Supporting Information for

Mutant of a Light-Driven Sodium Ion Pump Can Transport Cesium Ions

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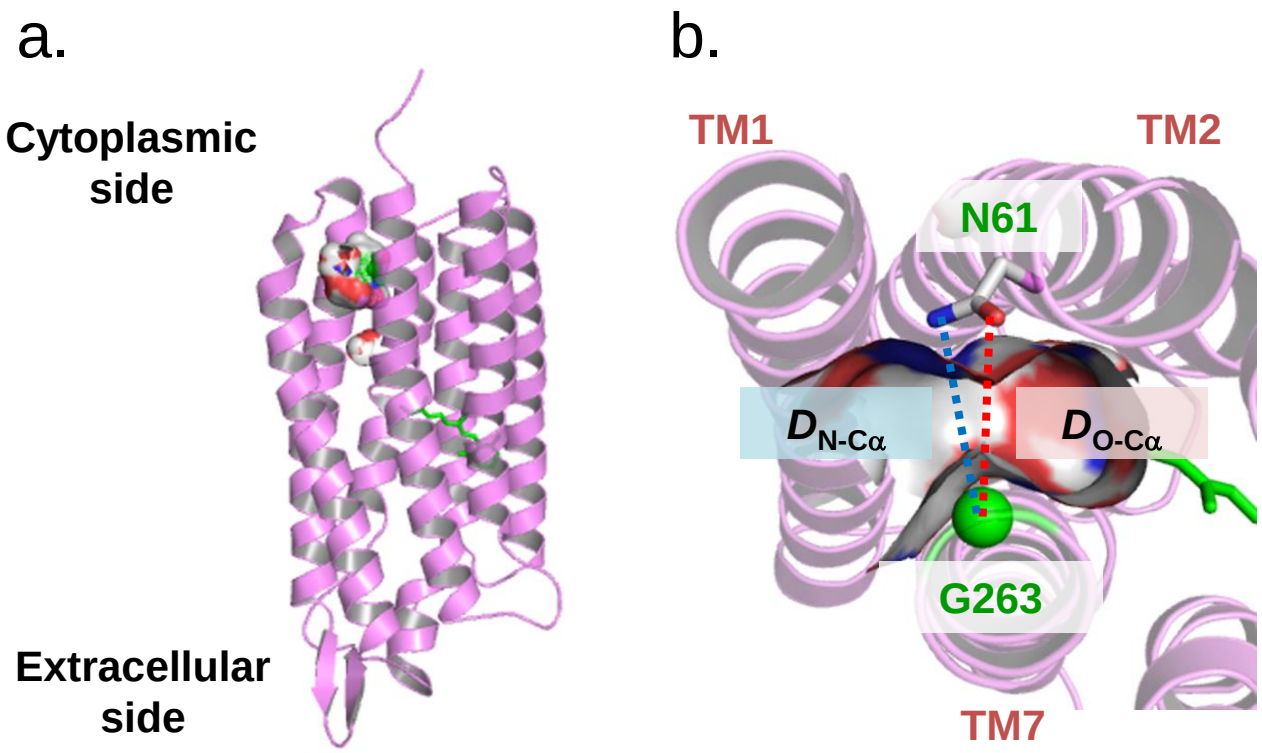
I. Supporting Experimental Procedure

Photocycle of $KR2_{Cs+}$ in *E.coli* membrane.

$KR2_{Cs+}$ protein-expressed *E. coli* cells were collected by centrifugation. The cells were washed once, equilibrated for 10 minutes for three times and resuspended in the buffer containing 50 mM Tris-HCl (pH 8.5) and 100 mM salt (NaCl or CsCl). After the equibration, the cells were broken by sonication and lysozyme-hydrolysis. The sample solution was placed in a quartz cuvette and it was excited with a beam of second harmonics of a nanosecond pulsed Nd^{3+}-YAG laser (λ = 532 nm, INDI40, Spectra-Physics). The laser power was 3 mJ per pulse, and repetition rate was sufficiently slower than the rate of photocycle of rhodopsin to avoid photoexcitation of transient intermediates. The intensities of the transmitted probe light from a Xe arc lamp (L8004, Hamamatsu Photonics, Japan) were measured before and after laser excitation, and transient absorption spectra were obtained by calculating the ratio between them. Thirty identical spectra were averaged.

I. Supporting Experimental Procedure



c.

