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## Supplementary Material

# Novel phosphopeptides as surface-active agents in iron nanoparticle synthesis

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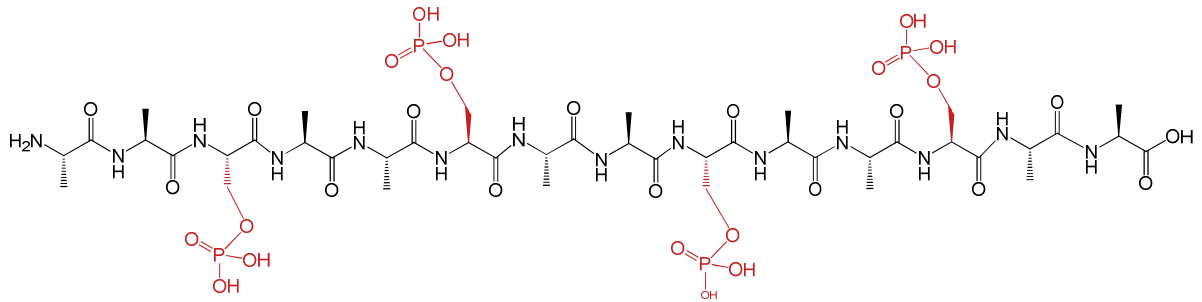
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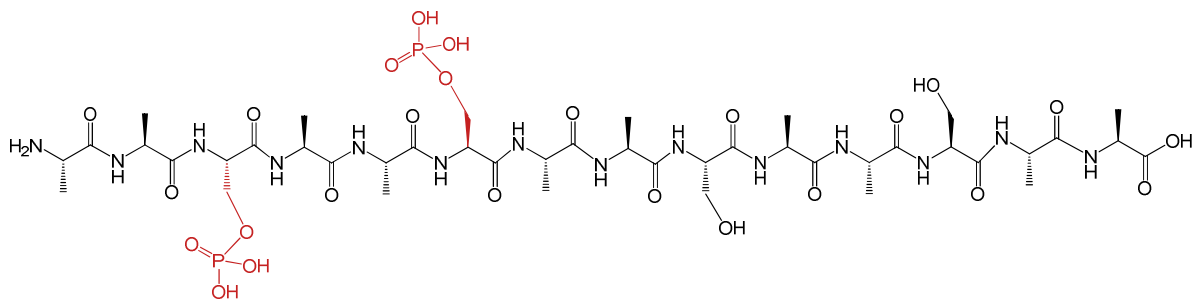
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## 1. Analogue Structures

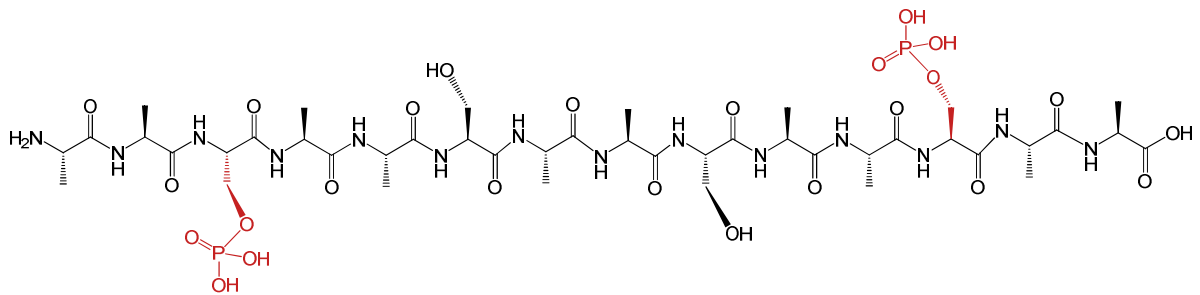
Analogue 1 AA**Sp**AA**Sp**AA**Sp**AA**Sp**AA



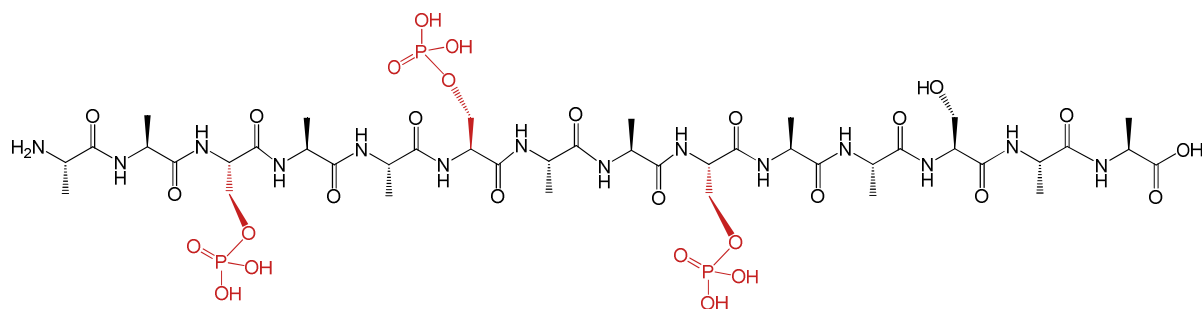
Analogue 2 AA**Sp**AA**Sp**AA**S**AA**S**AA



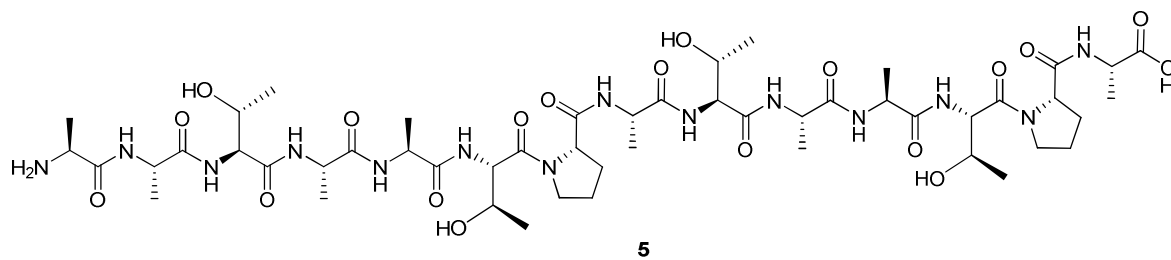
Analogue 3 AA**Sp**AA**S**AA**S**AA**Sp**AA



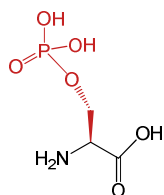
Analogue 4 AA<sup>Sp</sup>AA<sup>Sp</sup>AA<sup>Sp</sup>AASAA



Analogue 5 AATAATPATAATPA



Analogue 6 (Sp)



