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Time-Dependent DFT on Phytochrome Chromophores: A Way to the Right Conformer

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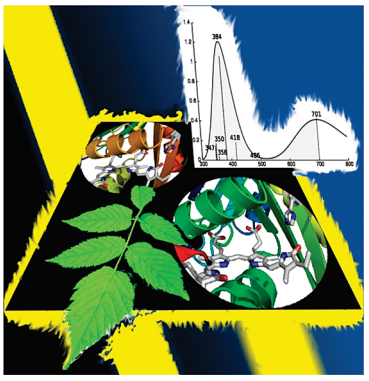
ABSTRACT A theoretical approach based on time-dependent density functional theory (TDDFT) together with a polarizable continuum model for incorporating the bulk effect of the surrounding environment is used to estimate the excitation energies for the phytochromobilin chromophore of plant phytochrome. The TDDFT results reproduce well the experimental values of the absorption maxima and those of the ratio of the spectroscopic bands (Q band over the Soret band or Q/S index). Our results suggest that the phytochromobilin within the P_r form of the phytochrome holoprotein should adopt a semicyclic ZZZssa structure as it does in bacteria.

SECTION Biophysical Chemistry



Phytochromes are photoreceptors in plants and are also found in nonoxygenic bacteria, cyanobacteria, and fungi. In plants, phytochromes regulate photomorphogenic responses as germination, de-elotation, shade avoidance, phototropism, chloroplast movement, stomatal opening, and flowering. The photocycle of the receptor involves a Z/E photoisomerization around a double bond of its phytochromobilin (PΦB) chromophore (Figure 1), directing the photoconversion between a physiological inactive red form called P_r to a physiological active far-red form called P_{fr}.[1] Phytochrome A absorbs in the UV−visible range, showing two prominent bands, the Soret band in the blue region and the Q band in the red region.[2,3] The Soret band normally absorbs near the 380 nm, but the Q band is variant and specific for each chromophore, for example, in the P_r of the plant phytochrome, this band has its maximum at around 666 nm.[3,4]

According to the pioneering work of Wagner et al. showing the crystallographic structure in proteobacteria,[5] the P_r form of the biliverdin (BV) chromophore adopts a ZZZssa conformation (C_5-Z, C_{10}-Z, C_{15}-Z, C_5-syn, C_{10}-syn, C_{15}-anti). In the case of the cyanobacterial phytochrome, theoretical calculations based on time-dependent density functional theory[6] (TDDFT) predicted satisfactorily a ZZZssa structure for its phycocyanobilin (PCB) chromophore conformation,[7] which was then confirmed by NMR solution experiments[8] and crystallographic[9] structures. However, no X-ray or NMR structural information is available for plant phytochrome, and thus, there is no consensus about which structure is adopted by its PΦB chromophore in the holoprotein. On the one hand, a semicyclic ZZZssa structure, as in proteobacteria, is supported by sequence identity;[1a,10] on the other hand, a stretched ZZZasa conformation is proposed based on resonance Raman (RR) evidence[11−14] (Figure 1), and it has been adopted in the most recent computational studies of the phytochrome system.[15,16] In this Letter, we elucidate which conformer should be found in plant phytochromes by means of calculating the electronic absorption spectra of both possible conformations, as well as that of the cyclic solution structure. The apoprotein is mostly transparent, not absorbing in the UV−vis, except for some aromatic residues such as tyrosine and tryptophan, which together generate the so-called protein band at 280 nm, that is, at around 100 nm to the UV side with respect the Soret band of the chromophore. Therefore, an efficient procedure consists of treating the chromophore alone with a full quantum description (TDDFT in our case) while the surrounding environment can be modeled in a much simpler way, for instance, with continuum models.[7] Such an approach is, for example, suggested in a recent review on the theoretical description of biomolecular spectroscopy by Neugebauer,[17] who points out the necessity to keep fixed parts of the chromophore at the crystal structure geometry, that is, considering implicitly the geometry imposed by the surrounding apoprotein. This treatment is a good shortcut to the more demanding one of sampling the ensemble of structures belonging to the conformational space;[18] moreover, it is preferred over the typical relaxation of the chromophore in vacuo since the latter method converges to a geometry, which being out of the conformations accessed by the system, delivers overestimated excitation energies.[7]

