Mathematical Biology and Bioinformatics
2025. V. 20. № 1. P. 47–53. doi: 10.17537/2025.20.47

========== **MATHEMATICAL MODELING** ==========

Molecular Dynamics of Twisted B-Sheet inside a Protein Dimer of TTHA1013 *Thermus thermophilus*

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Abstract. α-Helix and β-sheet are two basic secondary structure motifs of protein molecules. A true native protein state is realized in a water solution. Under these conditions the proteins carry out the dynamic properties and, what is important, fulfill their various functions. We recently studied molecular dynamics of α-helical fragment of the *T. thermophilus* TTHA1013 protein in a water solution. Here we consider dynamics of β-sheet in the dimer of this protein. Dynamics of only main chain Cα-atoms were studied. One of the most distinctive type of fluctuations was detected along four independent dynamic trajectories with a total time of 200 nanoseconds. This fluctuation, called a "butterfly wing", corresponds to the movements of Cα-atoms of the peripheral residues of the β-sheet fragment. While Cα-atoms of the central part of the sheet remain immobile because of hydrophobic interaction of the side chains within the molecular core.

Key words: *β-sheet; globular protein; molecular dynamics; molecular dynamics simulation program PUMA-CUDA, Trajectory Analyzer of Molecular Dynamics* (*TAMD*)

1. INTRODUCTION

The native proteins perform their function mainly in a water solution. A molecular dynamic simulation allows us to study in details the dynamic fluctuation of protein structure under such condition [1]. More essential that dynamic features of proteins in a water solution determine their function. For example, the contribution of dynamics in the signal transfer of the human tyrosine phosphatase PTP1B from an allosteric inhibitor to the active site of the enzyme was shown [2]. An evolutionary conservation of protein dynamics has also been revealed in the N-terminal 'peptidase' domain of Saccharomyces cerevisiae protein Spt16 and three of its homologs [3]. Molecular dynamic simulation highlighted conserved motions in all three homologous similar to the Spt16N protein. This appears to be a very important observation allowing us to take a new look at the behavior of protein molecules in their native condition. Using molecular dynamics simulations, it is possible to discover new features of proteins in solution and compare the protein structures in water and in crystalline states. Thus, the dynamics of standard motifs significantly extends our understanding of protein structure. α-Helix and β-sheet are two basic motifs in protein structure. We have recently studied molecular dynamics of Cα-backbone of α-helical fragments inside the poly-L-glutamic acid, in the proteins TTHA1013 from Thermus thermophilus, and in ribosomal protein L25 in a water medium [4–6]. In this communication we studied the molecular dynamics of the β-sheet which is also one of the basic structural motifs in globular proteins. Dynamics of only main chain Cα-atoms was studied. We observed the presence of one most populated type of fluctuation, which appears along the four independent trajectories with a total time 200 nanoseconds. This

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fluctuation, called a “butterfly wing”, corresponds to movements of the peripheral residues of the β-sheet.

2. MATERIALS AND METHODS

2.1. Selection of the protein structures with β-sheet motif

The protein structure of the selected globular protein must include a distinctive fragment of the β-sheet. We reviewed more than 70 suitable protein structures from the Protein Data Bank [7] using information retrieved from the PubMed Database [8] and the Database of Protein Structural Trees [9]. The crystal structure of a protein with a polypeptide chain length of less than 200 amino acid residues was chosen as the initial model. Most of such proteins exist as oligomers, and for accuracy, we should consider rather large structures. In other cases, such proteins were fixed by S-S bonds or the proteins were bound to metal ions. Finally, the protein dimer TTHA1013 from *Thermus thermophilus* was chosen as more suitable for our study [10–11]. In the crystal state, it forms tight contacts with the nearest molecule, and the final model is a dimer (Fig. 1). The dimer length is 144 residues.

