

Department of Bioengineering

Laboratory for Nanotherapeutics

May 26st 2011

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Nanotherapeutics Laboratory

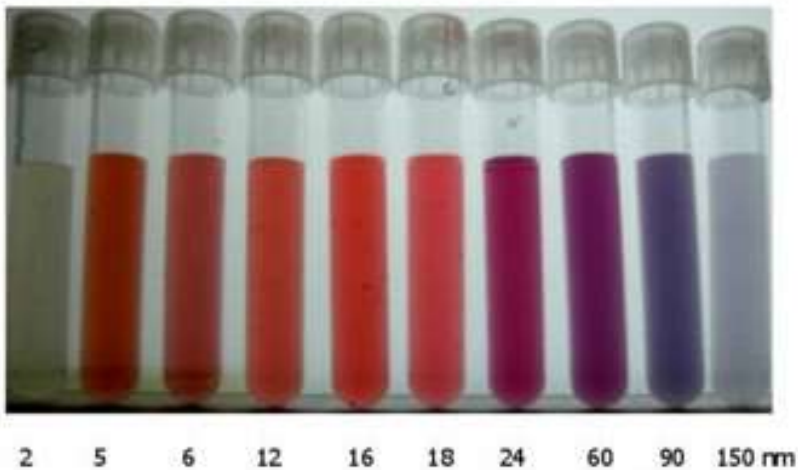
1. Use of plasmonically responsive NIR resonant gold nanoparticles:

light + nanoparticles → heat → therapeutic effect

- kill tumor cells directly
 - release drugs at specific location
2. Use gold nanoparticles (GNPs) as a sensor for biomarkers
 3. Development of novel drug vehicle using bio-polymer (chitosan) coated onto GNPs (2 patents filed)
 4. Development of innovative high yield synthesis methods for NIR absorbing GNPs

Why Gold Nanoparticles?

Different sizes of colloidal gold particles



Optically responsive

Gold is *biocompatible*

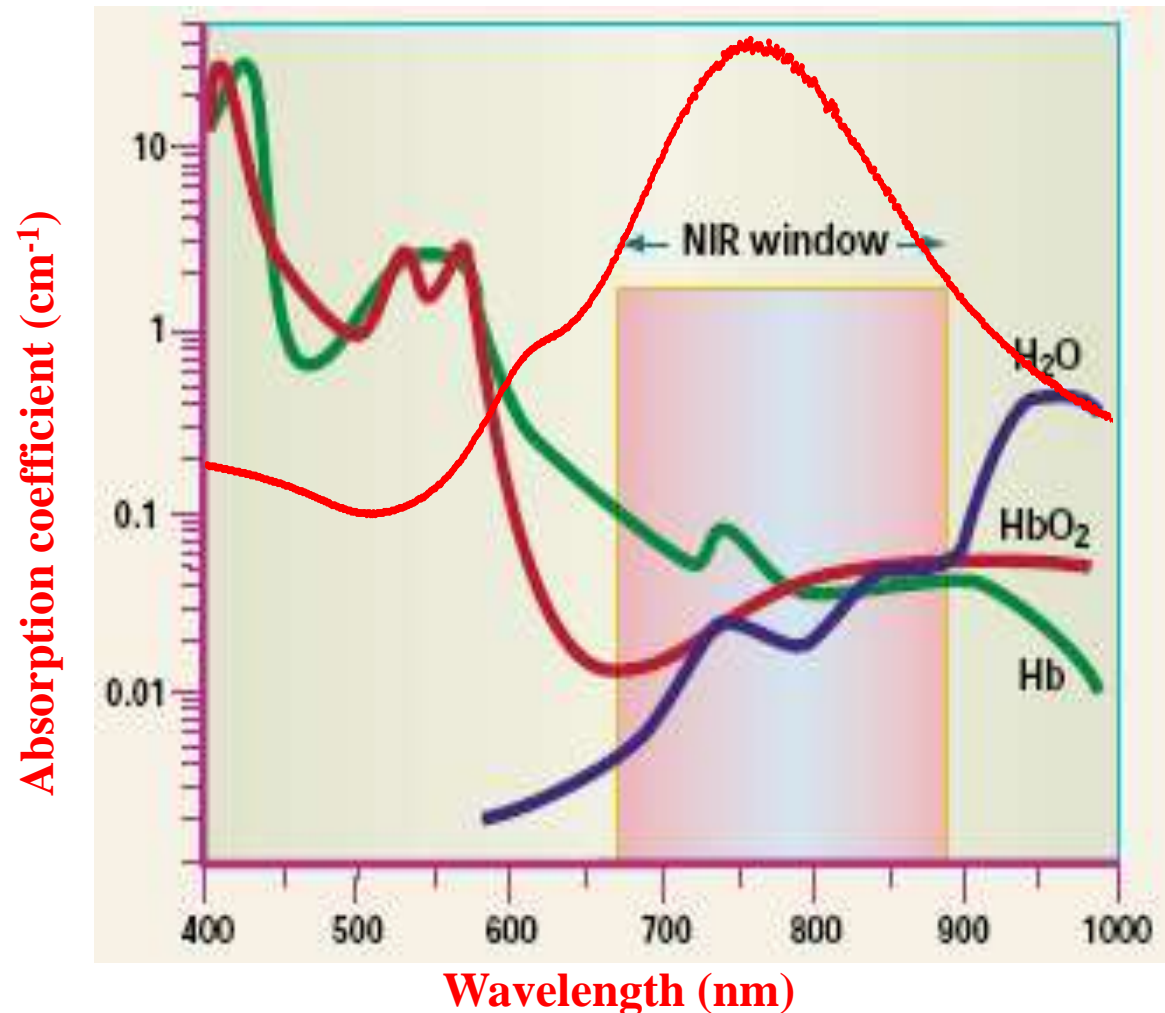
Gold is **Inert**

- conjugation via thiols (-SH)
- conjugation via charge



Nanoparticles + Near Infrared Light

- Extinction = Scattering + Absorption
- 650-900 nm → nIR Window
 - Low Absorption
 - High Transmission
- Exploit **NIR window** to develop photo-thermal therapies & imaging modalities



Goals of Nanotherapeutics Laboratory

- nIR resonant gold nanoparticles with multifunctional capabilities
 - 1) Strong nIR absorption for therapeutic benefit (cancer)
 - 1) Targeting ability (cancer)
 - 2) Particle as sensors (LOC Devices)
 - 3) Contrast properties for imaging (CT)
 - 4) Delayed drug delivery through external activation (LED)
 - 5) Platform for synthesis and separations to tailor chemical reactions

Goals of Nanotherapeutics Laboratory

- Investigate toxic effects of nanoparticles as part of development process
 - *In vitro*: Cell culture assays
 - *In vivo*: Bio-distribution
- *Optimize process for production & scaleup*
 - Translate to produce medically relevant technology

Nanotherapeutics Laboratory

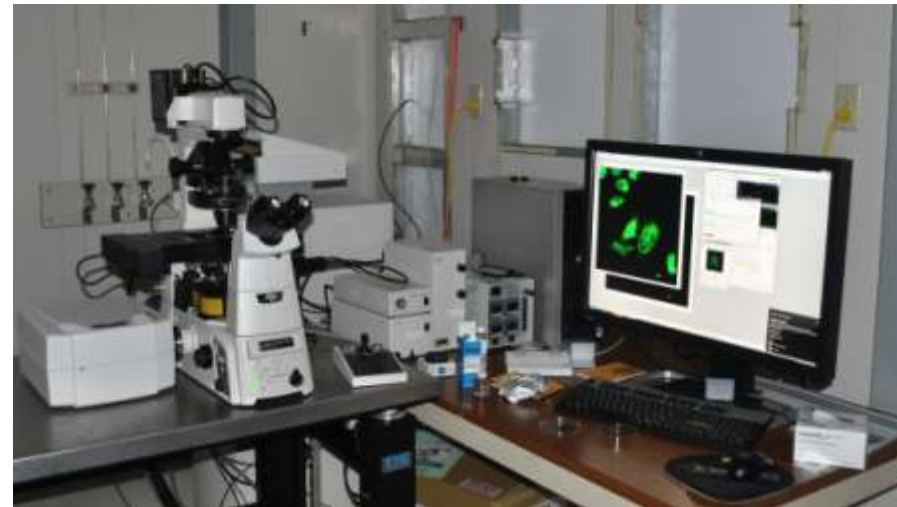
- So how do we fit in with the EPSCoR group

NL in relation to EPSCoR Group's Vision

- Use plasmonically responsive gold nanoparticles to enhance detection of cellular level events
 - Internalization and Tracking
 - Understand cellular internalization of nanoparticles
 - Cell-Surface and Molecular Binding Events
 - For detection of cells (alternative cytometer)
 - For biomarker detection of diseases
 - Diagnostic Platform
 - Creation of Hybrid photo-actuators
 - Creation of devices for cell capture and analysis

Effects of EPSCoR Funding to NL

- Instrument for size, zeta and isoelectric point measurement
 - Critical in nanoparticle characterization
- Confocal Microscope
 - Critical in tracking internalization of nanoparticle
- Dimatix Printer
 - For precise printing on nanoparticle suspensions unto MEMs devices



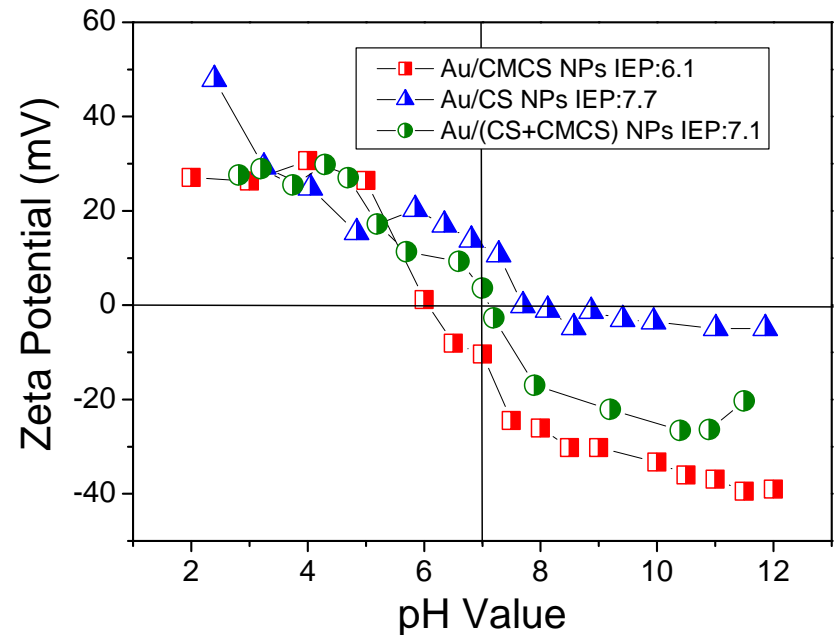
Unique Opportunities in this Arena

- Coating with **chitosan** to enhance binding & uptake to tumor cells
- Conjugation of targeting molecules (Ab)
- Conjugation of imaging molecules (Iodine)
- Improve Synthesis of GGS nIR absorbing nanoparticles

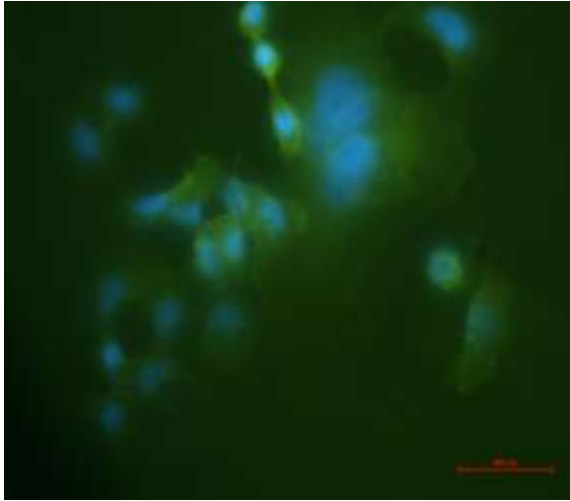
Flexible Nanoparticle Surfaces

- Coated GGS nanoparticles with CS & CMCS:
 - 2 forms of CMCS (Na & H)

