Supplementary Information for

Integration of Multiple Chromophores with Native Photosynthetic Antennas to Enhance Solar Energy Capture and Delivery

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Sequences of Native Peptides. The sequences of the LH1 (B875) α- and β-peptides are as follows:[19]

α from *Rb. sphaeroides* LH1 (B875)
MSKFYKIWMIFDPRRVFVAQGVFLFLLAVMI**H**LILLSTPSYNWLEISAAKYNRVAVAE

β from *Rb. sphaeroides* LH1 (B875)
ADKSDLGYTGLTDEQAQELHSVYMSGLWLFSAVAIVA**H**LAVYIWRPWF

Additional Experimental Procedures

Isolation of the α and β peptides from the Ser(−34β)Cys and Asp(−20α)Cys mutants. About 300 mg of lyophilized membranes from the mutant bacteria of *Rb. sphaeroides* containing Cys at postion −34 of the LH1 β peptide was extracted with 4 mL of $CHCl_3/CH_3OH$ (1:1) containing 0.1 M ammonium acetate (CMAc). After 5 min centrifugation in a tabletop centrifuge, the supernatant was carefully removed and the pellet was subjected to a second extraction with an additional 2 mL of CMAc. After centrifugation for 5 min, the supernatant was added to that of the first extraction, and the combined extract was applied to a LH60 column for separation of the RC, LH1 α- and β-peptides and pigments.[51,54] The separation of components is indicated in Fig. S1a. The region containing the α- and β-peptides was divided into three fractions: early, middle and late. Each pooled fraction was immediately evaporated to dryness under a stream of argon, further dried overnight on a lyophilizer, and then stored at −20 °C. A portion of each sample was dissolved in 10–30 μL of HFA with sonication; an equivalent volume of HPLC A/B solvent was added; the sample was centrifuged; and the supernatant injected into the HPLC.

For comparison, a comparable separation of components from native membranes of *Rb. sphaeroides* is shown (Fig. S1b). There is clearly a difference in the separation of the LH1 α- and β-peptides. Because of its smaller size, the mutant β-peptide would be expected to trail the α-peptide as it does in the profile for native membranes (Fig. S1b). We suspected that the Cys-containing mutant β-peptide had dimerized in the membrane by forming a disulfide bridge between adjacent β-peptides. This conclusion was further supported by comparing the behavior of the mutant peptide to that of the native peptide on HPLC. As shown in Figs. S2a-d, the mutant β-peptide eluted at 25 min compared to 19 min for the native β-peptide, consistent with expectation for a dimerized mutant peptide.

To reduce the putative disulfide linkage of the Ser(−34β)Cys mutant β-peptide, the CMAc extract was incubated with β-mercaptoethanol before application to the LH60 column. The resulting profile (Fig. S1c) was very similar to that observed for the extract of native membranes (Fig. S1b), and the HPLC results of the αβ fraction were also consistent with those of the native αβ fraction (c.f. Figs. S2a,b and S2e,f). This same procedure was used for isolation of the α-peptide from lyophilized membranes of the Asp(−20α)Cys mutant.

Preparation of peptides with covalently attached chromophores. The procedure followed was essentially that recommended by Invitrogen:Molecular Probes (Handbook of Fluorescent Probes and Research Products 2001) and is summarized here for conjugation of **OGR** to the synthetic β-peptide with Cys at position −34. A sample of the peptide (6.0 mg) was dissolved in 660 μL of *N,N*-dimethylformamide (DMF), and 140 μL of 100 mM Tris buffer (pH 7.3) was added with stirring. A sample of **OGR** (4.0 mg) was dissolved in 330 μL of DMF, and 70 μL of 100 mM Tris buffer (pH 7.3) was added. This latter solution was slowly added to the former with stirring and under a gentle flow of argon. After an additional 10 min under argon, the sample was stoppered and stirred for 2 h at room temperature in the dark. The reaction mixture was then dried under vacuum and stored at −20 °C. A portion of the dried material was dissolved in 35 μL of hexafluoroacetone trihydrate (HFA), to which was then added 35 μL of 1:1 HPLC A:B solvent, and the sample was injected into the HPLC (Waters system consisting of two 501 pumps, a system interface module, a 486 tunable absorbance detector, and a U6K injector). Perkin-Elmer HCODS C18 columns (150 x 4.6 mm) were used for all purifications. The HPLC solvent system consisted of (A) 0.1% trifluoroacetic acid (TFA) in water as the aqueous solvent and (B) 0.1 % TFA in 2:1 (v/v) acetonitrile/2-propanol as the organic solvent.[16]

