Structural Dynamics of Neighboring Water Molecules of N-Isopropylacrylamide Pentamer

Kenee Kaiser S. Custodio, Gil C. Claudio, and Ricky B. Nellas*

ABSTRACT: Poly(N-isopropylacrylamide) (PNIPAM) is a popular polymer widely used in smart hydrogel synthesis due to its thermo-responsive behavior in aqueous medium. Aqueous PNIPAM hydrogels can reversibly swell and collapse below and above their lower critical solution temperature (LCST), respectively. The present work used molecular dynamics simulations to explore the behavior of water molecules surrounding the side chains of a NIPAM pentamer in response to temperature changes (273–353 K range) near its experimental LCST (305 K). Results suggest a strong inverse correlation of temperature with water density and hydrophobic hydration character of the first coordination shell around the isopropyl groups. Integrity of the first and second coordination shells is further characterized by polygon ring analysis. Predominant occurrence of pentagons suggests clathrate-like behavior of both shells at lower temperatures. This predominance is eventually overtaken by 4-membered rings as temperature is increased beyond 303 and 293 K for the first and second coordination shells, respectively, losing their clathrate-like property. It is surmised that this temperature-dependent stability of the coordination shells is one of the important factors that controls the reversible swell-collapse mechanism of PNIPAM hydrogels.

1. INTRODUCTION

Poly(N-isopropylacrylamide)-based hydrogel (PNIPAM) is one of the most popular classes of hydrogels and has been used in various material fabrications because of its thermo-responsive property.1 Hydrogels of this class exhibit large volume phase transition at a specific temperature called the lower critical solution temperature (LCST). This property of PNIPAM hydrogels has been exploited in several applications such as in catalytic activity control,2 drug delivery and monitoring,3 novel compound synthesis,4,5 heavy metal sensing,6,7 and tissue engineering.8 At temperatures below the LCST, PNIPAM hydrogel swells to its maximum volume in aqueous solutions brought upon by the absorption of a relatively huge amount of water molecules. This stable swollen state collapses when the temperature is raised above its LCST. Upon collapse, the gel volume is observed to decrease dramatically as most of the absorbed water is removed from the gel’s interior. This phenomenon has been initially studied in an earlier work as the radius of gyration and hydrodynamic radius of the swollen PNIPAM hydrogel abruptly drop as the temperature increases past the LCST at 305 K.9

The thermo-responsive behavior of PNIPAM is not exclusive to cross-linked hydrogels. It was previously found that even single chain PNIPAM oligomers exhibit the same behavior at about the same temperature,10 signifying the independence of LCST on the degree of cross-linking of PNIPAM hydrogels. This provides a convenient way to study the LCST phenomenon using linear PNIPAM oligomers.

The role of water in the thermo-responsive behavior of PNIPAM is believed to be an integral part in understanding LCST. Interestingly, among popular pure solvent systems, only water exhibits LCST with PNIPAM.11 Thus a great interest is drawn towards the hydration behavior of water molecules in aqueous NIPAM pentamer.

Several theories attempting to explain the LCST phenomenon in PNIPAM hydrogels have been established.12 Most of these consider the hydrophobic interaction of the isopropyl group and the surrounding water molecules. In this study, this hydrophobic interaction is affirmed and the effect of temperature is also determined. Considering that the isopropyl group is directly bonded to the hydrophilic amide group in the side chains, results obtained in the present work is a consequence of the hydrophobic–hydrophilic coupling in the PNIPAM side chain.

Hydration of nonpolar groups in solutes is claimed to promote the formation of clathrate-like structures in water.13 These structures are responsible for the favorable hydration of these nonpolar groups. Consequently, their stability becomes an important factor in keeping a favorable interaction between these groups and water. For PNIPAM hydrogels, the
interaction of water with the isopropyl groups may provide insights on their thermo-responsive behavior.

In this work, we have used molecular dynamics to explore the temperature-dependent behavior of water molecules around the side groups of a NIPAM pentamer. We have studied the hydrogen bonding network of water around the isopropyl groups to probe clathrate-like behavior.

