Simultaneous optimisation of shape and magnetisation of nanoparticles synthesised using a green bioinspired route

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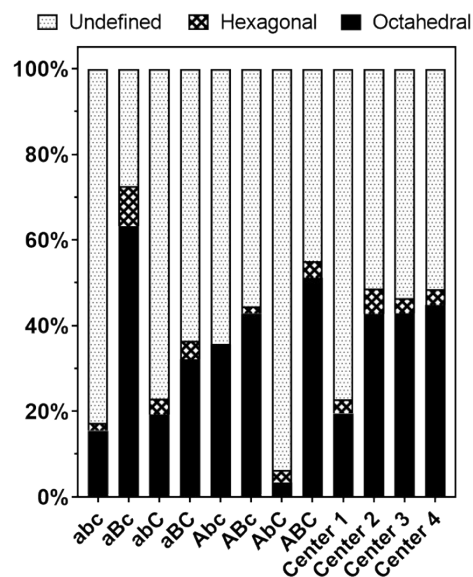
Design 1
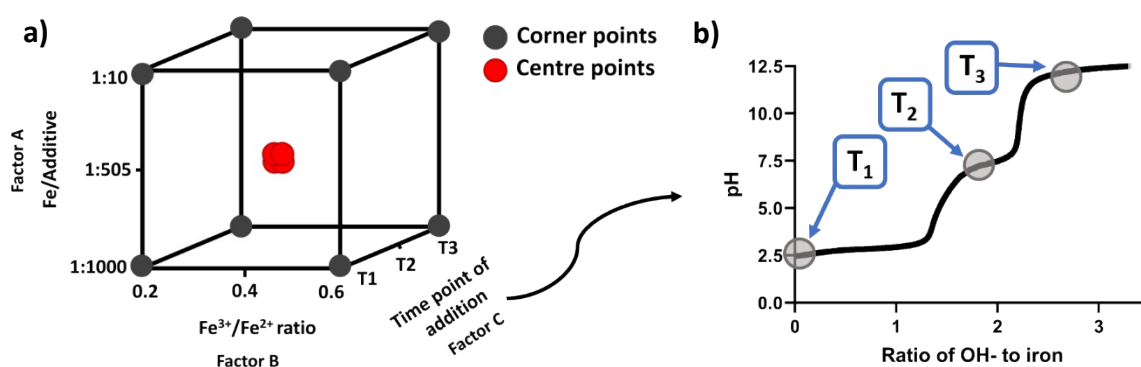
Figure S1. a) Three-factor study varying (A) Additive:iron ratio, (B) Fe^{3+}:Total Fe, and (C) additive time-point of addition, with the grey and red circles representing reaction conditions tested. **b)** pH titration curve from a 0.6 ratio reaction, showing the timepoints at which TEPA was added.

Table S1. Summary of the synthesis conditions with the experimental results from the first iteration of the factorial design

| Experiment number	Block	Factor A TEPA:iron ratio (mol/mol)	Factor B Fe(III):total Fe ratio (mol/mol)	Factor C Time of TEPA addition	% Faceted particles	Average size (nm)	Saturisation magnetism (emu g^{-1})
1	1	1:1000	0.2	T1	17.3	35.3	34.2
2	1	1:10	0.6	T1	44.5	21.0	70.6
3	1	1:10	0.2	T3	6.2	19.9	53.4
4	1	1:1000	0.6	T3	36.5	18.6	68.6
5	1	1:505	0.4	T2	22.7	33.9	62.0
6	1	1:505	0.4	T2	48.6	30.0	64.0
7	2	1:10	0.2	T1	35.7	24.4	67.5
8	2	1:1000	0.6	T1	72.6	21.7	75.0
9	2	1:1000	0.2	T3	23.0	27.4	67.2
10	2	1:10	0.6	T3	55.0	17.2	72.3
11	2	1:505	0.4	T2	46.5	31.2	72.6
12	2	1:505	0.4	T2	48.4	27.8	82.6

