Electronic Supplementary Material (ESI) for Soft Matter.

SUPPORTING INFORMATION FOR Unfolding mechanism and free energy landscape of single, stable, alpha helices at low pull speeds†

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1 Force-strain curves for individual runs at the lowest pull speed

Fig. 1 shows four individual force traces at the lowest pull speed. The large variations in force, even within the plateau region, are consistent with refolding-unfolding events (stick-slip events) visible in Fig. 4 in the main text.

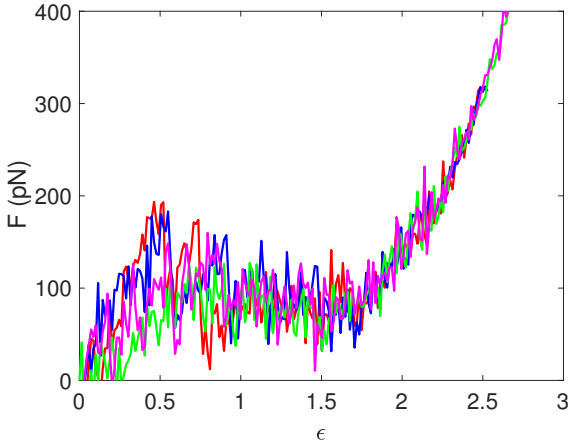

Fig. 1 Force-strain curves for individual runs at $v = 10^{-3}$ nm/ns, obtained from the molecular dynamics simulations. For ease of viewing, only every 10^{th} point of each simulation trajectory is displayed.

2 Hydrogen bonds and salt bridges vs. strain

The number of hydrogen bonds and salt bridges vs. strain depends on the pull speed: In SI Fig. 2 and SI Fig. 3 we show the number of hydrogen bonds and salt bridges as a function of strain for individual realizations at the highest and low pull velocities. The helix has initially ≈ 35 hydrogen bonds, of which a theoretical maximum of 24 can be backbone-backbone hydrogen bonds. The remaining ones are side-chain–side-chain hydrogen bonds, which effectively double as salt bridges because they are established between the negatively charged glutamic acid and the positively charged lysines and arginines. The number of hydrogen bonds and salt bridges oscillates within any chosen (small) range of ε, as expected given the high frequency of breaking and reformation events. For strains for which some helical structure still exists ($\varepsilon < 2$), simulations at the higher pulling speed show larger average number of hydrogen bonds but smaller fluctuations. This observation suggests that at large pull speeds the helix has less time to explore conformational rearrangements that facilitate breakage or reformation events. Hydrogen bonds thus reach steeper regions of their free energy basin before a breaking event occurs than at the low pulling speed.

