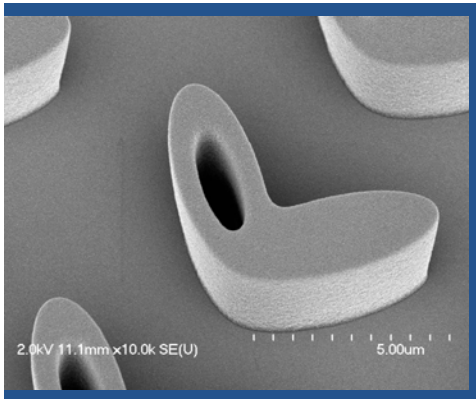

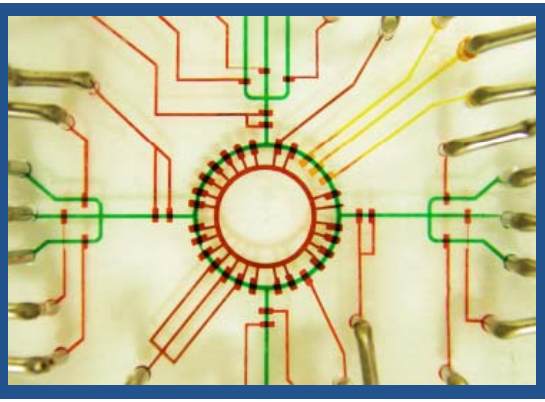
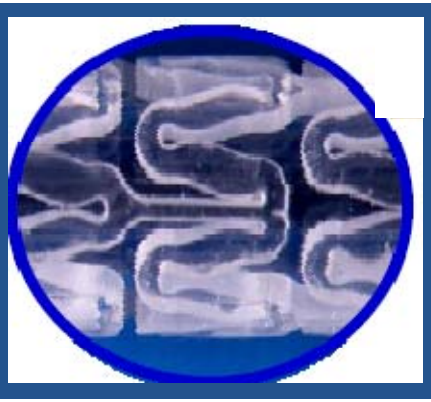
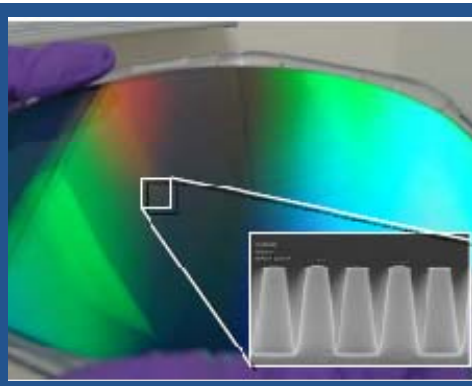

Design of Better Medicines and Vaccines Using Nanotechnology

Joseph M. DeSimone

Departments of Chemistry and Pharmacology
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Lineberger Comprehensive Cancer Center
Institute for Advanced Materials, Nanoscience & Technology
University of North Carolina at Chapel Hill

Department of Chemical & Biomolecular Engineering
North Carolina State University

Founder, Liquidia Technologies, Inc.

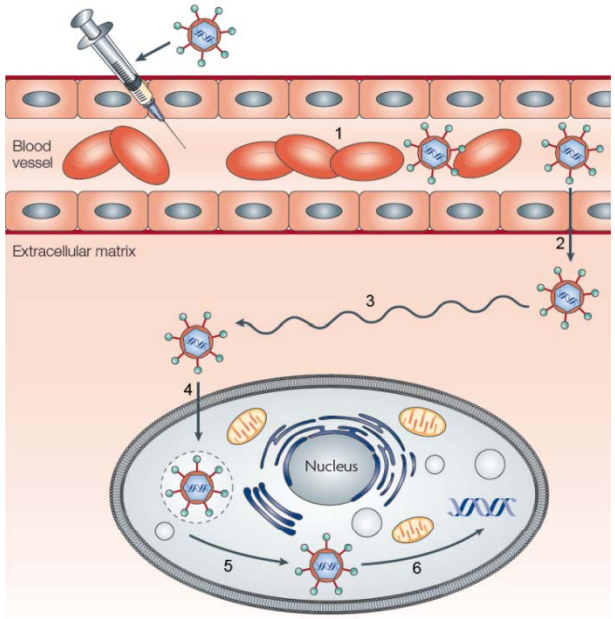
Financial Disclosure

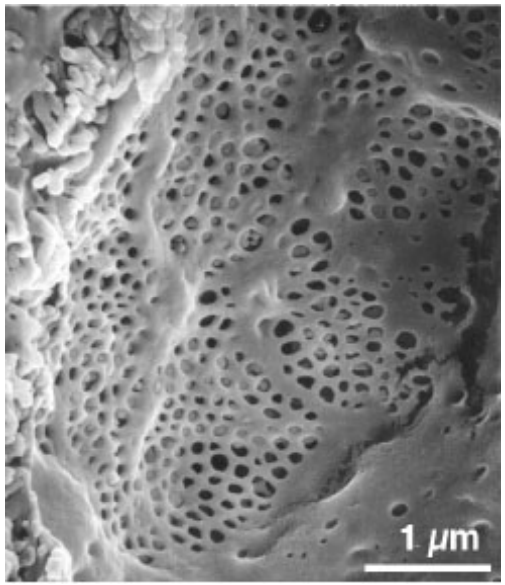
- Joseph DeSimone declares he has a financial relationship with Liquidia Technologies as a result of being a Founder and a member of the Board of Directors

Navigating Biological Barriers in Drug Delivery

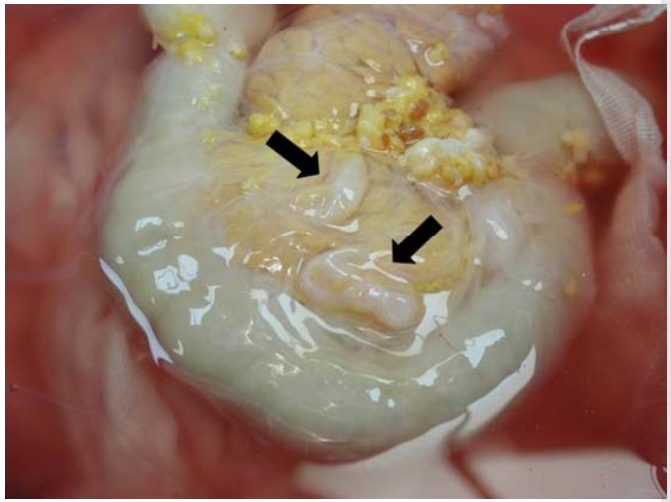
Barriers to systemic delivery of nanoparticles:

1. Filtration, degradation, and phagocytosis in bloodstream
2. Transport across vascular endothelium
3. Extracellular matrix diffusion
4. Cellular targeting and uptake
5. Endosomal escape
6. Intracellular trafficking to target

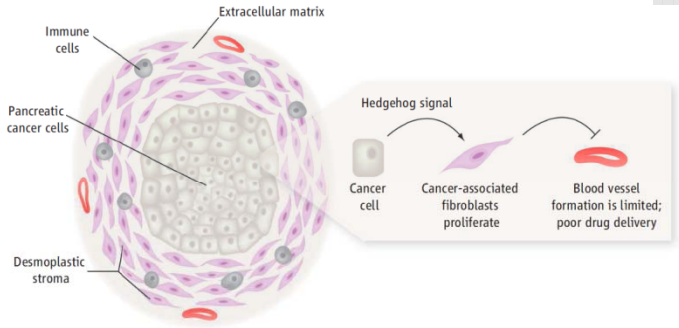




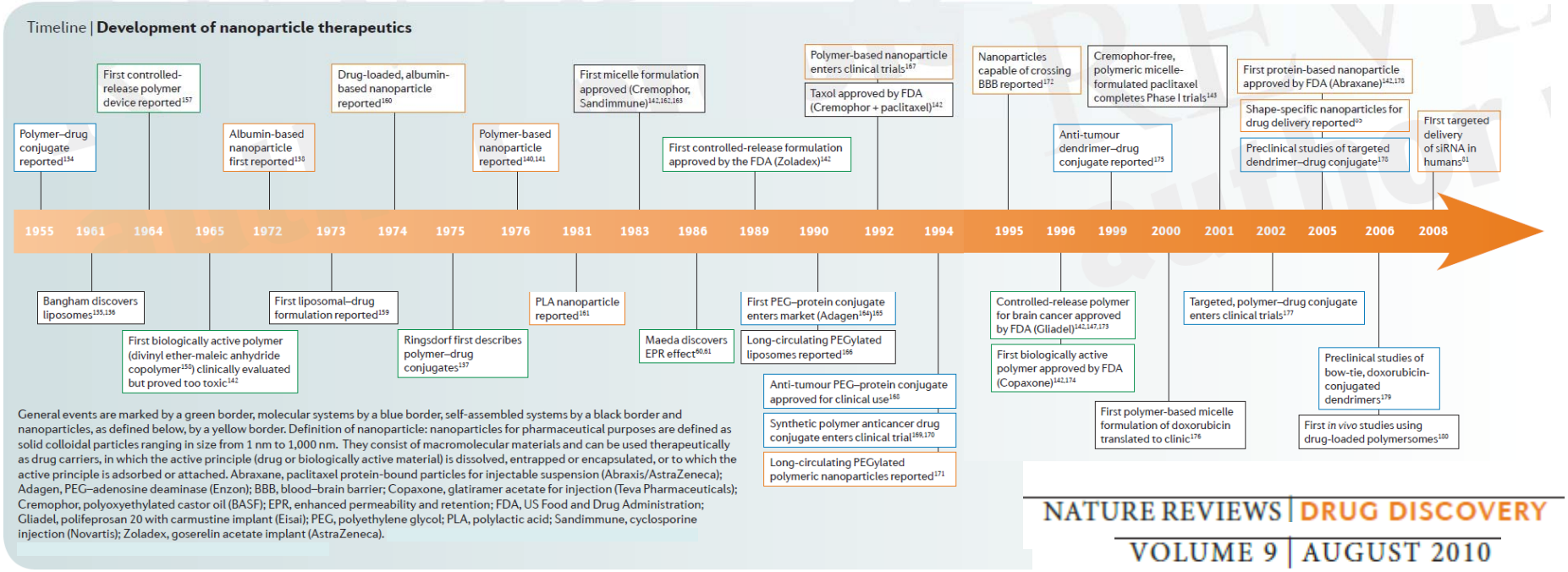
150 nm Fenestrations in Liver



Inhibition of Hedgehog Signaling Enhances Delivery of Chemotherapy in a Mouse Model of Pancreatic Cancer
Kenneth P. Olive, *et al.*
Science **324**, 1457 (2009);
DOI: 10.1126/science.1171362



Navigating Biological Barriers in Drug Delivery

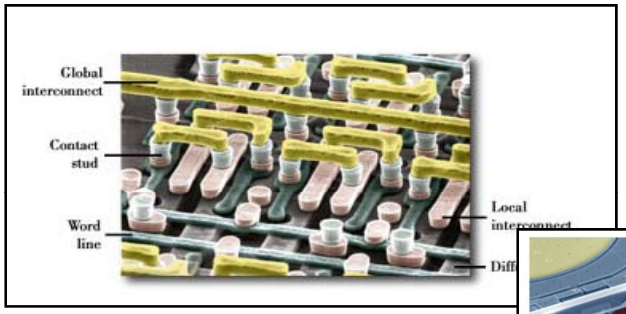


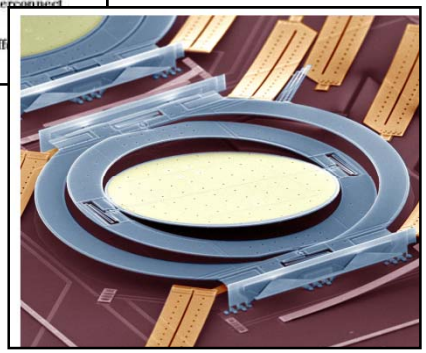
- Researchers use an extremely wide range of various fabrication techniques in order to produce nano-structures
  - → All are based on molecular conjugates or self-assembled structures

Nanofabrication

- Researchers use an extremely wide range of various fabrication techniques in order to produce nano-structures.

- Nanofabrication techniques:



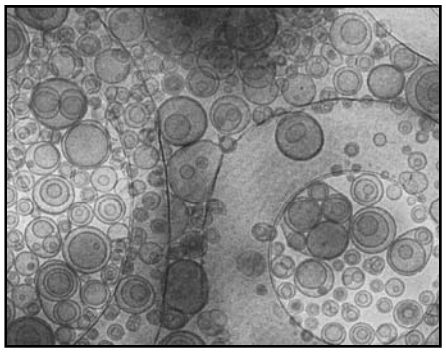


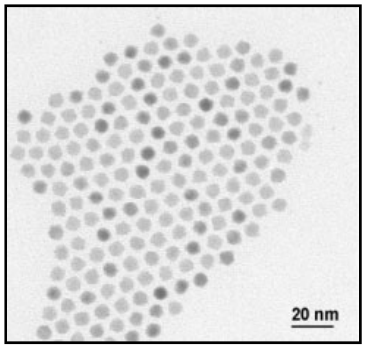
→ **Top-down**

- Remove or add material to a surface
  - ❖ Lithography, scanning probe methods

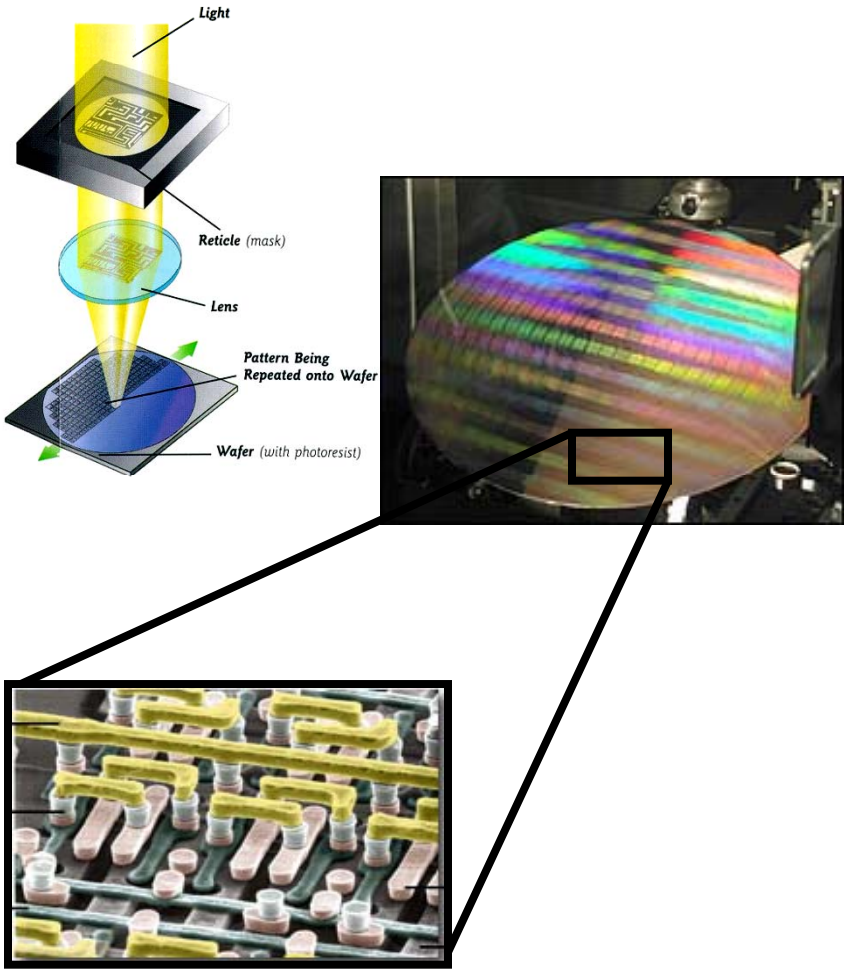
→ **Bottom-up**

- Construct nano-structures from molecules or atoms





Micro- and Nano-fabrication in the Semiconductor Industry



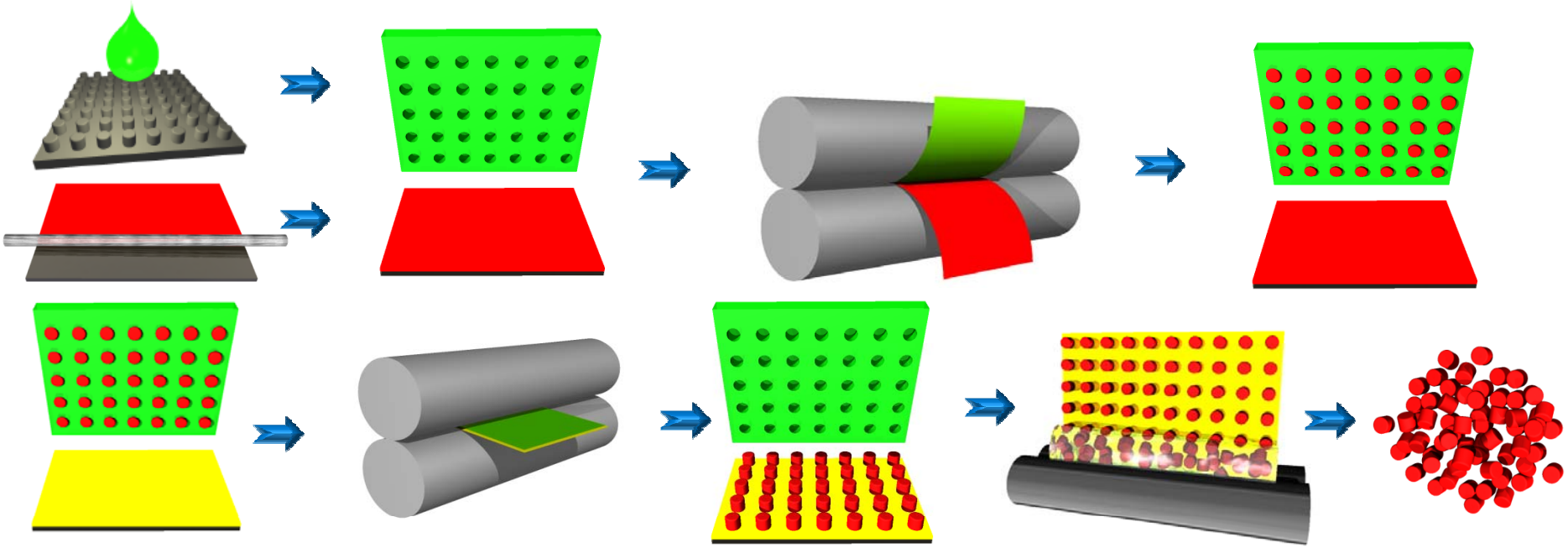
Year	Processor	Number of Transistors	Minimum Feature Size
1971	4004	2,300	10 micron
1972	8008	3,500	10 micron
1974	8080	6,000	6 micron
1978	8086	29,000	3 micron
1982	80286	134,000	1.5 micron
1985	80386	275,000	1.5 micron
1989	Intel 486	1.2 million	1.0 micron
1993	Pentium	3.1 million	800 nm
1997	Pentium II	7.5 million	350 nm
1999 (Feb)	Pentium III	9.5 million	250 nm
1999 (Oct)	Pentium III	28 million	180 nm
2000	Pentium IV	42 million	130 nm
2004	Itanium 2	592 million	90 nm
2007	Dual-Core Xeon	1.3 billion	65 nm

Translational Research Vision

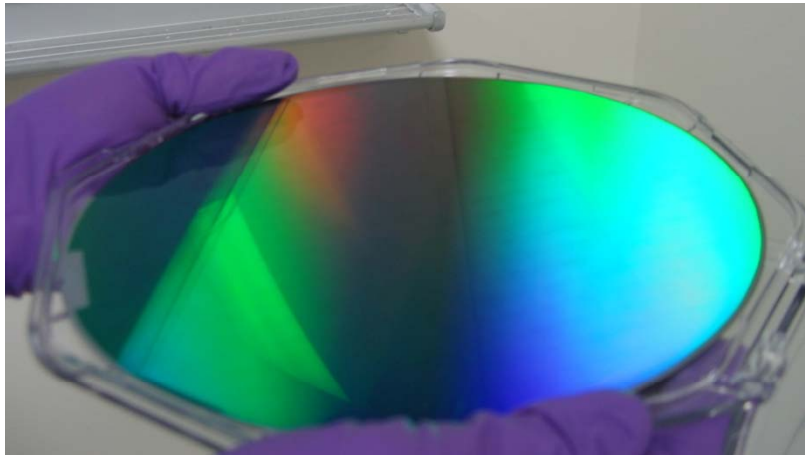
Adapt emerging techniques from the microelectronics industry to generate *and harvest* extremely versatile shape-specific, *organic* carriers for application in nano-medicine

