A Study of the Mechanism of Action of Zervamicin IIB Peptide Antibiotic by Molecular Dynamics Simulation

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6 figures

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Abstract.

We model mechanism of action of a channel-forming peptide antibiotic, zervamicin IIB, by molecular dynamics (MD) simulation. Interaction of this peptide with neutral and negatively charged lipid bilayers is investigated. It is found that charge of membrane surface influences the orientation of zervamicin IIB molecule, that may in turn effect its permeation into the membrane. On this basis we propose modifications to ZrvIIB structure that may increase its affinity towards the prokaryotic cellular membrane. Zervamicin IIB transmembrane channels are modeled as bundles consisting of 4, 5 and 6 individual peptide monomers. Our results suggest that four monomers don't form a stable water-filled ion channel. Thus the channel with the least number of monomers (and the lowest conductance level by literature data) is a pentamer.

Key words: molecular dynamics, antimicrobial peptide, zervamicin IIB, membrane channel

Introduction.

Antibiotic resistance and infectious diseases are now considered the global healthcare problem that demands development of new antimicrobial agents. Peptide antibiotic and antimicrobial peptides are the representative of such agents.

Zervamicin IIB (ZrvIIB) is an antimicrobial peptide which interacts with the membrane of the target cell and increases ion permeability. ZrvIIB, isolated from cultures of *Emericellopsis salmosynnemata,* is a member of the antibiotics peptaibol family, known for its activity against Gram-positive bacteria and lack of toxicity towards eucariotic cells . Peptaibols are known to be potentially useful in chemotherapeutic applications in oncology (Oh et al., 2002). Also zervamicin IIA and

zervamicin IIB appear to inhibit the locomotors activity of test mice , probably via its by effect on the brain. These effects of zervamicin IIA become apparent at lower dosages (0.05-2.0 mg/kg) as compared to zervamicin IIB (0.5-12.0 mg/kg). ZrvIIB consists of 16 amino acid residues and, like other peptaibols, it contains a high proportion of helix-promoting α,α-dialkylated amino acids. In planar lipid bilayer ZrvIIB forms voltage-dependent ion channels with multilevel conductance state . The exact channel structure is still unclear. But the conventional model for voltage-gated peptaibol channel action involves the formation of the water-filled pore by a bundle of parallel helices . Different conductance levels are thought to correspond to different numbers of helixes in a bundle . An understanding on atomic level of how these peptides interact with the membrane may promote the development of variants with desired properties (such as increased antimicrobial or anticancer activity).

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In this paper we investigate different stages of ZrvIIB channel formation in a lipid bilayer using a Molecular Dynamics (MD) simulation method. We use MD simulations of ZrvIIB with two membranes to investigate how lipid composition affects the peptide interaction with the membrane. Further we construct models of channels formed by four to six zervamicin molecules and investigate dynamics of water molecules in the channel pore and the ion penetration through the membrane channel. Finally, we propose modifications to ZrvIIB structure that may increase its affinity towards the prokaryotic cellular membrane.

Materials and Methods

The Molecular dynamics (MD) technique is based on numerical solution of classical Newtonian equations in respect to a many atom system (Levitt,

1983). By solving the equations of motion for a multiple atom system we can obtain its evolution in a time (on a time scale of up to 100 ns) and some properties. In most cases the simulation results are rather reliable and can be regarded as a computational experiment. Such problems as peptide-membrane interaction, channel forming and ion penetration through the membrane channel can be studied using different variations of MD simulation (Nadler W. et al., 1985) Unfortunately, many biological processes (such as protein synthesis, opening of membrane channel, action of toxins, antibiotics and other processes) take much more time than 100 ns. In such cases steered molecular dynamics (SMD) can be used . Depending on the problem, in SMD we apply definite extra forces to definite particles or apply extra boundary conditions. For the study of large molecular systems or processes which take much time in equilibrium MD the SMD approach is preferable .

Both MD and SMD methods in the scope of current work were applied. Protein Data Bank structure 1IH9 was taken as starting peptide structure. MD simulations were carried out for the following systems: ZrvIIB in a box of 1700 water molecules, in a box of 1700 methanol molecules, ZrvIIB at the surface of two different bilayers and three zervamicin channels (formed by 4, 5 and 6 peptides) in the negatively charged membrane.

