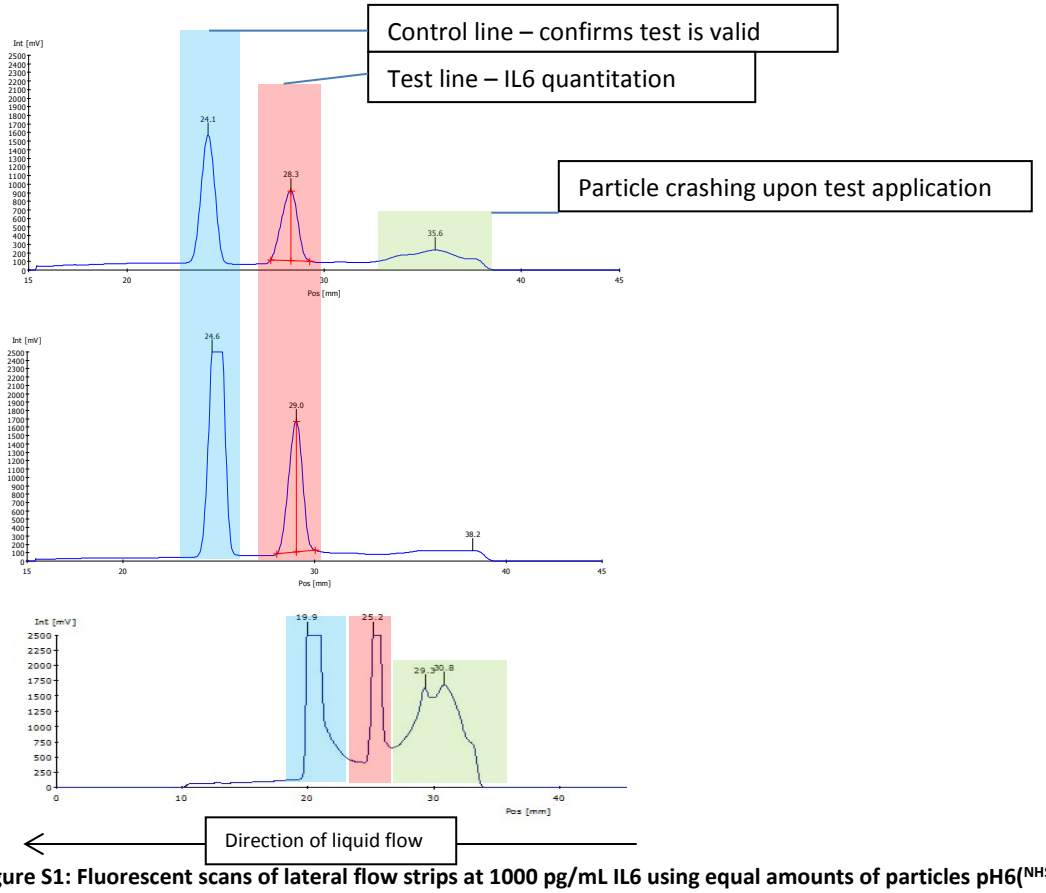

Figure S1: Fluorescent scans of lateral flow strips at 1000 pg/mL IL6 using equal amounts of particles pH6($^{NHS}/_{EDC}$)+BSA (upper trace) and pH5($^{NHS}/_{EDC}$)+BSA (middle trace). Significant particle crashing was observed for tests run with conjugate pH6($^{NHS}/_{EDC}$)+BSA, the lower trace is an example of a particle that was not blocked with BSA that displayed low stability in solution, resulting in large amounts of the particle crashing onto the nitrocellulose.

