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Corresponding Author: Mr. Miroslav Krajčí, Mgr.

Corresponding Author's Institution: Comenius University faculty of Mathematics, Physics and Informatics

First Author: Miroslav Krajčí, Mgr.

Order of Authors: Miroslav Krajčí, Mgr.; Ján Urban, Prof.; Pavel Mach, Dr.

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Abstract: The influence of the mechanical strain on the artificial protein L24 (acetyl-K2-L24-K2-amide) has been studied at the molecular mechanics (MM) level of theory. The effect of the surrounding environment (DPPC molecules) has been observed during the stretching or compressing of the L24. The calculations gave the view on the structural changes occurring during these processes. All calculations were done using the GROMACS code with the ffgmx forcefield enhanced with lipid-protein interaction potentials.

Response to Reviewers: Revision notes:

Reviewer 1:

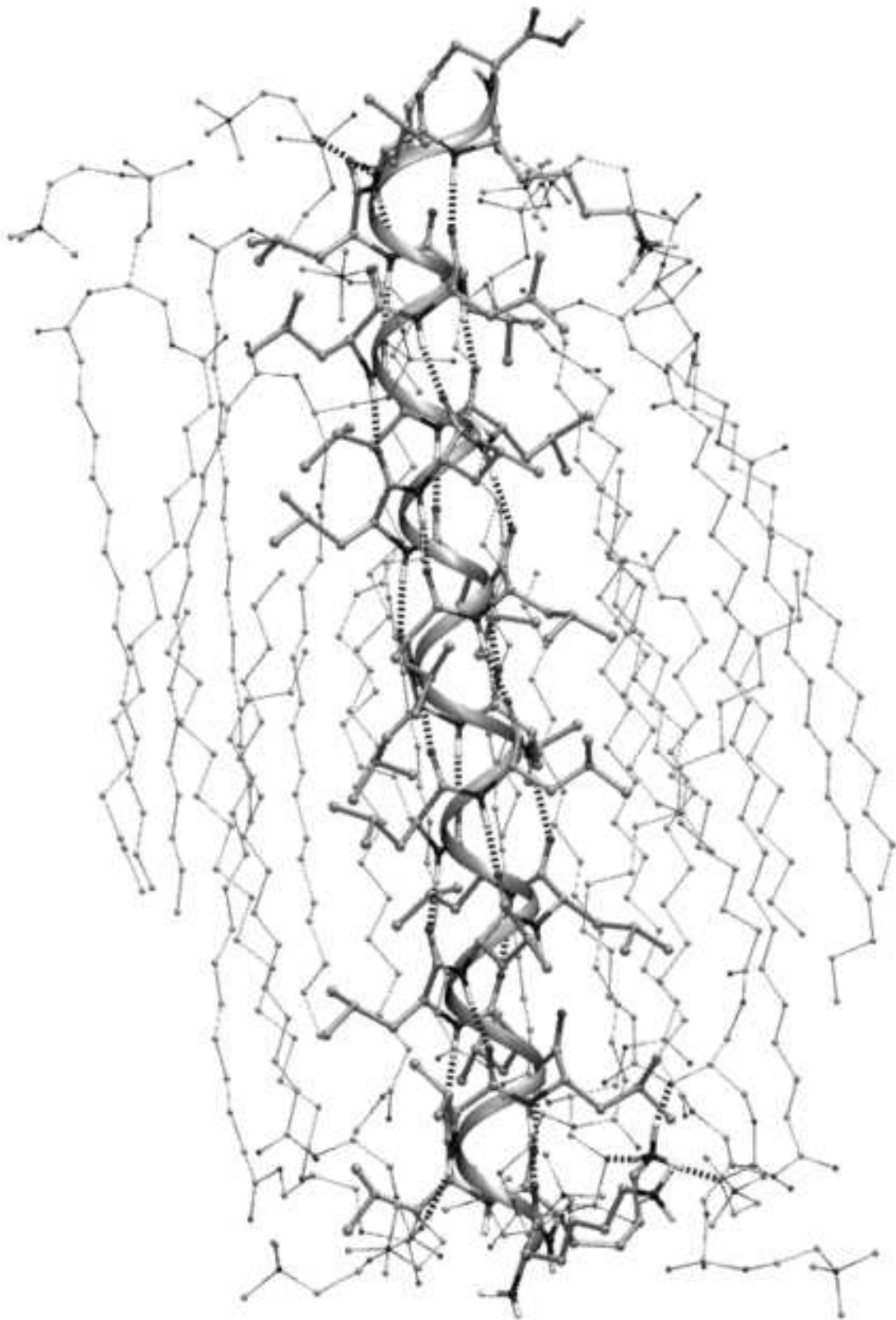
1. Grammar mistakes have been corrected
2. Changes have been made:
 - 2.1. section introduction - paragraph 3 has been corrected based on reviewer notes - coarse grained approach has been mentioned as a possible application
 - 2.2. section introduction - paragraph 3 has been corrected based on reviewer notes - added timescale microseconds - seconds
 - 2.3. question answered by point 1.
 - 2.4. section methodology - paragraph 2 has been corrected based on reviewer notes - information about number of atoms, boundary conditions, timestep, electrostatic scheme has been more explained
 - 2.5. answered by point 4.
 - 2.6. section discussion - paragraph 3 has been changed based on reviewer notes - used term „locally unfold" instead of „broken"
 - 2.7. section discussion - paragraph 5 has been corrected based on reviewer notes - mostly rewritten sentences to better clarify what actually happened
 - 2.8. Added „probably" in the end of section discussion - paragraph 2 to clarify statement. Note to reviewer: Figure 3 was added merely to demonstrate limit of method not for obtaining some conclusions from it, it indicates that at 10nm the simulation starts to behave differently and we should be very careful when we are discussing these results, since they may be beyond interpolation limit of forcefield used. We are not saying that peptide is breaking at that point, we are merely saying that

simulation is probably no longer meaningful. Stretching L24 peptide more than 10nm by this methodology may produce irrelevant results. Covalent bond breaking may happen later than that, but this can not be seen in our simulation due to inherent limitations of molecular mechanics methodology. Quantum mechanics simulations would be required for test this precisely.

Reviewer 2:

1. detailed introduction of system required: whole introduction paragraph 2 is about L24 peptide alone, including references to work of other people [1-4], stating its significance and reason for studying it.
2. studies about L24 that is already in literature: answered by point 1.
3. better understanding if more parameters were analysed: Since our work was not MD simulation, it was mere a consecutive set of minimizations at which we artificially added displacement, which simulated our force pulling. RMSD wouldn't give much sense. We thought images and force graphs give enough relevant information. We were actually analysing many of minimization trajectories individually (especially those where there was jump on the graph), observing many effects and conformation changes, however describing them all wouldn't give much more useful solid information. We have to choose which information is relevant since we had about 13GB of data from over 10000 trajectories. Many effects were irregular, caused by random surrounding DPPC environment or effects of ending Lysine anchors or just forcefield artifacts.
4. more discussion (how does the work fit to others work): section results and discussion - added paragraph about applications of the results of the work.
5. Other changes
 - 5.1. replaced as suggested
 - 5.2. replaced as suggested
 - 5.3. fixed as suggested
 - 5.4. replaced as suggested
 - 5.5. replaced as suggested
 - 5.6. replaced as suggested
 - 5.7. replaced as suggested
 - 5.8. replaced as suggested
 - 5.9. replaced as suggested
 - 5.10. replaced as suggested
 - 5.11. new figure 10c, 10d added to give better quantitative explanation of the process; in the section "discussion" - paragraph 2 the references to these figures have been added.

Abstract graphic
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Theoretical study of structural changes caused by applying mechanical strain on peptide L24

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Miroslav Krajčí[✉], Jan Urban, and Pavel Mach

Department of Nuclear Physics and Biophysics, FMFI UK, Mlynská dolina F1, 842 48
Bratislava, Slovakia

[✉]Email: miro.krajci@gmail.com

Abstract

The influence of the mechanical strain on the artificial protein L24 (acetyl-K2-L24-K2-amide) has been studied at the molecular mechanics (MM) level of theory. The effect of the surrounding environment (DPPC molecules) has been observed during the stretching or compressing of the L24. The calculations gave the view on the structural changes occurring during these processes. All calculations were done using the GROMACS code with the ffgmx forcefield enhanced with lipid-protein interaction potentials.

Keyword Molecular mechanics · Synthetic transmembrane peptides · Nanoelasticity · GROMACS · Ffgmx forcefield

Introduction

Proteins are the basic compounds from which the life itself is formed. Understanding of elementary processes that take place at the nano-scale has the crucial role in the understanding of their capabilities, such as folding, synthesis or their function itself. Proteins are by no means random statistical molecules. Their function usually involves mechanical movement of several parts while preserving configuration of others. Considering these parts are individual secondary structure sequences, their mechanical properties play fundamental role in the protein functionality.

For the purpose of the study of their elasticity properties we have chosen the L24 artificial peptide. This peptide belongs to the group of membrane peptides where the lipid-protein interactions are of special importance due to the wide variety of the functions they perform in the cells, such as *e.g.* receptor activity, energy transduction or active transport. In order to overcome the problem of the complicated structure of the integral proteins and their isolation and purification, chemically synthesized peptide models of specific regions of natural membrane proteins have been used in biophysical studies of the mechanisms of protein-lipid interactions [1-4]. Among others, the α -helical peptide acetyl-K2-L24-K2-amide (L24) (Fig. 1), has been successfully utilized as a model of the hydrophobic transmembrane α -helical segments of integral proteins [5]. This peptide contains a long sequence of hydrophobic leucine residues capped at both the N- and C-termini with two positively charged, relatively polar lysine residues. The central poly-leucine region of this peptide was designed to form an optimal stable α -helix which will incorporate strongly into the hydrophobic environment of the lipid core, while the dilysine caps were designed to anchor the ends of these peptides to the polar surface of the bilayer membrane (BLM) and to inhibit the lateral aggregation of these peptides. Detailed biophysical studies of the interaction of P24 or L24 [5, 6] or WALP peptides [7] with BLM revealed the fact that incorporation of these peptides into the phosphatidylcholine bilayers resulted in the decrease of the ordering of the bilayer in a gel state and increase of the ordering in a liquid crystalline state.

In our work we studied how the secondary structure of L24 would be resistant to applied force, what processes occur during the stretching (compressing) and how surrounding environment affects all of this. This kind of the study may provide basic data for designing or validating parameters of the coarse grain approach to protein simulations, which would take

secondary structure sequences as its fundamental elements. This may eventually lead to whole protein simulations on the sufficient timescale (microseconds – seconds). These simulations are required for the insight to the protein function mechanisms and are presently unavailable due to the lack of computer power needed for using current methodology.

