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

Supplementary Information

Single-molecule spectroscopy and femtosecond transient absorption studies on excitation energy transfer process in ApcE(1-240) dimers

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S1. Structure and absorption spectrum of APC trimer

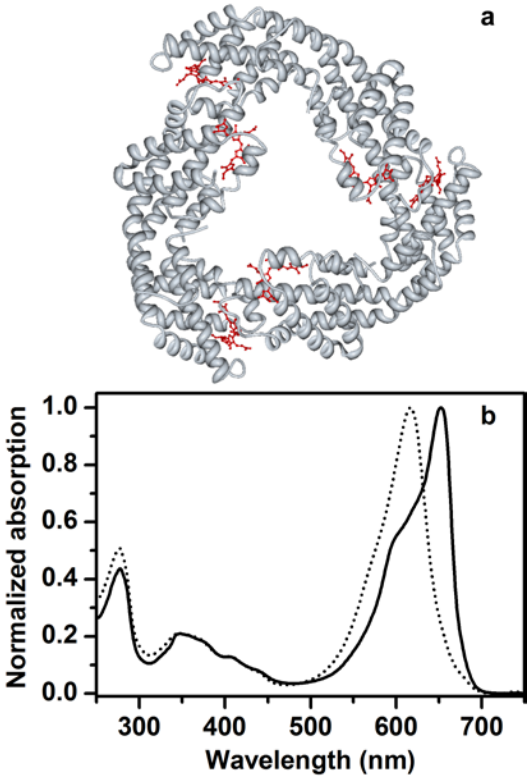

Figure S1. Structure and absorption spectrum of APC trimer. (a) Structure of APC trimer. Produced from protein data bank structure '1ALL'.[1] (b) Absorption spectra of APC trimer (solid line) and monomer (dotted line).

S2. Decomposition of absorption of ApcE(1-240) dimers

We assign the 660 nm peak and the 620 nm shoulder from the absorption spectrum of ApcE(1-240) dimers to the acceptor and the donor chromophores, respectively. Assuming mirror symmetry between the absorption and fluorescence spectra of the acceptor,[2] we can decompose the absorption spectrum of ApcE(1-240) dimers into individual spectra of

the donor and acceptor chromophores. We thus obtain the fraction of excitation at 605 nm about 70/30 for donor and acceptor chromophores, similar to the decomposition results of APC trimer.

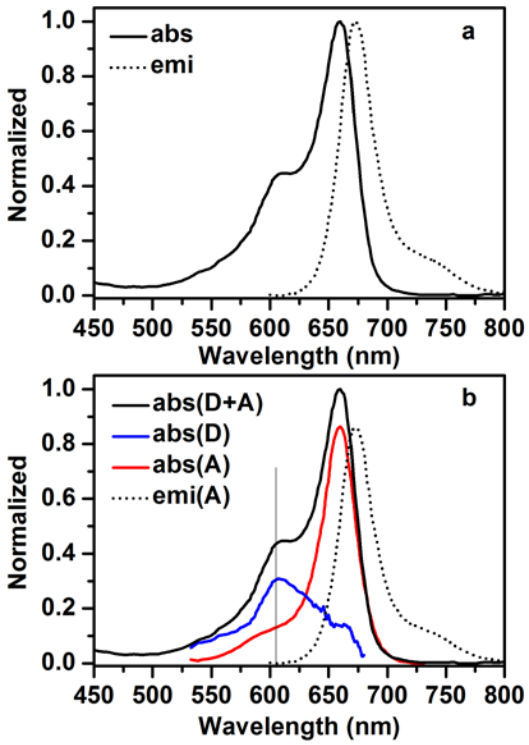

Figure S2. Decomposition of absorption of ApcE(1-240) dimers. (a) Steady-state absorption (solid line) and fluorescence (dotted line) spectra of ApcE(1-240) dimers in KPB buffer. (b) The calculated individual absorption (blue line for donor, and red line for acceptor), fluorescence spectra (dotted line), according to mirror symmetry between the absorption and fluorescence spectra of the acceptor. The thin vertical line at 605 nm is the pump wavelength for femtosecond transient absorption.

S3. Kinetics at selected wavelengths for showing the quality of global fitting

In order to show the quality of fitting of the data in Figure 5 in the Text, kinetics at selected wavelengths of 500, 535, 655, 665, 675, 687, 700 and 740 nm are plotted together with a global fit of all the collected time traces in Figure S3. The fraction (70/30) of excitation for donor and acceptor at 605 nm is used during fitting in Figure 5 in the Text.

