Electronic supplementary information (ESI) for:

Interplay between the "flipping" glutamine, a conserved phenylalanine, water and hydrogen bonds within a blue-light sensing LOV domain

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Content:

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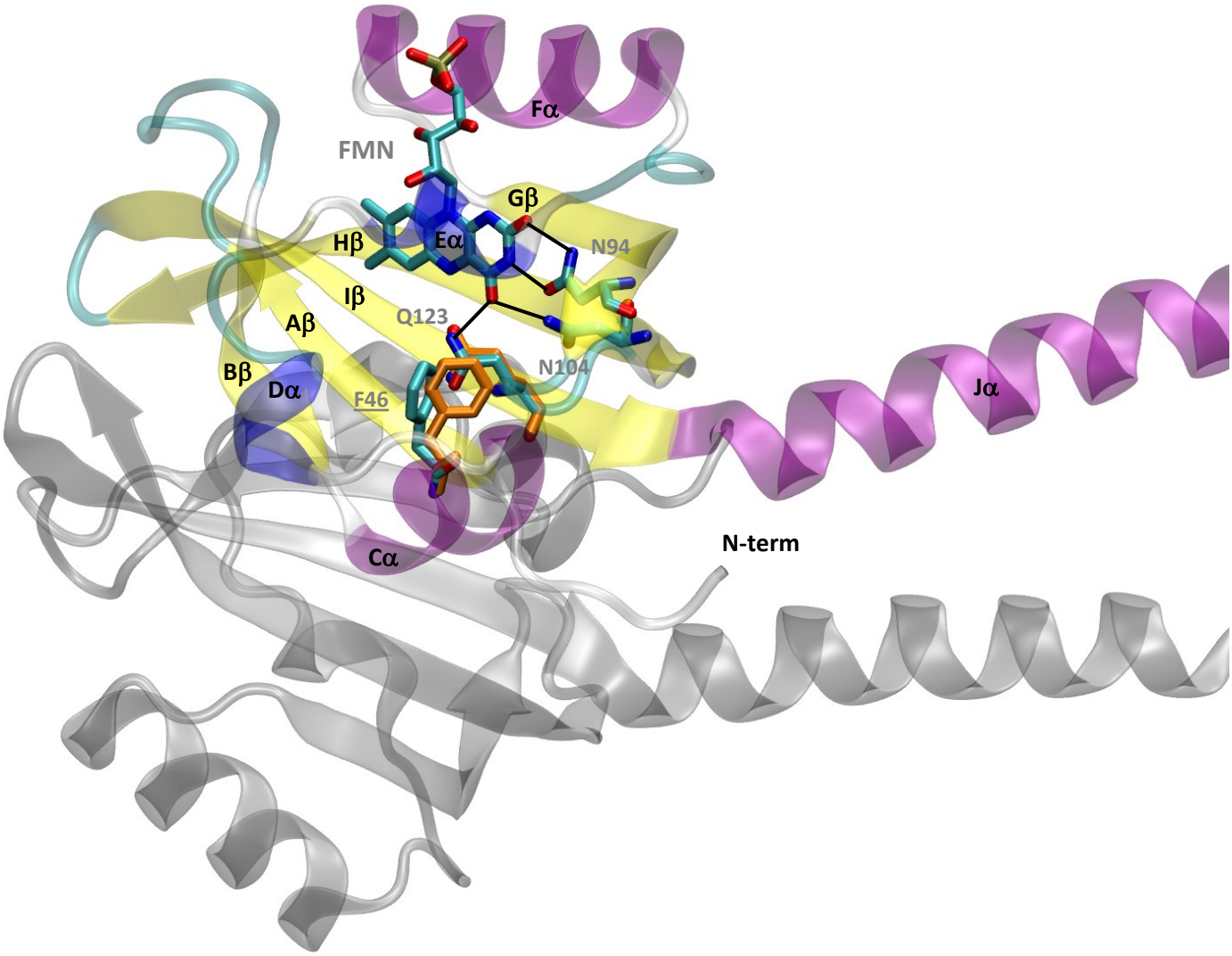

Figure S1. wt *Bs*YtvA-LOV crystal structure in the dimeric dark state (PDB id code 2pr5) showing the here mutated residue F46 (underlined) and the H-bonds network involving Q123, N94 and N104. The positions of Q123 and F46 in the light state are also shown (in orange). The A chain, used for MD simulations, is colored by secondary structure types. The conventional nomenclature of secondary structure elements is indicated (in black).

