Supporting Information

**Eumelanin-Fe₃O₄ hybrid Nanoparticles for Enhanced MR/PA Imaging - assisted Local Photothermolysis**

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†Electronic Supplementary Information (ESI) available.

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Figure S1. Photograph of precipitated pristine Fe$_3$O$_4$ NPs, as-prepared euMel-Fe$_3$O$_4$ NPs in aqueous solution after standing at room temperature for 24 h, and magnetic attraction for euMel-Fe$_3$O$_4$ NPs collection.

Table 1. Energy-dispersive X-ray (EDX) spectroscopy of euMel-Fe$_3$O$_4$ NPs.

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight %</th>
<th>Atomic %</th>
<th>Uncert. %</th>
<th>Correction</th>
<th>k-Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>O (K)</td>
<td>59.23</td>
<td>83.53</td>
<td>0.82</td>
<td>0.51</td>
<td>1.889</td>
</tr>
<tr>
<td>Fe (K)</td>
<td>40.76</td>
<td>16.46</td>
<td>0.51</td>
<td>0.99</td>
<td>1.401</td>
</tr>
</tbody>
</table>

Table 2. Relative tumor volume changes after first day treatment by euMel-Fe$_3$O$_4$ NPs.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>NPs only</th>
<th>Laser only</th>
<th>NPs+Laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 SD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.29558</td>
<td>0.18036</td>
<td>1.44259</td>
<td>1.31351</td>
</tr>
<tr>
<td>5</td>
<td>1.73621</td>
<td>0.00264</td>
<td>2.166</td>
<td>0.18211</td>
</tr>
<tr>
<td>7</td>
<td>2.35275</td>
<td>0.00809</td>
<td>2.48301</td>
<td>0.0347</td>
</tr>
<tr>
<td>9</td>
<td>2.78191</td>
<td>0.20528</td>
<td>2.75106</td>
<td>0.35605</td>
</tr>
<tr>
<td>11</td>
<td>3.48114</td>
<td>0.29323</td>
<td>3.17993</td>
<td>0.25547</td>
</tr>
<tr>
<td>13</td>
<td>3.82852</td>
<td>0.45987</td>
<td>3.65228</td>
<td>0.35834</td>
</tr>
<tr>
<td>15</td>
<td>4.32365</td>
<td>0.54282</td>
<td>4.21164</td>
<td>0.1536</td>
</tr>
</tbody>
</table>
Figure S2. EDX spectrum of euMel-Fe₃O₄ NPs.

Figure S3. XPS wide scans of euMel-Fe₃O₄ NPs.
Figure S4. Fe2p\textsubscript{1/2} and Fe2p\textsubscript{3/2} spectra of euMel-Fe\textsubscript{3}O\textsubscript{4} NPs.

Figure S5. C1s spectrum of euMel-Fe\textsubscript{3}O\textsubscript{4} NPs.
XPS analysis (Figure S4) showed two further peaks at 710.7 and 724.45 eV, attributed to Fe\(2p_{3/2}\) and Fe\(2p_{1/2}\), respectively, which is related to the magnetite in agreement with the XRD and literature results. Furthermore, the O1s spectrum (Figure S7) showed a peak with a binding
energy close to 529.95 eV, which is assigned to the Fe-O binding in Fe$_3$O$_4$. Besides, with respect to the carbon peak positions (C1s), a peak with a binding energy of 284.7 eV assigned to Fe-C was observed (Figure S5), which has the same value as a graphitic structure (aromatic ring).

Figure S8. Infrared thermal images of euMel-Fe$_3$O$_4$ NP aqueous solution (10 mM Fe) exposed to 808 nm laser with different power densities.

Figure S9. Temperature elevation of euMel-Fe$_3$O$_4$ NP aqueous solution (10 mM Fe) exposed to 808 nm laser with different power densities.
Figure S10. Plot of temperature change (ΔT) over a period of 5 min versus power densities.

Figure S11. The linearity of PA signal intensities under 800 nm laser irradiation as a function of the concentration of euMel-Fe₃O₄ NPs.
**Figure S12.** TEM image of euMel-Fe₃O₄ NPs and several commercially available magnetic nanoparticles at equivalent iron molar concentration (10 mM).

**Figure S13.** Representative photographs of U-87 MG tumor-bearing mice from different groups at day 1 before treatment and at day 15 after treatment.
**Figure S14.** Photothermal heating curves of euMel-Fe$_3$O$_4$ NPs (10 mM Fe) irradiated by 808 nm NIR laser at 2 W/cm$^2$ over one laser on/off cycle.

**Figure S15.** Photothermal heating curves of euMel-Fe$_3$O$_4$ NPs (10 mM Fe) irradiated by 808 nm laser at 2 W/cm$^2$ over five laser on/off cycles (on: 5 min, off: 6 min).
Figure S16. Linear time data versus $-\ln\theta$ obtained from the cooling period. Time constant ($\tau_s$) for heat transfer was determined to be 136.5 s.

Figure S17. Standard absorbance curve of eumelanin solution at 500 nm.
**Figure S18.** Standard absorbance curve of iron content at 510 nm. There were about 0.26 mg eumelanin for per mg Fe.