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Origin of Spectral Tuning in Rhodopsin—It Is Not the Binding Pocket**

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One of the most basic and unresolved puzzles in the chemistry of vision is the mechanism regulating the absorbance of the visual photoreceptors. Rhodopsin, the rod pigment that mediates black/white vision in the human eye, absorbs at 498 nm; the three cone pigments responsible for trichromatic (color) vision absorb at 425, 530, and 560 nm, respectively. Since the chromophore in these receptors is the same protonated Schiff base of 11-*cis*-retinal (pSb11), the spectra of these pigments are clearly a function of the protein environment the chromophore "sees"; in other words, the spectra are tuned by the protein.[1]

Three mechanisms are generally agreed to be involved in spectral tuning: 1) distortion of the chromophore as a result of steric interactions with the protein binding pocket; 2) interaction of the chromophore with the counterion balancing its positive charge; and 3) interaction of the chromophore with the remaining polar residues of the amino acids lining the binding pocket. However, the importance of these contributions could not be assessed quantitatively as long as realistic structures of the visual pigments were not available. This changed with the first X-ray crystal structure of rhodopsin,[2] now solved down to 2.2-Å resolution,[3] which made it possible to study the chromophore including its environment with high-quality quantum-mechanical methods.[4–7] Despite considerable insights gained from these studies, fundamental questions remain, in particular to what extent the amino acid residues of the binding pocket affect the spectrum of the chromophore.

Very recently the absorption cross section of pSb11 in the gas phase was determined by analyzing fragments of photochemically excited ions.[8] Devoid of the restraining forces and charges of the protein environment, the chromophore in the gas phase or vacuum appears tailor-made for high-level quantum-mechanical calculations. It also presents a well-defined point of reference for the analysis of spectral tuning in rhodopsin. Using multiconfigurational perturbation theory we have been able to reproduce the experimental absorption maximum of the bare rhodopsin chromophore (610 nm) with high accuracy.[9] Employing the same theoretical platform we show in the following that the three contributions discussed above add up quantitatively to the experimentally observed spectral shift of the chromophore on going from the vacuum to the rhodopsin molecule. By far the largest contributor to the shift is the counterion, while the role of the polar amino acid residues of the protein pocket, contrary to general consensus, is limited to modulating the spectrum.

The calculations were performed on three pSb11 model systems derived from the SCC-DFTB-refined[10] geometry of the rhodopsin binding pocket with 2.2-Å resolution shown in Figure 1, and they increasingly reflect the influence of the protein. Starting with the optimized vacuum structure $pSb11_{vac}$ described earlier,[9] changes in the geometry corresponding to the calculated rhodopsin structure were added which resulted in the bare distorted chromophore $pSb11_{dist}$. To this was attached the counterion E113 giving the ion pair structure $pSb11_{ip}$. Finally, electrostatic interactions between the chromophore and the polar amino acids constituting the protein pocket were added using quantum-mechanically calculated partial atomic charges, which yielded finally the protein-embedded 11-*cis*-retinal chromophore $pSb11_{pe}$.

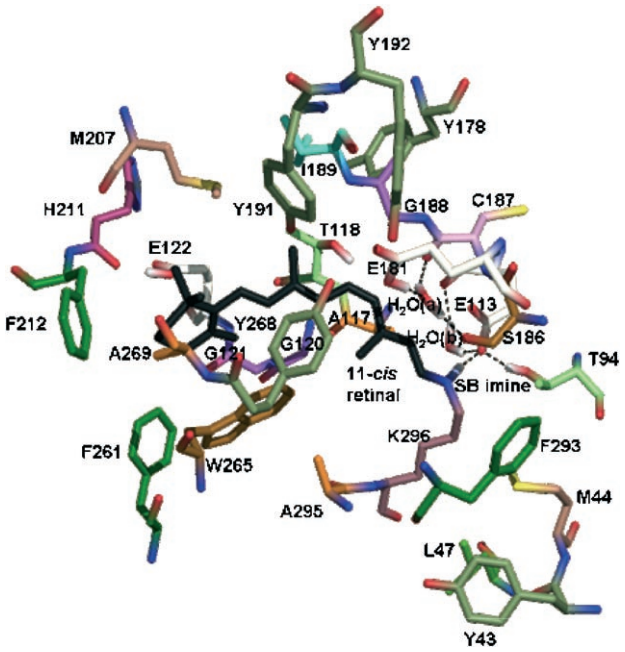

Figure 1. SCC-DFTB-optimized binding pocket of rhodopsin. The chromophore is shown in black. The 28 amino acids within 4.5 Å distance of the chromophore and two water molecules close to the counterion E113 are shown.

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Ground (S_0) and excited states (S_1 and S_2) of the three chromophore models were calculated using second-order perturbation theory (CASPT2[11]) within an atomic natural orbital (ANO) basis set expansion. Atomic point charges were obtained by a natural population analysis (NPA)[12]) of the B3LYP/6-31G** wave function of the complete binding pocket. Details are described in the Experimental Section. For reference purposes we also include data for $pSb11_{vac}$ taken from previously published work.[9]

A summary of the calculated spectral data of the four retinal model systems is given in Table 1. For completeness we have also included the results for the calculated and strongly

Table 1: CASPT2 energies (E), oscillator strengths (f), main contributing configurations with weight, and dipole moments (μ) for the ground state S_0 and the excited states S_1 and S_2 of chromophore models.

