vacuo and the residue was purified by column chromatography (silica, petroleum ether:ethyl acetate 9:1): mixture of **8a** (18%) and **9a** (46%). **8a**: colorless crystals, mp.: 174–175°C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 1.92$–2.21 (m, 3H), 2.42–2.55 (m, 1H), 3.52–3.59 (m, 1H, HC-Ph), 4.72 (s, 1H, $H_{bridgehead}$), 4.80 (s, 1H, $H_{bridgehead}$), 7.16–7.55 ppm (m, 10H, H_{aryl}); ^{13}C NMR (100 MHz, $CDCl_3$, TMS; Multiplicities of ^{13}C NMR signals were determined by the DEPT sequence and are reported as (+) for CH or CH_3, (−) CH_2, and (o) for C): $\delta =$ 35.00 (−), 36.25 (−), 45.29 (+), 60.33 (+), 64.87 (+), 125.38 (+), 126.95 (+), 127.07 (+), 128.31 (+), 128.85 (+), 129.15 (+), 131.52 (o), 140.26 (o), 156.71 (o, C=O), 156.96 ppm (o, C=O); MS (EI): *m*/*z* (%) 320 (15, $[M^+ + 1]$), 319 (59, $[M^+]$), 214 (100), 143 (20), 142 (19), 115 (17), 104 (43), 91 (30); IR: $\tilde{\nu}_{max} = 3056$, 3011, 1776, 1716, 1598, 1501, 1415, 735, 697 cm^{-1}. HRMS: calcd for $C_{19}H_{17}N_3O_2$ 319.1321; found 319.1321. **9a**: Colorless crystals, mp.: 187–189°C. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 2.49$–2.63 (m, 1H), 2.77–2.92 (m, 1H), 4.03–4.07 (m, 1H, HC-Ph), 4.74–4.84 (m, 1H, HC-N), 5.73–5.83 (m, 1H, HC=), 5.90–5.96 (m, 1H, HC=), 7.17–7.49 (m, 10H, H_{aryl}), 9.07 ppm (br s, 1H, HN); ^{13}C NMR ($CDCl_3$): $\delta = 35.26$ (−), 54.28 (+), 64.30 (+), 125.44 (+), 127.15 (+), 127.36 (+), 128.23 (+), 128.74 (+), 129.09 (+), 129.63 (+), 131.13 (o), 132.73 (+), 141.48 (o), 152.21 (o, C=O), 154.09 ppm (o, C=O); MS (EI): *m*/*z* (%) 320 (16, $[M^+ + 1]$), 319 (68, $[M^+]$), 215 (16), 214 (100), 143 (22), 142 (21), 104 (57), 91 (26); IR: $\tilde{\nu}_{max} = 3160$, 3059, 1772, 1687, 1595, 1493, 1378, 700, 676 cm^{-1}; HRMS: calcd for $C_{19}H_{17}N_3O_2$ 319.1321; found 319.1321.

[15] a) R. Giger, R. Rubinstein, D. Ginsburg, *Tetrahedron* **1973**, *29*, 2387; b) P. G. Gassman, R. C. Hoye, *J. Am. Chem. Soc.* **1981**, *103*, 2498.

Biomimetic Model of a Plant Photosystem Consisting of a Recombinant Light-Harvesting Complex and a Terrylene Dye**