Covalent attachment of **OGR** to the β-peptide from the Ser(−34β)Cys mutant gave a major peak on HPLC that contained **OGR** and protein (Fig. S3a). Similarly, attachment of **RR** to the α-peptide from the Asp(−20α)Cys mutant was isolated by HPLC, but as a minor peak (Fig. S3b). In the latter case, the amount of membrane material available was more limited because of a low yield of mutant cells, and the yield of purified α-peptide was also low compared to that of the β-peptide from the same cells. For the synthetic peptides, the yields of covalently modified peptide were greater and exhibited major peaks on HPLC (Figs. S3c and S3d).

Preparation of αβ-dyads and oligomers. The conditions for forming αβ-dyads (B820-type) and oligomers (LH1-type) have been previously described[19,20,21] and were followed with one change. After the solution mixture of peptides was brought to 0.90% octyl glucoside and before BChl *a* was added, the acidity of hexafluoroacetone (HFA) was neutralized. For each 10 μL of HFA used to dissolve the peptides, 15 μL of 3 M KOH was added. In general, the peptides were dissolved in small amounts of HFA and then taken up in 4.5% octyl glucoside,

which was later diluted to 0.90%. BChl *a* in acetone was added to the peptide-detergent solution until the BChl *a* concentration was approximately the same as that of the peptide as indicated by an increase in the ratio of absorbance at 780 nm compared to that at 820 nm (e.g., Fig. S4). Typically, this resulted in a combined absorbance ($A_{780\ nm} + A_{820\ nm}$) between 0.1 and 0.2. The detergent solution sample was then diluted to optimize formation of the αβ-dyad. LH1-type oligomers were formed by overnight incubation of the sample at 48 °C.

Calculation of chromophore/protein and chromophore/B820 ratios. In some cases, the ratio of chromophore to protein could be calculated from the absorption spectra of isolated peak fractions on HPLC. This was reasonably accurate for the β-peptide with **BC1** attached, which was clearly a distinct peak on HPLC (Fig. S3d), and the protein absorbance at 289 nm is not obscured (Fig. S4). The molar absorption coefficient at 289 nm for this type of peptide has been shown to be 3,400 $M^{-1}cm^{-1}$/Trp.[16,40] More relevant, however, is the ratio of chromophore to B820 observed during complex formation. Given that the molar absorption coefficients of the covalently linked chromophores are accurately known [ε of the Q_y band of **BC1** = 120,000 $M^{-1}cm^{-1}$,[21,41,42] ε of **OGR** = 77,000 $M^{-1}cm^{-1}$ and that of **RR** = 126,000 $M^{-1}cm^{-1}$ (Molecular Probes)] as well as those of B820 (86,000 $M^{-1}cm^{-1}$) and free BChl *a* (55,000 $M^{-1}cm^{-1}$),[19,40,51,52] the concentration of the chromophores and that of total BChl *a* can be calculated by evaluating the absorption of each under equilibrium conditions, typically at 0.75% octyl glucoside. The spectra at this percent octyl glucoside are chosen since a prominent B820 complex is usually formed and the data are acquired within about 3 min of the addition of BChl *a* to the peptide solution. Therefore, little decomposition of the BChl *a* has occurred.

Although the B820 complex is even better formed at 0.66% octyl glucoside, often some B820 association has begun to occur so that larger oligomers are forming, the wavelength maxima and molar absorption coefficients of which are not well known. For many of the complexes, as a first approximation, the assumption can be made that the free BChl *a*, along with that in B820, will become part of the LH1-type complex when the latter is formed. Evaluation of the absorbance of each species was done after correcting each spectrum for scattering. It is assumed that the absorption spectrum in the region 720 to 860 nm at 0.75% octyl glucoside consists of the sum of only two species, that of free BChl *a* and that of B820 which are in equilibrium.[19,51] The contribution of these two species to the spectrum was estimated by approximating the concentration of free BChl *a* by subtraction of B820 absorption at 780 nm according to the absorbance ratio of 780/820 = 0.24 in pure B820 preparations.[19,40] The resultant chromophore/B820 ratios are shown in Table S1.

