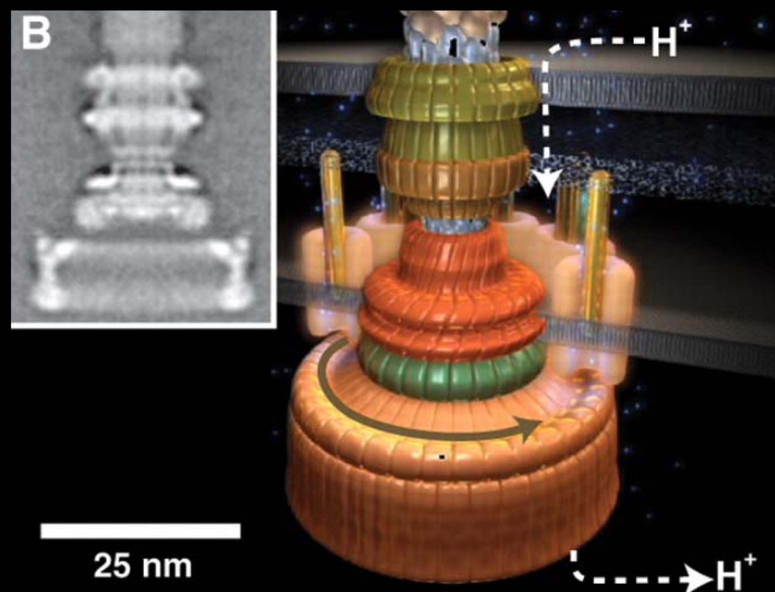
SAND2014-3883C

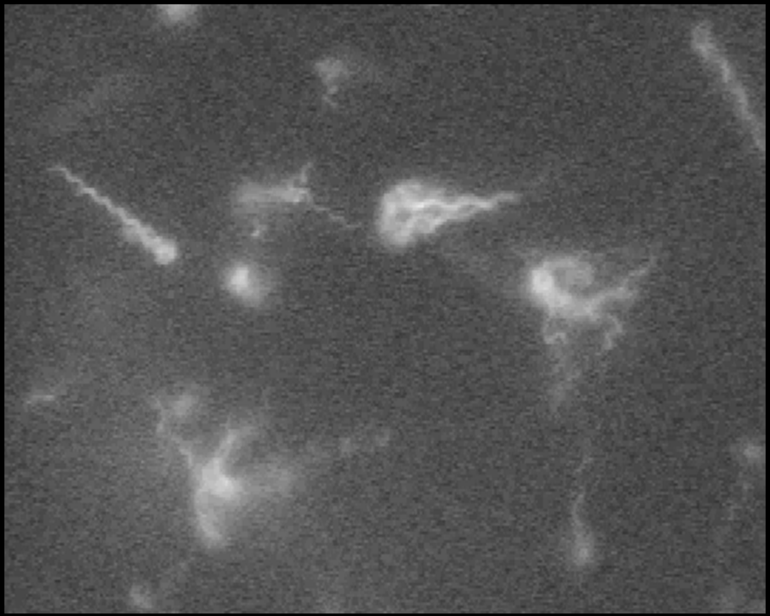
Biological building blocks for synthetic, life-like materials

Bryan Kaehr
Advanced Materials Laboratory
Sandia National Laboratories
NanoForum, University of Helsinki, May 16, 2014

Howard Berg, Harvard

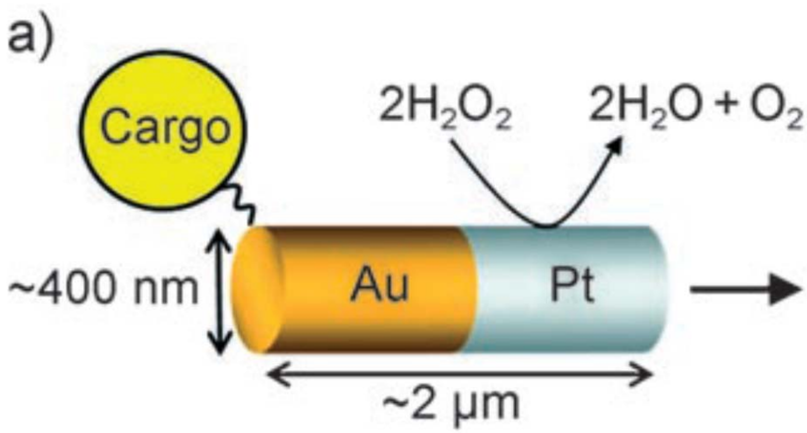


SCIENCE VOL 317 20 JULY 2007

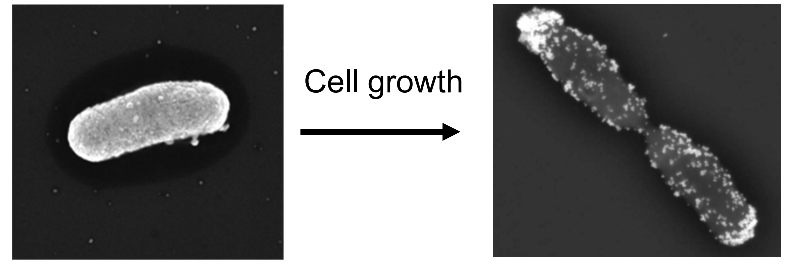


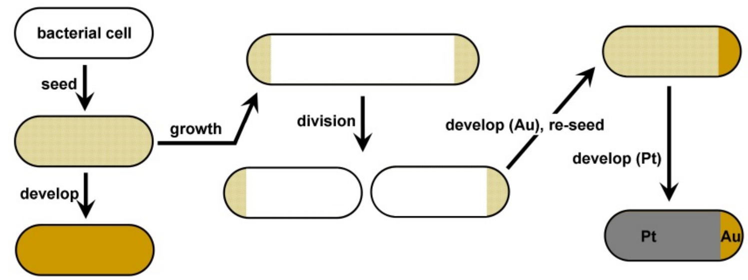
Howard Berg, Harvard

Catalytic (Ecoli-like) Nanomotors:
Paxton (CINT), Mallouk and Sen (Penn State)



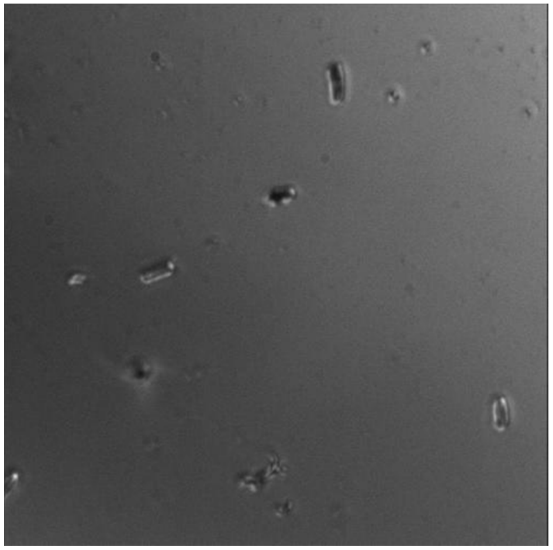
Angew. Chem. Int. Ed. 2008, 47, 8565 – 8566

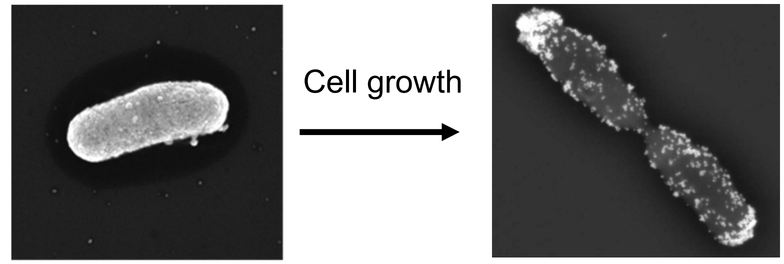


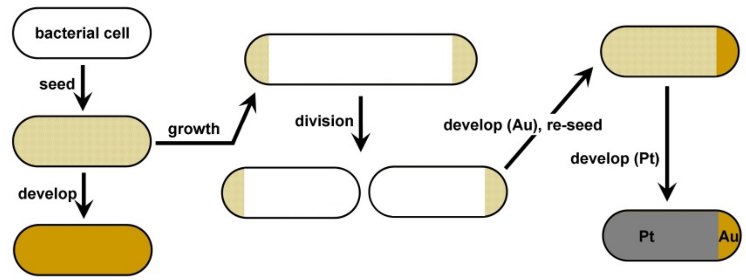


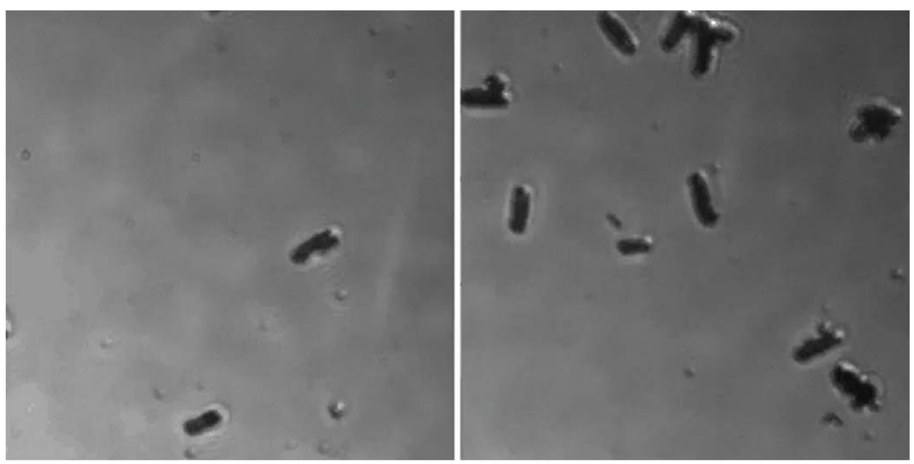
Xie Group, Harvard

Catalytic (Ecoli-like) Nanomotors: **Built from Ecoli**

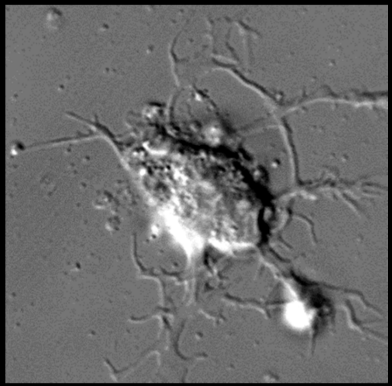


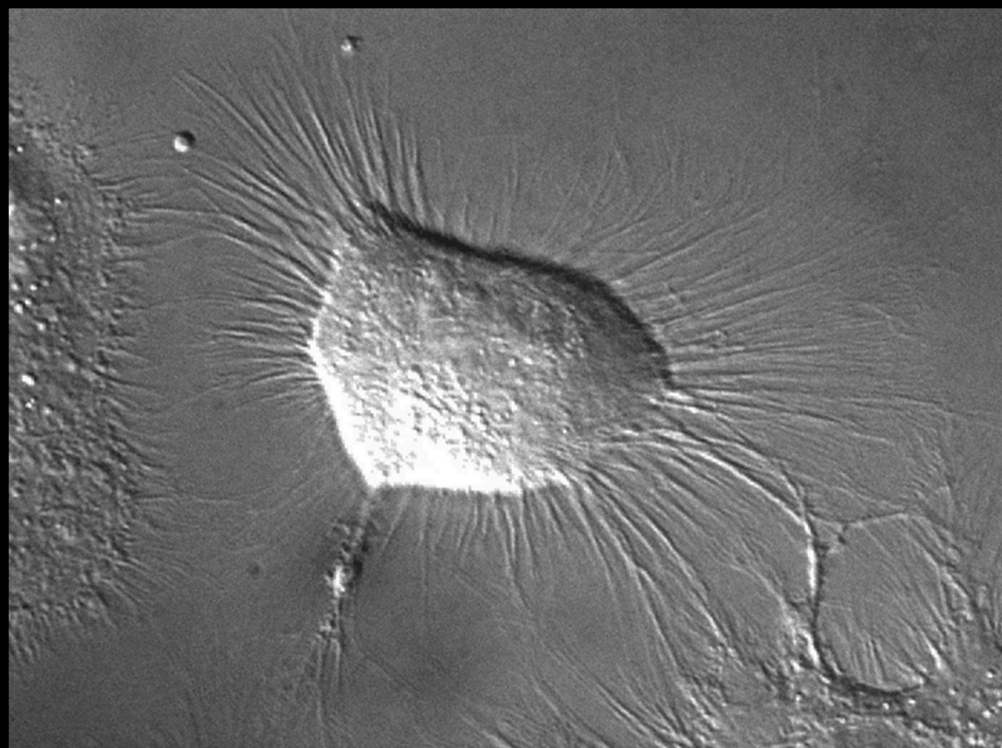


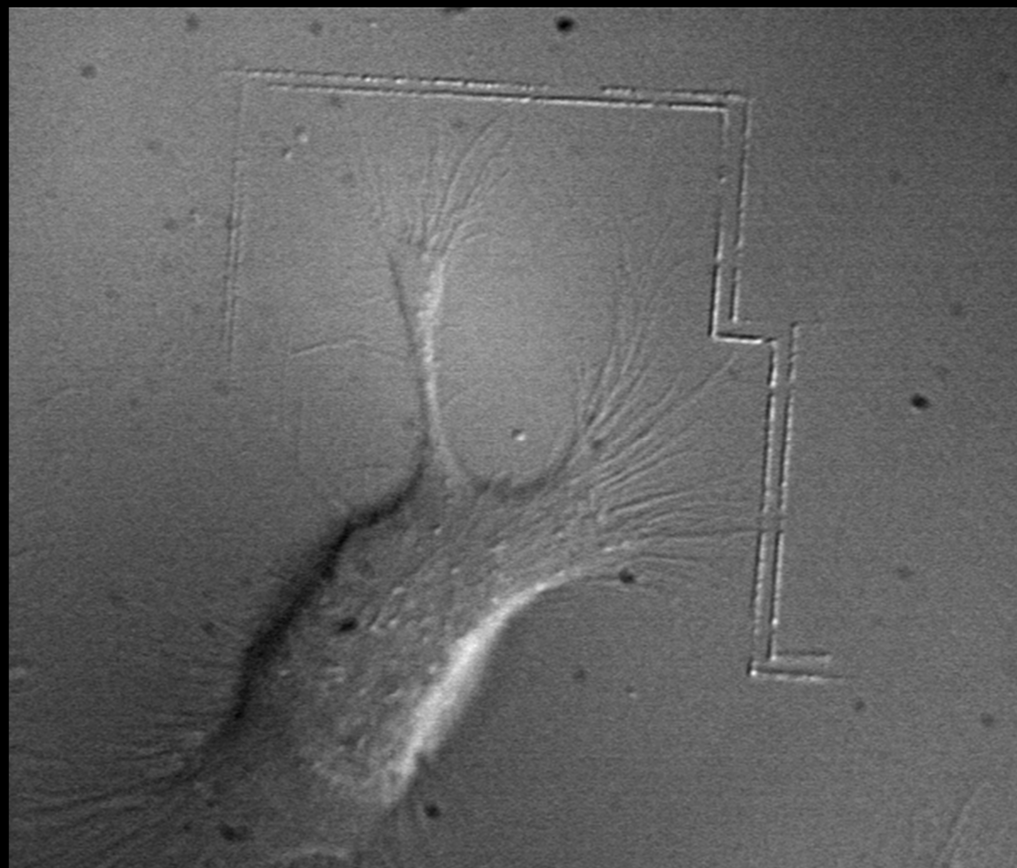


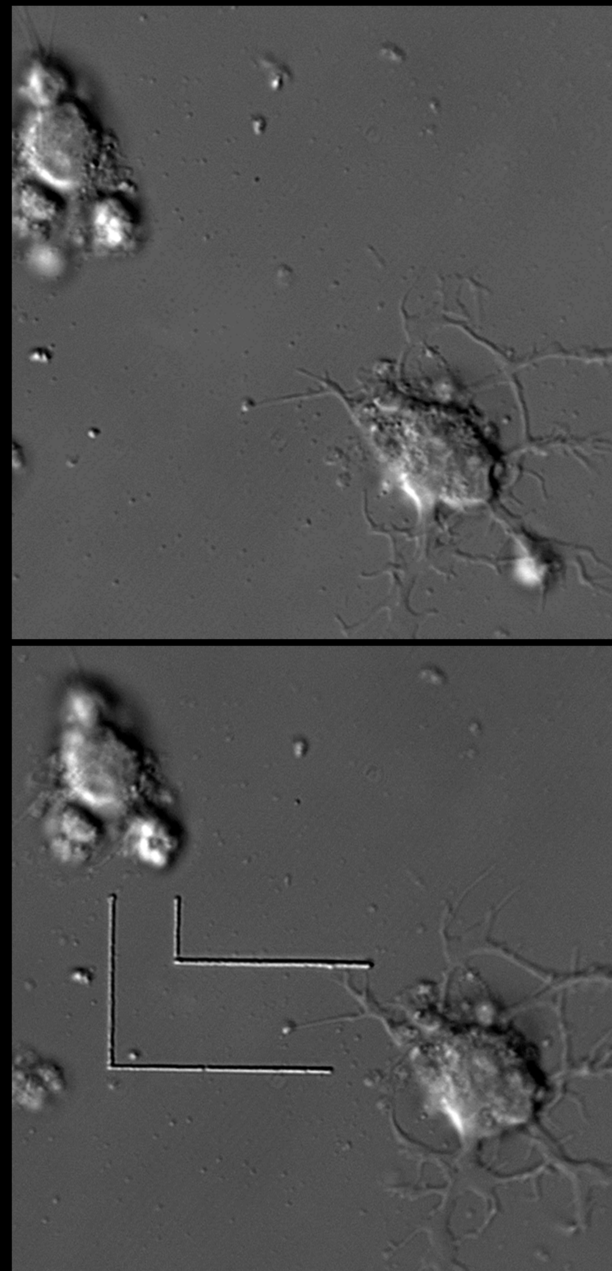


Chem. Commun.,(2010) 46, 5268-5270.

