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Antifouling Surface Layers for Improved Signal-to-Noise of Particle-Based Immunoassays

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Abstract

A ten fold improvement in the signal-to-noise (S/N) ratio of an optically encoded silica particle-based immunoassay was achieved through incorporating a protein resistant poly(ethylene glycol) (PEG) surface layer and optimizing antibody immobilization conditions. PEG was activated using 2,2,2-trifluoroethanesulfonyl chloride (tresyl) and required a minimum reaction time of 1.5 hrs. The activated PEG had a reactive half life of approximately 5 hrs when stored in acidified dimethyl sulfoxide (DMSO). By increasing the protein incubation time and concentration, a maximum antibody loading on the particle surface of 1.6×10^{-2} molecules per nm^2 was achieved. The assay S/N ratio was assessed using a multiplexed multicomponent optically encoded species-specific immunoassay. Encoded particles were covalently grafted or nonspecifically coated with either bovine or mouse IgG for the simultaneous detection of complimentary anti-IgG 'target' or uncomplimentary anti-IgG 'noise'. The versatility and potential as a serum-based assay platform was demonstrated by immobilizing either a polyclonal antibody or an engineered single-chain variable fragment (scFv) capture probe on particles for the detection of the ovarian cancer biomarker, mesothelin (MSLN). The MSLN antigen was spiked into PBS buffer or 50% human serum. Both capture probe orientations and media conditions showed similar low level detection limits of 5 ng/mL; however, a 40% decrease in maximum signal intensity was observed for assays run in 50% serum.

Keywords

Immunoassay; molecular diagnostic; signal-to-noise; cancer biomarker; scFv; bead-based assay

Introduction

Immunoassays use the specific interactions of antibodies and antigens for the discovery, detection and monitoring of biological processes which express a distinctive proteomic "biomarker" signature. They are increasingly important as medical and research tools and are commonly employed in the detection of pregnancy, diabetes¹ and HIV,² to mention only a few current applications. Of key interest, however, is the development of immunoassays for the early detection of cancer. Early diagnosis and treatment of cancer is believed to be the most

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promising means for improving patient quality of life and survival rates.^{3, 4} Early stage (pre-metastasis) treatment for a variety of cancers (melanoma, colon, cervical and ovarian cancers) is associated with better patient 5 year survival rates, a difference of less than 25% to greater than 90%.⁵ To date a number of cancer biomarkers have been approved by the Food and Drug Administration (FDA) for diagnostic and/or disease monitoring tests. For example, CA125^{6, 7} and Human Epididymis Protein 4 (HE4) are currently approved for monitoring for recurrences of ovarian cancer. Although not yet validated for ovarian cancer, other potential biomarkers such as mesothelin (MSLN) and secretory leukocyte peptidase inhibitor (SLPI) have been identified which may offer improved or stage dependent ovarian cancer detection.^{8–12} Unfortunately, all currently available biomarker immunoassays are plagued by issues regarding their diagnostic confidence and throughput speed, which has limited their wider use.

One of the most commonly employed proteomic molecular assays used in research and health care is double-determinant immunoassays, such as enzyme-linked immunosorbent assays (ELISA). Since their introduction over forty years ago, ELISA assays performed in 96-well plates have not changed significantly and still have a number of intrinsic platform limitations.¹³ They are typically considered to be slow, laborious and require large sample volumes due to the platform's single analyte per well analysis. Immunoassays are also often troubled by low signal-to-noise (S/N) ratios which gives rise to poor assay confidence. This can arise from the random orientation of nonspecifically immobilized capture antibody inhibiting the specific binding of target biomarkers, and the high background noise from the nonspecific adsorption of both target and non-target molecules onto the assay platform. Therefore, appreciable effort has been given to modifying and creating assay platforms with increased assay confidence and sample throughput rate.

