Supporting Information: Protein Unfolding by SDS: the Microscopic Mechanisms and the Properties of the SDS-Protein Assembly

David Winogradoff,[†,‡] Shalini John,[‡] and Aleksei Aksimentiev[*,†,‡,¶]

†*Center for the Physics of Living Cells,*

‡*Department of Physics,*

¶*Beckman Institute for Advanced Science and Technology,*

University of Illinois at Urbana–Champaign, Urbana, IL

E-mail: aksiment@illinois.edu

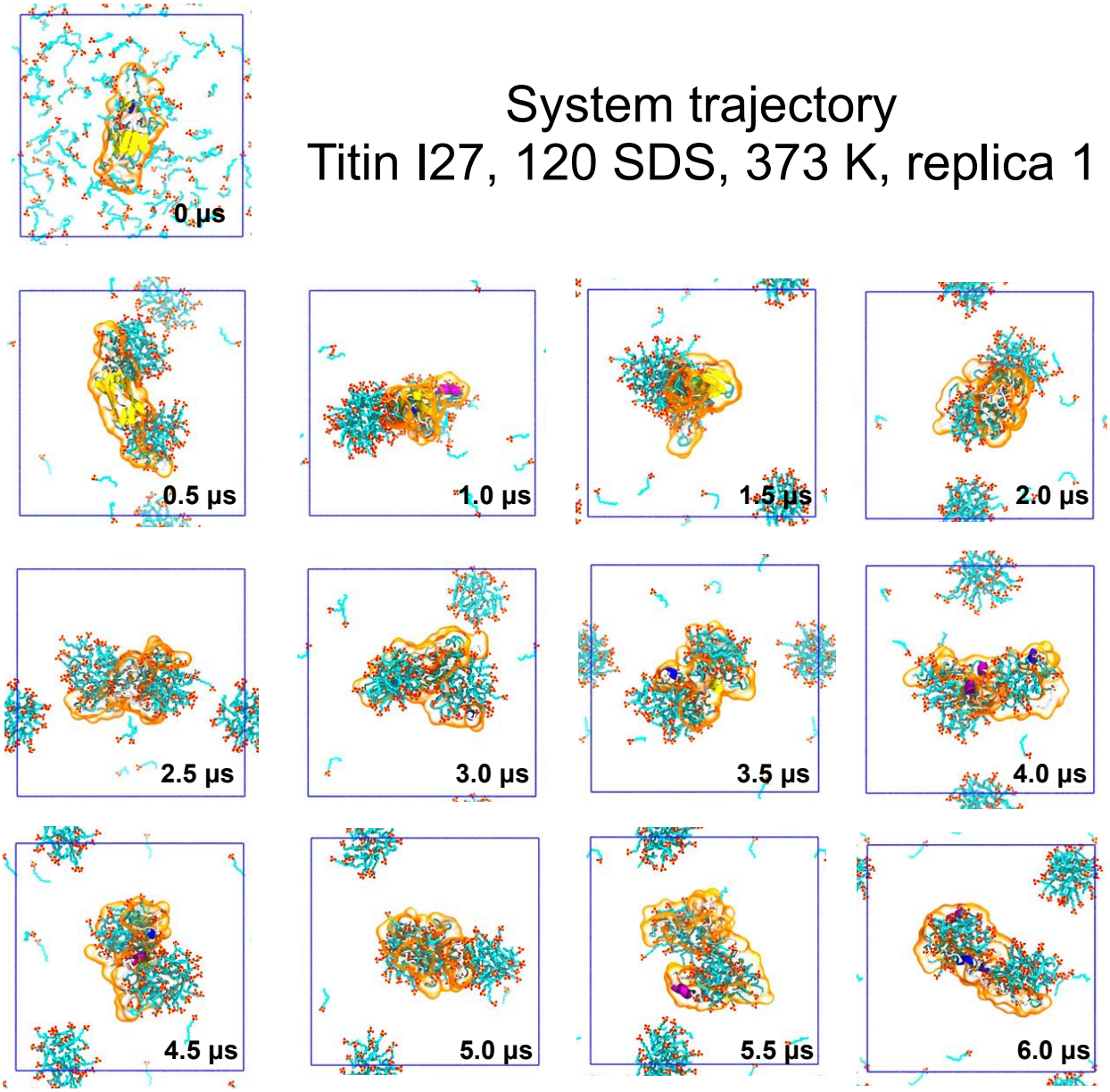


Figure S1 : Spontaneous unfolding of titin I27 at 373 K, replica 1. The snapshots illustrate a 6-μs MD trajectory of a system containing one I27 domain of titin (orange semi-transparent), 120 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S1 illustrates the same MD trajectory.

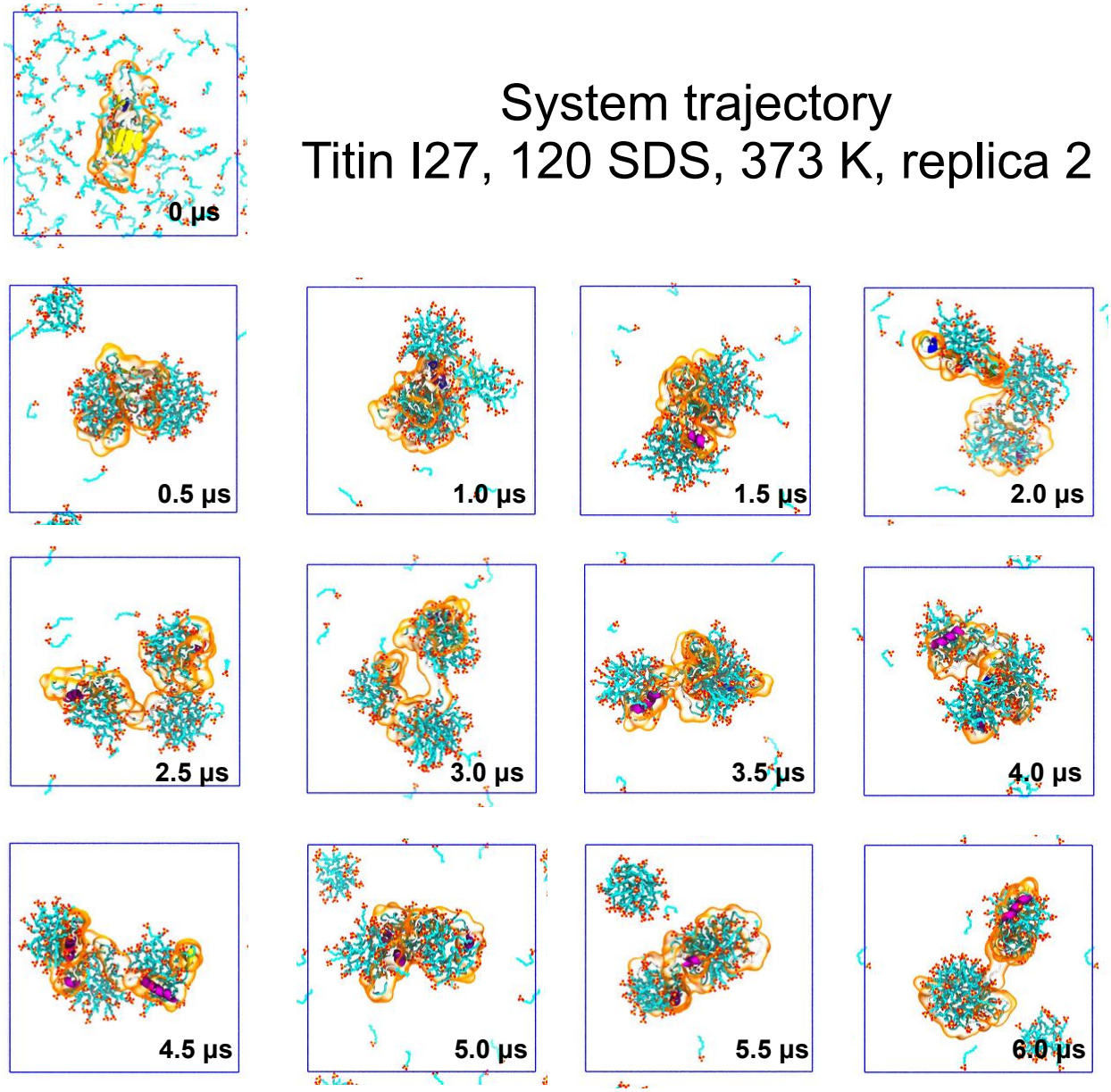


Figure S2 : Spontaneous unfolding of titin I27 at 373 K, replica 2. The snapshots illustrate a 6-μs MD trajectory of a system containing one I27 domain of titin (orange semi-transparent), 120 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S2 illustrates the same MD trajectory.

