Electronic Supplementary Material

The pigment binding behaviour of Water-Soluble Chlorophyll Protein (WSCP)

Philipp Girr[1,2], Jessica Kilper[1], Anne-Christin Pohland[1], Harald Paulsen[1,*]

[1] Institute of Molecular Physiology, Johannes Gutenberg-University Mainz, Johannes-von-Müller-Weg 6, 55128 Mainz, Germany

[2] current address: Department of Biology, University of York, Heslington, York YO10 5DD, United Kingdom

* paulsen@uni-mainz.de

Contents

Table S1: Fitted parameters of the pigment-binding of WSCPs from TX-100 micelles. CD Coefficient k in mdeg*l*µmol^{-1}, chlorophyll concentration in µM and dissociation constant K_D in µM.

Sample	k	[Chl]	K_D	R^2
LvWSCP Chl *a*	1.85±0.05	31.57±0.86	0.06±0.12	0.99
LvWSCP Chl *b*	7.16±0.19	17.66±0.42	0.53±0.12	0.99
BobWSCP Chl *a*	2.28±0.04	29.5±0.48	0.14±0.08	0.99
BobWSCP Chl *b*	4.75±0.08	16.64±0.26	0.09±0.05	0.99
LvWSCP PCPS Chl *a*	2.25±0.05	28.49±0.65	0.03±0-07	0.99
LvWSCP PCPS Chl *b*	5.57±0.21	17.5±0.49	0.09±0.14	0.99
LvWSCP Chlide *a*	2.27±0.53	11.97±2.52	2.56±1.17	0.98
LvWSCP Chlide *b*	1.39±0.25	26.27±3.91	3.71±2.28	0.98
BobWSCP Chlide *a*	1.61±0.69	11.27±4.37	1.6±1.77	0.91
BobWSCP Chlide *b*	1.59±0.31	12.56±2.2	1.34±0.84	0.98
LvWSCP PCPS Chlide *a*	2.74±0.42	10.83±1.51	0.52±0.43	0.97
LvWSCP PCPS Chlide *b*	1.61±1.52	17.88±14.88	24.67±15	0.99
LvWSCP Pheo *a*	3.22±1.11	21.72±8.24	72.95±11.42	0.99
BobWSCP Pheo *a*	1.37±0.25	20.4±3.21	2.71±1.58	0.98

Table S2: Fitted parameters of pigment-binding kinetics of WSCPs from TX-100 micelles. Amplitudes A in mdeg

Sample	A_0	A_1	τ_1	A_2	τ_2	R^2
LvWSCP Chl *a*	0.00±0.54	55.57±0.54	8.73±0.11	6.0±0.15	143.88±4.55	0.95
LvWSCP Chl *b*	-0.64±0.72	91.36±0.73	6.21±0.07	11.13±0.12	239.81±4.03	0.97
BobWSCP Chl *a*	2.89±0.59	46.78±0.62	2.73±0.35	1.5±0.17	90.5±13.43	0.79
BobWSCP Chl *b*	-2.44±0.03	73.27±0.15	6.45±0.08	4.49±0.11	268.1±11.5	0.92
LvWSCP PCPS Chl *a*	-3.5±0.55	75.25±0.56	3.17±0.06	4.25±0.09	197.24±6.61	0.94
LvWSCP PCPS Chl *b*	-3.24±0.74	83.14±0.74	7.5±0.08	8.36±0.11	393.26±6.88	0.96
LvWSCP Chlide *a*	3.51±0.17	20.58±0.18	4.08±0.1	2.74±0.08	59.07±1.92	0.94
LvWSCP Chlide *b*	-0.75±0.21	24.97±0.21	4.59±0.07	3.45±0.03	324.68±5.27	0.96
BobWSCP Chlide *a*	3.5±0.2	14.37±0.22	5.03±0.14	0.27±0.12	55.4±24±71	0.84
BobWSCP Chlide *b*	0.93±0.15	32.02±0.15	1.55±0.08	0.63±0.03	175.75±10.81	0.97
LvWSCP PCPS Chlide *a*	2.9±0.16	24.52±0.18	4.58±0.08	0.99±0.08	66.27±5.53	0.94
LvWSCP PCPS Chlide *b*	0.97±0.2	16.92±0.2	4.36±0.08	2.13±0.04	162.6±4.23	0.91
LvWSCP Pheo *a*	0.71±0.08	8.93±0.08	12.06±0.14	22.85±0.03	155.04±0.20	0.99
LvWSCP Pheo *b*	6.82±0.08	15.59±0.08	6.75±0.05	2.12±0.02	120.73±1.60	0.98
BobWSCP Pheo *a*	0.82±0.18	29.8±0.18	5.89±0.05	2.25±0.02	314.47±5.93	0.97
BobWSCP Pheo *b*	8.13±0.04	3.76±0.04	29.42±0.54	3.21±0.02	251.26±1.89	0.99

and time constants τ in s.

