Electronic Supplementary Material (ESI) for Chemical Communications

Multifunctional Nanoprobes for Pathogen-Selective Capture and Detection

Chantal Paquet, Shannon Ryan, Shan Zou, Arnold Kell, Jamshid Tanha, John Hulse, Li-Lin Tay, Benoit Simard

-Materials-

ACS grade sodium dodecyl sulphate (SDS), 99+%, glycidyl methacrylate (GMA), ethyl glycol dimethacrylate (EGDMA), potassium persulfate (KPS), cystamine, sodium borohydrate ($NaBH_4$), cadmium oxide (99.99 %), zinc oxide powder (99.9 %), sulfur powder (99.98 %), selenium powder (100 mesh, 99.999 %), tributylphosphine (TOP, 97 %), 1-octadecene (ODE, 90 %), oleic acid (OA, 90%) trioctylphosphine oxide (TOPO, 90 %), octadecylamine (ODA, 90 %), stearic acid (SA, 90%) and 5-aminopentanol were purchased from Sigma-Aldrich and used without further purification. Superparamagnetic iron oxide nanoparticles (SPIONs) coated with fatty acids were purchased from Ferrotec. The Ferrotec SPIONs were analyzed by TEM image analysis and found to have a diameter of 9.1 ±2.7 nm. Phosphate saline buffer powder was purchased from Invitrogen. Water filtered through a Millipore filtration system was used for all syntheses and experiments.

-Quantum dot synthesis-

CdSe-ZnS core-shell nanoparticles of CdSe-ZnS were prepared as described elsewhere.[1] For a typical reaction, a cadmium solution was prepared by mixing 0.0514 g of CdO, 0.456 g of SA, and 4.0 g of ODE in a 50 mL three-neck round bottom flask with a reflux condenser attached to one of the necks. The flask was evacuated and heated to 200 °C under nitrogen-flow until the solution became clear. The temperature was then lowered to 100 °C. In a separate flask, a selenium solution was prepared by mixing 0.3 mg of selenium powder with 1.2 mL of TOP. Once the selenium was dissolved, 3 mL of ODE was added. In a separate 50 mL three-neck round bottom flask, a mixture of 1.0 g of ODE, 3.2 g of ODA and 1.0 g of TOPO was stir under nitrogen flow at 100 °C. The cadmium solution was then transferred to the ODA mixture. A thermometer and a reflux condenser were then placed into the necks of the flask. The cadmium solution was heated; when the mixture reached 100–130 °C, the flask was evacuated and filled with nitrogen. The cadmium solution was then heated to 280 °C at which point the selenium solution was quickly injected. The heat was removed shortly after injection. When the solution reached about 50°C, hexane was added.

The ZnS shells of the CdSe were prepared as follows. A 0.4 M sulfur solution in ODE was heated under nitrogen-flow at 100 °C. A zinc solution was prepared by mixing of 0.228 g of ZnO, 6.43 g of OA, and 8.2 g of ODE in a 50 mL three-neck round bottom flask. The flask was evacuated and heated under nitrogen-flow to 250 °C. Once dissolved, the solution was cooled and 40 g of ODE was added. A mixture of 12 g of ODA, 40 g of ODE, and 6 g of CdSe nanocrystal dispersion was stirred in a 50 mL three-

neck flask. The hexane present in this mixture, which originated from the nanocrystal dispersion, was then removed by a rotary evaporator. A thermometer, reflux condenser and gas inlet were fixed to the flask. The flask was heated to 120 °C at which point the flask was evacuated and filled with nitrogen.
The mixture was then heated to 230 °C. At this point, 1.2 mL of the 0.4 M sulfur solution was injected followed by 2.5 mL of the zinc solution after 10 min. Eight more injections with 10 min intervals were made alternating between the sulfur and zinc solution with the volume of each injection increasing by about 1 mL. The temperature was increased to 260 °C for about 20 min. The mixture was then cooled and once the temperature reached 70 °C, hexane was added. The nanocrystals were precipitated by the addition of acetone, followed by centrifugation. The ligands of the nanocrystal were then exchanged with TOPO by redispersing the dispersion in excess TOPO and chloroform. The nanocrystals were purified by precipitation and redispersed into toluene. The quantum dots were ligand exchanged with 5-aminopentanol (stored in ethanol) as described elsewhere.[2]

-Nanoprobe synthesis-

Clusters of SPIONS were first prepared by dispersing SPIONS in toluene at a concentration of 0.10 g/mL. Using a Branson Sonifier 250 at a duty cycle of 50% for 120 seconds, 2mL of the SPION solution was ultrasonicated in 3.6mL of an aqueous solution containing 50 mM SDS. The emulsion was heated at 90°C for 2hrs to evaporate the toluene. Water was added intermittently to maintain a constant volume. The clusters of SPIONs were purified by magnetic purification.

