Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information

The photochemistry of sodium ion pump rhodopsin observed by watermarked femto- to submillisecond stimulated Raman spectroscopy

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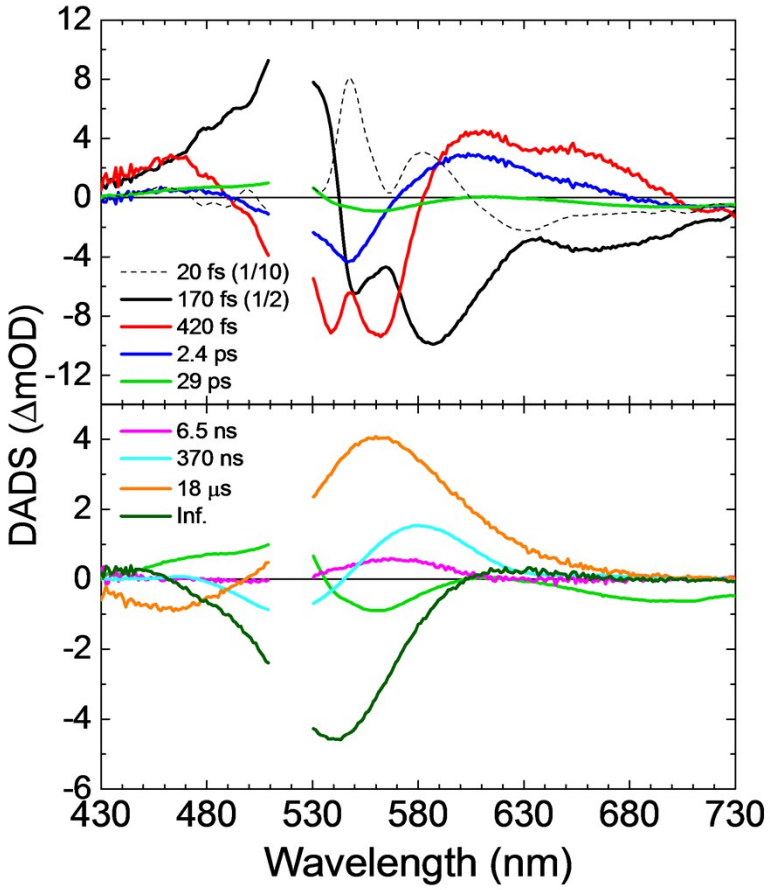

Figure S1. DADS of transient absorption spectra of WT KR2 in NaCl. DADS of WT KR2 in NaCl. The first five (top) and the following four (bottom) time components are shown separately. Spectra at 510–530 nm on the transient absorption spectra are cut because of the strong pump light scattering. The components of 20 fs and 170 fs are scaled down by a factor of 10 and 2, respectively. The 29-ps component is shown on both of the top and the bottom figures for reference.

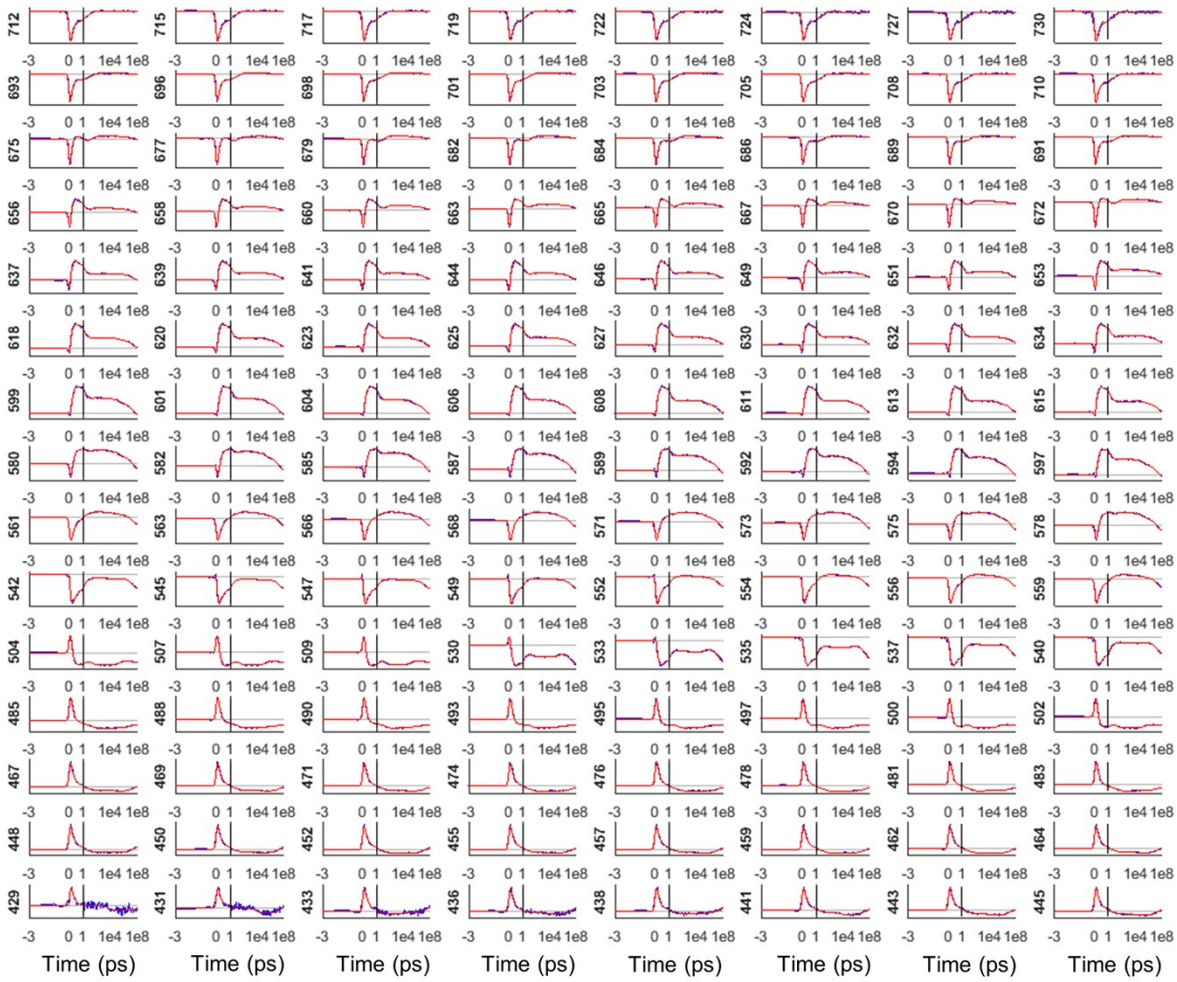

Figure S2. Selected time traces of transient absorption spectra of WT KR2 in NaCl. Raw data (blue line) and globally fitted time traces (red line) in ΔOD (normalized) are shown. The values written in bold on the vertical axis show wavelengths in nm. The time axis is linear until 1 ps, and logarithmic thereafter. The horizontal gray line shows ΔOD = 0.

