May 20th, 1:15 PM - 2:45 PM

Biosensing using Particle-(Bio)Polymer Sensor Arrays

Vincent Rotello
University of Massachusetts - Amherst

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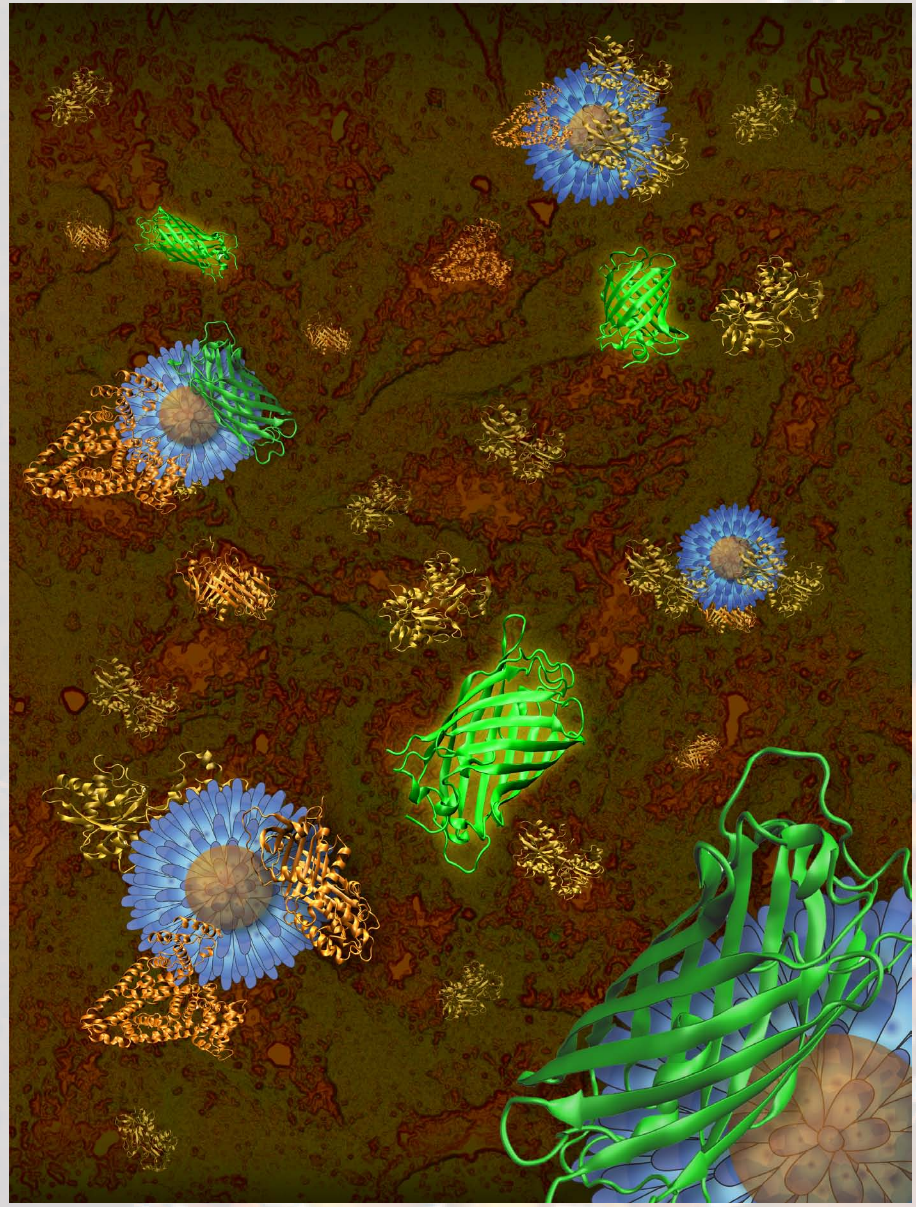


I) Sensing

a) Proteins

b) Bacteria

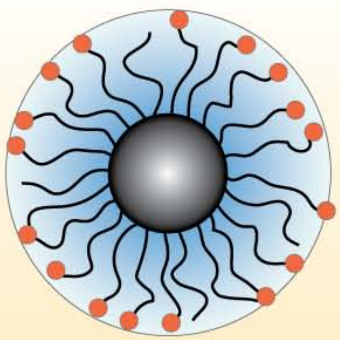
c) Mammalian cells

*DISCLOSURE

•I have no actual or potential conflict of interest in relation to this program or presentation.

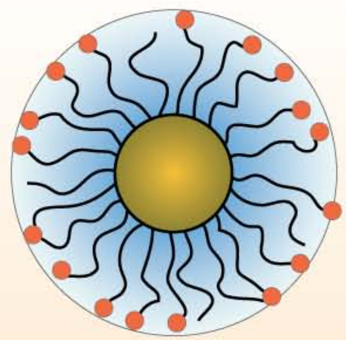
Nanoparticles have unique and useful properties

- nanoparticle behavior is very different from corresponding bulk material



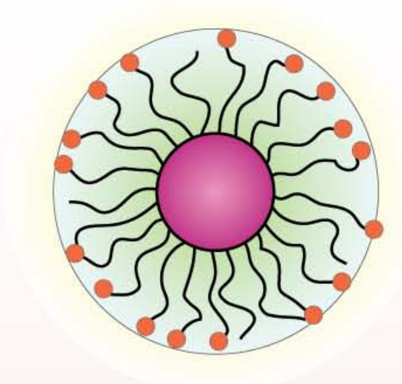
- Pd, Au, Ag

optics and electronics:
biomedical (vide infra)
electronics, sensors



- Fe_xO_y, M_xO_y, FePt

magnetic materials:
memory, ferrofluids,
MRI imaging,
hyperthermic therapies



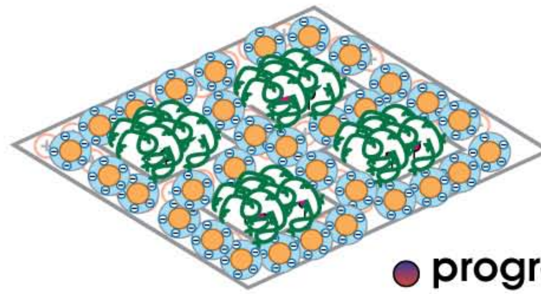
- CdSe, ZnSe

semiconductor and
fluorescent materials:
bioimaging, electronics
photovoltaics

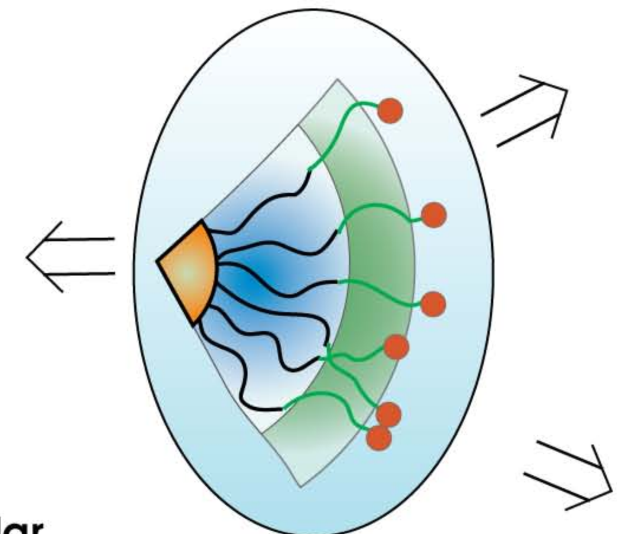
- how can we employ these materials in real-world applications?

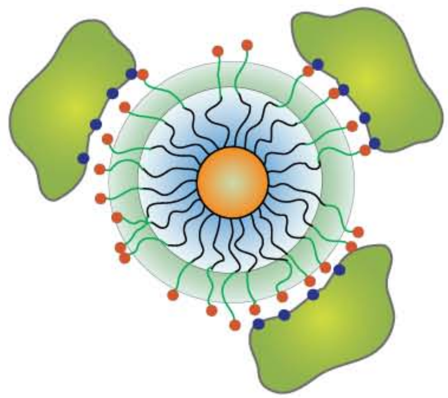
The key is engineering the particle interface

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

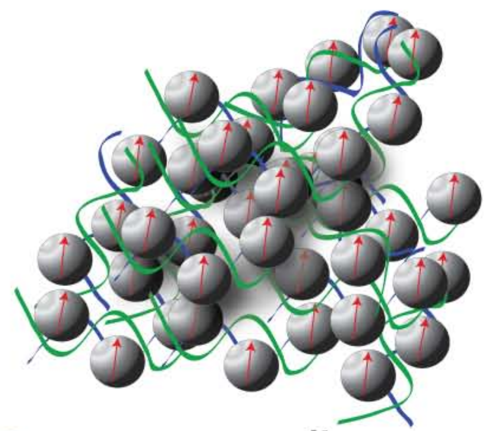


- programmed surface modification





- controlled biomolecular interactions

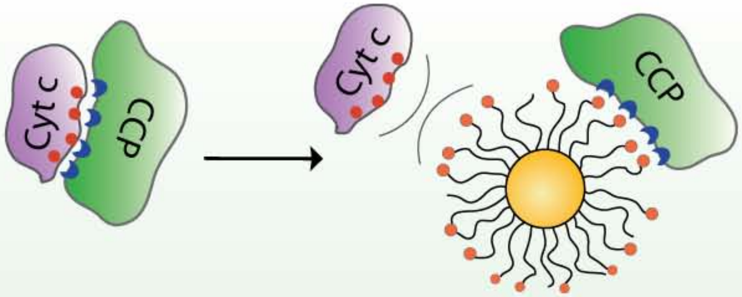


- nanocomposite assembly

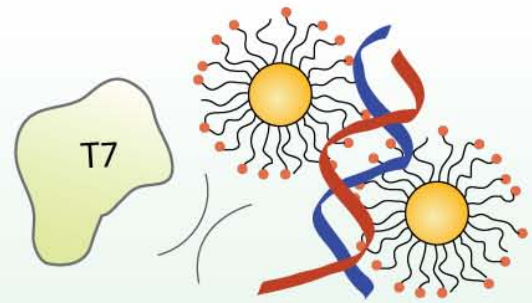
- of course we can mix and match...
- and lessons learned with one core can be generalized

Biomacromolecule surface recognition using nanoparticle receptors

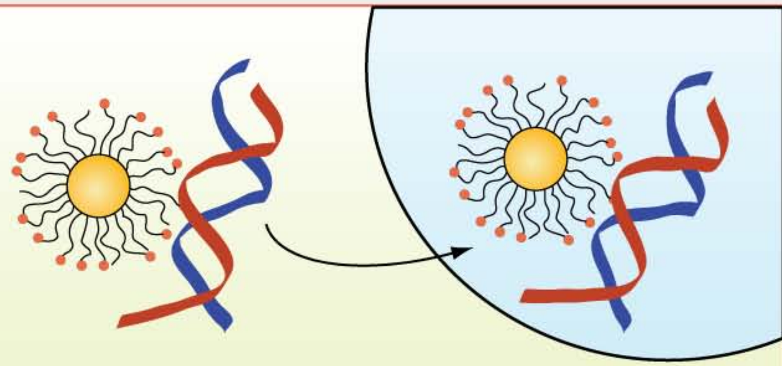
- why we want to bind biomacromolecule surfaces:



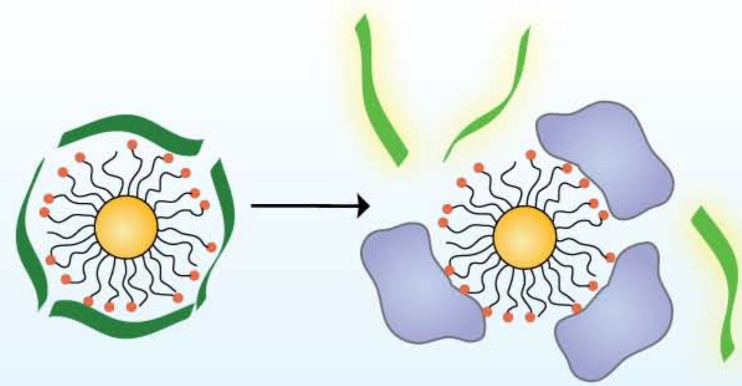
-inhibition of protein-protein interactions



-transcription regulation via DNA binding



-DNA, siRNA, and protein delivery



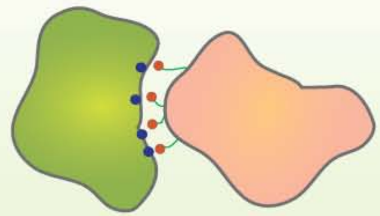
-sensors and diagnostics

The three challenges of biomacromolecule surface recognition

- "why is protein surface recognition so hard?" (Andrea Corchoran, Genentech)

1) a large surface is required

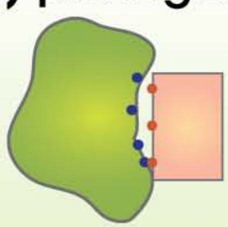
-active site inhibition: isolated and concave target



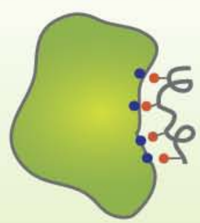
-surface recognition convex, solvent exposed (hint--protein protein interactions bury 600-1400 $Å^2$ per protein!)

