Supporting Information

Analysis of fluorescence intensity changes by a three state model

To analyse the non-linear profile of fluorescence intensity F_T *vs* pressure p, we hypothesized the presence in solution of three conformers, native (N), compact (C), and soft (S)), whose relative concentrations are pressure dependent.

$$N \underset{}{\overset{K_1}{\rightleftharpoons}} C \underset{}{\overset{K_2}{\rightleftharpoons}} S$$

The experimental data were fitted using the equation derived by Phillips et al. for a two-step model,[27] adapted to account for the fluorescence signal

$$F_T(p) = \frac{1 + F_1 K_1 \exp\left(-\frac{p\Delta V_1}{RT}\right) + F_2 K_1 K_2 \exp\left(-\frac{p\Delta V_2}{RT}\right)}{1 + K_1 \exp\left(-\frac{p\Delta V_1}{RT}\right) + K_1 K_2 \exp\left(-\frac{p\Delta V_2}{RT}\right)}$$

where K_1 and K_2 are the pressure-independent equilibrium constants, $\Delta V_1 = V_C - V_N$ and $\Delta V_2 = V_S - V_N$, are the changes in the reaction volume, and F_1 and F_2 are the relative fluorescence intensities of states C and S with respect to state N. Notably, the fitting procedure required fixing the F_2 parameter to converge. Under the assumption that Mut2 was fully converted to state S at 600 MPa and 279 K, experimental data yielded F_2=0.5 for this protein. Accordingly, we adopted this value for fitting all F_T *vs* p datasets for both Mut2 and Mut2Y. Notably, changing F_2 from 0.4 to 0.6 did not vary appreciably the fitting results, thus supporting the reliability of recovered parameters.

Table S1. Brightness and thermodynamic parameters for Mut2Y and Mut2 conformers

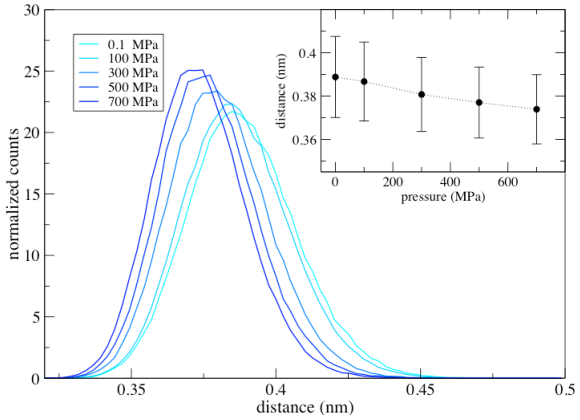
T (K)	F_1	ΔG_1 (kJ/mol)	ΔV_1 (ml/mol)	F_2	ΔG_2 (kJ/mol)	ΔV_2 (ml/mol)	F_1	ΔG_1 (kJ/mol)	ΔV_1 (ml/mol)	F_2	ΔG_2 (kJ/mol)	ΔV_2 (ml/mol)
	MUT2Y						MUT2					
279	1.2	5.3±2.6	-29±4	0.5	4.4±2.2	-34±5	1.1	3.7±1.2	-8±3.0	0.5	5.4±2.4	-38±5
300	1.8	7.0±3.5	-30±3	0.5	3.0±1.5	-36±6	1.3	7.5±2.5	-15±3.8	0.5	2.1±0.9	-35±5
321	2.0	7.5±4.0	-30±4	0.5	2.2±1.0	-38±5	2.0	8.6±2.8	-26±4.3	0.5	1.3±0.5	-34±4

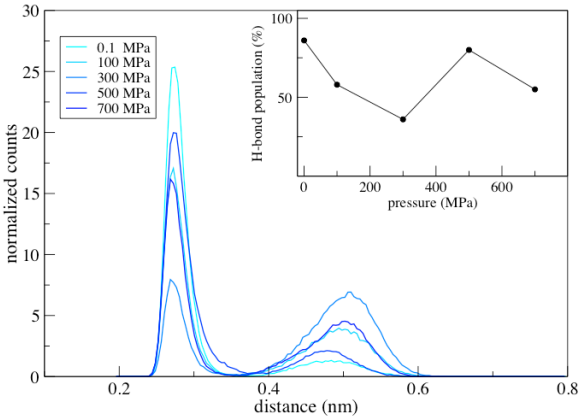
Additional Figures

Mut2Y

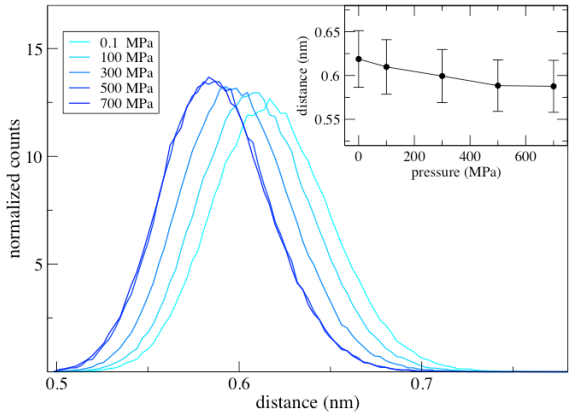
Mut2

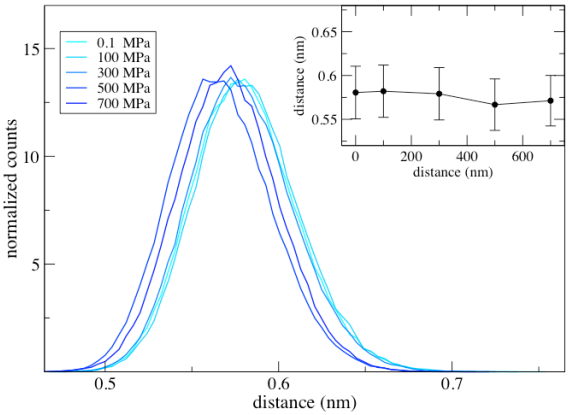
(a) Y203 – chromophore phenol ring/T203 – chromophore phenolate



