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Reversible Photocontrol of Peptide Conformation with a Rhodopsin-like Photoswitch

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Supporting Information

ABSTRACT: Reversible photocontrol of biomolecules requires chromophores that can efficiently undergo large conformational changes upon exposure to wavelengths of light that are compatible with living systems. We designed a benzylidene−pyrroline chromophore that mimics the Schiff base of rhodopsin and can be used to introduce light-switchable intramolecular cross-links in peptides and proteins. This new class of photoswitch undergoes an ∼10 Å change in end-to-end distance upon isomerization and can be used to control the conformation of a target peptide efficiently and reversibly using, alternately, violet (400 nm) and blue (446 nm) light.

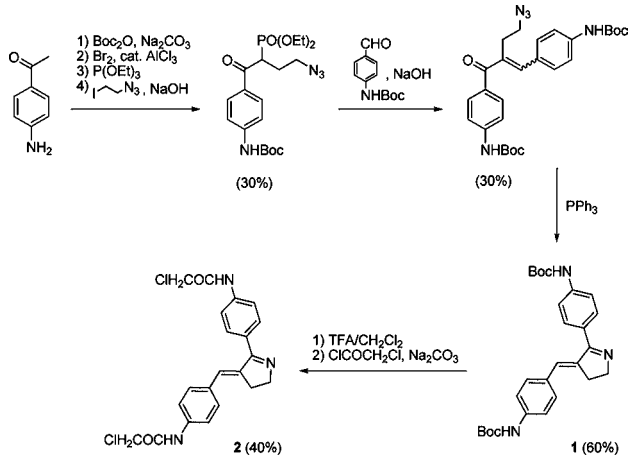
Molecular switches based on *E*/*Z* photoisomerization have been used in a variety of contexts to control diverse properties.[1] Biomolecular photocontrol places a number of demands on photoswitches. In particular, (i) they must undergo isomerization efficiently at wavelengths compatible with biological systems, and (ii) they must undergo substantial conformational change so that they can drive functional change in a biomolecule.[2] The discovery of new or alternative photoswitch types could expand the applicability of the switch concept to different and increasingly complex molecular environments.

The protonated retinal Schiff base chromophore of rhodopsins[3] constitutes an example of a very efficient *E*/*Z* photoswitch shaped by biological evolution. In bovine rhodopsin, selective photoisomerization of the 11-*cis* chromophore occurs rapidly (150 fs) via evolution of a single $\pi \rightarrow \pi^*$ excited state (S_1), providing the all-*trans* ground state (S_0) product with high efficiency (Φ = 67%).[3] While rhodopsin itself can be engineered as a photoswitch,[4] the limited information on the conformational changes that occur hampers the broad applicability of this approach. When removed from the opsin protein, the isolated retinal chromophore exhibits complex photochemistry, including isomerization at any of three double bonds.[5] However, a number of analogues of the rhodopsin chromophore designed to act as photoswitches in isolation have been explored both computationally and experimentally.[6−11] Computational work has shown that some of these molecules may work as efficient photoswitches for peptides,[12,13] but to date there has been no experimental test of the ability of this class of molecules to function as biomolecular photoswitches.

We report here the experimental photocontrol of peptide conformation by the newly designed rhodopsin-like photoswitch **1** (Scheme 1). We based our design on the previously described benzylidene−pyrroline (BP) and N-protonated BP (NHBP) systems.[6] The synthetic route permits the synthesis of a family of switches by modification of the starting aldehyde. Placing amino groups at both ends of the switch unit allowed for the synthesis of thiol-reactive chloroacetamide derivative **2**, which was suitable for site-specific introduction into a Cys-containing peptide as an intramolecular cross-link. The photochemical behavior of this new photoswitch was computed using the multiconfiguration complete active space second-order perturbation theory//complete active space self-consistent field (CASPT2//CASSCF)[6,14] strategy. The amido substituents, together with the added phenyl ring, were predicted to lead to longer-wavelength absorption and a higher isomerization quantum yield relative to the parent benzylidene−pyrroline (see the Supporting Information).