Semiconductor Clean Room

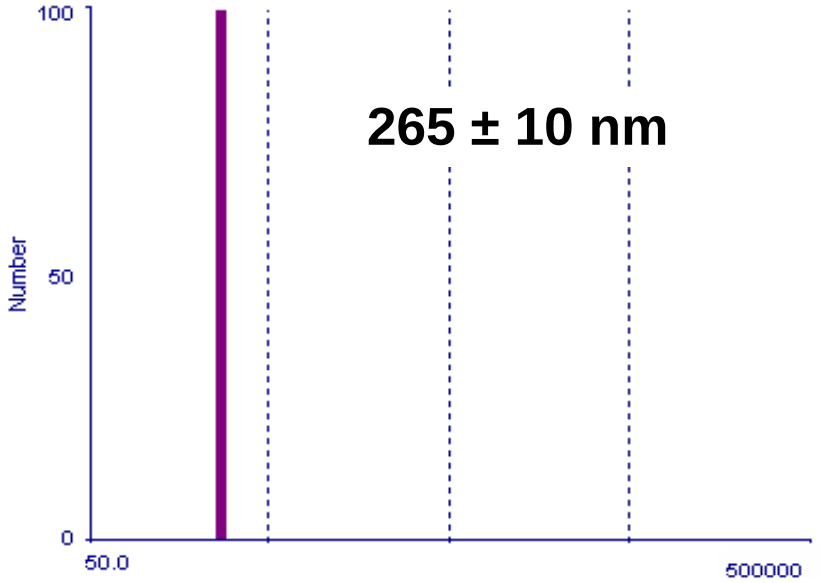
Particle Replication in Non-wetting Templates (PRINT® Platform)

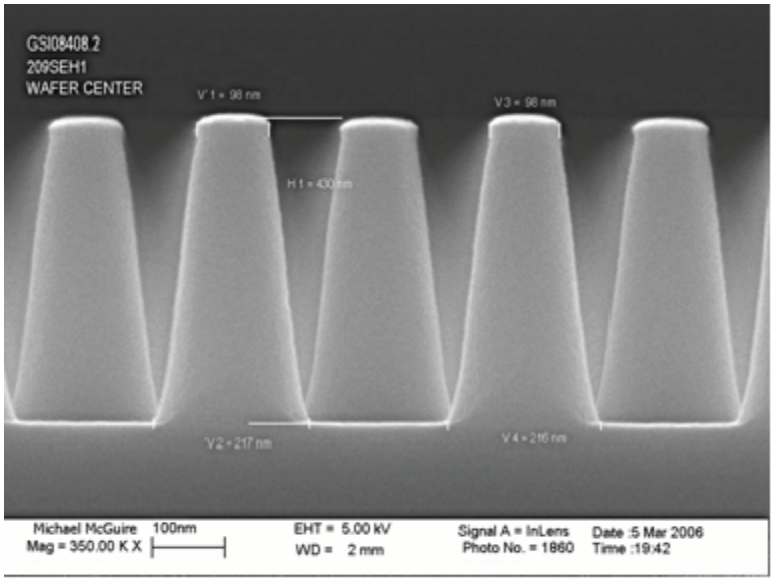


"Direct Fabrication and Harvesting of Monodisperse, Shape Specific Nano-Biomaterials"; Rolland, J. P.; Maynor, B. W.; Euliss, L. E.; Exner, A. E.; Denison, G. M.; DeSimone, J. M *J. Am. Chem. Soc.* **2005**, 127, 10096



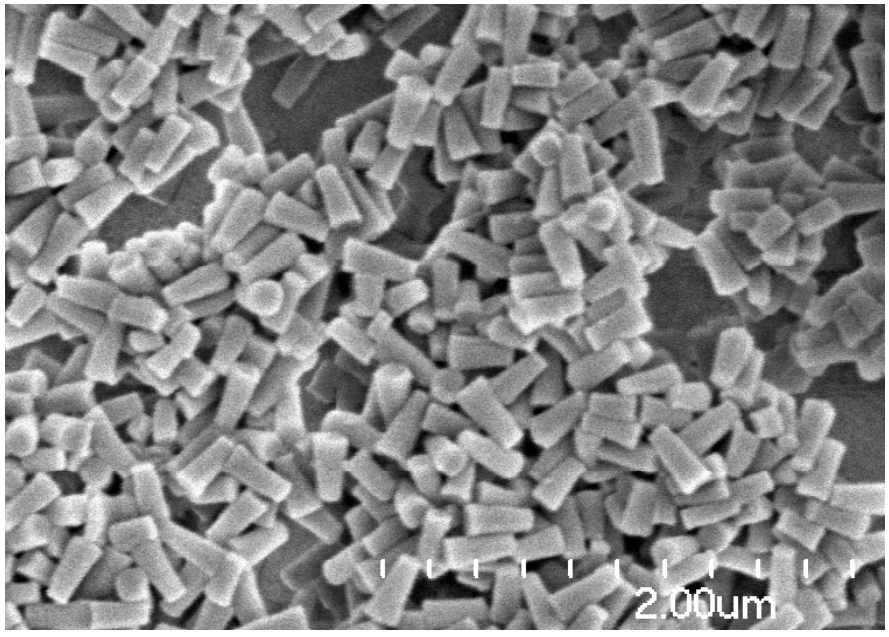
8 inch Masters:
< 200 nm PRINT Particles

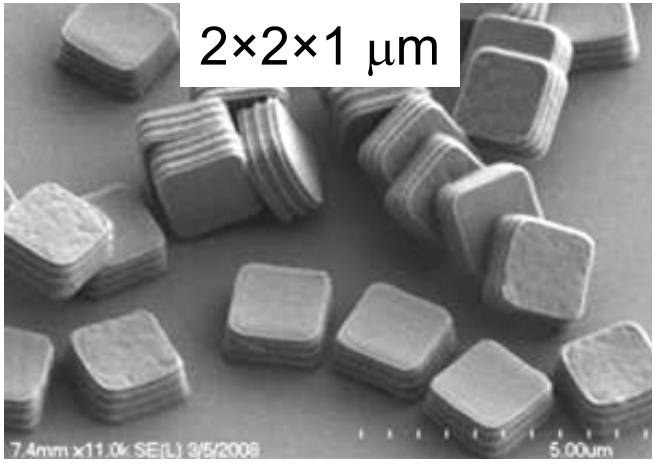


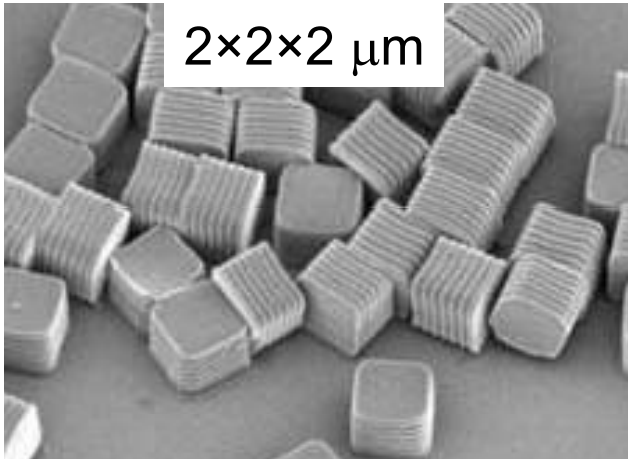


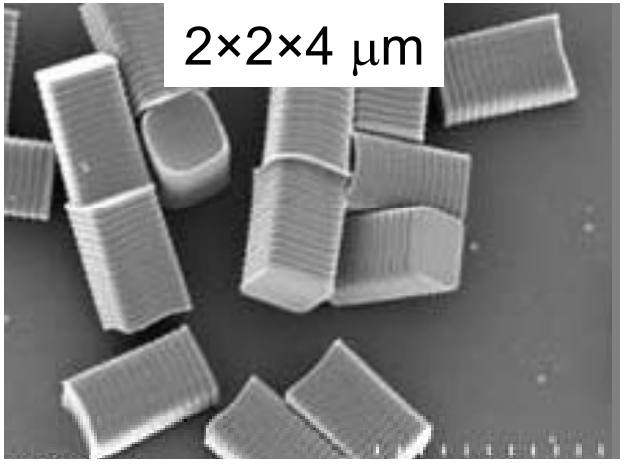
400 nm height

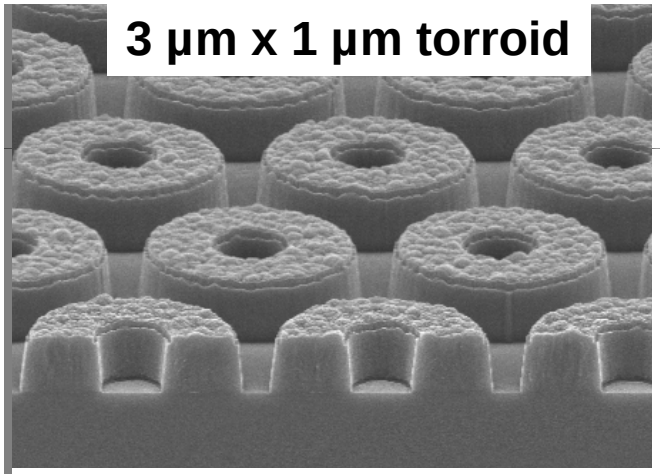
Sterically Stabilized PEG Particles

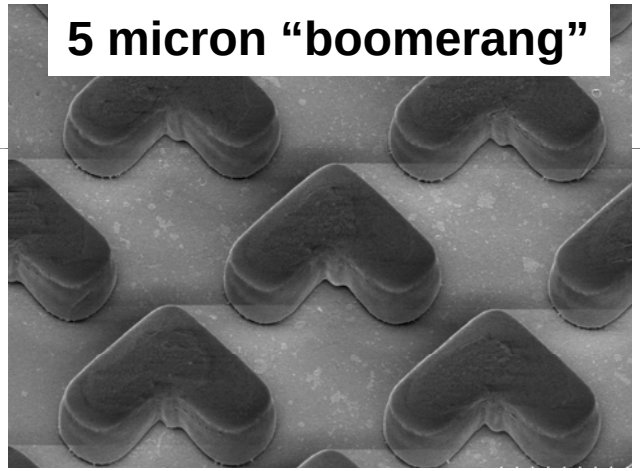


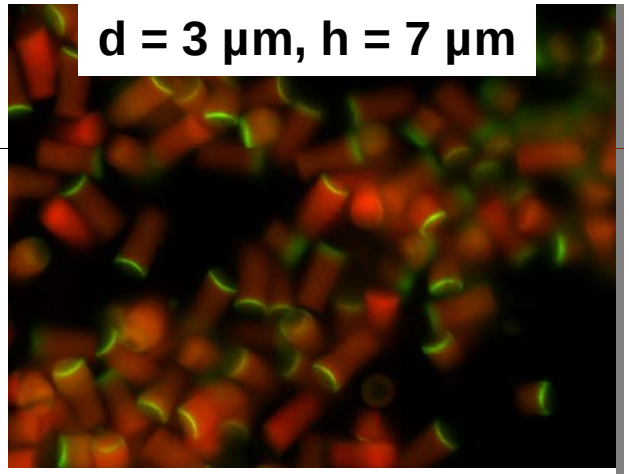

2×2×1 μm
7.4mm x11.0k SE(L) 3/5/2008
5.00um

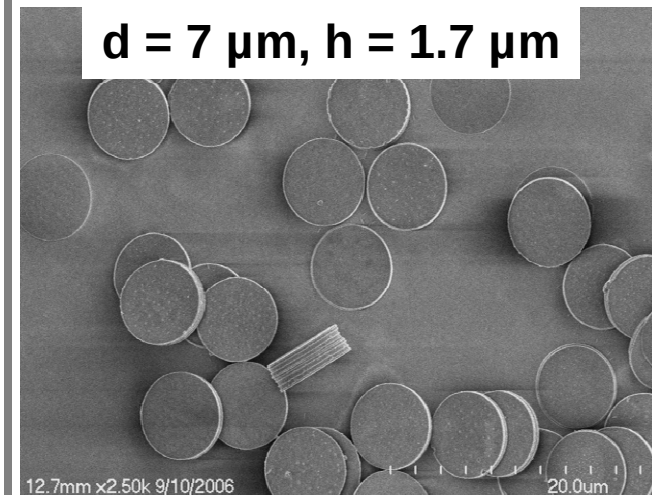

2×2×2 μm

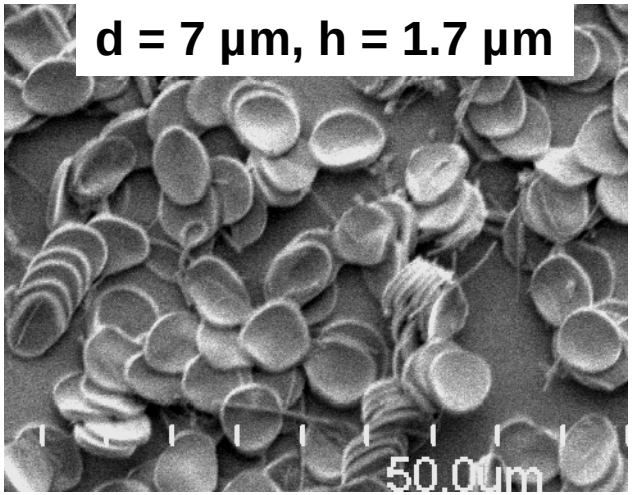

2×2×4 μm

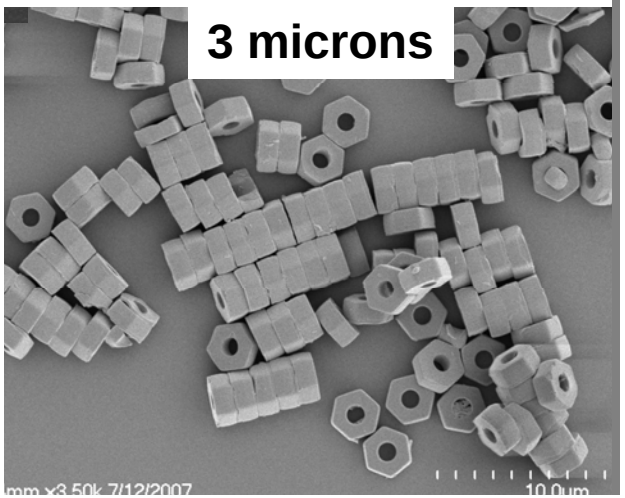

3 μm x 1 μm torroid


5 micron “boomerang”


d = 3 μm, h = 7 μm


d = 7 μm, h = 1.7 μm
12.7mm x2.50k 9/10/2006
20.0um


d = 7 μm, h = 1.7 μm
50.0um

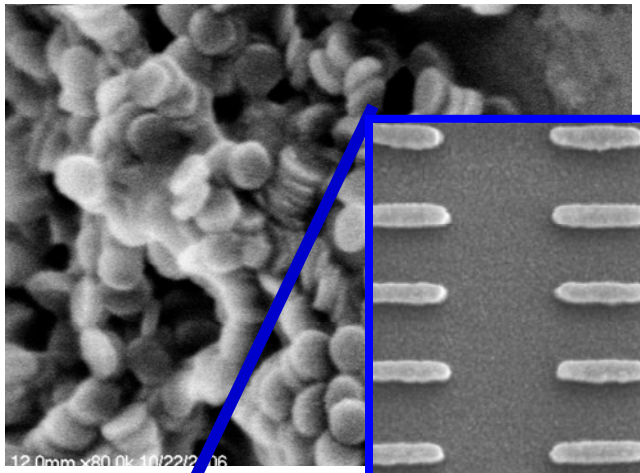

3 microns
10.0um

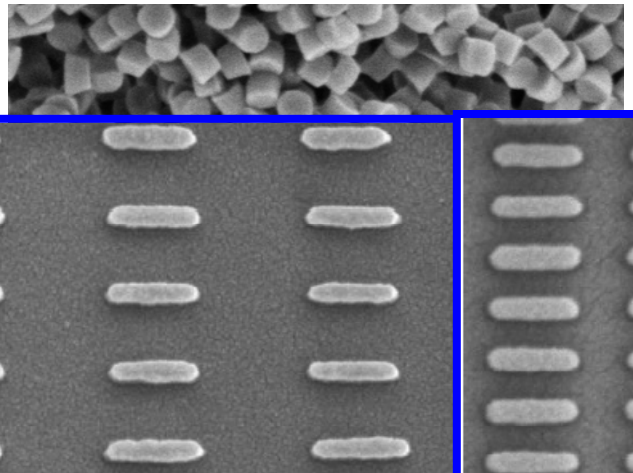
Unique PRINT Particle Shapes for Tailored PK/PD