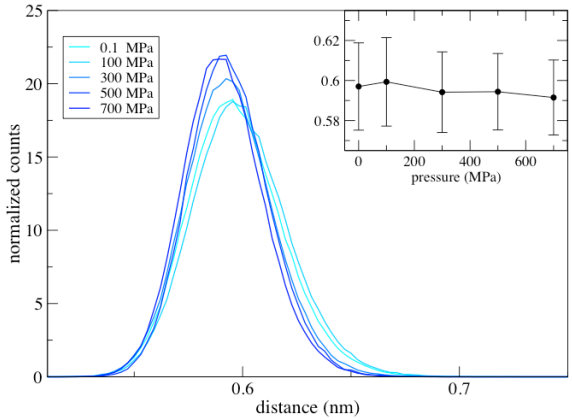


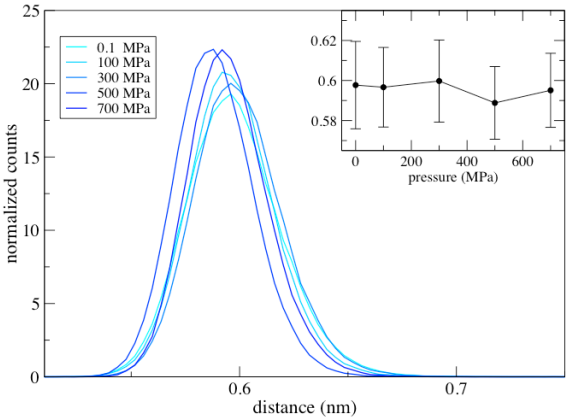
(b) F165 – chromophore phenol ring



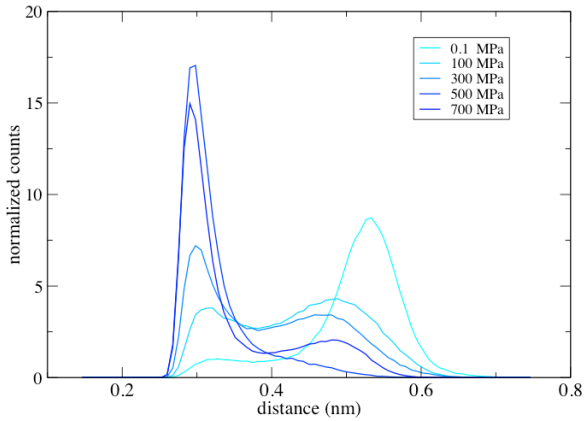


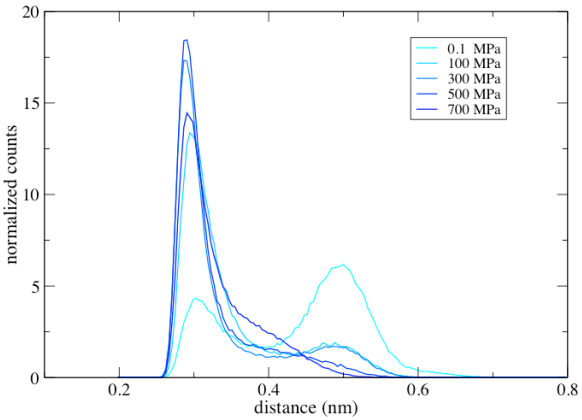
(c) I167 – chromophore phenol ring



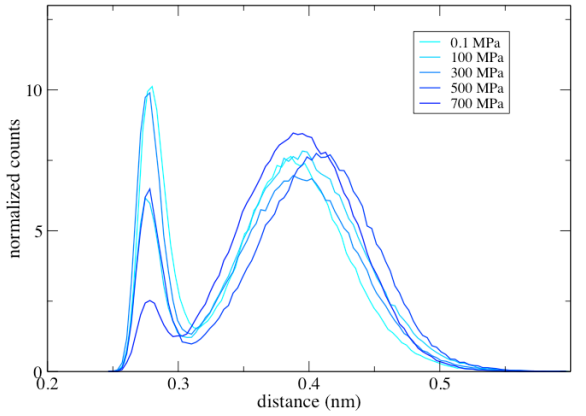


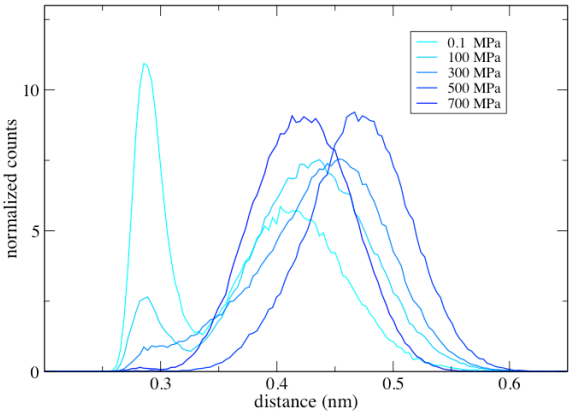
(d) Q94 – chromophore imidazolinone carbonyl