Encoded particles have recently generated considerable interest for use in an assay platform as they provide a number of advantages over plate and 2-D chip based assay platforms. Particles boast better mixing within biological media, have a high surface area to volume ratio, and offer the possibility of large numbers of particles in a small volume. These properties can help to improve the assay kinetics and statistical accuracy along with reducing the required sample volumes, as limited patient samples are often collected for disease diagnosis in the clinical setting. Encoding the particles allows the production of large multiplexed libraries of distinguishable particle populations, enabling the simultaneous analysis of multiple biomarker analytes in a high throughput fashion (>3000 particles per second) via flow cytometry.¹⁴ These encoded particle assays have been used previously as genomic¹⁵ and immunoassays along with protease substrate mapping of trypsin¹⁶ and the NS3Pro viral proteases of West Nile and Dengue viruses.¹⁷

Improving assay confidence is crucial as it can reduce undue medical procedures and patient stresses associated with false-positive or misdiagnosis. Significant improvement to assay confidence can be achieved by increasing the assay S/N ratio, however, this is difficult due to the complexity of the detection conditions.¹⁸ Immunoassays are expected to selectively and definitively detect very low biomarker concentrations, down to the attomolar level, within complex media such as sera. However, due to the presence of high concentrations of non-target proteins in sera, the nonspecific adsorption of these non-target molecules decrease the assay signal whilst increasing the background noise, thereby reducing assay confidence. Assay S/N ratios can be improved by increasing the amount and orientation of the immobilized capture antibodies as well as incorporating a protein resistant, anti-fouling coating on the assay surface.¹⁹

Currently, grafted poly(ethylene) glycol (PEG) surface layers are considered to be one of the most effective means of inhibiting nonspecific protein adsorption.^{20, 21} We have recently reported on the use of 2,2,4-tresyl chloride (tresyl) for the activation and covalent grafting of

five different MWs of PEG on the surface of organosilica particles to create protein resistant surfaces.²² Tresyl has been used previously for the covalent grafting of linear and star PEGs on amine modified silica surfaces²³ and for the immobilization of enzymes to chromatography columns.²⁴ Tresyl chloride reacts with hydroxyl groups, such as the terminal alcohol groups of PEG, to generate a reactive intermediate group which forms a covalent alkyl bond when reacted with a nucleophilic, e.g. amino- or sulfhydryl- containing ligand. The tresyl intermediate has the advantage of being highly reactive in a variety of solvents and conditions, thus giving rise to the ability to tailor the conditions of PEG and subsequent protein immobilization on a surface.

Herein we present the optimization of tresyl activation and antibody immobilization conditions on low fouling PEG modified encoded organosilica particles as a high throughput immunoassay. Activation of surface grafted PEG and subsequent Immunoglobulin gamma (IgG) incubation time and concentration were optimized to give the greatest IgG loading conditions. The increase in the assay S/N ratio due to the incorporation of the PEG layer was examined using a model, species specific (bovine or mouse) IgG assay. High throughput 'multiplexed' assay analysis was achieved by encoding the particles and using flow cytometric readout.^{14, 17} This developed immunoassay platform was then applied to a model spiked antigen assay in both PBS and human serum for the detection of the ovarian cancer biomarker MSLN.²⁵

Materials and Methods

Particle synthesis, surface modification and activation

4.60 μm organosilica particles were synthesized using 3-mercaptopropyl trimethoxysilane (MPS), purchased from Lancaster (UK), according to the protocol outlined in Miller *et al.*²⁶ Particles were optically encoded through the covalent incorporation of increasing concentrations of maleimide functionalized Atto-Tec 620 fluorescent dye. The particle surfaces were then subsequently amine modified by reacting with 3-aminopropyl trimethoxysilane (APS) and triethylamine (TEA) in ethanol for 2 hrs under constant agitation.

The alcohol groups at both terminal ends of the 3,400 M_W PEG were reacted with 2,2,2-trifluoroethanesulfonyl chloride (tresyl) and purified according to the protocol outlined in Sofia *et al.*²³, aiming to react with the nucleophilic amine modified organosilica particle surfaces for polymer grafting, and with biomolecule amine groups for their covalent immobilization. Tresyl-activated PEG was grafted onto the organosilica particle surfaces at a grafting concentration of 200 mg/mL, using anhydrous DMSO as the solvent. One mL of the PEG solution was added to 50 mg of particles, followed by 5 μL of TEA. The samples were reacted overnight under constant agitation at 4 °C. Ungrafted polymer was removed by washing twice in anhydrous DMSO and the free terminal alcohol group of the grafted PEG was regenerated by washing twice in water for 30 minutes. The grafted PEG was then reactivated for 90 minutes (unless otherwise stated) using equal molar amounts of tresyl chloride and TEA in anhydrous DMSO. These activated particles were washed twice in DMSO and if not used immediately were stored in acidified DMSO (0.154M HCl) at 4 °C. Otherwise the particles were washed three times with acidified water (0.154M HCl) and used immediately for antibody immobilization.

