Supporting Information for:

Development and Validation of Fluorinated, Aromatic Amino Acid Parameters for use with the AMBER ff15ipq Protein Force Field

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Supporting Figures

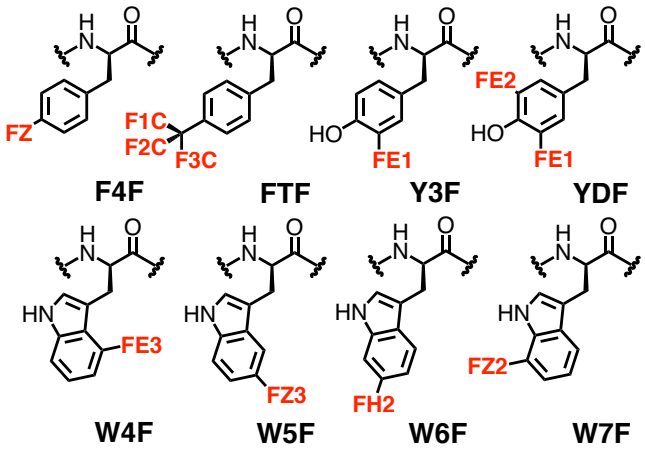


Figure S1. New fluorinated residue atom names presented in this work and their respective 3-letter identifier codes. The substituted fluorine atom is shown in red.

