Chemical nose biosensor design for differentiation of cancer cells using fluorescent compounds

Sukru Gokhan Elci
Assist. Prof.
Pamukkale University
Biomedical Eng. Dep.

Outline

- Introduction
  - Cancer diagnosis
  - Biosensors
- Experimental
  - Fluorescence measurements
- Results
  - Protein identification
  - Cancer diagnosis
- Conclusion

Cancer diagnosis

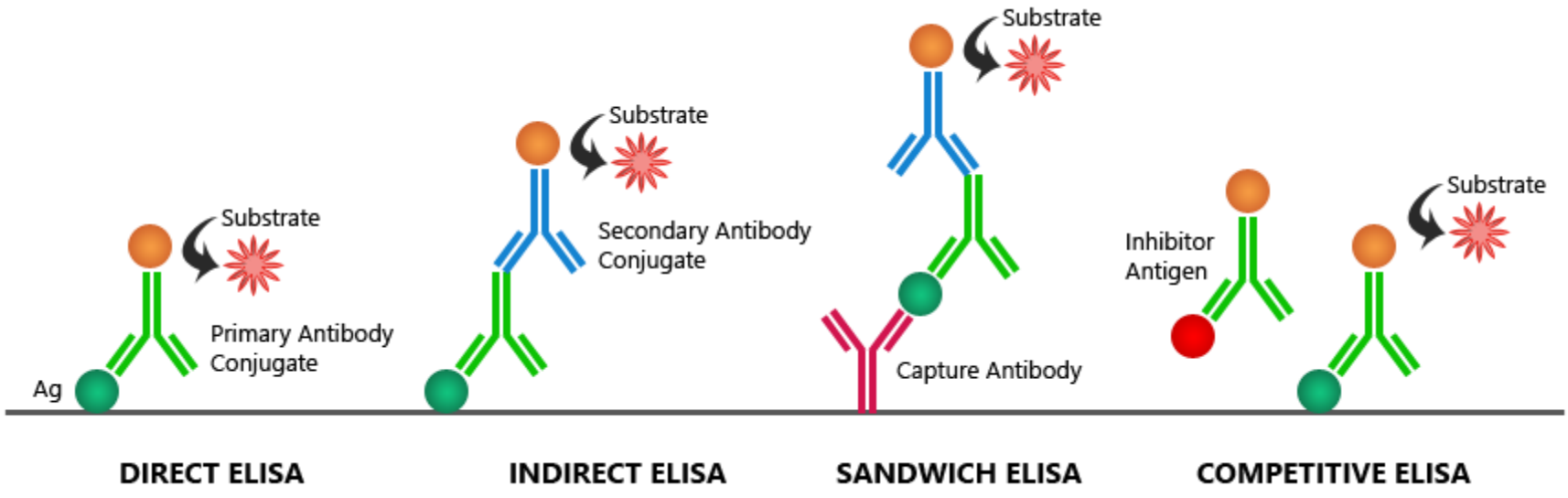
- Cancer diagnoses come in two forms:
- 1) Does the patient have cancer?
- 2) What kind of cancer (i.e. phenotype) is present?

- Both questions are important, however the answer to the second question is what dictates the course of therapy to be used.

- Cancer cells or proteins can be used for the diagnosis.

Conventional cancer diagnosis

- ELISA- Enzyme-linked immunosorbent assay

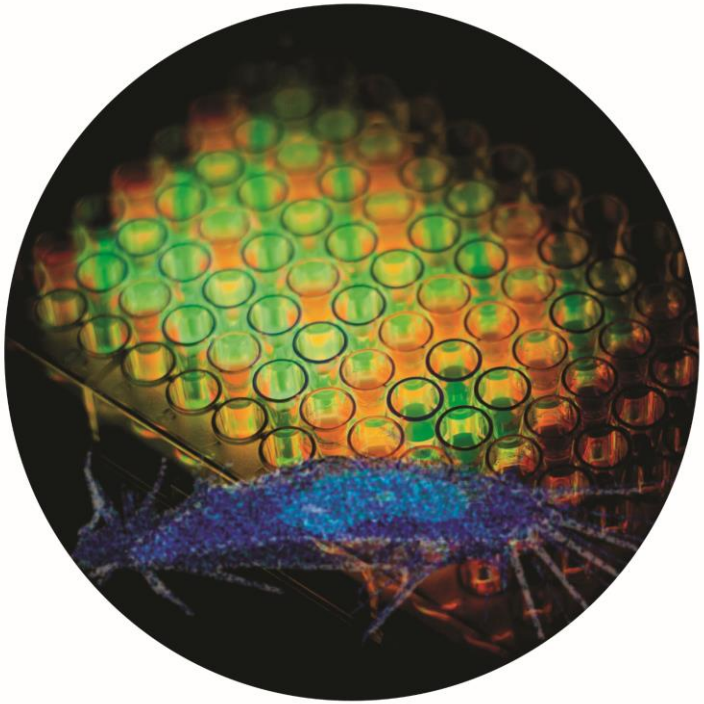


- It is based on "lock and key" specific recognition of specific biomarkers to provide diagnostic information.
- This biomarker-based approach is complicated by the highly variable and plastic nature of tumors and cancer cells.

Chemical nose sensor

- A ‘chemical nose’ sensor is broadly defined as an array based system that uses synthetic molecules and/or materials to mimic the mammalian olfactory systems.

- They are selective systems, rather then specific



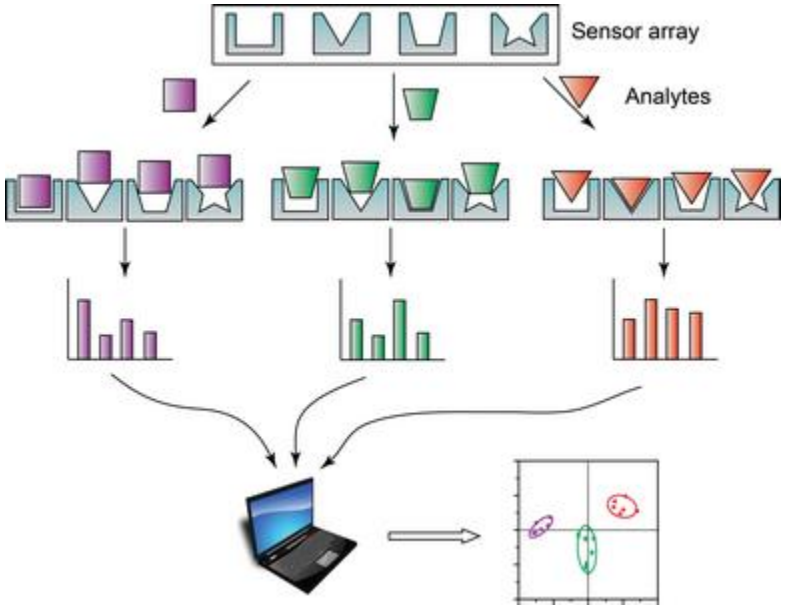
The term *sensing* refers to the detection of a particular entity (e.g matter, energy) by a specialized device (i.e sensor).

Elci et. al. Chem. Sci., **2013**, 4, 2076

Specific binding or selective binding?

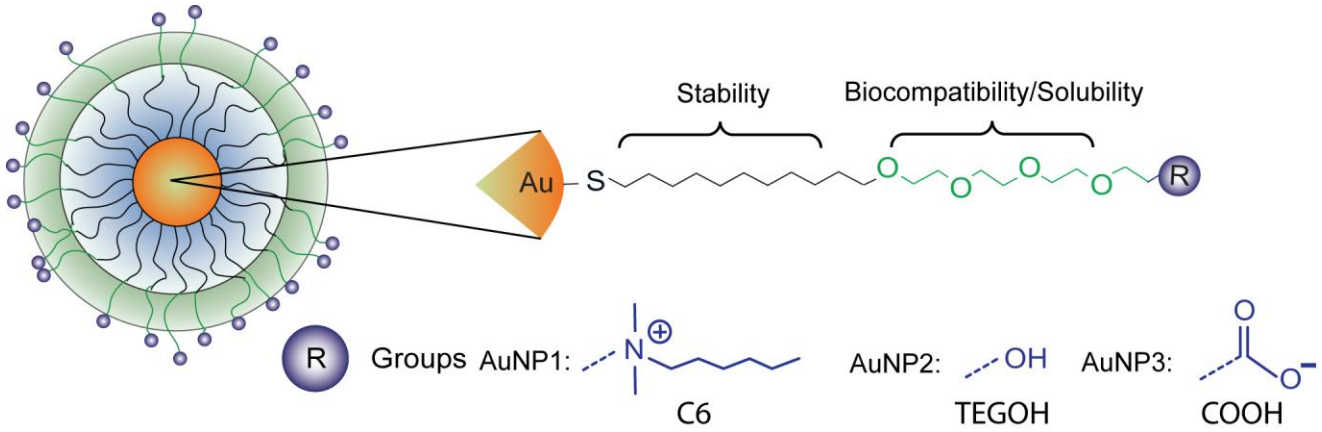
- Most biomolecular recognition processes in biology occur via specific interactions. Sensory processes such as taste and smell, however, use "differential" binding where the receptors bind to their analytes through selective rather than specific interactions.

