



“Integrated Nanosystems for Diagnosis and Therapy”

Program of Excellence in Nanotechnology

HL080729
05/01/2005 – 04/30/2010

Karen L. Wooley, P.I.

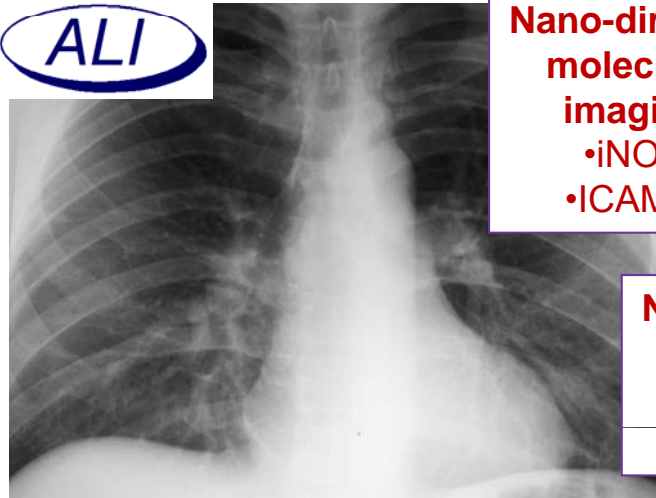
Washington University in Saint Louis (Abendschein, Achilefu,
Anderson, Brody, Gropler, Taylor, Welch, Woodard, Wooley,
Zheng)

University of California-Berkeley (Fréchet)
University of California-Santa Barbara (Hawker)

Main Goals/Objectives



Multi-functional Nanoparticles for Imaging and Therapy of Acute Pulmonary and Systemic Vascular Injury



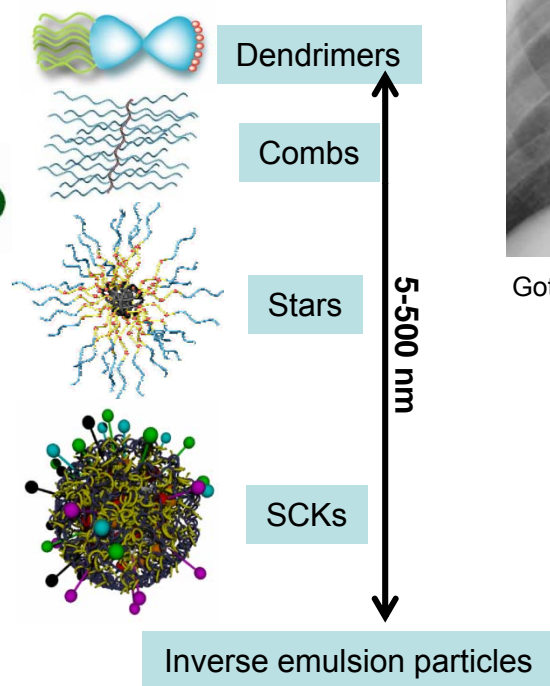
Nano-directed molecular imaging
 •iNOS
 •ICAM-1

Nano-targeted therapies
 •Antisense
 •Antibiotics

Gotway, M. B. et al. Radiographics 2002;22:S119-S135



Stealth
 Labeled
 Targeted
 Loaded
 Degradable



Atherosclerosis Progression
 Natriuretic Peptide C Receptor



Angiogenesis
 $\alpha_v \beta_3$ Receptors

Acute Vascular Injury
 Phosphatidylserine



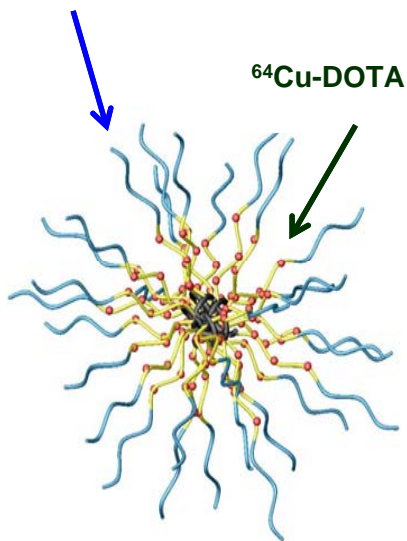
Modularity combined with prioritized, iterative, systematic development and evaluation of the systems *in vitro* and *in vivo*

In vivo Metabolism Study of Non-targeted ^{64}Cu -star-like Copolymer Nanoassemblies

Tetsuya Mori, Aviv Hagooly, Raffaella Rossin, Eric D. Pressly, Jasmine N. Hunt, Ashley M. Mynar, Ryosuke Sakai, Ken-ichi Fukukawa, Craig J. Hawker and Michael J. Welch

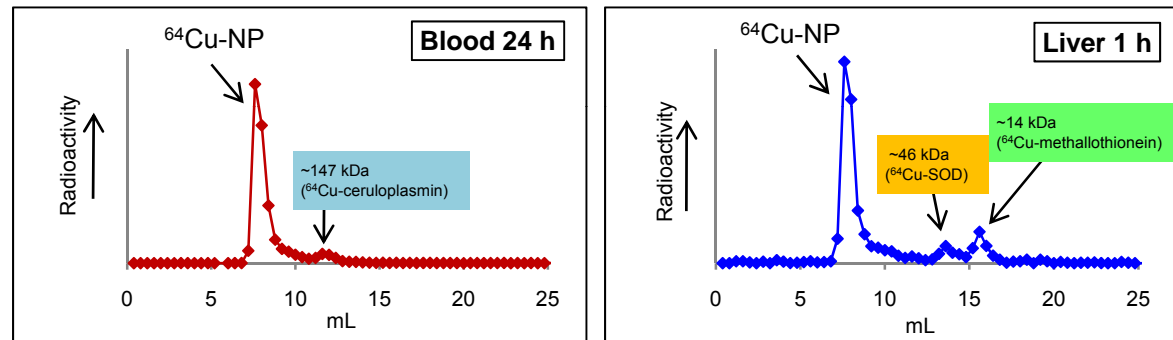
- More than 95% of ^{64}Cu -NP was intact at 24 h p.i. in the blood with one metabolite (^{64}Cu -ceruloplasmin)
- 80 % of original ^{64}Cu -NP was observed at 24 h p.i. in the liver with two metabolites, which corresponded to ^{64}Cu -SOD and ^{64}Cu -metallothionein

