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Supplementary information

Simultaneous quantification of oligo-nucleic acids and ferritin nanocage by size-exclusion chromatography hyphenated to inductively coupled plasma mass spectrometry for developing drug delivery systems

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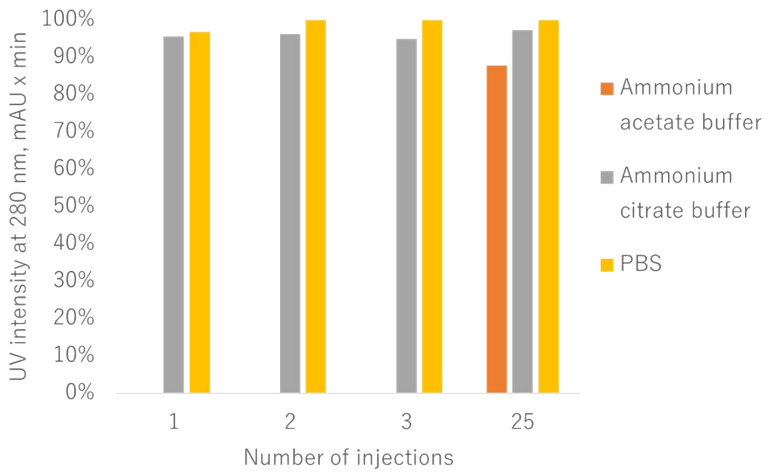
Table S1. The recovery rates of siRNA at different concentrations were measured by SEC-ICP-MS using the mobile

Concentration, µM	Ammonium citrate buffer (pH 7)	Ammonium acetate buffer (pH 7)
0.25	100%	68%
0.5	100%	73%
1	110%	86%
2.5	110%	82%



phases ammonium acetate buffer and ammonium citrate buffer.

Figure S1. Ratio of ferritin UV response at 280 nm measured using ammonium acetate buffer, ammonium citrate buffer, and phosphate buffered saline as mobile phase to the average of UV response using PBS.

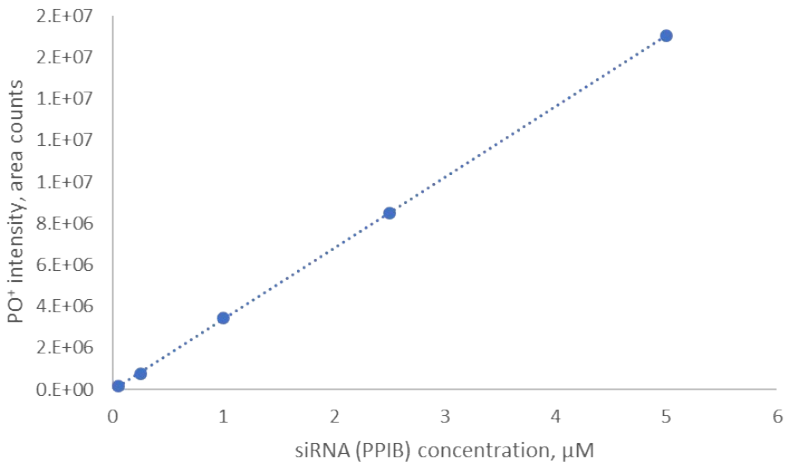


Figure S2. Plot of intensities (*m/z* 47) of peaks observed at the elution time of 19 min (free siRNA) with different concentrations of siRNA.

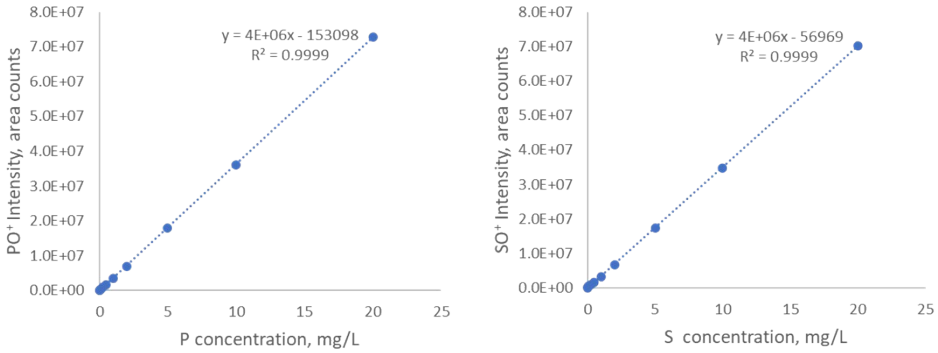


Figure S3. Calibration curves of P (PO^+, *m/z* 47) and S (SO^+, *m/z* 48) based on a series of elemental standards measured by SEC-ICP-MS.