Figure S2. Shape distribution of particles formed at various response levels as detailed in Table 1. The three factors are denoted by the letters a-c where a = Fe/additive ratio, b = Fe^{3+}/Fe^{2+} ratio, and c = time point of TEPA addition. Capital letters denotes a high factor level, whereas lowercase represents a low factor level (in the case of Fe/additive a high level denotes more additive present).
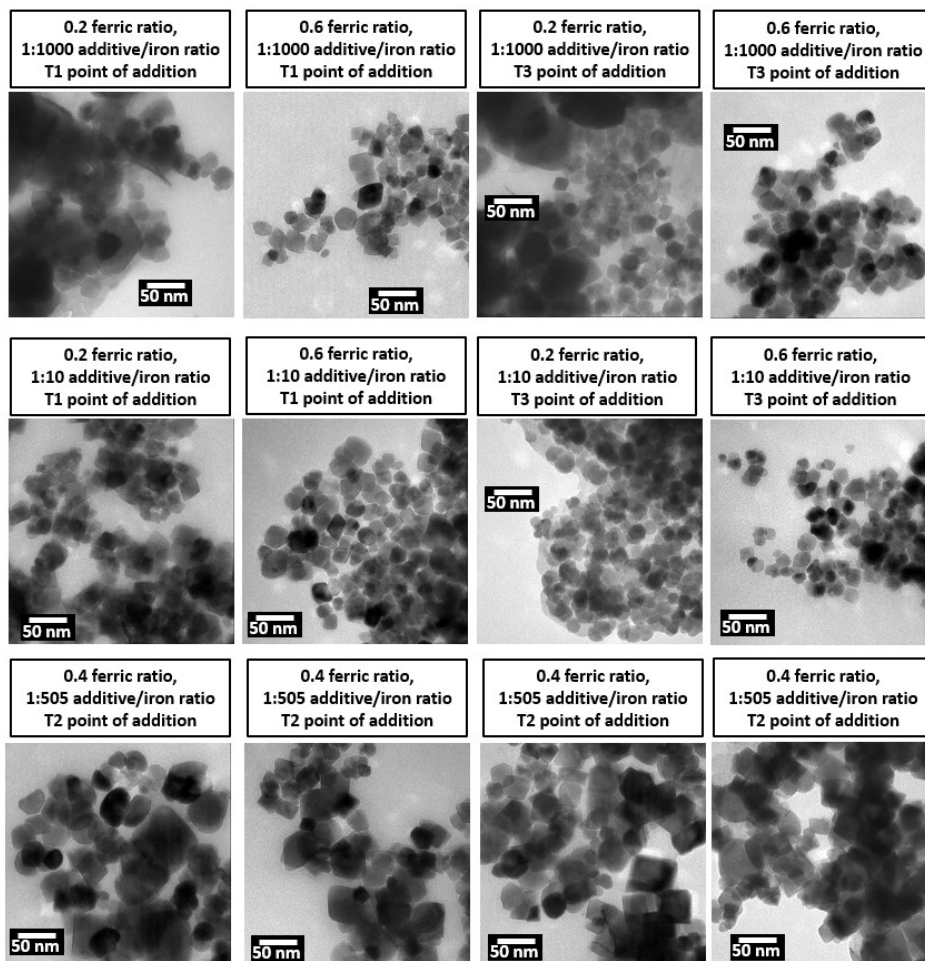
Figure S3. Representative TEM images of the particles formed in the first DoE iteration in order of experiment number
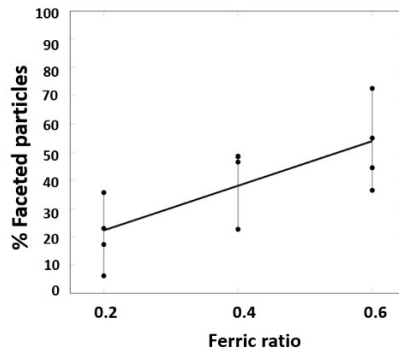
Figure S4. Variation of % faceted particles with ferric ratio for the first iteration of DoE. Black dots are the experimental values.
