1-1-2017

Aggregation of cyclic polypeptoids bearing zwitterionic end-groups with attractive dipole-dipole and solvophobic interactions: A study by small-angle neutron scattering and molecular dynamics simulation

Pu Du

Louisiana State University

Ang Li

Louisiana State University

Xin Li

Louisiana State University

Yueheng Zhang

Tulane University

Changwoo Do

Oak Ridge National Laboratory

See next page for additional authors

Follow this and additional works at: https://digitalcommons.lsu.edu/chemistry_pubs

Recommended Citation

Du, P., Li, A., Li, X., Zhang, Y., Do, C., He, L., Rick, S., John, V., Kumar, R., & Zhang, D. (2017). Aggregation of cyclic polypeptoids bearing zwitterionic end-groups with attractive dipole-dipole and solvophobic interactions: A study by small-angle neutron scattering and molecular dynamics simulation. *Physical Chemistry Chemical Physics*, 19 (22), 14388-14400. <https://doi.org/10.1039/c7cp01602f>

This Article is brought to you for free and open access by the Department of Chemistry at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.

Authors

Pu Du, Ang Li, Xin Li, Yueheng Zhang, Changwoo Do, Lilin He, Steven W. Rick, Vijay T. John, Revati Kumar, and Donghui Zhang

Aggregation of Cyclic Polypeptoids bearing Zwitterionic End-groups with Attractive Dipole-Dipole and Solvophobic Interactions: A Study by Small-Angle Neutron Scattering and Molecular Dynamics Simulation

Pu Du[†], Ang Li[†], Xin Li[†], Yueheng Zhang[§], Changwoo Do[‡], Lilin He[‡], Steven W. Rick^{††}, Vijay T. John[§], Revati Kumar^{*†} and Donghui Zhang^{*†}

[†]Department of Chemistry and Macromolecular Studies Group, Louisiana State University, Baton Rouge, Louisiana 70803, United States.

[§] Department of Chemical and Biomolecular Engineering, Tulane University, New Orleans, Louisiana 70118

^{††}Department of Chemistry, University of New Orleans, New Orleans, Louisiana 70118

[‡]Biology and Soft Matter Division, Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States.

Corresponding authors: Revati Kumar (revatik@lsu.edu) and Donghui Zhang (dhzhang@lsu.edu)

ABSTRACT

Aggregation behavior of cyclic polypeptoids bearing zwitterionic end-groups in methanol has been studied using a combination of experimental and simulation techniques. The data from SANS and cryo-TEM indicate that the solution contains small clusters of these cyclic polypeptoids, ranging from a single polypeptoid chain to small oligomers, while the linear counterpart shows no cluster formation. Atomistic molecular dynamics simulations reveal that the driving force for this clustering behavior is due to the interplay between the effective repulsion due to the solvation of the dipoles formed by the charged end-groups in each polypeptoid chain and the attractive forces due to dipole-dipole interactions and the solvophobic effect.

I. INTRODUCTION

Cluster formation is a common phenomenon that has been widely observed for solutions containing colloidal particles,^{1, 2} biomacromolecules (e.g., proteins³ and DNAs)⁴ as well as synthetic polymers. Clusters can exist in various morphologies and are often precursors to glass or gel formation.^{2, 5-7} Thus, cluster formation has significant implications for the practical uses of these solutions in the areas of food products, agricultural products and biopharmaceuticals.

The mechanism of cluster formation often varies and depends on the nature of the specific solutes in the solution and the interplay of various contributing forces. To form stable finite clusters, the presence of competing interactions is often required.^{2, 6, 8} The attractive interaction promotes the formation and growth of clusters, whereas the repulsive interaction will arrest and prevent indefinite cluster growth and further aggregation. The balance of these forces will lead to stable clusters consisting of finite number of particles. Colloidal particles (CPs) with short range attractive interaction and long range repulsive interaction (SALR) have been shown to form stable clusters with specific size and size distribution determined by the thermodynamics.⁷⁻¹⁰ Interestingly, it has also been shown that CPs with only attractive interaction (*e.g.*, depletion force) can also form stable clusters, suggesting that details of attractive mechanism are important.¹¹ With low attractive potentials, the clusters can bump into each other but quickly diffuse away from each other before they can reorganize and merge into larger clusters, thus allowing stable small clusters to exist. Even more intriguingly, theoretical work has indicated that particles with purely repulsive interactions can also form finite clusters, stabilized by a free energy competition due to the intra-cluster and inter-cluster interactions.¹²

