Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Design and Investigation of Targeting Agent Orientation and Density on Nanoparticles for Enhancing Cellular Uptake Efficiency

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Calculation of ligand density

The average number of ligands binding on the nanoparticle was calculated by a mathematical method. The ligand density (D_{ligand}, ligands per nm^2) on the nanoparticle surface could be obtained by the following equation:

$$M_{NP} = \frac{4\pi r^3 \rho}{3w}$$

$$S_{NP} = 4\pi R^2$$

$$D_{ligand} = \frac{M_{NP} \times Q \times N_A}{S_{NP} \times M} = \frac{r^3 \rho Q N_A}{3wMR^2}$$

Where, M_{NP} is the mass of one MSP-AOPB nanoparticle, r is the radius of Fe_3O_4 core measured by TEM, ρ is the density of Fe_3O_4, w is the mass fraction of Fe_3O_4 in the MSP-AOPB nanoparticle, S_{NP} is the surface area of one MSP-AOPB nanoparticle, R is the radius of MSP-AOPB nanoparticle measured by DLS, Q is the transferrin (Tf) binding amount on the MSP-AOPB nanoparticle, N_A is the Avogadro constant, M is the molecular weight of Tf.

Parameter	Value
r	120 nm
R	287 nm
ρ	5.18 g/cm^3
w	49.5%
N_A	6.02×10^{23}
M	79 kDa

Table S1 Hydrodynamic diameters, PDI and zeta potentials of MSP, MSP-PGMA, MSP-IDA and MSP-AOPB.

Sample	D_h (nm)	PDI	Zeta-potential (mV)
MSP	313	0.187	-22.3 ± 0.5
MSP-PGMA	457	0.057	-22.8 ± 0.4
MSP-IDA	512	0.015	-45.8 ± 0.9
MSP-AOPB	575	0.045	-36.5 ± 0.7

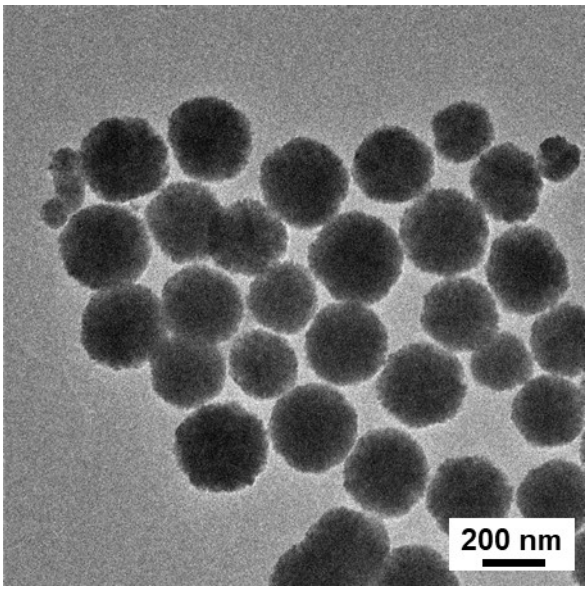

Figure S1 TEM image of MSP nanoparticles.

(a) (b)

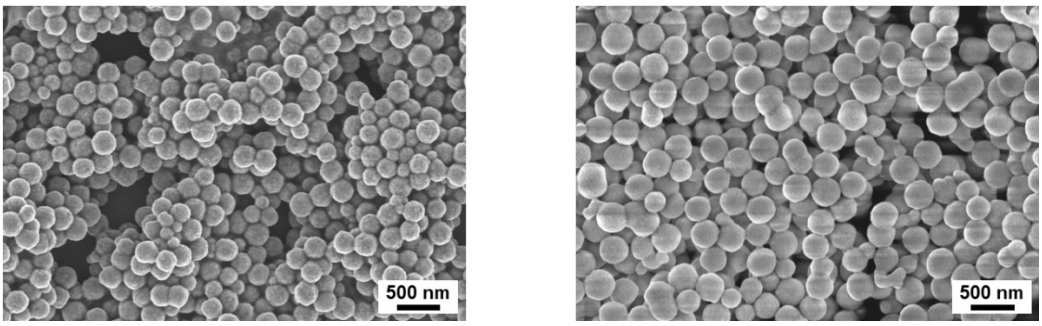

Figure S2 SEM images of (a) MSP and (b) MSP-AOPB nanoparticles.

