

Supporting Information

An Approach to Rapid Synthesis and Functionalization of Iron Oxide Nanoparticles for High Gene Transfection

Zachary R. Stephen¹, Christopher J. Dayringer¹, Josh J. Lim^{2, 4}, Richard A. Revia¹, Mackenzie V. Halbert¹, Mike Jeon¹, Arvind Bakthavatsalam³, Richard G. Ellenbogen^{4, 5}, and Miqin Zhang^{1, 4}*

¹ Department of Materials Science and Engineering, University of Washington, Seattle,
Washington 98195

² Department of Chemical Engineering, Stanford University, Stanford, California 94305

³ Department of Biochemistry, University of Washington, Seattle, Washington 98195

⁴ Department of Neurological Surgery, University of Washington, Seattle, Washington 98195

⁵ Department of Radiology, University of Washington, Seattle, Washington 98195

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*Miqin Zhang, Department of Materials Science & Engineering, University of Washington

Email: mzhang@uw.edu

302L Roberts Hall, Box 352120, Seattle, WA 98195

Fax: (206) 543-3100

Supporting Information

Supporting Methods

Quantitation of fluorescence intensity of IOCCP-Cy5. The fluorescence intensity of IOCCP-Cy5 synthesized over a range of CCP/CCP-Cy5 ratios were quantified by fluorescence spectrophotometry using a fluorescence excitation wavelength of 630ex/662em on a SpectraMax i3 microplate reader (Molecular Devices, Sunnyvale, CA). All samples were normalized to the same Fe concentration before measurement.

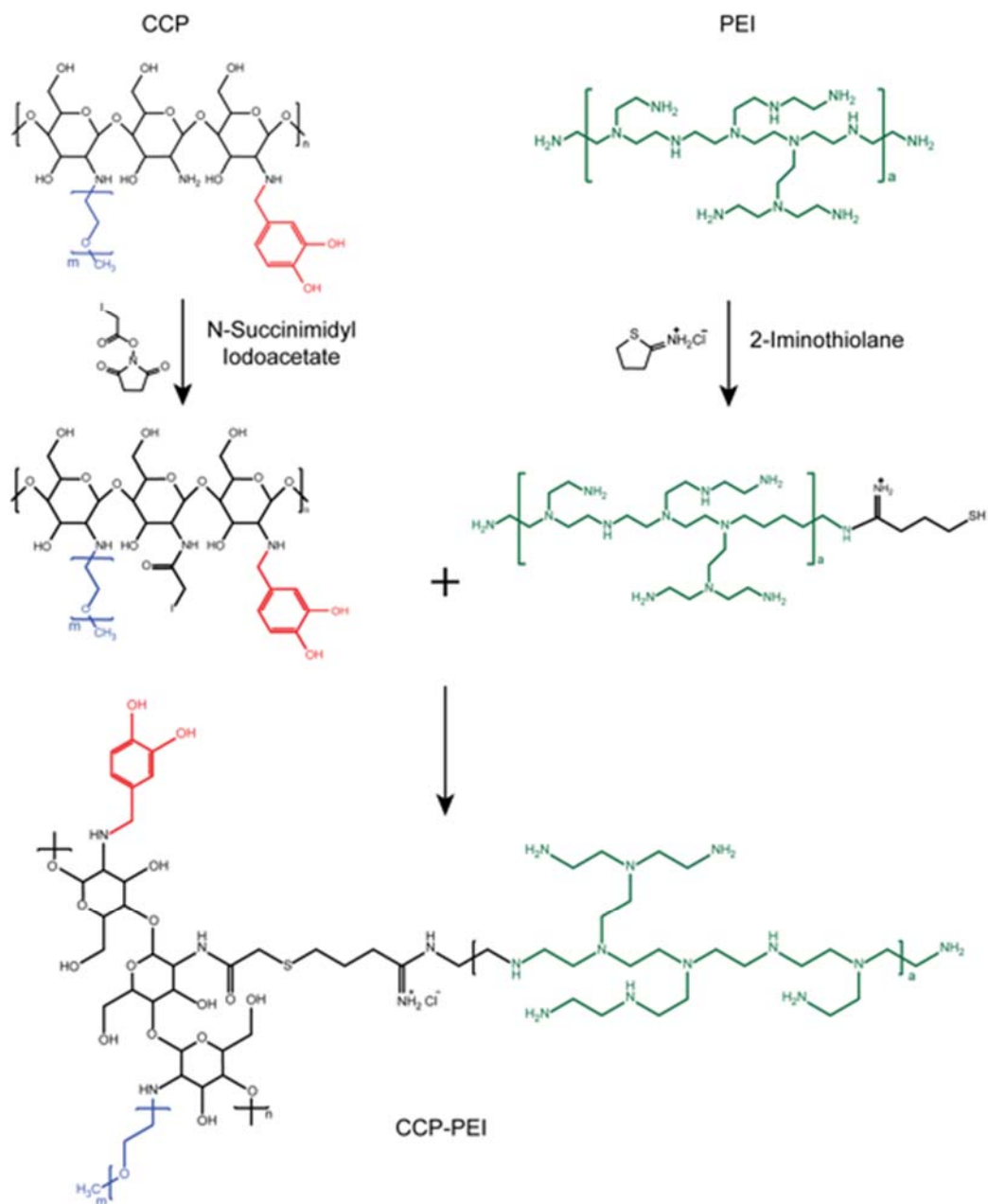
TEM and SAED analyses of SPION core. Samples were prepared by evaporating dilute suspensions on carbon-coated copper grids. TEM and SAED images were acquired with an FEI TECNAI F20 TEM (Hillsboro, OR) operating at 200 kV. SPION core diameters were analyzed with ImageJ software, and the size distribution, mean diameter, and standard deviation were calculated from 200 SPION measurements.