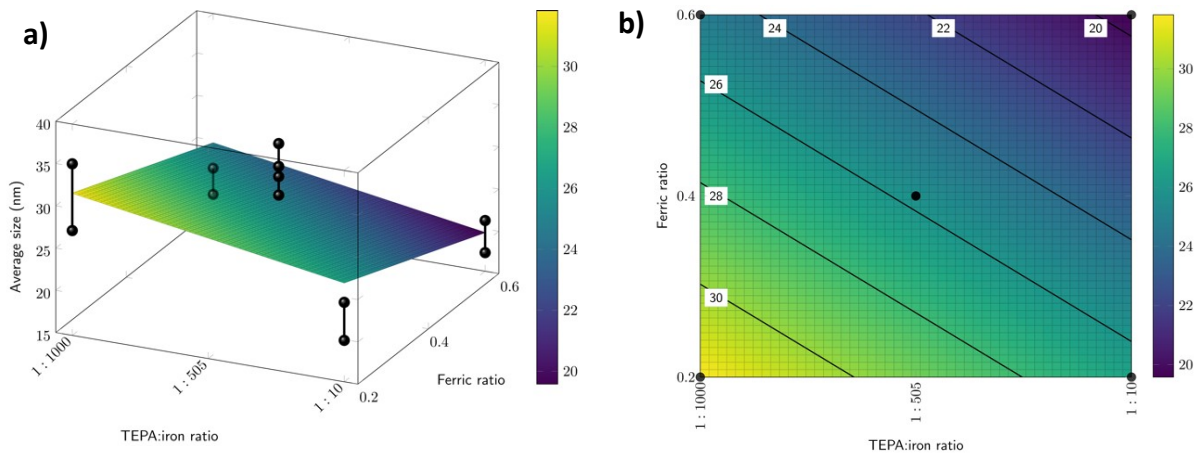
Figure S5. a) Surface plot of variation of average size with additive concentration and ferric ratio. Black spheres are experimental values; **b)** Contour plot of variation of average size with additive concentration and ferric ratio. Contour lines shown in black represent constant average size indicated in white box.
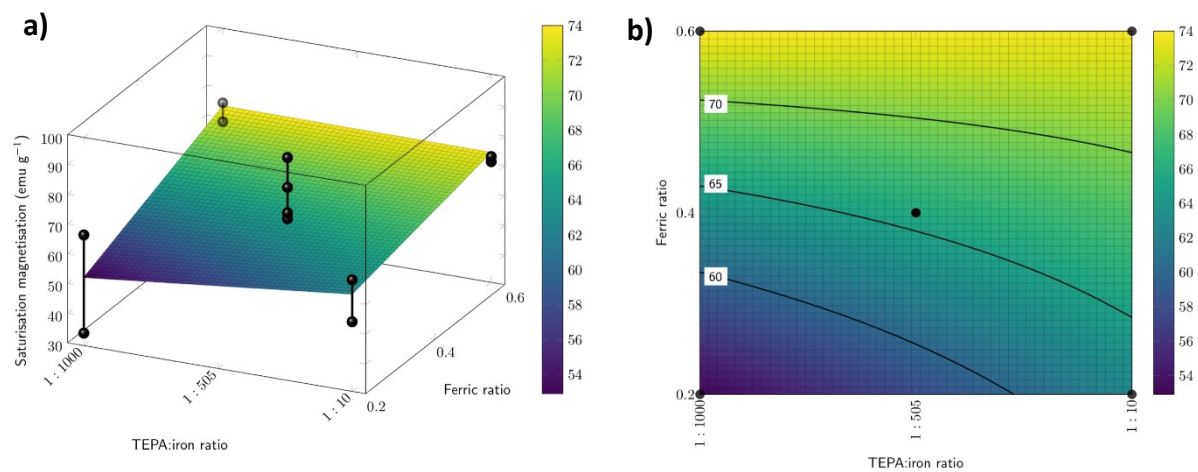
Figure S6. a) Surface plot of variation of saturisation magnetisation with additive concentration and ferric ratio. Black spheres are experimental values; **b)** Contour plot of variation of saturisation magnetisation with additive concentration and ferric ratio. Contour lines shown in black represent constant saturisation magnetisation indicated in white box.

