

## Introduction

Recently peptide-type foldamers have been used to mimic oxyanion hole. Oxyanion hole is very important motif in active side of enzymes. However large size foldamers are very hard to synthesize for the characterization purposes. Because of this finding 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 soluted systems within time scale

The main focus on this study was to create molecular dynamics (MD) model for the conformational behaviour of acetylfoldamer in solutions. The suitability of this model was put to test by comparing it with the experimental 1D NOE-spectrum

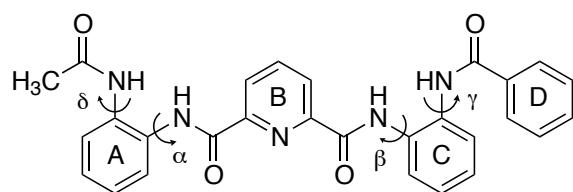
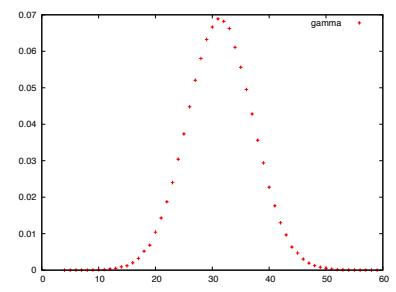
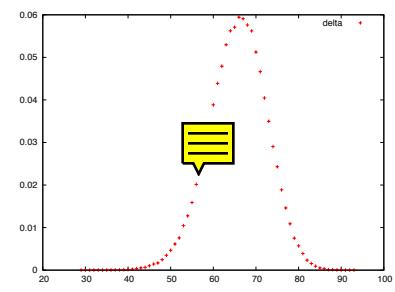
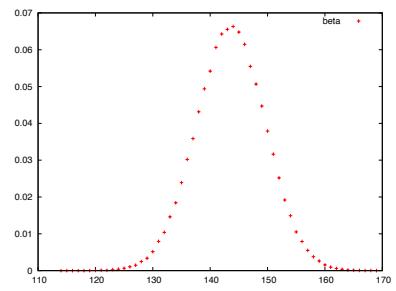
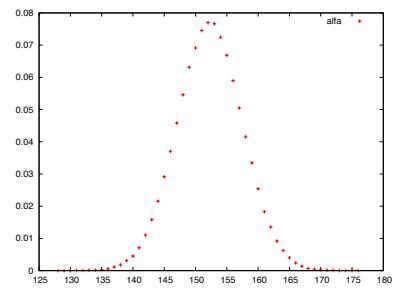
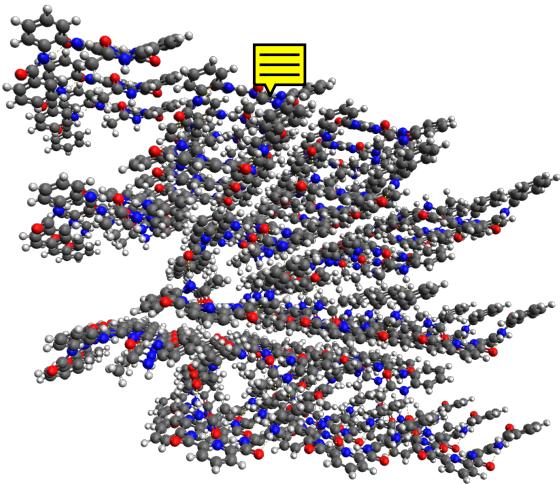
## Methods

### Molecular dynamics

Molecular dynamics (MD) simulations were made with GROMACS version 5.0.4 software<sup>1</sup>. Interactions of acetylfoldamer were modeled with OPLS-AA forcefield.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup>

