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

SUPPORTING INFORMATION

Ice-binding site of surface-bound type III antifreeze protein partially decoupled from water

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SUPPLEMENTARY FIGURES

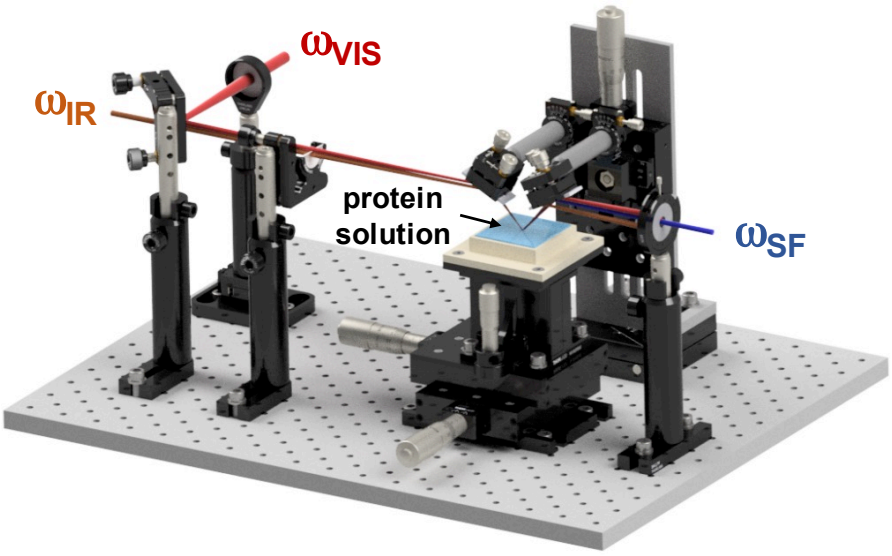

Fig. S1 Schematic 3D rendering of the SFG setup sample area and the generation of the SF signal from the AFP III monolayer adsorbed at the air-water interface.

