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Supplementary information

Cargo loading within ferritin nanocages in preparation for tumor-targeted delivery

In the format provided by the authors and unedited

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Cryo transmission electron microscope (cryo-TEM) characterization

For cryo-TEM characterization, 3.5 μL of HFn, HFn-His or M-HFn samples were embedded in vitreous ice using an FEI Vitrobot Mark VI. The frozen samples were imaged under an FEI 300-kV Titan Krios electron microscope. The microscope was operated at 300 kV and equipped with a Gatan Ultrascan 4000 (model 895) 16-megapixel CCD camera at the magnification of 96,000, and a total electron dose of about 25 $e^{-}/Å^{2}$.

Circular Dichroism (CD) spectra

CD spectra was acquired on an Applied Photophysics Chirascan Plus spectrometer at 25 ℃. The sample concentrations used were 0.25 mg/mL in PBS buffer. CD spectra of HFn nanocages and HFn encapsulated Fe_3O_4, Co_3O_4 and MnO_2 NPs were measured from 270 to 200 nm with 0.1 nm resolution in a quartz cell with a 1 cm path length.

Dynamic light scattering (DLS) analysis

DLS measurements were conducted to analyze the hydrodynamic diameter of HFn and HFn-Dox using a DynaPro Titan system DLS instrument. DLS data were collected at 25 ℃ and the concentration of the samples used was 0.25 mg/mL in PBS buffer. Measurements were collected at 10 s intervals. Each sample was run 10 times.

Size-exclusion chromatography (SEC) analysis

For SEC analysis, an aliquot of 500 μL of the prepared HFn (0.5 mg/mL) or HFn-His (0.3 mg/mL) protein was loaded onto a HiLoad 16/600 Superdex 200 prep grade size exclusion chromatography column (General Electric, cat. no. 28-9893-35) connected to an AKTA avant 150 system (General Electric, model no. 28-9308-42), with 20 mM Tris-HCl (pH 8.0, 100 mM NaCl) buffer as the mobile phase. The loaded sample was eluted by 1 column volume of buffer (flow rate: 1 mL/min), when the UV280 nm was monitored.

Size-exclusion HPLC analysis

Size-exclusion HPLC analysis was performed on a Superose 12 HR 10/30 column (Amersham Pharmacia Biotech, cat. no. 17-0538-01) using 20 mM Tris-HCl (pH 8.0, 100 mM NaCl) as the mobile phase at a flow rate of 0.6 mL/min with both in-line radioactivity and UV detection.

Reagents

- HNO_3 (Sinopharm Chemical Reagents, cat. no. 7697-37-2) **! CAUTION:** Causes severe burns. Do not inhale the vapor. Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Use in a fume hood and wear gloves, protective eye-wear and a lab-coat.
- Iron standard for ICP (Supelco, cat. no. 43149)
- Cobalt standard for ICP (Supelco, cat. no. 30329)
- Manganese standard for ICP (Supelco, cat. no. 74128)

Equipment

- ICP-OES (Thermo Scientific, model no. iCAP6300)
- Metal bath thermostat (OLABO, model no. OLB-DH100-I)
- Cryo-TEM (Thermo Fisher Scientific, model no. FEI Titan Krios)
- Dynamic light scattering (DLS) instrument (Wyatt Technology, model no. DynaPro-Titan system)
- CD Spectrometer (Applied Photophysics Ltd, model no. Chirascan ^{TM})
- HiLoad 16/600 Superdex 200 prep grade size exclusion chromatography column (General Electric, cat. no. 28-9893-35)
- AKTA avant 150 (General Electric, model no. 28-9308-42)
- Superose 12 HR 10/30 (size exclusion column; Amersham Pharmacia Biotech, cat. no. 17-0538-01)
- HPLC system with a UV and a γ-detector connected in series. HPLC system (Agilent, model no. 1260 Infinity II), γ- counter (USTC Chuangxin Co. Ltd., model

no. GC-1200)

Reagent Setup

Iron/Cobalt/Manganese standard solution

Dissolve the iron, cobalt or manganese standards in 2% nitric acid to a concentration of 0 mg/L, 0.1 mg/L, 0.3 mg/L, 0.5 mg/L, 1 mg/L, and 2 mg/L, respectively. The solution should be stored at a temperature less than 4 ℃ for no more than 3 months.

Quantifying metal content by ICP-OES

- **TIMING: ～3 h**

CRITICAL We have used the procedure below for quantifying the mineral content encapsulated within the cavity of HFn nanocage using ICP-OES.

