

## Conformational analysis of oligomers of non-ionic thermoresponsive polymers containing amide groups

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*Dedicated to Acad. Bogdan Kurtev on the occasion of his 100<sup>th</sup> birth anniversary*

The conformations of oligomers of 2-isopropyl-2-oxazoline (**IPOZ**) and N-isopropylacrylamide (**NIPAM**) were studied with molecular mechanics. Preferred geometries and inherent energy balances were determined. The computed data present significant differences in the conformational distributions of oligomers of **IPOZ** and **NIPAM** with the same degree of polymerization (hexamers). Only a few conformations of **IPOZ-6** have most of the computed population, whereas more than hundred conformations of **NIPAM-6** are in equilibrium. Two orders of magnitude larger number of conformations belong to the lowest-energy cluster of **NIPAM-6** compared with **IPOZ-6**. The intramolecular hydrogen bonds in **NIPAM-6** are the decisive factor for its preferred conformations.

**Key words:** oligomers of thermoresponsive polymers; conformations; molecular mechanics; cluster analysis

### INTRODUCTION

The development of synthetic building blocks that can selectively interact in water with a given substrate or self-assemble to yield highly responsive functional materials is a topic of increasing significance. Water-soluble oligomers have acquired importance due to the demand for water-based instead of the traditional solvent-based technological processes [1]. The non-ionic thermoresponsive oligomers are building units of polymeric nanoparticles possessing thermosensitive core and functional outer shell that manifest *smart* behavior – the inner core of the aggregates is sensitive to temperature variations and can solubilize and deliver hydrophobic substances, mostly biologically active compounds (*e.g.* drugs) under external stimuli. Such *stimuli-sensitive* systems (due to changes in temperature, pH, solvent, light, *etc.*) may have macroscopic properties that could be controlled at the macroscopic level by modifying the structure and organization of the polymeric chain. Besides, in particular, some of them could be considered also as candidates for thermo-driven single-molecule motors [2] and many other advanced applications.

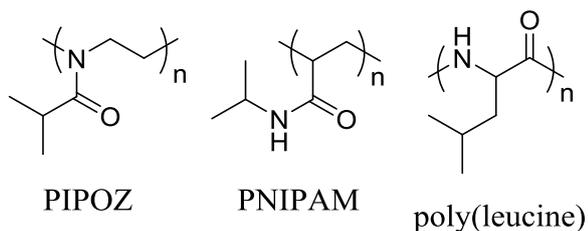
Most of the synthetic macromolecules become more soluble when heated. There are, however, water soluble polymers that separate from solution

upon heating (*inverse temperature-dependent solubility*) above the phase transition temperature (*lower critical solution temperature*, LCST). This phenomenon is explained to result from the balance between the enthalpic contribution from the energy stabilization due to hydrogen bonding of the polymer with the water molecules and the entropy gain of the system at higher temperature that outweighs the enthalpic preference at lower temperatures. Hydrogen bonding between the polymer and the water molecules lowers the free energy of dissolution. This effect becomes less important at higher temperature and accordingly, entropy effects become more important [1].

Polymers containing amide groups constitute the largest group among the thermoresponsive polymers. The most examined thermoresponsive polymers are poly (2-isopropyl-2-oxazoline) [**PIPOZ**] and poly(N-isopropylacrylamide) [**PNIPAM**], both isomeric to poly(leucine) (Fig. 1). The two polymers, **PIPOZ** and **PNIPAM**, could be considered as simplified, but appropriate models of a protein. Substantial difference between **PIPOZ** and **PNIPAM** from one side, and poly (leucine) from the other, is that poly(leucine) contains amide groups in the main chain and has nonpolar side-chain substituents; the main chain of **PNIPAM** is nonpolar with amide group in the side fragment, whereas **PIPOZ** is with polar backbone and amide group in the side chain. The amide groups of **PNIPAM** can be proton donors as well as proton

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acceptors, whereas the amide groups of **PIPOZ** can be only proton acceptors.



**Fig. 1.** Schematic structures of **PIPOZ**, **PNIPAM** and poly(leucine).

We report here results from molecular mechanics conformational search studies on the conformations of hexamers of 2-isopropyl-2-oxazoline (**IPOZ-6**) and N-isopropylacrylamide (**NIPAM-6**) with the aim to elucidate at atomistic level differences in the conformational distributions of the oligomers that may have effect on the particle's behavior at the macroscopic level. A dodecamer was studied also for one of the cases (**IPOZ-12**) in order to ascertain the effect of increasing the chain length on the preferred conformations of the oligomer.