Table S2. Analysis of variance for the response isotropic faceted particles (%) of the first DoE iteration

| Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-Value	P-Value
A (tepa:fe)	8.00	1	8	0.06	0.819
B (ferricratio)	1997.12	1	1997.12	15.48	0.029
C (time)	305.04	1	305.04	2.36	0.222
AB	15.68	1	15.68	0.12	0.750
AC	16.24	1	16.24	0.13	0.746
BC	0.40	1	0.40	0.00	0.959
Blocks (ABC)	925.76	1	925.76	7.17	0.075
Residual	387.09	3	129.03		
Lack-of-fit	49.88	1	49.88	0.30	0.641
Pure Error	337.21	2	168.61		
Total	3727.46	11			

Table S3. Analysis of variance for the response saturation magnetism (emu/g) of the first DoE iteration

| Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-Value	P-Value
A (tepa:fe)	44.69	1	44.686	2.53	0.21
B (ferricratio)	514.52	1	514.516	29.14	0.012
C (time)	25.38	1	25.376	1.44	0.317
AB	50.89	1	50.886	2.88	0.188
AC	190.07	1	190.069	10.77	0.046
BC	70.61	1	70.609	4	0.139
Blocks (ABC)	593.64	1	593.644	33.62	0.01
Residual	52.97	3	17.656		
Lack-of-fit	0.47	1	0.474	0.02	0.905
Pure Error	52.49	2	26.247		
Total	1663.49	11			

Table S4. Analysis of variance for the response average particle size (nm) of the first DoE iteration

| Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-Value	P-Value
A (tepa:fe)	433.65	1	433.65	30.05	0.012
B (ferricratio)	1881.91	1	1881.91	130.42	0.001
C (time)	87.78	1	87.78	6.08	0.090
AB	686.35	1	686.35	47.56	0.006
AC	44.65	1	44.65	3.09	0.177
BC	98.70	1	98.70	6.84	0.079
Blocks (ABC)	2.34	1	2.34	0.16	0.714
Residual	43.29	3	14.43		
Lack-of-fit	18.55	1	18.55	1.5	0.345
Pure Error	24.74	2	12.37		
Total	3711.33	11			

