Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for "Insight into the Structure and the Mechanism of the Slow Proton Transfer in the GFP Double Mutant T203V/S205A"

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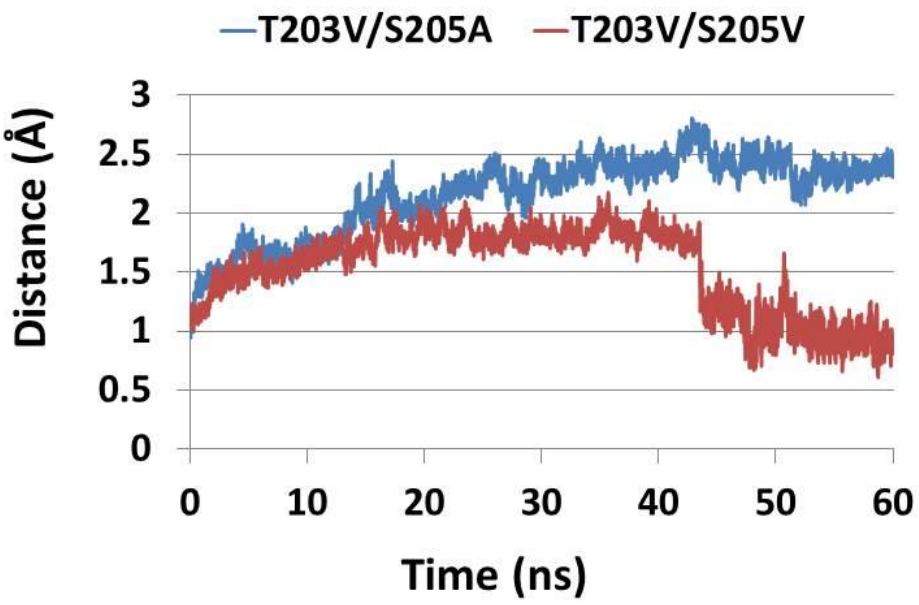

Figure S1: RMSD of T203V/S205A and T203V/S205V.

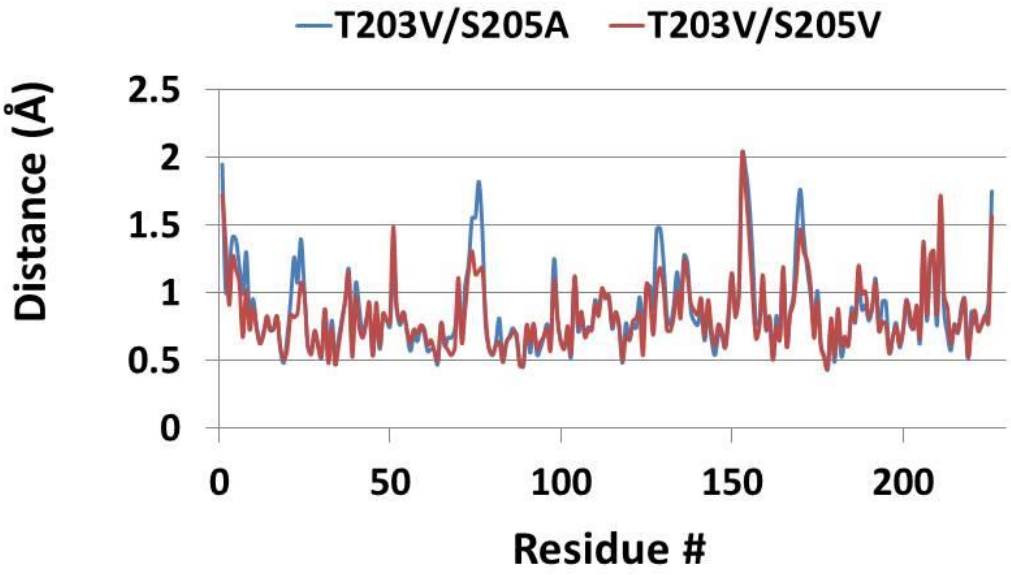

Figure S2: RMSF of T203V/S205A and T203V/S205AV.