Table S1. Energy-Transfer Donor Pigment/Dyad Ratios.

Antenna	Process	Donor pigment per αβ-dyad
Design 1		
A-1	**OGR** →B875	1.0
A-2	**RR**→B875	1.4
A-3	**OGR→RR**	
A-3	**RR**→B875	1.3
A-3	**OGR→RR**→B875	2.1
Design 2		
A-4	**OGR**→B875	2.4
A-5	**BC1**→B875	1.3
A-6	**OGR→BC1**	
A-6	**BC1**→B875	1.2
A-6	**OGR→BC1**→B875	0.8

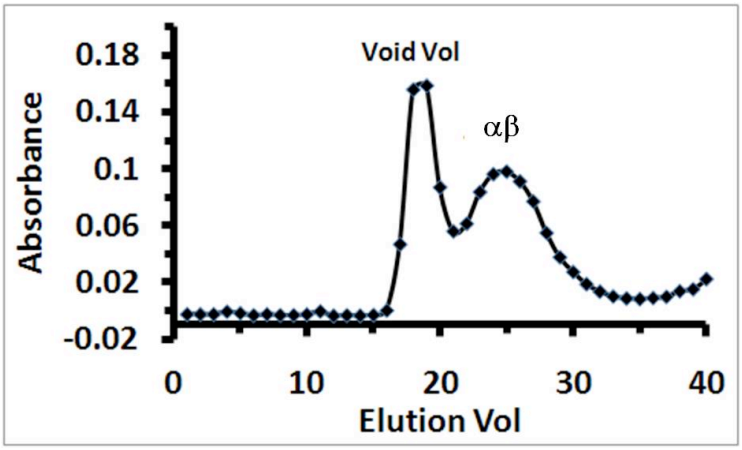

Figure S1a. LH 60 column profile for the CMAc extract from membranes of the Ser(–34β)Cys mutant. Not shown at longer elution times are the carotenoid and BChl *a* pigments.

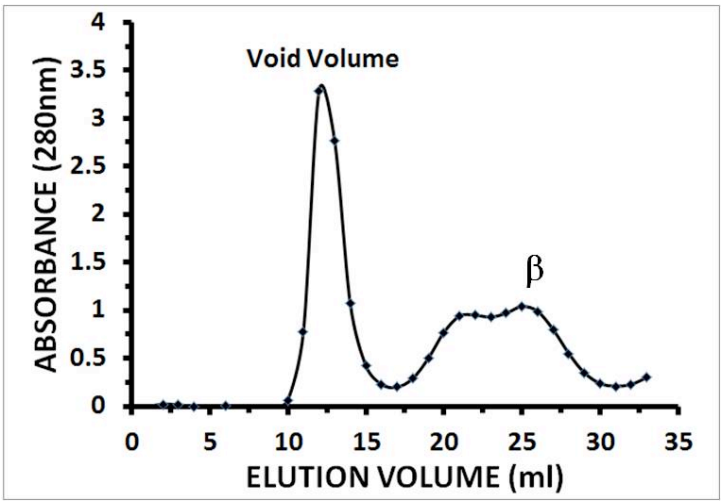

Figure S1b. LH 60 column profile for the CMAc extract from native *Rb. sphaeroides* membranes. Not shown at longer elution times are the carotenoid and BChl *a* pigments.

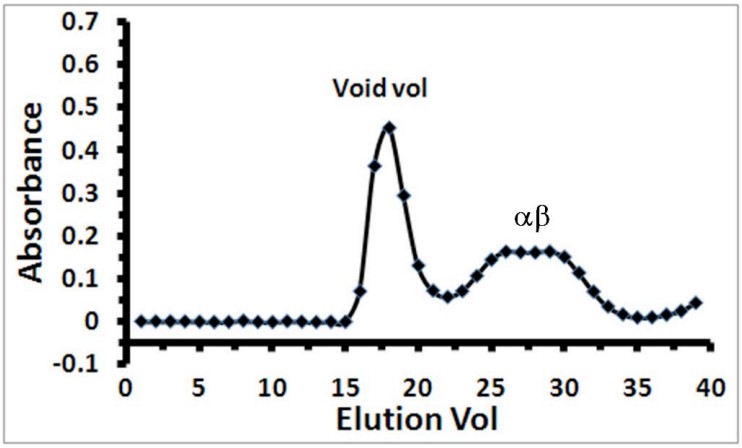

Figure S1c. LH 60 column profile for the CMAc extract of membranes from the Ser(−34β)Cys mutant after incubation with β-mercaptoethanol. Not shown at longer elution times are the carotenoid and BChl *a* pigments.