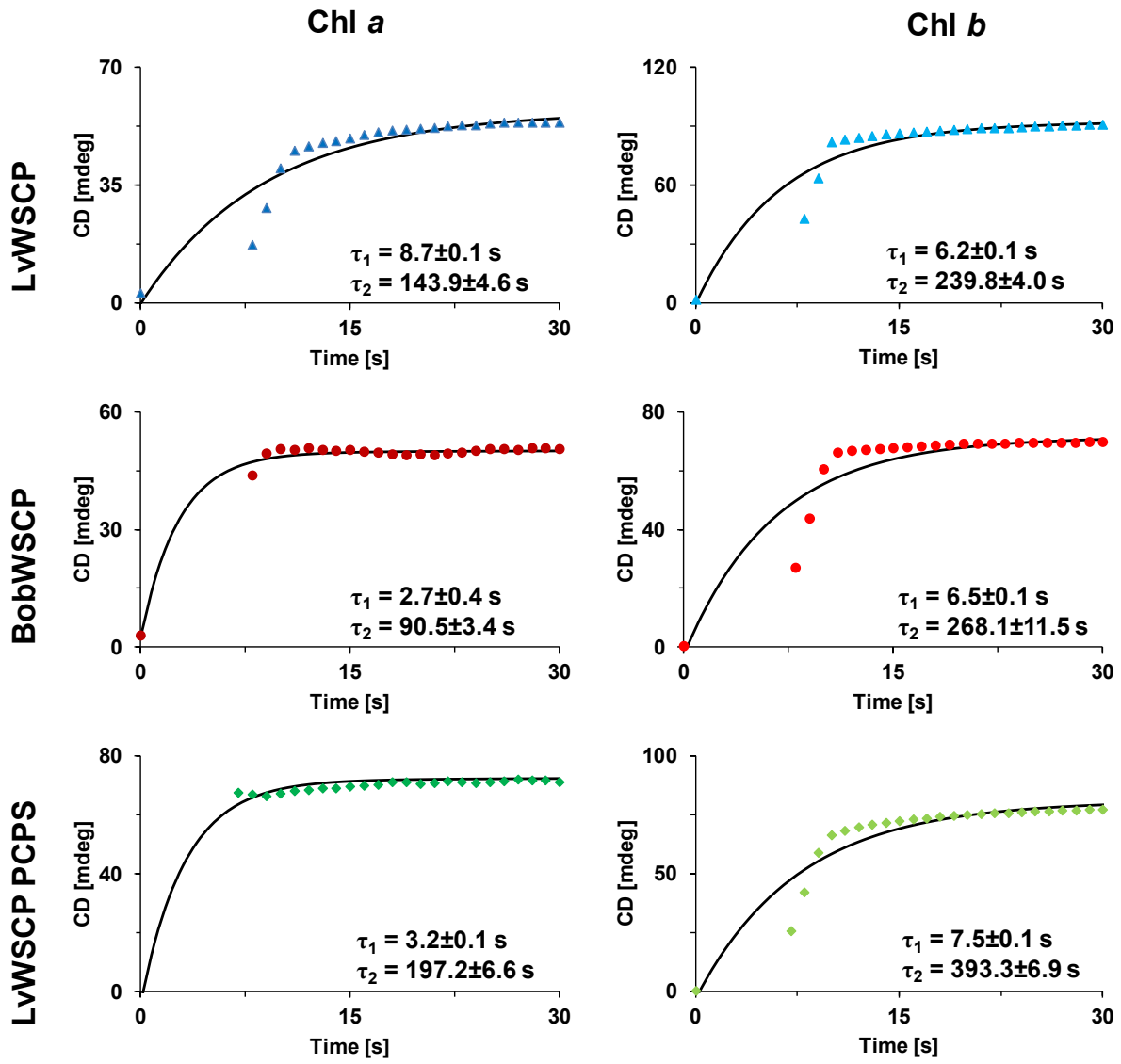

Figure S1: First 30 s of time-resolved CD measurements of the Chl *a*/*b* binding to LvWSCP (blue), BobWSCP (red) and LvWSCP PCPS (green) and the corresponding fits (black) (Fig. 2). 40 µM WSCP (final) were mixed with 20 µM Chl (final) in 0.1 % TX-100 (final) at RT. The experimental dead time amounts to 8 s for all measurements. The fitted time constants τ (in s) are indicated for each measurement.