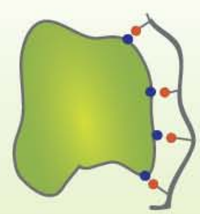
think: fingers in a glove vs palming a basketball

2) preorganized yet flexible receptor



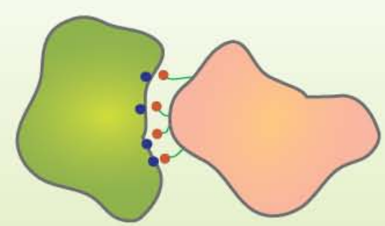
-rigid doesn't work





-too flexible, lose affinity and specificity

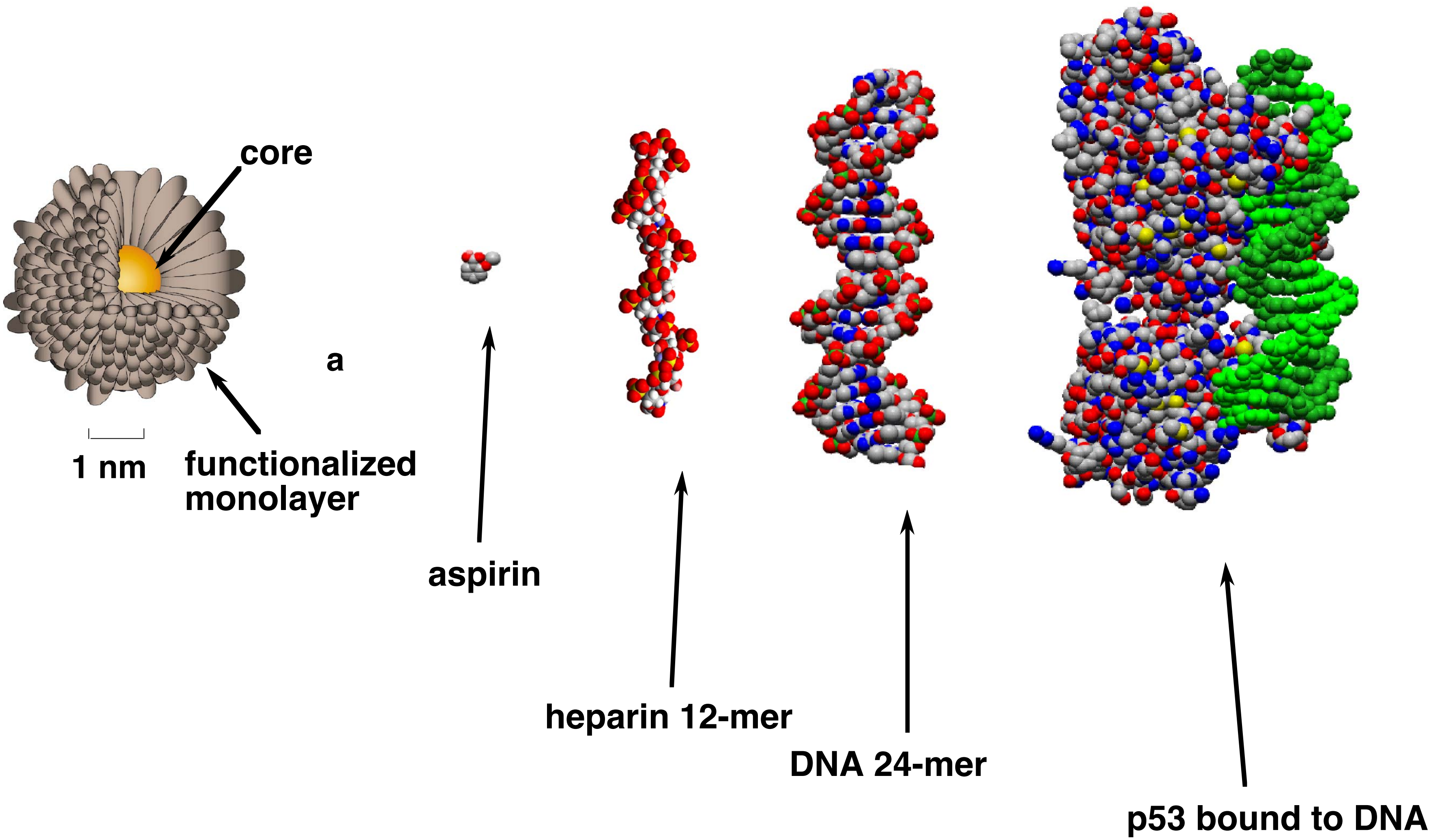
3) things have to line up



aka proper orientation of multivalent recognition elements

Nanoparticles provide *at least* two out of three (ain't bad!)

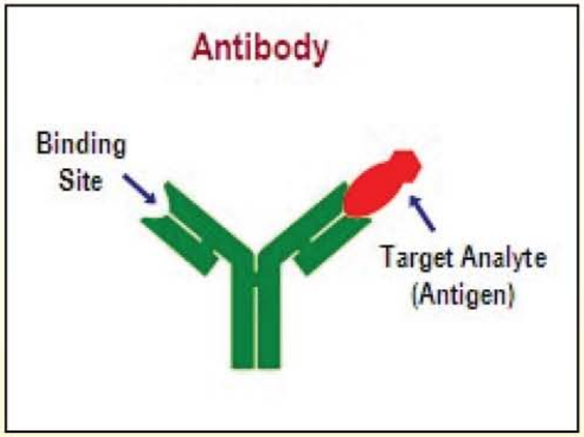
- SAM-covered nanoparticles provide regular shape
- and are the right size for biomacromolecule recognition



Specific or selective: Two different sensing paradigms

- one biomimetic, one not..

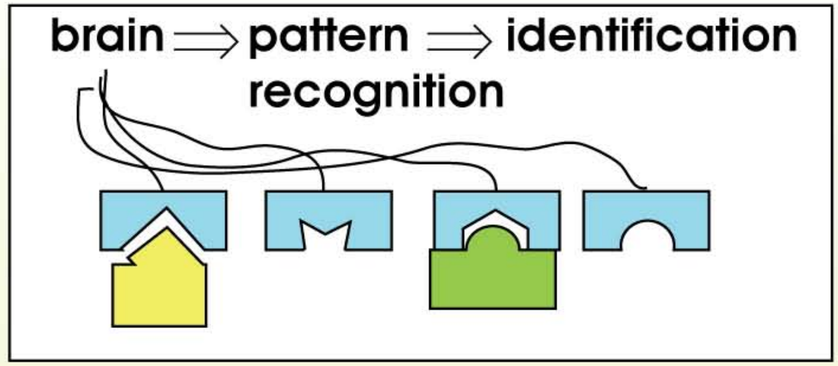
- specific recognition (e.g. ELISA)



- 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)



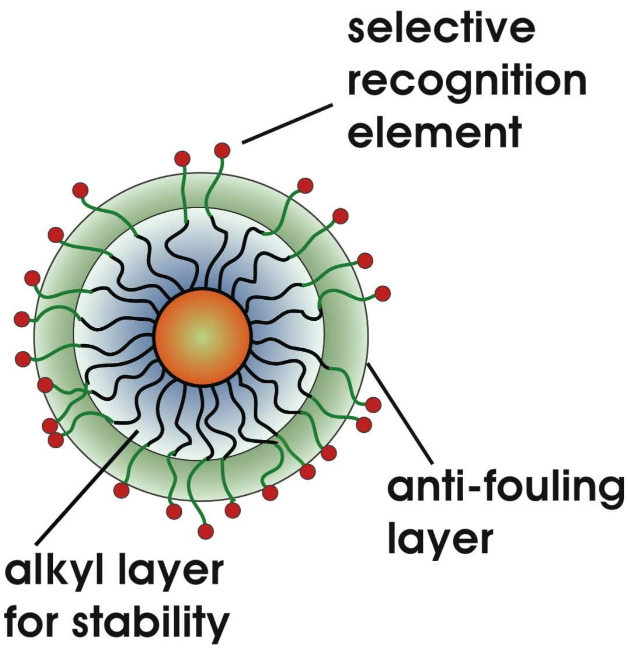
- strengths:
  - -simpler hardware
  - -excellent for complex mixtures
  - -trainable for new "odors"

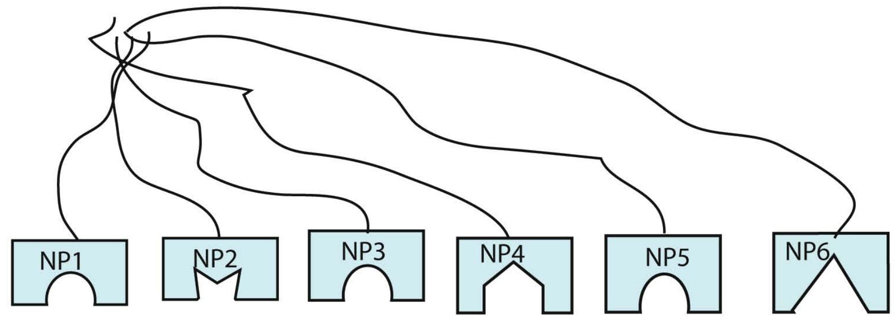
- challenges:
  - - more complex software
  - -structural diversity required

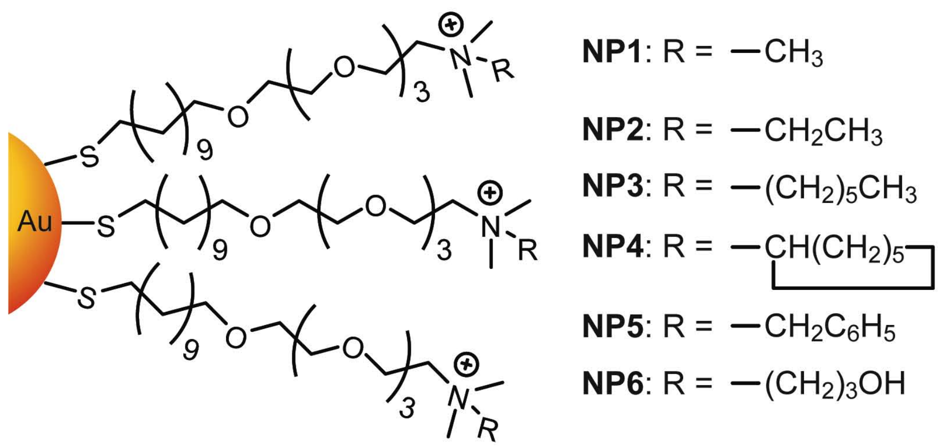
- can we create nose-type sensors for proteins?