As a template for PΦB in the semicyclic ZZZssa model, we use either the X-ray structure of the BV chromophore found in the P_r form of the bacteriophytochrome of *Deinococcus radiodurans* (PDB: 2O9C)[19] or the P_r form of the PCB chromophore found in the cyanobacterial Cph1 phytochrome from the cyanobacterium *Synechocystis* 6803 (PDB: 2VEA).[9] For the stretched ZZZasa model, we use the PCB found in the α-84

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subunit of the X-ray structure of the *C*-phycocyanin (a light-harvesting antenna system) from the cyanobacterium *Synechoccus elongatus* (PDB: 1JBO).[20] This latter choice is motivated by the fact that the *C*-phycocyanin has the same chromophore but a different function, namely, to capture photons and transport them very efficiently to the photosynthetic reaction centers. In order to preserve the crystallographic conformations but still allow for some refinement of the bond distances and angles, the former three chromophore backbones were also optimized at the B3LYP/6-31G* level of theory,[21] keeping the dihedrals frozen. The model ZZZsss for PΦB in solution was constructed by rotating the single bond $C_{14}-C_{15}$ of the ZZZssa (BV template) model and then optimizing the structure at the B3LYP/6-31G* level of theory[21] in vacuo. A Hessian was calculated to ensure that a true minimum was found. In all of these models, the cysteine linkage was replaced with a hydrogen and the propionic acid side chains on the rings B and C were not considered as they are not part of the π-conjugated system and they have no effect on the calculations.[7] The hydrogens were added and then relaxed together with the ethyl and vinyl side chains of the rings A and D, respectively, taking into account the structural differences between the BV, PCB, and PΦB structures (Figure S1 of Supporting Information (SI)).

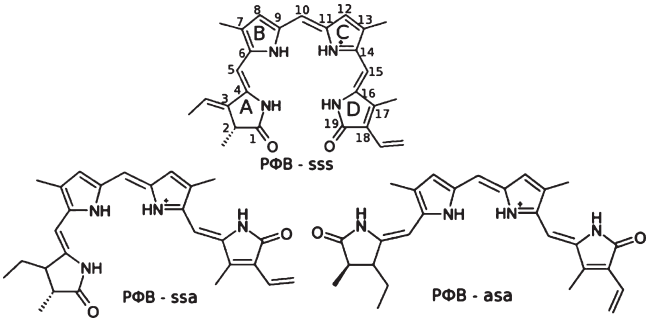

Figure 1. Phytochromobilin (PΦB) models for ZZZsss, ZZZssa, and ZZZasa conformers.

The excitation energies and associated oscillator strengths were calculated for eight states with TD-B3LYP. Since TD-DFT is less basis-set-dependent than post-Hartree−Fock methods,[22] a medium size basis set with polarization, 6-31G(d), has been chosen.[22] The calculations have been performed with the Gaussian03 set of programs.[23] To mimic the effect of the surrounding environment, we used the polarizable continuum model (PCM)[24] with a dielectric constant of 4.0 for the ZZZssa and ZZZasa models (as previously suggested in refs 25 and 26) and with the dielectric constant of water for the ZZZsss model in solution. As reviewed in ref 17, continuum models with a proper dielectric constant represent the average electrostatic interaction with the apoprotein in a reasonable manner, except for specific interactions with surrounding residues, which are not taken into account in this work. The theoretical absorption spectra were generated by convoluting Gaussian functions using the GaussSum2.1[27] program and a full width of 4000 cm^{-1} at half-maximum.