Huge progress in the experimental nano techniques involving AFM microscopy make it also possible to verify theoretical results, since it is now possible to observe and manipulate individual molecules [8-14]. This type of experiments is ideal for supporting the theoretical models and for the testing of their predictions. Experimental works have been done to test several different theoretical approaches when studying elasticity. Good agreement was found with the theory of polymer entropic elasticity [15], when small force has been applied to single DNA molecule. Applying bigger force (~10 pN) however showed some differences between the theory and experiments, caused by inelastic deformation of the molecule skeleton [16]. These differences were even bigger when the force (~300 pN) was applied to molecule using AFM tip [8]. It was shown that in this case the difference occurred even between models that involved elasticity of skeleton [16, 17]. Based on these observations, new models involving equilibrium thermodynamics [18-20] and molecular dynamics simulation have been formed, trying to explain this phenomenon [21]. In these models, kinetic aspect was included, which explained additional extension of the skeleton with the conformation change. It has been shown that this model is able to explain visco-elastic properties of different types of polymers. There have been experiments for example with the molecule of titin (muscle protein) [9] and dextran (polysaccharide) [8] where multi level conformational characteristics of molecules studied by Monte Carlo simulations have been compared with experimental values. The kinetic aspect of simulations and reversibility of the whole process has been also observed.

These types of studies and fact that chemistry of primary structure of peptides is quite well known, suggest that molecular mechanics simulation based on intermolecular potentials could be an usefull tool to investigate peptide properties at reasonable computing speed with minimum restrictions.

Methodology

Due to the facts mentioned in the introduction we performed our simulations at the molecular mechanics level of theory. We used GROMACS code [22] with forcefield ffgmx as a simulation engine. This forcefield was originally designed for the calculations that include proteins only, therefore to compute contributions of environment (DPPC) to overall properties we had to provide additional interaction-potentials that would enable us to do such calculations. For this purpose we altered forcefield based on study made by group of authors [23], who were studying proteins integrated in phospholipid membranes and tested this approach.

Our method of implying force on molecule consisted of 2 steps. At first we did homogeneous stretch (compress) of rescaled coordinates of all L24 atoms in Z direction (the direction of lateral axis of peptide). Then we equilibrated system at zero temperature in vacuum with the ends of the molecule fixed to its coordinates. The length of one stretch or compression step was 0.002 nm. Since we performed only a set of minimizations to investigate structural and energetic changes, there was no time parameter involved. We repeated the same procedure with free DPPC molecules organized to bilayer as environment and also with DPPC fixed in Z direction. Fig. 1, Fig. 2 present the images of our starting equilibrated geometries. Two highlighted atoms at the ends of peptide are those carbons which are being fixed during the simulations. In the simulation in vacuum the system consists only of L24 peptide (see Fig. 1) (547 atoms). In the simulation with DPPC environment system consists of L24 peptide and 12 DPPC molecules formed in two rings (see Fig. 2) (totally 2107 atoms). System was simulated in cluster boundary conditions – no periodic box was present. Electrostatic charges were obtained from aminoacid database which is a part of the used forcefield. Charges in amino acids correspond to natural cell environment (pH ~ 5.5) and were constant in all minimizations.

To test MM methodology and the conditions, we were observing the energy distribution among forcefield terms in our simulations. Doing this we found the limits of our approach. Original L24 was 3.86 nm long and during the simulation it was stretched up to 10.5 nm, however as Fig. 3 shows, the results beyond 10.0 nm are not realistic and normally bond break would occur since too many energy is distributed to bond terms. After some testing runs, where we optimized the simulation step and convergence criteria we obtained the

following results (Fig. 4 - 9). These graphs were obtained from analytic derivation of precisely computed potential energy.

Results and discussion

Figs. 4 - 9 show that the effect of applied force is not continuous. Each jump on graphs is caused by sudden conformation change caused by applied force. Example of such change observed in simulation of stretching without DPPC is presented on Fig. 10a, Fig. 10b. As we can see the highlighted H-bond, which stabilize the molecule, is broken, new one is formed and system changes the conformation. This change cannot be normally undone. After releasing ends of the helix, it stays in this new conformation. This is the prime reason why the stretching and compression is not elastic according to the Hook's theorem and why there are so many irregular jumps on the graphs.

We could distinguish several stages of conformational changes, in which different types of H-bonds jumps occurred when we were observing L24 stretched alone. The type shown on pictures (Fig. 10a, Fig. 10b) responds to 1-3 to 1-2 jump. Better quantitative insight of this jump is given by graphs Fig. 10c, Fig. 10d. This type is responsible for periodical force jumps (Fig. 4) in the range 5.5 nm-7.5 nm. Untwisting and H-bond jumping occurs from N-end of the peptide. This end is probably less stable due to inherent asymmetry of peptide chain of the molecule. According to Fig. 3, MM method is not suitable for stretching larger than 10.0 nm or as in our case for applying force larger than ~2500 pN, since the bonds are stretched to the extend, where the classical description in terms of forcefield loses its validity and normally covalent bond breaking would probably occur.

When applying the compression to L24 alone, molecule first undergoes an elastic bent. After being compressed to the size 2.8 nm (1 nm compress from its original size) it broke in middle into two parts (see Fig. 5). Force required for compressing is much smaller than for stretching, this is due to fact that molecule was actually not compressed like helical spring, but rather bent like a stick and then locally unfold (Fig. 11a, Fig. 11b). H-bonds have stabilizing effect not only when the stretching is applied. They maintain certain helix size and they make the molecule rather rigid than flexible.

Generally DPPC surroundings put high degree of irregularity to the problem and gave L24 higher level of stability. It discarded step-wise look of the stretch process and different types of structure changes occur in apparent random order, depending on starting position of DPPC molecules. Simulation with fixed DPPC caused huge increase of force required for stretching (Fig. 6), this was due to the fact that big force has been required for the pulling of the polar ends out of membrane. When we repeat the simulation with free DPPC the increase is not that obvious (Fig. 8), but this behavior is rather strange, since the surrounding DPPC were attached to the ends of L24 and moved with them as L24 was stretching (Fig. 12a, Fig. 12b). In this case untwisting and H-bond breaking occurred from the middle of peptide, where no DPPC was present.

The effect of the compression of L24 when DPPC was fixed in the Z direction gave also very interesting results. The environment had again stabilizing effect, which can be seen at the Fig. 07. The force which is required for compressing is almost five times bigger than without DPPC. L24 didn't unfold into two rigid parts like in Fig. 5. The character of the compression has been completely changed - it resembles as if only the two atoms we kept fixed were affected by force – pushing them closer, despite of the fact that all L24 atom coordinates were transformed before each minimization. The peptide completely unfolded from its N-end *i.e.* all stabilizing H-bonds have been disturbed in this end. The destabilized part behaved like free polymer thread, while the rest of the peptide remained in its helical form. This again demonstrates the size of the stabilizing effect of the surrounding DPPC on the helical structure of the peptide. In the process of compression L24 peptide pushed away two DPPC molecules as can be seen in Fig. 13a and Fig. 13b. Anomalous behavior can be seen on Fig. 7 for the range 2.0-2.5 nm which shows numeric limits of our force calculating method. The compression with free DPPC caused all DPPC molecules to follow polar ends just like in the free stretching and big lipid globule around the bended L24 has been formed (Fig. 9).

These and the similar results can be further used for the better qualitative understanding of mechanical properties of the secondary structure fragments of the peptides. Quantitatively it can be used for designing or validating the appropriate coarse grained parameters.

Conclusions

The application of the MM method for the study of the model peptides can be an useful tool for the explanation of the force effect on molecules. We have shown that the inelastic change of the conformation of the peptides causes abrupt changes in the structure of the peptides. The influence of the force (stretching, compressing) is reduced by stabilizing effect of H-bond. This effect is quite high, causing that peptide resembles rather rigid stick more than elastic spring. Interaction with neighbor lipid molecules further stabilized rod-like structure of this peptide. The lysine anchor groups effectively interact with polar heads of surrounding lipids, thus when embedded into the membrane the size mismatch can cause the local deformation of the membrane.

Acknowledgments

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Figure captions

- Fig. 1** L24 initial equilibrated geometry. Two emphasized carbons are those being fixed in simulations, what simulate applying force
- Fig. 2** L24 initial equilibrated geometry with surrounding 12 DPPC molecules. Two emphasized carbons are those being fixed in simulations, what simulate applying force. In simulations with DPPC fixed also DPPC atoms are fixed, but only in direction of stretch (compression) - in Z direction. This should simulate integrity of membrane
- Fig. 3** Energy distribution among forcefield terms observed in simulation of stretching L24 in vacuum
- Fig. 4** Force required for stretching calculated from potential energy as observed in simulation of stretching L₂₄ in vacuum
- Fig. 5** Force required for compressing calculated from potential energy as observed in simulation of compressing L₂₄ in vacuum
- Fig. 6** Force required for stretching calculated from potential energy as observed in simulation of stretching L₂₄ with surrounding DPPC fixed in Z direction
- Fig. 7** Force required for compressing calculated from potential energy as observed in simulation of compressing L₂₄ with surrounding DPPC fixed in Z direction
- Fig. 8** Force required for stretching calculated from potential energy as observed in simulation of stretching L₂₄ with surrounding DPPC free in all directions
- Fig. 9** Force required for compressing calculated from potential energy as observed in simulation of compressing L₂₄ with surrounding DPPC free in all directions

- Fig. 10**
- a) Starting geometry of example of 1-3 to 1-2 H-bond jump. H-bond O(0) to H on N(3) will be broken and new one will be formed with O(0) to H on N(2). This change is coupled with change of conformation, what results in sudden release of strain as observed in all force graphs
 - b) Final geometry of example of 1-3 to 1-2 H-bond jump. H-bond O(0) to H on N(3) was broken and new one was formed with O(0) to H on N(2)
 - c) Step 1034 minimization. Example of H-bond H1-A1, H2-A1 lengths change corresponding to Fig. 10a, 10b. A1 resembles oxygen in our case, H1, H2 are two different hydrogens on which the H-bond jump occurs
 - d) Step 1034 minimization. Example of H-bond HDA angle change corresponding to Fig. 10a, 10b. D1, D2, H1, H2 resembles two different nitrogens (hydrogens), A1 resembles oxygen on which the H-bond jump occurs
- Fig. 11**
- a) Intermediate step of compressing of L₂₄ in vacuum in range about 0.8 nm compressed. Bending is already apparent
 - b) Intermediate step of compressing of L₂₄ in vacuum in range about 1.1 nm compressed. Helical structure is broken into two separated parts, but both of them keep its original helical structure
- Fig. 12**
- a) L₂₄ initial equilibrated geometry with surrounding 12 DPPC molecules (free in all directions) prepared for stretching
 - b) L₂₄ geometry with surrounding 12 DPPC molecules (free in all directions) after applied stretching. Untwisting of L₂₄ take place in middle part where no stabilizing effect of DPPC is present. This figure also demonstrate how Lysine anchors stick to polar heads of DPPC molecules, which in effect follow stretched molecule
- Fig. 13**
- a) L₂₄ initial equilibrated geometry with surrounding 12 DPPC molecules (fixed in Z direction) prepared for compressing. Length marked shows starting distance between two DPPC molecules
 - b) L₂₄ geometry with surrounding 12 DPPC molecules (fixed in Z direction) after applied compression. As can be seen two DPPC molecules are pushed away (their distance increases) and only one end of L₂₄ is in motion. Untwisted part resembles rope, while the rest of the molecule keeps its original helical form. Force required

for this behavior was quite high as can be seen on Fig. 7 what suggest high stabilizing effect of DPPC environment