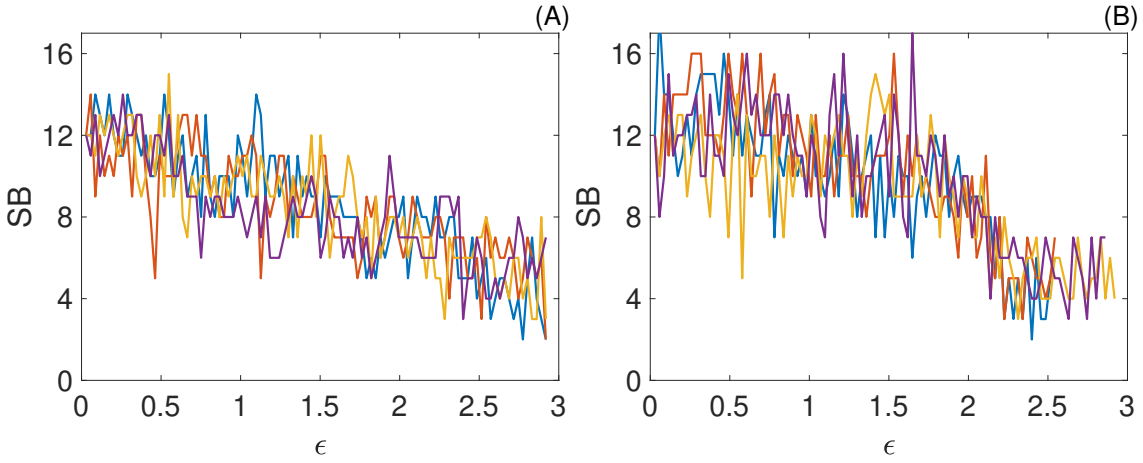

Fig. 2 Number of salt bridges as a function of strain, for each of the four simulations done at (A) $v = 1\,\text{nm/ns}$ and (B) $v = 10^{-3}\,\text{nm/ns}$. Salt bridges exist if the distance between the N and O atoms in the side chains of basic and acidic amino acids is below 0.4 nm. For ease of viewing, only every 10^{th} point of each simulation trajectory is displayed.