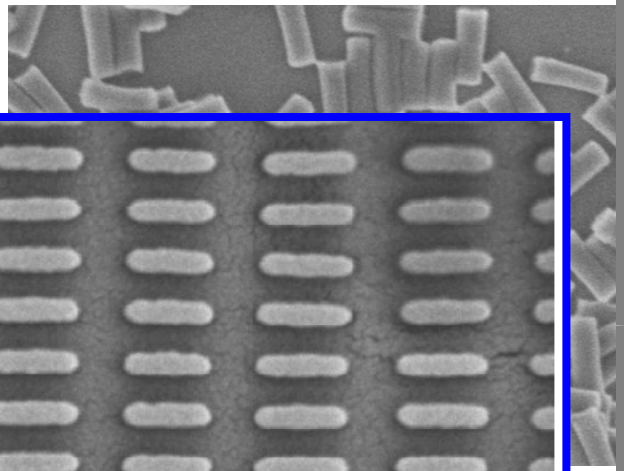
D = 113 nm, L = 36 nm

D = 200 nm, L = 200 nm

D = 95 nm, L = 280 nm

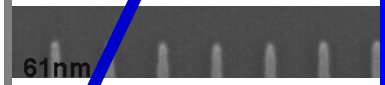






80x320 nm Low Density

80x320 nm High Density



New Masters

D = 80 nm, L = 90 nm

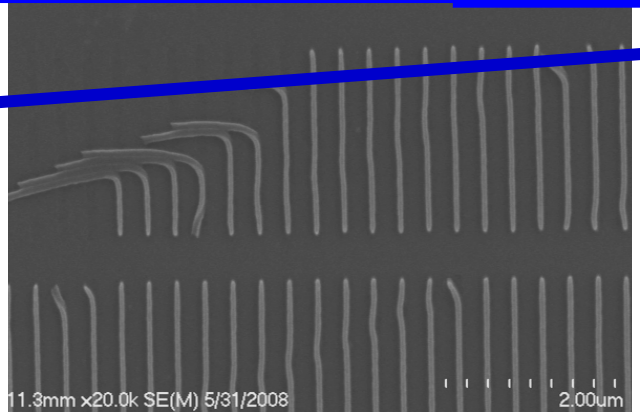
D = 80 nm, L = 320 nm

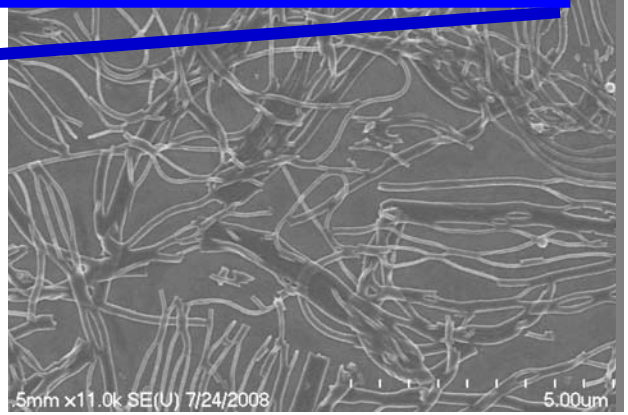
D = 80 nm, L = 500 nm

D = 80 nm, L = 2000 nm

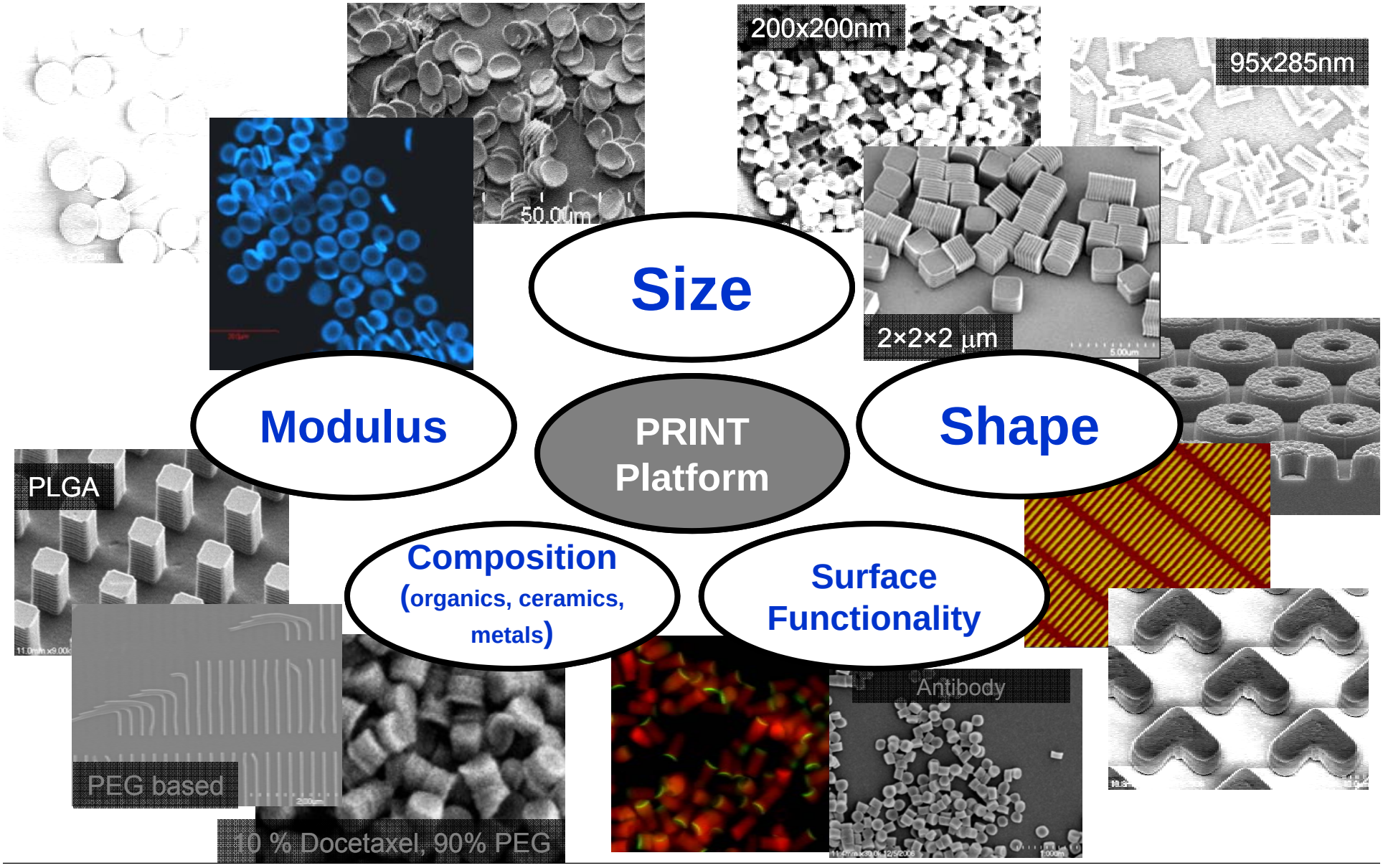
D = 80 nm, L = 3000 nm

D = 80 nm, L = 5000 nm

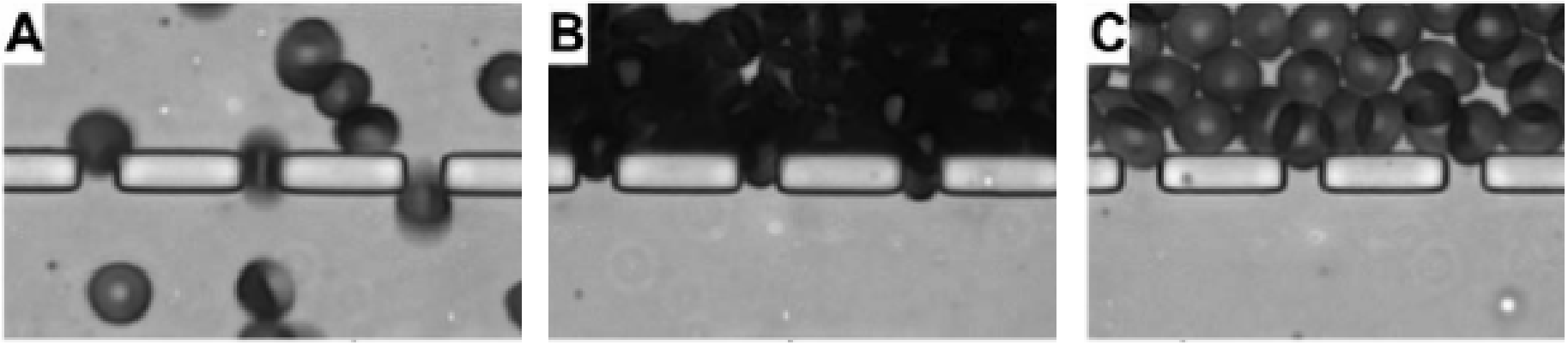




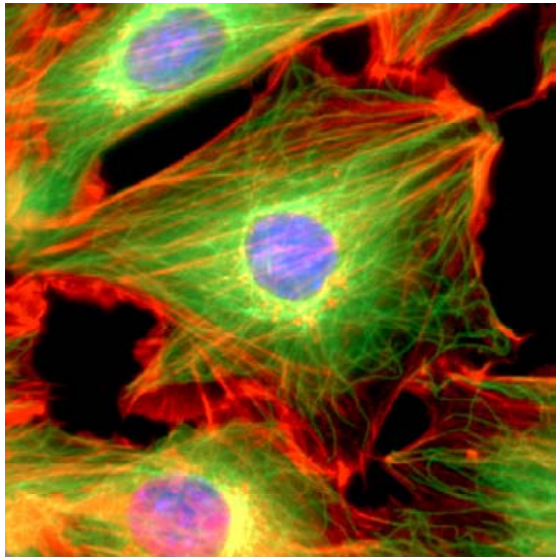
PRINT Platform



Biomimetic Design: Mechano-biology of RBCs and Metatatic Cancer Cells



Shevkoplyas, et.al. *Lab Chip* **2006**, *6*, 914

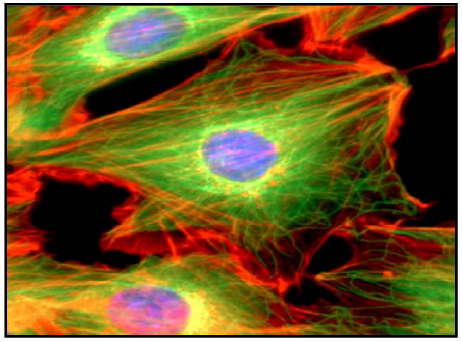


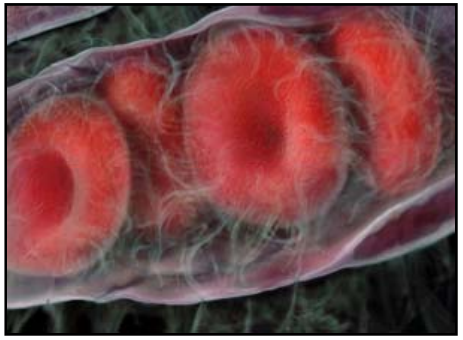
- **"Cancer cells are an order of magnitude more deformable (lower in effective stiffness) than normal cells, due to reorganization of the cytoskeleton"**
- **"Cancerous MCF-7 cells deform more than noncancerous MCF-10 cells. Deformability of metastatic modMCF-7 cells significantly higher than that of MCF-7."**
- **"An increase in cell deformability directly correlates with the progression of the transformed phenotype from a nontumorigenic cell into a tumorigenic, metastatic cell"**

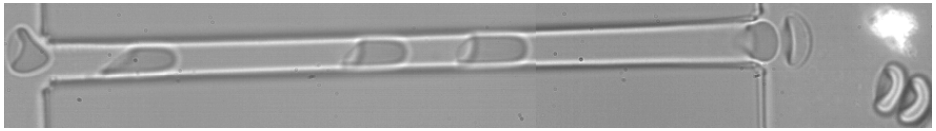
Subra Suresh "Biomechanics and Biophysics of Cancer Cells Acta Materialia 55 (2007) 3989

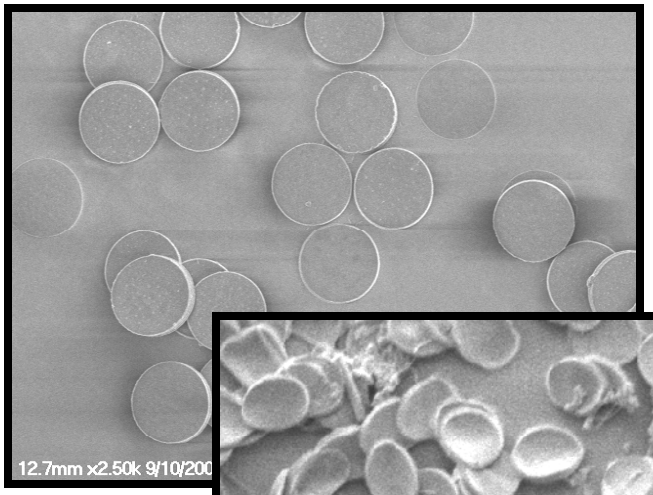
Role of Mechano-biology in Biodistribution:

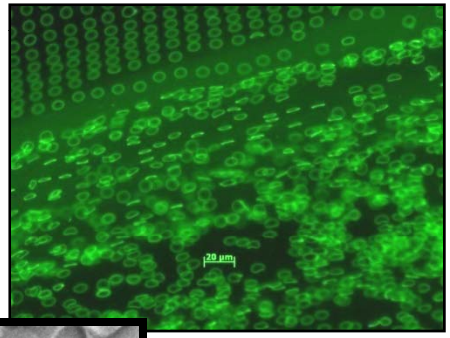
Inspired by Red Blood Cells and Metastatic Cancer Cells