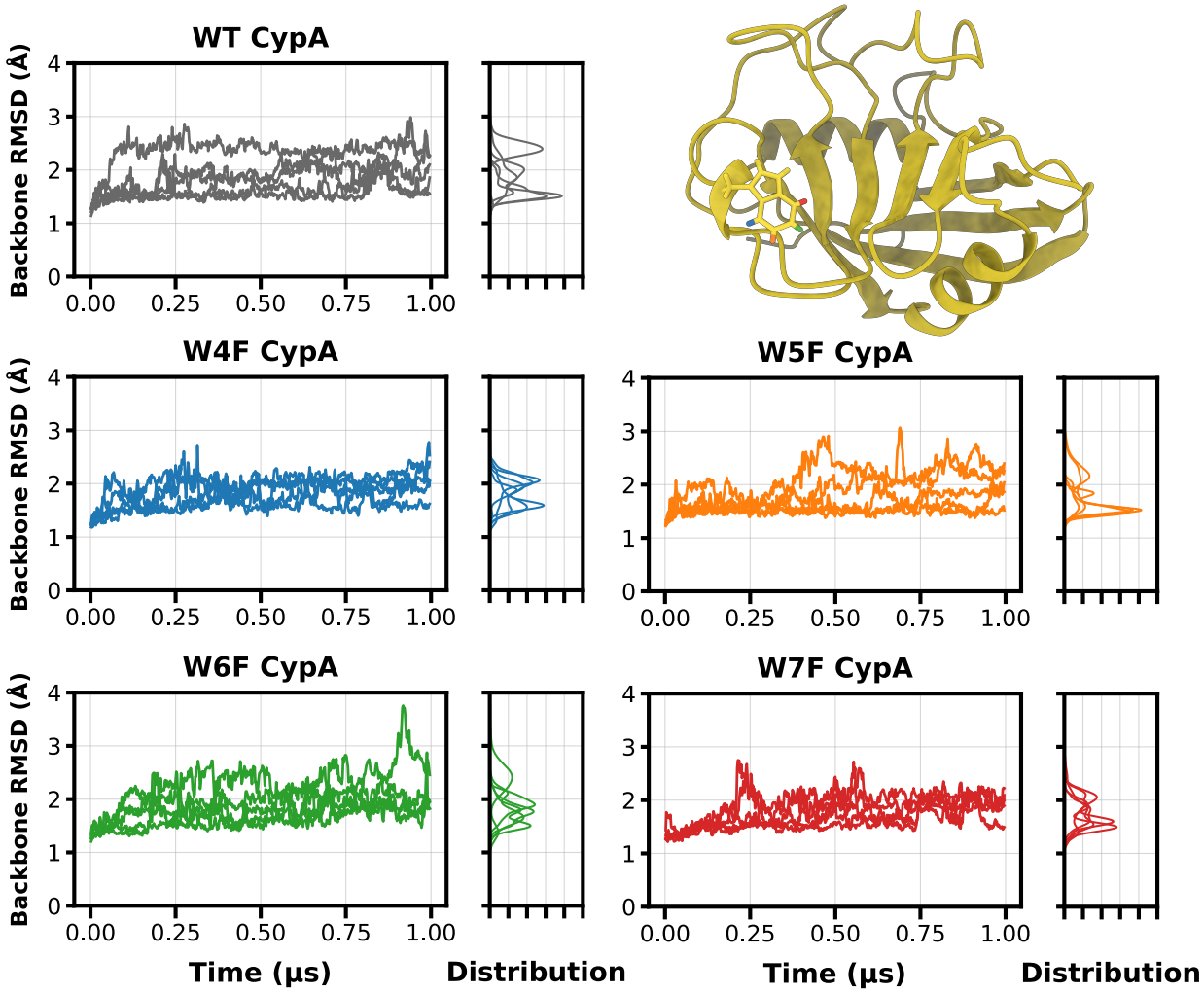


Figure S2. Backbone RMSD values with respect to the experimental X-ray structure (PDB: 3K0N)[63] coordinates for wild-type and fluorinated CypA proteins extracted from each of the five independent 1 μs simulation replicates. The distribution of RMSD values is shown for each protein. The protein X-ray structure is shown in ribbon representation in the upper-right with individual fluorine positions on the Trp121 indole ring color coded like the trajectory plots.

