Supporting Information

Evaluation of high-yield purification methods on monodisperse PEG-grafted iron oxide nanoparticles

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Materials

Iron(0)pentacarbonyl (99,99% trace metal basis), oleic acid (≥93% technical grade), dioctyl ether (>99%), dopamine hydrochloride (≥98%), sulfuric acid (95-98%), sodium nitrite (≥99%), 4-Methyl-morpholine (99% ReagentPlus), (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU, 97%), hydrochloric acid (37% ACS reagent), Sephadex G75 (superfine), bovine calf serum (sterile filtered, for cell cuture) and phosphate buffered saline (PBS 10x concentrate BioPerformance certified) were purchased from Sigma Aldrich, Methoxy-PEG acetic acid (MeO-PEG-COOH, Mw 5000) from JenKem Technology, EtOH (>96% technical grade), DMF (>99,9% ACS reagent), CHCl3 (≥99.5% containing 100-200ppm amylenes as stabilizer), n-hexane (≥95% chromasolv plus for HPLC) and petroleum ether (30-50°C bp, p.a.) were obtained from Carl Roth. Ultrapure Milli-Q water (Millipore USA, R=18MΩcm) was used for all experiments. 1000kD MWCO Float-A-Lyzer regenerated celluose dialysis membranes were purchased from Spectrum Labs Netherlands.

All chemicals were used as received without further purification.

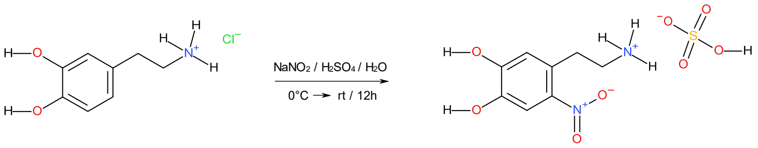
Experimental Details and spectral data

Synthesis of oleic acid-capped iron oxide cores

Table S1. Iron oxide cores synthesized using a slightly modified method developed by Hyeon et al.[1] Core size was controlled by varying the amount of precursor to surfactant.

NP diameter (nm)	$Fe(CO)_5$: OA (molar ratio)
3.5 ± 0.3	1:1.6
4.9 ± 0.3	1:1.9
7.1 ± 0.4	1:2.5
9.6 ± 0.4	1:3.9

Synthesis of 6-Nitrodopamine-Hemisulfate



NDA was synthesized using a slightly modified procedure published earlier.[2] 5g dopamine hydrochloride (DA-HCl) and 6.3g sodium nitrite ($NaNO_2$) were dissolved in 150mL deionized water and cooled in an ice/NaCl bath. 25mL of 20% sulfuric acid were added dropwise under vigorous stirring to the cooled solution maintaining the temperature below 10°C. After complete addition the reaction mixture was slowly warmed to room temperature and reacted overnight. The resulting yellow precipitate was collected by filtration and washed excessively with ice-cold water, once with MeOH and a 50:50 mixture of $EtOH:Et_2O$. NDA-HSO_4 was obtained as an intense yellow powder in 65% yield. NDA-HCl was characterized by ^{1}H and ^{13}C-NMR, MS, IR and UV/Vis

^{1}H-NMR (d_6-DMSO, 200MHz, ppm): 7.86 (s b, 1H, OH), 7.54 (s, 1H, Ph), 6.80 (s, 1H, Ph), 5.92 (s b, 1H, OH), 3.04 (s, 4H, CH_2)

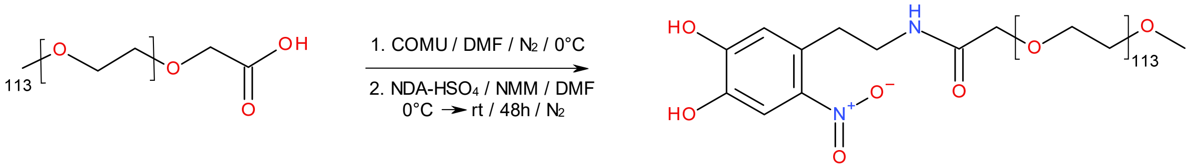
^{13}C-NMR and DEPT (d_6-DMSO, 200MHz, ppm): 151.80 (C), 144.63 (C), 139.47 (C), 125.91 (C), 118.47 (CH), 112.52 (CH), 39.52 (CH_2), 30.97 (CH_2)