The thiol-reactive photoswitch **2** was coupled to the peptide SS-11 (see below), which confers aqueous solubility to the

Scheme 1. Synthesis of the Photoswitch (*E* Form)



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molecule. The UV/vis spectrum of the photoswitch in aqueous solution in the dark-adapted *E* form is shown in Figure 1a. The

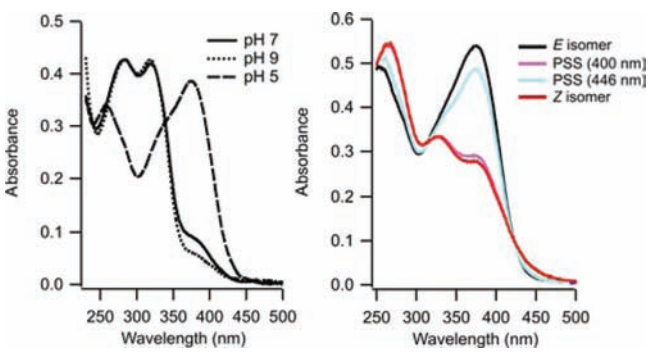

Figure 1. (a) UV−vis spectra of the SS-11 peptide cross-linked with **2** in the dark-adapted *E* form in aqueous solution at different pHs (as indicated) at 20 °C. (b) Photoisomerization of the SS-11 peptide cross-linked with **2** at pH 5.0. Spectra of the pure *E* and *Z* forms of the cross-linked peptide (isolated by HPLC) are shown. Photostationary states (PSSs) at 400 and 446 nm are 7/93 *E*/*Z* and 78/22 *E*/*Z*, respectively.

chromophore (in both isomers) is predicted to have a pK_a of 7.4 ± 0.2 (ACD/iLabs; see the Supporting Information), a value significantly lower than that of the Schiff base in rhodopsin.[15] Lowering the pH to 5.0 caused a red shift in the spectrum consistent with the protonation of the Schiff base moiety,[15−17] while this longer-wavelength band appeared only partially at pH 7.0. (Figure 1a). Presumably, the pK_a of the photoswitch could be raised so that the Schiff base would be protonated at physiological pH by the appropriate introduction of electron-donating substituents. Alternatively, the amino group could be quaternized to maintain a permanent positive charge, as has been done previously with Schiff base analogues.[6,17] The molar extinction coefficient was calculated to be 24 700 M^{-1} cm^{-1} at 375 nm, a value that compares well to those of azobenzene photoswitches, which typically are ∼20 000 M^{-1} cm^{-1}.[18]

Irradiation with violet light (400 nm) efficiently produces a photostationary state (PSS) of the cross-linked peptide that is 93/7 *Z*/*E*. (Figure 1b). The *Z* isomer has decreased absorbance at 375 nm and exhibits a long-wavelength tail. This long-wavelength tail enables photoswitching of the chromophore back to the *E* isomer with blue light (446 nm). Reversible photoswitching was also possible at pH 7.0 (see the Supporting Information), although the process was less efficient since the photoswitch is only partially protonated at this pH. Multiple rounds of photoisomerization gave no evidence of photobleaching/photooxidation (Figure 2).

The *E*-to-*Z* isomerization quantum yield of **1** was measured to be ∼0.6 for the unprotonated chromophore alone in acetonitrile and ∼0.24 for the protonated chromophore attached to a peptide in water using *trans*-azobenzene or a *trans*-azobenzene-modified peptide[19] as an actinometer (see the Supporting Information). The value for the quantum yield of the isolated chromophore is significantly larger than those reported for some biarylidenes (0.07−0.55).[20] The overall spectroscopic behavior of **1** compares well with predictions from the CASPT2//CASSCF theory (see the Supporting Information for details).[6]