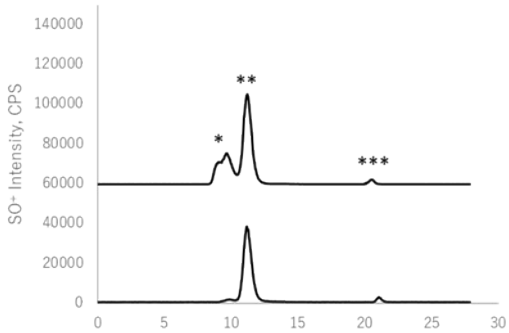


Figure S4. Mass chromatograms of siRNA-ferritin complex immediately after encapsulation (top) and siRNA-ferritin complex purified by SEC (bottom). x-axis: intensities of *m/z* 48 (cps of SO^+), y-axis: elution time (min).

* ferritin aggregates, ** ferritin, *** salts in sample solution

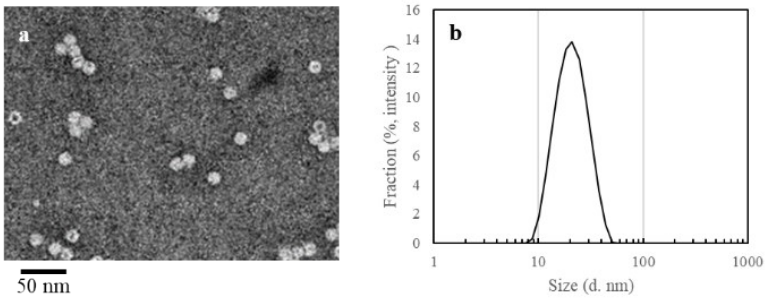


Figure S5. (a) Image of ferritin captured by transmission electron microscopy and (b) hydrodynamic diameter by dynamic light scattering measurements of the siRNA-ferritin complex.

	Average radius of inertia, nm
DAL F DNA	1.92±0.10
DAL R DNA	1.89±0.12
dsDNA	1.96±0.03
siRNA	2.29±0.08

Table S2. Average radius of inertia

Reagents

Ferritin production by bacteria

The ferritin heavy chain (FTH, UniProtKB accession; P02794) was produced by a previously method with some modifications.[Ref 1] *Escherichia coli* BL21(DE3) was transformed with an FTH expression plasmid, pET-FTH, and was cultivated in LB medium (Luria-Bertani, Becton, Dickinson and Company, Sparks, MD, USA) with 100 µg/mL ampicillin at 37 °C for 24 h. Harvested bacteria were disrupted by an ultrasonic wave (140 W for 5 min) and centrifuged at 5,000 × g for 5 min. The supernatant was then boiled at 60 °C for 20 min. After removing the debris by centrifugation at 8,000 × g for 15 min and 0.22 µm filtration, a clear supernatant was loaded into an anion-exchanger column (HiPrep Q HP 16/10, Cytiva, Marlborough, MA, U.S.A.). FTH was eluted with an NaCl gradient (0 to 0.7 M) in 50 mM Tris-HCl buffer (pH 8.0) at a flow rate of 3.0 mL min^{-1}. After concentration of the eluate containing FTH by ultrafiltration (Vivaspin 20-100K, MWCO 100 kDa, Cytiva), FTH was purified using a gel filtration column (HiPrep 26/60 Sephacryl S-300 HR, Cytiva) equilibrated with 10 mM Tris-HCl (pH 8.0) at a flow rate of 1.3 mL min^{-1}. The FTH yield was 50 mg L^{-1} of broth.

Characterization of ferritin

Protein concentration and purity. The concentration and purity of ferritin were measured by reverse phase chromatography using PLRP-S 300A (3 µm, 150 mm × 4.6 mm, Agilent Technologies, Santa Clara, CA, USA). Samples were applied to the reverse phase column at a flow rate of 1.0 mL min^{-1} with a linear elution gradient from 24% to 56% acetonitrile containing 0.1% trifluoroacetic acid (TFA) at 70 °C. The eluate was analyzed using a Chromaster HPLC system (Hitachi, Japan) and monitored at 220 nm absorbance. A recombinant human ferritin heavy chain protein (ab78877, Abcam, UK) was used as a standard.

Transmission electron microscopy (TEM) imaging. Nanostructures of ferritin-encapsulated oligonucleotides were analyzed using high-resolution TEM images (JEM-2200FS, JEOL, Japan). Ferritin solution (0.2 mg mL^{-1}, 2 µL) was dropped on a carbon-coated copper grid. After 1 min, the excess solution was wicked with filter paper and washed with 2 µL of water. The samples were stained with 1% phosphotungstic acid (PTA).

Dynamic light scattering (DLS) and zeta potential analyses.

For DLS analysis, a solution containing 0.5 mg mL^{-1} ferritin was measured in 50 µL PBS (pH 7.4) at 25 °C using a Zetasizer Nano (Malvern Instruments, U.K.). The zeta potential of ferritin was also analyzed using a Zetasizer Nano. Phosphate buffer (50 mM, pH 7.0) containing 0.1 mg mL^{-1} ferritin was measured in 750 µL of the buffer at 25 °C.