

Introduction

Recently peptide-type foldamers have been observed to form certain conformations that can be used to mimic oxyanion hole. The structure of oxyanion hole is formed by a net of negatively charged oxygen and two peptide hydrogens. Oxyanion hole is very important motif in active side of enzymes. To efficiently mimic an enzyme the size of the foldamer should be large enough, though. However large size foldamers are very hard to synthesize for the characterization purposes. Because of this finding an accurate computational model for these foldamers is important. Quantum mechanical computational methods are accurate but rather expensive, therefore unpractical to simulate larger systems. Additionally QM-methods are not suited to model solution systems within the scale. Instead of with molecular dynamics it is possible to efficient way simulate large solution systems with long time scale.

The main focus on this study was to create validated MD model for the foldamers, which can be used for the designing of the foldamers in the future. This was done by choosing well characterized system (acetylfoldamer) for the MD simulation. With well characterized system it is simple to say is the model accurate or not.

Methods

Molecular dynamics

Molecular dynamics (MD) simulations were made with GROMACS version 5.0.4 software¹. Interactions of acetylfoldamer were modeled with OPLS-AA forcefield.² Topology files for Gromacs are available as supporting information. Two groups of simulations were carried out. The first was a simulation of the acetylfoldamer crystal, which was performed to test the validity of the OPLS-AA forcefield parameters, as an experimental reference structure is available.³ Crystal structure was made from 27 acetylfoldamer unitcells. This system was first energy minimized using steepest descent for 500 steps and then simulated for 100 ns in vacuum with the temperature maintained at 173,15 K using the v-rescale thermostat.⁴

In second group of simulations single foldamer in crystal structure conformation (5-S-1) was placed in 46.1 nm³ (diameter = 1.63 nm) dodecahedron periodic box and system maximum force was minimized below 500.0 kJ mol⁻¹ nm⁻¹ with the steepest descent method. Minimized acetylfoldamer in 46.1 nm³ dodecahedron box was solvated by 462 chloroform^{5;6} molecules. Total mass of the solvated system is 55646.158 Da. Maximum force of the solvated system was minimized below 250.0 kJ mol⁻¹ nm⁻¹ with the steepest descent of 1000 steps. System was equilibrated first with 100 ps NVT ensemble and then with NPT ensemble in 2 fs steps and finally 1 μ s production MD simulation was done in 1 fs steps. Pressure was maintained at 1 atm with Parrinello-Rahman^{7;8} barostat and system compressibility was set same as chloroform^{5;6} (2.16 GPa⁻¹). Temperature was coupled to 300 K with v-rescale thermostat. For short-range nonbonded interactions verlet cutoff-scheme was used with 0.005 kJ mol ps⁻¹ buffer. For long-range electrostatic interactions was applied the particle mesh Ewald (PME) method with grid spacing of 0.16 nm and fourth-order interpolation⁹. Snapshots were taken from simulations every 0.5 ps for analysis. Simulation of 1 μ s was done in methanol^{5;6} with the same parameters. Different conformations were identified with cluster analysis, which was performed with gmx cluster program. Structures were added to the same cluster if their root-mean-square deviation (RMSD) was less than 0.8 nm. Dihedral angles of conformations were calculated with GROMACS gmx angle program. Obtained conformations were DFT optimized using Gaussian 09 program¹⁰ at ω B97D-X/6-311G(d,p) level of theory. They also was minimized with Low-memory Broyden-Fletcher-Goldfarb-Shanno quasi-

