Supporting Information for

" Mutagenic Induction of an Ultra-Fast Water-Chain Proton Wire"

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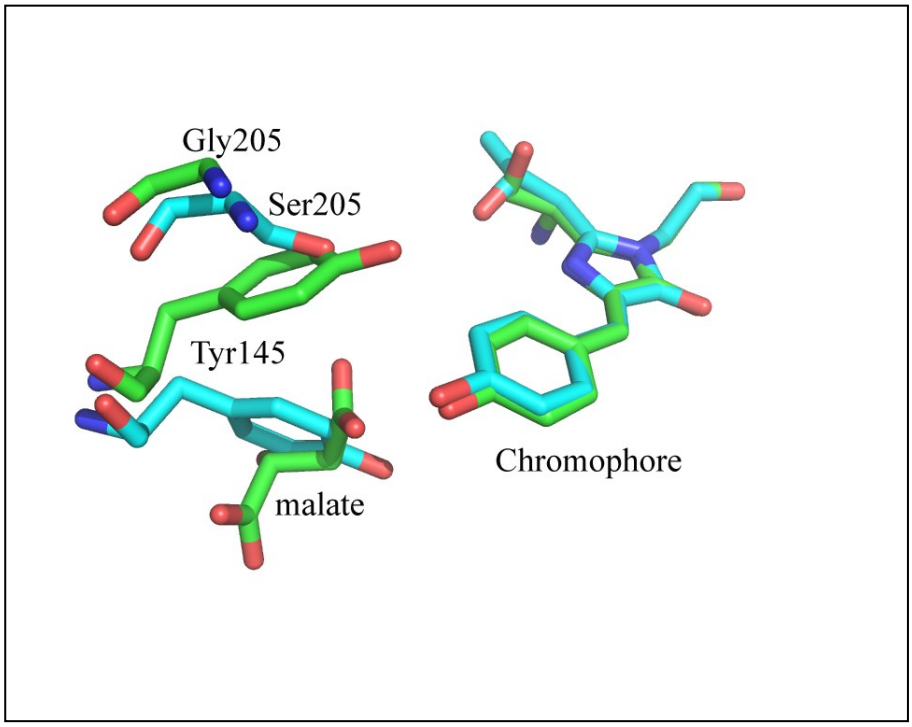

Figure S1: Superposition of chromophore and nearby residues in WT GFP (blue bonds) and GFP S205G (green bonds). The side chain of Tyr145 is displaced by malate and the main chain at position 205 moves out to accommodate the malate molecule (lower left). Oxygen atoms are red, nitrogen is blue and carbon is cyan or green.

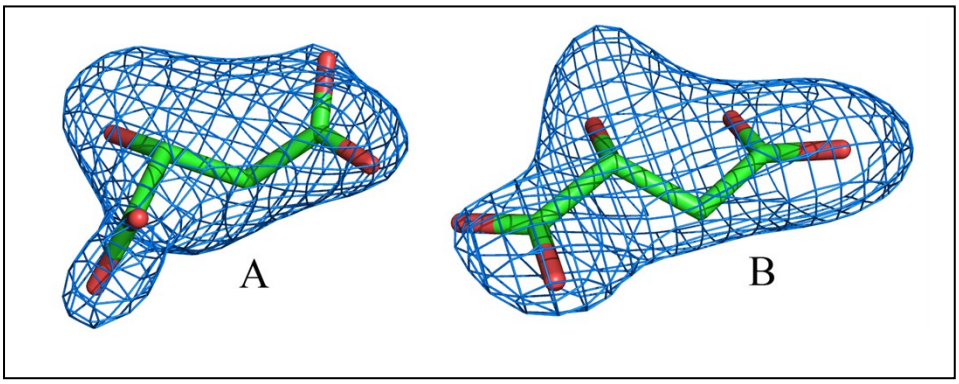

Figure S2: Final atomic model of presumed malate ligands in the active site of GFP S205G, shown superimposed on the Fo-Fc "omit" difference map, contour level 2.8 standard deviations. The presumed malate molecules were removed from the final model, which was then subjected to 10 rounds of crystallographic refinement to remove model bias, and then the Fo-Fc difference coefficients were prepared from the resulting model. A and B refer to the two protein chains comprising the asymmetric unit.

