Folding of the human Pin1 WW domain using molecular dynamics simulations

Aristea-Marina Zigkou, Nicholas M. Glykos

Structural and computational biology laboratory,
Department of Molecular Biology & Genetics, Democritus University of Thrace,
Alexandroupolis, Greece, ✉ Marina.Zigkou@gmail.com, glykos@mbg.duth.gr

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Εφαρμοσμένη Βιοπληροφορική και Ανάλυση Δεδομένων

Introduction

In recent years, there has been significant progress in the field of Molecular Dynamics Simulations, providing us with the ability to understand the behavior and dynamics of molecular systems at the atomic level. The challenges and failures encountered by researchers in their efforts to create effective force fields have been insightful. Understanding the folding process of a β-sheet, which is a dominant secondary structure, is of great importance. In this communication, the possibilities of this process are explored using the well-studied β-sheet fold with three antiparallel β-strands of the Fip mutant, which is part of the WW domain of the Pin1 protein. The objective is to analyze and confirm whether the applied force fields and parameters can successfully simulate the protein's folding process.

Methods

Two independent MD simulations were performed to investigate the folding of the Fip protein, starting with the peptide chain in an unfolded state. A 10 μs simulation was performed to attempt the folding of a 35-residue mini protein using the ff99SB-ILDN force field [1], with the Fip35 mutant as the reference structure. Additionally, a 15 μs simulation was carried out to attempt the folding of the same mini protein using the ff99SB*-ILDN force field [2], with the same reference structure. Both simulations utilized the explicit TIP3P water model, and the systems were equilibrated at 360K and 1 atm.

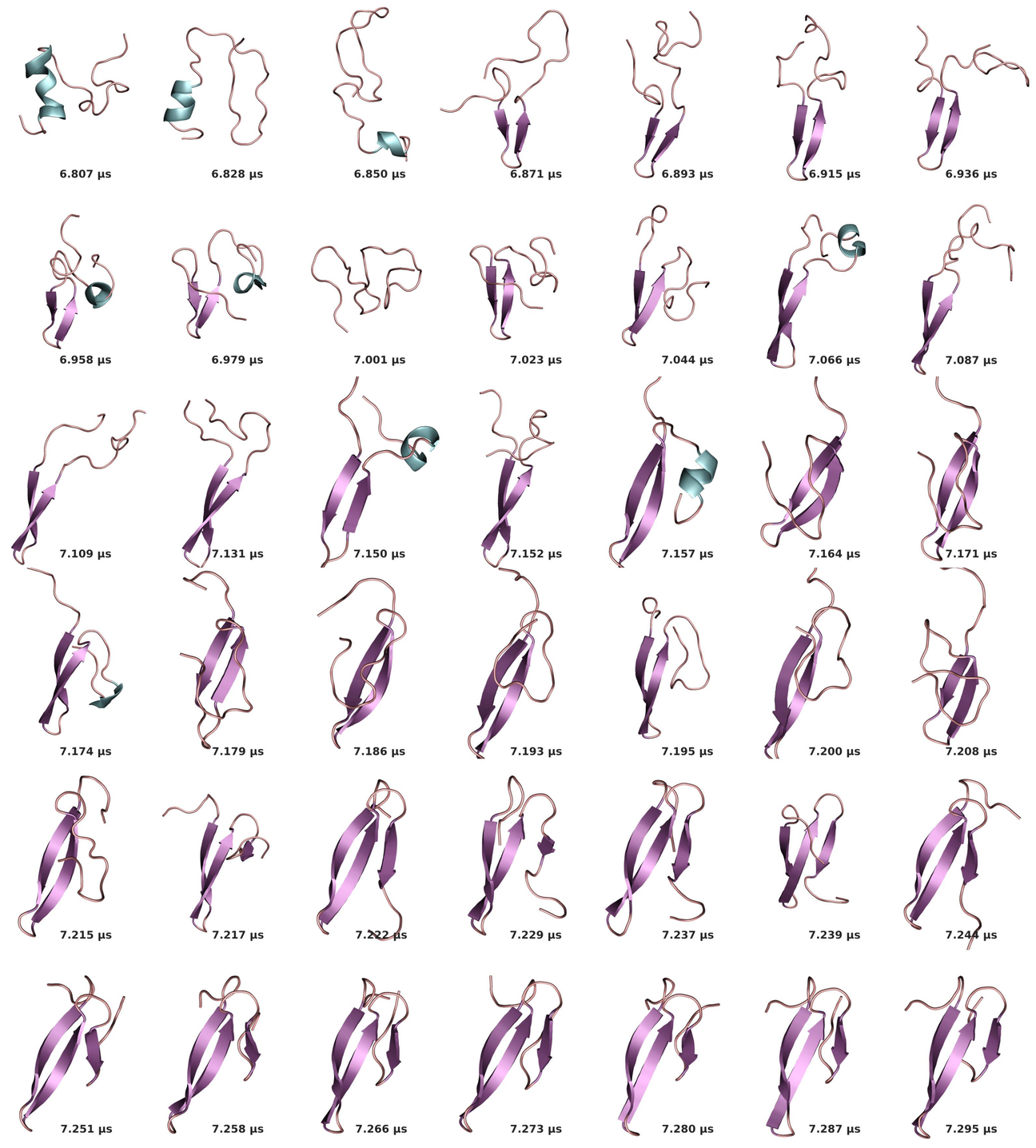

Figure 1. Representation of the entire folding process, illustrating the initial formation of the β-turn-β motif leading to the creation of the β-sheet (final conformation). The representation begins with low-resolution snapshots of the folding process and transitions to a detailed high-resolution focus on the formation of the third strand of the β-sheet. The peptide chain, upon achieving its final conformation, does not remain static but demonstrates significant structural fluctuations. The snapshots presented illustrate a small but representative sample of the structural dynamics observed throughout the simulation.

