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Proton Dynamics in the Light-driven Bacterial Chloride Pump Nmhr

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NmHR, a halorhodopsin from the marine bacterium Nonlabens marinus, is an inward chloride pump that was discovered less than 10 years ago. Although NmHR and the other two known halorhodopsins, HsHR and NpHR, transport the same anion, NmHR shows a higher sequence similarity to the outward sodium pump rhodopsin KR2. The latter two share a similar conserved motif of residues involved in ion transport.

Here we report the insertion of the unnatural amino acid p-cyano-phenylalanine (pCNF) via Amber stop codon suppression. The pCNF probe is an optical probe sensitive to local environmental changes, as the C=N stretching vibration of the cyano group can be used as an infrared marker of the local environment due to its sensitivity to hydrogen bonding and local electric field. We inserted the pCNF by replacing two conserved tryptophans, Trp-99 and Trp-201, which are close to the chromophore and involved in the retinal isomerization and chloride pathway. In this way, we try to elucidate the environmental changes around the retinal.

This was done using spectroscopic techniques such as time-resolved UV-Vis spectroscopy and steady-state FTIR.

In addition, using ultrafast UV-Vis spectroscopy, we are trying to elucidate the putative role of the two tryptophans of NmHR in the very early phase of the photocycle.