Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Energy Transfer Dynamics and Mechanism of Biohybrid Photosynthetic Antenna Complexes Chemically-Linked with Artificial Chromophores

Yusuke Yoneda[1,2,*], Tomoyasu Noji[3, a], Naoto Mizutani[3], Daiji Kato[3], Masaharu Kondo[3], Hiroshi Miyasaka[1], Yutaka Nagasawa[4,*], Takehisa Dewa[3,*]

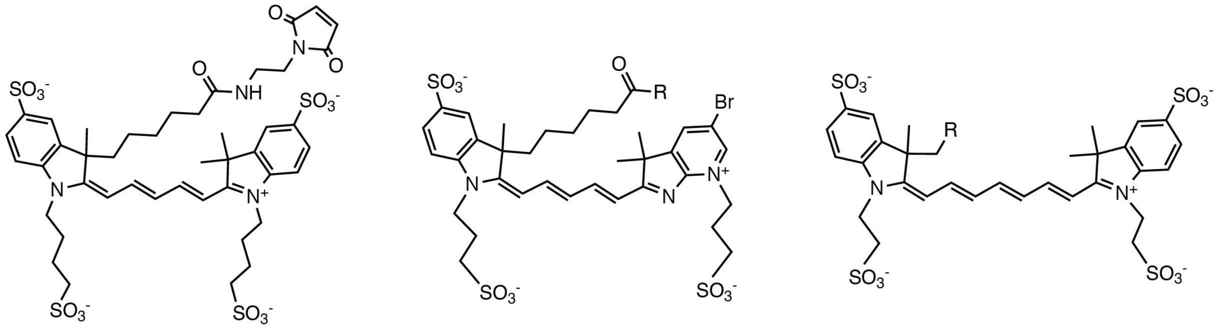
[1]*Graduate School of Engineering Science, Osaka University, Toyonaka Osaka 560-8531, Japan*

[2]*Research Center of Integrative Molecular Systems, Institute for Molecular Science, National Institute of Natural Sciences, Okazaki, Aichi, 444-8585, Japan*

[3]*Department of Life Science and Applied Chemistry, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, Aichi 466-8555, Japan*

[4]*College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

[a]*Present Address: Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8904, Japan*



Alexa647
(A647)

Alexa680
(A680)

Alexa750
(A750)

Scheme S1. Structures of Alexa647 (left, with maleimide group), Alexa680 (middle), and Alexa750 (right). The moieties denoted as R, which corresponds to a part including functionalized group, seem to be not disclosed.