Step 1--selective receptors for proteins

- a wide variety of different nanoparticles can be made quickly
- the key--engineering the protein-particle interface





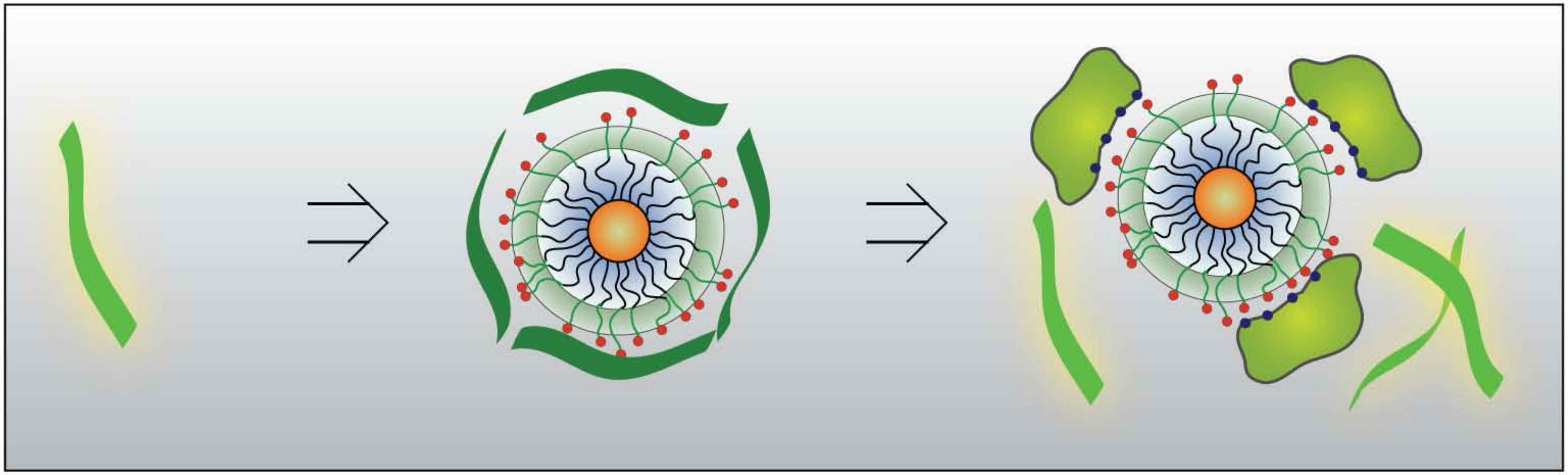


- recognition elements should provide selectivity
- how do we transduce the signal?

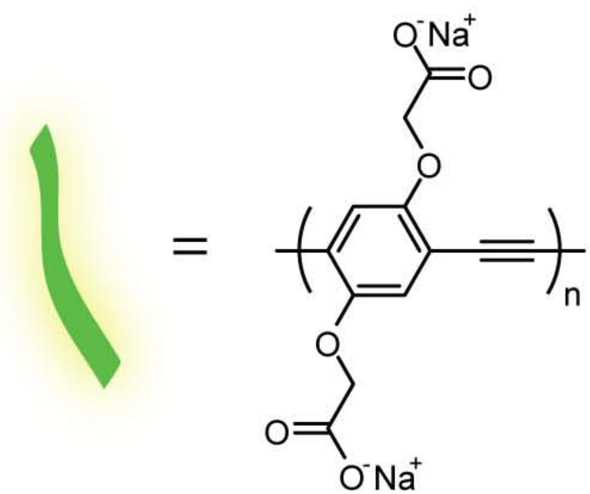
You, C.-C.; Miranda, O. R.; Gider, B.; Ghosh, P. S.; Kim, I. –B.; Erdogan, B.; Krovi, S. A.; Bunz, U. H. F.; Rotello, V. M. *Nature Nanotech*, 2007, *2*, 318-323.

Step 2--transduction

- long experience shows nanoparticle+protein looks like nanoparticle
- gold nanoparticles are great fluorescence quenchers, though....

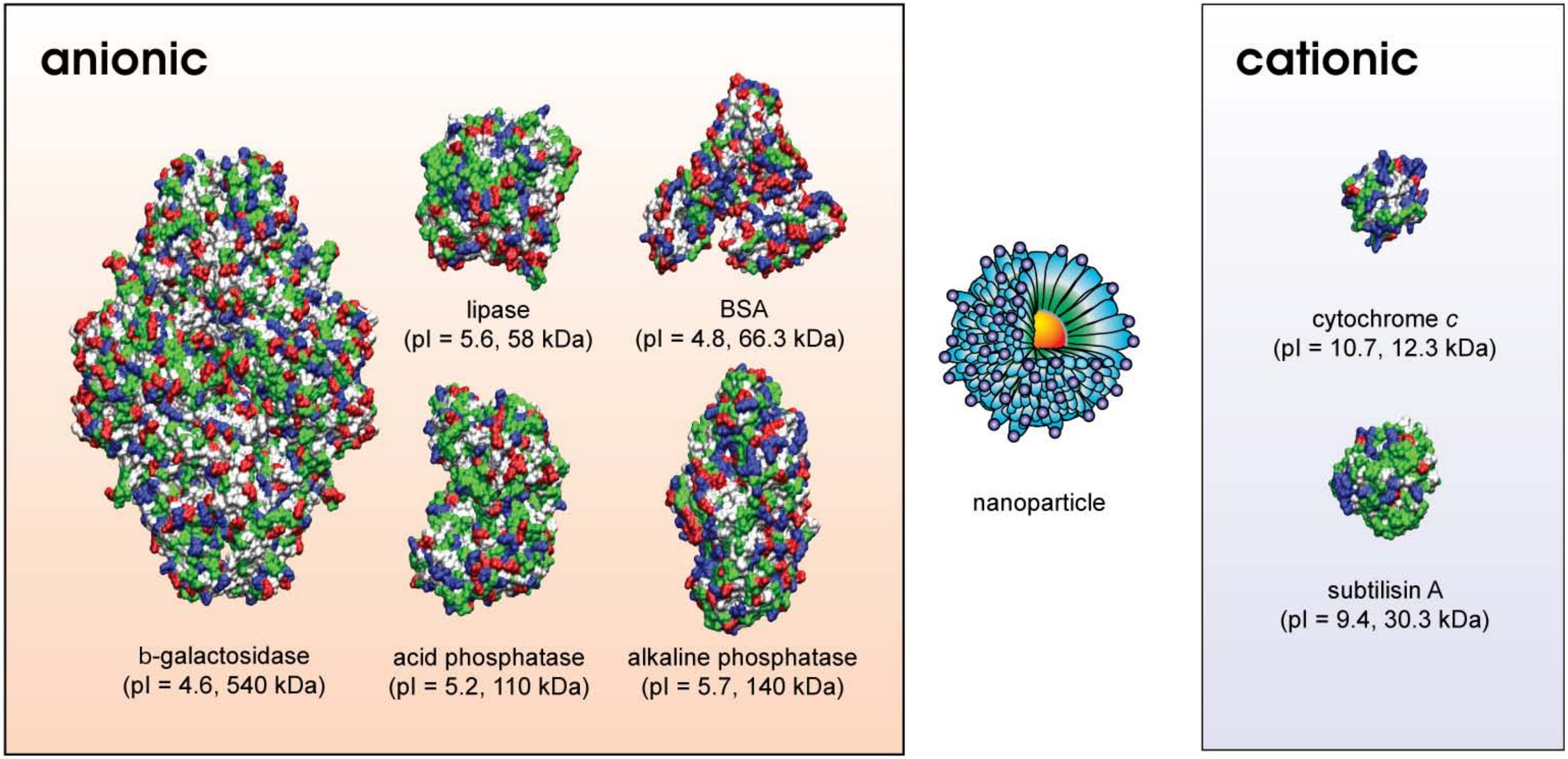


- key features of fluorophore
  -anionic to bind cationic particle
  -multivalent (sticky) for selectivity
- the answer--anionic PPEs provided by Uwe Bunz (Georgia Tech)



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
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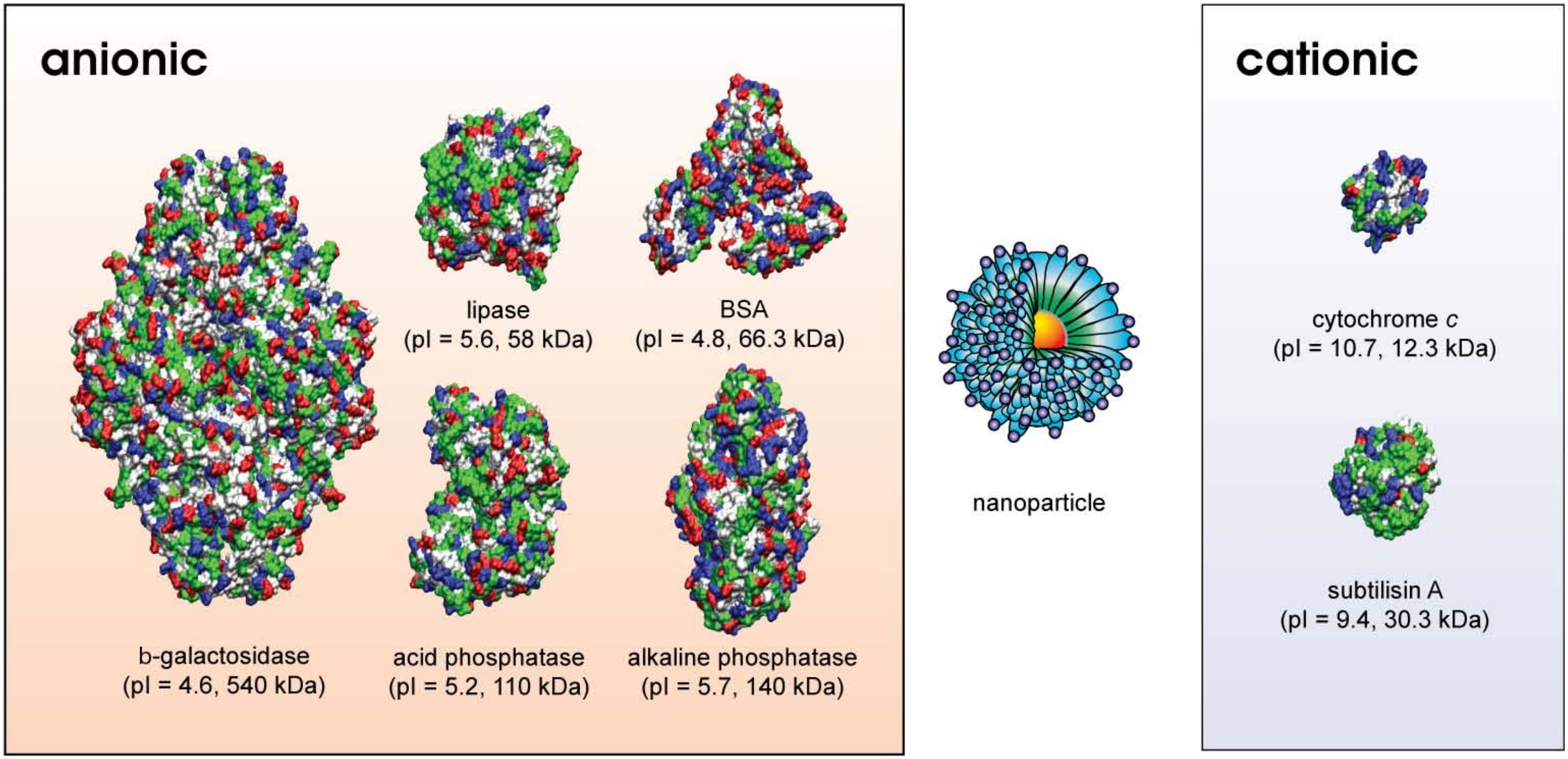
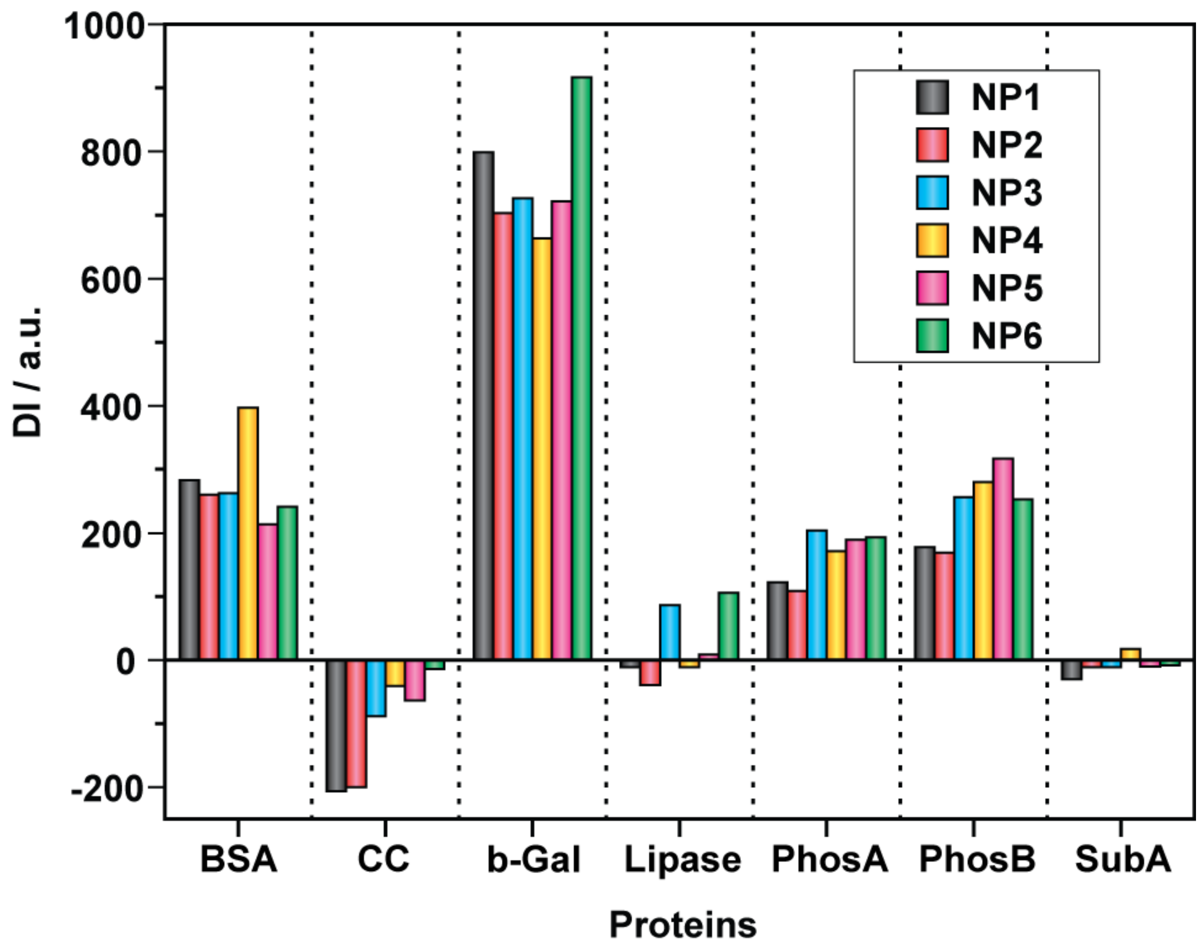


