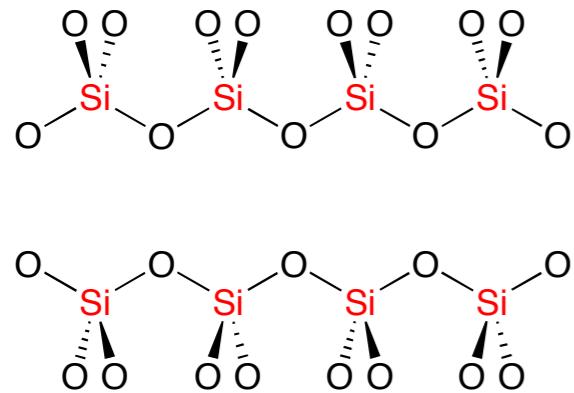


# Porous Silica Particles in Biology: Studies on Cellular Uptake, Molecular Delivery, and Imaging

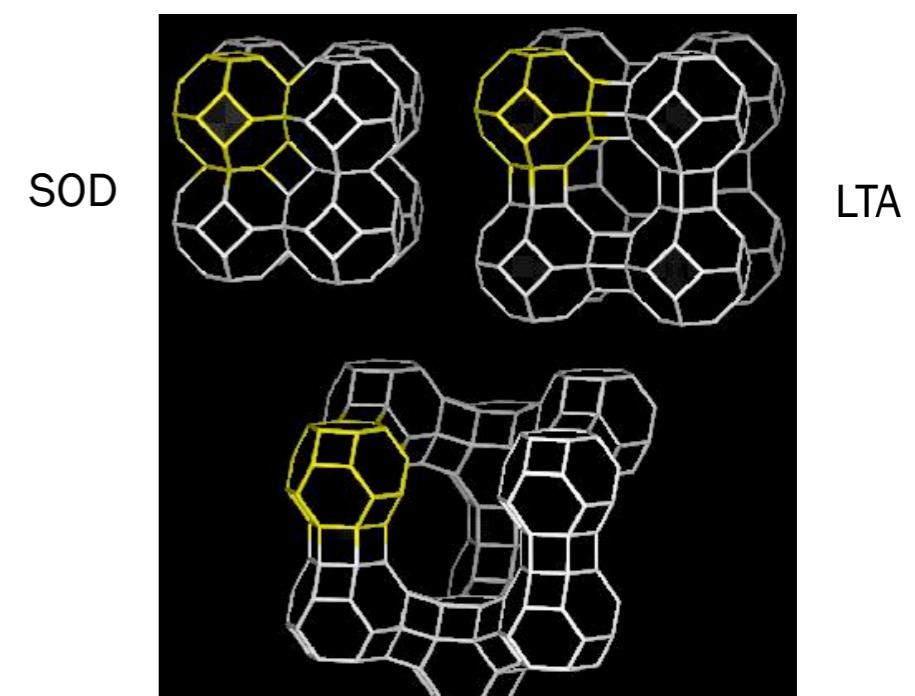
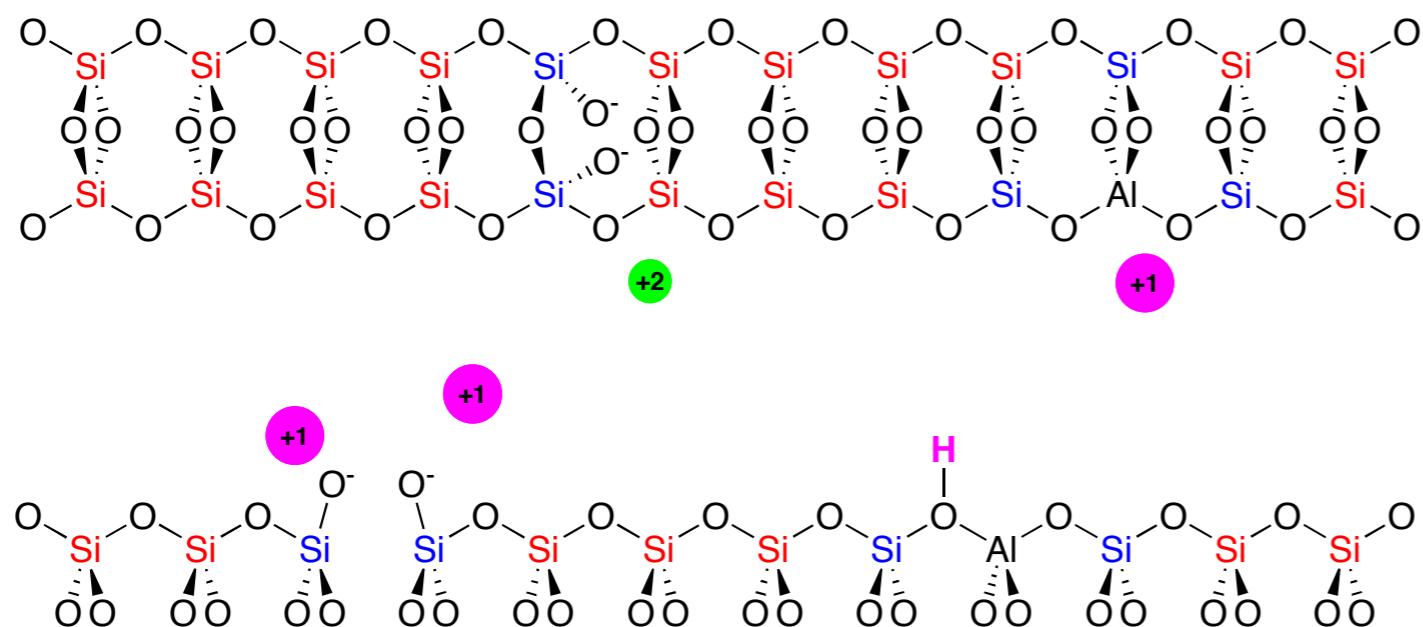
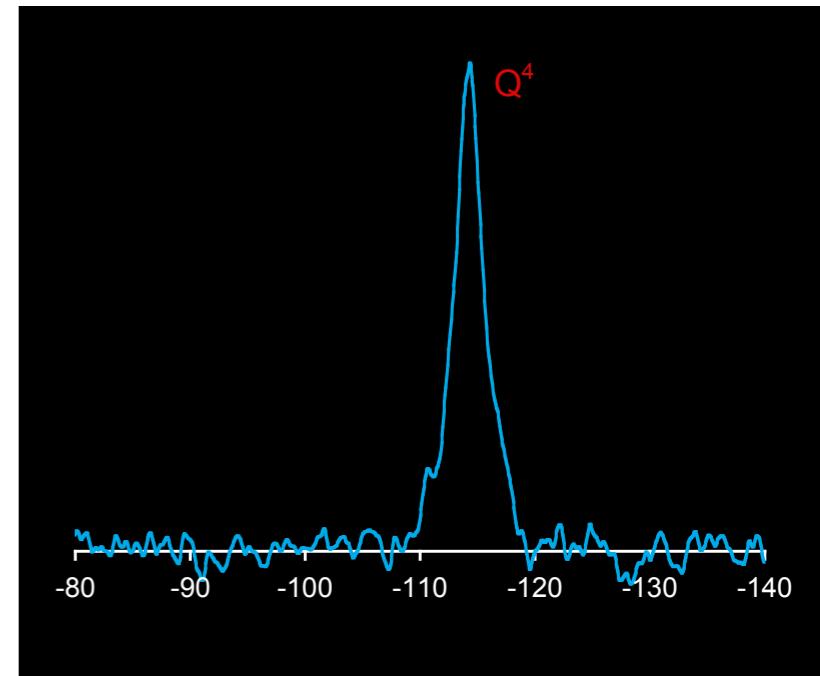
Christopher Landry  
Department of Chemistry  
University of Vermont

Geological Society of America  
Bretton Woods, NH  
March 19, 2013

# Zeolites

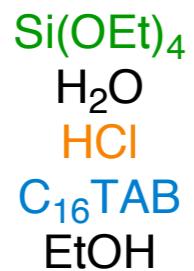


$Q^4$  = fully polymerized  $\text{SiO}_2$   
 $\delta \approx -110$  ppm in  $^{29}\text{Si}$  SSNMR  
 $Q^3$  = incompletely polymerized  $\text{SiO}_2$  (bulk or surface defect)  
 $\delta \approx -100$  ppm ( $\text{Si-O}^-$ ); -90 ppm ( $\text{Si-O-M}$ )



FAU (LTY)

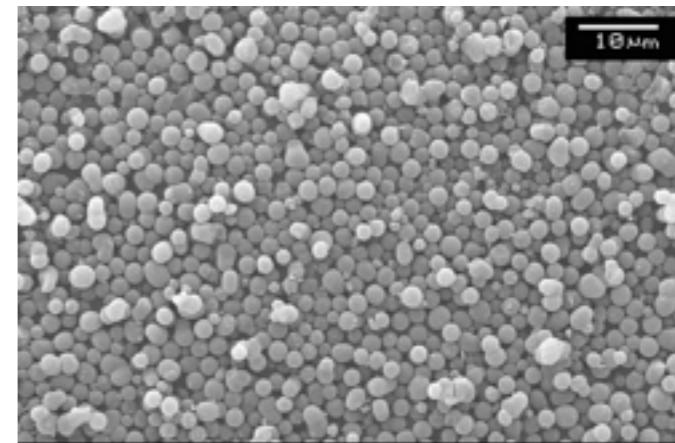
# Acid-Prepared Mesoporous Spheres (APMS)