Magnetic characterization. Magnetometry measurements were performed using a Microsense EV9 vibrating sample magnetometer (VSM) in applied magnetic fields of -20 to 20 kOe.

Plasmid DNA preparation. The plasmid pDsRed-Max-N1 (pRFP) vector was purchased from Addgene (Cambridge, MA) and contained RFP encoding DNA under control of the cytomegalovirus (CMV) promoter, and was propagated in DH5 α *E. Coli* and purified using a Plasmid Giga Kit (Qiagen, Valencia, CA).

SPION/DNA complex formation. For DNA ratio optimization based on hydrodynamic size and zeta potential, DNA was prepared at appropriate concentrations in 20mM HEPES buffer pH 7.4 and incubated for 5 minutes at room temperature to produce SPION/DNA ratio of 2, 5, 10 and 20:1 SPION to pRFP with SPIONs added at a final concentration of $100 \mu\text{g Fe mL}^{-1}$. After addition of SPION, the solution was mixed by micropipette and incubated for 15 minutes at room temperature before measurements were taken. For *in vitro* cell transfections, SPION /DNA complexes at $1 \mu\text{g DNA}/50 \mu\text{L}$ were formed by first incubating pRFP in 20mM HEPES buffer pH 7.4 for 5 minutes at room temperature. SPIONs were then added at appropriate volume to yield a 10:1 w/w ratio with pRFP, mixed thoroughly by micropipette and incubated for 15 minutes at room temperature.

Supporting Schemes and Figures



Scheme S1. Modification of CCP with 25 k MW PEI. CCP was reacted with SIA to produce a thiol reactive iodoacetyl functionalized CCP intermediate, while PEI was reacted with Traut's reagent to yield a free thiol. Thiol functionalized PEI and iodoacetyl functionalized CCP were coupled in 0.1 M Na bicarbonate, 5 mM EDTA buffer, at pH 8.0.