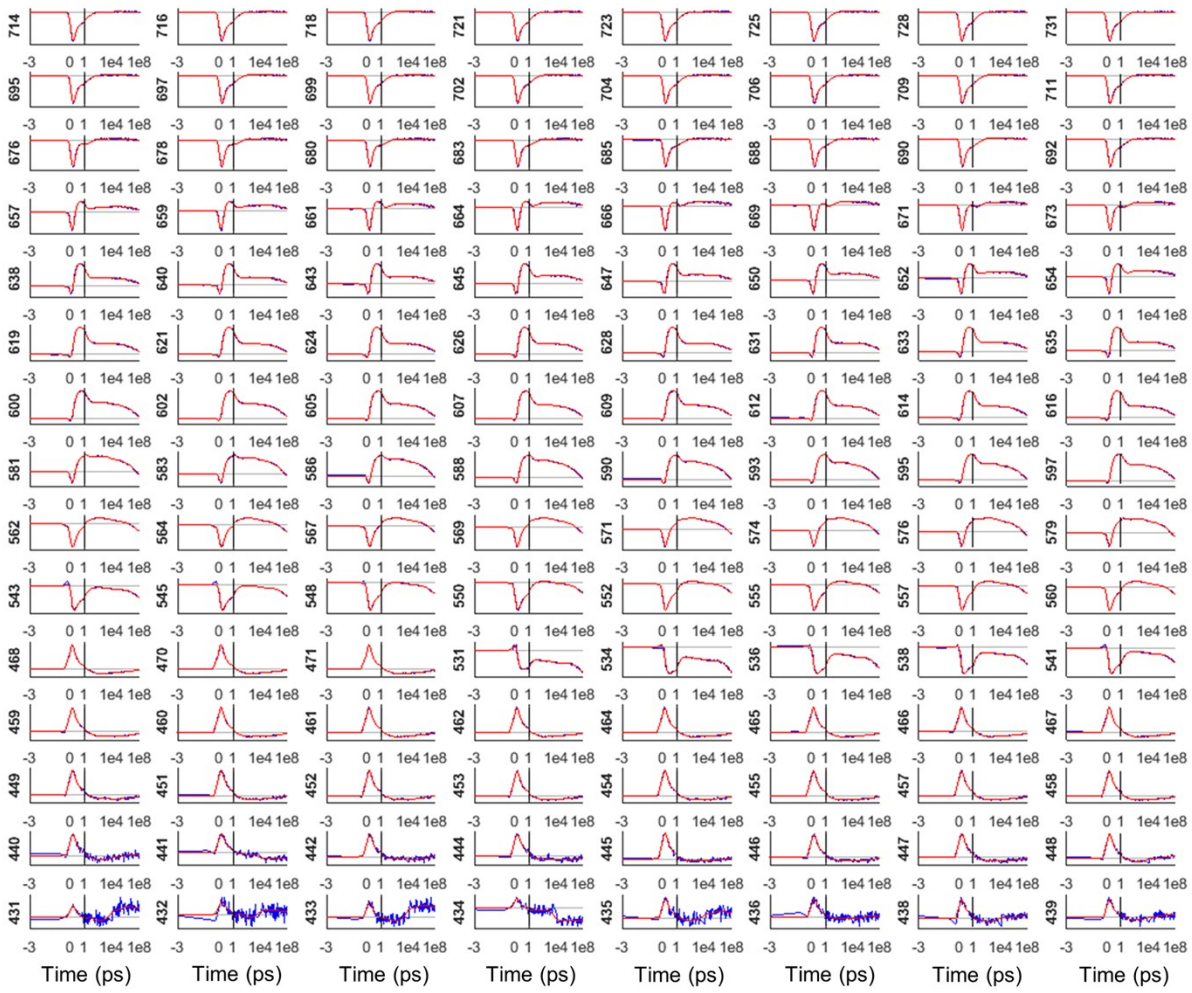

Figure S3. Selected time traces of transient absorption spectra of KR2/D102N in NaCl. Raw data (blue line) and globally fitted time traces (red line) in ΔOD (normalized) are shown. The values written in bold on the vertical axis show wavelengths in nm. The time axis is linear until 1 ps, and logarithmic thereafter. The horizontal gray line shows ΔOD = 0.

