Supporting Information

In vitro & *In vivo* Analysis of Indocyanine Green-Labeled Panitumumab for Optical Imaging – A Cautionary Tale

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Experimental Procedures

Reverse-phase HPLC (RP-HPLC). ICG-sulfo-OSu was analyzed by using a Beckman system Gold HPLC (Fullerton, CA) equipped with 126 solvent module and 168 UV detector (λ = 254 nm) controlled by 32 Karat software and Grace Vydac C_{18} semi-prep column (10 x 250 mm, 300 Å pore size). The flow rate was 2.5 mL/min, and the gradient mobile phase was isocratic with 90 % A (0.1 % TFA in H_2O) and 10 % B (0.1 % TFA in acetonitrile) at 0 – 5 min, followed by a gradient mobile phase going from 10 % B at 5 min to 100 % B at 15 min. The mobile phase was then isocratic with 100 % B at 15 – 25 min.

Fluorescence microscopy. HER1-positive A431 cells (2.5×10^4/well, American Type Culture Collection (ATCC), Rockville, MD) were cultured in 8-well chamber slides for 72 h in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FetalPLEX (Gemini Bio-Products, West Sacramento, CA), and 10 mM glutamine solution in a humidified atmosphere of 5% CO_2 at 37 °C. Thereafter, cells were treated with 2 μg of the HPLC-purified ICG-sOSu-panitumumab conjugates (**1**, **2**, and **3**) in 400 μL culture media, and incubated for another 24 h. ICG-sOSu-HuM195 was used as a negative control. Cells were then fixed with 4% formalin in PBS for 10 min. at room temperature. Fluorescence microscopy was performed using a Zeiss AxioObserver Z1 microscope (Carl Zeiss Microscopy, Thornwood, NY) equipped with a 63x Plan-apochromat (N.A. 1.4) oil immersion lens and the following filter set: excitation ET710/75x, dichroic T760lpxr, and emission ET810/90m (Chroma Technology Corp., Bellows Falls, VT). Images were acquired using an Axiocam MRm CCD camera operated in NIR sensitivity mode with a Zeiss Zen software. Differential interference contrast (DIC) images were also captured.

Animal model used in Figure S4. Metastatic peritoneal LS-174T colorectal cancer model was established by intraperitoneal (i.p.) injection of 1×10^8 LS-174T cells in 1 mL of the media as previously described.[1]

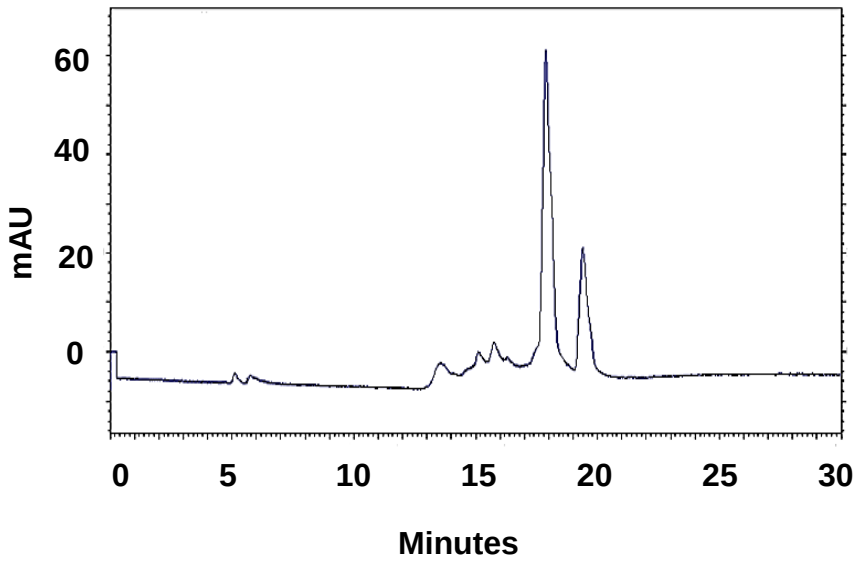

Figure S1. RP-HPLC profile of ICG-sulfo-OSu dissolved in DMSO.