- can we differentiate ‘em--especially the tough ones?

We can differentiate the proteins qualitatively

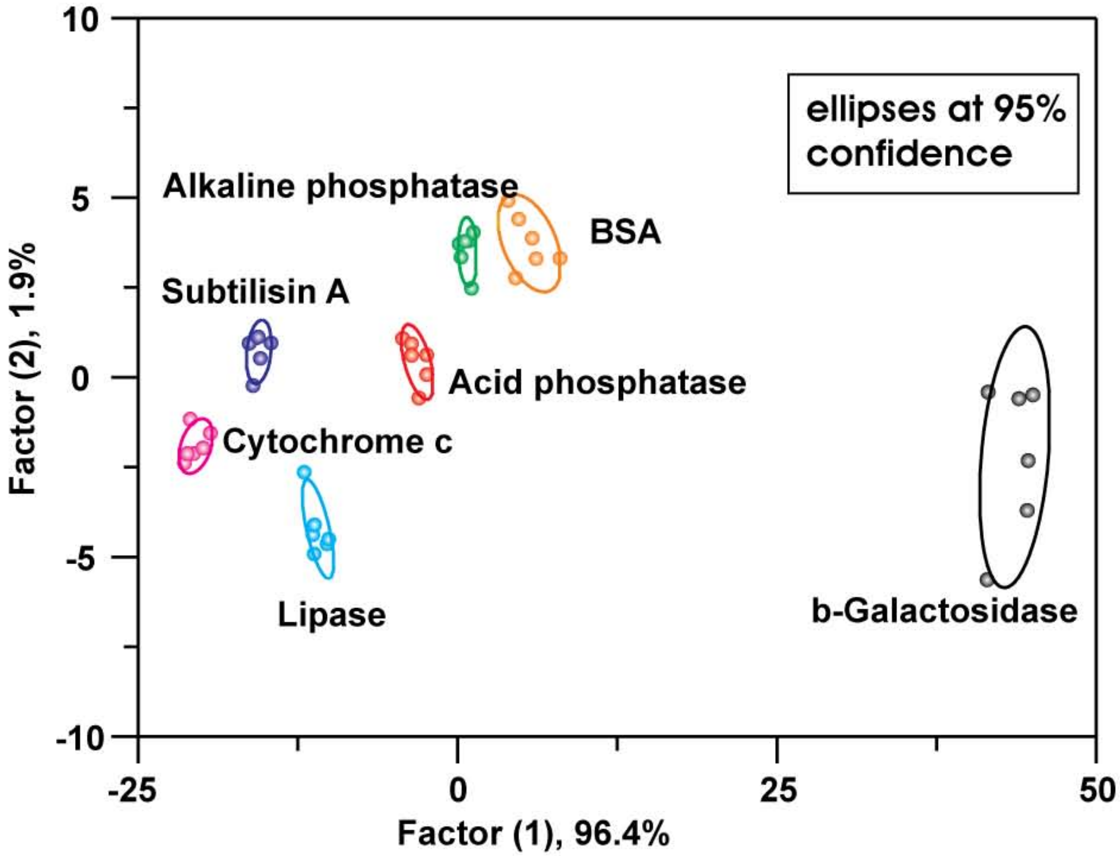
- different nanoparticles show different selectivity...
- ...providing a different pattern for each protein



- 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

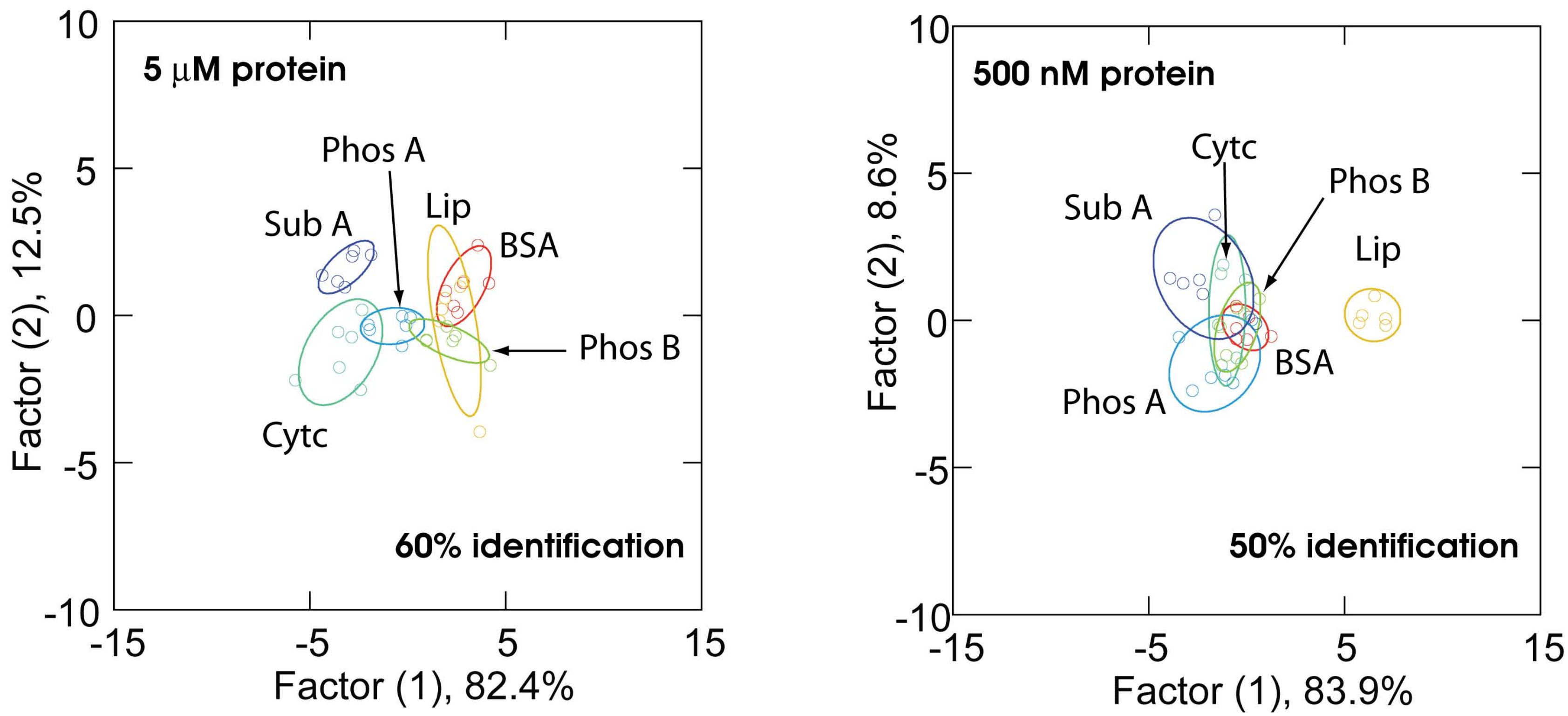


- the test: 56 samples randomly chosen from training set
- the outcome: 96% accuracy in identification!
- ongoing studies: biofluids (serum looks promising!)

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!

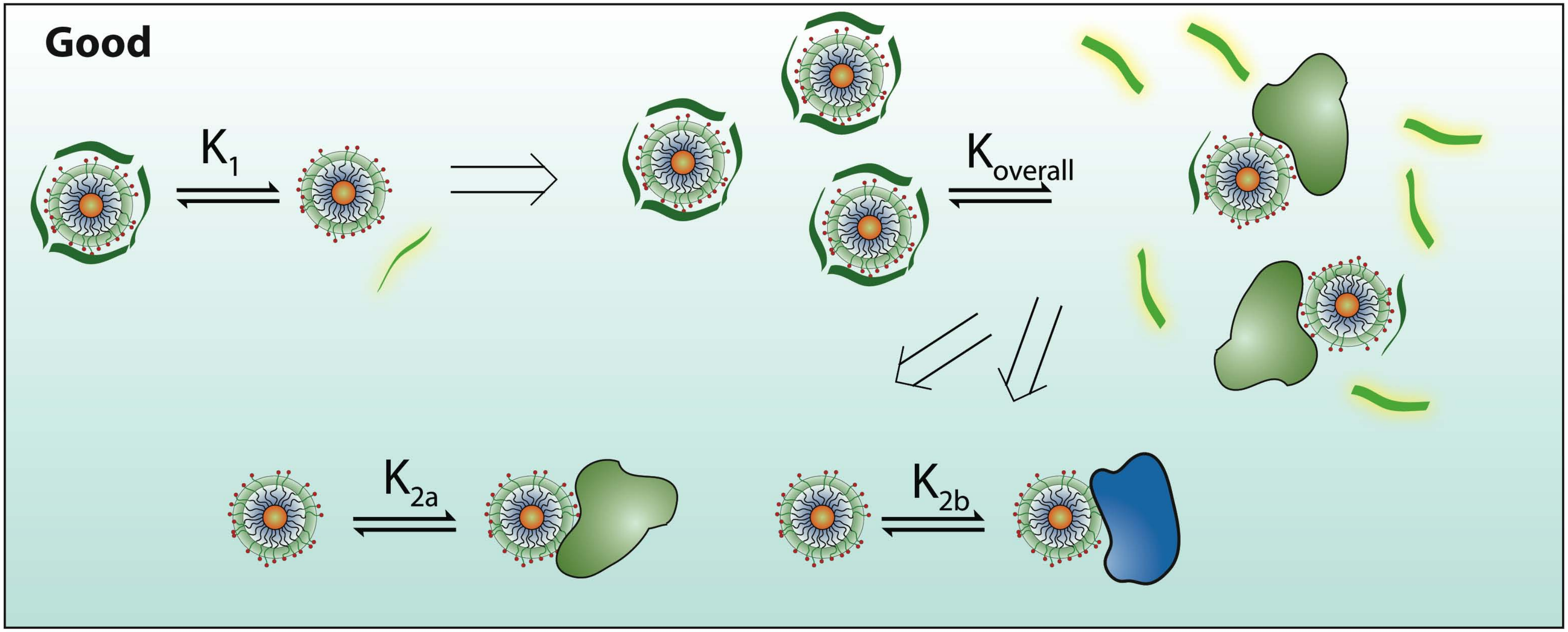
- proteins 'spiked' into undiluted human serum



