Supporting Information for

Endosomal escape of peptide-photosensitizer conjugates is affected by amino acid sequences near the photosensitizer

Yuichi Miyoshi[1], Maho Kadono[1], Shigetoshi Okazaki[2], Ayano Nishimura[3], Mizuki Kitamatsu[3], Kazunori Watanabe[1,4], Takashi Ohtsuki[1,4*]

[1] *Department of Medical Bioengineering, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan*

[2] *Preeminent Medical Photonics Education and Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

[3] *Department of Applied Chemistry, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan*

[4] *Department of Interdisciplinary Science and Engineering in Health Systems, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan*

*Corresponding author:

E-mail: ohtsuk@okayama-u.ac.jp

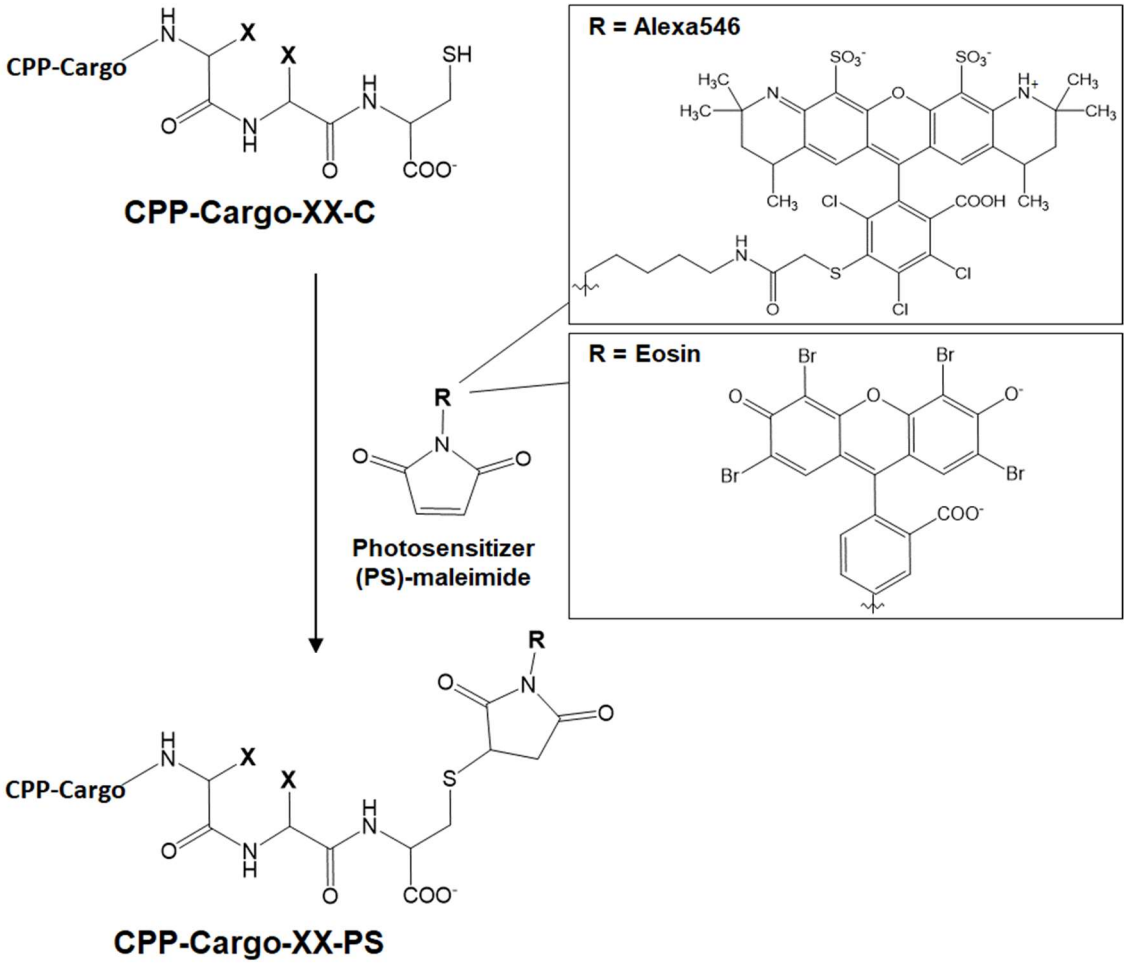
Supplementary Results and Discussion

Absorption and fluorescence excitation spectra of XX-eosin molecules

Slight differences were observed among the absorption spectra of eosin-maleimide and XX-eosin molecules, with absorption peaks ranging from 519.5 to 525.0 nm (Fig. S3). In contrast, the fluorescence excitation spectra of these molecules were very similar, with peaks around 519 nm. For each XX-eosin molecule, the wavelength of the absorption peak was approximately 1-6 nm longer than that of the fluorescence excitation peak. These results indicate that each XX-eosin molecule has structural species with absorption at a longer wavelength than the fluorescence excitation peak, but weak/no fluorescence. These structural species with weak/no fluorescence might be dimers and/or multimers.

