Supporting Information

for

Evidence for coherent mixing of excited and charge-transfer states in the major plant light-harvesting antenna, LHCII

Charusheela Ramanan[a]*, Marco Ferretti[a], Henny van Roon[a], Vladimir Novoderezhkin[b], Rienk van Grondelle[a]*

[a]*Department of Physics and Astronomy and Institute for Lasers, Life, and Biophotonics, Faculty of Sciences, VU University Amsterdam, De Boelelaan 1081, 1081HV, Amsterdam, The Netherlands*

[b]*A.N. Berlozersky Institut of Physico-Chemical Biology, Moscow State University, Leninskie Gory 1, 119992, Moscow, Russia*

Preparation of LHCII Trimer Samples

LHCII trimers were isolated from spinach as per previously established protocol, with some modifications.[1,2] PSII grana membranes were first isolated from thylakoid membranes. Spinach leaves, obtained from a greenhouse, were depetiolated, washed and ground in a blender in a grinding buffer containing 50 mM MES (pH 7.5), 0.4M NaCl, 2mM $MgCl_2$, 1.0mM EDTA and 2.0mg/ml BSA. After filtration through a nylon cloth the material was centrifuged for 10 min at 6 000 x g. The pellet was re-suspended and washed in a buffer containing 50mM Hepes (pH 7.5), 0.15M NaCl and 4.0mM $MgCl_2$ by centrifugation for 10 min at 10 000 x g.

The chloroplast pellet was re-suspended in a buffer containing 50mM Mes (pH 6.0), 15mM NaCl, 5mM $MgCl_2$ and 1mM Sodium-ascorbic acid until a final concentration of 2.5 mg Chl/ml. Triton X-100 was added slowly from a stock solution to a final concentration of 20 mg/mg Chl. After 15 min incubation at 4 °C in the dark, the suspension was centrifuged for 25 min at 40 000xg. The PSII membrane particles were re-suspended in BTS400: 20mM BisTris (pH 6.5), 20mM $MgCl_2$, 5mM $CaCl_2$, 10mM $MgSO_4$ and 0.4 M sucrose, centrifuged for 25min at 40 000xg. to wash the membranes, after which they were re-suspended again in BTS400 and stored at –80 °C. To separate PS II core and LHCII particles, PSII membranes were diluted in BTS buffer: 20mM BisTris (pH 7), 20mM $MgCl_2$, 5mM $CaCl_2$, 10mM $MgSO_4$ and 0.4 M sucrose to a concentration of 2 mg.Chl/ml. 1/7 volume of a 10 % (w/v) β-DM (β-dodecyl maltoside) solution in BTS buffer was added to a final concentration of 1.25 % (w/v) detergent and 1.75 mg Chl/ml). The suspension was incubated at room temperature for 20 min. gently stirred. By centrifugation for 20 min. at 40.000 x g. min the non-solubilized material was removed. The supernatant was transferred to a Q-Sepharose column (Pharmacia LKB,), equilibrated with BTS400+ β-DM 0.03% to separate LHC II from the PS II core. The enriched LHCII was eluted with BTS buffer + 20mM MgSO4+β-DM 0.03% from the column. The LHCll enriched eluent was then applied on a continuous sucrose gradient and gel filtration to separate the LHCII trimers and monomers. For the sucrose gradient 0.5M sucrose in BTT buffer (20mM BisTris, pH 7, 20mM $MgCl_2$, 5mM $CaCl_2$, 10 mM $MgSO_4$) was frozen at -50 ºC and thawed at RT to create the gradient of sucrose. The separation of the trimers and monomers occurred during centrifugation on the sucrose gradient for 17h at 40000 rpm at 4°C. For the final purification the lowest part of the thick LHCII band was subjected to gel filtration chromatography as described previously[3] using a Superdex 200 HR 10/30 column (GE Healthcare) with 20 mM BisTris (pH 7), 5mM $MgCl_2$ and

0.03% β -DM as mobile phase and a flow rate of 25 ml/h. For detection a Shimadzu LC-10AT VP diode array detector was used. The LHCII trimers peak appeared at 29 min and the monomer peak at 32 min. and could be verified with the spectra and maximum at 675 nm. The particles were kept in dark during the complete isolation procedure.

