Angela M. Belcher
Debadyuti Ghosh, Hyunjung Lee, Aditya Kohli, Kim Kelly

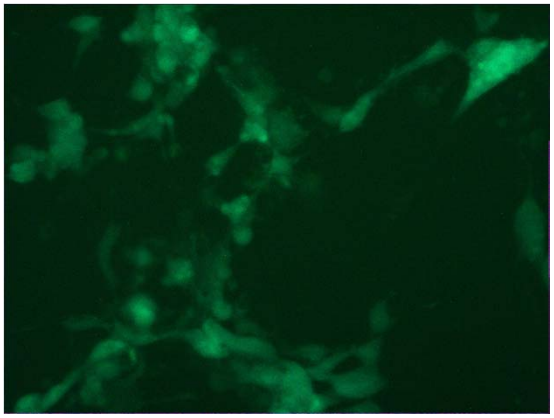
Biological Engineering
Materials Science and Engineering
The Koch Institute
MIT

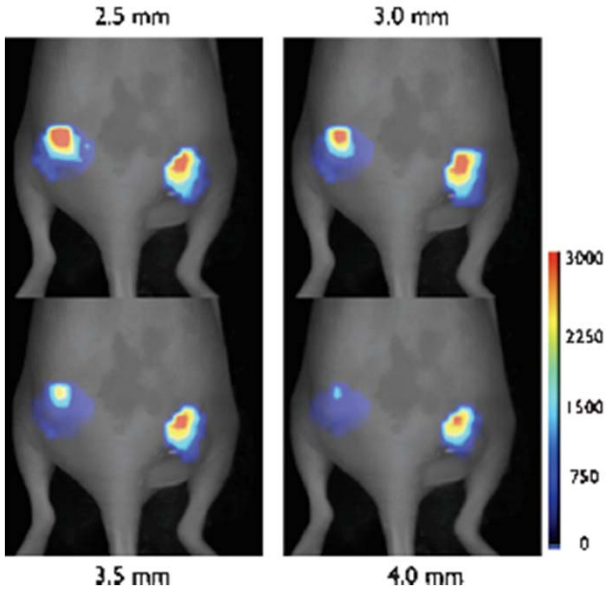
Massachusetts Institute of Technology

KOCH INSTITUTE for Integrative Cancer Research at MIT

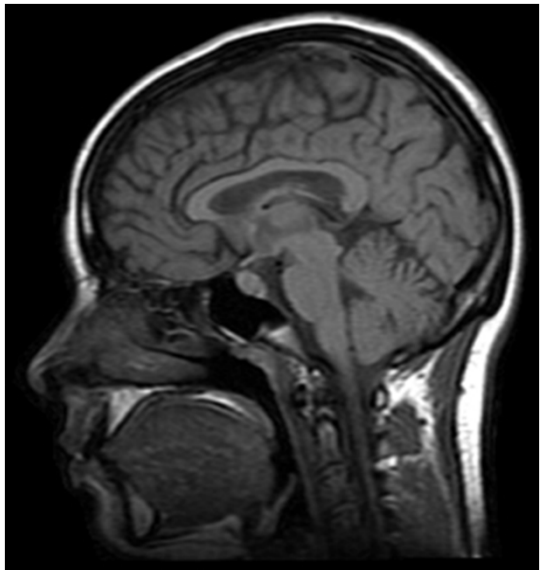
NATIONAL CANCER INSTITUTE

The scale of things: The solution of nanotechnology - from a single cell to the body





Kelly et al, Neoplasia (2006) (Weissleder group)



Courtesy of National Institutes of Health

Nanotechnology is a powerful tool because it can be used from the nano- to macro- scale to look at single cell events all the way to entire systems *in vivo*

CCNE Members

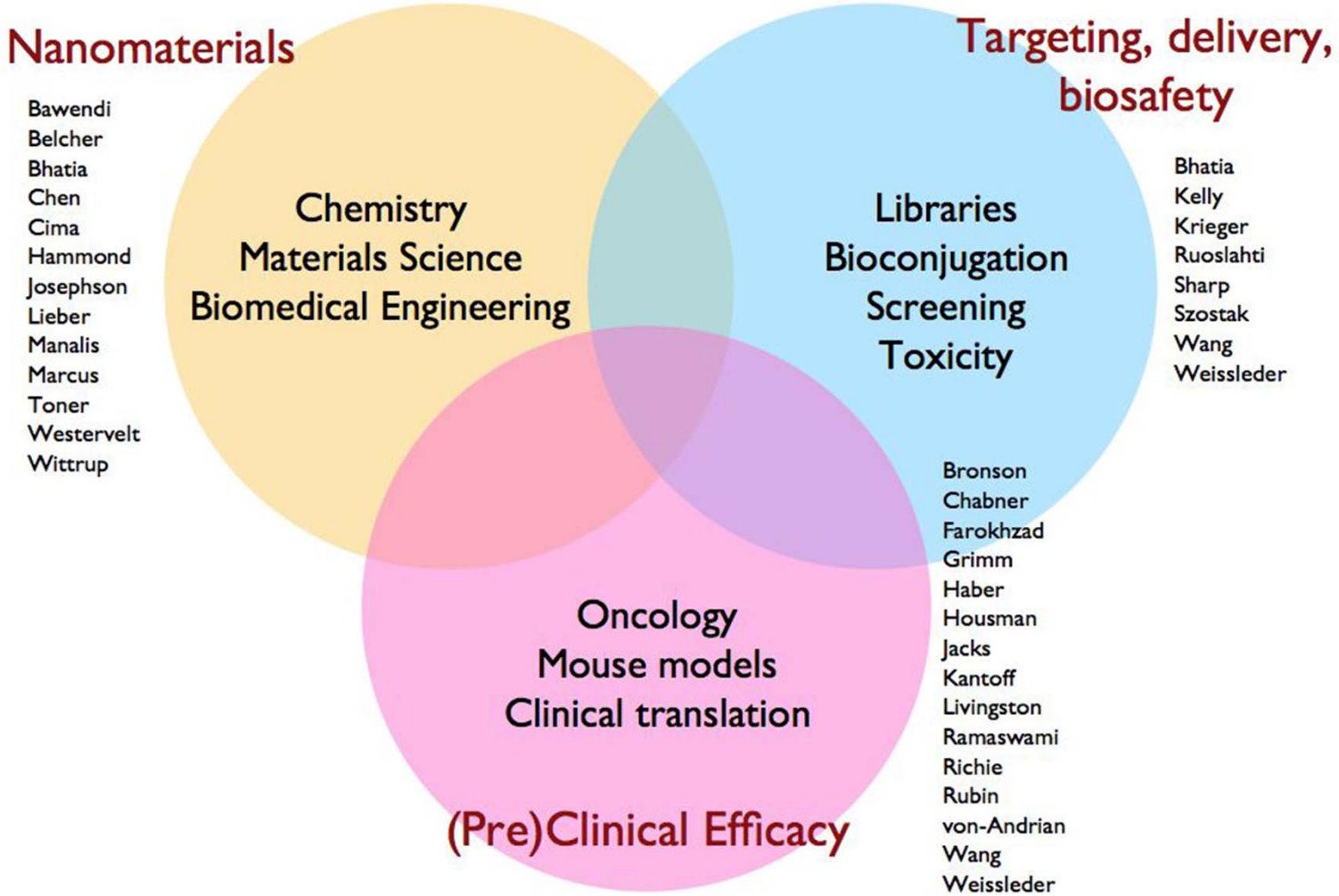
Principal Investigators: Robert Langer & Ralph Weissleder

Mounji Bawendi, PhD
Angela Belcher, PhD
Sangeeta Bhatia, MD, PhD
Rod Bronson, PhD
Bruce Chabner, MD
Alain Charest, PhD
Jianzhu Chen, PhD
Michael Cima, PhD
Omid Farokhzad, MD, PhD
Jan Grimm, MD
Daniel Haber, MD
Paula Hammond, PhD
David Housman, PhD
Yu Huang, PhD
Tyler Jacks, PhD
Lee Josephson, PhD
Phil Kantoff, MD
Kimberly Kelly, PhD
Monty Krieger, PhD
David Livingston, MD
Scott Manalis, PhD
Jerome Richie, PhD
Mark Rubin, PhD
Erkki Ruoslahti, PhD
Phillip A. Sharp, PhD
Jack Szostak, PhD
Uli Von-Andrian, PhD
Yz Wang, PhD
Robert Westervelt, PhD
Dane Wittrup, PhD

The Burnham Institute

MIT-Harvard NanoMedical Consortium

Core Competencies



New Funding Which Leveraged CCNE Work

The work supported by this CCNE has lead to many new funding opportunities for many participants. Additionally, MIT received a gift from David Koch:

October 2007: David H. Koch made a $100 million gift to establish the David H. Koch Institute for Integrative Cancer Research and to build a state-of-the-art research facility. The Koch Institute broke ground for the new building on March 7th, 2008.

