

Nanoparticle Imaging Probes for Molecular Imaging with Computed Tomography

Ryan K. Roeder

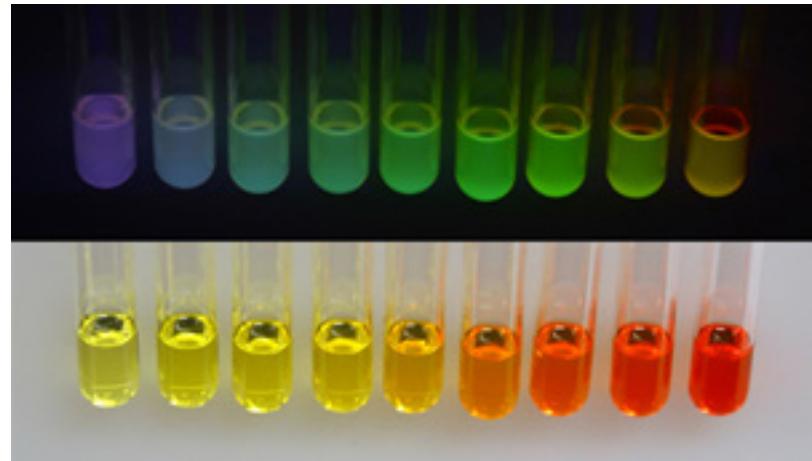
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Nanoparticle Contrast Agents

- luminescence, fluorescence
(e.g., QDs, Au NPs)
- optical coherence tomography
(e.g., Au NPs)



- electron microscopy
(e.g., immunogold)
- radiography, computed tomography
(e.g., Au NPs)
- magnetic resonance imaging
(e.g., SPIO or Fe_3O_4)



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Computed Tomography (CT)

Clinical Significance

- 75% of clinical diagnostic imaging procedures use X-rays. *Yu et al., 1999*
- 70 million CT scans per year in U.S. *de González et al., 2009*



Advantages

- non-invasive 3D anatomic imaging
- high spatial and temporal resolution
- low cost and wide availability

Elliott, 2005; Kircher et al., 2012; Goldman, 2009

Limitations

- low attenuating tissues require contrast agents for imaging
- lower sensitivity (mM) compared to PET and MRI (μM)
- molecular imaging not (yet) possible

Willmann et al., 2008; Kircher et al., 2012



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Renaissance in X-Ray Contrast Agents

1895 - 1st X-Ray



Roentgen

1920s - BaSO₄



1950s - iodine



White et al., 2001

2010s - Au NPs



*Hainfield
et al., 2004*

2010s + Bi, Gd₂O₃, HfO₂, Ta₂O₅, WO₃, Yb₂O₃ and other NPs!



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Au NPs as X-ray Contrast Agents

Milestones

~2004 1st demonstrations as a blood pool contrast agent

Hainfield et al., 2004, 2006

~2010 1st demonstrations of targeted delivery *in vivo*

Sun et al., 2009; Chanda et al., 2010; Cormode et al, 2010; Eck et al., 2010

Advantages

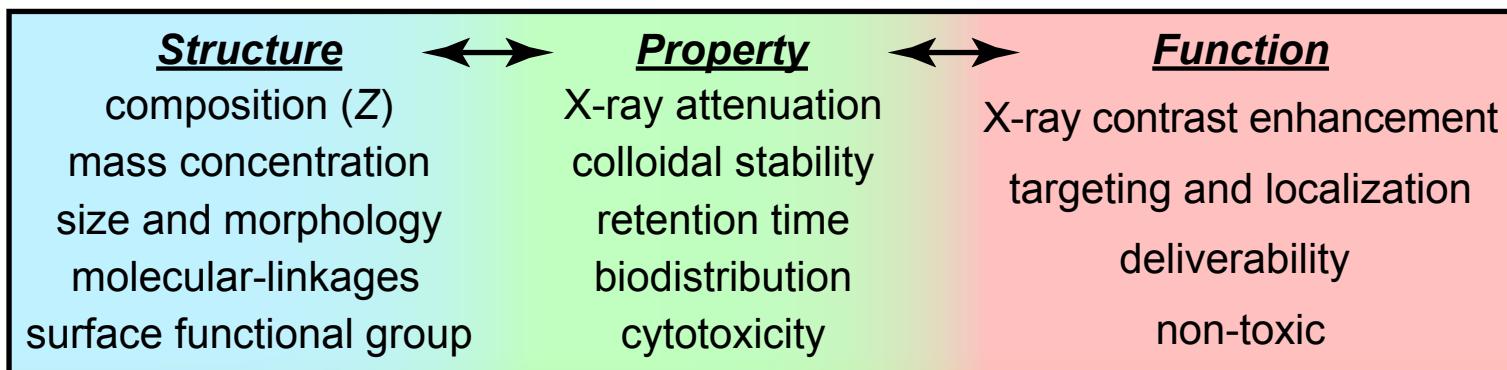
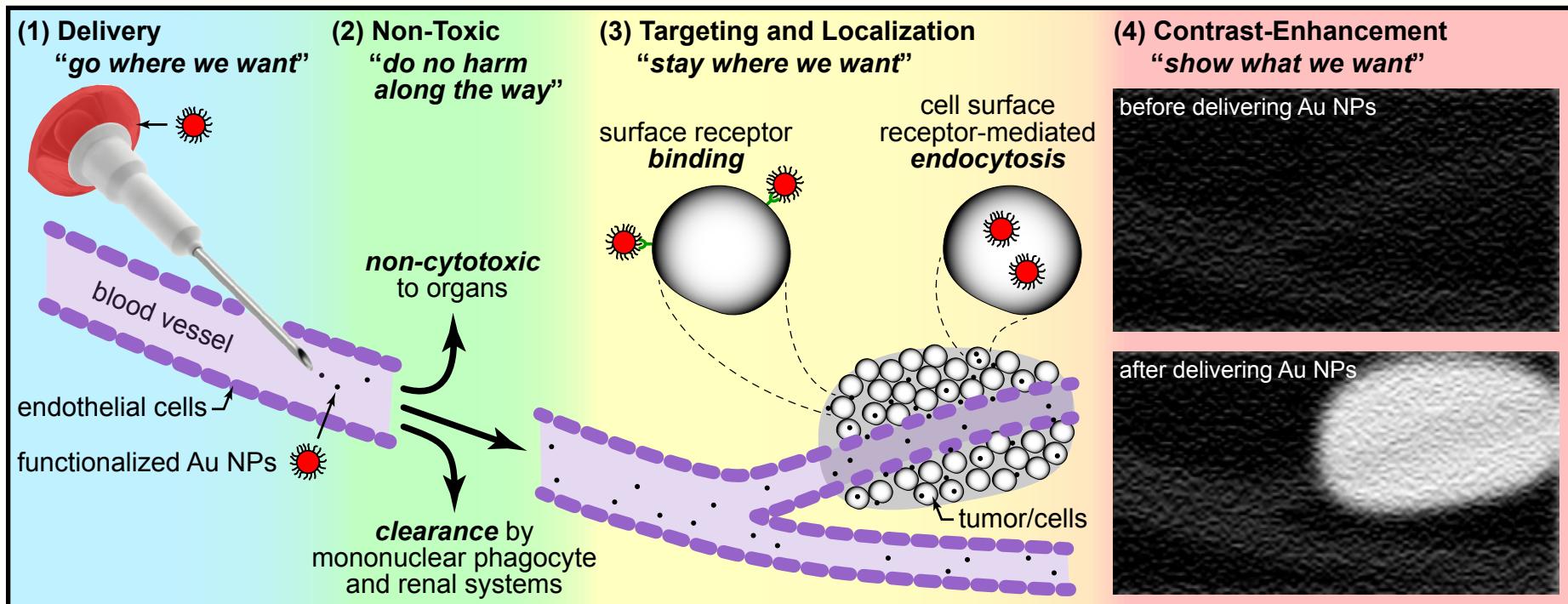
- high X-ray attenuation
 - high mass concentration per particle
 - k -edge at 80.7 keV
- non-toxicity
- facile synthesis of monodispersed NPs
- facile surface functionalization leveraging thiol and amine linkages
 - colloidal stability
 - vascular retention
 - targeted delivery



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Design of Targeted NP Imaging Probes

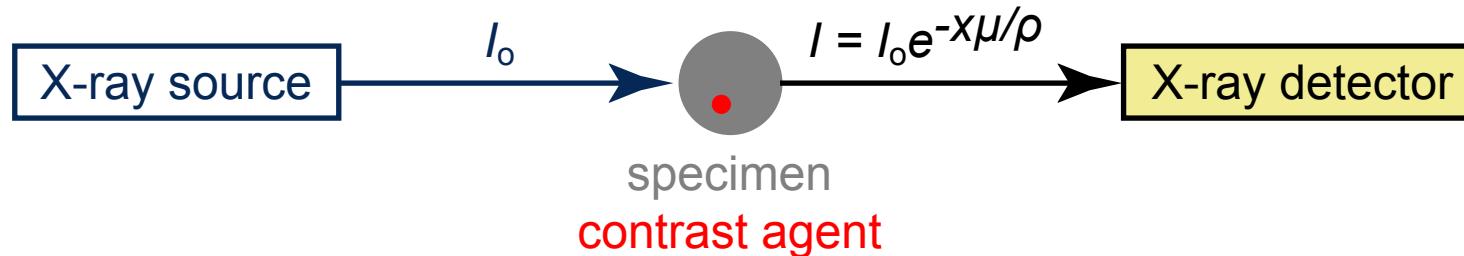


Cole et al., *Nanomedicine*, 2015



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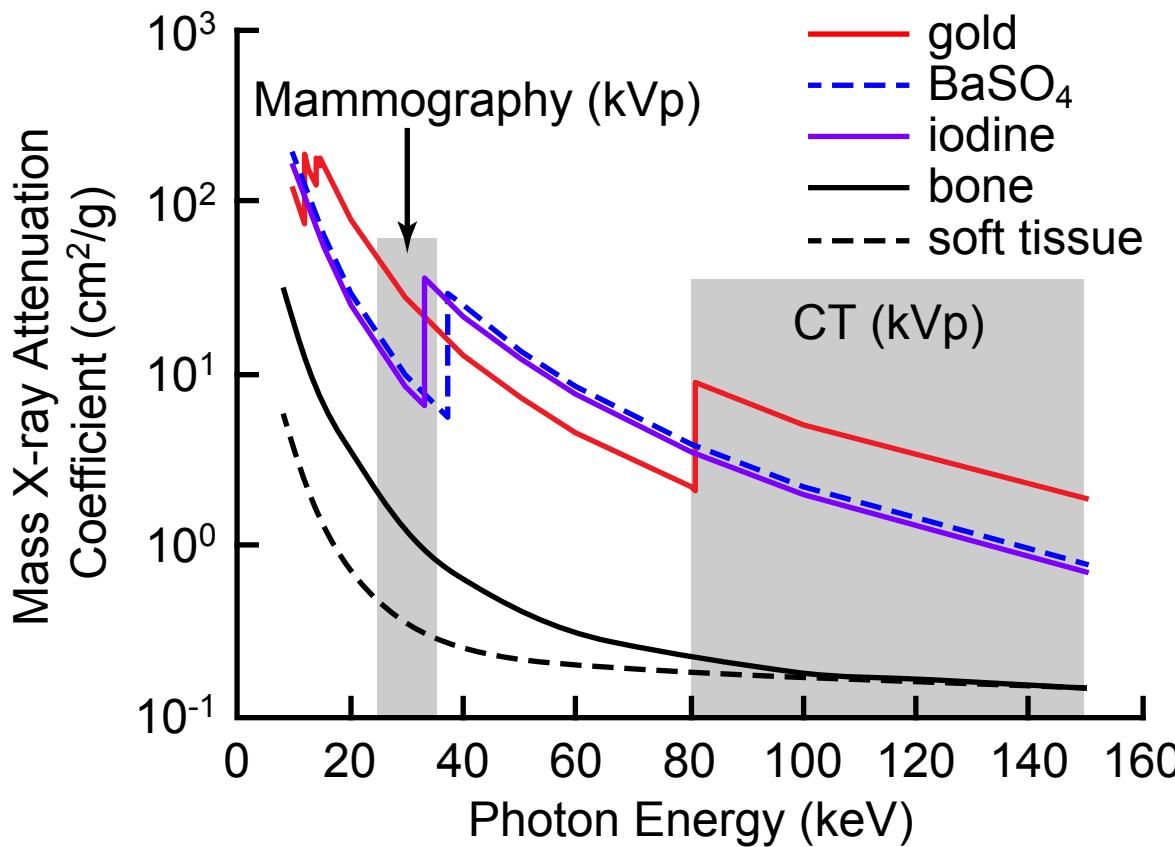
Contrast-Enhancement & X-ray Attenuation



- contrast agent X-ray attenuation (μ , HU)
 - atomic number (Z), density, absorption edges
 - X-ray source energy (kVp) and spectra
 - contrast agent mass concentration
- background X-ray attenuation (μ , HU)

$\Delta HU \geq 30$ between contrast agent and background required for detection.

X-Ray Attenuation of Contrast Agents

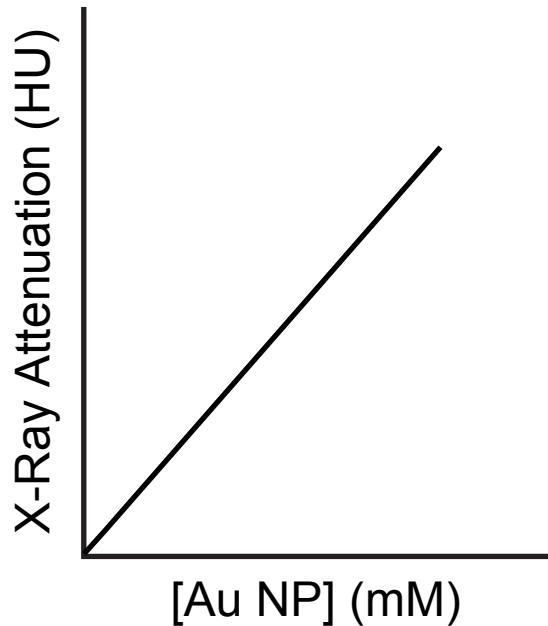


Au NPs provide significantly greater X-ray attenuation at photon energies relevant to clinical imaging.

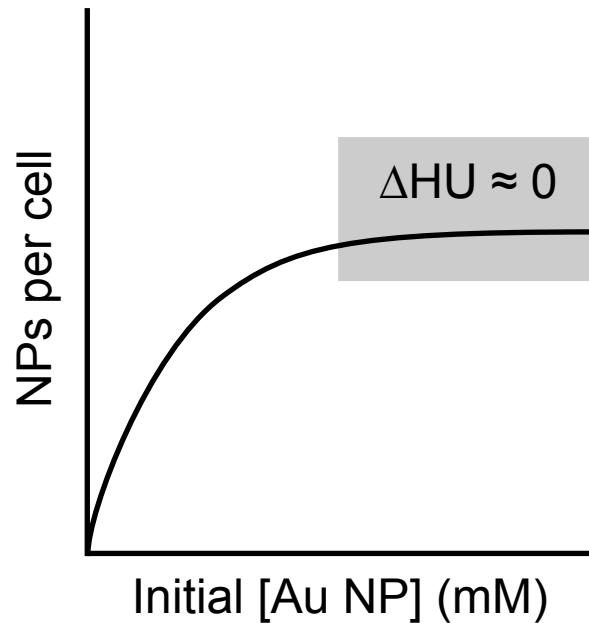
Comparative experiments must consider the X-ray source spectra produced by a system at a given tube potential (kVp) relative to absorption edges.

Contrast-Enhancement by Targeted Au NPs

Image contrast is all about delivered dose.



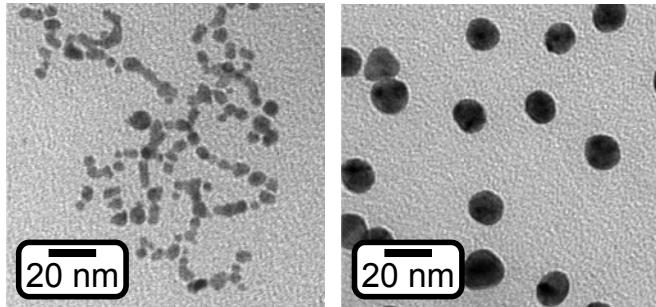
Delivered dose is not directly related to administered dose.



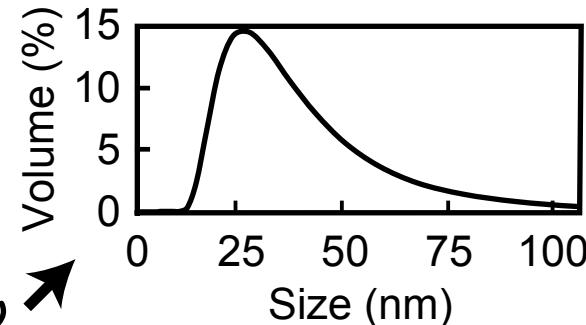
- clearance vs. targeting
- stability
 - size
 - molecular surface functionalization
 - delivery method

Au NP Characterization

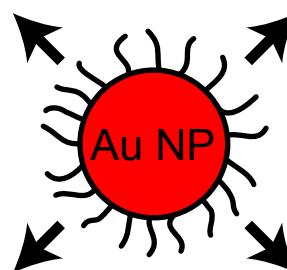
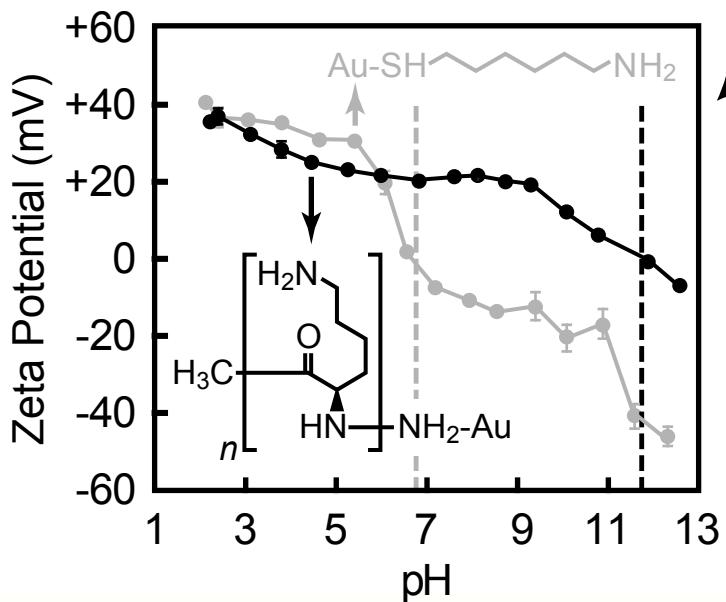
TEM → size and morphology



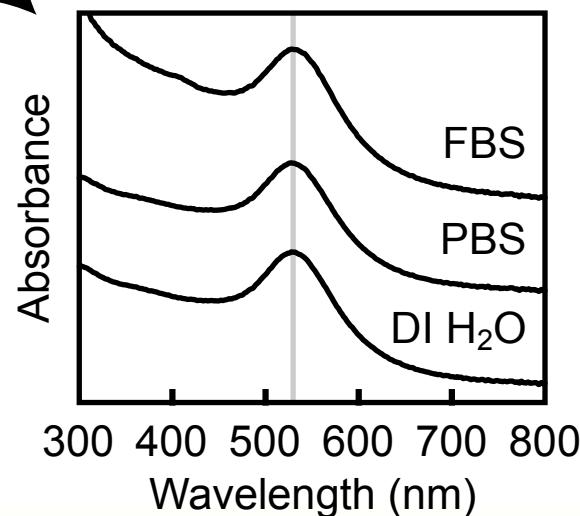
DLS → hydrodynamic diameter



DLS → zeta potential



UV-vis → SPR peak

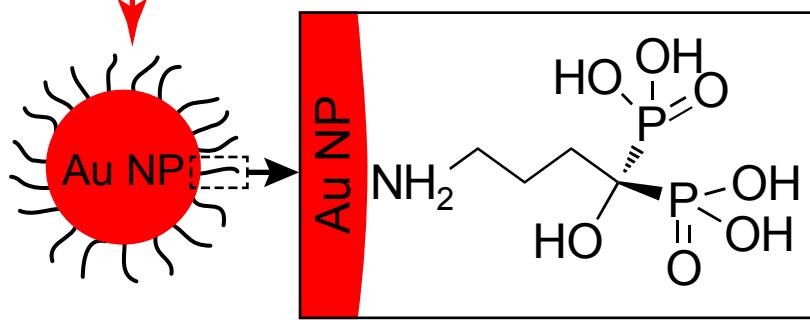
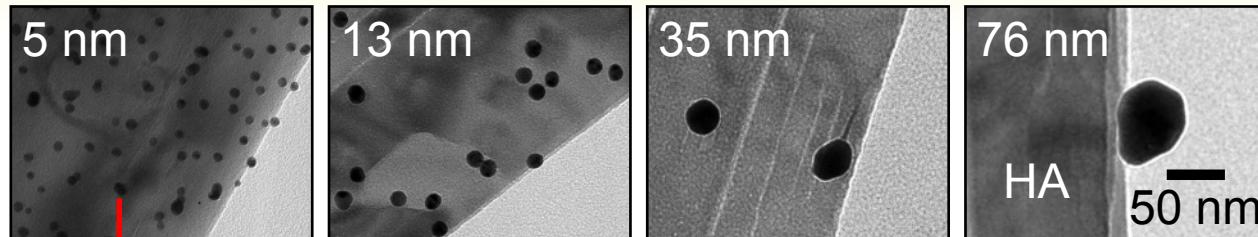


Cole et al., Nanomedicine, 2015

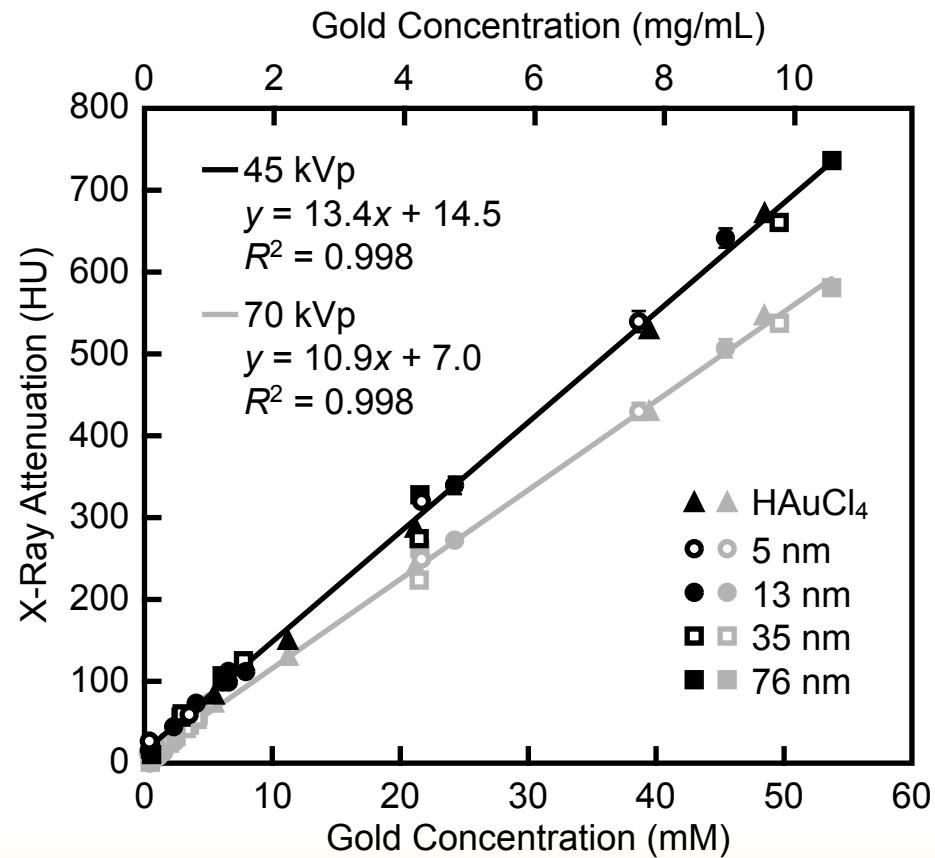


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Effects of Au NP Size



↓ Au NP size → = X-ray attenuation per mass Au
↑ # Au NPs/m² HA
↓ mg Au/g HA



Effects of Au NP Size

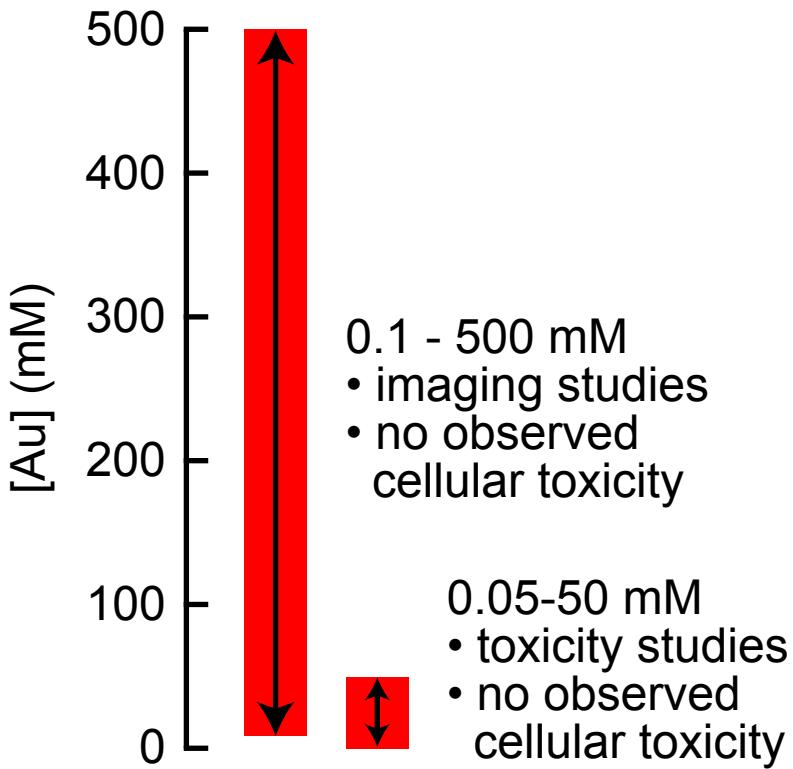
	Reference	Size (nm)	# Au NPs/cell	ng Au NPs/cell
Passive targeting to cells		14	$3 \cdot 10^3$	$8.31 \cdot 10^{-8}$
	<i>Chithrani et al. 2006</i>	30	$4.5 \cdot 10^3$	$1.23 \cdot 10^{-6}$
	Citrate-stabilized Au NPs	50	$6 \cdot 10^3$	$7.58 \cdot 10^{-6}$
	HeLa cells	74	$4 \cdot 10^3$	$1.64 \cdot 10^{-5}$
		100	$2 \cdot 10^3$	$2.02 \cdot 10^{-5}$
Xu <i>et al.</i> 2009		4	$1 \cdot 10^7$	$6.46 \cdot 10^{-6}$
	2-mercaptosuccinic acid	20	$1 \cdot 10^5$	$8.08 \cdot 10^{-6}$
	stabilized Au NPs	40	$2 \cdot 10^4$	$1.29 \cdot 10^{-5}$
	HeLa cells	60	$1 \cdot 10^4$	$2.18 \cdot 10^{-5}$
	Reference	Size (nm)	# Au NPs/g mineral	mg Au NPs/g mineral
Active targeting to mineral substrate		5	$9.3 \cdot 10^{15}$	11.0
	<i>Ross et al. 2014</i>	13	$5.1 \cdot 10^{14}$	11.3
	Bisphosphonate	35	$7.0 \cdot 10^{13}$	29.3
	functionalized Au NPs	76	$1.6 \cdot 10^{13}$	62.5