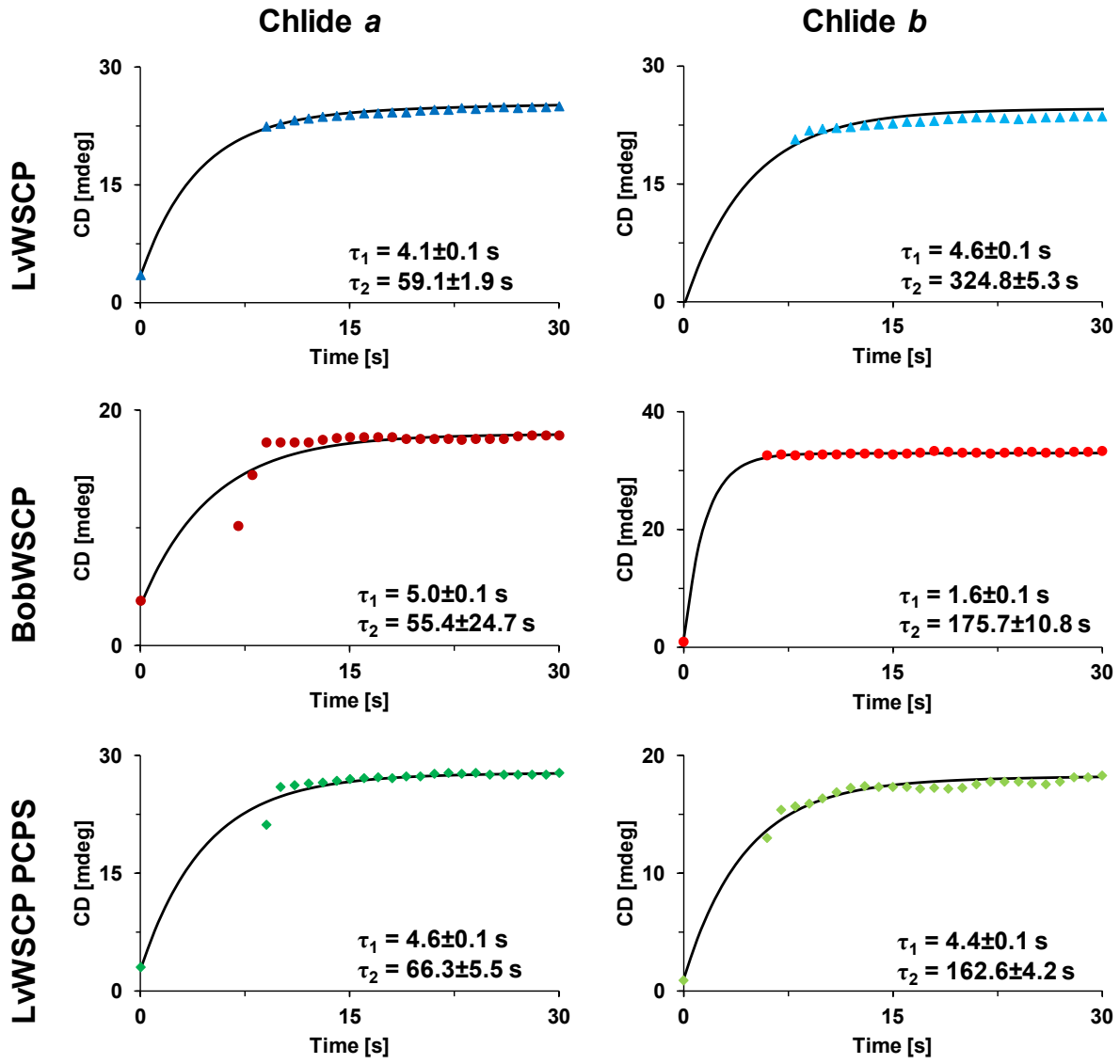

Figure S2: First 30 s of time-resolved CD measurements of the Chlide a/b binding to LvWSCP (blue), BobWSCP (red) and LvWSCP PCPS (green) and the corresponding fits (black) (Fig.4). 40 µM WSCP (final) were mixed with 20 µM Pheo (final) in 0.1 % TX-100 (final) at RT. The experimental dead time amounts to 6 s for BobWSCP Chlide b and LvWSCP PCPS Chlide b, to 7 s for BobWSCP Chlide a, to 8 s for LvWSCP Chlide b, and to 9s for LvWSCP Chlide a and LvWSCP PCPS Chlide a. The fitted time constants τ (in s) are indicated for each measurement.