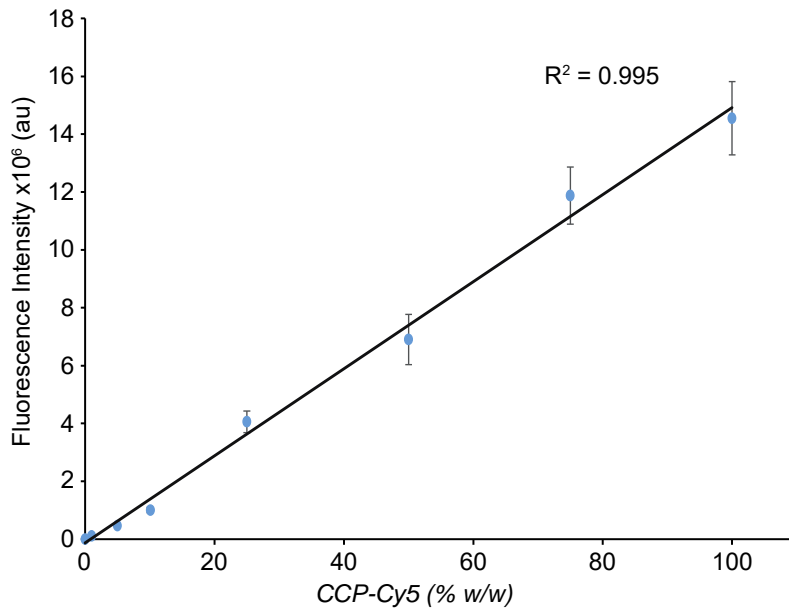


Figure S1. Evaluation of change in weight % CCP-Cy5 on the fluorescence intensity of IOCCP-Cy5. IOCCP-Cy5 produced by incubation with 1, 5, 10, 25, 50, 75, and 100 % CPP-Cy5 w/w. The mean fluorescence intensity for each ratio was determined from three separate batches of IOCCP-Cy5. Error bars = standard deviation.

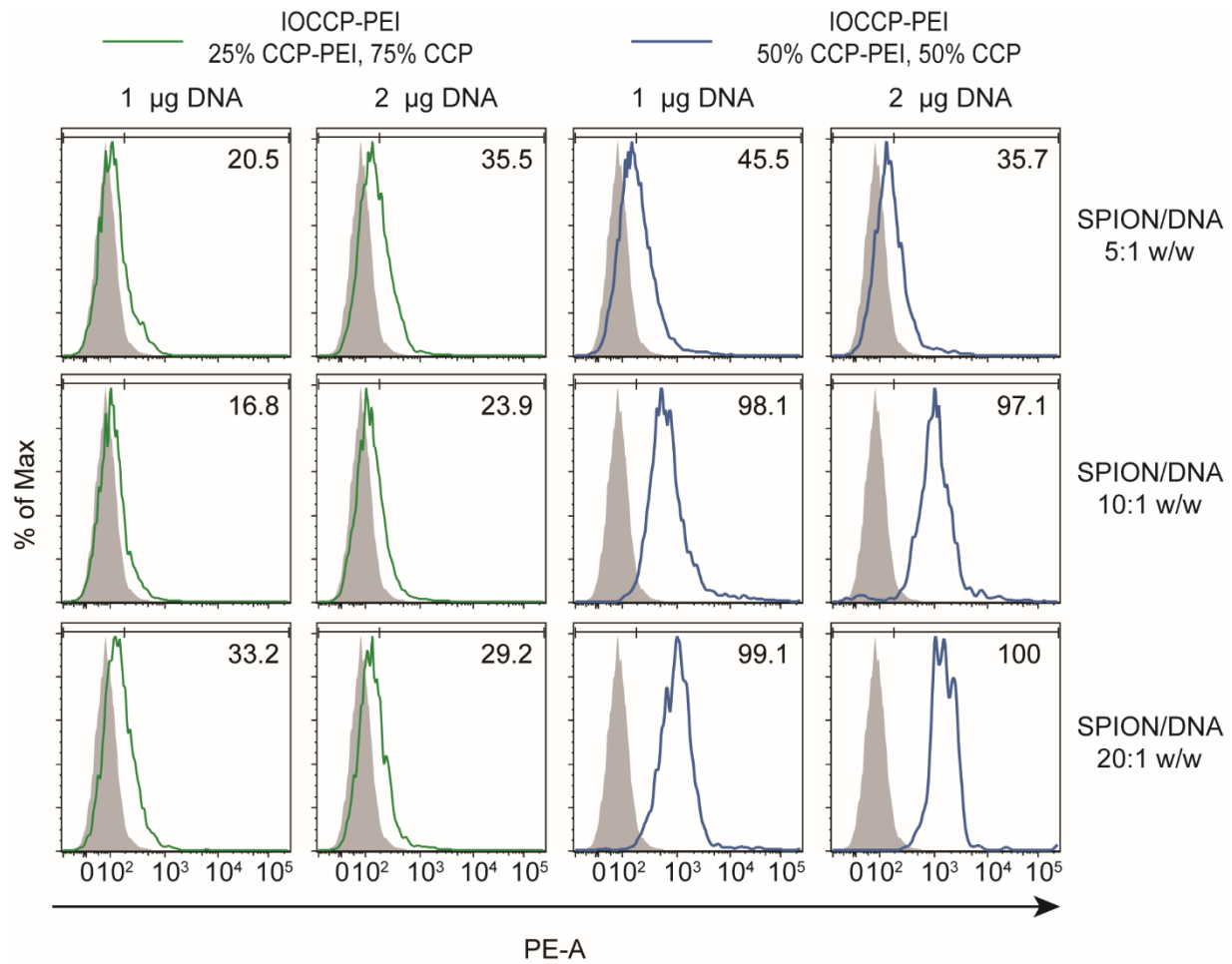


Figure S2. Initial assessment of transfection efficiency of IOCCP-PEI synthesized with 25% CCP-PEI or 50% CCP-PEI. SPIONs were complexed as described in the Experimental Section at 5, 10, and 20:1 w/w SPION/pRFP and evaluated at doses of 1 and 2 μg pRFP. IOCCP-PEI synthesized with 50% CCP-PEI had far greater transfection efficiency at all evaluated SPION/DNA ratios.