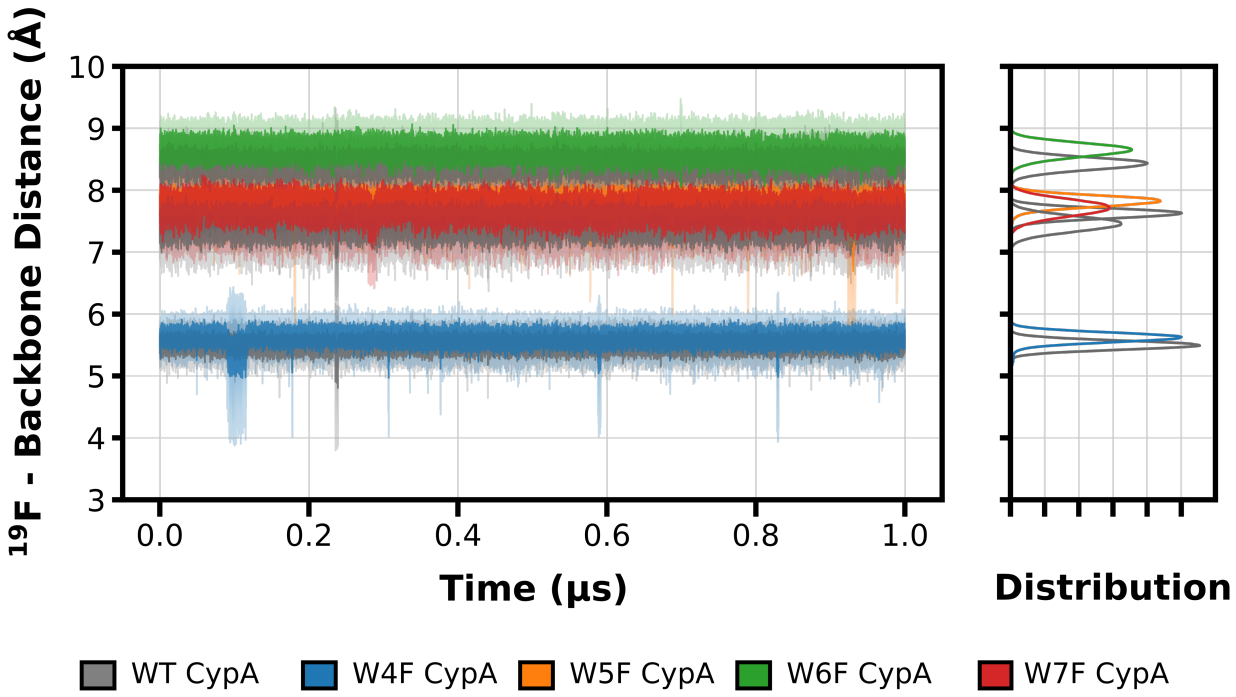
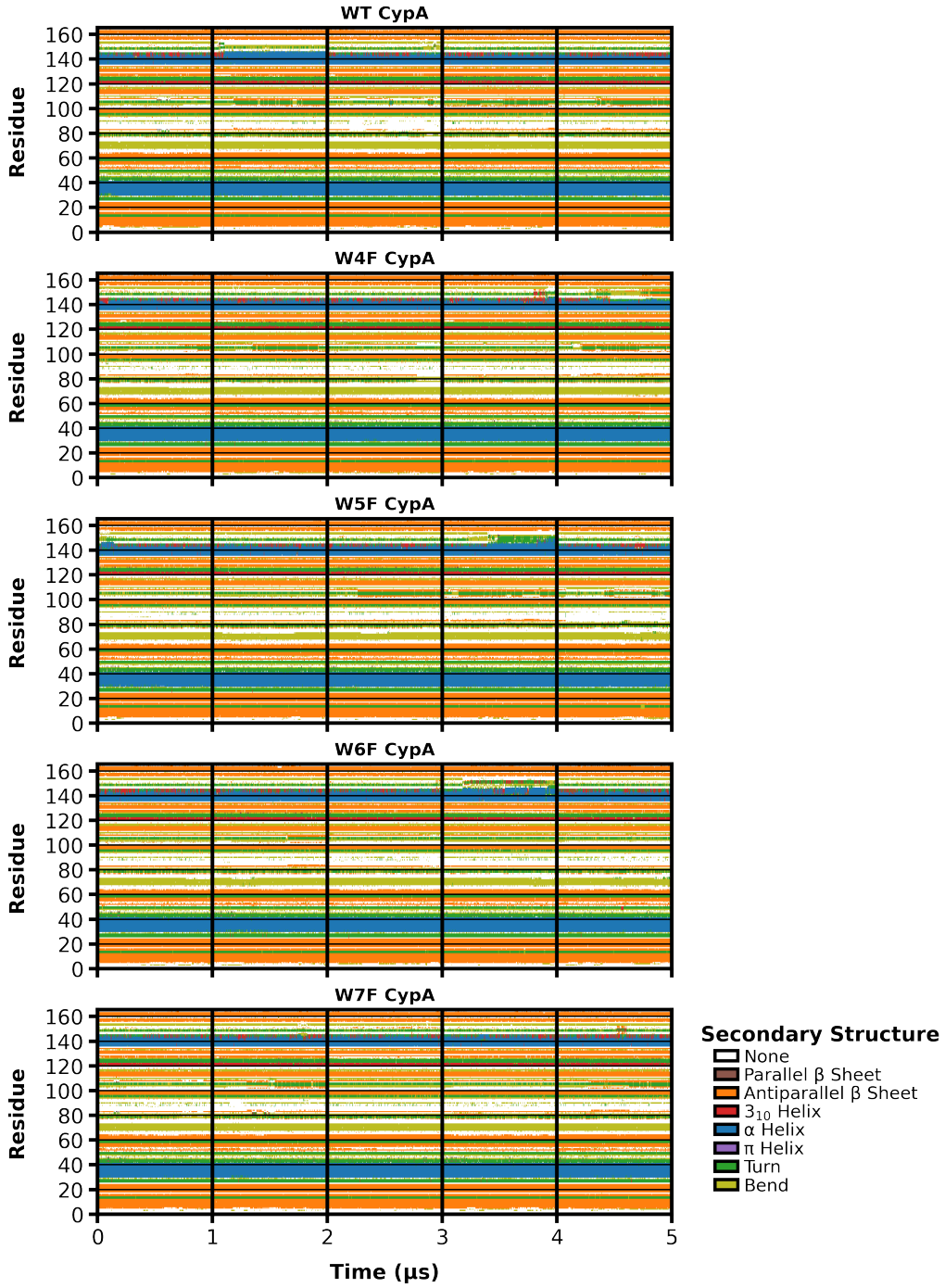


Figure S3. Average distance between the center of mass of the fluorine atom and the backbone carbonyl ± the standard deviation over the simulation time course for the fluorinated CypA variants. The distance for the hydrogen atom and the backbone carbonyl for wild-type CypA is shown in grey. This data represents all five independent replicate simulations of 1 *µ*s each for each system.