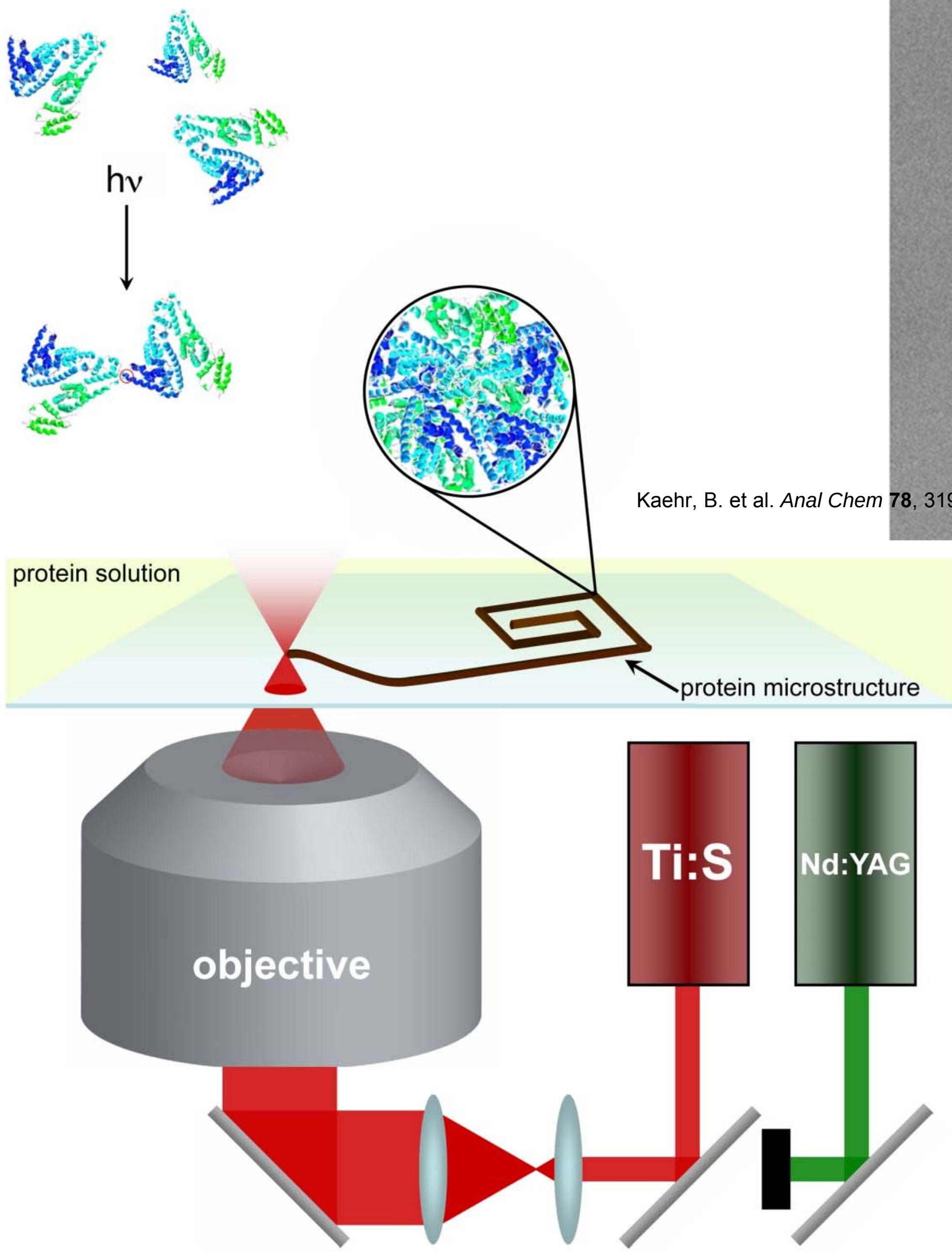


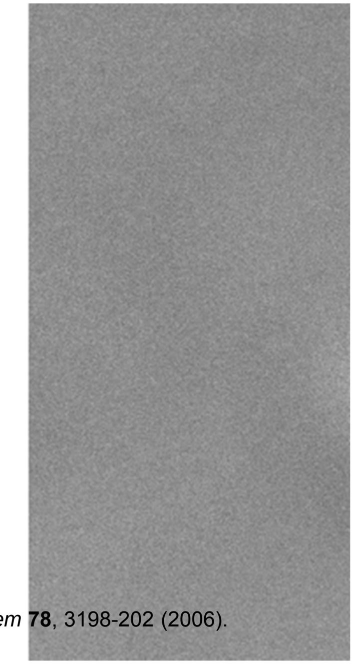






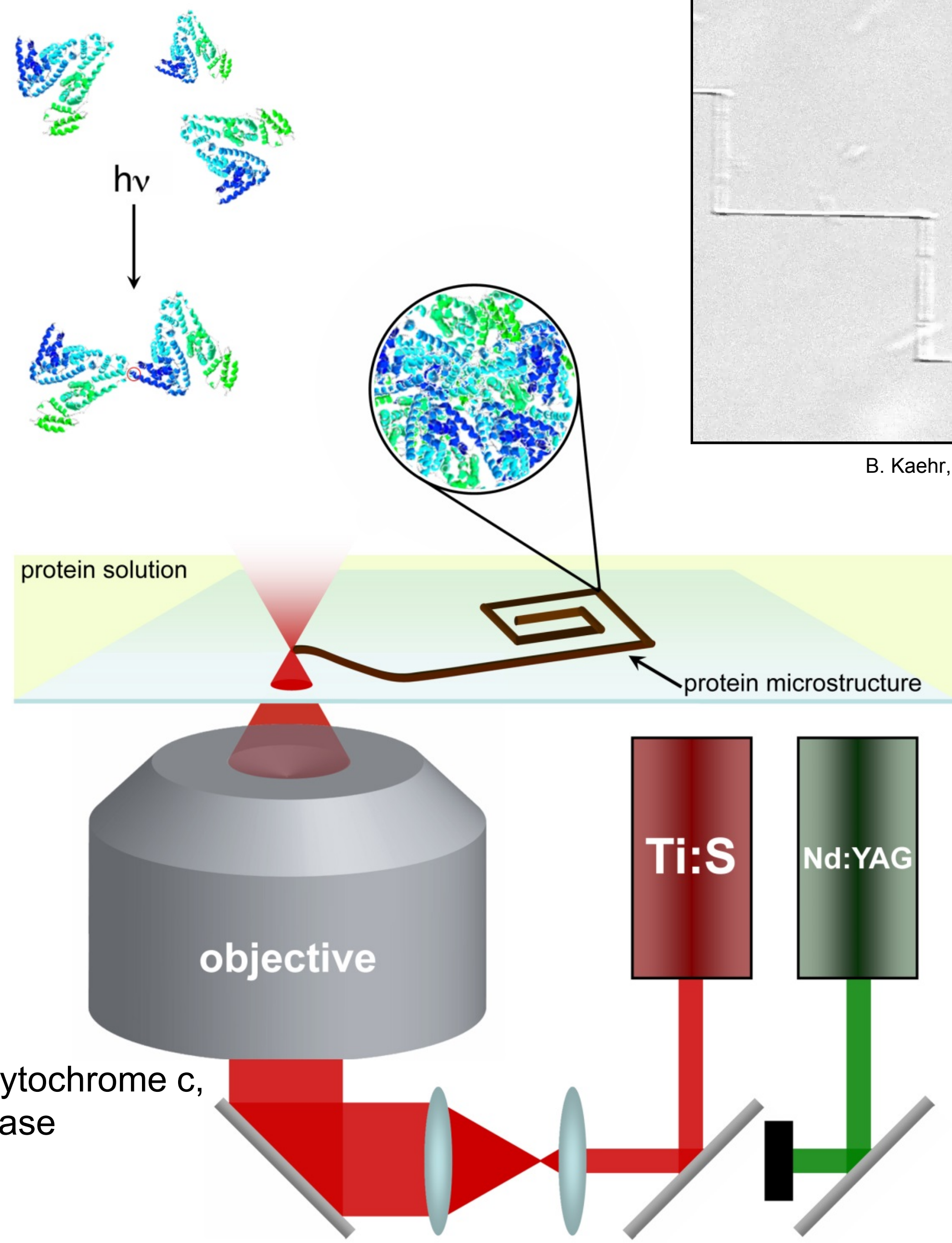
MPE Protein Photocrosslinking

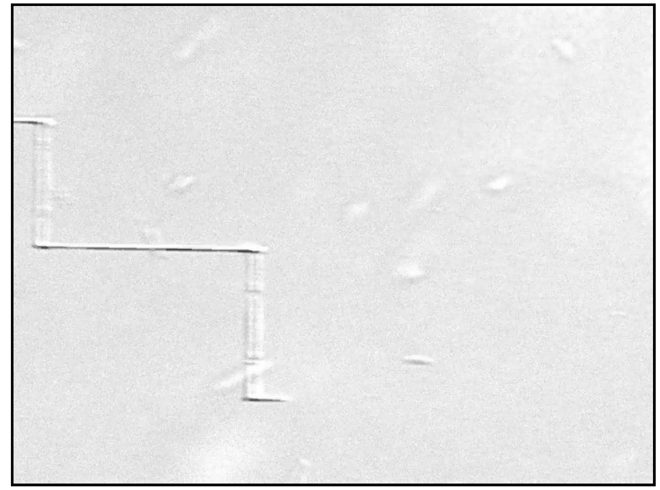




Kaehr, B. et al. *Anal Chem* **78**, 3198-202 (2006).

MPE Protein Photocrosslinking





B. Kaehr, J. Shear, *Lab on a Chip* **2009**, *9*, 2632.

Sensitizers

Rose Bengal, Methylene Blue, Porphyrins, Flavins

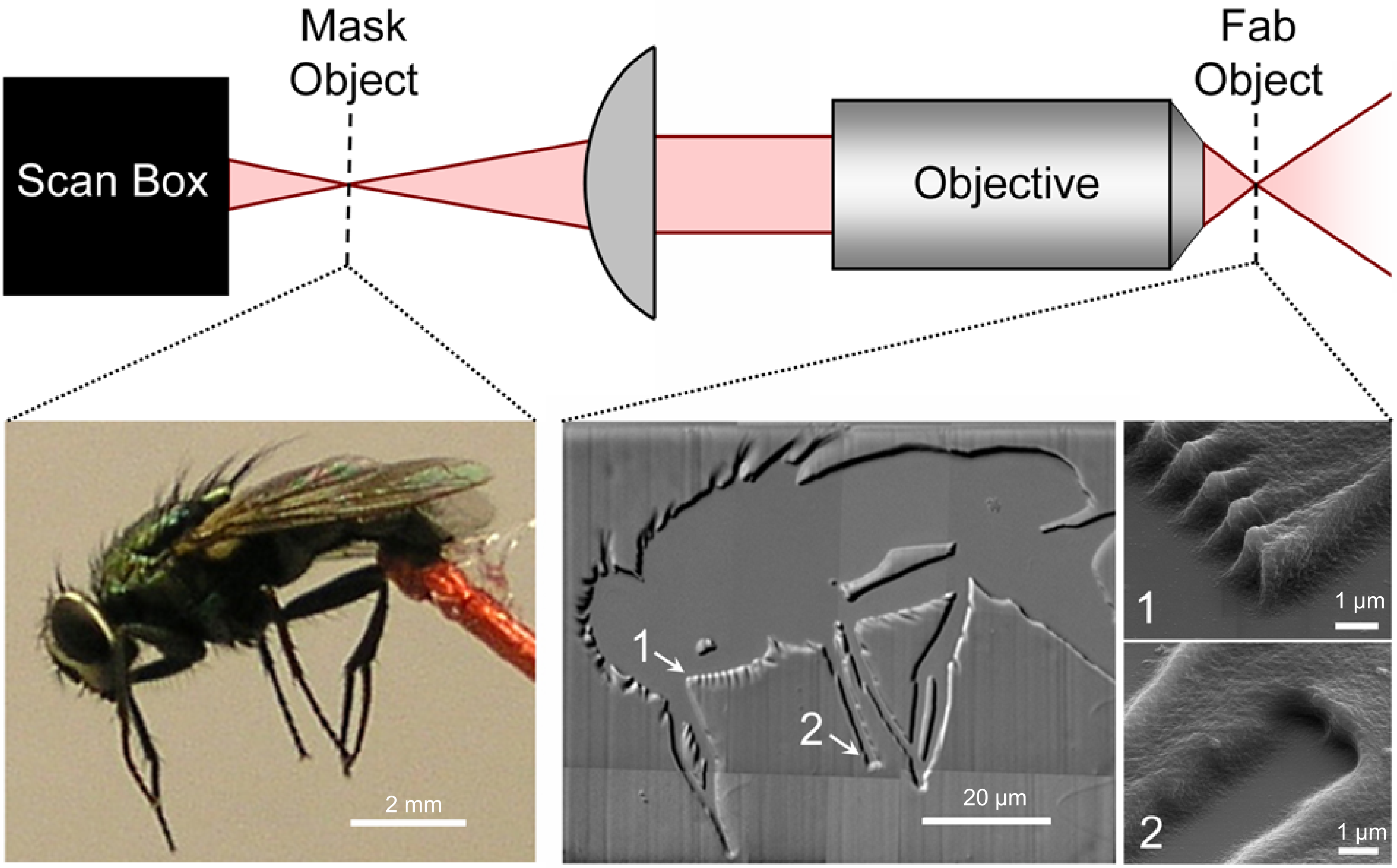
Reactive Residues

Tyr, His, Lys, Cys, Trp

Proteins

BSA, avidin, lysozyme, cytochrome c, GDH, GOx, Con A, Catalase

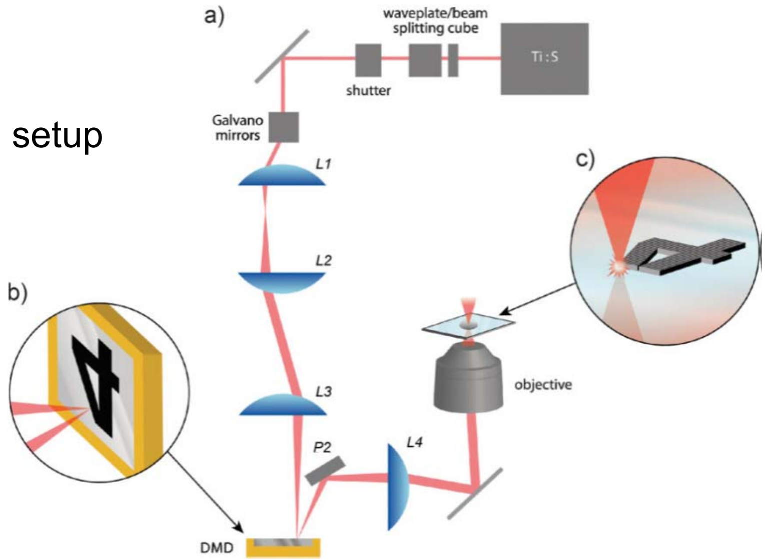
Mask-Directed Multiphoton Lithography



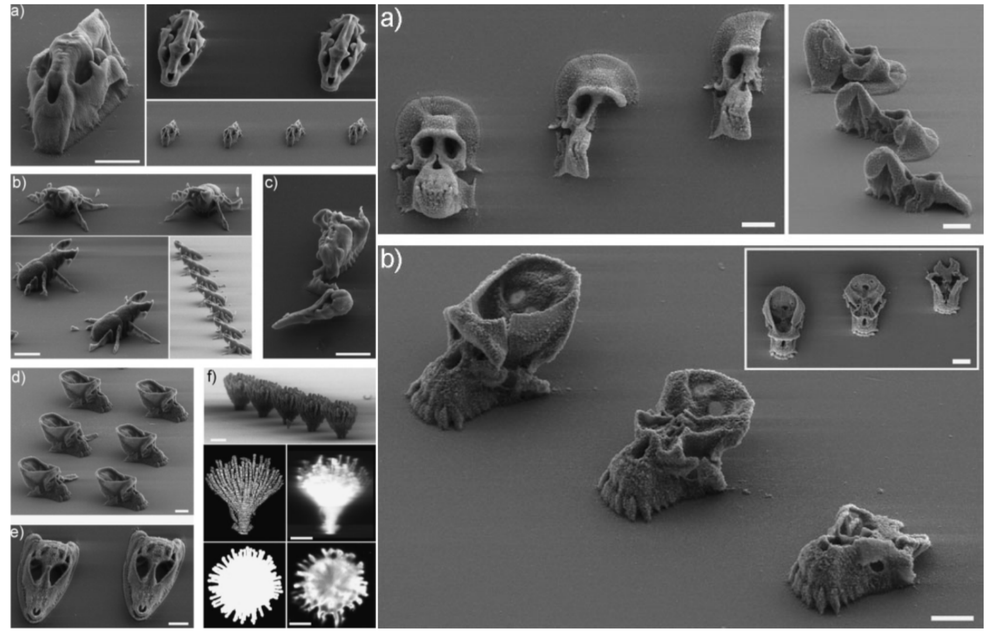
B. Kaehr and J. B. Shear, *J. Am. Chem. Soc* 2007 129 (7),1904-1905.

Dynamic-Mask Multiphoton Lithography

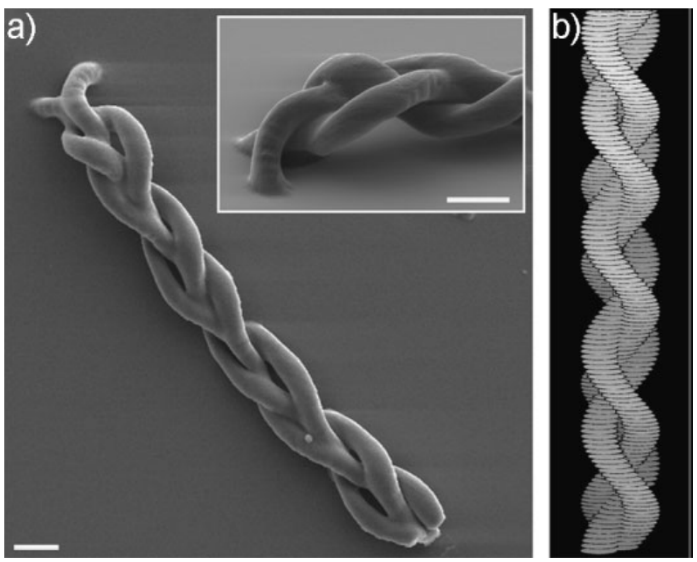
setup



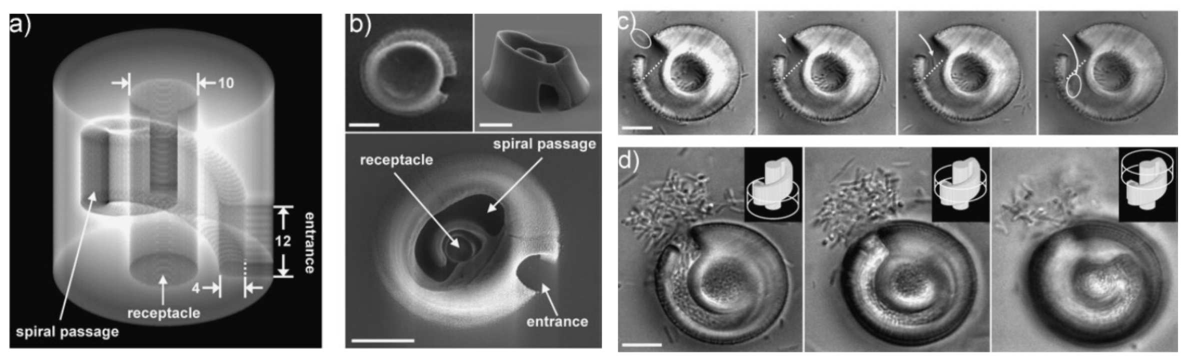
Mask Inputs



Tomographic data



Animations

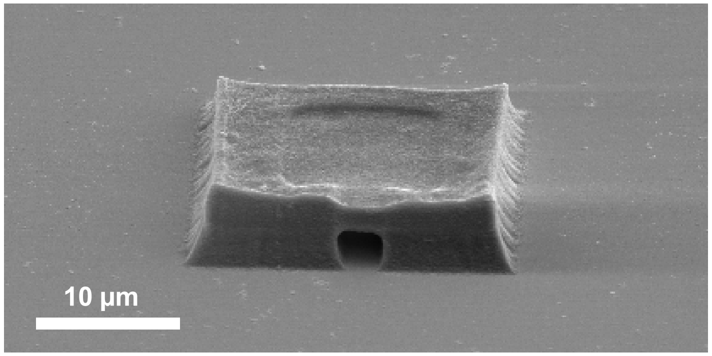


CAD data

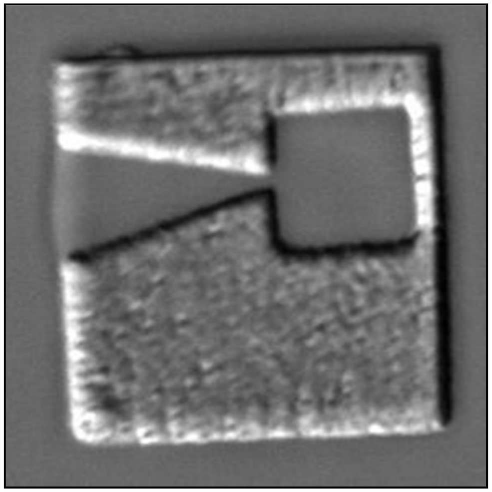
Nielson, Kaehr and Shear, *Small*, 2009, **5**, 120-125

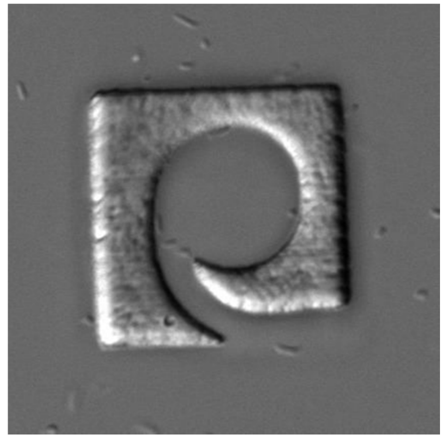
All scales, 5 μm

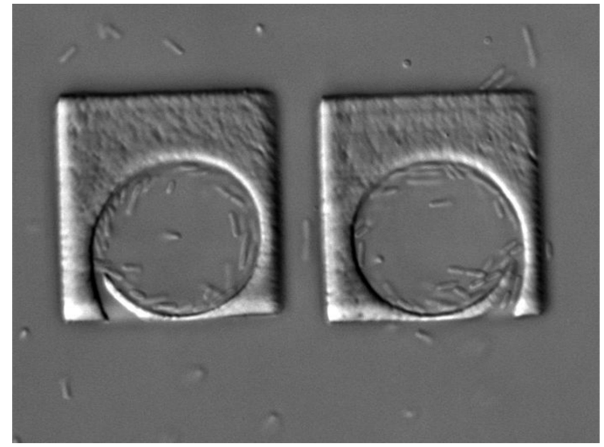
Static Structures to Direct Cell Behaviors

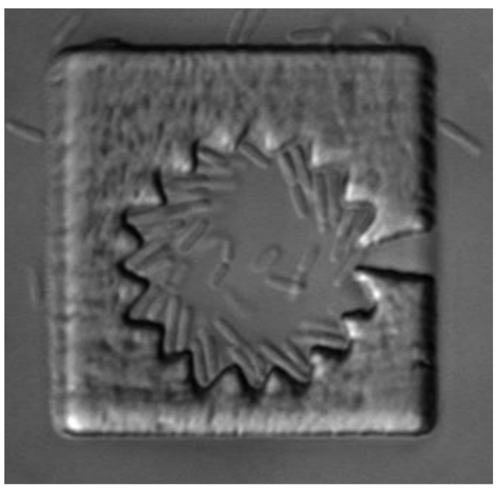


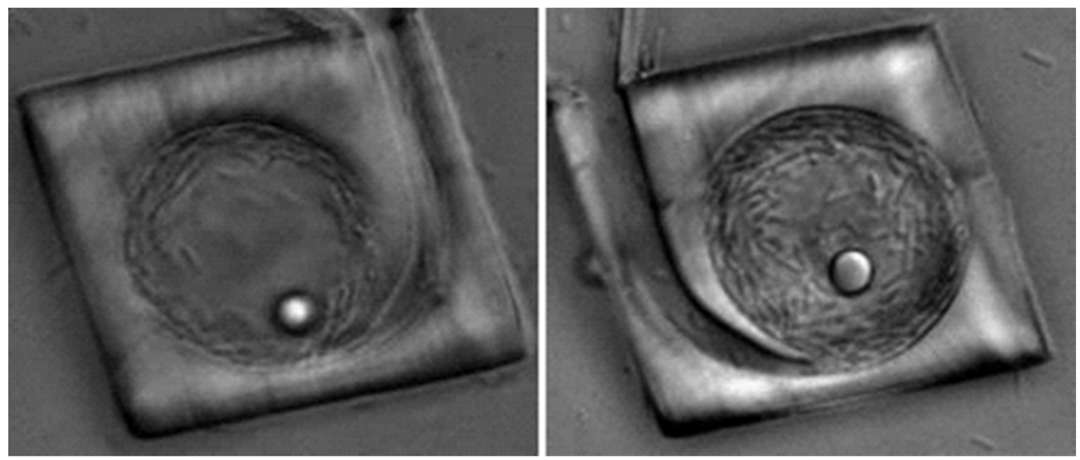
Static Structures to Direct Cell Behaviors