References for sample preparation:

1 D. A. Berthold, G. T. Babcock and C. F. Yocum, *FEBS Lett.*, 1981, **134**, 231–234.

2 P. J. van Leeuwen, M. C. Nieveen, E. J. van de Meent, J. P. Dekker and H. J. van Gorkom, *Photosynth. Res.*, 1991, **28**, 149–153.

3 H. van Roon, J. F. L. van Breemen, F. L. de Weerd, J. P. Dekker and E. J. Boekema, *Photosynth. Res.*, 2000, **64**, 155–166.

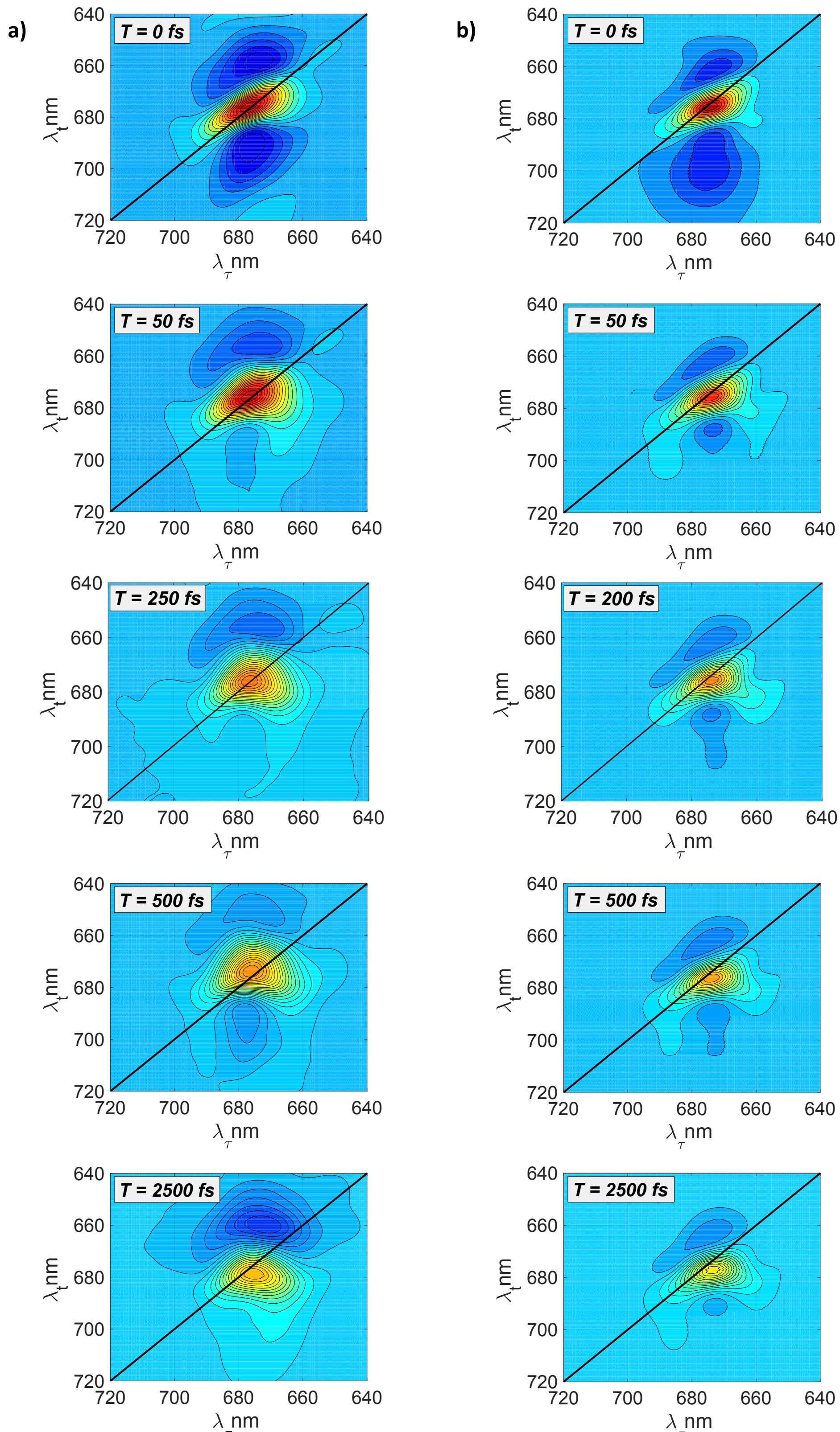

Figure S1. Real total (rephasing plus non-rephasing) 2D spectra at different population times *T* for measurements done at **a)** RT and **b)** 77K.

