Electronic Supplementary Material (ESI) for Chemical Communications

One-pot synthesis of water soluble iron nanoparticles using rationally-designed peptides and ligand release

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Supporting Information

Table of Contents

1. General Information

1.1 Materials and methods

Flash Chromatography

Flash chromatography was performed using Davisil® chromatographic silica (LC60Å 40-63 micron) (Grace GmbH & Co.KG) with indicated solvents. Analytical thin layer chromatography was performed on 0.2 mm aluminium plates of silica gel 60 F_{254} (Merck) and compounds were visualised by ultra-violet fluorescence.

NMR

Nuclear magnetic resonance spectra were recorded on either a Bruker AVANCE DRX300 spectrometer operating on 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei or on a Bruker AVANCE DRX400 spectrometer operating on 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei.

Mass Spectrometry

Electrospray ionisation mass spectra were recorded on a Thermo Finnigan Surveyor MSQ Plus spectrometer or a Bruker micrOTOF-Q II spectrometer. Samples were introduced using direct flow injection at 0.1-0.2 mL/min into an ESI source in positive mode. Major and significant fragments are quoted in the form *x* (mass to charge ratio).

Microwave reactor

Microwave-enhanced click reactions were performed in a sealed glass reaction vessel on a CEM Discover 908010 microwave reactor with IR-monitored temperature control. The peptides were pre-purified by solid phase extraction using Alltech C18-LP 900 mg bed cartridges, freeze-dried and then purified by semi-preparative RP-HPLC.

HPLC

Semi-preparative RP-HPLC was performed on a Dionex Ultimate 3000 system using the Phenomenex Gemini C_{18}, 5 μm, 10 mm × 250 mm column at a flow rate of 5 mL/min. Analytical RP-HPLC was performed on a Dionex P680 system using the Waters XTerra MS C_{18}, 5 μm, 4.6 mm × 150 mm column at a flow rate of 1 mL/min (unless otherwise stated). A linear gradient of 0.1% trifluoroacetic acid-water (A) and 0.1% trifluoroacetic acid-acetonitrile (B) was used with detection at 210 nm.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy images, electron diffraction patterns and energy-dispersive spectroscopy data were acquired digitally with a JEOL 2010 operated at an acceleration voltage of 200 keV and equipped with an Oxford Inca EDS detector. TEM samples were prepared by suspending the NPs in ethanol using sonication, depositing a few drops of the suspension on carbon-coated copper TEM grids and allowing the solvent to evaporate under ambient conditions.

Magnetic measurements

Magnetisation measurements were carried out on a dried powder in a cylindrical gelatin holder on a MPMS-XL Quantum Design SQUID magnetometer with applied fields up to 60 kOe.

UV/Vis Absorption

UV/Vis absorption spectra were measured in water on a Shimadzu UV-2101PC spectrophotometer with 1 cm path length quartz cuvettes.

Circular Dichroism

Circular dichroism measurements were carried out in water at ambient temperature using an Applied Photophysics PiStar-180 spectrometer. 0.1 cm path length quartz cuvettes were used and the values were expressed in terms of mean residue ellipticity (θ_{MRE}) (deg cm^2/ dmol).

Dynamic Light Scattering (DLS)

Dynamic light scattering measurements were carried out using a Malvern Zetasizer Nano ZS at a 173° backscatter angle.

1.2 Solvents and reagents

All reagents were purchased as reagent grade and used as supplied. Solvents were used as supplied or dried according to standard methods.[1] RP-HPLC solvents were purchased as HPLC grade and used without further purification. *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Advanced ChemTech. *N*,*N*-Dimethylformamide (DMF) (synthesis grade), di-sodium hydrogen phosphate and acetonitrile (HPLC grade) were purchased from Scharlau. Piperidine, guanidine hydrochloride, 3,6-dioxa-1,8-octanedithiol, triisopropylsilane (TIS), 4-(dimethylamino)pyridine (DMAP), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and 4-methylmorpholine (NMM) were purchased from Aldrich. *N,N'*-diisopropylcarbodiimide (DIC) was purchased from GL Biochem. Trifluoroacetic acid (TFA) was purchased from Halocarbon, $CuSO_4 \cdot 5\ H_2O$ from Ajax Finechem and copoly(styrene-1%-divinylbenzene) resin (Bio beads S-X1) 200-400 mesh from Bio-Rad.
L-amino acids were used in every case. Fmoc-amino acids were purchased from either CEM corp. or GL Biochem with the following side chain protection: Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Arg(Pbf)-OH. Fmoc-L-Lys(Boc)-OCH_2PhO-$CH_2CH_2CO_2H$ was purchased from PolyPeptide Group. The building blocks for the CuAAC cycloaddition reaction Fmoc-L-propargylglycine (*pra*)[2, 3, 4] and 2-azidoethylphosphonic acid[5, 6] were synthesised according to published procedures.

2. Synthesis of the peptides 1and 2

Solid phase peptide synthesis based on Fmoc protection strategy was performed on a 0.05 mmol scale using aminomethylated polystyrene resin[7] (loading 1.0 mmol/g) derivatised with Fmoc-L-Lys(Boc)-OCH_2PhO-$CH_2CH_2CO_2H$ (**method 1**).

The peptide chains were then assembled using either manual Fmoc SPPS (**method 2**) or a Tribute^{TM} peptide synthesiser (**method 3**) and cleaved from resin according to **method 4.**

2.1 General methods

1: Attachment of Fmoc-L-Lys(Boc)-$OCH_2PhOCH_2CH_2CO_2H$ to resin

The resin was swollen in DCM (5 mL) for 15 min and then the solvent was drained. Fmoc-L-Lys(Boc)-OCH_2PhOCH_2-CH_2CO_2H (2 eq) was dissolved in 1 mL of DCM, DIC (2 eq) was added and the reaction mixture was added to resin followed by agitating for 1h. The mixture was drained and the resin was washed with DMF (3 ×) and DCM (3 ×).

2: Manual Fmoc SPPS

N^{α}-Protected standard amino acids (5 eq) were dissolved in 2 mL of 0.23 M HBTU/ DMF (4.6 eq), 0.5 ml of 2M NMM/ DMF (10 eq) were added and the mixture was transferred to the reaction vessel. The mixture was shaken for 45 min, filtered and washed with DMF (3 ×) and DCM (3 ×). The N^{α}-protecting group was removed by 20% piperidine solution in DMF (3 mL, 2 × 5 min), filtered and washed with DMF (3 ×) and DCM (3 ×).

N^{α}-Protected Fmoc-L-propargylglycine (50 mg, 0.15 mmol, 1.5 eq), HATU (55 mg, 0.145 mmol, 1.45 eq) and HOAt (20 mg, 0.145 mmol, 1.45 eq) were dissolved in 2 mL of DMF, 2,4,6-collidine (80 µL, 0.6 mmol, 6 eq) and DMAP (cat., 10 µL of a stock solution of 1.22 mg DMAP in 122 µL DMF) were added and the mixture was transferred to the reaction vessel. The mixture was shaken for 1 h, filtered and washed with DMF (3 ×) and DCM (3 ×). The N^{α}-protecting group was removed by 20% piperidine solution in DMF (3 mL, 2 × 5 min), filtered and washed with DMF (3 ×) and DCM (3 ×).

3: Automated Fmoc SPPS, Tribute™ peptide synthesiser

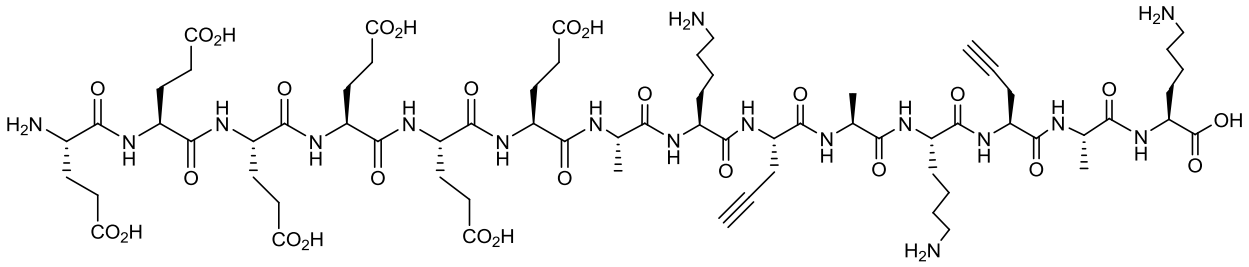
Couplings of N^{α}-Fmoc-protected amino acids (5 eq) were carried out in 45 min at room temperature in the presence of HBTU (4.6 eq) and NMM (10 eq) in DMF. The N^{α}-protecting group was removed by 20% piperidine solution in DMF (3 mL, 2 × 5 min).

