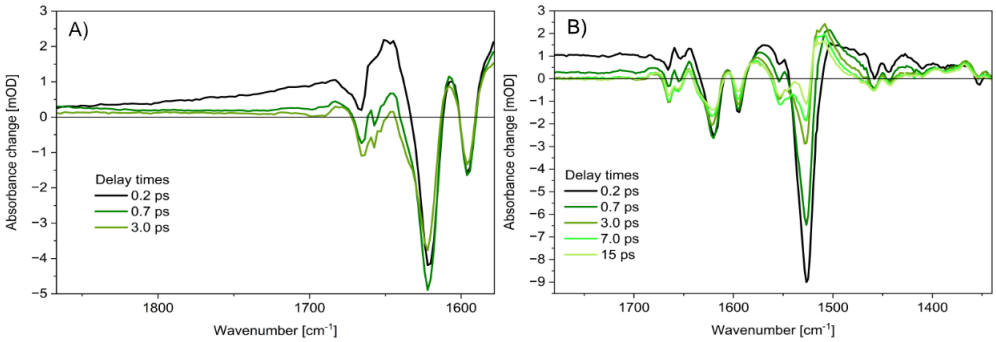
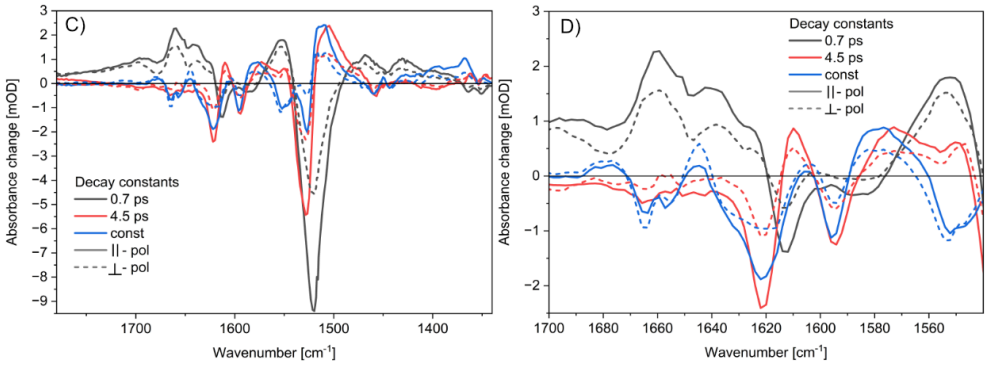
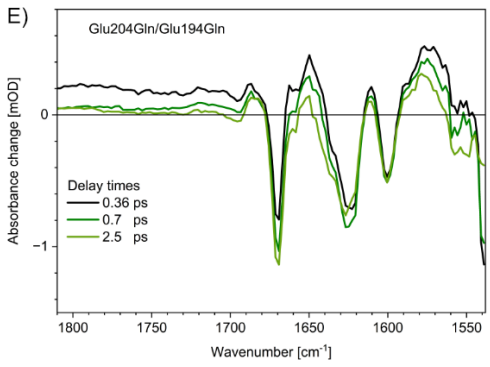
Extended Data Figure 4: Continuum band measurements

All presented datasets show *Hs*BR dynamics in D_2O solution. In A) absorbance difference spectra from 1867 cm^{-1} to 1580 cm^{-1} (data for transient in Fig. 4 inset) are shown for parallel polarization, i.e. pump and probe beams are parallel polarized. The CB is clearly visible as a strong, broad, and mainly structureless band from 1850 cm^{-1} to at least 1700 cm^{-1}. The positive CB signal is strongest at early delay times (0.2 ps black line) and decays to zero on a sub-picosecond time-scale. We cannot determine how much influence the CB has in the spectral range below 1700 cm^{-1}, because of other overlapping positive contributions from C=ND+ and C=C stretching vibrations in the excited state (ES). In B) absorbance difference spectra for isotropic polarization (A_{iso}) as in Fig. 4 are shown.



Decay associated spectra from a global fit of the dataset in B) are presented in C), and D) for parallel ($A_{\parallel}$, solid lines) and perpendicular ($A_{\perp}$, dashed lines) polarization. Global fitting gives time constants of 0.7 (1) ps and 4.5 (3) ps. From the dichroic ratio $D=A_{\parallel}/A_{\perp}$ one can calculate the relative angle between electronic transition dipole moment and vibrational transition dipole moment. D ranges from 3 to 0.5 corresponding to angles from 0° to 90°.[65] Polarization resolved data around 1640 cm^{-1} indicate a blue-shifted $\nu(C=ND^{+})^*$ vibration in the ES, probably due to increased electron density at the SB, and a red-shifted absorption in the J and K intermediate. Upon photoexcitation a band pair is visible at 1554(-) / 1540(+) cm^{-1} assigned to Trp vibrations that is persistent in J and K photoproduct.[23,37]





In E) isotropic absorption difference spectra of the Glu204Gln/Glu194Gln variant of *Hs*BR are presented for delay times 0.36 ps (0.32 – 0.4 ps), 0.7 ps (0.68 – 0.72 ps), and 2.5 ps (2.3 -2.8 ps). The CB around 1780 cm^{-1} is clearly visible and decays to nearly zero within a picosecond. Signal strengths are similar to *Hs*BR WT, except for the amide I region around 1665 cm^{-1} where the signals are stronger in the variant.[45]