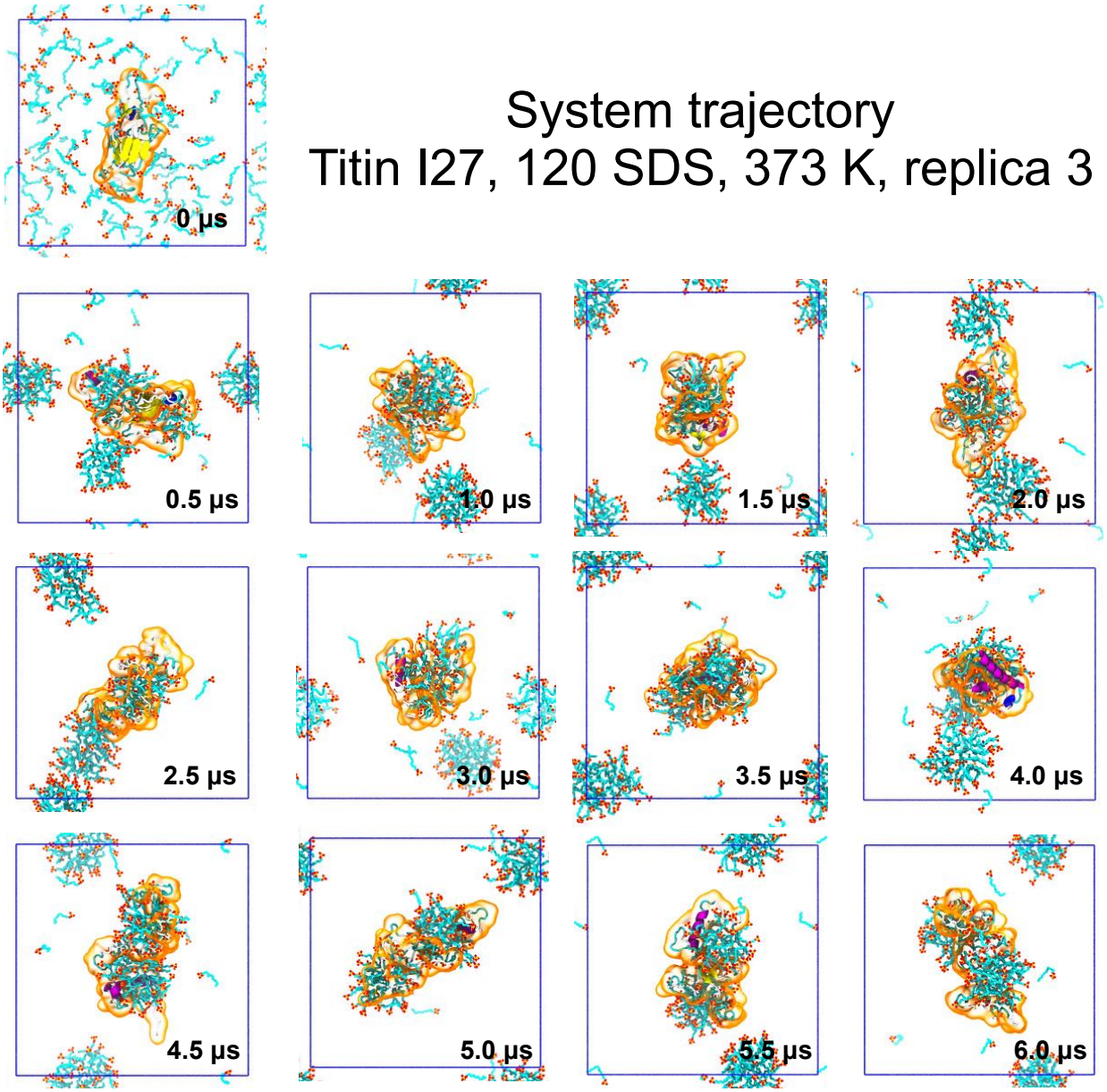


Figure S3 : Spontaneous unfolding of titin I27 at 373 K, replica 3. The snapshots illustrate a 6-μs MD trajectory of a system containing one I27 domain of titin (orange semi-transparent), 120 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S3 illustrates the same MD trajectory.

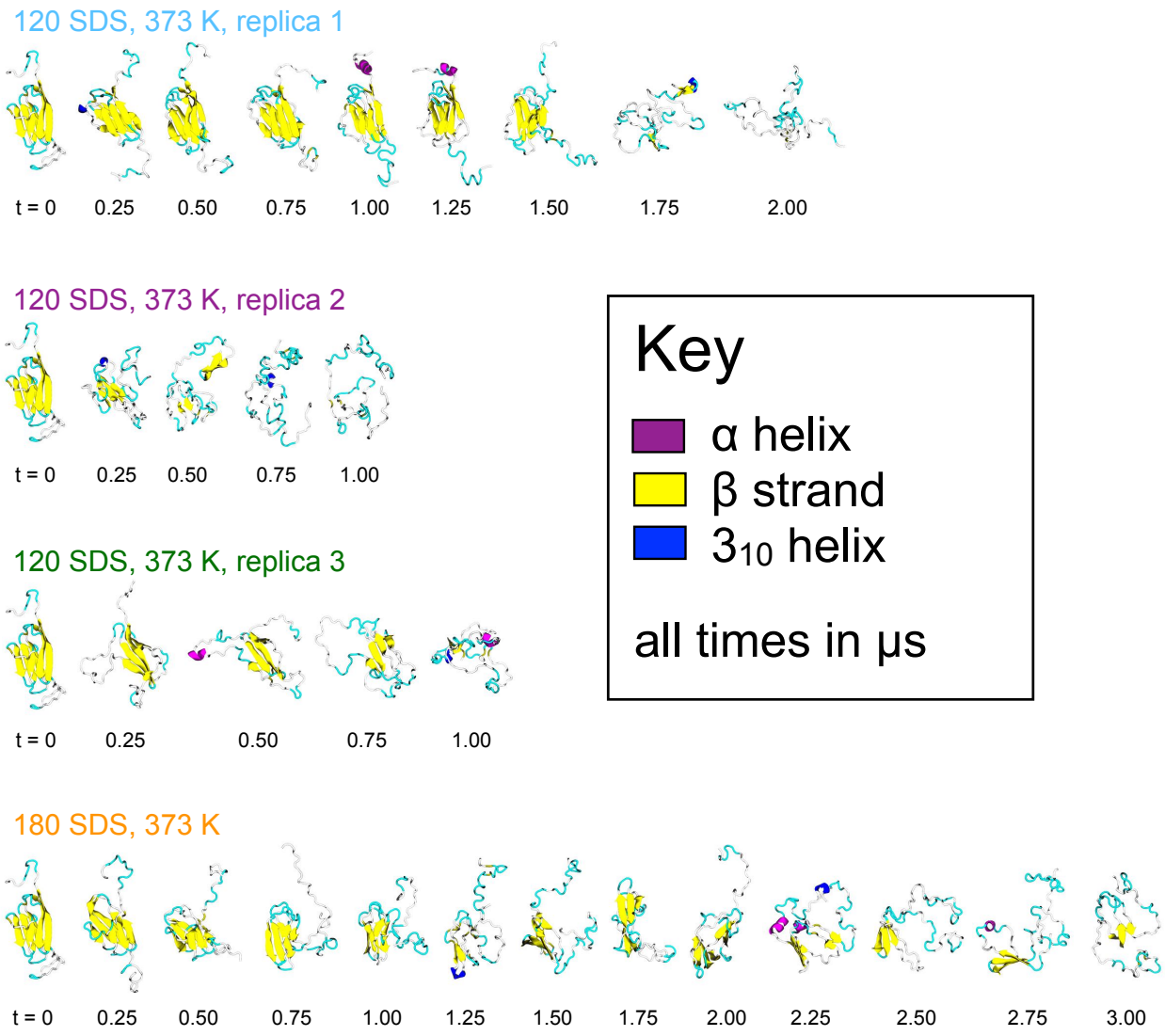


Figure S4 : SDS-induced melting of titin I27 secondary structure. Each sequence of snapshots shows the secondary structure of the protein every 0.25 μs, aligned to its starting conformation, until the moment of complete unfolding. The secondary structure assignment was done by STRIDE in VMD. The key specifies the colors used to identify each type of secondary structure. The same MD trajectories are featured in Figures S1–S3 and S8.

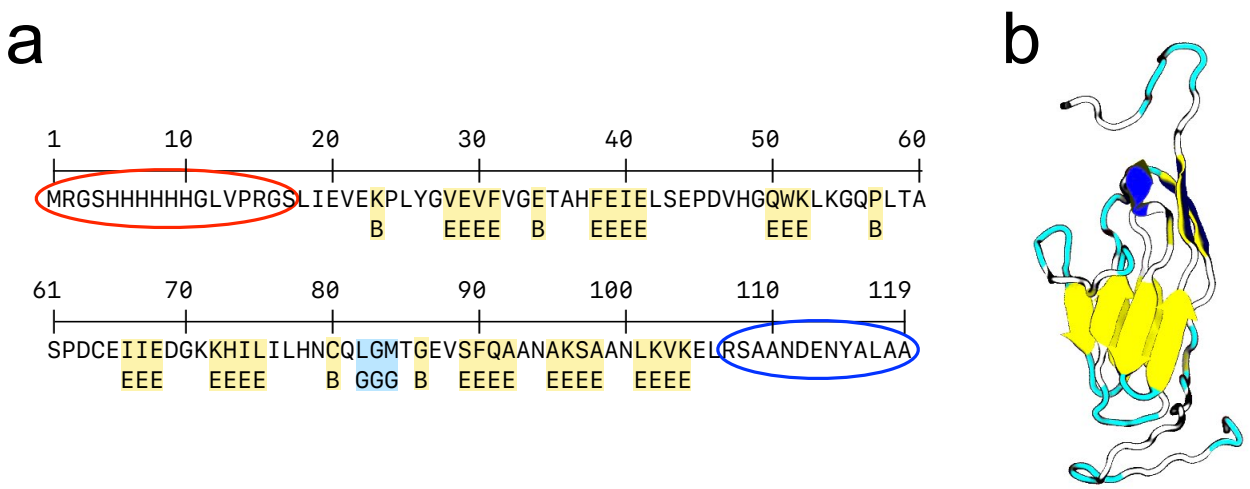


Figure S5 : Titin I27 structure. (a) Titin I27's amino-acid sequence, i.e. primary structure, with the folded protein's secondary structure highlighted by color: β strands and bridges in yellow, and the lone 3_{10} helix in blue. N- and C-terminal tag sequences circled in red and blue, respectively. (b) Cartoon representation of titin I27, secondary structure determined by STRIDE in VMD, matching the assignments in panel a. This structure was based on PDB ID 1TIT; N- and C-terminal tags were added on to the PDB structure.

