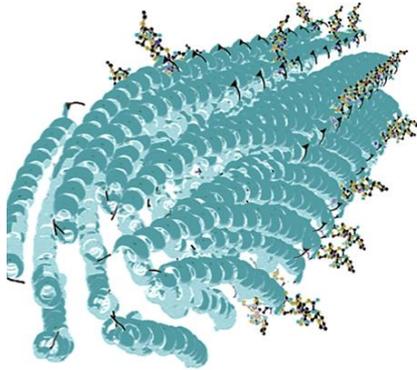




ENGINEERING M13 BACTERIOPHAGE NIR-II PLATFORMS FOR TUMOR IMAGING APPLICATIONS



UYANGA TSEDEV

BIOMOLECULAR MATERIALS GROUP

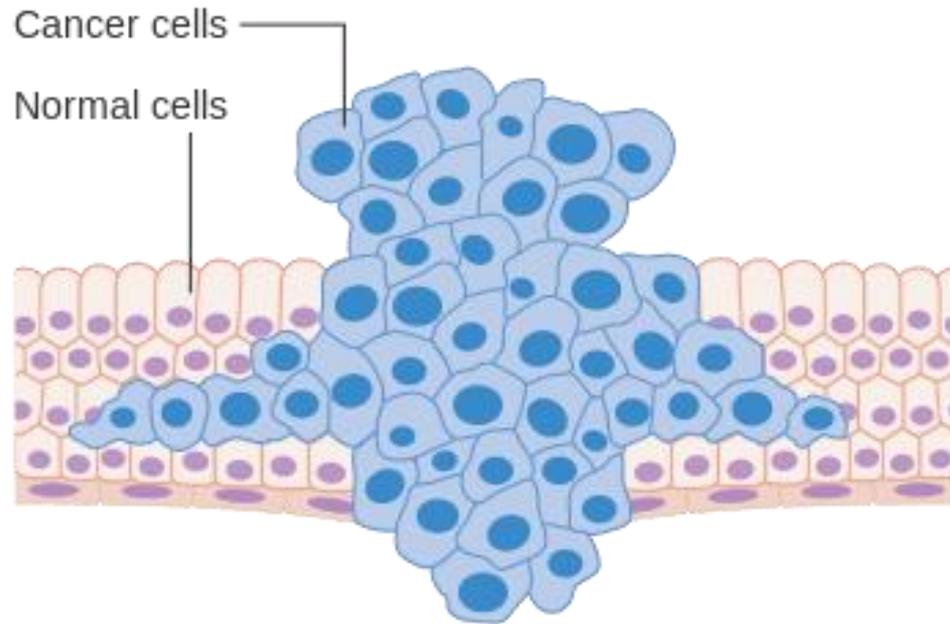
PI: ANGELA BELCHER

GIRLS WHO BUILD

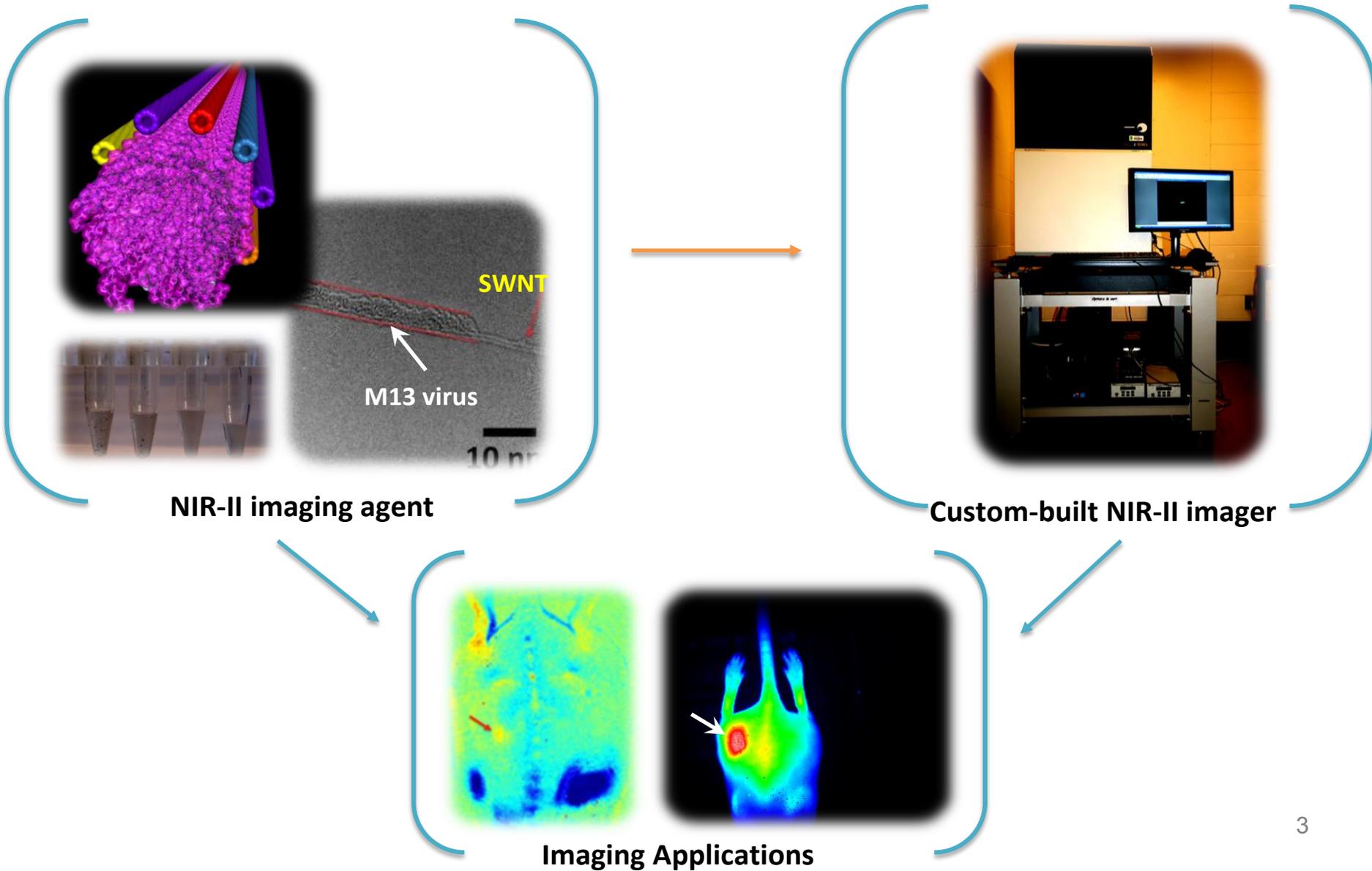
MIT LINCOLN LABORATORY

JUNE 4, 2016

Finding Tumors



Our Tools