Genetically-templated multimodal scaffolds for amplifiable targeted imaging

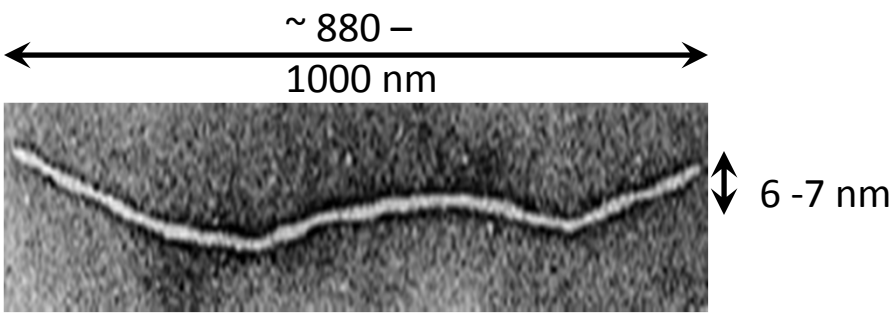
Motivation

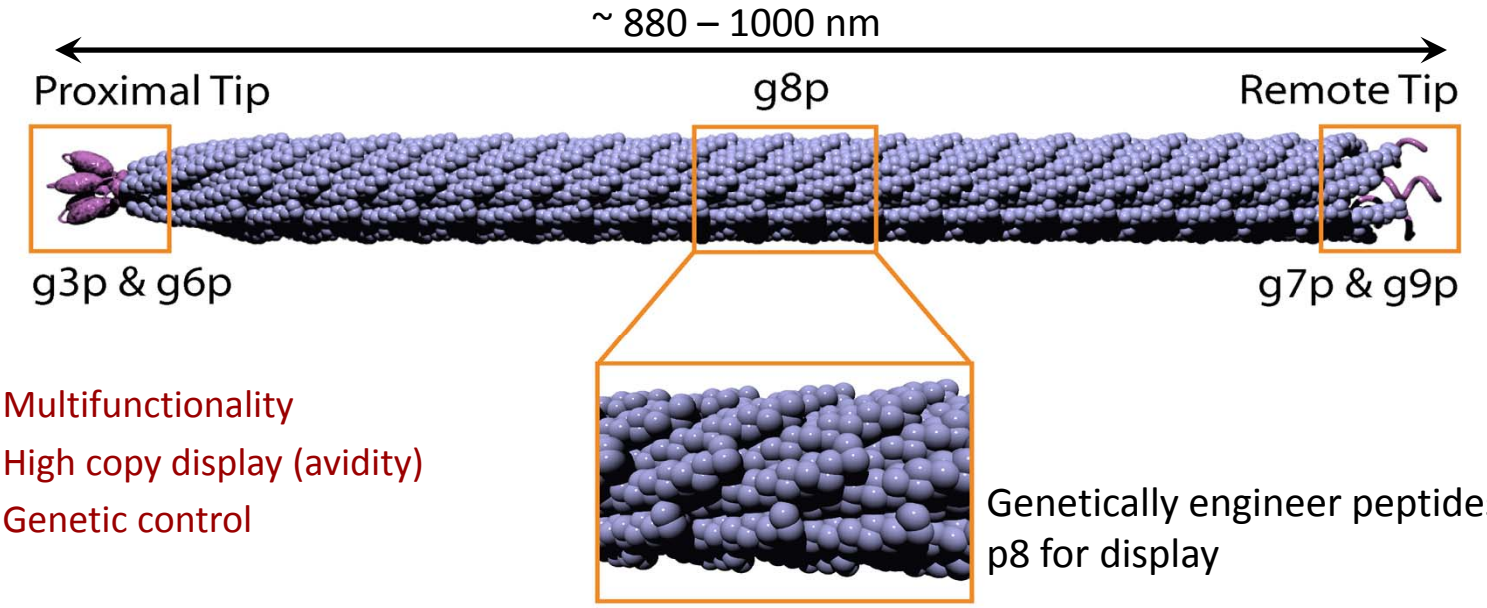
- need for early cancer detection: ~90% of deaths occur due to metastasis
- single functionalized nanoparticle (NP) probes for magnetic imaging
- NP clearance due to protein adsorption and opsonization
- filamentous structures stay in circulation *in vivo* longer
- active tumor targeting with antibodies (Ab) against known biomarkers--difficult to identify early stage markers and generate Abs; production also laborious
- Ideal to have 'all-in-one' platform for targeting and imaging

M13 virus: a template for material assembly

Consists of single stranded DNA + protein coat (capsid)

Long template-1D material
(Length dependent on genome size)--

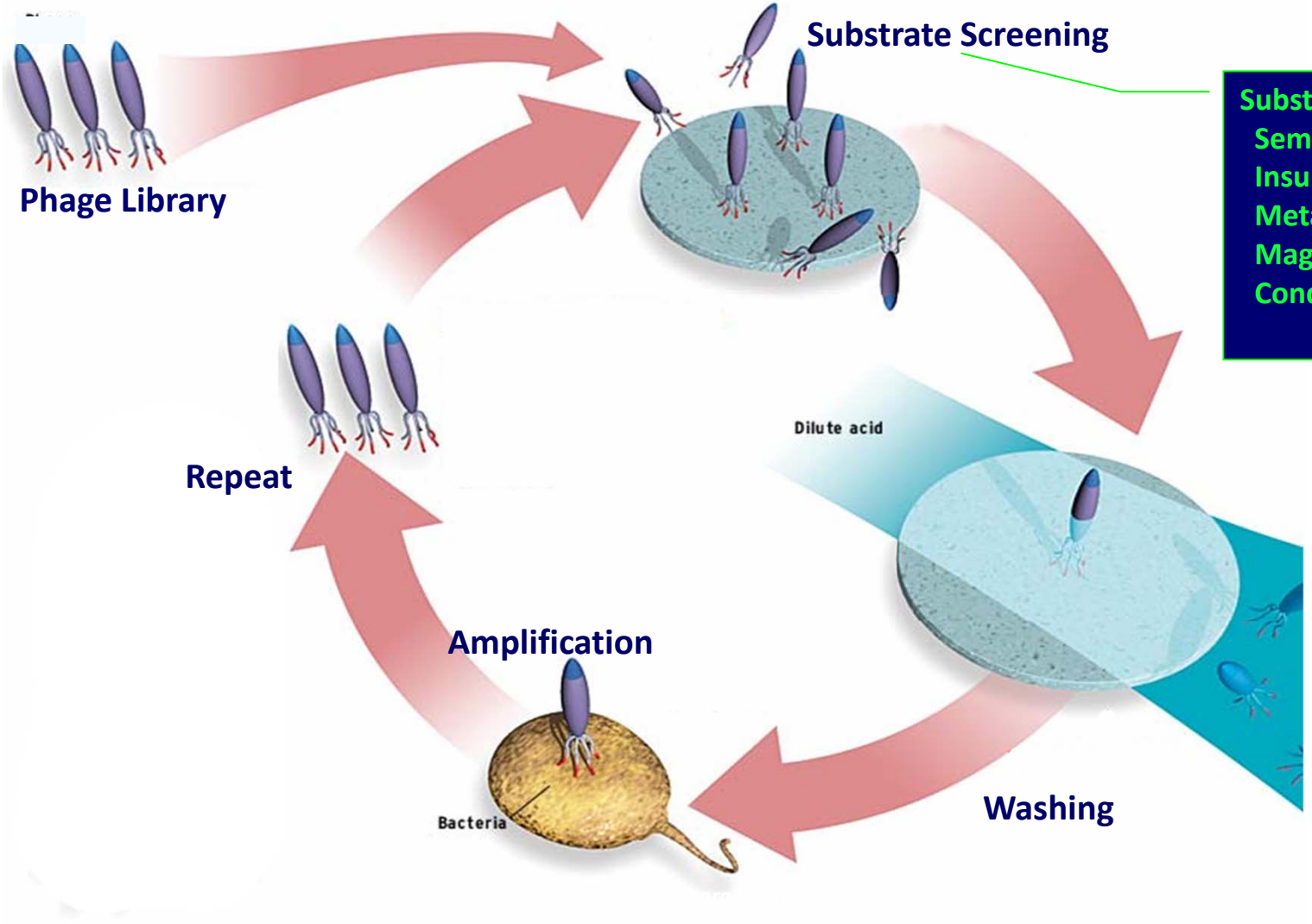




- Multifunctionality
- High copy display (avidity)
- Genetic control

Genetically engineer peptides on p3 and p8 for display

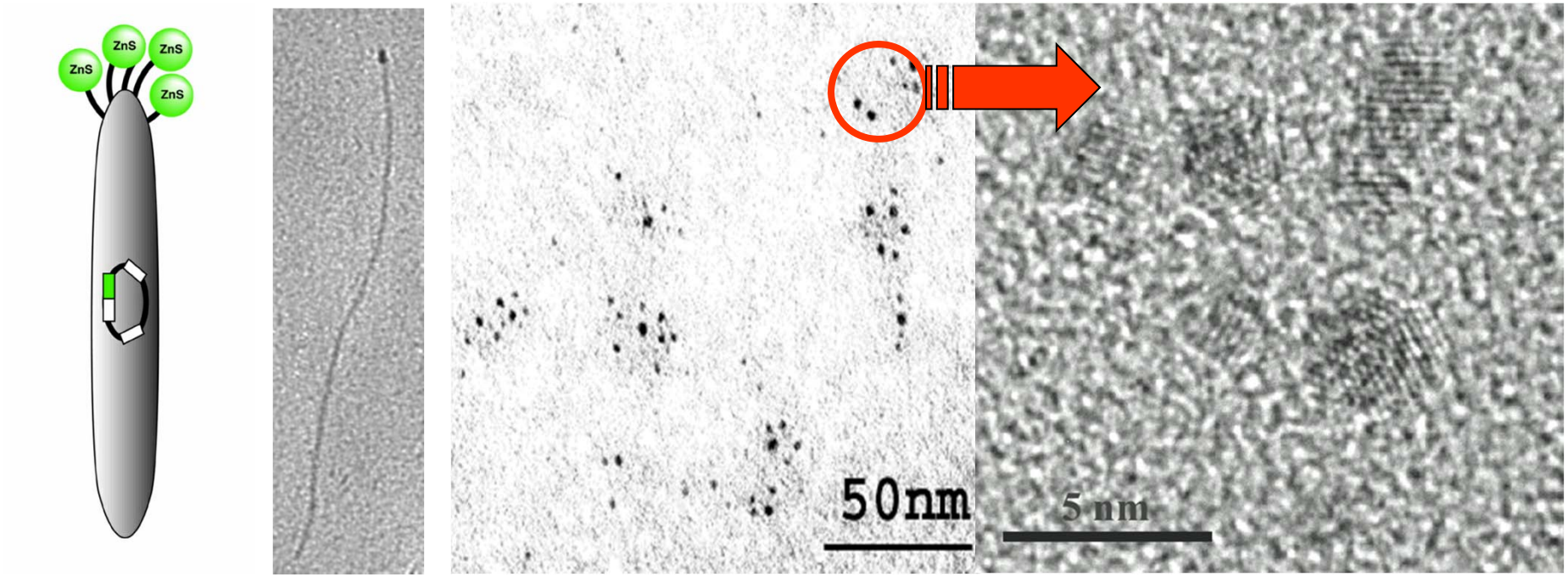
Peptide Libraries & Selection



Science, 296, 891,2002
Science, 303, 213, 2004
Science, 312,885, 2006
Science , 324,1051, 2009

