Electronic Supplementary Information for:

Straightforward phase-transfer route to colloidal iron oxide nanoparticles for protein immobilization

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This ESI file includes a detailed description of experiment and characterization methods, and Figs. S1 to S8.

Experimental

Starting materials

Iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, 99%, Sigma-Aldrich), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, 99%, Sigma-Aldrich), sodium oleate ($C_{18}H_{33}NaO_2$, 82%, Sigma-Aldrich), oleic acid ($C_{18}H_{34}O_2$, 99%, TCI), aqueous ammonium hydroxide solution (NH_4OH, 28-30%, Sigma-Aldrich), cyclohexane (C_6H_{12}, 99.8%, Sigma-Aldrich), *n*-hexane (C_6H_{14}, 99%, Sigma-Aldrich), 1-octadecene ($C_{18}H_{36}$, 90%, Sigma-Aldrich), absolute ethanol (C_2H_5OH, analytical reagent grade, Fisher Scientific), potassium hydroxide (KOH, 90%, Sigma-Aldrich), 2-propanol ($(CH_3)_2CHOH$, 99.8%, Sigma-Aldrich), *n*-(trimethoxysilylpropyl)ethylene diamine triacetic acid (COOH–silane, 45% in water, ABCR), toluene ($C_6H_5CH_3$, 99.8%, Sigma-Aldrich), trimethylamine ($(CH_3)_3N$, 99.5%, Sigma-Aldrich), acetone (CH_3COCH_3, 99.5%, Sigma-Aldrich) potassium bromide (KBr, 99%, IR grade, Acros), sodium phosphate dibasic (Na_2HPO_4, 99%, Sigma-Aldrich), sodium phosphate monobasic dihydrate ($NaH_2PO_4{\cdot}2H_2O$, 99%, Sigma), 2-(*N*-morpholino)ethanesulfonic acid (MES, 99%, Sigma-Aldrich), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, Thermo Fisher Scientific) and *N*-hydroxysulfosuccinimide (sulfo-NHS, Thermo Fisher Scientific), bovine serum albumin (BSA, Sigma-Aldrich) were used as received without further purification. Ultrapure water having pH 5.3 used in the study was produced using a Milli-Q Advantage A10 system (Millipore).

Hydrothermal synthesis

Hydrothermal synthesis of hydrophobic nanocolloid. Colloidal Fe_3O_4 NPs were prepared via hydrothermal synthesis followed by washing and purification. A glass vial was charged with sodium oleate (0.500 g, 2 mmol), 8 mL of freshly degassed water and a magnetic stir bar. The vial was closed, placed on a hot plate set at 50 °C, and the mixture was stirred until complete dissolution of sodium oleate was observed, forming yellowish transparent solution. In parallel, a PTFE cup (total volume 45 mL) was loaded with $FeCl_2{\cdot}4H_2O$ (1.590 g, 8 mmol), $FeCl_3{\cdot}6H_2O$ (3.784 g, 14 mmol), 10 mL of freshly degassed water and a magnetic stir bar. The solution was stirred until complete dissolution of iron precursors was achieved, forming a dark-brown transparent solution. After removing the magnetic stir bars, the solution of sodium oleate was slowly poured into the PTFE cup with the Fe-containing solution, forming a light brown milky suspension. Next, 15 mL of concentrated aqueous ammonium hydroxide solution was added to the suspension to initiate the coprecipitation reaction turning the mixture black. The PTFE cup with the resultant black suspension was sealed by a PTFE cover, and fitted into a 4744 general purpose acid digestion vessel (Parr Instrument). The vessel was hand shaken and placed into the heating oven. Best results were achieved when the reaction was performed at 200 °C for 24 h under an autogenous pressure.

