Cancer TNT
Cancer Translational Nanotechnology Training

Raman Endoscopy for Delineation of Non-Muscle-Invasive Bladder Cancer

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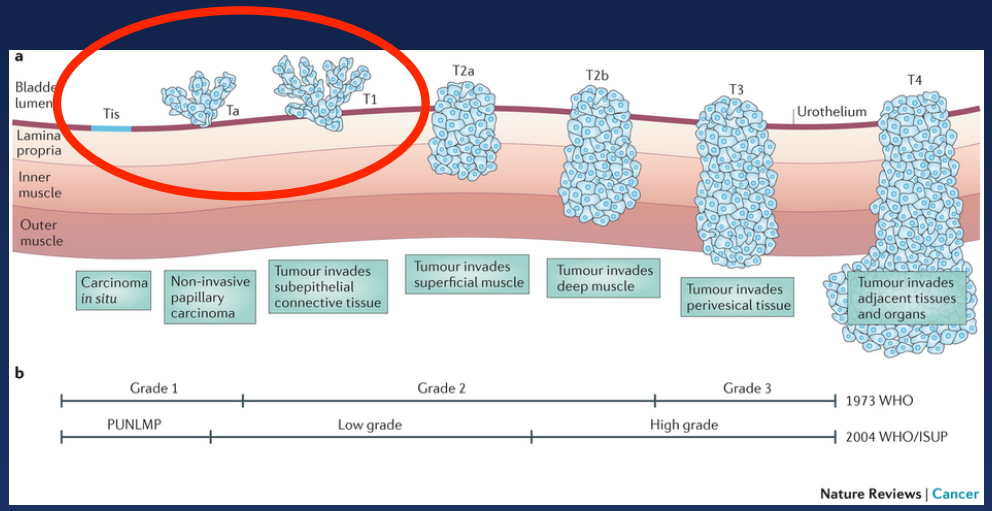
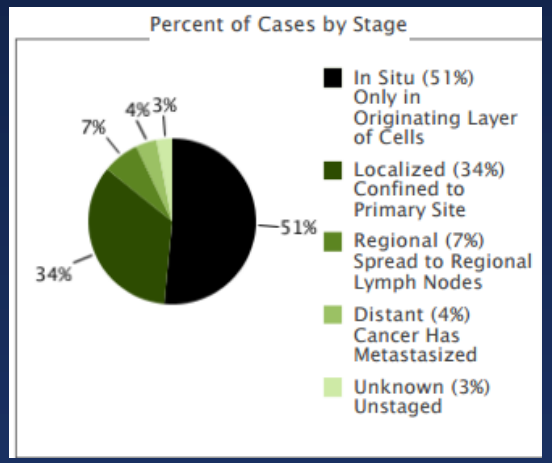
Bladder cancer is a worldwide public health concern:

- 165,000 people died of bladder cancer worldwide in 2012
- In 2012, 5 year prevalence was 1,300,000 people
- Early stage bladder cancer is treated with transurethral resection



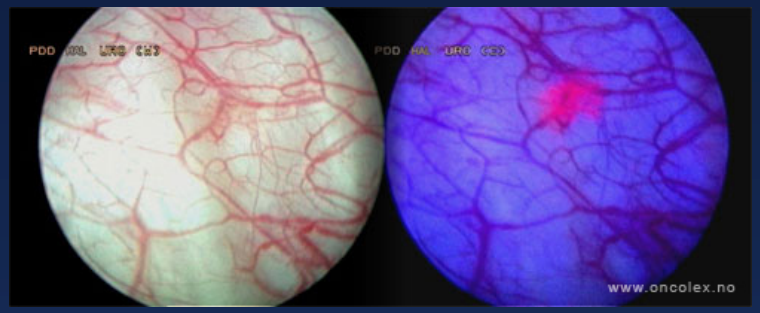


http://www.nature.com/nrc/journal/v15/n1/fig_tab/nrc3817_F1.html

http://seer.cancer.gov/statfacts/html/urinb.html

Bladder cancer has one of the highest recurrence rates of all cancers

- Disease free interval between recurrences is often 1-2 years.
- Recurrence is caused by disease left behind after transurethral resection or field defect.
- Several prospective clinical trials have shown that improved detection (lower miss rate) of fluorescence cystoscopy (FC) compared to white light cystoscopy (WLC) reduces the recurrence rate.