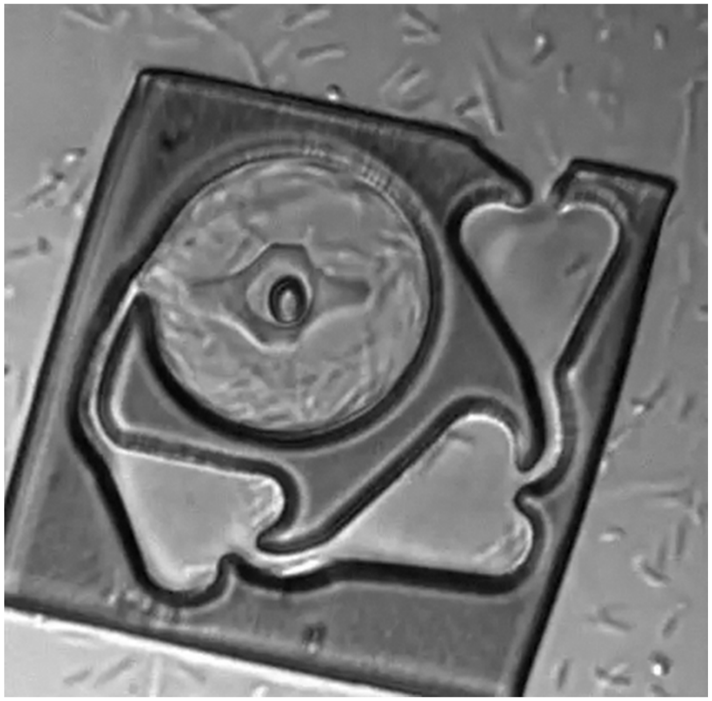






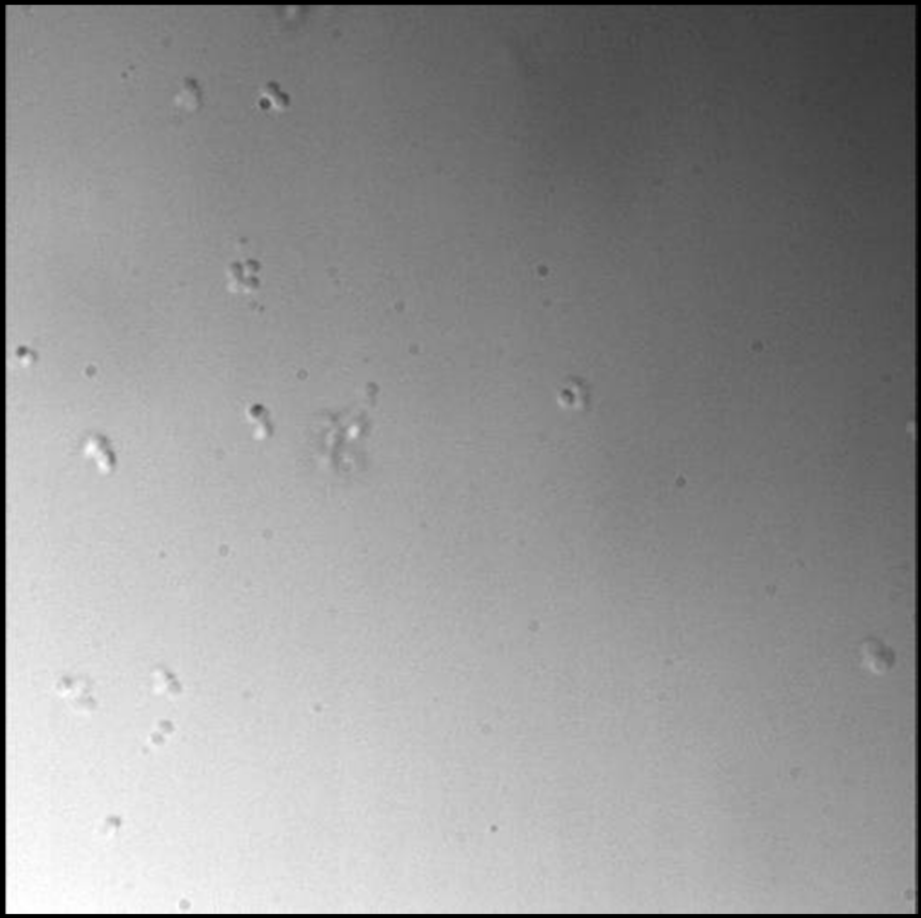
Kaehr & Shear (2009) *Lab on a Chip*, 9, 2632-2637

Static Structures to Direct Cell Behaviors



Kaehr & Shear (2009) *Lab on a Chip,* 9, 2632-2637

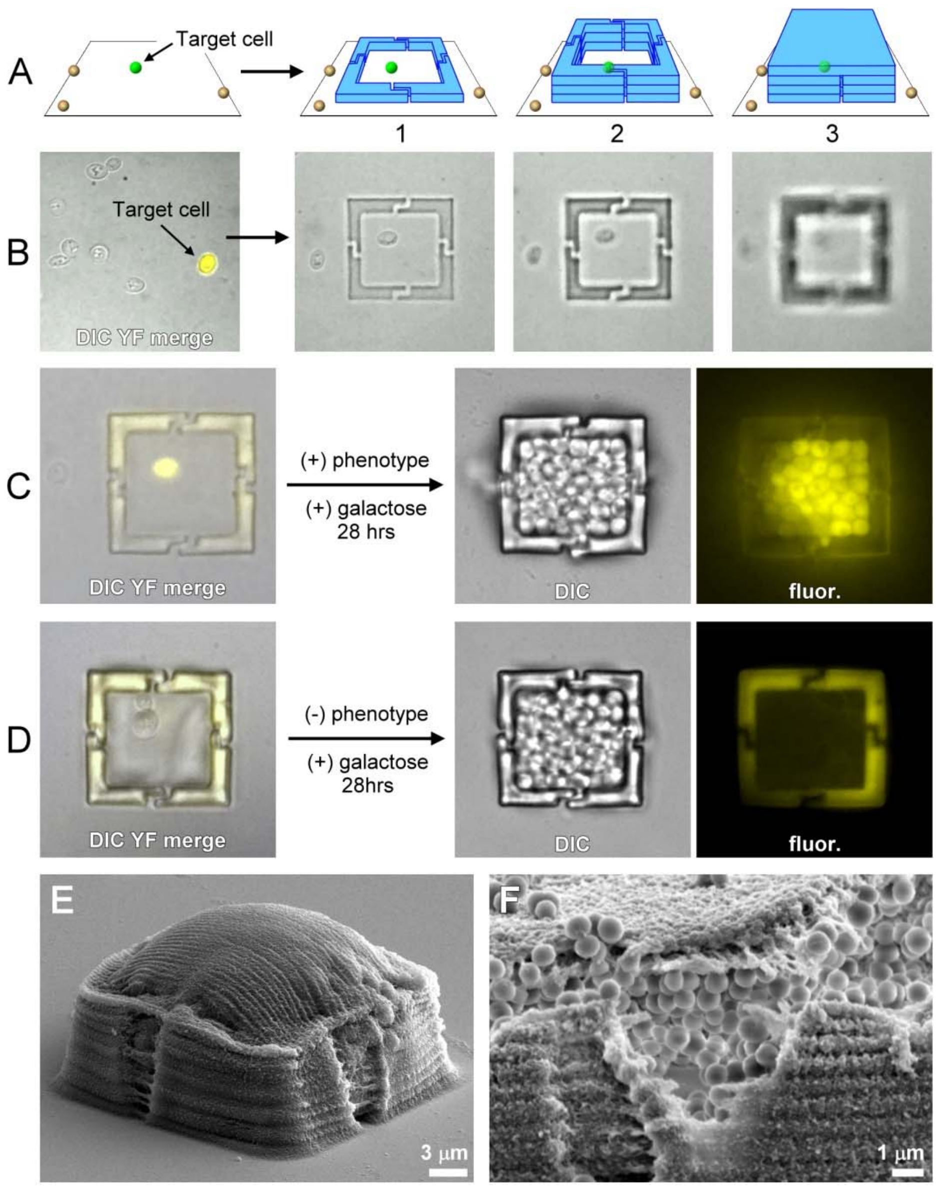
In situ cell confinement using biocompatible microfabrication

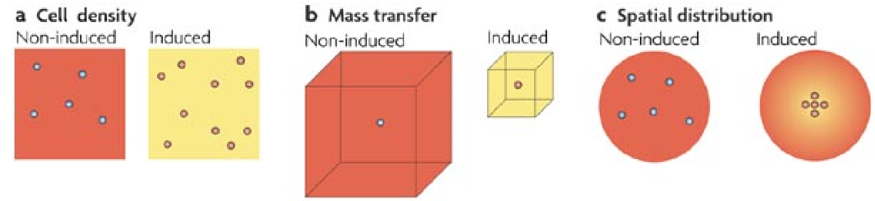


S. aureus,
BSA ~ 320 mg/ml
MB ~ 4 mM

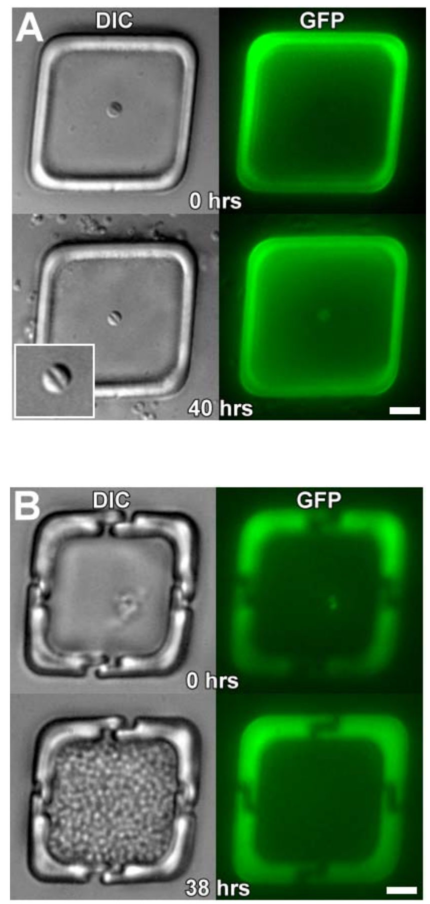
In situ cell confinement using biocompatible microfabrication

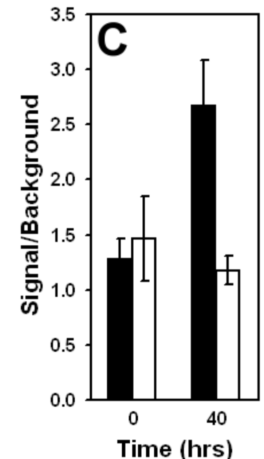
Cell communication



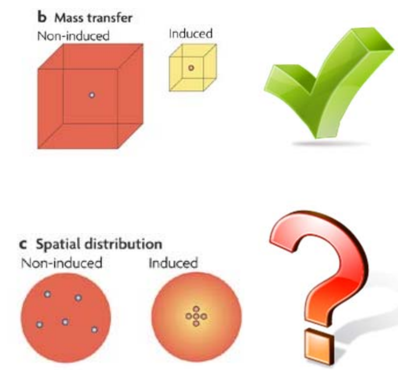


Nature Reviews Microbiology 5, 230-239 (March 2007)



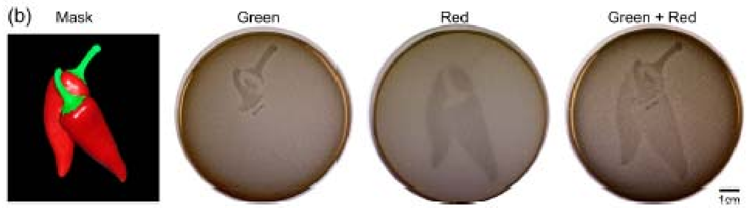


low diffusivity (black)
high diffusivity (white)

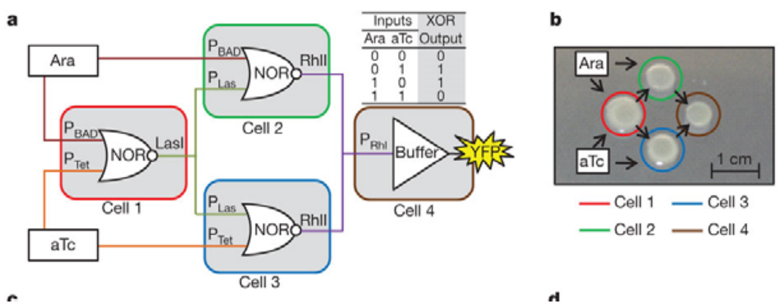


With Jason Harper SNL/UNM

Extensions

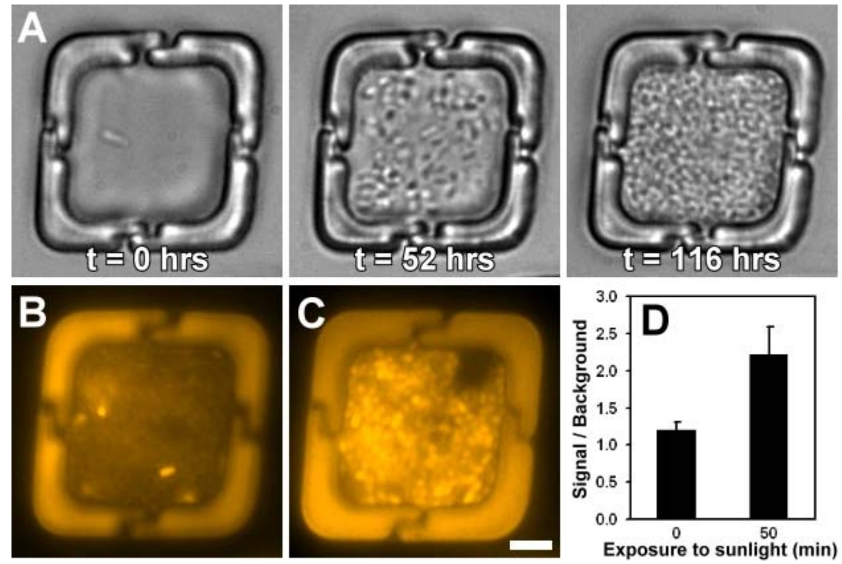


J. Mol. Biol. (2011) 405, 315–324



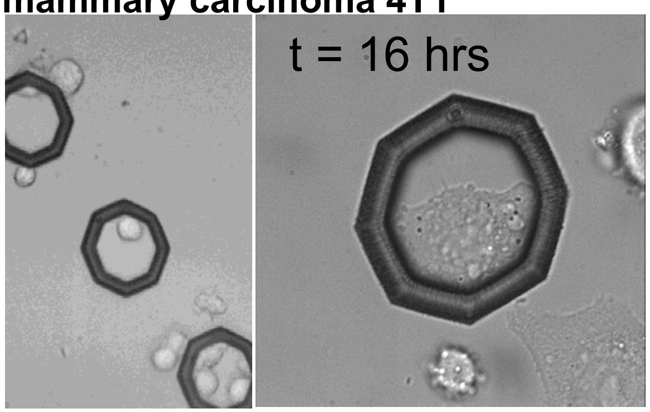
Jeff Tabor (Rice)

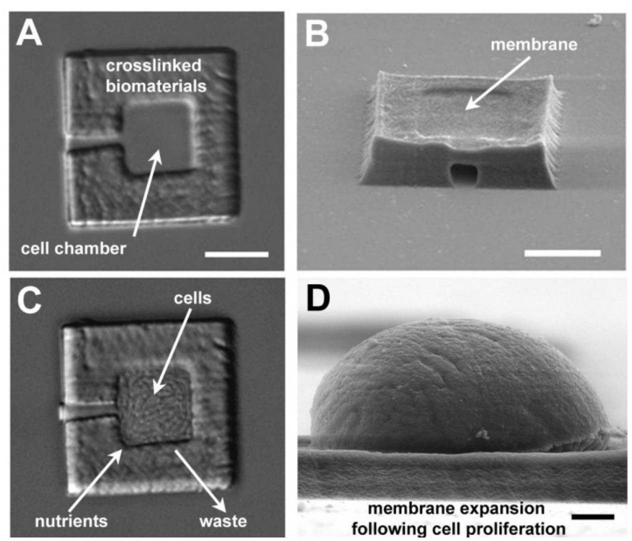
A Tamsir *et al. Nature*, 1-4 (2010) doi:10.1038



In vitro tumor, cancer cell differentiation

mammary carcinoma 4T1

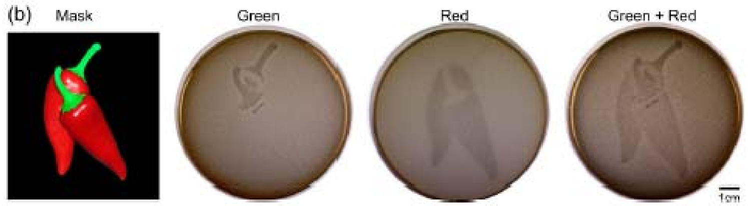




- What environmental factors contribute to cancer cell differentiation within primary tumors?
- Can we build a tractable, in vitro model for metastasis?

Jason Townson, UNM

Extensions



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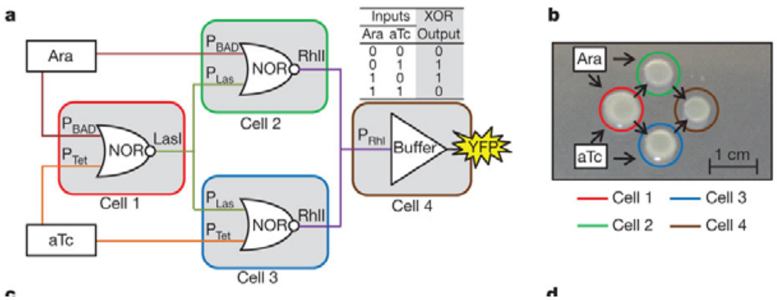

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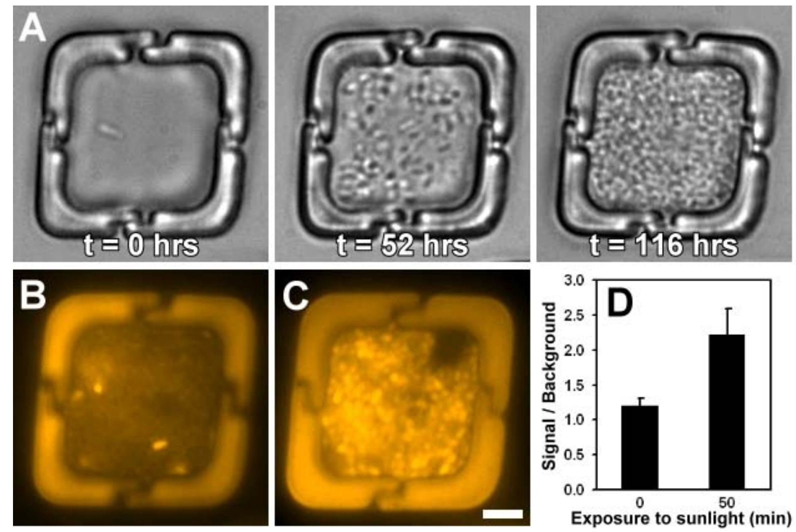