In one of the recent studies on PNIPAM oligomers, the effect of the polymer chain length has been studied in response to temperatures below and above the LCST. It was found that changes in the local density of water near the polymer significantly varies with chain length. Specifically, the 30-mer PNIPAM exhibits more pronounced changes in water density compared to 3-, 5-, and 10-mer oligomers. However, we have noted that one of the effects of using longer chain oligomers is the increase in the intramolecular interactions at temperatures higher than the LCST due to folding. This decreases the effective surface area of the polymer−water interface resulting to a decrease in the local water density. In the present study, we focused solely on the structural dynamics of water molecules near the PNIPAM−water interface. To amplify the observed behavior of water, the interface is maximized by using a short chain oligomer of PNIPAM and applying consistent conformational bias towards the coil state at all temperatures around the LCST.

2. RESULTS AND DISCUSSION

2.1. Water Density around the NIPAM Pentamer Side Chains. Water densities surrounding the side chains of NIPAM pentamer show possible first and second coordination shells around the isopropyl groups, denoted by characteristic water oxygen atom peaks at 0.5 and 0.75 nm in its \( g(r) \) plot with respect to the isopropyl carbon atom (Figure 1a). The peaks for the water hydrogen atoms appear at almost the same radial distances, implying that a significant number of O−H vectors of water molecules are oriented along respective coordination shells. This O−H vector orientation signifies the hydrophobic interaction between water and the isopropyl groups.

Peaks found at the \( g(r) \) plot of water molecules around the amide nitrogen atoms (Figure 1b) show relatively weak intensities at 0.3 and 0.35 nm for the water oxygen and hydrogen atoms, respectively. This arrangement of the first peaks is consistent with the hydrogen bond donor capability of the N−H bond of the amide group, where the oxygen of water is expected to be closer to the nitrogen than the water hydrogen atoms. However, the weak intensities denote that this N−H···O−H hydrogen bond between the amide N−H and water oxygen does not persist over time and may be considered weak and less stable.

The \( g(r) \) plot for water with respect to the amide oxygen atoms (Figure 1c) also shows consistent hydrogen bond acceptor tendency. The nearest peaks for the \( g(r) \) of water oxygen and hydrogen atoms are found at around 0.3 and 0.2 nm.
nm with respect to the amide oxygen atoms of the NIPAM pentamer, respectively. This suggests that the carbonyl oxygen of the amide group forms C=O···H–O hydrogen bond with water. The 0.1 nm difference in distance between the first peaks implies that the water O–H vectors are oriented directly towards the amide oxygen. The sharp peaks strongly suggest that the C=O···H–O hydrogen bond is strong and stable.

Water oxygen g(r) peaks are found at the same radial distances at different temperatures within the 273–353 K range as shown in Figure 1d–f. However, a decreasing trend in the intensities with increasing temperature is observed. This trend implies that increasing the temperature decreases the ordering of the coordination shells around the isopropyl groups as well as the destabilization of the hydrogen bonds formed between water and the amide groups.

2.2. Isopropyl–Water Interface. The orientation of water molecules in the coordination shells around the isopropyl groups is determined by measuring the angle formed between the O–H bond vectors of water molecules and the O–C vector formed by the water oxygen atom and the isopropyl carbon atom (see Supporting Information). Figure 2a shows that only the interfacial water (0.3–0.6 nm) molecules exhibit preferred orientation. Notable peaks are found at cos(θ) values −0.925 and 0.400, corresponding to 157.7 and 66.4° respectively. The latter is consistent with the g(r) findings in Figure 1a where, compared to oxygen, the peak for the hydrogen atoms is slightly closer to the isopropyl carbon. The former, on the other hand, implies that the interfacial water molecules have O–H bonds that are oriented away from the surface of the isopropyl groups. These orientations conform with an earlier work on the hydration of hydrophobic surfaces.15

Peak heights in the angular parameter distribution provide quantitative comparison on the extent of the hydrophobic character of hydration. Temperature dependence of the degree of hydrophobic hydration is observed from the overlay of 0.3–0.6 nm cos(θ) distributions at different temperatures (Figure 2b). Decreasing trend of the peak heights with increasing temperature implies a decrease in the hydrophobic character of the first coordination shell of the isopropyl groups of the NIPAM pentamer side chains.

