SUPPORTING MATERIAL

for

"Gloeobacter Rhodopsin, Limitation of Proton Pumping at High Electrochemical Load"

Arend Vogt, Jonas Wietek, and Peter Hegemann

From Institute of Biology, Experimental Biophysics, Humboldt-Universität zu Berlin, Invalidenstraße 42, 10115 Berlin, Germany

*Correspondence: hegemape@rz.hu-berlin.de

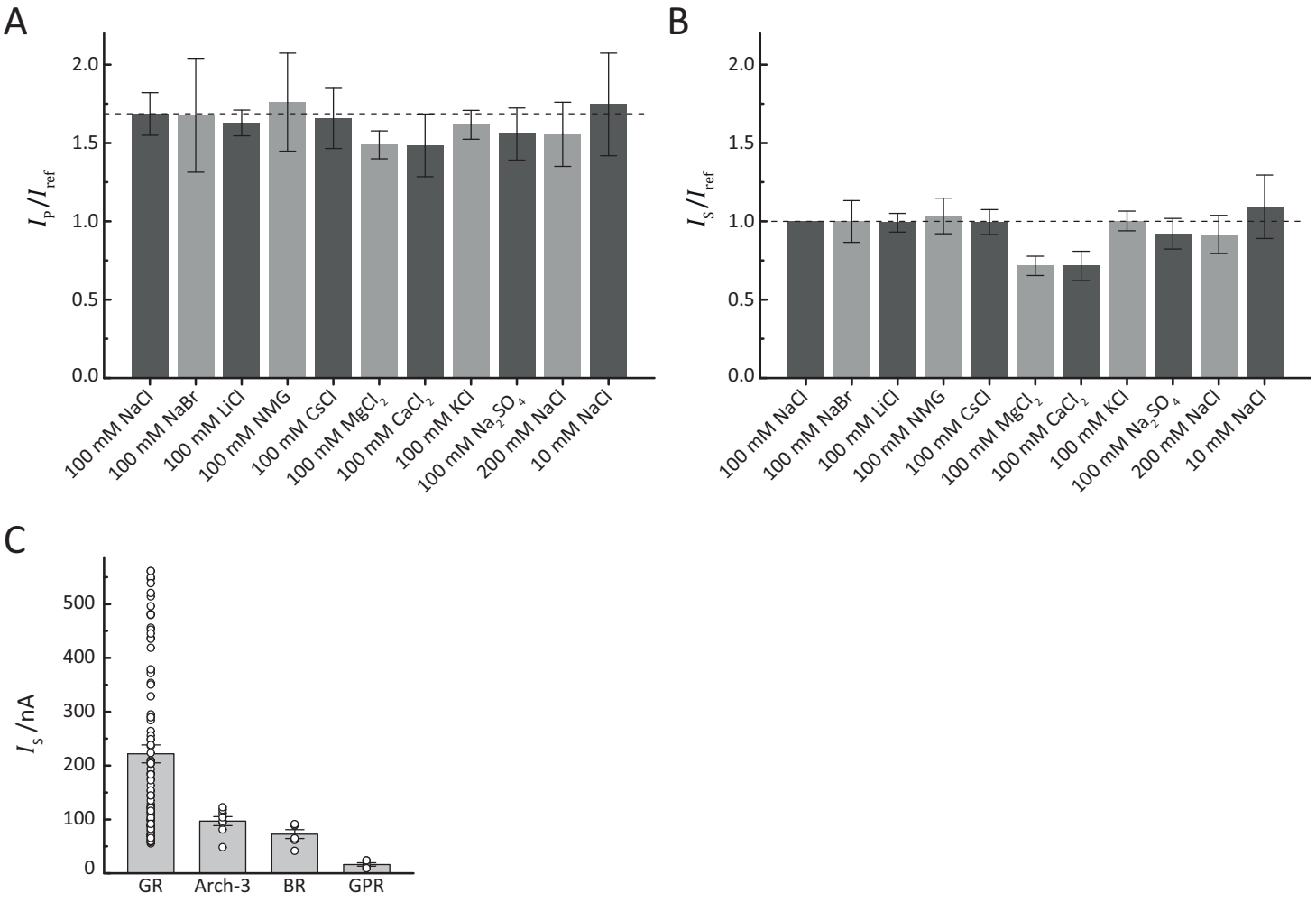

Figure S1. Ion selectivity and absolute photocurrent amplitudes of Gloeobacter Rhodopsin. Oocytes expressing GR-WT were activated by green light of 550±25 nm and photocurrents were recorded at a holding voltage of 0 mV. Different extracellular buffers were tested to test ion selectivity. No crucial differences were observable for peak currents (A) as well as stationary currents (B). Small current reduction in case of high concentrations of divalent cations can be explained by a surface charge effects. The results confirm that GR transports only protons. The extracellular buffer contained the following additional components [mM]: 1 $MgCl_2$, 0.1 $CaCl_2$ and 5 MOPS (pH_o 7.5, adjusted with NMG) (mean ± SD, n ≥ 8). C. Comparison of stationary photocurrent amplitudes (mean ± SE) of different proton pumping rhodopsins. Open dots indicate single measurement values. All proton pumps were expressed and measured under the same conditions (pH_o 7.5, 0 mV and 550±25 nm). Only GPR was excited with twofold increased light intensity (400-600 nm filter) due to low photocurrent amplitudes. This comparison underlines the high photocurrents of GR compared to GPR. GR: Gloeobacter Rhodopsin from *Gloeobacter violaceus* (n=89), Arch-3: Archaerhodopsin-3 from *Halorubrum sodomense* (n=8), BR: Bacteriorhodopsin from *Halobacterium salinarum* (n=6), GPR: Green Proteorhodopsin from γ-proteobacterium EBAC31A08 (n=5).