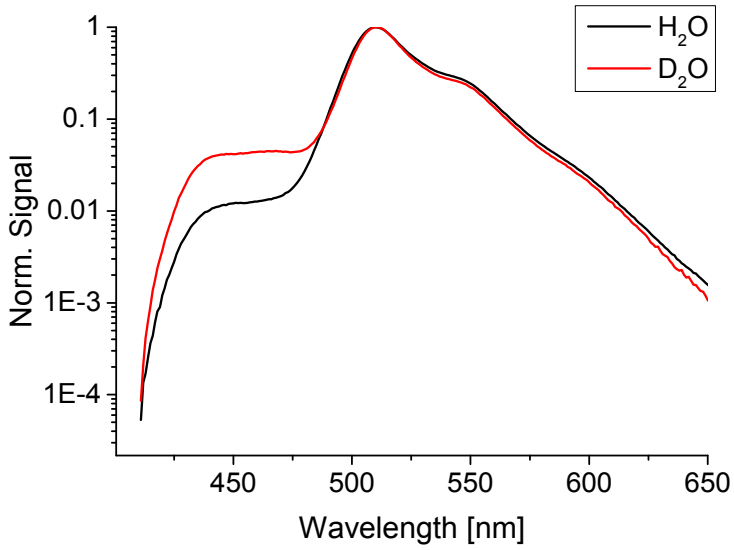

Figure S3: Steady state fluorescence spectrum of S205G in H_2O and D_2O on a normalized semilogarithmic scale.

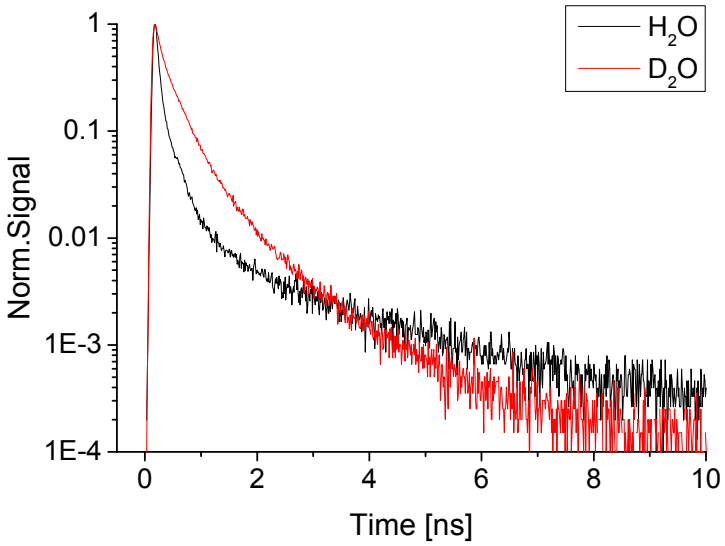

Figure S4: Time-resolved fluorescence of the ROH form of S205G mutant measured at 460nm in both H_2O and D_2O solutions.

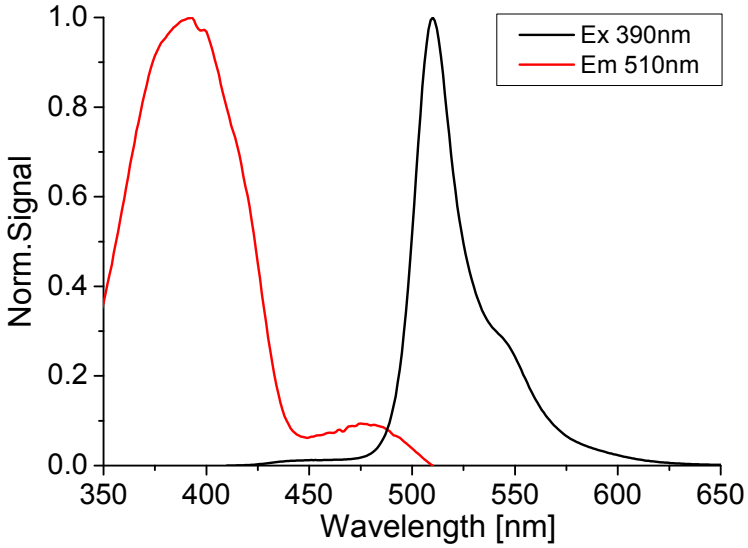

Figure S5: Steady state excitation and emission spectra of the S205G mutant in HEPES pH~7 buffer solution.