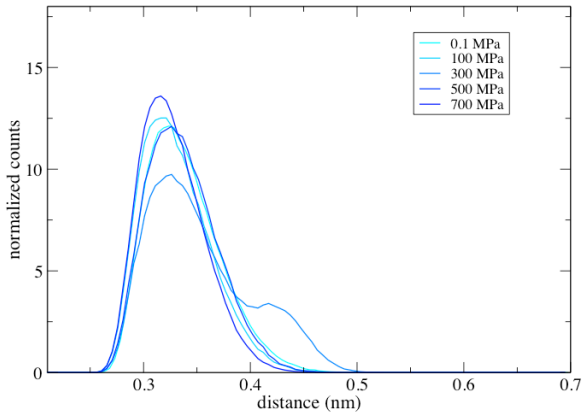


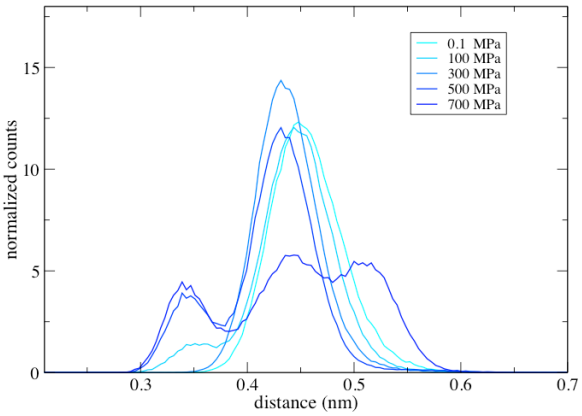
(e) Q69 – chromophore imidazolinone carbonyl



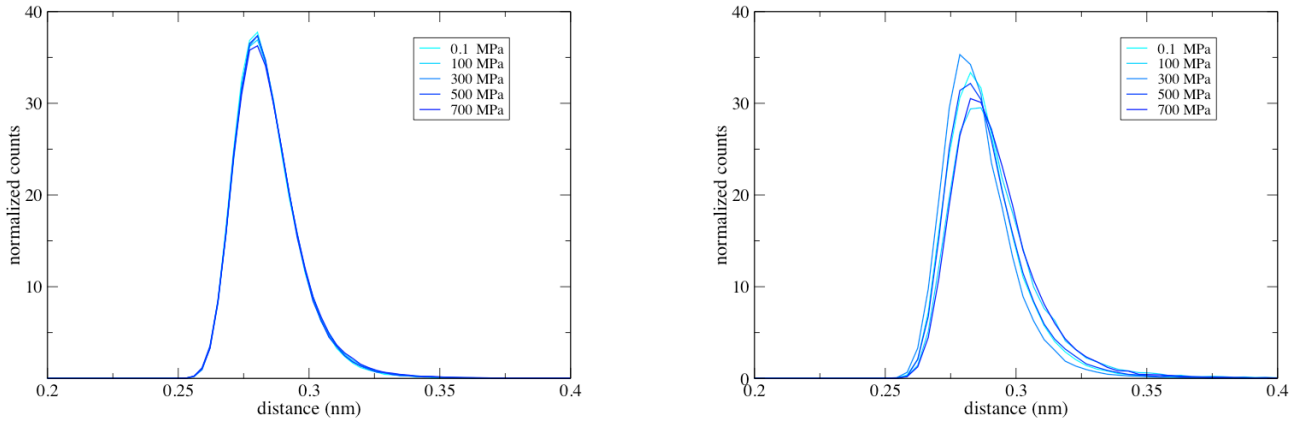


(f) E222 – chromophore imidazolinone





(g) H148 – chromophore phenol oxygen



(h) R96 – chromophore imidazolinone carbonyl

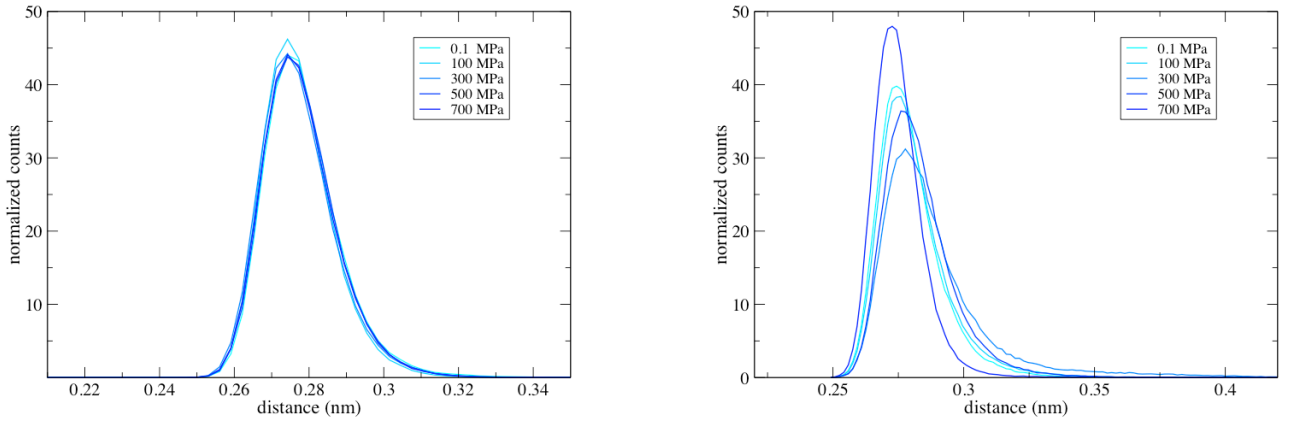
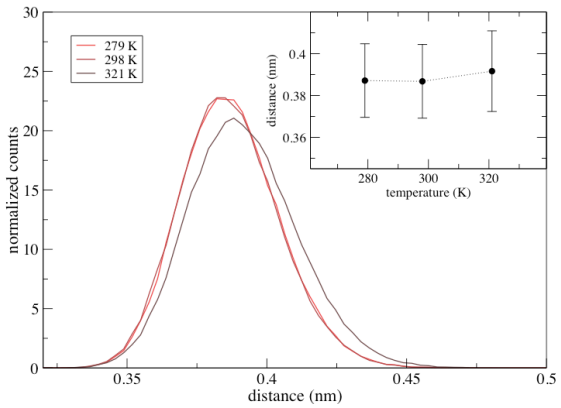