PDB accession code	$d_{N\text{-}C\alpha}$ (Å)	$d_{O\text{-}C\alpha}$ (Å)
3X3B	5.4	5.5
3X3C	5.3	5.6
4XTL	5.2	4.7
4XTN	4.8 - 5.9	4.6 - 6.1
4XTO	4.5 - 5.0	4.0 - 5.6

Figure.S1. Structural information of the cavity near N61 and G263 in KR2.
a. Overall structure of KR2 (3X3C).
b. Transverse section viewed from intracellular side (3X3C).
c. Distance between Cα of G263 and the side-chain oxygen ($d_{N\text{-}C\alpha}$) /nitrogen ($d_{N\text{-}C\alpha}$) of N61.
Protein structures of KR2 are referred from Kato et al (2015; 3X3B at pH 4.0 and 3X3C at pH 7.5 – 8.5) [1] and Gushchin et al (2015; 4XTL in the monomeric form at pH 4.3, 4XTN in the pentameric form at pH 4.9 and 4XTO in the pentameric form at 5.6) [2].

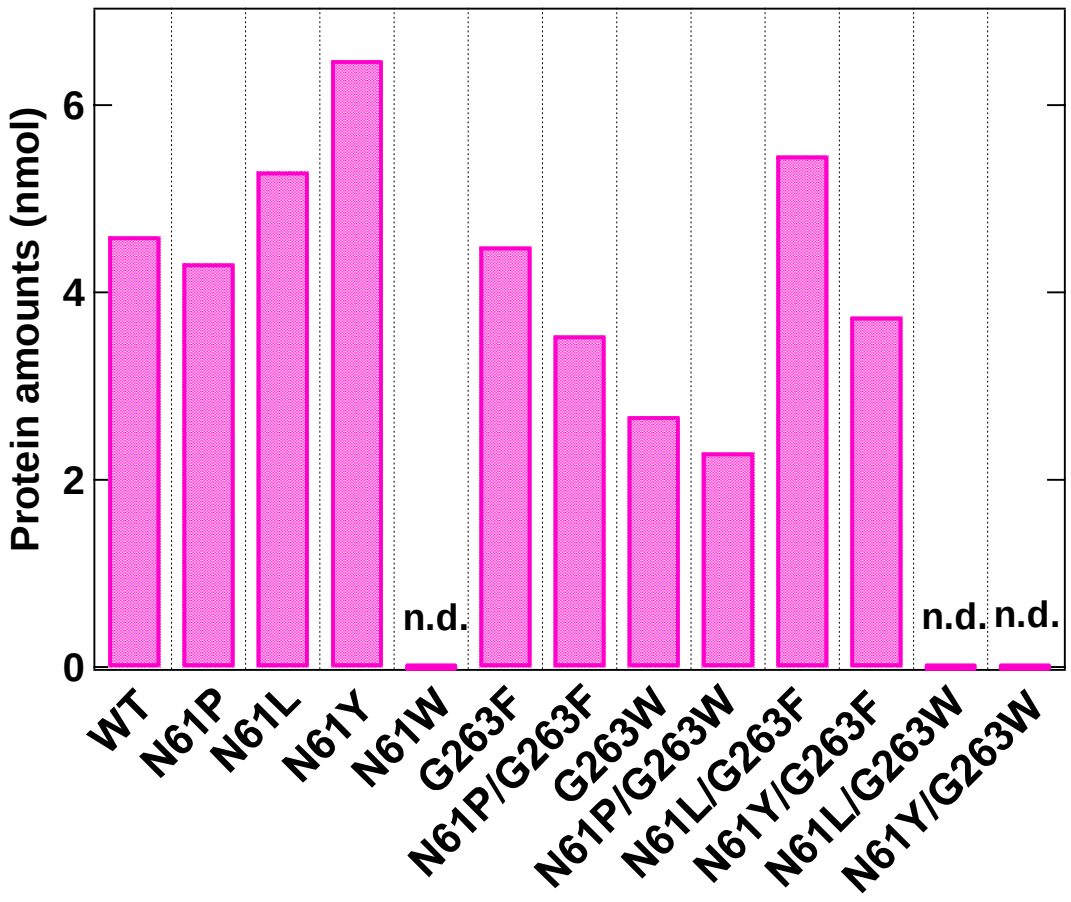


Figure S2. Protein amount existed in the *E. coli* cells used for the measurements of pumping activity of KR2 WT and mutants. n.d.; not detectable.

