

Supporting Information:

Converting a light-driven proton pump into a light-gated proton channel

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Table S1. Absorption maxima, opsin shifts and retinal configurations of the wild-type AR3 and its mutants.

Table S2. Absorption maxima, opsin shifts, retinal configurations and directions of the proton pumping of the wild-type *Hw*BR and the triple mutant.

Table S3. Absorption maxima, opsin shifts, retinal configurations and directions of the proton pumping of the wild-type GR and the triple mutant.

Figure S1. Difference FTIR spectra of wild-type AR3 and AR3-T (middle) and the chimeric ChR (C1C2) in 1595-850 cm⁻¹ region.

Figure S2. The correlation of the absorption maxima (λ_{max} s) and the frequencies of retinal C=C stretching vibrations of microbial rhodopsins.

Figure S3. Transient absorption spectra of wild-type AR3 and AR3-T in the *E. coli* membrane.

Figure S4. The absorption spectra and proton transport activity of wild-type *Hw*BR, GR and their mutants.

Supporting Information References

(49) Kandori, H.; Shimono, K.; Sudo, Y.; Iwamoto, M.; Shichida, Y.; Kamo, N., *Biochemistry* **2001**, *40*, 9238.

Table S1. Absorption maxima (λ_{\max}), opsin shifts ($\Delta\nu$) and retinal configurations of the wild-type AR3 and its mutants. $\Delta\Delta\nu$ indicates the difference from the value expected from the sum of $\Delta\nu$ s of single mutations comprising respective double and triple mutants.

Opsin type	λ_{\max} [nm]	$\Delta\nu$ [cm ⁻¹]	$\Delta\Delta\nu$ [cm ⁻¹]	ATR [%]
wild-type	552 ^a	-	-	53 ± 2 ^a
M128A	536 ^a	541 ^a	-	68 ± 1 ^a
G132V	526	895	-	65 ± 3
A225T	540	403	-	70 ± 2
M128A/A225T	518	1189	245	51 ± 3
G132V/A225T	508	1569	271	75 ± 2
M128A/G132V/A225T	455	3862	2023	65 ± 3

^a From Sudo et al., (2013) J. Biol. Chem., n.d. = not determined.

Table S2. Absorption maxima (λ_{\max}), opsin shifts ($\Delta\nu$), retinal configurations and directions of the proton pumping of the wild-type *HwBR* and the triple mutant.

Opsin type	λ_{\max} [nm]	$\Delta\nu$ [cm ⁻¹]	ATR [%]	Direction
wild-type	552 ^a	-	79 ± 2 ^a	Outward ^a
M126A/G130V/A223T	472	3070	70 ± 1	Inward

^a From Sudo et al., (2013) J. Biol. Chem.,

Table S3. Absorption maxima (λ_{\max}), opsin shifts ($\Delta\nu$), retinal configurations and directions of the proton pumping of the wild-type GR and the triple mutant.

Opsin type	λ_{\max} [nm]	$\Delta\nu$ [cm ⁻¹]	ATR [%]	Direction
wild-type	540	-	90 ± 2	Outward
M158A/G162V/A256T	474	2579	64 ± 3	Outward