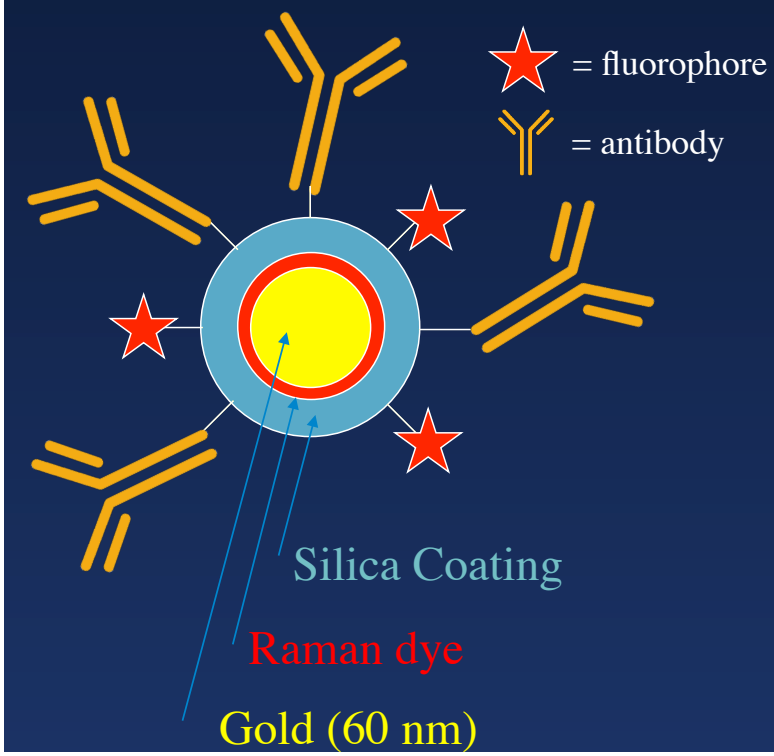


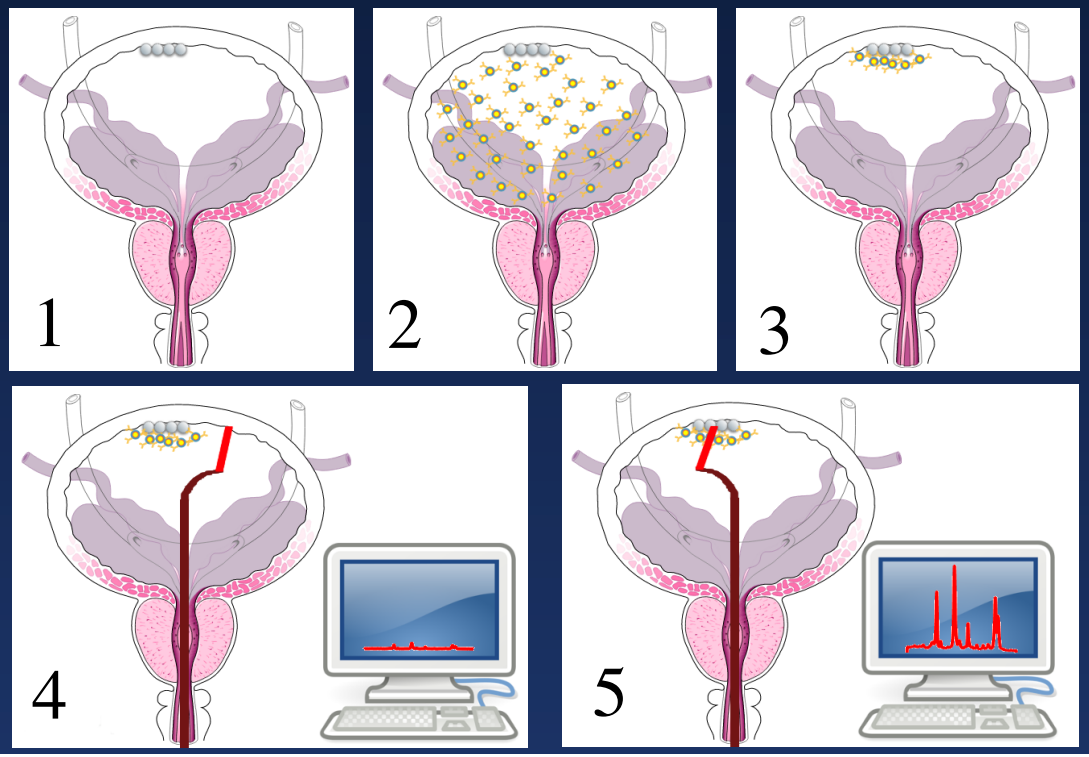
White Light Cystoscopy

Fluorescence Cystoscopy

New and improved cystoscopy techniques are <u>essential</u> for improving bladder cancer delineation and reducing recurrence

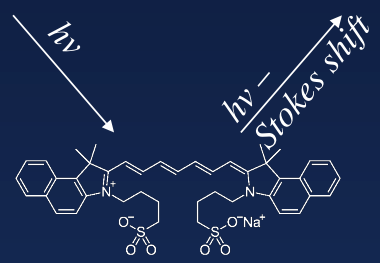
We propose a molecular cystoscopy strategy based on Surface Enhanced Raman Scattering nanoparticles to improve delineation of bladder tumors

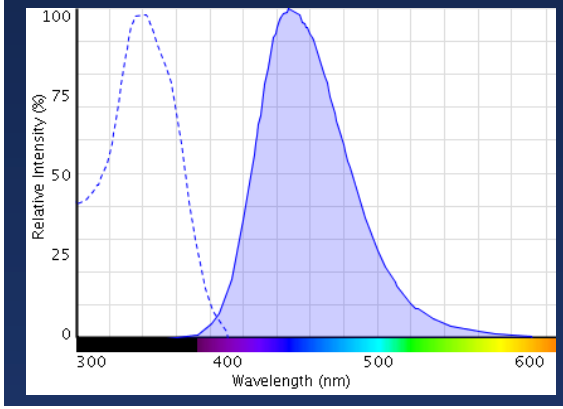




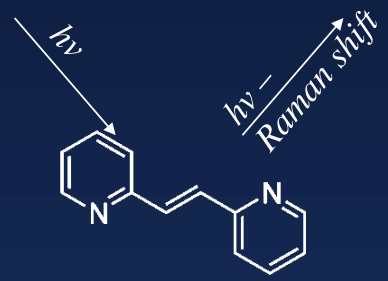
Raman Scattering is the inelastic scattering of light by vibrational modes of molecular bonds