Antibody materials and immobilization

Bovine IgG, rabbit anti-bovine IgG, mouse IgG and goat anti-mouse IgG were purchased from Sigma Aldrich as lyophilized powders and resuspended in PBS buffer (pH 7.4). Anti-bovine and anti-mouse IgGs were fluorescently labeled with Atto-Tec 350 and Fluorescein Isothiocyanate (FITC) dyes, respectively, and purified by dialysis against PBS. The degree of

fluorophore tagging was determined spectroscopically, using a NanoDrop UV-Vis spectrophotometer. It was calculated that anti-bovine and anti-mouse IgG contained an average of 2.3 and 1.7 dye molecules per protein molecule, respectively. MSLN antigen (40 kDa) was produced by methods previously described in Scholler *et al.*²⁵ Recombinant anti-mesothelin single-chain variable fragment (scFv, 27 kDa) which was previously described in Bergan *et al.*²⁷ was cloned into a novel vector p416-BCCP²⁸ that permits the secretion of scFv in yeast supernatant and adds a linker containing 3 lysines in the N-terminal of the soluble scFv. The MSLN polyclonal antibody (pAb) was purchased from R&D systems (USA).

Bovine and mouse IgGs were immobilized separately onto four optically encoded particle populations (P1–P4); two with tresyl-activated PEG (P1 & P2) and two were unPEGylated organosilica particles (P3 & P4). Unless otherwise stated, the standard immobilization conditions onto PEGylated particles were as follows: 5 million of the tresyl-activated particles dispersed in acidified water were incubated with 250 μ L of a 100 μ g/mL solution of IgG, MSLN pAb or scFv in PBS for 24 hrs. The volume of the activated PEG particles added to the protein solution was kept to a minimum (10 μ L of a 500 million particles per mL suspension was added to 250 μ L of capture probe in PBS) to ensure that pH and solvency conditions during immobilization would not alter or denature the protein of interest. Nonspecific IgG immobilization on P3 and P4 was achieved by mixing a 10 μ L suspension of ~500 million particles per mL with 250 μ L of a 100 μ g/mL bovine or mouse IgG solution and incubating overnight.

IgG immunoassays

Multiplexed immunoassays were conducted on a mixed suspension of 10,000 particles comprised of equal amounts of the four populations, in a total volume of 250 μ L PBS. Equal yet increasing concentrations (1 – 30 μ g/mL) of fluoro-tagged anti-bovine and anti-mouse were incubated with the multiplexed suspensions for two hours prior to analysis. The assay S/N ratio was calculated by dividing the maximum amount of captured complementary anti-IgG target by the maximum amount of nonspecifically adsorbed uncomplimentary anti-IgG non-target. Improvement in the S/N ratio due to immobilization methodology was determined by dividing the S/N ratios of the two techniques: covalently immobilized onto PEGylated particles (P1 & P2) versus nonspecifically immobilized on particles (P3 & P4).

Model ovarian cancer biomarker immunoassays

For each assay, 5000 particles coated with either MSLN pAb or scFv were incubated in 250 μ L of PBS buffer or 50% PBS buffer and 50% human serum with increasing concentrations of MSLN antigen for one hour at room temperature. The antigen captured particles were then washed in PBS buffer and incubated with either a FITC labeled version of the MSLN pAb or scFv at 5 μ g/mL for 30 minutes.

All immunoassays were characterized by flow cytometry using a Dako Cytomation MoFlo with three laser excitation lines and 6 fluorescence detectors; a Coherent Innova 90C Argon ion laser (emission: 366 nm, power: 50 mW) with corresponding 450 $\pm$ 32 nm and 530 $\pm$ 20 nm detectors, an iCyt Visionary Bioscience Inc. (emission: 488 nm, power: 100 mW) with corresponding 530 $\pm$ 20 nm and 580 $\pm$ 10 nm detectors, and a 635 nm diode laser at 12 mW power with corresponding 670 $\pm$ 15 nm and 710 $\pm$ 10 nm detectors, and forward and side scatter detectors. Fluorescent histograms were composed of at least a thousand particles and were analyzed using Summit V4.1 software. The mean fluorescence intensity signal generated by flow cytometry was quantified according to the calibration method outlined in Kozak *et al.*²⁹

