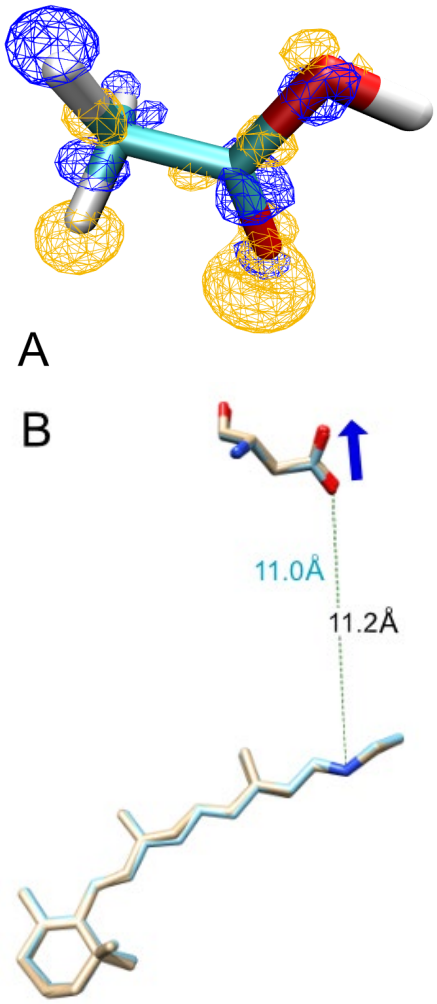
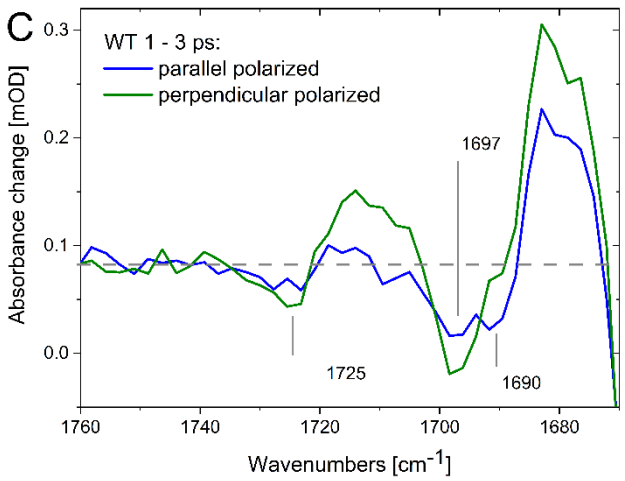
Extended Data Figure 9: Impact of electric-field jump on the protein



The crystal structure (6ga7.pdb) provide the oxygen positions of Asp96, but not of the hydrogen.[23] Our experimental data and simulations support protonation of the upper oxygen in Asp96 closer to the cytoplasmic side (Asp96.O1). Our simulations show an ED decrease at the lower oxygen (Asp96.O2) in the ES due to the influence of the electric field change, and an ED increase at the carbon (see A). This is in line with a Coulomb force moving the carboxylic group of Asp96 towards the cytoplasmatic side (B, blue arrow). Indications for such a tiny structural change are given by comparing the refined structure of *Hs*BR in the ground state (6ga7.pdb) with the refined structure at 240 fs (6ga1.pdb).[23] The distance between the lower oxygen Asp96.O2 and the nitrogen of the SB Lys216.N7 increases from 11.04 Å in the ground state to 11.22 Å in the ES (see B).

IR spectroscopy is very sensitive to changes of hydrogen-bonded carboxylic groups. Figure 5c, and Figure C show polarization resolved IR absorbance difference data in the region of the C=O stretching vibrations in H_2O in the ES, and in D_2O for the product state, respectively. At 1740 cm^{-1} in Figure 5c and at 1725 cm^{-1} in C, the negative signals for perpendicular polarization are stronger than for parallel polarization. This indicates a relative angle between the electronic transition dipole moment (tdm) of the retinal and the vibrational transition dipole moment (vtdm) of the carbonyl group in the range of 56° and 90°. The vtdm of a carbonyl vibration is roughly parallel to the C=O orientation. This angle is consistent with the Asp96 conformation Asp96 ii) depicted in A and Figure 3b, but still compatible with the conformation Asp96 ii) (Figure 3b). In C it is also clearly visible that positive signals for perpendicular polarization (green line) around 1717 and 1704 cm^{-1} are stronger compared to parallel polarized signals (blue line) demonstrating relative angles in the range of 56° and 90° between retinal's tdm and carbonyl's vtdm in the product state.



We interpret this IR signal alterations as Asp96 side chain movement via an electric-field jump in the ES that is stabilized by adjacent hydrogen-bond partner, i.e. Thr46, and persists in the K intermediate.[64] The electric-field jump occurs at the retinal and affects protein side chains 11 Å away. This supports our calculations showing negligible electric field screening on a time-scale of several picoseconds. The electric-field change decays with the ES on a time-scale of 0.5 ps. However, we observe long-lasting changes after the decay of the electric-field change. Hence, we interpret these observations by electric-field jump induced persistent modifications in the protein structure.