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Please come to the **Research in Progress Seminar Series**

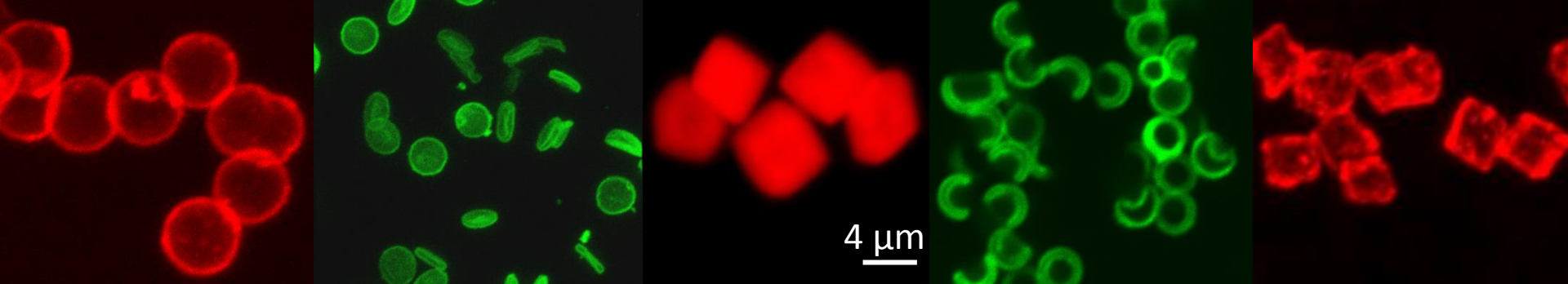
WONDERFUL WORLD OF TECHNOLOGY

eventfeaturing

Eugenia Kharlampieva, PhD
Assistant Professor,
Nanomaterials/Polymer Chemistry

Polymeric Nanomaterials for Controlled Delivery

Thursday, September 4, 2014,
12:00 – 1:00 PM
SHEL 515, 1825 University Blvd.



Polymeric nanomaterials for controlled delivery

Eugenia Kharlampieva

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Center for Nanoscale Materials & Biointegration*

University of Alabama at Birmingham, Birmingham, AL, USA
<http://www.kharlampievagroup.com/>



CHEMISTRY

BLAZING A TRAIL OF DISCOVERY

<http://www.uab.edu/cas/chemistry/>

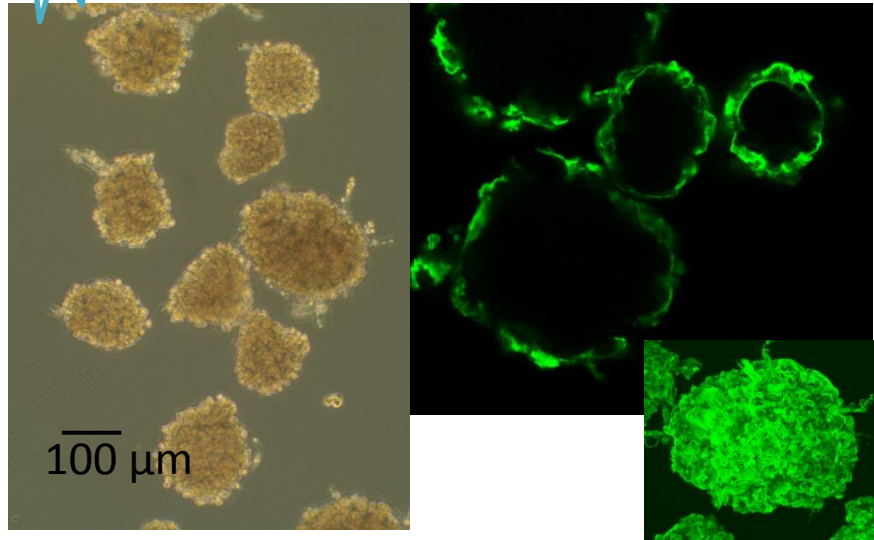
Excellent research opportunities in

- Polymer Synthesis
- Polymer Thin Films and Devices
- Biomimetic and Biopolymer Materials
- Inorganic Polymers
- Nanocrystals
- Photophysics
- Coordination Compounds
- Electron Transfer Reactions
- NMR Spectroscopy
- Main Group Inorganic Synthesis
- Drug Design
- Metallocrown Ethers
- Matrix Assisted Laser Desorption Spectroscopy (MALDI)
- Nonlinear Optical Materials
- Biochemistry and Spectroscopy
- Quantum Chemistry
- DNA Microarrays
- Chemical Education

Nanostructured functional polymeric coatings and containers



Encapsulated cells to protect from immune injury for transplantation



Islet transplantation for diabetes treatment

Hubert Tse, Microbiology
Tony Thompson, Surgery, UAB
Diabetes Research Center

Type I Diabetes:

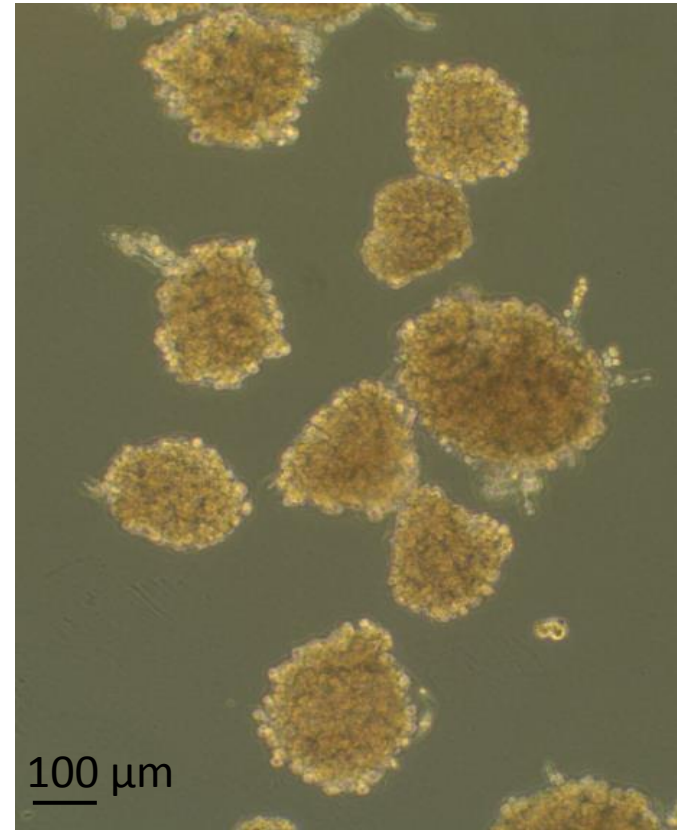
Islet-reactive immune cells destroy insulin-producing β -cells

- T cells release inflammatory cytokines
- Macrophage- and T cell-produce ROS inducing inflammatory cytokine synthesis

Disease treatment:

Transplantation of insulin-producing pancreatic islets

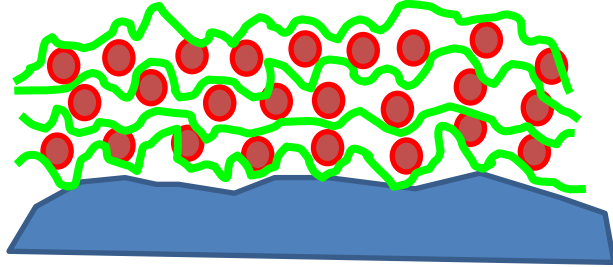
- Modulation of immunogenic reactions
- Stabilization of islets morphology and functionality



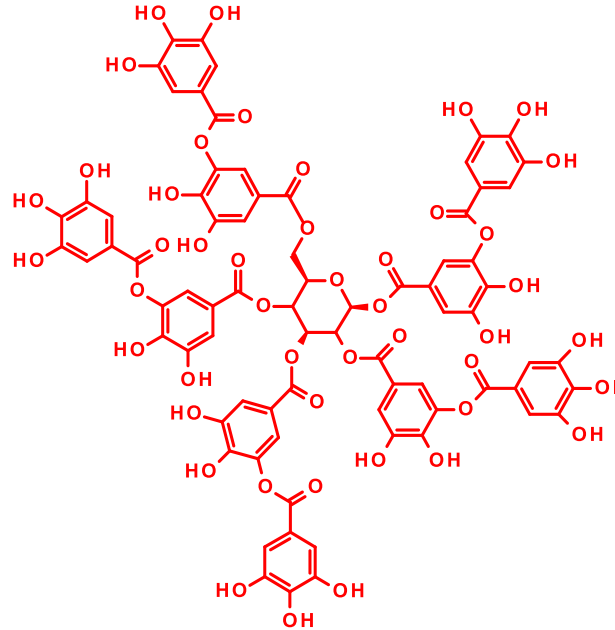
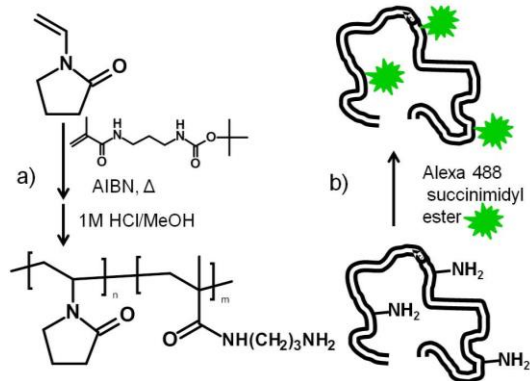
Human pancreatic islets - Isolated 'microorgans'

Hydrogen-bonded layer-by-layer coatings for encapsulation of islets

Multilayer films

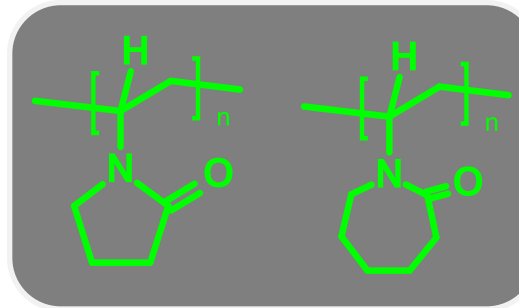


Fluorescently labeled
PVPON* or PVCL*



Tannic Acid (TA)

- Rich in phenolic content
- H-bonding $2 < \text{pH} < 9$



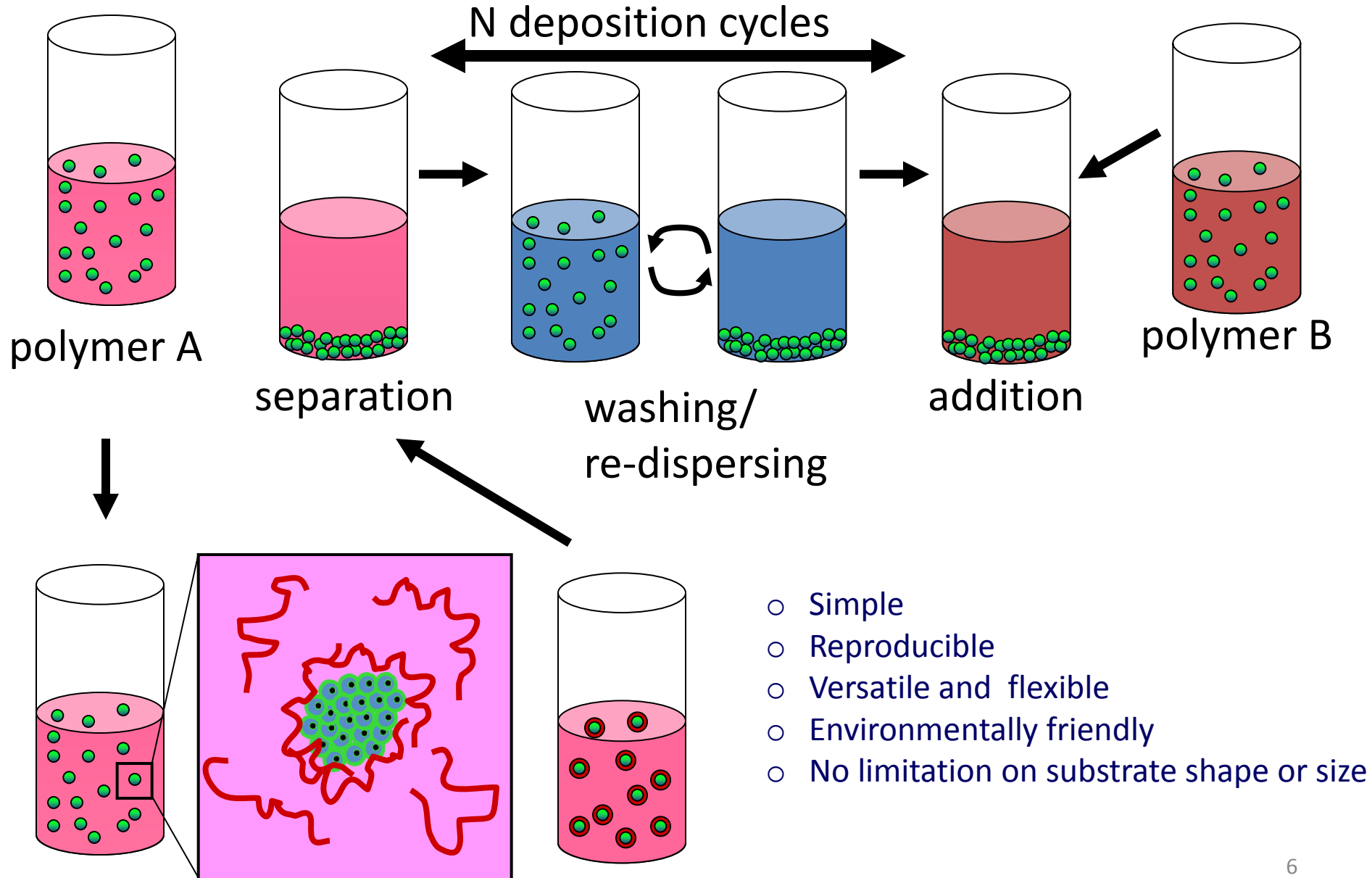
Poly(N-vinylpyrrolidone)
(PVPON),
 $M_w \sim 1,300,000 \text{ g mol}^{-1}$

- Hydrophilic, anticoagulant properties
- Non-charged, non-toxic

Kozlovskaya, Zavgorodnya, Chen, Ellis, Tse, Cui, Thomson, Kharlampieva, *Adv. Funct. Mater.* 2012, 22, 3389