Fluorescence



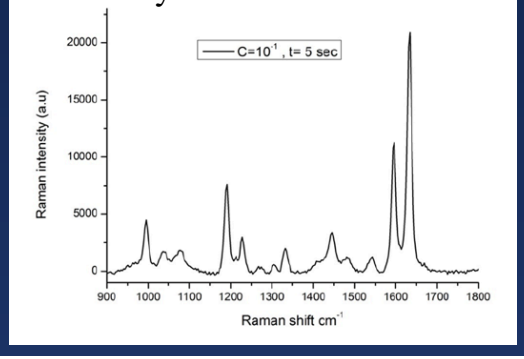


Raman Scattering

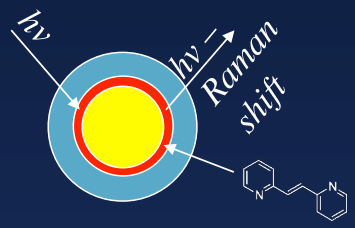


Raman scattered: ~1:10 million

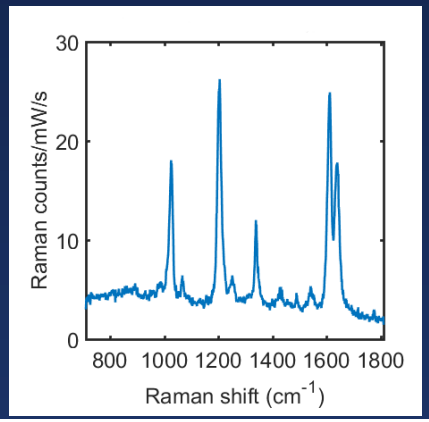
Khaywah M. et al. 2015



Surface Enhanced Raman Scattering

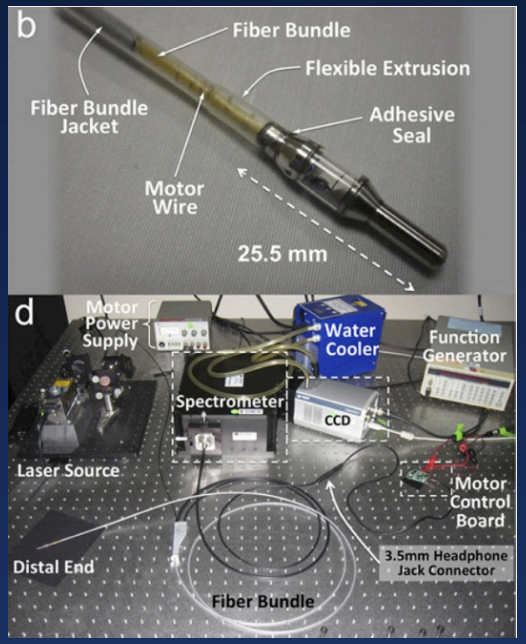


Raman scattered: ~1:10-1:100



Gambhir/Contag labs have a endoscope and microscope capable of detecting Raman scattering

Raman microscope

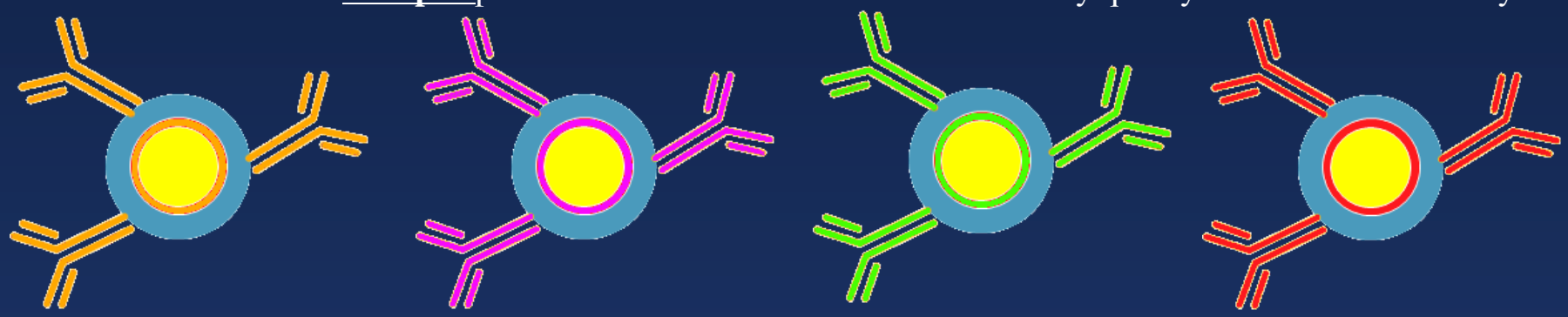


Raman Endoscope. Designed by Ellis Garai in Contag lab.