Sample No.	Coating Composition	Average Surface Charge (mV)
1	Bare Au NP, no coating	-46.0
2	Au/(Na-CMCS) (without dialysis)	-30.4
3	Au/PEG	-13.3
4	Au/(Na-CMCS) (dialyzed)	-3.2
5	Au/(25%CS+75%CMCS)	18.4
6	Au(H-CMCS)	19.2
7	Au/(50%CS+50%CMCS)	26.8
8	Au/(CS+PEG)	30.5
9	Au/CS	38.5

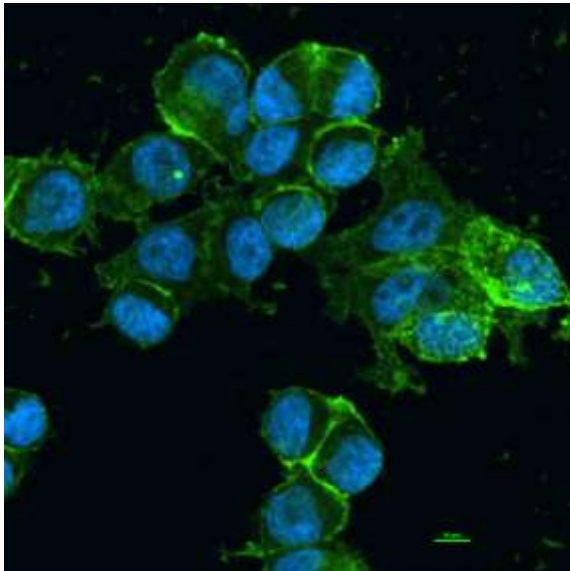


Imaging of Ab Targeting of GNPs via Chitosan

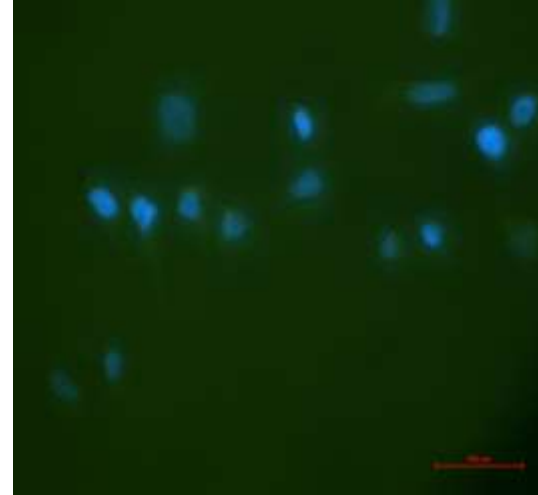


HER-2 Positive
cells (SKBR-3)

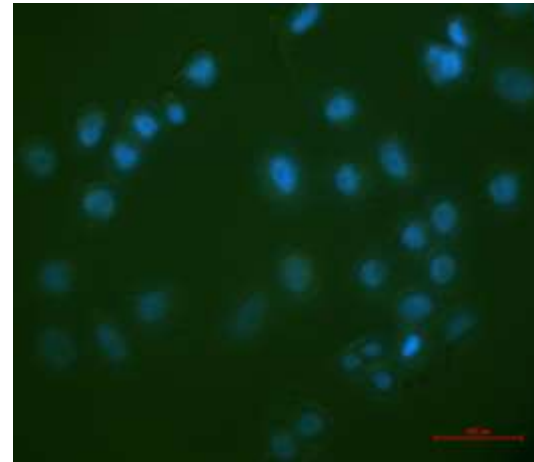
Targeted with
mouse-anti
HER-2 GNPs or
control (no Ab)



Imaged with
FITC-anti-
mouse (2°)



CS/CMCS-AuNPs



PBS-Control

Collaborations

- At **University of Louisville**
 - Robert Martin, M.D. / Ph.D.
 - Target and Eliminate Esophageal Cancer & Diagnose Barrett's Esophagus
 - Cindy Harnett, Ph.D. (ECE)
 - Enhancing Detection of Cells via gold NP
 - Create photo activated MEMS devices for Bio applications
 - Palaniappan Sethu, Ph.D. (BE)
 - Devices to separate gold nanoparticles, LOC diagnosis platform
 - Robert Keynton, Ph.D.; Andrea Gobin, Ph.D.; Robert Cohn, Ph.D.
 - NASA: Diagnosis & Mitigation of Human Exposure to Radiation
 - Detection of astronauts exposure to cosmic rays

Funding

■ Current

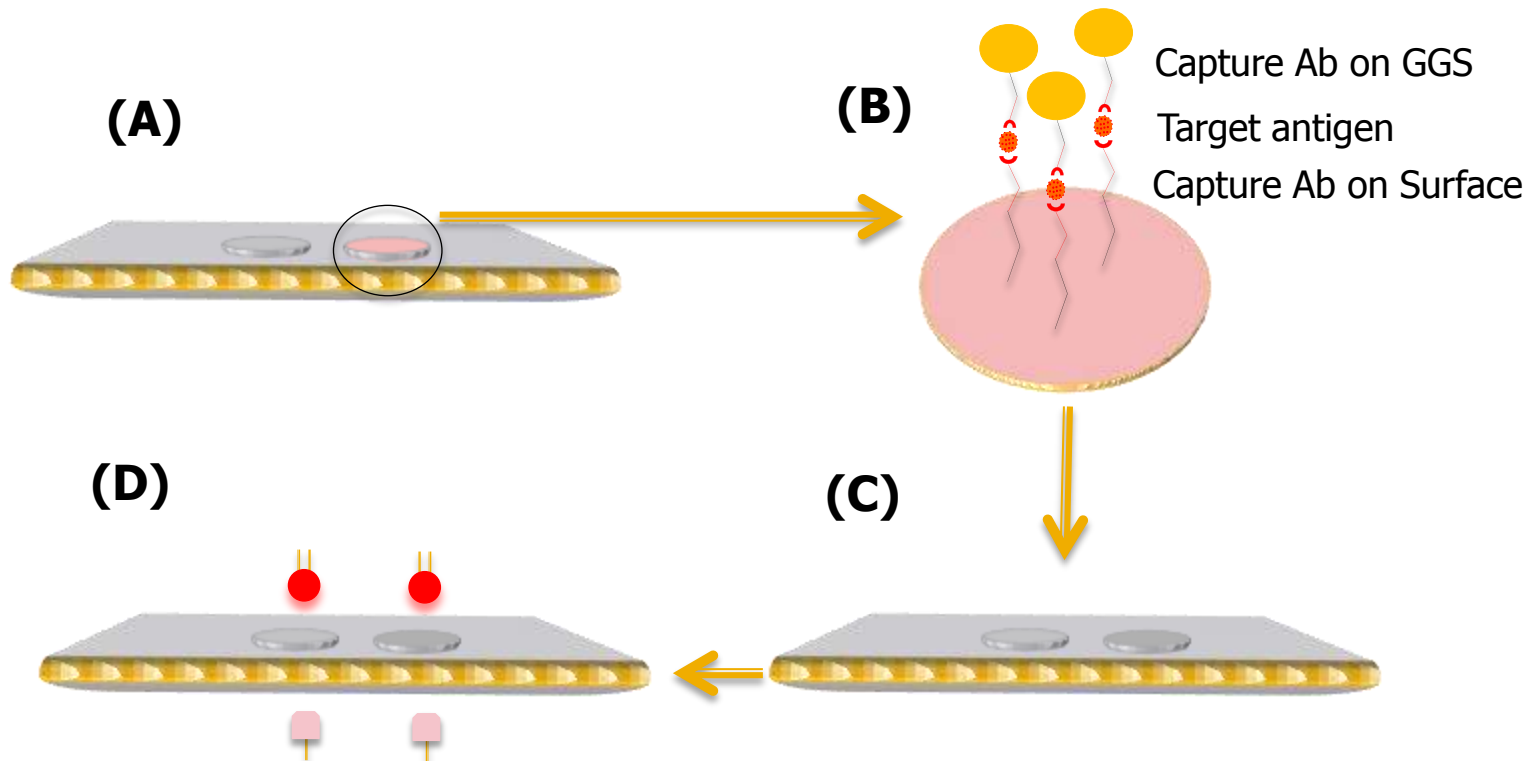
- **Wallace H. Coulter Foundation Phase 2 Translational Research Award in Biomedical Engineering** "Chitosan coated GNP for treatment of Esophageal Adenocarcinoma & Barrett's Esophagus" (08/2010 – 08/2012), **\$260,000** total, PI: André Mathura Gobin – 10% Effort
- **Clinical & Translational Science Pilot Grant Program's Basic Award – University of Louisville** "Chitosan Coated Gold Nanoparticles: Flexible Nanoparticles for Therapeutic and Imaging Applications", (03/2010 – 03/2011) **\$49,980** PI: Andre Gobin, No Effort
- **Clinical & Translational Science Pilot Grant Program's Basic Award – University of Louisville** "Light-absorbing nanoparticles to induce localized tumor tissue heating in esophageal cancer therapy", (06/2010 – 06/2011), **\$49,980** PI: Yan Li, co-PI: Andre Gobin, No Effort
- **NASA** "Diagnosing and mitigating Human Exposure to Radiation Using Micro / Nanotechnology", (09/2010 – 09/2011), **\$2,000,000**; PI: Robert Keynton, co-PIs: Andre Gobin – 30% Effort, Palaniappan Sethu, Andrea Gobin, Eric Rouchka, Co-Is: Robert Cohn, Balaji Panchapakesan John Eaton, Bill Ehringer.

■ Pending

- **KSEF, RDE 14:** "Light Powered Hybrid Microactuators for Biotechnology", 2 Years, **\$99,996**, PI: Cindy Harnett, co-PI: Andre Gobin



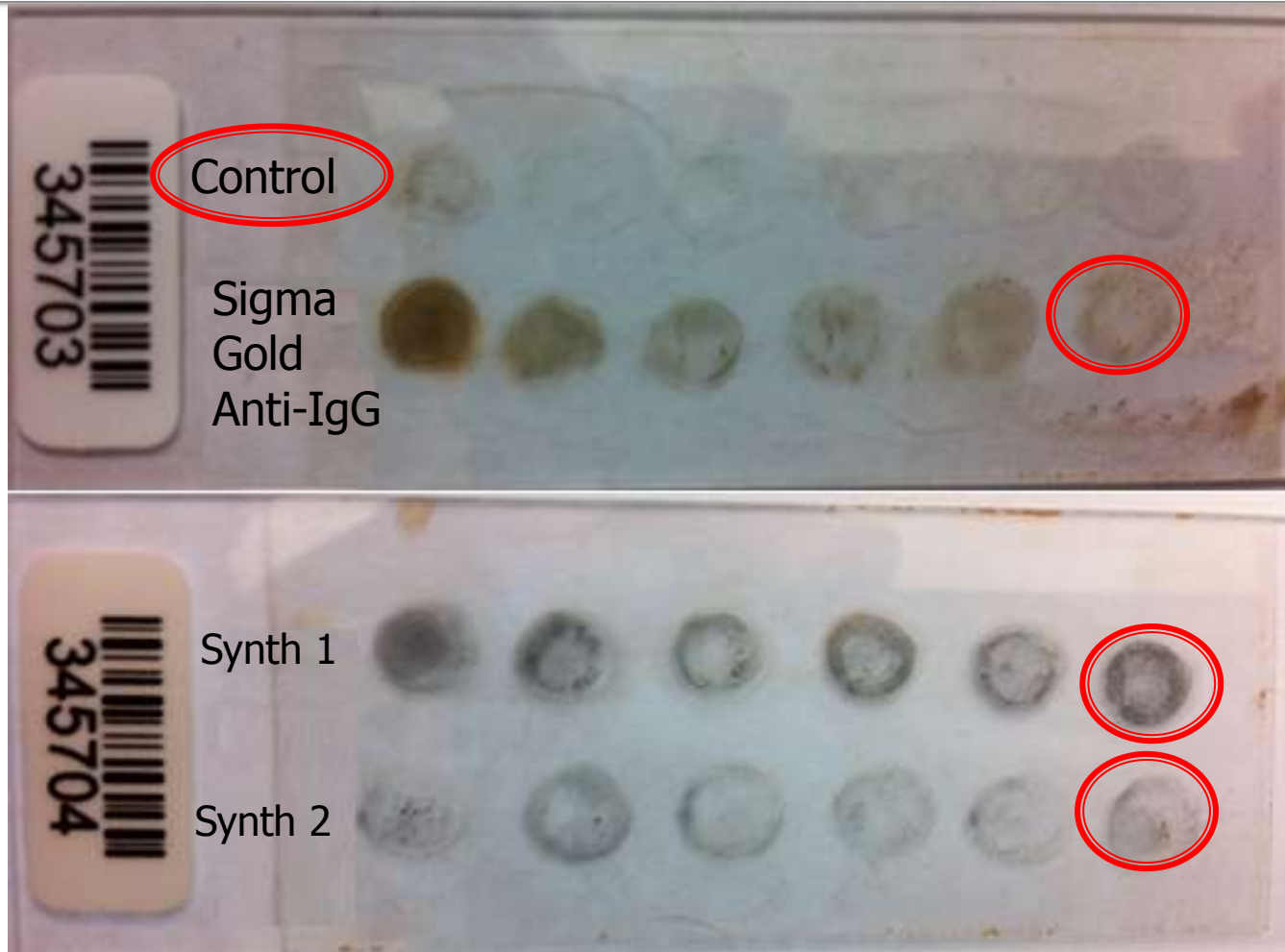
Application: Detection Platform



- (A) Capture vs. control surfaces
- (B) Close-up of capture scheme
- (C) Develop with silver enhancer
- (D) Detect via LED / photodetector (or with image & grayscale analysis)