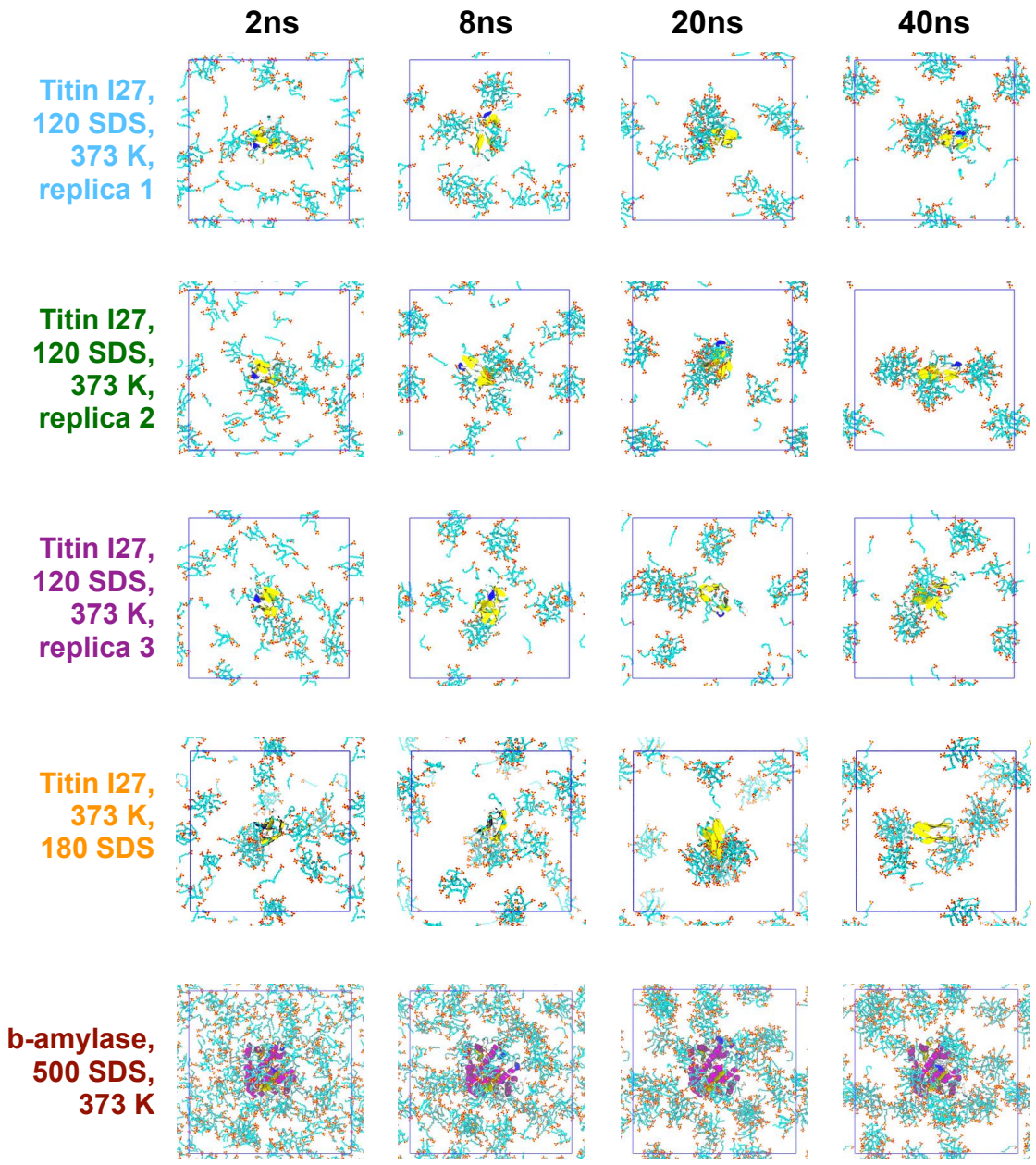


Figure S6 : Visualization of initial SDS micelle formation. Snapshots from all simulations performed of proteins started fully-folded in an ionic solution containing randomly-placed SDS monomers at 2, 8, 20 and 40 ns. Each row refers to a specific system, and each column to a certain point in time. Proteins shown as cartoons colored by secondary-structure; SDS molecules as cyan and red bonds. The periodic images of some SDS are shown to illustrate micelles.

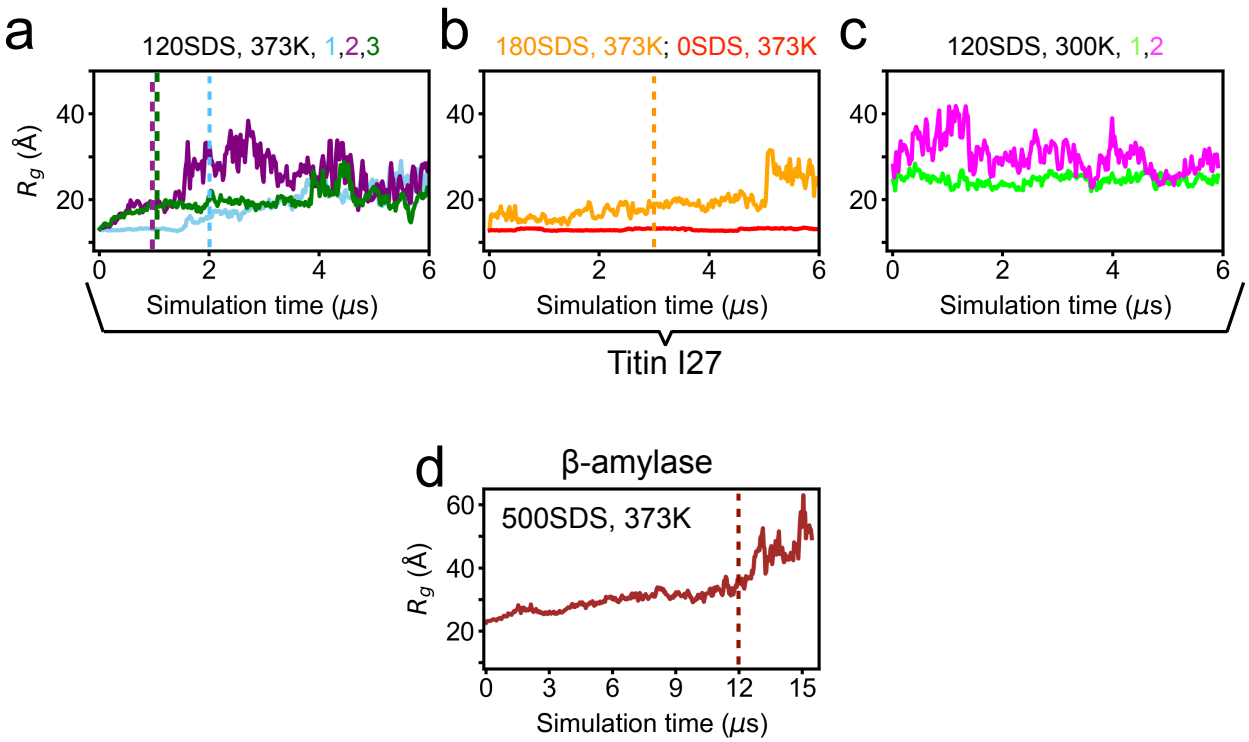


Figure S7 : Protein R_g versus simulation time. Titin I27 R_g in solutions containing (a) 120 SDS molecules and 0.4 M NaCl at 373 K, (b) either 180 SDS (orange) or no SDS molecules (red) and 0.4 M NaCl at 373 K, and (c) 120 SDS molecules and 0.4 M NaCl at 300 K. (d) β-amylase R_g in a solution containing 500 SDS molecules and 0.4 M NaCl at 373 K. Vertical dashed lines in panels a, b and d indicate the moment when the protein became unfolded unfolded (i.e. $Q < 0.1$). Note, the purple and green dashed lines overlap in panel a. R_g was calculated from the positions of all Cα atoms.