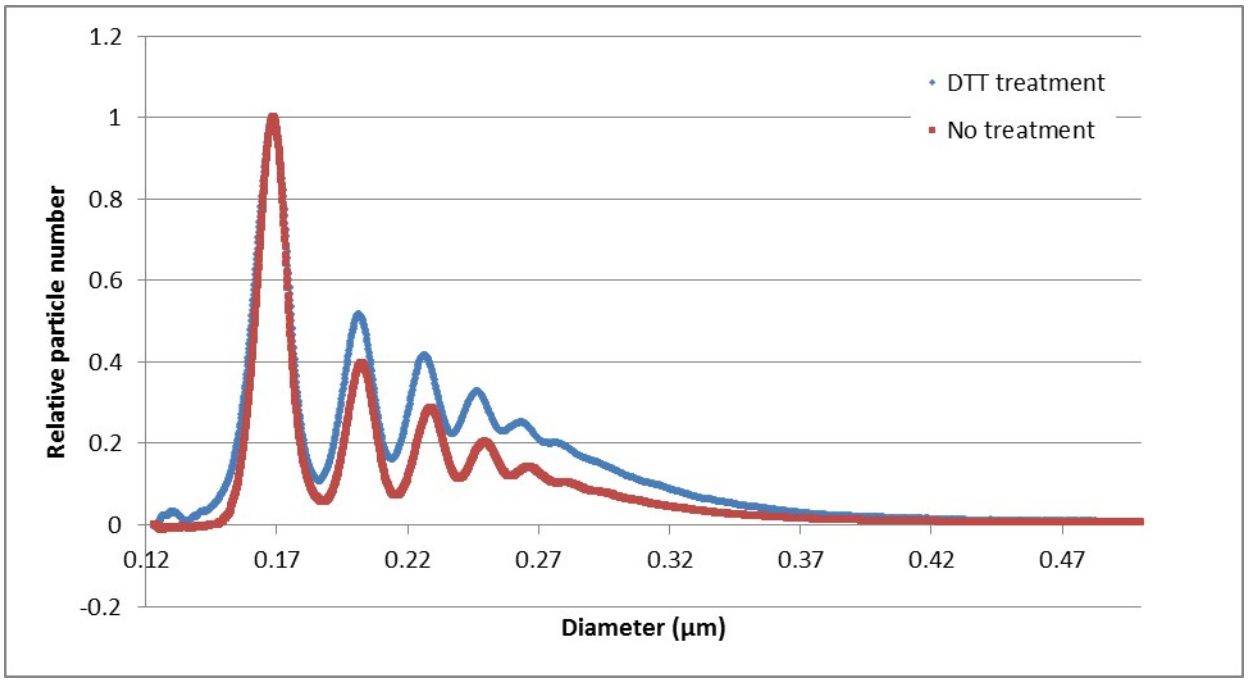

Figure S2: DCS profiles for pH6($^{NHS}/_{EDC}$)+BSA 100 nm particles incubated with, or without 10 mM DTT for 30 minutes prior to analysis. No reduction in the number of oligomer peaks was observed.