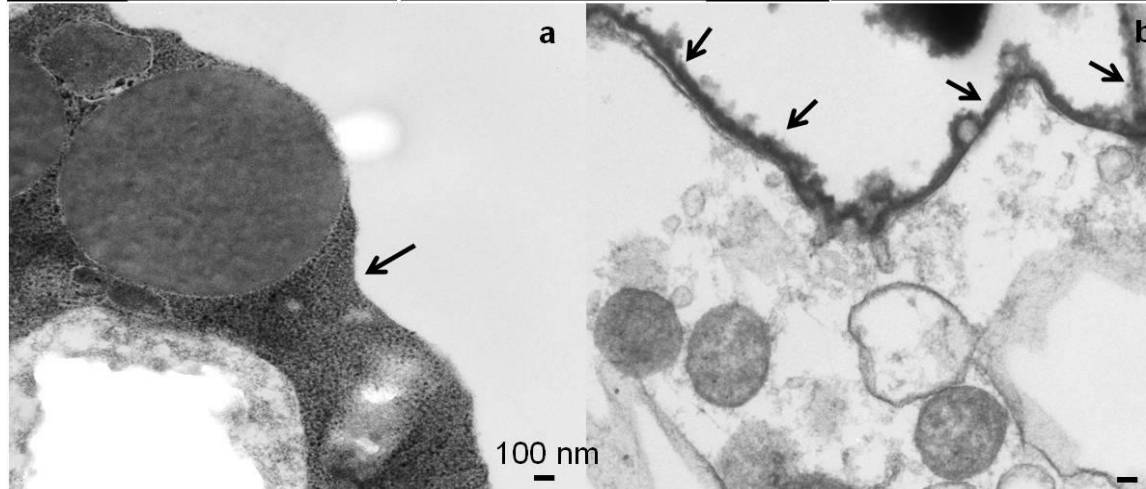
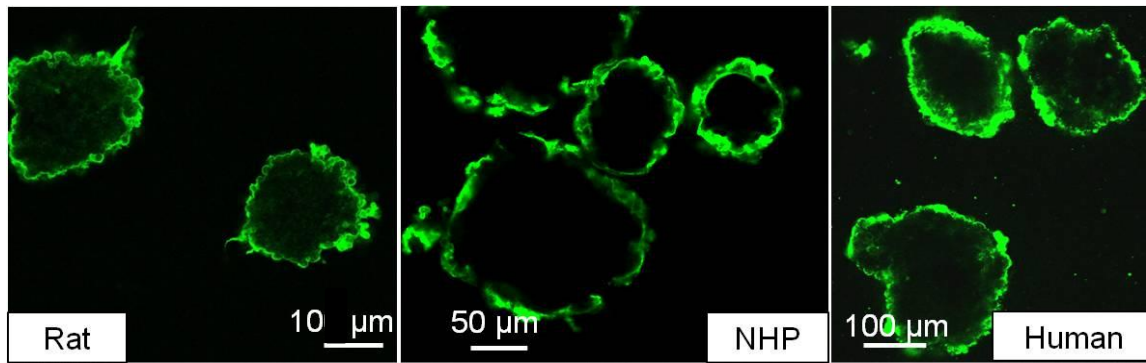
Chen, Kozlovskaya, Campos-Gomez, Saeed, Kharlampieva, *Biomacromolecules* 2013, 14, 3830

Layer-by-layer assembly on islets



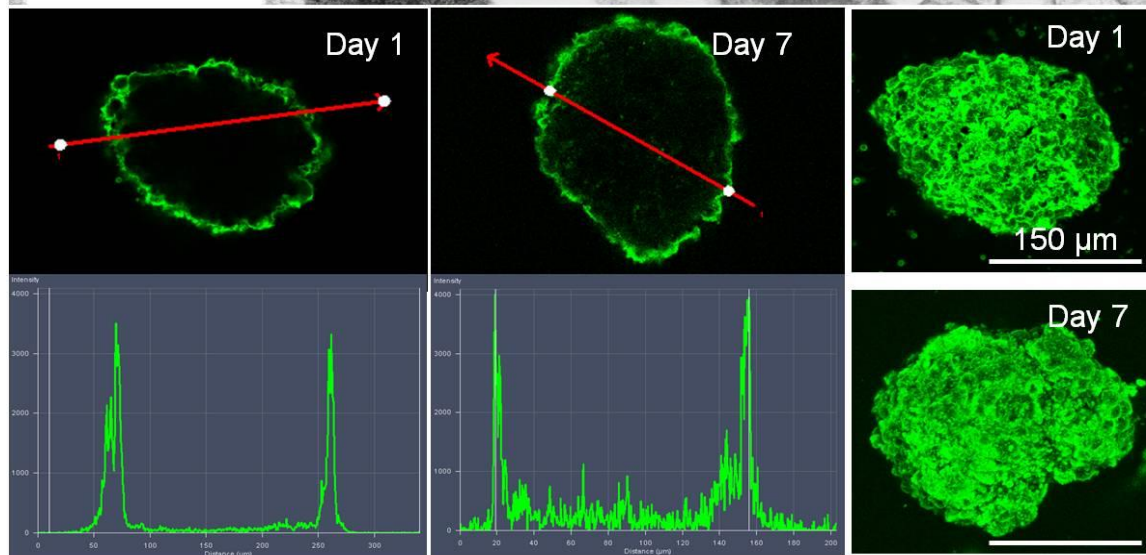
Integrity and stability of the coating

$(\text{PVPON}/\text{TA})_4\text{PVPON}$



7nm/bilayer thick

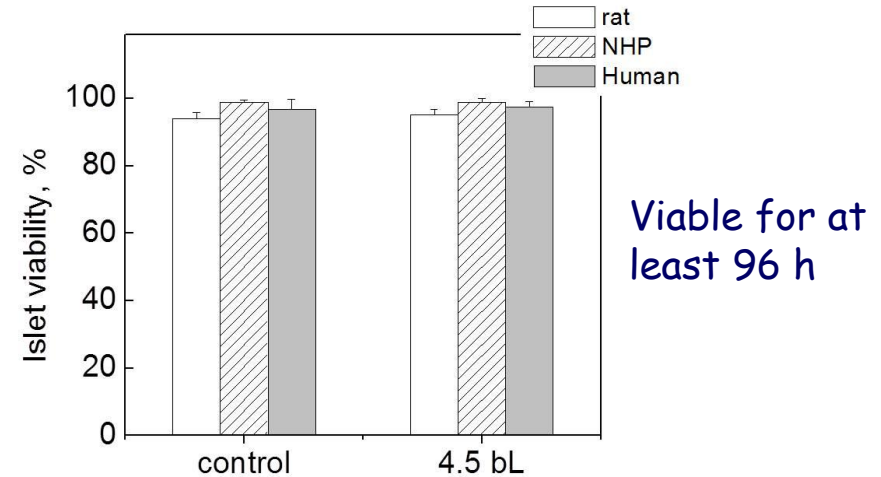
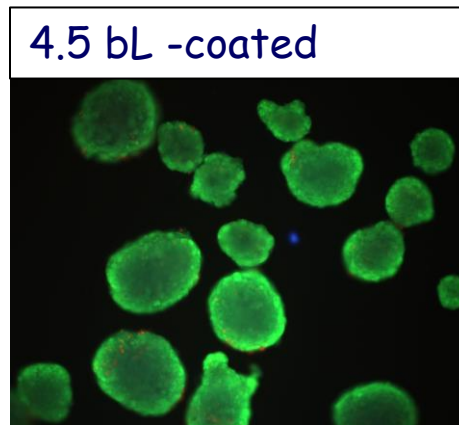
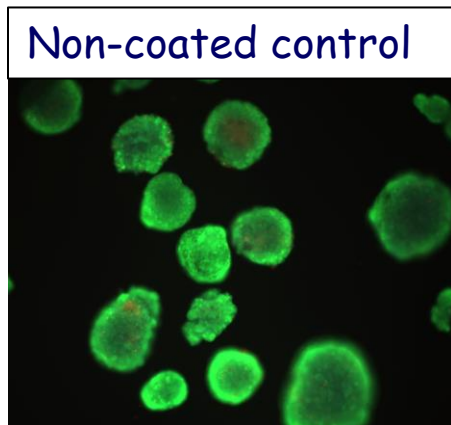
NHP, $(\text{PVPON}-\text{TA})_{4.5}$ -
PVPON-Alexa



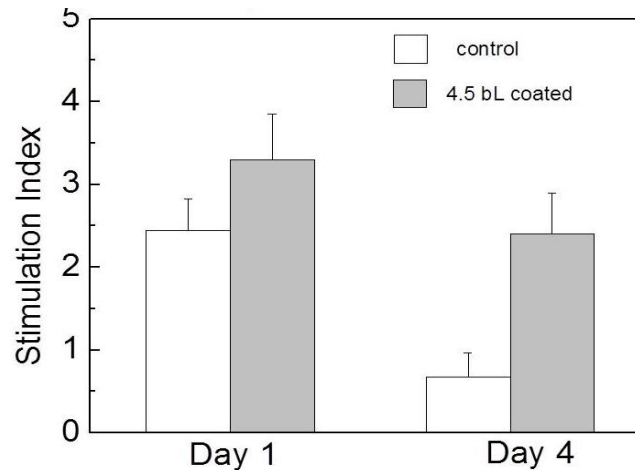
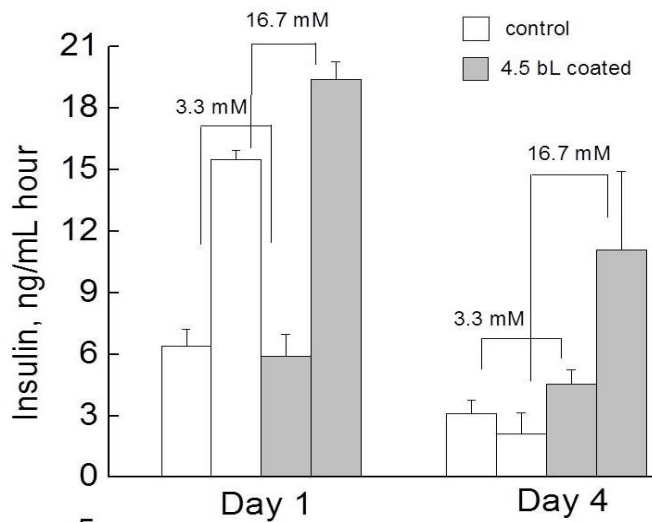
Cultured in Miami 1A, 25°C

Viability and Function *in vitro*

Viability Assay: Propidium iodide (red-dead)/fluoresceine diacetate (green-live)



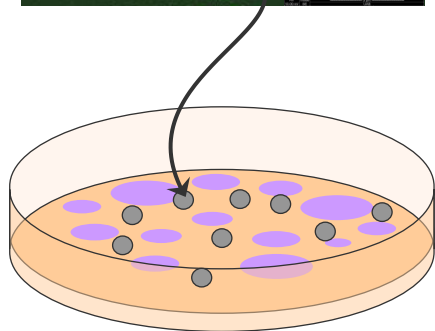
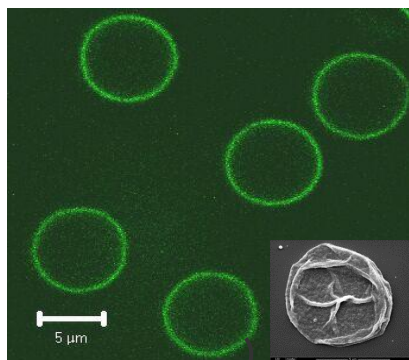
Functioning: Glucose-stimulated insulin secretion (GSIS)



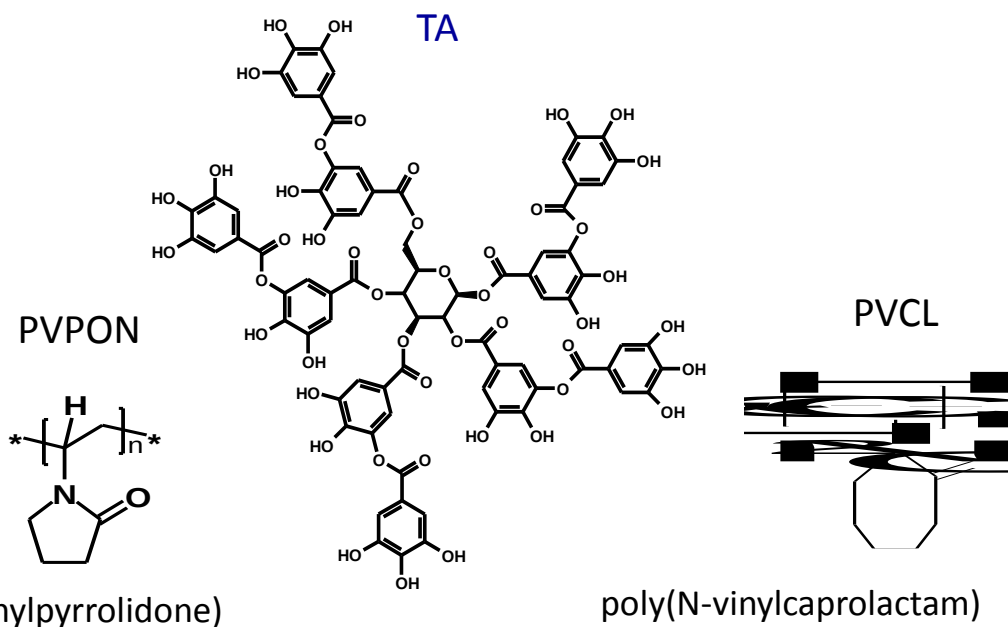
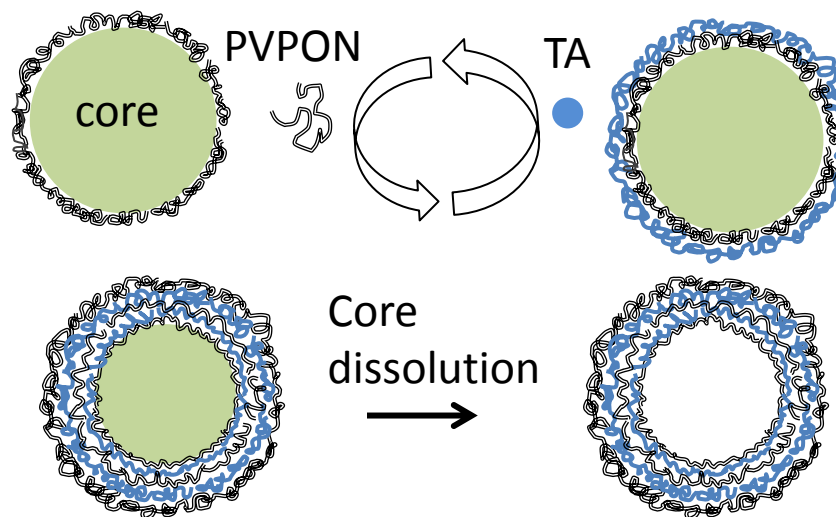
- Static incubation in Miami Medium 1A at 25°C
- Coated islets preserve β -cells functioning for at least 96 hours *in vitro*
- Crucial requirement for islet shipping and transplantation