Probe: M13 Bacteriophage

Figure . Structure of the M13 bacteriophage

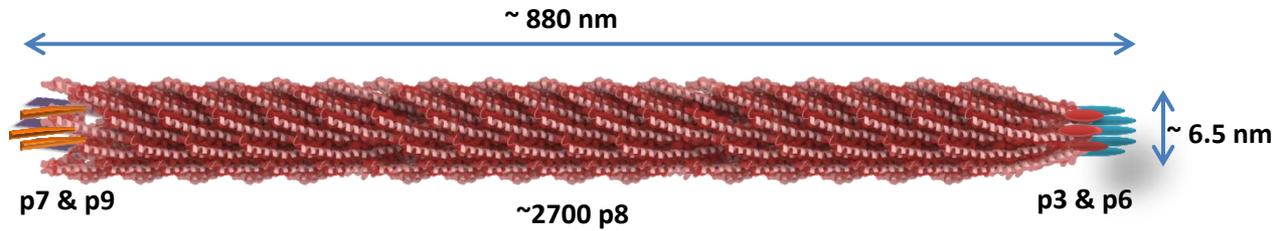


Figure . AFM Image of M13 bacteriophage

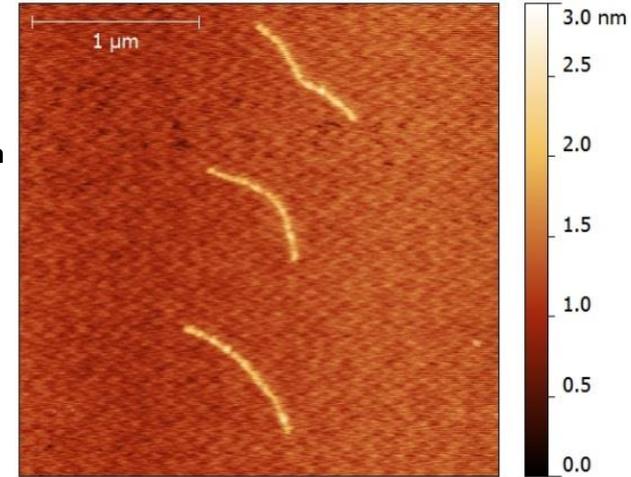
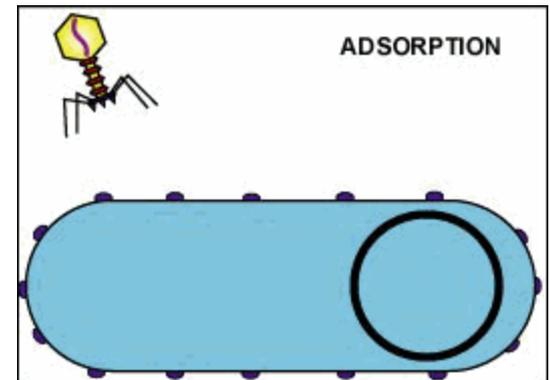
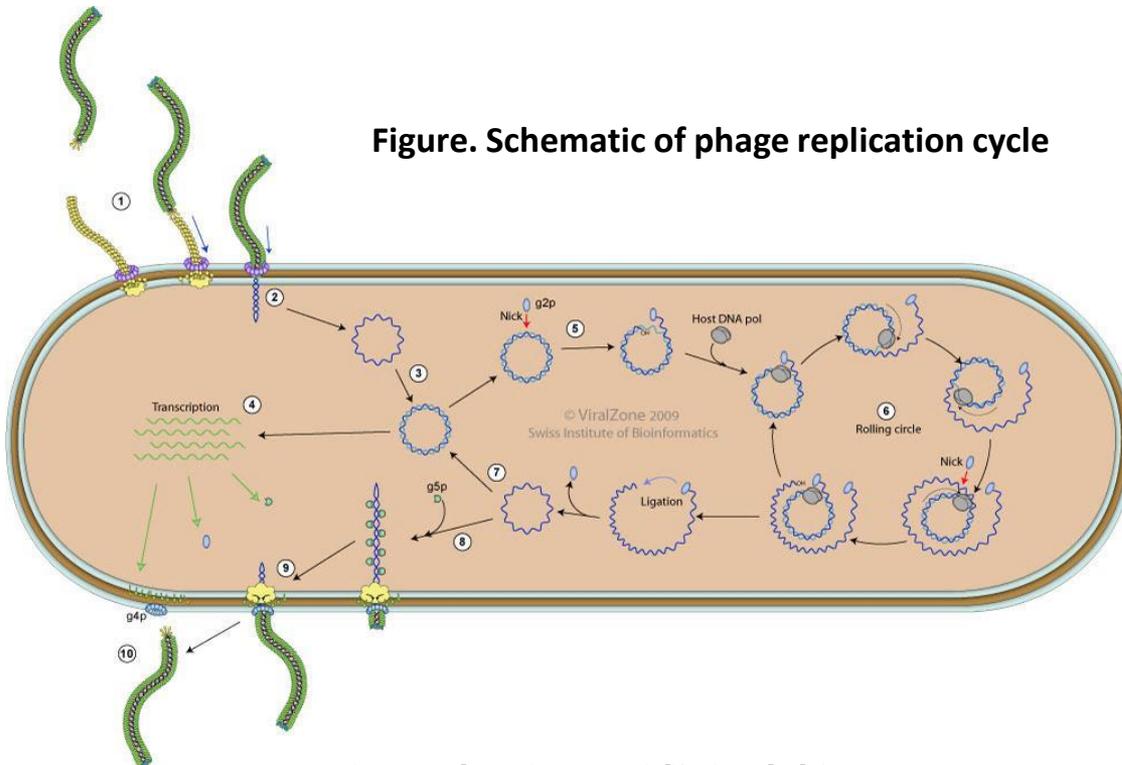
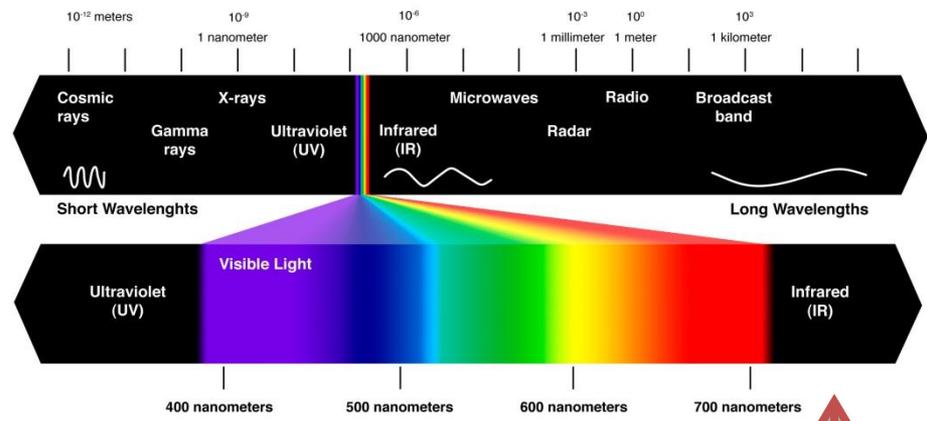
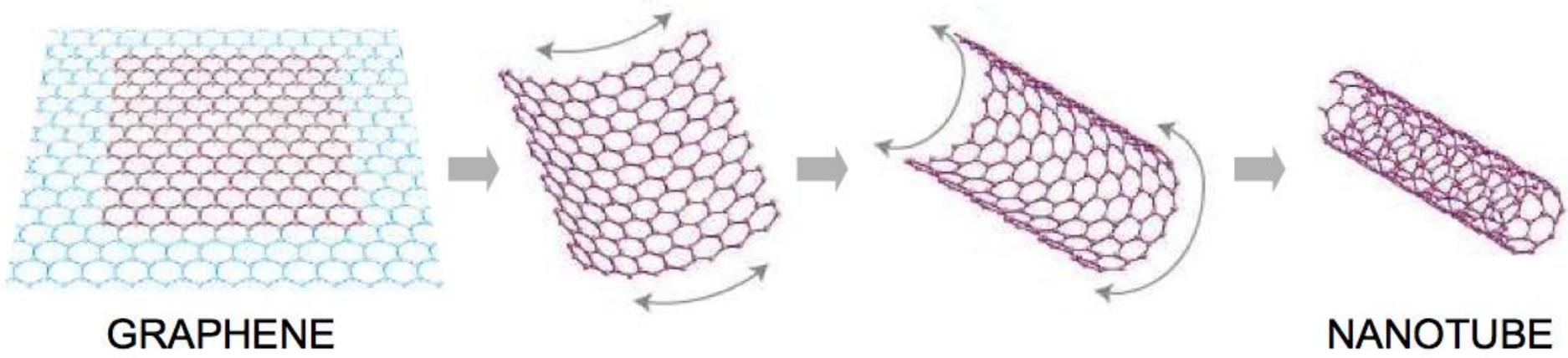