4: Cleavage from resin (Fmoc SPPS)

100 μL TIPS, 250 μL H_2O, 250 μL 3,6-dioxa-1,8-octanedithiol and 9.4 mL TFA were added to the resin and the mixture was agitated for 2 h at room temperature. The TFA solution was filtered and the peptide was precipitated by addition of hexane/ diethyl ether (1:1). After centrifugation and washing with hexane/ diethyl ether (1:1) the crude peptides were lyophilised from 0.1% trifluoroacetic acid-water.

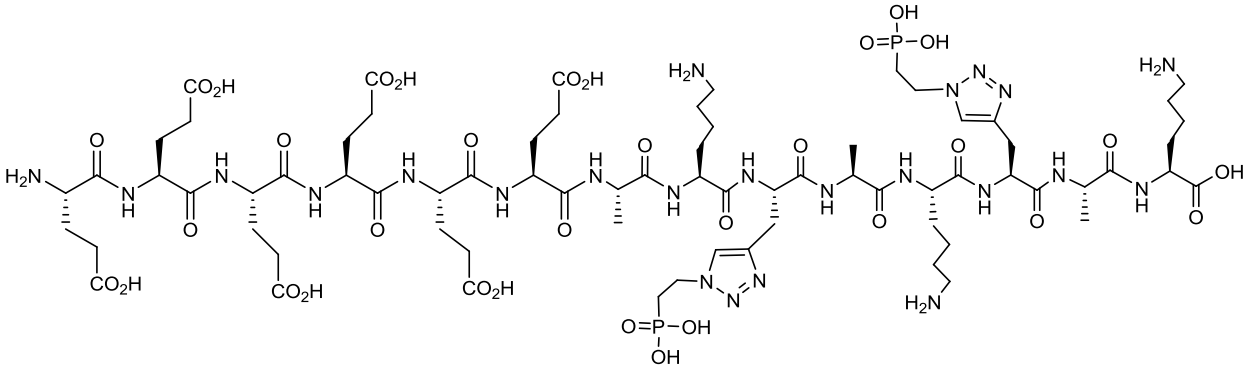
2.2 Synthesis of analogue 1: E_6AKXAKXAK

Synthesis of precursor E_6AK*pra*AK*pra*AK



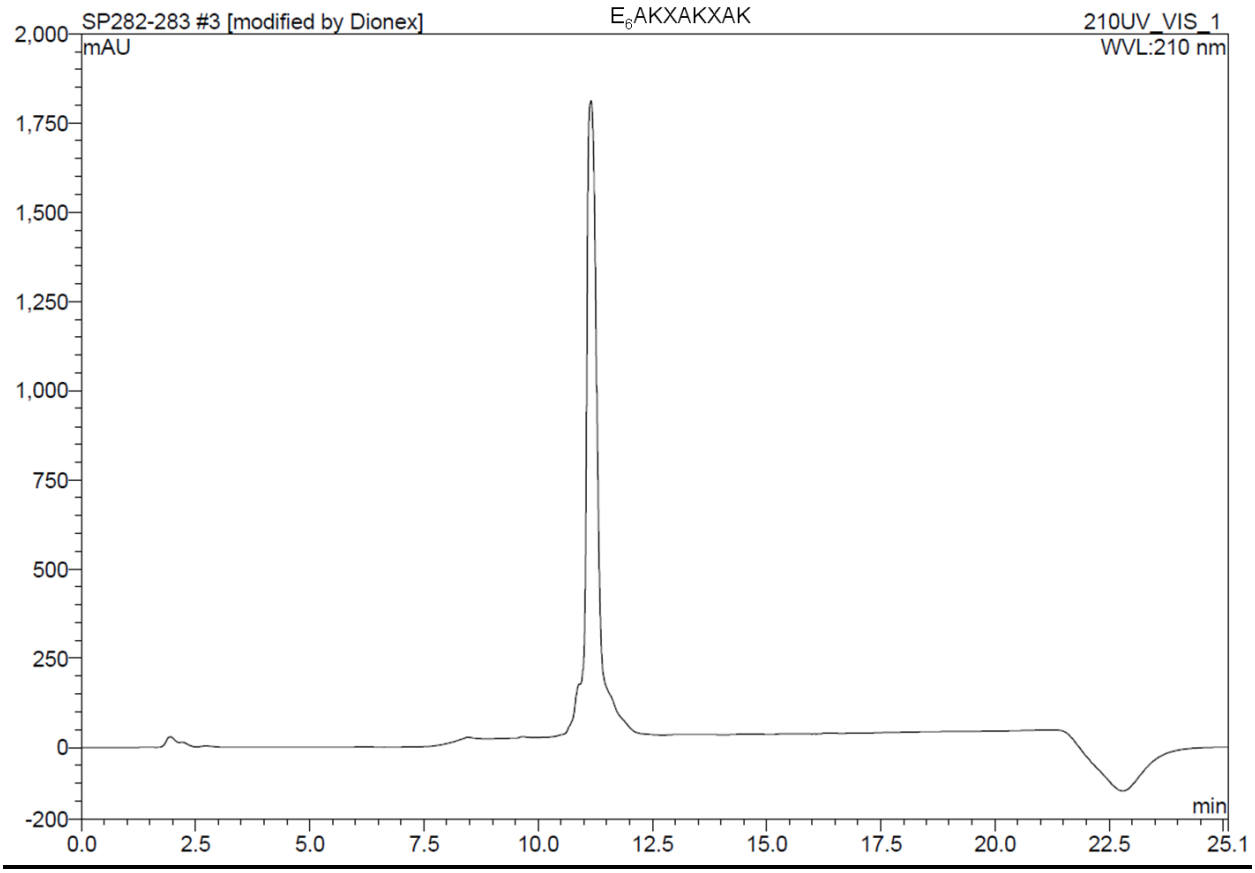
The first residue (Lys) was attached to the aminomethylated polystyrene resin (0.05 mmol) according to **method 1** and then the peptide chain was assembled according to **method 2** (residues EEEEEEAKpraAKpraA). Cleavage from resin according to **method 4** yielded 80.9 mg of crude product. *m/z* (ESI-MS): $[M+H^+]$ calculated mass = 1581.6, observed mass = 1581.7; $[M+2H^+]$ calculated mass = 791.3, observed mass = 790.9; $[M+3H^+]$ calculated mass = 527.9, observed mass = 527.6.