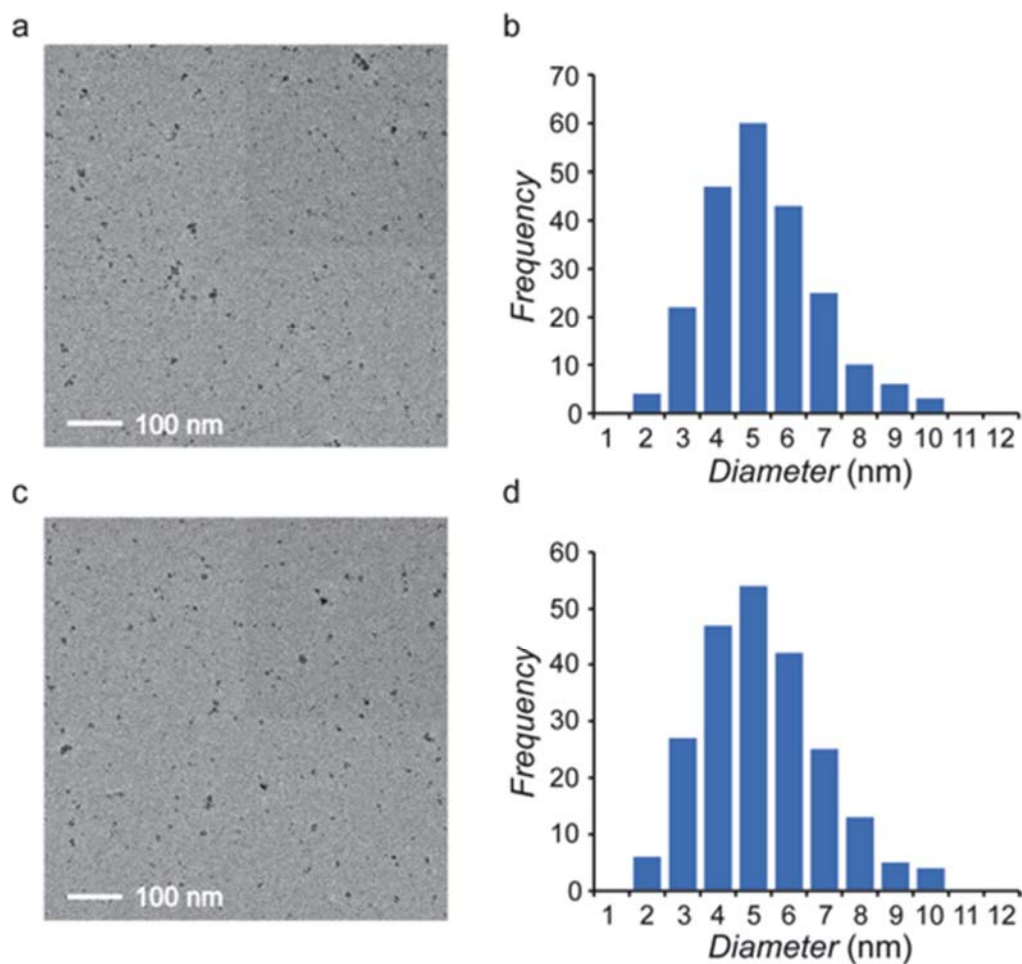


Figure S3. Determination of IOCCP and IOCCP-PEI core size. a) Representative TEM image of IOCCP. b) Distribution of IOCCP core diameter yielding a mean of 5.2 ± 1.4 nm. c) Representative TEM image of IOCCP-PEI. d) Distribution of IOCCP-PEI core diameter yielding a mean of 5.3 ± 1.7 nm. Histograms were produced from 200 independent core measurements using ImageJ software.

