Supporting information

A Comparative Study on Albumin-binding Molecules for Targeted Tumor Delivery through Covalent and Noncovalent Approach

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Conformational change analysis

1 mg of Albumin, Alb-PEP-Cy5.5, Alb-PA-Cy5.5 or Alb-MI-Cy5.5 was dissolved in 1 ml of DIW before they were analyzed by a circular dichroism detector (Chirascan plus, Applied Photophysics Ltd, Surrey, UK). To assess the amount of PEP-Cy5.5, PA-Cy5.5, or MI-Cy5.5 in albumin complex, the UV-absorbance of Alb-PEP-Cy5.5, Alb-PA-Cy5.5, or Alb-MI-Cy5.5 (0.05 mg/ml in DIW) was obtained from UV-vis spectrophotometer (Optizen 3220UV, Mecasys Co., Ltd., Daejeon, Korea). Using the absorbance value, the content of Cy5.5 moieties in albumin complex was calculated based on weight ratio (wt%). In the case of hydrodynamic size analysis, the size of conjugated albumin in saline was characterized using dynamic light scattering (DLS; Zetasizer Nano ZS Malvern Instruments, Worcestershire, UK).

Binding simulation with albumin

The crystal structure of human serum albumin (protein data bank [PDB] code, 1AO6) was previously reported.[1] The human serum albumin can be complexed with hexadecanoic acid (palmitic acid), and the combined structure of albumin and hexadecanoic acid was also published.[2] The molecular structure of the conjugates was visualized by ChemBioDraw Ultra 12.0 (Cambridge Soft Corporation) and PyMOL 1.7.0.1 (DeLano Scientific). Its molecular structure without water and salt molecules was used for docking test. For the docking simulation with AutoDock Vina, all bonds of PEP-Cy5.5 and PA-Cy5.5 were considered as active torsional bonds.[3]

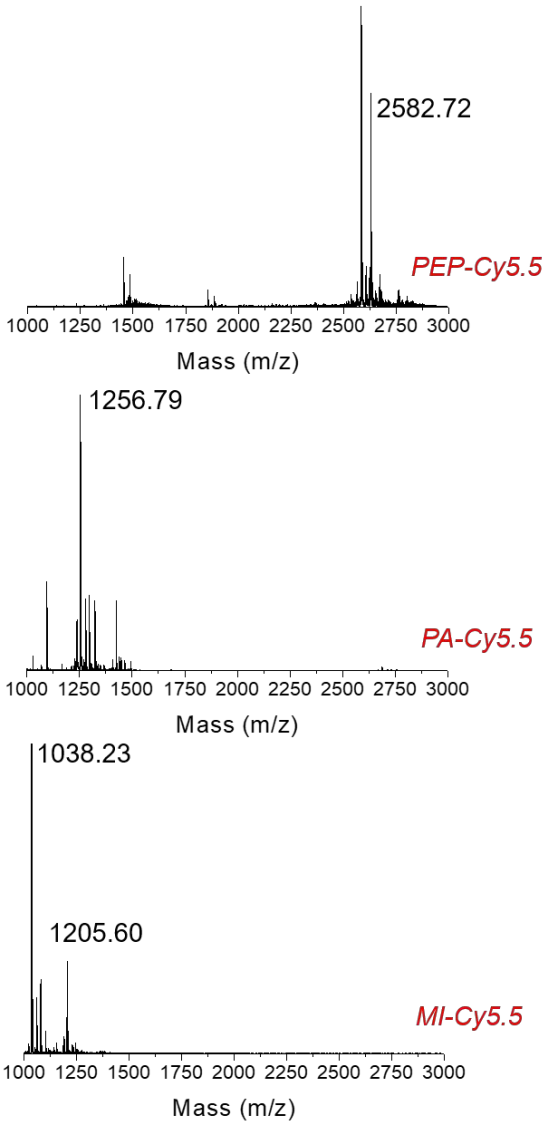

Figure S1. The molecular weight of PEP-Cy5.5, PA-Cy5.5 or MI-Cy5.5 was confirmed using matrix-assisted laser desorption/ionization (MALDI) analysis.

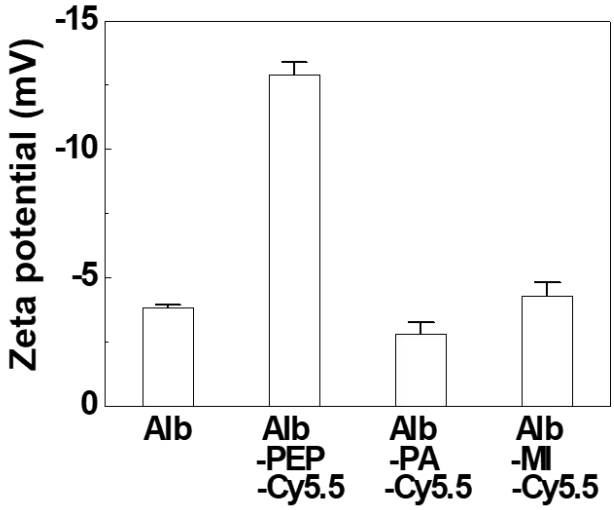

Figure S2. Zeta potential of albumin-Cy5.5 complexes including Alb-PEP-Cy5.5, Alb-PA-Cy5.5 and Alb-MI-Cy5.5