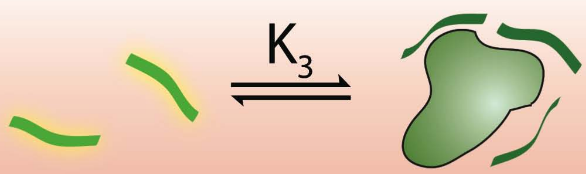
- the first attempts using original polymer/particle mixture--not great
- it's a modular system--let's switch the polymer!

A closer look at the sensing process

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



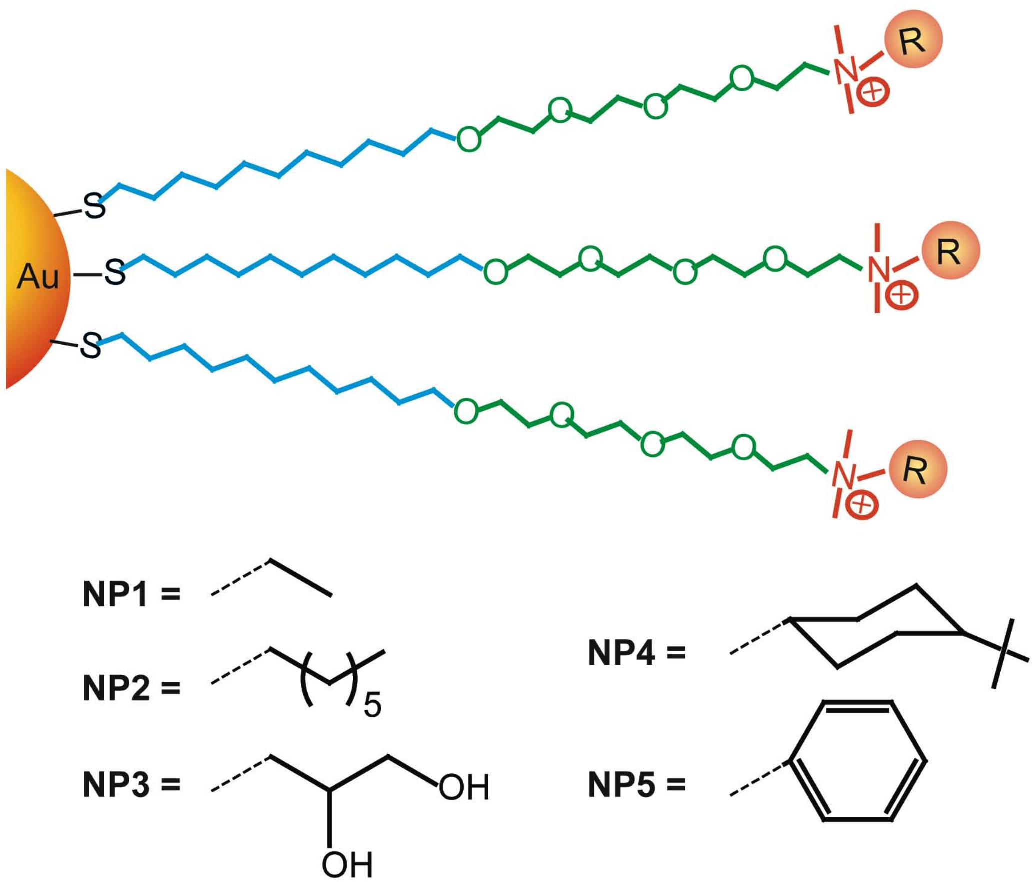
Bad

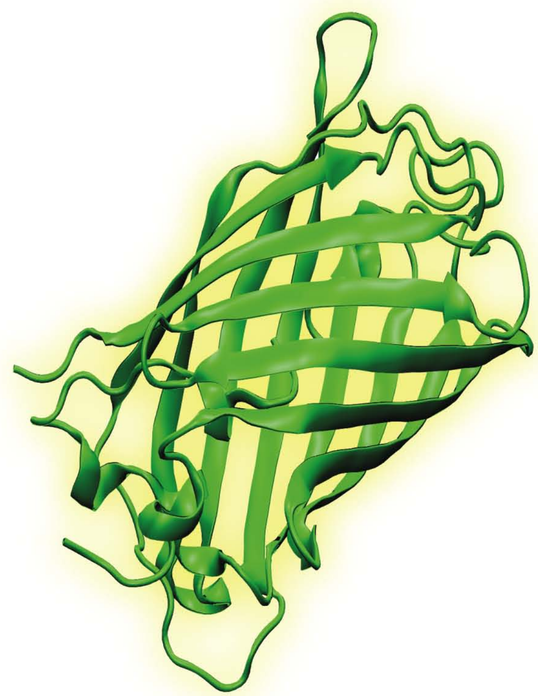


- quenching reduces signal
- can we shut down K_3 ?

Instead of a polymer, what about a biopolymer transducer?

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



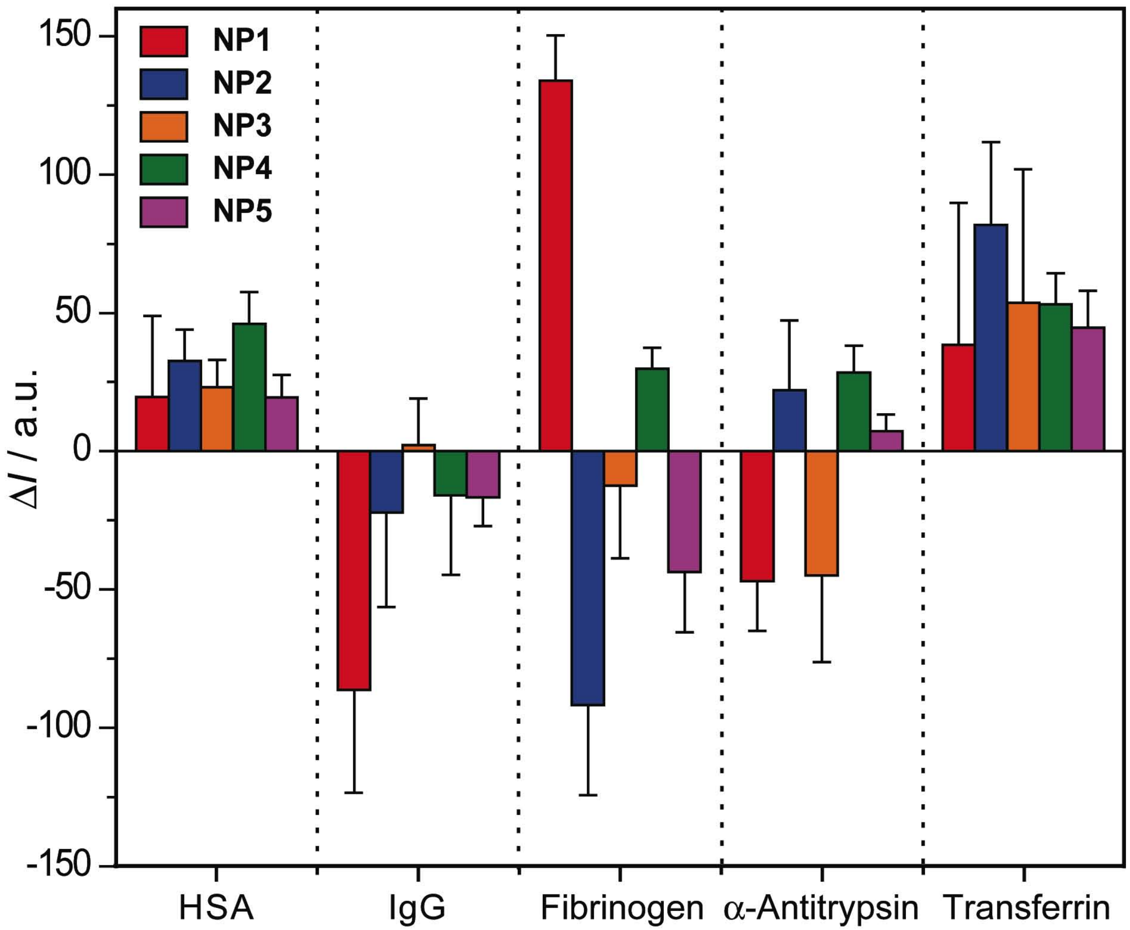


Green Fluorescent Protein
MW = 27 KDa, pI = 5.92

- 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

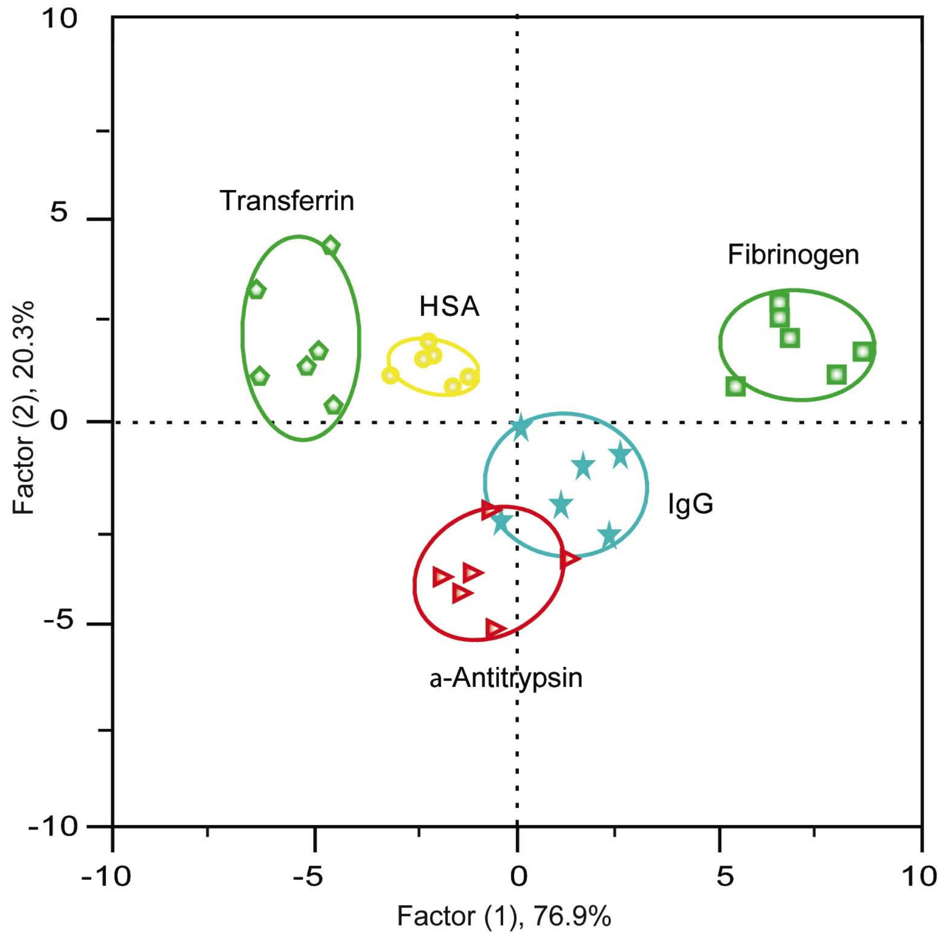


- analyte proteins look different...