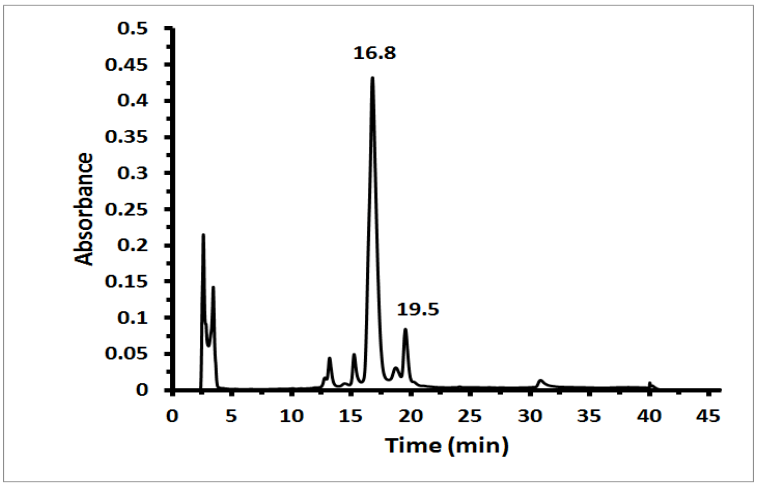

Figure S2a. HPLC of the early part of the αβ band from the LH 60 column of the CMAc extract from native *Rb. sphaeroides* membranes (Fig. S1b). The peak at 16.8 min is that of the α-peptide.

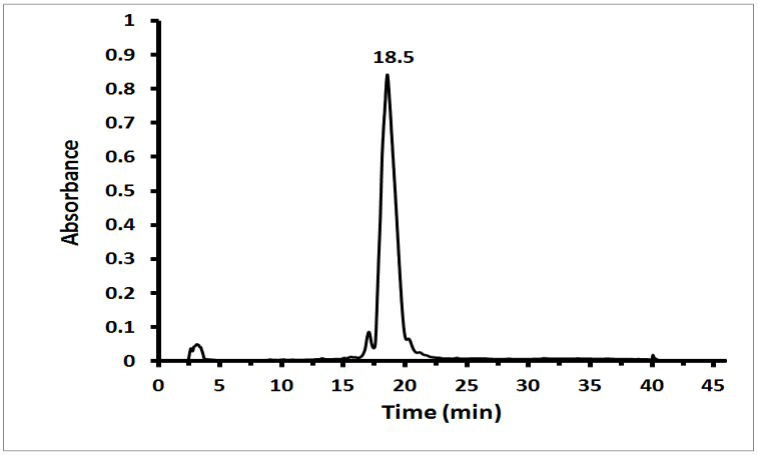

Figure S2b. HPLC of the late part of the αβ band from the LH 60 column of the CMAc extract from native *Rb. sphaeroides* membranes (Fig. S1b). The peak at 18.5 min is that of the β-peptide.

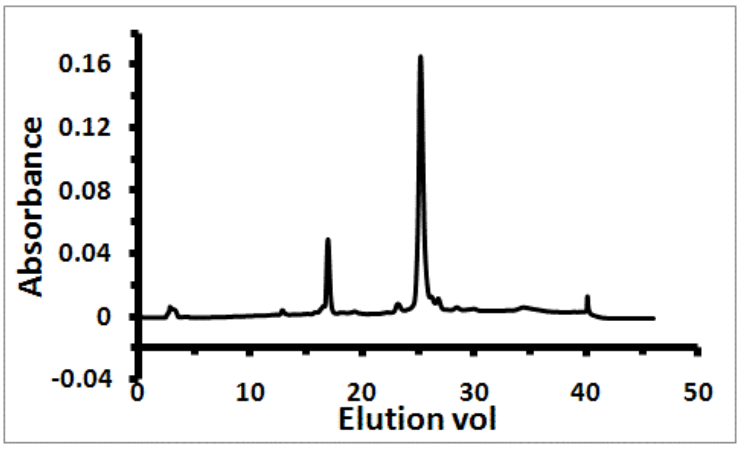

Figure S2c. HPLC of the early part of the αβ band from the LH 60 column of the CMAc extract from membranes of the Ser(–34β)Cys mutant (Fig. S1a). The peak at 25 min is that of the β-peptide.