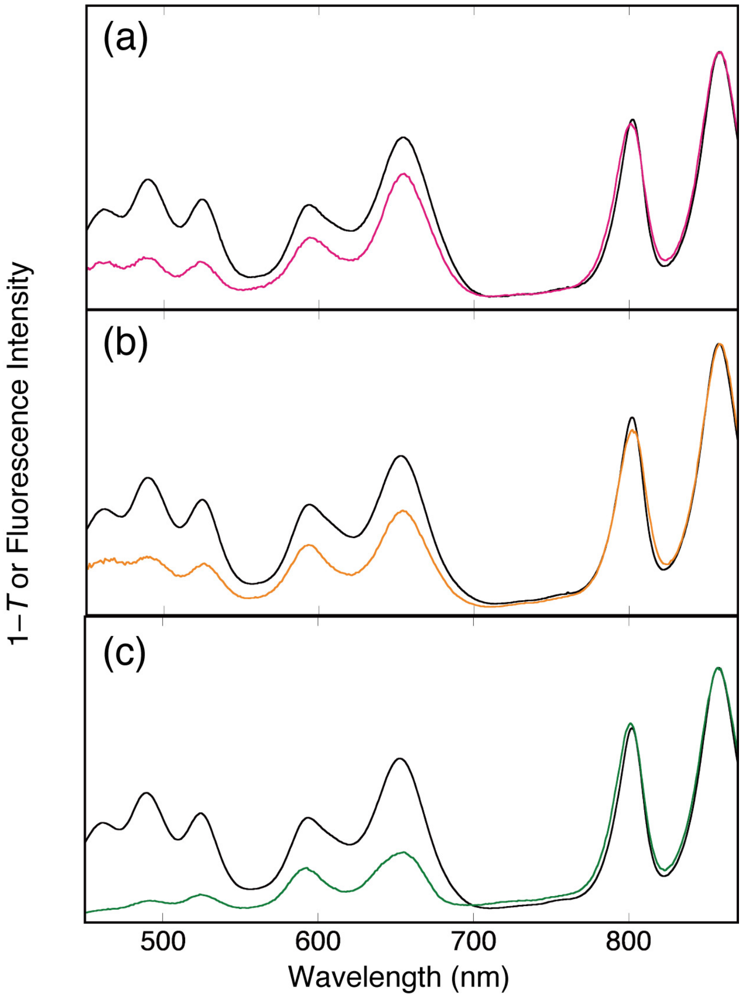


Figure S1. Fluorescence excitation spectra of LH2-LL-A647 (a), LH2-SL-A647 (b), and LH2-DL-A647 (c) monitored at 880 nm. The excitation spectra indicated by colored lines were compared with absorptance ($1 - T$, T: transmittance) spectra (black lines), normalized at the maximum of B850. The conjugates were dissolved in a TL buffer solution (Tris-HCl, 20 mM, pH 8.0, containing 0.1 wt% LDAO). All spectra were acquired at room temperature.

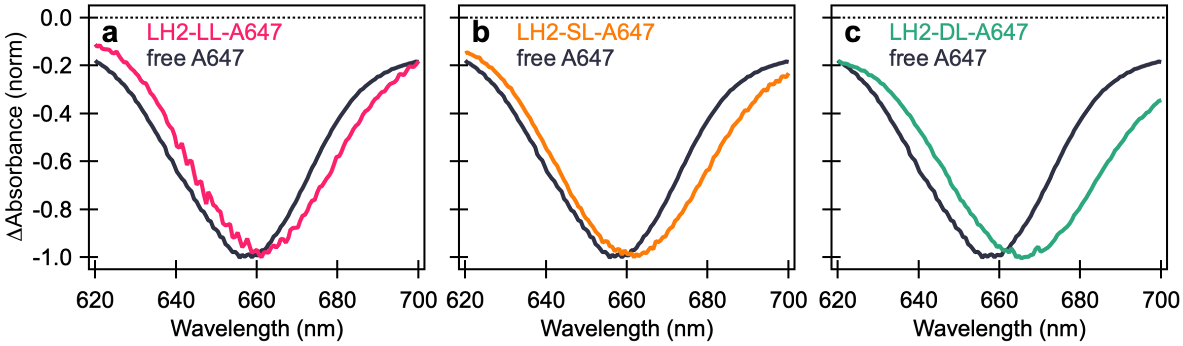


Figure S2. Transient absorption spectra of LH2-LL-A647, LH2-SL-A647 and LH2-DL-A647 at 50 ps in the range of 620–700 nm which is overlap of ground state bleach and stimulated emission of A647. Transient absorption spectrum of free A647 is also shown in black curve for comparison.