Results and Discussion

Optimization of Antibody Immobilization onto Particles

Maximizing the assay capture probe loading whilst minimizing nonspecific biomolecule adsorption is typically considered the easiest and most efficient method to increase assay signal and confidence. Therefore, this study has focused on optimizing the activation and subsequent immobilization of capture of IgGs onto a low fouling PEGylated particle-based assay support, using tresyl chloride as a rapid activation intermediate. Previous studies have shown that tresyl chloride undergoes a more rapid and efficient coupling reaction than other activation methods such as the use of tosyl chloride or EDC.^{30, 31} In this study, tresyl chloride was used to activate the terminal hydroxyl groups of 3,400 M_w PEG for grafting and covalently immobilizing biomolecules, such as proteins, onto the organosilica particles. The effects of tresyl chloride reaction time, activated tresyl-PEG lifetime, IgG immobilization time and concentration on assay loading were investigated. IgG loading was assessed by the amount of fluorescent IgG immobilized onto the particles using flow cytometry.

Using similar PEG grafting conditions we previously demonstrated that greater than 1 mg/m² of tresyl activated 3,400 M_w PEG could be grafted onto the particles. This grafted amount corresponds to more than 0.17 PEG molecules per nm². At these grafting densities, the distance between PEG molecules (~1.3 nm) is less than the radius of gyration (2–4 nm)³² of the polymer forcing the polymer chains to overlap. Under these conditions it becomes favorable for the PEG chains to extend and form a polymer 'brush' on the surface. Our previous study showed that such PEG brushes form a good anti-fouling surface, reducing nonspecific IgG and bovine serum albumin adsorption to lower than the flow cytometric detection limit, being below 0.02 mg/m².²² Consequently, this low fouling PEG surface will reduce assay background noise while providing a flexible, functionalized linker suitable for covalent antibody immobilization.

Tresyl activation of the free terminal hydroxyl groups of the surface grafted PEG as a function of tresyl reaction time exhibited an initial lag time of 15 min, as shown in Figure 1.A. Before 15 min minimal PEG was activated for IgG immobilization. This lag is likely due to tresyl chloride reaction kinetics with the hydroxyl groups of PEG and possible tresyl reaction with trace water present in the reaction. After 15 min, PEG activation rapidly increased and reached a maximum IgG immobilization of 1.6×10^{-2} molecules per nm² at 1.5 hrs. A similar 1.5 hr time period for PEG activation had been suggested in the protocol outlined by Sofia *et al.*²³

The longevity of the tresyl reactive PEG intermediate was examined as a function of storage in acidified DMSO (0.154M HCl) at 4 °C, as shown in Figure 1.B. Cold acidified storage conditions were chosen as they are expected to increase the longevity of the tresyl-activated PEG intermediate.³³ The tresyl reactivity of particles stored in acidified DMSO at 4 °C was stable for the first 0.5 hr, immobilizing 1.6×10^{-2} molecules of IgG per nm². This IgG loading equated to an IgG for every 10 PEG molecules on the particle surface. Beyond 0.5 hr storage time, the activity of the tresyl-PEG began to decrease. At 24 hrs of storage, the amount of IgG immobilized decreased by 75% to 4.0×10^{-3} molecules per nm². Thus, storage in acidic DMSO beyond 24 hrs did not effectively prevent tresyl-activated PEG from undergoing side reactions such as hydrolysis as was expected. The greater than zero immobilized amount of IgG beyond 24 hrs is most likely due to nonspecific IgG adsorption onto the particles, rather than the continued covalent immobilization via the tresyl activated PEG linker. This is supported by finding that after 20 hours of IgG incubation 0.003 IgG molecules / nm² could be attributed to nonspecific adsorption onto PEGylated particles which had not been tresyl activated (Figure 1.C). It has been widely shown that although highly effective over short time periods (i.e., hrs), the effectiveness of low fouling PEG brushes diminish with increasing contact time (i.e., days) with a biological solution.²⁴

The amount of IgG immobilized onto the activated particles was observed to rapidly increase in the initial 5 hrs of incubation reaching 1.2×10^{-2} molecules per nm^2 (Figure 1.C). Incubation beyond 5 hrs showed a slower immobilization rate with greater than 20 hrs required to reach the maximum IgG loading of 1.6×10^{-2} molecules per nm^2 . This reduction in the immobilization rate is believed to be due to the deactivation of the trysel-PEG by hydrolysis and steric effects from the increased IgG packing density at the surface. Interestingly the decreased rate of immobilization at 5 hrs also corresponded to an increase in nonspecifically adsorbed IgG on the surface which may also explain the observed change in IgG immobilization rate.