Figure 1
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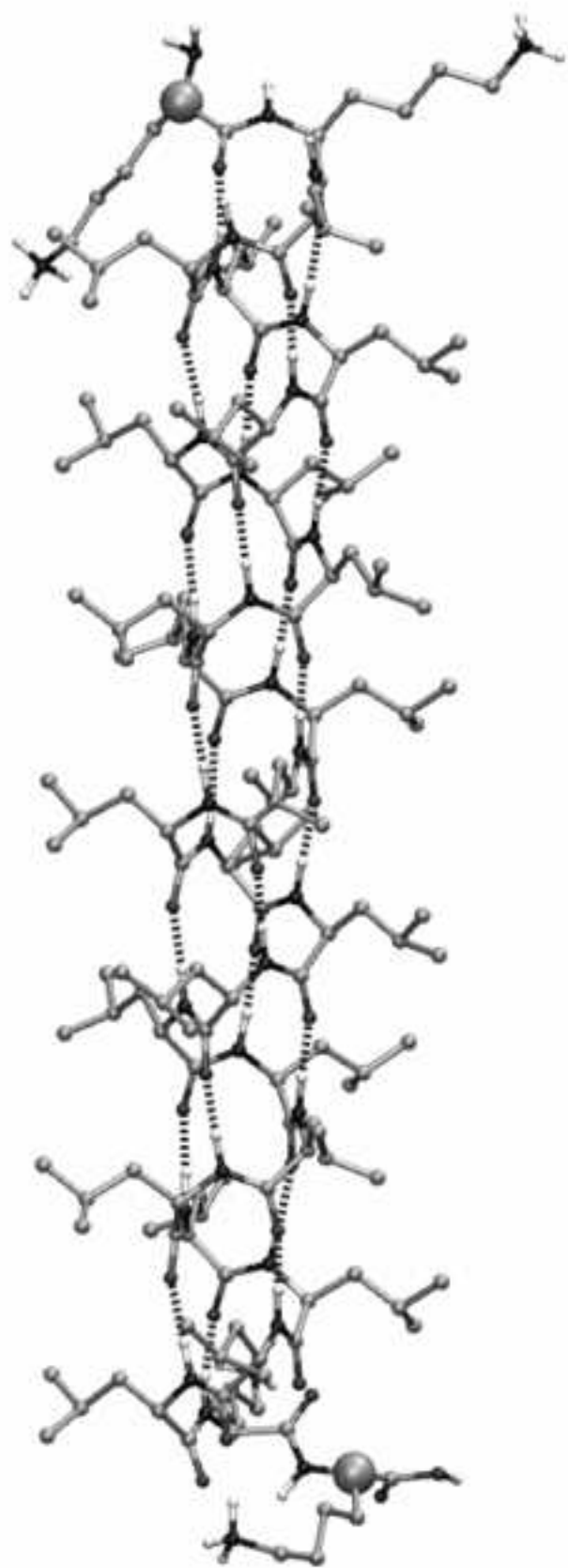


Figure 2
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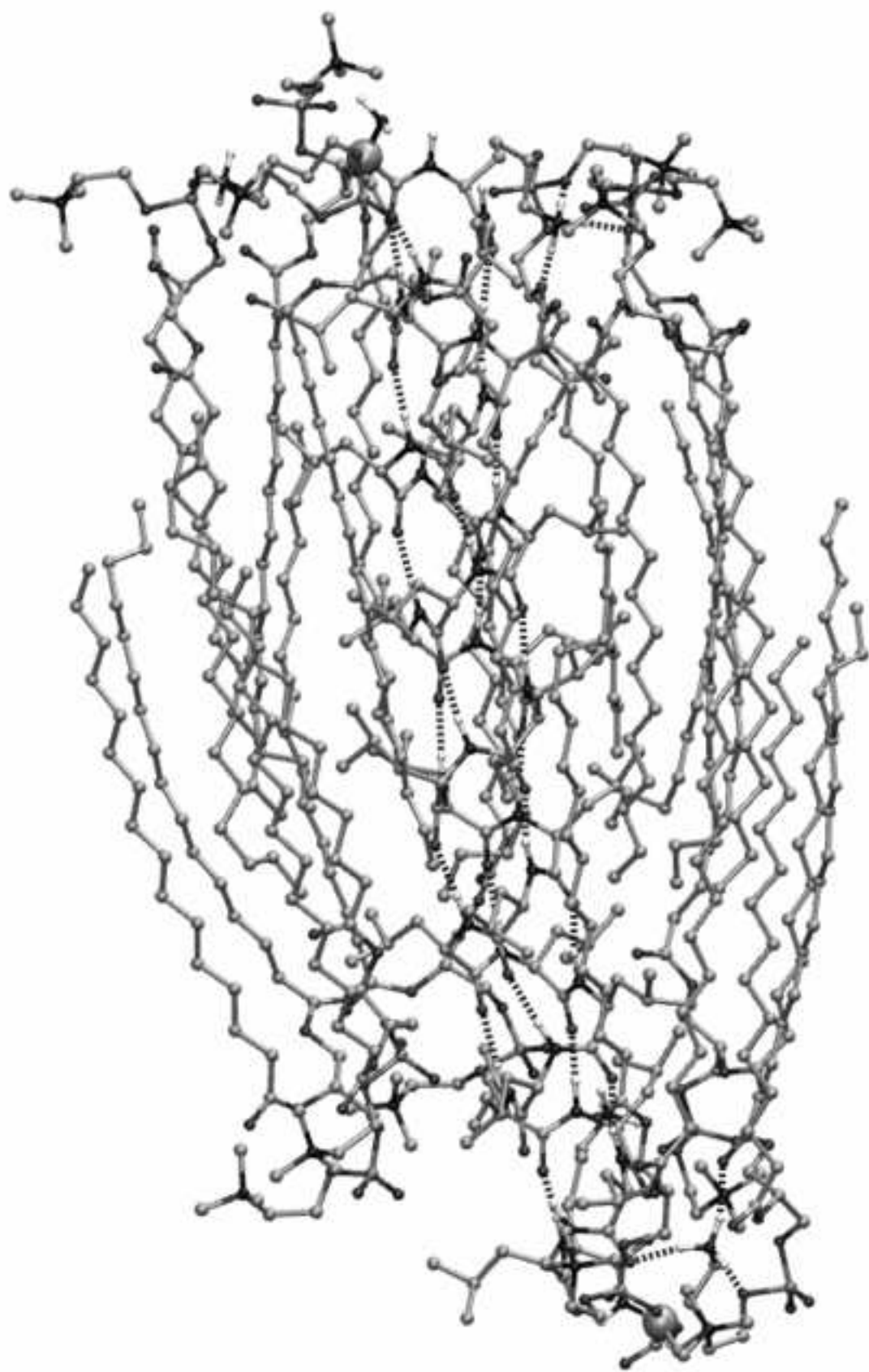


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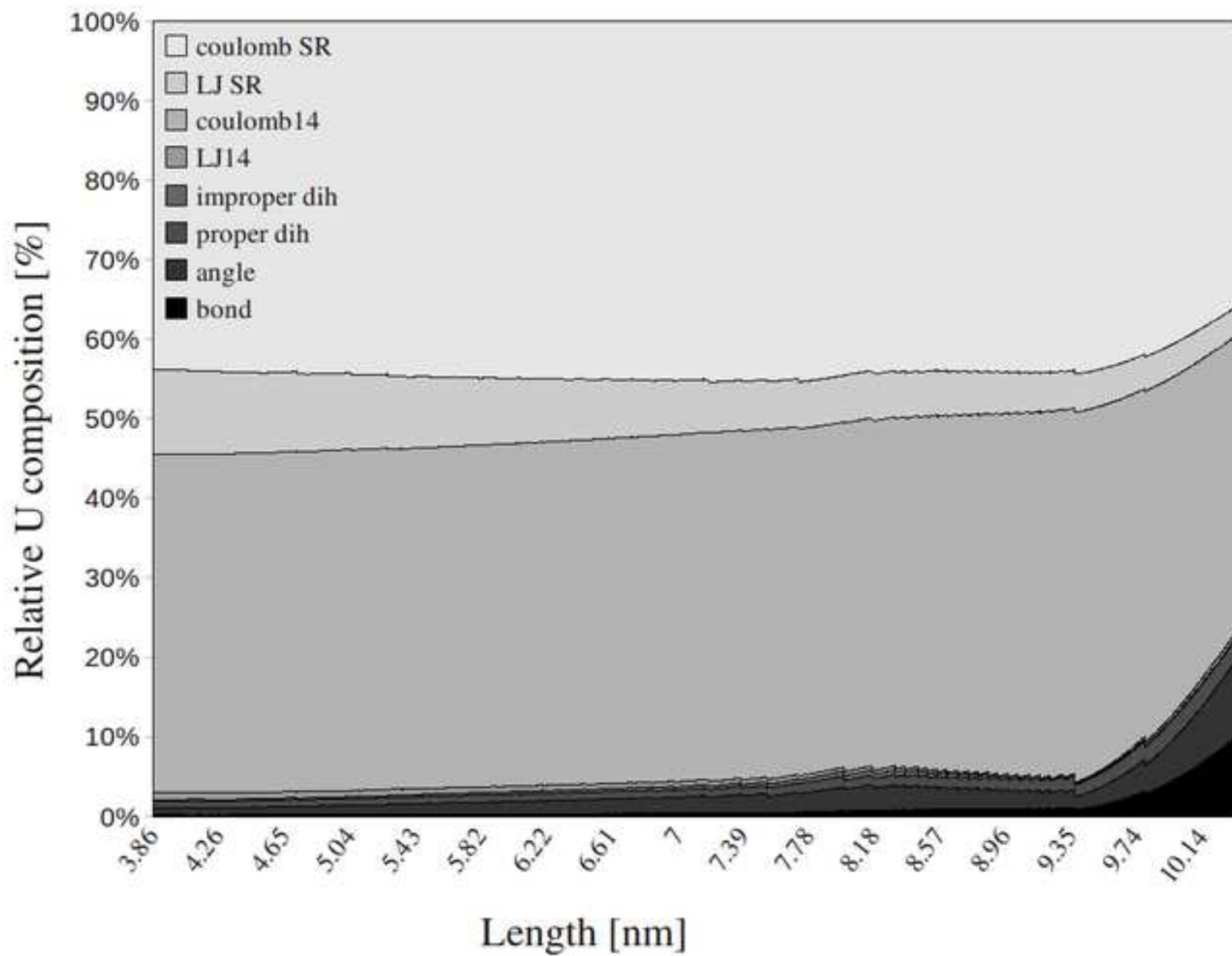