Figure. Schematic of phage replication cycle



7ci fhYgmcZJ]fU'NcbY""I gYX'k]h' dYfa]gg]cb"

Probe: Second Window Near-Infrared NanoTube



Our wavelengths of interest

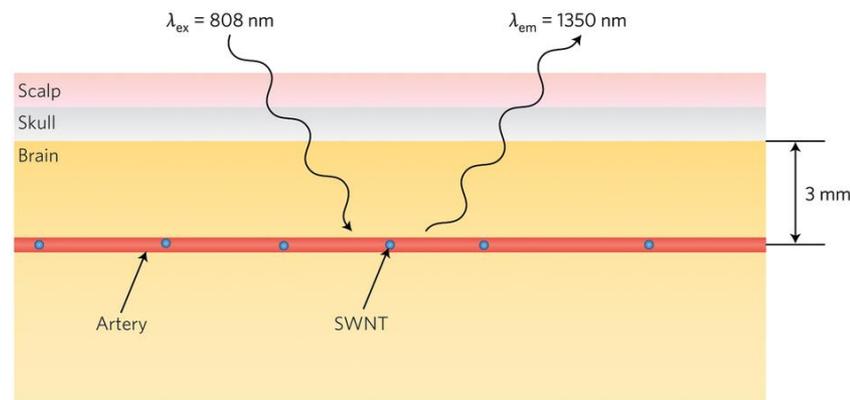
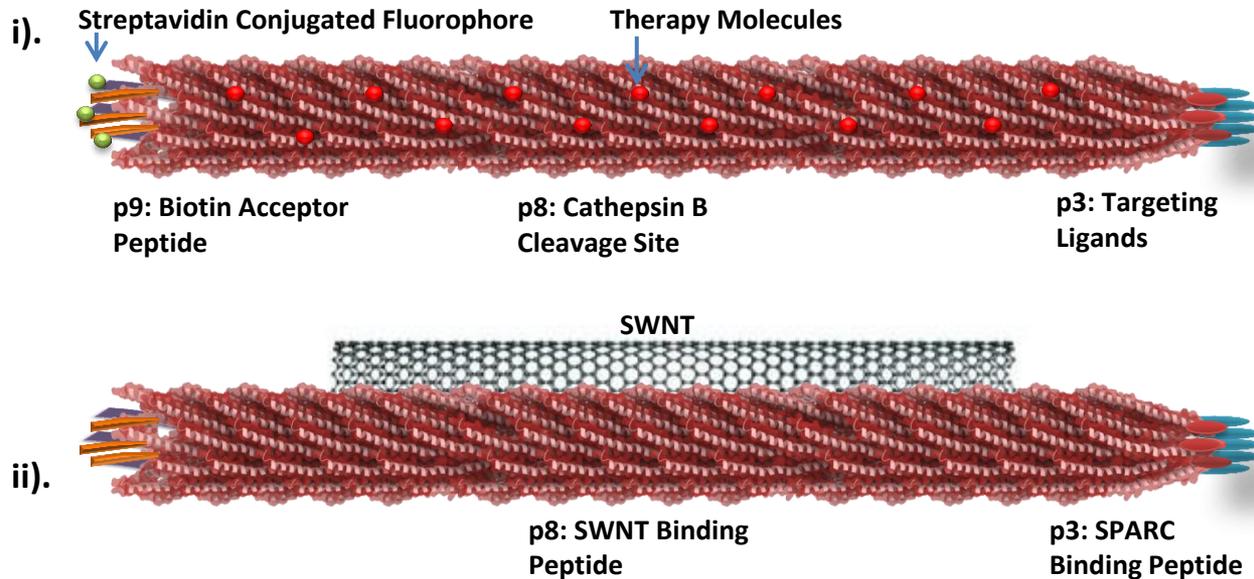