We use Raman spectroscopy because of its potential to multiplex several molecular targets

Pros/cons of Surface-enhanced Raman Scattering (SERS) nanoparticles for cystoscopy

- Pros
  - **Multiplexing** (sharp spectral lines)
  - **Low autofluorescence** contamination
  - Can detect **1-10 pM** particles on tissue
- Cons
  - Requires nanoparticle
  - Requires specialized cystoscope/endoscope
  - Limited by quality of biomarker/antibody



Anti-CD47
Raman Dye #1

Anti-c-MET
Raman Dye #2

Anti-CA9
Raman Dye #3

Control antibody
Raman Dye #4

Overview of methods/results

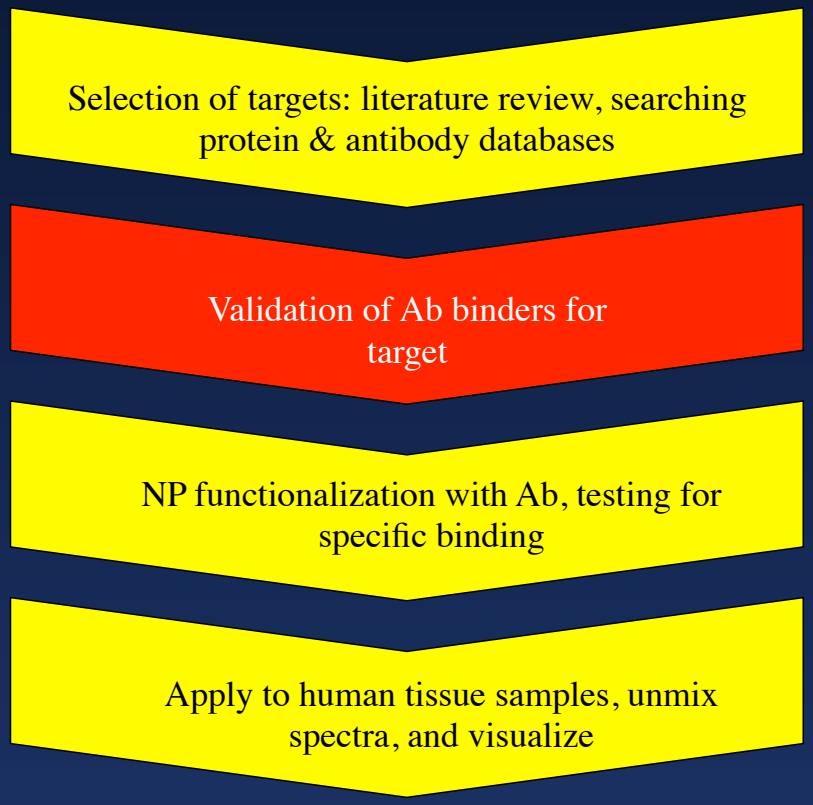
Selection of targets: literature review, searching protein & antibody databases

Validation of Ab binders for target

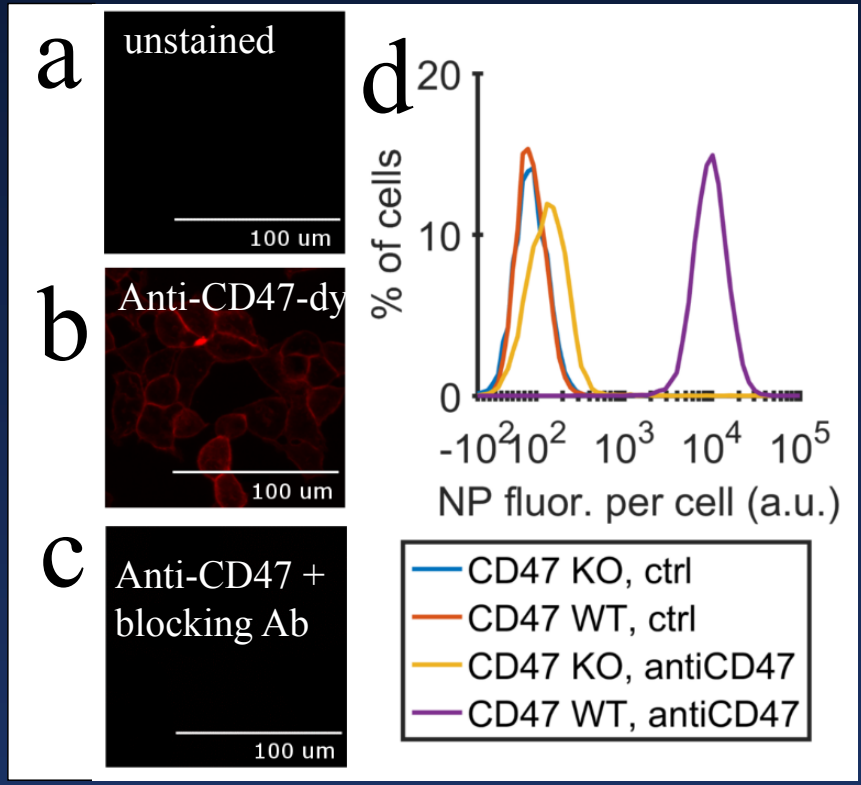
NP functionalization with Ab, testing for specific binding