The obtained TDDFT spectra shown in Figure 2 reproduce well the experimental spectroscopic bands,[2,4,19,28−30] with the Soret band in the blue region and the Q band in the red

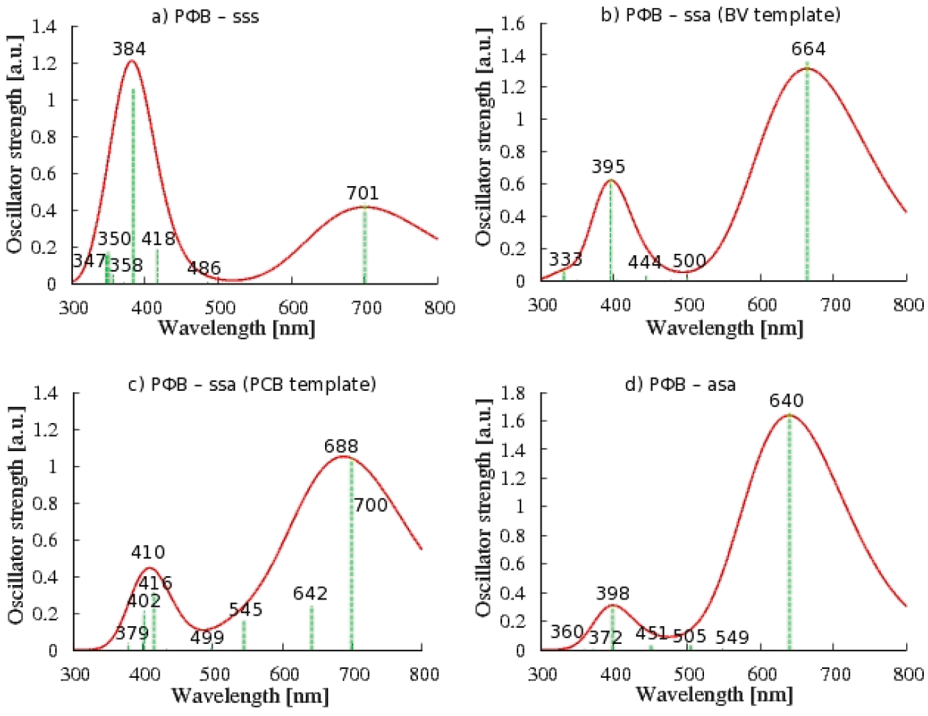

Figure 2. TDDFT absorption UV−vis spectra for the phytochromobilin in the conformations PΦB-ZZZsss (a), PΦB-ZZZssa from the BV template (b), PΦB-ZZZssa from the PCB template (c), and PΦB-ZZZasa (d).

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Table 1. Calculated TDDFT Absorption Maxima (in nm) and Q/S Ratios[a]

	absorption maxima		Q/S	
model	exptl.[b]	TDDFT	exptl.[c]	TDDFT[d]
BV-sss	377, 696,710[e]	383, 712	0.29[f]	0.26
PCB-sss	375, 692	368, 675	0.33, 0.43	0.37
PΦB-sss	386, 700	384, 701		0.34
BV-ssa	380, 702	417, 712	2.69[g]	1.99
PCB-ssa	380, 659	394, 661	1.17	2.28
PΦB-ssa	380, 666	395[h] (382)[h], 664[h] (633)[h], 410[i] (382)[i], 688[i] (611)[i]	1.36, 1.45	2.12[h] (2.02)[h], 2.36[i] (1.90)[i]
PCB-asa	380, 618	385, 614	4.1	5.26
PΦB-asa		398 (383), 640 (619)		5.27 (4.26)

[a] Values in parentheses correspond to structures refined with a constraint optimization (frozen dihedrals). [b] Taken from refs 2, 4, 19, and 28−30. [c] Taken from refs 33, 35, and 40−42. [d] Dielectric constant $\varepsilon = 4.0$ for ssa and asa conformations and $\varepsilon = 78.4$ for sss conformation. [e] Q band maximum for the protonated Biverdin dimethyl ester ($BVEH^+$) in solution corresponding to the helically coiled conformation according to Braslavsky et al.[28] [f] This value is obtained as an average of the oscillator strengths of the two peaks contributing to the Q and S bands, respectively.[33] [g] This value is likely to be overestimated since it is obtained[42] as the ratio between the heights of the Q and S bands and not the areas below the bands, that is, the oscillator strength. [h] These values have been obtained using the BV template. [i] These values have been obtained using the PCB template.