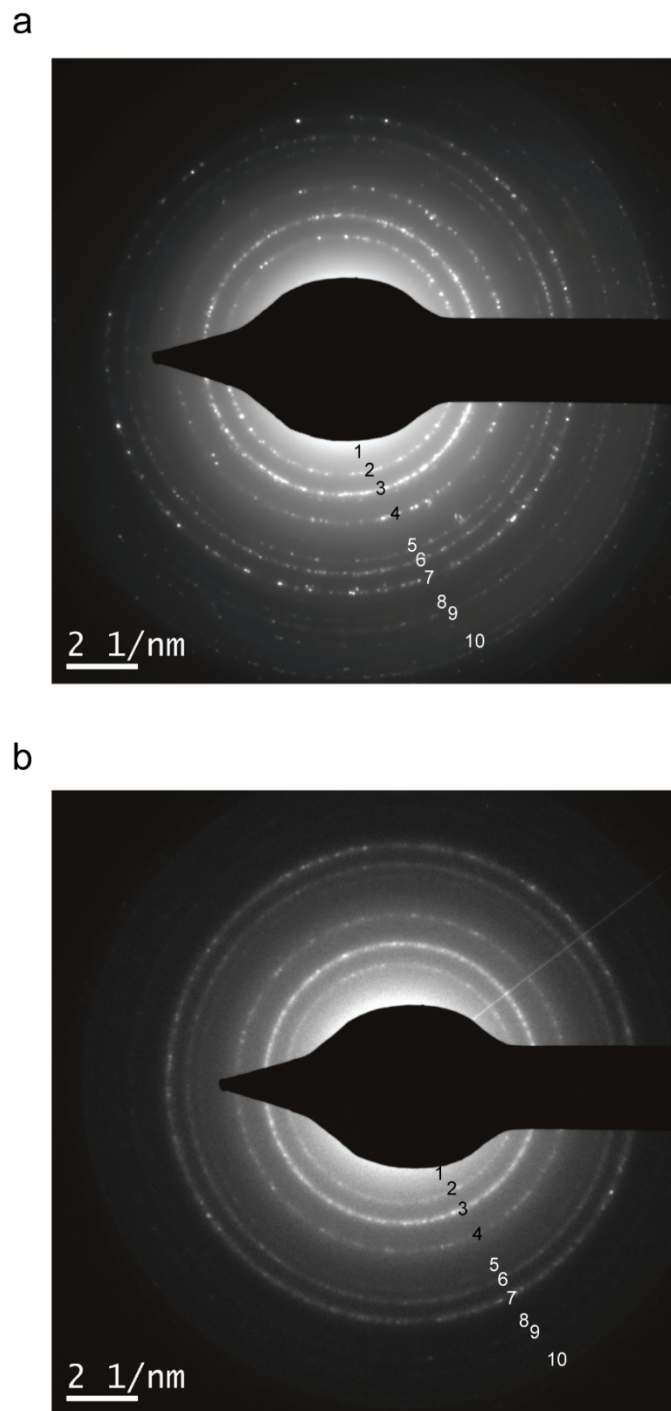


Figure S4. SAED of IOCCP (a) and IOCCP-PEI (b) acquired with an FEI Tecnai TEM instrument using a double tilt holder. Ring numbers are annotated and listed in **Table 1**.

Table 1. Diffraction data d-spacings in Å, based on the rings (**Figure S4**) and standard atomic spacing for Fe₃O₄ along with their respective hkl indices.

	<i>Ring</i>									
	1	2	3	4	5	6	7	8	9	10
<i>SAED</i> *	4.87	2.96	2.54	2.11	1.74	1.63	1.50	1.33	1.30	1.21
<i>Fe₃O₄</i> **	4.86	2.98	2.54	2.12	1.73	1.63	1.50	1.34	1.29	1.22
<i>hkl</i>	111	220	311	400	422	511	440	620	533	444

