Color Tuning

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# Clay Mimics Color Tuning in Visual Pigments**

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Humans have four photoreceptive proteins; one for twilight vision, and three for color vision.[1] The first, rhodopsin, is present in rod cells ($\lambda_{max} \approx 500$ nm). The other three are present in cone cells, and called by their absorbing colors, such as human blue ($\lambda_{max} \approx 425$ nm), human green ($\lambda_{max} \approx 530$ nm), and human red ($\lambda_{max} \approx 560$ nm).[2,3] In all cases, the chromophore is the protonated retinal Schiff base in the 11-*cis* isomeric state (RSB-11) that is bound to a lysine residue at the seventh helix of the opsin (Figure 1 a).[4] No structures have been determined for color pigments, though the fundamental architecture is believed to be similar to that of bovine rhodopsin. Protein structures composed of 7-transmembrane helices are common not only for the visual proteins but also for thousands of G-protein coupled receptors. Color originates from the energy gap of the protonated RSB-11 between its electronically excited and ground states. It is generally accepted that the mechanism of color tuning is primarily in the interaction between RSB-11, protonated at the Schiff base, and its counterion; when the interaction is weaker, the spectrum shifts to longer wavelengths.[1,5] Although hydrophobic amino acid residues surround the β-ionone ring and polyene chain of RSB-11, the retinal Schiff base region is highly hydrophilic. Polar residues and internal water molecules must participate in the stabilization of the ion-pair state,[5] though the protein inside is normally hydrophobic (the dielectric constant is about 4). In solution, the cationic chromophore and the anion present are free and their short separation gives the energy state corresponding to a $\lambda_{max}$ at 430–460 nm.[6] In contrast, the position of counterion in the protein is controlled so as to change the interaction, leading to maxima suited for acquisition of visible light (400–700 nm; $\lambda_{max}$ from 425 to 560 nm). In bovine rhodopsin, the oxygen atoms of Glu113 are located at a 3–4-Å distance from the Schiff base nitrogen atom (Figure 1 a),[4] and $\lambda_{max}$ is shifted to 498 nm.

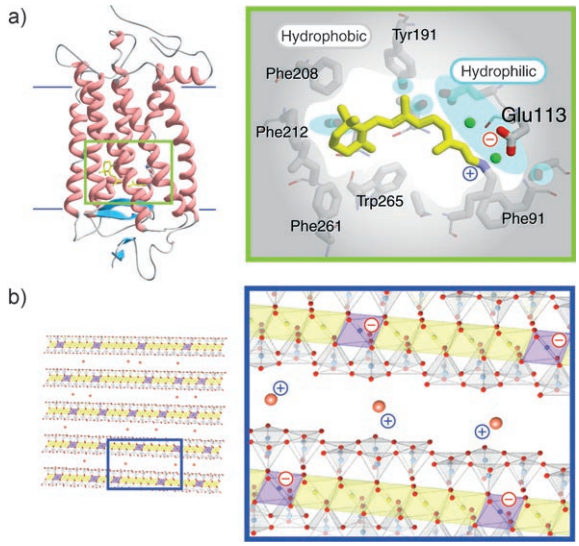


***Figure 1.*** a) Left: Protein structure of bovine rhodopsin with chromophore RSB-11 (shown in yellow). The region in the green box is enlarged (right), highlighting the structure of RSB-11 (yellow) and the protein environment. Protonated Schiff base nitrogen atom is blue, internal water molecules green, and negatively charged Glu113 is indicated (oxygen red). The hydrophilic regions are shown by light blue shading. b) The structure of the clay montmorillonite. Two $SiO_2$ tetrahedral sheets (gray) sandwich an $AlO_6$ octahedral sheet (yellow). Substitution of some aluminum ions, primarily by magnesium ions (purple), results in negative charge in the layer, which is neutralized by interlayer cations (red). Small spheres are Al (yellow), O (red) and Si (blue).

Although artificial construction of wide color tuning of the rhodopsin chromophore in other materials had long been unsuccessful, Sasaki and Fukuhara reported that $\lambda_{max}$ of *all-trans* RSB was found at 530 nm when mixed with a montmorillonite (Kunipia-F) modified by dimethyloctadecylamine (DOA) in benzene solution.[7] Montmorillonite is a natural clay (Figure 1 b). The negative charges of the silicate layers are compensated by interlayer cations ($Na^{I}$, $K^{I}$, $Ca^{II}$, $Mg^{II}$, etc.) that intervene in adjacent layers with some water molecules. The properties of clays are determined by substitution of ions in the backbone layer and interlayer cations. Unique characteristics are exhibited, which depend on the clay source and from the common architecture. Exchange of interlayer cations, which is easily achieved in aqueous solution with cationic surfactants, such as DOA, presumably leads to a great affinity for organic molecules,[8] and hence *all-trans* RSB was intercalated and a proton was supplied from DOA. While the color-tuning mechanism is yet to be understood, clay was seen to be a potential protein-like model matrix. A similar approach was reported in an application as a photonic device.[9] Herein we report that RSB-11 exhibits various colors when adsorbed onto mont-