The clusters were diluted in 100 mL of 4mM SDS in a 250 mL round bottom flask with a condenser, temperature probe and nitrogen inlet connected to it. The clusters were heated to 80ºC under nitrogen. Once the temperature stabilized, 3 mg of KPS and 0.200 mL of a 90:10 mixture of glycidyl methacrylate and ethylene glycol methacrylate were injected. The monomer was left to react for 2 hours before 100mL of borate buffer was added (pH 8.9) along with a large excess of cystamine (~1g). The mixture was left to react overnight. The particles were centrifuged 3 times and diluted to 25mL to give a solid content of 6 mg/mL.

Using 5 mL of the cystamine-functionalized particles, the particles were reduced using 0.1 g $NaBH_4$ under nitrogen. After 1 hour, the particles were magnetically purified under nitrogen. One fifth of the particles were then diluted in ethanol to form a final 1:1 solution of water:ethanol. Using dropwise addition, 0.100mL of a 1 μM QD solution was added to these particles and stirred for 3 hrs. At this point it is possible to add poly(ethylene glycol) maleimide if it is desired. The particles were centrifuged, redispered, magnetically purified and redispersed in 10 mL of water containing an excess of 5-aminopentanol.

The particles were functionalized with the molecular recognition agents by first transferring the nanoparticles to phosphate saline buffer. The proteins or antibody were added in a 100 times excess to the number of particles. After mixing for 2 hrs, the particles were magnetically purified three times.

Particle concentrations (number of particles/mL) were determined based on an estimated density of the particles and the solid content of the solutions. Details can be found elsewhere.[3]

-Instrumentation-

TEM imaging was performed on an Hitachi HD-2000. A Fluorolog byYvon-Jobin Fluorolog Tau-3 Lifetime System was used to acquire fluorescence spectra and lifetimes of the particles. Spectra ranging from 565 to 665 nm using an excitation wavelength of 555 nm and slits of 4.0 nm were used to acquire spectra of quantum dots. Hydrodynamic diameters were determined on a Nanosight LM10-HS particle sizer. All AFM images were taken using the Nanowizard ® II BioAFM (JPK Instruments, Berlin, Germany) mounted on an Olympus 1X81 inverted fluorescence microscope, operating in contact mode. Silicon nitride cantilevers (DNP-S, Veeco, CA) and silicon cantilevers (Ultrasharp NSC12, MikroMasch, Estonia) were used in contact and intermittent contact mode imaging, respectively. The spring constants, which was typically in the range of 0.06-0.28 N/m for DNP tips and 3.2-7.9 N/m for Ultrasharp tips, were determined by the thermal noise method after the determination of the tip sensitivity (nm/V) by indenting the AFM tip against a hard reference glass substrate. All AFM images were plane-fit (1st order) using the JPK Image Processing Software (JPK Instruments, Berlin, Germany). Fluorescence and optical images were taken using an Olympus 1X81 inverted optical microscope. Fluorescence images used 633 nm laser excitation with a Cy5 filter set (Chroma Technology) equipped with Cascade 512 CCD camera (Photometrics, US) and a 100x/1.45 NA Plan Apochromat objective. XPS (X-ray photoelectron spectroscopy) was performed with PHI5500 XPS system. Measurements were performed with neutralizer to minimize the surface charge.

- X-ray photoelectron spectroscopy-

Blank colloids were prepared (no SPION core) in order to analyze the modifications in the surface composition using x-ray photospectroscopy. Blank colloids were prepared by reacting 0.900mL of methyl methacylate and ethylene glycol dimethacrylate (90:10) and 6 mg of potassium persulfate in 100 mL of water under nitrogen at 80ºC. After 30 minutes, 0.100 mL of glycidyl methacrylate was added. After 3 hours, the dispersion was centrifuged and redispersed in PBS buffer. The dispersion was divided into four aliquots and manipulated in the following manner:

XPS-1: left unreacted
XPS-2: reacted with cystamine and purified.
XPS-4: reacted with cystamine, purified, reacted with $NaBH_4$ and purified.