stir 5 min  
RT

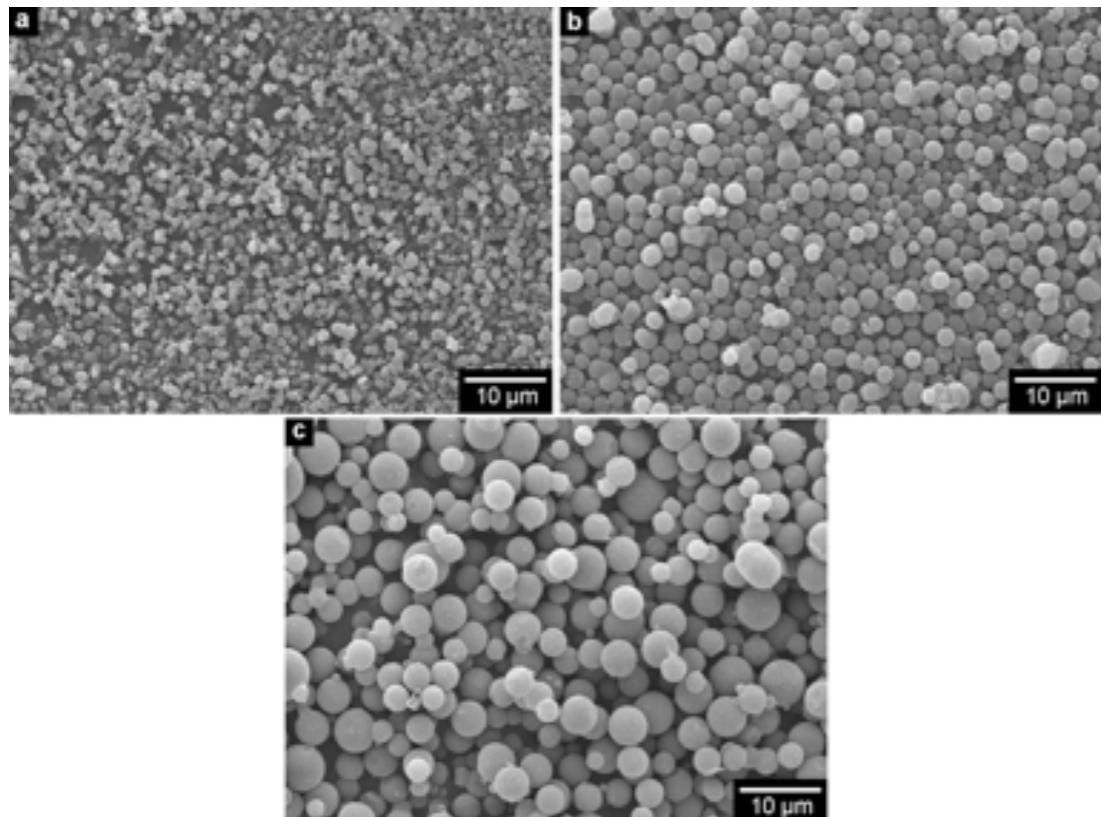
NaF  
stir 7 min  
RT

(precipitation  
begins)  
40 min  
100 °C



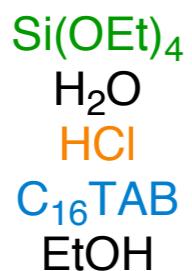
EtOH: slows polymerization rate of  $\text{Si(OEt)}_4$   
F<sup>-</sup>: increases number of nucleation sites  
heat: increases polymerization rate

adjusting variables relative to each other  
allows control over diameter of spheres



	particle diameter (μm)	NaF (mg)	EtOH (wt %)	$S_{\text{BET}}$ (m <sup>2</sup> /g)	$V_{\text{pore}}$ (cm <sup>3</sup> /g)	$d_{\text{pore}}$ (nm)
<b>a</b>	0.5 - 1.0	200	30	1022	1.35	3.9
<b>b</b>	1.5 - 2.5	100	20	894	1.20	4.8
<b>c</b>	2.5 - 5.0	50	10	672	1.15	6.6

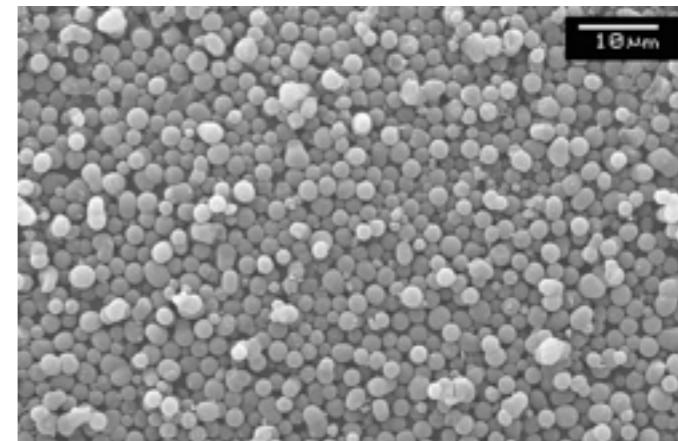
# Acid-Prepared Mesoporous Spheres (APMS)



stir 5 min  
RT

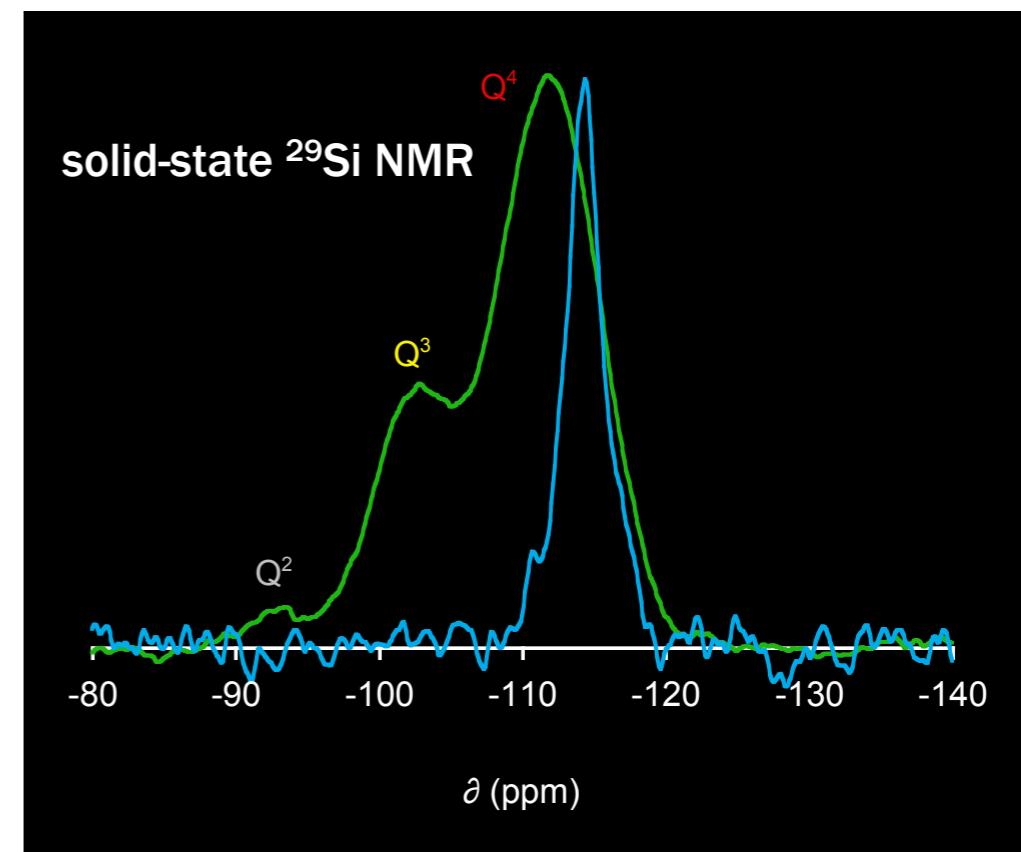
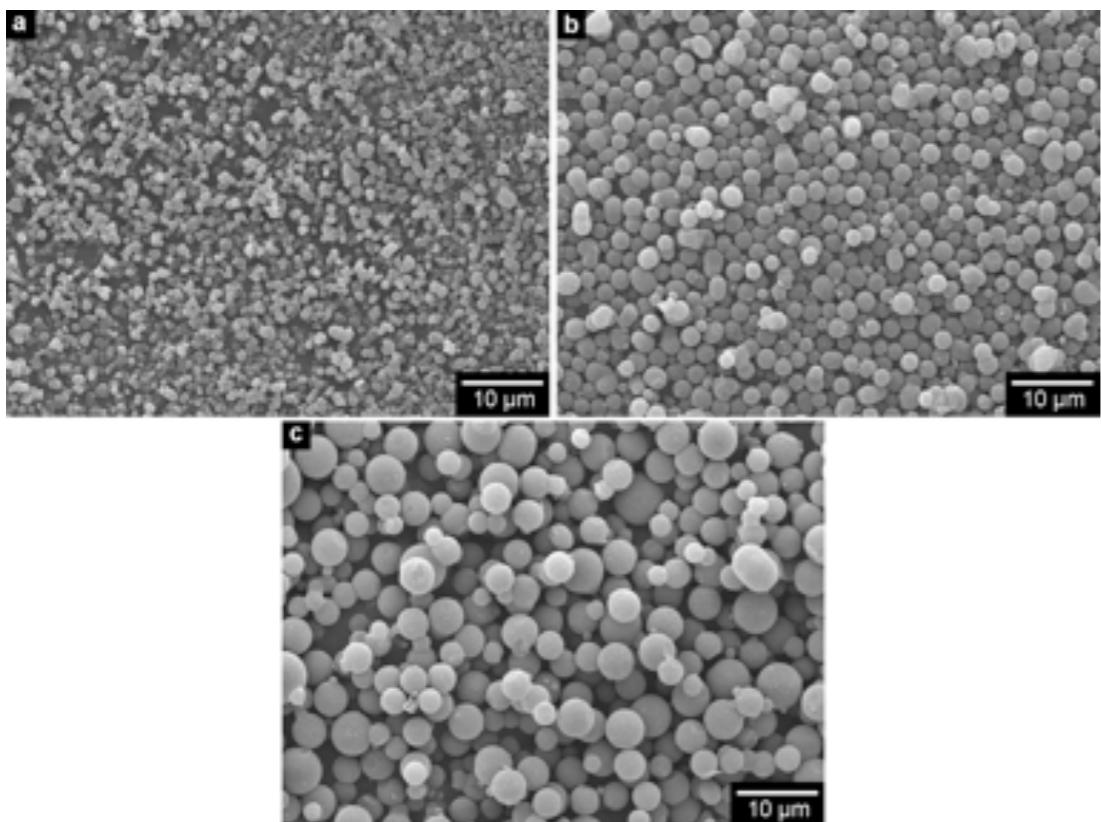
NaF  
stir 7 min  
RT