5 kDa PEG chain

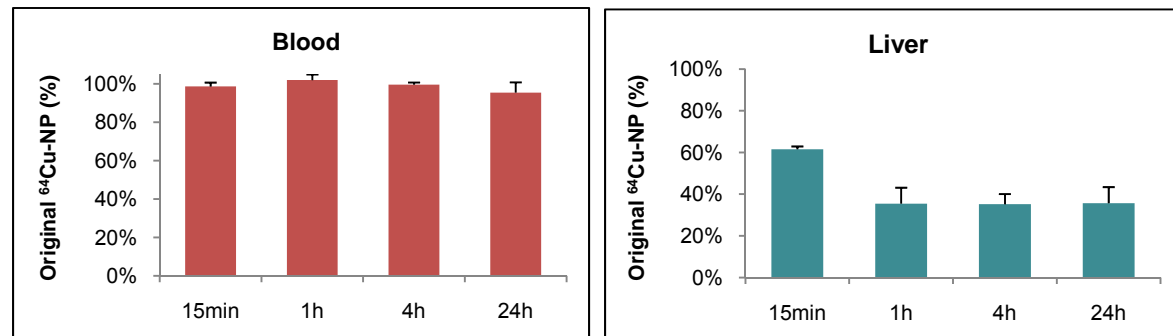


Non-targeted star-NP with ^{64}Cu -DOTA in the core

▪ Typical FPLC chromatograms of extracted samples from rat blood and liver



▪ Original ^{64}Cu -star-like-copolymer in rat blood and liver samples*

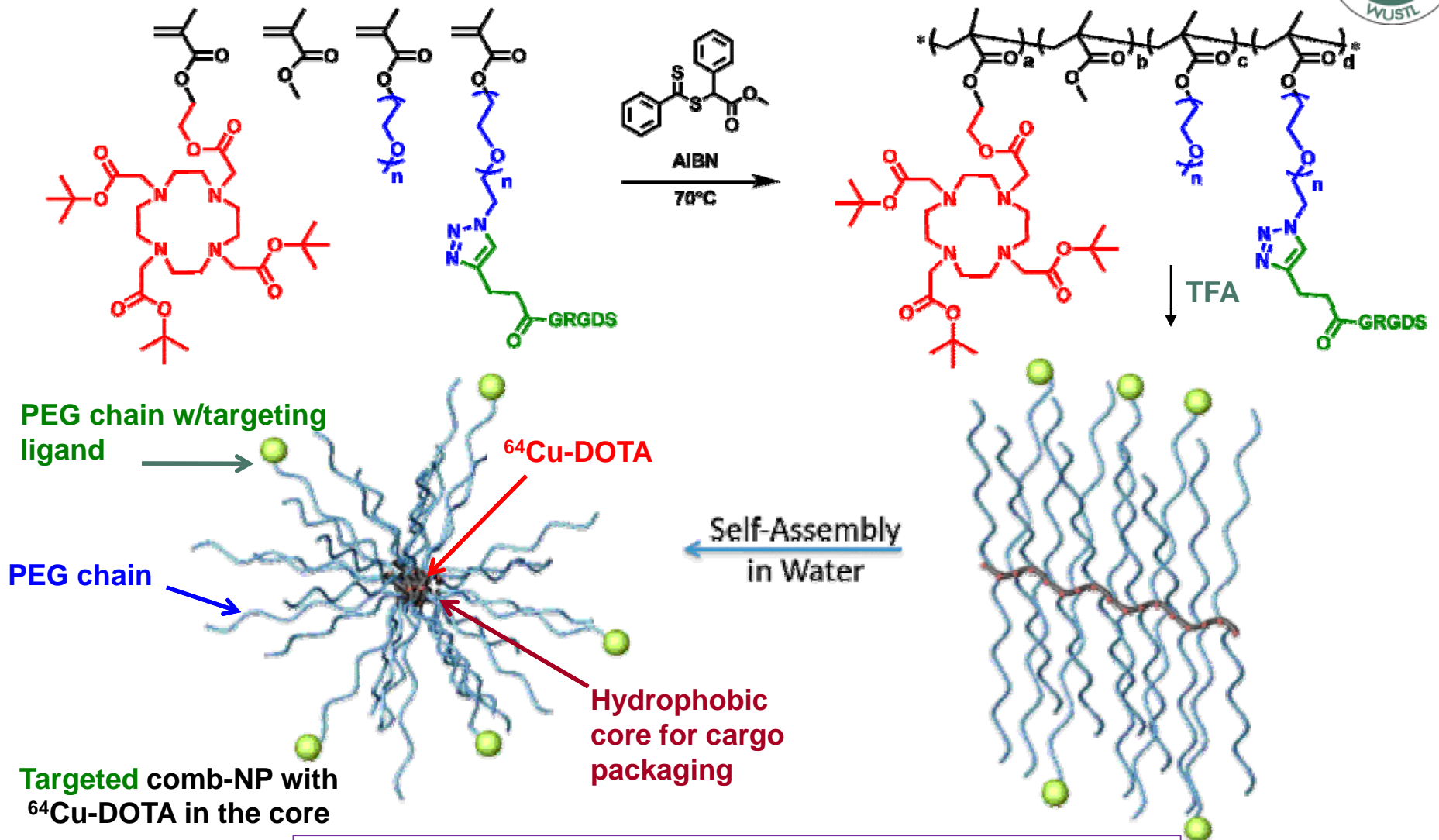


- The values were calculated from extraction efficiency and FPLC results.
- The extraction efficiencies of liver were lower than those of blood

