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Electronic Supplementary Information Elucidating the role of structural fluctuations, intermolecular and vibronic interactions in the spectroscopic response of a bacteriophytochrome.

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S1 Electronic spectra calculation

Homogeneous lineshape The homogeneous lineshape of the biliverdin was described with the second-order cumulant expansion.[1] Within this formalism, the linear absorption lineshape of a chromophore with due to one excited state e can be written with the following time-domain integral:

$$\sigma_{eg}(\omega - \omega_{eg}) = \Re \int_0^\infty \mathrm{d}t \, e^{i(\omega-\omega_{eg})t - g_{eg}(t)} \tag{S1}$$

where ω_{eg} is the *vertical* excitation energy from the ground state to state e, and $g_{eg}(t)$ is the *lineshape function*, which, in the second-order cumulant expansion, is derived from the spectral density $J(\omega)$:

$$g_{eg}(t) = -\int_0^\infty \mathrm{d}\omega \frac{J_{eg}(\omega)}{\omega^2} \left[\coth\left(\frac{\beta\hbar\omega}{2}\right) \cdot \big(\cos(\omega t) - 1\big) \; - \; i\,(\sin(\omega t) - \omega t) \right] \tag{S2}$$

For a discrete set of harmonic oscillators with frequencies ω_k, linearly coupled to the e-th excitation through the Huang-Rhys factors $S_k^{(eg)}$, the spectral density can be written as in equation (1) of the main text. To account for the finite lifetime of vibrational states, we broaden the spectral density peaks with Lorentzian functions with a HWHM of 4 cm^{-1}.

Inhomogeneous contribution to the electronic spectra To compute the effect of inhomogeneous disorder on the absorption and CD spectra, we consider the inhomogeneous distribution of both excitation energies and dipole strengths computed along the MD trajectory (after chromophore optimization), which is convoluted with the homogeneous lineshape $\sigma_{eg}(\omega - \omega_{eg}^{(j)})$. For a finite number of excitation energies computed along the MD, the total absorption spectrum reads:

$$A(\omega) = \omega \sum_e^{(\text{states})} \frac{1}{N_{\text{frames}}} \sum_j^{(\text{frames})} |\boldsymbol{\mu}_{eg}^{(j)}|^2 \sigma_{eg}(\omega - \omega_{eg}^{(j)}) \tag{S3}$$

where $\omega_{eg}^{(j)}$ and $\boldsymbol{\mu}_{eg}^{(j)}$ are, respectively, the excitation energy and transition dipole moment to state e in frame j. Analogously, the circular dichroism spectrum is calculated as:

$$CD(\omega) = \omega \sum_{e}^{(\text{states})} \frac{1}{N_{\text{frames}}} \sum_{j}^{(\text{frames})} R_{eg}\sigma_{eg}(\omega - \omega_{eg}^{(j)}) \tag{S4}$$

where R_{eg} is the rotatory strength of the e-th excitation from the ground state.