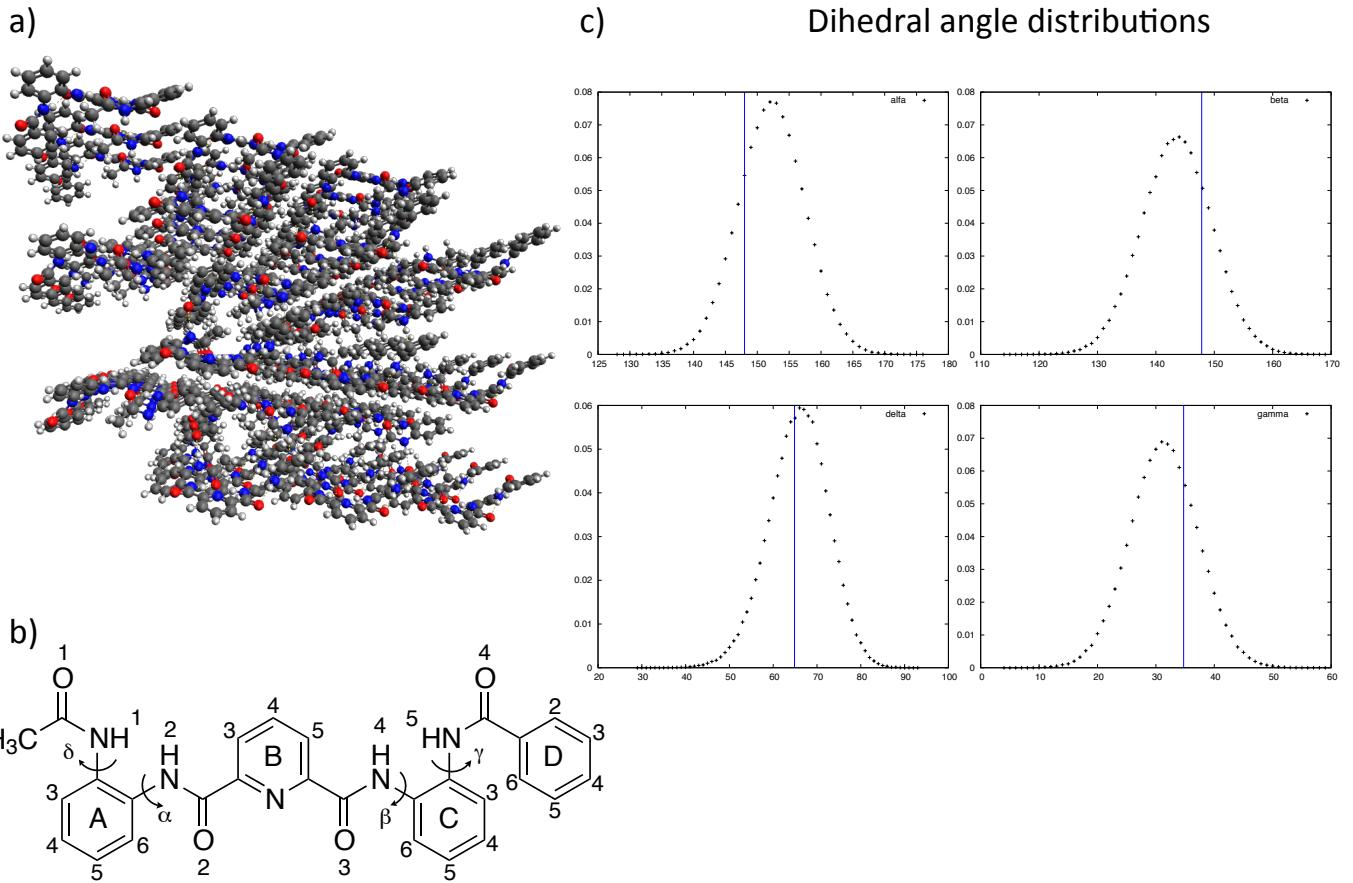


Figure 1: Picture of simulated crystal structure (a). Numbering of atoms and dihedral angles in the acetylfoldamer (b). Dihedral angle distribution of the crystal structure simulation. The blue line represents the dihedral angle value from experimental x-ray structure (c).

Newtonian minimizer (L-BFGS) algorithm in OPLS-AA forcefield. The hydrogen bond distances were calculated with GROMACS gmx distance program for the NOE active atoms pairs as identified by Kortelainen et al³. Notation of the conformations was taken from previous article.³

Results and discussion

The dihedral angle distributions of the crystal structure simulation (Figure 1) indicate that the structure stays intact through the 100 ns simulation, therefore OPLS-AA forcefield seems to be suitable for conformational analysis of the acetylfoldamer.