^{64}Cu -star-like copolymer nanoassemblies with DOTA in the core were stable in circulation

Optimized Strategy for Synthesis of Targeted Comb Copolymers and Nanoassemblies`

AVI FUND

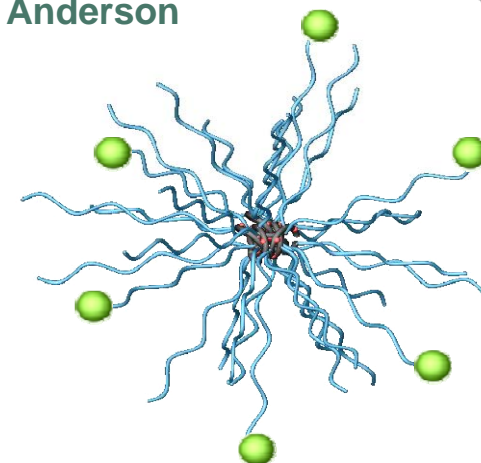
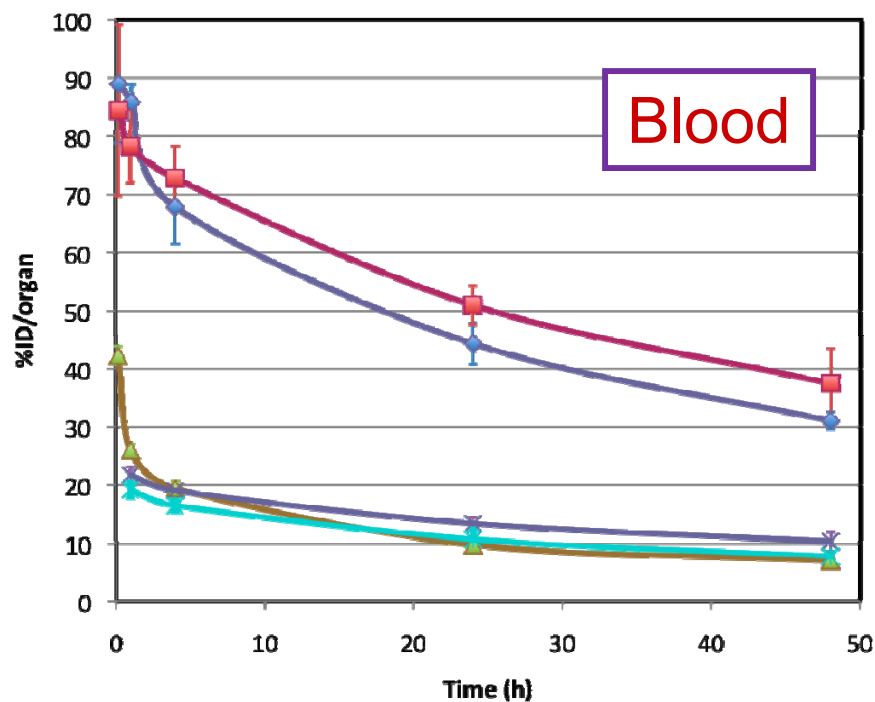


Functional monomers are the key components

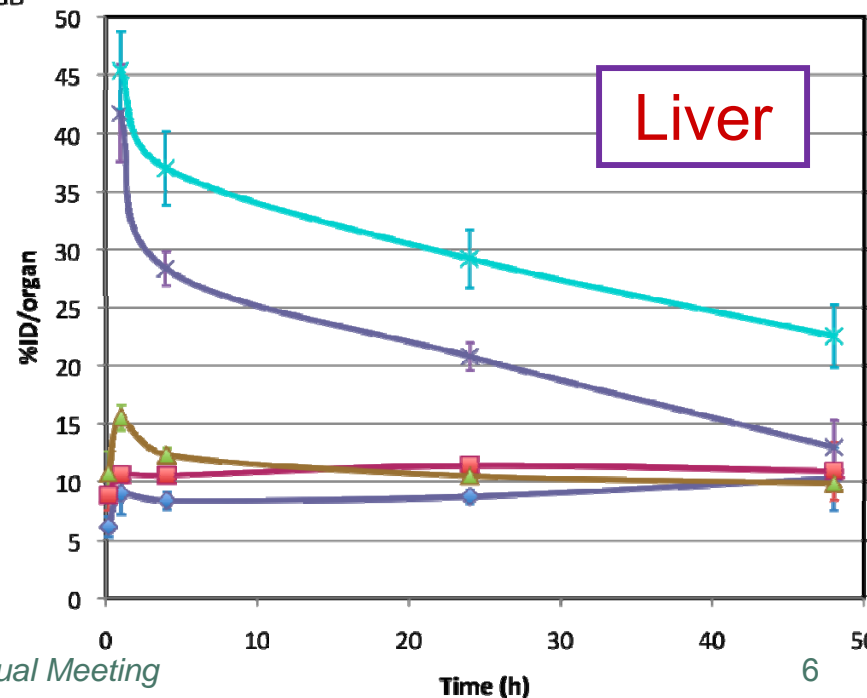


In Vivo Biodistributions for the RGD-Comb Nanoassemblies

Eric D. Pressly, Monica Shokeen, Aviv Hagooly, Ashely Fiamengo
Craig J. Hawker, Michael J. Welch, Carolyn J. Anderson



The synthetic control exerted over the number of peptides allows for tuning of the bioD and receptor targeting abilities.