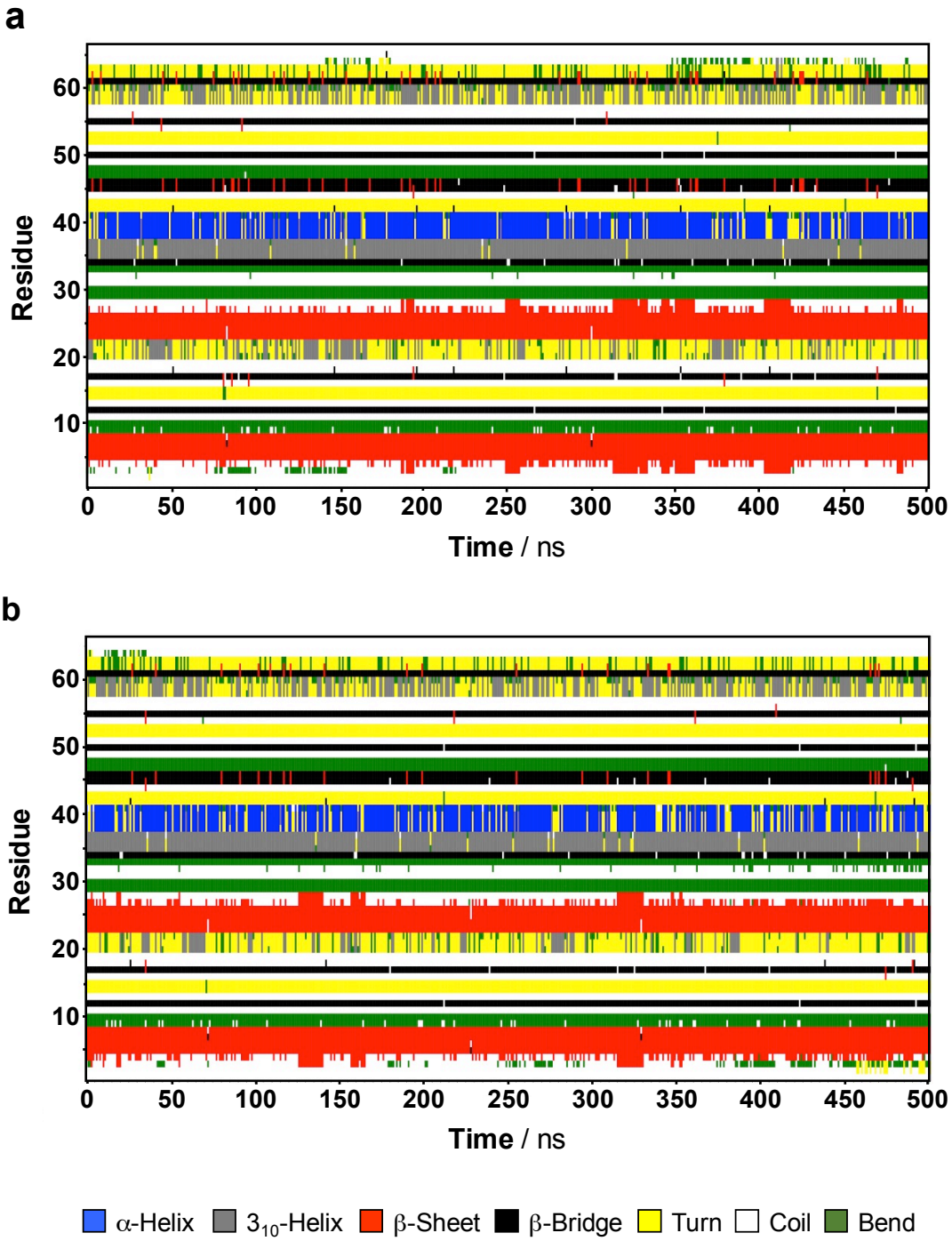

Fig. S2 Time evolution of secondary structures (DSSP analysis) of trials 10 from (a) AFP III and (b) T18N AFP III over 500 ns simulation runs. The analysis starts with structures equilibrated prior to the production run.

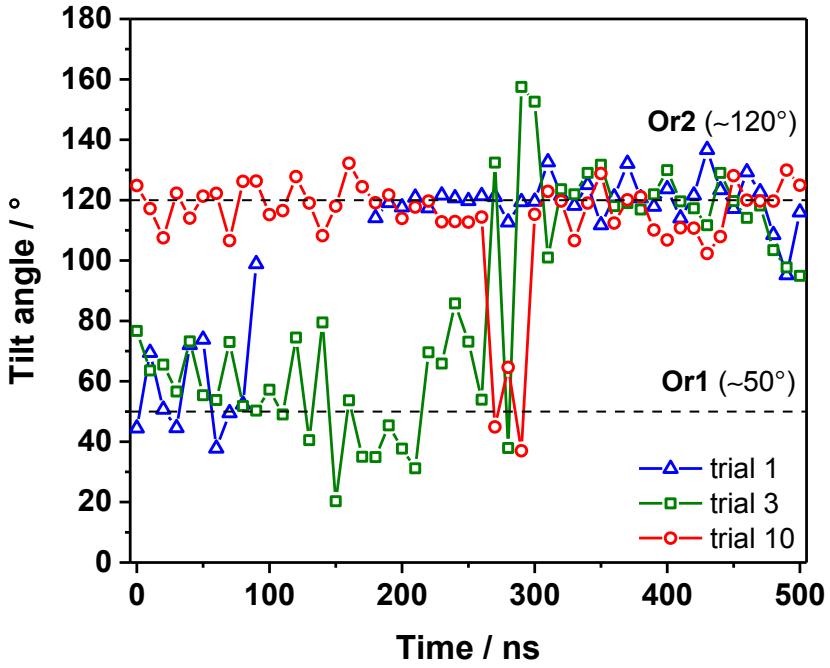

Fig. S3 Tilt angle of mutant T18N AFP III adsorbed at the air-water interface over 500 ns simulation runs. For clarity, simulation data is given at every 10 ns step. Breaks in the data indicate that occasionally the protein briefly desorbed such that the tilt angle could not be calculated. The protein was simulated until it remained adsorbed for 50 ns. Of the 10 trials for wild-type protein, five initially adsorbed to **Or1** and the other adsorbed to **Or2** over 50 ns of simulation. Launching trials 4, 6, and 10 for an additional 500 ns, **Or2** was the only conformation stable over these long timescales. For the mutant protein, 4 of the simulations adsorbed to **Or1**, and 6 adsorbed to **Or2**. Again, after launching trials 1,3, and 10 for an additional 500 ns, **Or2** remained to be the only stable conformation on these timescales.