The majority of studies on clustering are focused on the CPs. Several globular proteins have been recently shown to have characteristics of CPs with short-range attractive interaction and long-range repulsive interactions, giving rise to liquid-liquid phase transitions and some critical phenomenon.^{3, 13} Synthetic polymers including neutral polymers,¹⁴ polyelectrolytes¹⁵ and dendrimers/star polymers¹⁶⁻¹⁸ have been described as soft colloids and were shown to form clusters driven by the interplay of various interactions.^{16, 18-21} For examples, high molecular weight poly(ethylene oxide) (PEOs) have been shown to form clusters driven by attractive solvophobic interactions of chain ends with the backbone.^{22, 23} The effect of counter ions in the case of charged polyelectrolytes has also been explored,²⁴ with larger counter ions giving rise to

finite clusters due to steric and short-range electrostatic effects.²⁵ The interplay between hydrophobic and electrostatic forces in the aggregations of ionomers, another class of charged polymers, has received attention due to its implication in PEM materials.²⁶

Investigations of cluster formation in macromolecular systems have been carried out using a number of experimental tools including small angle neutron and X-ray scattering (SANS and SAXS), optical microscopy as well as dielectric spectroscopy.^{3, 7, 11, 22, 27} Features in the low Q region in neutron and X-ray scattering present a signature of clustering in macromolecular systems. Large aggregates that extend to the micron region can be observed in optical microscopy experiments, and presence of slow modes in DLS suggests the formation of aggregates. While these are extremely useful in analyzing the mechanism of clustering, one has to resort to theoretical and computational studies to tease out the subtle forces involved in the formation of clusters in various macromolecular systems. The bulk of these computational studies involve molecular dynamics or Monte Carlo simulations of coarse-grained models of the polymer that lack atomistic level detail.^{1, 9, 12, 14, 15, 28} The use of coarse-grained models arises due to the slow dynamics of aggregation in these systems, which gives rise to inadequate sampling when using atomistic simulation techniques. A number of coarse-grained Monte Carlo simulations of clustering of colloidal particles describe the inter-particle interactions in terms of an attractive component described by a Lennard-Jones type interaction and a longer-range repulsive Yukawa interaction.^{1, 2} In addition, mean field type approaches as well as classical density functional theory based methods wherein a free energy functional is constructed and minimized with respect to component densities have also been explored.^{12, 20, 25, 26, 29}

Polypeptoids, a class of polymers with N -substituted polyglycine backbones, are structural mimics of polypeptides.^{30, 31} Because of the N -substitution, polypeptoids lack extensive hydrogen bonding or main chain chirality that is characteristic of polypeptides. As a result, polypeptoids tend to have more flexible backbone conformation^{32, 33} and enhanced proteolytic stability³⁴ and solubility in various solvents relative to polypeptides.^{30, 31} Early studies have shown that the physicochemical properties of polypeptoids (e.g., conformation,³⁵⁻³⁷ solubility and crystallinity)³⁸⁻⁴⁰ are strongly dependent on the sidechain structures.^{30, 31}

In this study, we investigate the cluster formation of cyclic polypeptoids bearing zwitterionic end-groups (Figure 1 a) in solution using a synergistic combination of experimental

tools, namely small-angle neutron scattering (SANS), DLS and cryoTEM, as well as computational methods like molecular dynamics (MD) simulations combined with umbrella sampling (US), an enhanced sampling method. SANS, DLS and cryoTEM characterization provide experimental support for the formation of small clusters based on the cyclic polypeptoids in dilute methanol solution. By contrast, no cluster formation is observed for the linear counterparts (Figure 1 a). The simulations reveal that the subtle interplay between the attractive dipole-dipole and the solvophobic interactions along with the repulsive forces due to solvation of the dipoles provide the driving force for the cluster formation of the cyclic polypeptoids.