Oligomers of different length (up to 50-units) of PNIPAM have been studied by molecular dynamics simulations below and above its LCST [3–7]. The expected behavior has been observed where the backbone folds onto itself above the LCST in order to minimize the amount of hydrophobic surface in contact with water [3, 7]. The decisive interactions in the isolated oligomer constitute an extensive intra-chain hydrogen bonding, responsible for the adoption of more compact structures. Isolated water molecules buried inside the polymer contribute to stabilize the conformation of chain fragments [3]. Computational studies on oligomers of PIPOZ are not known to us.

## COMPUTATIONAL DETAILS

Two problems are of particular importance when molecular mechanics is used for modelling conformations of large molecules: (i) the proper choice of a force field for modelling the system under study; (ii) reliable and effective conformational search of low-energy conformations when many degrees of freedom for intramolecular rotations determine the conformational flexibility of the molecule. The force field used in the present study is MMFF94 [8–14], whereas conformational search approach [15, 16], implemented in CONFLEX version 5.0 Rev. A.2 and version 6.89

[17] was applied for searching for low-energy conformations. The CONFLEX algorithm was specifically developed for searching conformations of macro-rings, but it has also an efficient module for stepwise rotations to generate new rotamers during the conformational search. Thirty rotatable bonds were detected for the two hexamers by the 'rotatable bond finder' procedure in CONFLEX, and twice as much for **IPOZ-12**. These were, accordingly, the bonds with rotations about which was searched for low-energy conformations and used also for conformation comparison when eliminating identical conformers. The default values of the options were used when the steric energies and the dihedral angles were compared in the redundancy test. A gradual increase to a search limit of 10.0 kcal mol<sup>-1</sup> determined the energy range from which the initial structures for the search were selected [15, 16]. The full-matrix Newton-Raphson minimization procedure was used. Electrostatic interactions were estimated with the value 1.0 of the dielectric parameter as recommended for the MMFF94 force field. The searches ended with 4546, 10280 and 14947 conformations stored with relative energies less than 25.0 kcal mol<sup>-1</sup>, for **IPOZ-6**, **IPOZ-12** and **NIPAM-6**, respectively.

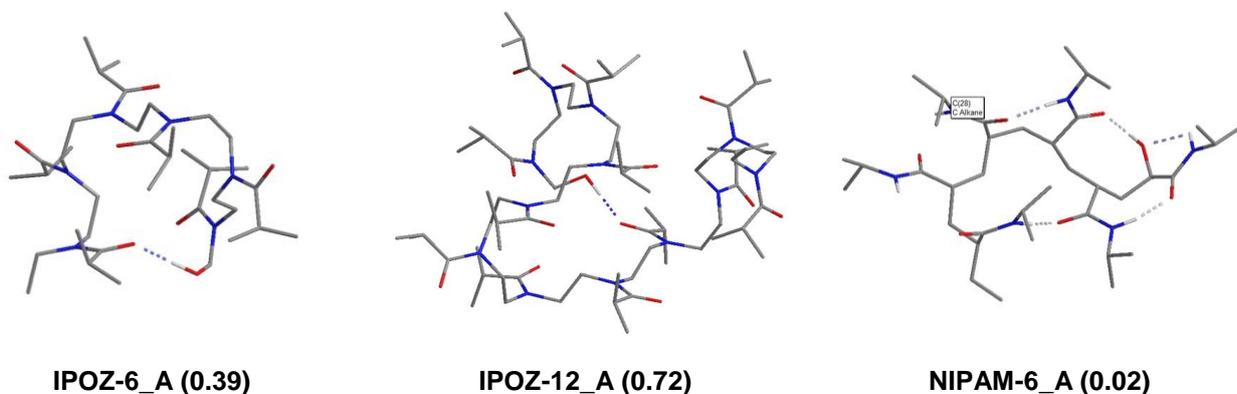
The complete sets of conformations (4552, 15378, 14947, respectively) were subjected to a cluster analysis [18]. The single linkage clustering method was used with torsional distance as similarity (distance) index, distance threshold 10.0°, and distance definitions (number of torsions for the clustering) 17, 34 and 10 for **IPOZ-6**, **IPOZ-12** and **NIPAM-6**, respectively. The main chain torsional angles were used for the clustering in all cases. Populations of conformations within a cluster and distribution (population) of clusters were estimated at 300 K following Boltzmann distribution. Computed data for some low-energy clusters are presented in Table S1 in Supplementary Data. An identity number has been given to each conformation designating the sequential number of appearance of a new conformation during the conformational search (Fig. S1 and Table S1).

