

Supplemental information

Glypican-3 (GPC3) Targeted Fe₃O₄ Core/Au Shell Nanocomplex for Fluorescence/MRI/Photoacoustic Imaging-Guided Tumor Photothermal Therapy

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Determination of photothermal conversion efficiency

The photothermal conversion efficiency (η) of FANP was measured according to the literature^{1, 2}. The aqueous solution of the FANP was under continuous irradiation of the laser (630 nm, 1 W/cm²) until a steady state temperature was reached. Subsequently, the laser was shut off, and the temperature decrease of the aqueous solution was recorded to measure the rate of heat transfer from the FANP solution system to the environment. The η value was calculated as follows:

$$\eta = hs(T_{\max} - T_{\text{surr}}) - Q_{\text{Dis}} / I(1 - 10^{-A_{630}})$$

where h is the heat transfer coefficient, s is the surface area of the container, and the value of hs was gained from Figure S6 ($t = 123.2 \ln(\theta) - 7.47$). and τ_s was calculated as 120 s. $T_{\max} - T_{\text{surr}}$ is the temperature change of the FANP solution at the maximum steady (56.37°C)-environmental temperature (25°C), I is the power of the laser (1 W/cm²), A_{630} is the absorbance of FANP at 630 nm (1.7631), and Q_{Dis} expresses heat dissipated from light absorbed by the solvent and the container (19.3).

Serum half-life of GBP-FANP in mice

To determine in vivo nanoparticle kinetics of GBP-FANP in mice, iron concentrations were measured. Briefly, seven weeks old nude mice ($n = 3/\text{group}$) were injected intravenously with GBP-FANP at 10 mg/kg of equivalent dose of Fe in H₂O. At different time points, (0.1, 1, 2, 4, 24, 48 and 72 hours) after injection, these mice were sacrificed. Blood was collected by terminal heart puncture and then centrifuged for 10 minutes at 5,000 rpm to separate the plasma. To track the iron concentrations, 100 μL of such serum samples were incubated in 1 mL of nitric acid. Iron concentrations in mouse blood as well as in the IONP solution were determined by colorimetric analysis using 1,10-phenanthroline. A calibration curve was created using standard solutions containing the iron/1,10-phenanthroline complex in water with gradient iron concentrations

ranging from 0.1 $\mu\text{g/mL}$ to 5 $\mu\text{g/mL}$. Absorption spectra were obtained using a scanning UV-VIS spectrophotometer. Blood circulation time was defined as the time over which the GBP-FANP level reduced to less than 5%ID/g in blood^{3,4}. It is believed that the prolonging circulation in blood provides possibility of GBP-FANP accumulation in tumors and therefore improves the therapeutic efficiency.