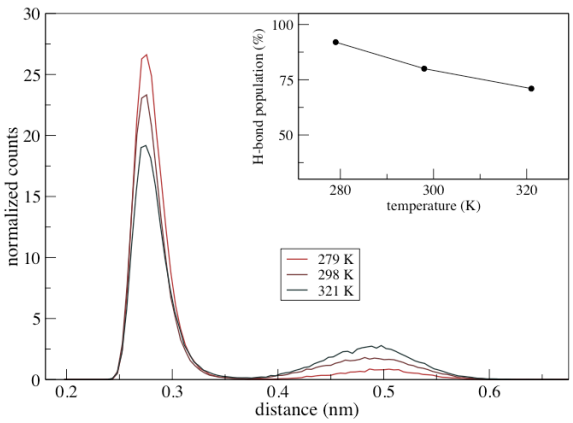
Figure S1: Distribution from MD simulations of Mut2Y (left) and Mut2 (right) at the indicated pressure values (and room temperature) of the distance between (a) chromophore phenolate ring and Y203 ring/ chromophore phenolate and T203 hydroxyl (already reported in the main text) (b) F165 phenol ring and chromophore phenolate ring (c) I167 side-chain center of mass and chromophore phenol ring (d) Q94 NH_2 and chromophore imidazolinone carbonyl oxygen (e) Q69 NH_2 and chromophore imidazolinone carbonyl oxygen (f) E222 protonated oxygen and chromophore imidazolinone nitrogen atom (g) H148 protonated nitrogen and chromophore OH (h) R96 NH_2 and chromophore imidazolinone carbonyl oxygen (see Fig. 5 in the main text). The inset, when present, shows the average distance, with error bands indicating the standard deviation.

Mut2Y

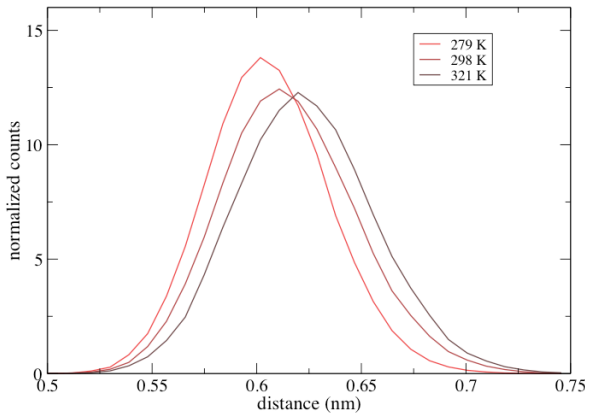
Mut2

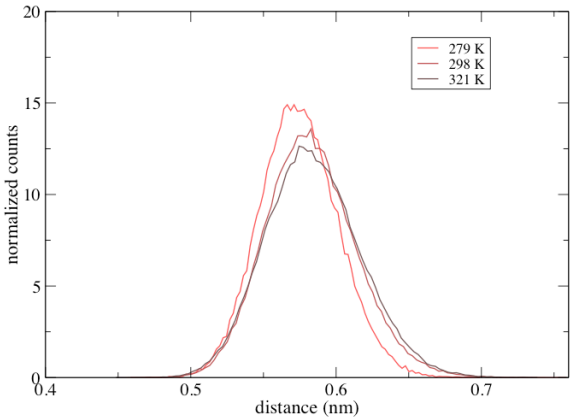
(a) Y203 – chromophore phenol ring/T203 – chromophore phenolate