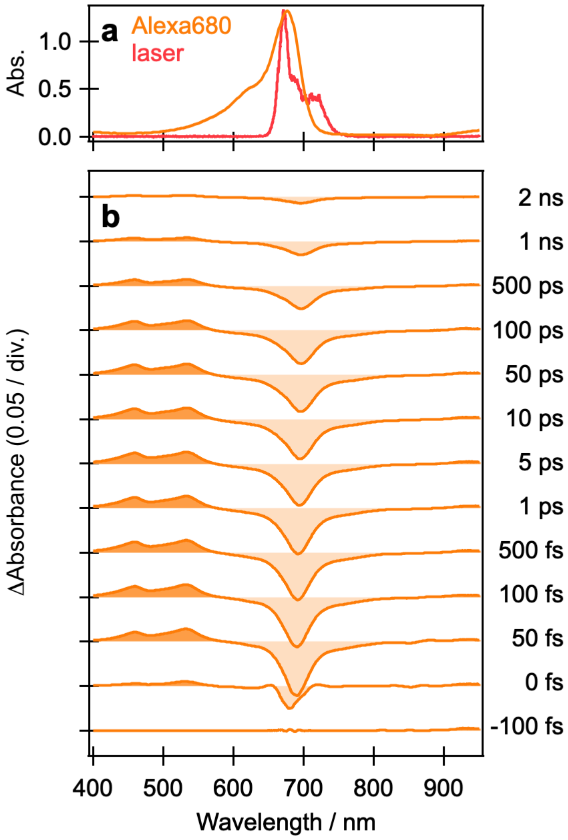


Figure S3. (a) Steady-state and (b) transient absorption spectra of free Alexa Fluor 680.

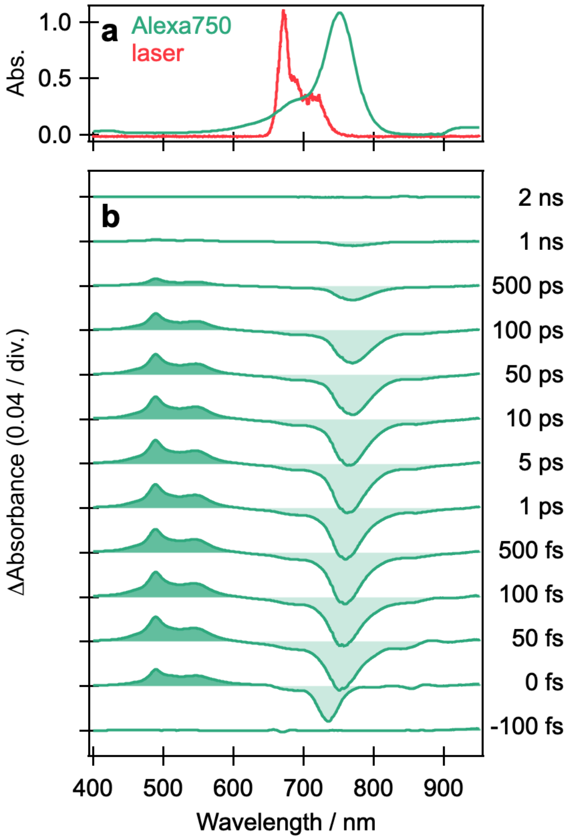


Figure S4. (a) Steady-state and (b) transient absorption spectra of free Alexa Fluor 750.

