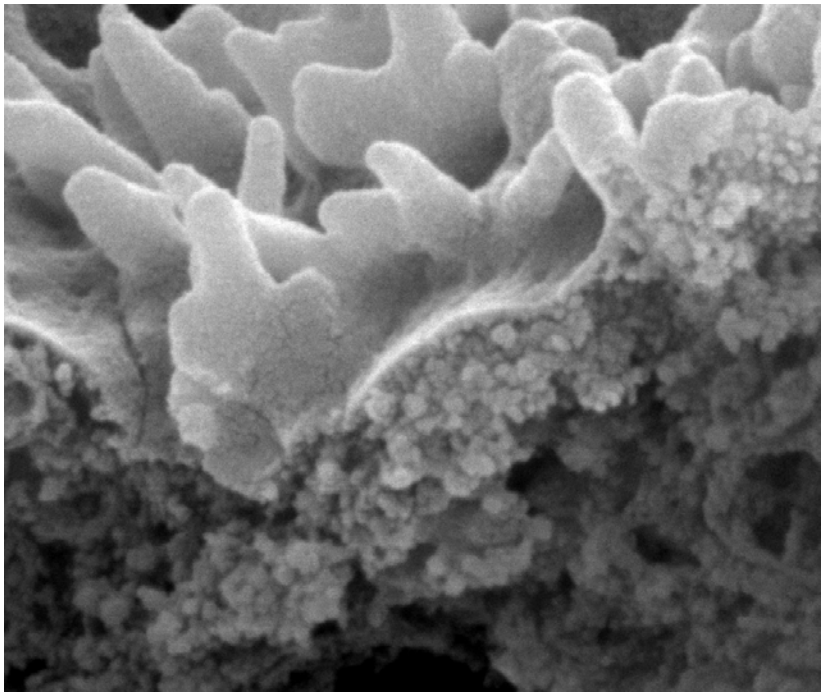
Water Replacement Hypothesis – Can silicic acid replace/displace hydrogen-bonded interfacial water at cellular/biomolecular interfaces and be concentrated and catalyzed by proximal membrane associated proteins, carbohydrates (other components) to form stable interfacial silica network in self-limited process?

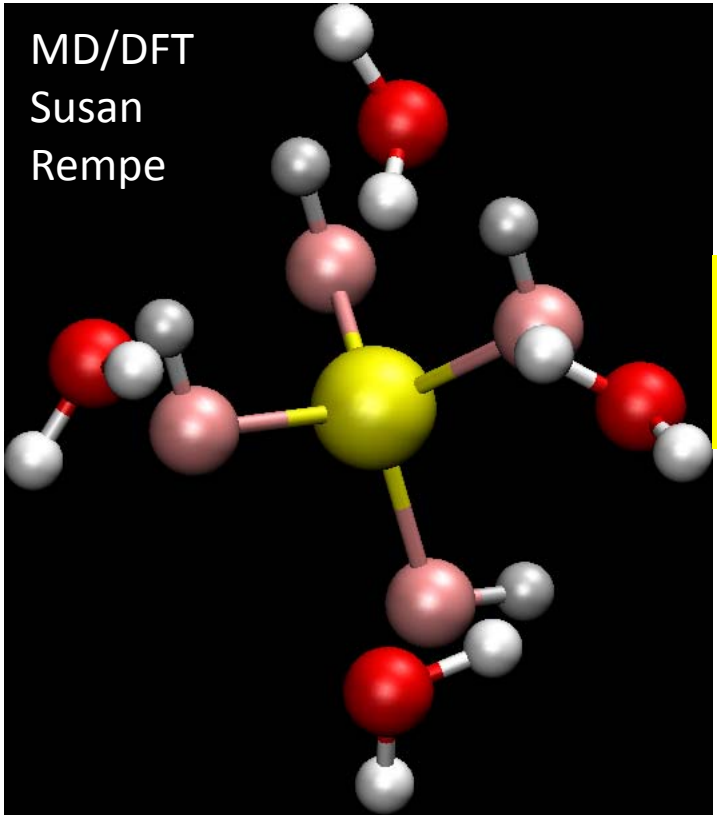
Do water associated surfaces represent an optimized mechanically connected network that if replaced with silica would result in dimensional stability?

Water Replacement Hypothesis – Can silica (like trehalose) hydrogen bond with and preserve cellular function upon drying within robust inorganic host