*Measured values

**Standard Fe₃O₄ powder diffraction data¹

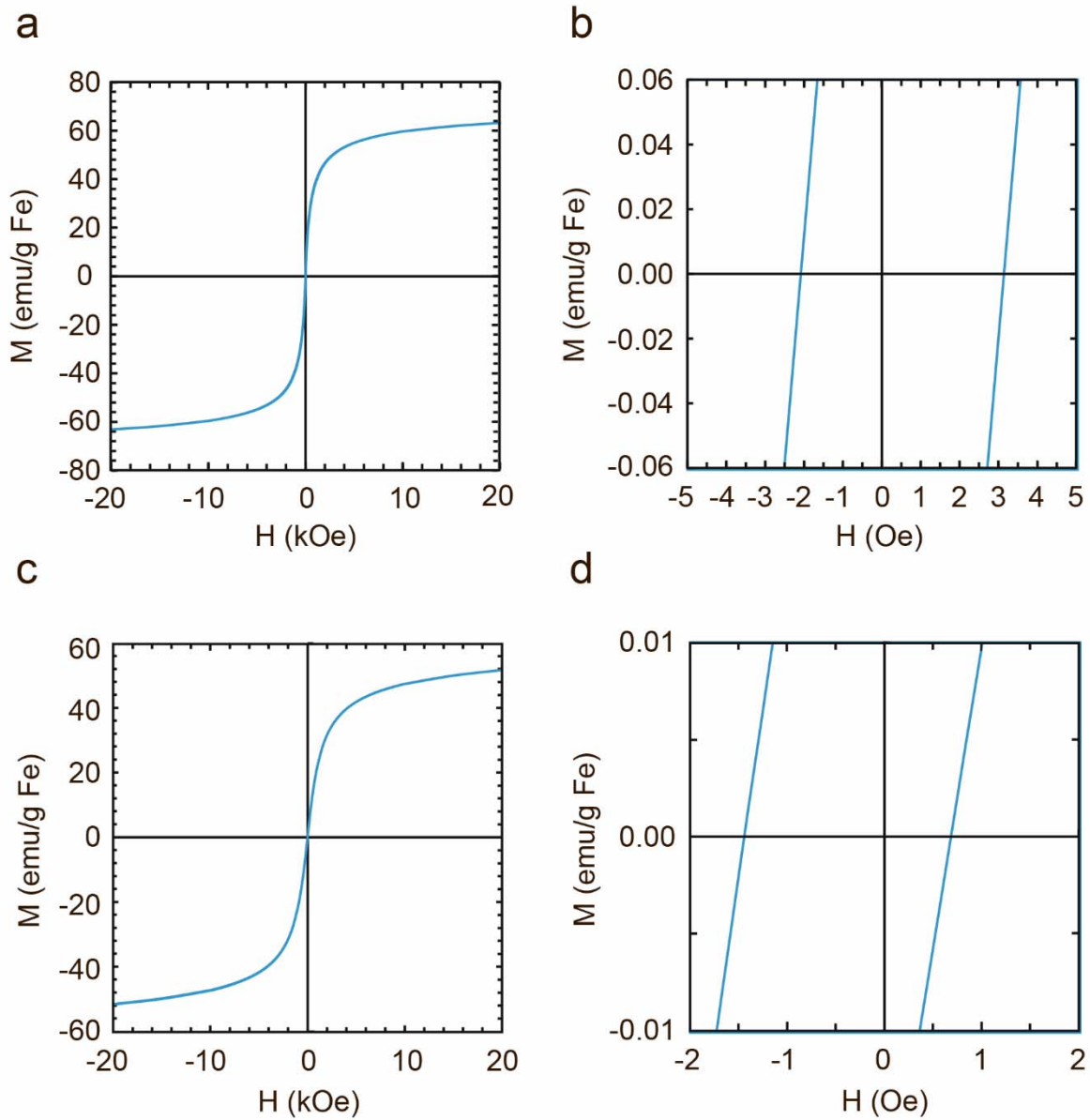


Figure 5. VSM analysis of IOCCP and IOCCP-PEI. Magnetic hysteresis curves at room temperature for IOCCP (a) and IOCCP-PEI (c). Details of the hysteresis curves in the region near 0 Oe for IOCCP (b) and IOCCP-PEI (d) indicate near zero coercivity.