Design 2
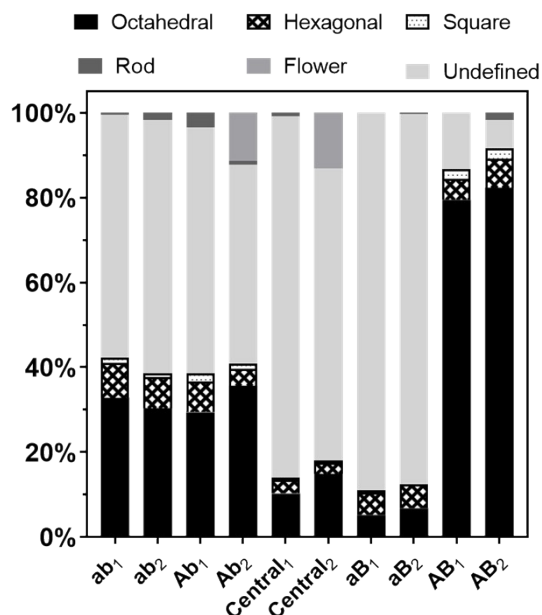
Figure S7. Shape distribution of particles formed in the second factorial design. The two factors are denoted by the letters a-b where a = Fe/additive ratio, b = Fe^{3+}/Fe^{2+} ratio. Capital letters denotes a high factor level, whereas lowercase represents a low factor level (in the case of Fe/additive a high level denotes more additive present and subscript 1 and 2 represent the two repeats of the experiments).
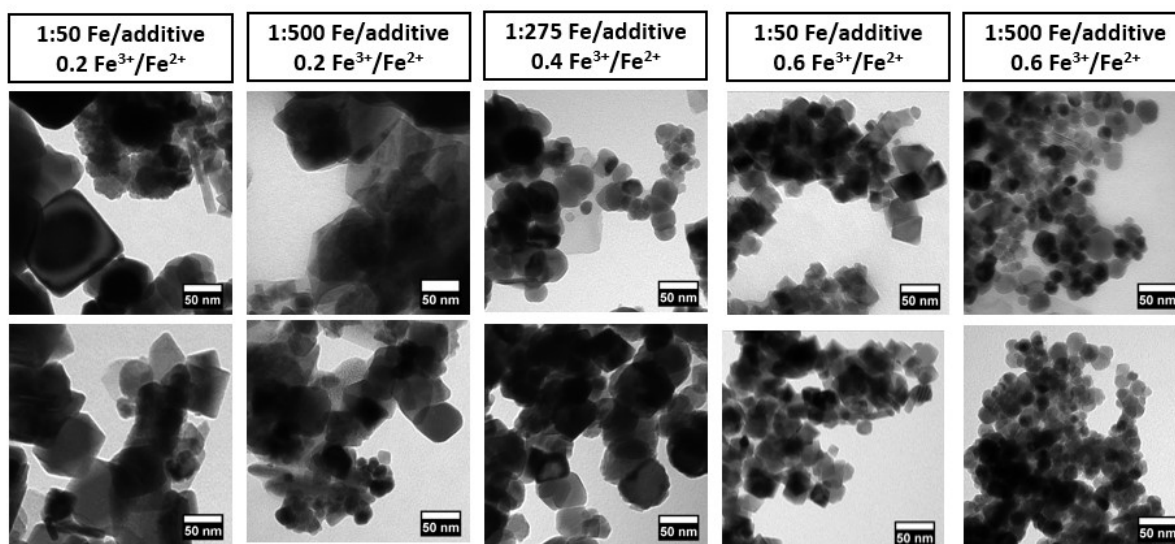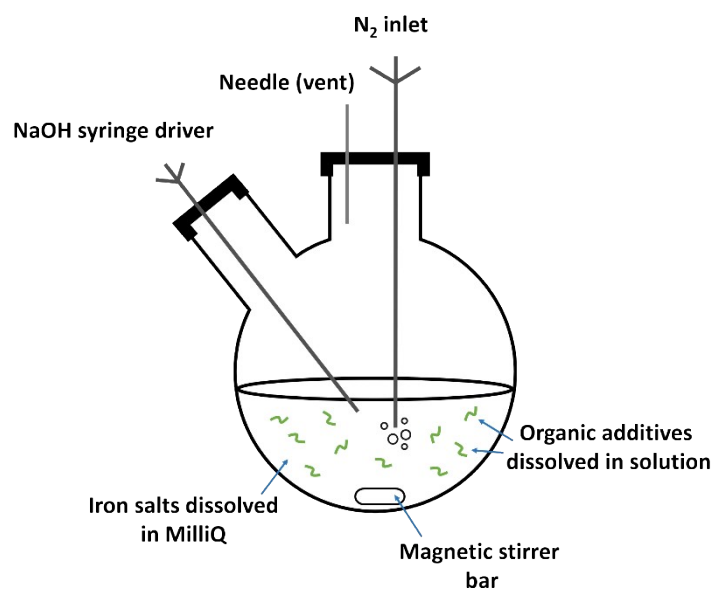

Figure S8. Representative TEM images of the particles formed in the second DoE iteration.