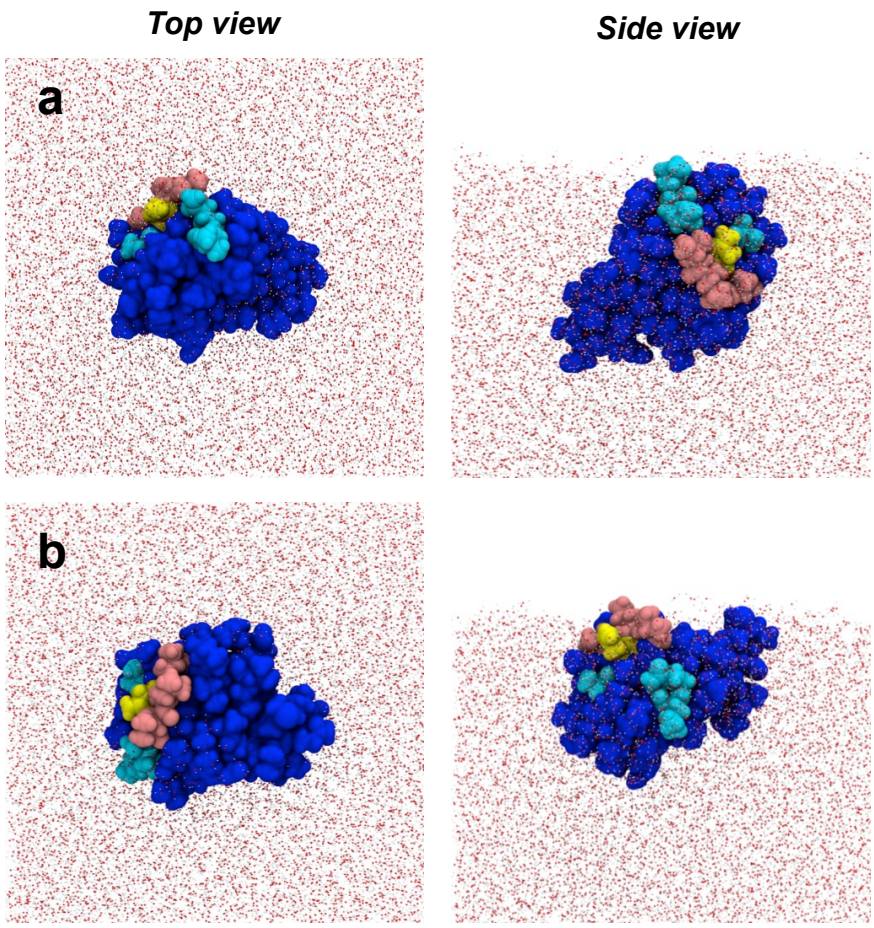

Fig. S4 Simulation snapshots of mutant T18N AFP III adsorbed at the air-water interface. Top and side views of (a) orientation 1 (**Or1**) and (b) orientation 2 (**Or2**). The residues of the IBS forming the regions binding to the primary prism and pyramidal ice planes are highlighted in magenta and cyan, respectively. The mutated residue T18N in the IBS is colored in yellow.