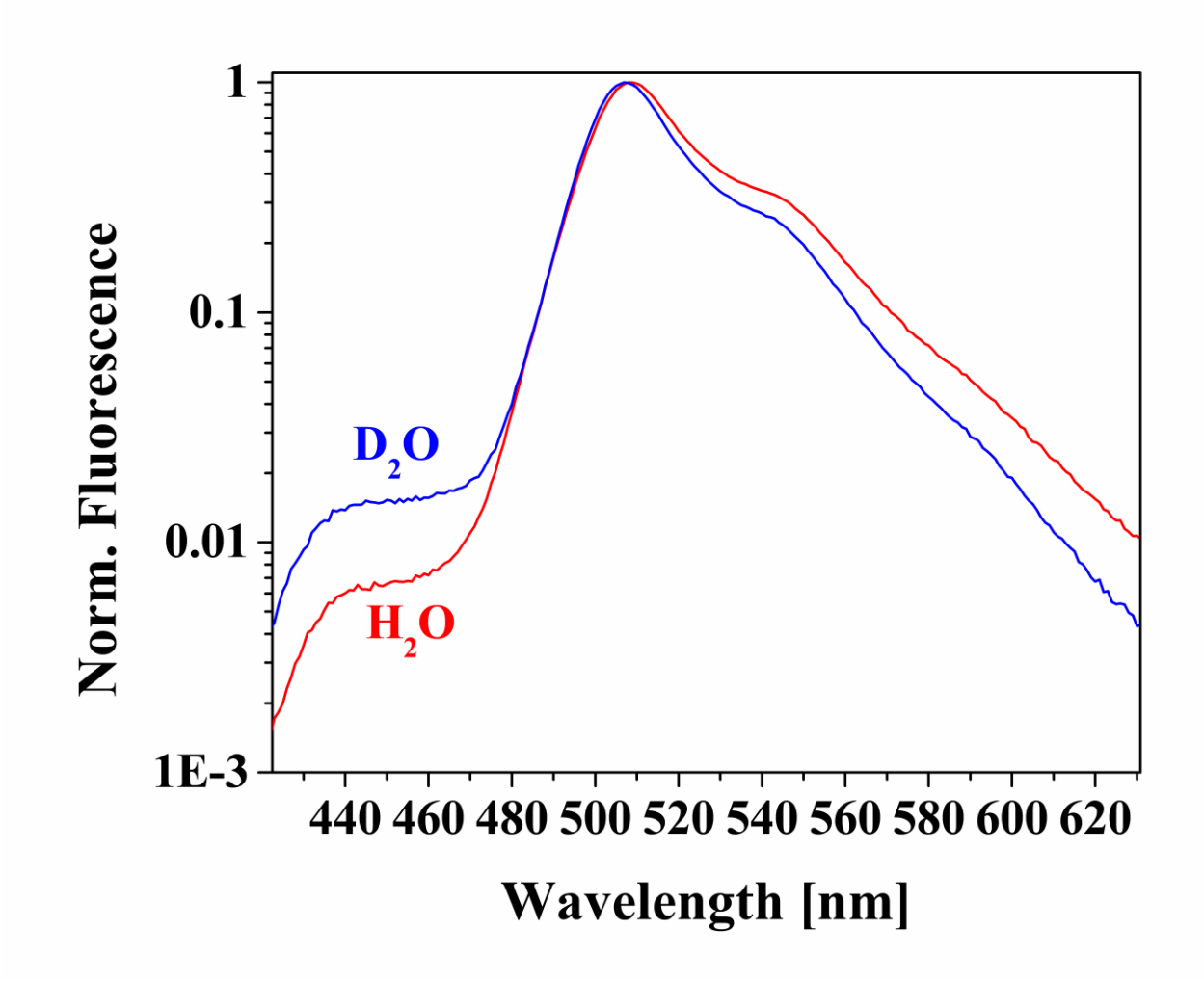

Figure S3: The steady-state emission of wt-GFP in H_2O and D_2O. This figure shows that the KIE of the wt-GFP is ≈5.