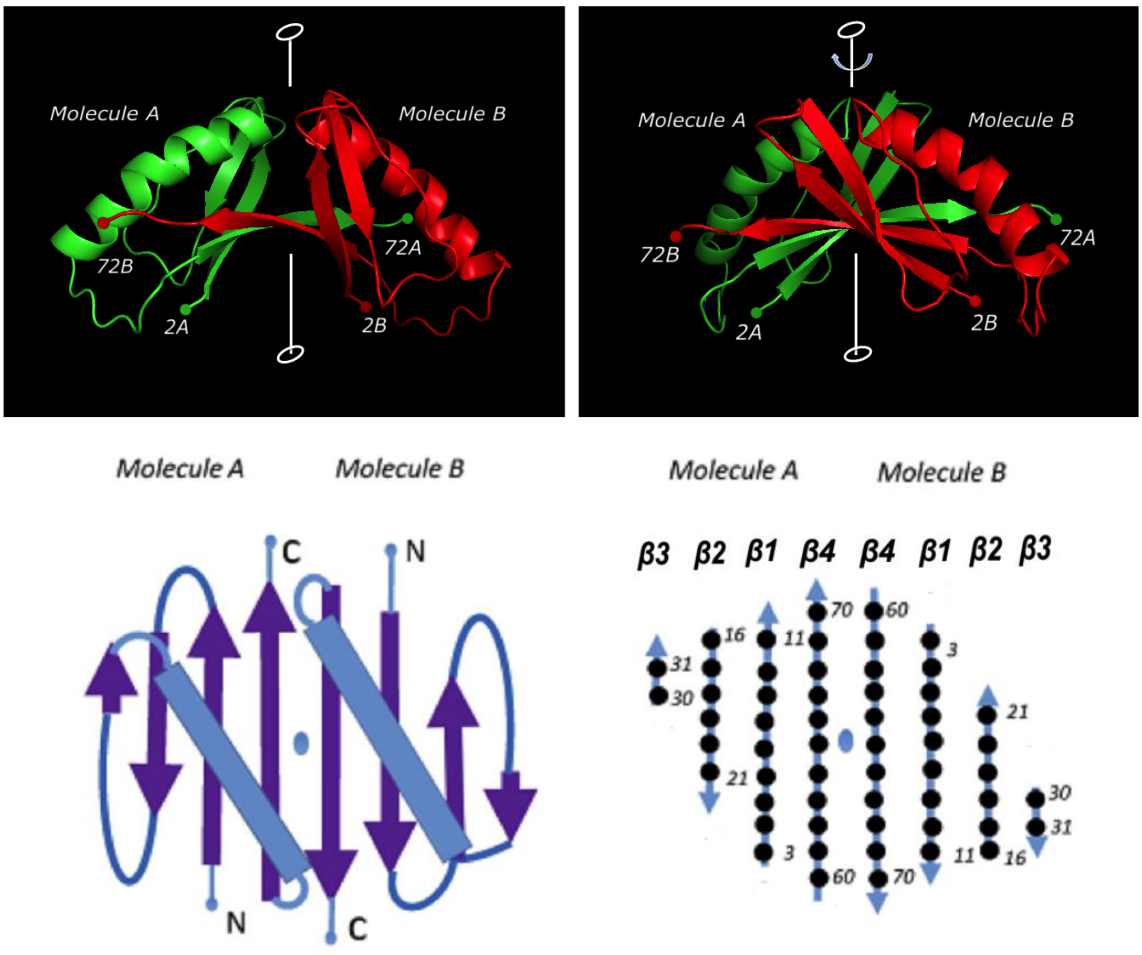

Fig. 1. Dimer structure of protein TTHA1013. Upper: Two views where the monomers are related by twofold rotational symmetry axis. Lower: Pathway of the main chain of protein dimer and amino acid residues in the β-sheet fragment.

The atomic coordinates of the protein in a crystal state were used from the Protein Data Bank file pdb ID:1wv8. The protein model was extended by addition of hydrogen atoms. The molecule was then placed in a box 59 × 53 × 86Å, filled with water molecules. The protein structure includes two α-helices with 4.5 turns and one twisted β-sheet with eight extended strands. Pathway of polypeptide main chain of the protein dimer is shown in Figure 1. Inner

atomic groups of side chain residues of the helical and sheet fragments contact with side groups of hydrophobic core of the molecule. They are immobile.