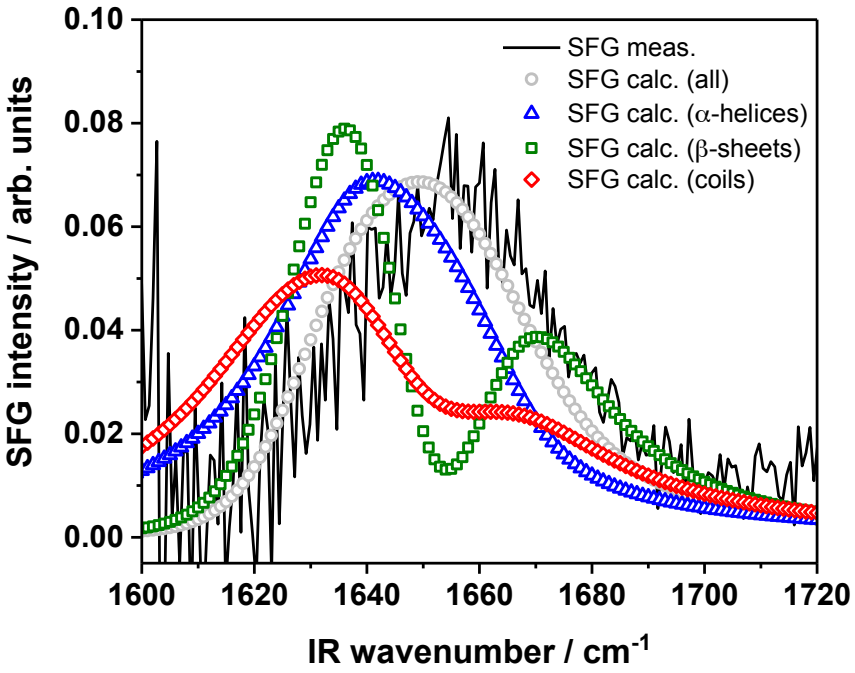

Fig. S5 Calculated spectral responses from three types of secondary structures (α-helices, β-sheets, and coils) for AFP III in **Or2**. Measured and calculated amide I SFG spectra are also shown. The spurious noise observed at low frequencies in the experimental SFG spectrum is due to the normalization procedure.