The strong contribution of oxidized sulfur in all three samples originates from the presence of sulfonate groups that arise from the thermo-initiator, potassium persulfate.

Fitting results:

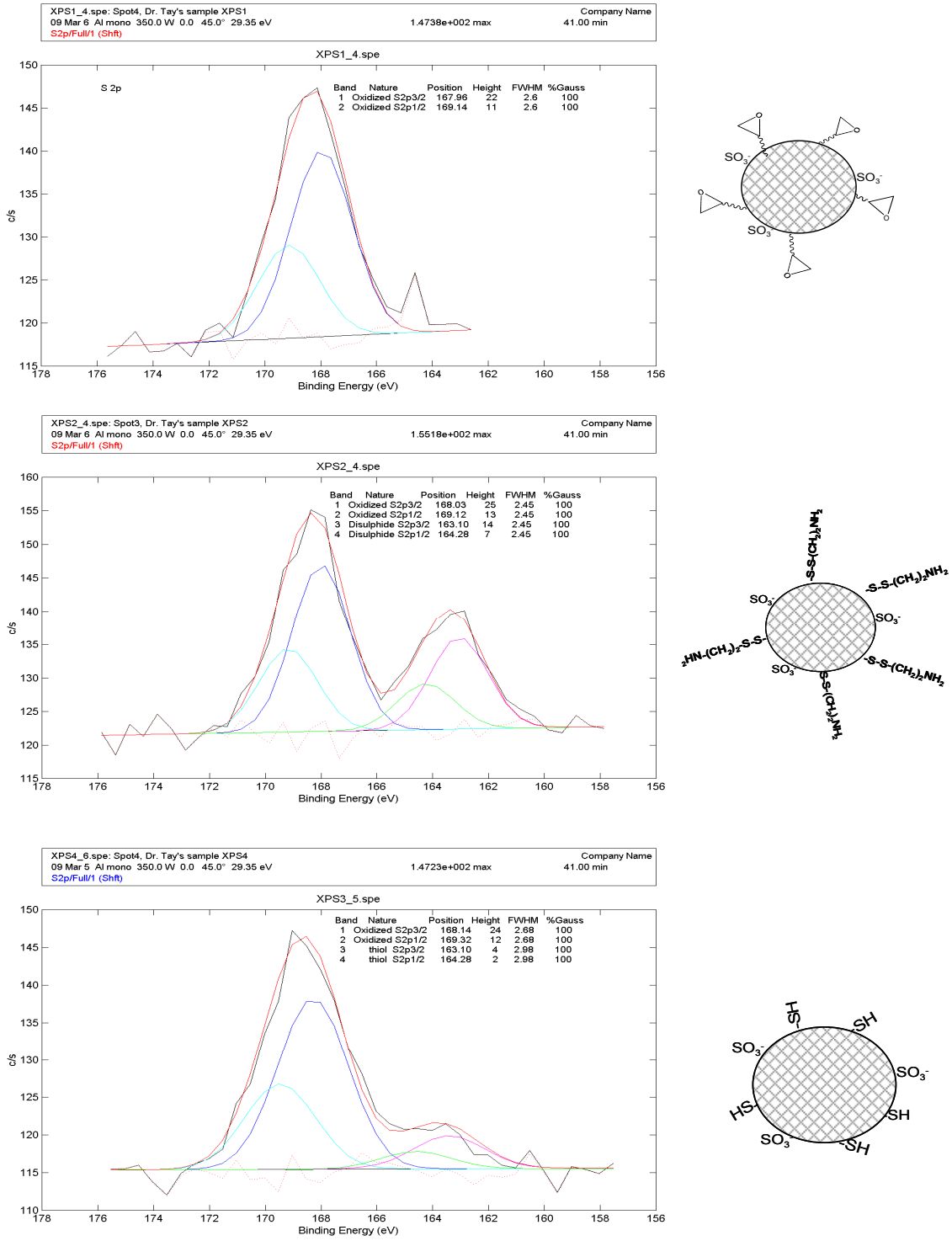

Figure ESI_1 Fitting of S 2p spectrum for XPS1, XPS2 and XPS4

(1) Sample 'XPS1'

Table ESI_1 Fitting results of XPS-1.