2.2. Molecular dynamics simulation

Calculations were carried out using standard molecular dynamic simulation PUMA software [12, 13] and the PUMA-CUDA compatible with the GPGPU graphics processing unit. Force field AMBER [14] was used, as well as TIP3P water model [15]. At first, molecular dynamics simulation was carried out during 50ns. The relaxation time for this object is about 1ns. Energy of all system goes to the plateau during this time. We have controlled energy (Coulomb, Van-der-Waals, kinetic parts) every 0.1 ps. Last coordinates of the first stage will be initial coordinates for the second stage. At the second stage, molecular dynamics simulation of four independent trajectories during 50ns have been carried out. All trajectories originate from one point. However, due to the use of random numbers in the thermostat, these trajectories quickly diverge in phase space. The resulting trajectories of molecular dynamics were investigated by the Trajectory Analyzer of Molecular Dynamics TAMD [16–18]. The analysis of the dynamics of Cα atoms is carried out in the polypeptide chain of a protein using molecular dynamics (MD) simulations. To analyze conformational changes, we used an original method of time dependence of Cα-shifts between two sequential pairs of time points [6]. Its main significant features are described below.

Dynamics of Cα atoms: The study examines the dynamics of only the Cα atoms, which are part of the polypeptide backbone. The shifts of these atoms (referred to as Cα-shifts) are calculated between two consecutive time points, separated by 1 nanosecond. All atoms were included in the MD simulations, but only the motions of the Cα-atoms were analyzed for conformational changes.

Superposition of structures: To properly measure these shifts, we aligned the protein models at different times using a moments of inertia tensor, which ensures that both structures are in the same internal coordinate system. The ellipsoid of inertia was calculated, and its origin was set at the center of mass of the Cα atoms. This critical step allowed precise measurements of Cα-shifts to be made.

Example of β-sheet observation: We paid special attention to the dynamics of specific regions, such as the β-sheet structure, by monitoring the Cα-shift of residue ValB61. Changes in protein structure were controlled by measuring Cα-Cα distances, typically 3.80 Å with an RMSD of 0.16 Å in standard polypeptide chains.

Geometric consistency: The internal rotation angles ϕ and ψ were analyzed using a Ramachandran plot, and for 95 % of the residues, the angles were within the acceptable range. In the β-sheet region, approximately 90 % of the residues remained in the allowed region, indicating that the geometry of the β-sheet remained stable despite dynamic fluctuations.

This approach allows to better understand how the dynamics of individual amino acid residues contribute to overall structural changes in the protein.

3. RESULTS AND DISCUSSION

3.1. Molecular dynamics of β-sheet in the protein dimer TTHA1013 in a water solution

The process of the protein structure relaxation of the whole system is completed in one nanosecond. Then we can analyze the molecular dynamics events. Consider the first trajectory at 1–50 ns. In this time interval we have observed the different dynamic profiles. One most frequently appeared profile was found in all four trajectories for a total time 200 ns. Examples of this profile for the first trajectory are presented in Figure 2. The averaged displacements of Cα-atoms were: in the loop region about 7.0 Å, in α-helices about 4.0 Å, and in the β-sheet about 1.0 Å. Many details of protein dynamics can be obtained after interpretation Cα-shift diagrams. Assigned diagram of time 12–13 ns is presented in Figure 3.