In the experiment with surface, binding peptide was oriented parallel to the lipid bilayer with the convex side of peptide facing the membrane. Two lipid bilayers were used for the comparative analysis. The first one consists of 64 palmitoyloleoyl phosphatidylcholine (POPC) lipids, and the other one consists of 44 palmitoyloleoyl phosphatidylethanolamine (POPE) and 12 palmitoyloleoyl glycerol (POPG). Both bilayers were solvated with 2500 water molecules. 12 Na^+ ions were added in the second system to compensate for

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the negative charge of the lipid bilayer.

The lipid system was energy minimized and equilibrated for 500ps, after that the 10 ns trajectories were calculated under the following MD-protocol:

- simulation software: Gromacs 3.2.1
- force field: OPLS-AA
- time step: 1fs
- temperature coupling: stochastic dynamics
- system temperature: 300K
- time constant for coupling: 2 fs
- coulomb cut-off: 2nm
- van-der-waals cut-off: 2nm
- pressure coupling: Berendsen (semiisotropic) for the systems with membrane and without pressure coupling for the zervamicin in the water and methanol
- time constant for coupling: 10ps
- pressure: -50 bar (x/y direction), 1 bar (z direction)

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Results

Zervamicin IIB in the water and methanol solvent

Most of peptaibols do not have a definite structure in aqueous solution outside of a lipid bilayer . Dynamics and structure of these peptides strongly depend on the environment. Unlike these long peptaibols (for example, Alamethicin), Zervamicin IIB maintains helical structure not only near the bilayer surface, but also in water and methanol solvents. A measure of structural stability for zervamicin molecules in different systems was obtained by comparing Cα atom root mean square

deviation (RMSD) from the starting structure. RMSD for zervamicin IIB in water and methanol was ~ 15×10^{-2} nm. RMSD for ZrvIIB near the membrane surface changed from ~ 2×10^{-1} nm in the beginning of the simulation to ~ 3×10^{-1} nm in the end for the POPE+POPG bilayer and from ~ 18×10^{-2} nm to ~ 35×10^{-2} for the POPC bilayer. These high RMSD values for ZrvIIB with membrane are explained by rotation of peptide molecule relative to the bilayer surface.

Stability of the hinge region was investigated by means of probability distribution of distance between N- and C- ends (Fig. 1). Only minor hinge-bending motions were detected. In both solutions length alteration was less than 1 nm, in agreement with the NMR experimental data . This helix stability implies absence of significant conformational rearrangement during the embedding of the peptide into the membrane.

Surface binding of zervamicin IIB.

At the onset of the simulation ZrvIIB was placed on its convex side onto the membrane surface. In the simulation with a POPC bilayer, ZrvIIB remained parallel to the surface at the distance ~ 8×10^{-1} nm. Gln3 and Gln11 formed hydrogen bond with lipid heads, and stabilized parallel orientation of the zervamicin IIB. However, in this situation, both Gln residues were situated on the same zervamicin side, which caused some conformation changes in helical structure of peptide (Fig. 3).

Because of the identical orientation of intermolecule h-bonds, zervamicin IIB molecule has a dipole moment equal to approximately 50D that corresponds to point charges of + 0,4e and -0,4e placed at the N and C-ends respectively. During the simulation with the POPE+POPG bilayer the N-end of the peptide, which has a local positive charge, moved to the negatively charged surface of the bilayer, and C-end moved away from the membrane. This orientation was stabilized by hydrogen

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bonds between Gln3 and lipid heads. So as a result in the end the concave side of ZrvIIB was facing the membrane (Fig. 2). Presumably, this orientation is more appropriate for the subsequent embedding of the peptide into the membrane, because of the embedding of zervamicin begins on the N-end.

The conclusion from these simulation results is that ZrvIIB peptide is loosely bound to the surface of lipids bilayeres, and hydrophobic surface of the helix does not penetrate into the bilayer core. The traditional model of peptaibol interaction with membranes is that helix stabilization is due to partitioning of hydrophilic and hydrophobic side chains between the aqueous phase and the hydrophobic core of the bilayer, respectively. It means that the peptide must be located deep in the interfacial region, but it is not so in case of the loose complex described here.

