Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

One-step construction of ferritin encapsulation drugs for cancer chemotherapy

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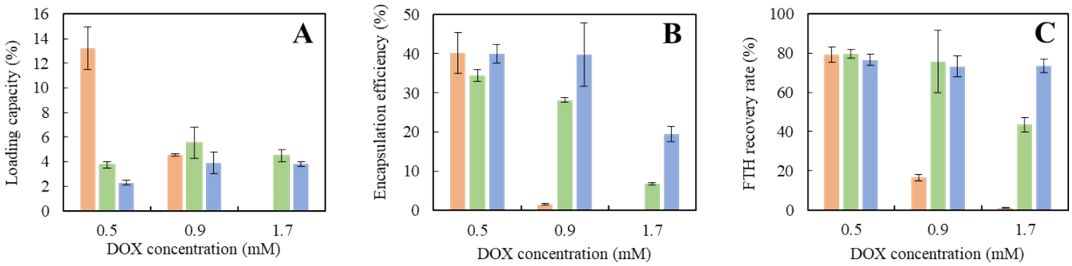

Figure S1. FTH concentration dependency of the one-step method. (A) The loading capacity, (B) DOX-encapsulation efficiency and (C) FTH recovery rate (means ± standard deviation, n = 3). DOX was added to 50 mM Tris-HCl buffer (pH 9.0) with FTH [1 mg mL^{-1} (red bar), 5 mg mL^{-1} (green bar), 10 mg mL^{-1} (blue bar)]. After 1 h incubation at 60 °C, DOX-FTH was purified using ultrafiltration (Vivaspin 500-100K).

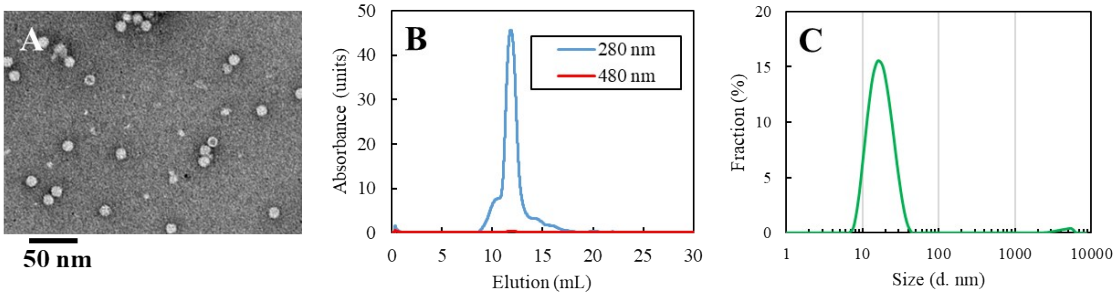

Figure S2. Characterisation of apo-ferritin nanoparticles. (A) TEM image of apo-ferritin. (B) SEC analyses of apo-ferritin. FTH protein was detected using absorbance at 280 nm (blue line) and 480 nm (red line). (C) DLS analysis of apo-ferritin size distribution. Diameter of apo-ferritin was 16.19 nm using intensity mode. The Pdi value was 0.150.

