Mathematical Biology and Bioinformatics
2025. V. 20. № 2. P. 369–378. doi: 10.17537/2025.20.369

===================== MATHEMATICAL MODELING =====================

Molecular dynamics of the α-helical hairpin and its surrounding inside the protein S16 from *Thermus thermophilus*

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Abstract. Dynamics of the α-helical hairpin inside the ribosomal protein S16 from *Thermus thermophilus* has been considered. The analysis of polypeptide chain dynamics was carried out on five independent trajectories with a total time of 370 ns. It was shown that relaxed fluctuations of Cα-atoms of the polypeptide backbone at the final part of the trajectory of 60–80 ns become constant: in the β-sheet – 0.4 Å, in helix1 – 0.8 Å, in helix2 – 0.6 Å, and in loops – 1.1 Å. The predicted dynamic profile of the Cα-atom shifts clearly indicates a decreasing in mobility of the α-hairpin in the regions where hydrophobic clusters are located. The restriction factor of such movements is directly related to the interaction of their residues with the other protein residues.

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

Abbreviations: *MDS: molecular dynamic simulation; NMR: nuclear magnetic resonance, TAMD: Trajectory Analyzer of Molecular Dynamics*

1 INTRODUCTION

A native state of globular protein is realized in a water solution where its structural behavior and functions are determined by dynamic properties. Earlier we have studied the molecular dynamics of two basic structural motifs of the protein molecule: α-helix and β-sheet [1, 2]. Here we consider the dynamics of the more complex motif, α-helical hairpin, which consists of the two helical fragments connected by the short peptide linker and is widespread in the proteins [3]. The first systematic study of about one hundred α-hairpin motifs has been carried out [4]. All hairpins were divided into five groups according to different linkers, ranging in length from 2 to 4 residues. One of the most populated hairpin motif groups includes 22 proteins. Linker of this group consists of four residues and has Ramachandran map conformation $\gamma\alpha_L\beta\beta$. The helical fragments are almost antiparallel: an angle between the helices is about 140 degrees. Both hairpin helices have averaged lengths of 14 residues. Later a database of approximately 1500 proteins with α-hairpin motifs was analyzed and the classification was expanded to ten protein groups [5], confirming the result described above.

A water solution protein model and crystal structures of two protein containing α-hairpin motifs are presented in Figure 1. There is a significant difference between the structure of protein in aqueous solution and in crystalline state. In water some of amino acid residues are in contact with the aqueous environment, while the others are tightly packed within the molecule. In the crystalline state almost all protein residues and even part of structural water are fixed

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because of crystal packing. Thus, a widespread crystal protein structures cannot give us full information on the protein dynamics. For example, in a water solution of the protein with α-hairpin there is another factor limiting the mobility of residues: the interaction of the hairpin with contacting part of the rest protein body.

Consider now the examples of proteins with α-helices and hairpin. The first one is the Rop protein, which is involved in the control of replication of plasmids of the ColE1 family [6]. This small protein consists of 56 residues and its structure is just a single α-hairpin motif (Figure 1). Suppose one protein unit is placed in a water solution. It contains two α-helices with 27 and 24 residues, respectively. The helices of hairpin can form the inner hydrophobic contacts. The second protein is the alkyl hydro peroxidase D like protein PA0269 from *Pseudomonas aeruginosa*, which is involved in the antioxidant defense of bacterial cells [7, 8]. Four catalytic residues on the external side of helix α4 of the hairpin motif form the active site. The main difference for two hairpins is a different environment: a water solution in the first case and a protein body in the second one. The difference in surrounding affects the dynamics of the proteins and hairpins, respectively. The aim of this study is to investigate ~~a~~ molecular dynamics of α-hairpin of the particular protein molecule in a water solution in order to elucidate its unique dynamic characteristic.