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Channel models.

The structure of zervamicin channel is still unknown. But we can construct three channel models according to the Barrel-stave model (Molle G. et al., 1987), there peptides form bundles of 4, 5 and 6 molecules, so that the helices within a bundle were all parallel to one another, and Gln11 of all ZrvIIB were directed toward the lumen of the channel. These models will be designated in the foregoing as N4, N5 and N6 respectively. We investigated conformation stability of ZrvIIB molecules in the bundles and dynamics of water molecules in the channel pores.

In order to estimate conformation stability of different channel models in this study, we measured Cα-atom root mean square deviation (RMSD) from the starting conformation (Fig. 4). Within 2 ns the RMSD for tetrameric bundle (N4) increased to a value of 27×10^{-2} nm, whereas RMSD for N5 and N6 raised more slowly to ~ 16×10^{-2} nm and 15×10^{-2} nm respectively. In the N5 bundle peptide molecules coiled up into a superhelix. In case of N6 bundle, peptides remained

parallel. It accounted for the small difference in the RMSD values. The reason of greater conformation drift for N4 could be the absence of the water molecules in the channel pore. As shown in Fig. 5B, C, and D, N4 does not contain a continuous water column. There are well-defined columns of water for the other two bundles. Water molecules in the channel pore form hydrogen bonds with the atoms of zervamicin molecules. These bonds as well as the hydrophobic interaction between peptide molecules and lipids stabilize channel conformation.

The pore radius profiles were calculated using the program HOLE which uses a Monte-Carlo algorithm to find maximal radius spherical probe that will fit in the in the channel pore. Profiles of all three channels are shown in Fig. 5A. The N4 bundle has a pore with a radius of only 2×10^{-3} nm, which is too small to accommodate an ion. This confirms that N4 bundle can't form an ion channel.

After a 2ns relaxation period, N5 and N6 bundles formed two narrow gate regions inside the pore of the channel (narrowest diameters of 27×10^{-2} nm and 25×10^{-2} nm for N5, 38×10^{-2} nm and 37×10^{-2} nm for N6), that correspond to positions of the Gln3 and Gln11 glutamine rings. (Peptides in N5 form a superhelix, so the exact length of the channel and the specific position of the narrowest region differ slightly from N6).

The Na+ migration through the channel

We researched migration of Na+ through the N5 bundle. N4 bundle does not form ion channel and the pore of N6 is large enough for the channel interior not to influence the ion movement. We, therefore, chose N5 bundle to investigate the details of ion migration.

In the SMD digital experiment the ion moved through the channel pore driven by the external force (4 kcal/mol*Å) and electric potential. Particular attention was

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paid to the alteration of interaction energy between ion and surrounding atoms during ion migration. Energy profile of sodium ion transfer along the pore axis is represented in Fig. 6A. Energy was calculated as sum of LJ and coulomb interaction between ion and peptide molecules and water. During its migration there were two zones where ion motion slowed down. These zones corresponded to the two narrowest regions of the pore (glutamine rings). In the water and near the mouth of the channel 6 water molecules hydrated Na+ and in the area of the first glutamine ring it lost 4 molecules. Then, hydrated by two water molecules, it moved to the next glutamine ring and further on the opposite side of the lipid bilayer.

Energy of interaction between sodium and zervamicin IIB molecules had two local minima (Fig. 6B), which were the result of the glutamine oxygen coordinating the ion. Interaction energy of ion with peptides was opposite to the interaction energy between ion and water molecules, which means that glutamine oxygen replaced hydrating water molecules. Therefore, glutamines play an important role in the ion migration through the N5 zervamicin channel. In channels formed by more than 5 peptide molecules, the pore radius is so big that pore interior does not influence the ion movement.

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Discussion.

