Supporting Information

Direct detection of the substrate uptake and release reactions of the light-driven sodium-pump rhodopsin

Keisuke Murabe,[†] Takashi Tsukamoto,[‡,§] Tomoyasu Aizawa,[‡,§] Makoto Demura,[‡,§] and

Takashi Kikukawa[*,‡,§]

[†]Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan

[‡]Faculty of Advanced Life Science, Hokkaido University, Sapporo 060-0810, Japan

[§]Global Station for Soft Matter, Global Institution for Collaborative Research and Education, Hokkaido University, Sapporo 001-0021, Japan

*To whom correspondence should be addressed.

Takashi Kikukawa

Email: kikukawa@sci.hokudai.ac.jp

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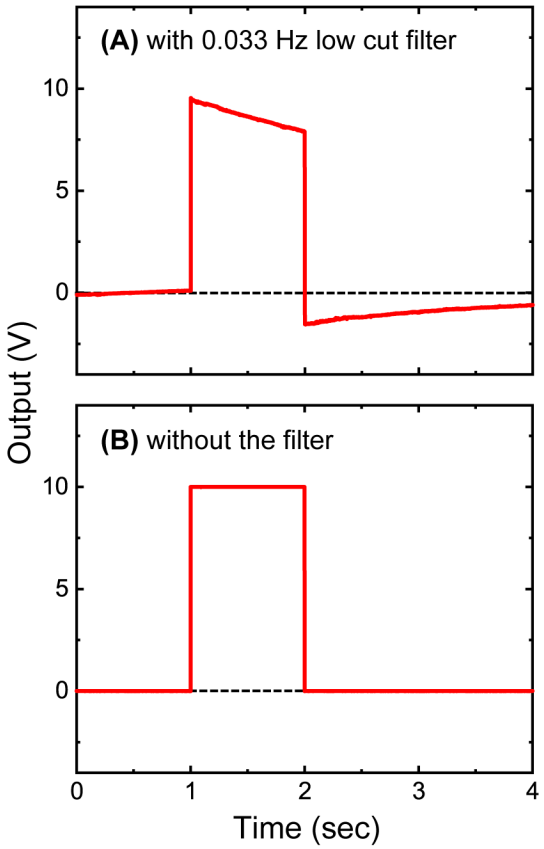

Figure S1. The response of the voltage amplifier for the input of square wave voltage. This amplifier is equipped with a removable 0.033 Hz low cut filter. Panels A and B show the output voltages with and without the filter, respectively. Here, we input the square wave voltage. The output with the filter was slightly differentiated.

