Jan 24th, 12:00 PM

Nanoparticles in Biomedicine: Delivery and Sensing

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Nanoparticles in Biomedicine: Delivery and Sensing

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I) Nanoparticle therapeutics

a) Supramolecular triggering

b) Immunomodulation

II) Delivery

a) Nanoparticle capsules

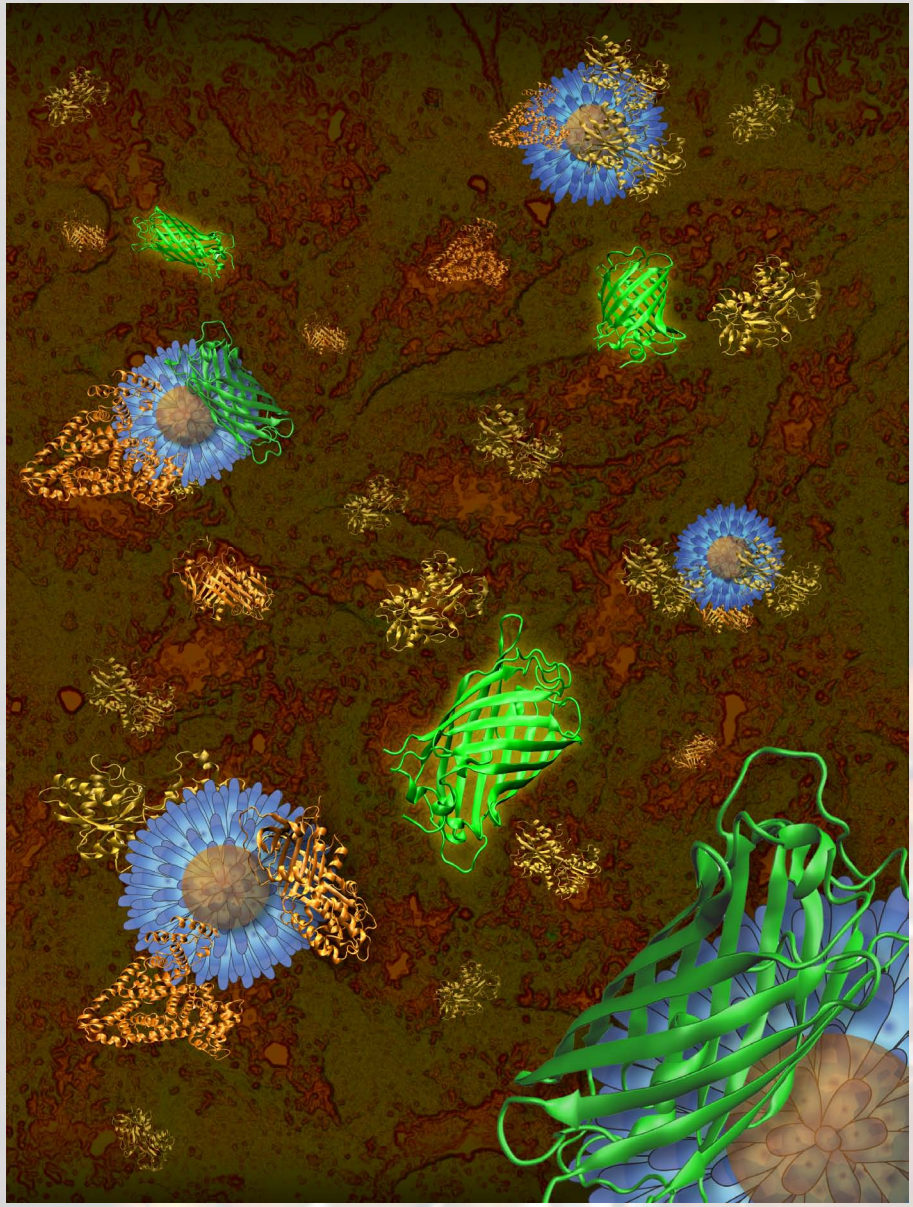
b) Protein delivery

II) Sensing

a) Proteins

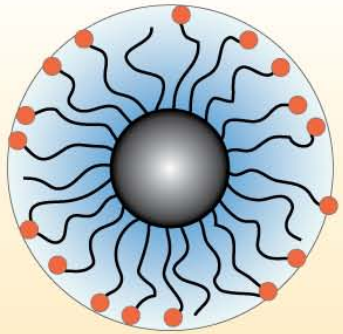
b) Cell surfaces

c) Tissues

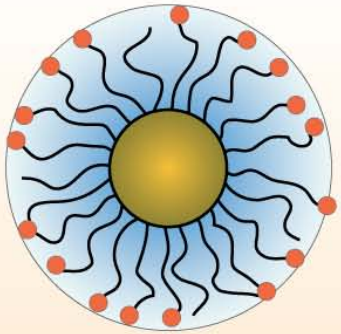


Nanoparticles have unique and useful properties

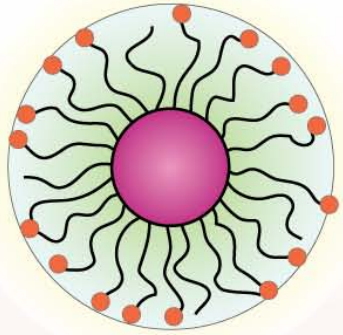
- nanoparticle behavior is very different from corresponding bulk material



- Pd, Au, Ag
  optics and electronics:
  biomedical (vide infra)
  electronics, sensors



- Fe_xO_y, M_xO_y, FePt
  magnetic materials:
  memory, ferrofluids,
  MRI imaging,
  hyperthermic therapies

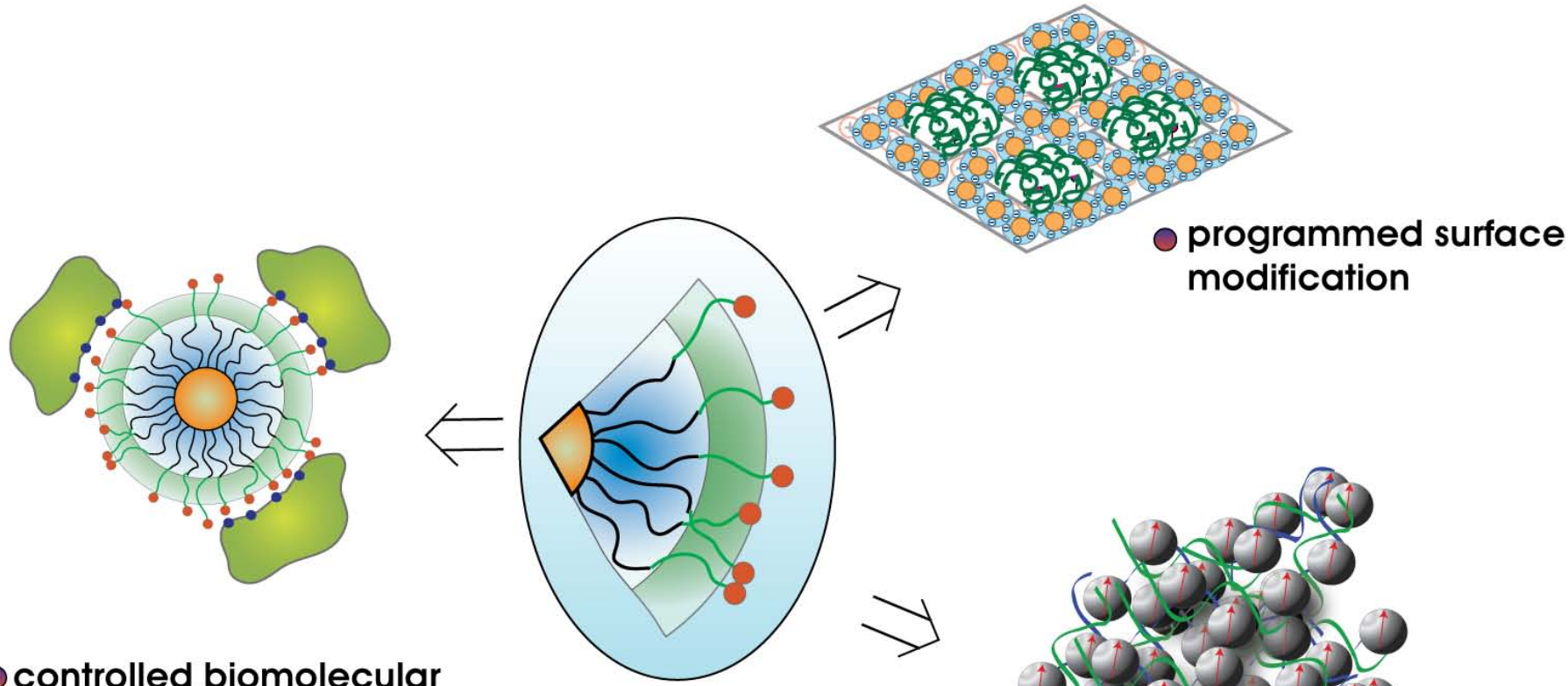


- CdSe, ZnSe
  semiconductor and
  fluorescent materials:
  bioimaging, electronics
  photovoltaics

- how can we employ these materials in real-world applications?

The key is engineering the particle interface

- our goal: use the atomic-level structural control of synthetic chemistry to control particle interactions and self-assembly



- programmed surface modification
- controlled biomolecular interactions
- nanocomposite assembly

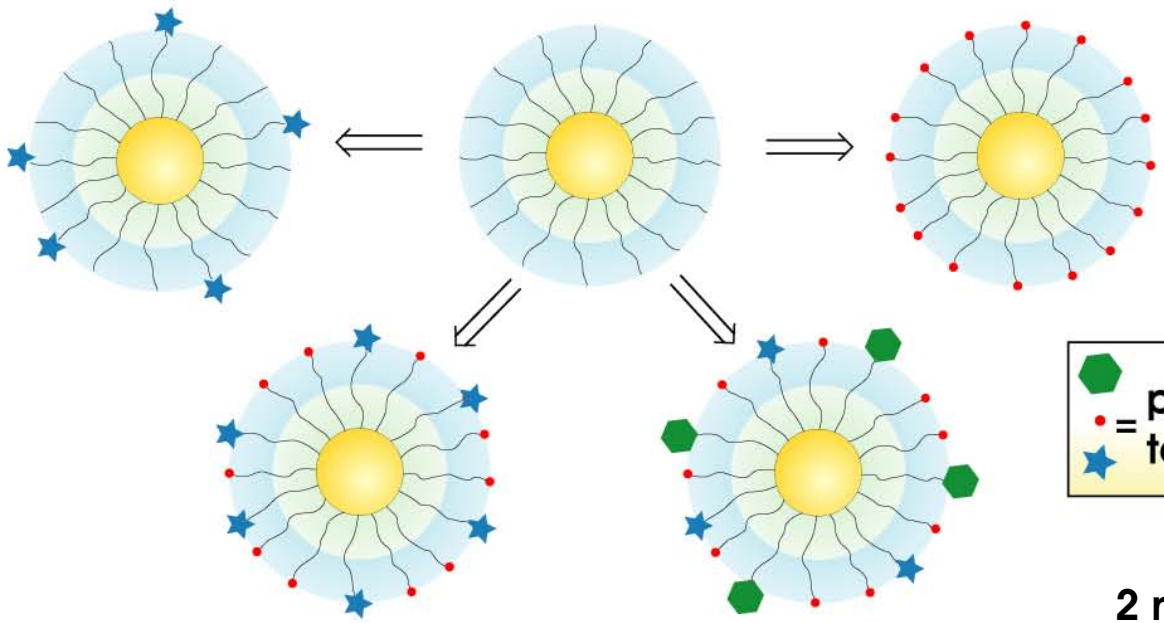
- of course we can mix and match...
- and lessons learned with one core can be generalized

Delivery with gold nanoparticles

- why does the world need another DDS?

1. gold has low toxicity and reasonable clearance
   -excellent compatability with appropriate coverage (i.e. OEG)

2. rapid, efficient creation of diverse delivery agents (think tinkertoy...)



-one step
-reproducible
-scalable

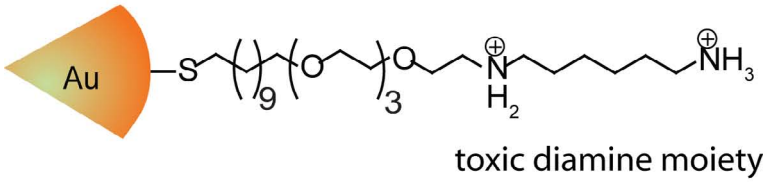
= prodrug, surface modifier, targeting functionality, etc

2 nm core diameter Au particles
-stable in biofluids (inc serum)
-redispersible

Duncan, B.; Kim, C.; Rotello, V. M. *J. Control. Release*, **2010**, *148*, 122-127.

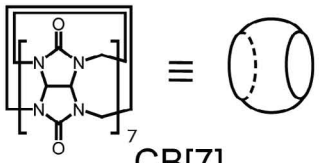
What about nanoparticles as therapeutics?

- we know we can create toxic particles
- can we harness that toxicity using supramolecular chemistry?



toxic diamine moiety

AuNP-NH_2



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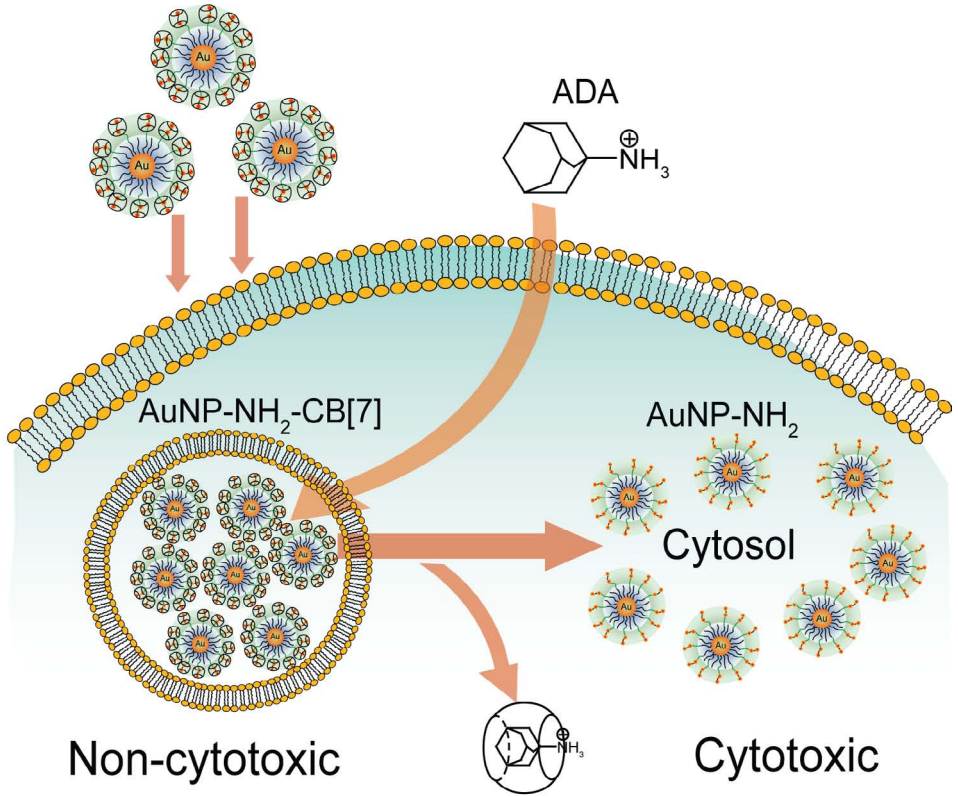
CB[7]

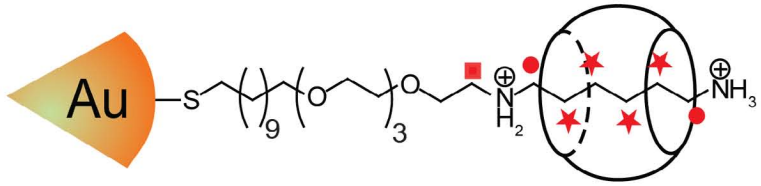


- our hypothesis: CB[7] should mask cationic functionality...
- ...reducing lytic activity and toxicity

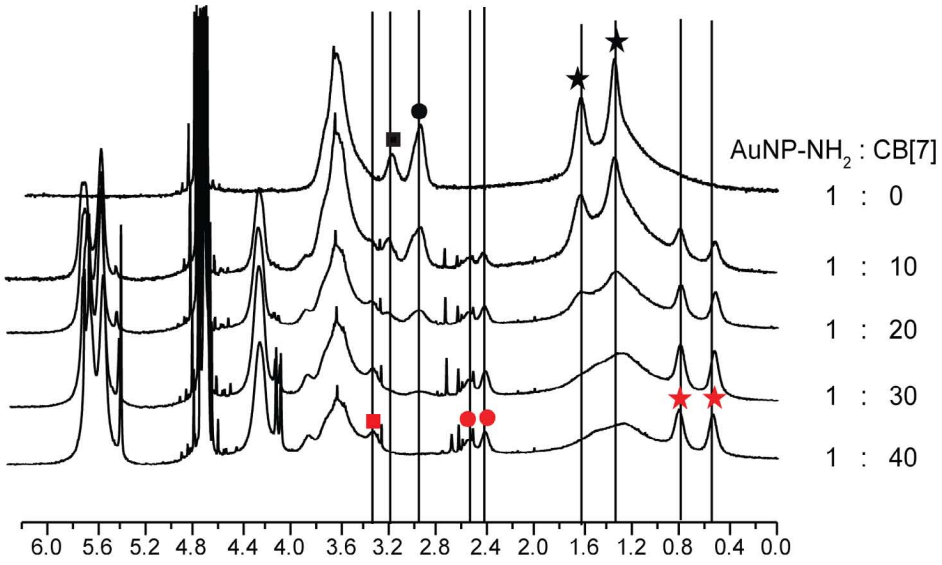
Kim, C.-K.; Agasti, S. S.; Zhu, Z.J.; Isaacs, L.; Rotello, V.M. *Nature Chem.*, 2010, *2*, 962-966.

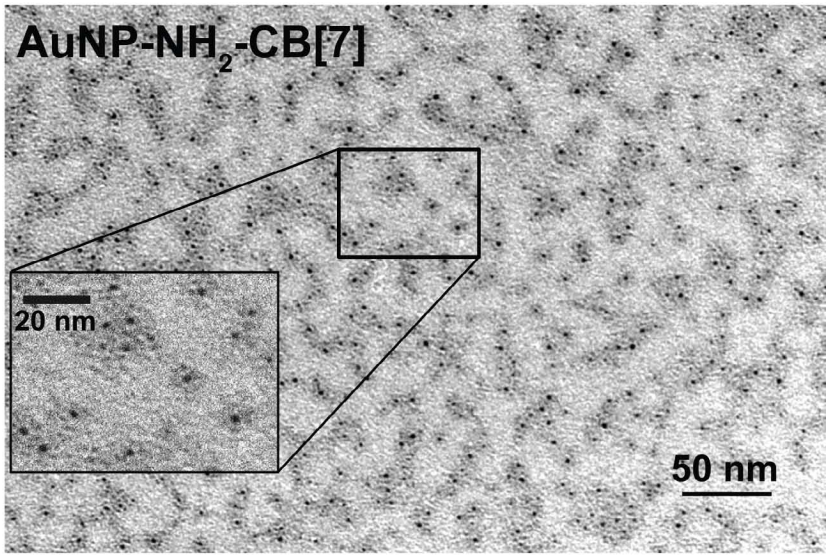
AuNP-NH_2 binds CB[7]

- NMR shows characteristic shifts, providing affinity and stoichiometry
- CB[7] visible in TEM, looks cool



AuNP-NH_2-CB[7]

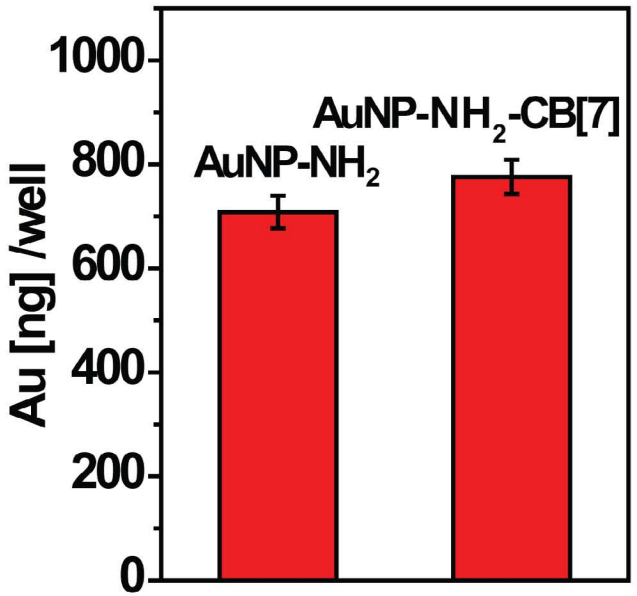




- ~40 CB[7] per NP, K_a 1.0x10^8, high enough for biological applications
- what about cell uptake?