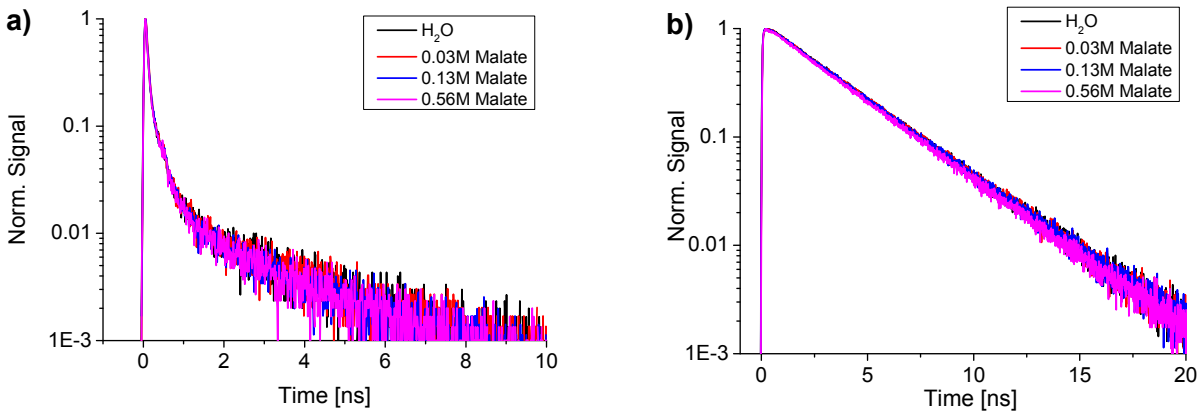

Figure S6: TCSPC time-resolved emission of the S205G mutant, in water and solutions of various malate concentrations (a) ROH form, measured at 460 nm. (b) RO^- form, measured at 520 nm. Figures are shown on a semilog scale.

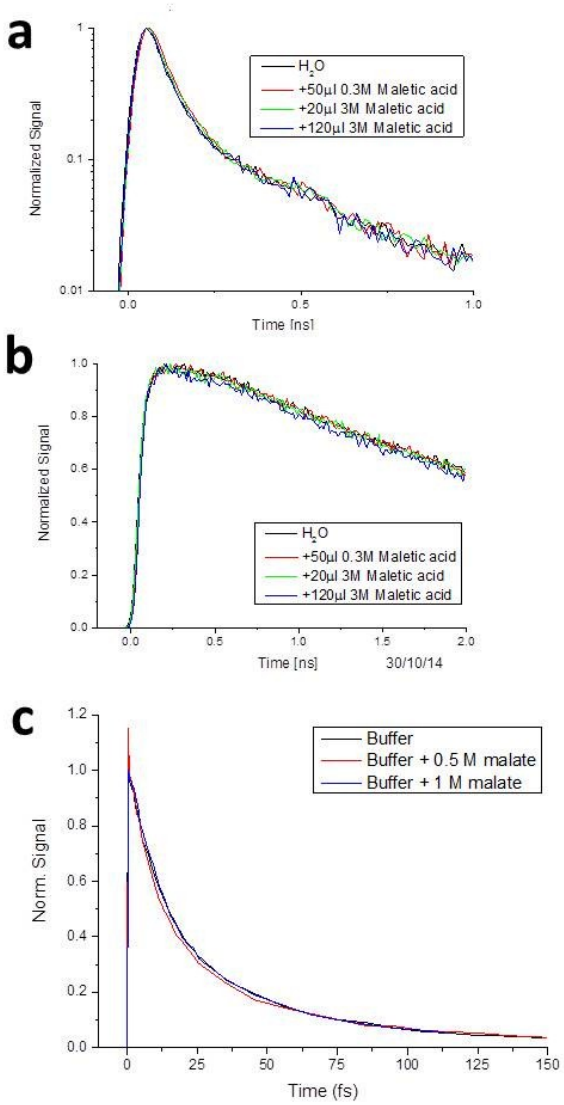

Figure S7: S205G mutant time-resolved measurements in water and solutions of various malate concentrations TCSPC time-resolved emission of (a) ROH form, measured at 460 nm (b) RO- form, meas-ured at 520 nm (c) Fluorescence up-conversion of the ROH form, measured at 460 nm.

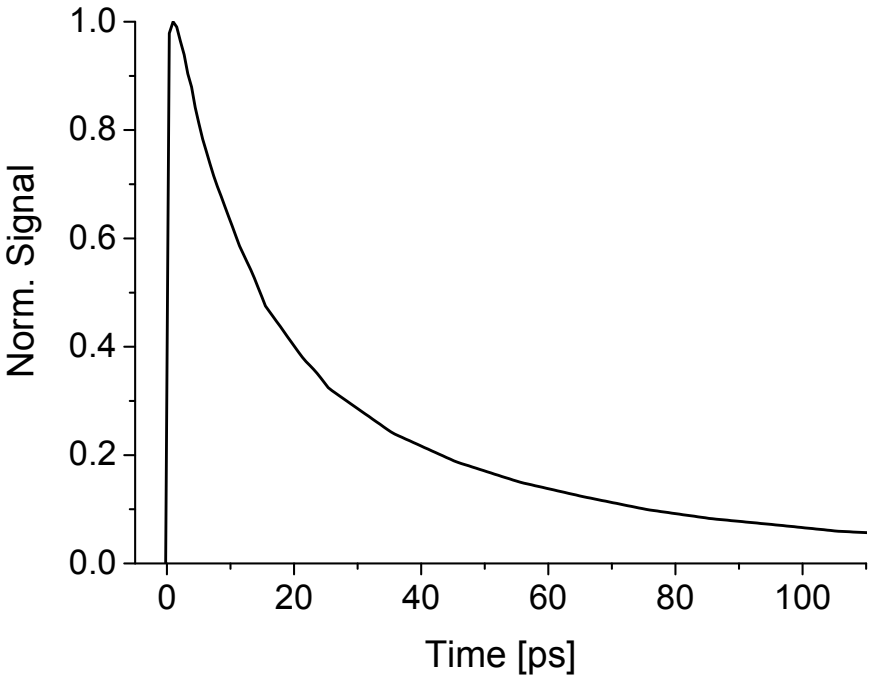

Figure S8: Fluorescence up-conversion of the S205G mutant, a buffer solution with 1M malate concentration.