Effects of Au NP Size

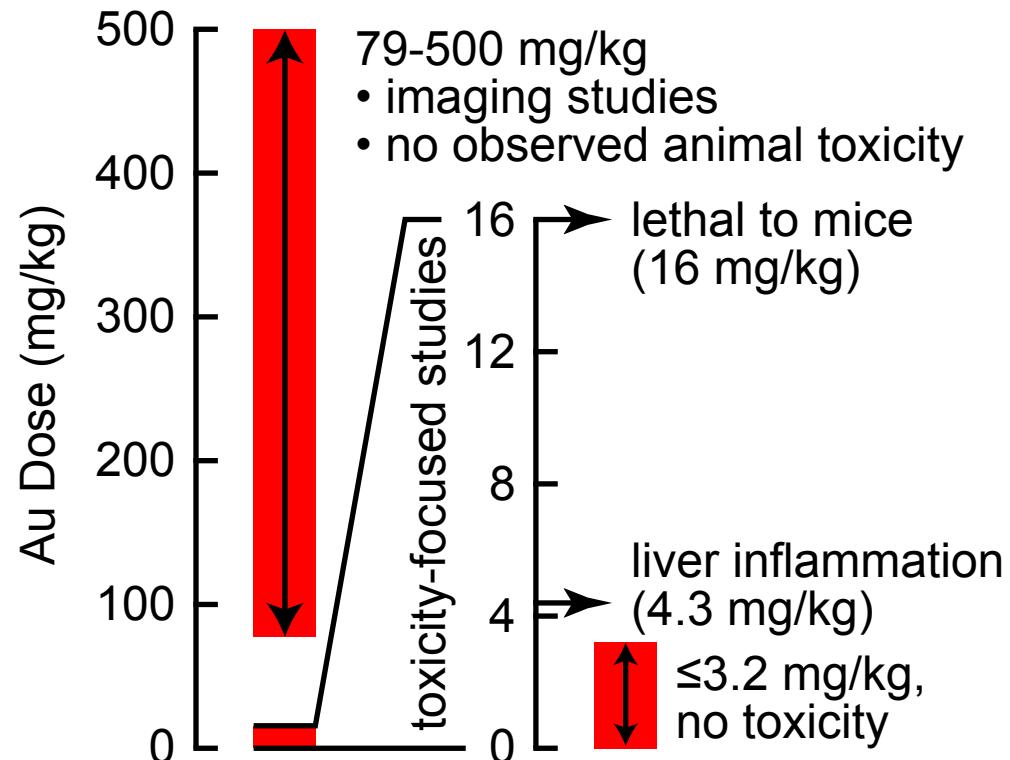
- No direct effect on X-ray attenuation.
- Indirectly affects X-ray contrast via delivery of a high mass concentration to the site of interest.
- Want as large as possible to maximize delivered mass concentration, but not too large to compromise colloidal stability, blood retention, cellular uptake, or targeting.
- <10 nm – more rapidly cleared, greater risk of toxicity
- 10-30 nm – more retention, non-toxic at $<10^{12}$ NPs/mL

Au NP (Non-)Toxicity

in vitro

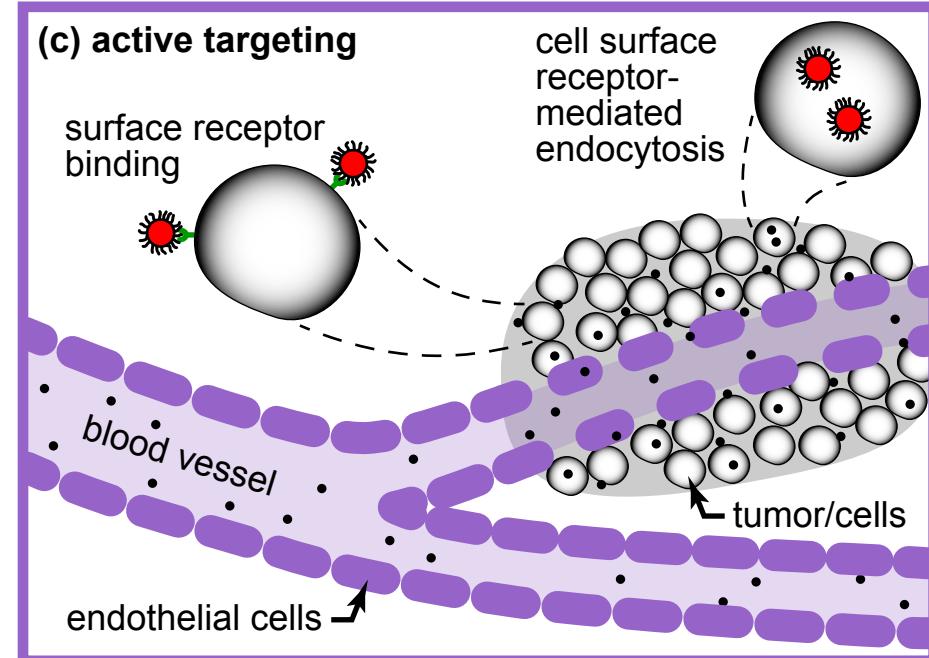
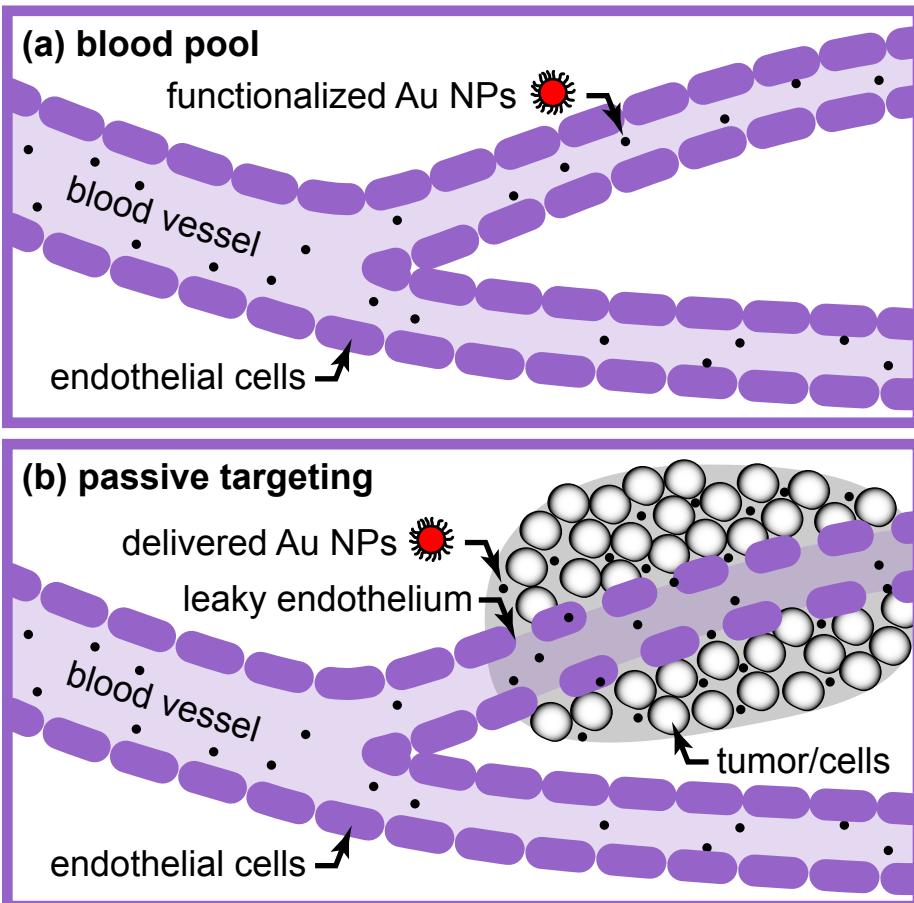


in vivo

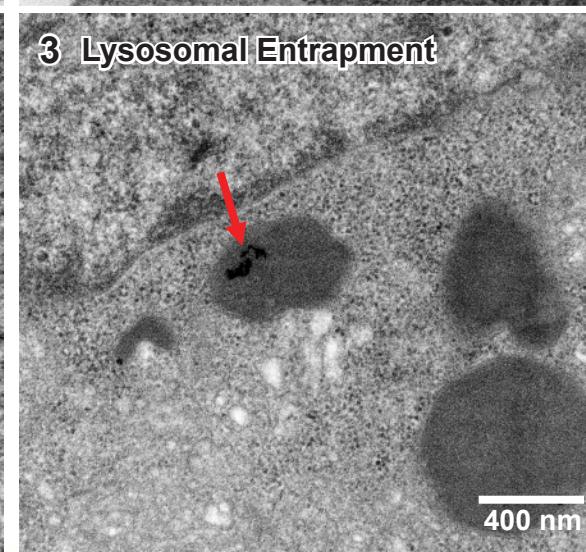
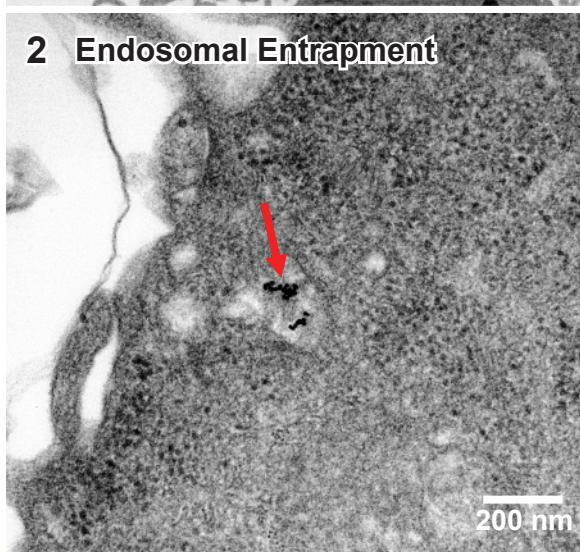
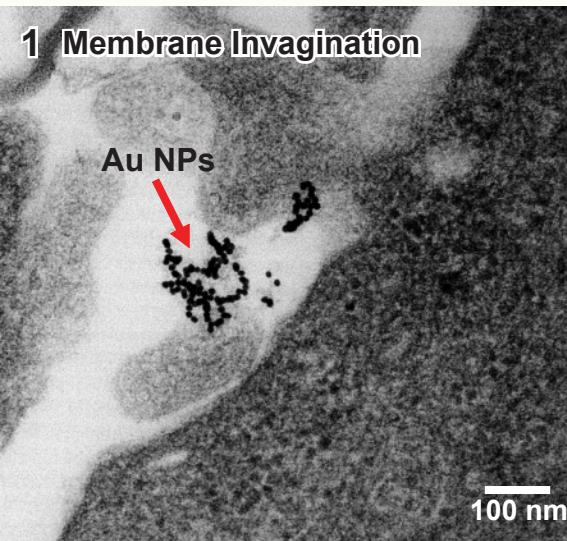
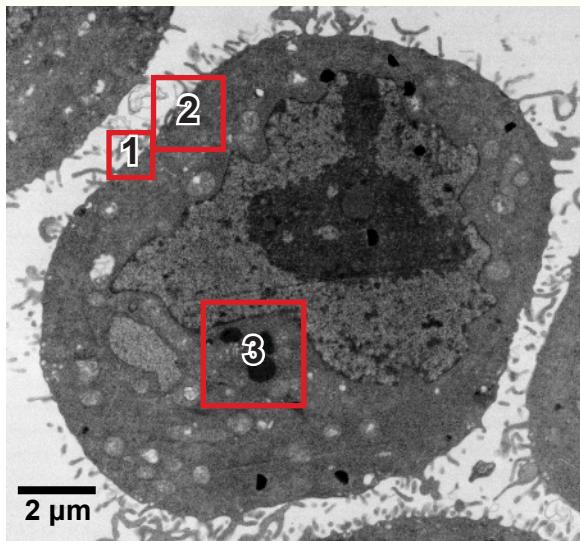


Dosing studies are critical to determine the minimum dose required to enhance contrast without inducing cytotoxicity.

Applications of Au NPs as X-ray Contrast Agents in Diagnostic Imaging

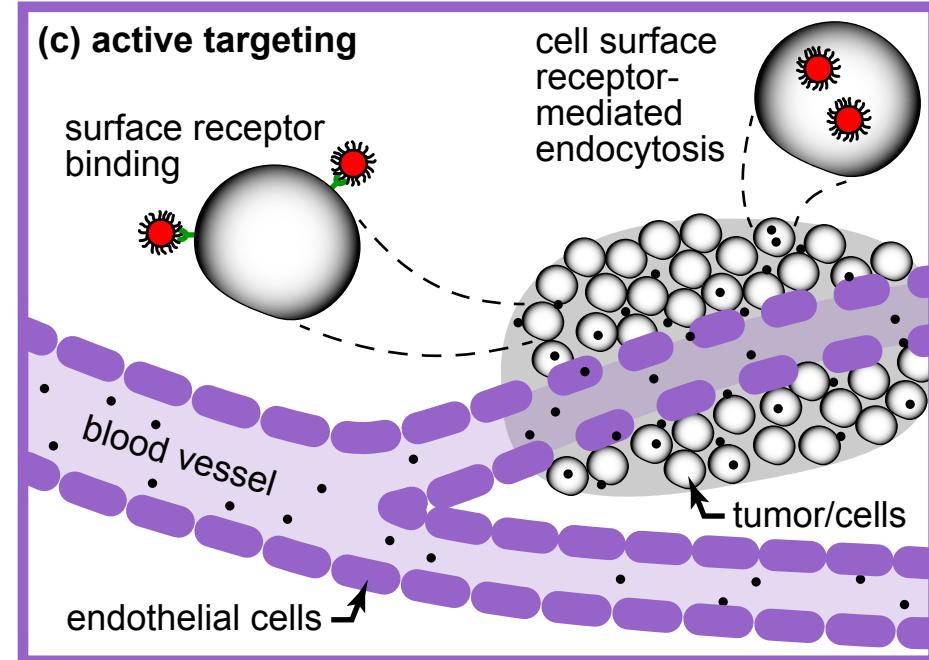
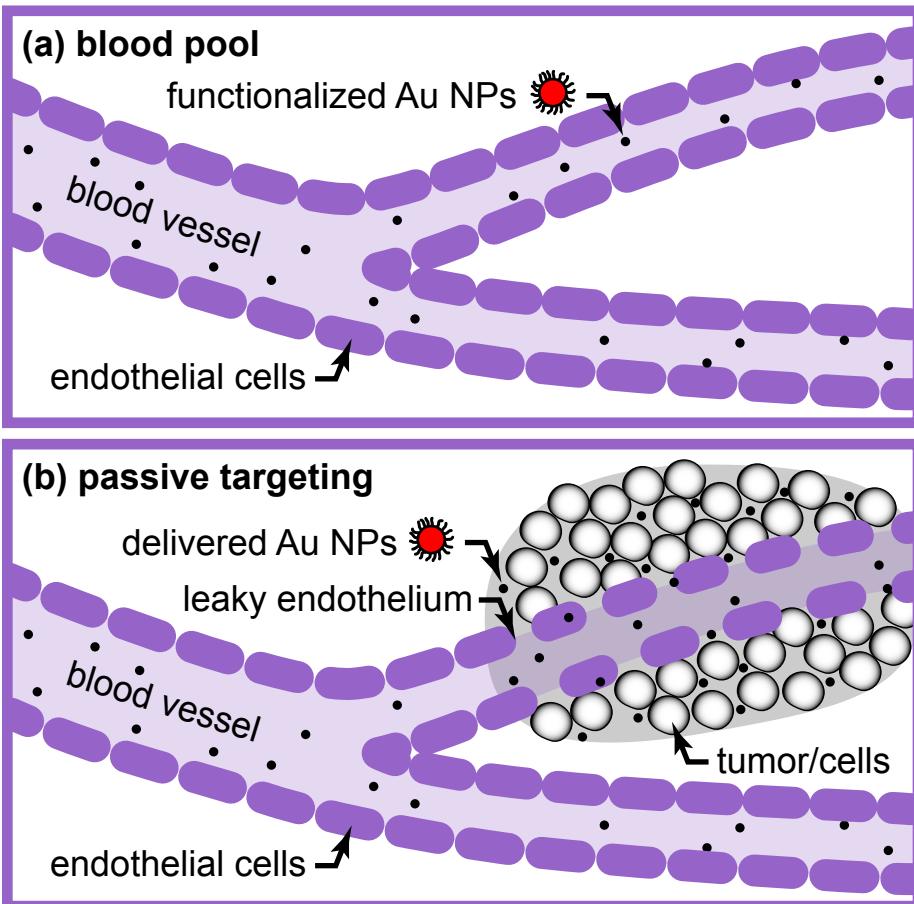


Cellular Uptake of Au NPs



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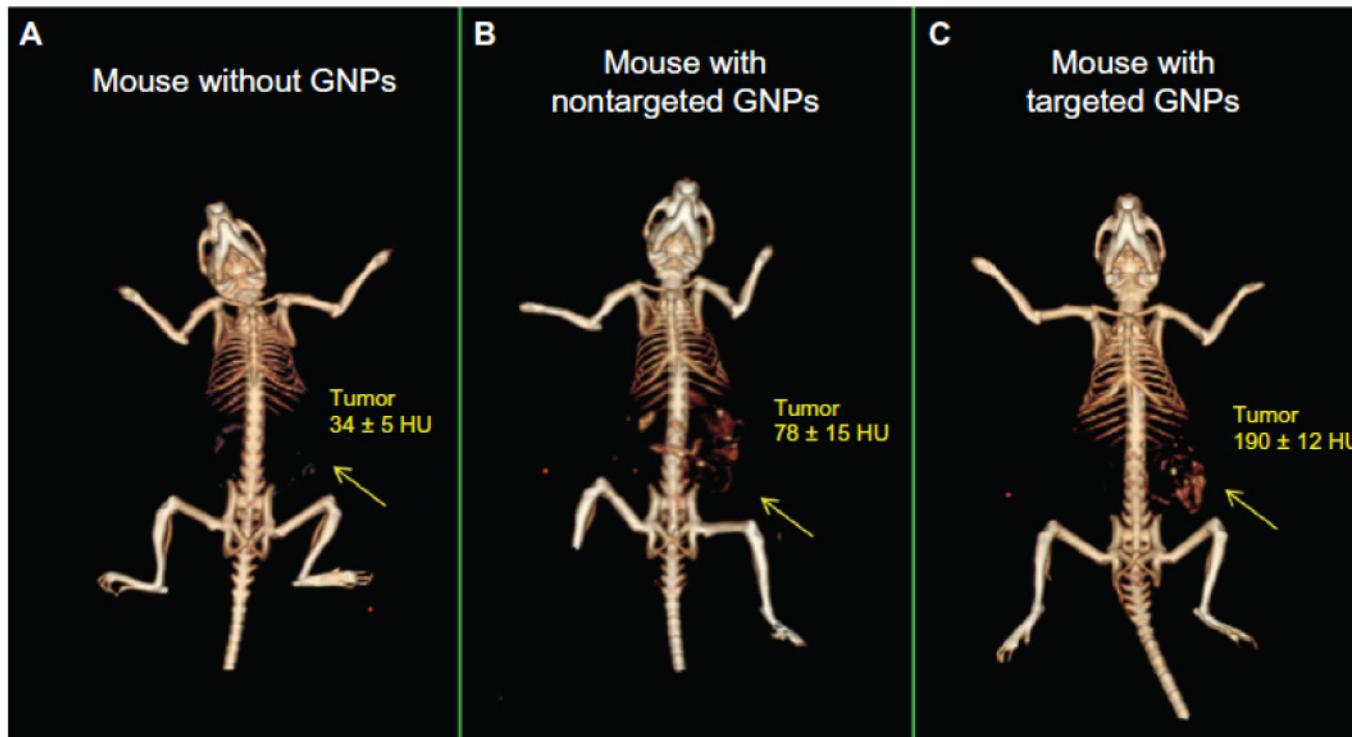
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Targeted Au NP X-ray Contrast Agents

Active Targeting: binding of a functional molecule to specific sites

- ligands (e.g., -COOH, -NH₂, -PO(OH)₂, etc.)
- biomolecules (e.g., folic acid, heparin, bombesin, insulin, etc.)
 - antibodies (e.g., anti-HER, **anti-EGFR**, anti-CD4, etc.)



Reuveni et al., 2011



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What Would You Fight For?

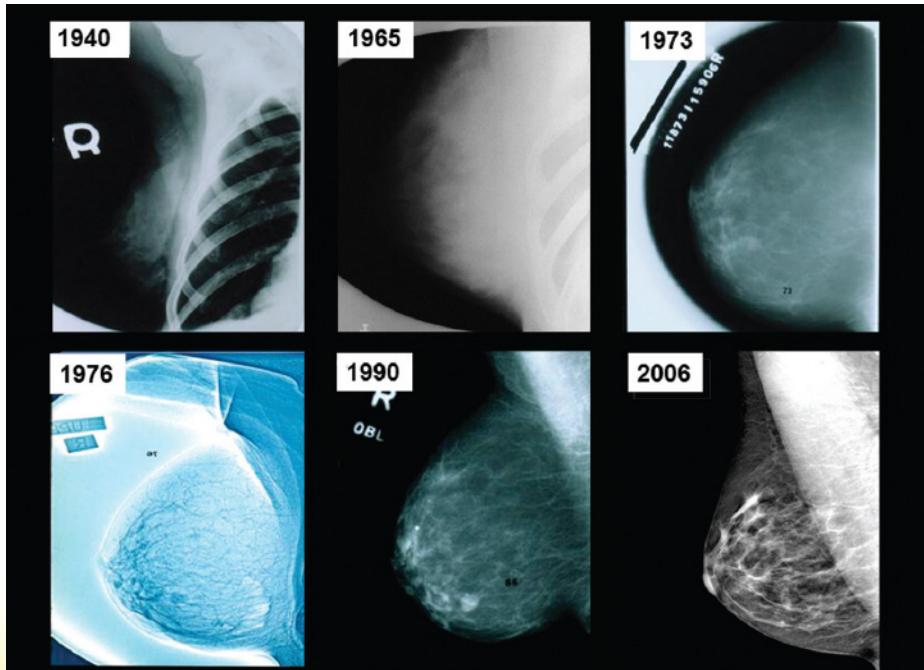


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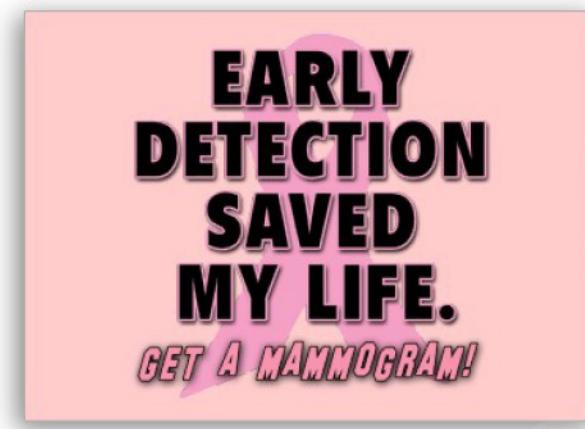
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Breast Cancer and Mammography

- Most commonly diagnosed cancer and 2nd leading cause of cancer deaths. Siegel *et al.* 2012
- Mammographic screening has decreased mortality by 30%. Tabar *et al.* 2010



Smith *et al.* 2012



- The most commonly detected abnormality is microcalcifications which are deposits of hydroxyapatite (HA) and are coincident with 30-50% of breast cancers detected by mammography. Cheng *et al.* 2003



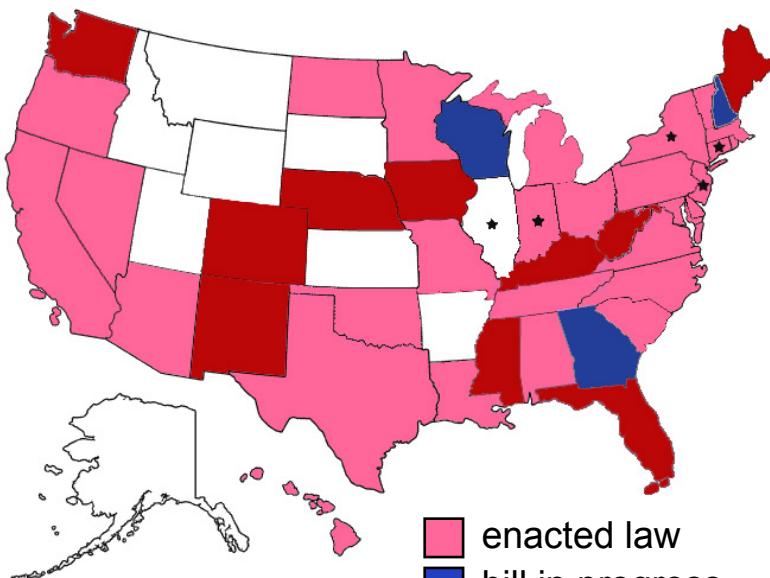
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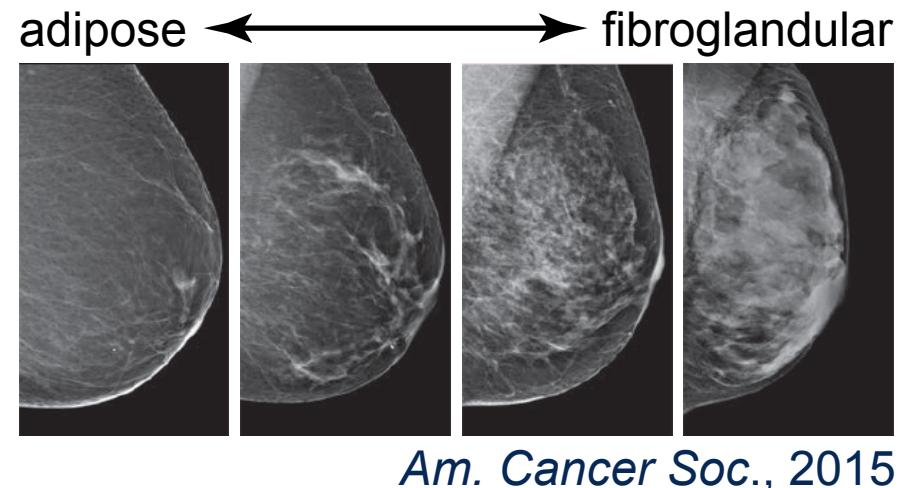
Breast Cancer and Mammography

- Breast cancer detection remains limited by the sensitivity of mammography; 10-30% of breast cancer lesions are missed during routine screening.

Smith *et al.* 2012



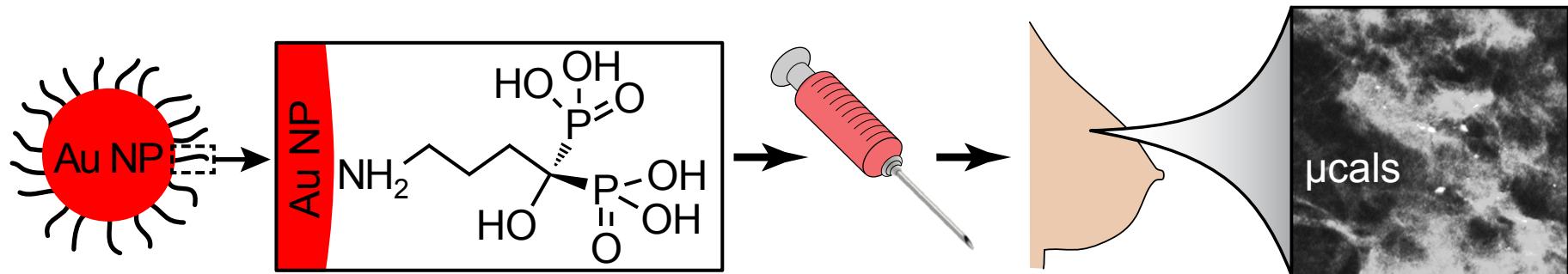
Are You DENSE?
ADVOCACY
because your life matters®



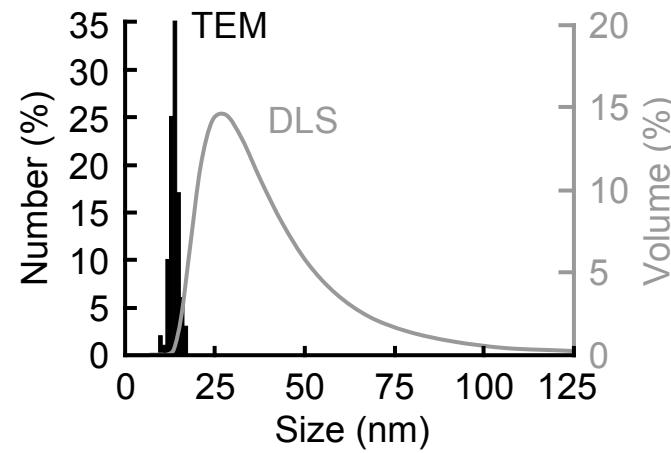
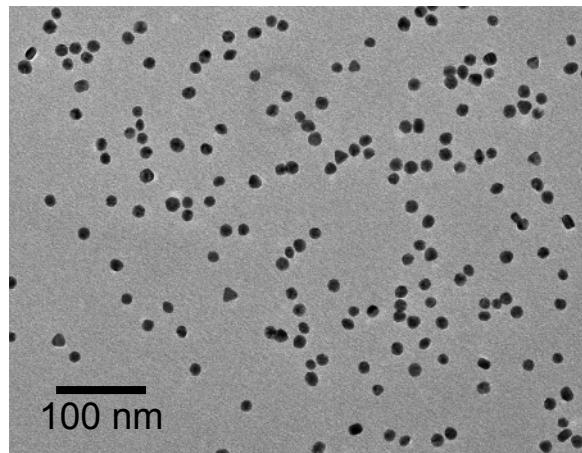
- Women with dense breast tissue are at 4 to 6-fold greater risk for developing breast cancer while mammography is 3-fold less likely to provide accurate detection.