(A)


WT CypA
W4F CypA
W5F CypA
W6F CypA
W7F CypA
Residue
0
20
40
60
80
100
120
140
160
0
1
2
3
4
5
Time (μs)
Secondary Structure
None
Parallel β Sheet
Antiparallel β Sheet
3$_{10}$ Helix
α Helix
π Helix
Turn
Bend

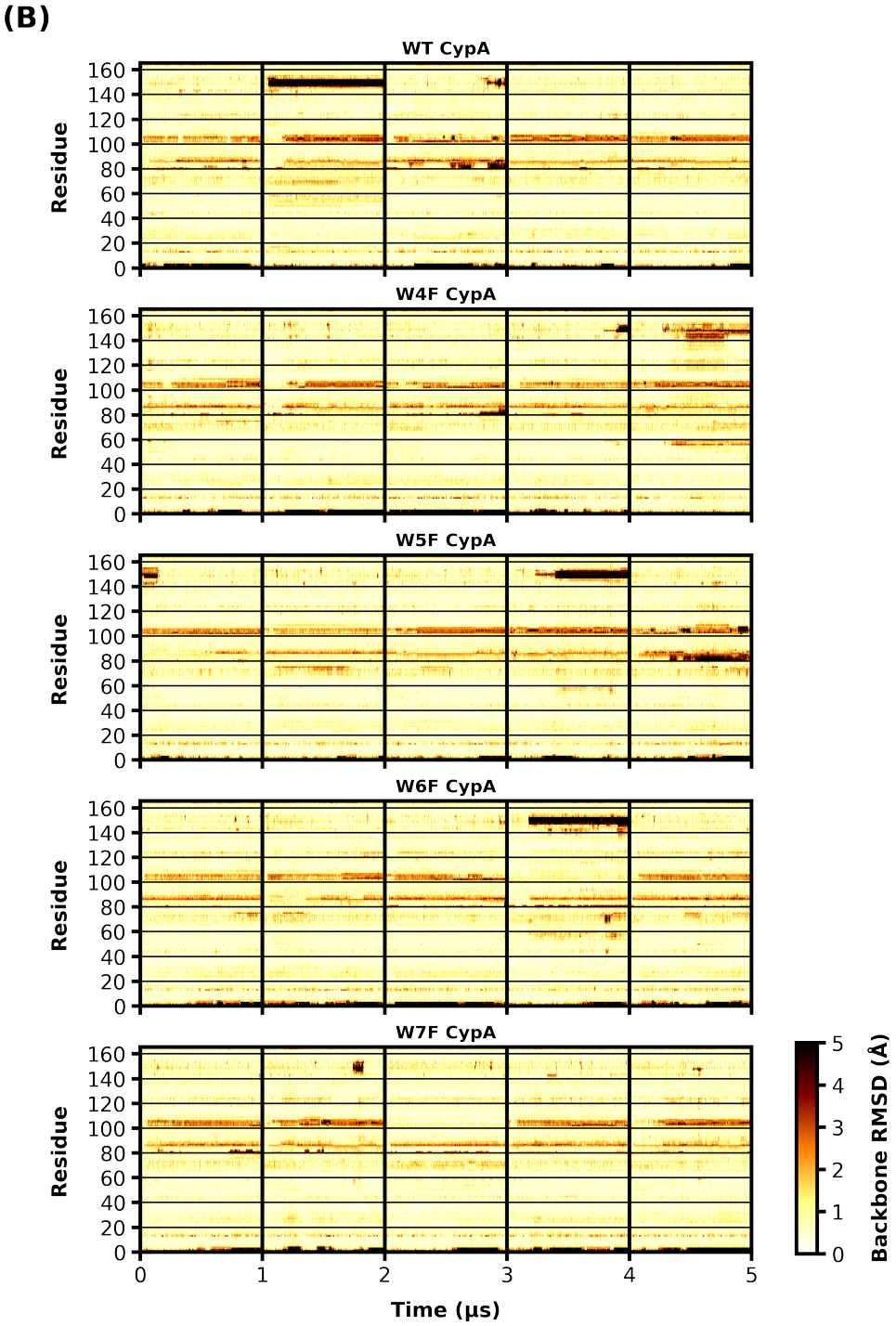
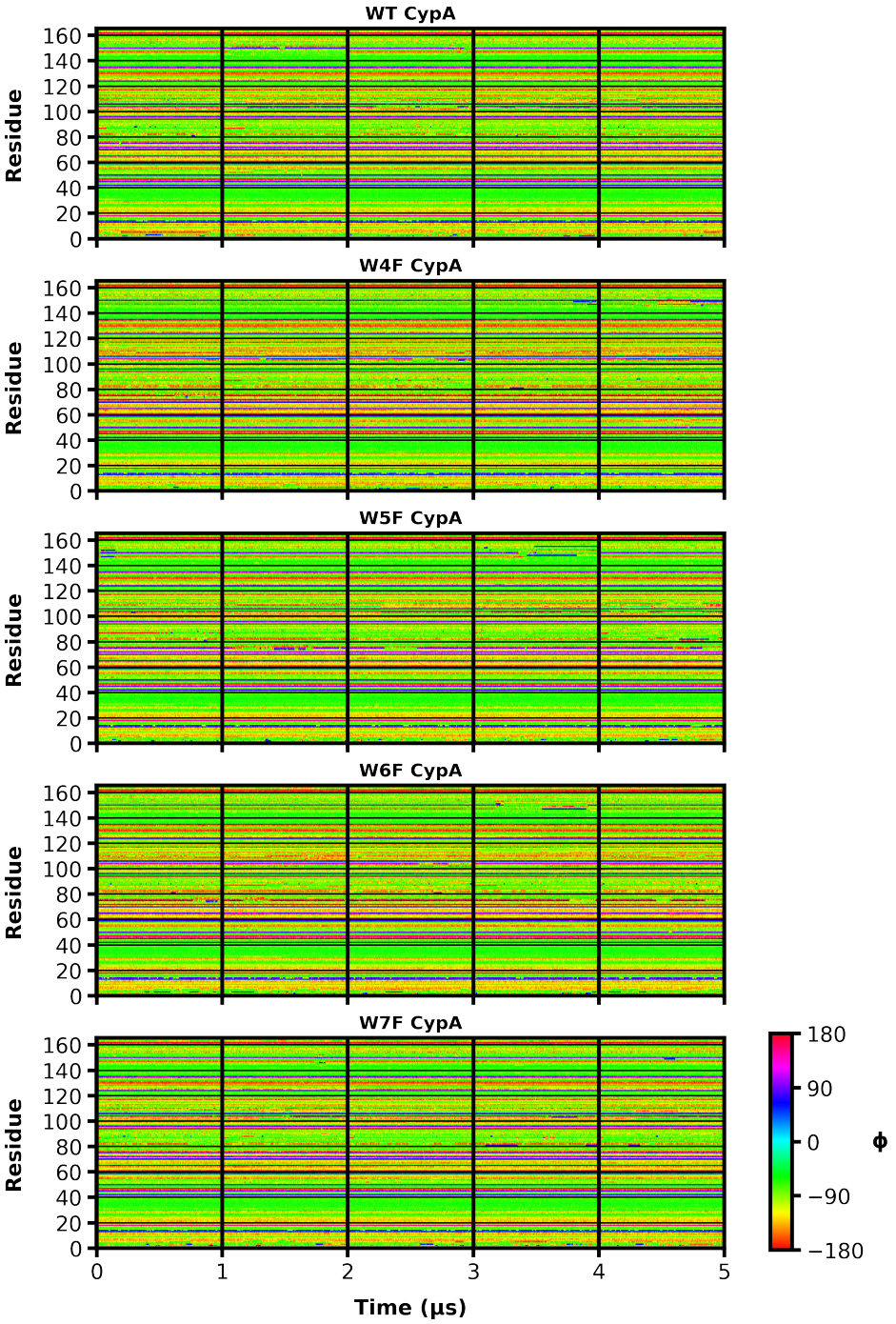