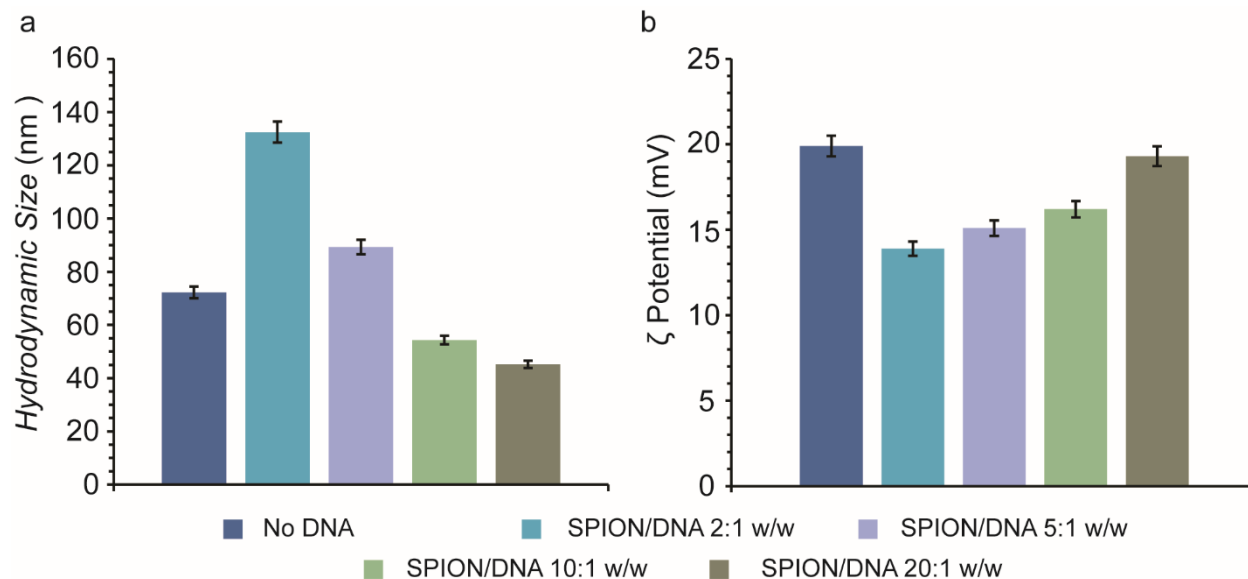


Figure S6. Physicochemical properties of IOCCP-PEI complexed at various SPION to DNA ratios. (a) Hydrodynamic size and (b) ζ potential in HEPES buffer pH 7.4 of IOCCP-PEI with no DNA and with DNA bound at a 2, 5, 10 or 20 to 1 SPION to DNA ratio.

SPION/DNA complexes at ratios (w/w) of 0, 2, 5, 10, and 20 to 1, NP to red fluorescent protein encoding plasmid DNA (pRFP) were evaluated for their hydrodynamic size and ζ potential. SPION/DNA complexes increased in size at low ratios of SPION to DNA but trended toward smaller size with decreased amount of DNA (**Figure S6a**), while ζ potentials reduced significantly for low ratios of SPION to DNA and trended in the positive direction with decreased amount of DNA (**Figure S6b**). Interestingly, IOCCP-PEI complexed with DNA at ratios of 10:1 and 20:1 w/w were smaller in size than the native IOCCP-PEI most likely due to efficient packing of bulky PEI facilitated by DNA binding on the surface of the SPION.

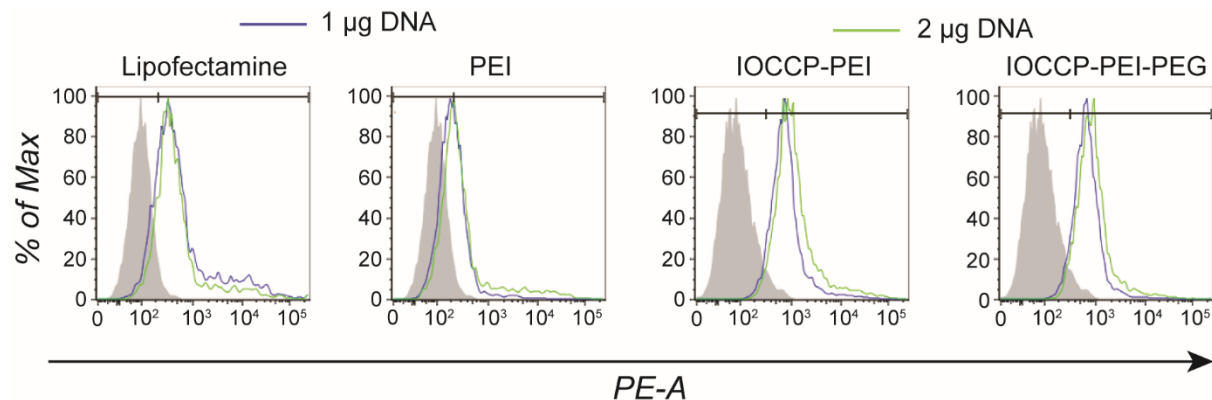


Figure S7. Assessment of transfection efficiency of Lipofectamine, PEI, IOCCP-PEI and IOCCP-PEI-PEG complexed as described in the Experimental Section, at doses of 1 and 2 µg pRFP. Transfection efficiency was minimally improved by increasing the DNA dose from 1 to 2 µg pRFP. 1 µg pRFP was chosen as the optimal treatment dose, due to the decreased cell viability of all transfection agents at 2 µg pRFP as compared to 1 µg pRFP (main text **Figure 6**).