Washing and purification of hydrothermally-synthesized hydrophobic nanocolloid. After cooling to room temperature (RT), the black product of the hydrothermal synthesis was collected by centrifugation at 9,000 rpm for 10 min and washed by water to remove water soluble byproducts. Specifically, the resultant pellet from centrifugation was treated in 150 mL of water using propeller EUROSTAR (IKA) stirring at 500 rpm for 30 min with 15 min ultrasonication in-between using an Elmasonic P bath (Elma). Black suspension thus obtained was centrifuged at 9,000 rpm for 1 h. The resulting solid was washed 2 more times with water using the same workup as described above, to yield a black wet solid mass. This mass was dried *in vacuo* overnight at RT to give a black solid.

The as-derived NPs were further purified from extra oleate by ethanol and from non-dispersible solids via centrifugation. Notably, due to the presence of large ferrimagnetic NPs in the sample at this stage of the synthesis, extraction of the product using a permanent magnet (instead of centrifugation) could not be used to avoid low yield of the colloidal NPs. Instead, the resultant black powder was redispersed in 50 mL of cyclohexane using a propeller stirring at 500 rpm for 30 min followed by 30 min ultrasonication to produce a black NPs dispersion. This dispersion was added to 150 mL of ethanol to precipitate the NPs, followed by 5 min ultrasonication. The NPs were collected by centrifugation at 6,000 rpm for 10 min. This purification yields dark-brown wet solid mass. This mass was dried *in vacuo* for 15 min at RT and redispersed in 30 mL of cyclohexane using ultrasonication. The resulting NP dispersion was purified from non-dispersible solids (large NPs, aggregates) by centrifugation at 3,000 rpm for 10 min, which afforded 0.914 g (68% yield) of colloidal superparamagnetic Fe_3O_4 NPs as a black stock dispersion in cyclohexane. The as-synthesized sample is labeled as **OL-HT**, where **OL** stands for oleate capping and **HT** stands for hydrothermal method of the synthesis.

Thermal decomposition synthesis

Monodisperse hydrophobic nanocolloids with different particle sizes were prepared by a modification of the thermal decomposition of iron(III) oleate complex, $Fe(OL)_3$, procedure outlined by Park and co-authors [Ref. 9, MS].

$Fe(OL)_3$ preparation. A 1-necked 500 mL bottom round flask was charged with a stir bar, 140 mL of hexane, 80 mL of absolute ethanol, and 60 mL of water. Next, 31.5g sodium oleate and 10.8 g $FeCl_3{\cdot}6H_2O$ were added in small portions while stirring. After complete dissolution of the starting reagents, the solution turns to shiny black. Next, the flask was immersed in a silicon oil bath and attached to a condenser. The system was heated to 70 °C and allowed to reflux at this temperature while stirring for 4 h. Afterwards, the heating bath was removed and the solution was allowed to cool to RT while stirring. The contents of the flask were transferred to a separatory funnel and the $Fe(OL)_3$ product was rinsed one time with hexane into the funnel. Black organic and muddy aqueous layers were separated, and then the organic layer was washed three more times with 200 mL of water. Finally, the solvent from the resultant organic extract was removed by rotary evaporation at 50 °C under reduced pressure, resulting in stable viscous brown product. The formation of $Fe(OL)_3$ was confirmed by FTIR spectroscopy.

Thermal decomposition synthesis of monodisperse hydrophobic nanocolloids. A 3-necked 50 mL round bottom flask was charged with glass-coated stir bar and (*i*) 1.0 g of $Fe(OL)_3$, 0.15 g of oleic acid, and 5 mL of 1-octadecene for synthesis of 9.3-nm iron oxide NPs, (*ii*) 2.0 g of $Fe(OL)_3$, 0.6 g of oleic acid, 10 mL of 1-octadecene for synthesis of 13.2-nm iron oxide NPs, and (*iii*) 2.0 g of $Fe(OL)_3$, 0.15 g of oleic acid, 5 mL of 1-octadecene for synthesis of 15.8-nm iron oxide NPs. The central neck of the flask was equipped with a condenser, and connected to the 5-port Airfree Schlenk inert gas vacuum manifold (Chemglass) via rubber tube using side-port stopcock adapter. Right-side neck of a 3-necked reaction flask was covered with a rubber septum, and then K-type thermocouple (Omega) was immersed into the reaction mixture through the septum. During the experiment, the tempering was realized by heating mantle controlled by CSi8DH series benchtop temperature controller (Omega). Left-side neck of a 3-necked reaction flask was equipped with straight stopcock adapter and connected to the Schlenk line using rubber tube.