Cytoprotective (PVPON/TA) capsules

(PVPON/TA)₄ capsules

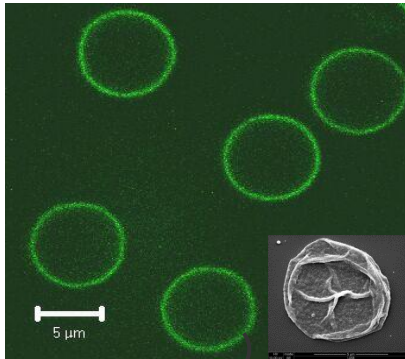


- Macrophages and T cells can kill islets by releasing the proinflammatory cytokines
- Synthesis of cytokines by activated macrophages and T-cells in the presence of capsules

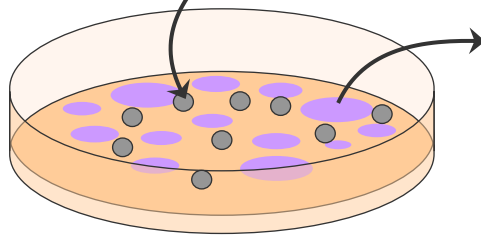


Immunomodulatory properties of (PVPON/TA) capsules

(PVPON/TA)₄ capsules

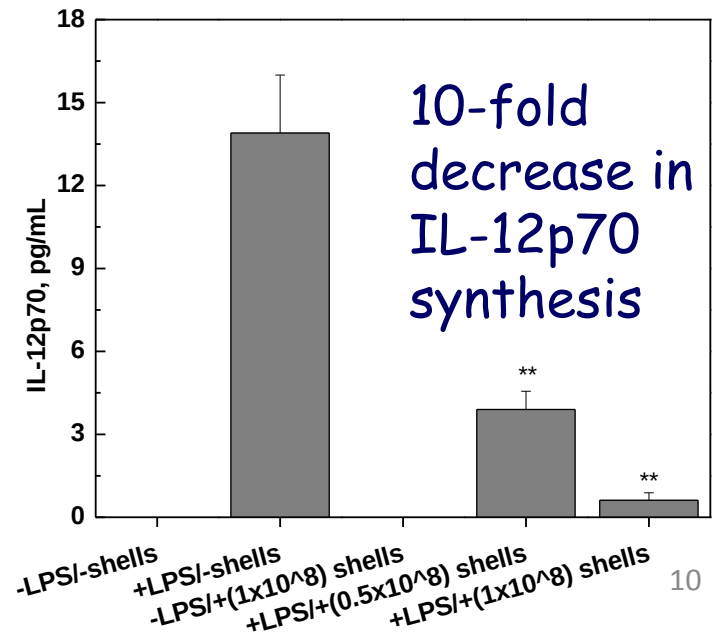
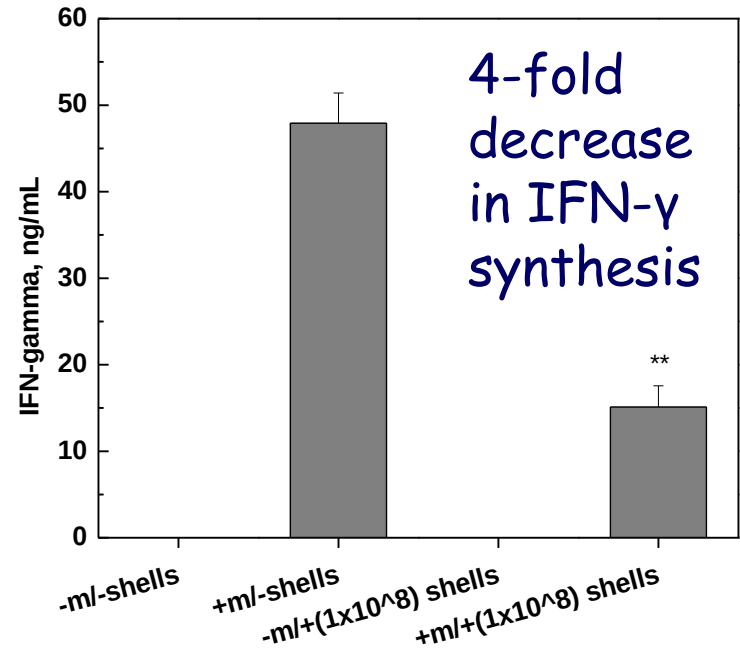


pH = 7.2
Miami M1



Bone marrow-
derived
macrophages (LPS
from E.Coli)

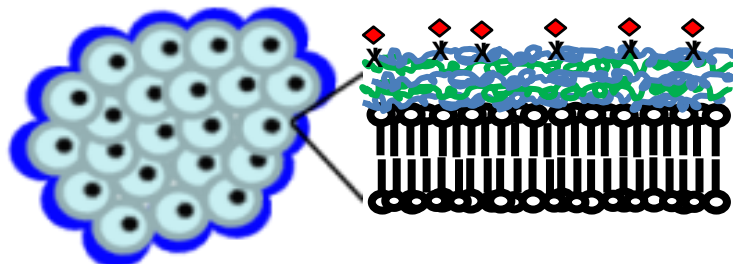
- The activated T cells (adaptive immune response) and macrophages (innate immune response) co-cultured with capsules
- Suppression of lymphocyte maturation factor (IL-12p70) and interferon-gamma (IFN-γ) synthesis as compared to capsule-free media
- Suppression is controlled by capsule amount
- Capsules are not immunogenic



Summary

Hydrogen bonded TA-containing multilayer is an effective material for *prolonging islet viability and function*:

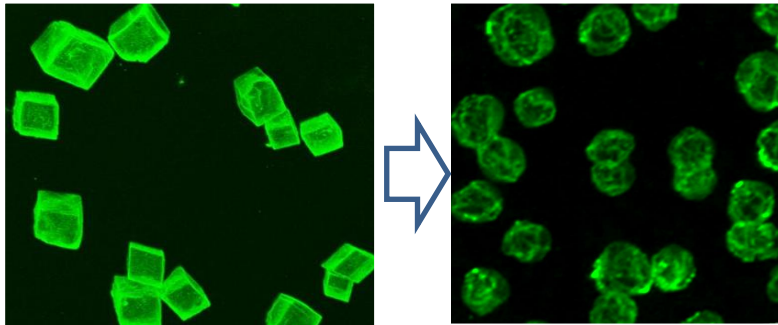
- Conformal coverage of the entire islet surface
- Stable for at least 7 days *in vitro*
- Nontoxic and non-immunogenic
- Prolonged islet functionality
- Versatile: Can be applied to rat, NHP, and human islets
- Suppresses synthesis of pro-inflammatory cytokines involved in modulation of both innate and adaptive immune response *in vivo*
- Can be easily modified
- Scavenges ROS and RNS
- Tackles islet transportation and transplantation issues



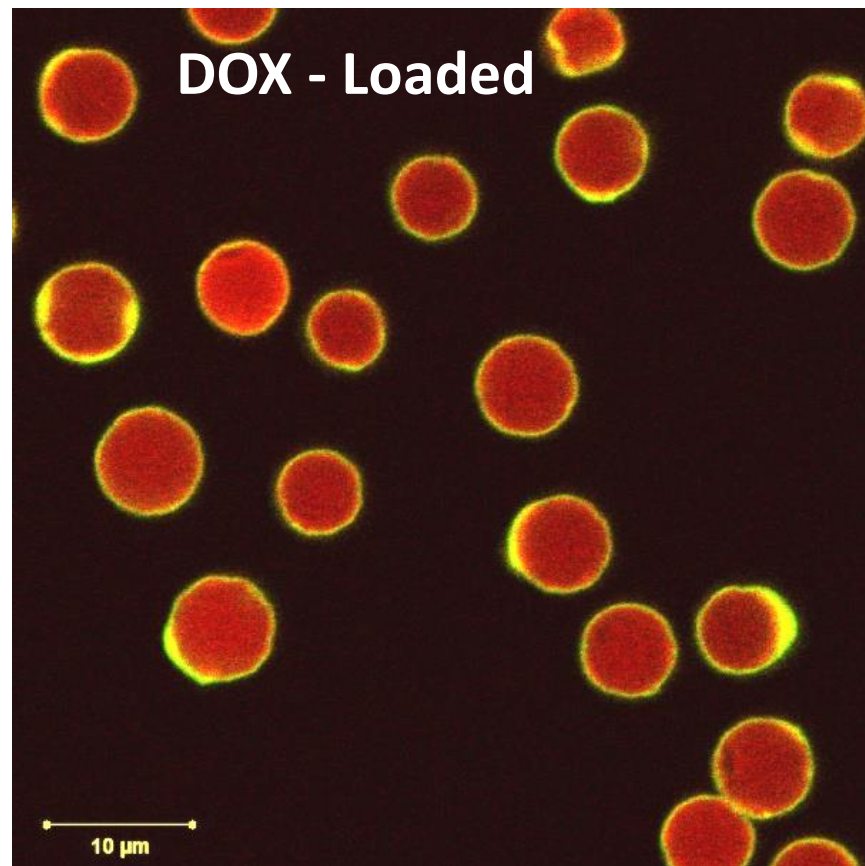
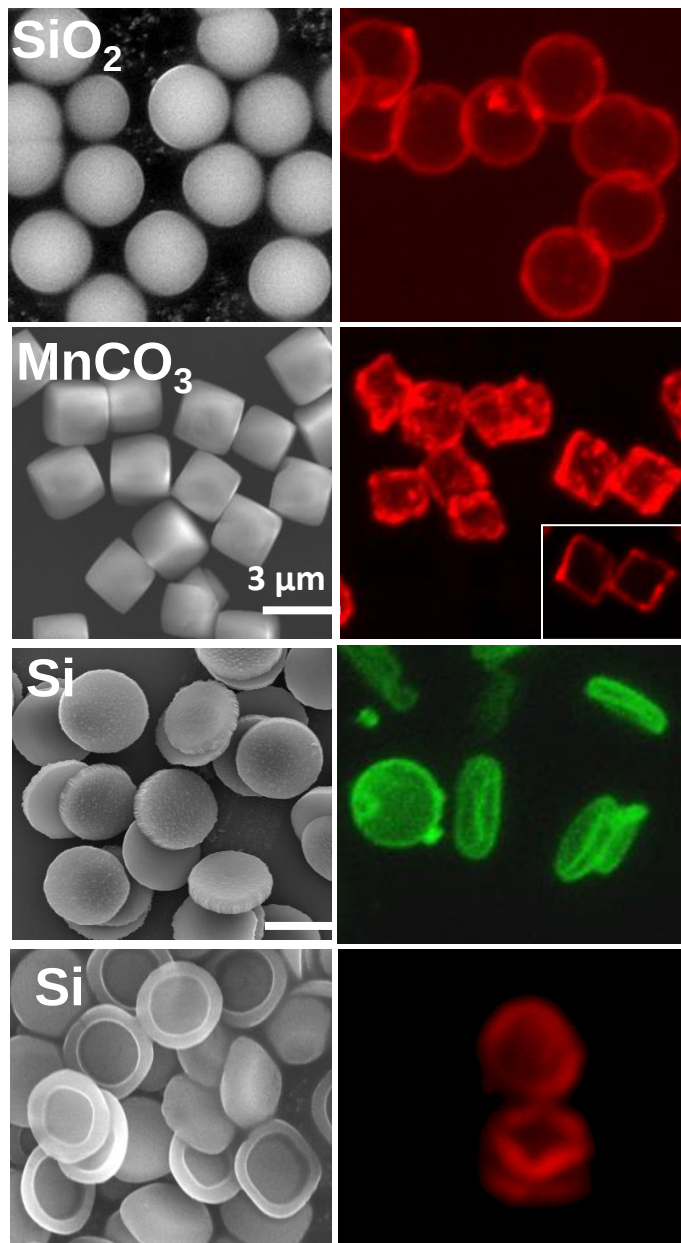
Nanostructured functional polymeric coatings and containers



