

# Detection and differentiation of normal, cancerous, and metastatic cells using nanoparticle-polymer sensor arrays

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**Rapid and effective differentiation between normal and cancer cells is an important challenge for the diagnosis and treatment of tumors. Here, we describe an array-based system for identification of normal and cancer cells based on a “chemical nose/tongue” approach that exploits subtle changes in the physicochemical nature of different cell surfaces. Their differential interactions with functionalized nanoparticles are transduced through displacement of a multivalent polymer fluorophore that is quenched when bound to the particle and fluorescent after release. Using this sensing strategy we can rapidly (minutes/seconds) and effectively distinguish (i) different cell types; (ii) normal, cancerous and metastatic human breast cells; and (iii) isogenic normal, cancerous and metastatic murine epithelial cell lines.**

fluorescence | gold nanoparticle | sensor | conjugated polymer

Each cell type has unique molecular signatures that distinguish between healthy and diseased tissues (1). In the case of cancers, the distinctions between normal vs. tumor and benign vs. metastatic cells are often subtle. The identification of cellular signatures for early cancer cell detection is a major hurdle for cancer therapy; the earlier these signatures can be established, the more effectively they can be treated (2). Cancerous cells are differentiated from noncancerous ones on the basis of intracellular or extracellular (cell surface) biomarkers. Detection methods based on specific recognition of intracellular biomarkers (e.g., DNA/RNA/Proteins) require previous knowledge of specific mutations in DNA/RNA (3) or changes in the regulation of protein expression inside the cells. Similarly, detection methods based on specific recognition of extracellular (cell surface) biomarkers such as histopathology (4), bioimaging (5), antibody arrays require prior knowledge of biomarkers on cell surfaces. Observation of overexpressed antigens (6) on tumor cells using antibody-based platforms have been explored using ELISA (7), surface plasmon resonance (8, 9), nanoparticles (10–13), microcantilevers (14), carbon nanotubes (15, 16), and expression microarrays (17). Antibody arrays provide an effective but complex approach for cancer detection, diagnosis and prognosis (18), however, there is no single marker or a combination of biomarkers that has sufficient sensitivity and specificity to differentiate between normal, cancerous, and metastatic cell types (19). Here, we describe a detection system that is based on selective noncovalent interactions between cell surface components and nanoparticle-based sensor elements that does not require any previous knowledge of intracellular or extracellular biomarkers.

The cell membrane surface consists primarily of a thin layer of amphipathic phospholipids, carbohydrates and many integral membrane proteins. The amount and types of which differ between species and according to function of cells (20, 21). This results into distinct cell membrane composition in different cell types. Therefore, one can predict, however, that there will be physicochemical (i.e., charge, hydrophobicity etc.) differences

between cell types and between healthy and cancerous cells. Such physicochemical differences could potentially be detected by an array-based “chemical nose” approach that relies on selective interactions between multiple reporter elements and the target cell.

In the chemical nose approach, an array of different sensors is used where every element in the sensor array responds to a number of different chemicals or analytes (22). A distinct pattern of responses produced from a set of sensors in the array provide a fingerprint that allows classification and identification of the analyte (23). The collection of sensors should contain chemical diversity to respond to largest possible cross-section of analytes. The specific interactions involved between the reporter elements and the analyte are noncovalent and reversible. This approach provides an alternative to “lock–key” specific recognition (24) and has been used to detect metal ions (25), volatile agents (26), aromatic amines (27), amino acids (28, 29), and carbohydrates (25). In recent research we have demonstrated that the displacement of fluorescent polymers from differentially functionalized gold nanoparticles with concomitant restoration of fluorescence provides an effective array-based method for the identification of proteins (30). More recently, we have shown that this methodology can be used to differentiate between bacterial species and even between different strains of the same species (31). We report here a particle-polymer array that distinguishes between healthy, cancerous and metastatic human breast cells, and differentiates isogenic healthy and transformed cells.

## Results and Discussion

Our detection system is based on conjugates between 3 structurally related cationic gold nanoparticles (NP1–NP3, Fig. 1A and Fig. S1) and the poly(*para*-phenyleneethynylene) (PPE) polymer PPE-CO<sub>2</sub> featuring charge multivalency (32) and molecular wire properties (33) (Fig. 1A). In these noncovalent conjugates, the nanoparticle quenches the fluorescence of the polymer. The interactions between nanoparticles and anionic polymers are noncovalent, and predominantly electrostatic. When mammalian cells were incubated with these nanoparticle-polymer complexes, there is competitive binding between nanoparticle-polymer complexes and cell types (Fig. 1B). Because of their cationic surface, nanoparticles are expected to interact with phospholipids, membrane proteins and carbohydrates of the cell surface through both electrostatic and hydrophobic interactions.

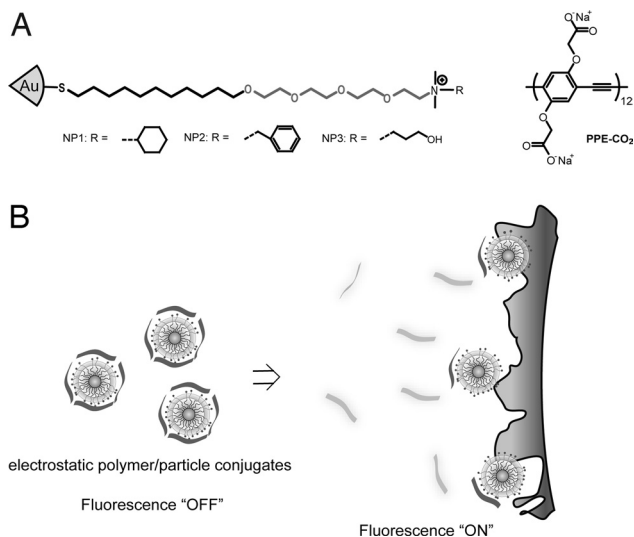
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**Fig. 1.** Molecular structures of nanoparticles and polymers, and schematic of fluorophore displacement cell detection array. (A) Molecular structures of the cationic gold nanoparticles (NP1-NP3) and the fluorescent polymer (PPECO<sub>2</sub>). (B) Displacement of quenched fluorescent polymer (dark green strips, fluorescence off; light green strips, fluorescence on) by cell (in blue) with concomitant restoration of fluorescence.