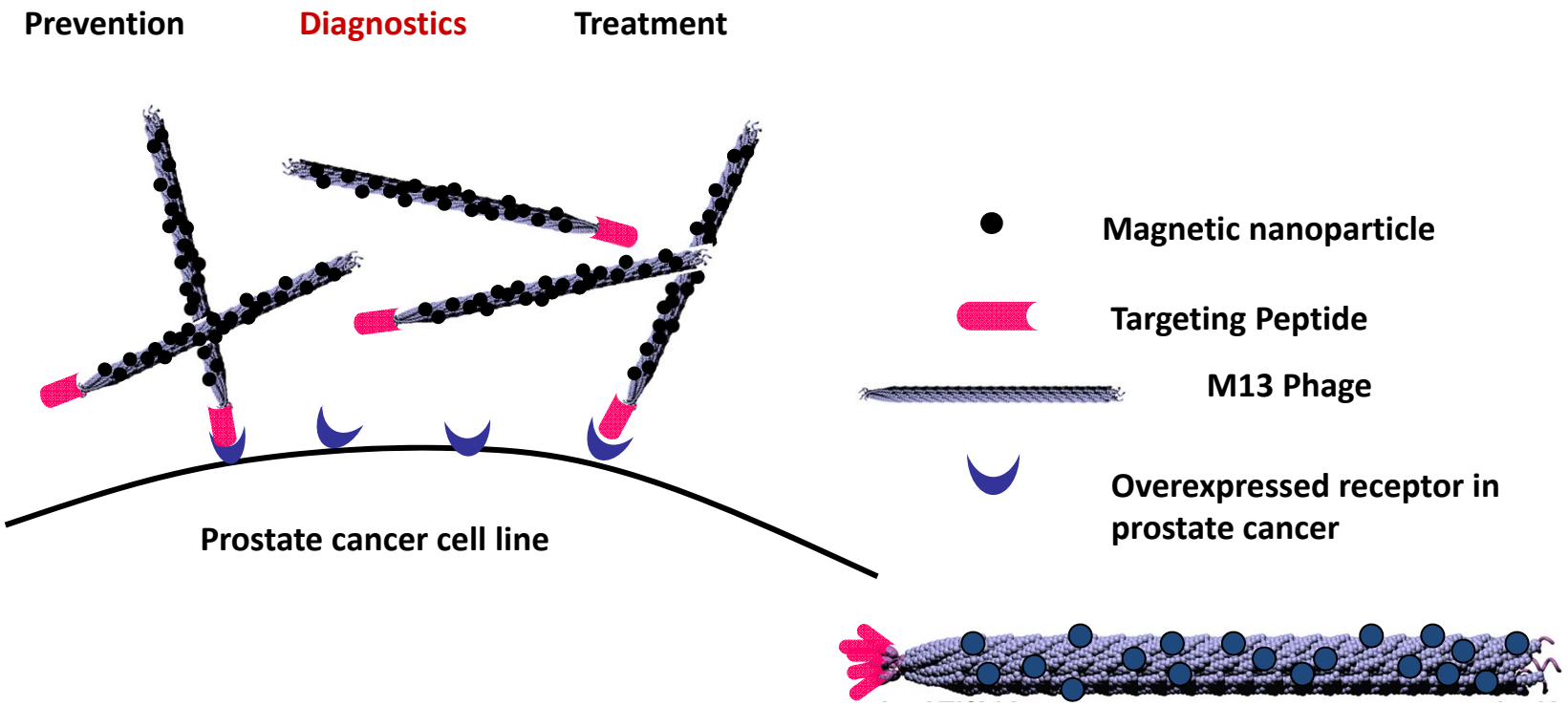
Nature,405, 665, 2000
Nature Materials,4,496 2005
Nature Materials, 5,234,2006
Nature Nanotechnology, 2010

Specific peptide binding to nanocrystals



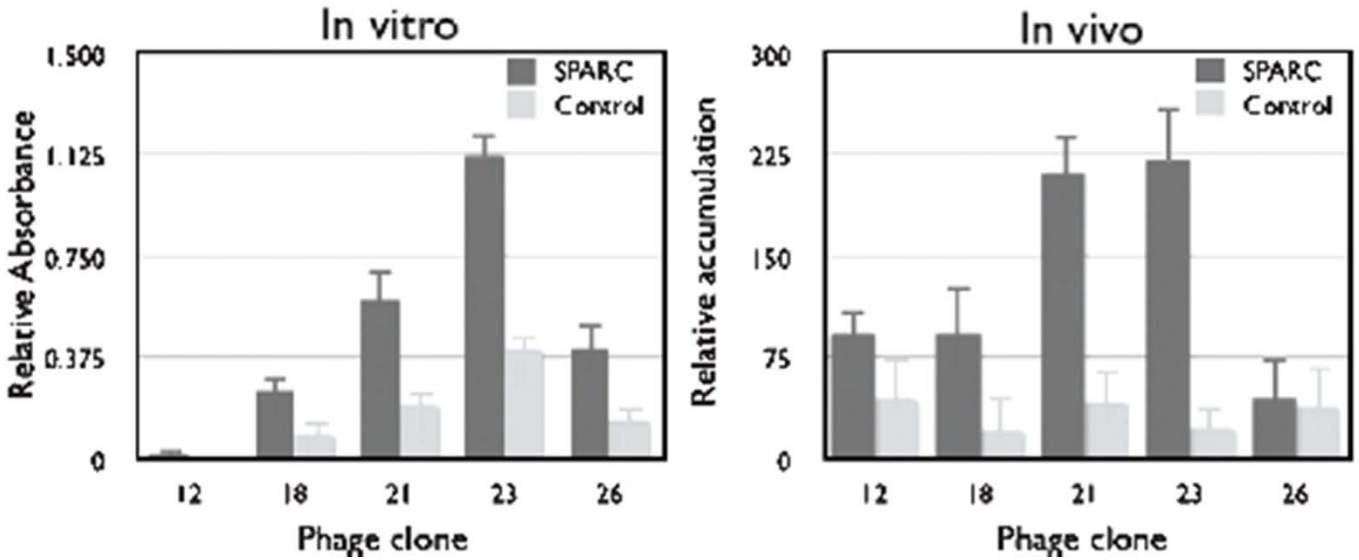
Lee, Mao, Flynn, Belcher, Science, 296, 891,2002

Our approach: M13 as a multifunctional genetic scaffold for targeting MRI contrast agent



Allows for genetic control, specificity and avidity!

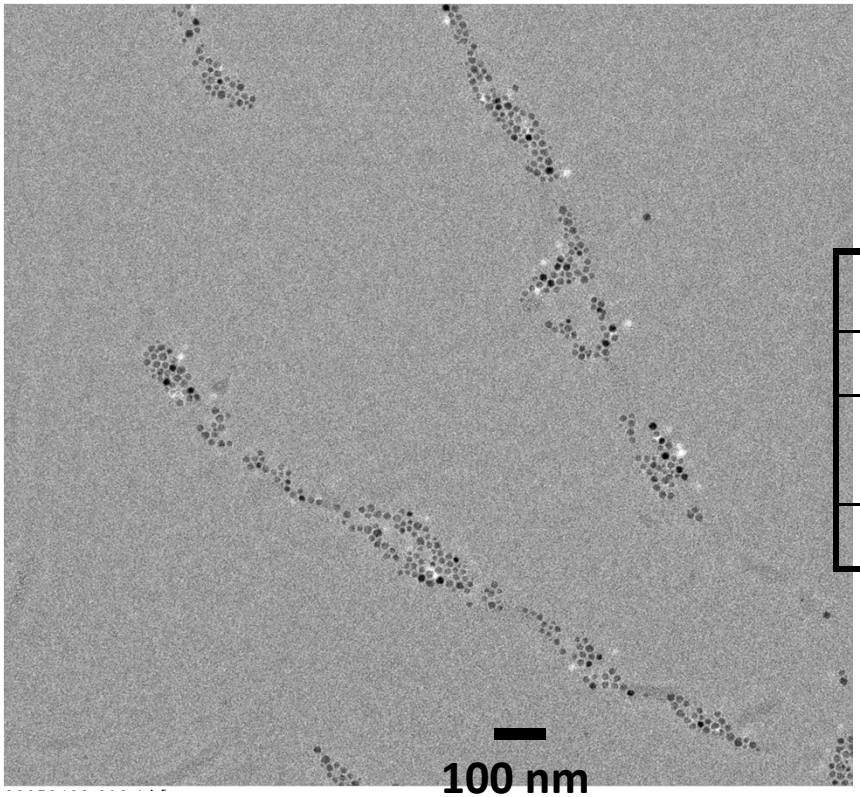
SPARC: discovery of a biomarker in metastases



Kelly et al , Neoplasia,8, 1011, 2006 (Weissleder group)

- peptide clone #23 binds *in vitro* and *in vivo*
- identified against secreted protein, acidic and rich in cysteine (SPARC)
- SPARC is protein overexpressed in many cancers; high levels are associated with bone metastases

