

Supporting information

Investigation on the Thermodynamic Dissociation Kinetics of Photosystem II Supercomplexes to Determine the Binding Strengths of Light-harvesting complexes

Eunchul Kim¹, Ryutaro Tokutsu^{1,2,3}, and Jun Minagawa^{1,2,3*}

¹*Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki 444-8585, Japan*

²*Department of Basic Biology, School of Life Science, Graduate University for Advanced Studies, Okazaki 444-8585, Japan.*

³*Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan.*

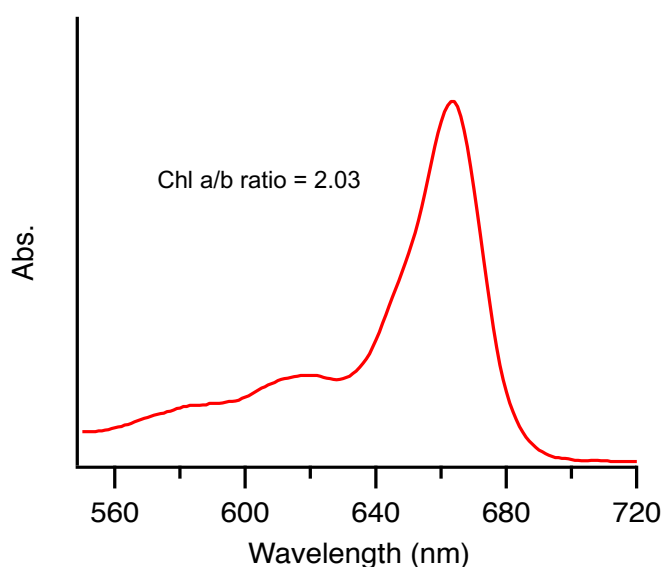


Figure S1. Absorption spectrum and calculated Chl *a/b* ratio of extracted pigments from the PSII supercomplex fraction in 80 % aqueous ethanol. The Chl *a/b* ratio was calculated based on Porra et al.¹.

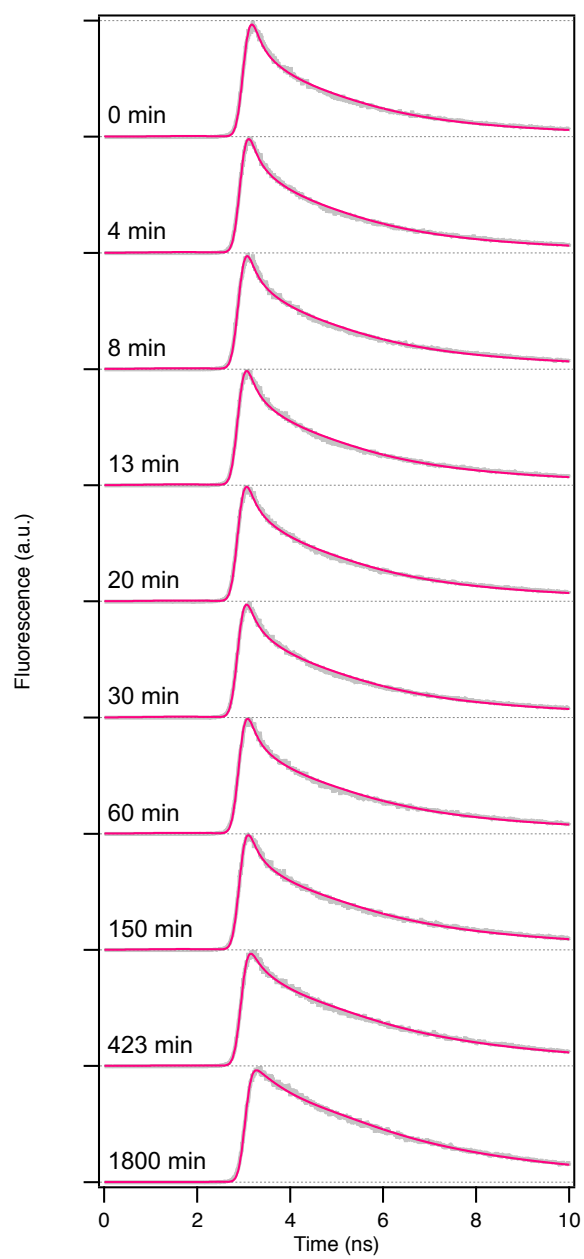


Figure S2. Fluorescence decays measured at 18 °C and fitting curves. Gray and pink lines represent raw data and fitting lines, respectively.

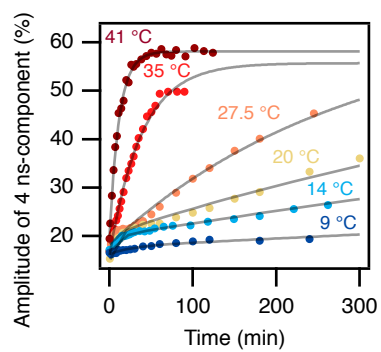


Figure S3. Traces of the 4 ns-fluorescence lifetime component amplitudes at 9, 14, 20, 27.5, 35 and 41 °C. Gray lines represent fitting lines of a bi-exponent function.