## 2. Synthesis of analogues 1-5

### Method

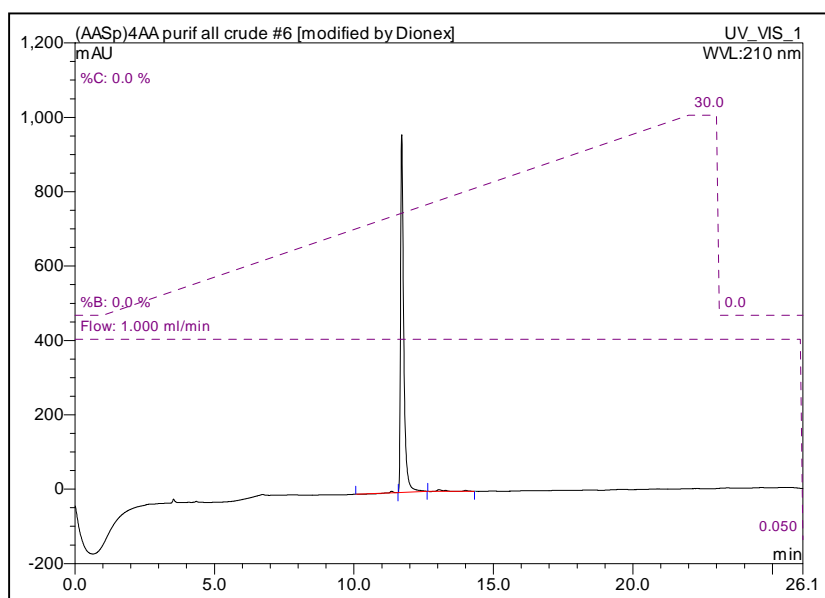
Fmoc-Ala-Wang-resin was prepared by adding Fmoc-Ala-Wanglinker-OH (Polypeptide Group) (0.2 mM) in DCM (3 mL) and DIC (42  $\mu$ L) to 0.1 mM of TentaGel S NH<sub>2</sub> resin (RAPP Polymere) previously swollen in DCM. The suspension was shaken gently for one hour, the resin washed with DCM and dried under nitrogen. Analogues **1-4** were then synthesized using solid phase peptide synthesis performed using a Tribute™ Peptide Synthesizer (Protein Technologies, Inc) using the Fmoc/*t*Bu strategy. The Fmoc group was deprotected with 20% v/v piperidine in DMF for 5 minutes followed by a second deprotection for 15 minutes at room temperature. The coupling steps were performed with 5 equivalents of Fmoc protected amino acid in DMF (0.25 M), 4.5 equivalents of HBTU in DMF (0.24 M) and 10 equivalents of NMM in DMF (2 M). Standard amino acids couplings were performed for 40 minutes at room temperature. The coupling of the phosphorylated serine was performed with 5 equivalents of Fmoc-Ser(HPO<sub>3</sub>Bzl)-OH (LC Sciences) using the same conditions as the natural amino acids but the coupling was performed for one hour. The coupling of the amino acids following the phosphorylated serines was also performed for one hour. In the synthesis of analogues **1-4**, Boc-Ala-OH was used as the last residue and coupled as a standard amino-acid. Following completion of the sequence, the peptide was cleaved from the resin with concomitant removal of protecting groups by treatment with TFA/TIPS/H<sub>2</sub>O (95/2.5/2.5, v/v/v) at room temperature for three to five hours as required. The crude peptide was precipitated with cold diethyl ether, isolated by centrifugation, washed with cold diethyl ether, dissolved in 1:1 (v/v) acetonitrile-water containing 0.1% TFA and lyophilized. The crude peptide products were analysed for purity by analytical RP-HPLC

(Dionex P680) at 210 and 254 nm using Gemini C18 (4.60 x 250 mm, 110Å, 5μ) column (Phenomenex). The solvent system used was A (0.1% TFA in H<sub>2</sub>O) and B (0.1% TFA in MeCN). Final purification was performed using a Waters 600 reverse-phase HPLC using a Gemini C18 (10.00 x 250 mm, 110Å, 5μ) column (Phenomenex). The solvent system used was A (0.1% TFA in H<sub>2</sub>O) and B (0.1% TFA in MeCN). Final purity was determined by analytical RP-HPLC (Dionex P680) using the same conditions as for the crude product. Peptide masses were confirmed by LC-MS (Dionex Ultimate 3000 equipped with a Thermo Finnigan Surveyor MSQPlus spectrometer) using ESI in the positive mode.

Synthesis and characterisation of analogue **5** was described in previously published work.<sup>1</sup>

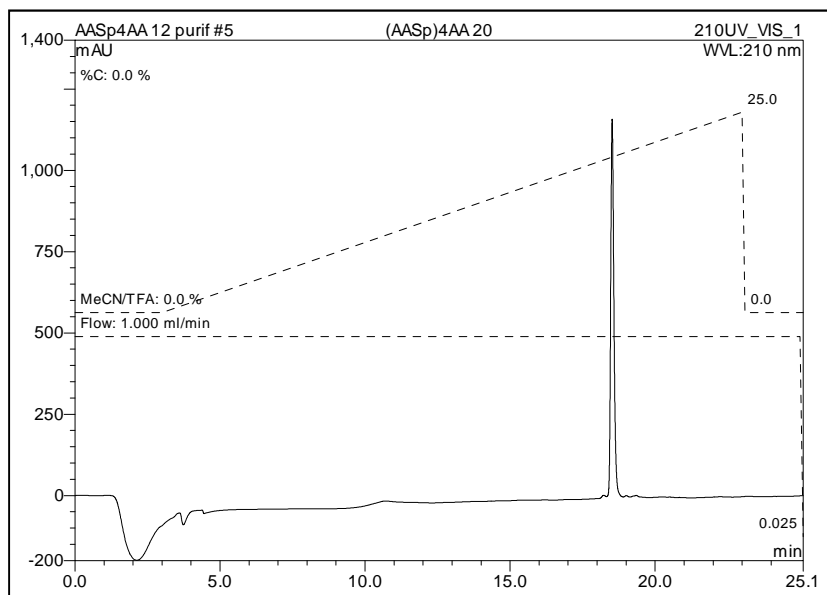
## Characterisation of analogues 1-5

### Analogue 1



H-[AASpAASpAASpAASpAA]-OH: C<sub>42</sub>H<sub>76</sub>N<sub>14</sub>O<sub>31</sub>P<sub>4</sub>; MW = 1397.2 g.mol<sup>-1</sup>; *m/z* (ESI)  
1397.25 [M+H]<sup>+</sup>; 699.19 [M+2H]<sup>2+</sup> .

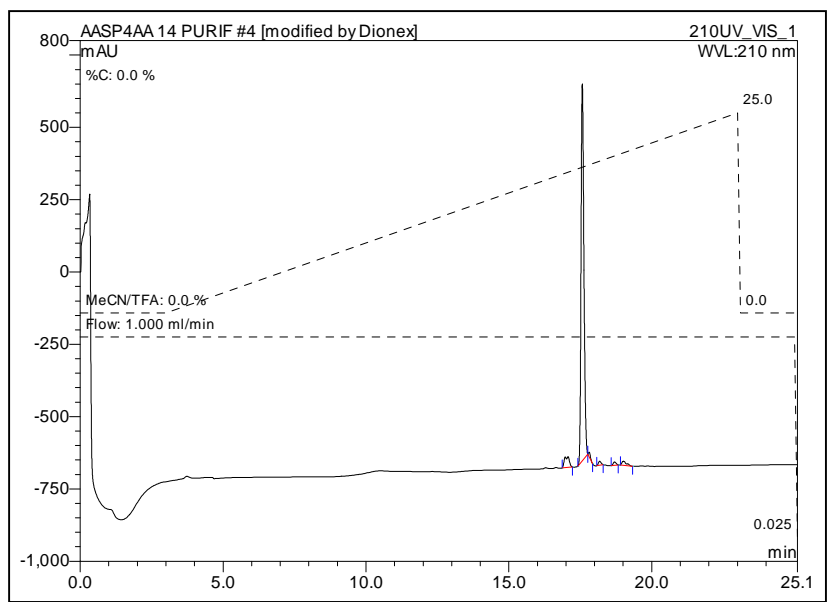
## Analogue 2



H-[AASpAASpAASAASAA]-OH:  $C_{42}H_{74}N_{14}O_{25}P_2$ ; MW = 1237.06 g.mol<sup>-1</sup>; *m/z* (ESI) 1237.44

[M+H]<sup>+</sup>; 619.22 [M+2H]<sup>2+</sup> .