Magnetic Virus nanowire as *enhanced* MR contrast agent



Contrast agent	Relaxivity (R2) $[mMs]^{-1}$
Commercial CLIO	~30-40
Iron oxide nanoparticle (12 nm)	65.1
magnetic phage	58.7

phage with multiple attached functionalized FeOx nanoparticles

Nanoparticles specifically attach to phage and cover the pVIII major coat

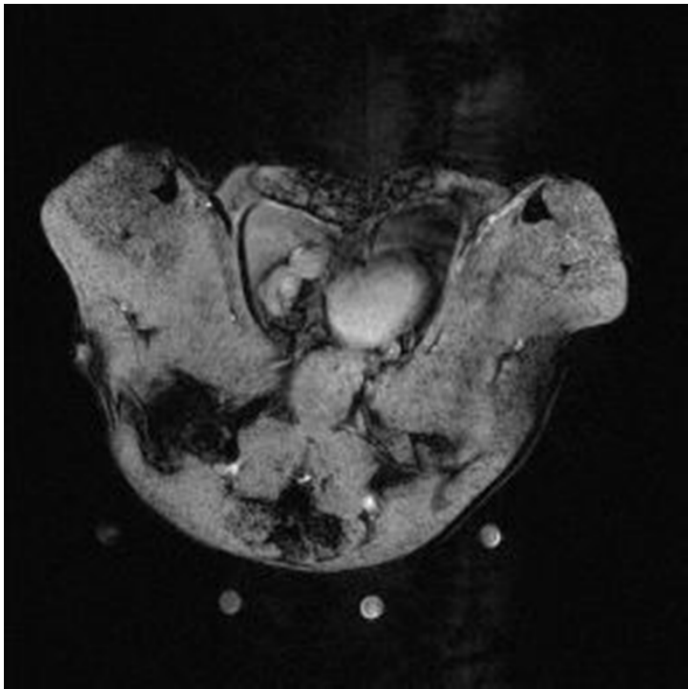
Specificity of targeted magnetic nanowires *in vitro*

- SPARC-targeted nanowires were tested for specificity against a prostate cancer cell line that highly expressed SPARC (C42B) versus a low-expressing negative control prostate cancer cell line (DU145)

- there is improved uptake of magnetic nanowires towards SPARC-positive C42B

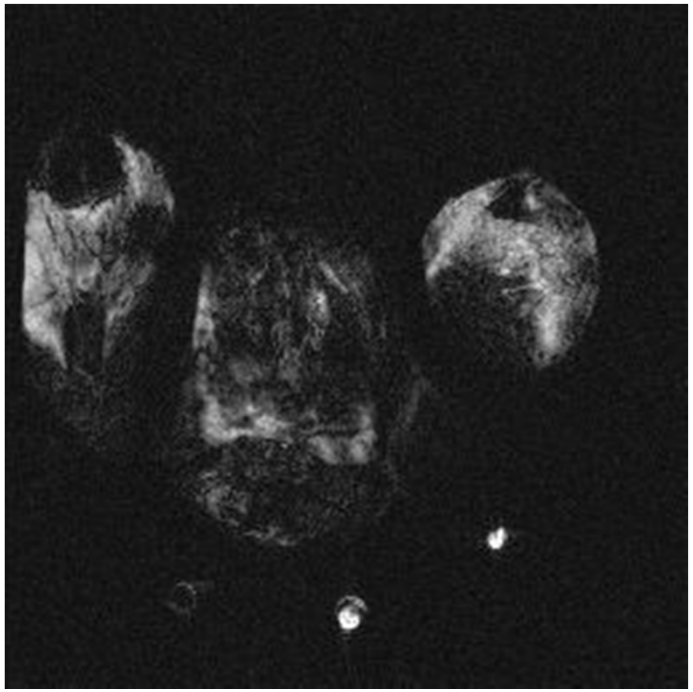
- at both nanowire concentrations specific targeting is observed

MR imaging of SPARC negative tumor

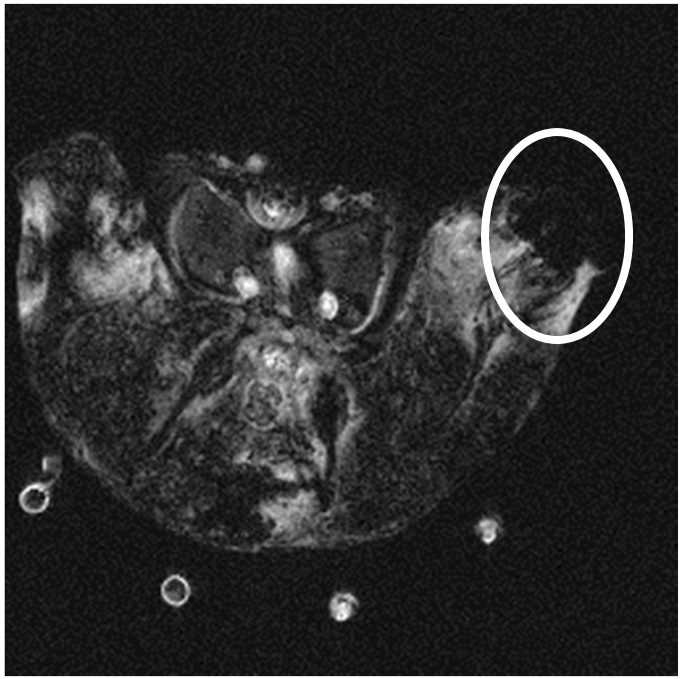


No ‘dark contrast’

Targeted imaging *in vivo*: after delivery of magnetic GEMS

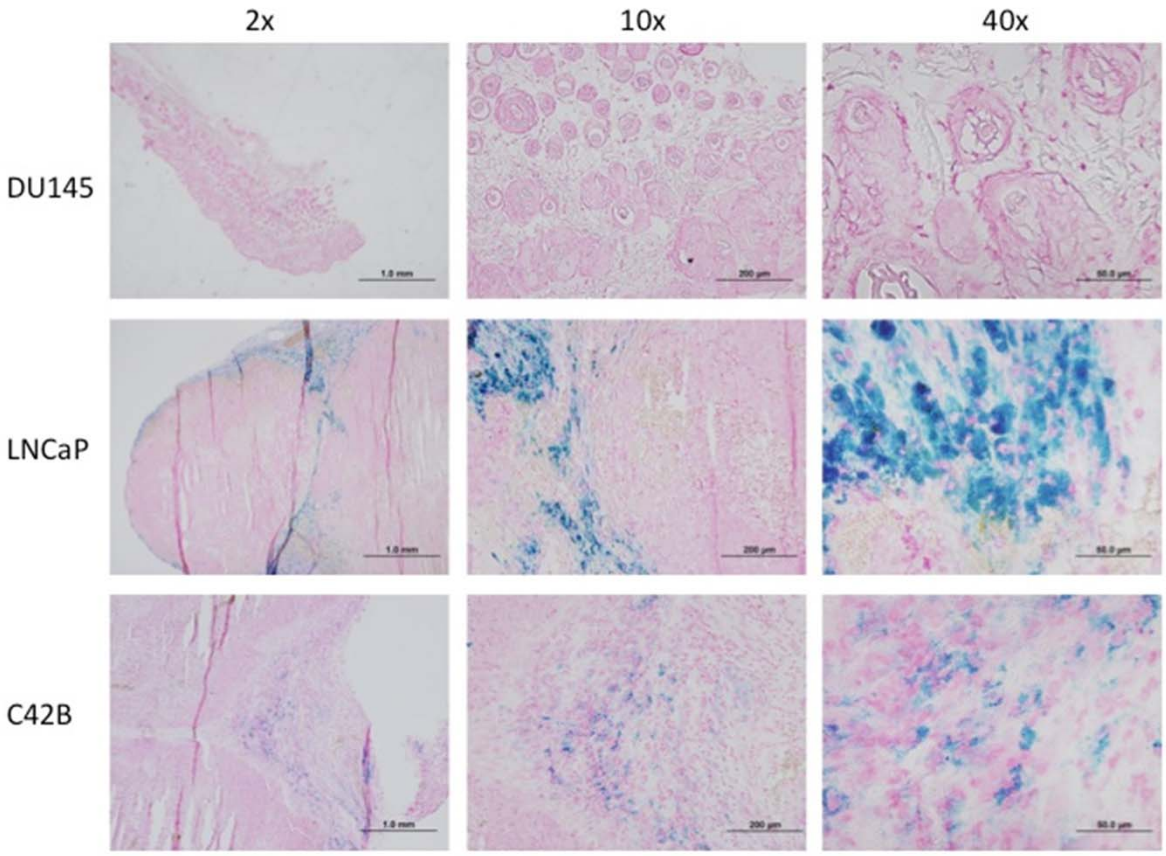


after addition to SPARC expressing tumor



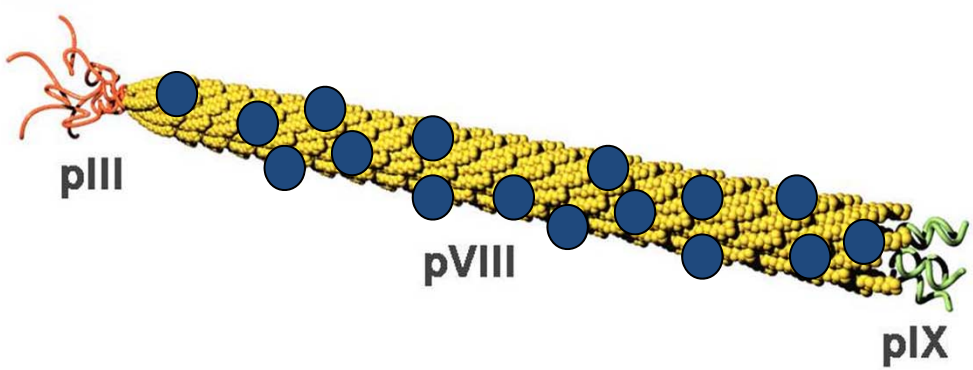
‘dark contrast’ image

Targeting *in vivo*: histology



- control, SPARC- DU145, SPARC+ LNCaP and C4-2B sub-Q xenograft tumors
- stain for iron using Perl's reagent
- targeting and specificity achieved; iron only present in SPARC positive tumors