Binding Affinity and Cell Internalization Trends in RGD-Conjugated Novel Nanoparticles for $\alpha_v\beta_3$ Targeting

Monica Shokeen, Adah Almutairi, Eric D. Pressly, Nikko L. Ramos, Tyler Mains, Alex Zheleznyak, Jean M. J. Fréchet, Craig J. Hawker, Carolyn J. Anderson

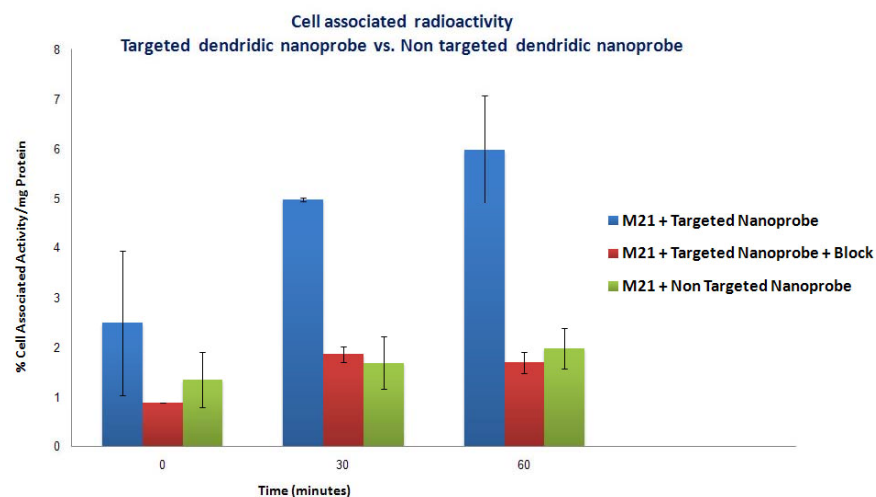


Dendrimers

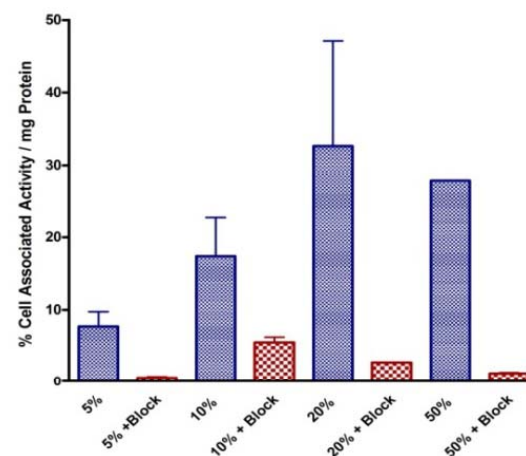
	IC ₅₀ / [NP] $\alpha_v\beta_3$ (nM)	IC ₅₀ / [NP] $\alpha_v\beta_5$ (nM)
Monovalent targeting peptide	10.4	921
Targeted dendrimer	0.18	10
Non-targeted dendrimer	> 8000	> 8000

Combs

Targeted Combs	5%	10%	20%	50%	Control Non-targeted
RGD's per Comb	7	14	28	70	0
$\alpha_v\beta_3$ IC ₅₀ /Comb nM	78.46	12.48	5.206	1.079	> 1,000



Cell Associated Fraction at 30 min @ 37 °C (5%, 10%, 20%, 50% Combs)



- 50-fold enhanced binding affinity to $\alpha_v\beta_3$ (*c.f.* RGD peptide small molecule)
- 6-fold increase in $\alpha_v\beta_3$ receptor mediated endocytosis (*c.f.* non-targeted) in M21 cells
- These data were validated in *in vivo* models

- Improved binding and cellular internalization were observed with increasing numbers of targeting peptides
- 70-fold improvement in binding with only a 10-fold increase in peptide loading

Validation of the Hindlimb Ischemia Model in Mice for Detection of Nanoparticle Binding to Upregulated $\alpha_v\beta_3$ Receptors

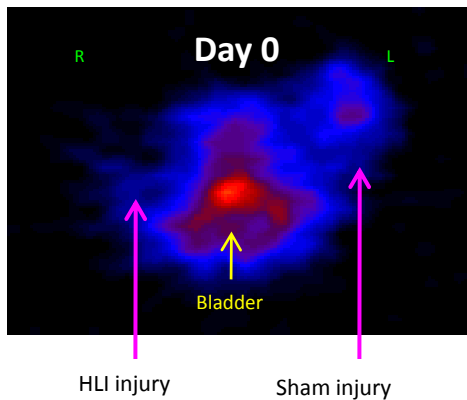


Kal A. Clark, Aviv Hagooly, Susannah A. Grathwohl, Tetsuya Mori, Carolyn J. Anderson, Michael J. Welch, and Dana R. Abendschein

