Supplementary Materials

A Plasmonic Gold Nanostar Theranostic Probe for *In Vivo* Tumor Imaging and Photothermal Therapy

Yang Liu[1,2,3†], Jeffrey R. Ashton[2†], Everett J. Moding[4], Hsiangkuo Yuan[1,2], Janna K. Register[1,2], Andrew M. Fales[1,2], Jaeyeon Choi[5], Melodi J. Whitley[4], Xiaoguang Zhao[5], Yi Qi[6], Yan Ma[7], Ganesan Vaidyanathan[5], Michael R. Zalutsky[5,6], David G. Kirsch[4,7], Cristian T. Badea[6*], Tuan Vo-Dinh[1,2,3*]

[1] Fitzpatrick Institute for Photonics, Duke University, Durham, NC, 27705, United States

[2] Department of Biomedical Engineering, Duke University, Durham, NC, 27705, United States

[3] Department of Chemistry, Duke University, Durham, NC, 27705, United States

[4] Department of Pharmacology and Cancer Biology, Duke University, Durham, NC, 27705, United States

[5] Department of Radiology, Duke University Medical Center, Durham, NC, 27710, United States

[6] Center for In Vivo Microscopy, Department of Radiology, Duke University Medical Center, Durham, NC 27710, United States

[7] Department of Radiation Oncology, Duke University Medical Center, Durham, NC, 27710, United States

† These authors contributed equally to this work

* Address correspondence to: tuan.vodinh@duke.edu, cristian.badea@duke.edu

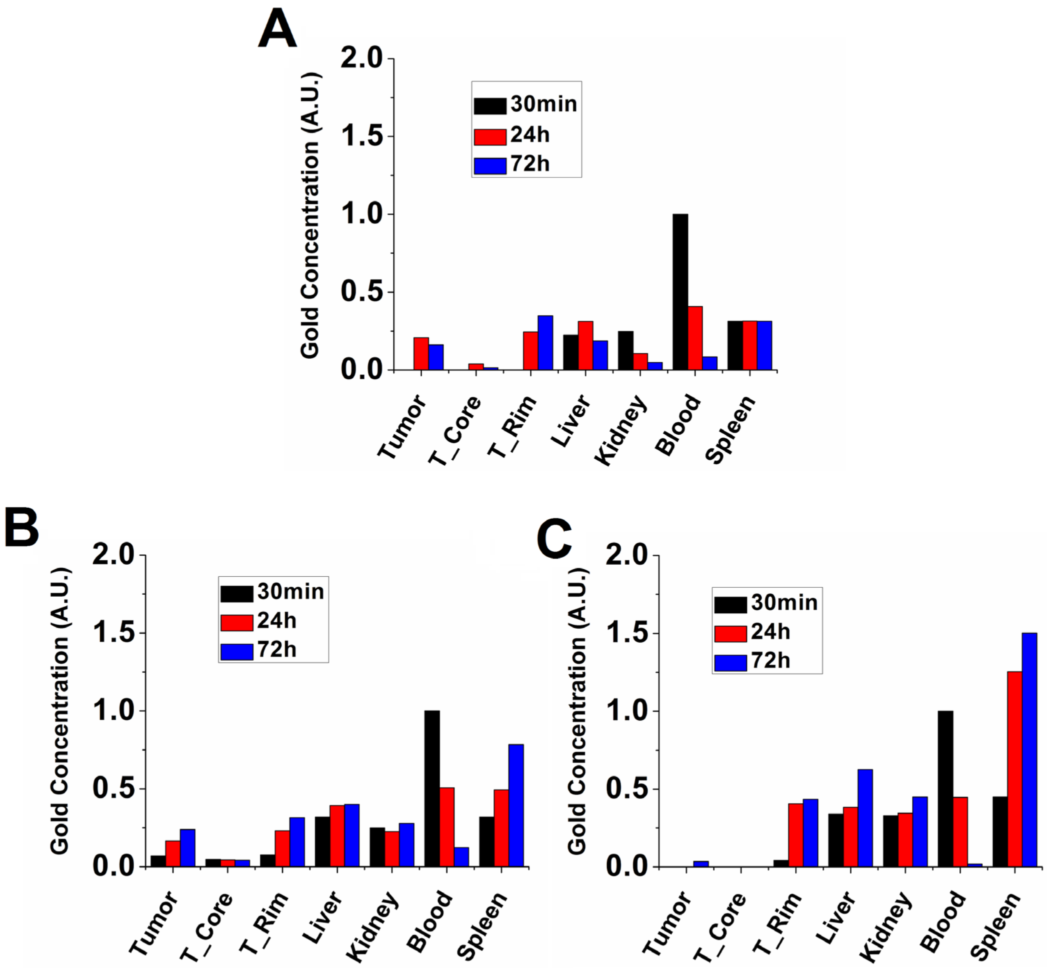

Figure S1: Dynamic biodistribution data for (A) 12-nm nanospheres, (B) 30-nm GNS, and (C) 60-nm GNS at 30 minutes, 24 hours, and 72 hours after intravenous tail vein injection based on gold measurements from dual-energy CT scans. The gold concentration is normalized to the concentration of gold in blood at 30 min. T_Core refers to the tumor core, and T_Rim refers to the tumor rim.