As expected, increasing the concentration of the IgG solution, from 0.0 to 50 $\mu\text{g}/\text{mL}$, increased the amount of IgG immobilized on the particles, as shown in Figure 1.D. IgG immobilization exhibited characteristics similar to a Type I isotherm, as would be expected for, monolayer limited, attachment to an activated surface. IgG immobilization saturated the particle surface at 10 $\mu\text{g}/\text{mL}$ with greater concentrations giving no increase in IgG loading. At saturation, a maximum IgG probe loading of 4.2 mg per gram of particles was achieved, which equates to 1.6×10^{-2} IgG molecules per nm^2 based upon a particle surface area of $1 \text{ m}^2/\text{g}$ and IgG molecular weight of 150 kDa. This equates to a mixed orientation of the attached IgG on the PEGylated particles assuming the hydrodynamic dimensions of IgG are $8.5 \times 14.5 \times 4 \text{ nm}$. A monolayer composed entirely of end on vertical attachment or all laying flat on the surface is expected to give rise to 3.1×10^{-2} or 0.8×10^{-2} molecules per nm^2 , respectively.³⁴ As most IgG molecules have similar physical properties regardless of their function or species of origin, the IgG loading was found to be similar, as expected, for both the bovine and mouse IgGs.

Signal-to-Noise Ratio of a Multiplexed Particle Based Immunoassay

Assay S/N ratio was examined by quantifying the amount of fluorescently tagged 'target' anti-bovine to the amount of 'non-target' anti-mouse IgG captured. This was achieved in a high throughput simultaneous fashion as a multiplexed multicomponent assay consisting of four particle populations two without PEG layers modified with non-specifically adsorbed bovine IgG or mouse IgG and two PEGylated particle populations with bovine IgG or mouse IgG covalently attached to the PEG layer. These were incubated with anti-bovine and anti-mouse IgGs tagged with distinguishable fluorophores. The S/N ratio was calculated from the amount of anti-IgG complementary 'target' to uncomplimentary 'noise' captured by the respective IgG coated particles. By these means the generated S/N ratio is the result of a competitive reaction between multiple analytes, as would be expected for assay conditions within a biological sample.

Figure 2 shows the assay S/N ratio for the bovine IgG coated particles with increasing concentrations of anti-bovine IgG 'target' and anti-mouse IgG 'non-target'. Typically, an assay S/N ratio of ≥ 10 is considered to demonstrate a successful immunoreaction with very low background non-target binding noise.³⁵ One of the most commonly applied methods for achieving this is to coat the assay surface with a surface blocking agent or an inert polymer such as PEG to prevent the non-specific binding of non-target proteins. IgG immobilized to the PEG modified particles gave rise to a S/N ratio of 30.7, capturing approximately 0.014 molecules / nm^2 of the target anti-IgG and a near negligible amount (4.7×10^{-4} molecules / nm^2) of the non-target anti-IgG. In contrast, unmodified 'high' fouling particles with non-specifically immobilized antibodies had a lower target signal and a significantly higher non-target signal, being of 0.013 and 0.004 molecules / nm^2 , respectively, giving rise to a S/N ratio of 3.1. This corresponds to an approximately ten fold increase in the S/N ratio due to IgG probe immobilization on the low fouling PEG surface layers compared to native silica. Similar S/N results were observed with the mouse IgG coated particles (figure not shown).

Model Ovarian Cancer Biomarker Immunoassay

Immunoassays are seen as a vital tool for the early and accurate detection of diseases such as cancers.⁴ Increasingly, immunoassay development is increasingly taking advantage of recombinant protein synthesis technology along with immunogenetic and naïve non-immune responsive libraries to isolate and create highly specific antibodies and antibody fragments.^{36–39} Generating considerable interest are single-chain variable fragments (scFvs) for antigen capture and display.^{40–42} ScFvs have the advantages of reduced size as compared to whole antibodies (~27 kDa and ~150 kDa, respectively) representing the smallest functional domain with no significant loss in antigen affinity or specificity,⁴³ and they can be genetically engineered to include other desired peptide domains.⁴¹