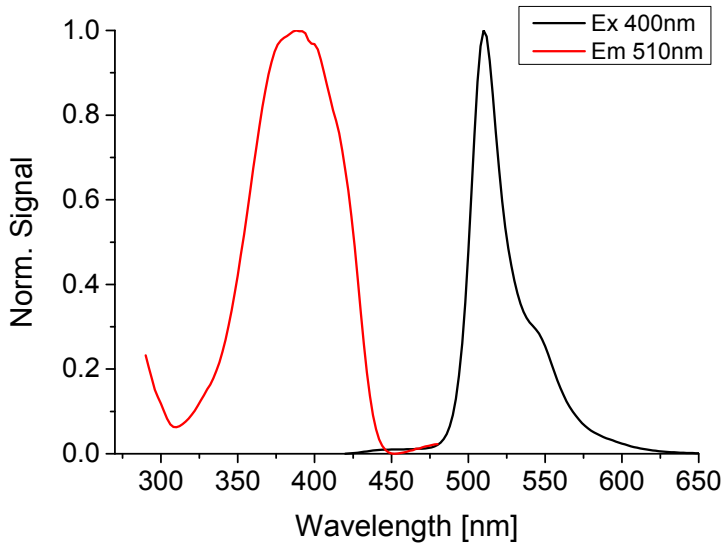

Figure S9: steady-state excitation and emission spectrum of the S205G mutant in pH=7 buffered aqueous solutions in the presence of ~0.5 M malate.

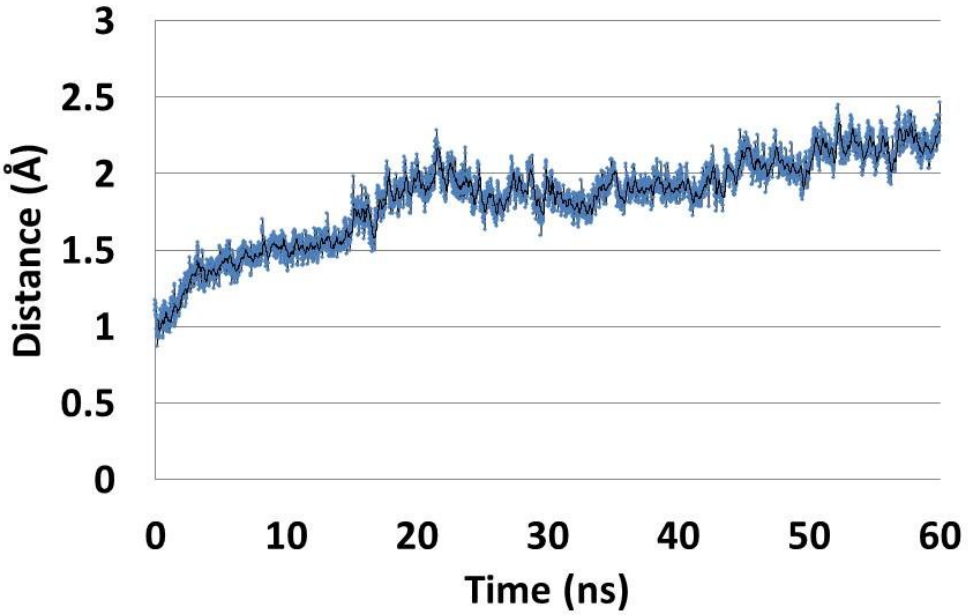

Figure S10: RMSD of the simulated crystal structure of GFP S205G along the MD simulations show a convergence of the structure in the last 20 ns of the simulations.