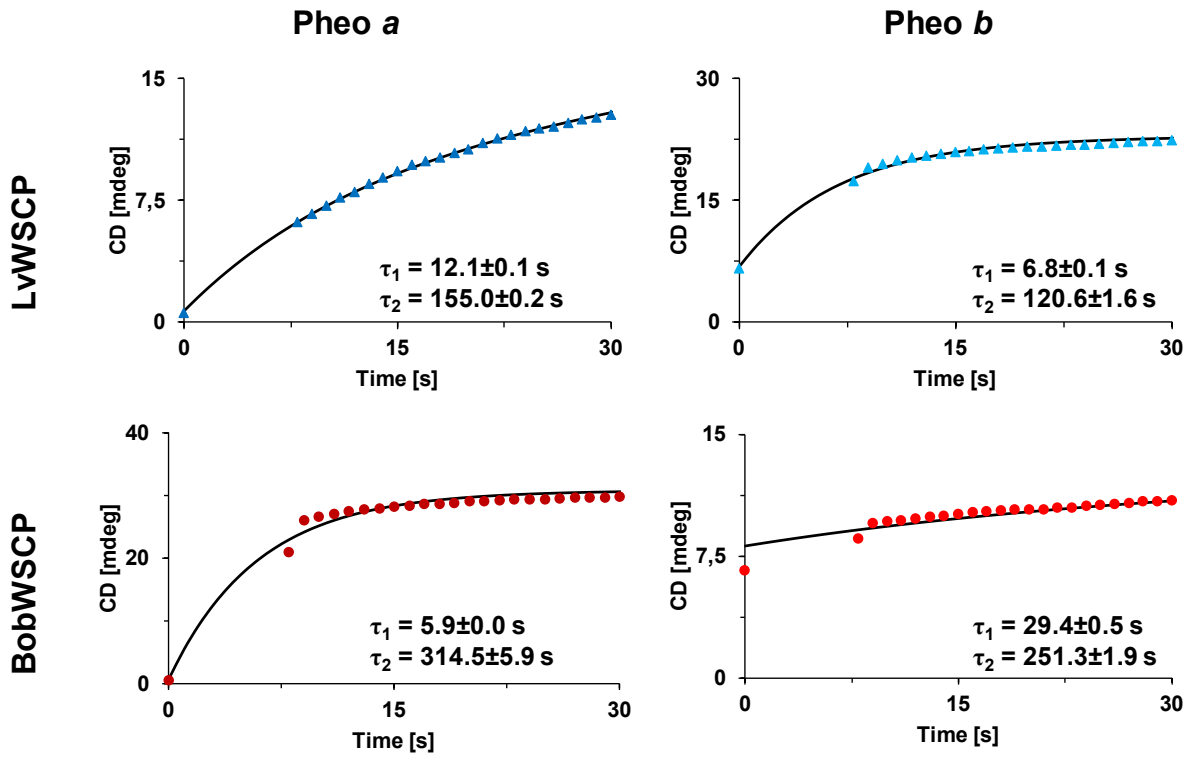

Figure S3: First 30 s of time-resolved CD measurements of the Pheo *a*/*b* binding to LvWSCP (blue) and BobWSCP (red) with and the corresponding fits (black) (Fig. 6). 40 µM WSCP (final) were mixed with 20 µM Pheo (final) in 0.1 % TX-100 (final) at RT. The experimental dead time amounts to 6 s for BobWSCP Pheo a, and to 8 s for all other measurements. The fitted time constants τ (in s) are indicated for each measurement.

