

## Supplementary Information

# Fluorinated PEG-PEI Coated Magnetic Nanoparticles for siRNA Delivery and CXCR4 Knockdown

Yixiang Cao <sup>1</sup>, Shiyin Zhang <sup>2</sup>, Ming Ma <sup>1,\*</sup> and Yu Zhang <sup>1,\*</sup>

<sup>1</sup> State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Sciences and Medical Engineering, Southeast University, Nanjing 210096, China; 220195143@seu.edu.cn

<sup>2</sup> Nanjing Nanoeast Biotech Co., Ltd., Nanjing 211000, China; [syzhang@nanoeast.net](mailto:syzhang@nanoeast.net)

\* Correspondence: maming@seu.edu.cn (M.M.); zhangyu@seu.edu.cn (Y.Z.)

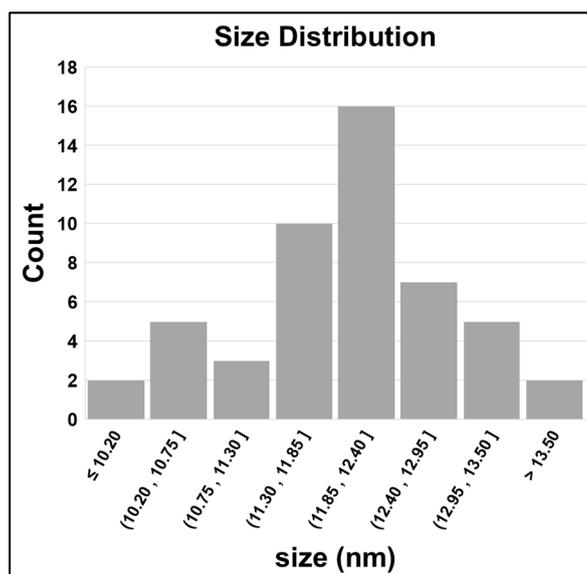
## Supplementary Tables and Figures

**Table S1.** Relative Content of C, O, F Elements in F<sub>7</sub>-PEG-COOH measured by EDS

Elements	Percentage of weight	Percentage of atoms
C	57.62%	64.94%
O	36.44%	30.83%
F	5.94%	4.23%

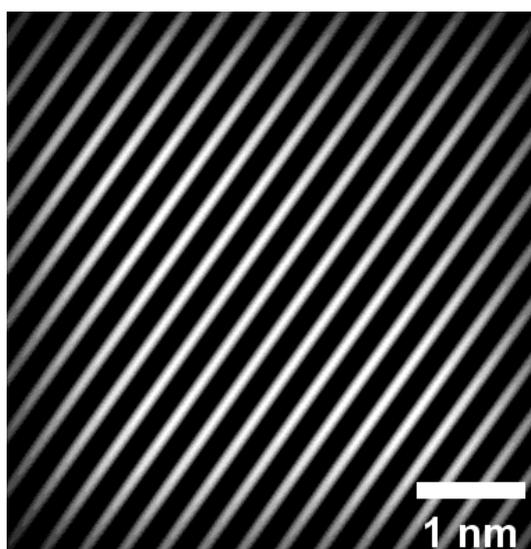
**Table S2.** Lattice spacing measured by HRTEM and SAED. The results were compared with the Standard lattice spacing of each plane (According to MDI Jade)

Planes (Fe <sub>3</sub> O <sub>4</sub> )	Standard <i>d</i> (nm)	SAED <i>d</i> (nm)	HRTEM <i>d</i> (nm)
(111)	0.467	0.439	
(220)	0.286	0.297	0.295
(311)	0.244	0.254	
(222)	0.234		
(400)	0.202	0.215	
(422)	0.165	0.168	
(511)	0.156	0.154	
(440)	0.143		
(533)	0.123		