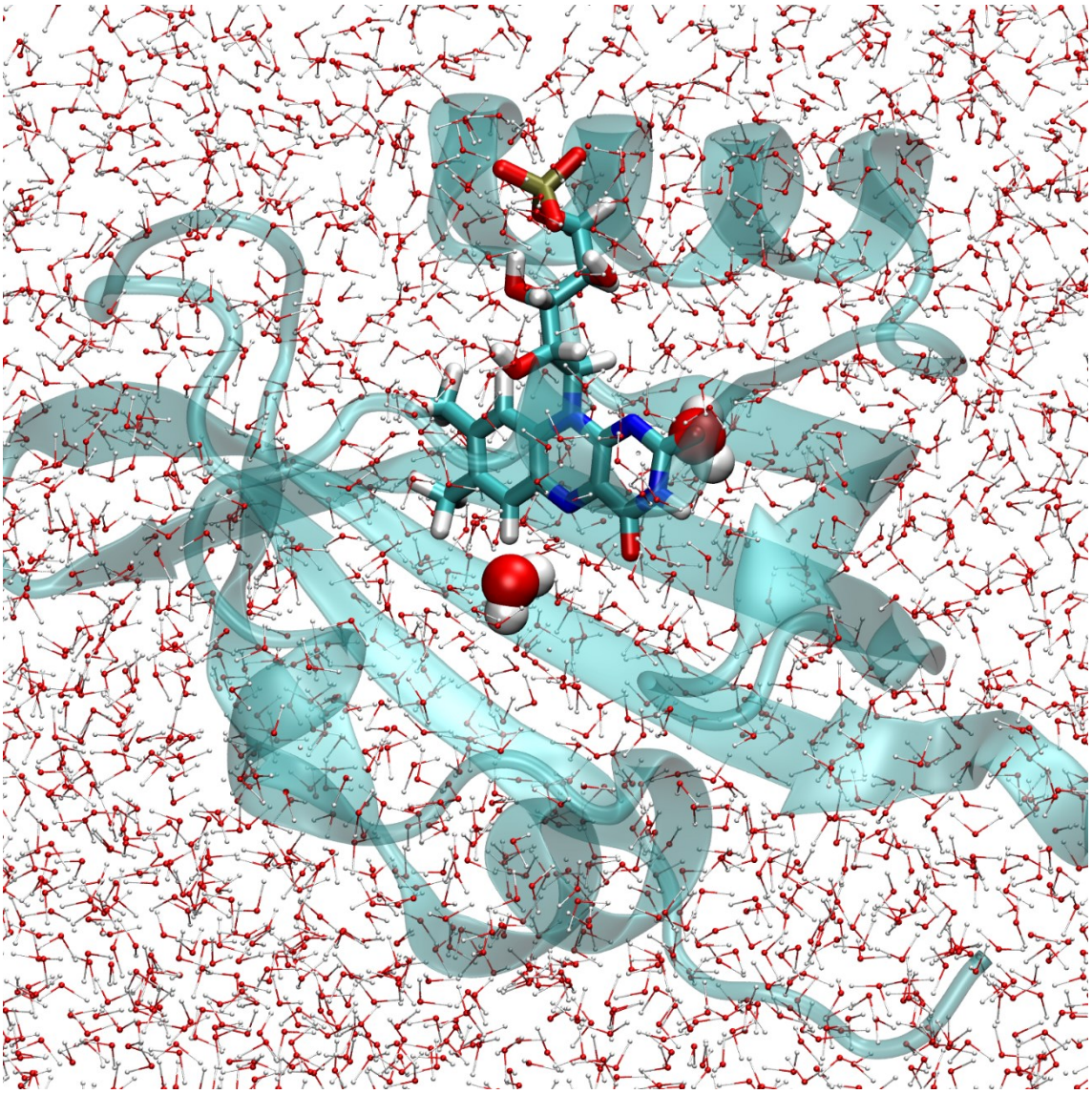

Figure S2. Example of starting structure for MD simulations with protein embedded in a box of water (in thin sticks). The only two molecules that are present in the binding site are represented in van der Waals spheres.