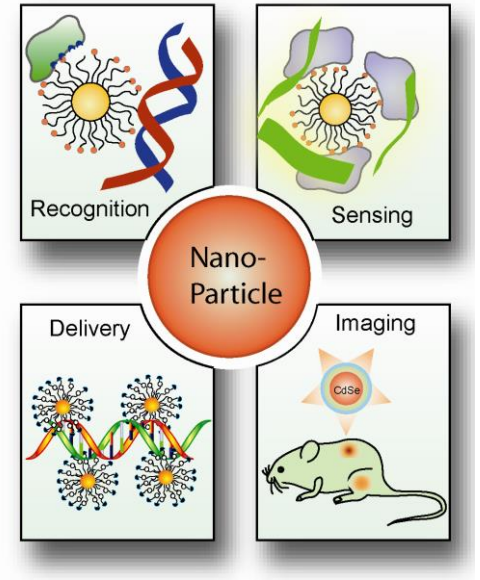
- Inspired by human olfactory system, chemical nose sensors are designed and developed as nanosensors for on a broad range of bioanalytes: proteins, bacteria and mammalian cells.



AuNPs

- Au is inherently non-toxic
- Easily tunable
- Various surface functionalities are available
- Quenches fluorescence



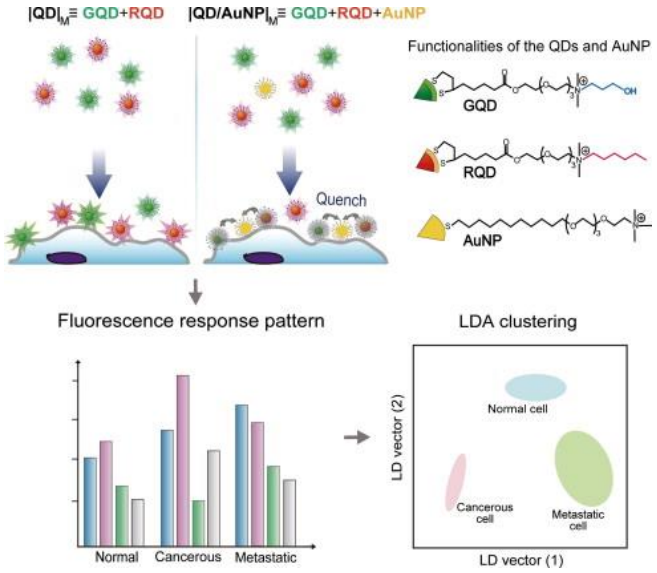


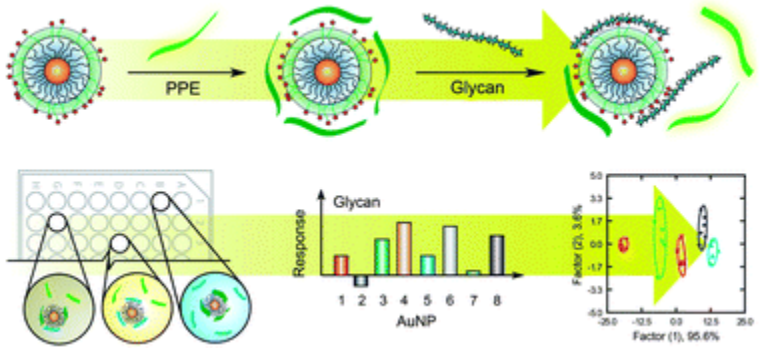
Elci et. al. Chem. Sci., **2013**, 4, 2076

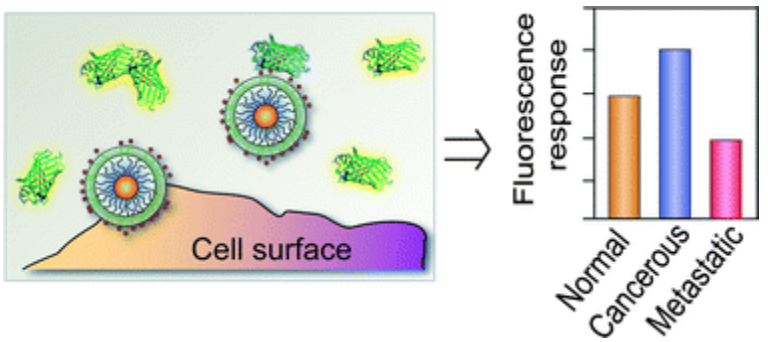
AuNP-Fluorescent probe chemical nose sensors

- Fluorescent proteins
- Fluorescent polymers
- Fluorescent nanomaterials

- All of these materials can interact with the AuNP to create a chemical sensor array to obtain varying signals that can be differentiated



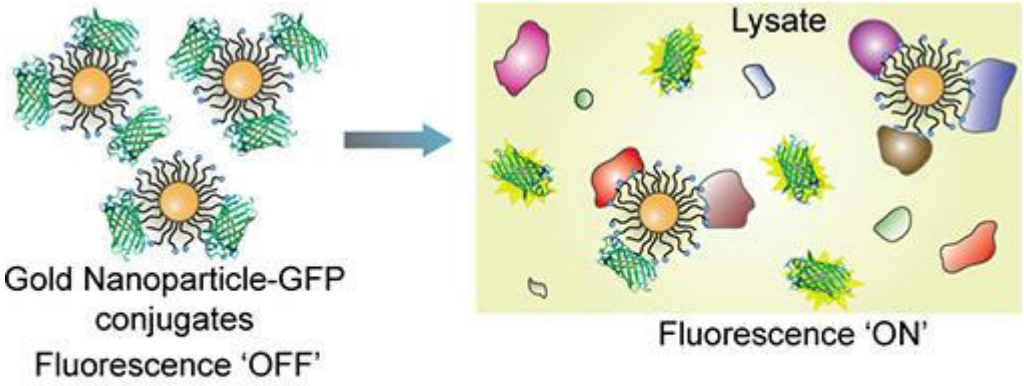


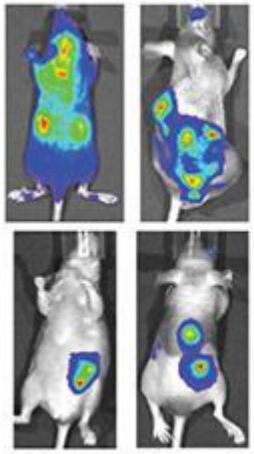


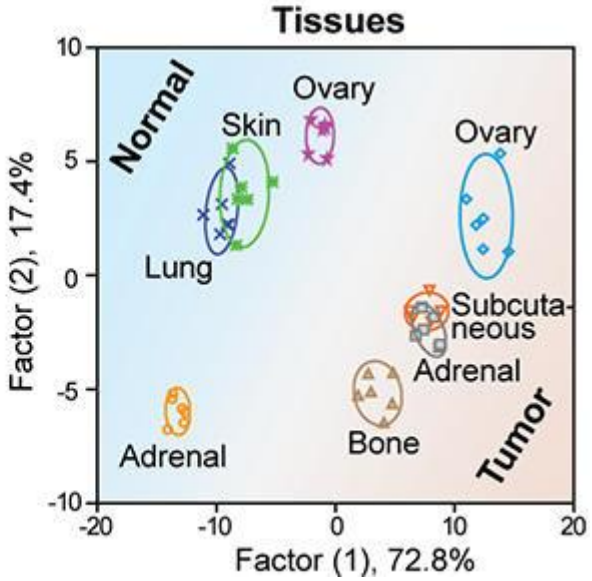
Rotello and Bunz. Angew. Chem. Int. Ed. **2010,** 49, 3268 – 3279

