Highly Monodisperse Water-Dispersable Iron Oxide Nanoparticles for Biomedical Applications

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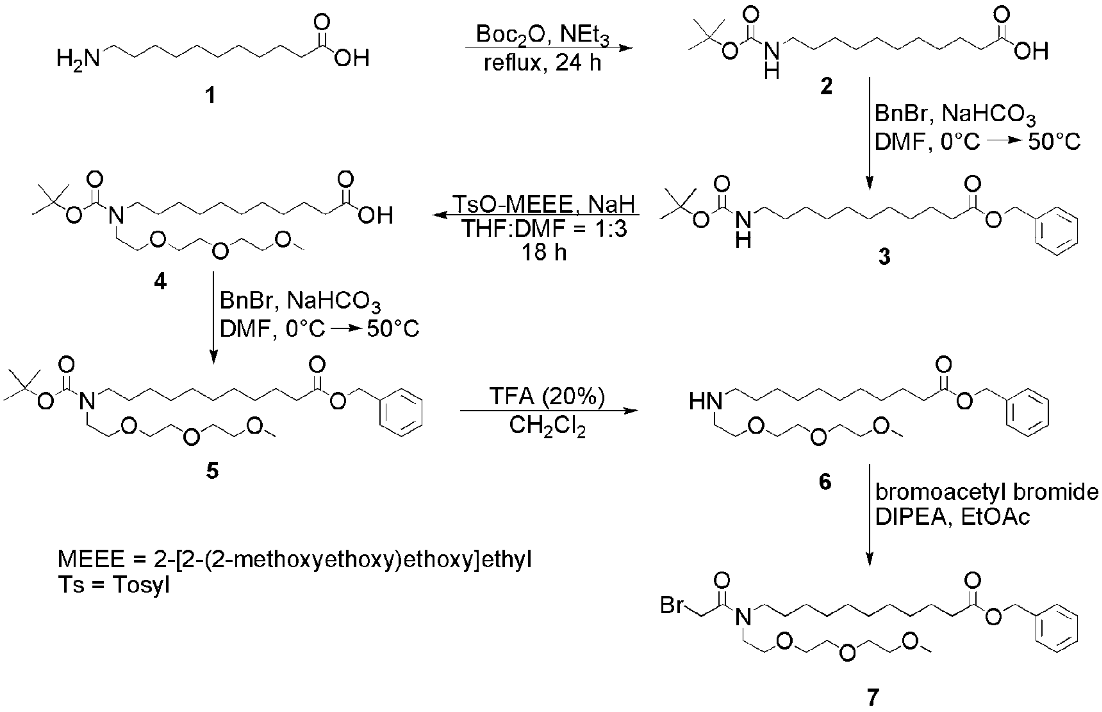
Supporting Information

1. Experimental details

a) The reaction schemes for the preparation of ligand (2) are shown in Figure 1-SI and Figure 2-SI.



MEEE = 2-[2-(2-methoxyethoxy)ethoxy]ethyl
Ts = Tosyl

Figure 1-SI. Synthesis of the key intermediate **7**.