S2 Additional figures

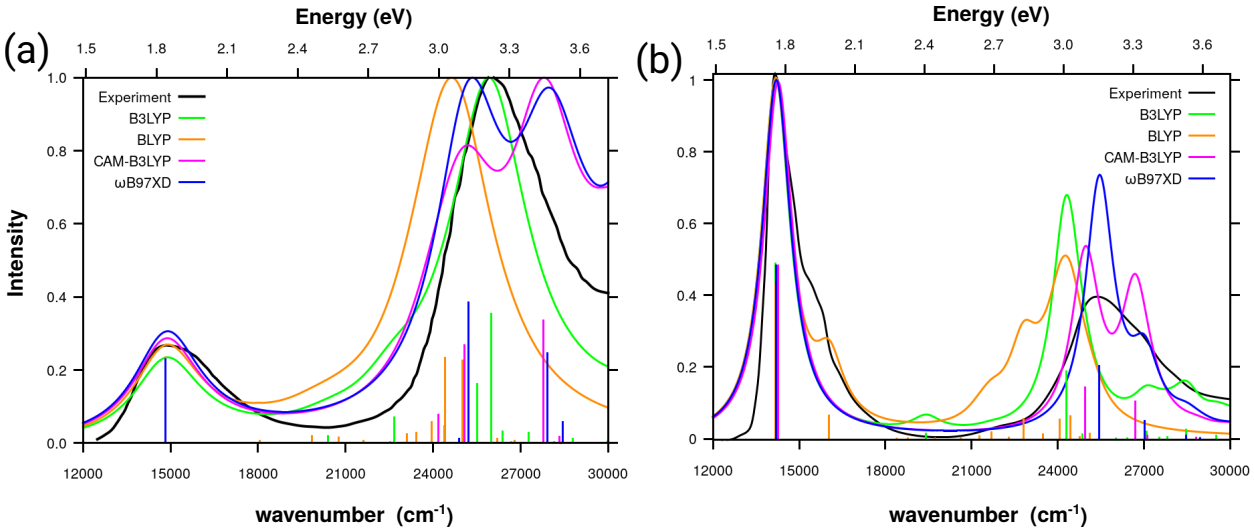

Figure S1: (TD)DFT absorption spectra and excitation energies computed with different functionals for the minimum energy conformer of biliverdin in chloroform using PCM (a) and on the refined crystal structure (b). The basis set chosen for all the functionals is 6-31+G(d).The calculated spectra in Chloroform and in the crystal have been homogeneously shifted to match the experimental[2] lowest energy Q band (the shifts are of the order of 0.08 and 0.2 eV using B3LYP and BLYP, -0.1 using CAM-B3LYP or ωB97XD in Chloroform; while in the crystal environment the shifts are of the order of -0.1 and 0.02 and -0.2 eV respectively). The position of the sticks correspond to the electronic transition energy and the height is proportional to the square of the transition dipole moment. All the spectra have been finally normalized with respect to the most intense peak.