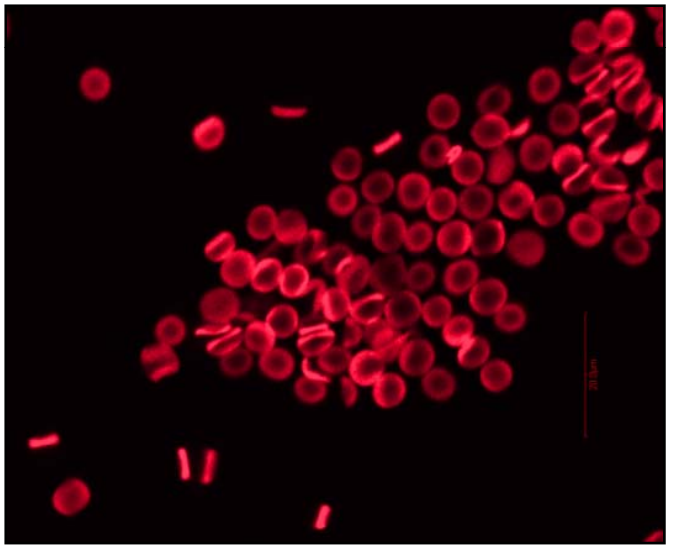


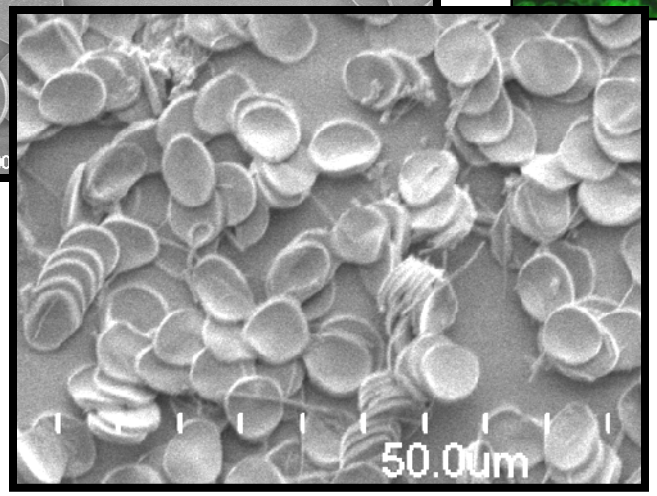


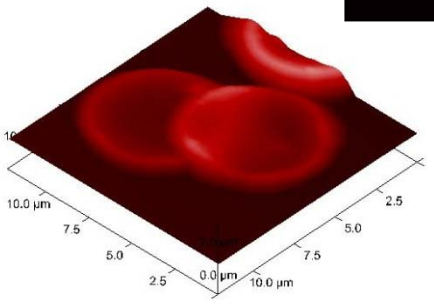






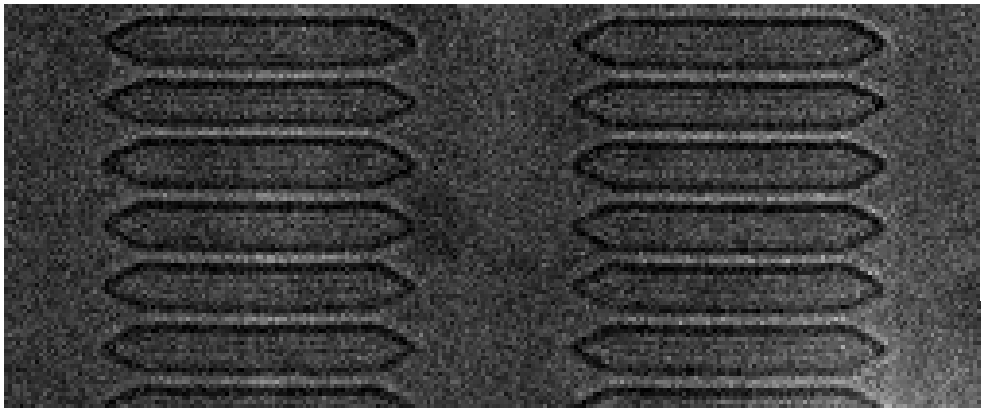


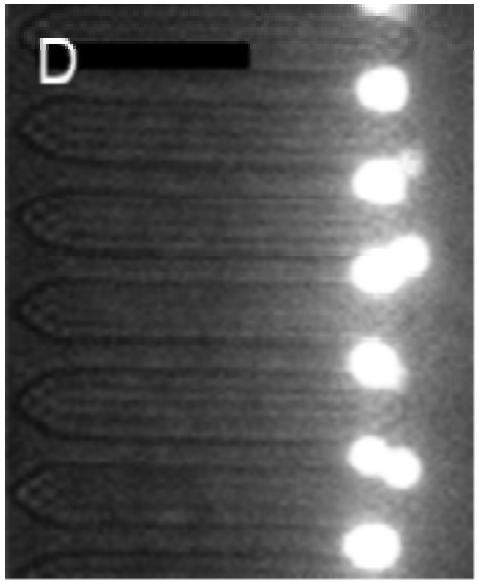


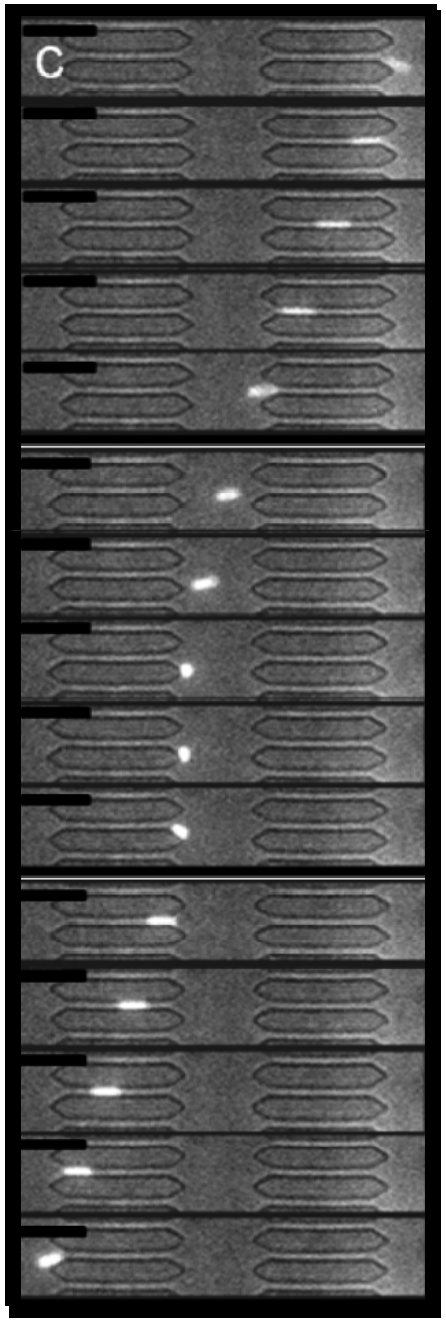


Opportunities for synthetic blood, imaging agents & long circulating carriers

Mimicking Red Blood Cells

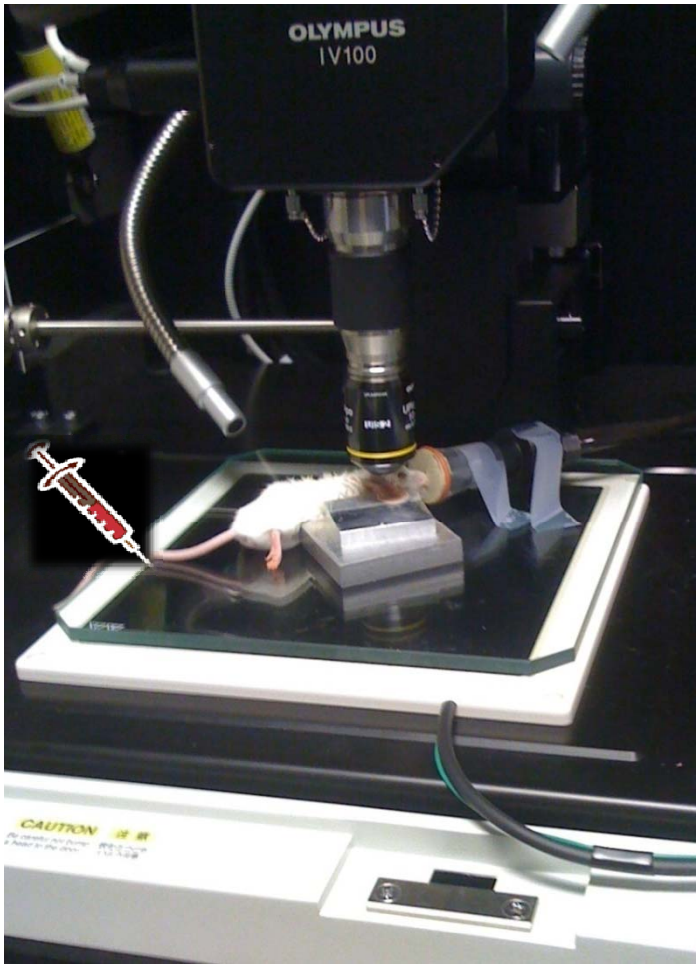


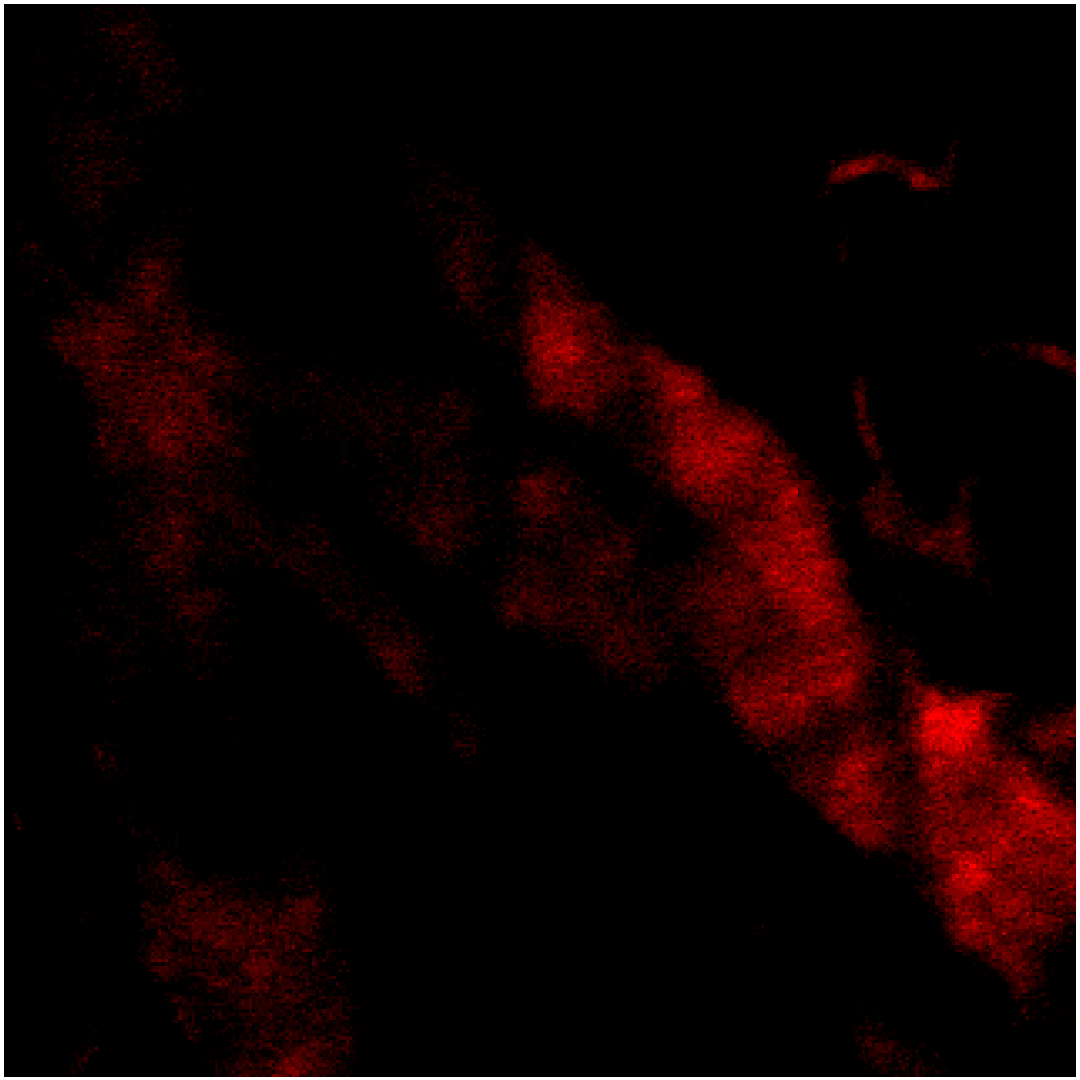




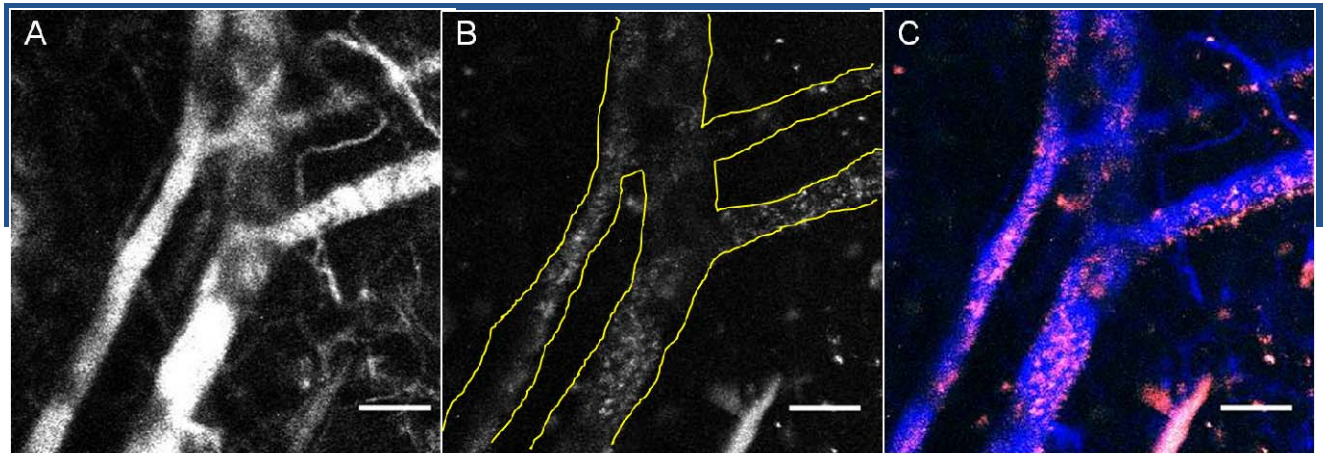
Role of Mechano-biology in Biodistribution:

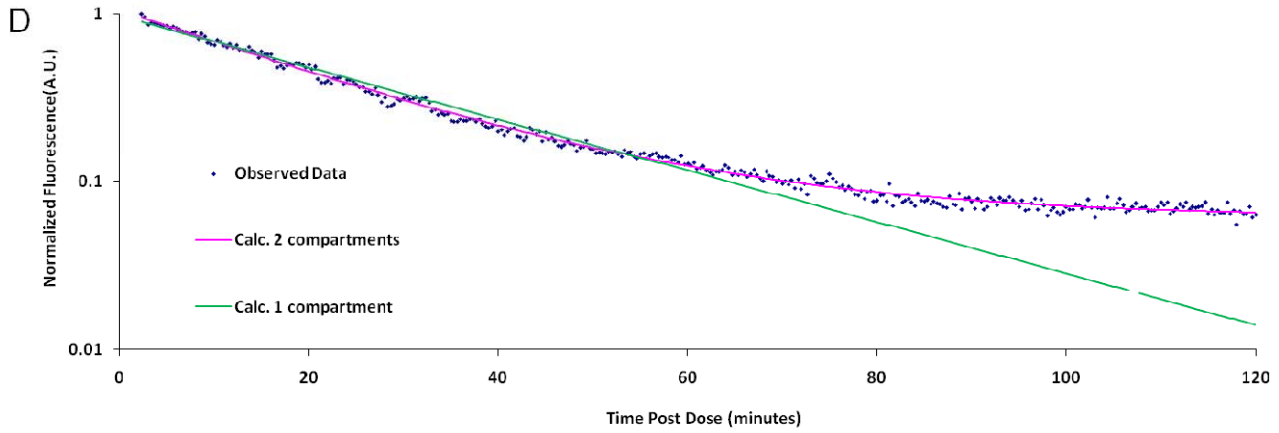
Inspired by Red Blood Cells and Metastatic Cancer Cells





Intravital Results and PK analysis





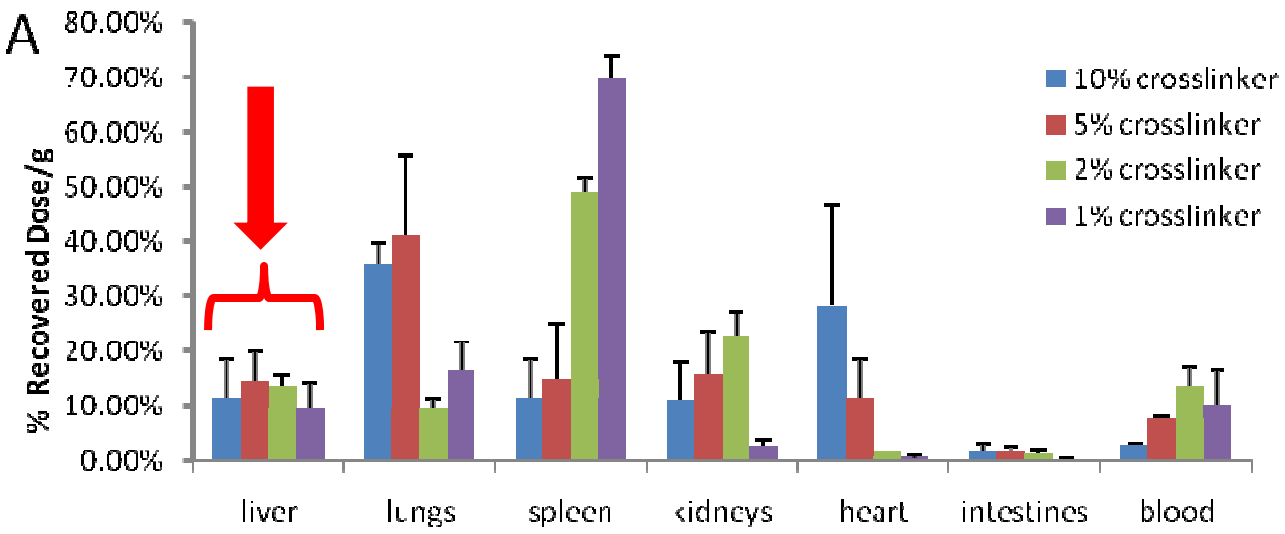
Blood draw out to 5 days post injection.

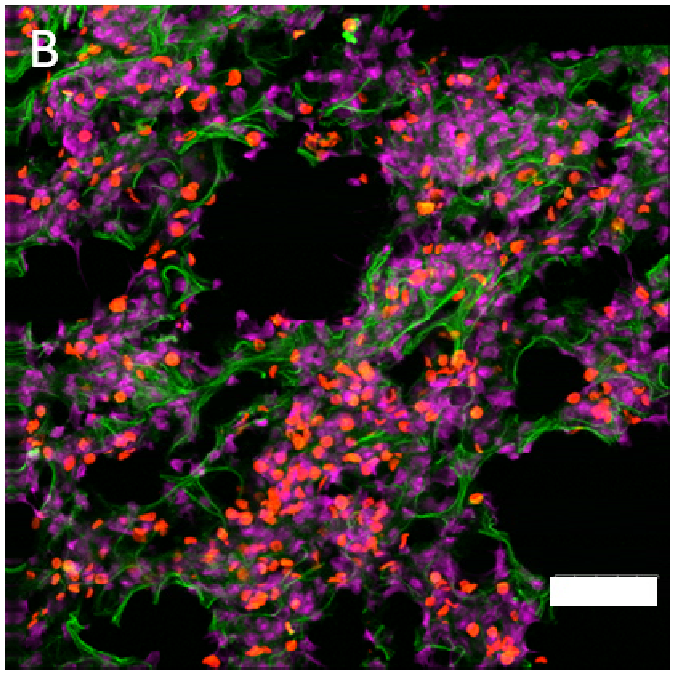
Full pk analysis from 2 compartment model

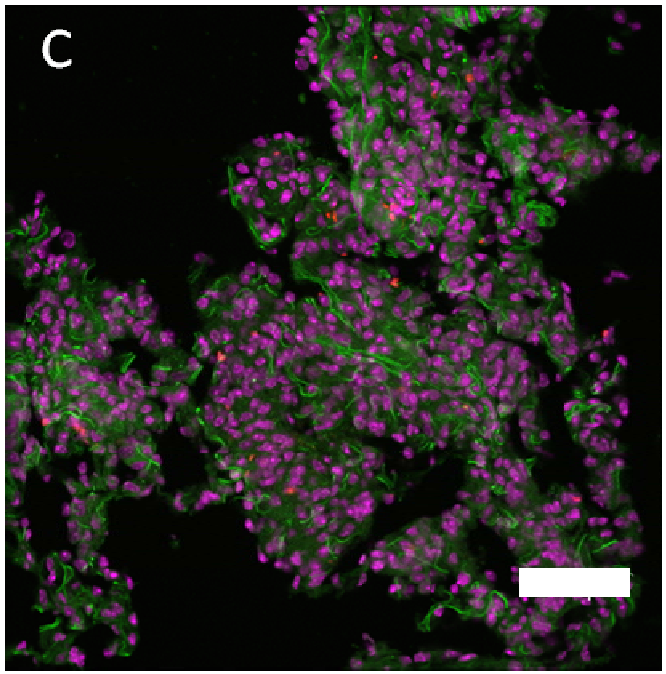
5% of the injected dose remaining in the blood after 5 days

% Crosslinker	Modulus of Bulk Material (kPa)	Distribution Half-life (hours)	Elimination Half-life (hours)
10%	63.9 ± 15.7	0.038 ± 0.0012	2.88 ± 0.92
5%	39.6 ± 10.4	0.066 ± 0.036	5.12 ± 2.17
2%	16.9 ± 1.7	0.15± 0.025	7.12 ± 0.82
1%	7.8 ± 1.0	0.35± 0.13	93.29± 31.09

Biodistribution data





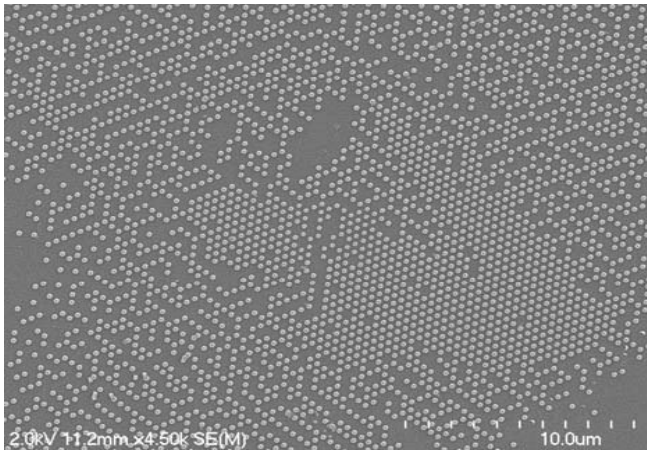


Trends with decreasing modulus:

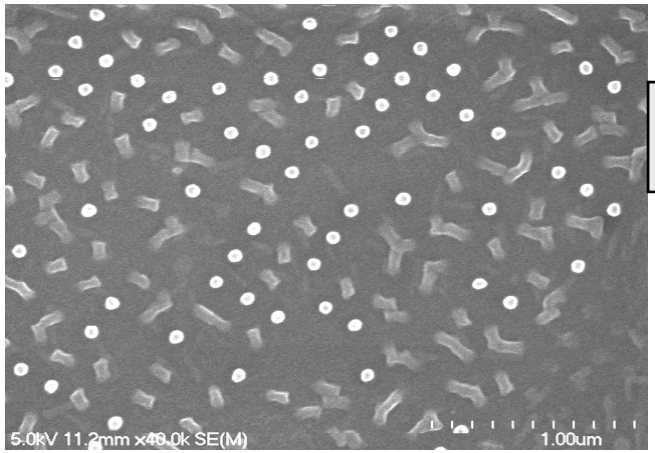
- Spleen accumulation increases
- Heart accumulation decreases
- Lung accumulation is largely avoided
- Kidney accumulation increases, but drops drastically with the softest particle
- Relatively low liver up-take as well

Very Deformable Nanometer Scale Particles

200x200 nm

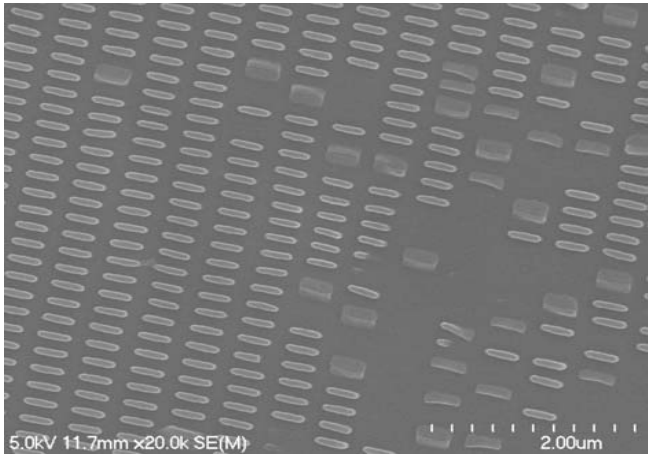


80x90 nm

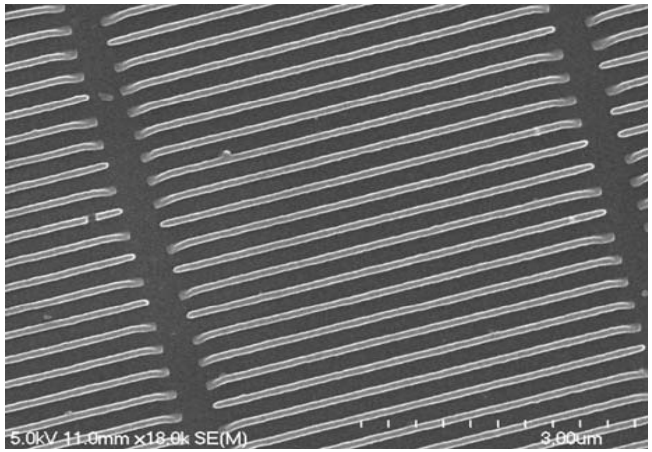


10 wt% PEG_{700}-Diacrylate
90 wt% Hydroxy PEG_4Acrylate

80x320 nm



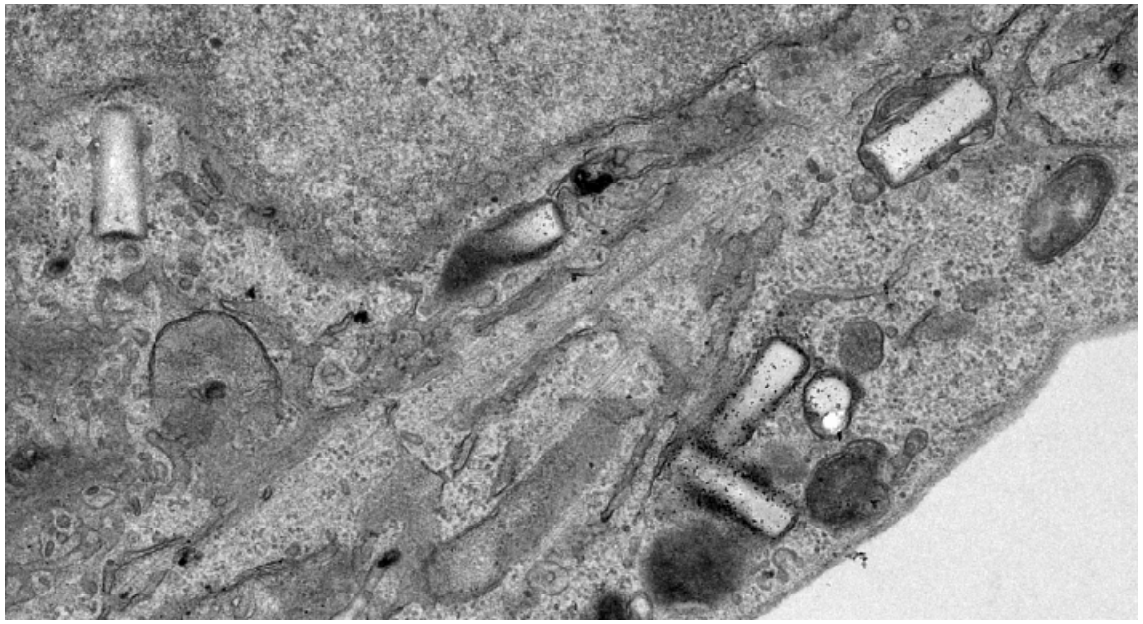
80x5000 nm

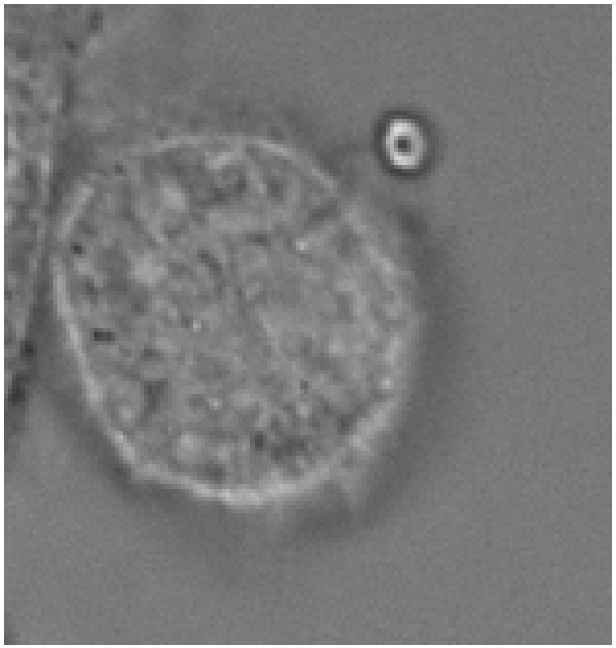


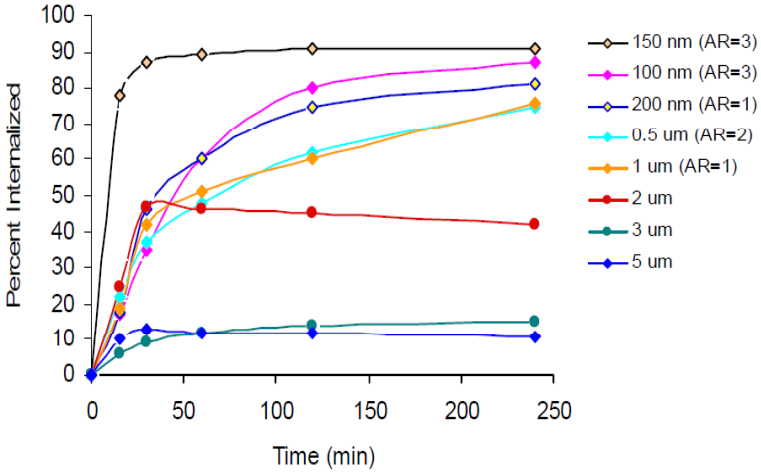
Jillian Perry

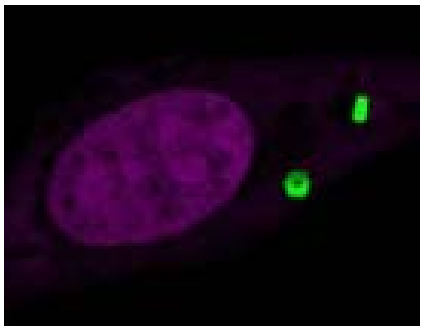
Role of Size and Shape on Particle Cellular Internalization