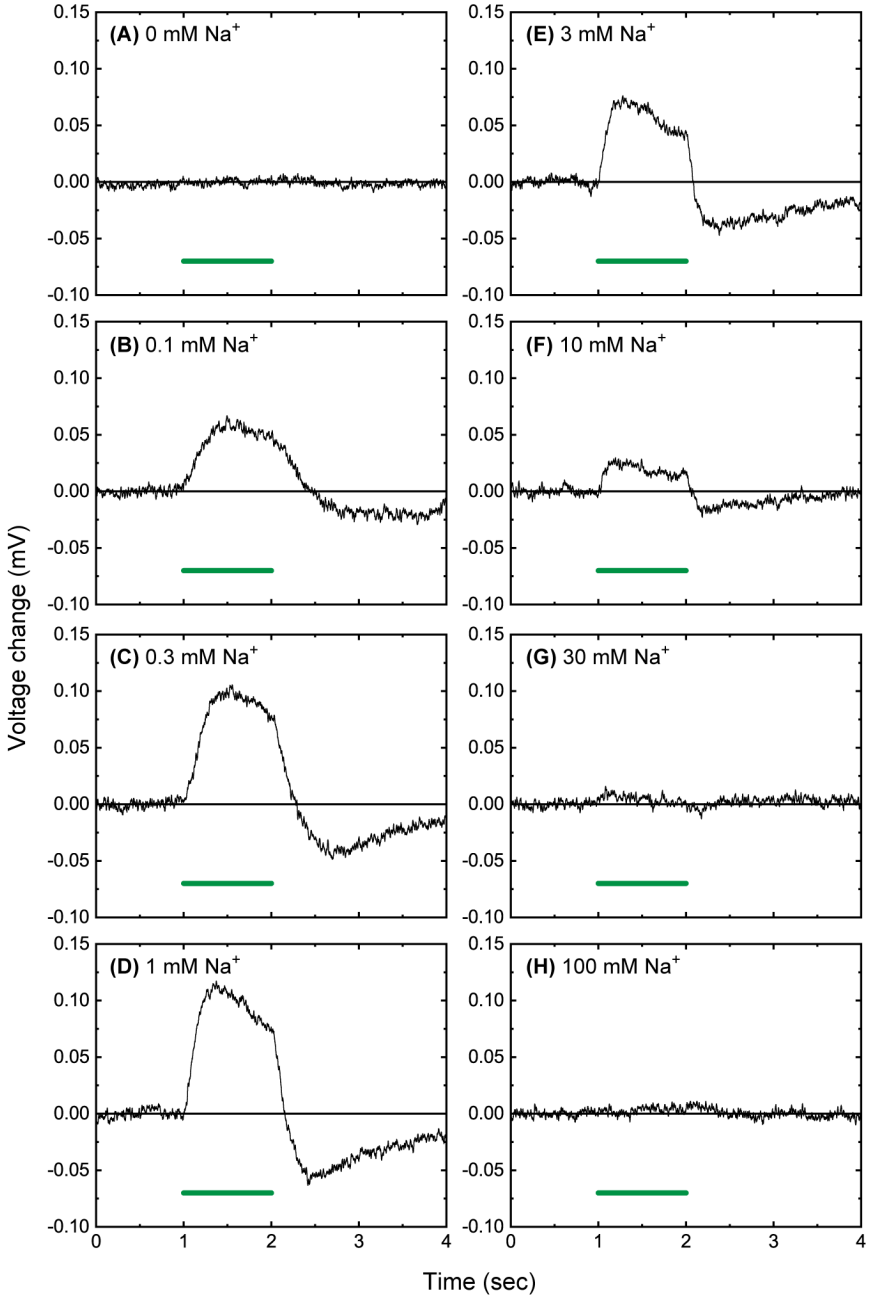

Figure S2. Light-induced potential changes of a Na^+ selective membrane at various Na^+ concentrations. The lipid-reconstituted NaR was deposited on the membrane surface facing the sample chamber. The green bar indicates the period of illumination.

