Electronic Supplementary Material (ESI) for Nanoscale.

Ca^{2+} Incorporated Self-assembly of Apoferritin Nanostructure for Nucleic Acid Drugs Delivery

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Methods

Preparation of Ca^{2+}-siRNA complexes

Equal volumes of siRNA (8.75 μM) and $CaCl_2$ (20 mM-1M) were mixed together in an equal volume of pH8.0 buffer (20 mM Tris, 0.15 M NaCl, pH 8.0) by vortexing for 30 s and incubating for 20 min at room temperature (RT). The Ca^{2+}-siRNA nanoparticles were treated with 3 mg/mL RNase A at 37 °C for 30 min, followed by a treatment with 5mg/mL Proteinase K at 37 °C for 30 min. 2% agarose gel electrophoresis (AGE) was used to demonstrate the successful loading and protection of siRNA.

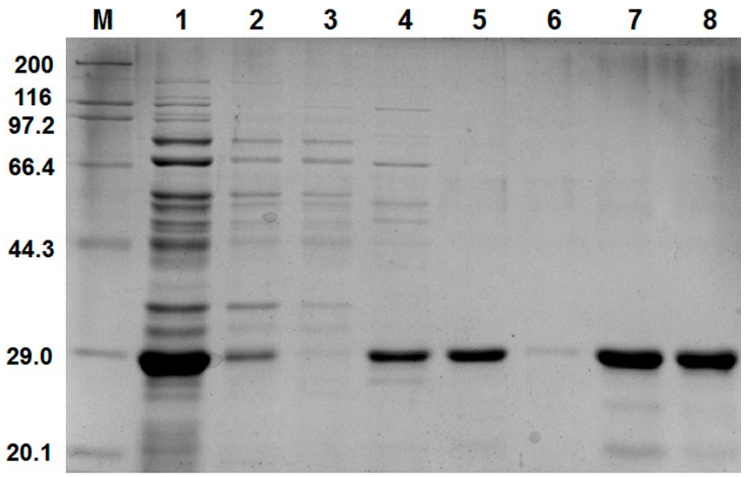

Figure S1. The purification of HFn was analyzed by 12 % SDS-PAGE. Lane M: Protein marker; Lane 1:supernatant after heat; Lane 2: sample effluent; Lane 3: effluent washed by binding buffer; Lane 4 - 8: HFn effluent eluted by 30 mM, 50 mM, 70 mM, 90 mM and 300 mM imidazole respectively.

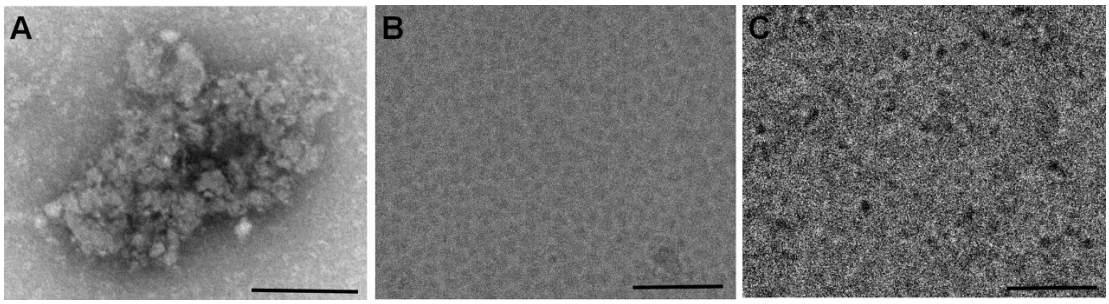

Figure S2. (A) TEM photograph of HFn in pH 2.0 buffer (20 mM Tris, 0.15 M NaCl). **(B)** Cryo-TEM images of HFn@siRNA NPs. **(C)** Cryo-TEM images of HFn@Ca NPs. Scale bar of A, 100 nm, Scale bar of B and C, 50 nm.