To evaluate the potential existence of dimers or multimers, we measured the absorption spectra of diluted XX-eosin molecules, using EE-eosin and WW-eosin as examples (Fig. S4). If the longer absorption peaks of 1 μM EE-eosin (524 nm) and WW-eosin (523.5 nm) were due to intermolecular interactions (dimer or multimer), diluting the samples should cause these peaks to decrease in wavelength and shift closer to the fluorescence excitation peaks (518.4 nm for EE-eosin and 519.2 nm for WW-eosin). However, the absorption peak wavelengths were identical in 1 μM and 0.1 μM for each XX-eosin. These results indicate that the absorption peak shift was not due to intermolecular interactions (dimer or multimer), but rather to monomeric structural species with intramolecular interactions that reduce fluorescence. Such structural species might be a reason for the low $^1O_2$ quantum yield of most of XX-eosin molecules.

Supplementary Scheme and Figures



Scheme S1. Synthesis of CPP-Cargo-XX-PS.

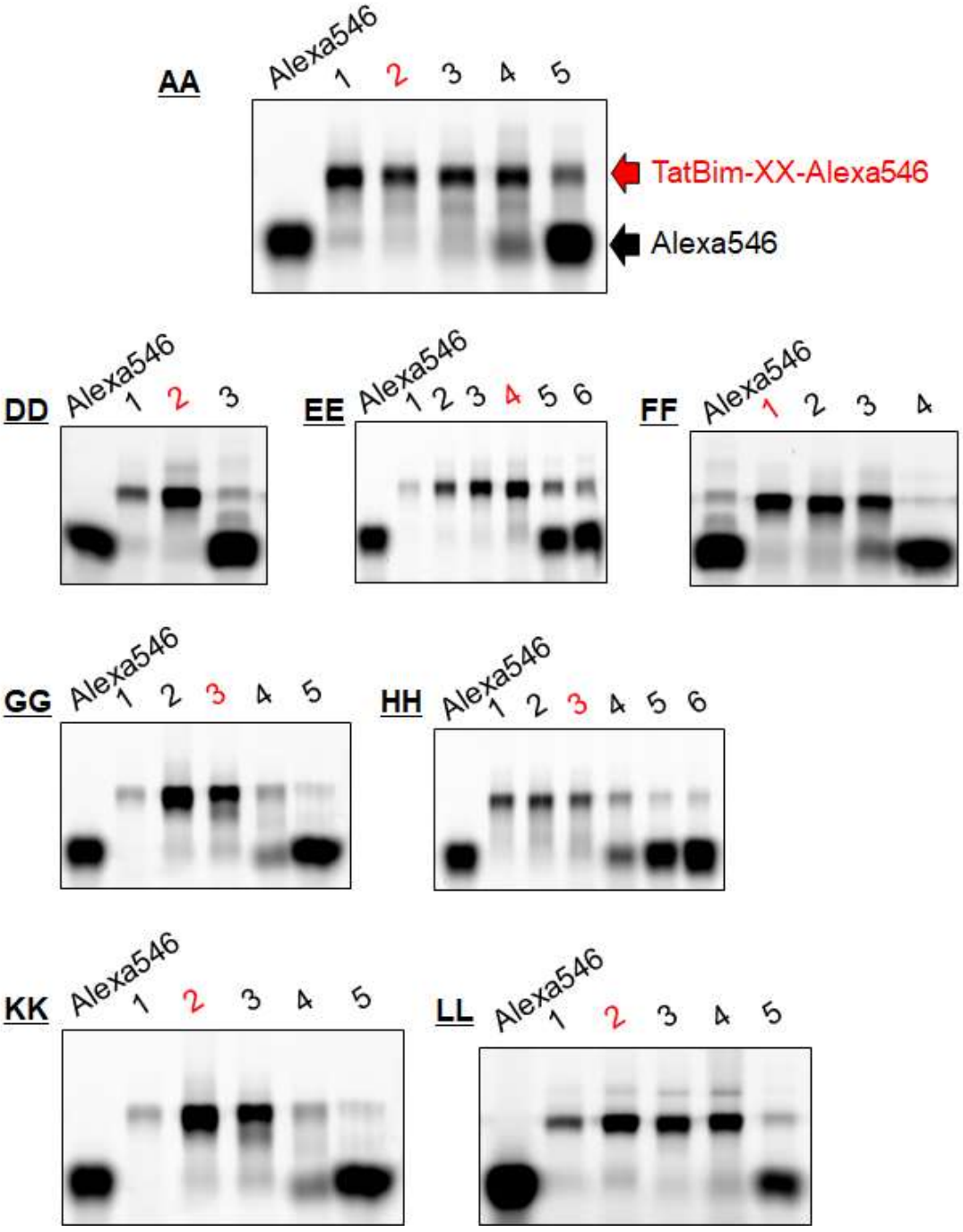


Figure S1. Analysis of TatBim-XX-Alexa546 molecules by 18% SDS-PAGE. The reaction mixture including TatBim-XX-C and Alexa Fluor 546 C5 maleimide was loaded on the reverse-phase HPLC, the HPLC peak fractions were analyzed. Red peak number indicates a fraction which we used as TatBim-XX-Alexa546 for the cell treatment. Alexa546 images of the gels were acquired using an FLA-9000 (Fujifilm, Japan).