Design 3
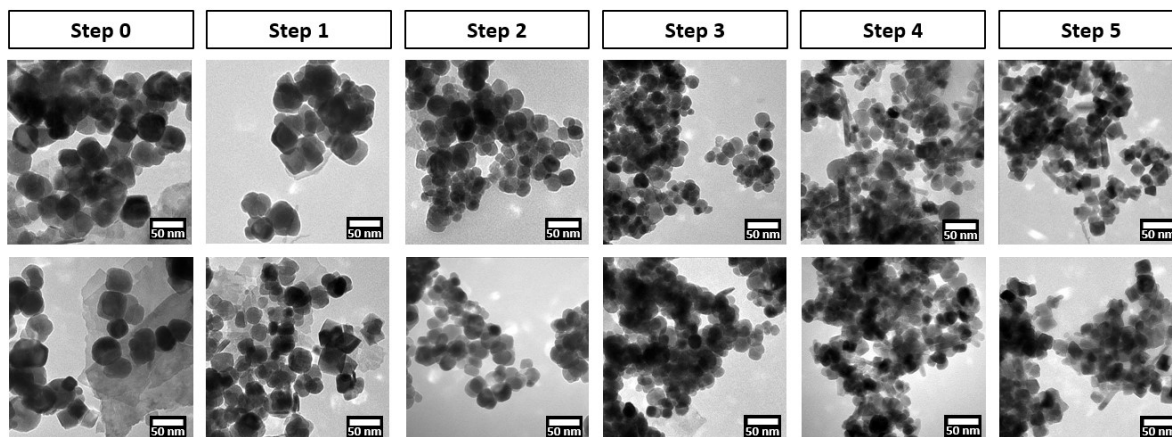
**Figure S9.** Representative TEM images of the particles formed in the third DoE iteration.

Miscellaneous

Figure S10. Schematic of the reaction set-up used to synthesise magnetic nanoparticles in each co-precipitation reaction.

Particle Sizing Methodology:

Particle measurements are taken across the longest axis of each particle to ensure consistency between measurements using ImageJ software. Approximately 200 measurements are taken from each sample to get an accurate measure of mean size given the variety in particles per sample. To avoid human bias in which particles are selected for analysis, particles are analysed from a single "area" of a TEM image to prevent small or large particles being selectively measured. Several images are analysed for each sample to ensure a
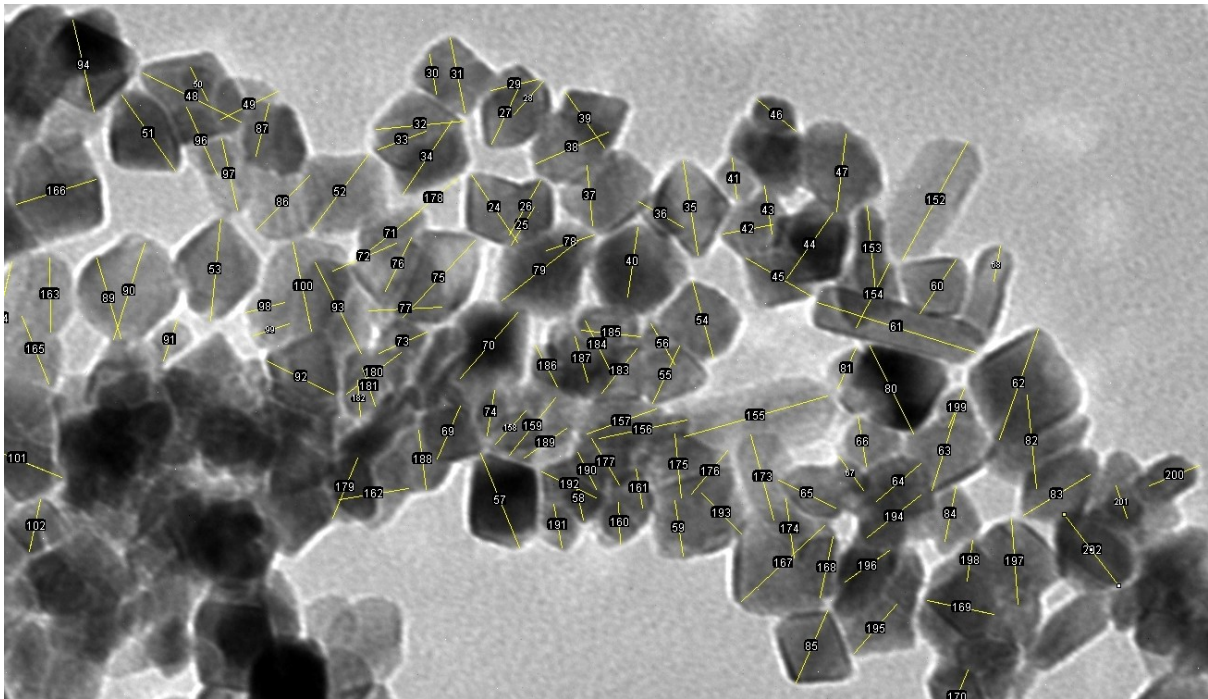


representative sample is taken.