Single channel chemical nose sensor array (GFP)

- Cell lysate can be obtained from the lysis of both normal and tumorigenic cell lines
- Differentiating different cell lysate samples can give information about patient`s status



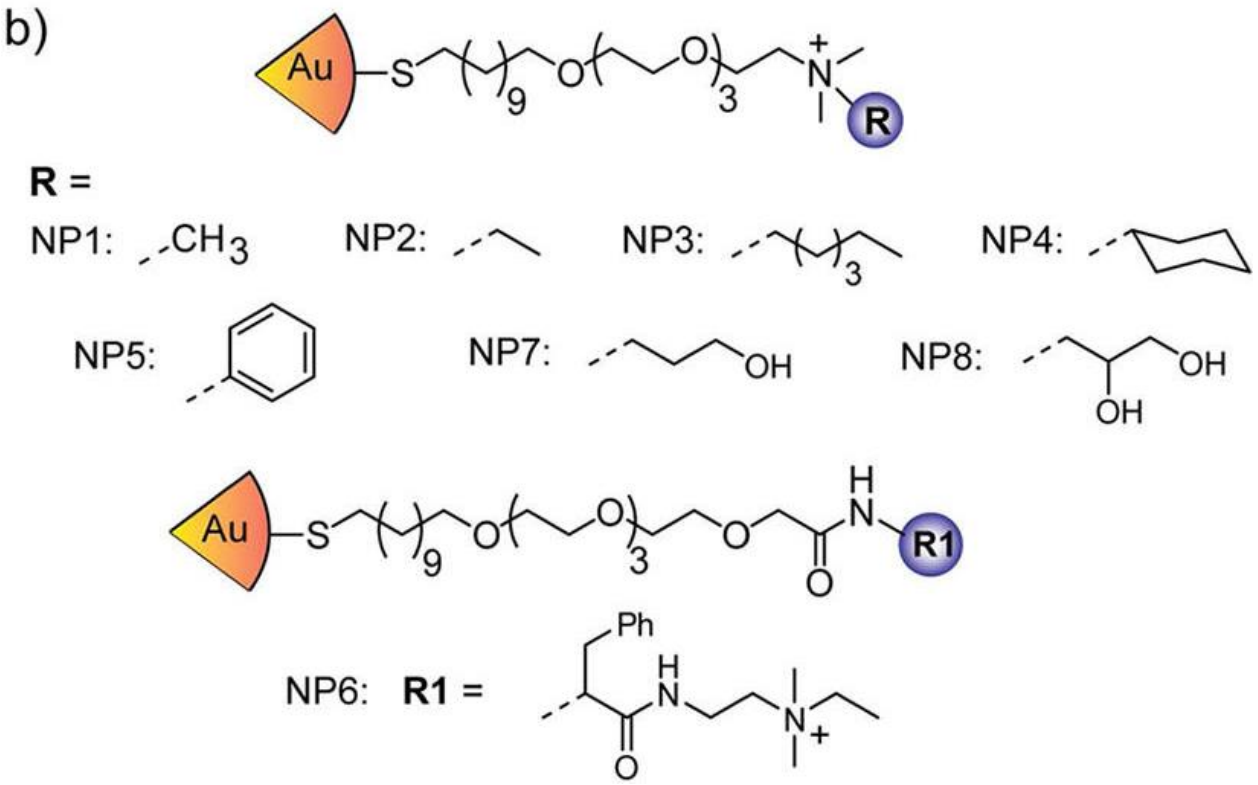

Generation of metastases



Rana et. al. *ACS Nano*, **2012**, *6* (9), 8233–8240

Single channel chemical nose sensor array (GFP)

- 8 different AuNP structure is selected as recognition element for the sensor design



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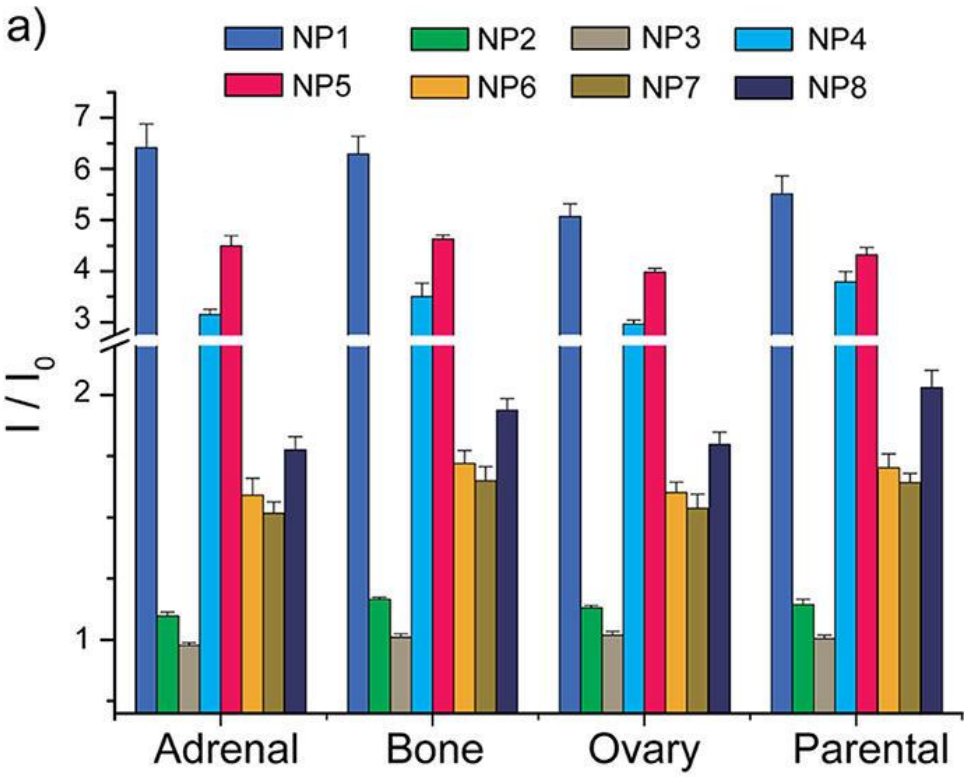
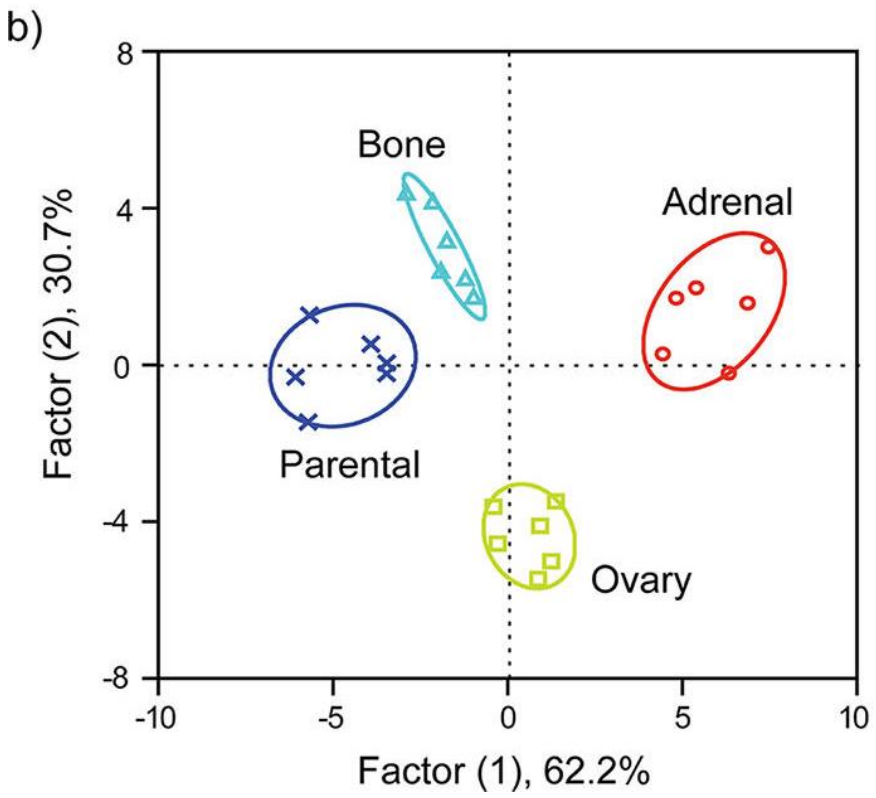
Single channel chemical nose sensor array (GFP)