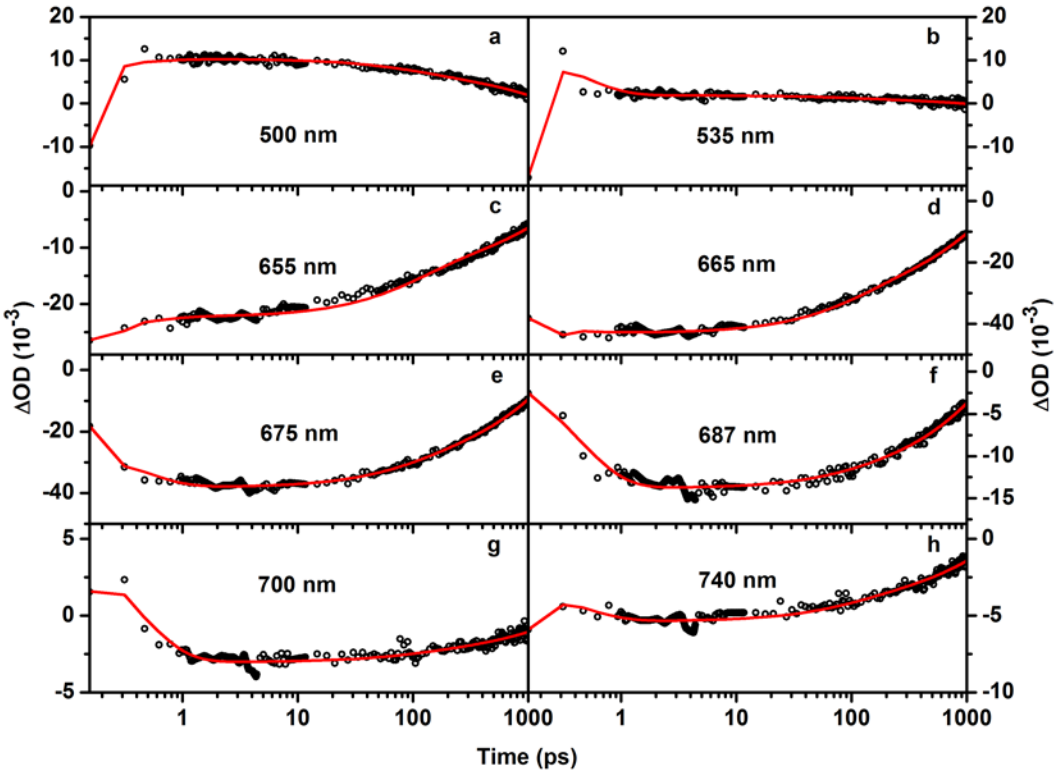

Figure S3. Kinetics at selected wavelengths for showing the quality of global fitting. (a-h) Kinetics at selected wavelengths of 500, 535, 655, 665, 675, 687, 700 and 740 nm are plotted (dot) together with a global fit (red line) of all the collected time traces. The traces are fitted with the target model described in *Inset* of Figure 5a in the Text. The fraction (70/30) of excitation for donor and acceptor at 605 nm is used during fitting.

As a comparison, we have also tried the target global fitting procedure based on the proposed kinetic model with the 100% of direction excitation of donor chromophore at 605 nm, similar results as that with the fraction (70/30) of excitation for donor and acceptor, respectively, from target global fitting for SADS and time-dependent concentrations of transient species are obtained as shown in Figure S4 and S5. The fitted parameters are shown in Table S1.

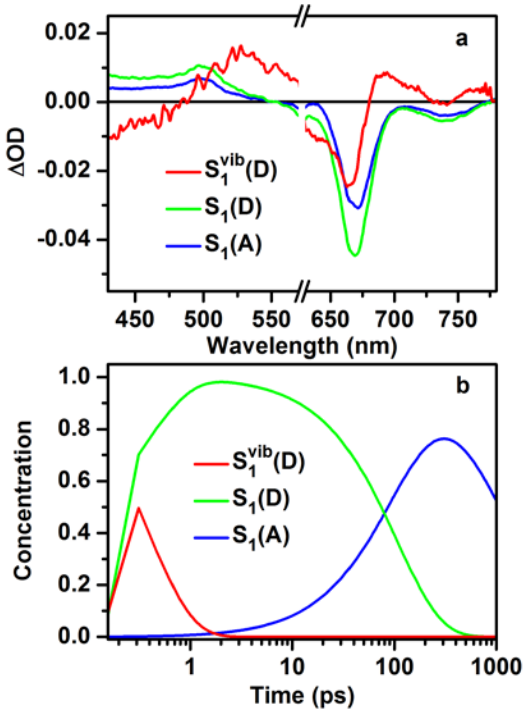

Figure S4. Global analysis of the transient absorption spectra based on the FRET model. (a) SADS obtained from target global fitting according to the FRET model described in *Inset* of Figure 5a in the Text. (b) Time-dependent concentrations of the transient species. Excitation (100%) of donor at 605 nm is used during fitting.