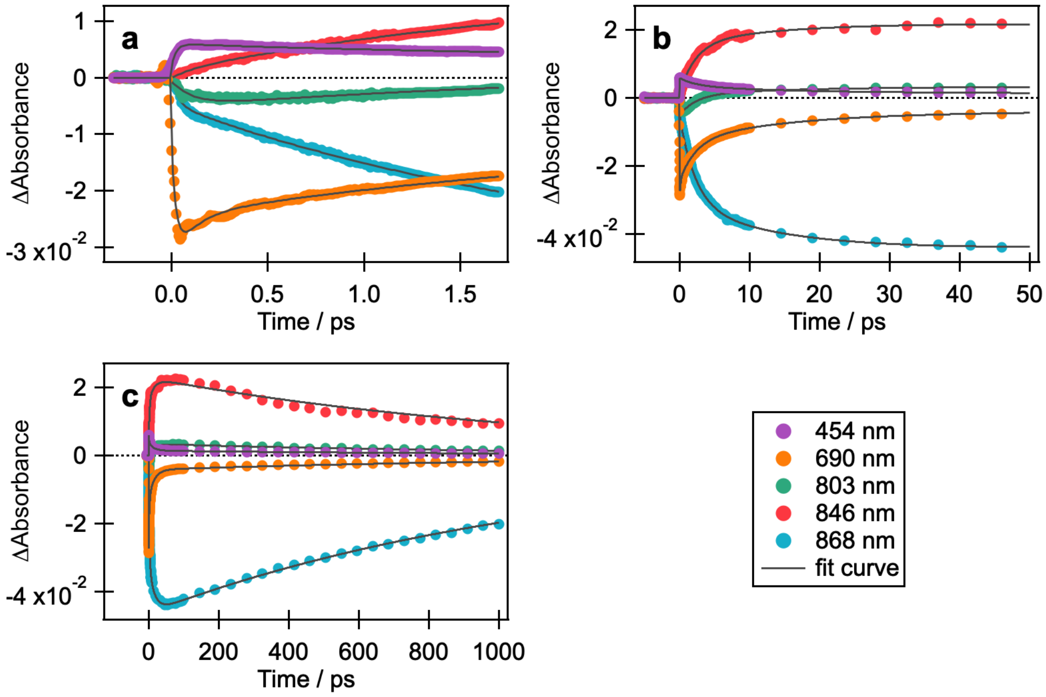


Figure S5. Time traces of transient absorbance of LH2-LL-A680 at various wavelengths. The fit result of global analysis is also shown in black curve.

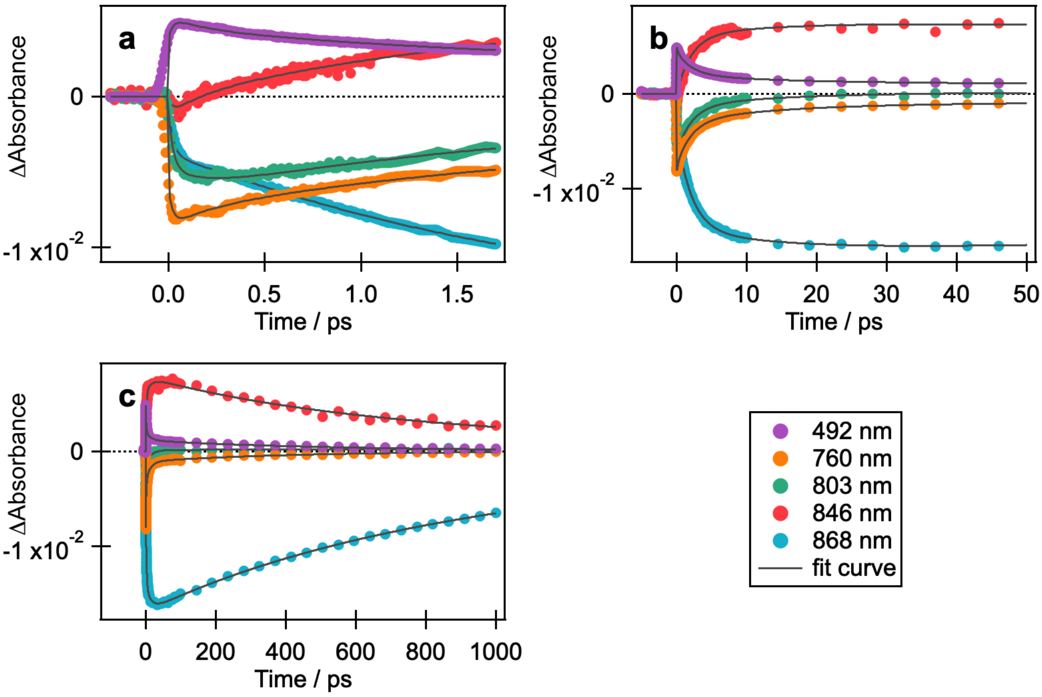


Figure S6. Time traces of transient absorbance of LH2-LL-A750 at various wavelengths. The fit result of global analysis is also shown in black curve.