M13 for targeted drug delivery AND imaging

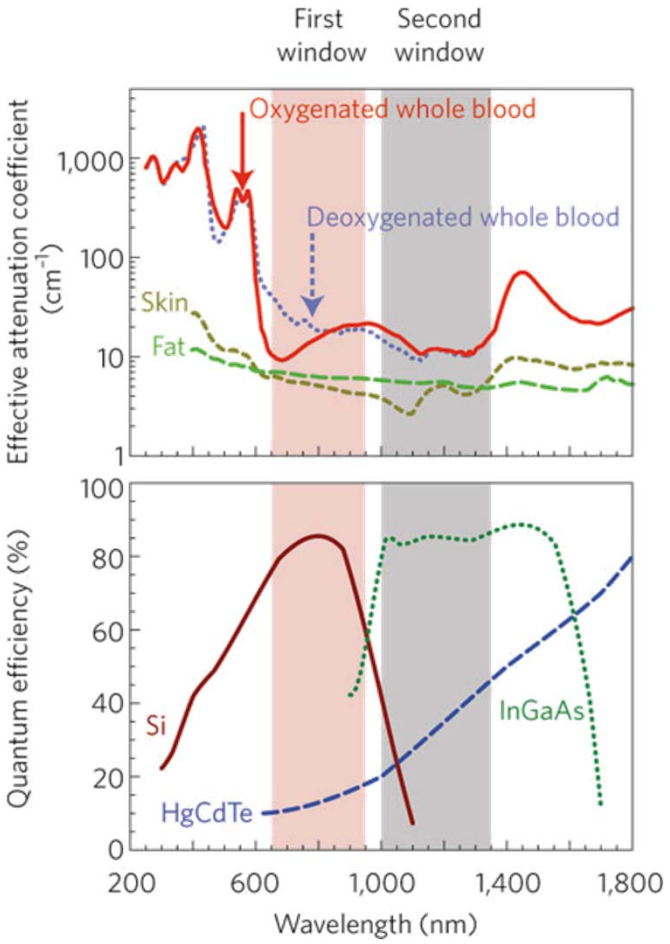


- have demonstrated imaging
- use M13 to couple chemotherapeutics along with imaging for targeted delivery using re-engineered M13

- targeted M13 conjugated with hygromycin

- initial attempts show decrease in cell proliferation activity

Single walled carbon-nanotubes for *in vivo* imaging



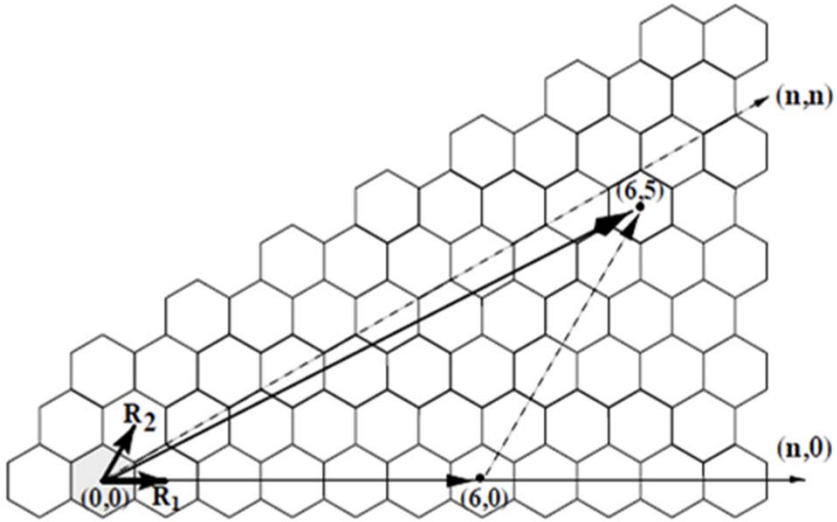
- Near infrared light (~700-2500 nm) can penetrate tissue without absorption or scattering

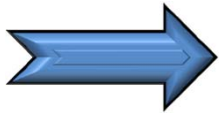
- From 650-950 nm allows for optical imaging *in vivo*
  - suffers from tissue autofluorescence
  - limited penetration depth (1-2 cm)

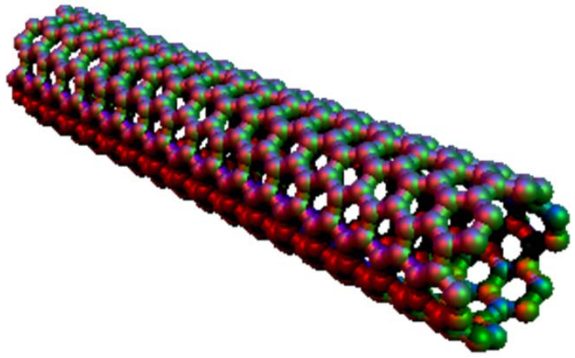
- Can improve signal to noise (tissue background) ~100 fold using fluorescent probes that emit at longer wavelengths (950 nm- 1350 nm)

Smith, Mancini, and Nie, *Nature Nanotech.*, **4**: 710-711, 2009

SWNTs







PRL **81**, 2506 (1998)

- A rolled-up graphene sheet
- A chiral vector, (n,m), defines a chirality of SWNT

: if (n-m)=3 × integer: metallic, if not, semiconducting

➔1/3 is metallic and 2/3 is semiconducting

➔Only semiconducting and non-bundled SWNTs can emit light

- The wavelength of emitted light from SWNTs resides in the second window

Motivation for SWNT/Why use M13-SWNT

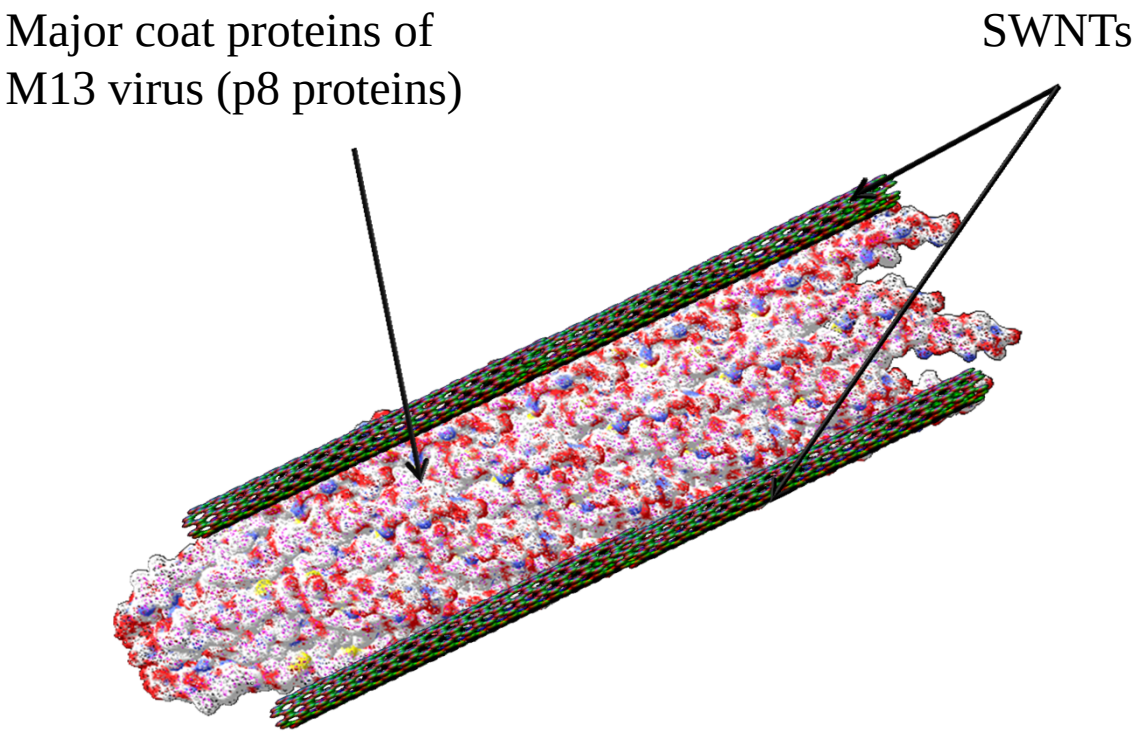
- Issues for SWNTs for bio-imaging applications

  - Biocompatible and efficient dispersants
  - Stability of SWNTs in aqueous solutions, buffers, and serums
  - Non-covalent functionlization of SWNTs keeping optical properties of pristine SWNTs
  - Brightness of fluorescence: quenching through bundling, defect creation
  - Possible toxicity of SWNTs: controversial

- Motivation for M13-SWNT

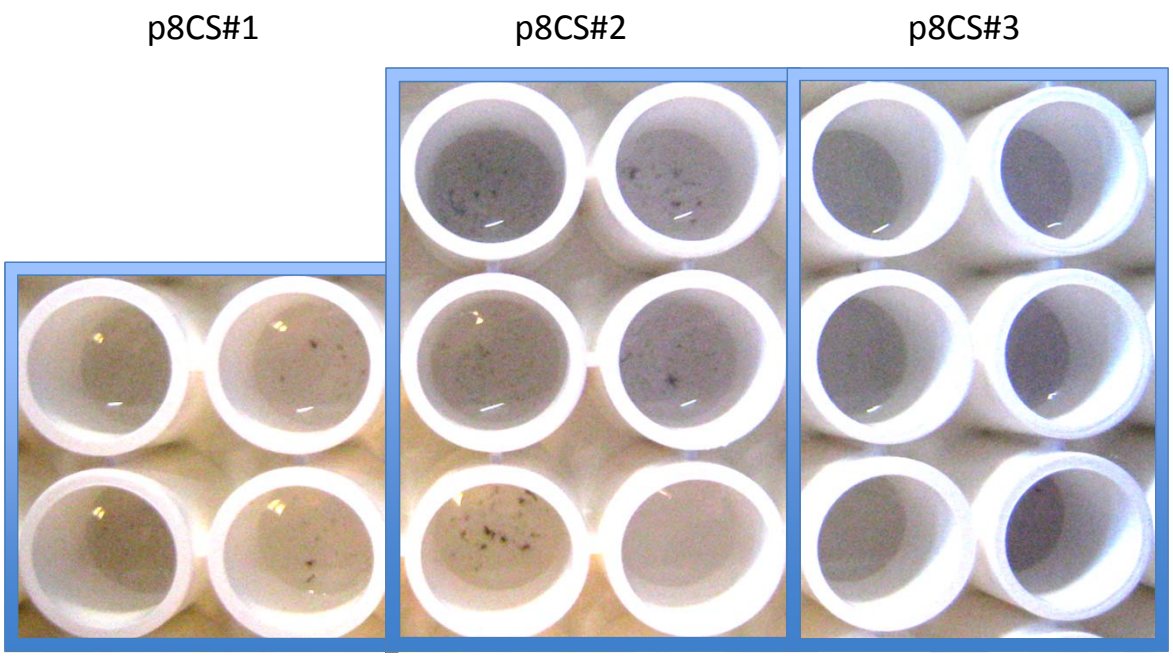
  - M13 with binding affinity toward SWNTs can disperse SWNTs in aqueous solution
  - M13 is already bio-compatible dispersant
  - Various capsid proteins can be independently engineered further
    → versatility and easiness of functionalization of SWNT through M13 engineering
  - Physical separation of targeting and imaging functionality: high ratio of SWNT to binding sites compared to SWNT-wrapping schemes → 2:5 vs. 1: 50
  - improved sensitivity