Figure. Schematic of SWNT fluorescence through brain tissue

7ci fhYgmicZBUhi fY'D\cncb]Vg"l gYX`k Jh`dYfa]gg]cb"

Probe: M13 Bacteriophage

Figure . M13 bacteriophage designs. i). Tumor targeting at p3 end, fluorescent imaging agent at p9, and doxorubicin/drug release sites at p8. ii). Tumor targeting at p3 end and single walled carbon nanotube (SWNT—a deep tissue, near infrared imaging agent) binding at p8.



- Biocompatibility
- Extended blood circulation time, accumulation in diseased tissue
- Successful targeting to sites of biomedical interest

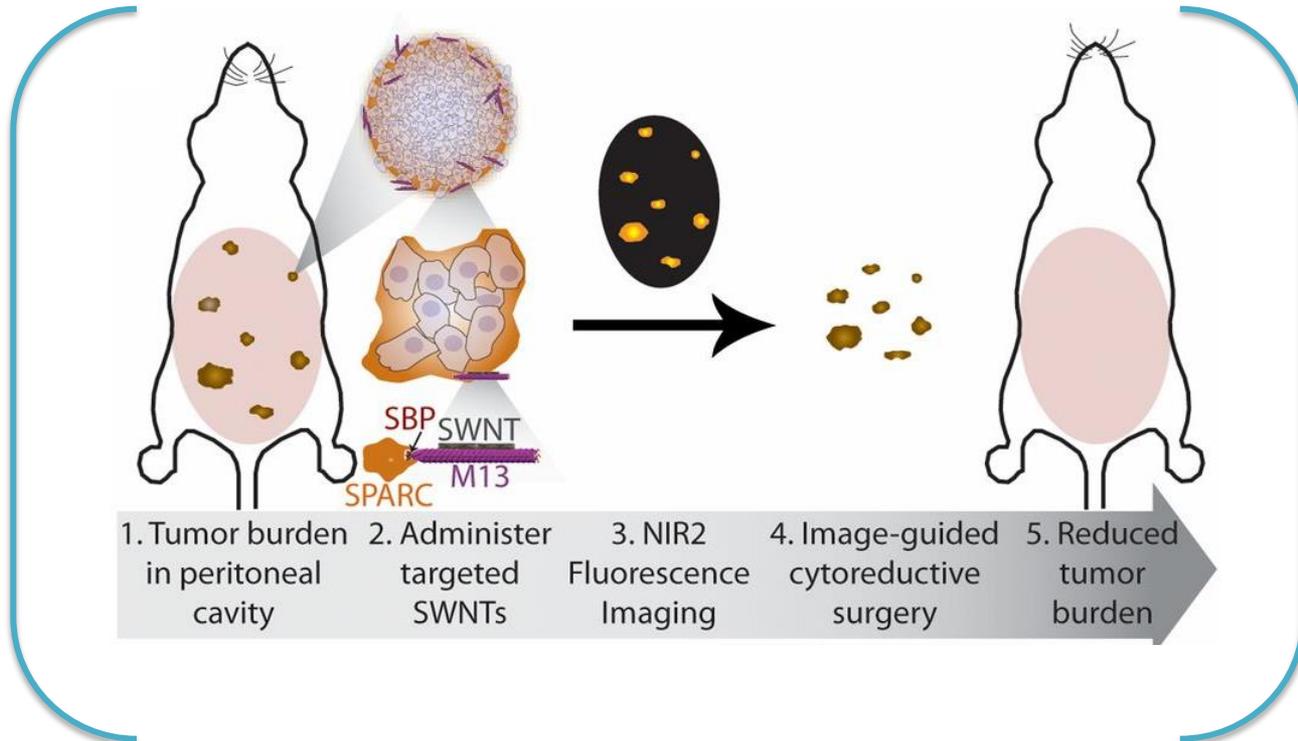
Real Time Intraoperative NIR-II Imaging System

Ovarian Cancer Model

~225,000 women diagnosed annually

~140,000 death toll

- Early diagnosis is key, before metastatic stage
- Thorough therapy, small tumor detection prolong survival



7ci fhYgmicZBUhcbU`5WUXYa mcZGVWYbWgZl`"G"5"l gYX`k]h`dYfa]gg]cb"

Gci fW. ; \cg\z8YVUXmi h]z`5`Yl UbXYf: ""6U[`Ynz`Mci b[`>Ycb[`BUz`A]WkUY`>""6]ffYfz`GUb[YYHU`B""6\Uh]Uz`UbX`5b[`Y`U`A " 6Y`WkYf""8YYdz`bcb]bj Ugjj `Y`ja U[]b[`UbX`gi f[]W`[i]XUbW`cZgi Va]`ja YHf`hi a cfg`i g]b[`hUf[YHYX`A % !ghUV]]nYX g]b[`Y!k U`YX`WfVcb`bUbch VYg""DfcWYX]b[g`cZ`h `Y`BUh]cbU`5WUXYa mcZGVWYbWg`%`%`z`bc"" , `f&\$%(`L`%`-`(`,`!`%`-)`"

Gen-1 Application: Image-Guided Surgical Planning

SWNT NIR-II image guidance (pre-surgical planning): helps in better excision of sub-mm tumors