In second group of simulations single foldamer in crystal structure conformation was placed in 46.1 nm<sup>3</sup> (diameter = 1.63 nm) dodecahedron periodic box and system maximum force was minimized below 500.0 kJ mol<sup>-1</sup> nm<sup>-1</sup> with the steepest descent method. Minimized acetylfoldamer in 46.1 nm<sup>3</sup> dodecahedron box was solvated by 462 chloroform<sup>5;6</sup> molecules. Total mass of the solvated system is 55646.158 Da. Maximum force of the solvated system was minimized below 250.0 kJ mol<sup>-1</sup> nm<sup>-1</sup> 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  $\mu$ s production MD simulation was done in 1 fs steps. Pressure was maintained at 1 atm with Parrinello-Rahman<sup>7;8</sup> barostat and system compressibility was set same as chloroform<sup>5;6</sup> (2.16 GPa<sup>-1</sup>). Temperature was coupled to 300 K with v-rescale thermostat. For short-range nonbonded interactions Verlet cutoff-scheme was used with 0.005 kJ n $\text{m}^{-1}$ s<sup>-1</sup> buffer. For long-range electrostatic interactions was applied the particle mesh Ewald (PME)<sup>9</sup> method with grid spacing of  $\text{nm}$  and fourth-order interpolation<sup>9</sup>. Snapshots were taken from simulations every 0.5 ps for analysis. Simulation of 1  $\mu$ s was done in methanol<sup>5;6</sup> 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 conformation<sup>10</sup> calculated with GROMACS gmx angle program. Obtained conformations were DFT optimized using Gaussian 09 program<sup>10</sup> at  $\omega$ B97D-X/6-311G(d,p) level of theory. The hydrogen bond distances were calculated with GROMACS gmx distance program for the NOE active atoms pairs as identified by Kortelainen et al<sup>3</sup>.