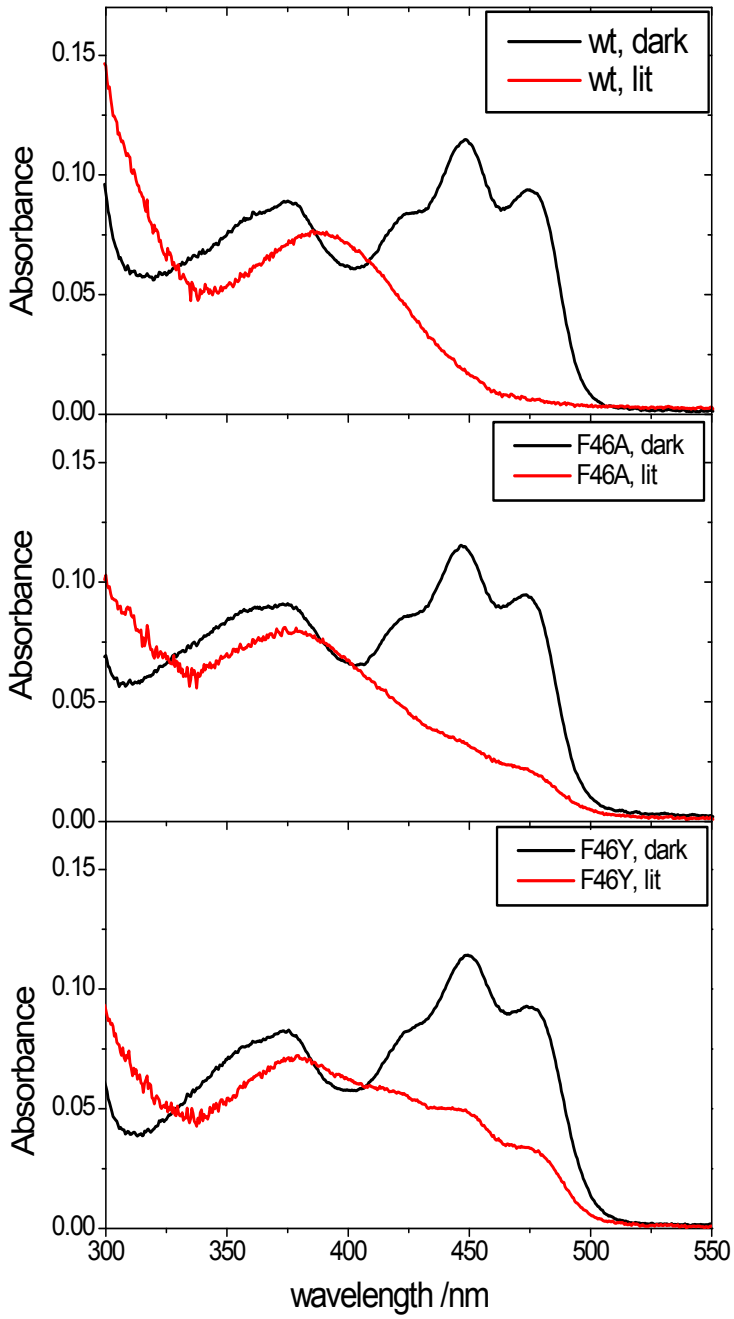

Figure S3. Absorption spectra of dark and lit states in BsYtvA and its F46 variants, in Na-phosphate buffer 10 mM, NaCl 10 mM, ph =8. For F46A and F46Y the fast recovery does not allow accumulation of the lit state. Note the blue shift of the absortion maximum of the lit state.

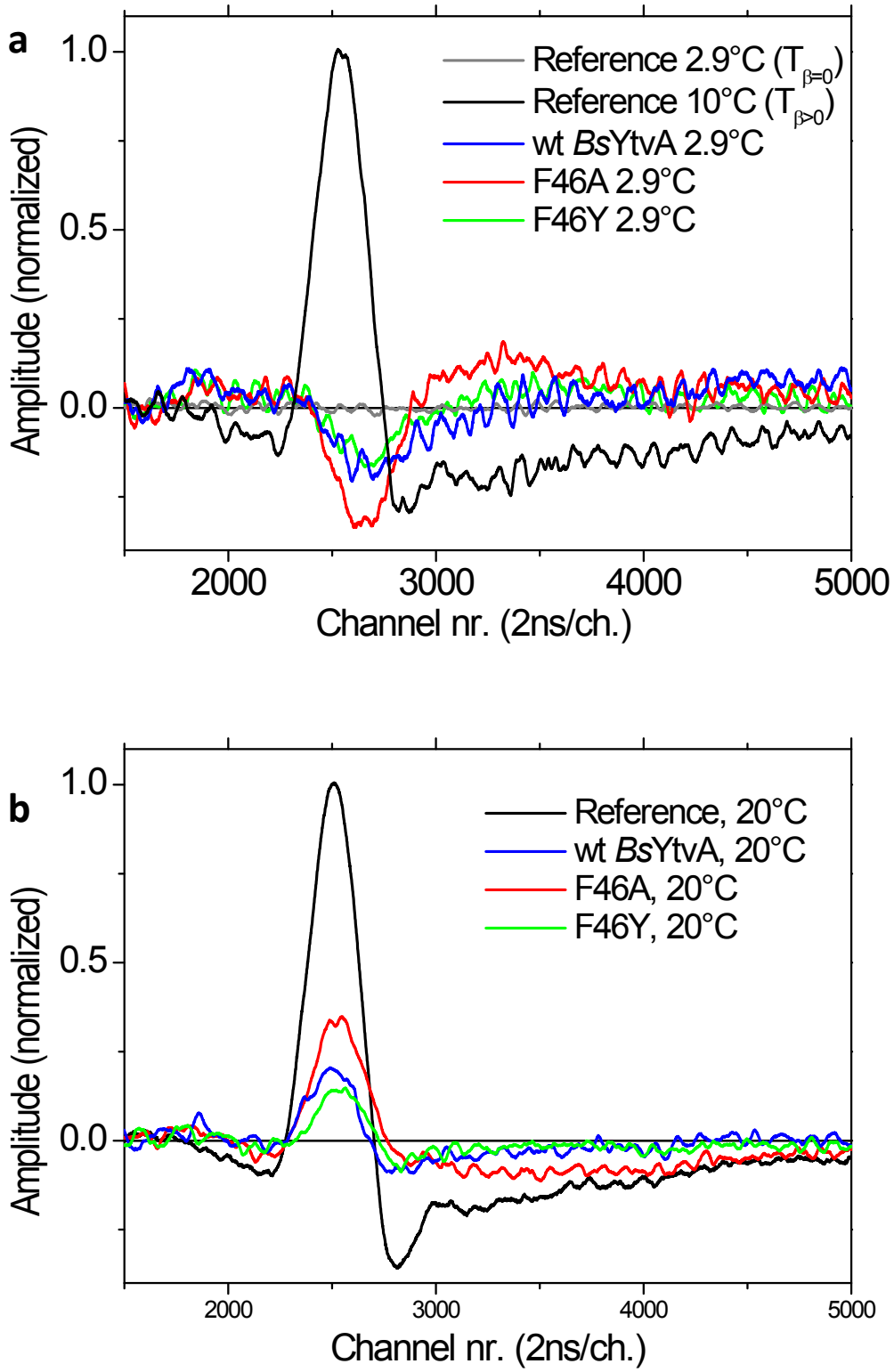

Figure S4. a. PA signals at $T_{\beta=0}$ = 2.9°C and, for the reference, also at $T_{\beta>0}$ = 10°C. This last signal was used for deconvolution; **b**. PA signals at $T_{\beta=0}$ = 2.9°C and, for the reference, also at $T_{\beta>0}$ = 10°C. This last signal was used for deconvolution.

