

Casein-coated Iron Oxide Nanoparticles for High MRI Contrast Enhancement and Efficient Cell Targeting

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Supporting Information

Experimental details:

1. The standard Bradford assay was performed to determine the casein concentration on the nanoparticles, with non-casein coated nanoparticles as the control. The iron concentration was determined by the 1,10-phenanthroline colorimetric method.¹ Then the nanoparticle concentration was calculated based on the assumption that the reported IO nanoparticle is spherical with a bulk magnetite density of 5.18 g/cm³.

2. The low-frequency alternative-current magnetic susceptibility (χ_{ac}) of magnetic iron oxide nanoparticles was measured using a χ_{ac} analyzer (XacQuan, MagQu) with the driving frequency ranged from 10 Hz to 25 kHz. Samples of CNIO and SPH15 were prepared at the same concentration of 1.0 mg Fe/mL. This AC magnetic susceptibility measurement has been used to determine the biodistribution of ferritin and iron oxide nanoparticles.^{2,3}

Results and Figures:

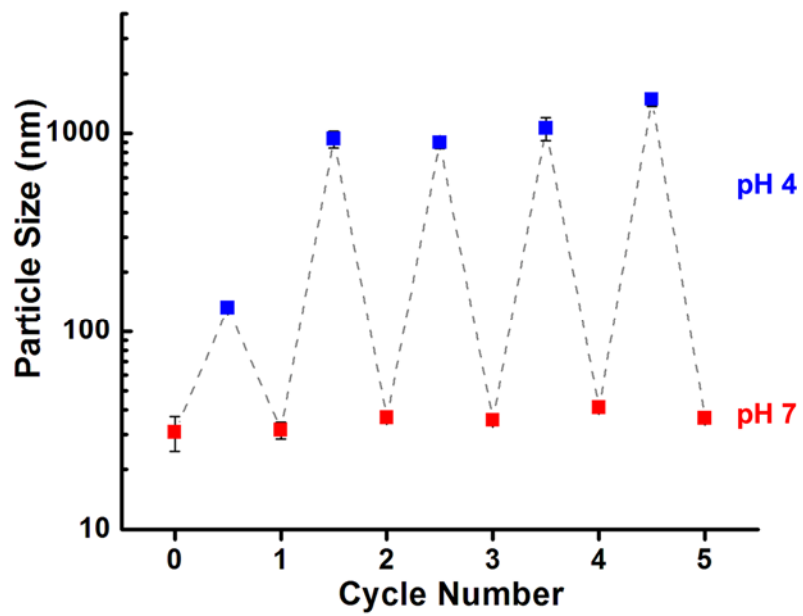


Figure S1. Reversible size change of CNIOs between pH 4.0 and 7.0. Particle size (diameter) was measured by DLS in the term of a number-weighted diameter.