Britton *et al.* 2012; Boyd *et al.* 2011

Detection of Breast Microcalcifications

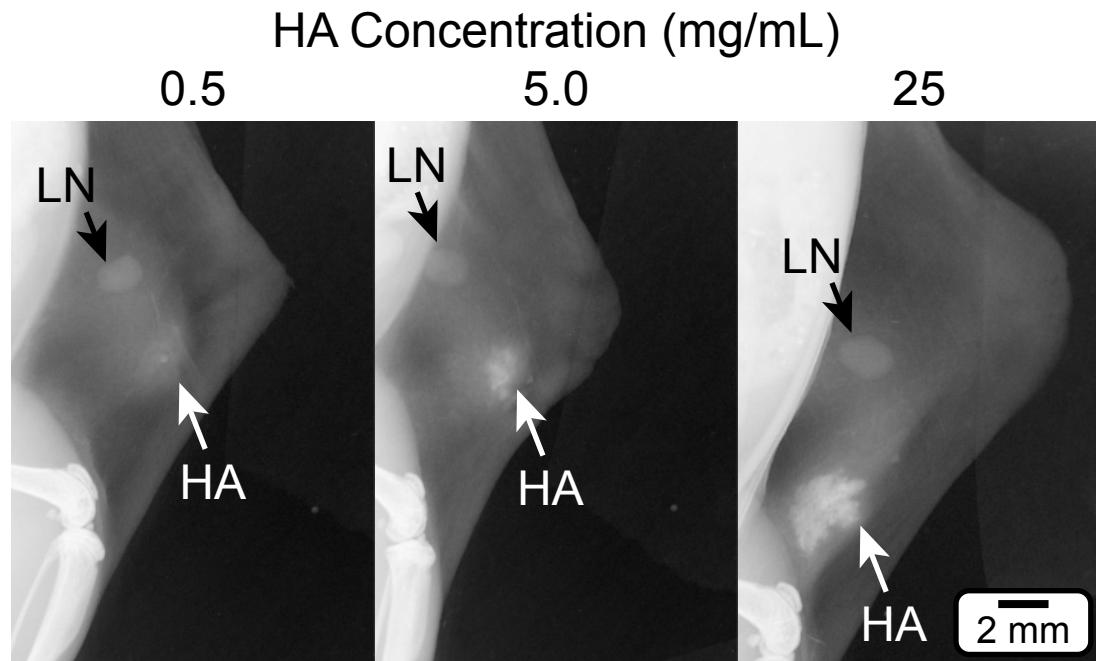
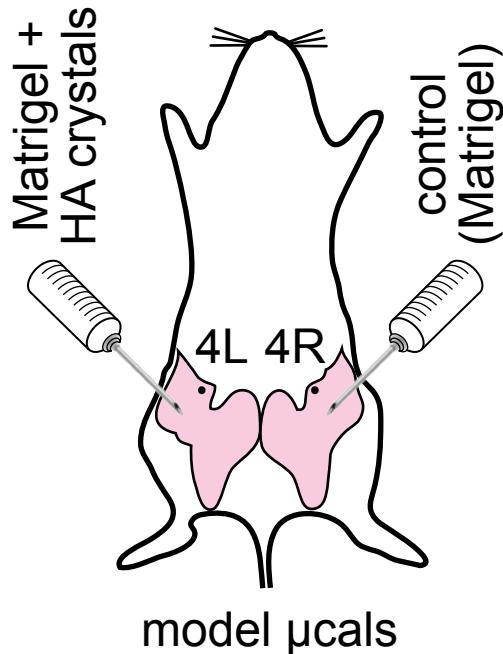


Bisphosphonate-Functionalized Gold Nanoparticles (BP-Au NPs)



Detection of Breast Microcalcifications

Murine Model of Microcalcifications



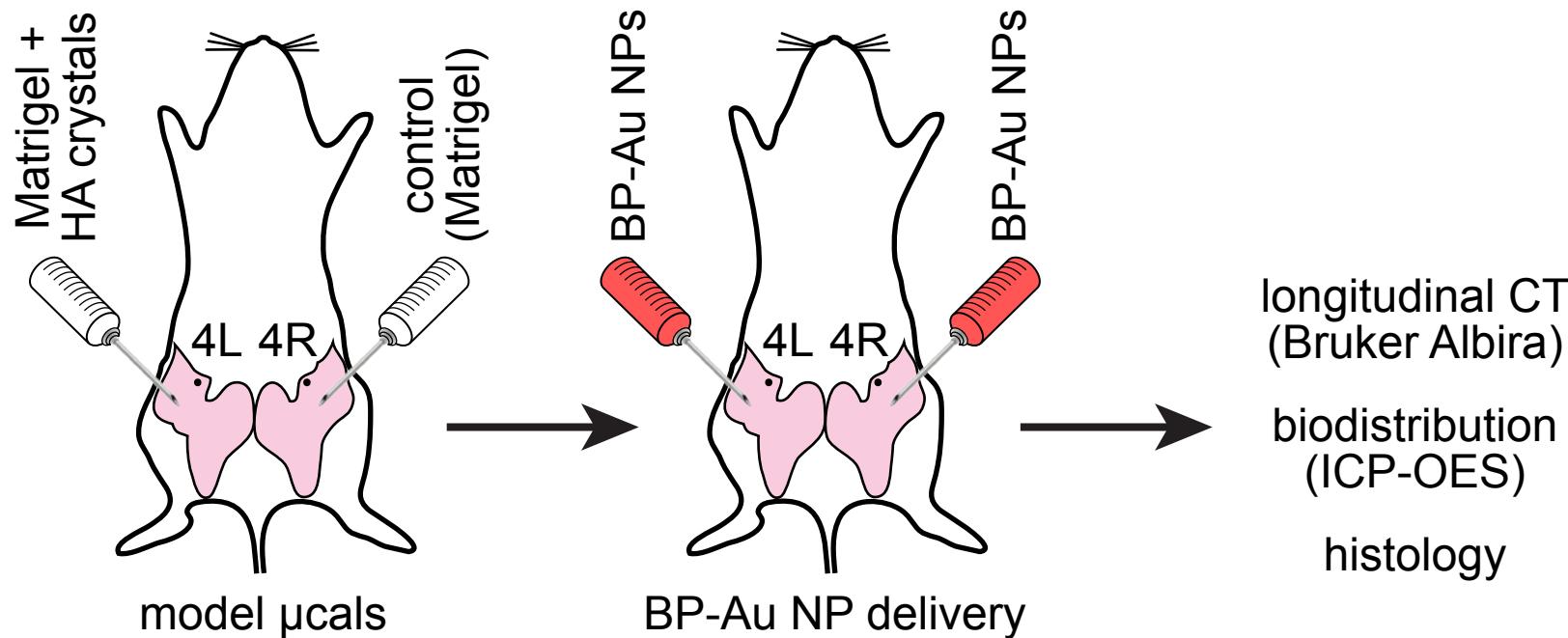
Cole et al., ACS Nano, 2014, 2015



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Detection of Breast Microcalcifications

Murine Model of Microcalcifications

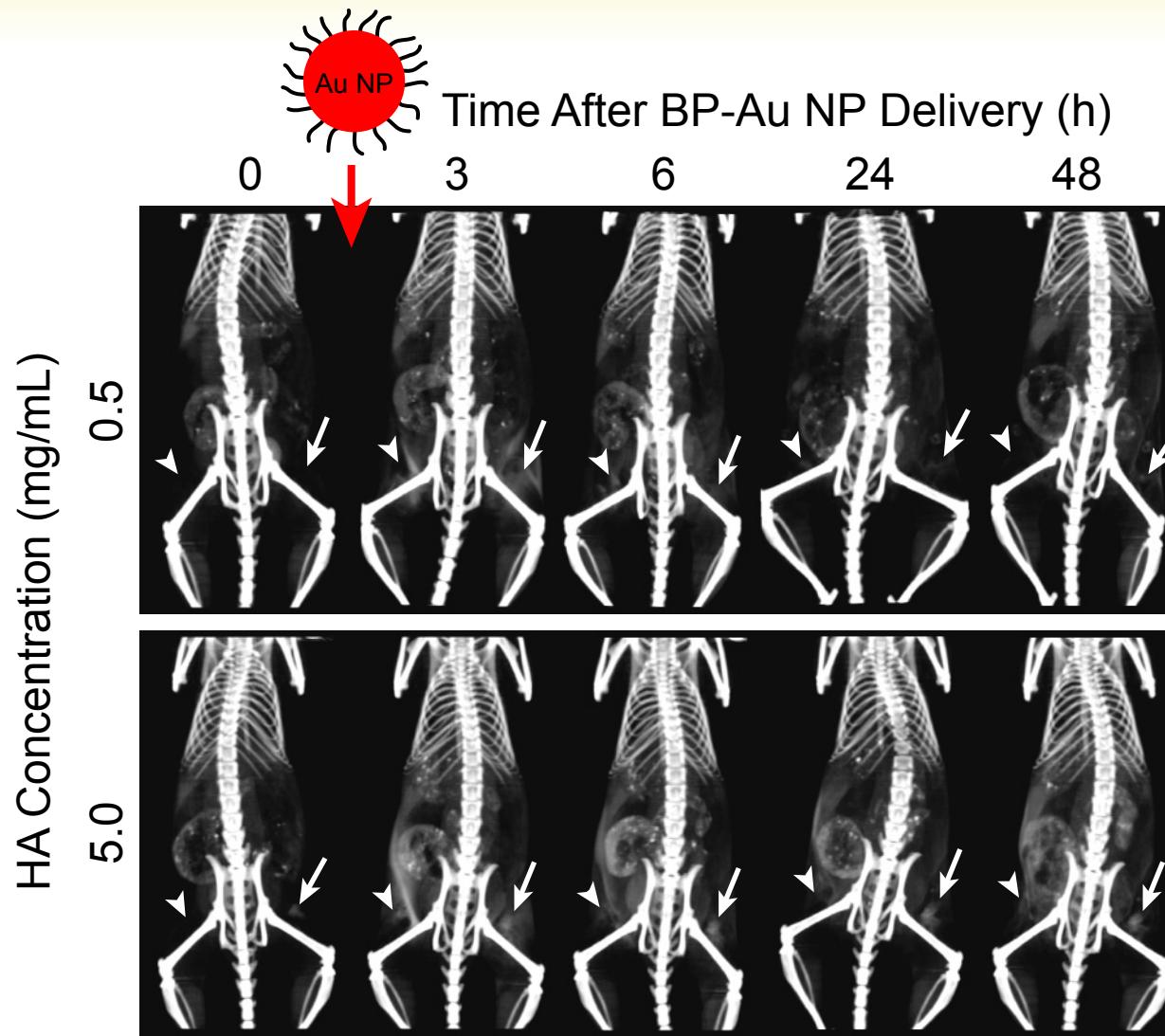


Cole *et al.*, ACS Nano, 2014, 2015



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Detection of Breast Microcalcifications

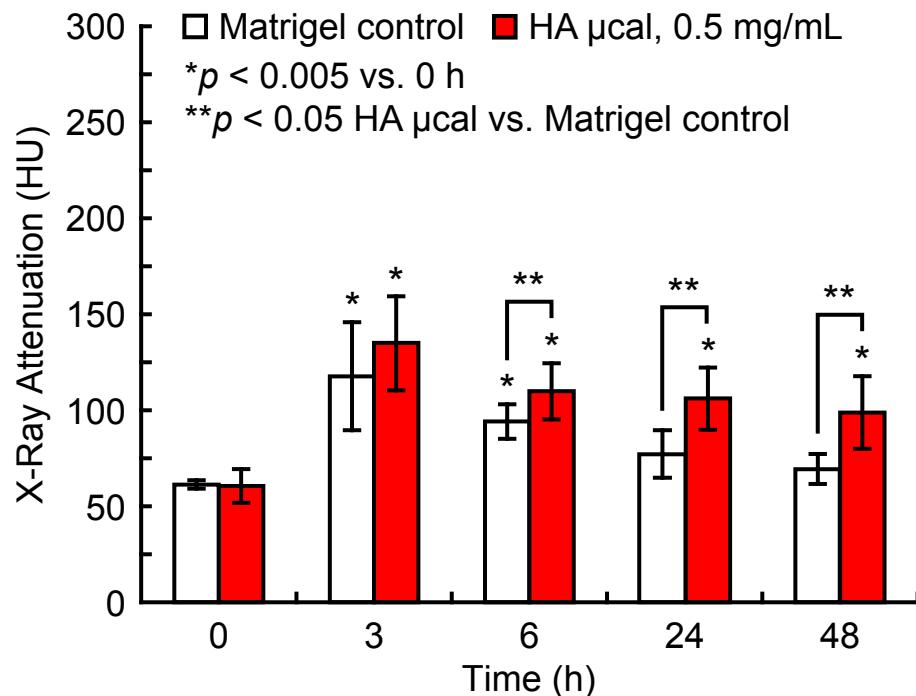


Cole et al., ACS Nano, 2014



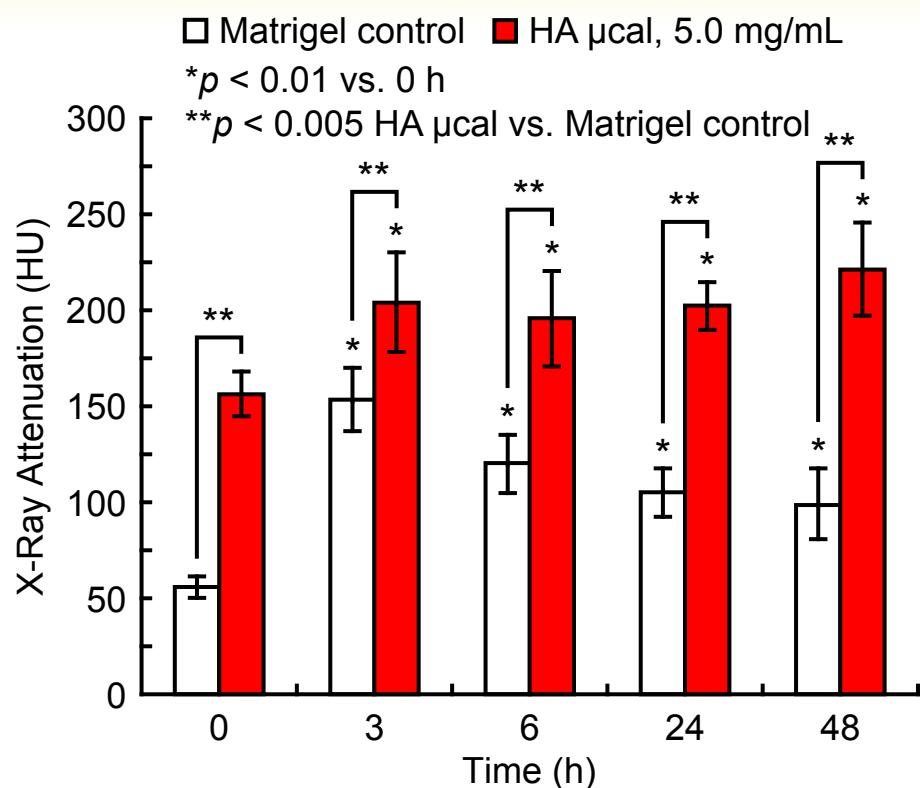
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Detection of Breast Microcalcifications



suggests improved sensitivity

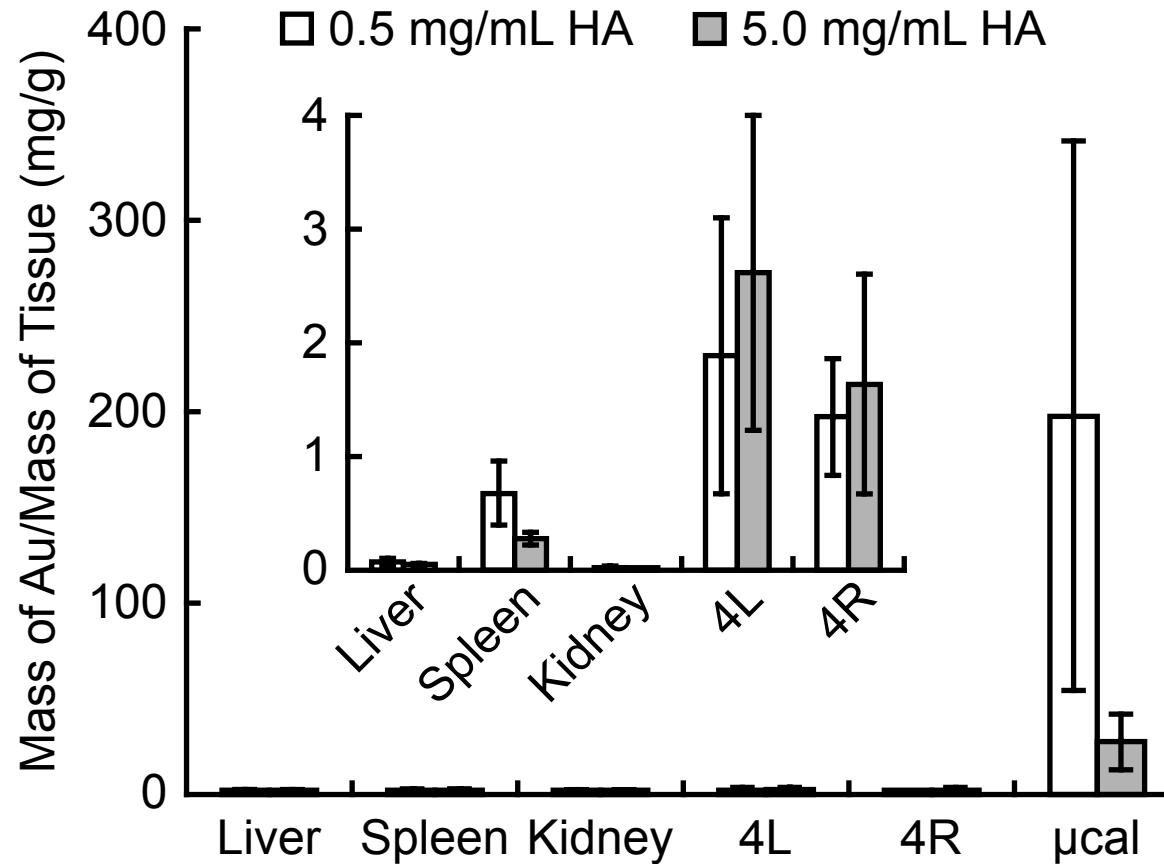
$\mu\text{cal} < 100 \mu\text{m}$ in size
[Au] within $\mu\text{cal} = 5\text{-}10 \text{ wt\%}$ or 2.5 mg/cc ; theoretically need $\sim 0.3 \text{ wt\%}$



suggests improved specificity

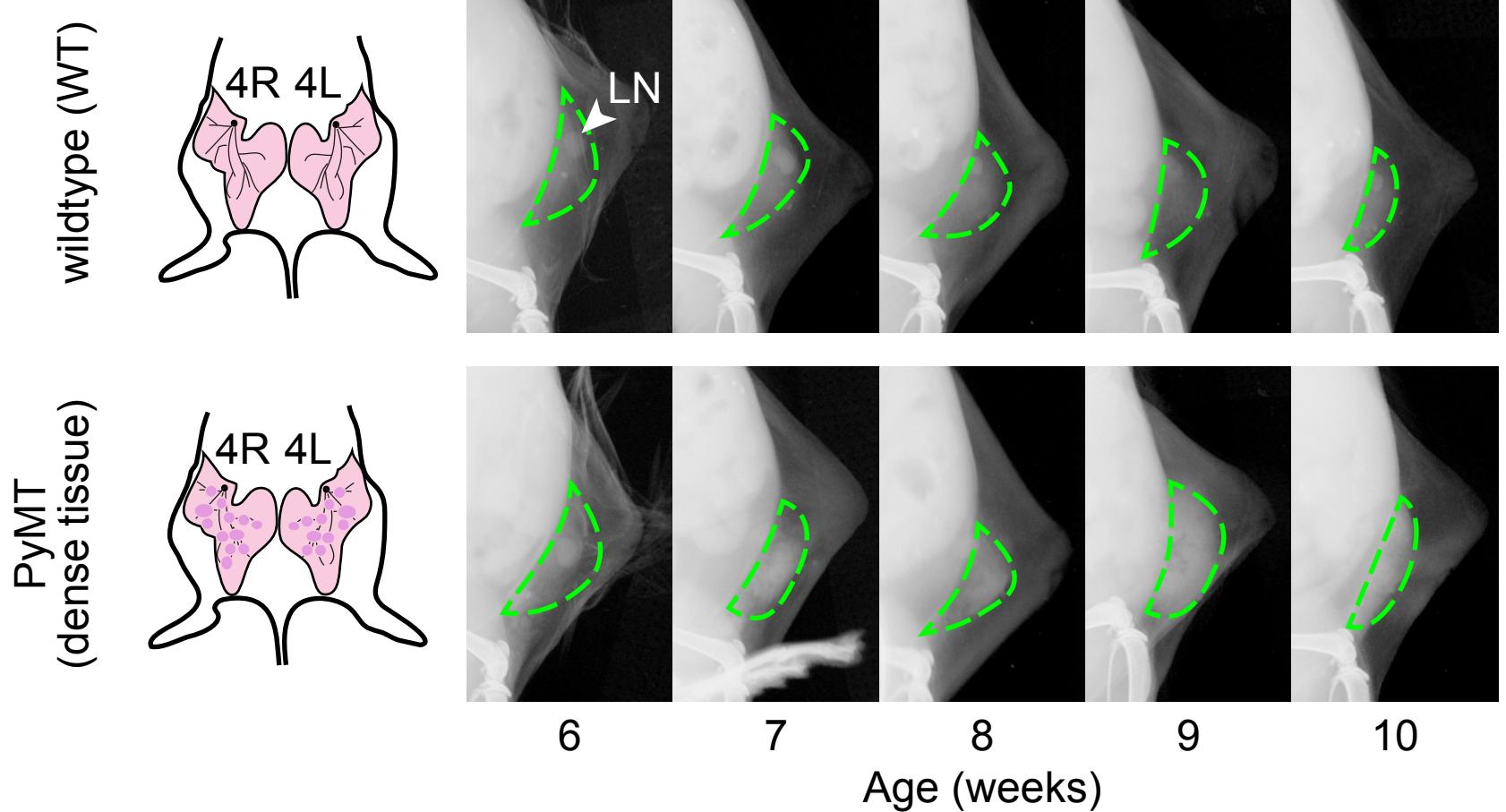
Detection of Breast Microcalcifications

Biodistribution of BP-Au NPs



Detection of Breast Microcalcifications

Transgenic Murine Model of Dense Mammary Tissue

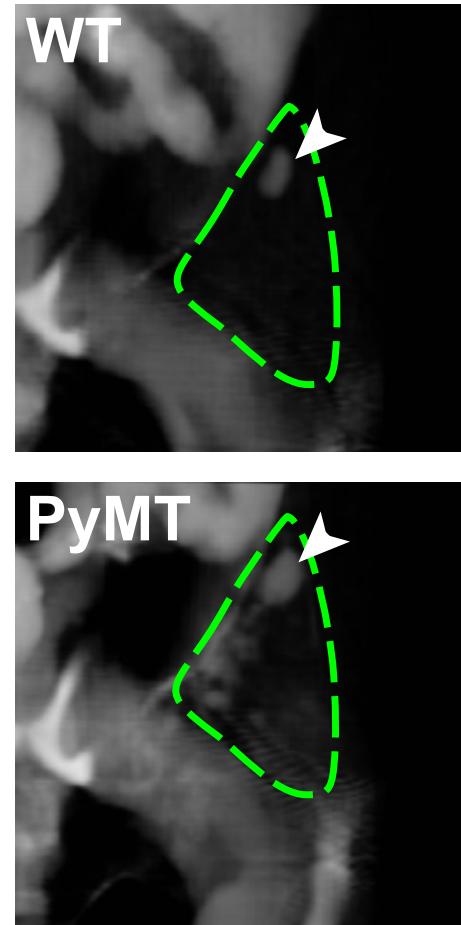
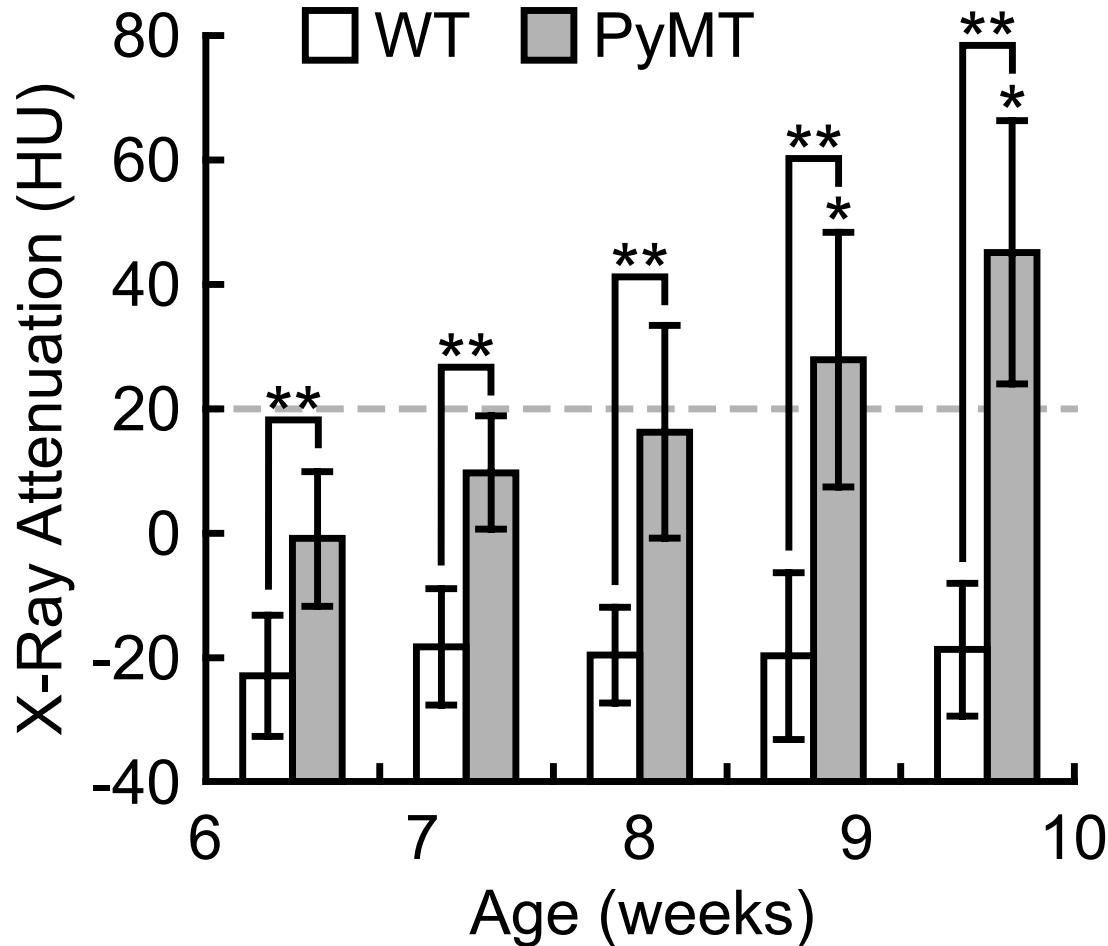