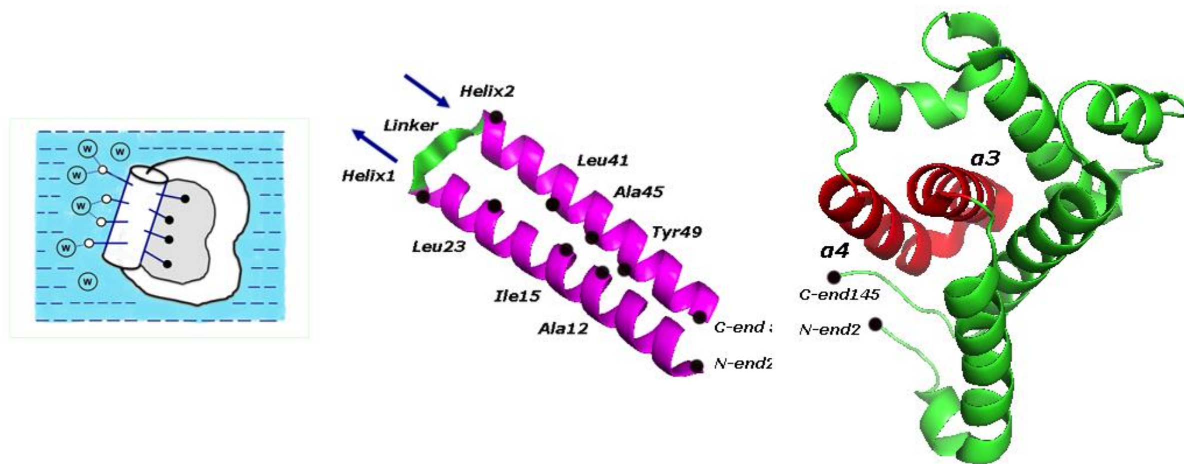

Fig. 1. Water solution protein model and two crystal structures of the proteins with an α-helical hairpin motif. *Left* – protein model in a water solution. *Middle* – Rop protein of the ColE1 family, PDB ID 1rop. *Right* – alkyl hydro peroxidase D like protein from *Pseudomonas aeruginosa,* PDB ID 2o4d.

2 MATERIALS AND METHODS

2.1 The selected protein with α-hairpin motif

To obtain more detailed data on the α-hairpin motif dynamics we chose small ribosomal protein S16 from *Thermus thermophilus* [9]. Its NMR structure in aqueous solution and amino acid sequence are shown in Figure 2. The protein consists of 88 amino acid residues. It has α-hairpin motif with helices α1 (residues 52–61) and α2 (residues 67–75) connected by linker (residues 62–66). The linker includes five residues with conformation $\gamma\alpha_L\beta\beta\beta$ which is similar to the linker conformation $\gamma\alpha_L\beta\beta$ of the α-hairpin group [4]. Nonpolar residues on one side of the hairpin bind with the hydrophobic core of the molecule. Three residues of another side of the hairpin forms hydrophobic cluster Ala56-Leu74-Phe 80, where the last residue belongs to the small loop 4 at C-terminal end of the molecule. Loops 1–3 are rather long, and supposed to be movable. The molecule has four stranded β-sheet which is situated on the opposite side of hydrophobic core.