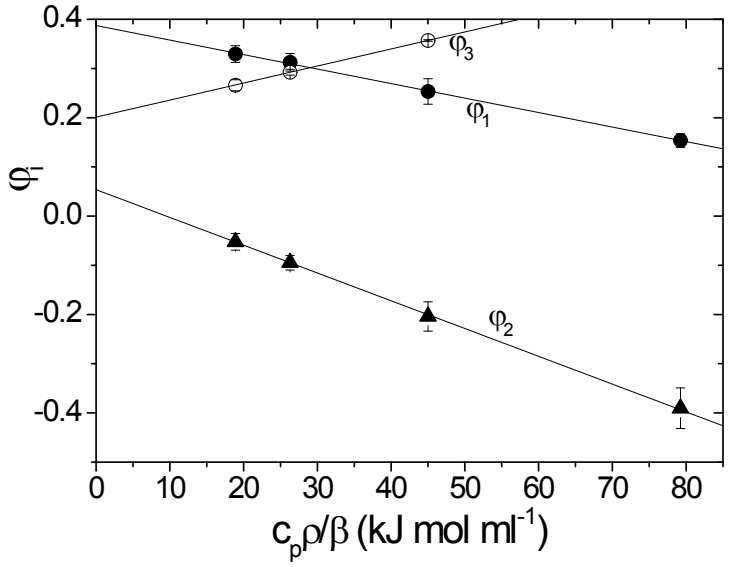

Figure S5. ST plot for *Bs*YtvA-F46A: filled circles, φ_1, τ_1 < 20 ns, not time-resolved; triangles, φ_2, τ_2 (see table ; open circles, φ_3, τ_3

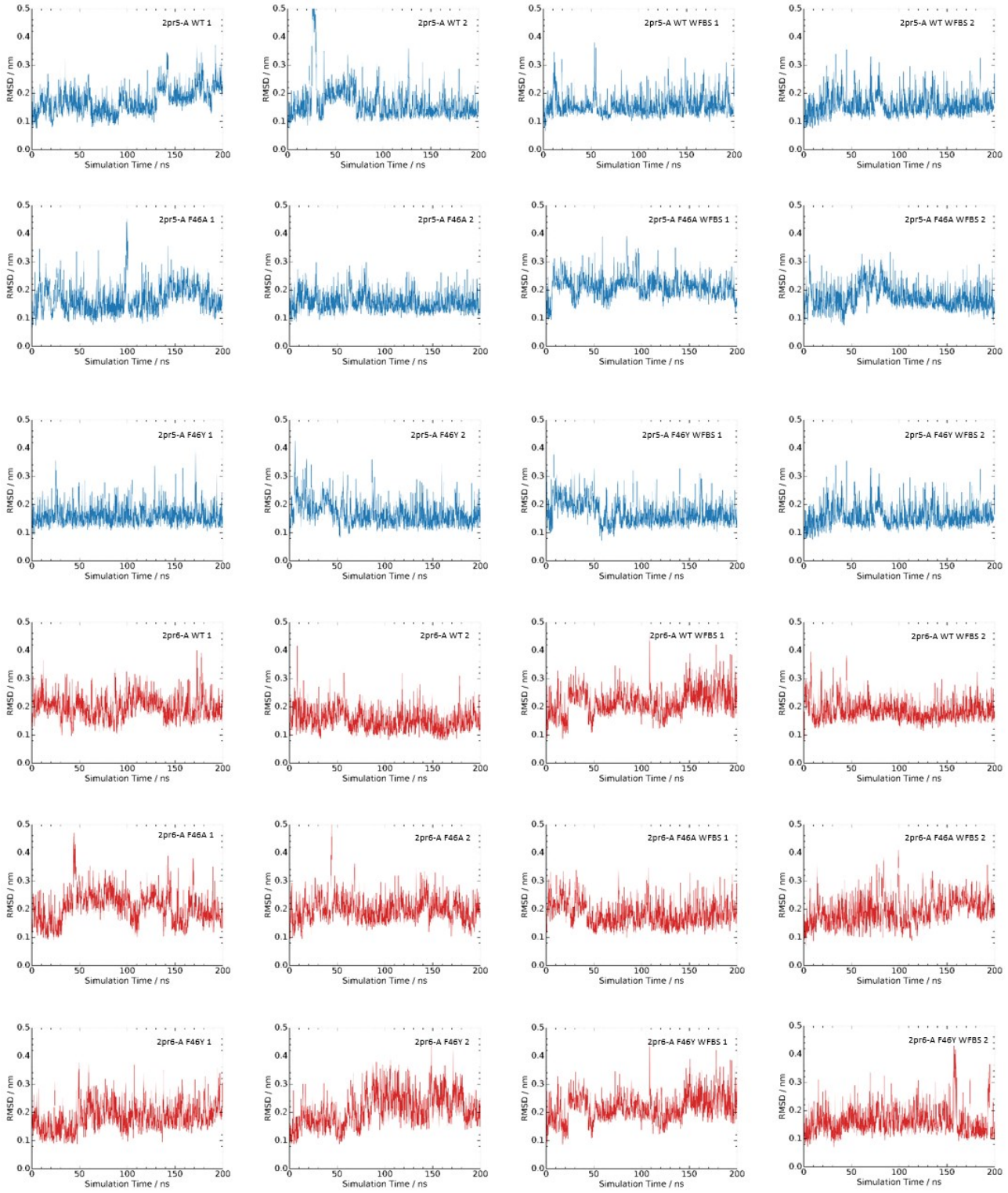

Figure S6: RMSD plots of Ca atoms of LOV domain from wt BsYtvA and F46A and F46Y variants in the dark- (blue lines) and light-adapted (red lines) states. WFBS = water free binding site.

