Golden Opportunities: Gold Nanocrystals for Biomedical Applications

Catherine J. Murphy and her group
University of Illinois at Urbana-Champaign

Walton Lecture, Purdue University, Fall 2019

Art Before Science

Lycurgus Cup, on a visit to the
Art Institute of Chicago

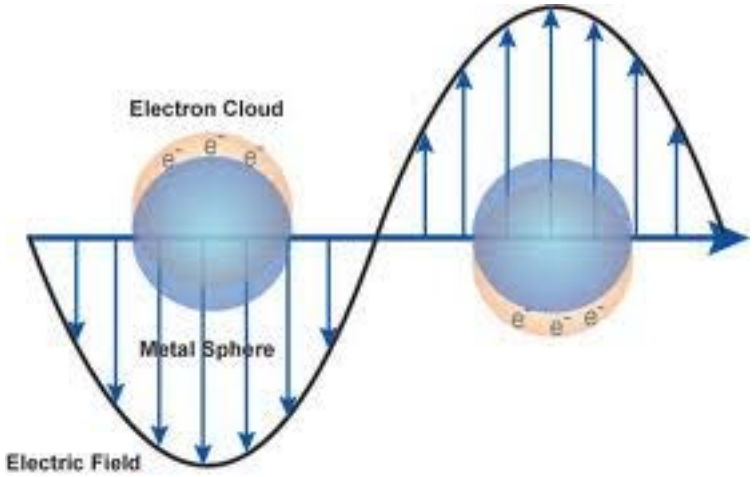
Michael Faraday's colloidal gold
Royal Institution, London

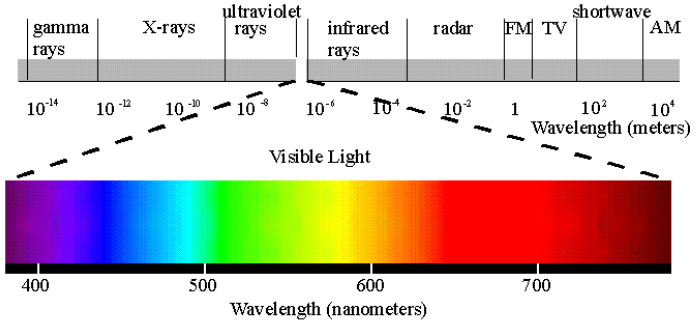
the plasmon

- mean free path of e^- in metal: ~ 10-100 nm

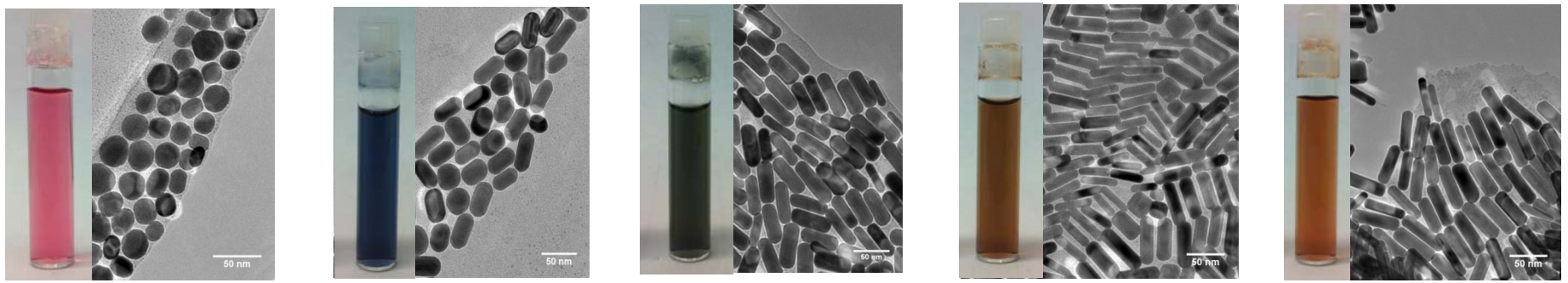
- G. Mie (1908): light impinges upon the “small conducting sphere”

- down to ~4 nm for gold/silver, particles still large enough to support a conduction band; *plasmon* = coherent oscillation of conduction band electrons





Gold Nanorods: strong absorbers and scatterers



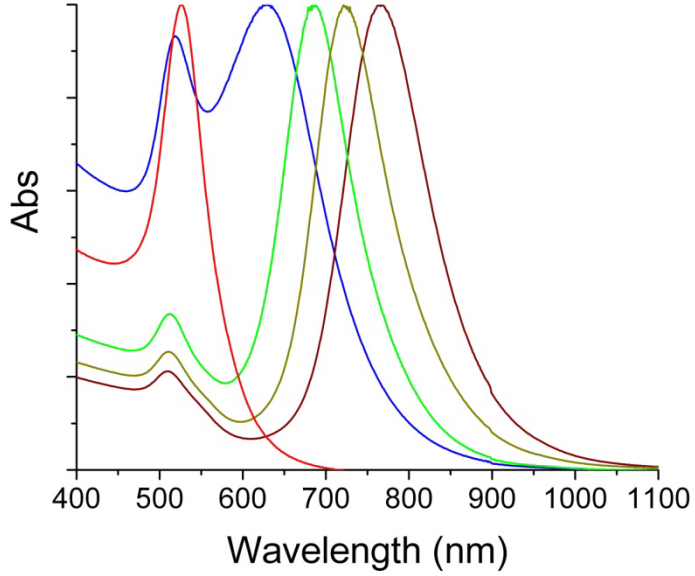
Long Axis 20.8 ± 5.1 nm
Short Axis 17.3 ± 2.9 nm

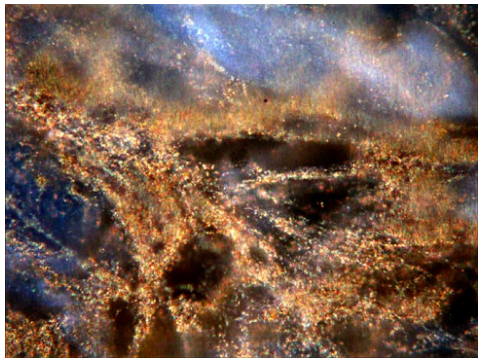
Increasing Aspect Ratio

Long Axis 56.2 ± 3.9 nm
Short Axis 16.3± 1.6 nm

Au nanorods:
Aspect ratio 1-20

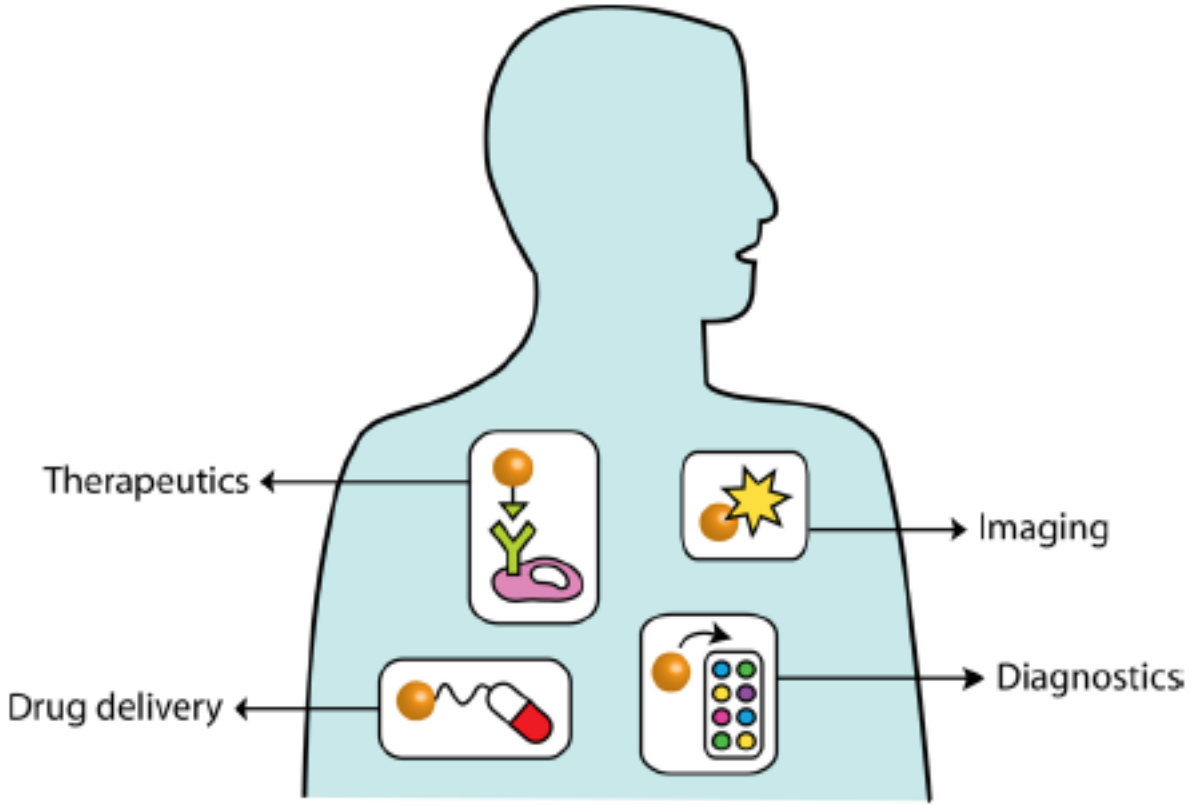
Au nanowires:
Aspect ratio > 20





The Promise of (Gold) Nanotechnology for Human Health

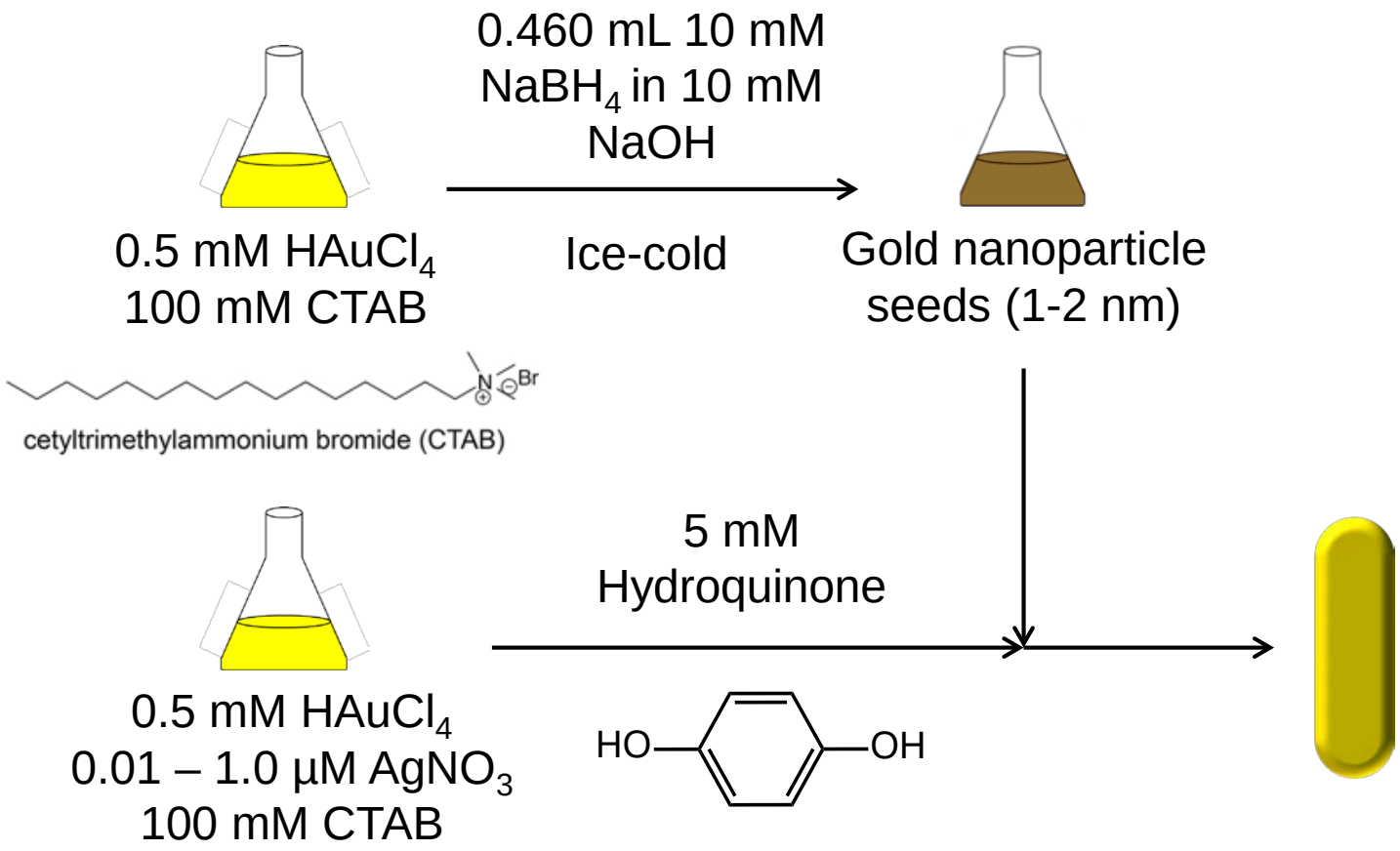
Photothermal properties



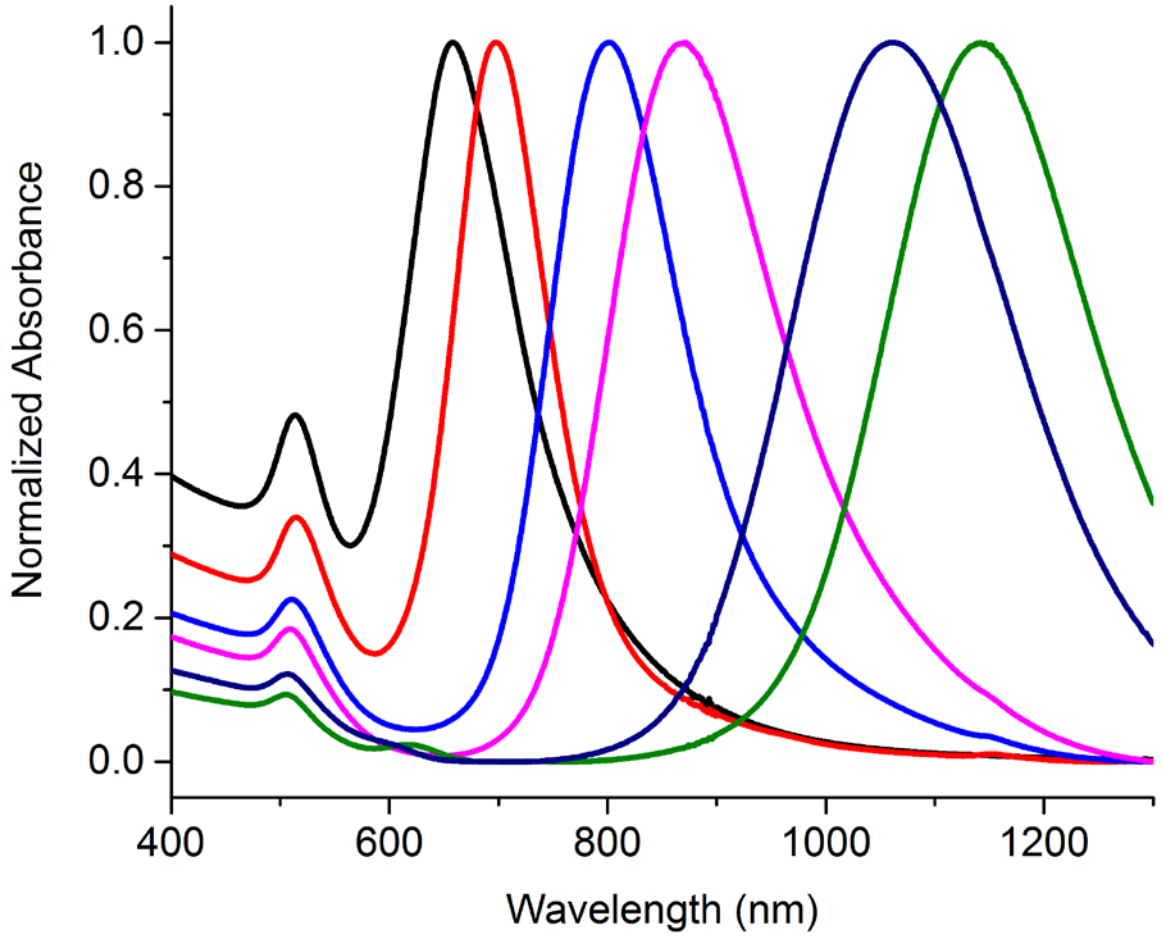
Surface-enhanced spectroscopies

Murphy et al, *ACS Central Science* **2015**, *1*, 117-123.

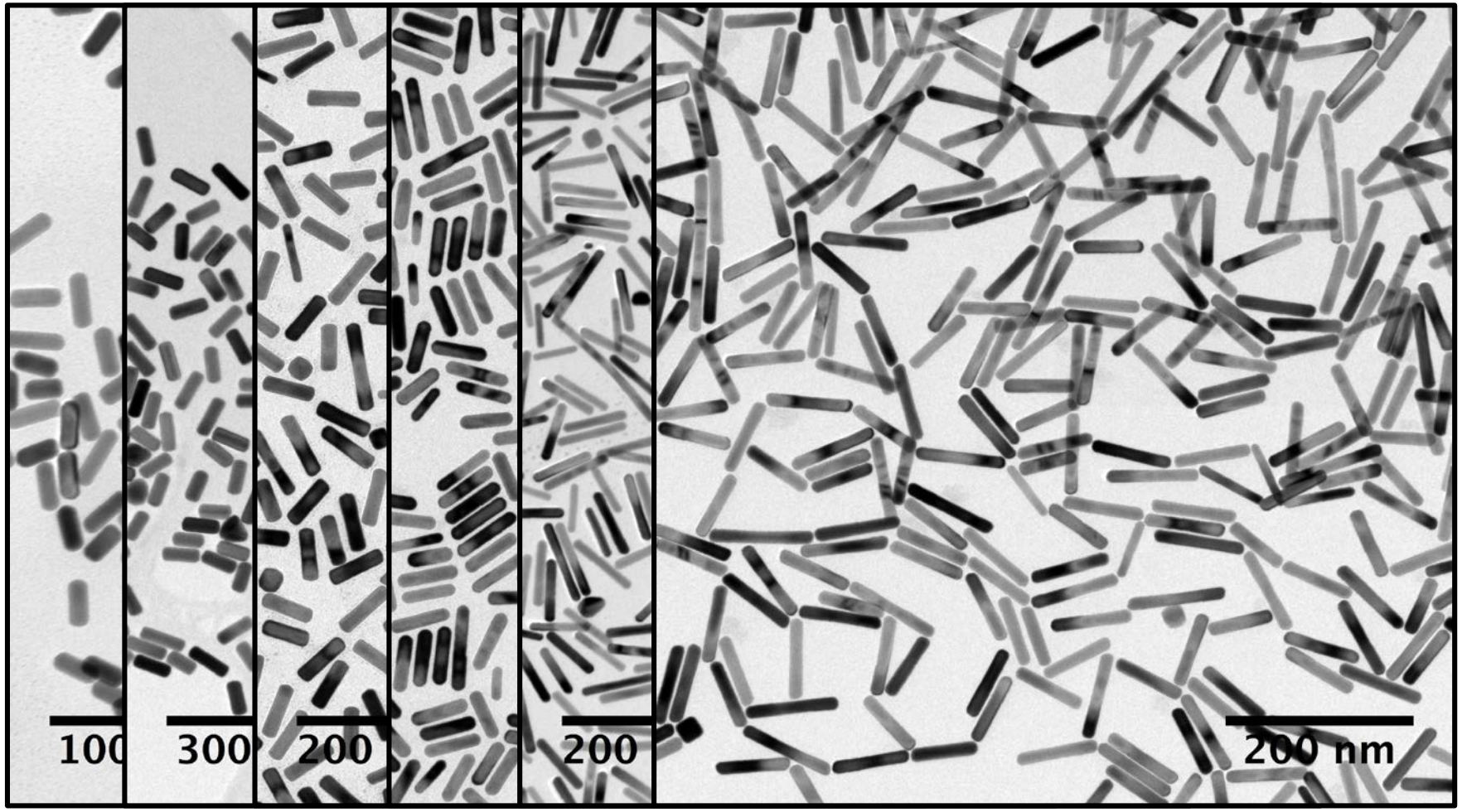
Seed-Mediated Growth



Pushing plasmons further out



Beautiful particles!