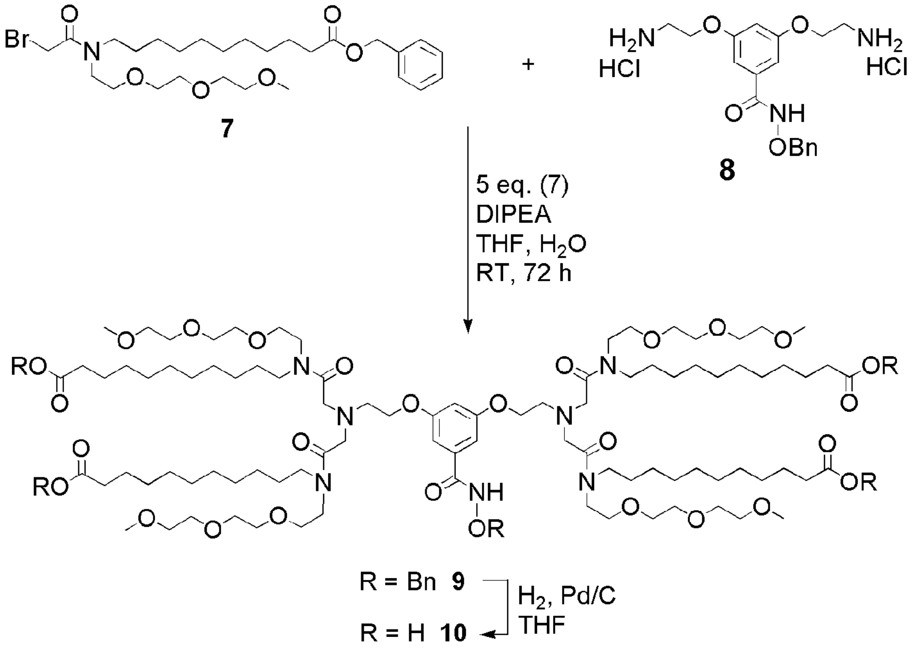


Figure 2-SI. Synthesis of the hydroxamic acid **10**.

Synthesis of 11-(tert-butoxycarbonylamino)undecanoic acid (2): ^{1}**H-NMR** (400 MHz, CDCl$_3$): δ / ppm = 10.88 (br, 1H), 4.54 (br, 1H), 3.08 (m, 2H), 2.33 (t, J = 7.5 Hz, 2H), 1.65-

1.58 (m, 2H), 1.50-1.37 (m, 2H), 1.43 (s, 9H), 1.34-1.20 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 179.3 (s), 156.0 (s), 79.0 (s), 40.6 (t), 34.0 (t), 30.0 (t), 29.4 (t), 29.24 (t), 29.16 (t), 29.1 (t), 29.0 (t), 28.4 (q, 3C), 26.7 (t), 24.7 (t).

*Synthesis of benzyl-11-(tert-butoxycarbonylamino)undecanoate (**3**)*: m.p. 31-32 °C. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ / ppm = 7.36-7.32 (m, 5H), 5.11 (s, 2H), 4.53 (br, 1H), 3.11-3.06 (m, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.67-1.58 (m, 2H), 1.47-1.40 (m, 2H), 1.43 (s, 9H), 1.31-1.22 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 173.6 (s), 155.9 (s), 136.1 (s), 128.5 (d, 2C), 128.1 (d, 3C), 78.9 (s), 66.0 (t), 40.6 (t), 34.3 (t), 30.0 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 29.0 (t), 28.5 (q, 3C), 26.7 (t), 24.9 (t).

*Synthesis of 11-[tert-butoxycarbonyl-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-undecanoic acid (**4**)*: **^{1}H-NMR** (400 MHz, $CDCl_3$): δ / ppm = 10.63 (br, 1H), 3.62-3.50 (m, 10H), 3.34 (s, 3H), 3.34-3.26 (m, 2H), 3.20-3.14 (m, 2H), 2.27 (t, J = 7.5 Hz, 2H), 1.62-1.54 (m, 2H), 1.48-1.38 (m, 2H), 1.41 (s, 9H), 1.31-1.14 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 179.4 (s), 178.8 (s), 79.3 (s), 71.8 (t), 70.5 (t), 70.4 (t), 70.3 (t), 69.5 (t), 58.9 (q), 47.9 (t), 46.6 (t), 34.2 (t), 29.4 (t), 29.3 (t, 3C), 29.1 (t), 29.0 (t), 28.4 (q, 3C), 26.7 (t), 24.8 (t).

