Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Long-distance perturbation on Schiff base–counterion interaction by His30 and the extracellular Na^+-binding site in *Krokinobacter* rhodopsin 2

Arisu Shigeta [a, †], Shota Ito [b, †], Rina Kaneko [c], Sahoko Tomida [b], Keiichi Inoue [b, d, e], Hideki Kandori [b, e, ††], Izuru Kawamura [a, c, ††]

[a] Graduate School of Engineering, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan,

[b] Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466-8555, Japan,

[c] Department of Chemistry, Chemical Engineering, and Life Science, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan,

[d] OptoBioTechnology Research Center, Nagoya Institute of Technology, Showa-ku, Nagoya 466-8555, Japan,

[e] PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan

† A. S and S. I. contributed equally to this paper.

††Corresponding authors: Izuru Kawamura, izuruk@ynu.ac.jp

Hideki Kandori, kandori@nitech.ac.jp

Materials and Methods

Sample preparation

Wild-type (WT) and H30A KR2 was overexpressed in *Escherichia coli* strain C41(DE3) cultured in M9 minimal medium and then purified as previously described. To perform NMR analyses, we labeled samples with stable isotopes to obtain [U-^{15}N]WT-KR2 and [U-^{15}N]H30A-KR2. Samples were separately reconstituted into widely used 1-palmitoyl-2-oleoylsn-glycero-3-phosphoethanolamine (POPE)/1-palmitoyl-2-oleoyl-sn-glycero-3-phospho(1′-rac-glycerol) (POPG) membranes (3:1 POPE:POPG ratio, 1:20 protein:lipid ratio) for the study of the bacterial membrane proteins as previous work characterizing KR2.[19] Subsequently, each samples were suspended in 10 mM Tris-H_3PO_4 buffer at pH 8.0 with 100 mM LiCl, 100 mM NaCl, 100 mM KCl, 100 mM RbCl, 100 mM CsCl or 0 mM NaCl.

Solid-state NMR measurements

Sample suspended with each buffer was packed in a 4.0 mm zirconium sample tube. ^{15}N NMR experiments were then performed at 278 K, and the magic angle spinning (MAS) speed was adjusted to 10.0 kHz on a Bruker Avance III spectrometer operated at 14.1 T (600 MHz as the ^{1}H Larmor frequency) with an E-free probe. ^{1}H-^{15}N cross-polarization contact time was 2.0 ms and Spinal 64 proton decoupling of 80 kHz was employed during acquisition. ^{15}N chemical shifts were referenced to glycine powder at 11.59 ppm (NH_4NO_3 at 0.0 ppm[20]).

UV-Vis measurements

Lipid reconstituted KR2 WT and H30A samples were suspended in 10 mM Tris-H_3PO_4 buffer at pH 8.0 with 100 mM LiCl, 100 mM NaCl, 100 mM KCl, 100 mM RbCl, 100 mM CsCl or 0 mM NaCl. Absorption spectra of each samples were recorded with UV-vis spectrometer (V650, JASCO) equipped with an integrating sphere at room temperature.

FTIR measurements

Light-induced FTIR difference spectra at 77 K was performed as described previously.[29] The lipid reconstituted KR2 WT and H30A samples were suspended in 1 mM NaCl or CsCl and 2 mM Tris buffer at pH 8.5. The dried films of 0.1 mg sample were prepared and hydrated with D_2O. Hydrated samples were replaced on a cryostat (Optistat DN, Oxford) coupled to a FTIR spectrometer (FTS-40; Bio-Rad). Samples stabilized at 77 K were illuminated 2 min using 500 ± 10 nm band-pass filter for the formation of $KR2_K$ and also illuminated 1 min using >600 nm light for photo-reversed to KR2. 128 interferograms were accumulated and 40 repeated recordings were averaged.

Flash-photolysis measurements

Transient absorption change of KR2 H30A was measured by nanosecond laser flash photolysis method as previously described.[1] The absorption of POPE/POPG reconstituted sample with a 1:50 protein-to-lipid molar ratio in 100 mM NaCl or CsCl, 20 mM Tris-HCl (pH 8.0) were adjusted to be 0.8-0.9 with 1-cm optical path-length. The sample was photo-excited by a nanosecond pulse of second harmonics (SHG) of Nd^{3+}: YAG laser (λ = 532 nm, INDI40, Spectra-Physics) with an excitation laser power was 3 mJ/(cm^{2}· pulse). The intensities of the transmitted probe light from a Xe arc lamp (L8004, Hamamatsu Photonics, Shizuoka, Japan) were measured using a multichannel detector (C9125, Hamamatsu Photonics, Shizuoka, Japan) before and after laser excitation, and transient absorption spectra were obtained by calculating the ratio between them. Ninety homologous spectra were averaged to obtain higher signal-to-noise ratio.