The reaction mixture was degassed under high vacuum at 120 °C for 1 h employing constant magnetic stirring, and then was kept under an argon atmosphere throughout the experiment. After degasing, the resultant brown solution was quickly heated to 320 °C, and kept at this temperature while vigorously stirring for 30 min. Under this conditions, the thermal decomposition of $Fe(OL)_3$ is taken place, encouraging the formation of monodisperse iron oxide NPs. Next, the heating mantle was removed and the resultant black hot reaction mixture was naturally brought to RT to afford a back oil-like mixture product. This product was diluted with hexane, ultrasonicated for 5 min, transferred from the flask to the beaker, and then the iron oxide NPs were precipitated by 200 mL of ethanol. The NPs were collected by centrifugation at 8,000 rpm for 10 min, and washed copiously three times. The washing includes redispersion of the NPs in 20 mL of hexane, their precipitation by 60 mL of ethanol with subsequent isolation by centrifugation at 8,000 rpm for 10. The as-washed NPs were redispersed in 10 mL of cyclohexane, and finally purified from non-dispersible solids by centrifugation at 3,000 rpm for 10 min, which afforded colloidal hydrophobic iron oxide NPs as a black stock dispersion in cyclohexane.

Procedure for base-bath-assisted phase transfer

The phase transfer of magnetite NPs from apolar solvent to aqueous solution was accomplished by novel base-bath-assisted procedure. For this purpose, 5 mL of stock dispersion of hydrophobic **OL-HT** iron oxide NPs in cyclohexane (20 g/L) was placed into a 50 mL PTFE centrifugation tube. Then 40 mL of base-bath cleaning solution (5 L of water, 5 L of 2-propanol, 500 g of KOH) was added to PTFE tube and the resultant mixture was ultrasonicated for 30 min with brief stirring in-between using a vortex mixer (VWR). Next, the mixture was left at RT for 24 h, after which time the NPs were collected by centrifugation at 6,000 rpm for 10 min. The resultant brown solid mass was washed from the byproducts via redispersion in ethanol (3×50 mL) followed by centrifugation at 6,000 rpm for 10 min. Finally, the recovered product was redispersed in 20 mL of water, and purified from non-dispersible solids by centrifugation at 3,000 rpm for 10 min, which afforded aqueous magnetic nanocolloid in 69% yield. The as-prepared aqueous nanocolloid is labeled as **OL-HT-BB**, where **BB** stands for base bath.

The control experiments of KOH base-bath-assisted phase transfer of hydrophobic monodisperse iron oxide NPs synthesized by thermal decomposition method was carried out in the same manner as in the case of the described above **OL-HT-BB** nanocolloid.

Procedure for reference COOH-terminated NPs

The reference COOH-terminated aqueous magnetic nanocolloid for control EDC/sulfo-NHS covalent protein immobilization experiment was prepared by the phase-transfer protocol adapted from that of Bloemen and co-workers (Ref.