## Dihedral angle distributions



Figure

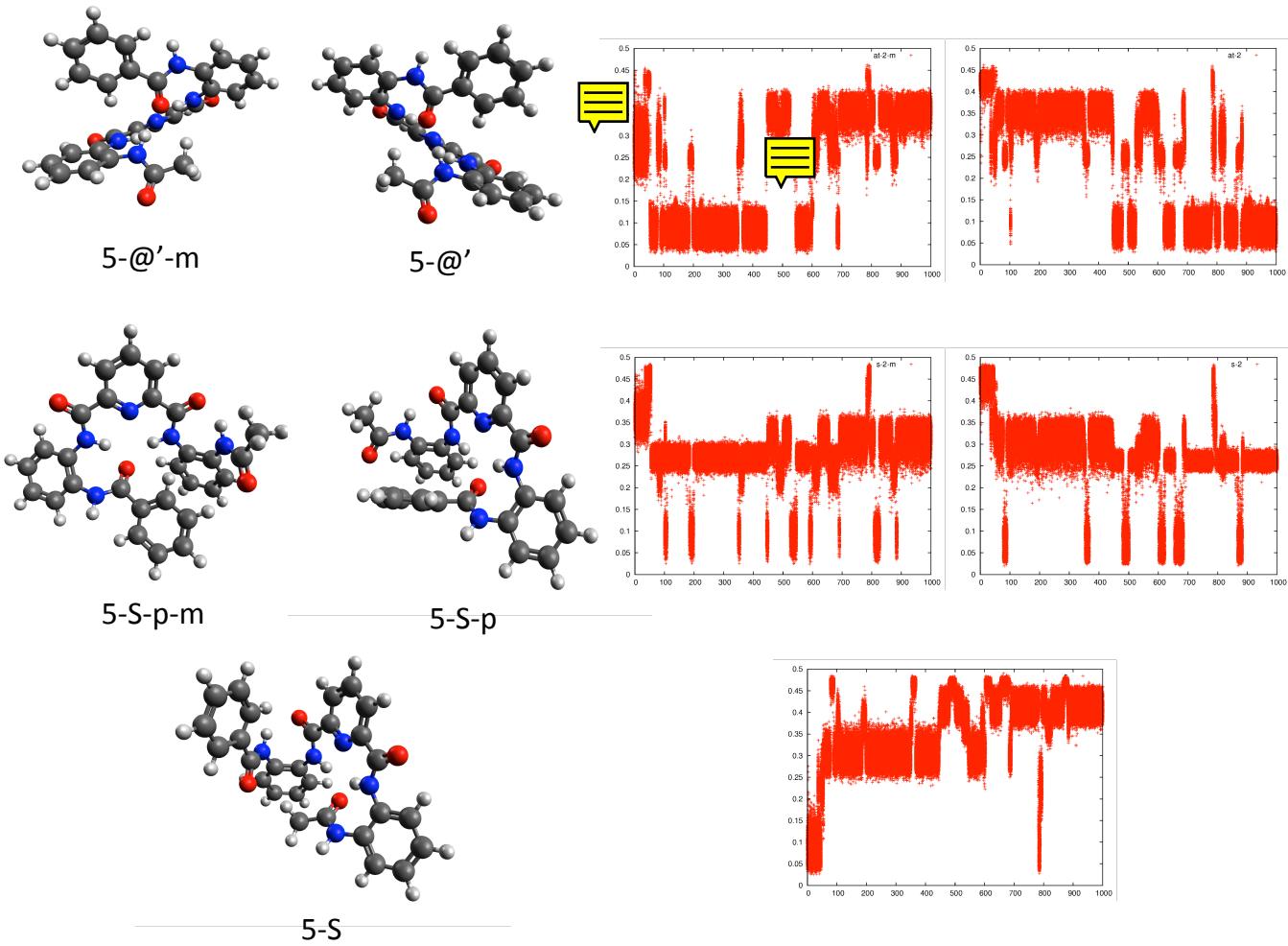


Figure 2:

## Results and discussion

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

Total of six different conformations were found with cluster analysis in chloroform simulation (Figure 2). The six conformations can be structurally divided in @- and S-conformations. Number 1 in all conformations means that acceptor oxygen is O, not O4 which is acceptor in conformations with number 2. With S-conformations, the acceptor oxygen O (5-S-1) or O4 (5-S-2 or 5-S-2-m) will only be bonded with hydrogens HN2 and HN4, while with @-conformations the oxygens O or O4 are also bonded with either hydrogen HN1 or HN5. @' conformations are like @, but O4 or O are bonded to methyl group and H2D (5-@'-1) or to hydrogens H3A and H2D (5-@'-2), while in helix-conformers these oxygens are only bonded to HN2 (5-helix) or HN4 (5-helix). Moreover the A-ring is positioned perpendicularly to D-ring. The conformation 5-@-2 also appears as a mirrored form (5-@-2-m). Their molecular forces are identical and therefore their total electronic energies and dipolemoments are also the same (Table 1). 5-@'-2 and 5-helix-p conformations are seen only in mirrored form. Even though the conformations 5-S-2 ja 5-S-2-m also seem to be mirrored at first, we can see that their torsional angles are twisted unsymmetrically and that is why their intramolecular forces also differ.

Conformation	E <sub>tot</sub> (Ha)	E <sub>totmc</sub> (Ha)	dipole moment	$\alpha$	$\beta$	$\gamma$	$\delta$
5-S-1	-1655.4576984	-1655.4576981	1.4363	156.063	-51.384	81.4	54.3
5-@-1	-1655.457294	-1655.4544988	8.4150	147.273	126.304	113.905	59.885
5-@-2	-1655.4557366	-1655.4557182	7.8949	167.7	144.6	-126.5	-132.5
5-@-2-m			7.8982	-167.6	-144.6	126.5	132.6
5-S-2	-1655.4611423	-1655.4612251	3.0724	-52.1	141.4	-131.1	-145.0
5-S-2-m	-1655.4595914		2.5523	48.5	-156.2	132.4	136.5
5-@'-1	-1655.4597942	-1655.459794	3.7225				
5-@'-2-m	-1655.4585488	-1655.458484	3.1939				
5-helix-p-m	-1655.4592239	-1655.457342	4.1760	176.404	136.871	119.597	130.441
5-helix	-1655.4622932	-1655.462293	2.0605	155.136	-154.729	52.332	50.053

Table 

from each other.

Acetylfoldamer favors completely different conformers in methanol and chloroform. As an unpolar solvent, chloroform doesn't bind with the oxygens on acetylfoldamer which makes appearance of @-conformers possible where either O or O4 oxygen is bonded with three peptide hydrogens. The 5-@-2 conformation folds into its mirrored conformation through the 5-S-2 ja 5-S-2-m conformations in chloroform. 