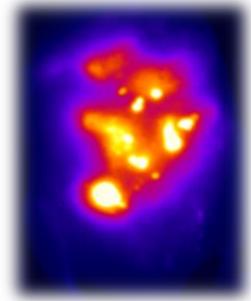
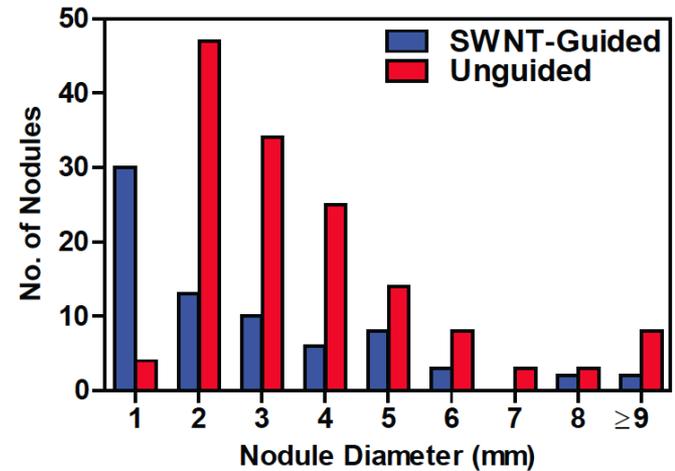


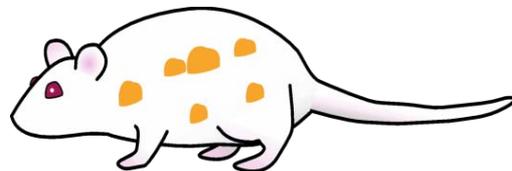
Image-guided surgery

Measure tumor nodules

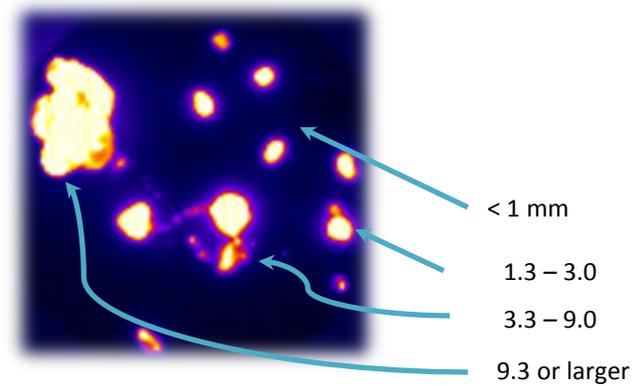


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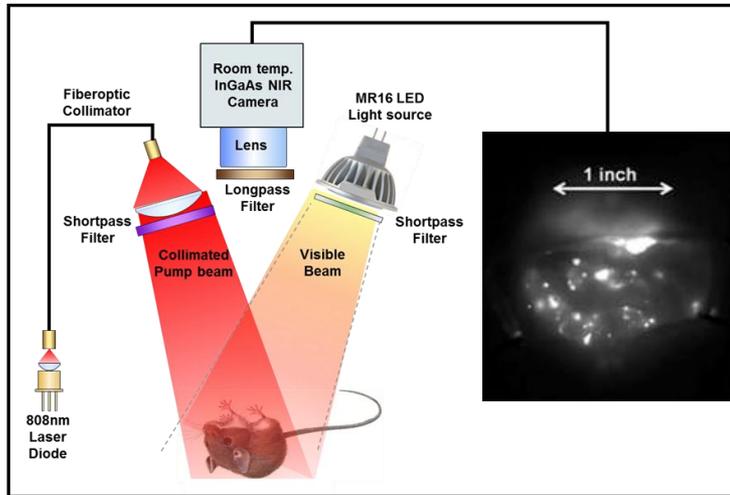
Randomize tumor cohort



Unguided surgery



Gen-2 Application: Real Time Intraoperative NIR-II Imaging System

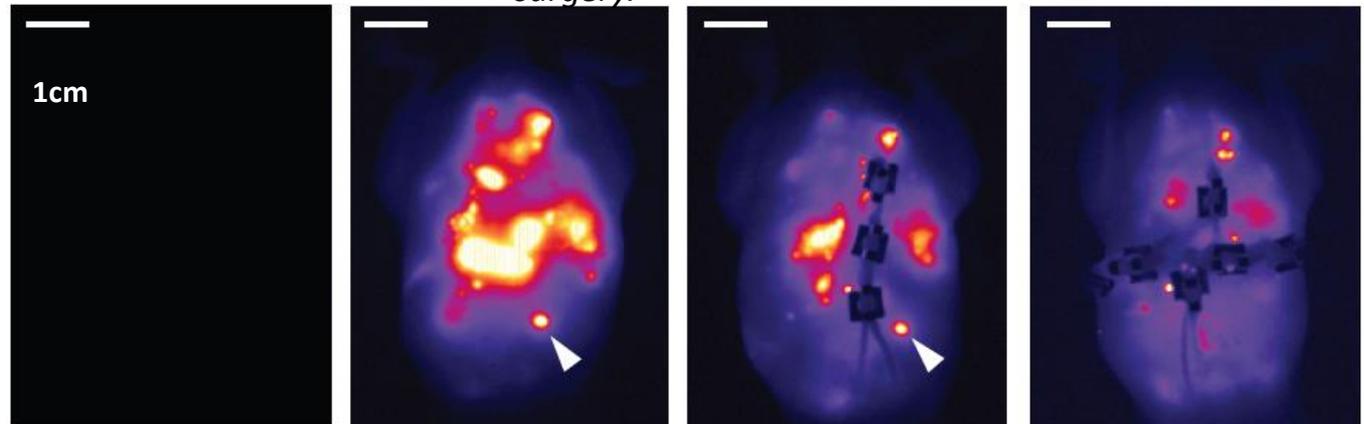


*In collaboration with MIT Lincoln Laboratory
Image courtesy of Dr. Andrew Siegel, Lincoln Lab*



Massachusetts General Hospital surgeon, Dr. Na, demonstrates the NIR-II imaging system, where camera detects probes attached to tumors, and displays their "glow" on a monitor as he performs surgery.