Previously total of eight conformations of acetylfoldamer (5-S-1, 5-S-2, 5-@-1, 5-@-2, 5-@'-1, 5-@'-2, 5-helix and 5-helix-2) were found using Monte Carlo (MC) conformational analysis with OPLS-2005 force field (figure 4) CIT. These structures were DFT optimized with the ω B97X-D/6-311++G(3df,3pd) level of theory. The conformations found with this method were also found with MD simulations. In the S-conformations, the acceptor oxygen O (5-S-1) or O4 (5-S-2 and 5-S-2-m)

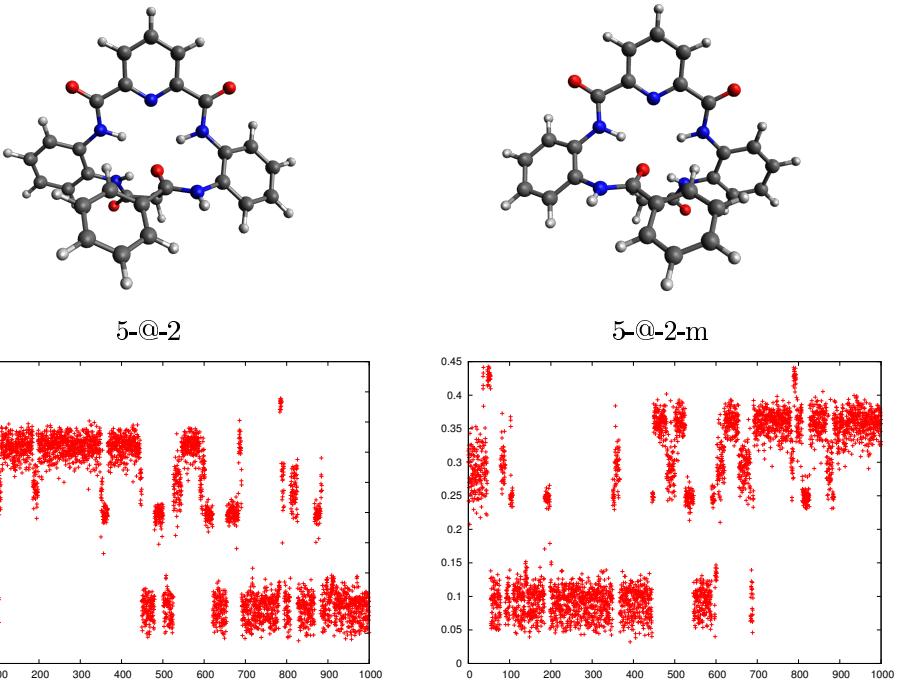


Figure 2: Structures and RMSD of the conformers 5-@-2 and 5-@-2-m, extracted from the chloroform simulation.

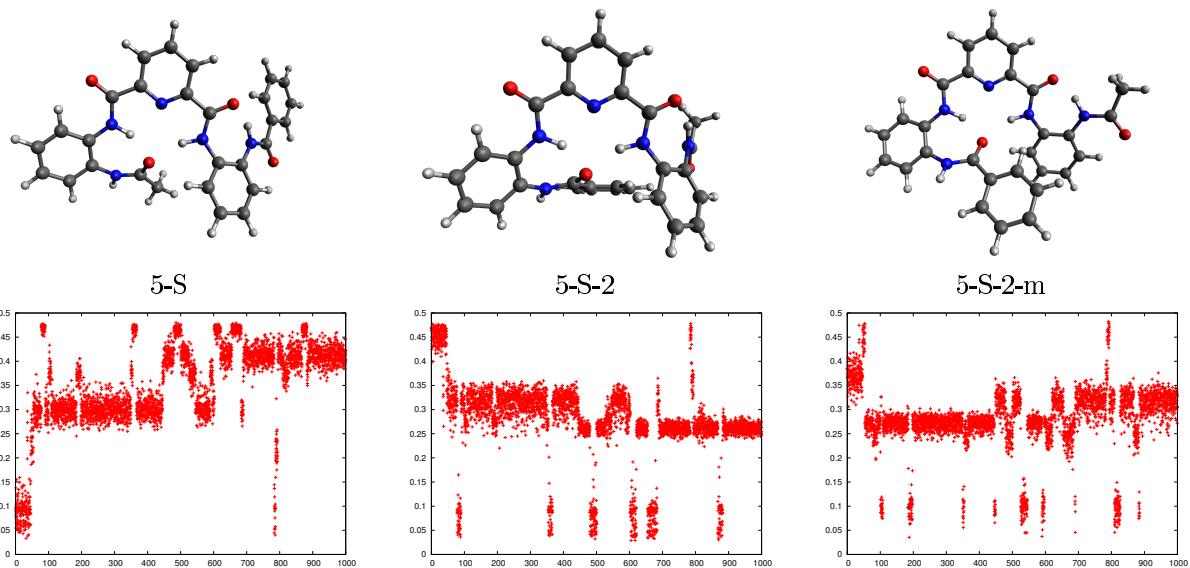


Figure 3: Structures and RMSD of the conformers 5-S, 5-S-2 and 5-S-2-m, extracted from the chloroform simulation.