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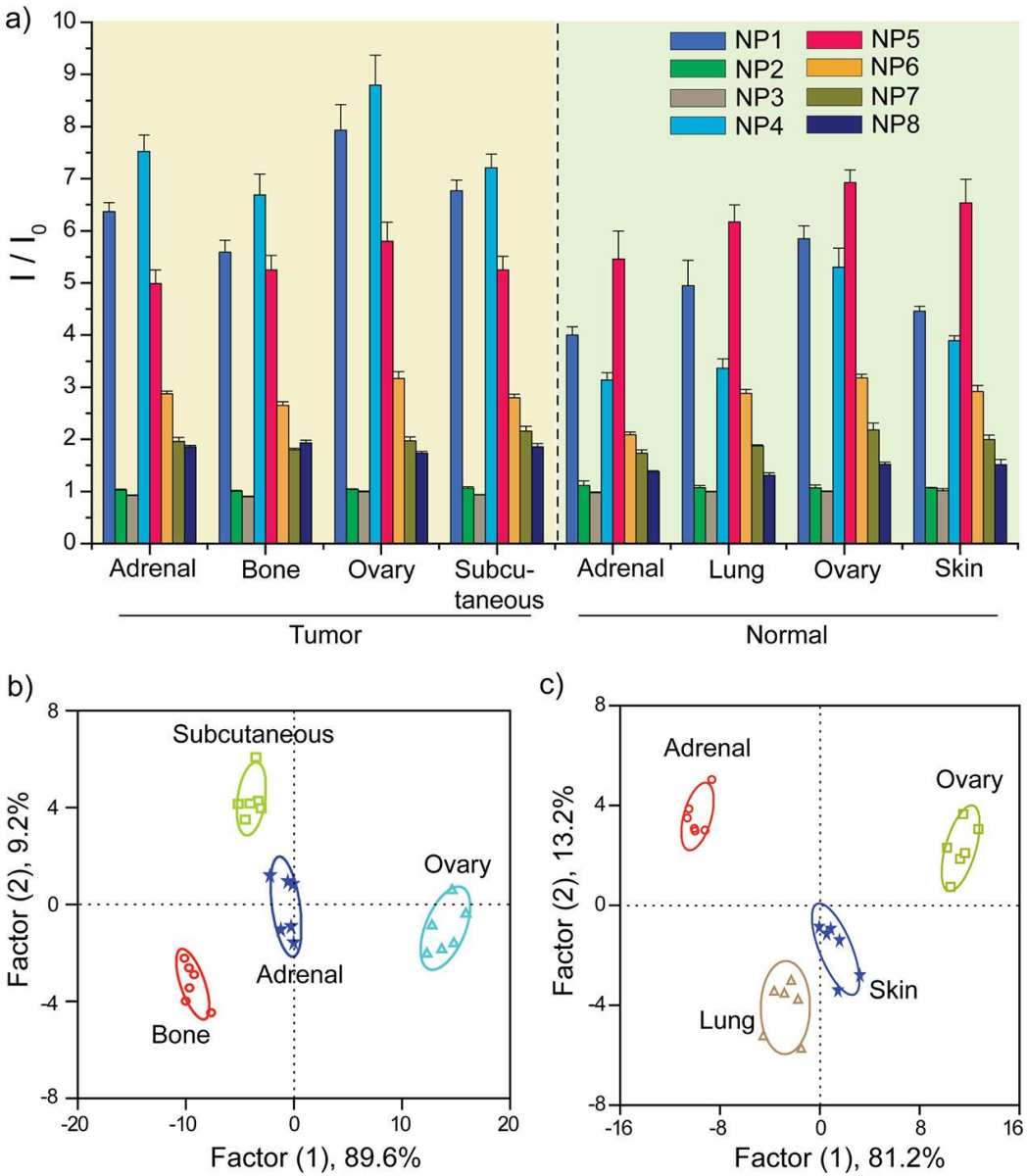
Single channel chemical nose sensor array (GFP)



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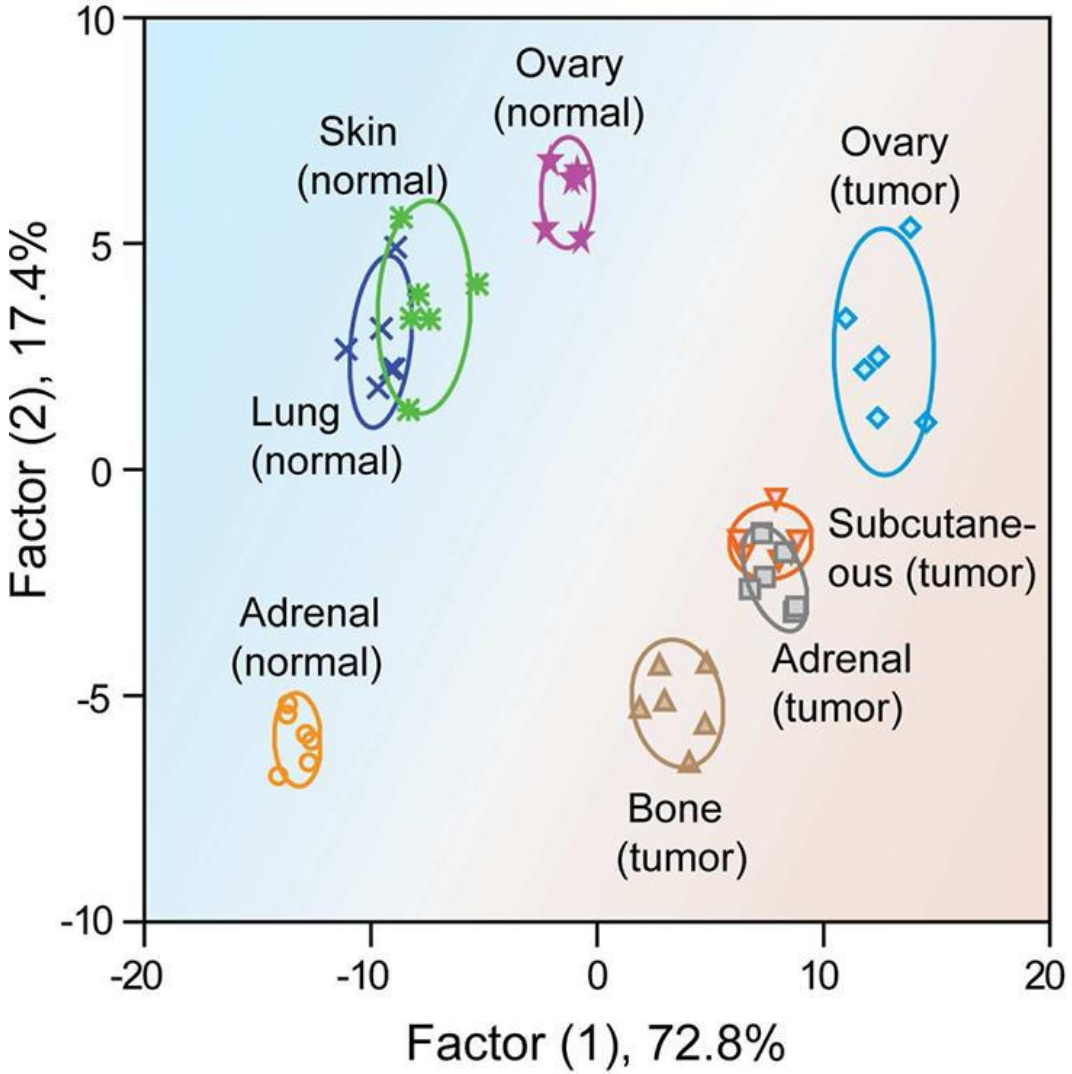

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Single channel chemical nose sensor array (GFP)

- This sensing approach provides a complementary strategy to traditional biomarker-based methods for diagnosis or prognostication.