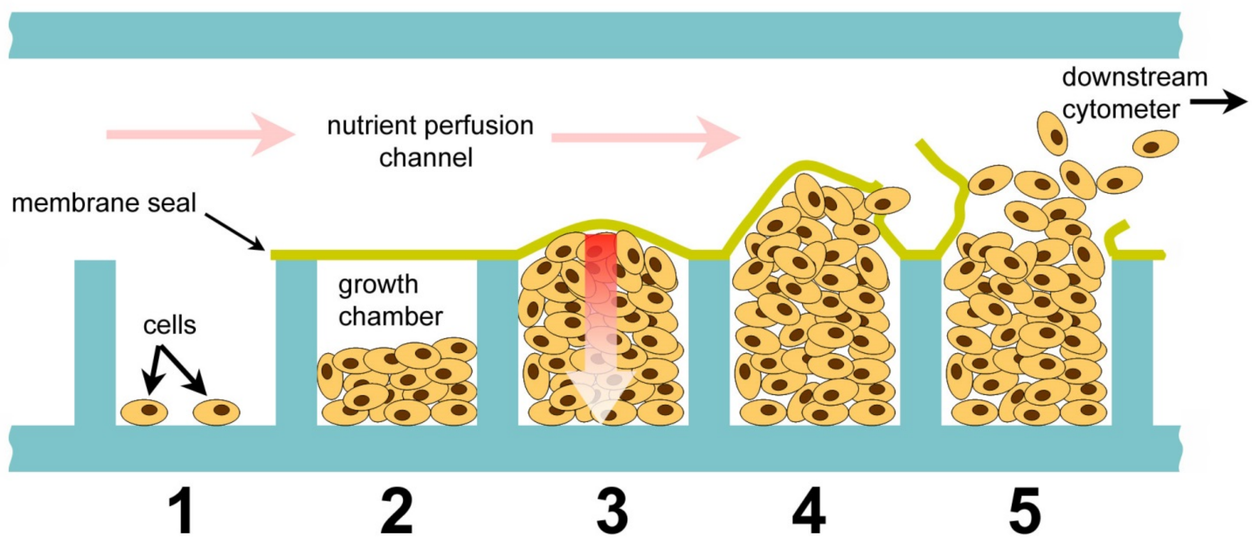

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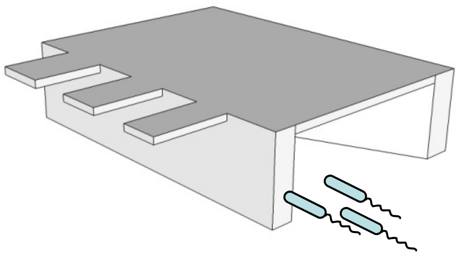
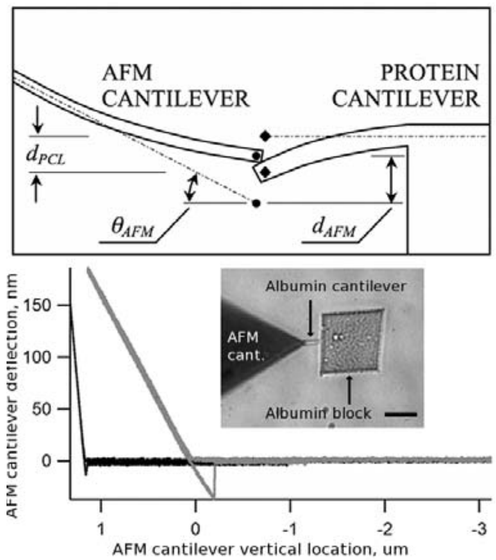
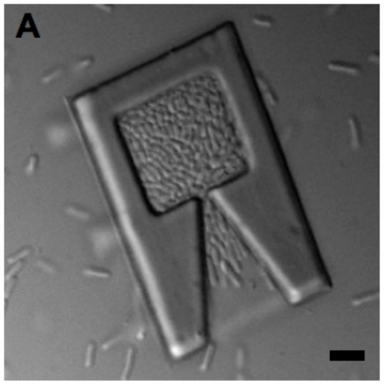
Jason Townson, UNM

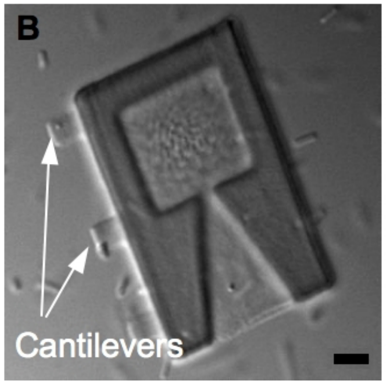
Measuring cell/materials interactions in situ



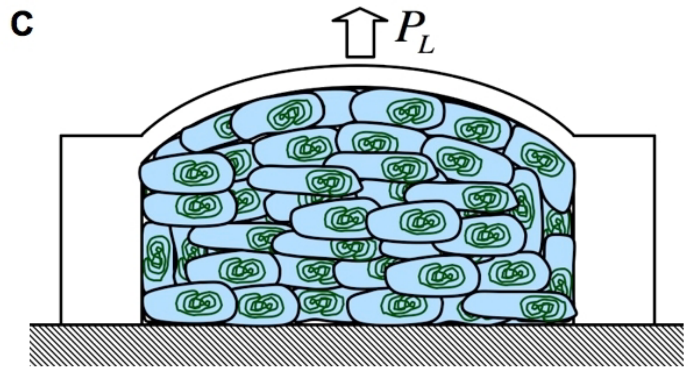


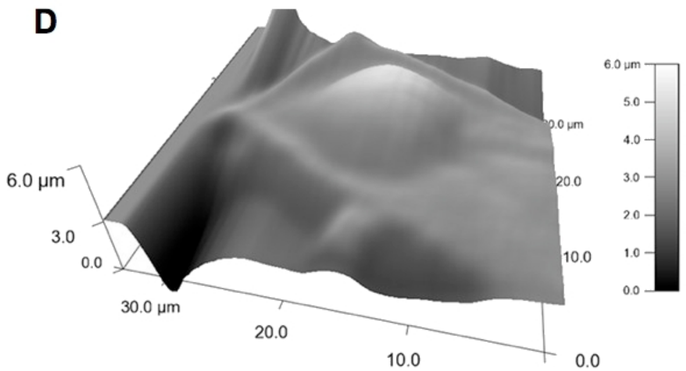


focus low



focus high

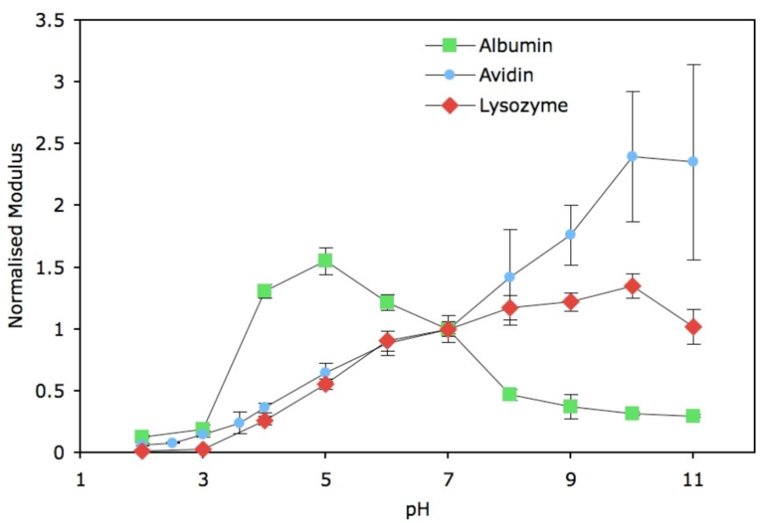


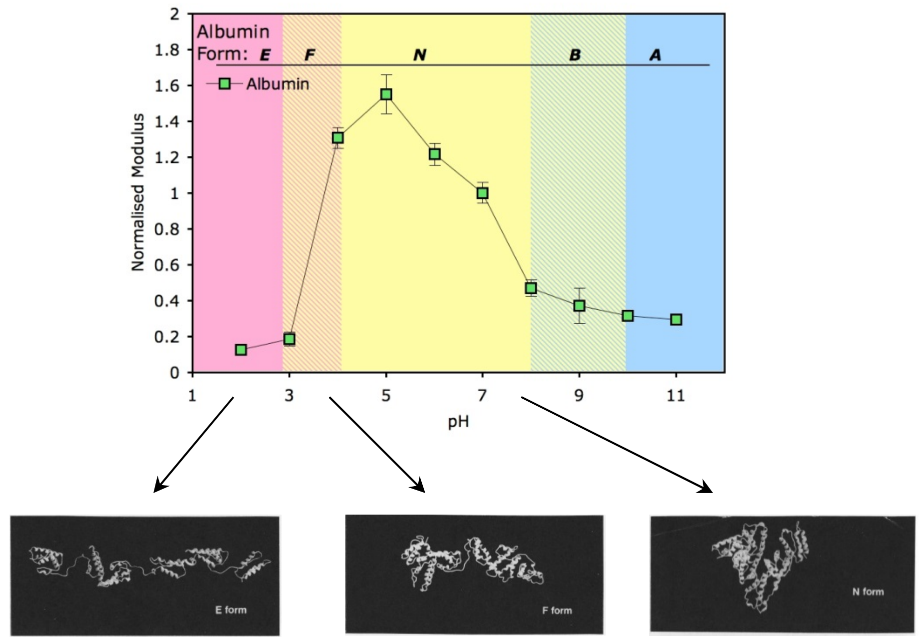


AFM image of chamber

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

Mechanical Properties of Protein Matrices

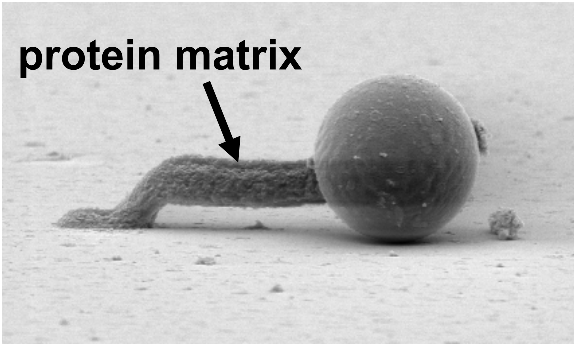


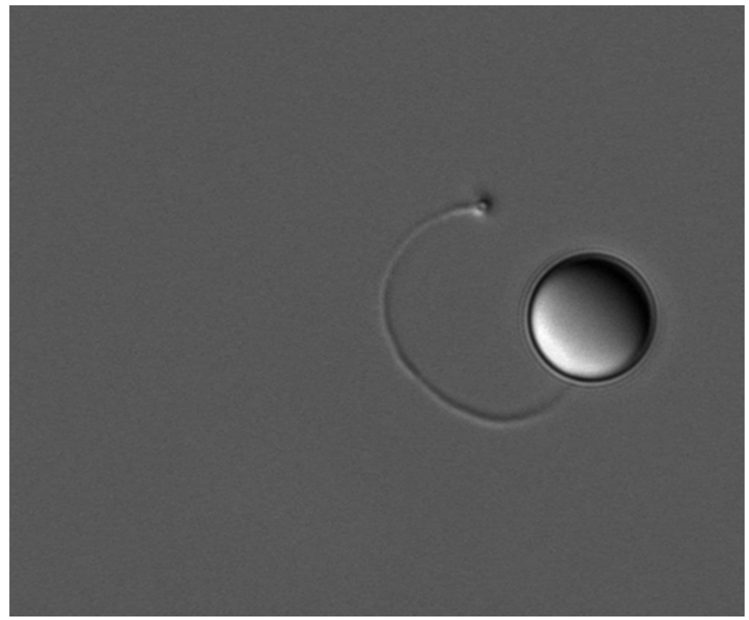


Images from Carter et al *Adv Prot Chem* 1994

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

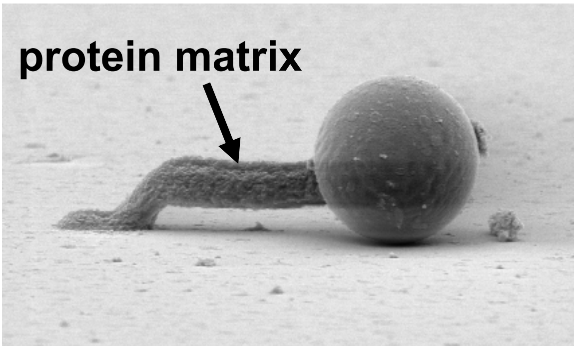
Stimuli-Responsive Behavior of Protein Matrices

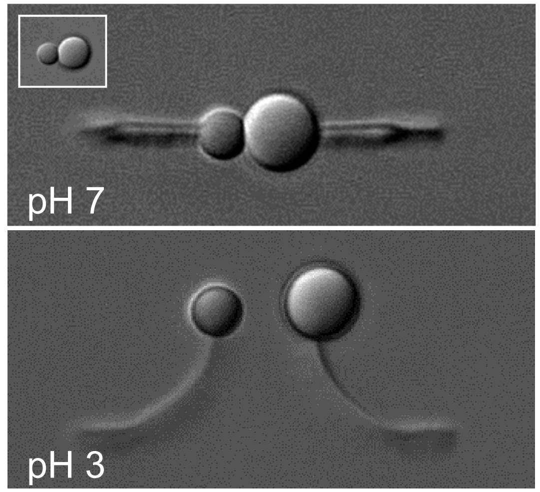




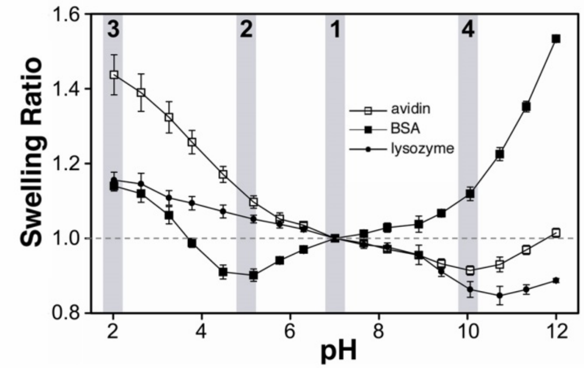
B. Kaehr and J. B. Shear, *Proc Natl Acad Sci U S A*, 2008, 105, 8850-8854.

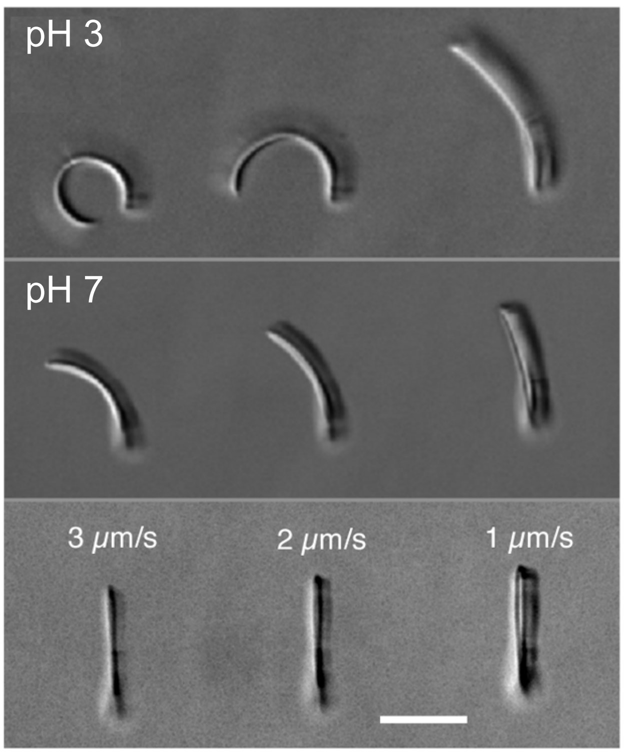
Stimuli-Responsive Behavior of Protein Matrices

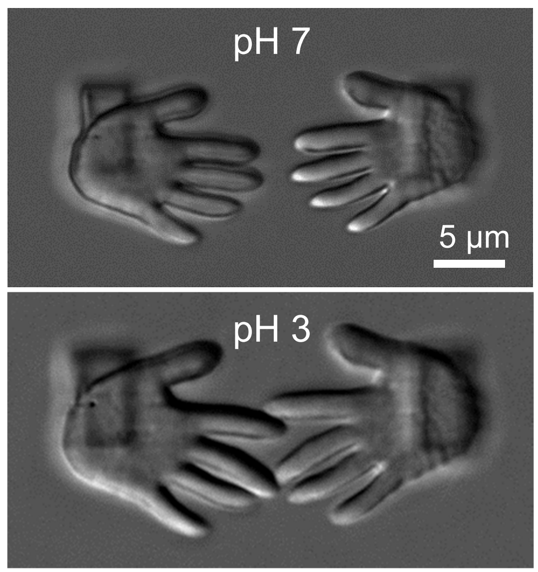


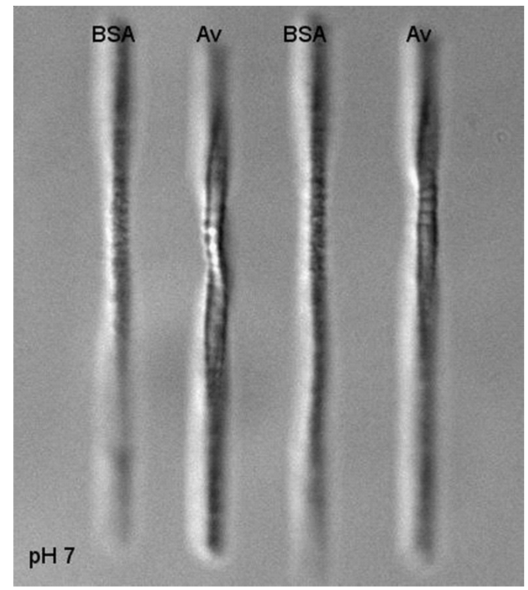


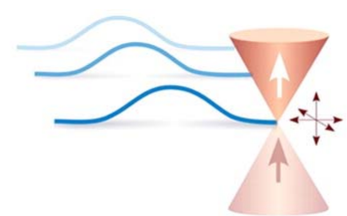
Protein Specific Response





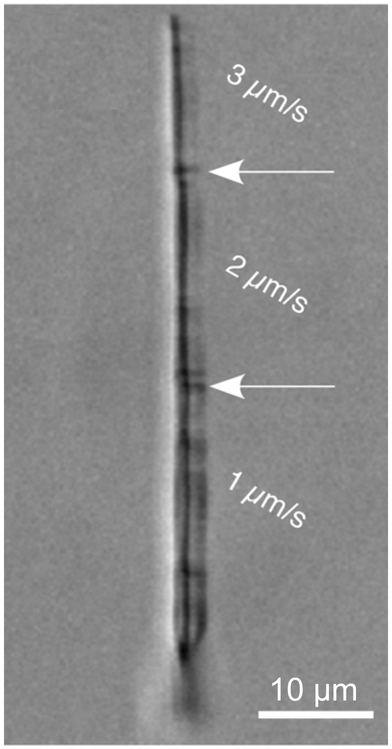


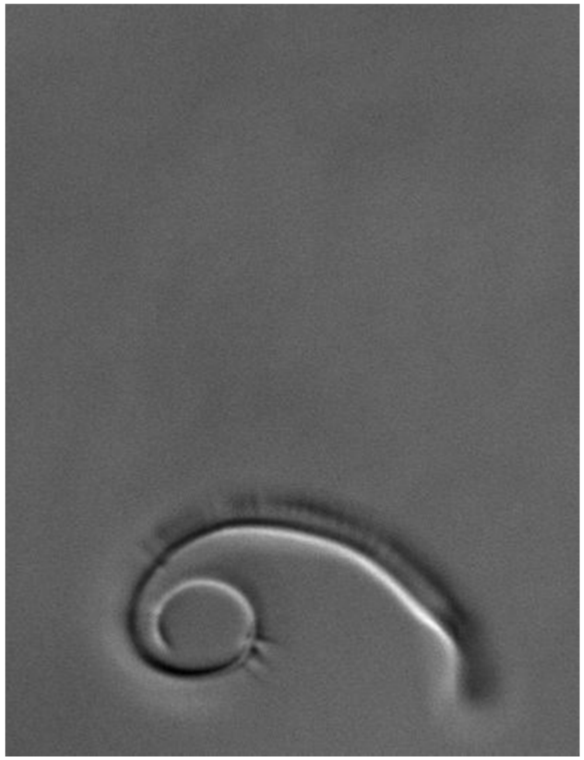


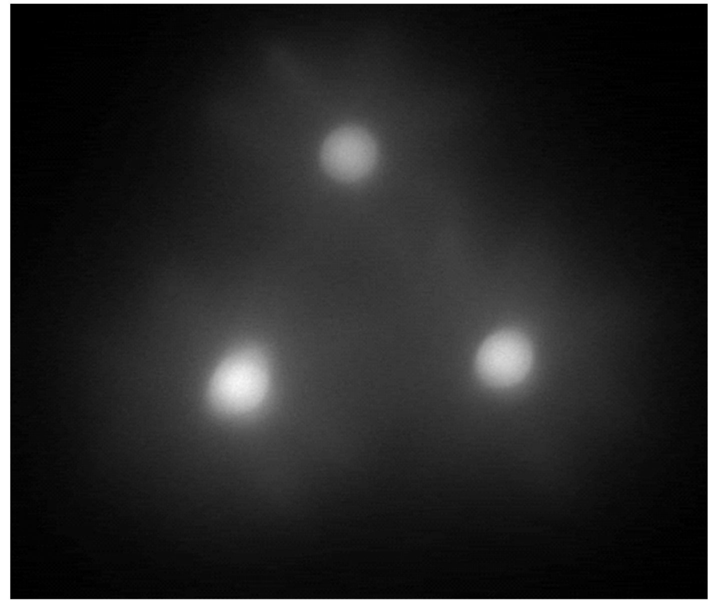


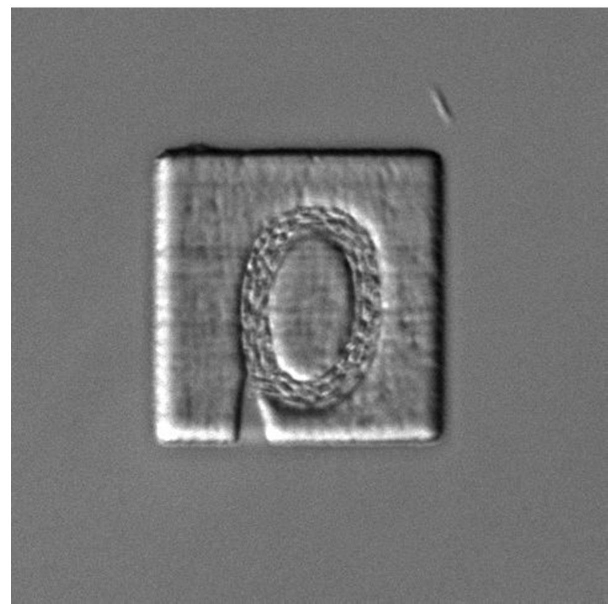
B. Kaehr and J. B. Shear, *Proc Natl Acad Sci U S A*, 2008, 105, 8850-8854.