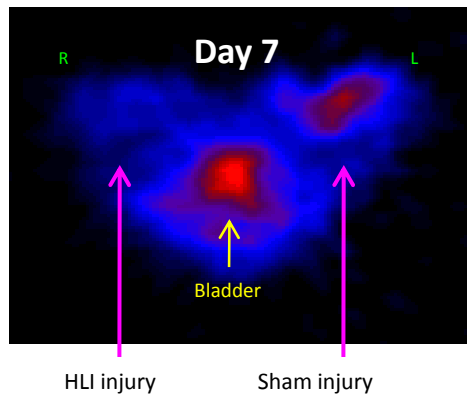


Micro PET

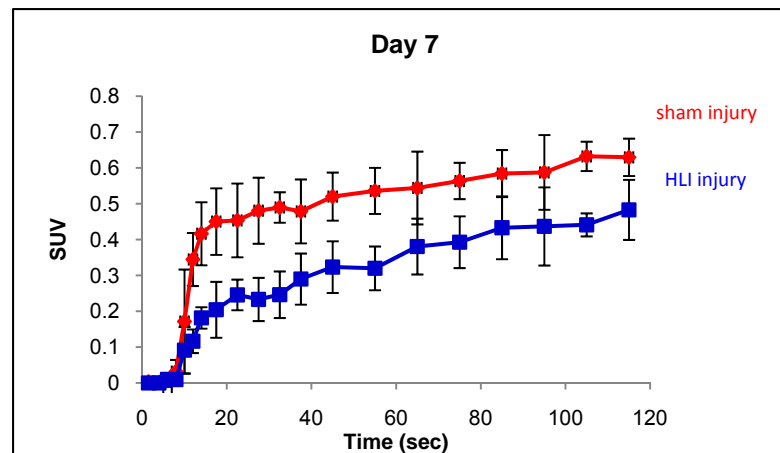
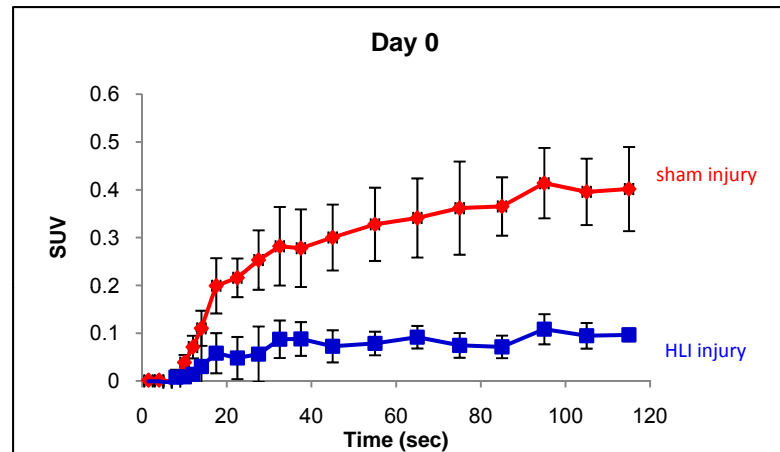
Transverse



Transverse



Representative Images from one mouse



Time activity curves of ROIs using $[^{15}\text{O}]$ water in C57BL/6 mice on days 0 and 7 after injury (n=3). The ROI were set on the right (HLI) and left (sham) thigh.

Using $[^{15}\text{O}]$ water, perfusion increase is observed after 7 days of HLI, consistent with angiogenesis

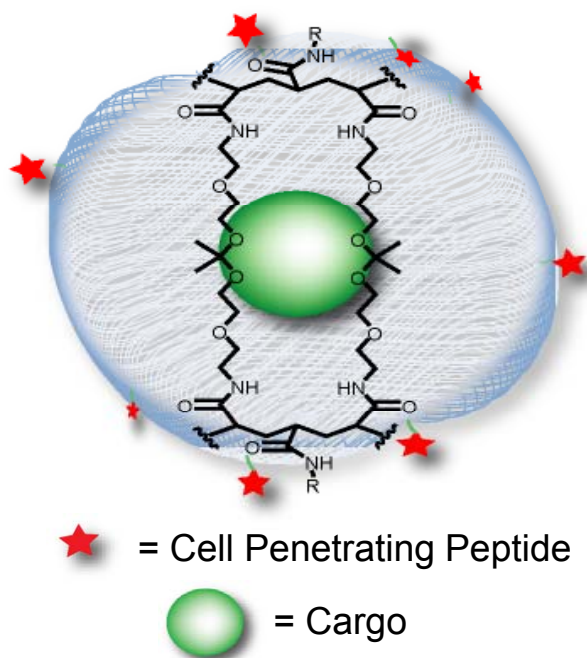
Enhanced Cell Penetration of Acid-Degradable Particles Functionalized with Cell-Penetrating Peptides