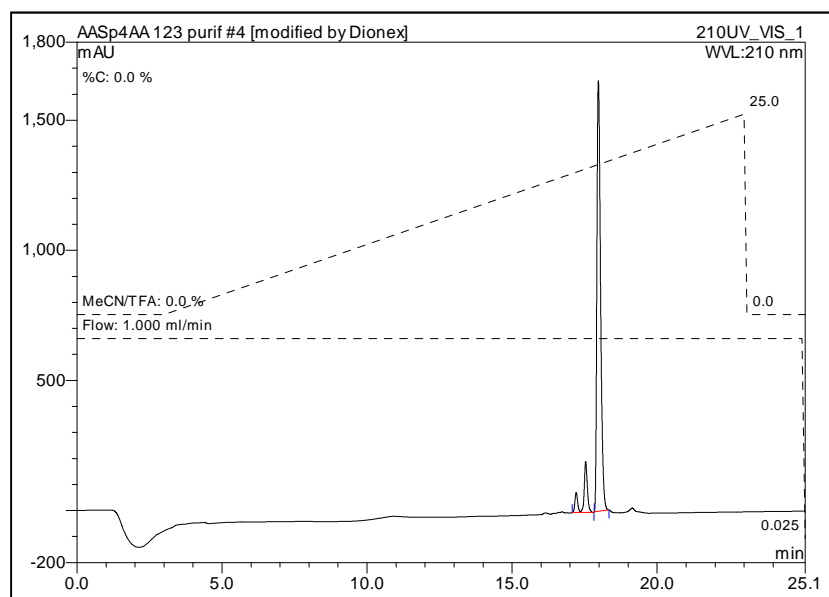
## Analogue 3



H-[AASpAASAASAASpAA]-OH:  $C_{42}H_{74}N_{14}O_{25}P_2$ ; MW = 1237.06 g.mol<sup>-1</sup>; *m/z* (ESI) 1237.41

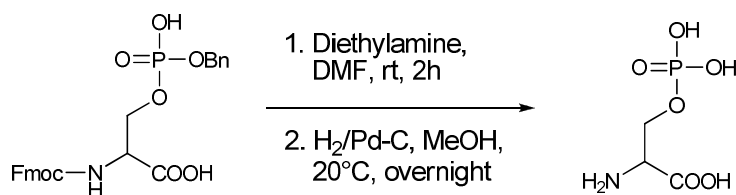
[M+H]<sup>+</sup>; 619.22 [M+2H]<sup>2+</sup> .

## Analogue 4



H-[AASpAASpAASpAASAA]-OH:  $C_{42}H_{75}N_{14}O_{28}P_3$ ; MW =  $1317.04 \text{ g}\cdot\text{mol}^{-1}$ ;  $m/z$  (ESI)  
1317.41  $[M+H]^+$ ; 659.21  $[M+2H]^{2+}$ .

### 3. Synthesis of phosphoserine 6



#### General :

All reagents were purchased as reagent grade and used without further purification. Analytical thin layer chromatography was performed using aluminum-backed silica plates, and compounds were visualised by staining with potassium permanganate solution, followed by heating the plate

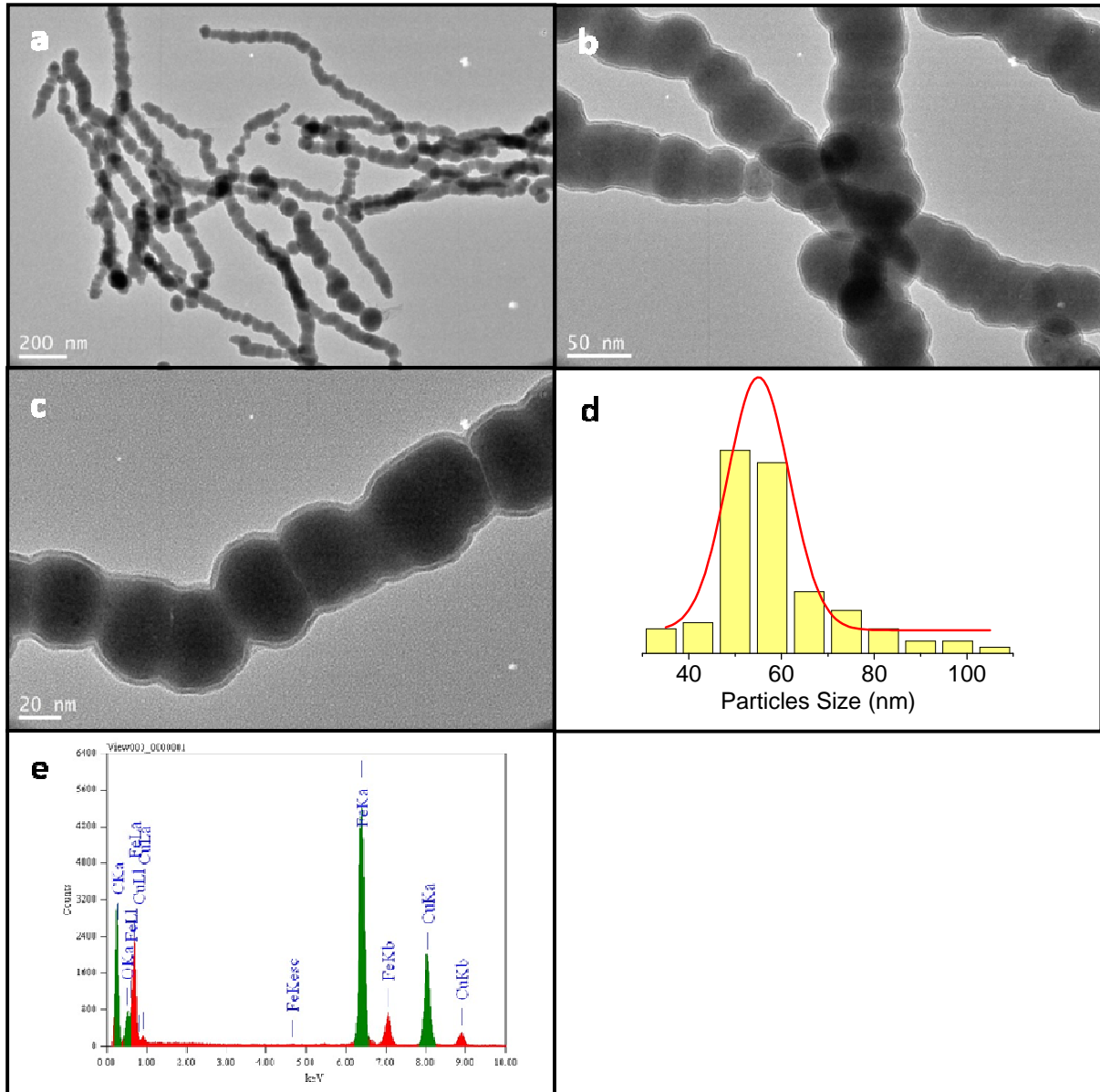


as appropriate. Infrared spectra were obtained on an FTIR spectrometer as neat samples and absorption maxima are expressed in wavenumbers ( $\text{cm}^{-1}$ ).  $^1\text{H}$  NMR spectra were recorded on a Bruker AC 300 (300 MHz) spectrometer at ambient temperature. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard and were reported as chemical shift ( $\delta$ ), relative integral, multiplicity (m, multiplet), coupling constant ( $J$  in Hz) and assignments. Mass spectra were recorded by LC-MS (Dionex Ultimate 3000 equipped with a Thermo Finnigan Surveyor MSQPlus spectrometer). The ionisation method employed was electrospray ionization (ESI) operating with an ionisation potential of 65 eV. Flow injection method was used with 100% MeOH as the solvent.