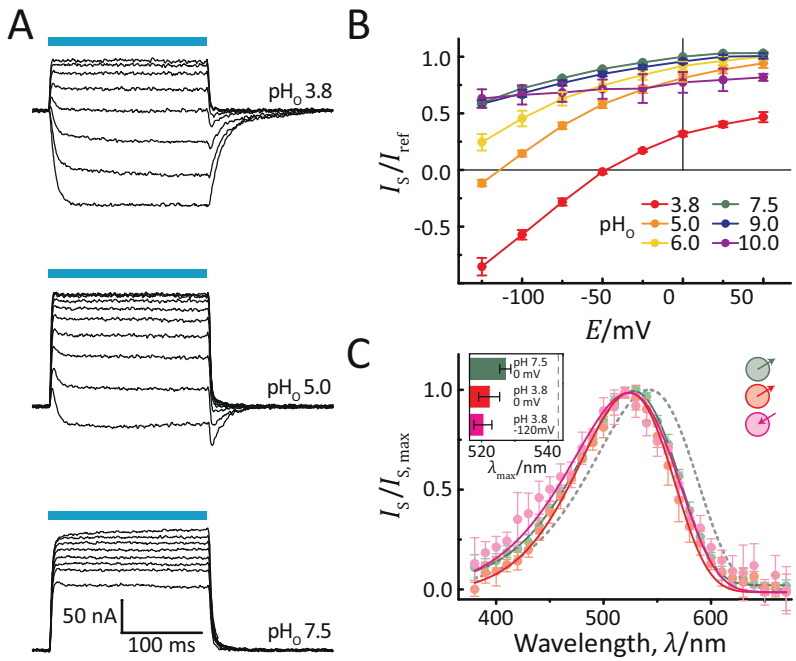

Figure S2. Characterization of GR-H87A.
The conserved Histidine residue was exchanged by Alanine to prevent potential coupling with the Schiff base counterion D121. A. Typical photocurrents at different extracellular pH conditions and holding voltages ranging from -125 mV to +75 mV. Stationary outward and inward directed currents are maintained whereby transient peak currents disappeared. Measurements were carried out under identical conditions as GR-H87M (Fig. 2 B-D). Even though, for photoactivation cyan light of 500±25 nm was used. This observation supports the conclusion that the conserved His87 is not crucial for inward currents. B. *I(E)*-Plot of stationary currents of GR-H87A with similar characteristics like GR-H87M as already mentioned before (Fig. 2C) (mean ± SE, n ≥ 6). C. Action spectra show a blue shift of about 16 nm compared to GR-WT (Fig. 1D). A distinct pH and voltage dependent difference could be not observed similar to GR-WT. The pH/voltage-dependence of GR-H87M might be a specific feature of the replacement by Methionine (Fig. 2D). (mean ± SD, n ≥ 6)

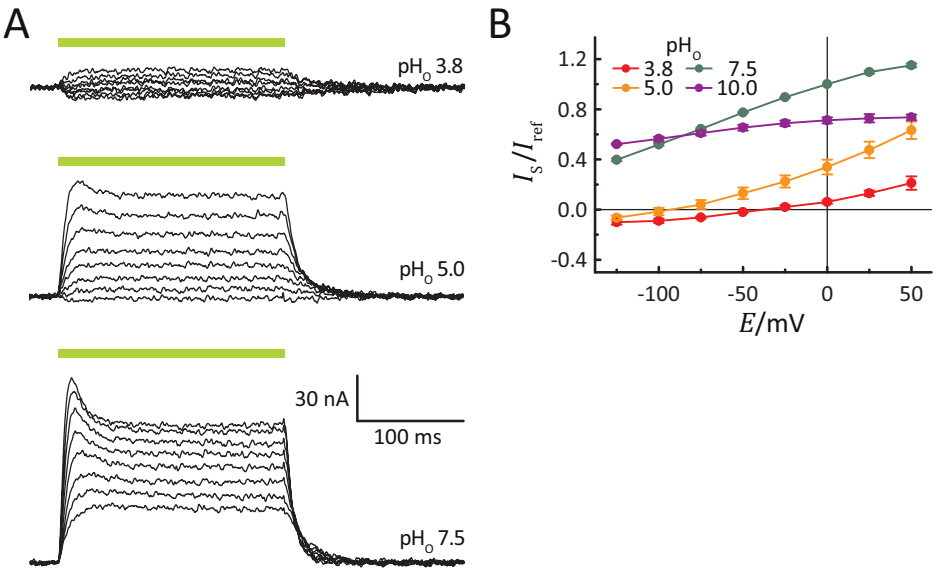

Figure S3. Characterization of GR-S77C.
A. Photocurrents were measured in oocytes similarly as for GR-E132D and GR-S77A (Fig. 3) at holding potentials between -125 mV and +75 mV. The currents showed similar characteristics as the other two mutants although replacement of S77 by cysteine is a conservative exchange. B. $I(E)$-Plot of stationary currents of GR-S77C (mean ± SE, n=4).