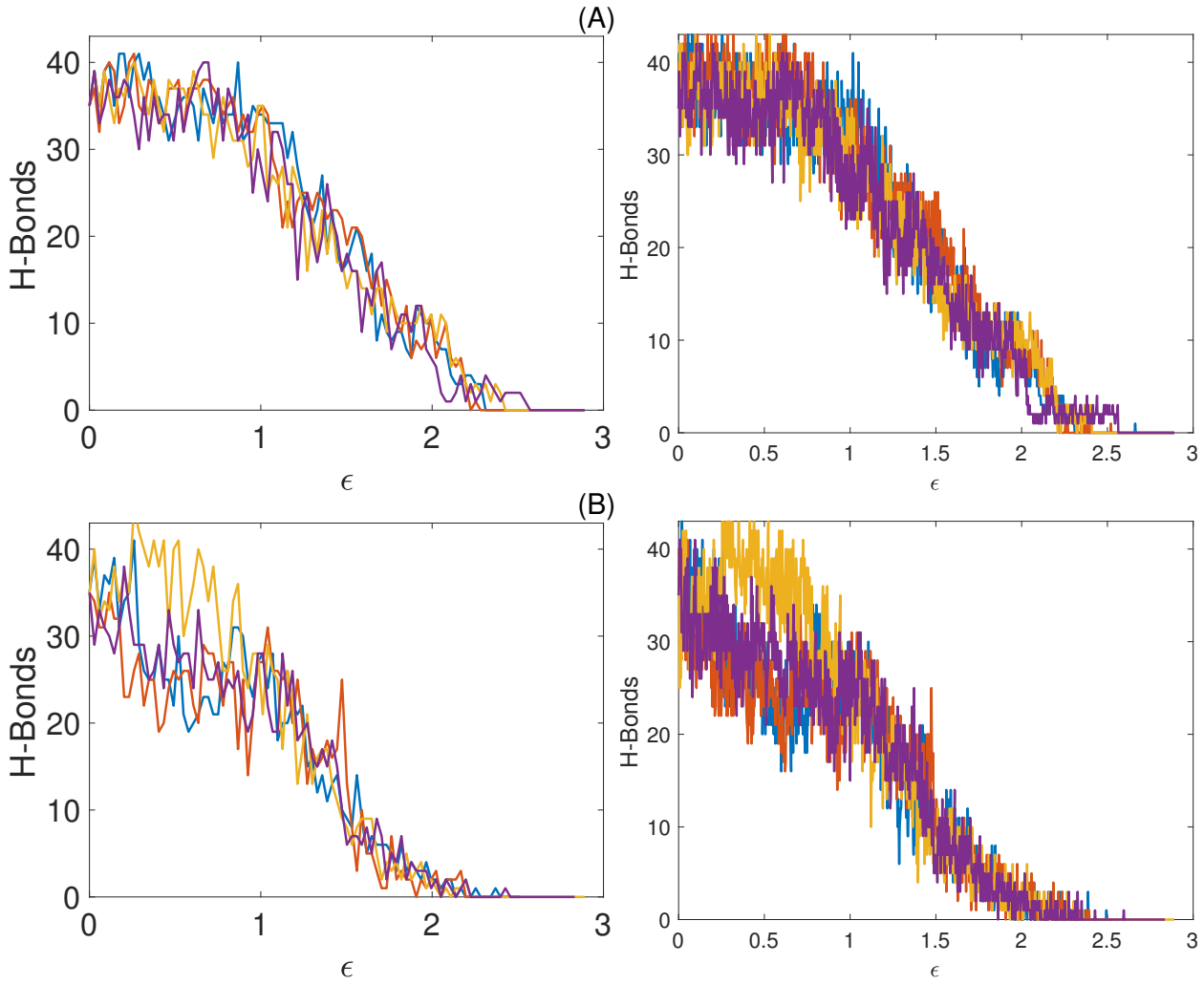

Fig. 3 Number of hydrogen bonds as a function of strain, for each of the four simulations done at (*top*) $v = 1\,\mathrm{nm/ns}$ and (*bottom*) $v = 10^{-3}\,\mathrm{nm/ns}$. Hydrogen bonds exist if the donor-acceptor distance is below 0.35 nm and the hydrogen-donor-acceptor angle is below 30°. The left panel shows only every 10$^{\mathrm{th}}$ point of each simulation; the right panel show all data points for the same simulation runs.

3 Ramachandran plots as a function of time

In SI Figs. 4, 5, 6 and 7 we show Ramachandran plots as a function of time for the residues forming the turn stabilized by the $(i, i+4)$ hydrogen bond time series shown in Fig. 5 in the main text. Comparison of these two sets of results illustrates that substantial changes in the ϕ angle of residue $i+4$, in the ψ angle of residue i, or in either angle of the residues in between these two necessarily force an $(i, i+4)$ hydrogen bond to break. The contrary is not true, however: that hydrogen bond can (and overwhelmingly does) break without inducing substantial changes in the backbone dihedrals. In two cases (Figs. 5 and 6) unfolding of a single amino acid lead to the breakage of the $i, i+4$ hydrogen bond. The other amino acids forming the helical turn stayed in their helical conformation.

