Beyond Biomarkers: Array-Based Profiling for Diagnostics and High-Throughput Screening

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ABSTRACT

Array-based sensing is a versatile alternative to biomarker-focused strategies for biosensing. This "chemical nose" approach generates signatures that can be used for classifying complex systems for both diagnostic and screening applications. The use of nanoparticle-fluorophore sensing arrays for diagnostic and high content screening will be discussed.

Keywords: nanoparticles, diagnostics, sensors, serum

1 INTRODUCTION

The "chemical nose/tongue" approach presents a potential alternative to specific biomarker approaches. In this strategy a sensor array is generated to provide differential interaction with analytes via *selective* receptors, generating a stimulus response pattern that can be statistically analyzed and used for the identification of individual target analytes and also for profiling of complex mixtures. In our research, we have applied this methodoloy to sensing of proteins and cell surfaces, focusing on areas of biomedical importance.

2 RESULTS AND DISCUSSION

2.1 Serum Sensing

While sensing of proteins in buffer described above provides excellent proof of concept, real-world diagnostics for serum require the ability to address complex mixtures. The sensitivity of array-based sensing to subtle changes in analyte profile generates an excellent platform for sensing complex mixtures. In prior studies that provide the foundation of our proposed research, we developed a fluorophore displacement strategy for sensing of proteins in undiluted human serum using green fluorescent protein (GFP), a strategy that allowed us to identify proteins "spiked" into serum at concentrations of 500 nM in undiluted serum (serum features *~1 mM* total protein concentration) (Figure 1).[1] This sensitivity corresponds to the ability to sense 0.06-5% changes in the five most abundant proteins These studies demonstrate the ability of array-based sensing to discriminate minute changes in serum protein levels, i.e. we have a very sensitive "nose" .

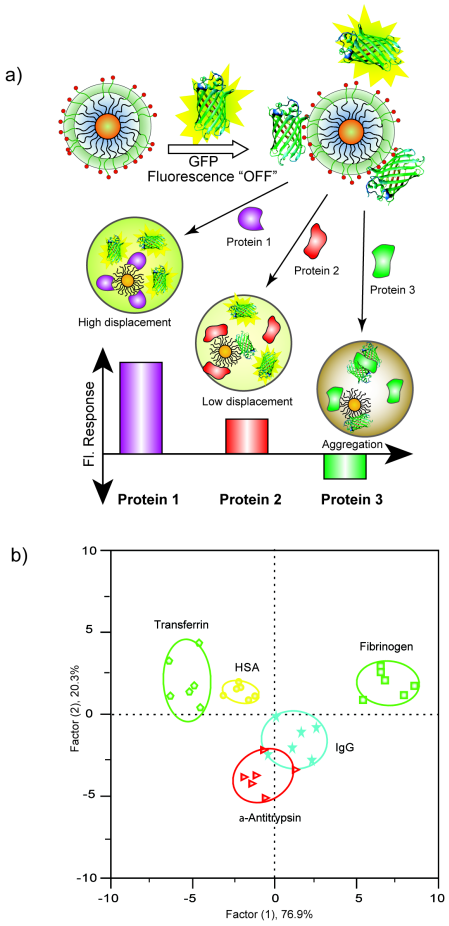

Figure 1. a) Competitive binding between protein and quenched GFP-NP complexes and protein aggregation leading to sensor response. b) Fluorescence responses for five most abundant serum proteins (500 nM) spiked into undiluted human serum c) Canonical score plot for the fluorescence patterns of the GFP-NP adducts.

2.2 Cell Surface Sensing

Our initial studies in cell surface sensing focused on differentiation of cancer cell genotypes using chemical nose sensors (Figure 7).[2] In these studies we used an array of nanoparticle-fluorescent polymer sensors. The differential interactions of the functionalized nanoparticles with the different cell genotypes was 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 could rapidly (minutes/seconds) and effectively distinguish: 1) different cell types; 2) normal, cancerous and metastatic human breast cells; 3) *isogenic* normal, cancerous and metastatic murine epithelial cell lines. (Figure 2 c,d).

