

Supporting Information

Sizing by Weighing: Characterizing Sizes of Ultra-small-sized Iron Oxide Nanocrystals Using MALDI-TOF Mass Spectrometry

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Detailed Experimental Procedures:

Chemicals

90% oleic acid, 98% iron chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 1-octadecene and 9-nitroanthracene were purchased from Aldrich. 95% sodium oleate, diphenyl ether and oleyl alcohol were purchased from TCI. Acetone, *n*-hexane and chloroform (CHCl_3) were purchased from Samchun Chem.

Synthesis of ultra-small-sized iron oxide nanocrystals

Small-sized iron oxide nanocrystals were prepared using the heat-up process.¹ Iron-oleate complex was prepared by reacting sodium oleate and iron(III) chloride hexahydrate.² For the synthesis of 3 nm-sized nanocrystals, 1.8 g (2 mmol) of iron-oleate complex, 0.57 g (2 mmol) of oleic acid and 1.61 g (6 mmol) of oleyl alcohol were dissolved in 10 g of diphenyl ether at room temperature. The mixture was heated to 250 °C at a heating rate of 10 °C/min, and then kept for 30 min under inert atmosphere. After the reaction, the reaction vessel was rapidly cooled to room temperature, and washed by adding 5 mL of *n*-hexane and 50 mL of acetone. The nanocrystals were washed twice and dispersed in 10 mL of chloroform. To obtain 2 nm-sized nanocrystals, 1.8 g (2 mmol) of iron-oleate complex and 3.22 g (12 mmol) of oleyl alcohol were dissolved in 10 g of diphenyl ether. The mixture was heated to 250 °C at heating rate of 10 °C/min, and then kept at that temperature for 30 min. The washing step was the same as the 3 nm scheme. When the aging was performed at 200 °C, 1.5 nm-sized nanocrystals were generated. To obtain 4 nm-sized nanocrystals, 1.8 g (2 mmol) of iron-oleate complex, 0.57 g (2 mmol) of oleic acid, and 1.61 g (6 mmol) of oleyl alcohol were dissolved in 10 g of 1-octadecene. The mixture was heated to 280 °C at heating rate of 10 °C/min, and then kept at that temperature for 30 min. The 3 nm-sized iron oxide nanocrystals shown in Figure 3a were synthesized by thermal decomposition of 0.9 g (1 mmol) of iron-oleate complex in the presence of 0.57 g (2 mmol) of oleic acid and 1.61 g (6 mmol) of oleyl alcohol in 10 g of diphenyl ether at 250 °C for 30 min.

Small-sized iron oxide particles were also obtained in the absent of oleyl alcohol. 1.8 g (2 mmol) of iron-oleate complex and 0.57 g (2 mmol) of oleic acid were dissolved in 10 g of 1-octadecene. The mixture was heated to 300 °C at heating rate of 3.3 °C/min. When the reaction mixture was aged for 30 min and 35 min, 1 and 2.5 nm-sized nanocrystals were synthesized, respectively. When the mixture was heated to 320 °C at heating rate of 10 °C/min, and aged at that temperature for 30 min, 4.3 nm-sized nanocrystals were obtained.

Characterization with MALDI-TOF MS

MALDI-TOF MS was performed on a Voyager-DE™ STR Biospectrometry Workstation manufactured by Applied Biosystems Inc. in National Center for inter-University Research Facilities. 9-nitroanthracene was used as a matrix. Nanocrystals were dispersed in chloroform and mixed with chloroform solution of 9-nitroanthracene in a ratio of 1:1 and spotted onto a target plate. Desorption and ionization of the nanocrystals were achieved by absorbing pulsed nitrogen laser (337 nm, 3 ns pulses). The spectra were measured with the laser between 40% and 50% full power. After desorption, a 20 kV potential accelerated the ions into a 2.0 m flight tube (linear mode), which yielded a resolution of 3-5 m/z in the mass spectrum. The mass spectrum was smoothed with simple average of 100 data point.

Nanocrystal characterization

TEM images were obtained with JEOL-2010 electron microscope. Powder X-ray diffraction (XRD) pattern was collected with a Rigaku D/Max-3C diffractometer, equipped with a Cu K α radiation source ($\lambda = 0.15418$ nm). Fourier-transform infrared (FT-IR) spectra were obtained with a JASCO FT/IR 200. Optical absorption was characterized using a JASCO V-550 UV-VIS spectrometer. Magnetic studies were carried out using a Vibrating Sample Magnetometer (VSM) equipped within Physical Property Measurement System (PPMS®) manufactured by Quantum Design. Thermogravimetric analysis (TGA) was performed with a Q-5000 IR (TA Instrument).