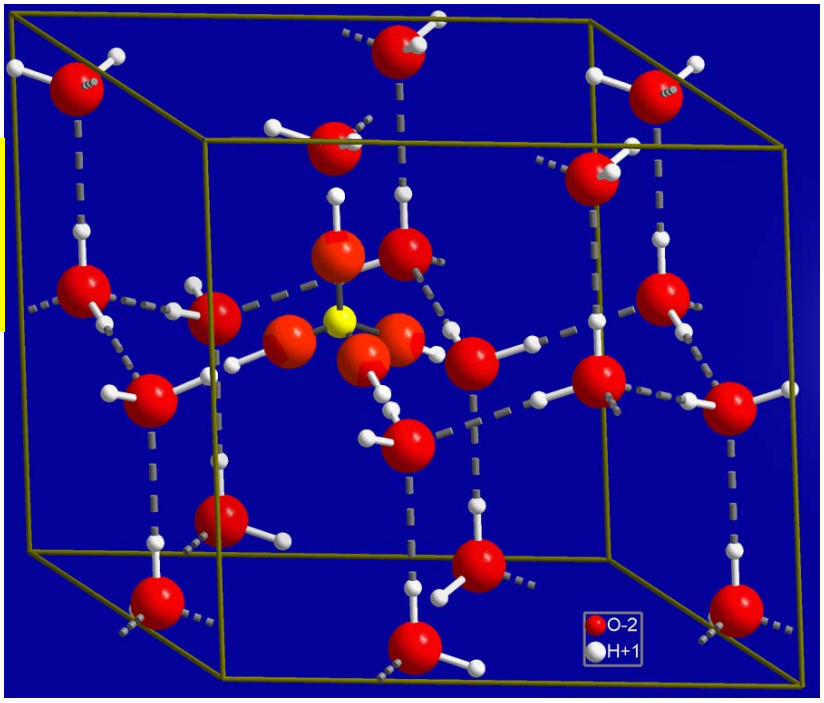
-Retained enzymatic activity

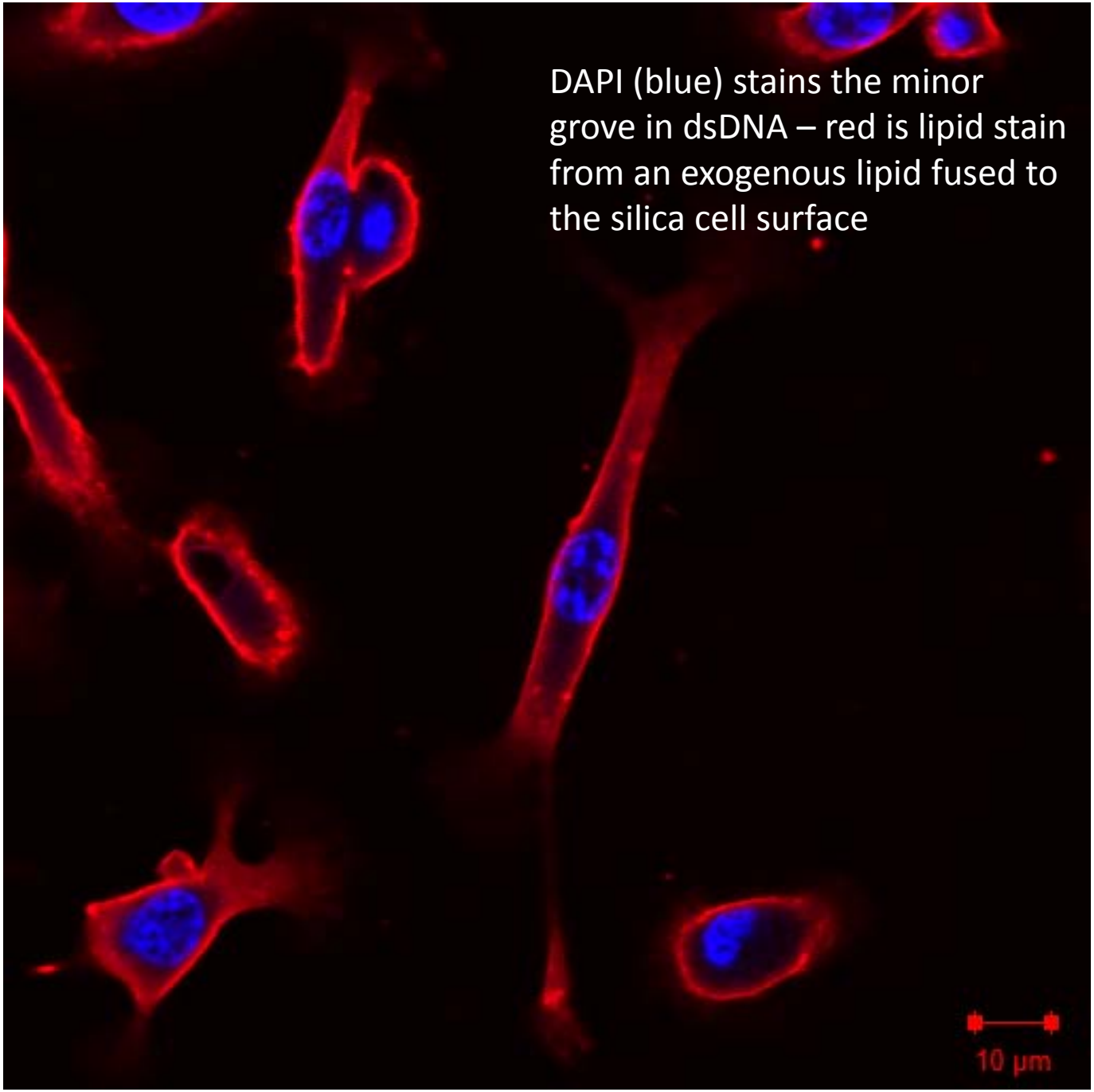
-Replace cryo-preservation?





H-bond strengths comparable




DAPI (blue) stains the minor grove in dsDNA – red is lipid stain from an exogenous lipid fused to the silica cell surface
10 µm

Conventional Microtome preparation shows morphological details nearly indistinguishable from the parent cells