*Stimuli-responsive shaped
hydrogels for controlled delivery*

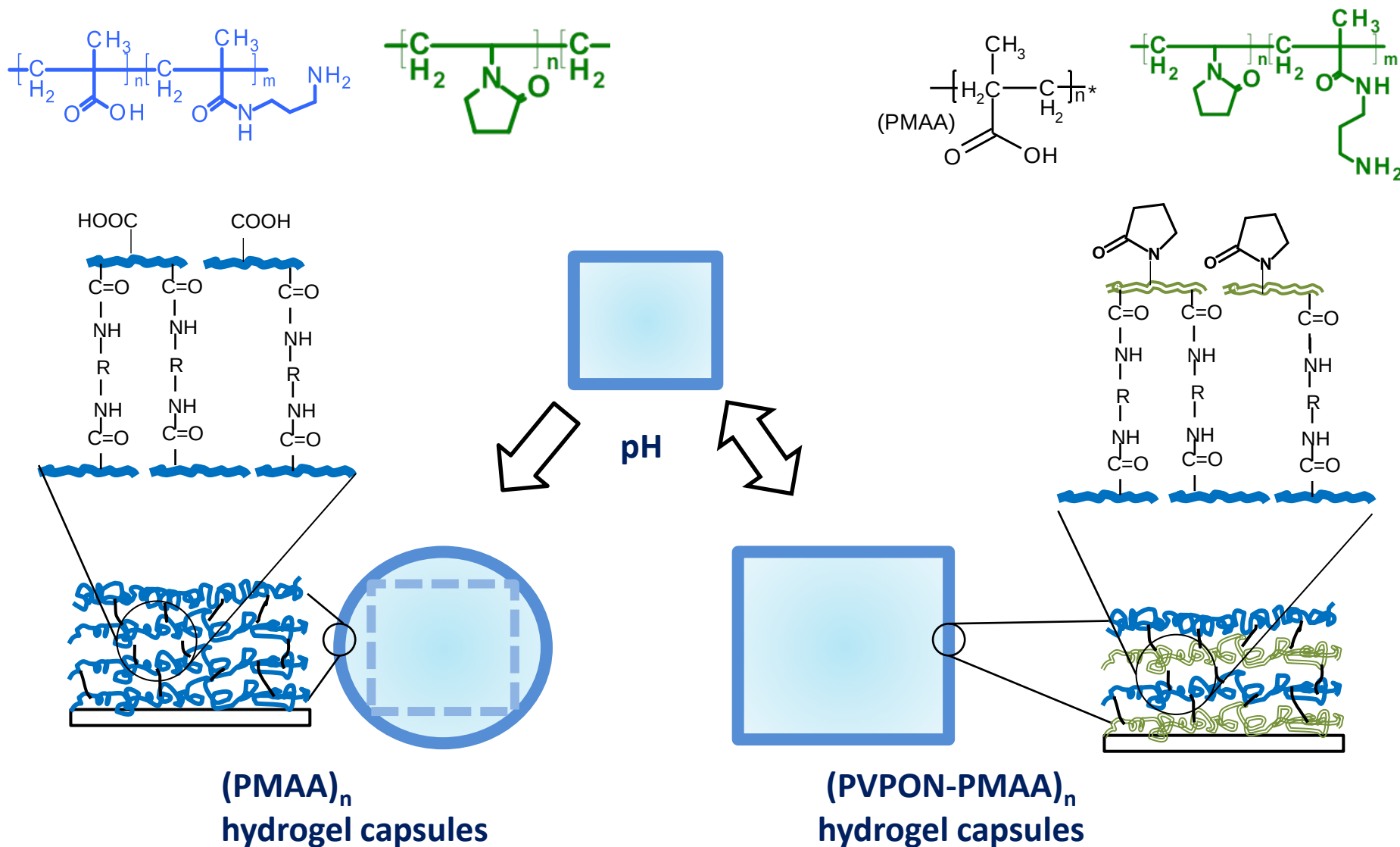


Shaped ultrathin multilayer capsules for drug delivery

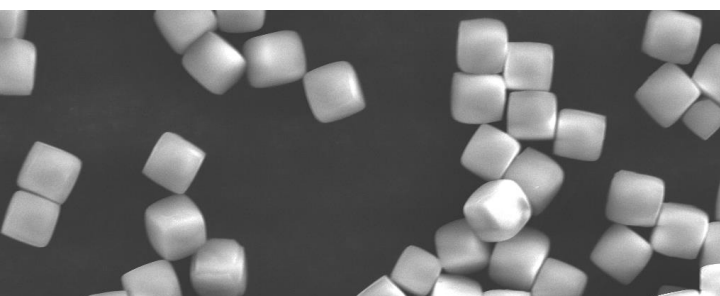


Liang et al. *Chemistry of Materials* 2012, 24, 3707
Kozlovskaya et al. *ACS Macro Letters* 2012, 1, 384
Chen et al. *Biomacromolecules* 2013, 14, 3830
Kozlovskaya et al. *ACS Nano* 2014, 8, 5725
Liu et al. *Soft Matter*, in print

pH-Triggered responses in cubical PMAA-based microcapsules

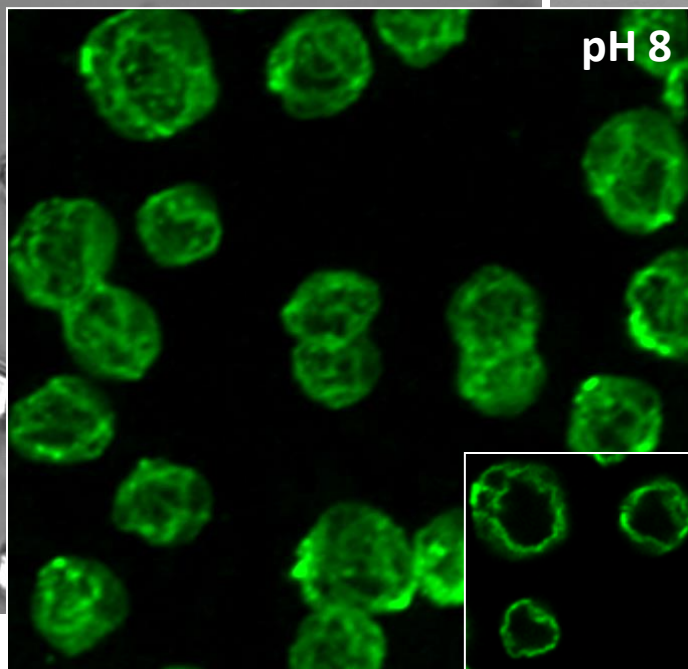
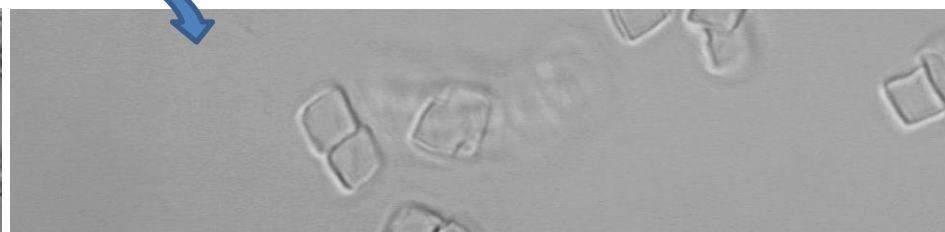


pH-induced shape switch in cubical (PMAA) microcapsules

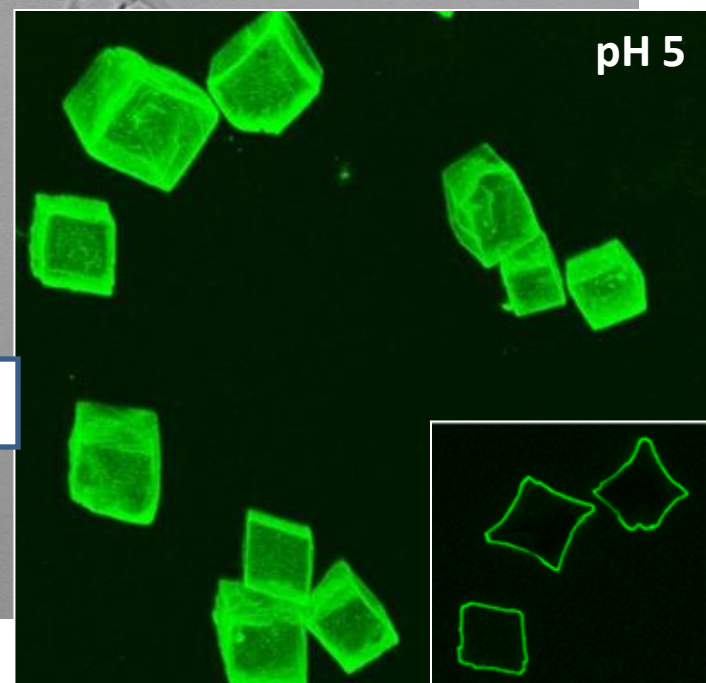


MnCO₃ templates coated with (PMAA)₂₀
multilayer hydrogels

Template dissolution

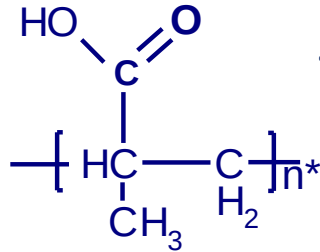


Δ pH



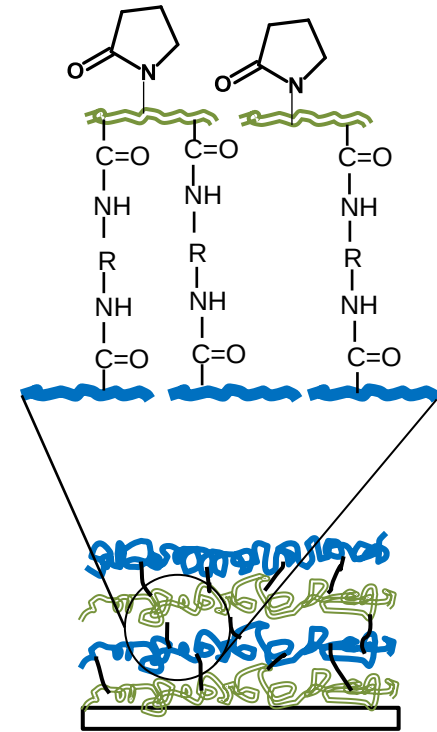
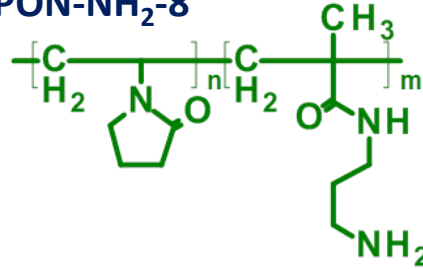
pH-induced responses of cubical (PMAA/PVPON)₅ microcapsules

PMAA

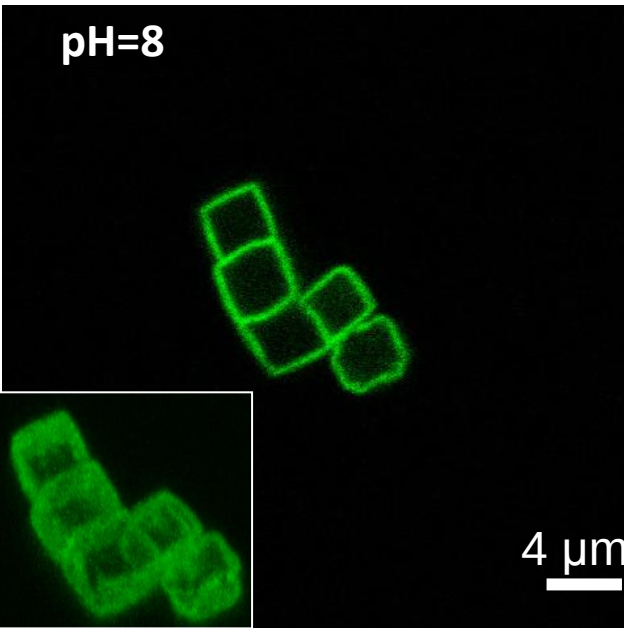


+

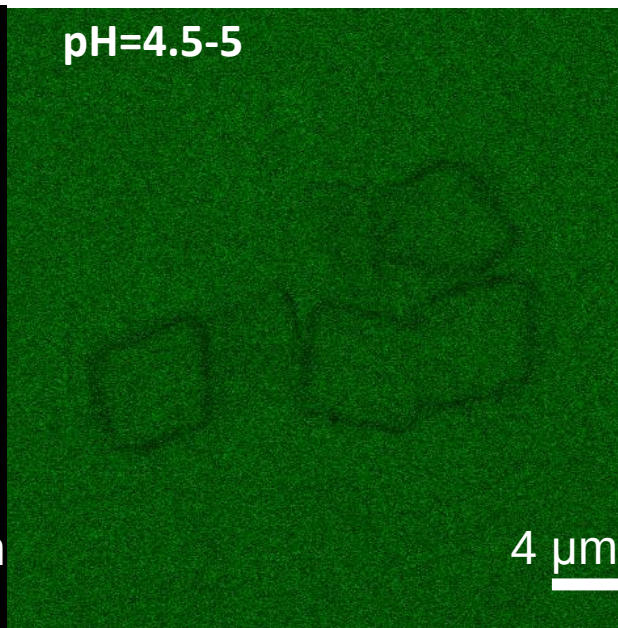
PVPON-NH₂-8



pH=8



pH=4.5-5



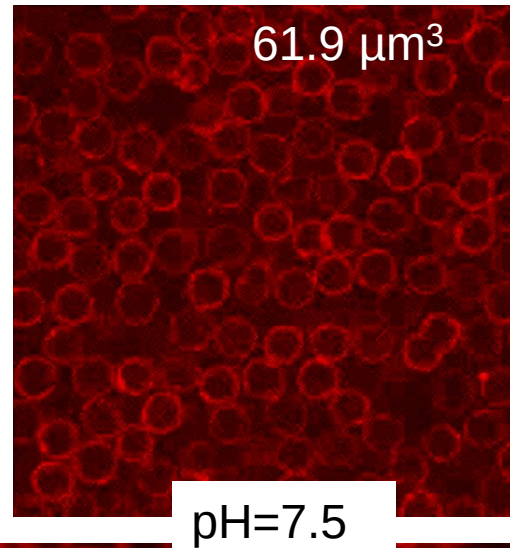
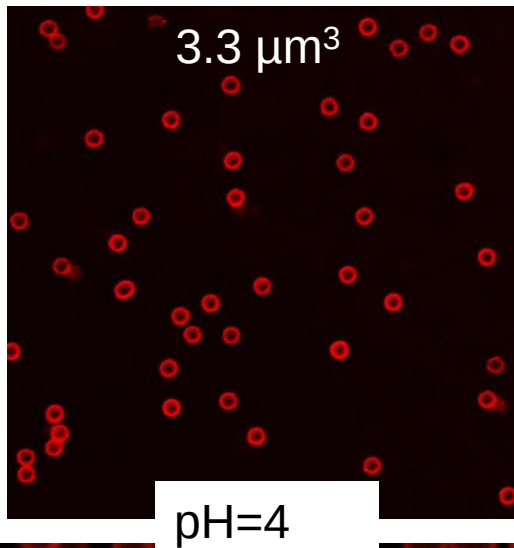
Swelling ratio is ~1.4
($D_{\text{pH}=8}/D_{\text{pH}=5}$)