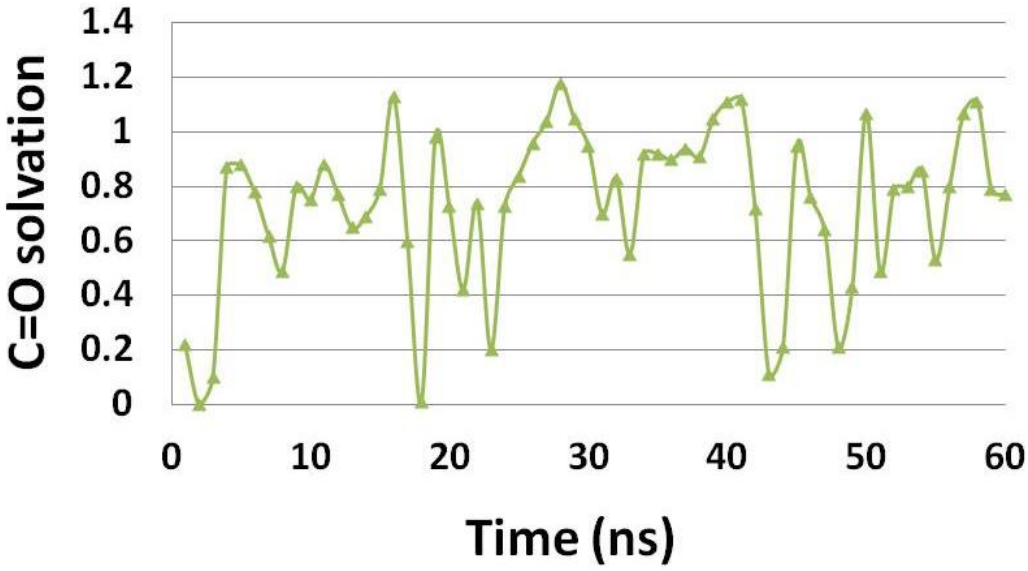

Figure S4: The average number of water molecules around each side chain Cβ carbon (within 4 Å) of Glu222 for the GFP double mutant T203V-S205A along the MD simulations.

