Supplementary Material (ESI) for Nanoscale

Supporting Information

Rapid detection of trace amounts of surfactants using nanoparticles in fluorometric assays

Harri Härmä*[a], Susana Laakso[a], Sari Pihlasalo[a], Pekka Hänninen[a]
Bertrand Faure[b], Subhasis Rana[b], Lennart Bergström[b]

[a] Laboratory of Biophysics and Medicity, University of Turku, Tykistökatu 6A,FI-20520 Turku, Finland
[b] Materials Chemistry Research Group, Dept. of Physical, Inorganic and Structural Chemistry, Stockholm University, SE-106 91, Stockholm, Sweden
[c] Sensor and Actuator Division, Central Glass & Ceramic Research Institute, Kolkata 700 032, India
* Corresponding author

Materials: All chemicals were commercially available. The following surfactants were studied: SDS (sodium dodecyl sulphate from Fisher Scientific, Loughborough, UK), Tween 20 (Polysorbate 20, from AppliChem, Darmstadt, Germany), Triton X-100 (from Fluka, St. Louis, MO) and CTAB (cetyl triammonium bromide, from Fluka). Europium-chelate-doped FluoroMax™ particles with a reported diameter of 92 nm were purchased from Seradyn Inc. (Indianapolis, IN). N-hydroxysuccinimide-activated Alexa Fluor 680 was a product of Invitrogen (Carlsbad, CA). $FeCl_3.6\ H_2O$, sodium-oleate, oleic acid, bovine serum albumin (BSA) and bovine gammaglobulin (IgG) were obtained from Sigma-Aldrich (St. Louis, MO). Dipyrrylmethene-BF_2 530 was prepared in our laboratories as described previously.[1] The NAP-5 column was from GE Healthcare (Uppsala, Sweden). The reaction buffer throughout the study was 5.0 mM phosphate-buffered citrate with a pH of 4.0. The PBS buffer used as a medium during the labeling was from Lonza (Walkersville, MD).

Instrumentation:
Nanoparticle separation was carried out with a Beckman LK-80 ultra centrifuge (Beckman-Coulter, Fullerton, CA). An ultrasound horn (Vibra cell VCX 130, SONICS) was used for the phase transfer of the maghemite nanocrystals. High resolution transmission electron microscope (TEM) images of the maghemite nanoparticles were obtained using a JEOL JEM-3010 microscope operating at 300 kV (Cs = 0.6 mm, point resolution 0.17 nm). Thermogravimetric analysis data were collected using a Perkin Elmer Thermogravimetric Analyzer TGA-7 at the heating rate of 5 ^{o}C/min in air. The FTIR spectra were recorded with a Bruker IFS55 spectrometer in transmission mode. The Eu-PSCOOH particles were imaged with a JEOL JSM-7000F scanning electron microscope operating at 1.5kV.

The fluorescence was monitored with a 1420 multilabel counter VICTOR^2 (PerkinElmer, Wallac, Turku, Finland). Fluorescence energy transfer was measured with a red-sensitive photomultiplier tube (R2949, Hamamatsu, Japan).

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Synthesis, characterization and preparation of the water-dispersible iron oxide maghemite nanoparticles:

The synthesis of maghemite nanoparticles is a modification of the non-hydrolytic thermal decomposition method [2] where a precursor, iron (III) oleate (synthesized from iron chloride and sodium oleate), was thermally decomposed by heating in 1-octadecene in the presence of oleic acid. The obtained oleic-acid capped maghemite nanocrystals were separated from the solvent using addition of excess ethanol followed by centrifugation at 30 000 rpm for 30 min at room temperature, yielding a black nanoparticles concentrated paste.

The oleic acid capping agent was removed from the surfaces of the maghemite iron oxide nanoparticles by a controlled leaching and phase transfer procedure. The oleate-capped iron oxide nanoparticles were dispersed in equal volumes of tetrahydrofuran (THF) and nitric acid at pH 1. The two-phase dispersion was mixed for 60 minutes with the ultrasonic horn, allowed to phase separate and the aqueous phase was retrieved with a separatory funnel. The concentration of nanoparticles in the aqueous dispersion was determined by thermogravimetric analysis from the residual mass at 550°C. The complete removal of the oleate capping upon phase transfer was confirmed by FTIR spectroscopy from the disappearance of the C=O absorption band at 1710 cm^{-1} (Fig. S1).