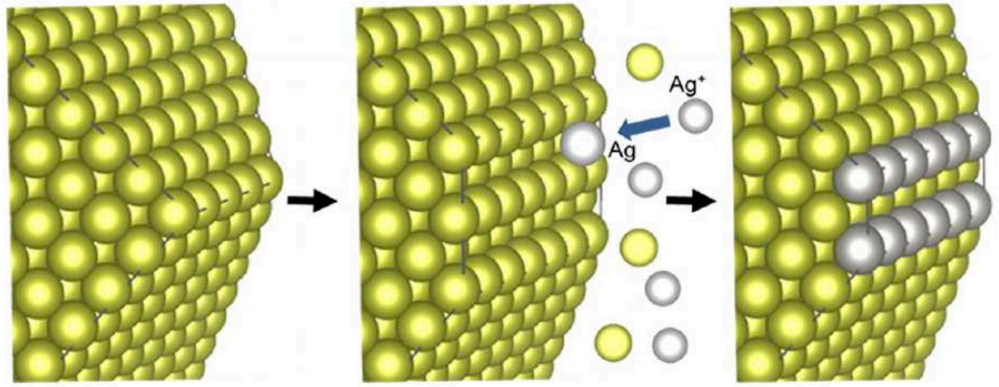
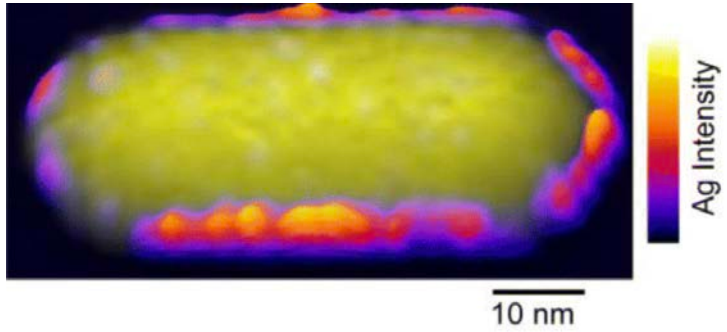


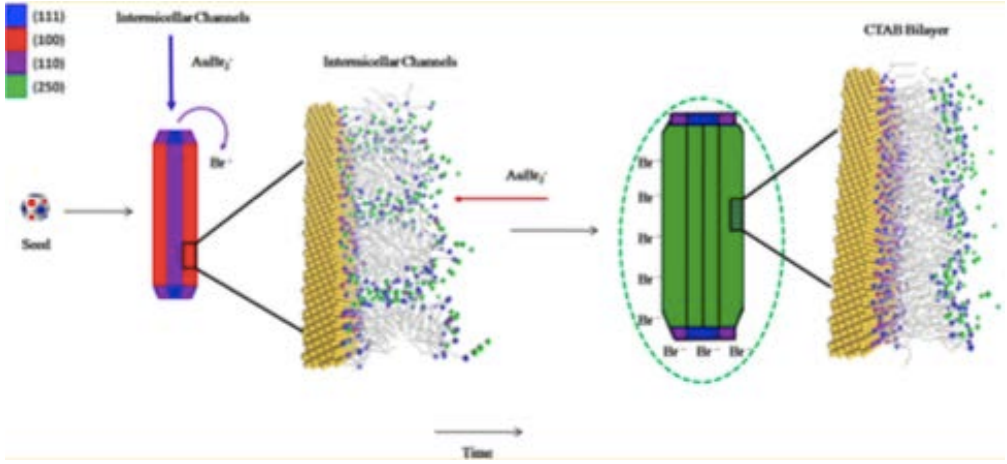
Figure 6. Schematic depiction of the formation of a truncating surface and its stabilization by Ag UPD. The facet size at which Ag deposition can begin is dependent on the $[HAuCl_4]$:$[AgNO_3]$ ratio.

Funston et al, *Acc. Chem. Res*. **2017**, *50*, 2925-2935; 4 nm minimum particle size postulated to break symmetry by exhibiting these facets to favor rods with silver UPD…



…but silver is all over??
JACS **2014**, *136*, 5261-5263, Rosenthal, Wright et al …

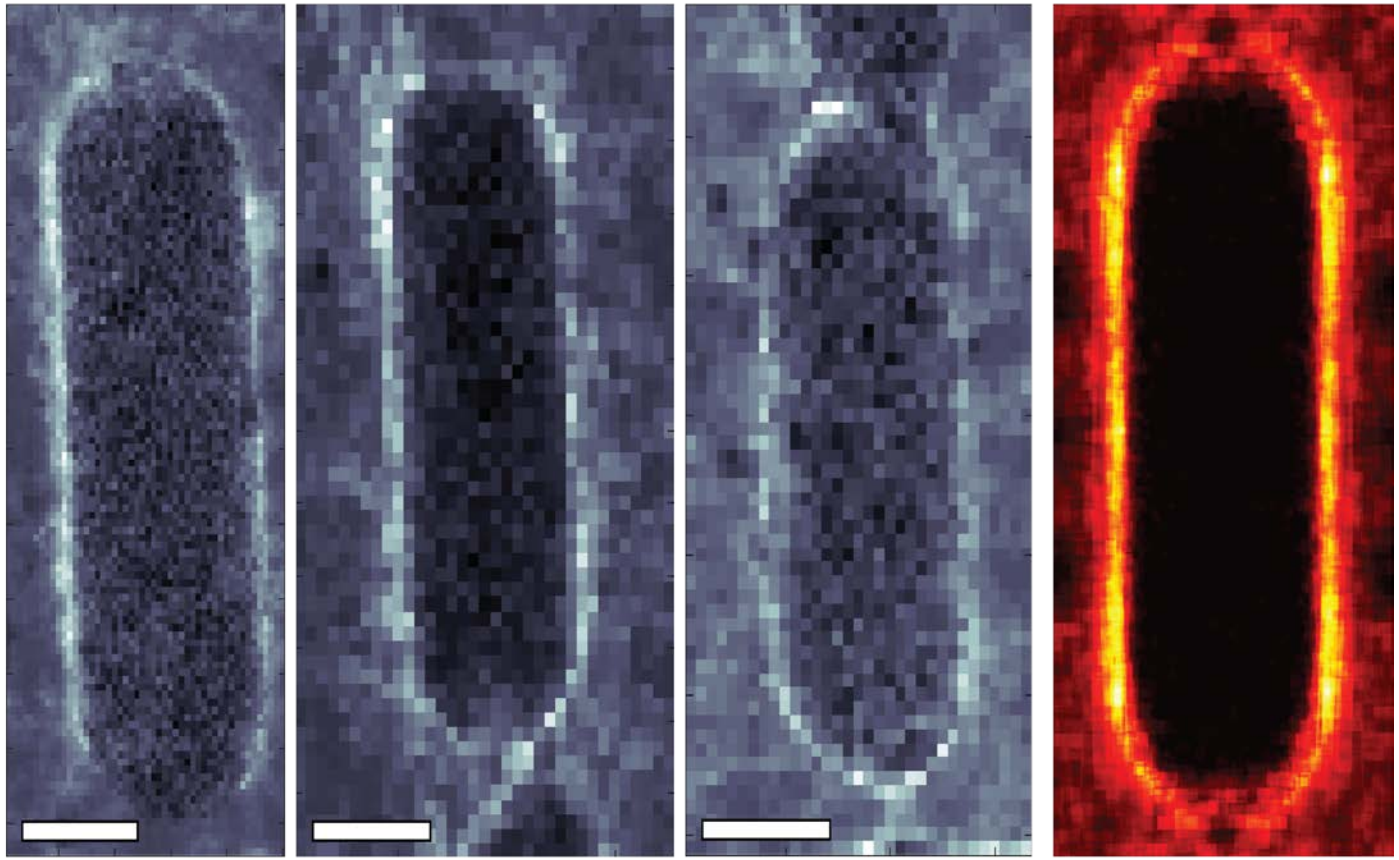
…but maybe silver is mobile?

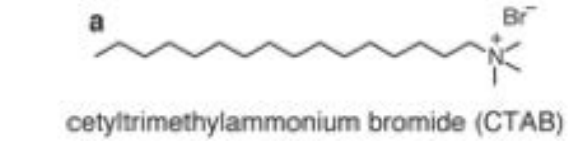


Micellar nature of CTAB important on surface: “channels” for $[AuBr_2]^-$ transport to surface. MD simulations, *Langmuir* **2018**, *34*, 366-375.

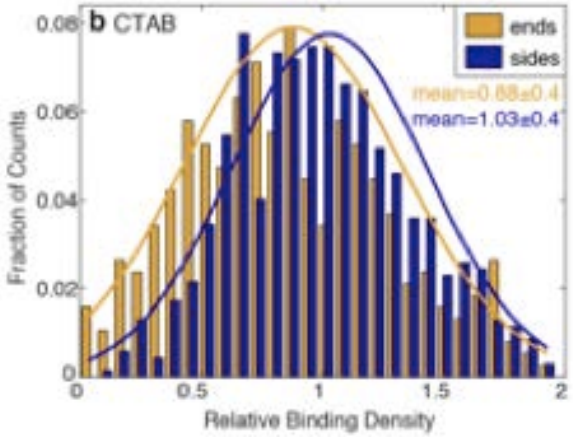
How close are we to the dream (*in situ* imaging of NP growth at atomic level with elemental analysis information)?

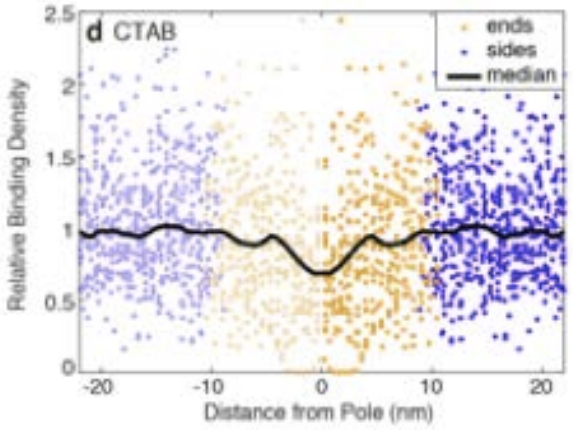
How close are we to the dream (*in situ* imaging of NP growth at atomic level with elemental analysis information)?





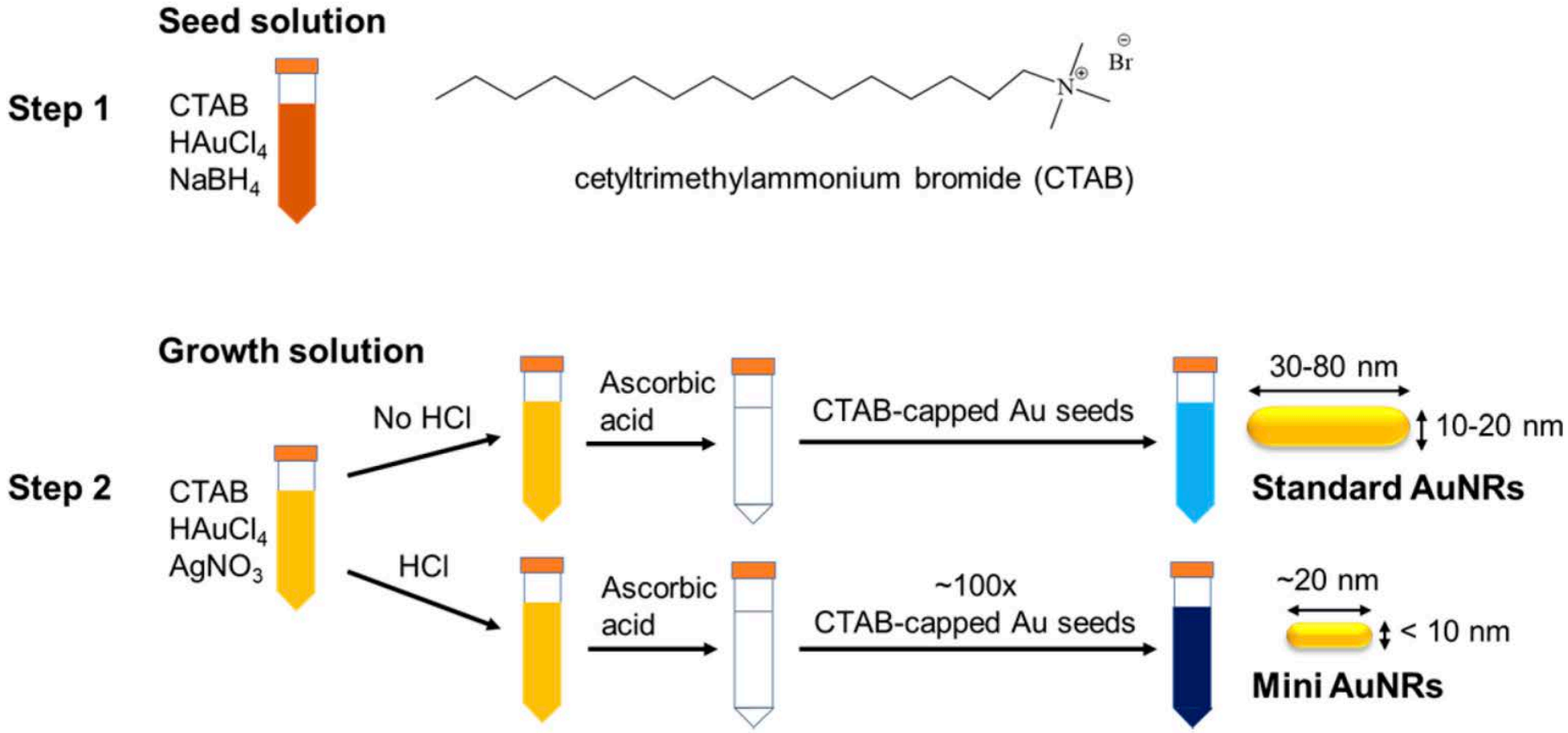
cetyltrimethylammonium bromide (CTAB)





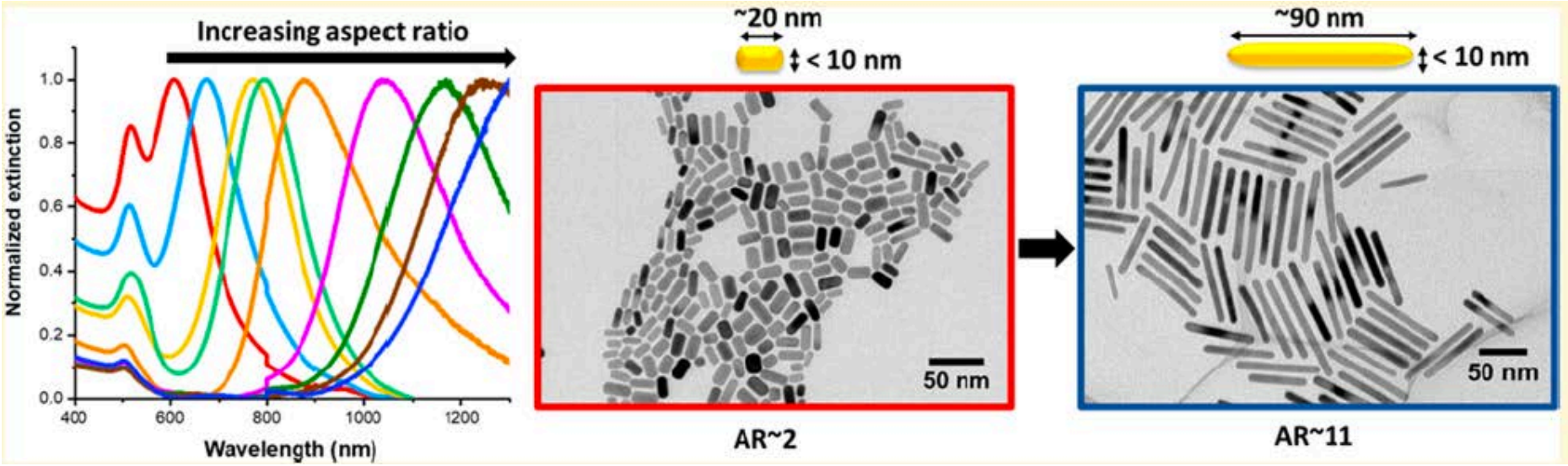
Quantitative EELS mapping of carbon signal compared to single layer graphene grid!

Mini rods



Chem. Mater. **2018**, *30*, 1427-1435.

Mini rods



"Regular" rods: short axes 12-20 nm
"Mini" rods: short axes 5-8 nm

Chem. Mater. **2018**, *30*, 1427-1435.

Sustainability bonus!

15-fold less gold per particle

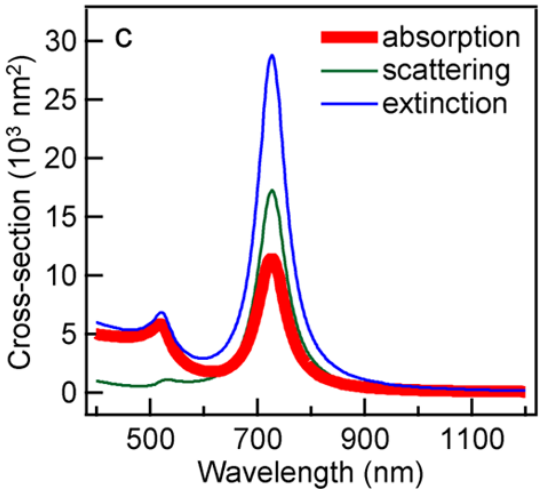
79-100% yield compared to ~25%

Extinction coefficients decrease 10-fold, but absorption proportion up

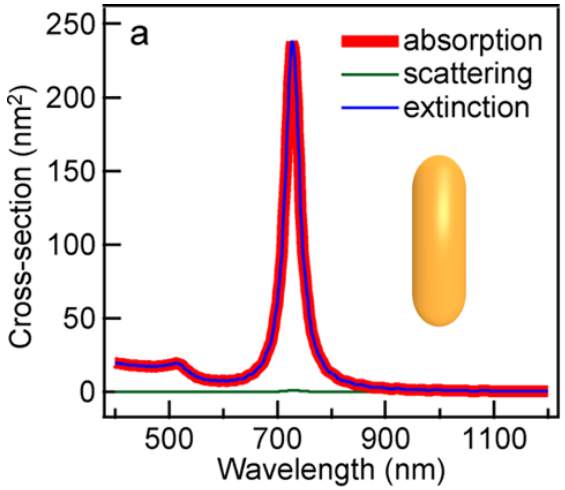
Mini rod bio-bonuses

Photothermal therapy

Short AuNRs:
16.6 ± 1.3 x 40.2 ± 2.8 nm



Mini AuNRs:
6.0 ± 0.6 x 16 ± 3 nm

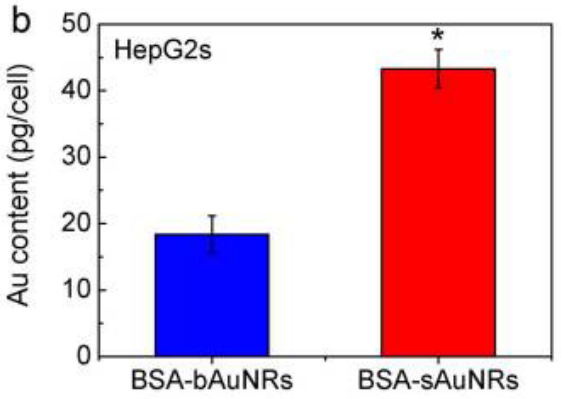


Finite-difference time-domain (FDTD) calculations:
Mini AuNRs are absorption dominant

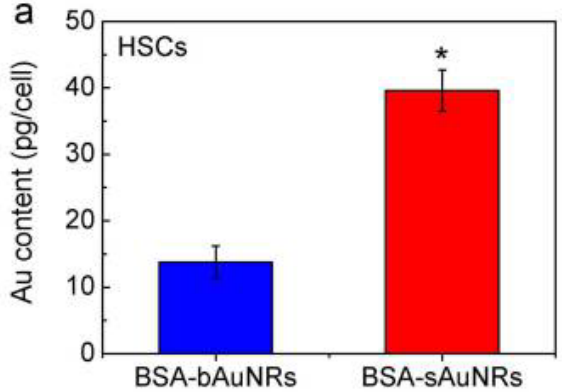
Jia, H. et al. *Langmuir* **2015**, *31*, 7418-7426.