*Synthesis of benzyl-11-[tert-butoxycarbonyl-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]undec-anoate (**5**)*: **^{1}H-NMR** (400 MHz, $CDCl_3$): δ / ppm = 7.33-7.27 (m, 5H), 5.07 (s, 2H), 3.64-3.49 (m, 10H), 3.34 (s, 3H), 3.37-3.27 (m, 2H), 3.22-3.16 (m, 2H), 2.31 (t, J = 7.5 Hz, 2H), 1.63-1.56 (m, 2H), 1.41 (s, 9H), 1.50-1.39 (m, 2H), 1.29-1.19 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 173.7 (s), 155.5 (s), 136.0 (s), 128.4 (d, 2C), 128.0 (d, 3C), 79.0 (s), 71.8 (t), 70.5 (t), 70.4 (t, 2C), 70.2 (t), 69.5 (t) 65.9 (t), 58.9 (q), 48.2 (t), 46.6 (t), 34.3 (t), 29.4 (t), 29.2 (t, 2C), 29.1 (t), 29.0 (t), 28.3 (q, 3C), 26.7 (t), 24.9 (t). **HR-MS (+ESI)**: m/z calculated for $C_{30}H_{51}NNaO_7$ ($[M + Na]^+$): 560.3563. Found: 560.3559.

*Synthesis of benzyl 11-[2-[2-(2-methoxyethoxy)ethoxy]ethylamino]undecanoate (**6**)*: **^{1}H-NMR** (400 MHz, $CDCl_3$): δ / ppm = 7.35-7.28 (m, 5H), 5.10 (s, 2H), 3.64-3.52 (m, 10H), 3.37 (s, 3H), 2.76 (t, J = 5.2 Hz, 2H), 2.58 (t, J = 7.3 Hz, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.71 (br, 1H), 1.65-1.58 (m, 2H), 1.50-1.42 (m, 2H), 1.33-1.15 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 173.6 (s), 136.1 (s), 128.5 (d, 2C), 128.1 (d, 3C), 71.9 (t), 70.6 (t), 70.5 (t), 70.4 (t), 70.2 (t), 66.0 (t), 59.0 (q), 50.0 (t), 49.3 (t), 34.3 (t), 30.1 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.0 (t), 27.3 (t), 24.9 (t).

Synthesis of benzyl 11-[(2-bromoacetyl)-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-undecanoate (7): **^{1}H-NMR** (400 MHz, $CDCl_3$): δ / ppm = 7.37-7.29 (m, 5H), 5.09 (s, 2H), 3.98 (s, 1H), 3.84 (s, 1H), 3.62-3.58 (m, 8H), 3.58-3.49 (m, 3H+2H), 3.38-3.29 (m, 3H), 3.362 + 3.359 (2 x s, 3H), 2.34 + 2.33 (t, J = 7.5 Hz, 2H), 1.66-1.47 (m, 4H), 1.32-1.18 (m, 12H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ / ppm = 173.59 (s), 173.55 (s), 176.3 (s), 166.5 (s), 136.0 (s, 2C), 128.4 (d, 4C), 128.1 (d, 6C), 71.84 (t), 71.81 (t), 70.74 (t), 70.53 (t, 2C), 70.45 (t), 70.43 (t), 70.38 (t), 68.78 (t), 68.75 (t), 66.0 (t, 2C), 59.0 (q, 2C), 50.1 (t), 48.4 (t), 46.26 (t), 46.20 (t), 34.2 (t, 2C), 29.4 (t, 2C), 29.24 (t, 2C), 29.22 (t, 2C), 29.1 (t, 2C), 29.00 (t), 28.99 (t), 27.3 (t), 27.1 (t), 26.70 (t, $BrCH_2$), 26.67 (t, $BrCH_2$), 26.3 (t), 24.84 (t), 24.82 (t). **HR-MS (+ESI)**: m/z calculated for $C_{27}H_{44}BrNNaO_6$ ($[M + Na]^+$): 580.2250. Found: 580.2251.