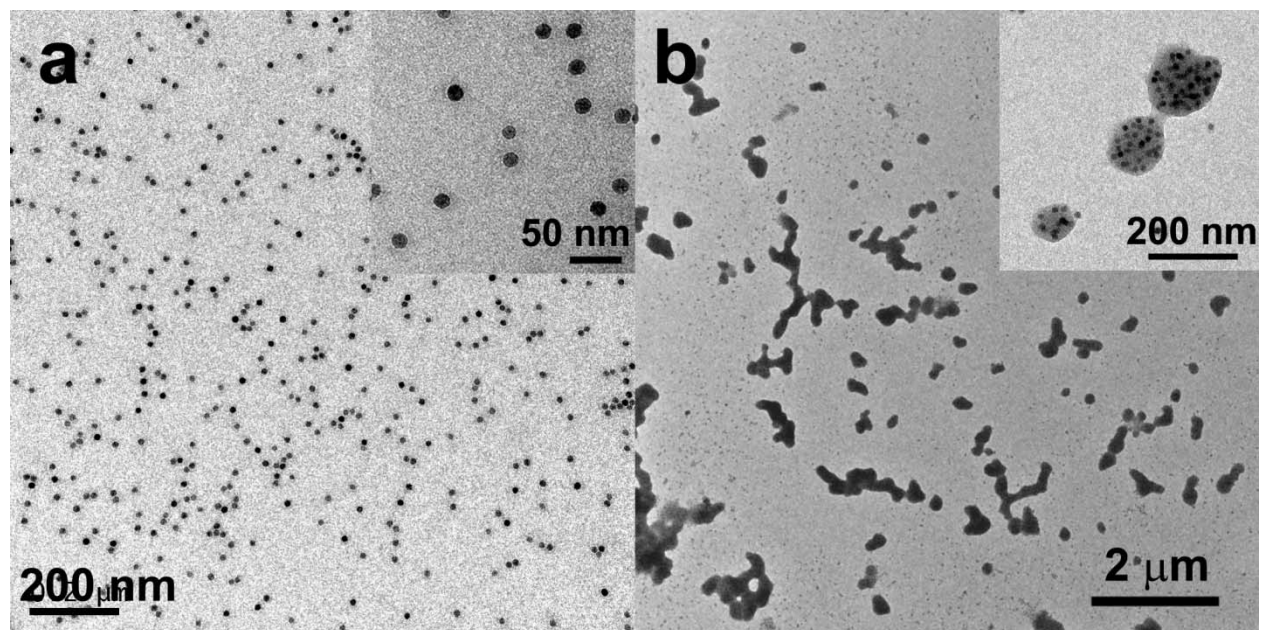


Figure S2. TEM images of CNIOs at: a) pH 7.0, and b) pH 4.0. Inserts are the corresponding magnified images.

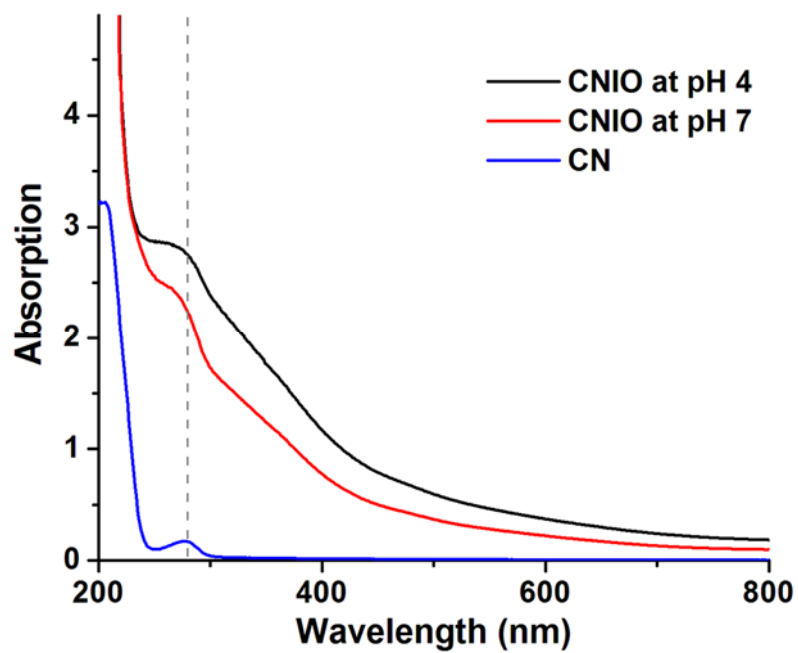


Figure S3. UV-vis absorption spectra of casein (CN) and CNIOs solutions at pH 7.0 and pH 4.0.