Peak	Band	Position	Height	Area	FWHM	%Gauss
S 2p in Oxidized S	1	167.96	22	55.2	2.60	100
	2	169.14	11	27.6	2.60	100

(2) Sample 'XPS2'

Table ESI_2 Fitting resultsXPS2 obtained using medium resolution mode

Peak	Band	Position	Height	Area	FWHM	%Gauss
S 2p in Oxidized S	1	168.03	25	64.8	2.45	100
	2	169.21	13	32.4	2.45	100
S 2p in Disulphide	3	163.10	14	36.5	2.45	100
	4	164.28	7	18.2	2.45	100

4) For sample XPS4

Table 3 Fitting results for XPS4 obtained using medium resolution mode

Peak	Band	Position	Height	Area	FWHM	%Gauss
S 2p in Oxidized S	1	168.29	23	71.6	2.98	100
	2	169.47	11	35.8	2.98	100
S 2p in thiol	3	163.42	4	12.6	2.98	100
	4	164.60	2	6.3	2.98	100

-Fluorescence-

A. Fluorescence of the core-shell particles

The fluorescence spectra of the QDs before and after they were deposited on the particles were measured. The spectra, found in ESI _2, shows that the QDs fluorescence decreases by ~ 10% and is blue shifted by about 2 nm with respect to the QDs before deposition. The spectrum of the QDs that were not deposited on the particles (supernatant solution) is also shown in the inset and demonstrates that only marginal amounts of QDs did not bind to the particles.

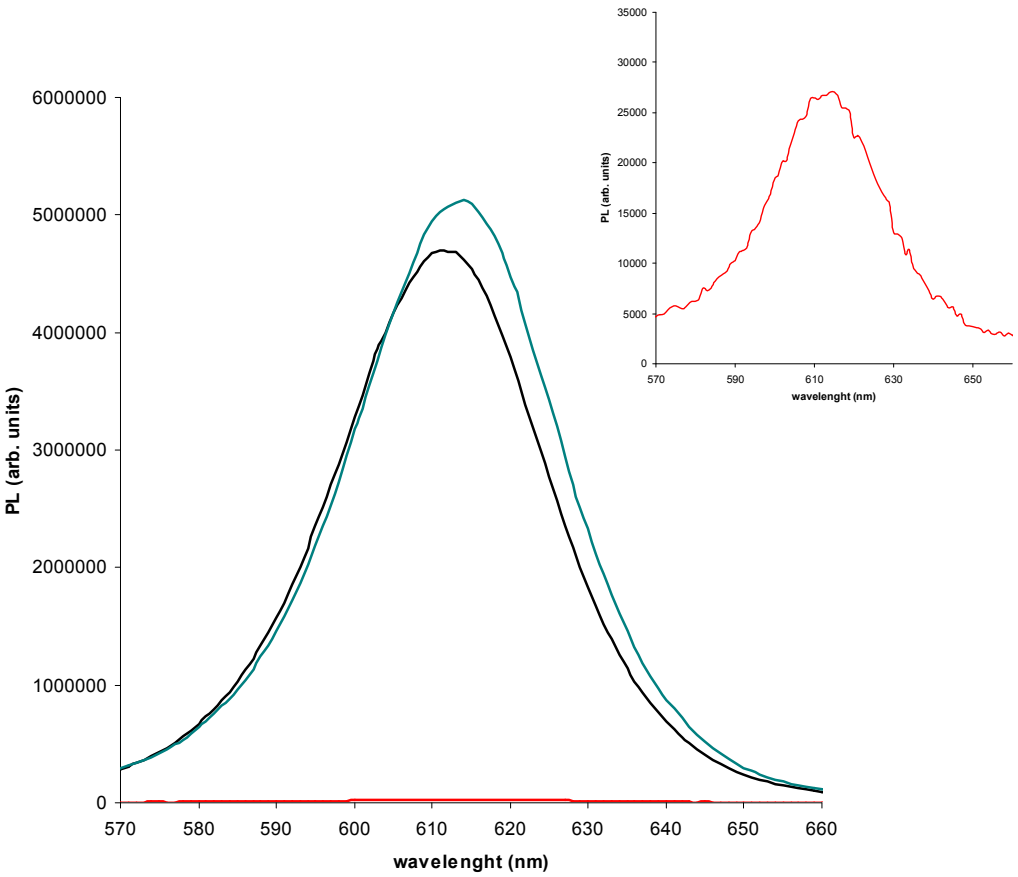

Figure ESI_2: Photoluminescence of the QDs before (blue) and after (black) the deposition onto the particles. The red curve in the inset is the fluorescence of the QDs that did not bind to the particles**.**

