# OPTICALLY ENCODED PARTICLES AND FLOW CYTOMETRY AS A HIGH-THROUGHPUT PLATFORM FOR THE ANALYSIS OF PROTEIN RESISTANT SURFACES, PROTEASE EFFICACY AND BIOMARKER DETECTION

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The assessment of physiological conditions and events at the molecular level is increasingly seen as the future of medical diagnosis for the early detection and more effective treatment of diseases.[1] The development of molecular diagnostics based upon mixed (multiplexed) suspensions of distinguishable particles gives rise to the posibility of creating libraries of chemical and biological moities which can be anaylised simultaneously and competitively in a high-throughput format not prevoiusly possible.[2] In this study, fluorescently doped, optically encoded organosilica particles have been syntheised and surface functionalised for the multiplexed analysis of - i) protein resistant surfaces,[3, 4] ii) the enzymatic degredation of a focused random peptide library[5] and, iii) the detection of genetic[6] and proteomic biomarkers. Organosilica particles offer the advantages of chemical stability under a range of conditions and of being capable of acting as both a synthetic support for the organic synthesis of polymers, peptides and DNA along as well as serving as a high-throughput biological assay platform, Figure 1. Flow cytometric analysis of particle based assays offers the advantage of interogating multiple parameters on an individual particle basis using small volumes. This enables flow cytometry to distinguish between the individual encoded particles whilst monitoring biologicaly mediated changes to those particles.

In this study, the high-throughput multiplexed and multicomponent screening of protein resistive surfaces was examined by the adsorption of three proteins: bovine serum albumin (BSA), bovine immunoglobulin gamma (IgG) and fibrinogen, onto five encoded and surface modified particle populations. By uniquely labeling each protein and particle polulation with spectrally distinguishable fluorescent dyes, the adsorption process was ‘multiplexed’ allowing for simultaneous screening of multiple adsorbent (particle surface) and adsorbate (protein) interactions, Figure 2. This in turn enabled the characterisation of both competitve and cooperative protein adsorption, along with assessing the relative adsorption affinities of different modified surfaces. The combitorial, small volume analysis of this technique allowed for not only the rapid examination of a number of surface modificatons, but also for the inexpensive analysis of otherwise costly or exotic proteins or surfaces.

The enzymatic activity of the closely related NS3 proteases of West Nile and Dengue viruses were screened against a small, focused random peptide library immobilized on 14 optically distinguishable particle populations. Characterisation of the peptide substrate specificity of these and all viral proteases has been seen as a key step in the design of inhibitors and strategies toward their successful treatment. WNV and Den proteases both displayed fast kinetic activity and a narrow substrate specificity toward basic amino acids. It was observed that WNV preferentially cleaved the substrate containing lysine whereas Den favoured the substrate with arginine. The assay platform presented here is easily scalable and consequently has a broad potential application in the mapping of any biological site-specific peptide interactions.

Encoded multiplexed suspensions of chemical and biological libraries offer a unique high-throughput analysis platform with advantages not rivaled by other more common analysis techniques. It offers great potential in the screening and detection of biologically significant

conditions and events, specifically for the early diagnosis of diseases. However, these platforms and methodologies can be easily transferred to other systems or disciplines where the interaction of moieties of interest against libraries of complementary moieties is required.

**References:**

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**Figures:**

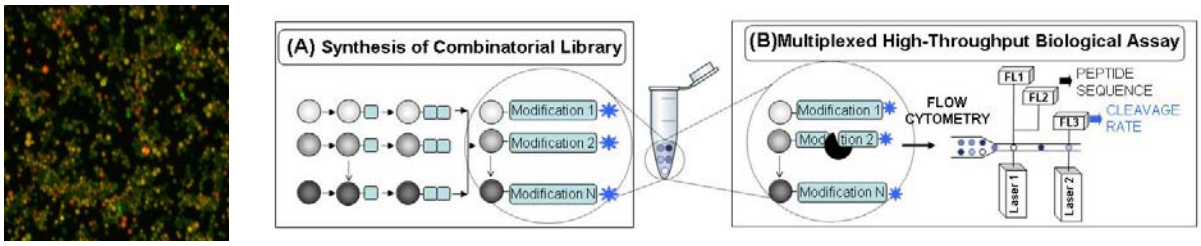


**Figure 1**: Dual purpose optically encoded organosilica particles for the organic synthesis of chemical and biological libraries for multiplexed high-throughput biological assay analysis

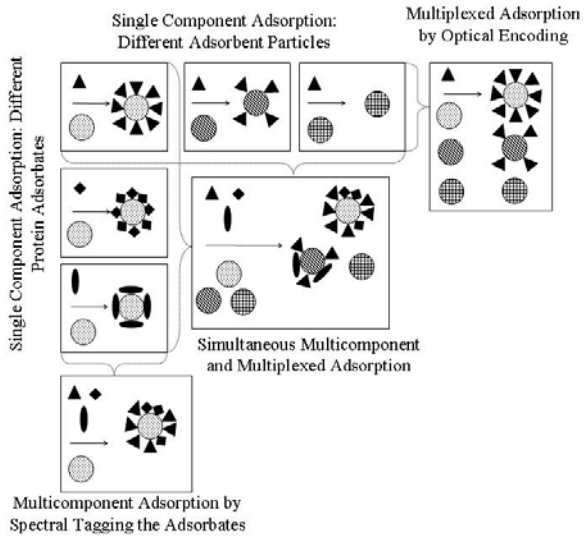


**Figure 2:** Flow cytometry analysis of multicomponent and multiplexed adsorption of proteins (▲), (♦) and (ו) onto surface modified adsorbent particles (O) in a high-throughput simultaneous and competitive manner.