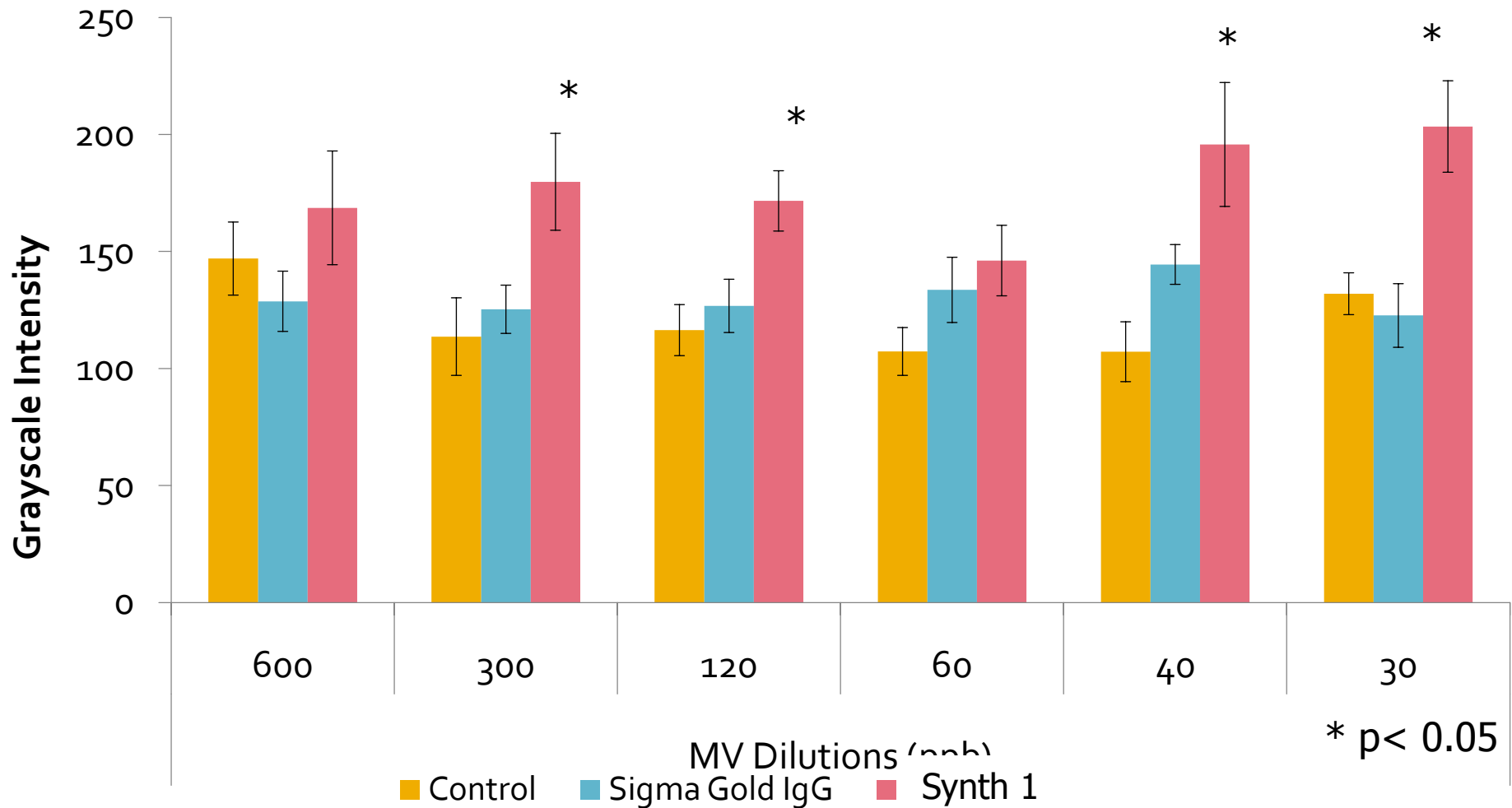
Application: Detection of Biomarkers

Surface ELISA Based



Decreasing concentration

Application: Detection of Biomarkers Surface ELISA Based



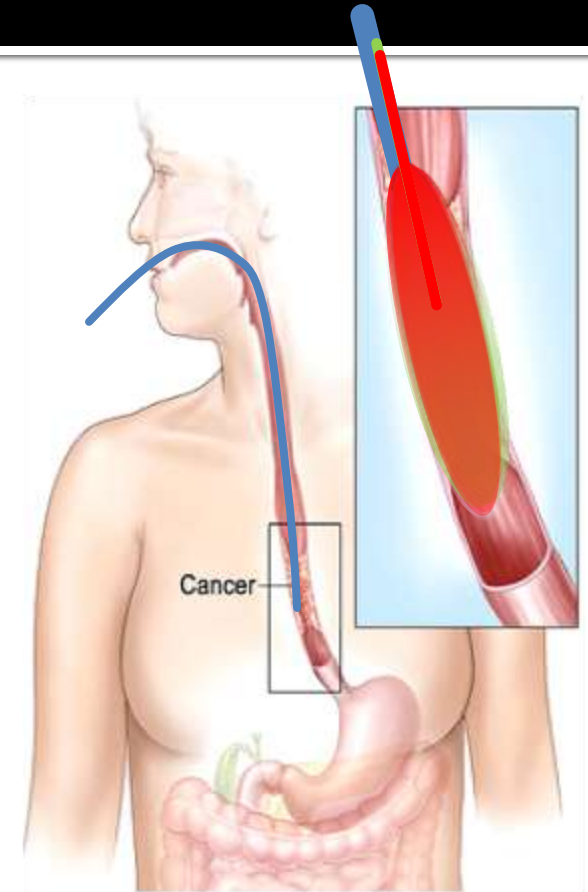


Application: E. Adenocarcinoma

- Current Treatment:
 - Surgical resection:
 - Limitations:
 - tolerance to surgery;
 - tumor must resectable & not metastasized
 - Radiotherapy:
 - Limitation:
 - local and systemic antineoplastic activity.
 - The concept **using a pulse laser to ablate cancerous tissue** without damaging healthy tissue could be a solution to create a competitive advantage to the current therapeutic strategies

Improved Technology for Treatment of EA

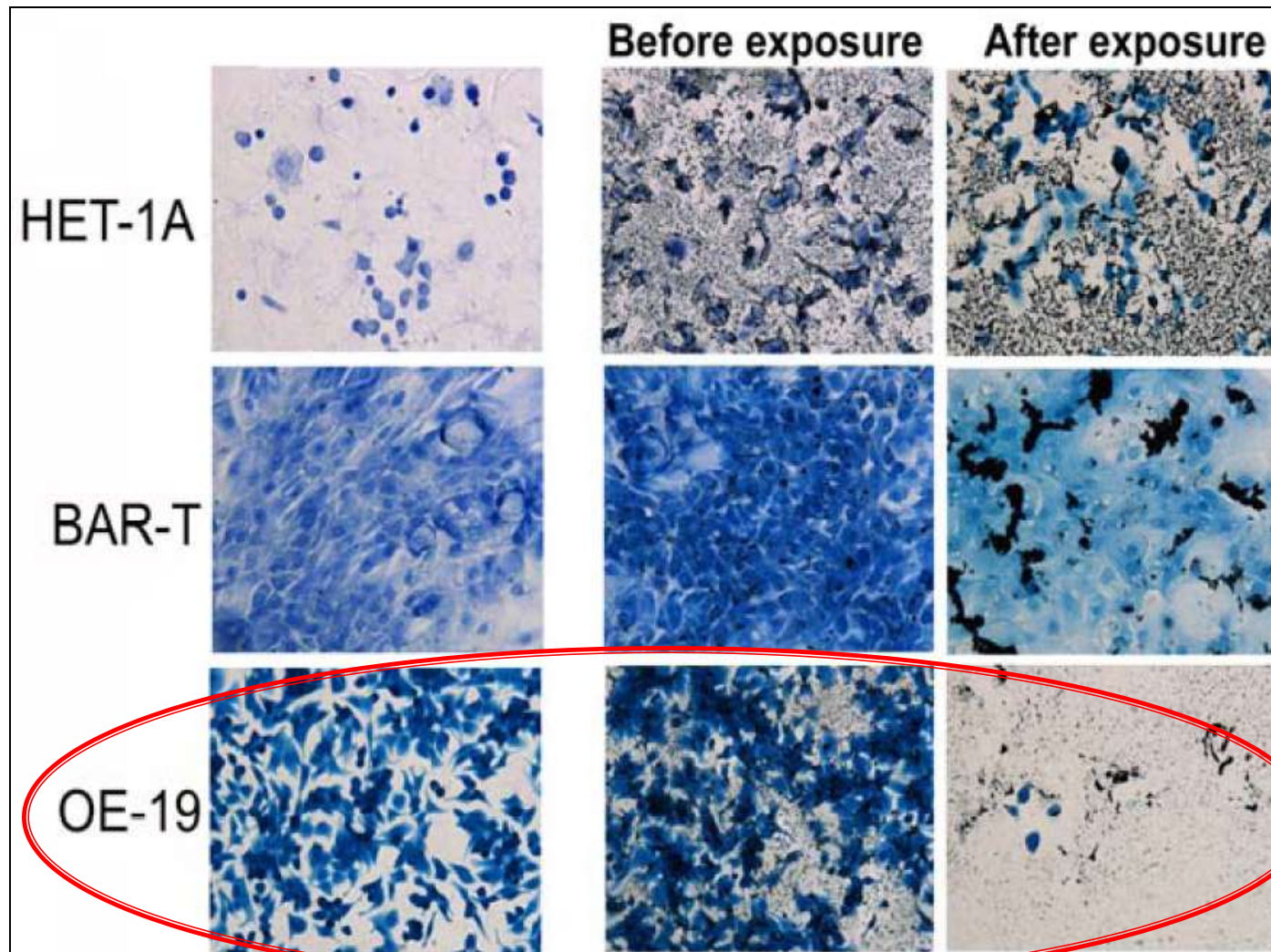
- **Chitosan Gold nanoparticles**
 - Easy manufacturing (scalability & tunability)
 - Easy conjugation (flexibility)
 - High affinity for Esoph. Adeno. (binding)
 - High Light Absorbing Efficiency (efficacy)
- **Photo-ablation via Endoscopic Procedure**
 - Current Techniques
 - Current Instruments
 - All-in-one procedure
 - Deep light penetration in target tissue