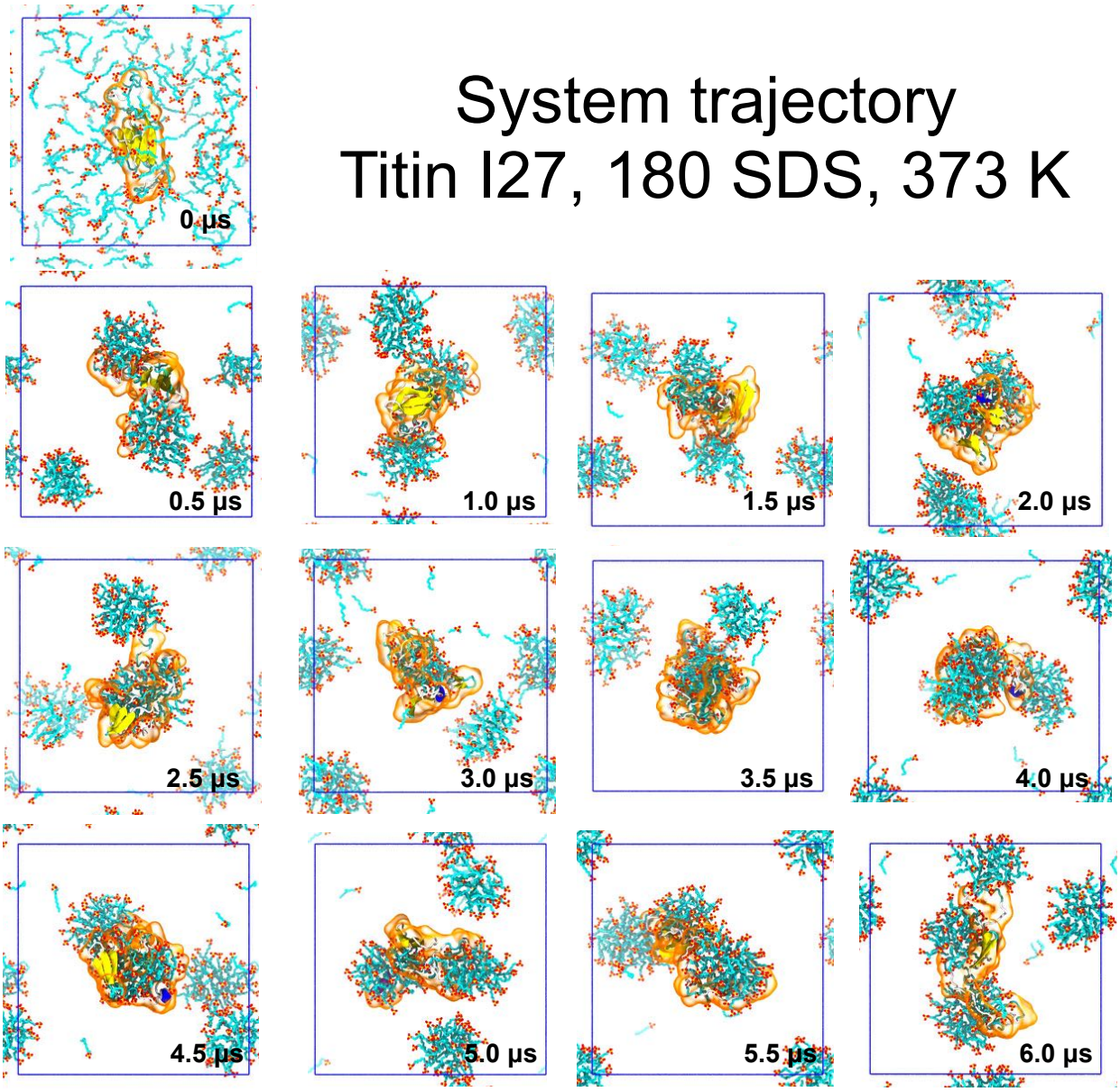


Figure S8 : Spontaneous unfolding of titin I27 at 373 K and elevated concentration of SDS. The snapshots illustrate a 6-μs MD trajectory of a system containing one I27 domain of titin (orange semi-transparent), 180 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S4 illustrates the same MD trajectory.

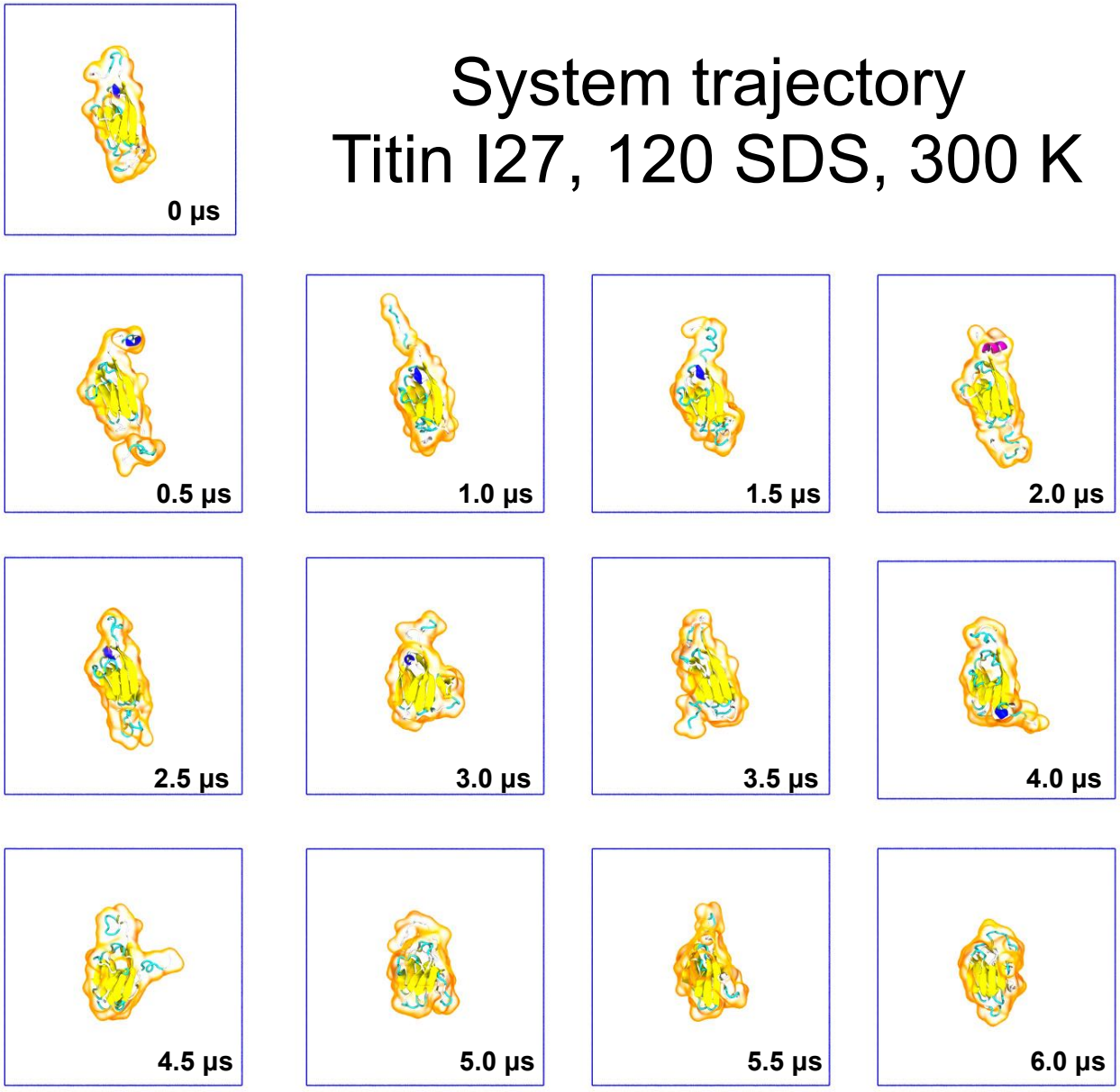


Figure S9 : Control simulation of titin I27 at 373 K in the absence of SDS. The snapshots illustrate a 6-μs MD trajectory of a system containing one I27 domain of titin (orange semi-transparent), and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S5 illustrates the same MD trajectory.

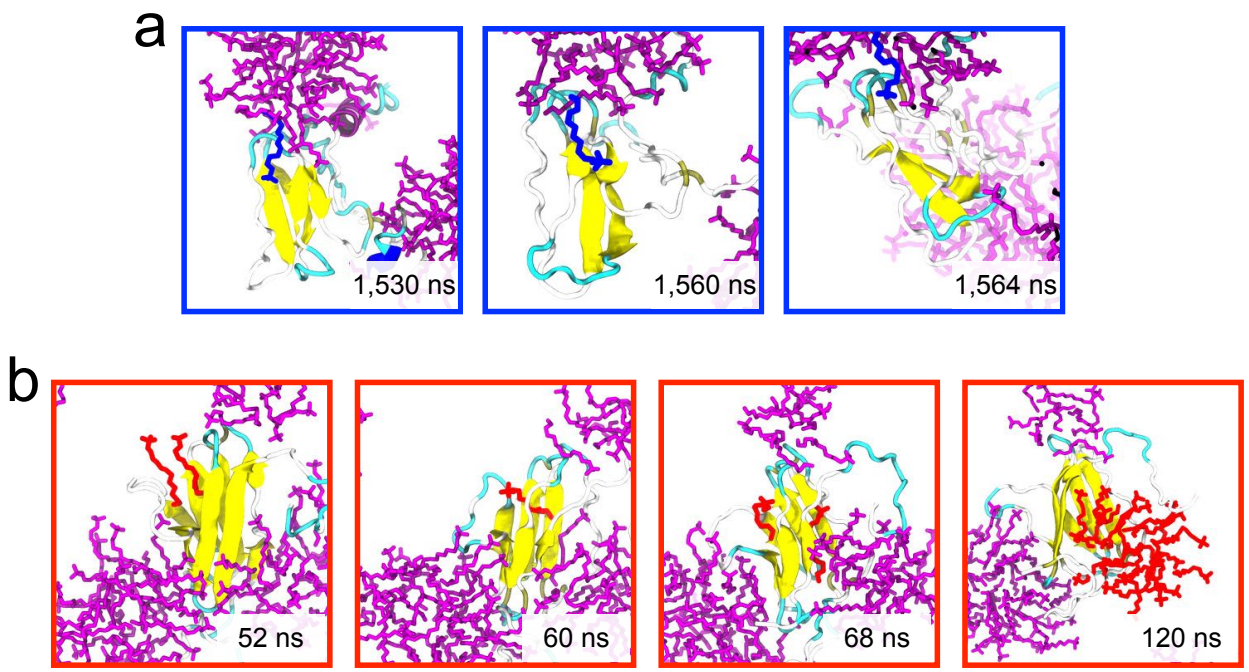


Figure S10 : Specific examples of the unfolding mechanisms. (a) For replica 1 of titin I27 in a boiling solution containing 120 SDS, an individual SDS molecule (in blue) migrates away from a protein-bound micelle (1,530 ns), to lie directly on top of a specific pair of β-strands (1,560 ns), causing the specific hairpin to dissolve (1,564 ns). (b) For replica 2, under the same conditions, two specific SDS molecules begin to insert tail-first into titin I27's central fold (52 ns), only one successfully staying inside in the near future (60 ns), followed by another, different SDS molecule inserting (68 ns), leading the way for an entire SDS micelle to wedge titin's sandwiched β sheets apart (120 ns).