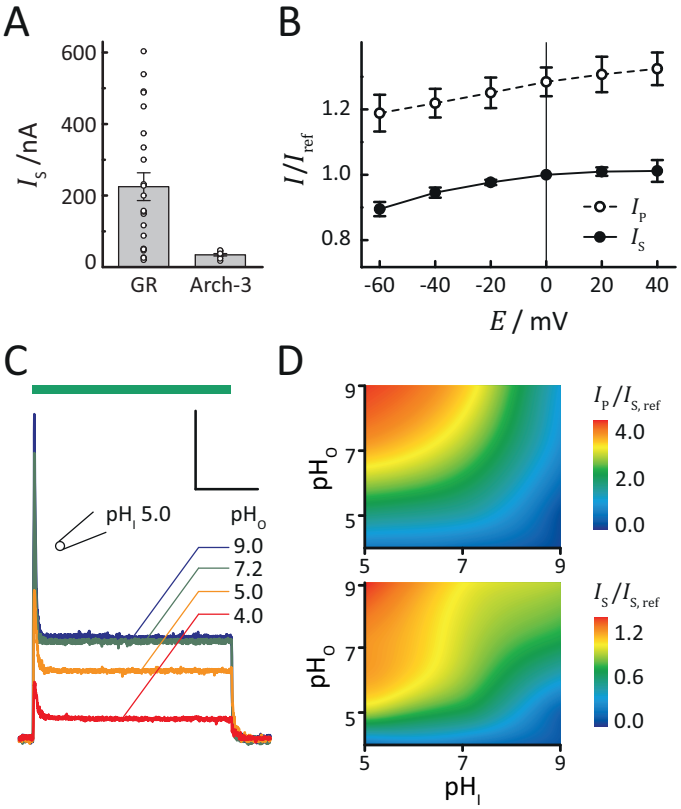

Figure S4. Whole-cell measurements of GR-WT-expressing HEK-293 cells.
A. Comparison of stationary photocurrent amplitudes (mean ± SE) of GR (n=23, 540±7 nm) and Arch-3 (n=9, 565±7 nm) at a holding voltage of 0 mV. Photocurrents of GR were about five fold higher compared to Arch-3. B. *I*(*E*)-plot of peak and stationary currents of GR-WT at pH_o 7.2 (n=8) normalized to stationary photocurrent at 0 mV. Current-voltage relation is similar to oocyte measurements (see Fig. 1B). C. Photocurrent traces of GR-WT expressing HEK-293 cell at different pH_o while the intracellular pH_i was 5.0, measured at 0 mV holding potential. Scale bar: 70 pA, 50 ms. The photocurrent amplitudes decrease with increasing extracellular proton concentrations as expected for a proton pump. D. Peak and stationary currents at various pH_o (4.0, 5.0, 7.2 and 9.0) as well as different pH_i (5.0, 7.2 and 9.0). The holding potential was kept at 0 mV (n=3 for each pH_o/pH_i pair). Data points between measured values are linearly interpolated. Photocurrents were normalized to the stationary current at symmetric proton concentrations (pH_o=pH_i).

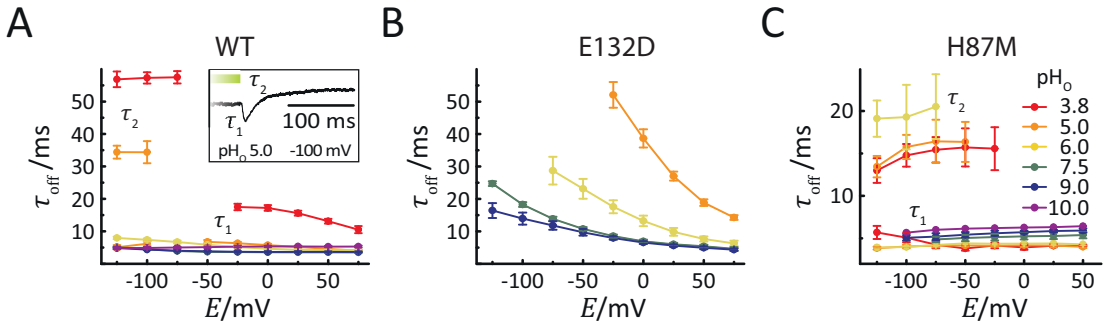

Figure S5. Kinetic properties of GR-WT, GR-E132D and GR-H87M
Characteristics of τ_{off} for GR-WT (A), GR-E132D (B) and GR-H87M (C) at different pH$_o$-values and holding voltages. The inset (A) shows biphasic kinetic decay at low pH$_e$ where τ_1 corresponds to a fast off-kinetics of the active pump mechanism and τ_2 refers to the kinetic of the passive inward proton flux. For determination of kinetic parameters only current amplitudes with $I_S \geq 60$ nA were analyzed (mean ± SE, n ≥ 9 (WT), n ≥ 6 (E132D), n ≥ 6 (H87M)).