Slow thermal relaxation of the *Z* isomer of the cross-linked peptide to the thermodynamically more stable *E* form was seen

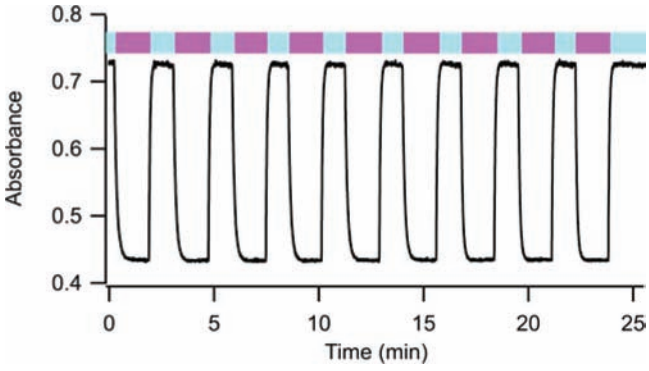

Figure 2. Multiple rounds of photoswitching of the SS-11 peptide cross-linked using **2** with alternating violet (400 nm) and blue (446 nm) light in aqueous solution at 20 °C.

with a half-life of ∼10 h at 50 °C (see the Supporting Information). This slow relaxation permitted separation of the *E* and *Z* isomers by HPLC (see the Supporting Information). The spectra of the pure *E* and *Z* forms at pH 5.0 are shown in Figure 1b.

The change in end-to-end distance of **2** upon isomerization was estimated using molecular dynamics simulations (see the Supporting Information). Figure 3 shows that *E*/*Z* isomerization produces a large (∼10 Å) change in the mean end-to-end distance with very little overlap between isomers. The size of this conformational change is significantly greater than those in many azobenzene-based photoswitches.[2] Azobenzene derivatives specifically designed to undergo large changes in end-to-end distance upon photoisomerization have exhibited lower photoisomerization efficiencies or more complex conformational distributions than those exhibited by **2** (Figure 3a).[21,22]

Photocontrol of peptide α-helical structure can be achieved by matching the end-to-end distance of a photoswitchable cross-linker in one isomeric state with the distance between cross-linker attachment points in the peptide.[23,24] The end-to-end distance of the *E* isomer matches well with the distance between Cys side chains with an *i*, *i* + 11 sequence spacing in a peptide α-helix (Figure 3a), whereas the *Z* isomer is too short to permit normal helix formation with this Cys side-chain spacing. The peptide SS-11, studied previously as an ideal helix-forming sequence in water,[25] was chosen as target for photocontrol by **2**. The peptide is long enough to confer aqueous solubility on the relatively hydrophobic cross-linker. Since **2** is not a symmetrical molecule, two distinct species result when it reacts with a peptide to form an intramolecular cross-link. Since the end-to-end distance change caused by isomerization is the same for both species, we did not attempt to characterize these isomers separately for conformational analysis (see the Supporting Information).

Cross-linking the SS-11 peptide with the *E* form of **2** leads to stabilization of the α-helical content, as judged by circular dichroism (CD) spectroscopy (see the Supporting Information), and the peptide is essentially fully helical at 20 °C. Irradiation with 400 nm light to produce the 7/93 *E*/*Z* PSS leads to a marked decrease in helicity, as predicted (Figure 3b). Since the cross-linker is near the N-terminus of the peptide, conformational distortion is likely to be focused there, with the rest of the peptide remaining helical (Figure 3d,e). It is unlikely that the photoswitch itself contributes significantly to the observed CD change, since no induced CD signal was seen at