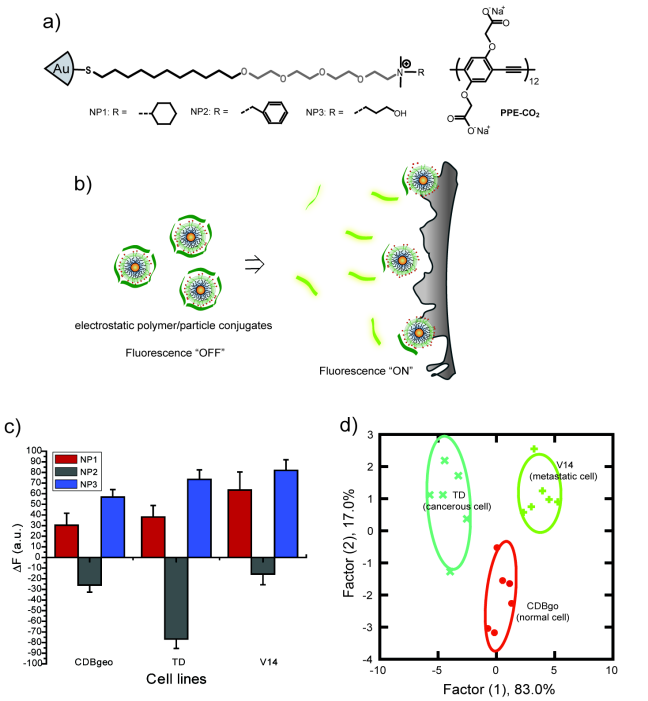

Figure 2. Array-based sensing of cells. a) polymer and particles used for sensing. b) The sensing process c) Fluorescence output of sensor array using three isogenic breast cell lines derived from BALB/c mouse. d) LDA plot for the fluorescence response patterns.

Multi-channel sensing and cell-surface phenotyping are key enabling technologies for our research. We recently introduced a simple and powerful high-throughput multi-channel sensor platform comprised of supramolecular complexes of a gold nanoparticle with three fluorescent proteins. (Figure 3a).[3]

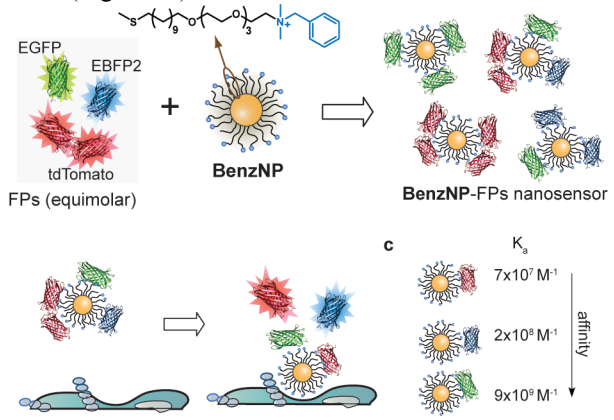

Figure 3. Working principle of the three-channel multiplexed sensor.

This sensor is engineered for selective cell surface recognition, allowing direct measurement using cell culture plates, (Figure 4a). We tested this sensor system using anti-cancer drugs. Our studies demonstrated that the sensor system responds reproducibly to physicochemical changes at the cell surface induced by these drugs, generating characteristic patterns that identify specific mechanisms of cell death (Figure 4b). This capability overcomes hurdles in developing chemotherapeutics, providing phenotypic profiling of drug mechanisms as a strategy for greatly increasing throughput in the drug discovery process. Notably, this whole-cell based high-throughput technique uses a *single well* of a microplate for drug treatment and analysis, with rapid (minutes) determination of chemotherapeutic mechanism.

In our study we used the sensor to:

1) Identify the mechanism of blinded drugs with high (90%) accuracy.
2) Differentiate between "new" mechanisms and ones in the training set using statistical methods.
3) Determine when synergistic combination therapies proceeded through the mechanism of one of the components or through a new mechanism.

The sensor platform is generalizable to any other type and does not require processing steps such as extracting biomarker or labeling cells prior to analysis.

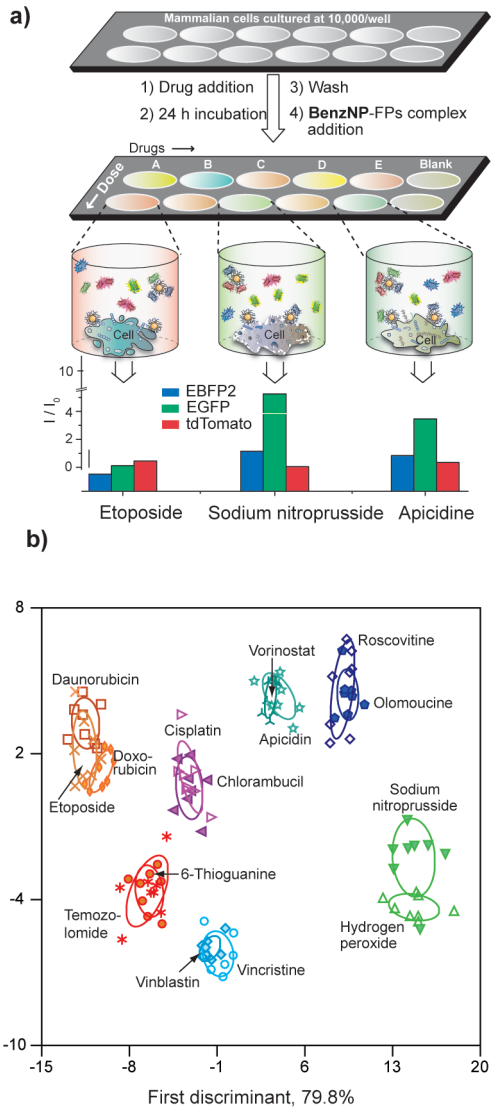

Figure 4. a) Drug screening workflow. The fluorescence outputs corresponding to the three drug candidates are actual experimental results. b) Linear discriminant analysis of the fluorescence responses plotted with 95% confidence level ellipses around the centroid of each group.

References

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