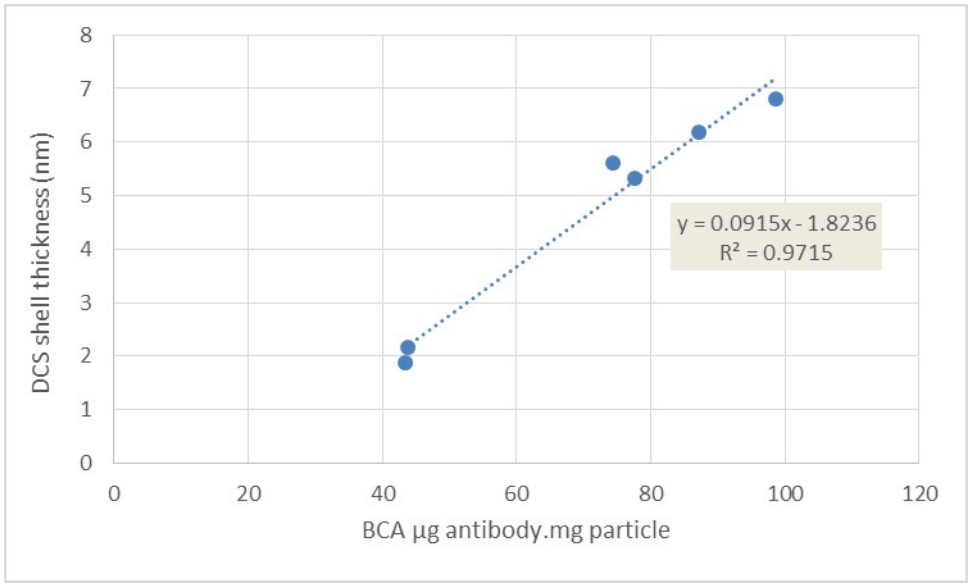

Figure S3: Correlation between the amount of antibody bound to the particle (as determined using the BCA assay) and the thickness of the antibody corona (as determined by the DCS analysis).

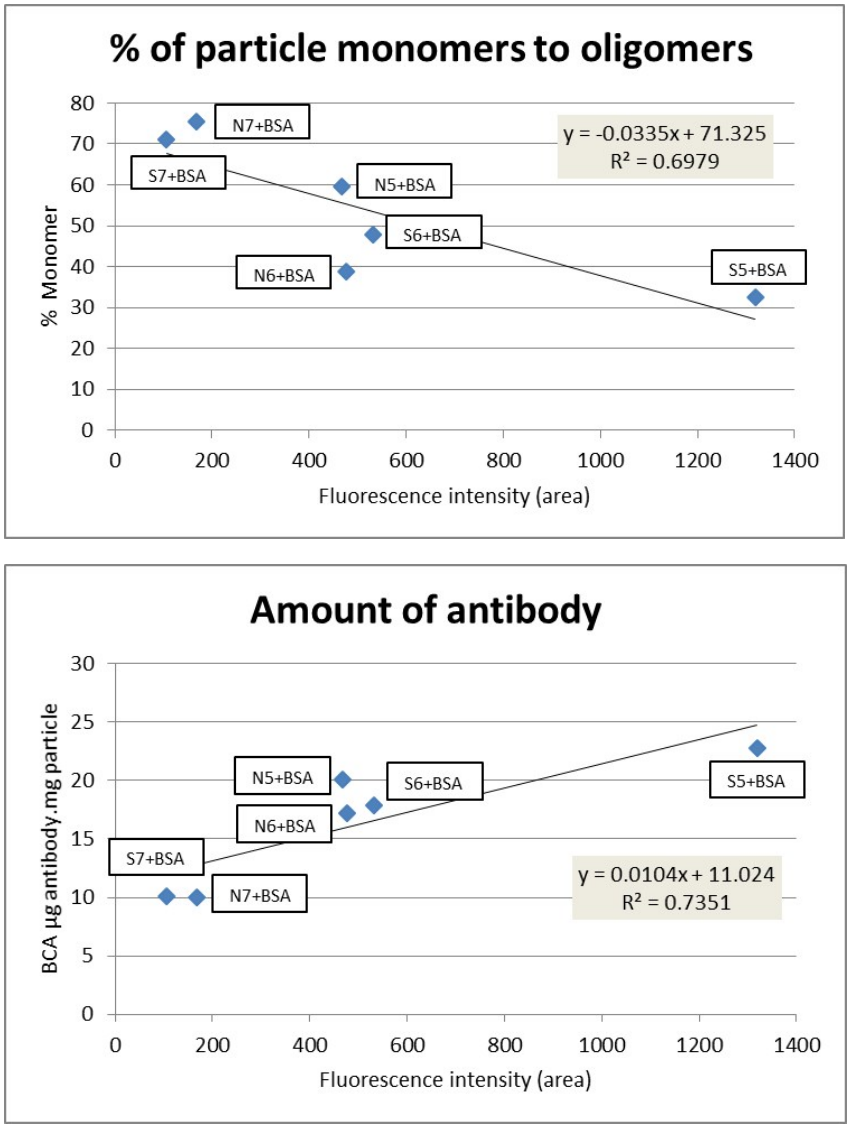

Figure S4: Correlation between assay response (fluorescence intensity area) at 1000 pg/mL and % monomer (upper figure) and amount of antibody bound per particle (lower figure) for each conjugate.