Apply to human tissue samples, unmix spectra, and visualize

Overview of methods/results

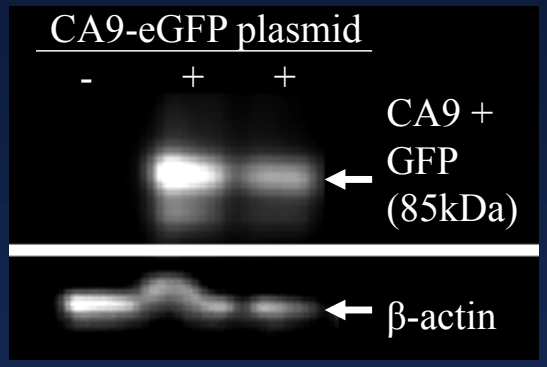


Anti-CD47 validation studies were supportive of active targeting to CD47



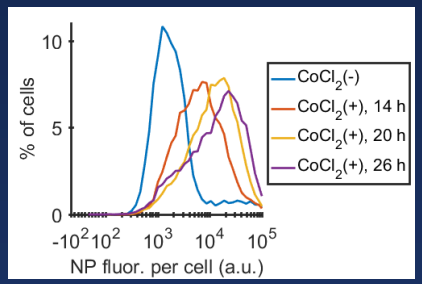
Anti-CA9 validation studies were supportive of active targeting to CA9

a



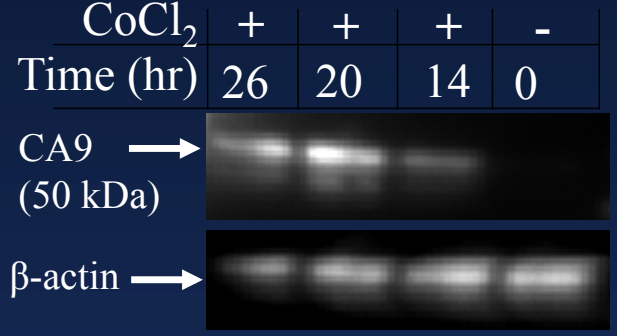
a) Western blot of HCT116 cells transfected with CA9+eGFP fusion

b



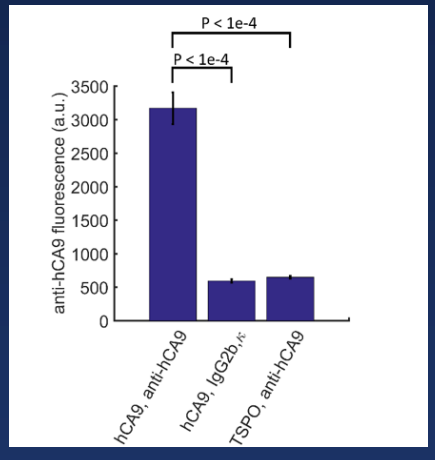
b) Flow cytometry of HeLa cells induced to express CA9 with $CoCl_2$

c



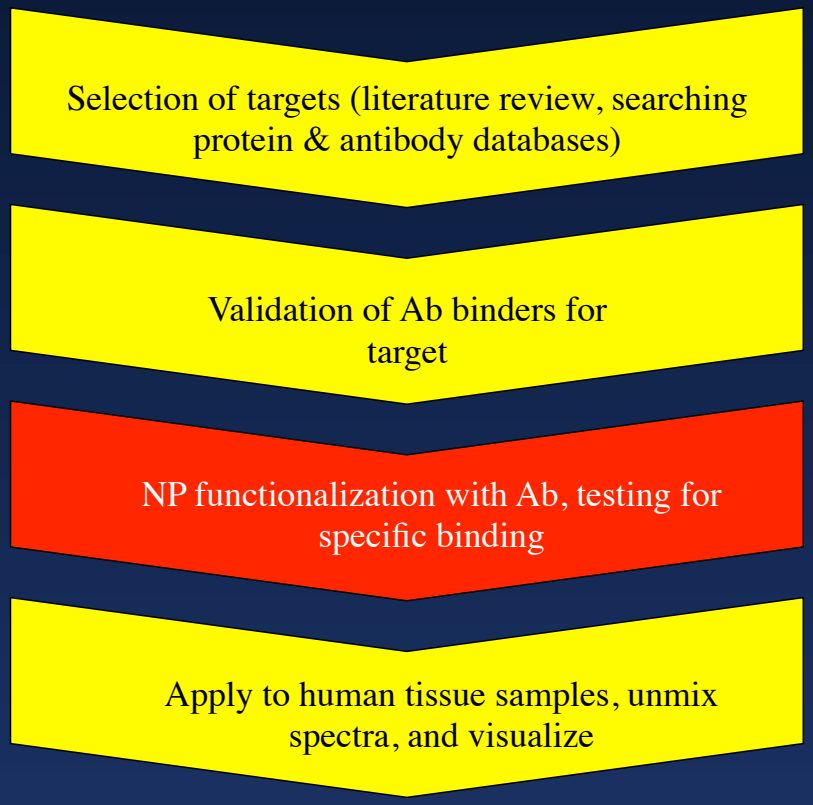
c) Western blot of HeLa cells induced to express CA9 with $CoCl_2$