17, MS). This protocol is based on oleate ligand exchange with COOH–silane, namely, *n*-(trimethoxysilylpropyl)ethylene diamine triacetic acid. Briefly, 5 mL of **OL-HT** stock dispersion in cyclohexane (20 g/L) was added to 50 mL of toluene in a 100 mL PYREX bottle, and the solution was ultrasonicated for 1 min. Next, 2.5 mL of trimethylamine 0.05 mL of water and 0.5 mL of COOH–silane were added, and the mixture was ultrasonicated for 5 h at 50 °C. The resultant suspension was diluted with 50 mL of cyclohexane. The NPs were collected by a NdFeB permanent magnet, and were washed with acetone (3×10 mL). The resultant product was dried for 10 min *in vacuo*. Finally, the recovered product was redispersed in 10 mL of water, and purified from non-dispersible solids by centrifugation at 3,000 rpm for 10 min, which afforded aqueous magnetic nanocolloid in 78% yield. The as-prepared aqueous nanocolloid is labeled as **OL-HT-COOH**, where **COOH** stands for carboxylic termination of the NPs.

Immobilization of BSA onto NPs

OL-HT-COOH reference NPs for control experiment were mixed with freshly prepared EDC solution to activate carboxylic functional groups followed by the addition of freshly prepared sulfo-NHS. After a 30 min of incubation at RT while stirring, the mixture was centrifuged at 25,000 rpm for 15 min to eliminate the excess of EDC/sulfo-NHS reagents, and then redispersed in aqueous BSA solution at 4 °C for 3 h while stirring.

OL-HT-BB NPs were incubated in a similar manner but solely with the BSA solution (pH 5.3) without EDC/sulfo-NHS. The supernatant containing unanchored protein fraction was isolated by centrifugation at 25,000 rpm for 15 min. Further, this supernatant was used to estimate the amount of protein that interacted with the NPs by means of the Bradford protein assay. Statistical differences between the amounts of immobilized BSA in **OL-HT-COOH** and **OL-HT-BB** groups were estimated using Mann-Whitney test with 0.05 as criterion of significance.

Procedure for powder specimen preparation

For structural, spectroscopic, magnetic and thermogravimetric measurements, where specimens in form of the powder are required, the NPs were precipitated from the stock dispersion by excess of ethanol. The NPs were isolated by centrifugation at 6,000 rpm for 10 min, dried *in vacuo* overnight at RT, and ground using agate mortar and pestle.

Characterization

STEM

High-angle annular dark-field scanning transmission electron microscopy (HAADF–STEM) and electron energy loss spectroscopy in STEM mode (STEM–EELS) studies were performed using probe aberration-corrected Titan ChemiSTEM (FEI) electron microscope, operated at 200 kV and equipped with Gatan Energy Filter. The samples for TEM were prepared by dropping an ultrasonically dispersed diluted dispersion of the NPs onto a holey carbon-coated Au grid (400 mesh) followed by the evaporation of the solvent *in vacuo*.

TGA

The thermal behaviour of the products was studied by means of thermogravimetry and differential scanning calorimetry (TGA-DSC) using TGA/DSC 1 STAR^e system (Mettler-Toledo). The powdered samples were heated from 25 to 1000 °C at 10 °C/min under a continuous Ar flow of 50 mL/min. The concentration of NPs in the dispersions was also determined using TGA. The nanocolloids were heated from 25 to 130 °C at 3 °C/min and from 130 to 700 °C at 10 °C/min under a continuous Ar flow of 50 mL/min.

FTIR

Fourier transform infrared (FTIR) spectra were collected using VERTEX 80v (Bruker) and Nicolet 6700 (Thermo Scientific) spectrometers. For FTIR study of protein loading, the test samples were lyophilized from the respective aqueous solutions and subjected to the analysis. KBr was used as reference material.

Zeta potential

Zeta potentials of the diluted **OL-HT-BB** nanocolloid at different pH were measured at RT using SZ-100 nanoparticle analyser (Horiba). The data were averaged over three scans.

XRD

Powder X-ray diffraction (XRD) data were collected on a X'Pert PRO diffractometer (PANalytical) set at 45 kV and 40 mA, and equipped with Cu $K\alpha$ radiation (λ=1.541874 Å) and a PIXcel detector. Data were collected using Bragg-Brentano geometry in the 15 to 80° 2θ range with a scan speed of 0.006 °/s. The XRD patterns were matched to International Centre for Diffraction Data (ICDD) PDF-4 database using HighScore software package (PANalytical).