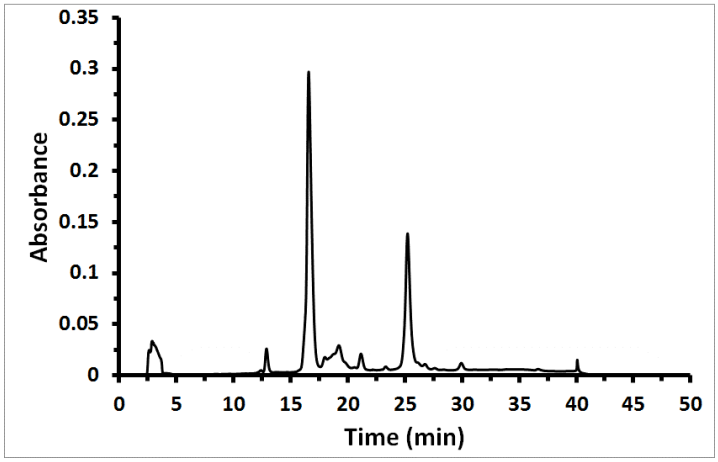

Figure S2d. HPLC of the late part of the αβ band from the LH 60 column of the CMAc extract from membranes of the Ser(–34β) mutant (Fig. S1a). The peak at 16 min is the α-peptide.

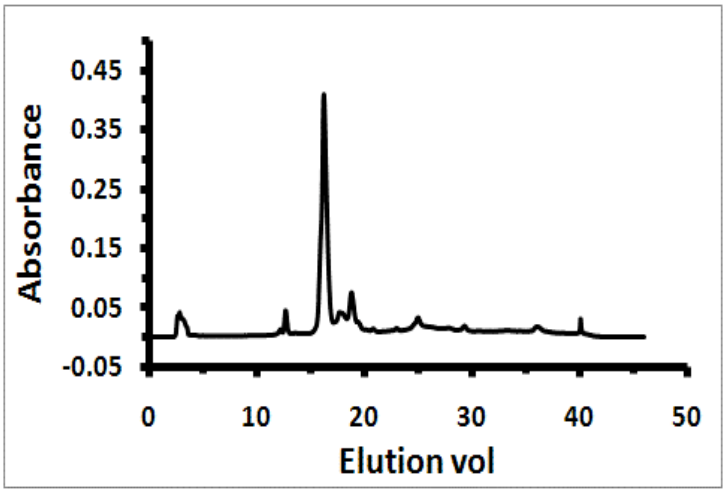

Figure S2e. HPLC of the early part of the αβ band from the LH 60 column of the CMAc extract from membranes of the Ser(–34β)Cys mutant after incubation with β-mercaptoethanol (Fig. S1c). The peak at 16 min is that of the α-peptide.

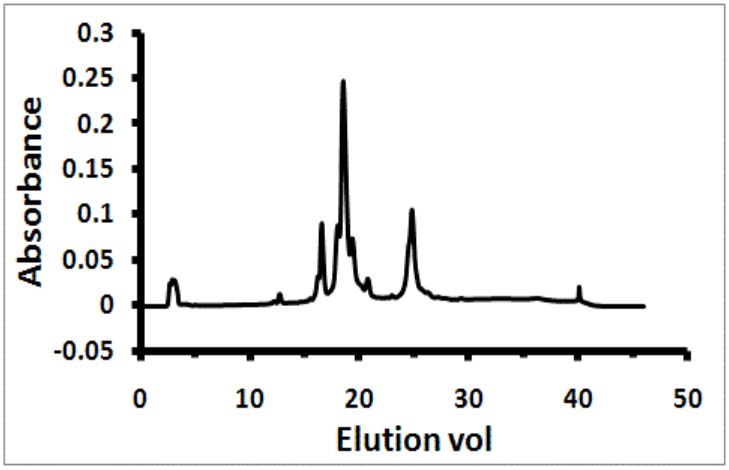

Figure S2f. HPLC of the late part of the αβ band from the LH 60 column of the CMAc extract from membranes of the Ser(–34β)Cys mutant after incubation with β-mercaptoethanol (Fig. S1c). The peak at 19 min is that of the β-peptide.