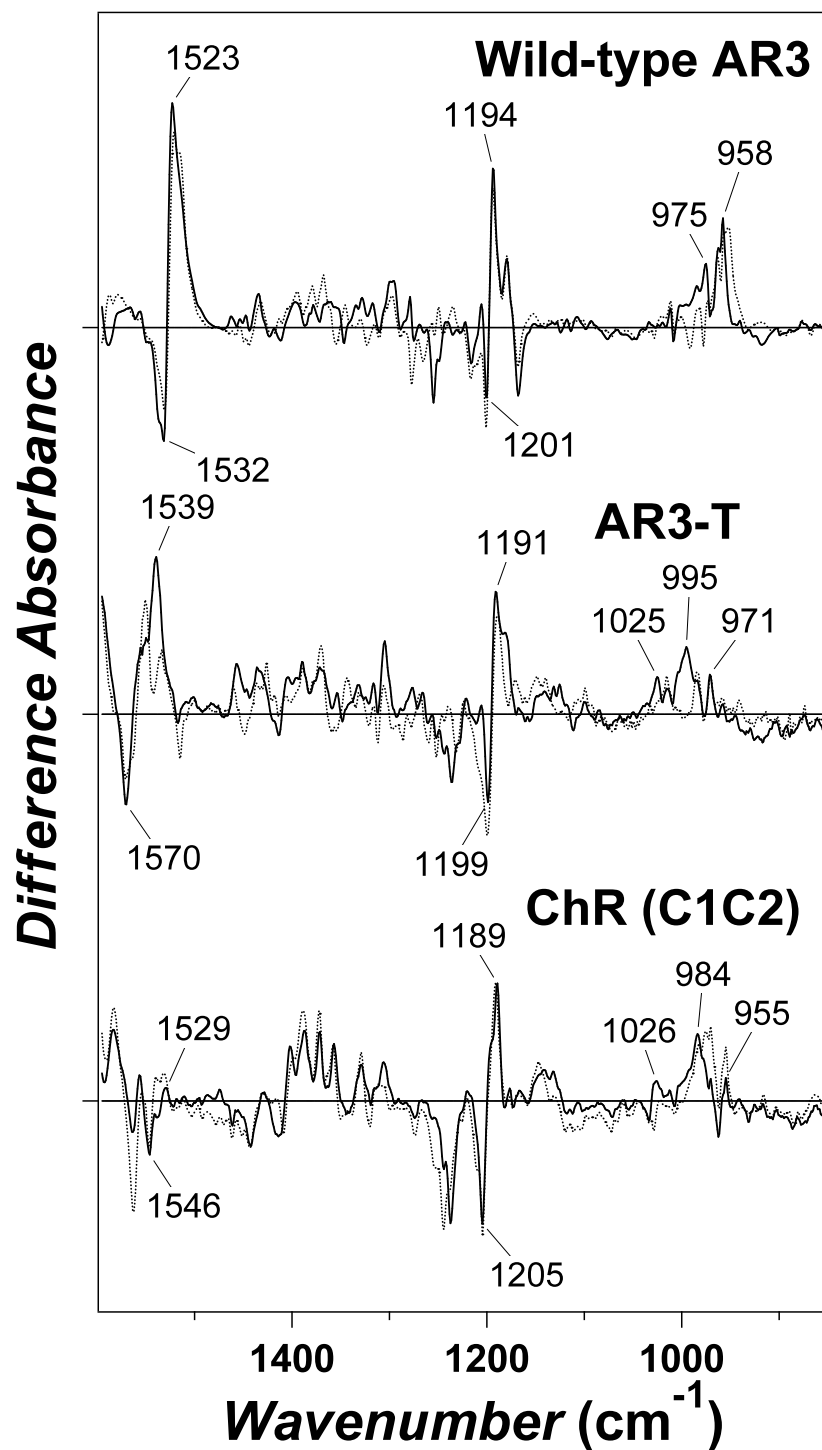


Fig. S1.

Difference FTIR spectra of wild-type AR3 and AR3-T and ChR (C1C2) in the 1595-850 cm^{-1} region. Difference FTIR spectra of wild-type AR3 (upper), AR3-T (middle) and the chimeric ChR (C1C2) (lower, reproduced from Ref.¹⁵) at 77K in the 1595-850 cm^{-1} region. Solid and dotted lines are the spectra of films hydrated with H_2O and D_2O , respectively.