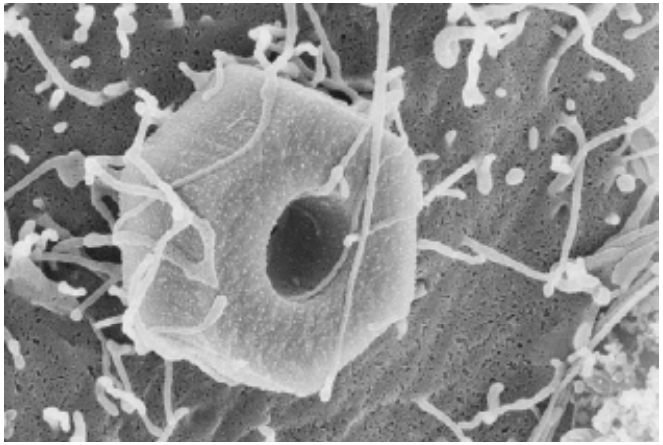
DeSimone *et.al. Proceedings of the National Academy of Sciences* **2008**, 105, 11613







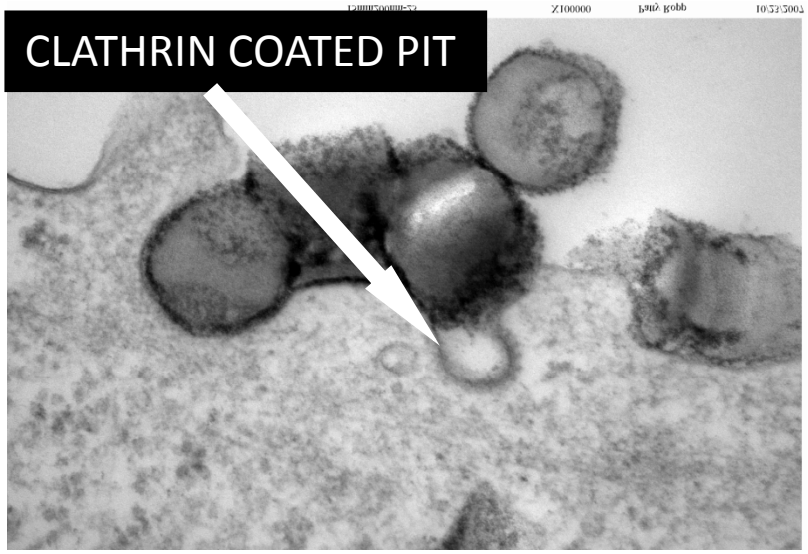


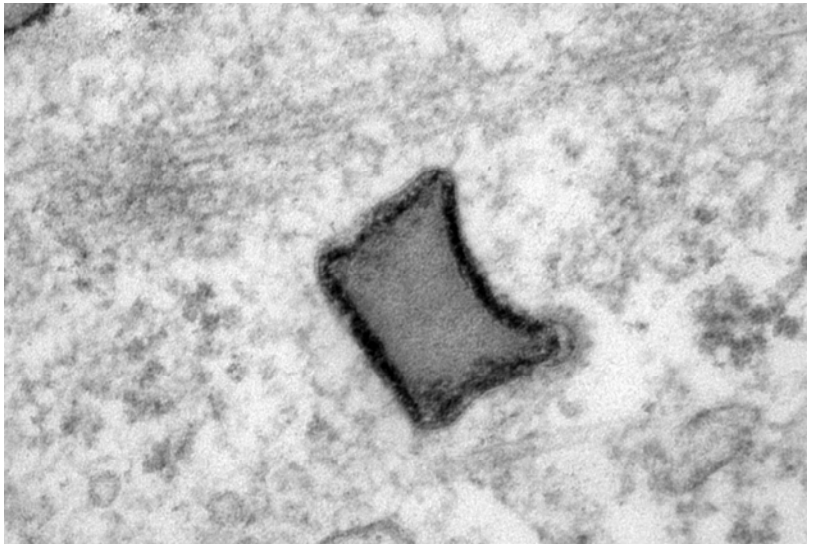


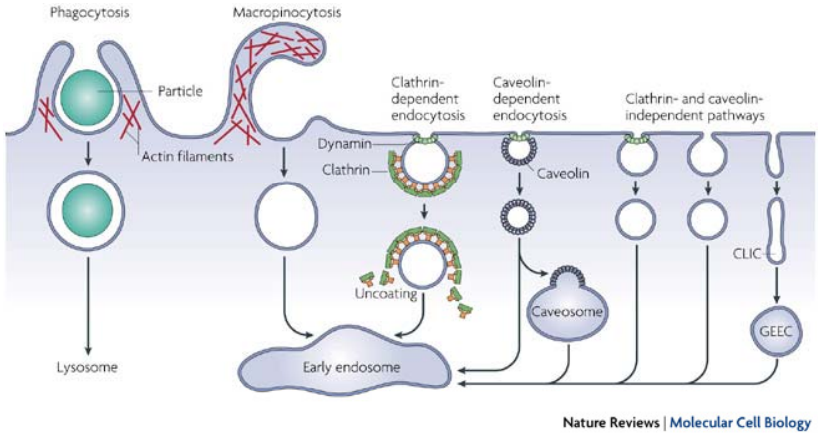
Role of Size and Shape on Particle Cellular Internalization

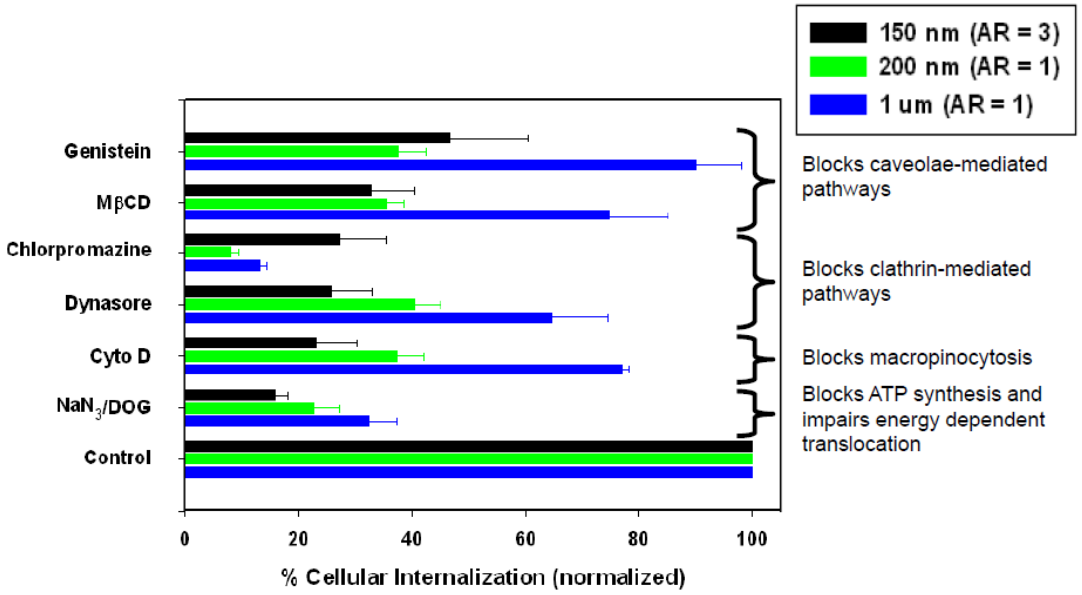
DeSimone *et.al. Proceedings of the National Academy of Sciences* **2008**, 105, 11613

1 Hour

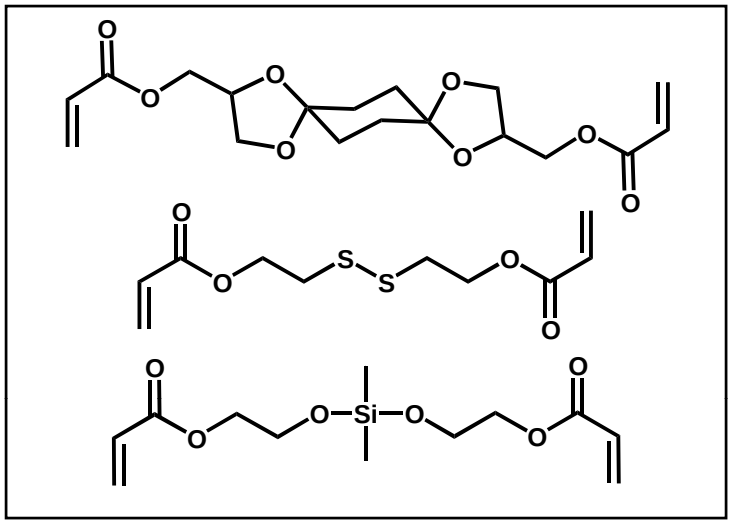


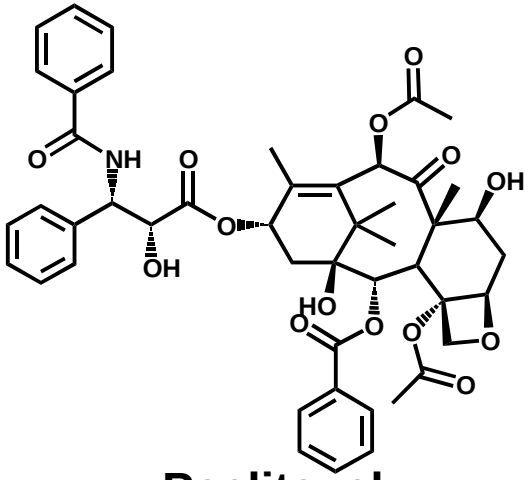






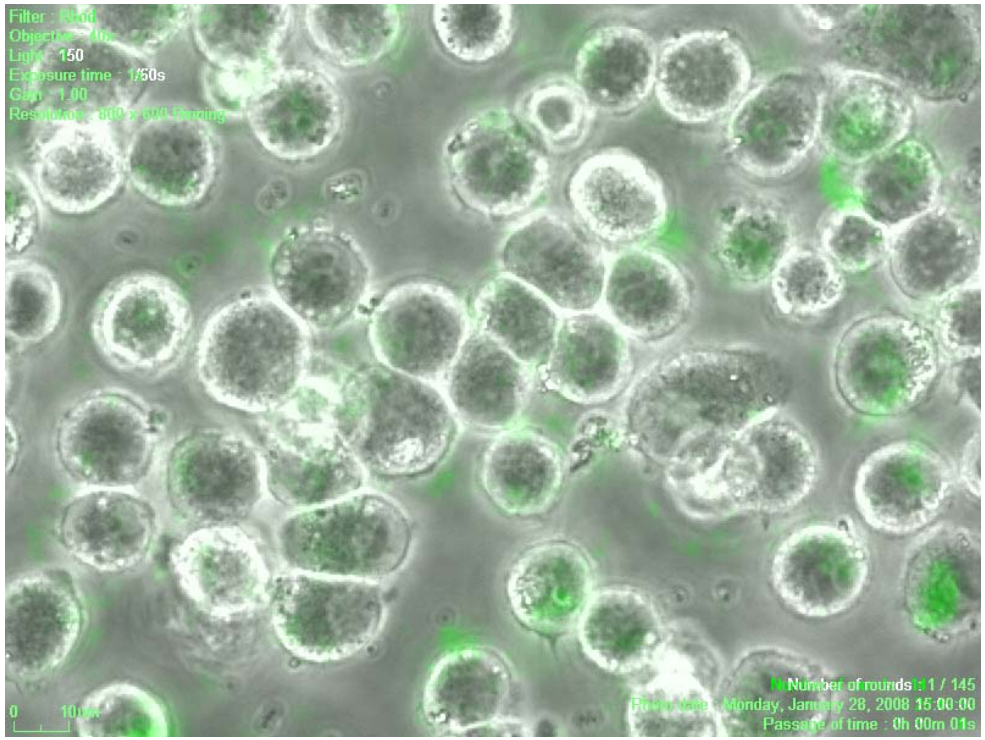
Particle Degradation and Chemotherapeutic Release





Paclitaxel

Monomer	wt%	wt%	wt%
PEG8DES	74.4	67.4	60.6
APMAM	18.1	18.1	18.1
DEAP	0.6	0.6	0.6
PACLITAXEL	**6.9**	**13.8**	**20.7**
Total	100	100	100

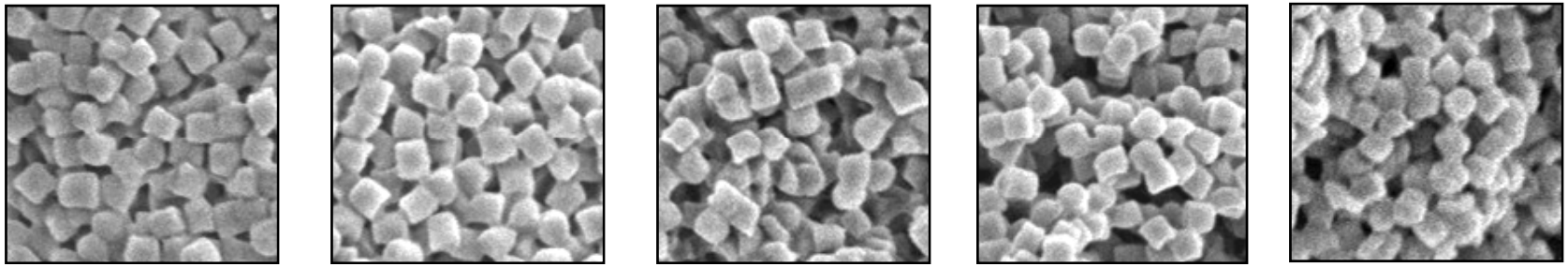


"Reductively Labile PRINT Nanoparticles for the Delivery of Doxorubicin to HeLa Cells"; Petros, R. A.; Ropp, P. A.; DeSimone, J. M.; *J. Am. Chem. Soc.* **2008**, *130*, 5008-5009

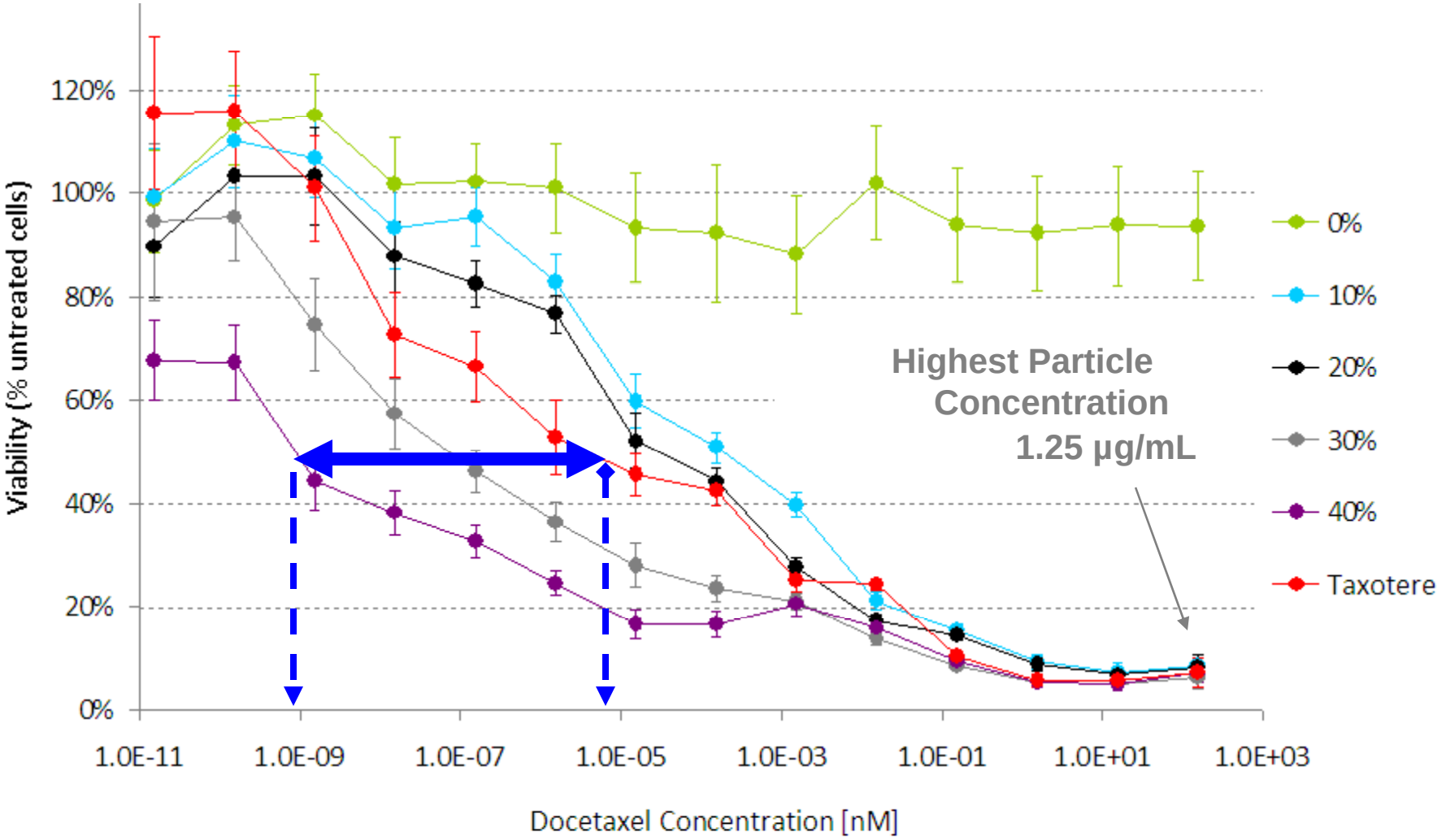
PLGA / Docetaxel PRINT Particles

Theoretical Loading	Encapsulation Efficiency	Size (nm)	Zeta Potential (mV)
0%	--	268	-23
10%	93%	284	-20
20%	91%	252	-22
30%	97%	256	-20
40%	99%	255	-22

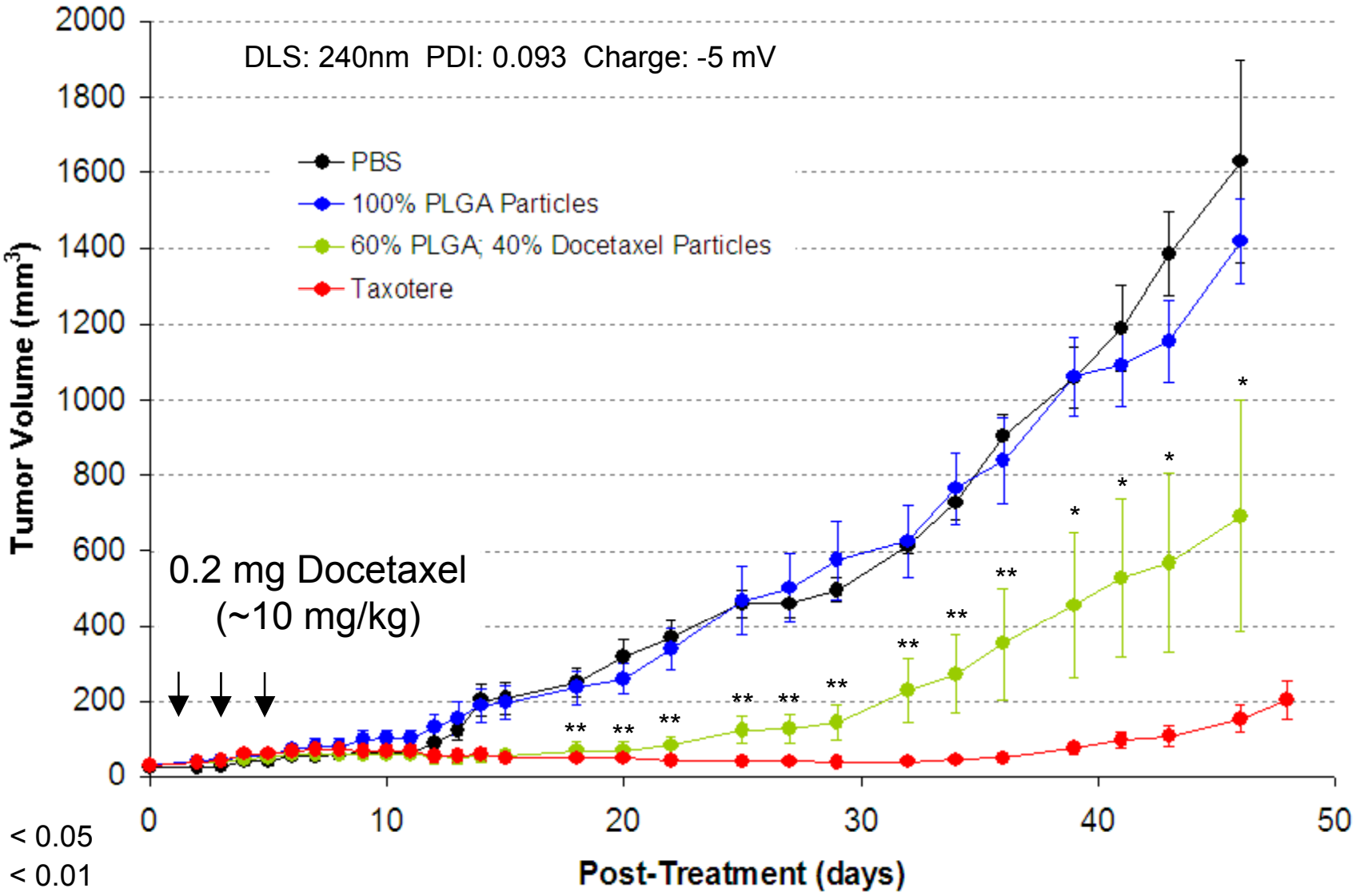
0% **10%** **20%** **30%** **40%**



PLGA / Docetaxel *In Vitro* Toxicity H460



PLGA PRINT® Docetaxel In Vivo I.T. Therapy



* $p < 0.05$
** $p < 0.01$

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

“If you could get RNA to the target you could have some really cool therapeutics. Delivery is the major issue in all of it.”