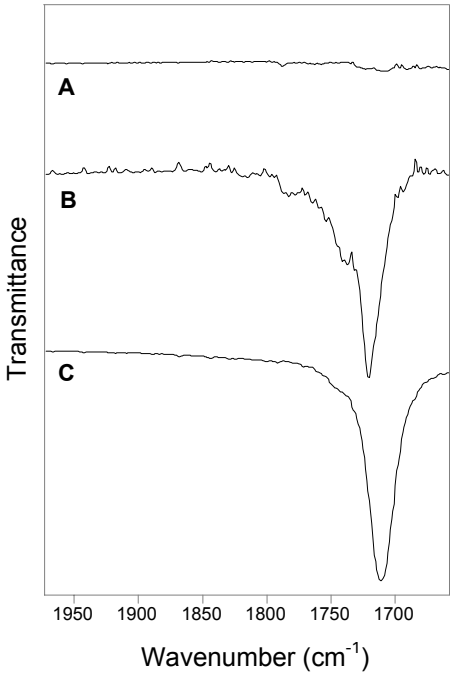

Fig. S1 FTIR spectra of the transferred nanoparticles collected by drying the aqueous dispersion (A); the oleate-capped maghemite nanoparticles dispersed in 1-octadecene (octadecene spectrum subtracted) (B); and pure oleic acid (C). The difference in maghemite concentration between the spectra a and b was compensated by matching the transmittance at 630 cm^{-1} corresponding to Fe-O lattice vibration.[3]

Protein labeling: N-hydroxy succinimide-activated Alexa Fluor 680 was conjugated to gammaglobulin (500 µg) using a 30-fold excess of Alexa dye dissolved in DMF. The

reaction was carried out in 100 µl of 50 mM phosphate buffer pH 7.4 for 30 min. Non-conjugated dye was removed using a NAP-5 column and the labeled IgG-Alexa680 was eluted with PBS. N-hydroxy succinimide-activated dipyrrylmethene-BF_2 530 was coupled to BSA (BF_2 530-BSA) as described previously.[4]

Surfactant detection: The excitation and emission wavelengths for the Alexa Fluor 680 acceptor label were 340 and 730 nm and the measurement delay and integration times were 75 and 50 µs, respectively. The fluorescence of BF_2 530 was detected at 572 nm using excitation wavelength of 530 nm. All measurements were performed in a citrate-phosphate buffer, 5.0 mM, with a pH at 4.0 in duplicates or triplicates and an average of the results is presented. The fluorescence signal intensity decrease or increase of 10% from the fluorescence intensity of the zero concentration was set as a detection limit in the Eu-PSCOOH or maghemite sensors, respectively.

The preparation of the dispersions for the fluorescence energy transfer measurements using the Eu-PSCOOH nanoparticles were carried out in microcentrifuge tubes with a total volume of 175 µl. Solutions with a know concentration of surfactants were performed from a stock solution by dilution with sodium citrate buffer, to a total volume of 150 µl. This solution was mixed with 20 µl of the nanoparticle dispersion containing 2×10^7 Eu nanoparticles. Thereafter, 1.5 ng of IgG-Alexa680 (5 µl) were added to the dispersion, mixed well before 70 µl of the dispersion was transferred to a microtiter plate well for the time-resolved fluorescence measurement.

The dispersions for the measurements using maghemite nanoparticles were prepared by mixing 5 µl of maghemite dispersion containing 4×10^{11} nanocrystals with 175 µl of surfactant in sodium citrate buffer. 20 µl of the fluorescent protein, BF_2 530-BSA, having a concentration of 5.9 nM, was added to the suspension and mixed thoroughly before 70 µl of the mixture was retrieved and transferred to a 96-well microtiter plate for the fluorescence measurement.

Control experiments on dispersions of Eu-PSCOOH nanoparticles containing high concentration of surfactants without the acceptor-labeled protein resulted in a negligible change in the fluorescence intensity at 730 nm, compared with the strong signal change observed when nanoparticle – fluorescently-tagged protein pair is utilized.

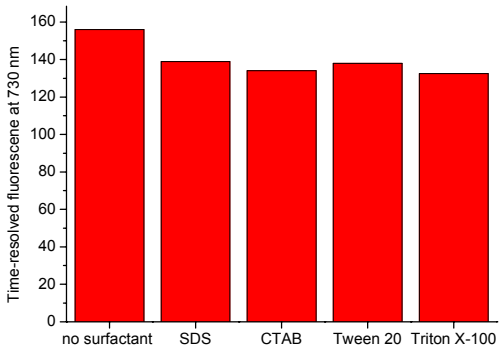

Fig. S2. Time-resolved fluorescence signal stemming from the Eu(III)-doped polystyrene nanoparticles in the absence of dye-labeled proteins. Very high surfactant concentrations (1 $g.l^{-1}$ of each surfactant) were added to the nanoparticle dispersions in the control experiments and compared to the fluorescence in a surfactant-free solution.

References:

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