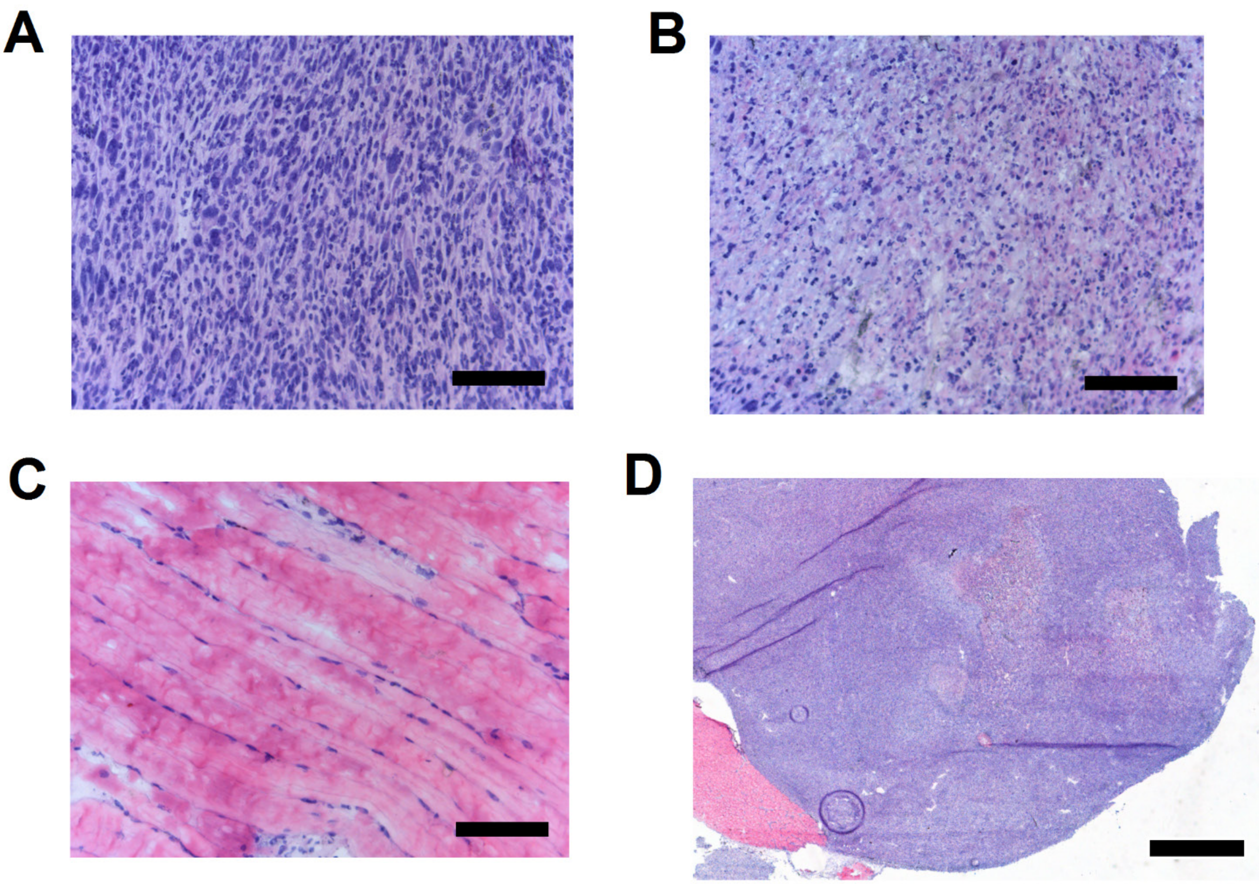

Figure S2: H&E histopathology of a primary sarcoma. (A) viable tumor (near the tumor margin); (B) necrotic tumor (in the tumor core); (C) neighboring muscle; (D) tumor slice with low magnification. Scale bar, 100 μm for A-C and 2 mm for D.