(precipitation  
begins)  
40 min  
100 °C

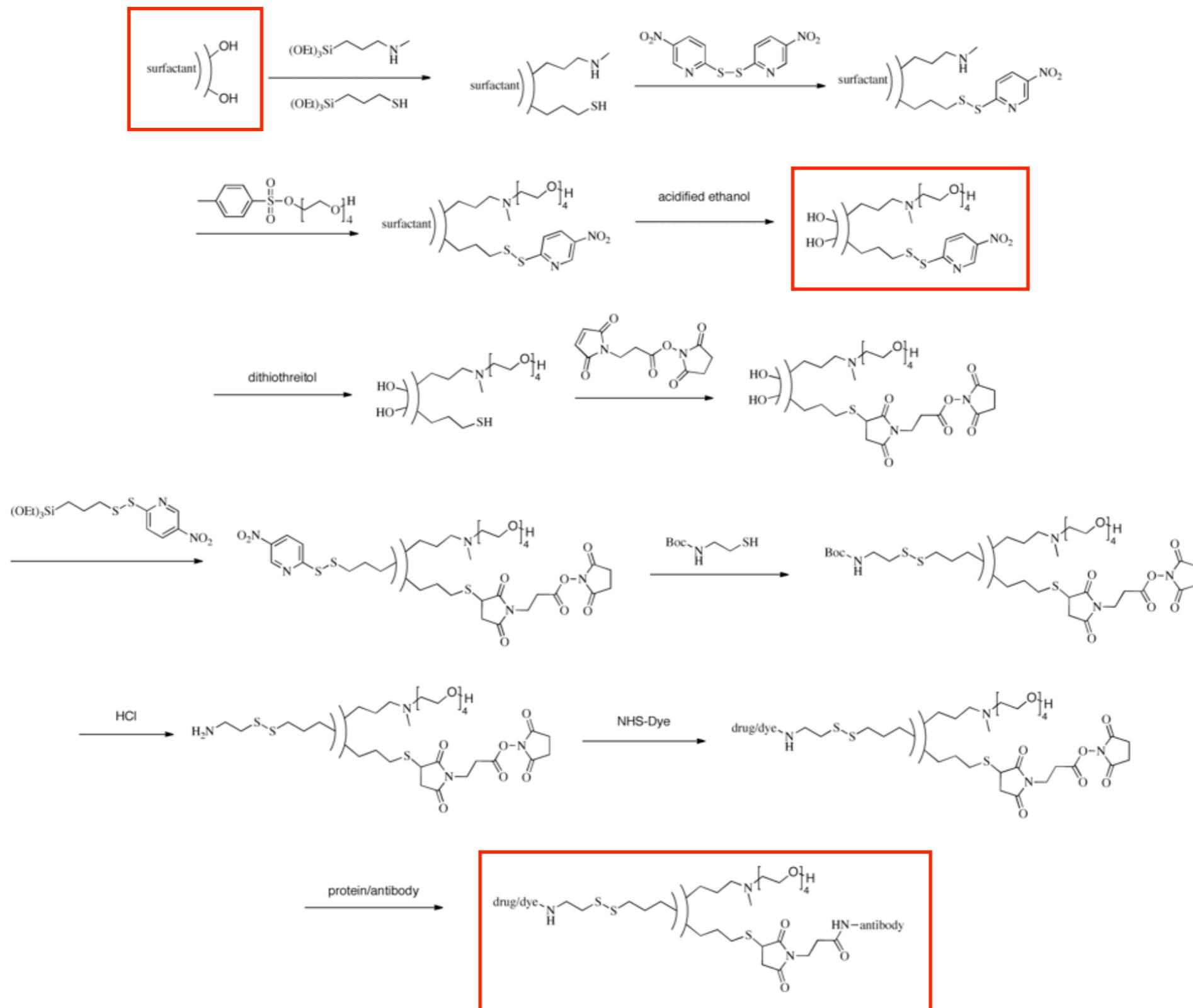


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F<sup>-</sup>: increases number of nucleation sites  
heat: increases polymerization rate

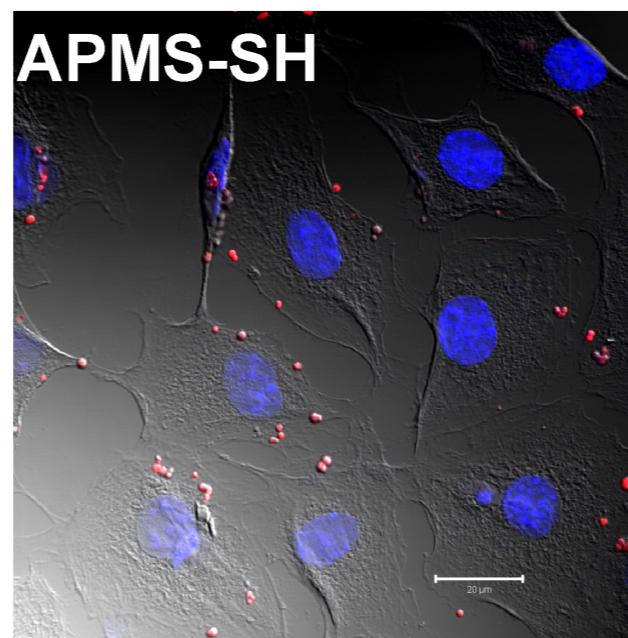
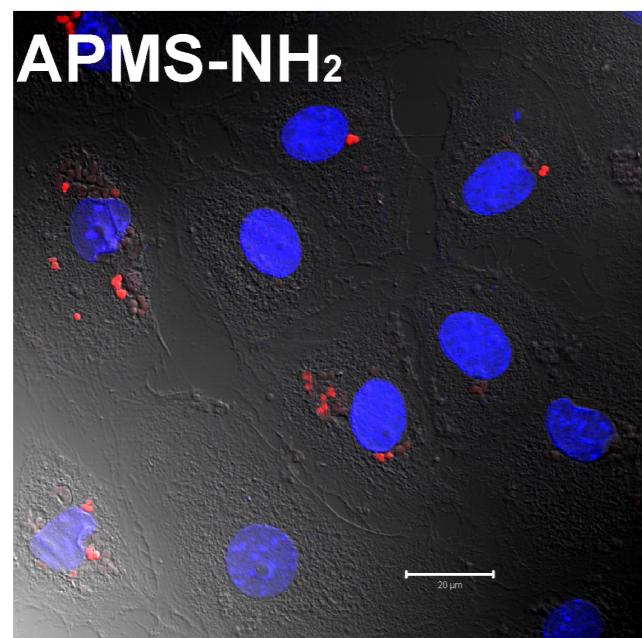
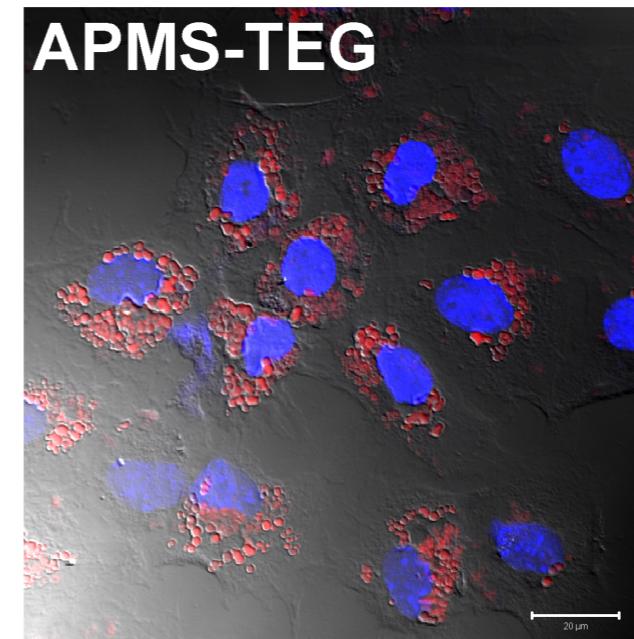
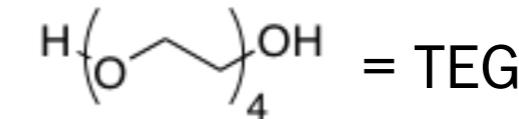
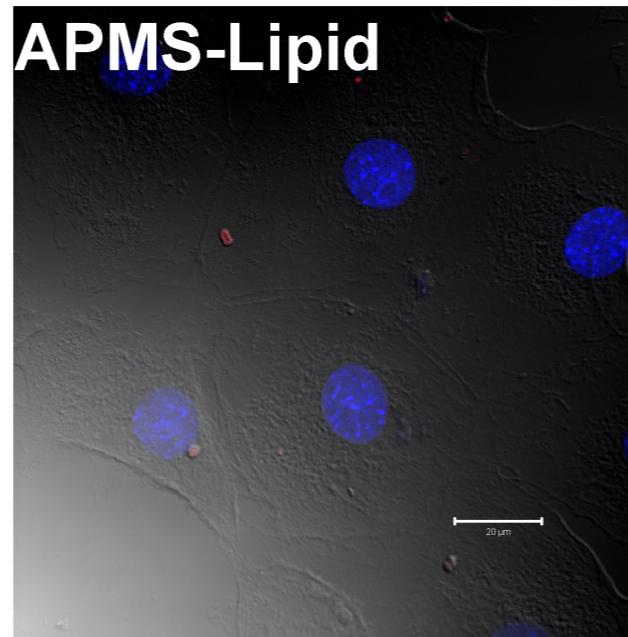
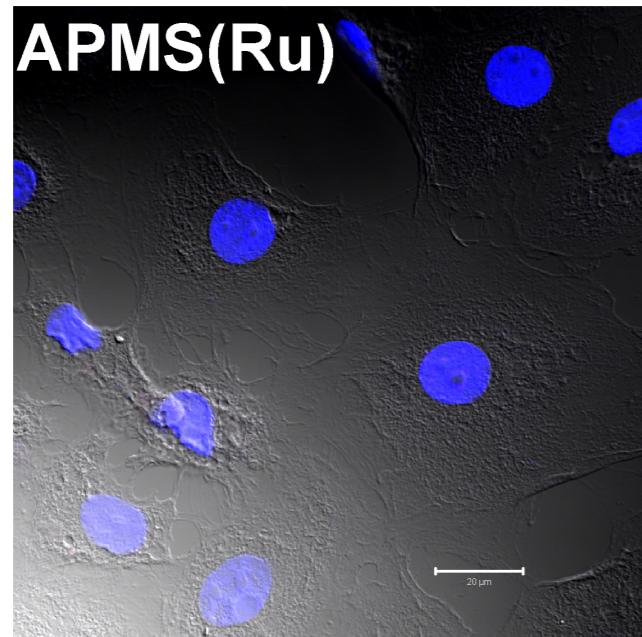
} adjusting variables relative to each other  
allows control over diameter of spheres