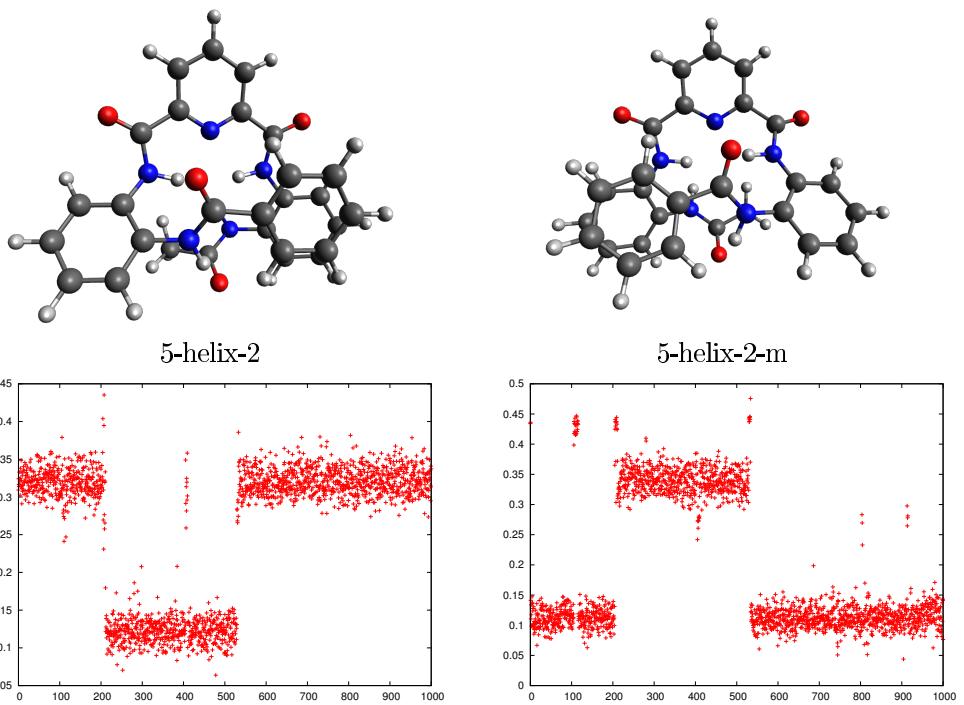


Figure 4: Structures and RMSD of the conformers 5-helix-2 and 5-helix-2-m, extracted from the methanol simulation.