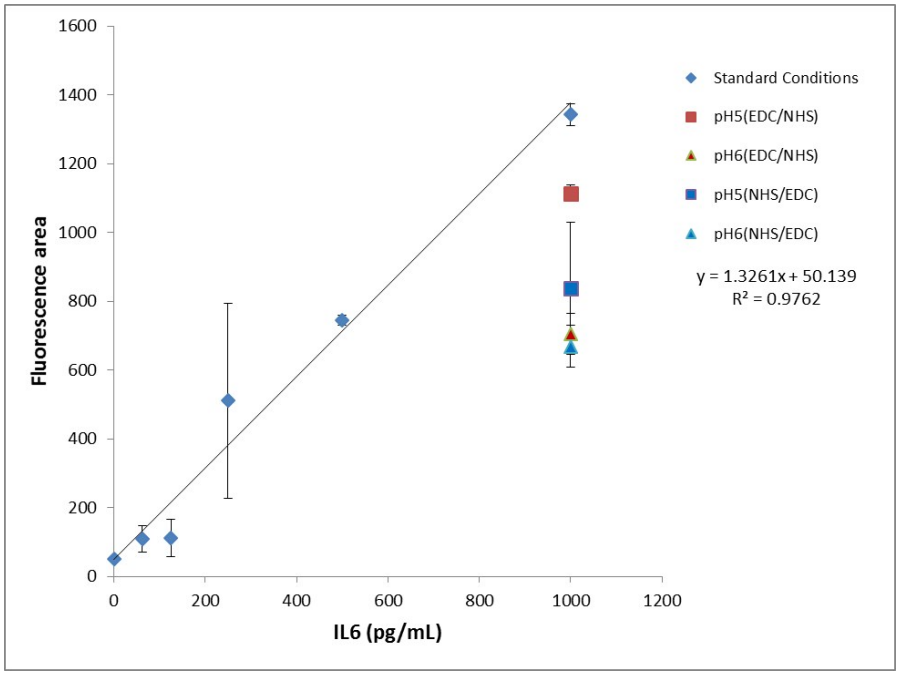

Figure S5: Response curve for IL6 detection using 100 nm nanoparticles. At 1000 pg/mL IL6 the performance of each of the particle conjugate prepared using different conditions was compared. Each data point represents the mean response and standard deviation from two independent lateral flow assays. Standard conditions refers to an independent batch of particles prepared using the method designated pH5($^{EDC}/_{NHS}$)+BSA.

Particle size as measured by DCS

	DCS (monomer peak)
Particle	Mode (nm)
YG200-washed	206.1 ± 2.3
pH5($^{EDC}/_{NHS}$)+BSA	273.5 ± 1.1
pH6($^{EDC}/_{NHS}$)+BSA	266.4 ± 2.1
pH7($^{EDC}/_{NHS}$)+BSA	251.8 ± 1.8
pH5($^{NHS}/_{EDC}$)+BSA	273.5 ± 1.0
pH6($^{NHS}/_{EDC}$)+BSA	269.4 ± 1.3
pH7($^{NHS}/_{EDC}$)+BSA	255.1 ± 1.8
YG200 + BSA-Passive	251.8 ± 1.8

Table S1. Modes of the monomer peak of the particle size distributions as measured by DCS modelling the particles as homogeneous spheres of density 1.054 g/cm^3.