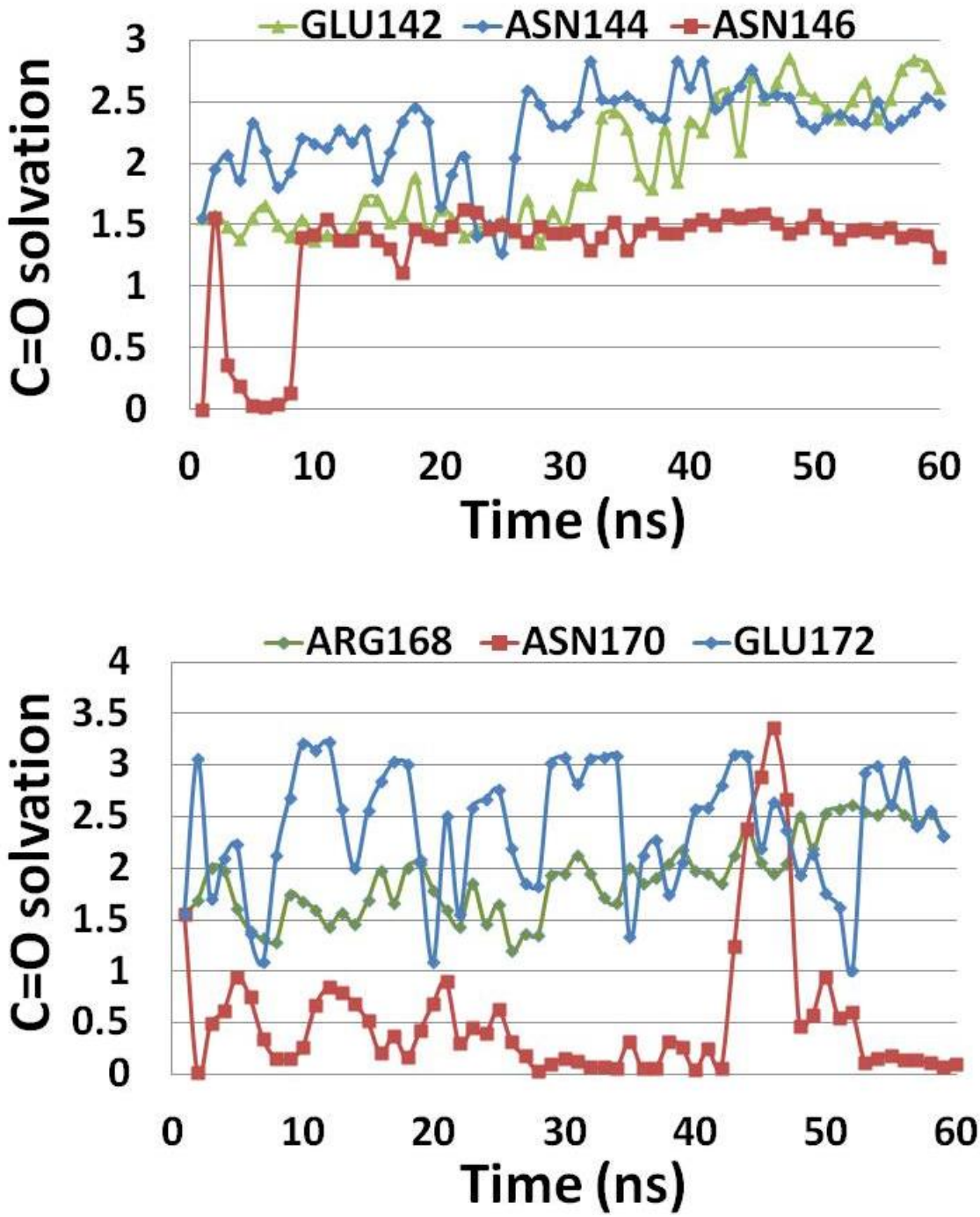

Figure S5: The average number of water molecules around each side chain Cβ carbon (within 4 Å) of residues along the two β-strands in the "hole" domain for the GFP double mutant T203V-S205A along the MD simulations.

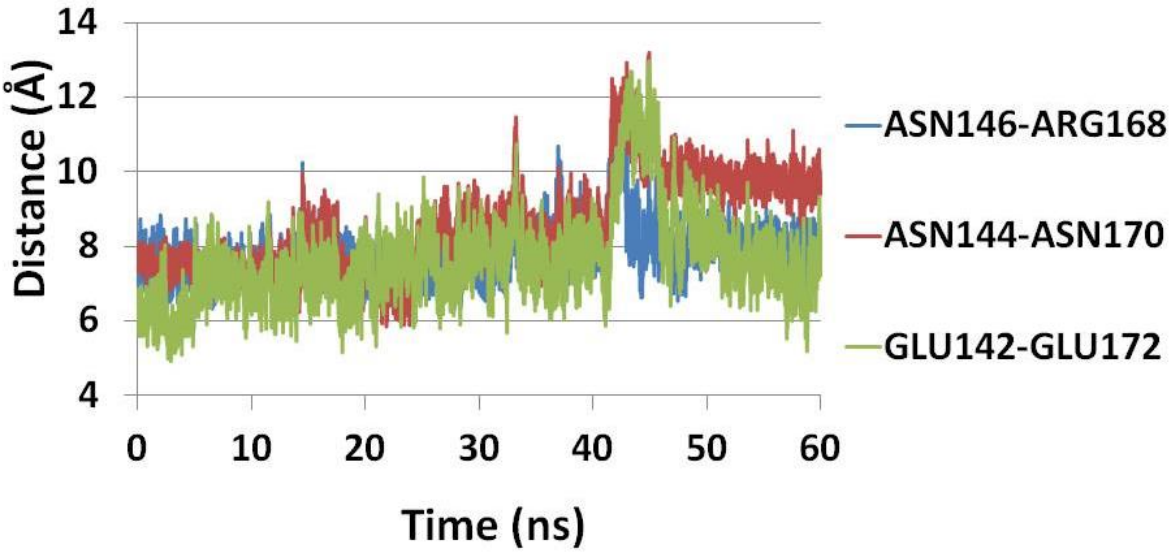

Figure S6: The Cα backbone-backbone distances between two opposite residues in the two β-strands for the GFP double mutant T203V-S205A during the MD simulations.