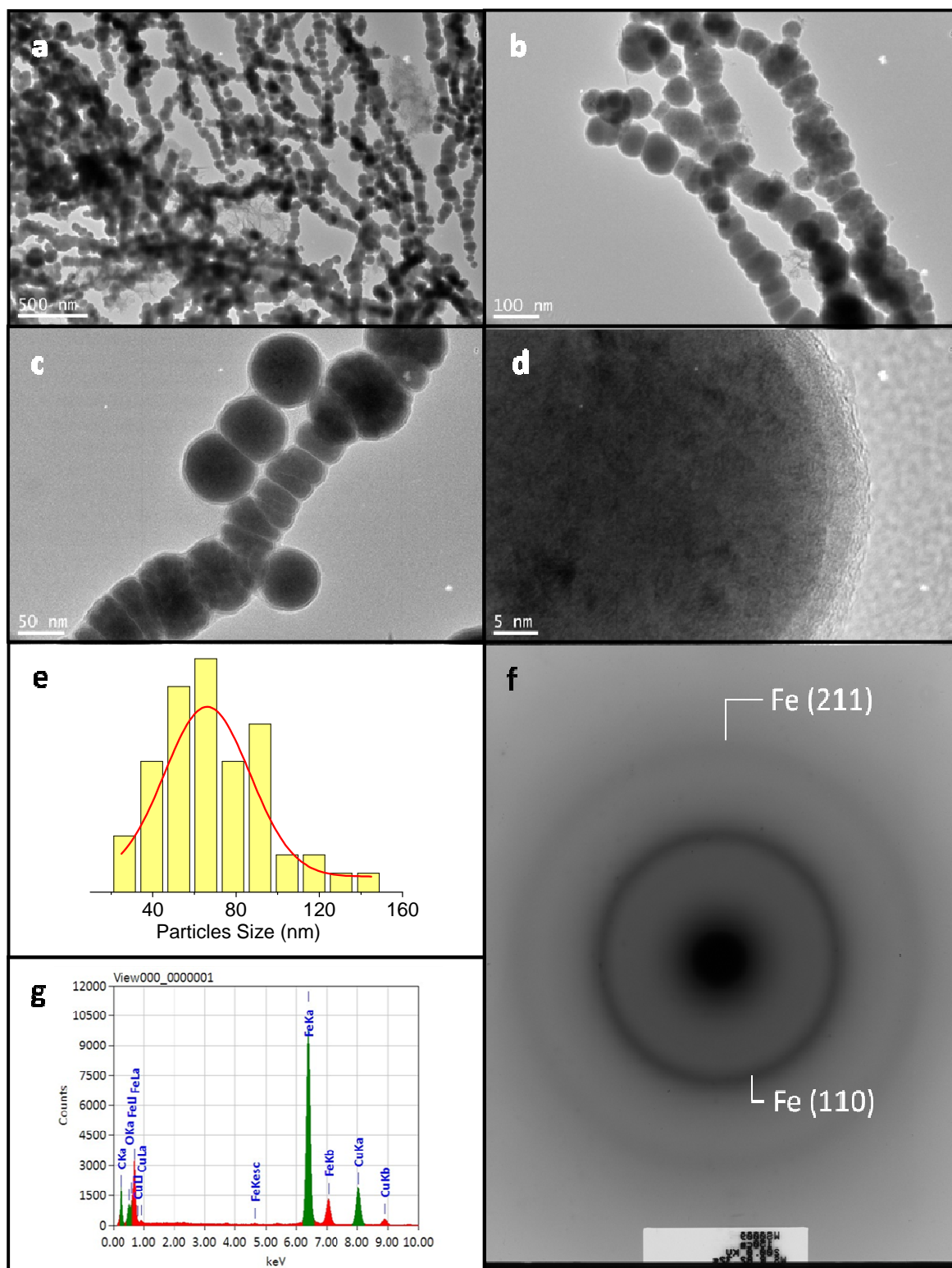
#### Preparation of HN-Ser( $\text{H}_2\text{PO}_3$ )-OH **6** :

Commercially available Fmoc-Ser( $\text{HPO}_3\text{Bzl}$ )-OH (200 mg, 0.4 mmol) was dissolved in a mixture of 20% diethylamine in DMF (2 mL) and stirred for two hours at room temperature. The solvent was then removed under reduced pressure. Without further purification, the product was dissolved in methanol (2 mL), 10% palladium on carbon (10 mg) was added and the reaction was left to stir overnight with  $\text{H}_2$  gas bubbling into the mixture. The suspension was then filtered through Celite and the solvent evaporated under reduced pressure. After redispersion in water (3 mL), the suspension was washed with diethyl ether ( $2 \times 3$  mL) and ethyl acetate ( $2 \times 3$  mL). The aqueous phase was then collected and the water evaporated under reduced pressure. The resulting residue was recrystallised from a mixture of water, cold diethyl ether and methanol to afford the *title compound 6* as a white powder.  $\nu_{\text{max}}$  (neat)/ $\text{cm}^{-1}$  3002 (br), 1629, 1516, 1088, 987, 760.  $\delta_{\text{H}}$  (300 MHz,  $\text{D}_2\text{O}$ ) 4.18-4.06 (m, 2H,  $\text{H}_\beta$ ), 3.94-3.91 (m, 1H,  $\text{H}_\alpha$ ).  $m/z$  (EI,  $[\text{M}-\text{H}]^-$ ) found at 184.00 and (EI,  $[\text{M}_2-\text{H}]^-$ ) found at 369.01. The data obtained were in agreement with that reported in the literature.<sup>2</sup>