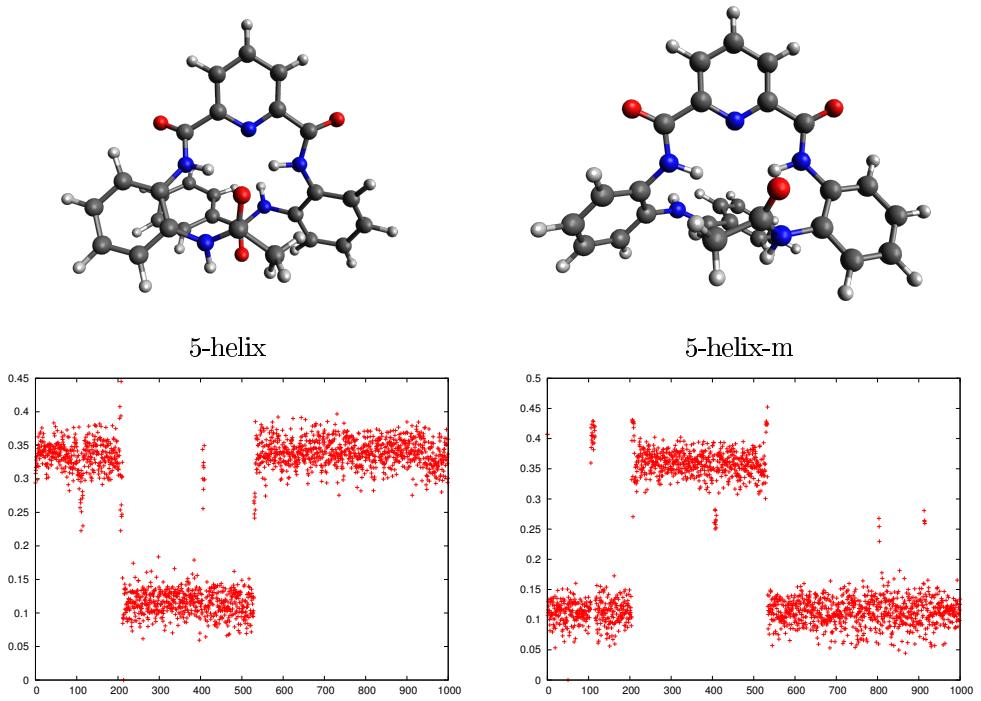


Figure 5: Structures and RMSD of the conformers 5-helix and 5-helix-m, extracted from the methanol simulation.

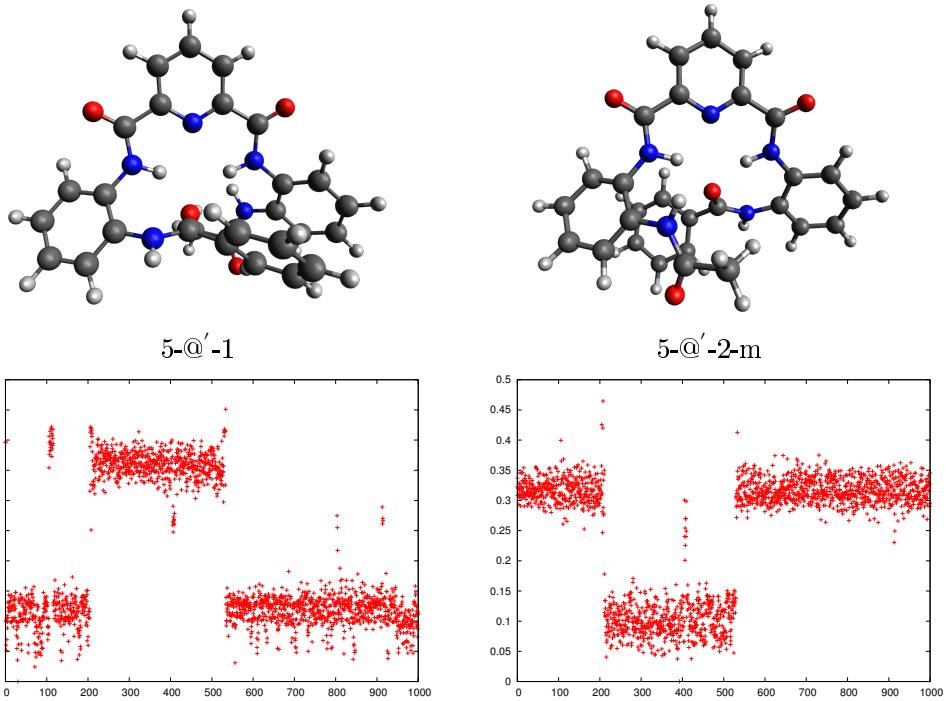


Figure 6: Structures and RMSD of the conformers 5-@'-1 and 5-@'-2-m, extracted from the methanol simulation.