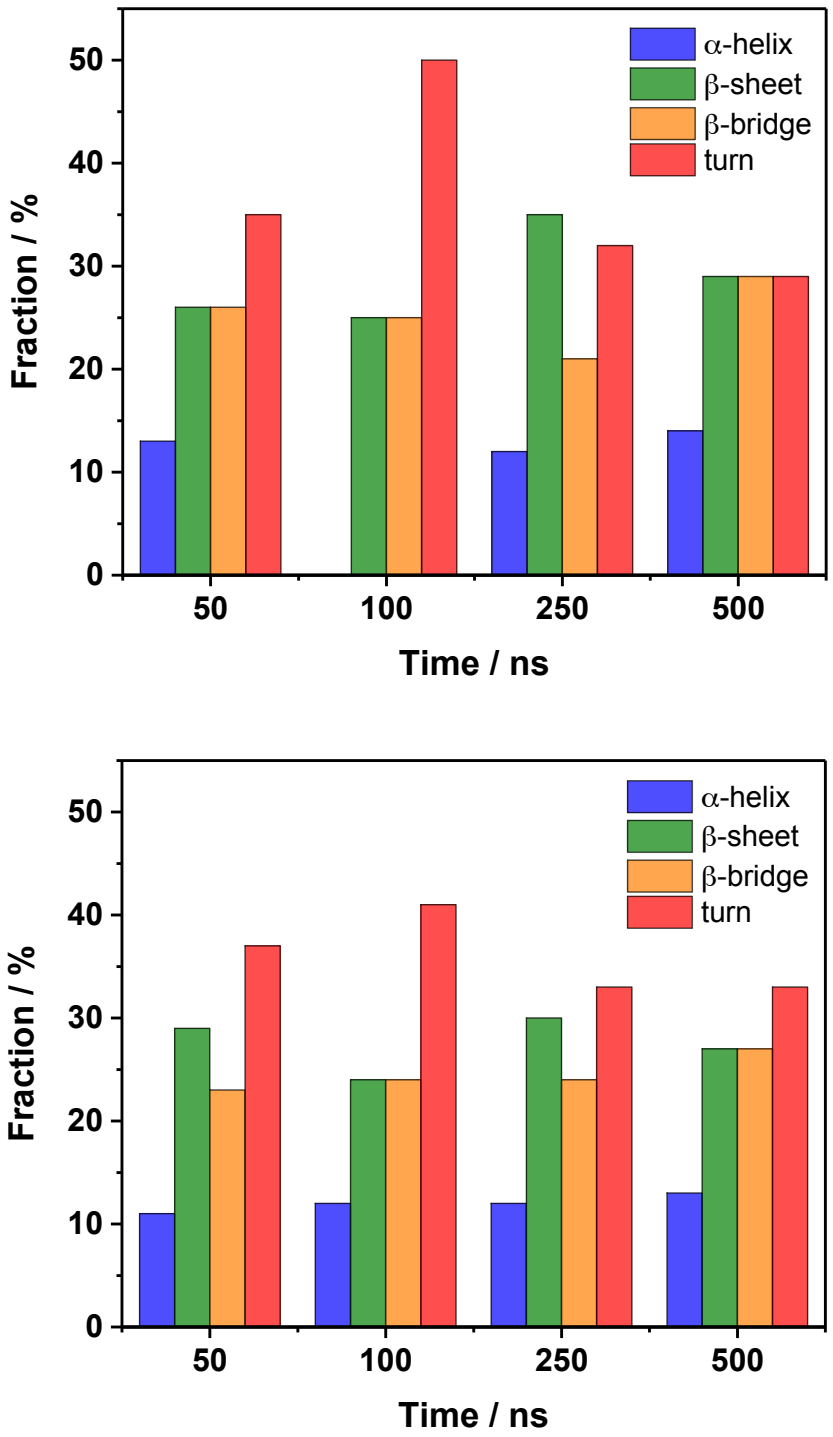

Fig. S6 Secondary structure fractions calculated from DSSP analysis of wt (top) and T18N (bottom) AFP III (both trial 10) before and after adsorption to the air-water interface. The adsorption of wt AFP occurred at 151 ns, binding of T18N occurred at ~3 ns.

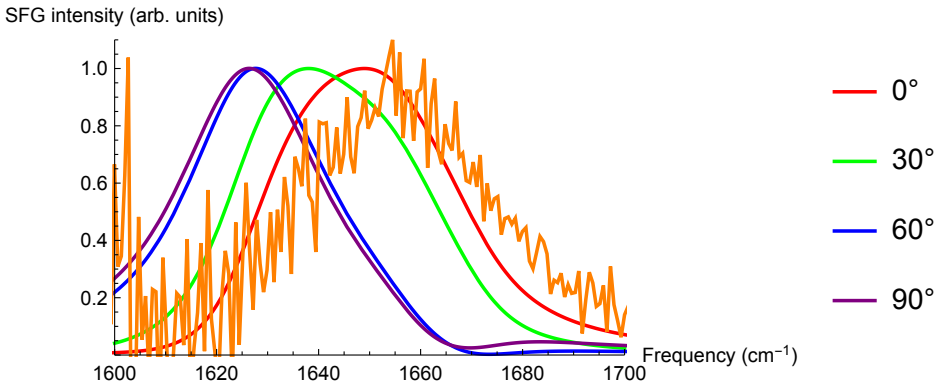

Fig. S7 Calculated spectra for the AFPIII structure in **Or2** in different orientations show the sensitivity of the spectra calculations to the protein orientation. The spectra calculated for 60° and 90° are quite similar to the spectra obtained for the **Or1** state of AFPIII