http://en.wikipedia.org/wiki/File:Esophagael_stent.jpg

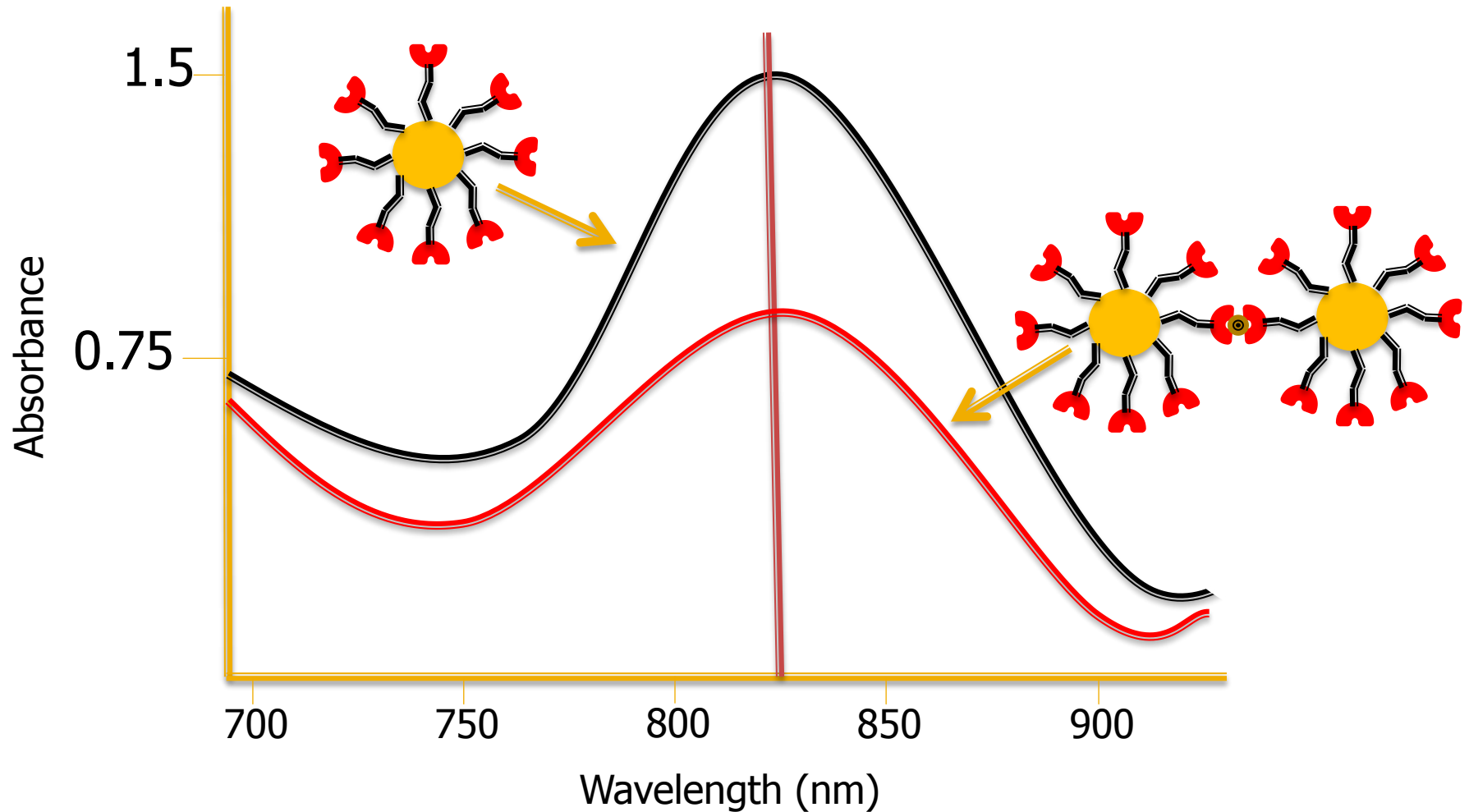
Efficacy of Chitosan/GGS Nanoparticles: Esophageal Model

- HET-1A & BAR-T controls shows little damage
- OE-19 cells shows almost complete ablation



Application: Detection of Biomarkers

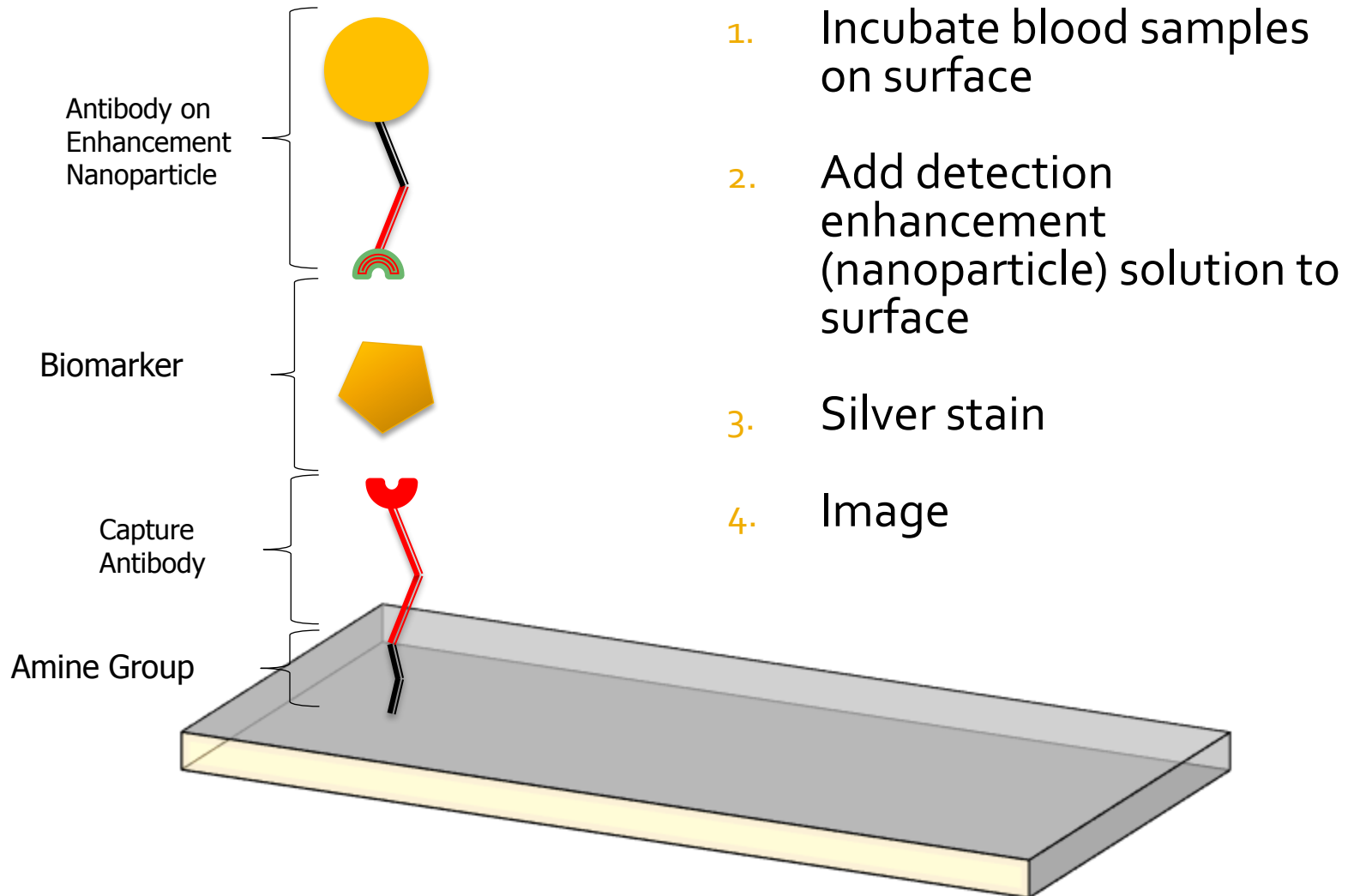
Method 1 – Liquid Based



* Hirsch et. al.

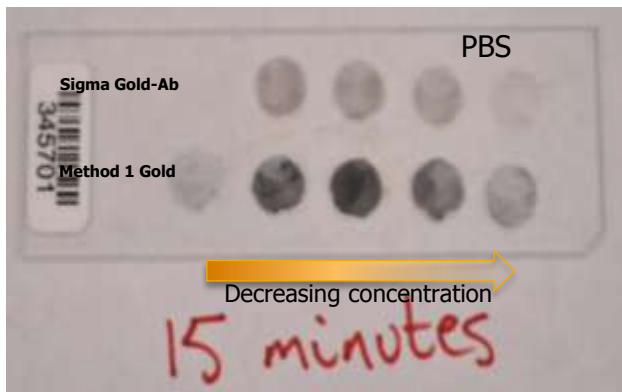
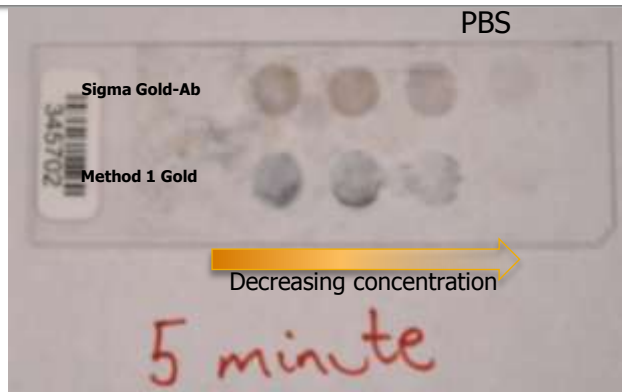
Application: Detection of Biomarkers