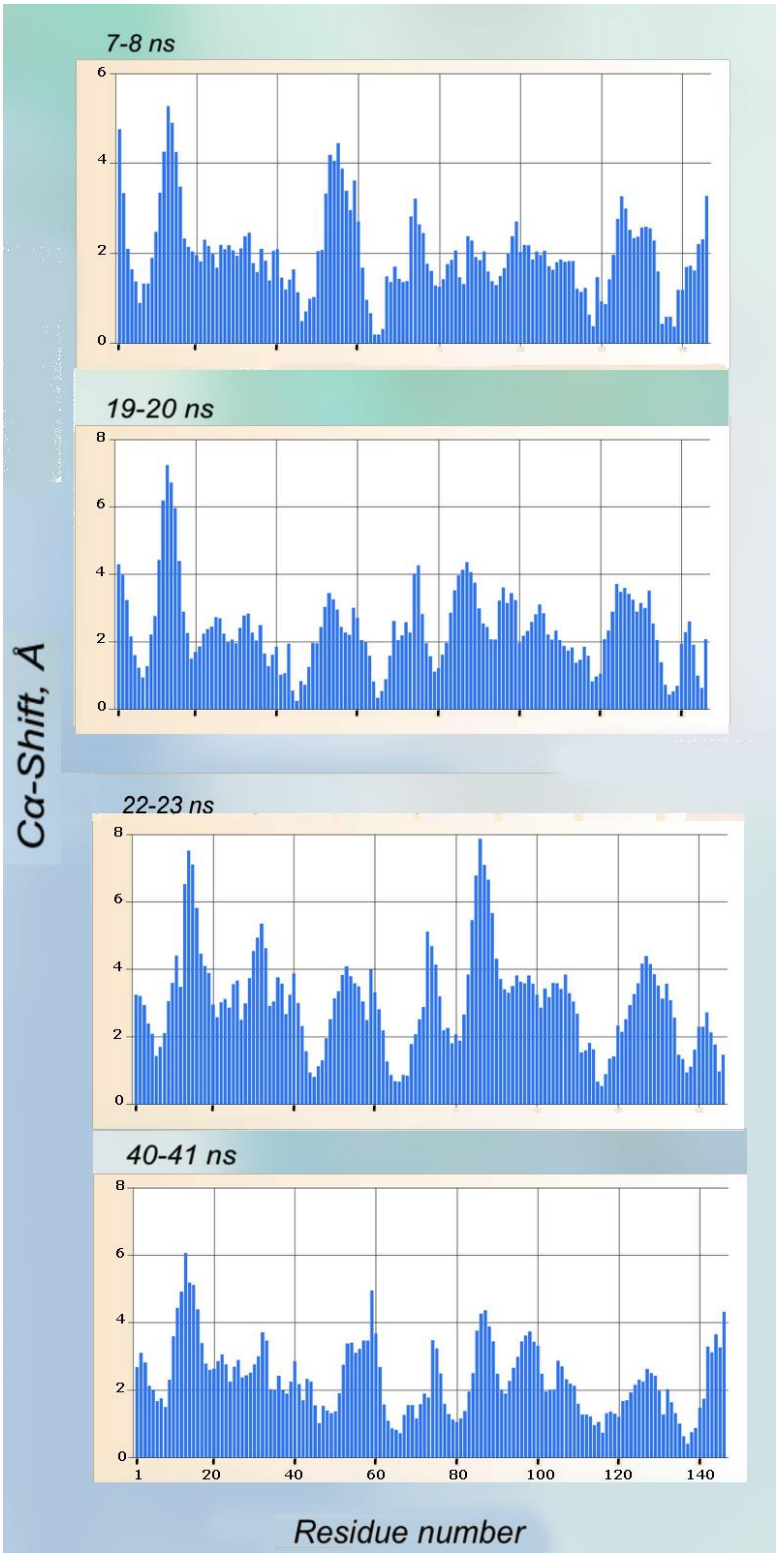

Fig. 2. Four Cα-shift diagrams of polypeptide chain for the most populated fluctuation type “butterfly wing”.

Here we see the most populated type of β-sheet fluctuation, which we call the “butterfly wing”: it is marked with black arrows. Here we concentrated on dynamics of the β-sheet fragment. It should be noted that adjacent β-strands can be sometime related to far located parts of the sequence. Displacements of Cα-atoms in the fluctuation type of the β-sheet fragment at 12–13 ns are shown in Figure 4. Here the large displacements occur in the peripheral Cα-atoms of the β-sheet while Cα-atoms of the central part of the sheet remain immobile because of the

hydrophobic contacts of the side chains with the molecular core. In order to estimate the frequency of occurrence for this fluctuation during the time we used as a check point the values of Cα-shift of the boundary residue.