Both bound and unbound AuNP-NH_2 are taken up effectively

- essentially identical uptake with or without CB[7]--strange coincidence
- bound particle stuck in endosome, unbound particle escapes
- ADA triggers endosomal release

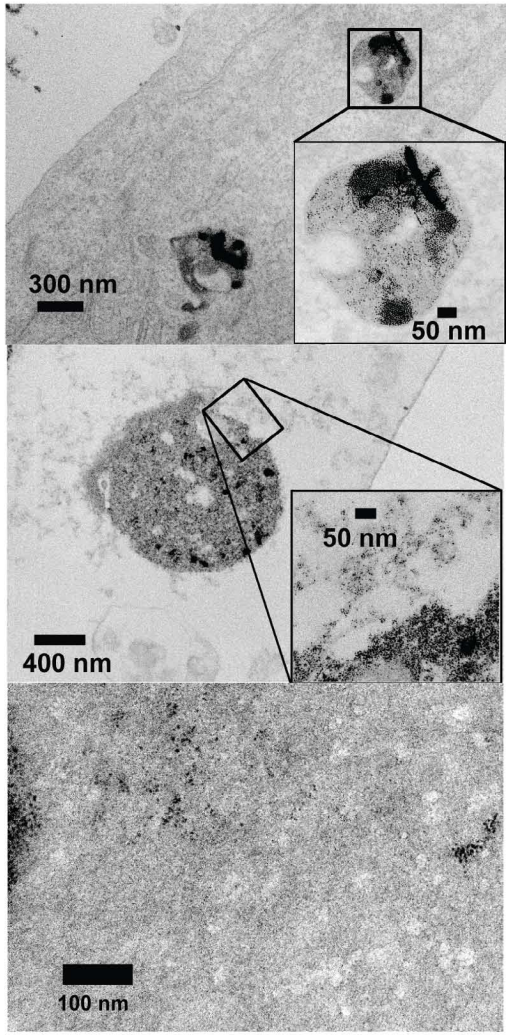


- ICP-MS quantification of uptake

+CB[7]
all in endosome

-CB[7]
dispersed

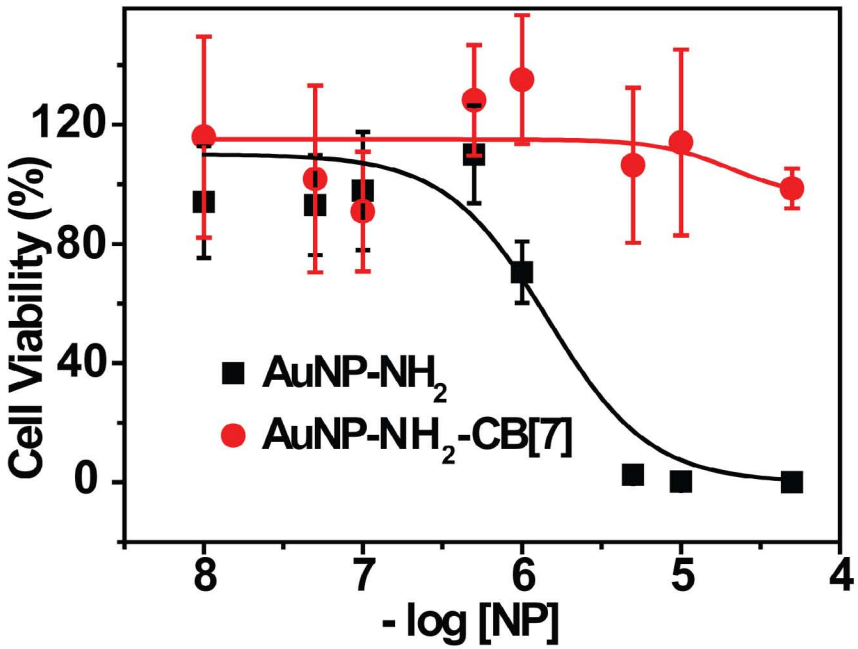
+CB[7], then ADA
dispersed

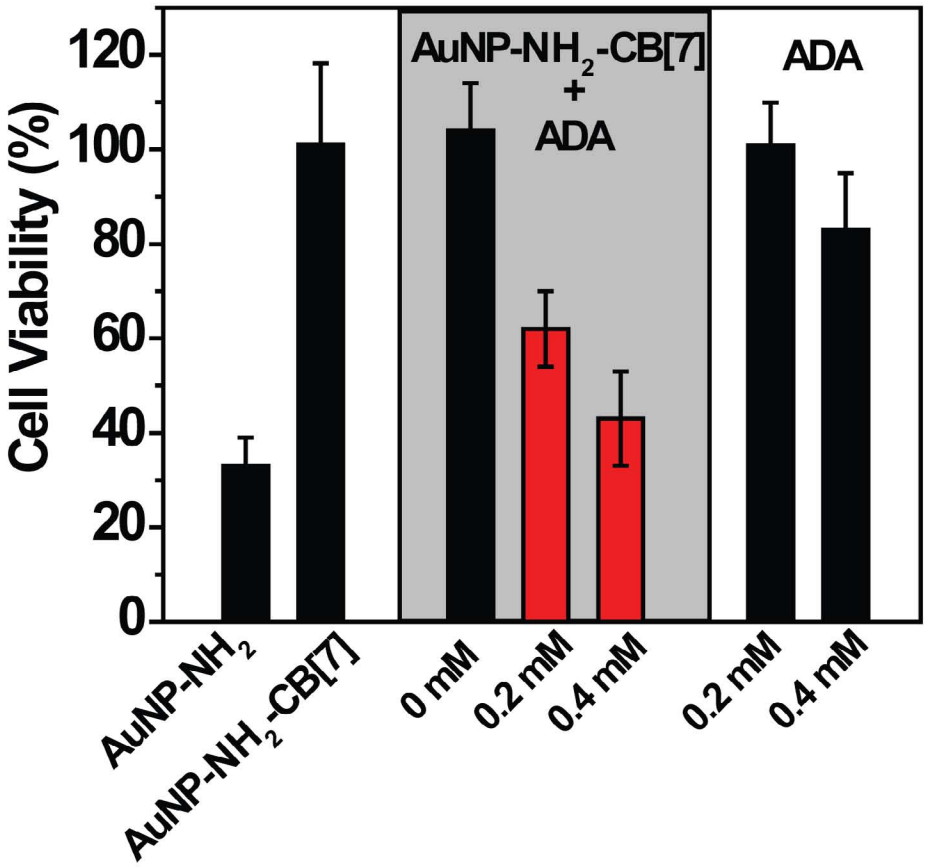


- synthetic host-guest chemistry inside the cell!

Particle release triggers toxicity

- CB[7]-bound particle is non-toxic (it's stuck in the endosome)
- free AuNP is toxic...and so is ADA-released CB[7]

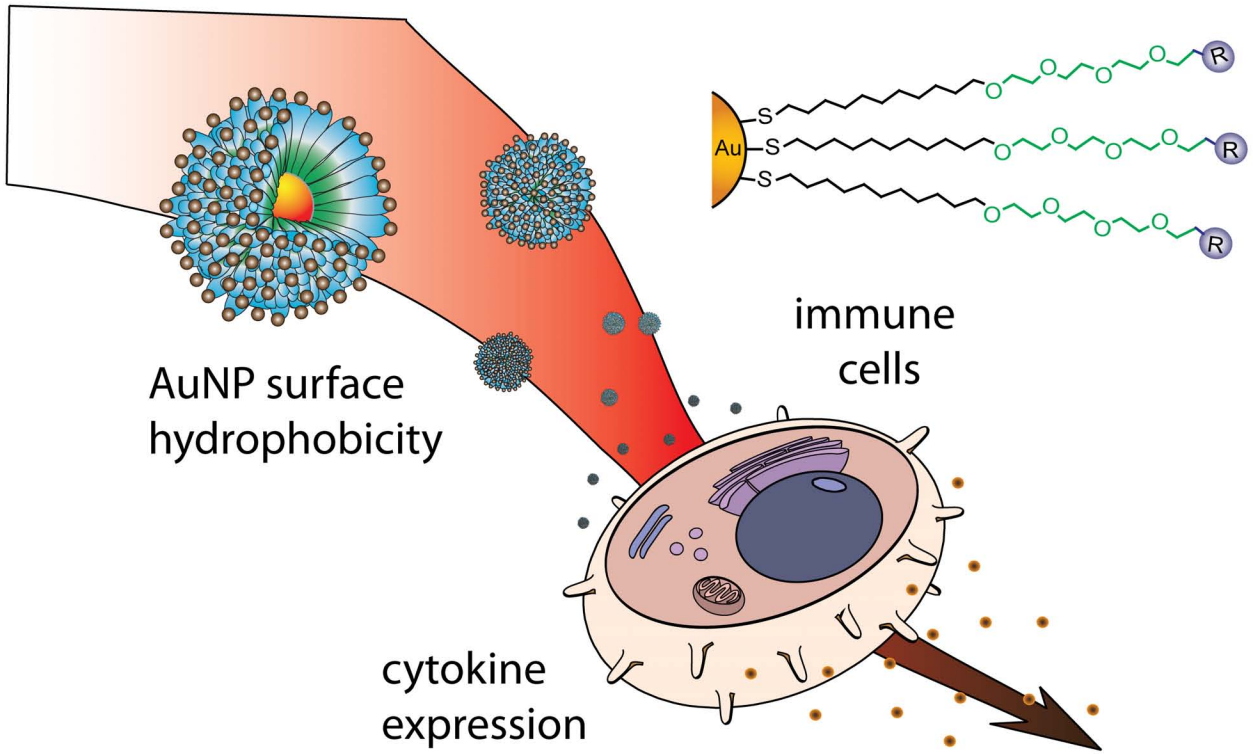


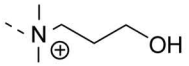
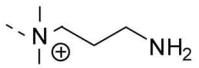
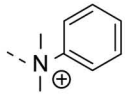
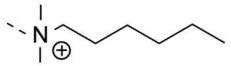
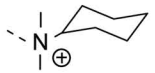


- supramolecular activation of nanoparticle therapeutic

Nanoparticle surface properties and immune response

- nanoparticles provide a tunable scaffold for presentation of surfaces...
- ...to probe the role of hydrophobicity in innate immune response

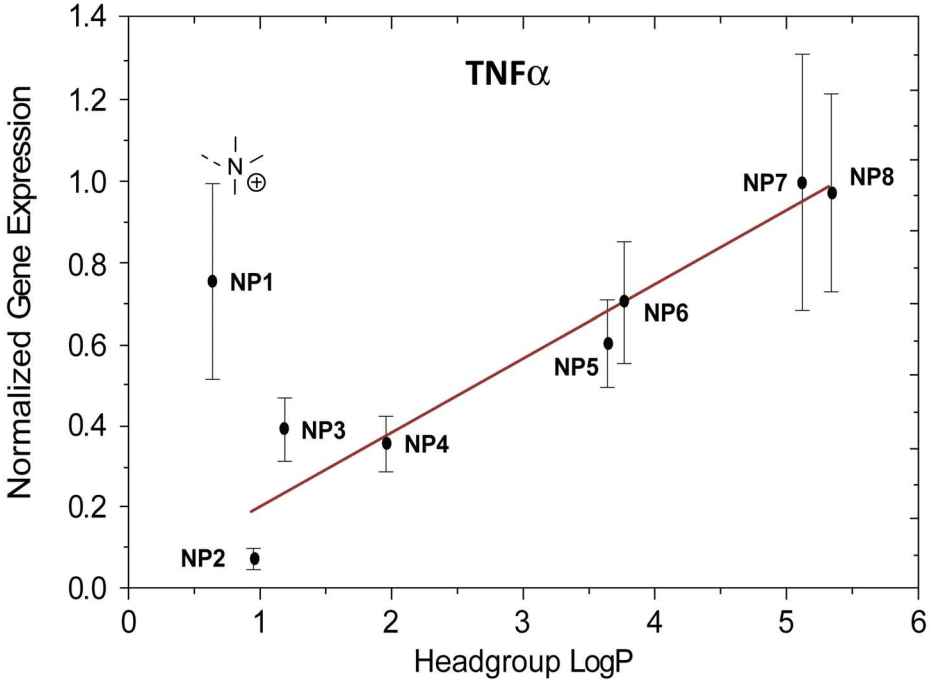


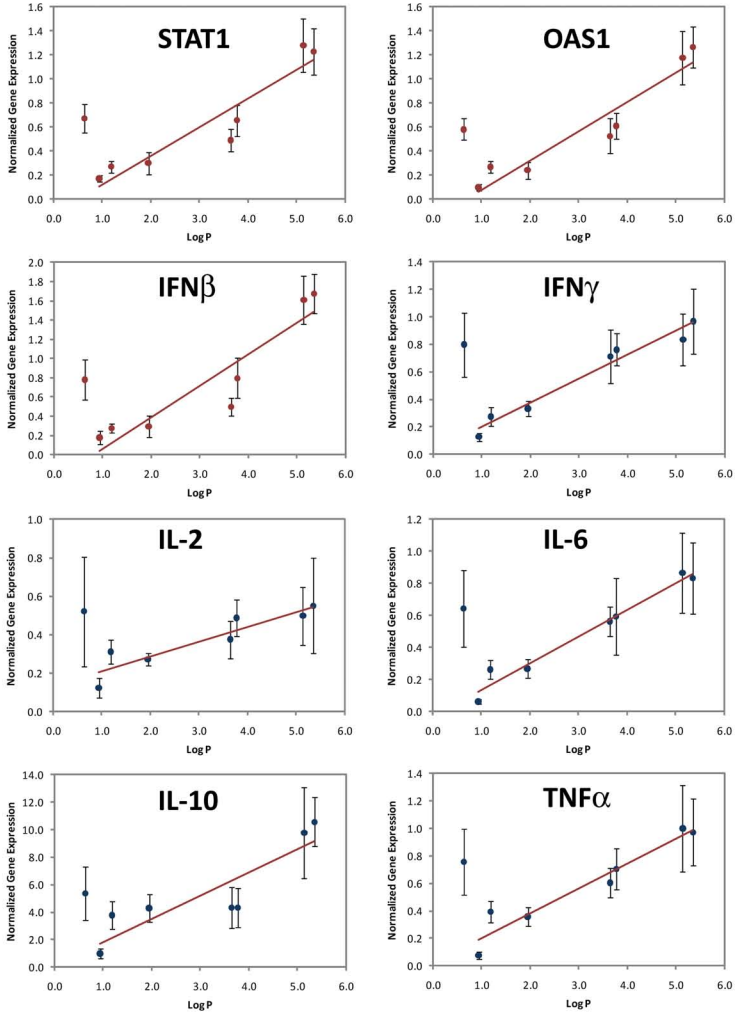
	R Group	Log P
NP1		0.63
NP2		0.94
NP3		1.19
NP4		1.95
NP5		3.65
NP6		3.77
NP7		5.14
NP8		5.35
NP9		

- a test for the “hyppo” innate immune response (inflammation, vaccines)
- can’t use polymers, liposomes, etc.--hydrophobicity changes structure

Hydrophobicity and cytokine response strongly correlated

- in vitro mRNA response of splenocytes used to quantify expression
- cells incubated with 10 μM NP for 2h

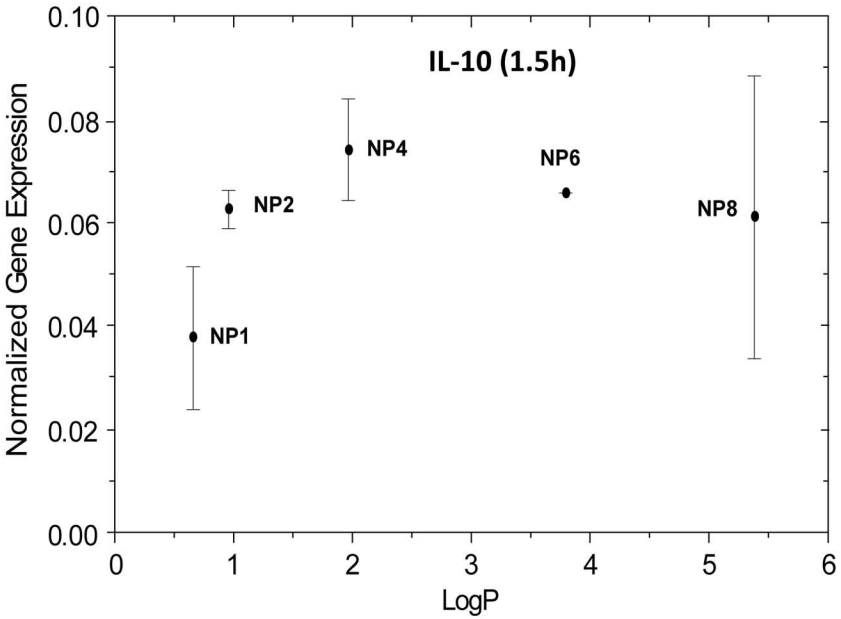


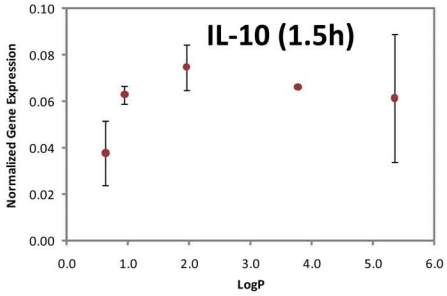


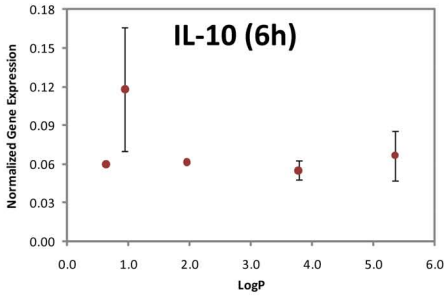
- linear correlation of cytokine expression
- except for NP1, suggesting alternative activation mechanism for this NP

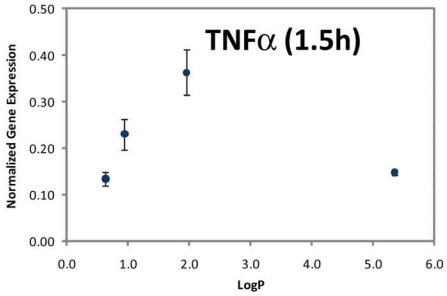
Hydrophobicity dictates immune response in vivo

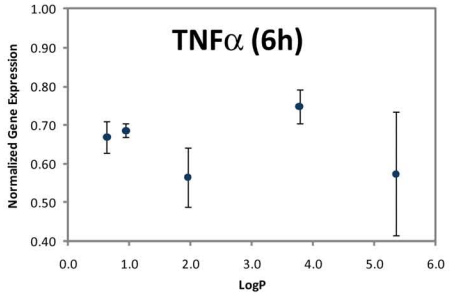
- mouse model, 5 mg NP/kg
- mice sacrificed after 1.5h, 6h









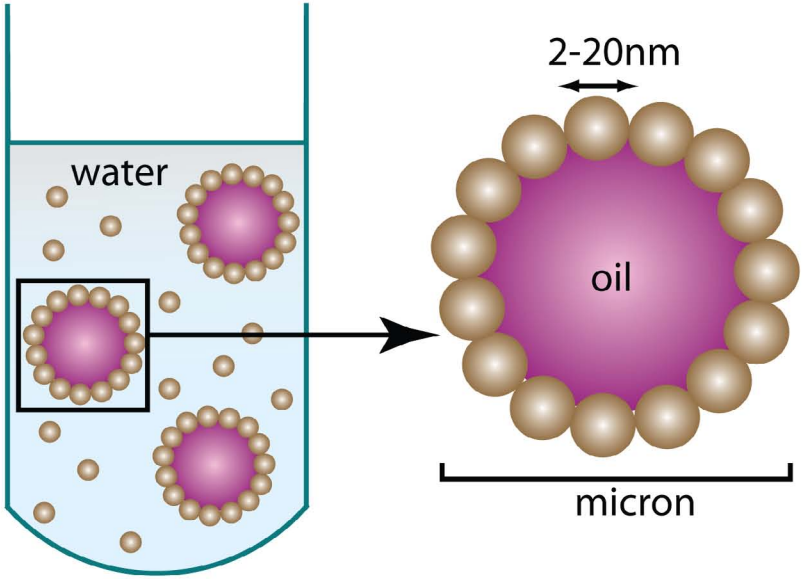


- strong effect after 1.5 h, no correlation after 6h
- increasing hydrophobicity=increasing cytokine response...up to a point
- leveling off most likely due to biodistribution effects (hydrophobic=sticky)
- immune response both cautionary and potentially useful