Raman spectroscopy

Raman spectroscopy experiments were performed at RT in a back scattering geometry on an alpha300 R confocal Raman microscope (WITec) using a 532 nm Nd:YAG laser for excitation. The system was operated with an output laser power of ca. 100 μW to avoid sample degradation due to laser-induced heating. The laser beam was focused on the powder by a ×50 lens (Zeiss); the spectra were collected with a 600 groove/mm grating using 100 acquisitions with a 5 s acquisition time.

VSM

Magnetic properties were measured on powdered samples using an EV7 (MicroSense) vibrating sample magnetometer (VSM). The magnetization data is presented in units of emu per gram of iron oxide [emu/g(iron oxide)], i.e., the masses of the test samples were corrected for the non-magnetic-shell contribution, e.g, oleate ligand. TGA was used to determine the mass of the shell present on a magnetic NPs. The powdered samples were heated from 25 to 700 °C K at 10 °C/min under a continuous Ar flow of 50 mL/min.

Tables

Table S1. Assignment of FTIR bands

Position (cm^{-1})	Assignment
Samples OL-HT / OL-HT-BB	
630, 585, 564	Fe–O
1096	–CH–O–
1384	–CH$_3$ umbrella deformation
1630	–COO$^-$
2850	–CH$_2$–CH$_3$
2925	–CH$_2$–CH$_2$–
2960	–CH$_3$
Sample OL-HT-BB-BSA	
630, 585, 564	Fe–O
1245	Amide III (C–N stretch N–H bend)
1397	C–O–H bend
1453	–CH$_3$
1541	Amide II (C–N stretch, N–H bend)
1652	Amide I (C=O stretch)
2850	–CH$_2$–CH$_3$
2925	–CH$_2$–CH$_2$–
2960	–CH$_3$ asymmetric stretch
3295	Amide A (N–H stretch)

Figures

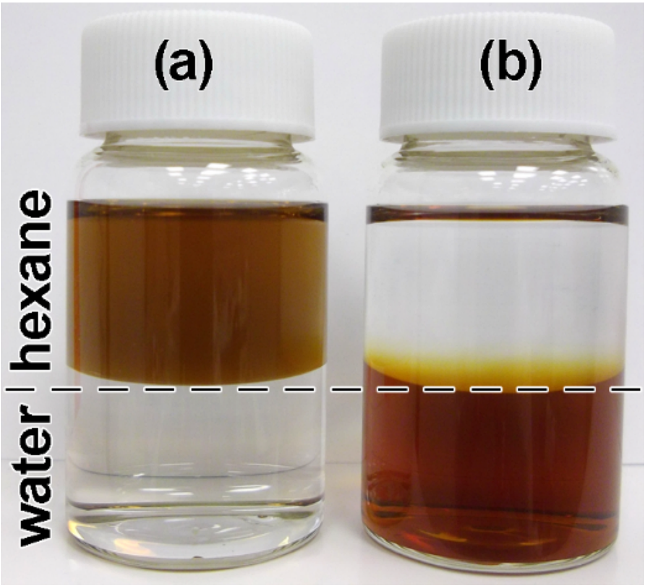

Fig. S1. Image showing initial hydrothermally-prepared OL-coated **OL-HT** magnetite NPs (a) and the resultant **OL-HT-BB** NPs (b) after base-bath-assisted phase transfer, which form stable colloidal dispersions in hexane and water, respectively.