Cole et al., ACS Nano, 2015



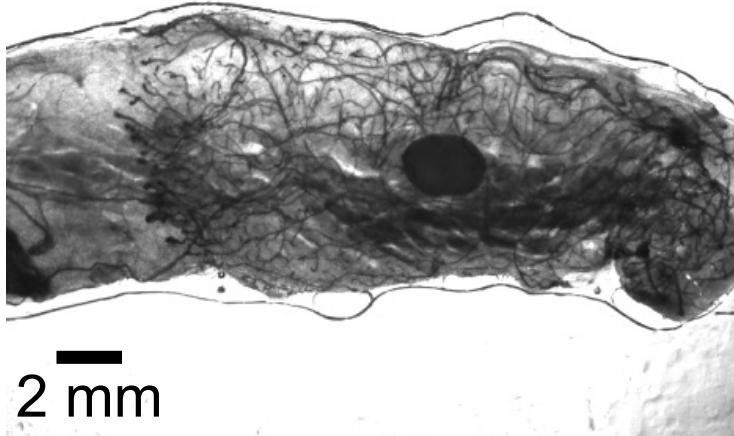
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Dense Tissue Model

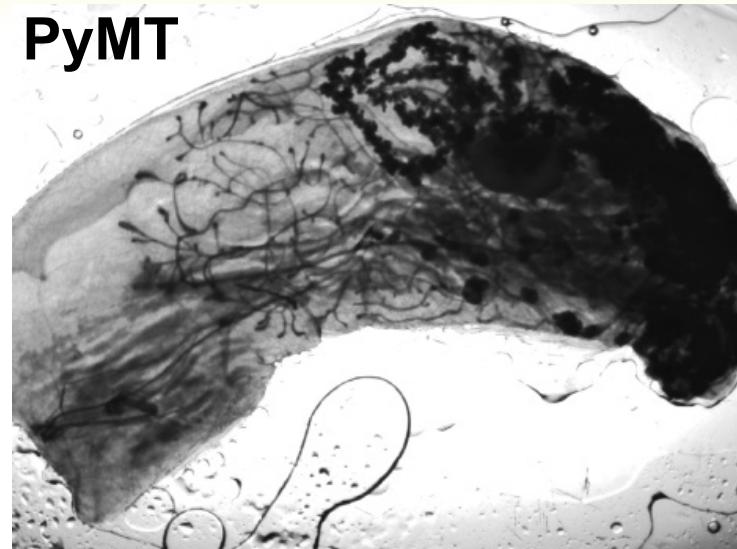


Dense Tissue Model

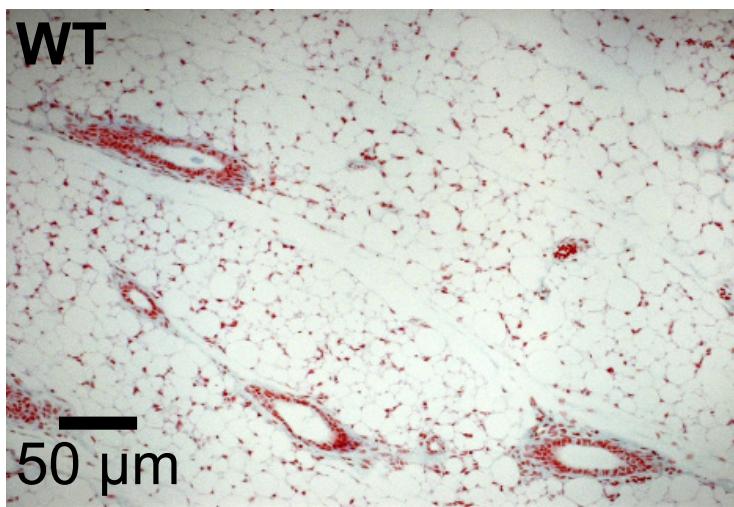
WT



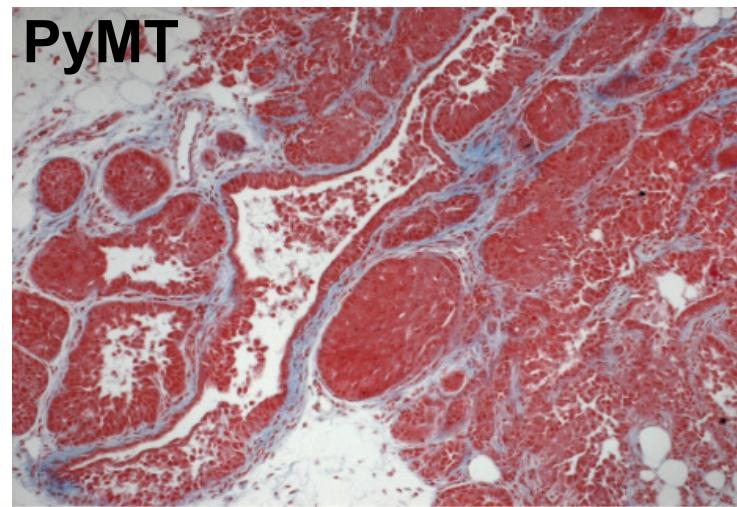
PyMT



WT



PyMT



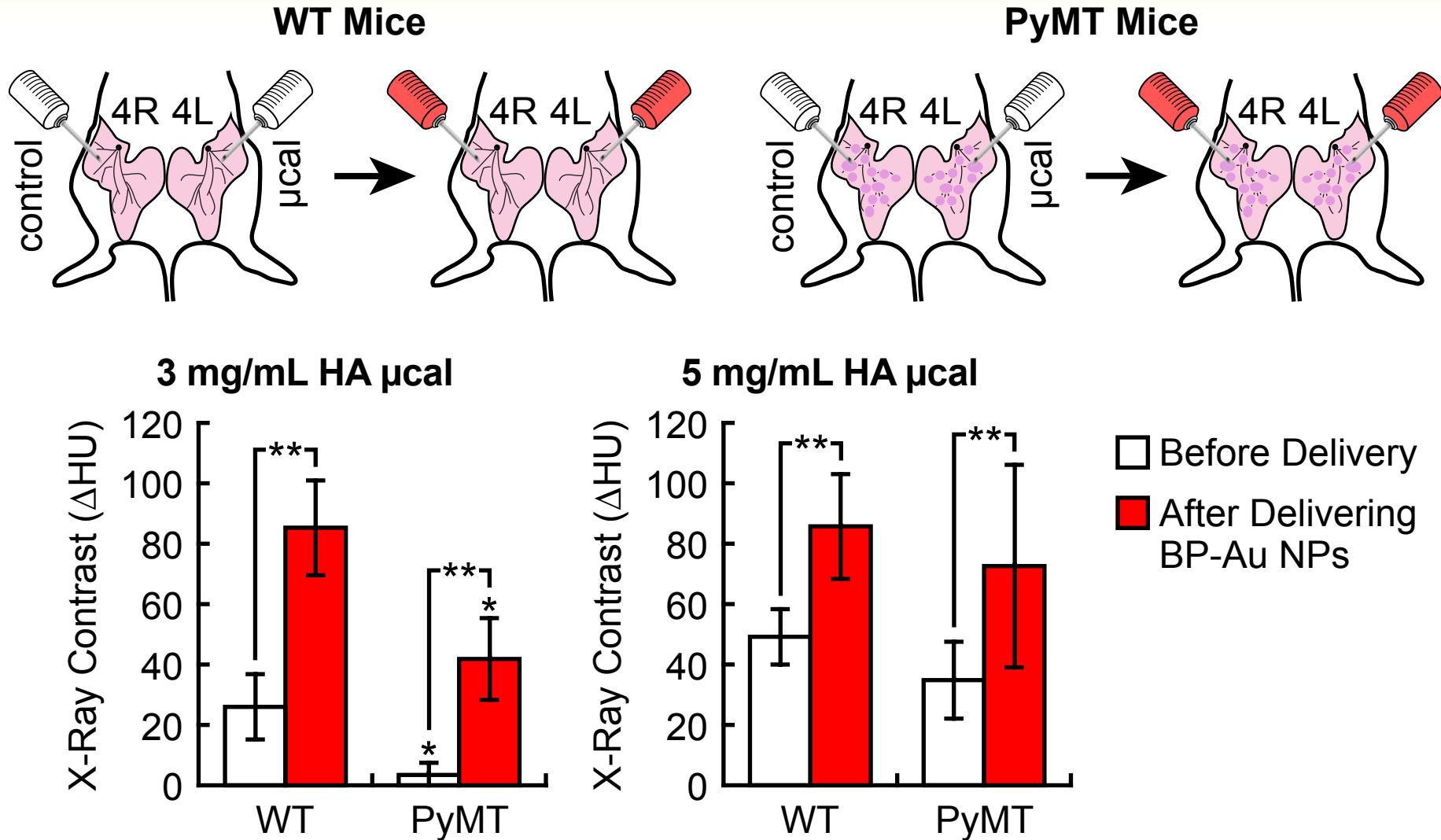
Cole et al., ACS Nano, 2015



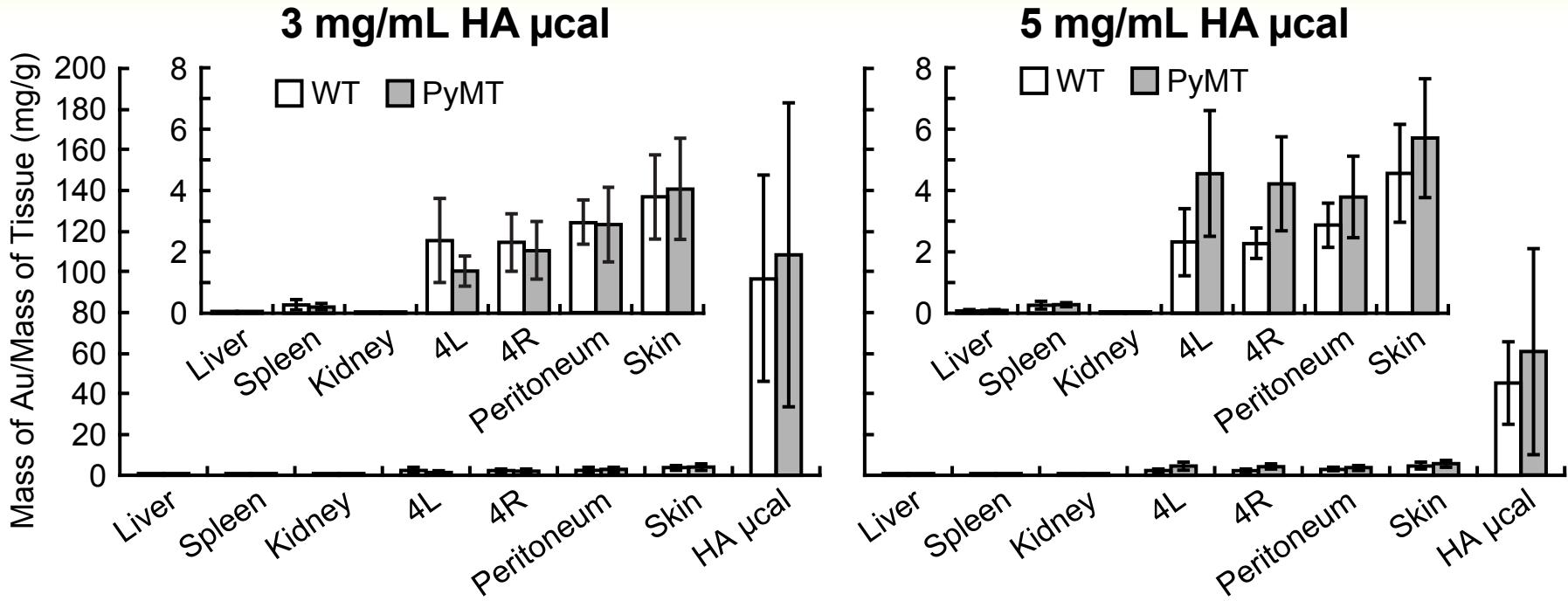
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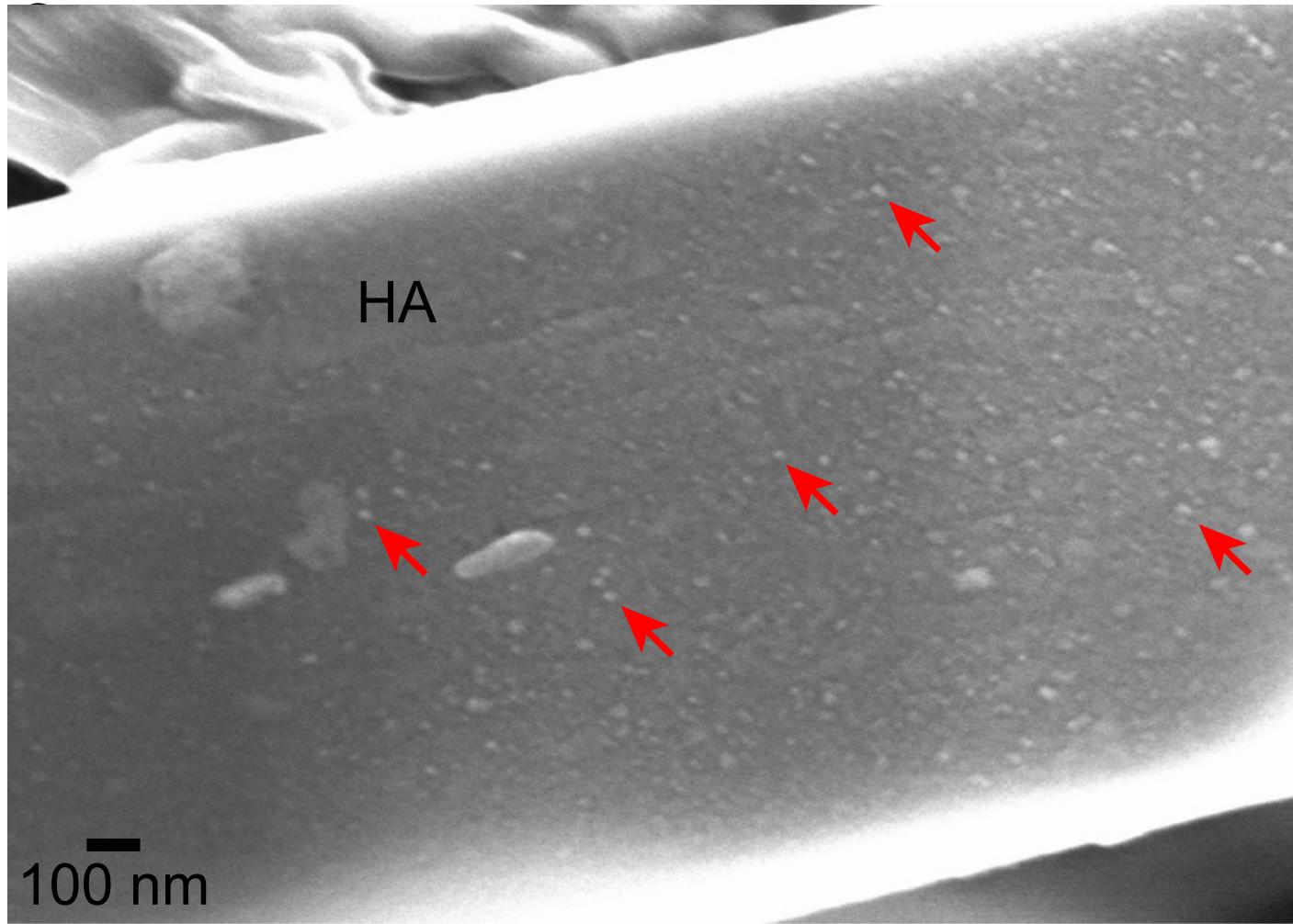
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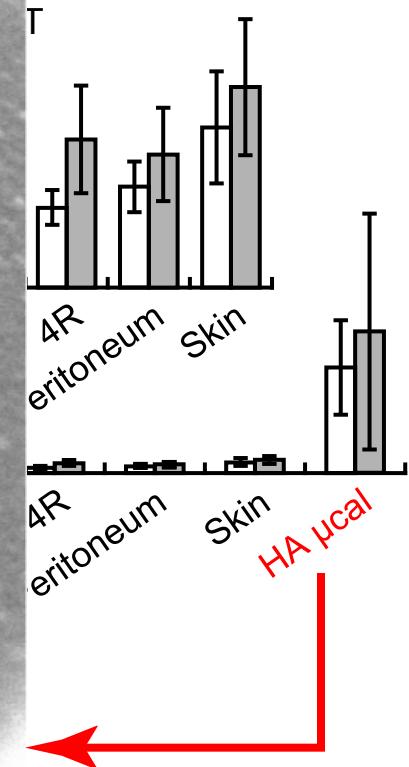
Biodistribution of Au NPs



Biodistribution of Au NPs



5 mg/mL HA μ cal

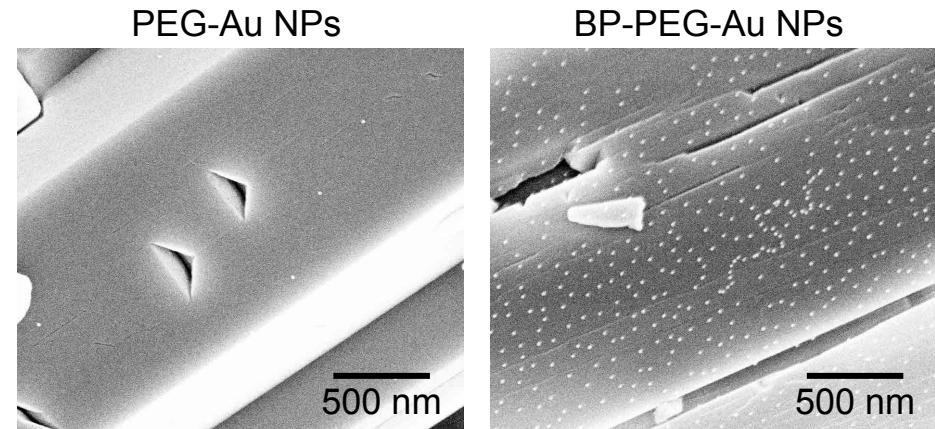
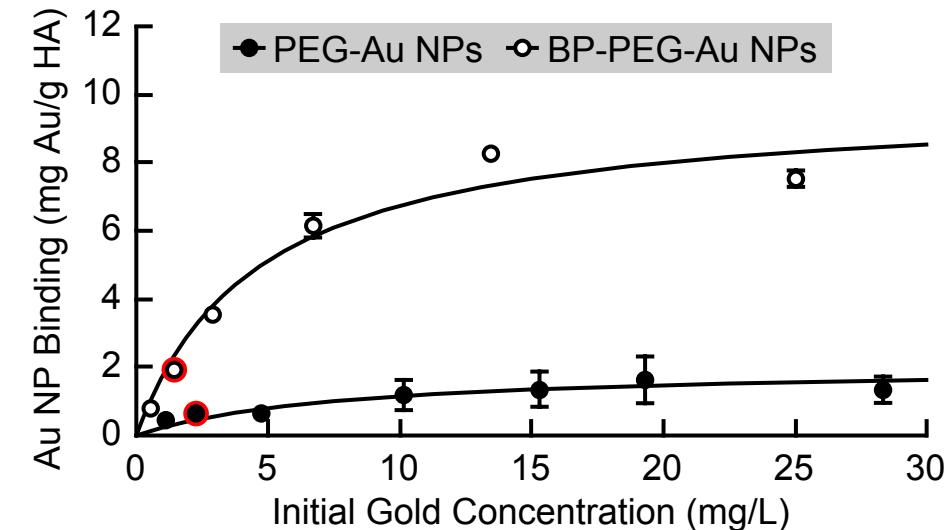


Cole et al., ACS Nano, 2015



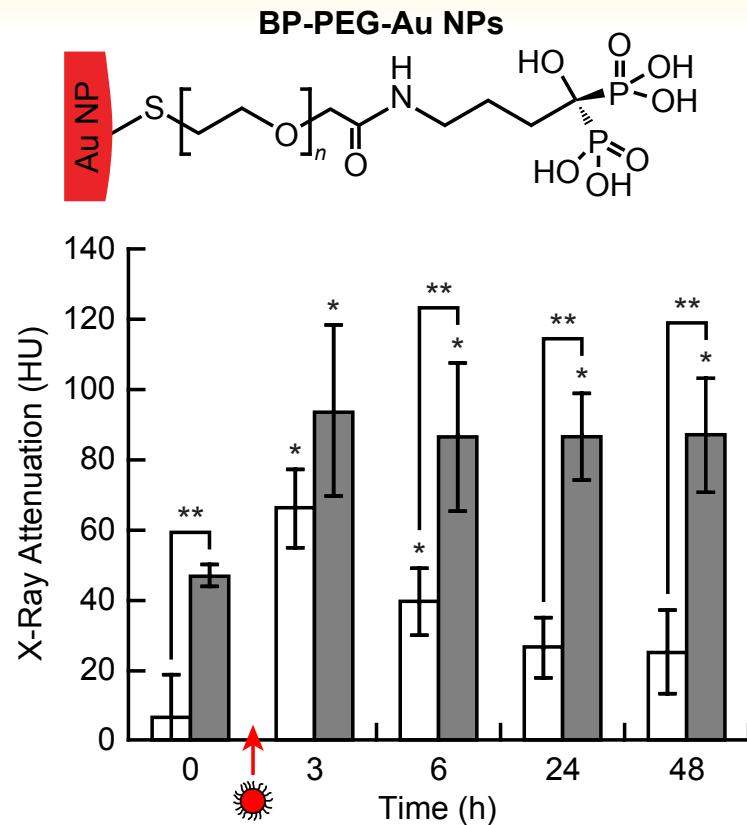
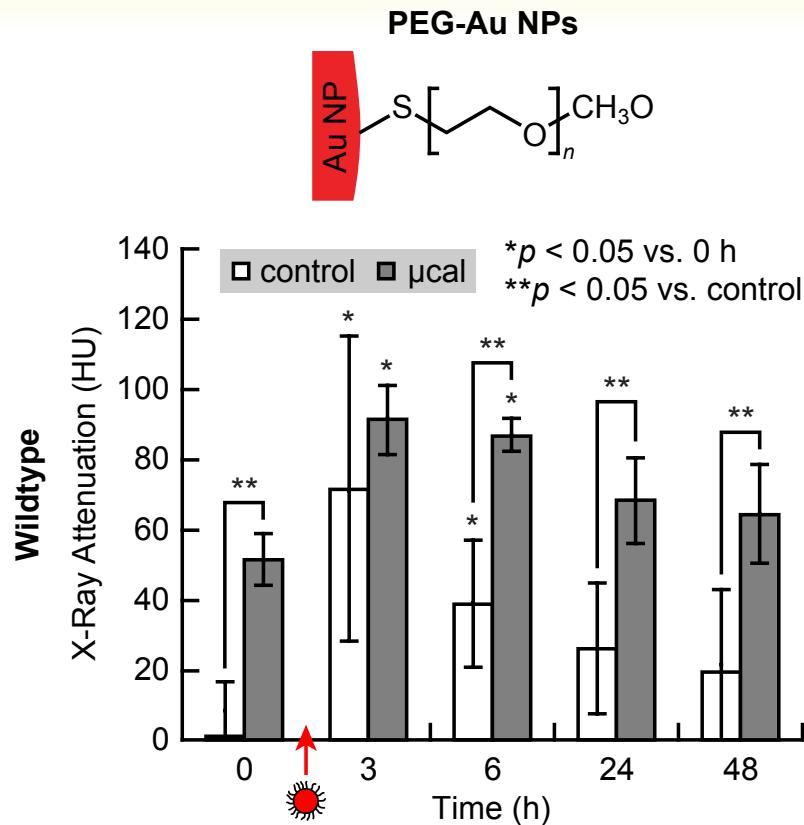
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BP- vs. PEG- vs. BP-PEG Au NPs

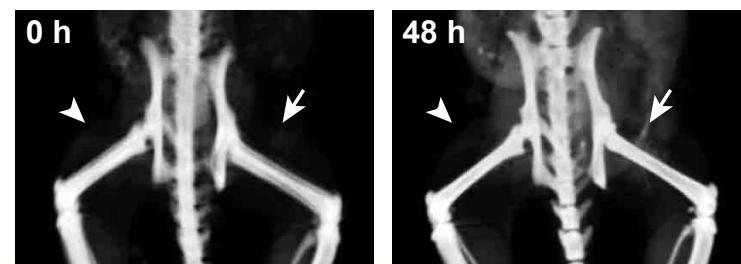
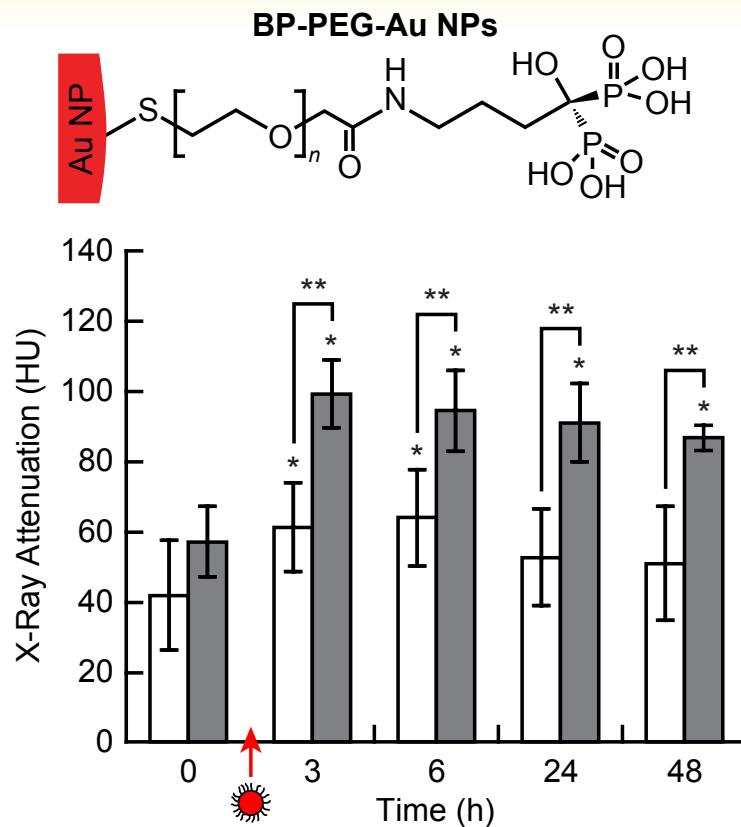
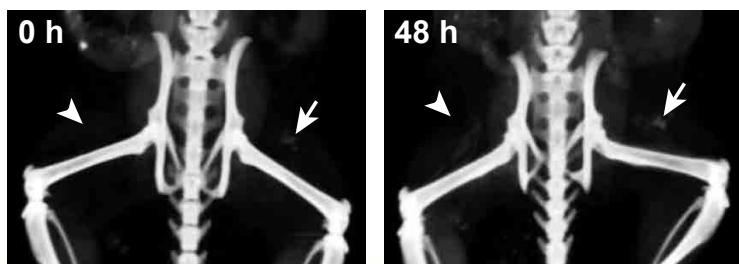
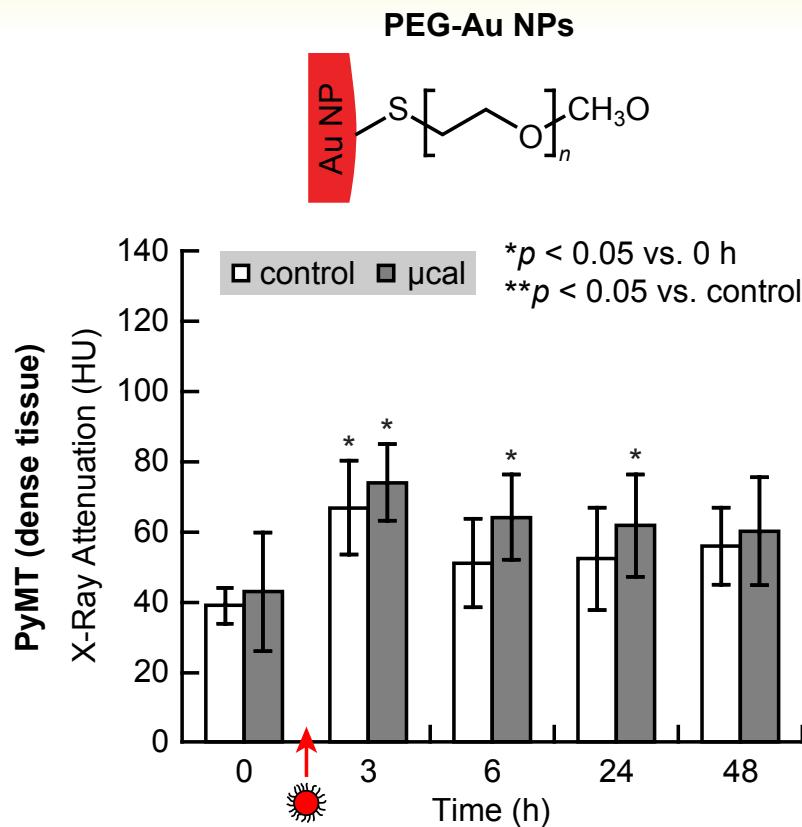