CuAAC reaction on precursor E_6AK*pra*AK*pra*AA



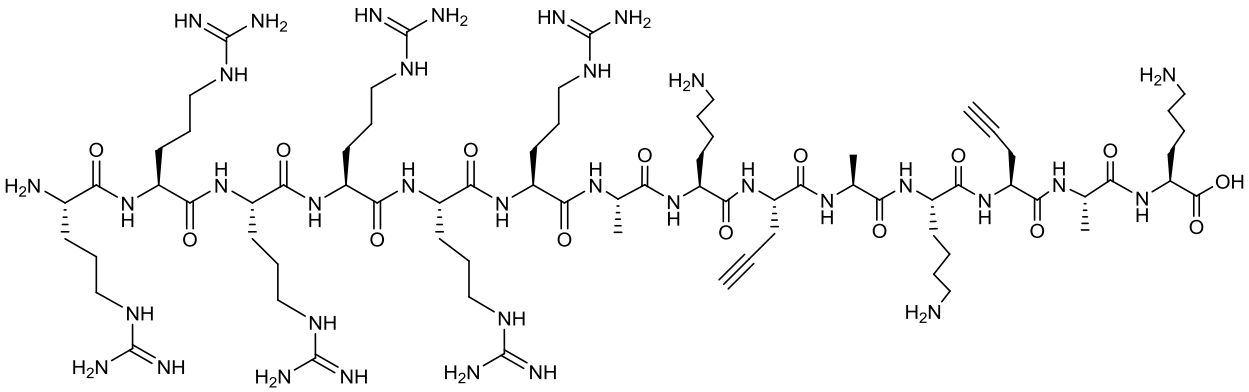
Crude propargylated peptide E_6AK*pra*AK*pra*AK (26.2 mg, 0.0166 mmol, 3 eq) was dissolved in a degassed aqueous solution of 6 M GnHCl/ 0.2 M Na_2HPO_4 (5.533 mL) containing TCEP hydrochloride (885.2 μL of 0.5 M aqueous solution, pH = 7, 80 eq) and $CuSO_4 \cdot 5\ H_2O$ (885.2 μL

of 0.5 M aqueous solution, 80 eq). After 30 min of incubation at 55°C, 2-azidoethylphosphonic acid (15.05 mg, 0.0996 mmol, 18 eq) was added (1.505 mL of a stock solution of 10 mg 2-azidoethylphosphonic acid in 1 mL H_2O) and the reaction carried out under argon with microwave irradiation (20 W, 60°C, 2 h). The reaction mixture was acidified to pH = 1 with conc. HCl, purified by solid phase extraction and lyophilised. Purification by semi-preparative RP-HPLC at a flow rate of 5 mL/min using a linear gradient of 0% to 50% B over 20 min and lyophilisation yielded the purified peptide (7.50 mg, 24%) as a white solid in ca. 95% purity according to analytical HPLC. R_t 11.2 min (0-40% B over 16 min, 1 mL/min); *m/z* (ESI-MS): [M+2H$^+$] calculated mass = 942.4, observed mass = 942.5; [M+3H$^+$] calculated mass = 628.6, observed mass = 628.9; [M+4H$^+$] calculated mass = 471.7, observed mass = 471.9.



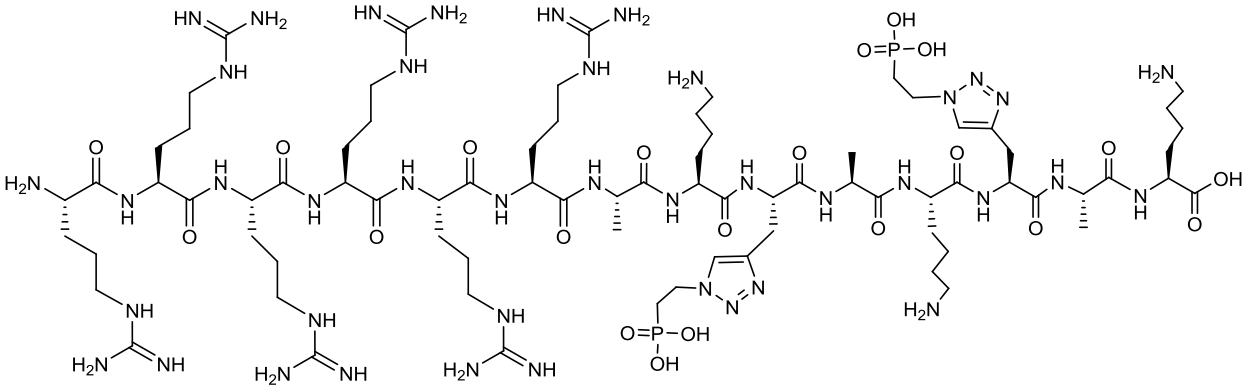
2.3 Synthesis of analogue 2: R_6AKXAKXAK

Synthesis of precursor R_6AK*pra*AK*pra*AK

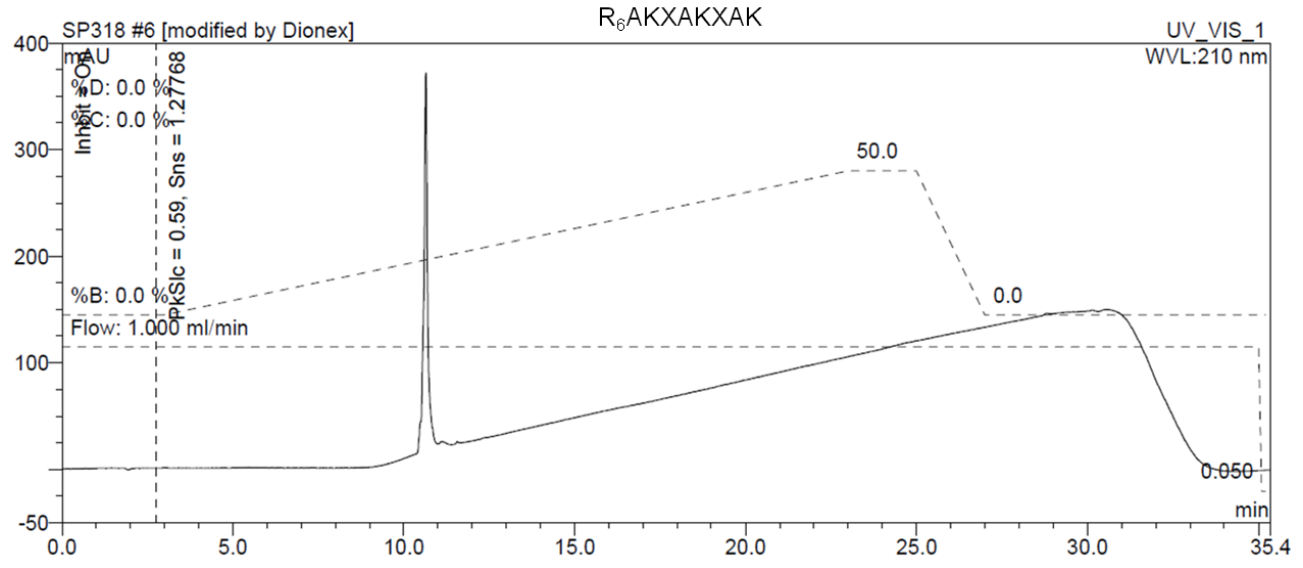


The first residue (Lys) was attached to the aminomethylated polystyrene resin (0.05 mmol) according to **method 1** and the peptide chain was assembled according to **method 2** (residues AK*pra*AK*pra*A) and **method 3** (R_6). Cleavage from resin according to **method 4** yielded 81.4 mg of crude product. *m/z* (ESI-MS): $[M+3H^+]$ calculated mass = 582.0, observed mass = 581.4; $[M+4H^+]$ calculated mass = 436.8, observed mass = 436.7.

CuAAC reaction on precursor R_6AK*pra*AK*pra*AK



Crude propargylated peptide R_6AK*pra*AK*pra*AK (28.9 mg, 0.0166 mmol, 3 eq) was dissolved in a degassed aqueous solution of 6 M GnHCl/ 0.2 M Na_2HPO_4 (5.533 mL) containing TCEP hydrochloride (885.2 μL of 0.5 M aqueous solution, pH = 7, 80 eq) and $CuSO_4 \cdot 5\ H_2O$ (885.2 μL of 0.5 M aqueous solution, 80 eq). After 30 min of incubation at 55°C, 2-azidoethylphosphonic acid (15.05 mg, 0.0996 mmol, 18 eq) was added (1.505 mL of a stock solution of 10 mg 2-azidoethylphosphonic acid in 1 mL H_2O) and the reaction carried out under argon with microwave irradiation (20 W, 60°C, 2 h). The reaction mixture was acidified to pH = 1 with conc. HCl, purified by solid phase extraction und lyophilised. Purification by semi-preparative RP-HPLC at a flow rate of 5 mL/min using a linear gradient of 0% to 50% B over 20 min and lyophilisation yielded the purified peptide (3.1 mg, 9%) as a white solid in ca. 97% purity according to analytical HPLC. R_t 10.7 min (Phenomenex Gemini C_{18}, 3 µm, 4.6 mm × 150 mm column, 0-50% B over 20 min, 1 mL/min); *m/z* (ESI-MS): $[M+2H^+]$ calculated mass = 1023.6, observed mass = 1023.0; $[M+3H^+]$ calculated mass = 682.8, observed mass = 682.4.