- In the present sensing, the discrimination relies upon the phenotypic differences within the overall proteomic signatures of the respective cells and tissues.

- Using the lysates for these sensors offers distinct advantages compared to whole cell sensing, such as increased homogeneity of the test samples leading to reduced error in identification, increased reproducibility, and higher sensitivity.

Rana et. al. *ACS Nano*, **2012**, *6* (9), 8233–8240

Differentiating cancer cells

- Comparison between a healthy cell and a cancerous cell shows that the cancerous cells have a different surface morphology than the healthy cells

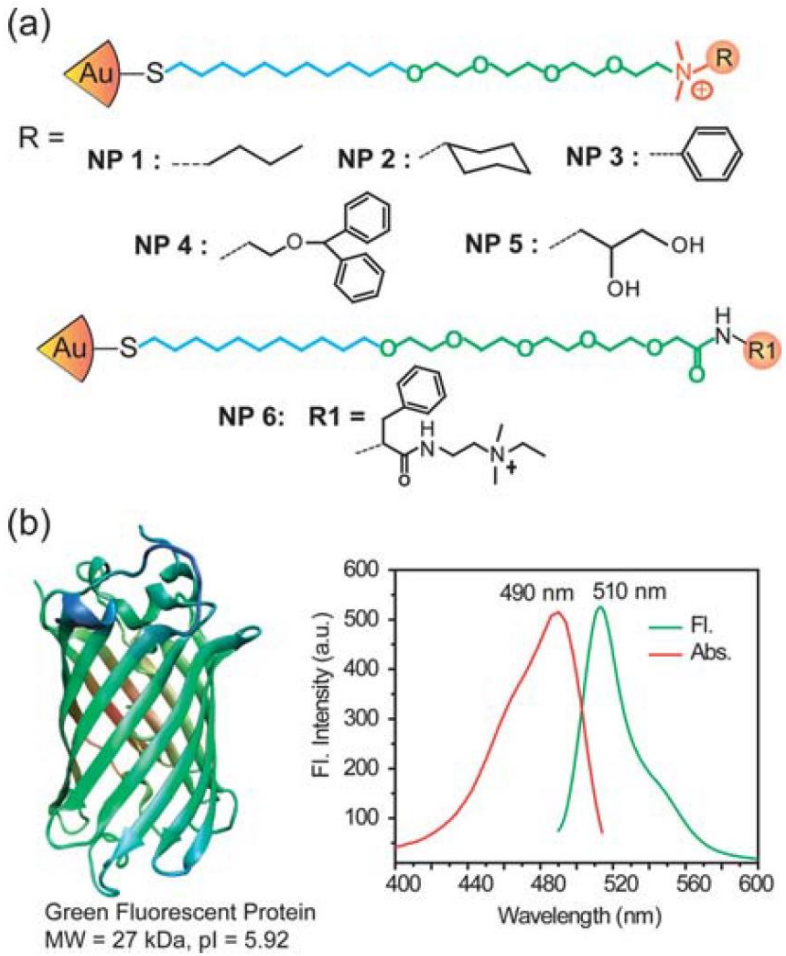
- This observation is due to the changes on the glycan structures seen on the surface of the cells

- Besides this changes, the proteins expressed in the cells differ drastically in both cells

Bajaj et. al. *PNAS* **2009,** 106 (27), 10912-10916

Single channel chemical nose sensor array (GFP)

- 6 different cationic NPs were selected that differed in hydrophobicity
- Negatively charged GFP used to interact with the nanoparticles individually



Bajaj et. al. *Chem. Sci.*, **2010,** 1, 134–138

Single channel chemical nose sensor array (GFP)

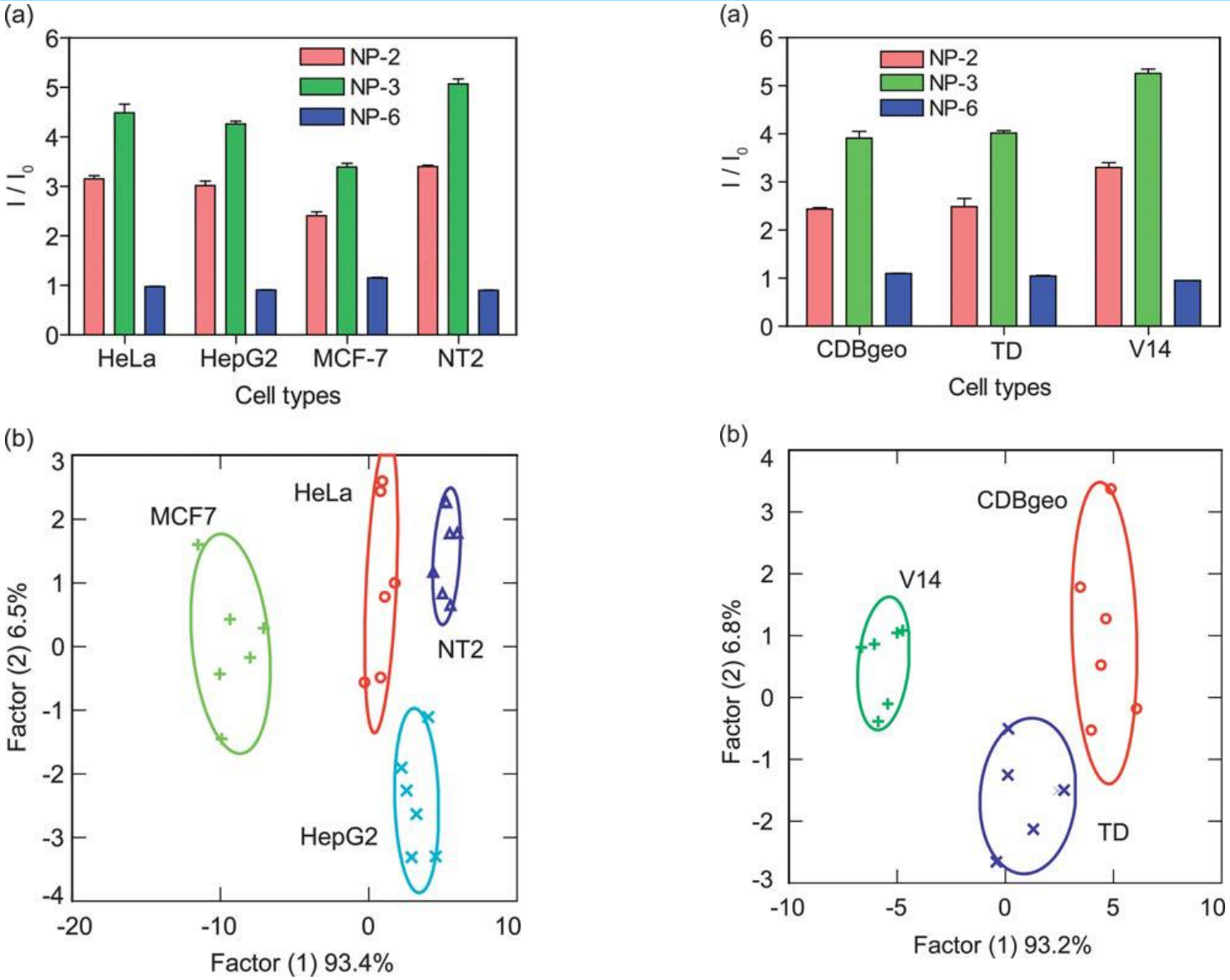
- 6 different cells lines selected to be diagnosed using AuNP-GFP sensor array

- Cell lines came from both human and mouse cell lines to compare the various cancer cell types and also the healthy lines from cancerous ones

Human cell lines	Cervix	HeLa	Cancerous
	Breast	MCF-7	Cancerous
	Testis	NT2	Cancerous
	Liver	HepG2	Cancerous
Mouse cell lines	Breast	CDBgeo	Normal immortalized
	Breast	TD	Cancerous
	Breast	V14	Metastatic

