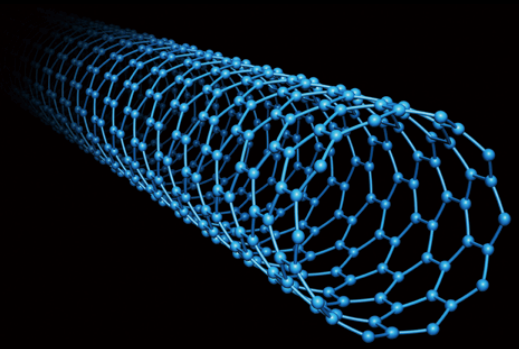
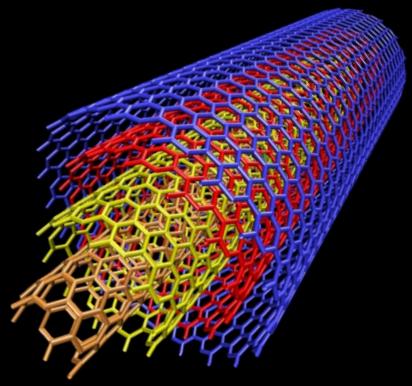
Single-walled carbon nanotubes as diagnostic sensors in vivo

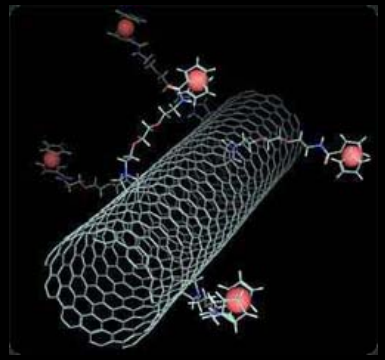




Audrey Fahrny

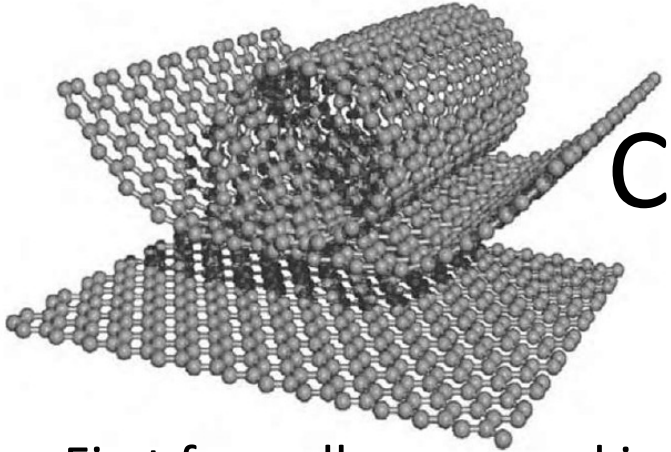
07.10.14

Division of Infectious Diseases &
Hospital Epidemiology, USZ



Outline

- CNTs & SWNTs
- SWNT functionalization for biomedical applications
- Current applications
- Paper 1
- Paper 2
- Conclusion & Outlook



Carbon Nanotubes (CNTs)

- First formally prepared in 1991 by Iijima
- Nano-scale hollow cylinders comprised of rolled up graphene sheets
  - Graphene sheet = hexagonal arrangement of covalently bonded carbon atoms
  - High aspect-ratio → Quasi 1-D → distinct optical & electrical properties
  - Single-walled or multi-walled carbon nanotubes (SWNTs / MWCTs)

Φ ≥0.48nm

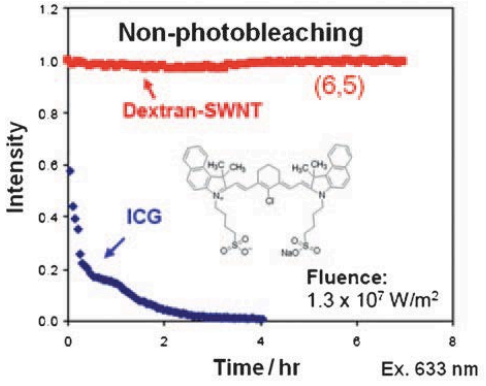
Φ: 5- 100nm

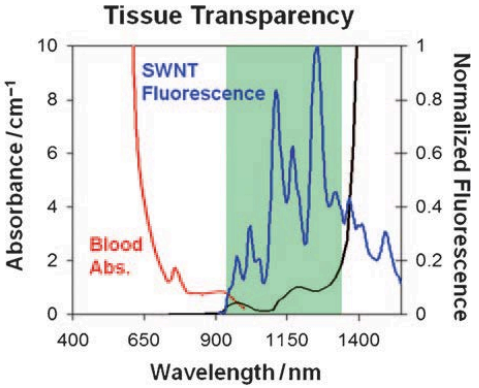
Vardharajula et al. Int. J. Nanomedicine (2012); Boghossian et al. Chem. Sus. Chem. (2011)

Properties of SWNTs

- Physical properties:
  - Strong & flexible
  - Highly hydrophobic surfaces→ insoluble in most organic & aqueous solvents
  - Interact with cell membranes and penetrate various biological tissue
  - High surface-to-volume ratio and reduced surface → Electronic properties of SWNTs are very sensitive to their environment

- Electrical properties:
  - CNT can be metallic or semiconducting, depending on chirality
  - Electrical conductivity six orders of magnitude higher than copper
  - Excellent field emitter





- Optical properties:
  - Unique NIR-fluorescence properties
    - Absorption in NIR range: can be utilized for photo thermal therapy & photoacoustic imaging
    - Emission in NIR range: 800-2000nm fluorescence
    - Photostability (no bleaching & no blinking)
  - Distinct resonance enhanced Raman signatures for Raman spectroscopy

Vardharajula et al. Int. J. Nanomedicine (2012); Boghossian et al. Chem. Sus. Chem. (2011); Liang & Chen. Curr. Med. Chem. (2010)

Properties of SWNTs

Main advantages for biosensing:

• NIR intrinsic fluorescence & photostability

• Electronic and optical properties of SWNTs are very sensitive to their environment

--> inherent biosensors with unlimited lifetime

Main issues:

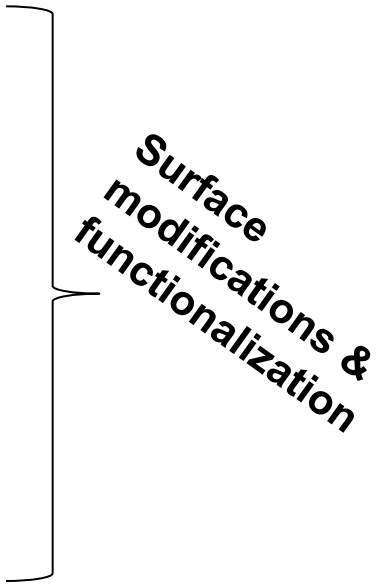
•Solubility

•Toxicity & biocompatibility

- Bare SWNTs exhibit toxicity in vitro & in vivo
- Depend on size and morphology of CNTs, surface chemistry, process of purification and functionalization.

•Pharmacokinetics

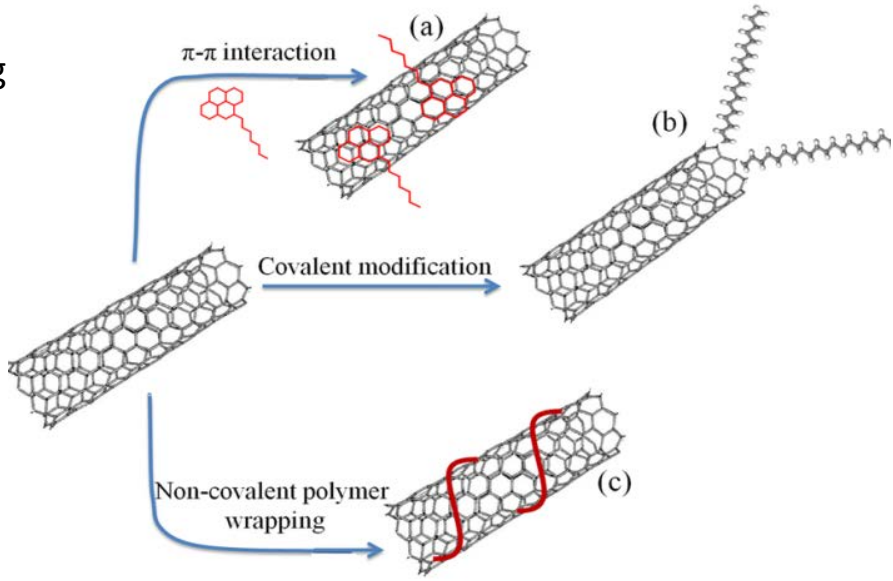
- Blood circulation half-life & clearance
- Organ bio-distribution and accumulation: after systemic administration, pristine CNTs predominantly localized in liver, lungs and spleen, exhibiting toxic effects



Vardharajula et al. Int. J. Nanomedicine (2012); Boghossian et al. Chem. Sus. Chem. (2011); Liang & Chen. Curr. Med. Chem. (2010)

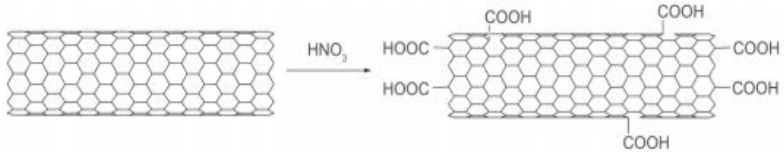
Functionalization of SWNTs: a pre-requisit for biological applications

Chemical modifications to solubilize and disperse CNTs; improving biocompatibility and biodegradability, reducing toxicity



1. **Surfactant coating** of CNTs for dispersion: Highly hydrophobic CNT surface interacts with surfactants

2. **Covalent modifications**: Attachment of chemical groups through reactions onto the SWNT skeleton
   - CNT oxidation
   - Intrinsic electrical & optical properties of CNTs often destroyed