Polar methanol can form hydrogen bonds with peptide oxygens and hydrogens. This prevents oxygens to form a tight intramolecular bonding network like in @-conformers. This explains why the simulation with methanol clearly favors @'-conformers. There is also minor shifts to 5-helix-p-m conformation, in the cluster where 5-@'-1 conformer occurs. This is caused by the hydrogen bonds between acetylfoldamer and methanol. Methanol forms hydrogen bonds to O and HN1 destroying the bonding network of HN2-O-HN4 which enables the oxygen O4 to form a bond with HN4. There is also a minor appearance of 5-helix conformation in the cluster where the 5-@'-2 conformer is dominant. The 5-helix conformation is produced by the same way as with the shiftings to 5-helix-p conformer, by hydrogen bondings to methanol. In both methanol and chloroform solutions the conformation folds also back to starting 5-S-1 conformation few times. Maybe because there is no changes between mirror forms in methanol, there is no appearance of 5-S-2 conformation and only very minor changes to 5-S-2-m conformation. 

Previously the conformations of acetylfoldamer were found using Monte Carlo conformational analysis with OPLS-2005 force field (figure 4) CIT. These structures were DFT optimized with the  $\omega$ B97X-D/6-311++G(3df,3pd) level of theory. All the conformations that were found with this method, were also found with MD simulations. The energies of conformers from the MD simulation differ only slightly with all conformers except the 5-@-1 and 5-helix-p-m conformers, where the energy differences are more significant. Minimum energies of 5-@-1 and 5-helix-p-m conformers are lower in MD-simulation than the minimum energies obtained from the Monte Carlo analysis. This remark implies that the 5-helix-p-m is not actually mirrored conformation of 5-helix-p, but rather completely new conformation. Moreover 5-S-2-m conformation was only  found 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  $\text{CDCl}_3$  are listed in Table 2. The distance values in Table 2 are taken from the DFT optimized conformations found in MD simulation. The values of less than 5 Å show correlation that can be seen in the NOE spectrum. NOE-coupling between H1-HN5Also these two conformations are the only ones with the NOE couplings 



Table 2:

Table 3: H4C ja H5C puuttuu?

HN1-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1	5-helix	5-helix-p	5-@'-1	5-@'-2	
H2D	<b>7.6</b>	4.7	<b>7.9</b>	2.7	4.7	3.9	4.6	4.7	3.8	2.7
H3A	2.4	3.6	3.5	3.4	2.6	2.4	4.7	2.2	3.6	2.4
H3C	<b>7.3</b>	<b>9.4</b>	<b>9.7</b>	<b>5.9</b>	<b>8.0</b>	4.7	6.1	6.6	6.9	<b>5.9</b>
H4C	7.5	11.3	11.5	7.6	9.4	6.4	6.2	8.1	8.6	
H5C	6.8	11.3	11.4	7.7	9.5	7.2	7.6	8.6	8.4	
H4A	4.6	<b>5.4</b>	<b>5.3</b>	<b>5.3</b>	4.7	4.6	5.3	4.5	5.4	4.6
H6D	<b>6.5</b>	<b>5.9</b>	3.7	<b>5.1</b>	<b>8.3</b>	3.2	5.6	5.2	6.1	3.7
HN2	3.6	3.6	3.7	2.1	3.6	3.5	2.2	3.7	2.3	2.1
HN4	4.7	<b>5.9</b>	<b>6.0</b>	3.2	<b>5.4</b>	4.0	4.2	5.0	3.9	3.2
HN5	<b>7.1</b>	<b>7.3</b>	<b>7.7</b>	4.9	5.0	4.2	4.0	5.8	6.1	4.9
H3B	<b>7.5</b>	4.1	4.0	<b>6.5</b>	<b>7.0</b>	7.6	5.8	7.7	5.6	4.0
H4D	<b>9.4</b>	4.9	<b>6.8</b>	<b>6.3</b>	<b>7.1</b>	5.6	6.7	6.1	7.0	4.9
H5A	<b>5.7</b>	<b>5.8</b>	<b>5.8</b>	<b>5.8</b>	<b>5.7</b>	5.7	5.7	5.7	5.8	
H6A	<b>5.3</b>	4.7	4.7	4.8	<b>5.4</b>	5.4	4.7	5.4	4.6	
H6C	<b>5.8</b>	<b>9.4</b>	<b>9.4</b>	<b>6.3</b>	<b>8.1</b>	6.5	6.2	7.7	7.2	
H5B	<b>8.4</b>	<b>7.4</b>	<b>7.4</b>	<b>7.7</b>	<b>8.3</b>	8.1	6.4	8.8	7.0	
H4B	<b>8.9</b>	<b>6.4</b>	<b>6.3</b>	<b>7.7</b>	<b>8.4</b>	8.8	6.9	9.2	7.1	
H5D	<b>8.2</b>	<b>5.6</b>	4.3	<b>6.0</b>	<b>8.5</b>	4.8	6.6	5.9	7.3	
H3D	<b>9.1</b>	4.3	<b>8.2</b>	4.4	5.0	5.3	5.8	5.5	5.5	
Me	3.1	2.2	3.4	3.2	2.2	2.2	2.4	2.2	2.3	2.2