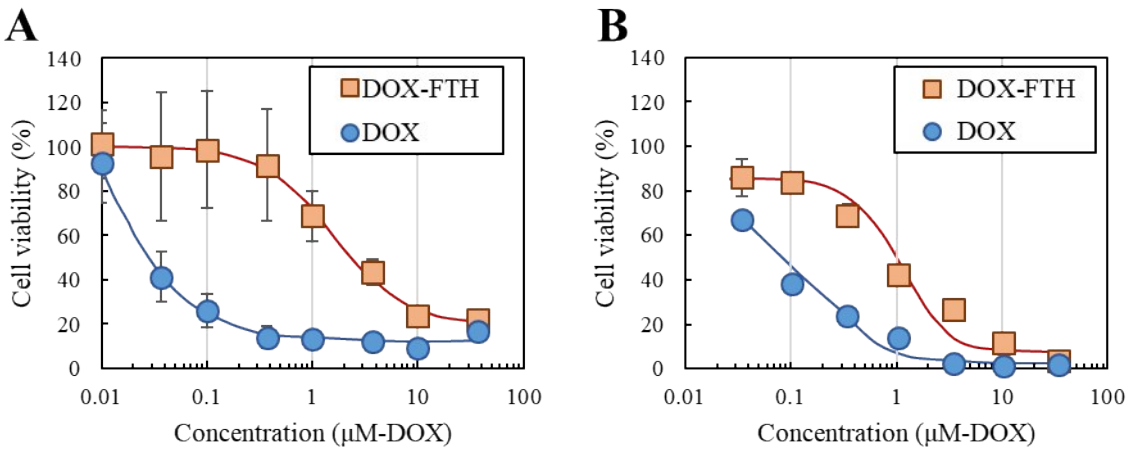

Figure S3. Dose-dependent cytotoxicity of DOX-FTH. Cell viability of (A) HEK293 cells (n = 3) and (B) colon-26 cells (n = 2) treated with DOX-FTH and free DOX measured by WST-8.