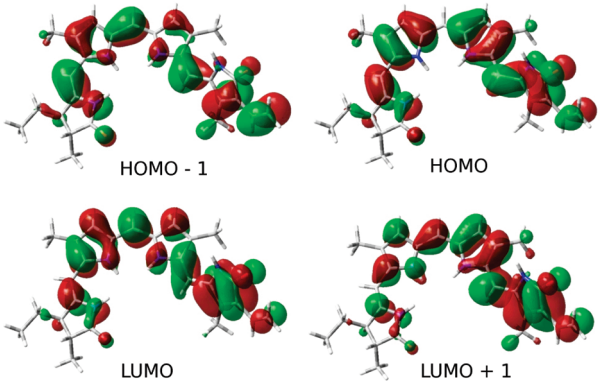

Figure 3. HOMO−1, HOMO, LUMO, and LUMO+1 orbitals involved in the TDDFT absorption spectrum given in Figure 2b.

region (Table 1). In all cases, the Q band is mainly composed of a HOMO to LUMO transition, whereas in the Soret band, there are four main orbitals involved in the transitions, that is, the HOMO−1, HOMO, LUMO, and LUMO+1 (see Figure 3 and Table S1 of SI). These same four orbitals were found in the Soret band of the cyanobacterial phytochrome,[7] in analogy with the four-orbital model of Gouterman for the Soret band in porphyrins.[31]

Two spectra are obtained for the semicyclic ZZZssa conformer, depending on the underlying template, either BV (Figure 2b) or PCB (Figure 2c). In the spectrum based on BV, the Q band absorbing at 664 nm is in excellent agreement with the experimental Q band absorption value of 666 nm. In the spectrum based on PCB, the Q band absorbs at 688 nm, that is, it shows a bathochromic shift of 24 nm, which can be explained by looking at the template structures. The ring D of BV is tilted by 44° over the plane formed by the other three pyrrol rings[19] (Figure 4A), while in the PCB, it is tilted only 26° (Figure 4B).[9] The latter arrangement achieves better conjugation within the π system, thus lowering the excitation energy and inducing the concomitant bathochromic shift in the spectrum. It has been suggested that the difference in the torsional angle of the ring D of both crystals can be explained by the lack of one domain (PHY domain) in the X-ray structure of the phytochrome with BV, which could destabilize the ring D in BV.[32] Hypothetically, both positions of the ring D could be accessed at room temperature by torsional movement in solution. However, if we assume a different torsion of the ring D between proteobacteria and cyanobacteria, in view of the better approximation of 664 versus 688 nm compared to the experimental value of 666 nm, our results would suggest that PΦB adopts a conformation similar to that of BV in proteobacteria and not like PCB in cyanobacteria. This conclusion is not so clear if one analyzes the excitations obtained using partially optimized geometries. As seen from the values in parentheses in Table 1, the excitation energies of the constraint geometries for PΦB-ssa (BV template) and PΦB-asa are blue-shifted with respect to those of the unrefined crystal structures. With values of 633 and 619 nm, it is not straightforward to assign the Q band of PΦB to the ssa or asa conformer. Moreover, taking into account that the Q band obtained for the crystal of the ZZZasa conformer (Figure 2d) is blue-shifted only by 24 nm with respect to that of the ZZZssa structure, a stretched structure cannot be unequivocally discarded.

To get additional information, we shall use the ratio of the oscillator strengths of the Q band over the Soret one, or the so-called Q/S index, to discriminate between both conformers. Q/S indexes have been invoked in the literature for a long time, first, using semiempirical methods, as in the work of Chae and Song[33] and Wagnière et al.[34,35] (see also the review by Scheer[36]), and later experimentally in vitro using synthesized adducts of tetrapyrrole locked in different conformations.[37,38] Afterward, Q/S indexes were revisited through AM1 semiempirical studies.[39,40] In our calculation of the Q/S ratios, we have also included the cyclic conformer (Figure 2a) besides the semicyclic and stretched ones.

