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Crystal structure of the natural anion-conducting channelrhodopsin *GtACR1*

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Supplementary Discussion

Water molecules around the Schiff base region

In the *Gt*ACR1 structure, several $2F_o - F_c$ and $F_o - F_c$ electron densities of putative water molecules were detected near the Schiff base. Since the densities are not identical among chains A-D and the resolution of present structure is moderate (2.9 Å), we modeled just 4 water molecules in the final structure. 2 water molecules coordinated by Asp-234 and Tyr-72, were detected in chains B and C. Another 2 water molecules coordinated by Arg-94, were detected in chains A and B (EDFig. 2c). Their electron densities and B-factors are very reasonable for well-ordered water molecules (B-factors: 54.87, 52.64, 50.28, and 59.19, respectively). However, there remain unassigned densities in chains A and B (EDFig. 2c). Especially in chain B, there is relatively strong positive $F_o - F_c$ electron density between the Schiff base nitrogen (Asp-234) and Trp-98, and it is possible that weakly coordinated water or hydroxyl ion binds near the Schiff base. Further studies, including the determination of higher-resolution structure and additional spectroscopic analysis, will be needed to more fully reveal water distribution in the Schiff base region.