Group	V_{\max} (mg/g)	V_{\max}^* (mg/m ²)	$V_{\max}^{\#}$ (#/ μm^2)	R^2
PEG-Au NPs	2.1 [1.2, 2.9]	0.4	17	0.62
BP-PEG-Au NPs	9.8 [8.8, 10.9]	1.7	86	0.96
BP-Au NPs [26]	7.7 [6.9, 8.6]	1.4	65	0.95
Au NPs [26]	0.4 [0.2, 0.5]	0.1	3	0.75

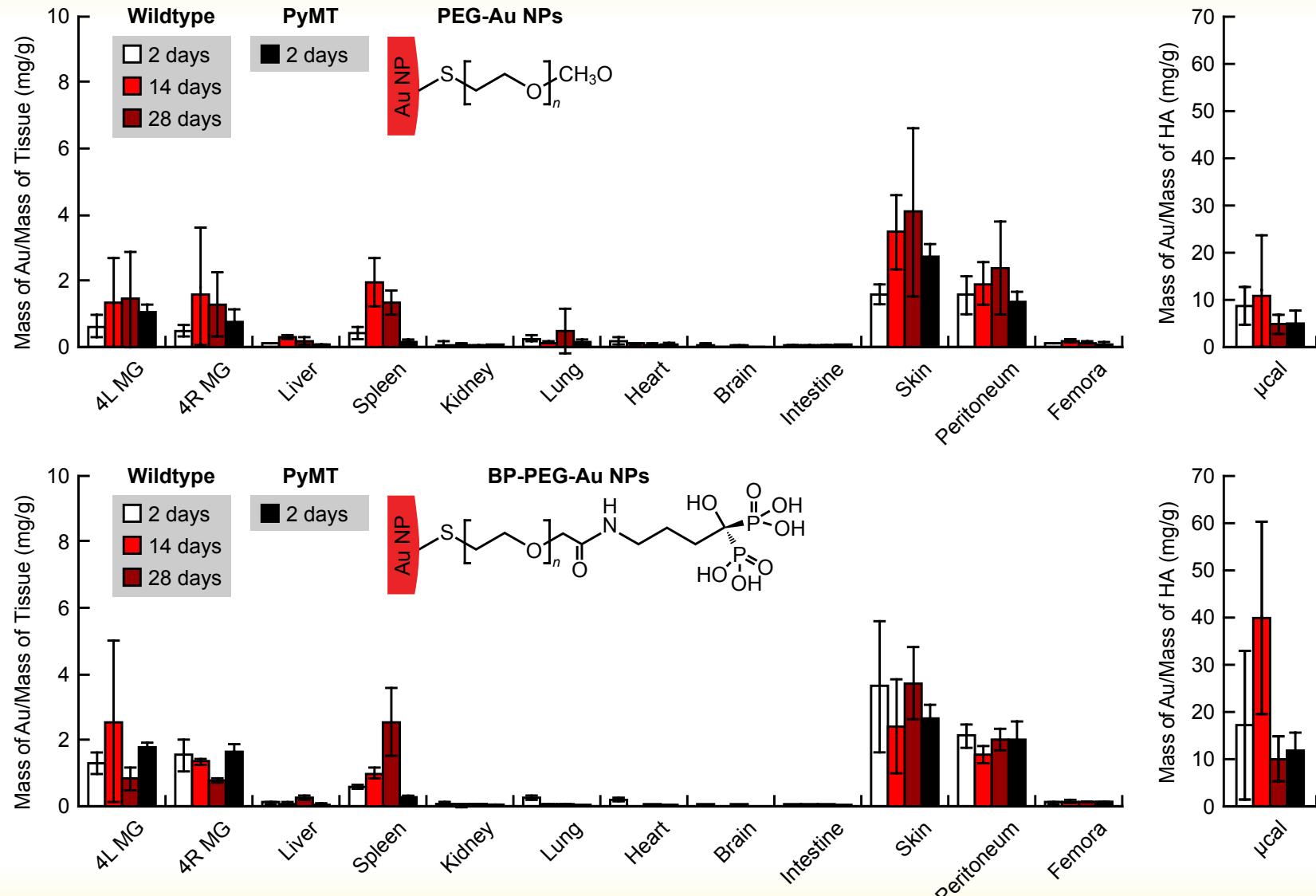
BP- vs. PEG- vs. BP-PEG Au NPs



BP- vs. PEG- vs. BP-PEG Au NPs



BP- vs. PEG- vs. BP-PEG Au NPs

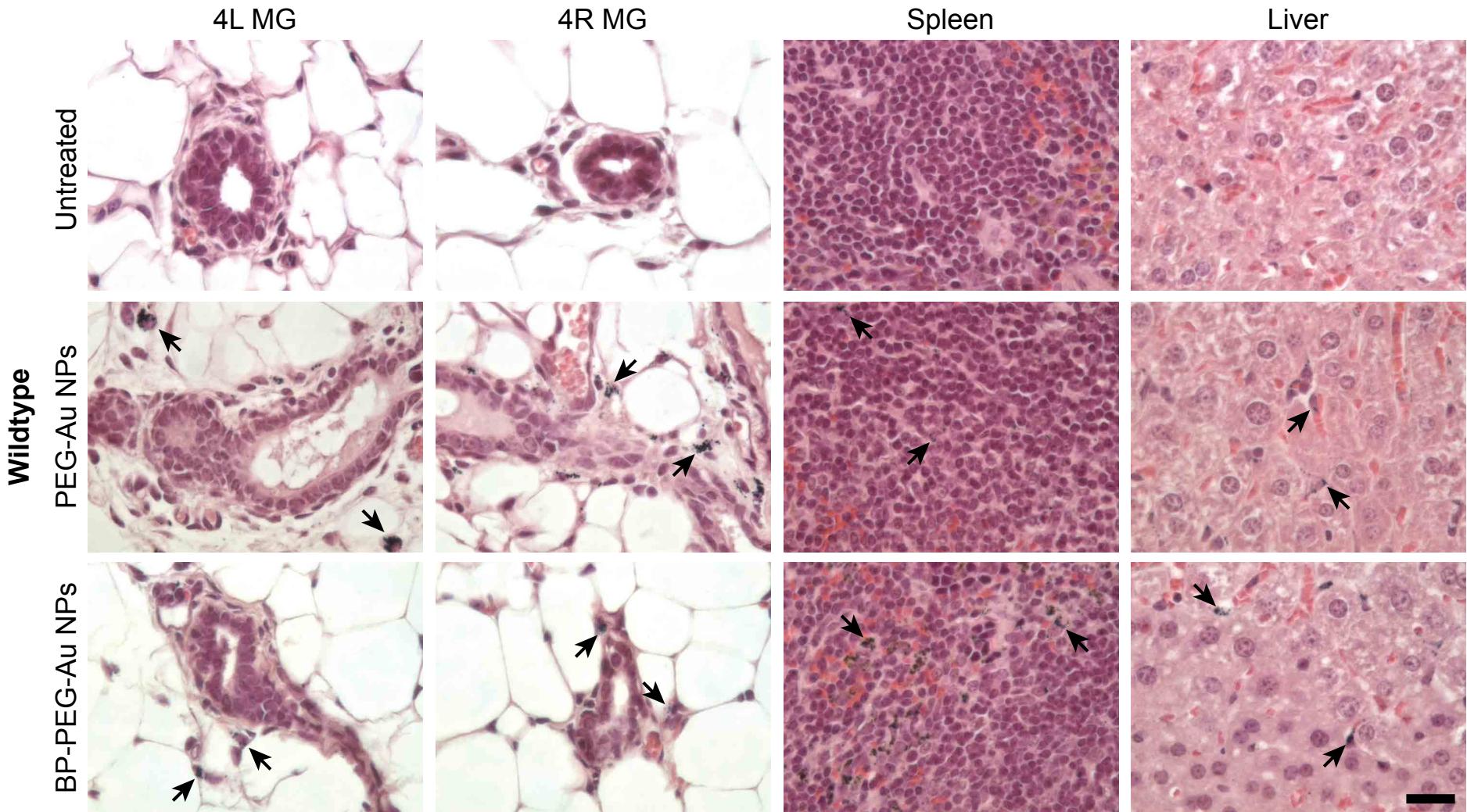


Cole et al., *Acta Biomaterialia*, 2018

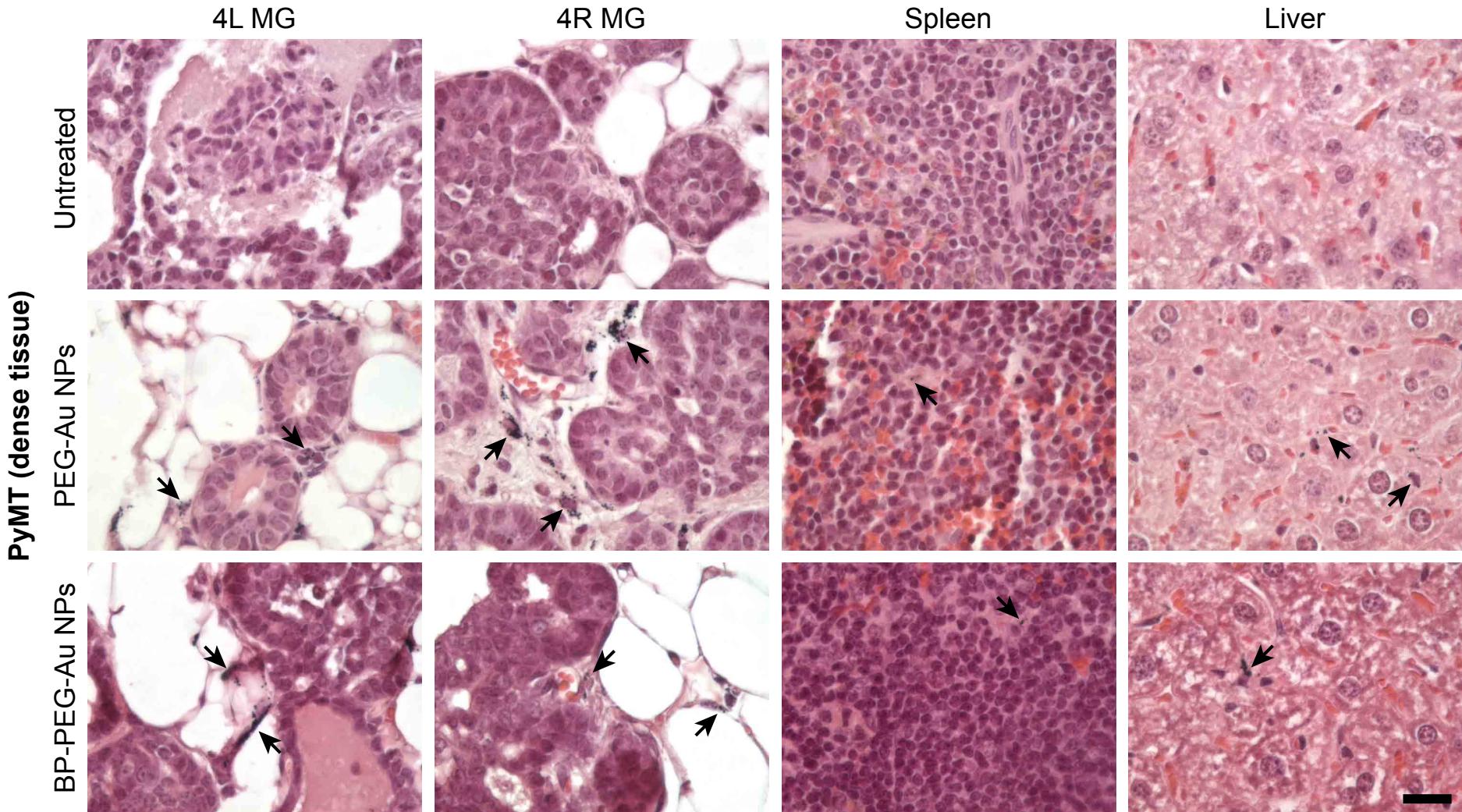


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Histology after 14 days



Histology after 14 days

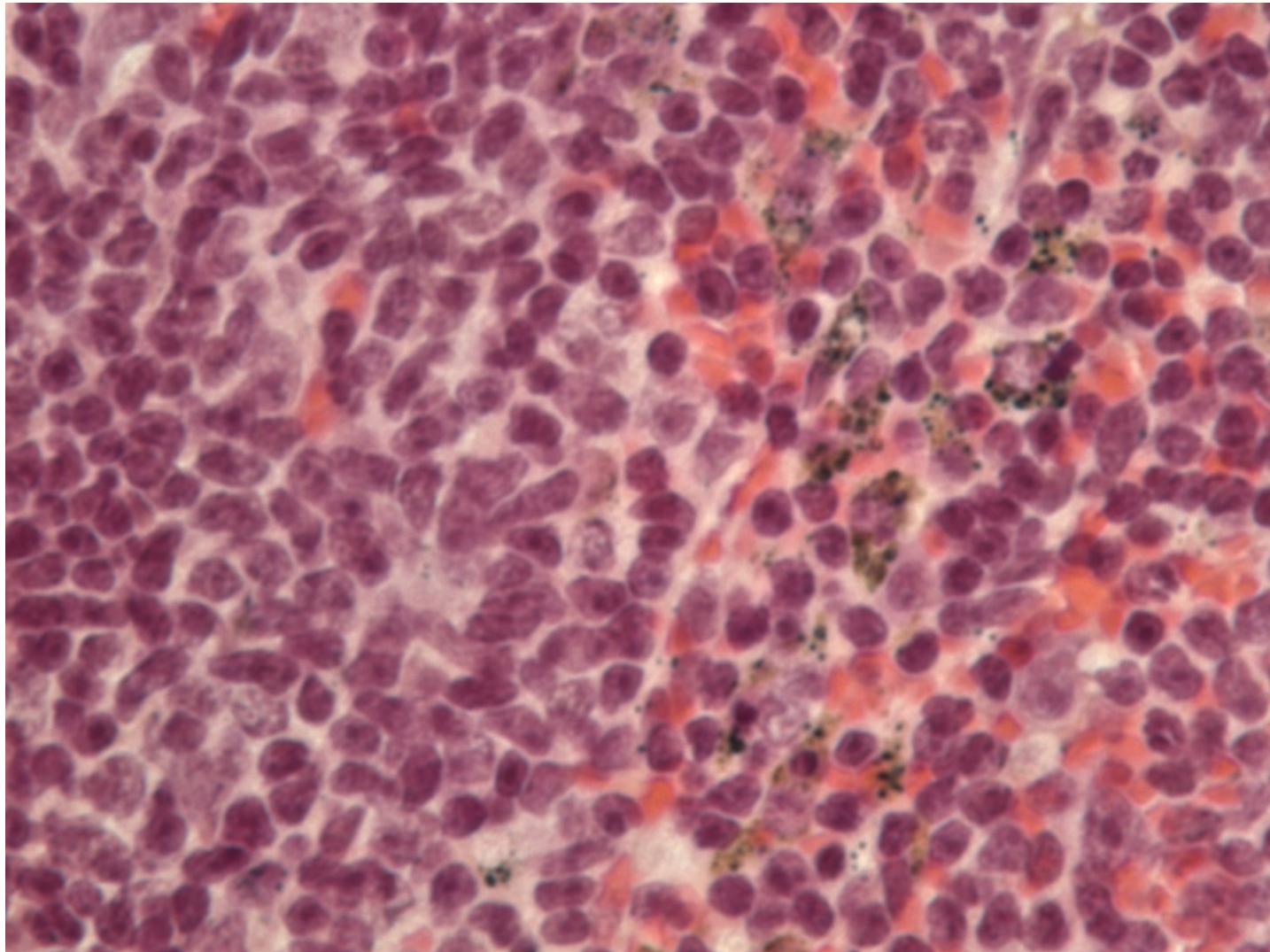


Cole et al., *Acta Biomaterialia*, 2018



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Accumulation in the Spleen (28 d)

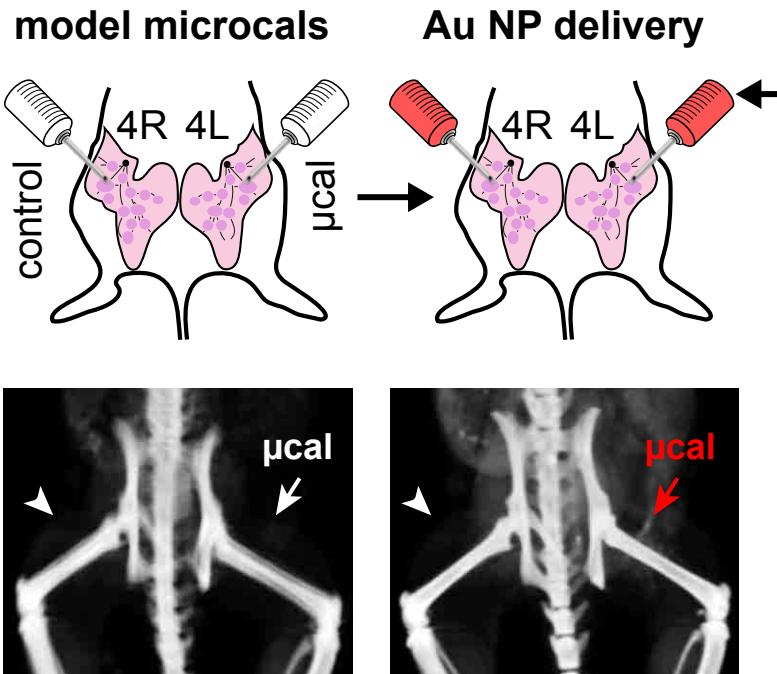


Cole et al., *Acta Biomaterialia*, 2018



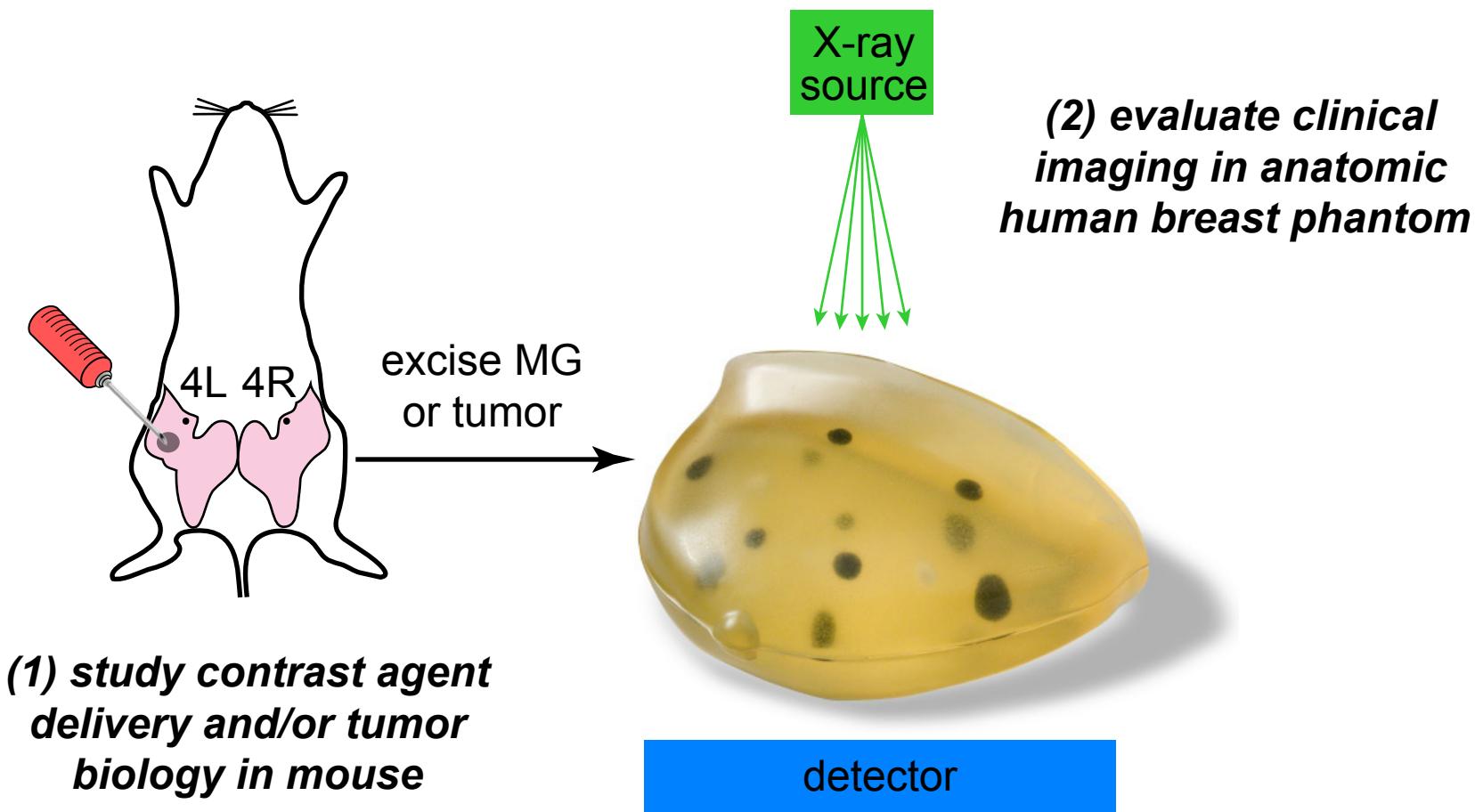
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BP- vs. PEG- vs. BP-PEG Au NPs



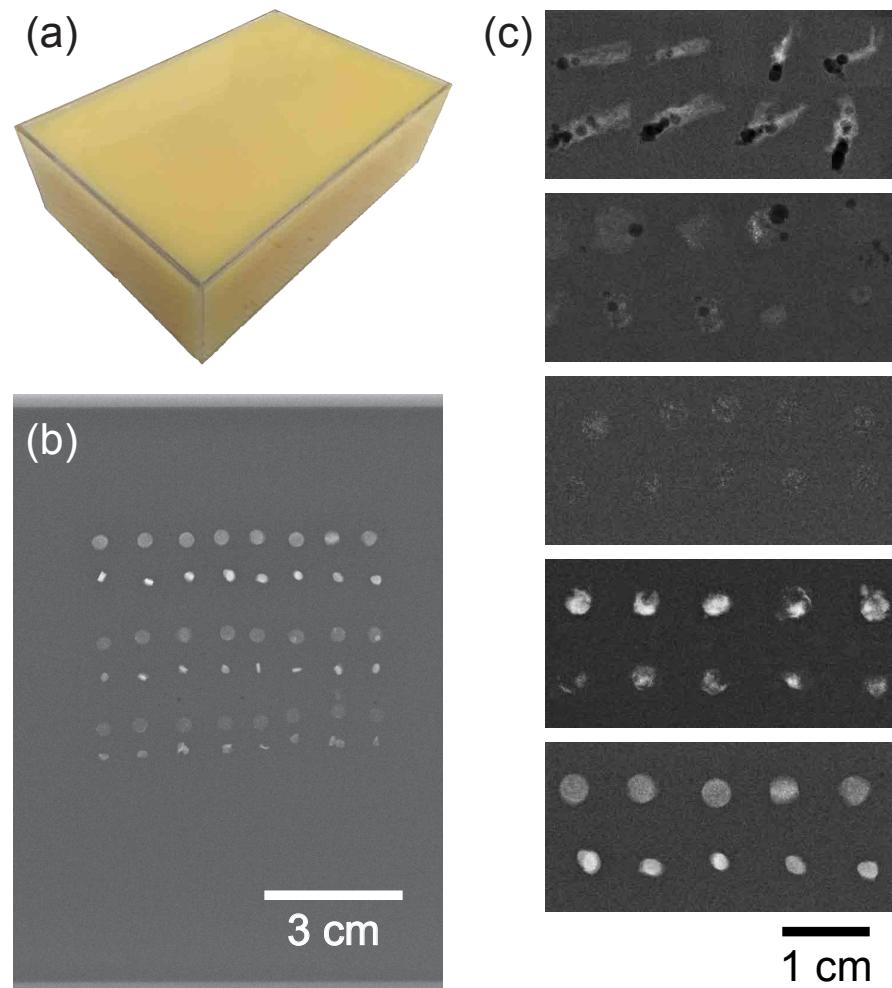
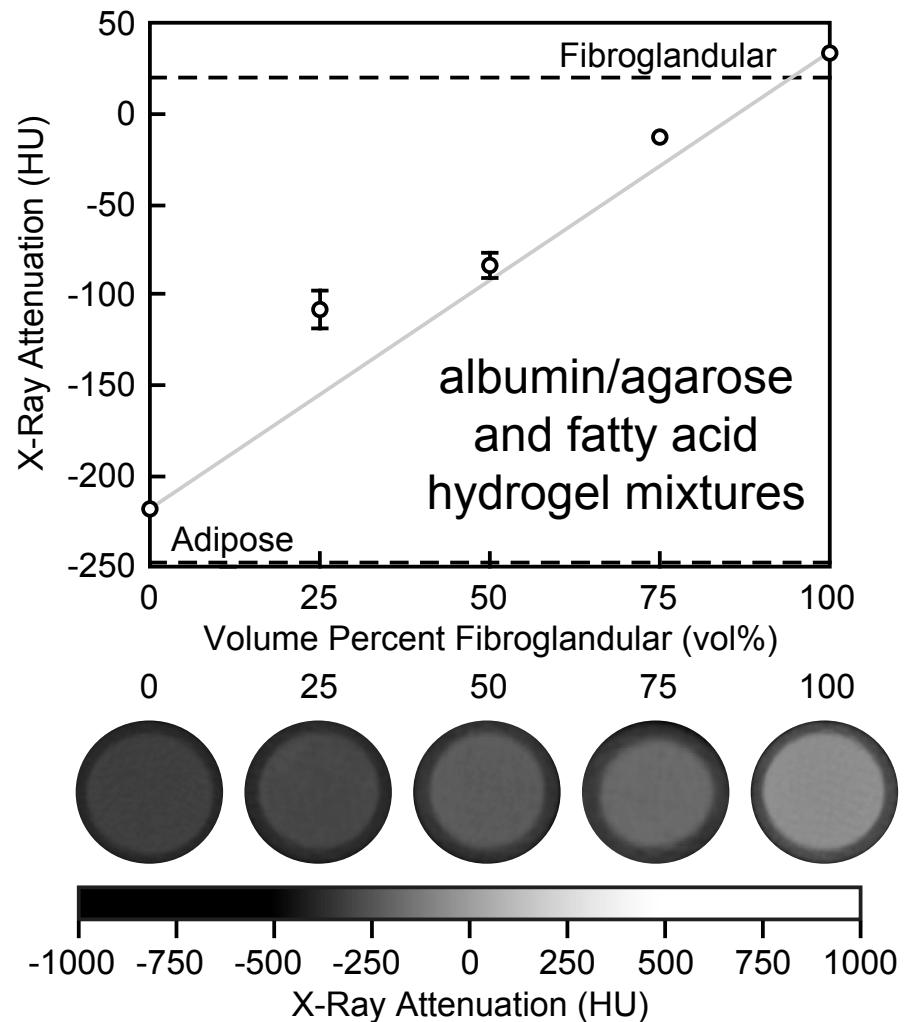
Functionalized Au NPs	targeting	clearance
PEG-Au NPs 	none	most
BP-PEG-Au NPs 	strong	most
BP-Au NPs 	strong	least