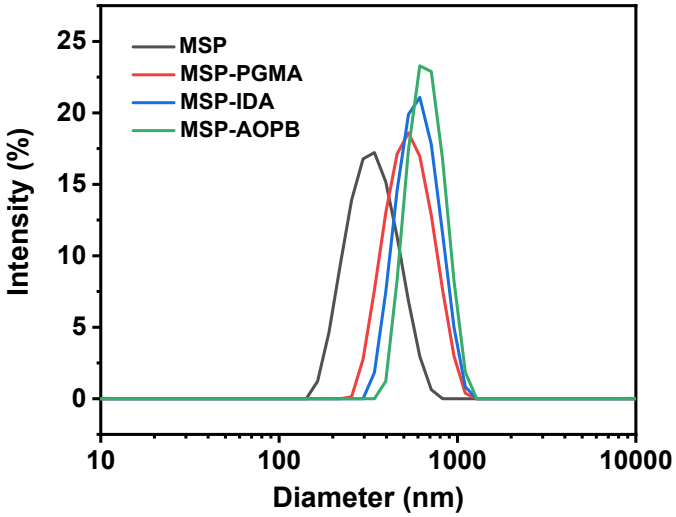

Figure S3 DLS plots of MSP, MSP-PGMA, MSP-IDA and MSP-AOPB dispersions.

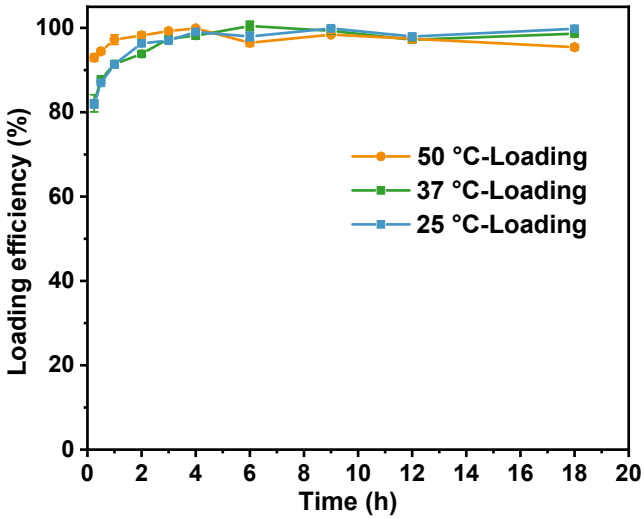

Figure S4 Loading efficiency of Tf on MSP-AOPB NPs at different temperatures and time. Results are presented as mean ± SD, n = 3 per group.

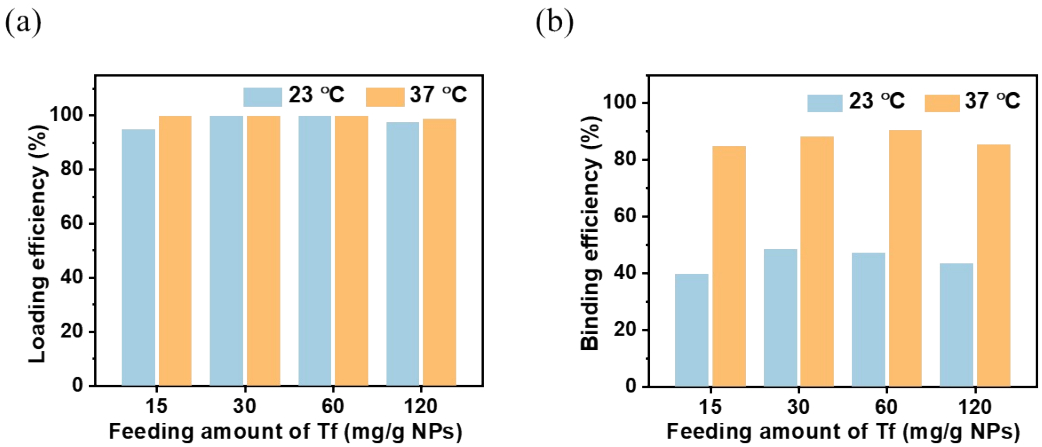

Figure S5 (a) Loading efficiency and (b) binding efficiency of Tf on MSP-AOPB NPs at different initial feeding amounts of Tf and temperatures.