-Photoluminescence of the mixed QDs-SPION clusters-

Clusters made from a mixture of QDs and SPIONs were prepared with a range of QD to SPION ratios and studied for their photoluminescence properties. This structure places the mixture of QDs and SPIONs in a densely packed structure and as a result the two types of nanoparticles are in close spatially proximity, with the ligands of the nanoparticles separating them. A TEM image of the mixed QD-SPION cluster is found in Figure ESI_3. The fluorescence and lifetime measurements of these clusters as a function of the QD to SPION ratios are shown in Figure ESI_4 and Figure ESI_5. In Figure ESI_4, the fluorescence is normalized against the fluorescence of QDs when the SPION fraction is zero. The fluorescence of the clusters is then compared to the fluorescence of QD when the same ratio of QDs and SPIONs are in solution (in toluene). Figure ESI_4 shows that the fluorescence decreases nonlinearly as the ratio of QD to SPION decreases. A linear decrease is observed however when the two types of particles are in solution (and spatially separated). These results suggest that when QDs are in close proximity to the SPIONs, fluorescence is loss to energy transfer to the

SPIONs and that this loss increases with increasing fraction of SPIONs. Lifetime measurements on these clusters further support that energy transfer from the QDs to the SPIONs is occurring, as shown in Figure ESI_5. The results show that when the fraction of QDs in the clusters decreases with respect to SPIONs, the fraction of the fast component of the lifetime decreases and also shortens in time. The fraction of the slow component increases while its lifetime also decreases in time. The horizontal black lines in the figure show the lifetimes of QDs in solutions while the grey line represents the lifetime of the nanoprobes where the QDs are assembled on the surface the particles separated from the SPION core by a polymer layer.

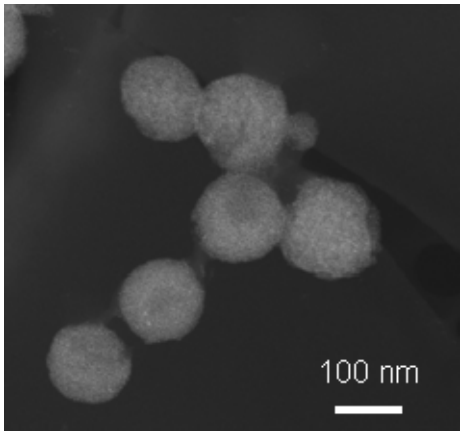

Figure ESI_3: TEM image of the clusters made from a 1: 4 mixture of SPIONs to QDs.

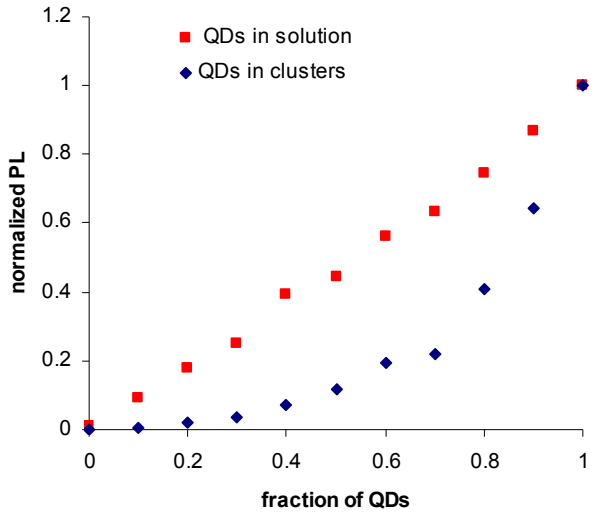

Figure ESI_4: Normalized fluorescence (PL) of clusters (blue diamond) and solutions (red squares) containing varying ratios of QD to SPION.