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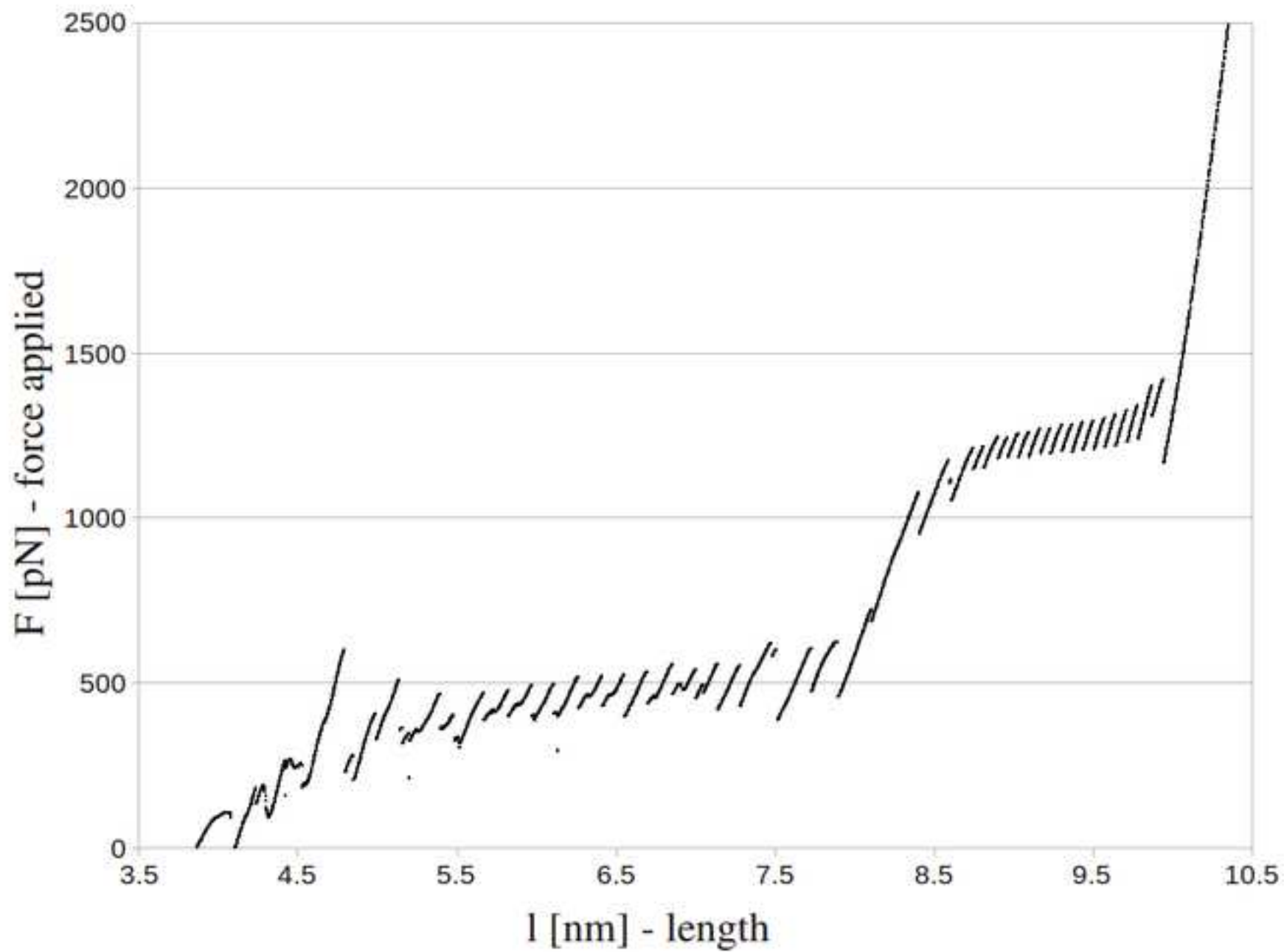


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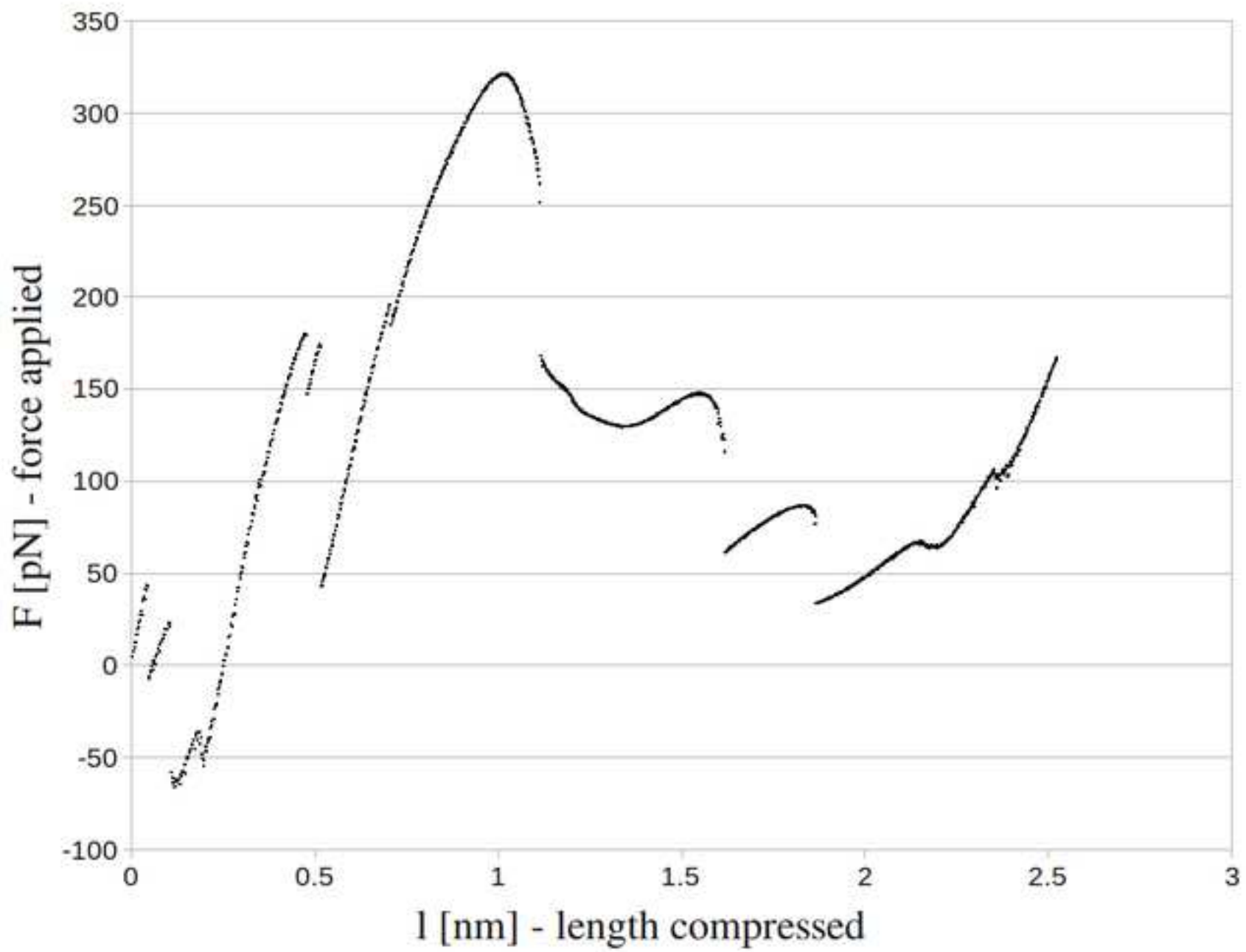


Figure 6
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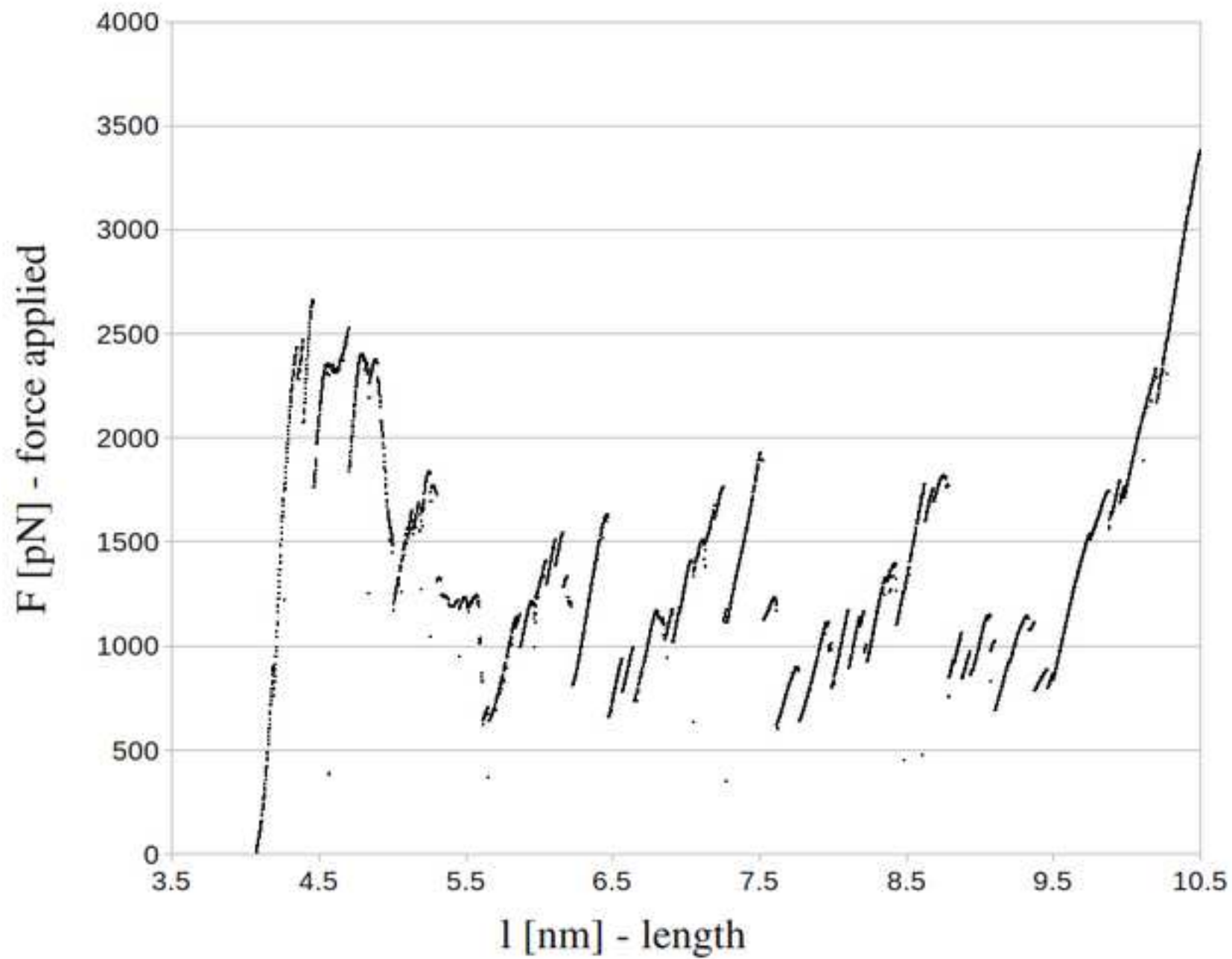