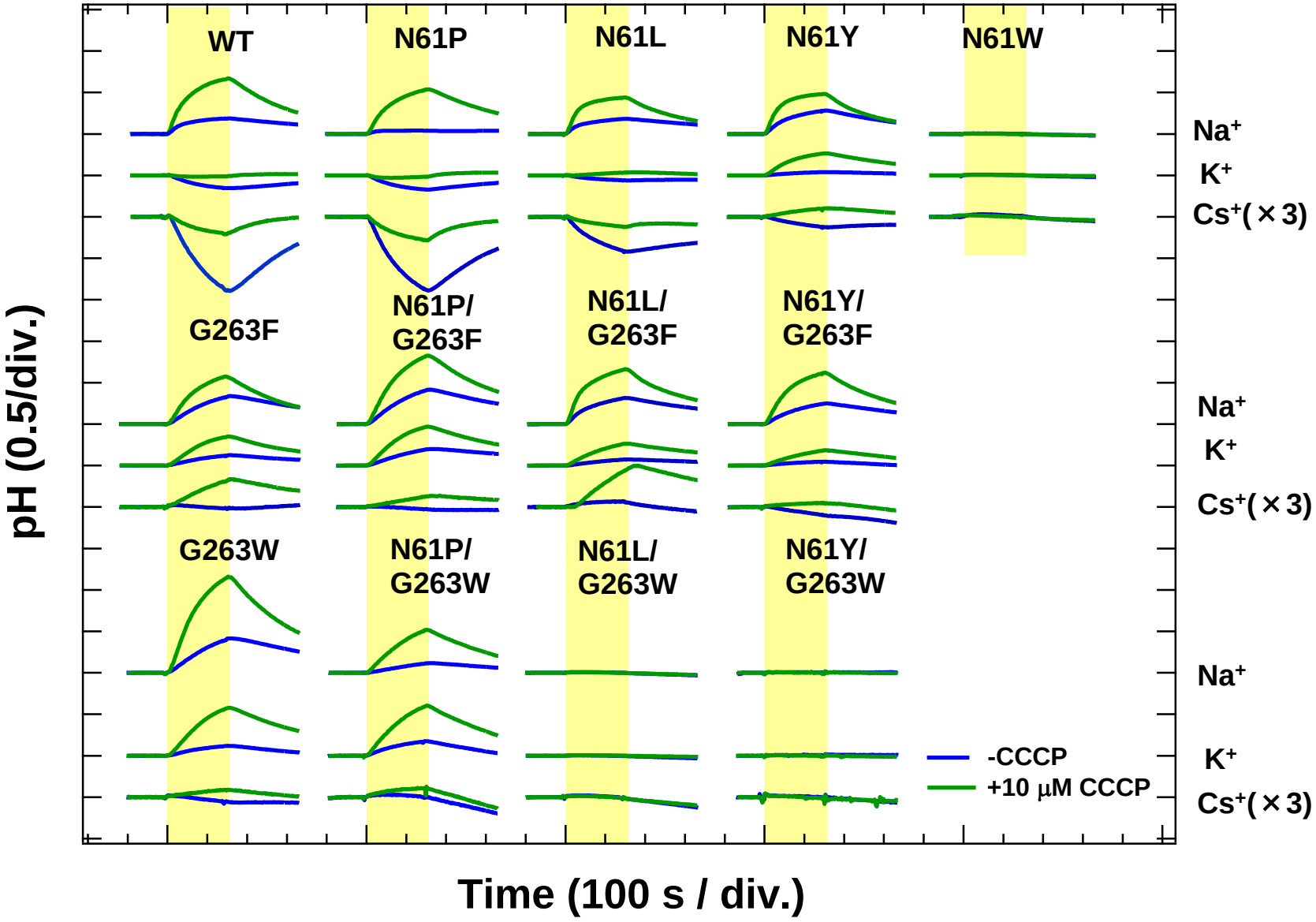


Figure S3 Pump activities of KR2 WT and mutants enlarged from Figure 1. The traces in 100 mM CsCl were expanded 3-folds.

