

Light-Powered Transport of Organic Anions by Microbial Rhodopsins

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Cite This: *J. Phys. Chem. Lett.* 2025, 16, 10528–10535



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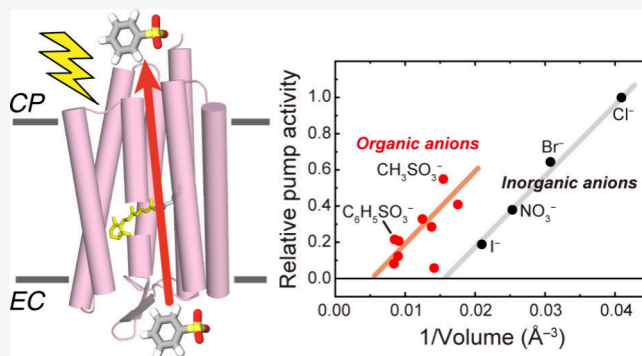


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Supporting Information

ABSTRACT: Microbial rhodopsins are photoactive membrane proteins known for transporting small inorganic ions such as H^+ , Cl^- , and Na^+ . Their compact structure—comprising seven transmembrane helices—has long been thought to limit their substrate range to such ions. Here, we report that several anion-pumping rhodopsins can also transport organic anions. In particular, a rhodopsin from cyanobacteria transports bulky organic anions, including those with a benzene ring, with volumes up to $\sim 120 \text{ \AA}^3$ —five times larger than Cl^- . These anions bind in the dark state and are translocated upon photoactivation, via a mechanism similar to Cl^- . Notably, only anions with pK_a values below 2 are transported, suggesting that negative charge is essential for binding. This study provides the first evidence that naturally occurring proteins can use light to transport organic compounds across membranes. These findings broaden the functional scope of microbial rhodopsins and open new possibilities for light-driven transport of organic ions.



Membrane transport proteins are indispensable for cellular function, enabling the selective movement of ions and molecules across biological membranes. Among them, microbial rhodopsins stand out for their ability to harness light energy to drive ion transport.^{1–5} Since the discovery of the first light-driven H^+ pump in the 1970s,^{6,7} many ion-translocating variants have been identified.^{1–5} Their light-activated nature has made them valuable model systems for studying membrane transport and, more recently, powerful tools for optical manipulation of cells.^{8,9} Channelrhodopsins, which function as light-gated ion channels, are the most widely used in optogenetics,^{10,11} while ion-pumping rhodopsins have also demonstrated strong potential.^{9,12} These proteins now enable light-based control over diverse cellular processes, including the activation or inhibition of excitable cells, modulation of signaling pathways, and induction of programmed cell death.^{9,12,13} To date, all characterized microbial rhodopsins have been shown to transport only small inorganic ions. Representative examples are H^+ , Cl^- , or Na^+ , although anion-transporting rhodopsins (both pumps and channels) are also known to accommodate a wider range of anions, such as Br^- , NO_3^- , and I^- .^{11,14–20} These inorganic ions, while effective for electrical or pH modulation, lack the intrinsic biological activity of organic compounds. Expanding the substrate scope of microbial rhodopsins to organic ions could open new avenues for light-driven transport applications. Here, we demonstrate for the first time that certain microbial rhodopsins can transport organic anions.

Microbial rhodopsins contain the chromophore retinal within the protein.^{1–5} Figure 1A shows the structure of a Cl^- -pumping rhodopsin (ClR) from the cyanobacterium *Synechocystis* sp. PCC 7509,²¹ referred to here as SyHR. Upon light absorption, retinal undergoes isomerization, triggering a conformational change in the protein. These structural changes proceed through a cyclic series of intermediates known as the photocycle, ultimately returning the protein to its ground state. Each rhodopsin performs a specific function during this cycle within its native host.

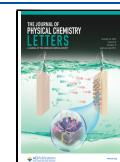
Although many ion-translocating microbial rhodopsins have been identified over the past 50 years, they have been thought to transport only small inorganic ions. An assumption consistent with their compact structure. Membrane transporters for organic molecules—such as the Major facilitator superfamily, Multidrug and toxic compound extrusion, and many ATP-binding cassette transporters—typically involve two homologous transmembrane domains (each with six helices).^{22–24} In contrast, typical ion-transporting microbial rhodopsins possess a single transmembrane domain composed