Structure[a]	State	E[b,c]	f	Config.[d]	μ[e]
$pSb11_{vac}$[f]	S_0	−871.2306	–	$(6a)^2(7a)^0$ *75*	22.75
	S_1	2.05 (**606**)	1.12	$(6a)^1(7a)^1$ *61*	6.70
	S_2	2.84 (436)	0.13	$(6a)^0(7a)^2$ *28*	19.45
$pSb11_{dist}$	S_0	−871.2185	–	$(6a)^2(7a)^0$ *74*	16.41
	S_1	1.93 (**643**)	0.96	$(6a)^1(7a)^1$ *60*	11.42
	S_2	2.77 (447)	0.14	$(6a)^0(7a)^2$ *27*	13.34
$pSb11_{ip}$	S_0	−1060.0803		$(6a)^2(7a)^0$ *65*	10.22
	S_1	2.55 (**486**)	0.82	$(6a)^1(7a)^1$ *62*	20.79
	S_2	2.93 (423)	0.00	$(6a)^0(7a)^2$ *29*	10.03
$pSb11_{pe}$[g]	S_0	−1060.7835		$(6a)^2(7a)^0$ *65*	9.64
	S_1	2.47 (**502**)	0.79	$(6a)^1(7a)^1$ *62*	21.59
	S_2	2.89 (429)	0.00	$(6a)^0(7a)^2$ *29*	9.64

[a] See text for abbreviations used. [b] S_0 energies in au, S_1 and S_2 energies relative to S_0 in eV and (in parentheses) in nm. [c] Optical absorption in bold. [d] Only π-type orbitals are counted; weights in %. [e] Dipole moments in Debye. [f] Data taken from Ref. [9]. [g] Charges calculated using NPA. Results obtained with Mulliken and CHARMM charges are shown in the Supporting Information.

forbidden S_2 state, but here we will discuss only the $S_0 \rightarrow S_1$ excitation, which corresponds to the optically allowed transition. Turning first to the bare chromophore in vacuo, $pSb11_{vac}$, we recall the very good agreement between the calculated absorption maximum (606 nm) and the experimental value obtained from measurements in the gas phase (610 nm).[8] The agreement is by no means accidental. When the same particular set of CASPT2 parameters is used, quantitative agreement is obtained with other retinal derivatives in charged and uncharged environments as well covering a wavelength range from 600 to 350 nm.[9]

Binding by the protein enforces steric constraints by way of nonplanar distortions of the chromophore, in particular about the pivotal C11=C12 bond, where the dihedral angle increases from 8° in $pSb11_{vac}$ to 17° in $pSb11_{dist}$. The sum of all distortions adds up to a red shift of the absorbance by 37 nm. In addition to the spectral shift we note a reduction of the oscillator strength and of the ground state dipole moment, both a consequence of the somewhat compressed geometry of the bound chromophore relative to the fully stretched geometry in vacuo. We further note that for both $pSb11_{vac}$ and $pSb11_{dist}$ the dipole moment is significantly less in the S_1 state than in the S_0 state, a consequence of the massive flow of negative charge from the conjugated carbon chain towards the positive nitrogen atom following excitation to the ionic S_1 state.[13]

Major changes take place when the counterion is attached to the chromophore ($pSb11_{ip}$). The energy gap between S_0 and S_1 increases significantly by 0.62 eV, which shifts the absorbance of the ion pair (486 nm) into the range of the rhodopsin absorption maximum (498 nm). The decrease of the oscillator strength indicates that the motion of the electrons being excited is further restricted. This is conceivable considering the fact that in the ion pair this charge must be shifted against the negatively charged counterion. This shift is also the reason for the increased dipole moment of the S_1 state relative to that of S_0 as we have discussed before.[14]

The major part of the calculated blue shift arises from the destabilization of the S_1 state in the presence of the counterion caused by increased electrostatic repulsion. There is another effect, specifically, the increased double-bond fixation of the conjugated chain as the positive charge of the chromophore becomes localized by the counterion. Calculations on model retinals have shown, however, that this effect contributes no more than 10% to the total shift.[14]

The spectral tuning is completed by the addition of the electrostatic interactions with the amino acids to the chromophore/counterion pair ($pSb11_{pe}$). A look at Table 1 reveals that the perturbation due to the charges is rather small. There is a red shift of the absorbance of 0.08 eV or 16 nm, and also the charge distribution as measured by the dipole moments does not change significantly. The net effect of the charges is, however, to bring the calculated absorbance very close to the experimental absorption maximum of rhodopsin.