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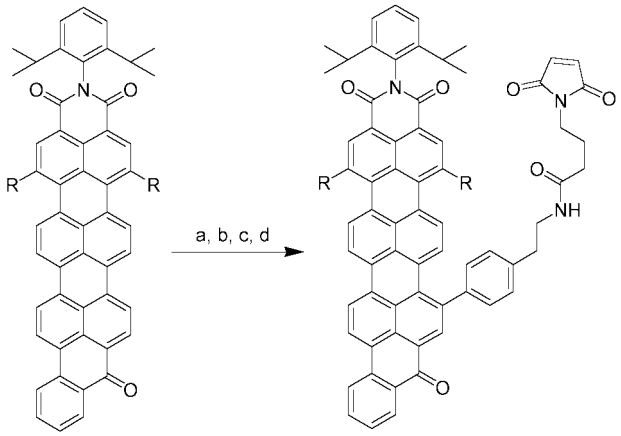
The light-harvesting chlorophyll (Chl) *a*/*b* antenna in higher plants contributes to photosynthesis by absorbing light energy and funneling it into the photosynthetic reaction centers where the conversion into an electrochemical potential takes place. The components of this photosynthetic antenna, the light-harvesting Chl *a*/*b* complexes (LHC), fulfil this task with the help of numerous protein-bound pigments, carotenoids, and Chl *a* and Chl *b*, which exchange absorbed energy rapidly and with high efficiencies.[1] The most abundant Chl *a*/*b* complex is the major LHC of photosystem II, LHCIIb, which comprises roughly 50% of the total Chl in higher plants. This complex can be reconstituted in vitro from its protein and pigment components, using either denatured thylakoid proteins[2] or recombinant LHCIIb apoprotein, Lhcb1.[3] Recombinant LHCIIb exhibits structural, biochemical, and spectroscopic properties very similar to those of native LHCIIb.[3–5]

The in vitro reconstitution of recombinant LHCIIb opens up the possibility of introduce useful modifications into the structure by altering the amino acid sequence. Thus, anchors have been generated for immobilizing the complex or for site-specific fluorescence labeling.[6] This property makes recombinant LHCIIb a promising candidate for designing hybrid biological–chemical structures that contain an ordered arrangement of fluorophores.

Herein we show that recombinant LHCIIb can be coupled to an artificial energy trap, benzoylterrylene-3,4-dicarboximide (BTI). This NIR dye collects, by efficient energy transfer, a large fraction of the light energy absorbed by the LHCIIb pigments, which makes the LHCIIb–BTI construct a simple model of a photosystem consisting of a light-harvesting pigment–protein complex and an energy trap.

To couple BTI (**1**)[7] to cysteine side chains in the protein, a maleimido derivative of the dye was constructed (Scheme 1). Maleimido BTI (**2**) was synthesized in a four step reaction



Scheme 1. R = *tert*-butylphenol; a) Br_2, $CHCl_3$, RT, 16 h, 78%; b) (4-pinacolylborono)-Ph$(CH_2)_2$NBOC, $Pd(PPh_3)_4$, K_2CO_3(aq), toluene, 110°C, 18 h, 84%; c) TFA/CH_2Cl_2, RT, 2 h, 93%; d) *N*-succinimidyl-4-maleimidobutyrate, Et_3N, CH_2Cl_2, RT, 6 h, 78%. BOC = *tert*-butyloxycarbonyl, TFA = trifluoroacetic acid.

from **1**. Bromination of **1** followed by Suzuki coupling with the BOC-protected (4-pinacolylborono)phenylethylamine afforded the corresponding BOC derivative of phenylethylamino BTI. After acidic removal of the BOC protecting group, the phenylethylamino BTI was transformed into **2** with *N*-succinimidyl-4-maleimidobutyrate.

Maleimido BTI (**2**) was attached to a single cysteine residue near the N terminus of the Lhcb1 mutant S3C. The site specificity of the labeling reaction was verified by the fact that another Lhcb1 mutant containing no cysteine at all (C79S) did not bind any BTI maleimide (not shown). Lhcb1–BTI was purified by preparative electrophoresis, in which it migrated more slowly than the nonlabeled protein. Subsequently the

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protein was reconstituted with pigments[5,6] to form LHCIIb–BTI. This construct was approximately as stable towards dissociation in a detergent environment as the nonlabeled LHCIIb containing the wild-type protein and exhibited virtually 100% energy transfer from complex-bound Chl *b* to Chl *a* (not shown).