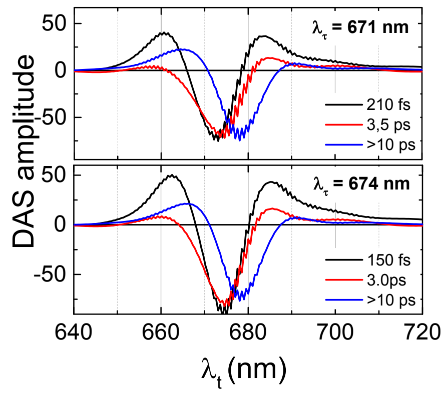

Figure S2. Decay associated spectra (DAS) from global analysis of 2D spectra along selected excitation wavelengths (λ_τ) as indicated.

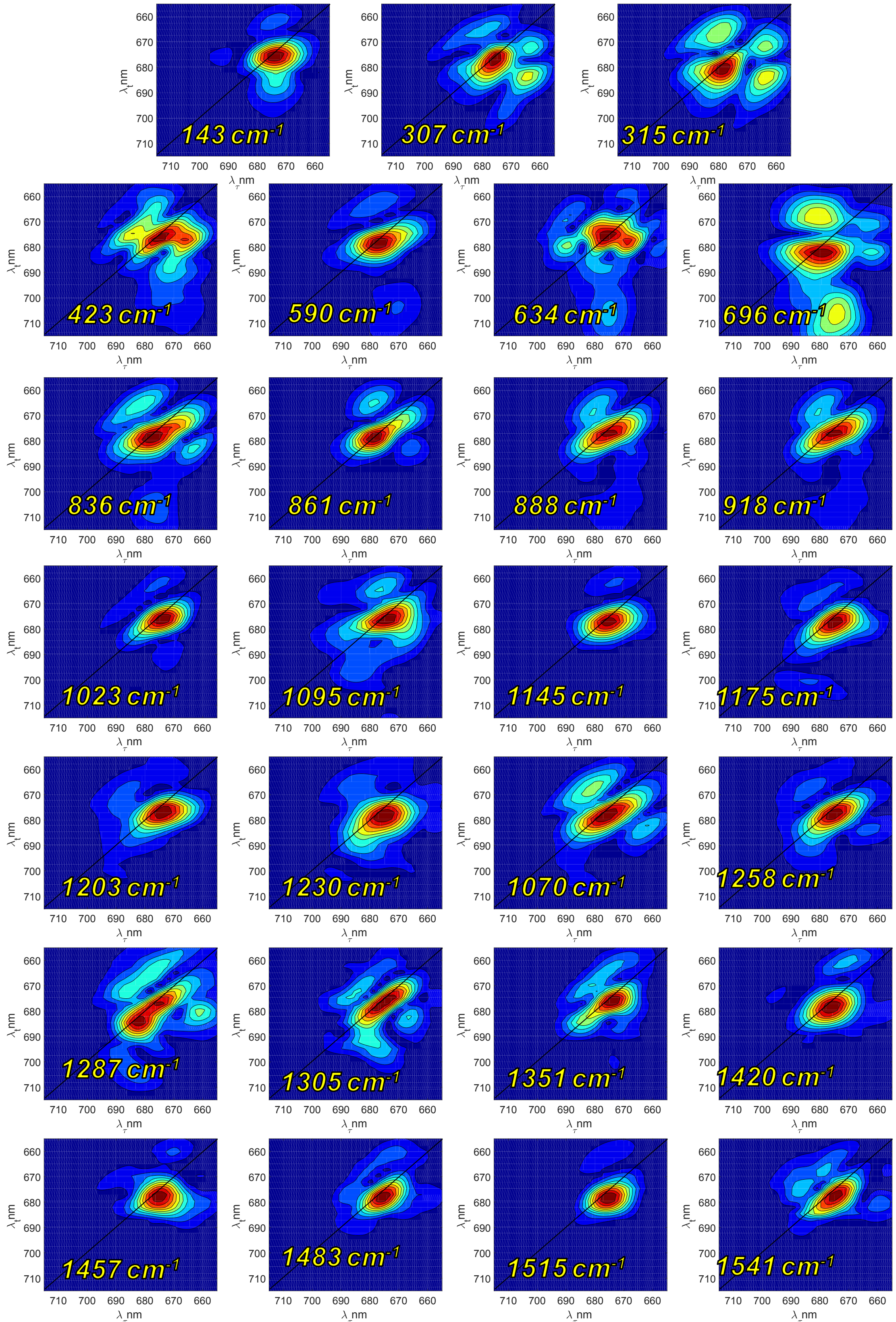

Figure S3. 2DFT maps of LHCII measured at 77K at