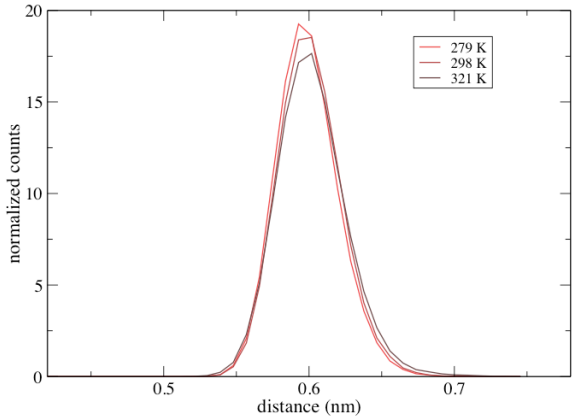


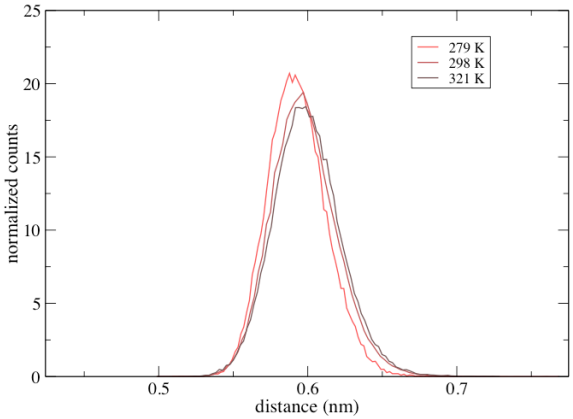
(b) F165 – chromophore phenol ring



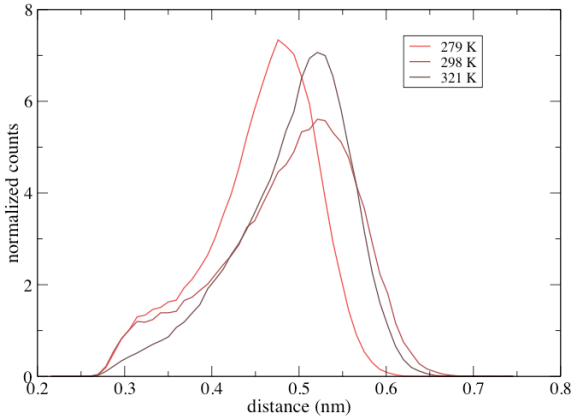


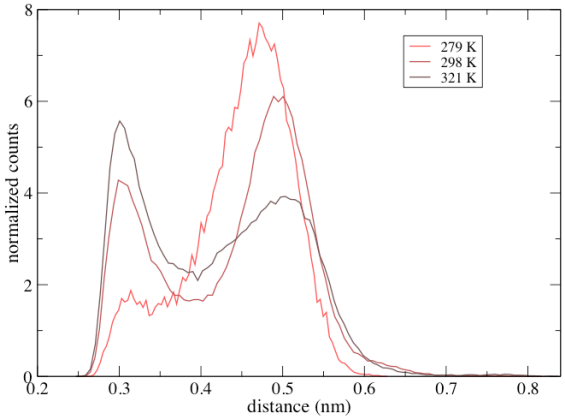
(c) I167 – chromophore phenol ring



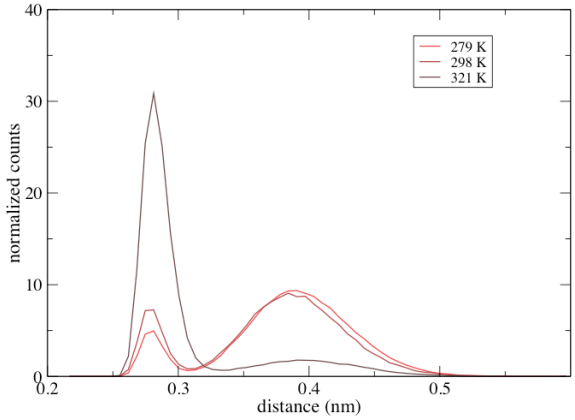


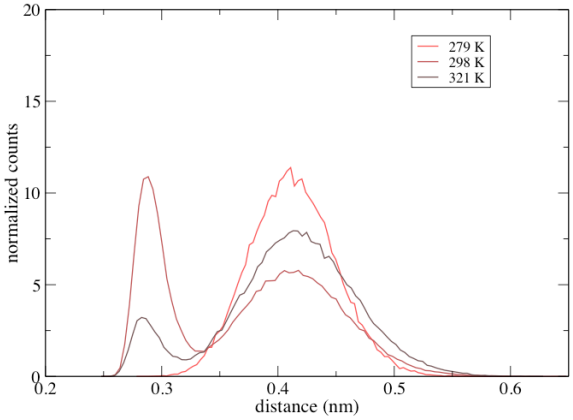
(d) Q94 – chromophore imidazolinone carbonyl



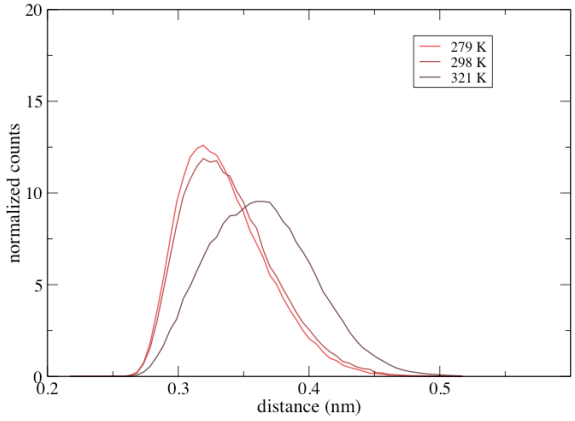


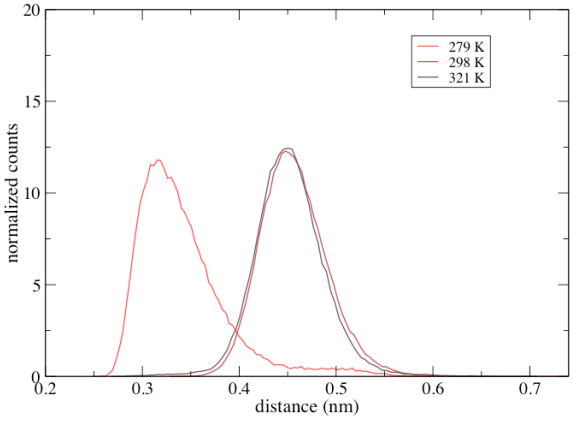
(e) Q69 – chromophore imidazolinone carbonyl