Effect of excited state relaxation

The regression analysis for the B850 population dynamics by the double exponential function indicates that the time constant of the energy transfer in the LH2-LL-Alexa systems seems to plateau at approximately 2 ps (Table 4). This time scale is comparable to that of the excited-state relaxation process (a few ps). FRET theory assumes that the energy transfer occurs after the excited-state relaxation (the overlap integral is calculated between the thermally relaxed fluorescence and absorption spectra). Thus, FRET theory may not always be applicable to ultrafast energy transfer dynamics, in which excited-state relaxation can affect either the spectral overlap integral or emission intensity (radiative decay rate). However, we think that this effect is minor because of the small Stokes shift between absorption and emission maxima of the conjugated dye.

It is well known that an emission spectrum shifts to longer wavelengths in the course of structural relaxation or solvation in the excited state (dynamic Stokes shift), which will certainly change the spectral overlap with time. Radiative decay rate may also change when the excited state population moves out of the Franck-Condon region. We have previously shown that emission intensity is significantly reduced in the course of intramolecular charge separation (CS) reaction for 10-cyano-9,9'-bianthryl.[1] The emission from the CS state is weaker than that from the locally excited state because the wave function of the CS state is a mixture of emissive excites state and nonemissive radical-ion-pair state. This difference caused a contradiction between the interpretation of the results of TA spectroscopy and that of time-resolved fluorescence spectroscopy. However, this may not be the case for the present dyes, because they do not have CS character in the excited state (small Stokes shift between the absorption and emission maxima).

The time scale of excited state relaxation can be obtained by analyzing the peak shift of the negative bands of Alexa dyes in buffer solutions, which is a mixture of SE and GSB (Fig. S7). The peak shift of the short time range < 50 ps can be reproduced by two exponential functions, and the fitting parameters are summarized in Table S1. The peak shift of Alexa molecules showed fast (0.20 - 1.0 ps) and slow (9.1 - 14 ps) components. The global analysis of TA dynamics of Alexa dyes in buffer solution also suggests similar time scales (Table S1 and Fig. S8). Note that these values are measured in buffer solutions, while the recent report suggests that chromophore-protein interactions through linker molecule make excited state relaxation slower (1.0 - 5.5 times slower at outside position).[2]

The amount of the shift is the smallest for A647, and it is the largest for A750, *i.e.*, 2 nm for A647, 6 nm for A680, and 13 nm for A750. As can be seen from Fig. 1, only a minor change is expected for the overlap of spectra from such a slight spectral shift. For A750 with the most significant shift, the emission spectrum overlaps with the strong absorption of B800 on the longer wavelength side. Hence, the spectral shift could affect the overlap between the spectra. According to Table S1, 73 % of the change occurs with a time constant of 13 ps, while 71 % of the energy transfer occurs with 1.8 ps (Table 4). Thus, in early times, energy transfer could occur with a smaller spectral overlap. However, according to our calculation, such an effect was minor even for A750.

Time dependence of the emission intensity could be qualitatively assumed from the shape of DAS shown in Fig. S8. For A647 and A680, DAS with a short lifetime exhibits a negative band at 651 and 685 nm, respectively, with only a minor positive band at longer wavelengths, indicating that the relaxation of the excited state reduces the emission intensity. However, the time scale of the relaxation is shorter than the energy transfer for A647 and A680, suggesting that energy transfer occurs mainly after the reduction of emission intensity, and the energy transfer rate calculated from steady-state spectra is valid in this case. For A750, DAS with shorter lifetimes exhibit a derivative-shaped feature with negative and positive bands on shorter and longer wavelengths, indicating that the relaxation only causes a red shift of the emission. Therefore, time-dependent emission intensity is also expected to have only a minor effect on the EET rate.