Higher cellular uptake

Uptake of 7 x 35 and 14 x 56 nm BSA AuNRs

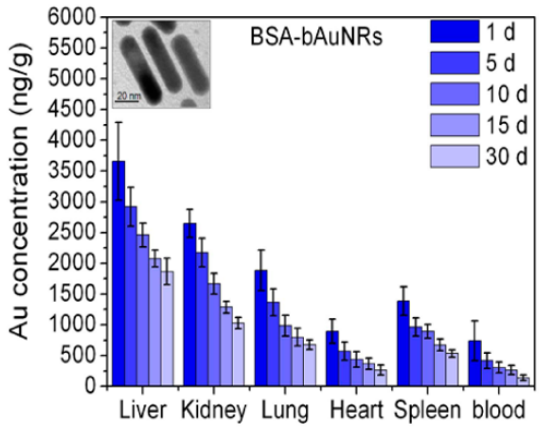


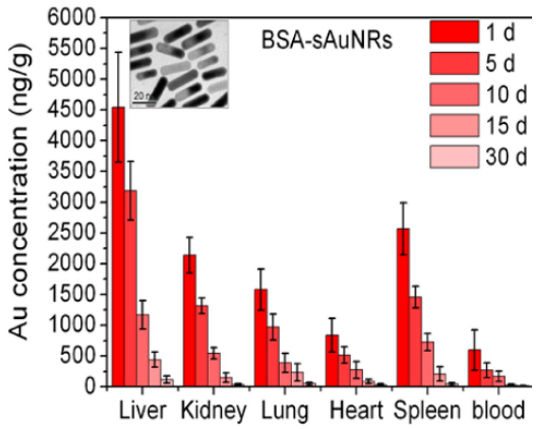
Hepatocellular carcinoma cells



Hepatic stellate cells

Faster organ clearance

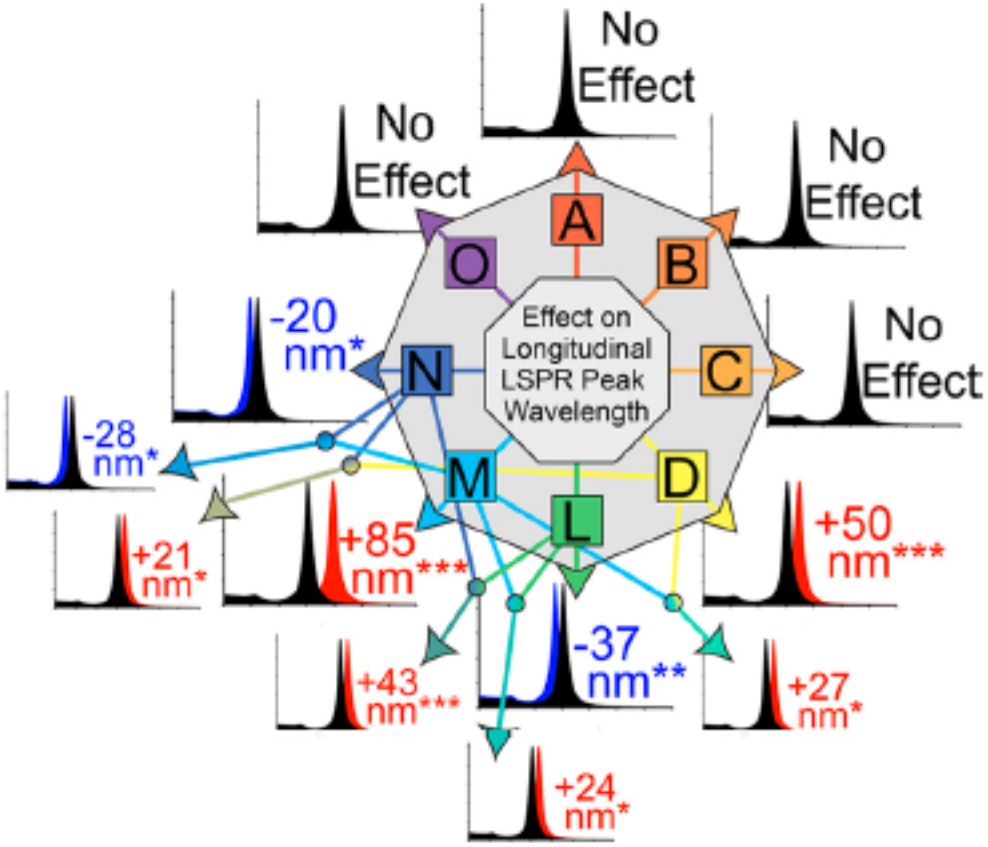




Li, Z, et al. *ACS Biomater. Sci. Eng.* **2016**, 2, *789-797.*

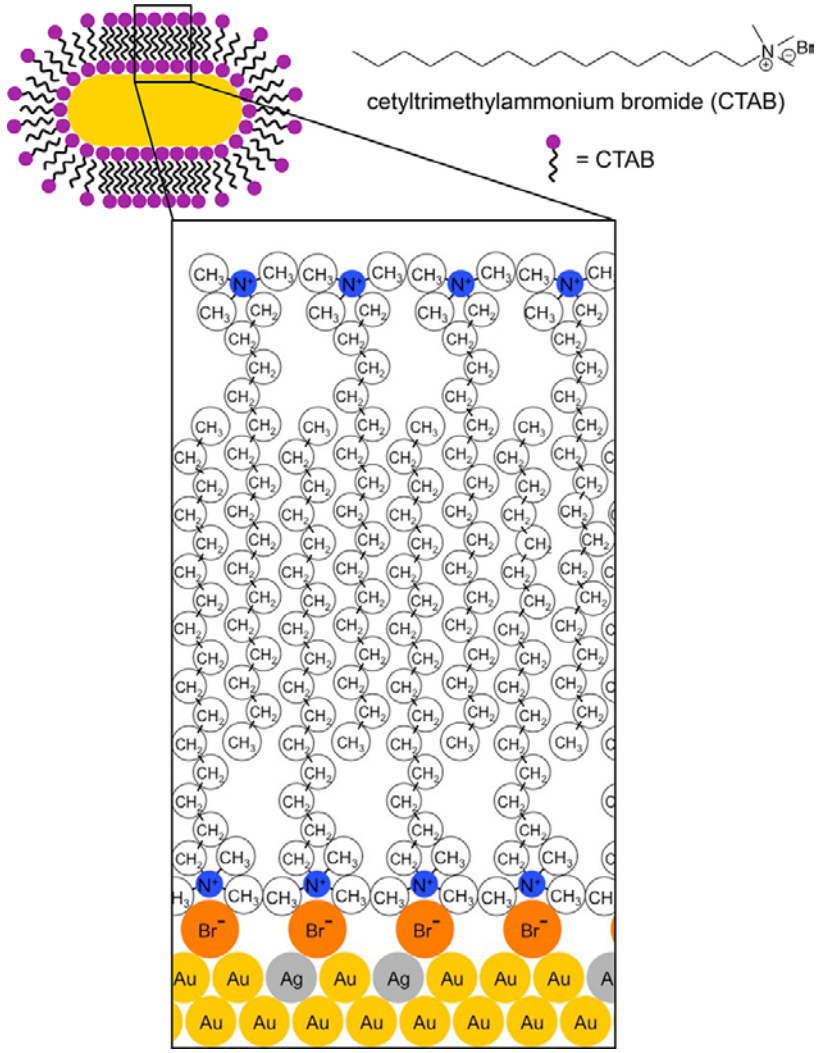
Why are nanoparticle preps so picky?

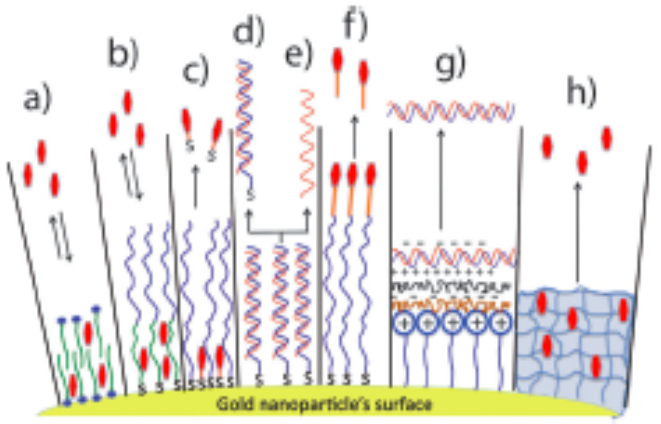
	factors	standard protocol	low (−)	high (+)
A	amount of $NaBH_4$	0.0378 g	0.0378 g	0.0450 g
B	rate of stiring seed solution		260 rpm	750 rpm
C	age of seed solution	1 h	1 h	5 h
D	amount of seeds	12 μL	12 μL	60 μL
L	temperature	room	26 °C	50 °C
M	amount of silver (0.0100 M)	variable	40 μL	90 μL
N	amount of ascorbic acid (0.100 M)	55 μL	55 μL	70 μL
O	age of reduced solution		1 min	30 min



Langmuir **2017**, *33*, 1891-1907.

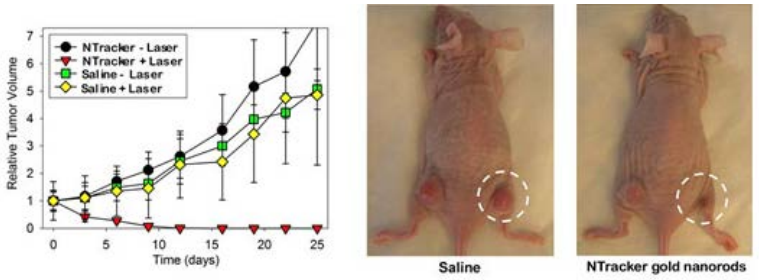
Final surface: bilayer of quaternary ammonium surfactant; many options for next reactions



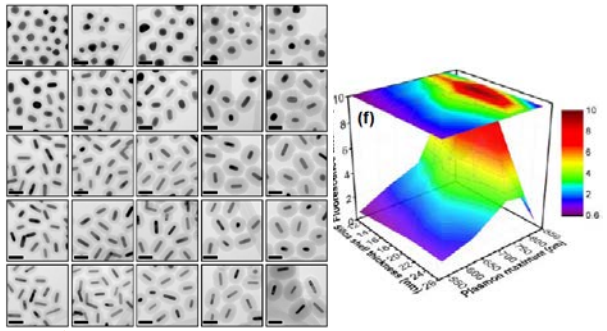


Dreaden, E.; Huang, X.; Alkilany, A. M.; Murphy, C. J.; El-Sayed, M. A. "The Golden Age: Gold Nanoparticles for Biomedicine," *Chem. Soc. Rev*. **2012**, *41*, 2740-2779.

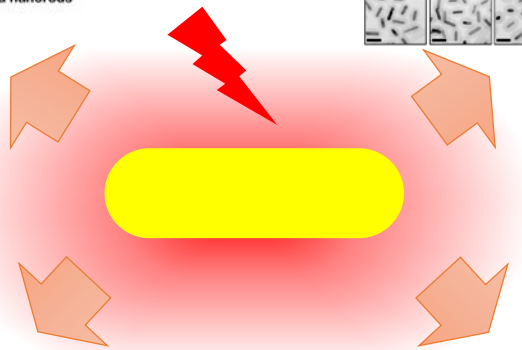
Applications: Get electric fields, light, heat upon resonant illumination

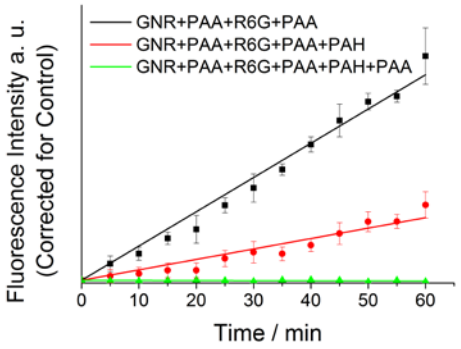


Photothermal cancer therapy:
Companies/clinical trials;
NanoPartz

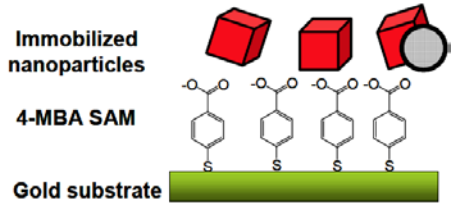


Imaging agents:
ACS Nano **2014**, *8*, 8392.

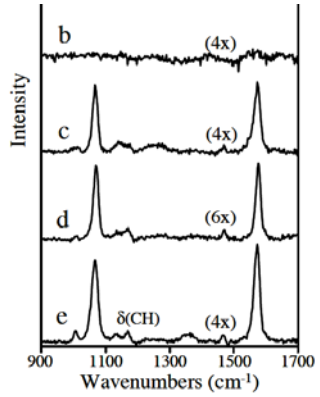




Controlled molecular delivery:
Nano Lett. **2012,** *12*, 2982.



Diagnostics:
Anal. Chem. **2005**, *77*, 3261.



In addition to plasmons: Practical advantages of colloidal gold

- Detection: ICP-MS with almost zero environmental background; large Z for TEM of complex samples; SERS tags with Raman probe

- Stability: gold should not oxidize under most biological conditions

- Size and shape control: spheres, rods, prisms, stars…

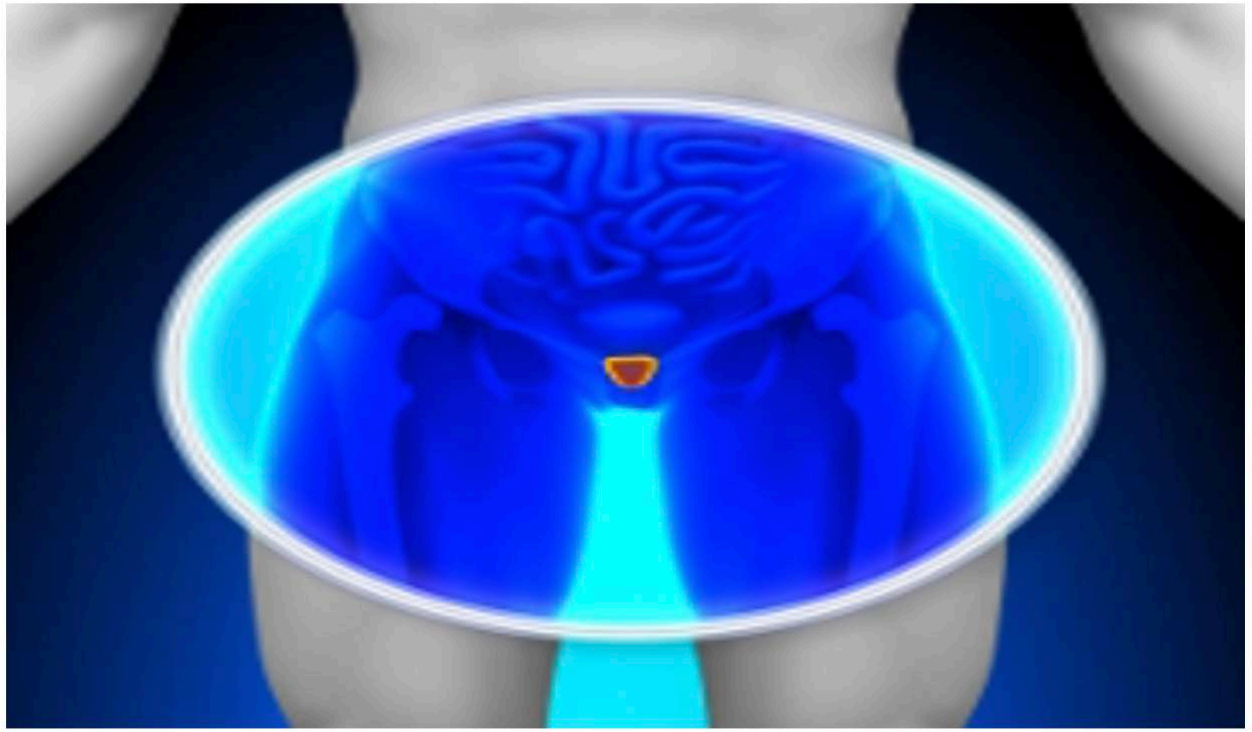
- Surface chemistry: many possibilities

Brachytherapy

https://www.urologists.org/article/treatments/brachytherapy-gold-seed-implant

Brachytherapy (Gold Seed Implant)

SHARE Tweet



Brachytherapy is a type of radiation treatment used to treat various types of cancers; but in urology, it is commonly used to destroy cancerous cells that affect the prostate. Also known as internal radiation therapy, brachytherapy involves placing high-energy (radioactive) material inside the body to kill cancer cells and halt the growth of tumors. The radioactive material is sealed within catheters, wires, seeds, or needles and placed either near or directly inside of the mass.

Commercial colloidal gold nanocrystals

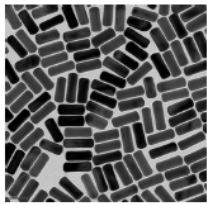
NANOPARTz™

Product Release

Gold Nanorods

August 28, 2007 420 Chipeta Dr. Salt Lake City, UT 84108-1256

Nanopartz, Inc. released a new line of highly monodisperse gold nanorods.



Monodisperse Nanorods

Utilizing exclusively licensed patent pending technologies developed by Dr. Cathy Murphy at the Univ. of South Carolina and Dr. Eugene Zuberev at Rice University, Nanopartz, Inc. released a new line of highly monodisperse gold nanorods. These nanorods are particularly suited to diagnostics as well as biomedical imaging and photothermal therapy applications. Specifically, nanorods may be used to selectively destroy solid cancer tumors. The nanorods are delivered systemically and then activated by a near-infrared laser outside the body, resulting in the thermal destruction of the tumor and the blood vessels supplying them without significant damage to healthy tissue. In addition to the elimination of solid tumors, potential applications of nanorods include cancer detection, the rapid, sensitive detection of biomolecules and biodefense agents, surface-enhanced Raman scattering, the treatment of macular degeneration, laser tissue welding, microfluidic devices, and optical protection.

Gold nanoparticles are one of the most widely used classes of nanomaterials for chemical, bioanalytical, biomedical, optical and nanotechnological applications. While there are numerous methods known for the synthesis of gold nanoparticles, the ability to control the size, shape and monodispersity for gold nanoparticles is one of the important areas in which few standard protocols have been established to allow preparation of gold nanoparticles of desired sizes, shapes and monodispersity in a systematic way. Such ability is critical for many applications. Nanopartz can manufacture nanorods with aspect ratios from 1.67 to 3.8, resulting in absorptions from 560 nm to 800 nm.

Compared to other types of nanoparticles including spheres and shells, nanorods are more favorable for in vivo applications due to their tunable optical resonance in the NIR region. Moreover, their relative scattering to absorption contribution can be easily tuned by a change in their dimensions. Gold nanorods

NanoHYBRIDS

ADVANCED IMAGING SOLUTIONS

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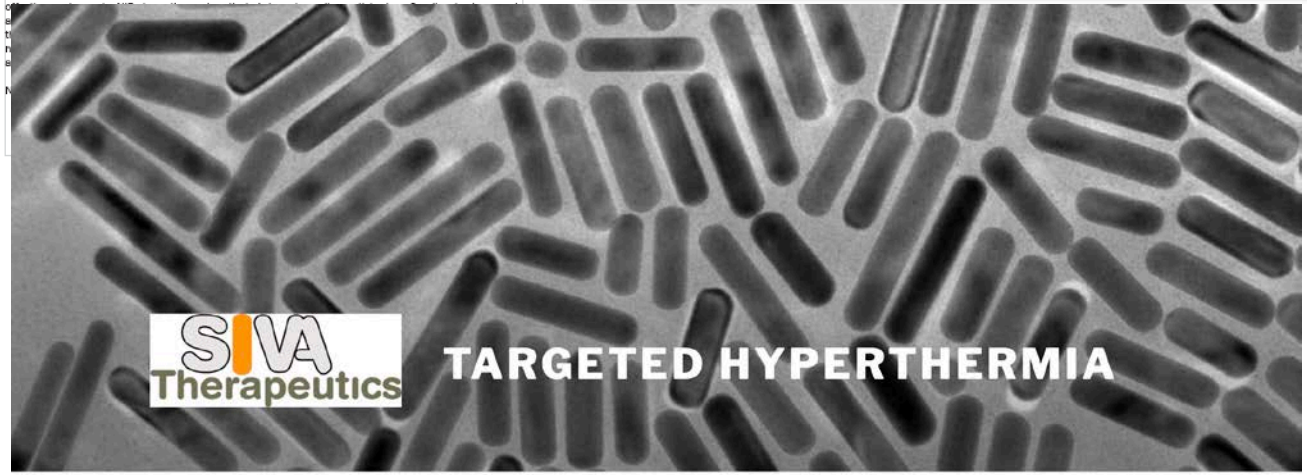
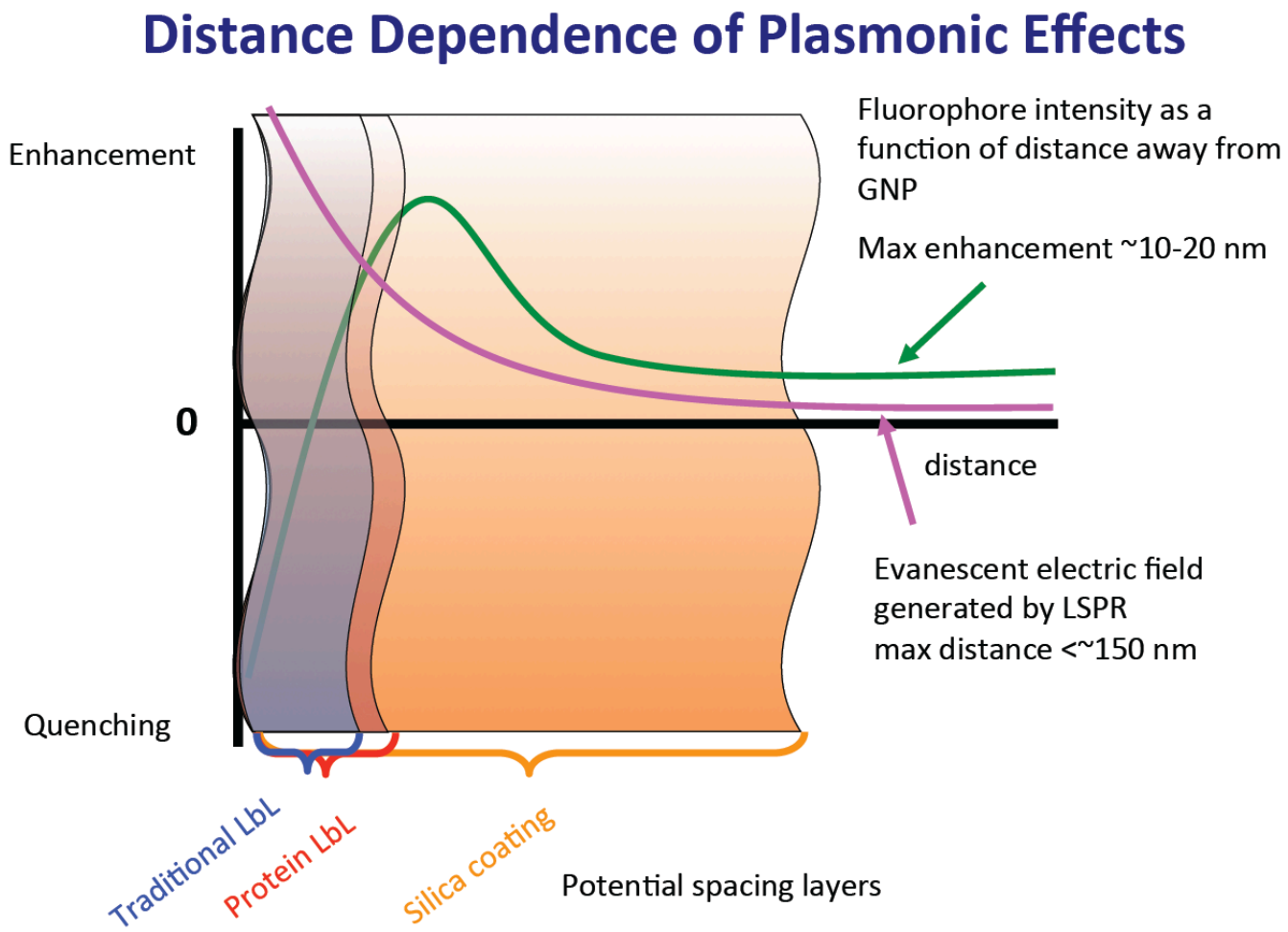
Premium, Monodisperse Gold Nanoparticles for Researchers

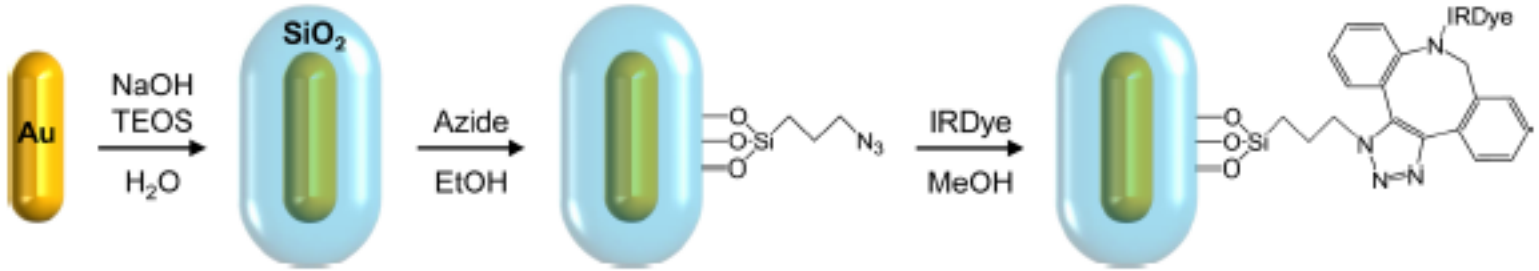


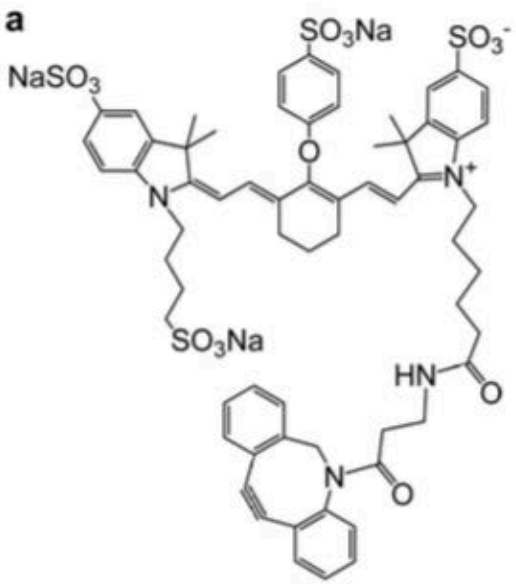
Colloidal gold: Sold as nutritional supplement (!)