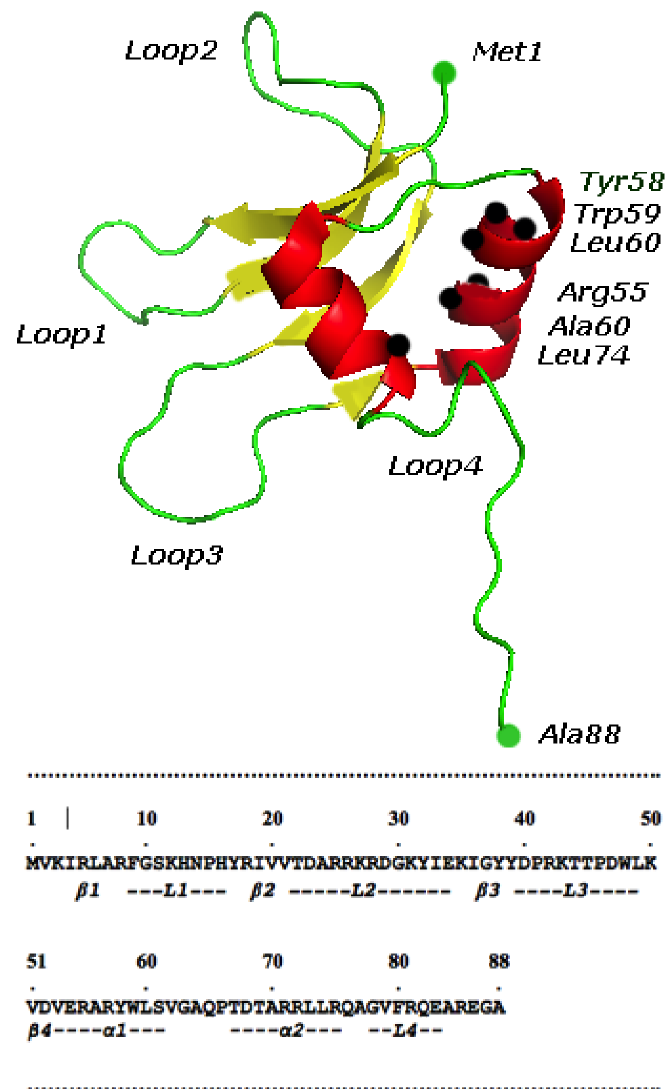

Fig. 2. The structure and sequence of the ribosomal protein S16 *Thermus thermophilus*.

2.2 Molecular dynamics simulation

All dynamic calculations were carried out using MDS PUMA software [10, 11] and the PUMA-CUDA accelerated by NVIDIA CUDA graphics processor. The AMBER force field [12] and the TIP3P water model [13] were used. The first trajectory of molecular dynamics simulation was carried out during 0–50 ns. The relaxation time was about 1ns, and then the energy of the system became constant. We have monitored the electrostatic, Van-der-Waals, and kinetic energy every 0.1 ps. Last atomic coordinates of the first stage have been used as the initial coordinates for the second and other stages. After the first stage, molecular dynamics simulation of four independent trajectories during 80 ns have been carried out. Thus, the total time of dynamic observation was 370 ns. All trajectories originate from one point. However, due to the use different streams of random numbers in the thermostat, these trajectories quickly diverge in a phase space. The resulting trajectories of molecular dynamics were investigated by the Trajectory Analyzer of Molecular Dynamics TAMD [14–16].

All protein atoms were included in the dynamic simulations, but only dynamic profile of Cα-atoms of the protein polypeptide chain was analyzed. We used the Cα-atom shifts between two sequential time points with a step of one nanosecond. For measurement accuracy, we aligned the protein model at different times in the same internal coordinate system which was built on the moments of inertia tensor of the molecule. The ellipsoid of inertia was calculated,

and its origin was set at the center of mass of the Cα atoms. This allows us to obtain the correct values of the Cα-shift dynamics profile [1].

3 RESULTS AND DISCUSSION

3.1 Molecular dynamics of α-hairpin using Cα-atom shifts

Now consider molecular dynamics of α-helical hairpin structure of the ribosomal protein S16 from *Thermus thermophilus* in a water solution. Examples of the typical Cα-shift diagrams for the first trajectory T1, time period 1–50 ns, are shown in Figure 3. Differences of dynamics profiles for the various structural blocks are clearly seen. It is important to note that dynamic profiles of Cα-shifts remain practically the same along the whole time of observation.