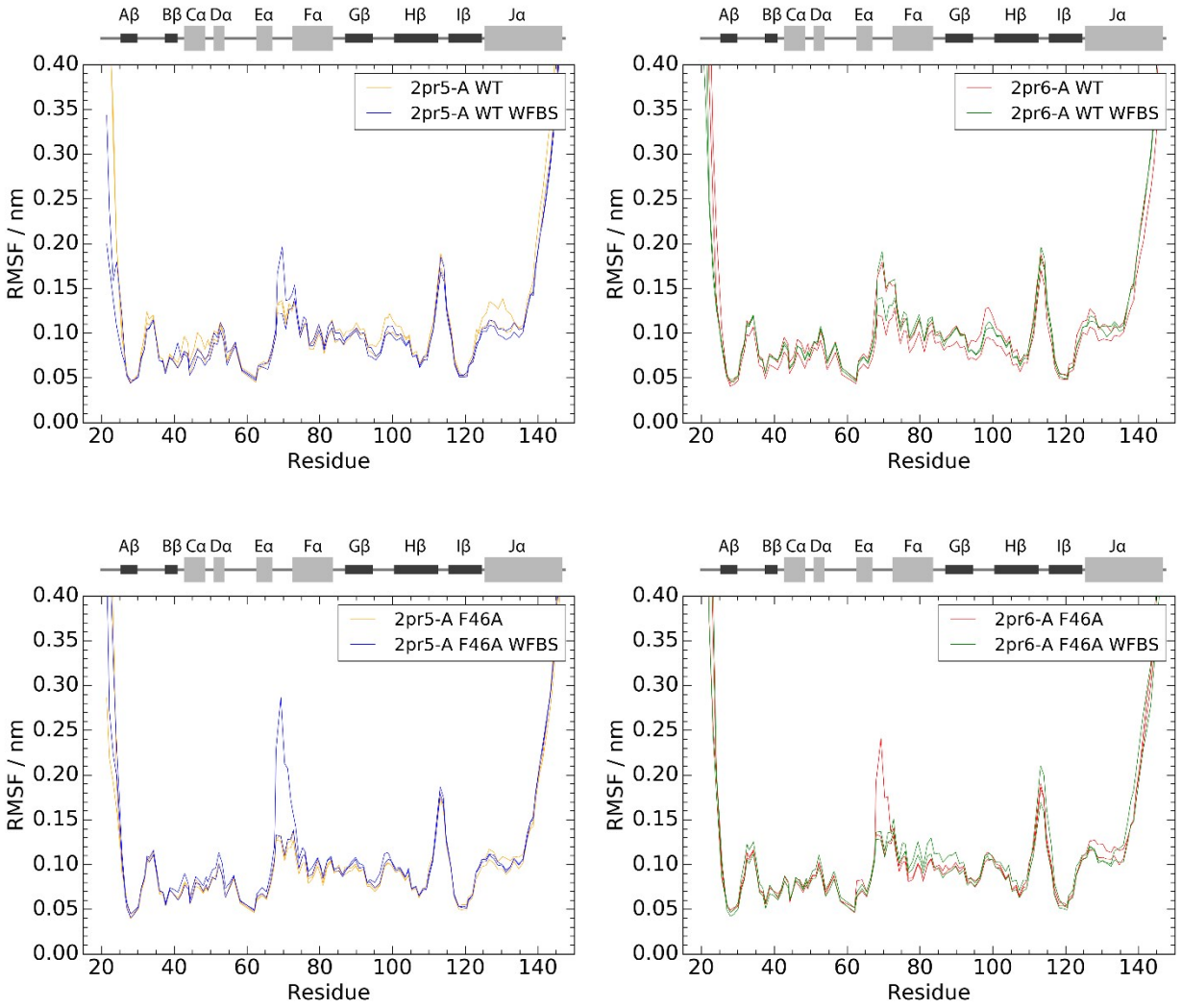

Figure S7. RMSF of protein Cα atoms during simulations of *Bs*YtvA-LOV wt and F46A variants in the dark- (left) and light-adapted (right) states. For each variant, the simulations with a starting water-free binding site (WFBS) are depicted in blue and green respectively. The corresponding secondary structure elements are indicated at the top of each plot.