Bajaj et. al. *Chem. Sci.*, **2010,** 1, 134–138

Single channel chemical nose sensor array (GFP)



Bajaj et. al. *Chem. Sci.*, **2010,** 1, 134–138

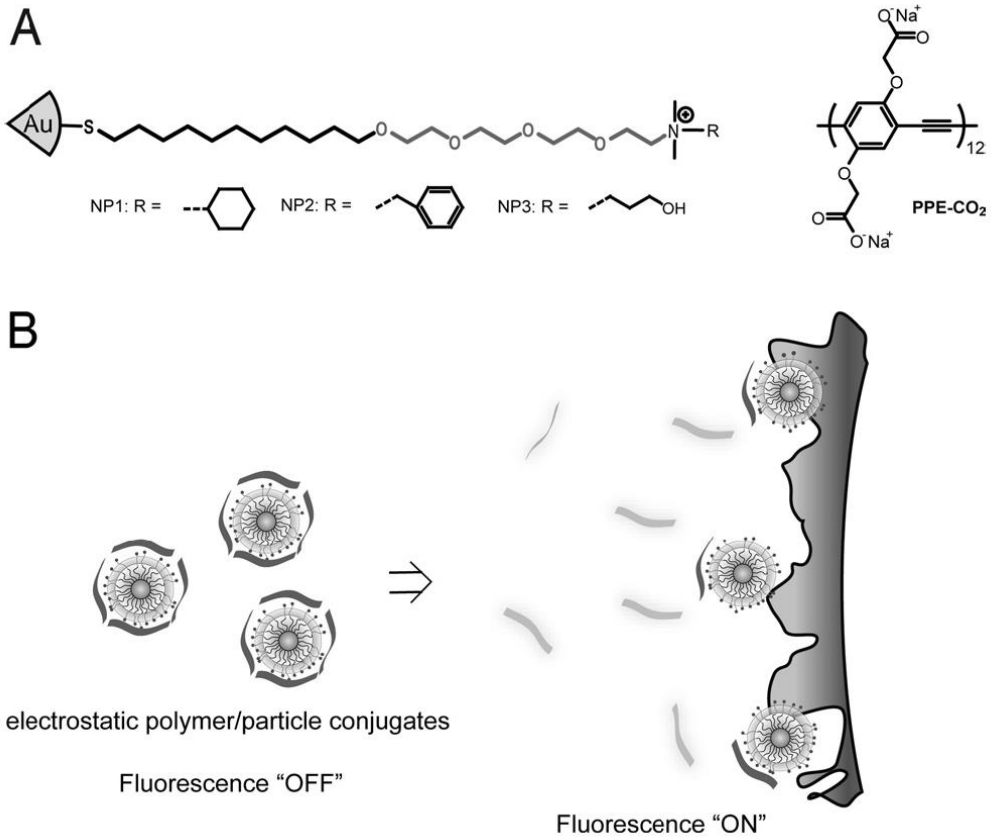
Single channel chemical nose sensor array (GFP)

- The sensor array efficiently discriminates normal, cancerous and metastatic isogenic cells
- The use of GFP as the reporter fluorophore provides a four-fold enhancement of sensitivity relative to prior NP-polymer sensors
- This sensor was highly successful at differentiating single cell states. Clearly differentiation of cells in heterogeneous mixtures remains a challenge
- Besides use of GFP reduces the stability of the sensor system

Bajaj et. al. *Chem. Sci.*, **2010,** 1, 134–138

Single channel chemical nose sensor array (PPE)

- To create a more solid sensor design, GFP is replaced with fluorescent polymer, poly(para-phenyleneethynylene),PPE.



Bajaj et. al. *PNAS* **2009,** 106 (27), 10912-10916

Single channel chemical nose sensor array (PPE)

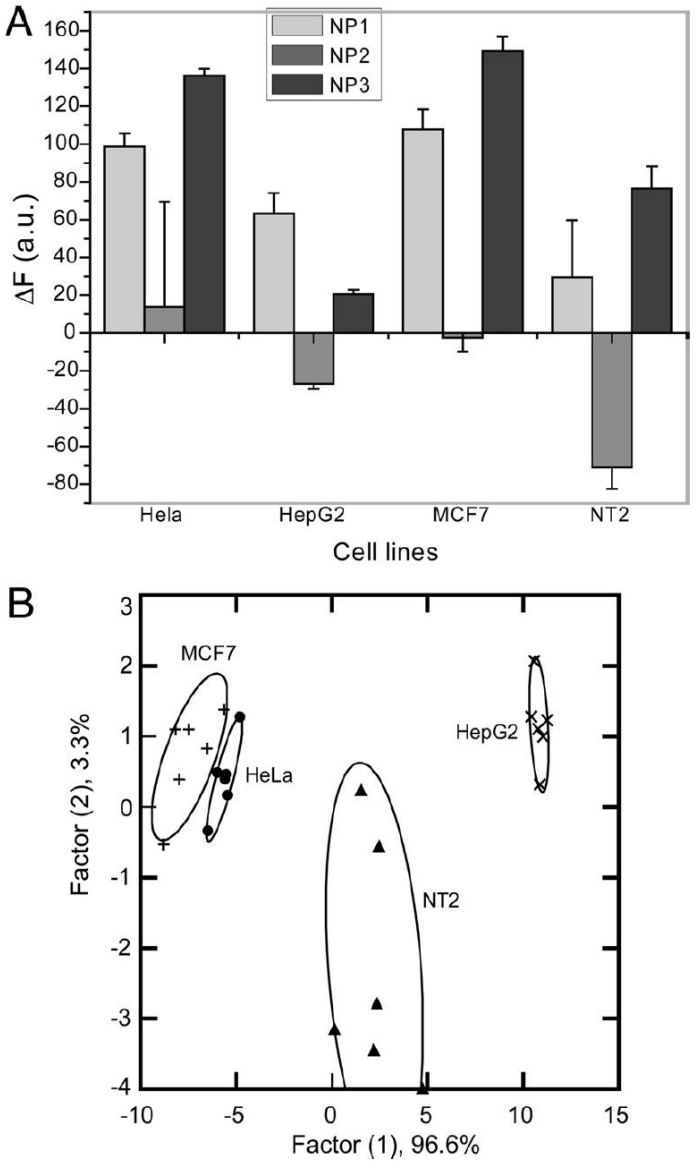
- Same cell lines studied in this study
- A human healthy cell line is added for comparison

Cell line	Liver	HepG2	Cancerous
Human	Cervix	HeLa	Cancerous
	Testis	NT2	Cancerous
	Breast	MCF10A	Normal immortalized
		MCF-7	Cancerous
		MDA-MB-231	Metastatic
Mouse	BALB/c mice (breast)	CDBgeo	Normal immortalized
		TD	Cancerous
		V14	Metastatic

Bajaj et. al. *PNAS* **2009,** 106 (27), 10912-10916

Single channel chemical nose sensor array (PPE)

- Similar to the GFP analysis, polymer system can also differentiate between the cell lines with a great success.