Tracking formation mechanism

1.8 g (2 mmol) of iron-oleate complex, 0.57 g (2 mmol) of oleic acid and 1.61 g (6 mmol) of oleyl alcohol were dissolved in 10 g of diphenyl ether at room temperature. The mixture was heated to a 250 °C at a heating rate of 10 °C/min, and then kept for 60 min under inert atmosphere. To study growth mechanism, 0.1 mL of reaction mixture was collected by syringe during the heat-up process. The sample aliquots were washed twice with the mixed solvent of 1 mL of acetone and 0.1 mL of hexane. The samples were separated by centrifugation and dispersed in 0.1 mL of chloroform.

Supplementary Text:

Formulating eq 2

The total mass (M) is denoted as the sum of the core and ligand masses as follows:

$$M = M_{\text{core}} + M_{\text{ligand}} \quad (\text{S1})$$

The M_{core} is the product of density and volume, and the M_{ligand} is the product of packing density of ligand and molecular mass of ligand and surface area. Assuming that the particles are spherical, the total mass of the nanocrystal is expressed as a third order formula with respect to the diameter as follows:

$$M = \frac{\rho N_A \pi D^3}{6} + \sigma m \pi D^2 = aD^3 + bD^2 \quad (\text{S2})$$

where ρ is the core density (4.87×10^{-21} g nm $^{-3}$ for maghemite), N_A is Avogadro's number (mol $^{-1}$ = Da g $^{-1}$), D is the nanocrystal diameter, σ is the packing density of the ligand and m is the molecular mass of the ligand (281.5 Da for oleate). All coefficients except σ were known. Assuming that σ is constant, it can be derived from the data set of core mass fraction.

The core fraction (f) is represented as M_{core}/M :

$$f = \frac{M_{\text{core}}}{M} = \frac{\frac{\rho N_A \pi D^3}{6}}{\frac{\rho N_A \pi D^3}{6} + \sigma m \pi D^2} \quad (\text{S3})$$

Taking the reciprocal of both sides, we obtain the following:

$$\frac{1}{f} = \frac{6\sigma m}{\rho N_A D} + 1 \quad (\text{S4})$$

The coefficient σ deduced from the slope of f^1 (obtained from TGA) versus D^{-1} (measured by TEM) plot was 3.93 nm^{-2} (Figure S6), which well agreed with the reported value, 3.8 nm^{-2} .³ Substituting the σ value, the third- and second-order coefficients (a and b , respectively) of D are obtained: $a = 1.54 \times 10^3 \text{ Da nm}^{-3}$, $b = 3.46 \times 10^3 \text{ Da nm}^{-2}$.

$$M = \frac{\rho N_A \pi D^3}{6} + \sigma m \pi D^2 = aD^3 + bD^2 \quad (\text{S5})$$

Eq S5 is then rearranged as follows:

$$aD^3 + bD^2 - M = 0 \quad (\text{S6})$$

The cubic equation with respect to D can be solved using Cardano's method. We reduce eq S6 by substituting D with $(t - b/3a)$ to obtain a depressed cubic equation.

$$a\left(t - \frac{b}{3a}\right)^3 + b\left(t - \frac{b}{3a}\right)^2 - M = 0 \quad (\text{S7})$$

The equation is rearranged in the descending order of t .

$$t^3 - \frac{b^2}{3b}t + \frac{2b^3}{27a^2} - M = 0 \quad (\text{S8})$$

To facilitate the calculation of the cubic equation, the coefficients in eq S8 are substituted with p and q :

$$t^3 + pt + q = 0 \quad (\text{S9})$$

where $p = -b^2/3a$ and $q = 2b^3/27a^2 - M$.