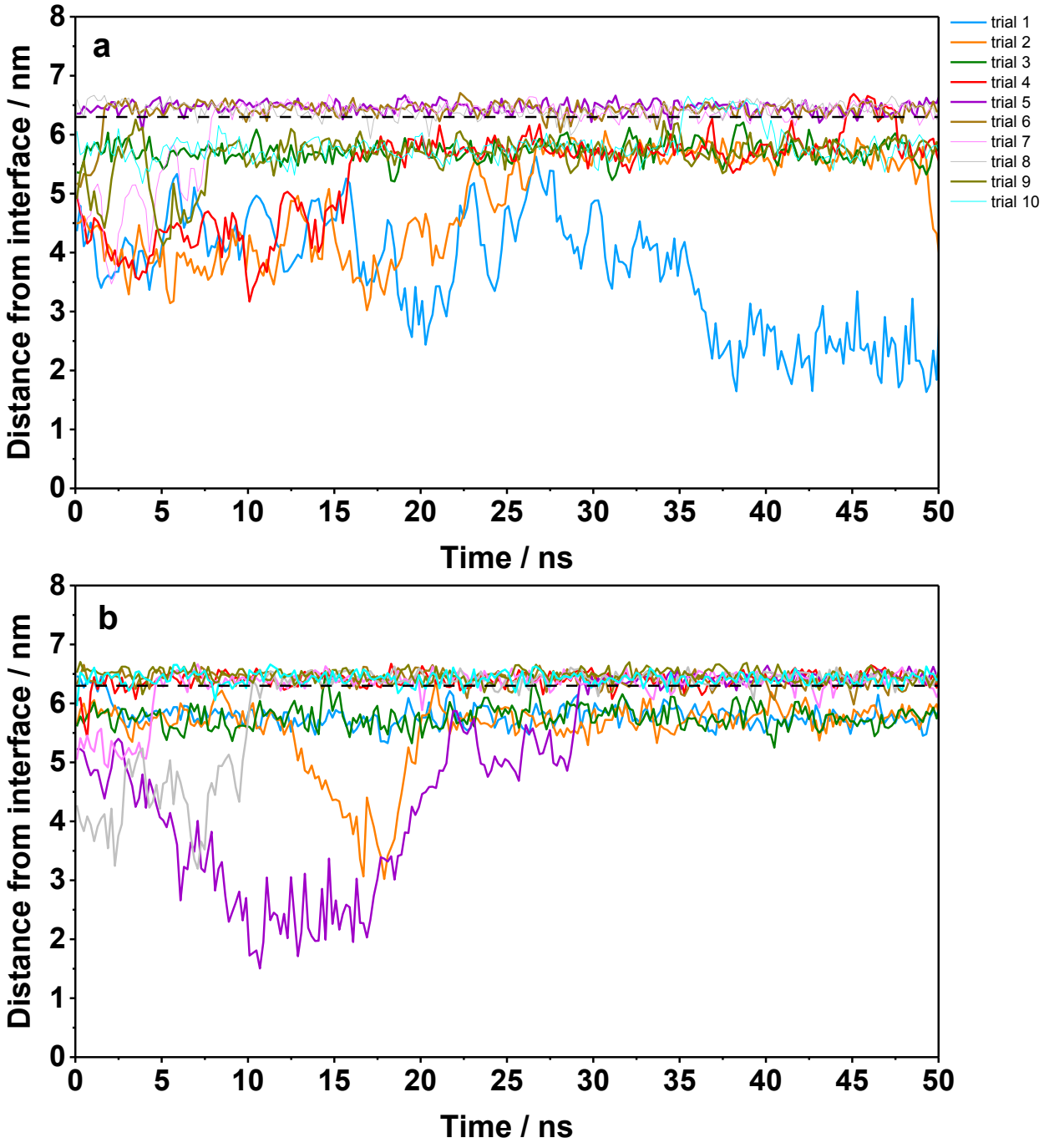

Fig. S8 Distance of residue 18 from the air-water interface for (a) wt and (b) T18N AFP III (trials 1–10) over the first 50 ns of a simulation run. For clarity, the simulation data has been averaged at every 10 data points. The position of the air-water interface (6.3 nm) is indicated by a dash line. The periodic boundary conditions of the simulation box are located at 0 and 6.3 nm.