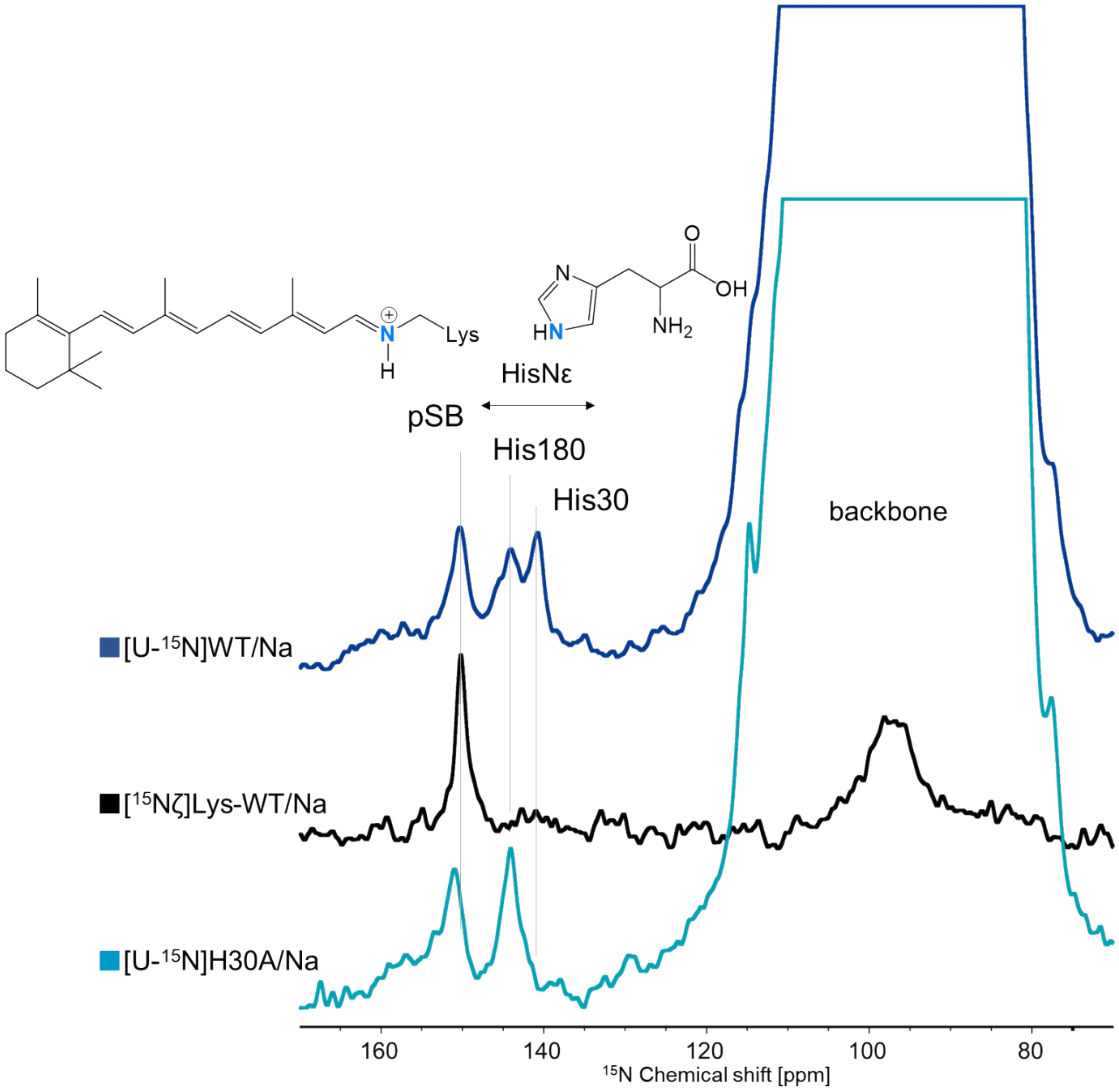

Figure S1. Assignment of Schiff base and His signals. The peak of Schiff base was previously assigned with [^{15}Nζ]Lys-WT-KR2. At higher field of Schiff base signal, HisNε signals were observed showing two peaks of 140.7 ppm and 144.0 ppm which corresponds to two residues in KR2, His30 and His180. As the signal at 140.7 ppm disappeared in the spectrum of H30A, thus, the other remained peak was assigned as His180.