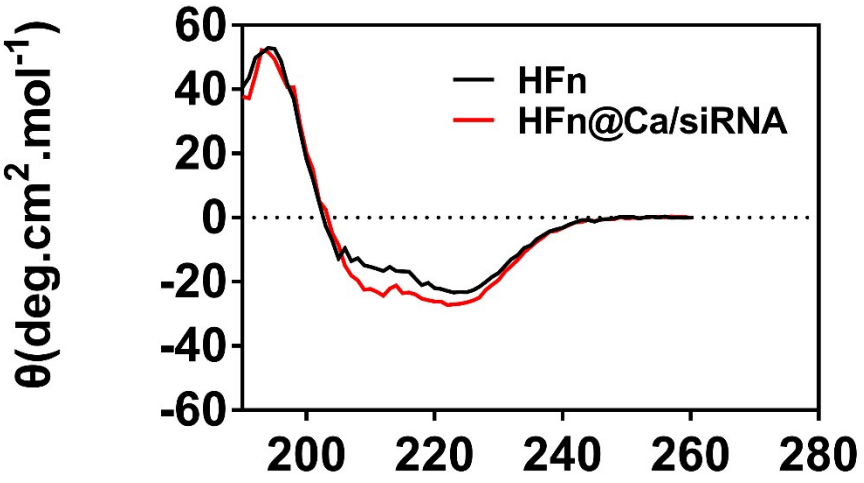

Figure S3. CD spectra results of HFn and HFn@Ca/siRNA NPs.

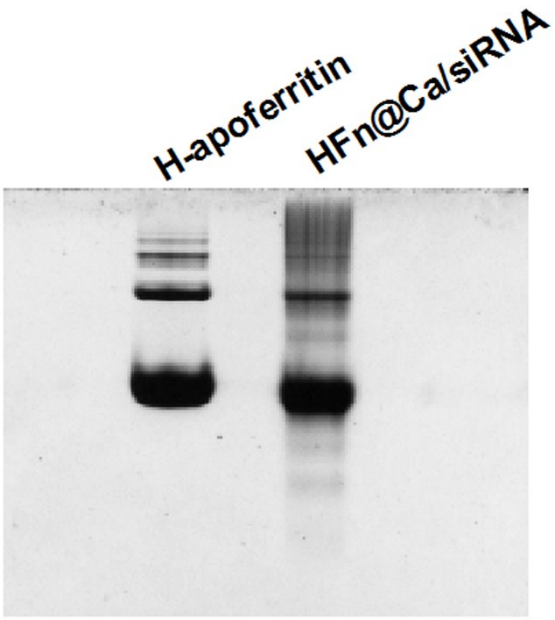

Figure S4. Characterization of the reassembly protein cage of HFn and HFn@CaP/siRNA with 6 % continuous native gel electrophoresis.

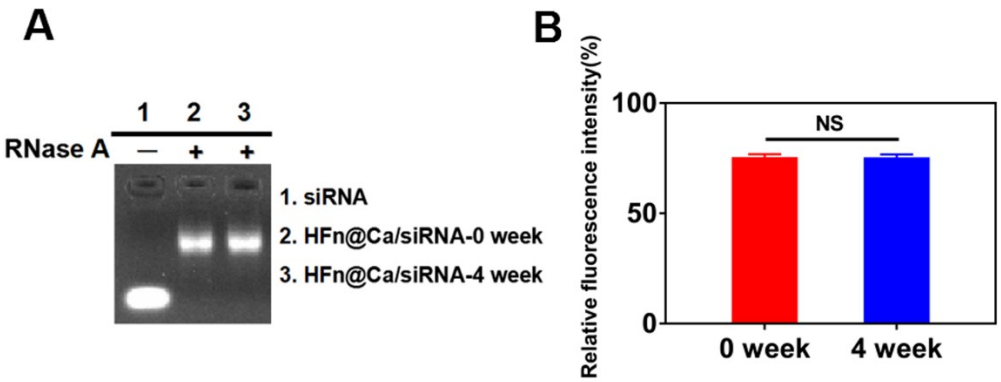

Figure S5. (A) Stability of HFn@Ca/siRNA (1 : 8) NPs before and after stored at 4 °C for four weeks, detected by AGE. **(B)** The fluorescence intensity analysis of HFn@Ca/siRNA NPs according to section A.

