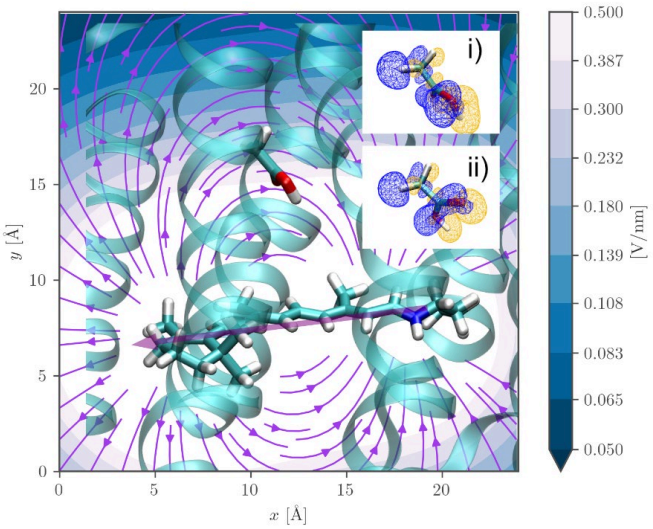
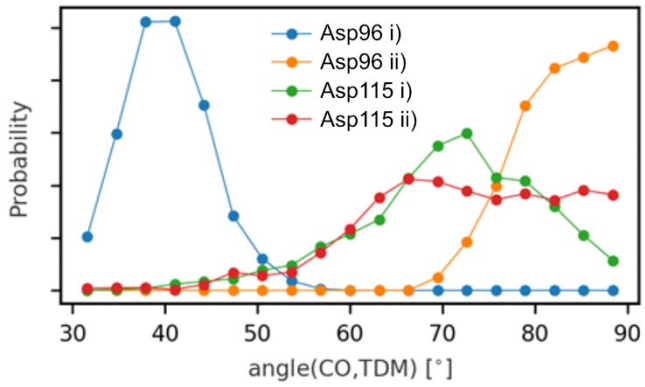
Extended Data Figure 8: Simulated IR spectra of Asp115

The same procedure as explained in EDF 7 for Asp96 was performed for Asp115. Asp115 is located closer to the retinal between SB and ß-ionone ring. The electric field change is directed roughly perpendicular to the carboxylic acid plane or close to 70° to 90° (see Figure). This is in contrast to Asp96, where the electric field lines are mainly parallel to the carboxylic acid plane. Thus, we expect a much smaller polarizability of the carbonyl and OH stretching vibration upon ultrafast electric field change. Upon electric field change conformation Asp115 i) and Asp115 ii) show an ED change with increase ED (blue mesh) towards the protein backbone. Both conformations of Asp115 show broader distributions of C=O orientations in the electronic ground state compared to Asp96, as shown in the graph below. Here, the probability of the relative angle between the carbonyl CO group and retinal's electronic tdm is plotted for Asp115 and Asp96 conformations. The conformations of the carboxylic acid of Asp96 are clearly better localized in the ground state compared to the carboxylic acid of Asp115. We assume that a broad distribution of orientations leads to a poorly defined polarization dependence. Since we observe a well-defined polarization dependence at the bleaching band around 1740 cm^{-1} in H_2O and around 1724 cm^{-1} in D_2O, our experimental data support assignment to Asp96.





Simulated difference IR spectra of conformation Asp115 i) (left panel) and Asp115 ii) (right panel) in the ground state without (negative signals) and with electric field change (positive signals). Contributions parallel (blue lines) and perpendicular (orange lines) to retinal's tdm are plotted. Simulations show negligible negative bands around 1700 cm^{-1} for both conformations, in contrast to our observed experimental spectra (Fig. 5).