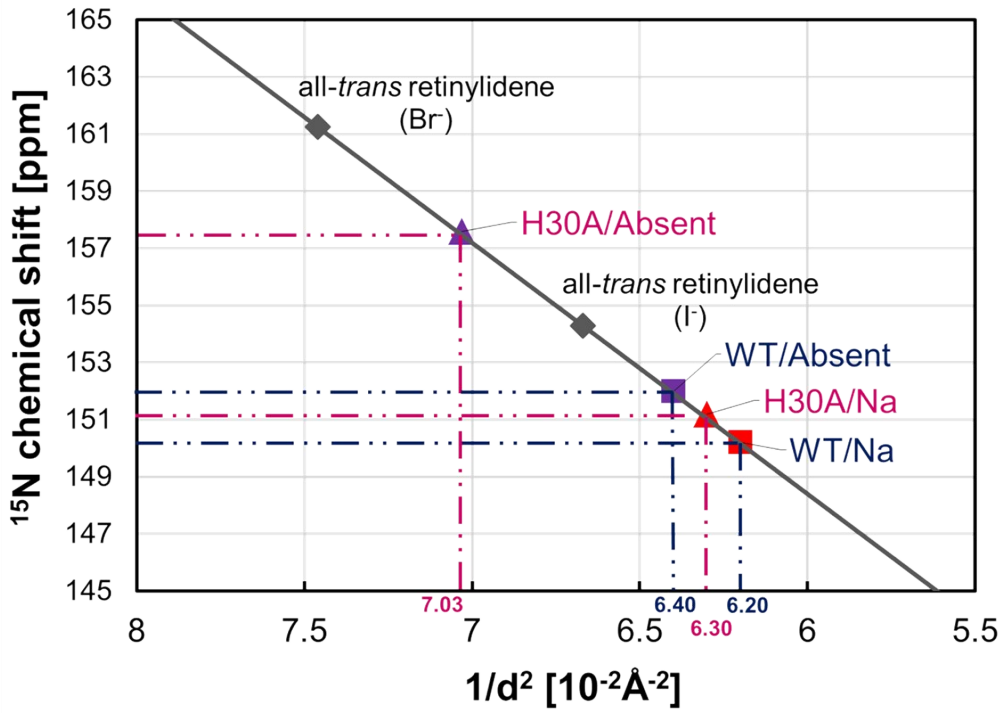

Figure S2. Correlation of the ^{15}N chemical shifts of the protonated all-*trans* retinylidene-butyl-^{15}N-imides (Br^-, I^-) versus the inverse of the center-to-center distance squared ($1/d^2$) of the crystallographic radii of N^{3-} and the halide counterion. From the obtained chemical shift of Schiff base, distance between positive charge at Schiff base and negative charge counterion was estimated.[23,24]