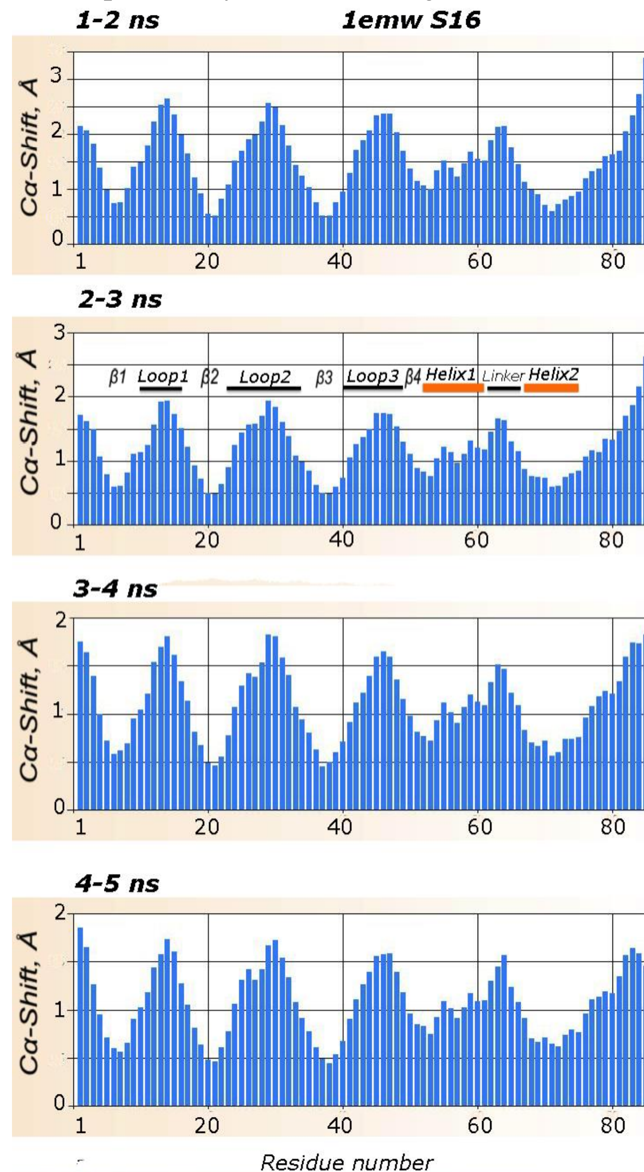

Fig. 3. Dynamic profiles of Cα-shifts of the protein S16 at four time intervals.

Consider now the molecular dynamics of S16 protein along the longer trajectory T2 with time period 1–80 ns. We analyzed the dynamics of the selected residues which are the check points of a certain structural block of the molecule. The check point residues were chosen as follows:

Residue14 .... the middle of the loop1
Residue19 .... the middle of the extended β2-strand
Residue60 .... the middle part of the helix1

Residue74 .... the C-terminal end of the helix 2

Corresponding Cα-shifts along the trajectory T2 are presented in Figure 4.

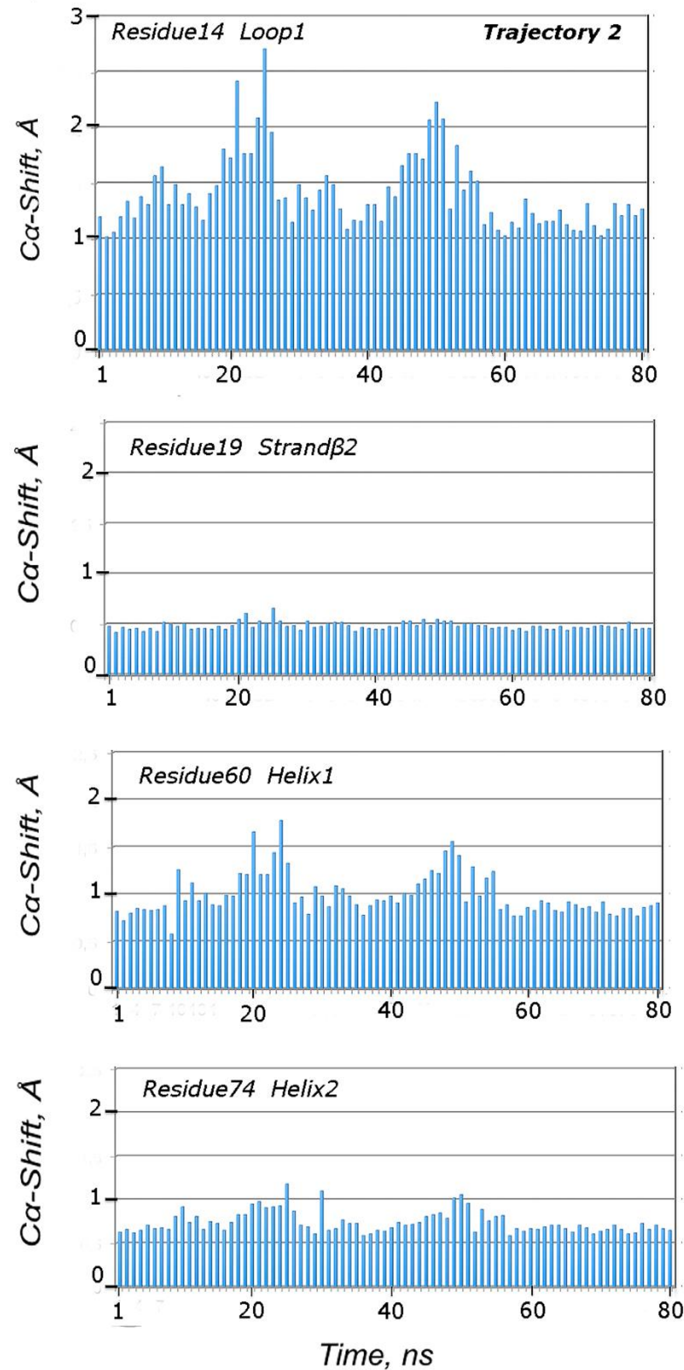

Fig. 4. Molecular dynamics of S16 protein for the residues selected as the check points of the definite structural blocks. Time duration 1–80 ns.