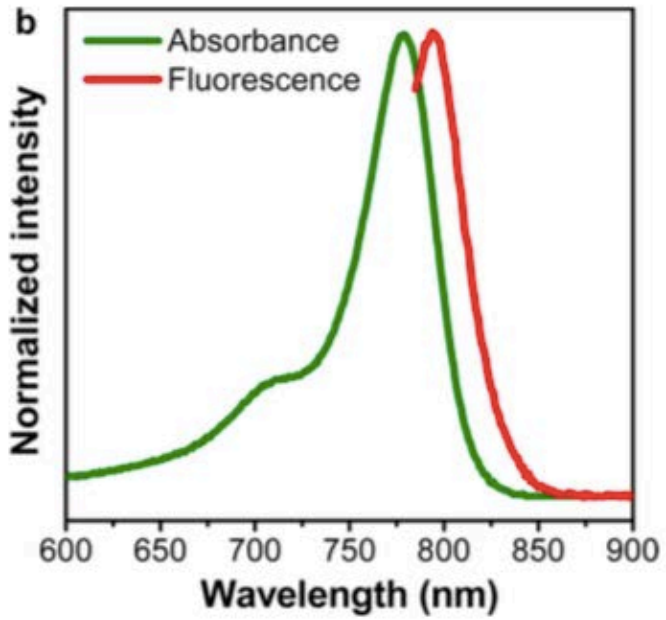
Plasmon-enhanced bioimaging agents

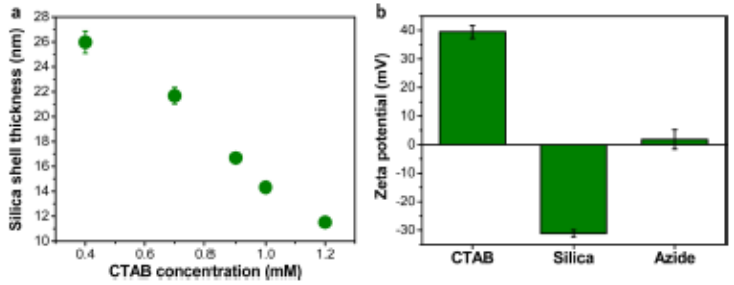


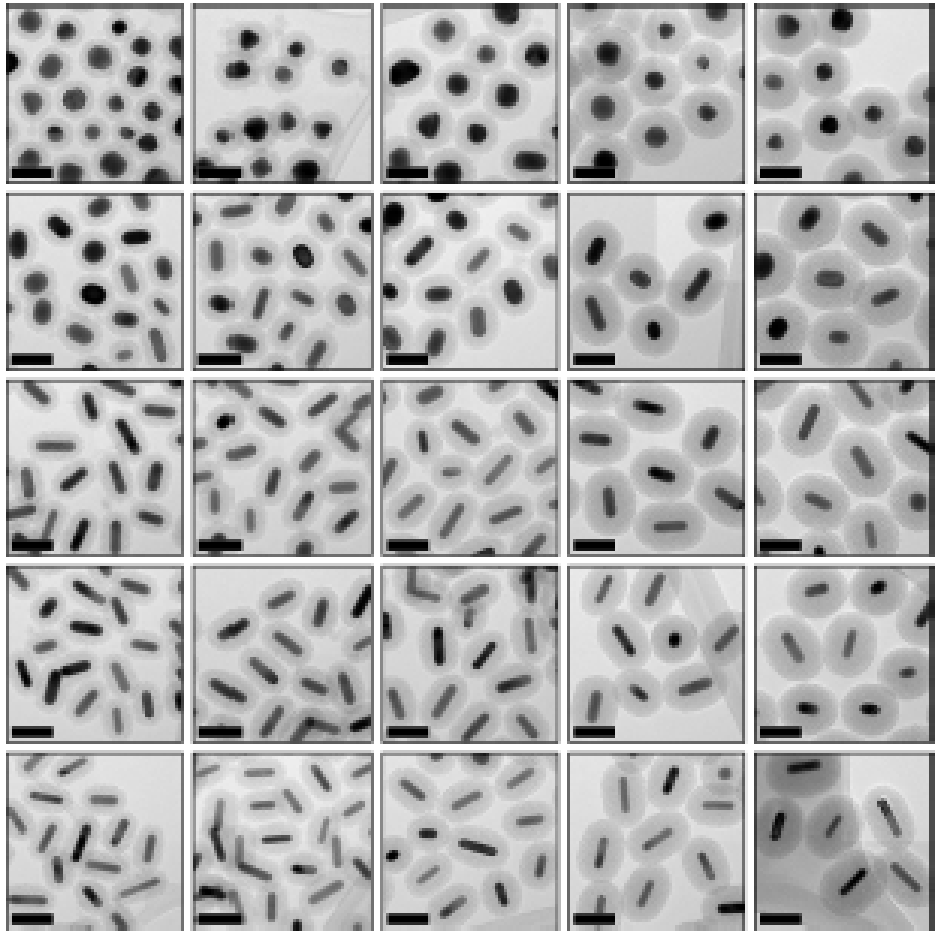
Silica spacer layer

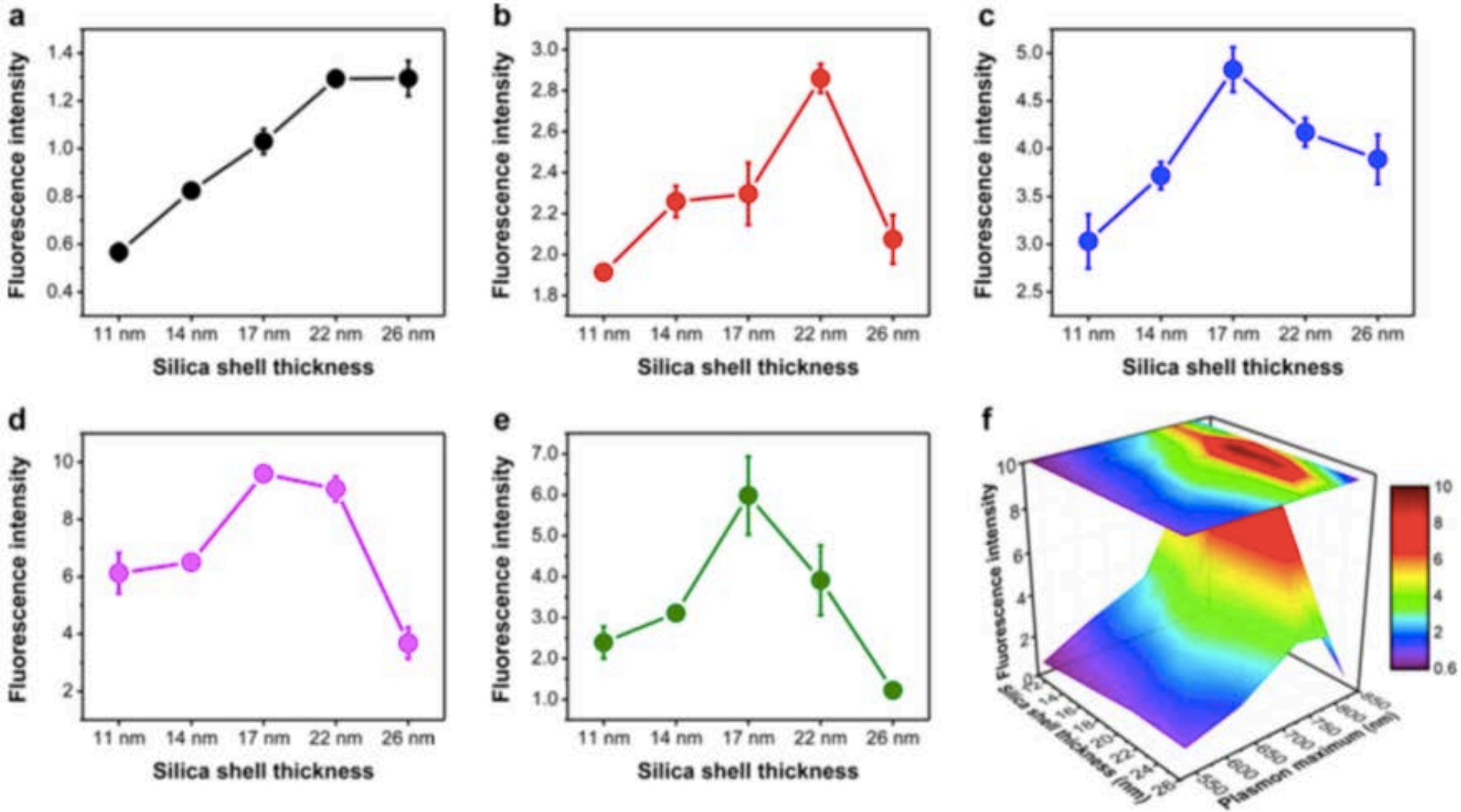







a
Silica shell thickness (nm)
CTAB concentration (mM)
b
Zeta potential (mV)
CTAB
Silica
Azide





abcde = plasmon band at 535, 650, 720, 776, 820 nm (corrected for AuNR absorption)

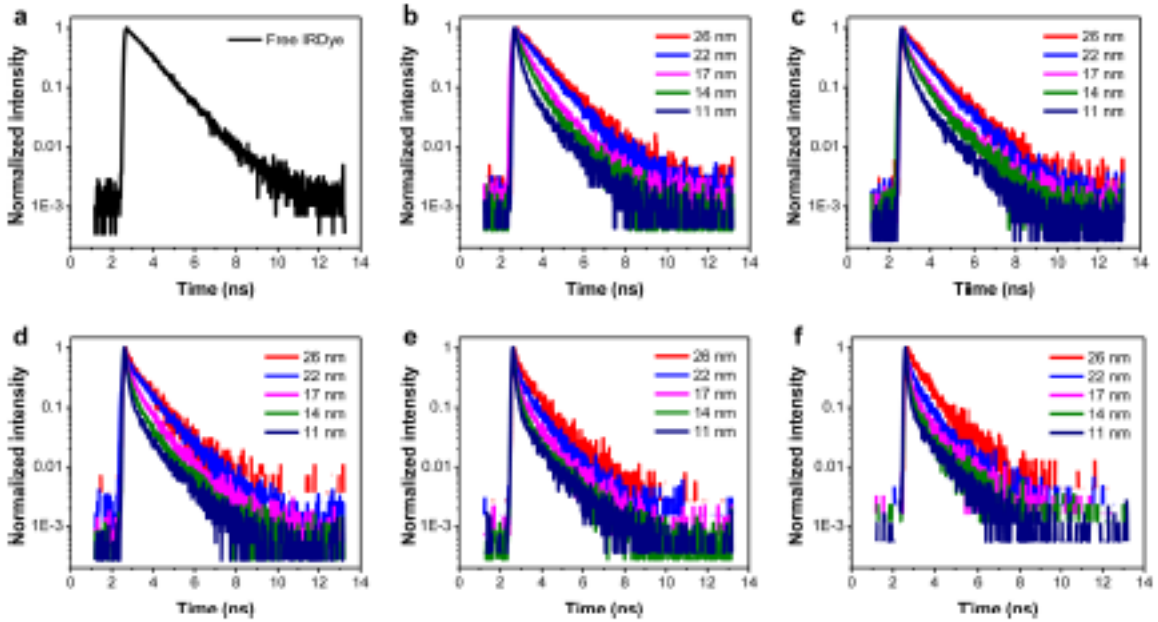
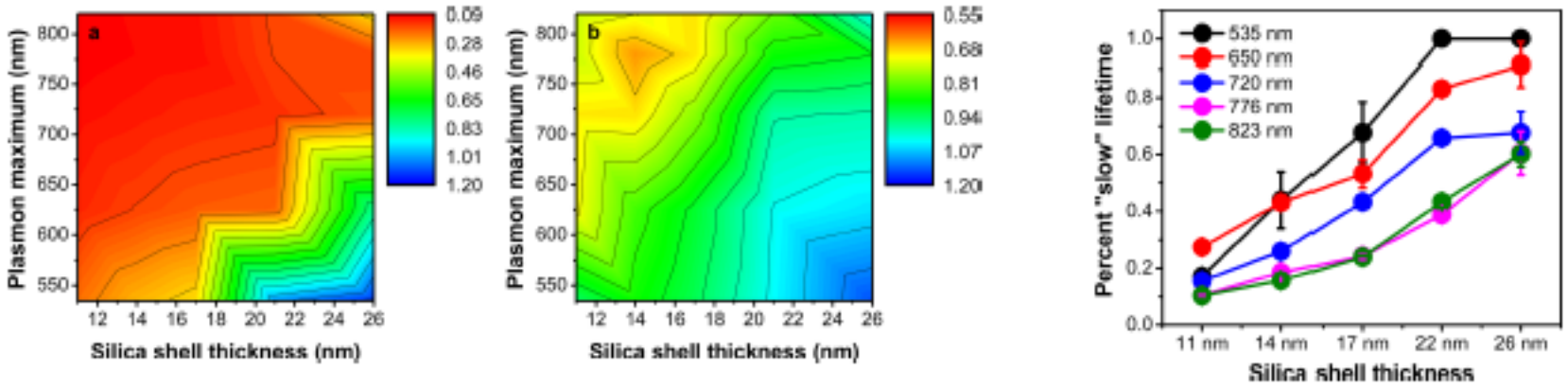


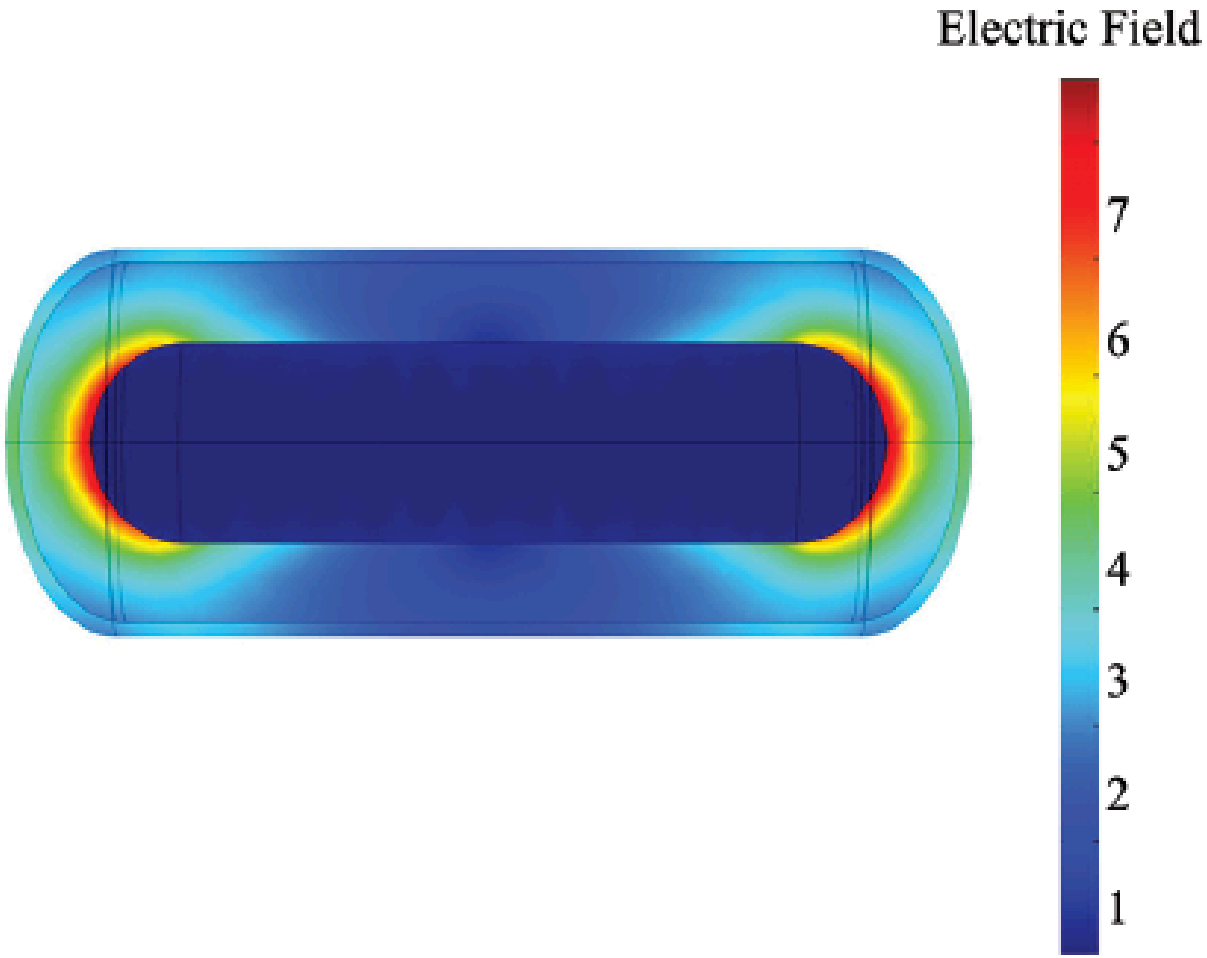
Figure 6. Fluorescence decay curves of (a) free IRDye and IRDye bound to gold nanorods as a function of silica shell thickness with plasmon band maxima located at (b) 535 nm, (c) 650 nm, (d) 720 nm, (e) 776 nm, and (f) 823 nm.

Lifetime changes:
Faster decays
as get closer.
kr, knr vary in
complex manner
as f(distance).