# Acid-Prepared Mesoporous Spheres (APMS)



# TEG-APMS Are Endocytosed By Lung Epithelial Cells



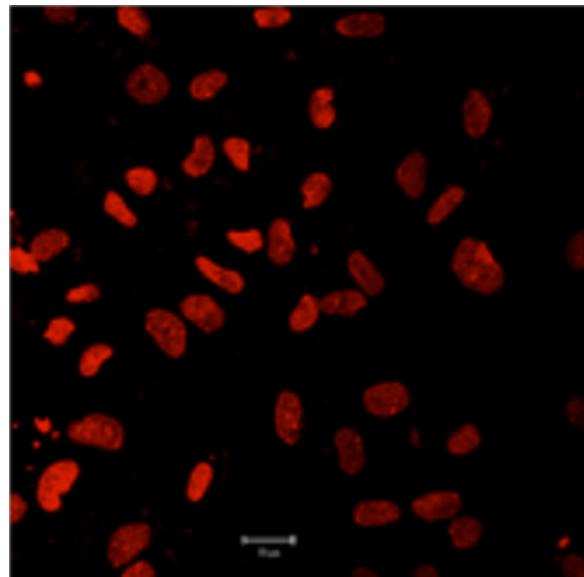
TEG facilitates uptake  
of APMS by C10 cells

- Label with **Alexa 568** or doped with **Ru(bpy)<sub>2</sub>**
- Incubate with C10 cells for 24 h *in vitro*
- Fix, stain nuclei with **DAPI**

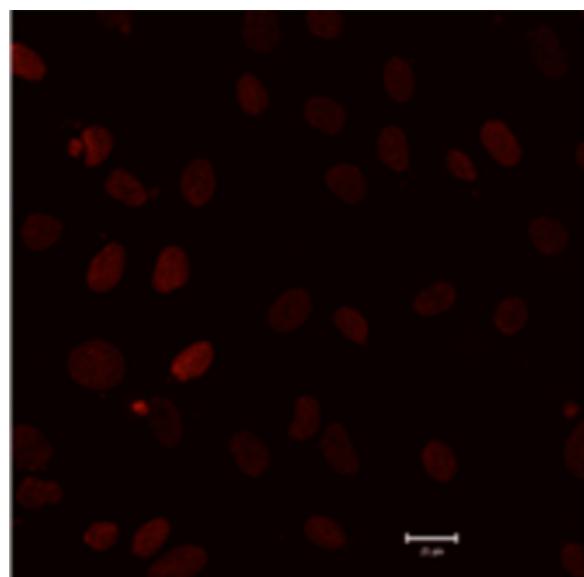
# Doxorubicin Is Delivered To MM Cells

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APMS+Dox



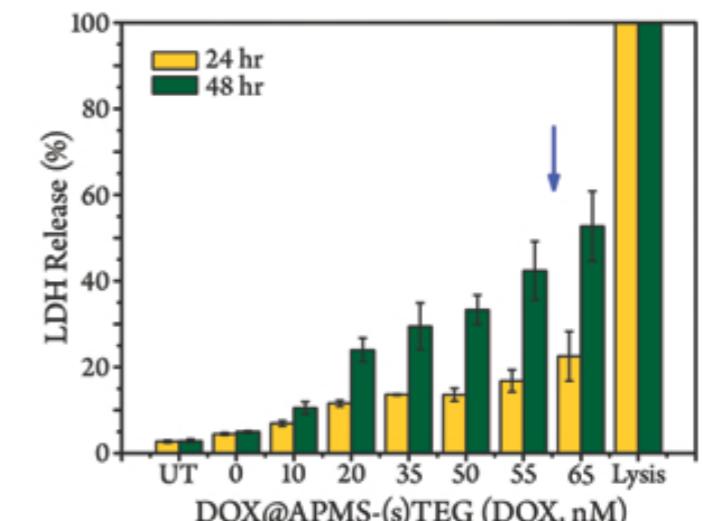
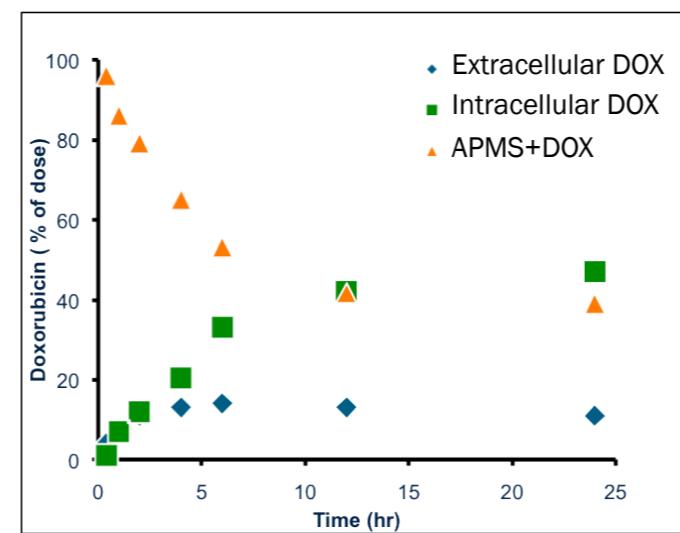
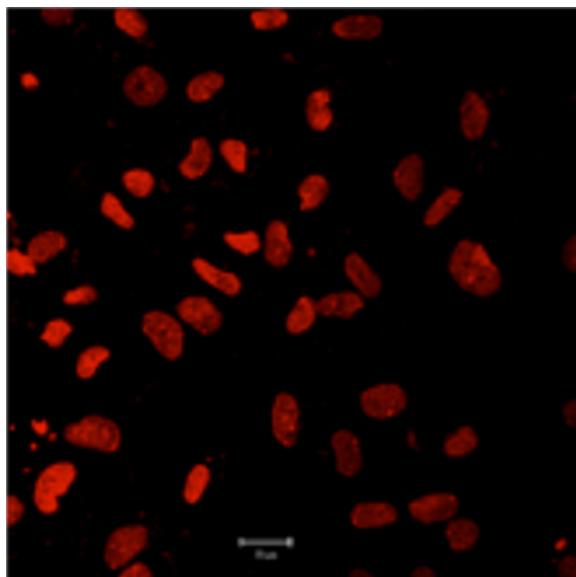
Dox alone



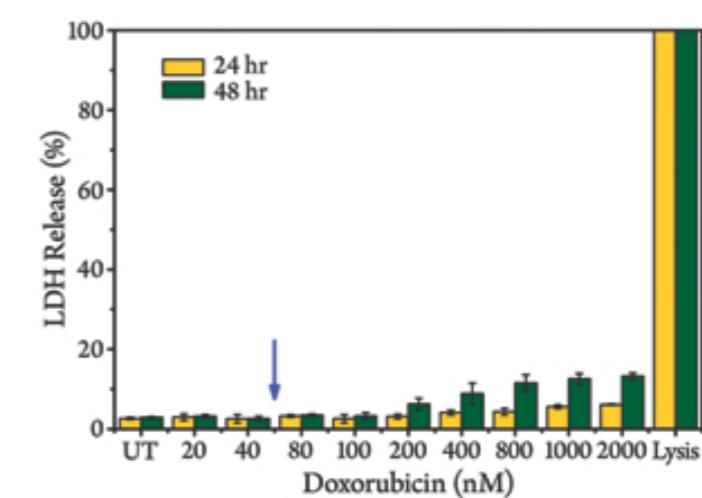
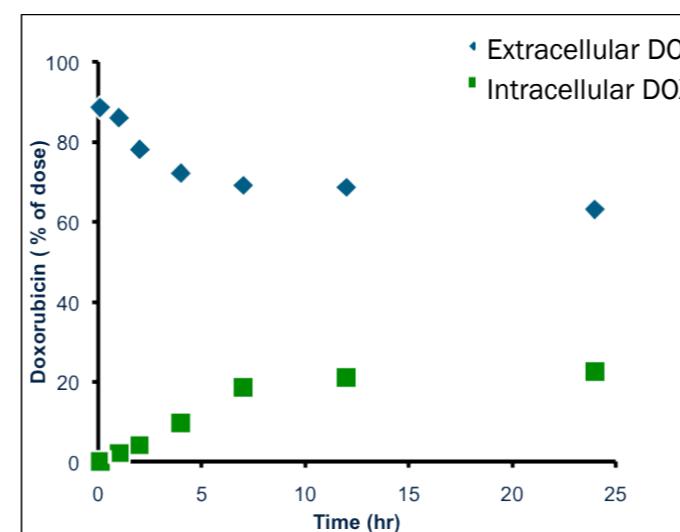
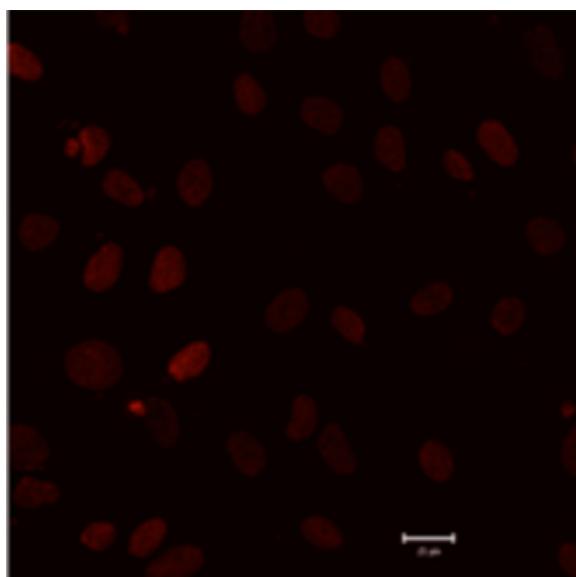
microscopy, 8 hr