Table S1. Rate constants estimated from global target analysis of ApcE(1-240) dimers.

k_1	k_2	k_3	k_4
$(386 \pm 30\ \text{fs})^{-1}$	$(114 \pm 10\ \text{ps})^{-1}$	$(1.55\ \text{ns})^{-1}$	$(1.50\ \text{ns})^{-1}$

The excitation (100%) of donor at 605 nm is used during fitting.

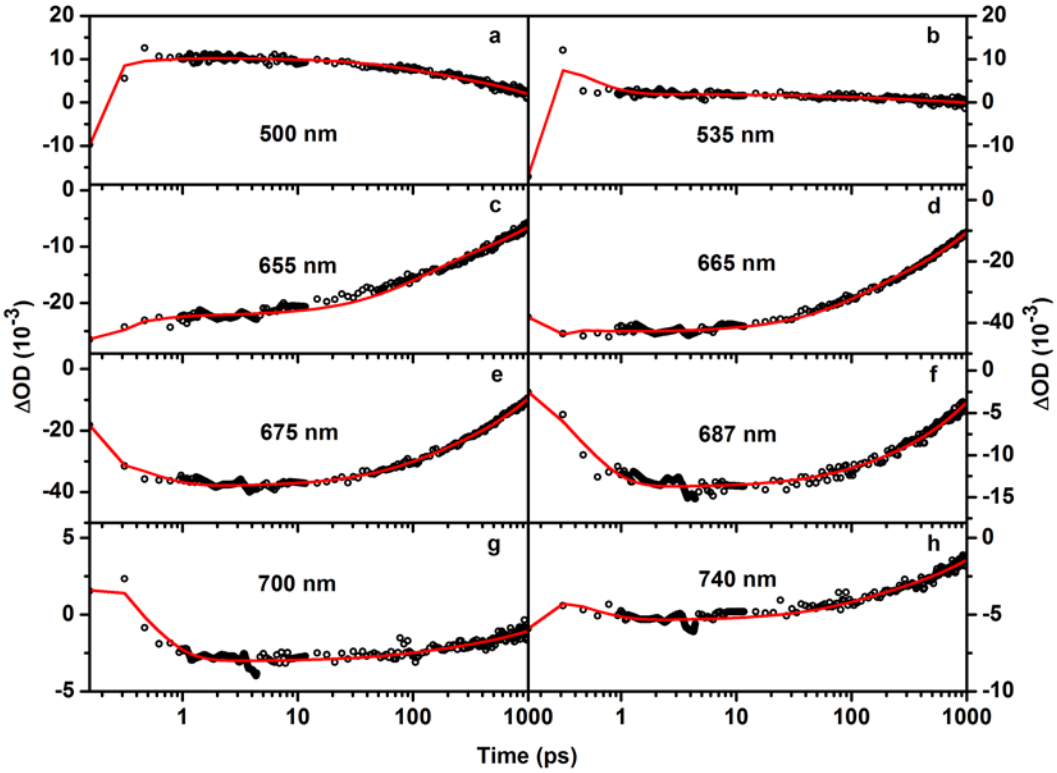

Figure S5. Kinetics at selected wavelengths for showing the quality of global fitting. (a-h) Kinetics at selected wavelengths of 500, 535, 655, 665, 675, 687, 700 and 740 nm are plotted (dot) together with a global fit (red line) of all the collected time traces. The traces are fitted with the target model described in *Inset* of Figure 5a in the Text. Excitation (100%) of donor at 605 nm is used during fitting.

References

1 K. Brejc, R. Ficner, R. Huber and S. Steinbacher, *J. Mol. Biol.*, 1995, **249**, 424-440.

2 D. Loos, M. Cotlet, F. De Schryver, S. Habuchi and J. Hofkens, *Biophys. J.*, 2004, **87**, 2598-2608.