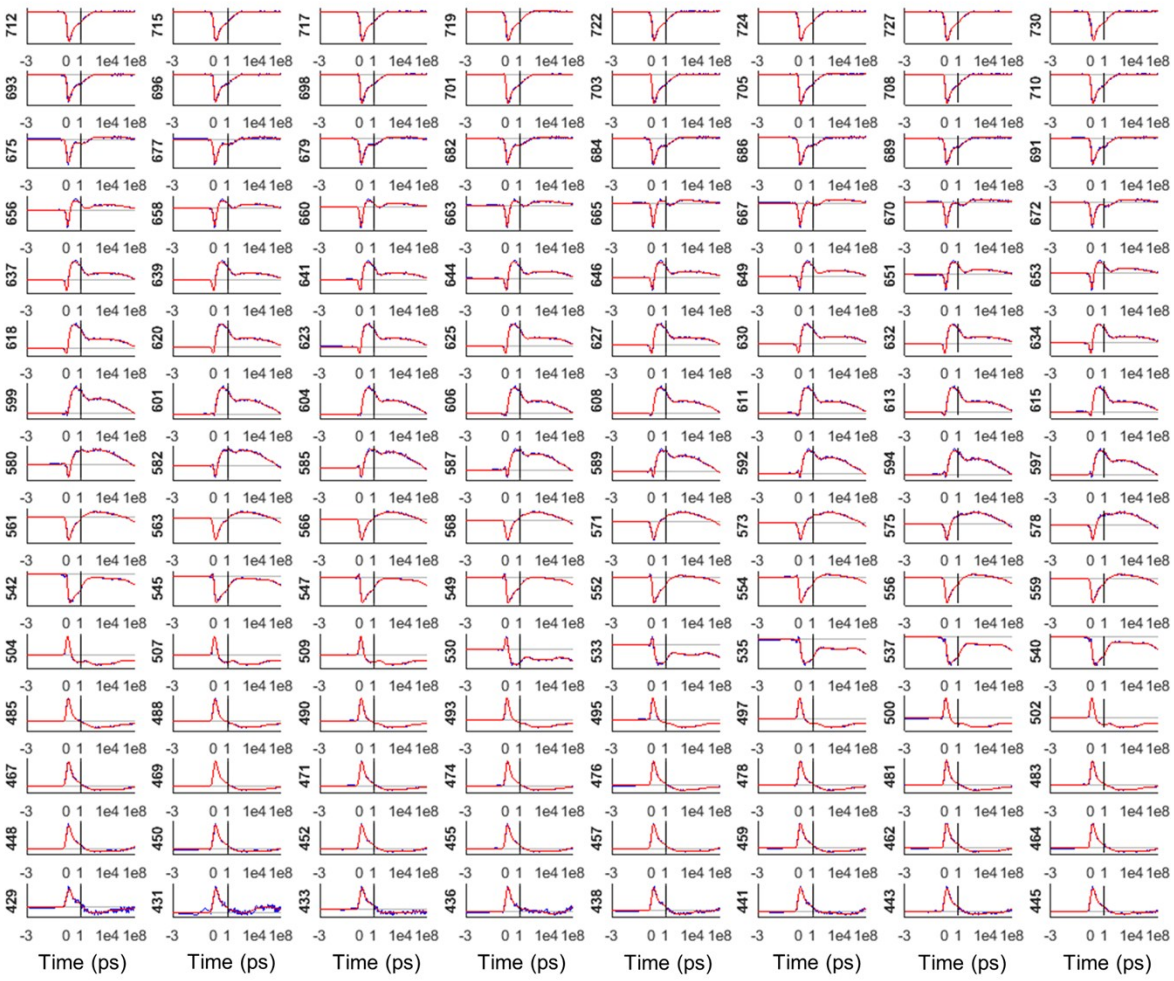

Figure S4. Selected time traces of transient absorption spectra of WT KR2 in CsCl. Raw data (blue line) and globally fitted time traces (red line) in ΔOD (normalized) are shown. The values written in bold on the vertical axis show wavelengths in nm. The time axis is linear until 1 ps, and logarithmic thereafter. The horizontal gray line shows ΔOD = 0.

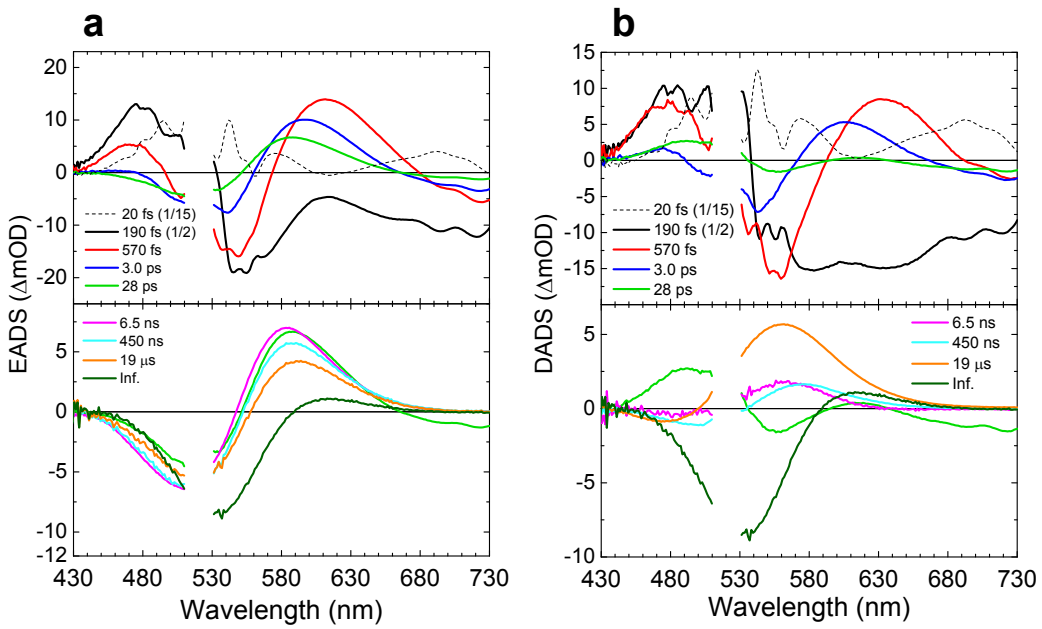

Figure S5. Globally fitted transient absorption spectra of KR2/D102N in NaCl at 430–730 nm. (**a**) EADS and (**b**) DADS of transient absorption KR2/D102N in NaCl. The first five (top) and the following four (bottom) time components are shown separately. Spectra at 510–530 nm are cut because of the strong pump light scattering. The first and the second components of are scaled down. The 28-ps component is shown on both of the top and the bottom figures for reference.