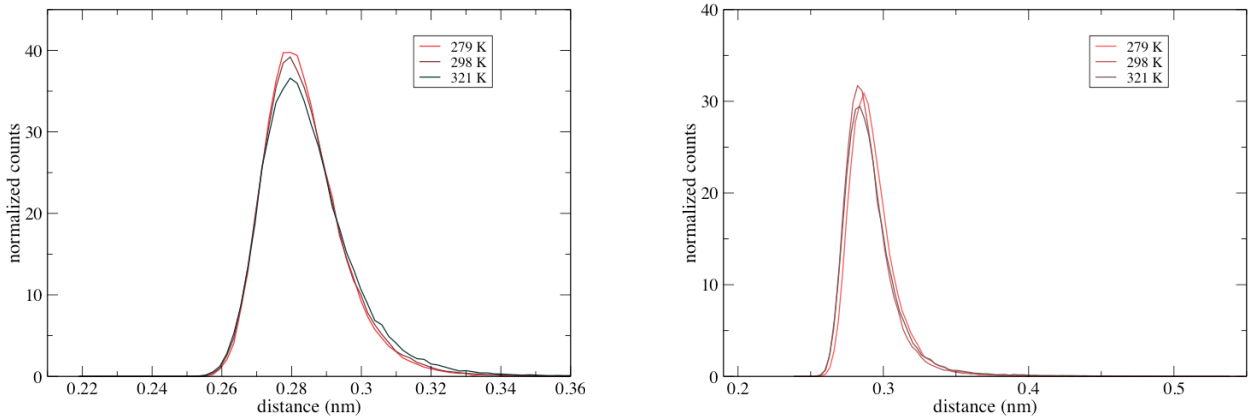


(f) E222 – chromophore imidazolinone





(g) H148 – chromophore phenol oxygen



(h) R96 – chromophore imidazolinone carbonyl

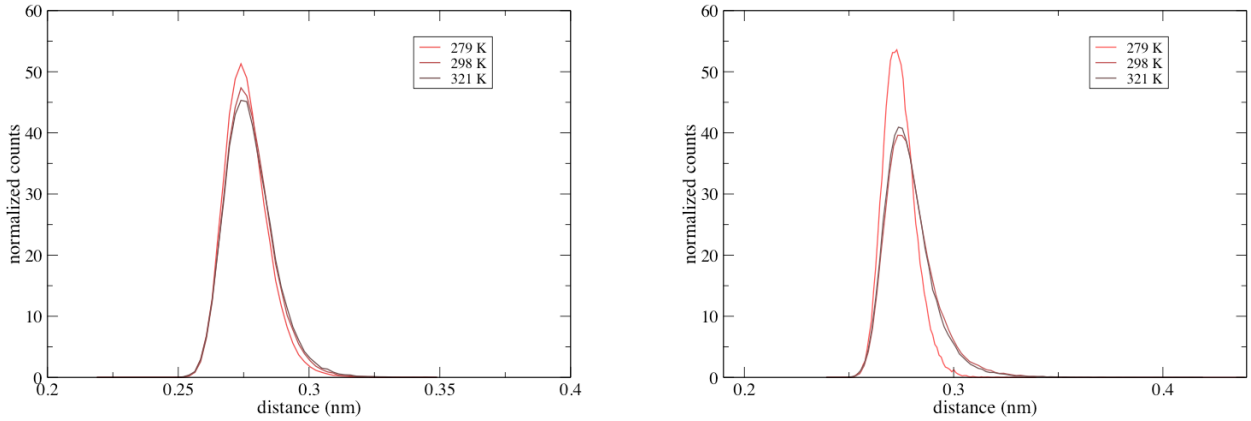


Figure S2: Distribution from MD simulations of Mut2Y (left) and Mut2 (right) at the indicated temperature values (at 0.1 MPa) of the distance between (a) chromophore phenolate ring and Y203 ring/ chromophore phenolate and T203 hydroxyl (already reported in the main text) (b) F165 phenol ring and chromophore phenolate ring (c) I167 side-chain center of mass and chromophore phenolate ring (d) Q94 NH_2 and chromophore imidazolinone carbonyl oxygen (e) Q69 NH_2 and chromophore imidazolinone carbonyl oxygen (O2) (f) E222 protonated oxygen and chromophore nitrogen (g) H148 protonated nitrogen and chromophore phenolate (h) R96 NH_2 and chromophore imidazolinone carbonyl oxygen (see Fig. 5 in the main text).

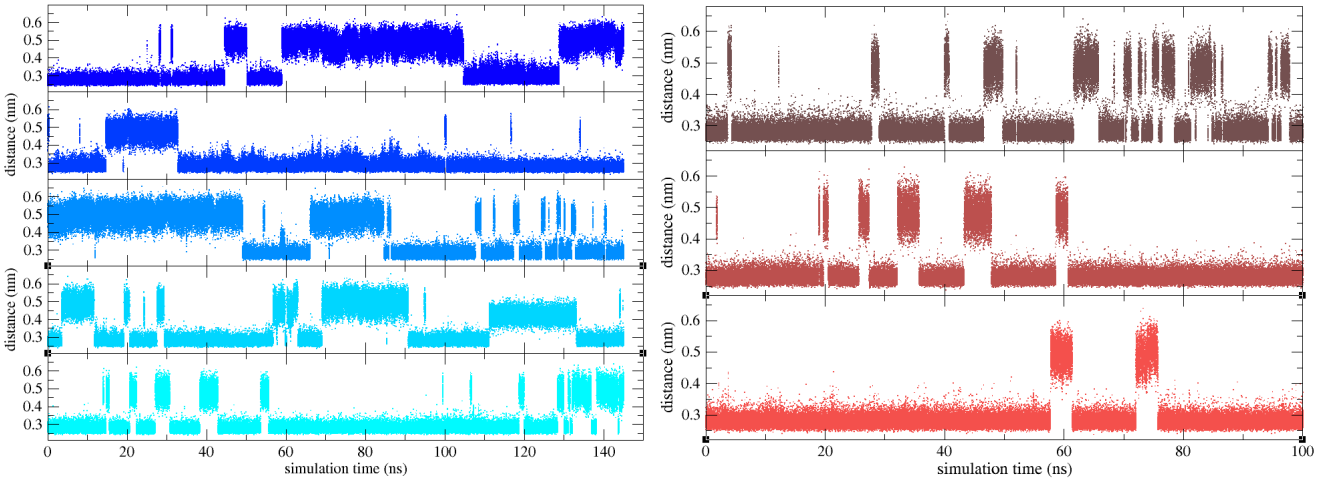


Figure S3: T203-phenolate distance during the MD trajectories at different pressure (left) and temperature (right). Same color code for temperature and pressure values as previous figures.