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)

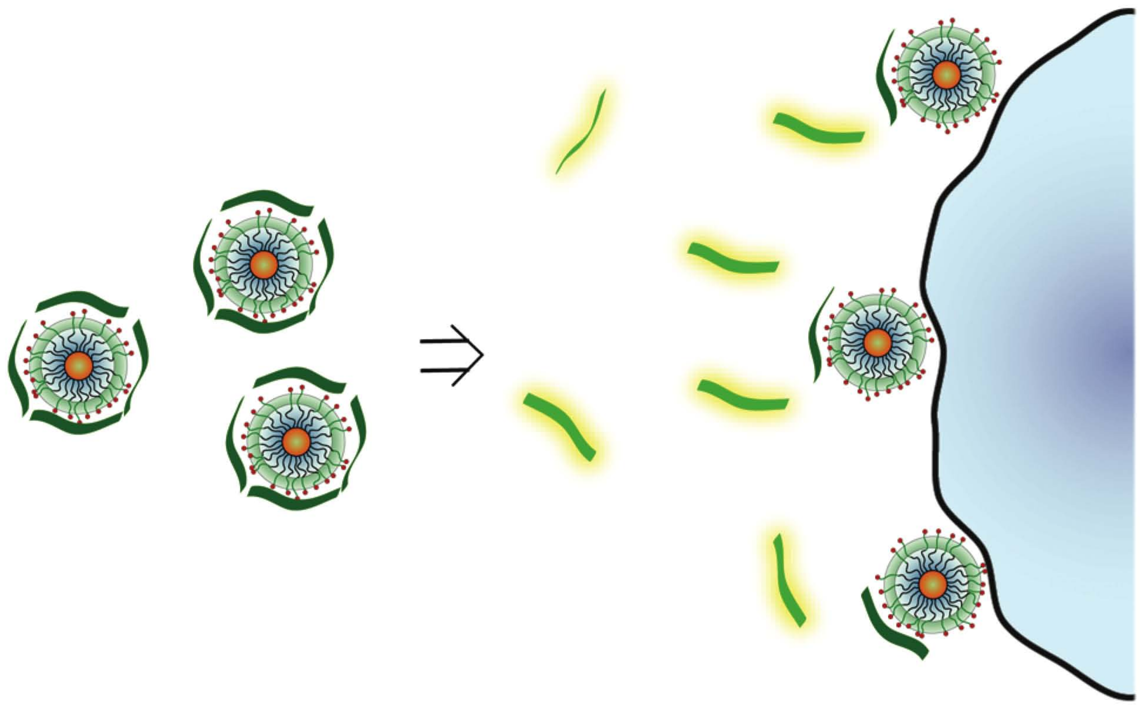


Protein	%Molar change
Albumin	0.06
IgG	0.75
Transferrin	1-2
Fibrinogen	8.4
α-Antitrypsin	5.2

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

How about something bigger--identification of bacteria!

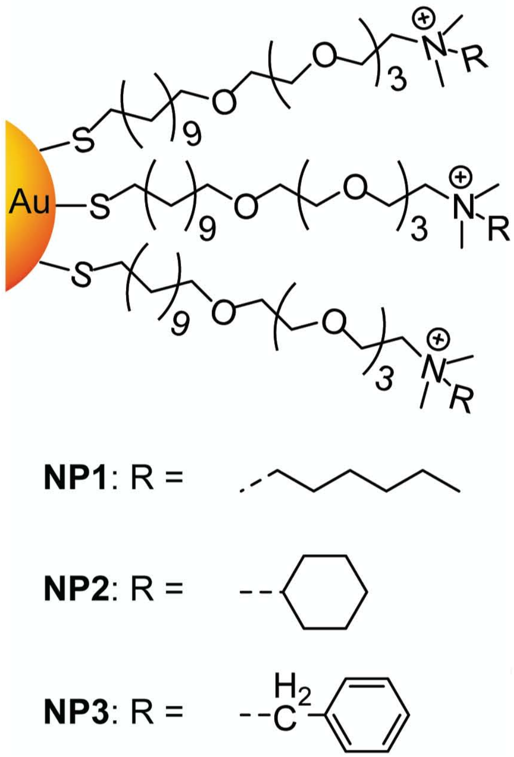
- bacteria pose environmental, bioterrorism, food and water safety hazards
- these are all ostensibly “clean” environments
- allowing us to use our “nose” to identify!

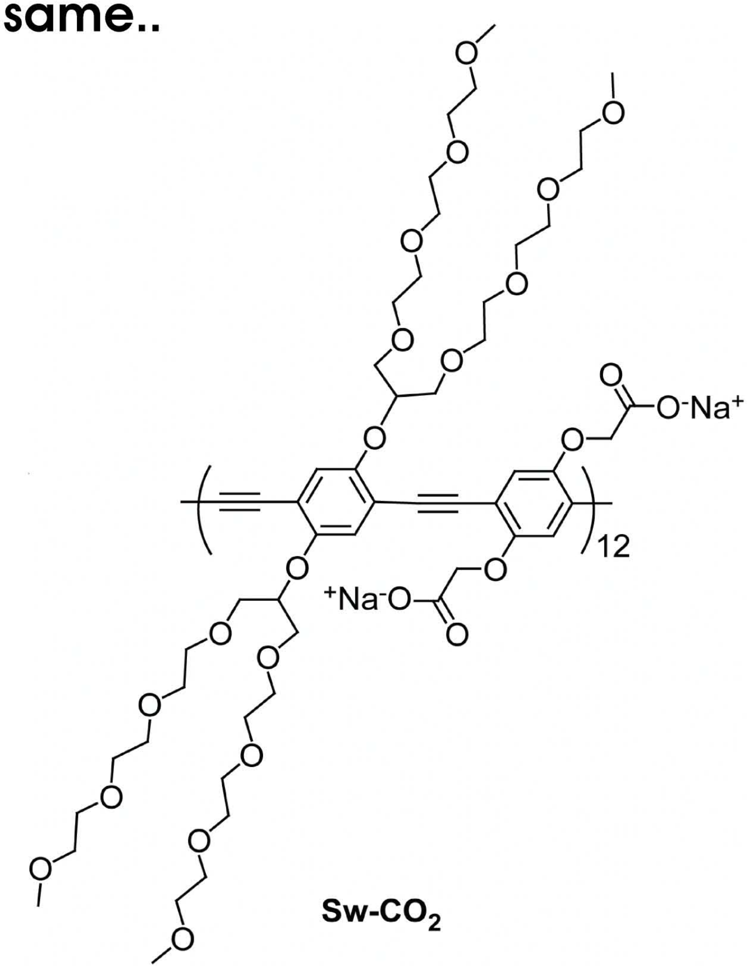


- the first attempts using original polymer/particle mixture=disaster
- hypothesis: polymer interacts too strongly with bacteria
- let’s test!

Same particles, different polymer

- “swallowtail” polymer designed to reduce non-specific interactions...
- the particles, however, remain the same..

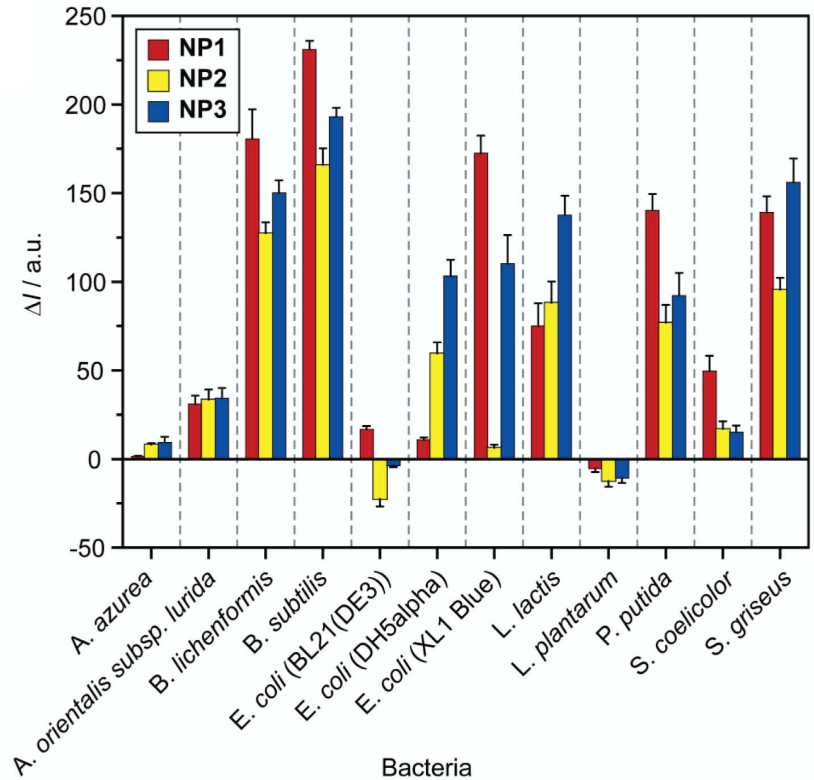


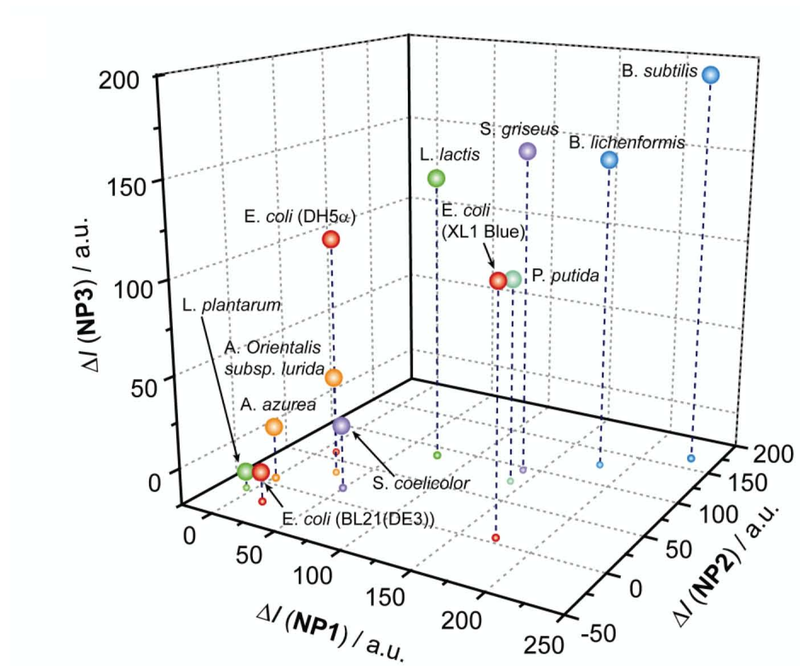


- notice--only three particles...because--

Three particles differentiate 13 bacteria!

- Gram +/- no problem
- we can even differentiate between strains!