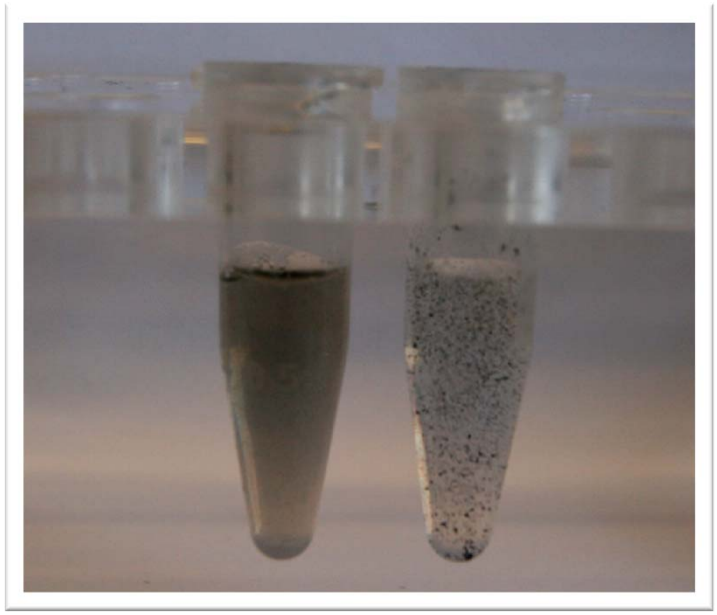
Proposed scheme of virus-SWNT complexes



SwissPDB viewer, Source: 2C0W.pdb

Stability of M13-SWNT complexes



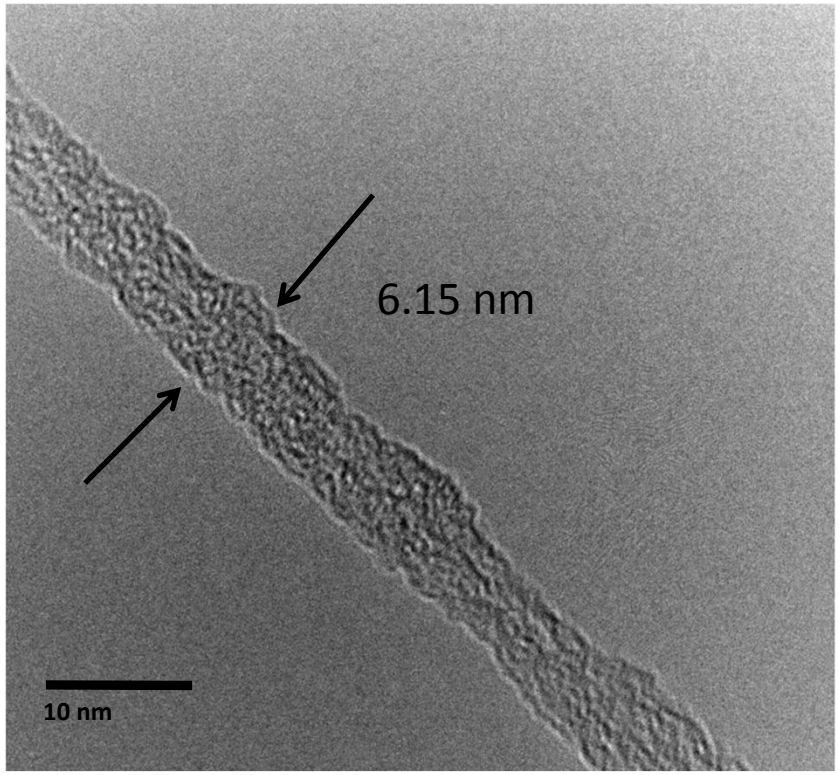


SWNTs dispersed with various M13 virus

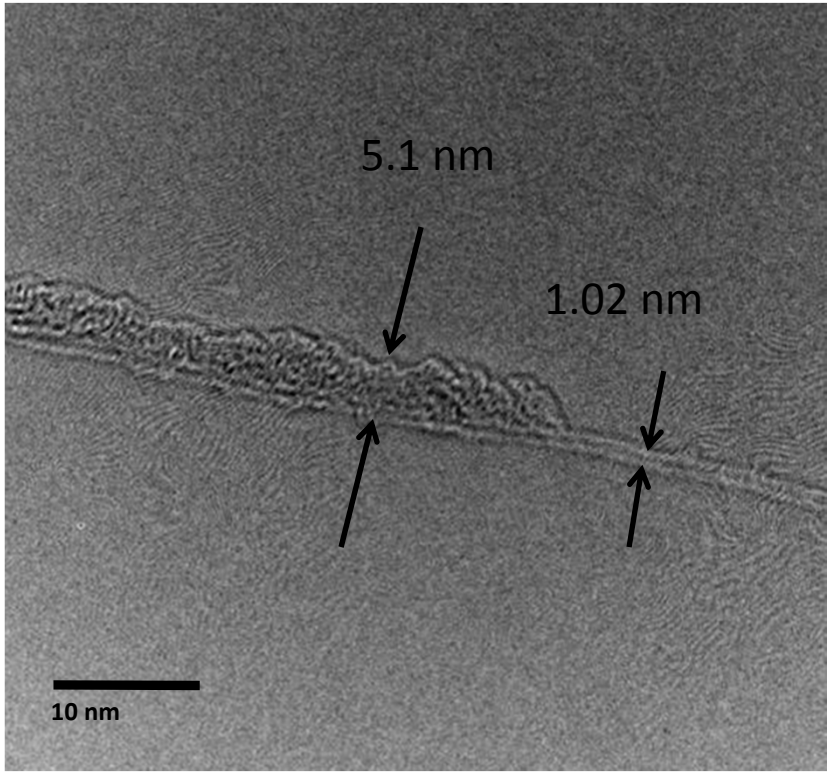
Left: SWNT bound to M13 (p8CS#3)

