

## **Intramolecular proton transfer in channelrhodopsins**

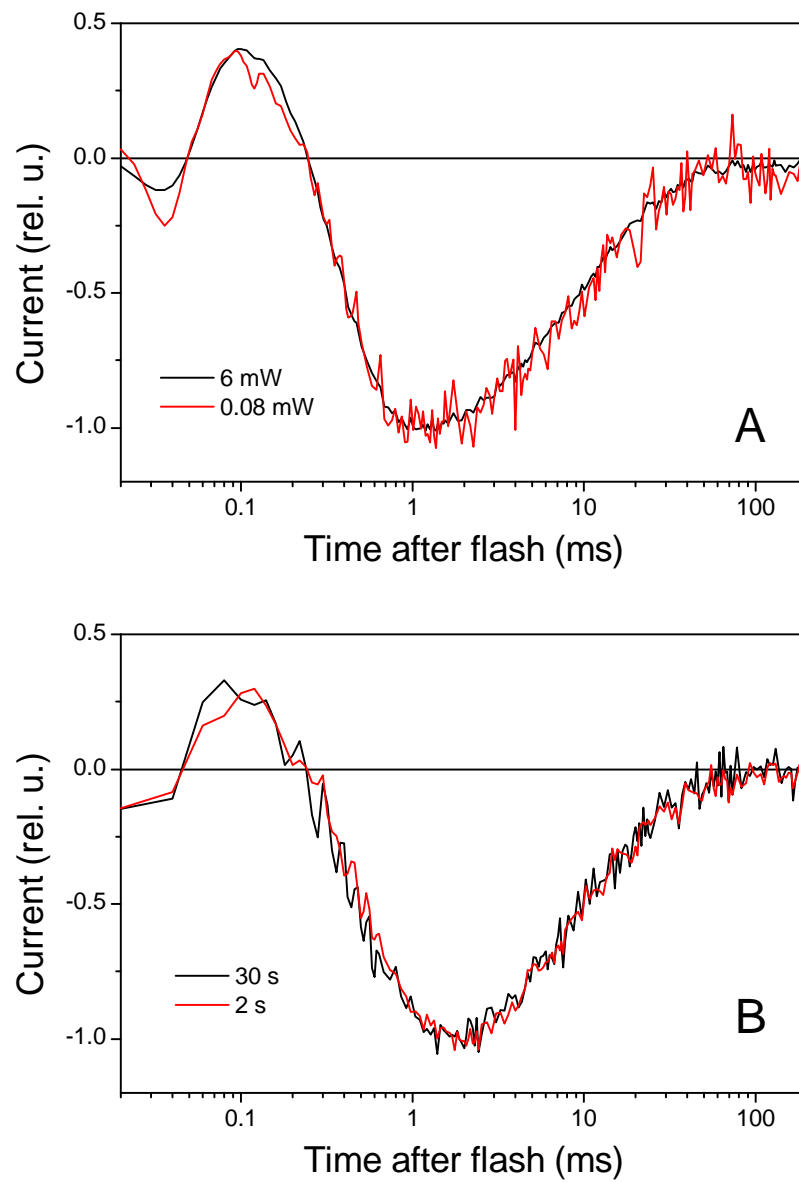
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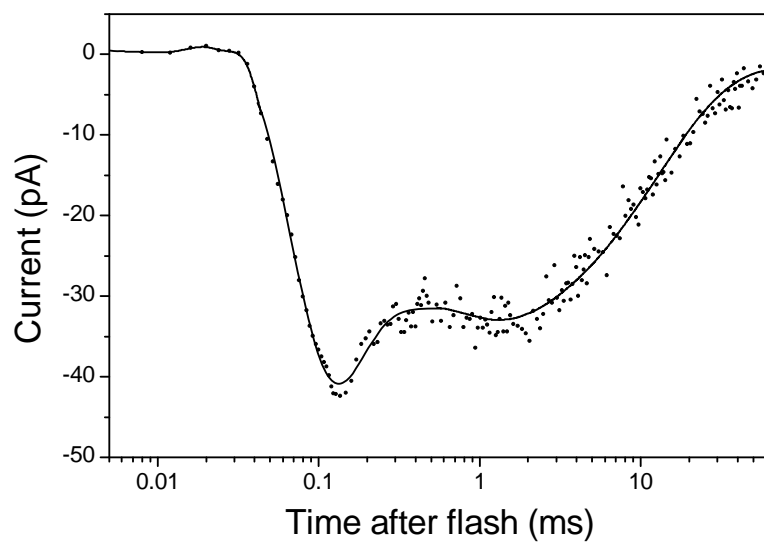
### **SUPPORTING MATERIAL**