To estimate energy flow from Chl *a* to BTI, the fluorescence emission spectrum of LHCIIb–BTI was compared to that of nonlabeled LHCIIb at 297 K and 77 K (Figure 1). The

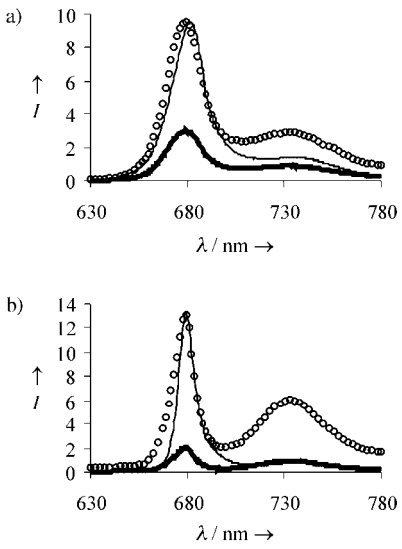

Figure 1. Fluorescence emission of LHCIIb (thin line) and LHCIIb–BTI (bold line) at 297 K (a) and 77 K (b). Purified LHCIIb and LHCIIb–BTI were brought to exactly (±1%) the same absorption at 410 nm. Fluorescence emission spectra were measured of these solutions using a Fluoromax-2 (Jobin Yvon, Grasbrunn, Germany) with the following instrument parameters: excitation wavelength 410 nm, excitation and emission bandwidths 3 nm, integration time 0.1 s. The spectra were corrected for fluctuations in excitation light intensity and wavelength-dependent changes in photomultiplier sensitivity. All LHCIIb samples had an optical density of < 0.1 over the entire visible spectrum, to minimize fluorescence reabsorption. For measurements at 77 K, the samples were dissolved in 60% (v/v) glycerol. To visualize the BTI contribution (emission maximum at about 735 nm) to the LHCIIb–BTI emission spectrum, the latter is also shown normalized to the maximum signal of LHCIIb emission (circles).

excitation wavelength was 410 nm, near the absorption maximum of Chl *a*. The emission spectra of LHCIIb–BTI (bold lines in Figure 1) exhibit a maximum at 678 nm, near the emission maximum of Chl *a*, and a shoulder at 730 nm, near the emission maximum of BTI. The emission of Chl *a* in LHCIIb–BTI is significantly reduced compared to the emission of Chl *a* in nonlabeled LHCIIb at the same concentration (thin lines in Figure 1). From fluorescence quenching of the donor (Chl *a*), an apparent energy transfer efficiency was calculated to be 70 ± 4% at 297 K (Figure 1a) and 85 ± 4% at 77 K (Figure 1b). Sensitized acceptor (BTI) fluorescence was expected to be low because of the low fluorescence quantum yield of protein-bound BTI, 3 ± 0.3%, measured in detergent solution at 297 K. The BTI emission band is more clearly visible in the LHCIIb–BTI fluorescence emission spectra only when these are normalized to the Chl *a* emission maximum of nonlabeled LHCIIb (circles in Figure 1).

The quenching of donor (Chl *a*) fluorescence in LHCIIb–BTI is caused by energy transfer to the acceptor (BTI), as the quenching effect ceases when donor and acceptor are separated by trypsin digestion of LHCIIb–BTI. Trypsin cleaves 35 amino acids from the N terminus of LHCIIb, leaving the pigment-binding domain of the protein intact.[8] Upon trypsin digestion, the Chl *a* fluorescence of LHCIIb–BTI rose to the level of that of nonlabeled LHCIIb (Figure 2).

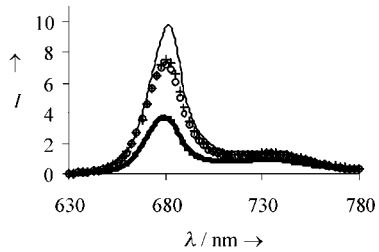

Figure 2. Fluorescence emission of LHCIIb (thin line; after trypsin digestion: crosses) and LHCIIb–BTI (bold line; after trypsin digestion: circles) before and after protein digestion with trypsin. Purified LHCIIb and LHIIb–BTI were brought to the same Chl *a* absorption at 410 nm and trypsin digested.[6] Emission spectra (excitation at 410 nm) were taken before and after digestion (same instrument parameters as in Figure 1).