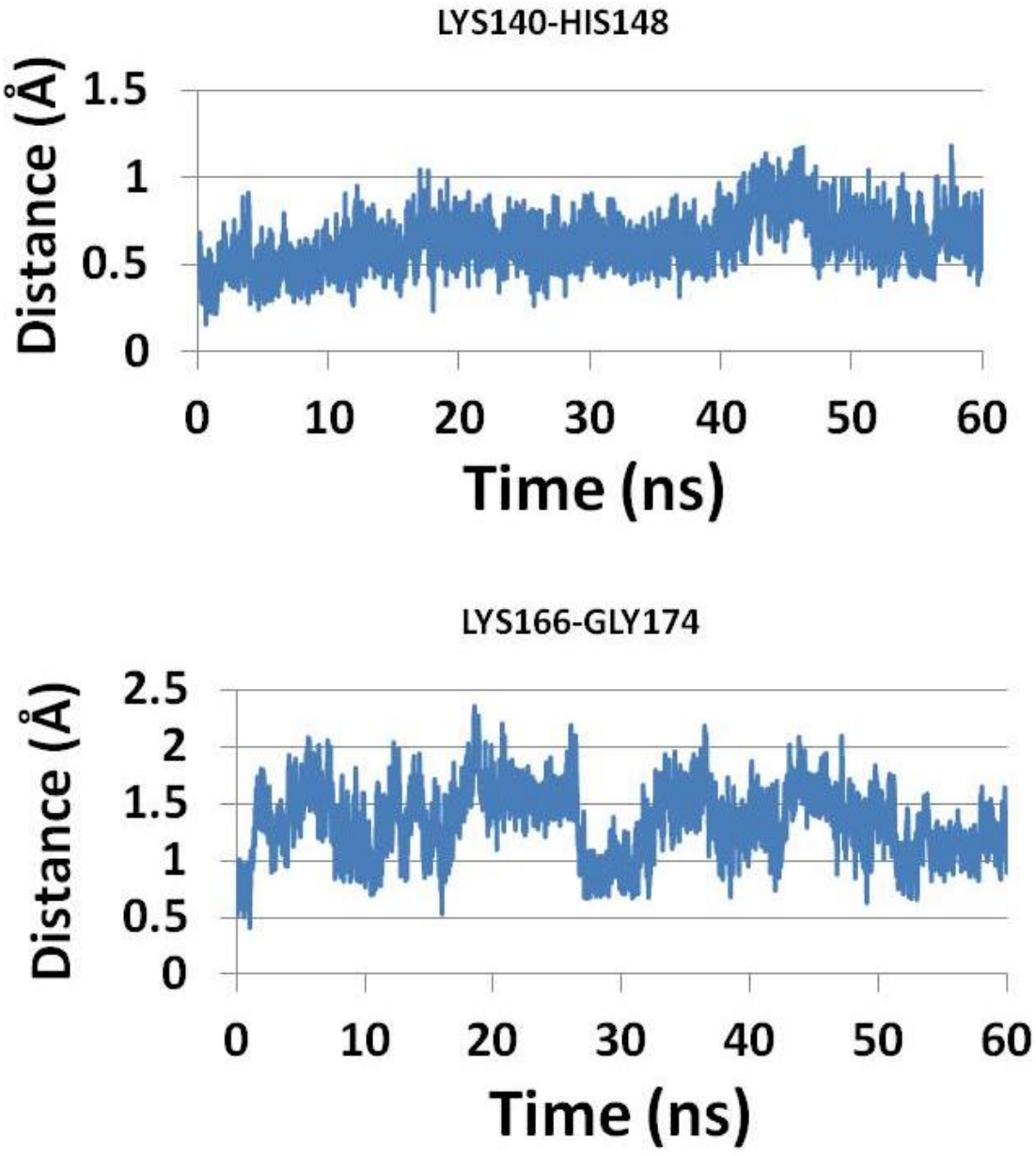

Figure S7: RMSD of the fragments of the two β-strands in the "hole" domain of the GFP double mutant T203V-S205A along the MD simulations.