Translational Model



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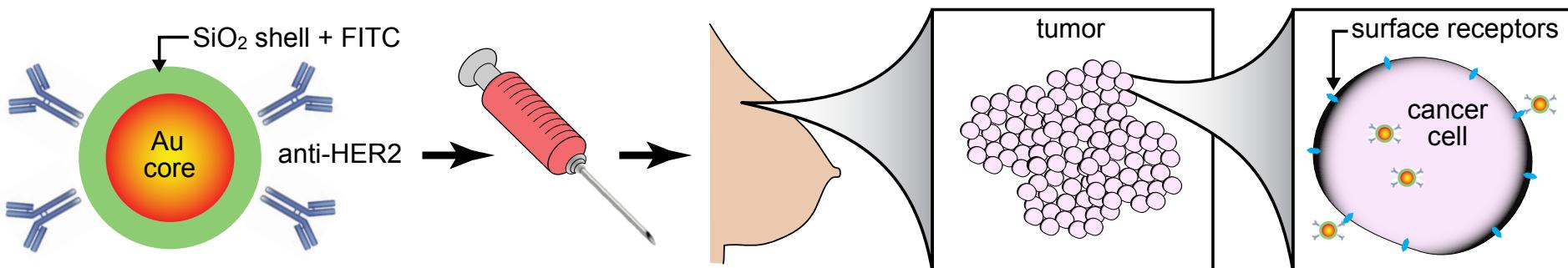
Mammographic Breast Phantom



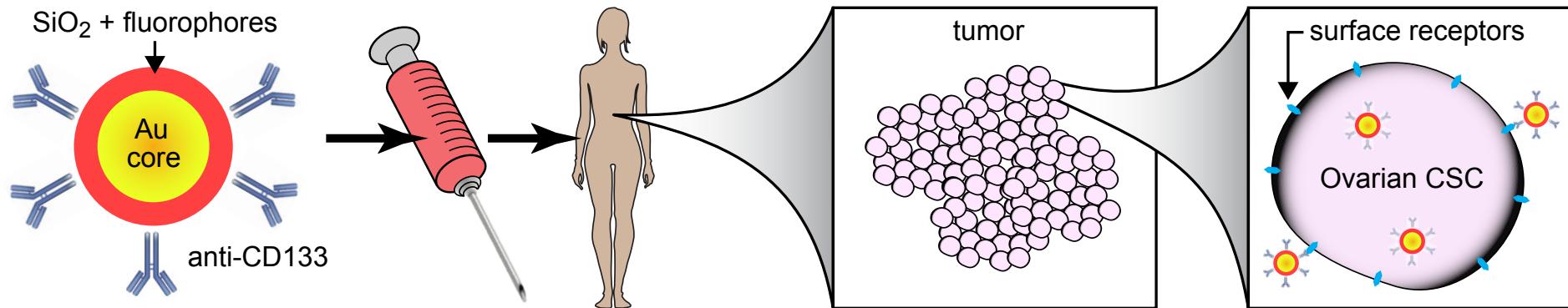
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Immunotargeted NP Probes for Spectral CT

preoperative staging & monitoring therapeutic response of HER2+ breast cancer



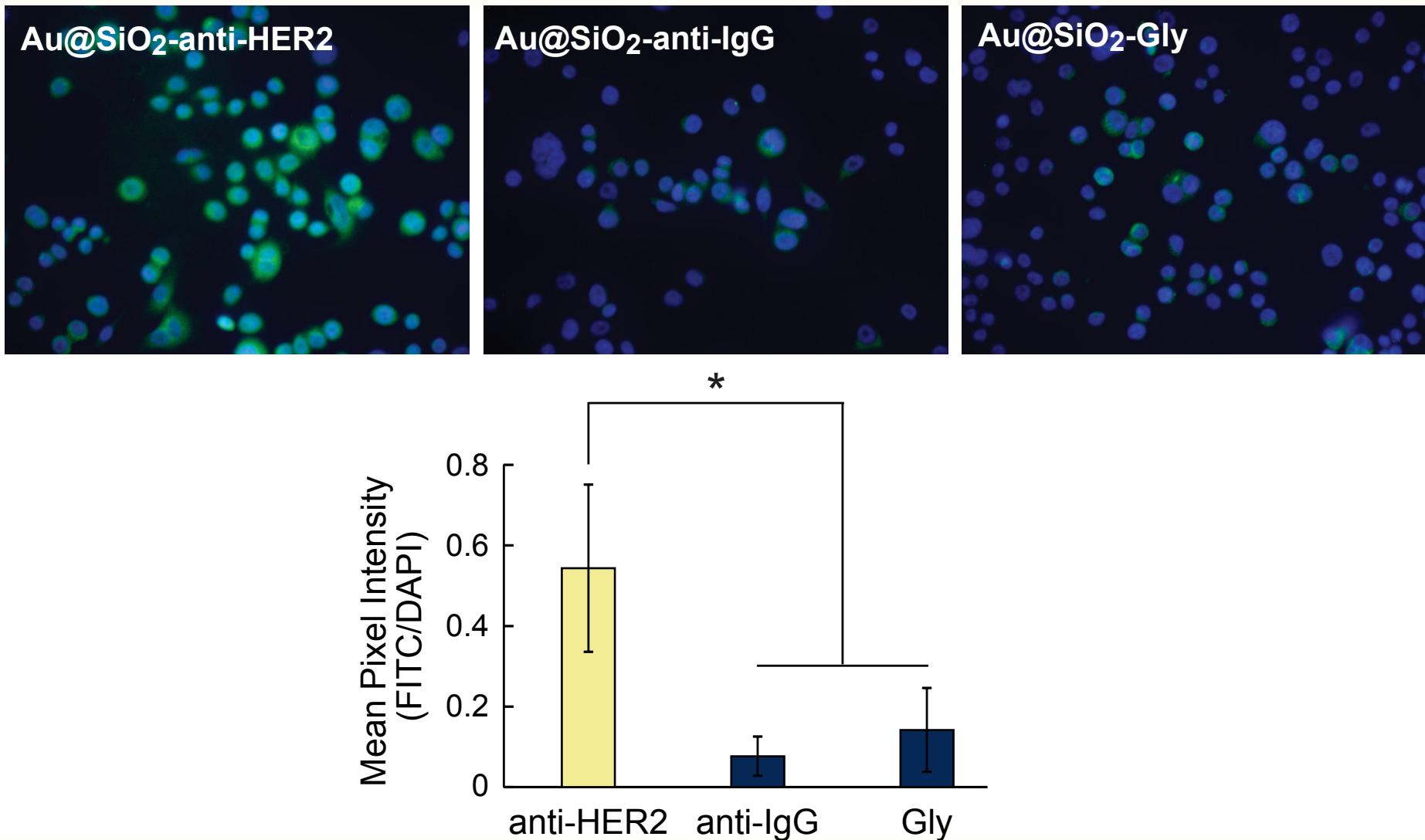
detection of primary tumors and recurrence of ovarian cancer



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Immunotargeted NP Probes for Spectral CT

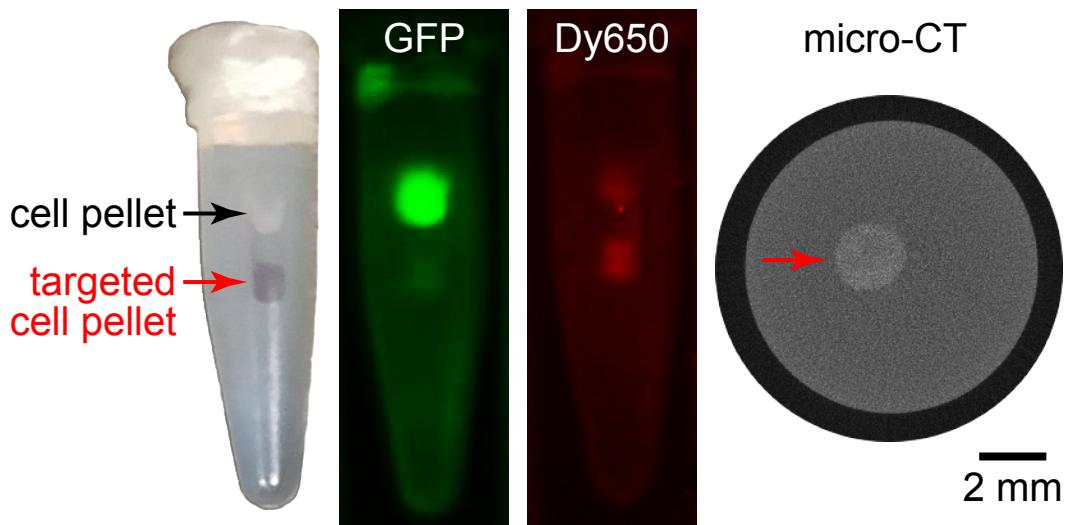
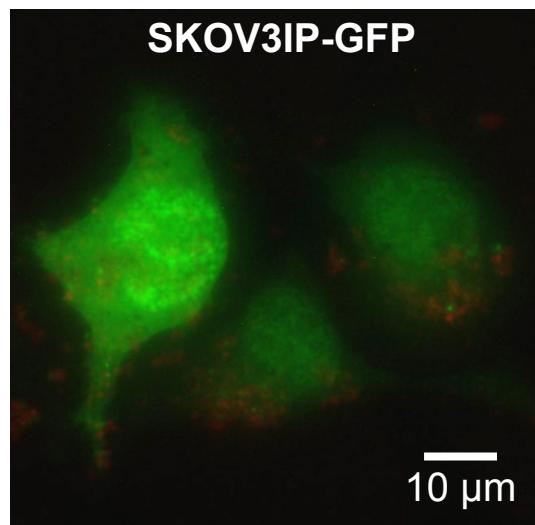
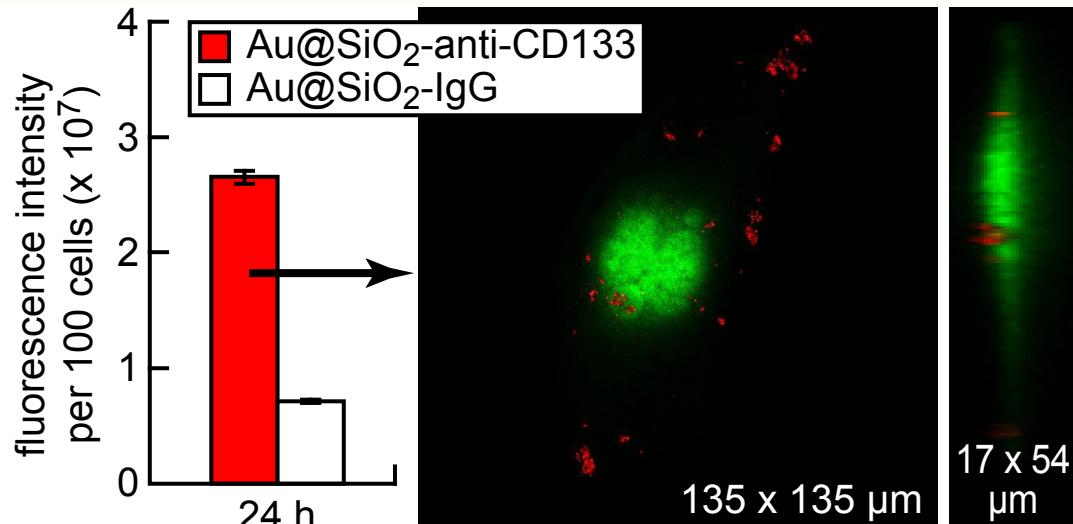
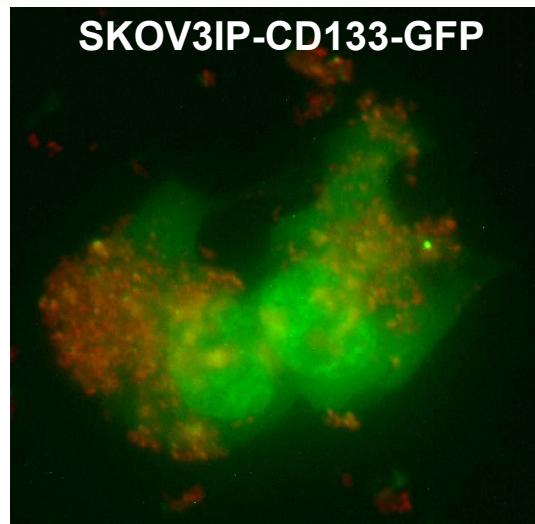


McGinnity *et al.*, *Trans. SFB*, 2017

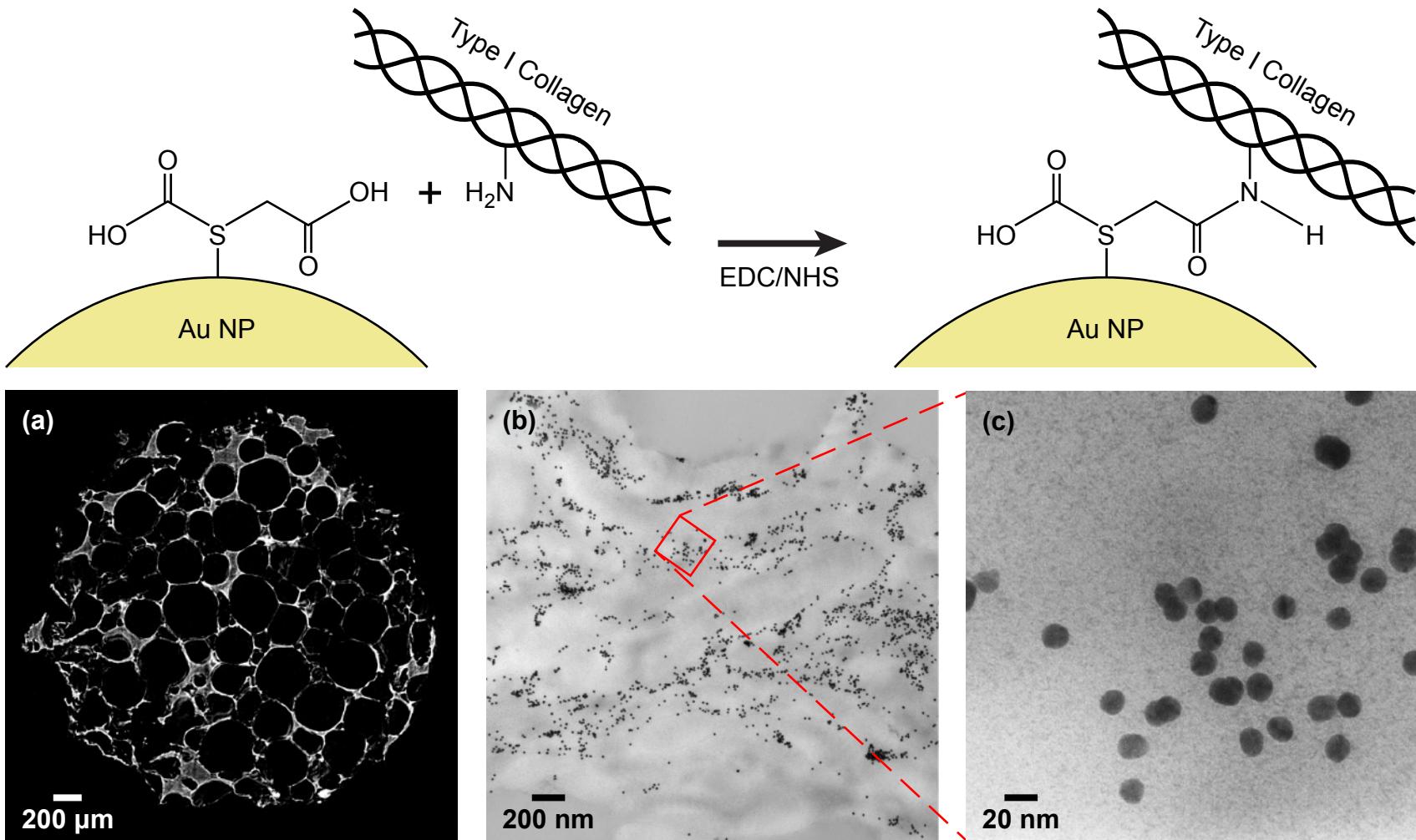


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Immunotargeted NP Probes for Spectral CT



Collagen Scaffold Degradation Measured Using CT and Au NPs

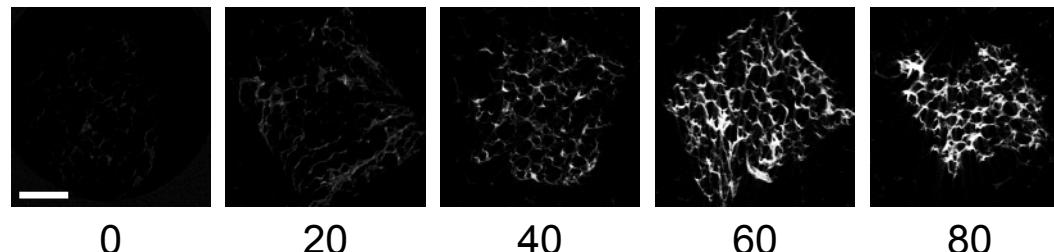


Finamore *et al.*, in preparation

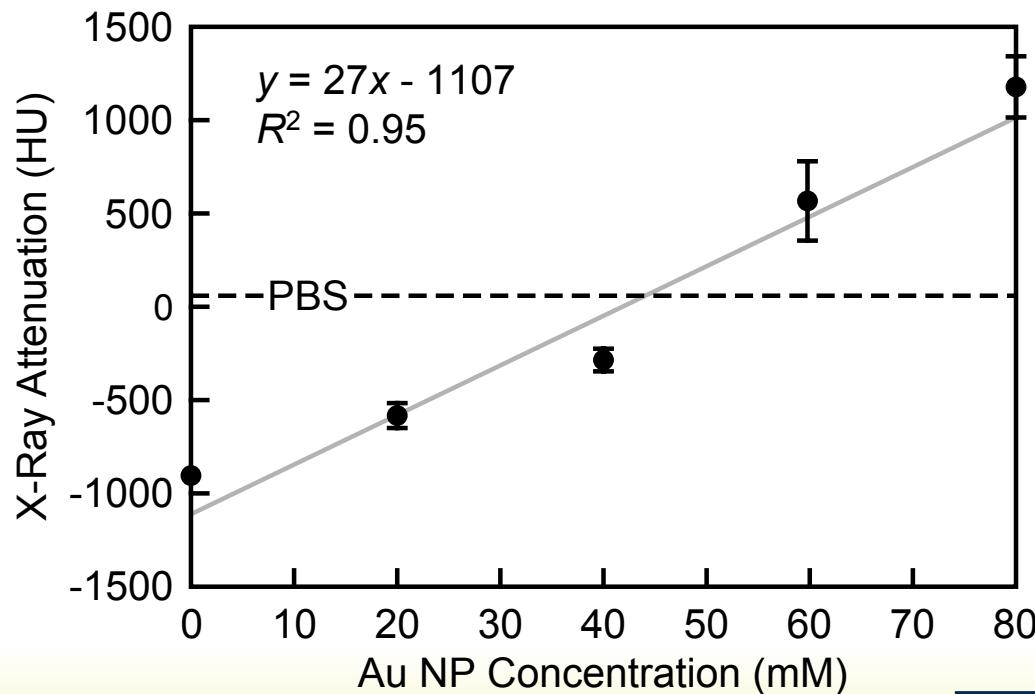


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Collagen Scaffold Degradation Measured Using CT and Au NPs



0 20 40 60 80
Au NP Concentration (mM)

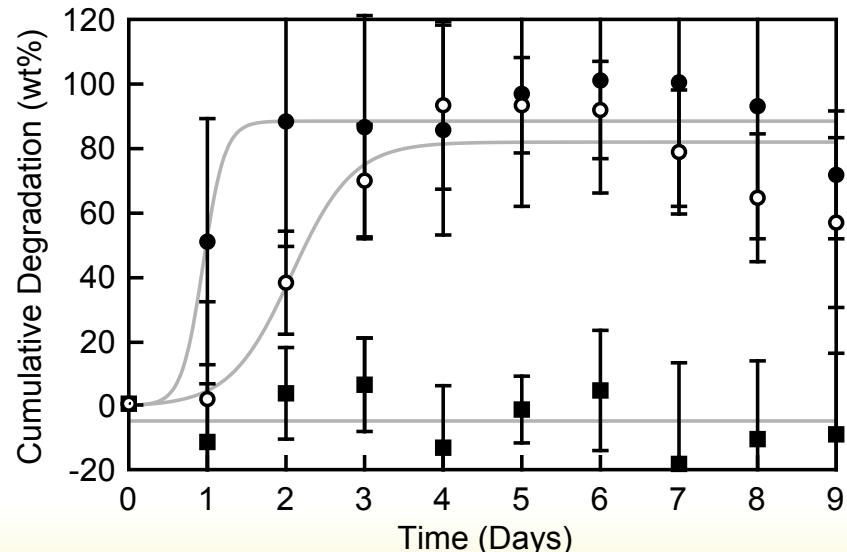
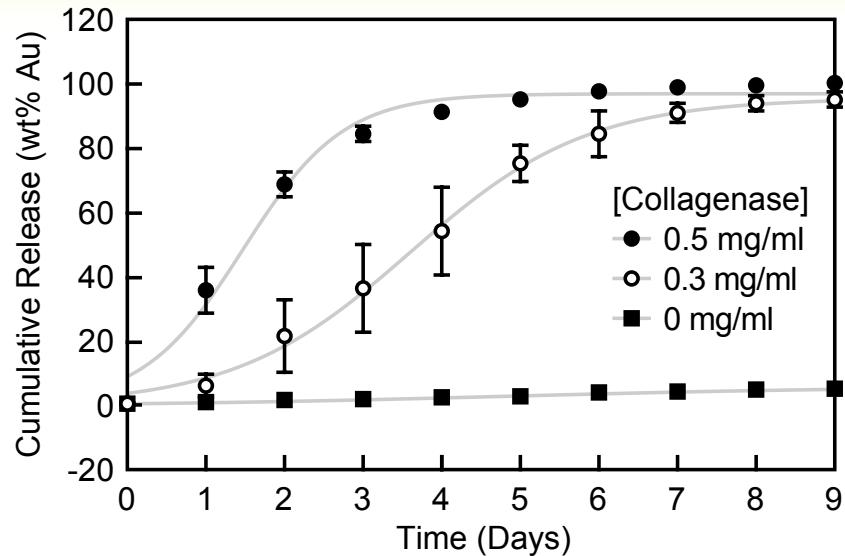
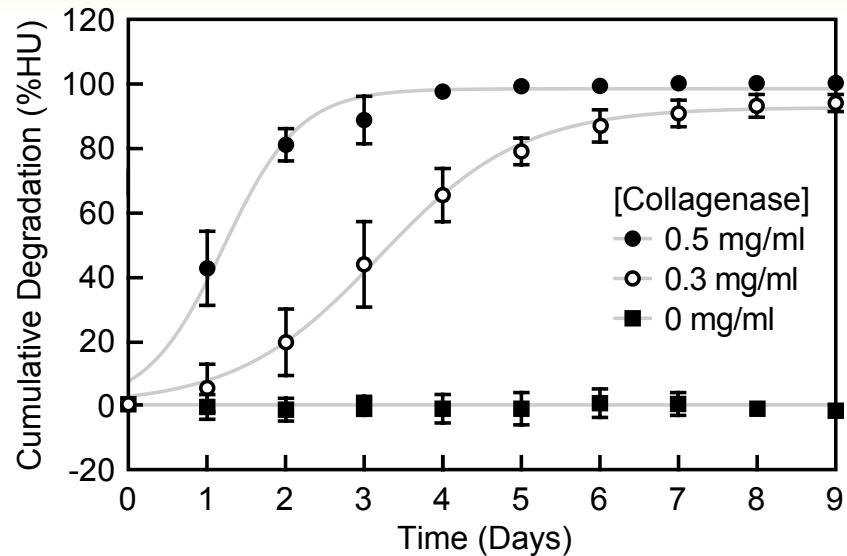


Finamore *et al.*, in preparation



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Longitudinal Collagen Scaffold Degradation



Finamore *et al.*, in preparation



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The Future of CT is Color! (Spectral CT)

We've watched color television since the 1950s...

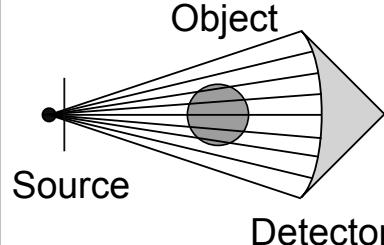
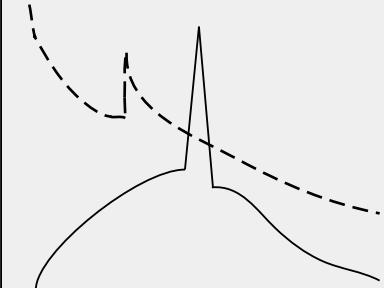
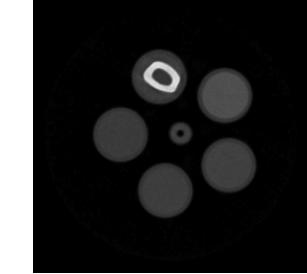
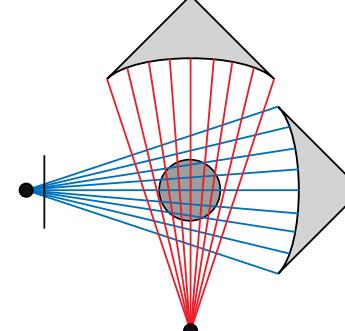
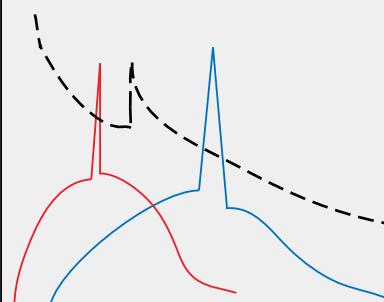
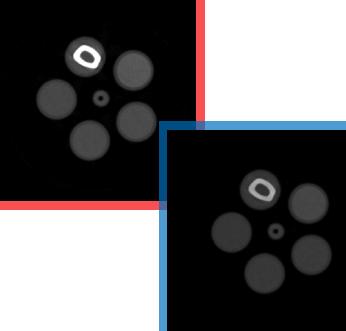
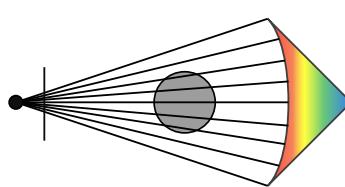
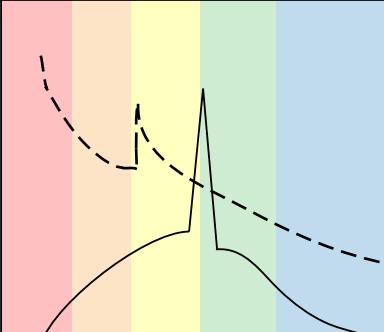
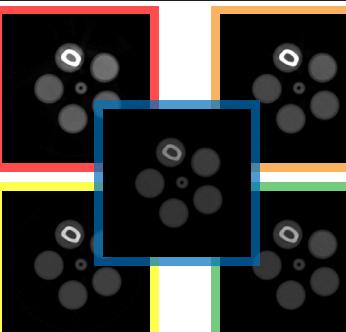


...yet radiology remains primarily black and white in 2018!