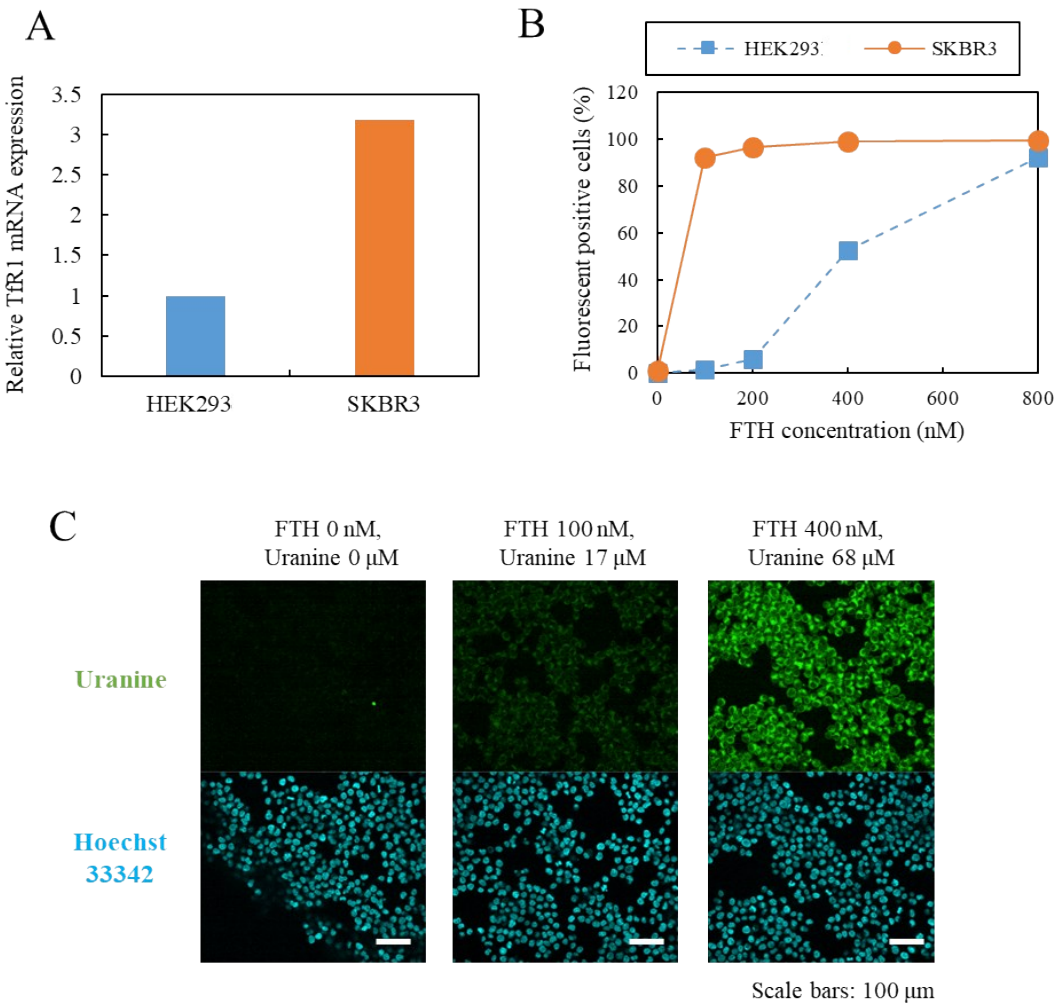

Figure S4. Comparison of FTH-uptake efficiencies of two cell lines expressing TfR1 at different levels. HEK293 (TfR1-low) and SKBR-3 (TfR1-high) cell lines were used. (A) qPCR analysis of TfR1 mRNA expression levels of HEK293 and SKBR-3 cells. Mean values of relative expression levels are shown (n = 2). (B) Flow cytometric analysis of FTH-uptake by the cells. Fluorescence-positive fractions were quantified in HEK293 (blue) and SKBR-3 (orange) treated with 0–800 nM Fluo-FTH. (C) Confocal microscopic images of HEK293 cells incubated with 0–400 nM of uranine dye (green)-loaded FTH for 24 h at 37 °C. Nuclei were labelled with Hoechst33342 (cyan).