Method 2 – Surface ELISA Based

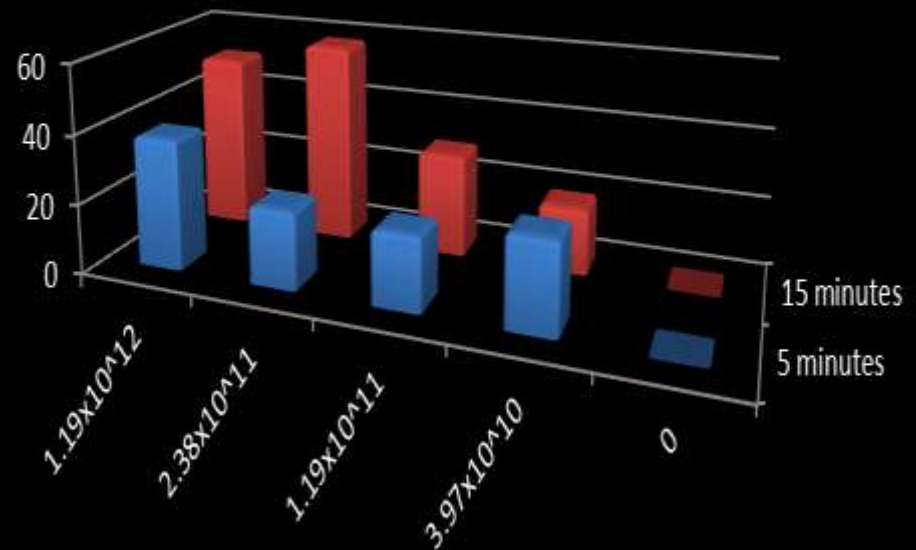


Application: Detection of Biomarkers

Method 2 – Surface ELISA Based

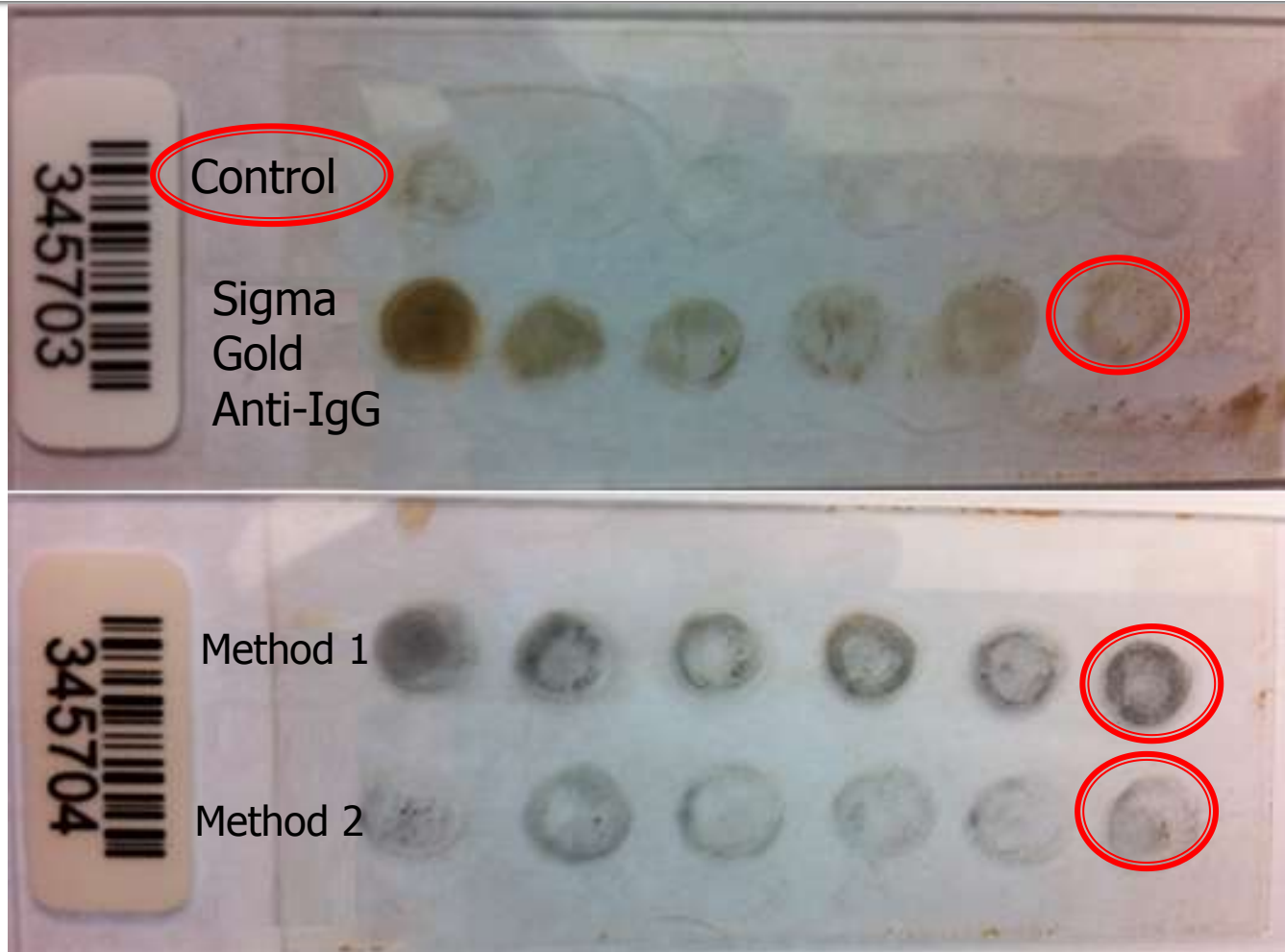


Average Intensities using Synthesized gold-IgG



Application: Detection of Biomarkers

Method 2 – Surface ELISA Based

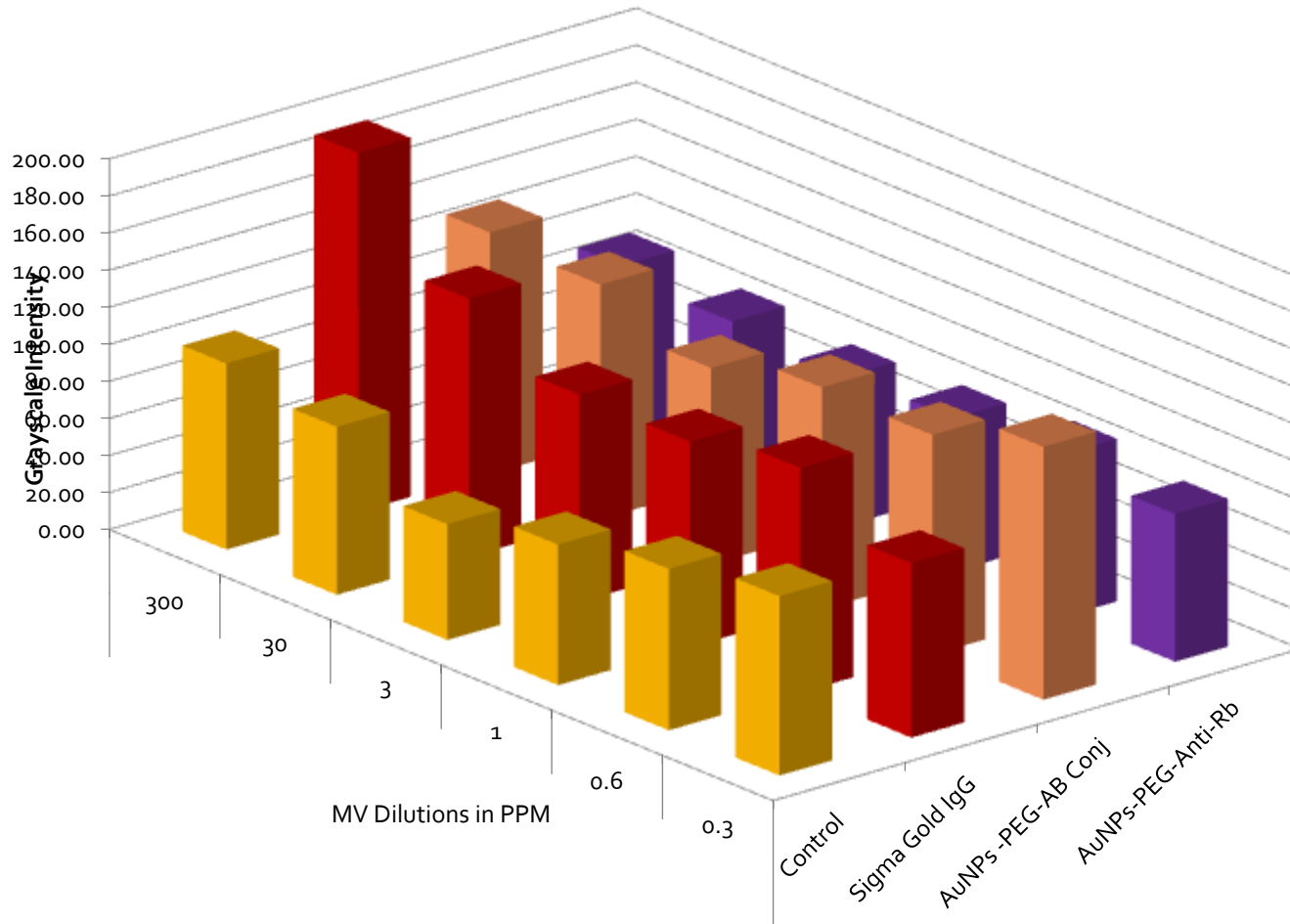


Decreasing concentration

Application: Detection of Biomarkers

Method 2 – Surface ELISA Based

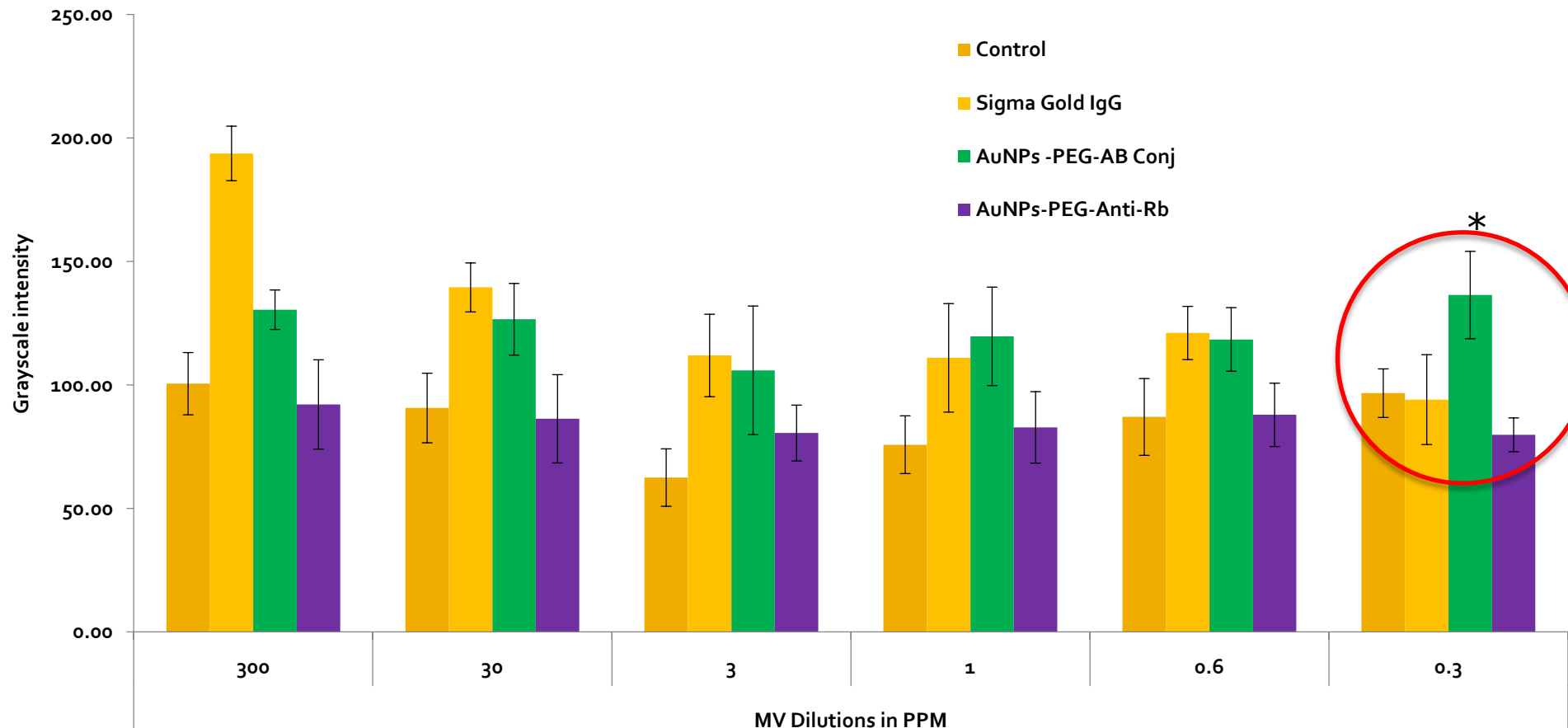
Image analysis of Silver stain



Application: Detection of Biomarkers

Method 2 – Surface ELISA Based

Image analysis of Silver stain

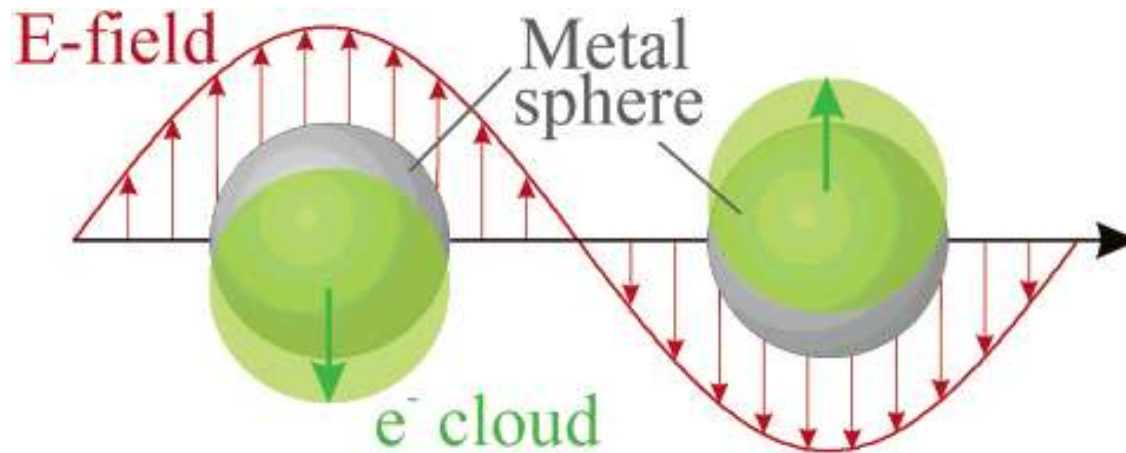


* $p < 0.05$

Optical Properties of Gold Nanoshells

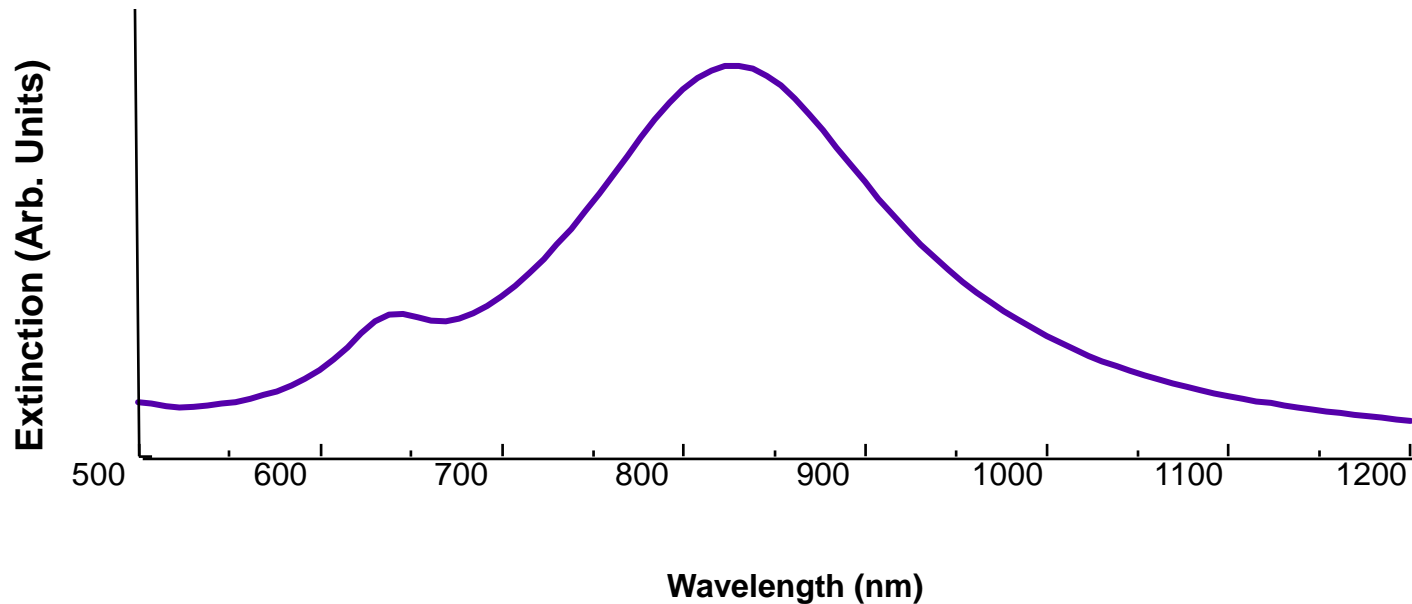
Surface Plasmon Resonance (SPR):

