Electronic Supplementary Information

Manganese-ferritin nanocomposite as ultrasensitive T_2 contrast agent

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Experimental details:

Mineralization of AfFtn-AA with manganese:

Manganese ($MnCl_2$ solution) was added stepwise to 0.1 mg/ml AfFtn-AA solution (in 100 mM HEPES buffer, pH 7.5 with 50 mM NaCl) to final molar ratio of 2400 Mn per 24-meric ferritin cage and incubated for one hour at room temperature, followed by overnight incubation at 4°C. Unbound manganese was removed by buffer exchange using a 100-kD MWCO Amicon centrifugal filter device (Millipore) followed by desalting in Sephadex G-25 desalting column (GE Healthcare). Manganese content of the (Mn)AfFtn-AA was determined by inductively coupled plasma (ICP) spectrometer (Perkin Elmer).

Characterization of (Mn)AfFtn-AA:

Self-assembly of ferritin subunits upon metal binding was studied by monitoring the change of hydrodynamic diameter measured by dynamic light scattering (DLS) technique at 25°C using a zetasizer instrument (Zetasizer Nano ZS; Malvern Instruments). The measurements were done with 1 mg/ml protein concentration and the samples were pre-equilibrated at 25°C for 5 minutes. Molecular size of AfFtn-AA and (Mn)AfFtn-AA were calculated from their elution volumes through superdex 200 10/300 GL size exclusion

column (GE Healthcare) using 50 mM HEPES buffer with 50 mM NaCl, pH 7.5. Size exclusion chromatography (SEC) was performed in AKTA Explorer FPLC system (GE Healthcare) and the elution volumes were compared with a set of standard protein of known molecular sizes.

Manganese content of the (Mn)AfFtn-AA nanocomposite was quantified by inductively coupled plasma spectrometer (ICP-OES Optima 2000 DV, Perkin Elmer). Elemental analysis of the platinum-coated sample was performed with an energy dispersive X-ray (EDX) spectrometer (JSM-6700F, JEOL). Acquisition parameters used were as follows: acceleration voltage 20.0 kV; probe current 2.562 nA; PHA mode T4; real time 63.16 sec; live time 60.00 sec; dead time 5%; counting rate 523 cps; energy range 0 – 20 keV.

The structure of the nanocomposite was studied by transmission electron microscopy (TEM) of the samples with and without negative staining. (Mn)AfFtn-AA solutions (0.1mg/ml) were applied directly onto carbon-coated copper grids and allowed to adhere for one minute. Excess solution was removed carefully by wicking with filter paper and grids were allowed to air dry for a few minutes. For staining, the air dried grids were treated with 1.5 % uranyl acetate for one minute, excess stains were removed by wicking using filter paper and allowed for air drying. The grids were air dried for another 24 hours before the specimens were examined in a TEM (JEM-1400, JEOL) at an accelerating voltage of 100 kV.

The stability of the manganese core of (Mn)AfFtn-AA was studied in presence of iron. A final concentration of 30 µM and 100 µM of ferrous sulphate solution (10 mM stock prepared in 0.1% HCl) was added to 10 ml of 0.1 mg/ml (Mn)AfFtn-AA solution in

100 mM HEPES buffer pH 7.5 with 50 mM NaCl and incubated at 37°C. The control experiment was done without addition of any iron solution. After 24 hours the solution was concentrated to 2.5 ml and free manganese was removed using a 100-kD MWCO Amicon centrifugal filter device (Millipore) followed by desalting in Sephadex G-25 desalting column (GE Healthcare). Remaining metal concentration was measured by ICP spectrometer (Perkin Elmer).

Aliquot of (Mn)AfFtn-AA was buffer exchanged with water and lyophilized. Magnetization of the lyophilized (Mn)AfFtn-AA was characterized in a 1.5-T vibrating sample magnetometer (VSM; 7300 series, Lakeshore) under magnetic field strength of up to 1.2 T at room temperature.