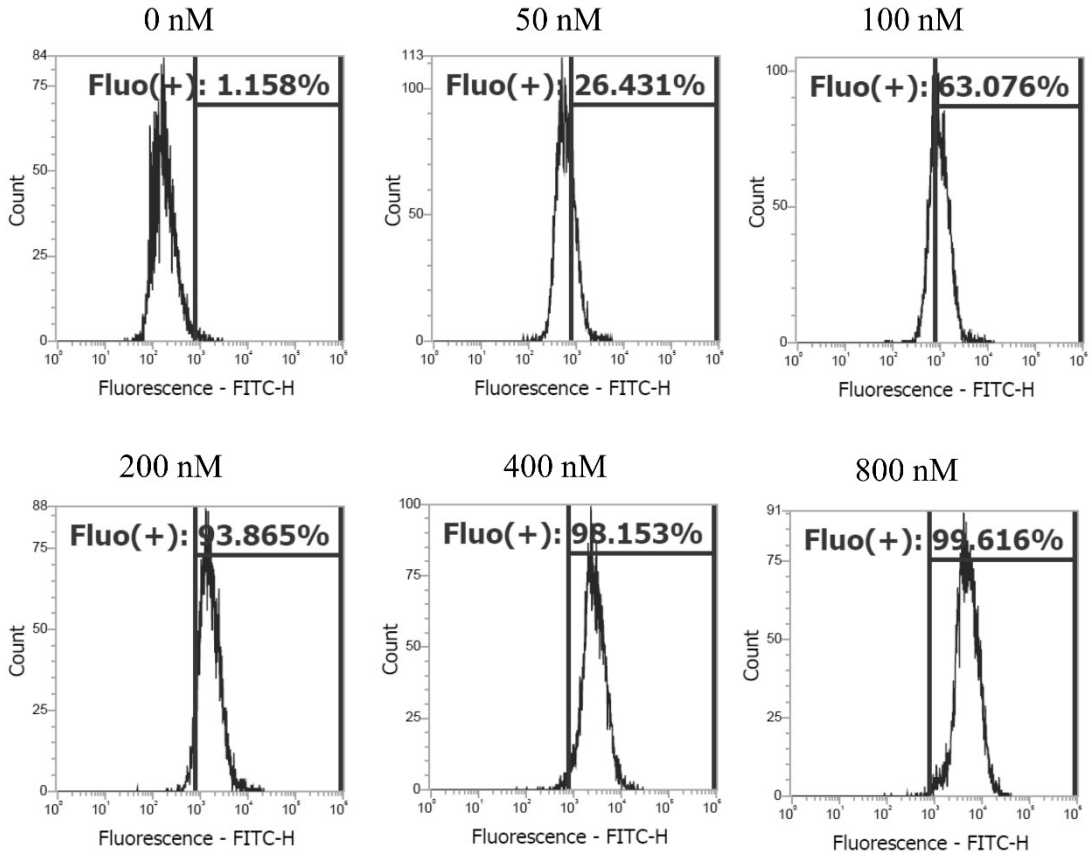

Figure S5. Flow cytometry analysis of SKBR-3 cells treated with 0–800 nM of Fluo-FTH. Fractions of fluorescence-positive cells were quantified.

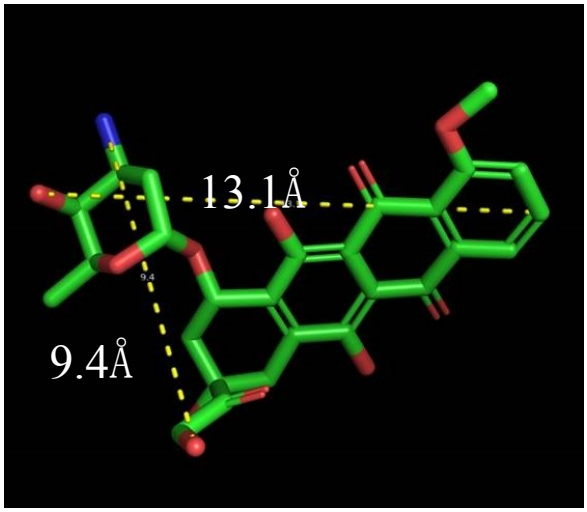

Figure S6. DOX structure demonstrating its size.

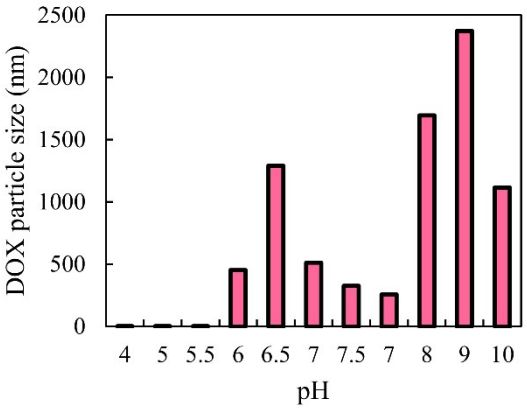

Figure S7. DLS analysis of DOX aggregate formulation. To observe DOX aggregates, 0.5 mM of DOX was incubated in various pH buffers at 60 °C for 60 min. After incubation, the size of DOX particles was measured by Zetasizer Nano.