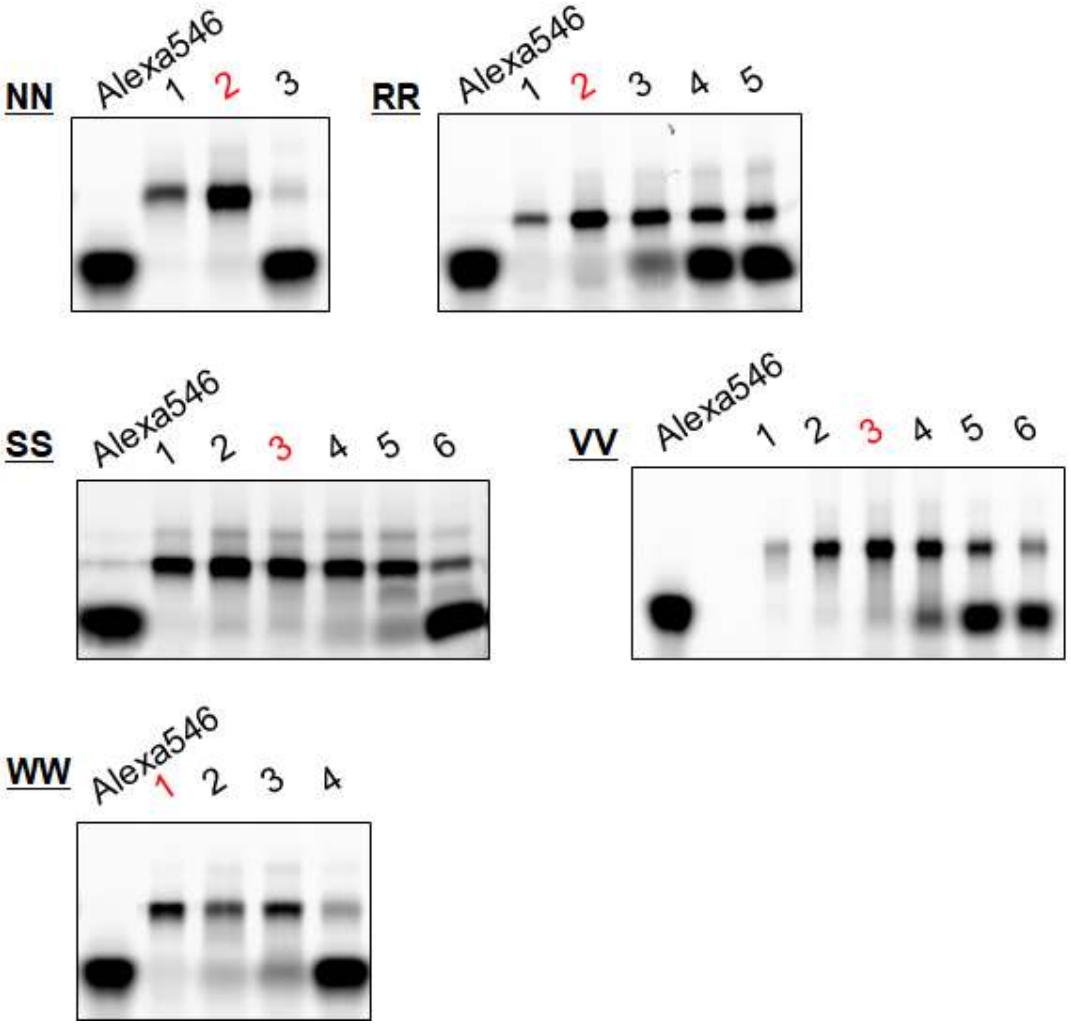


Figure S1. continued.

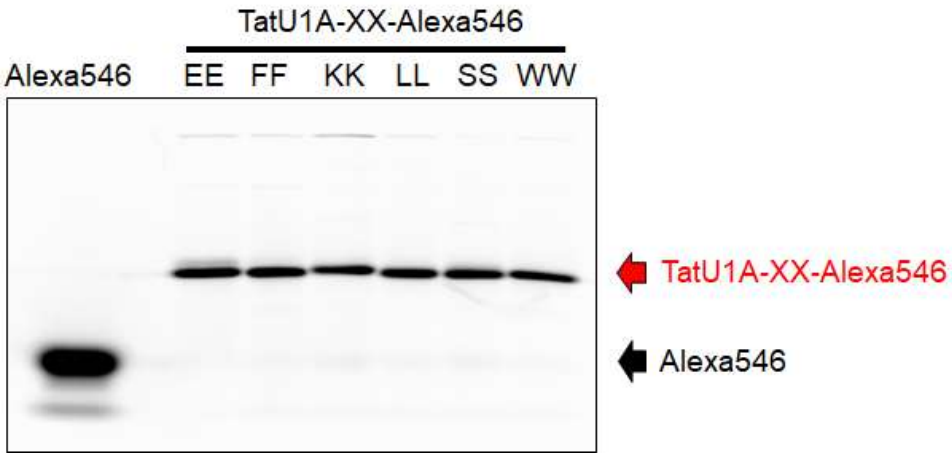


Figure S2. Analysis of TatU1A-XX-Alexa546 conjugates by 18% SDS-PAGE. Each conjugate was analyzed after purification of the conjugate using a Centri-Sep Spin Column. Alexa546 images of the gels were acquired using an FLA-9000 (Fujifilm).

