

## FUNCTIONALIZED SURFACES THAT LIMIT NON-SPECIFIC PROTEIN BINDING FOR BIOASSAY AND ARRAY PLATFORMS

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**INTRODUCTION:** Long-desired improvements to immunodiagnostic, protein micro array, and various biosensor device platforms require careful control of synthetic material/biomolecule interactions at surfaces. Frequently, current assay and array designs require tethering a biomolecule or signal transduction element to a surface in a manner that optimises surface density for signal production while maintaining bioactivity. Simultaneously, inhibition of non-specific biological adsorption is desired in order to minimize background noise. Optimal surface chemistry for bioanalytical applications thus provides a means for immobilizing specific biomolecules while minimizing non-specific binding, often including control over spatial patterning and repeat assay/surface renewal. This presentation will describe two different newly developed polymeric coatings formulated to provide these features and their properties under bioassay conditions. Two graft copolymers, a copolysiloxane and a terpolysiloxane, have been synthesized by grafting with dialkyl disulfide chains and methoxy-terminated poly(ethylene glycol) (PEG) chains. The terpolysiloxane also has grafted PEG sidechains terminated with the reactive ester, N-hydroxysuccinimide (NHS). These copolymers have been attached to gold surface plasmon resonance (SPR) assay surfaces, analysed using surface analysis and assessed for protein binding in both specific and non-specific modes in the SPR biosensing configuration.

A new commercial bioassay polymer coating, OptiChem™,\* was also tested for non-specific and specific binding on a variety of polymer, glass and metal substrates. This crosslinked organic polymer thin film surface, while proprietary, is derivatized with controlled amounts of either biotin or reactive vinyl sulfone ligands capable of bioimmobilization at high densities. Analysis using colorimetric and particle-based detection schemes both demonstrate the combination of high specific antibody and oligo-DNA capture in sandwich assay format, with low non-specific protein uptake from serum.

**RESULTS & DISCUSSION:** Both grafted polysiloxanes spontaneously form monolayers on gold surfaces with estimated thicknesses of 23Å and 31Å, respectively. Combined analysis with

angle-dependent X-ray photoelectron spectroscopy (XPS) and static time-of-flight secondary ion mass spectrometry (ToF-SIMS) support direct attachment of dialkyl disulfide sidechains to the gold surface, while also concentrating the grafted PEG chains at the outer surface. ToF-SIMS provided evidence for NHS group surface exposure as well. Single protein adsorption onto both polymer monolayers examined with SPR was below the method's detection limit (~1 ng/cm<sup>2</sup>). Under chemical coupling conditions, the NHS-grafted terpolymer monolayer immobilized significant amounts of antibody, reflecting NHS reactivity.

The OptiChem™ polymer surface is coatable from solvent, crosslinking into stable transparent <100nm-thick films independent of substrate chemistry. Streptavidin, antibody and serum protein adsorption is minimal (<5 ng/cm<sup>2</sup>) on "inert" (no ligand) OptiChem™ controls coated on thermoplastic, metal or metal oxide supports. Specific streptavidin binding on biotinylated OptiChem™ surfaces can be varied with biotin load, and actively promotes further biotinylated-antibody binding in the typical sandwich format. Biotinylated DNA probes also bind at high density.

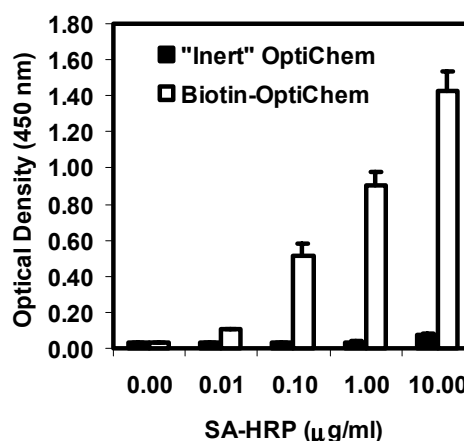


Figure: Colorimetric assay of streptavidin-HRP binding to biotinylated and inert OptiChem™ coatings on tissue culture polystyrene substrates. (n = 6, error bars +/- standard deviation in the set).

\*OptiChem™ surfaces are owned and licensed by Accelr8 Technology Corp. ([www.accelr8.com](http://www.accelr8.com))