Life-like behaviors from protein-based microfabricated structures



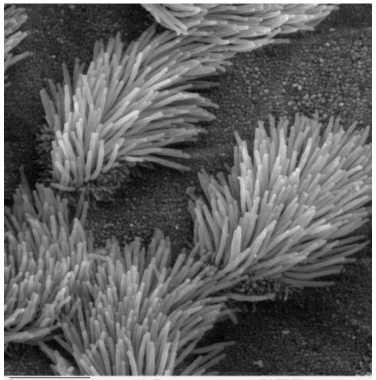


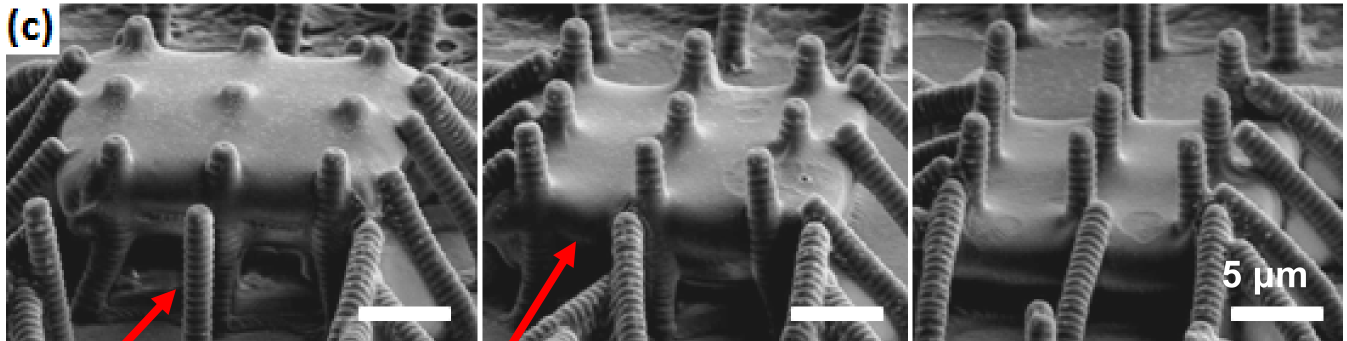




Integration into complex surfaces

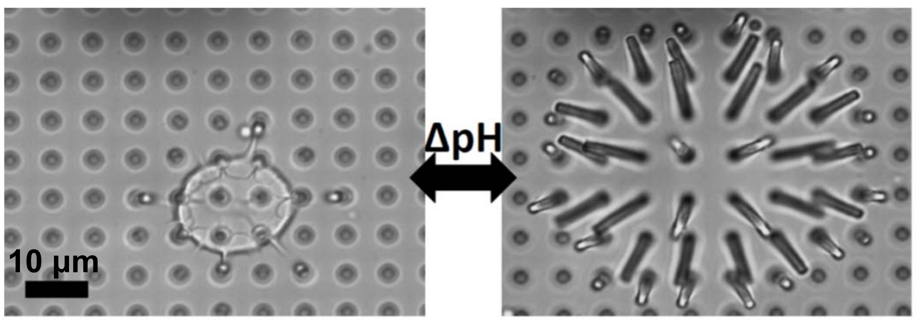
cilia

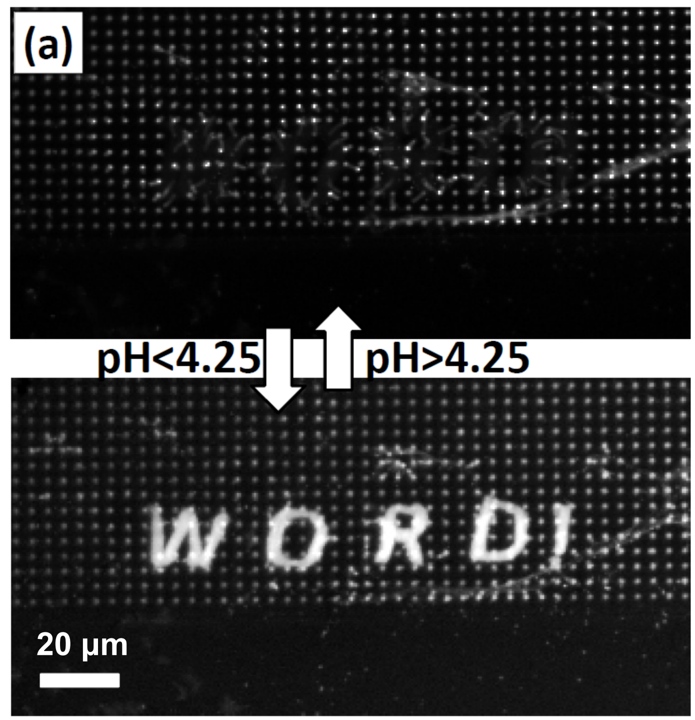




synthetic cilia

hydrogel micro-muscle





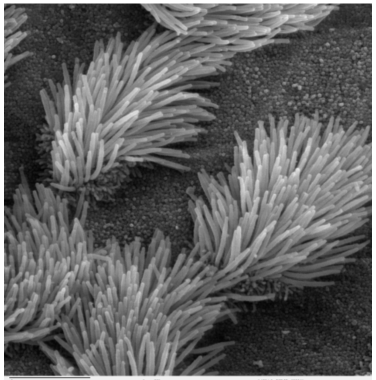
pH responsive hydrogel:
poly(acrylic acid-co-acrylamide) (poly(AAc-co-AAm).
20% AAm, 20% AAc, 2% bis-AAm, 1% Irgacure 819 in ethylene glycol

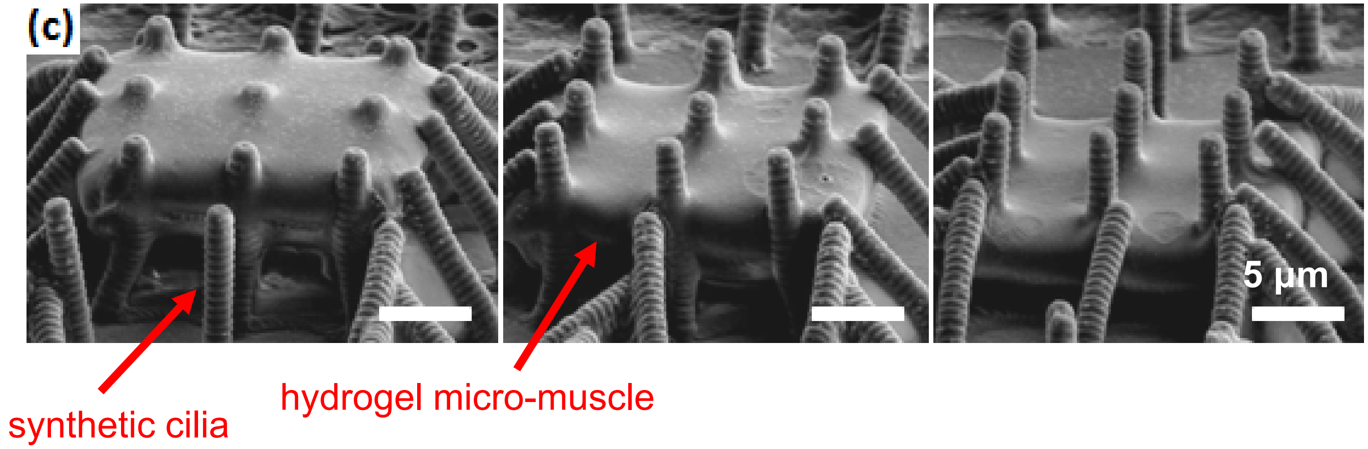
temperature-responsive hydrogel:
poly(N-isopropylacrylamide) (PNIPAM)
40% NIPAM, 2% bis-AAm, 1% Irgacure 819 in ethylene glycol (w/w)

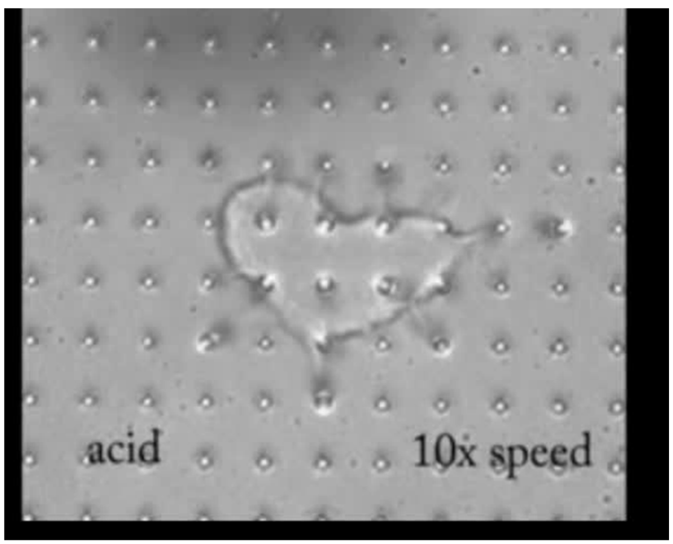
Zarzar, L.D., Kim, P., Kolle, M., Brinker, C.J., Aizenberg, J., Kaehr, B. (2011) *Angew. Chem. Int. Ed.*, 50, 9356–9360.

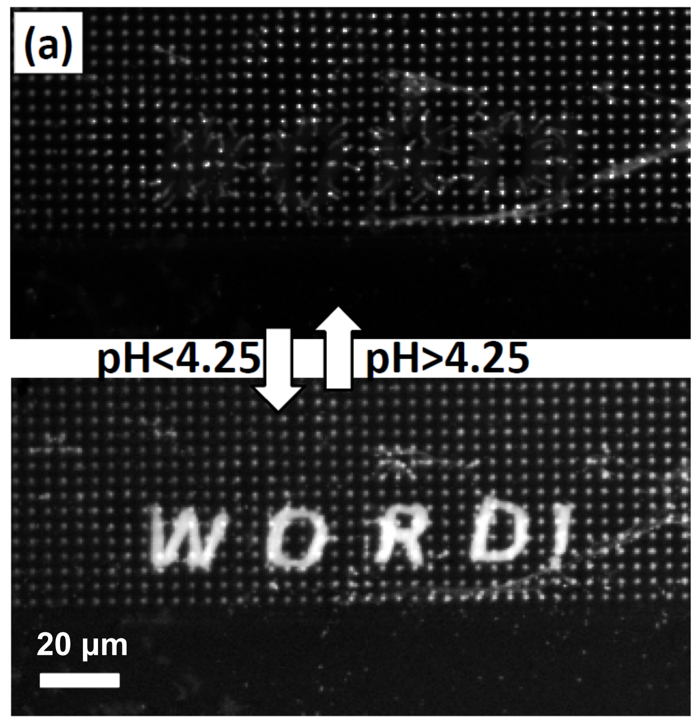
Integration into complex surfaces

cilia





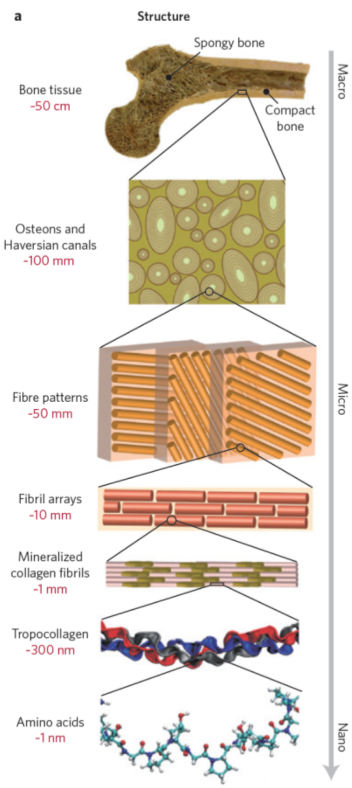




Zarzar, L.D., Kim, P., Kolle, M., Brinker, C.J., Aizenberg, J., Kaehr, B. (2011) *Angew. Chem. Int. Ed.*, 50, 9356–9360.

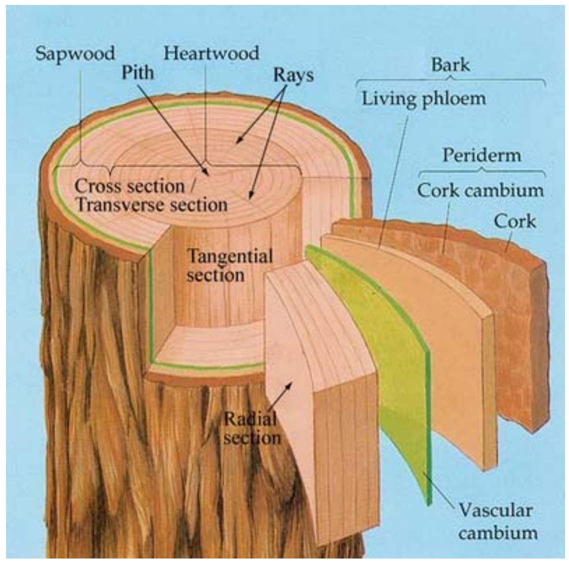
Nature's exceptional hierarchical structures

Bone



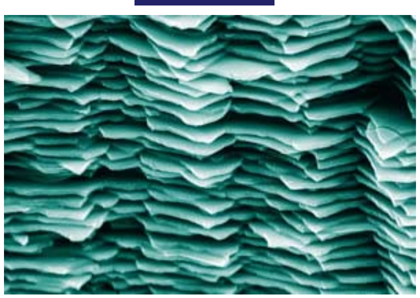
Lawrence Berkeley

Wood

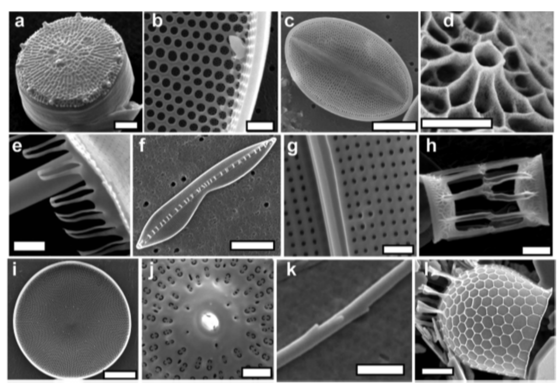


University of Cambridge

Shell



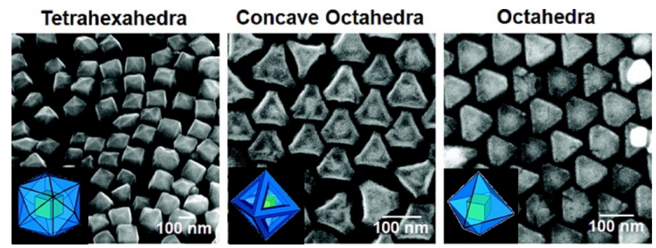
Diatoms



M. Hildebrand, *Chemical Reviews* **2008**, *108*, 4855.

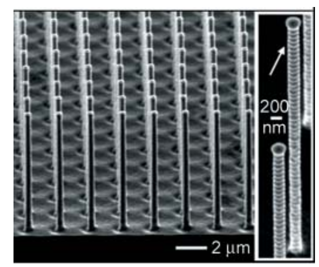
?

Synthetic Nanomaterials: particles, surfaces, porosity

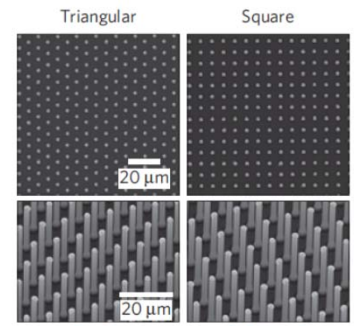


Au/Pd

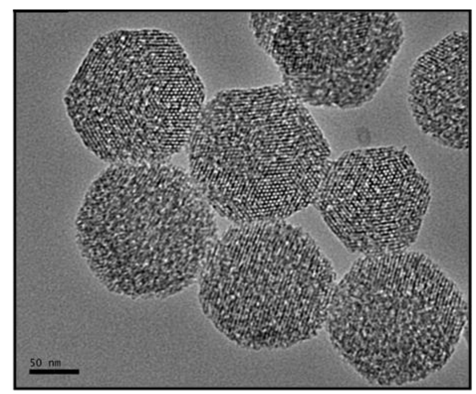
JACS, 2010, 132 (41), pp 14546–14553



Silicon columns (Aizenberg)

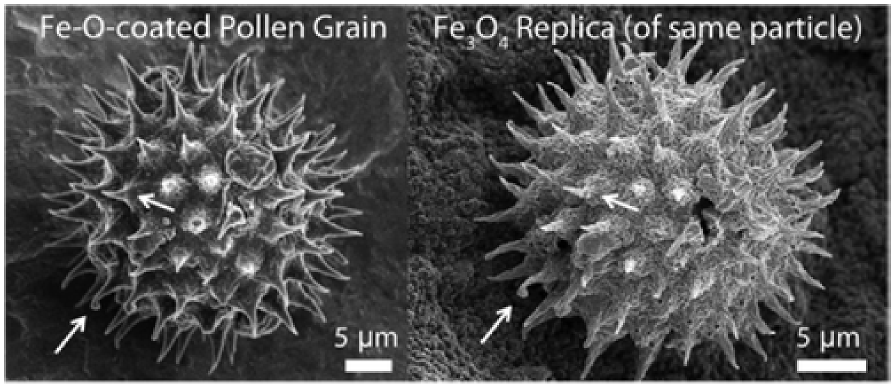


(Lewis, Atwater)

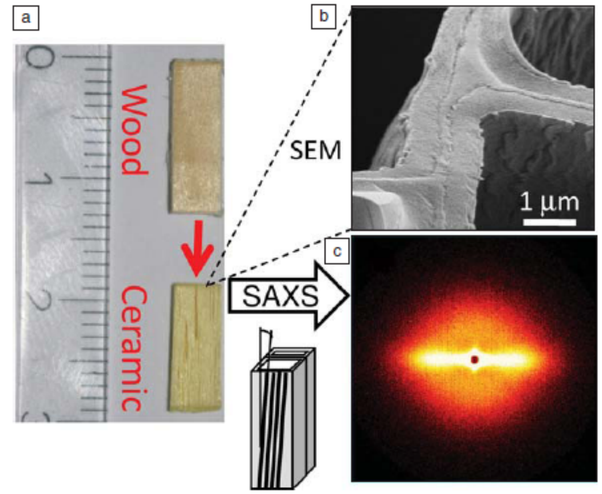


Yu-Shen Lin, CJ Brinker

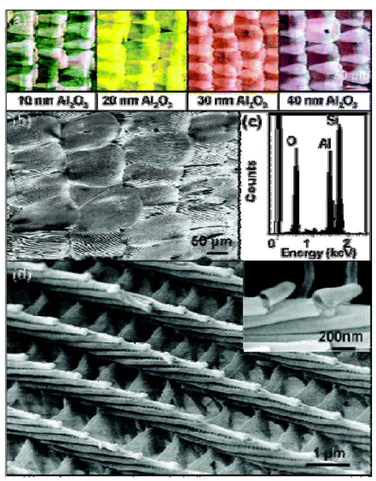
Enabling functional (photonic, electronic, catalytic, etc.) materials: *If you can't make 'em, use 'em.*



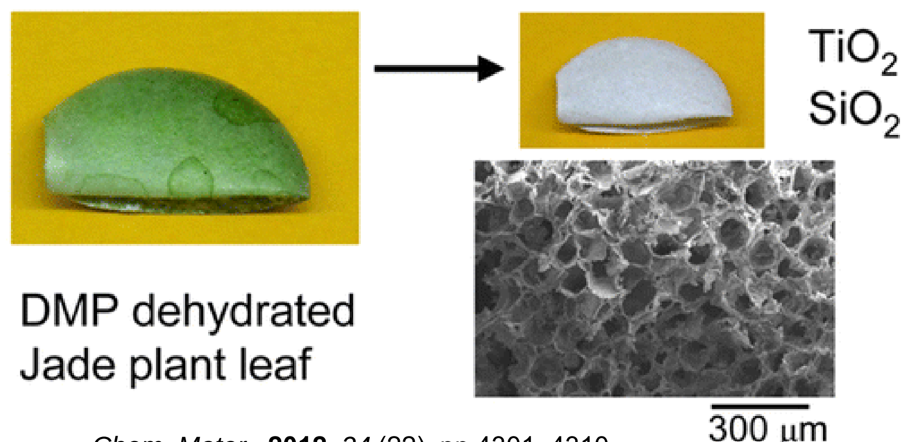
Chem. Mater., **2013**, *25* (22), pp 4529–4536



MRS Bulletin, **2010**, (35)3 pp 219-225



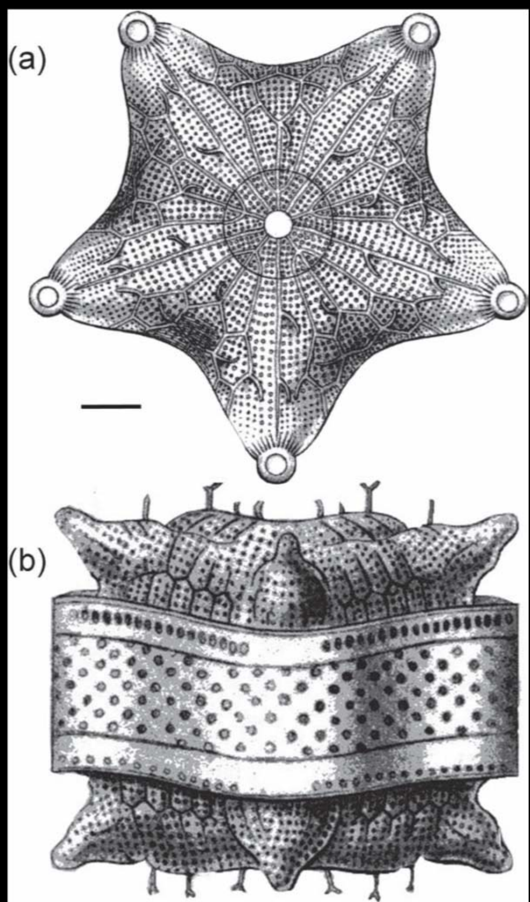
Nano Lett., **2006**, *6* (10), pp 2325–2331