Our modeling results are consistent with the experiment data in general:

1. The the membrane charge influences the orientation of zervamicin IIB molecule. This orientation promotes embedding of zervamicin IIB into the lipid bilayer and possibly defines ZrvIIB selective action on prokaryotic cells.
2. Our molecular modelling experiments show that four zervamicin IIB molecules cannot form a water-filled ion channel. However it can be a

nucleus for the formation more large assembles. But 5 and 6 peptides form the stable channels with the smallest radius 2.5 Å and 3.7 Å respectively. For the pentamer channel, we observe that ion diffusion through the pore is facilitated by coordination of the moving sodium ion by the two glutamine residues positioned inside the channel that compensate for the lost waters of hydration.

We want to stress that the main aspects of peptaibols interaction with lipid bilyers, mentioned above, can be useful for modification of their affinity to cell membrane. The peptide dipole moment plays a key role in this interaction. The alteration of either the number of h-bonds or the peptide length by protein engineering methods may critically effect the peptibol function. Also, addition of the negatively charged amino acids on the N-end can increase the affinity to the prokaryote membrane. On the other hand, increase in the number of glutamine residues, that form h-bonds with positively charged lipids, may make peptaibols more toxic to the eukaryotic cells.

This idea may be key to computer design of new antibiotics on the basis of ZrvIIB with specified selectivity and activity (such as improved antimicrobial and anticancer activity). The insight of how ZrvIIB interacts differently with neutral and charged lipids may also help to explain experimental observations (Balaram P. et al., 1992) on how membrane composition affects the function of antimicrobial peptides.

References.

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Fig. 1. Probability distribution of distance between N- and C- end of zervamicin IIB in the water and methanol surrounding.

Fig. 2. Z-coordinate of amino acid Cα-atoms in the beginning of the simulation and after 10 ns of the simulation (A. zervamicin II with POPC bilayer. B. zervamicin II with POPE and POPG bilayer)

Fig. 3. Zervamicin II conformation at the start of the simulation and after 10 ns of molecular dynamics in the water, near the POPC membrane surface and near the POPE and POPG membrane surface.

Fig. 4. RMSD from the starting model for N4, N5 and N6 channels.

Fig. 5. A. Pore radius profile after 2 ns of relaxation. Snapshots after 2ns of simulation of the water columns in the N4 (B), N5 (C) and N6 (D).

Fig. 6. A. Sodium ion transfer along the pore axis (z coordinate). B. Interaction energy of sodium ion with zervamicin molecules (solid line) and water molecules (dashed line).

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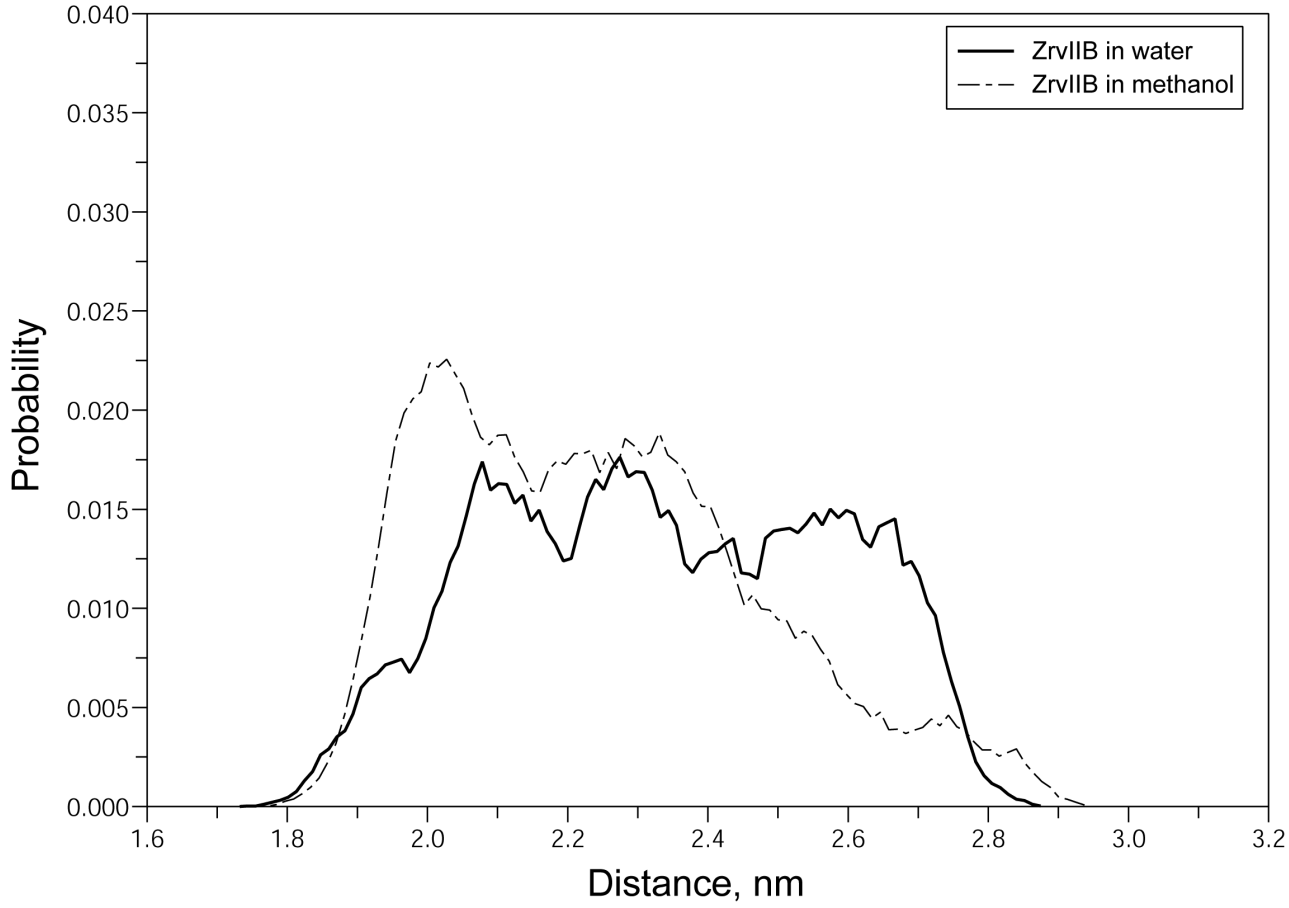