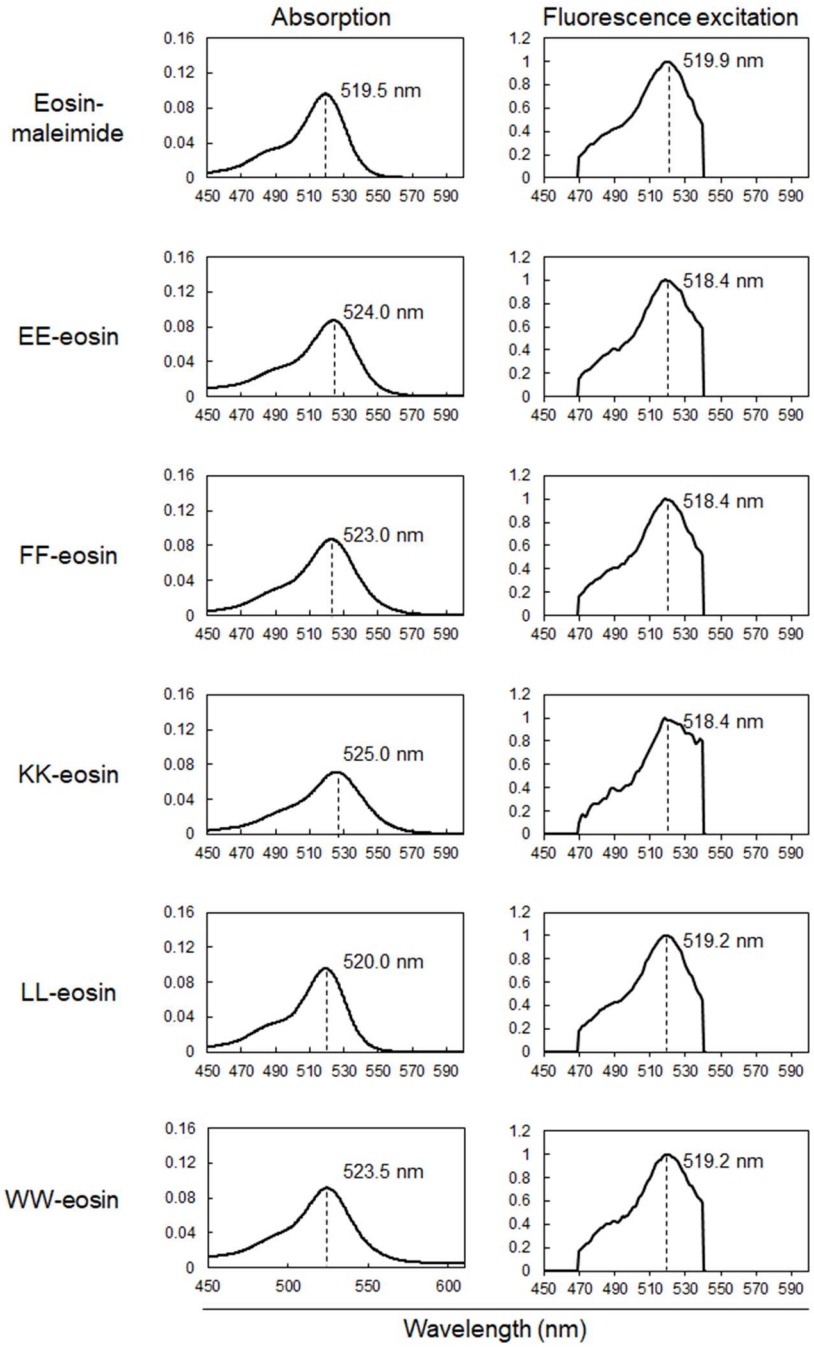


Figure S3. Absorption (left) and fluorescence excitation (right) spectra of eosin-maleimide and XX-eosin molecules. The number in each spectrum indicates the peak wavelength. Absorption and fluorescence excitation spectra of the eosin derivatives (1 μM) were measured in the D_2O solution including 100 mM NaOAc (pH 5.5) and 0.25% dimethyl sulfoxide. Fluorescence excitation spectra (470-540 nm) were