Supplementary Information

“In situ” observation of role of chloride ion binding to monkey green sensitive visual pigment by ATR-FTIR spectroscopy

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Figure S1. The absolute infrared absorption spectra of the MG sample adsorbed on the ATR crystal and soaked in NaCl solution (solid line). The spectrum without the MG sample is drawn with dotted line. Red line represents the infrared spectrum of MG sample.
Figure S2. (a) The ion-binding induced difference FTIR spectra of MG measured in the 10 mM NaCl (black) or 25 mM NaCl (red) and absence of NaCl containing 200 mM phosphate (pH 7.25) at room-temperature in the 1780-1120 cm\(^{-1}\) region. (b) The double difference spectrum between black and red curves in (a). One division of the y-axis corresponds to 0.0006 absorbance unit.
Figure S3. The ion-binding induced difference FTIR spectra of MG measured in the 10 mM NaCl (red) or NpHR measured in the 25 mM NaCl (black) containing 200 mM phosphate (pH 7.25) at room-temperature in the 1780-1120 cm\(^{-1}\) region. One division of the y-axis corresponds to 0.0002 absorbance unit.
Figure S4. (a) The ion-binding induced difference FTIR spectra between Cl⁻-minus-anion free on the 11-cis form of MG (solid curve) and that on the 9-cis form of MG (dotted curve) in the 1290-1170 cm⁻¹ region. (b) The double difference FTIR spectra between 9-cis form (dotted curve (a)) and 11-cis form (solid curve (a)). One division of the y-axis corresponds to 0.00002 absorbance unit.
Figure S5. The ion-binding induced difference FTIR spectrum of MG. Upper panel: Cl⁻ minus anion free difference spectra in H₂O (black curve) and D₂O (red curve) in the 1260-1180 cm⁻¹ region. Lower panel: difference spectra between red curve and black curve. One division of the y-axis corresponds to 0.0001 absorbance unit.
Figure S6. The ion-binding induced difference FTIR spectra in H₂O (black curve at pH 7.25) and D₂O (red curve at pD 7.25) of MG in the 1780-1700 cm⁻¹ region. The thick curves were fitted by one-component using a model of Gaussian distribution. One division of the y-axis corresponds to 0.00002 absorbance unit.
Figure S7. The comparison of ion-binding induced difference FTIR spectra of NpHR. Each spectra correspond to Cl$^-$ (25 mM) -minus- anion free (a), Br$^-$ (25 mM) –minus- anion free (b), NO$_3^-$ (25 mM) –minus- anion free (c), Cl$^-$ (25 mM) –minus- Br$^-$ (25 mM) (d), and Cl$^-$ (25 mM) –minus- NO$_3^-$ (25 mM) (e), respectively. One division of the y-axis corresponds to 0.009 absorbance unit.