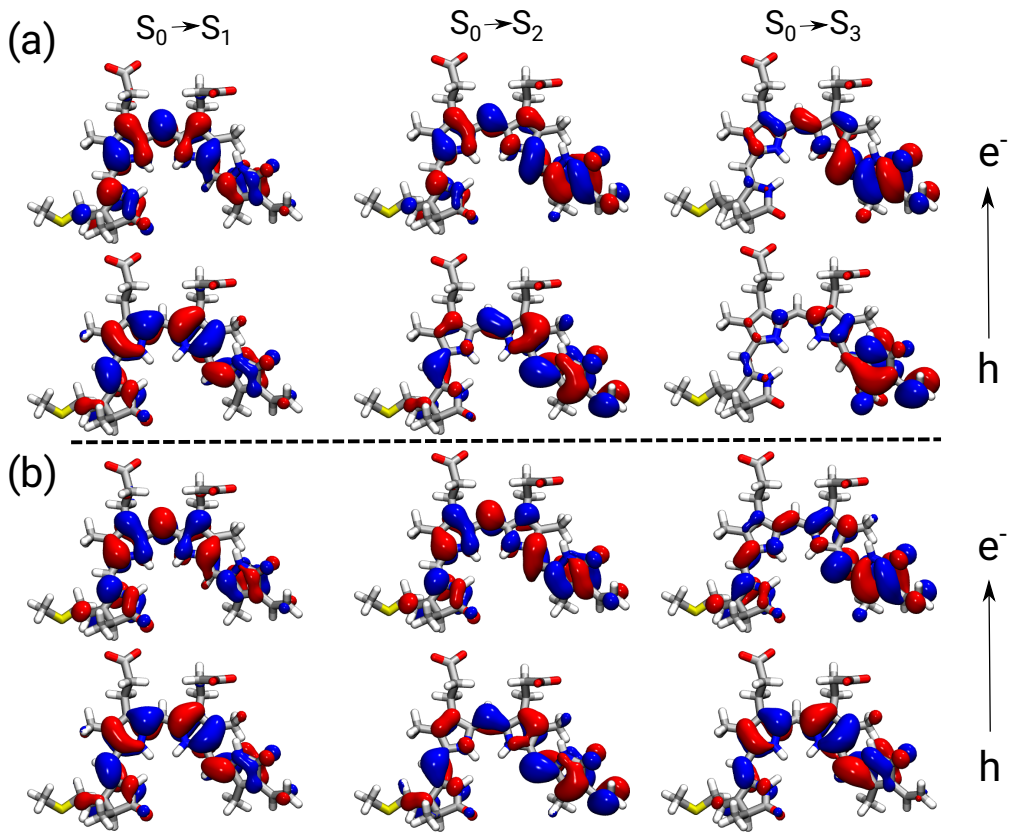

Figure S2: NTO orbital pairs, "hole" (h) and "electron" (e^-), computed with (a) ωB97XD and (b) CAM-B3LYP for the $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_3$ transitions.

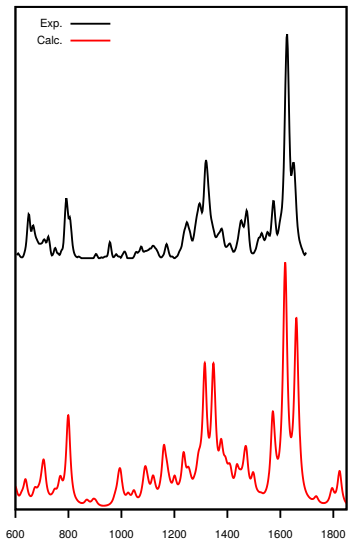

Figure S3: Comparison of experimental pre-resonance Raman spectrum[3] and calculated spectral density with ωb97XD at the crystal structure. The calculated SD frequencies were scaled by 0.95 for this comparison.[4]