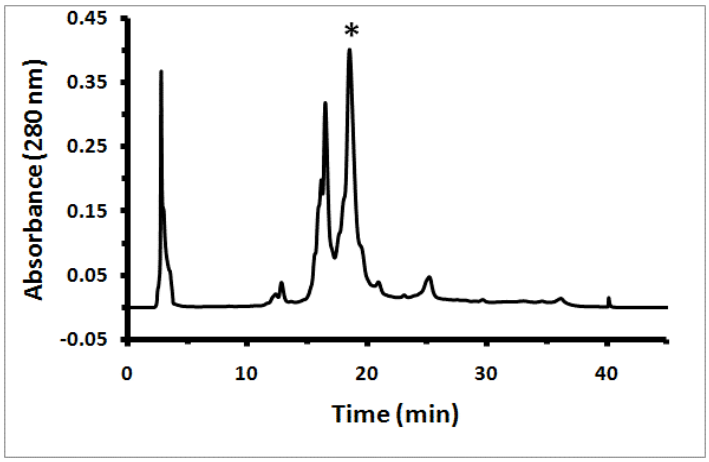

Figure S3a. HPLC of the reaction product from coupling the β-peptide from the Ser(−34β)Cys mutant with **OGR**. The peak at 19 min marked with the * is that of β-peptide with **OGR** attached while that at 16 min is that of the α-peptide.

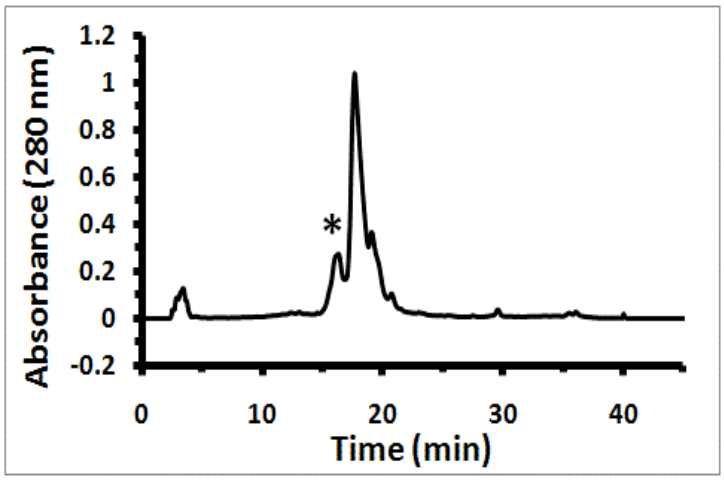

Figure S3b. HPLC of the reaction product from coupling the α-peptide from the Asp(−20α)Cys with **RR**. The peak at 19 min is that of the β-peptide while that marked with the * at 16 min is that of the α-peptide with **RR** attached.

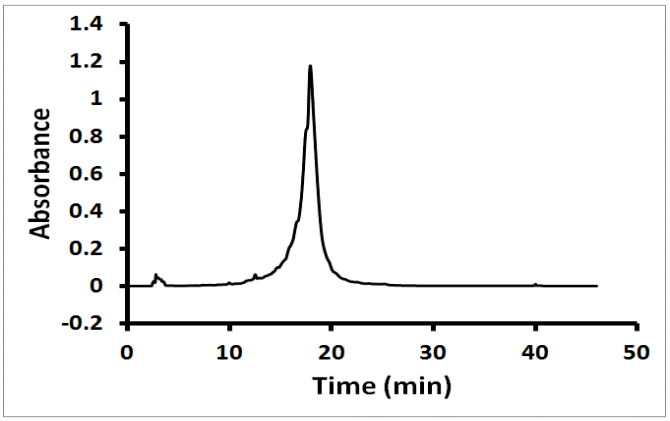

Figure S3c. HPLC of the reaction product from coupling **OGR** to the synthetic β-peptide containing Cys at position –34. The peak at 19 min is that of the covalently linked **OGR** product.