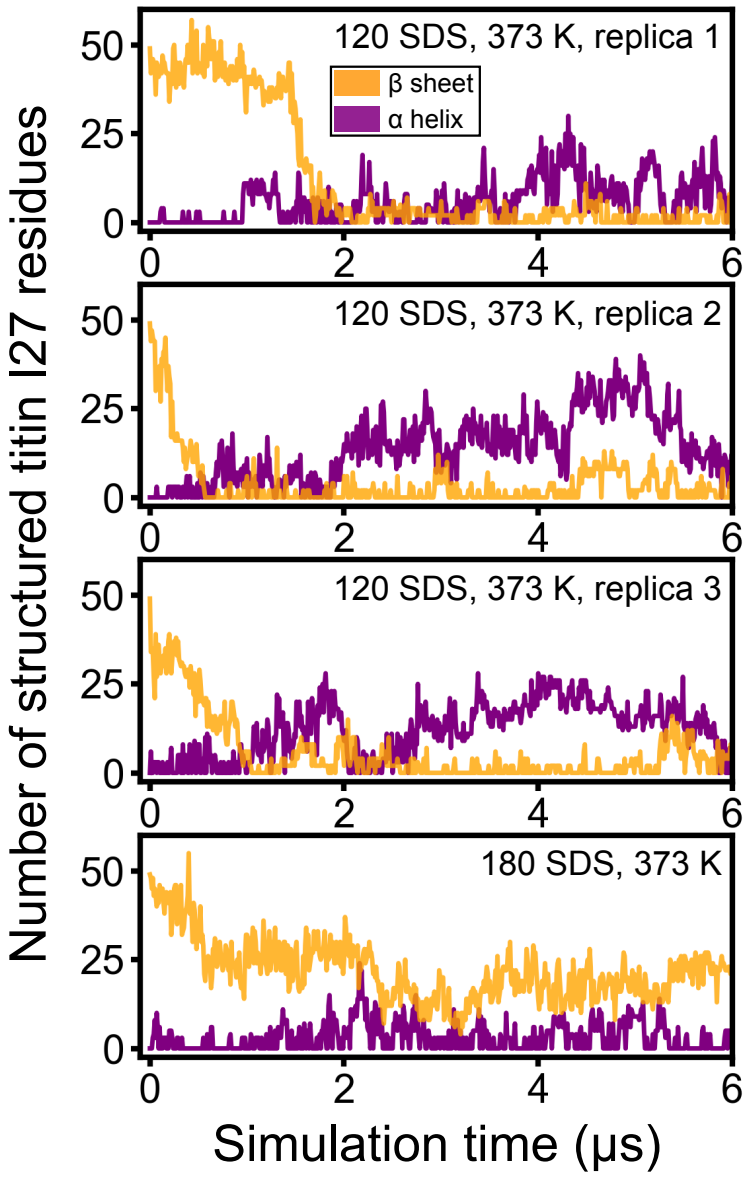


Figure S11 : Titin I27's β versus α secondary structure. The number of titin I27 residues classified as β sheet or α helical by STRIDE in VMD are plotted with respective to all-atom MD simulation time in orange and purple, respectively.

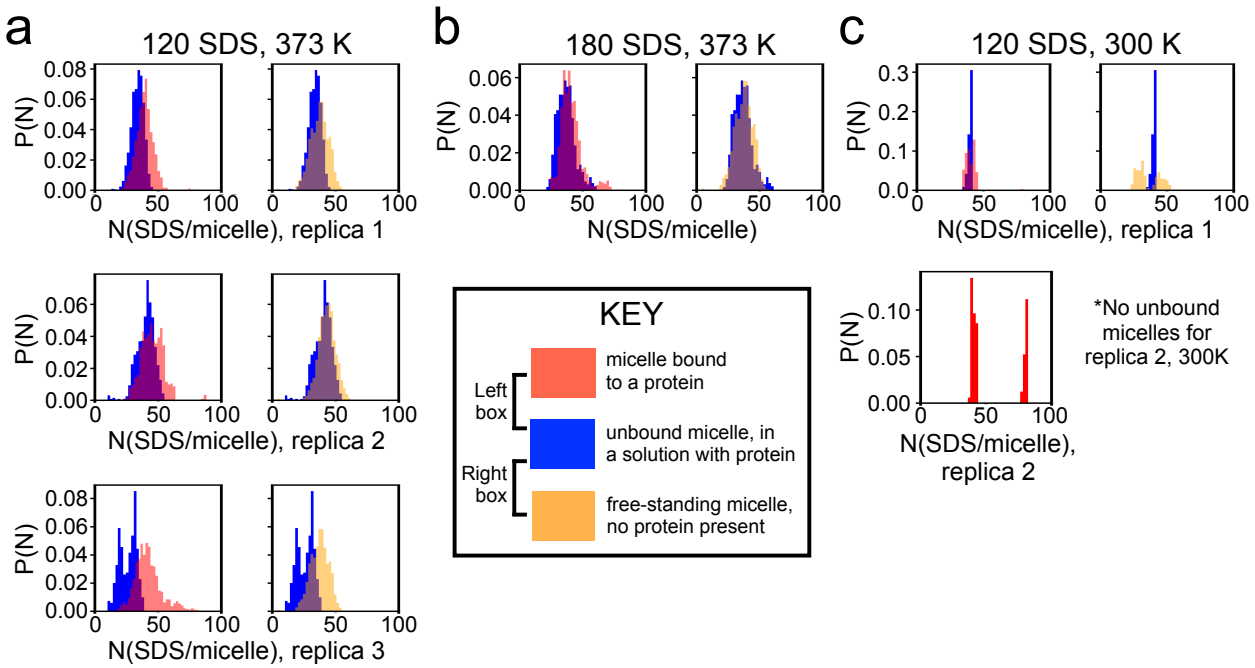


Figure S12 : The composition of SDS micelles. The plots specify the probability of observing the specified number of SDS molecules in an SDS micelle for the simulations performed at 120 SDS and 373 K (panel a), 180 SDS at 373 K (panel b), and 120 SDS at 300 K (panel c). In each panel, the left figure compares the number of SDS molecules in a micelle bound to a protein (red) with that in a free-standing micelle from the same simulations (blue). The right panel compares the number of SDS molecules in a free standing micelle observed in the presence of a protein (blue) or in a simulation carried out without any protein present (orange). The simulations of each SDS-only system were carried out in triplicate, each replica lasting 4.5 μs; the last 1.5 μs of each simulation was used for analysis. For the titin I27 systems, analysis was performed on the parts of the trajectories that followed complete protein unfolding. Axes labels include replica number (if applicable). Note, for the second replica of titin I27 at 300 K, all of the SDS micelles were found to be bound to the protein, so no comparison is made to free-standing micelles at 300 K.

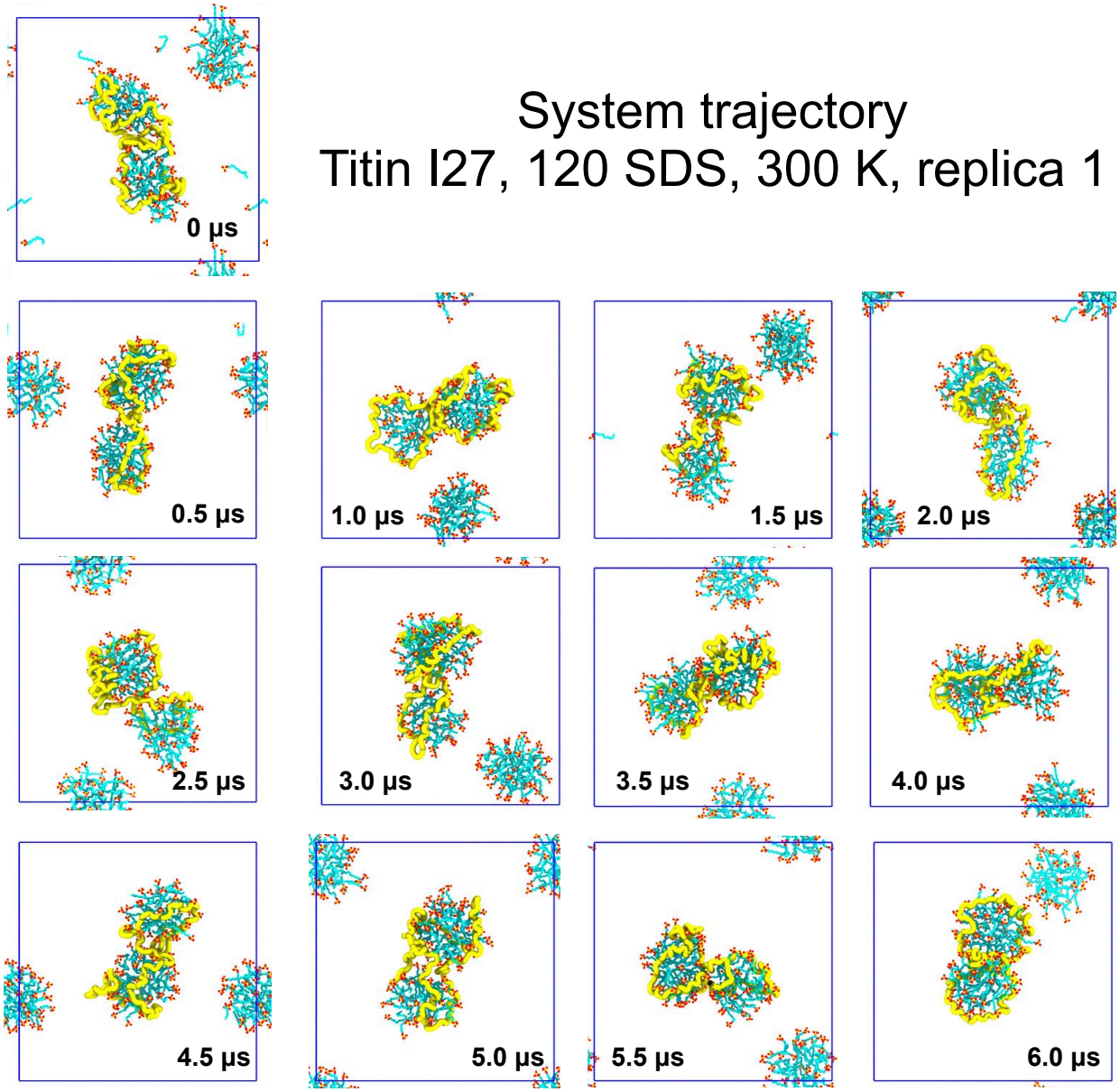


Figure S13 : Conformational dynamics of unfolded I27 domain of titin in complex with SDS at 300 K, replica 1. The snapshots illustrate a 6-μs MD trajectory of a system containing one unfolded titin I27 domain of titin (yellow tube), 120 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S6 illustrates the same MD trajectory.