# Doxorubicin Is Delivered To MM Cells

APMS+Dox



Dox alone

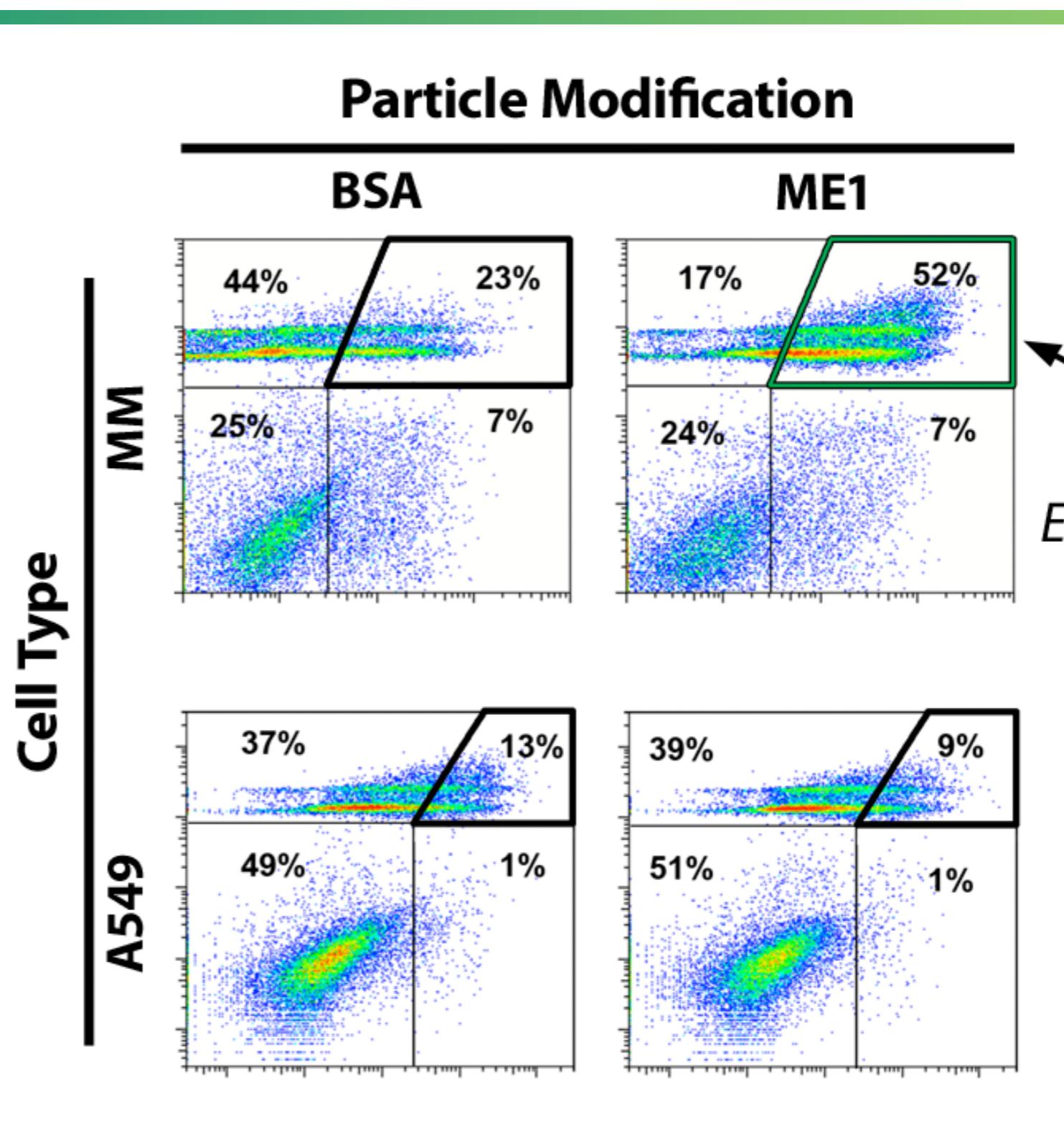
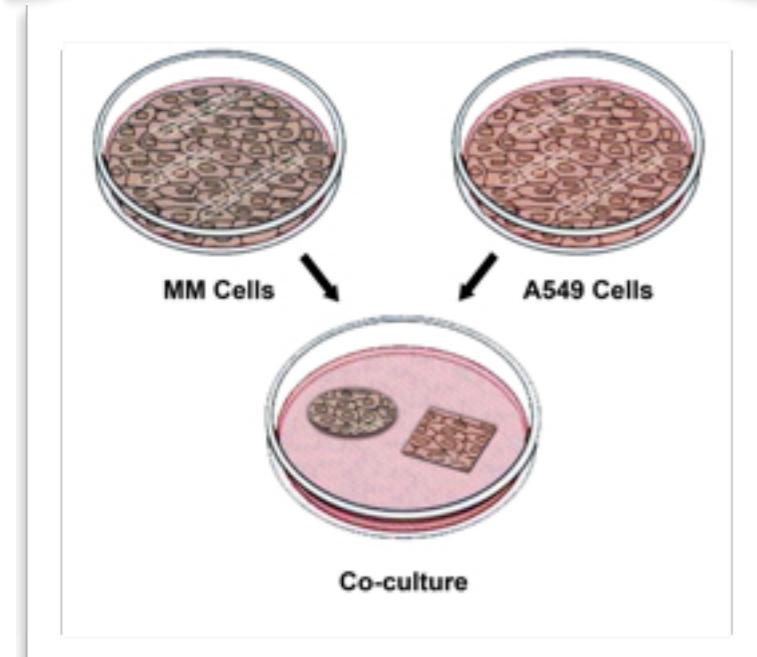
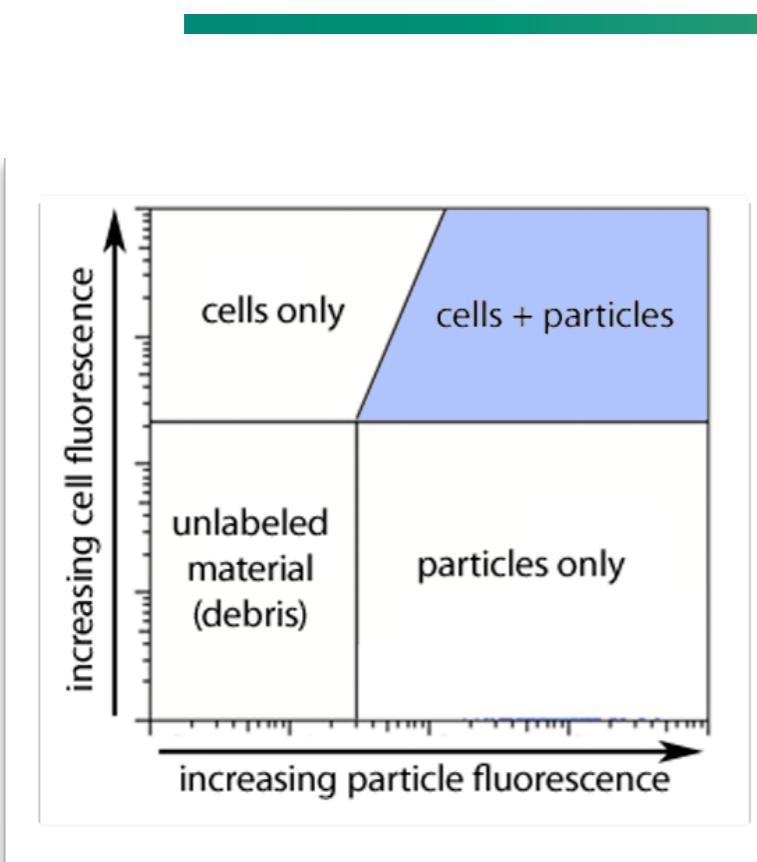