Natural population analysis is based on the transformation of the molecular wave function into natural bonding orbitals. It has been developed as a tool to obtain a molecular charge distribution that is less dependent on the choice of the basis set. We have also calculated the spectral properties of the embedded chromophore $pSb11_{pe}$ using the charges obtained from a conventional Mulliken population analysis and from CHARMM. While the former, like the NPA charges, are derived from an explicitly calculated wave function, the latter are transferable parameters. The calculated shifts are +20 nm using Mulliken charges and −7 nm for the CHARMM charges. The effect of the environment should be described better by the quantum-mechanically derived charges than by the environment-insensitive CHARMM charges. However, with the absolute shifts in all three cases being smaller than 0.1 eV, these calculations rule out the interaction between the chromophore and the neutral amino acids of the protein as a major contributor to spectral tuning in rhodopsin.

A red shift due to the charges of the protein pocket has been calculated elsewhere using a setup (CASPT2 with AMBER charges) similar to the one described here.[5] However, the magnitude of this shift, 0.53 eV, is according to our analysis much too large. It is only because the calculated absorbance of the bare chromophore in that study (comparable to our $pSb11_{dist}$ structure) is highly blue-

shifted relative to ours, that the calculated S_0/S_1 difference of the protein embedded chromophore is moderately close to the experimental value.

Employing the best structural data available, we have shown that the absorption spectrum of rhodopsin can be calculated ab initio and with great accuracy using a high-level quantum-mechanical platform. We have demonstrated that by far the largest effect is exerted by the counterion. Since the protein environment provides and stabilizes the chromophore distortion necessary for the selective and ultrafast transformation to bathorhodopsin, we conclude that this is its primary role and that spectral tuning by the binding pocket is not the goal pursued by evolution.

Experimental Section

For the calculations reported in this paper we have used the SCC-DFTB-optimized model for the binding pocket of rhodopsin, which is based on chain B of the crystal structure of rhodopsin with 2.2-Å resolution (PDB code 1U19) and has been described in detail.[3] The different pSb11 structures were obtained by removing from this model the complete protein shell to give $pSb11_{dist}$, and adding the counterion as a formiate ion to give $pSb11_{ip}$. For $pSb11_{pe}$, the wave function of the whole binding pocket including the chromophore was calculated with Gaussian[15] using DFT (B3LYP in a 6-31G** basis set) on which a natural population analysis was performed. Cartesian coordinates of the binding pocket and the charges can be found in the Supporting Information.

For the electronic structure calculations CASPT2 was employed from the Molcas set of routines.[16] The basis was an ANO set[17] contracted to 4s3p1d for the heavy atoms and 2s for hydrogen. The (12/12) active space included all π-type orbitals and electrons; the level shift was set uniformly at 0.3 au. Transition dipole moments were calculated in the velocity formalism and combined with CASPT2 energies to give oscillator strengths.

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[1] T. P. Sakmar, S. T. Menon, E. P. Martin, E. S. Awad, *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 443 – 484.
[2] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739 – 745.
[3] T. Okada, M. Sugihara, A. N. Bondar, M. Elstner, P. Entel, V. Buss, *J. Mol. Biol.* **2004**, *342*, 571 – 583.
[4] N. Ferre, M. Olivucci, *J. Am. Chem. Soc.* **2003**, *125*, 6868 – 6869.
[5] T. Andruniow, N. Ferre, M. Olivucci, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17908 – 17913.
[6] K. Fujimoto, J. Hasegawa, S. Hayashi, S. Kato, H. Nakatsuji, *Chem. Phys. Lett.* **2005**, *414*, 239 – 242.
[7] M. Schreiber, M. Sugihara, T. Okada, V. Buss, *Angew. Chem.* **2006**, *118*, 4380 – 4383; *Angew. Chem. Int. Ed.* **2006**, *45*, 4274 – 4277.
[8] L. H. Andersen, I. B. Nielsen, M. B. Kristensen, M. O. A. Ghazaly, S. Haake, M. Brondsted Nielsen, M. A. Petersen, *J. Am. Chem. Soc.* **2005**, *127*, 12347 – 12350.
[9] S. Sekharan, O. Weingart, V. Buss, *Biophys. J.* **2006**, *91*, L7 – L9.
[10] M. Elstner, D. Porezag, G. Jungnickel, J. Elsner, M. Haugk, T. Frauenheim, S. Suhai, G. Seifert, *Phys. Rev. B* **1998**, *58*, 7260 – 7268.
[11] K. Andersson, P.-A. Malmqvist, B. O. Roos, A. J. Sadley, K. Wolinski, *J. Phys. Chem.* **1990**, *94*, 5483 – 5488.
[12] A. R. Reed, R. B. Weinstock, F. Weinhold, *J. Chem. Phys.* **1985**, *83*, 735 – 746.
[13] P. Tavan, K. Schulten, *J. Chem. Phys.* **1986**, *85*, 6602 – 6608.
[14] J. Hufen, M. Sugihara, V. Buss, *J. Phys. Chem. B* **2004**, *108*, 20419 – 20426.
[15] M. J. Frisch et al., Gaussian **2003**, Revision B.04; Gaussian Inc, Pittsburgh PA.
[16] M. P. Fuelscher et al., Molcas **2004**, Version 6.2; University of Lund, Sweden.
[17] K. Pierloot, B. Dumez, P. O. Roos, B. O. Roos, *Theor. Chim. Acta* **1995**, *90*, 87 – 114.