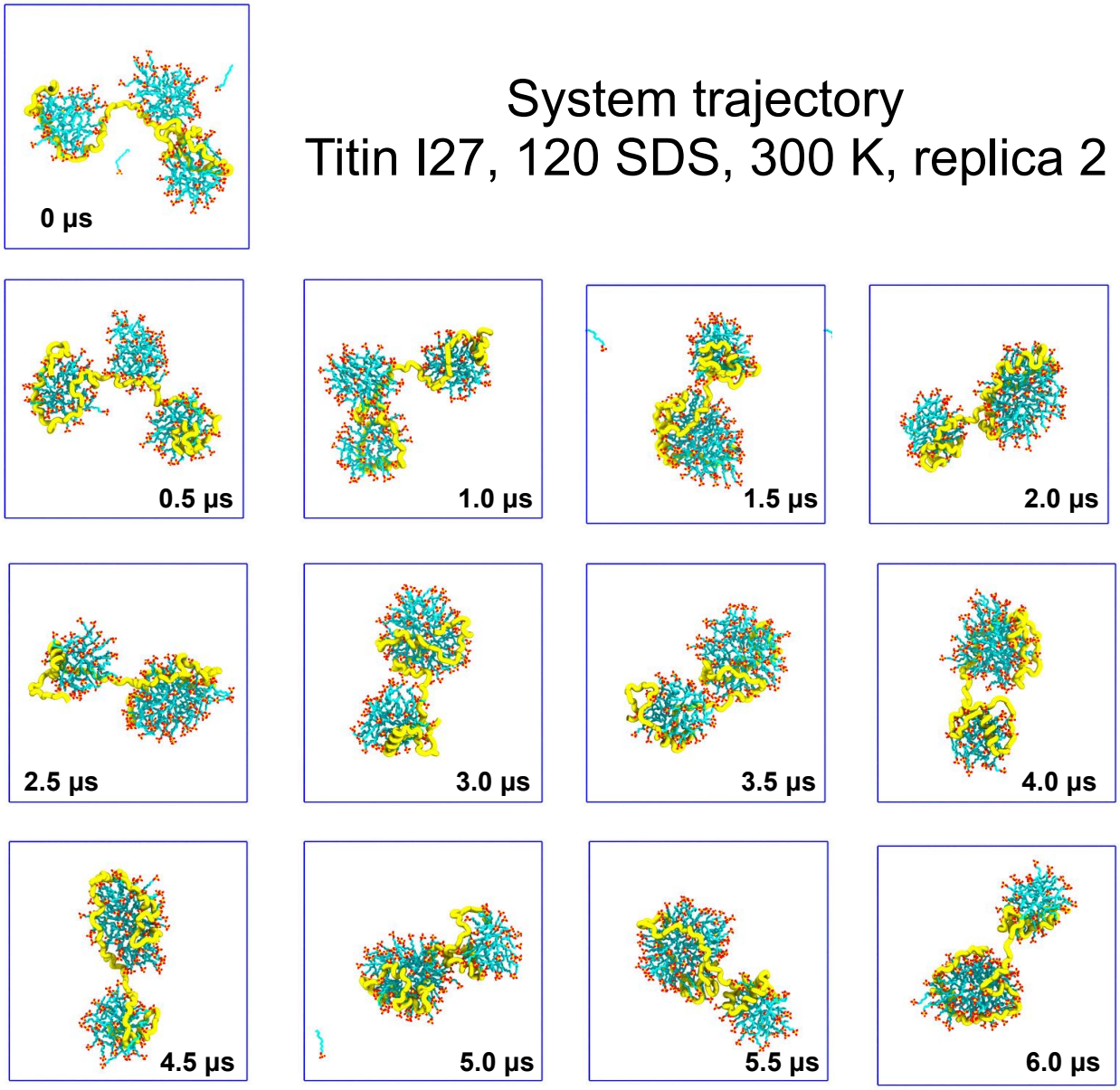


Figure S14 : Conformational dynamics of unfolded I27 domain of titin in complex with SDS at 300 K, replica 2. The snapshots illustrate a 6-μs MD trajectory of a system containing one unfolded I27 domain of titin (yellow tube), 120 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S7 illustrates the same MD trajectory.

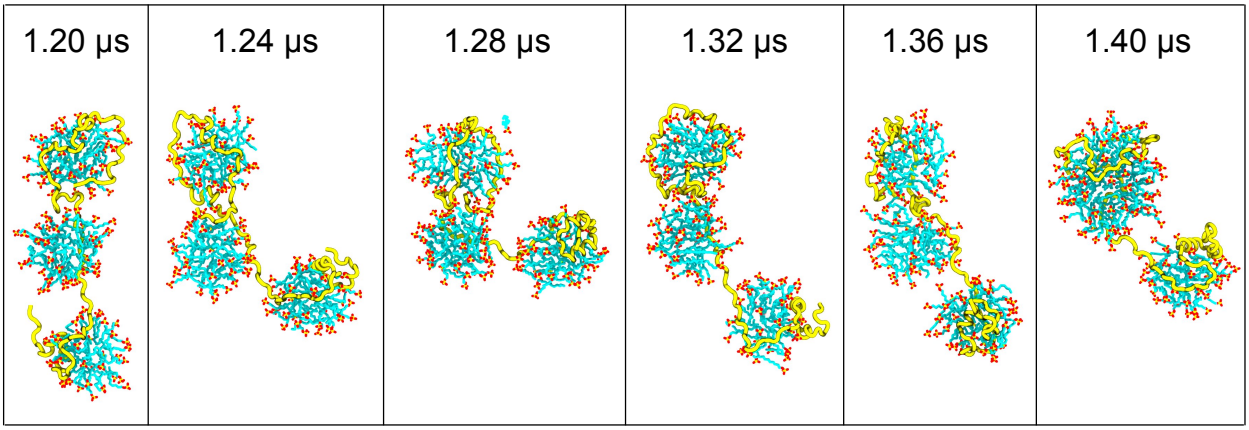


Figure S15 : Fusion of two micelles bound to titin I27. The snapshots illustrates a fragment of the MD trajectory presented in SI Figure S14. The unfolded I27 domain of titin is shown as yellow tube, 120 SDS molecules as colored bonds (cyan and red), water and ions are not shown.

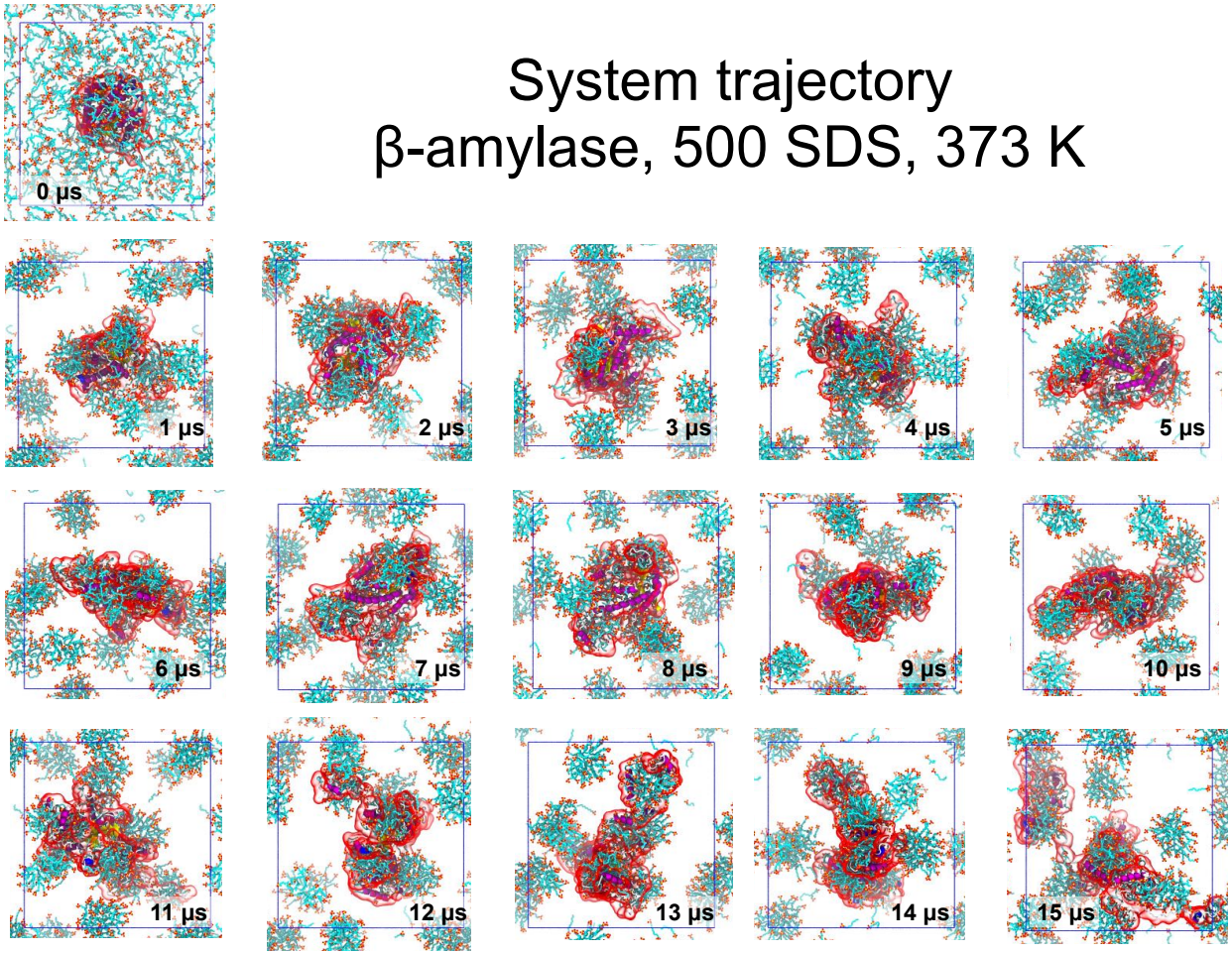


Figure S16 : Spontaneous unfolding of β-amylase at 373 K. The snapshots illustrate a 15-μs MD trajectory of a system containing one β-amylase protein (red semi-transparent surface), 500 SDS molecules (cyan and red bonds) and 0.4 M NaCl solution (not shown). Blue squares highlight the edges of the system. Periodic images of some SDS shown to distinguish micelles. Cartoon representation of the protein also shown, secondary structure assigned by STRIDE in VMD. Movie S8 illustrates the same MD trajectory.