microscopy, 8 hr

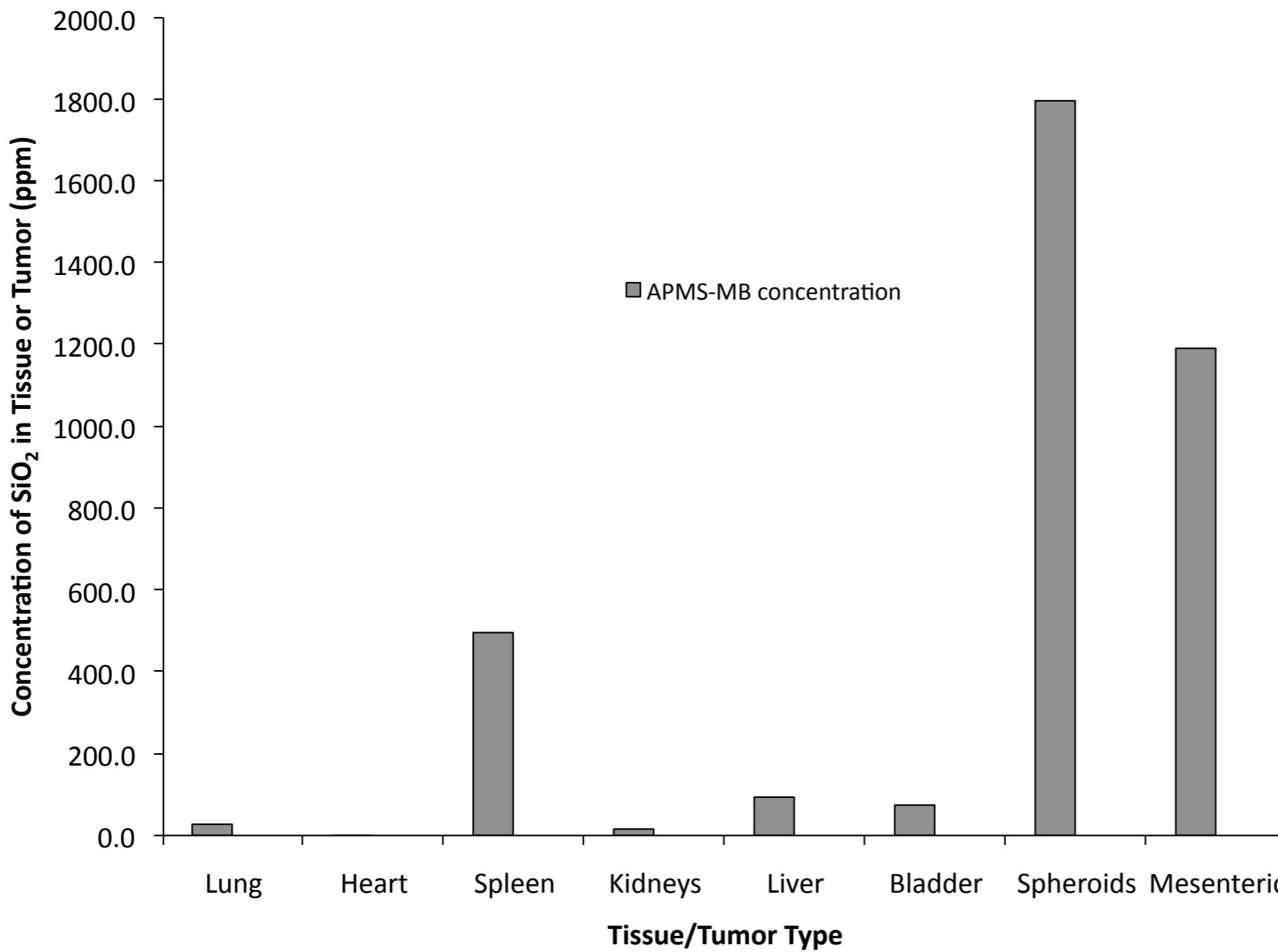
HPLC, 24 hr

dose-response

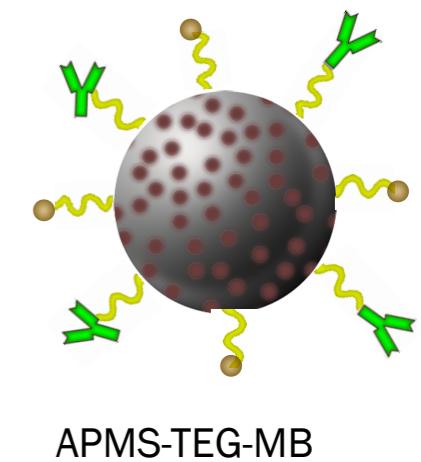
# APMS-TEG-Ab Is Selectively Endocytosed



# Targeted Particles Are Non-Immunogenic

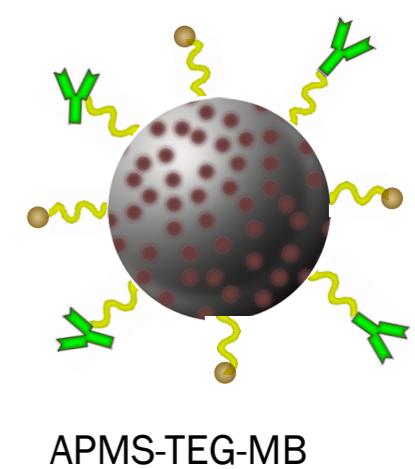
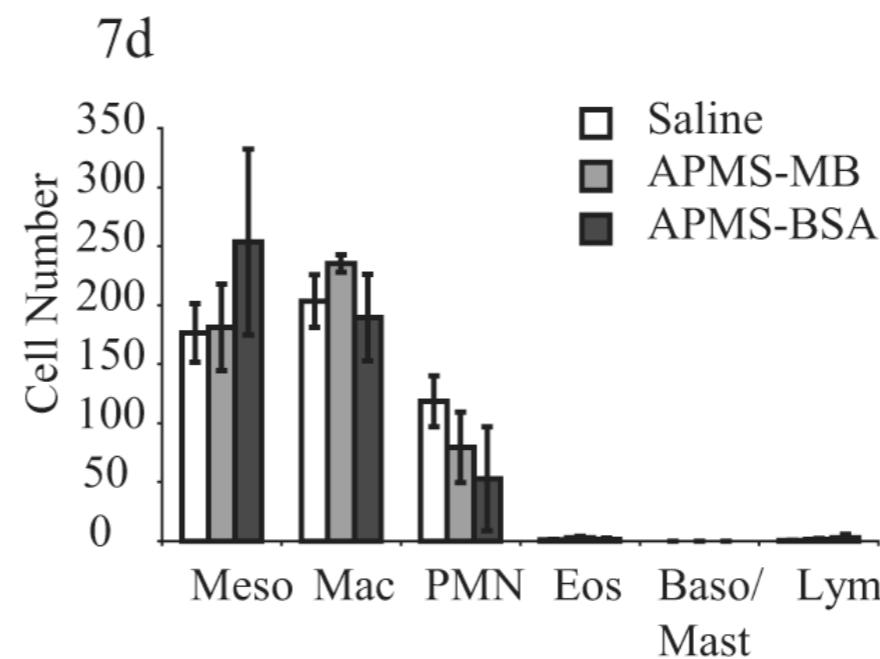
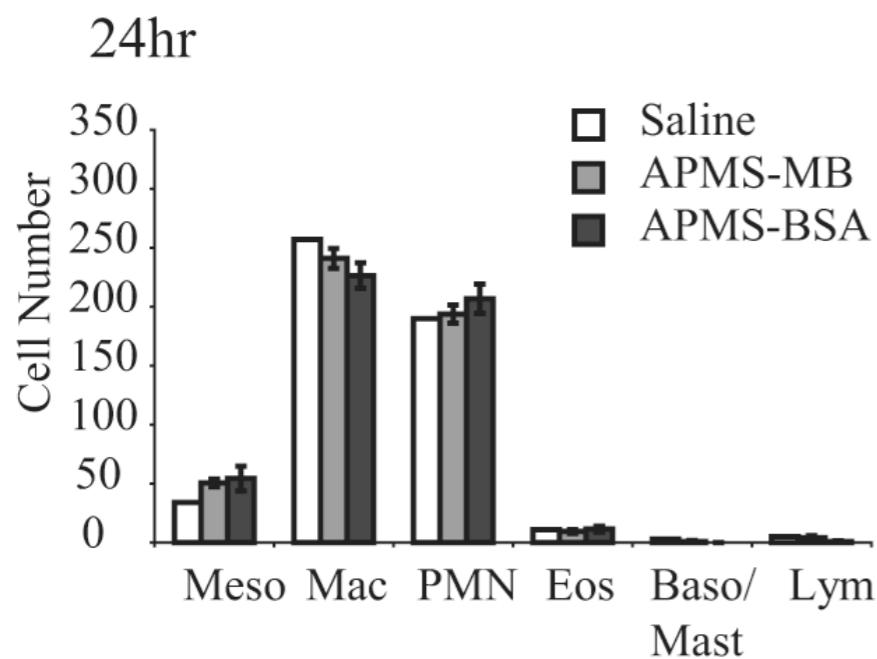


- Human MM tumors grown in mouse IP cavity
- After 4 weeks, **APMS-TEG-MB** (targeted) or **APMS-TEG-BSA** (control) injected into IP cavity
- Euthanasia after 24 h, organs and tumors collected
- Digestion in (CH<sub>3</sub>)<sub>4</sub>NOH followed by ICP-MS (Dartmouth)