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morillonite. The $\lambda_{max}$ of RSB-11 in benzene is at 356 nm (Figure 2), indicating that the Schiff base is not protonated. However, when various montmorillonites (Table 1) were mixed with the benzene solution, absorption in the UV

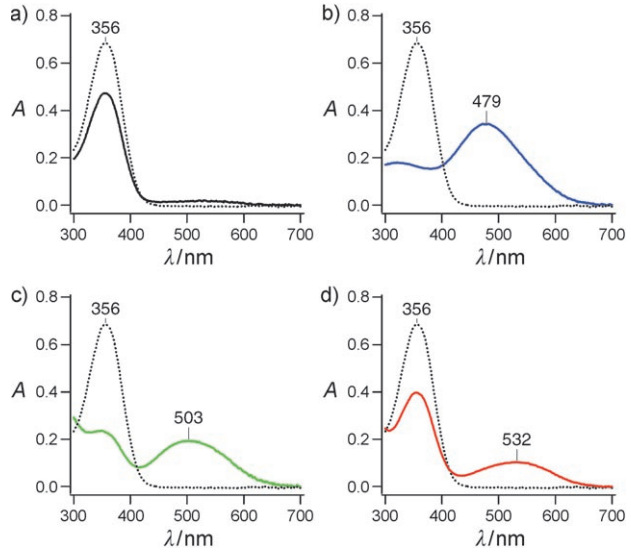


***Figure 2.*** Absorption spectra of RSB-11 mixed with montmorillonite in benzene solution. Black dotted lines in a)–d): absorption spectra of RSB-11 in benzene (without clay). Solid lines: absorption spectra of RSB-11 mixed with clay a) Kunipia-F, b) Bengel Bright 11, c) Mikawa, and d) Bengel A in benzene, where scattering effect of each clay is subtracted. For host materials used, see the Experimental Section.

region decreased with concomitant appearance of absorption in the visible region. Interestingly, the $\lambda_{max}$ and the absorbance were highly dependent on the clay used. In case of Kunipia-F, the absorption in the visible range is very small, and $\lambda_{max}$ not accurately determined (Figure 2a). On the other hand, clear absorption maxima in the visible region appeared at 400–700 nm in other clays, resembling the visual rhodopsins, with maxima at 479 nm for Bengel Bright 11 (Figure 2b), at 503 nm for Mikawa montmorillonite (Figure 2c), and at 532 nm for Bengel A (Figure 2d). The linear concentration dependence of the absorption increase (not shown) indicates

***Table 1:*** Four types of montmorillonites and their color-tuning characteristics.[a]

| Montmorillonite (CEC[b]) | RSB-11 | | *all-trans* RSB | |
|---|---|---|---|---|
| | $\lambda_{max}$ [nm] | fwhm[c] [$cm^{-1}$] | $\lambda_{max}$ [nm] | fwhm[c] [$cm^{-1}$] |
| Kunipia-F (119) | – | | – | |
| Bengel Bright 11 (78) | 479 | 6740 | 502 | 6110 |
| Mikawa (93) | 503 | 5930 | 524 | 5490 |
| Bengel A (94) | 532 | 5640 | 544 | 4550 |
| Other systems: | | | | |
| Benzene solution[6] | 455 | | 455 | |
| bovine rhodopsin | 498 | 4240 | | |

[a] For details of the clays used, see the Experimental Section. [b] Cation exchange capacities (meq/100 g clay). [c] fwhm = full-width half-maximum.

that RSB-11 does not form a complex such as a dimer, which is also the case in the visual protein. The strongly shifted $\lambda_{max}$ values in the visible region strongly suggest that RSB-11 is protonated through interaction with montmorillonite, and the interaction controls the value of $\lambda_{max}$ as for proteins in visual pigments.

Figure 3a shows the normalized absorption spectra of RSB-11 mixed with three clays. These spectra cover almost the entire visible region from 400 to 700 nm. The range of the $\lambda_{max}$ (479–532 nm) is narrower than those of proteins (425–560 nm), even though the entire visible region is covered. The reason is the broadened absorption spectra of RSB-11 in clay (Table 1). The full-width half-maximum (fwhm) is 6740, 5930,

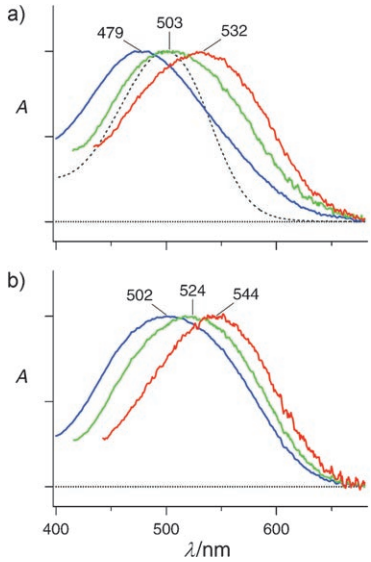


***Figure 3.*** a) Normalized absorption spectra of RSB-11 mixed with various clays in benzene solution (blue Bengel Bright 11, green Mikawa, red Bengel A). Black dashed line represents absorption spectrum of bovine rhodopsin. b) Normalized absorption spectra of *all-trans* RSB mixed with various clays in benzene solution (blue Bengel Bright 11, green Mikawa,; red Bengel A).

and 5640 $cm^{-1}$ in Bengel Bright 11, Mikawa, and Bengel A, respectively, and is clearly larger than that of visual rhodopsins (4240 $cm^{-1}$ for bovine rhodopsin, black dotted line in Figure 3a). In contrast, the greater degree of freedom in the two-dimensional interlayers of the clay presumably provides a multiple distribution of RSB-11.