3. Procedure for the synthesis of iron/iron oxide core/shell NPs

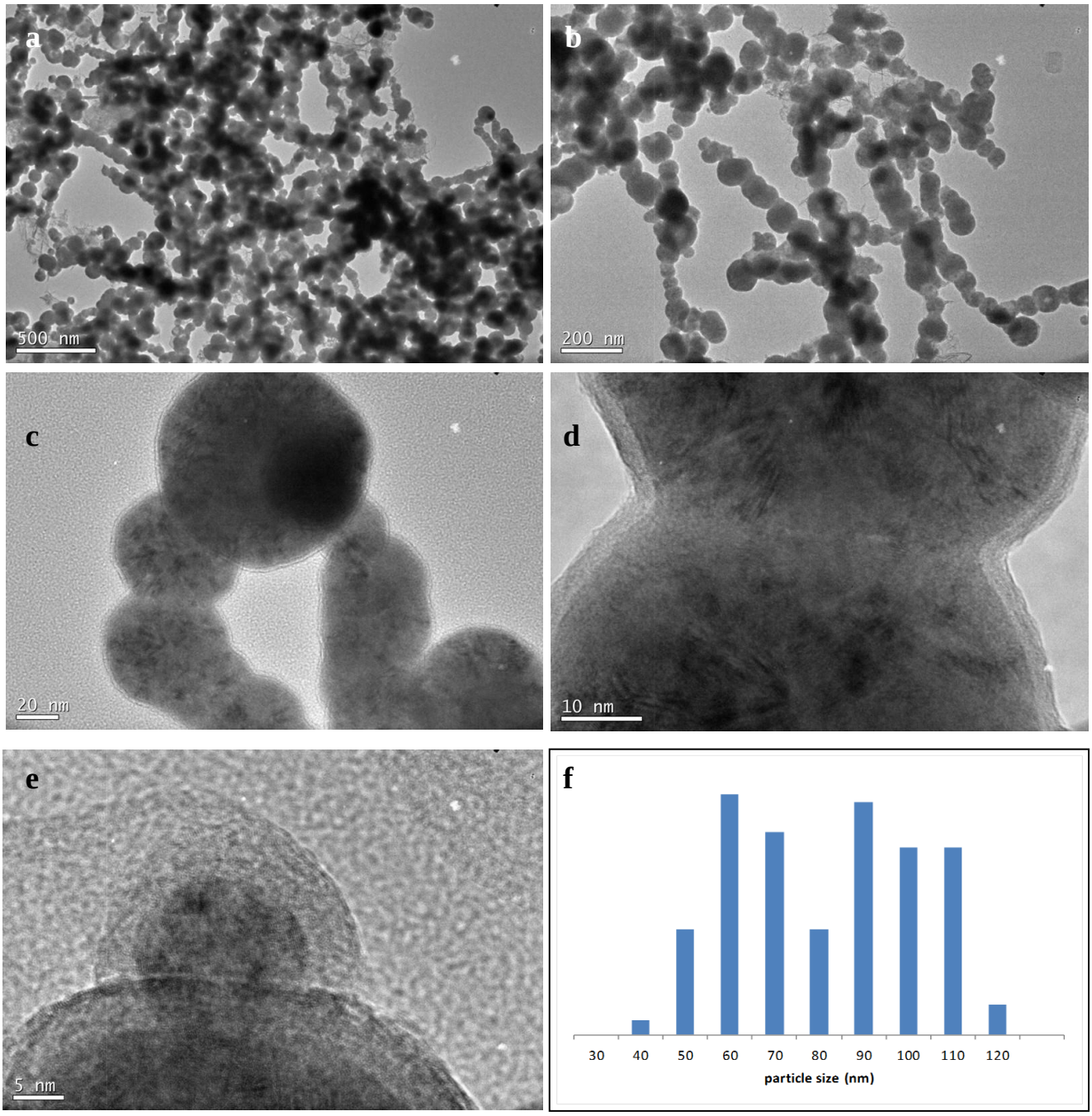
General procedure (Fe(II)/ peptide ratio = 20/1):

$FeSO_4 \cdot 7\ H_2O$ (1.95 mg, 7 μmol) and the peptide (0.35 μmol) were dissolved in 2 mL of previously degassed distilled water and stirred under nitrogen for 15 min. Then the $NaBH_4$ solution (40 μmol, 20 μL of a 2M solution in triethylene glycol dimethyl ether) was added all at once and the reaction solution stirred vigorously under nitrogen atmosphere (stirring speed 1000 rpm). A black precipitate immediately appeared and the mixture was stirred for 10 min. The reaction mixture was sonicated in order to separate all particles from the stirrer bar, centrifuged and the supernatant solution was decanted. The black precipitate was suspended in degassed ethanol, sonicated for 5 min, centrifuged and the supernatant decanted (2 ×) and then the particles were dried *in vacuo*.

For peptide **1** a Fe(II)/ peptide ratio of 10/1 was tested in a separate experiment additional to the general procedure. Doubling the amount of peptide **1** lead to the formation of the same iron/iron oxide core/shell NPs as the ones presented for the 20/1 ratio in section 4.2 of the ESI. However, the NPs were heavily covered with organic material, which was detrimental to the quality of the TEM images. For this reason those results are not shown. All of the results presented below refer to the general procedure with a ratio of Fe(II) precursor/ peptide = 20/1.