2.3. Polygon Distribution in the Water Network. Determination of hydration behavior using angular order parameter is shown to be limited only at the interface of isopropyl and water. Here we used polygon distribution analysis to characterize both the first and second coordination shells of the isopropyl groups of NIPAM pentamer. Analysis of the hydrogen bonding network of water molecules surrounding the isopropyl groups (Figure 3) shows predominance of non-

![Image](https://dx.doi.org/10.1021/acsomega.9b02898)

**Figure 3.** Representative configurational snapshot of the hydrogen bonding network of water forming polygon rings near the NIPAM pentamer within 0.6 nm from isopropyl carbon atoms. Water molecules (red and white lines) form hydrogen bonds (green broken lines) within the water HB network near the NIPAM pentamer (ball and stick structure).

short-circuited polygon rings with sizes n = {4, 5, 6}. Initially at 273 K, the number of occurrences for pentagons are higher than both the quadrilaterals and hexagons (Figure 4). As previously mentioned, this distribution of polygon rings is consistent with the clathrate-like behavior of water surrounding large hydrophobic surfaces.16

Increasing the temperature decreases the total number of occurrences of all three polygon rings in the first and second coordination shells (Figure 4a,b, respectively). However, pentagons apparently decrease at a higher rate than the quadrilaterals as temperature is increased. As a result, the number of pentagons start to dip lower than the quadrilaterals at 303 and 293 K for the first and second coordination shells, respectively. This implies that both coordination shells lose their clathrate-like behavior at these temperatures. Furthermore, the second coordination shell loses this property at a lower temperature than the first coordination shell. This appears as if the shells are sequentially destabilized, where the outer shell is peeled off first followed by the inner shell. Incidentally, the temperature limit of the clathrate-like behavior of the first coordination shell is close to the experimentally determined LCST of PNIPAM hydrogels at 305 K, showing a good correlation with its thermo-responsive behavior. Considering that this clathrate-like behavior is responsible for the stable hydration of hydrophobic surfaces, it is possible that losing this property may cause unstable hydration of the PNIPAM side chains ultimately leading to the collapse of the hydrogel.

3. CONCLUSIONS

PNIPAM hydrogels have been one of the most exploited material when it comes to smart hydrogels due to their thermo-responsive property. In this study, we have explored the behavior of water molecules interacting with NIPAM pentamer side chains in an attempt to discover possible insights on the LCST phenomenon. Molecular dynamics simulations were performed for an aqueous NIPAM pentamer at different temperatures around the experimental LCST.
Analyses show the presence of two coordination shells surrounding the isopropyl groups, where the densities of these shells decrease with increasing temperature. Orientation of water molecules in the first coordination shell signified consistency with the hydration of hydrophobic surfaces, also exhibiting a decrease in the hydrophobic character of the coordination shell as temperature is increased. Polygon distribution analyses further characterized the coordination shells and were shown to possess a clathrate-like behavior at lower temperatures, but subsequently losing it upon breaching a temperature threshold. This clathrate-like behavior may be responsible for the stability of the hydrophobic surface of the isopropyl group at temperatures below the LCST. It is surmised that the LCST is a threshold at which the clathrate-like behavior of the coordination shells can persist before the PNIPAM hydrogel collapses on itself. This supports the previous results done by Sumi and Koga\(^1\) where solvent-induced interactions play a dominant role in the stability of the unfolded state of chignolin, a protein having both hydrophilic and hydrophobic interactions in its native folded state.\(^1\) The findings presented in this work can provide insights on both the understanding of LCST in PNIPAM hydrogels as well as the intricacies of solvent behavior in studying stimuli-responsive properties of smart polymer hydrogels.

### 4. METHODS

#### 4.1. Computational Details

In this study, a model of NIPAM pentamer was prepared using the atom type format provided by the OPLSAA forcefield.\(^\)\(^1\)\(^9\) It was initially solvated with 2161 TIP4P water molecules in a \(4.0 \times 4.0 \times 4.0\) nm\(^3\) cubic box. MD simulations were performed using GROMACS v5.0.6.\(^2\)\(^0\) Leapfrog algorithm\(^2\)\(^1\) for integrating Newton’s laws of motion was used in all simulations. Potentials arising from bonded and nonbonded interactions were calculated using OPLSAA forcefield.\(^1\)\(^9\) All bonding interactions were constrained using the LINCS\(^2\)\(^2\) algorithm. Cut-off scheme was set to Verlet.\(^2\)\(^3\) Short range Coulombic and van der Waals interactions were cut off at 1.1 nm. A conformational bias towards the coil state was applied on the pentamer by adding non-bonded weak harmonic potentials between adjacent monomer side chains.

This increases the interfacial surface area between the monomer side chains and water.