Equation S1

Calculating the amount of PEG per chitosan and catechol per chitosan in CCP:

- MW of PEG: 2000
- MW of chitosan: 2300
- MW of catechol: 138.12
- NMR sample was made with: 9 mg CCP + 1.72 mg TSP in 1 mL D₂O (10 mM TSP)

TSP peak was integrated to 1, and the terminal –OCH₃ peak from the PEG was integrated to 0.10835 and the aromatic peaks from the catechol were integrated to 0.05498.

$$\text{Concentration of PEG} = \text{Concentration of TSP} \times \frac{\text{Integration of PEG/proton}}{\text{Integration of TSP/proton}}$$

$$\text{Concentration of PEG} = 10 \text{ mM} \times \frac{0.10835/3}{1/9} = 3.2505 \text{ mM}$$

$$3.2505 \text{ mM} = \frac{3.2505 \text{ mmol}}{1\text{L}} \times \frac{2000 \text{ mg}}{\text{mmol}} \times \frac{1\text{L}}{1000\text{mL}} = 6.501 \text{ mg/mL}$$

$$\text{Concentration of catechol} = \text{Concentration of TSP} \times \frac{\text{Integration of catechol/proton}}{\text{Integration of TSP/proton}}$$

$$\text{Concentration of catechol} = 10 \text{ mM} \times \frac{0.05498/3}{1/9} = 1.6494 \text{ mM}$$

$$1.6494 \text{ mM} = \frac{1.6494 \text{ mmol}}{1\text{L}} \times \frac{138.12 \text{ mg}}{\text{mmol}} \times \frac{1\text{L}}{1000\text{mL}} = 0.2278 \text{ mg/mL}$$

Concentration of chitosan = Concentration of CCP – Concentration of PEG – Concentration of catechol

$$\text{Concentration of chitosan} = 9 \text{ mg/ml} - 6.501 \text{ mg/ml} - 0.2278 \text{ mg/ml} = 2.271 \text{ mg/ml}$$

$$\# \text{ of PEG/chitosan} = \frac{\text{Concentration of PEG} / \text{MW of PEG}}{\text{Concentration of chitosan} / \text{MW of chitosan}}$$

$$\# \text{ of PEG/chitosan} = \frac{\frac{6.501 \frac{\text{mg}}{\text{ml}}}{2000 \frac{\text{mg}}{\text{mmol}}}}{\frac{2.271 \frac{\text{mg}}{\text{ml}}}{2300 \frac{\text{mg}}{\text{mmol}}}} = \sim 3 \text{ PEG/chitosan}$$

$$\# \text{ of catechol/chitosan} = \frac{\text{Concentration of catechol} / \text{MW of catechol}}{\text{Concentration of chitosan} / \text{MW of chitosan}}$$

$$\# \text{ of catechol/chitosan} = \frac{0.2278 \text{ mg/ml} / 138.12 \text{ mg/mmol}}{2.271 \text{ mg/ml} / 2300 \text{ mg/mmol}} = \sim 2 \text{ catechol/chitosan}$$

The amount of PEI on CCP-PEI was calculated by comparing the integration values from the PEI peaks from CCP-PEI to the integration values from the PEI peaks from a standard PEI sample.

- NMR sample was made with 9 mg CCP-PEI + 1.72 mg TSP in 1 mL D₂O (10 mM TSP)
- NMR standard was made with 9 mg PEI + 1.72 mg TSP in 1 mL D₂O (10 mM TSP)

In both spectra, the TSP peak was integrated to 1, and the PEI peaks were integrated to be 10.38184 for CCP-PEI and 14.34209 for the standard. A ratio of the integration values were used to calculate the amount of PEI on CCP-PEI.

Concentration of PEI in CCP – PEI

$$= \text{Concentration of PEI in standard} \times \frac{\text{Integration of PEI in CCP – PEI}}{\text{Integration of PEI in standard}}$$

$$\text{Concentration of PEI in CCP – PEI} = 9 \text{ mg/ml} \times \frac{10.38184}{14.34209} = 6.515 \text{ mg/ml}$$

References

1. Cornell, R. M., Schwertmann, U., *The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses*. 2nd ed.; Wiley-VCH: New York, **2003**; p 703.