4. Characterisation of the nanoparticles

4.1 Blank without addition of capping agent



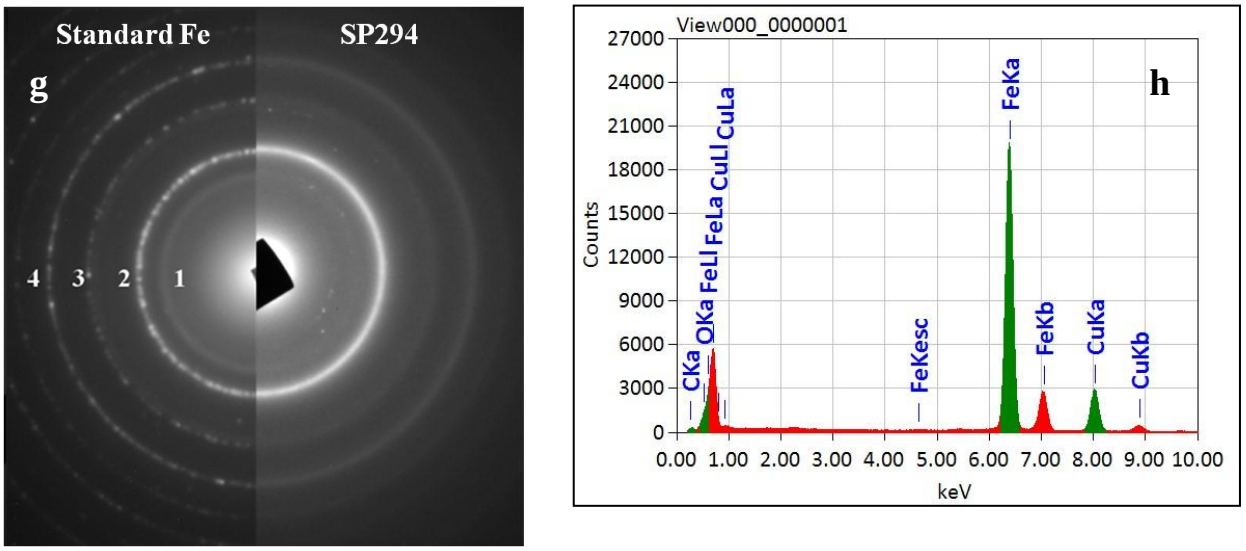
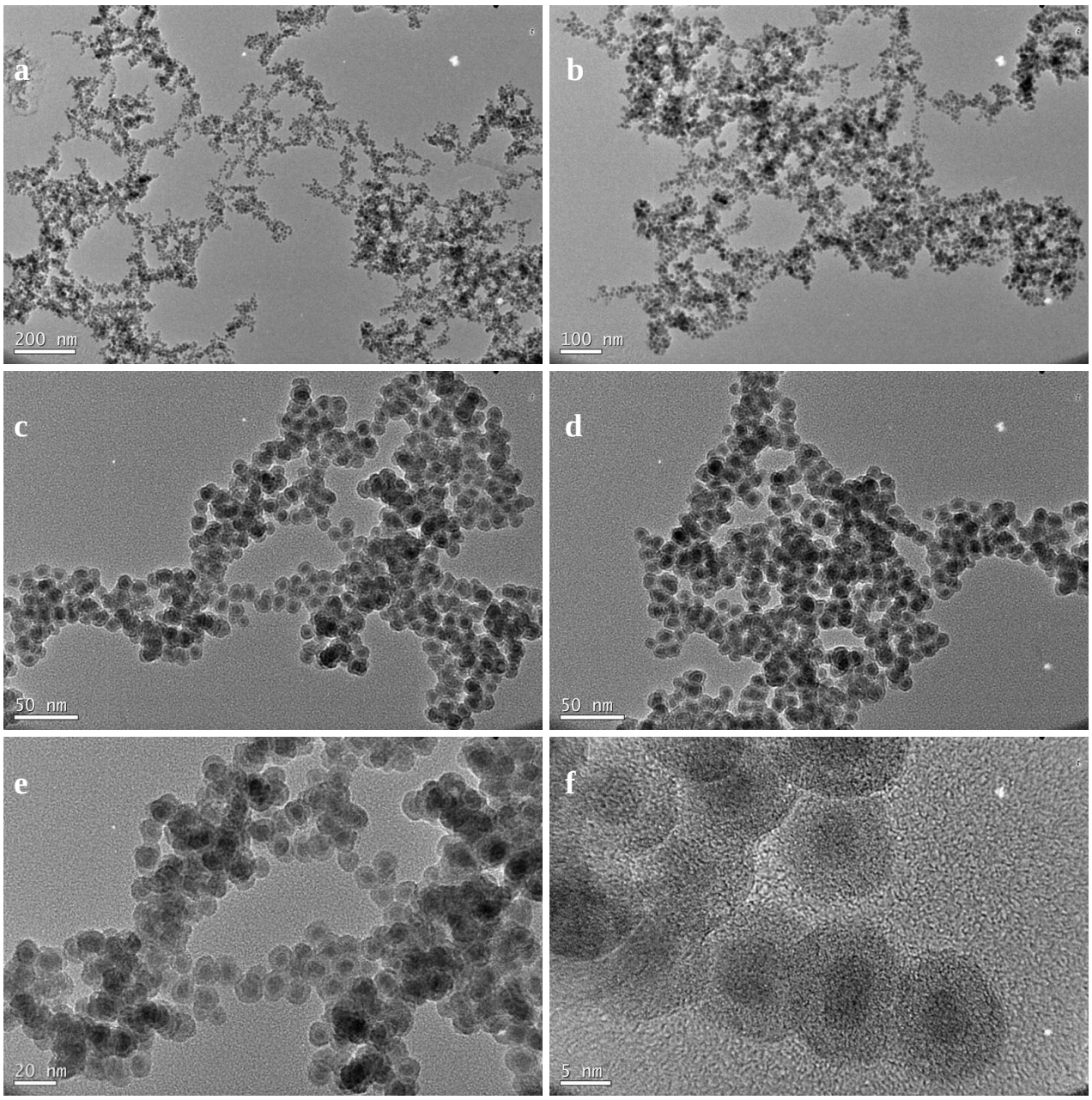


Figure S1: Analysis of particles. a-e) TEM pictures of particles at different magnifications; f) size distribution histogram; g) electron diffraction pattern; h) EDS measurement graph.

4.2 Capping agent 1: $E_6AKXAKXAK$



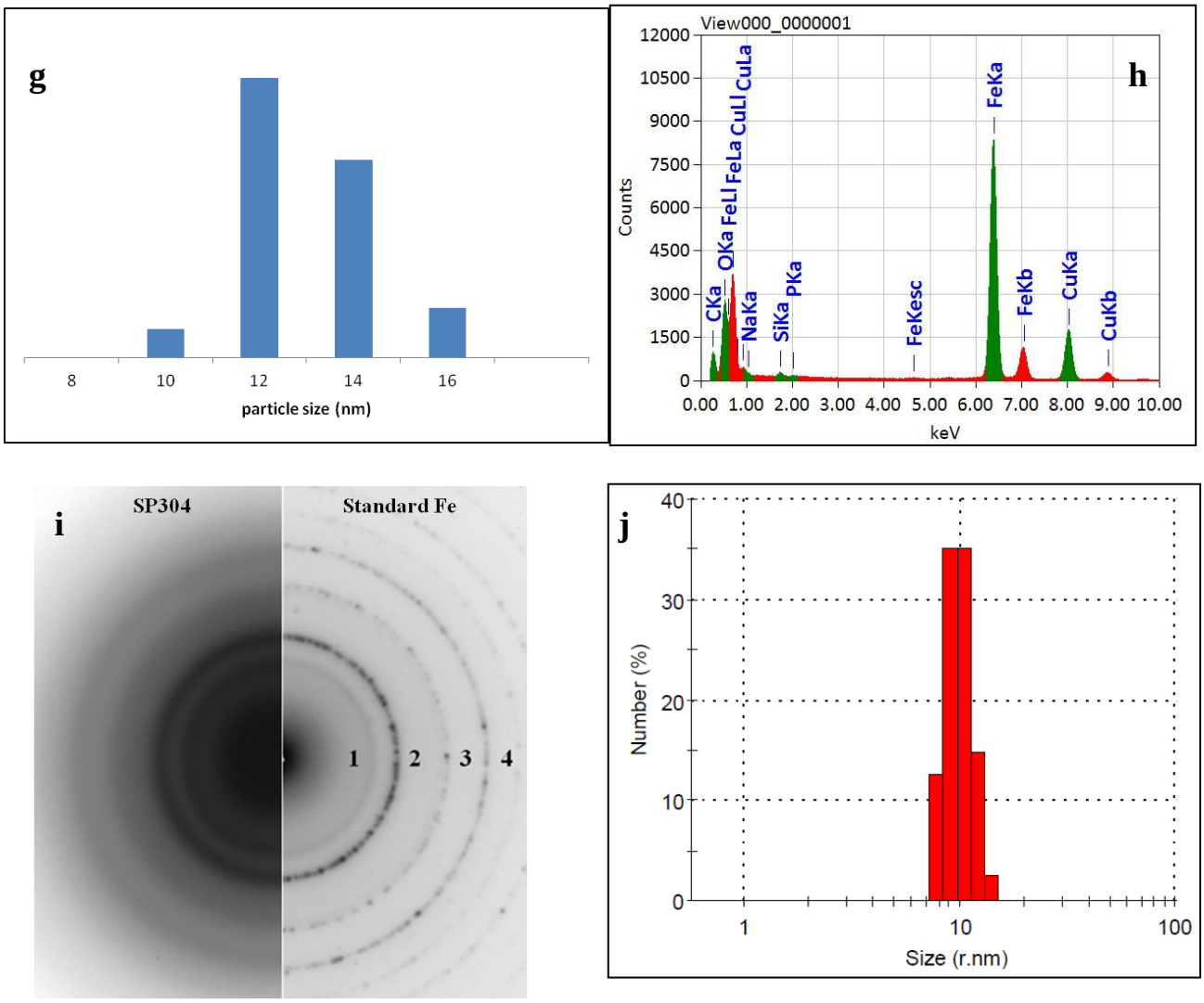
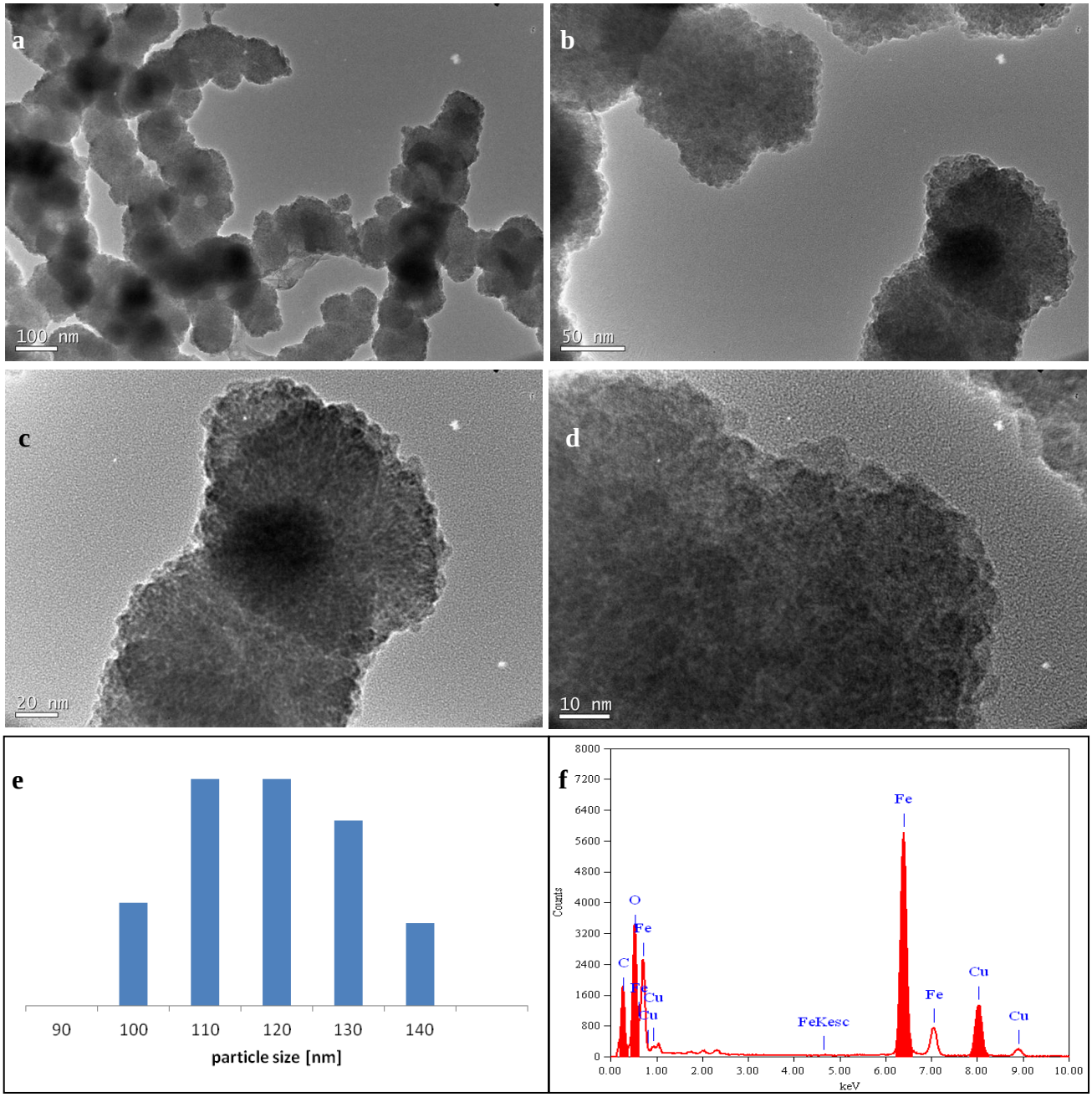