measured at emission wavelength from 550 nm to 680 nm using Absolute PL quantum yield spectrometer (C9920-02, Hamamatsu Photonics K.K., Hamamatsu, Japan).

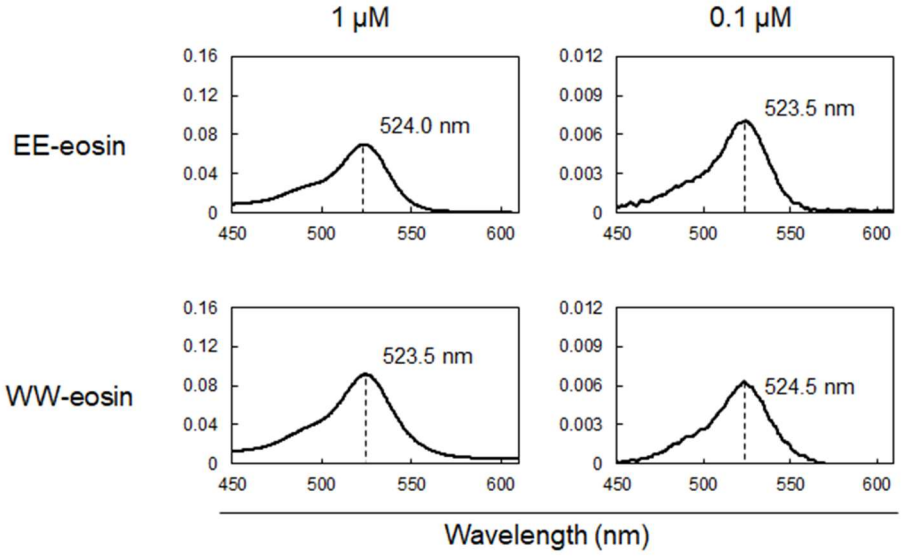


Figure S4. Absorption spectra of EE-eosin and WW-eosin at different concentrations (1 μM and 0.1 μM). The number in each spectrum indicates the peak wavelength. The absorption spectra were measured using an U-3500 spectrophotometer (Hitachi, Japan).