Moyano, D. F.; Goldsmith, M.; Solfiell, D. J.; Landesman-Milo, D.; Miranda, O. R.; Peer D.*; Rotello, V. M.* *J. Am. Chem. Soc.*, in press

Nanoparticle assembly at interfaces

- particles go to interfaces to minimize interfacial energy
- providing access to NP-based capsules and membranes



$$\Delta E = -\frac{\pi r^2}{\gamma_{o/w}}\left[\gamma_{o/w} - \left(\gamma_{p/w} - \gamma_{p/o}\right)\right]^2$$

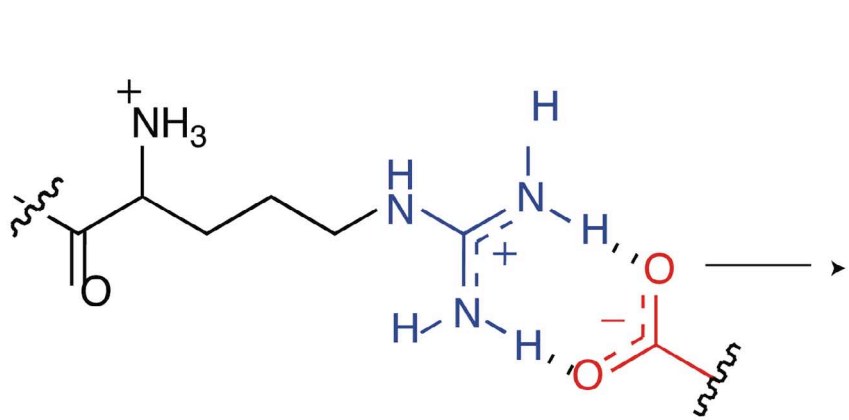
-smaller particles harder to assemble
-careful tailoring of wettability required
particle should be "amphiphilic":

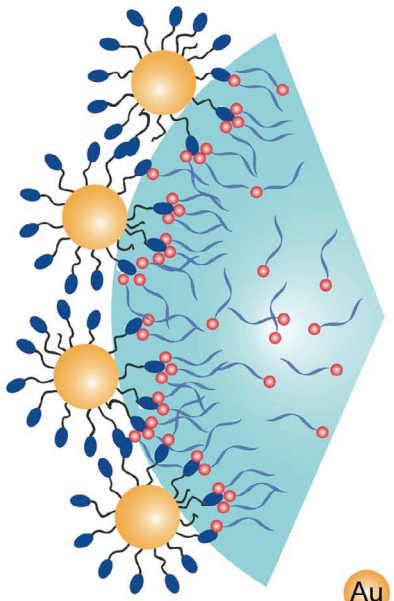
- the interface provides a template for particle assembly
- capsules provide functional systems...
- ...that are inherently multiscale

Patra, D.; Sanyal, A.; Rotello, V. M.
Chem.-Asian J., 2010, *5*, 2442-2453.

How do we make nano-scale nanoparticle capsules?

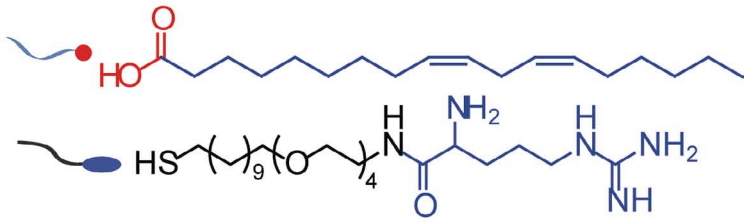
- modularity and functionality would provide great delivery vehicles
- current oil-in-water NPSCs are >1 μm -- smaller capsules are unstable
- smaller particles=higher Laplace pressure $\Delta P = 2\gamma_{o/w}/R_{capsule}$
- how do we make 'em small enough for tissue penetration (<150 nm)?





- can we cheat the system by making particles that really like both water and oil?
- guanidinium groups love carboxylates... ...and are very hydrophilic!

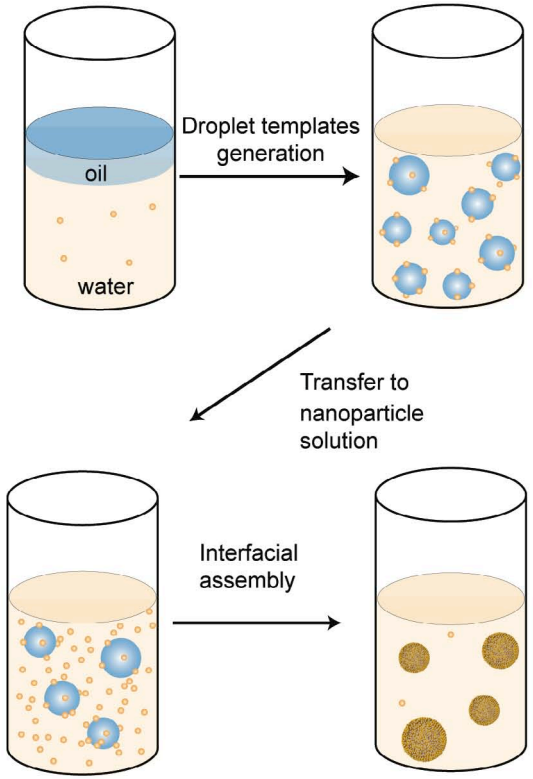
Au 2 nm Au particle;

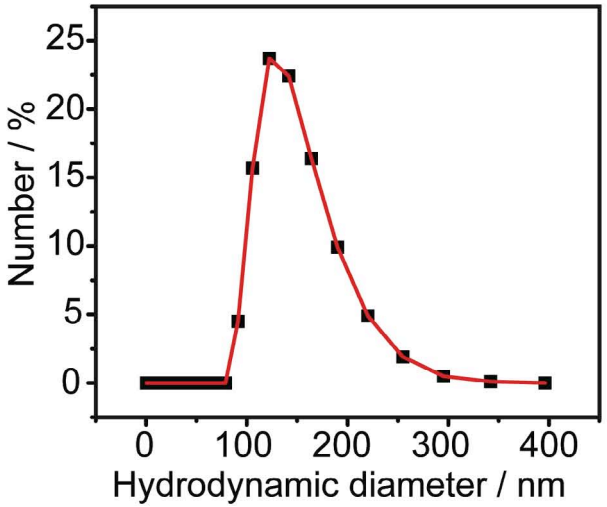


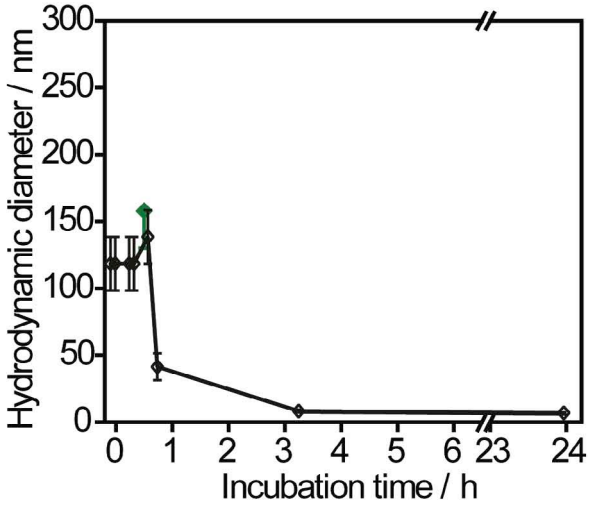
- maybe"superamphiphilicity" will pin particles to the interface...

Supramolecular interactions provide nano-scale NPSCs

- assembly provides ~120 nm capsules
- the good news: capsule are stable in buffer
- the bad news: capsules rapidly degrade in serum (bummer)



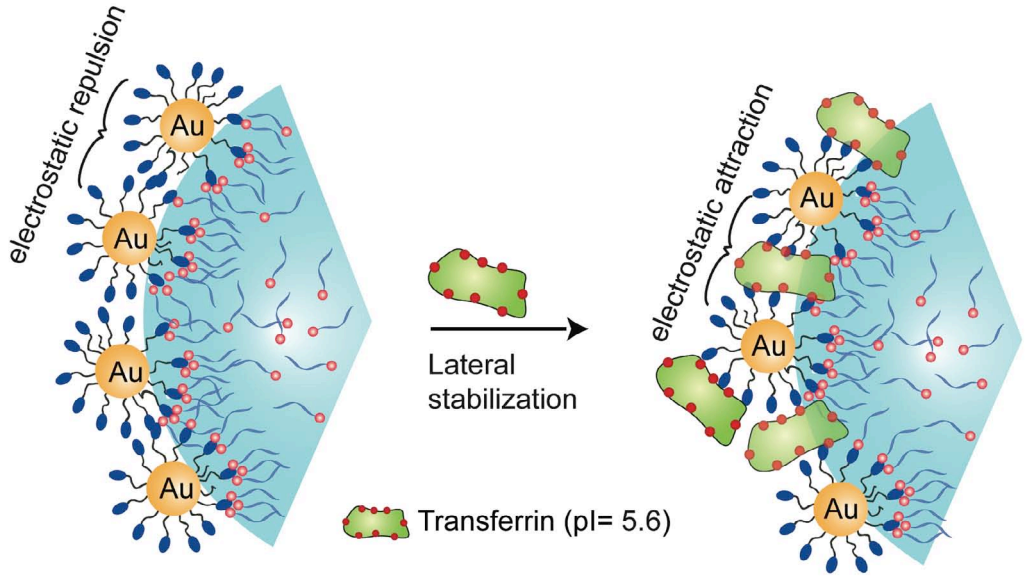


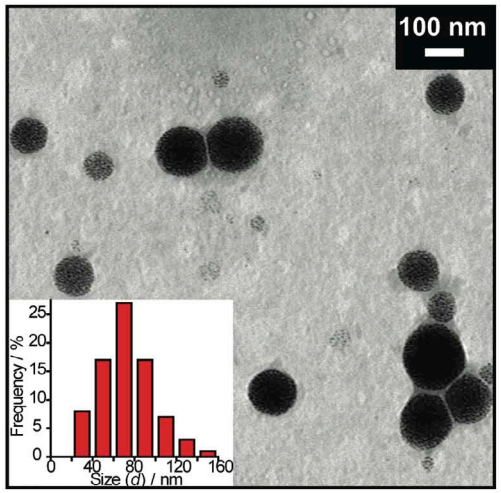


- let's take a closer supramolecular look to see why...

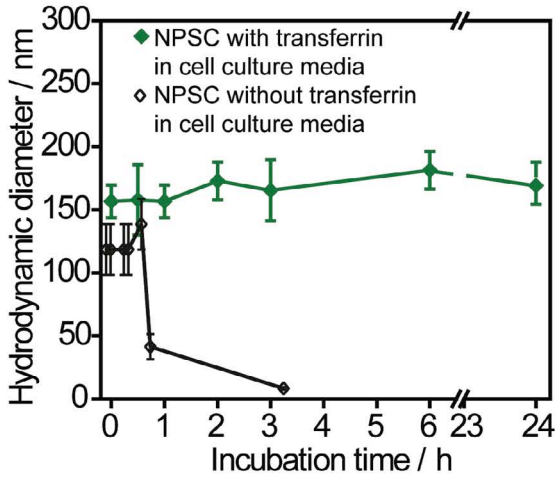
Lateral supramolecular interactions provide nano-NPSCs

- a whole lot of positively charged NPs probably doesn't help stability...
- proteins can provide anionic "mortar" to solve this problem



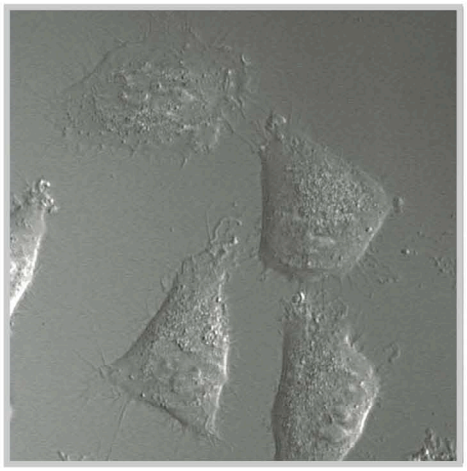


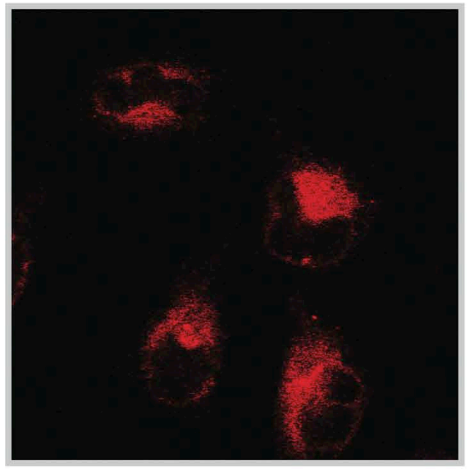
- stable capsules...
- next stop, delivery!

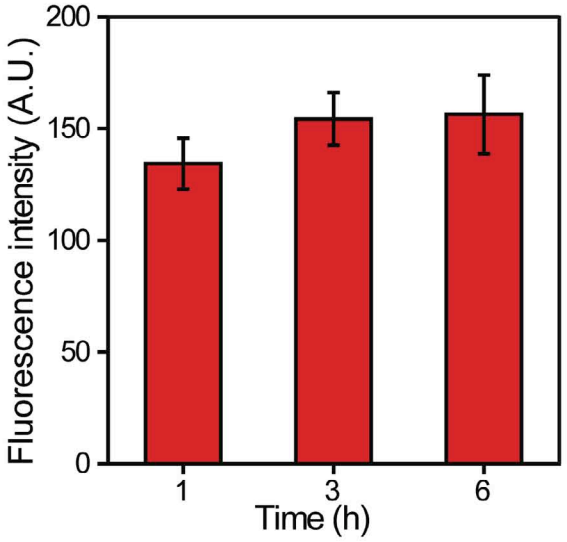


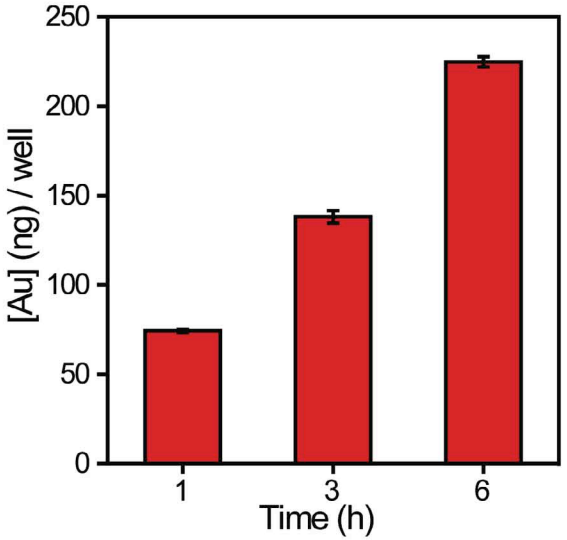
Hydrophobic dyes are delivered efficiently

- Nile Red provides easy to see drug analog
- dye enters the cells far faster than the particles...





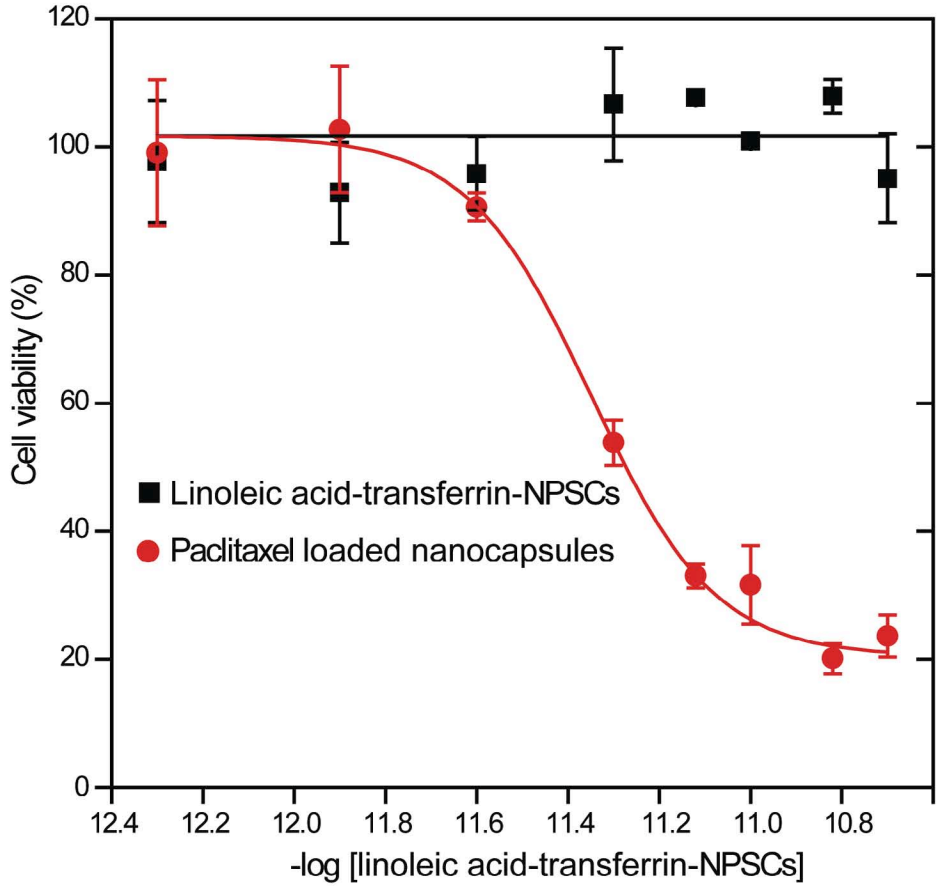




- Nile Red fluorescence
- Intracellular Au (ICP-MS)
- results suggest membrane fusion, not endocytosis

Drugs go in just fine too...

- paclitaxel--a nice hydrophobic drug
- non-toxic NPSC, loaded capsule kills cells dead!