## RESULTS AND DISCUSSION

The computed data are indicative for differences in the conformational distributions of the oligomers that originate from the inherent properties of the monomeric building blocks. Only one conformation was found for the monomeric unit of **IPOZ** whereas four conformations of **NIPAM-1** have the

**Table 1.** Conformational energies (in kcal mol<sup>-1</sup>) of oligomers of thermo-responsive polymers.

Energy range	Steric energy		Gibb's free energy	
	Number of conformations	Population	Number of conformations	Population
<b>IPOZ-1</b>				
1. 0.0 – 0.5	1	1.00	1	1.00
<b>IPOZ-6</b>				
1. 0.0 – 0.5	1	0.38	2	0.25
2. 0.5 – 1.0	0	-	4	0.17
3. 1.0 – 1.5	3	0.13	6	0.10
4. 1.5 – 2.0	6	0.12	18	0.13
5. 2.0 – 2.5	12	0.10	39	0.11
6. 2.5 – 3.0	25	0.10	67	0.08
7. 3.0 – 3.5	33	0.05	108	0.06
8. 3.5 – 4.0	42	0.03	83	0.04
9. 4.0- 10.0	3584	0.09	3457	0.06
<b>IPOZ-12</b>				
1. 0.0 – 0.5	1	0.72	2	0.51
2. 0.5 – 1.0	0	-	2	0.14
3. 1.0 – 1.5	0	-	3	0.12
4. 1.5 – 2.0	3	0.12	5	0.07
5. 2.0 – 2.5	3	0.06	9	0.06
6. 2.5 – 3.0	6	0.04	16	0.04
7. 3.0 – 3.5	6	0.02	20	0.02
8. 3.5 – 4.0	8	0.01	29	0.01
9. 4.0- 10.0	1451	0.03	1729	0.03
<b>NIPAM-1</b>				
1. 0.0 – 0.5	2	0.77	2	0.84
2. 0.5 – 1.0	2	0.20	1	0.11
3. 1.0 – 1.5	0	-	1	0.05
4. 1.5 – 2.0	1	0.02	0	-
<b>NIPAM-6</b>				
1. 0.0 – 0.5	10	0.14	8	0.07
2. 0.5 – 1.0	30	0.17	51	0.19
3. 1.0 – 1.5	76	0.20	100	0.19
4. 1.5 – 2.0	129	0.15	282	0.22
5. 2.0 – 2.5	236	0.12	433	0.14
6. 2.5 – 3.0	398	0.09	696	0.10
7. 3.0 – 3.5	595	0.06	855	0.05
8. 3.5 – 4.0	875	0.03	875	0.01
9. 4.0- 10.0	7790	0.04	8468	0.03



**Fig. 2.** Optimized geometries of the lowest-energy conformations of the most populated clusters of **IPOZ-6**, **IPOZ-12** and **NIPAM-6** (populations of conformers are given in parenthesis).

**Table 2.** Different energy contributions to the total steric energy of the lowest energy conformations of oligomers of thermo-responsive polymers (in kcal mol<sup>-1</sup>).

Energy term	Total steric energy					Steric energy per monomer unit		
	IPOZ-1	IPOZ-6	IPOZ-12	NIPAM-1	NIPAM-6	IPOZ-6	IPOZ-12	NIPAM-6
Stretch	1.3	11.9	24.1	1.0	9.6	2.0	2.0	1.6
Bend	4.4	26.2	53.6	1.3	15.8	4.4	4.5	2.6
Torsion	-3.1	-13.1	-24.6	-4.1	-10.1	-2.2	-2.1	-1.7
van der Waals	13.4	73.9	140.4	9.8	63.1	12.3	11.7	10.5
Electrostatic	-24.1	-149.9	-244.0	-27.6	-189.7	-25.0	-20.3	-31.6
Total steric	-7.7	-48.3	-45.0	-19.6	-110.2	-8.1	-3.7	-18.4

