Electronic Supplementary Information

An EGFRvIII targeted dual-modal gold nanoprobe for imaging-guided brain tumor surgery
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Figure S1. Fourier transform infrared spectroscopy (FTIR) of AuP-FAL and its intermediates. The strong peak at 1720 cm\(^{-1}\) was attributed to the C=O stretching in the DTPA chelators, scissor-like peaks at 1044 and 1193 cm\(^{-1}\) were assigned to the S=O stretching in the IR783B, a broad peak at 2800–2960 cm\(^{-1}\) was attributed to C–H stretching in PEGs, peaks at 1650 and 1550 cm\(^{-1}\) were assigned to typical amide bending in FAL peptides.

Figure S2. Characterization of AuP-PEG. (a) TEM image demonstrated the morphology of AuP-PEG with an average diameter about 60 nm. Insert: amplified image presenting the semi-transparent PEG coating. (b) AuP-PEG showed a hydrodynamic size of 60.7 nm and zeta potential of -21.1 mV.
Figure S3. Absorbance and Raman spectra of AuP-FAL (a) and AuP-PEG (b).

Figure S4. T1-weighted magnetic resonance phantoms (upper panel) and T1 maps (lower panel) of AuP-FAL (a) and AuP-PEG (b) in PBS.
Figure S5. Cellular uptake of nanoprobe in U87-EGFRvIII cell culture. (a) Confocal fluorescence microscopic images of the U87-EGFRvIII cells treated with 0.5 nM AuP-PEG or AuP-FAL for 2 h or 24 h. In the receptor competitive study, U87-EGFRvIII cells were pretreated with 200 nM FAL peptide for 30 min followed by AuP-FAL. Scale bar: 20 μm. (b) Mean intracellular fluorescence intensities (optical density per pixel) after nanoprobe treatment for 2 and 24 h at 37 °C. The values represent mean ± SD (n = 4). *p < 0.05 (Mann-Whitney U-test).

Figure S6. Cytotoxicities of AuP-PEG and AuP-FAL in human glioblastoma U87-EGFRvIII cell line (A) and mouse brain capillary endothelial bEnd.3 cell line (B). Cells were treated with nanoprobe for 24 h with final concentrations in a range of 0.016–50 nM. The CCK8 assay was applied to measure the cytotoxicity.
Figure S7. Bio-distribution of AuP-PEG or AuP-FAL in mice bearing 87-EGFRvIII tumor xenograft at 24 h post-injection. (A) *Ex vivo* fluorescence images of the excised mouse organs at 24 h post-injection of the nanoprobe. (B) Bio-distribution of the nanoprobe labeled with near-infrared fluorescent dye IR783B in tumor-bearing mice at 24 h post-injection. The values represent mean ± SD (n = 3).

Figure S8. Average number of nanoprobe distributed in a selected area of tumor periphery and normal brain tissue. Above data were calculated from TEM images. All data are presented as mean ± SD. *, $P < 0.05$. 
Figure S9. H&E staining of major organs (heart, liver, spleen, lung, kidney, brain) from healthy mice treated with PBS or AuP-FAL. The organs were harvested at 1 day or 7 days after intravenous injection, and then sectioned for histological staining. No obvious lesions were observed. Scale bar = 50 μm.

Table S1. Physical parameters of the gold nanoprobes.

<table>
<thead>
<tr>
<th>Nanoprobe</th>
<th>d (nm)a</th>
<th>PDI a</th>
<th>ζa</th>
<th>λabsb (nm)</th>
<th>Molar ratios c</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuP-FAL</td>
<td>61.2</td>
<td>0.178</td>
<td>-23.3</td>
<td>535</td>
<td>1/10780/1257/36,152/32</td>
</tr>
<tr>
<td>AuP-PEG</td>
<td>60.7</td>
<td>0.195</td>
<td>-21.1</td>
<td>535</td>
<td>1/10147/1308/37,916/N.A.</td>
</tr>
</tbody>
</table>

Diameters (d), polydispersity index (PDI) and zeta potentials (ζ) were measured by dynamic light scattering (DLS). Maximal absorption wavelength. The molar ratios of gold nanoparticle/Gd³⁺/IR783B/PEG/FAL peptide in the nanoprobe. Number of Gd³⁺ ions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The average number of IR783B, PEG or FAL peptide labeled on the nanoprobe was quantified by gravimetric analysis. N.A. means Not Applicable.