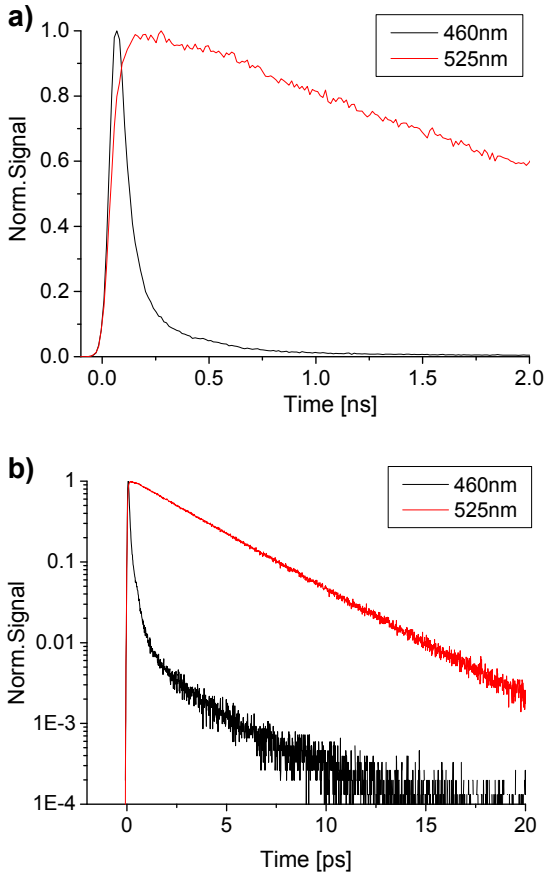

Figure S11: Time-resolved fluorescence of the S205G mutant measured at the ROH emission band maximum 460nm and near the RO^- emission band maximum at 525nm. a) linear scale. b) semilogarithmic scale.

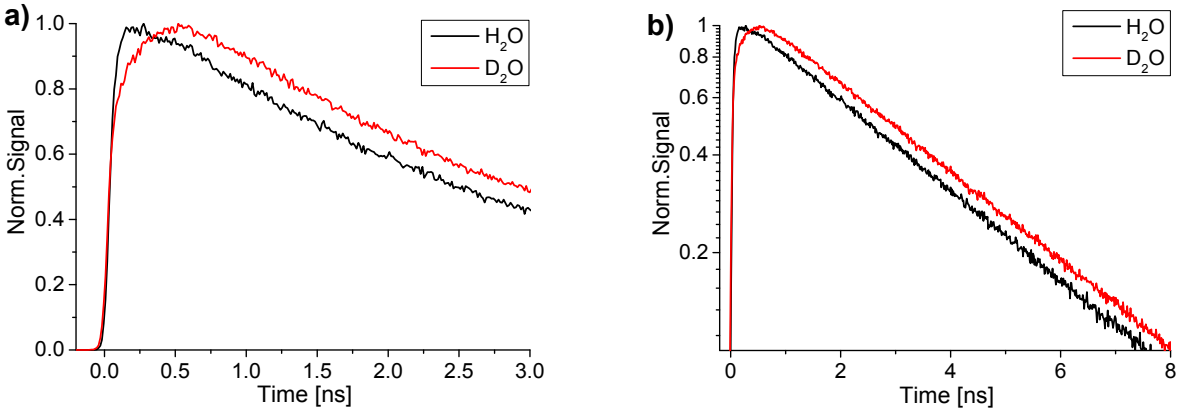

Figure S12: Time-resolved emission of S205G RO^- form in H_2O and D_2O measured at 520 nm. a. linear scale b. semi-logarithmic scale.