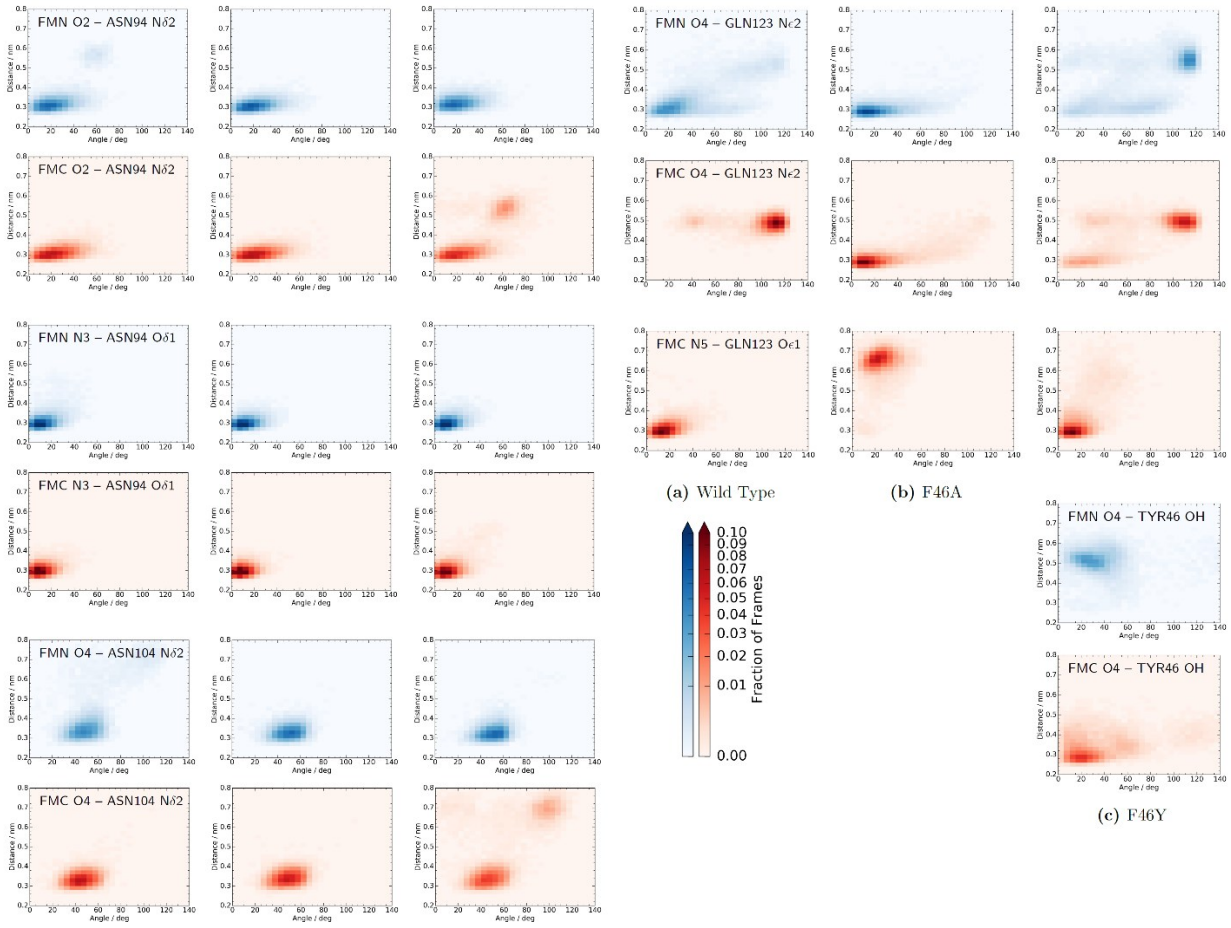

Figure S8. Distance/angle representation of the H-bond network involving Q123, N94 and N104, for the dark (blue) and light (red) state of *Bs*YtvA. Donor-acceptor distances and the angle that the bonded hydrogen atom forms with the donor – acceptor connection vector were determined for each frame. The four replicas of each variant (two with and two without water in the binding site starting structure) are grouped in the same plot. The H-bonds formed by Y46 with FMN/FMC (FMC = FMN bound to cysteine are also reported. A narrow, single spot represents a strong, conserved hydrogen bond, while a wider area stands for a weaker interaction since the bond is less capable to constrain the joint movement of the residues involved. The presence of two or more distinct spots indicates that the residue adopts alternative conformations whose frequency in the trajectories is related to colour intensity.

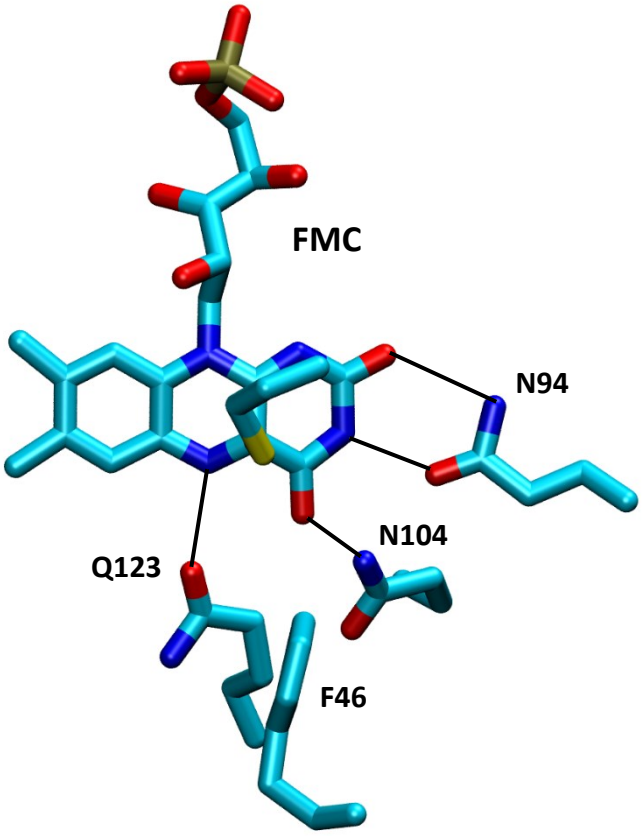

Figure S9. Representative central structures of binding site residues cluster analysis of wt *Bs*YtvA-LOV simulations in the light state. The side chain of Q123 is locked in its crystal-like conformation. Water is almost completely absent in all the trajectories.