Fig. 1

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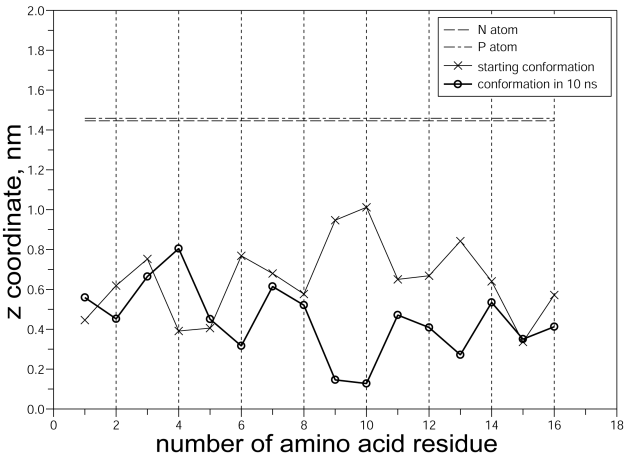
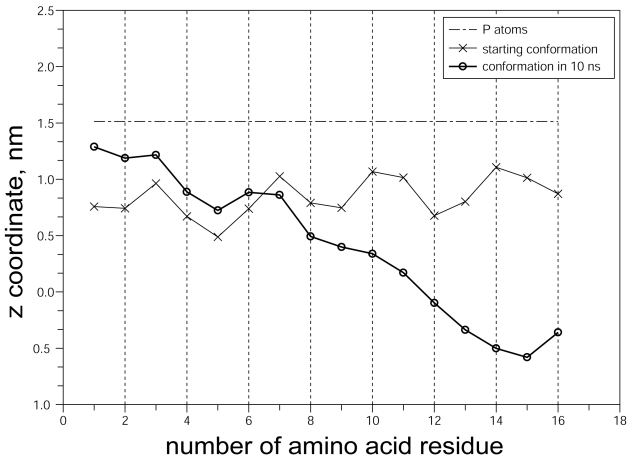