Figure S2: Analysis of particles. a-f) TEM pictures of particles at different magnifications; g) size distribution histogram; h) EDS measurement graph; i) electron diffraction pattern; j) Dynamic light scattering experiment conducted in $NaHCO_3/Na_2CO_3$ buffer in the presence of 0.1% (v/v) Triton X-100 (reduced form) at 65°C confirmed an average NP size of 10 nm.

4.3 Capping agent 2: R_6AKXAKXAK



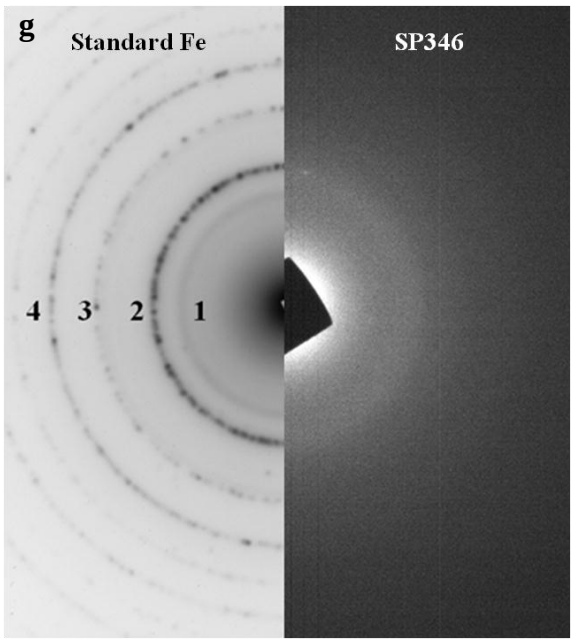


Figure S3: Analysis of particles. a-d) TEM pictures of particles at different magnifications; e) size distribution histogram; f) EDS measurement graph; g) electron diffraction pattern.

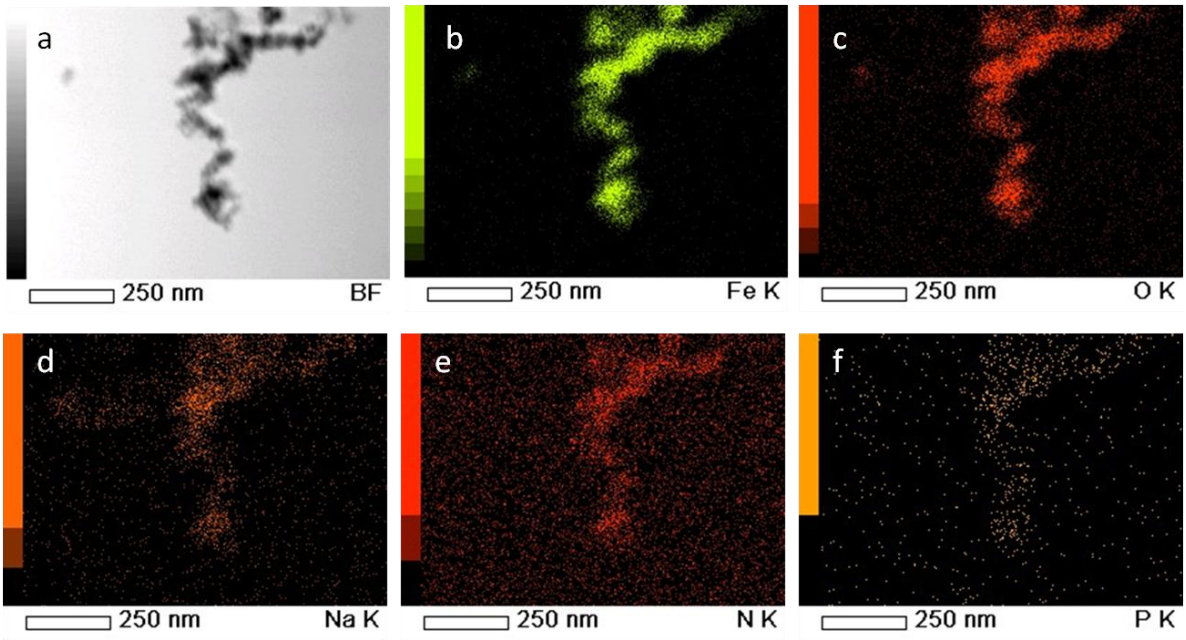


Figure S4: STEM-EDS on particles synthesized with peptide 1. a) STEM bright field image, b) iron EDS map, c) oxygen EDS map, d) sodium EDS map, e) nitrogen EDS map, f) phosphorous EDS map.