*Synthesis of benzyl-11-[[2-[2-[3-(benzyloxycarbamoyl)-5-[2-[bis[2-[(11-benzyloxy-11-oxo-undecyl)-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-2-oxo-ethyl]amino]ethoxy]phenoxy]ethyl-[2-[(11-benzyloxy-11-oxo-undecyl)-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-2-oxo-ethyl]amino]acetyl]-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]undecanoate (**9**)*: **^{1}H-NMR** (500 MHz, $CDCl_3$): δ / ppm =

10.71 (br, 1H), 7.45-7.41 (m, 2H), 7.34-7.23 (m, 23H), 7.10 (t, J = 13.1 Hz, 2H), 6.49 (s, 1H), 5.07 (s, 8H), 5.01 (s, 2H), 4.15-4.08 (m, 4H), 3.72-3.58 (m, 8H), 3.65-3.51 (m, 24H), 3.51-3.43 (m, 24H), 3.31 (s, 6H), 3.29 (s, 6H), 3.34-3.23 (m, 8H), 3.12-3.03 (m, 4H), 2.30 (t, J = 7.8 Hz, 8H), 1.63-1.55 (m, 8H), 1.52-1.42 (m, 8H), 1.28-1.16 (m, 48H). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ / ppm = 173.4 (s, 4C), 170.42 (s, 1C), 170.34 (s, 1C), 170.25 (s, 1C), 170.21 (s, 1C), 164.9 (s, 1C), 159.7 (s, 2C), 136.0 (s, 4C), 135.7 (s, 1C), 133.8 (s, 1C), 129.0 (d, 2C), 128.3+128.23+128.20+127.9 (d, 23C), 105.9 (d, 1C), 105.6 (d, 2C), 77.9 (t, 1C), 71.75 (t, 2C), 71.72 (t, 2C), 70.5+70.37+70.34+70.18 (t, 12C), 69.24 (t, 2C), 69.08 (t, 2C), 67.05 (t, 2C), 65.88 (t, 4C), 58.83 (q, 2C), 58.77 (q, 2C), 56.3 (t, 1C), 56.2 (t, 1C), 55.72 (t, 1C), 55.70 (t, 1C), 53.3 (t, 2C), 48.4 (t, 2C), 46.7 (t, 2C), 46.3 (t, 2C), 45.6 (t, 2C), 34.1 (t, 4C), 29.41+29.37+29.27+29.21+29.06+28.94 (t, 20C), 28.76 (t, 2C), 27.51 (t, 2C), 26.93 (t, 2C), 26.73 (t, 2C), 24.8 (t, 4C). **HR-MS (+ESI)**: m/z calculated for $C_{126}H_{195}N_7NaO_{28}$ ($[M + Na]^+$): 2278.3975. Found: 2278.3966. **HR-MS (+ESI)**: m/z calculated for $C_{126}H_{196}N_7O_{28}$ ($[M + H]^+$): 2256.4156. Found: 2256.4147. **HR-MS (+ESI)**: m/z calculated for $C_{126}H_{195}N_7Na_2O_{28}$ ($[M + 2Na]^{2+}$): 1150.6934. Found: 1150.6896.