ESI-MS ($CH_3CN/NH_4^+COO^-$ m/z (%)): $[M]H^+$ = 199.11 (100); electrostat. aggregates: 397.19 (55), 495.16 (50), 693.24 (20), 791.19 (10), 891.31 (20), 989.26 (35), 1089 (10), 1187.34 10), 1385.47 (10) (20), 1286.34 (

IR (powder, cm^{-1}): 3479, 3175, 3043, 2946, 1624, 1600, 1540, 1512, 1493, 1437, 1411, 1376, 1338, 1311, 1283, 1251, 1202, 1146, 1068, 1034, 978, 949, 906, 882, 853, 810, 753, 674, 621, 603, 557, 451

UV/VIS (MeOH, nm): 246, 305 , 353 , 425

Synthesis of PEG-NDA



6-Nitrodopamine-poly(ethylene glycol)(5kDa) (NDA-PEG) was synthesized via (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) mediated peptide-coupling reactions[3].

Typically 5 g MeO-PEG(5000)-COOH were dissolved in 30 mL DMF and pre-activated for 30 min at room temperature with 0.64 g COMU. The mixture was purged with N_2 for 20 min and cooled to 4°C. 0.24 g of NDA-H_2SO_4 were dissolved in 2 mL DMF and mixed with 257 µL n-

Methylmorpholine (nMM). After 10 min the amine containing solution was added dropwise to the activated PEG at 4°C under N_2. The solution was slowly warmed to RT and reacted for 48h. 90mL 3M HCl were added to the mixture and extracted 3x with $CHCl_3$. The solvent was removed under reduced pressure and the product was collected as a light yellow powder in 58% yield verified by ^{1}H-NMR.

^{1}H-NMR ($CDCl_3$, 300MHz, ppm): 7.65 (s, 1H, Ph), 7.25 (t, 1H, CONH), 6.8 (s, 1H, Ph), 4.2 (s, 2H, CH_2CONH) 3.65 (t, 452H, EG-CH_2), 3.40 (s, 6H, OCH_3)

Functionalization was calculated by comparing the integral ratios of CH_2 from the PEG-backbone to the aryl CH (NDA).

IR (powder, cm^{-1}): 2946 (EG-CH_2 str), 2883 (EG-CH_2 str), 2862 (EG-CH_2 str), 2807, 2740, 2694, 1755, 1729 (COOH), 1642 (CONH), 1532 (CONH), 1467 (EG-CH_2 sci, g), 1455 (NO_2, EG-CH_2 sci, g), 1359 (NO_2), 1341(EG-CH_2 wag, g), 1279(EG-CH_2 twist), 1240 (EG-CH_2 twist), 1146 (EG-CO, EG-CC str), 1103 (EG-CO, EG-CC str), 1060 (EG-CO, EG-CC str), 957 (EG-CH_2 rock, g), 947 (COOH), 841, 558, 528, 509

Purification methods - details

Figure S1. Column purification of 7.1 nm superparamagnetic iron oxide nanoparticles with NDA-PEG grafted shells.

Figure S2. Dialyzed NP dispersions after 1h, 2h, 6,5h, 24h and 48h. The color changes from brown to black due to removal of free PEG and results in almost black samples, indicating that also bound NDA-PEG has been ripped off the surface and leaving almost bare cores.

Figure S3. NPs purified by repeated precipitation and magnetic decantation. As the purification proceeds, the color of the sample changes to a darker brown which is attributed to the removal of free NDA-PEG.