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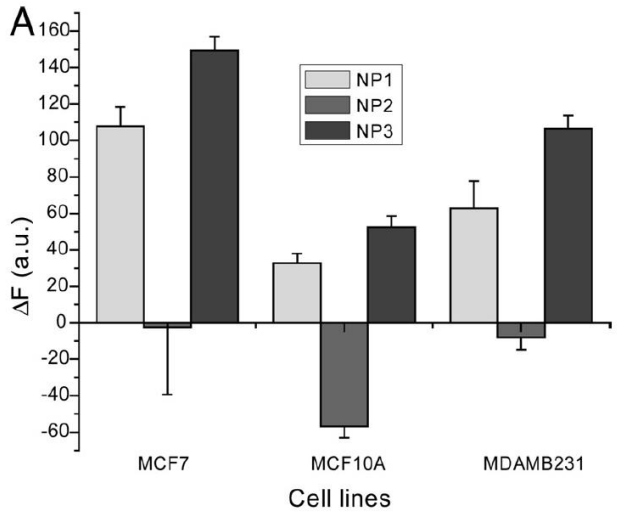
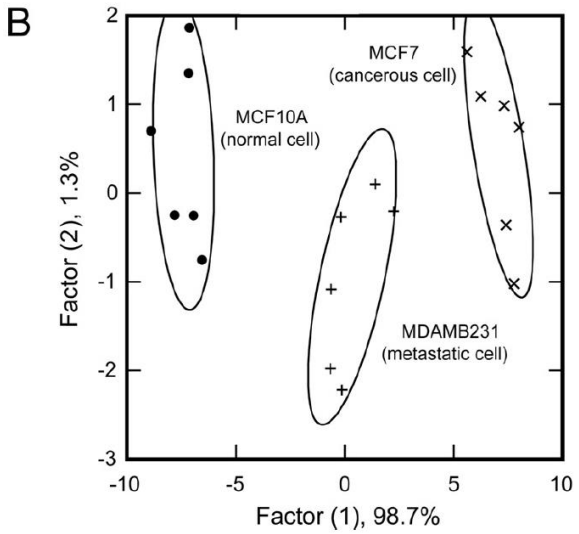
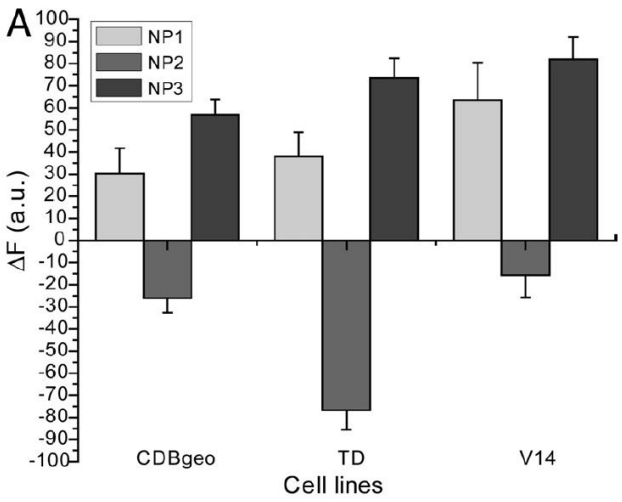
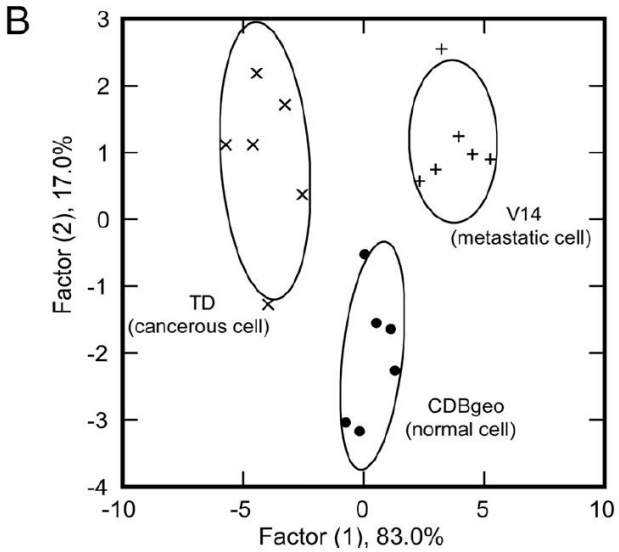








Bajaj et. al. *PNAS* **2009,** 106 (27), 10912-10916

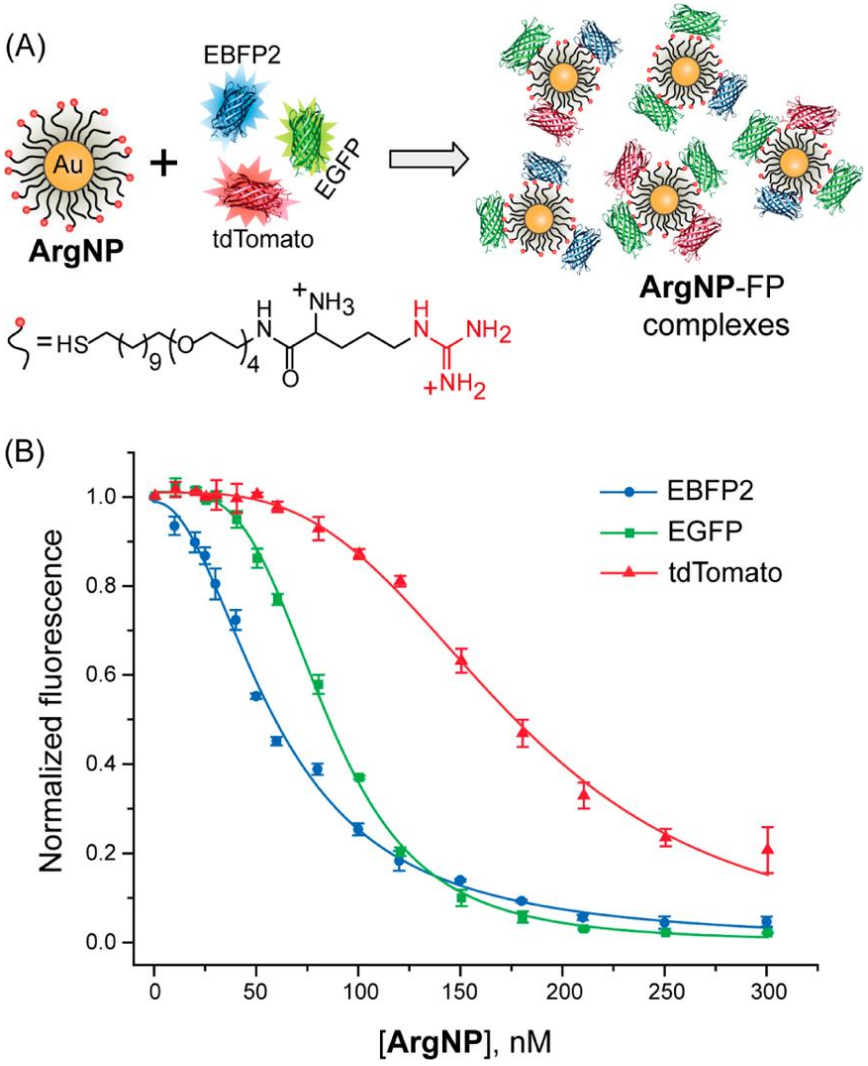
Single channel chemical nose sensor array (PPE)

- Significantly, full differentiation was achieved using only 3 nanoparticle-polymer dyads, indicating that a simple sensor array has ample diagnostic capacity when exposed to mammalian cells.
- These systems have the potential to help us understand the physical changes that occur on the surfaces of cells in various disease states.
- Taken together, "nose" based sensor systems are a fundamentally new way of looking into biodiagnostic, biophysical and surface science processes involving cell surfaces.