**Figure . Serial
cytoreduction of ovarian
tumor at sub-millimeter
resolution (scale—1cm)
i) Pre-injection
ii) Re-surgery
iii) Post unguided surgery
iv) Post SWNT-M13
guided surgery**

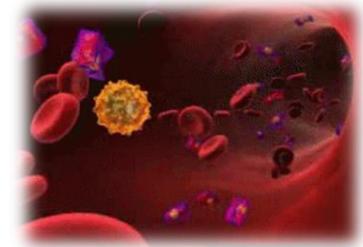


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6Y`W`Yf`"8Y`Ydž`bcb]bj Ugj] Y`ja U[]b[`UbX`gi f[]W`[i]XUbW`cZgi Va]`ja YHf`hi a cfg`i g]b[`Hf[YHXA`A`% !ghUV]`nYX
g]b[`Y!k U`YX`WfVcb`bUbchi VYg`"DfcWYX]b[g`cZh`Y`BUh]cbU`5WUXYa mcZGWYbWg`%`%ž`bc`" , `f&\$%(Ł`% -(, !% -)`" 9

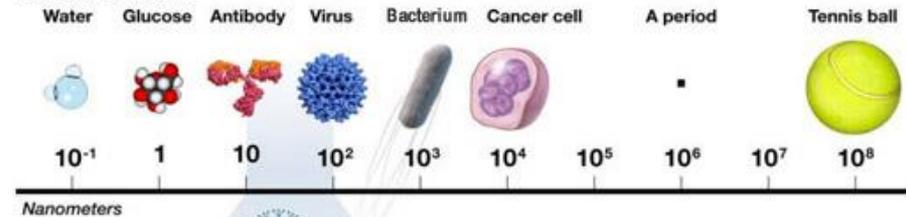
Reach Hard-to-Detect Tumors

Project 1. Small {Inho} Phage

Construction of smaller phage to improve on the blood trafficking of our M13 probe systems while retaining its multi-functionality which allows us to simultaneously target, detect, and deliver various agents to cancer masses.



How Small Is Small?



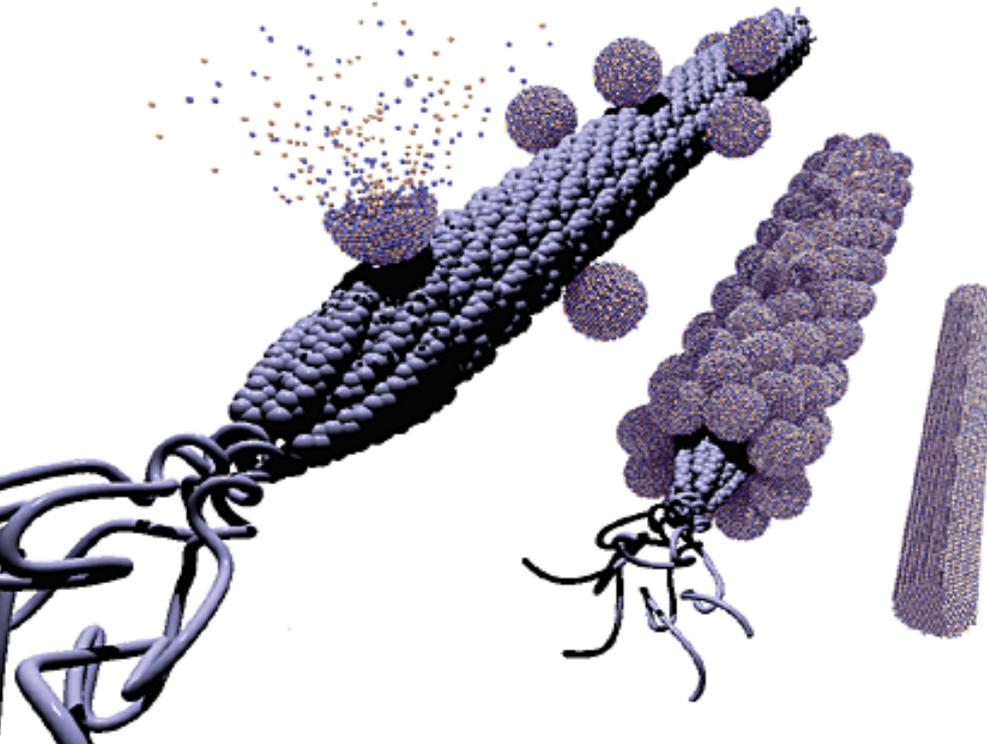
Nanometers



Nanodevices
Nanopores
Dendrimers
Nanotubes
Quantum dots
Nanoshells

Project 2. Glioma Phage

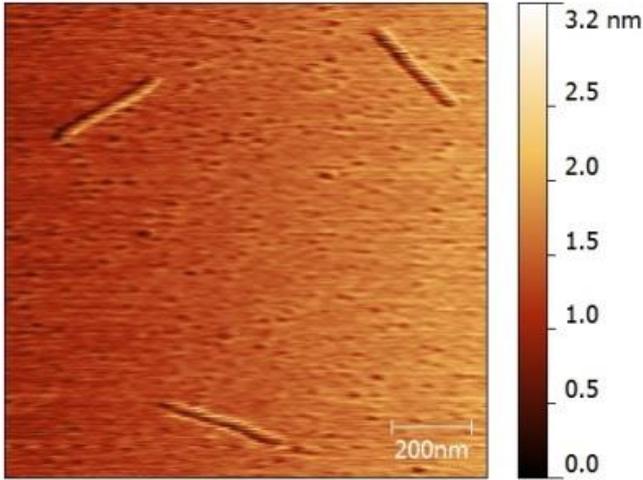
Cloning for peptide display on the tail p3 capsid protein of M13 to allow for passage across the blood-brain barrier and targeting to glioma cells.