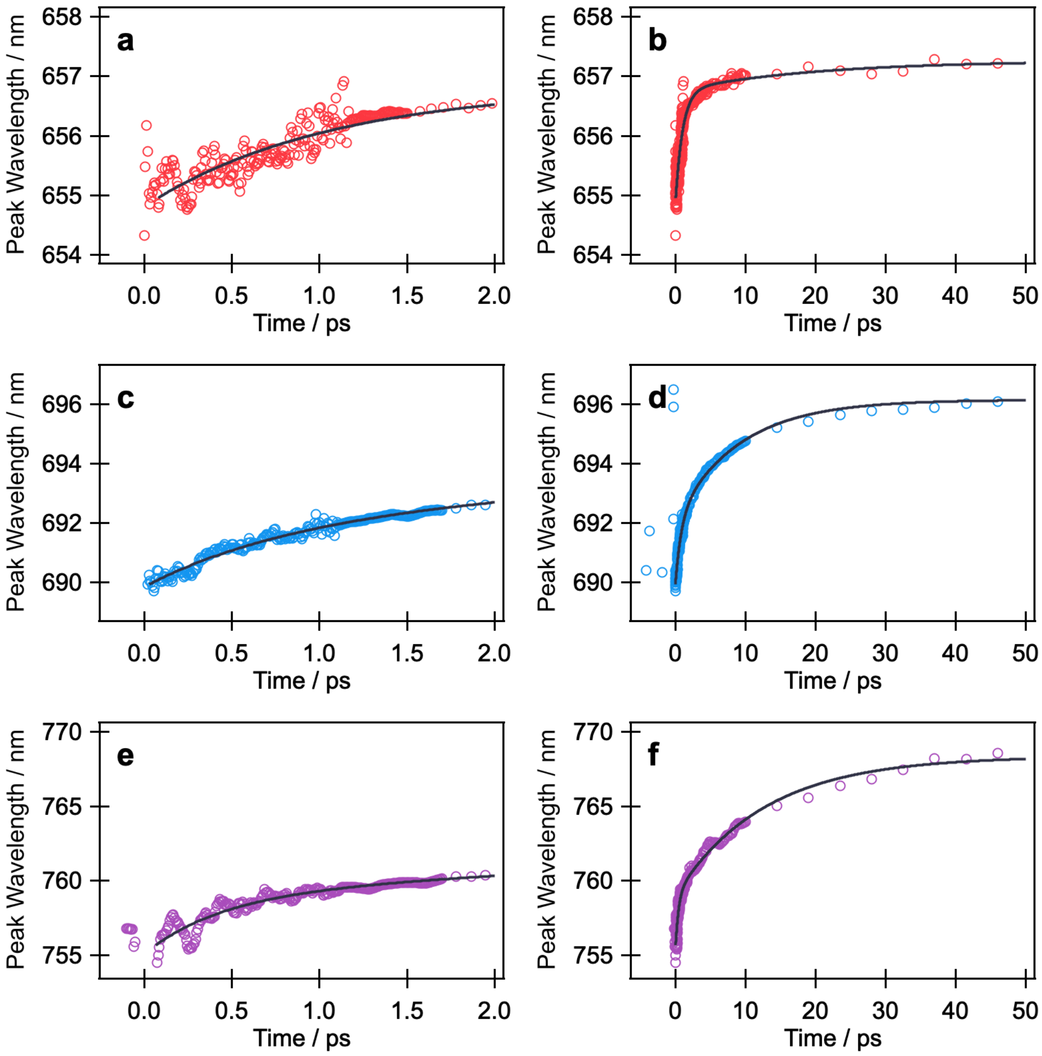


Figure S7. Peak shift dynamics of stimulated emission (and ground state bleach) of (a, b) A647, (c, d) A680 and (e, f) A750 in a buffer solution.