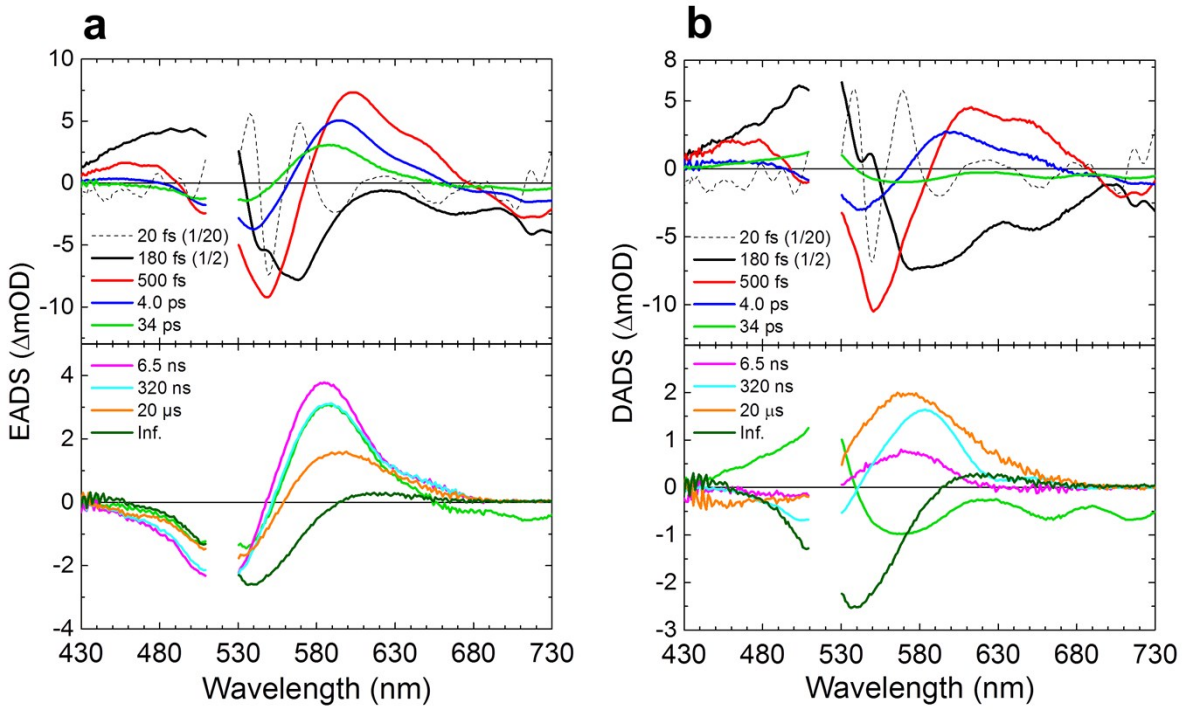

Figure S6. Globally fitted transient absorption spectra of WT KR2 in CsCl at 430–730 nm. (**a**) EADS and (**b**) DADS of transient absorption WT KR2 in CsCl. The first five (top) and the following four (bottom) time components are shown separately. Spectra at 510–530 nm are cut because of the strong pump light scattering. The first and the second components of are scaled down. The 34-ps component is shown on both of the top and the bottom figures for reference.

Table S1. Time constants of global fitting on transient absorption spectra in WT KR2 in NaCl, KR2/D102N in NaCl and WT KR2 in CsCl.

Transient absorption	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7	τ_8	τ_9
WT KR2 in NaCl	20 fs	170 fs	420 fs	2.4 ps	29 ps	6.5 ns	370 ns	18 μs	inf.
KR2/D102N in NaCl	20 fs	190 fs	570 fs	3.0 ps	28 ps	6.5 ns	450 ns	19 μs	inf.
WT KR2 in CsCl	20 fs	180 fs	500 fs	4.0 ps	34 ps	6.5 ns	320 ns	20 μs	inf.