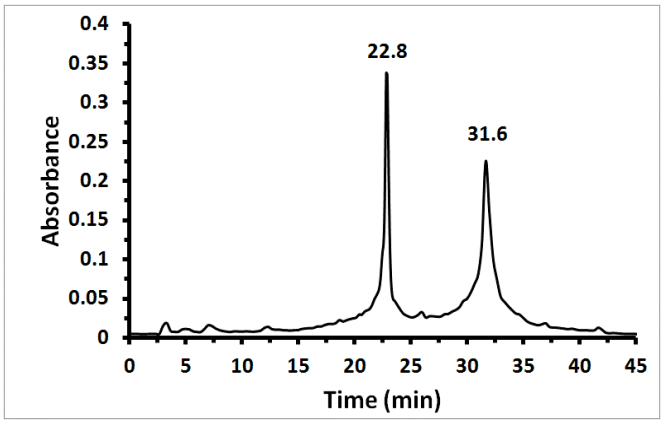

Figure S3d. HPLC of the reaction product from coupling BC1 with the synthetic β-peptide containing Cys at position –14. The peak at 22.8 min is that of free **BC1** and that at 31.6 min is that of the covalently linked **BC1** product.

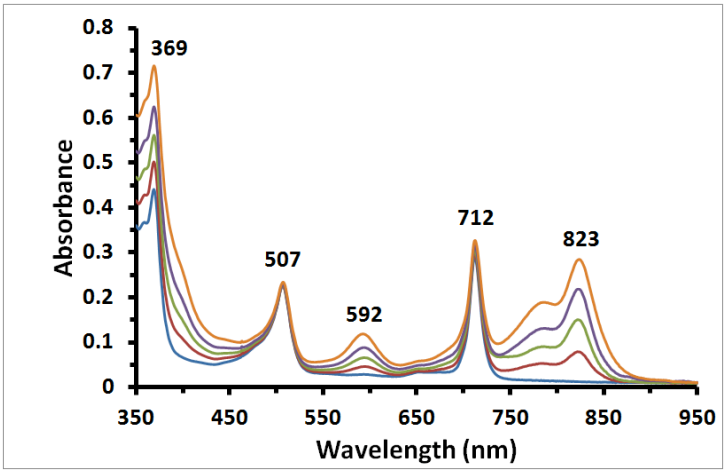

Figure S4. Absorption spectra taken as BChl *a* is added to a solution of approximately equal amounts of the **OGR** and **BC1** covalently linked β-peptides at 0.90% octyl glucoside. Curves are for 0 (blue), 10 (red), 20 (green), 30 (purple) and 40 μL (brown) of BChl *a* solution in acetone. The peak at 712 nm is that of **BC1** and that at 507 nm is that of **OGR**.

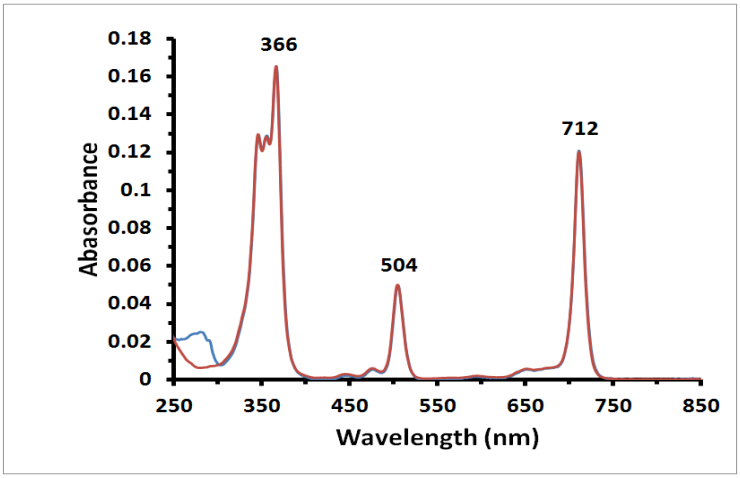

Figure S5. Absorption spectra of the 22.8 min peak (red) and 31.6 min peak (blue) material from the HPLC shown in Fig. S3d. The curves were normalized at 712 nm. Note the protein absorbance in the ultraviolet region for the 31.6 min peak material.