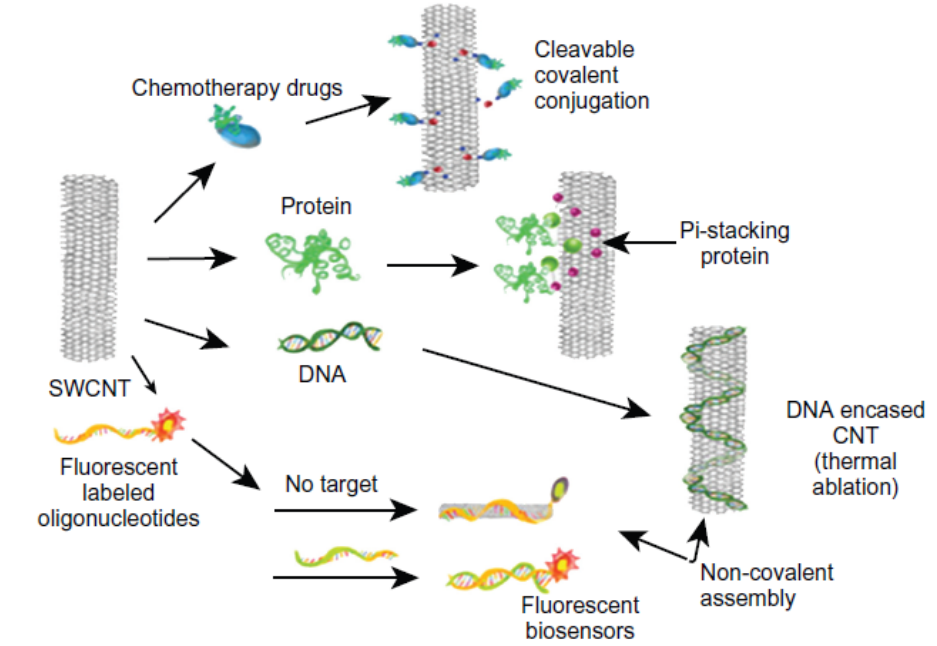
3. **Non-covalent modifications**: Supramolecular adsorption or wrapping of various functional molecules
   - CNTs interact with various molecules through weak interactions: surface adsorption onto sidewalls, π- π stacking, electrostatic interactions, hydrogen bonding and van der Waals force

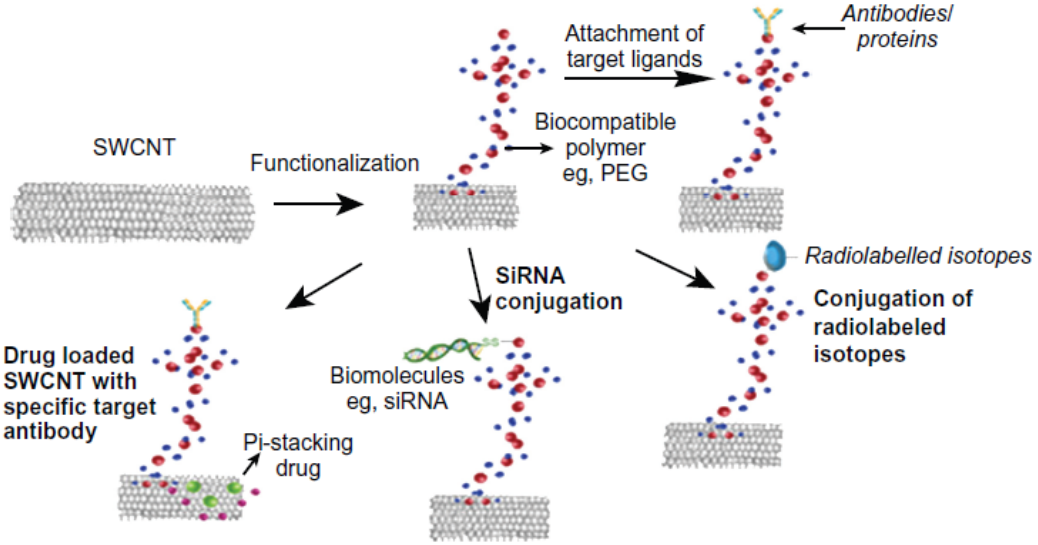
Vardharajula et al. Int. J. Nanomedicine (2012); Boghossian et al. Chem. Sus. Chem. (2011); Liang & Chen. Curr. Med. Chem. (2010)
Top image: Choudhary & Gupta. DOI: 10.5772/18423

Non-covalent functionalization of SWNTs

- Conjugation with DNA, proteins, hydrophilic polymers (eg PEG) have all proven to be effective at solubilizing CNTs and rendering them more biocompatible

- Further functionalization for specific targeting
- Huge versatility generated by functionalizing CNTs





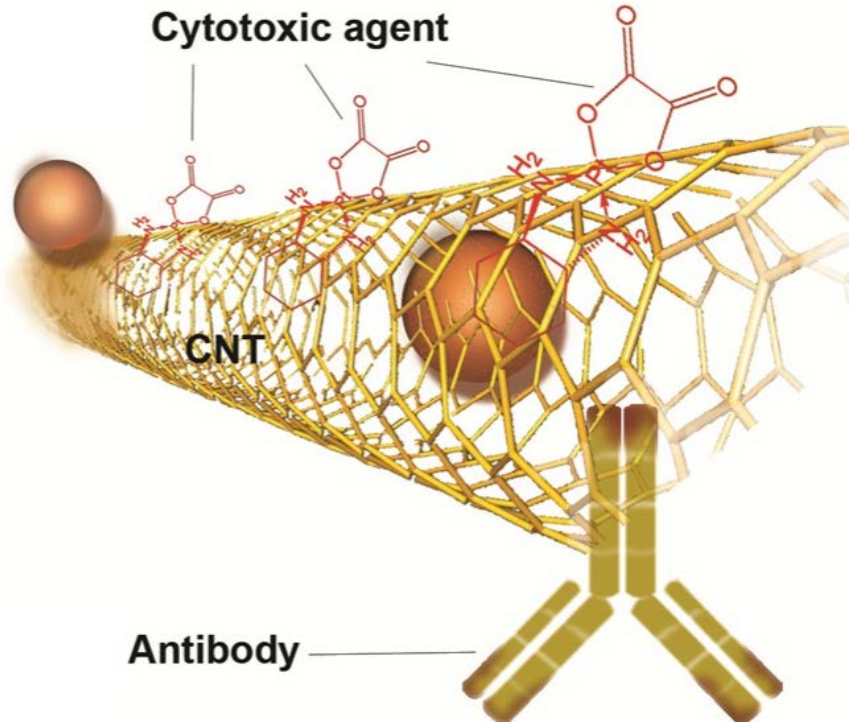
Vardharajula et al. Int. J. Nanomedicine (2012)

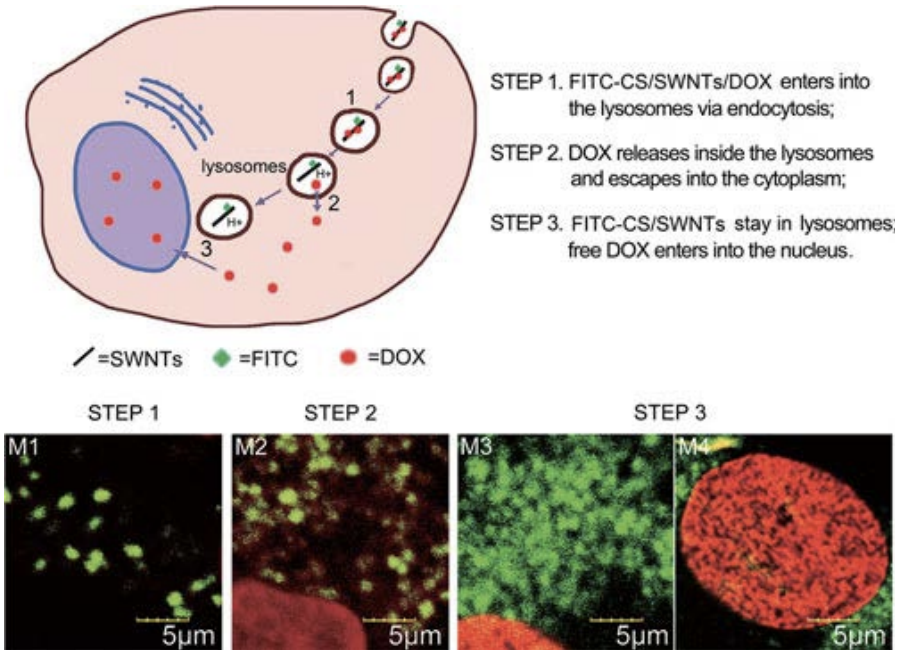
Current biomedical applications of SWNTs

- **Therapeutics:**
  - **Delivery vehicles**: shuttle various biological molecular cargoes into cells: eg drugs, oligonucleotide molecules

    (Z. *Liu et al. Drug delivery with carbon nanotubes for in vivo cancer treatment. Cancer Res. 2008; 68, 6652–6660)*
  - **Cancer therapy: eg** photothermal tumor ablation

    (X. *Liu et al., Optimization of surface chemistry on single-walled carbon nanotubes for in vivo photothermal ablation of tumors. Biomaterials 2011;32, 144–151.)*