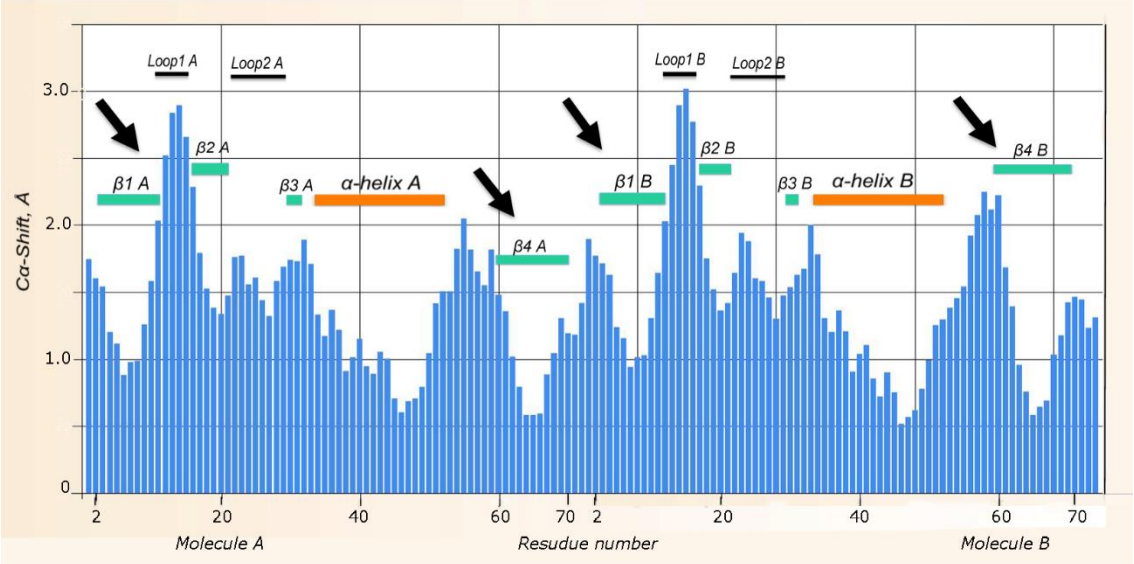

Fig. 3. Dynamic profile of the protein dimer TTHA1013 from in the form of Cα-shift diagram at 12–13 ns. Extended strands of the β-sheet are shown with black arrows.