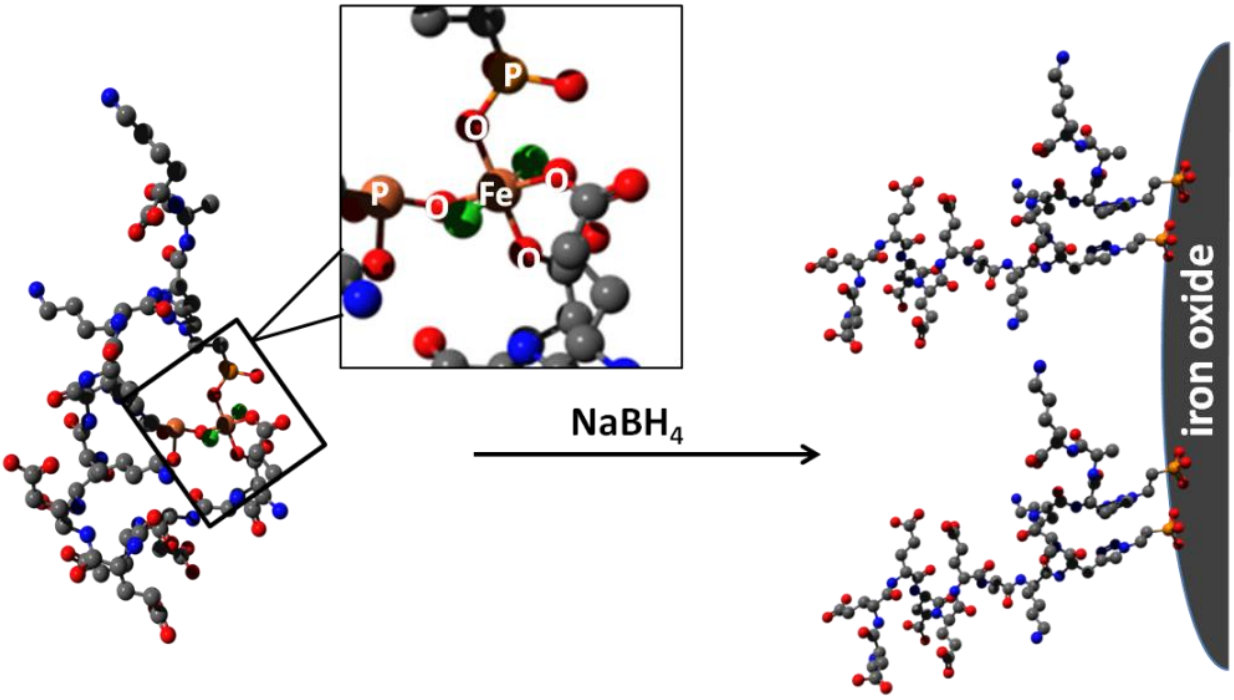
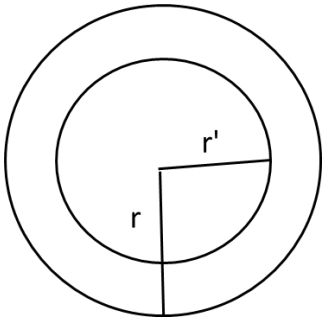


Figure S5: Avogadro model. (Left) Molecular model of peptide **1** binding to Fe^{II} in aq. solution via two phosphonate and two carboxylic acid side chains; (right) After reduction of Fe^{II} to Fe(0) by $NaBH_4$ peptides **1** are adsorbed to the surface of the formed NPs conferring steric and electrostatic stabilization to the colloid.

5. Magnetic measurements of NPs synthesized with peptide 1

Mass correction assuming a spherical nanoparticle consisting of a Fe(0) core and a Fe_3O_4 shell and given the following values:



NP sizes		**Densities**
Total NP radius	r = 6.0 nm	ρ [Fe(0)] = 7.87 g/cm^3
Fe core radius	r' = 2.9 nm	ρ [Fe_3O_4] = 5.18 g/cm^3
Oxide shell thickness	r - r' = 3.1 nm	

Calculation of the volumes of the Fe core and the iron oxide shell:

$$V_{core} = 4/3 \cdot \pi \cdot (r'^3) = 4/3 \cdot \pi \cdot (2.9\ nm)^3 = 102.16\ nm^3$$

$$V_{shell} = 4/3 \cdot \pi \cdot (r^3 - r'^3) = 4/3 \cdot \pi \cdot ((6\ nm)^3 - (2.9\ nm)^3) = 802.62\ nm^3$$

$$m_{core} = 7.87\ g/cm^3 \cdot 1.02 \cdot 10^{-19}\ cm^3 = 8.03 \cdot 10^{-19}\ g\ (per\ 12\ nm\ NP)$$

$$m_{shell} = 5.18\ g/cm^3 \cdot 8.03 \cdot 10^{-19}\ cm^3 = 4.16 \cdot 10^{-18}\ g\ (per\ 12\ nm\ NP)$$

Fe_3O_4 contains 72.36 wt% Fe → $m_{Fe\ in\ shell} = 3.01 \cdot 10^{-18}$ g

total weight of one NP:

$$m_{core} + m_{shell} = 4.96 \cdot 10^{-18} \text{ g}$$

total weight of Fe contained in one NP:

$$m_{core} + m_{Fe\ in\ shell} = (8.03 \cdot 10^{-19}) \text{ g} + (3.01 \cdot 10^{-18}) \text{ g} = 3.81 \cdot 10^{-18} \text{ g}$$

In a single iron/iron oxide NP the wt% of Fe content is:

$$\text{wt\%} = 76.8$$

23.35 mg of $FeSO_4 \cdot 7\ H_2O$ (contain 4.69 mg of Fe) and 7.91 mg of peptide **1** were used in the synthesis of the NPs used for the magnetic measurement. We assume that all of the iron atoms of the $FeSO_4 \cdot 7\ H_2O$ solution (addition of excess of reducing agent) will form Fe/Fe_3O_4 core/shell NPs and all the peptide molecules will be bound to the surface of the NPs:

4.69 mg Fe yield 6.11 mg of Fe core/ Fe3O4 shell NPs + 7.91 mg of peptide **1** = 14.02 mg

6.11 mg (Fe NPs) = 43.58 wt%
7.91 mg (peptide) = 56.42 wt%

The measured saturation magnetization M_s = 58 emu/ g corresponds to 43.58 wt% Fe NPs in the sample → mass corrected value of the saturation magnetization:

$$M_s \text{ (corr.)} = 58 \text{ emu/ g} \cdot (100/43.58) = \mathbf{133\ emu/\ g}$$