Variable t is denoted as the sum of two variables (u and v), which satisfies the condition:

$$uv = -\frac{p}{3} \quad (\text{S10})$$

Eq S9 is rearranged with respect to u and v .

$$(u + v)^3 - 3uv(u + v) + q = 0 \quad (\text{S11})$$

From eqs S10 and S11, we obtain eqs S12 and S13, respectively.

$$u^3 v^3 = -\frac{p^3}{27} \quad (\text{S12})$$

$$u^3 + v^3 = -q \quad (\text{S13})$$

Solving the simultaneous equation for u^3 and v^3 , we obtained:

$$u^3 = -\frac{p}{2} + \sqrt{\frac{q^2}{4} + \frac{p^3}{27}} \quad (\text{S14})$$

$$v^3 = -\frac{p}{2} - \sqrt{\frac{q^2}{4} + \frac{p^3}{27}} \quad (\text{S15})$$

Since t is the sum of u and v , we get

$$t = u + v = \sqrt[3]{-\frac{p}{2} + \sqrt{\frac{q^2}{4} + \frac{p^3}{27}}} + \sqrt[3]{-\frac{p}{2} - \sqrt{\frac{q^2}{4} + \frac{p^3}{27}}} \quad (\text{S16})$$

$$\mathbf{D} = t - \frac{b}{3a} = -\frac{b}{3a} + \sqrt[3]{-\frac{p}{2} + \sqrt{\frac{q^2}{4} + \frac{p^3}{27}}} + \sqrt[3]{-\frac{p}{2} - \sqrt{\frac{q^2}{4} + \frac{p^3}{27}}} \quad (\text{S17})$$

As the diameter is real number, complex solutions are not needed.

Substituting p with $(-b^2/3a)$ and q with $(2b^3/27a^2 - \mathbf{M})$, the solution is written as follows:

$$\mathbf{D} = -\frac{b}{3a} + \sqrt[3]{-\frac{b^3}{27a^3} + \frac{1}{2a}\mathbf{M} + \sqrt{-\frac{b^3}{27a^4}\mathbf{M} + \frac{1}{4a^2}\mathbf{M}^2}} + \sqrt[3]{-\frac{b^3}{27a^3} + \frac{1}{2a}\mathbf{M} - \sqrt{-\frac{b^3}{27a^4}\mathbf{M} + \frac{1}{4a^2}\mathbf{M}^2}} \quad (\text{S18})$$

For conciseness, we introduce two coefficients, α and β .

$$\mathbf{D} = \alpha + \sqrt[3]{\alpha^3 + \beta\mathbf{M} + \sqrt{2\alpha^3\beta\mathbf{M} + \beta^2\mathbf{M}^2}} + \sqrt[3]{\alpha^3 + \beta\mathbf{M} - \sqrt{2\alpha^3\beta\mathbf{M} + \beta^2\mathbf{M}^2}} \quad (\text{S19})$$

where

$$\alpha = -\frac{b}{3a} = \frac{-2\sigma m}{\rho N_A} \approx -0.754 \text{ (nm)} \quad (\text{S20})$$

$$\beta = \frac{1}{2a} = \frac{3}{\rho N_A \pi} \approx 3.25 \times 10^{-4} \text{ (nm}^3\text{Da}^{-1}) \quad (\text{S21})$$

Determination of particle size from TEM images

It is difficult to obtain high-quality TEM images of nanocrystals of < 4 nm due to ablation by electron beam and intrinsic uncertainty of TEM. To obtain size distribution data as accurately as possible based on the TEM images, we measured size of each nanocrystal twice and took geometric mean using image J program (NIH). We prepared 70 batches of iron oxide nanocrystals and obtained each size distributions based on 70-100 particles in their TEM images. Y-axis values of red dot in Figure 2c indicate the modes of the size distributions from TEM image.

References

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Complete reference 5

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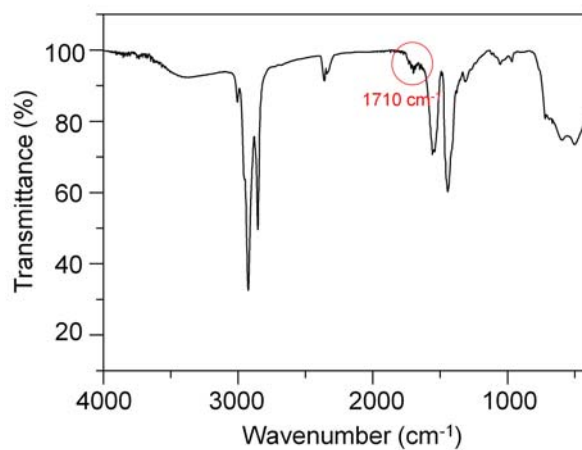


Figure S1. FT-IR spectrum of 3 nm-sized iron oxide nanocrystals. The spectra shows a very weak peak at 1710 cm^{-1} , demonstrating that almost all free oleic acid was removed through the washing process.