- 10^5 cells/mL

- and LDA? we don't need no stinking LDA!
- the fluorescence response is fully differentiated as-is

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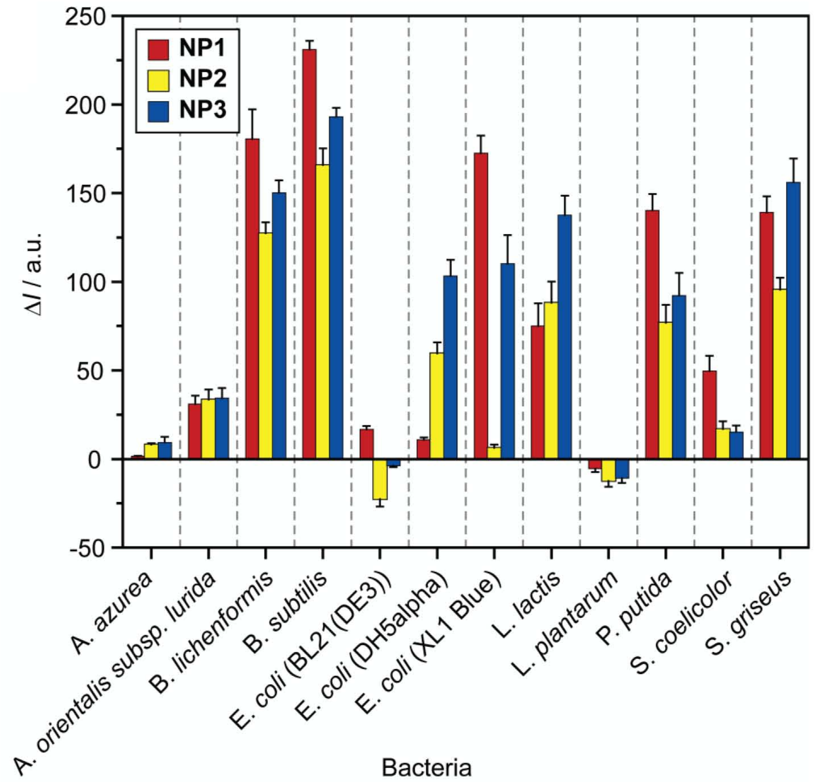

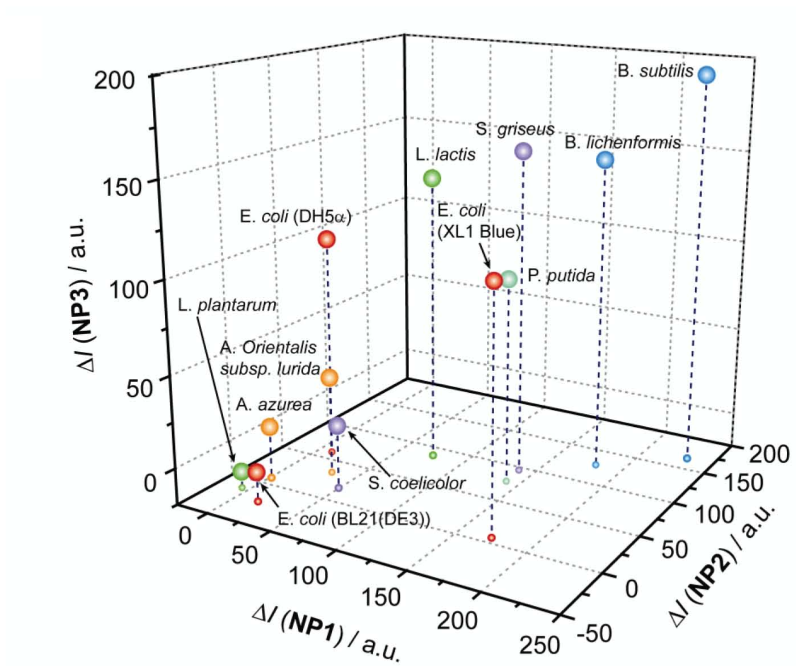

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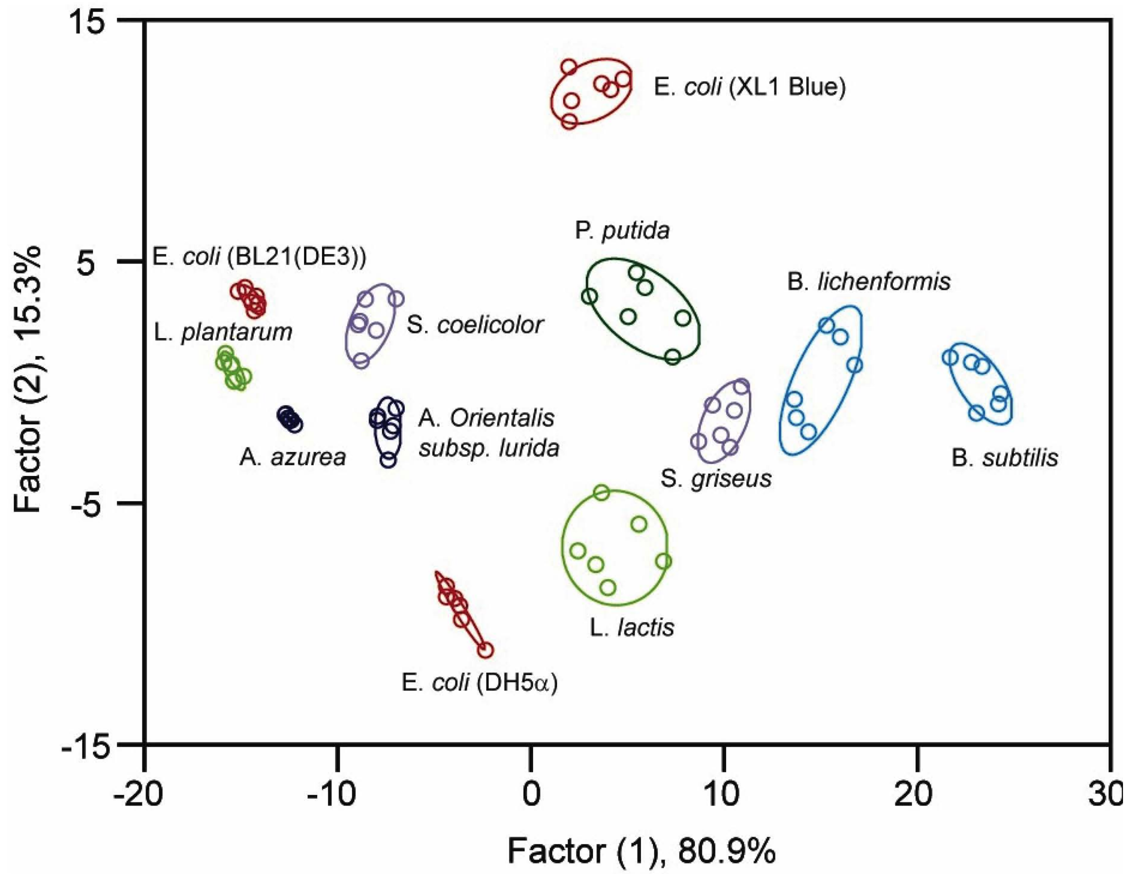
Phillips, R.L.;Miranda, O. R.; You, C.-C.; Rotello, V.M.;
Bunz, U.H.V. *Angewandte Chemie*, 2008. 47, 2590-2594

But we can do LDA if we want...

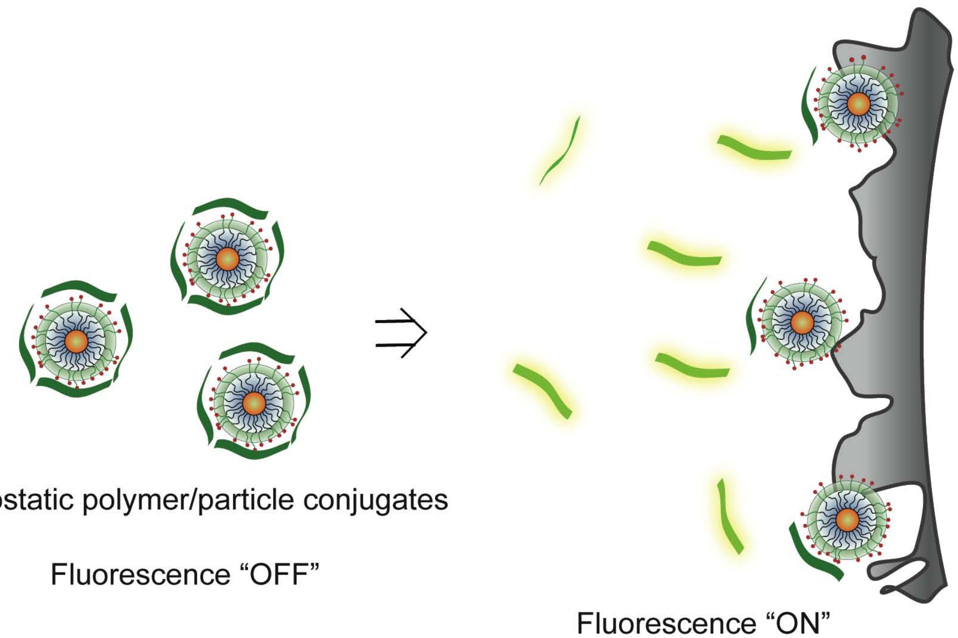
- full differentiation of bacteria
- we can differentiate between species and strains!

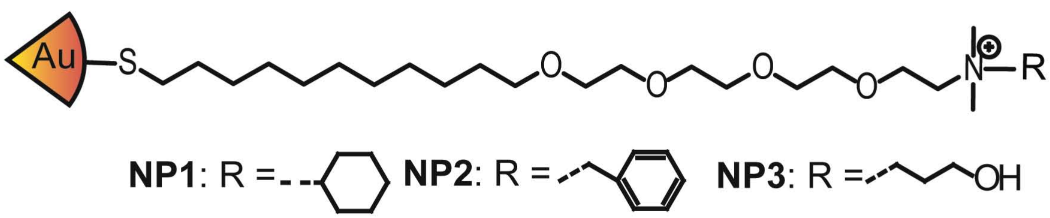


- identification of unknowns with >95% accuracy
- in minutes, with no sample preparation!

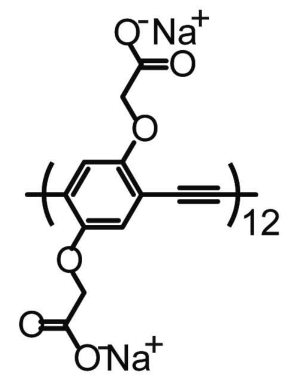
Identification of cancer via cell-surface interactions

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





- three particles (the ones that worked best)