Received: August 17, 2025

Revised: September 13, 2025

Accepted: September 25, 2025

Published: October 2, 2025



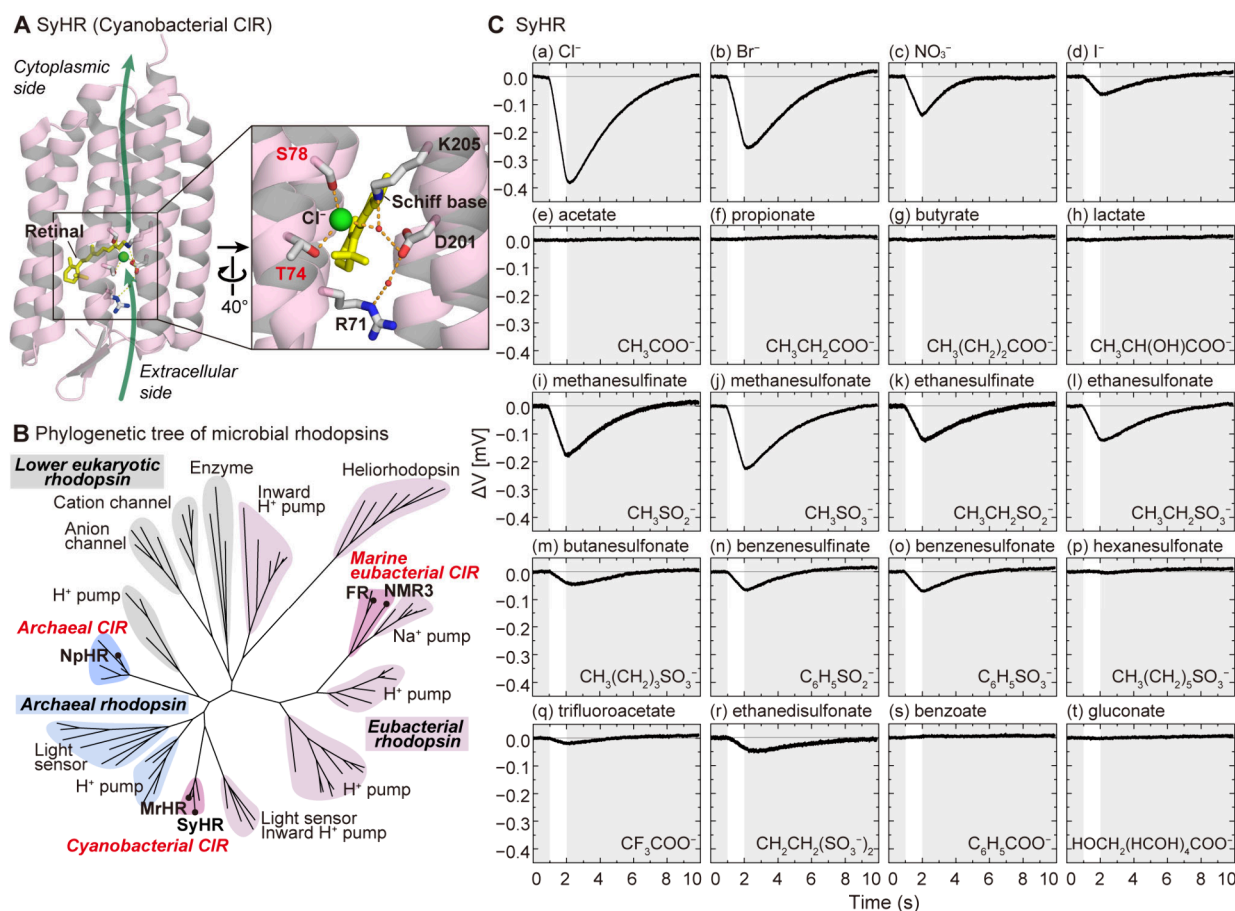


Figure 1. Tertiary structure, phylogenetic position, and anion transport activity of SyHR. (A) Tertiary structure of unphotolyzed SyHR in the Cl⁻-bound state (PDB ID: 7ZOU). Water molecules are shown as small spheres, and polar contacts within 3.2 Å are indicated by dashed lines. (B) Phylogenetic relationships among microbial rhodopsins. (C) Anion transport activity of SyHR. Each trace represents the average of 30–50 technical replicates using samples derived from the same *E. coli* culture and prepared under identical conditions. *E. coli* cells were suspended in 200 mM sodium salts of the respective anions with 10 μM CCCP. Actinic light was applied for 1–2 s.

of seven helices.^{1,2,4,5} This structural simplicity has fostered the widespread belief that microbial rhodopsins are specialized for small inorganic ions and are incapable of transporting organic ions. In this study, we focused on CIRs, which have also been shown to transport a few polyatomic inorganic anions, such as NO₃⁻, SCN⁻, and SO₄²⁻.^{14–20} Although these ions lack hydrocarbon groups, their volumes are close to those of organic anions like acetate and propionate—short-chain fatty acids (SCFAs) known for their bioactivity in animals. Contrary to expectations, we found that CIRs fail to transport SCFAs. However, several CIRs, and particularly SyHR, were capable of transporting other organic anions, including those with benzene rings.