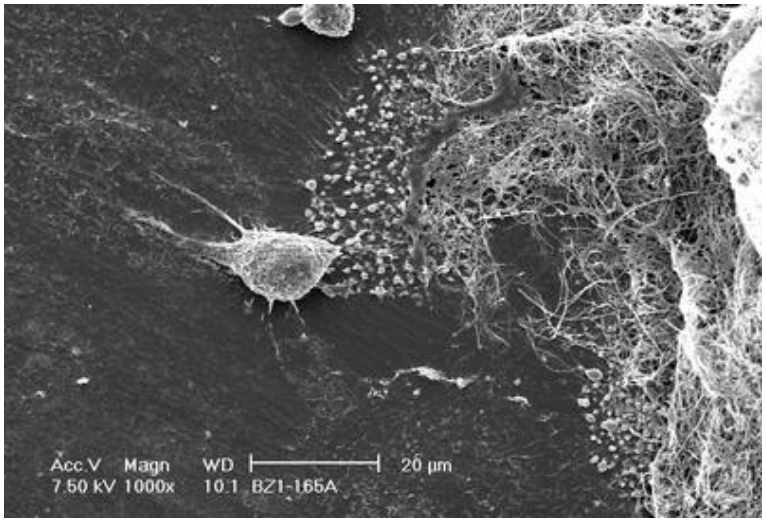


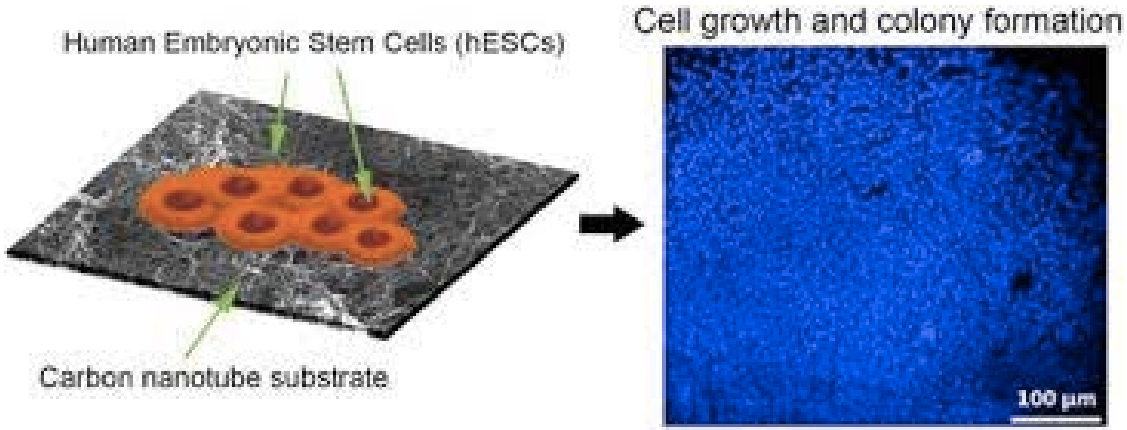


Current biomedical applications of SWNTs

- **Scaffold** for cell culture & tissue engineering
  - Collagen & polymer fCNT-based matrices

    (*RA. MacDonald et al. Collagen–carbon nanotube composite materials as scaffolds in tissue engineering. J Biomed Mater Res A*. 2005; 74:489–496)

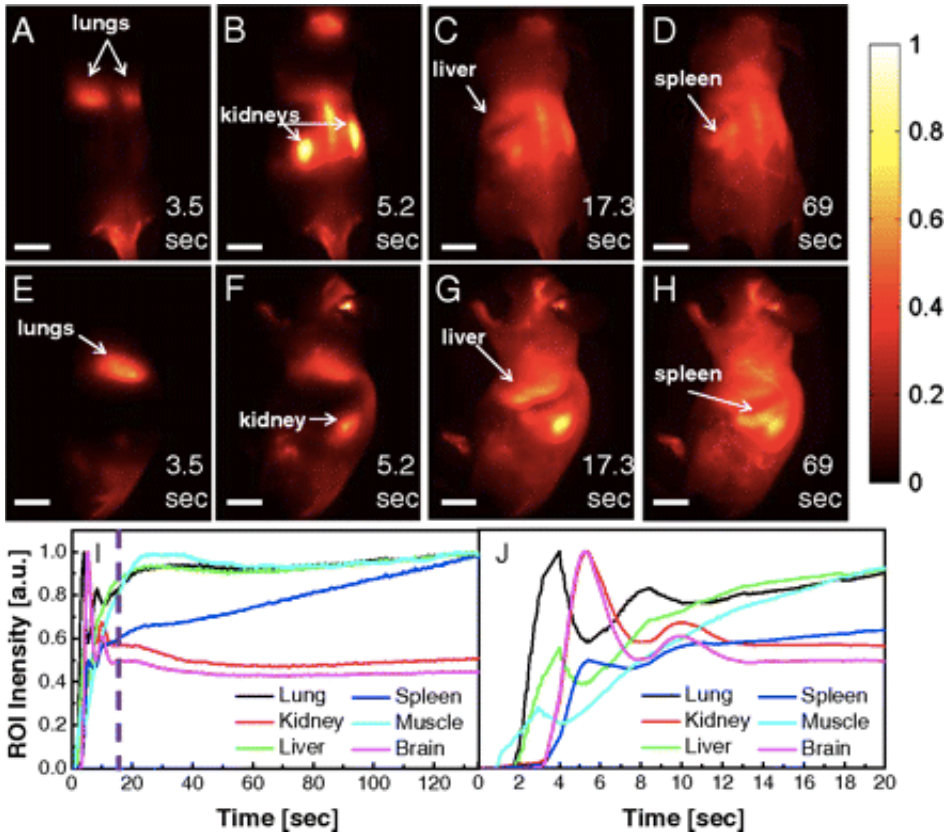




Current biomedical applications of SWNTs

- **Bioimaging**: SWNTs as optical tags or contrast agents

  (*K. Welsher et al. A route to brightly fluorescent carbon nanotubes for nearinfrared imaging in mice. Nature Nanotech. 2009; 4, 773–780)*



Current biomedical applications of SWNTs

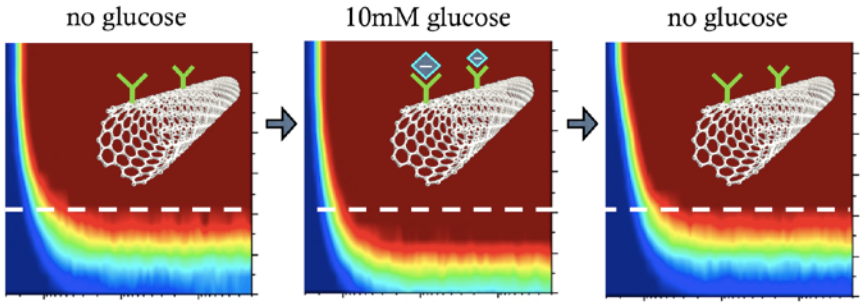
BIOSENSING: specific sensing of a wide variety of biological species

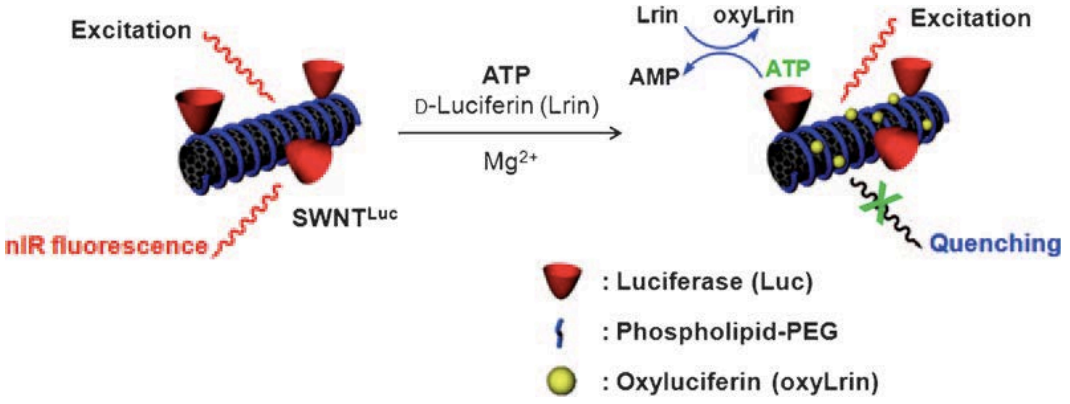
- Possibility to perform sensing operation without the use of any labels or complex reaction schemes
- DNA detection

  (*D. A Heller et al. Optical detection of DNA conformational polymorphism on single-walled carbon nanotubes. Science 2006; 311, 508–511.)*
- Protein detection

  (*J. H. Ahn et al. Label-free, single protein detection on a near-infrared fluorescent single-walled carbon nanotube/protein microarray fabricated by cell-free synthesis. Nano Lett. 2011; 11, 2743–2752.*)
- Analyte/single-molecule detection

  (*J. H. Kim et al. The rational design of nitric oxide selectivity in single-walled carbon nanotube near-infrared fluorescence sensors for biological detection. Nature Chem. 2009; 1, 473–481.)*





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ARTICLE

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Carbon nanotubes as *in vivo* bacterial probes

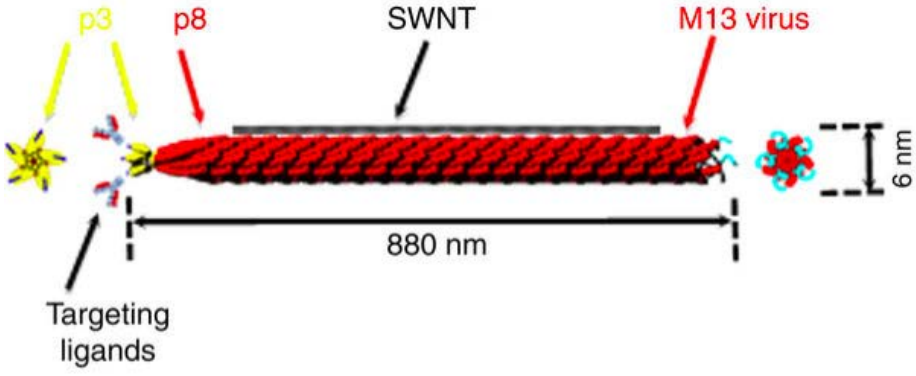
Neelkanth M. Bardhan[1,2], Debadyuti Ghosh[1,2,3] & Angela M. Belcher[1,2,4]

Introduction

- Gold standard nuclear medicine technique for imaging of infectious diseases: targeting bacteria via ex vivo radiolabelled autologous leukocytes
  - exposes patients to radiation, laborious to design and implement, requires specialized equipment and operator training.