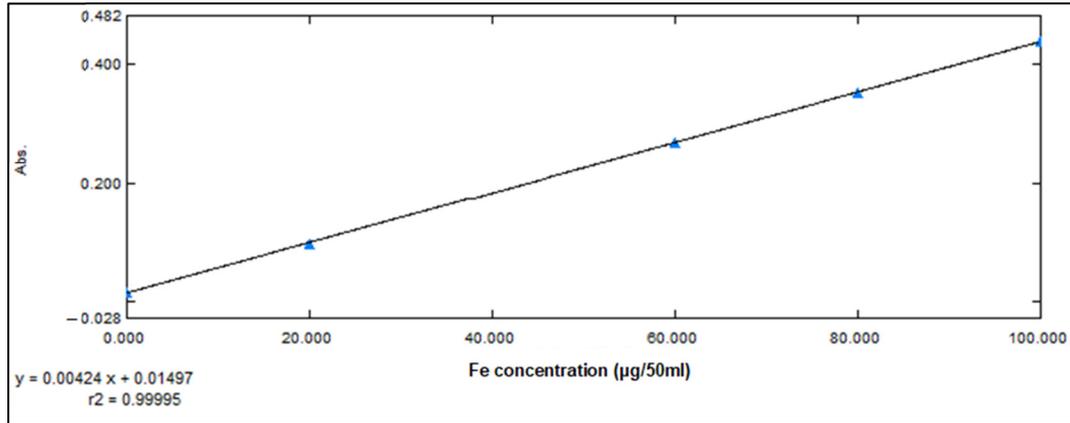


**Figure S1.** TEM size distribution of MNPs.

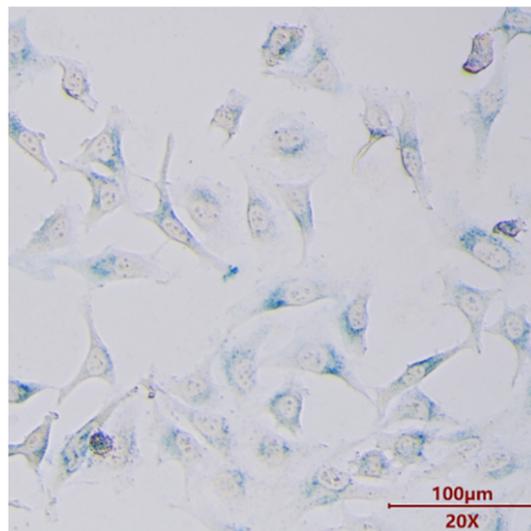
The mean size of prepared Fe<sub>3</sub>O<sub>4</sub> nanoparticles is 11.92 ± 0.94 nm



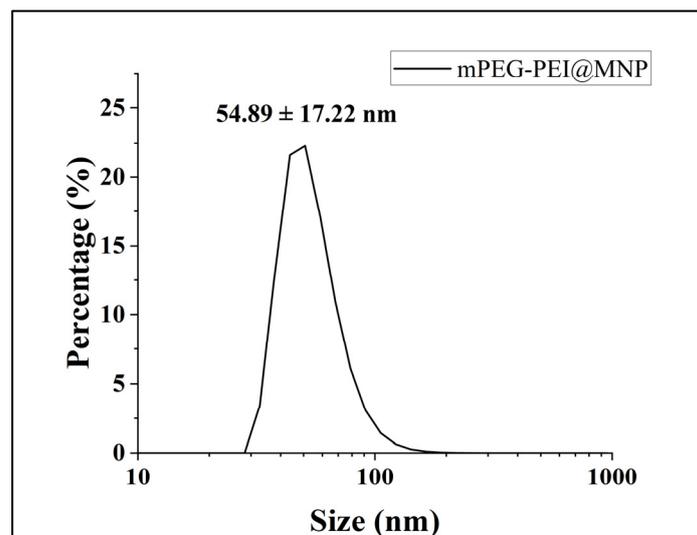
**Figure S2.** Filtered HRTEM image for measuring lattice spacing of Fe<sub>3</sub>O<sub>4</sub> NPs



**Figure S3.** Standard curve for measuring Fe concentration



**Figure S4.** Fe<sup>3+</sup> distribution in HeLa cells measured by Prussian blue Staining



**Figure S5.** DLS distribution of mPEG-PEI@MNPs

## Supplementary Methods

### Method S1. Preparation of mPEG-PEI@MNPs (mPP@MNPs):

- (1) The suspension of DMSA@MNPs (containing 5 mg of Fe), and 20 mg of methoxy-polyethylene glycol-polyethyleneimine (mPEG-PEI, Mw 2000–25000, Provided by Xi'an Ruixi Co. Ltd.) were mixed in 40 ml of pure water.
- (2) The above mixture was reacted under electric stirring at 500 rpm with 100 W ultrasonic vibration for 1 h.
- (3) Removal of excess mPEG-PEI: after step (2), the products were then purified by ultrafiltration (100 kDa NMWL) for 6 times, and kept in aqueous suspension. The DLS distribution of mPP@MNPs is shown in Figure S5.

### Method S2. Measurement of Fe concentration by UV-Vis Spectrophotometry:

- (1) 50  $\mu$ L of the sample was added into 2 mL of hydrochloric acid (6 mol/L), and 100 W ultrasonic vibration for 30 min was used.
- (2) 1 mL of hydroxylamine hydrochloride solution ( $\text{HONH}_2\text{Cl}$ , mass fraction 10%) was added, and then 100W ultrasonic vibration for 10 min was used.
- (3) 2 mL of 1,10-phenanthroline solution (mass fraction 0.1%) was added and mixed well.
- (4) 2 mL of NaOH solution (6 mol/L) was added and the solution was shaken quickly.
- (5) 5 mL of pH 5.0 HAc-NaAc buffer (17 g of NaAc with 15 mL of glacial acetic acid, made up to 62.5 mL) was added. The solution was shaken well and placed for 30 min.
- (6) The solution to be measured was made up to 50 mL with ultrapure water.
- (7) Baseline and Background: Ultrapure water was taken into a quartz cuvette as the background for baseline scanning (520 nm–500 nm), and the absorbance measured at the wavelength of 510 nm was set as 0 value.
- (8) Absorbance: The solution to be measured was added into a quartz cuvette, and the absorbance was measured at the wavelength of 510 nm.
- (9) The Fe concentration was determined according to the standard curve.

### Method S3. Prussian blue staining:

- (1) Cells were fixed with 4% paraformaldehyde solution for 30 min at 4 °C.
- (2) Staining solution preparation: 1M potassium ferrocyanide solution was mixed with 1 M hydrochloric acid in a 1:1 ratio.
- (3) Paraformaldehyde solution was removed, and the cells were rinsed for 3 times with PBS buffer.
- (4) Staining solution was added to the cells, and the cells were placed at room temperature for 30 min–60 min.
- (5) Prussian Blue Staining Solution was removed, and the cells were rinsed for 3 times with PBS buffer.
- (6) Nuclear Fast Red Staining Solution was used at room temperature for 3 min.
- (7) Nuclear Fast Red Staining Solution was removed, and the cells were rinsed for 3 times with PBS buffer.
- (8) Cells were observed under a light microscope.