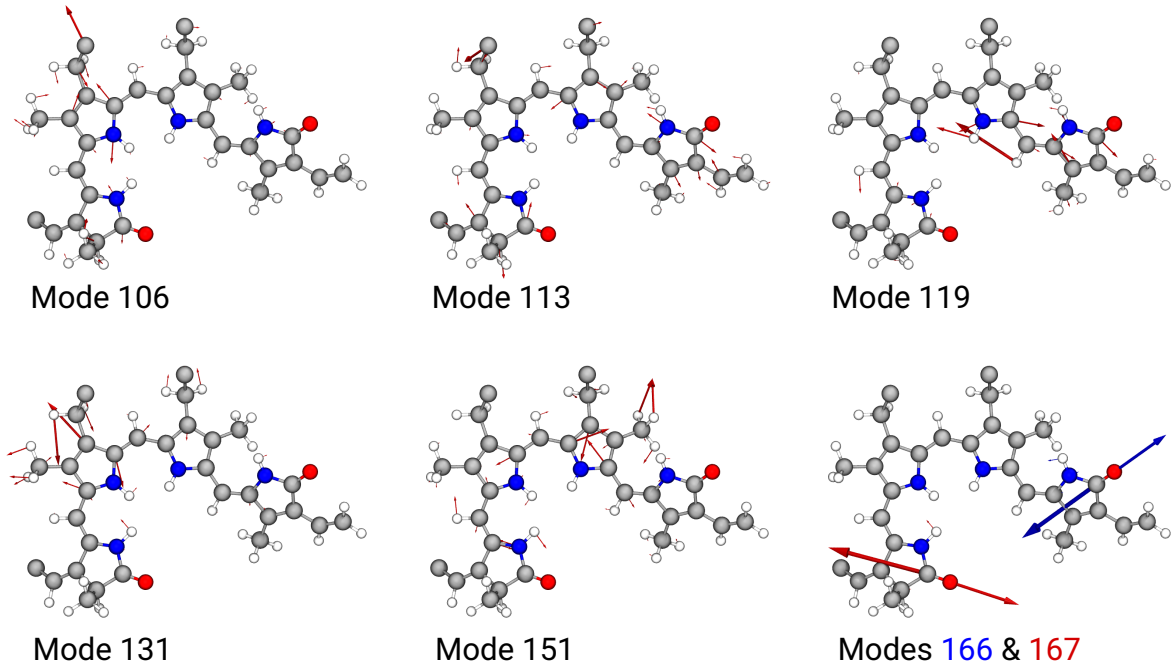

Figure S4: Other normal modes coupled to the excitation and contributing to the peaks in the calculated spectral density.

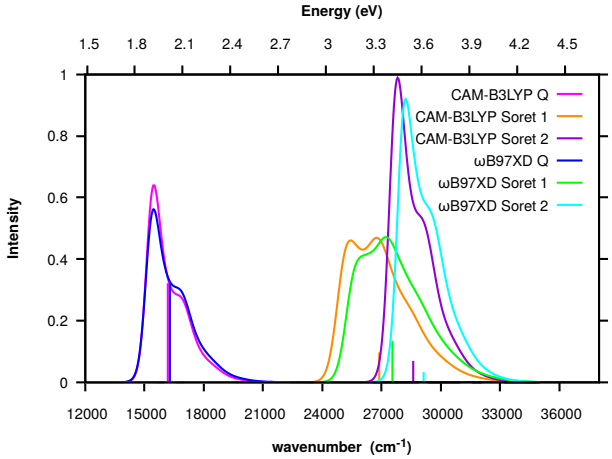

Figure S5: Spectral lineshapes obtained applying a gaussian broadening of 500 cm^{-1} to the spectral densities for $S_0 \rightarrow S_1$ (Q), $S_0 \rightarrow S_2$ (Soret 1) and $S_0 \rightarrow S_3$ (Soret 2) transitions computed with CAM-B3LYP and ωB97XD functionals. The position of the sticks correspond to the electronic transition energy and the height is proportional to the square of the transition dipole moment. Both spectral lineshapes and sticks were divided respectively by the same number.

