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

Supporting Information

Local Dynamics of the Photo-Switchable Protein PYP in Ground and Signalling State Probed by 2D-IR Spectroscopy of -SCN Labels

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I.

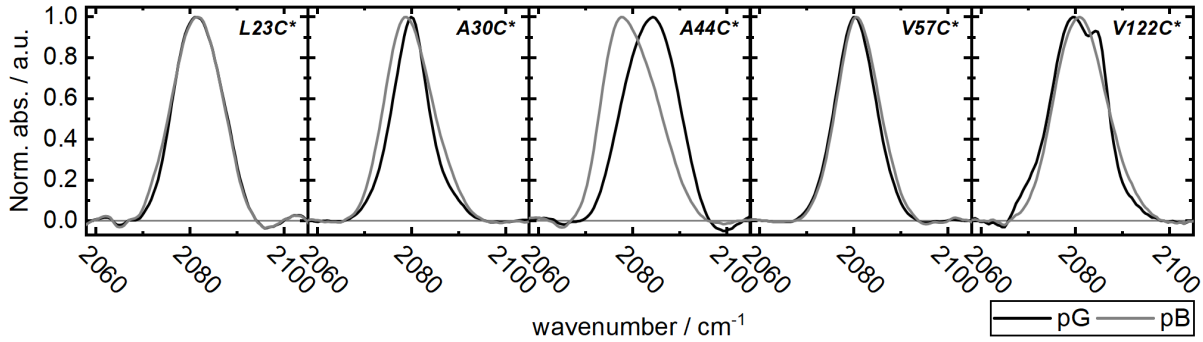

Fig. S1 Linear absorption spectra of -SCN in the studied mutants.[1] The pG state is shown as black and the pB state as grey line.

Tab. S1 Vibrational lifetimes T_1 for the probed SCN labels. These were obtained by time-resolved IR pump IR probe spectroscopy in deuterated buffer.[2]

Label / State		T_1 / ps
*L23C**	*pG*	55.5±1.2
	pB	56.0±1.5
*A30C**	*pG*	55.9±1.6
	pB	52.0±1.2
*A44C**	*pG*	54.1±0.9
	pB	48.0±2.9
*V57C**	*pG*	34.1±2.8
	pB	52.0±7.1
*V122C**	*pG*	50.2±3.6
	pB	50.1±2.4

II.

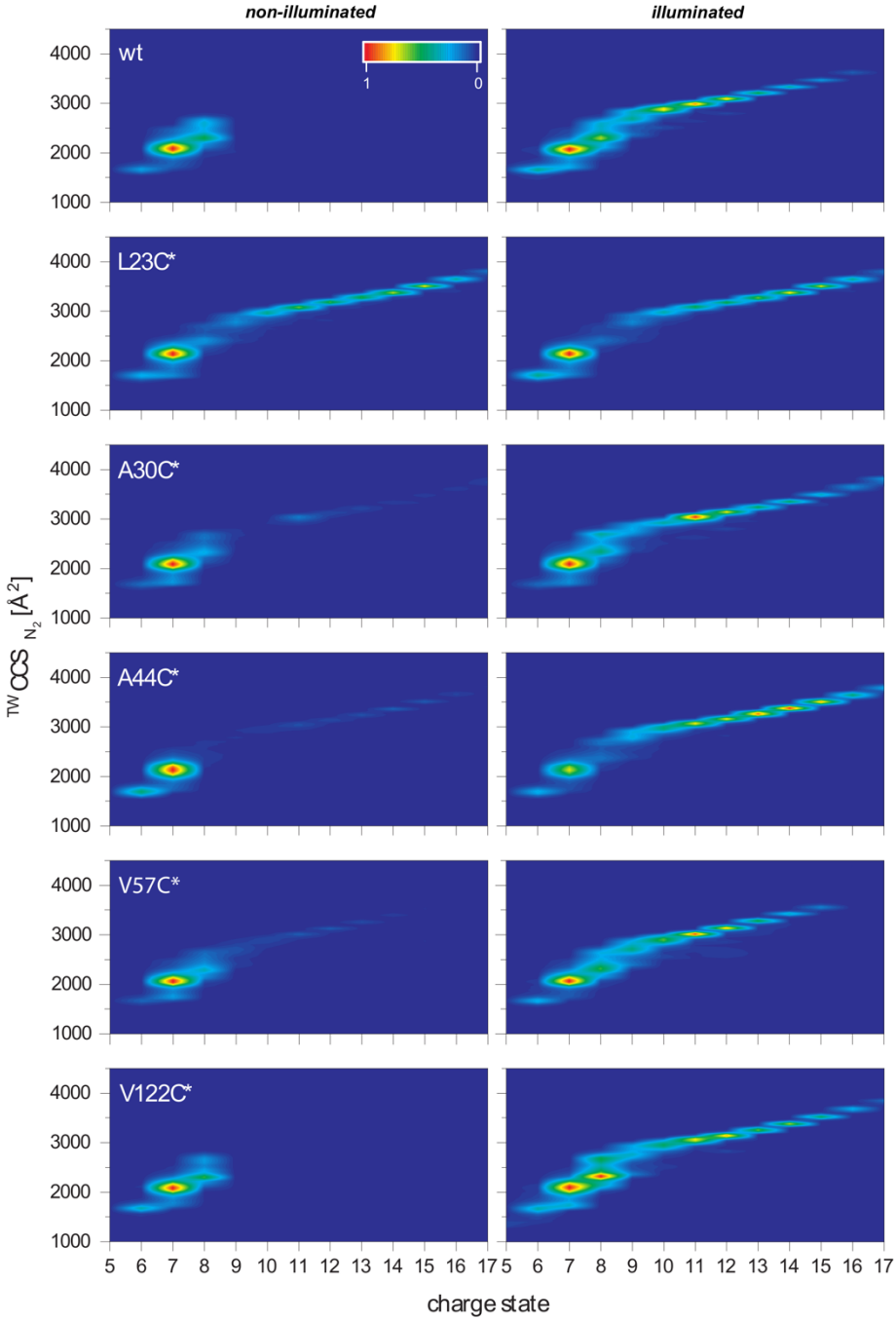

Fig. S2 Ion mobility data of PYP under non-illuminated and illuminated conditions. The extracted ion mobilograms of the non-irradiated PYP wildtype (wt) show high signal intensities at low charge states with collision cross sections about 2000 Å^2. Data acquisition of PYP-wt during nESI tip illumination results in a shift to higher charge states with an averaged collision cross section about 3000 Å^2. Remarkably, despite the observation of normal switching behaviour in FTIR spectroscopy,[1] the L23C* mutant shows negligible effects regarding sample illumination in the ion mobility experiment. Under both conditions a broad charge state distribution can be observed. The mutants A30C*, A44C*, V57C* and V122C* show a similar behaviour upon illumination as the wt.

References

1. L. Blankenburg, L. Schroeder, F. Habenstein, B. Błasiak, T. Kottke and J. Bredenbeck, *Phys. Chem. Chem. Phys.*, 2019, **21**, 6622–6634.
2. J. M. Schmidt-Engler, L. Blankenburg, B. Błasiak, L. J. G. W. van Wilderen, M. Cho and J. Bredenbeck, *Anal. Chem.*, 2020, **92**, 1024–1032.