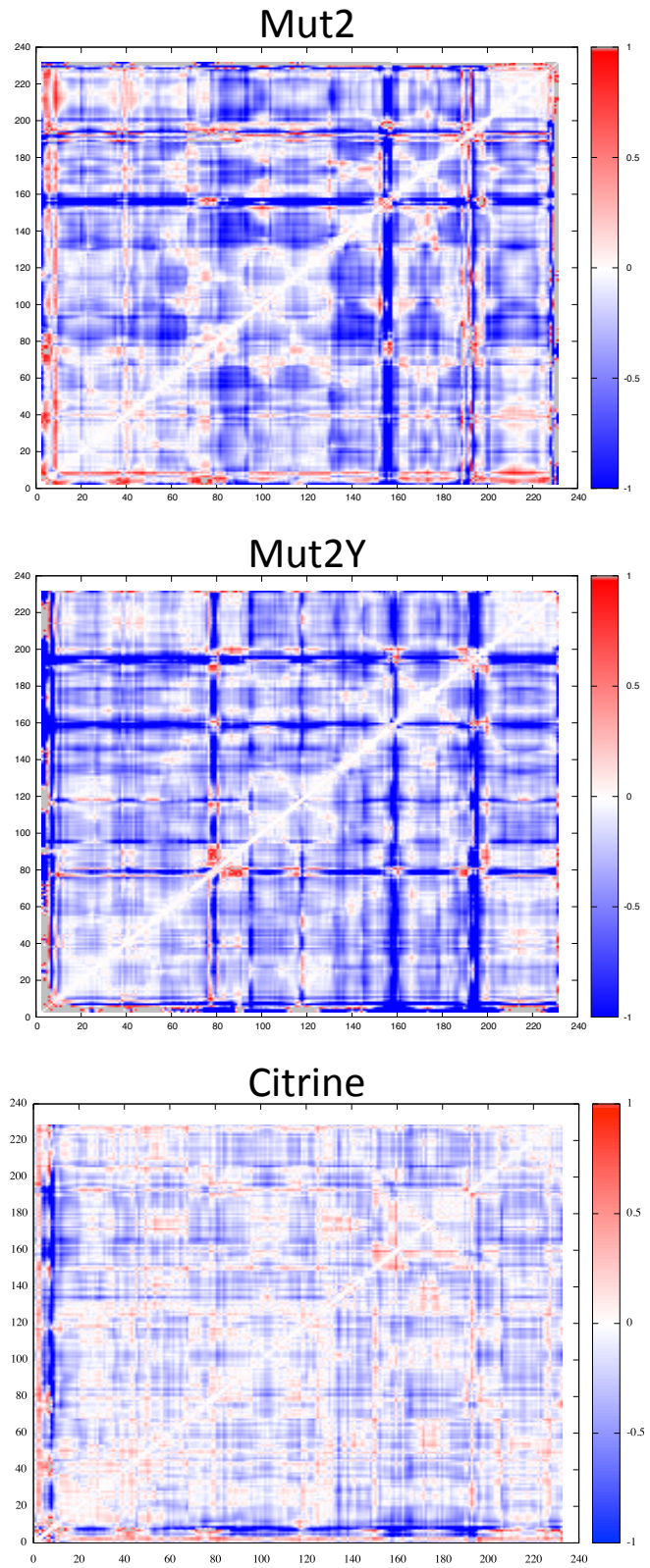


Figure S4: Difference distance matrix calculated for the CA atoms only. The blue color indicates a compression of the corresponding distance. Top: Mut2 MD averages at 0.1 and 500 MPa. Middle: Mut2Y MD averages at 0.1 and 500 MPa. Bottom: PDB structures of cryocooled Citrine at 0.1 and 400MPa (PDB codes 3DPW and 3DQ2 respectively).

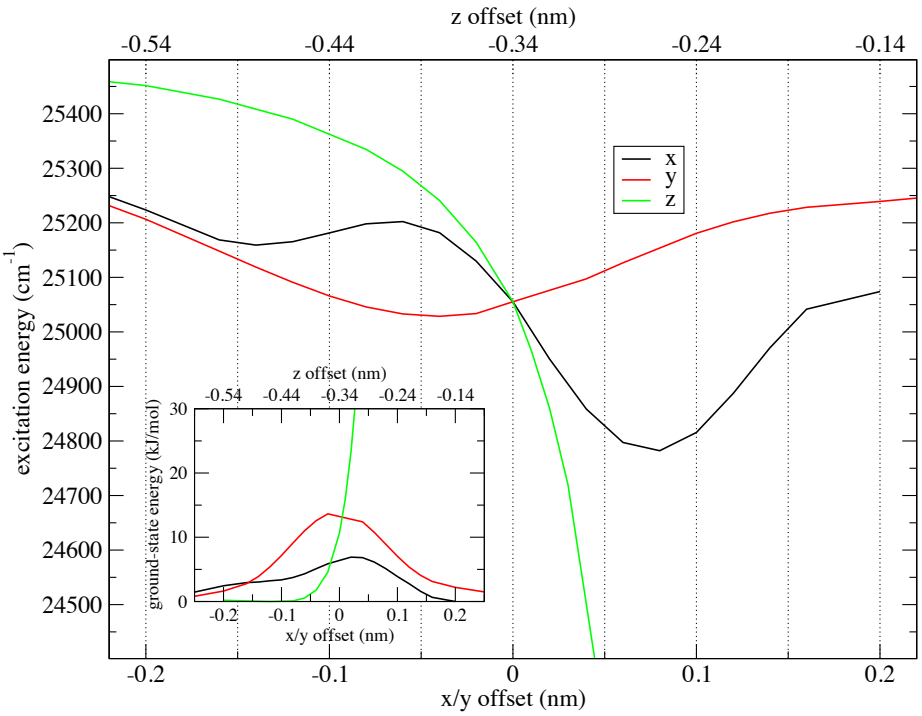


Figure S5: TD-DFT excitation energy (ground-state energy in the inset) as a function of the coordinates of the chromophore phenolate center of mass.