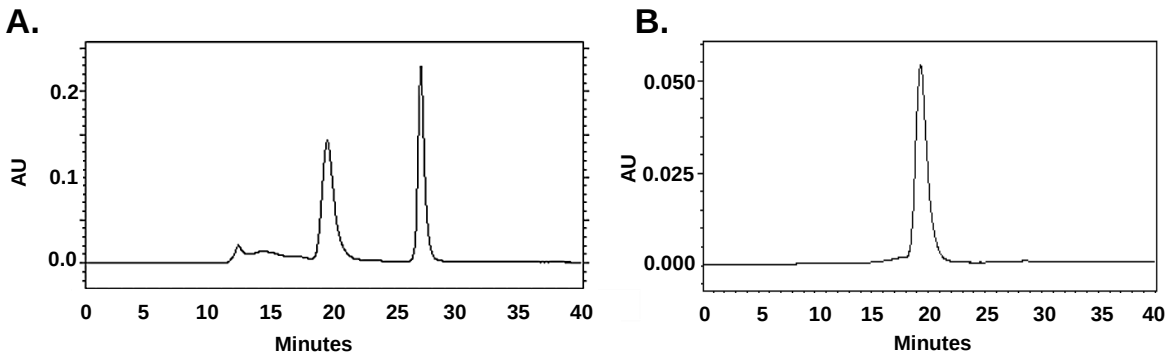

Figure S2. A. SE-HPLC profile of ICG-sOSu-HuM195 conjugation reaction mixture (**10**×). **B**. SE-HPLC profile of SE-HPLC-purified ICG-sOSu-HuM195 (**10**×).

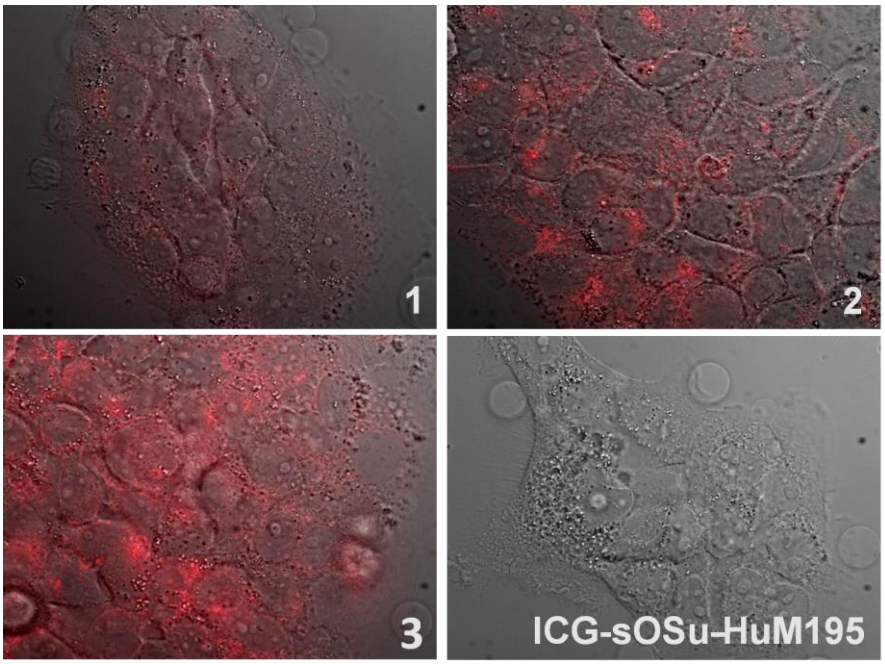

Figure S3. Representative fluorescence microscopy images, merged with DIC pictures, of SE-HPLC-purified ICG-sOSu-panitumumab (**1**, **2** and **3**) interacting with HER1-positive A431 cells. Cells were incubated with 2 μg of each bioconjugate in 400 μL culture media at 37 °C for 24 h. ICG-sOSu-HuM195 served as a negative control. Magnification: 63×.