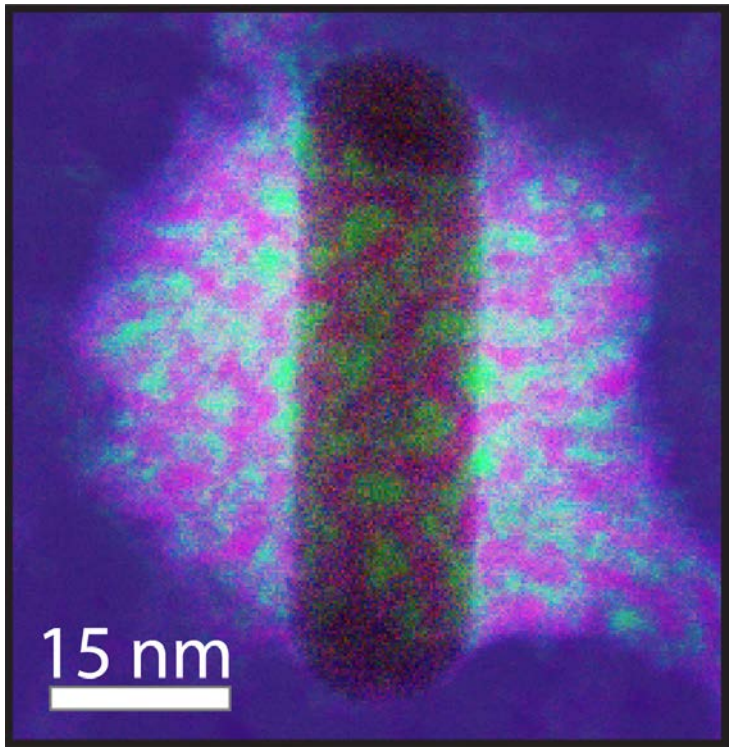
17 nm = “hot spot”

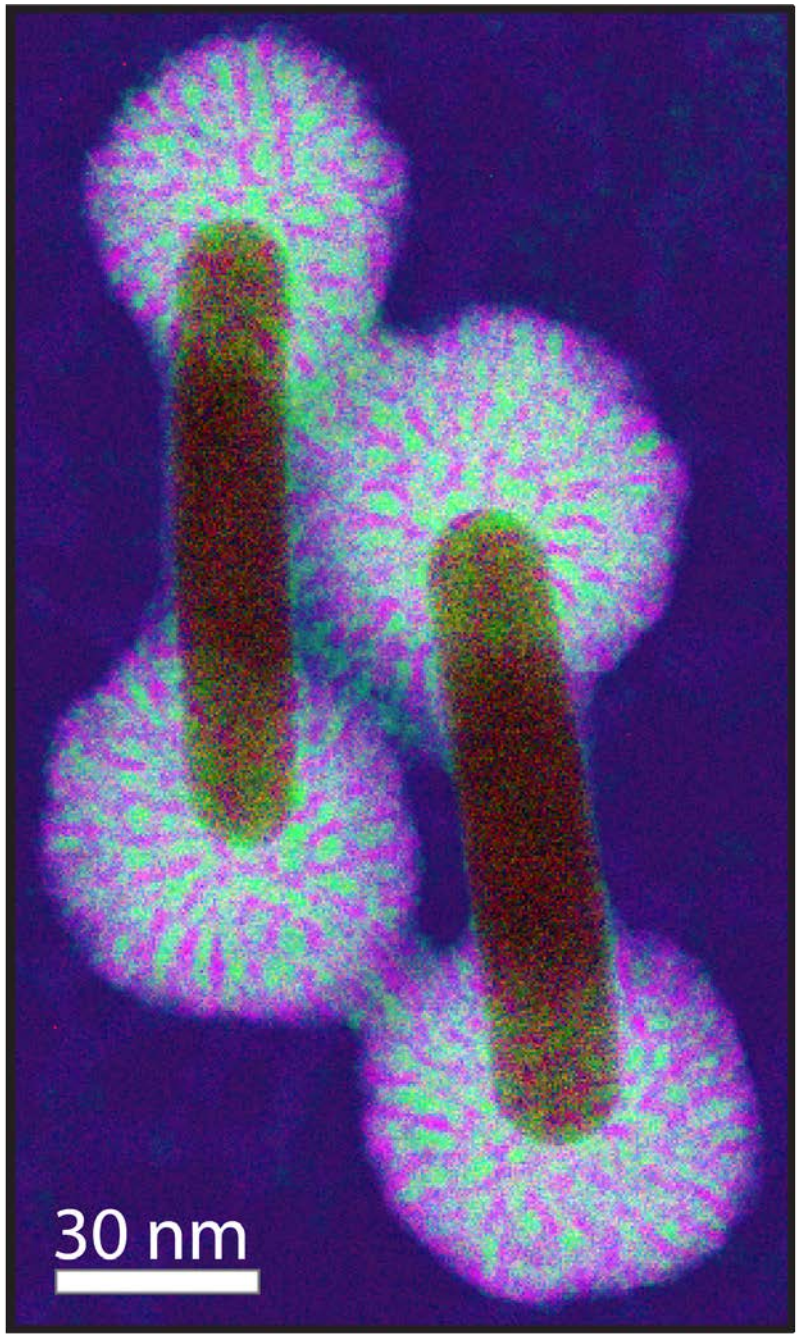


Biexponential decay: “fast” and “slow” components.

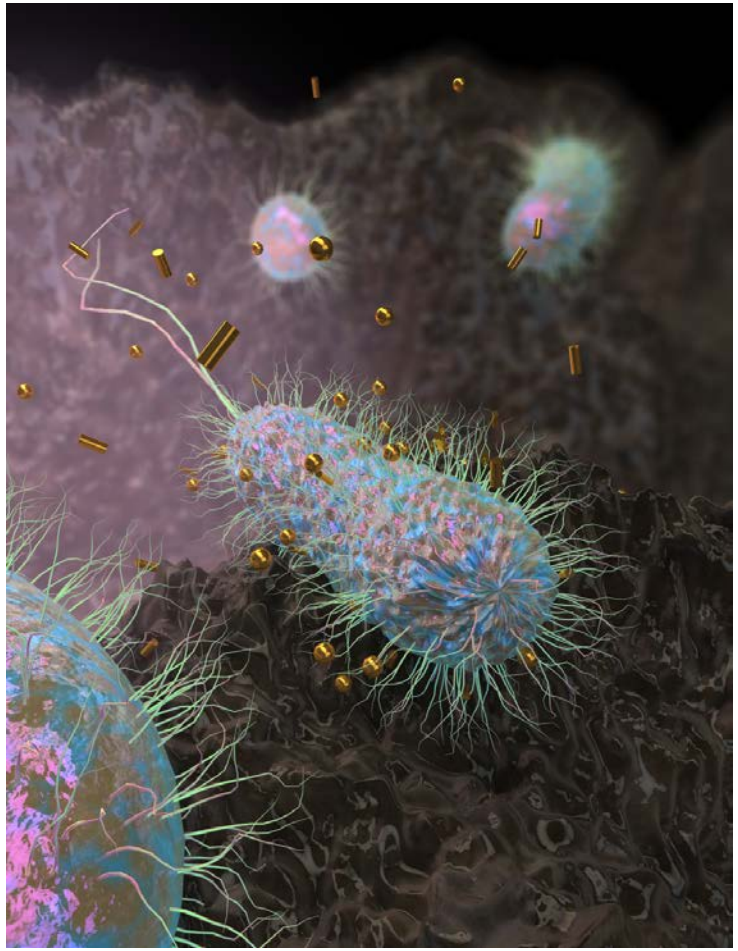

Electric Field
7
6
5
4
3
2
1

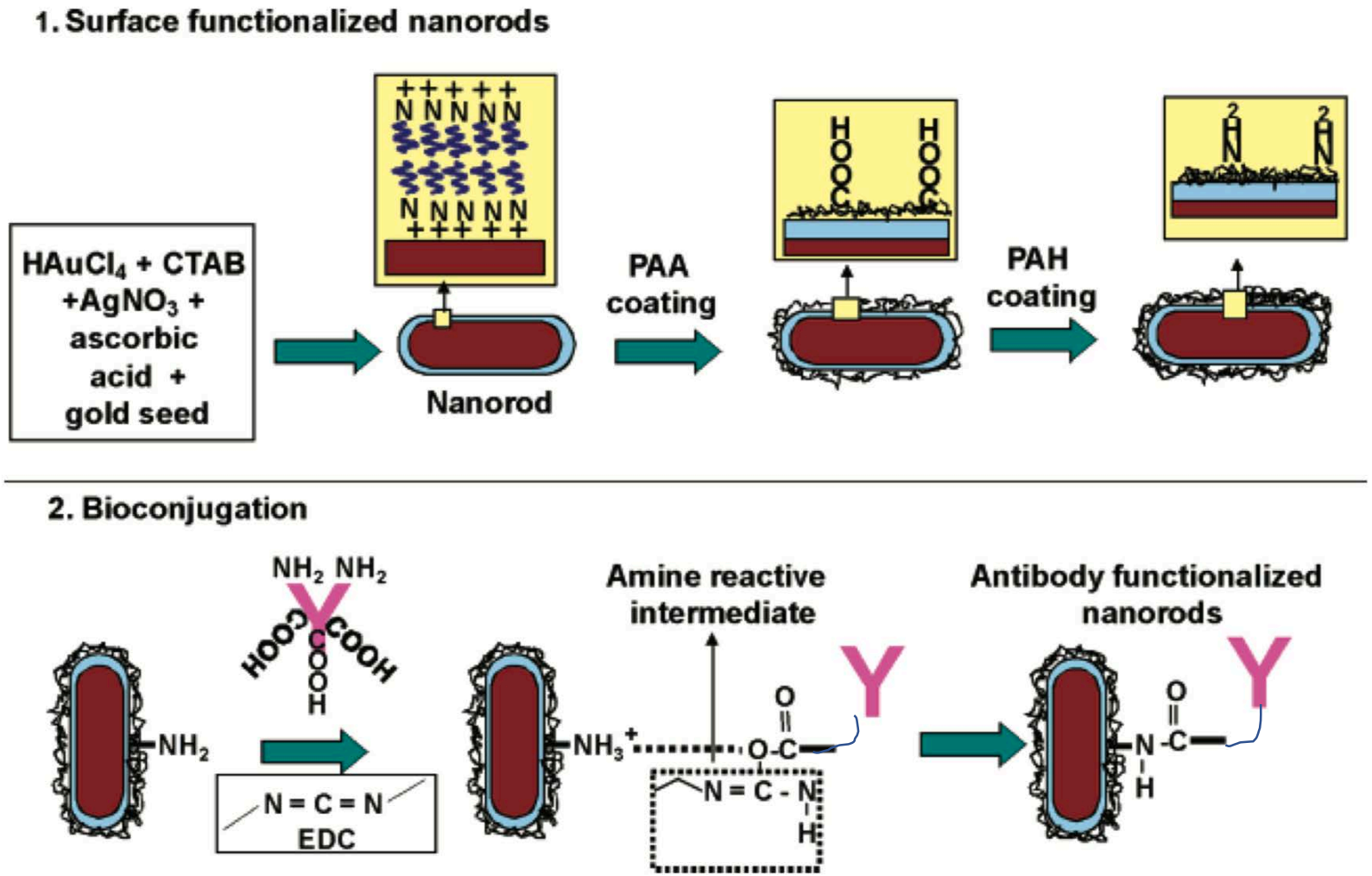
Color EELS spectrum images of the mesoporous silica coated particles. Carbon in green, silicon in blue, and oxygen in red (thus silica looks purple)



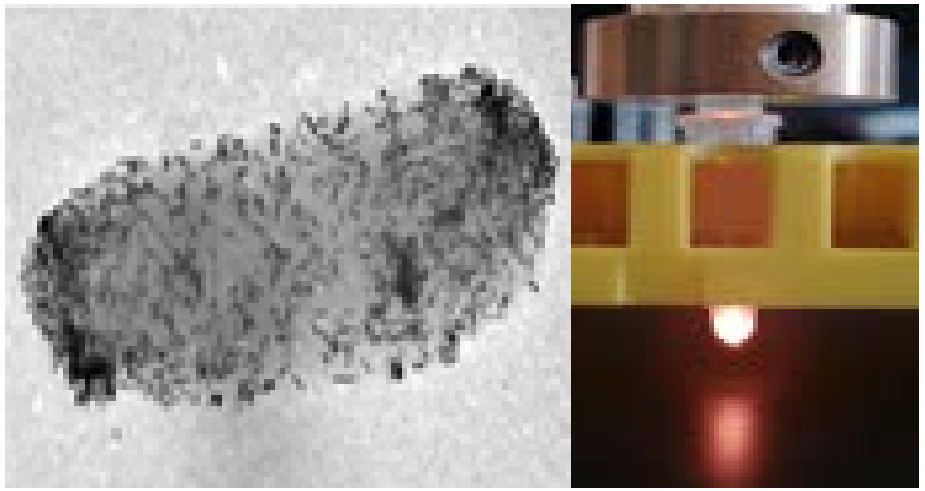


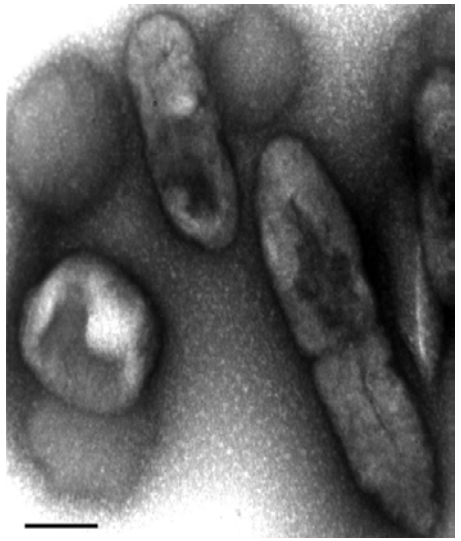
Photothermal therapy: shine light, kill “local” stuff

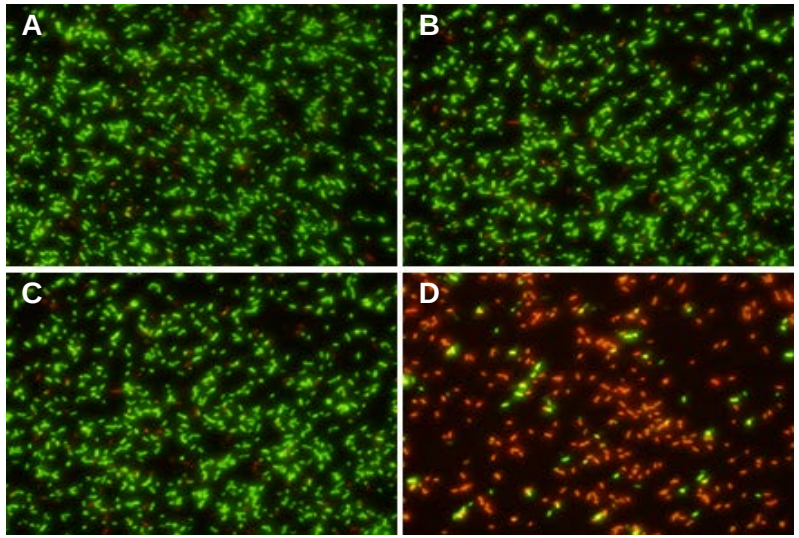


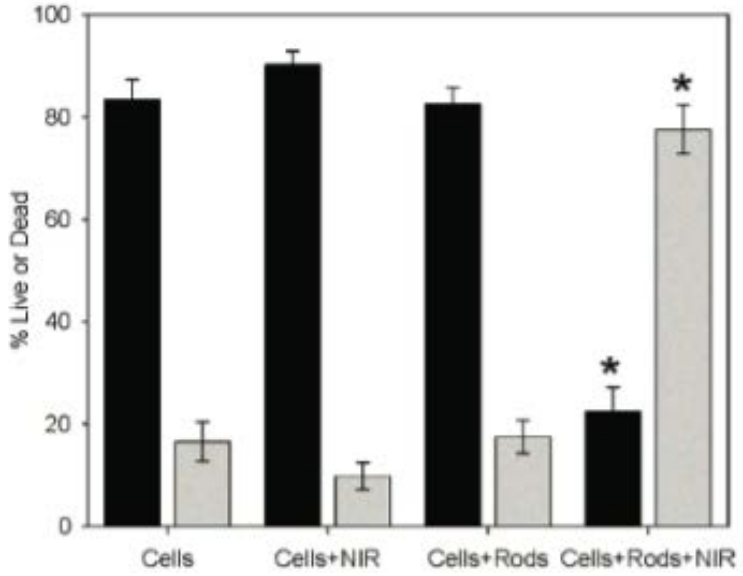


Norman, R. S.; Stone, J. W.; Gole, A.; Murphy, C. J.; Sabo-Attwood, T. “Targeted Photothermal Lysis of the Pathogenic Bacteria, *Pseudomonas aeruginosa,* by Gold Nanorods,” *NanoLetters* **2008**, *8*, 302-306.






A
B
C
D


100
80
60
40
20
0
% Live or Dead
*
*
Cells
Cells+NIR
Cells+Rods
Cells+Rods+NIR

What we didn’t do

- Multiple bacteria in pot to show specificity
- Biofilm as opposed to planktonic form

- For cancer: good thing about photothermal therapy is “lack of side effects”

A few words about photothermal molecular release

LeChatelier's Principle

Exothermic

A(*aq*) + B(*aq*) → AB(*aq*) + heat

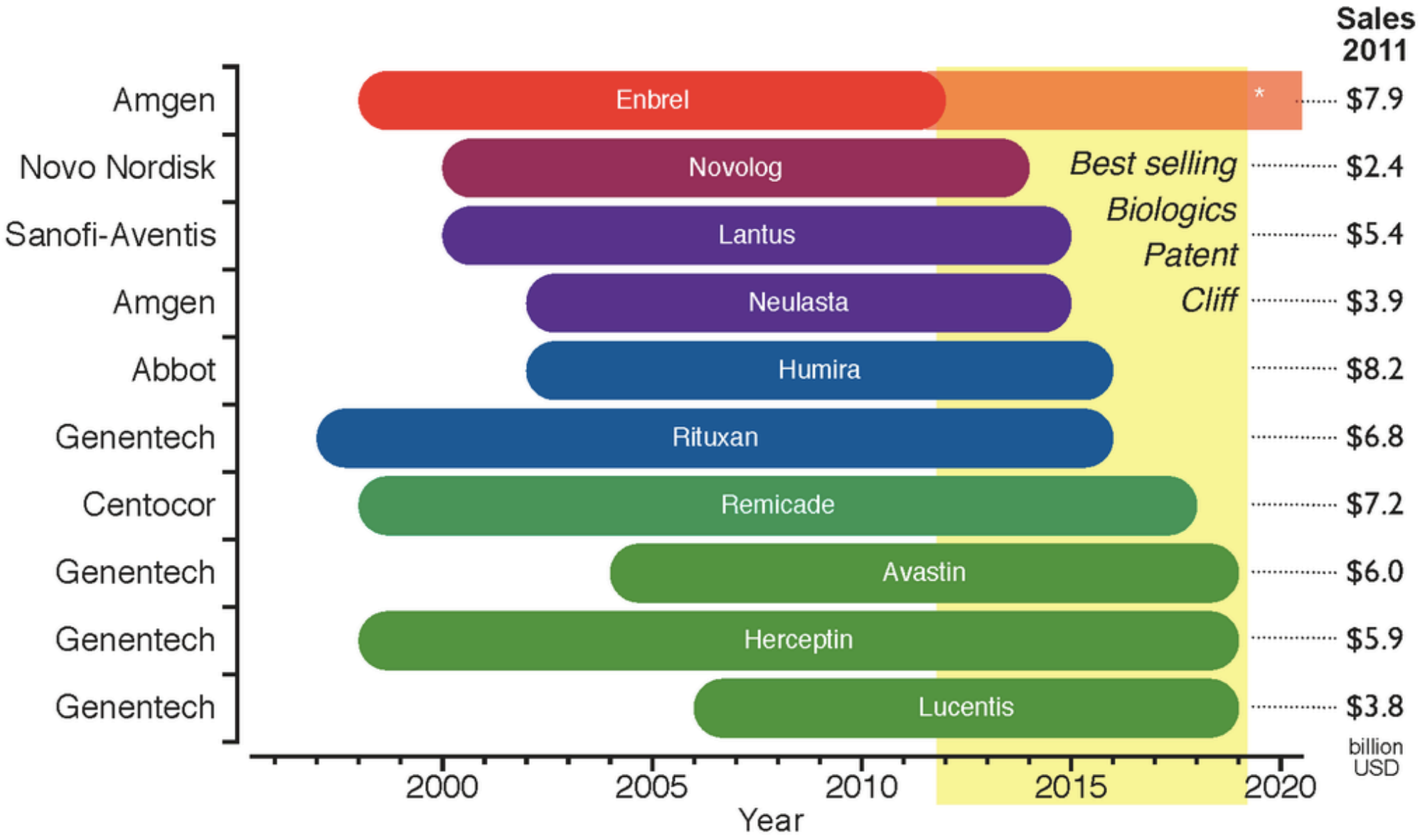
Raising the temperature leads to decomplexation

Endothermic

A(*aq*) + B(*aq*) + heat → AB(*aq*)

Raising the temperature leads to more complexation

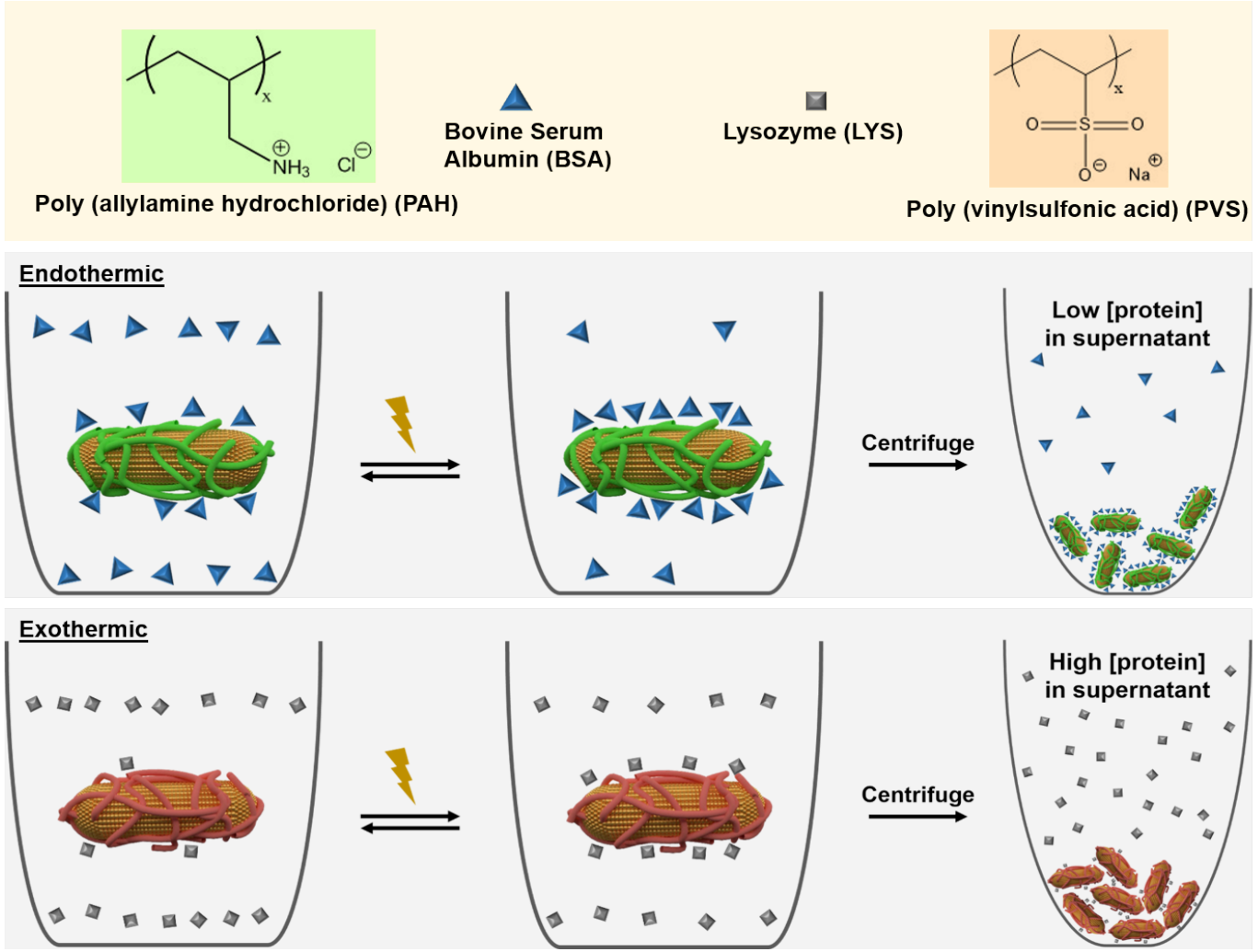
Biologics: pharmaceuticals that are derived from organisms/cells, includes proteins