To our knowledge, this is the first report demonstrating that naturally occurring proteins can use light energy to transport organic compounds across membranes.

Chloride-pumping rhodopsins (CIRs) can be phylogenetically classified into three distinct groups (Figure 1B):²⁵ archaeal CIRs,^{26,27} marine eubacterial CIRs,²⁸ and cyanobacterial CIRs.²⁹ To investigate their ability to transport organic anions, we selected one or two representative members from each group. Several CIRs are abbreviated as “HR” (e.g., SyHR), a term originating from “halorhodopsin,” the name given to the first CIR discovered in a halophilic archaeon.^{26,27}

All tested CIRs were heterologously expressed in *Escherichia coli* membranes. This enabled straightforward detection of

inward anion pump activity by monitoring light-induced pH increases in *E. coli* suspensions in the presence of a protonophore such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). In this setup, anion uptake by CIRs generates a negative membrane potential, which drives passive H⁺ influx and raises the extracellular pH. Conventional pH electrodes, which use 3.3 M KCl as an internal solution, risk contaminating samples with chloride ions. To avoid this, we used a custom electrochemical cell incorporating indium tin oxide (ITO) electrodes (Figure S1A).¹⁸ These transparent electrodes respond rapidly to pH changes. In our setup, the *E. coli* suspension is separated from a reference salt solution by a dialysis membrane. Illumination causes a pH increase only in the *E. coli* compartment, leading to a voltage difference (ΔV) between the two electrodes. A typical light-induced ΔV trace for a CIR from a marine eubacterium *Fulvimarina pelagi* (FR) is shown in Figure S1B. One second of illumination produces a negative ΔV, indicating a pH increase in the *E. coli* suspension. To improve the signal-to-noise ratio, measurements were averaged over approximately 17 to 50 replicates. All tested anions were used at concentrations of 200 mM.

Figure 1C displays the light-induced voltage changes (ΔV) for SyHR in the presence of various anions. Corresponding results for other CIRs are presented in Figure S2. In the top row of Figure 1C, robust pump activity was confirmed for typical inorganic anions: (a) Cl⁻, (b) Br⁻, (c) NO₃⁻, and (d)

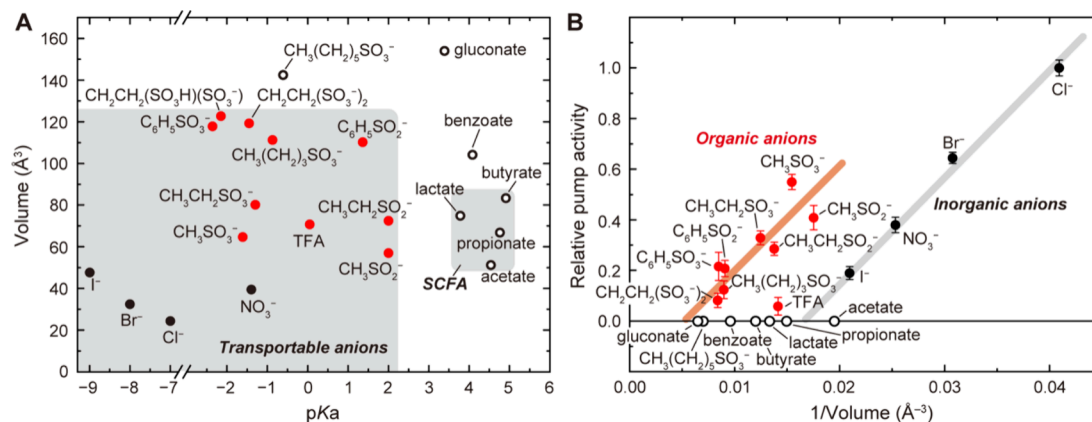


Figure 2. Dependence of anion transport activity on ion size and pKa. (A) pKa values and dehydrated volumes of the tested anions. Ethanedisulfonic acid has two pKa values; its lower and higher pKa are plotted in association with the dehydrated volumes of its monoanionic ($\text{CH}_2\text{CH}_2(\text{SO}_3\text{H})(\text{SO}_3^-)$) and dianionic ($\text{CH}_2\text{CH}_2(\text{SO}_3^-)_2$) forms, respectively. (B) Relationship between transport activity and the reciprocal of dehydrated ion volume. Transport activities were calculated from the slopes of individual traces shown in Figure 1C ($n = 30\text{--}50$) and corrected for buffer capacity. Error bars represent standard deviation (SD) values. The activity for ethanedisulfonate is plotted against the reciprocal volume of its dianionic form ($\text{CH}_2\text{CH}_2(\text{SO}_3^-)_2$). In both panels, data for inorganic anions are shown as black filled circles, transportable organic anions as red filled circles, and nontransportable organic anions as black open circles. In (B), gray and orange diagonal lines are shown as visual guides to highlight distinct trends for inorganic and organic anions, respectively.