Bajaj et. al. *PNAS* **2009,** 106 (27), 10912-10916

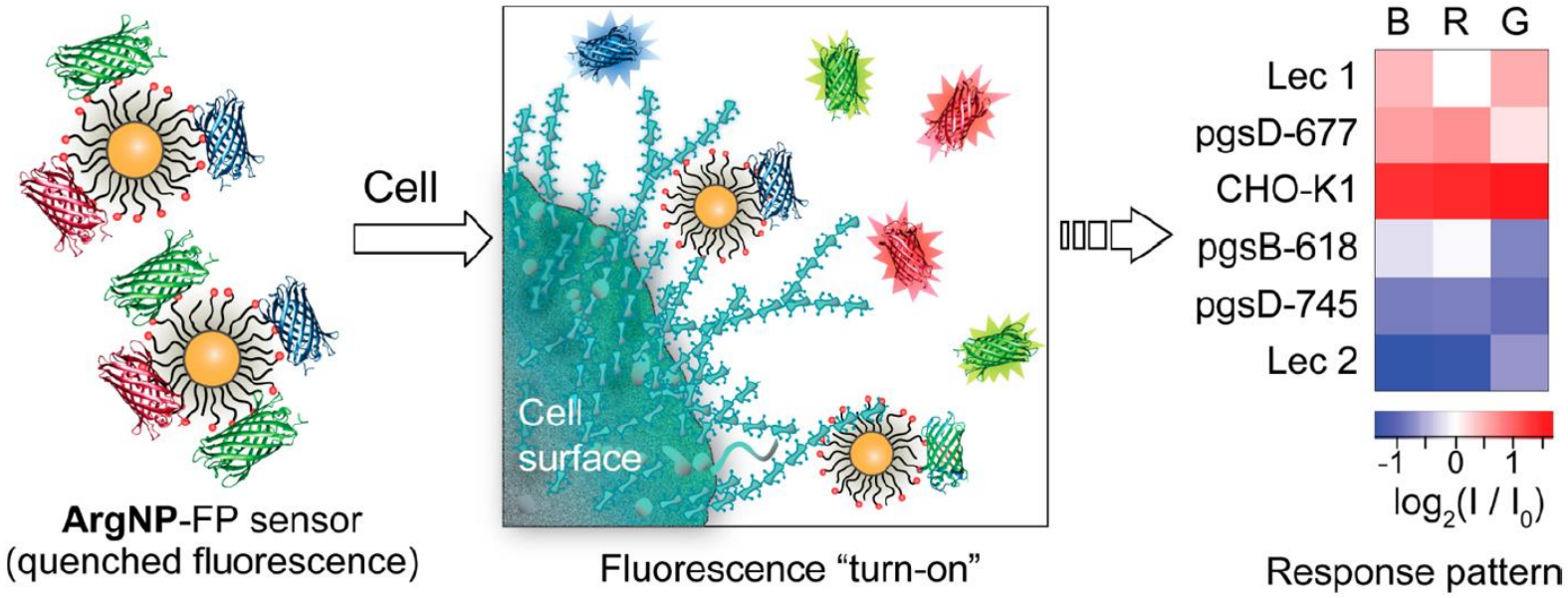
Multi-channel chemical nose sensor array (RFP-BFP-GFP)

- Despite the efficacy of array-based sensors in diagnostics, current systems are capable of producing only single channel measurements of the molecular recognition, requiring multiple spatially distinct sensor elements for identifying one analyte and limiting their application in rapid high-throughput screening of bioanalytes.



Rana et. al. *ACS Cent. Sci.*, **2015**, *1* (4), 191–197

Multi-channel chemical nose sensor array (RFP-BFP-GFP)



ArgNP-FP sensor
(quenched fluorescence)

Fluorescence "turn-on"

Response pattern

Rana et. al. *ACS Cent. Sci.*, **2015**, *1* (4), 191–197

Multi-channel chemical nose sensor array (RFP-BFP-GFP)

- 6 cell lines selected at differing surface glycan structures that also differ in tumorigenic and nontumorigenic

cell line	biochemical defect	glycan composition	cell status
CHO-K1	none	wild-type	tumorigenic
pgsB-618	galactosyltransferase I deficient	proteoglycan deficient (15% of wild-type cells)	tumorigenic
pgsA-745	xylosyltransferase deficient	proteoglycan deficient (8% of wild-type cells)	nontumorigenic
pgsD-677	lacks *N*-acetylglucosaminyltransferase and glucuronyltransferase activities	HS deficient; produces 3−4-fold higher CS than CHO-K1 cells[a]	nontumorigenic
Lec-1	*N*-acetyl-D-glucosamine (GlcNAc) transferase I deficient	does not synthesize complex- or hybrid-type *N*-linked oligosaccharides	tumorigenic
Lec-2	unable to translocate CMP-sialic acid to Golgi apparatus	*N*- and *O*-linked sialic acid deficient	tumorigenic

[a]HS: heparan sulfate. CS: chondroitin sulfate.

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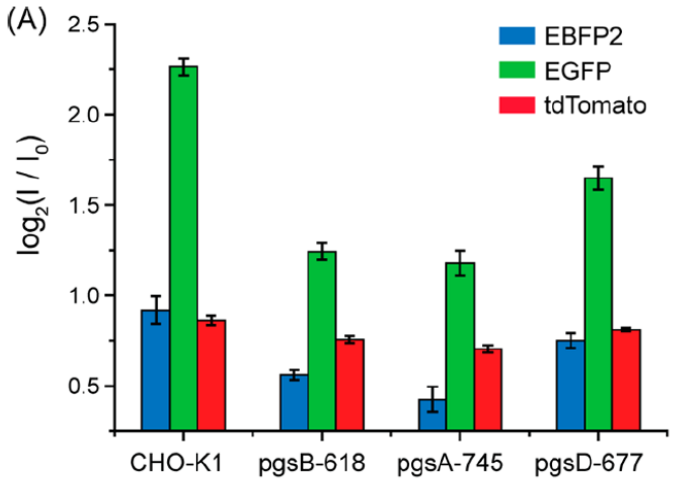
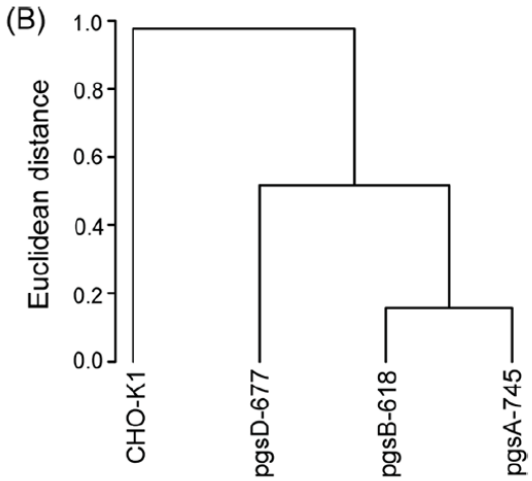
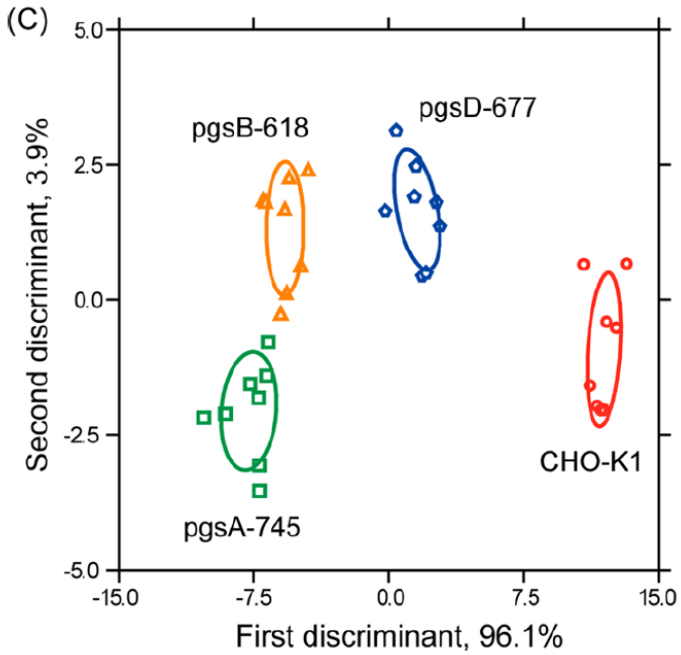
Multi-channel chemical nose sensor array (RFP-BFP-GFP)







(D)

Jackknifed classification matrix

	I	II	III	IV	%correct
I	8	0	0	0	100
II	0	8	0	0	100
III	0	0	8	0	100
IV	0	0	0	8	100
Total	8	8	8	8	100

I : CHO-K1 III : pgsA-745

II : pgsB-618 IV : pgsD-677

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Multi-channel chemical nose sensor array (RFP-BFP-GFP)

- This system responds to different glycan patterns, both charged and noncharged, on cell surfaces.
- Healthy and cancerous cells with the same genetic background and exhibiting different cell surface glycome signatures were effectively discerned within a single well of a microplate.
- Significantly, the present study demonstrated the role of glycans in identifying cell states using nonspecific sensors, an important step forward to designing effective signature-based biodiagnostics.
- Taken together, the ability to recognize cells combined with the high-throughput features of the sensor holds great promise in personalized screening of disease states, and cell-based profiling of the mechanisms of carbohydrate therapeutics.

Rana et. al. *ACS Cent. Sci.*, **2015**, *1* (4), 191–197

Conclusion

- Single channel or multichannel chemical nose sensor arrays are capable of differentiating different cell lines to diagnose cancers.

- Simple reliable and fast response time makes them a great candidate for replacement of ELISA based diagnosis tools.

Acknowledgment

Erasmus+

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BİYOMEDİKAL MÜHENDİSLİĞİ

Thank you for listening