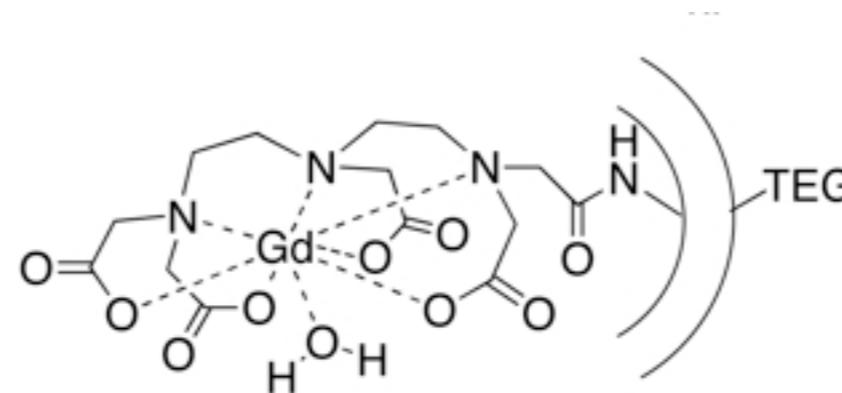
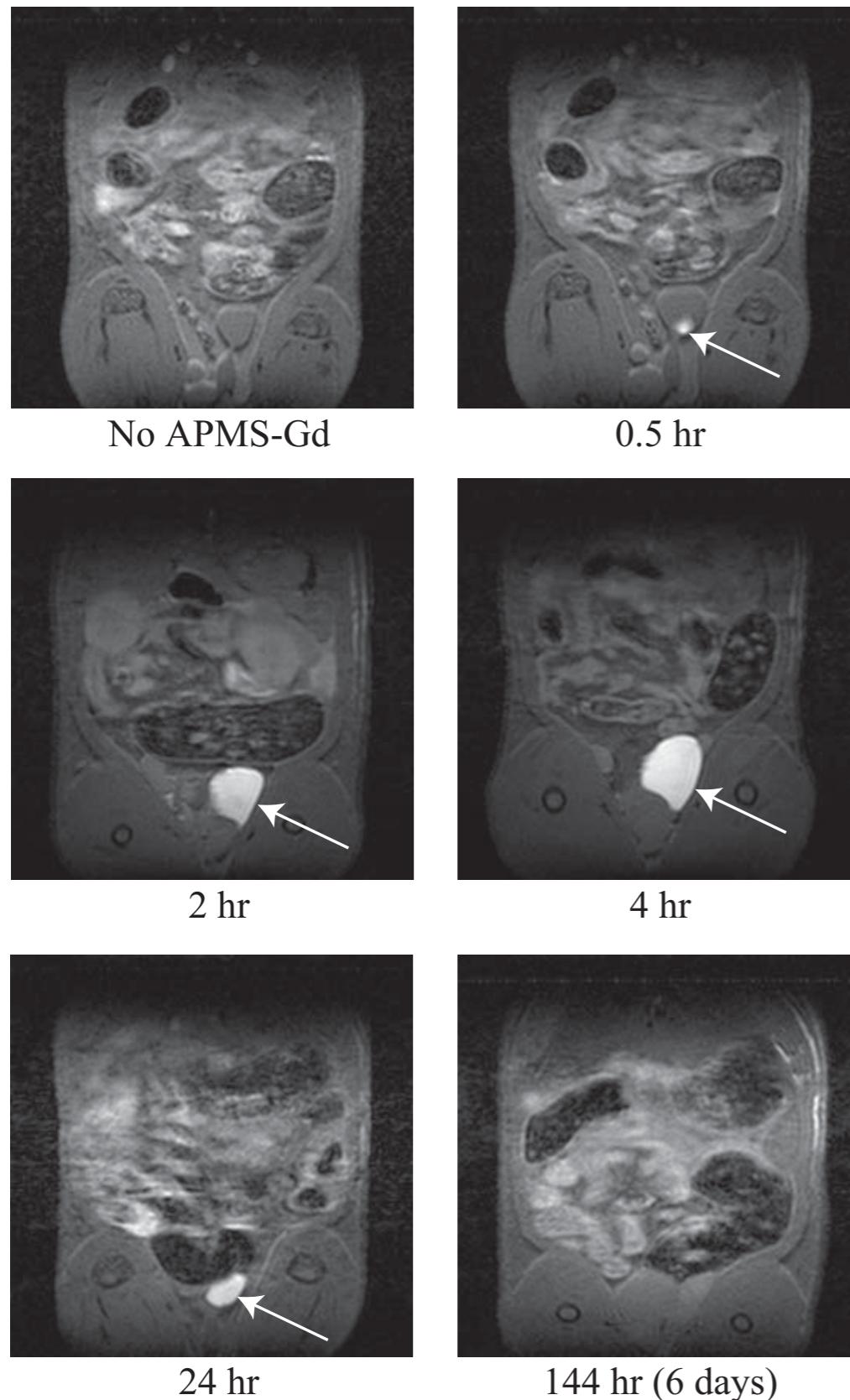


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# Excretion Of Microparticles Followed By MRI

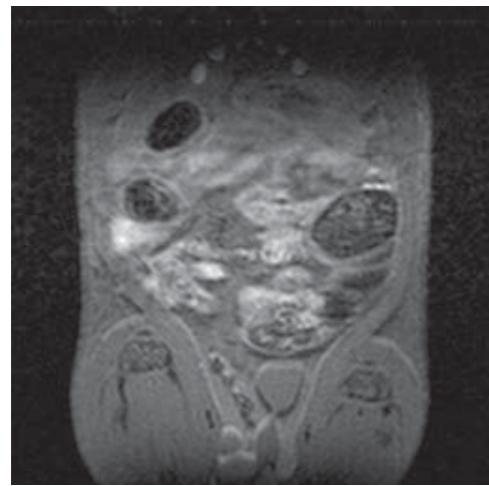


MRI shows that APMS-TEG is excreted in the urine by 24 hr. The bladder has returned to normal intensity after 6 days, indicating that APMS-TEG has been excreted.

Relaxivities ( $\text{mM}^{-1}\text{s}^{-1}$ ) at 1.5 T suspended in agar.

Sample	$r_1$ (per Gd)	$r_1$ (per particle)	$r_2$ (per Gd)	$r_2$ (per particle)
APMS/Gd	$2.8 \pm .1$	$\sim 3 \times 10^9$	$23 \pm 1$	$\sim 2 \times 10^{10}$
DTPA/Gd <sub>(aq)</sub>	$2.9 \pm .2$	-	$2.9 \pm .2$	-

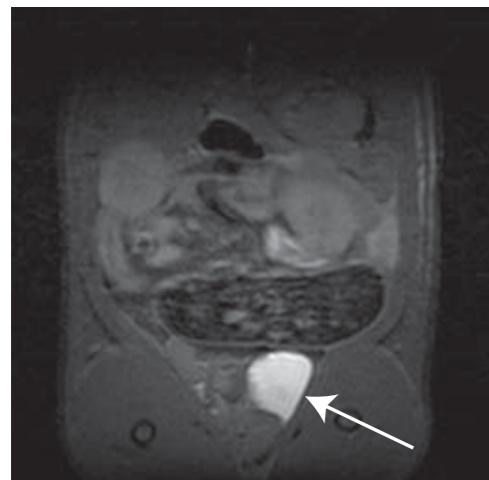
# Excretion Of Microparticles Followed By MRI



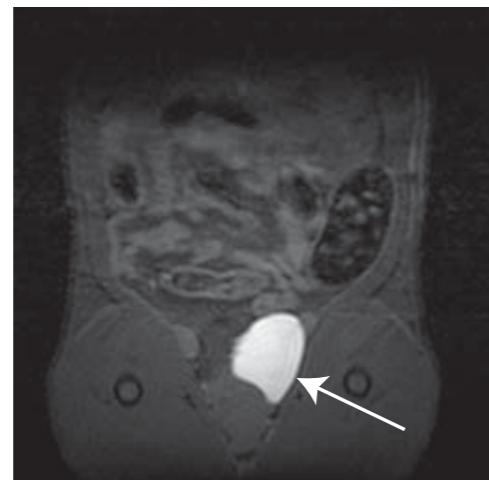
No APMS-Gd



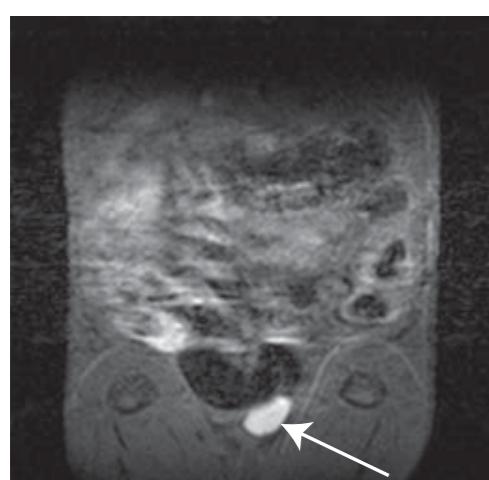
0.5 hr



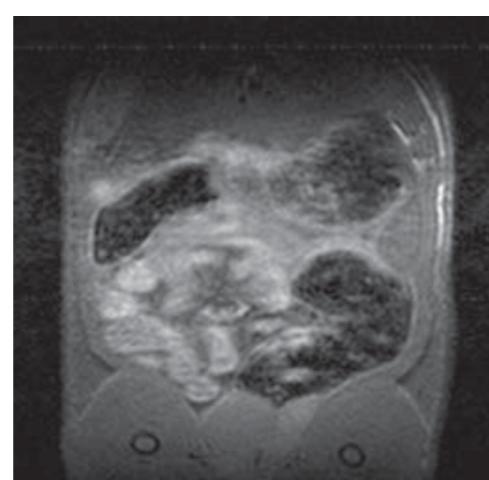
2 hr



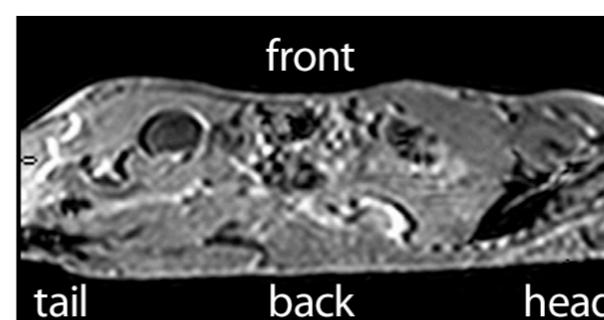
4 hr



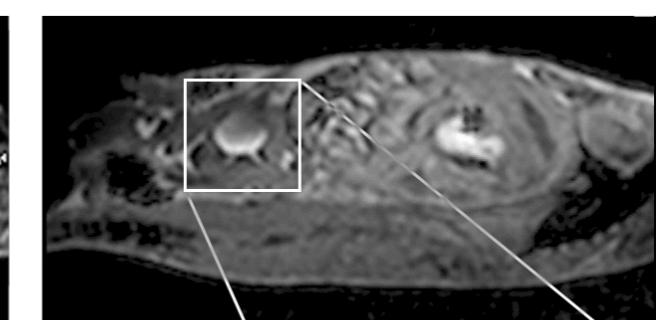
24 hr



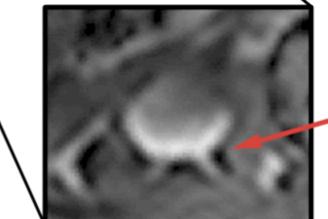
144 hr (6 days)