between HN1-H3C hydrogens. Furthermore the bonding between HN1-HN5 hydrogens is seen in 5-@-2 and 5-@-1. In the rest of the conformations, the shortest hydrogen bond distances of these hydrogen pairs are too great to be seen in NOE spectrum (figure 3), and because of that only 5-@-2, 5-@-2-m and 5-@-1 conformations explain the existence of these couplings. Therefore the conformation of the acetylfoldamer in chloroform solution will most likely be either in 5-@-2 or 5-@-1 conformation at some point. On the other hand, the distance between HN1-H3B and HN1-H4D hydrogens are always more than 6 Å in 5-@-2, 5-@-2-m and 5-@-1 conformations while the HN1-H4D coupling can be explained only with 5-S-2-conformer and HN1-H3B NOE coupling is possible for both 5-S-2-m and 5-S-2 conformations (figure 3). In conclusion both 5-S-2-m and 5-S-2 conformations are likely to be found in chloroform solution. Although acetylfoldamer was originally crystallized only in 5-S-1 conformation, the results show that the acetylfoldamer do not necessarily appear in 5-S-1 conformation while in chloroform solution. All the couplings shown in NOE spectrum can be identified with other conformations. Although none of the conformations include all the couplings, they all are presented at some point of the simulation.

Table 3 contains all the hydrogen couplings between HN5 and hydrogens found in experimental 1D NOE spectrum in  $\text{CDCl}_3$ . The coupling distances of the hydrogen HN5 to hydrogens H2D, H3C and HN4 are less than 5 Å with all the conformations. Conformations 5-@-2 and 5-@-1 methyl group hydrogens have direct NOE-coupling to HN5-hydrogen and 5-S-1 conformer have many vibrational states where HN5-Me distance is below 5 Å. This gives direct evidence that @-style conformers are presented in chloroform solution state of acetylfoldamer. Interestingly the coupling between HN5 and H5B is only present in 5-S-1 conformation, which has only minor population during the 1  $\mu\text{s}$  simulation. At the same time this coupling between hydrogens HN5 and H5B is only seen as a faint peak in the experimental NOE spectrum, which proves that rejection of the 5-S-1 conformer in the simulation is right assumption.

Table 4: HN5

HN5-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1
HN4	3.8	3.6	3.6	3.5	2.3
HN2	5.9	4.7	4.9	5.0	3.1
H2D	2.0	4.2	2.4	4.1	2.1
H6D	4.4	2.4	4.1	2.5	4.4
H3C	3.4	2.5	2.4	2.6	3.4
H3A	9.3	8.2	8.1	7.5	5.7
H4A	11.2	8.4	7.9	8.6	7.0
Me	4.3	7.3	8.5	7.2	4.9
H4D	5.9	5.8	5.7	5.8	5.8
H5A	11.2	7.4	6.7	8.4	7.1
H6A	9.3	6.0	5.6	7.0	5.8
H6C	4.8	5.3	5.3	5.3	4.9
H5B	4.2	7.1	7.5	6.9	6.5
H4B	6.4	8.5	9.0	8.2	7.7
H5D	5.9	4.5	5.6	4.6	5.9
H3D	4.4	5.7	4.5	5.7	4.4
H3B	7.3	8.1	8.6	7.7	7.0

Table 5: Hydrogens that are coupled to Me in experimental 1D NOE spectrum. The distance values are obtained from optimized DFT structures. The value represents the shortest distance to the corresponding hydrogen from any of the three hydrogens of methyl group.