Polymer contour length between the neighboring cross-links to the persistence polymer length:

$$L/l_p (\text{PMAA})_{20} > L/l_p (\text{PMAA-PVPON})_5$$

10-fold difference suggesting a more rigid system in the case of (PMAA-PVPON)_n

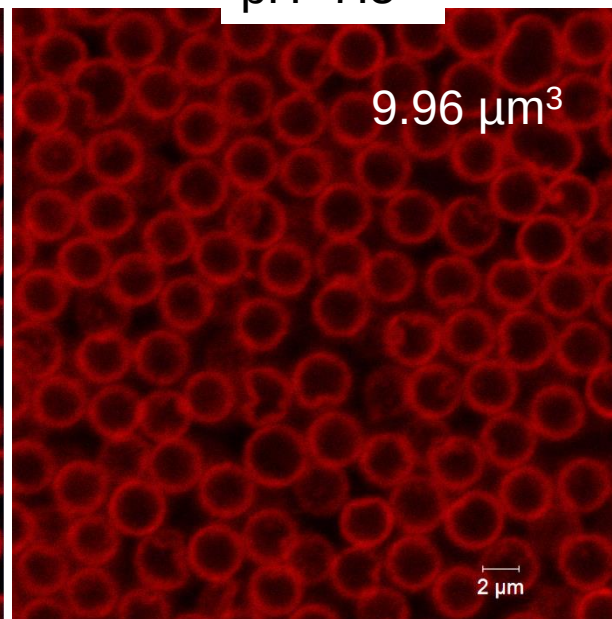
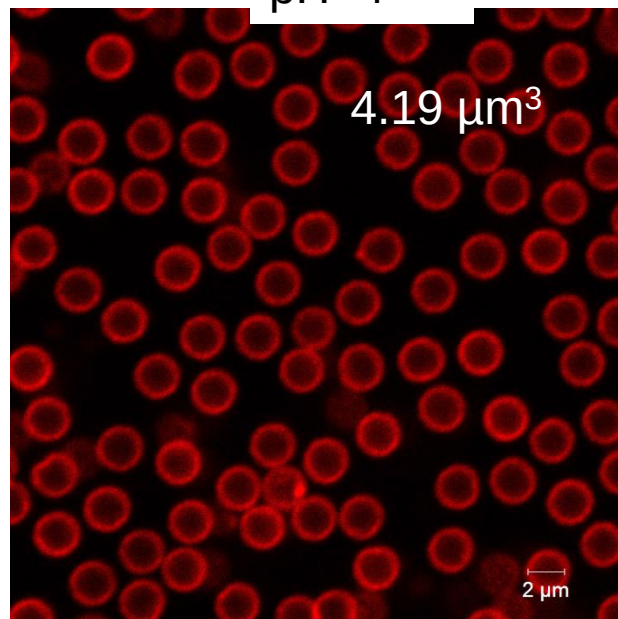
pH-response of spherical capsules: (PMAA) vs (PMAA-PVPON)



(PMAA)

Size increase: 2.5-fold

Volume increase: 19-fold



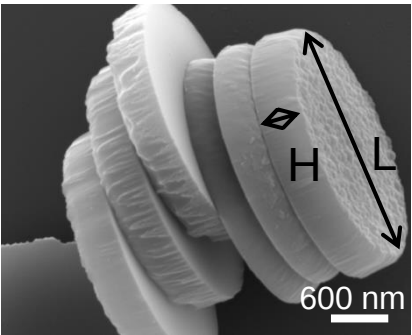
(PMAA-PVPON)

Size increase: 1.4-fold

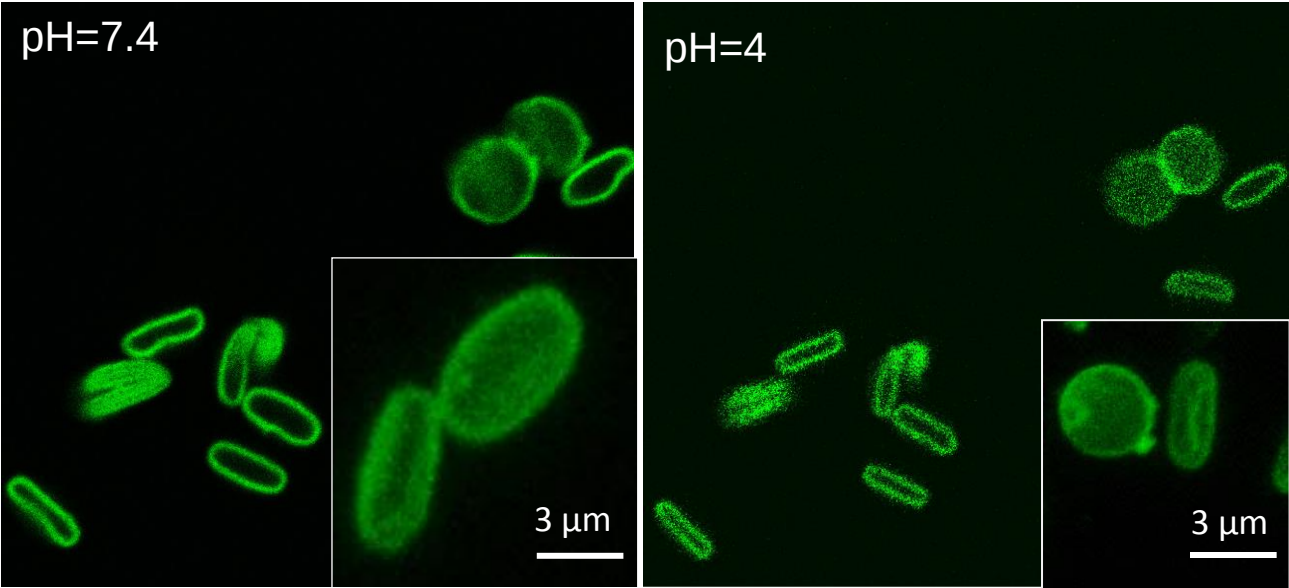
Volume increase: 2.4-fold

pH-Induced change in aspect ratio from discs to oblate ellipsoids

Silicon templates



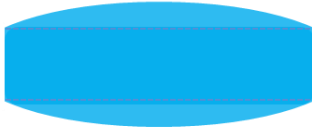
$$L/H \gg 1$$



Solid Si discs



pH=7.4



pH=4



(PMAA)₁₅ capsules

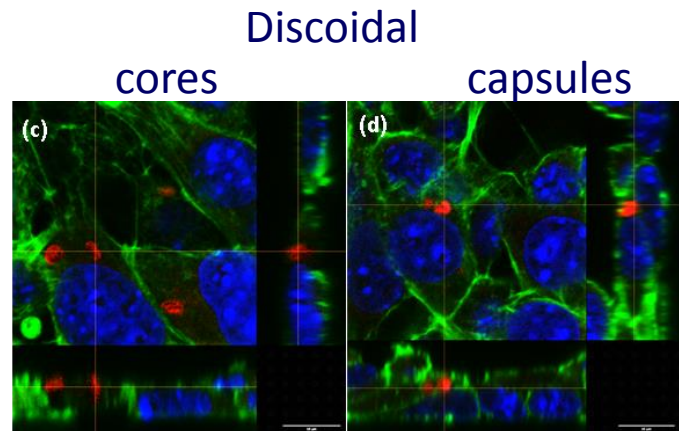
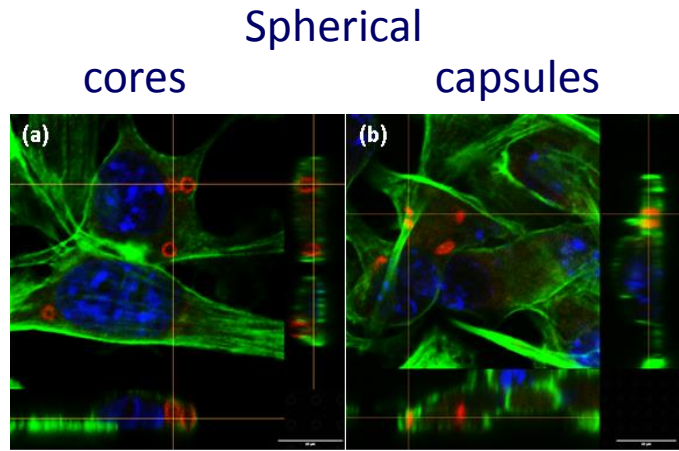
(PMAA-PVPON)₅ capsules



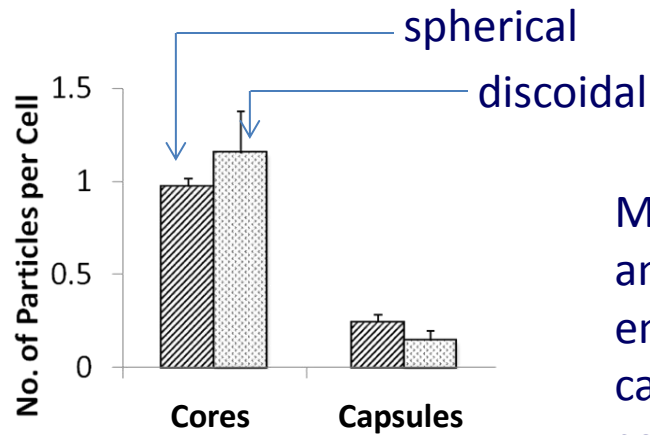
	Diameter/height, L/H		Volume change
	pH=7.4	pH=4	pH=7.4 to 4
Solution pH	pH=7.4	pH=4	pH=7.4 to 4
(PMAA) ₁₅	3.5±0.3	2.8±0.3	24-fold
(PMAA-PVPON) ₅	5.2±0.7	3.0±0.3	2.3

Shape-Dependent Cellular Internalization: Spheres vs Discs

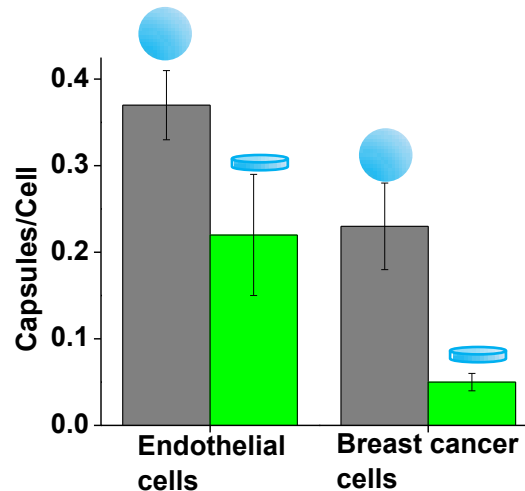
4T1 breast cancer cells
incubated for 24h



Macrophage J774A.1 and endothelial HMVEC cells

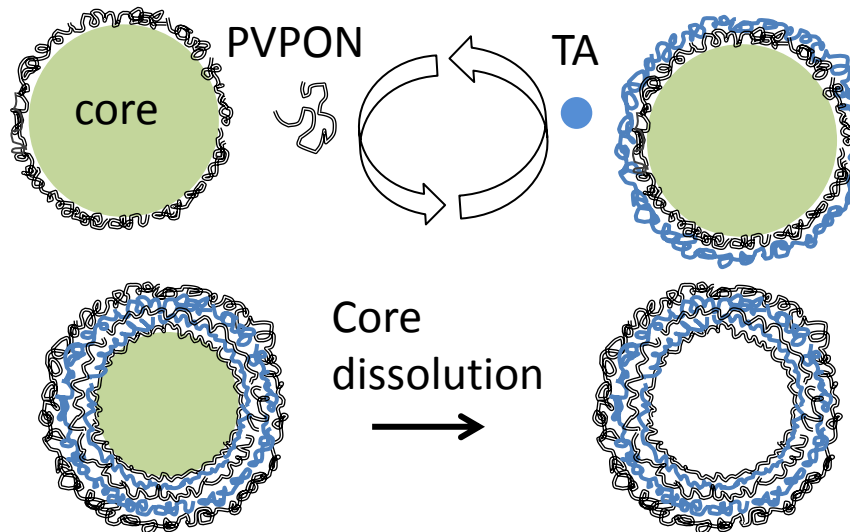
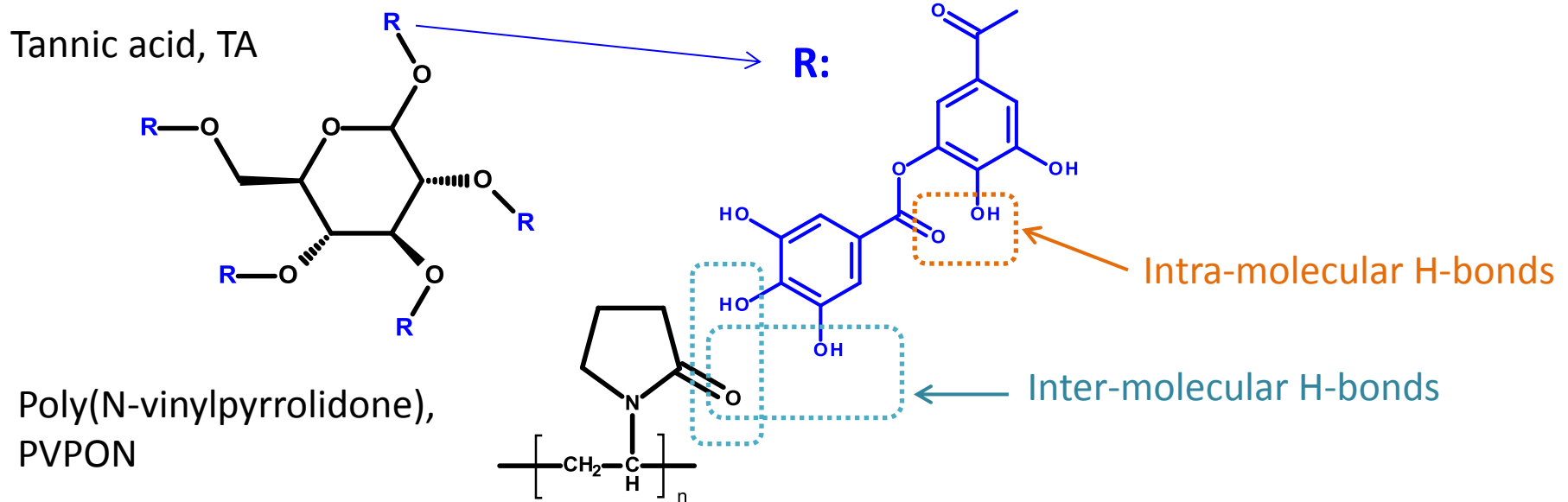


Minimized phagocytosis and interactions with endothelial cells of capsules compared to solid particles -> longer circulation times



Spherical and discoidal capsules decreased 2-fold in size upon internalization by HMVECs and 4T1 cells -> controlled release in the early endosomes of pH=5-6.5.