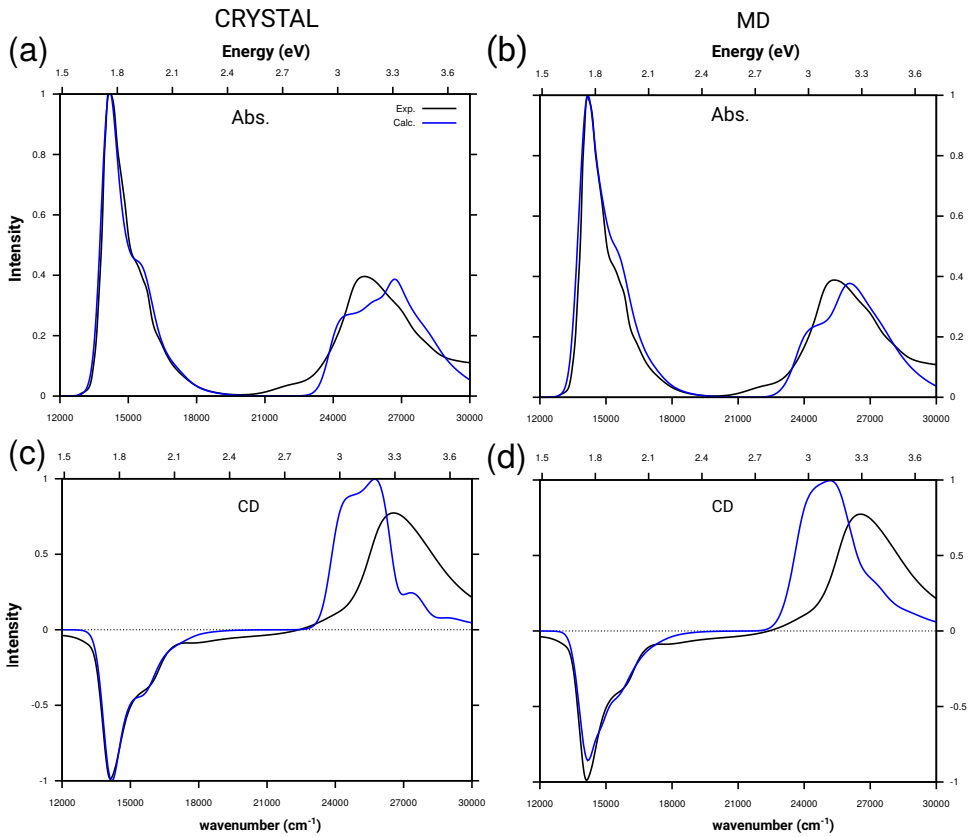

Figure S6: (TD)DFT absorption and CD spectra computed with wb97XD (a,c) on the crystal structure and (b,d) along the MD trajectory. In the latter case the spectra have been obtained as an average over 80 MD configurations (See the ESI† for details). All the calculated spectra have been homogeneously shifted by −0.18 eV for the crystal and −0.20 eV for the MD, to match the experimental[5] lowest energy Q band. All spectra are normalized to the Q-band maximum (minimum in the case of CD).

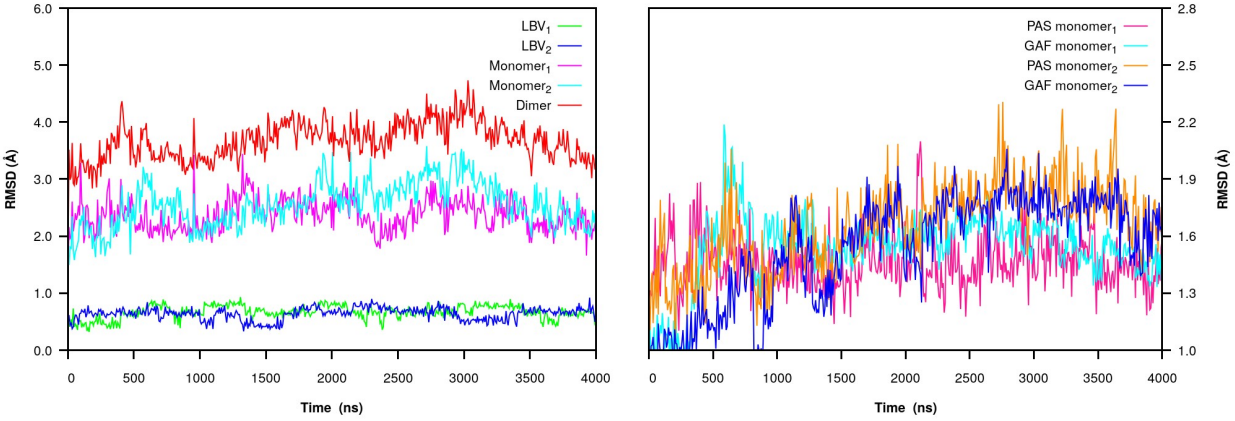

Figure S7: Left panel: Root mean square displacement (RMSD) on the protein backbone (C,O,N atoms) for the dimer and the two monomers and on the heavy atoms of the BV chromophore. Right panel: RMSD on the protein backbone (C,O,N atoms) for the PAS and GAF domains of the two monomers. All the RMSDs are computed along four μs of the production run and using as reference the refined crystal structure