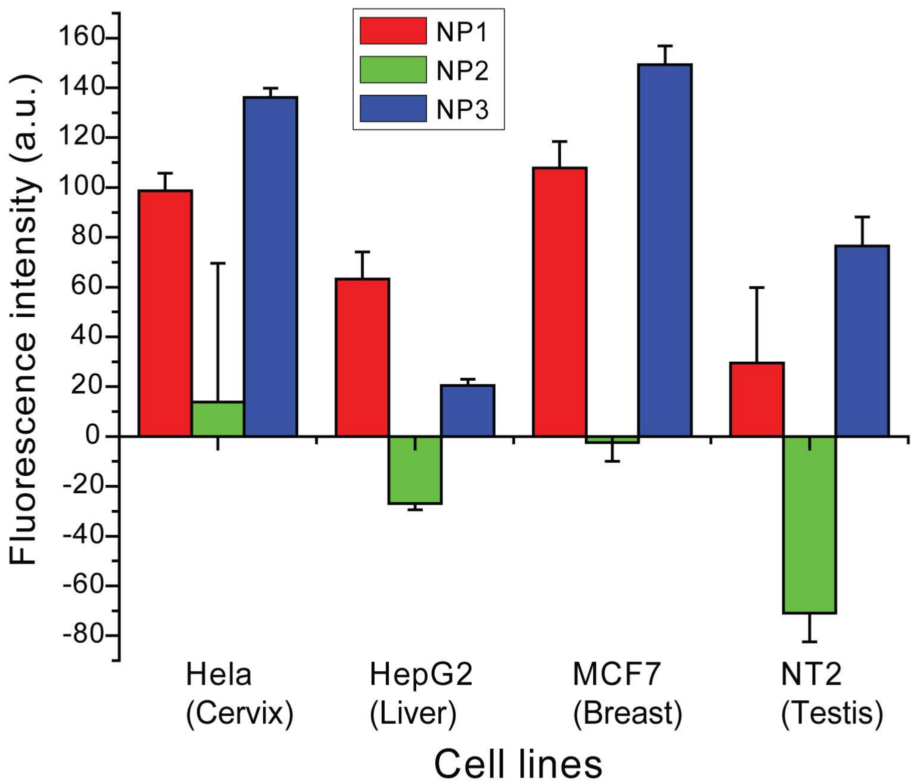


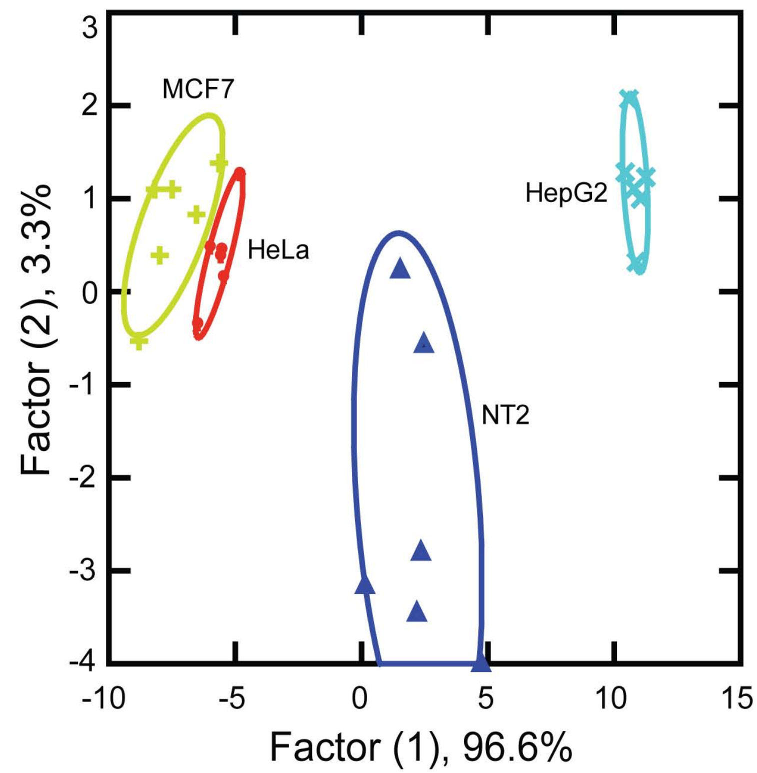
PPE-CO2

- one polymer (the original)

Starting easy--differentiating between cell types

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



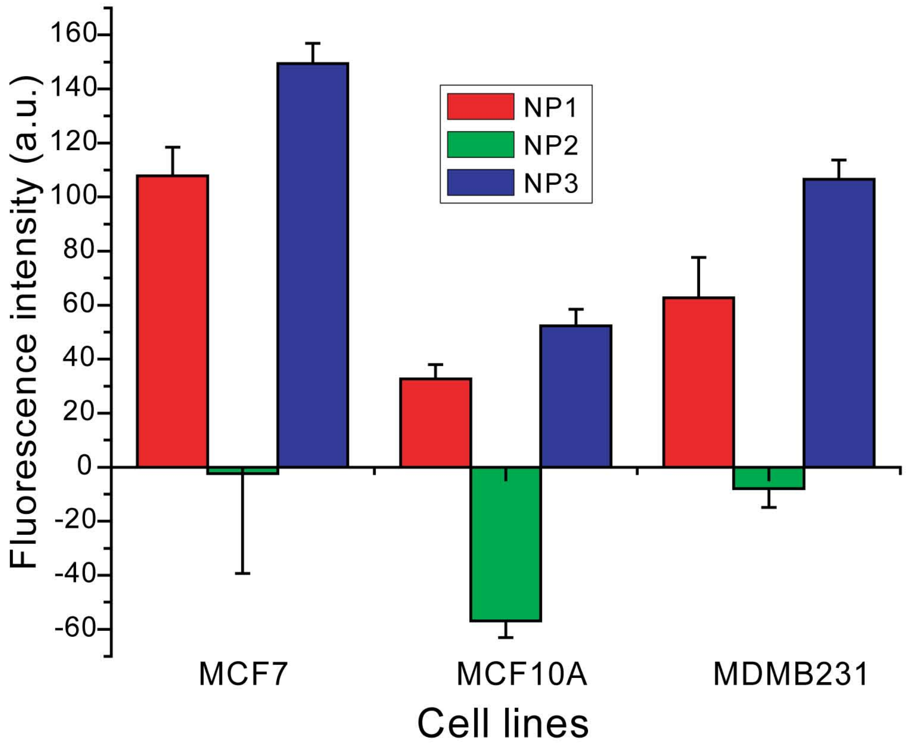


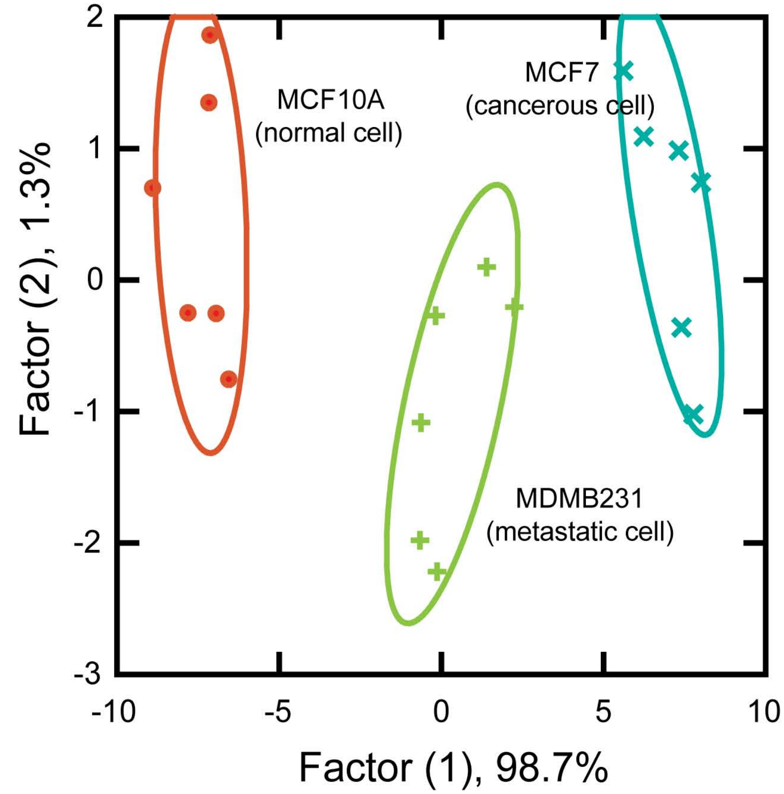
- complete differentiation
- now let's try something a bit more challenging

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?

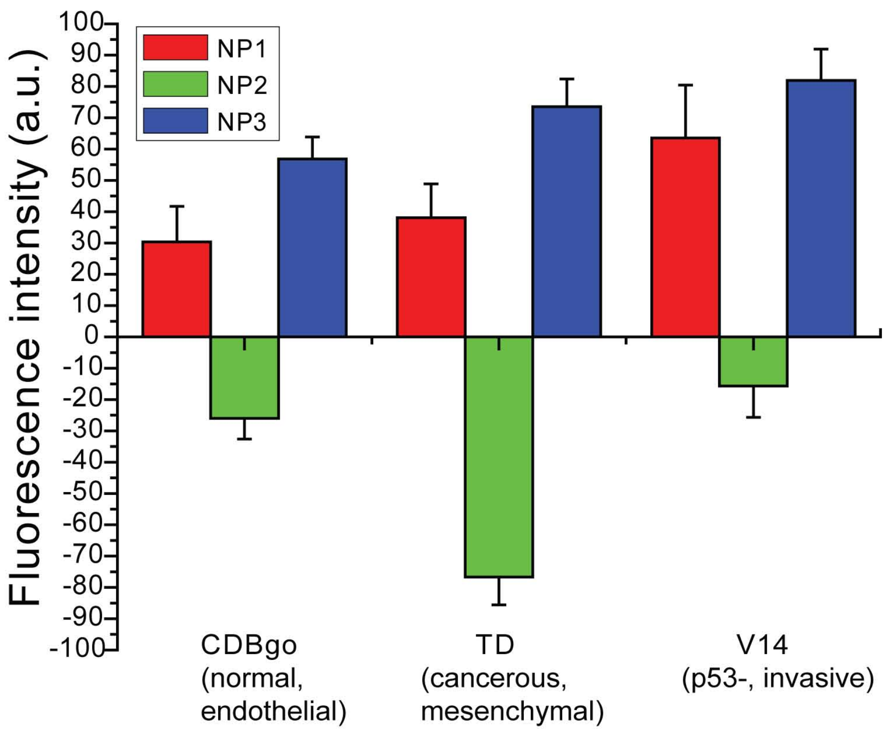


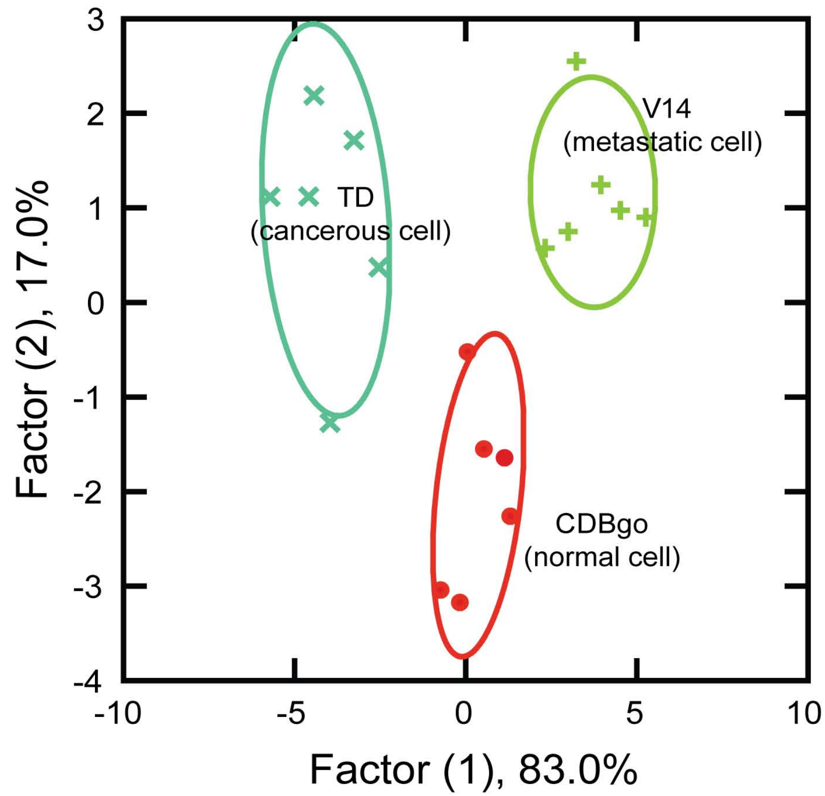


- once again, complete differentiation
- we can't celebrate yet: the three cell lines come from different people
- are we detecting cancer, or individual variations?

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

The “My Time is Up” Summary:

Nanoparticles provide:

- Effective “chemical nose” sensors for:
  - -Proteins
  - -Proteins in serum (500 nm in 1 mM total protein)
  - -Bacteria (species and strain)
  - -Cancer (metastatic vs non)

Acknowledgments:

Alumni: postdocs

Gilles Clavier
Allan Goodman
Alam Syed
Ulf Drechsler
C-C You
Amitav Sanyal
Tyler Norsten
Roy Shenhar
Belma Erdogan
"Pops" Arumugam
Amitav Sanyal
Yuval Ofir

Alumni: grad students

Bing Nie
Eric Breinlinger
Michael Greaves
Angelika Niemz
Robert Deans
Alex Cuello
Trent Galow
Faysal Ilhan
Eunhee Jeoung
Mark Gray
Andy Boal
Kanad Das
Kate Goodman
Kevin Bardon
Joe Simard
Ray Thibault
Joe Carroll
Oktay Uzun
Nick Fischer
Ben Frankamp
Rui Hong
Basar Gider
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Funding

NIH,NSF, ONR, DOE
NSF CHM-NSEC
NSF MRSEC, Army
Keck Foundation
BAE