To illustrate the versatility and diagnostic potential of the developed PEGylated particle technology, either MSLN pAbs or engineered tri-lysine tagged scFvs were covalently immobilized onto particles for the detection of the ovarian cancer biomarker MSLN. Mesothelin (MSLN, 40 kDa) is a promising biomarker candidate for early stage ovarian cancer detection due to its immunogenicity, over expression and presence in sera.^{11, 12} As a proof-of-concept validation of the particle-based immunoassay platform, low level (1 to 100 ng/mL) detection of MSLN from spiked PBS buffer and human serum were examined. The assay was conducted in two formats, using the pAb and scFv as either the capture or display molecules. ScFv coated capture particles provide the advantage of quantifying the number of full antibody display molecules using the flow cytometric calibration method outlined in Kozak *et al.*²⁹

As shown in Figure 3.A and 3.B, the detection limits of antigen spiked in PBS buffer and 50% human serum for both assay orientations were similar, being 5 ng/mL. At this antigen concentration, the assay signals were 68 and 75 mean fluorescent intensities (MFI) for the pAb capture orientation, and 2.5×10^{-3} molecules per nm^2 and 3.0×10^{-3} molecules per nm^2 for the scFv capture orientation, in PBS buffer and 50% human serum, respectively. In comparison, the signal of the nonspecific background noise generated by the display molecules were approximately half, being 32 MFI and 8×10^{-4} molecules per nm^2 , respectively. The maximum assay signals (antigen concentrations >100 ng/mL) were of similar values to the expected maximum assay signal as determined by the non-specific adsorption of display antibody onto unmodified APS particles. This indicates that the assay display signals were close to those exhibited when the surface is saturated. The maximum assay signals, however, were observed to decrease by up to 40% when the assay was performed in 50% human serum. This reduction in signal is most likely due to protein fouling of the assay surface blocking the number of available capture probes. However, this signal reduction can also occur due to the formation of protein complexes between the antigen of interest and other biomolecules within the sera. MSLN has been shown to form a complex with CA125, both of which are naturally found in all sera at low concentrations.²⁷ These complexes can affect assay signal by preventing antigen capture or display.

Conclusions

A ten fold increase in the signal-to-noise ratio was achieved through use of a protein resistant poly(ethylene glycol) (PEG) surface layer as well as through optimization of the antibody binding concentration. The terminal alcohol groups of PEG grafted, low fouling, organosilica particles were activated with tresyl chloride for subsequent antibody immobilization to create a multiplexed high throughput immunoassay with an improved S/N ratio. By increasing the tresyl reaction time, IgG immobilization concentration as well as incubation time, the amount of IgG immobilized on the particles was maximized. A maximum antibody loading of up to 1.7×10^{-2} molecules of IgG per nm^2 was achieved. A decrease in background noise and increase in signal was achieved through the use of the optimized protein antifouling polymer layer. The potential application of the technology as a disease diagnosis tool was demonstrated by

performing a model proteomic ovarian cancer biomarker assay using both antibody and scFv as antigen capture in both PBS buffer and 50% human serum.

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Figure 1. A, B, C & D. Optimization of assay loading via tresyl mediated immobilization of IgG
 A) Tresyl-activation of PEG with time and subsequent degree of immobilization of IgG onto the particle surface. B) Longevity of the activated PEG particles in acidified DMSO C) Incubation time of IgG with tresyl-activated PEG particles (▲) and non-specific adsorption onto non-activated PEG particles (■). D) Bovine (□) and Mouse (▲) IgG immobilization with increasing incubation concentrations. All curves are the combination of two independent experimental setups and errors represent the calculated experimental standard deviation of each point.

Figure 2. Signal-to-Noise Ratio of Multiplexed Antibody-anti-Antibody Immunoassay
 Species specific assay capturing a fluorescent target anti-bovine IgG and non-target anti-mouse IgG from solution on particles with non-specifically immobilized (adsorbed) Bovine IgG (● ○) and PEGylated particles with covalently attached Bovine IgG (▲, △) filled symbols represent target signal and open symbols non-target noise signal.

Figure 3. Mesothelin Immunoassay using

A) covalently immobilized pAb for antigen capture and fluorescently labelled scFv for display, and B) covalently immobilized scFv for capture and fluorescently labelled pAb as display of increasing concentrations of MSLN spiked in PBS buffer (■), and 50% human serum / 50% PBS buffer (▲). The assay dynamic ranges are determined by non-specific adsorption of display agents onto PEGylated particle platform surface (background noise, ◇), and unmodified particles (expected maximum signal, ◆)