d



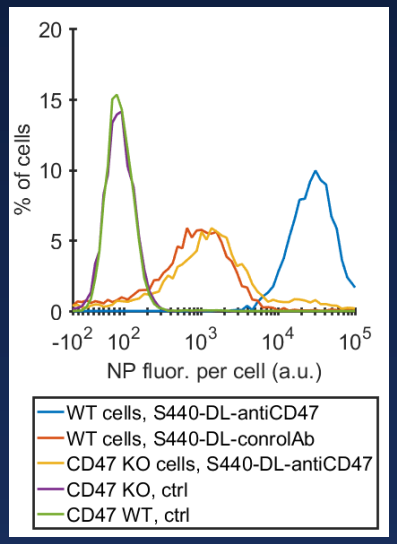
d) Flow cytometry of HCT116 cells transfected ith CA9-eGFP plasmid showed significant binding over controls.

Overview of methods/results

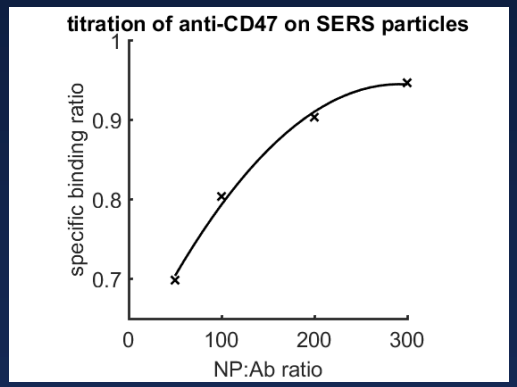


Nanoparticles functionalized with anti-CD47 actively targeted cells in suspension