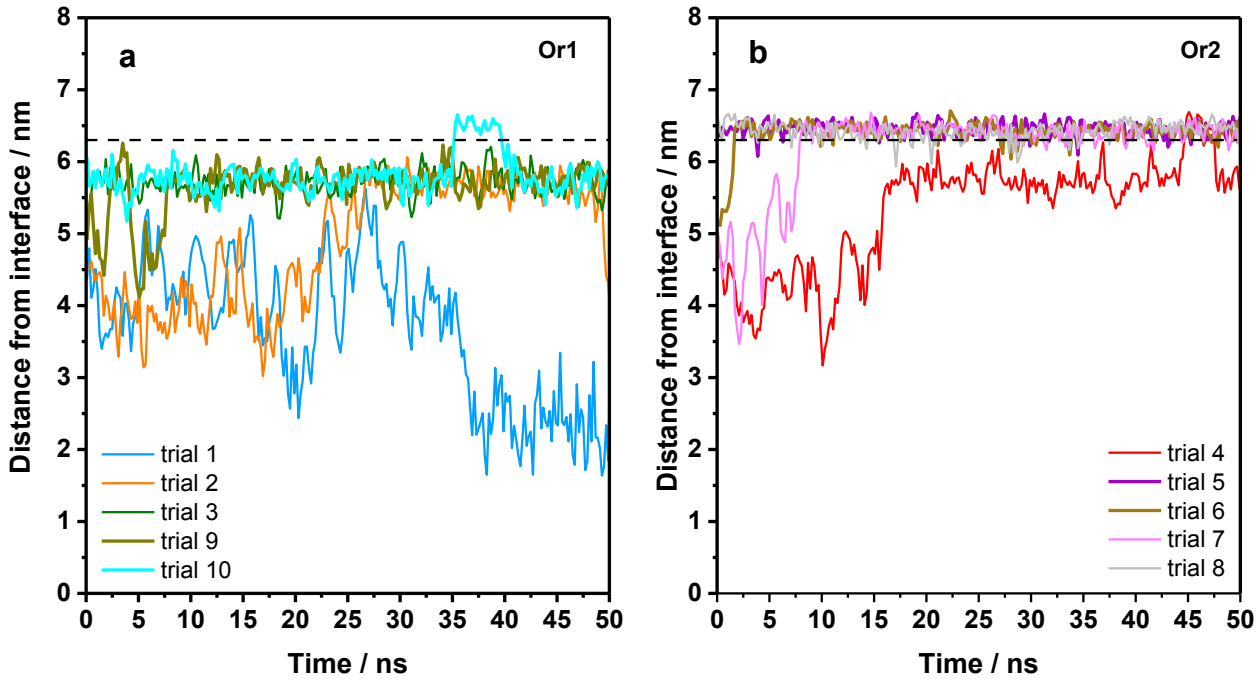

Fig. S9 Distance of residue T18 from the air-water interface for wt AFP III in **Or1** (trials 1–3, 9, 10) and **Or2** (trials 4–8) over the first 50 ns of a simulation run. For clarity, the simulation data has been averaged at every 10 data points. The position of the air-water interface (6.3 nm) is indicated by a dash line. The periodic boundary conditions of the simulation box are located at 0 and 6.3 nm.