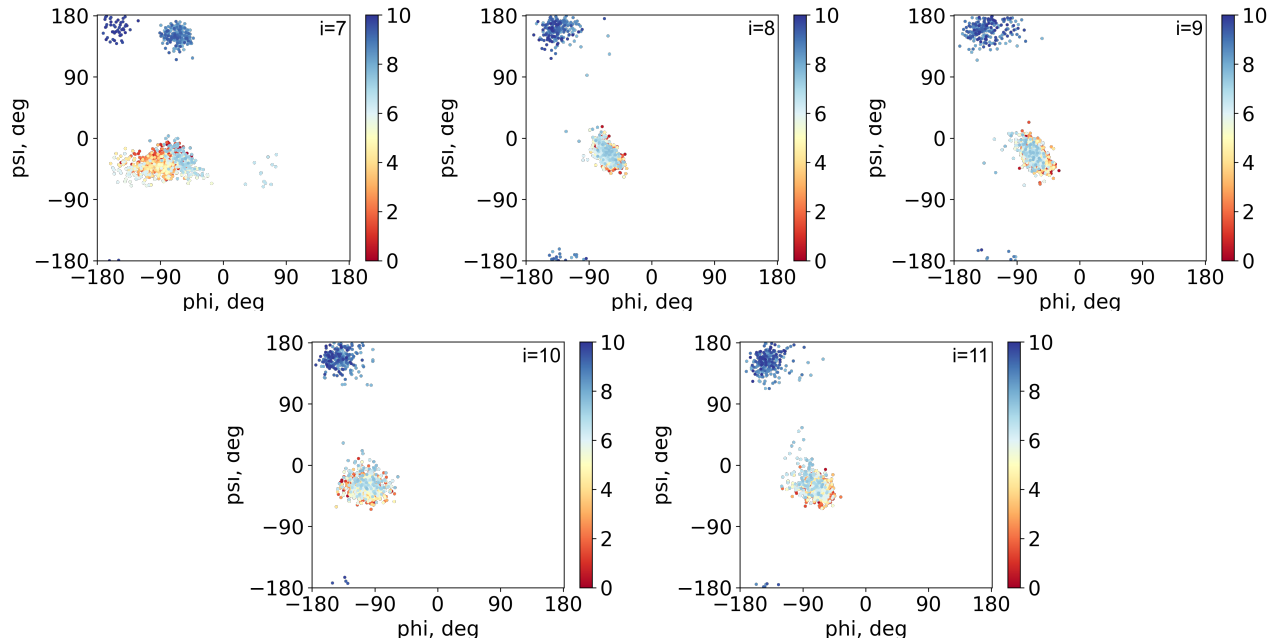

Fig. 4 Ramachandran plots for residues 7, 8, 9, 10 and 11 at $v = 1$ nm/ns. The alpha helical configuration of these amino acids is stabilized by a hydrogen bond between residues 11 and 7, which breaks permanently at ≈ 7.5 ns as shown in panel A of Fig. 5 in the main text. The color scale corresponds to time in nanoseconds. $t = [0, 10]$ ns corresponds to strain $\varepsilon = [0, 2.9]$.