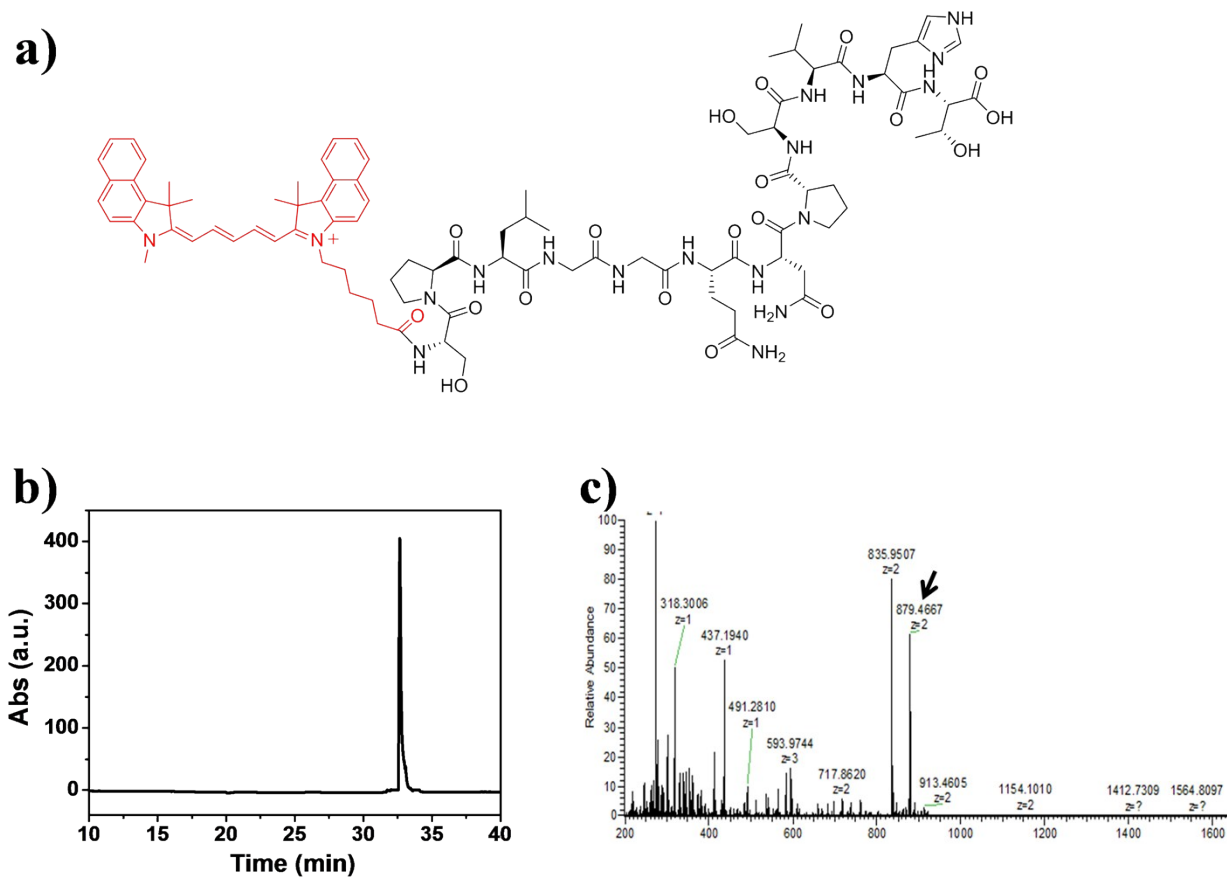
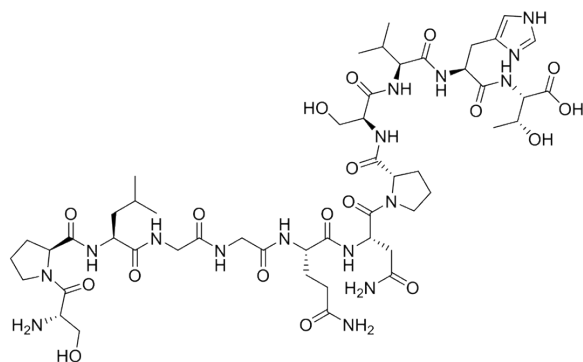
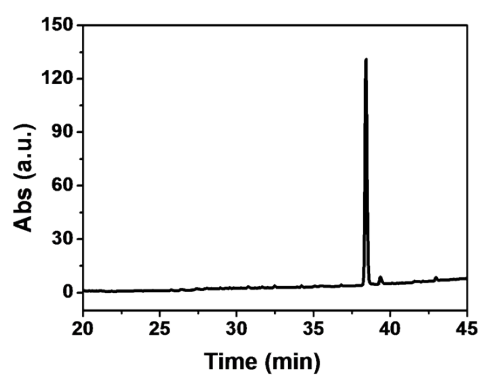


Figure S1. a) Chemical structure of Cy5.5 conjugated GBP. **b)** HPLC analysis of Cy5.5-GBP. The retention time of peptide is 32.66 min. **c)** Mass spectrum analysis of Cy5.5-GBP.

a)



b)



c)

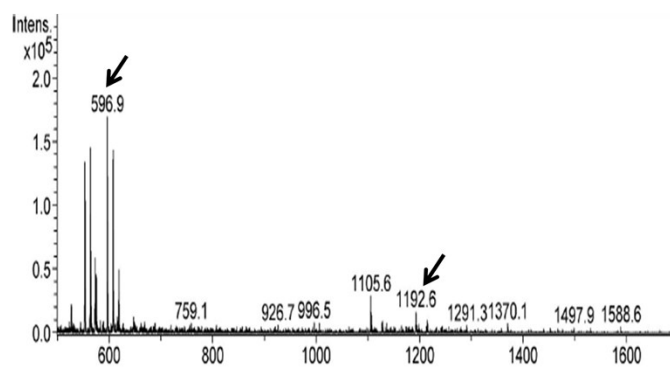
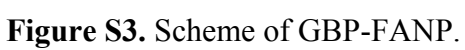


Figure S2. a) Chemical structure of GPC3 binding peptide, GBP. **b)** HPLC analysis of GBP. The retention time of peptide is 38.43 min. **c)** Mass spectrum analysis of GPC3 binding peptide.