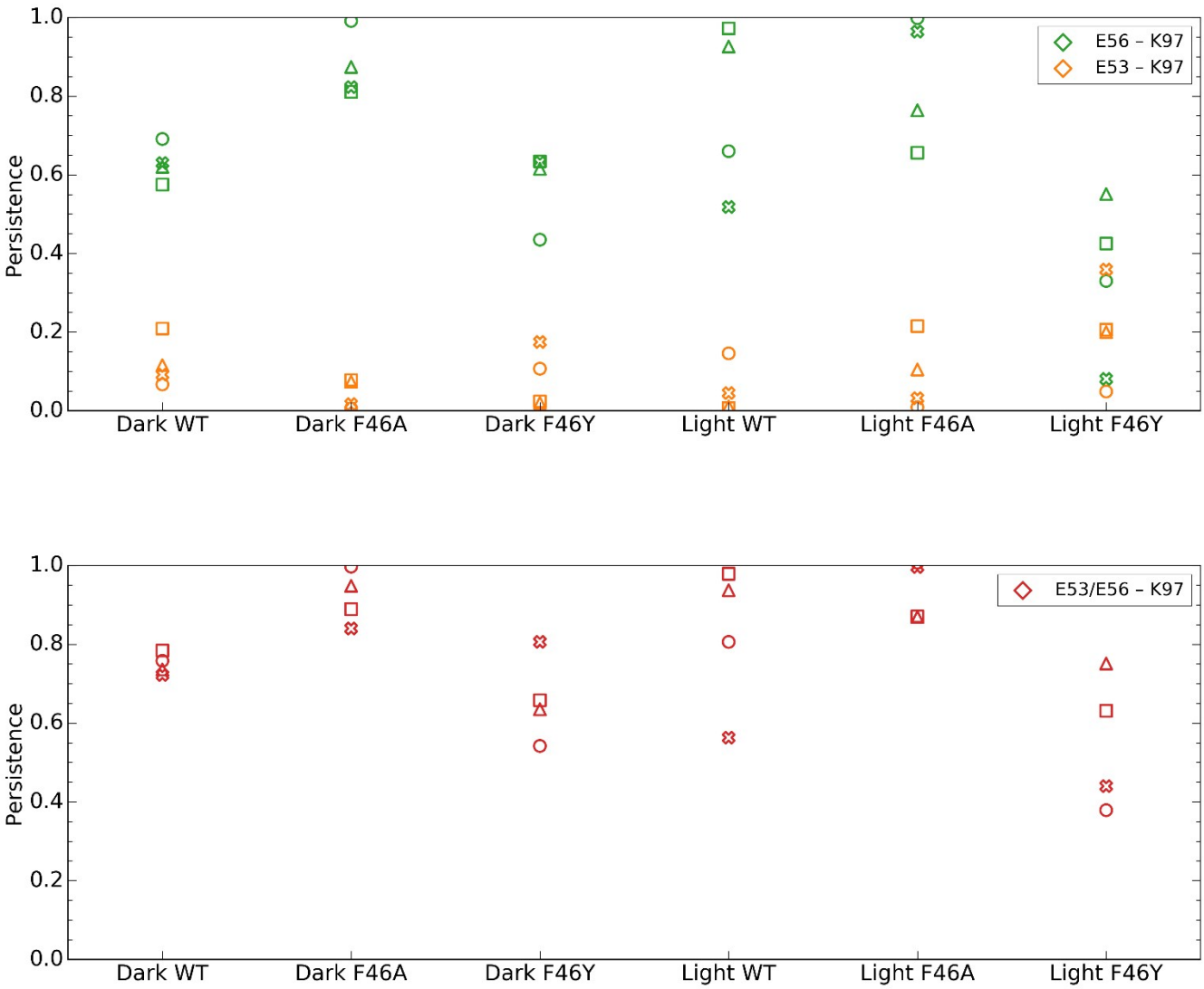

Figure S10. Top, individual persistence of salt bridges K97-E56 and K97-E53 in wt and mutated *Bs*YtvA-LOV structures in dark and light states. Only one of the two salt brideges is formed at a time; Bottom, total persistence of the salt bridges. Corresponding points of the top panel are added up.