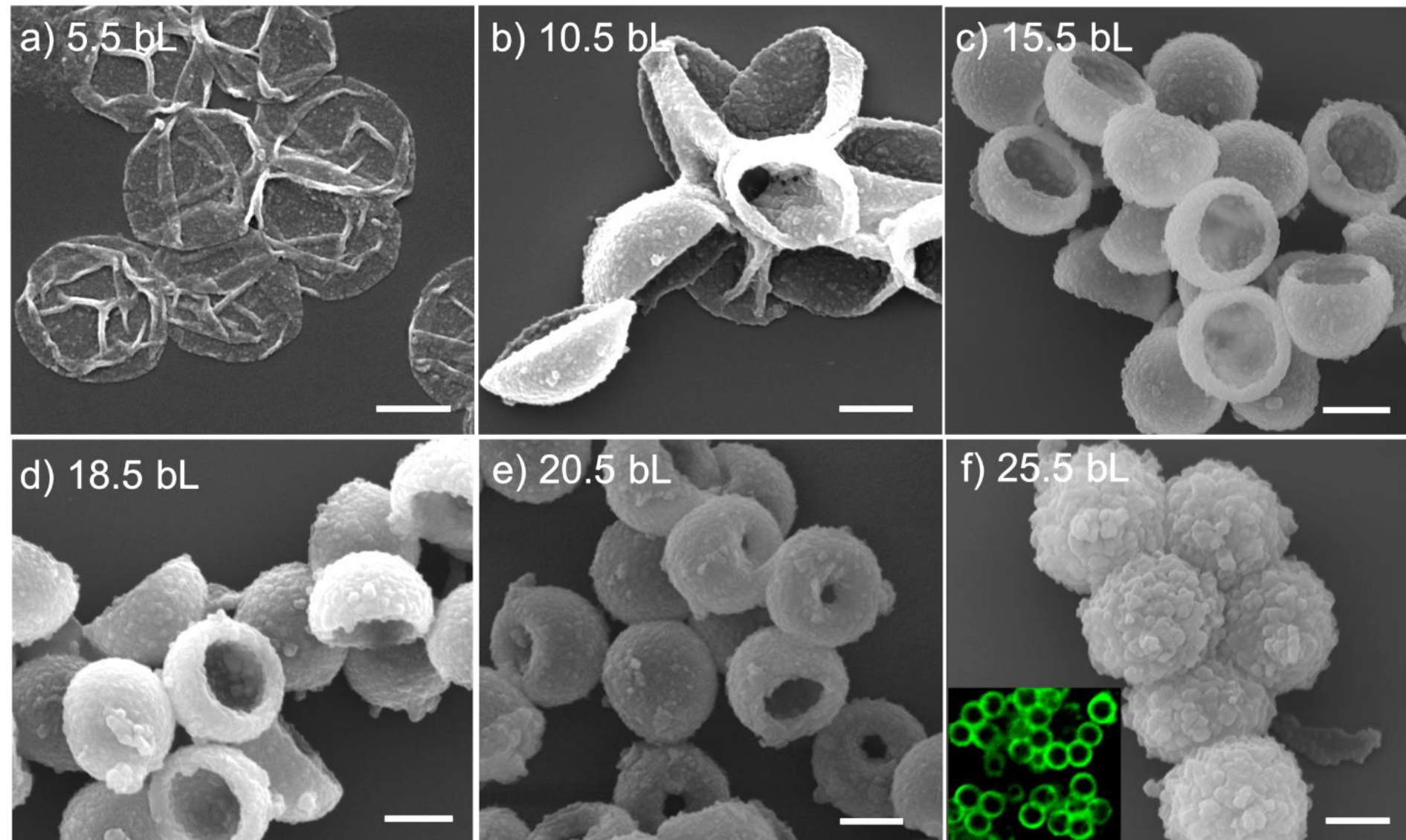
Capsules with Enhanced Structural Stability in Dry State



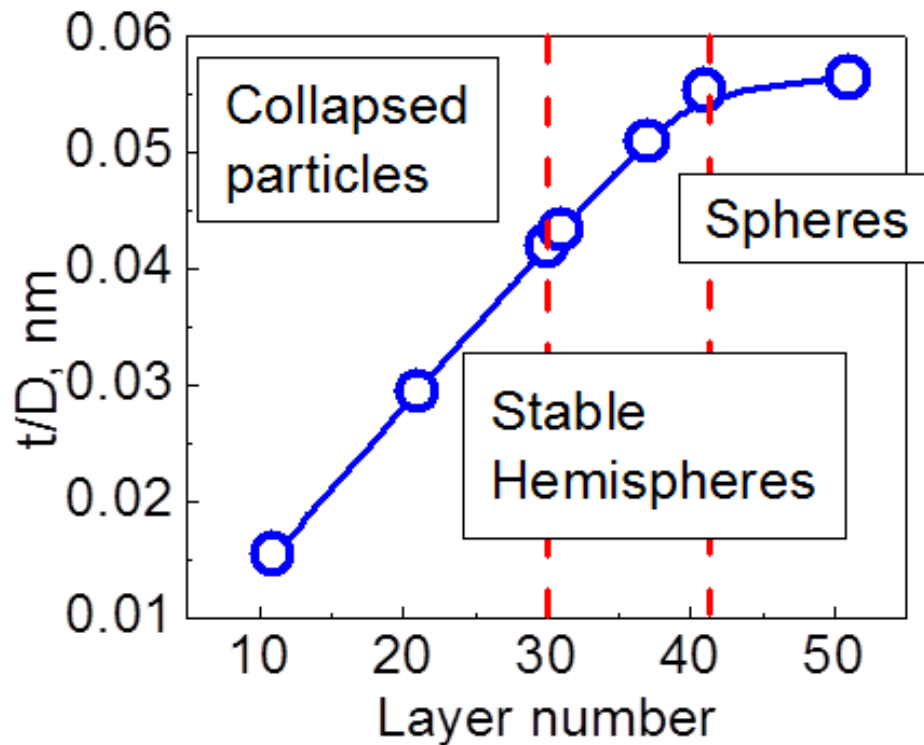
- Stable at physiological pH
- Antioxidant properties
- Immunomodulatory capability
- Antibacterial properties

Effect of Wall Thickness on Particle Shape

Tannic acid (TA) / Poly(N-vinylpyrrolidone) (PVPON)



Effect of Wall Thickness on (PVPON/TA)_n Shape



Hemispheres are produced when

- $15 < n < 20$
- the ratio of the shell thickness (t) to the particle diameter (D) lies in the region from $t/D > 0.041$ to $t/D < 0.055$

Hollow particles maintain their three-dimensional shapes in the dry if $n \geq 25.5$

$$k = \frac{4t^2 E}{r \sqrt{3(1-\nu)^2}}$$

The LbL shell deformation depends on the shell stiffness, k , which depends on:

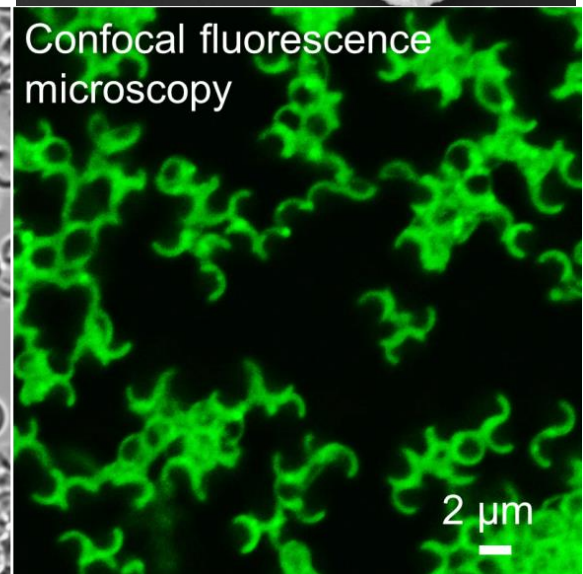
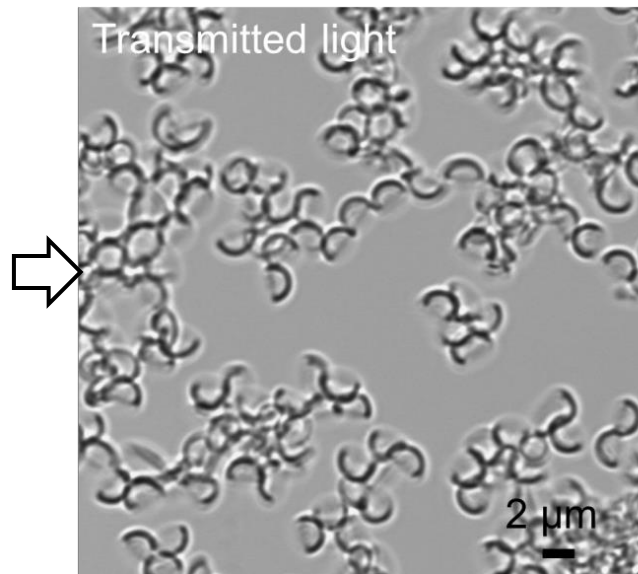
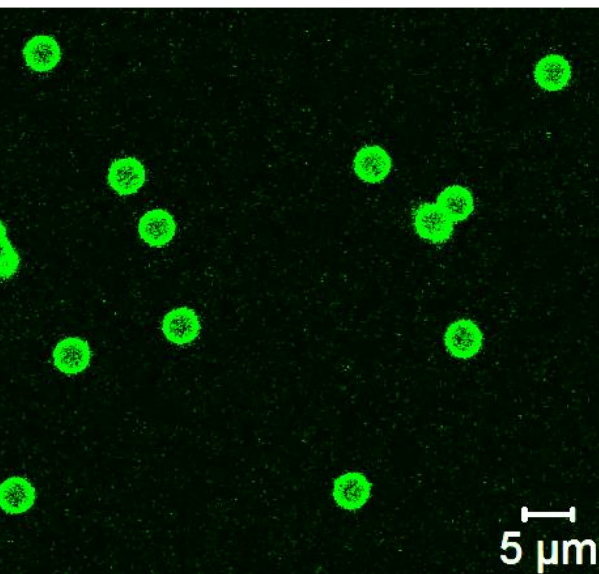
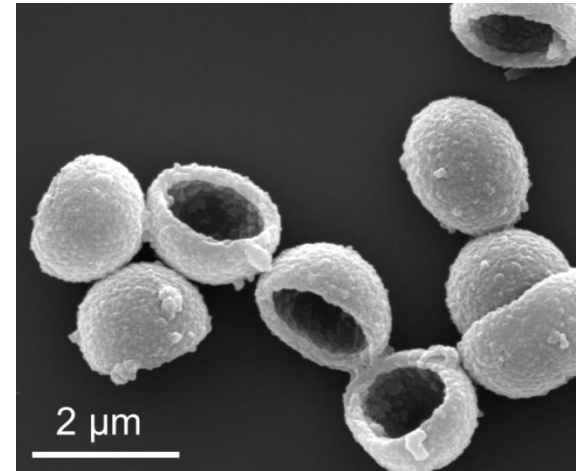
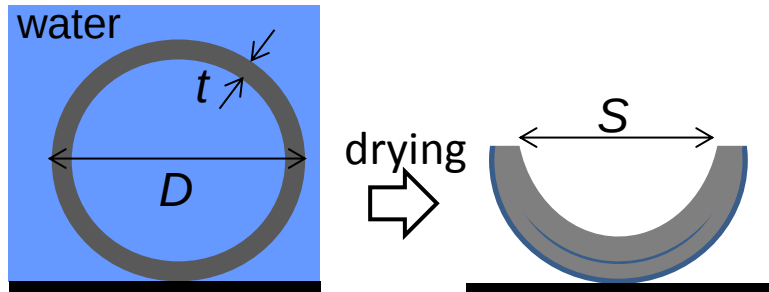
- Young's modulus, $E = 600$ MPa in dry state (vs 1 GPa for (PMAA/PVPON))
- Shell thickness, t
- Capsule radius, r
- Poisson ratio, ν

Gao et al. Eur. Phys. J. E 2001, 5, 21

Köhler et al. Macromolecules, 2004, 37, 9546

Elsner et al. Soft Matter, 2006, 2, 966

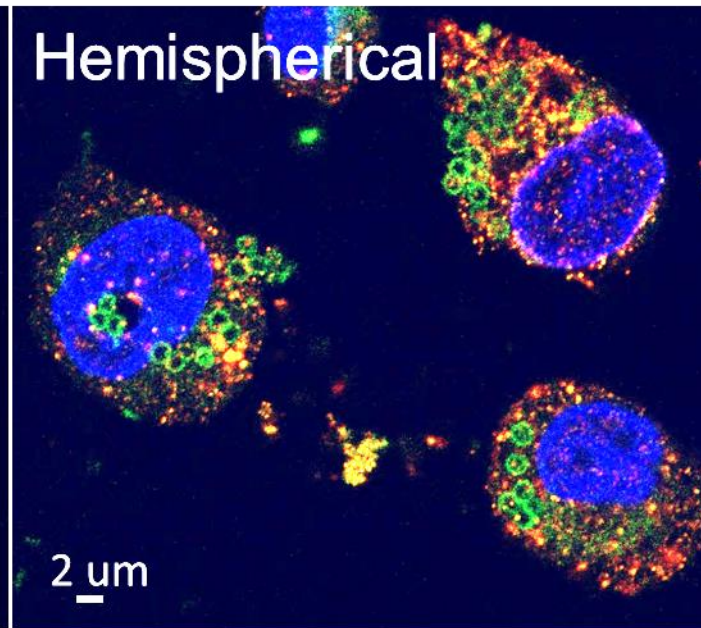
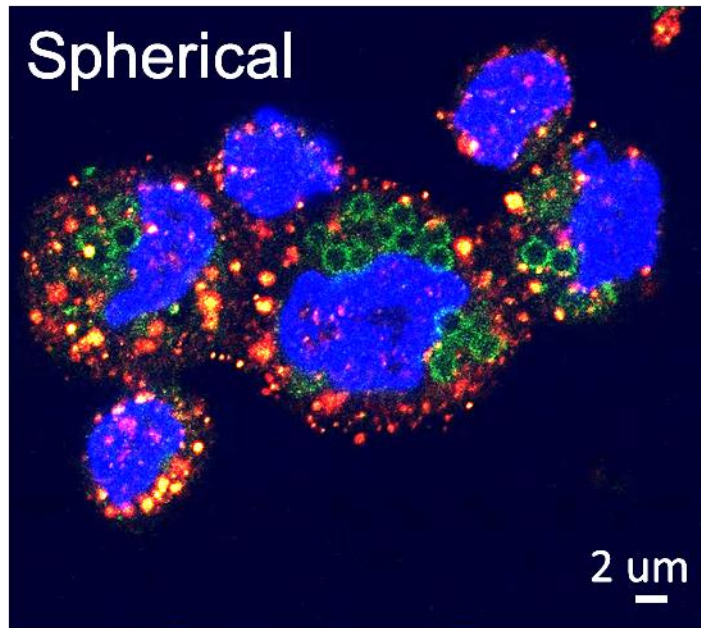
Formation of Hemispheres