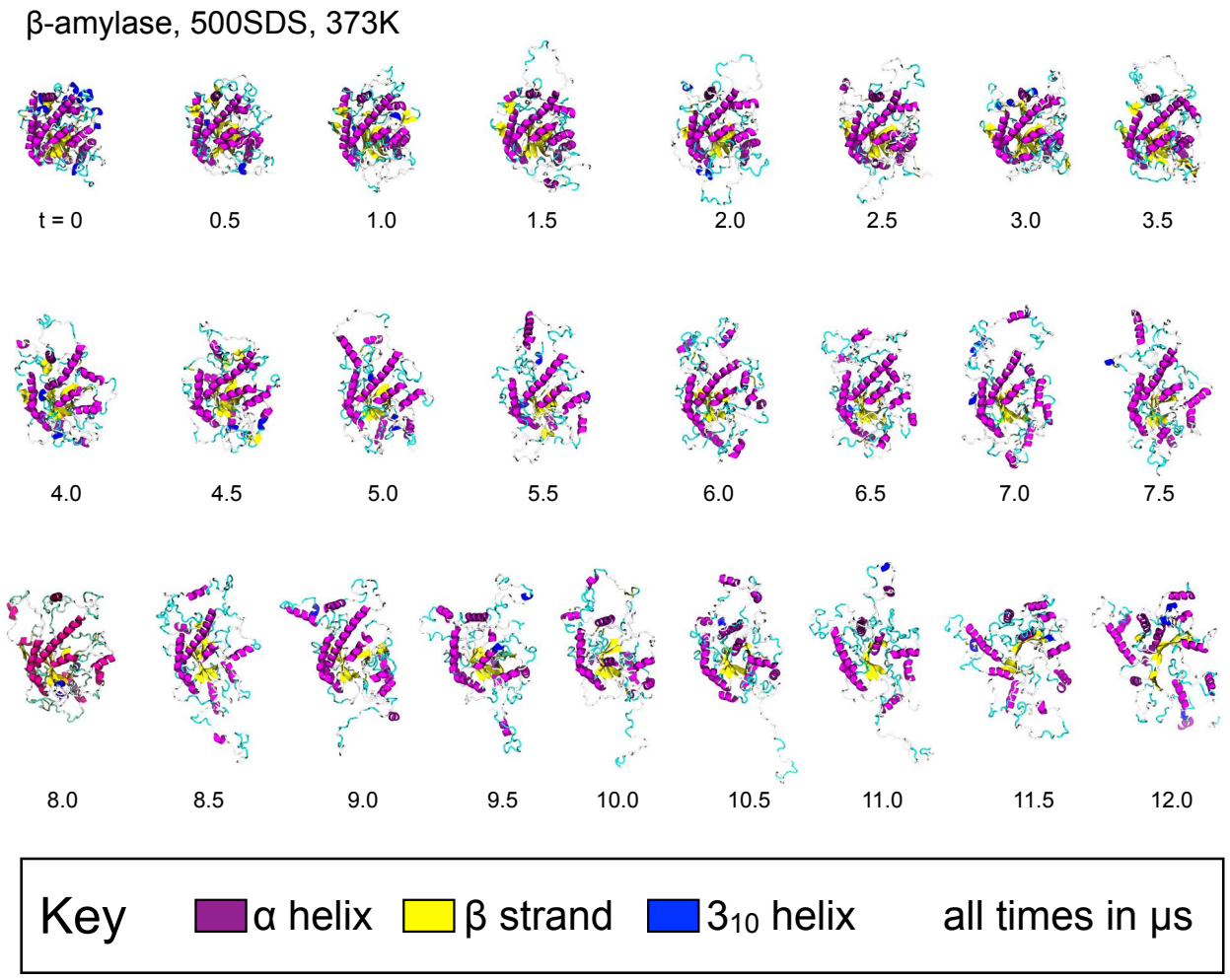


Figure S17 : SDS-induced melting of β-amylase secondary structure. The sequence of snapshots shows the secondary structure of the protein every 0.5 μs, aligned to its starting conformation, until the moment of complete unfolding. The secondary structure assignment was done by STRIDE in VMD. The key specifies the colors used to identify each type of secondary structure. The same MD trajectory is presented in Figure S12.

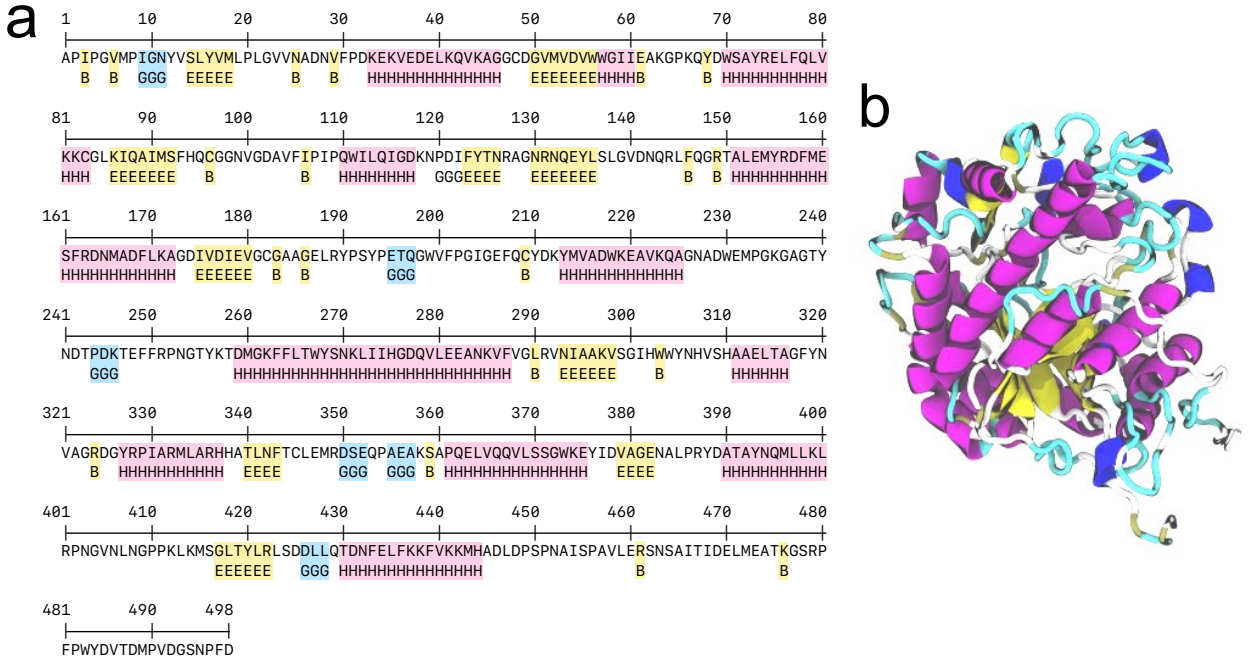


Figure S18 : **β-amylase structure.** (a) β-amylase's amino-acid sequence, i.e. primary structure, with the folded protein's secondary structure highlighted by color: β strands or bridges in yellow, α helices in purple, 3_{10} helices in blue. (b) Cartoon representation of β-amylase, secondary structure determined by STRIDE in VMD, matching the assignments in panel a. This structure was based on PDB ID 1FA2.

