Kinetics of immunoassays with particles as labels: effect of antibody coupling using dendrimers as linkers

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Nanoparticle synthesis – polydispersity index variations related to particle surface modifications

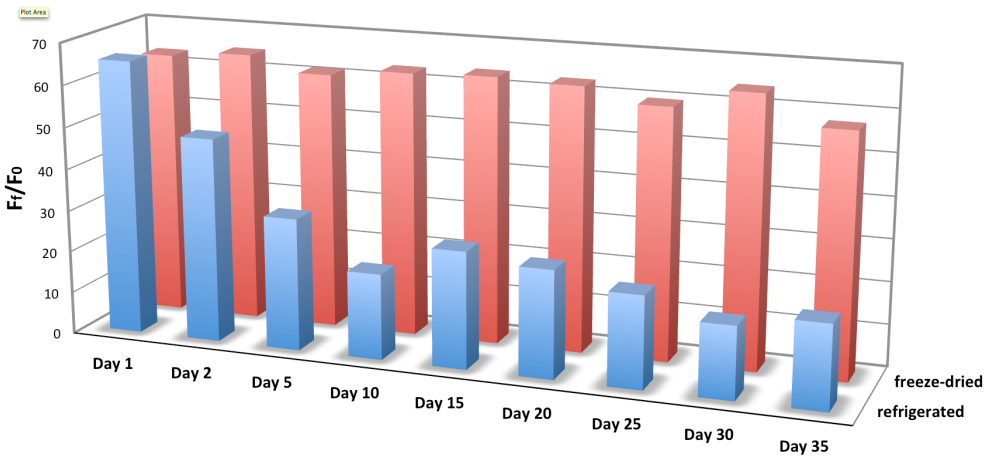
Colloidal stability of silica NPs has been previously improved by the addition of negatively charged non-reactive organosilanes in addition to organosilanes with functional groups (such as $–NH_2$ or –COOH groups) available for bio-immobilisation (ref. 12 and 17). The average particle size, as well as the polydispersity index can show small variations as efect of applied particle surface chemistry.

	Surface chemistry	*Polydispersity index*	*Average size*
Batch 1	10% -NH_2	0.118	77.5 ± 20.9
Batch 2	8% -NH_2	0.184	80.1 ± 21.6
Batch 3	4% -NH_2	0.274	84.2 ± 23.0
Batch 4	2% -NH_2	0.262	81.1 ± 22.1
Batch 5	100% -COOH	0.170	81.9 ± 21.1

ESI 1 *Variations in particle polydispersity index and average particle size across different batches of dye-doped silica nanoparticles, measured by dynamic light scattering (DLS).*

Storage of protein-sensitized nanoparticles

We have performed a simple experiment with GFP-G4.5-sensitized NPs to study the effect of storing conditions on ageing of particle-immobilized protein. Two sets of samples were prepared as described in the 'Material and Methods' section. One set of sample was aliquoted into 100uL fractions and stored in a refrigerator, the other set was aliquoted the same way and and freeze-dried. The conformational changes of the GFP immobilized via G4.5 PAMAM dendrimer on the NP surface were monitored over 35 days by means of fluorescence. We hypothesized that due to GFP-nanoparticle aggregation, frequent ultrasonication, temperature and other extrinsic factors, the fluorescence of the GFP in the sample stored in the fridge would be decreasing with time, as a consequence of GFP structural changes. On the other hand, the freeze-dried aliquots were easily reconstituted in water without the need of ultrasonication. As a result, the changes in the conformation of the GFP were minimal, which was demonstrated as reasonably constant fluorescence values over the 35 days (ESI 2). We have therefore conluded that the optimal way of storing the protein-sensitized-NPs is by freeze-drying smaller aliquots of the sample.



ESI 2 *Ageing studies using GFP-G4.5-sensitized NPs. One set of sample was aliquoted into 100uL fractions and stored in a refrigerator, the other set was aliquoted and freeze-dried. The conformational changes of GFP immobilized on the NP surface were monitored over 35 days by means of fluorescence.*

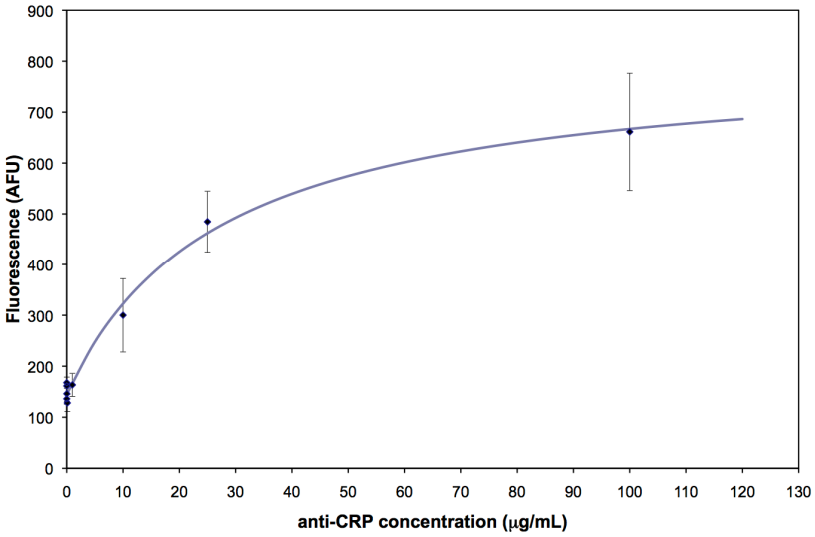
Washing the reaction mixture by ultrasonication

One of the simplest techniques to purify the prepared NPs and separate the antibody-sensitized NPs from the excess of the antibodies present in the reaction mixture is by centrifugation. The precipitated NPs must be then redissolved in BSA containing buffered solution. In order to obtain monodisperse NP solution, ultrasonication for 10 – 60 seconds is necessary. To ensure that no free antibody (or as little as possible) is present in the final sample, the centrifugation-ultrasonication steps must be repeated several times. This can obviously introduce some stresses to the attached antibodies. We have therefore investigated how many purification steps are necessary to remove the majority of the free antibody from the reaction mixture. Four samples with GFP-sensitized NPs were prepared as described in 'Materials and Method' section. The amount of washed GFP in the supernatant after each washing step was measured by fluorescence. The results are summarized in ESI 3. In general, two centrifugation-ultrasonication cycles are enough to remove ~99% of the free, unbound protein. Repeating the washing steps one more time removes > 99.5% of the free protein.

label	*Wash 1*	*Wash 2*	*Wash 3*	*Wash 4*
NP-G1.5	78.7	20.3 (99.0)	0.6 (99.6)	0.3 (99.9)
NP-G2.5	89.6	9.6 (99.3)	0.4 (99.7)	0.2 (99.9)
NP-G3.5	80.8	17.6 (98.4)	0.9 (99.3)	0.6 (99.9)
NP-G4.5	87.0	12.1 (99.1)	0.5 (99.6)	0.4 (100.0)

ESI 3 *Percentage (%) of excess of antibody, washed from the reaction mixture by means of centrifugation. The number in parentheses shows the accumulated amount of excess antibody washed from the reaction mixture.*

Measurement of equilibrium constant for antibody binding to the antigen-sensitised reaction well



ESI 4 *The equilibrium constant was estimated by measuring the fluorescence signal at equilibrium, resulting from incubation of the CRP-sensitised surface with lissamine rhodamine-labelled anti-CRP ScFv. The signal fitted to a Langmuir isotherm, with surface binding equilibrium constant (9.5±2)* $\times 10^5 M^{-1}$*. The measurement highlighted significant variability of the surface preparation.*