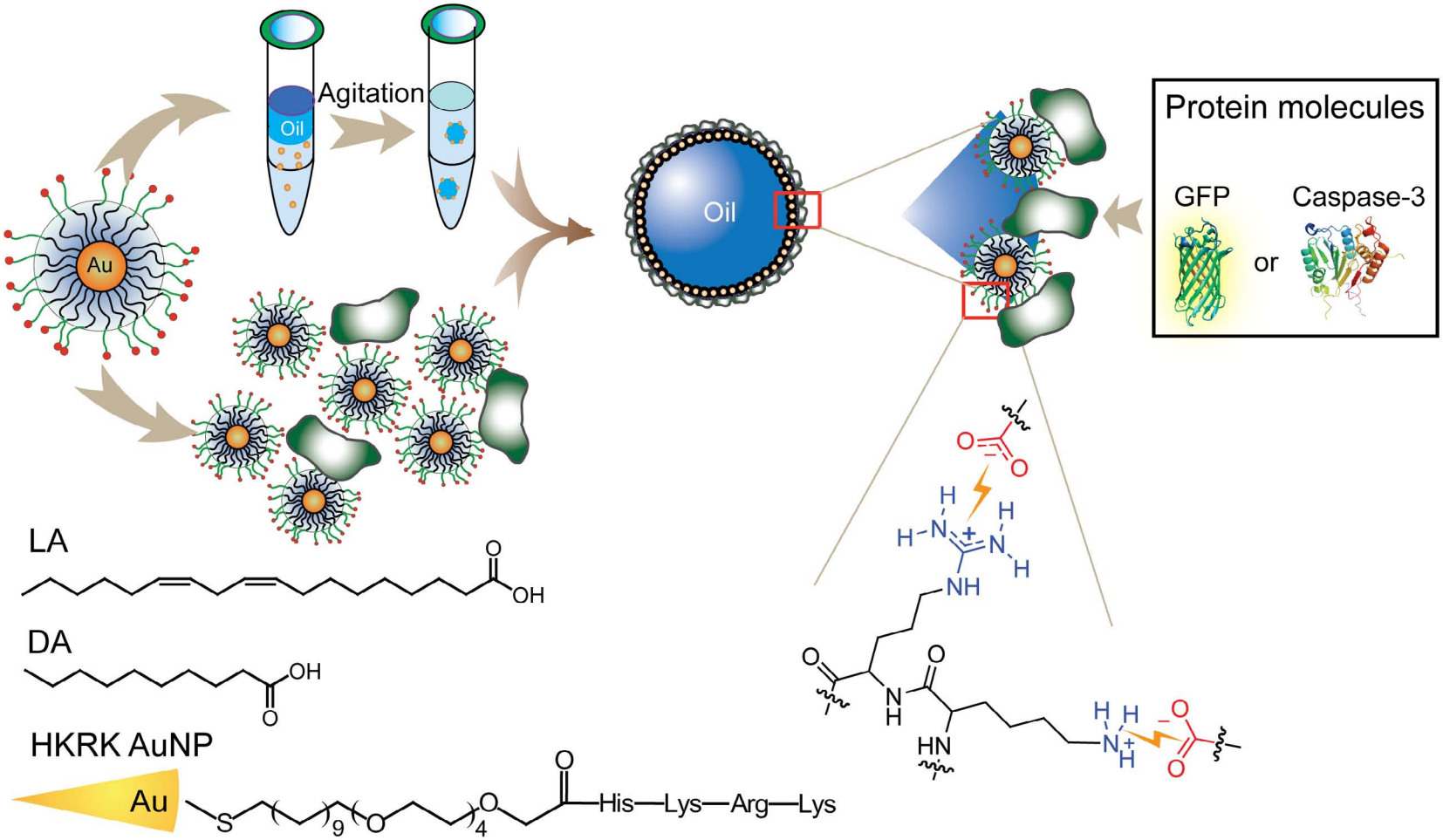


- capsules provide excellent vehicles for delivering hydrophobic drugs
- next up--targeting

Yang, X.-C.; Samanta, B.; Agasti, S. S,; Jeong, Y.; Zhu, Z.-J.; Rana, S.; Miranda, O.R.; Rotello, V. M." *Angew. Chem.*, *2011 5*0, 477-481 .

What about proteins?

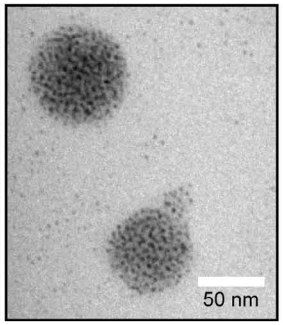
- protein therapeutics are a great idea...
- ...if you can get them into the cell cytosol



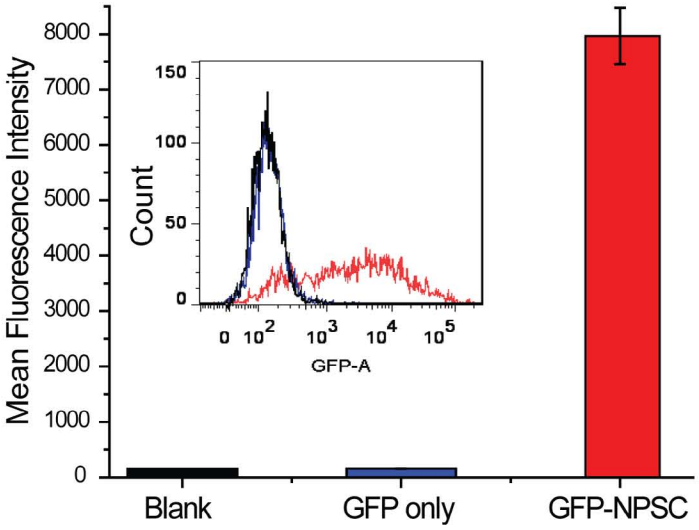
- let's see what tweaking our capsules can do for protein delivery

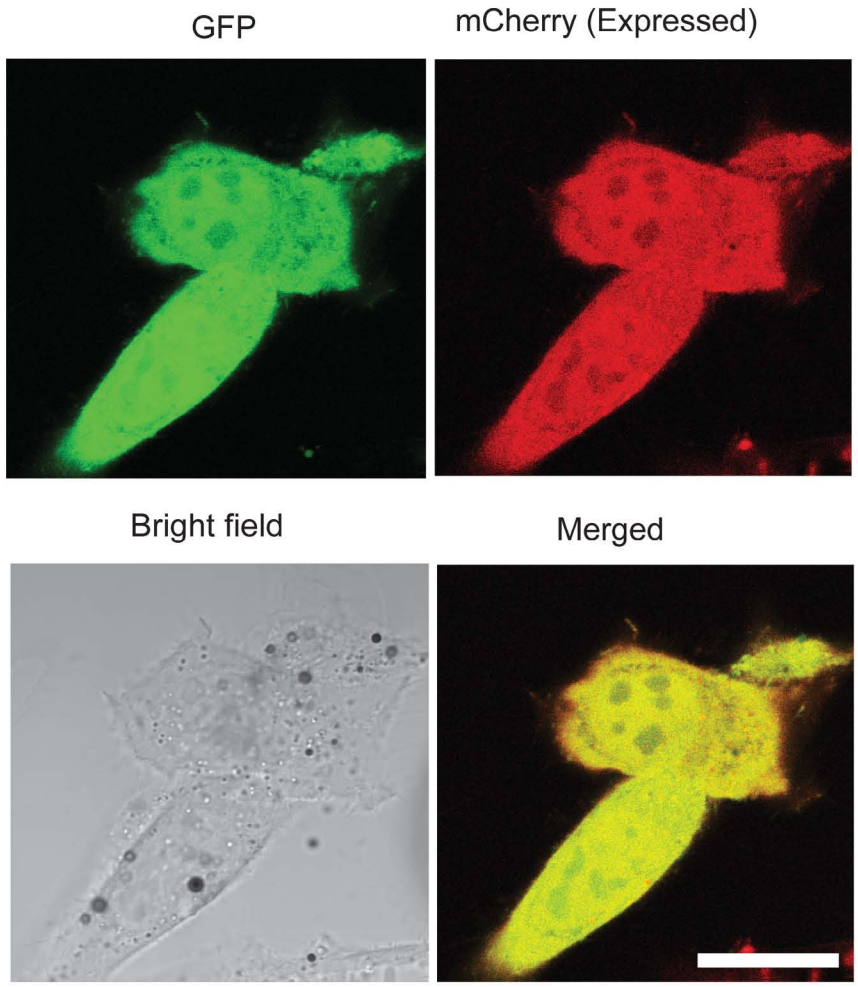
What about imaging?

- GFP--useful for imaging applications (and our work!)
- the testbed--RFP-expressing HELA cells



- pretty capsules...

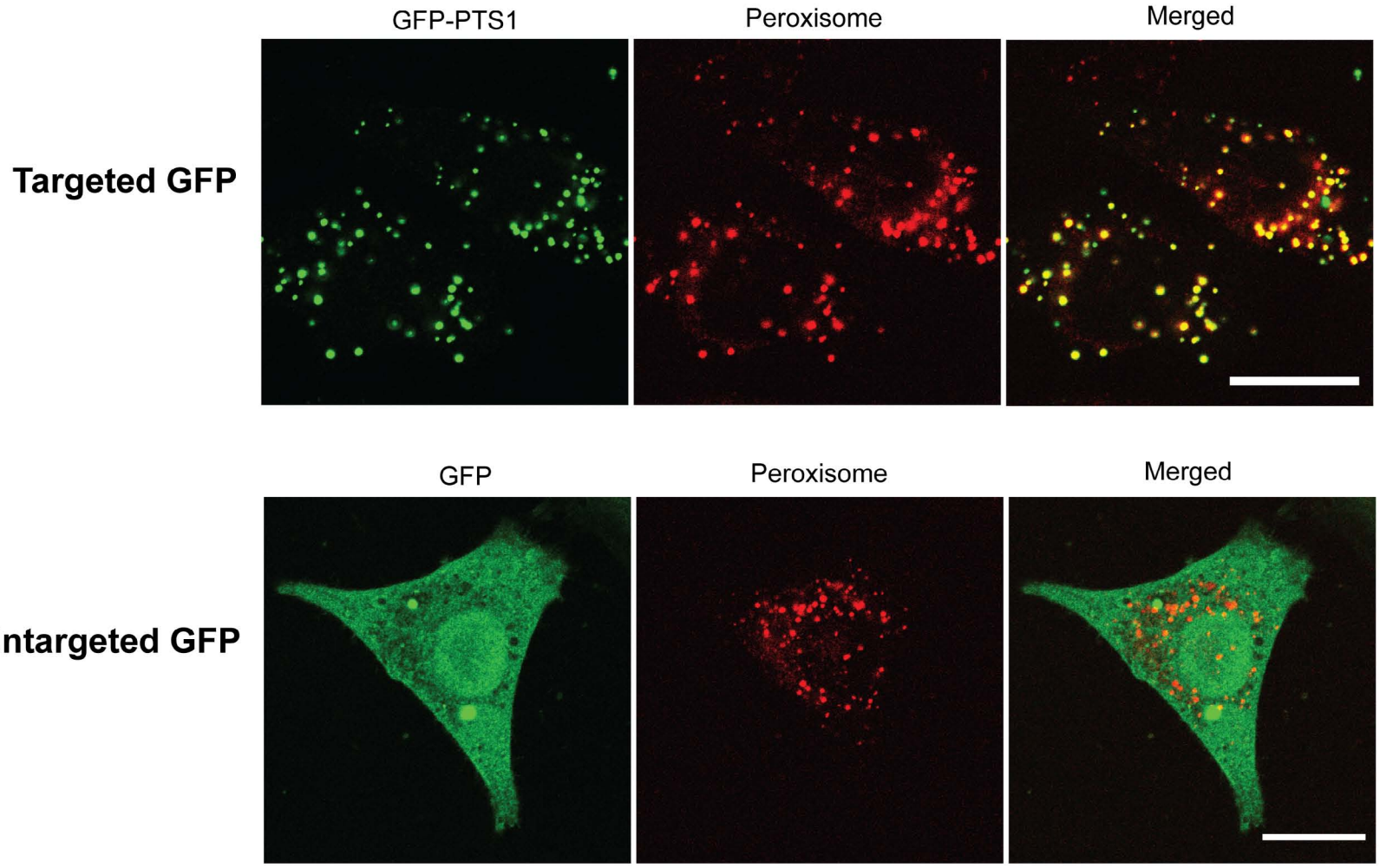




- efficient delivery and complete co-distribution

If we can get into the cytosol...we can target organelles

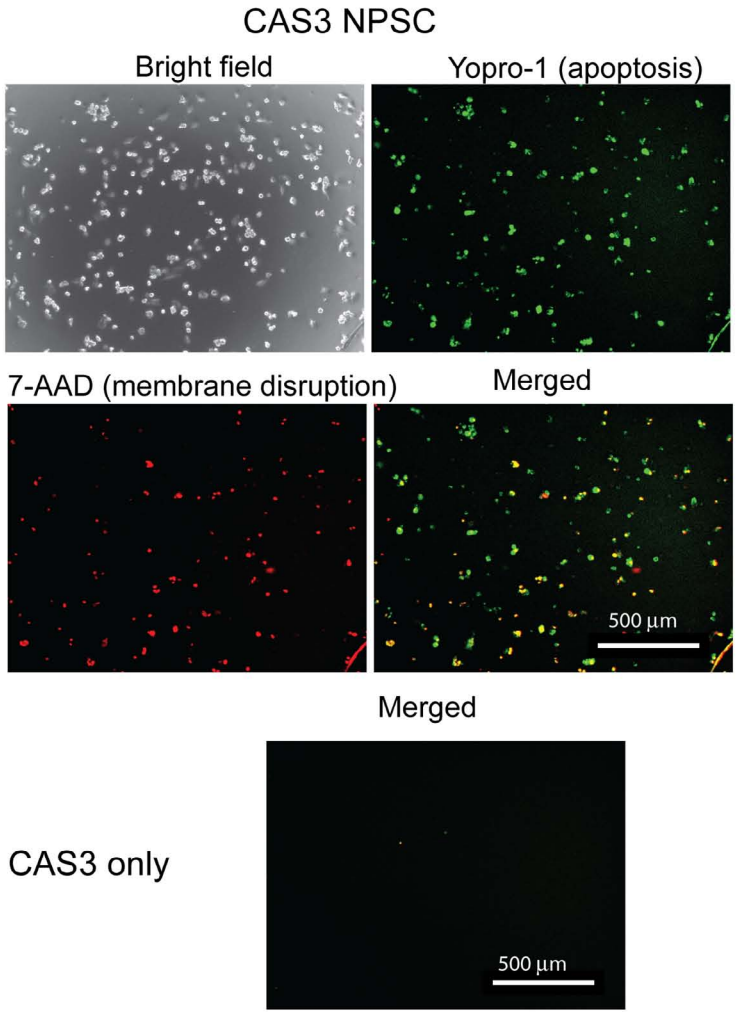
- a particularly stringent test for cellular delivery
- peroxisome targeting using PTS1-GFP fusion protein

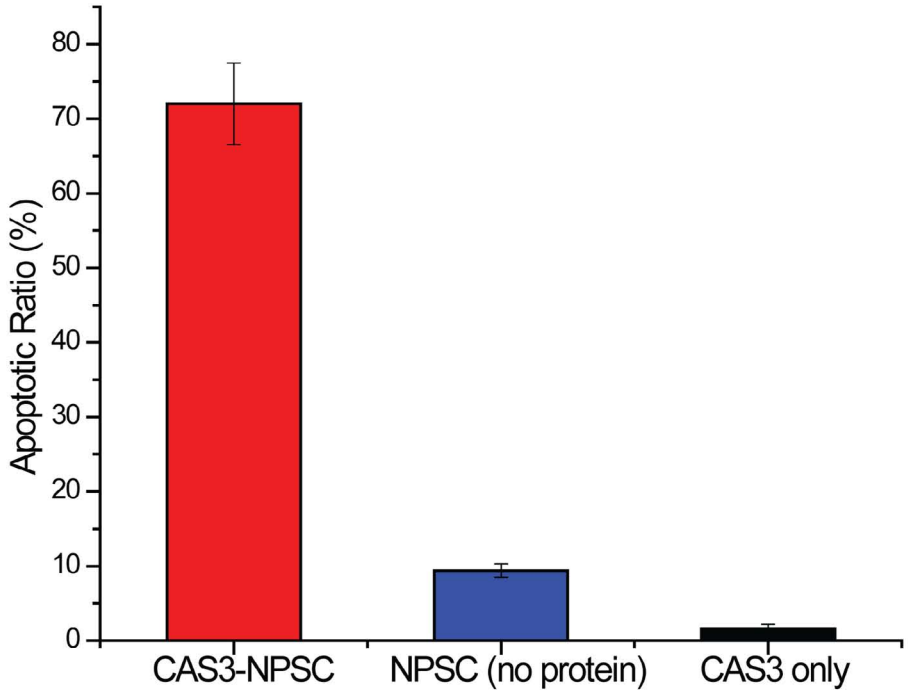


- targeted=localized, untargeted=diffuse, i.e. it works!

Enough of the pretty pictures--whaddabout therapeutics?

- caspase 3 induces apoptosis...
- ...and has been identified as a potential protein therapeutic



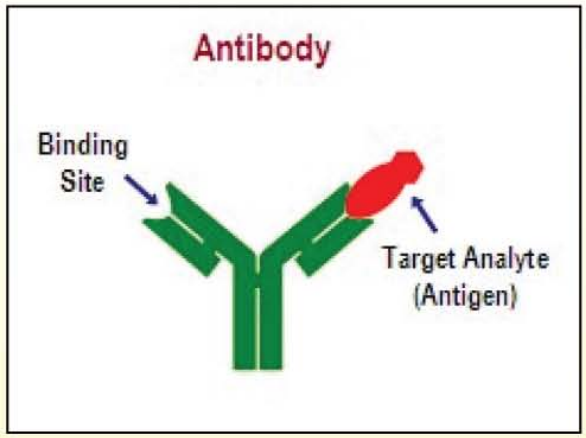


- CAS3: nada; NPSC alone:modest toxicity; CAS3 NPSC wholesale apoptosis

Specific or selective: Two different sensing paradigms

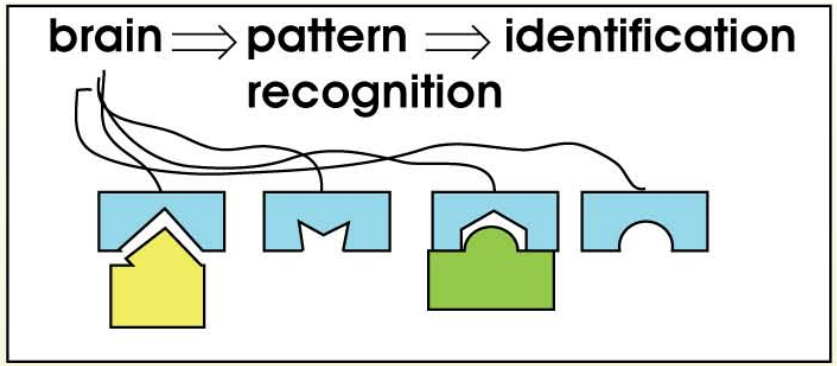
- one biomimetic, one not..

- specific recognition (e.g. ELISA)



- strengths:
  - -sensitive
  - -wide range of antibodies available
- challenges:
  - - new protein = new antibody
  - -difficult to quantify (i.e. not holistic)

- selective recognition (e.g. the nose)

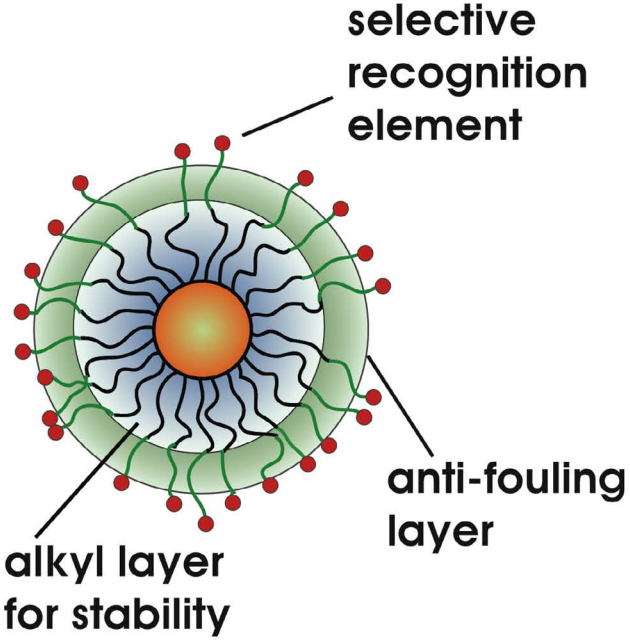


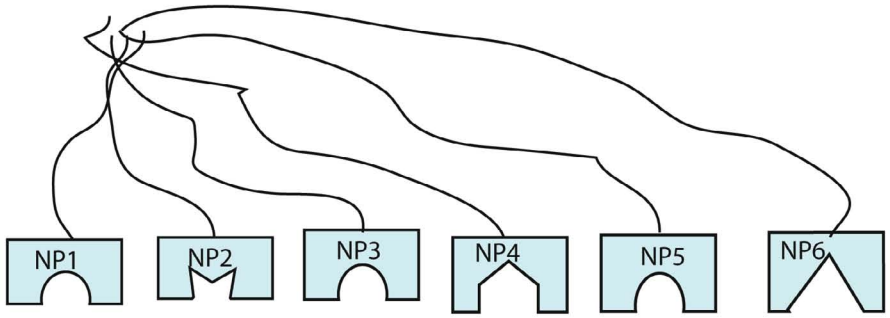
- strengths:
  - -simpler hardware
  - -excellent for complex mixtures
  - -trainable for new “odors”
- challenges:
  - - more complex software
  - -structural diversity required

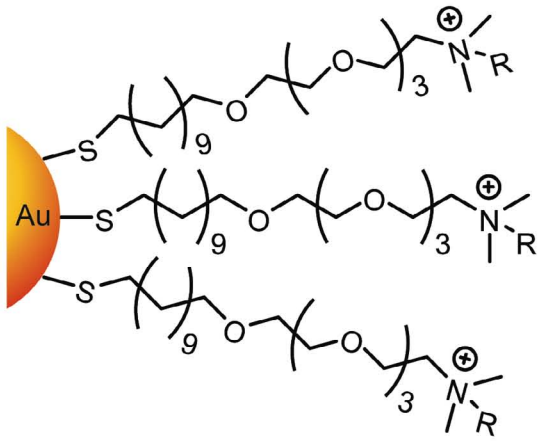
- can we use this strategy for cell surface sensing?