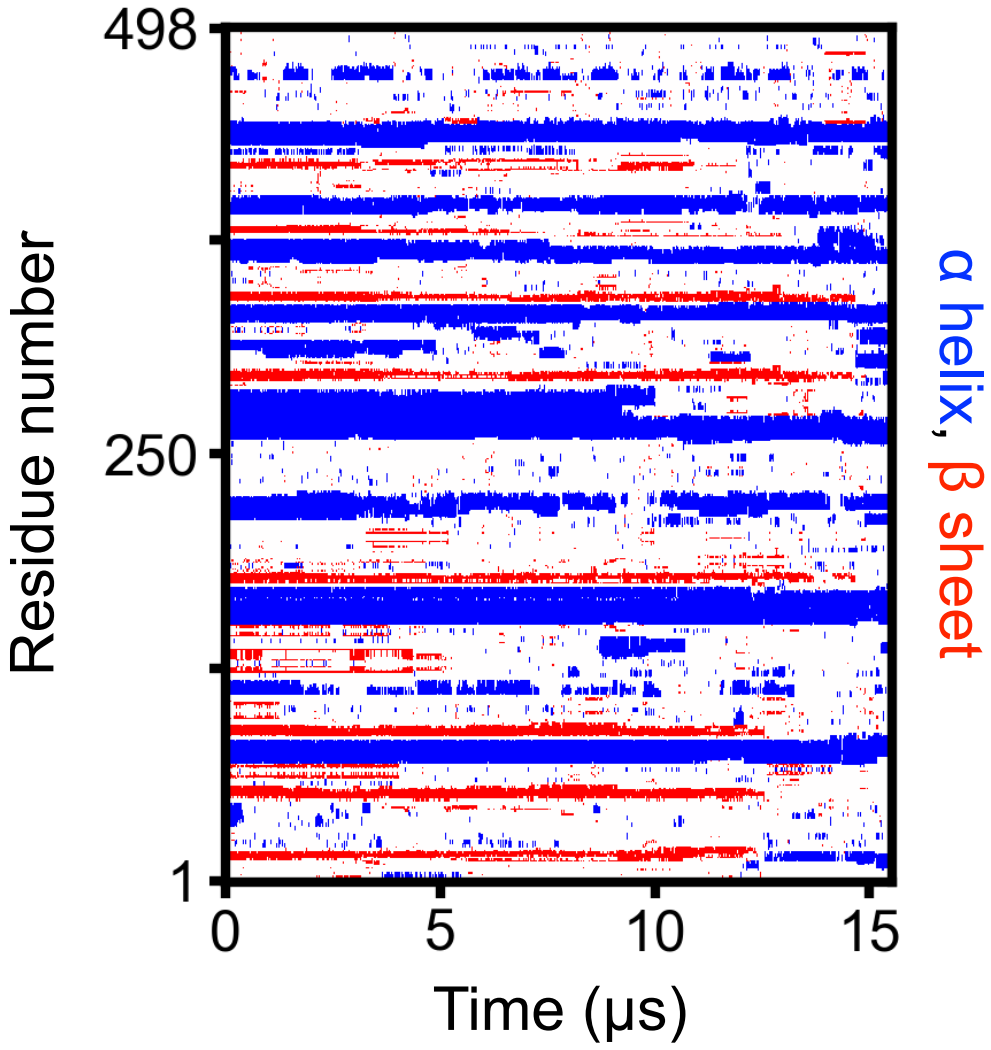
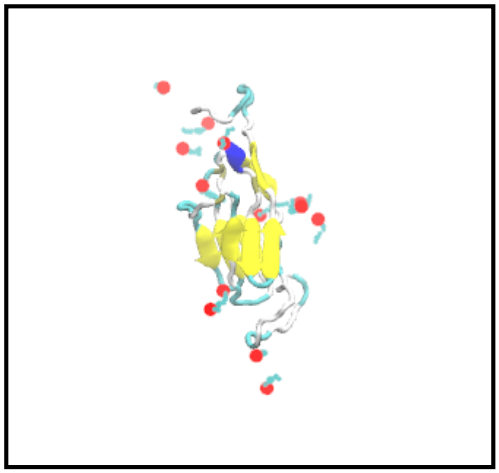
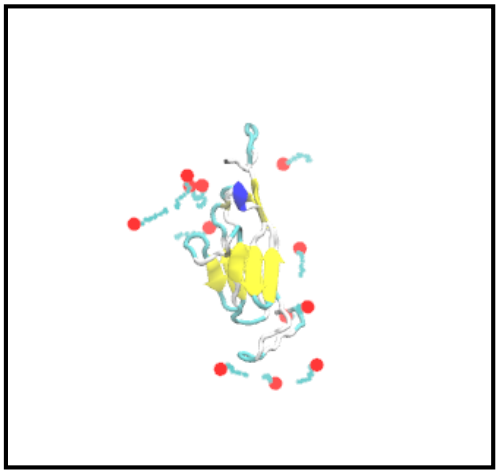


Figure S19 : β**-amylase residue secondary structure vs simulation time.** α helices in blue and β sheets in red. The secondary structure assignment was done by STRIDE in VMD.

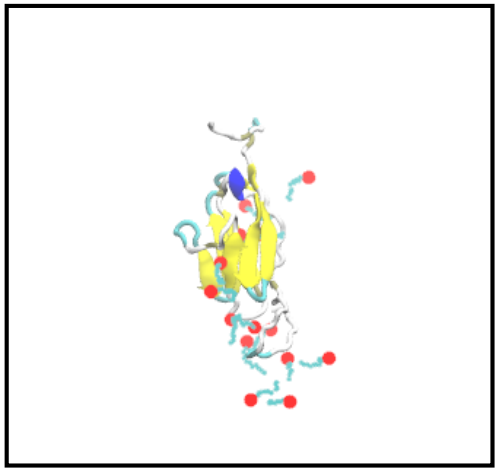
Captions for SI Movies.



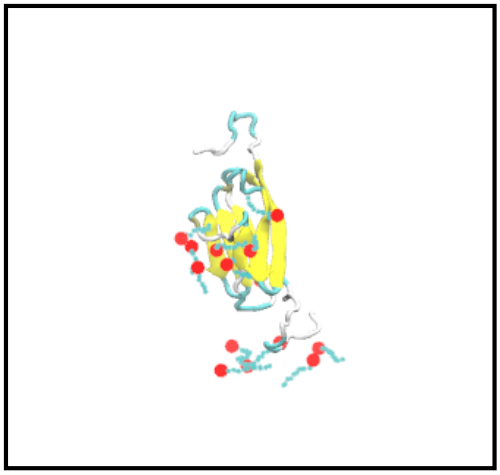
Movie S1 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 120 SDS at 373 K, replica 1. The I27 domain of titin (cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K with 120 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing and alignment may have introduced some minor visual artifacts.



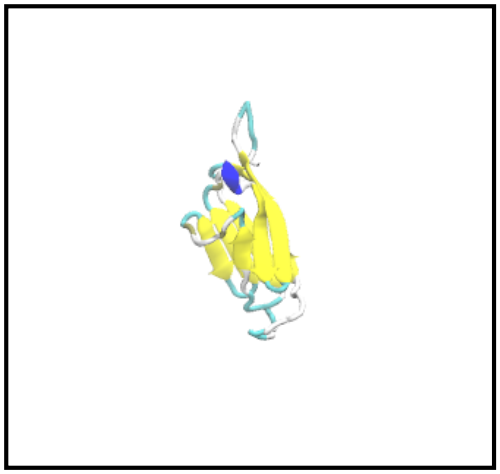
Movie S2 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 120 SDS at 373 K, replica 2. The I27 domain of titin (cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K with 120 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing and alignment may have introduced some minor visual artifacts.



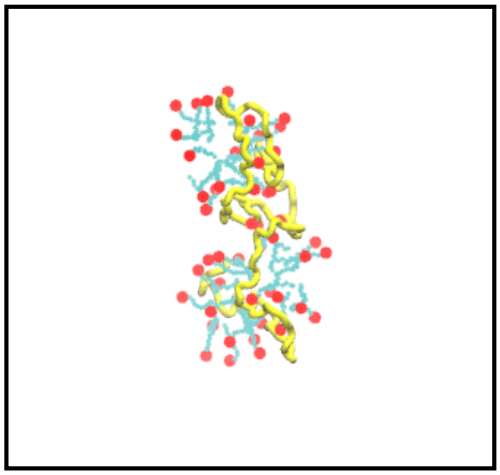
Movie S3 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 120 SDS at 373 K, replica 3. The I27 domain of titin (cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K with 120 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing and alignment may have introduced some minor visual artifacts.



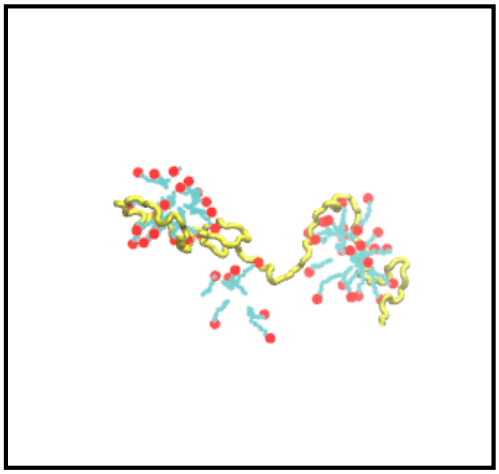
Movie S4 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 180 SDS at 373 K. The I27 domain of titin (orange semi-transparent surface, plus cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K with 180 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing and alignment may have introduced some minor visual artifacts.



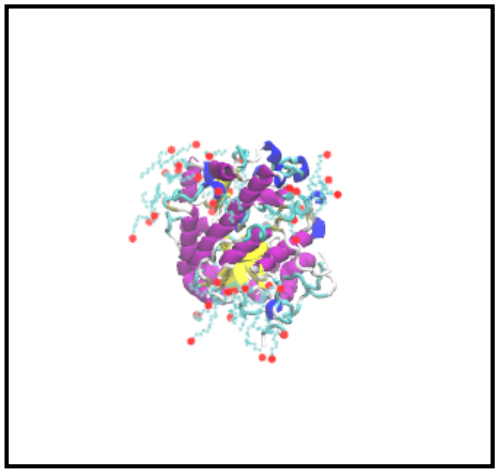
Movie S5 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl without SDS at 373 K. The I27 domain of titin (cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K without any SDS molecules present. Trajectory smoothing and alignment may have introduced some minor visual artifacts.



Movie S6 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 120 SDS at 300 K, replica 1. The I27 domain of titin (yellow tube) in an aqueous 0.40 M NaCl solution (not shown) at 300 K with 120 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing may have introduced some minor visual artifacts, no alignment was performed.



Movie S7 : Movie illustrating a 6 μs MD simulation of titin I27 in 0.40 M NaCl with 120 SDS at 300 K, replica 2. The I27 domain of titin (yellow tube) in an aqueous 0.40 M NaCl solution (not shown) at 300 K with 120 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing may have introduced some minor visual artifacts, no alignment was performed.



Movie S8 : Movie illustrating a 15 μs MD simulation of β-amylase in 0.40 M NaCl with 500 SDS at 373 K. β-amylase (cartoon representation of the protein) in an aqueous 0.40 M NaCl solution (not shown) at 373 K with 500 SDS molecules (cyan and red spheres), only showing SDS when directly bound to the protein for clarity. Trajectory smoothing and alignment may have introduced some minor visual artifacts.