ALI FUND

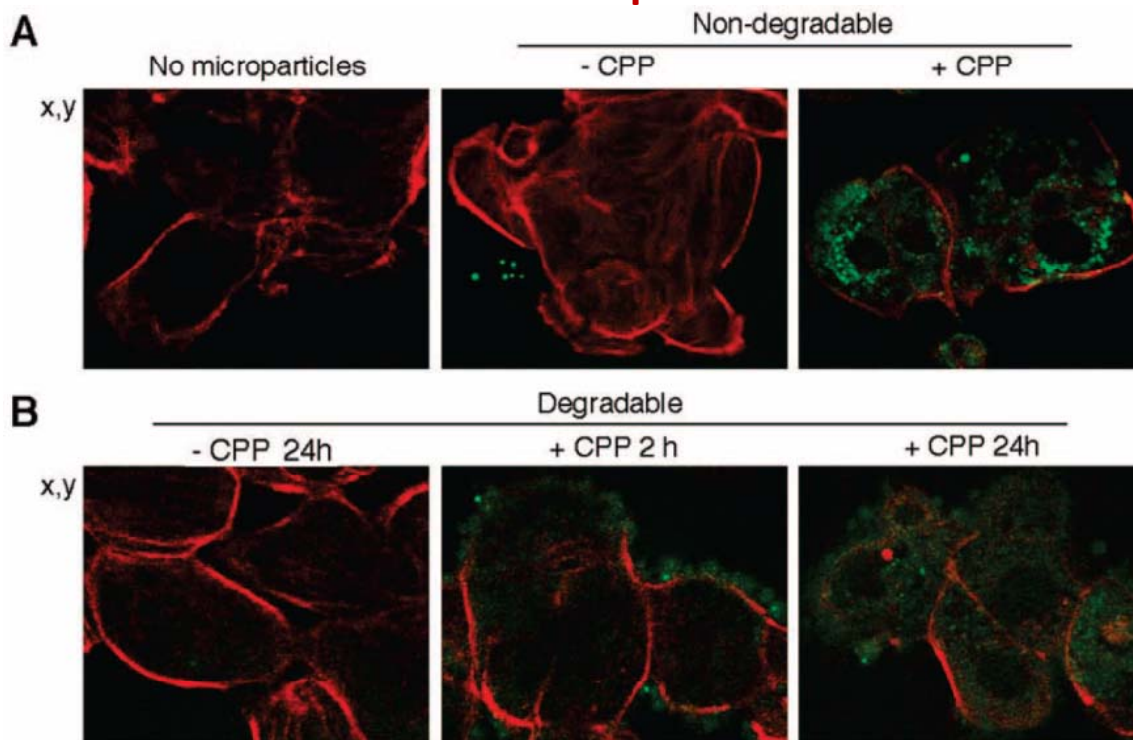
Jessica L. Cohen, Adah Almutairi, Joel Cohen, Yongjian Liu, Matt Bernstein,
Steven L. Brody and Jean M. J. Fréchet



Polymeric Particles



In Vitro Cell Uptake Studies

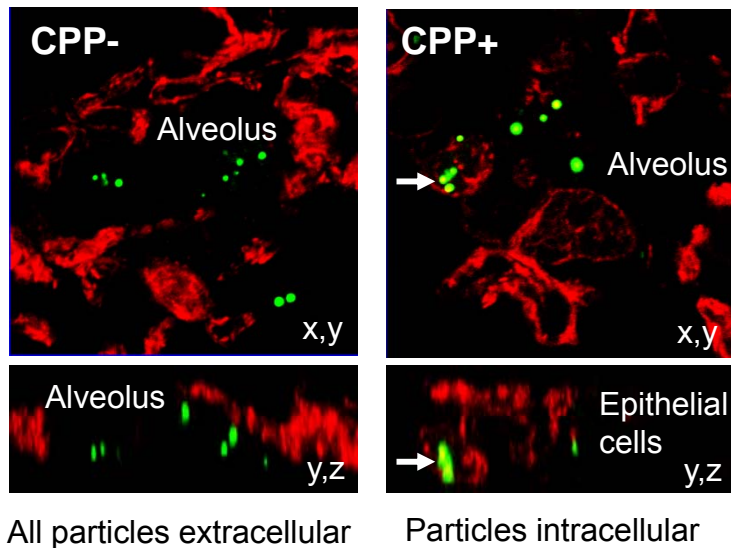


Acid-degradable CPP+ microparticles were prepared and evaluated *in vitro* and *in vivo*:
Safe and effective uptake in non-phagocytic cells (BEAS-2B, a lung epithelial cell line)
Slower clearance and longer retention profile in the lungs than do CPP- particles
Adequate activity for lung imaging via PET following IT administration

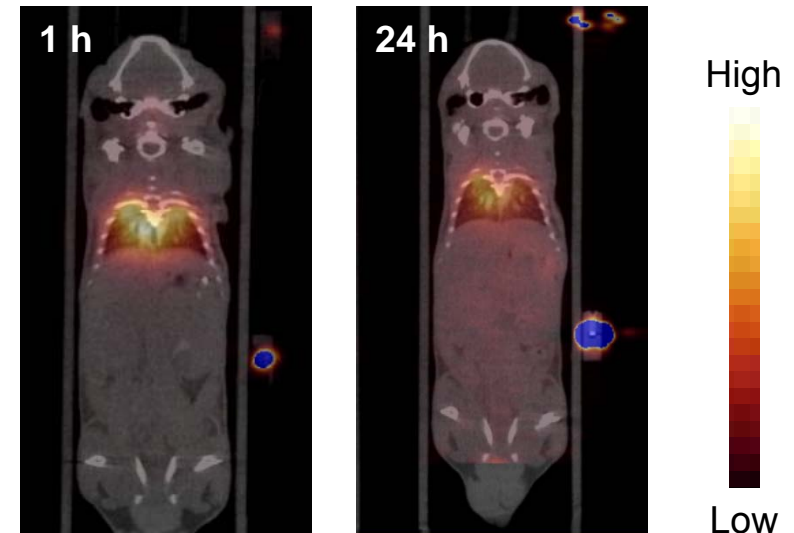
Evaluation of Cell Penetrating Peptide-modified Particles for Lung Drug Delivery and Imaging

Yongjian Liu, Aviv Hagooly, Aida Ibricevic, Jessica L. Cohen, Joel A. Cohen, Ke Zhang, Sean P. Gunsten, Michael J. Welch, Steven L. Brody, Michael J. Walter, Jean M. J. Fréchet and Karen L. Wooley