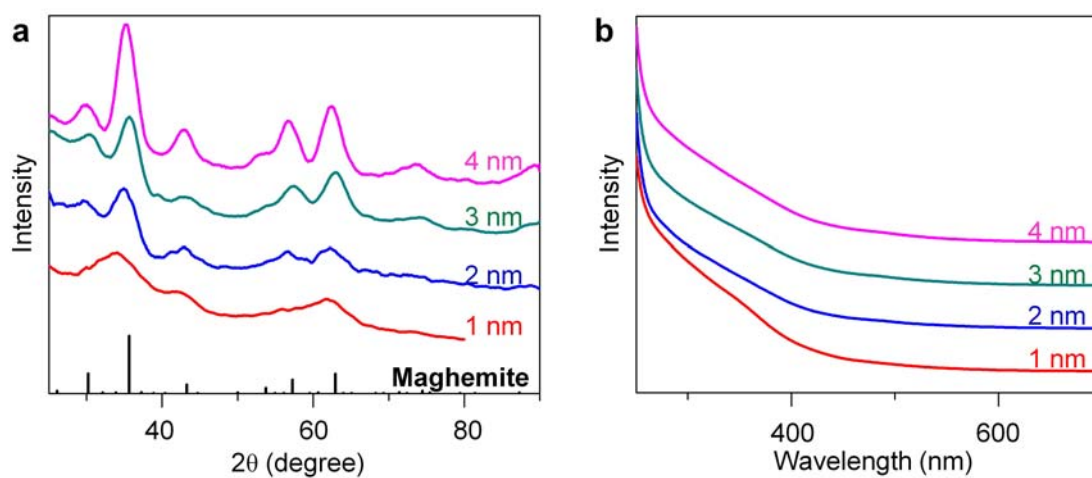


Figure S2. (a) XRD patterns, and (b) UV-visible spectra of 1, 2, 3, and 4 nm-sized iron oxide nanocrystals. The XRD patterns revealed the maghemite crystal structure. The UV-visible spectra showed no size-dependent optical properties.

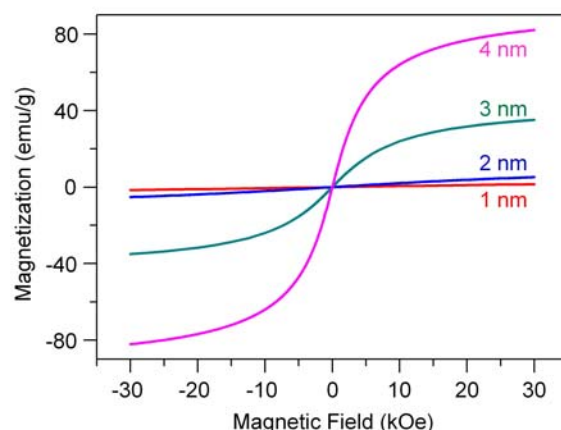


Figure S3. Field dependent magnetization curves (M-H) at 300 K for 1, 2, 3, and 4 nm-sized iron oxide nanocrystals measured at room temperature. The iron oxide nanocrystals showed highly size-dependent magnetic property. 1 and 2 nm-sized iron oxide nanocrystals exhibited nearly paramagnetic behavior, whereas 3 and 4 nm-sized nanocrystals were weakly superparamagnetic.