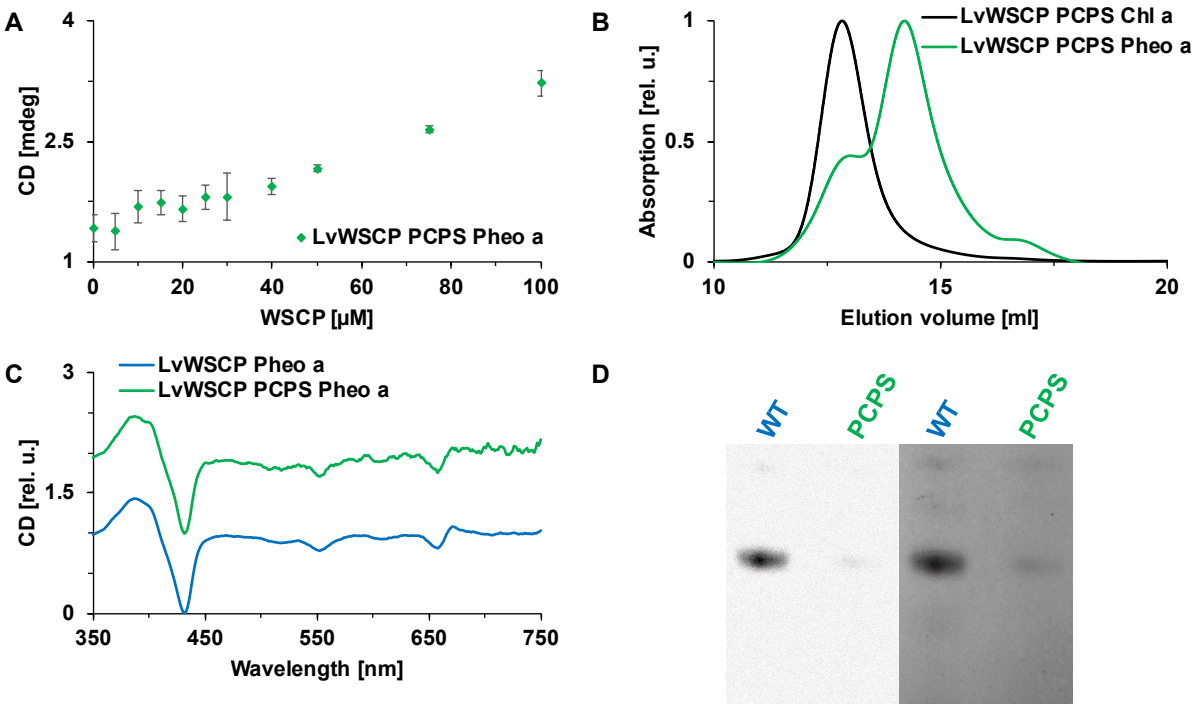

Figure S4: Pheo *a* binding to LvWSCP PCPS. **A** Titrations of Pheo *a* with LvWSCP PCPS. Data points are the means of three measurements and the errors are given as standard deviations. **B** SEC elution diagram of LvWSCP PCPS reconstituted with Pheo *a* (green) in comparison with LvWSCP PCPS Chl *a* tetramers (black). Protein absorption was detected at 280 nm. **C** CD spectra of purified LvWSCP Pheo *a* (blue) and LvWSCP PCPS Pheo *a* (green). D Native PAGE of LvWSCP Pheo *a* (WT) and LvWSCP PCPS Pheo *a* (PCPS). Tetramers were detected via fluorescence (left panel) and by coomassie staining (right panel).