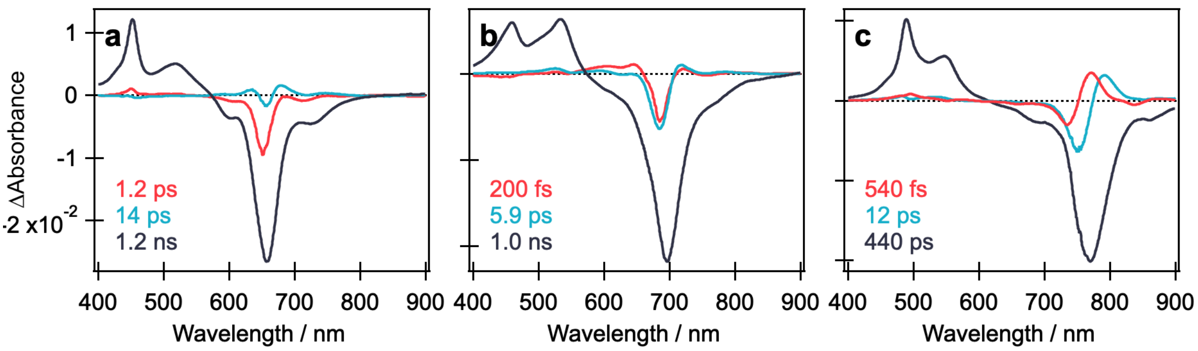


Figure S8. Decay associated spectra of (a) A647, (c) A680 and (e) A750 in a buffer solution. The transient absorption dynamics was analyzed by 3 components, fast and slow excited state relaxation (red, cyan) and life time (black).