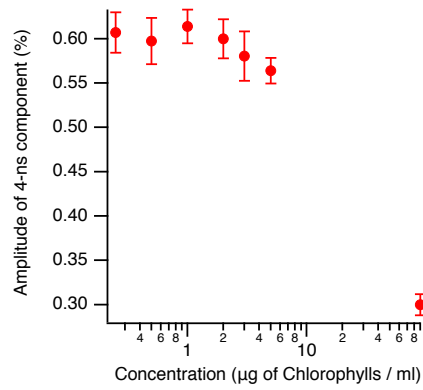


Figure S4. The relationship between concentration of proteins and the amplitude of 4 ns-component on the equilibrated state of dissociation at 23 °C. High density of samples suppress the dissociation. The concentration (2 μg of chlorophylls per ml) was able to induce full dissociation.

Table S1. The number of chlorophylls and chlorophyll a/b ratio of a C₂S₂M₂ PSII supercomplex and a LHCII trimer.

	Total [Chl]	Chl a/b ratio	References
PSII core dimer	70		[2], [3]
CP29 and CP26	13	1.9 and 2.25	[4-6]
LHCII trimer	42	1.33	[7], [8]

Table S2. Estimated the number of chlorophylls and chlorophyll a/b ratio of PSII supercomplexes, and the ratio of LHC.

	Total [Chl]	Chl a/b ratio	[Chl] ratio of LHCII
C ₂ S ₂ M ₂ L ₄	490	1.90	0.69
C ₂ S ₂ M ₂ L ₃	448	1.95	0.66
C ₂ S ₂ M ₂ L ₂	406	2.02	0.62
C ₂ S ₂ M ₂ L ₁	364	2.10	0.58
C ₂ S ₂ M ₂	322	2.20	0.52
C ₂ S ₂ M ₁	280	2.33	0.45
C ₂ S ₂	238	2.51	0.35

Table S3. Global analysis results for fluorescence decays of isolated PSII supercomplexes at 18 °C. Fluorescence decays were obtained by excitation at 441 nm. Each value indicates relative amplitude.

τ (ns)	Time (min)									
	0	4	8	13	20	30	60	150	423	1800
4.00	15.72	18.41	19.80	20.86	21.53	22.54	24.22	29.27	44.87	62.97
1.55	41.45	40.11	40.40	40.69	38.17	37.84	34.26	32.12	30.90	35.15
0.17	42.83	41.48	39.80	38.45	40.31	39.62	41.52	38.61	24.24	1.88

References in Supporting information

1. Porra, R. J.; Thompson, W. A; Kriedemann, P. E. Determination of Accurate Extinction Coefficients and Simultaneous Equations for Assaying Chlorophylls *a* and *b* Extracted with Four Different Solvents: Verification of the Concentration of Chlorophyll Standards by Atomic Absorption Spectroscopy, *Biochim. Biophys. Acta*, **1989**, 975, 384-394.
2. Guskov, A.; Kern, J.; Gabdulkhakov, A.; Broser, M.; Zouni, A.; Saenger, W. Cyanobacterial Photosystem II at 2.9-Å Resolution and the Role of Quinones, Lipids, Channels and Chloride. *Nat. Struct. Mol. Biol.*, **2009**, 16, 334-342.
3. Umena, Y.; Kawakami, K.; Shen, J. R.; Kamiya, N. Crystal Structure of Oxygen-Evolving Photosystem II at a Resolution of 1.9 Å, *Nature*, **2011**, 473, 55-60.
4. Pan, X.; Li, M.; Wan, T.; Wang, L.; Jia, C.; Hou, Z.; Zhao, X.; Zhang, J.; Chang, W. Structural Insights into Energy Regulation of Light-Harvesting Complex CP29 from Spinach. *Nat. Struct. Mol. Biol.*, **2011**, 18, 309-315.
5. Caffarri, S.; Passarini, F.; Bassi, R.; Croce, R. A Specific Binding Site for Neoxanthin in the Monomeric Antenna Proteins CP26 and CP29 of Photosystem II. *FEBS Lett.*, **2007**, 581, 4704-4710.
6. Croce, R.; Muller, M. G.; Bassi, R.; Holzwarth, A.R. Chlorophyll *b* to Chlorophyll *a* Energy Transfer Kinetics in the CP29 Antenna Complex: A Comparative Femtosecond Absorption Study between Native and Reconstituted Proteins. *Biophys. J.*, **2003**, 84, 2508-2516.
7. Liu, Z.; Yan, H.; Wang, K.; Kuang, T.; Zhang, J.; Gui, L.; An, X.; Chang, W. Crystal Structure of Spinach Major Light-Harvesting Complex at 2.72 Å Resolution. *Nature*, **2004**, 428, 287-292.
8. Caffarri, S.; Croce, R.; Breton, J.; Bassi, R. The Major Antenna Complex of Photosystem II has a Xanthophyll Binding Site not Involved in Light Harvesting. *J. Biol. Chem.*, **2001**, 276, 35924-35933.