- Aim: Targeted non-invasive fluorescence imaging of bacterial infections in vivo, using functionalized SWNT probes

- Strategy: Non-covalent conjugation of SWNTs to engineered-M13 virus phage



Dispersion & M13-functionalization of SWNTs

1. Commercially manufactured “plain” SWNTs

2. SWNTs dispersed in 2%(w/v) aqueous **sodium cholate** (SC-SWNTs)

3. Functionalized with **M13**:
   - M13: natural binding affinity towards strains of E. coli (F’-positive)
   - M13 surface capsid proteins genetically engineered to display peptides with multiple functionality (*targeting motifs, molecule carriers, fluorescent probes*)
   - Phage p8 library constructed to find best SWNT-binding M13 candidate

Engineered M13 as a multifunctional platform

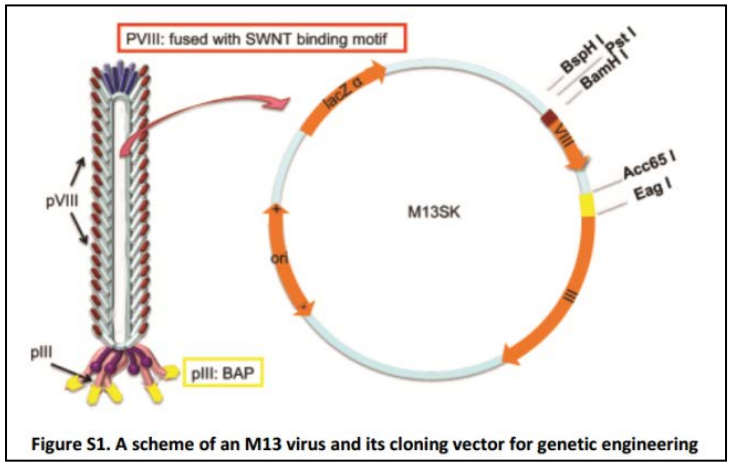
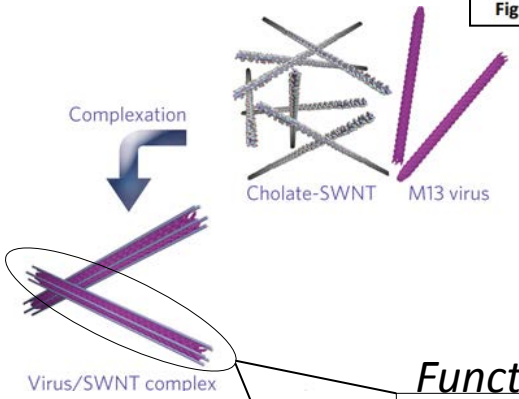
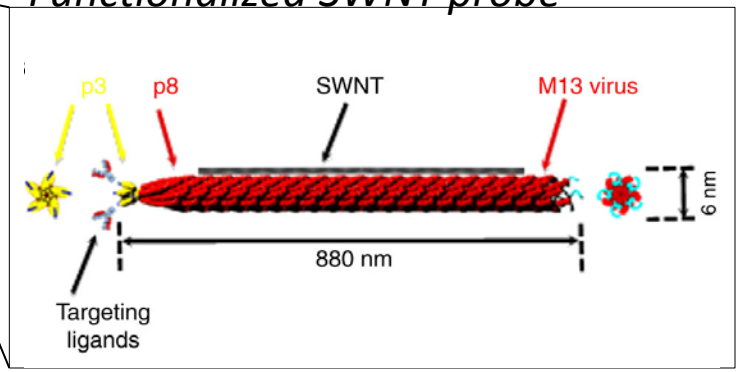


Figure S1. A scheme of an M13 virus and its cloning vector for genetic engineering



Functionalized SWNT probe



Dang et al. Nat. Nanotechnol. (2011)

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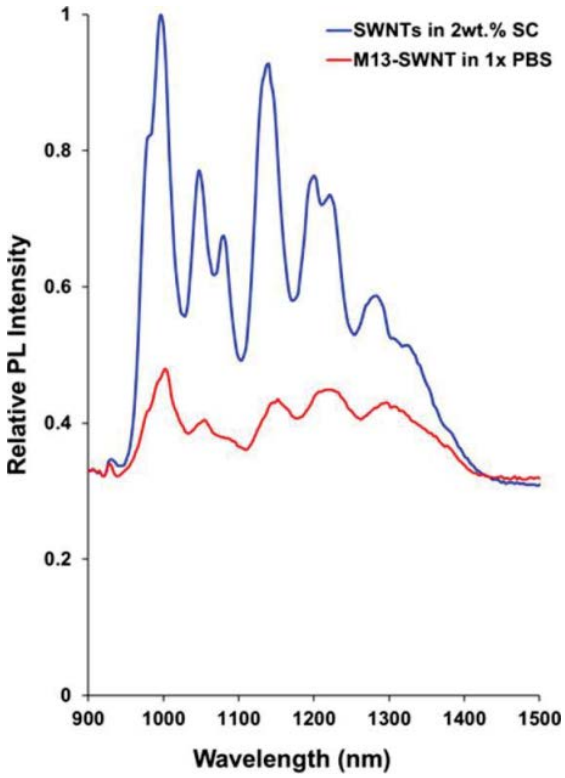
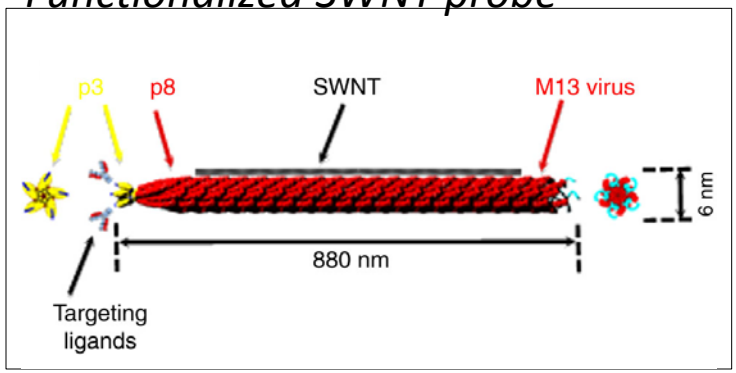


SWNT retains its photoluminescence characteristics upon M13 functionalization

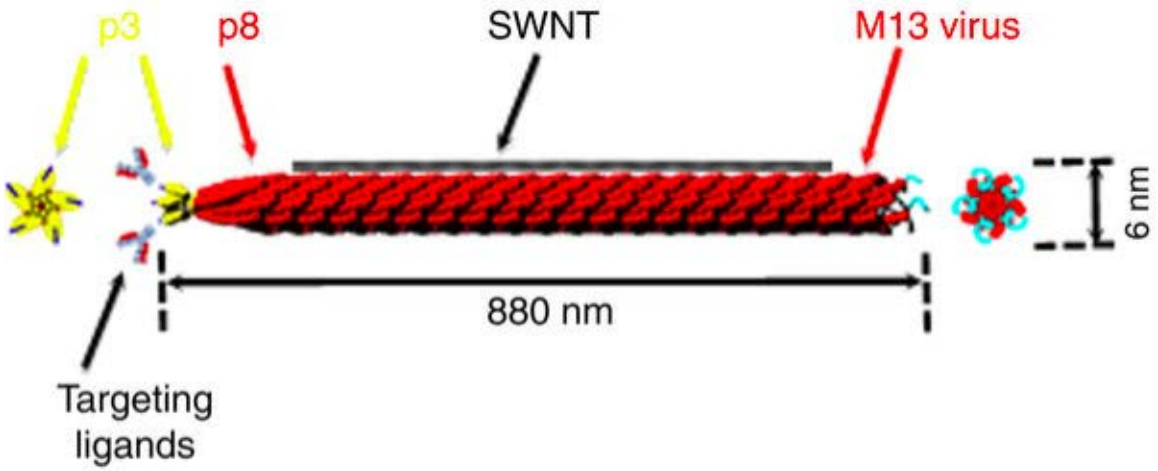
Functionalized SWNT probe



Dang et al. Nat. Nanotechnol. (2011)

Further functionalization of M13-SWNT probe

- *Antibody-M13-SWNT* probes for targeting specific bacterial infections
- Modular one-step functionalization of M13 → Antibody-binding system incorporated on p3 coat protein:
  - Express biotin acceptor peptide (BAP) on the p3 coat protein
  - Biotinylation of BAP-M13 → site-specific conjugation of streptavidin-modified targeting moieties
  - **Attach specific anti-bacterial antibodies** to target specific bacterial infections.

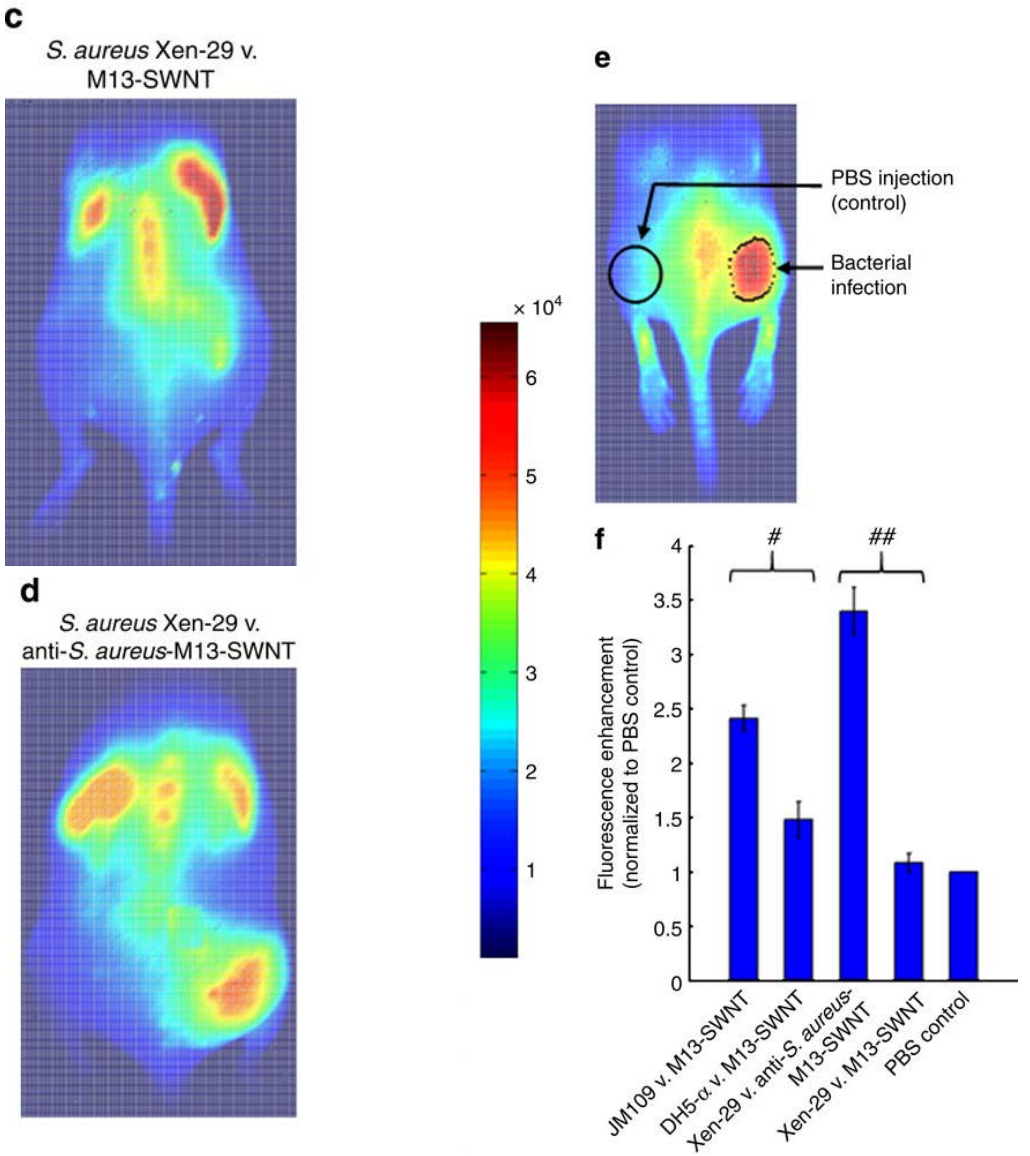


M13-SWNT probes specifically target and allow imaging of various bacterial strains in vivo