Light-induced delivery (or not!) of proteins

BSA + PAH + Δ → BSA/PAH complex (endothermic)

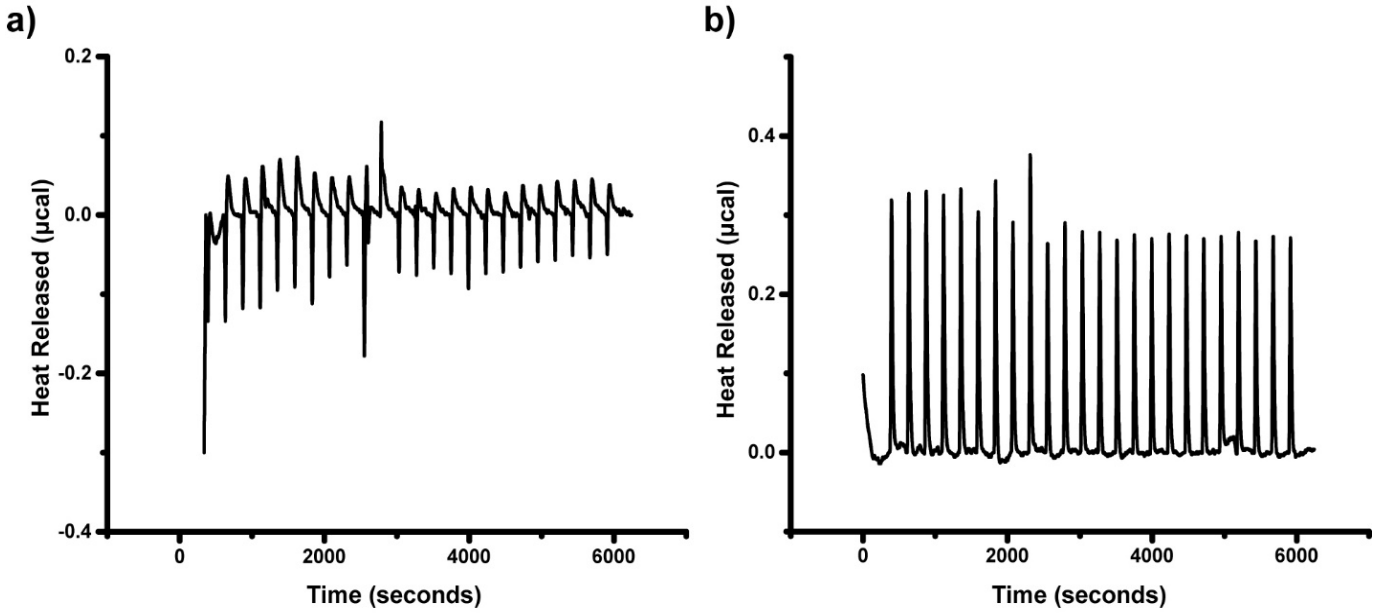
LYS + PVS → LYS/PVS complex + Δ (exothermic)



Isothermal titration calorimetry

BSA + PAH + Δ → BSA/PAH complex (endothermic)

LYS + PVS → LYS/PVS complex + Δ (exothermic)

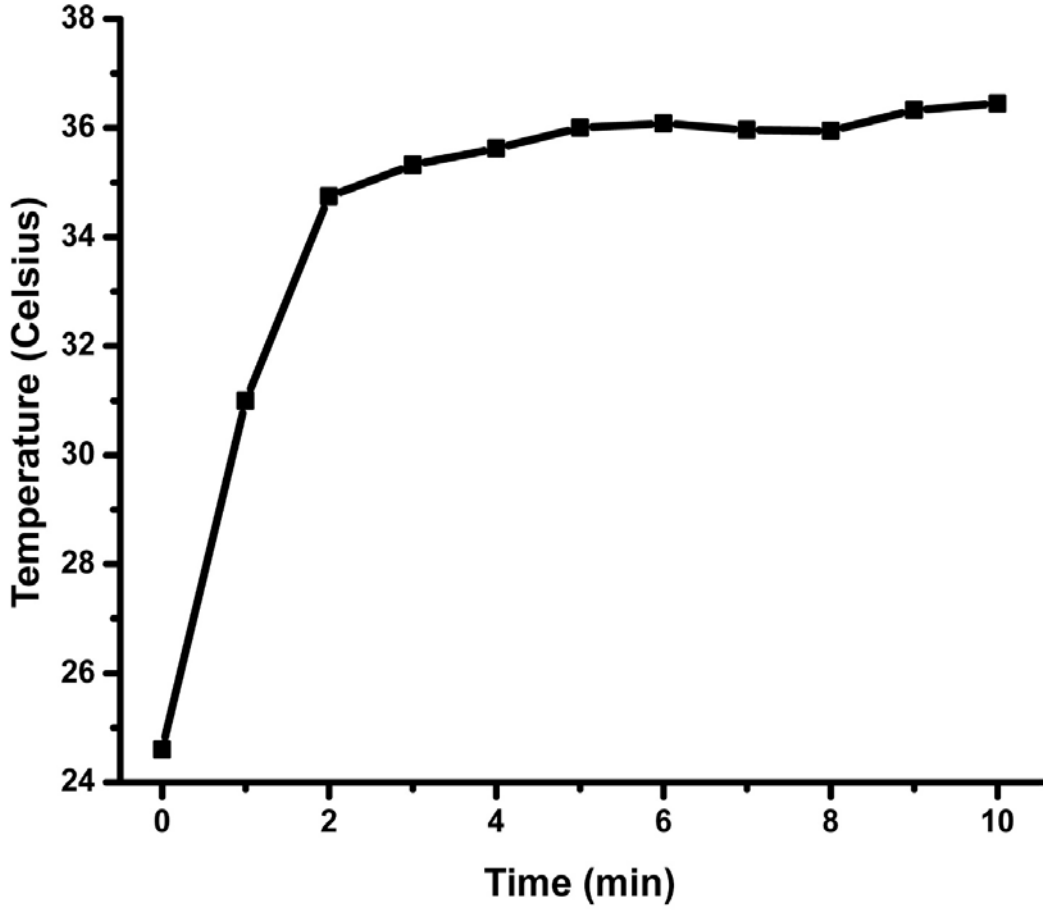


14 nM rods, 60 uM protein, 20 mM HEPES, pH 7

ΔH = +3.2 kJ/mol protein
(polymer only: +400 kJ/mol

ΔH = -10.3 kJ/mol protein
polymer only: -64 kJ/mol)

Au NRs that absorb at 808 nm

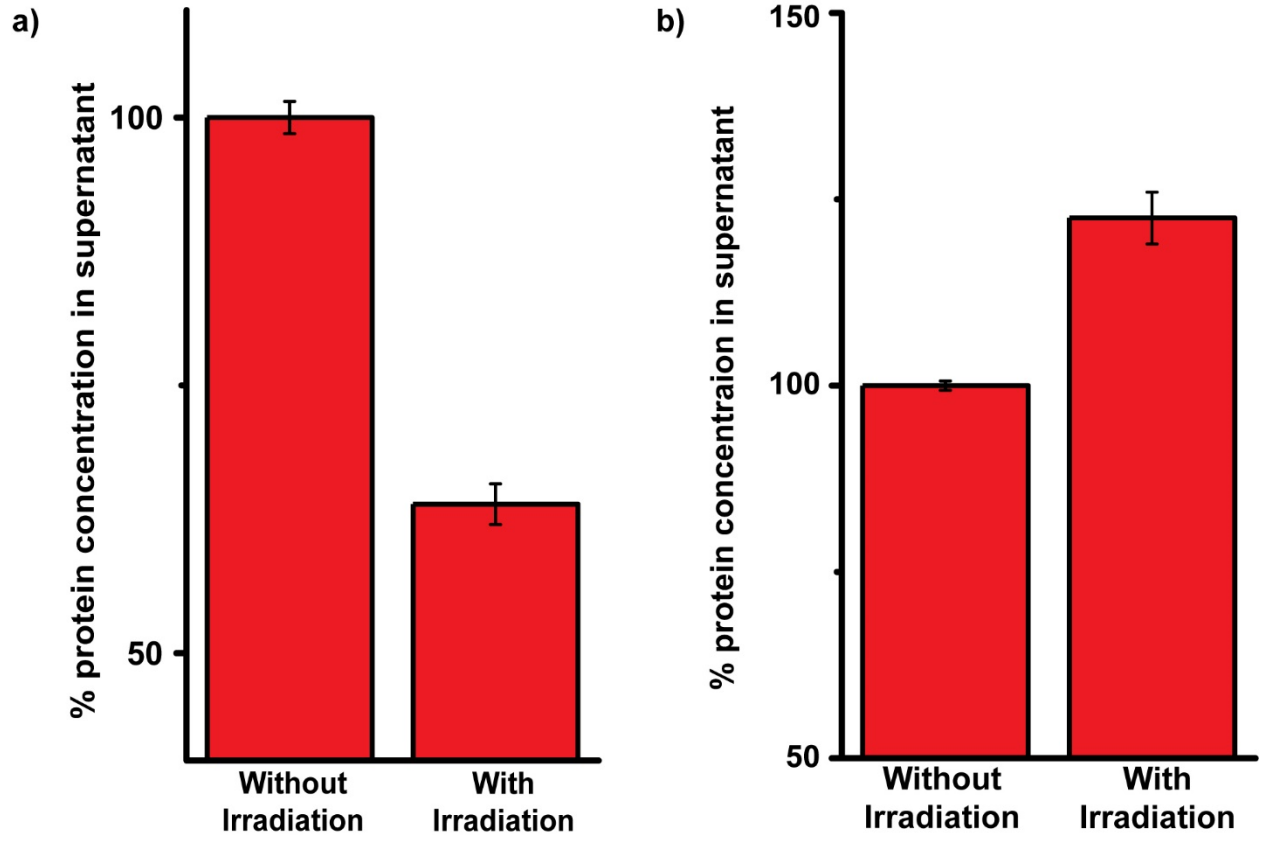


100 µL of 0.1 nM CTAB Au NRs, 0.5 W/cm^2, laser spot size is 0.75 cm^2

Pop on, pop off

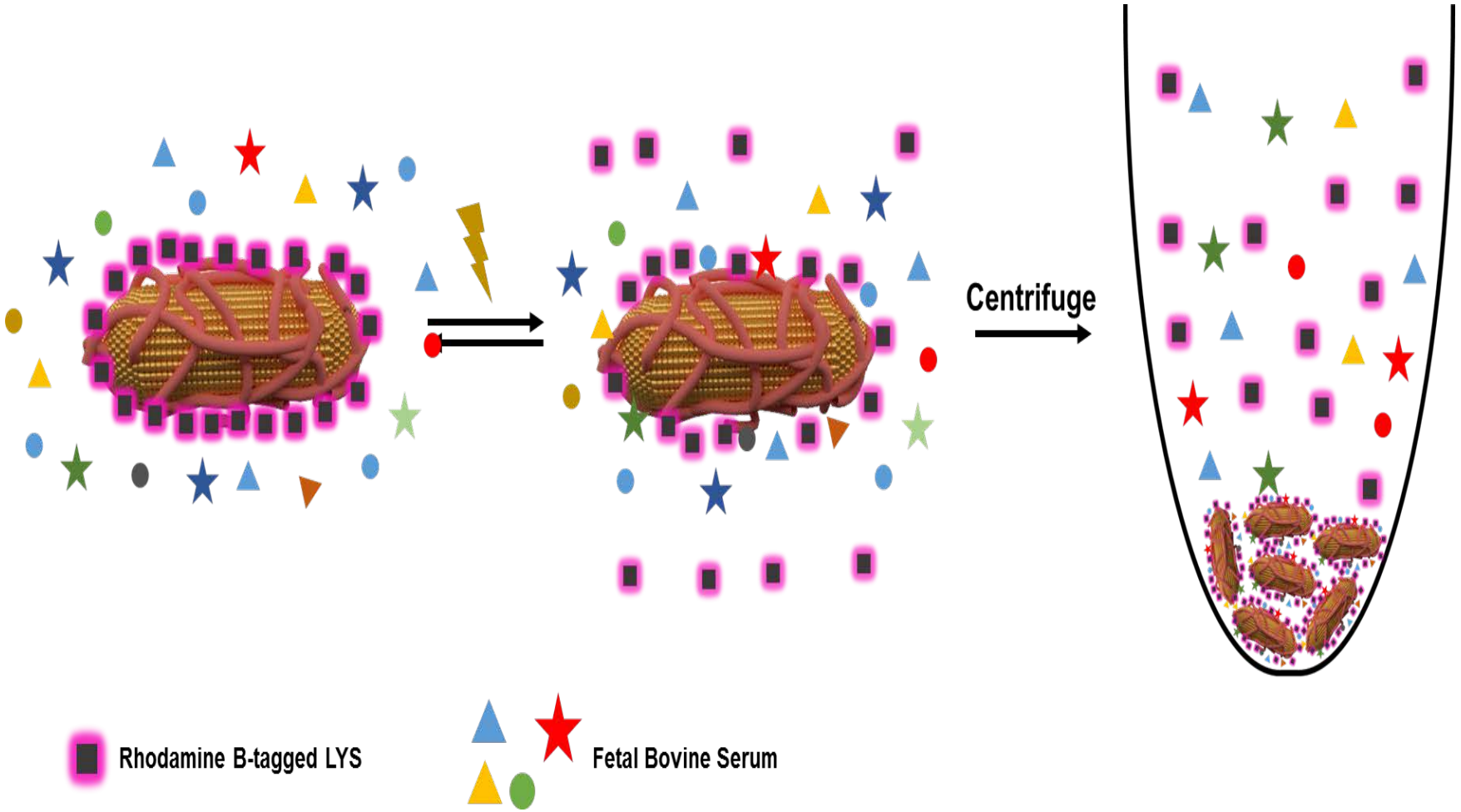
BSA + PAH + Δ → BSA/PAH complex
(endothermic, with gold nanorods)

LYS + PVS → LYS/PVS complex + Δ
(exothermic, with gold nanorods)



BCA and fluorescence protein assays for [protein] in supernatant

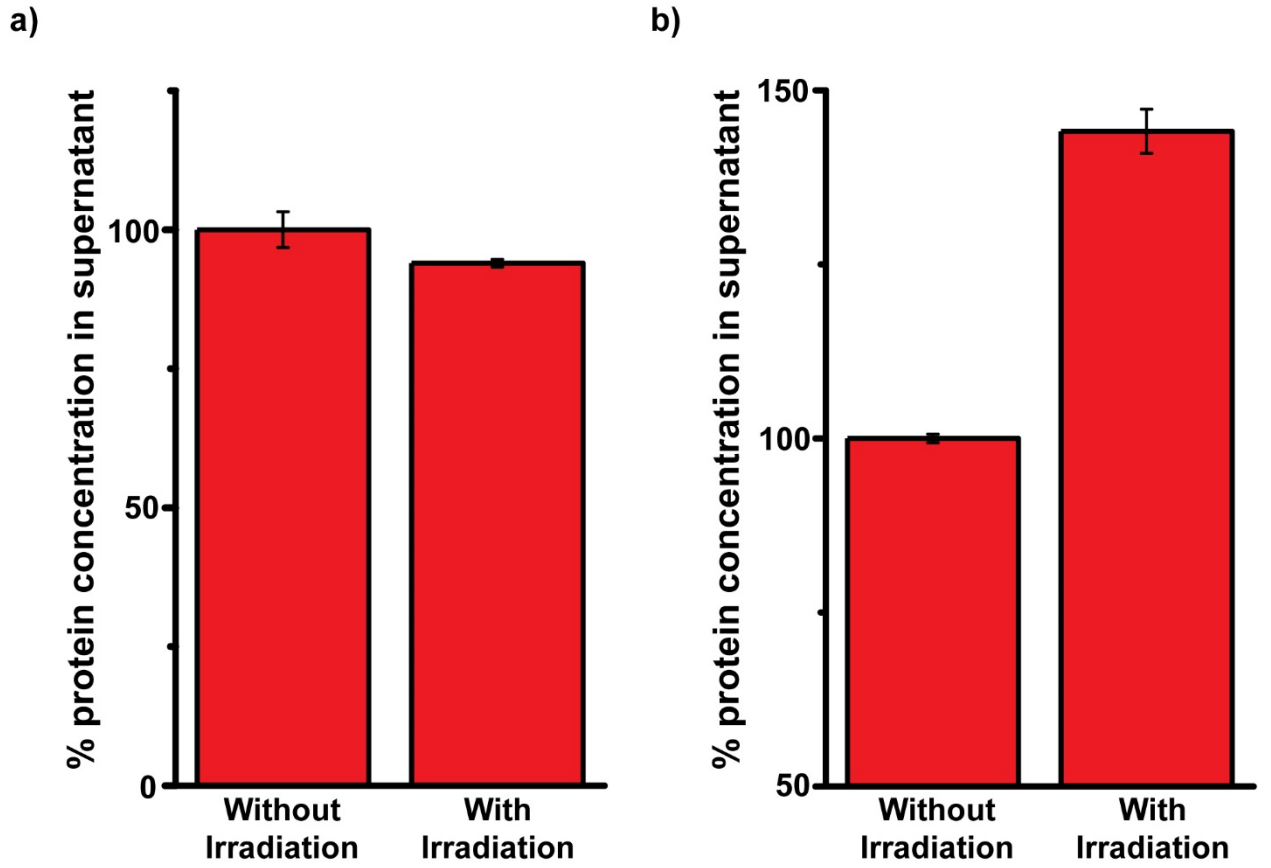
Can we predict protein adsorption/desorption in complex media?



FITC-BSA-rods and rhodamine-LYS-rods in 10% FBS

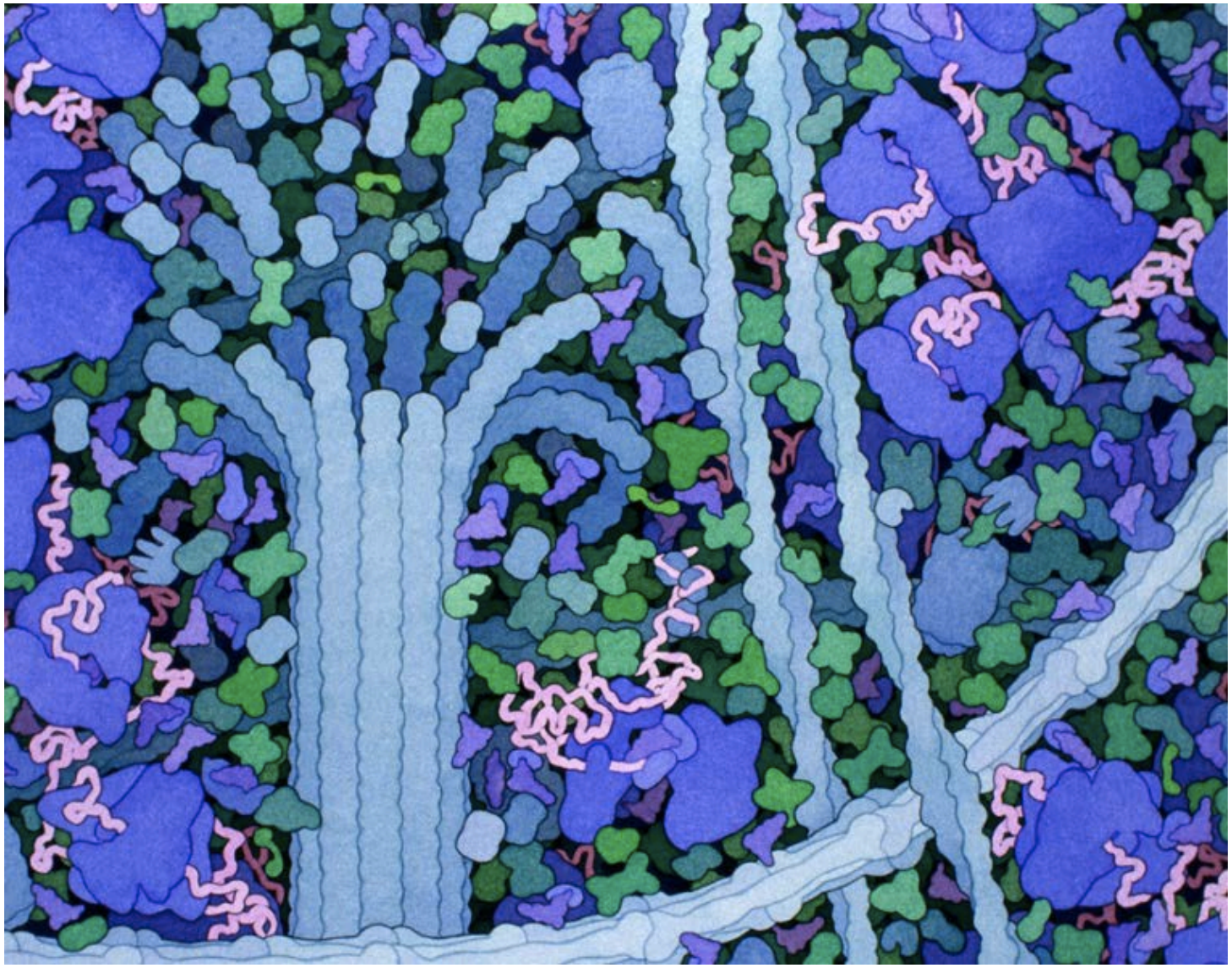
BSA + PAH + Δ → BSA/PAH complex
(endothermic with gold nanorods)