Synthesis of 11-[[2-[2-[3-[2-[bis[2-[(11-hydroxy-11-oxo-undecyl)-[2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl]-amino]-2-oxo-ethyl]amino]ethoxy]-5-(hydroxycarbamoyl)phenoxy]-ethyl-[2-[(11-hydroxy-11-oxo-undecyl)-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]-2-oxo-ethyl]amino]acetyl]-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]amino]undecanoic acid ***(10)***: **^{1}H-NMR** (400 MHz, $CDCl_3$/DMSO-d_6): δ / ppm = 8.9 (br s, 1H), 6.96 (br s, 2H), 6.36 (br s, 1H), 4.00-3.80 (m, 4H), 3.65-3.25 (m, 48H), 3.25-3.05 (m, 12H+8H), 3.05-2.85 (m, 4H), 2.10 (t, J = 7.4 Hz, 8H), 1.49-1.29 (m, 8H+8H), 1.28-0.95 (m, 48H). **^{13}C-NMR** (100 MHz, $CDCl_3$/DMSO-d_6): δ / ppm = 176.4 (s, 4C), 170.55 (s, 1C), 170.48 (s, 1C), 170.40 (s, 1C), 170.30 (s, 1C), 164.3 (s, 1C), 159.8 (s, 2C), 133.8 (s, 1C), 105.9 (d, 1C), 105.6 (d, 2C), 71.4 (t, 4C), 70.4+70.1+70.34+70.05+70.00+69.9 (t, 12C), 68.9 (t, 2C), 68.7 (t, 2C), 66.6 (t, 2C), 58.52 (q, 2C), 58.49 (q, 2C), 56.1 (t, 1C), 55.9 (t, 1C), 55.44 (t, 1C), 55.36 (t, 1C), 52.9 (t, 2C), 48.1 (t, 2C), 46.5 (t, 2C), 46.0 (t, 2C), 45.4 (t, 2C), 33.8 (t, 4C), 29.9+29.0+28.9+28.8+28.6 (t, 20C), 28.4 (t, 2C), 27.1 (t, 2C), 26.6 (t, 2C), 26.4 (t, 2C), 24.5 (t, 4C). **HR-MS (+ESI)**: m/z calculated for $C_{91}H_{167}N_7O_{28}$ ($[M + 2H]^{2+}$): 903.5940. Found: 903.5912. **HR-MS (+ESI)**: m/z calculated for $C_{91}H_{166}N_7NaO_{28}$ ($[M + H + Na]^{2+}$): 914.5850. Found: 914.5797.

b) Materials: The solvents ($CHCl_3$, DMF, Et_2O, ethyl acetate, hexane, methanol, THF) were purchased in technical quality, purified by distillation, and dried according to standard procedures[1] if mentioned as dry solvent. 11-Aminoundecanoic acid (Fluka, 98%), benzyl bromide (Acros, 98%), bromoacetyl bromide (Fluka, 98%), Di-tert-butyldicarbonate (ABCR, 97%), DMEM and DMEM/FCS (Sigma-Aldrich), HCl_{aq} (p.a.), Et_3N (Acros, 99%), NaCl (Grüssing, 99.5%), NaH (Adrich, 60% in mineral oil), $NaHCO_3$ (Fluka, 99.5%), Na_2SO_4 (Fluka, 99%), tetrabutylammonium iodide (Fluka, 99%), trifluoroacetic acid (Acros, 99%), Pd/C (Fluka, 10% Pd), $^{i}Pr_2NEt$ (ABCR, 99%).

The preparation of compound **8** is described in reference [2].

2. Spectroscopic results

a) An infrared spectrum of the oleic acid protected iron oxide nanoparticles is shown in **Figure 3-SI** . The IR vibrations of the iron oxide nanocrystals with oleic acid as well as those of the nanoparticles functionalized with the ligands L-1 to L-5 are presented in **Table SI-1.**

