Supporting Information

Synthesis of magnetic polystyrene nanoparticles

60 mmol of $FeCl_2$-$4H_2O$ and 90 mmol of $FeCl_3$-$6H_2O$ were mixed in 100 mL of deionized water with mechanical stirring. 40 mL of concentrated ammonium hydroxide solution were added drop-wise over 5 min at room temperature. Afterwards, 5.0 g of oleic acid were added and the mixture was heated to 70 °C with constant stirring. 1 h later, the temperature was raised to 110 °C and the reaction continued for 2 h, keeping the volume constant by adding water sporadically. The resulting black precipitate was washed several times with water and dried overnight under vacuum at 40 °C.

Afterwards, 1.0 g of the oleate-capped iron oxide nanoparticles was dispersed in 1.0 g of octane in an ultrasonication bath during 30 min. Then, 24 mL of an aqueous solution of SDS (0.15 wt%) was added and the system was emulsified by ultrasonication with a tip sonifier for 4 min (50% amplitude, 10 s pulse, 5 s pause) under ice cooling. The obtained miniemulsion was transferred to a two-necked round bottom flask and stirred mechanically at room temperature. Separately, 1.2 g of styrene were mixed with 20 mg of *n*-hexadecane and 24 mL of an aqueous SDS solution (0.10 wt%). The mixture was then stirred at 1000 rpm for 1 h, ultrasonicated with a tip sonifier (1 min, 10% amplitude, 5 s pulse, 5 s pause), and mixed with the initial iron oxide-containing miniemulsion. Argon was bubbled for 10 min through the reaction mixture and after the addition of 20 mg of KPS, the system was heated to 80 °C under mechanical stirring. After 1 h, 50 mg of sodium *p*-styrenesulfonate were added and the reaction proceeded for further 14 h. After cooling down, the particles were washed and purified several times with water using a permanent magnet and discarding the supernatant. The precipitate was dispersed in 1.0 mL of an aqueous SDS solution (0.2 wt.%) and diluted with 5.0 mL of water (solid content = 1.6 wt%, inorganic content 75 wt% determined by TGA, M_w = 24,100 g mol^{-1}, polydispersity index – PDI = 2.0).

Synthesis of magnetic polystyrene nanoparticles labeled with a fluorescent dye

Fluorescently labeled magnetic particles were prepared following the same procedure as described above. The oleate-capped iron oxide nanoparticles (1.0 g) were mixed with 1.0 mg of the boron-dipyrromethene BODIPY dye (Fig. S1) and dispersed in 1.0 g of octane in an ultrasonication bath during 30 min. The rest of the procedure was kept unchanged.

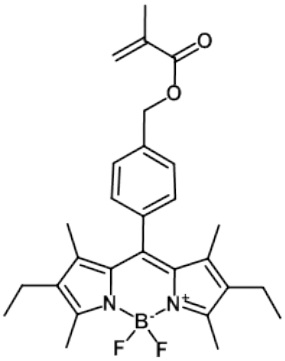

Fig. S1. Molecular structure of the fluorescent BODIPY dye used to label the particles, λ_{ex} 523 nm, λ_{em} 536 nm.

Silica coating of magnetic Fe_3O_4/polystyrene nanoparticles

Silica coating on the magnetic iron oxide/polystyrene nanoparticles was performed by the Stöber method. Under the selected experimental conditions, a homogeneous silica shell of *ca*. 40 nm around the particles was attained (sample **1b**, Table 1). However, the shell thickness can be adjusted by changing the initial amount of TEOS and the pH of the reacting medium (samples **1a-c**, Table 1).

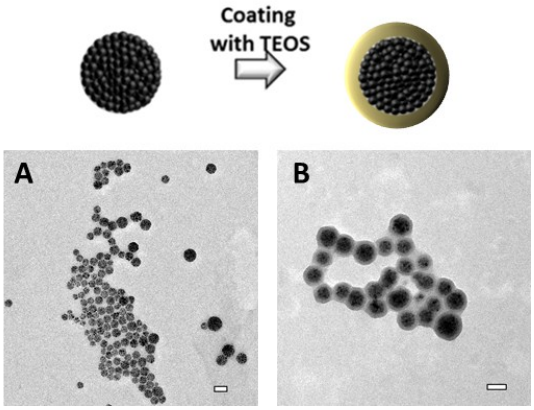

Fig. S2. TEM images of magnetic hybrid nanoparticles before (A) and after (B) coating them with a silica shell of ca. 40 nm (sample **1b**). The scale bars represent 100 nm.