Me-	5-S-1	5-S-2	5-S-2-m	5-@-2	5-@-1	shortest dist.
HN1	2.2	2.4	2.5	2.1	2.2	2.1
HN2	4.0	5.3	5.7	3.6	3.7	3.6
HN4	3.2	7.2	8.0	3.3	3.7	3.2
HN5	5.4	8.7	9.8	4.5	3.7	3.7
H3A	4.2	4.3	4.5	4.5	4.5	4.2
H2D	6.5	5.1	10.0	4.5	4.4	4.4
H6D	8.1	7.9	5.7	5.1	8.0	5.1
H3C	5.0	10.8	11.9	4.6	5.6	4.6
H4C	4.5	12.7	13.6	5.9	6.6	4.5
H5C	3.4	12.6	13.4	6.1	6.6	3.4
H6C	2.7	10.6	11.3	5.3	5.1	2.7
H3B	7.7	4.7	4.8	8.4	6.8	4.7

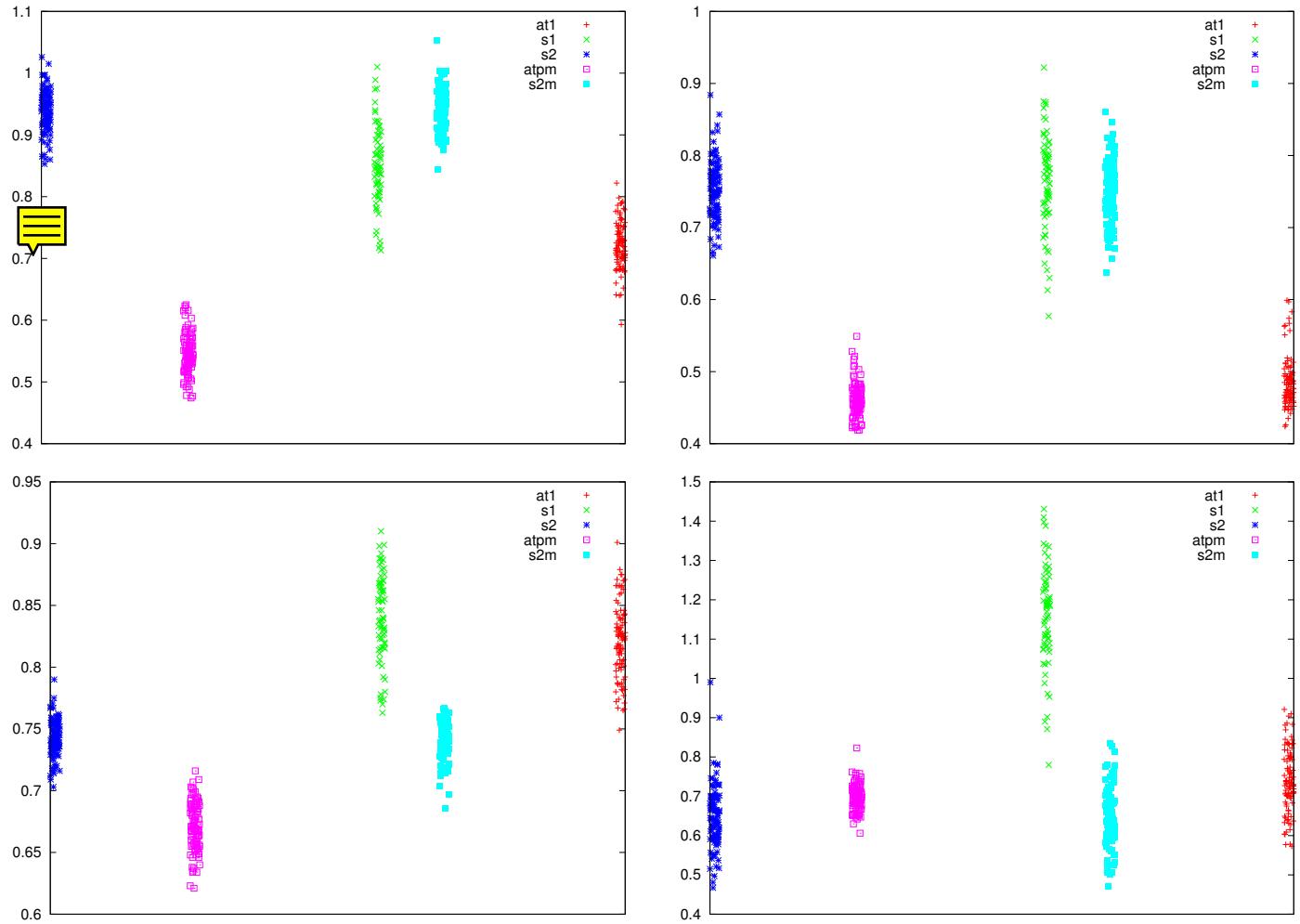


Figure 3: 5 ns snapshots for all conformations taken from simulation trajectory.

DFT-optimized conformers shortest distances between acetylfoldamers methyl group hydrogens and hydrogens which are presented in the experimental 1D NOE spectrum in  $\text{CDCl}_3$  are listed in Table 4. The NOE distances of Me to HN2 and HN4 are less than 5 Å in 5-S-1, 5-@-2 and 5-@-1 conformations. The couplings between Me-HN5 and Me-H2D are only present in 5-@-2 and 5-@-1 conformations so yet again these two conformations are proved to most likely to appear in soluted acetylfoldamer. Distance between hydrogens Me-H6D in minimum energy state is more than 5 Å in all the conformations, but there is a lot of states where distance is below 5 Å in conformations 5-@-2 and 5-S-2-m, which makes simulation match with the experimental NOE spectrum. The 5-S-1 conformation appears to be the only one where H4C, H5C and H6C are coupled to Me hydrogens. Additionally the Me-H3C hydrogen coupling is seen in both conformations 5-S-1 and 5-@-2. Also here we have the same kind of situation with hydrogen H6C as we had before with H6D. The distances between Me-H6D in the conformations 5-@-2 (5.3 Å) and 5-@-1 (5.1 Å) are both just around the value of 5.0 Å and are likely to explain the peak in the NOE spectrum along with the 5-S-1 conformation. Finally the Me-H3B coupling is only present in 5-S-2 and 5-S-2-m conformations and the distance values in all states in rest of the conformations are way too high to be seen in NOE.