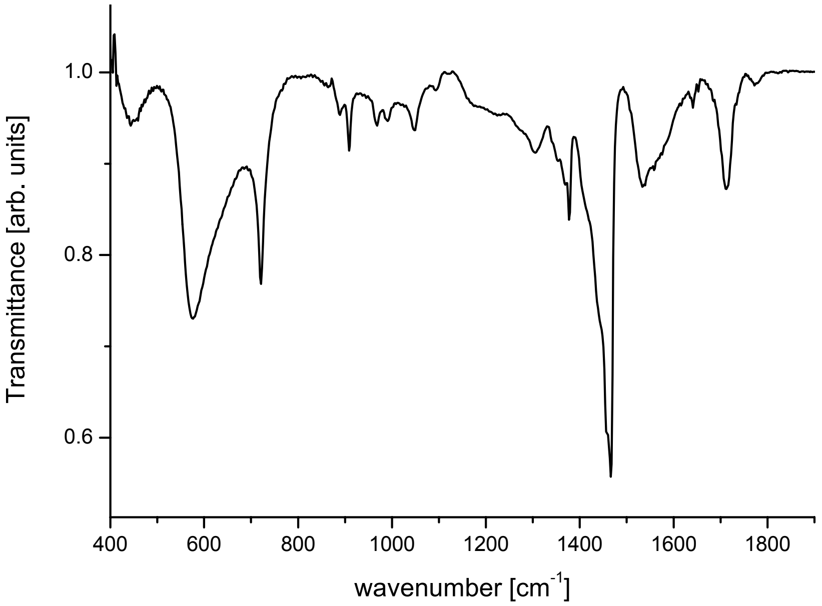


Figure 3-SI. Infrared spectrum of the oleic acid capped Fe_3O_4 nanoparticles (NP-OA).

It cannot be concluded without any ambiguity if the double bond in oleic acid is stable under the experimental conditions. Considering the high temperatures during the growth process saturation of the molecule cannot be fully excluded. A similar discussion was given by Willis et al. for maghemite particles, which were synthesized from iron pentacarbonyl and oleic acid at 350 °C.[3]

Table SI-1. IR vibrations of the iron oxide nanocrystals.[4-10]

sample	wave number [cm^{-1}]	explanation
NP-OA	3004	ν_{vinyl} C-H (free oleic acid)
	2954	ν_{as} CH_3
	2925	ν_a CH_2
	2855	ν_s CH_2
	1711	ν C-O (free oleic acid)
	1466	δ_s C-H
	1377	δ_s CH_3
	966	δ_{oop} C=C (free oleic acid)

	722	r CH$_2$
NP-1, NP-2, NP-3	1726	carboxylic acid (NP-2 and NP-3)
	1654-1650	Amid-I (ν C=O; ν C-N)
	1580-1500	Amid-II (ν C=O; ν C-N; δ N-H)
	1340-1330	Amid-III (δ N-H rocking)
	1200-1050	alkyl ether
	1170	ν_{as} C-O-C
	1110	ν_{as} C-O-C
	985	γ_{oop} C-H
NP-4	1700	carboxylic acid functionality
	1255	ν_{as} C-O-C
	1250	$\delta_{as,s}$ C-H (aryl ether)
	1022	ν_{as} C-O-C
NP-5	1706	carboxylic acid functionality
	1583	hydroxamic acid group
	1524	hydroxamic acid group
	1428	hydroxamic acid group

b) The time dependence of the absorption spectra of sample NP-3 in 0.05 M HCl solution is shown in **Figure 4-SI**.