1. Established probe specificity & sensitivity in vitro

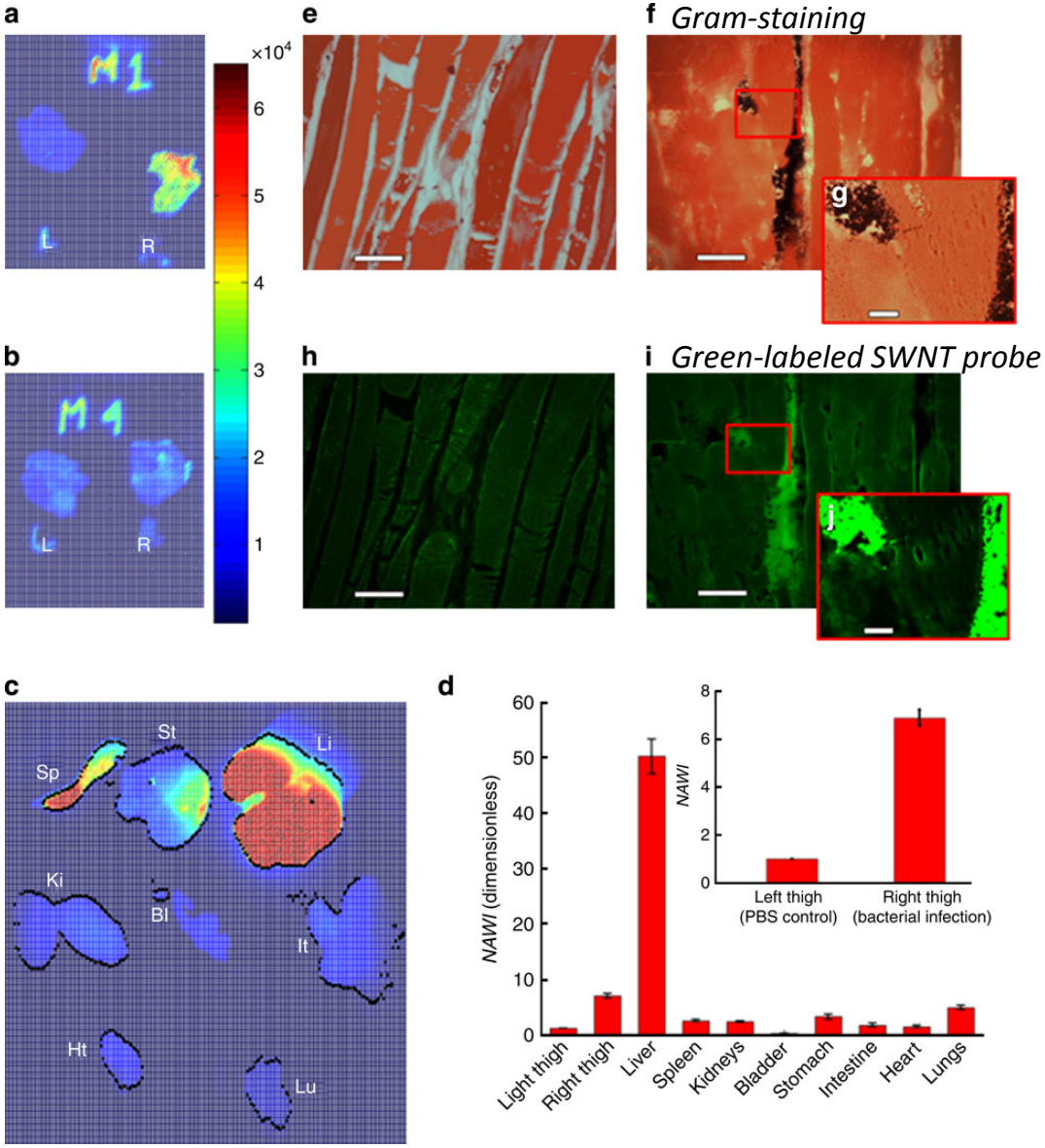
2. Testing anti S. aureus-M13-SWNT probe using a mouse infection model of S. aureus:

   - Targeted probe offers 3.1x enhancement in signal over the untargeted case
   - Tested probe with lower thresholds of bacterial infection: sensitivity remains with one order of magnitude less bacteria
   - Using SWNT probe dosage up to an order of magnitude lower than that used in other reported in vivo applications



Ex vivo validation: anti S. aureus-M13-SWNT probe specially targets and localizes at the site of bacterial infection

- 6.9x increase in probe localization at site of infection compared to control
- **Co-localization** of bright-field (gram-staining) & fluorescence (SWNT probe) images
  - Correlation coefficient of 0.704±0.053
- Relative probe distribution of SWNT in various organs
  - Maximum probe uptake in liver and spleen
  - Probe in stomach, kidneys and intestine: clearance of fSWNTs

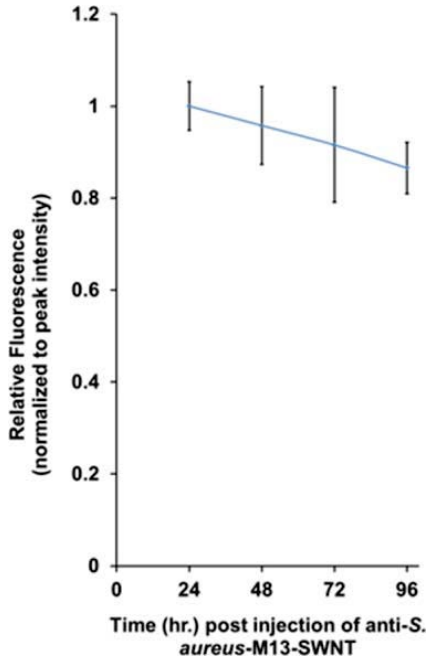


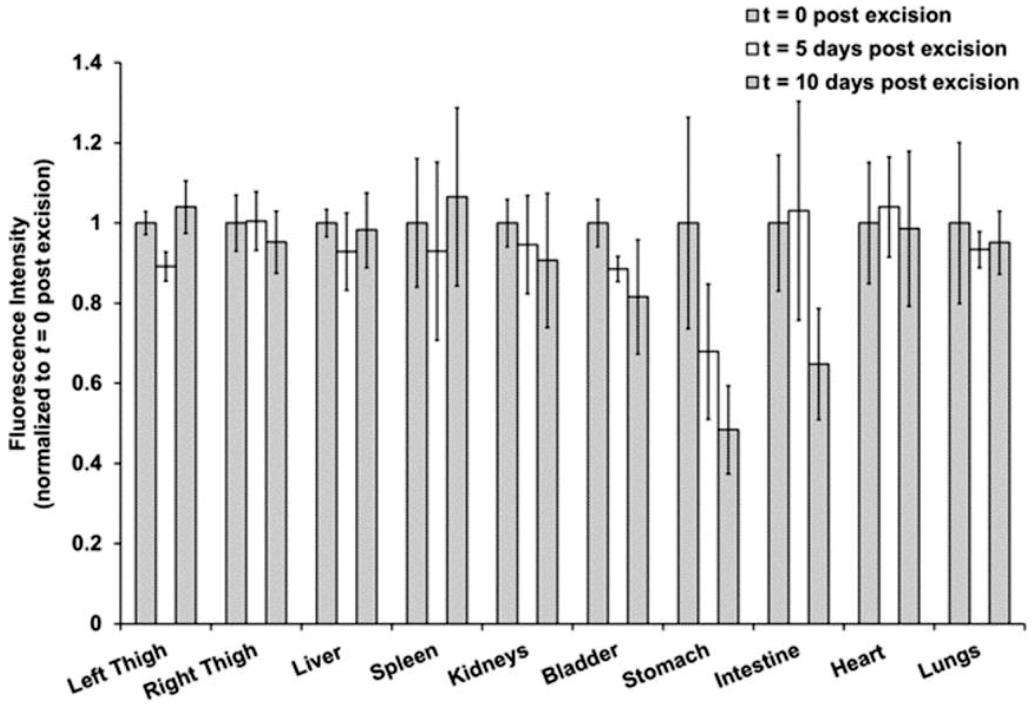
Fluorescence stability

- In vivo fluorescence signal decreases with time BUT :
- Over 96hrs SWNT probe is clearly distinguishable, with fluorescence signal ~85% of peak intensity.