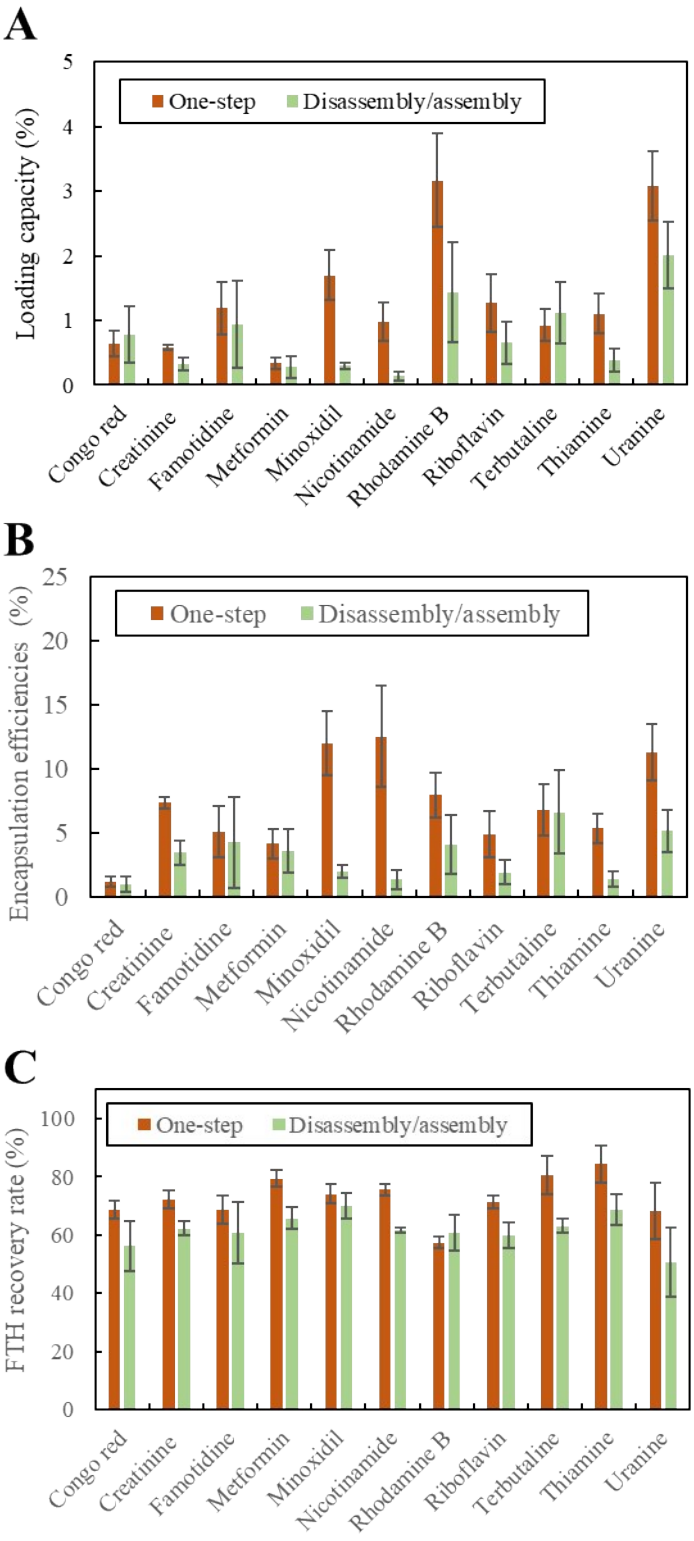

Figure S8. Comparison of the one-step method and conventional method with various chemical molecules. (A) Loading capacities, (B) drug-encapsulation efficiency and (C) FTH recovery rate (means ± standard error, n = 3~4).