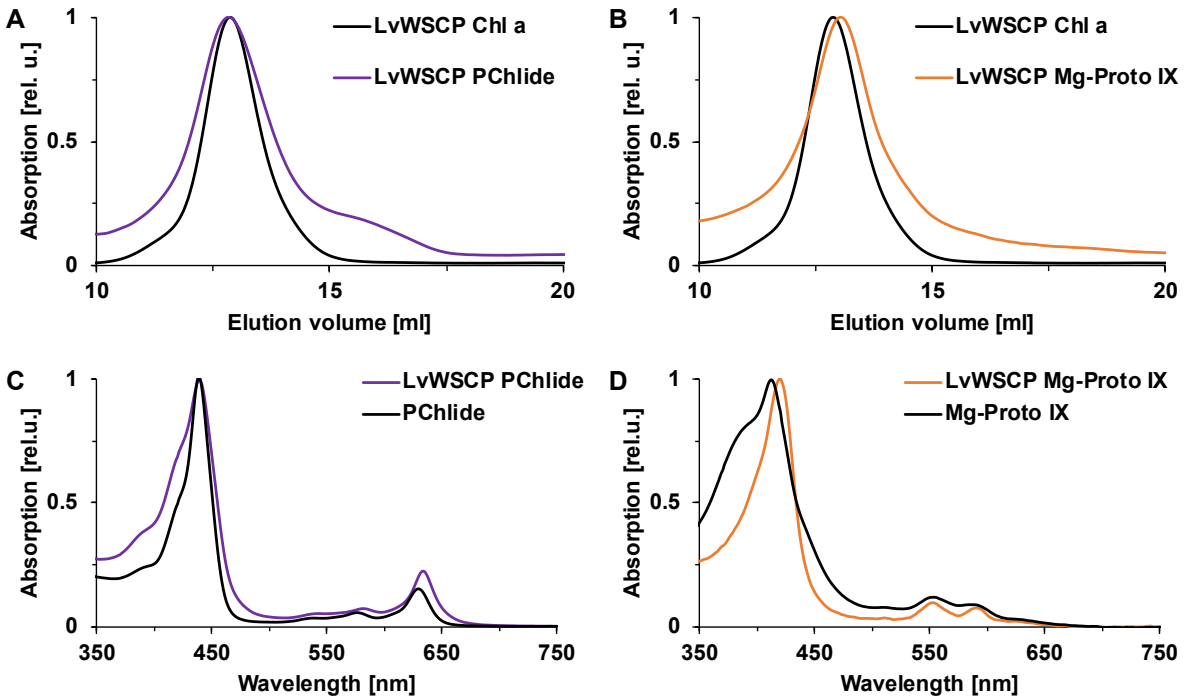

Figure S4: PChlide and Mg-Proto IX binding to LvWSCP. **A** SEC elution diagram of LvWSCP reconstituted with PChlide (purple) in comparison with LvWSCP Chl *a* tetramers (black). Chl *a* absorption was detected at 670 nm and PChlide absorption at 440 nm. **B** SEC elution diagram of LvWSCP reconstituted with Mg-Proto IX (orange) in comparison with LvWSCP Chl *a* tetramers (black). Chl *a* absorption was detected at 670 nm and PChlide absorption at 415 nm. **C** Absorption spectrum of purified LvWSCP PChlide (purple) in comparison with PChlide resolved in 2 % TX-100 (black). **D** Absorption spectrum of purified LvWSCP Mg-Proto IX (orange) in comparison with Mg-Proto IX resolved in water (black).

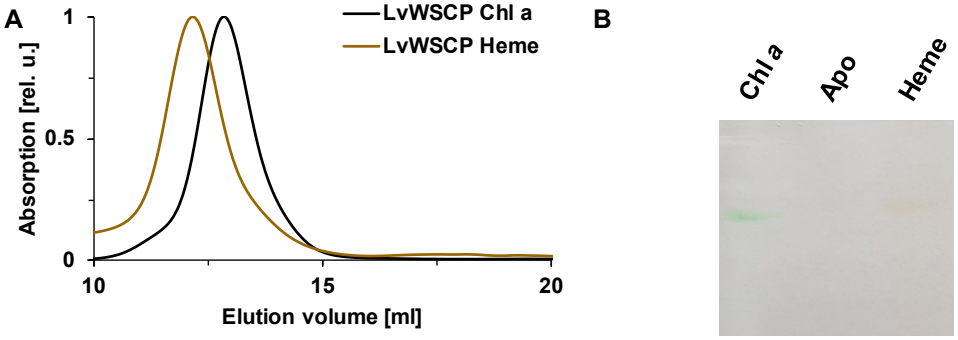

Figure S5: Heme *b* binding to LvWSCP. **A** SEC elution diagram of LvWSCP reconstituted with heme (brown) in comparison with LvWSCP Chl *a* tetramers (black). Chl *a* absorption was detected at 670 nm and heme absorption at 420 nm. **B** Native PAGE of LvWSCP heme reconstitution in comparison with LvWSCP Chl *a* and LvWSCP apoprotein. Unstained and unedited photograph of the gel. LvWSCP Chl *a* is visible as a green band and LvWSCP heme as a red band.