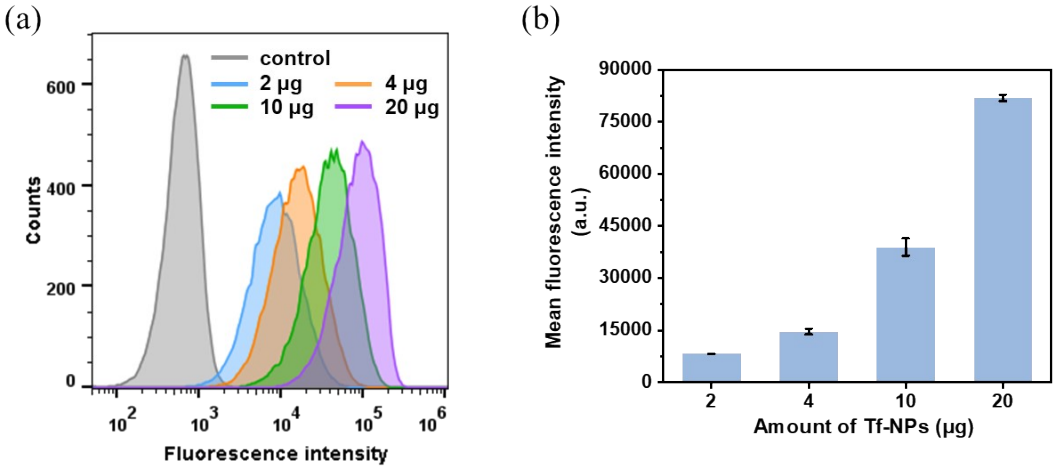

Figure S6 Cellular uptake of Tf-MSP-AOPB (abbreviated as Tf-NPs) determined by flow cytometry. (a) Fluorescence intensity of Tf-NPs in HepG2 cells. The amount of Tf-NPs incubated with HepG2 cells was 2 μg, 4 μg, 10 μg and 20 μg. (b) Quantitative fluorescence intensity analysis of panel (a). Results are presented as mean ± SD, n = 3 per group.

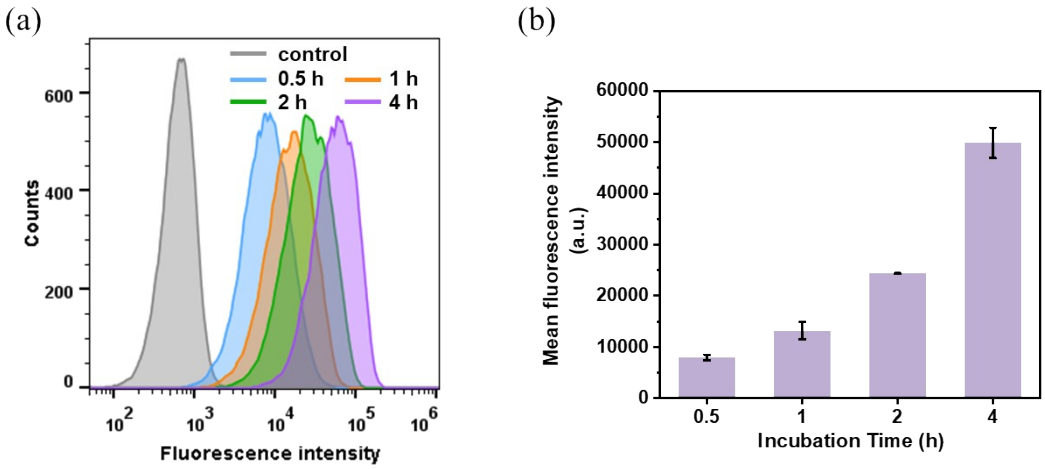

Figure S7 Cellular uptake of Tf-MSP-AOPB (abbreviated as Tf-NPs) determined by flow cytometry. (a) Fluorescence intensity of Tf-NPs in HepG2 cells. Incubation time for Tf-NPs and HepG2 cells was 0.5 h, 1 h, 2 h and 4 h. (b) Quantitative fluorescence intensity analysis of panel (a). Results are presented as mean ± SD, n = 3 per group.

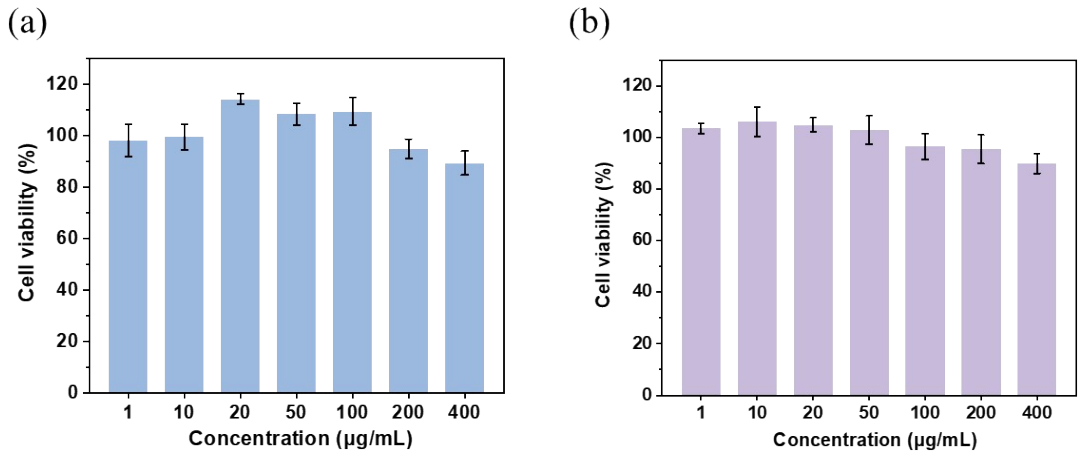

Figure S8 Cell viability of MSP-AOPB NPs to (a) HEK 293T cells and (b) HepG2 cells after incubation for 24 h. Results are presented as mean ± SD, n = 6 per group.