Confocal images of *in vivo* cell uptake



MicroPET/CT imaging the lung

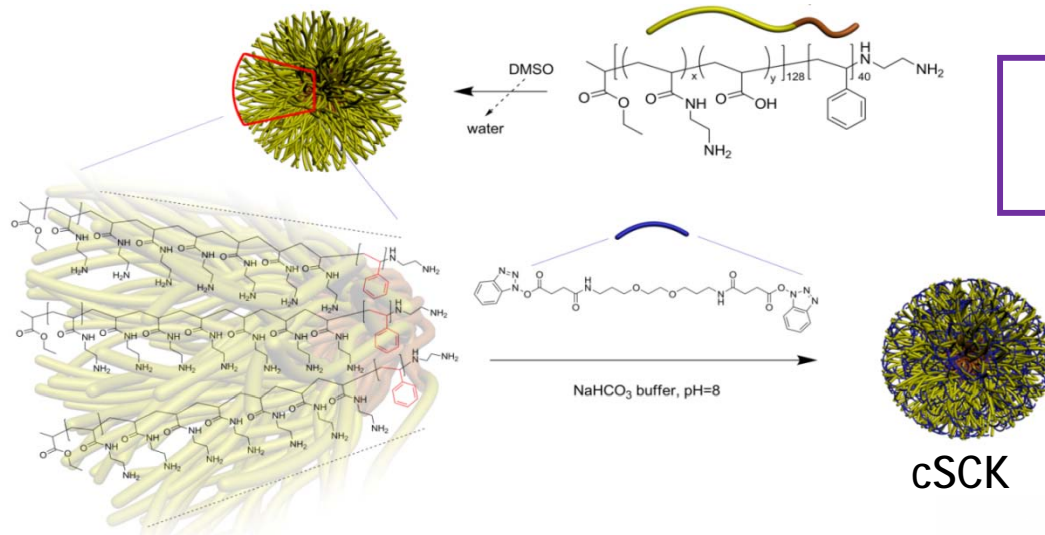


- Surface decoration with cell penetrating peptide (CPP) can deliver micro/nano particles into cells both *in vitro* and *in vivo* with mild/no inflammation.
- *In vitro* dynamic imaging shows a time-dependent and concentration-independent cell uptake of CPP+ particles.
- Bio-D studies show a slower clearance profile for CPP+ particles in the lung relative to CPP- particles.

Cationic SCKs (cSCKs) for Highly Efficient Delivery of DNA, PNA and siRNA to Mammalian Cells



Ke Zhang, Huafeng Fang, John-Stephen A. Taylor and Karen L. Wooley

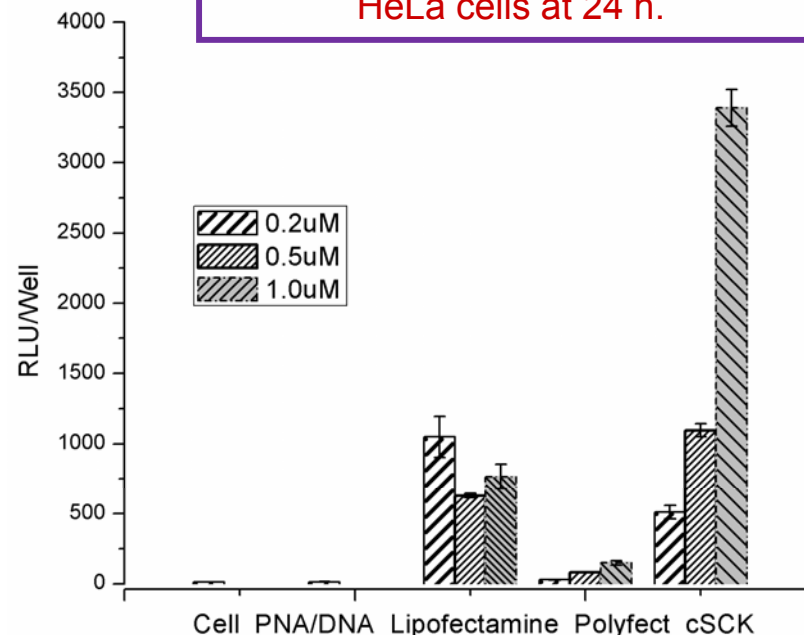
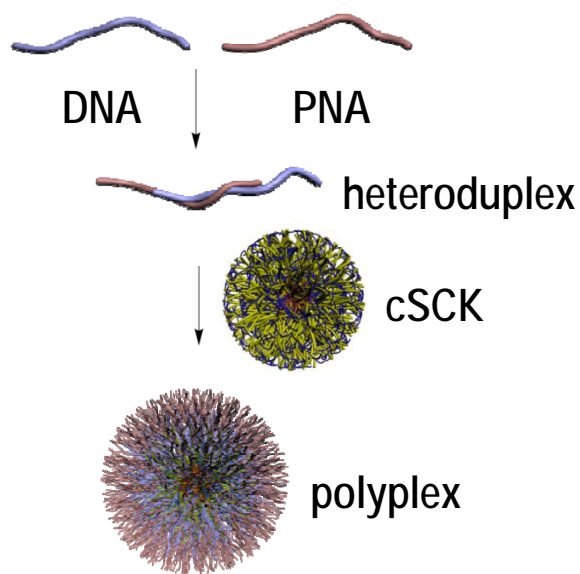


Positively-charged, cationic SCKs (cSCKs) were prepared from PAEA-*b*-PS.

Superior transfection efficiencies were achieved for cSCKs, in comparison to commercial agents, as measured by a luciferase splice correction assay with pLuc705 HeLa cells at 24 h.

Several strategies were employed to associate DNA, PNA and siRNA to the cSCK.

One PNA-delivery strategy involved formation of DNA/PNA heteroduplexes to facilitate complexation with cSCK by electrostatic interactions.