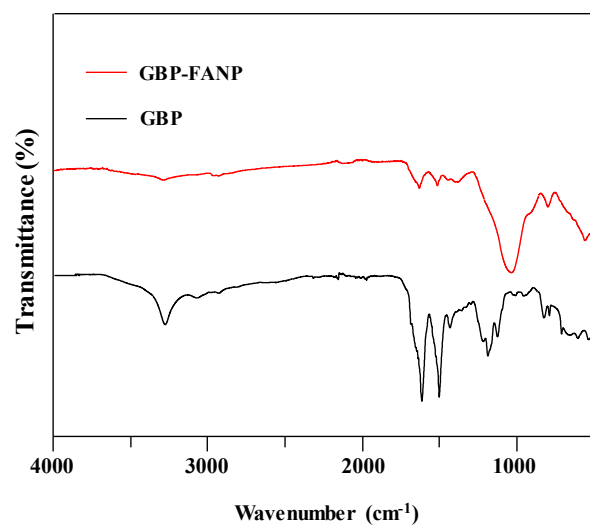


Figure S4. Fourier Transform Infrared Spectroscopy of GBP and GBP functionalized FANP.

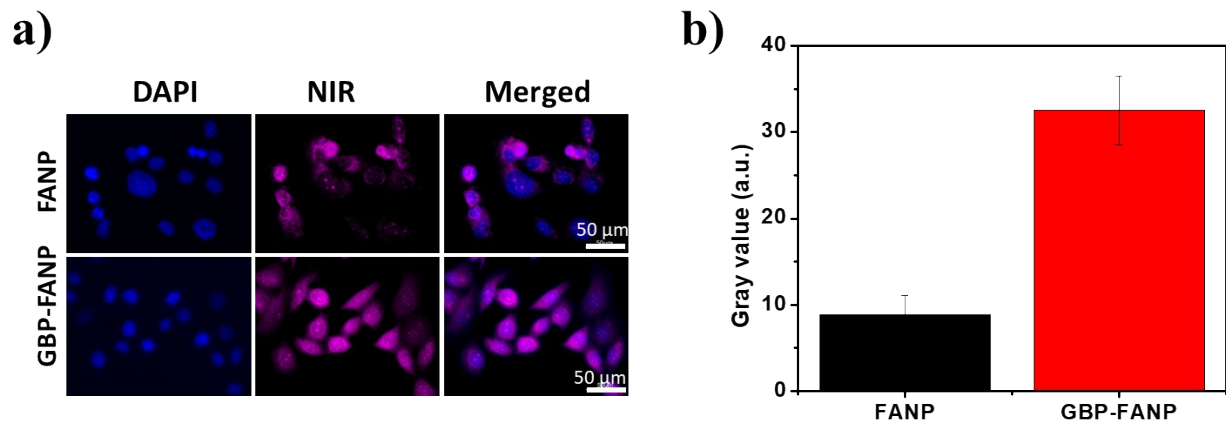


Figure S5. In vitro cellular imaging to verify GBP mediated more HepG2 cell accumulation of FANP, compared with non-targeted FANP. **a)** visualization of FANP in HepG2 cells; **b)** quantification analysis of **a)**.