Γ. Next, we evaluated SCFAs, including (e) acetate, (f) propionate, (g) butyrate, and (h) lactate. However, no pump activity was detected for SyHR or the other CIRs (Figure S2).

Although acetate (51.3 Å³) is close in size to iodide (47.7 Å³), its significantly higher pK_a (4.5 vs −9.0) may explain the absence of transport activity. Other SCFAs also exhibit relatively high pK_a values (3.8 to 4.9), in contrast with those of the inorganic anions tested (−9.0 to −1.4). Figure 2A summarizes the dehydrated volumes and pK_a values of all anions examined (see Experimental Methods for details). Given the hydrophobic environment of microbial rhodopsin transmembrane regions, weak acids like SCFAs likely remain predominantly uncharged within the protein and thus fail to interact strongly with it, thereby preventing transport.

To test this hypothesis, we examined anions derived from stronger acids containing sulfinic ($-\text{SO}_2^-$) and sulfonic ($-\text{SO}_3^-$) groups, which have pKa values below 2 (Figure 2A). In the third and fourth rows of Figure 1C, clear pump activities were detected for (i) methanesulfinate, (j) methanesulfonate, (k) ethanesulfinate, and (l) ethanesulfonate. Although transport efficiency decreased for larger anions (fourth row), SyHR still exhibited activity toward bulkier substrates, including (n) benzenesulfinate and (o) benzenesulfonate, both containing a benzene ring. In contrast, negligible activity was observed for (p) hexanesulfonate, likely because its size ($142.4 \text{ \AA}^3$) exceeds the upper limit for transport. We also tested additional anions with varying properties: (q) trifluoroacetate (TFA), (r) ethanedisulfonate, (s) benzoate, and (t) gluconate. Although TFA ($70.8 \text{ \AA}^3$) is smaller than ethanedisulfonate ($\text{CH}_2\text{CH}_2(\text{SO}_3^-)_2$, $119.3 \text{ \AA}^3$), it exhibited lower transport activity, possibly due to its relatively high pKa of 0.05. In this study, “ethanedisulfonate” refers to the dianionic form; however, it remains uncertain whether the monoanionic or dianionic form is actually transported. Benzoate ($104.2 \text{ \AA}^3$) is smaller than benzenesulfonate ($\text{C}_6\text{H}_5\text{SO}_3^-$, $117.9 \text{ \AA}^3$), but was not transported, likely due to its high pKa of 4.1. Gluconate, which has both a high pKa (3.4) and a large volume ($153.9 \text{ \AA}^3$), was also not transported.

Pump activity for sulfonic anions was also tested in other CIRs (Figure S2). The archaeal CIR from *Natronomonas*

pharaonis (NpHR) showed only a faint response to methanesulfonate (CH_3SO_3^-) and none to larger sulfonates, while the marine eubacterial FR transported methanesulfonate but lost activity with increasing substrate size, disappearing for benzenesulfonate. Both were far weaker than SyHR, which was therefore chosen for detailed characterization.

As shown in Figure 1C, SyHR exhibited the strongest activity with Cl^- , while transport efficiency decreased for larger anions. However, the size dependence was not straightforward. For example, the pump activity for methanesulfonate (CH_3SO_3^-) (j) was greater than that for NO_3^- (c) and I^- (d), despite its larger molecular volume ($64.7 \text{ \AA}^3$ vs 39.5 and $47.7 \text{ \AA}^3$) (Figure 2A). To better understand this relationship, we plotted the relative pump activities against the reciprocal of ion volume ($1/\text{Volume}$) in Figure 2B. The y-axis represents the initial slope of the light-induced voltage change, adjusted for the buffer capacity of each sample (see Experimental Methods and Figure S3). This representation facilitates the visualization of an inverse correlation, in which larger anions are expected to encounter greater steric hindrance within the protein's binding pocket and translocation pathway. These steric effects are likely to reduce both binding affinity (analogous to K_m in Michaelis–Menten kinetics) and translocation rate (analogous to $V_{\max}$). Consistent with this reasoning, the plot reveals a general trend: for both inorganic and organic anions, transport efficiency decreases as ion volume increases. However, this dependency differs between the two groups, highlighted by the gray and orange trendlines, respectively. These results indicate that ion size alone cannot fully explain the differences in transport activity. Other factors—such as the ion's chemical structure or interaction with the protein—appear to critically influence transport efficiency.