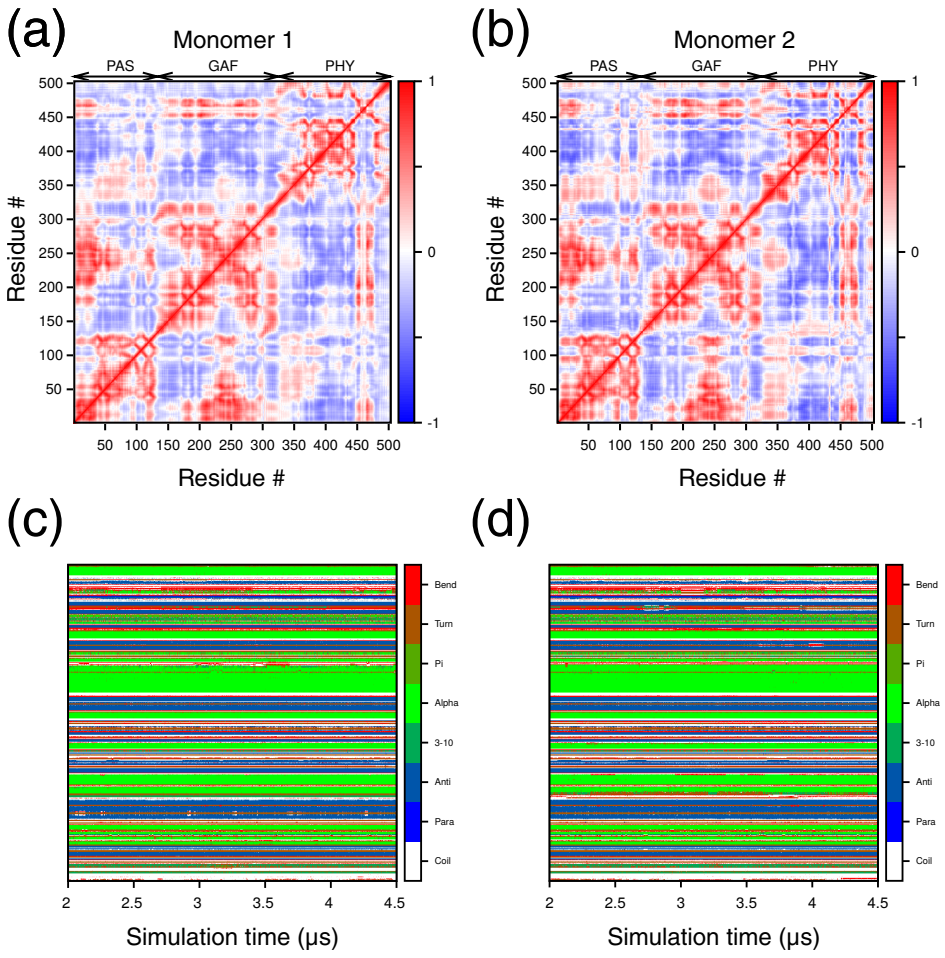

Figure S8: (a,b) Dynamic cross-correlation and (c,d) secondary structure calculated on the Cα along the 2-4 μs interval of the simulation.