Coherent oscillation of the conduction band electrons induced by interaction with an electromagnetic field.

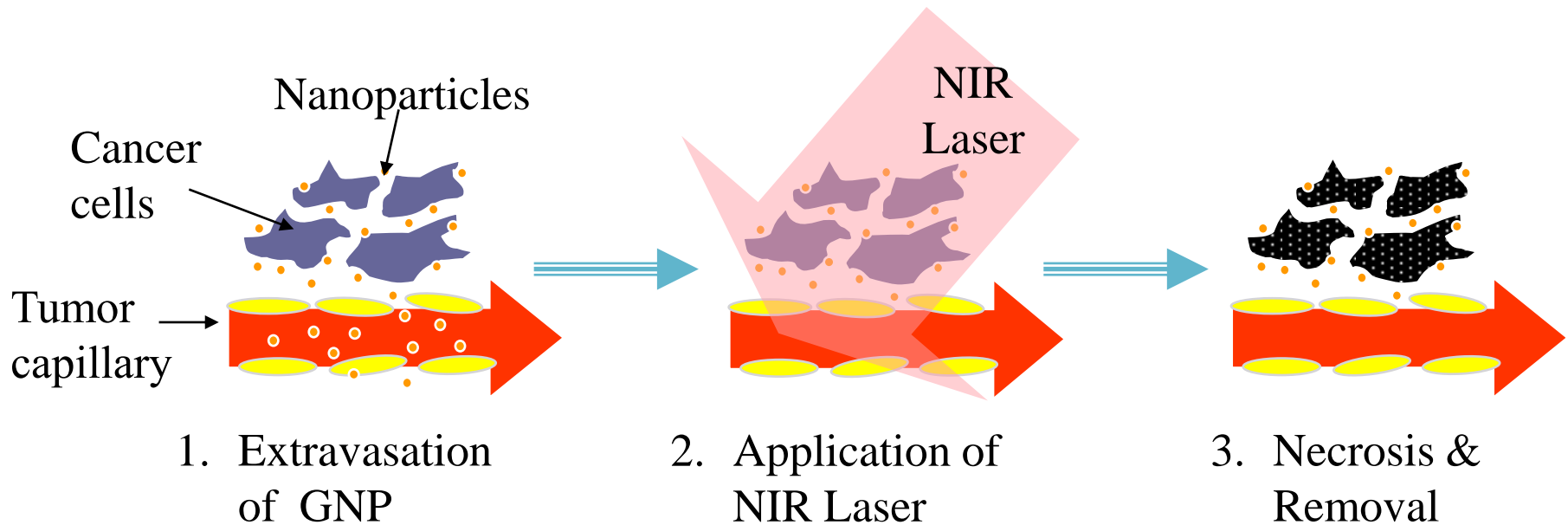


Optical Properties of Gold Nanoshells

- Maximal resonance (peak resonance) depends strongly on wavelength



Gold Nanoparticle Based Photothermal Therapy

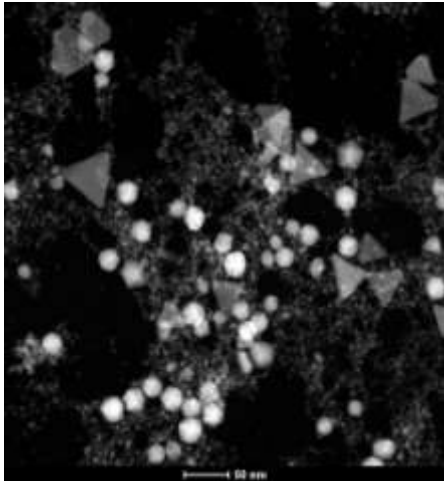


Optically Tunable Nanoparticles

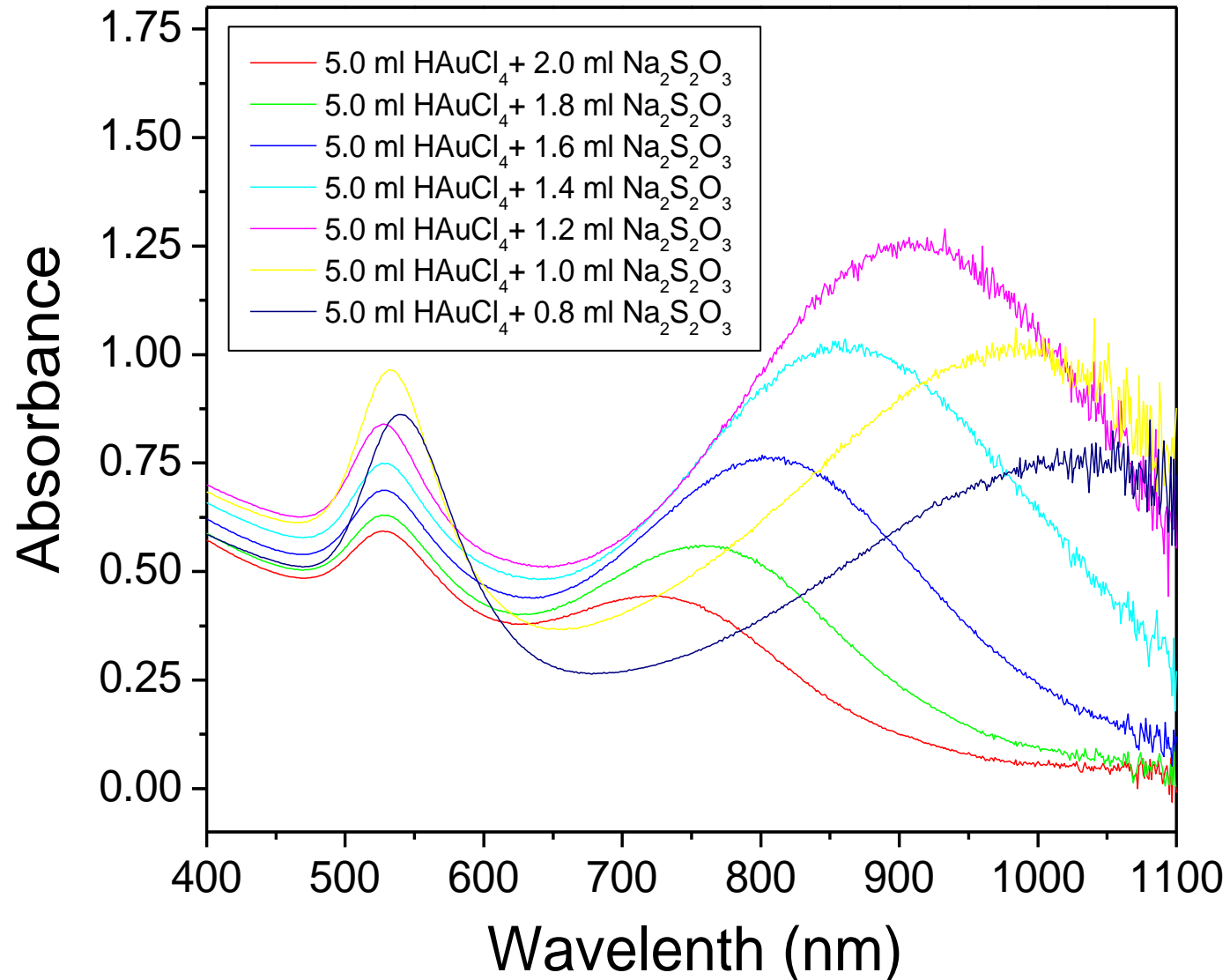
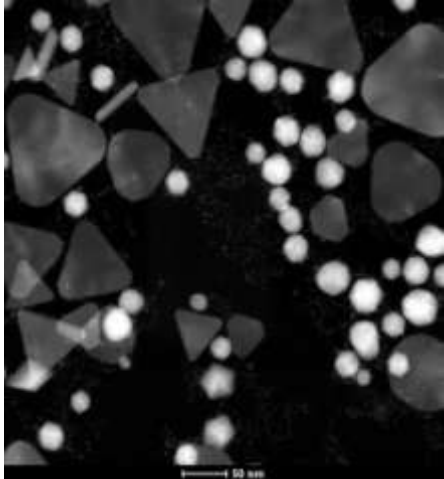
- Gold nanoparticles produced through self assembly of gold and sulfur
- Able to adjust the optical properties by changing the reaction conditions
- Result → Very strongly absorbing nanoparticles out of GOLD / GOLD SULFIDE

Optically Tunable Nanoparticles

Condition 1



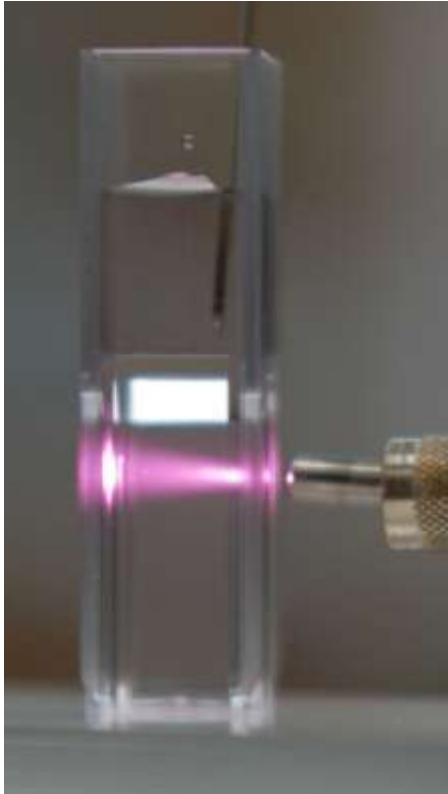
Condition 2



Focus: gold / gold sulfide nanoparticles

- Smaller and more efficient at converting light to heat
 - → Deeper penetration into tumors
 - → Lower light dosage for therapy
- Easier manufacturing process
- Gold/Gold-sulfide nanoparticles achieve **higher temperatures** at **lower** laser power at equivalent optical density compared to Gold/Silica nanoshells