Shell Characterization - IR

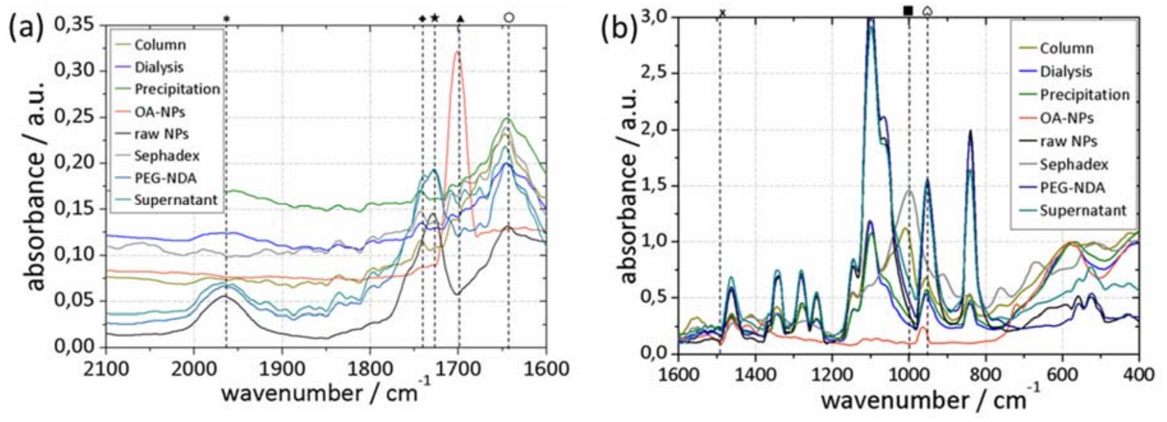


Figure S4. (a) High wavenumbers with amide CO-stretch at 1640 cm^{-1} (o), C=O-stretch of free OA at 1702cm^{-1} (Δ), residual COOH from free, non-coupled PEG at 1728cm-1 (★) found only in supernatants, raw NPs and pure NDA-PEG; bands at 1740 cm^{-1} can be attributed to COOR bonds of NDA-PEG (+) and the peak at 1960 cm^{-1} was attributed to a combination vibration OH bend of COOH of unbound PEG-NDA (*).

(b) Low wavenumbers with NP bands (780-400 cm^{-1}), ((C=O)-OH) stretch from free OA (ϖ) at 1702 cm^{-1}, sugar-bands (■) (COC & CO at 1130-974 cm^{-1}) originating from column material, characteristic CH_2 (1345-1240; <965 cm^{-1}), and CO / CC(1144-1060 cm^{-1}) vibrations of the PEG backbone and a peak that was attributed to the vibration of NO_2 of surface-bound PEG at 1491cm^{-1} (x).

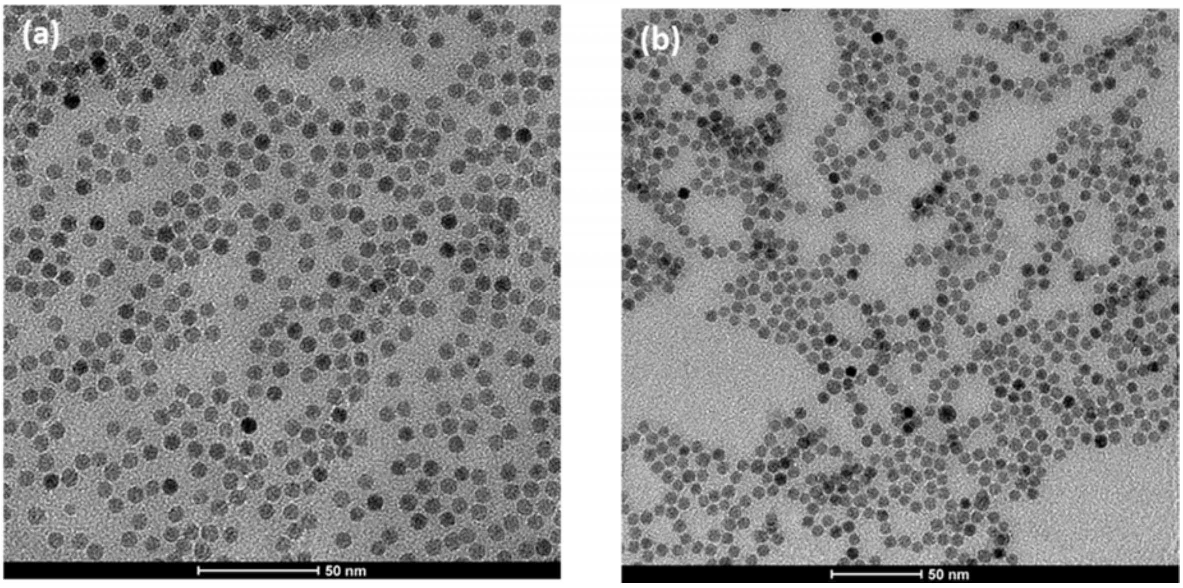


Figure S5. TEM micrographs of 7.1 nm NPs dispersed in **(a)** H_2O and **(b)** PBS at 4mg/mL, stored for 4 months at 4°C. No aggregation occurred after 4 months, the inter-NP distance is comparable to as-prepared NP dispersions