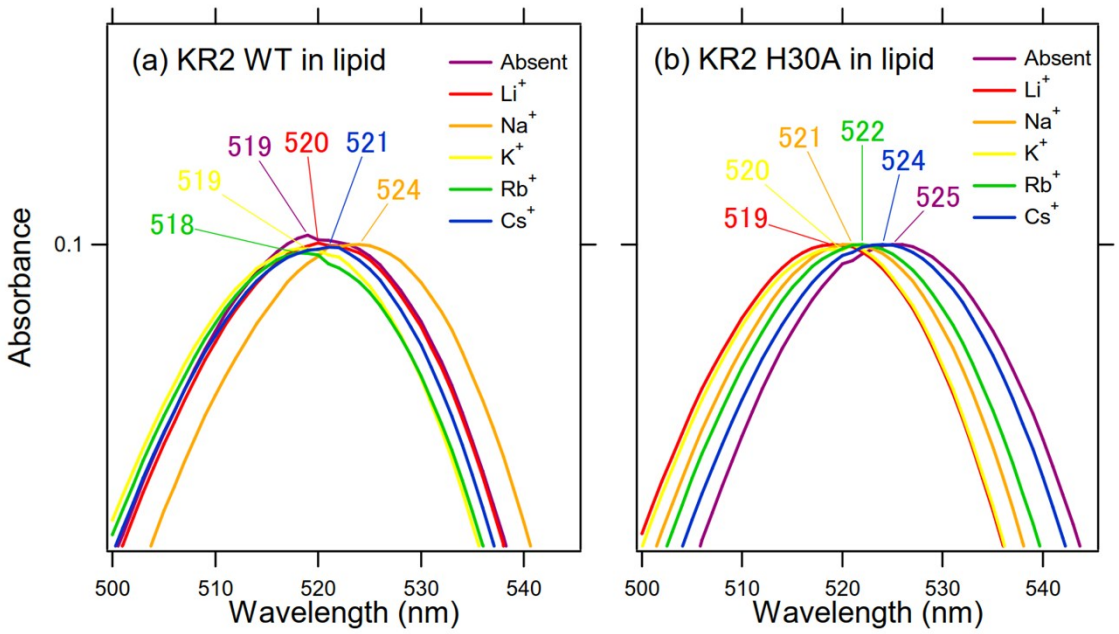

Figure S3. Maximum absorption wavelength of WT-KR2 and H30A-KR2 in lipid membrane. UV-vis absorption spectra of lipid reconstituted WT-KR2 and H30A-KR2 in 100 mM each cations or no salt conditions.

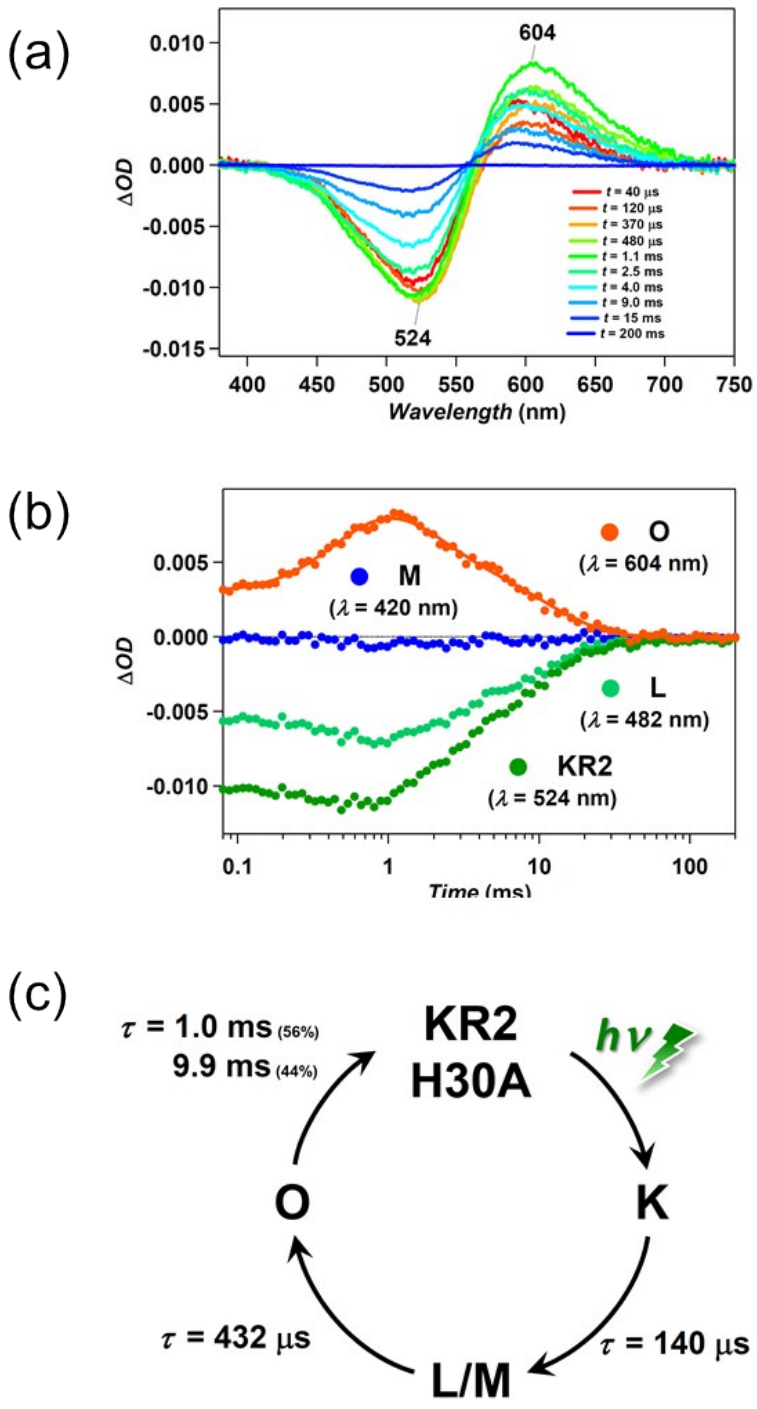

Figure S4. Photocycle of KR2 H30A reconstituted in POPE/POPG and 100 mM NaCl. Transient absorption spectra of KR2 H30A reconstituted in POPE/POPG liposome (1:50 protein-to-lipid molar ratio) in solution containing 100 mM NaCl and 20 mM Tris-HCl (pH 8.0) (a) and time traces of absorption change (b) at specific probe wavelengths. (c) Photocycle scheme of KR2 H30A in the presence of Na^+ determined from the analysis of the results shown in a, b.

