Supporting Information

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

Weiwei Fei, Xiuli Wang, Jia Guo, and Changchun Wang*

State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, and Laboratory of Advanced Materials, Fudan University, Shanghai 200433, P. R. China.

*Corresponding author: ccwang@fudan.edu.cn (C.C. Wang)
**Calculation of ligand density**

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

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

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

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

Where, $M_{\text{NP}}$ is the mass of one MSP-AOPB nanoparticle, $r$ is the radius of Fe$_3$O$_4$ core measured by TEM, $\rho$ is the density of Fe$_3$O$_4$, $w$ is the mass fraction of Fe$_3$O$_4$ in the MSP-AOPB nanoparticle, $S_{\text{NP}}$ 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>$r$</td>
<td>120 nm</td>
</tr>
<tr>
<td>$R$</td>
<td>287 nm</td>
</tr>
<tr>
<td>$\rho$</td>
<td>5.18 g/cm$^3$</td>
</tr>
<tr>
<td>$w$</td>
<td>49.5%</td>
</tr>
<tr>
<td>$N_A$</td>
<td>$6.02 \times 10^{23}$</td>
</tr>
<tr>
<td>$M$</td>
<td>79 kDa</td>
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Table S1 Hydrodynamic diameters, PDI and zeta potentials of MSP, MSP-PGMA, MSP-IDA and MSP-AOPB.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$D_h$ (nm)</th>
<th>PDI</th>
<th>Zeta-potential (mV)</th>
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<tbody>
<tr>
<td>MSP</td>
<td>313</td>
<td>0.187</td>
<td>-22.3 ± 0.5</td>
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<tr>
<td>MSP-PGMA</td>
<td>457</td>
<td>0.057</td>
<td>-22.8 ± 0.4</td>
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<tr>
<td>MSP-IDA</td>
<td>512</td>
<td>0.015</td>
<td>-45.8 ± 0.9</td>
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<tr>
<td>MSP-AOPB</td>
<td>575</td>
<td>0.045</td>
<td>-36.5 ± 0.7</td>
</tr>
</tbody>
</table>
**Figure S1** TEM image of MSP nanoparticles.

**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.