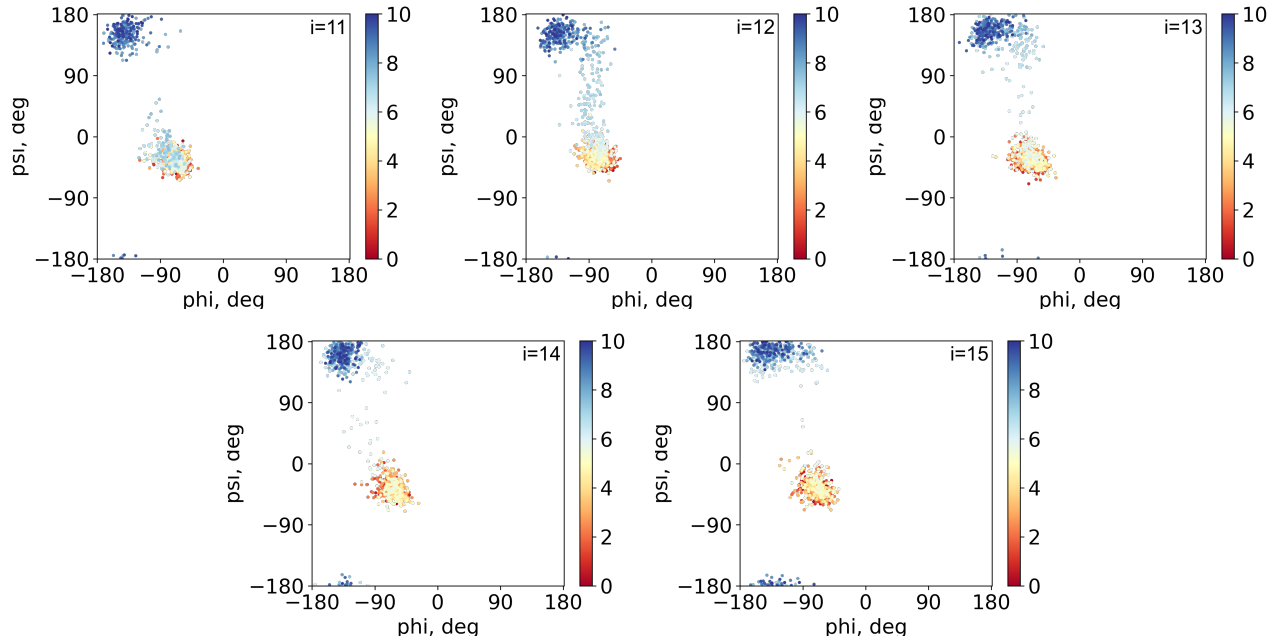

Fig. 5 Ramachandran plots for residues 11, 12, 13, 14 and 15 at $v = 1$ nm/ns. The alpha helical configuration of these amino acids is stabilized by a hydrogen bond between residues 11 and 15, which breaks permanently at ≈ 4 ns, as shown in panel B of Fig. 5 in the main text. The color scale corresponds to time in nanoseconds; $t = [0, 10]$ ns corresponds to strain $\varepsilon = [0, 2.9]$.

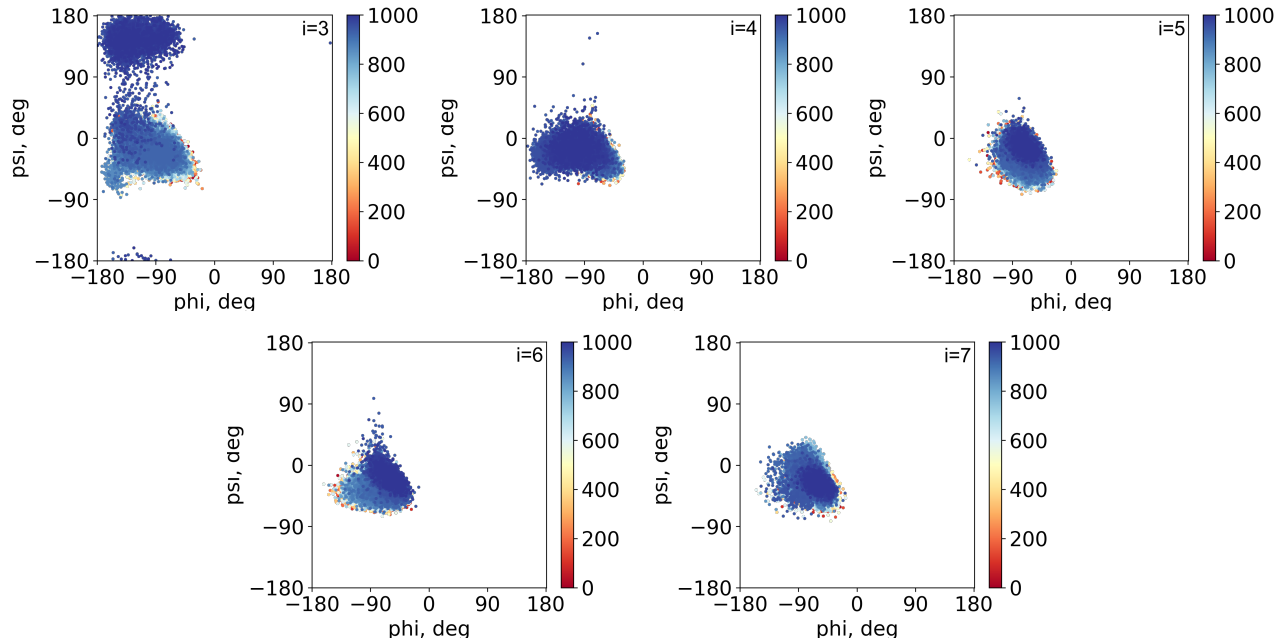

Fig. 6 Ramachandran plots for residues 3, 4, 5, 6 and 7 at $v = 10^{-3}$ nm/ns. The alpha helical configuration of these amino acids is stabilized by a hydrogen bond between residues 7 and 13, which breaks permanently at ≈ 9 ns, as shown in panel C of Fig. 5 in the main text. The color scale corresponds to time in nanoseconds; $t = [0, 1000]$ ns corresponds to strain $\varepsilon = [0.5, 0.8]$. The time resolution is 10 ps.