Table 1 collects the calculated and experimental[2,4,19,28−30] absorption maxima together with the experimental[33,35,40−42] and TDDFT Q/S ratios. As we can see, the calculated Q/S ratios reproduce qualitatively the experimental ones, keeping the

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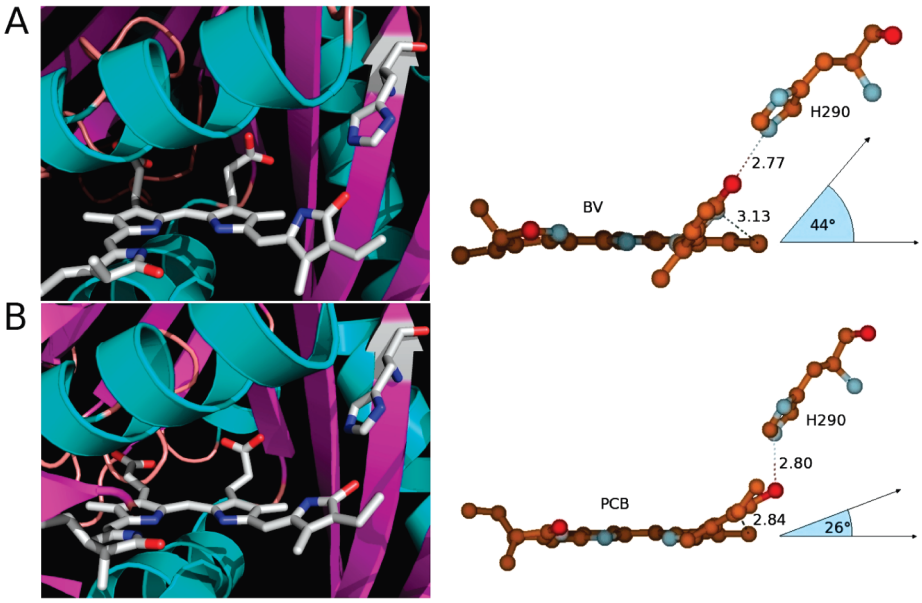

Figure 4. Structure of the chromophore inside of the crystallographic structure. (A) Biliverdin (BV) in bacteriophytochrome of D. radiodurans and (B) phycocyanobilin (PCB) in the cyanobacterial phytochrome Cph1 of Synechocystis 6803.

same trend; the lowest Q/S values are found for the cyclic conformers. Moreover, the higher the Q/S value the more stretched the conformer. With this in mind, it is straightforward to assign that the experimental indexes of 1.36 and 1.45 found in plant phytochrome[35,41] must correspond to the ZZZssa conformation since the calculated ratio for ZZZssa (2.12 or 2.02) is closer to experiment than the calculated one for the ZZZasa conformer (5.27 or 4.26). This fact suggests that the PΦB chromophore in plant phyochrome should adopt the semicyclic structure ZZZssa and not the stretched structure ZZZasa, as predicted by RR spectroscopy and calculations performed on fully relaxed chromophores.[11−14] Such early studies suggested that the phytochrome chromophore in cyanobacteria and in plants should both have the same conformation, being this the stretched ZZZasa.[13,14] A recent report claims that only by the explicit consideration of the apoprotein environment, which keeps the crystallographic conformation, RR predictions can be reconciled with the ZZZssa structure found in the crystal of the cyanobacterial phytochrome.[43] Our calculations, especially those done in unrelaxed X-ray geometries, are then consistent with these findings, and most importantly, they consolidate the proposal of Lagarias et al. of a ZZZssa conformation based on sequence alignment studies.[1a,10] The TDDFT spectra predict well the experimental values in phytochrome, even though the expansion with Gaussians has often the inconvenience of losing the identification of band shoulders. An improved spectrum can be obtained either by dealing with vibronic states[44] or sampling an ensemble with molecular dynamics and TDDFT.[18]

SUPPORTING INFORMATION AVAILABLE Structural differences between the BV, PCB, and PΦB structures, electronic excitations of the PΦB-ssa conformation, and Cartesian coordinates of the phytochromobilin models. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) (a) Rockwell, N. C.; Su, Y. S.; Lagarias, J. C. Phytochrome Structure and Signaling Mechanisms. *Annu. Rev. Plant Biol.* **2006,** *57*, 837–858. (b) Ulijasz, A. T.; Cornilescu, G.; Cornilescu, C. C.; Zhang, J.; Rivera, M.; Markley, J. L.; Vierstra, R. D. Structural Basis for the Photoconversion of a Phytochrome to the aActivated P_{fr} Form. *Nature* **2010,** *463*, 250–254.