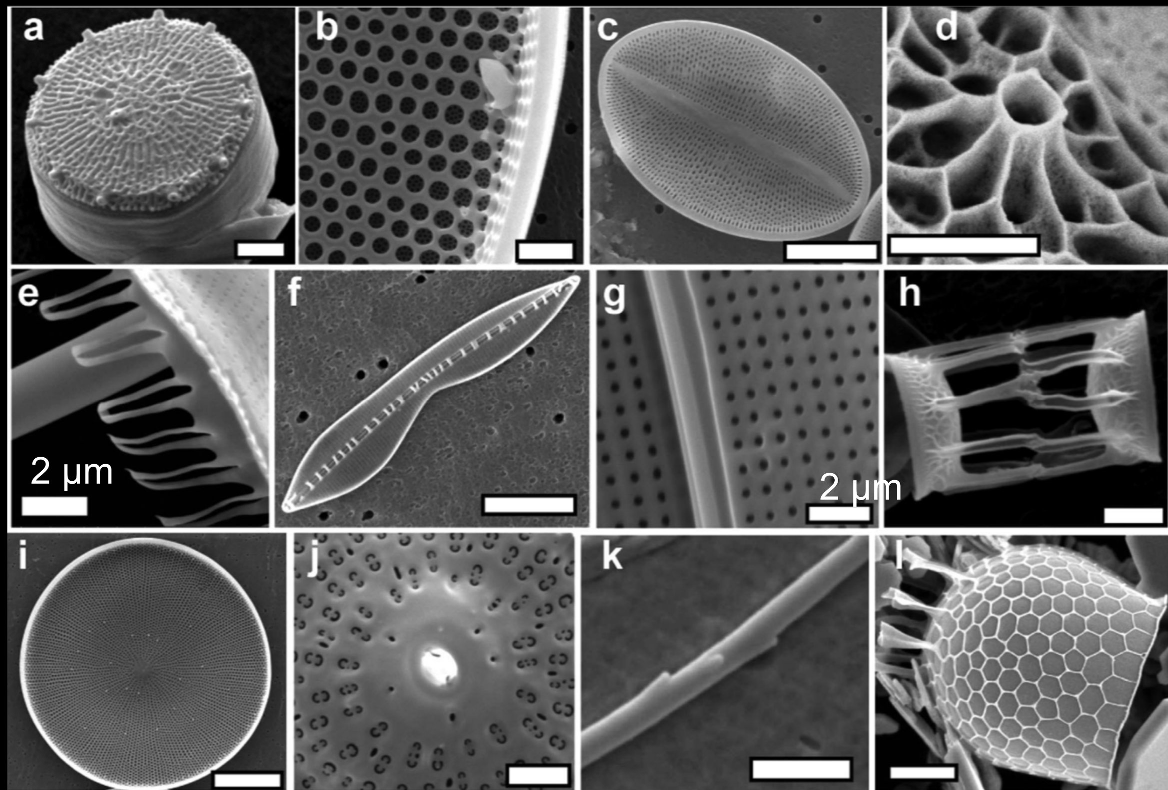
Chem. Mater., **2012**, *24* (22), pp 4301–4310

Static, mechanically stable structures

Biogenic silica (e.g., diatoms, radiolaria, sponges)

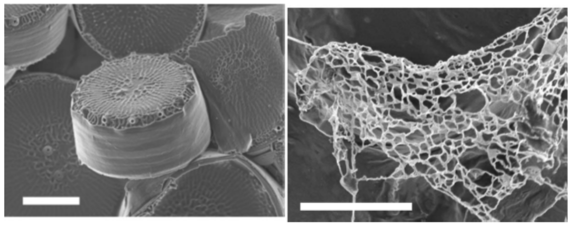


Haeckel, 1904

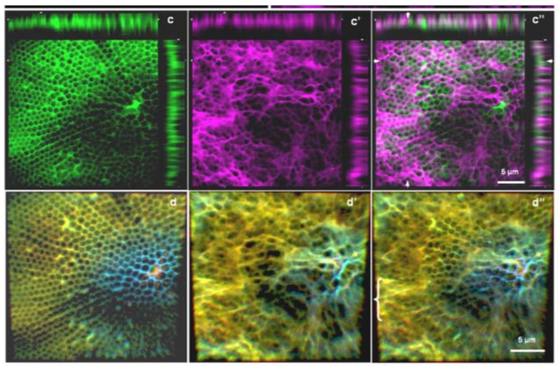


M. Hildebrand, *Chemical Reviews* **2008**, *108*, 4855.

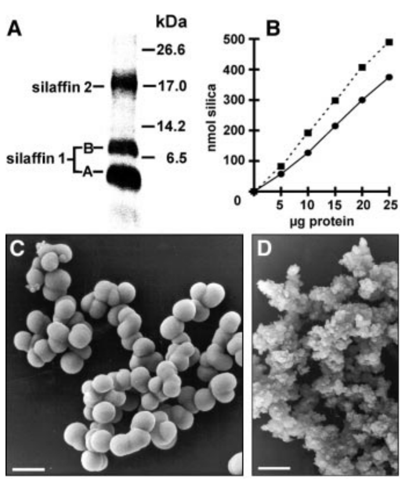
Silica polymerization and organization using peptides/proteins



Angew. Chem. Int. Ed. 2009, 48, 9724 –9727



Tesson B, Hildebrand M (2010). PLoS ONE 5(12)



N. Kroger, R. Deutzmann, M. Sumper, *Science* **1999**, *286*, 1129.

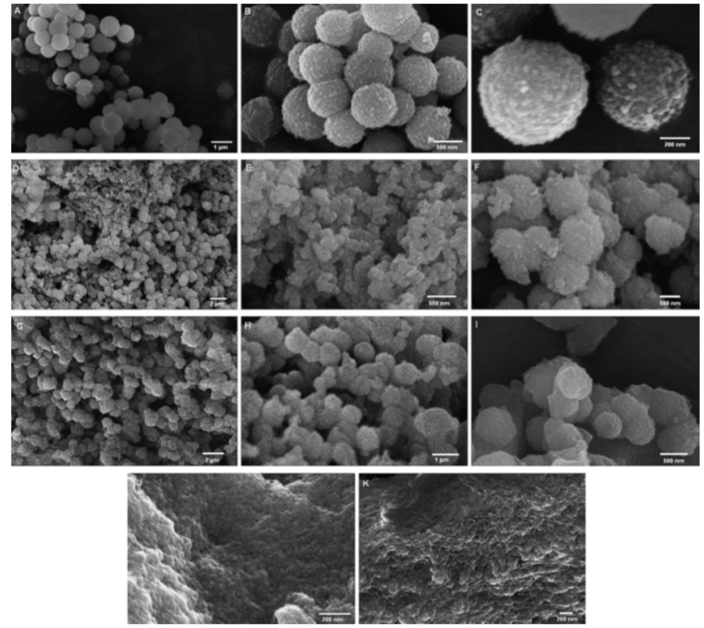


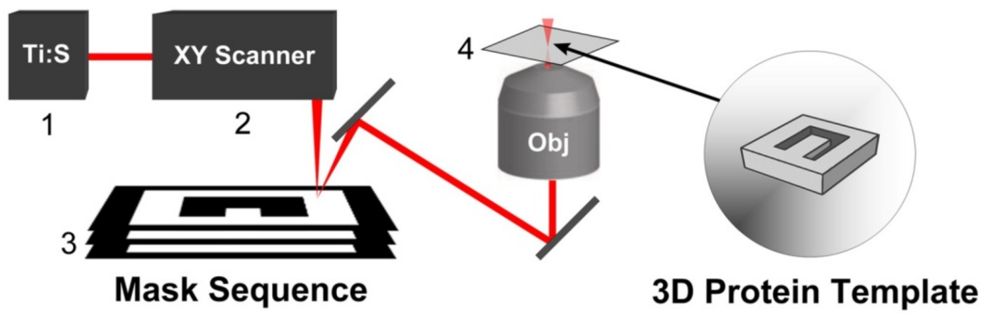
Table 1 Silica precipitating ability of various enzymes

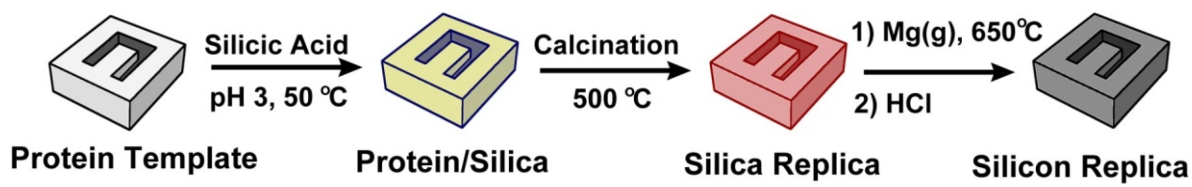
Enzyme	pI	Product	Physical state of solid silica	Time
Negative control	—	Gel		9 hours
Trypsin	10.5	Solid	Bimodal nanoparticles 100–200 nm + 700–950 nm	10 minutes
Papain	8.8–9.6	Solid	Nanoparticles 500–650 nm	15 minutes
Bromelain	9.6	Solid	Monolith	25 minutes
Tritirachium album proteinase K	8.9	Solid	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

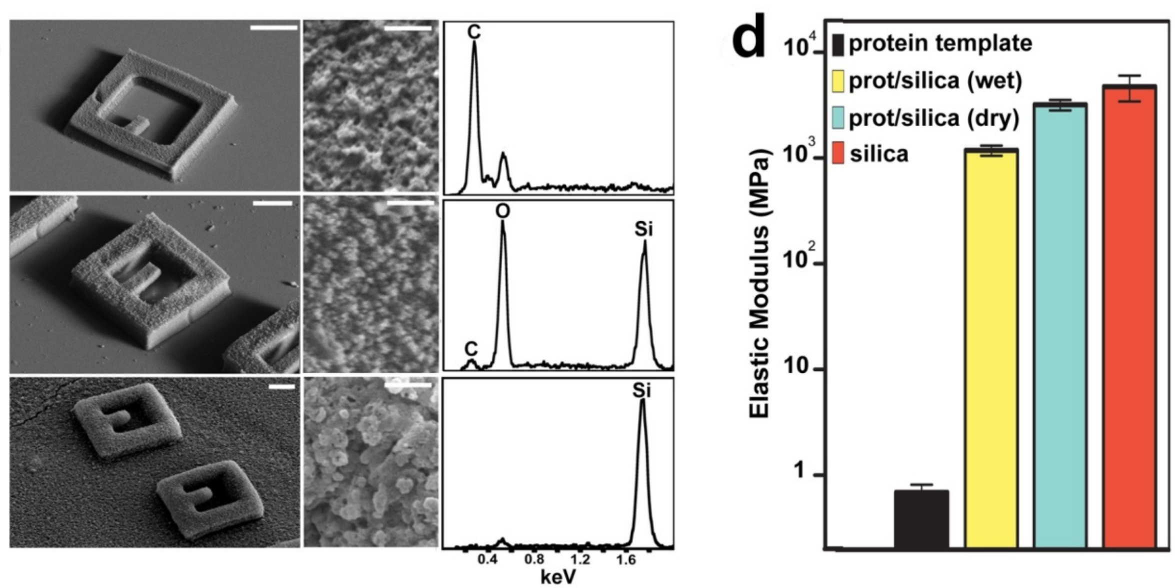
works

works not

J. Mater. Chem., 2009, 19, 7606–7609

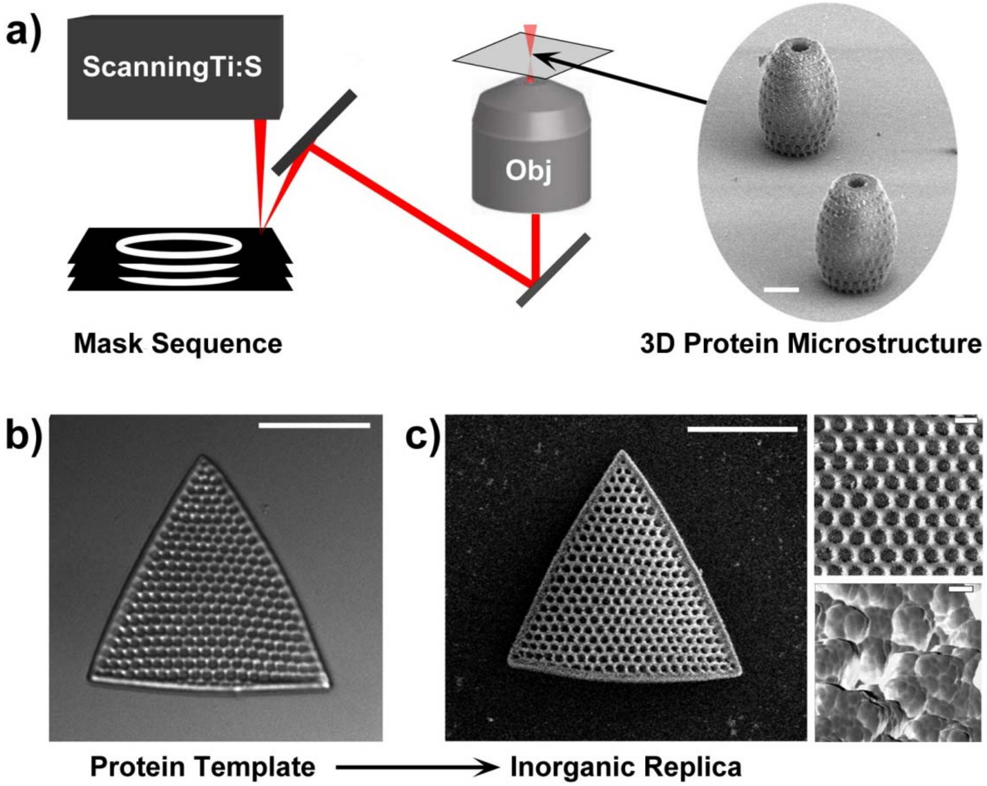


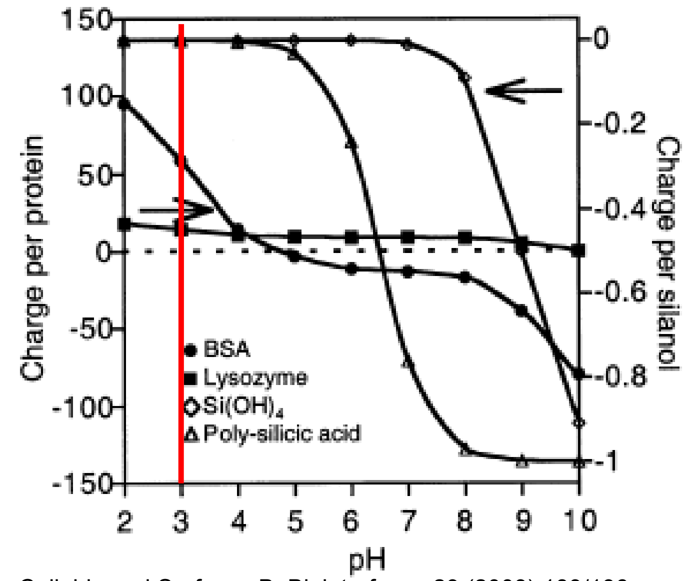




Khripin, C.Y., Pristinski, D., Dunphy, D.R., Brinker C. J., Kaehr, B. (2011) ACS Nano, 5, 1401-1409.

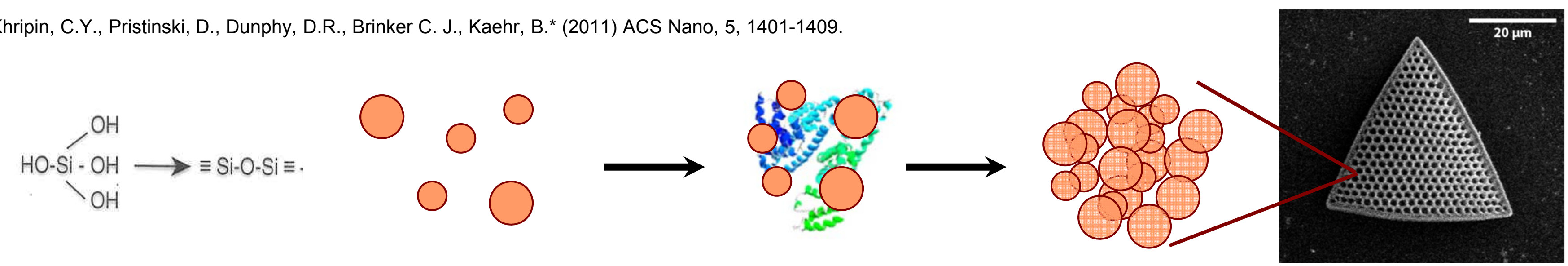
Protein scaffolds direct silica deposition –regardless of protein identity?!





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

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



Primarily monomers, oligomers, some particles

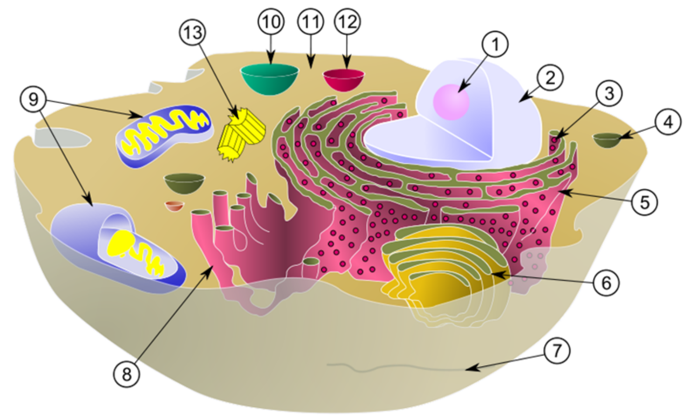
nucleation, flocculation, growth, and coarsening

silica network fusion

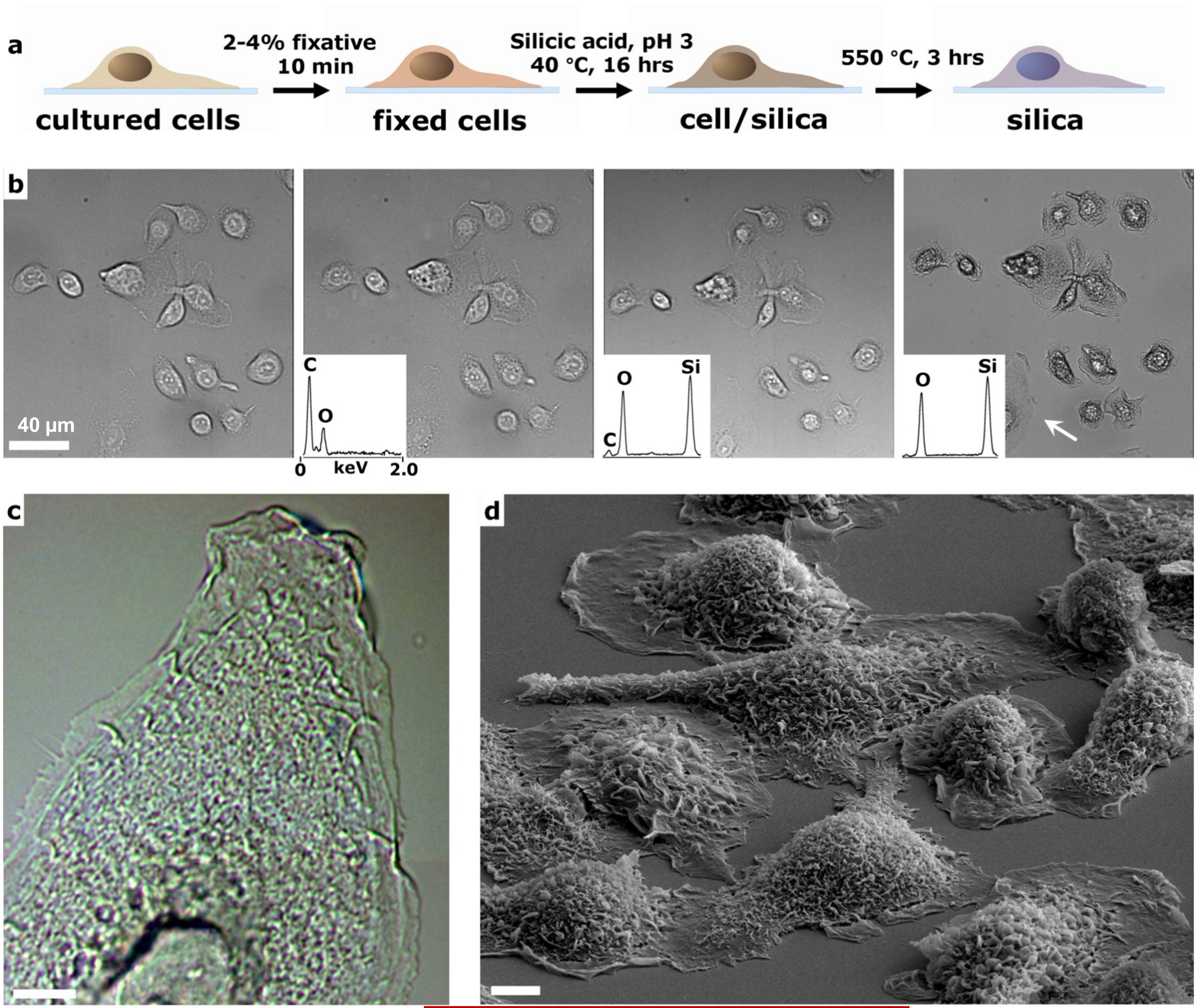
Protein scaffolds direct silica deposition –regardless of protein identity?!