Titration of the available reactive functional groups

The amount of surface-available amino groups was estimated by titration with fluorescamine (Fig. S3A). On the other hand, for the estimation of the available alkene groups a thiol-ene reaction with cysteamine was performed (Fig. S3B). The amount of introduced NH_2 groups with the cysteamine gives a trend for the estimation of the number of alkene groups present on the surface of the nanoparticles.

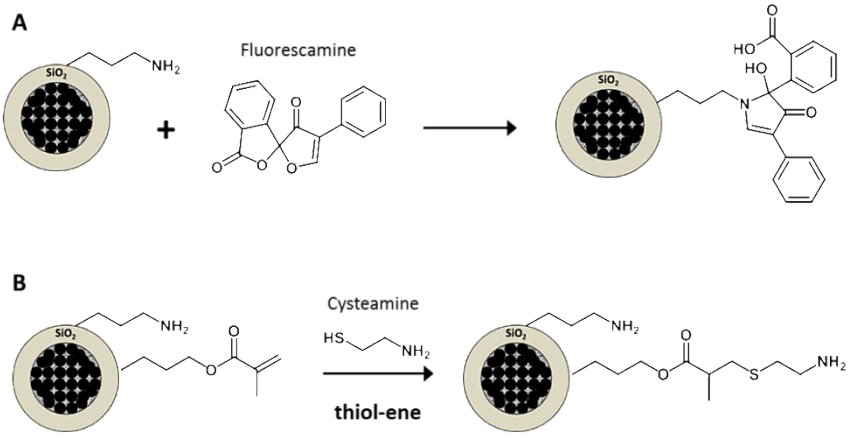

Fig. S3. (A) Quantification of NH_2 groups was performed by titration with fluorescamine, which reacts selectively with primary amines to produce a fluorescent compound (λ_{ex} = 420 nm, λ_{em} = 480 nm). (B) The amount of alkene groups can be assessed by a thiol-ene reaction with cysteamine.

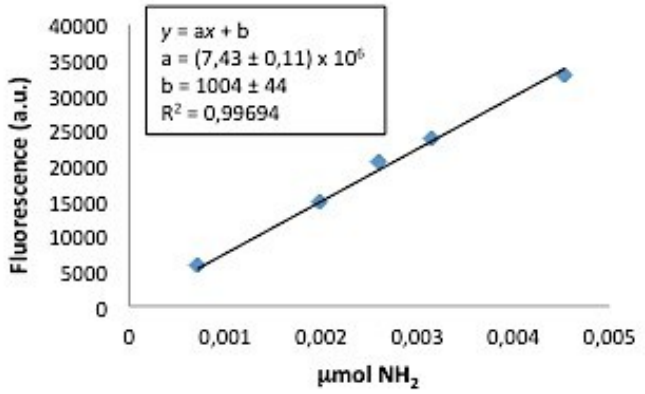

Fig. S4. Calibration curve from APTES for the estimation of accessible amino groups present on the surface of the silica particles.

Table S1. Estimation of the amount of reactive amino and alkene groups present on the surface of the multifunctional silica particles by fluorescence measurements.

Entry	Functionalization[a]			μmol amino groups / mg particles[b]	μmol alkene groups / mg particles[c]
	APTES	TPM	Betaine		
2a	1 %	-	-	0.04 ± 0.03	-
2b	2 %	-	-	0.08 ± 0.05	-
2c	3 %	-	-	0.12 ± 0.05	-
3a	2 %	10 %	-	0.09 ± 0.05	0.51 ± 0.05
4a	2 %	10 %	15 %	0.07 ± 0.02	0.34 ± 0.09
4b	2 %	10 %	37,5 %	0.06 ± 0.01	0.35 ± 0.08

[a] Initial amounts of alkoxysilanes used for the surface functionalization in mol% respect to the TEOS amount used for the silica coating. [b] Estimated amount of amino groups determined by titration with fluorescamine. The error bars represent standard deviation of the values obtained from three independent samples. [c] Estimation of alkene groups carried out by thiol-ene reaction with cysteamine and quantification of resulting amino groups as before.