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Evolution of Computed Tomography

	Geometry	Photon Spectrum	Reconstructions
Conventional			
Dual Energy CT (DECT)			
Photon-Counting Spectral CT			

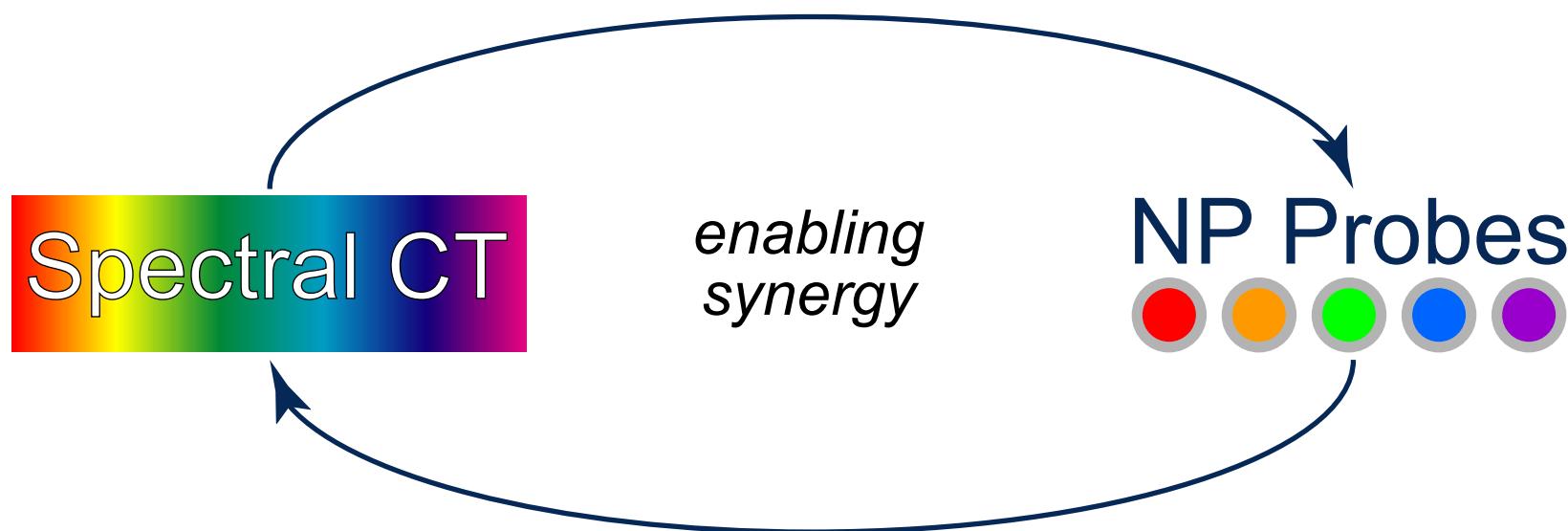


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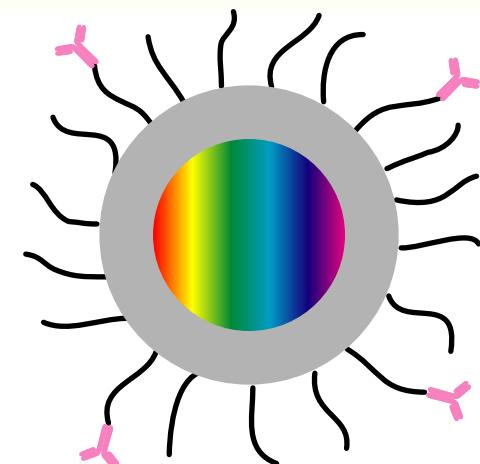
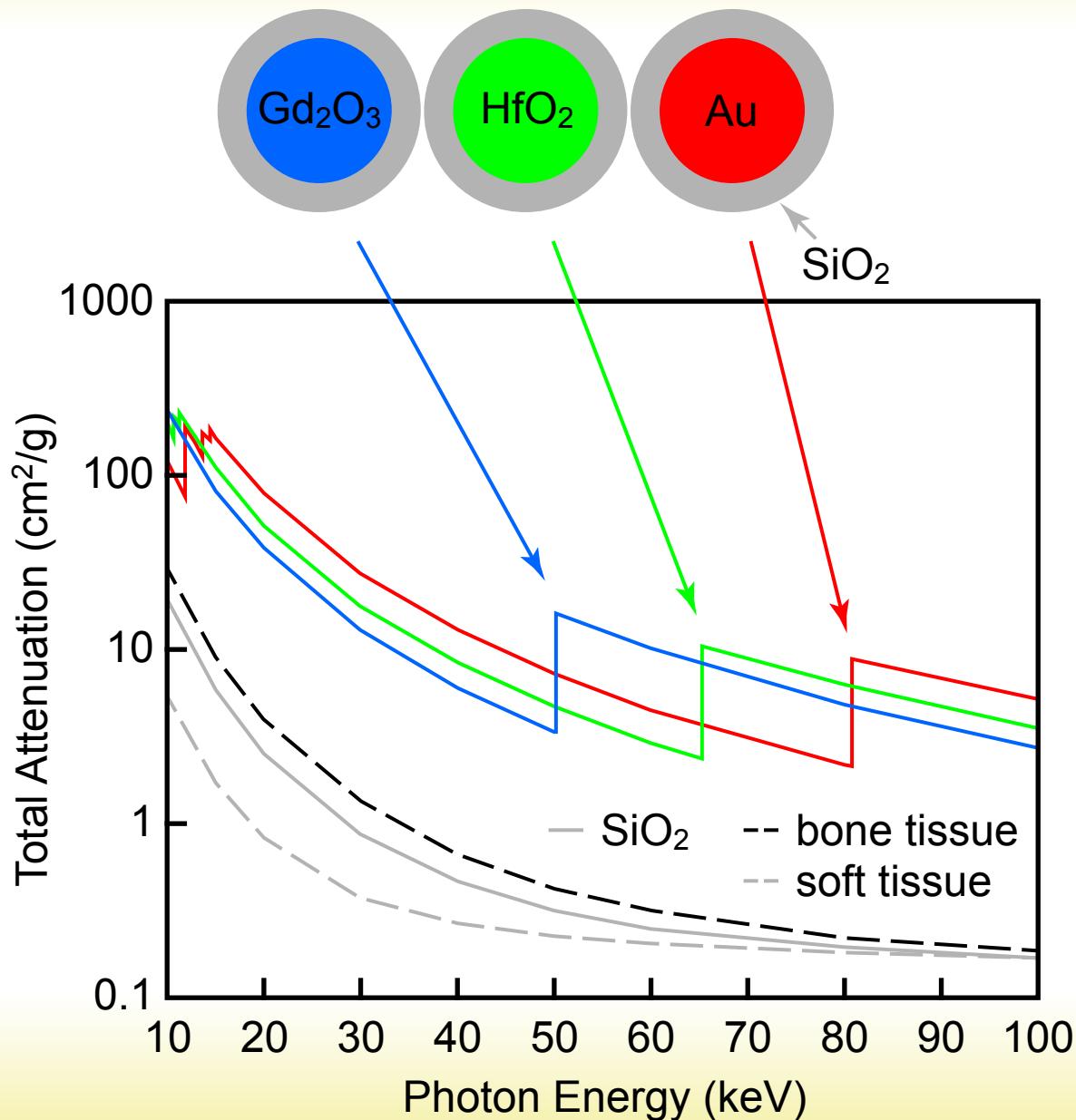
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Potential Applications for Spectral CT

- improved CNR and contrast in CT
- decreased X-ray dose
- metal artifact elimination
- quantitative material decomposition
- multi-agent, multi-material imaging
- decomposition of multiple, mixed (spatially coincident) materials
- improved sensitivity for contrast-enhancement (k -edge imaging)
- molecular imaging with CT



Spectral Library of NP Imaging Probes



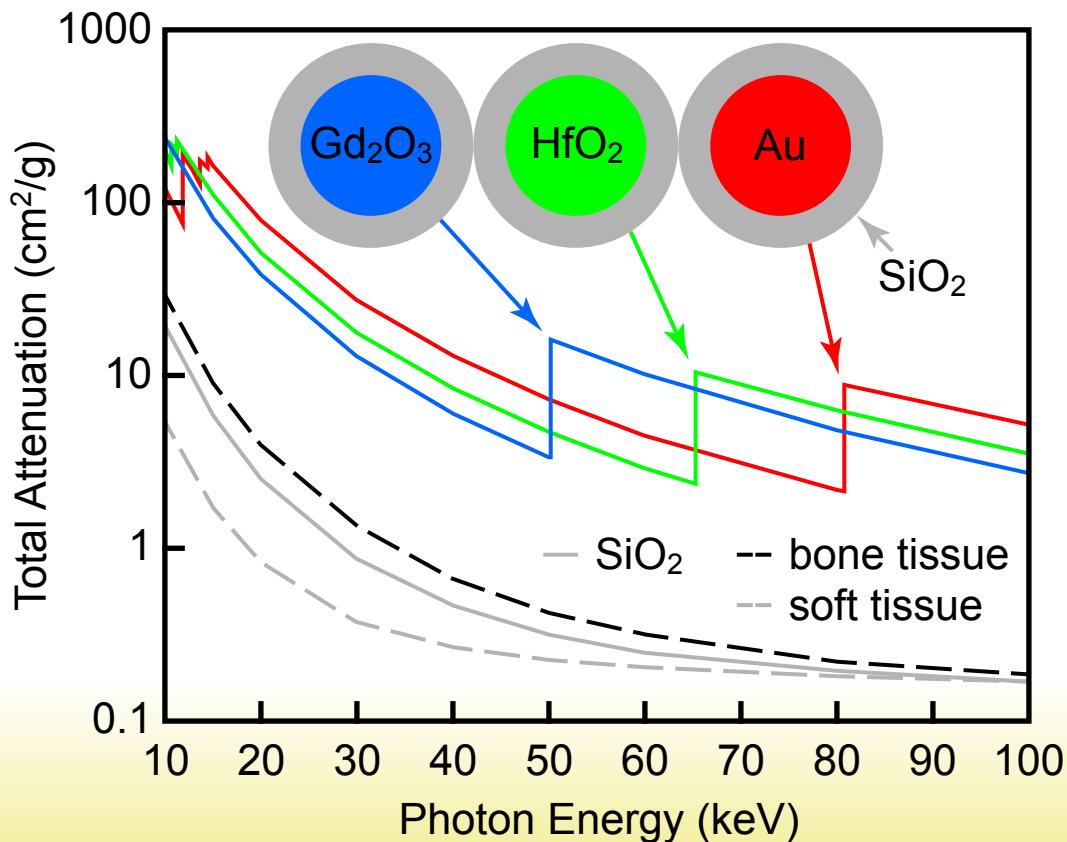
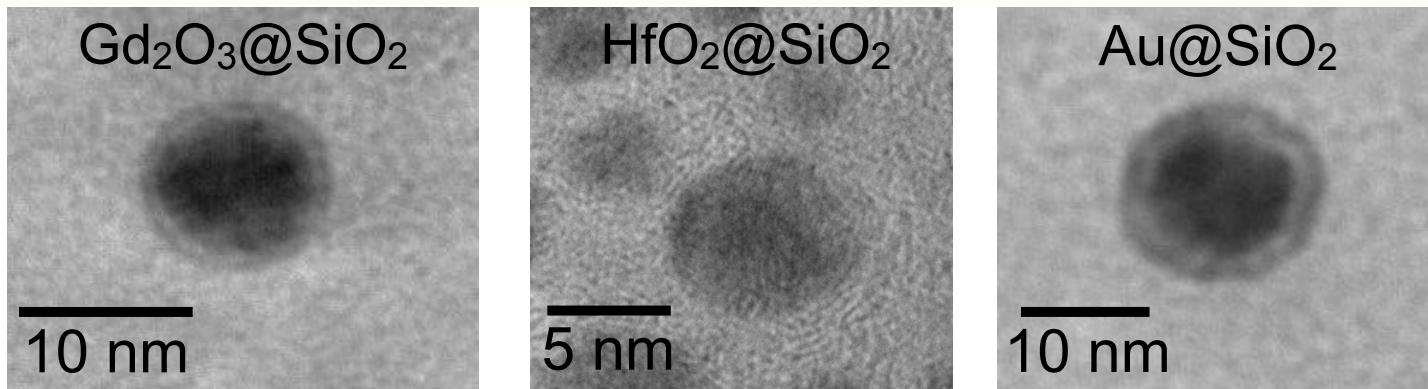
Design:

- 10-15 nm high-Z core
- K-edges spaced in CT energy range
- SiO_2 shell for stability and facile molecular surface functionalization (e.g., immunotargeting)



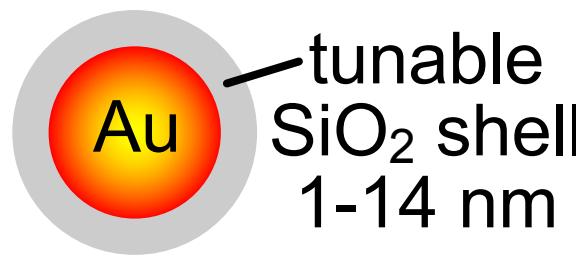
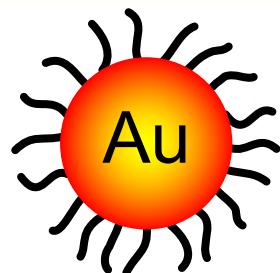
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Spectral Library of NP Imaging Probes

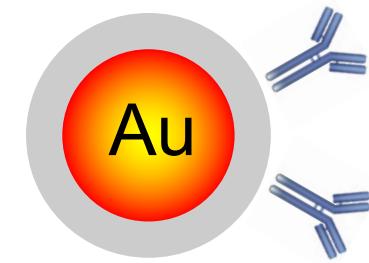


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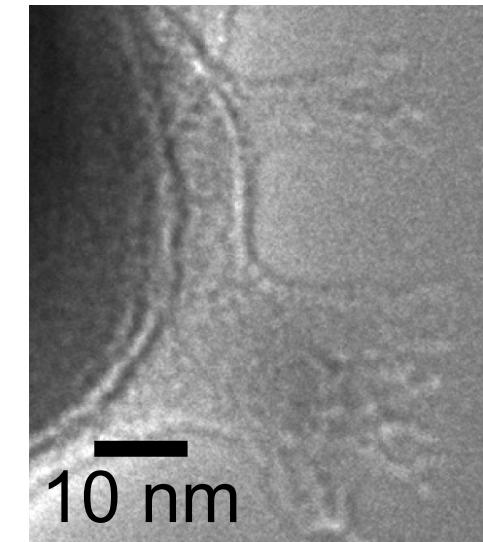
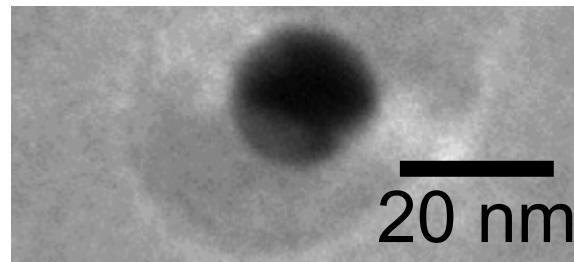
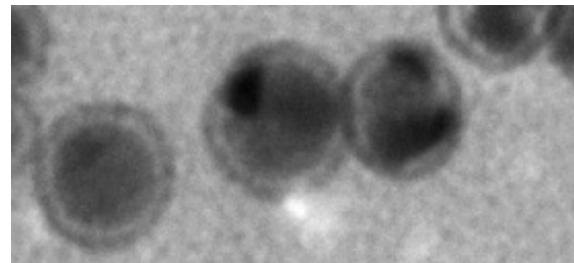
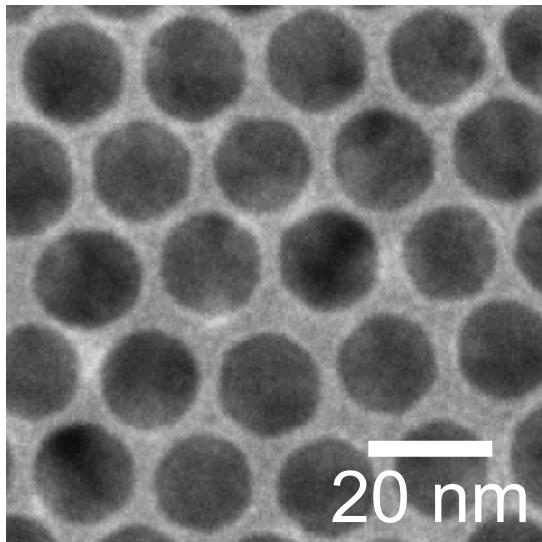
Tailored Core-Shell NP Imaging Probes



+ molecular payload

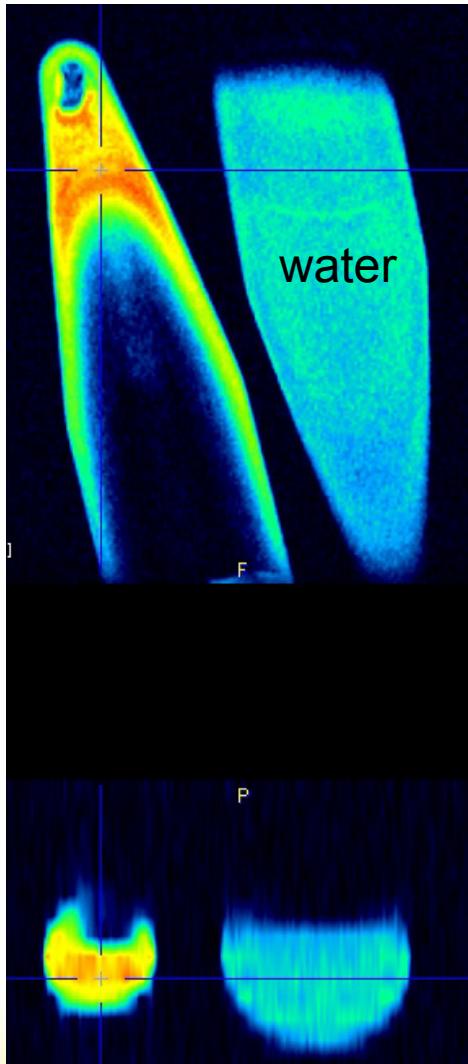


+ antibodies



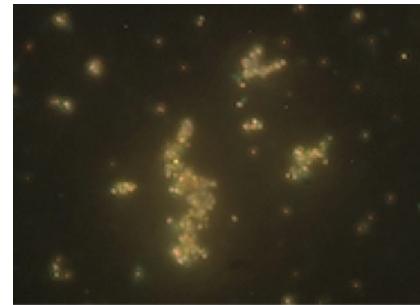
Multimodal Imaging

T1 MRI of Si-Gd₂O₃ NPs

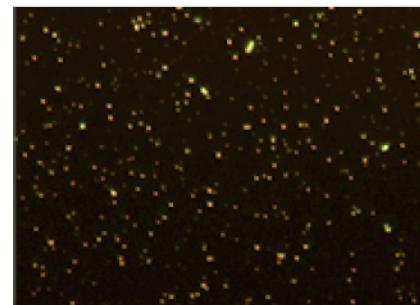


Au NPs with fluorescently labeled SiO₂

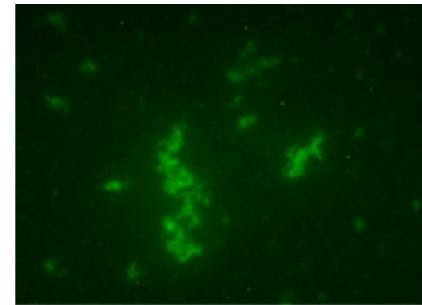
FITC



RITC



dark field



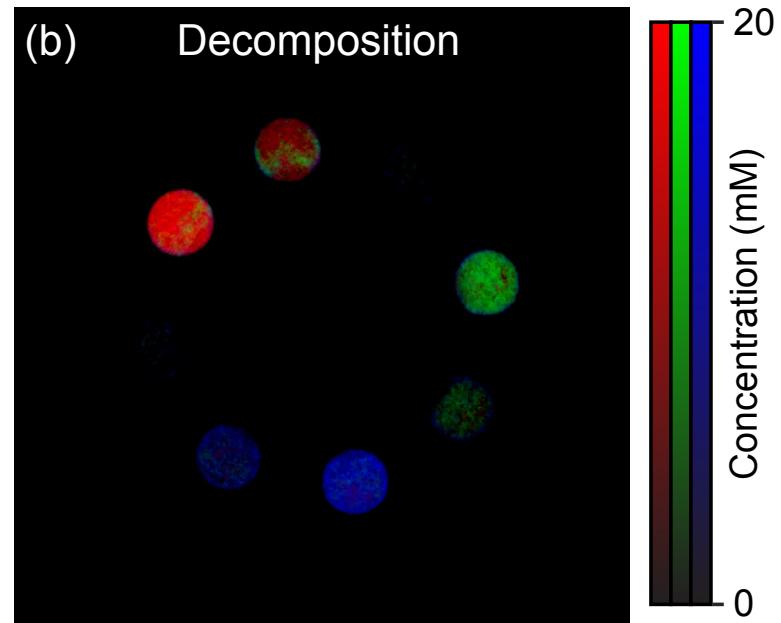
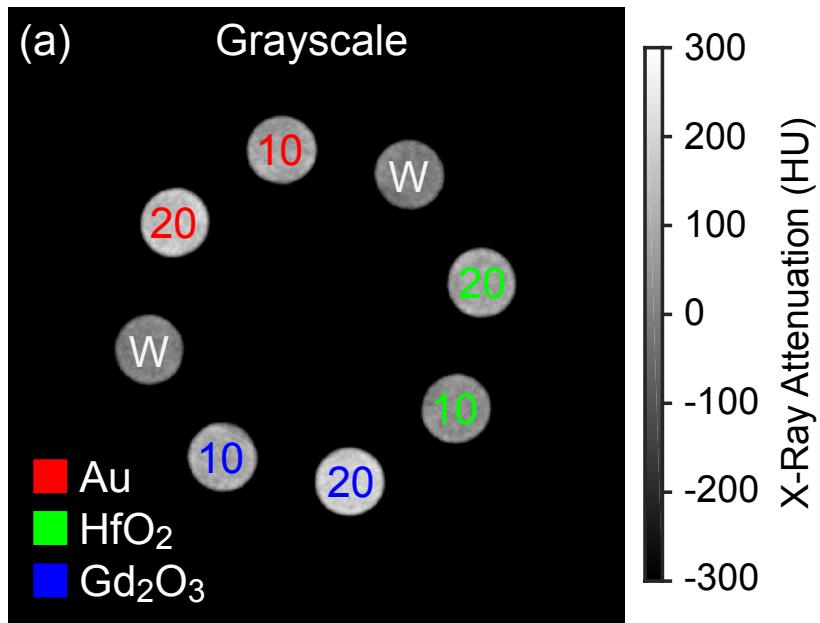
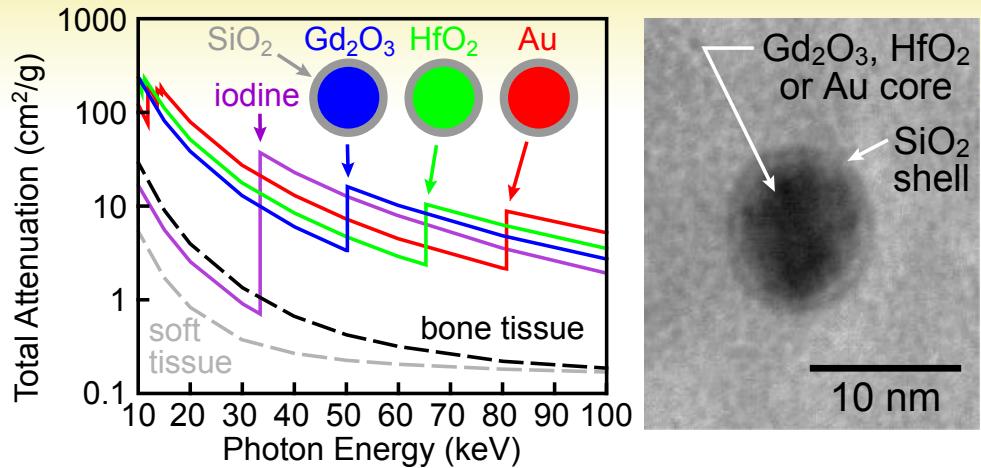
fluorescence



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Multi-Agent Imaging with Spectral CT

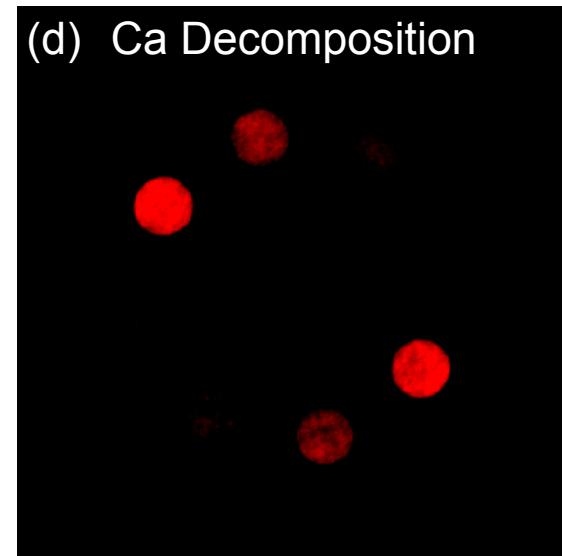
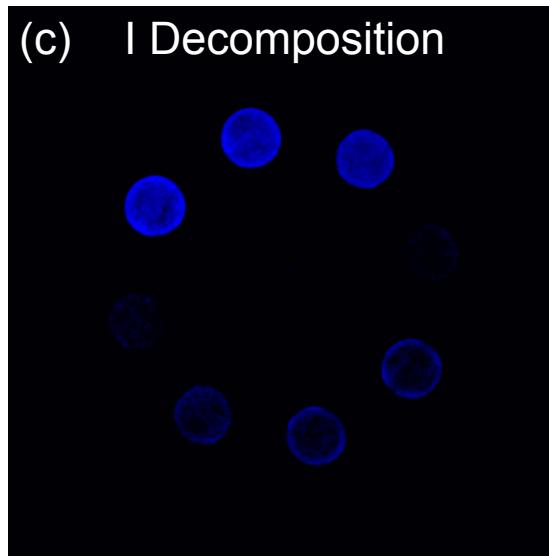
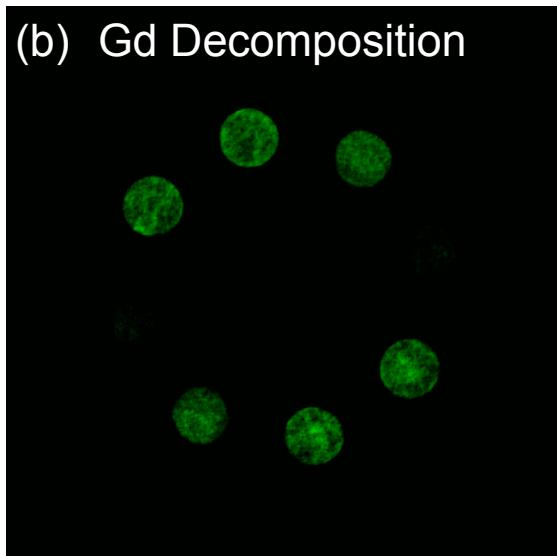
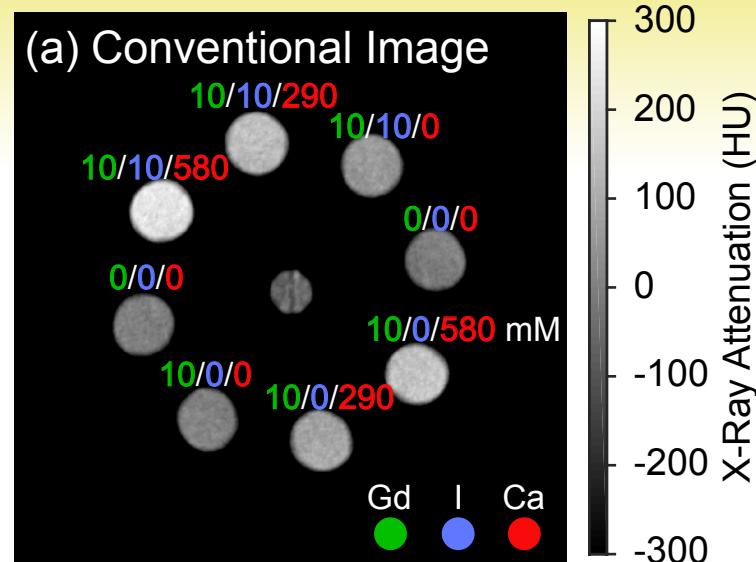


Curtis *et al.*, in preparation

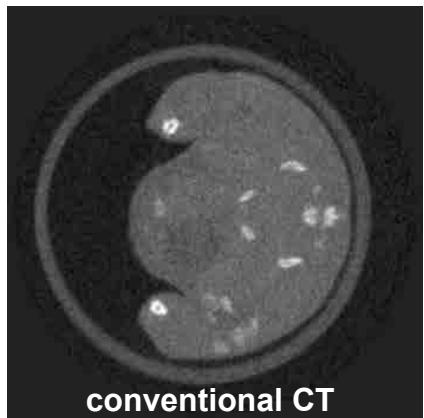


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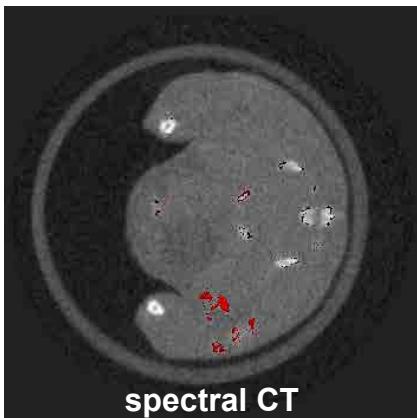
Quantitative Imaging of Mixed Compositions with Spectral CT



Mouse Imaging

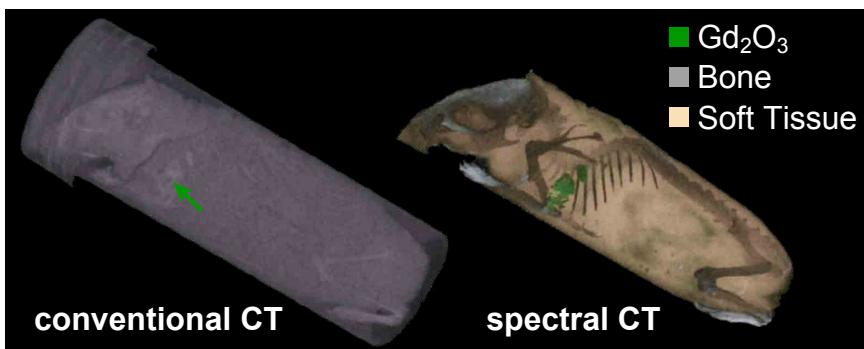


conventional CT

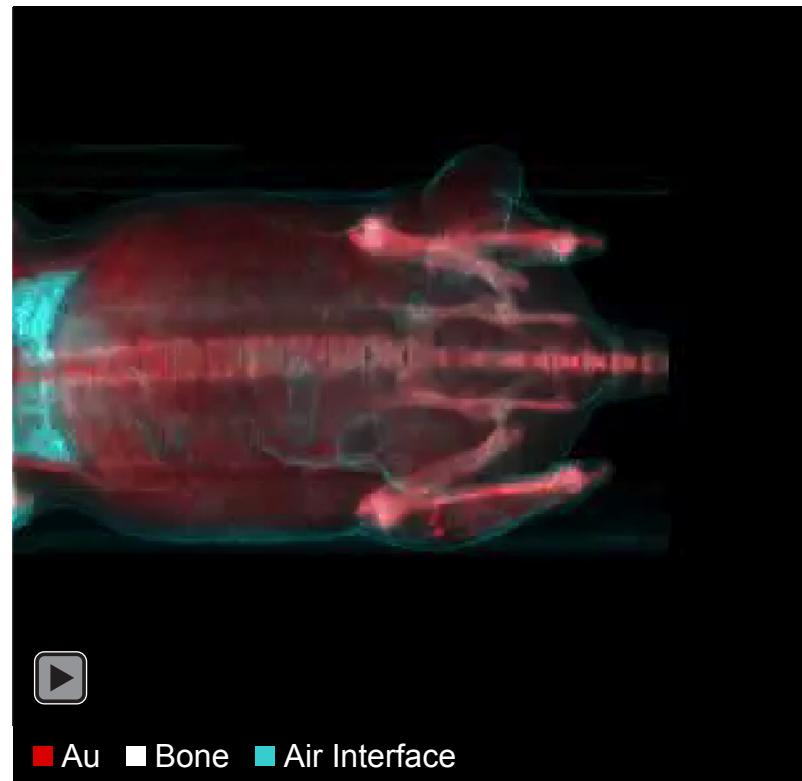


spectral CT

100 μL 50 mM Au NPs s.c.