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

Schematic of a typical animal cell



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

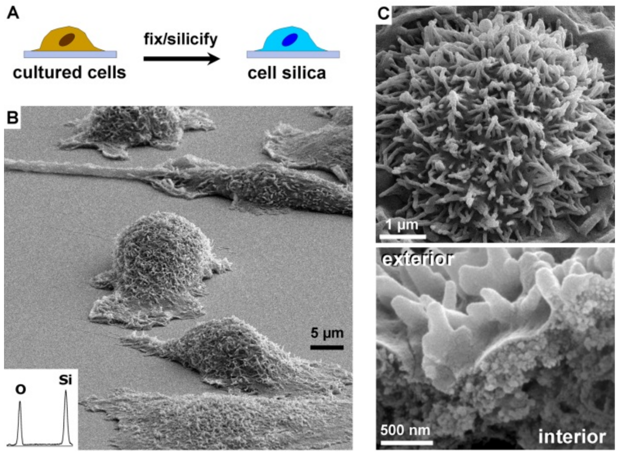


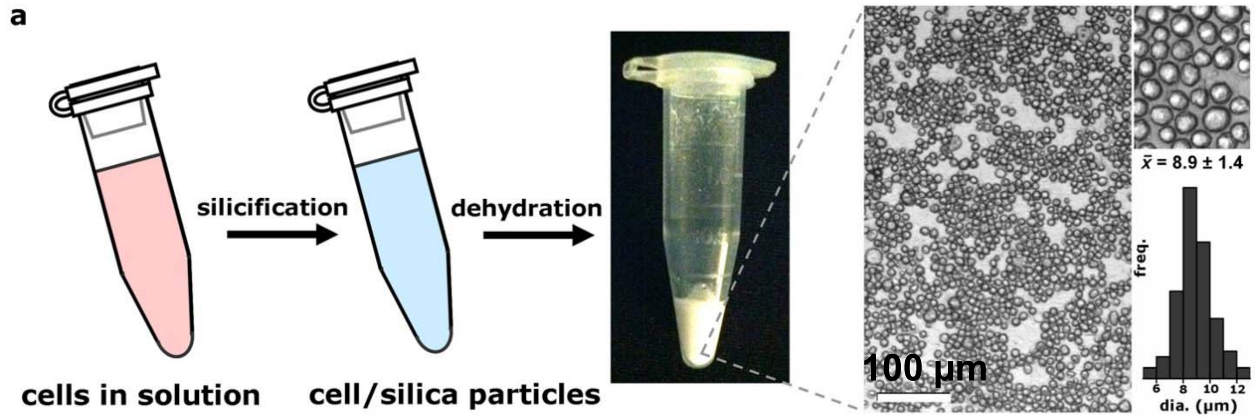
Silica Bio-Replication (SBR)

AsPC1 (pancreas)

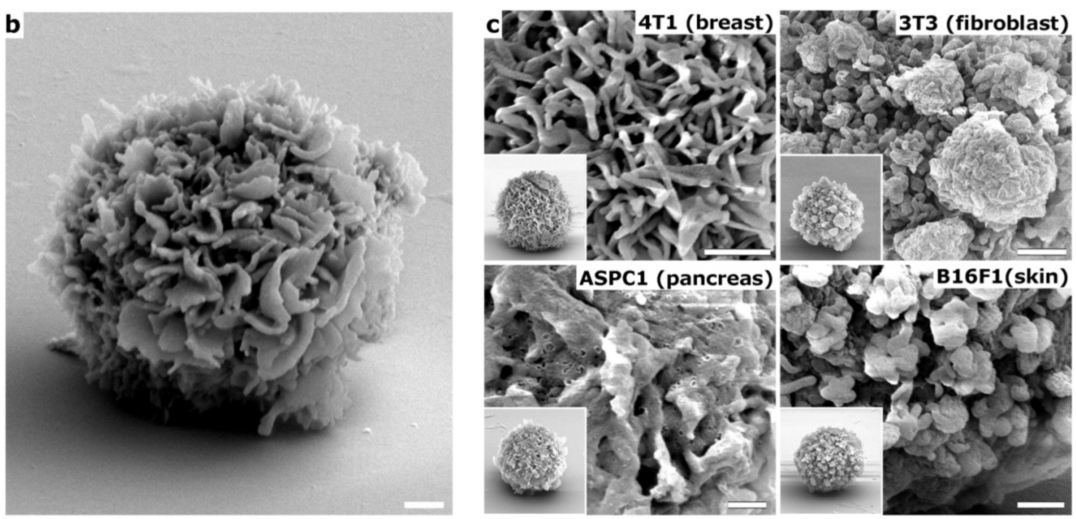
SBR: interior, exterior, solution processing

- **High throughput**
- **Monodisperse**

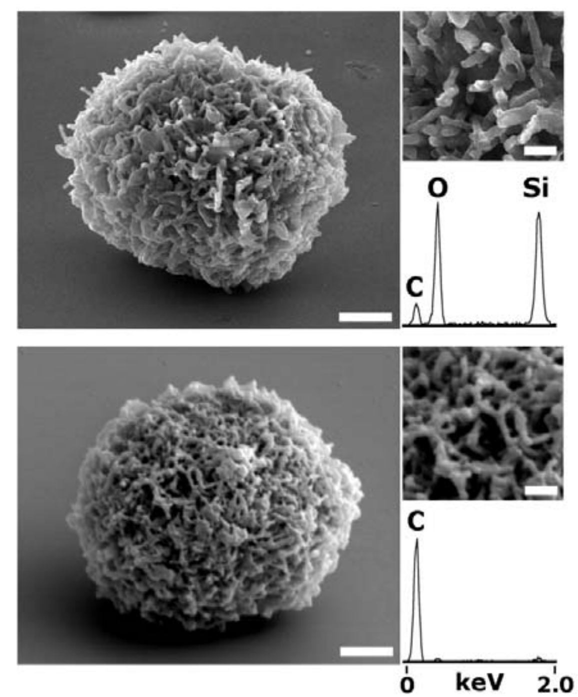




- **Generalizable**
- **Widely varying surface morphology**



N_2, 900C
4 hrs



scale bars = 1 µm

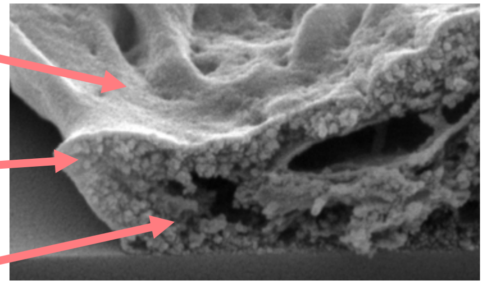
Features of silica cell replicas (Si-cells)

silica templated by:

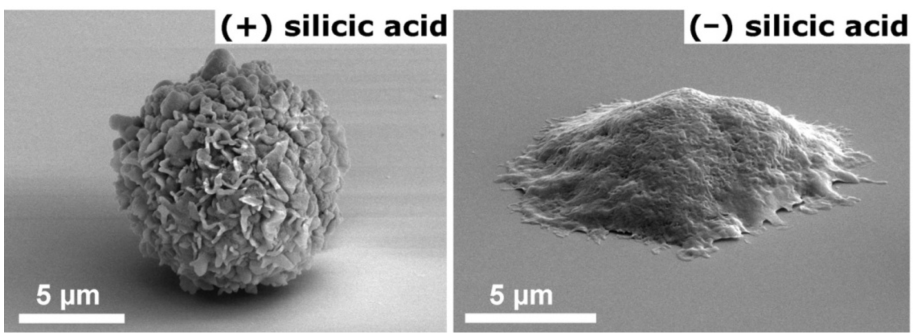
phospholipids, carbohydrates

cytoskeleton

protein complexes

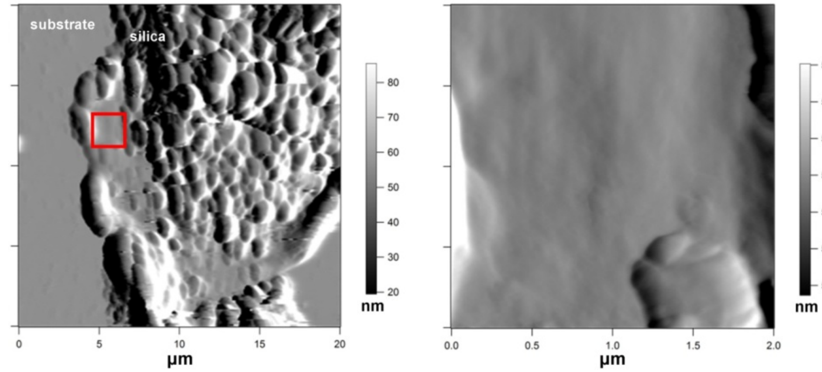


- Stable silica solution (pH 3)
- Minor electrostatic interaction, thus H-bonding, etc.
- Deposition targeted to cellular structures
- Resolution < 10 nm

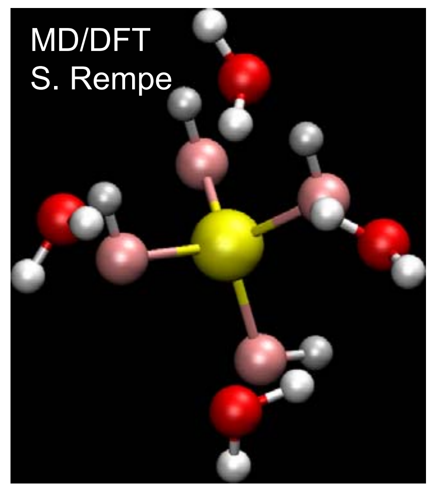


- Mechanical stability upon drying, calcination
- No appreciable dimensional change

AFM of AsPC-1 silica



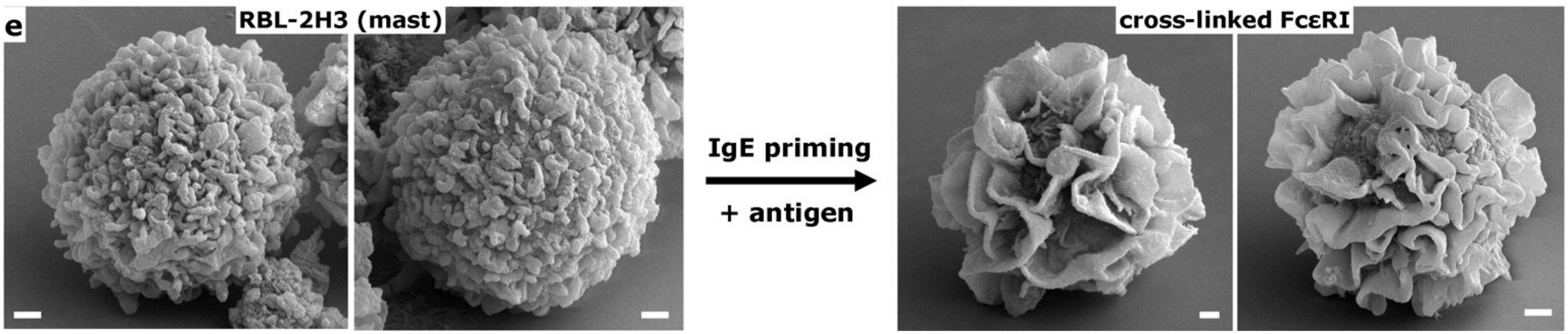
- No detectable primary feature/particle (surface roughness σ = 1 nm, within error of tip radius)



Hypothesis: silicic acid diffuses throughout cell, replaces/displaces hydrogen-bonded interfacial water at cellular/biomolecular interfaces to be concentrated and catalyzed by proximal membrane associated proteins, carbohydrates (other components) to form stable interfacial silica network in self-limited process?

Low MW silica precursors, self limiting replication of features, mechanically stable

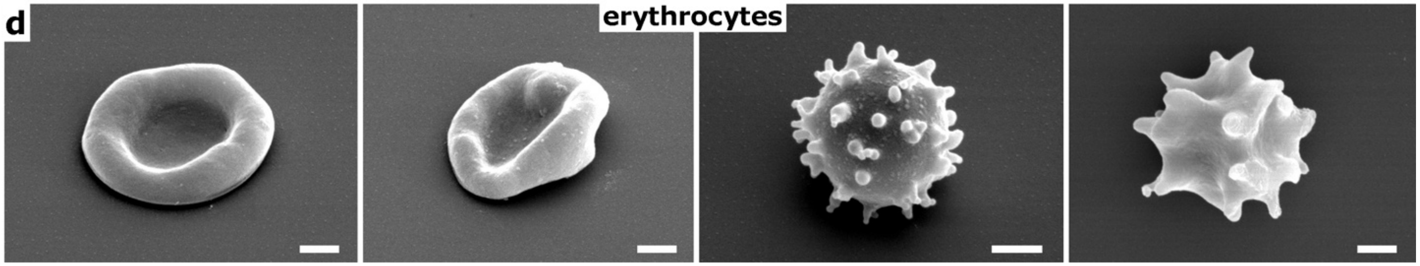
Using cellular behaviors to tune material properties



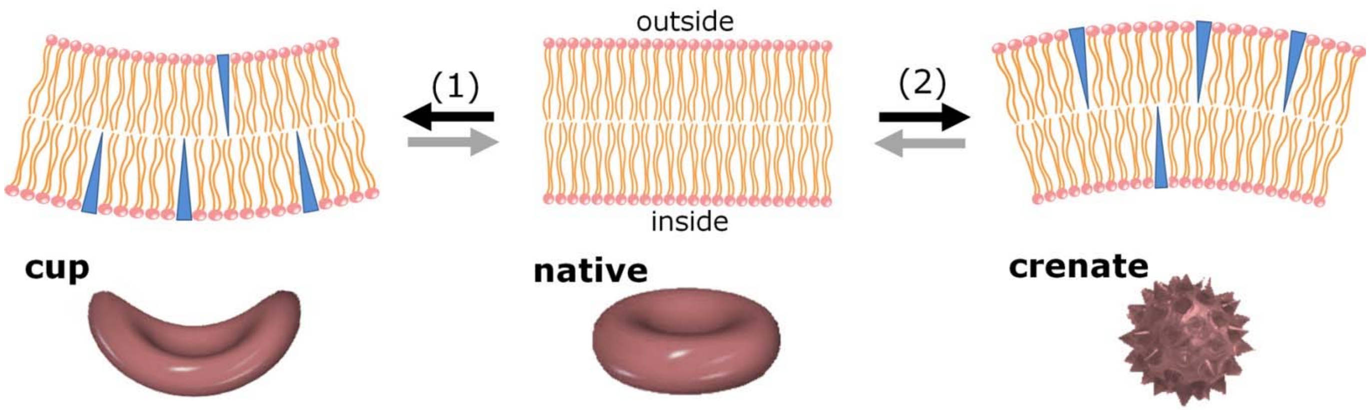
Proc Natl Acad Sci U S A. 2012 23;109(43):17336-41.

Diane Lidke, Janet Oliver (UNM)

Mechanically encoded shapes of red blood cells

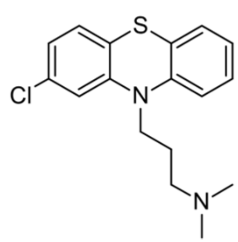


Proc Natl Acad Sci U S A. 2012 23;109(43):17336-41.

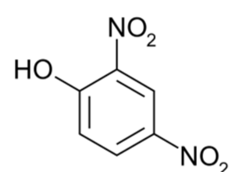


1) cationic amphipaths
low salt
low pH
cholesterol depletion

2) anionic amphipaths
high salt
high pH,
ATP depletion,
cholesterol enrichment



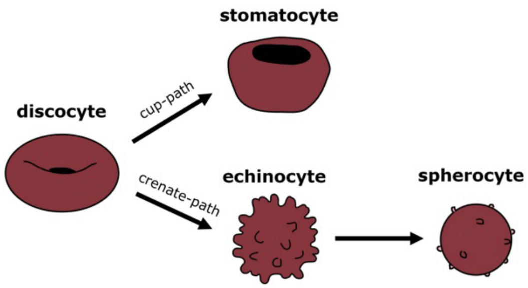
Chlorpromazine (pKa ~9)



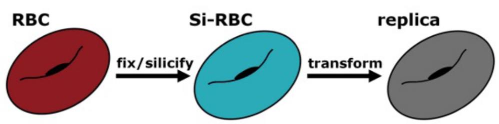
2,4-Dinitrophenol (pKa ~4)

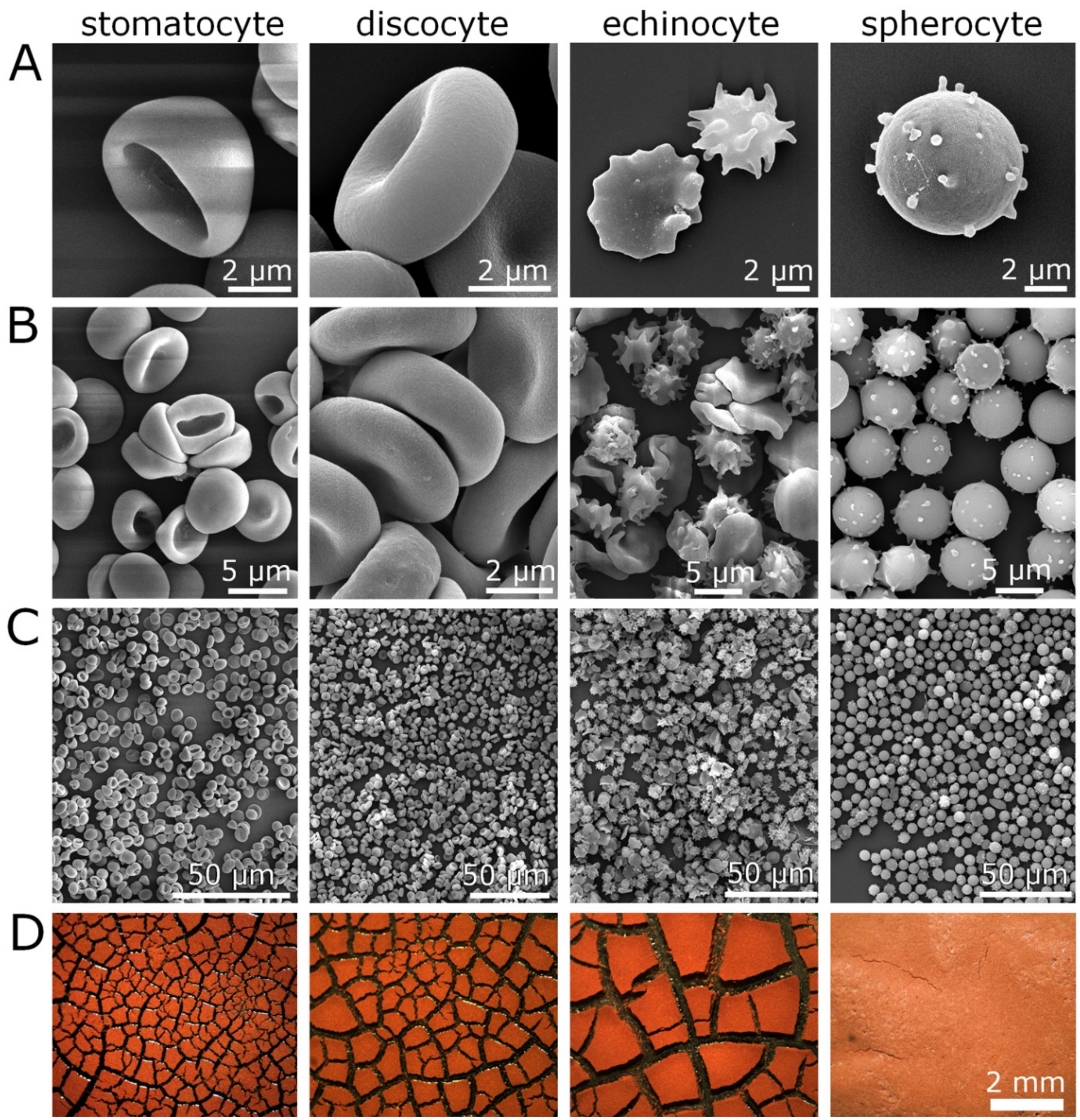
Translation of encoded shapes into composities: **Si-RBCs**

generate shape



Translate to Si-RBC



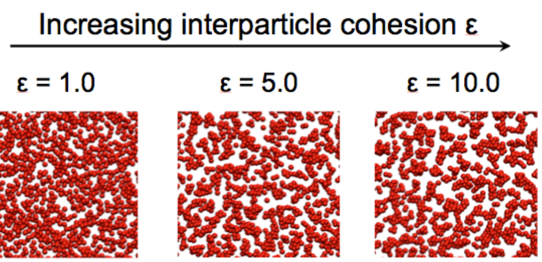


4.5-5 mls of whole blood yields 1 gram of dry dispersed particles (~10^{10} particles/gram)

Kristin Meyer (UNM-undergrad)