1. Add 3 mL nitric acid into a tube containing 0.2 mL mineralized HFn (M-HFn).
2. Heat up to 150 ℃ in a metal bath thermostat for 10 min to dissolve the protein and the metal oxide nanoparticle inside the cavity.
3. Add ddH_2O to make the reaction volume up to 10 mL.
4. Set the instrumental parameters of ICP-OES as below: the radio frequency power is 1.0 KW, the carrier gas is Argon, the plasma flow is 15 L/min, the auxiliary gas flow is 1.5 L/min, the nebulizer gas flow is 0.75 L/min, the detector mode is axial mode, and the calibration type is linear. The duration of the process is about 15 min.
5. Analyse the sample using ICP-OES.
6. Calculate the standard curve by measuring the standard metal samples (Reagent setup) at the concentration of 0 mg/L, 0.1 mg/L, 0.3 mg/L, 0.5 mg/L, 1 mg/L, and 2 mg/L.
7. Measure the M-HFn samples and calculate the metal content according to the standard curve.

   ▲ **CRITICAL STEP**: If the measuring value of the M-HFn sample exceeds the range of the standard curve, dilute the M-HFn sample with nitric acid until the ICP-OES reading falls within the limits of the calibration graph.

Supplementary Figures

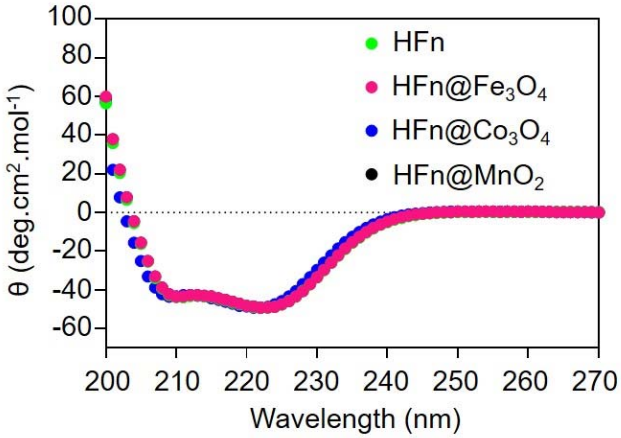

Figure S1 CD spectra of HFn nanocages and HFn encapsulated Fe_3O_4, Co_3O_4 and MnO_2 NPs showing no secondary structure change to the HFn protein nanocage after metal loading.

Supplementary Tables

Supplmentary Table 1 Values for data points in Table 4.

Ferritin	Cargo	Entry	Number of loaded cargos per ferritin	Average number of loaded cargos per ferritin	The standard deviation (s.d.) of the content of cargos	Number of loaded cargos per ferritin
HFn	Fe	1	3399	3186	188	3186±188
		2	3040			
		3	3121			
HFn	Co	1	2505	2814	268	2814±268
		2	2956			
		3	2981			
HFn	Mn	1	1996	2200	342	2200±342
		2	2594			
		3	2011			

HFn	Doxorubicin	1	68			
		2	48	53	12	53±12
		3	45			
HFn-His	Gd^{3+}	1	54			
		2	44	48	5	48±5
		3	46			
HFn-His	$64Cu^{2+}$	1	1.1			
		2	1.3	1.1	0.2	1.1±0.2 μCi/μg
		3	0.9			

Ferritin	Cargo	Entry	Protein recovery yield (%)	Average number of protein recovery yield (%)	The standard deviation (s.d.) of protein recovery yield (%)	Protein recovery yield (%)
HFn	Fe	1	87	87	9	
		2	78			87±9
		3	96			
HFn	Co	1	92	81	12	
		2	84			81±12
		3	68			
HFn	Mn	1	86	73	11	
		2	68			73±11
		3	66			
HFn	Doxorubicin	1	91			
		2	81	84	6	84±6

		3	80			
HFn-His	Gd^{3+}	1	98			
		2	90	91	6	91±6
		3	86			
HFn-His	$64Cu^{2+}$	1	82			
		2	95	90	7	90±7
		3	94			

Ferritin	Cargo	Entry	Cargo recovery yield (%)	Average number of cargo recovery yield (%)	The standard deviation (s.d.) of cargo recovery yield (%)	Cargo recovery yield (%)
HFn	Fe	1	65	64	4	
		2	60			64±4
		3	68			
HFn	Co	1	61	56	5	
		2	57			56±5
		3	50			
HFn	Mn	1	51	44	7	
		2	45			44±7
		3	37			
HFn	Doxorubicin	1	45			
		2	33	35	9	35±9
		3	27			
HFn-His	Gd^{3+}	1	12			
		2	17	13	3	13±3
		3	10			

HFn-His	$64Cu^{2+}$	1	13			
		2	21	17	4	17±4
		3	18			

Supplementary Table 2 Values for data points in Figure S1.