The decrease in LHCIIb emission upon trypsin treatment is in part caused by dilution of the complex during the experiment.

The apparent energy transfer efficiencies from Chl *a* to BTI (70% and 85% at 297 K and 77 K, respectively) are quite high but may nevertheless be underestimated because of energy back-transfer from BTI to the Chls. The critical Förster distance (R_0) for the donor–acceptor pair (Chl *a*–BTI) is estimated to be 58 Å (at 297 K, assuming an orientation factor κ^2 of $\frac{2}{3}$). The R_0 value for the reversed donor–acceptor pair is estimated to be 34 Å, which is well within the possible range of distances between Chls and BTI in LHCIIb–BTI. If energy back-transfer does limit the apparent energy transfer efficiency from Chl *a* to BTI, the latter could be further increased by decreasing the fluorescence lifetime of the acceptor, for instance, by engaging the acceptor dye in a fast photochemical reaction.

A large number of biomimetic models of photosystems have been described, including self-assembling arrays of bacteriochlorophyll *c*, which mimic chlorosomes of green sulfur bacteria,[9] and porphyrins covalently attached to other chromophores.[10] To our knowledge, the LHCIIb–BTI construct is the first biomimetic model of a plant photosystem that contains a self-assembling recombinant light-harvesting complex of the photosynthetic apparatus in higher plants. This model mimics the efficient transfer of absorbed light energy to an acceptor but differs from a photosystem in that light energy is not converted into charge separation. Experiments to integrate this feature into our model are underway.

Experimental Section

Analytical data of **2**: green solid; m.p. > 300 °C; ^{1}H NMR (500 MHz NMR, $C_2D_2Cl_4$, 313 K): $\delta = 9.44$ (d, $^3J = 7.9$ Hz, 1H); 8.96 (d, $^3J = 8.6$ Hz, 1H); 8.75 (d, $^3J = 7.9$ Hz, 1H); 8.54 (d, $^3J = 7.9$ Hz, 1H); 8.52 (d, $^3J = 7.9$ Hz, 1H); 8.44 (d, $^3J = 7.9$ Hz, 1H); 8.38 (s, 1H); 8.30 (d, $^3J = 8.0$ Hz); 8.22 (s, 1H); 8.17 (s, 1H); 7.76 (d, $^3J = 9.2$ Hz, 1H); 7.68 (tr, $^3J = 7.3$ Hz, 1H); 7.53 (tr, $^3J = 7.3$ Hz, 1H); 7.47–7.43 (m, 5H); 7.38–7.35 (m, 3H); 7.24–7.20 (m, 5H); 7.14 (d, $^3J =$

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6.8 Hz); 7.05 (d, $^3J = 9.2$ Hz, 2H); 6.62 (s, 2H); 3.53–3.49 (m, 4H); 2.87–2.86 (m, 2H); 2.64 (sept, $^3J = 8.6$ Hz, 2H); 2.13–2.10 (m, 2H); 1.90–1.87 (m, 2H); 1.32 (s, 9H); 1.29 (s, 9H); 1.08 ppm (d, $^3J = 8.6$ Hz, 12H); ^{13}C-NMR (500 MHz NMR, $C_2D_2Cl_4$, 313 K): $\delta = 185.41$ (C=O), 175.49 (C=O), 165.86 (C=O), 165.78 (C=O), 164.71 (C=O), 164.69 (C=O), 157.28, 155.35, 155.26, 150.55, 150.48, 148.06, 147.72, 142.29, 139.03, 137.79, 136.64, 136.61, 135.65, 134.77, 134.23, 133.58, 133.49, 133.44, 133.37, 133.21, 132.86, 132.41, 132.22, 132.12, 131.97, 131.53, 131.24, 131.15, 131.01, 130.59, 130.46, 130.33, 130.12, 130.04, 129.95, 129.74, 129.08, 128.29, 128.09, 126.69, 126,51, 125.51, 125.90, 125.27, 125.17, 125.12, 124.87, 124.26, 124.06, 121.85, 121.70, 117.78, 117.34, 45.10, 39.87, 37.28, 34.21, 32.08, 31.79, 31.44, 26.79, 24.66 ppm; UV/Vis ($CHCl_3$): λ_{max} (ε) = 263 (60100), 272 (61300), 445 (4500), 651 (47800), 705 (89400) nm; field desorption (FD)-MS (8 kV): m/z (%): 1289.0 (100) [M^+]; anal. calcd for $C_{87}H_{73}N_3O_8$: C 81.10, H 5.71, N 3.26; found: C 81.23, H 5.79, N 3.22