Here are the main dynamic characteristics of the residues selected as checkpoints of a certain structural blocks:

- Dynamic profiles of Cα-shift diagrams in the time interval 1–60 ns are very similar. Profile picture remains unchanged also in the time interval 60–80 ns. However, for all structural blocks with except of β-sheet region, the absolute value of shifts increases by approximately 30 % in time intervals of 19–26 ns and 46–55 ns.

- Dynamics of protein S16 shows a continuous fluctuation until the end of the calculated time 80 ns. The fluctuation shifts of Cα-atoms of the polypeptide backbone in the final period of trajectory 60–80 ns are:
- β2-strand – 0.45Å, helix1 – 0.8Å, helix2 – 0.6Å, loops 1-3 – 1.1Å.
- We can note the high stability of the β-sheet region and a slight displacement of its Cα atoms. The value of Cα-shift is about twice higher in the α-helices and 2.5 higher in the loop blocks.

3.2 Restriction factor of Cα-atoms of polypeptide backbone

A simple atomic protein model can be considered as a system of N point masses of Cα-atoms. It has 3N-6 internal resonance vibrational type of movements, which can be excited by a Brownian chaotic motion of the water molecules. However, there is an essential restriction for the displacements of Cα-atoms. The restriction factor of Cα-atom is determined by the contacts with all the surrounding atoms inside the protein molecule. Because of this we should use a more real protein model where the atoms are represented as Van der Waals spheres. Thus, the movement of Cα-atoms is limited by the close packing of the residues side chains. Every structural block of the protein has its own average value of the Cα-atom displacement. This value can be taken from the dynamics of the check point residues at the final part of trajectory obtained in Section 3.1. The estimated absolute shifts for loops 1–3 are equal 1.1 Å, and for helices 1 and 2 the shifts are 0.8 and 0.6 Å, respectively. In a simple approximation the restriction factor of Cα-atom is proportional to the number of these atomic contacts and the energy of interactions between atomic groups.

Table 1. Non-polar contacts and restriction status of the Cα-atoms in the α-hairpin block of S16 protein structure

Residue Number	Non-polar atomic contacts	Non-polar residue contacts	Side chains residue contacts	Restriction status of Cα-atoms
helix1				
52Asn	1	1	55Arg	weak
53Val	4	1	79Val	middle
55Arg	11	3	34Glu 36Leu 58Tyr	high
56Ala	5	2	36Ile 74Leu	weak
58Tyr	21	3	55Arg 59Trp 62Val	very high
59Trp	46	5	21Val 33Ile 36Ile	very high
			58Tyr 62Val	
60Leu	7	3	66Pro 74Leu 79Val	middle
linker				
62Val	4	2	59Trp 64Ala	weak
64Ala	3	2	59Trp 62Val	weak
65Gln	5	2	3Lys 66Pro	weak
66Pro	7	3	60Leu 71Arg 74Leu	weak
helix2				
67Thr	3	3	5Arg 69Thr 70Ala	weak
69Thr	3	2	6Leu 67Thr	weak
70Ala	2	2	19Ile 67Thr	weak
71Arg	3	2	66Pro 80Phe	weak
72Arg	2	1	75Arg	weak
73Leu	12	3	19Ile 39Tyr 48Trp	middle
74Leu	12	3	66Pro 79Val 80Phe	middle
75Arg	5	2	72Arg 80Phe	weak
C-terminal end				
76Gln	4	1	49Leu	weak
77Ala	5	3	49Val 51Val 79Val	weak

Notes: 1. Helix1 residues 52-61, helix2 67-75, linker 62-66.
2. Calculated contacts are less than 4.2Å.

The stability of globular protein in a water solution is mainly determined by hydrophobic interaction of the side chains of the amino acid residues. This results in the formation of the hydrophobic core inside the protein. The core of ribosomal protein S16 is formed by the residues of one α-hairpin and one four-stranded β-sheet (Figure 1). Thus, it can be assumed that restriction status of Cα-atoms for the internal and external sides of the α-helix and β-sheet are significantly different. The difference depends on the number of nonpolar atomic contacts of the surrounding residues. Therefore, we can estimate the Cα dynamic shifts for the hairpin or any other part of polypeptide chain.