250 μL of 20 mM Gd₂O₃ NPs s.c.



100 μL 60-80 mg/mL Au NPs i.v.

Roeder *et al.*, Proc. SPIE, 2017; Nallathamby *et al.*, 2018

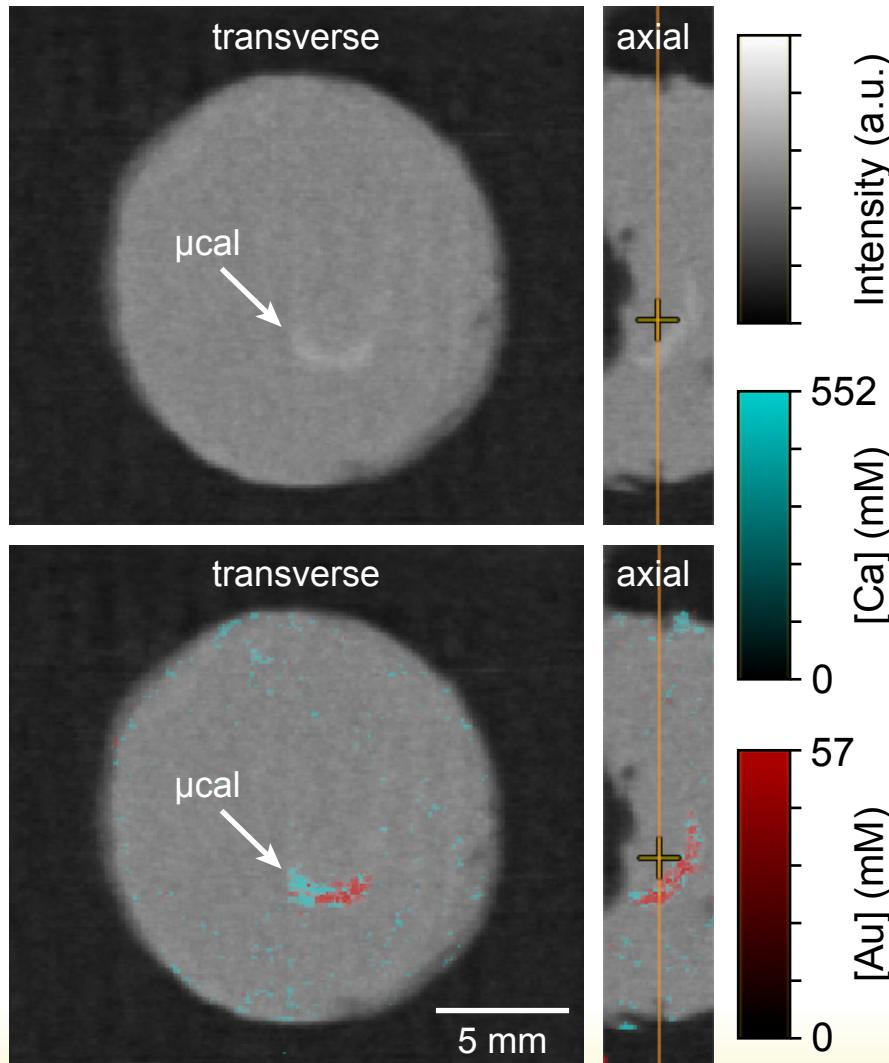


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Detection of Breast Microcalcifications

Quantitative Molecular Imaging with Photon-Counting Spectral CT

conventional CT



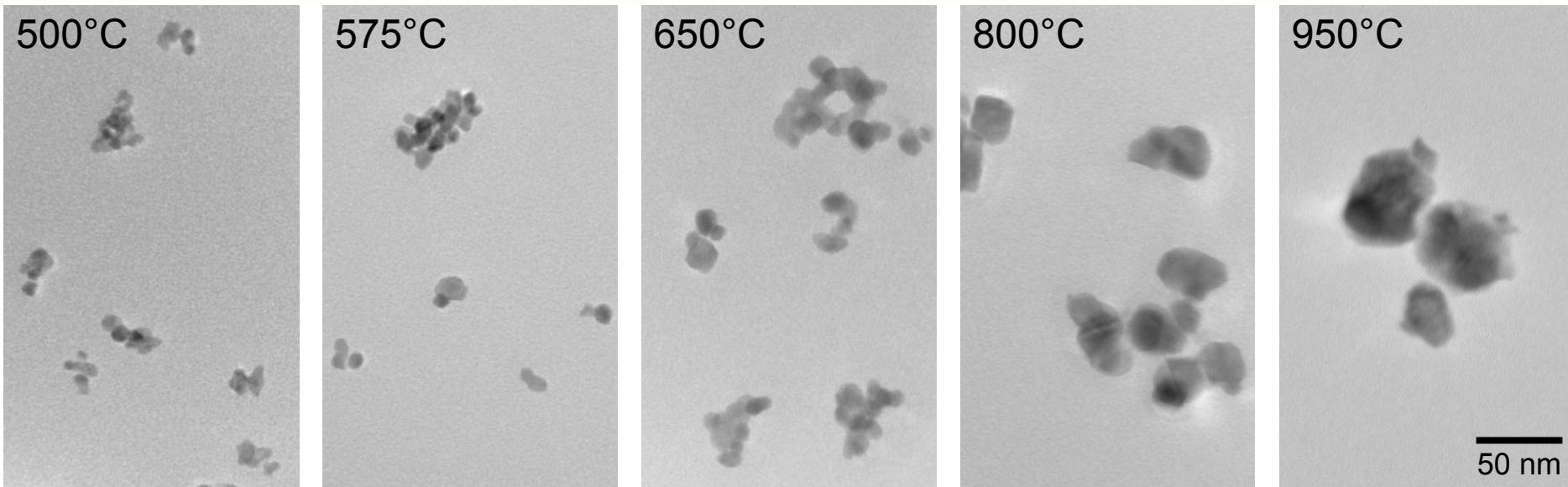
- removed MG from mouse with μ cal targeted by BP-Au NPs
- imaged using MARS CT with Medipix 3RX + CZT
- 7-30.9, 30.9-50, 50-60, 60-73, and 73-120 keV energy bins
- used MAP estimator for material decomposition
- calibrated to known Ca and Au concentrations

Roeder et al., Proc. SPIE, 2017



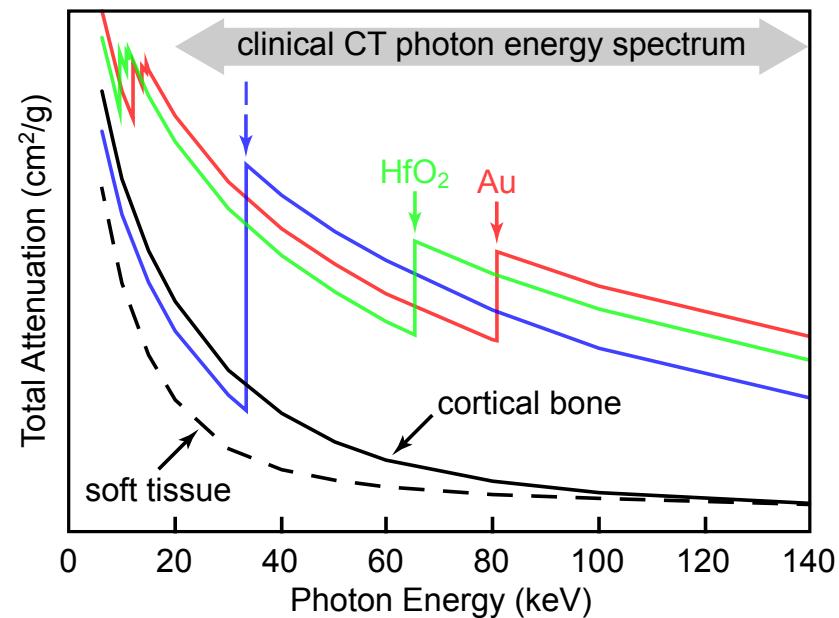
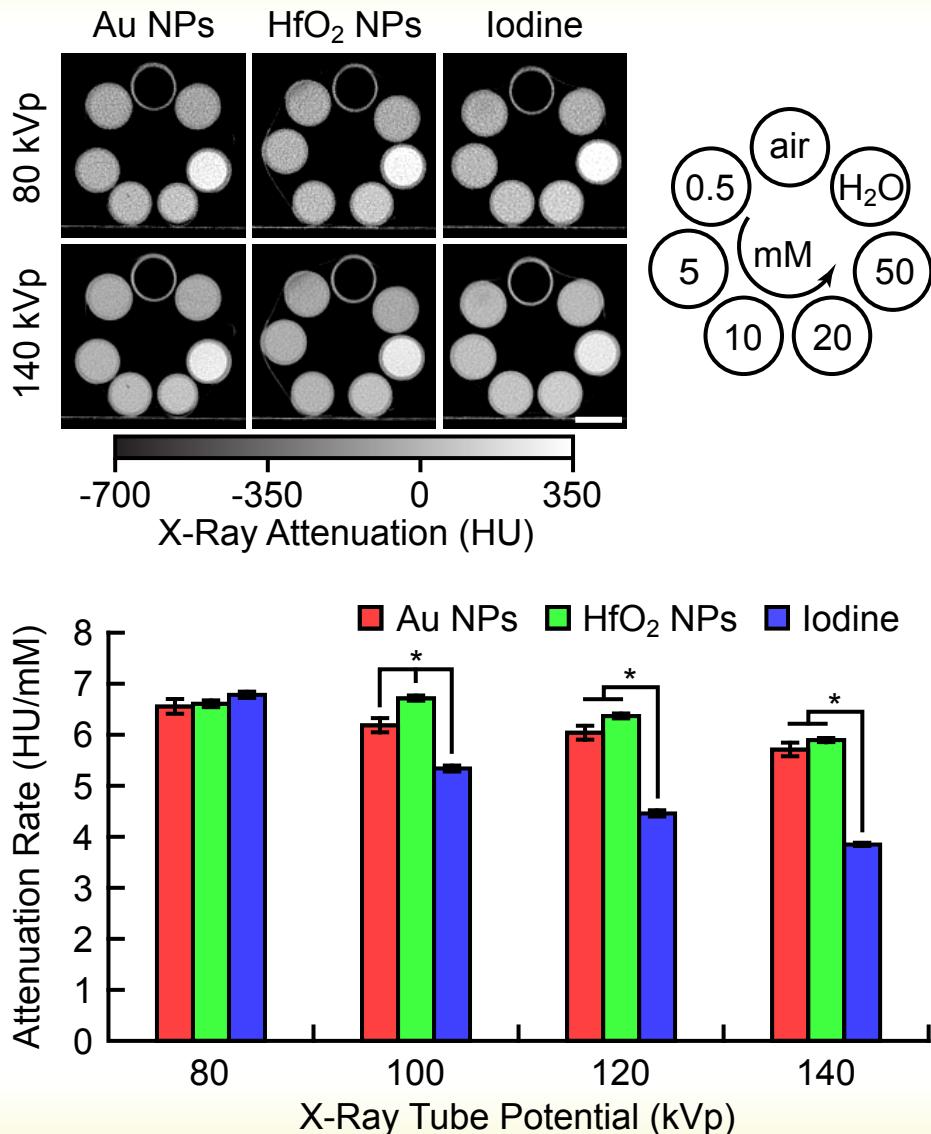
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Hafnia (HfO_2) Nanoparticles



Temperature (°C)	XRD		TEM	
	Crystallite Size (nm)		Diameter (nm)	Aspect Ratio
500	7.2 (0.8) ^a		7.4 (1.6) ^a	1.3 (0.2) ^{a,b}
575	8.4 (1.0) ^a		9.1 (2.3) ^a	1.3 (0.2) ^a
650	12.3 (1.0) ^b		12.5 (3.2) ^b	1.2 (0.2) ^b
800	21.2 (2.0) ^c		22.5 (5.6) ^c	1.2 (0.1) ^b
950	32.8 (1.3) ^d		31.0 (8.2) ^d	1.2 (0.1) ^{a,b}

Hafnia (HfO_2) Nanoparticles - DECT

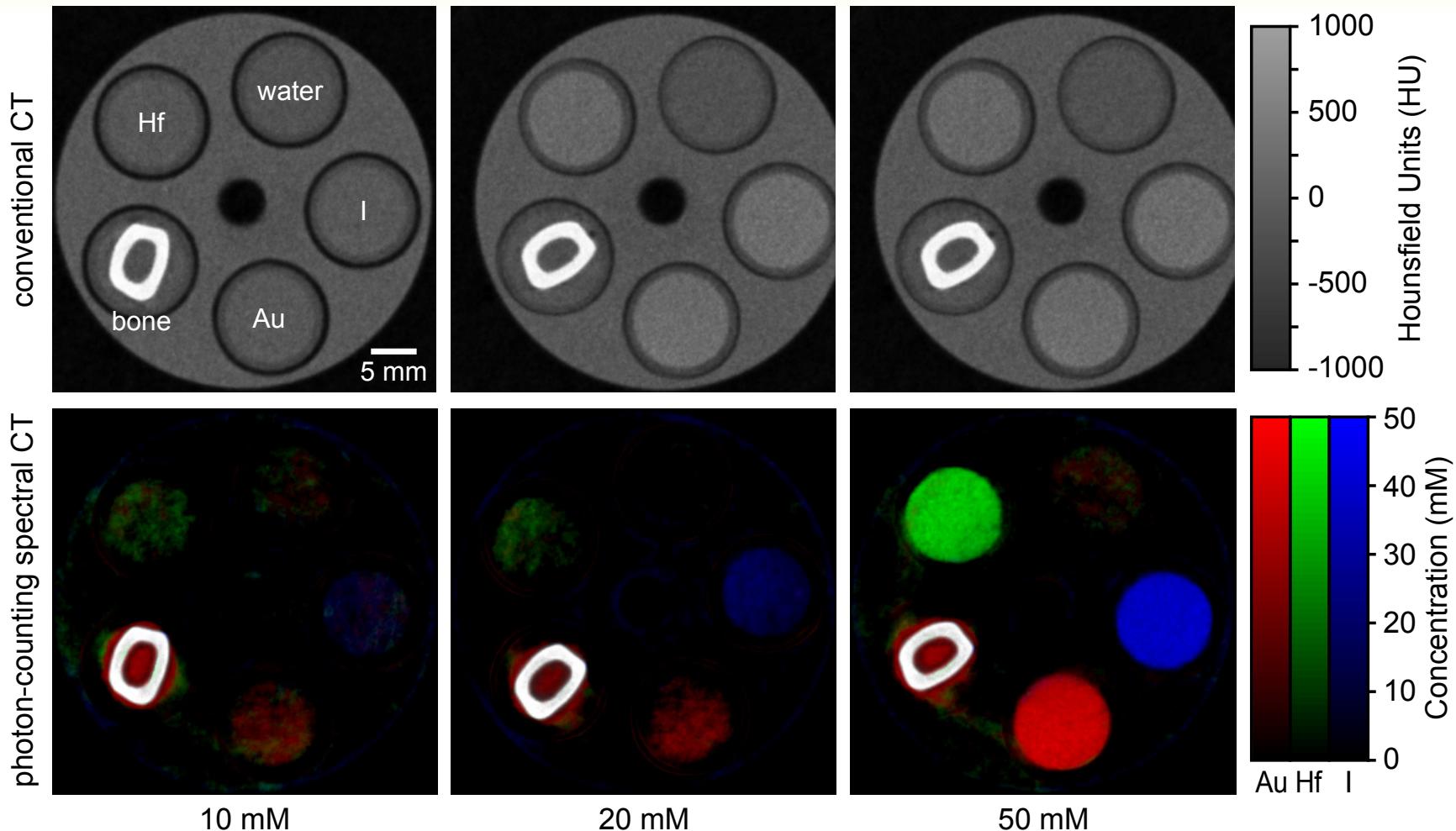


McGinnity et al., *Nanoscale*, 2016



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Hafnia (HfO_2) Nanoparticles - Spectral CT

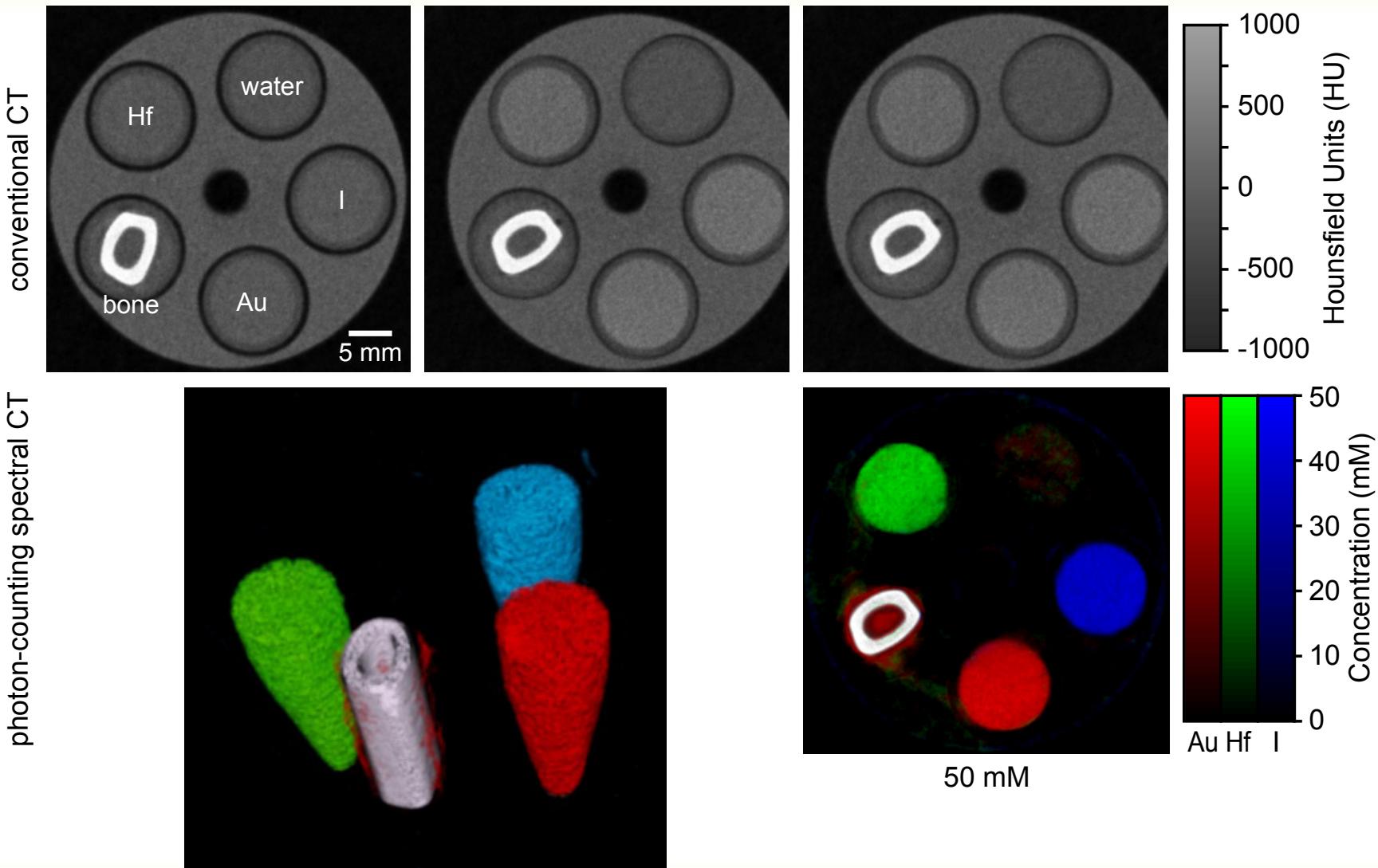


McGinnity *et al.*, *Nanoscale*, 2016;
Roeder *et al.*, *Proc. SPIE*, 2017



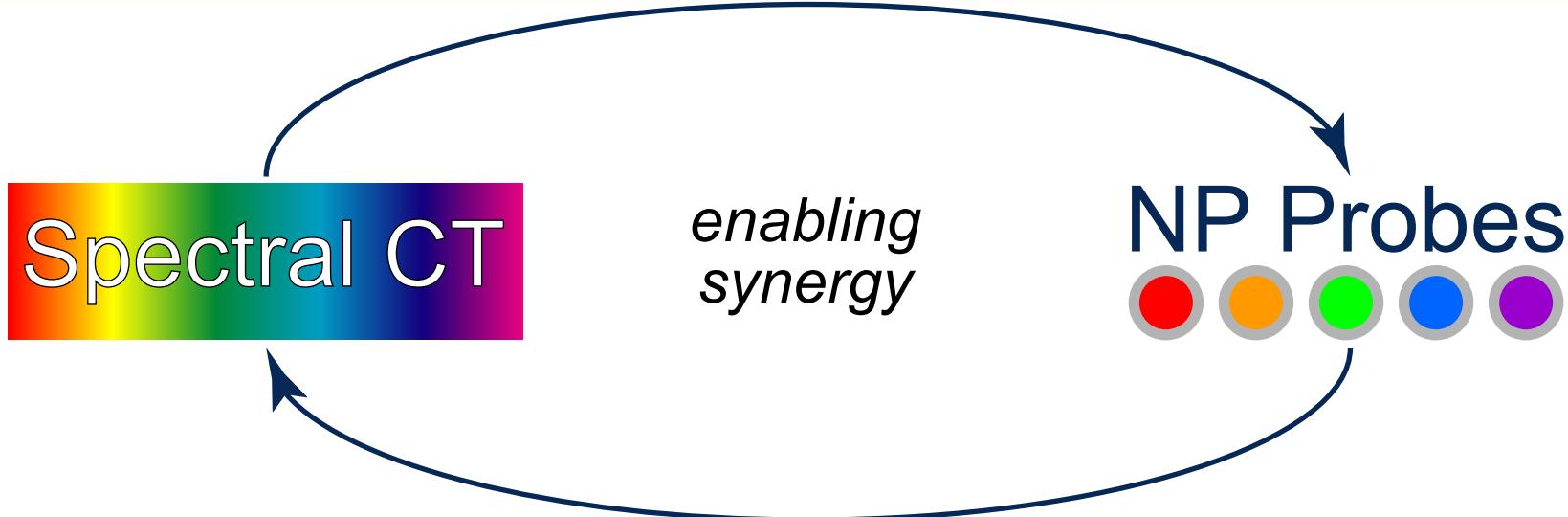
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<http://www.nd.edu/~bioeng>

Hafnia (HfO_2) Nanoparticles - Spectral CT



McGinnity *et al.*, *Nanoscale*, 2016;
Roeder *et al.*, *Proc. SPIE*, 2017

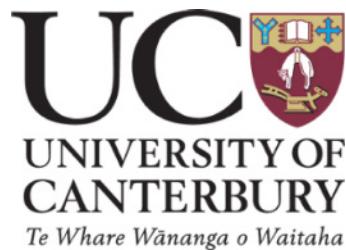
Take Home Message



- Photon-counting spectral CT and NP imaging probes can have an immediate impact on preclinical biomedicine by enabling quantitative molecular imaging in CT.
- In the future, targeted NP imaging probes and molecular imaging with CT may also act synergistically to realize precision diagnostic imaging.



Roeder Lab Funding and Collaborations



Offen im Denken



Acknowledgments

Current Trainees

Prakash D. Nallathamby (Postdoc)
Tracie McGinnity (PhD)
Felicia Roland (PhD)
Tyler E. Curtis (PhD)
Tyler A. Finamore (PhD)
Lisa E. Irimata (MS)

Facilities

Harper Cancer Research Institute
Notre Dame Integrated Imaging Facility
Freimann Life Science Center
Notre Dame Center for Nanoscience
 & Nanotechnology
Center for Environmental Science
 & Technology
Advanced Photon Source, Argonne
National Laboratory

Selected Alumni

Lisa E. Cole, PhD 2015
→ Postdoc, Northwestern University
Matthew J. Meagher, PhD 2015
→ Postdoc, Rush Univ. Medical Center
Ryan D. Ross, PhD 2011
→ Faculty, Rush Univ. Medical Center

Key Collaborators

Tracy Vargo-Gogola, IUSM-SB
Karen Cowden Dahl, IUSM-SB
Clodia Osipo, LUMC
Epple, Prymak, Sokolova, Loza
 University of Duisburg-Essen
Anthony J. Hoffman, Notre Dame



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