These interactions are responsible for displacement of the fluorophore polymer from the nanoparticle-polymer complexes generating a fluorescence response. The nanoparticles are expected to possess different affinities for dissimilar cell surfaces depending on cell membrane composition and surface of nanoparticles. Selective displacement of the polymer from the particle by the cell surface regenerates fluorescence, transducing the binding event in a “turn on” fashion.

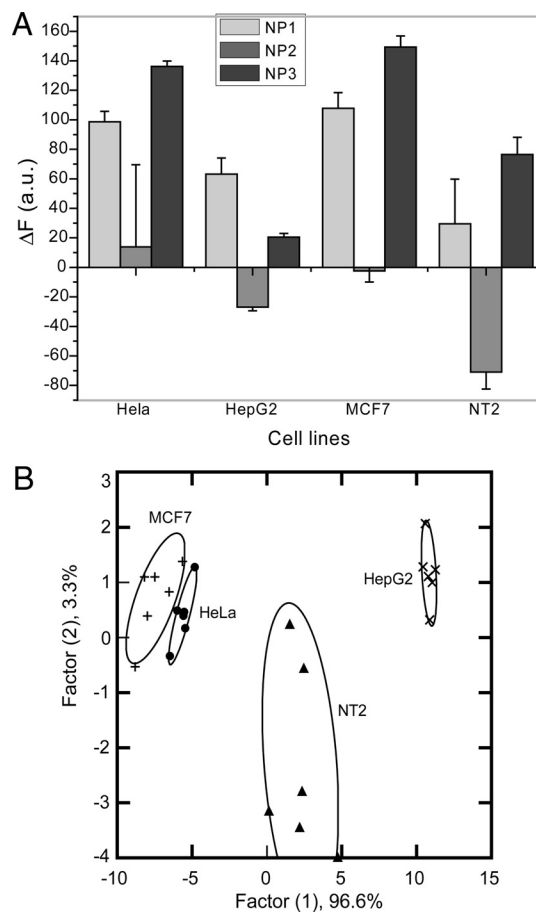
The complex stability constants ( $K_S$ ) and association stoichiometries ( $n$ ) for the nanoparticle-polymer dyads were obtained through nonlinear least-squares curve-fitting analysis (34). Complex stabilities vary within 1 order of magnitude ( $\Delta\Delta G \approx 4.5 \text{ kJ}\cdot\text{mol}^{-1}$ ), and the binding stoichiometry ranges from 2.5 for NP2 to 0.9 for NP3 (Fig. S2). After determining the saturation point for fluorescence quenching (Fig. S3), the appropriate stoichiometries of particle and polymer were mixed in 5 mM phosphate buffer (pH = 7.4) to yield nanoparticle-PPECO<sub>2</sub> complexes with a final concentration of polymer of 100 nM and of nanoparticles 10–40 nM. The complexes of PPECO<sub>2</sub> and NP1-3 were then incubated with different cell types to determine changes in fluorescence intensities. We observed increases and decreases in fluorescence intensities depending on the cell type and the nature of nanoparticle-polymer complexes. Increased fluorescence intensities are due to the displacement of the PPECO<sub>2</sub> polymer from the NP-PPECO<sub>2</sub> complexes by cell surfaces (Fig. 1B), whereas decreases in the fluorescence intensities are due to the quenching of the residual PPECO<sub>2</sub> fluorescence by the cell surfaces. These differences in the fluorescence patterns depend on the cell type and are reproducible. We have performed array-based sensing using 9 gold nanoparticles that possess different head groups and interact differently with polymers (Fig. S3a). We studied their interactions with the different cell types listed in Table 1, focusing on which particle set can best differentiate between different particles. (see below). From studies, we have observed the maximum differentiation grouping using 3 nanoparticles NP1-NP3, as established through jackknifed analysis (Fig. S3b).

**Detection of Differences in Cell Types.** As an initial test of our method we used 4 different types of human cancer cells: HeLa

**Table 1. Origin and nature of the normal, cancerous and metastatic cell lines used in this study.**

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

(Cervical), HepG2 (Liver), NT2 (Testis) and MCF-7 (Breast). Fig. 2A presents the change in the fluorescence response for the nanoparticle-polymer supramolecular complexes upon addition of the different cancer cell types. Linear Discriminant Analysis (LDA) was used to statistically characterize the fluorescence changes. This analysis reduced the size of the training matrix (3 nanoparticles  $\times$  4 cell types  $\times$  6 replicates) and transformed them into canonical factors that are linear combinations of the response patterns (3 factors  $\times$  4 cell types  $\times$  6 replicates). The 2 canonical factors contain 96.6% and 3.3% of the variation,



**Fig. 2.** Detection of human cancerous cell lines. (A) Change in fluorescence intensities ( $F - F_0$ ) for 4 different cancer cell lines HeLa (Cervical), MCF7 (Breast), HepG2 (Liver) and NT2 (Testes) using nanoparticle-polymer supramolecular complexes. Each value is average of 6 parallel measurements. (B) Canonical score plot for the two factors of simplified fluorescence response patterns obtained with NP-PPECO<sub>2</sub> assembly arrays against different mammalian cell types. The canonical scores were calculated by LDA for the identification of 4 cell lines.





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