The recombinant Lhcb1 mutants used in this work were derivatives of the clone D7f.3 coding for pea wild-type Lhcb1.[3,11] A single cysteine residue in position 79 of mutant C79S was exchanged with a serine residue, and mutant S3C carried the additional mutation of cysteine in place of serine in position 3.

BTI was covalently coupled to the N-proximal cysteine in the Lhcb1 mutant S3C. Lhcb1 (1 nmol) in buffer (25 μL; 1 g L^{-1} sodium dodecylsulfate, 10 g L^{-1} *n*-octyl-β-D-glucopyranoside and 20 mM sodium phosphate pH 7.0) was reduced for 15 min at 37 °C with 2 mM tris(2-cyanoethyl)phosphane and then mixed with BTI maleimide (12.5 nmol), dissolved in THF (2 μL). After 2 h incubation at 37 °C, the protein was precipitated with 80 % (v/v) acetone and 10 mM acetic acid. The protein pellet was dissolved in sodium dodecylsulfate (20 μL; 20 g L^{-1}), 100 mM Tris-HCl (Tris = tris(hydroxymethyl)aminomethane; pH 9.0), heated to 100 °C for 2 min and then centrifuged (5 min at 16000 g) to remove insoluble material.

In LHCIIb–BTI constructs, energy transfer from Chl *a* to the dye was calculated from the quenching of donor (Chl *a*) emission in the presence of the acceptor. Samples of LHCIIb with and without bound BTI were made up to the same Chl *a* absorption at 410 nm. The dye absorption was negligible at this wavelength. Energy transfer efficiency was estimated by comparing Chl *a* fluorescence emissions with and without energy transfer to the dye:

Energy transfer [%] = [1 − (maximum Chl *a* emission in LHCIIb–BTI)/(maximum Chl *a* emission in LHCIIb)] 100.