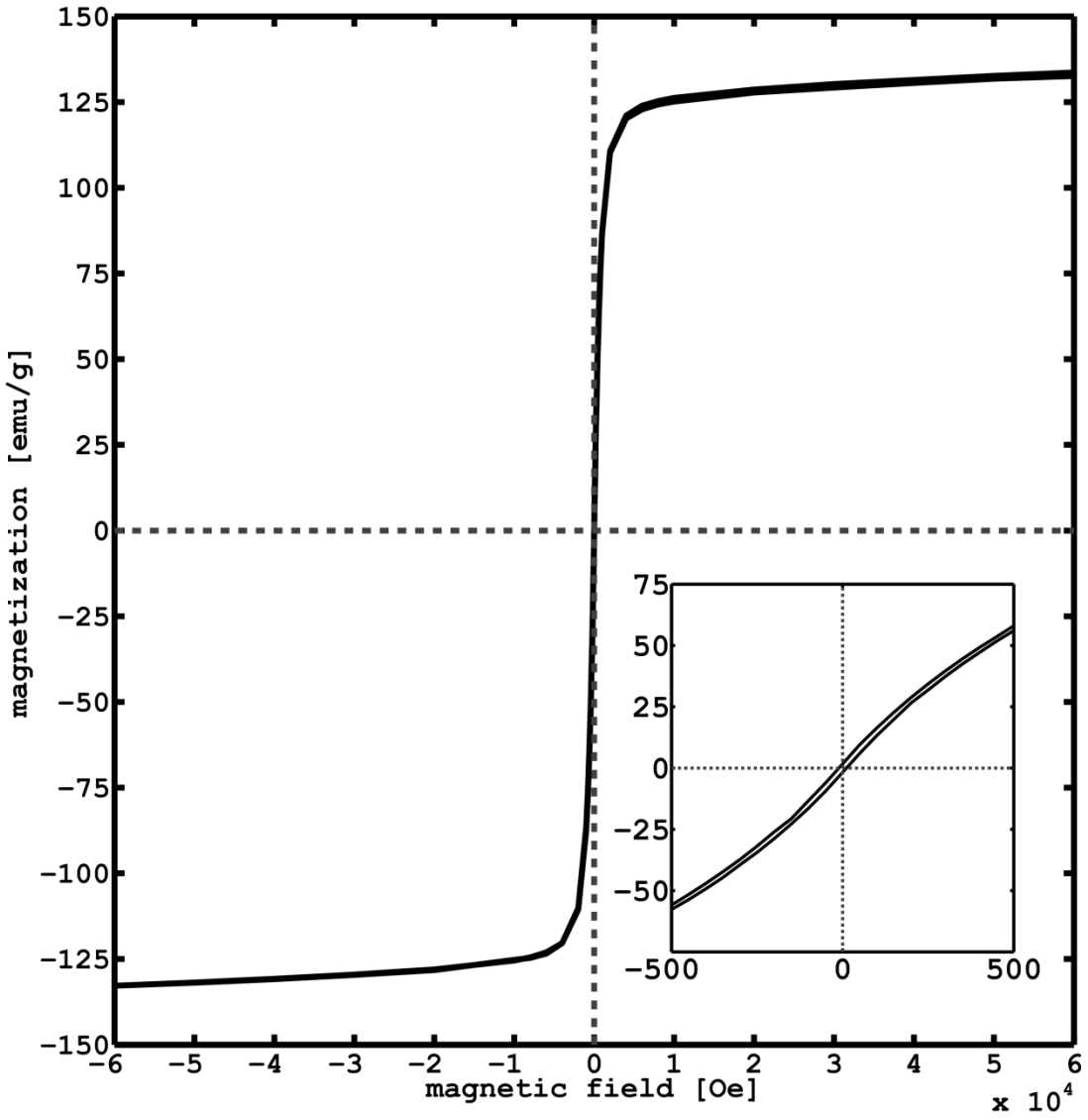


Figure S6: The M-H measurement at 300K yielded a mass corrected saturation magnetization value of M_s = 133 emu/g. Superparamagnetic character is proven by a remanent magnetization of only 1.7 emu/g and coercive field of 11 Oe, which both lie within the uncertainty of the SQUID magnetometer at low fields of ~ 10 Oe.

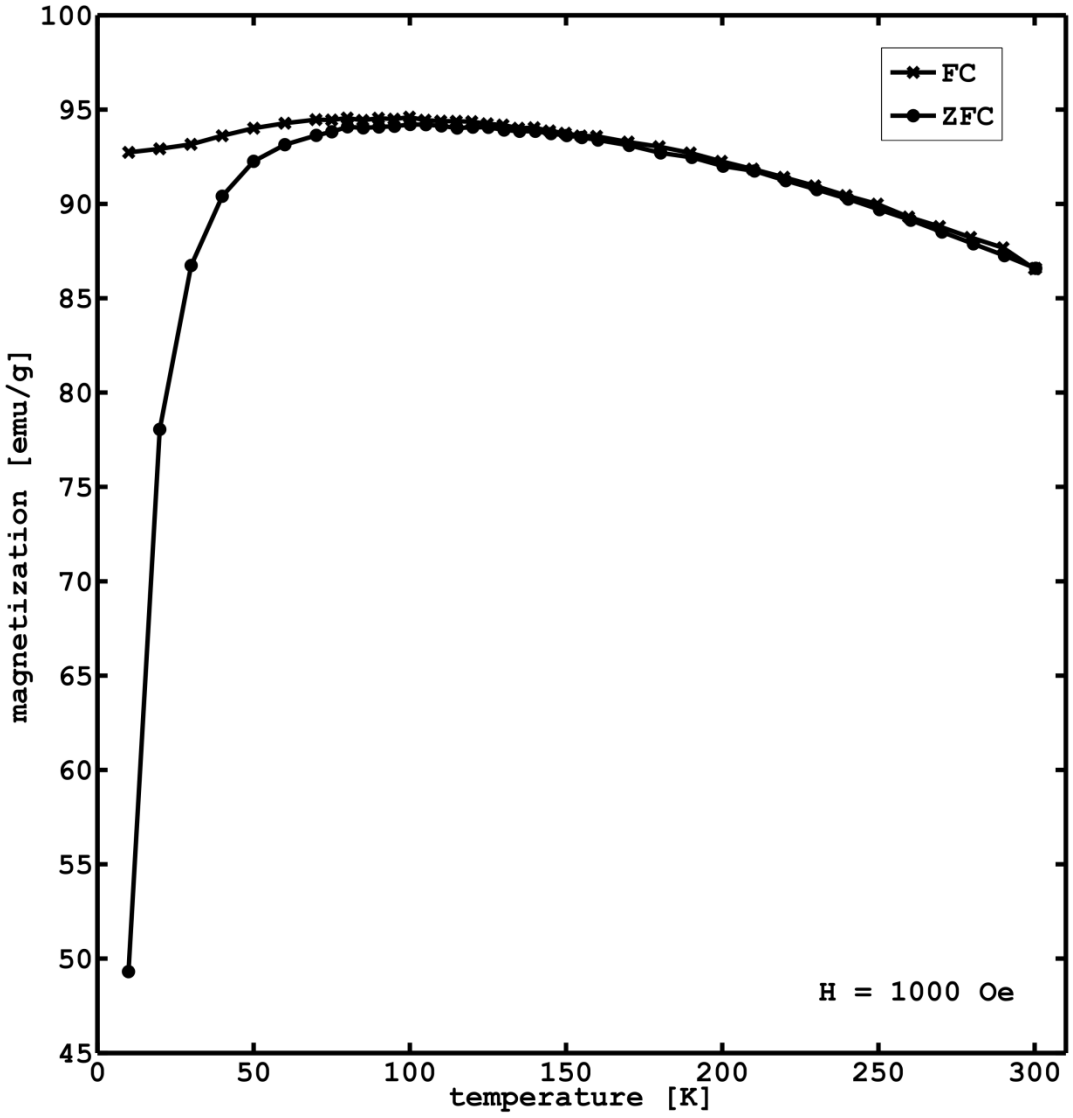


Figure S7: The M-T measurement was conducted at a set field of 1000 Oe in a temperature range from 10K-300K. Again the superparamagnetic character is shown by a clearly observable branching of the ZFC/FC measurements below room temperature at a blocking temperature of T_B = 105 K.

[1] D. D. Perrin, D. R. Perrin, W. L. F. Armarego, Purification of Laboratory Chemicals, Pergamon Press Ltd., Oxford, 2nd edn, **1980**.
[2] Lee, D. J.; Mandal, K.; Harris, P. W. R.; Brimble, M. A.; Kent, S. B. H. *Org. Lett.* **2009**, *11*, 5270-5273.
[3] Hung, K.-y.; Harris, P. W. R.; Brimble, M. A. *J. Org. Chem.* **2010**, *75*, 8728-8731.
[4] Jensen, K. J.; Meldal, M.; Bock, K. *J. Chem. Soc. Perkin Trans. 1* **1993**, 2119-2129.
[5] Brunet, E.; Juanes, O.; Jiménez, L.; Rodríguez-Ubis, J. C.*Tetrahedron Lett.* **2009**, 50, 5361-5363.
[6] Alexandrova, L. A.; Skoblov, A. Y.; Jasko, M. V.; Victorova, L. S.; Krayevsky, A. A. *Nucleic Acids Res.* **1998**, *26*(3), 778-786.
[7] Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43* (14), 2845-2852.