Ex vivo:

- Most tissues retain up to >91% and >82% of their initial fluorescence signal intensity at *t* = 5 and 10 days respectively.
- Superior fluorescence stability of the SWNT probe compared to conventional dye fluorophores such as fluorescein derivatives (fluorescence half-lives ~10s of seconds)



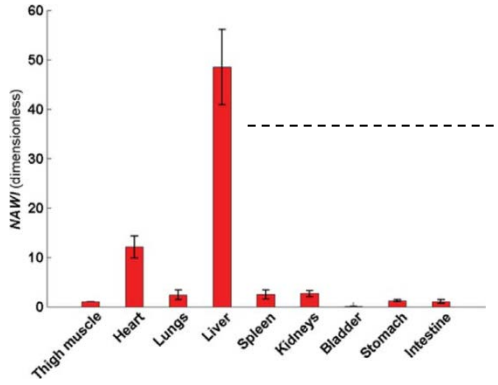


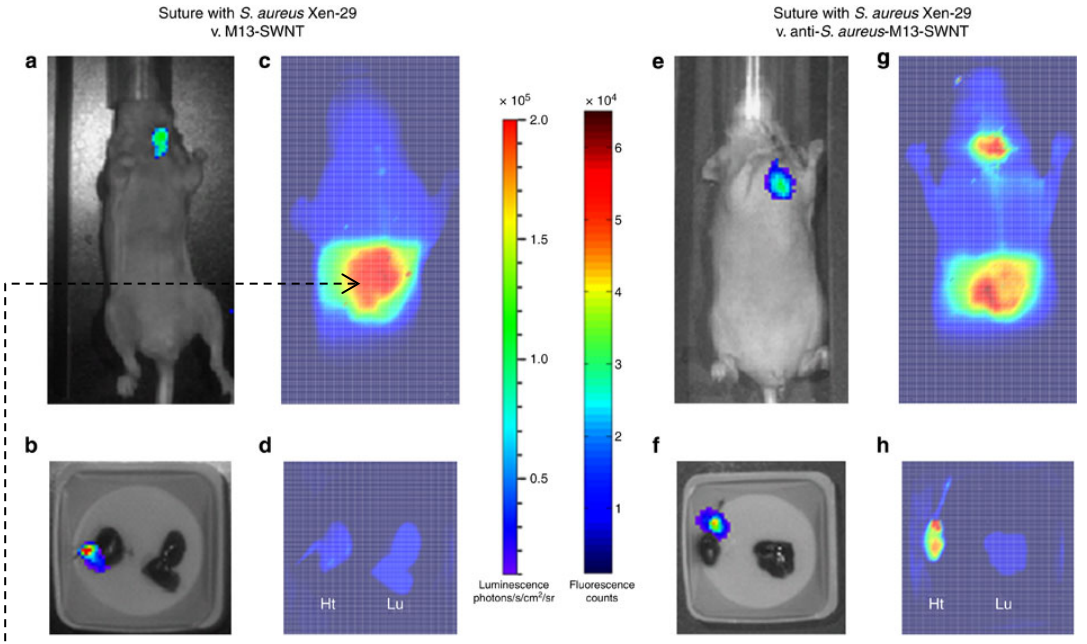
Deep-tissue in vivo imaging of bacterial endocarditis

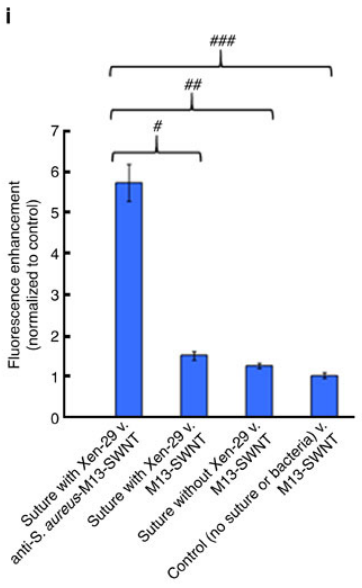
- Established model of S. aureus endocarditis
- Bioluminescence from Xen-29 S. aureus strain vs. NIR-II fluorescence from SWNT probe

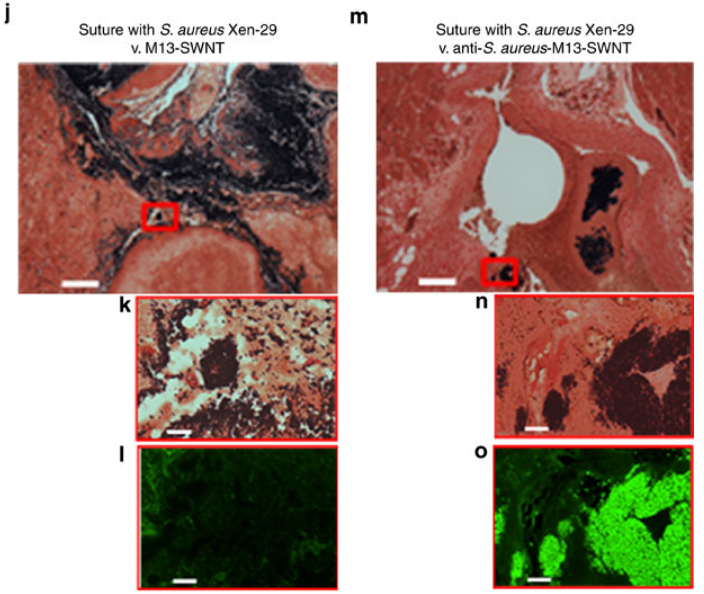
With targeted probe:

- Highly specific detection of deep-tissue infection
- 3.8x enhancement in the fluorescence intensity over the nonspecific case
- Co-localization of ex vivo fluorescence signal from <u>targeted probe</u> with gram-staining









Summary

New method using tunable **SWNT probes for in vivo targeting and fluorescence optical imaging of bacterial infections:**

- Biologically functionalized whilst retaining high NIR-photoluminescence
  - Engineer M13 for detection of a wide range of pathogens
  - Low dose achieving high contrast detection for minimizing patient exposure

- SWNTs probes as a non-ionizing, relatively less expensive alternative NIR-II imaging modality for non-invasive detection and monitoring of infectious diseases in the body

Drawbacks:

- Pharmacokinetics & biodistribution
- Strong signal in liver: may hinder imaging

nature nanotechnology

ARTICLES

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In vivo biosensing via tissue-localizable near-infrared-fluorescent single-walled carbon nanotubes

Nicole M. Iverson[1,2], Paul W. Barone[1], Mia Shandell[1], Laura J. Trudel[2], Selda Sen[1,3], Fatih Sen[1,4], Vsevolod Ivanov[2], Esha Atolia[2], Edgardo Farias[2], Thomas P. McNicholas[1], Nigel Reuel[1], Nicola M. A. Parry[5], Gerald N. Wogan[2] and Michael S. Strano[1]*

Introduction

- Current technology allows for in vivo NO detection using an electrochemical probe surgically implanted in a rat's brain

  --> Does not permit longterm or non-invasive NO detection

Aim: functionalized tissue localizable SWNTs as sensors of inflammation in vivo, using NO as a marker

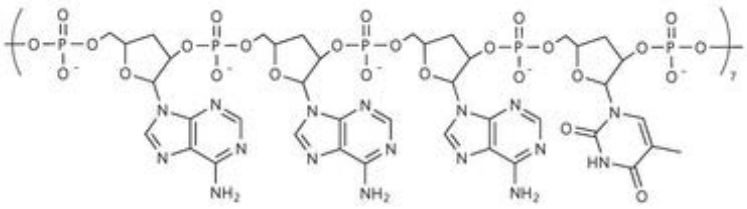
Strategy: Conjugation of SWNTs with ssDNA molecule for selective binding to NO

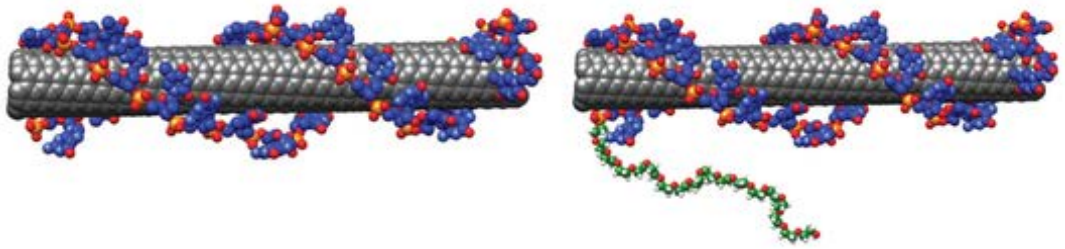
ssDNA functionalization of SWNTs for inflammation sensing

- Model molecule: Nitric oxide (NO), produced during inflammation
  - Steady-state concentration of NO in tissues
  - Biologically relevant concentrations ranging over three orders of magnitude (~1nM-500nM)

- SWNTs have demonstrated single-molecule sensitivity (in vitro) and can be functionalized to selectively detect a variety of molecules
  - Certain DNA sequences attached to SWNT have selective NO binding abilities in vitro

→ Functionalization: ssDNA oligonucleotide $d(AAAT)_7$ conjugated with PEG segment (*PEG-DNA*)
  - ssDNA for selective molecular recognition
  - PEGylation for biocompatibility and stability
- Optical signal transduction: NO binding perturbs nanotube electronic structure → Fluorescence quenching

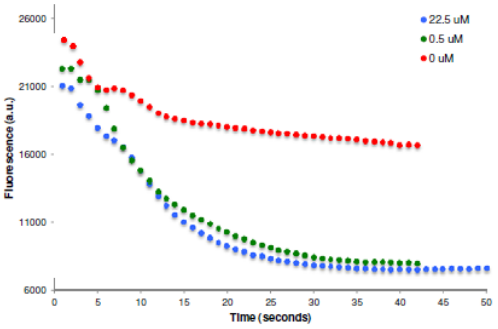


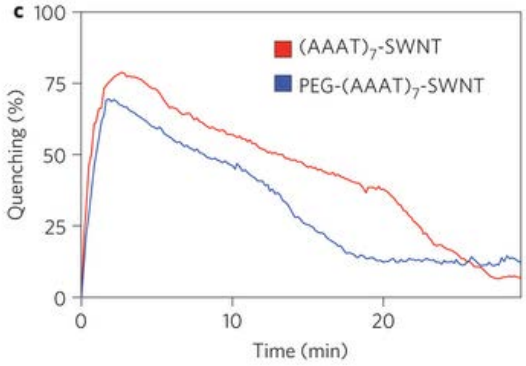


PEGylation of DNA-SWNT critical for i.v. stability

In vitro:

- Sensitivity: NO detection limit < 1uM
- Rapid fluorescence quenching





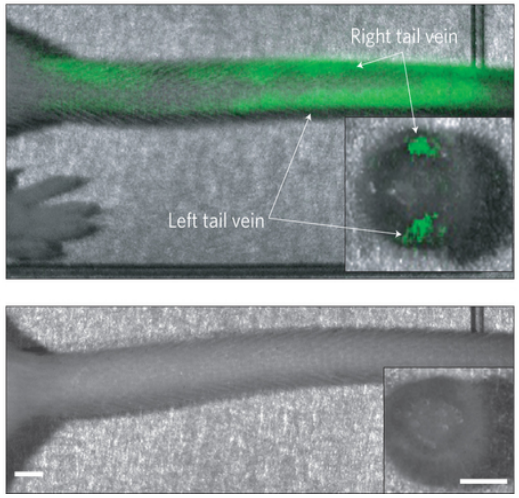
In vivo (*tail vein injection*):

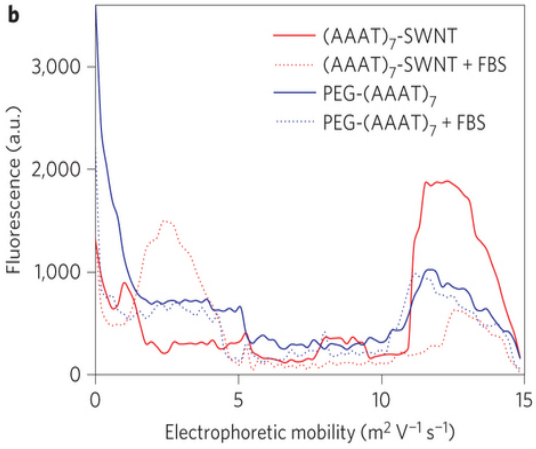
- Vein occlusion due to aggregates of serum proteins adsorption to *DNA-SWNTs*

- PEG ligation to *DNA-SWNTs* critical for in vivo circulation
- PEG moiety prevents adsorption

DNA-SWNT injected = accumulation

PEG-DNA-SWNT injected= clearance

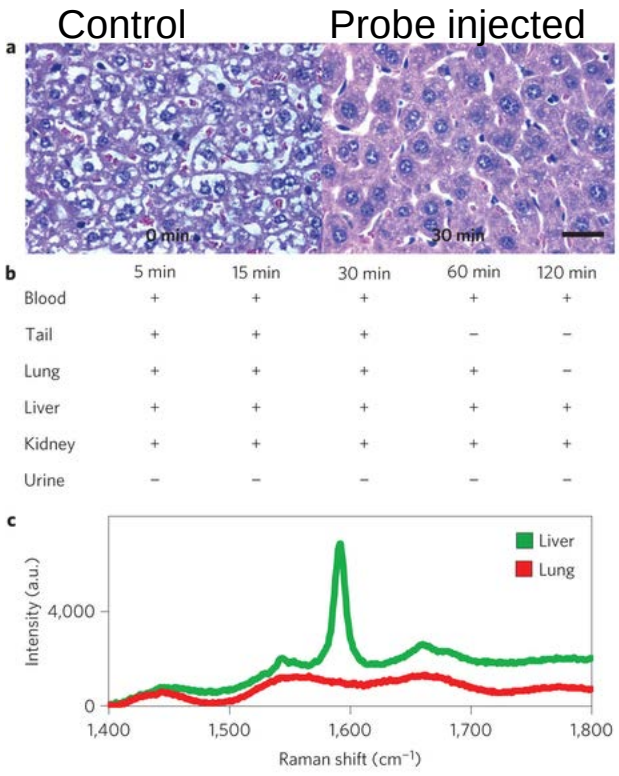




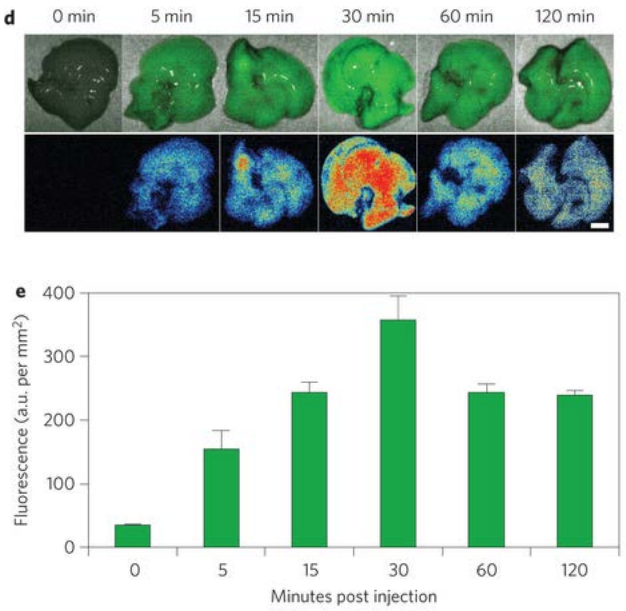
In vivo: Circulation time & Biodistribution

- Biocompatibility: Staining shows lack of inflammatory cell recruitment
- SWNTs remain in vivo for at least 2hrs
- Accumulation in liver (peak after 30mins, then constant)
- SWNTs detectable in lung 5 mins after injection & cleared within 2 hrs
  - PEG-DNA-SWNTs can penetrate restrictive capillary networks without causing occlusion

→ biocompatibility of PEG-DNA-SWNTs

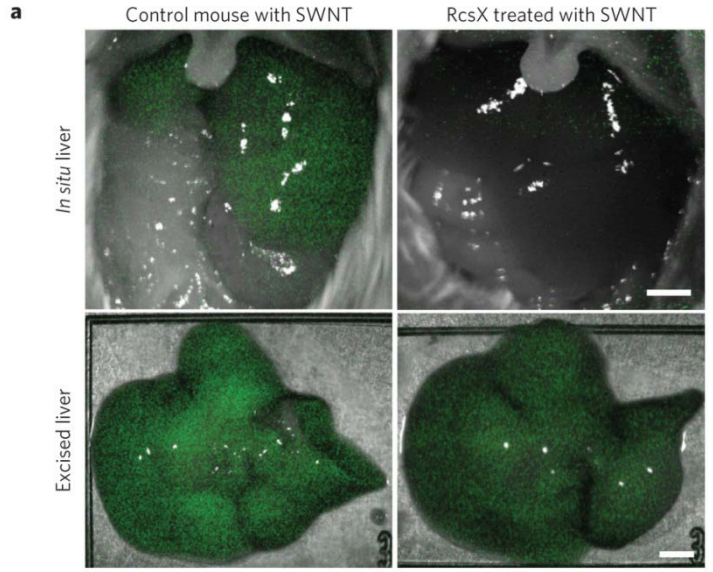


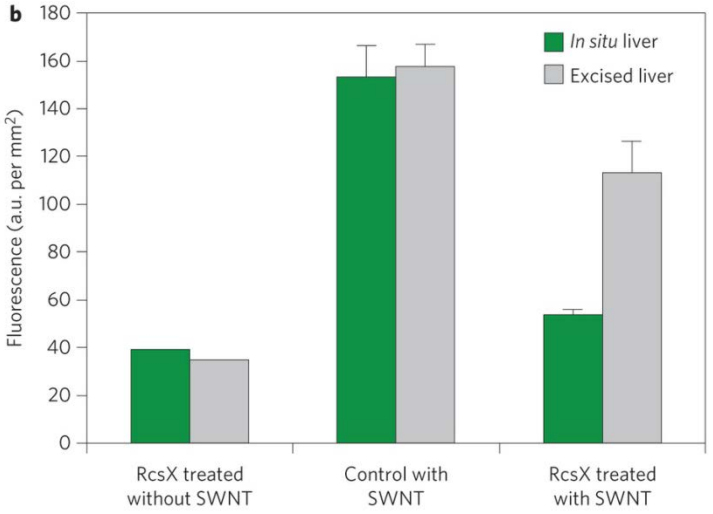
Fluorescence of PEG-DNA-SWNTs in mouse livers



Diagnosis of inflammation in mice using PEG-DNA-SWNT sensors

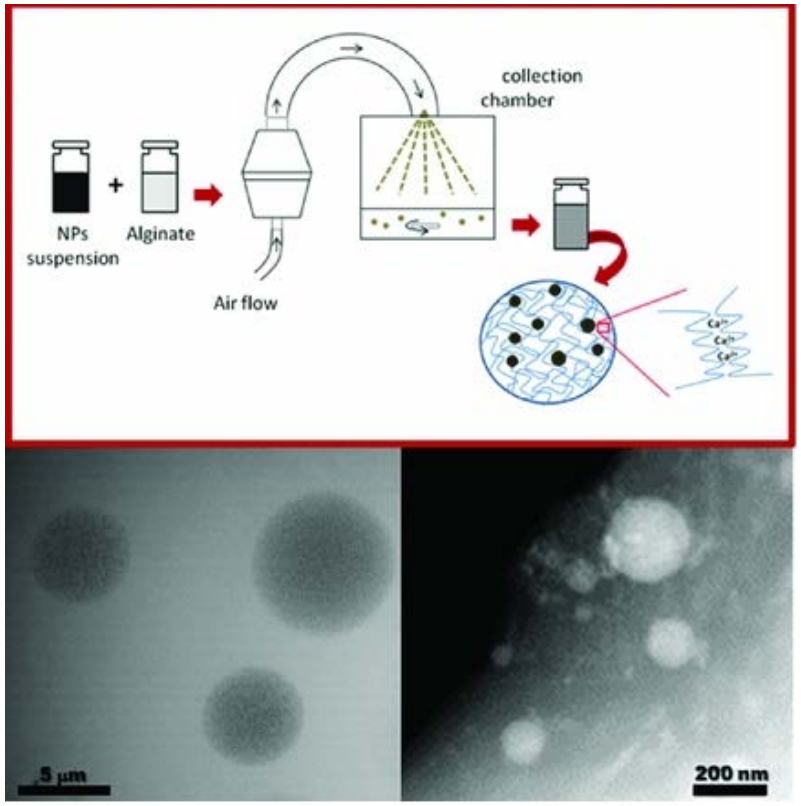
- ***SJL mouse model****: inject tumor cells → intense inflammatory response → massive overproduction of NO over a predictable time course*
- Absence of fluorescence in **in-situ** images due to NO production during inflammation
- Need to make incision in abdominal cavity

