Electronic Supplementary Information:

The femtosecond-to-second photochemistry of red-shifted fast-closing anion channelrhodopsin PsACR1

Yusaku Hontani¹, Matthias Broser², Arita Silapetere², Benjamin S. Krause², Peter Hegemann²  
and John T.M. Kennis¹*

¹Department of Physics and Astronomy, Vrije Universiteit Amsterdam, Amsterdam 1081 HV, De Boelelaan, The Netherlands  
²Institut für Biologie, Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany  

*Corresponding author: j.t.m.kennis@vu.nl
Fig. S1. All-trans retinal structure and PsACR1 homology model. (a) Protonated retinal Schiff base (RSB) of all-trans retinal. (b) A homology model of PsACR1 based on the crystal structure of cation channelrhodopsin C1C21 (PDB ID: 3UG9) built by the Robetta server (robetta.bakerlab.org). All-trans retinal is shown in magenta. Glu64 and Asp230 are shown in green sticks.
Fig. S2. Dark-state absorption of \( \text{PsACR1} \) and its mutants in different anion conditions at pH 7.

Table S1. Visible absorption maxima of \( \text{PsACR1} \) and the mutants in different salt conditions at pH 7.

<table>
<thead>
<tr>
<th></th>
<th>( \lambda_{\text{max}} ) (150 mM NaCl)</th>
<th>( \lambda_{\text{max}} ) (&lt;1 mM NaCl)</th>
<th>( \Delta \lambda_{\text{max}} )</th>
<th>( \lambda_{\text{max}} ) (100 mM Na(_2)SO(_4))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>534 nm</td>
<td>534 nm</td>
<td>0 nm</td>
<td>---</td>
</tr>
<tr>
<td>E64Q</td>
<td>534 nm</td>
<td>537 nm</td>
<td>3 nm</td>
<td>537 nm</td>
</tr>
<tr>
<td>D230N</td>
<td>523 nm</td>
<td>530 nm</td>
<td>7 nm</td>
<td>530 nm</td>
</tr>
<tr>
<td>E64Q/D230N</td>
<td>526 nm</td>
<td>536 nm</td>
<td>10 nm</td>
<td>---</td>
</tr>
</tbody>
</table>
Fig. S3. Decay-associated difference spectra (DADS) of fs-ps transient absorption for wild-type \textit{PsACR1} at pH 8. Normalized dark-state absorption is shown in the gray line, which is inverted for comparison with the GSB. The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.
Fig. S4. EADS and DADS of fs-ps transient absorption spectra of PsACR1/E64Q at pH 8. The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.
Fig. S5. EADS and DADS of picosecond-submillisecond transient absorption spectra of PsACR1/E64Q at pH 8. The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.
Fig. S6. Global-fitted flash photolysis results for WT PsACR1 and the E64Q, D230N and E64Q/D230N mutants at pH 8.
References