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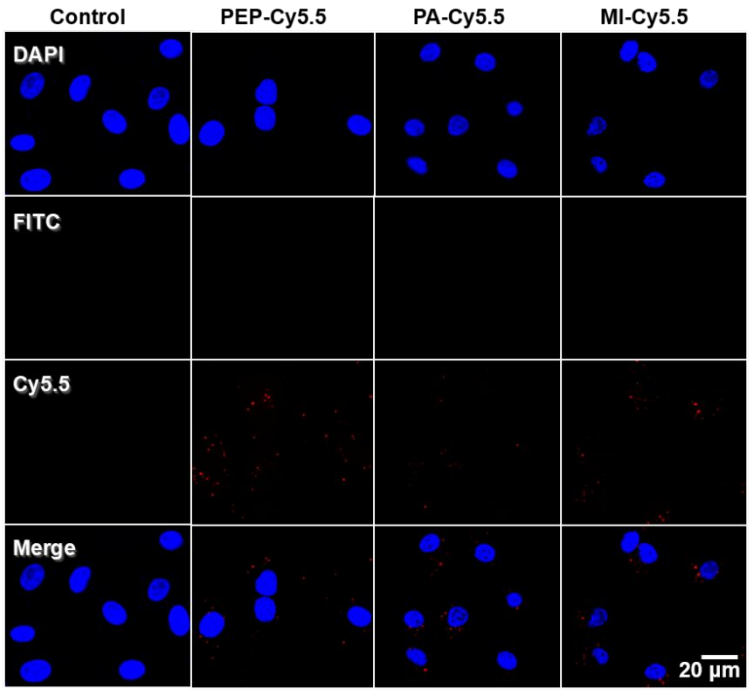

Figure S3. Cellular uptake of Cy5.5 conjugates to bovine aortic endothelial cell (BAEC)

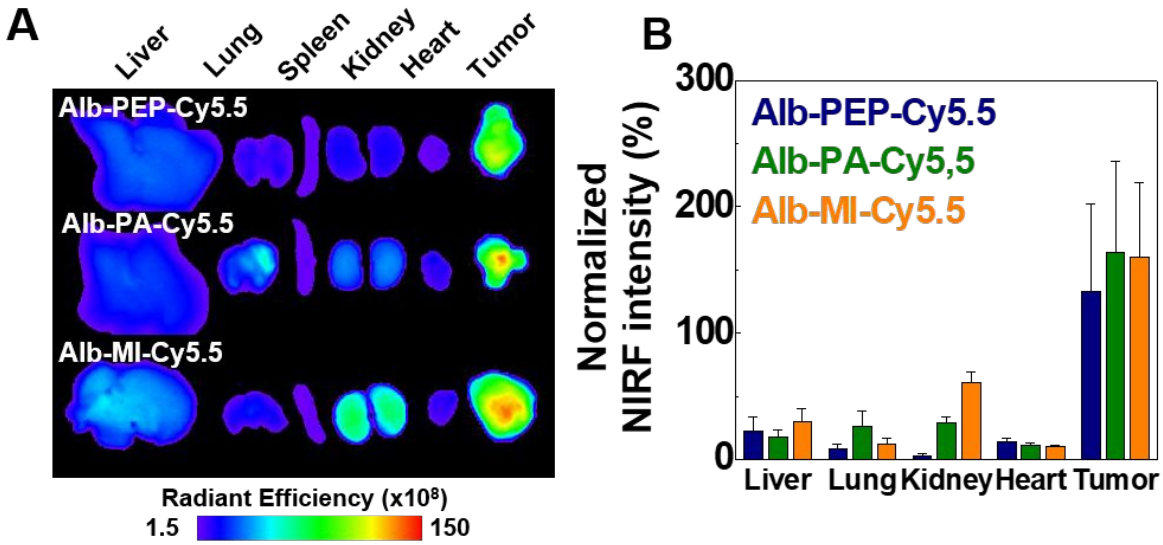

Figure S4. *In vivo* time-dependent biodistribution of prebound albumin-Cy5.5 complex (A) *Ex vivo* fluorescence images of the tumor and other organs (B) Quantitative analysis of fluorescence intensity of albumin-Cy5.5 complexes in the organs and tumor. Error bar represents standard error (n = 3)

References

(1) Sugio, S., Kashima, A., Mochizuki, S., Noda, M., and Kobayashi, K. (1999) Crystal structure of human serum albumin at 2.5 A resolution. *Protein Eng. 12*, 439-46.

(2) Bhattacharya, A. A., Grune, T., and Curry, S. (2000) Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. *J. Mol. Biol. 303*, 721-32.

(3) Trott, O., and Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem. 31*, 455-61.