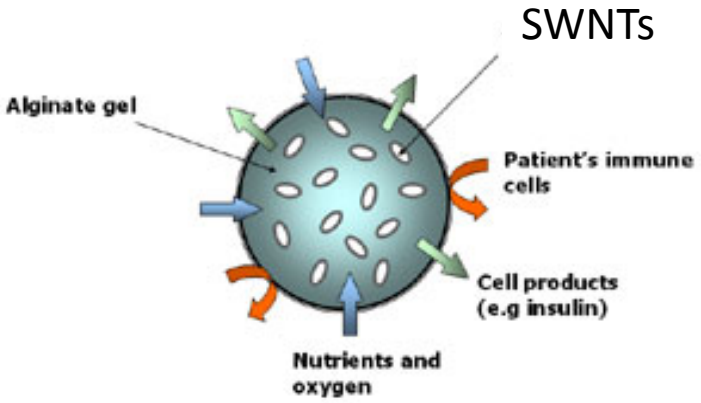




Encapsulation of DNA-SWNTs for tissue-specific localization

- Alignate is a natural biocompatible and biodegradable polymer
- Encapsulation of DNA-SWNT sensor in alignate-hydrogel

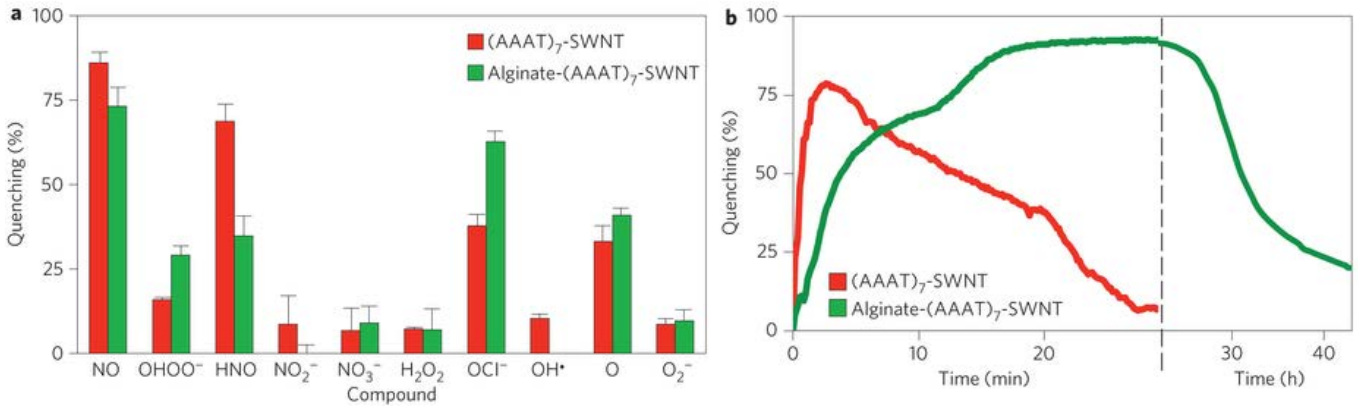




Epidermal tissue inflammation monitoring

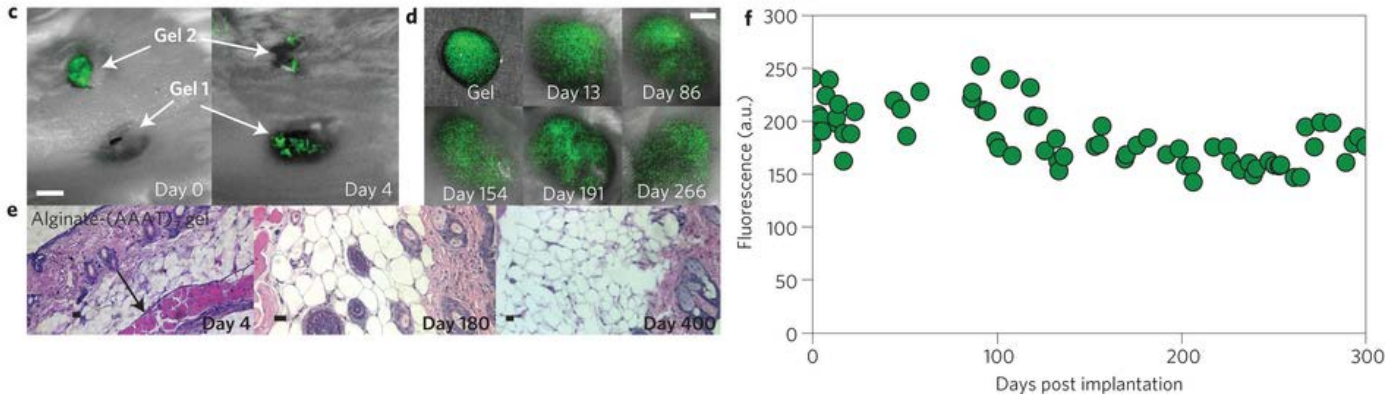
In vitro:

- *Alignate-DNA-SWNT* sensor retains NO specificity
  - Dynamic responses of encapsulated sensor to NO slower



In vivo:

- SubQ implantation of alignate-encapsulated DNA-SWNTs for long-term NO-sensing
- Total signal quenching after 20mins → *quenching by burst of NO in wound bed*
- In longitudinal implant study, gel remains intact and the signal is largely invariant (14% variability in intensity)



Summary

- PEG-DNA conjugation of SWNTs results in an biocompatible, stable probe for sensitive in vivo sensing of NO
- Injectable SWNT-sensor allows in situ inflammation imaging in mice liver
  - Half-life for liver retention: 4hrs; lung clearing: < 2 hrs
- Implantable SWNT-sensor holds potential as minimally invasive longitudinal inflammation monitor
  - No intrinsic inflammation or adverse responses detectable for >400 days

Limitations & outlook:

- Need to expose liver for in situ imaging → tuning of functionalization to overcome this
- More detailed pharmacokinetic studies (accumulation in liver)

Conclusion: SWNTs as in vivo biosensors

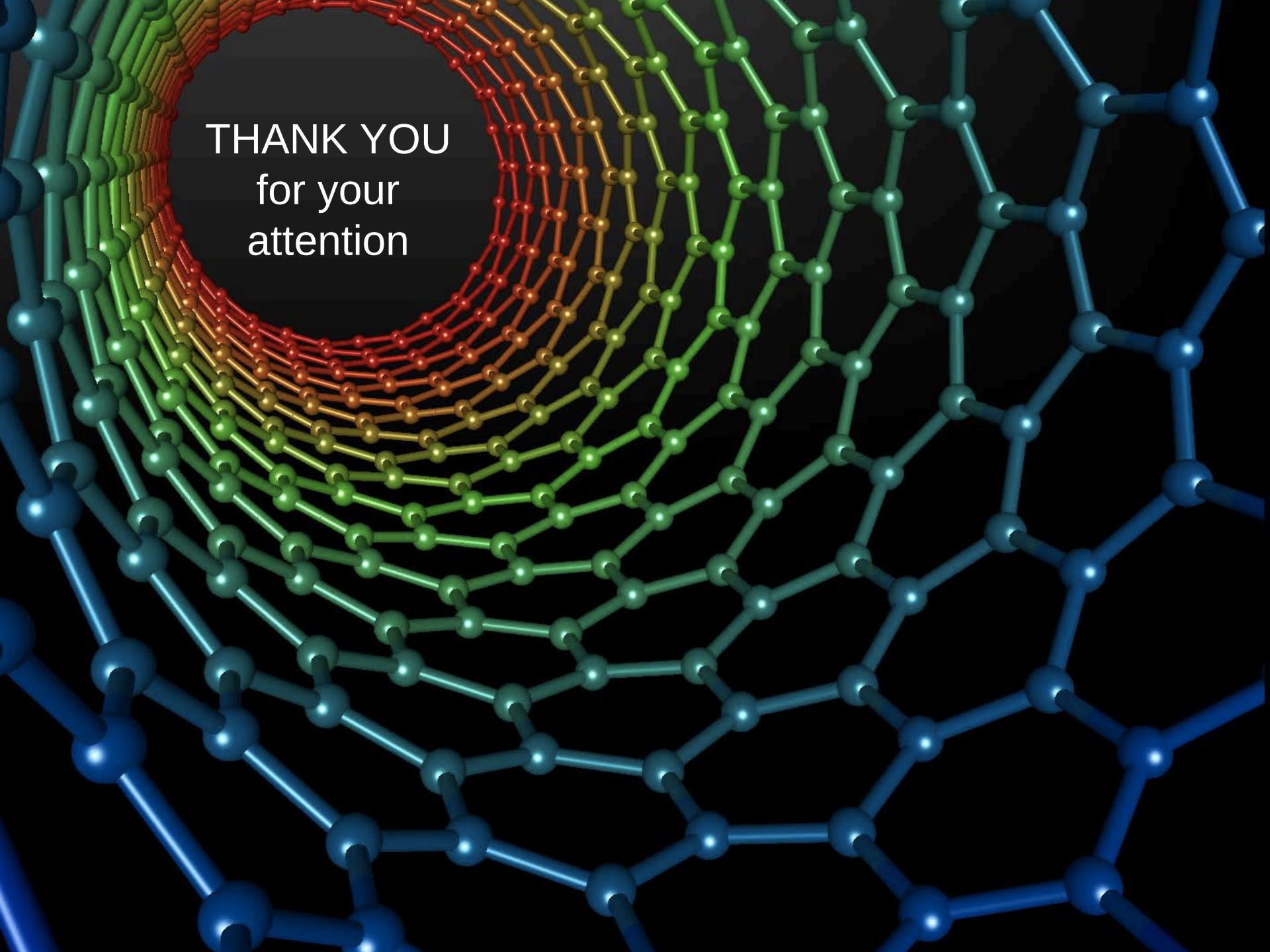
- Unique physical properties of CNTs allow for direct detection in vivo
- Functionalization of CNTs gives rise to a highly variable and selective, biocompatible probes

Issues

- SWNT inherently insoluble and toxic, need to be functionalized and require extensive biocompatibility and toxicity tests
- Pharmacokinetics & biodistribution: safety for use in humans

Outlook:

- Develop new specialized functionalizations of SWNT to detect new targets
- SWNT sensors loaded with, for example, drugs for “on site response” to analyte detection


THANK YOU
for your
attention