Supporting information

An Apoferritin-Hemagglutinin Conjugate Vaccine with Encapsulated Nucleoprotein Antigen Peptide from Influenza Virus Confers Enhanced Cross Protection

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Supporting method

Structure Characterization of AFt Following Heat-Treatment. AFt solution (0.5 mg/mL) was heated to different temperatures ranging from 25 to 80 °C and remained for 30 min. After cooling down to room temperature (RT), samples were analyzed by high-performance size-exclusion chromatography (HPSEC), circular dichroism (CD) spectra and dynamic laser scattering (DLS), respectively.

The HPSEC was performed on an Agilent 1100 high-performance liquid chromatography (HPLC) series system (Agilent, Santa Clara, CA, USA), equipped with a TSKgel G3000 SW_{XL} column (7.8 mm I.D.×30 cm, TOSOH Corp., Tokyo, Japan). For all measurements, 100 μL samples (0.5 mg/mL) were injected and eluted with phosphate buffered saline (PBS, 50 mM PB containing 0.1 M Na_2SO_4, pH 7.4) at 0.5 mL/min for 30 min.

CD spectra of AFt (0.1 mg/mL) were recorded on Jasco-810 spectrometer (JASCO Corp., Tokyo, Japan) at 25 ℃ using a quartz cuvette with 0.1 cm cell length. For each measurement, the spectra were averaged from three scans in the wavelength range from 190 to 260 nm with a scanning speed of 50 nm/min. The molar ellipticity ($deg \cdot cm^2 \cdot dmol^{-1}$) was calculated on the basis of a mean residue molecular weight of 110 in reference of CD spectra by Dr. J. T. Yang.

DLS was performed on a Malvern Zetasizer Nano ZS (Malvern Panalytical Ltd., Malvern, UK) at 25 ℃ using a quartz cuvette with 1 cm cell length. Three measurements were performed for each sample at a concentration of 0.5 mg/mL in 10 mM PB buffer (pH 7.4), and the results were exported in number percent (%) -size distribution (d. nm).

The Verification of NP Loading inside AFt. AFt nanoparticles were known to undergo acidolysis to oligomeric, dimeric or monomeric subunits at pH between 2.2 to 2.6[1]. To verify the successful loading of NP inside the nanocage of AFt, the pH of AFt+NP solution was adjusted to 2.6 with HCl and incubated at RT for 60 h to release the encapsulated NP. The resultant solution was subjected to Bruker autoflex Ⅲ matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Billerica, MA, USA) and RP-HPLC. The MALDI-TOF MS measurement was performed with α-Cyano-4-hydroxycinnamic acid (CHCA) matrix at linear positive-ion mode, operated with an accelerating voltage of 20 kV and a mass-to-charge ratio (m/z) range from 0 to 25,000. FlexControl and flexAnalysis software were used for data acquisition and analysis, separately. For RP-HPLC analysis, 7 μL sample (0.5 mg/mL) was injected into an Agilent ZORBAX 300SB-L-C8 column (2.1×150 mm, 5 μm, Agilent, Santa Clara, CA, USA) coupled to a Waters 2695 Alliance system (Waters Corp., Milford, MA, USA). The mobile phase (A) (0.1% (v/v) trifluoroacetic acid (TFA) in water) and mobile phase (B) (0.1% (v/v) TFA in acetonitrile) were used to establish the 45 min gradient elution comprising of 20 min of 20-80% B, 10 min of 100% B, and 15 min of 20% B at flow rate of 0.5 mL/min.

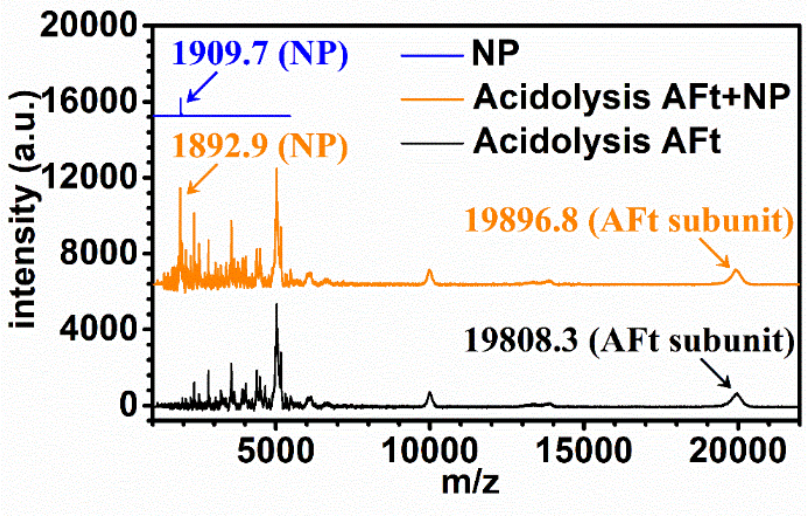

Figure S1. The MALDI-TOF spectra of native NP, AFt+NP and AFt after acidolysis. The AFt+NP samples were prepared by thermal-encapsulating NP inside AFt at 50 □ for 45 min. The "acidolysis" treatment of AFt was carried out at pH 2.6 for 60 h.

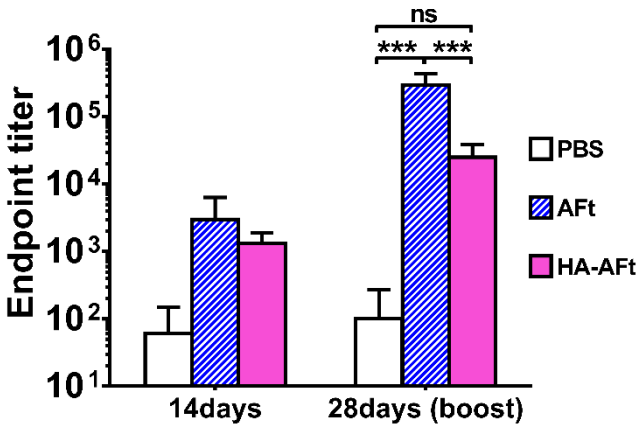

Figure S2. AFt-specific IgG titers in serum of mice collected 2 weeks after each immunization. (n =8; ***P<0.001)

Reference

(1) Kim, M., Rho, Y., Jin, K. S., Ahn, B., Jung, S., Kim, H., and Ree, M. (2011) pH-dependent structures of ferritin and apoferritin in solution: disassembly and reassembly, *Biomacromolecules 12*, 1629-1640.