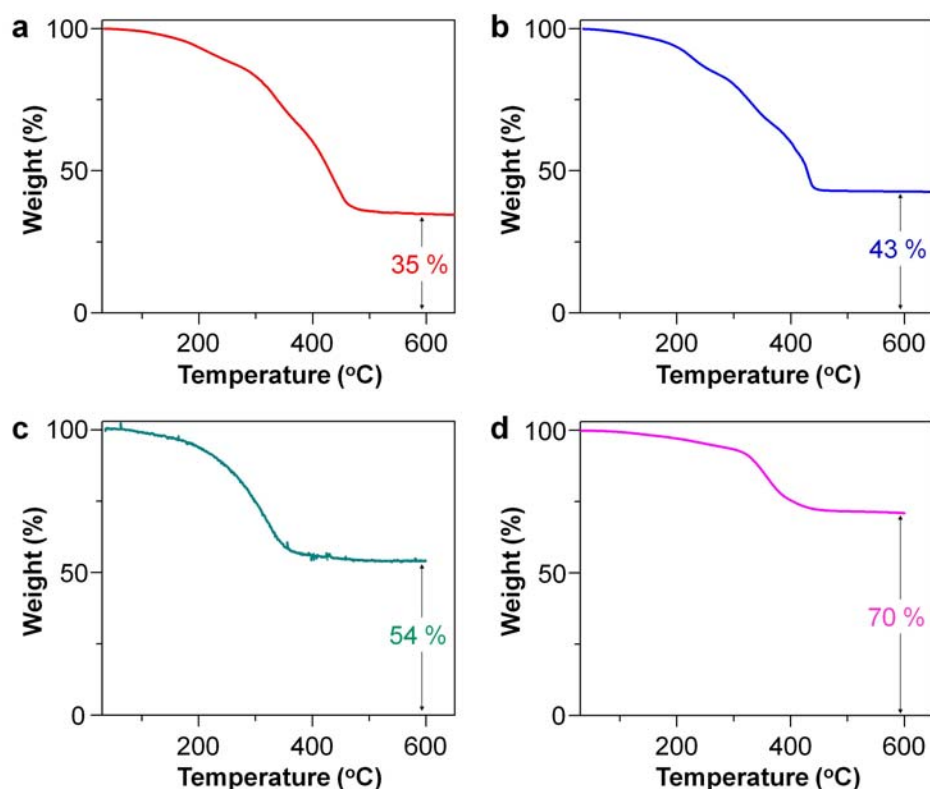


Figure S4. TGA data of (a) 1, (b) 2, (c) 3, and (d) 4 nm-sized iron oxide nanocrystals.

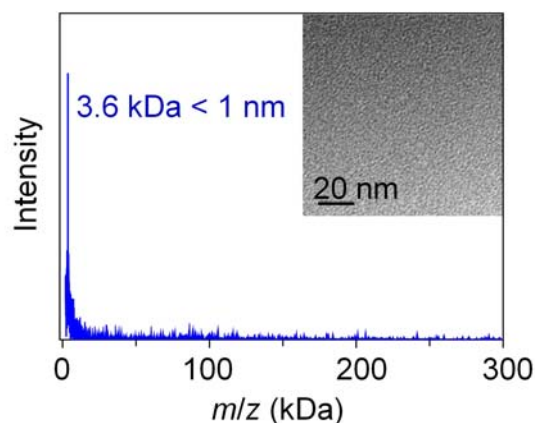


Figure S5. MALDI-TOF mass spectrum of sub-nanometer clusters, and (inset) TEM image of the same sample.

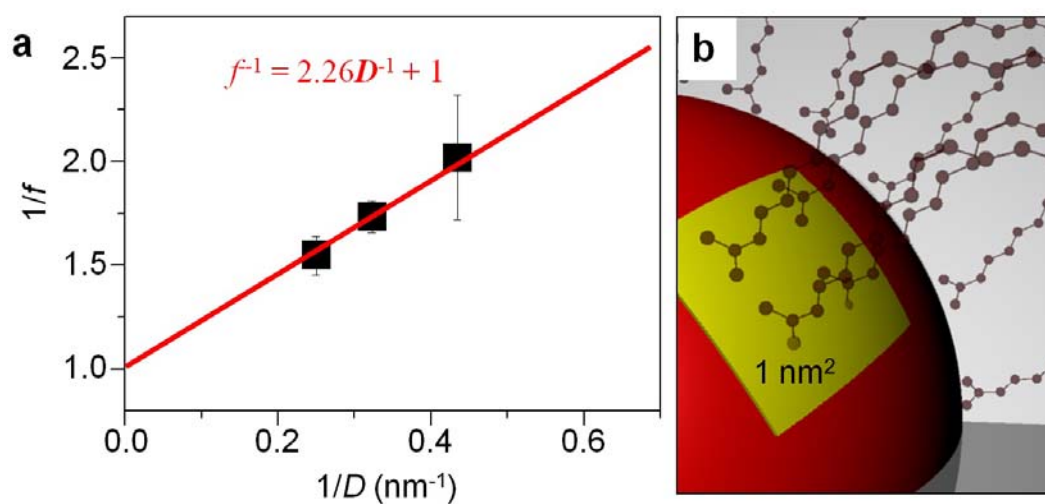


Figure S6. (a) Graph indicating the correlation between inverse diameter ($1/D$) and inverse core fraction ($1/f$). The slope was $2.26 \text{ nm} \pm 0.19 \text{ nm}$, approximated by least square analysis. (b) Schematics describing the coordinated ligand on the iron oxide nanocrystal surface with the packing density of ligand of 3.93 nm^{-2} .