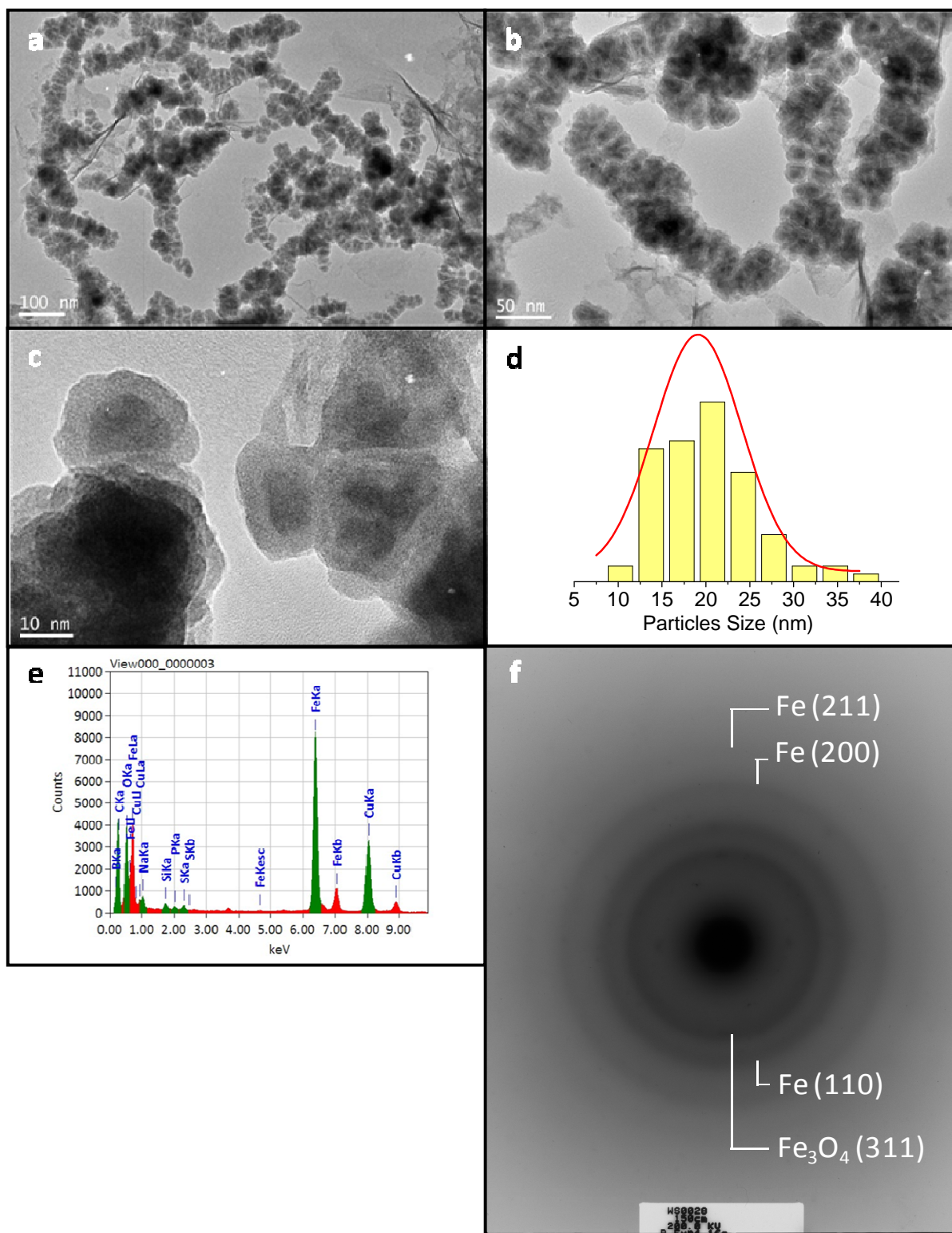
#### 4. Characterisation of the nanoparticles



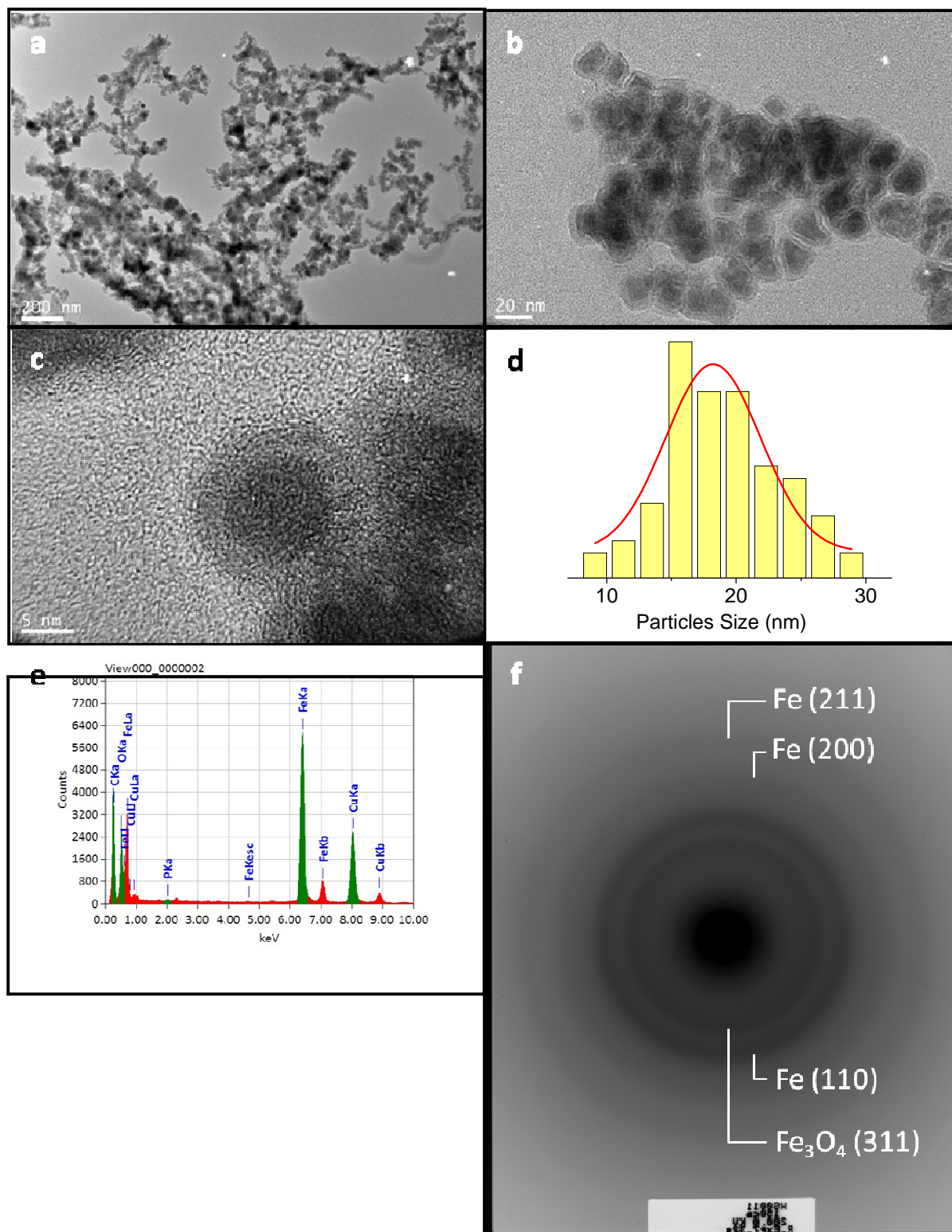
**Figure S1. Analysis of particles prepared in experiment *a*.** a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS measurement graph.