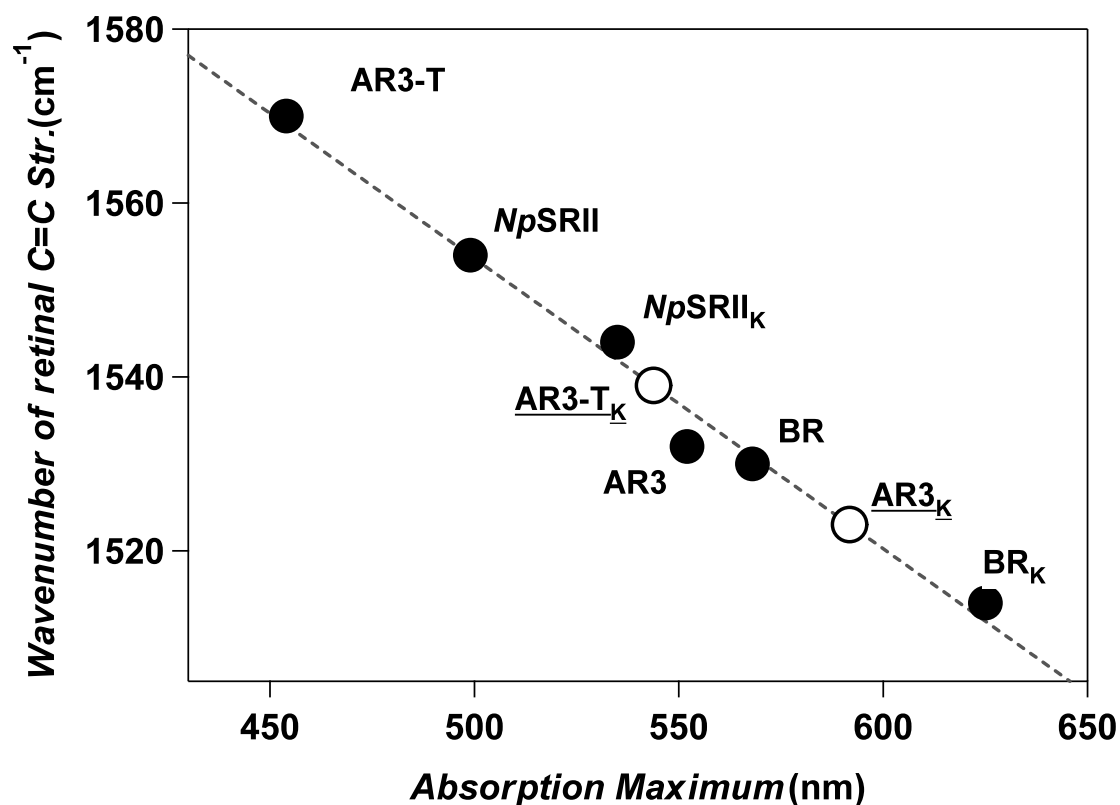


Fig. S2.

Correlation of the absorption maxima (λ_{\max}) and vibrational frequencies of retinal C=C stretching vibrations of microbial rhodopsins. The reported values for BR, NpSRII, the K intermediate of BR (BR_K) and NpSRII (NpSRII_K) and the values for AR3 and AR3-T in the dark determined in this study are shown by filled circles⁴⁹. The wavenumbers of retinal C=C stretching vibration of AR3_K and AR3-T_K (open circles) were calculated with their λ_{\max} s and the linear regression curve for the data shown by filled circles (dashed line).

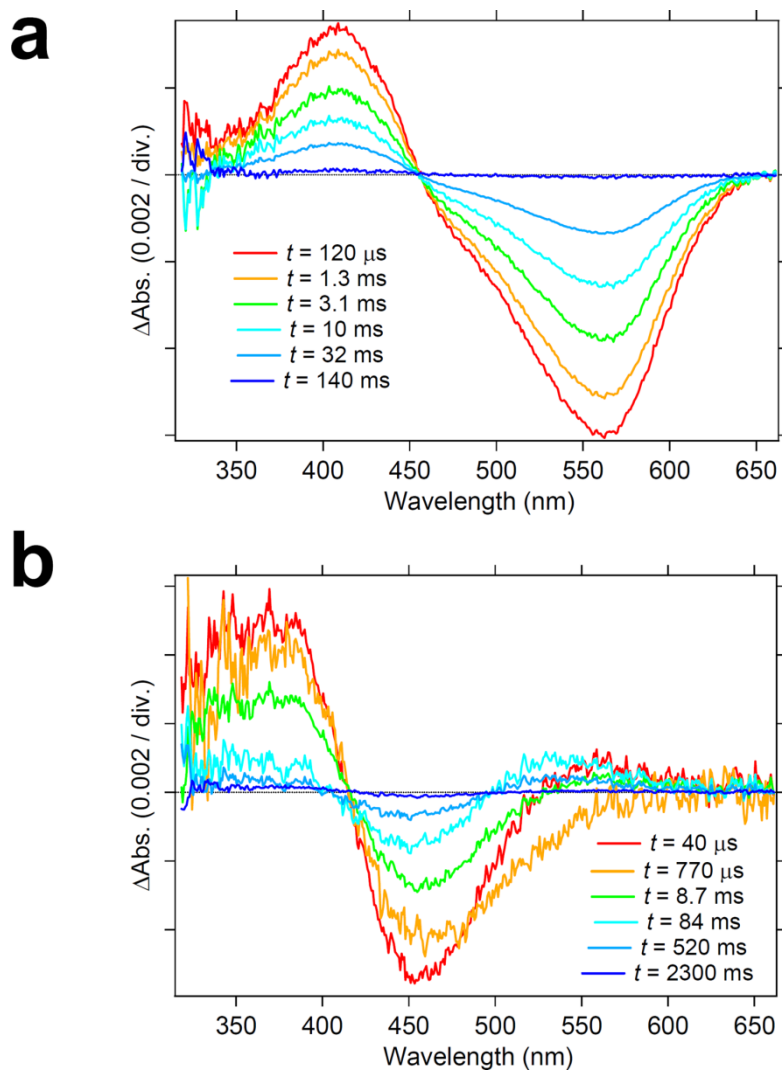


Fig. S3.