Andrew Fire
Stanford

"All the challenges are in delivery, Other issues, such as chemical stability, immune stimulation, and off-target effects are vastly overrated by comparison”

Phillip Zamore
UMass

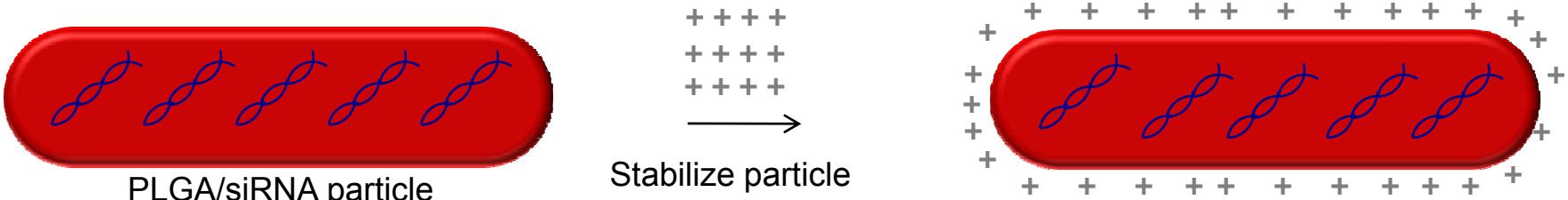
Andrew Z. Fire
Stanford University
School of Medicine

Craig C. Mello
UMass
Medical School

“The principal challenge that remains in achieving the broadest application of RNAi therapeutics is the hurdle of delivery.”

- “Interfering with disease.” Nature Reviews Drug Discovery, June 2007

PLGA Particle Preparation for siRNA Delivery



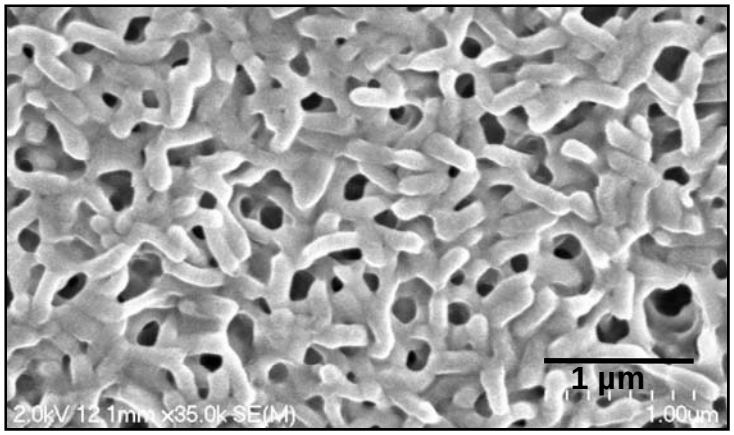
Options for Transfection agent:

1) Cationic lipids 2) Cationic polymers 3) Fusogenic peptides

Particle composition

Compound	Function	Weight%
PLGA	Matrix	95
siRNA	Cargo	5
DOTAP:DOPE	Transfection agent	Excess

SEM of 80 x 320 nm particles

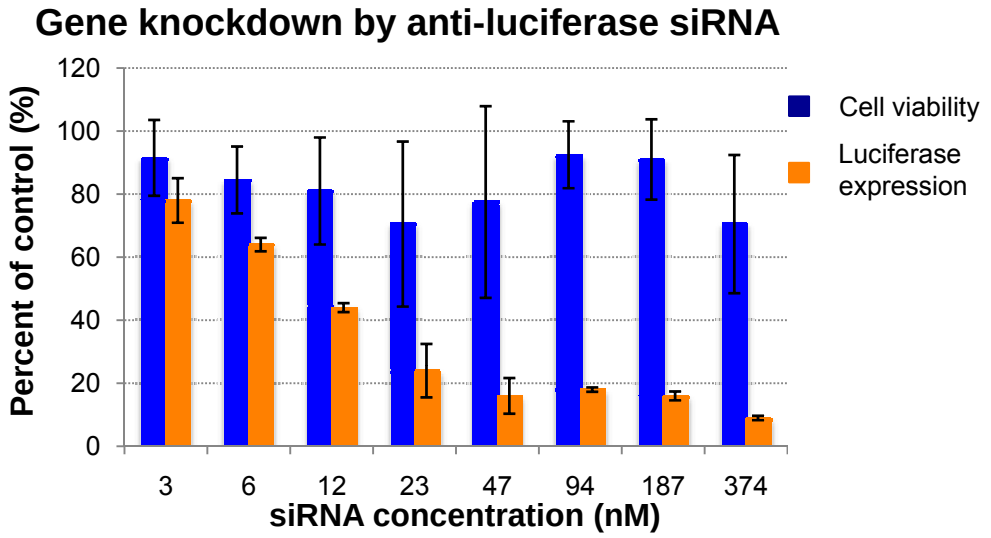


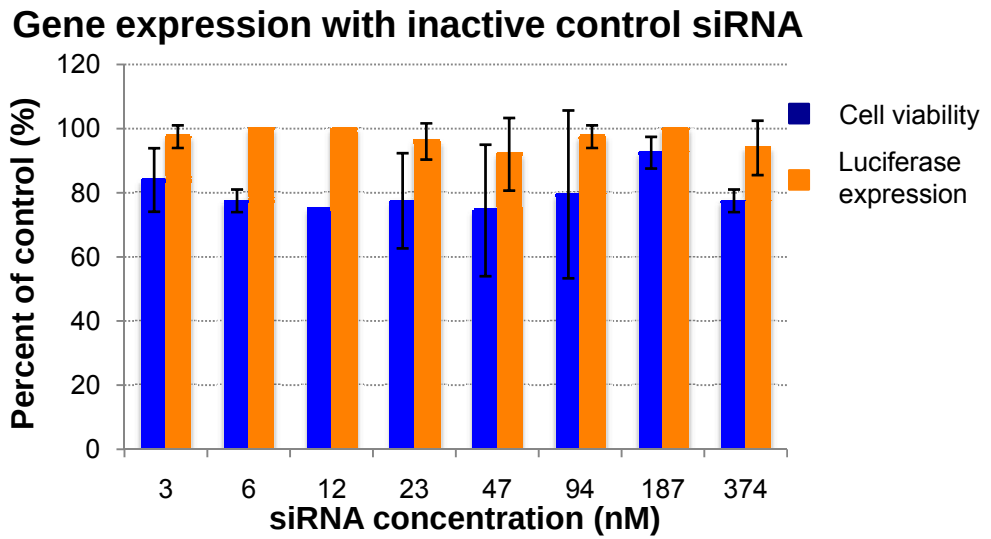
D_z = 210 nm, PDI = 0.15

ζ-potential = +15 mV

PLGA Particle Preparation for siRNA Delivery

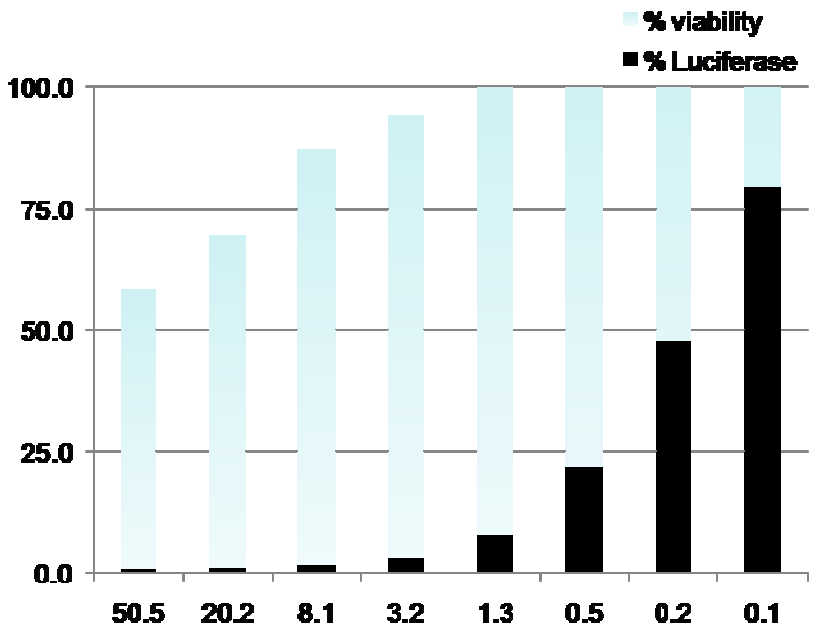
- Internalization of particles by HeLa cells
- Cell viability maintained
- High gene silencing efficiency (EC_{50} = 10 nM)
- Knockdown absent with control siRNA





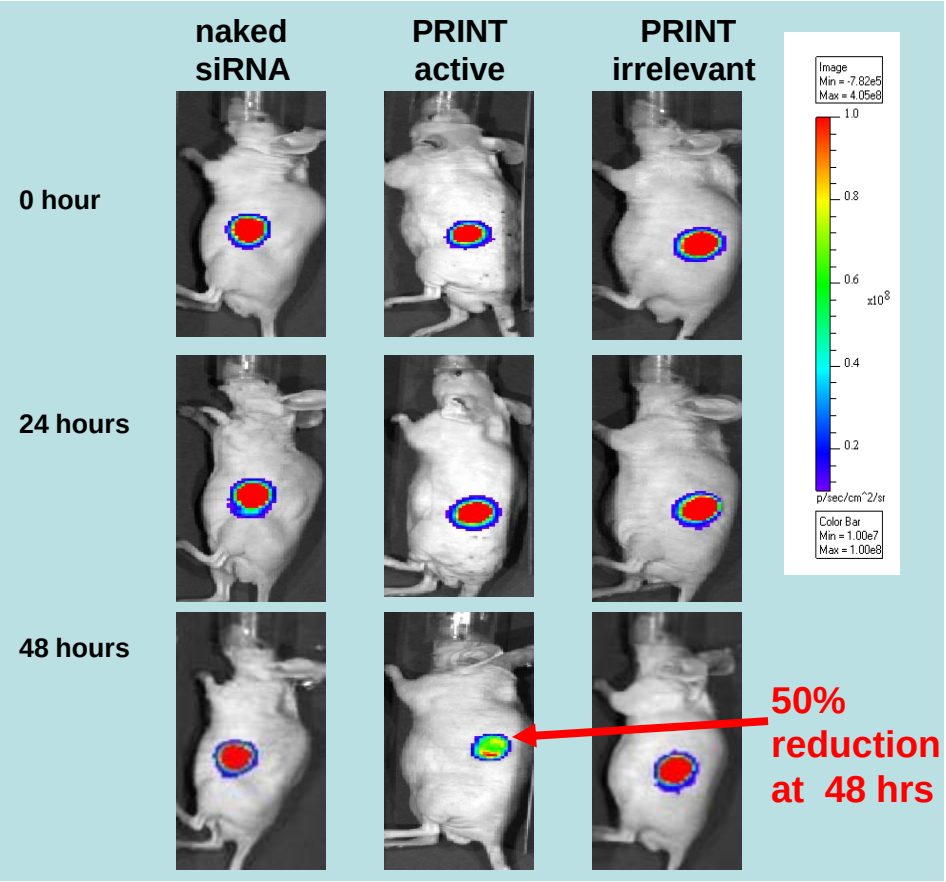
In vitro and *In vivo* siRNA Delivery

In vitro Anti-Luciferase Model

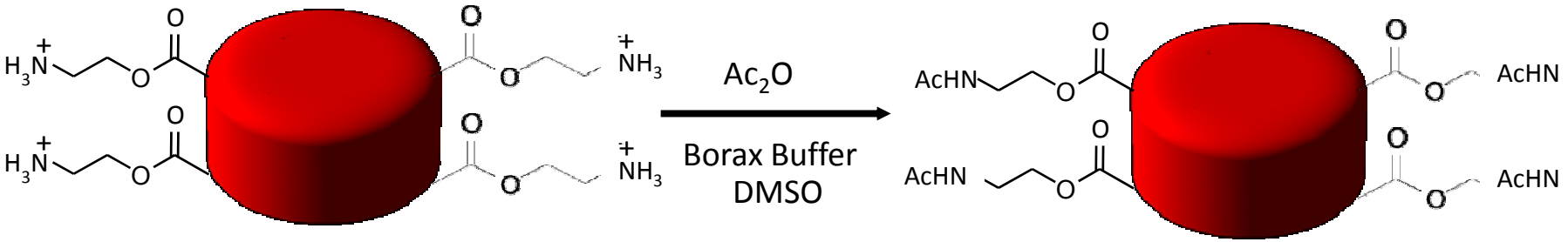


EC-50 = ~200 pM

In Vivo PC-3 Xenograft Tumor Model



Effect of Charge on Cellular Internalization



+34.78 ± 3.04 mV

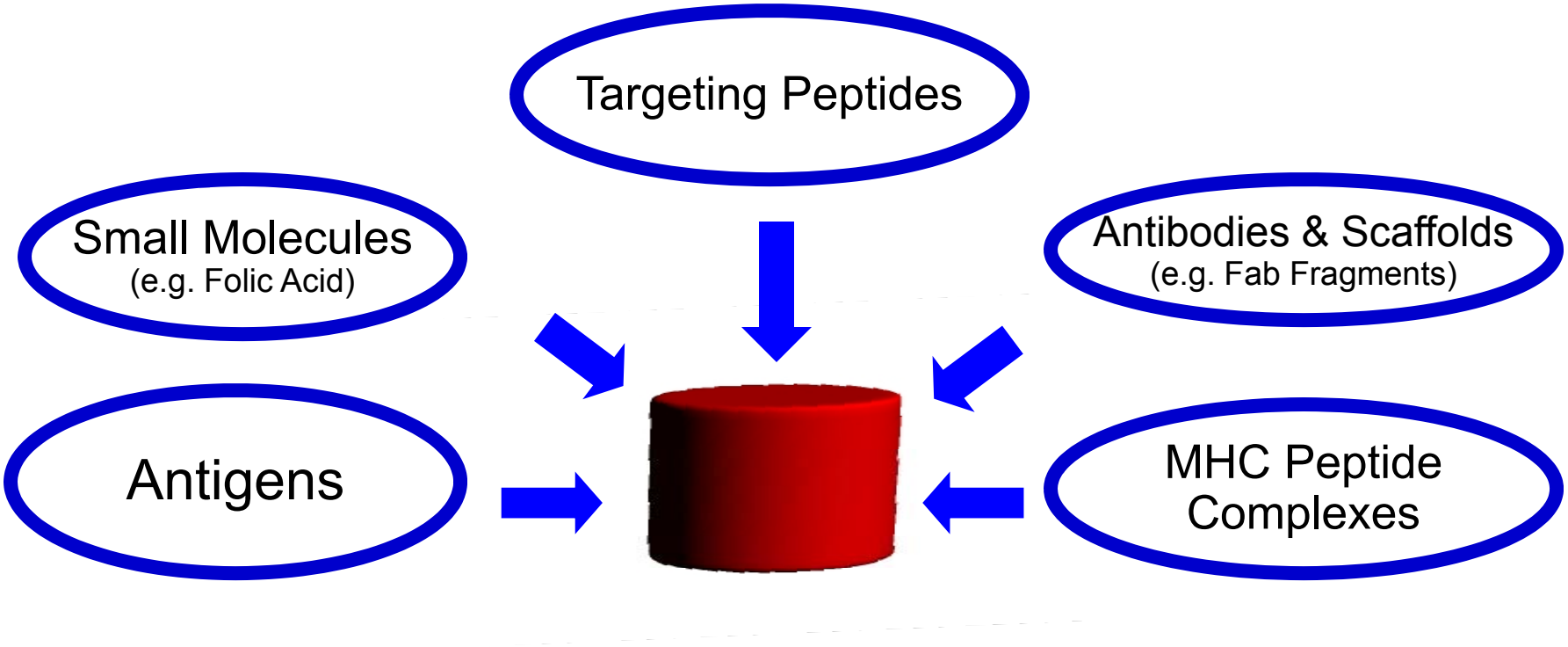
-33.69 ± 2.31 mV

Particle Internalization After 4 hours of Incubation:

Can we over-ride the negative zeta-potential with ligands that induce receptor-mediated endocytosis to increase specificity?

Gratton, S. E. A., *et al*, *Proc. Natl. Acad. Sci.*, 2008, *105*, 11613-11618.