total of 97% population (Table 1). Accordingly, 50% of the steric energy population of **IPOZ-6** is given by four conformations, whereas the same population in **NIPAM-6** is distributed over 116 local minima conformations. The conformational preference in **IPOZ** shifts further to a strongly populated conformation with increasing the chain length. Thus, in comparison with **PIPOZ**, the **PNIPAM** oligomers should have better pronounced conformational changes upon temperature variations. At the same time, the larger number of hydrogen bonds in **NIPAM-6**, in comparison with **IPOZ-6** (Fig. 2), as well as the greater number of conformations representing the lowest-energy cluster **A** of **NIPAM-6** (Table S1 in Supplementary Data; 4764 for **NIPAM-6** vs. 55 for **IPOZ-6**), makes the **PNIPAM** chain less amenable to deformations upon external stimuli. The values for the number of clusters and the maximum size of cluster provide clear indications for the completely different conformational distributions in the two species (Table S1 in Supplementary Data).

The energy contributions per monomer unit, related to the intramolecular coordinates - stretch, bend and torsion, become more disadvantageous with increasing the number of monomer units, whereas the non-bonded terms (van der Waals and electrostatic) have more complex behavior (Table 2; compare data for monomers, **IPOZ-1** and **NIPAM-1**, with averages for monomer units of **IPOZ-6** and **NIPAM-6**, respectively). The van der Waals interactions become more favourable for **IPOZ-6**, and less advantageous in **NIPAM-6** with increasing the chain length and this originates from the differences in the hydrogen bonding patterns in the two systems – the hydrogen bonding is at the expense of worsening the van der Waals interactions (Fig. 2). Hydrogen bonding interactions in the MMFF94 force field are considered as being essentially electrostatic in nature and are modeled by adjusting key van der Waals and electrostatic parameters to better fit scaled intermolecular interaction energies and geometries obtained from HF/6-31G\* calculations [9].

The electrostatic energy contributions manifest trend opposite to the van der Waals interactions, in a way as the contributions from the two intramolecular interactions balance each other. Additivity of the contributions from different energy terms is evident from comparing the steric energy components of **IPOZ-6** and **IPOZ-12** (Table 2). The **IPOZ-6** and **NIPAM-6** have substantially different conformational distributions and this may reflect on differences in flexibility in water of the two polymeric chains.

Fig. 2 presents the lowest-energy conformations of the most populated clusters (**A**) of the three oligomers. The lowest-energy local minima of some other less populated clusters are given in Fig. S1 in the Supplementary Data. The end OH group in the most populated conformation of cluster **A** (39%) of **IPOZ-6**, **IPOZ-6\_A**, closes 21-membered hydrogen bonded ring with the carbonyl at the other end of the chain. All side fragments point outwards from the macroring thus being exposed for interaction with the solvent molecules. The macroring presents opened cavity. The representative conformations of clusters **B** and **C** closely resemble the one of cluster **A** (21-membered hydrogen bonded rings), the macroring in **B** being more elongated. 18-ring with the carbonyl of the 5<sup>th</sup>-monomer unit is formed in cluster **D**. Clusters **E**, **G**, **I** and **J** are similar to cluster **A** with regard to the type and size of the 21-membered hydrogen bonded ring. A 15-membered ring is formed with the carbonyl of the 4<sup>th</sup> monomer unit in cluster **F**, with a side fragment to the macroring composed of two monomer units. The last presented cluster, **IPOZ-6\_K** is similar to cluster **D**. There is clearly expressed tendency for forming the largest possible hydrogen bonded macroring – the most favourable appears the largest 21-membered ring, next comes the 18-membered ring, followed by a 15-membered ring, representing hydrogen bonding of the end hydroxyl group with the carbonyls of the 6<sup>th</sup>, of the 5<sup>th</sup> and of the 4<sup>th</sup> monomer units, respectively.

How changes the situation when increasing the chain length with additional six monomer units, **IPOZ-12**? Enhances the population of the preferred cluster **A** - 72% population is computed for the lowest-energy conformation. The backbone acquires helix-like form with a 30-membered hydrogen bonded ring closed by O—H ... O=C hydrogen bond between the end hydroxyl and the carbonyl of the 9<sup>th</sup> monomer unit. Three monomer units constitute a side fragment to the macroring. The geometries of the conformations of clusters **B** and **C** are also characterized by the presence of 30-membered hydrogen bonded rings. Completely different geometry presents cluster **D**. The O—H ... O=C hydrogen bonding is with participation of the carbonyl of the 7<sup>th</sup> monomer unit and the side fragment contains five monomer units bent over the macroring. The overall shape of the macroring is boat-like.