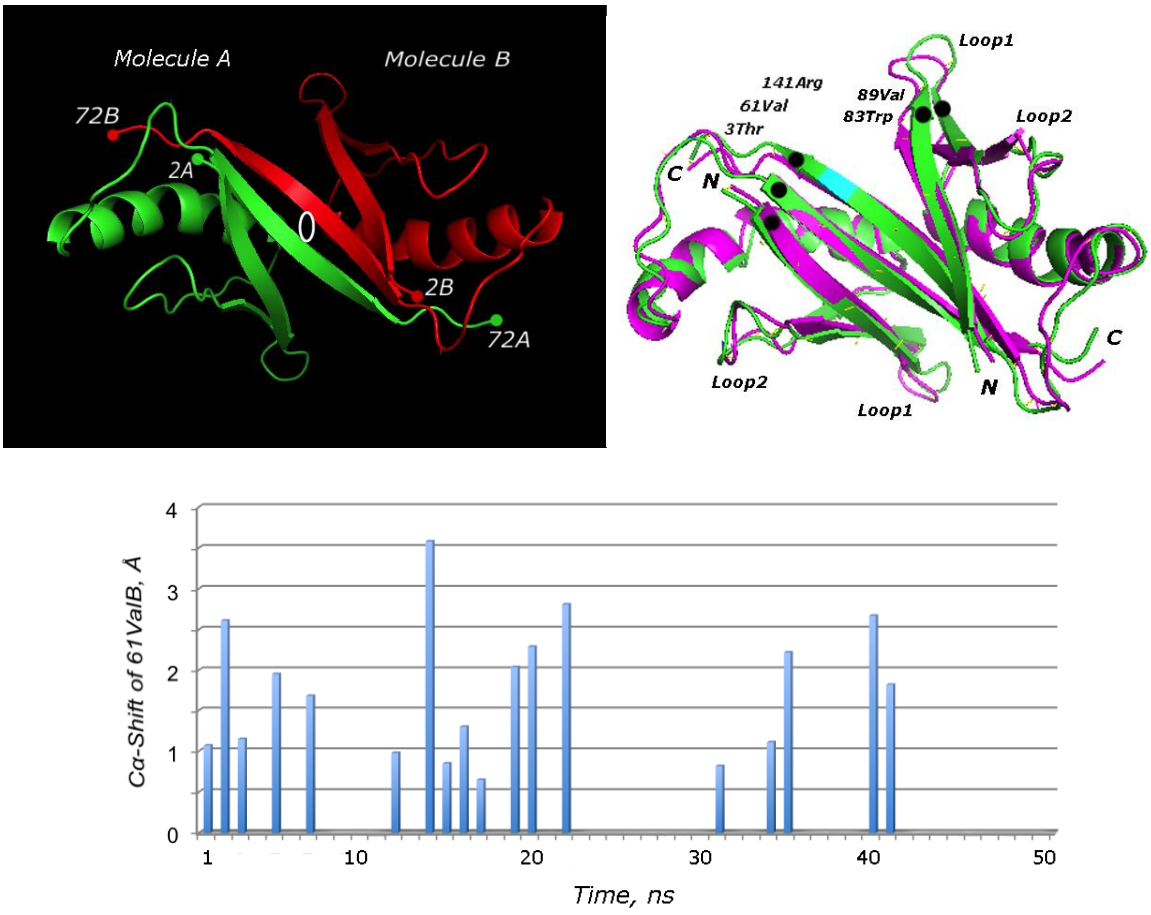

Fig. 4. Upper left: View of the β-sheet in the protein dimer TTHA1013. Upper right: Dynamic alterations in a "butterfly wing" fluctuation type at 12–13 ns, colored green and red. Lower: Cα-shifts of 61ValB of the protein dimer which appear along the first trajectory**.**

61ValB depending on the time (Fig. 4). After analyzing this diagram, we conclude that the fluctuations “butterfly wing” appears randomly. They have average amplitude of atomic displacements of about 1.5 Å ranging from 0.6 to 3.5 Å. This fluctuation type was observed at about 38 % of total time for the first trajectory, and 26, 22 and 22 % for three other trajectories.

4. CONCLUSION

In this work we used a novel approach to study the dynamic events in the protein structure. We analyzed the *dynamic profile of polypeptide chain* of the protein in the form of Cα-shifts diagrams in the structures at two nearest time points. In our case molecular dynamic trajectory was analyzed for the interval 1–200 ns at every one nanosecond. That means we must consider the same amount of protein structures and describe their differences time by time. The used dynamic profile allows us to make it very quickly and simple by visual inspection of Cα-shift diagrams in a few hours just after taking the results from supercomputer job. We can also increase resolution of time in one hundred times from 1ns up to 10 ps, if required. The method has already been applied to study the polypeptide and protein structures [4–6]. To date the dynamics of basic motifs of the protein structure, α-helix and β-sheet, has been considered. In the future, it will also be possible to study the molecular dynamics of many other more complex structural motifs. We believe that such an assessment of protein structure is becoming very relevant at present. We hope that the presence of the molecular dynamic simulation data in a water solution for the protein structure will be also announced from the Protein Data Bank in addition to the X-ray crystal and nuclear magnetic resonance water solution data.

This work was supported by Institute of Protein Research RAS [FFRN-2024-0007] and Keldysh Institute of Applied Mathematics RAS [FFMN-2025-0024]. Numerical calculations were performed on the hybrid supercomputer K-60 at the Keldysh Institute of Applied Mathematics, Russian Academy of Sciences.

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Received 21.01.2025.
Revised 11.02.2025.
Published 14.02.2025.