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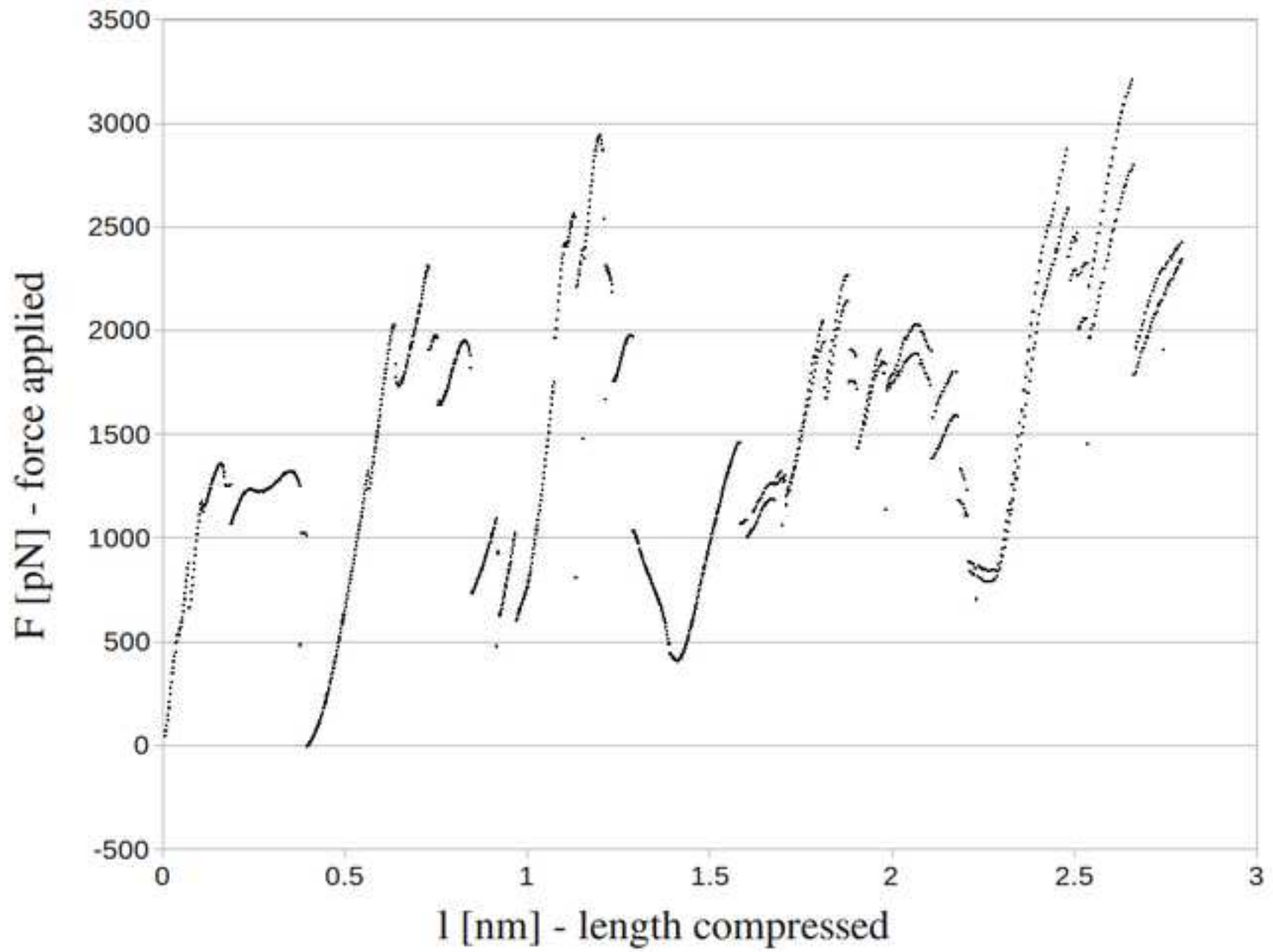


Figure 8
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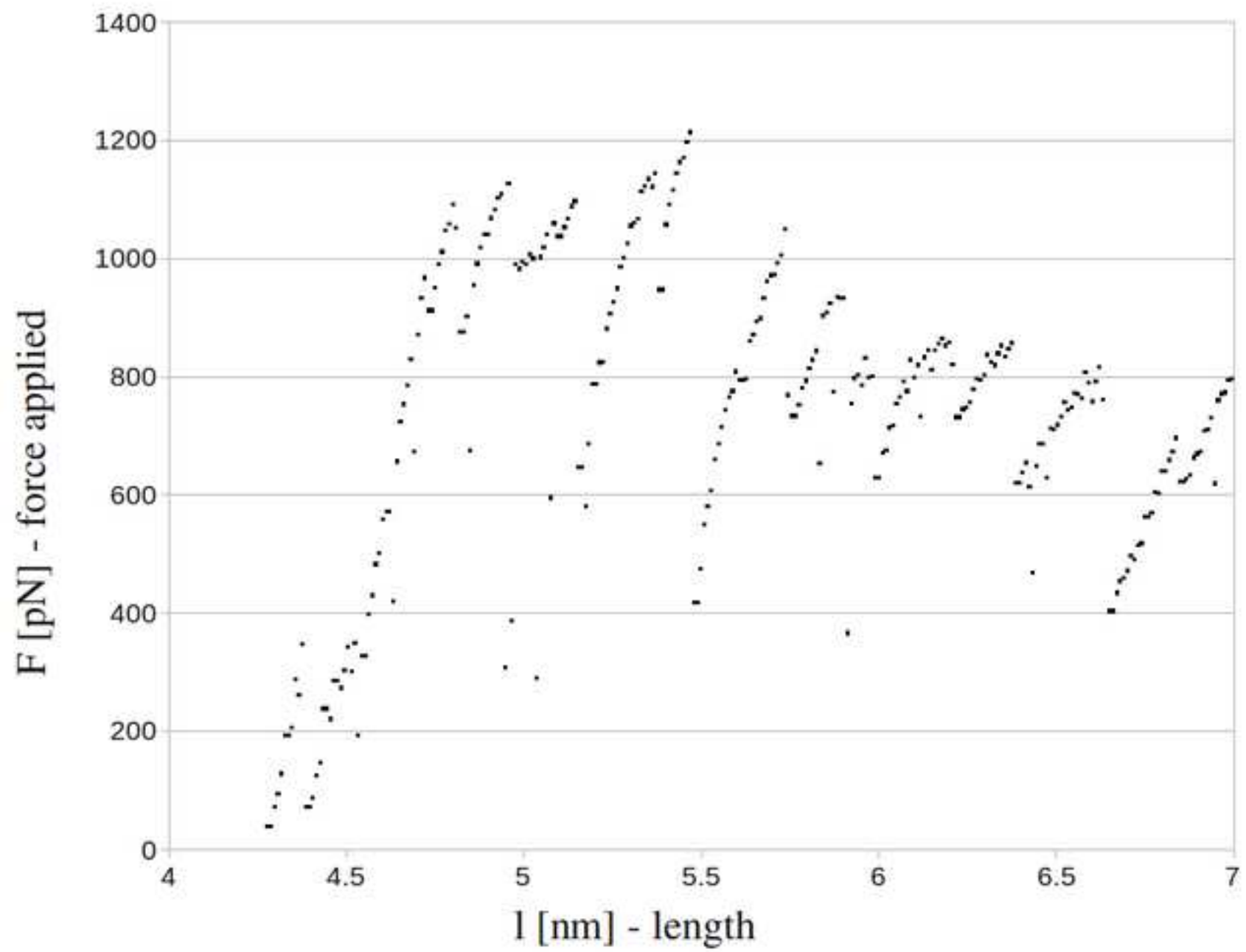


Figure 9
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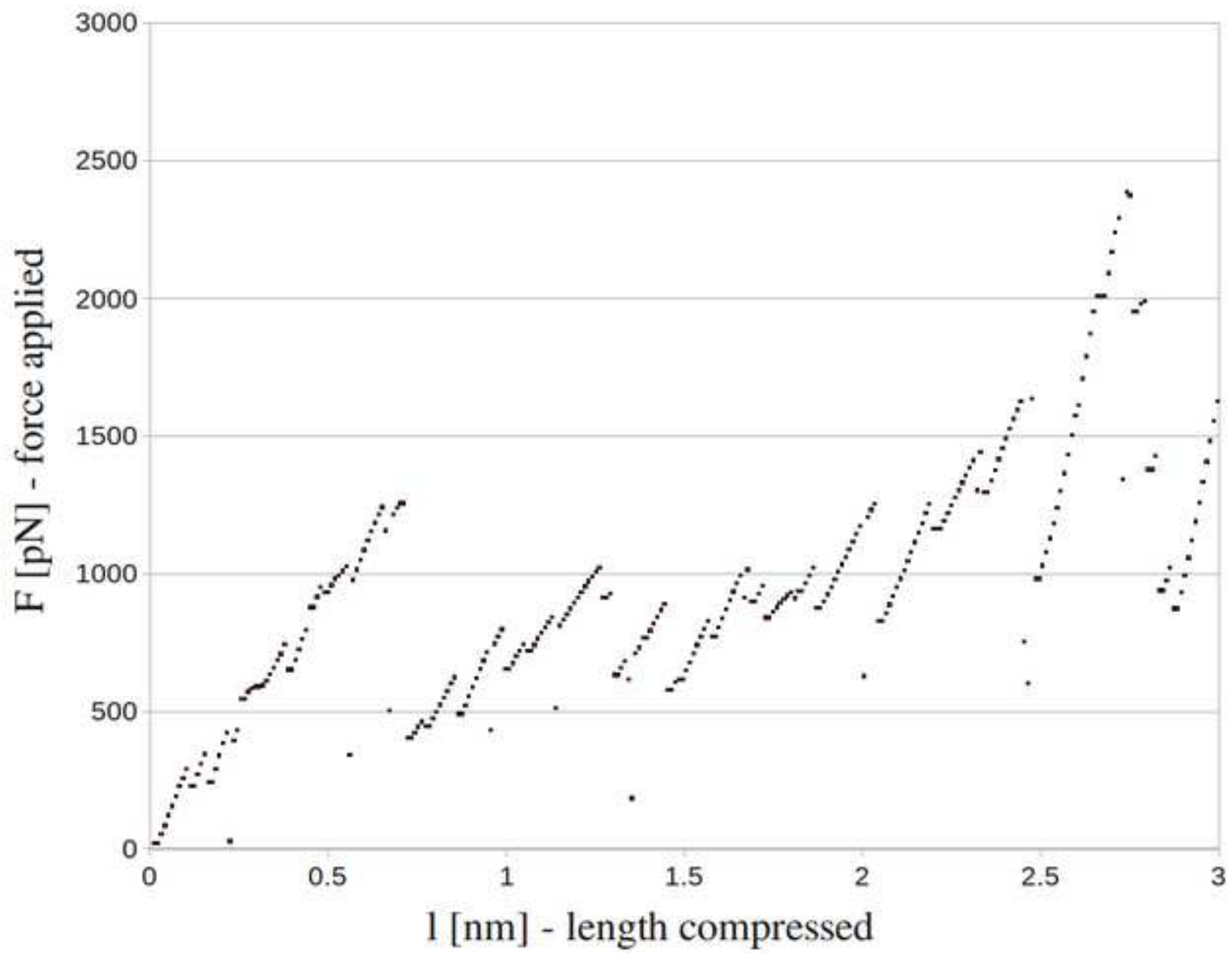