MR imaging and relaxivity measurement:

MRI samples were prepared with different concentrations of (Mn)AfFtn-AA embedded in a 0.8% agarose matrix in 24-well tissue culture plates. Samples were scanned in a 3.0 Tesla whole-body scanner (Magnetom Verio, Siemens) using a dedicated knee coil. T_1-weighted images were acquired with different inversion time (T_R = 2800 ms T_E = 8.5 ms, T_I = 30, 50, 100, 200, 400, 500, 600, 800, 100, 1200, 1800, 2000 and 2500 ms, matrix = 512 × 512, slice thickness = 3.0 mm, number of averages = 1, field of view = 27 cm) and T_2-weighted images were taken with different echo times (T_R = 1000 ms T_E = 10, 15, 20, 40, 60, 80, 100, 150, 200 ms, matrix = 512 × 512, slice thickness = 3.0 mm, number of averages = 1, field of view = 27 cm). The T_1 and T_2 values for each sample were calculated from mean signal intensity of MR images by using MATLAB. For the calculation of T_1 values, a nonlinear fit of the intensity data (T_1-weighted images) to the

inversion-recovery signal equation was performed by using cftool-fitting in customized equation. The T_2 of each sample was calculated by fitting the intensities of T_2 weighted images to a mono-exponential decay curve using the same method. Relaxivity (R_1 and R_2) of each sample was derived from the T_1 and T_2 values at the varying concentrations of (Mn)AfFtn-AA nanocomposites. Gradients of the linear fit were obtained by plotting inverse relaxation times ($1/T_1$ and $1/T_2$) against metal concentration indicate longitudinal (R_1) and transverse (R_2) relaxivities, respectively.

Figure:

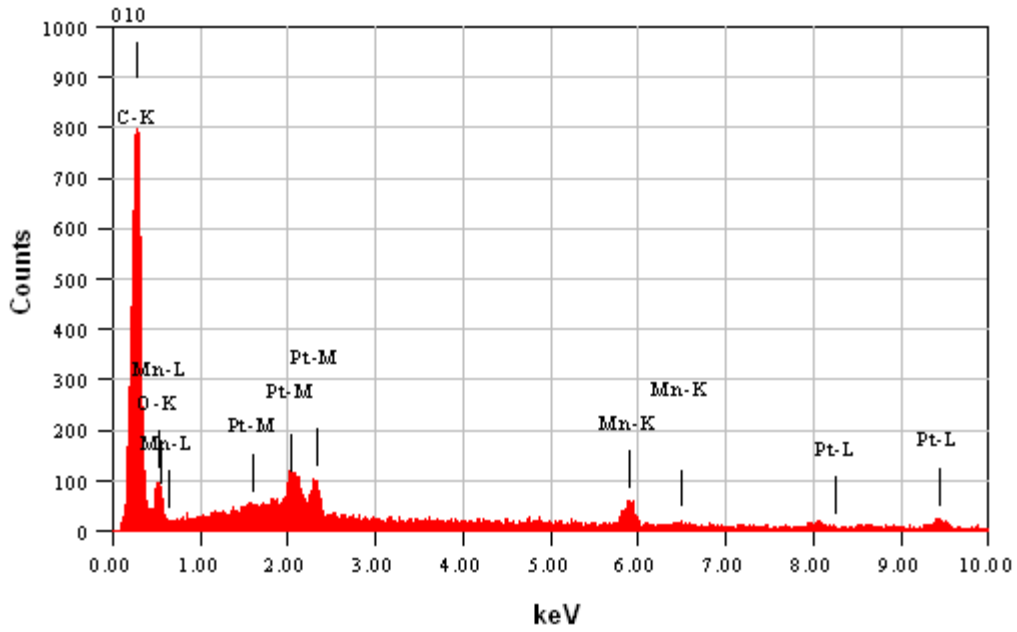

Figure S1. EDX spectrum of (Mn)AfFtn-AA sample (Pt-coated).