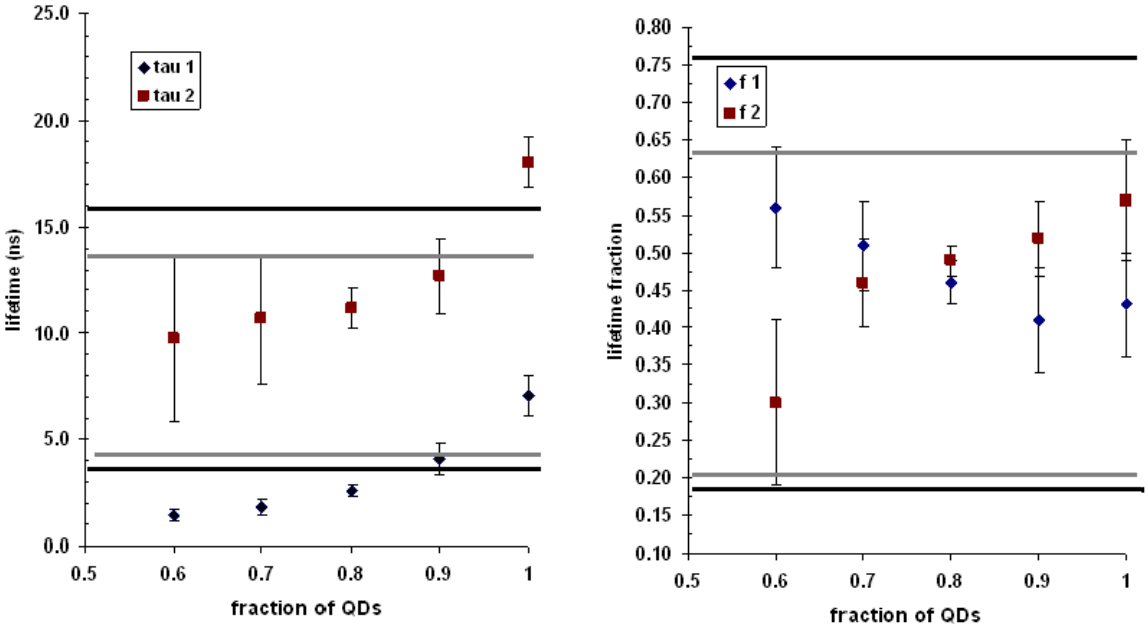

Figure ESI_5: Fluorescence lifetimes of clusters containing varying ratios of QDs to SPIONs. As a reference, the lifetime of QDs in toluene (black line) and the lifetime of QDs on nanoprobes with a core-shell structure (grey line) are included.

-Particle sizing-

A. Particle size with surface functionalization

The size distributions in Figure ES1_6 exemplify changes in sizes that occur after each modification. The sizes represent hydrodynamic diameters determined with a Nanosight instrument. The technique of laser light scattering microscopy generates number-averaged hydrodynamic diameters. Clusters of 113 nm in diameter were coated with a polymer coating, yielding particles of 138 nm. The modification of the particles with quantum dots and single-domain antibodies increased the size of the particles marginally, with diameters of 148 nm and 158 nm, respectively.

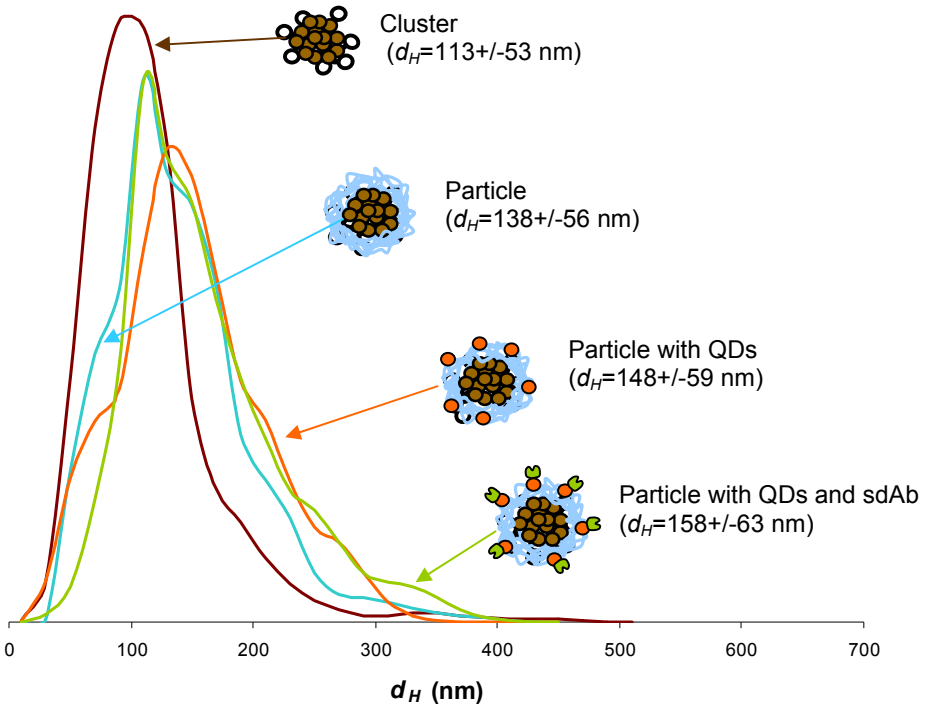

Figure ESI_6: Particle size distributions. The mean hydrodynamic diameters for clusters of iron oxide nanoparticles, polymer coated clusters (particles), particles with QDs, and, particles with QDs and sdAb was 113, 138, 148 and 158 nm, respectively.