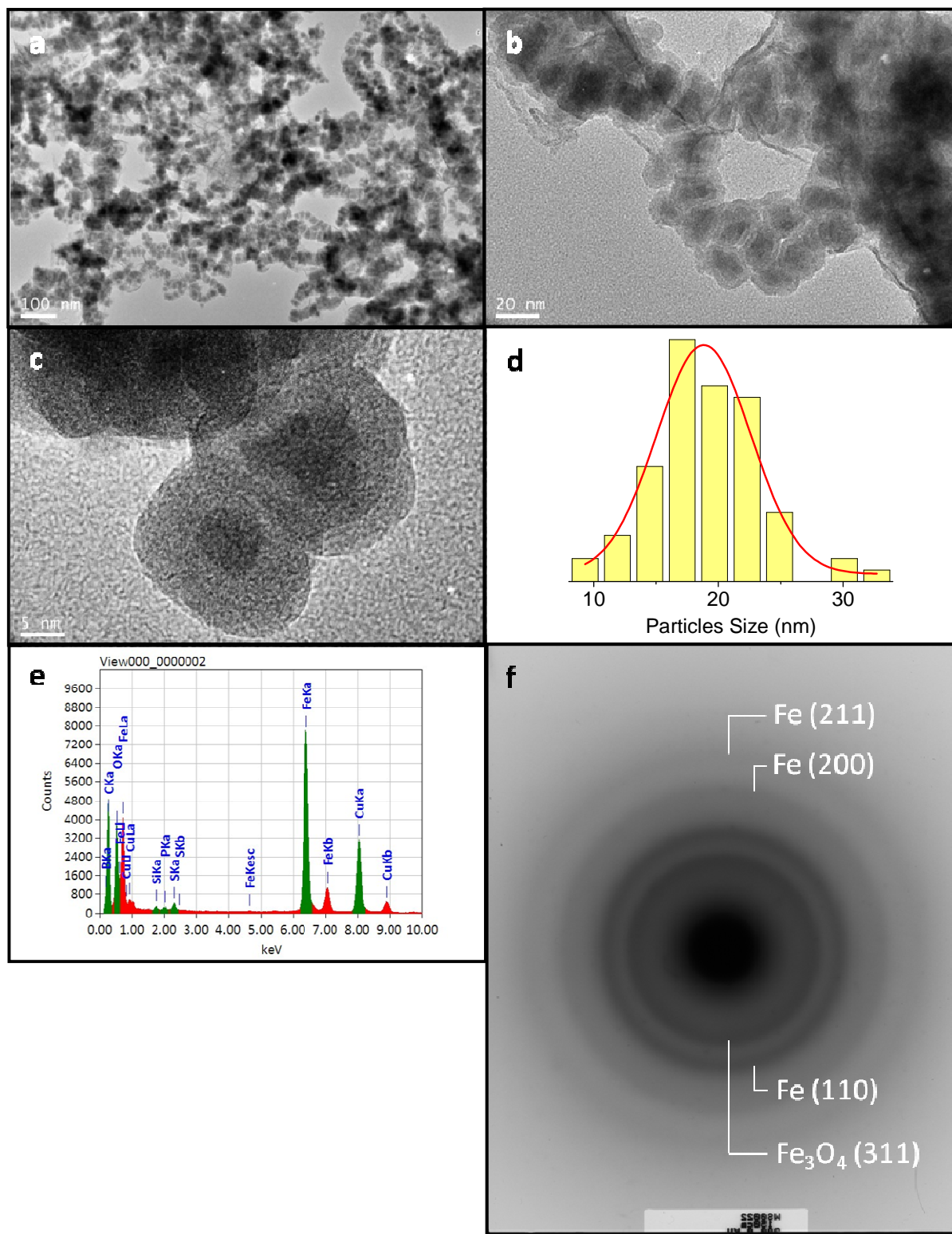
**Figure S2. Analysis of particles prepared in experiment *b*.** a-d) TEM pictures of particles at different magnifications; e) histogram of particles size; f) EDS spectra; g) electron diffraction pattern.



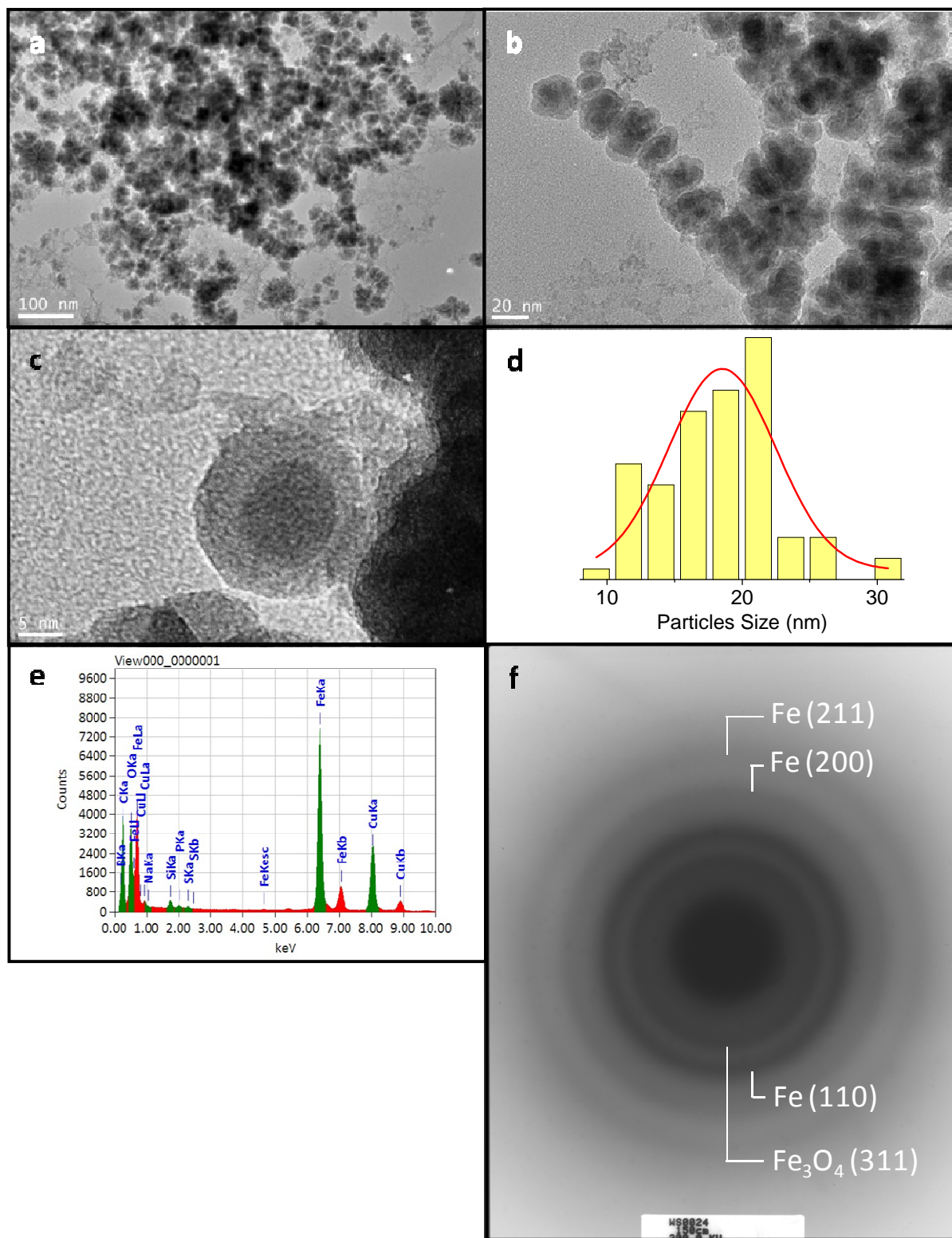
**Figure S3. Analysis of particles prepared in experiment c.** a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS spectra; f) electron diffraction pattern.