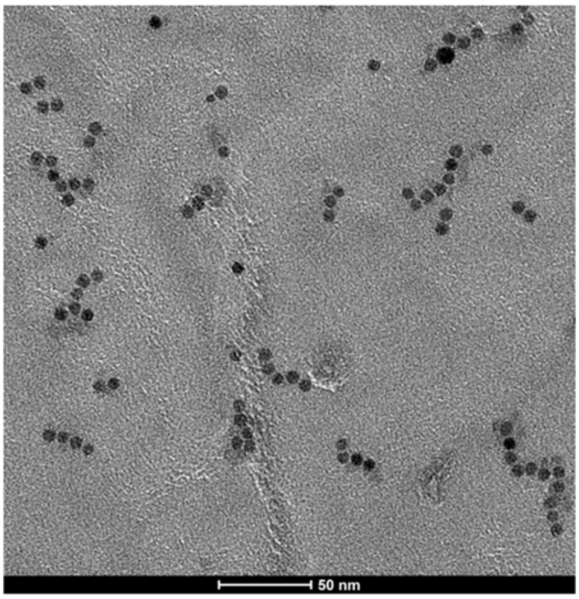


Figure S6. TEM micrograph of 7.1 nm NPs in PBS/FCS 10% v/v after 2 month storage. No aggregation is visible, underlining the exceptional stability of the NPs.

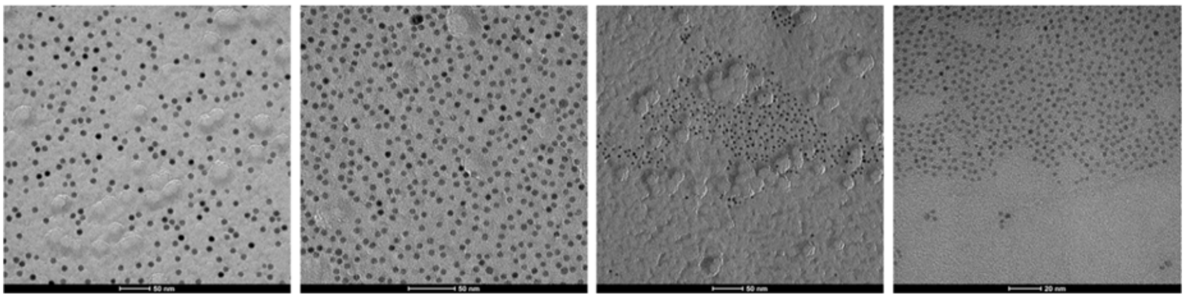


Figure S7. TEM micrographs of NPs purified by precipitation. 9.6, 7.1, 4.9 and 3.5 nm (from left to right. The blobs in the 1st and 3rd image originate from inhomogeneity of the TEM grid-surface.

References

1. Hyeon, T.; Lee, S. S.; Park, J.; Chung, Y.; Na, H. B., Synthesis of Highly Crystalline and Monodisperse Maghemite Nanocrystallites without a Size-Selection Process. *Journal of the American Chemical Society* **2001,** *123* (51), 12798-12801.

2. (a) Bixner, O.; Lassenberger, A.; Baurecht, D.; Reimhult, E., Complete Exchange of the Hydrophobic Dispersant Shell on Monodisperse Superparamagnetic Iron Oxide Nanoparticles. *Langmuir* **2015**, Ahead of Print; (b) Napolitano, A.; D'Ischia, M.; Costantini, C.; Prota, G., A new oxidation pathway of the neurotoxin 6-aminodopamine. Isolation and characterization of a dimer with a tetrahydro[3,4a]iminoethanophenoxazine ring system. *Tetrahedron* **1992,** *48* (39), 8515-22.

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