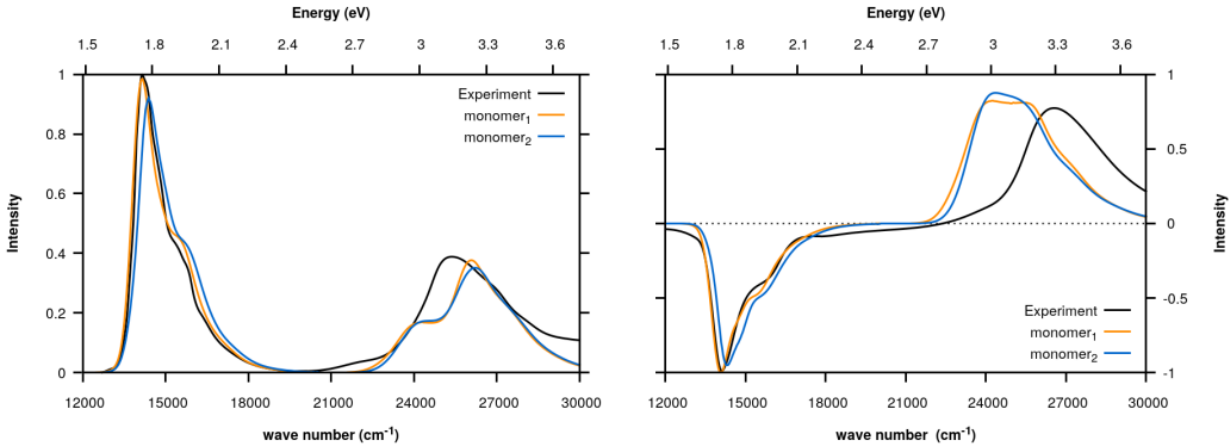

Figure S9: (TD)DFT absorption (a) and CD (b) spectra computed on monomer 1 and monomer 2 computed over the 2-4 microseconds interval of the Pr form simulation production run. To better compare the spectra of the two monomers they were shifted (by $\sim$1550 cm^{-1}) of the same energy and normalized by dividing both by the same quantity.

S3 Additional Tables

Table S1: (TD)DFT excitation energy and squared transition dipole moment for the first excitation, computed with different functionals for the minimum energy conformer of biliverdin in chloroform using PCM, and in the refined protein crystal structure using a MMpol description for the environment.

	Chloroform		Protein (CRY)	
Functional	Energy (cm^{-1})	μ^2 (a.u.)	Energy (cm^{-1})	μ^2 (a.u.)
B3LYP	14 182	6.9	15 246	29.3
BLYP	12 974	7.0	13 938	26.0
CAM-B3LYP	15 707	6.9	16 185	29.0
ωB97XD	15 933	6.9	16 291	29.0
Experiment	14 900	–	14 285	–

Table S2: Assignment of the normal modes most coupled to the excitation. The frequencies of the modes were scaled by 0.95 to aid the comparison with Figure 2(a) of the main text.

Mode	Scaled Freq. (cm^{-1})	Assignment
59	640	OOP C_{16} bending
71	713	OOP C_{14} bending
77	771	C_{14}-C_{15}-C_{16} bending
79	804	HOOP C_{15}-H bending
94	998	C-C bending (rings B and C)
106	1091	C-N stretching (ring B)
113	1161	C-C stretching (delocalized)
119	1231	C_{15}-H IP bending + N_C-H IP bending
127	1309	N_B-H IP bending + N_C-H IP bending
131	1352	C-C stretching (delocalized)
134	1380	Umbrella CH_3 (ring D), Wagging CH_2 (ring C)
135	1381	Umbrella CH_3 (ring D), Wagging CH_2 (ring C)
151	1466	Ring B breathing
152	1472	Ring B asymmetric stretch
159	1567	N_B-H IP bending + N_C-H IP bending
162	1615	C_9=C_{10} stretching
163	1656	C_{15}=C_{16} stretching
166	1791	C_{19}=O stretching
167	1821	C_1=O stretching

Table S3: H-bond interaction probabilities along the 2-4.5 μs range of the simulation.

H-bond donor	H-bond acceptor	Prob. Mon. 1 (%)	Prob. Mon. 2 (%)
HIS290-NH	C=O (ring D)	21	20
H_2O	C=O (ring D)	2	6
BV-NH	HIS260-N_δ	0	4.4
H_2O	HIS260-N_δ	89	65
TYR263-OH	ASP207-O	93	89
ARG466-NH	ASP207-O	99	99

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(3) Wagner, J. R.; Zhang, J.; von Stetten, D.; Günther, M.; Murgida, D. H.; Mroginski, M. A.; Walker, J. M.; Forest, K. T.; Hildebrandt, P.; Vierstra, R. D. *Journal of Biological Chemistry* **2008**, *283*, 12212–12226.

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