Step 1--selective receptors

- a wide variety of different nanoparticles can be made quickly
- the key is tuning the interface







NP1: R = —CH_3

NP2: R = —CH_2CH_3

NP3: R = —$(CH_2)_5CH_3$

NP4: R = —$CH(CH_2)_5$

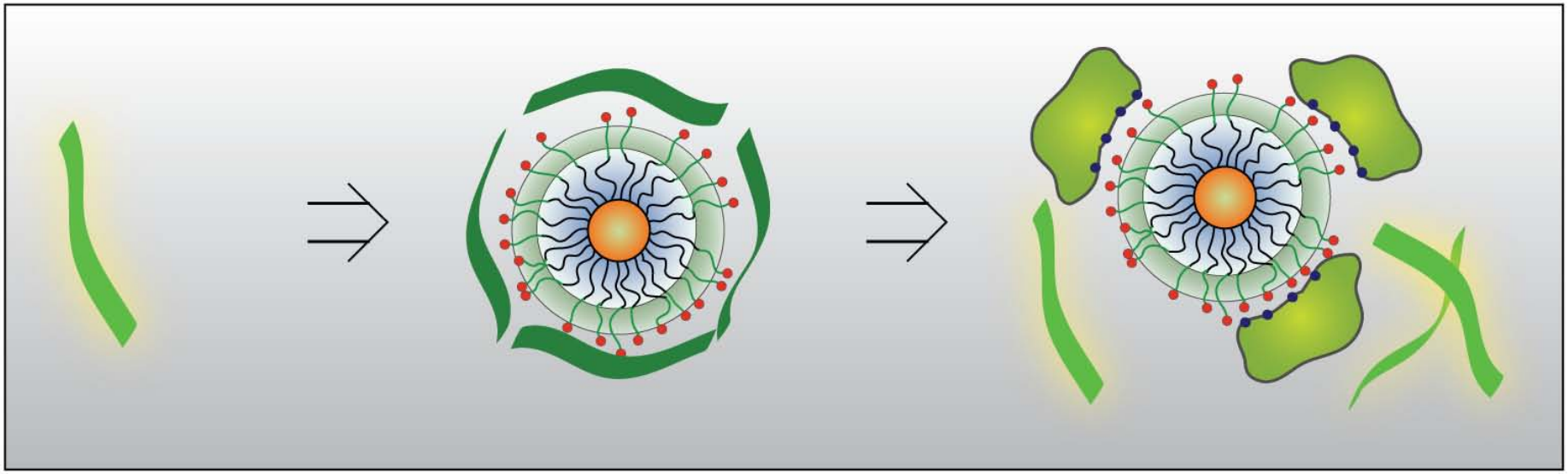
NP5: R = —$CH_2C_6H_5$

NP6: R = —$(CH_2)_3OH$

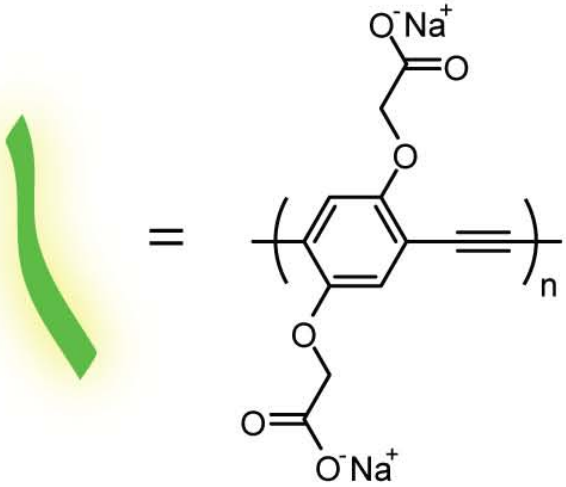
- recognition elements should provide selectivity
- how do we transduce the signal?

Step 2--transduction

- Au nanoparticles bound to analytes don't look much different than unbound
- gold nanoparticles are great fluorescence quenchers, though....

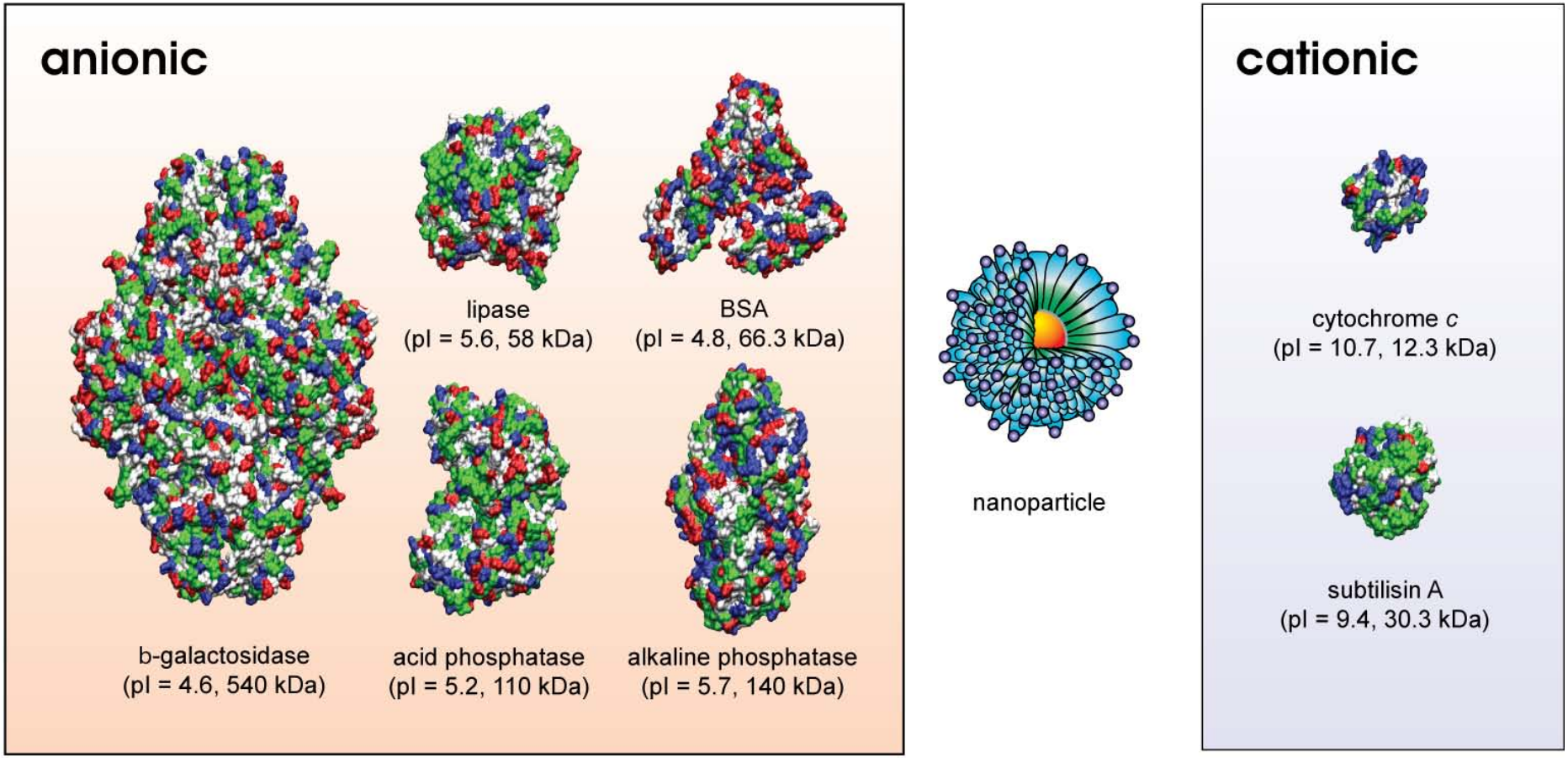


- key features of fluorophore
  - -anionic to bind cationic particle
  - -multivalent (sticky) for selectivity
- the answer--anionic PPEs provided by Uwe Bunz (Georgia Tech)



The targets

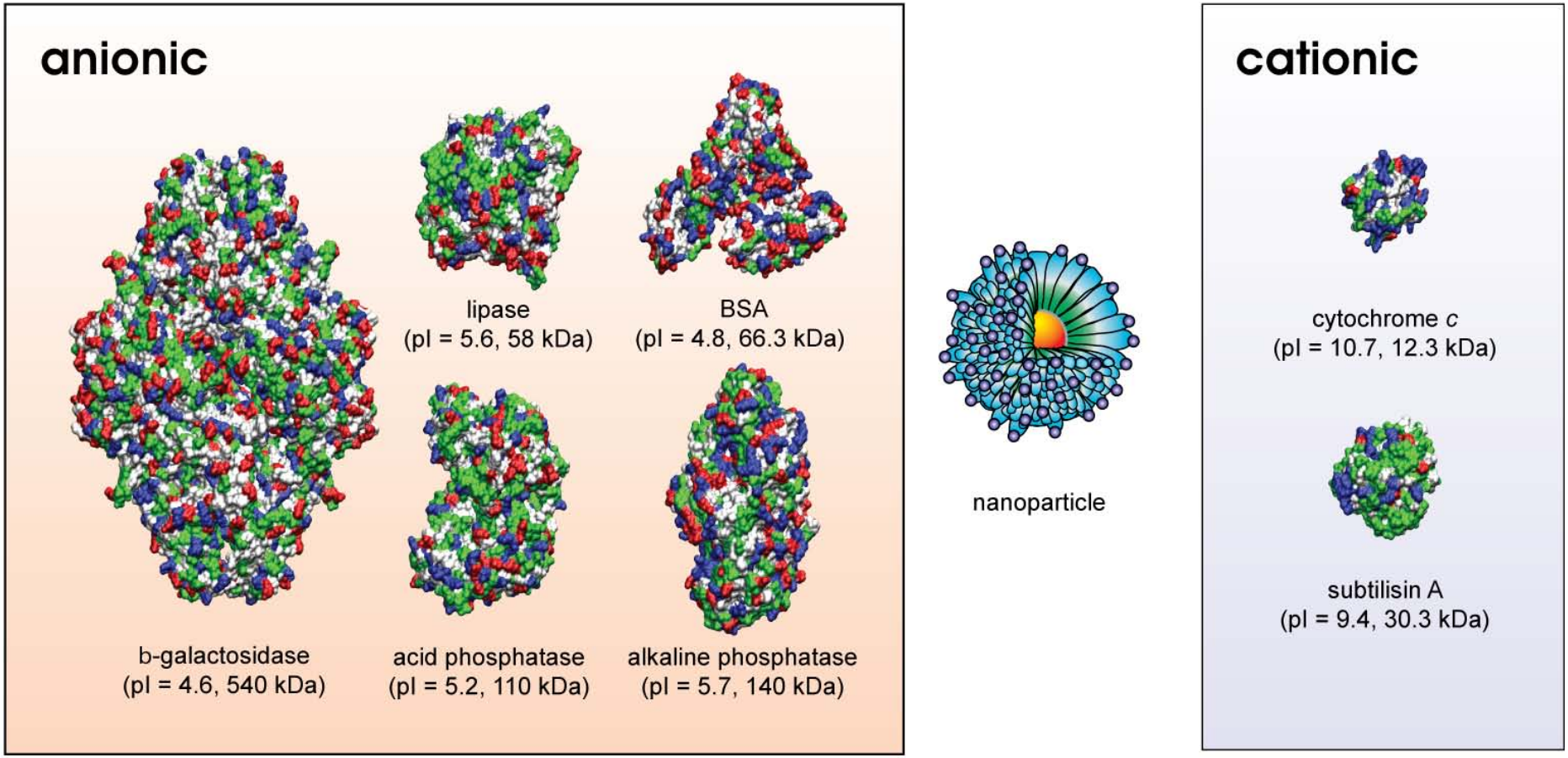
- commercially available proteins used as proof of concept
- proteins chosen to provide a range of size and charge



- can we differentiate ‘em--especially the tough ones?

The targets

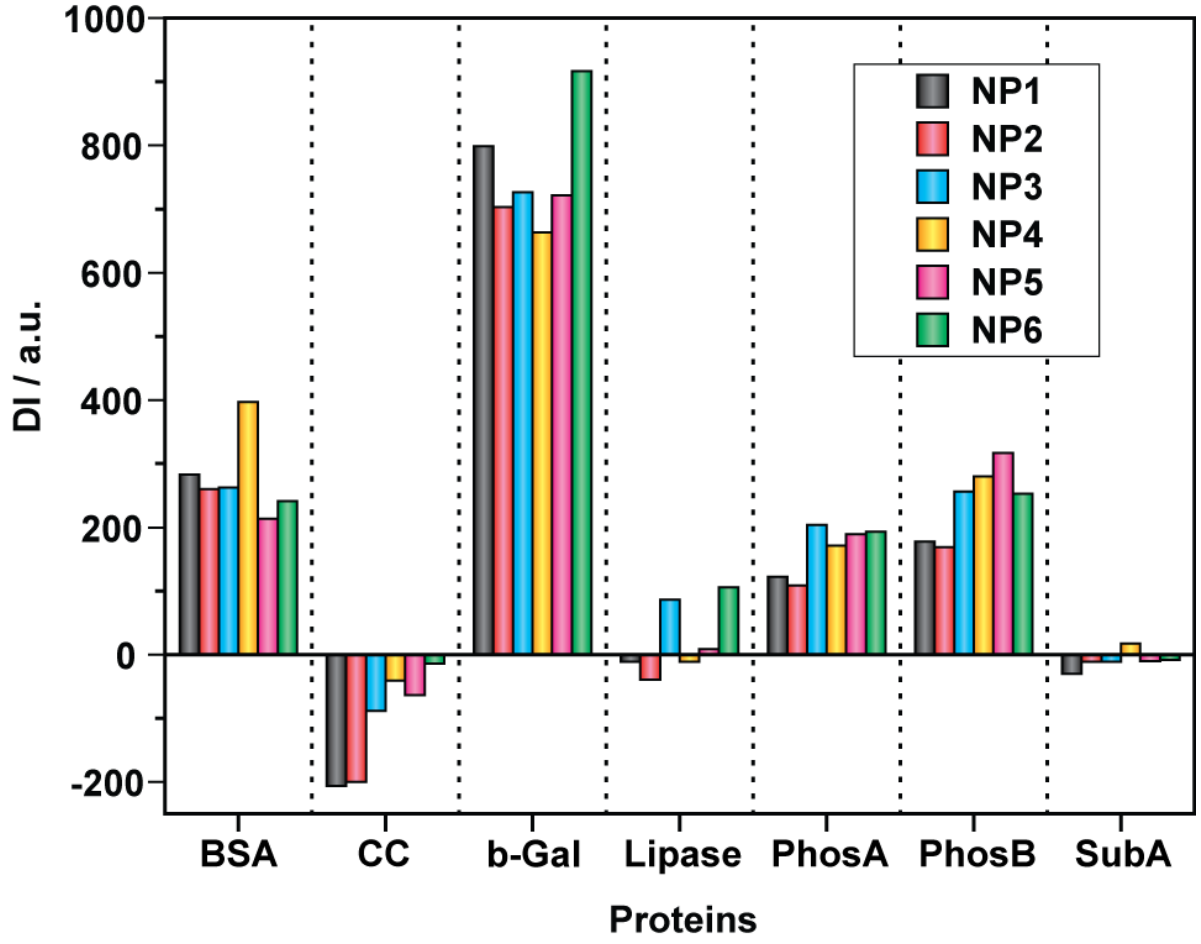
- commercially available proteins used as proof of concept
- proteins chosen to provide a range of size and charge



- can we differentiate ‘em--especially the tough ones?

We can differentiate the proteins qualitatively

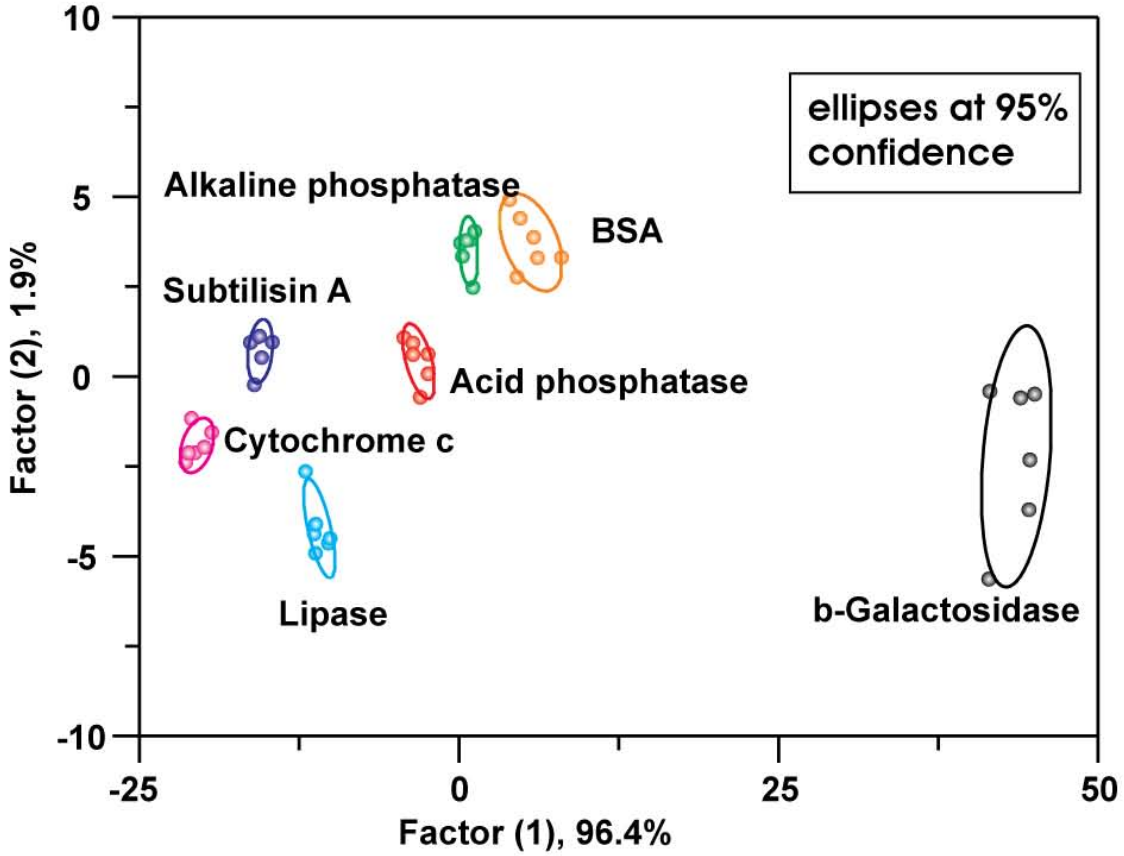
- different nanoparticles show different selectivity...
- ...providing a different pattern for each protein



- can this pattern be used to identify proteins?