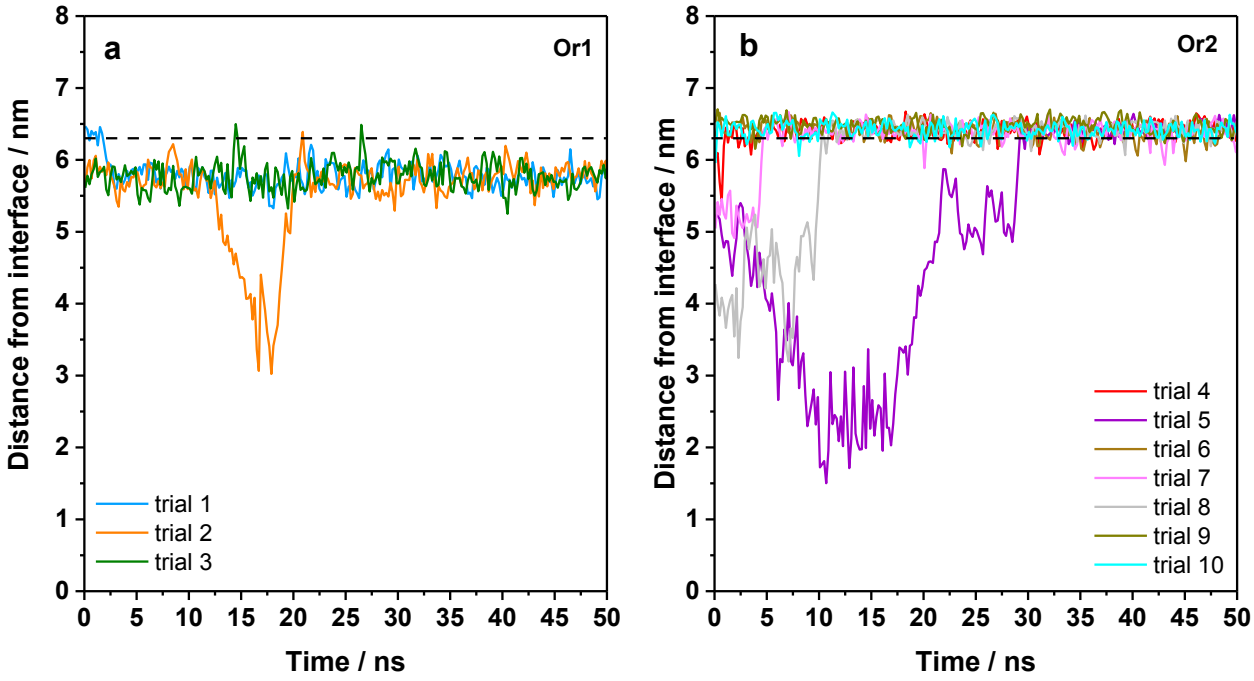

Fig. S10 Distance of residue N18 from the air-water interface for mutant T18N AFP III in **Or1** (trials 1–3) and **Or2** (trials 4–10) over the first 50 ns of a simulation run. For clarity, the simulation data has been averaged at every 10 data points. The position of the air-water interface (6.3 nm) is indicated by a dash line. The periodic boundary conditions of the simulation box are located at 0 and 6.3 nm.

SUPPLEMENTARY TABLES

Table S1 Secondary structure fractions of AFP III at different temperatures analyzed with the Dichroweb online server using the CDSSTR deconvolution method.[1,2]

Temperature (°C)	% Fraction [a]						
	Helix 1	Helix 2	Strand 1	Strand 2	Turn	Unordered	Total [b]
2	-0.02	0.00	0.15	0.06	0.09	0.70	0.98
6	-0.02	-0.01	0.20	0.09	0.11	0.60	0.97
10	-0.02	-0.01	0.18	0.09	0.15	0.57	0.96
16	-0.02	-0.01	0.17	0.08	0.13	0.63	0.98
20	-0.02	-0.01	0.18	0.07	0.05	0.69	0.96

[a] The numbering 1 and 2 for helices and strands refers, respectively, to regular and distorted structures.[3]

[b] In the CDSSTR method, each fraction is greater than -0.03 and the sum of fractions is between 0.95 and 1.05.[4]

Table S2 Peak fitting parameters (Eqn (2)) for the amide I SFG spectrum of AFP III at the air-water interface (Fig. 3a).

Fitting parameter				
A_{NR} (arb. units)	ϕ_{NR} (rad)	$A_{R,1}$ (arb. units)	ω_1 (cm^{-1})	Γ_1 (cm^{-1})
0.03	0.00*	6.2	1651.5	23.1

* The value is smaller than 10^{-13}.

Table S3 Tilt angles (with respect to the surface normal) of AFP III and mutant T18N initially adsorbed at the top air-water interface for different simulation runtimes. Tilt angles of ~50° and ~120° correspond, respectively, to AFP III orientations 1 (**Or1**) and 2 (**Or2**). Subscripts *b*, *t*, and *d* refer, respectively, to bottom and top adsorption, and desorption.

Trial	**Runtime** (ns)			
	50	100	250	500
	AFP III			
1*	52.1_b	51.5_b	–	–
2*	$–_d$	75.3_b	–	–
3	45.1_t	38.5_t	–	–
4*	53.8_t	108.0_b	116.5_b	114.0_b
5	106.4_t	–	–	–
6	125.7_t	117.7_t	112.5_t	125.6_t
7	127.3_t	–	–	–
8	91.2_t	–	–	–
9	45.6_t	–	–	–
10	34.6_t	55.8_t	116.9_t	123.6_t
	T18N AFP III			
1*	73.9_t	$–_d$	119.8_b	116.0_b
2	44.9_t	–	–	–
3	55.4_t	57.2_t	73.1_t	94.9_t
4	120.9_t	–	–	–
5	119.5_t	–	–	–
6	117.9_t	–	–	–
7	120.9_t	–	–	–
8	98.4_t	–	–	–
9	118.8_t	–	–	–
10	121.3_t	115.2_t	112.7_t	124.9_t

* In these runs, the protein initially adsorbed at the top air-water interface, eventually desorbed and re-adsorbed at the bottom air-water interface.

References

1. L. Whitmore and B. A. Wallace, *Nucleic Acids Res.*, 2004, **32**, W668-W673.
2. L. Whitmore and B. A. Wallace, *Biopolymers*, 2008, **89**, 392-400.
3. W. Kabsch and C. Sander, *Biopolymers*, 1983, **22**, 2577-2637.
4. N. Sreerama and R. W. Woody, *Anal. Biochem.*, 2000, **287**, 252-260.