Gold/Silica Nanoshells Exhibit Higher Scattering due to Size



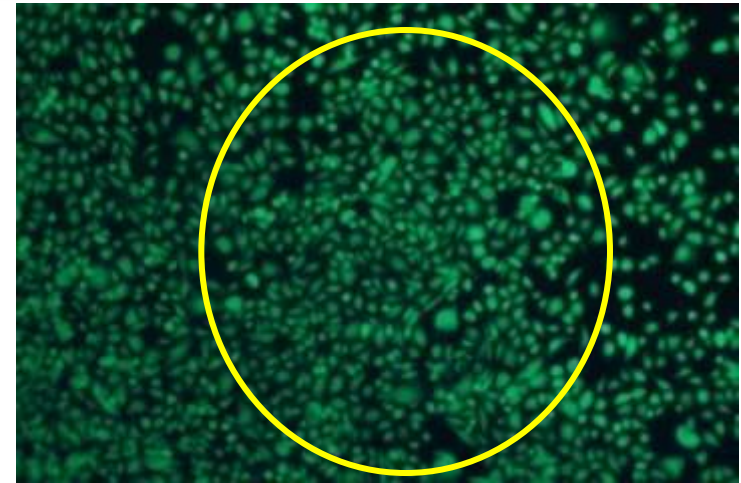
Gold/Gold-sulfide + Laser
Visibly **lower** scattering of
laser light



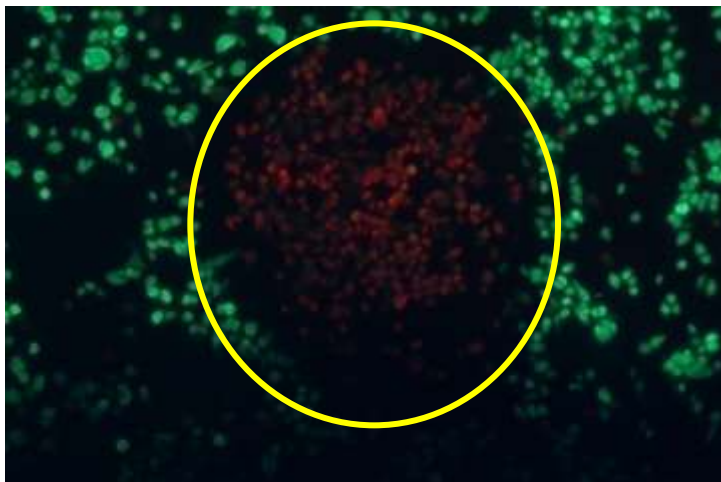
Gold/Silica + Laser
Visibly **higher**
scattering of laser light

Gold-Gold Sulfide Nanoshells Kill Cells with NIR Laser Exposure in *In Vitro* Experiments

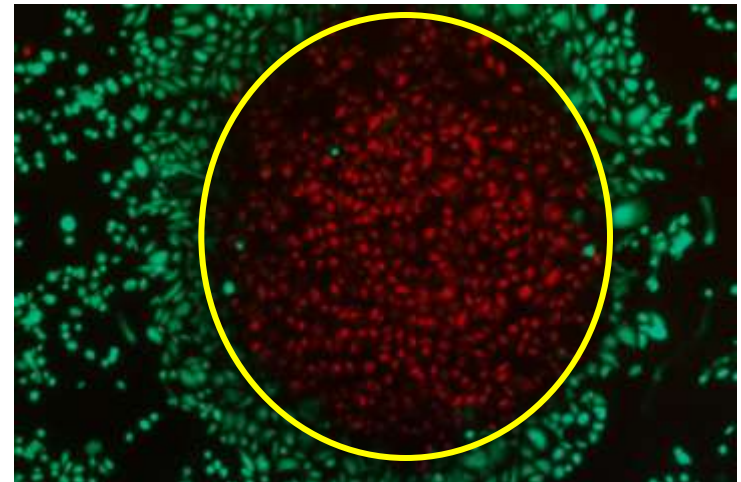
- Expose cells to nanoparticles
- Allow nanoparticles to bind
- Rinse cells of excess GNP
- Exposure to nIR light
- Stain for viability



No Nanoparticles + Laser



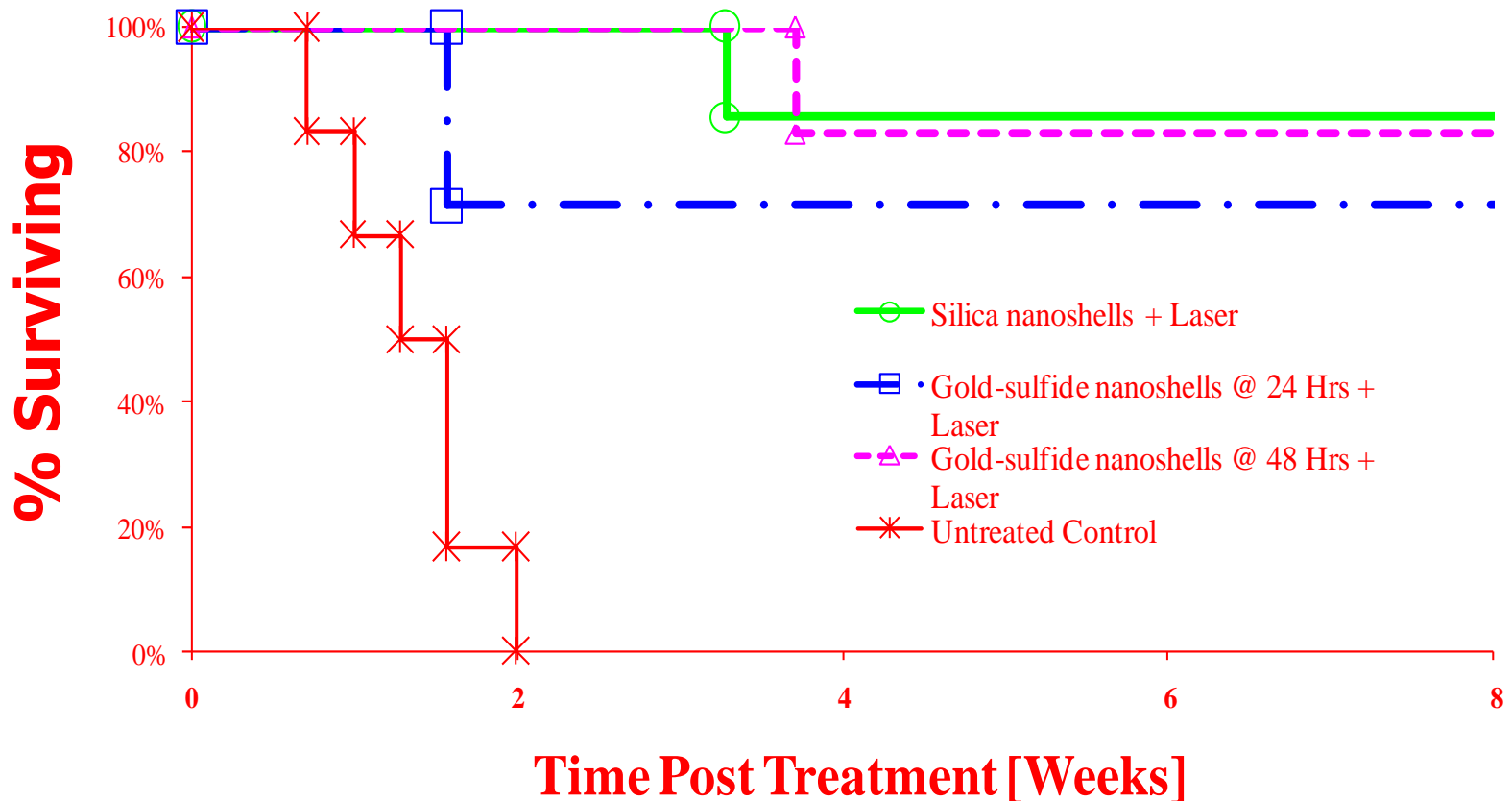
Silica-Gold Nanoshells + Laser



Gold / gold sulfide + Laser

Gold / Gold sulfide Shows Photothermal Therapeutic efficacy *in vivo*

- > 80 % survival after treatment



Current Research

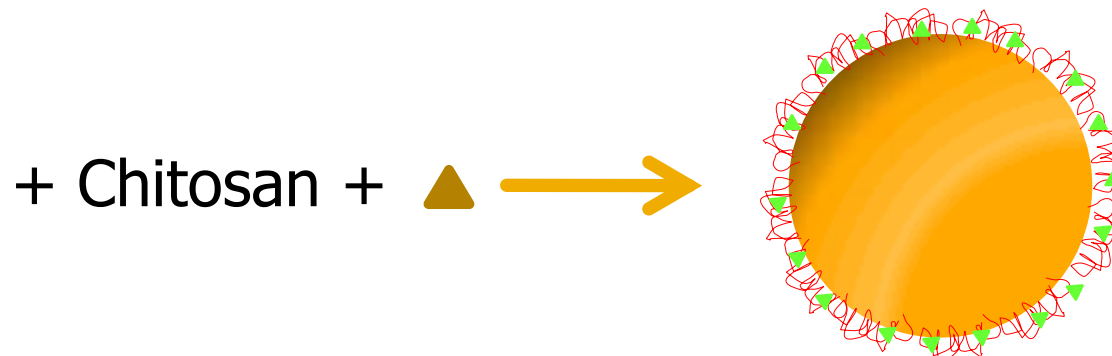
- Naturally derived polymer: Chitosan
 - Chitin: from shrimp & Crab-shells
 - De-acetylated chitin → chitosan
 - Free amine groups → positions for conjugation
 - Strong positive charge based on pH
 - Easy modification schemes
- Combine this natural polymer with our gold nanoparticles to yield new properties