The question arises as to whether RSB-11 is located between the layers in clay. The RSB-11 molecules may be simply attached to the surface of clay, not to interlayers, and the clay-dependent interaction might control the $\lambda_{max}$. To test this possibility, we measured absorption spectra of the *all-trans* form (RSB-AT). It is noted that protonated RSB-11 and RSB-AT has identical $\lambda_{max}$ in benzene (Table 1, 455 nm),[6] as the $\lambda_{max}$ is not significantly influenced by the isomeric form in solution. Figure 3b shows the normalized absorption spectra of RSB-AT mixed with three different clays. The distinct shifts of $\lambda_{max}$ observed between RSB-11 and RSB-AT in clay (23 nm for Bengel Bright 11, 21 nm for Mikawa, and 12 nm

  

for Bengel A) strongly suggest that the chromophore is not exposed to the solvent. The interlayer distance of approximately 0.5 nm, which was determined by subtracting the thickness of silicate layer (0.96 nm) from the observed basal spacings (data not shown), also suggests that RSB-AT molecules are embedded in the interlayer.

What is the mechanism to stabilize the RSB chromophore in the clay interlayers? In the case of RSB-AT in montmorillonite modified by detergent, our FTIR study suggested protonation of RSB-AT, where the Schiff base proton is probably supplied from DOA.[7,10] In contrast, the present system is simpler, where the Schiff base proton must be supplied from the clay. Clay is known to have hydroxide groups at the end of the layers, and such acidic groups may be important as the proton donor. It was also mentioned that the interlayer surface is negatively charged because of the replacement of $Al^{III}$ by $Mg^{II}$.[11] Clay contains interlayer cations and water in aqueous solution, while the RSB chromophore must be stabilized in the layer together with benzene. The effect of the cation on color tuning in each clay will be reported elsewhere.

The intercalation of dyes into the interlayer space often results in spectral changes as a result of host–guest interactions.[12] Large spectral shifts may occur in the presence of charge-transfer interactions between guest and host. However, to our knowledge, large spectral shifts similar to those achieved in the present system have never been observed by the intercalation into alkali- and alkaline-earth-ion exchanged clays.

In summary, we report herein the absorption spectra of RSB-11 mixed with three clays of the identical backbone structure. The observed spectra cover the entire wavelength region of visible light. Essentially similar structure but fine structural modification yields color tuning in clay, which is also the case in proteins. Thus, protein and clay, completely different matrices, have a similar effect on RSB, the chromophore molecule of our vision. Further efforts on both proteins and clay interlayers will lead to better understanding of the color-tuning mechanism of RSB in our vision.

## Experimental Section

Four kinds of natural montmorillonite clay were tested. Kunipia-F was obtained from Tsukimoto, Japan (Kunimine Kogyo Co., Japan; reference clay sample of The Clay Science Society of Japan). Bengel Bright 11 was obtained from Wyoming, USA (Hojun Ind. Co., Japan). Mikawa is the name of the mine in Japan, which is also the reference clay sample of The Clay Science Society of Japan. Bengel A was obtained from China (Hojun Ind. Co., Japan). These aqueous suspensions all gave pH values of about 9–10. In the experiments, each clay (20 mg) was dried for 20 h, then dissolved in benzene (3.125 mL) containing RSB-11 or *all-trans* RSB (each $2.0 \times 10^{-2}$ mM). The RSB sample was prepared by mixing 11-*cis* or *all-trans* retinal with an excess of *n*-butylamine as described previously.[7,10] Benzene was used as a solvent because its refractive index is equivalent to that of the clay (ca. 1.5),[7] resulting in the substantial reduction of Rayleigh scattering, as in the case of zeolite.[13] UV/Vis spectra were measured using a Photonic Mulitchannel Spectral Analyzer PMA-11 C8808-01, which contains a CCD linear detector, with a CW xenon lamp L8004 as a light source (Hamamatsu Photonics K.K., Japan). We accumulated the absorption spectra once every second, and accurate spectra were thus obtained for the gradually sedimented clay–RSB samples. We repeated 3 independent measurements from drying each clay, which all provided the identical $\lambda_{max}$. X-ray powder diffraction patterns were obtained by a Mac Science MXP3 diffractometer (monochromatic Cu KR) for the characterization of the products and a Mac Science M03XHF22 diffractometer (Mn-filtered Fe KR) for the measurement of low diffraction angles as described previously.[14]

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