ClRs are known to bind inorganic anions even in the dark state and subsequently transport them upon illumination^{25,28–30} Figure 1A illustrates this initial binding state for SyHR, with Cl[−] depicted as a green sphere. We next asked whether organic anions also bind in the dark. The binding site for inorganic ions is located near the protonated Schiff base (Figure 1A), and their binding typically induces shifts in the

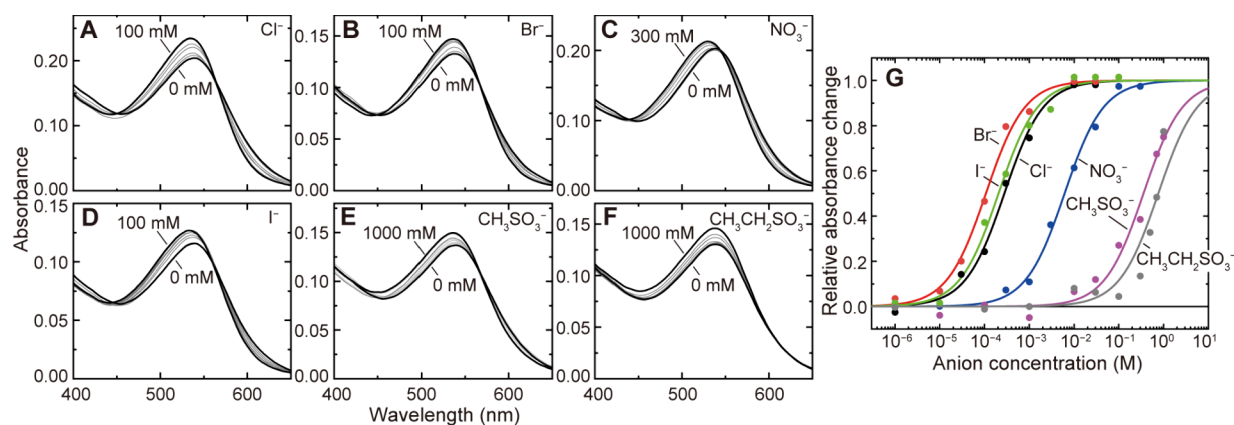


Figure 3. Anion binding to SyHR in the dark state. (A–F) Absorption spectral shifts of SyHR upon binding transportable anions. Lipid-reconstituted SyHR was used for all samples. Buffer solution contained 50 mM citric acid (pH 6) with various concentrations of sodium salts of the respective anions and sodium gluconate. Sodium gluconate was added to maintain constant ionic strength. (G) Absorbance changes at the wavelengths showing maximal responses were plotted against anion concentration and fitted with the Hill equation ($n = 1$) to determine dissociation constants, which are listed in Table S1. Relative absorbance changes are shown, along with best-fit curves. Absorbance values were taken from the following wavelengths: Cl^- (535 nm), Br^- (537 nm), NO_3^- (526 nm), I^- (533 nm), CH_3SO_3^- (536 nm), and $\text{CH}_3\text{CH}_2\text{SO}_3^-$ (539 nm).

absorption spectrum. These spectral shifts for inorganic anions are shown in Figure 3A–D, where a blue shift was observed as the ion concentration increased. Figures 3E and 3F present the corresponding data for smaller sulfonate ions—methanesulfonate (CH_3SO_3^-) and ethanesulfonate ($\text{CH}_3\text{CH}_2\text{SO}_3^-$). Clear spectral changes were observed for both. However, the wavelength shift was much smaller for ethanesulfonate. Its larger volume and nonspherical structure may differently influence the retinal environment compared to inorganic anions.

To estimate the dissociation constants (K_d), absorbance changes at wavelengths showing maximal response were plotted against ion concentration (Figure 3G). The resulting K_d values are listed in Table S1. For inorganic anions, K_d values ranged from 0.11 to 6.15 mM, indicating that nearly all SyHR molecules were in the ion-bound state at 200 mM—the concentration used for transport activity measurements (Figure 1C). For methanesulfonate and ethanesulfonate, saturation was not clearly observed (Figure 3G), but K_d values were provisionally estimated at 356 mM and 761 mM, respectively. This suggests that at 200 mM, only about 36% and 21% of SyHR molecules were in the ion-bound state for methanesulfonate and ethanesulfonate, respectively. Although these affinities are markedly weaker, the data confirm that both organic anions can bind to SyHR in the dark.