LYS + PVS → LYS/PVS complex + Δ
(exothermic with gold nanorods)



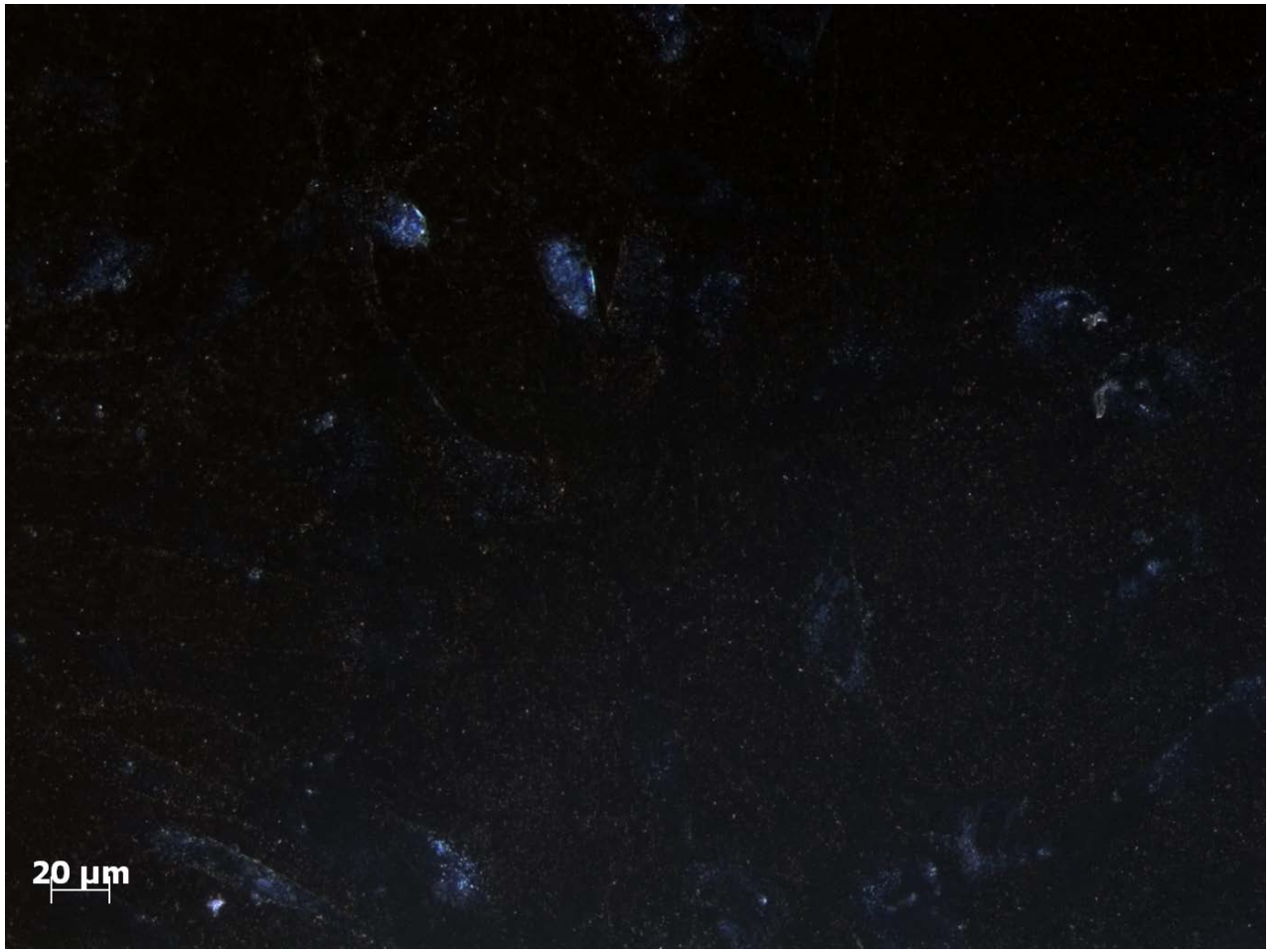
ACS Central Science **2017**, *3*, 1096-1102.

Biomolecules to cells

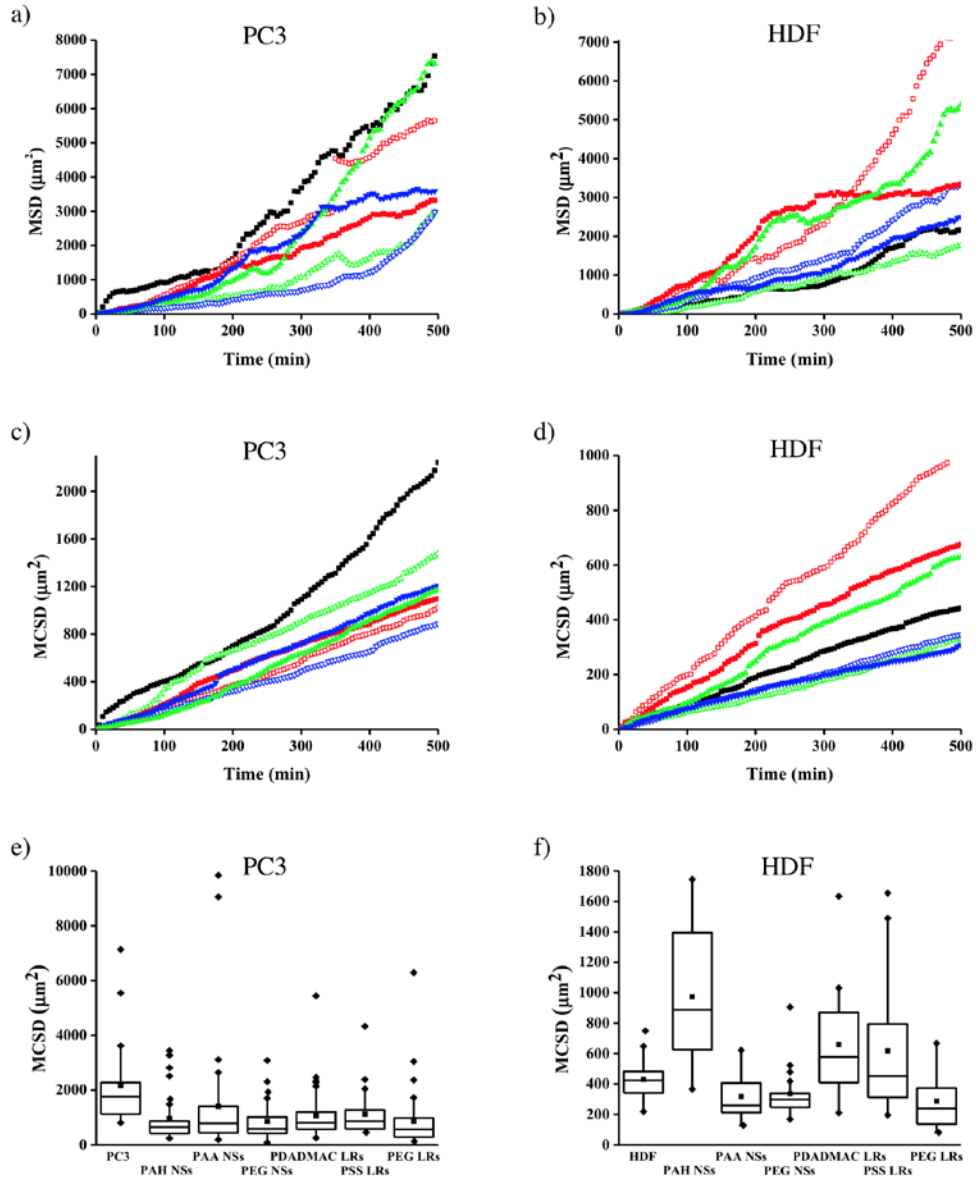


David Goodsell, *Cytoplasm*

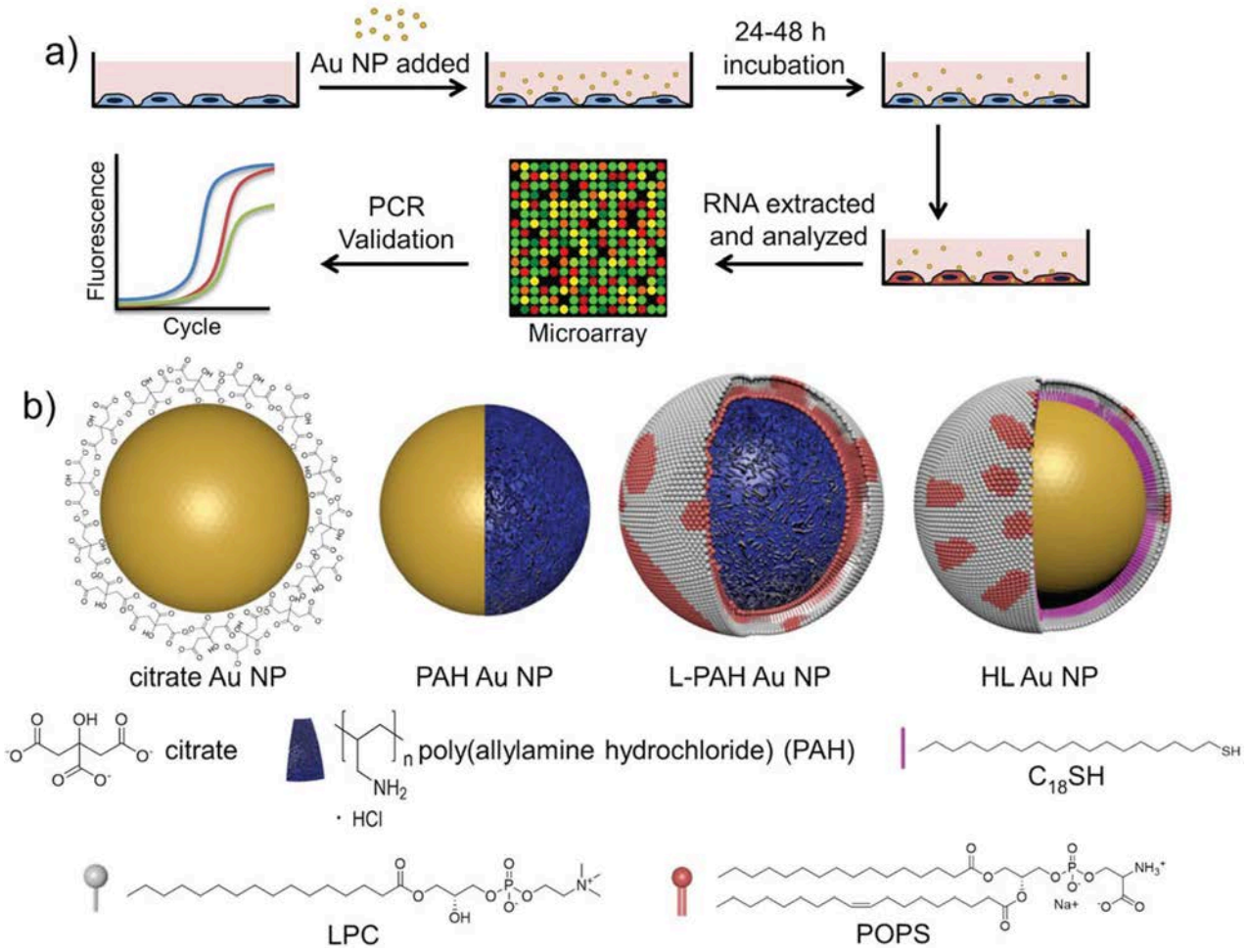
Darkfield imaging of nanoparticle accumulation



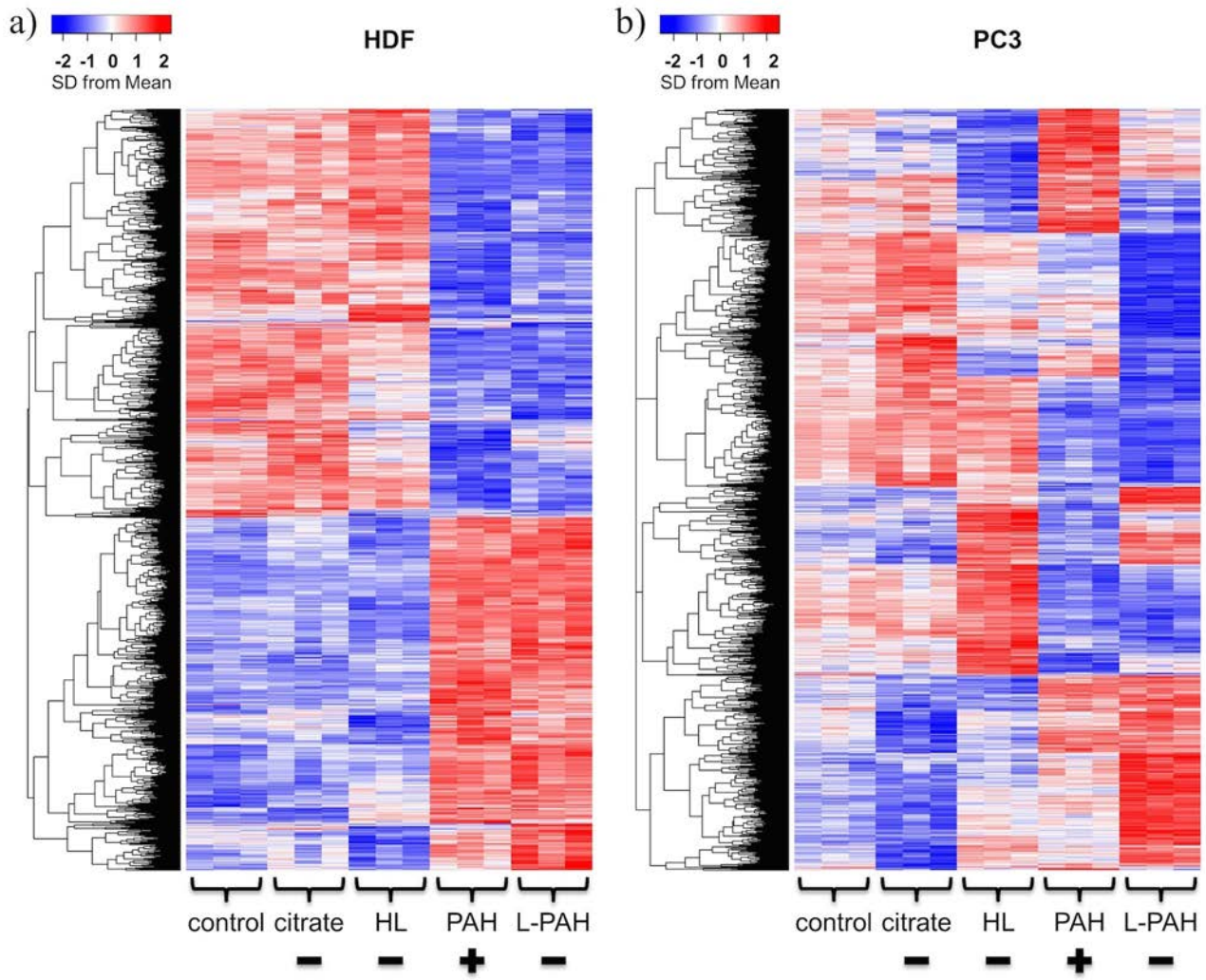
Yang et al, *Nano Lett*. **2013**, *13*, 2295-2302.


a)
PC3
MSD (μm²)
Time (min)
b)
HDF
MSD (μm²)
Time (min)
c)
PC3
MCSD (μm²)
Time (min)
d)
HDF
MCSD (μm²)
Time (min)
e)
PC3
MCSD (μm²)
PC3
PAH NSs
PAA NSs
PEG NSs
PDADMAC LRs
PSS LRs
PEG LRs
f)
HDF
MCSD (μm²)
HDF
PAH NSs
PAA NSs
PEG NSs
PDADMAC LRs
PSS LRs
PEG LRs

Hmmm. Side Effect: Changing Cell Behavior!

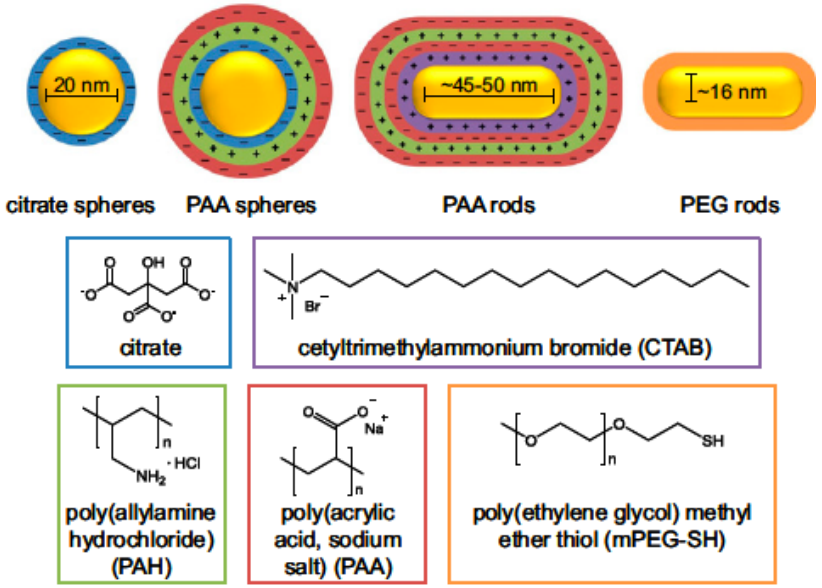


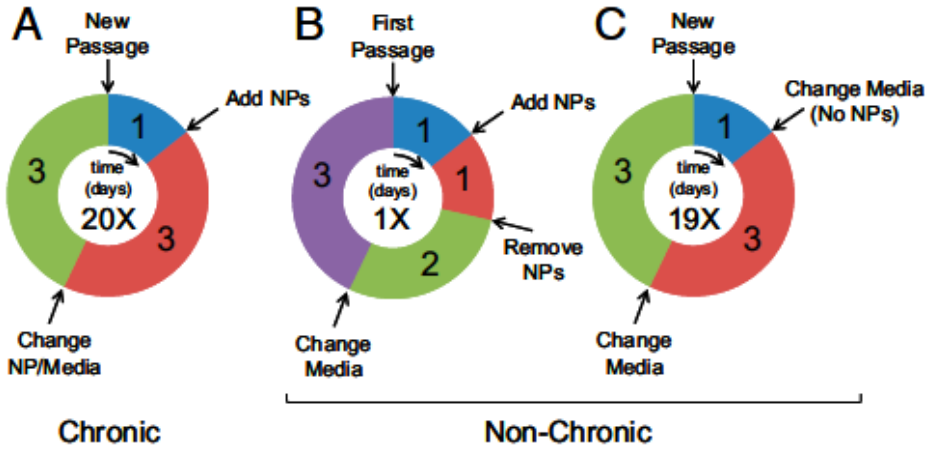
Nanoscale **2015**, *7*, 1349-1362.



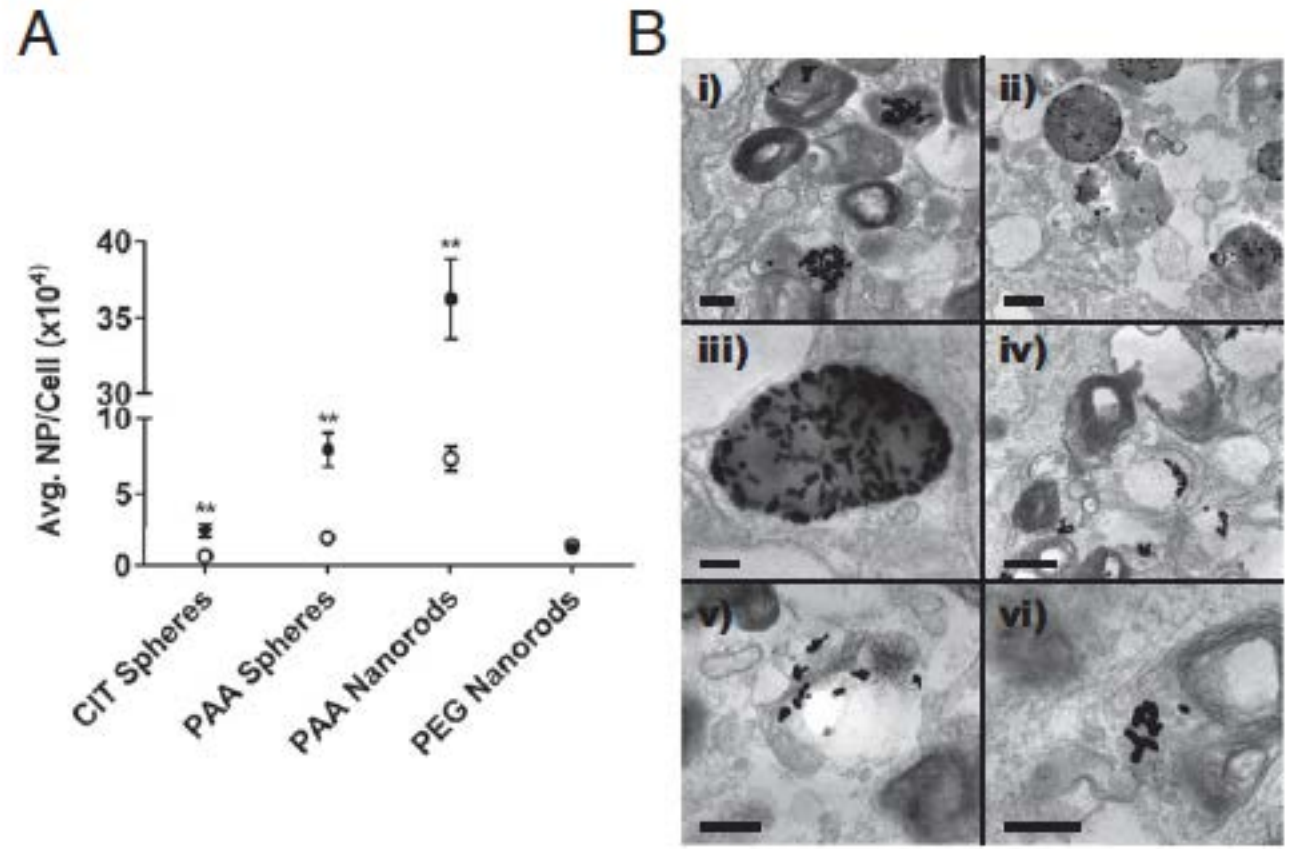
Nanoscale **2015**, *7*, 1349-1362.