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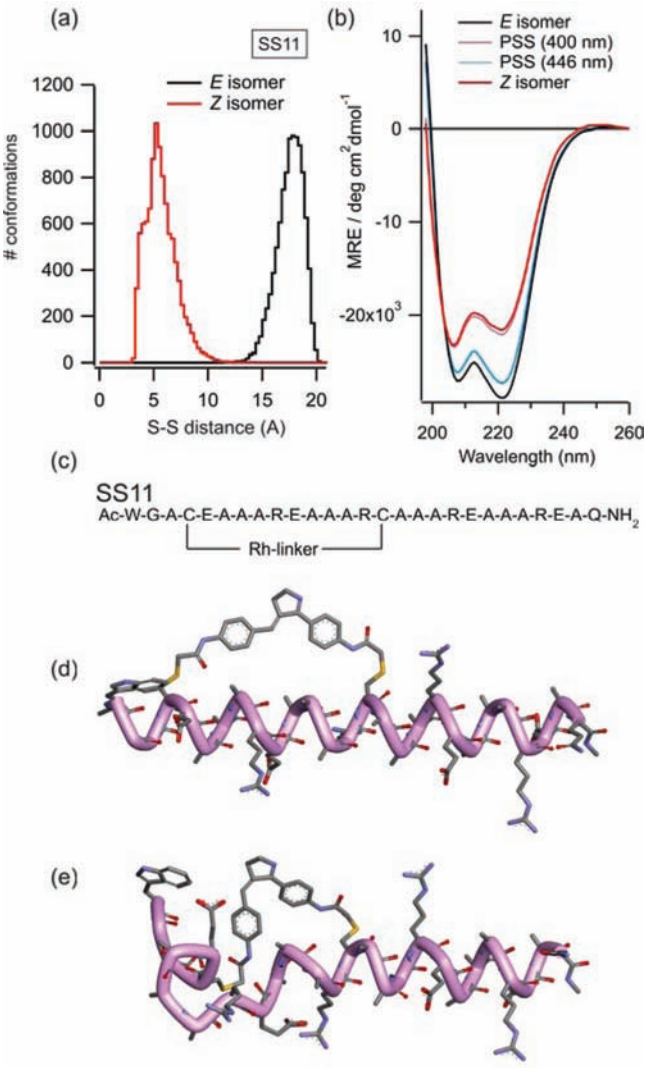

Figure 3. (a) Calculated change in the end-to-end distance between S atom attachment points for the *E* and *Z* forms of the photoswitch. (b) CD spectra of the peptide SS-11 (c) cross-linked with **2**. (d) Illustration of the cross-linked peptide with the photoswitch in the helix-stabilizing *E* form. (e) Illustration showing disruption of the helical structure by the *Z* form of the photoswitch.

wavelengths where only the photoswitch absorbs (see the Supporting Information).

The observed conformational change was fully reversible to the 78/22 *E*/*Z* PSS upon irradiation with blue light (Figure 3b). Thus, the designed switch functions effectively for the photocontrol of peptide conformation in an aqueous environment using visible wavelengths. The slow thermal relaxation process enables each conformational isomer to be produced with a brief light pulse and maintained on time scales of biological interest. The large conformational change that occurs upon isomerization and the efficiency of the photoswitching make this rhodopsin-like photoswitch an attractive candidate for peptide and protein photocontrol that can be applied to a wide variety of targets. Further elaboration may make even longer wavelength switching possible, as is observed with natural rhodopsin.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Computational details, synthetic procedures, UV/vis and CD data, and HPLC characterization of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) *Molecular Switches*; Feringa, B., Ed.; Wiley-VCH: Weinheim, Germany, 2001.

(2) Beharry, A. A.; Woolley, G. A. *Chem. Soc. Rev.* **2011**, *40*, 4422–4437.

(3) Mathies, R. A.; Lugtenburg, J. In *Handbook of Biological Physics*; Stavenga, D. G., de Grip, W. J., Pugh, E. N., Eds.; Elsevier: Amsterdam, 2000; Vol. 3, pp 56–90.

(4) Airan, R. D.; Thompson, K. R.; Fenno, L. E.; Bernstein, H.; Deisseroth, K. *Nature* **2009**, *458*, 1025–1029.

(5) Brown, P. K.; Wald, G. *J. Biol. Chem.* **1956**, *222*, 865–877.