The amount of surface-available amino groups determined here shows good agreement with previous reports (references 54-55 of the main text). The values are in the same order of magnitude (ca. 0.02 μmol amino/mg particles) as already determined with the fluorescamine assay in silica nanoparticles, using APTES solutions as standards for the calibration curve.

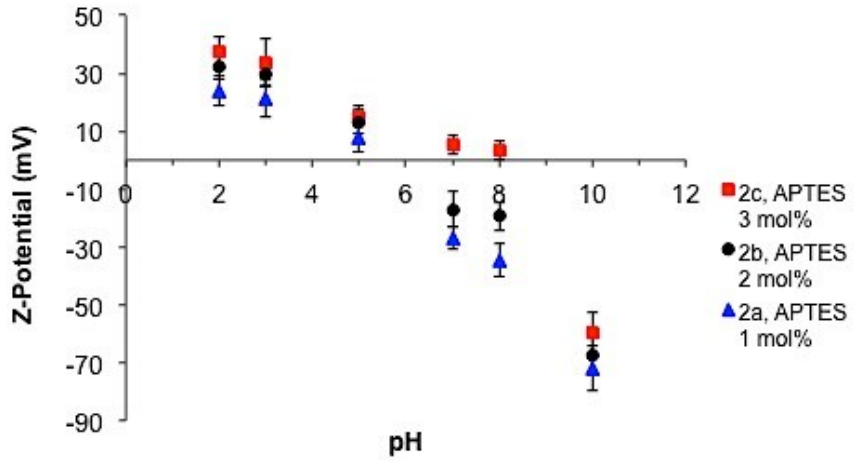

Fig. S5. Surface charge at different pH values determined by zeta potential measurements for the amino functionalized samples **2a-c**.

DLS data of multifunctional particles mixed with serum

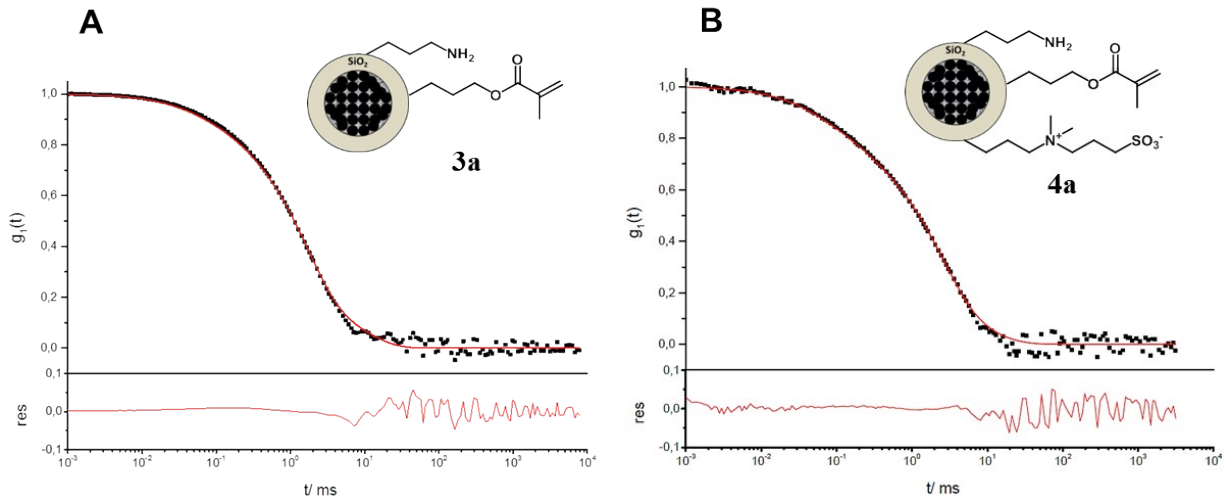

Fig. S6. Upper graphs: autocorrelation functions $g_1(t)$ (black squares) at $\theta = 90°$ of multifunctional particles **3a** (A) and **4a** (B) mixed with FBS. The red line represents the forced fit generated from the sum of the individual components. Lower graphs: corresponding residuals resulting from the difference between the fit and the data points.