The number of non-polar contacts of the hairpin residues in the protein S16 is presented in Table 1. There are 160 nonpolar atomic helical contacts in total, and 67 of them form large cluster between the hairpin and hydrophobic core of protein. All these and other contacts are responsible for a disposition of the hairpin in the protein structure. There is one external hairpin cluster Ala56-Leu74-Phe 80 that also stabilizes the structure of hairpin (Figure 2). In fact, Table 1 reflects the spatial arrangement of hairpin in the protein structure. It allows to evaluate the restriction status of every residue by the number of the nonpolar atomic contacts with other protein residues. Thus, we can predict a dynamic profile of the hairpin.

Dynamic displacement of Cα atoms of the protein in a water solution is strongly related with local packing of the amino acid side chains. And the packing is determined by the number of the surrounding atomic groups and the type of interaction. The non-hydrogen protein model consists of three types of atoms: nonpolar, polar uncharged, and polar charged. The hydrophobic interactions of the nonpolar atoms are ones of the most important for the stability of protein structure. Another significant type of interaction is a hydrogen bonds, which are involved in the formation of compact structural blocks. And the third one is an electrostatic interaction.

3.3 Prediction of dynamic profile of polypeptide chain in α-hairpin of S16 protein

The structure in time interval of globular protein in a water solution is a spatial pathway of polypeptide main chain with the residue side chains. Non-polar residues (Ala, Leu, Phe, Trp) are concentrated in the hydrophobic core. The residues with polar charged atomic groups (Asp, Glu, Lys, Arg) are situated on the external surface of the protein molecule. Energy of hydrophobic interactions can be determined by measuring the heat capacity of proteins in water solution [17]. Unfortunately, the accurate values of the hydrophobic potential energies of any residues cannot be determined using this method, since many residues include both non-polar and polar groups of atoms. Therefore, we should consider interaction energy between every pair of atoms. The energy of the hydrogen bonds is 3 kcal/mol. Energy of electrostatic interaction of polar charged atomic groups is 2 kcal/mol. The energy of the hydrophobic interaction of non-polar atomic groups is about ten times smaller; it is approximately 0.2 kcal/mol. However, number of non-polar contacts is large and, as result, total hydrophobic interactions of the residues determine the stability of the protein structure in a water solution. The dynamic shift S of Cα-atom for the given structural block can be expressed by the simplified empirical formula.

$$S = \frac{S_{\text{block}}}{R}$$

where S_{block} – relaxed fluctuation value of structural block,
R – restriction factor of Cα-atom.

A restriction factor is a power of limitation of the Cα-atom dynamic movement due to a presence of other residues. It is directly related to the energy of interaction of the residue with the neighbor ones. Since the hydrophobic interaction has the greatest contribution, we can calculate the value of R, using the data in Table 1, as:

$$R = N_{\text{res}} \bullet E_{\text{hb}}$$

where N_{res} – number of contacting residues,
E_{hb} – energy of hydrophobic interaction of the contacting residues.

Parameters of the hairpin blocks that determine their dynamics are given in Table 2. There is a significant difference in local residue status of the loop, β-sheet and hairpin block. For example, the loop residues are surrounded by water molecules, while the hairpin residues form peptide hydrogen bonds with other protein residues. The amplitude coefficient of the Cα-shift are varies among different structural motifs. We used the values of Cα-shifts of the relaxed fluctuation in the final interval of trajectory (Section 3.1).

The observed and predicted Cα-shift diagrams for α-hairpin inside the protein S16 are presented in Figure 5. We can see the restriction of Cα-atom shifts in the regions of hydrophobic clusters between the residues of the hairpin and the protein body. There are four such regions:

55Arg, 56Ala; 58Tyr, 59Trp, and 60Leu; 66Pro, 67Thr; and 73Leu, 74Leu.

The important result is that a predicted dynamic profile shows very clearly a decreasing of Cα-shifts of those residues which have the hydrophobic contacts with the other protein residues.