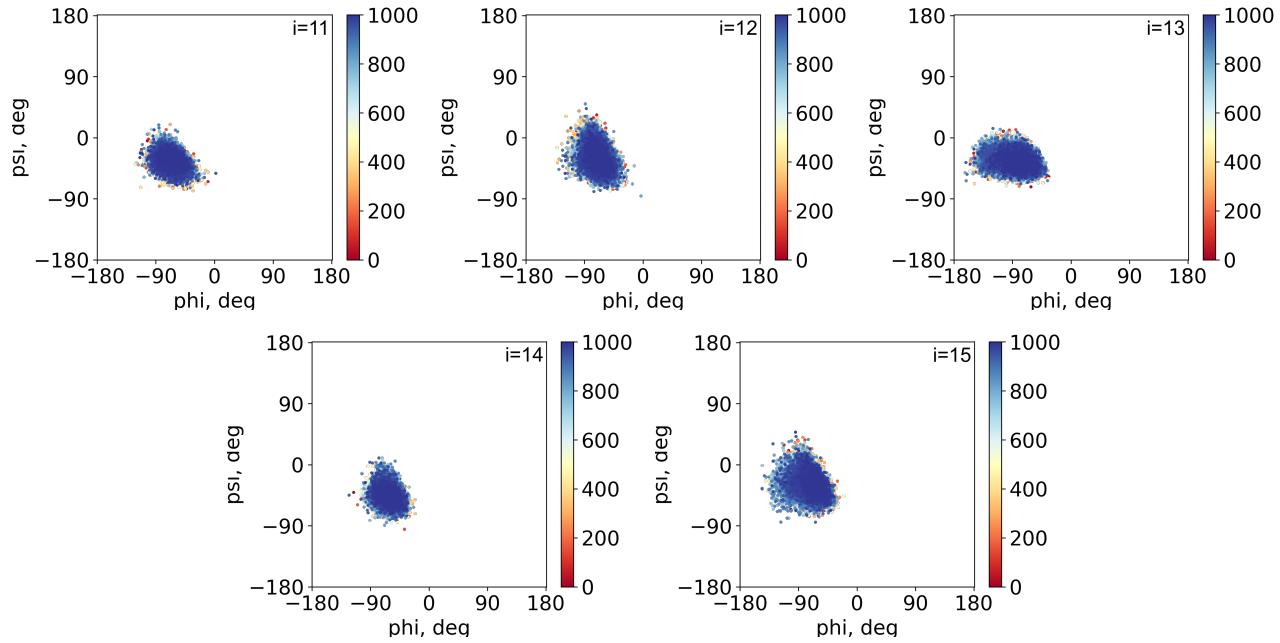

Fig. 7 Ramachandran plots for residues 11, 12, 13, 14 and 15 at $v = 10^{-3}$ nm/ns. The alpha helical configuration of these amino acids is stabilized by a hydrogen bond between residues 15 and 11, which does not permanently break during the simulation, as shown in panel D of Fig. 5 in the main text. The color scale corresponds to time in nanoseconds; $t = [0, 1000]$ ns corresponds to strain $\varepsilon = [0.5, 0.8]$. The time resolution is 10 ps.

4 Secondary structure vs. time from umbrella sampling simulations

SI Fig. 8 shows the secondary structure of the helix as a function of time, for two fixed values of strain. The structure of the helix changes substantially via events that occur infrequently within 50 ns, suggesting that some relaxation processes are slow enough that pulling at the lowest pull speed does not occur under quasi static conditions.