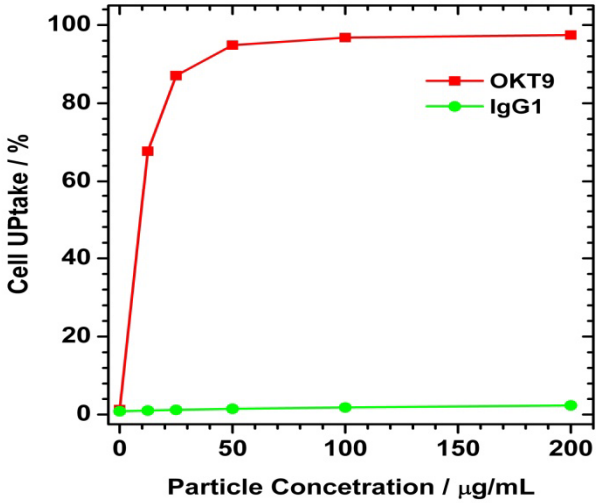
Targeted PRINT Particles: Range of Functionalization Opportunities

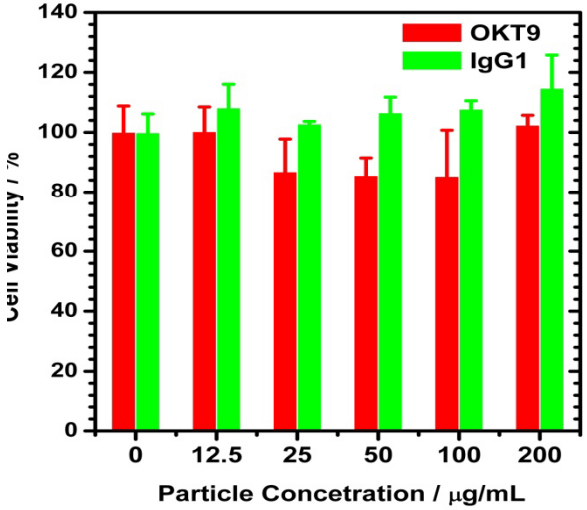


Ligand Class	**Targeting Ligand**	**Control**
Oligo-peptide	Sup-B8 Targeting Octapeptide; Lys-Glu	Scrambled Octapeptide; Lys-Glu protected
Protein	Human Tf	Bovine Tf
Antibody	TfR mAb (OKT9); PSMA	Isotype Control Ab

Solid Tumor Targeting to Transferrin Receptor

HeLa

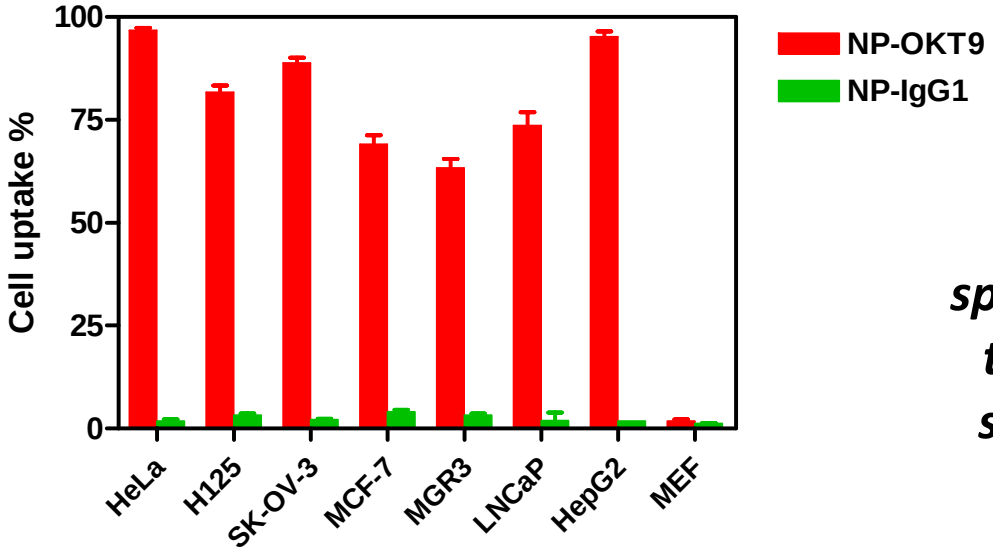




200 x 200 nm cylinders

4h dosing at 37°C

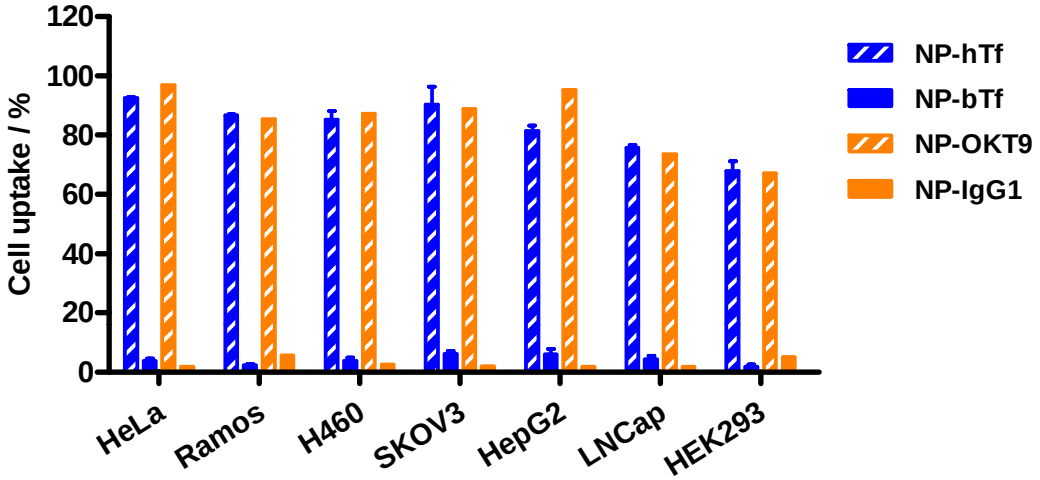
72h incubation

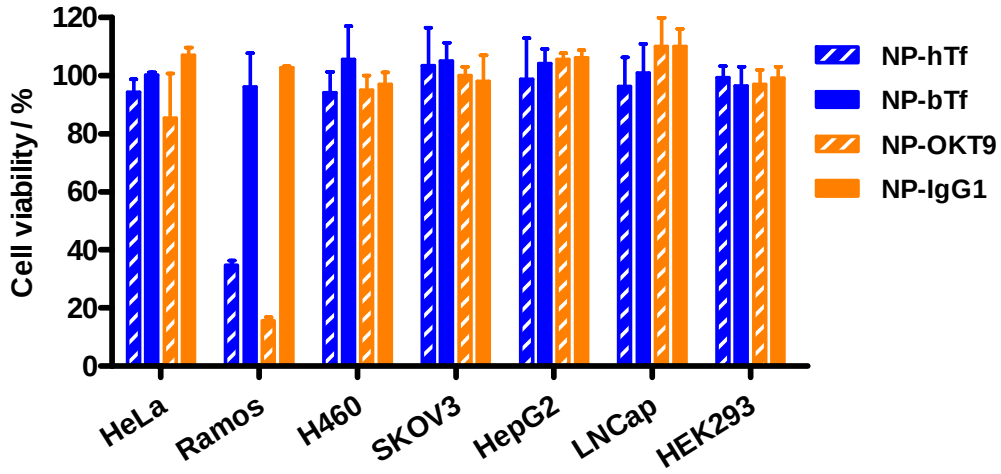


OKT9-PRINT® particles were specifically internalized by all solid tumor cell lines studied without showing significant cytotoxicity.

"The Complex Role of Multivalency in Nanoparticles Targeting the Transferrin Receptor for Cancer Therapies"; Wang, Tian, Petros, Napier, DeSimone *J. Am. Chem. Soc.* **2010**, in press

Targeting Transferrin Receptor with Transferrin or TfR Antibody Conjugated PRINT® Nanoparticles





HeLa: cervical carcinoma

Ramos: Burkitt's B cell lymphoma

H460: lung adenocarcinoma

SK-OV-3: ovarian adenocarcinoma

HepG2: hepatocellular carcinoma

LNCap: prostate adenocarcinoma

HEK293: human embryonic kidney cell

200 x 200 nm cylinders

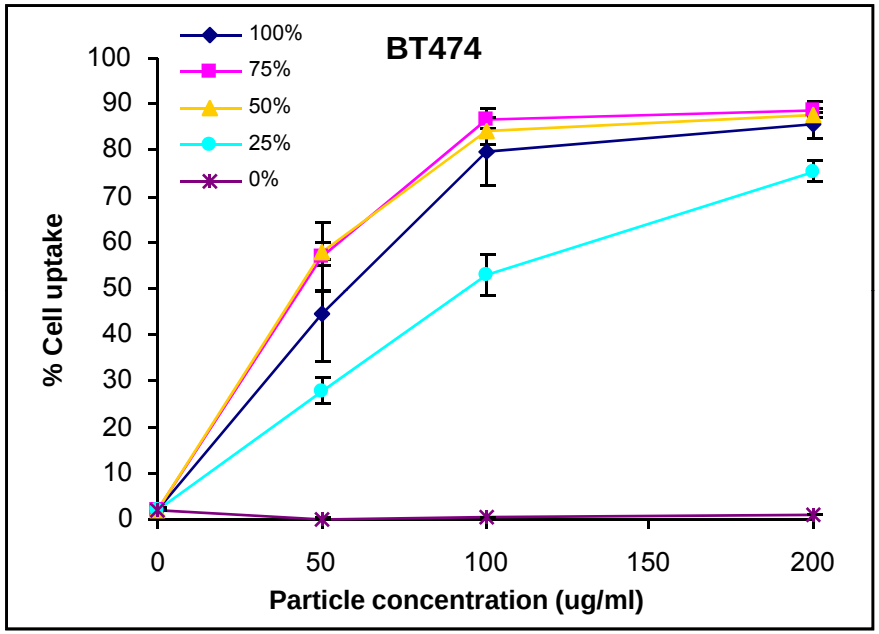
100 µg/ml

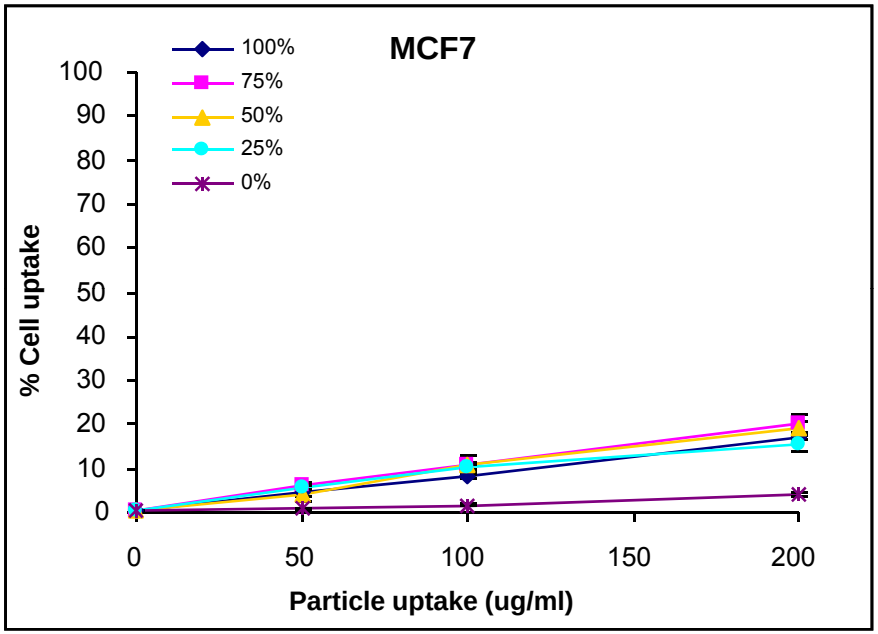
Flow cytometry for uptake after 4 hr dosing at 37°C

MTS assay for cytotoxicty after 72 hr further incubation at 37°C

"The Complex Role of Multivalency in Nanoparticles Targeting the Transferrin Receptor for Cancer Therapies"; Wang, Tian, Petros, Napier, DeSimone *J. Am. Chem. Soc.* **2010**, in press

Herceptin Conjugated to PRINT Particles





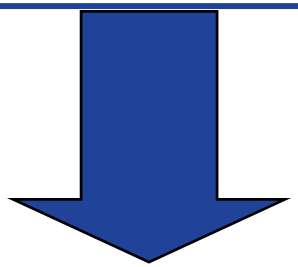
BT474 (HER2 positive breast cancer) and MCF7 (HER2 negative breast cancer) were treated with varying concentrations of particles for 4h prior to analysis by flow cytometry. Cells were analyzed in the presence of trypan blue to block external particle fluorescence. Particles contain FITC.

Interventional Oncology

interventional oncology

The Problem

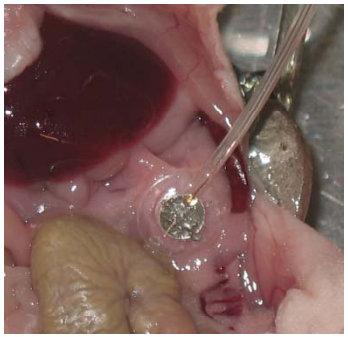
Inability to deliver therapeutics in a highly targeted and efficient manner to isolated or difficult-to-treat solid tumors

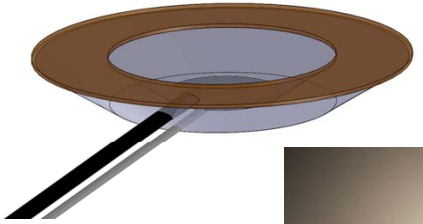


Concept Statement

Leverage existing medical device technologies to enable highly targeted and efficient delivery of therapeutic and diagnostic compounds to isolated or poorly vascularized solid tumors

interventional oncology





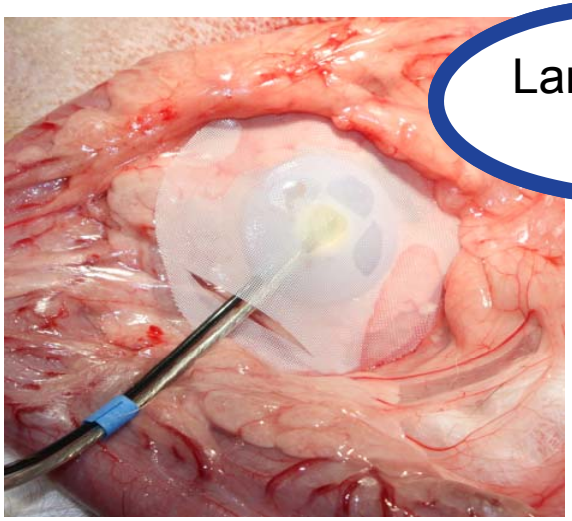
Proof-of-Concept

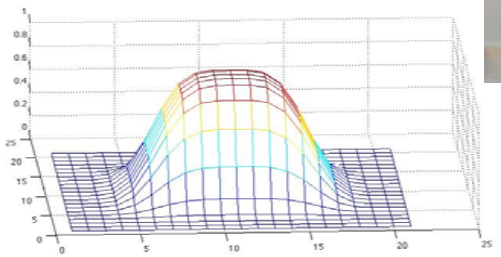
Small Animal Studies

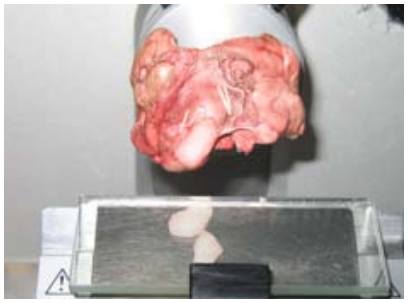
Engineering

Large Animal Studies

Pharmaco-kinetics

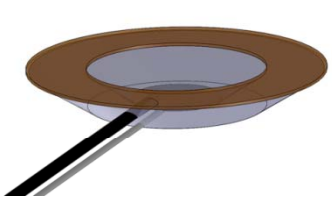


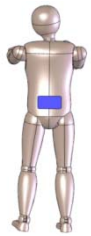


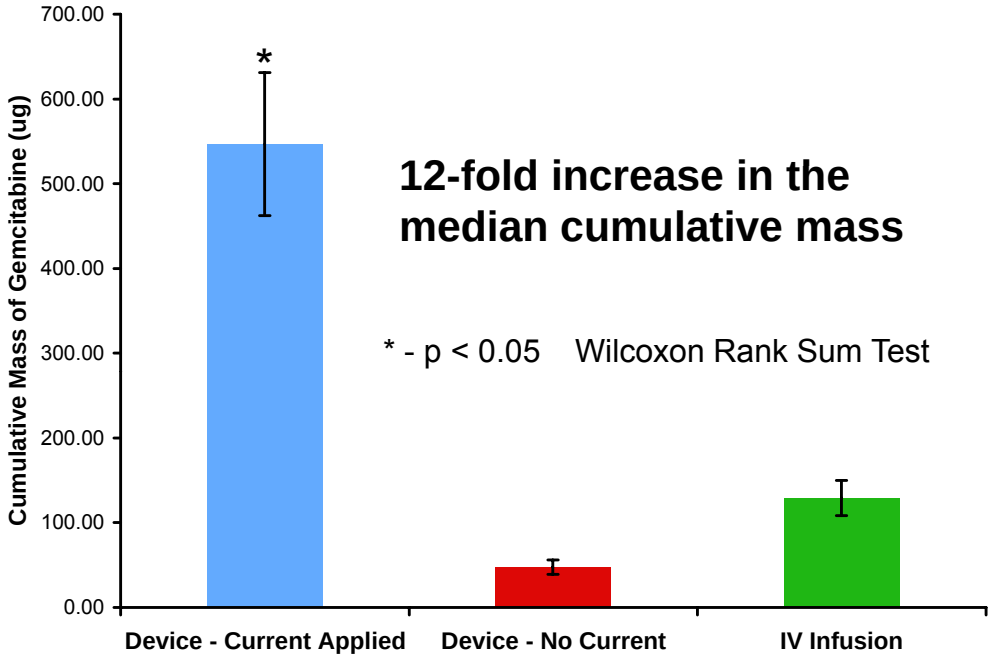


Analysis of Tissue

- Originating Electrode Position:
  - Patch on pancreas
- Counter Electrode Position:
  - Back of Pancreas
  - Back of Animal
  - Inferior Vena Cava





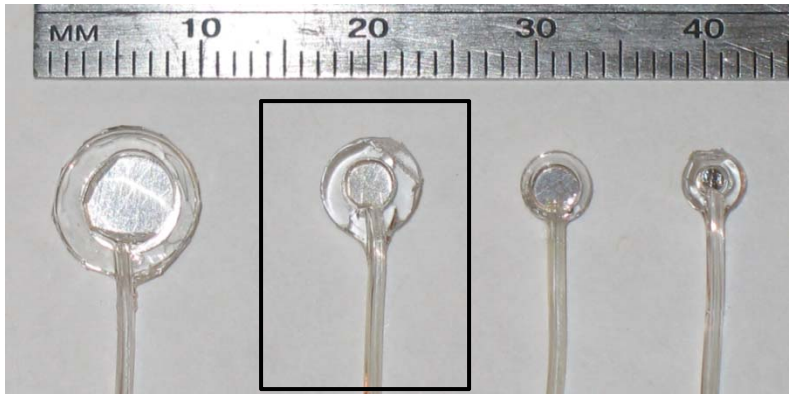


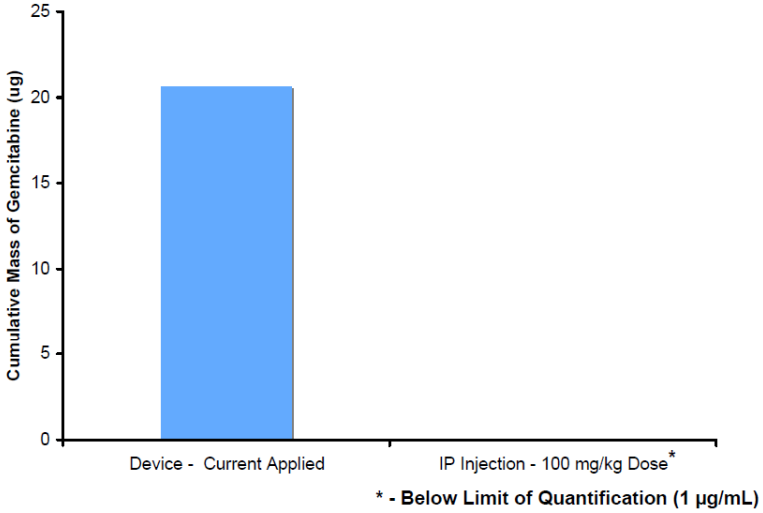
	Sample	IV Infusion Gem Concentration (ug/mL)	Experimental (w/ current) Gem Concentration (ug/mL)	Control (w/o current) Gem Concentration (ug/mL)
	-15	*	*	*
Start of therapy →	0	*	*	*
	15	37.02 ± 9.38	*	*
	30	54.11 ± 8.22	*	*
	45	36.88 ± 1.95	*	*
End of therapy →	60	30.19 ± 2.06	*	*

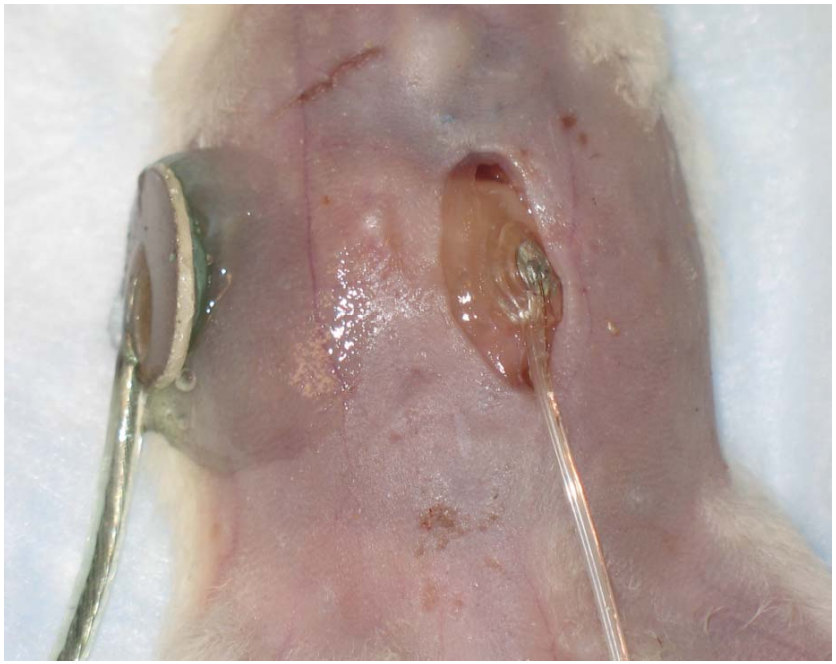
No gemcitabine detected in plasma for the device with current or without current applied

Device Studies on Patient-Derived Xenograft Tumors

	Device – Current Applied	IP Injection – 100 mg/kg Dose
Current	1 mA	-
Time of Administration	10 minutes	Bolus and sacrifice after 4 hours
Sample Size	1	1