A



B

Fig. 2

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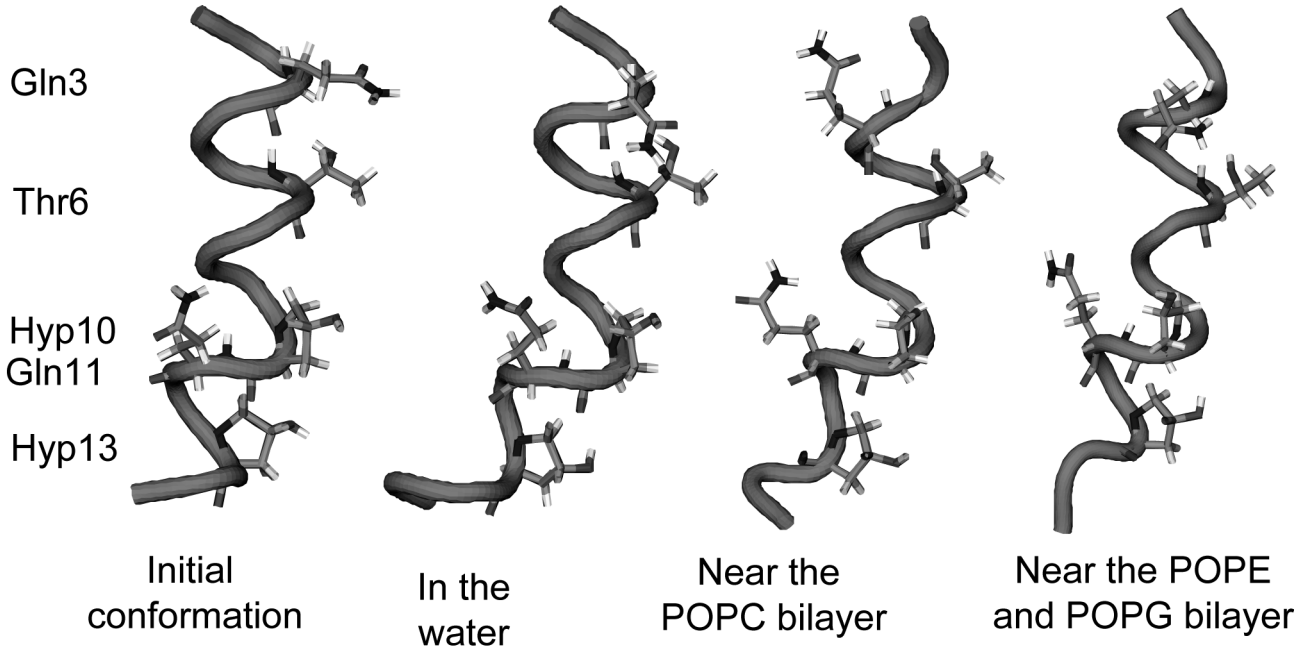