Current & Future Research

- Self assembled coating of chitosan unto negative surface of gold nanoparticles

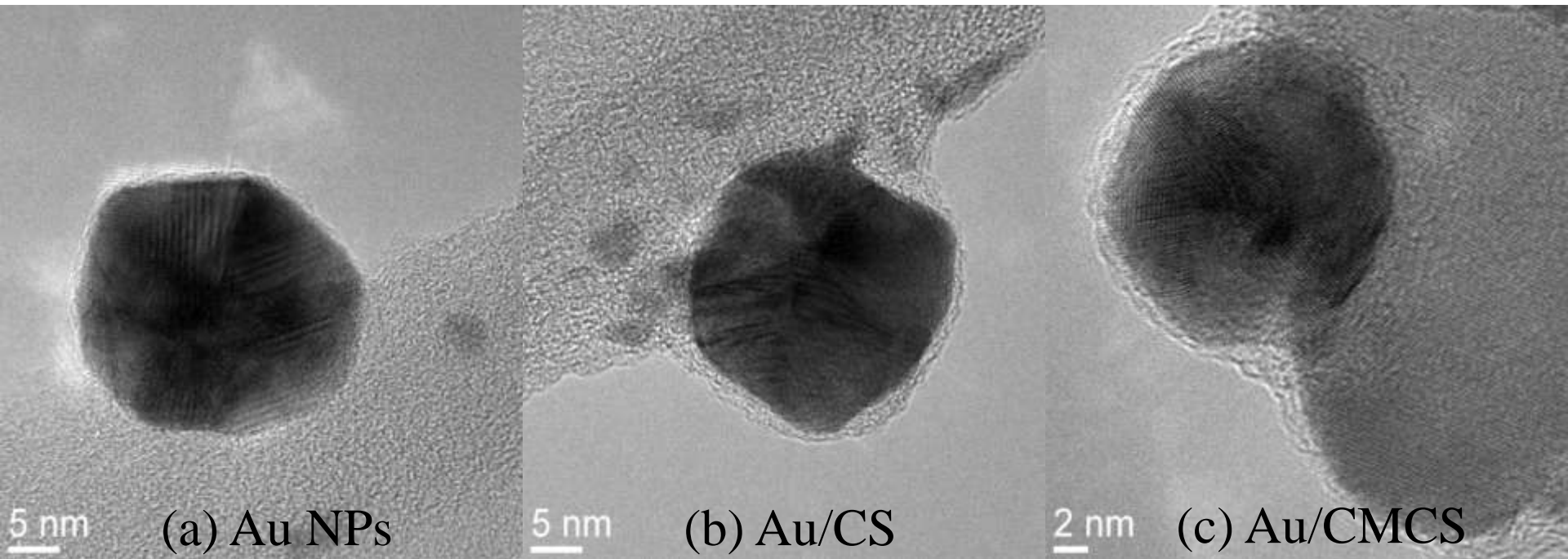


- + non covalent drug interactions



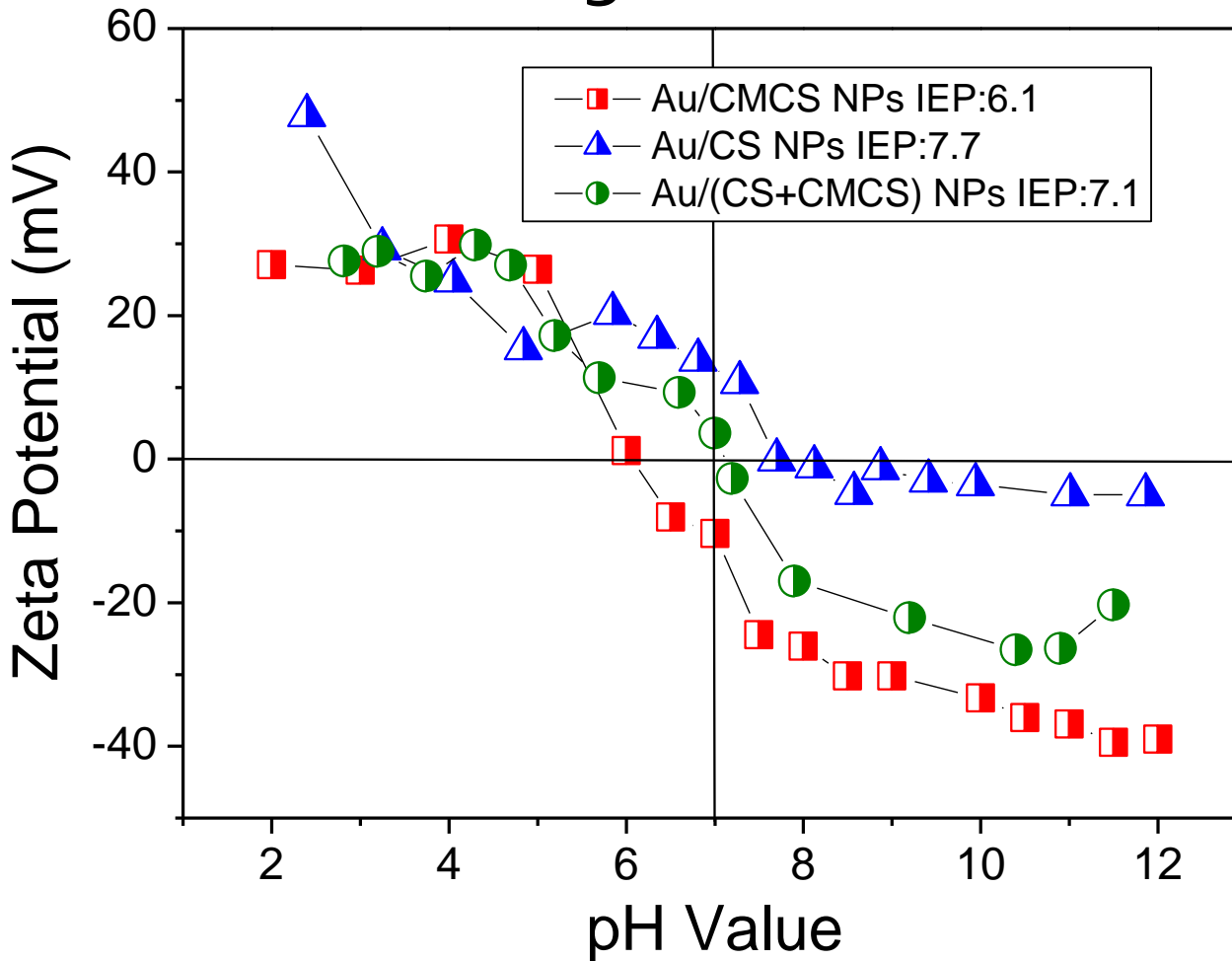
Chitosan coated gold / gold sulfide nanoparticles

- Soft chitosan coating visible on surface of nanoparticle after TEM



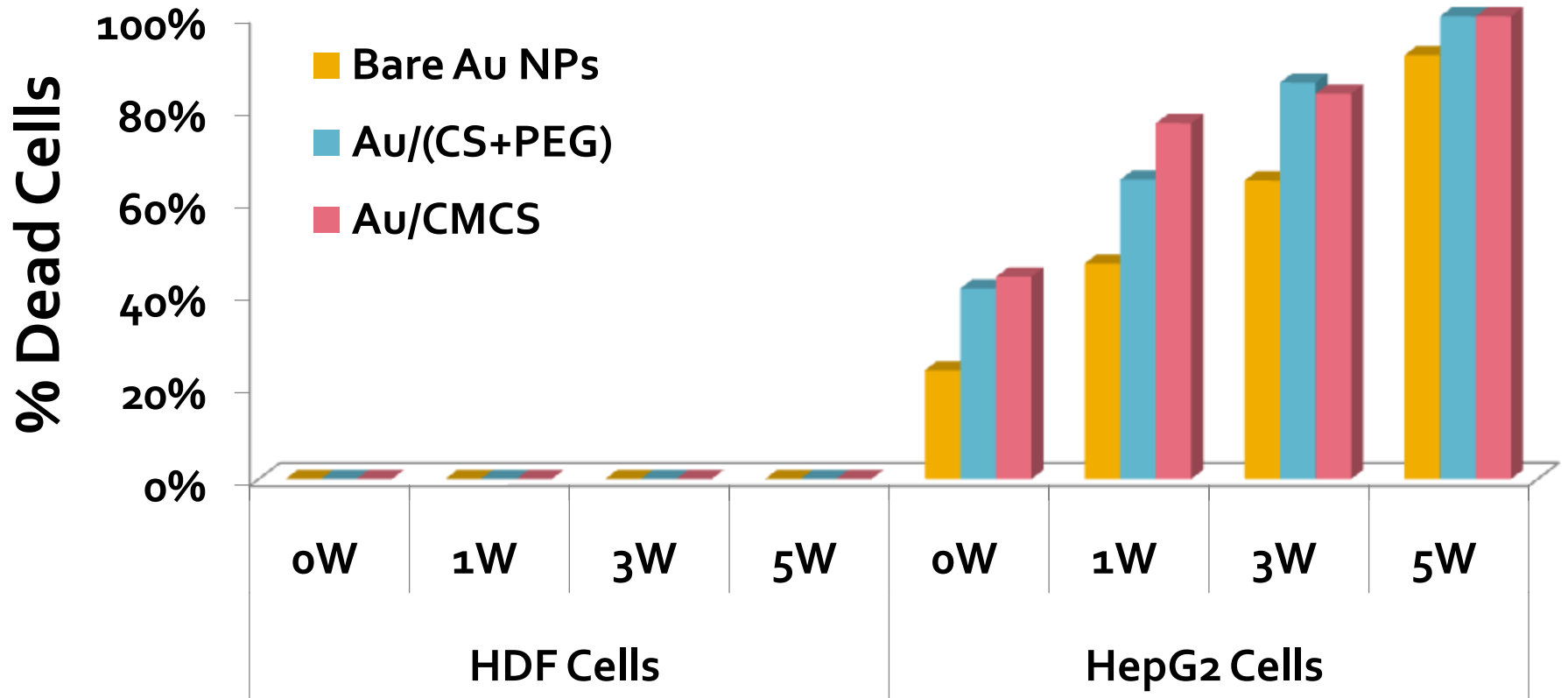
Surface Charge Tunability of Hybrid Chitosan/GGS Nanoparticles

- Tunable surface charge \rightarrow increased stability



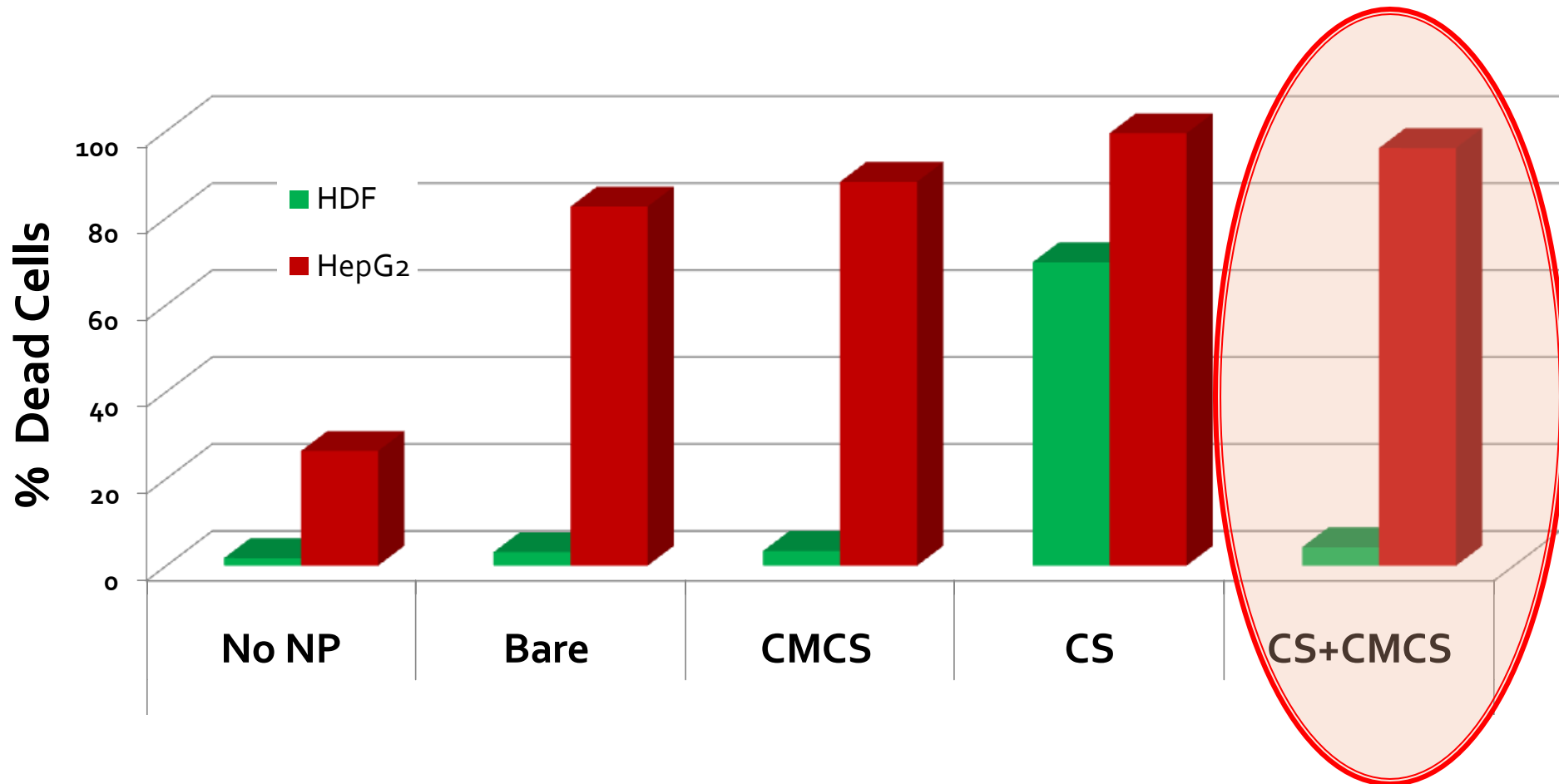
Efficacy of Chitosan/GGS Nanoparticles: Liver Cancer Model

- Laser @ 5W for 2 minutes



Efficacy of Chitosan/GGS Nanoparticles: Liver Cancer Model

- Hybrid Nanoparticles are specific to HCC in *co-culture*



Currently at U of L

- Chitosan Coated gold / gold sulfide nanoparticles as a therapeutic option for treatment of esophageal carcinoma.
 - Funding from Coulter Foundation (Phase I)
 - 2 - \$50k grants to aid with translation efforts (U of L)
 - Provisional Patent filed (09/09)
 - Full Patent Filing (09/10)
 - **Startup Company through partnership with Metacyte**
 - Invited for Coulter Phase II Proposal (June 2010)

Collaborations

- Outside UofL
 - Lakeshia Taite, Ph.D. (GATech)
 - Hybrid nanoparticle for synthesis & separation of polymer systems
 - Amanda Lowery, Ph.D. (Vanderbilt)
 - Enhancement of radiation treatment with gold nanoparticles

Acknowledgements

- Funding:
 - Coulter Foundation: Early Career Phase I
 - University of Louisville: Startup Funds & Clinical & Translational Science Program Basic Award
 - NSF – EPSCoR
- Lab Members
 - Guandong Zhang- Ph.D.
 - Ines Mullic – B.S., MEng.
 - Dhru Patel – B.S.
 - Deborah Hickman (KBRIN student)
 - Marcus Bradbury (KBRIN student)