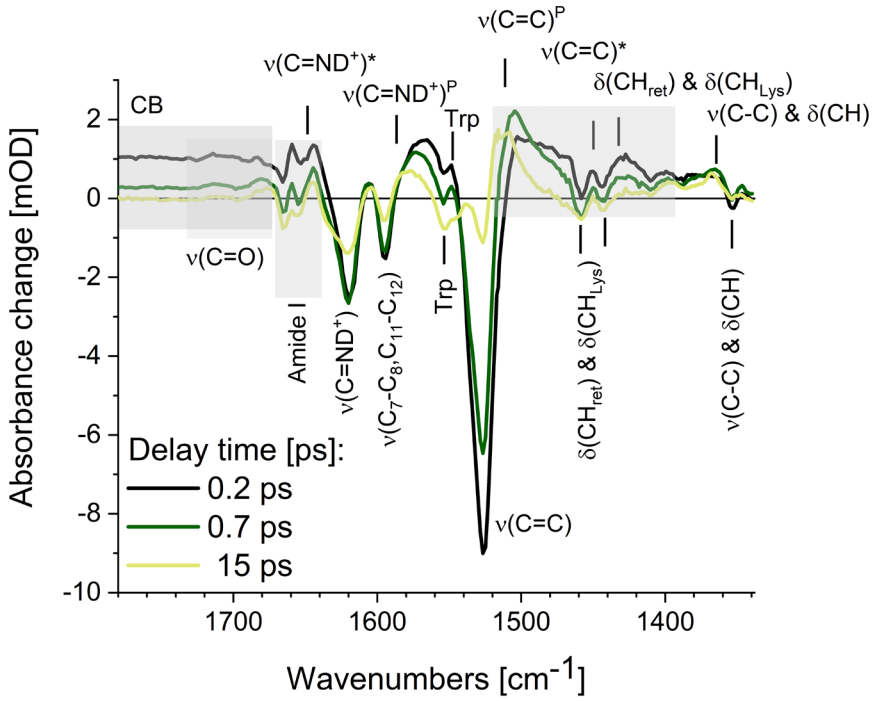
Extended Data Figure 3: Assignment of vibrations

Using QM/MM vibrations for *all-trans* (ground state) and *13-cis* retinal (K intermediate) were calculated in protein surrounding (see supporting information). With a scaling factor of 0.955 we found the ν(C=NH$^+$) at 1641 and 1607 cm^{-1} and the ν(C=C) at 1525 and 1518 cm^{-1} for GS, and K, respectively. This matches well with the observed peaks in H_2O (see EDF 6), and D_2O, where the ν(C=ND$^+$) vibration is red-shifted by 20 cm^{-1} (Figure below). These are the two strongest retinal vibrations. A weaker ν(C_7-C_8, C_{11}-C_{12}) retinal vibration is found at 1599 and 1586 cm^{-1} for GS, and K, respectively. From our calculations we found two adjacent CH bending vibrations of lysine and retinal at 1441 and 1429 cm^{-1} and 1425 and 1416 cm^{-1} for GS and K, respectively. Another retinal ν(C-C)&δ(CH) vibration was found at 1343 and 1348 cm^{-1} for GS and K, respectively. We assigned these vibrations in the figure below (absolute accuracy 4 cm^{-1}).

Protein contributions are observed in the ES prior isomerization. We observe a broad continuum band (CB) above 1700 cm^{-1} that extends up to the OH/OD stretching vibration. Moreover, amide band pairs at 1676(+)/1664(-) cm^{-1} and at 1654(-)/1643(+) cm^{-1}, as well as Trp vibrations at 1554(-)/1540(+) cm^{-1} are observed from protein groups. Additionally, contributions from carboxylic acid side chains are traced around 1700 cm^{-1} (see Fig. 5). The CB decays with or faster than the ES, while the other protein contributions persist in the K intermediate. Our assignment is in line with previous investigations.[16,44-48]



Upon photoexcitation the shift of the negative charge along the retinal backbone reduces the C=C double bond character in the ES, resulting in a red-shifted ν(C=C)* stretching vibration.[16] The ν(C=C)* appears instantaneously in Fig. 4, strongly red-shifted, from ~ 1515 cm^{-1} to ~ 1460 cm^{-1} at 0.2 ps. With the decay of the ES and formation of 13-cis retinal photoproduct J the ν(C=C) band narrows and blue-shifts significantly. Relaxation from J to K intermediate with 3 ps is followed by a further narrowing and blue-shift of the ν(C=C) stretching vibration absorption band. The ν(C=ND$^+$) vibration is found around 1578 cm^{-1} in the J and K intermediate, also narrowing and blue-shifting from J→K transition.