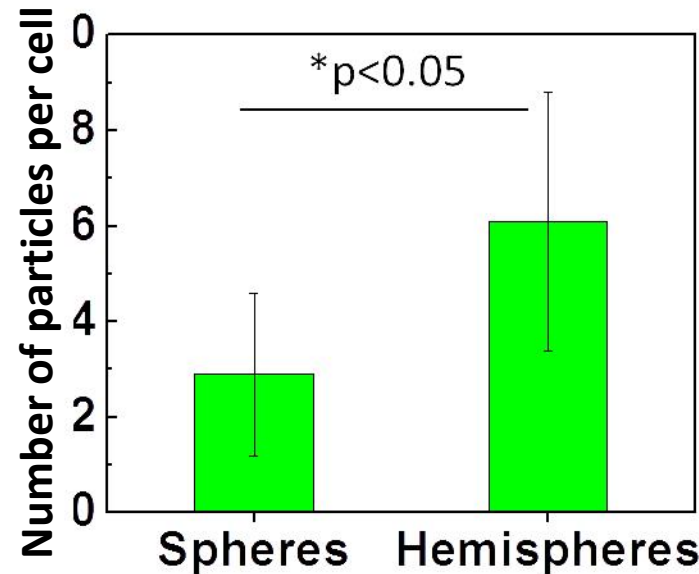
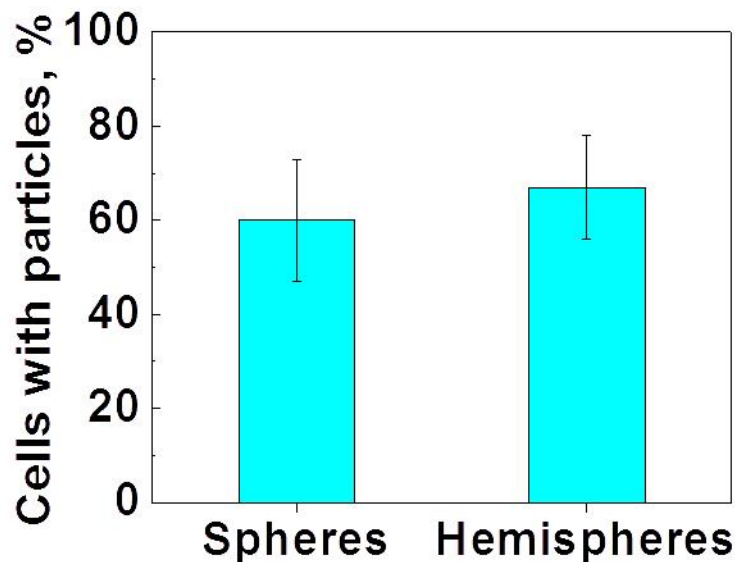
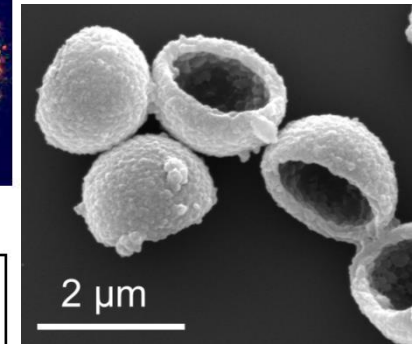
(PVPON/TA)_{15.5}
hollow multilayer
capsules in DI water

After being dried

Shape-Dependent Cellular Internalization: Spheres vs Hemispheres

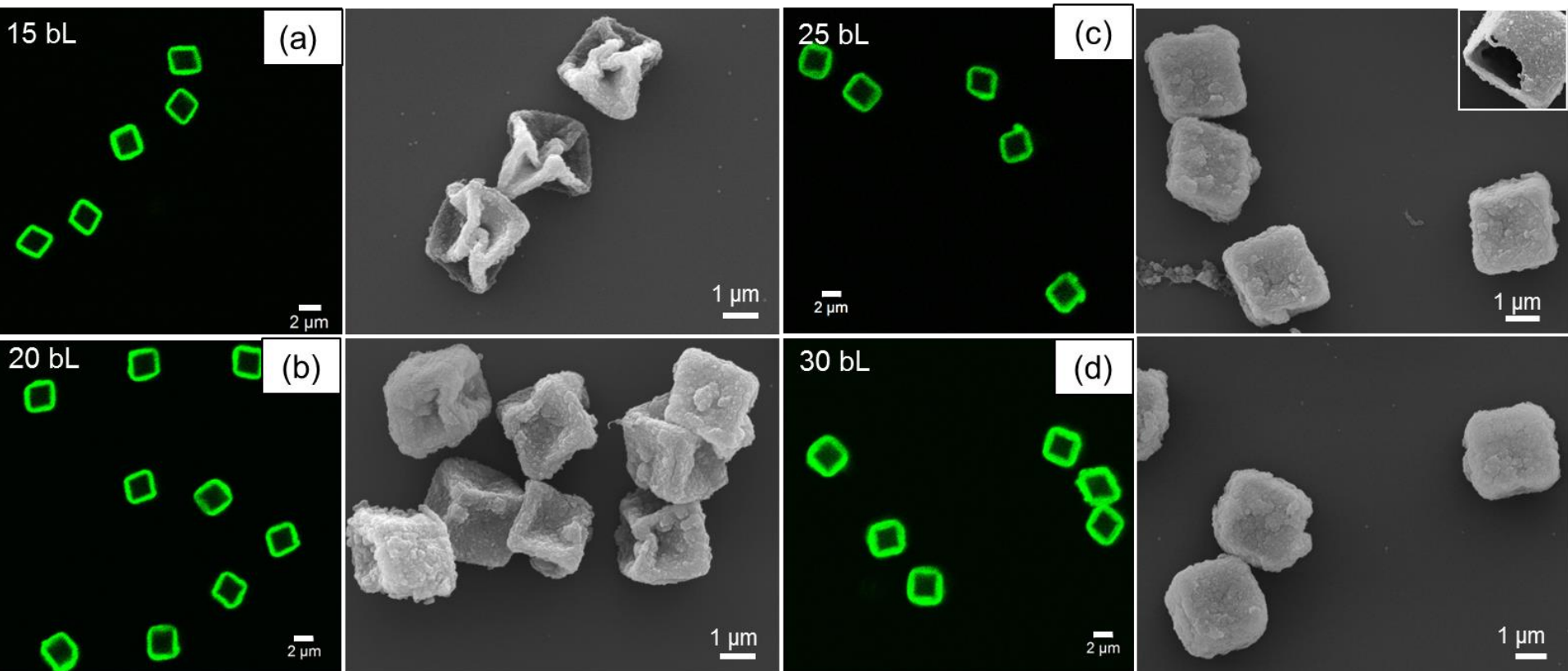


CLSM of THP-1 macrophage cells with internalized (PVPON/TA)_{15.5}



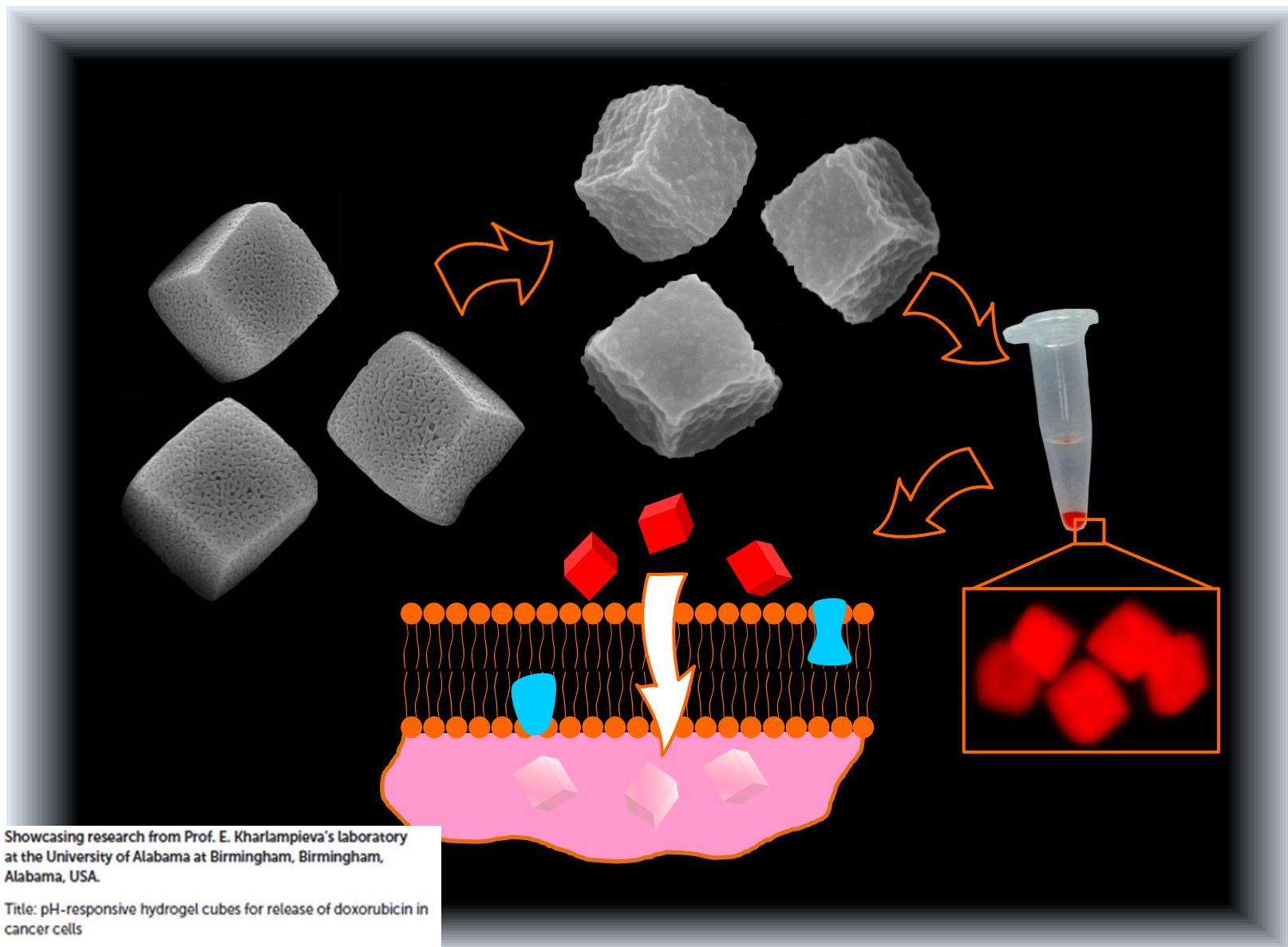
Uptake of the hemispheres per cell is two times more efficient

Effect of Wall Thickness on Particle Shape

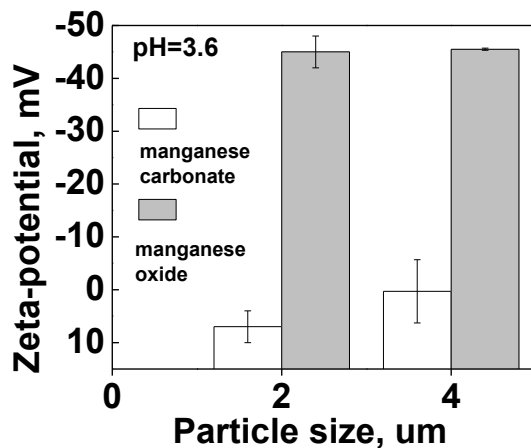
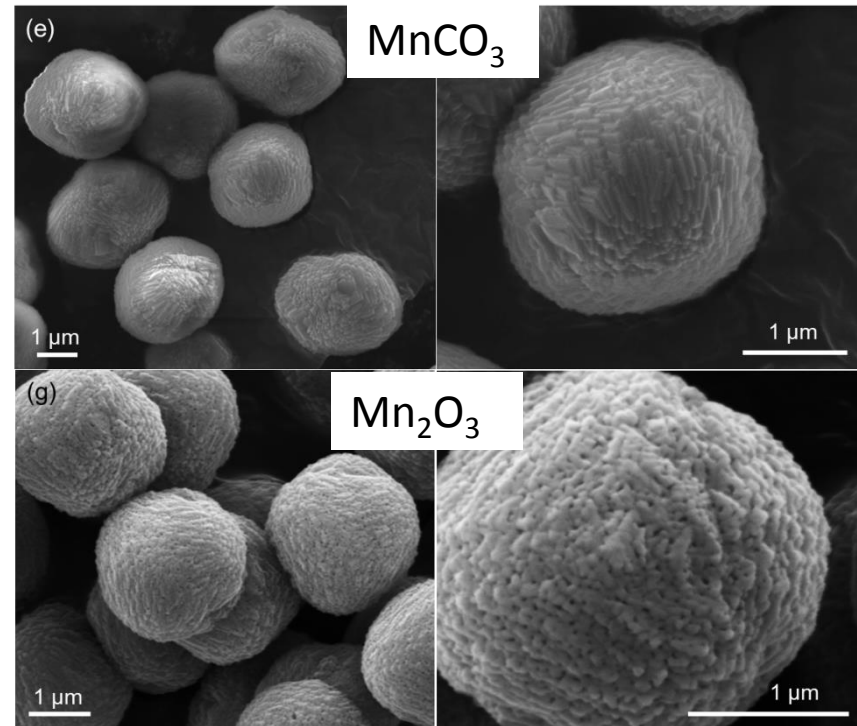
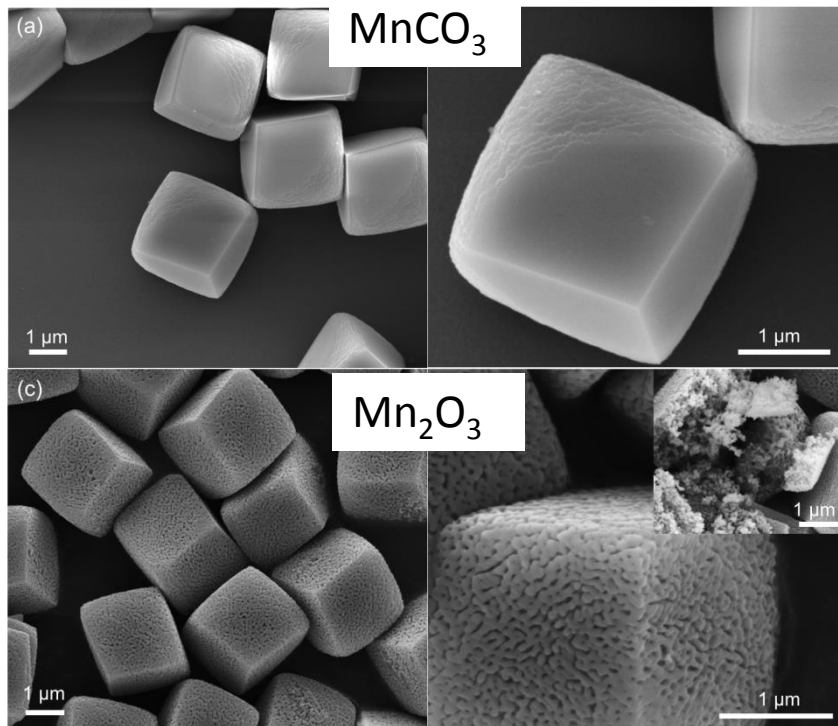