B. Stability of particles in various buffers

The particles with QDs were stored in solutions of HCl (pH 4.5), phosphate saline (pH 7.4) and borate (pH 8.9) for 20 days. Figure ESI_7 compares the size distribution of the particles stored in these three solution for 20 days with those stored in water. The curves show that the size distribution does not change significantly over this time scales.

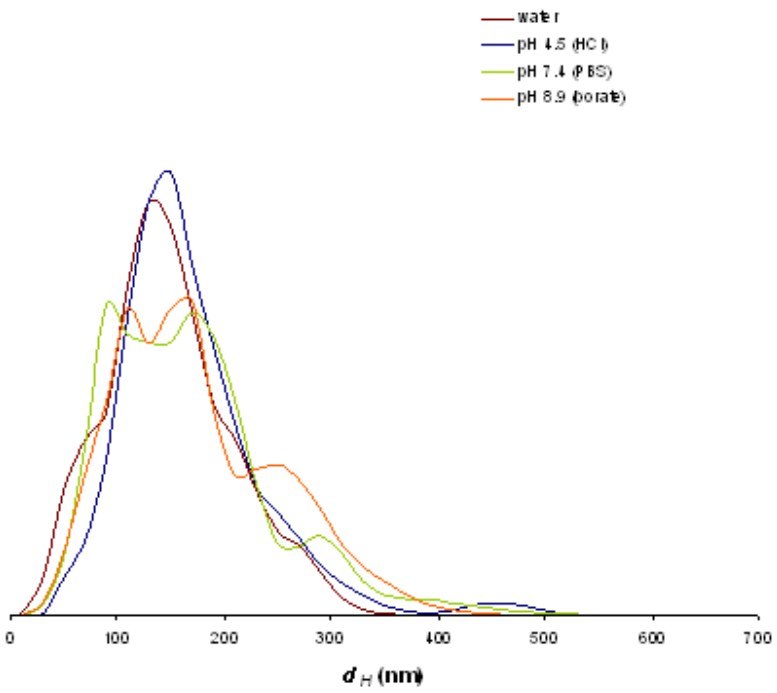

Figure ESI_7: Size distributions of particles in water (brown), and, after 20 days in solutions of pH 4.5 (blue), 7.4 (green) and 8.9 (orange).

-Magnetic capture and imaging-

Using a ratio of 66 nanoprobes: salmonella cells, the particles and bacteria were diluted in PBS with 0.1% tween to give a final cell concentration, expressed as colony forming unit (CFU)/mL, of $1x10^8$. The cells were mixed for 30 minutes, then magnetically confined against a rare early magnet and redispersed in diluted PBS solutions (0.001X PBS) three times before fixing to cleaned microscope slides.

Similar magnetic manipulations were performed with salmonella cells and particles containing no TSP. In this case, no salmonella cells could be confined.

-Microagglutination Assay-

The microagglutination assays were performed as follows:

***Staphylococcus aureus* preparation:**

1) Streaked a BHI plate from frozen stock and incubated overnight at 37ºC.
2) Innoculated 10 mL of BHI broth with a colony from plate and incubated 37ºC, 150 rpm overnight.
3) Spun down culture and resuspended in PBS buffer for an OD_{600} = 1.0 = ~10^8 cfu/ml.

***Salmonella typhimurium* preparation:**

1) Streaked a nutrient broth (NB) plate from frozen stock. Grew O/N at 37ºC.
2) Inoculated a 10 ml NB broth culture and grew O/N at 37ºC, 150 rpm.
3) Spun down culture at 6000 rpm, 10 min in Sorval centrifuge.
4) Resuspended the pellet in PBS, and adjust to OD_{600}=1.0=~10^8 cfu/ml.