Fig. 3

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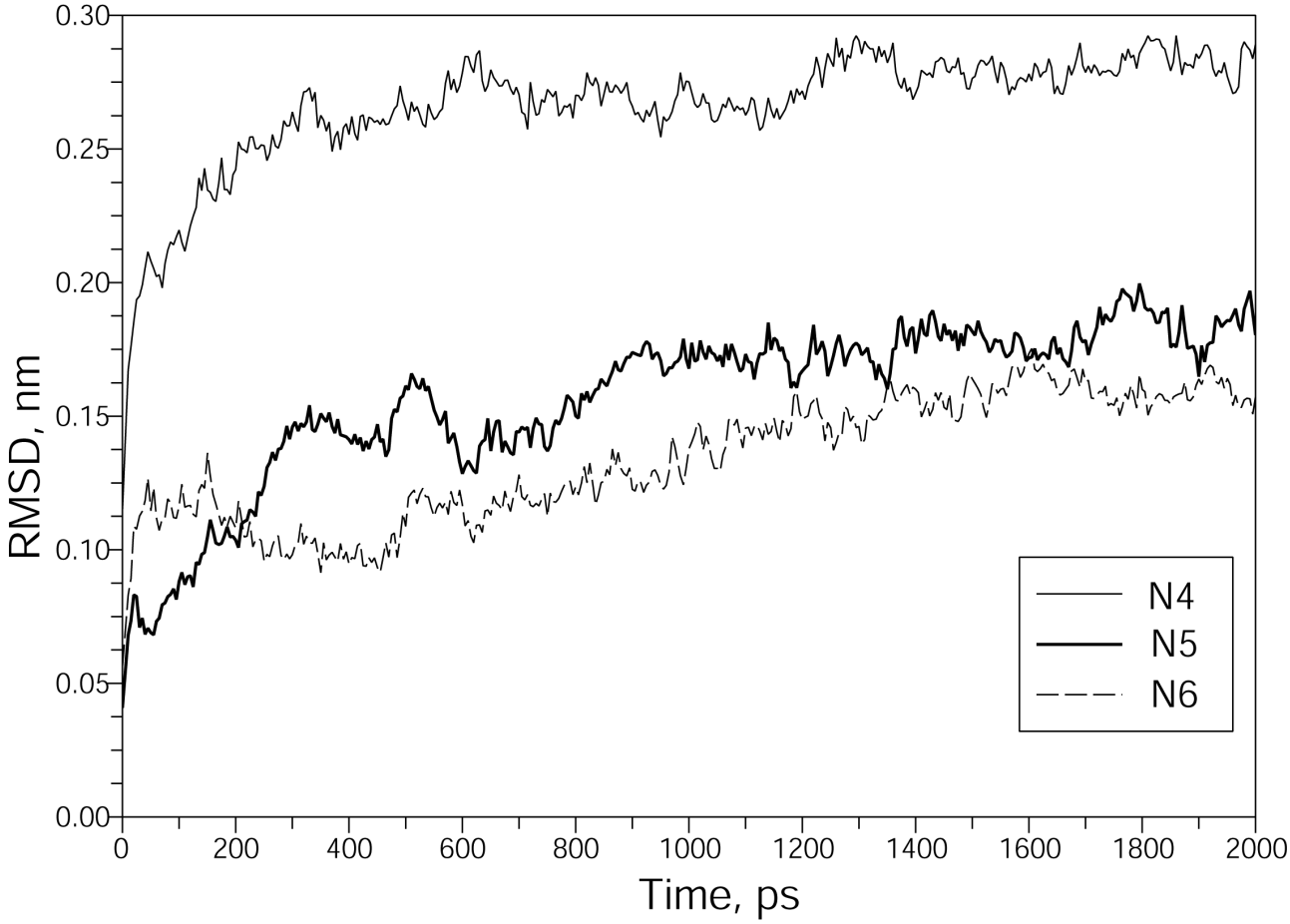