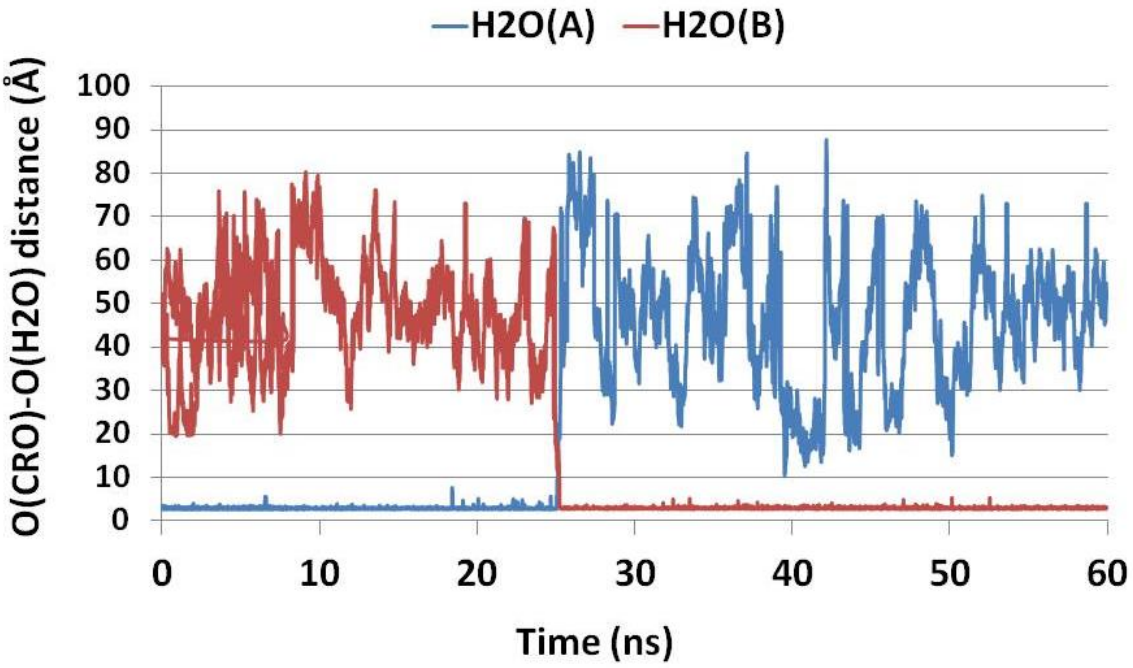

Figure S8: The distance between the O atom of the hydroxyl phenol group of the chromophore and the nearby O atom of the water molecule during the MD simulations. Two water molecules that are replaced during the simulations of 60 ns for T203V/S205A.

