Supporting Information for

## Facile non-hydrothermal synthesis of oligosaccharides coated sub-5 nm magnetic iron oxide nanoparticles with dual MRI contrast enhancement effect

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## **METHODS**

Simulation of MRI Contrast and Image Data Analysis.  $T_1$  and  $T_2$  values of all pixels were calculated using an in-house Matlab program (Mathworks, Natick, MA, USA). Colored  $T_2$  maps were generated for presenting the changes of  $T_2$  in the entire organs. Longitudinal relaxation times,  $T_1$ , of different nanoparticles were obtained by performing non-linear fitting to the signal intensities based on the Eq. [1]:<sup>1</sup>

$$SI = A \cdot \left| 1 - B \cdot e^{-TI/T1} \right|$$
[1]

where A, B and  $T_1$  are free parameters to fit. The transverse relaxation times,  $T_2$  were derived from non-linear fitting based on the Eq. [2] :<sup>1</sup>

$$SI = \sqrt{D^2 + (C \cdot e^{-TE/T^2})^2}$$
 [2]

where C, D and  $T_2$  are free parameters to fit.

In order to better understand the signal intensity change as a function of  $r_1$  and  $r_2$  of IONPs with different sizes, a simplified simulation was performed. Specifically, the signal intensity of MR images was simulated based on a basic spin echo sequence using the Equation [3] at a given TE and TR.

$$SI \propto e^{-TE/T_2} (1 - e^{-TR/T_1})$$
 [3]

In order to simulate  $T_1$  and  $T_2$  values for different IONPs, the empirical linear relaxivity approximation described in Eq. [4] and [5] was used for the simulation at any given concentration.<sup>1</sup>

$$\frac{1}{T_1} = \frac{1}{T_{1,0}} + r_1[Fe]$$

$$\frac{1}{T_2} = \frac{1}{T_{2,0}} + r_2[Fe]$$
[5]

where  $T_{1,0}$  and  $T_{2,0}$ , in the unit of s, are the baseline relaxation times of tissue or solution without the influence of IONP;  $r_1$  and  $r_2$  are the longitudinal and transverse relaxivities of a given IONP in the unit of mM<sup>-1</sup>s<sup>-1</sup>; and [Fe] denotes the IONP concentration in mM.

Using the simulated [Fe] of 0.1 mM,  $T_{1,0}$  of 3 s,  $T_{2,0}$  of 2.5 s, and  $r_1$  and  $r_2$  ranging 0.01-1000 mM<sup>-1</sup>s<sup>-1</sup>, the calculated  $T_1$  and  $T_2$  values were calculated using Eq. [4] and [5], and then substituted into Eq. [3]. As a reference, the  $r_1$  and  $r_2$  of SIO-3 were 4.1 and 16.4 mM<sup>-1</sup>s<sup>-1</sup>, respectively; and the  $r_1$  and  $r_2$  of SIO-20 were 8.4 and 252.1 mM<sup>-1</sup>s<sup>-1</sup>, respectively.



**Figure S1.** As synthesized IONPs with different sizes. TEM images of (a-d) hydrophobic IONPs, (e-h) after modified with oligosaccharides, and (i-l) the corresponding size distribution.



**Figure S2.** Hydrodynamic sizes of SIO-3, 5, 10, 15 and 20 measured by dynamic light scattering (DLS).



**Figure S3.** Representative photos for the SIO-3, 5, 10, 15 and 20 (from left to right) aqueous solutions after 2 months storage. All solutions were at the concentration of 10 mM Fe.



**Figure S4.** Photos of product solution after reacted in different solvent at bright field (a) and under UV light (b). From left to right, the solvent was octadecene (ODE), diethylene glycol (DEG), dimethyl sulfoxide (DMSO), and dimethylformamide (DMF).



**Figure S5.** Complex magnetic susceptibility spectra of SIO3, SIO10 and SIO20 recorded at 298K. The real part of complex susceptibility  $\chi'$  for SIO3 is 0.0004 at the frequency 1000 Hz, while  $\chi'$  is 0.013 and 0.055 for SIO10 and SIO20, respectively. The result suggested that SIO20 has the highest sensitivity to an external magnetic field, which is responsible for its highest transverse relaxivity  $r_2$  measured by MRI.



**Figure S6.** MTT based cytotoxicity assay for SIO3 after incubation of 24 h with RAW264.7, showing that they are non-toxic at substantial high dose as to 200  $\mu$ g/mL (no significant difference to control cells).



**Figure S7.** Chemical and histological analysis of biodistribution of SIO nanoparticles in mouse major organs. Iron concentration in (a) liver and (b) spleen determined by phenathroline-colorimetric method (n = 3). Prussian blue staining of the liver (c-e) and spleen (f-h) sections collected 3 weeks after administered SIO-3 (c, f), SIO-20 (d, g) and SHP20 (e, h), respectively.

## **References:**

1. O. M. Girard, J. Du, L. Agemy, K. N. Sugahara, V. R. Kotamraju, E. Ruoslahti, G. M. Bydder and R. F. Mattrey, *Magn. Reson. Med.*, 2011, **65**, 1649-1660.