Figure S4. Secondary structure (**A**) and per residue backbone RMSD values (**B**) with respect to the crystal structure for both native CypA and the fluorinated variants. Each 1 *μ*s simulation was replicated five times (separated by vertical lines) for a discontinuous simulation time of 5 *μ*s for each system.

(A)


WT CypA
W4F CypA
W5F CypA
W6F CypA
W7F CypA
Residue
0
20
40
60
80
100
120
140
160
0
1
2
3
4
5
Time (μs)
180
90
0
−90
−180
ϕ

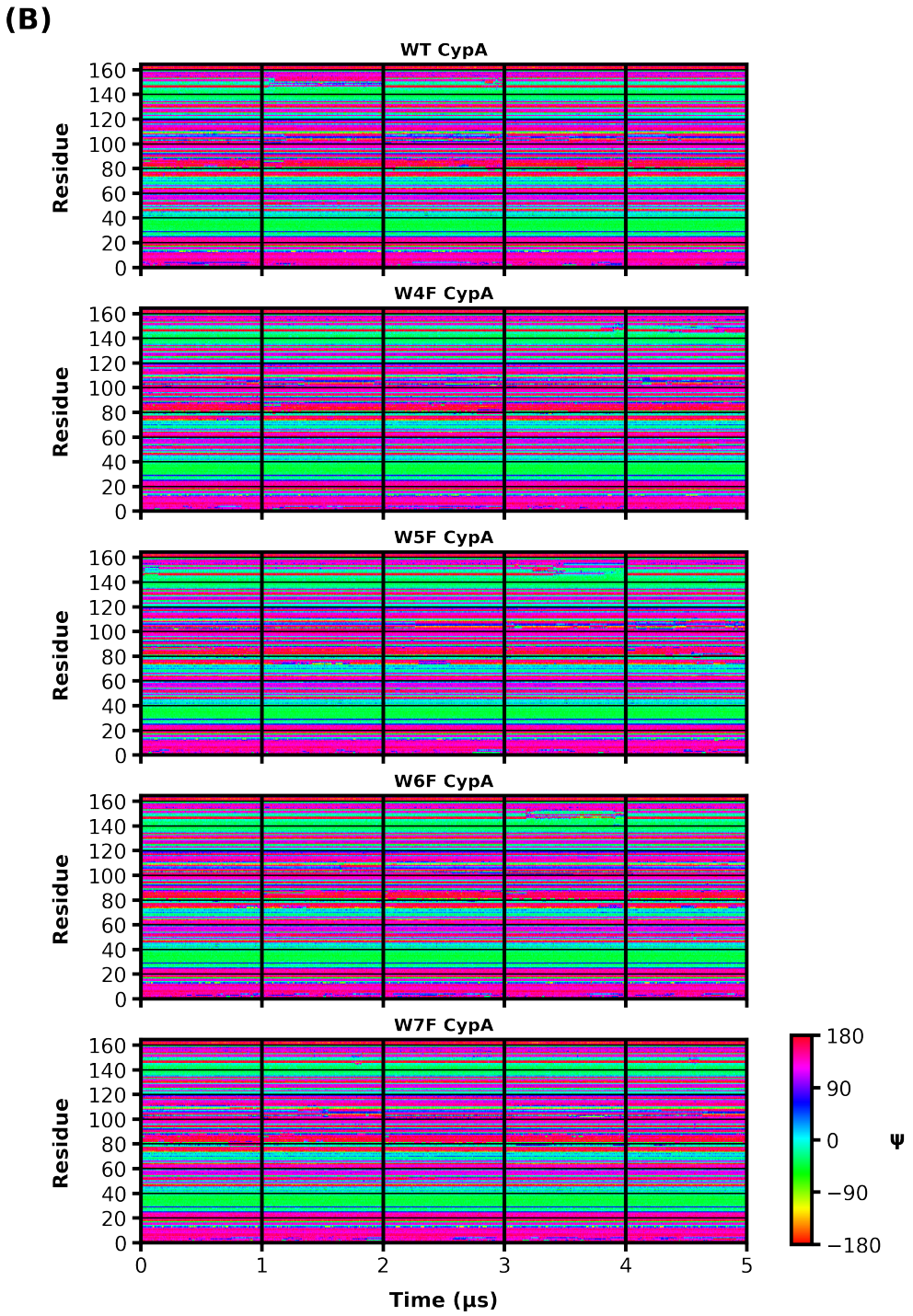


Figure S5. The per-residue Φ (**A**) and Ψ (**B**) dihedral angle variations for wild-type CypA and the fluorinated variants. Each 1 *μ*s simulation was replicated five times (separated by vertical lines) for a discontinuous simulation time of 5 *μ*s for each system.