Table 2. Dynamic Cα-shift parameters of polypeptide chain in a water solution

Local status of amino acid residue	Surrounding medium	Structural block	Dynamic shift of Cα-atomic, Å
Free, water solution	Water molecules	loops	$1.1/R_{loop}$
Part of protein	Protein residues	β-sheet	$0.45/R_{sheet}$
Part of protein	Protein residues	helix1	$0.8/R_{helix1}$
Part of protein	Protein residues	helix2	$0.6/R_{helix2}$
Part of protein	Protein residues	linker	$1.1/R_{linker}$

Note. R – restriction factor of the residue which is proportional to the number of the contacts with the surrounding residues.

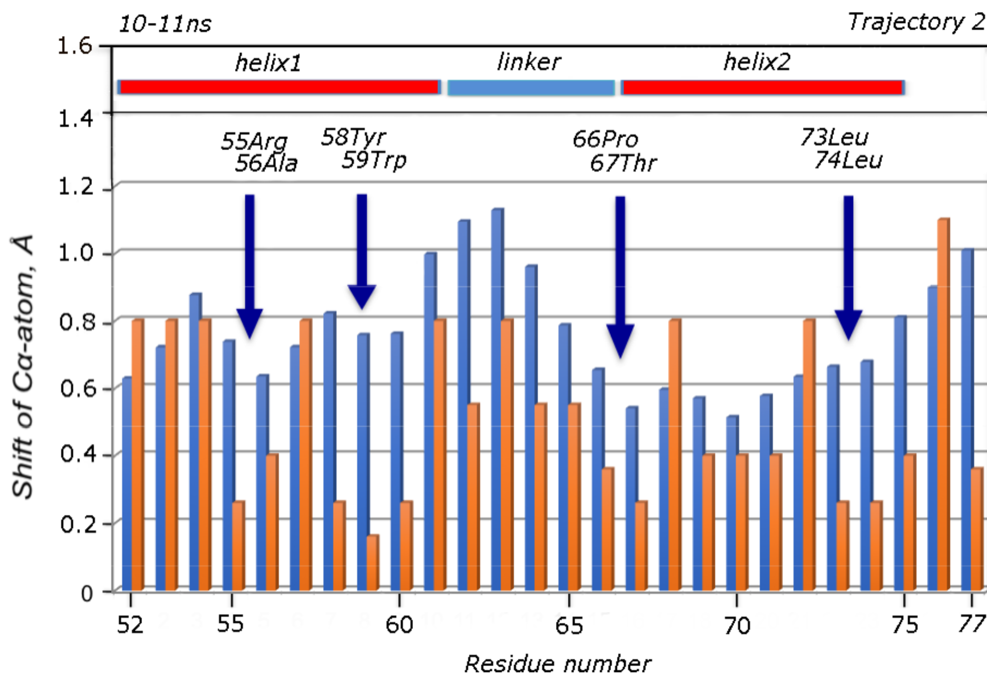

Fig. 5. Comparison of the Cα-displacement in the time interval 10-11ns in trajectory T2 (blue color) with the predicted Cα-shift (pink color) for the α-hairpin in S16 protein.

4. CONCLUSION

Analysis of dynamic profile of ribosomal protein S16 shows that relaxed fluctuation in time interval longer than 60ns remains constant. Here we faced an important feature of the globular protein: the protein molecule displays a constant dynamic status. This feature is directly related to the protein function. The α-hairpin motif shows its dynamic profile is similar to that of a single helical fragment. However, dynamic profile of hairpin is more complicated due to a limitation of the residue mobility in the regions where the hydrophobic clusters are situated. Thus, the main result of current study of the S16 protein is: restriction of the mobility of Cα-atoms is determined by the hydrophobic interaction between the residues of the hairpin block and the rest protein body.

Authors would like to thank Prof. Alexei V. Finkelstein, Prof. Alexander V. Efimov, and Mrs. Evgenia A. Khar'kina, Institute of Protein Research, Russian Academy of Sciences, for the valuable and very useful discussions. The calculations were performed on the hybrid supercomputer K60 installed in the Supercomputer Centre of Collective Usage of KIAM RAS. Authors are appreciated very much to the excellent work of the computer' staff accompanying our experiment.

This work was supported by Institute of Protein Research RAS [FFRN-2024-0007] and Keldysh Institute of Applied Mathematics RAS [FFMN-2025-0024].

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Received 22.07.2025.
Revised 27.08.2025.
Published 09.09.2025.