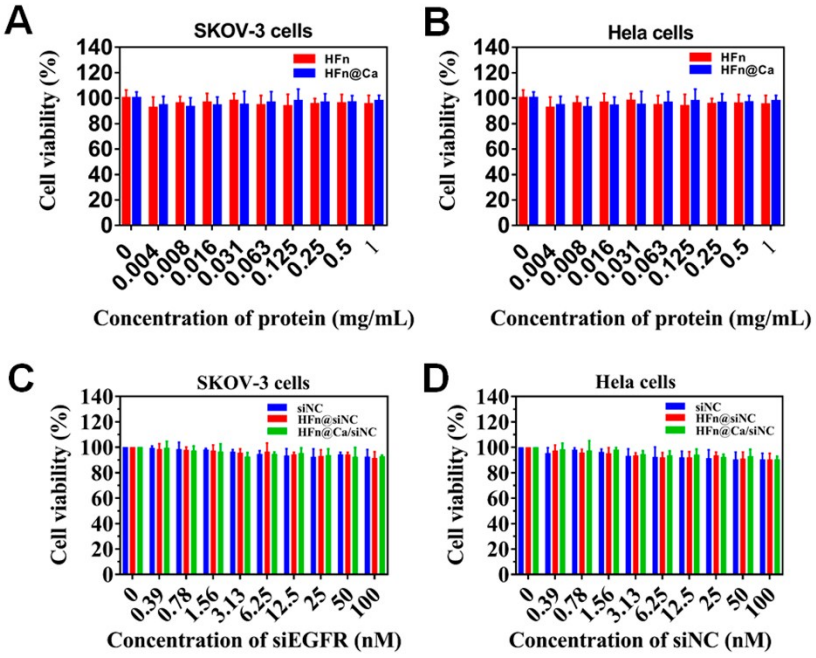

Figure S6. Cytotoxicity evaluation of HFn and HFn@Ca NPs in SKOV-3 cells **(A)** and Hela cells **(B)** after 48 h incubation. Cytotoxicity of siNC, HFn@NC and HFn@Ca/siNC NPs at different concentrations in SKOV-3 cells **(C)** and Hela cells **(D)** after incubation for 48 h.

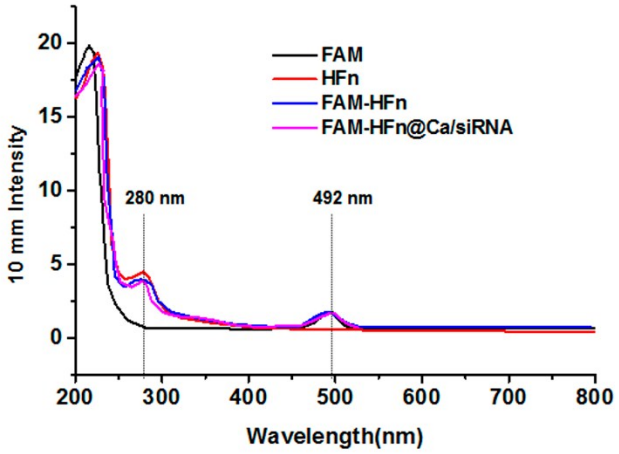

Figure S7. UV vis absorption spectra of FAM, HFn, FAM-HFn, and FAM-HFn@Ca/siRNA NPs.

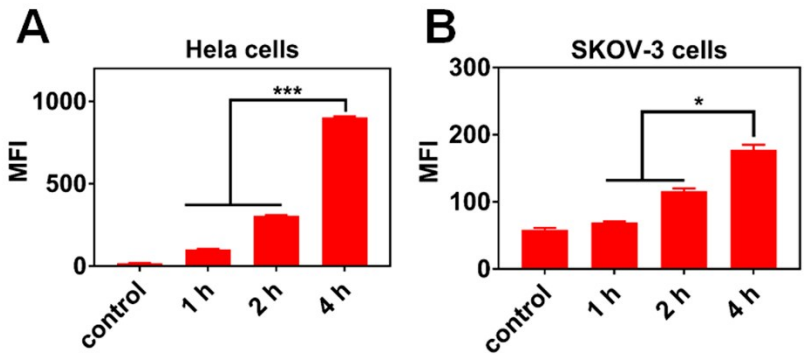

Figure S8. *In vitro* cellular uptake of FAM-HFn@Ca NPs incubated with Hela cells **(A)** or SKOV-3 cells **(B)** by flow cytometry. *p<0.05, ***p<0.001.