Periodic boundary conditions were applied in the three Cartesian coordinate axes. Long range interactions were treated using the particle mesh Ewald method.\(^2\)\(^4\) Temperature and pressure coupling were performed by applying velocity rescaling\(^2\)\(^5\) and Parrinello–Rahman\(^2\)\(^6\) methods, respectively. Timestep was set at 1 fs and configurations were recorded every 0.5 ps. The simulation box was pre-equilibrated at 1 bar and 500 K for 10 ns. This temperature is significantly higher than the experimental PNIPAM LCST and was selected to set an initial coil conformation of the pentamer as the starting configuration. Velocity generation was turned off after retrieval of coordinates of the last snapshot of the pre-equilibration. This snapshot was used as the starting configuration for 50 ns equilibration at nine temperatures (273, 283, 293, 303, 313, 323, 333, 343 and 353 K) under isothermal isobaric (NPT) conditions. Trajectory sampling for analysis was done for the last 10 ns of each of the 50 ns simulations.

#### 4.2. Methods of Analysis

##### 4.2.1. Radial Distribution Analysis

Interaction of water molecules with the NIPAM pentamer side chains were characterized by measuring radial distribution functions,\(^2\)\(^7\) \(g(r)\), of the oxygen and hydrogen atoms of water molecules with respect to atoms of interest which includes the amide nitrogen, amide oxygen, and isopropyl carbon atoms.

##### 4.2.2. Angular Order Parameter

Angles formed between the water \(\text{O}–\text{H}\) vectors with respect to the vector connecting the water oxygen atom to the isopropyl carbon atoms are obtained. From here, we monitored distributions of these angles to measure the degree of hydrophobic hydration as implemented in a previous work\(^2\)\(^8\) where these \(\text{O}–\text{H}\) vectors tend to orient tangentially to the solute surface along the interface while the others are pointed away from the solute (see Supporting Information).

##### 4.2.3. Polygon Analysis of the Hydrogen Bond Network

Structures formed by water molecules in the hydrogen bond network may be used to probe hydration behavior. One of these structures are polygon rings formed by water molecules connected through hydrogen bonding. We have used the hydrogen bonding parameters from a theoretical study on

**Figure 4.** Polygon distribution profile of the water network around the isopropyl group of NIPAM pentamer within 273–353 K range. Polygon ring counting from 20,000 frames (10 ns trajectory) showed polygon sizes with \(n = \{4, 5, 6\}\) the most dominant. Decreasing trend in the total number of occurrences of each polygon size is observed for both the first (a) and second (b) coordination shells of the isopropyl groups.
hydrogen bond dynamics in water. In an earlier work, the number of rings formed was used to describe both hydrophobic and hydrophilic type of hydration. The distinction was made between these two types of hydration based on the predominance of pentagons in its network. Pentagons are indicative of a clathrate-like behavior, characteristic of a favorable hydrophobic hydration. On the other hand, hydrophilic hydration is observed when the number of pentagon occurrences becomes less than the quadrilaterals. This distinction serves as a basis in determining clathrate-like behavior of water in the coordination shells.

The hydration behavior of the neighboring water molecules of the NIPAM pentamer is probed by counting occurrences of non-short-circuted rings within hydration shells surrounding the isopropyl groups. To test for clathrate-like behavior, a ring is only counted if the difference between radial distances from the isopropyl carbon atoms of the nearest and farthest water oxygen atom is less than 0.2 nm. This is to ensure that the faces of the rings counted are oriented towards the isopropyl group. The radial distances of the rings are measured by calculating the distance of the geometric center of each ring with respect to the nearest isopropyl carbon atom of the NIPAM pentamer.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02898.

Computational model of the solvated NIPAM pentamer, simulation parameters using GROMACS, determination of radial distribution function of water molecules using GROMACS, definition of the angular order parameter and its determination, and detailed algorithm for ring detection using the brute force method (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
Ricky B. Nellas – University of the Philippines Diliman, Quezon City, Philippines; Phone: +63 2 981 8500 loc 3652; Email: rbnellas@up.edu.ph; Fax: +63 2 920 5432

**Author Notes**

Kenee Kaiser S. Custudio – University of the Philippines Diliman, Quezon City, Philippines; Email: ckcustudio@up.edu.ph

Gil C. Claudio – University of the Philippines Diliman, Quezon City, Philippines

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b02898

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