Long-term cellular exposures

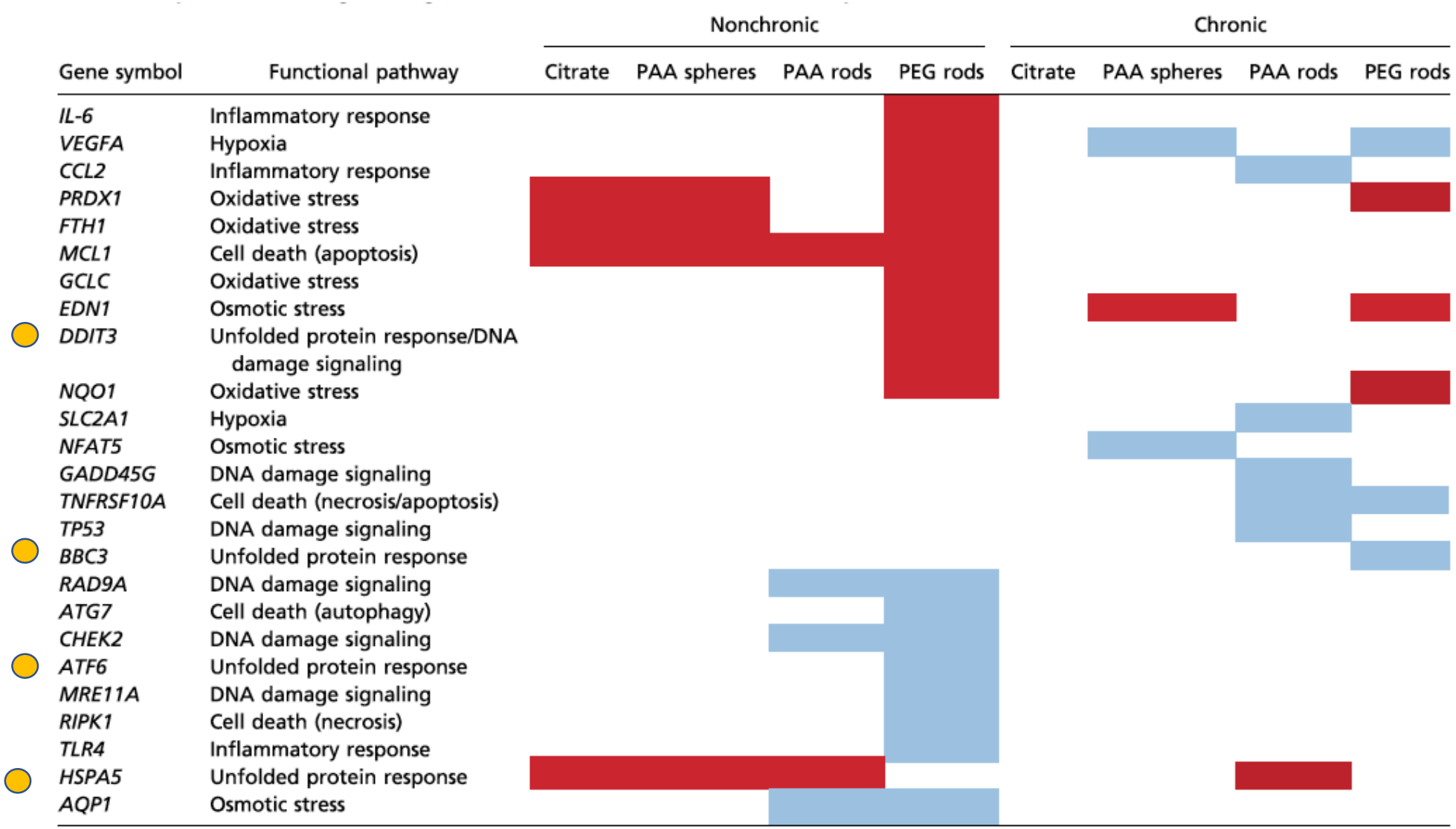


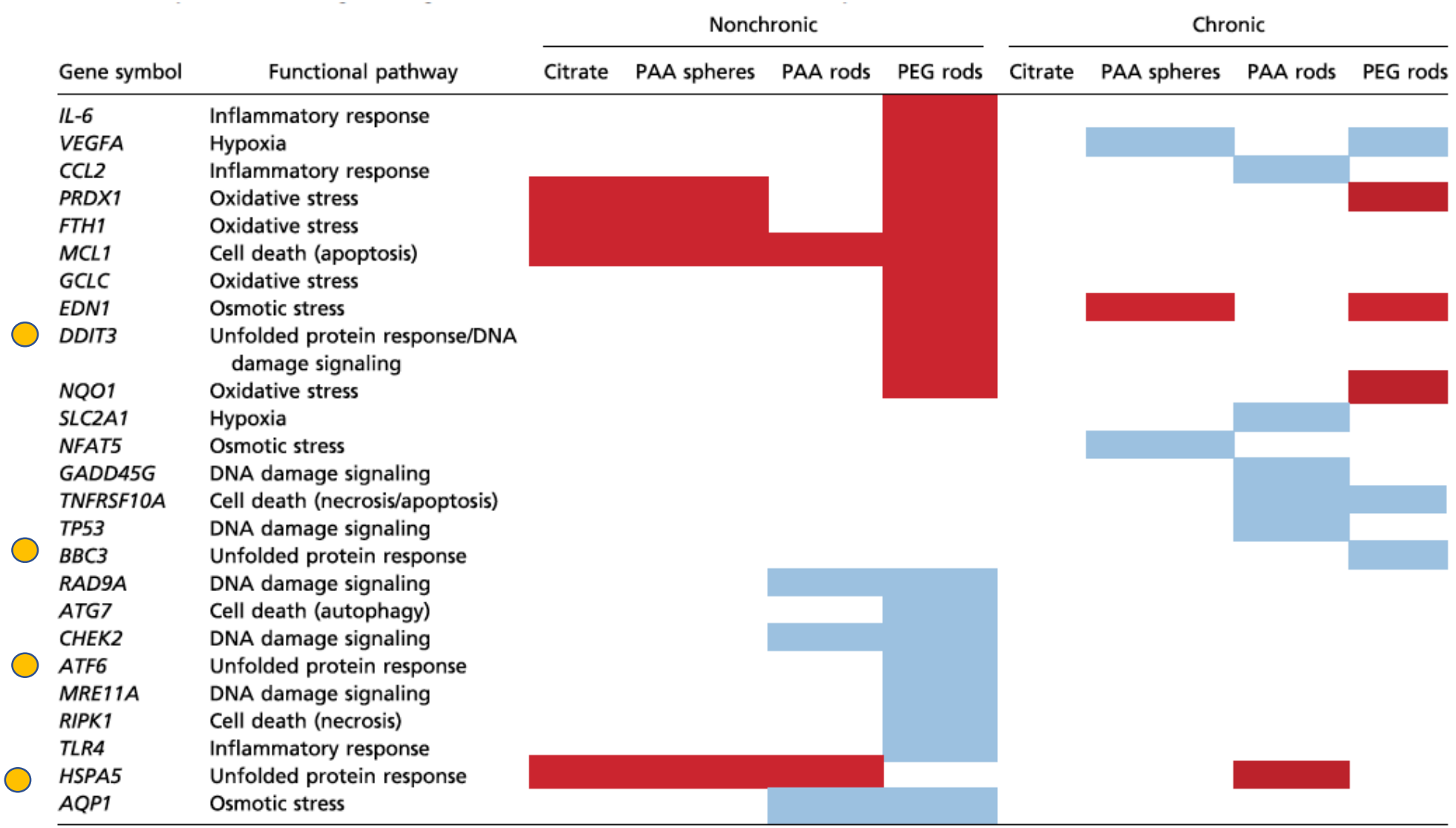


HDF cells. *PNAS* **2016**, *113*, 13318-13323.



Open circles: 72 h
Filled circles: 20 weeks
Charged rods: largest accumulation.


Nonchronic
Chronic
Gene symbol
Functional pathway
Citrate
PAA spheres
PAA rods
PEG rods
Citrate
PAA spheres
PAA rods
PEG rods
IL-6
Inflammatory response
VEGFA
Hypoxia
CCL2
Inflammatory response
PRDX1
Oxidative stress
FTH1
Oxidative stress
MCL1
Cell death (apoptosis)
GCLC
Oxidative stress
EDN1
Osmotic stress
DDIT3
Unfolded protein response/DNA damage signaling
NQO1
Oxidative stress
SLC2A1
Hypoxia
NFAT5
Osmotic stress
GADD45G
DNA damage signaling
TNFRSF10A
Cell death (necrosis/apoptosis)
TP53
DNA damage signaling
BBC3
Unfolded protein response
RAD9A
DNA damage signaling
ATG7
Cell death (autophagy)
CHEK2
DNA damage signaling
ATF6
Unfolded protein response
MRE11A
DNA damage signaling
RIPK1
Cell death (necrosis)
TLR4
Inflammatory response
HSPA5
Unfolded protein response
AQP1
Osmotic stress

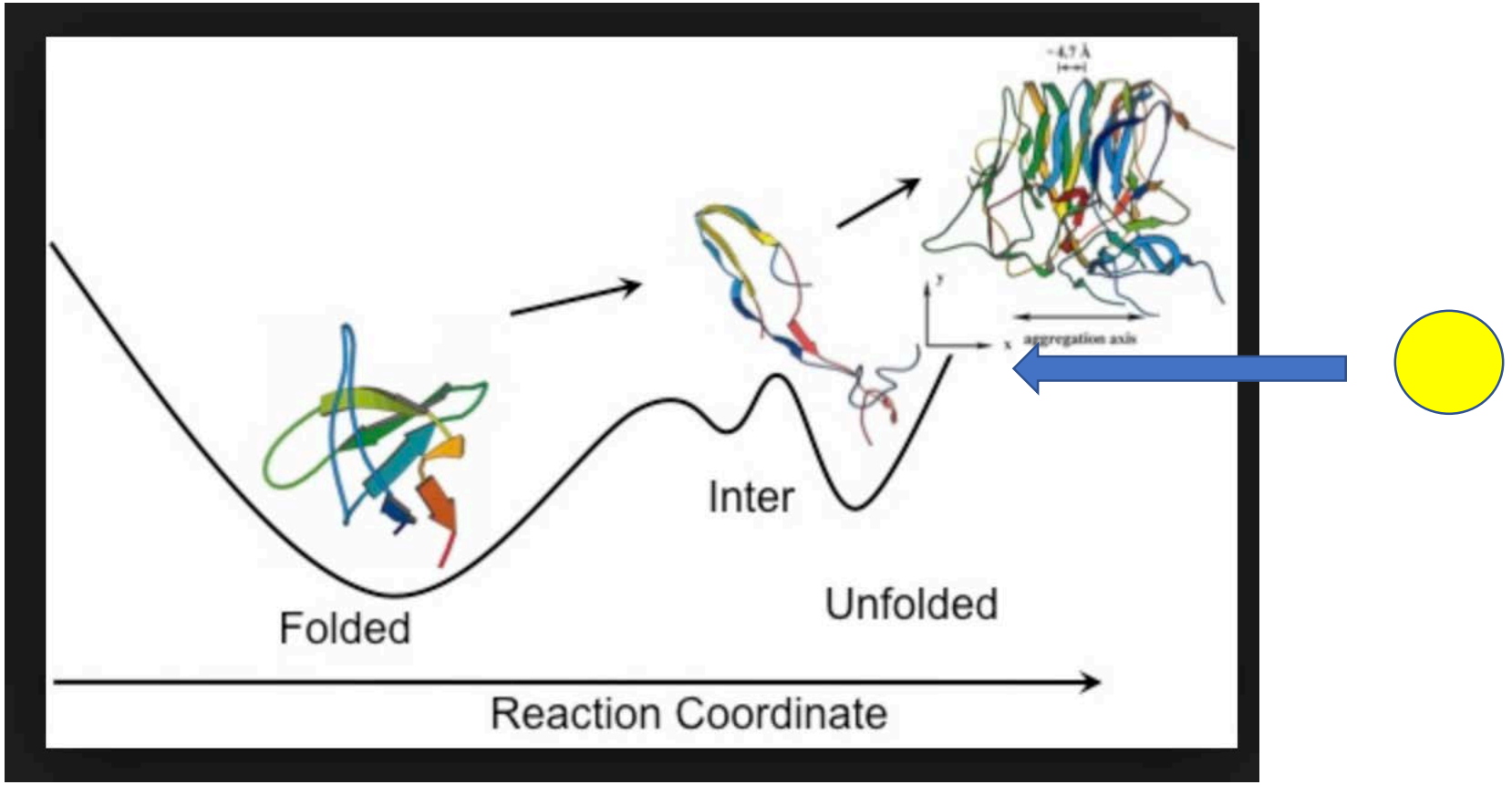


Big conclusion: cells can adapt over time to NP exposure

Mechanism(s)?

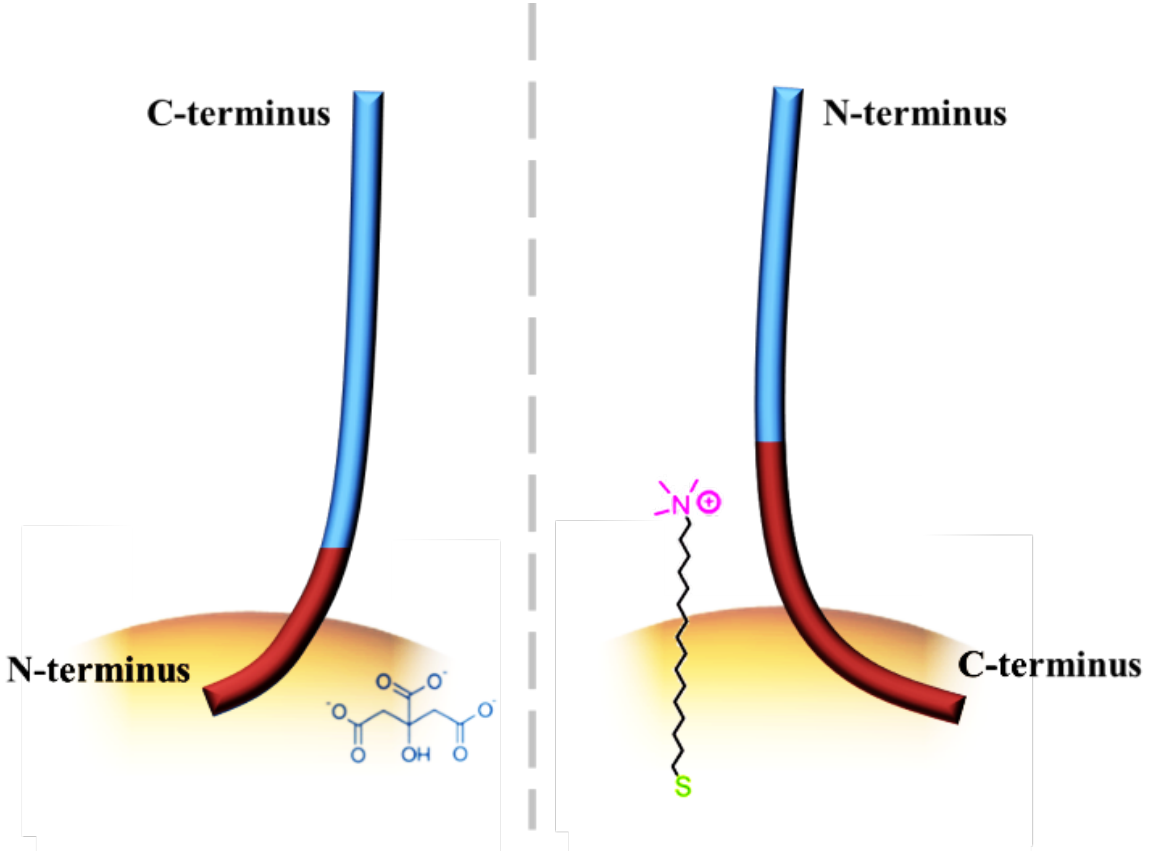
- Protein corona: physisorption of components in media changes local concentrations
- Mechanical alteration of the matrix (mechanotransduction)
- Nanoparticles interfere with protein-protein interactions that are important in cellular function?

Omics data can lead to testable hypotheses



http://dlab.clemson.edu/?p=186

Molecular control of protein display



Protein: alpha synuclein

Evidence: NMR, mass spectral footprinting

What have we learned?

- We have relatively robust methods to control metal crystal growth on the nanoscale
- Plasmons provide local electric fields for molecular photophysical enhancements
- Plasmons provide heat for triggered molecular delivery or sequestration (depending on enthalpy of interaction), and for killing pathogenic cells
- Initial surface chemistry of NPs influences protein orientation
- Side effects: NP exposure causes genetic changes in cells; cells adapt over time

Thank You

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Patrick Sisco, John Stone
Alaaldin Alkilany

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Nathan Burrows
Elissa Grzincic, Nardine Abadeer
Wayne Lin, Priscila Falagan Lotsch
Huei-Huei Chang

Collaborators:
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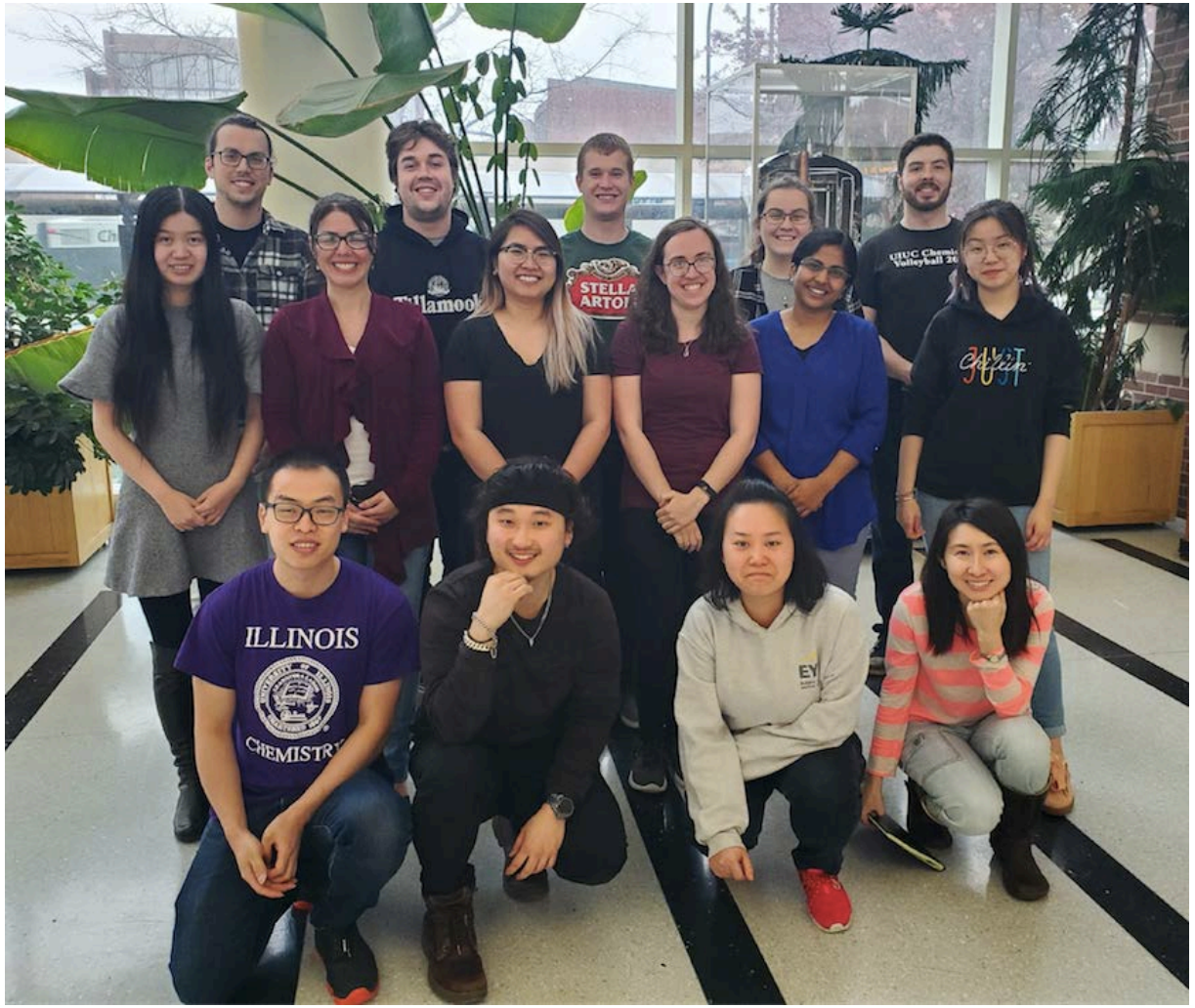
Chad Rienstra, Julia George
Pinshane Huang, Blanka Janicek



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