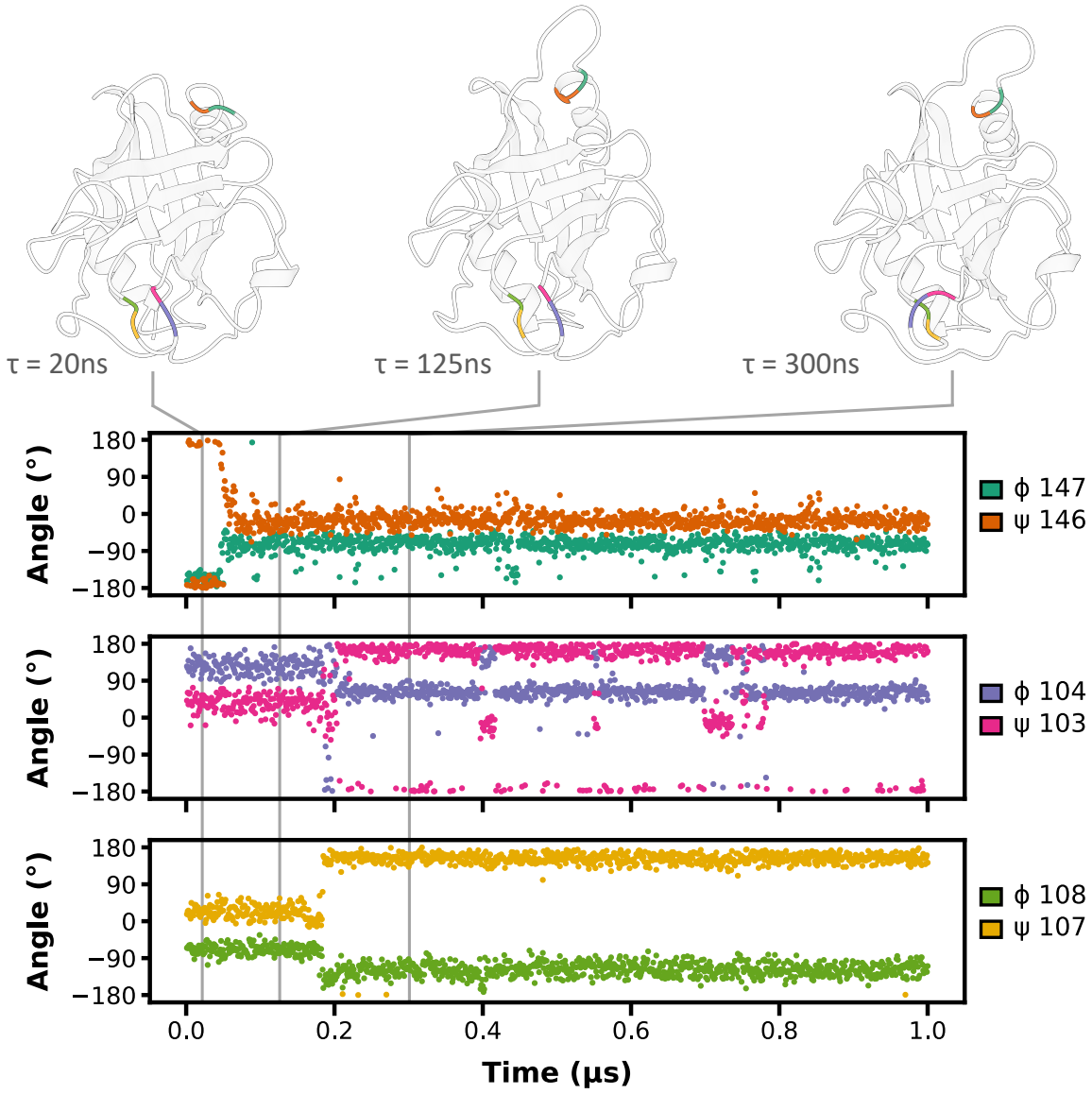


Figure S6. The Φ and Ψ dihedral angles of key neighboring residues around the amino acid exhibiting the jumps in backbone RMSD in Figure S4, taken from a representative 1 *μ*s simulation of the non-fluorinated, wild-type CypA protein. Changes in adjacent dihedral angles are in opposite direction to the original jump, indicative of peptide-plane flips. The structures of relevant timepoints are shown in ribbon representation with the respective dihedrals of interest colored according to the legend.