DFT-optimized conformers shortest distances between acetylfoldamers methyl group hydrogens and hydrogens which are presented in the experimental 1D NOE spectrum in  $\text{CDCl}_3$  are listed in

Table 4. The NOE distances of Me to HN2 and HN4 are less than 5 Å in 5-S-1, 5-@-2 and 5-@-1 conformations while some vibrational states are present under 5 Å in 5-S-2 conformation, too. The couplings between Me-HN5 and Me-H2D are present in 5-@-2 and 5-@-1 conformations so yet again these two conformations are proved to most likely to appear in soluted simulation of acetylfoldamer. We also have to keep in mind that 5-S-1 conformer has some vibrational states where distances of Me-HN5 are below 5 Å although the value in the table is more than 5 Å. The coupling distance between hydrogens Me-H6D is more than 5 Å in all the conformations but then again the distances for some of the states in conformations 5-S-2-m and 5-@-2 are low enough to be seen in NOE spectrum. The 5-S-1 conformation appears to be the only one where H4C, H5C and H6C are coupled to Me hydrogens but also conformations 5-@-2 and 5-@-1 have enough vibrational states where the distance goes below 5 Å. Additionally the Me-H3C hydrogen coupling is seen straightaway in both conformations 5-S-1 and 5-@-2 and also parts of 5-@-1 conformation are seen in the spectrum. Finally the Me-H3B coupling is only present in 5-S-2 and 5-S-2-m conformations and the distance values in rest of the conformations are way too high to be seen in NOE.



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