(2) Cornejo, J.; Beale, S. I.; Terry, M. J.; Lagarias, J. C. Phytochrome Assembly. The Structure and Biological Activity of 2(R),3(E)-Phytochromobilin Derived from Phycobiliproteins. *J. Biol. Chem.* **1992,** *267*, 14790–14798.

(3) Song, P. S. The Molecular Topography of Phytochrome. Chromophore and Apoprotein. *J. Photochem. Photobiol. B* **1988,** *2*, 43–57.

(4) Chai, Y. G.; Singh, B. R.; Song, P. S.; Lee, J.; Robinson, G. W. Purification and Spectroscopic Properties of 124-kDa Oat Phytochrome. *Anal. Biochem.* **1987,** *163*, 322–330.

(5) Wagner, J. R.; Brunzelle, J. S.; Forest, K. T.; Vierstra, R. D. A Light-Sensing Knot Revealed by the Structure of the Chromophore-Binding Domain of Phytochrome. *Nature* **2005,** *438*, 325–331.

(6) Casida, M. E. *Recent Advances in Density Functional Methods. Part I*; World Scientific: Singapore, 1995.

(7) Matute, R. A.; Contreras, R.; Pérez-Hernández, G.; González, L. The Chromophore Structure of the Cyanobacterial

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Phytochrome Cph1 As Predicted by Time-Dependent Density Functional Theory. *J. Phys. Chem. B* **2008,** *112*, 16253–16256.

(8) Cornilescu, G.; Ulijasz, A. T.; Cornilescu, C. C.; Markley, J. L.; Vierstra, R. D. Solution Structure of a Cyanobacterial Phytochrome GAF Domain in the Red-Light-Absorbing Ground State. *J. Mol. Biol.* **2008,** *383*, 403–413.

(9) Essen, L.-O.; Mailliet, J.; Hughes, J. The Structure of a Complete Phytochrome Sensory Module in the P_r Ground State. *Proc. Natl. Acad. Sci. U.S.A.* **2008,** *105*, 14709–14714.

(10) Rockwell, N. C.; Lagarias, J. C. The Structure of Phytochrome: A Picture is Worth a Thousand Spectra. *Plant Cell* **2006,** *18*, 4–14.

(11) Kneip, C.; Hildebrandt, P.; Schlamann, W.; Braslavsky, S. E.; Mark, F.; Schaffner, K. Protonation State and Structural Changes of the Tetrapyrrole Chromophore During the $P_r \rightarrow P_{fr}$ Phototransformation of Phytochrome: A Resonance Raman Spectroscopic Study. *Biochemistry* **1999,** *38*, 15185–15192.

(12) Mroginski, M. A.; Murgida, D. H.; von Stetten, D.; Kneip, C.; Mark, F.; Hildebrandt, P. Determination of the Chromophore Structures in the Photoinduced Reaction Cycle of Phytochrome. *J. Am. Chem. Soc.* **2004,** *126*, 16734–16735.

(13) Mroginski, M. A.; Murgida, D. H.; Hildebrandt, P. The Chromophore Structural Changes During the Photocycle of Phytochrome: A Combined Resonance Raman and Quantum Chemical Approach. *Acc. Chem. Res.* **2007,** *40*, 258–266.

(14) Murgida, D. H.; von Stetten, D.; Hildebrandt, P.; Schwinte, P.; Siebert, F.; Sharda, S.; Gartner, W.; Mroginski, M. A. The Chromophore Structures of the P_r States in Plant and Bacterial Phytochromes. *Biophys. J.* **2007,** *93*, 2410–2417.