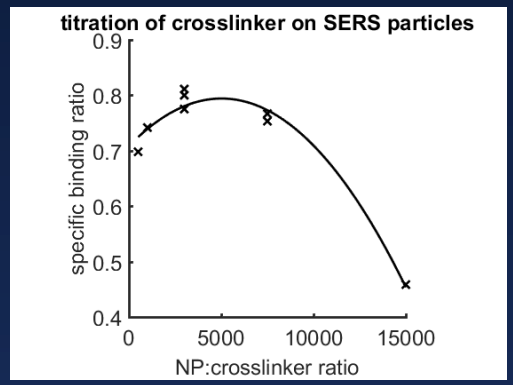
a



b

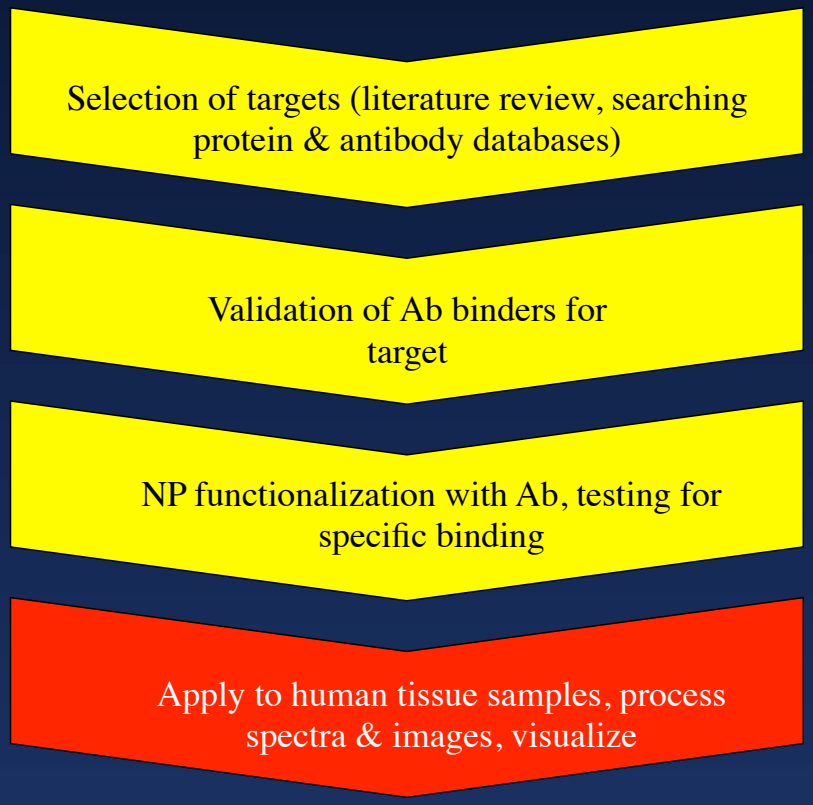


c

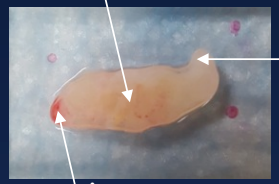
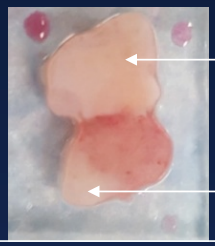
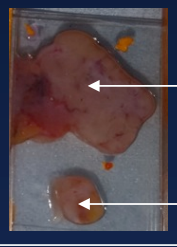
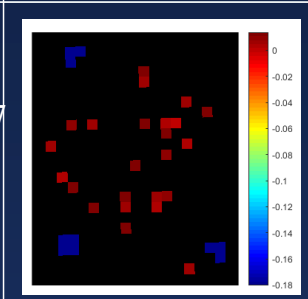
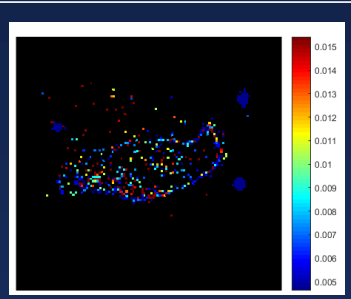
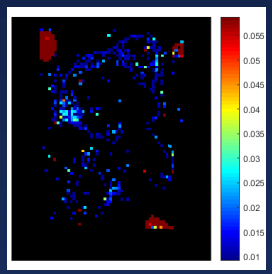


a) Binding of CD47-targeted NPs to cells in suspension. KO of CD47 or control antibody caused 38 and 25 times less NP binding (median fluor.) compared to positive control. b&c) Titrations of crosslinker and Ab concentrations were performed to optimize Specific binding ratio (SBR = (WT-KO)/WT).

Overview of methods/results



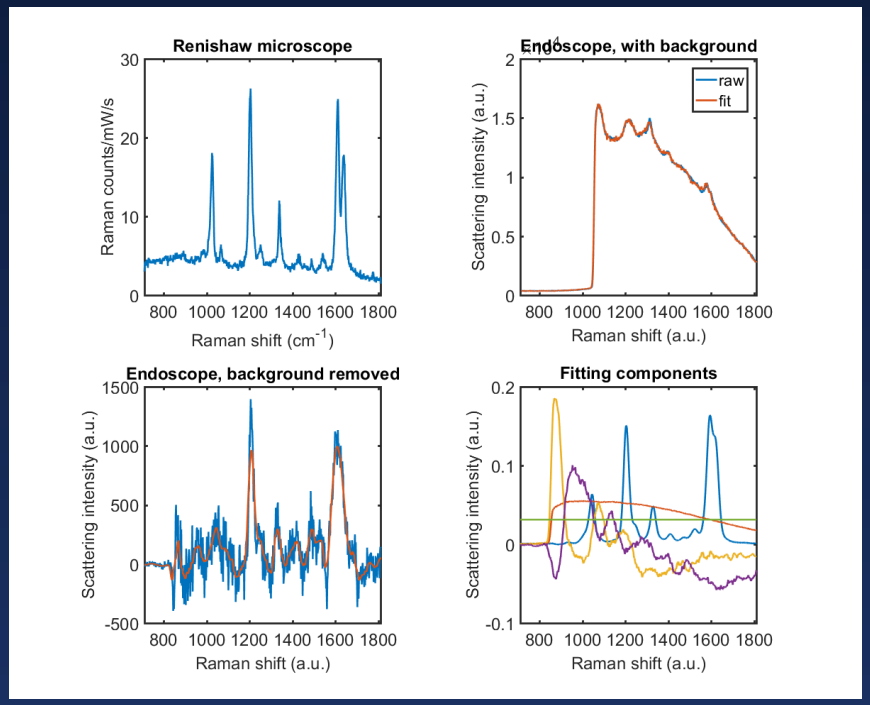
Summary of Bladder tissue samples

	Sample 1	Sample 2	Sample 3	Sample 4
photo				
Raman (antiCD47 minus control)				No nanoparticles used. Measured background signal
Surgeon's report	Normal urothelium and muscle	Tumor (t), possible inflammation (i), healthy/muscle (h)	Tumor (t), healthy/ muscle (h)	Tumor (t), healthy/ muscle (h). (no H&E)