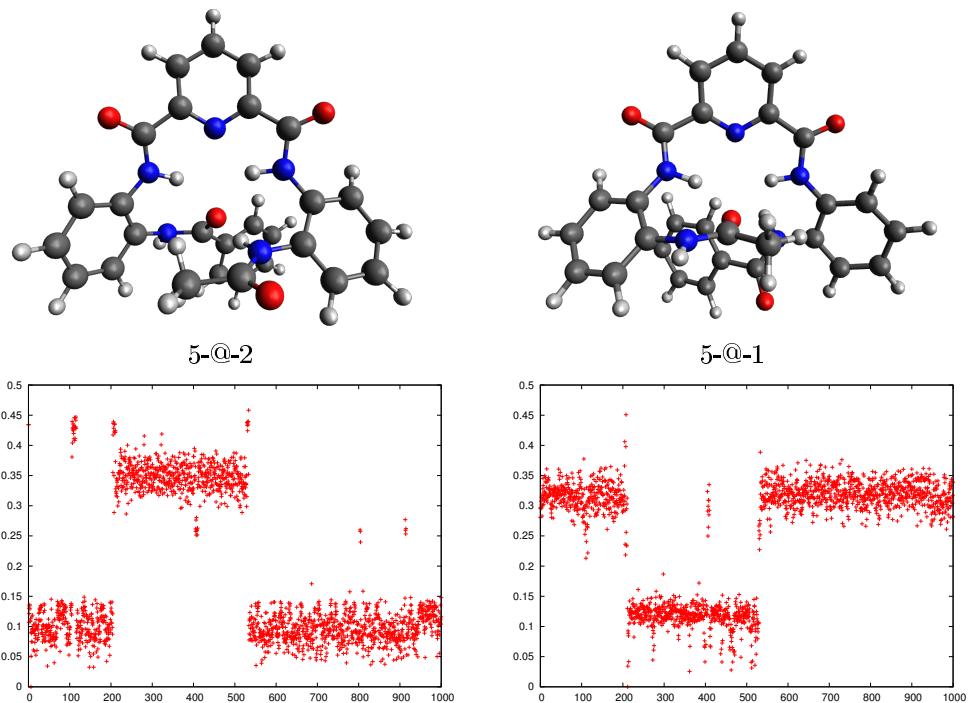


Figure 7: Structures and RMSD of the conformers 5-@-2 and 5-@-1-m, extracted from the methanol simulation.

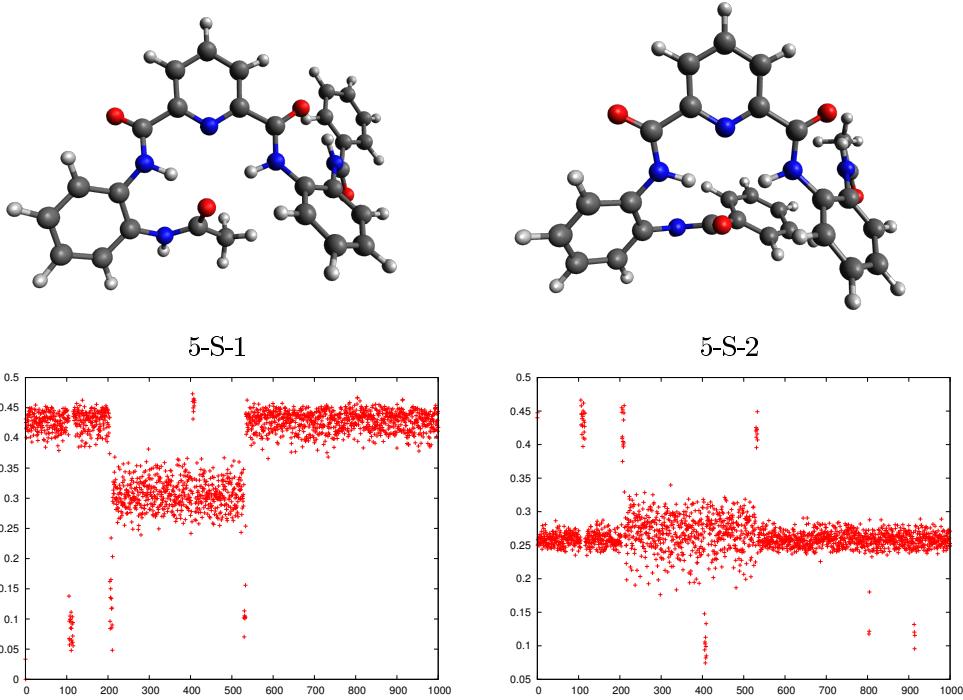


Figure 8: Structures and RMSD of the conformers 5-S-1 and 5-S-2, extracted from the methanol simulation.

will only be bonded with hydrogens HN2 and HN4, while in the @-conformations the oxygens O or O4 are also bonded with either hydrogen HN1 or HN5. @' conformations are structurally close to @ conformations, but O4 or O are bonded to methyl group and H2D ($5-\text{@}'-1$) or to hydrogens H3A and H2D ($5-\text{@}'-2$), while in helix-conformers these oxygens are only bonded to HN2 (5-helix) or HN4 (5-helix-2). Moreover the A-ring is positioned perpendicularly to D-ring. Number 1 in all conformations means that the acceptor oxygen is O and number 2 means that the acceptor oxygen is O4.