Figure S11. Screenshot of ImageJ particle sizing showing particles are analysed in clusters.

Particle Shape Analysis Methodology:

Images are manually analysed using drawing software (Inkscape, Paint.net, etc) by marking each particle a shape with a set colour (figure S12). A minimum of 300 samples are marked from several different images to ensure a representative sample is selected. The particles are then counted, at which point the shape assignment is checked a secondary time to maximise accuracy. Sample particle shapes can be seen in figure S13.
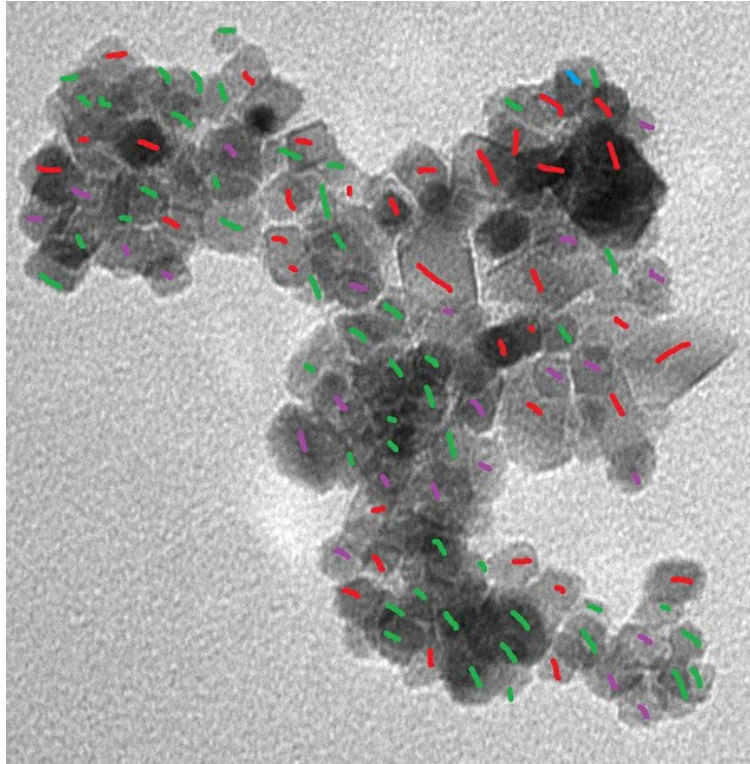
Figure S12. Example image analysis showing octahedral (red), undefined (green), and hexagonal (purple) particles.
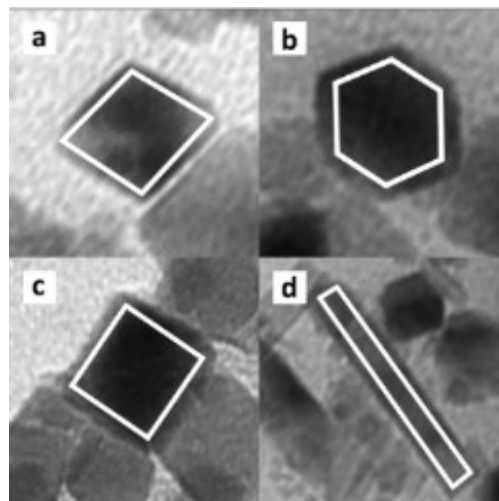
Figure S13. Sample particle shapes from TEM analysis; **a)** Octahedral; **b)** Hexagonal; **c)** square; **d)** rod/elongated.