Figure 10a
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a

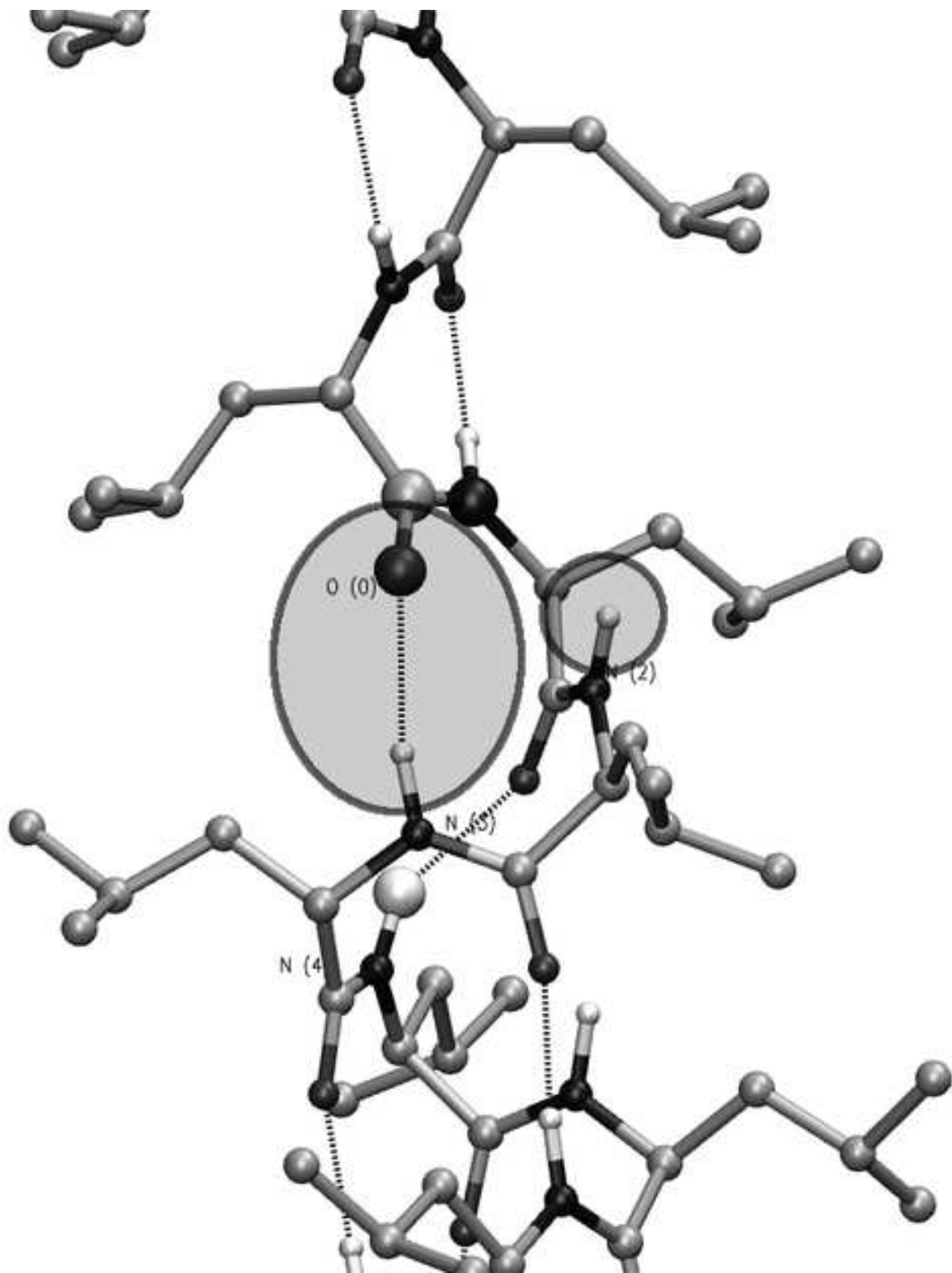


Figure 10b
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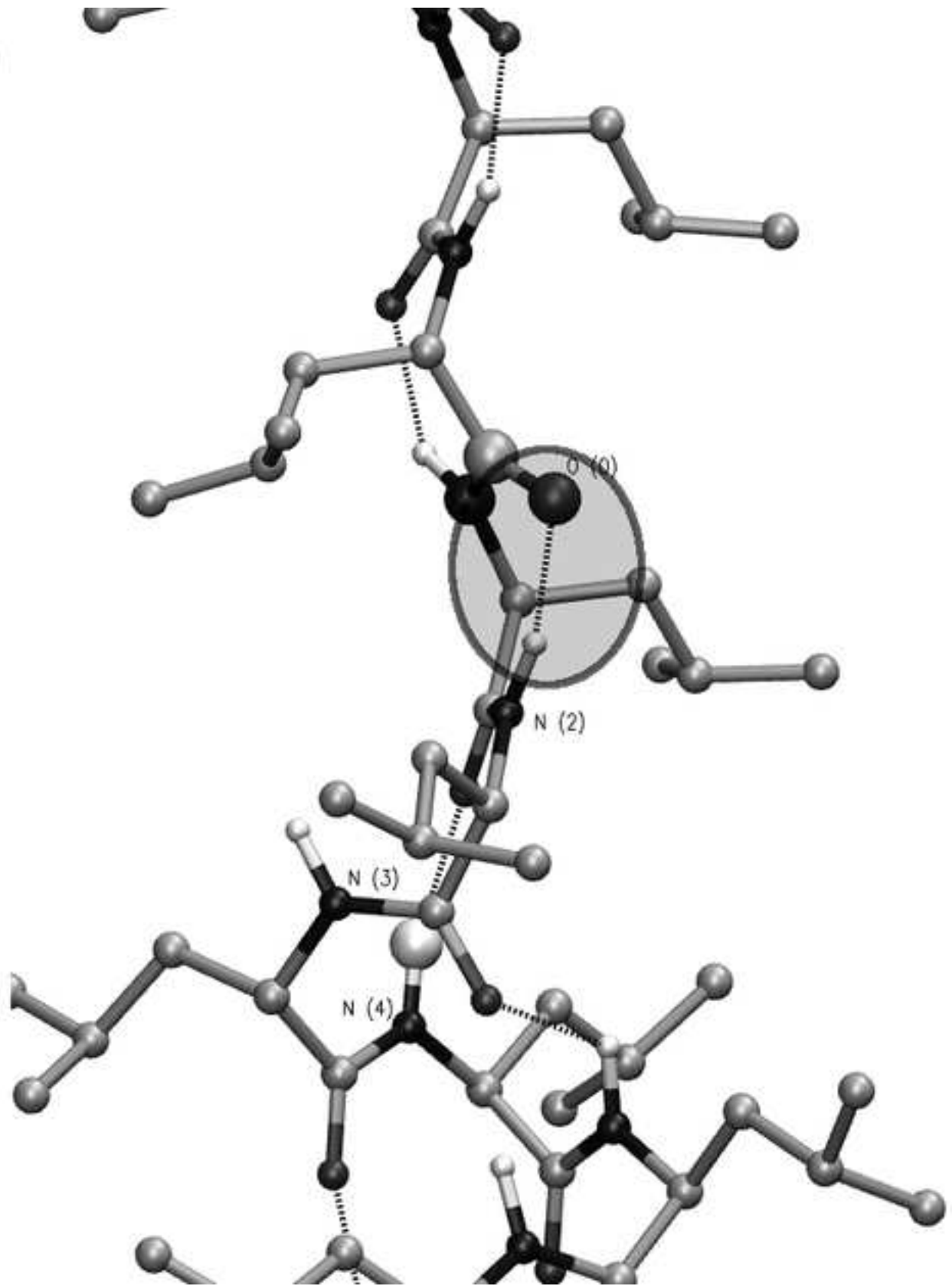