Total of four of these conformations (5-S-1, 5-S-2, 5-@-1 and 5-@-2) were found with cluster analysis from the chloroform simulation and one new conformation (5-S-2-m). This simulation clearly favors 5-@-2 conformation. Conformer 5-@-2 also appears as a mirrored form (5-@-2-m). Their intramolecular forces are identical and therefore their total electronic energies and dipolemoments are also the same (Table 1). The 5-@-2 conformation folds into its mirrored conformation through the 5-S-2 and 5-S-2-m conformations. Even though the conformations 5-S-2 and 5-S-2-m also seem to be mirrored structures between each other at first, we can see that their torsional angles are twisted unsymmetrically and that is why their intramolecular forces also differ from each other. Therefore 5-S-2-m is totally new conformation. There is also very short appearance of 5-@-1 conformer. The reason why 5-@-2 conformation is much more populated than 5-@-1 can be explained by energetics. It seems that in the case where conformations are structurally similar the simulation prefers conformer which has lower energy under the OPLS-AA forcefield (Table 1).

In methanol simulation total of eight different conformations (5-S-1, 5-S-2, 5-@'-1, 5-@'-2, 5-helix, 5-helix-2, 5-@-2, 5-@-1) were found. Simulation consists of two major clusters. In the more populated cluster the conformation changes rapidly between 5-@'-1 and 5-@-2 conformers. There is also very short shifts to mirrored forms of 5-helix and 5-helix-2 conformers, which both seem to be some kind of intermediates³ between 5-@'-1 and 5-@-2 conformers. The other cluster consist of 5-@'-2 and 5-@-1 conformers which are both only present in mirrored forms. Also in this case there are helix

Table 1: Energy and dihedral angle data of all conformations found in MD simulations.

Conformation	E_{tot} (Ha)	E_{totmc} (Ha)	$E_{gromacs}$ (kJ)	α	β	γ	δ
5-S-1	-1655.4576984	-1655.4576981	-456.044128	156.1	-51.4	81.4	54.3
5-@-1	-1655.457294	-1655.4544988	-455.966919	147.3	126.3	113.9	59.9
5-@-2	-1655.4557366	-1655.4557182	-469.767639	167.7	144.6	-126.5	-132.5
5-@-2-m				-167.6	-144.6	126.5	132.6
5-S-2	-1655.4611423	-1655.4612251	-472.050537	-52.1	141.4	-131.1	-145.0
5-S-2-m	-1655.4595914		-471.672729	48.5	-156.2	132.4	136.5
5-@'-1	-1655.4597942	-1655.459794	-461.614380	166.2	149.6	-54.4	-139.9
5-@'-2-m	-1655.4585488	-1655.458484	-460.913086	-138.7	-148.0	128.5	45.9
5-helix-2	-1655.4592239	-1655.457342	-467.861938	176.4	136.9	119.6	130.4
5-helix	-1655.4622932	-1655.462293	-466.487854	155.1	-154.7	52.3	50.1

Notation: E_{tot} refers to total electronic energy of the found conformations computed at ω B97X-D/6-311++G(3df,3pd) level of theory and $E_{gromacs}$ refers to energies obtained with minimizing structures with Low-memory Broyden-Fletcher-Goldfarb-Shanno quasi-Newtonian minimizer (L-BFGS) algorithm in GROMACS, E_{totmc} (Ha) refers to total electronic energy of the conformations found with Monte Carlo analysis computed at ω B97X-D/6-311++G(3df,3pd) level of theory.

intermediates (5-helix and 5-helix-2) between the two main conformations. The conformation also folds back to starting 5-S-1 conformer few times.

Acetylfoldamer favors completely different conformers in methanol and chloroform. As an polar solvent methanol can form hydrogen bonds with peptide oxygens and hydrogens. This makes it possible for methanol to break oxyanion holes in @-conformers, which then leads to formation of @'-conformers. In case of chloroform there is no interactions between the solvent and the acetylfoldamer. This prevents the breaking of oxyanion holes and the formation of @'-conformations.

The energies of conformers from the MD simulation differ only slightly with all conformers to MC conformations except the 5-@-1 and 5-helix-2 conformers, where the energy differences are more significant. Minimum energies of 5-@-1 and 5-helix-2 conformers are lower in MD-simulation than the minimum energies obtained from the Monte Carlo analysis. Moreover 5-S-2-m conformation was only observed from the MD simulation. It seems that more lower energy conformations can be found with MD-simulations in OPLS-AA forcefield than with the Monte Carlo conformational search.