(6) Sampedro, D.; Migani, A.; Pepi, A.; Busi, E.; Basosi, R.; Latterini, L.; Elisei, F.; Fusi, S.; Ponticelli, F.; Zanirato, V.; Olivucci, M. *J. Am. Chem. Soc.* **2004**, *126*, 9349–9359.

(7) Lumento, F.; Zanirato, V.; Fusi, S.; Busi, E.; Latterini, L.; Elisei, F.; Sinicropi, A.; Andruniów, T.; Ferré, N.; Basosi, R.; Olivucci, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 414–420.

(8) Rivado-Casas, L.; Blanco-Lomas, M.; Campos, P. J.; Sampedro, D. *Tetrahedron* **2011**, *67*, 7570–7574.

(9) Rivado-Casas, L.; Campos, P. J.; Sampedro, D. *Organometallics* **2010**, *29*, 3117–3124.

(10) Melloni, A.; Rossi Paccani, R.; Donati, D.; Zanirato, V.; Sinicropi, A.; Parisi, M. L.; Martin, E.; Ryazantsev, M.; Ding, W. J.; Frutos, L. M.; Basosi, R.; Fusi, S.; Latterini, L.; Ferré, N.; Olivucci, M. *J. Am. Chem. Soc.* **2010**, *132*, 9310–9319.

(11) Sinicropi, A.; Martin, E.; Ryazantsev, M.; Helbing, J.; Briand, J.; Sharma, D.; Léonard, J.; Haacke, S.; Cannizzo, A.; Chergui, M.; Zanirato, V.; Fusi, S.; Santoro, F.; Basosi, R.; Ferré, N.; Olivucci, M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17642–17647.

(12) Andruniow, T.; Fantacci, S.; De Angelis, F.; Ferre, N.; Olivucci, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 6077–6081.

(13) Sinicropi, A.; Bernini, C.; Basosi, R.; Olivucci, M. *Photochem. Photobiol. Sci.* **2009**, *8*, 1639–1649.

(14) Sampedro, D. In *Photochemistry: UV/VIS Spectroscopy, Photochemical Reactions, and Photosynthesis*; Maes, K. J., Willems, J. M., Eds.; Nova Science Publishers: Hauppauge, NY, 2011.

(15) Kolodner, P.; Lukashev, E. P.; Ching, Y. C.; Rousseau, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11618–11621.

(16) Nielsen, I. B.; Petersen, M. A.; Lammich, L.; Nielsen, M. B.; Andersen, L. H. *J. Phys. Chem. A* **2006**, *110*, 12592–12596.

(17) Petersen, M. A.; Nielsen, I. B.; Kristensen, M. B.; Kadziola, A.; Lammich, L.; Andersen, L. H.; Nielsen, M. B. *Org. Biomol. Chem.* **2006**, *4*, 1546–1554.

(18) Rau, H. In *Photochromism: Molecules and Systems*; Durr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; pp 165–192.

(19) Borisenko, V.; Woolley, G. A. *J. Photochem. Photobiol., A.* **2005**, *173*, 21−28.
(20) Feringa, B. L. *J. Org. Chem.* **2007**, *72*, 6635−6652.
(21) Standaert, R. F.; Park, S. B. *J. Org. Chem.* **2006**, *71*, 7952−7966.
(22) Zhang, F.; Sadovski, O.; Woolley, G. A. *ChemBioChem* **2008**, *9*, 2147−2154.
(23) Burns, D. C.; Flint, D. G.; Kumita, J. R.; Feldman, H. J.; Serrano, L.; Zhang, Z.; Smart, O. S.; Woolley, G. A. *Biochemistry* **2004**, *43*, 15329−15338.
(24) Flint, D. G.; Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Chem. Biol.* **2002**, *9*, 391−397.
(25) Samanta, S.; Woolley, G. A. *ChemBioChem* **2011**, *12*, 1712−1723.