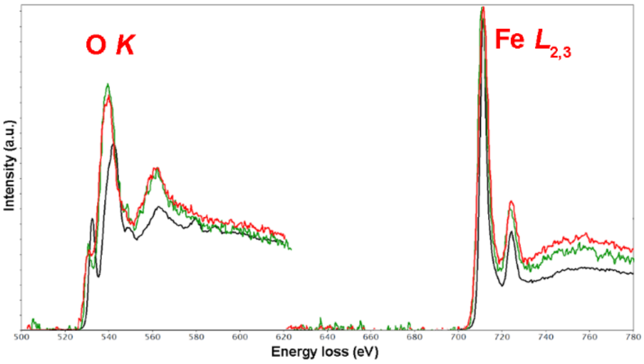

Fig. S2. Comparison of EELS spectra showing the O *K*-edge and the Fe $L_{2,3}$-edges of initial **OL-HT** NPs (green) and phase transferred **OL-HT-BB** NPs (red), together with the reference EELS spectrum for γ-Fe$_2$O$_3$ from GATAN EELS database (http://www.gatan.com).

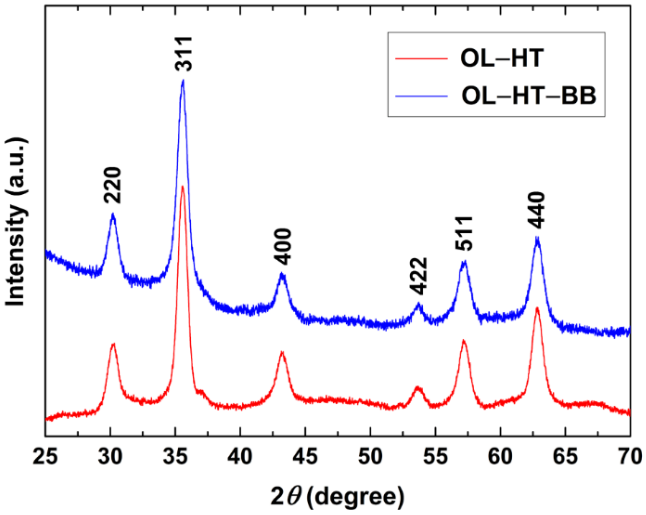

Fig. S3. Comparison of powder X-ray diffraction patterns of **OL-HT** and **OL-HT-BB** iron oxide nanocolloids, showing no significative differences.