frequencies which exhibit only vibrational coherences (along the diagonal).

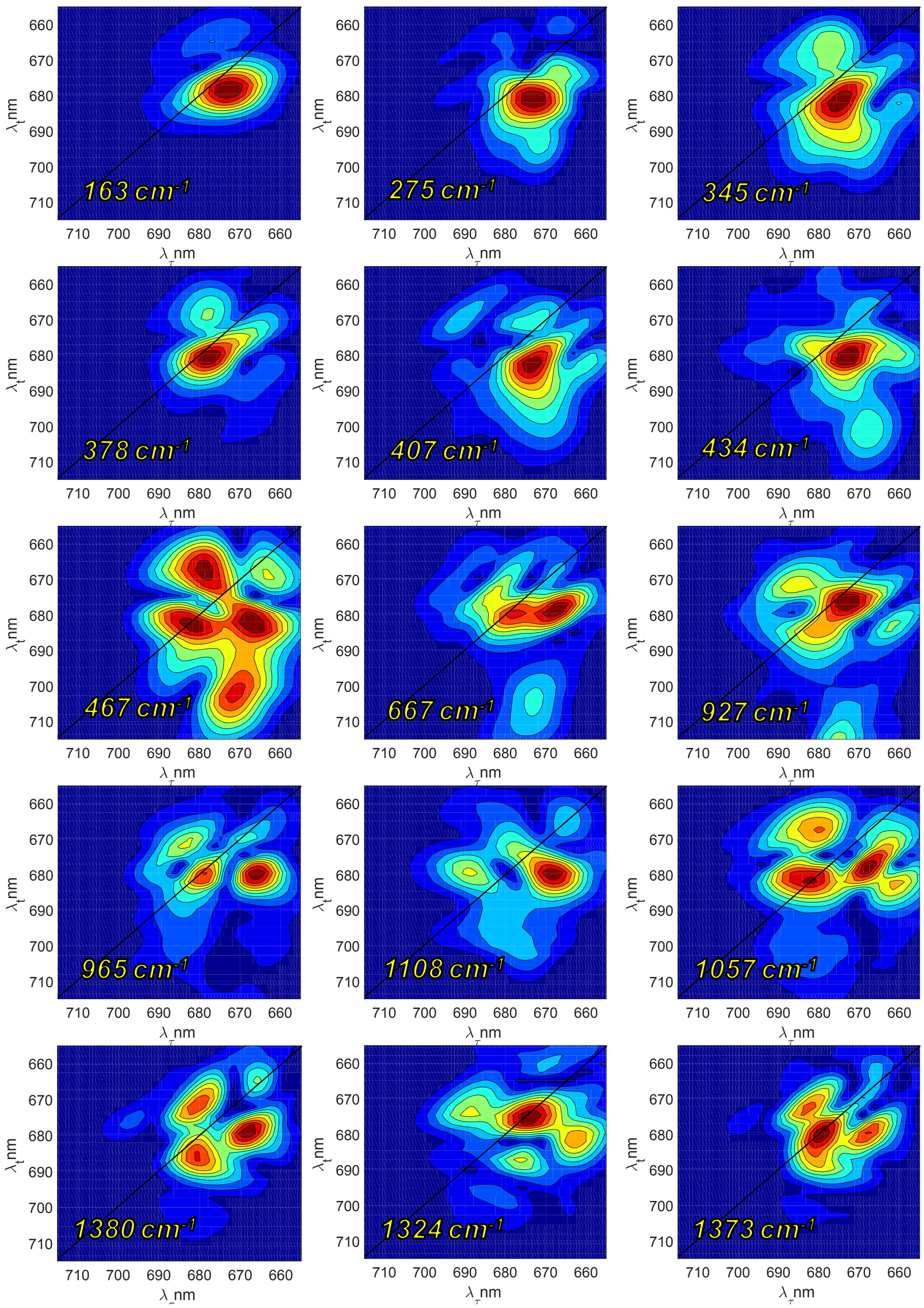

Figure S4. 2DFT maps of LHCII measured at 77K at frequencies which exhibit predominantly vibrational coherences, possibly with some excitonic contribution.