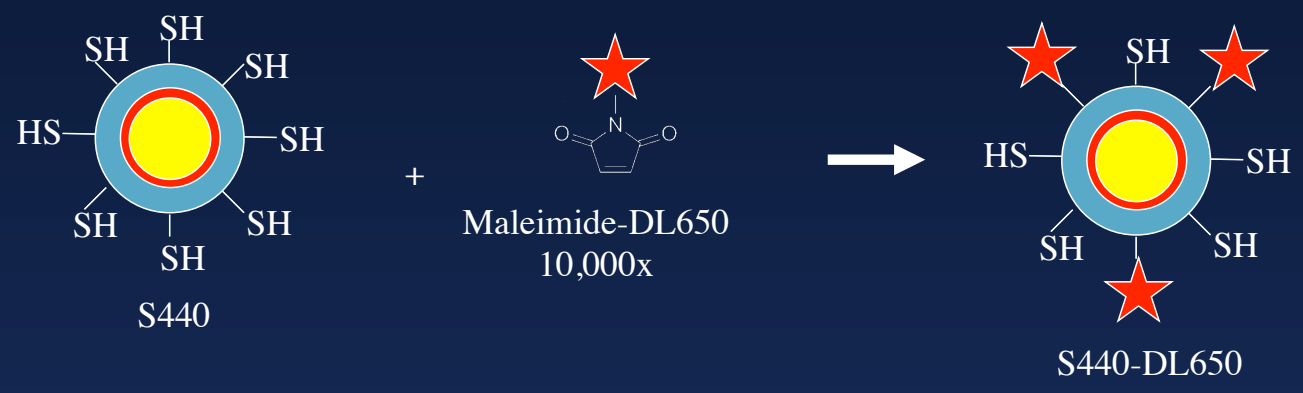
Conclusions

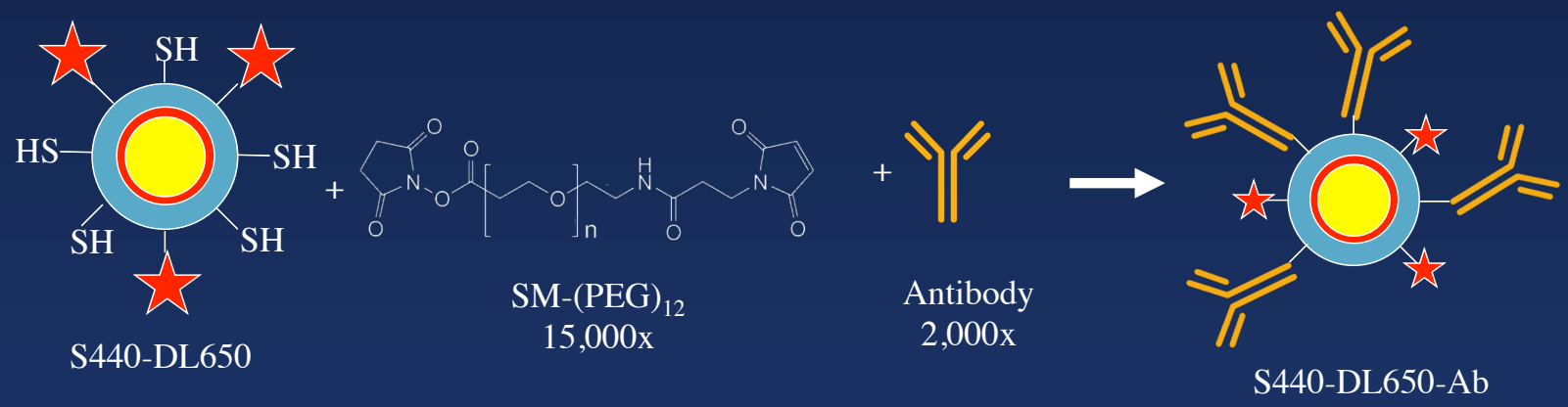
- Binders successfully target CD47 and CA9 in flow and Western blot assays, c-MET binder in progress
- Conjugating CD47 binder to SERS particles actively targets CD47
- Nanoparticles bind to tissue, but further work is needed to see if binding correlates to cancer on histology

Microscope and endoscope can detect particles, although endoscope has ~10x lower SNR.

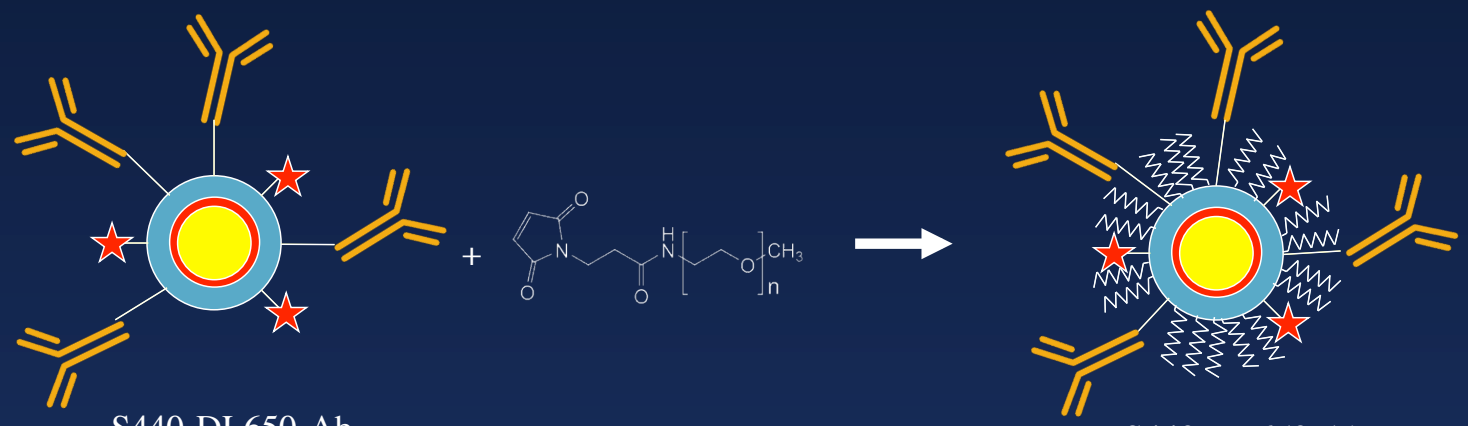


Two main possibilities for NP/Ab conjugation





Potential solution: PEGgylate surface and use longer crossilnker



S440-DL650-Ab (using PEG12 crosslinker instead of PEG4)

S440-DL650-Ab (using PEG12 crosslinker instead of PEG4)

I believe this will improve the stability and specific binding ability of my particles!