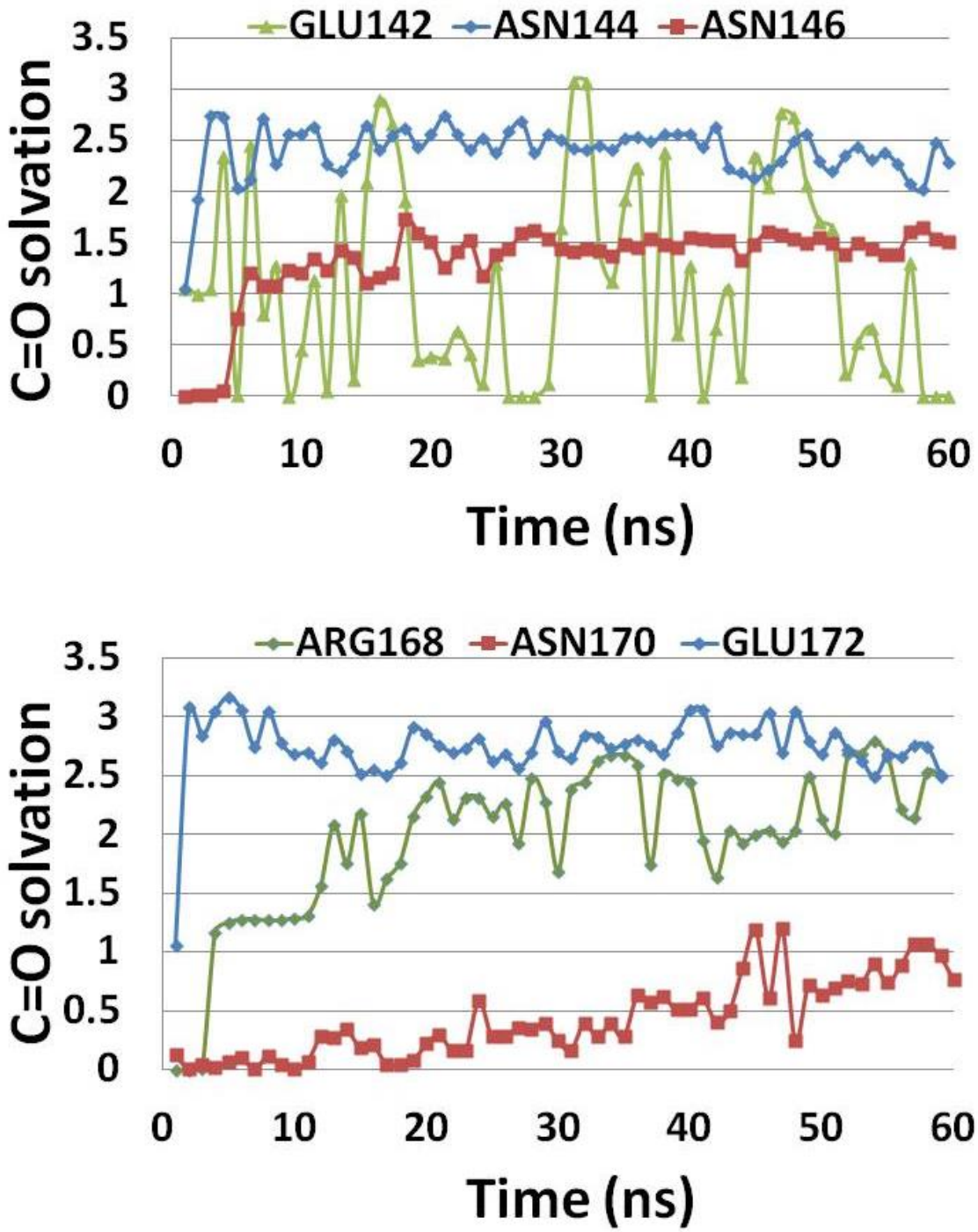

Figure S9: The average number of water molecules around each side chain Cβ carbon (within 4 Å) of residues along the two β-strands in the "hole" domain for the wt-GFP.