(15) Durbeej, B. On the Primary Event of Phytochrome: Quantum Chemical Comparison of Photoreactions at C_4, C_{10} and C_{15}. *Phys. Chem. Chem. Phys.* **2009,** *11*, 1354–1361.

(16) Altoè, P.; Climent, T.; De Fusco, G. C.; Stenta, M.; Bottoni, A.; Serrano-Andrés, L.; Merchán, M.; Orlandi, G.; Garavelli, M. Deciphering Intrinsic Deactivation/Isomerization Routes in a Phytochrome Chromophore Model. *J. Phys. Chem. B* **2009,** *113*, 15067–15073.

(17) Neugebauer, J. Subsystem-Based Theoretical Spectroscopy of Biomolecules and Biomolecular Assemblies. *ChemPhysChem* **2009,** *10*, 3148–3173.

(18) Schaffer, L. V.; Groenhof, G.; Klingen, A. R.; Ullman, G. M.; Boggio-Pasqua, M.; Robb, M. A.; Grubmuller, H. Photoswitching of the Fluorescent Protein asFP595: Mechanism, Proton Pathways, and Absorption Spectra. *Angew. Chem., Int. Ed.* **2007,** *46*, 530–536.

(19) Wagner, J. R.; Zhang, J.; Brunzelle, J. S.; Vierstra, R. D.; Forest, K. T. High Resolution Structure of Deinococcus Bacteriophytochrome Yields New Insights into Phytochrome Architecture and Evolution. *J. Biol. Chem.* **2007,** *282*, 12298–12309.

(20) Nield, J.; Rizkallah, P. J.; Barber, J.; Chayen, N. E. The 1.45A Three-Dimensional Structure of C-Phycocyanin from the Thermophilic Cyanobacterium Synechococcus elongatus. *J. Struct. Biol.* **2003,** *141*, 149–155.

(21) (a) Becke, A. D. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1998,** *38*, 3098–3100. (b) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle−Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988,** *37*, 785–789.

(22) Jacquemin, D.; Perpète, E. A.; Ciofini, I.; Adamo, C. Accurate Simulation of Optical Properties in Dyes. *Acc. Chem. Res.* **2009,** *42*, 326–334.

(23) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; et al. *Gaussian 03*, revision C.02.; Gaussian, Inc.: Wallingford, CT, 2004.

(24) Tomasi, J.; Menucci, B.; Cammi, R. Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005,** *105*, 2999–3094.

(25) Blomberg, M. R. A.; Siegbahn, P. E. M.; Babcock, G. T. Modeling Electron Transfer in Biochemistry: A Quantum Chemical Study of Charge Separation in *Rhodobacter sphaeroides* and Photosystem II. *J. Am. Chem. Soc.* **1998,** *120*, 8812–8824.

(26) Borg, O. A.; Durbeej, B. Relative Ground and Excited-State pK_a Values of Phytochromobilin in the Photoactivation of Phytochrome: A Computational Study. *J. Phys. Chem. B* **2007,** *111*, 11554–11565.

(27) O'Boyle, N. M.; Tenderholt, A. L.; Langner, K. M. cclib: A Library for Package-Independent Computational Chemistry Algorithms. *J. Comput. Chem.* **2008,** *29*, 839–845.

(28) Braslavsky, S. E.; Holzwarth, A. R.; Schaffner, K. Solution Conformations, Photophysics, and Photochemistry of Bile Pigments; Bilirubin and Biliverdin, Dimethyl Esters and Related Linear Tetrapyrroles. *Angew. Chem., Int. Ed. Engl.* **1983,** *22*, 656–674.

(29) Lamparter, T; Esteban, B.; Hughes, J. Phytochrome Cph1 from the Cyanobacterium Synechocystis PCC6803. Purification, Assembly, and Quaternary Structure. *Eur. J. Biochem.* **2001,** *268*, 4720–4730.

(30) Mimuro, M.; Fulglistaller, P.; Rumbeli, R.; Zuber, H. Functional Assignment of Chromophores and Energy Transfer in C-Phycocyanin Isolated from the Thermophilic Cyanobacterium Mastigocladus laminosus. *Biochim. Biophys. Acta* **1986,** *848*, 155–166.