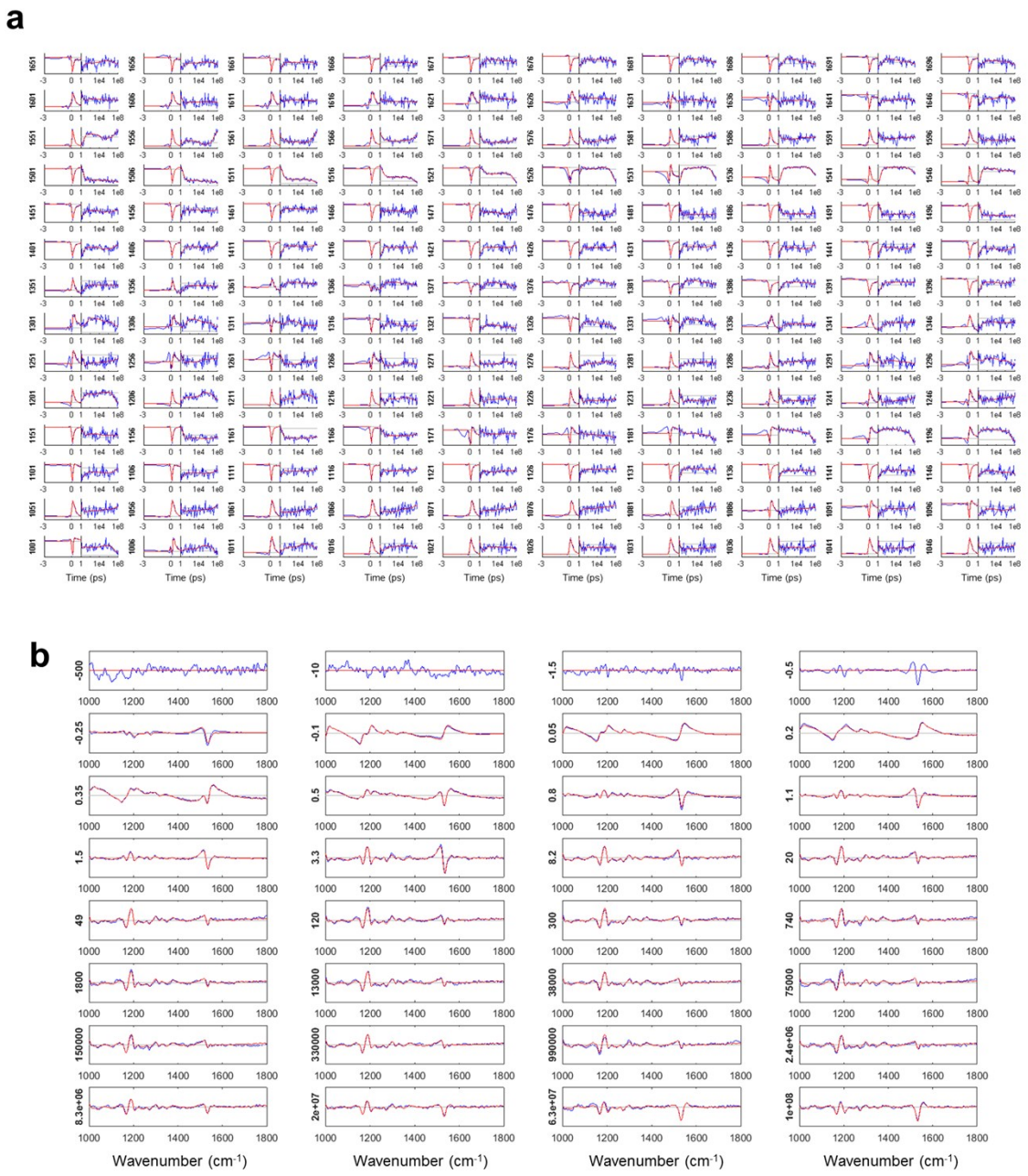

Figure S7. Selected time traces and transient spectra of transient stimulated Raman experiments in WT KR2 in NaCl. a, Selected time traces with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show wavenumbers in cm^{-1}. The time axis is linear until 1 ps, and logarithmic thereafter. Note that the vertical axis is normalized differently before and after 1 ps. The horizontal gray line shows ΔOD = 0. **b,** Selected transient stimulated Raman spectra with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show time delays in ps. The horizontal gray line shows ΔOD = 0.

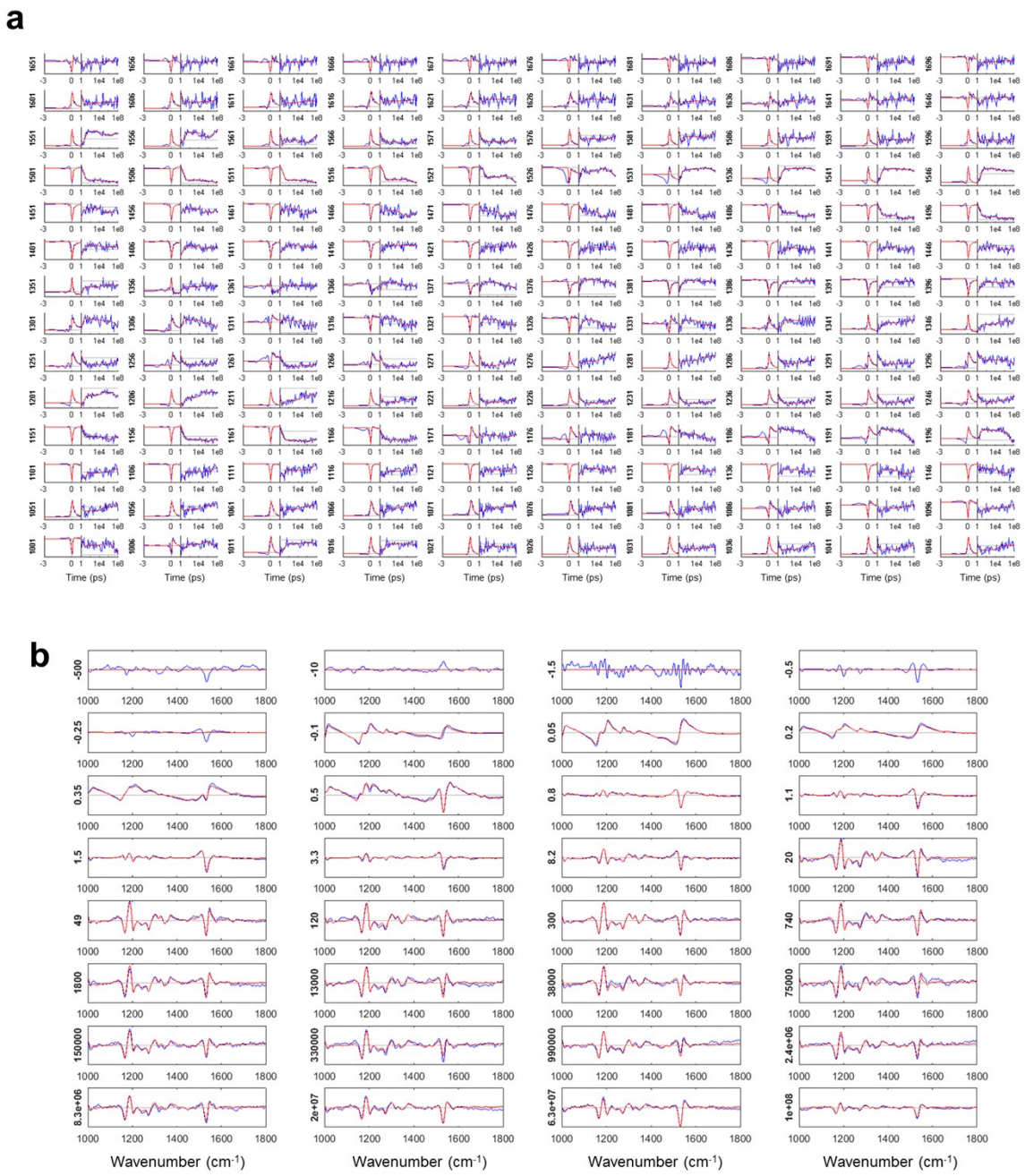

Figure S8. Selected time traces and transient spectra of transient stimulated Raman experiments in KR2/D102N in NaCl. a, Selected time traces with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show wavenumbers in cm^{-1}. The time axis is linear until 1 ps, and logarithmic thereafter. Note that the vertical axis is normalized differently before and after 1 ps. The horizontal gray line shows ΔOD = 0. **b,** Selected transient stimulated Raman spectra with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show time delays in ps. The horizontal gray line shows ΔOD = 0.