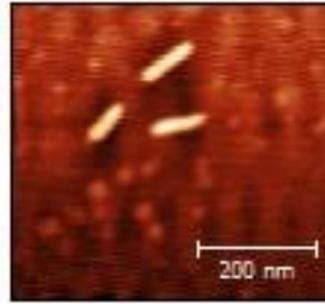
M13 Small {Inho} Phage

Figure . Atomic force microscopy inho1960, 475, 285 images

A) Inho1960

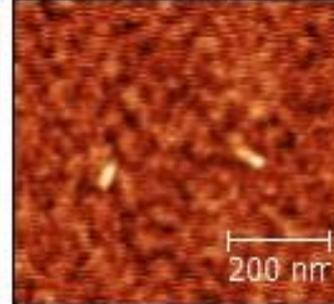


B) Inho475



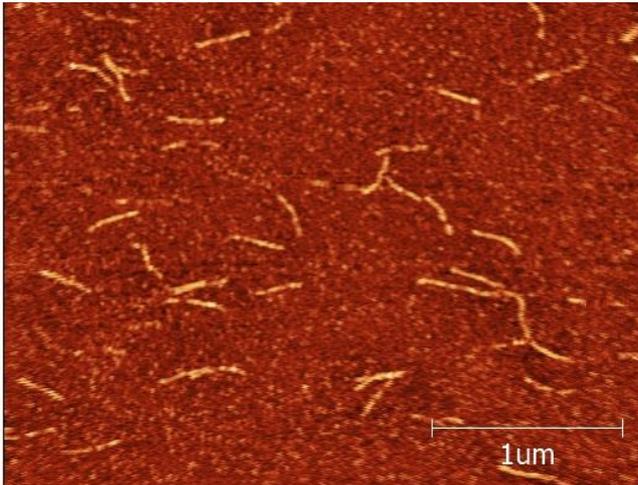
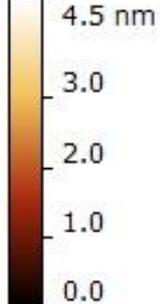
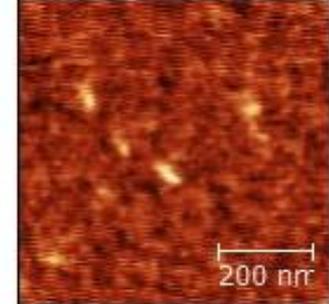
BASE SIZE ~100nm

C) Inho285



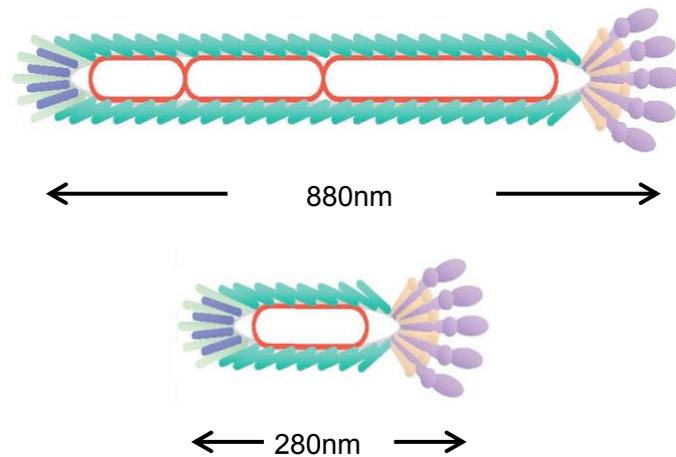
BASE SIZE ~50nm

Inho285



BASE SIZE ~280nm

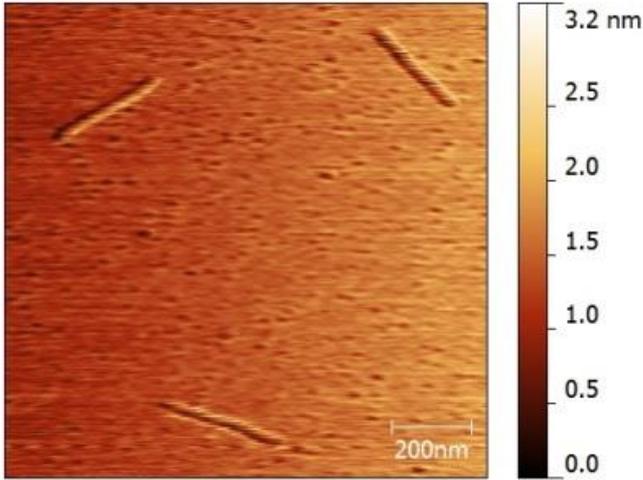
D)



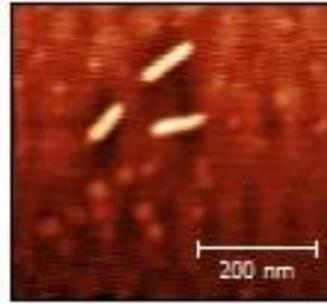
M13 Small {Inho} Phage

Figure . Atomic force microscopy inho1960, 475, 285 images

A) Inho1960

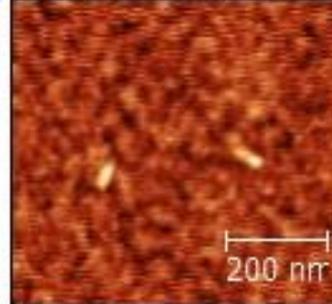


B) Inho475



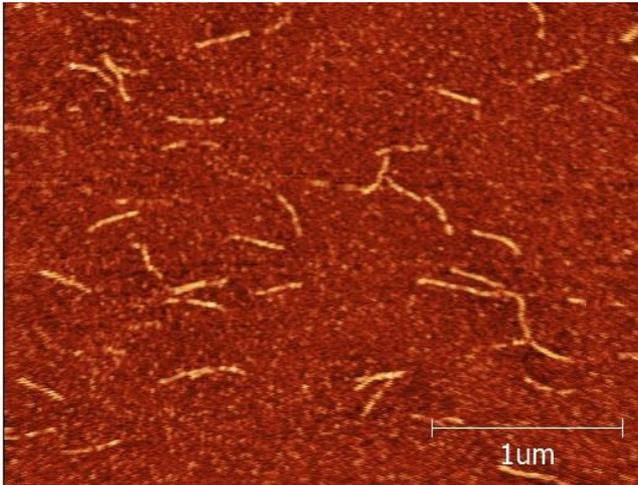
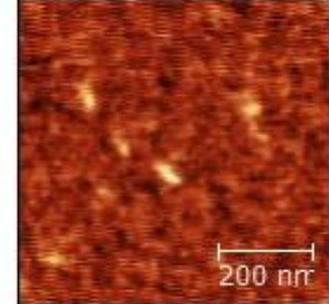
BASE SIZE ~100nm

