Tongue Refolding in the Knotless Cyanobacterial Phytochrome All2699

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Primer	Sequence		DNA
P1	5'	GCCATATGTCACCGACCGCTAAAC 3'	gaf1
P2	5'	GCCTCGAGATGACTTTGGGCGAT 3'	gaf1
P3	5'	GTCATATGTCACCGACCGCTAAACC 3'	gaf1-gaf2
P4	5'	CACTCGAGTTCGTTAAAGGCTTGTACT 3'	gaf1-gaf2
P5	5'	GATGCCAAAAAGTCACAAGCTCA 3'	R387P
P6	5'	GGGCCATAAATTAAATGATACACG 3'	R387P
P7	5'	CATAAATTTCATCCAGATGGAAGCG 3'	Ү55Н
P8	5'	AATCATCACTCGGTCAGTTCCCAAG 3'	Ү55Н

Table S1. Primers used for cloning gaf1 and gaf1-gaf2 with wild-type and mutated sequences.



Figure S1. IR absorption spectrum of a dried sample of ¹³C-labeled GAF1-GAF2. The amide I band (C=O stretch of the backbone) is shifted from ~1650 cm⁻¹ to 1614 cm⁻¹ by the ¹³C-labeling. Similarly, the amide II band is shifted from ~1550 cm⁻¹ to 1532 cm⁻¹. The large extent of these shifts supports a complete labeling of the protein moiety with ¹³C.



Figure S2. Light-induced FTIR difference spectrum of GAF1-GAF2 in D₂O after illumination with red light at 632 nm. The signals of tongue refolding shift from 1621 (-) / 1654 (+) cm⁻¹ to 1621 (-) / 1644 (+) cm⁻¹. Accordingly, the H/D exchange in the α -helix is much more pronounced than in the β -sheet. The signal increase of the latter is explained by the downshift of a compensating, positive contribution to 1607 cm⁻¹ in D₂O. The signals of the D-ring carbonyl downshift from 1736 (-) / 1712 (+) cm⁻¹ to 1729 (-) / 1706 (+) cm⁻¹ upon H/D exchange as an indication for a moderate hydrogen bond strength in Pr and Pfr states.



Figure S3. UV-vis spectra of the Pr and Pfr states of GAF1-GAF2-Y55H. The sample was photoconverted by illumination at 590 nm. The maxima of absorbance are close to those of the wild-type GAF1-GAF2 with 638 and 705 nm, respectively. Accordingly, the exchange does not interfere with formation of the Pfr state.