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[1] H. van Amerongen, R. van Grondelle, *J. Phys. Chem. B* **2001**, *105*, 604 – 617.
[2] F. G. Plumley, G. W. Schmidt, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 146 – 150.
[3] H. Paulsen, U. Rümler, W. Rüdiger, *Planta* **1990**, *181* , 204 – 211.
[4] K. V. Cammarata, F. Plumley, G. W. Schmidt, in *Current Research in Photosynthesis, Vol. 2* (Ed.: M. Baltscheffsky), Kluwer Academic, Dordrecht, **1990**, pp. 341 – 344.
[5] S. Hobe, S. Prytulla, W. Kühlbrandt, H. Paulsen, *EMBO J.* **1994**, *13*, 3423 – 3429.
[6] a) H. Rogl, K. Kosemund, W. Kühlbrandt, I. Collinson, *FEBS Lett.* **1998**, *432*, 21 – 26; b) K. Kosemund, I. Geiger, H. Paulsen, *Eur. J. Biochem.* **2000**, *267*, 1138 – 1145; c) H. Paulsen, B. Finkenzeller, N. Kühlein, *Eur. J. Biochem.* **1993**, *215*, 809 – 816.
[7] F. O. Holtrup, G. R. J. Müller, H. Quante, S. De Feyter, F. C. De Schryver, K. Müllen, *Chem. Eur. J.* **1997**, *3*, 219 – 225.
[8] A. Kuttkat, R. Grimm, H. Paulsen, *Plant Physiol.* **1995**, *109*, 1267 – 1276.
[9] a) H. Tamiaki, T. Miyatake, R. Tanikaga, A. R. Holzwarth, K. Schaffner, *Angew. Chem.* **1996**, *108*, 810 – 812; *Angew. Chem. Int. Ed.* **1996**, *35*, 772 – 774; b) T. Miyatake, H. Tamiaki, A. R. Holzwarth, K. Schaffner, *Helv. Chim. Acta* **1999**, *82*, 797 – 810.
[10] a) V. S. Y. Lin, S. G. Dimagno, M. J. Therien, *Science* **1994**, *264*, 1105 – 1111; b) R. W. Wagner, J. S. Lindsey, *J. Am. Chem. Soc.* **1994**, *116*, 9759 – 9760; c) G. Kodis, P. A. Liddell, L. de la Garza, P. C. Clausen, J. S. Lindsey, A. L. Moore, T. A. Moore, D. Gust, *J. Phys. Chem. A,* **2002**, *106*, 2036–2048.
[11] H. Dilly-Hartwig, J. F. Allen, H. Paulsen, H. L. Race, *FEBS Lett.* **1998**, *435*, 101 – 104.

Triboracyclopropanates: Two-Electron Double Aromatic Compounds with Very Short B–B Distances**

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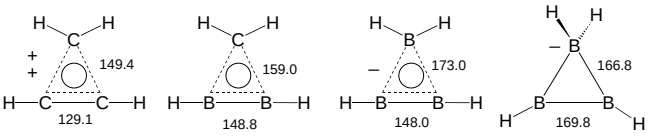
Dedicated to Professor Walter Siebert on the occasion of his 65th birthday

According to computations, the isoelectronic, two-electron double aromatic compounds **1u**–**3u**[1–4] (Scheme 1) have one extremely short and two relatively long bonds in the ring. This results from the fact that all of the ring atoms are connected



Scheme 1. Two-electron aromatic compounds, each containing a 3c–2e π (circles) and σ bond (dashed triangle). Solid lines symbolize a 2c–2e bond. The numbers indicate the distances in pm, computed at the MP2/6-31G* level.[1–3]

by one three-center, two-electron (3c–2e) π as well as one 3c–2e σ bond and that two ring atoms are connected by an additional 2c–2e σ bond. The planar compound **3u** is stabilized by 54.9 kcal mol^{-1} with respect to the classical structure **3u***.[3b] We present here the syntheses and crystal structures of **3a** and **3b** (see Scheme 2), the first derivatives of the triboracyclopropanate **3u**, as well as DFT computations[5] for model compounds **3c**–**f** (see Figure 2).

The monoanion **3a** is obtained from the reaction of two equivalents of lithium naphthalenide in THF with **4**, which forms upon treatment of **5**[6] with $(CH_3)_3SiCH_2Li$ (Scheme 2). The dianion **3b** results when **5** is treated with lithium in Et_2O at −80 °C.[7] Compounds **3a** and **3b** react with electrophiles to tetraboranes(6). Treatment of **3a** with hexachloroethane results in the regeneration of **4**, and **3b** reacts with methyl iodide[8] to form **6**, which spontaneously transforms into **7** in solution with elimination of 1,2-bis(trimethylsilyl)ethene. The constitutions of the new compounds are in agreement with their NMR data (Table 1), and the structures of **3a**, **3b**, and **6** have been confirmed by crystal structure analysis.[9]

The anion **3a** forms a contact ion pair with a lithium ion, which is coordinated to the B2–B3 bond as well as to two THF

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