Supporting Tables

Table S1. Bond, angle, and torsion terms for the current set of fluorinated amino acid parameters.

Bond	**Force Constant (kcal/mol/Å^2)**	**Bond Length (Å)**	
3C-CA	317.4204	1.5100	
3C-F	367.0233	1.3800	
Angle	**Force Constant (kcal/mol/rad^2)**	**Angle (°)**	
CB-CA-F	109.3865	133.44	
CN-CA-F	53.6158	118.76	
C –CA-F	55.5980	120.15	
3C-CA-CA	70.9193	119.54	
CA-3C-F	96.9340	111.90	
F—3C-F	98.1332	107.08	
Dihedral	**Barrier Height (kcal/mol)**	**Phase Angle (°)**	**Periodicity**
F -3C-CA-CA	3.83580	0.0	2.0

Table S2. PDB identifier codes of protein structures containing fluorinated amino acids. Of these protein structures, over 73% were solved to better than 2 Å resolution. The average resolution of all structures is 1.60 ± 0.37 Å.

W4F	5O7W	4B2J	6SZZ				
W5F	6YGW	6YH2	1NEY	6YYZ	6YGM	6YHD	6YGO
	5O7V	6YGP	6YGX	5OLX	6YGL	6YH3	6YGY
	6YHE	5K3C	6YH1	1NF0	4NAM	5CVT	1I45
	6YGZ	5CTJ	5FWG	5OLY	7JT4	7M8Q	6V5T
	7M8R	5OLW	6V64	2ZNX	2JT8	2JTZ	
W6F	4EAR						
W7F	5O7U						
F4F	6T00	6OS8	1ZNT	2MUZ			
FTF	7RFD						
Y3F	4QZS	1XIL	6OG8	1RRX	3FYG		
YDF	6XLS	6BPU	6BPV	6BPR	6BPS	5CI0	6BPT

Table S3. Average ± one standard deviation, minimum, and maximum ΔΔG values of the relative free energy difference of the tetrapeptide with a fluorine substituted residue, compared to the same peptide with a non-fluorinated amino acid.

Peptide	Avg ΔΔG (kcal/mol)	Min ΔΔG (kcal/mol)	Max ΔΔG (kcal/mol)
W4F	0.158 ± 0.162	-0.312	0.637
W5F	0.089 ± 0.137	-0.378	0.530
W6F	-0.007 ± 0.139	-0.626	0.547
W7F	0.062 ± 0.137	-0.484	0.474
Y3F	0.088 ± 0.196	-0.457	0.651
YDF	0.030 ± 0.228	-0.554	0.551
F4F	0.273 ± 0.186	-0.367	0.881
FTF	0.203 ± 0.268	-0.534	0.859

Table S4. Experimental NMR principal components of the CSA tensor, reduced anisotropy, and asymmetry parameters for fluoro-substituted tryptophan powders[78] . These values were used to calculate the relaxation rates from MD simulations for each respective CypA variant.

	σ_{11} (ppm)	σ_{22} (ppm)	σ_{33} (ppm)	δ_σ (ppm)	η
4F-DL-Trp	11.2	-48.3	-112.8	62.8	0.9
5F-DL-Trp	4.8	-60.5	-86.1	52.1	0.5
6F-DL-Trp	12.9	-51.2	-91.6	56.2	0.7
7F-DL-Trp	4.6	-48.3	-123.3	67.6	0.8

Table S5. Comparison between the experimental ^{19}F NMR relaxation rates and those extracted from MD simulations. Each value ± one standard deviation is listed.

	Experimental		Average ± σ from MD – All protons < 3Å	
System	R_1 (s^{-1})	R_2 (s^{-1})	R_1 (s^{-1})	R_2 (s^{-1})
W4F CypA	1.99 ± 0.05	109.1 ± 4.9	1.89 ± 0.55	121.3 ± 4.94
W5F CypA	1.19 ± 0.02	64.8 ± 2.5	0.94 ± 0.27	68.1 ± 2.47
W6F CypA	1.25 ± 0.01	63.0 ± 3.9	0.97 ± 0.27	84.1 ± 2.47
W7F CypA	1.20 ± 0.01	109.6 ± 1.9	0.99 ± 0.31	120.9 ± 2.82