Fig. 4

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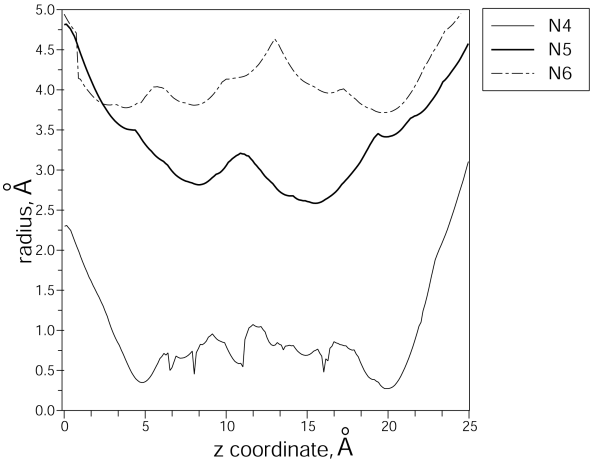
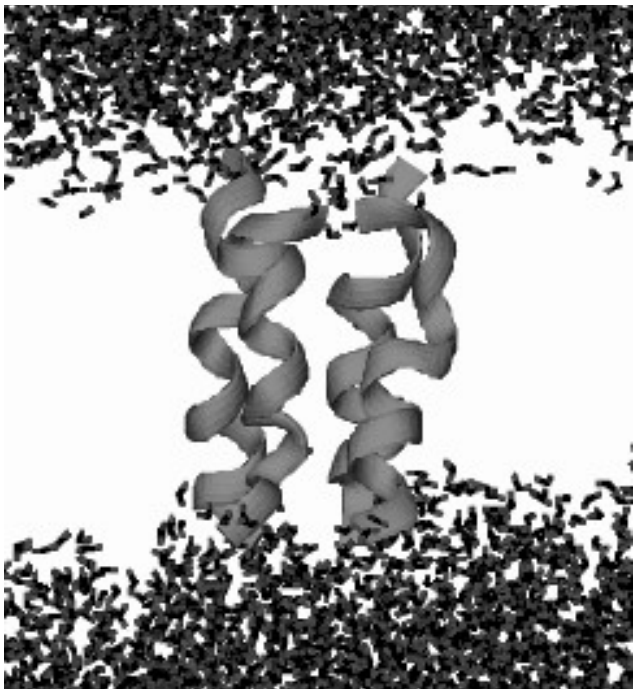
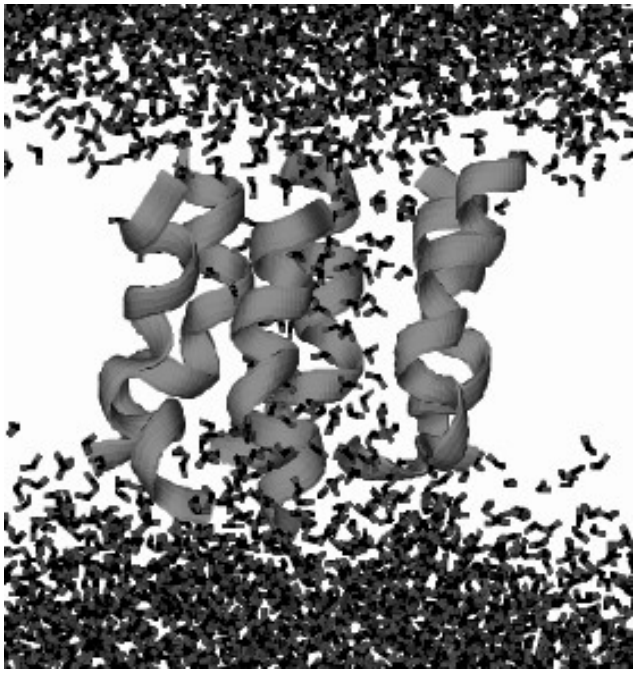


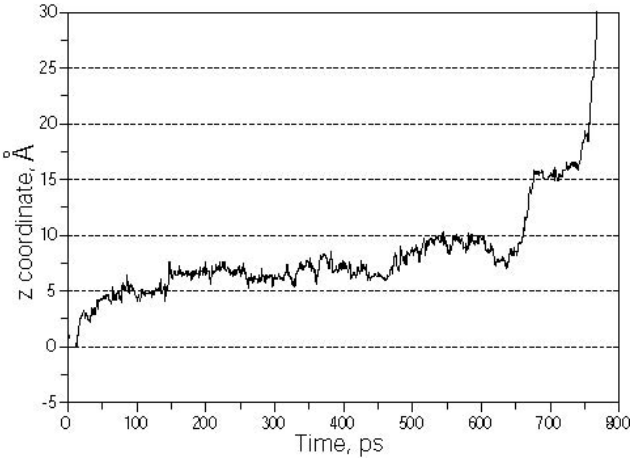


A B



C D

Fig. 5



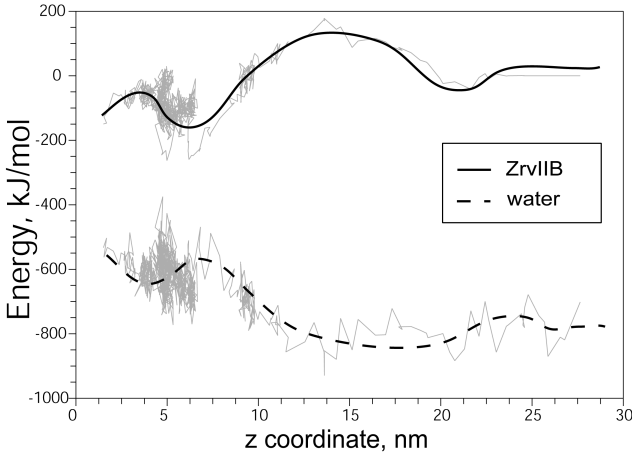


Fig. 6

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