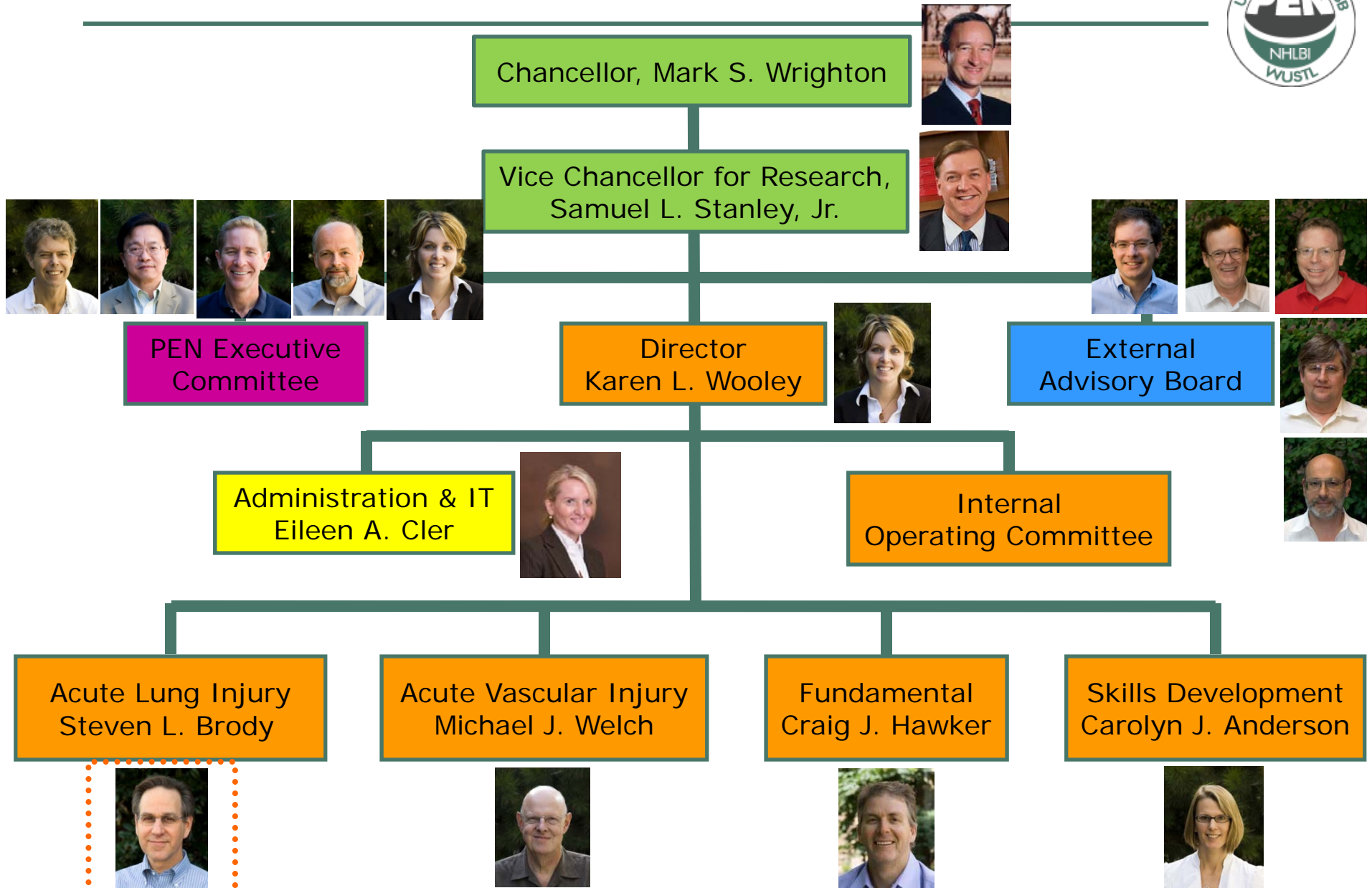


Strengths/Expertise of our PEN



- Diverse nanoparticle platforms
- Multiple imaging modalities
- Identification of novel targeting strategies
- Cardiovascular and pulmonary disease models
- Active and creative skills development
- Focused and integrated team having multi-disciplinary expertise, with efforts directed toward specific aims

New Organizational Structure



October 10-11, 2008

Inter-PEN 3rd Annual Meeting

Our Presentations



- **Craig J. Hawker (UCSB)**, *Syntheses of nanoparticles having variable structures, sizes and shapes, each designed for targeted in vivo imaging of ALI or AVI*
- **Michael J. Welch (WashU)**, *PET and optical imaging with nanoparticle probes*
- **Carolyn J. Anderson (WashU)**, *Biomarkers and targeting nanoparticle ligands for imaging cardiovascular and pulmonary diseases*
- **Steven L. Brody (WashU)**, *In vivo approaches for targeting particles in acute lung injury*
- **Dana R. Abendschein (WashU)**, *Developing animal models for nanoparticle imaging and therapy*
- **Monica Shokeen (WashU)**, *Diverse nanotechnology-centered educational activities, from K-12 outreach through graduate course curriculum and community awareness*

Six Specific Aims



- Specific Aim #1: **Preparation** and assembly of programmed, **integrated nanosystems**.
- Specific Aim #2: Application of nanostructures for **imaging at increased levels of sensitivity**.
- Specific Aim #3: **Direct imaging of gene expression** by recognition of mRNA transcription products.
- Specific Aim #4: Application of the nanostructures for **therapy**.
- Specific Aim #5: **Cross disciplinary education and training** of medical and materials scientists.
- Specific Aim #6: Serve a leadership role in the **dissemination and translation of nanotechnology** developments to unmet medical needs.