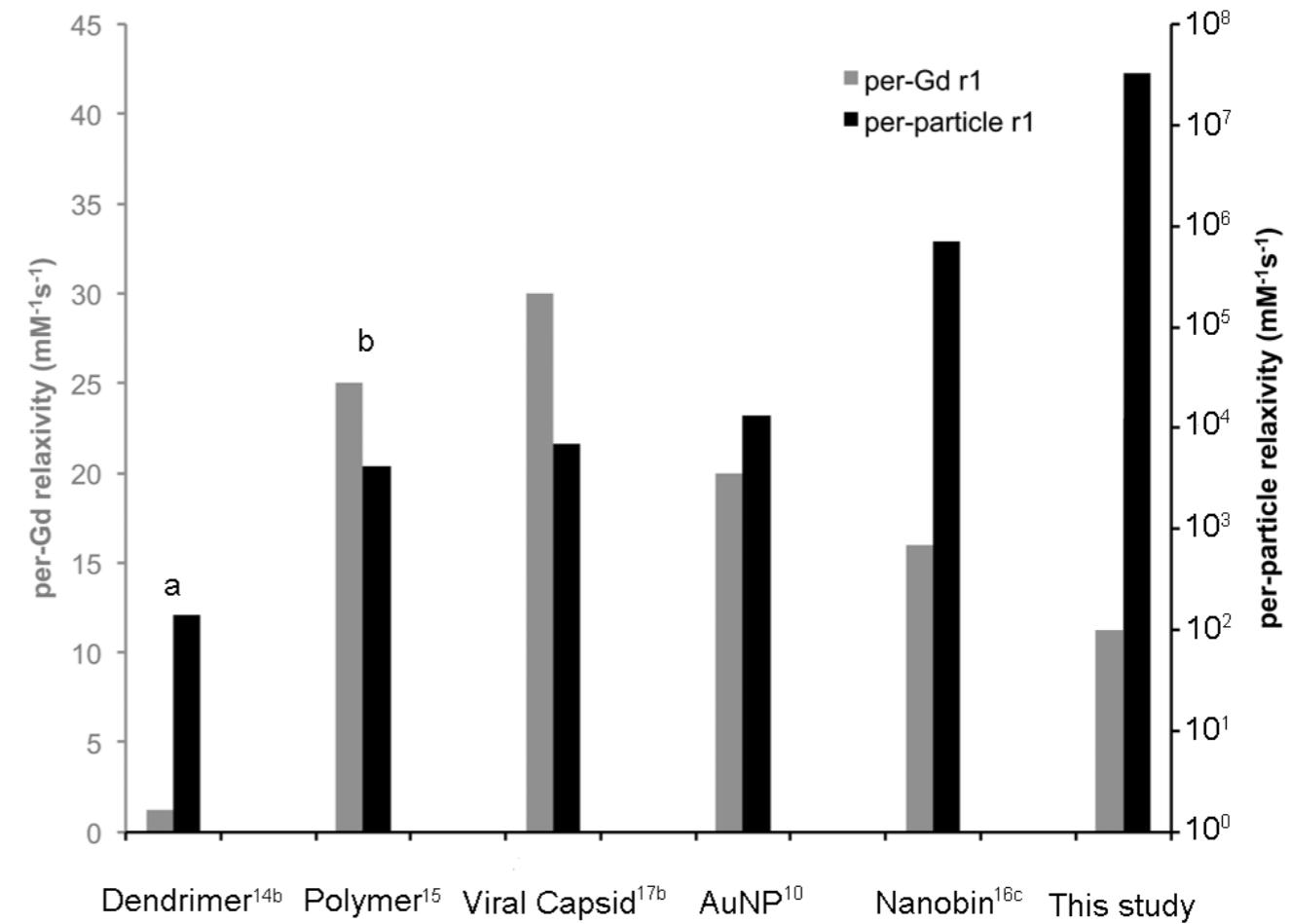
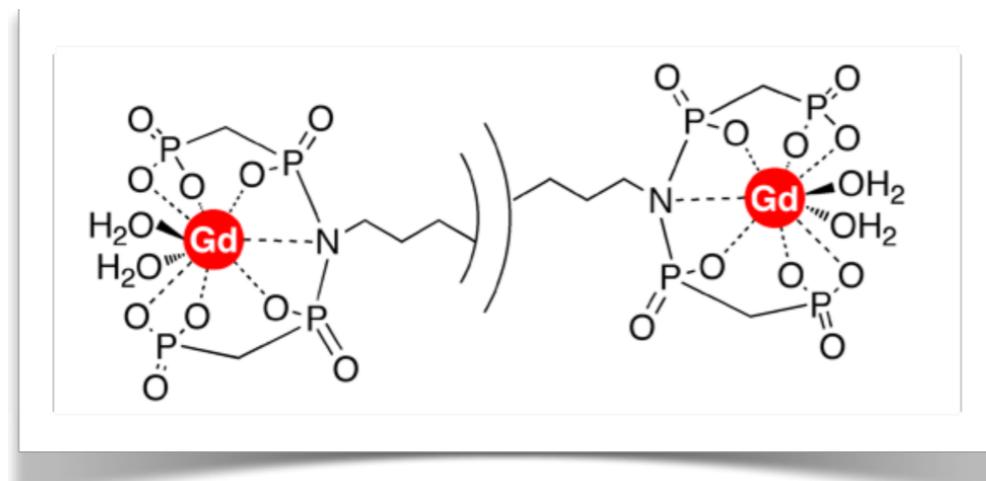
Pre-Injection



2 cm  
30 minutes



# New Gd Chelates Have High Relaxivities

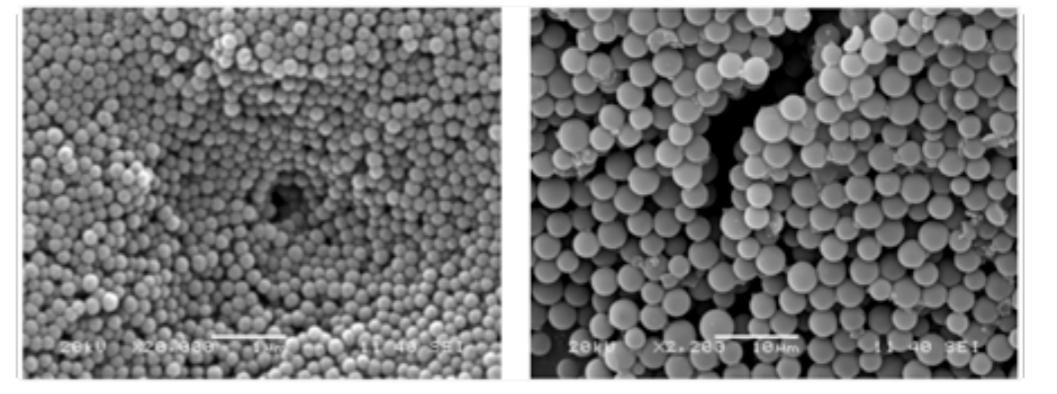


	Sample	as-made			functionalized			per-Gd relaxivity		per-particle relaxivity	
		$d_{pore}$ (Å)	$SA_{BET}$ ( $\text{m}^2/\text{g}$ )	$V_{pore}$ ( $\text{cm}^3/\text{g}$ )	$d_{pore}$ (Å)	$SA_{BET}$ ( $\text{m}^2/\text{g}$ )	$V_{pore}$ ( $\text{cm}^3/\text{g}$ )	$r_1$ ( $\text{mM}^{-1}\text{s}^{-1}$ )	$r_2$ ( $\text{mM}^{-1}\text{s}^{-1}$ )	$r_1$ ( $\text{mM}^{-1}\text{s}^{-1} \times 10^7$ )	$r_2$ ( $\text{mM}^{-1}\text{s}^{-1} \times 10^7$ )
standard synthesis	<b>1</b>	36	790	0.87	33	380	0.41	4.6	15	3.3	11
rehydroxylated	<b>2</b>	39	780	0.65	34	350	0.35	6.0	17	1.7	5.0
pore-expanded	<b>3</b>	55	690	0.97	45	380	0.56	10	23	2.4	5.4
	<b>Gd-DOTA<sup>2</sup></b>	-	-	-	-	-	-	3.0	3.3	-	-
	<b>Gd-DTPA<sup>2</sup></b>	-	-	-	-	-	-	3.5	3.9	-	-

# Ongoing Studies

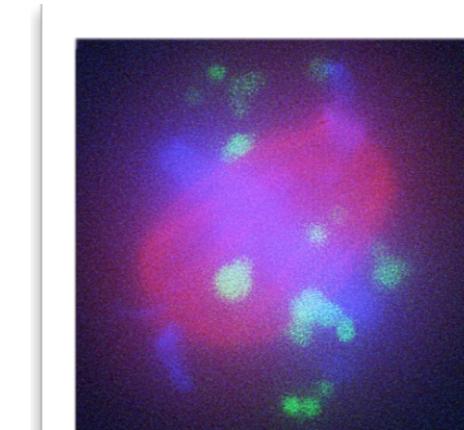
## 1. Effect of particle size on rate of uptake and trafficking of particles

- 200 , 1000, 3000 nm
- 0 - 100 % ethylene glycol
- Vesicular, mitochondrial, and microtubule staining



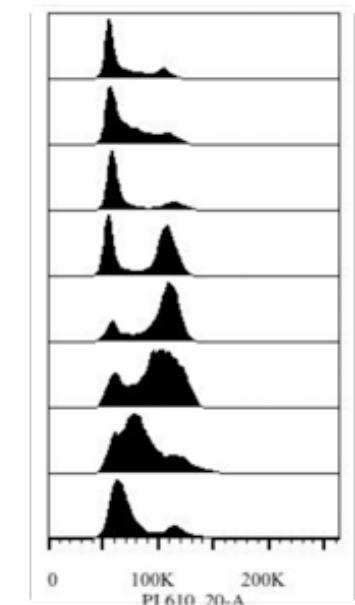
## 2. Trafficking of particles during mitosis

- DNA and microtubule staining
- Live cell imaging over 2 to 8 hours



## 3. Delivery of CDK inhibitors through cleavable covalent bonds

- Cell viability assays
- Flow cytometry analysis of cell cycle progression



# Acknowledgements

Chemistry (Past):

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Dr. Dino El-Boubou

Doug Fox

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Alex Duncan

Eric Chase

Dan Ciabattoni

U.C. Berkeley:

Ken Raymond

Piper Klemm

Pathology:

Prof. Brooke Mossman

Prof. Arti Shukla

Prof. Nick Heintz

Prof. Doug Taatjes

Ted James, M.D.

Dr. Steve Blumen

Dr. Jedd Hillegass

Sherrill Lathrop

Stacie Buschel

Dartmouth:

Risto Kauppinen

ITQ:

Dr. Avelino Corma Canos

Dr. Pablo Botella Ascunción

Dr. Manuel Quesada Vilar

Carlos Muniesa Lajara