Do organic anions share the same binding site as inorganic ones? As shown in Figure 1A, the binding of inorganic ions is stabilized by hydrogen bonds with Thr74 and Ser78. If both types of ions bind at the same site, mutations at these residues would be expected to reduce transport activity for both groups. Figure S4 shows the light-induced voltage changes detected by ITO electrodes for the T74A and S78A mutants. In Figure 4, the initial slopes of these traces—representing transport activity—are plotted as open circles. For comparison, the corresponding data for wild-type SyHR (reproduced from Figure 2B) are shown as filled circles. Mutant data were corrected for differences in protein expression levels relative to wild-type SyHR (see Experimental Methods and Figure S5). As shown in Figure 4A and 4B, both mutants exhibited clearly reduced transport activity for both inorganic and organic

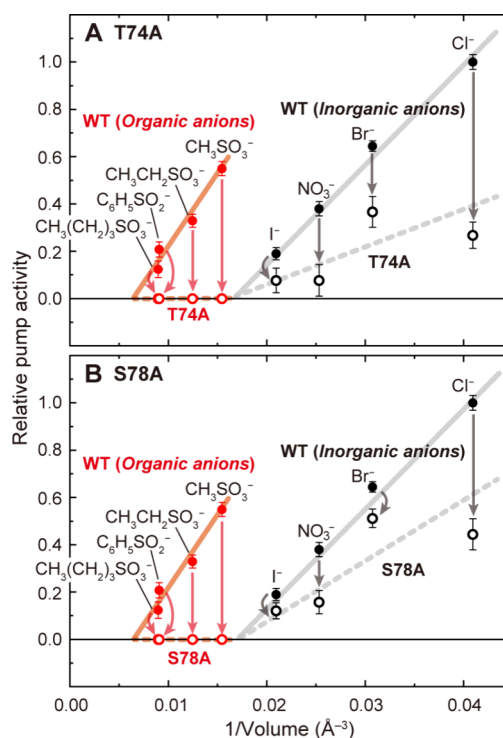


Figure 4. Reduced anion transport activity in SyHR mutants. Data for the T74A (A) and S78A (B) mutants are shown as open circles. For comparison, the corresponding data for wild-type SyHR are shown as filled circles (identical to those in Figure 2B). Error bars represent SD values ($n = 18$ –25 for T74A; $n = 17$ –25 for S78A). Mutant data were corrected for differences in expression levels relative to wild-type SyHR.

anions. This suggests that both ion types share the same binding site. However, transport activity for organic anions was nearly abolished in both mutants, indicating that the functional importance of these residues differs between the two groups. Inorganic anions can still bind and be transported in the absence of either residue, albeit less efficiently. In contrast, both residues appear essential for stable binding of organic anions.

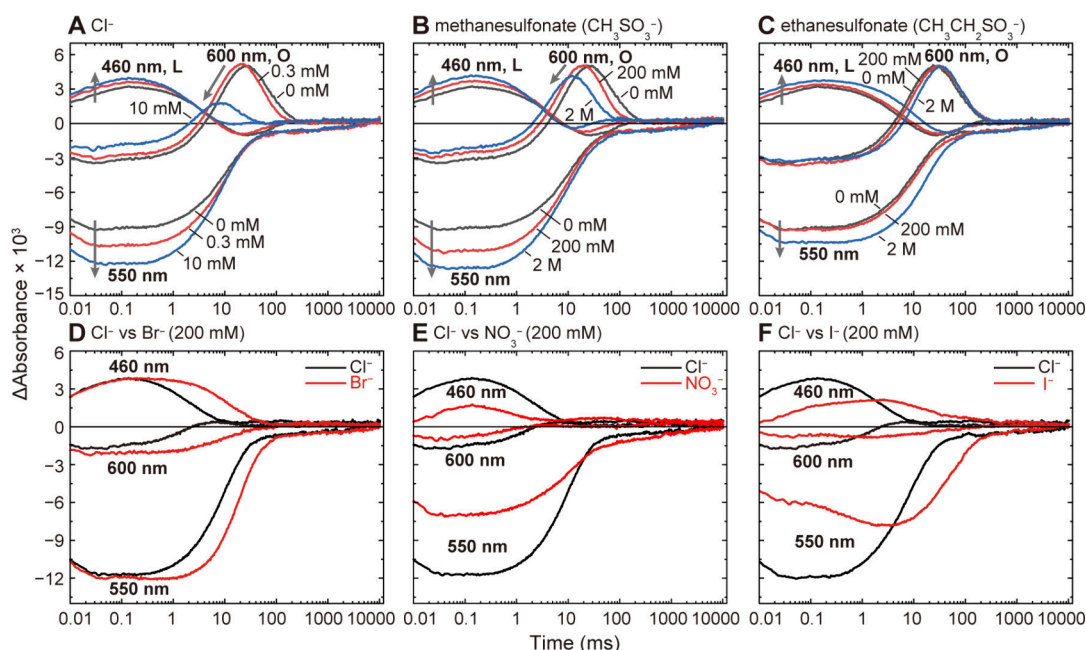


Figure 5. Photocycles of SyHR with various anions. Flash-induced absorbance changes at three representative wavelengths are shown for each condition. Each trace represents the average of 20 measurements (technical replicates). (A–C) Comparison of photocycles in the absence (black) and presence of (A) Cl^- , (B) methanesulfonate, and (C) ethanesulfonate. (D–F) Comparison of the Cl^- transport photocycle (black) with those of (D) Br^- , (E) NO_3^- , and (F) I^- . SyHR concentration was kept constant across all samples. Sample and buffer conditions were essentially the same as those used in Figure 3.