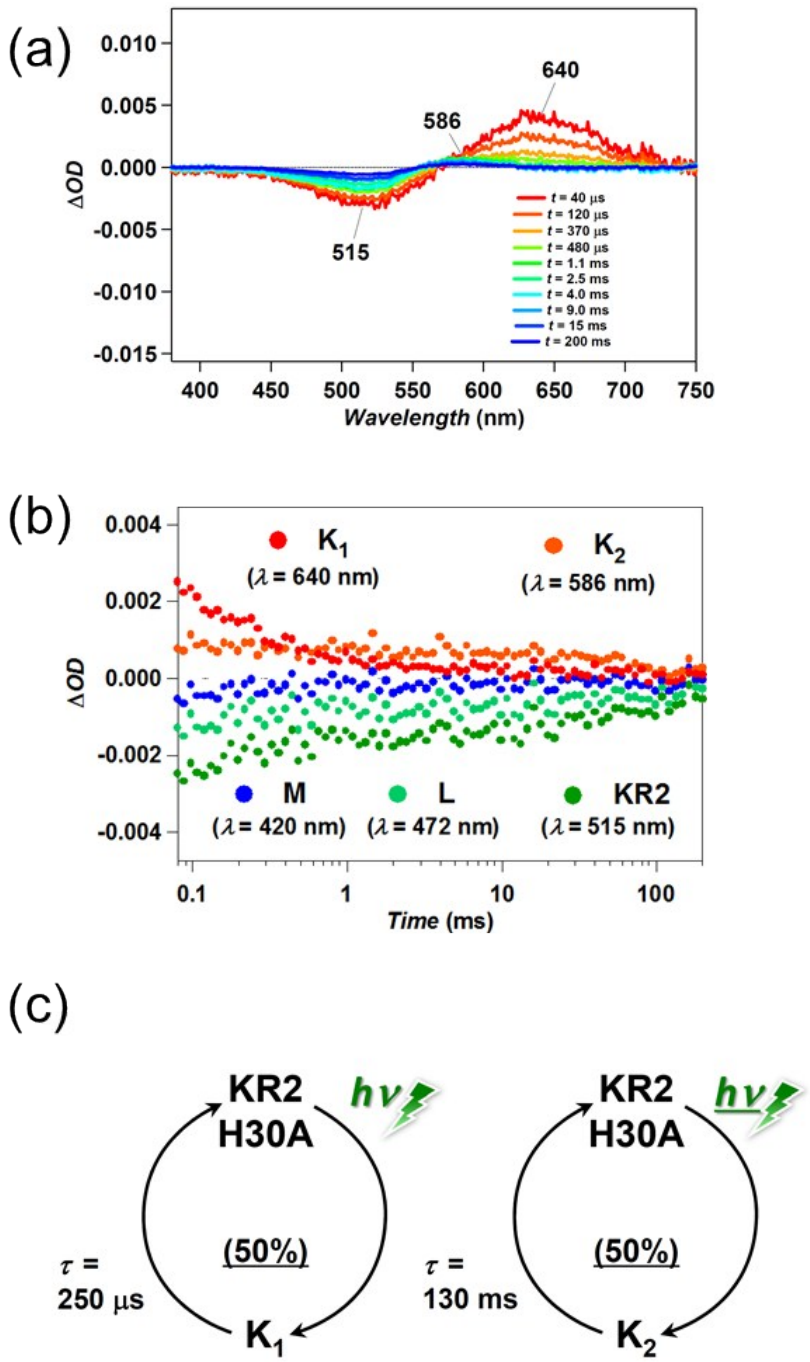

Figure S5. Photocycle of KR2 H30A reconstituted in POPE/POPG and 100 mM CsCl. Transient absorption spectra of KR2 H30A reconstituted in POPE/POPG liposome (1:50 protein-to-lipid molar ratio) in solution containing 100 mM CsCl and 20 mM Tris-HCl (pH 8.0) (a) and time traces of absorption change (b) at specific probe wavelengths. (c) Photocycle scheme of KR2 H30A in the presence of Cs^+ determined from the analysis of the results shown in a, b.