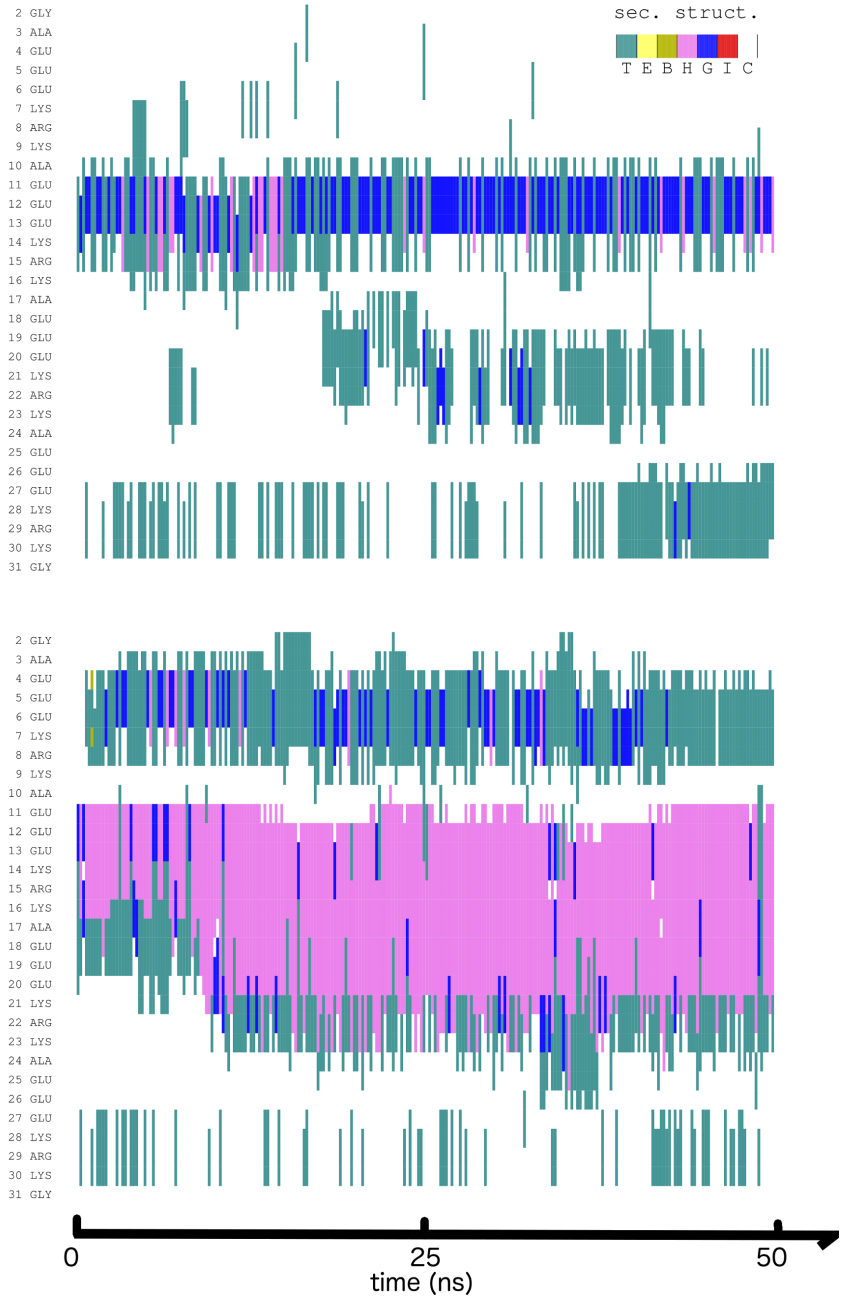

Fig. 8 Secondary structure as a function of time for two umbrella sampling simulations, at (*top*) $\varepsilon = x/L_0 = 1.5$ and (*bottom*) $\varepsilon = 1.3$. Showing every 20$^{\text{th}}$ point of 5000 saved configurations for ease of viewing. The color/letter code specifies the secondary structure for each amino acid, determined using STRIDE [1]: turn (T); extended configuration (E); isolated bridge (B); a helix (H); 3_{10} helix (G); π-helix (I) and coil (C).

Notes and references

1 D. Frishman and P. Argos, *Proteins: Struct., Funct., Bioinf.*, 1995, **23**, 566–579.