All CIRs undergo photocycles even in the absence of transportable anions, although these differ from the active ion-pumping cycles and reflect only internal photoisomerization events. A similar distinction is anticipated for SyHR when comparing photocycles with and without organic ion transport. Figures 5A–C illustrate these differences by showing flash-induced absorbance changes at three representative wavelengths: in the absence of transportable ions (black lines in all panels) and in the presence of Cl^- (Figure 5A), methanesulfonate (Figure 5B), and ethanesulfonate (Figure 5C). SyHR concentrations were kept constant across all samples. Representative light-minus-dark difference spectra are provided in Figure S6.

As discussed below, the photocycles associated with methanesulfonate and ethanesulfonate transport are similar to that of Cl^- , and all three differ from the photocycle observed without transportable anions. To ensure sufficient binding, we increased the concentrations of methanesulfonate and ethanesulfonate up to 2 M. At these highest concentrations, $\sim 85\%$ and $\sim 72\%$ of SyHR molecules are estimated to be bound, respectively, based on their dissociation constants (Table S1).

In the dark state, SyHR exhibits a maximum absorbance at 530–540 nm, irrespective of whether Cl^- or organic anions are bound (Figure 3A, E, F). Accordingly, in Figure 5A–C, the 550 nm traces display an early negative deflection, reflecting depletion of the ground state. As the concentration of Cl^- or organic anions increases, the magnitude of this deflection also grows, likely indicating a higher quantum efficiency of the substrate-bound form initiating the photocycle.

Positive absorbance changes at 460 and 600 nm correspond to the formation of two major intermediates observed in all conditions (Figure 5A–C). Based on previous studies, the earlier intermediate at 460 nm is assigned to the L state, while the later one at 600 nm corresponds to the O state.¹⁷

Increasing substrate concentration enhances the 460 nm signal, consistent with greater accumulation of the L state. The O intermediate also exhibits clear substrate dependence under Cl^- and methanesulfonate conditions (Figure 5A, B). At higher concentrations, O accumulation decreases, suggesting that O represents the substrate-releasing state and exists in a quasi-equilibrium with L—implying that L still retains the substrate. Moreover, the decay of O accelerates as Cl^- and methanesulfonate concentrations rise, likely reflecting rebinding of the substrate from the extracellular side.

In contrast, ethanesulfonate showed little effect on O accumulation, even at 2 M (Figure 5C). Its larger molecular size may weaken binding at the site in the L state, driving the L–O equilibrium substantially toward O, i.e., the substrate-releasing state. Moreover, the bulky structure may hinder uptake from the extracellular side, resulting in no detectable acceleration of O decay.

Following O decay under all three conditions (Figure 5A–C), a small negative signal persists at 550 nm. This signal corresponds to the slow decay of the SyHR' intermediate, a precursor to the ground state. Notably, the decay rate of SyHR' was independent of substrate concentration and remained consistent across all three photocycles. These results are consistent with the previous reports for SyHR itself and other microbial rhodopsins that the decays of final intermediates, the precursors to the initial states, do not involve the ion transfer processes and associate with structural rearrangements to recover the initial states.^{31–33}

At 200 mM, nearly all SyHR molecules are in the ion-bound state for inorganic anions (Figure 3G). Nevertheless, SyHR transports larger inorganic anions less efficiently (Figures 1C and 2B). This difference in transport activity is likely linked to variations in their respective photocycles. In Figure 5D–F, flash-induced absorbance changes for 200 mM Cl^- are compared with those for 200 mM Br^- , NO_3^- , and I^- ,

respectively. SyHR concentrations were kept constant across all samples, and the corresponding difference spectra are presented in Figure S7.

As shown in Figure 5D, the photocycle for Br^- closely resembles that for Cl^- . However, L decay is noticeably slower with Br^- . In contrast, NO_3^- and I^- induce markedly distorted photocycles (Figures 5E, F). In both cases, the signals at 550 and 460 nm were significantly reduced, indicating that a smaller fraction of SyHR molecules undergoes a productive photocycle. Unlike Cl^- , the binding of NO_3^- and I^- may not enhance the quantum efficiency for initiating the photocycle. Similar substrate dependences were previously reported for the archaeal Cl^- pump NpHR.³⁴ Besides the smaller signals, both showed distorted photocycles. For I^- , L formation and decay were markedly slower. For NO_3^- , kinetics resembled the Cl^- transport photocycle. However, the prolonged negative signal at 550 nm is clearly larger despite the reduced signal intensity at early times. A prolonged component also remains at 460 nm (see also Figure S7A,C), suggesting equilibration between SyHR' and L. These altered photocycles likely underlie the diminished transport activity observed for larger inorganic anions.