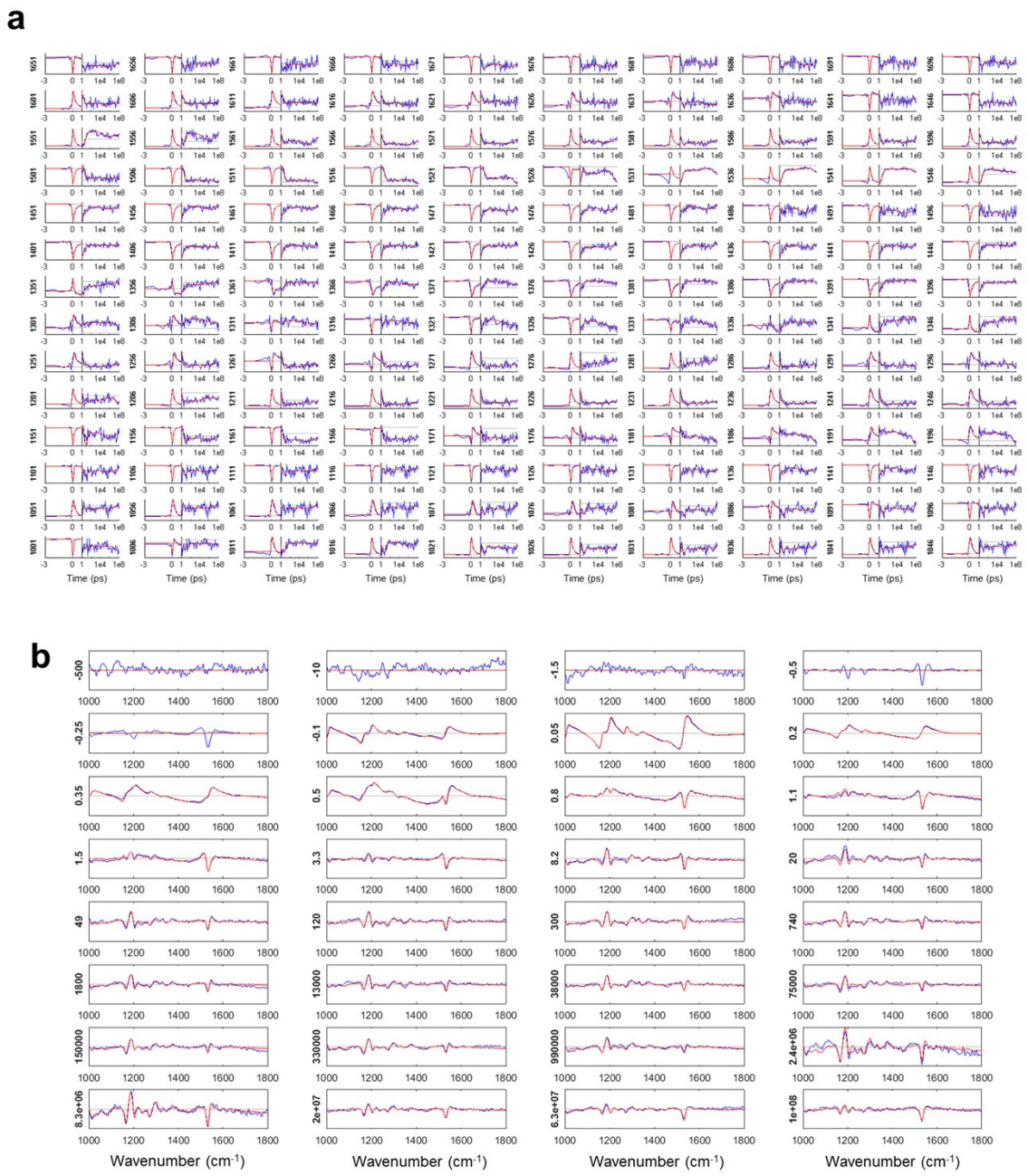

Figure S9. Selected time traces and transient spectra of transient stimulated Raman experiments in WT KR2 in CsCl. a, Selected time traces with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show wavenumbers in cm^{-1}. The time axis is linear until 1 ps, and logarithmic thereafter. Note that the vertical axis is normalized differently before and after 1 ps. The horizontal gray line shows ΔOD = 0. **b,** Selected transient stimulated Raman spectra with raw data (blue line) and globally fitted data (red line) in ΔOD (normalized). The values written in bold on the vertical axis show time delays in ps. The horizontal gray line shows ΔOD = 0.

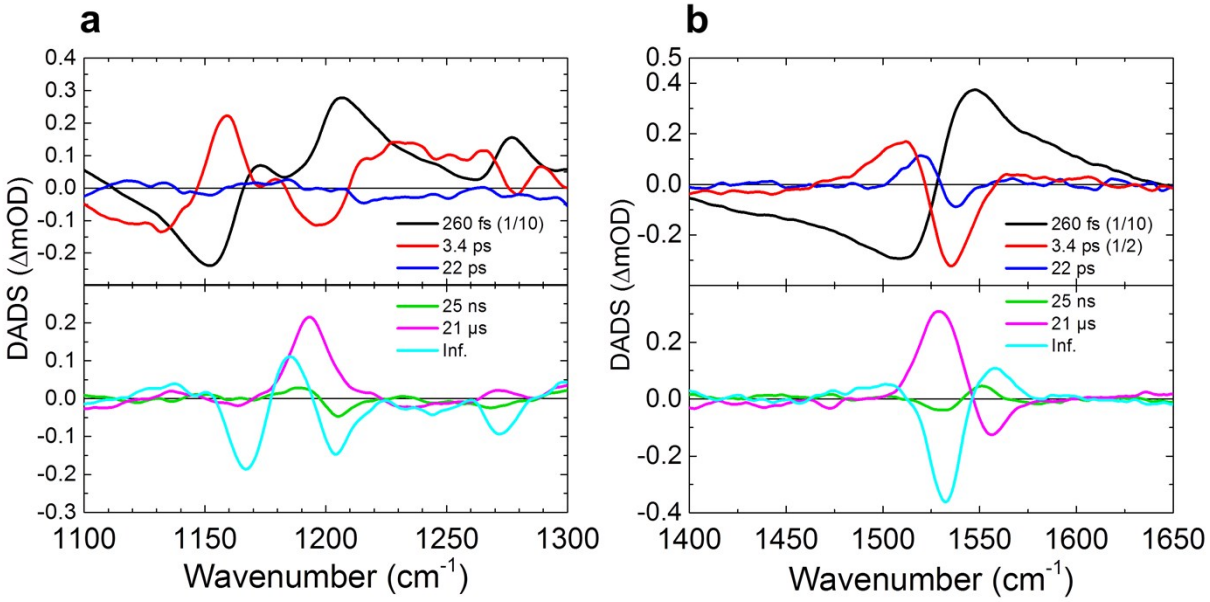

Figure S10. DADS of transient stimulated Raman spectra of WT KR2 in NaCl at selected wavelengths. The signal evolves as 260 fs (black), 3.4 ps (red), 22 ps (blue), 25 ns (green), 21 μs (magenta) and an infinite time constant (cyan) in order. The 260-fs and 3.4-ps components are scaled down. The first three (top) and the following three (bottom) time components are shown separately.