## SUPPORTING METHODS

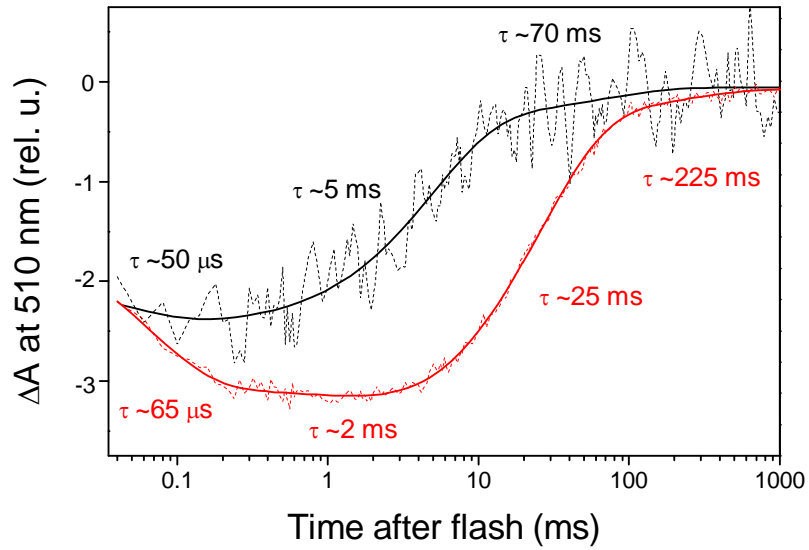
Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA). First strand cDNA was synthesized with the SMARTer RACE cDNA amplification kit (Clontech Laboratories, Mountain View, CA). Degenerate primers for *P. subcordiformis* were designed according to the conserved regions of previously known channelopsins. First PCR (polymerase chain reaction) was carried out with primers TCGCGNTGGGAGGAGRTNTA and KCCCTCRGKBCCCARBAGGAAS, after which the product mixture was subjected to another round of PCR with the nested forward primer TACGSIGAITGGYTICTIACITGCCC. PCR products of the appropriate size were gel-purified, cloned into the TOPO TA cloning vector (Invitrogen, Grand Island, NY) and sequenced. For the fragment that showed homology with channelopsins, 3' and 5' RACE (rapid amplification of cDNA ends) was performed using the SMARTer RACE cDNA amplification kit. Overlapping RACE fragments were joined by fusion PCR to obtain full-length cDNA, which was cloned into the TOPO vector and sequenced. The 7TM domain of *D. salina* channelopsin was cloned using the reverse primer GAGGGACGCGCGTGTGTTGAGTGCCG designed according to the sequence information provided by Jon K. Magnuson (Pacific Northwest National Laboratory).



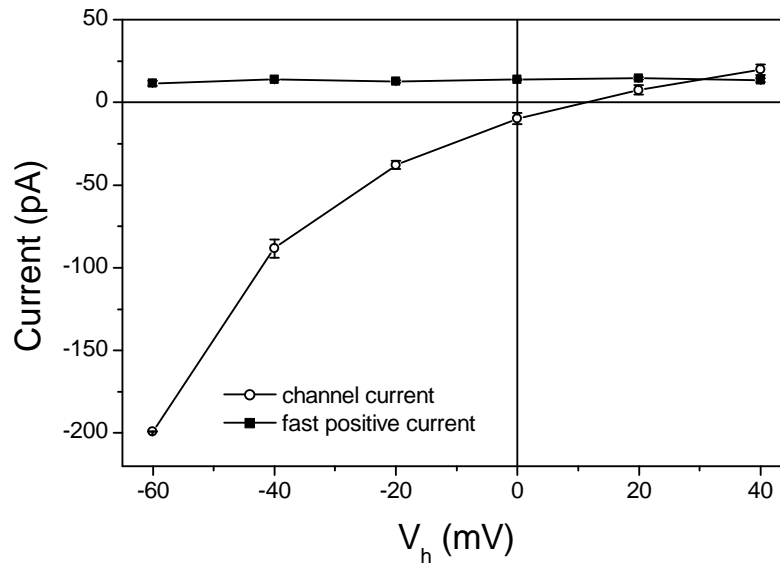
**FIGURE S1.** Currents generated by *CaChR1* in HEK293 cells in response to laser flashes of (A) different intensity (black line, 6 mW and red line, 0.08 mW), and (B) different time interval between successive flashes (black line, 30 s and red line, 2 s). Signals were normalized at the peak value of the channel current.



**FIGURE S2.** A differential signal calculated by subtraction of traces recorded at -60 and 20 mV upon laser flash excitation of *CaChR1* expressed in HEK293 cells.



**FIGURE S3.** Absorbance changes at 510 nm of *CaChR1* in response to a laser flash (532 nm) in intact *Pichia* membranes (black lines) and in detergent (red lines). The maximal absolute absorbance change in membranes was  $\sim 1$  mOD. Absorbance changes in purified pigment were arbitrarily scaled for better visualization of the difference in the rates of photocycles.



**FIGURE S4.** Voltage dependence of the mean fast positive current calculated between 30 and 50  $\mu$ s (filled squares) and mean channel current calculated between 2 and 4 ms (empty circles) generated by  $V_c$ ChR1 expressed in HEK293 cells upon laser flash excitation. Data points are mean values  $\pm$  SEM of experiments in 3 cells.

Protein	$\tau_1$	$\tau_2$	$\tau_3$	$\tau_4$	$\tau_5$
WT	20 $\mu$ s	110 $\mu$ s	3 ms	32 ms	200 ms
E169Q	65 $\mu$ s	350 $\mu$ s	5 ms	140 ms	1.2 s
D299N	1.5 $\mu$ s	75 $\mu$ s	3.3 ms	400 ms	$\sim$ 2 s

**TABLE S1.** Deconvolution of *CaChR1* M intermediate concentration changes into 5 kinetics components. The first 3 components define accumulation of the intermediate, whereas the last two components define its disappearance.