Agglutination plate set-up:

1) Added 50 µl of PBS buffer to each of the wells except the first well in the series.
2) To the first well, added 100 µl of the appropriate nanoparticle sample (initial concentration =$6.5x10^{11}$ particles/mL).
3) Did 2-fold dilutions of the sample (50 µl volume) down the row for 11 wells. Left the last well in PBS buffer alone.
4) Added 50 µl of appropriate bacteria to each well.
5) In row G, column 10-12, added 50 µl of samples #5-7, without addition of bacteria as a visual control of particle behavior.
6) Incubated plate overnight at 4ºC.

Microagglutination assays are performed by incubating a constant number of cells with decreasing numbers of nanoprobes across a row of a microtiter plate. When the nanoprobes interact with the bacteria, a nanoprobe-pathogen aggregate forms and a pathogen, they fail to form a nanoprobe-pathogen aggregate and precipitate at the bottom of the well as a pellet. Using this approach, it is possible to compare the efficiency of the interactions with the pathogen as measured by the minimum agglutination concentration (MAC) of the particles (i.e. the minimum concentration of nanoprobes that is required to agglutinate the bacteria).

The agglutination results found in the photograph of Figure ESI_8 show that agglutination occurs between *S. aureus* and sdAb-nanoprobes up to a particle concentration of $2x10^{10}$ particles/mL.

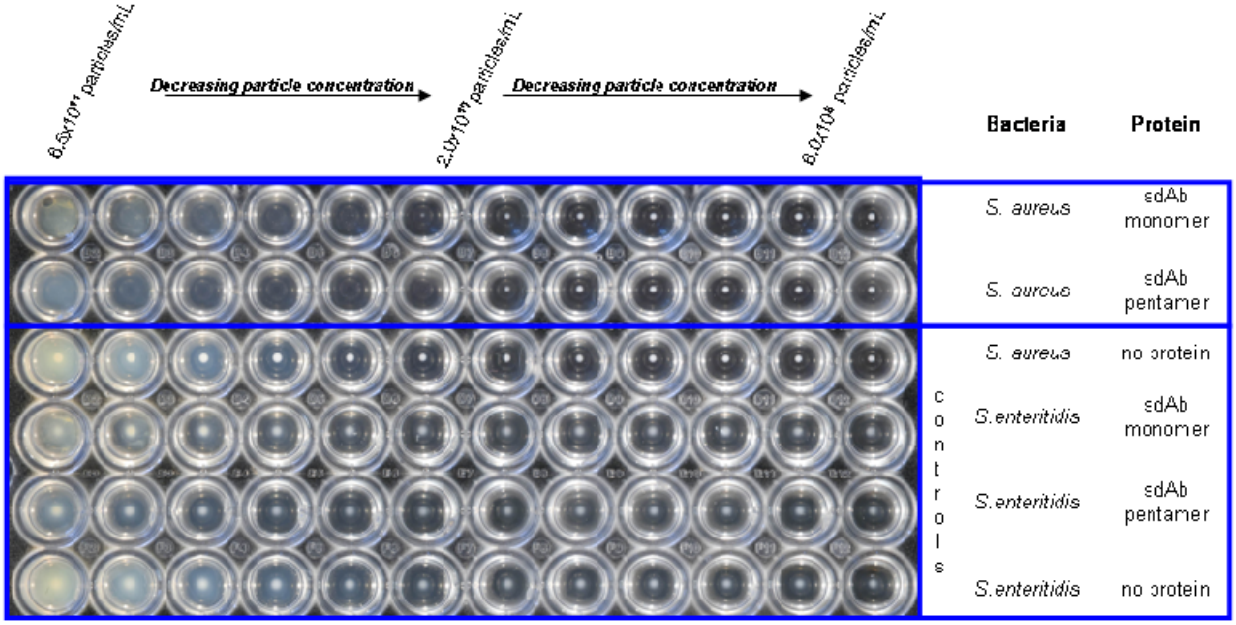

Figure ESI_8: Microagglutination assay of sdAb particles against S. aureus and S. enteritidis.

References

1. C. Paquet and E. Kumacheva, *Advanced Functional Materials*, 2007, **17**, 3105-3110.
2. Y. Chan, J. P. Zimmer, M. Stroh, J. S. Steckel, R. K. Jain and M. G. Bawendi, *Advanced Materials*, 2004, **16**, 2092-2097.
3. C. Paquet, H. W. De Haan, D. M. Leek, H. Y. Lin, B. Xiang, G. Tian, A. Kell and B. Simard, *ACS Nano*, **5**, 3104-3112.