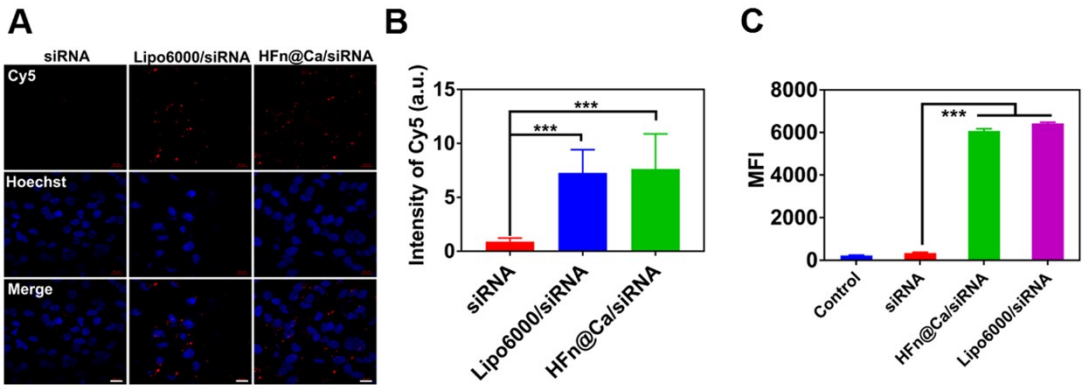

Figure S9. (A) Cellular uptake studies in Hela cells incubated with siRNA, Lipo6000/siRNA and HFn@Ca/Cy5-siRNA NPs for 4 h by CLSM. **(B)** Analysis of Cy5 intensity by Image J according to section A. **(C)** Cellular uptake of HFn@Ca/Cy5-siRNA NPs incubated with Hela cells by flow cytometry. The scale bar was 20 μm. $^{***}p<0.001$.

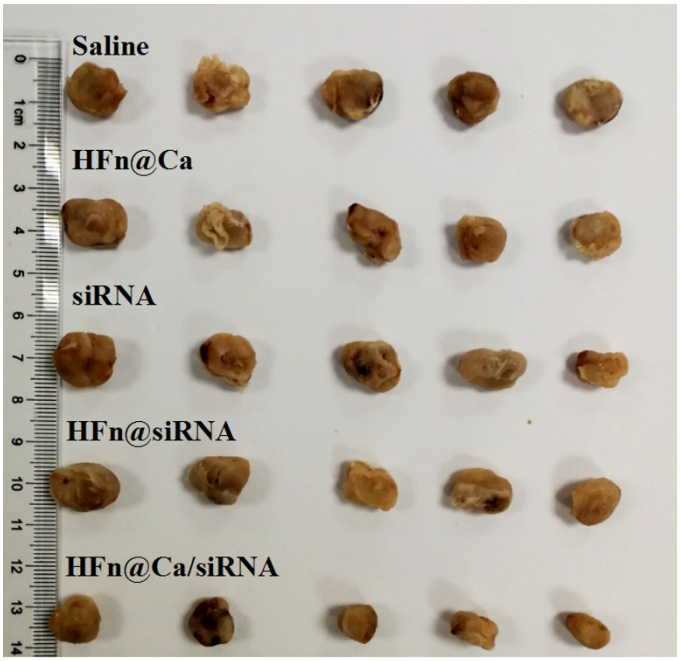

Figure S10. Images of excised tumors of each group with saline, HFn@Ca, siRNA, HFn@siRNA and HFn@Ca/siRNA after last injection treatment (n = 5).