Zeta Potential Measurements

	Zeta Potential (mV)		
Sample	100 mM MES pH 5.0	100 mM MES pH 6.0	100 mM MES pH 7.0
($^{EDC}/_{NHS}$) activated YG200	-31.78 ± 2.45	-34.54 ± 2.6	-32.16 ± 0.57
Anti-IL6 (clone 6708)	0.68 ± 2.18	-2.57 ± 1.26	-3.11 ± 1.62
Mouse IgG	3.47 ± 0.38	-0.12 ± 1.95	-3.25 ± 1.29

Table S2: Zeta potential measurements for EDC/NHS activated particles and mouse antibodies in the three reaction buffers of different pH. Zeta potential measurements were performed at 22°C on a Nano-ZS 3600 using a DTS 1070 disposable folded capillary cell at 40 V. The data shown is the mean and standard deviation of five measurements, where each measurement consisted of 40 runs. The EDC/NHS activated particles were analysed at 0.002 % (w/v) and the antibodies were analysed at 0.2 and 1 mg/mL for the anti-IL6 and mouse total IgG respectively.

S1: Calculation of protein shell thickness by DCS

Acquisition of a protein layer on a particle changes both the particle volume and the particle density. The thickness of the protein layer can be measured by DCS using a spherical core/shell model for the particle and assuming the densities of the protein shell and the particle core. Detailed descriptions and examples of this approach are available in references [1] and [2]. Briefly, for a particle with core diameter D_0 and density ρ_0 in a fluid of average density ρ_f, acquiring a shell of density ρ_s, the thickness of the shell is given by solving for T in Equation (1) which equates the sedimentation times for a core-shell particle with that of a particle of diameter and homogenous density.

$$(\rho_1 - \rho_f)D_1^2 = (\rho_s - \rho_f)(D_0 + 2T)^2 + \frac{(\rho_0 - \rho_s)D_0^3}{(D_0 + 2T)} \quad (1)$$

D_1 is the diameter measured by DCS for the particle if it is assumed to have homogenous density ρ_1. D_0 must be measured by DCS in a separate experiment before the particles acquire the protein shell.

Equation 1 is derived by starting from the basic equation for DCS:

$$t = \frac{\alpha}{(\rho_P - \rho_f)D_P^2} \quad (2)$$

With:

$$\alpha = \frac{18\eta ln\left(\frac{R_f}{R_0}\right)}{\omega^2} \quad (3)$$

D_p is the total particle diameter, ρ_P is the total particle density, ρ_f is the fluid density and t is the sedimentation time between radii R_0 and R_f at angular frequency ω and fluid viscosity η.

If a particle (D_0, ρ_0) acquires a shell of density ρ_s, the total diameter and density can be expressed as:

$$D_p = D_0 + 2T \quad (4)$$

$$\rho_p = \frac{\rho_0 V_0 + \rho_s V_s}{V_0 + V_s} = \frac{\rho_0 D_0^3 + \rho_s\left((D_0 + 2T)^3 - D_0^3\right)}{(D_0 + 2T)^3} = \frac{(\rho_0 - \rho_s)D_0^3}{(D_0 + 2T)^3} + \rho_s \quad (5)$$

Where V_0, and V_s are the volumes of the particle core and the shell respectively.

Equating the sedimentation times, leads to:

$$(\rho_1 - \rho_f)D_1^2 = \left(\frac{(\rho_0 - \rho_s)D_0^3}{(D_0 + 2T)^3} + \rho_s - \rho_f\right)(D_0 + 2T)^2 \quad (6)$$

And therefore to Equation 1.

S2: Calculation of protein shell density using DCS

The protein shell density is calculated by re-arranging Equation 1 to determine the shell of density ρ_s. The variables D_0 and D_1 the total particle diameter in the absence and presence of the protein shell are determined using DCS, ρ_P is the total particle density that can be determined from D_0 using the method described by Minelli et al. [3], or from the manufacturer's data. The protein shell thickness T is determined using DLS data from monomeric non-coated and passively protein coated particle preparations.

References

[1] N. C. Bell, et al., *Analytical Methods*, 2013, **5**, 4591-4601.

[2] Walczyk, D., et al., *Journal of the American Chemical Society*, 2010, **132**, 5761-5768.

[3] C. Minelli, et al., *Surface and Interphase Analysis*, 2014, **46**, 663-667.