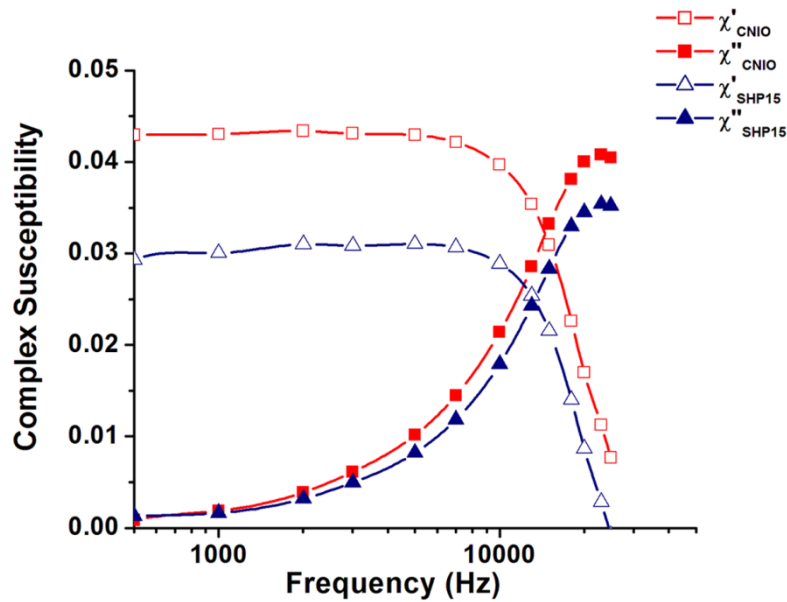


Figure S4. Complex magnetic susceptibility spectra of CNIO and SHP15 recorded at 298K. The real part of complex susceptibility for CNIO is 0.043 at the frequency 1000 Hz, where the susceptibility has reached a plateau, at the level 1.4 times higher than that of SHP15 (~0.030). The result suggested that CNIOs have higher sensitivity to an external magnetic field, which is responsible for its higher relaxivity measured by MRI. Using the crossing point of the real and imaginary susceptibility curves, the hydrodynamic sizes of the magnetic nanoparticles can be estimated based on the ideal Debye model for the Brownian rotation of particles in solvent.⁴ Given viscosity of 1 mPas, the calculated corresponding hydrodynamic diameters of CNIOs and SHP15 are 30.4 nm and 31.3 nm, respectively. These are in accordance with the results obtained from the dynamic light scattering (DLS) measurement.

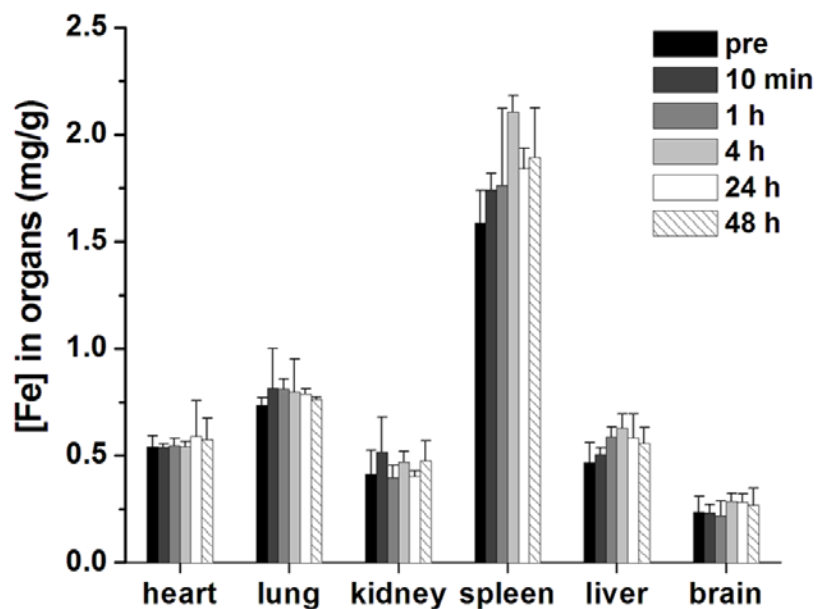


Figure S5. Biodistribution of CNIOs in different organs of mice (n=3/time point) determined by chemical analysis of iron concentrations in the collected tissue samples. Organs were collected from mice after injection of CNIOs at a dosage of 2.5 mg Fe/kg per mouse body weight. Controls were obtained from organs collected from mice without getting CNIOs. Iron concentrations were measured by the phenanthroline colorimetric method after each sample was lyophilized and acid digested.