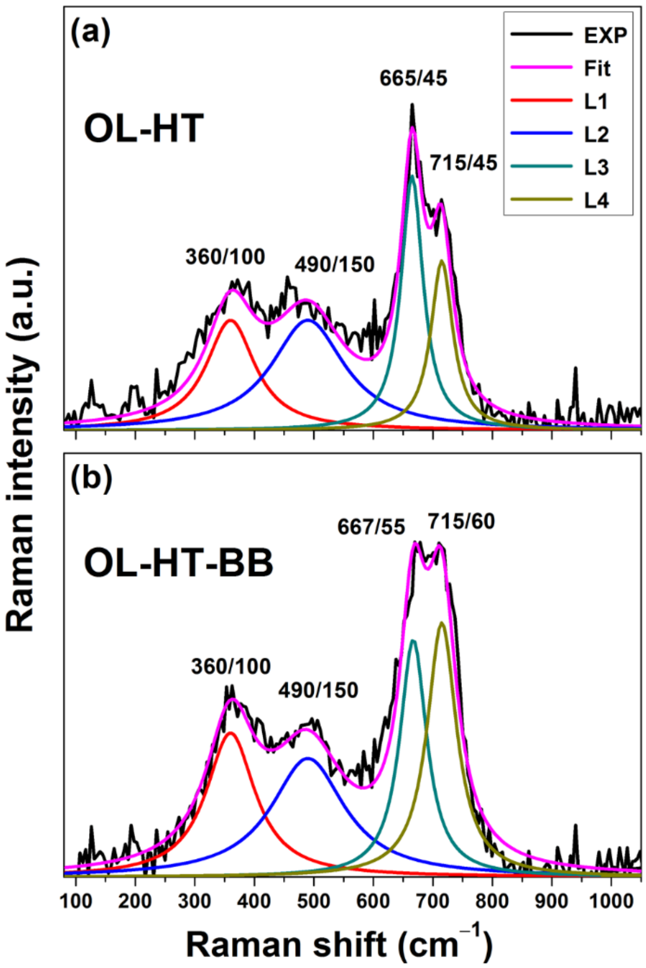

Fig. S4. Lorentzian fit (pink line) of the experimental Raman data (black line) for hydrophobic **OL-HT** (a) and hydrophilic **OL-HT-BB** (b) iron oxide samples. Peak positions (ω_o) and full width at half-maximum (FWHM) values are marked as ω_o/FWHM (cm^{-1}) for each component. Note the increase of the A_{1g} Raman mode associated to γ-Fe_2O_3 in the **OL-HT-BB**.

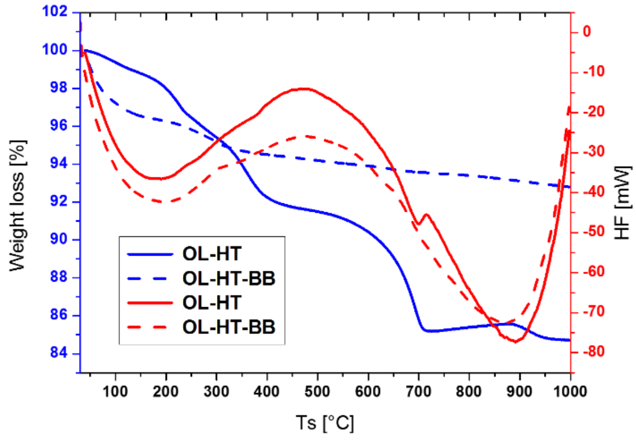

Fig. S5. Comparison of thermogravimetry and differential scanning calorimetry (TGA-DSC) analysis results for the powdered initial **OL-HT** and phase transferred **OL-HT-BB**, showing the intensive thermal degradation of oleate coating above 300 °C under Ar atmosphere in the case of **OL-HT** sample.

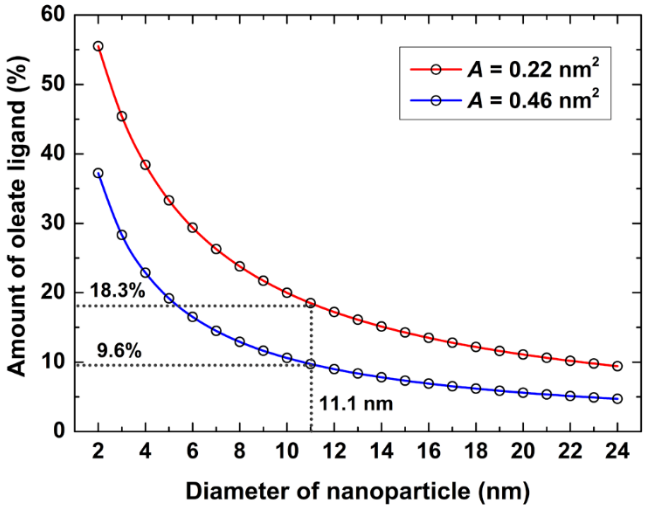

Fig. S6 Theoretically calculated amount of oleate surface capping as a function of the particle size. Two oleate configurations with different cross sectional areas of 0.22 and 0.46 nm^2 are used. The expected percentages of oleate capping for 11.1-nm **OL-HT-BB** NPs are indicated with the dotted lines.