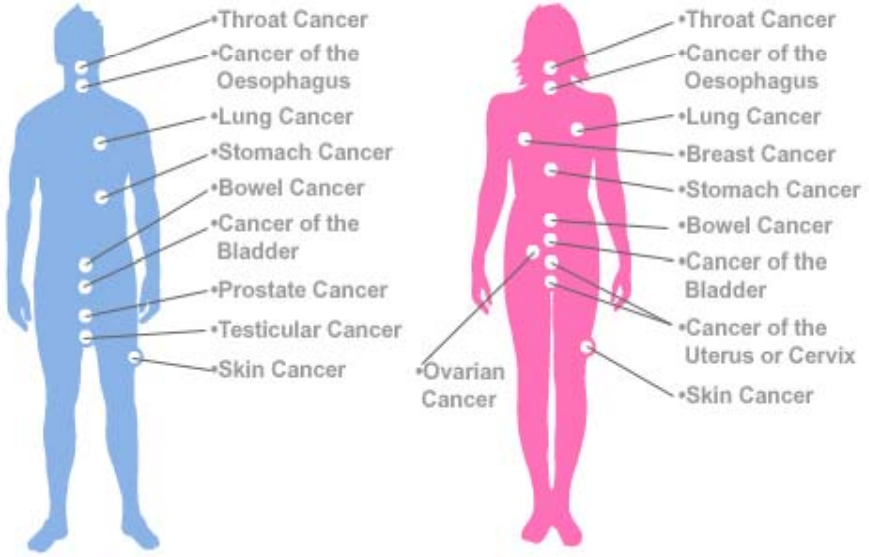




- Originating electrode on pancreas
- Counter electrode on back

Opportunities for Interventional Oncology

- Accessible Cancer Targets
  - → Pancreatic cancers
  - → Head & Neck cancer
  - → Liver cancers
  - → Pancreatic-derived liver metastases
  - → Colorectal cancers
  - → Colorectal-derived liver metastases
  - → Esophageal cancers
  - → Lung cancers
  - → Ovarian / Cervical / Uterine cancers

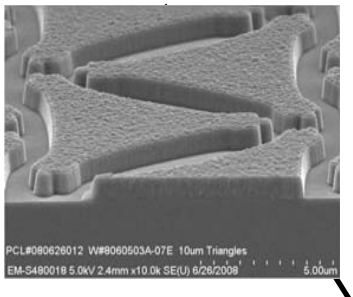


PRINT Inhaled Therapeutics

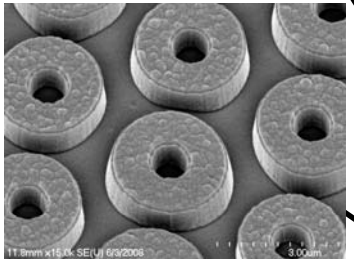
Engineering Pulmonary Delivery for Enhanced Therapeutic Index

- Tunable targeting and release
- Neat drug (biologics or small molecules)
- Precise drug combinations

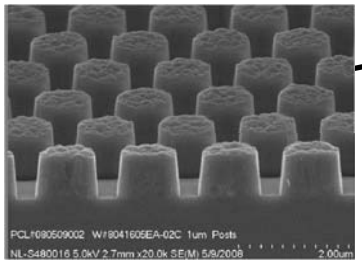
<u>Pollen</u>
Dg: 10µm
Da: 3.7µm

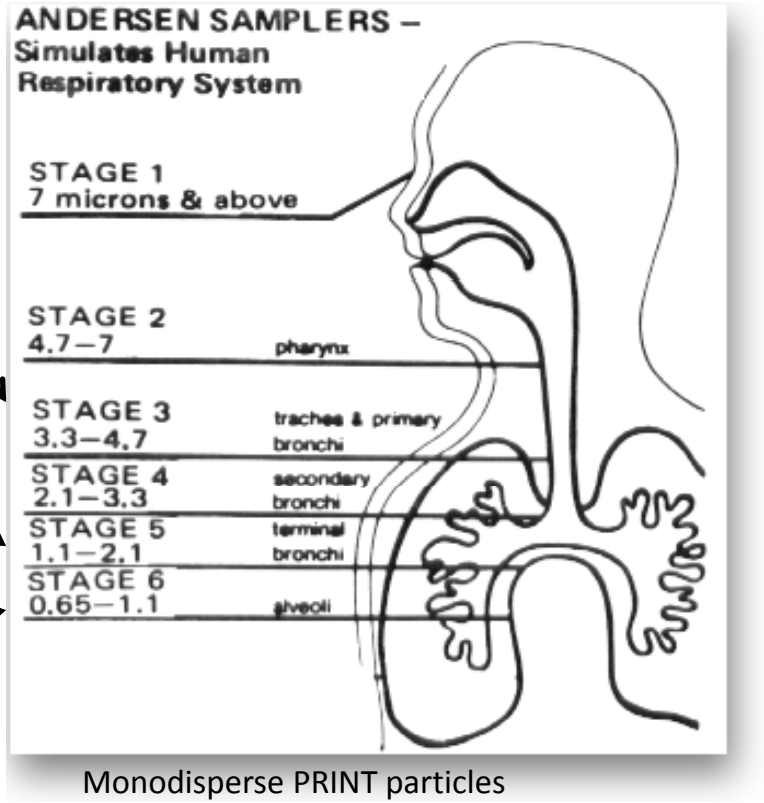


<u>Donut</u>
Dg: 3µm
Da: 1.9µm



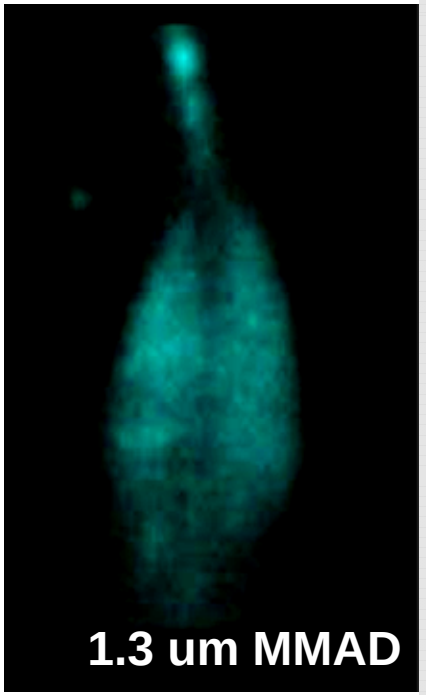
<u>Cylinder</u>
Dg: 1µm
Da: 1µm



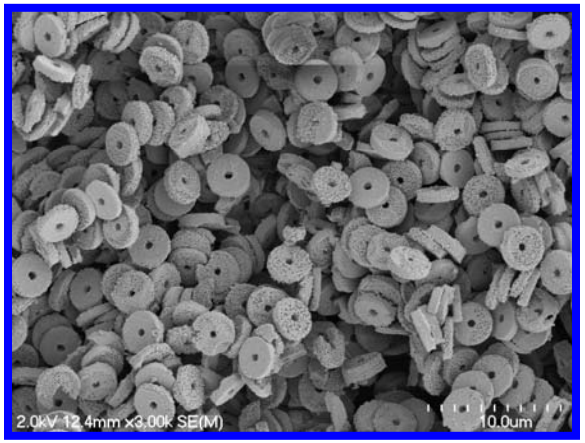


Monodisperse PRINT particles enable targeted lung delivery

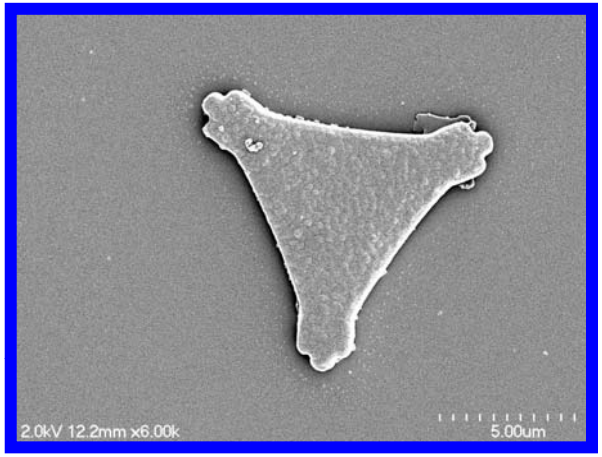
PRINT particles achieve robust canine lung delivery



Pure Drug PRINT Particles for Inhalation

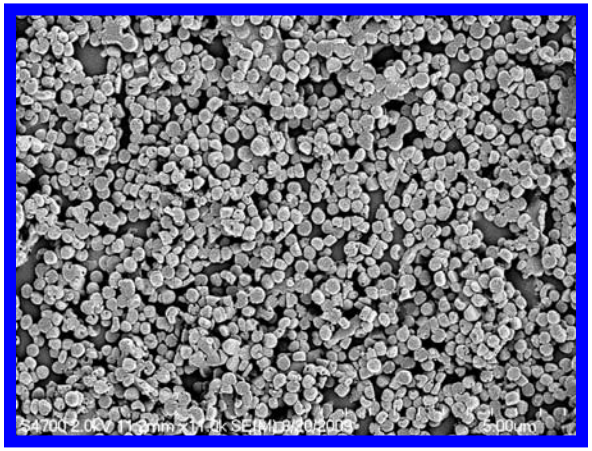


Itraconazole

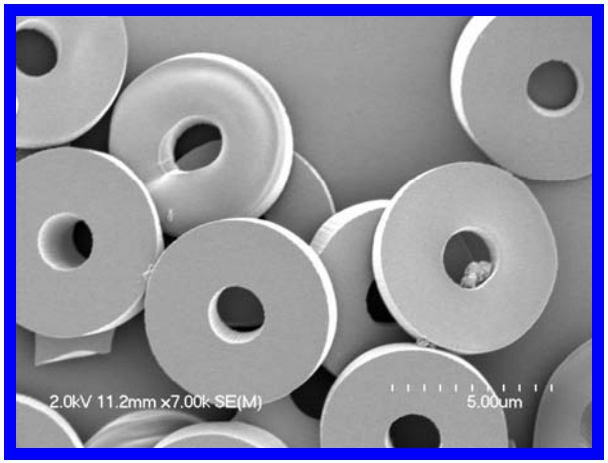


Cyclosporine

Beclomethasone Dipropionate



Partner Steroid



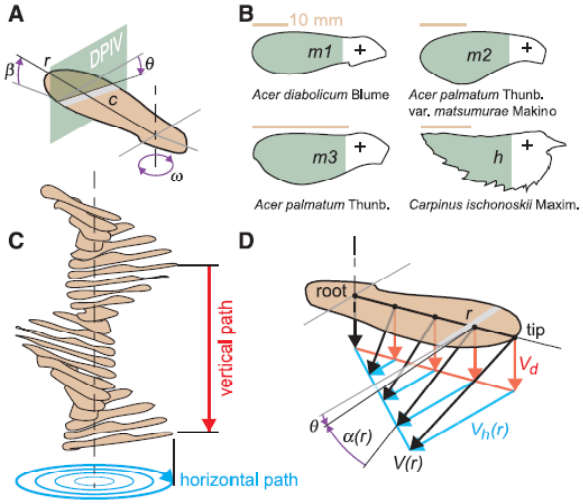
94 w/w % siRNA : 6 w/w % Leucine

Science

Leading-Edge Vortices Elevate Lift of Autorotating Plant Seeds

Science **324**, 1438 (2009)

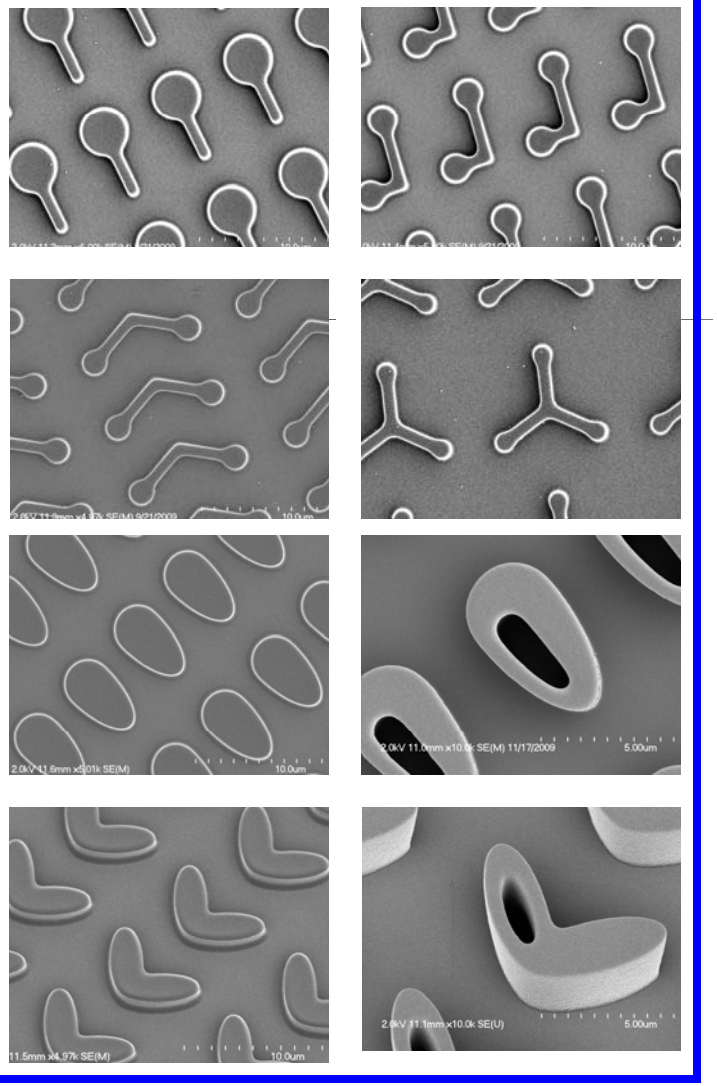
D. Lentink,[1*] W. B. Dickson,[2] J. L. van Leeuwen,[1] M. H. Dickinson[2]



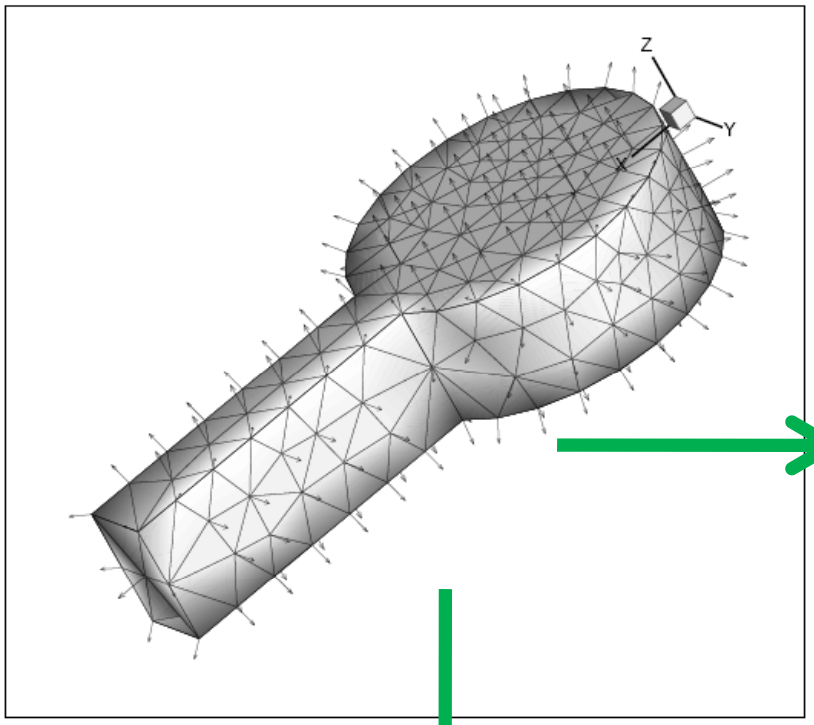
"…LEVs represents a convergent aero-dynamic solution in the evolution of high-performance flight of both animal wings and plant seeds…"

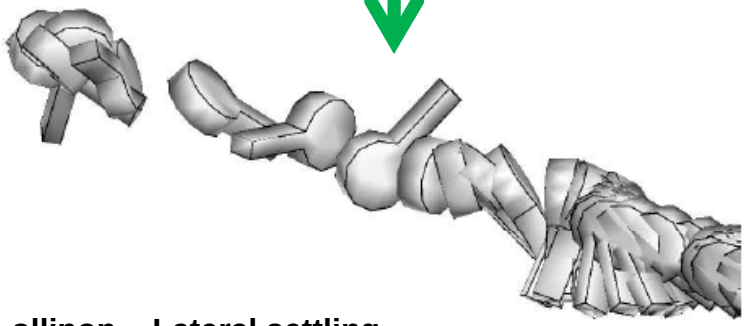
- Interplay between 3-dimensional inertial and aerodynamic properties
  - → Auto-rotating designs can generate high lift forces despite small size and low velocity
  - → Generation of leading edge vortex key
  - → Reynolds number (ratio of inertial to viscous stress) approximately 10^3

Engineered PRINT Aerosols



Computational Fluid Dynamics with Sorin Mitran (Applied Mathematics at UNC)

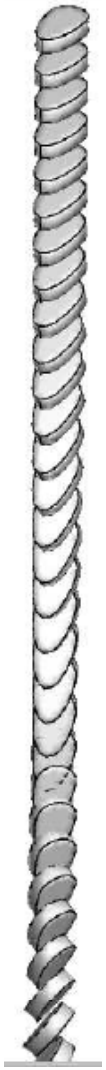




Lollipop – Lateral settling

Lollipop

Lorenz

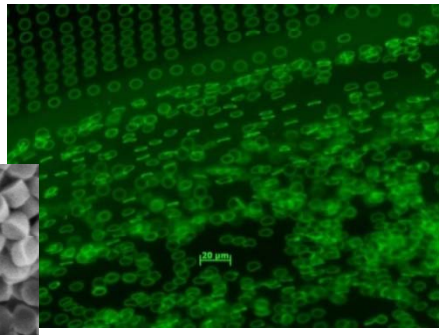


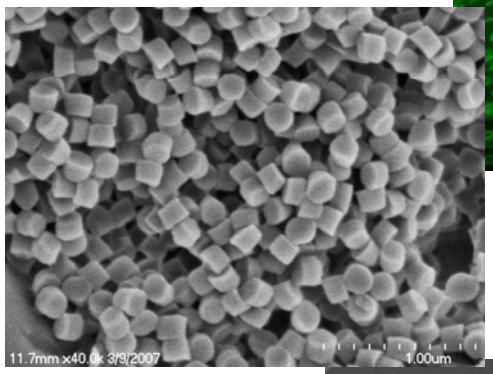
Ellipsoid

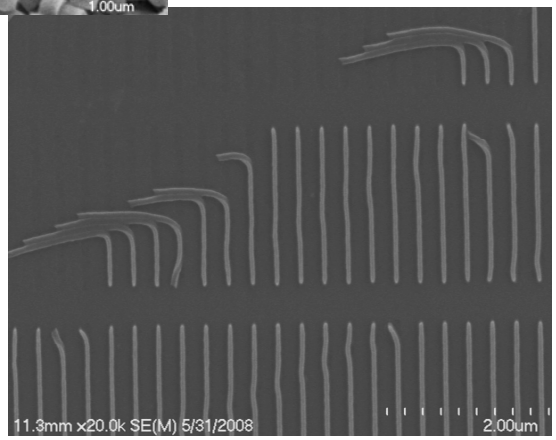
Ellipsoid with cut-out

Summary

- PRINT affords a systems engineering approach important for customizing therapeutics and vaccines
- **Tight control over key design parameters**
  - Size
  - Shape
  - Matrix chemistry
  - Deformability
  - Surface chemistry
- **A platform technology that provides:**
  - Calibration Quality Particles
  - Continuous manufacturing
  - Independent tuning of key variables
  - Quality by design







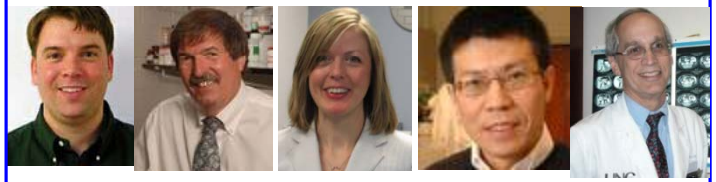
Innovation and Diversity

Semiconductor Clean Room

Collaborators

Mary Napier Jason Rolland Ben Maynor Jen Jen Yeh Jim Bear

Neal Fowler Billy Kim Richard Stack Ric Boucher



Bill Zamboni Shelley Earp Bolyn Hubby Rihe Liu Joel Tepper

DeSimone Team

Carolina Center of Cancer Nanotechnology Excellence

NCI Alliance for **Nanotechnology** in Cancer

DARPA

NIH Director's Pioneer Award

National Science Foundation

Office of Naval Research S&T NR

Revolutionary Research . . . Relevant Results

Liquidia Technologies

Solar Fuels & Next Generation Photovoltaics

Energy Frontier Research Center

The University of North Carolina at Chapel Hill