Zeta potential data of multifunctional particles mixed with BSA and lysozyme

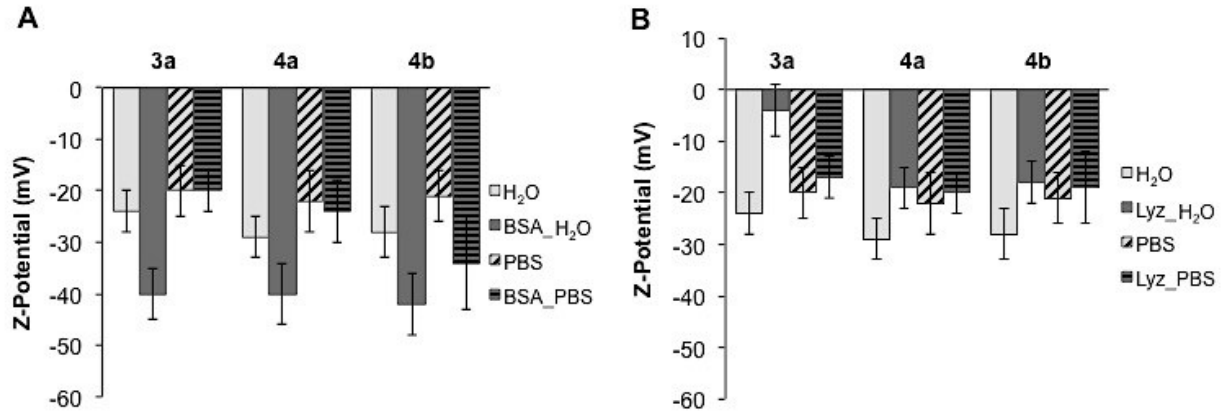

Fig. S7. Zeta potential of multifunctional amino/alkene particles without (**3a**) and with zwitterionic surface functionality (**4a-b**), measured in water (H_2O) and in phosphate buffer (PBS). Changes in the surface charge are elicited after incubation of the particles with BSA (A) and with lysozyme (B).

The net surface charge of the amino/alkene nanoparticles **3a** remains approximately unchanged when the zwitterion is added to the surface (samples **4a-b**). However, the functionalization with betaine gives a slightly more negative zeta potential in comparison to the sample **3a**, possibly because less amino groups are present on the surface of the particles **4a-b**. The surface charge of all the particles depends on the nature of the medium in which the dispersions are prepared. In general, the surface charge becomes more negative after incubation with BSA (Fig. S7A) and more positive when incubated with lysozyme (Fig. S7B) for dispersions in water and phosphate buffer.

Aggregation kinetics multifunctional particles

The multifunctional particles were mixed with BSA and lysozyme and their aggregation rates were determined by time resolved DLS measurements. None of the particles **4a-b** and **5a** showed evident aggregation in the buffer or in the presence of the protein. As an example, Fig. S5 shows the data for the particles **4a** dispersed in PBS buffer (red curve) and after the addition of lysozyme (black curve). These samples were characterized as kinetically inert.

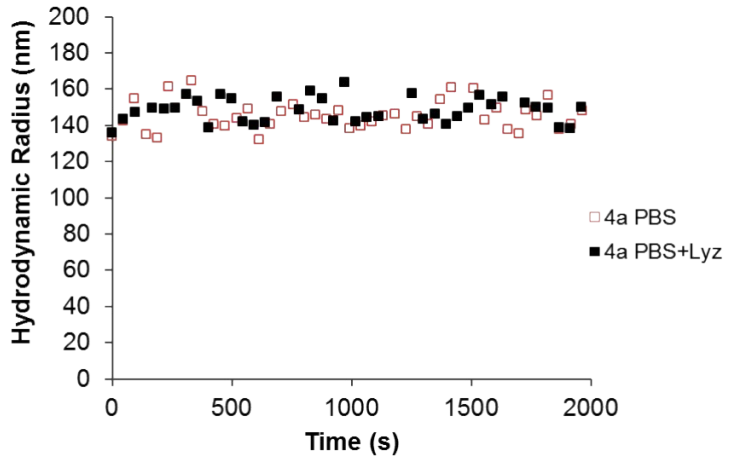

Fig. S8. Time resolved dynamic light scattering of dilute **4a** nanoparticle suspensions, in the presence (solid black symbols) and absence (open red symbols) of lysozyme.

Synthesis of azide mouse IgG derivative

To 360 μL of mouse IgG antibody (2.5 mg mL^{-1}, Life Technologies), were added 2.0 μL of HCl 0.1 M, reaching a pH value of 7.6. Afterwards, 0.018 μmol of an NHS ester azide derivative (NHS-PEG_4-N_3, in anhydrous DMSO) were added and the mixture was stirred at 25 ºC for 1 h.