Pattern recognition methodology provides protein identification

- Linear Discriminant Analysis (LDA) provides a tool for data analysis
- LDA maximizes the ratio of between-analyte and within-analyte variance

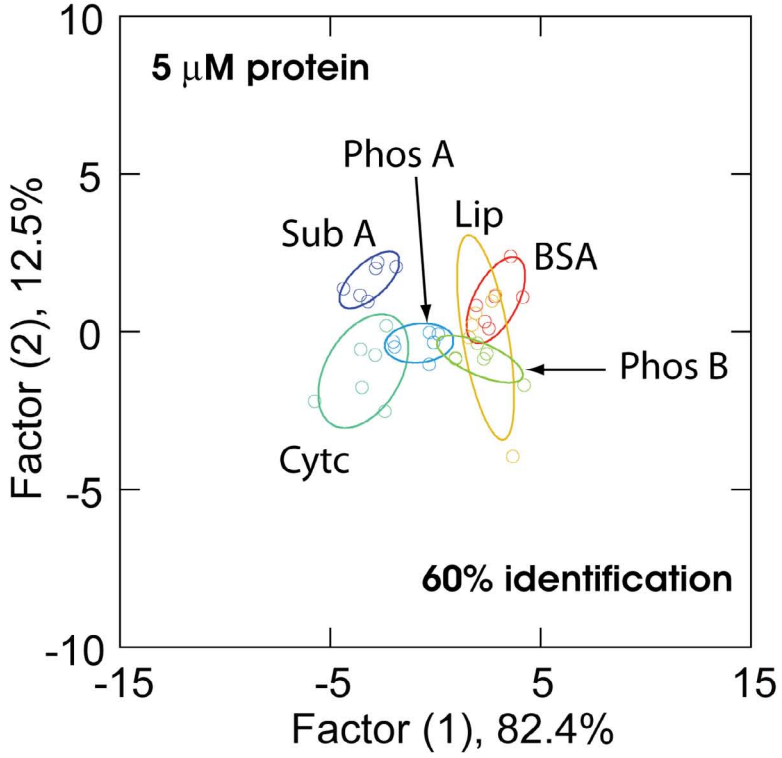


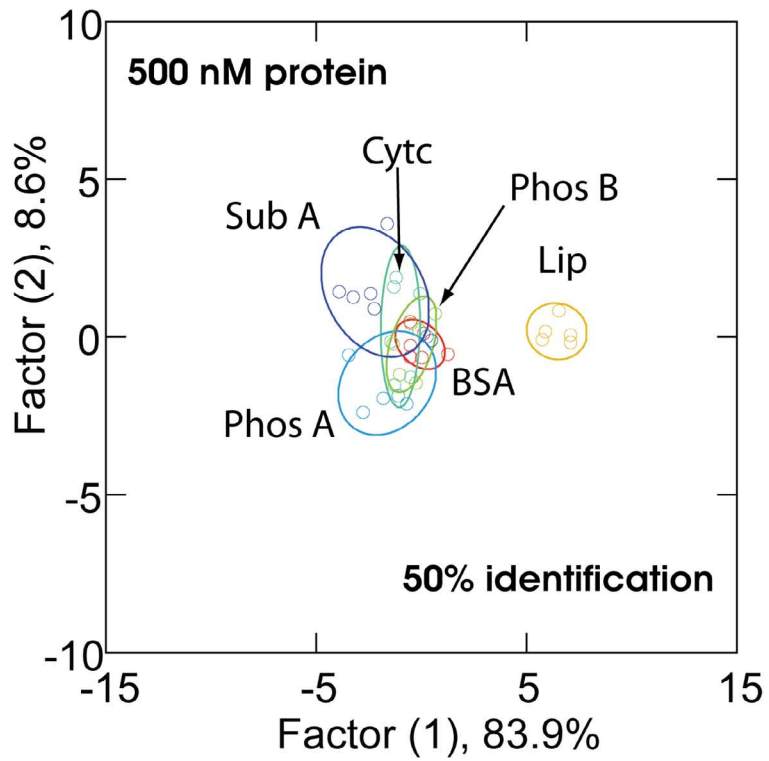
- the test: 56 samples randomly chosen from training set
- the outcome: 96% accuracy in identification!
- ongoing studies: biofluids (serum looks promising!)

Closer to the real world--sensing in serum

- Sensing protein levels in serum is an important diagnostic tool
- the challenge: serum albumin: 50 mg/mL (700 μM)
- it's like looking for needles in a haystack!

- proteins 'spiked' into undiluted human serum

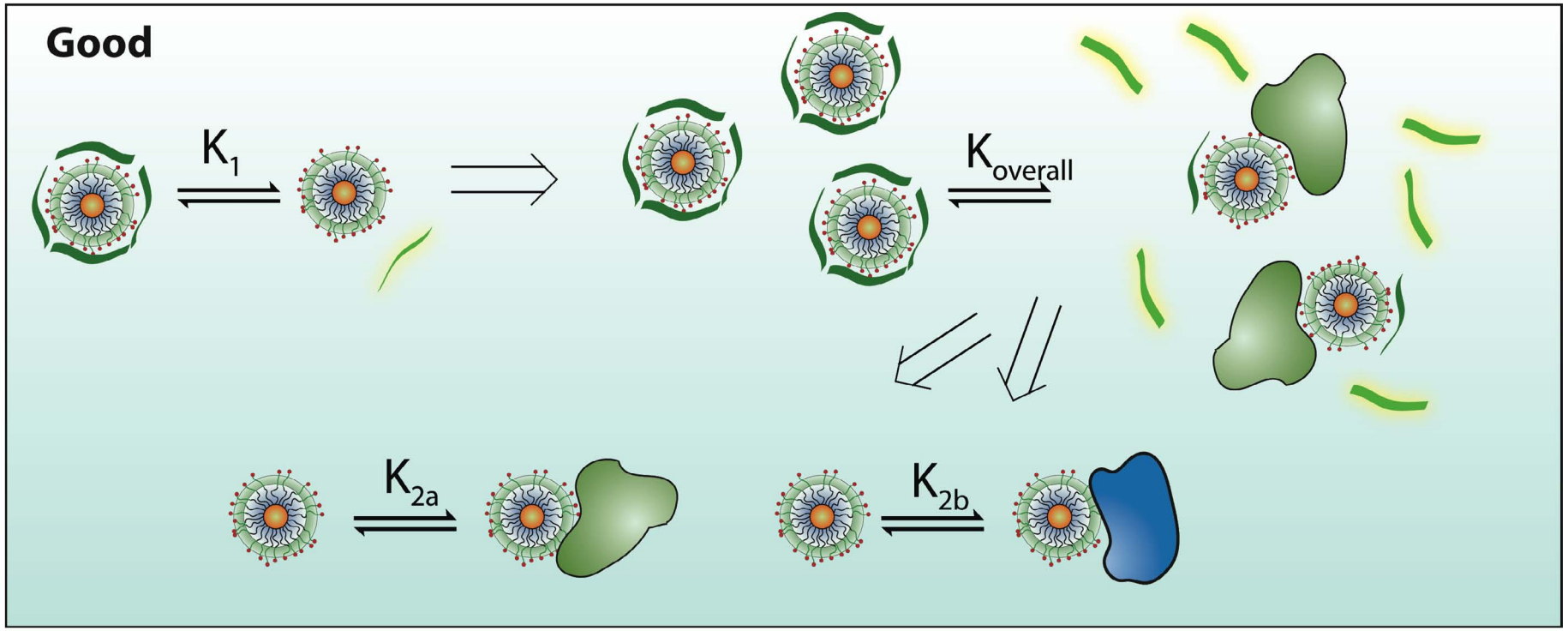


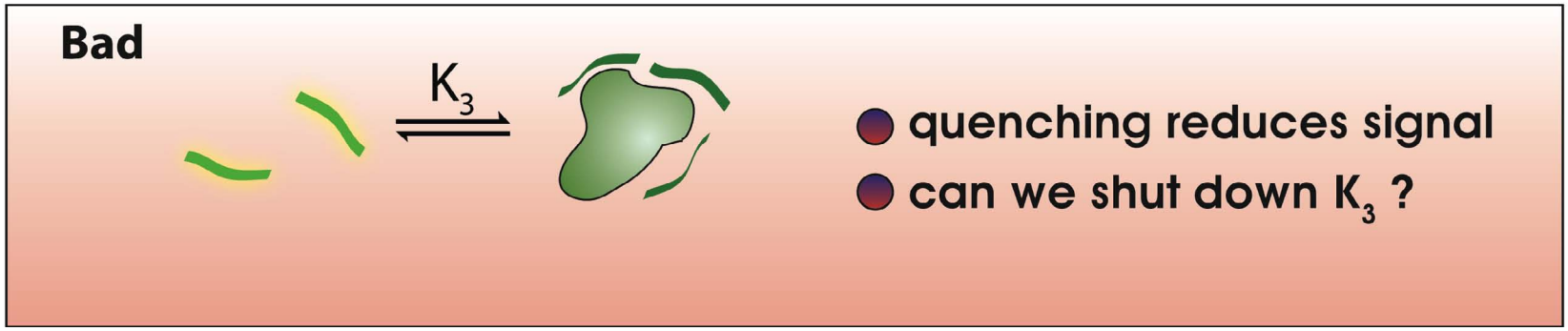


- the first attempts using original polymer/particle mixture--not great
- it's a modular system--let's switch the polymer!

A closer look at the sensing process

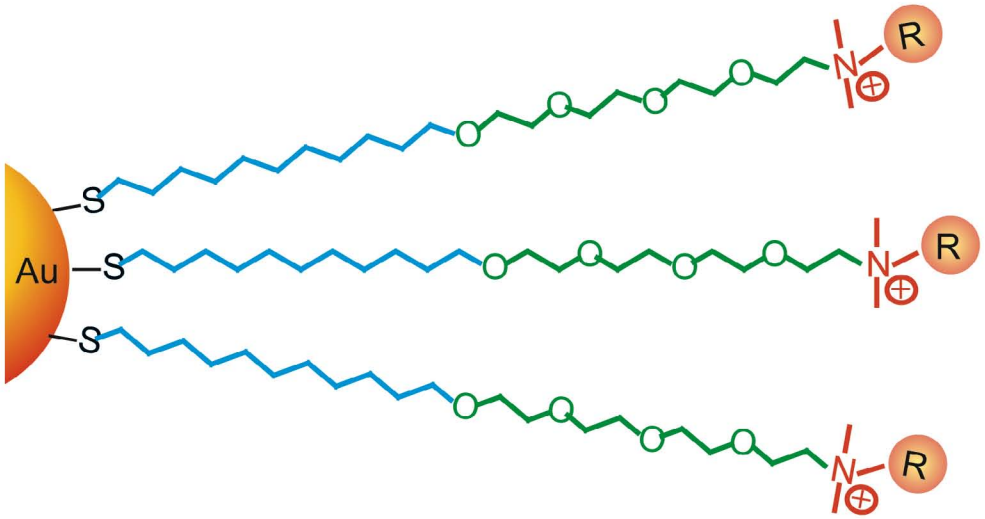
- multiple equlibria involved in sensing
- some good, some bad...





Instead of a polymer, what about a biopolymer transducer?

- fluorescent proteins come in many shapes, sizes and colors...
- and are inherently biocompatible!



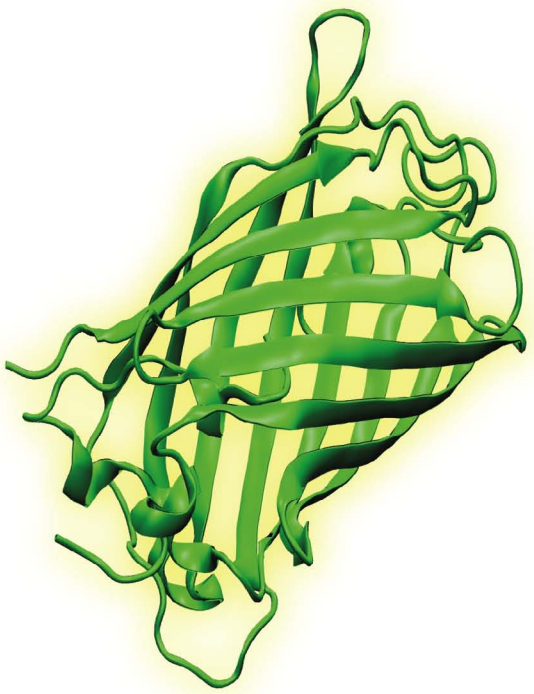
NP1 =

NP2 = $(CH_2)_5$

NP3 = OH, OH

NP4 =

NP5 =

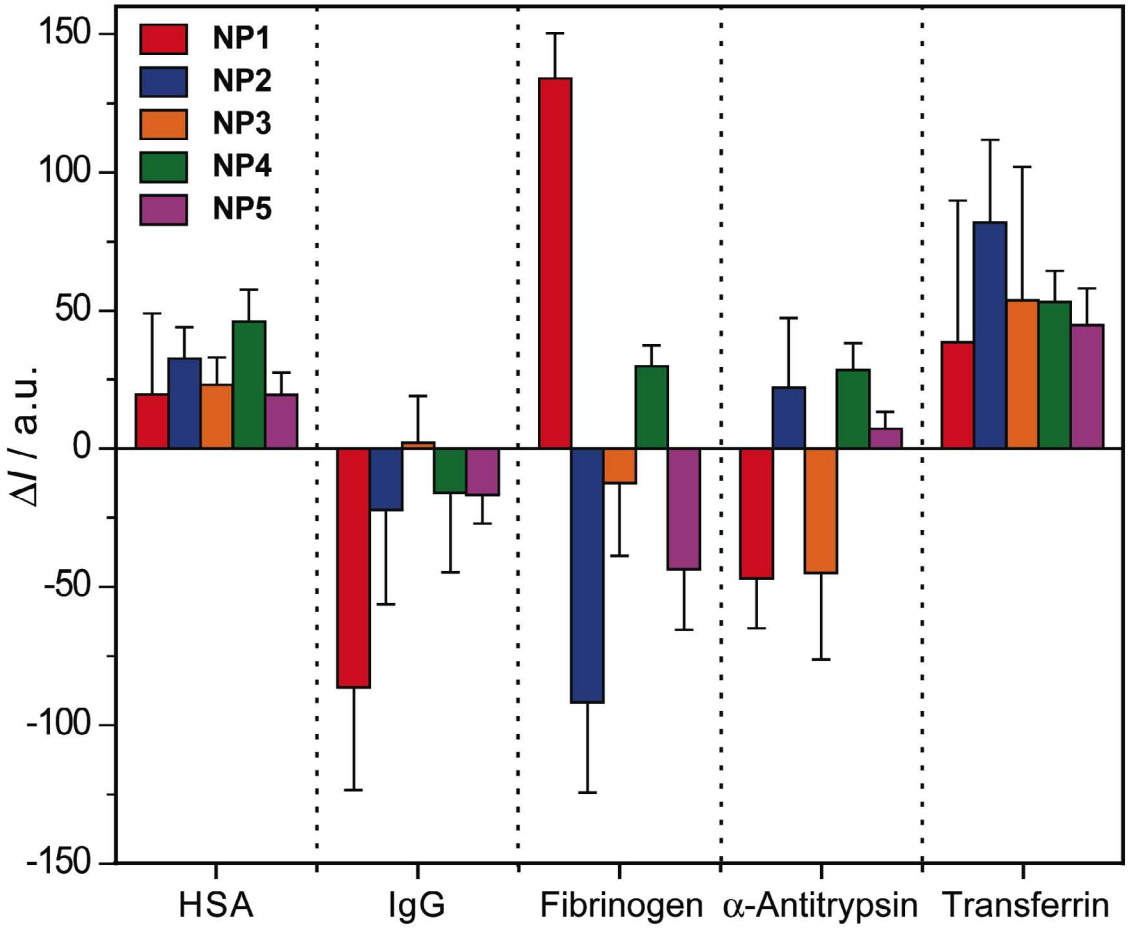


Green Fluorescent Protein
MW = 27 KDa, pI = 5.92

- the five particles that worked (trust me on this...)

Step 2: Fluorescence response from protein "spiking"

- analyte proteins added at 500 nM
- constant total protein concentration maintained

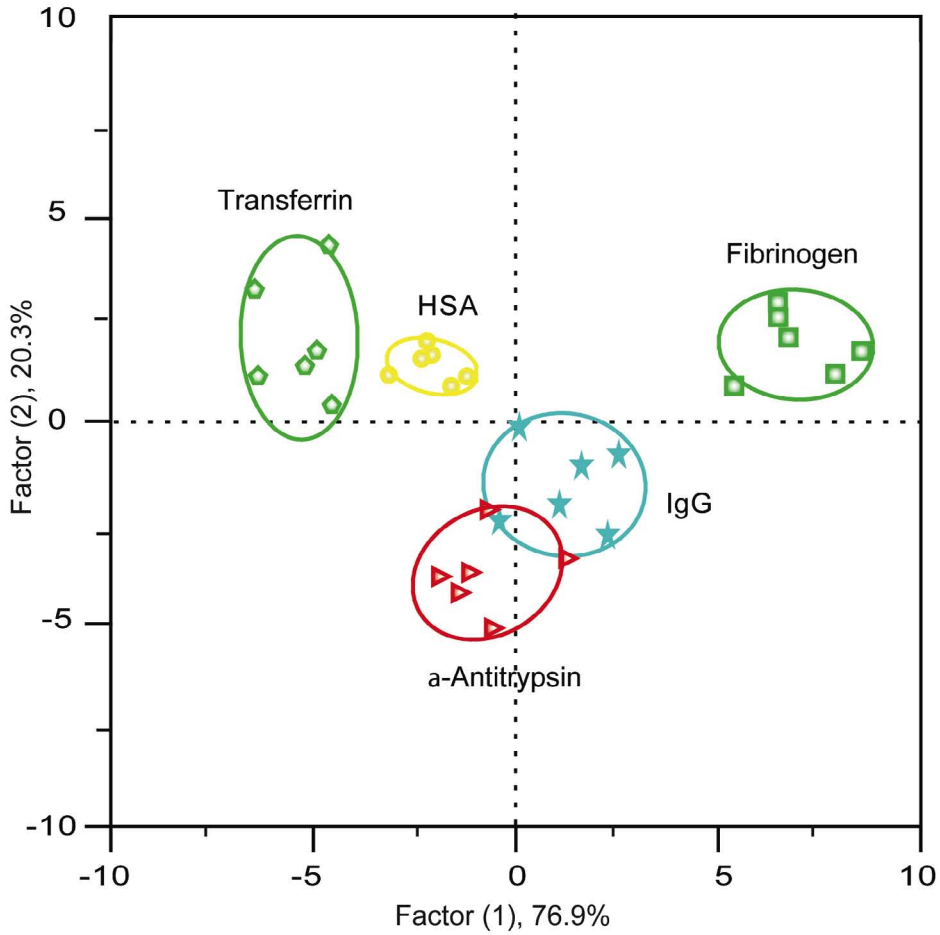


- analyte proteins look different...

De. M.; Rana, S.; Akpinar, H.; Miranda, O.R.; Arvizo, R. R.; Bunz, U. H. F.; Rotello, V. M. *Nature Chem.* 2009, *1*, 461-465.

...Because they are each distinct!