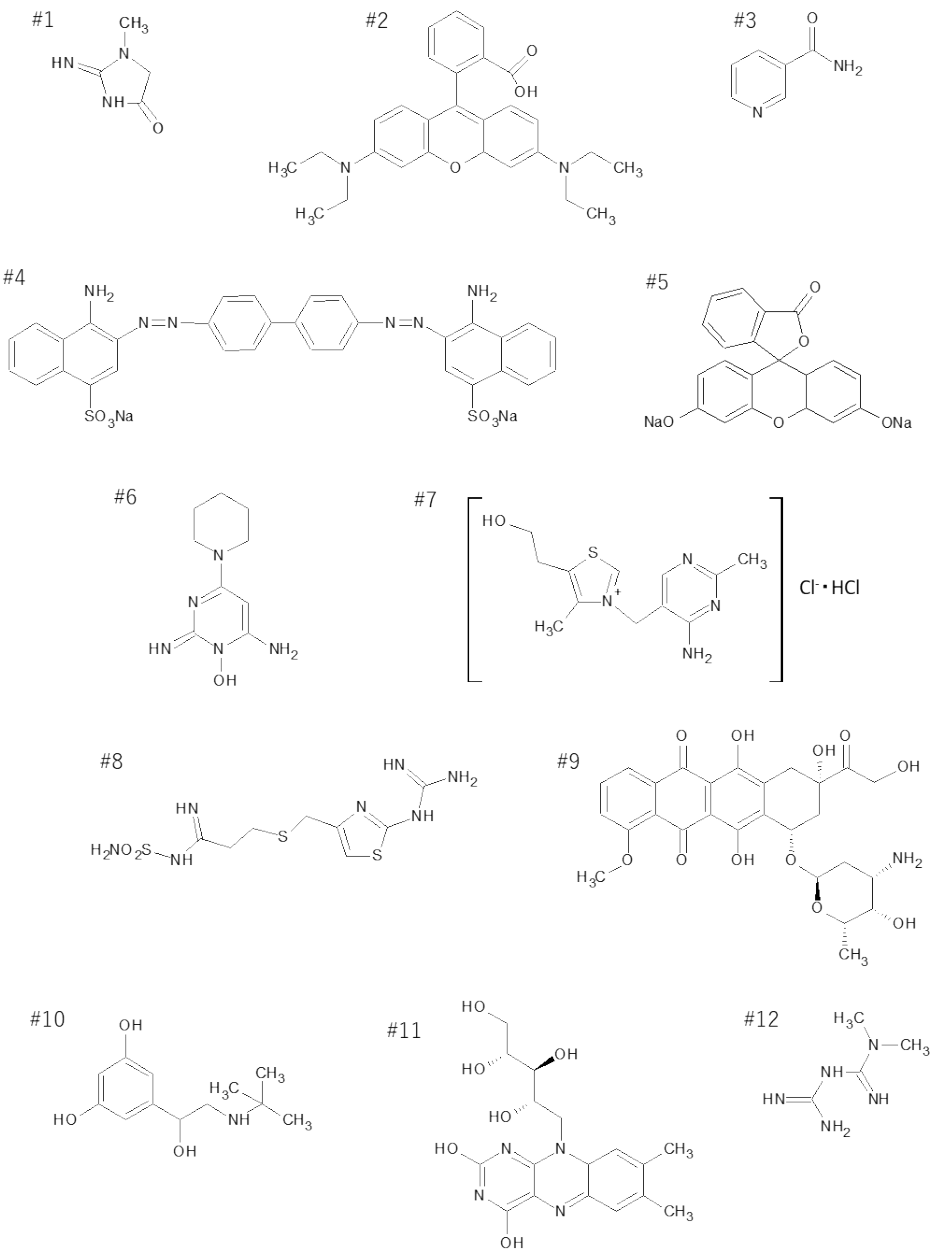

Figure S9. Chemical structure of molecules encapsulated in ferritin. #1; creatinine, #2; rhodamine B, #3; nicotinamide, #4; Congo red, #5; Uranine, #6; minoxidil, #7; thiamine, #8; famotidine, #9; doxorubicin, #10; terbutaline, #11; riboflavin and #12; metformin. Numbers on structures corresponds with the drugs in Figure 6.

Table S1. Reaction conditions for the one-step method for various chemicals

Chemical	Buffer, pH	Temp. (°C)
Creatinine	Acetate, pH4	60
Nicotinamide	Acetate, pH4	40
Rhodamine B	Acetate, pH5	60
Uranine	Acetate, pH5	40
Congo red	Phosphate, pH6	60
Famotidine	Phosphate, pH6	60
Minoxidil	Phosphate, pH6	40
Thiamine	Phosphate, pH6	40
Metformin	Tris-HCl, pH8	60
Terbutaline	Carbonate-bicarbonate, pH10	60
Riboflavin	Carbonate-bicarbonate, pH10	40

Table S2. Primer list

Gene	Primer sequence (5′–3′)
TFRC	F: TGGCAGTTCA AATGATGGA
	R: AGGCTGAACCGGGTATATGA
18S rRNA	F: TGAGAAACGGCTACCACATC
	R: TTACAGGGCCTCGAAAGAGT

Table S3. Cytotoxicity of DOX-FTH.

	SKBR3	HEK293	Colon26
DOX IC_{50} (μM)	0.66	0.03	0.07
DOX-FTH IC_{50} (μM)	0.87	3.01	0.81
Rate	1.3	92.8	11.6