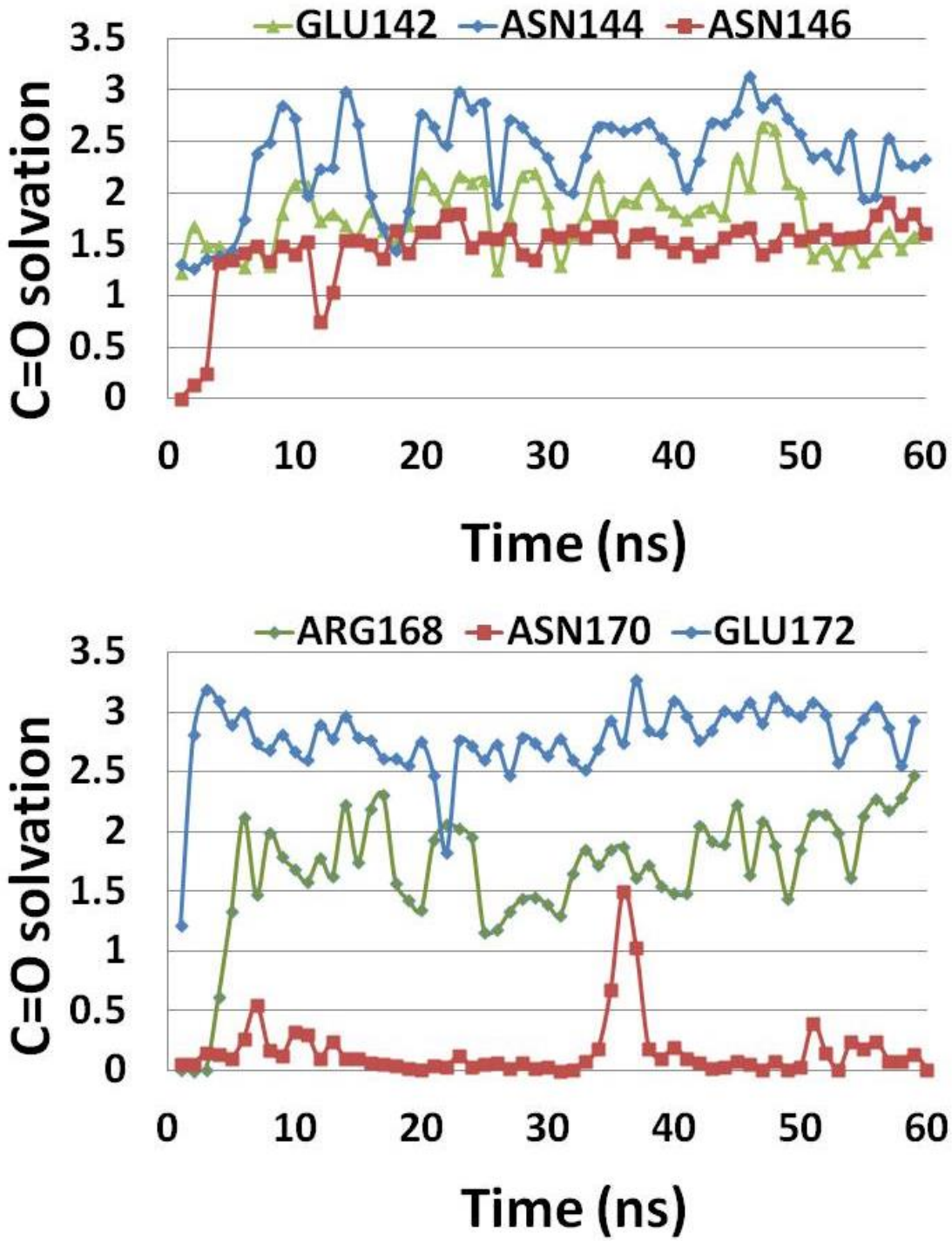

Figure S10: The average number of water molecules around each side chain Cβ carbon (within 4 Å) of residues along the two β-strands in the "hole" domain for the GFP double mutant T203V/S205V.

Estimation of pK_a of Asn146

It is extremely difficult to estimate the "apparent pK_a" of a given amino acid in a protein, because in addition to factoring in changes due to the local side-chain environment, one has to average over all the possible charge states of all interacting titratable groups, as a function of pH, in order to determine long-range effects.

However, it is possible to estimate the shift in "intrinsic pK_a" for Asn146, due to its immediate environment. We used the solvation/desolvation thermodynamic cycle procedure and the APBS package[1-3] to estimate the change in Asn146 intrinsic pK_a. The shift values that were obtained are positive and between 0.2 and 1.0, depending on the particular force field used to assign partial atomic charges (CHARMM, AMBER and PARSE). All other parameters were left at the recommended values[1-3]. Therefore, one can add to the textbook value for the Asn side chain in solution (16-17) this small value to roughly estimate the Asn146 pK_a to be greater than 16.

As an independent check of this result, we further estimated the side chain pK_a of a hypothetical mutation, Asn146 to Asp146 *in silico*, using the programs PROPKA[4-7] and PDB2PQR[8-9] (which use completely different procedures to estimate pK_a values) and the result agrees with that obtained from APBS for Asn146. The pK_a of the hypothetical Asp146 is estimated by PROPKA to be 4.3, and is thus elevated by less than one unit compared to the model amino acid side chain, assumed by PROPKA to be 3.8.

In summary, as expected because of the overall large negative charge on GFP (about -7 at pH 7.0) and the immediate environment of Asn146, there is no possibility that Asn146 is charged under ordinary circumstances.

References

1. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. Proc. Natl. Acad. Sci. USA 98, 10037-10041 2001.

2. M. Holst and F. Saied, Multigrid solution of the Poisson-Boltzmann equation. J. Comput. Chem. 14, 105-113, 1993.

3. M. Holst and F. Saied, Numerical solution of the nonlinear Poisson-Boltzmann equation: Developing more robust and efficient methods. J. Comput. Chem. 16, 337-364, 1995.
4. Hui Li, Andrew D. Robertson, and Jan H. Jensen "Very Fast Empirical Prediction and Interpretation of Protein pKa Values" Proteins, 2005, 61,704-721.
5. Delphine C. Bas, David M. Rogers, and Jan H. Jensen "Very Fast Prediction and Rationalization of pKa Values for Protein-Ligand Complexes" Proteins, 2008, 73, 765-783.
6. Mats H.M. Olsson, Chresten R. Søndergard, Michal Rostkowski, and Jan H. Jensen "PROPKA3: Consistent Treatment of Internal and Surface Residues in Empirical pKa predictions" Journal of Chemical Theory and Computation, 2011 7 (2), 525-537
7. Chresten R. Søndergaard, Mats H.M. Olsson, Michaz Rostkowski, and Jan H. Jensen "Improved Treatment of Ligands and Coupling Effects in Empirical Calculation and Rationalization of pKa Values" Journal of Chemical Theory and Computation, 2011 7 (7), 2284-2295.
8. Dolinsky TJ, Czodrowski P, Li H, Nielsen JE, Jensen JH, Klebe G, Baker NA. PDB2PQR: Expanding and upgrading automated preparation of biomolecular structures for molecular simulations. Nucleic Acids Res, 35, W522-5, 2007.
9. Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA. PDB2PQR: an automated pipeline for the setup, execution, and analysis of Poisson-Boltzmann electrostatics calculations. Nucleic Acids Res, 32, W665-W667, 2004.