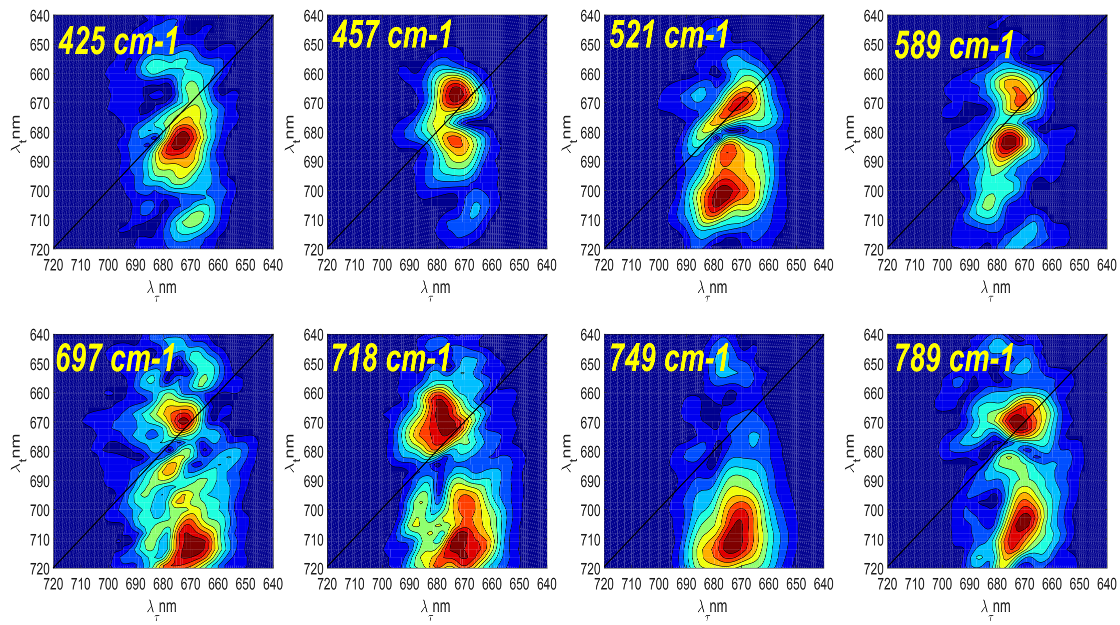

Figure S5. 2DFT maps of LHCII at RT show how the excitonic features are most pronounced at 521 and 749 cm^{-1}.

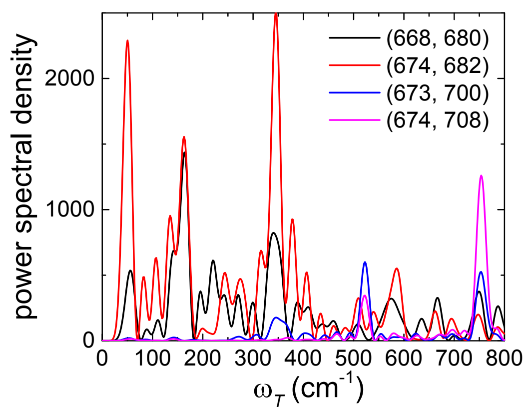

Figure S6. Power spectral density of beating frequencies at the cross peak positions identified in the main text as having significant excitonic contribution.

Table S1. Frequencies from FFT of LHCII 2D spectra at RT compared with literature values for vibrational frequencies of LHCII and Chl *a*.