Two kinds of hydrogen bonds can be formed in the model structure of the **NIPAM** hexamer - O—H ... O=C and N—H ... O=C. Thus variety of hydrogen bonds could be formed that determine large manifold of different clusters. The highest computer population of a cluster is less than 5%. Five hydrogen bonds characterize cluster **A**. The 1<sup>st</sup> and the 5<sup>th</sup> monomer units close 14-membered N—H ... O=C hydrogen bonded ring, which is further stabilized by additional two N—H ... O=C bonds between the 3<sup>rd</sup> and the 4<sup>th</sup>, and the 5<sup>th</sup> and the 6<sup>th</sup> monomer units, plus two consecutive N—H ... O—H ... O=C bonds with participation of the side chains of the 4<sup>th</sup> and the 6<sup>th</sup> monomer units and the end hydroxyl group. As a result, two 8-membered hydrogen bonded rings are formed. 14-membered hydrogen bonded ring is formed also in cluster **B**. Five more hydrogen bonds are present that constitute interrelated system connecting residues 2-3-4-6-5. The 4<sup>th</sup> and the 6<sup>th</sup> monomer units here are also connected by N—H ... O—H ... O=C bonds. Thus in addition to the 14-membered ring are formed one 9-membered, three 8-membered and one small 5-membered rings, *i.e.* in comparison with cluster **A**, one 8-membered ring is present in addition. Cluster **C** displays extended backbone stabilized by six N—H ... O=C hydrogen bonds. Three of them are between 1,3-monomer units closing three ten-membered rings. The end OH-group participates in a 9-membered O—H ... O=C ring with the 3<sup>rd</sup> residue. 8-membered ring (N—H ... O=C) is formed between the first and the second monomer units.

## CONCLUSION

We examined with the aid of molecular mechanics the conformations of oligomers of 2-isopropyl-2-oxazoline (**IPOZ-6**, **IPOZ-12**) and N-isopropylacrylamide (**NIPAM-6**) with the purpose to gain insight into their preferred geometries and inherent energy balances that may have effect on the particle's behavior at the macroscopic level. The computed data are indicative for significant differences in the conformational distributions of oligomers of **IPOZ** and **NIPAM** with the same degree of polymerization. Only a few conformations of **IPOZ-6** have most of the computed population whereas more than hundred conformations of **NIPAM-6** are in equilibrium. Two orders of magnitude larger number of conformations belongs to the lowest-energy cluster of **NIPAM-6** compared with **IPOZ-6**. The larger number of intramolecular hydrogen bonds in **NIPAM-6** is the decisive factor for its preferred conformations, and determines most probably different responses of the two molecules when merged in water.

The intrachain hydrogen bonds in PNIPAM will greatly influence the mode of solution-dissolution process in PNIPAM aqueous medium below and upon phase transition temperature, which leads to crucial difference with PIPOZ [19].

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Electronic Supplementary Data available here. 

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## КОНФОРМАЦИОНЕН АНАЛИЗ НА ОЛИГОМЕРИ НА НЕЙОНОГЕННИ ТЕРМОЧУВСТВИТЕЛНИ ПОЛИМЕРИ СЪДЪРЖАЩИ АМИДНИ ГРУПИ

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(Резюме)

С молекулна механика са изследвани конформациите на олигомери на 2-изопропил-2-оксазолин (**ИРОЗ**) и N-изопропил акриламид (**НИРАМ**). Определени са предпочетени геометрии на конформери и енергетични баланси на вътрешномолекулни взаимодействия. Изчислените данни представят значителни разлики в конформационните разпределения на олигомери на **ИРОЗ** и **НИРАМ** с една и съща степен на полимеризация (хексамери). Изчислената конформационна населеност при **ИРОЗ-6** е свързана с малък брой структури, докато над сто конформации са в равновесие при **НИРАМ-6**. В сравнение с **ИРОЗ-6**, на два порядъка по-голям брой конформации определят клъстера с най-ниска енергия на **НИРАМ-6**. Вътрешномолекулните водородни връзки са определящ фактор за предпочетеността на конформациите на **НИРАМ-6**.