Translation of encoded shapes into composities: **Si-RBCs**

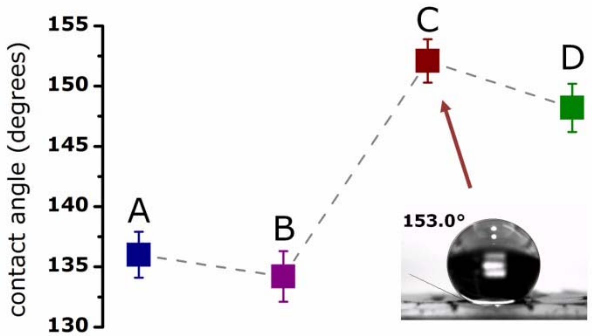
Salient features using simple models



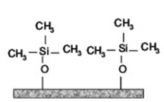
Lennard-Jones inter-particle potential for drying simulations

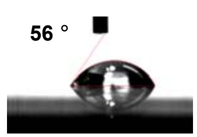
$$E = 4\varepsilon\left[\left(\frac{\sigma}{r-r_0}\right)^{12} - \left(\frac{\sigma}{r-r_0}\right)^{6}\right]$$

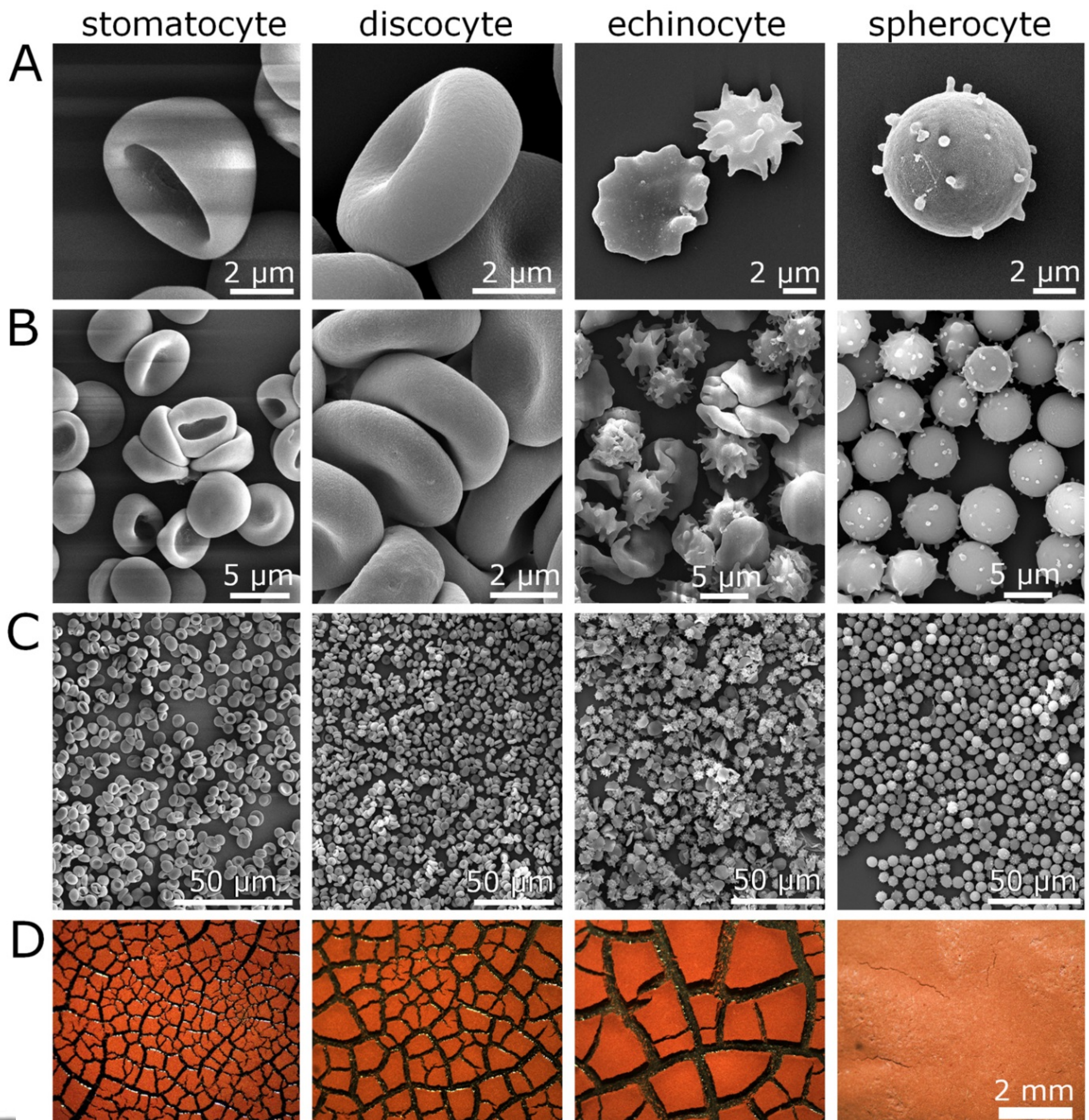
Surface energy/roughness



HMDS reacted on coverglass

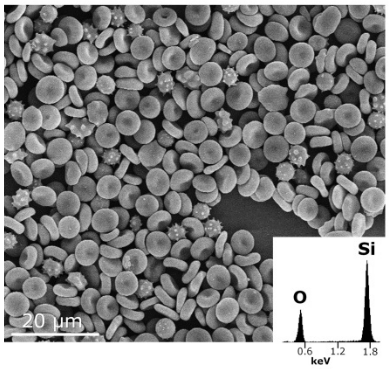


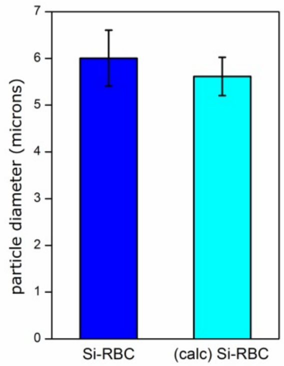


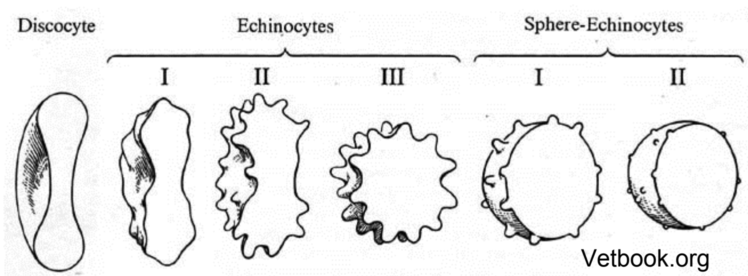


Using biological response to tune mesoporosity

Porous particles following calcination (550 C, 4hrs)

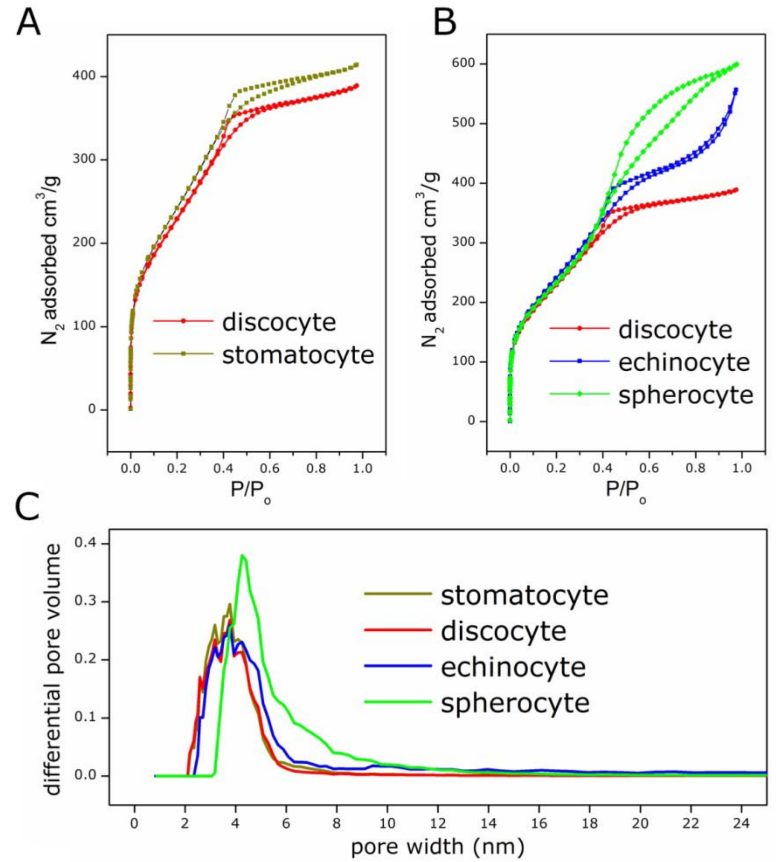


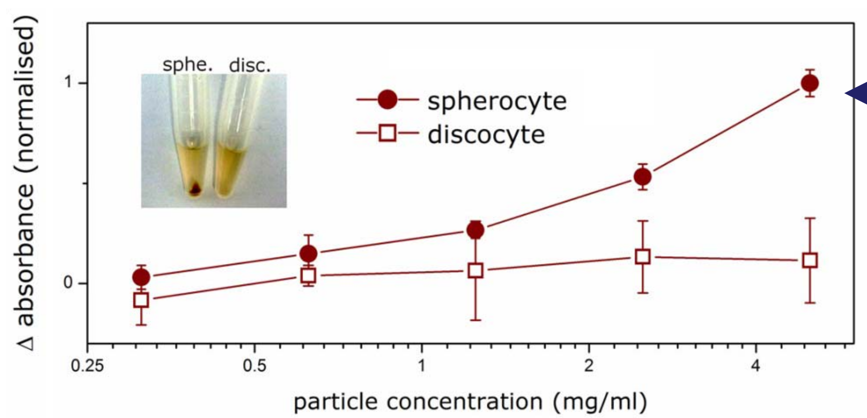




Increasing intracellular density

N_2 sorption of calcined Si-RBCs

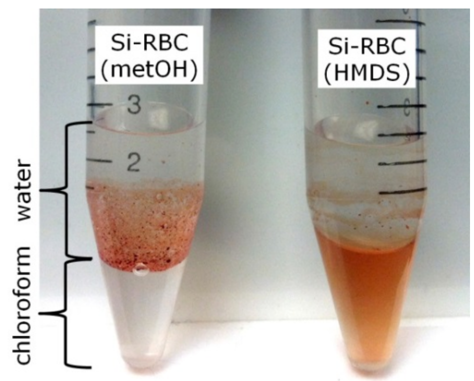




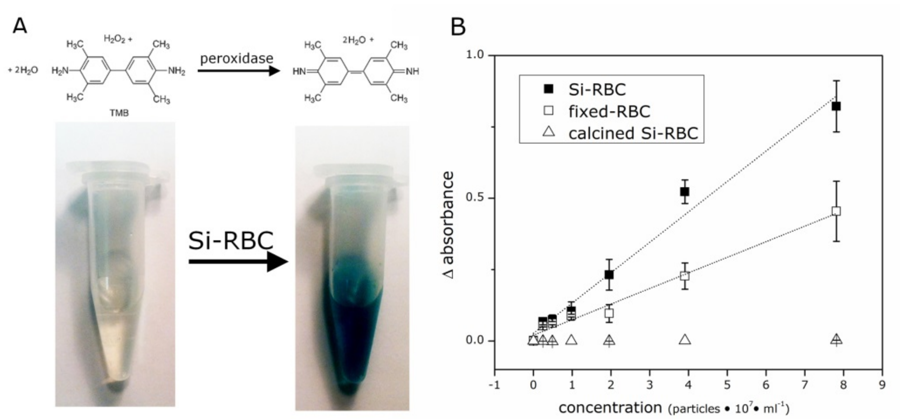
Myoglobin uptake: ~10^8 myo / cell

Features of Si-RBCs and follow-on work

Silanization for use across solvents

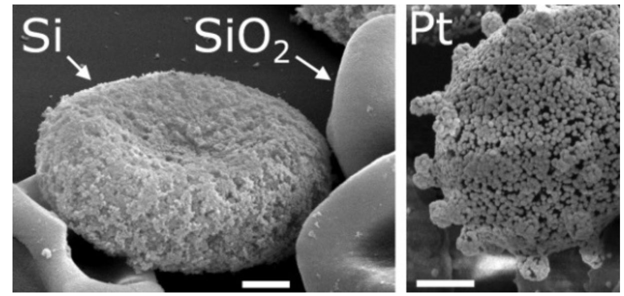


Stabilization of enzymatic activity

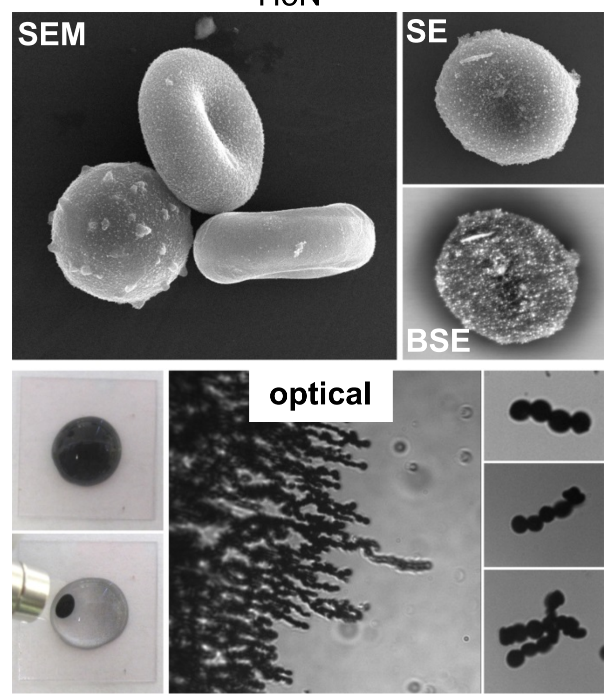


Shape-preserving conversion to other materials

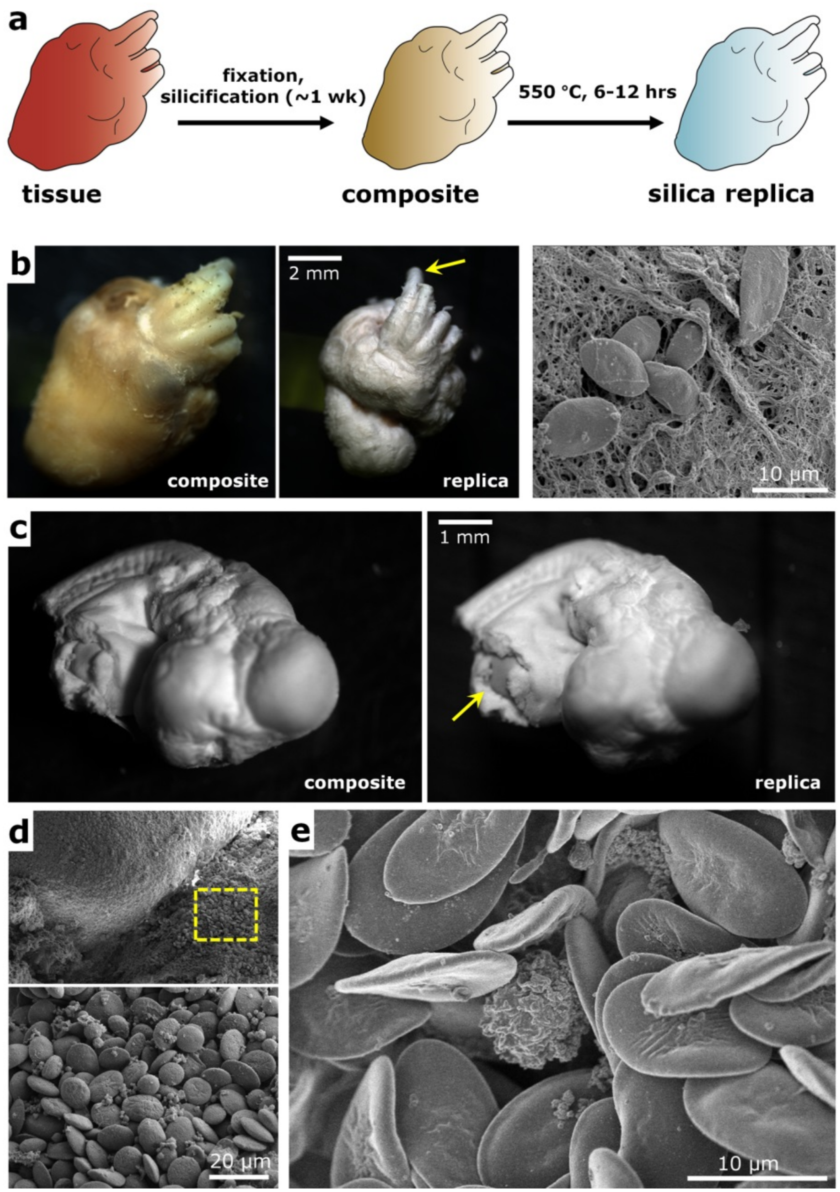
$$2Mg(g) + SiO_2 \rightarrow 2MgO(s) + Si(s)$$



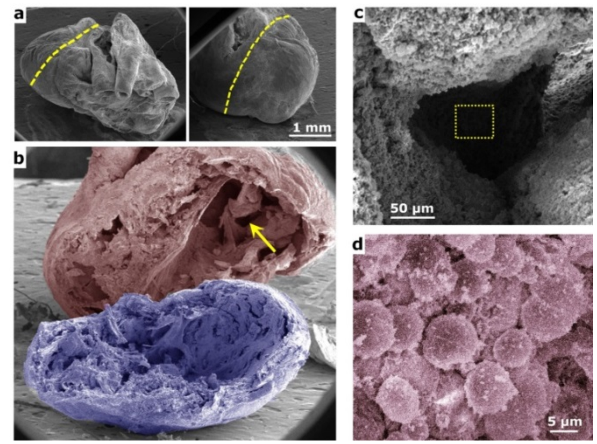
$$\text{Si-RBC} + Fe(CH_3CO_2)_2 \xrightarrow[\text{H5N}]{700\ °C} \text{FeC-RBC}$$



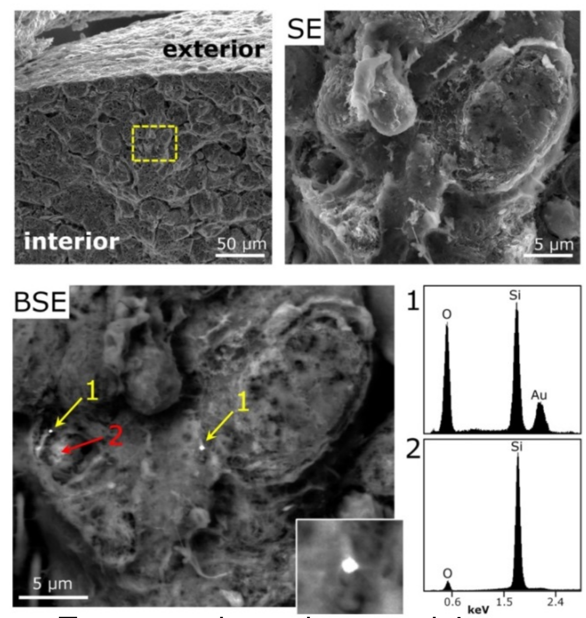
Shape-preserving transformation of tissues/organisms into inorganic and conductive specimens



SEM imaging and sectioning w/o conductive coating



Detection of single ingested AuNPs



Jason Townson, chx embryo model

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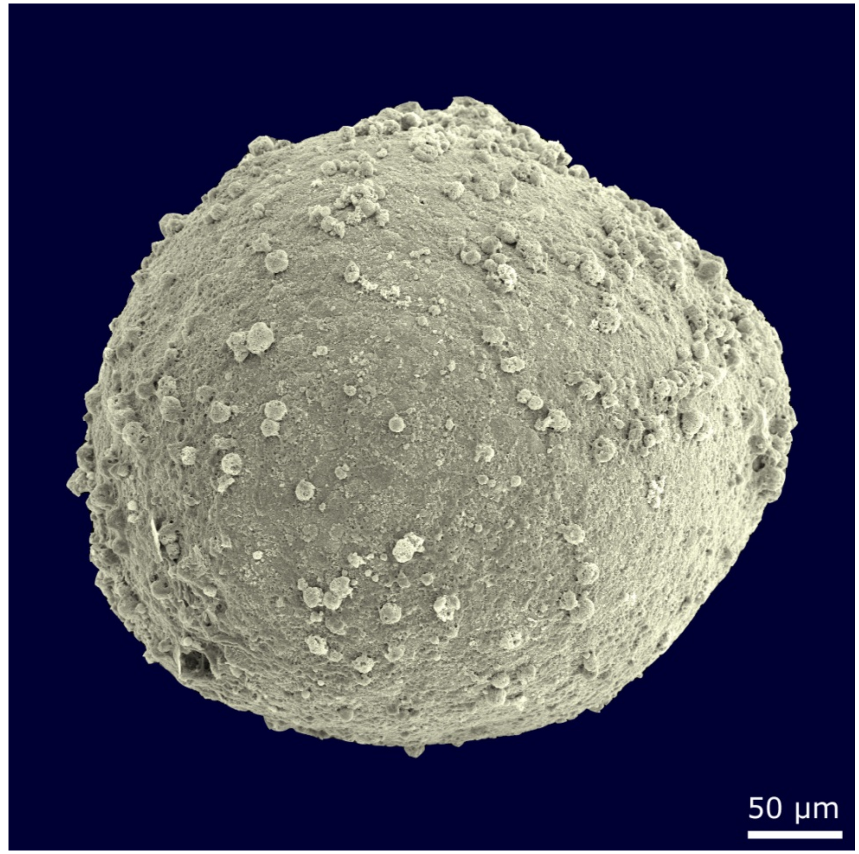

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