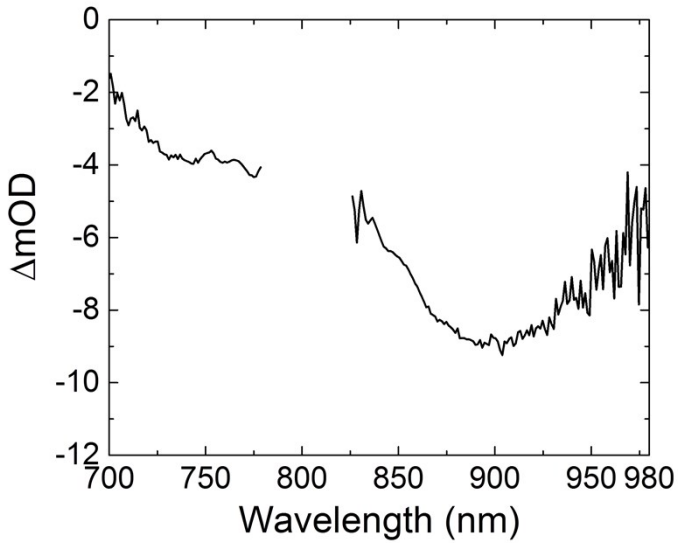

Fig. S11 Transient absorption spectra of WT KR2 in NaCl with a 150-fs time delay in the near-infrared spectral region. The signal at 780–825 nm is cut.

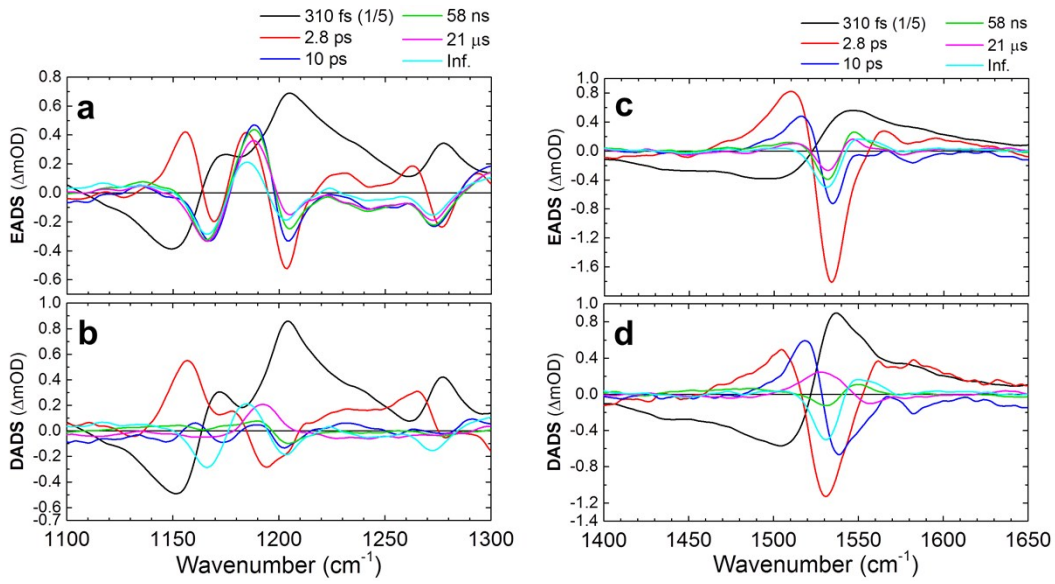

Figure S12. Globally fitted transient stimulated Raman spectra of KR2/D102N in NaCl. (**a**) EADS and (**b**) DADS at 1100–1300 cm^{-1}. (**c**) EADS and (**d**) DADS in 1400–1650 cm^{-1}. The first component (decaying with 20 fs) is not shown on the figures. The 310-fs component is scaled down by a factor of 5.

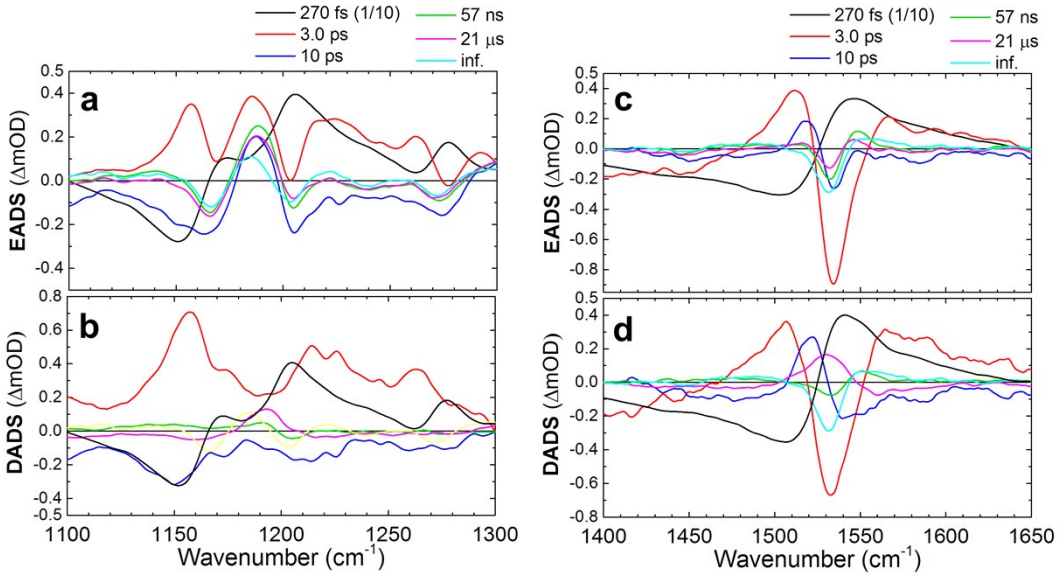

Figure S13. Globally fitted transient stimulated Raman spectra of WT KR2 in CsCl. (**a**) EADS and (**b**) DADS in 1100–1300 cm^{-1}. (**c**) EADS and (**d**) DADS in 1400–1650 cm^{-1}. The first component (decaying with 10 fs) is not shown on the figures. The 270-fs component is scaled down by a factor of 10.