C) Inho285



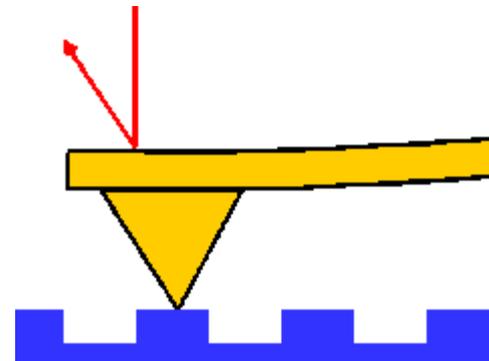
BASE SIZE ~50nm

Inho285



BASE SIZE ~280nm

D)



Glioma Targeted Phage Project

Functionalization of M13 phage to cross the blood-brain barrier

- Expand phage probes for usage with our NIR-II deep imager (10cm depths)
- Utilize the internalization of phage and localization near the nucleus to consider gene therapies (siRNA)

Figure. M13 phage shuttles localizes to the brain tumor mass.

Phage shuttles (carrying red dye) have selectively gathered at the brain tumor site (expressing green dye).

GFP – Tumor

Cy3 – Phage

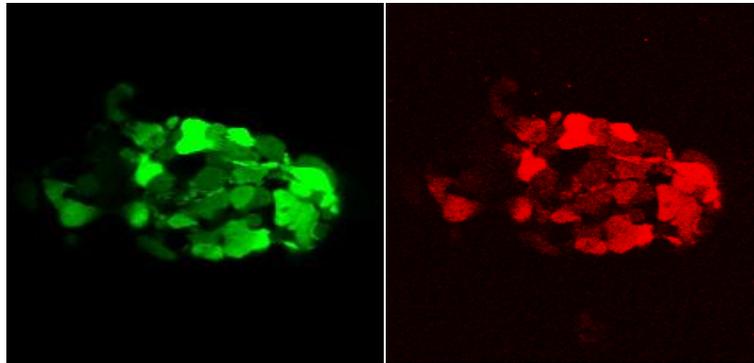
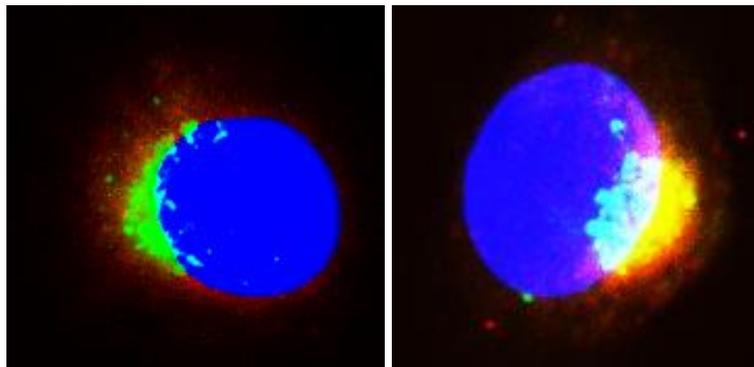


Figure. Phage Internalization. Internal cellular localization of glioma targeting phage (red) in relation to Golgin-97 (green) and the nucleus (blue) in human glioma cell line U87MG

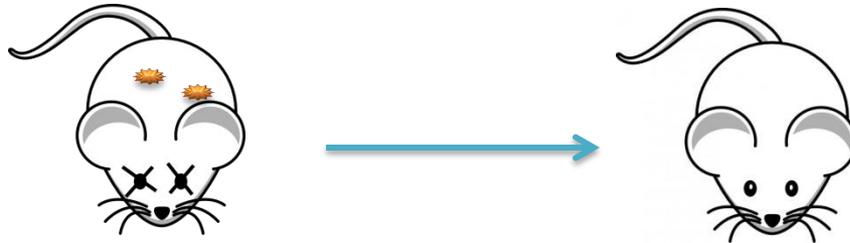
6hrs post incubation

24hrs post incubation



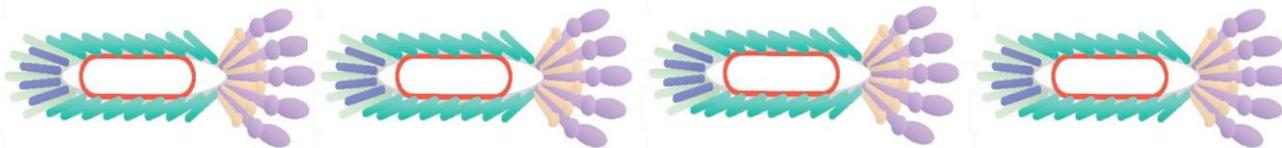
In Summary

Demonstrated the medical impact of tumor de-bulking surgery guided by NIR-II imaging and targeted M13-CNT probes



Two new platforms for early detection and treatment of hard-to-reach tumors

1. Inho-phage retains the multi-functional structure of M13 bacteriophage and will allow us to explore the benefits of new smaller geometries in trafficking to and extravasation into tumors as well as various other materials applications.



2. Glioma targeted phage can induce passage across the blood-brain barrier, target brain tumor cells, and internalize to the golgi region of cancerous cells.

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- ❖ Dr. Briana Dun

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Lincoln Labs

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Commonly Used Imaging Modalities

Technique	Advantages	Disadvantages
MRI	Good Resolution; Good Penetration Depth	Costly; Long Imaging Time
CT	Penetration Depth	X-ray radiation; Only Used for Bone and Lungs
PET	Good Penetration Depth	Radioactive Material; Long Imaging Time; Expensive
Ultrasound	Good Resolution; Cost Effective	Poor Penetration (cm)
Near-Infrared (650-950 nm)	Low Tissue Absorption; Safe Light Source; multiplex imaging	Autofluorescence; scattering
Second Window Near-Infrared (950-1650 nm)	Long Wavelength; Penetration Depth (10 cm); Non-Radioactive; Inexpensive; Quick Imaging Time (seconds-min)	Requires Sensitive Equipment for Detection

Adapted from Weissleder and Pittet, Nature 452 (2008)

Resource: Girls Who Build Cameras

Kristen Railey, Bob Schulein, Olivia Glennon, Leslie Watkins, Alex Lorman, Carol Carveth, Sara James

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