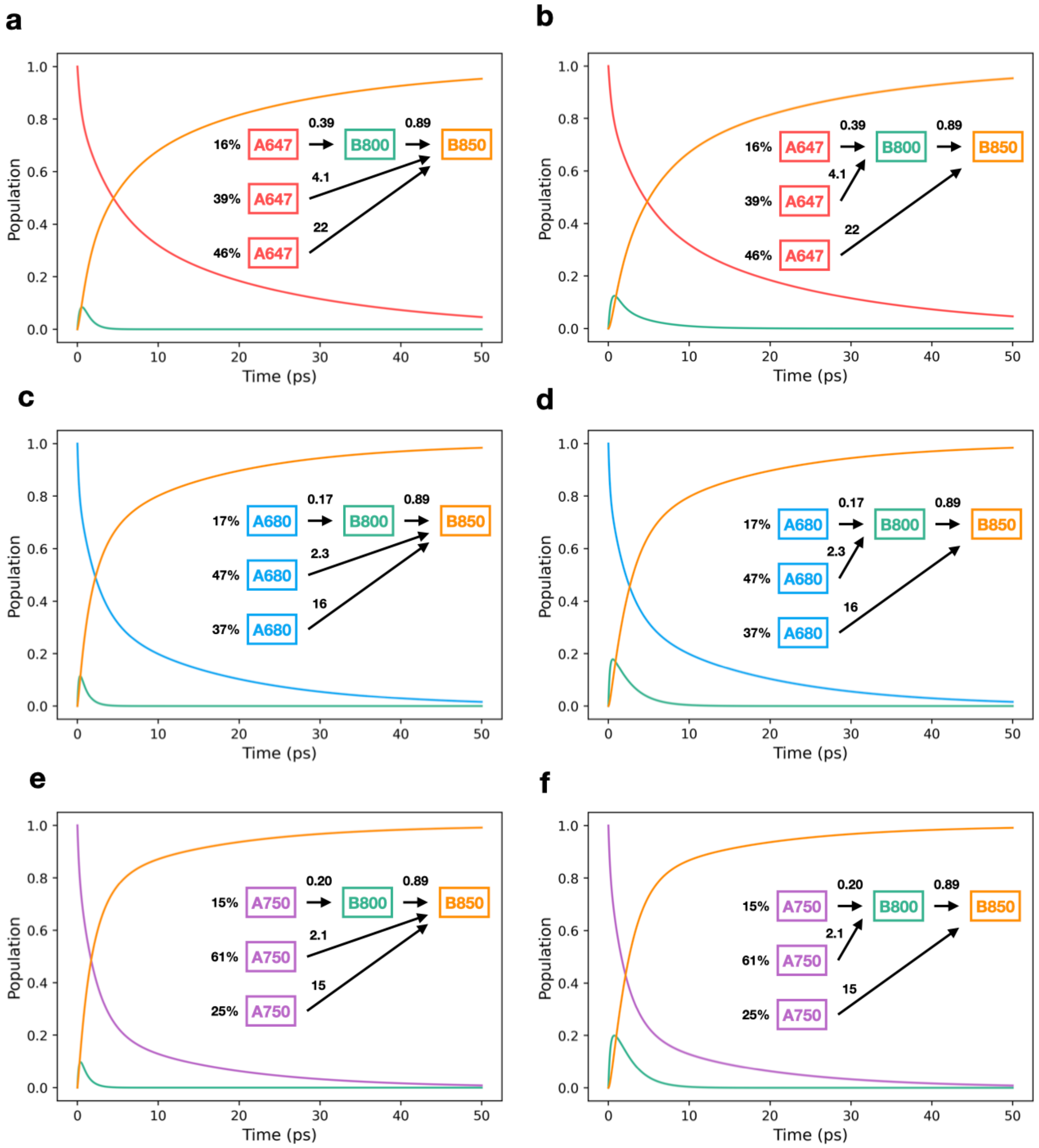


Figure S9. Simulated population dynamics of (a, b) LH2-LL-A647, (c, d) LH2-LL-A680 and (e, f) LH2-LL-A750. Kinetic models are shown in the inset. Parameters are based on the results of global analysis (Table 1). The time constant for B800 to B850 transfer (0.89 ps) was taken from the literature.[3] Provided that the B800-mediated pathway mainly contributed to the EET (b, d, f), the maximum population of B800 shows twice the amplitude for LH2-LL-A750 compared to that for LH2-LL-A647. On the other hand, in the case of the B800-mediated pathway is minor (a, c, e), the maximum population of B800 is essentially the same for all conjugates (~10%).

Table S1. Fitting parameters for peak shift dynamics at stimulated emission (and ground state bleach) of Alexa molecules.

	A647		A680		A750	
	Peak shift	DAS	Peak shift	DAS	Peak shift	DAS
1st [ps]	1.0 (0.78)	1.2	0.85 (0.35)	0.20	0.44 (0.27)	0.54
2nd [ps]	20 (0.22)	14	9.1 (0.65)	5.9	13 (0.73)	12
Average	5.2	-	6.4	-	9.4	-

1 E. Takeuchi, M. Muramatsu, T. Katayama, Y. Yoneda, S. Ito, Y. Nagasawa and H. Miyasaka, Sub-100 fs charge separation and subsequent diffusive solvation observed for asymmetric bianthryl derivative in ionic liquid, *J. Phys. Chem. C*, 2016, **120**, 14502–14512.

2 M. Delor, J. Dai, T. D. Roberts, J. R. Rogers, S. M. Hamed, J. B. Neaton, P. L. Geissler, M. B. Francis and N. S. Ginsberg, Exploiting Chromophore-Protein Interactions through Linker Engineering to Tune Photoinduced Dynamics in a Biomimetic Light-Harvesting Platform, *J. Am. Chem. Soc.*, 2018, **140**, 6278–6287.

3 Y. Yoneda, T. Noji, T. Katayama, N. Mizutani, D. Komori, M. Nango, H. Miyasaka, S. Itoh, Y. Nagasawa and T. Dewa, Extension of Light-Harvesting Ability of Photosynthetic Light-Harvesting Complex 2 (LH2) through Ultrafast Energy Transfer from Covalently Attached Artificial Chromophores, *J. Am. Chem. Soc.*, 2015, **137**, 13121–13129.