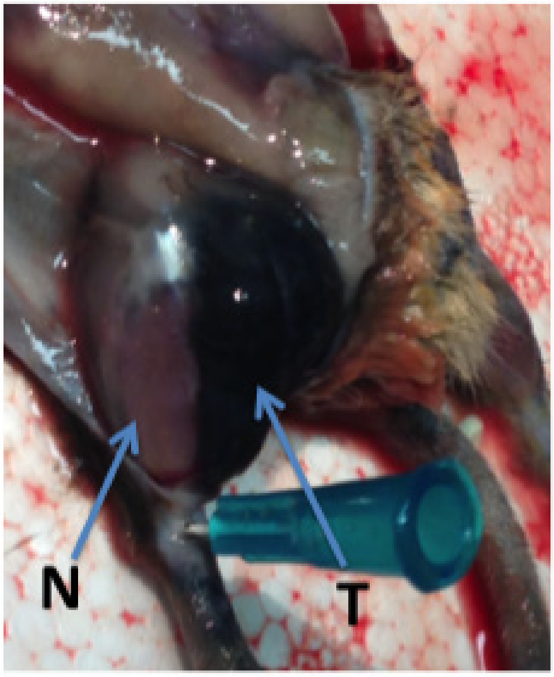

Figure S3. Mouse with a primary sarcoma 3 days after 30-nm GNS injection. After removing the overlying skin, there is a clear visible boundary between sarcoma (T) and adjacent muscle (N).

GNS Photothermal Heating Theoretical Calculations

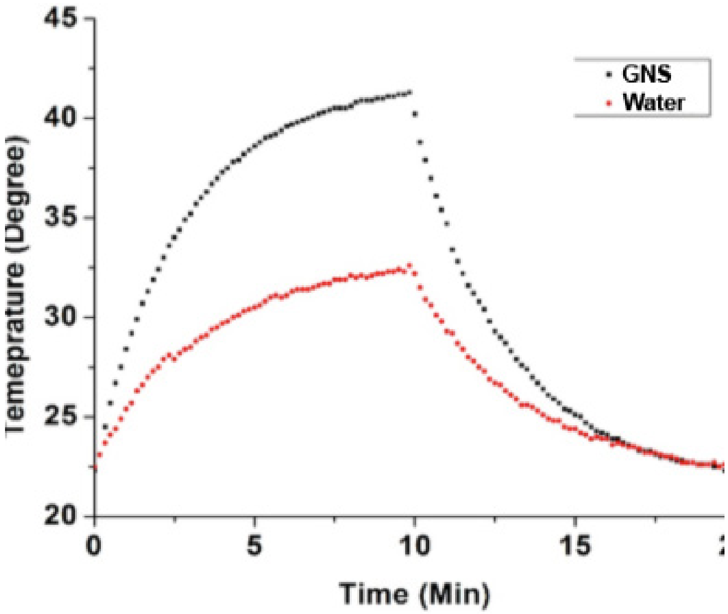

Figure S4: Temperature profile with 0.8 W/cm^2 laser irradiation. GNS, 0.8 ml of 0.2 nM 30 nm GNS solution. Water, 0.8 ml of water. Vial surface area with laser irradiation is 1.1 cm^2.

We quantitatively estimated the photothermal effect of developed 30 nm gold nanostars. In this *in vitro* test, the room temperature is measured to be 22 °C. The maximum temperature is 42 °C for 0.8 gram of 0.2 nM 30 nm GNS solution after 10 min 0.8 W/cm^2 laser irradiation, while that of pure DI water is 32 °C. After stopping laser irradiation, it takes 580 s for the GNS solution to cool from 42 °C to 22.1 °C. The heat capacity of water and gold is 4.18 J/(°C g) and 0.129 J/(°C g), respectively.

According to Newton's law of cooling,

$$-\Delta T \times m \times C = (T - T_{Enviro}) \times K \times \Delta t$$

Solving equation (1)

$$\ln(42\ °C\ - 22\ °C) - \ln(22.1\ °C\ - 22\ °C) = \frac{K \times 580s}{0.8g \times 4.18J \cdot °C^{-1}g^{-1}}$$

The constant K is calculated to be 0.0305 J/(°C s)

When the system reaches the maximum temperature, the input heat flux equals the output heat flux. As a result,

$$P_{input} = (T_{max} - T_{Enviro})) \times K \quad (2)$$

For pure water system, the total heating power input is calculated to be 0.305 W.

For GNS solution, the total heating power is calculated to be 0.71 W. As a result, the heating power input from GNS is 0.71 W–0.305 W = 0.305 W.

In the GNS solution, there is 27 μg of GNS and 0.8 g of water. We define an index, heat flux mass density (D), which is the heat flux per mass unit.

For water, the D is calculated to be 0.305W/0.8g = 0.38 W/g.

For GNS, D = 0.305 W/27 μg = 1.1×10^4 W/g.

With the assumption that there is no heat loss, we can calculate the temperature increase speed by dividing the heat flux mass density by the heat capacity.

For water, that is 0.38 W/g divided by 4.18 J/(°C·g), which equals to 0.091 °C/s.

For GNS, that is 1.1×10^4 W/g divided by 0.129 J/(°C·g), which equals to 8.5×10^4 °C/s.

GNS has much higher heat flux density and lower heat capacity than water. As a result, it can be estimated that the GNS temperature should be extremely high and water molecules near GNS would evaporate immediately under laser irradiation. The high temperature generated by GNS can be used for killing cancer cells.