SUPPORTING INFORMATION

Rational Approach to Select Small Peptide Molecular

Probes Labeled with Fluorescent Cyanine Dyes for in

vivo Optical Imaging

Mikhail Y. Berezin^a, Kevin Guo^a, Walter Akers^a, Joseph Livingston^a, Metasebya Solomon^{a,b}, Hyeran Lee^a, Kexian Liang^a, Anthony Agee^a and Samuel Achilefu^{a,b,c}*

Departments of ^aRadiology, ^bBiomedical Engineering, ^cBiochemistry & Molecular Biophysics,
Washington University, St. Louis, MO 63110

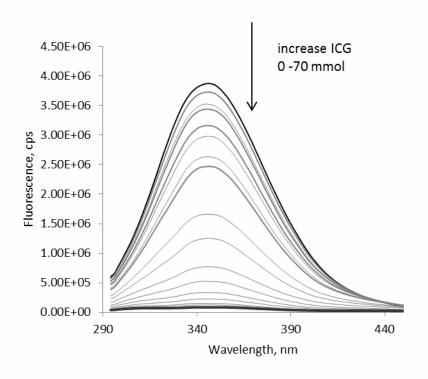


Figure S1: Quenching of tryptophan fluorescence by addition of ICG, ex/em 279/294-450 nm.

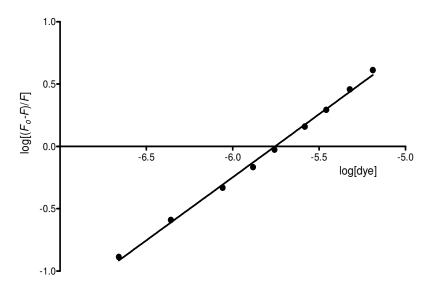


Figure S2: Double logarithmic plot of ICG binding to BSA in PBS buffer. Excitation 279 nm, emission 345 nm. Slope $n=1.012 \pm 0.0212$, ($R^2=0.997$) indicates that only one site on albumin molecules is occupied at a time

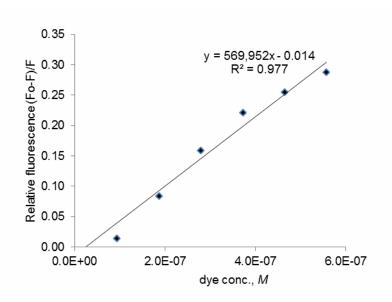


Fig. S3: Relative fluorescence as a function of dye concentration of ICG in HSA (ex/em 279/345nm, HSA conc.: $1.4 \times 10^{-6} \text{ M}$ in PBS). Slope of the trend lines corresponds to the binding constant (K = 570,000 M⁻¹)

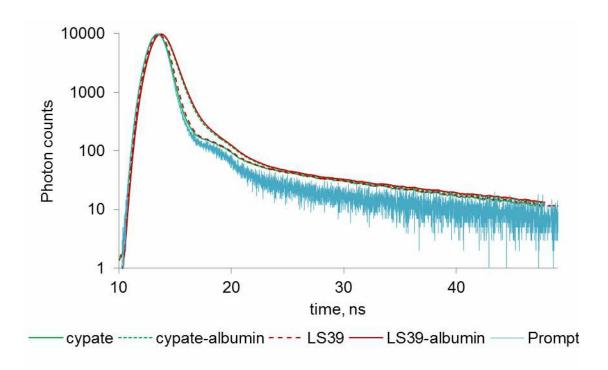


Figure S4: Model of kurtosis. Extravasation Fluorescence lifetime decays (fits) of probes in PBS buffer (free) and albumin/PBS solution (bound) (ex/em. 773/820 nm). Prompt: Ludox in water (ex/em. 773/773 nm).

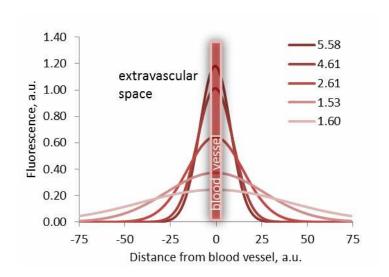


Figure S5: Model of kurtosis. Extravasation of a fluorescent probe into the tissue causes a decrease in kurtosis from 5.58 to 1.6. Kurtosis approaches zero when no difference between fluorescence in extravascular space and blood vessel is detected (model was based on Gaussian distribution and kurtosis was calculated using MATLAB).

Complete reference 3:

(3) Kelloff, G. J.; Krohn, K. A.; Larson, S. M.; Weissleder, R.; Mankoff, D. A.; Hoffman, J. M.; Link, J. M.; Guyton, K. Z.; Eckelman, W. C.; Scher, H. I.; O'Shaughnessy, J.; Cheson, B. D.; Sigman, C. C.; Tatum, J. L.; Mills, G. Q.; Sullivan, D. C.; Woodcock, J. *Clin Cancer Res* **2005**, *11*, 7967-85.