Right: SWNT without virus

High-resolution transmission-electron-micrographs

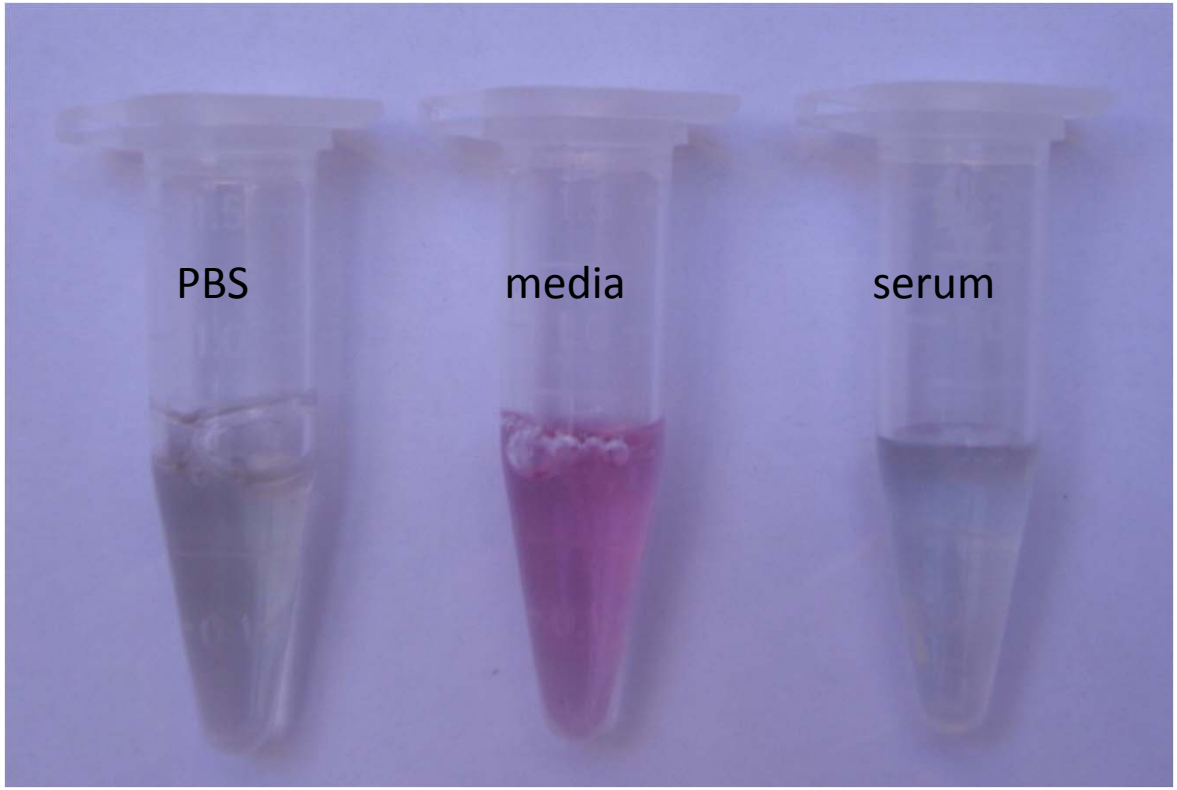


Virus-only

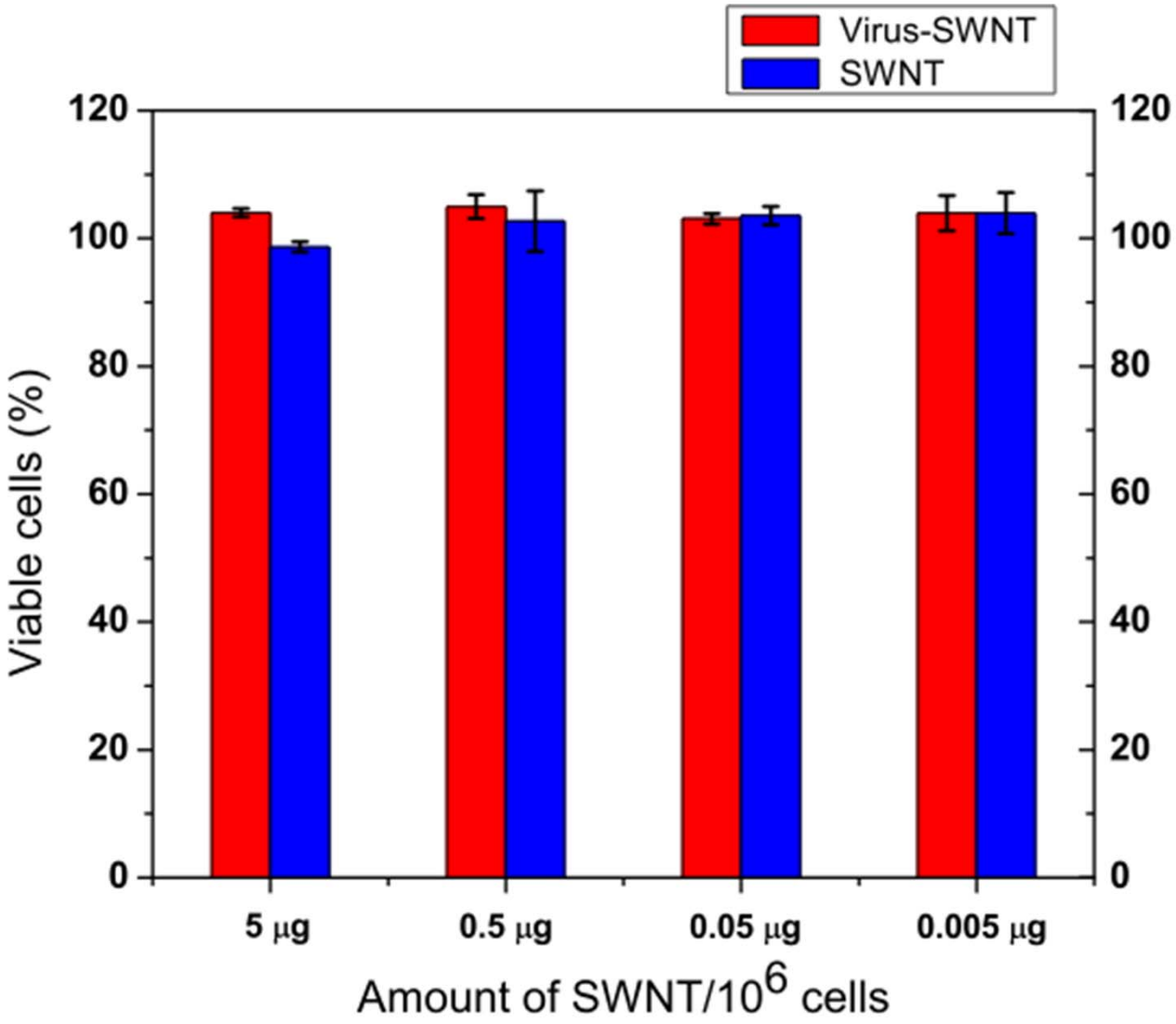


Virus-SWNT complex

Stability of virus-SWNT complex in various media

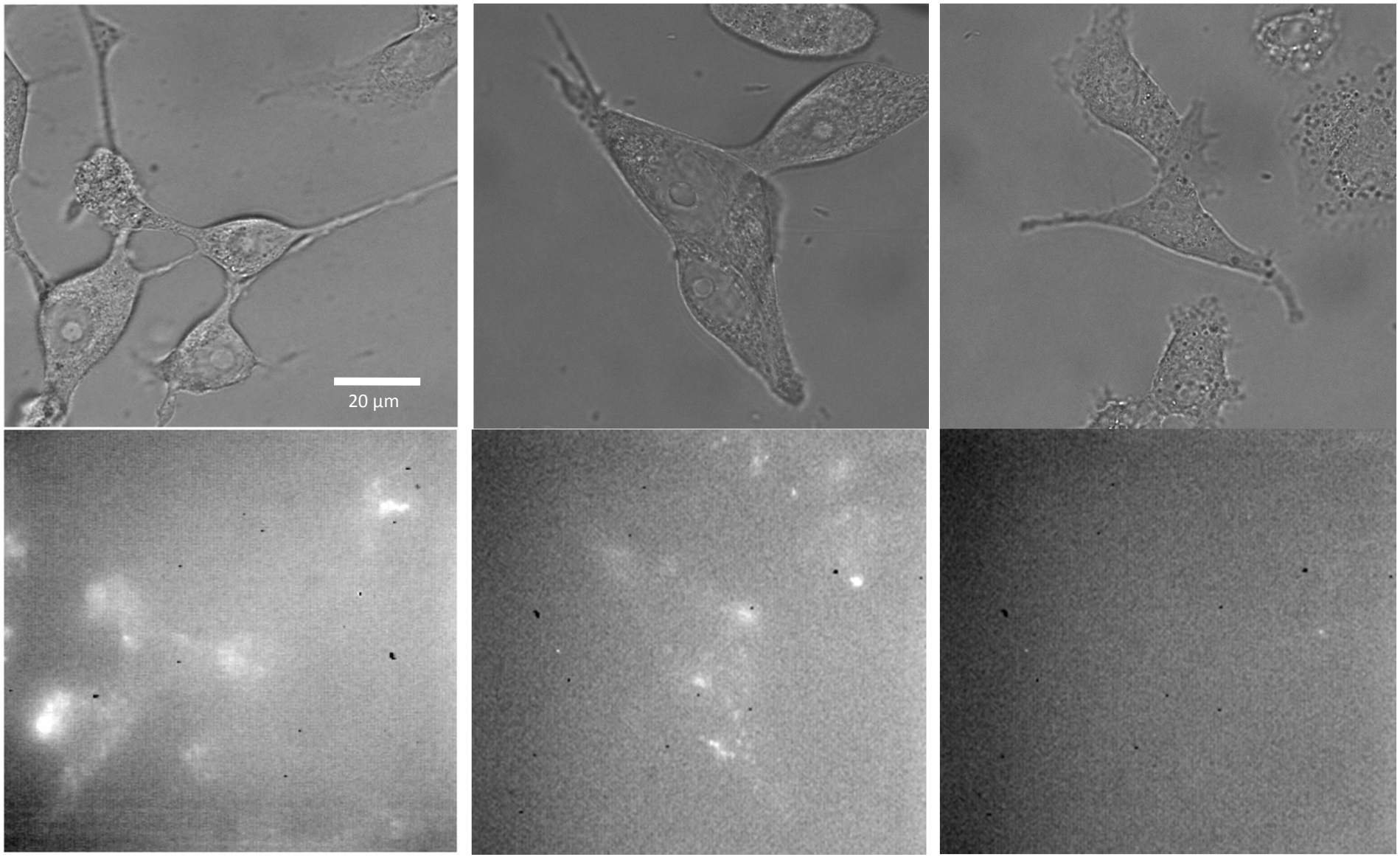


Cytotoxicity test of virus-SWNT complex

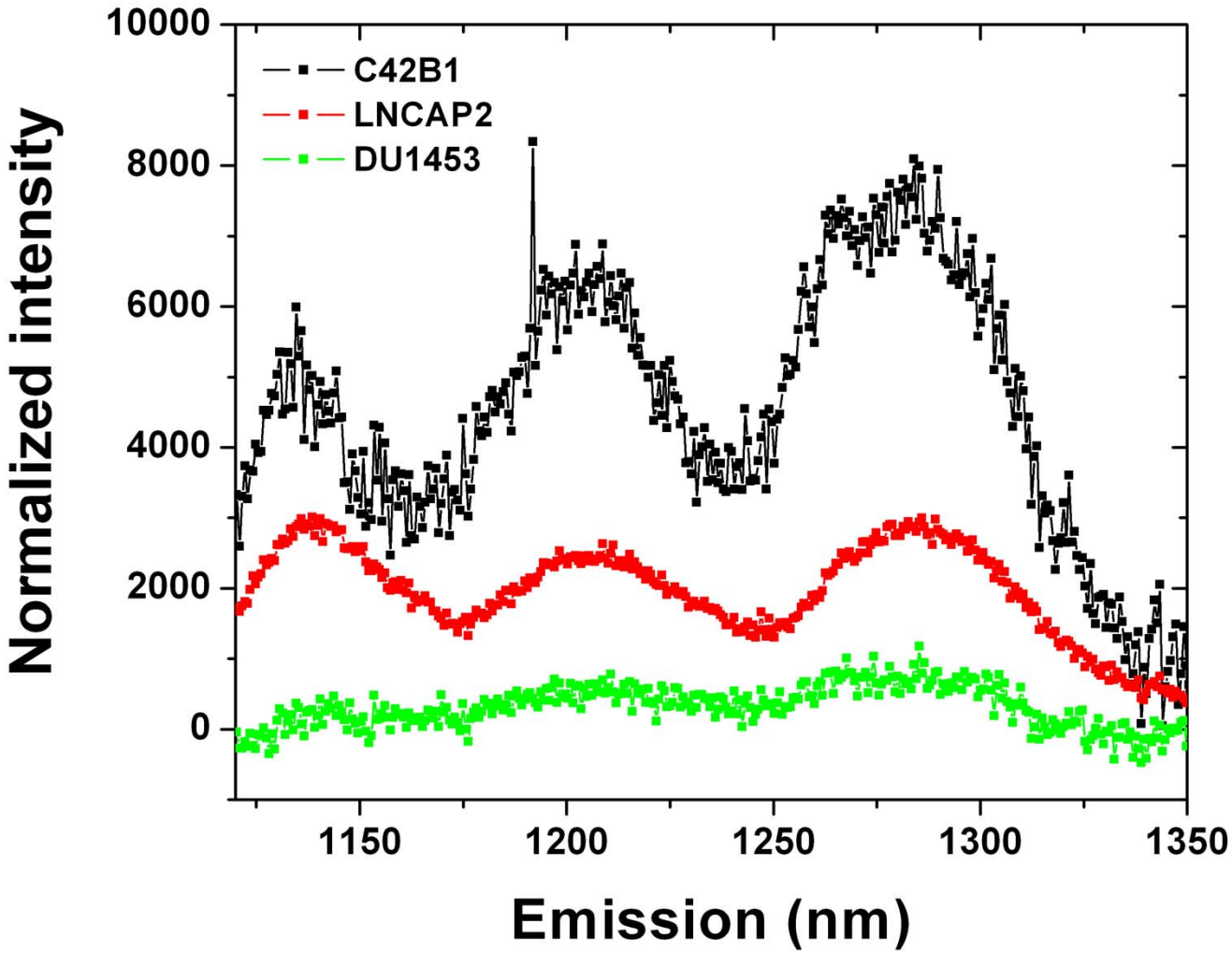


PL images: cells incubated with virus-SWNT at 37°C for 12 hr and washed with DPBS for 3X