- complete identification of analyte proteins
- verified by unknown analysis (93% accuracy)

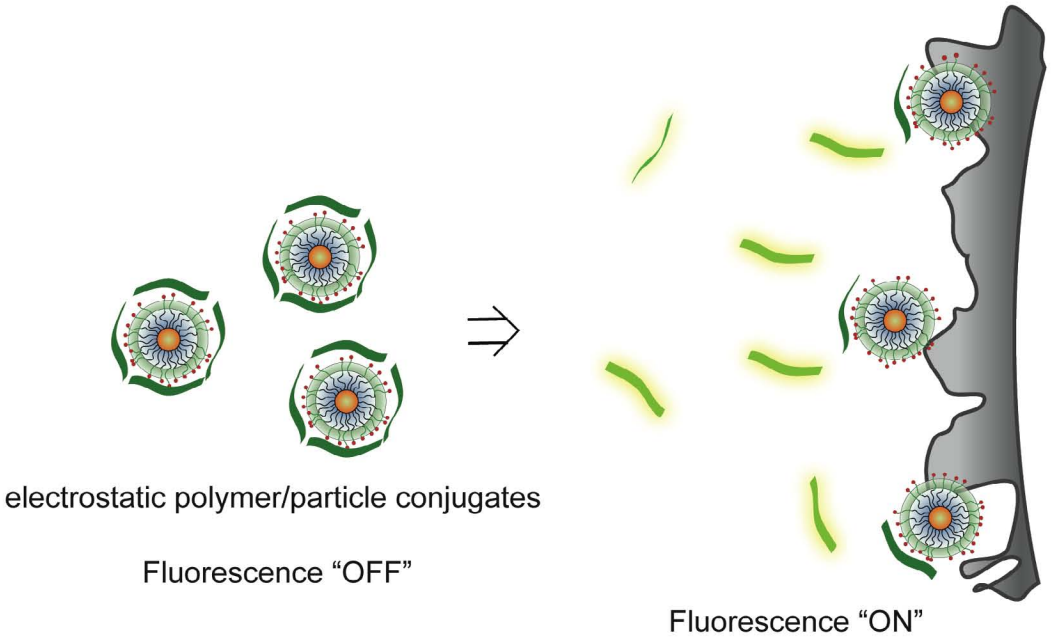


Protein	%Molar change
Albumin	0.06
IgG	0.75
Transferrin	1-2
Fibrinogen	8.4
α-Antitrypsin	5.2

- we are sensitive enough--
- ongoing studies exploring real-world serum samples

Identification of cancer via cell-surface interactions

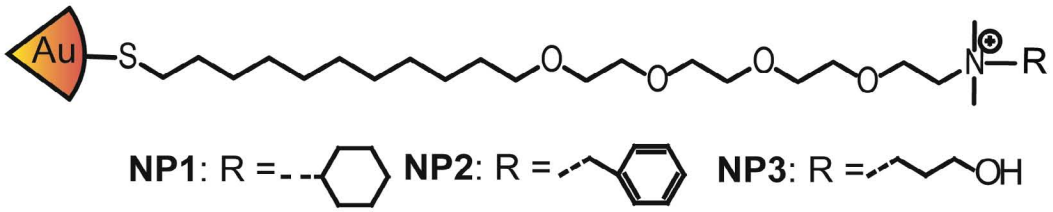
- Challenge 1: differentiating cancerous from non-cancerous cells
- Challenge 2: distinguishing aggressive and non-aggressive cancer cells



electrostatic polymer/particle conjugates

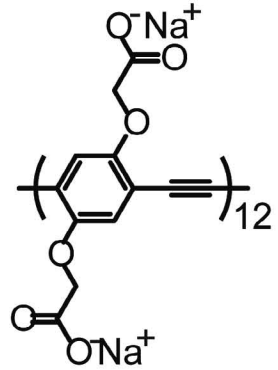
Fluorescence "OFF"

Fluorescence "ON"



NP1: R = **NP2**: R = **NP3**: R =

- three particles (the ones that worked best)

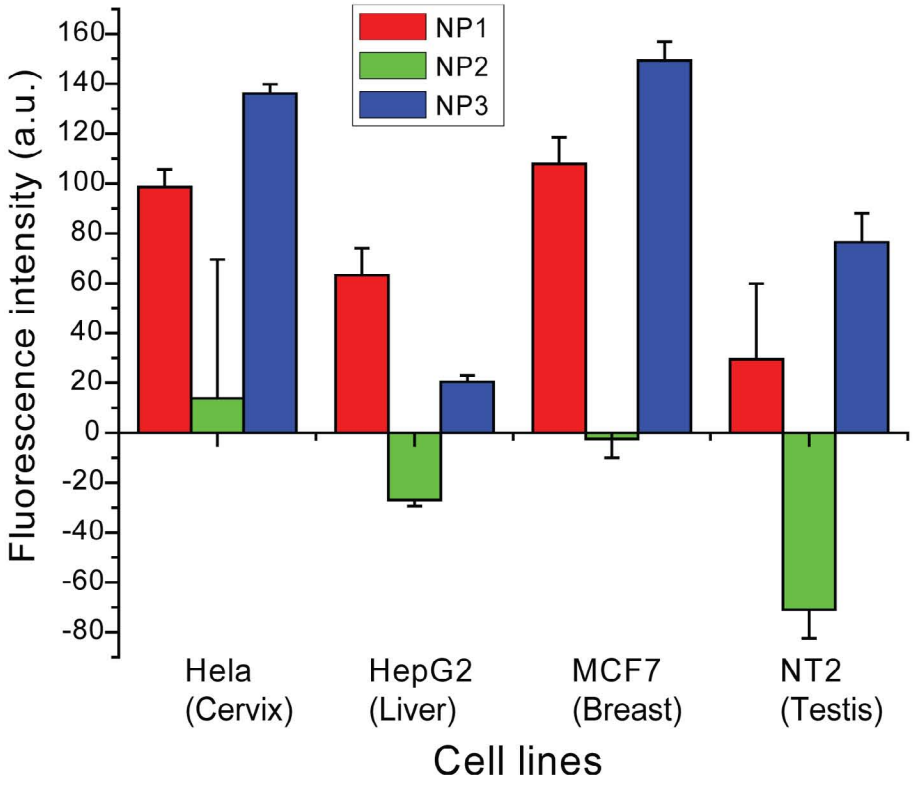


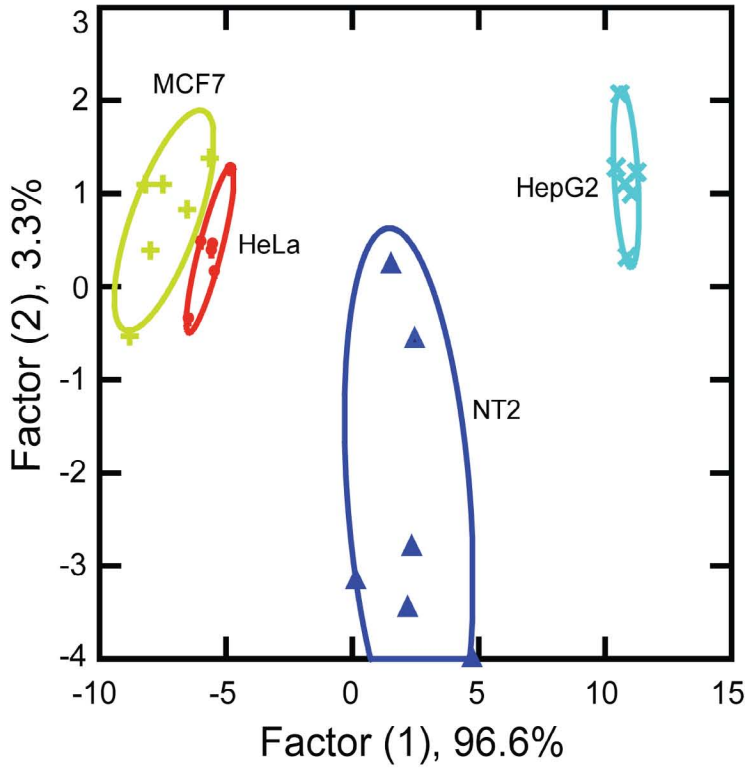
PPE-CO2

- one polymer (the original)

Starting easy--differentiating between cell types

- different cells should have different surfaces...
- ...based on their function



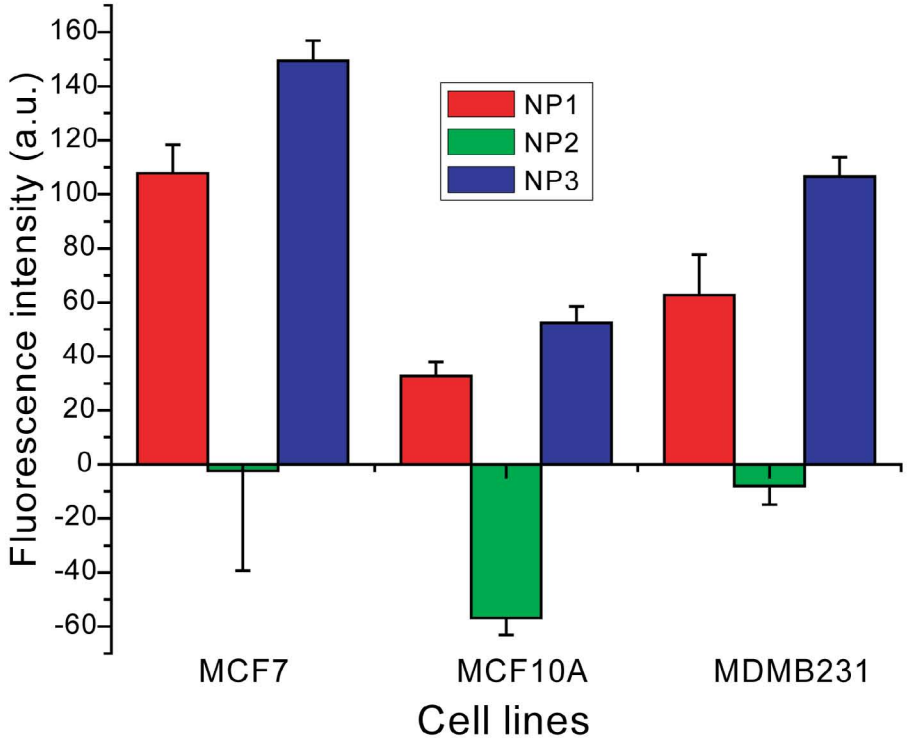


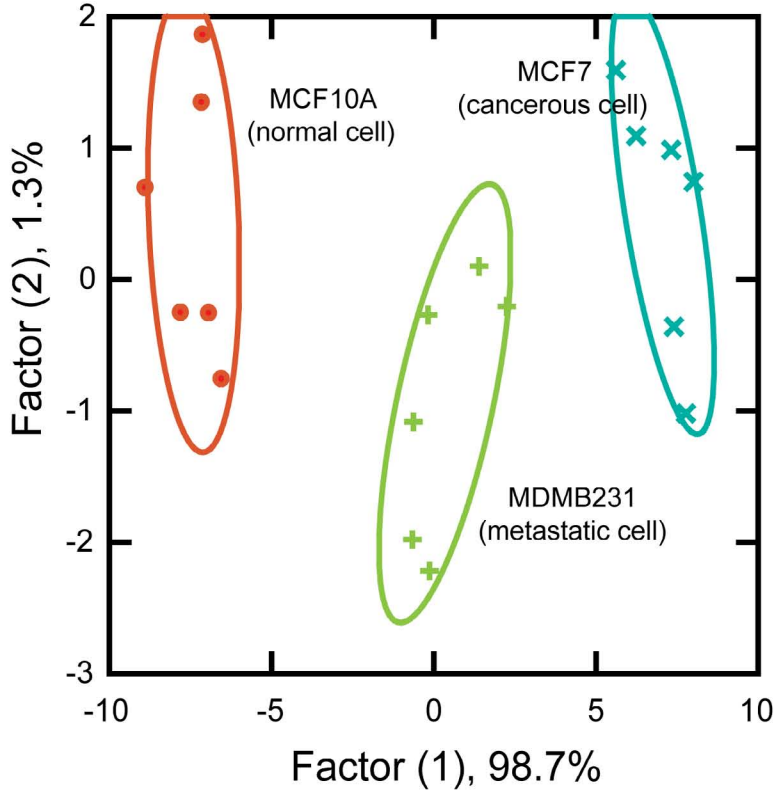
- complete differentiation
- now let's try something a bit more challenging

Bajaj, A.; Miranda, O. R.; Kim, I.-K.; Phillips, R. L.; Jerry, D. J.; Bunz, U. H. F.; Rotello, V. M. *Proc. Nat. Acad. Sci.*, 2009, *106*, 10912-10916.

Step 2--same cell type, healthy vs cancerous vs metastatic

- three different human breast cell lines
- can we detect cancer?

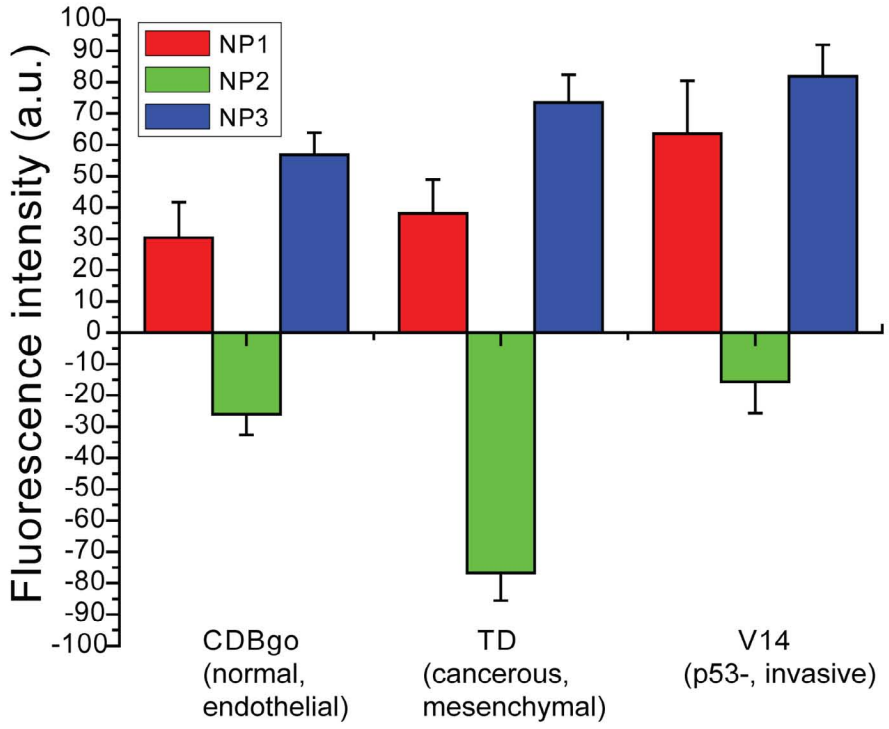


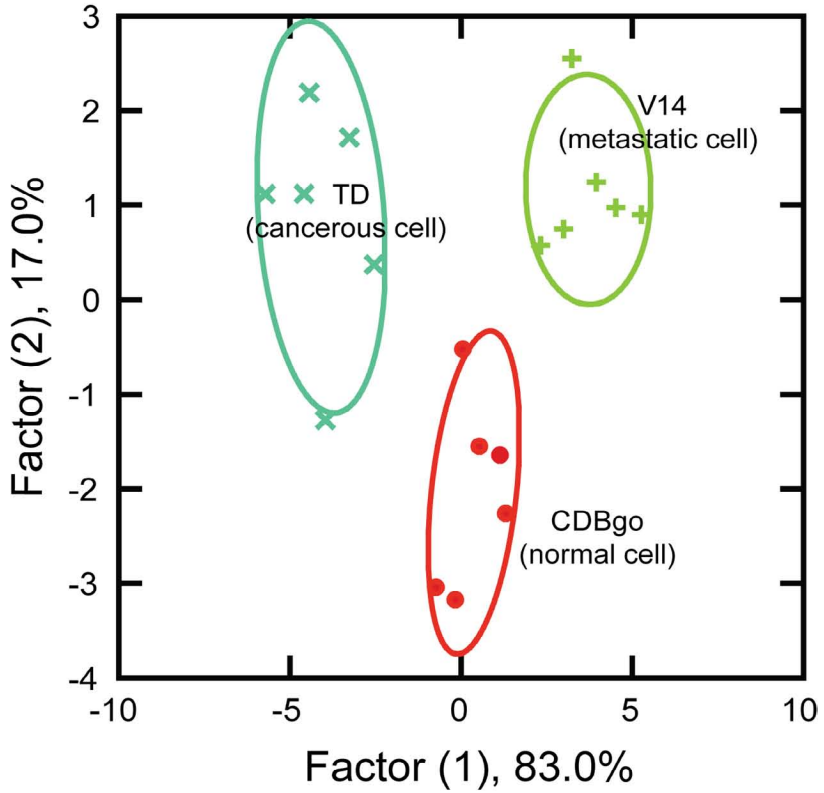


- once again, complete differentiation
- we can't celebrate yet: the three cell lines come from different people
- are we detecting cancer, or individual variations?

The answer--3 isogenic cell lines from BALB/c mice

- identical starting point eliminates individual variations
- isogenic cell lines provide a particularly stringent test

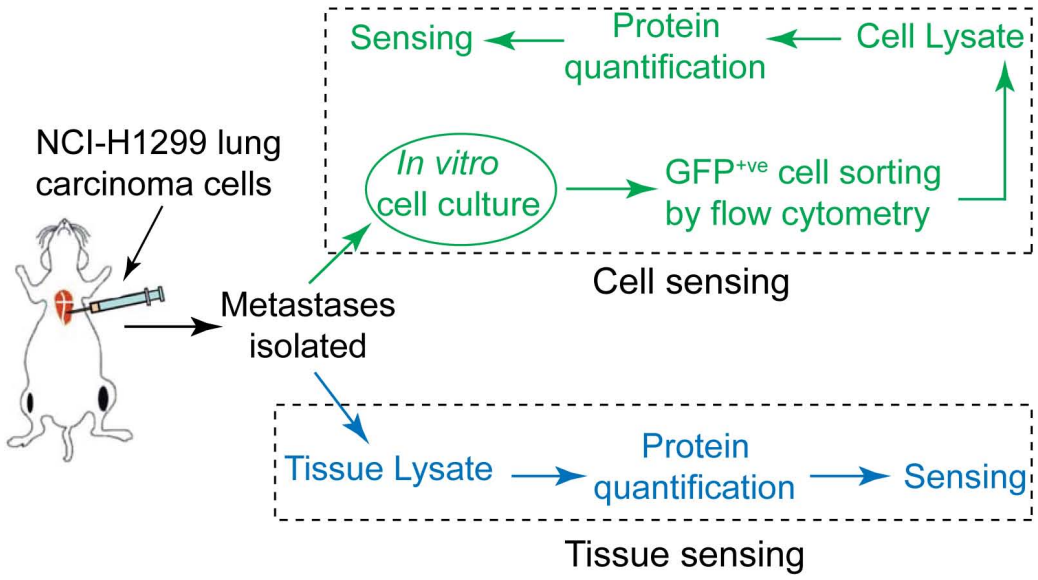


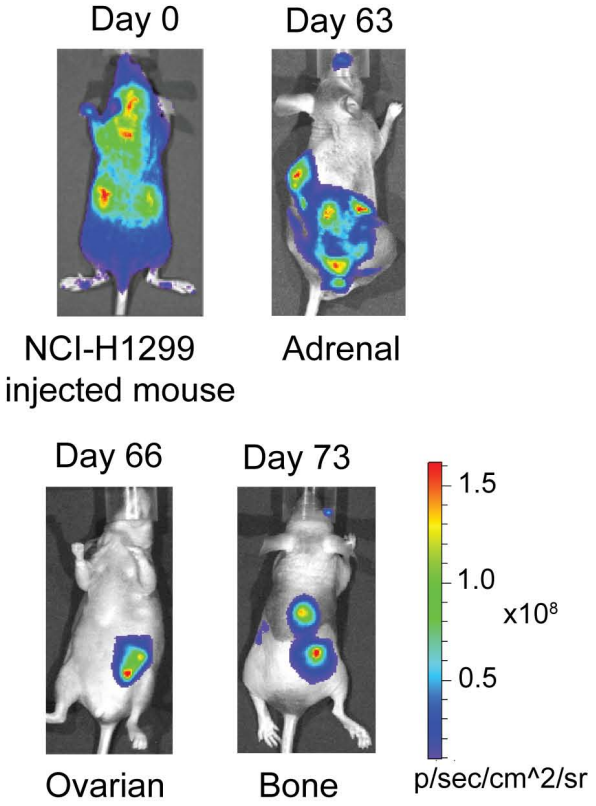


- once again, complete differentiation
- in a matter of minutes, based on cell-surface variations

What about in vivo?

- cells are complicated, tissues much more so
- step 1: tumor metastases by Frank Jirik and Arvind Singla
- step 2: culturing of isolated metastases, biopsies of tissues

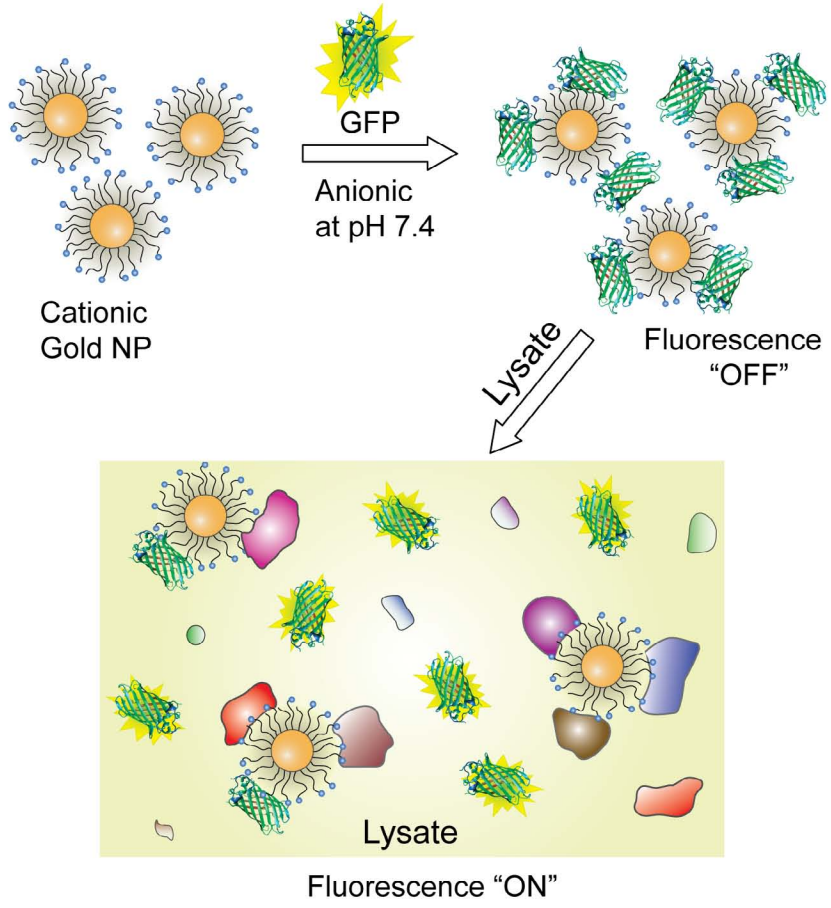


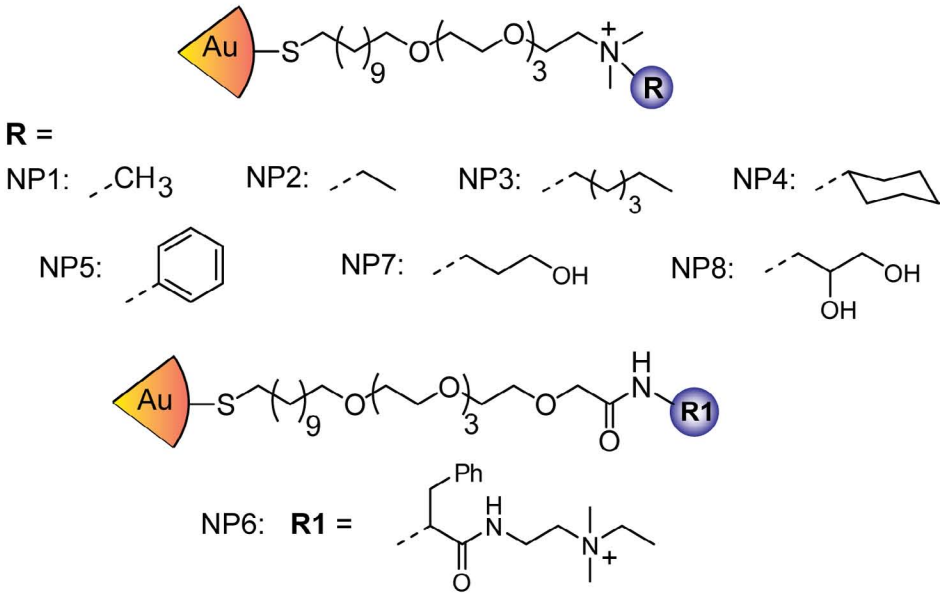


- n.b.: induced, not implanted metastases--i.e., the real deal!