Transient absorption spectra of wild-type AR3 and AR3-T in the *E. coli* membrane. (a) Transient absorption spectra of wild-type AR3 at $t = 120 \mu\text{s}$ to 140 ms. (b) Transient absorption spectra of AR3-T at $t = 40 \mu\text{s}$ to 2,300 ms.

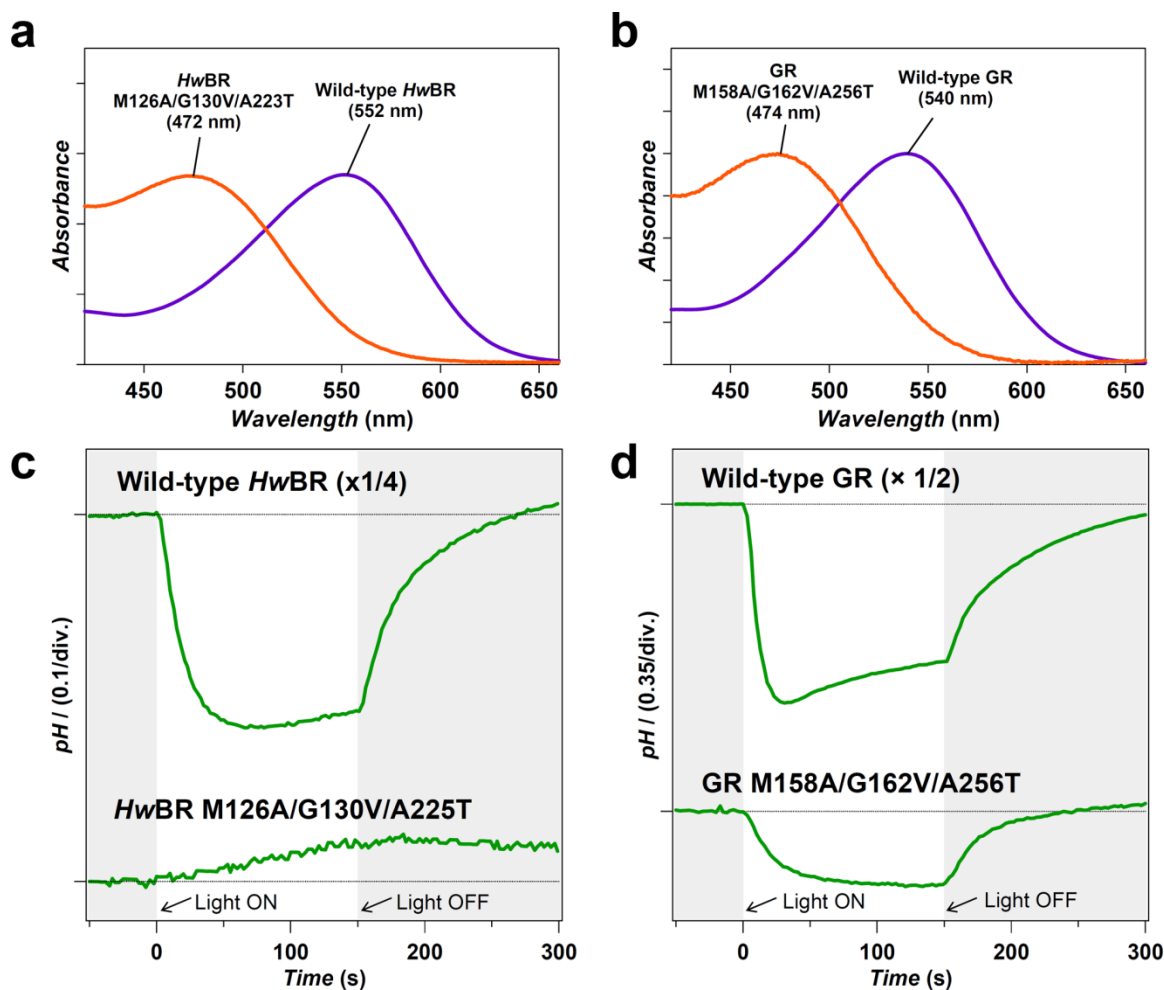


Fig. S4.

Absorption spectra and proton transport activity of *HwBR*, GR and their mutants. (a and b) Absorption spectra of *HwBR* and its triple mutant (a) and GR and its triple mutant (b). (c and d) Proton transport activity of wild-type *HwBR* and its triple mutant (M126A/G130V/A225T) (c), and wild-type GR and its triple mutant (M158A/G162V/A256T) (d).