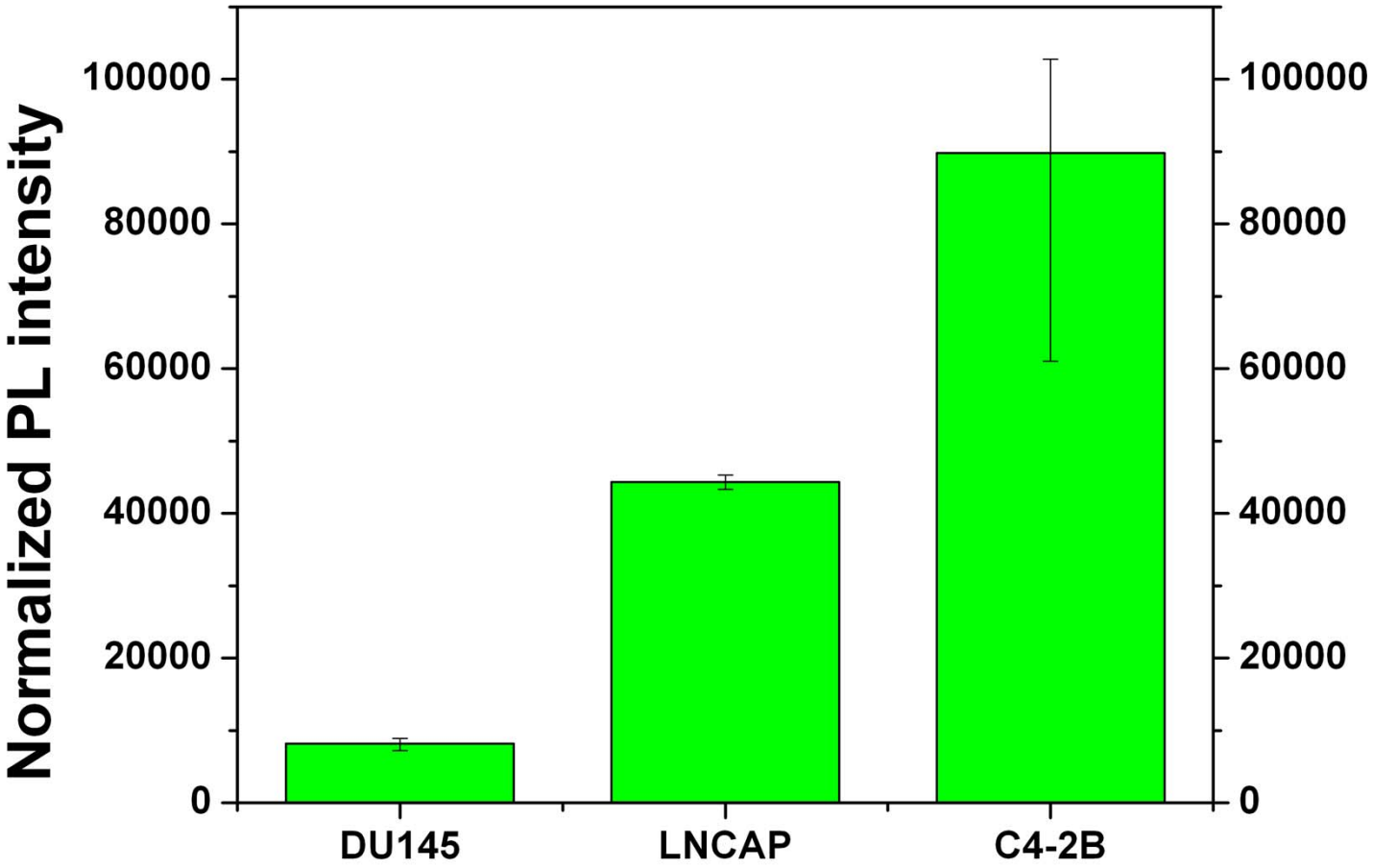
C4-2B LNCAP DU145



PL spectra from virus-SWNT incubated cancer cells



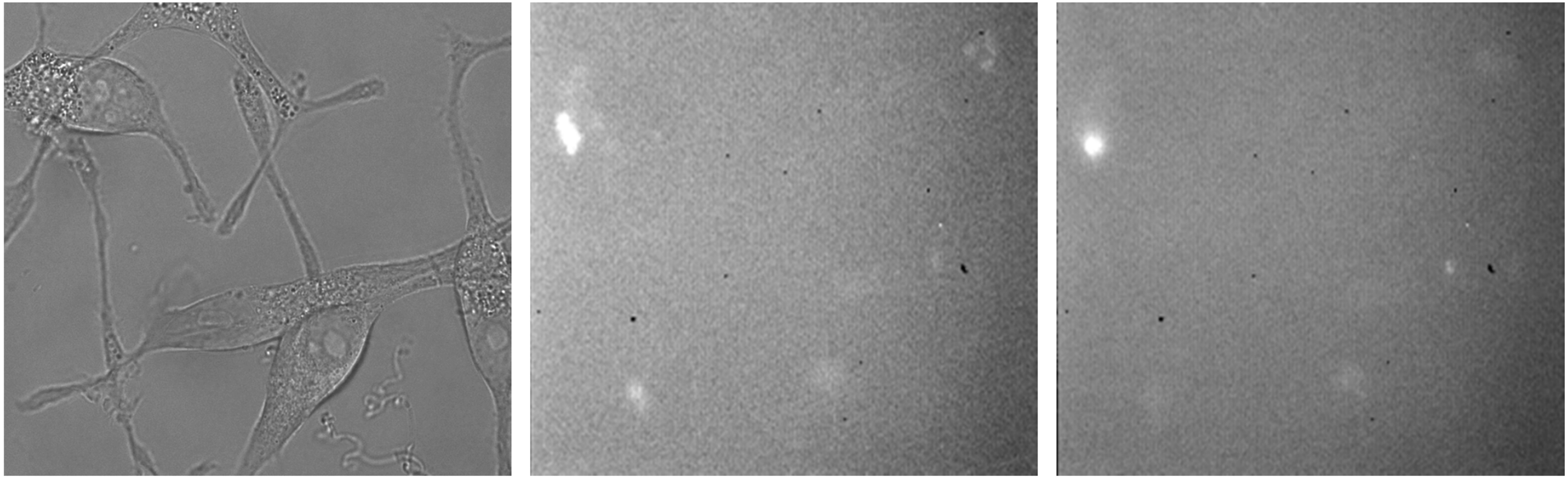
Comparison of PL intensity from various cancer cells



C4-2B: Experiments with whole blood

Before addition of whole blood

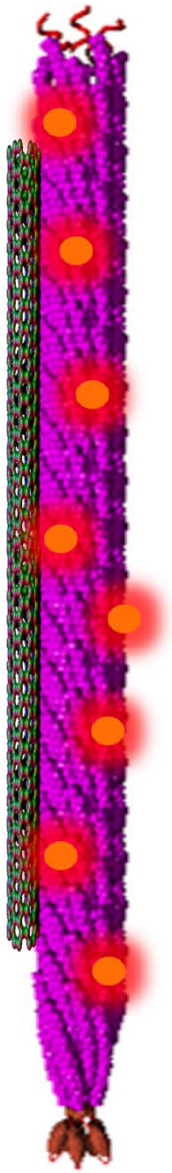
After



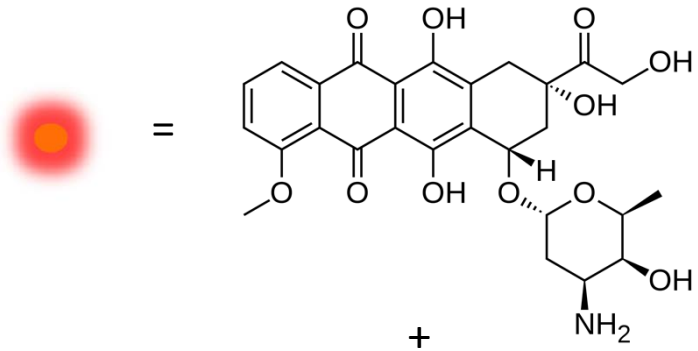
- Left: Bright field image of C4-2B before addition of whole blood
- Middle: Fluorescence image before addition of whole blood
- Right: Fluorescence image after addition of whole blood taken at the same position

The fluorescence image can be detected even after addition of whole blood.
It is noticeable that the image becomes slightly blurry.

Conjugation of drug onto p8 proteins of virus



Doxorubicin



+

COO^- of Asp (D2) or Glu (E5) of p8cs#3

EDC and Sulfo-NHS

Virus-SWNT-Drug complex

MTT Cell Proliferation Assay result

EC_{50} from dose-response curves

1) C4-2B (+) vs. DU145 (-) for virus-SWNT-drug

C4-2B	DU145	DU148/C4-2B
0.27 µg/ml	0.43 µg/ml	1.6

2) Virus-SWNT-Drug complex vs. free drug

virus-SWNT-drug	free drug	DU148/C4-2B
0.27 µg/ml	1.58 µg/ml	5.9