Hollow cubes at $n \geq 25$ bL

Hydrogel Cubes for Drug Storage and pH-Triggered Release



Shaped Porous Templates



Highly negative net surface charge

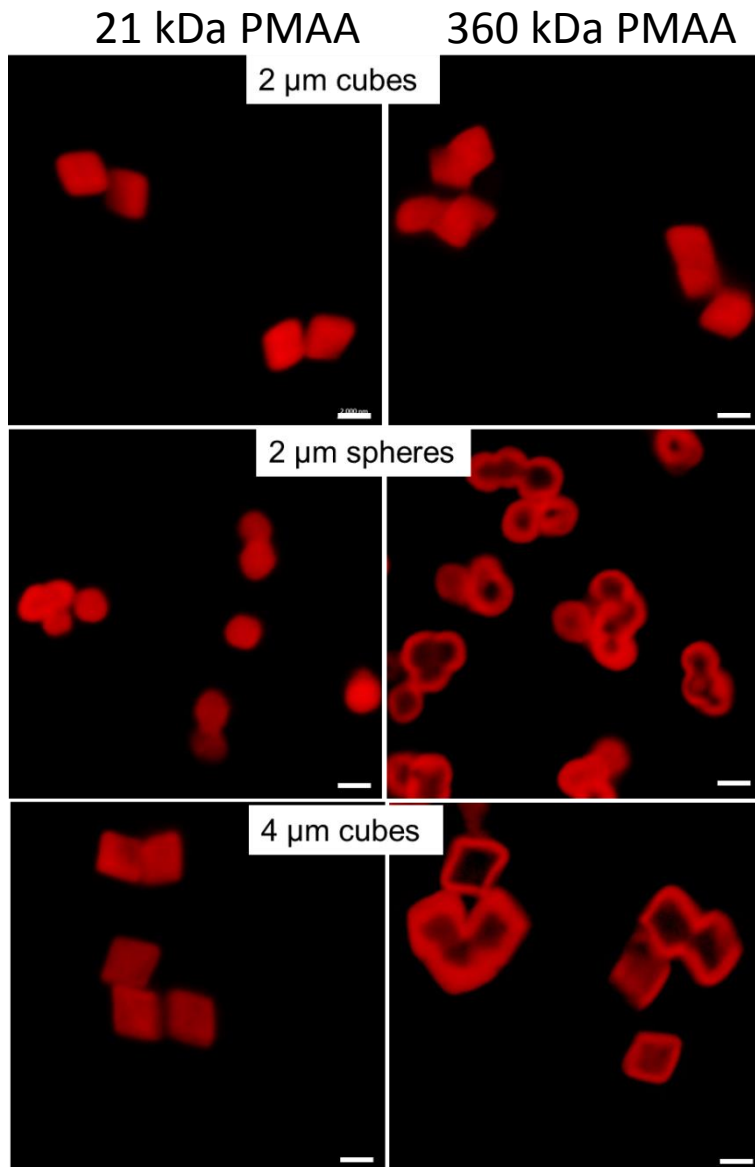
Particles	Average Pore Size ^a (nm)	Surface Area ^b ($\text{m}^2 \text{g}^{-1}$)	Pore Volume ^c ($\text{cm}^3 \text{g}^{-1}$)
2 μm cubic	44	17.5±0.4	0.192±0.002
4 μm cubic	40	17.5±0.5	0.175±0.004
2 μm spherical	43	19.3±0.5	0.205±0.005

Large average pore size

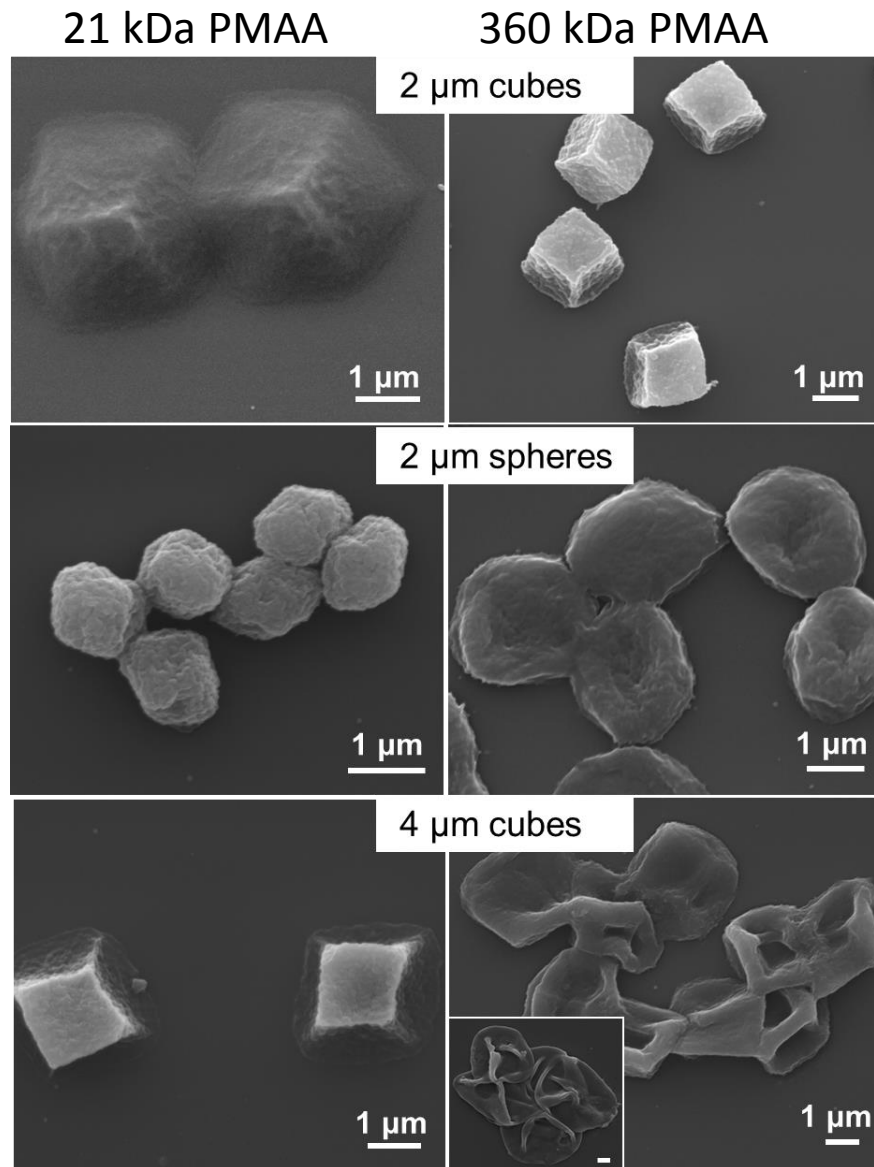
With Dr. Lungu, UAB

Hydrogel Particle Structure: Dry vs in Solution

CLSM

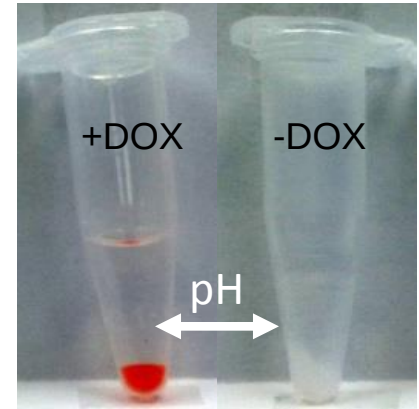
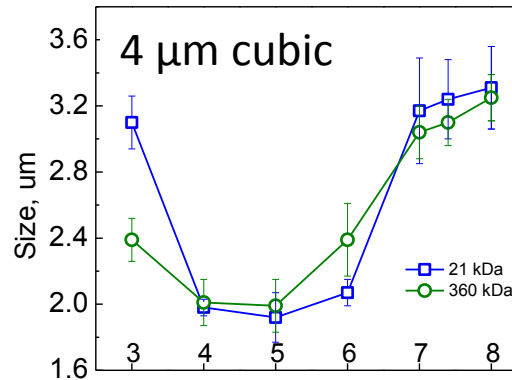
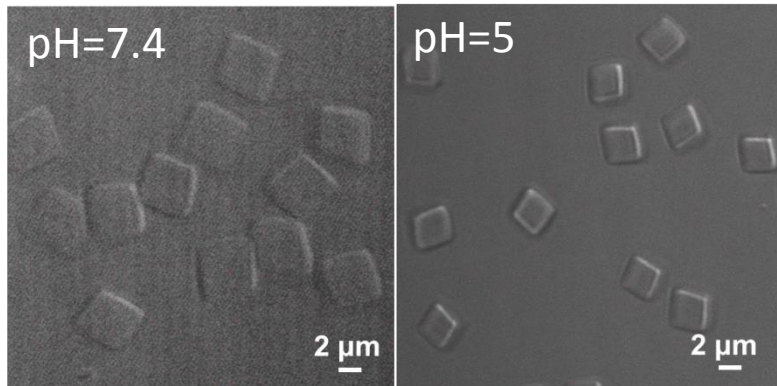


SEM

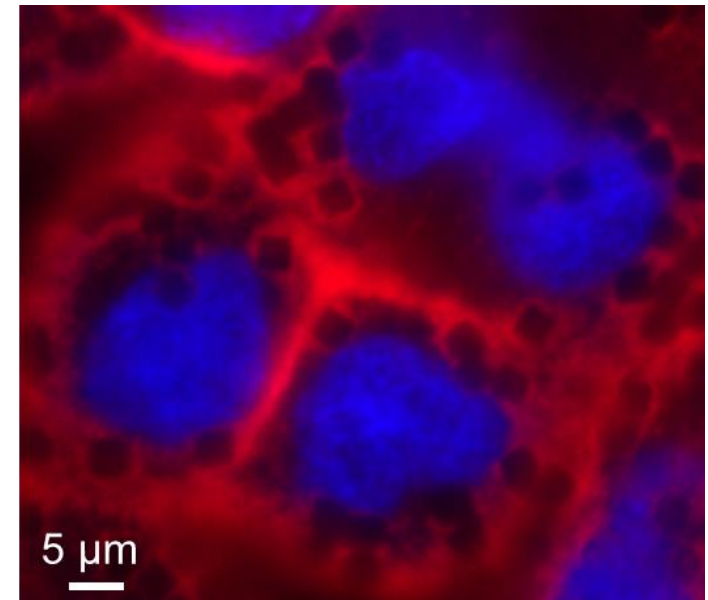
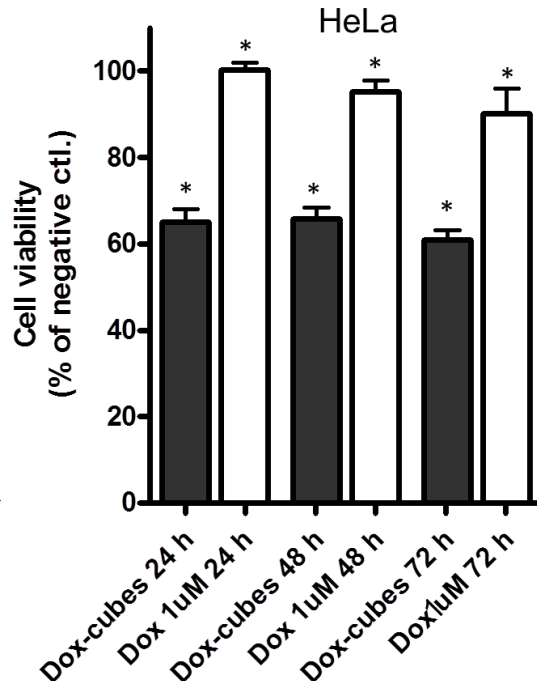
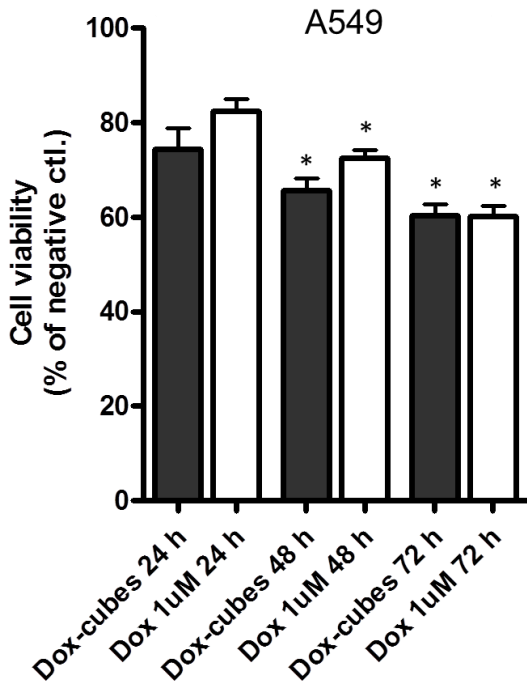


pH-Responsive Cubic Hydrogels for DOX delivery

Doxorubicin loaded at pH=7.2 released at pH<5



Viability of A549 and HeLa cancer cells decreased to 60% after 72 h of incubation with 2-μm DOX-loaded hydrogel cubes



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Thank You!