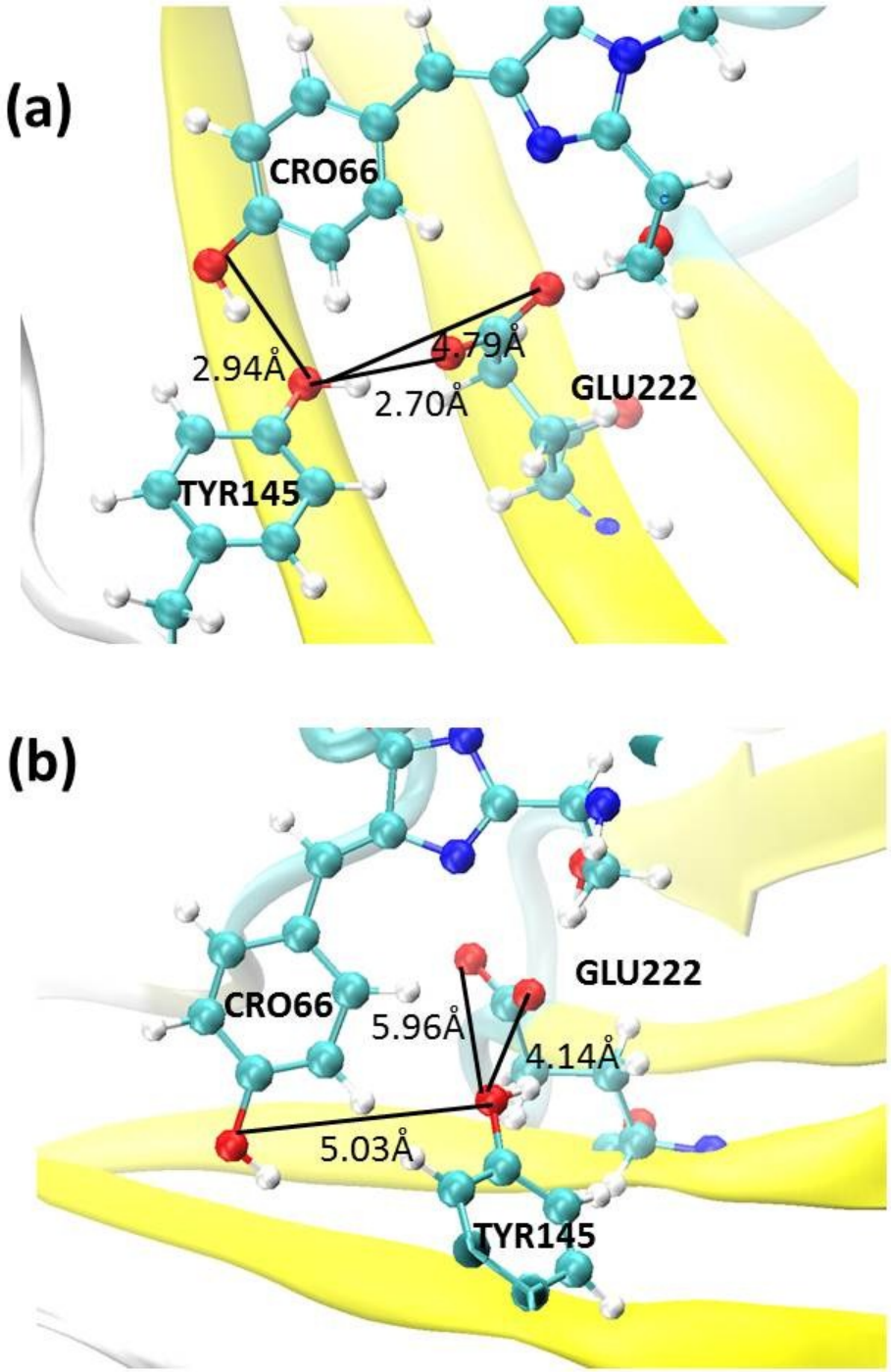

Figure S13: The change of the "proton wire" domain of the crystal structure of GFP S205G mutant.