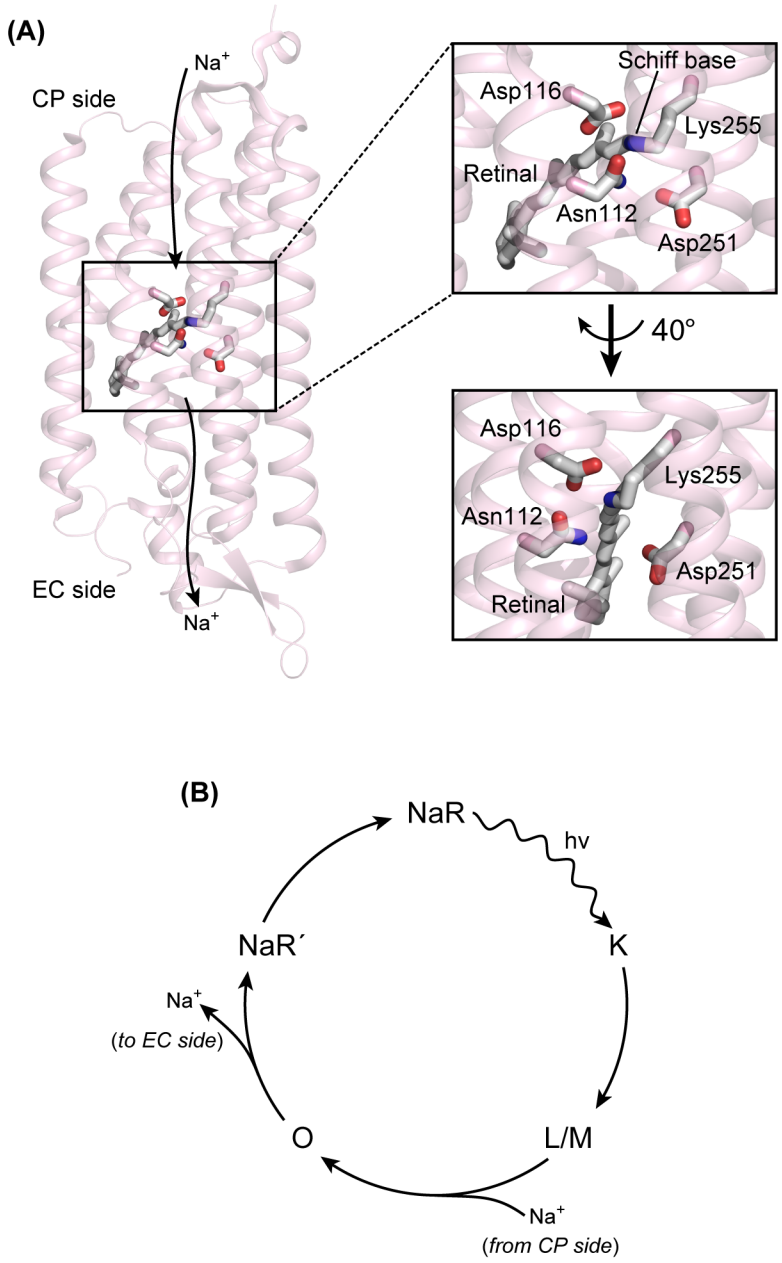

Figure S3. Positions of key residues for putative Na^+ binding sites and the photocycle scheme of NaR. In Panel A, the crystal structure of NaR from *Krokinobacter eikastus* (PDB code: 3X3C) is shown with the retinal and the nearby amino acid residues. Panel B shows a typical photocycle scheme, which also indicates the timing of Na^+ uptake and release that were determined in this study. L/M denotes the equilibrium of the L and M intermediates.

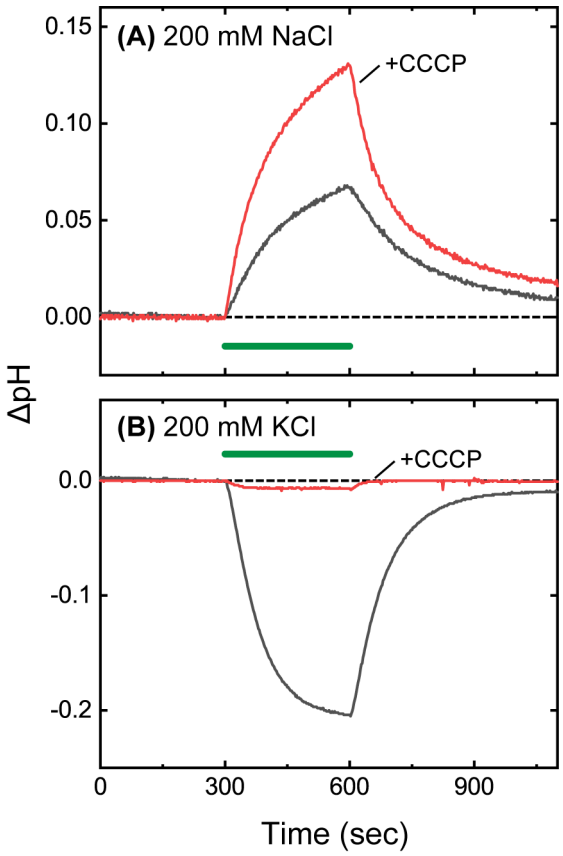

Figure S4. Light-induced pH changes of suspensions of *E. coli* cells expressing GLR. After harvest, the cells were starved in 200 mM NaCl or KCl overnight, respectively. These cells were washed again with the same salt solutions and then suspended so that the OD values at 660 nm became 0.5. The green bar indicates the period of green light illumination. In the presence of Na^+ (Panel A), a pH increase was observed, indicating the outward Na^+ pumping activity of GLR. This activity creates an inside negative potential, which drives a H^+ influx and then induces a pH increase. In the presence of 10 μM CCCP, the pH increase became large, reflecting the facilitation of the membrane penetration of H^+. On the other hand, in the absence of Na^+ (Panel B), a pH decrease was observed due to the outward H^+ pumping activity of GLR. This pH change disappeared after the addition of 10 μM CCCP, indicating that H^+ was indeed pumped by GLR.