(31) Gouterman, M.; Wagnière, G. H.; Snyder, L. C. Spectra of Porphyrins: Part II. Four Orbital Model. *J. Mol. Spectrosc.* **1963,** *11*, 108–127.

(32) Fischer, A. J.; Lagarias, J. C. Harnessing Phytochrome's Glowing Potential. *Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101*, 17334–17339.

(33) Chae, Q.; Song, P. S. Linear Dichroic Spectra and Fluorescence Polarization of Biliverdin. *J. Am. Chem. Soc.* **1975,** *97*, 4176–4179.

(34) Wagnière, G.; Blauer, G. Calculations of Optical Properties of Biliverdin in Various Conformations. *J. Am. Chem. Soc.* **1976,** *98*, 7806–7810.

(35) Pasternak, W.; Wagnière, G. Possible Interpretation of Long-Wavelength Spectral Shifts in Phytochrome P_r and P_{fr} Forms. *J. Am. Chem. Soc.* **1979,** *101*, 1662–1667.

(36) Scheer, H. Biliproteins. *Angew. Chem., Int. Ed. Engl.* **1981,** *20*, 241–261.

(37) Nesvadba, P.; Gossauer, A. Synthesis of Bile Pigments. 14. Synthesis of a Bilindionostilbenoparacyclophane as a Model for Stretched Bile Pigment Chromophores of Biliproteins. *J. Am. Chem. Soc.* **1987,** *109*, 6545–6546.

(38) Iturraspe, J. B.; Bari, S.; Frydman, B. Total Synthesis of "Extended" Biliverdins: The Relation between their Conformation and their Spectroscopic Properties. *J. Am. Chem. Soc.* **1989,** *111*, 1525–1527.

(39) Guimaraes, C. R. W.; Neto, J. D. D.; De Alencastro, R. B. Phytochrome Structure: A New Methodological Approach. *Int. J. Quantum Chem.* **1998,** *70*, 1145–1157.

(40) Goller, A. H.; Strehlow, D.; Hermann, G. The Excited-State Chemistry of Phycocyanobilin: A Semiempirical Study. *ChemPhysChem* **2005,** *6*, 1259–1268.

DOI: 10.1021/jz900432m | *J. Phys. Chem. Lett.* **2010,** *1*, 796–801

(41) Parker, W.; Goebel, P.; Ross, C. R.; Song, P. S.; Stezowski, J. J. Molecular Modeling of Phytochrome Using Constitutive C-Phycocyanin from Fremyella diplosiphon as a Putative Structural Template. *Bioconjugate Chem.* **1994,** *5*, 21–30.
(42) Wagner, J. R.; Zhang, J.; von Stetten, D.; Gunther, M.; Murgida, D. H.; Mroginski, M. A.; Walker, J. M.; Forest, K. T.; Hildebrandt, P. Mutational Analysis of Deinococcus radiodurans Bacteriophytochrome Reveals Key Amino Acids Necessary for the Photochromicity and Proton Exchange Cycle of Phytochrome. *J. Biol. Chem.* **2008,** *283*, 12212–12226.
(43) Mroginski, M. A.; von Stetten, D.; Escobar, F. V.; Strauss, H. M.; Kaminski, S.; Scheerer, P.; Gunther, M.; Murgida, D. H.; Schmieder, P.; Bongards, C.; Gartner, W.; Mailliet, J.; Hughes, J.; Essen, L. O.; Hildebrandt, P. Chromophore Structure of Cyanobacterial Phytochrome Cph1 in the P_r State: Reconciling Structural and Spectroscopic Data by QM/MM Calculations. *Biophys. J.* **2009,** *96*, 4153–4163.
(44) Spillane, K. M.; Dasgupta, J.; Lagarias, J. C.; Mathies, R. A. Homogeneity of Phytochrome Cph1 Vibronic Absorption Revealed by Resonance Raman Intensity Analysis. *J. Am. Chem. Soc.* **2009,** *131*, 13946–13948.

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