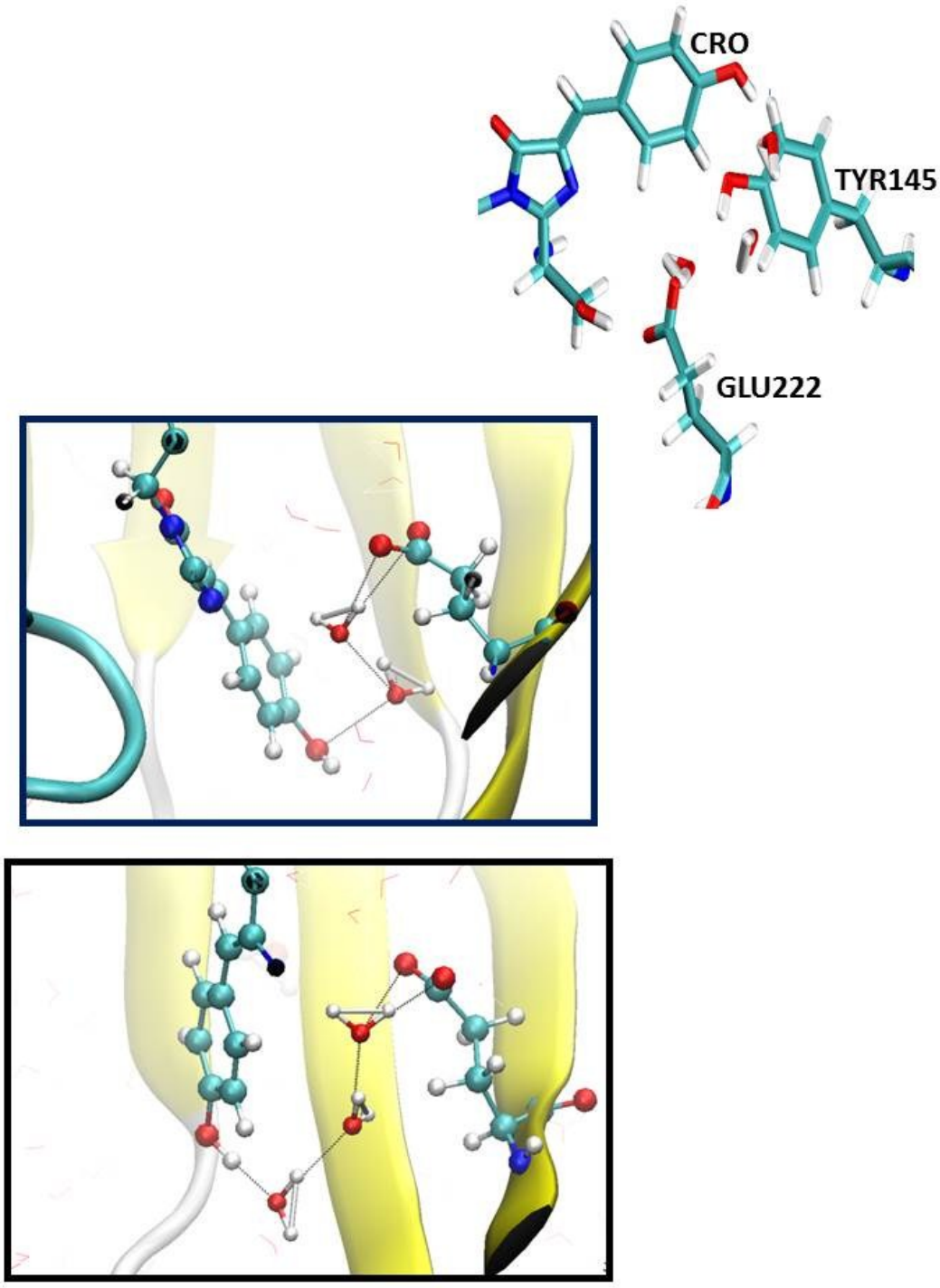

Figure S14: Snapshots from the MD simulations of the newly crystal structure mutant GFP S205G. The Tyr145 fluctuates, forming a "hole" and thus allows to water molecules to accommodate this "hole".