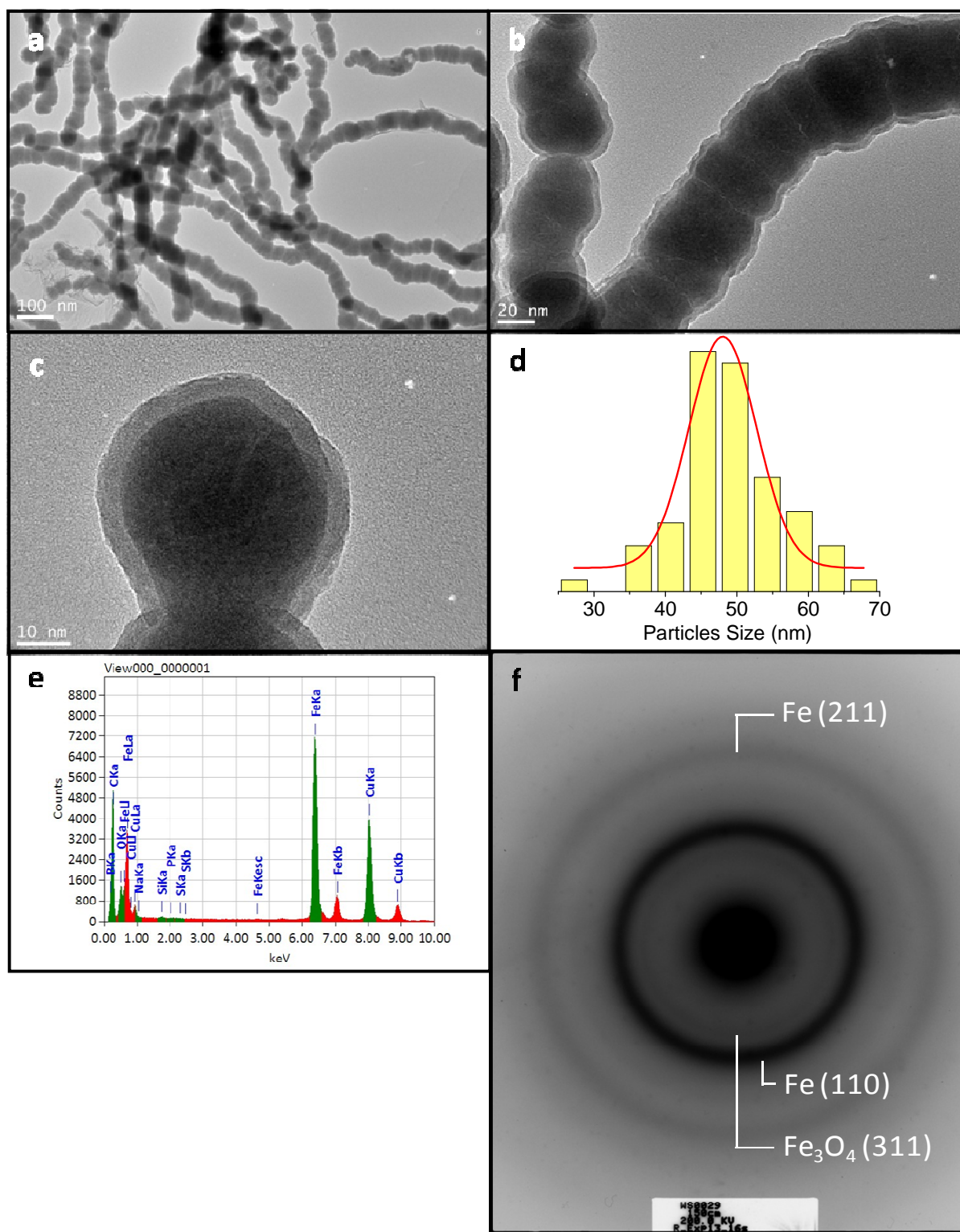
**Figure S4.** Analysis of particles prepared in experiment *d*. a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS spectra; f) electron diffraction pattern.



**Figure S5. Analysis of particles prepared in experiment *e*.** a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS spectra; f) electron diffraction pattern.

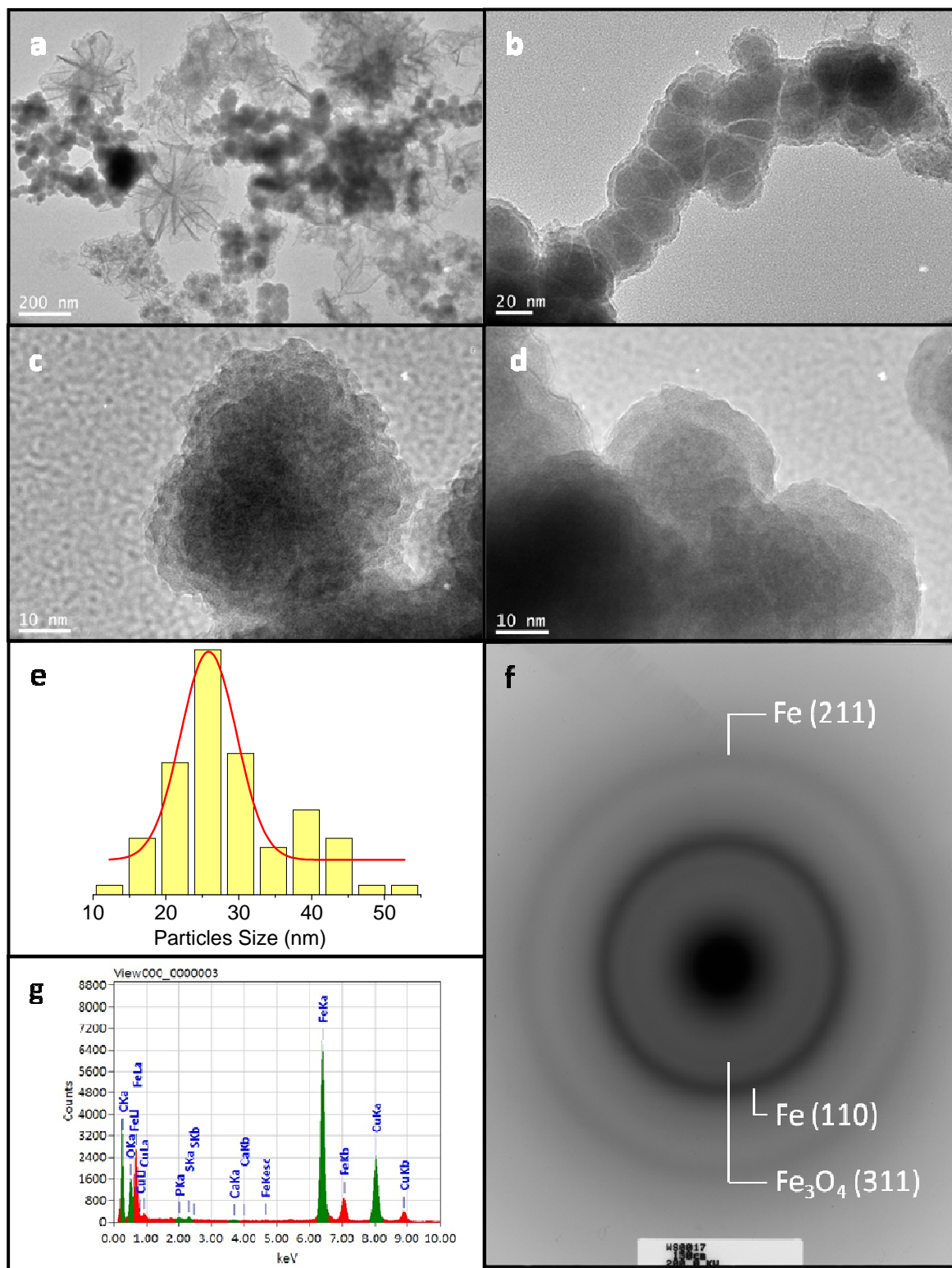


**Figure S6. Analysis of particles prepared in experiment *f*.** a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS spectra; f) electron diffraction pattern.