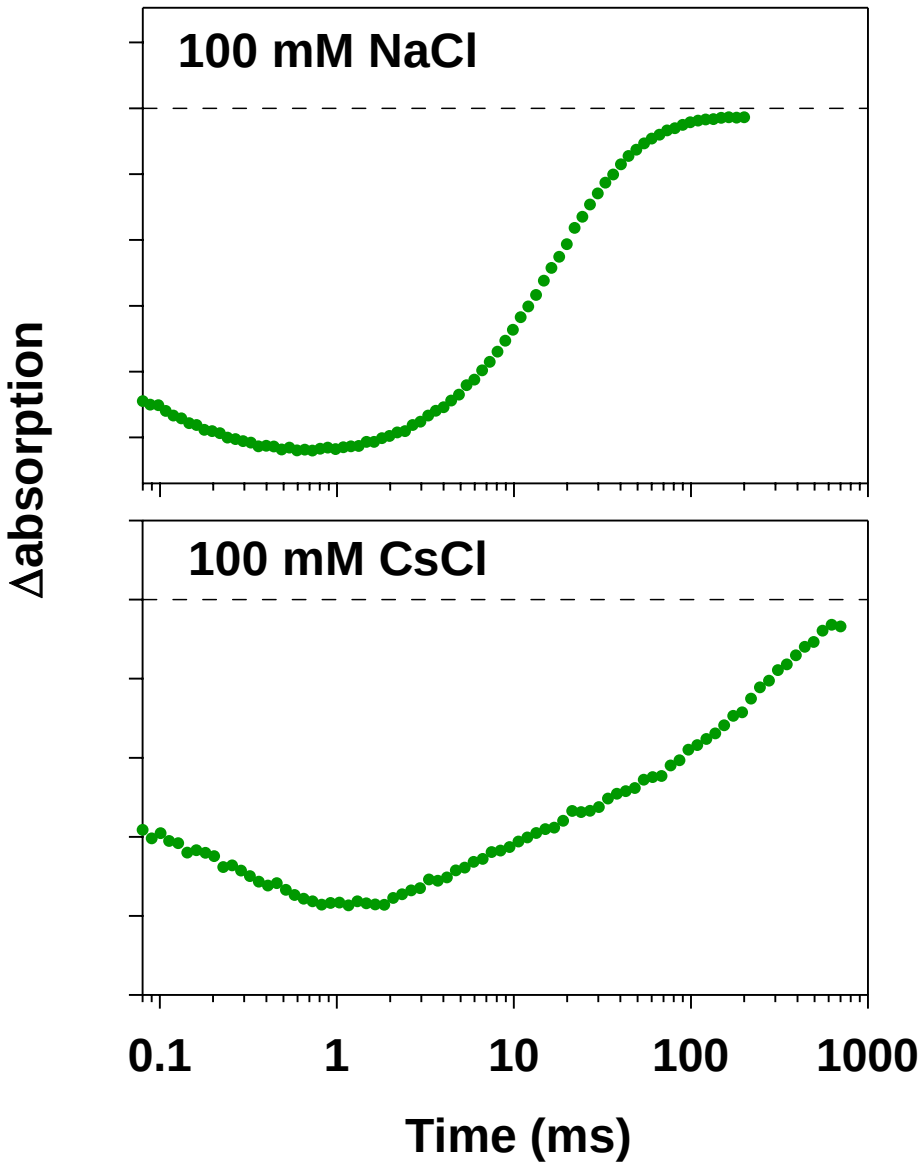


Figure.S4. Photocycle of $KR2_{Cs+}$ in *E.coli* membrane. Time trace of absorption changes represent the depletion of the initial state of $KR2_{Cs+}$ in NaCl (upper panel) or CsCl (lower panel) probed at 532 nm.

I. References

1. Kato, H. E.; Inoue, K.; Abe-Yoshizumi, R.; Kato, Y.; Ono, H.; Konno, M.; Ishizuka, T.; Hoque, M. R.; Hososhima, S.; Kunitomo, H.; et al. Structural Basis for Na^+ Transport Mechanism by a Light-Driven Na^+ Pump. *Nature* **2015**, 521 (755), 48-53.

2. Gushchin, I.; Shevchenko, V.; Polovinkin, V.; Kovalev, K.; Alekseev, A.; Round, E.; Borshchevskiy, V.; Balandin, T.; Popov, A.; Gensch, T.; et al. Crystal Structure of a Light-Driven Sodium Pump. *Nat. Struct. Mol. Biol.* **2015**, 22 (5), 390-395.