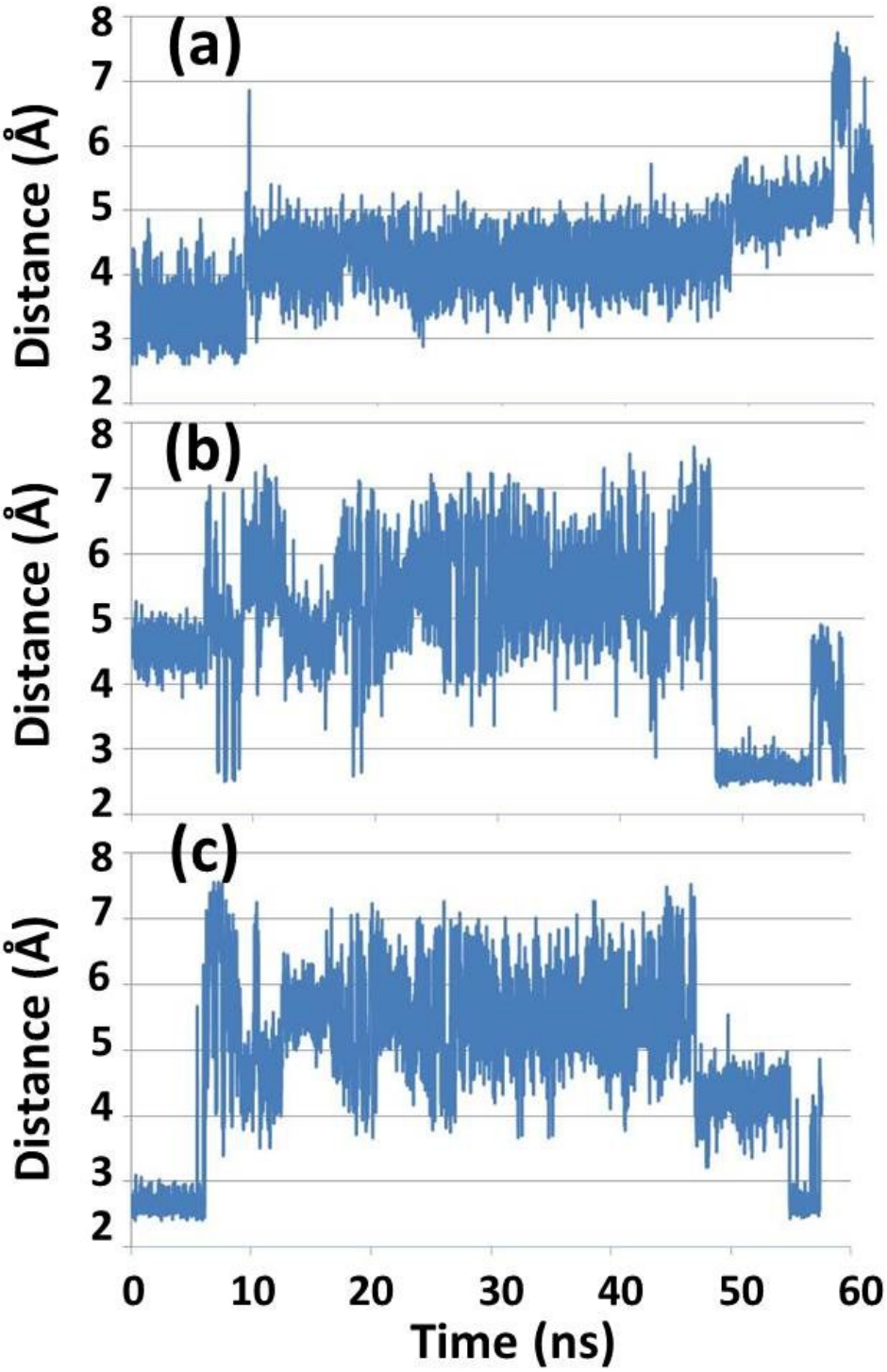

Figure S15: (a) The O(Tyr145)-O(CRO), (b) O(Tyr145)-O1(Glu222) and O(Tyr145)-O1(Glu222) distances along the MD simulations.

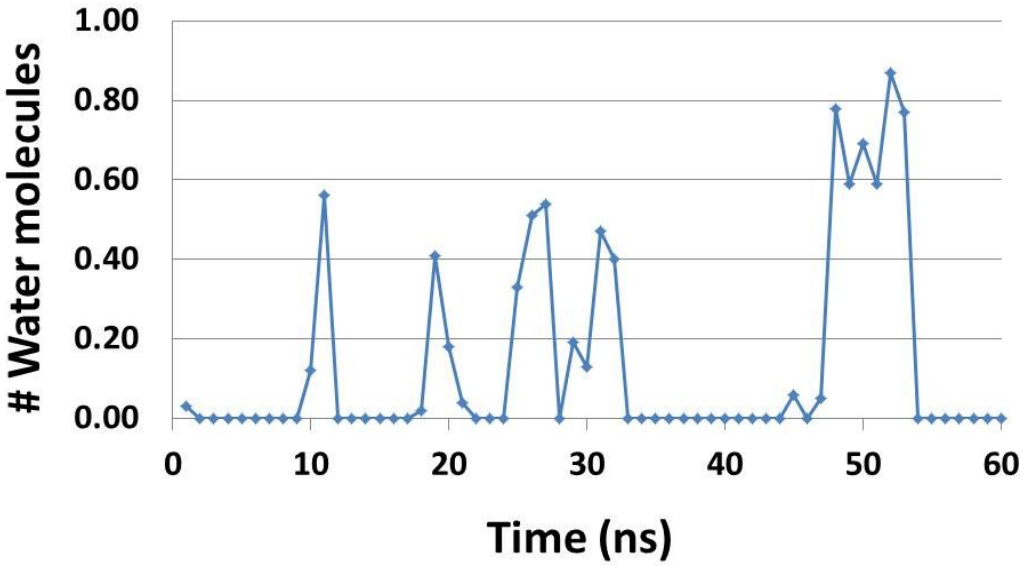

Figure S16: The backbone solvation of the Glu222 along the MD simulations.

Table S1: Multiexponential fit function parameters for the fluorescence decay of the ROH forms of wt-GFP and the S205G mutant [a]

	a_1	τ_1[fs]	a_2	τ_2[fs]	a_3	τ_3[ps]
wt-GFP	0.37	2600	0.49	8800	0.14	60
S205G	0.08	4600	0.78	18500	0.14	120

[a] $y = \sum_{i=1}^{3} a_i \cdot \exp(-t/\tau_i)$

Table S2: Crystallographic data, refinement and atomic model statistics

Mutation	S205G
Total reflections	280,842
Unique reflections observed	13,474
Space Group	C 2 2 21
Cell dimensions (a, b, c) (Å)	66.03, 96.28, 151.59
Resolution (Å)	50-2.49 (2.54-2.49)
Completeness[a] (%)	96.9 (12.4)
Average I/σ[a]	13.8 (1.6)
R_{merge}[a,b]	0.10 (0.43)
Atomic model statistics	
Asymmetric unit	2 protein chains (A,B)
Crystallographic *R*-factor[c]	0.178 (0.33)
R-free	0.277 (0.31)
R-factor, all data combined	0.180 (0.33)
Average B-factors, Protein atoms ($Å^2$)	39
Average B-factors, Solvent	32
rms bond lengths (Å)	0.014
rms bond angles (degrees)	1.8

[a] Values in parentheses indicate statistics for the highest resolution shell.

[b] Rmerge = $\Sigma_i\Sigma_j(I_{ij} - \langle I\rangle_i)/\Sigma_i\Sigma_j\langle I\rangle_i$, where I_{ij} is the amplitude of the jth observation of reflection i and $\langle I\rangle_i$ is the mean value of observations $I_{ij.}$

[c] *R*-factor = $\Sigma||F_o|-|F_c||/\Sigma|F_o|$, where F_o and F_c are the observed and calculated structure amplitudes.