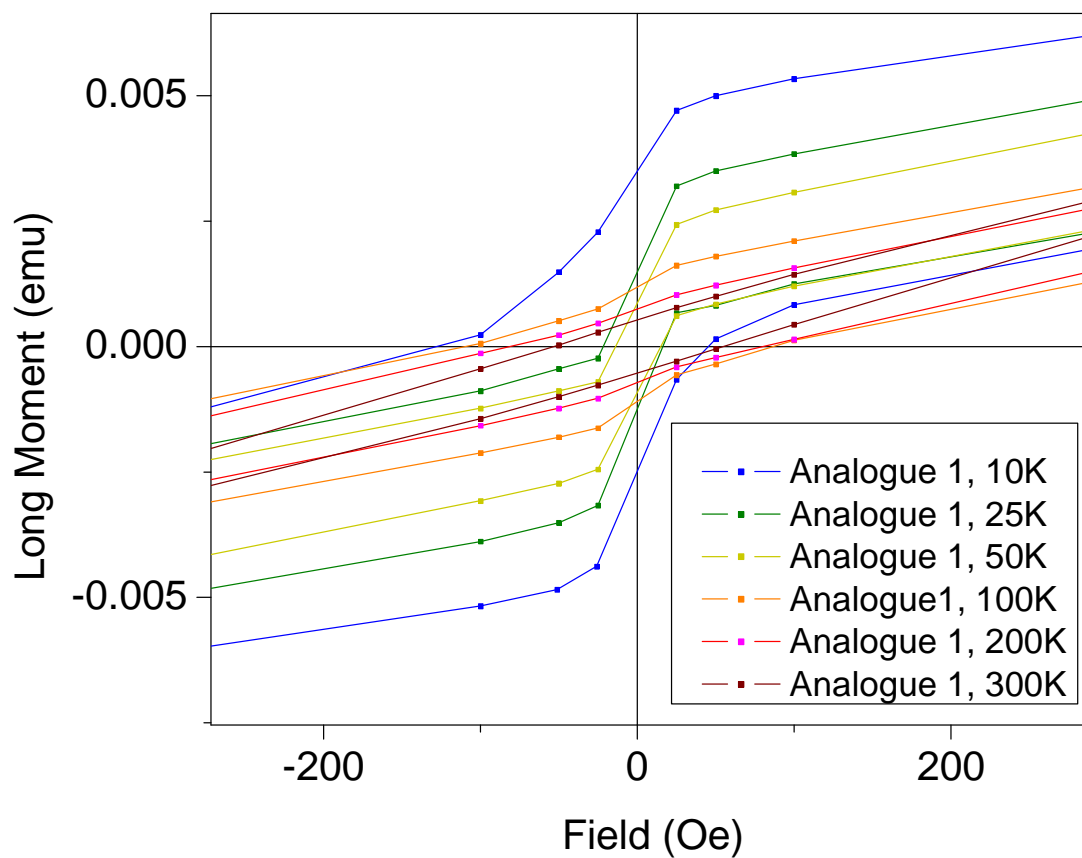
**Figure S7. Analysis of particles prepared in experiment g.** a-c) TEM pictures of particles at different magnifications; d) histogram of particles size; e) EDS spectra; f) electron diffraction pattern.



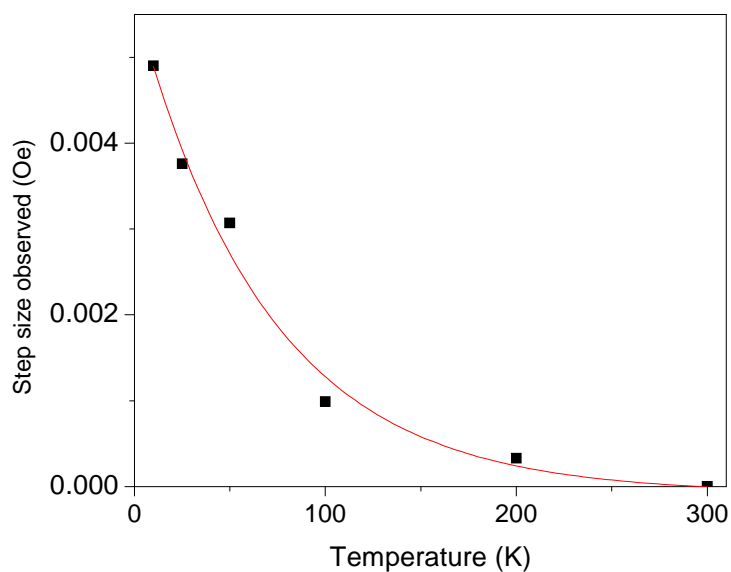


**Figure S8. Analysis of particles prepared in experiment *h*.** a-d) TEM pictures of particles at different magnifications; e) histogram of particles size; f) EDS spectra; g) electron diffraction pattern.

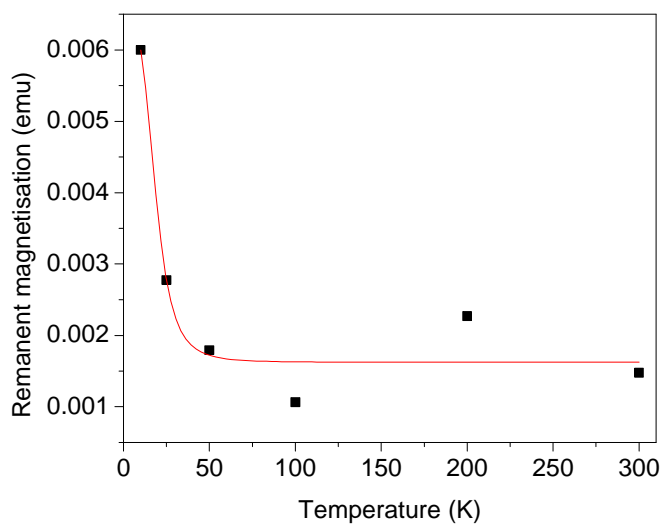
## 5. Magnetic Measurement



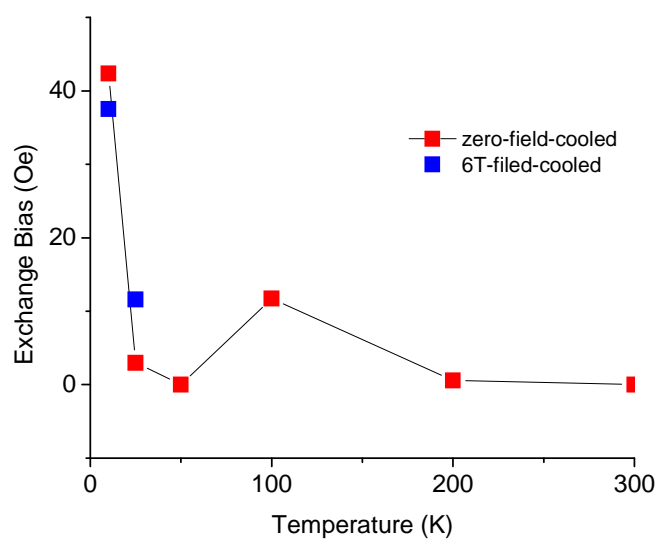
**Figure S9.** Evolution of the magnetic moment steps as a function of temperature as observed in the M(H) plot of the particles prepared in the presence of analogue **1** as an additive.



**Figure S10.** Size of the observed steps as a function of temperature for the particles prepared in presence of analogue **1** as an additive.



**Figure S11.** Remanent magnetisation as a function of temperature for the particles prepared in presence of analogue **1** as an additive.



**Figure S12.** Exchange bias field as a function of temperature for the particles prepared in presence of analogue **1** as an additive. The red values were obtained by cooling the system at zero field whereas the blue values were obtained by cooling down the system under an applied field of 6T.

## 6. References

1. R. Peltier, C. W. Evans, A. L. DeVries, M. A. Brimble, A. J. Dingley and D. E. Williams, *Cryst. Growth Des.*, 2010, **10**, 5066-5077.
2. L. D. Arnold, R. G. May and J. C. Vederas, *J. Am. Chem. Soc.*, 1988, **110**, 2237-2241.