The sensor array

- GFP used a a transducer--very biocompatible (no aggregation)
- step 1: screen library of ~70 particles
- step 2: find the ones that work

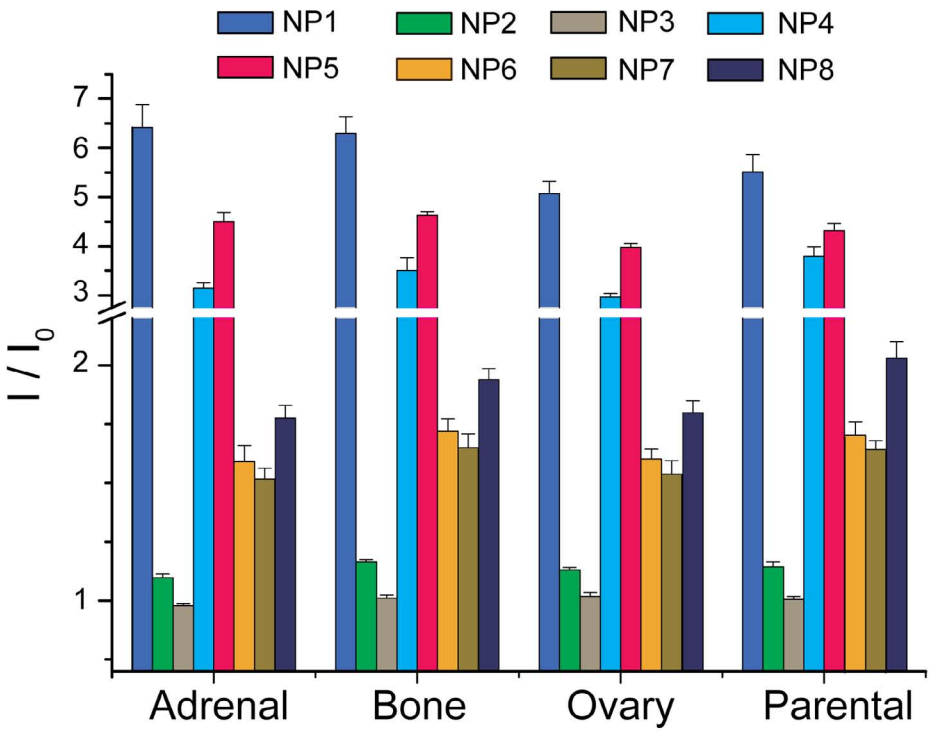


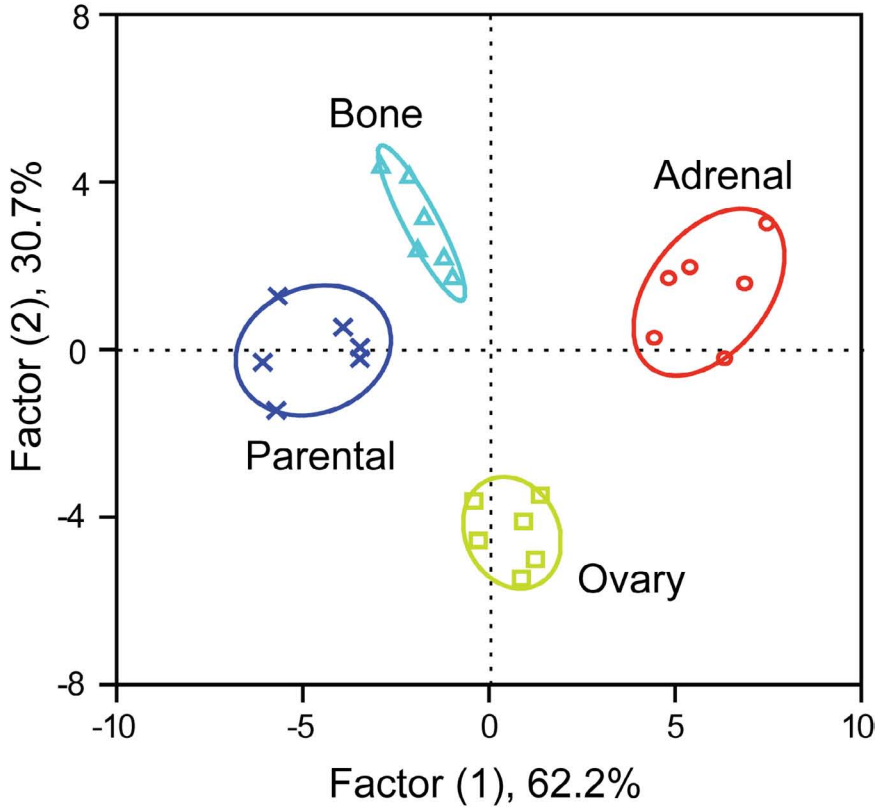


- if we picked 'em, they must have worked!

The sensor differentiates cultured cells

- a bit of warm-up--daughter cells clearly separated
- 200 ng lysate=~1000 cells=high sensitivity

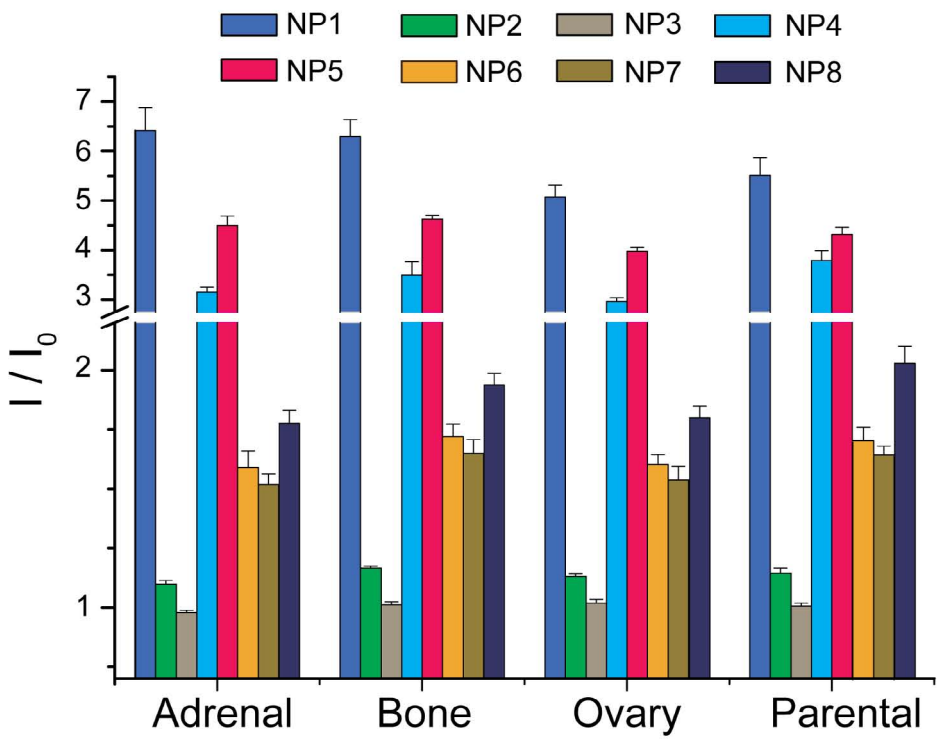


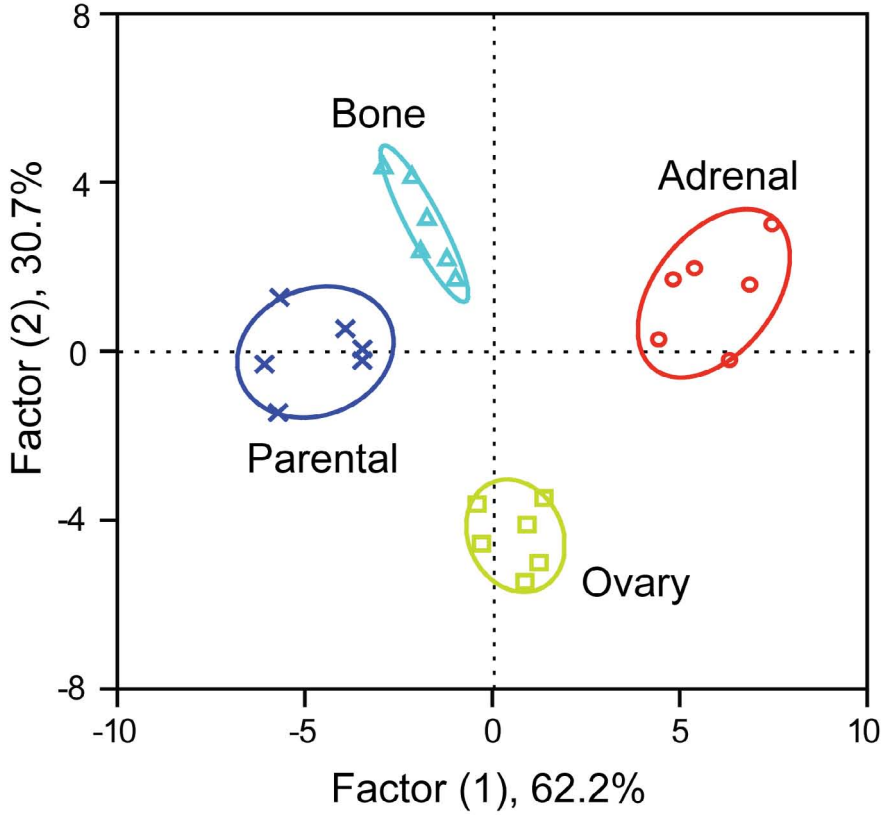


- nice start--what about in vivo?

The sensor differentiates daughter metastases

- a bit of warm-up--daughter cells clearly separated
- 200 ng lysate=~1000 cells=high sensitivity

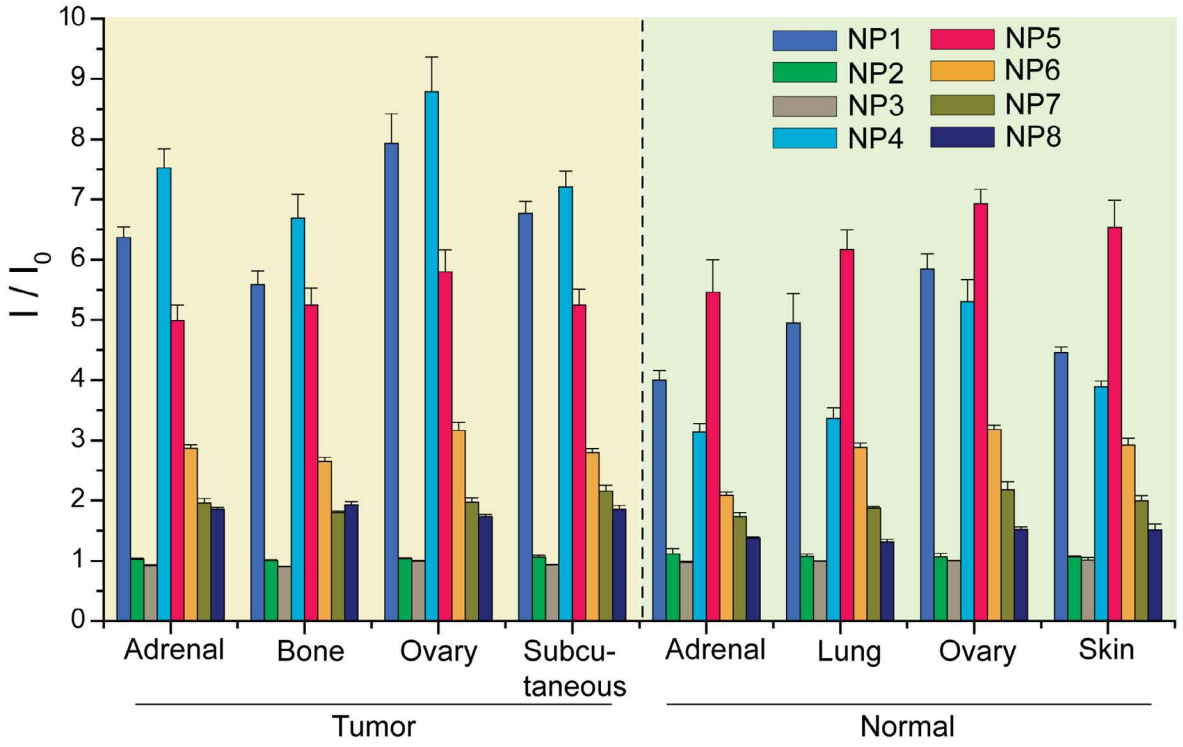


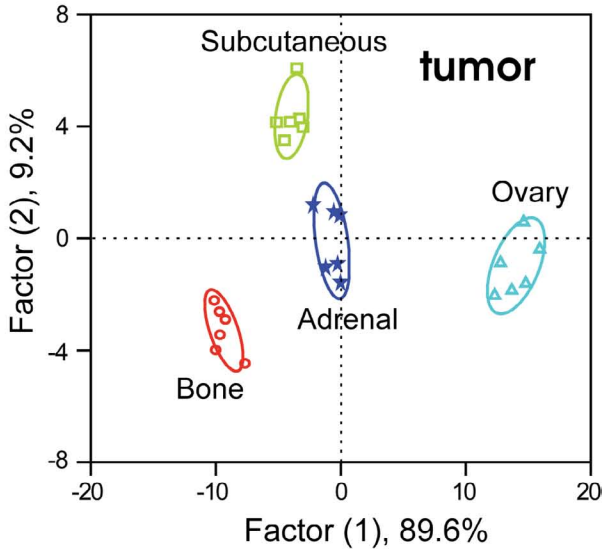


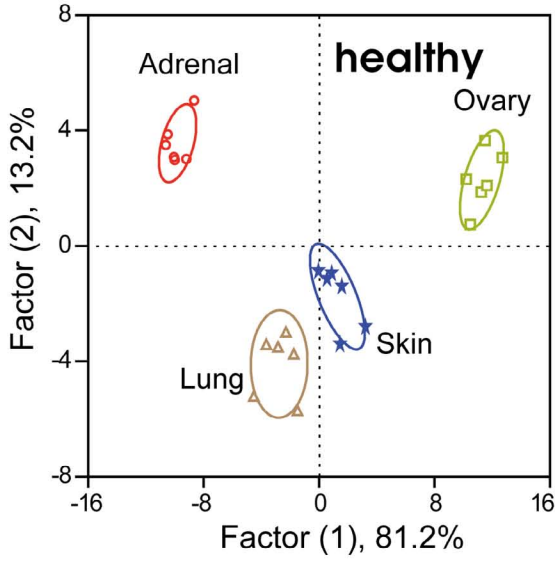
- nice start--what about in vivo?

Both tumors and tissues can be differentiated

- different organs/tumors have different lysate
- microbiopsy: 200 ng lysate=~1000 cells



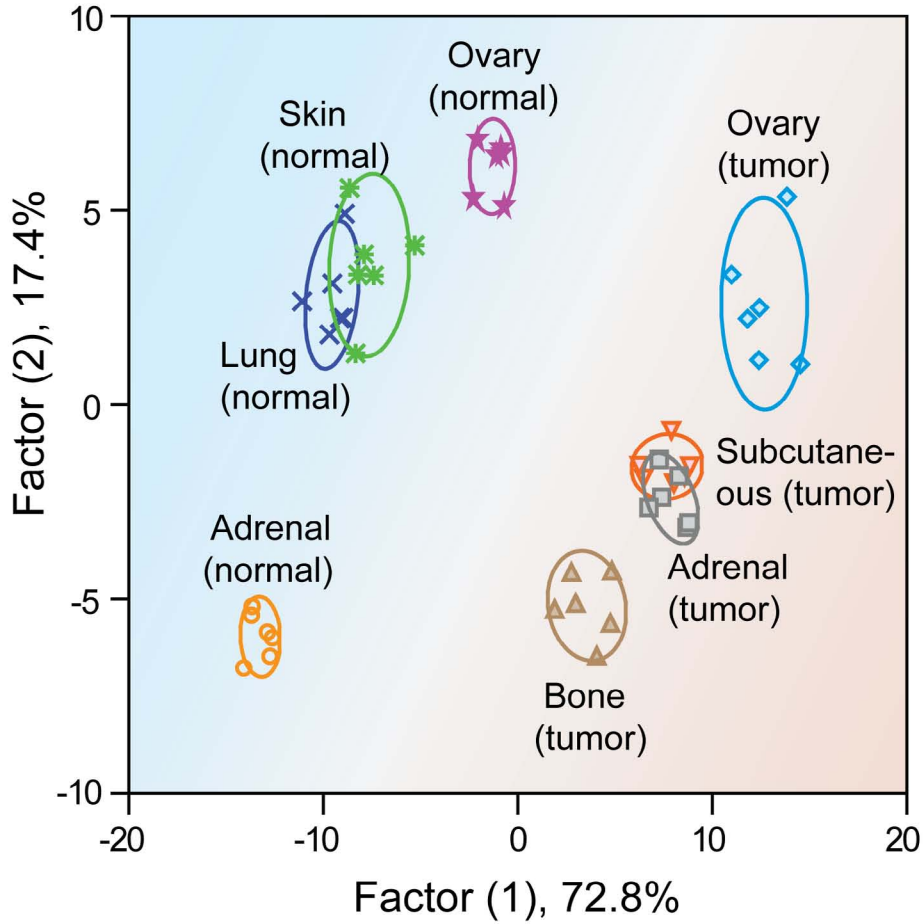




- looking better...
- ...but can we differentiate tumor and healthy?

Healthy and tumor tissues provide distinct clusters

- direct differentiation of healthy tissue and metastases
- metastases look more like parent than host organ



- chemical noses work in vivo, providing potential diagnostics
- tantalizing hints on cancer biology

The “out of time” summary:

Gold nanoparticles provide:

- Potential therapeutics
  -triggered cytotoxicity
  -tunable immunomodulation

- Building blocks for nanocapsules
  -for drug and protein delivery
  -direct to the cytosol!

- Effective “chemical nose” sensors for:
  -cancer cells (healthy, cancerous, metastatic)
  -metastases (induced—the real thing!)

Acknowledgments:

Alumni: postdocs

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Tyler Norsten
Roy Shenhar
Belma Erdogan
"Pops" Arumugam
Amitav Sanyal
Yuval Ofir

Alumni: grad students

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Eric Breinlinger
Michael Greaves
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