Wavelength	HFn	HFn@Fe_3O_4	HFn@Co_3O_4	HFn@MnO_2
270	0.168908	0.037704	0.013691	0.039204
269	0.150641	0.082325	0.038704	0.083825
268	0.169082	0.113587	0.083325	0.115087
267	0.303309	0.173234	0.114587	0.174734
266	0.272728	0.108582	0.174234	0.110082
265	0.224641	0.219166	0.109582	0.220666
264	0.274394	0.068794	0.220166	0.070294
263	0.162241	0.264768	0.069794	0.266268
262	0.097438	0.318216	0.265768	0.319716
261	0.200065	0.431895	0.319216	0.433395
260	0.190752	0.385401	0.432895	0.386901
259	0.374275	0.370261	0.386401	0.371761
258	0.227431	0.395721	0.371261	0.397221
257	0.167692	0.394569	0.396722	0.396069
256	0.185352	0.380297	0.395569	0.381797
255	0.200277	0.395941	0.381297	0.397441
254	0.225585	0.477814	0.396941	0.479314
253	0.285979	0.461237	0.478814	0.462737
252	0.139505	0.380954	0.462237	0.382454
251	0.149964	0.433354	0.381954	0.434854
250	0.097961	0.394756	0.434354	0.396256
249	0.016007	0.196049	0.395756	0.197549
248	-0.12433	0.130716	0.197049	0.132216
247	-0.29033	-0.097091	0.131716	-0.09559
246	-0.52698	-0.32524	-0.09609	-0.32374
245	-0.85598	-0.70581	-0.32424	-0.70431
244	-1.42941	-1.06842	-0.70481	-1.06692
243	-2.05462	-1.70664	-1.06742	-1.70514
242	-2.88655	-2.52525	-1.70564	-2.52375
241	-3.74407	-3.38621	-2.52425	-3.38470
240	-5.07811	-4.69907	-3.38521	-4.69757
239	-6.46819	-6.17006	-4.69807	-6.16856
238	-8.42520	-8.03671	-6.16906	-8.03521

237	-10.4972	-10.0792	-8.03571	-10.0777
236	-13.0832	-12.6041	-10.0782	-12.6026
235	-15.7512	-15.4231	-12.6031	-15.4216
234	-18.8971	-18.6976	-15.4221	-18.6961
233	-22.4501	-22.2109	-18.6966	-22.2094
232	-26.0531	-25.9545	-22.2099	-25.9533
231	-29.8766	-29.7817	-25.9535	-29.7802
230	-33.645	-33.5819	-29.7807	-33.5804
229	-37.2491	-37.1414	-33.5809	-37.1399
228	-40.5268	-40.6069	-37.1404	-40.6054
227	-43.5485	-43.4855	-40.6059	-43.4845
226	-45.7693	-45.8659	-43.4845	-45.8644
225	-47.6716	-47.5953	-45.8649	-47.5938
224	-48.8435	-48.7974	-47.5943	-48.7959
223	-49.2079	-49.2063	-48.7964	-49.2048
222	-49.3693	-49.3086	-49.2053	-49.3071
221	-48.9629	-48.6976	-49.3076	-48.6961
220	-48.2852	-48.2847	-48.6966	-48.2832
219	-47.5609	-47.2381	-48.2837	-47.2366
218	-46.6734	-46.3047	-47.2371	-46.3032
217	-45.6654	-45.3289	-46.3037	-45.3274
216	-44.7045	-44.4085	-45.3279	-44.4071
215	-43.7379	-43.5189	-44.4075	-43.5174
214	-43.4122	-43.1392	-43.5179	-43.1377
213	-43.0006	-42.8463	-43.1382	-42.8445
212	-43.3545	-42.8632	-42.845	-42.8615
211	-43.8925	-43.4312	-42.862	-43.4297
210	-43.8337	-43.6019	-43.4302	-43.6004
209	-42.7923	-42.2645	-43.6009	-42.2631
208	-39.4508	-38.9429	-42.2635	-38.9414
207	-33.9839	-33.1591	-38.9419	-33.1576
206	-25.5621	-25.1019	-33.1581	-25.1004
205	-16.3072	-15.6693	-25.1009	-15.6678
204	-5.59126	-4.63489	-15.6683	-4.63339
203	6.56519	7.823353	-4.63389	7.82485
202	20.3202	21.96201	7.824353	21.96351
201	35.8936	37.86764	21.96301	37.86914
200	56.4448	59.92212	57.34523	58.92362