Table S2. Time constants of global fitting on transient stimulated Raman spectra in WT KR2 in NaCl, KR2/D102N in NaCl and WT KR2 in CsCl.

Transient Raman	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7
WT KR2 in NaCl	20 fs	260 fs	3.4 ps	22 ps	25 ns	21 μs	inf.
KR2/D102N in NaCl	20 fs	310 fs	2.8 ps	10 ps	58 ns	21 μs	inf.
WT KR2 in CsCl	10 fs	270 fs	3.0 ps	10 ps	57 ns	21 μs	inf.

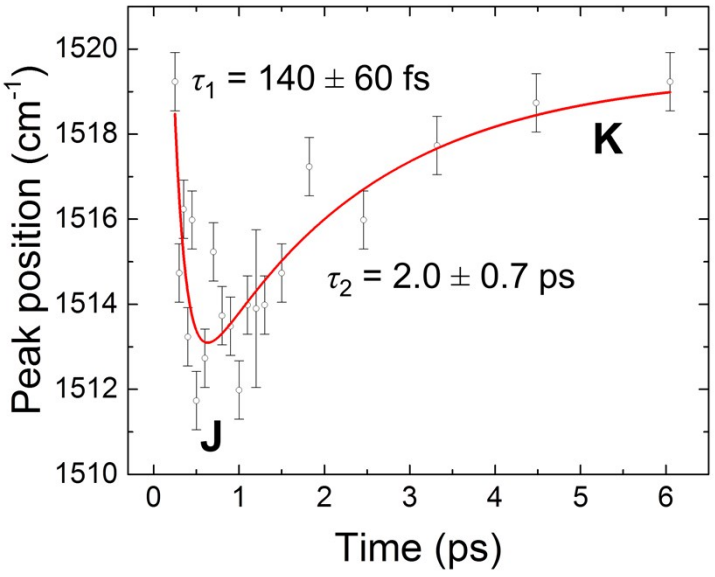

Figure S14. Time trace of Raman peak position at 1510–1521 cm^{-1} up to 6 ps. Time trace of the Raman peak position in the 1510–1521cm^{-1} spectral region up to 6 ps is plotted. The plots were fitted (red line) with bi-exponential components; 140 ± 60 fs and 2.0 ± 0.7 ps.

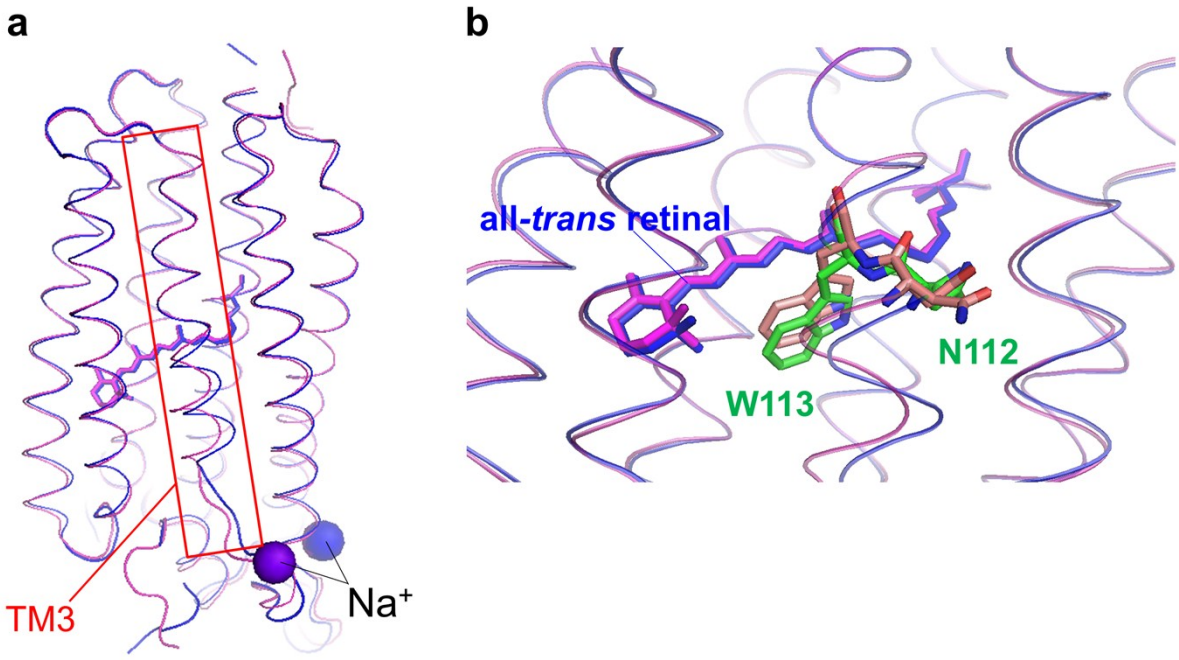

Figure S15. Structural difference with/without Na^+ binding to Asp102[1] (PDB ID: 4XTL and 4XTN). **a,** Overlapping structures of the monomeric form (blue) at pH 4.3 and the pentameric form (magenta) at pH 4.9. **b,** Close-up crystal structures near all-*trans* retinal. Asn112, Trp113 (green in monomeric form and orange in pentameric form) and all-*trans* retinal are shown.

Reference

1. I. Gushchin, V. Shevchenko, V. Polovinkin, K. Kovalev, A. Alekseev, E. Round, V. Borshchevskiy, T. Balandin, A. Popov, T. Gensch, C. Fahlke, C. Bamann, D. Willbold, G. Buldt, E. Bamberg and V. Gordeliy, *Nat. Struct. Mol. Biol.*, 2015, **22**, 390-395.