Table S1. Extended PA results for the TT method in PA

***Bs*YtvA**	**-wt**	**-F46A**	**-F46Y**	**FMN**
α_1	**0.30 ± 0.01**	**0.34 ± 0.04**	**0.44 ± 0.02**	**0.29 ± 0.01**
α_2	0.20 ± 0.05	0.20 ± 0.08	0.17 ± 0.03	0.18± 0.03
ΔV_1/ml einstein^{-1}	-0.57 ± 0.14	-0.43 ± 0.20	-0.53 ± 0.16	-0.80± 0.01
ΔV_2 /ml einstein^{-1}	-5.8 ± 1.3	-2.16 ± 0.10	-1.71 ± 0.07	+1.05± 0.03
*τ_2/ns (2.9°C)	2400 ± 200	270 ± 15	720 ± 10	2200 ± 100
*Φ_T (= Φ_1)	0.68 ± 0.02	0.71 ± 0.06	0.60 ± 0.03	0.66 ± 0.02
§ΔV_T /ml mol^{-1}	-0.84 ± 0.20	-0.60 ± 0.30	-0.90 ± 0.30	-1.20 ± 0.03
Φ_2	≥ 0.40± 0.07	≥ 0.44± 0.07	≥ 0.36 ± 0.06	≥ 0.36

*: for wt BsYtvA this represents the decay of FMN triplet into the FMN-Cys adduct; fro free FMN the decay of the triplet into a long-lived product, most probably singlet oxygen. For the F46A and F46Y see text for discussion.

Table S2. PA parameters from the ST method, errors within 10%

***Bs*YtvA**	**-F46A**	**-F46Y**
Amplitude analysis (PA signal of sample/reference)		
α_{tot}	0.57	0.62
ΔV_{tot}/ml einstein^{-1}	-2.81	-1.83
$\Phi \times E_{stored}$ /kJ mol^{-1}	82	67
Deconvolution analysis		
α_1 (τ_1 < 20ns)	0.39	0.63
α_2, τ_2 ($\tau_{T,1}$)	0.05	
α_3, τ_3($\tau_{T,2}$)	0.20	0.18
Φ_2	$\geq$ 0.44$\pm$ 0.07	$\geq$ 0.36 $\pm$ 0.06
Φ_1 E_1 /kJ mol^{-1}	130	64
Φ_2 E_2/kJ mol^{-1}	116	
Φ_3 E_3/kJ mol^{-1}	63	17
$\Phi_1\Delta V_1$/mL mol^{-1}	-0.8	-2.0
$\Phi_2\Delta V_2$/mL mol^{-1}	-1.5	
$\Phi_3\Delta V_3$ /mL mol^{-1}	+ 0.9	-0.4
$\Phi_1 = \Phi_T$	0.65	?
$\Delta V_1 = \Delta V_T$ /mL mol^{-1}	-1.2	?
Φ_2= ?	$\geq$ 0.58	$\geq$ 0.32
$\Phi_3 = \Phi_{390,2}$	$\geq$ 0.31	$\geq$ 0.08

Table S3. Overview of the performed simulations on LOV domains from wt *Bs*YtvA and F46A and F46Y variants

State	Structure *Bs*YtvA	Simulation	[a]LOV domain RMSD /nm	[a]LOV core RMSD /nm
Dark	-wt	2pr5-A WT 1	0.17 ± 0.05	0.07 ± 0.01
		2pr5-A WT 2	0.17 ± 0.06	0.07 ± 0.01
		2pr5-A WT [b]WFBS 1	0.16 ± 0.03	0.08 ± 0.01
		2pr5-A WT WFBS 2	0.16 ± 0.04	0.07 ± 0.01
	-F46A	2pr5-A F46A 1	0.17 ± 0.05	0.07 ± 0.01
		2pr5-A F46A 2	0.16 ± 0.03	0.07 ± 0.01
		2pr5-A F46A WFBS 1	0.21 ± 0.03	0.07 ± 0.01
		2pr5-A F46A WFBS 2	0.18 ± 0.04	0.09 ± 0.02
	-F46Y	2pr5-A F46Y 1	0.16 ± 0.03	0.08 ± 0.01
		2pr5-A F46Y 2	0.17 ± 0.04	0.08 ± 0.01
		2pr5-A F46Y WFBS 1	0.17 ± 0.04	0.08 ± 0.01
		2pr5-A F46Y WFBS 2	0.17 ± 0.04	0.07 ± 0.01
Light	-wt	2pr6-A WT 1	0.20 ± 0.04	0.07 ± 0.01
		2pr6-A WT 2	0.15 ± 0.04	0.07 ± 0.01
		2pr6-A WT [a]WFBS 1	0.17 ± 0.04	0.07 ± 0.01
		2pr6-A WT WFBS 2	0.19 ± 0.03	0.08 ± 0.01
	-F46A	2pr6-A F46A 1	0.21 ± 0.06	0.07 ± 0.01
		2pr6-A F46A 2	0.20 ± 0.04	0.07 ± 0.01
		2pr6-A F46A WFBS 1	0.18 ± 0.04	0.07 ± 0.01
		2pr6-A F46A WFBS 2	0.19 ± 0.05	0.07 ± 0.01
	-F46Y	2pr6-A F46Y 1	0.18 ± 0.05	0.09 ± 0.02
		2pr6-A F46Y 2	0.21 ± 0.06	0.09 ± 0.02
		2pr6-A F46Y WFBS 1	0.22 ± 0.04	0.09 ± 0.03
		2pr6-A F46Y WFBS 2	0.16 ± 0.05	0.07 ± 0.02

a: RMSD of protein Cα atoms was calculated both for the LOV core (aa 25-125) and for the extended LOV domain (aa 21-147) with respect to the energy minimized structure and averaged over the last 195 ns of simulation, where a stable structure was reached; [b]: WFBS = Water Free Binding Site