RT							
(653,675)	(665,685)	(670,710)	(675,685)	(690,695)	FLN of LHCII 4K[a]	Chl a acetone RT TG and PE[b]	Chl a 2 propanol 5K[c]
85	104	85	85	85			100
137	150	137	137	137	138		
182				182			190
221	228	215	221	208	213		
						259	262
				267	260		
299	299	313			298	345	350
							375
345	345	352	339	345	342		390
391	397	391	404	404	388		424
						456	
430		430		436	425	518	520
475	456	475	449	469			
521		514		521	518	579	573
			534		546		
			573		573		607
	586	586			585		
605			612	599	604		
	638		645	638			
658	684	664	684				
690		703	716	690	700	740	
729					722		747
					742	789	799
		749	755	749	752		
768	762						
	814	801	801		795	913	
820		827		833			
879	866	872	866	879		987	
	905		905		916		
944				924			
	977		977	964	986		
					995		

a. Peterman, E. J. G., et al. *J. Phys. Chem. B* **101,** 4448–4457 (1997).

b. Kosumi, D., et al. *J. Photochem. Photobiol. A Chem.* **313,** 72–78 (2015).

c. Ratsep, M., Linnato, J., Freiberg, A. *J. Chem. Phys.* **130,** 194501 (2009).

Table S2a. Frequencies from FFT of LHCII 2D spectra at 77K compared with literature values for vibrational frequencies of LHCII, Chl *a*, and Chl *b*.

(668, 680)	(674, 682)	(673, 700)	(675, 675)	(679, 679)	FLN LHCII 4K[a]	Chl *a* ether 5K S1[b]	Chl *b* ether 5K S1[b]	Chl *a* acetone RT TG and PE[c]	Chl *a* 2-propanol 5K[d]
55	50		57	59					
88	83				97				100
111	107		111	116					
143	135	142	140	145	138				
164	163		163	164					
195			190	190					190
220			215	213	213				
243	244		241	243			245		
270	275	272		283	260	263	255	259	262
299	316	308	306	317	298		310		
340	345	345	353	345	342	348	345	345	350
389	378		378	376	388	390	375		375
410	407	404					405		390
438	435	443	423	426	425				424
459		467	452	451		465	465	456	
508	509	493	508	479					
		522		508	518	515	515	518	520
	540	555		537	546				
575		581	562	563	573	570	560	579	573
	586	592	589	589	585	583	595		
					604	600			607
635		623	635			635	625		
667	662	671	656	656		670	680		
	695	697	684	687	700	688	700		
			719	715	722		732		
					742	740	742	740	
749	747	754	750	754	752	748	750		747
						765	765		
788			806	807	795	785		789	799

a. Peterman, E. J. G., et al. *J. Phys. Chem. B* **101,** 4448–4457 (1997).

b. Avarmaa, R. A. & Rebane, K. K *Spectrochim. Acta Part A Mol. Spectrosc.* **41,** 1365–1380 (1985).

c. Kosumi, D., et al. *J. Photochem. Photobiol. A Chem.* **313,** 72–78 (2015).

d. Ratsep, M., Linnato, J., Freiberg, A. *J. Chem. Phys.* **130,** 194501 (2009).

Table S2b. Frequencies from FFT of LHCII 2D spectra at 77K compared with literature values for vibrational frequencies of LHCII and Chl *a*, and Chl *b*.

(668, 680)	(674, 682)	(673, 700)	(675, 675)	(679, 679)	FLN LHCII 4K[a]	Chl *a* ether 5K S1[b]	Chl *b* ether 5K S1[b]	Chl *a* acetone RT TG and PE[c]
	837		837	835			832	
	861		861	861		880	885	
890	889		887	887		890		
	918		915	915	916	910		913
928						925	923	
965					986	984	980	987
1030	1024		1022	1022	995	1005	1003	
1058			1048		1052	1030	1040	
	1071		1074	1073	1069	1075	1070	
			1099	1095			1090	
1108				1120	1110	1110	1120	
1144	1146		1146	1146	1143	1135	1140	1147
1175	1174		1170	1170	1181	1168		
					1190	1195	1190	
1204	1203		1201	1201	1208			
					1216		1217	
1235	1230		1232	1230	1235	1243		1241
					1252	1250	1253	
1261	1258		1260	1258	1260			
			1287	1286	1286	1275	1275	
		1305	1305	1307	1304			
1325			1330	1331	1322	1325		
					1338	1345	1330	1327
1351			1359	1362	1354			
	1374					1372	1370	
1380					1382	1395	1390	
1406	1419		1419	1421		1415		
1460	1457		1453	1453	1439			
	1483		1489	1488	1487		1480	
1520	1515		1519	1517	1524	1510	1515	
1546	1541		1543	1543	1537	1530	1540	