Figure 10c
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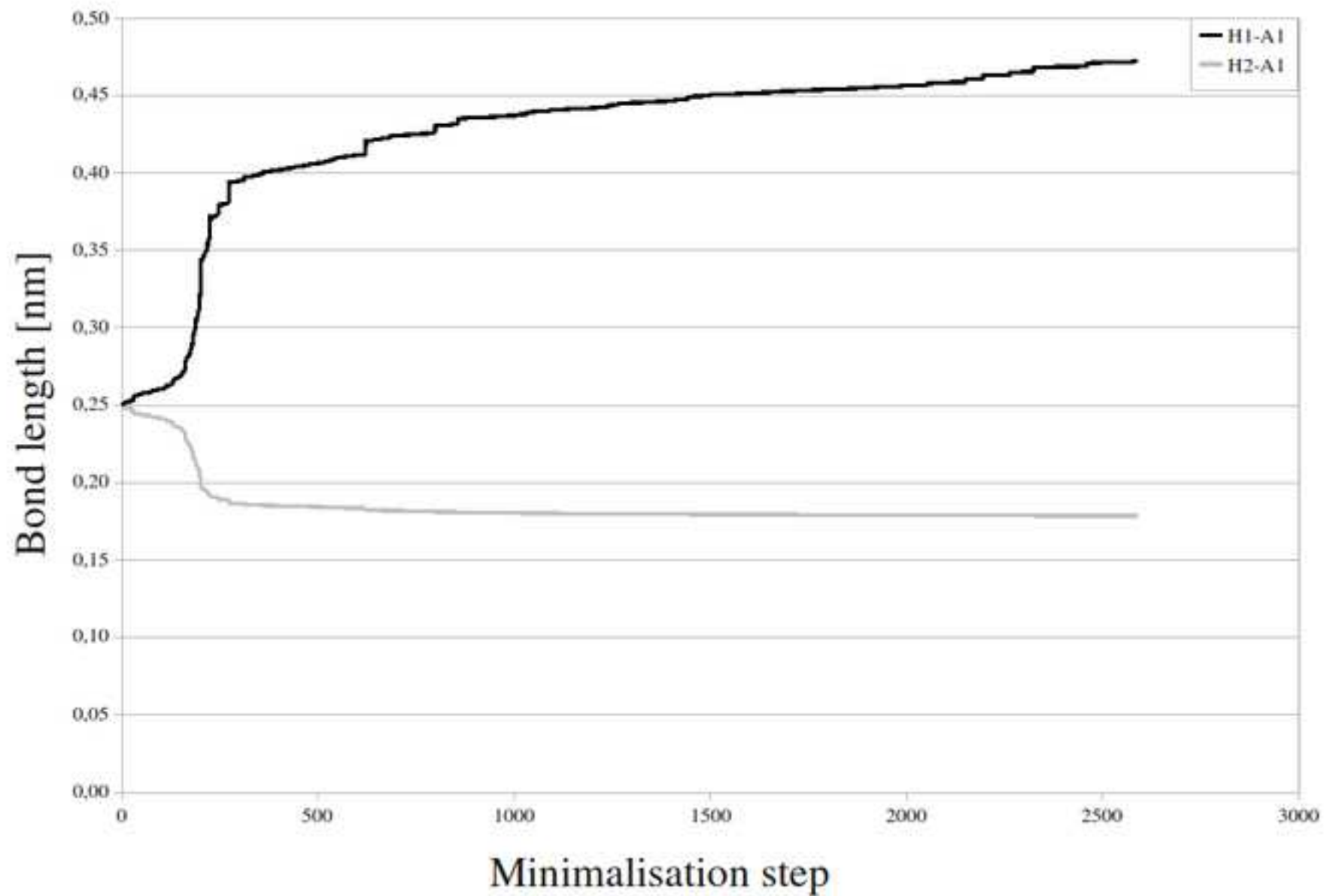
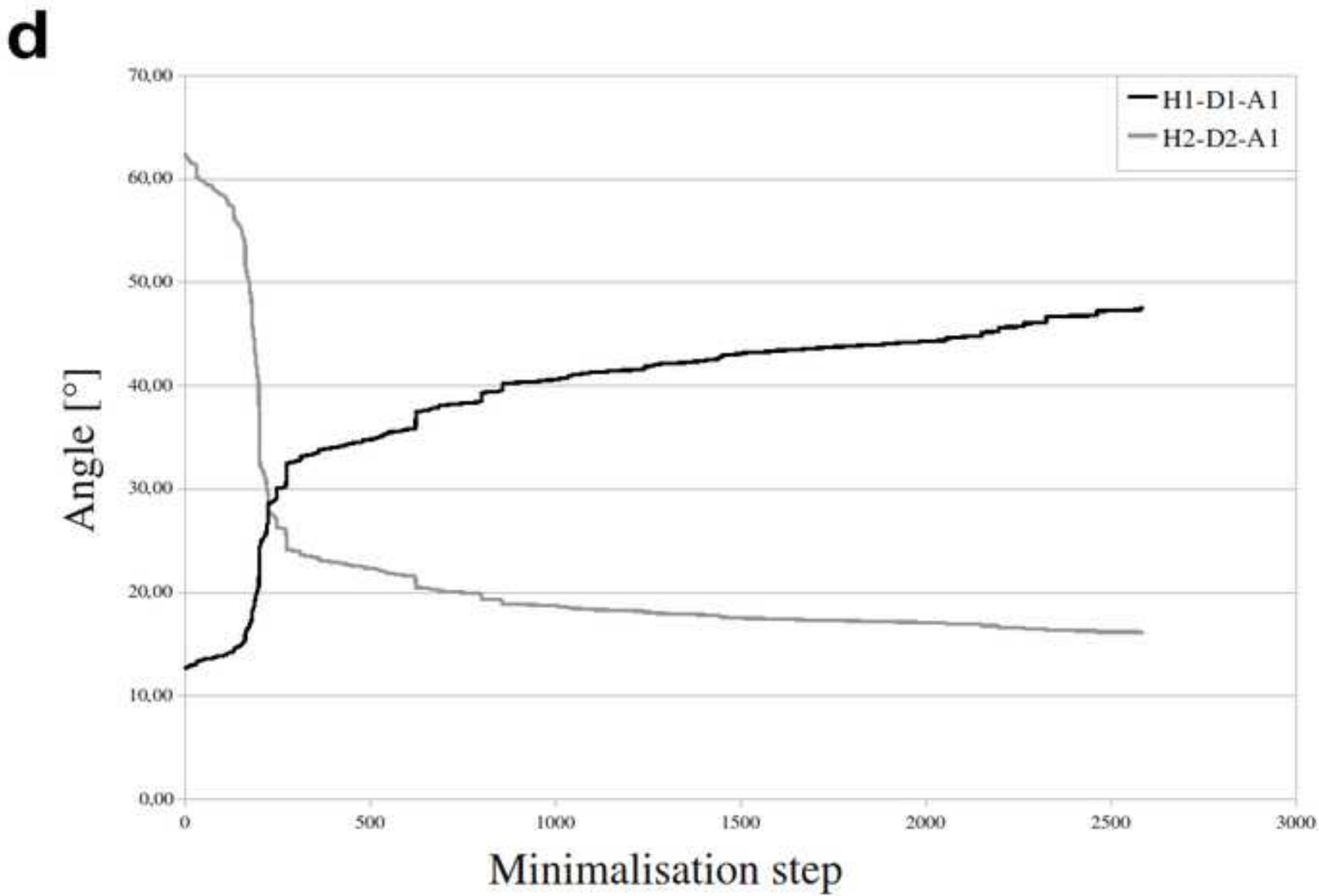
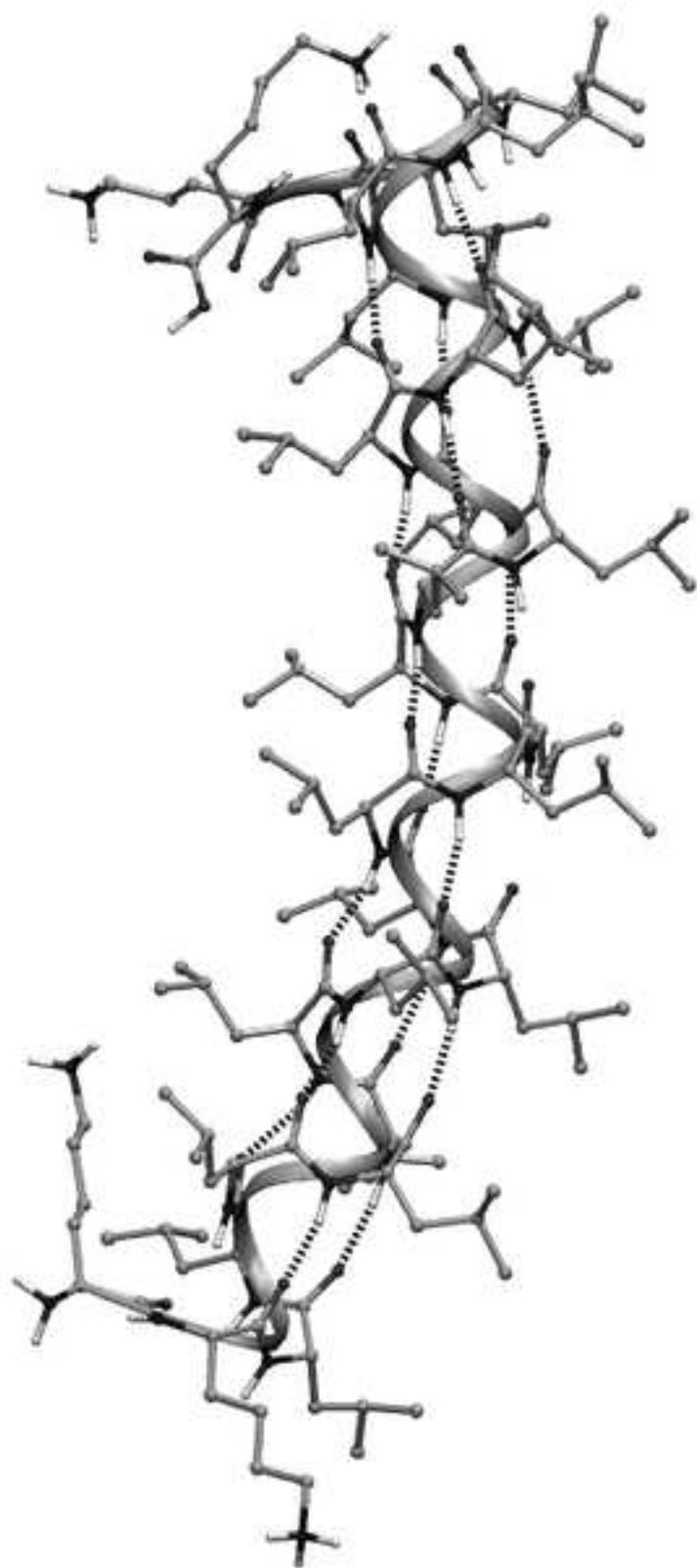