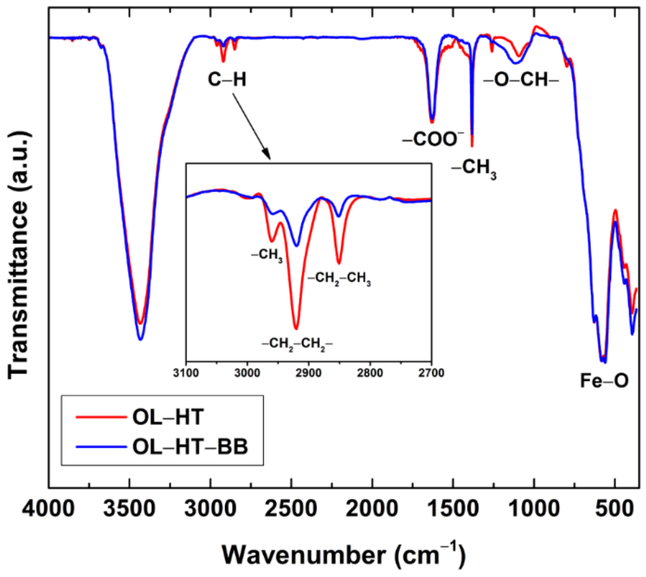

Fig. S7. Comparison of transmission spectra obtained by FTIR for the initial hydrophobic **OL-HT** nanocolloid and the resultant hydrophilic **OL-HT-BB** nanocolloid prepared by base-bath-assisted phase transfer. The enlarged region clearly shows weakening of characteristic oleate bands for **OL-HT-BB**, illustrating effective removal of the ligand by base-bath treatment.

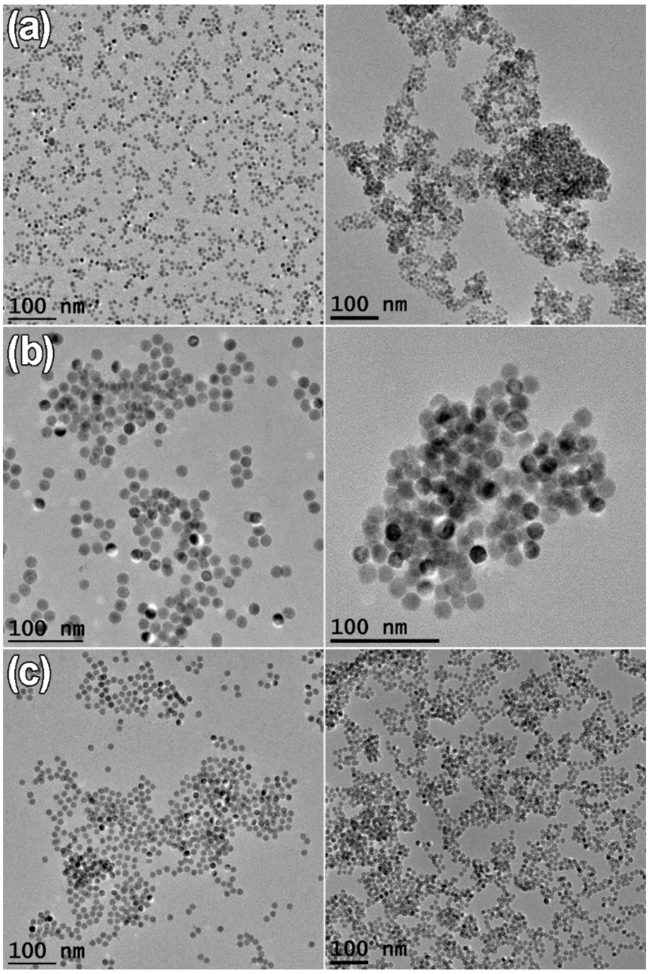

Fig. S8. Low magnification TEM images showing a series of control phase-transfer experiments for monodisperse iron oxide NPs with sizes of 9.3 ± 1.2 nm (a), 8.7 ± 1.2 nm (b), 13.2 ± 1.1 nm (c), 13.2 ± 1.4 nm (d), 15.8 ± 1.7 (e), and 14.8 ± 1.7 nm (f) prepared by thermal decomposition of $Fe(OL)_3$. Left panels (a, c, e) demonstrate the initial hydrophobic NPs, whereas right panels (b, d, f) show the respective NPs after phase transfer to aqueous media using base-bath treatment. The confidence levels are 95.45%, i.e. 2σ.