For both inorganic and organic anions, SyHR appears to transport only those capable of binding to the protein in the dark state. Thus, transport efficiency is determined by two principal factors: (1) the proportion of SyHR molecules in the ion-bound dark state, and (2) the efficiency of the transport reaction following photoactivation. For inorganic anions, nearly all SyHR molecules are bound at 200 mM. However, larger inorganic ions exhibit inefficient photoreactions, characterized by reduced photocycling and distorted photocycles, which likely account for their diminished transport activity. In contrast, SyHR molecules bound to organic anions undergo productive photoreactions. Although detailed photocycle analysis was feasible only for methanesulfonate and ethanesulfonate due to their relatively weak binding, both displayed photocycles are largely comparable to that of Cl^- . While O decay was slower for these organic anions, the subsequent decay of the SyHR' intermediate—the rate-limiting step of the photocycle—occurred at rates comparable to that observed for the Cl^- -pumping photocycle. This suggests that the overall photocycling rates (turnover rates) are similar among the photocycles for Cl^- , methanesulfonate, and ethanesulfonate. Therefore, for organic anions, the lower transport activity of larger substrates is primarily attributable to the smaller fraction of SyHR molecules in the ion-bound state prior to illumination.

We demonstrated that several CIRs, especially SyHR, can transport organic anions with molecular volumes up to 119.3 Å³ (e.g., ethanedisulfonate, $\text{CH}_3\text{CH}_2(\text{SO}_3^-)_2$). All transported anions had pKa values below 2, whereas those with higher pKa values were not transported—even when smaller in size. This suggests that retaining a negative charge within the protein's hydrophobic environment is a prerequisite for transport.

Like inorganic ions, certain organic anions—at least methanesulfonate and ethanesulfonate—can bind to unphoto-lyzed SyHR. Mutations at residues forming the Cl^- binding site impaired the transport of both inorganic and organic anions, indicating that they share the same binding site and that initial binding is essential for transport. This binding may require a low pKa, as the local pH near the protonated Schiff base may be close to 2—implying that anions with pKa values

above 2 would remain uncharged and therefore unable to bind, even if their molecular volumes are small.

The strong binding of inorganic anions suggests close size and shape complementarity to Cl^- . Yet, SyHR exhibits inefficient photocycles when bound to larger inorganic anions. In contrast, organic anions, despite weaker binding, still support productive photoreactions. This indicates that strong binding alone does not guarantee effective transport, highlighting a need to dissect the interplay between substrate binding and photocycle dynamics.

Beyond SyHR, we found that FR is also capable of transporting organic anions, suggesting such capability may be more widespread among CIRs than previously thought. Expanding the search may uncover CIRs capable of transporting even larger organic anions. SyHR and FR belong to the same rhodopsin groups as CIRs from *Mastigocladopsis repens* (MrHR) and *Nonlabens marinus* S1-08^T (NM-R3), respectively.^{16,17,28,29} However, MrHR and NM-R3 were unable to transport even smaller organic anions (Figure S8). Among them, sequence identity between FR and NM-R3 is moderate (36.1%, similarity 54.2%), but the identity between SyHR and MrHR is particularly high (66.8%, similarity 80.3%). These observations suggest that the ability to transport organic anions is determined by differences in specific residues and/or structural features, rather than by broad or complex sequence divergence. Identifying these determinants and engineering CIRs with expanded substrate ranges represent exciting avenues for future research. Possible ecological implications of light-driven organic anion transport and structure-based discussions of the SyHR transport pathway are provided in the Supporting Information (Supplemental Discussion 1 and 2).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpcllett.5c02551>.

Experimental methods; anion transport activities of SyHR mutants and other anion-pumping rhodopsins; detailed data on the photochemical cycles of SyHR; dissociation constants of SyHR for various anions; Possible ecological implications of organic anion transport by CIRs; Structure-based discussions of the SyHR transport pathway (PDF)

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Author Contributions

*S.S., S.A., J.W., and M.E. made equal contributions.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by JSPS KAKENHI Grant Numbers JP22H02579 to T.T. and T.K. and JP22H05389 to T.K.; the Steel Foundation for Environmental Protection Technology Grant Number C-40-56 to T.K.; and the JKA Foundation Grant Number 2025M-319 to T.K. Additional support was provided by the G-7 Scholarship Foundation Grant Number 7102400053 to Y.S.

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