Figure 10d
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a



b

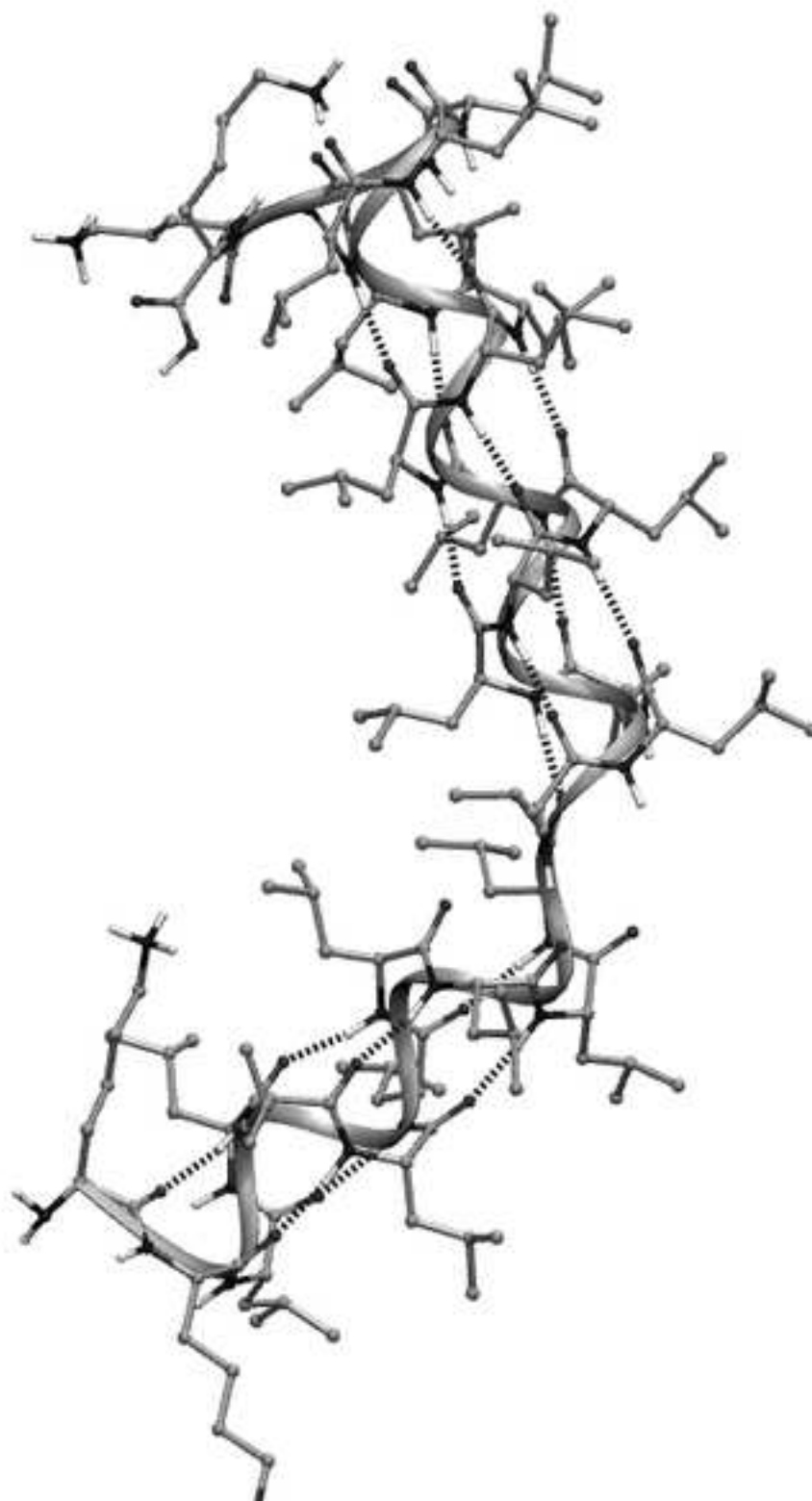


Figure 12a
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a

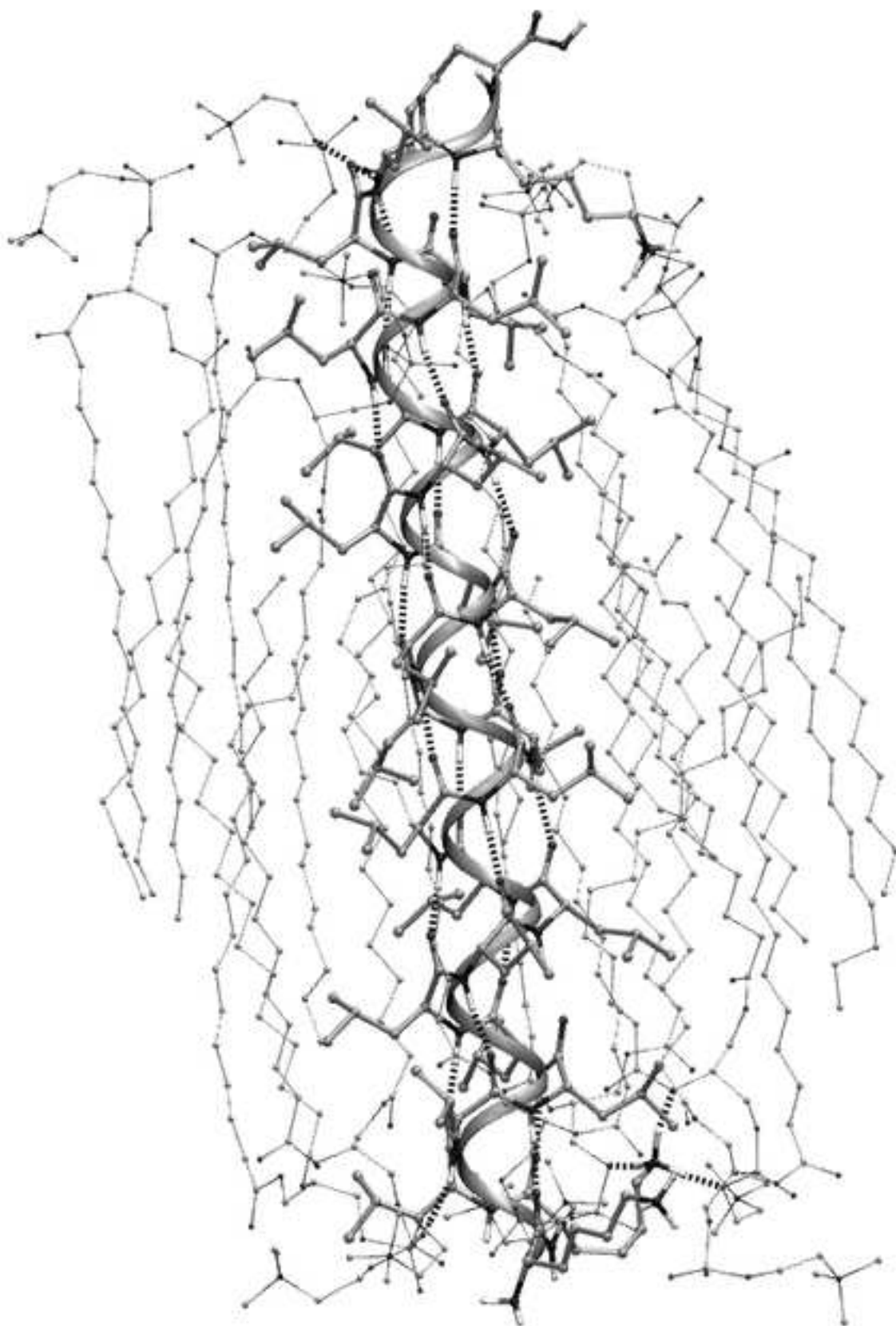


Figure 12b
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b

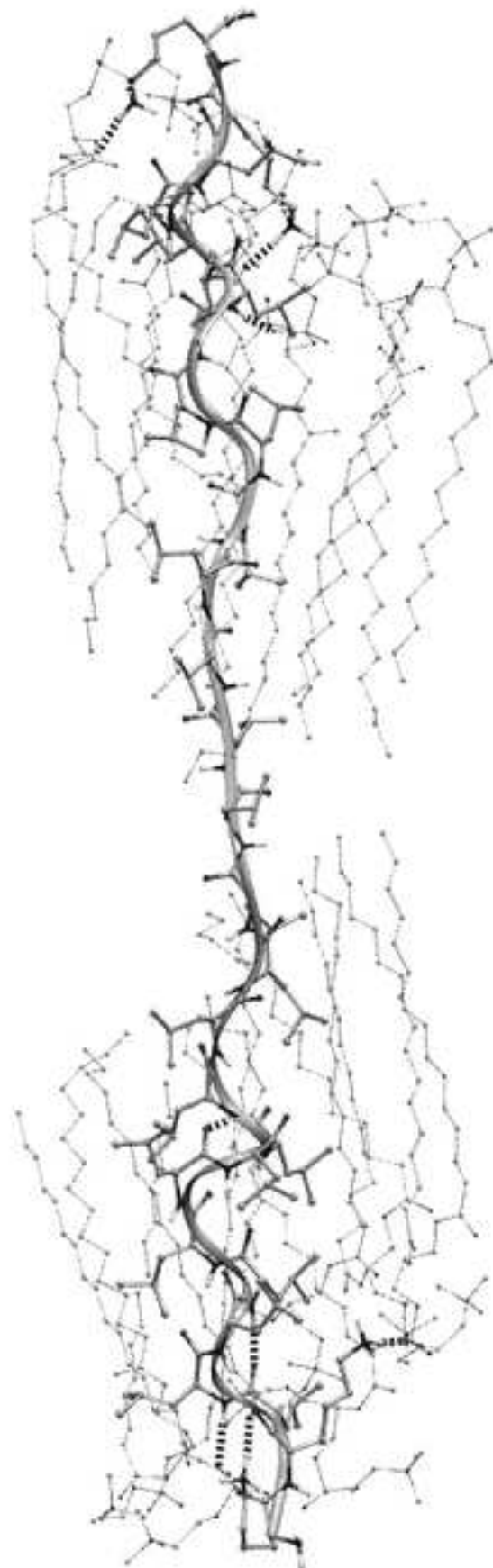


Figure 13a
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a

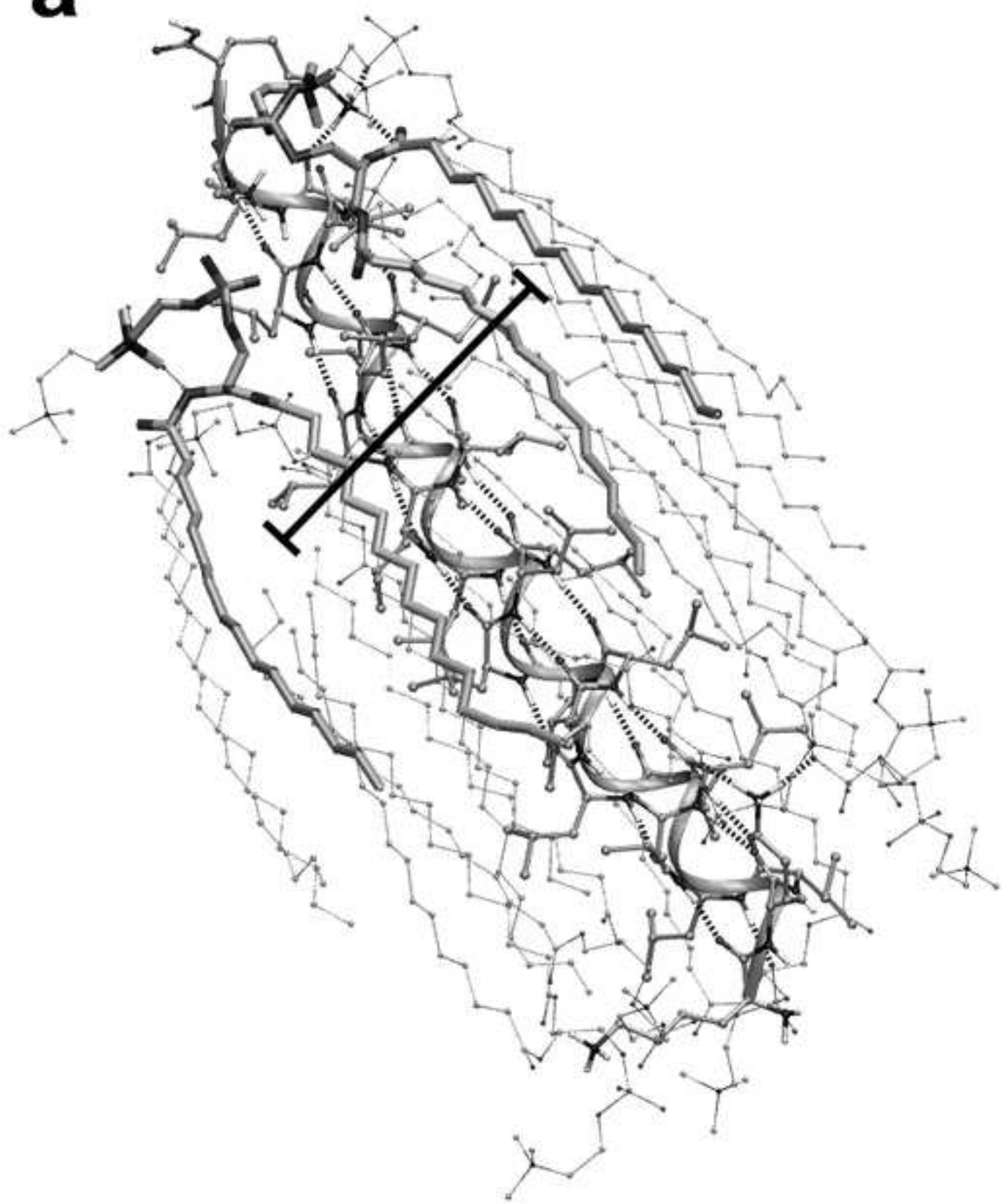


Figure 13b
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b