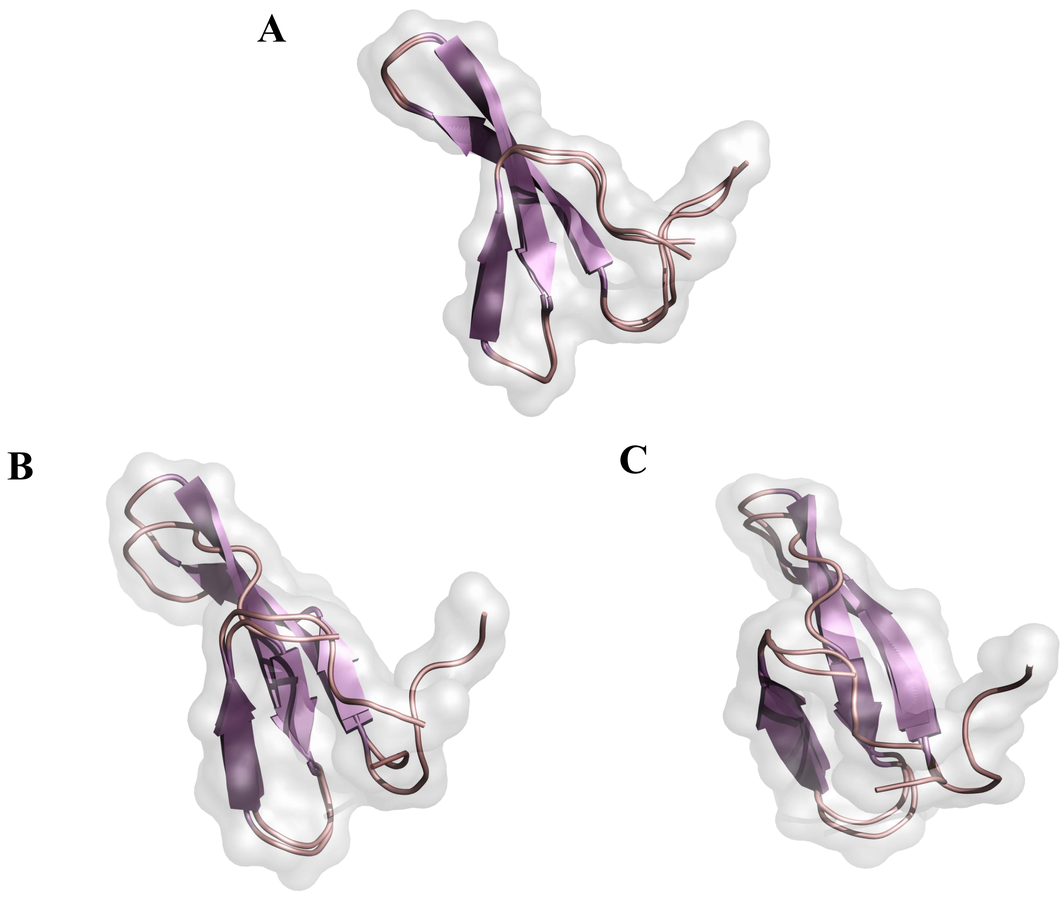

Figure 2. **A)** Superimposition of the representative structures of Clusters 1 and 2 identified through the 3D dPCA analysis. Despite minor structural differences, which account for the variations observed in their corresponding principal components, the alignment between the two structures affirms that both clusters represent the chain's final conformation. **B)** Superimposition of the representative structure from Cluster 1 and the WW domain of the Fip mutant (PDB entry: 2F21). **C)** Superimposition of the representative structure from Cluster 2 and the WW domain of the Fip mutant (PDB entry: 2F21).

Results

In Figure 1, at 6.871 μs of the simulation, the formation of the β-turn-β motif is observed, and this conformation reaches its final size at 7.066 μs. The formation of the third β-strand begins at 7.217 μs and achieves its maximum size much faster than the β-turn-β motif, at 7.222 μs. From that point onward, and for the remaining 7.8 μs, the structure maintains its β-sheet conformation.

Figure 2 illustrates a graphical representation of the similarities between the two main clusters (1 and 2) and their resemblance to the experimental structure used as the reference. The structures of Clusters 1 and 2 exhibit high similarity, as indicated by the very low RMSD value of 0.65 Å. This suggests that these states are closely related and could represent transitional phases during the folding process. The superimposition of Cluster 2 and the experimental structure shows a very low RMSD value (1.28 Å), indicating that Cluster 2 resembles the experimental structure more closely compared to Cluster 1 (RMSD = 1.81 Å). An RMSD (Root Mean Square Deviation) value below 2 Å typically signifies a high degree of structural similarity between two molecular structures. This suggests that both Cluster 1 and Cluster 2 closely resemble the experimental structure, with minimal deviations. Such a low RMSD indicates that the clusters likely represent conformations that are native-like, supporting the reliability of the simulation in capturing realistic protein folding.

Conclusions and Future Work

Remarkably, the ff99SB*-ILDN force field, in contrast to the ff99SB-ILDN force field, successfully folded our protein in approximately half the total simulation time (around 7.2 μs). Our ultimate goal is to achieve force field transferability, which means the ability to fold all types of secondary structures and their combinations, not just limited to one β-sheet and two-three α-helices.

References

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