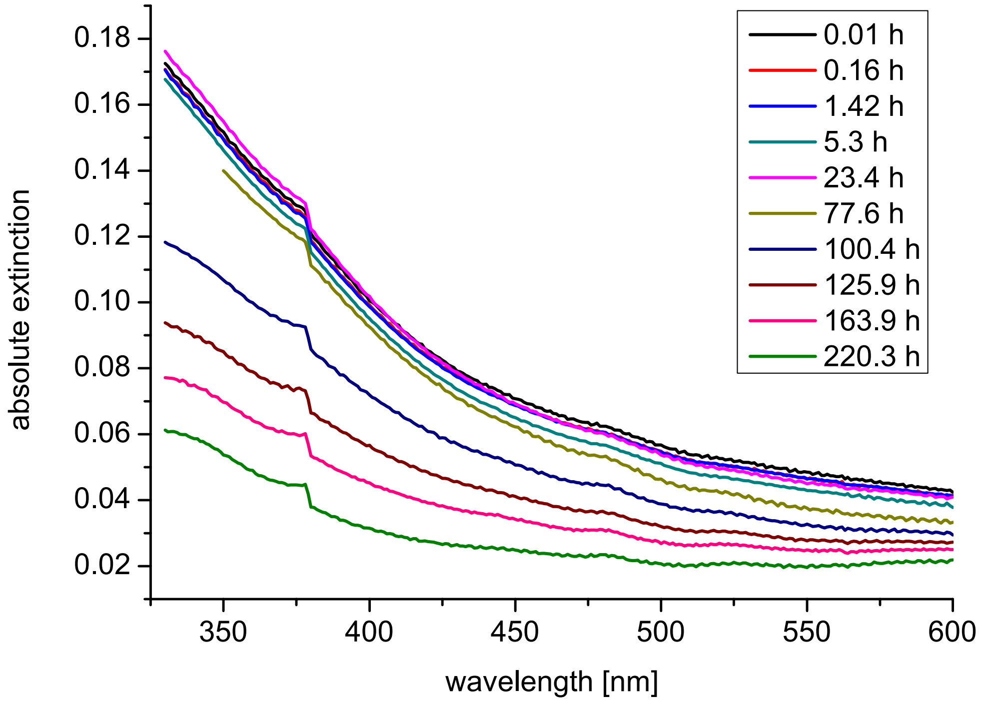


Figure 4-SI. The time dependence of the measured absorption spectra is shown for NP-3 in 0.05 M HCl solution. A lamp change in the spectrometer at 380 nm is causing a step in all spectra.

c) The Raman spectrum of the oleic acid protected iron oxide nanoparticles is compared to the spectra of sample NP-3 and magnetite (bulk) in **Figure 5-SI**.

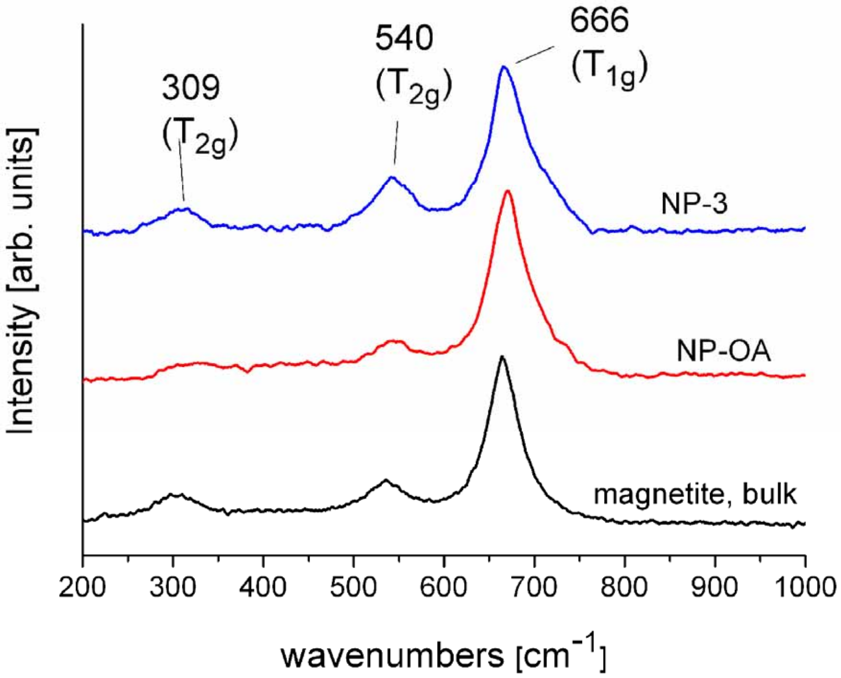


Figure 5-SI. Raman spectra of the oleic acid capped iron oxide particles (NP-OA) and of iron oxide particles after a ligand exchange reaction with ligand L-3 (see Figure 1, NP-3). A Raman spectrum of pure bulk magnetite (Fe_3O_4) is included for a comparison. The dominant features are assigned to the spectra as described in ref. 64 in the article for bulk magnetite.

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