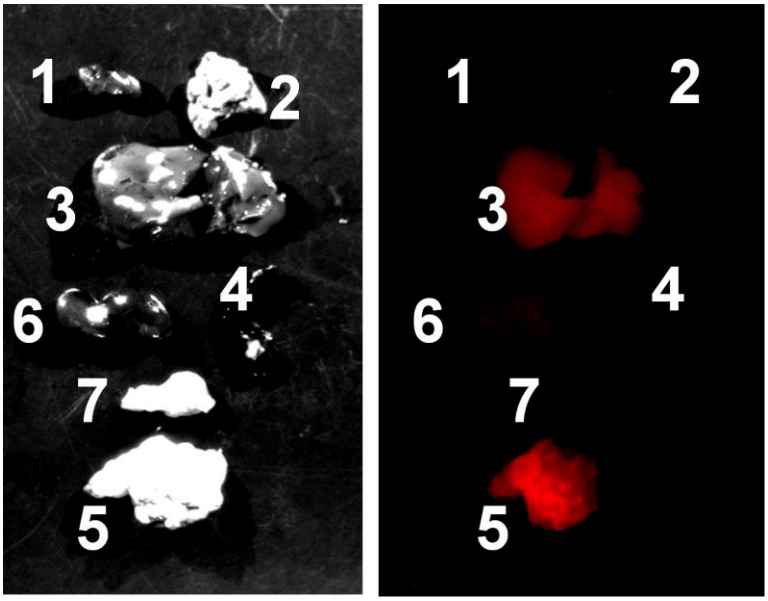

Figure S4. Representative *ex vivo* NIR fluorescence image (right) of the dissected organs from athymic mice bearing HER1-positive peritoneal LS-174T tumor xenografts at day 3 post-injection of ICG-sOSu-panitumumab (**2**, 20 μg, i.v.). White light image (left) served as a reference. The highest fluorescence signal was shown in tumor followed by liver. Labels: 1: heart; 2: lung; 3: liver; 4: spleen; 5: tumor; 6: kidney; 7. Intestine.

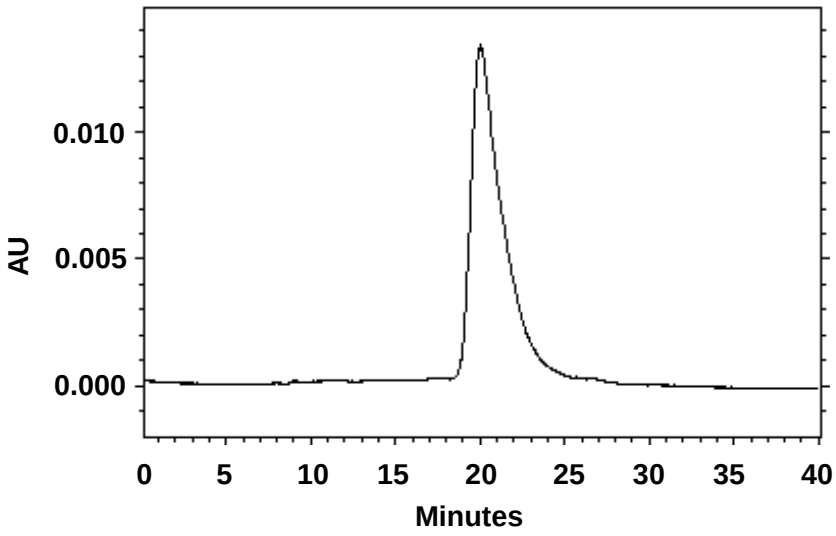

Figure S5. SE-HPLC profile of the doubly-purified ICG-sOSu-panitumumab (**5**).

References

[1] Milenic, D. E., Garmestani, K., Brady, E. D., Baidoo, K. E., Albert, P. S., Wong, K. J., Flynn, J., and Brechbiel, M. W. (2008) Multimodality therapy: potentiation of high linear energy transfer radiation with paclitaxel for the treatment of disseminated peritoneal disease. *Clin. Cancer Res. 14*, 5108-5115.