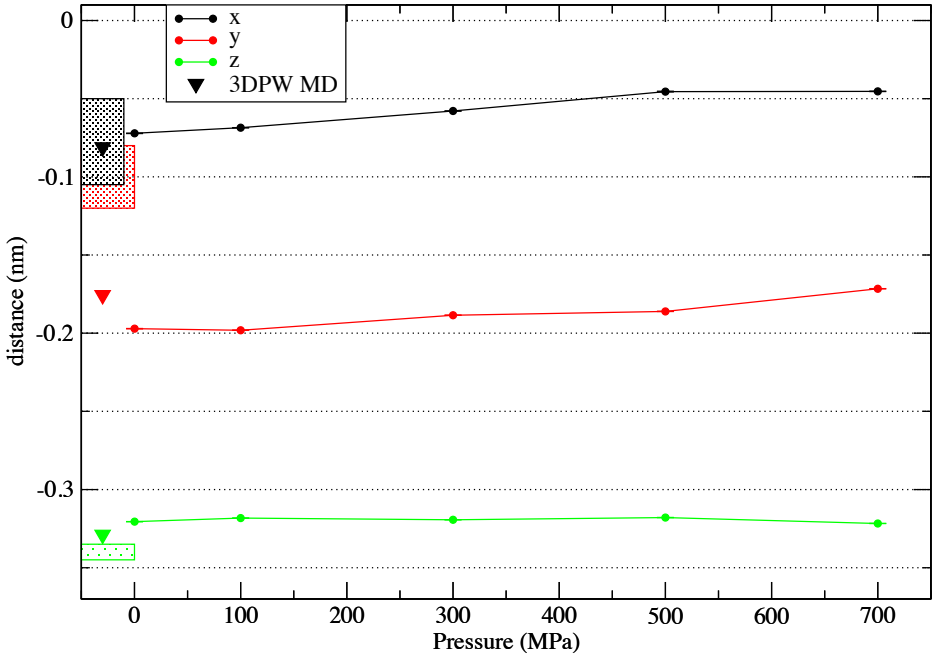


Figure S6: Average center of mass coordinates of the chromophore phenolate ring in Mut2Y as a function of pressure, in the coordinate system indicated in Fig.5c of main text. Shaded areas indicate the range of variation in high-pressure X-ray structures from Barstow et al (ref 14 in the main text). The triangles indicate the results of MD on the ambient pressure Citrine structure (pdb code 3DPW).

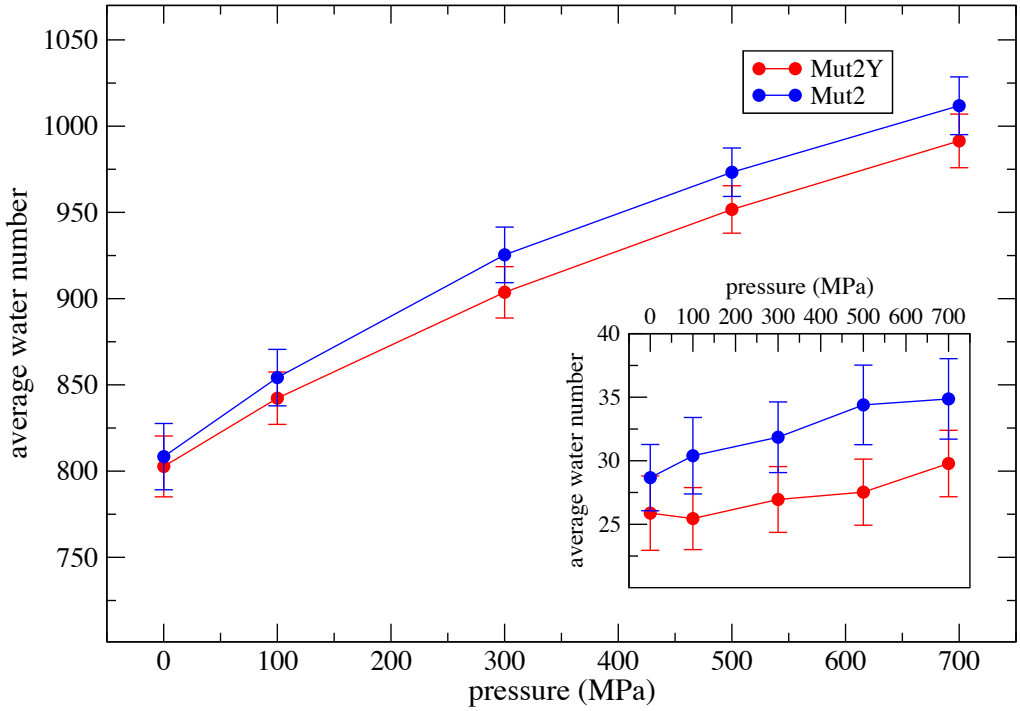


Figure S7: Average number of water molecules at a distance shorter than 0.35 nm from the protein during the MD simulations. The inset shows the average number of "internal" waters, defined as those within 0.35 nm of the side chains of residues inside the β-barrel (residue # 8 10 12 14 16 18 20 22 27 29 31 33 35 37 40 42 44 46 48 53-72 74 83-85 88 92 94 96 98 100 103 104 106 108 110 112 119 121 123 125 127 130 136 137 141 145 148 150 152 161 163 165 167 169 177 179 181 183 185 199 201 203 205 207 218 220 222 224 226).