All hydrogens which are coupled to HN1 in experimental 1D NOE spectrum in $CDCl_3$ are listed in Table 2. If distance between hydrogens is less than 5 Å there is correlation that can be seen in the NOE spectrum. During the simulation the distances from HN1 to hydrogens H5A, H4C, H5C, H4B and H5B are always more than 5 Å. However the peaks of all these hydrogens (H5A, H4C and H5C are overlapped) are seen in the NOE-spectra. H5A hydrogen is at para position with HN1 so on account of bond lengths the distance between these two cannot be under 5 Å. According to the simulation the HN1-H5A distance varies around 5.5-6.0 Å the whole time so probably in this case the distance is still short enough for the peak to appear in NOE-spectra. The appearance of H4B and H5B peaks is harder to rationalize, for the distance to HN1 is more than 6.0 Å during the whole simulation. Then again it is difficult to imagine a conformation where the HN1 would be positioned so a coupling to either H4B or H5B would take place. Therefore these peaks in NOE-spectra are most likely just artefacts. The rest of the peaks in NOE-spectra can be explained by the conformations of the simulation. None of the conformations alone fulfills the peaks in NOE-spectra which directly indicates that there are changes between the conformations during the simulation in chloroform. The peak of hydrogen HN5 is a proof that at least one @-style conformation is present in the simulation in chloroform because only in 5-@-2 and 5-@-1 conformations the distance between HN1-HN5 is short enough to be seen in NOE-spectra. The same goes with the H3B and H4D peaks that are only possibly if S-2 conformation is present.

Table 2: Conformations from chloroform simulation which gives peak in HN1 NOE-spectrum (marked with x). Marked with green if correlation is seen in experimental HN1 NOE-spectrum.

HN1-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1
HN2	x	x	x	x	x
HN4	x			x	x
HN5				x	x
H3A,H3D,H5D	x	x	x	x	x
H4A	x				x
H6A		x	x	x	
H3B		x	x		
H4B					
H5B					
H3C,H6C	x			x	
H2D, H6D		x	x	x	x
H4D		x	x		
Me	x	x	x	x	x

Table 3: Conformations from chloroform simulation which gives peak in HN5 NOE-spectrum (marked with x). Marked with green if correlation is seen in experimental HN5 NOE-spectrum.

HN5-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1
HN2		x	x	x	x
HN4	x			x	x
H3A,H3D,H5D	x	x	x	x	x
H4A					
H5A,H4C,H5C?		x	x	x	
H6A					
H3B					
H4B					
H5Bcon	x				
H3C,H6C	x	x	x	x	x
H2D, H6D	x	x	x	x	x
H4D					
Me	x			x	x

All the peaks in the HN5 and Me NOE-spectras can be explained by the conformations that are found in the simulation. Only in the 5-S-1 conformation the distance between HN1-HN5 is short enough to appear in the spectra. This particular peak is moderately weak which indicates that the acetylfoldamer visits the 5-S-1 conformation rarely in chlorofo214000rm, just like in the simulation.

Conclusion

In MD simulations on total of five different conformations of acetylfoldamer were found in chloroform solution and seven in methanol solution. One of the five conformations (5-S-2-m) in chloroform was not previously found with any method. Also one of the conformation in chlorofo
and one in methanol solution have lower DFT energies than the corresponding energies found with Monte Carlo analysis in previous article.³ The reason for the different conformational behaviour of acetylfoldamer in chloroform and in methanol is based on the separate interactions between the molecule and the solvent. In addition the simulation with chloroform matches well with the experimental CDCl₃1D NOE-spectra.

As stated in previous article, foldamers have been observed in conformations that contain oxyanion

Table 4: Conformations from chloroform simulation which gives peak in Me NOE-spectrum (marked with x). Marked with green if correlation is seen in experimental Me NOE-spectrum.

Me-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1
HN2	x	x	x	x	x
HN4	x			x	x
H3A,H3D,H5D	x	x	x	x	x
H4A					
H5A,H4C,H5C	x			x	x
H6A					
H3B		x	x		
H4B					
H5B					
H3C,H6C	x			x	x
H2D, H6D	x	x	x	x	x
H4D		x	x		

holes. We have also observed presence of oxyanion holes in @-conformations found in the simulations. These conformations are presented in both solvents (methanol and chloroform).

In conclusion, we have found an efficient way to simulate acetylfoldamer in two solution states (chloroform and methanol). We assume that this MD model is also well suitable for the simulation of larger foldamers.

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