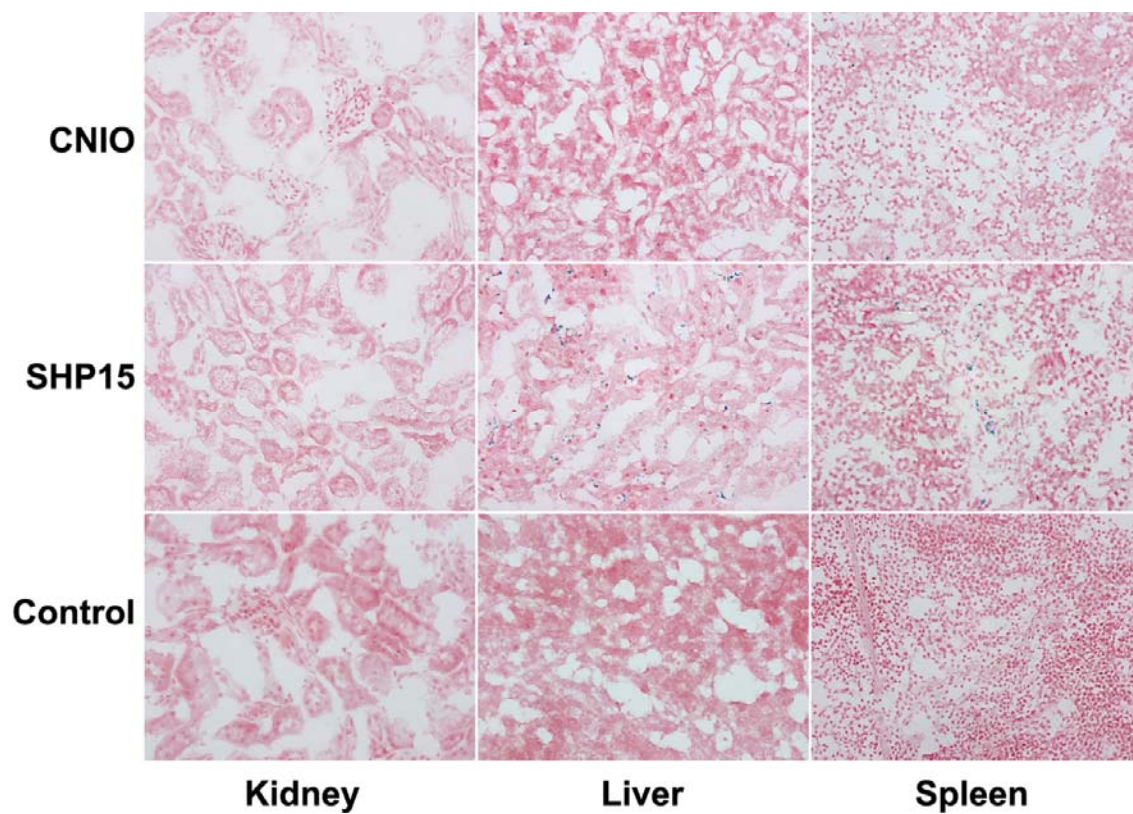


Figure S6. Biodistribution of CSIOs in the mouse kidney, liver and spleen tissues as shown in Prussian blue staining are compared with those from the mice received SHP15 and mice without any injection. The organs were collected 24 h after the injection at a dosage of 2.5 mg Fe/kg per mouse body weight.

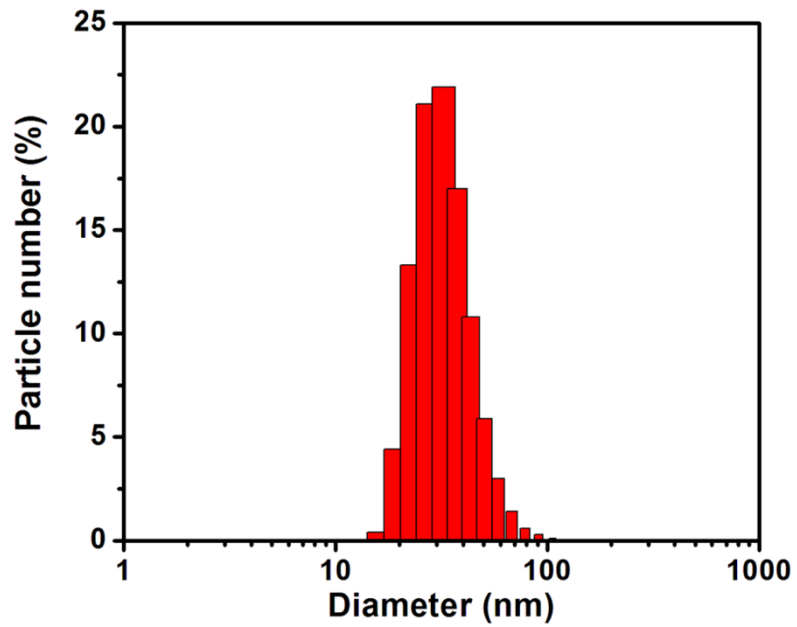


Figure S7. The number-weighted hydrodynamic size (~ 35.0 nm, in diameter) of ScFvEGFR-CNIO measured by DLS.

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