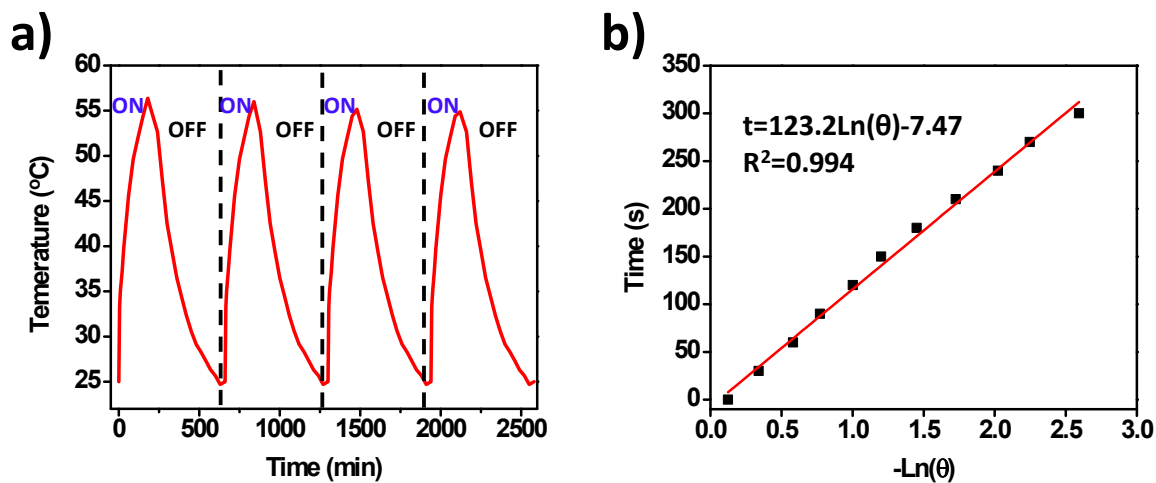


Figure S6. a) Cyclic photothermal stability tests of GBP-FANP (0.4 mg/mL) under 630 nm laser (1 W/cm²) irradiation as a function of irradiation time. **b)** Plot of time versus $-\ln(\theta)$ (θ is the driving-force temperature) from the data recorded during the cooling period of the experiment outlined in (a).

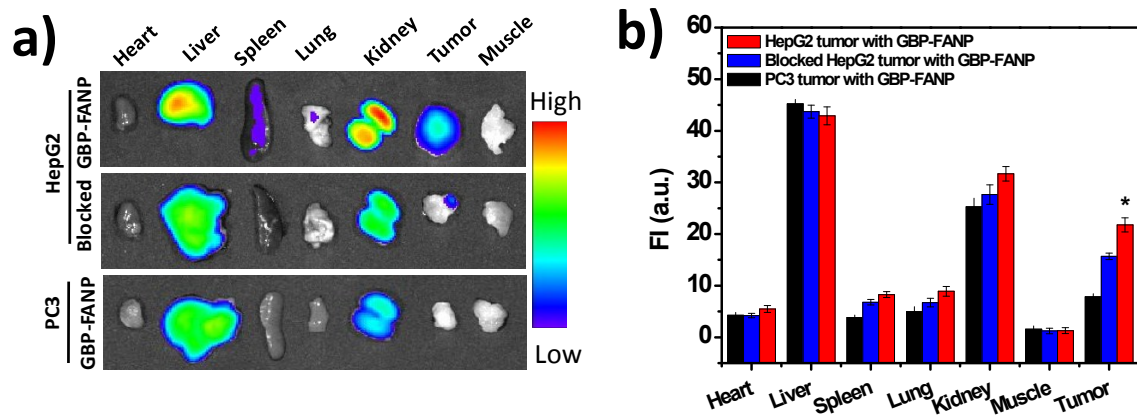


Figure S7. Biodistribution of GBP-FANP in HepG2 and PC3 tumor bearing mice model. **a)** Ex vivo imaging of GBP-FANP in tumors and normal organs. **b)** Quantification of tumors and normal organs with GBP-FANP.

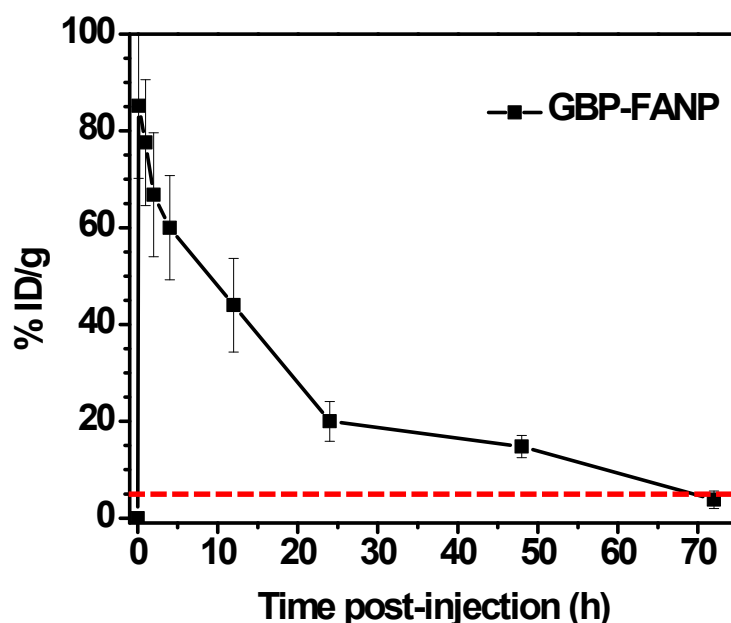


Figure S8. Serum half-life of GBP-FANP after tail vein injection of equivalent dose of 10 mg Fe/kg of mouse body weight. Iron concentration in serum was measured by spectrophotometry. The error bar is the standard deviation with three mice in each time point. Blood circulation time was defined as the time over which the GBP-FANP level reduced to less than 5%ID/g in blood.

References:

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