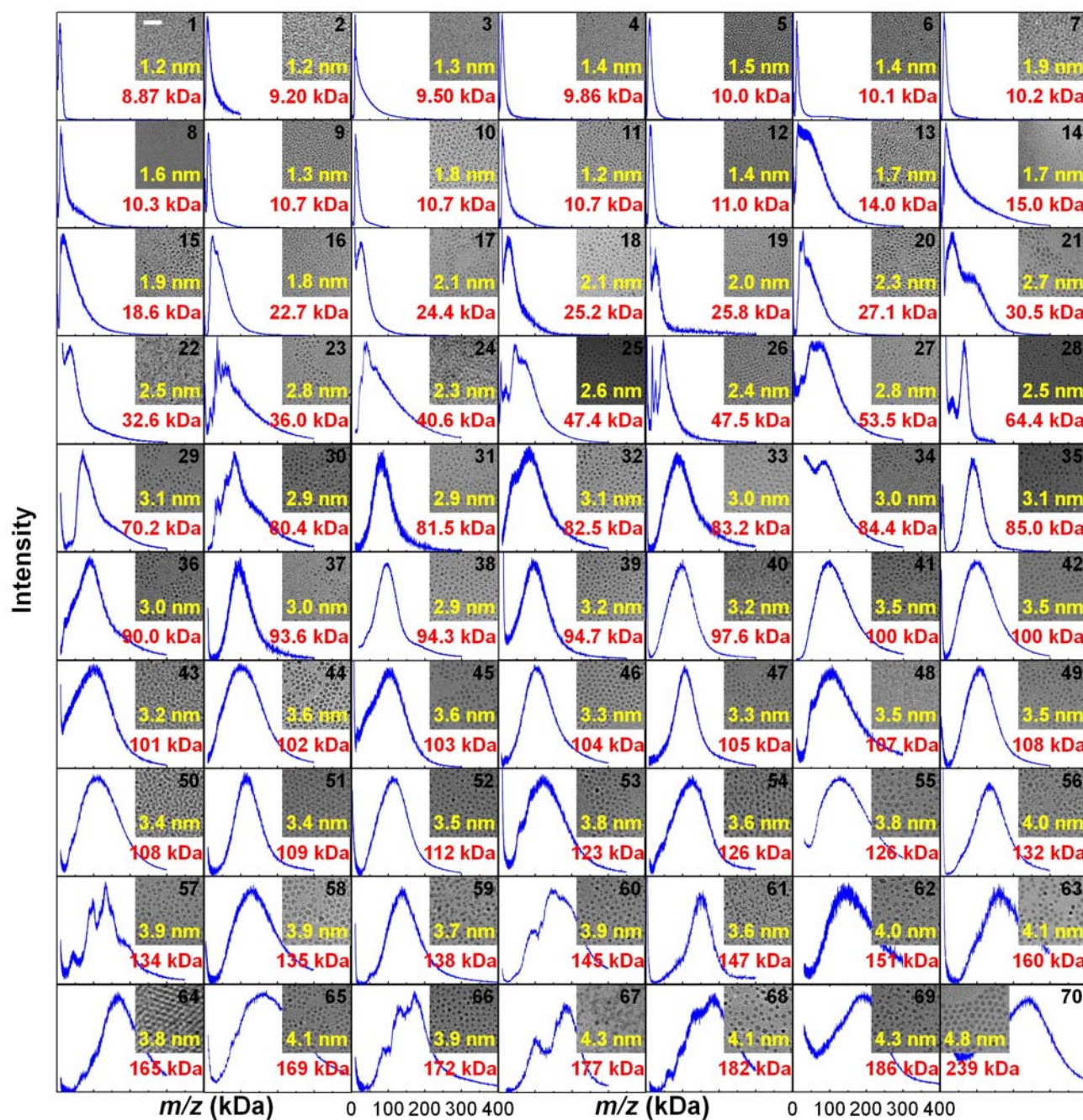


Figure S7. A total of 70 MALDI-TOF mass spectra and TEM images of iron oxide nanocrystals from 70 different batches in different synthetic conditions. Scale bar is 20 nm. Red numbers are peak positions from mass spectra and yellow numbers are the mean sizes from TEM images. The results are listed in Table S2 and plotted in Figure 2a.

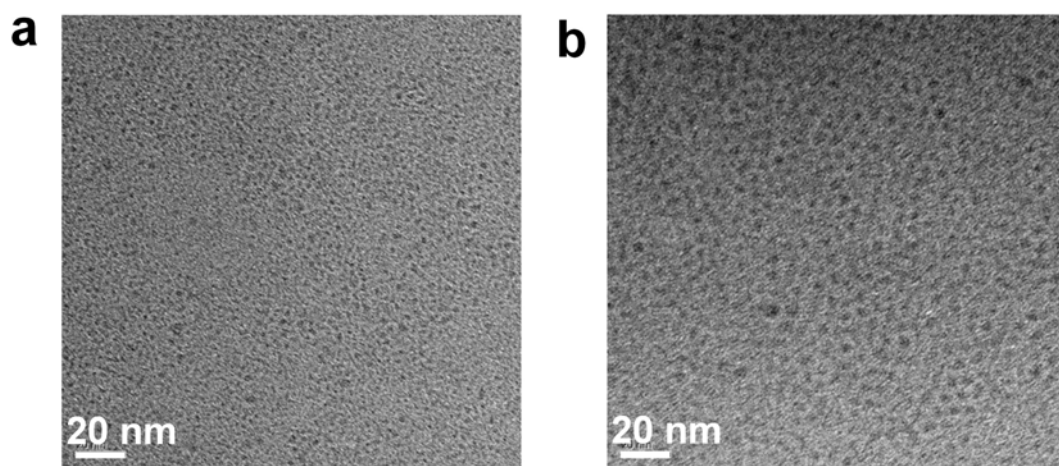


Figure S8. TEM images of (a) 1 nm and (b) 3 nm-sized iron oxide particles, which were used to prepare polydisperse mixture. Mass spectra of the mixture are shown in Figure 2e.

Table S1. Mass to diameter conversion data calculated from eq 2.

Mass (kDa)	Calculated Diameter (nm)
10	1.34
20	1.79
30	2.11
50	2.59
70	2.95
100	3.39
150	3.96

Table S2. The peak positions of MALDI-TOF mass spectra and the modes of size distributions from TEM images for different 70 batches of iron oxide nanocrystals. The mass spectra and TEM images is provided in Figure S7.

#	Mass (kDa)	Size from TEM (nm)	#	Mass (kDa)	Size from TEM (nm)
1	8.87	1.2	36	90.0	3.0
2	9.20	1.2	37	93.6	3.0
3	9.50	1.3	38	94.3	2.9
4	9.86	1.4	39	94.7	3.2
5	10.0	1.5	40	97.6	3.2
6	10.1	1.4	41	99.8	3.5
7	10.2	1.9	42	100	3.5
8	10.7	1.6	43	101	3.2
9	10.7	1.3	44	102	3.6
10	10.7	1.8	45	103	3.6
11	11.3	1.2	46	104	3.3
12	14.0	1.4	47	105	3.3
13	14.6	1.7	48	107	3.5
14	15.0	1.7	49	108	3.5
15	18.6	1.9	50	108	3.4
16	22.7	1.8	51	109	3.4
17	24.4	2.1	52	112	3.5
18	25.2	2.1	53	123	3.8
19	25.8	2.0	54	126	3.6
20	27.1	2.3	55	126	3.8
21	30.5	2.7	56	132	4.0
22	32.6	2.5	57	134	3.9
23	36.0	2.8	58	135	3.9
24	40.6	2.3	59	138	3.7
25	47.4	2.6	60	145	3.9
26	47.5	2.4	61	147	3.6
27	53.5	2.8	62	151	4.0
28	64.4	2.5	63	160	4.1
29	70.2	3.1	64	165	3.8
30	80.4	2.9	65	169	4.1
31	81.5	2.9	66	172	3.9
32	82.5	3.1	67	177	4.3
33	83.2	3.0	68	182	4.1
34	84.4	3.0	69	186	4.3
35	85.0	3.1	70	239	4.8