II. Methods

A. Experiment

Materials. 1, 8-Diazabicycloundec-7-ene (DBU) was dried over CaH_2 and purified by distillation before use. All solvents used were purchased from Sigma-Aldrich and purified by passing through alumina columns under argon (Innovative Technology, Inc.) and stored under a nitrogen atmosphere. All other chemicals were purchased from Sigma-Aldrich and used as received. *N*-butyl *N*-carboxyanhydride (Bu-NCA), *N*-propargyl *N*-carboxyanhydride (Pg-NCA), and *N*-methyl *N*-carboxyanhydride (Me-NCA) were synthesized by following reported procedures.⁴¹⁻⁴³ All cyclic and linear poly (*N*-methyl glycine) (*c*-PNMG and *l*-PNMG) bearing a DBU initiating moiety as well as the cyclic and linear bottlebrush copolymers bearing poly(*N*-butyl glycine-*r*-*N*-propargyl glycine) backbone and polyethylene glycol (PEG, $M_n=550 \text{ g}\cdot\text{mol}^{-1}$) and butyl sidechains (in a 6:1 molar ratio) [*c*-P(NBG-*r*-NPgG)-*g*-PEG and *l*-P(NBG-*r*-NPgG)-*g*-PEG] were synthesized and characterized by using previously reported procedures.^{41, 42, 44}

Representative procedures for the synthesis of the cyclic PNMG bearing a DBU initiating moiety (*c*-PNMG). In a glovebox, Me-NCA (33 mg, 0.29 mmol) was dissolved in anhydrous acetonitrile (700 μL). A stock solution of DBU in THF (30 μL , 2.9 μmol , 95.2 mM) was added to the reaction flask. The flask was sealed and stirred at room temperature for 16 h. The polymerization was terminated by adding diethyl ether (8 mL). The precipitated polymer was isolated by decantation and dried under vacuum (18 mg, 86% yield).

Representative procedures for the synthesis of the linear PNMG bearing a DBU initiating moiety (*l*-PNMG). In a glovebox, the cyclic PNMG bearing a DBU initiating moiety (10 mg, 1.4 μmol) was dissolved in anhydrous THF (250 μL). A THF stock solution of acetyl

chloride (AcCl) (156 μ L, 0.14 mmol, 0.9 M) was added to the reaction flask. The flask was sealed and stirred at room temperature for 16 h. The volatiles were removed under vacuum to yield the final polymer product (10 mg, 100 % yield).

Representative procedures for the synthesis of the linear bottlebrush copolymer (*l*-P(NBG-*r*-NPgG)-*g*-PEG). In a glovebox, the cyclic bottlebrush polymer precursor (10 mg, 0.08 μ mol) obtained by a published procedure⁴⁴ was dissolved in anhydrous THF (250 μ L). A stock THF solution of acetyl chloride (9 μ L, 8 μ mol, 0.9 M) was added to the reaction flask. The flask was sealed and stirred at room temperature for 16 h. The volatiles were removed under vacuum to yield the final polymer product (10 mg, 100 % yield).

Small-Angle Neutron Scattering. The small-angle neutron scattering (SANS) experiments were performed on GP-SANS at the High Flux Isotope Reactor (HFIR) in Oak Ridge National Laboratory (ORNL),⁴⁵ and NGB30m SANS at the NIST Center of Neutron Scattering (NCNR) in National Institute of Standards and Technology (NIST).⁴⁶ Four types of polypeptoids-based samples were measured: i.e., the cyclic and linear poly(*N*-methyl glycine) (*c/l*-PNMG), homopolymer as well as the cyclic and linear P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymers, all at the number average degree of polymerization (DP) targeted at 100, 200, 300, and 400. The real DP values are shown in Table 1, and in the text, we will use the targeted DP values to label the polymers for convenience and to be consistent with the values used in MD simulation. The SANS measurements of cyclic polypeptoid homopolymers and bottlebrush copolymers were performed first, and then AcCl was added to react with both of them to convert to their linear analogs with identical DPs, respectively.⁴¹ The scattering profiles of the cyclic polypeptoids were measured at GP-SANS using the wavelength of 4.75 Å at 1 m and 12m, with the Q range covering 0.006 - 0.5 Å⁻¹, and the polypeptoids after AcCl treatment were measured at NGB30m SANS using the wavelength of 6 Å at 1 m, 4m and 13m, to cover the Q range of 0.004 - 0.5 Å⁻¹. The polymers were directly dissolved in deuterated methanol at 1wt% concentration at room temperature, and then the solution samples were loaded into 2 mm thick banjo cell for measurements. The transmission values of all the samples are larger than 0.85, indicating a negligible multiple scattering contribution. At GP-SANS, the scattering intensities were scaled to absolute values using a secondary standard sample (Al-4), and at NGB30m SANS the absolute intensity was obtained automatically using the Igor Macro for NCNR SANS data reduction due to their calibration of attenuators.⁴⁷

Cryo-Transmission Electron Microscopy. Sample preparation for CryoTEM was carried out on FEI Vitrobot. The bottlebrush copolymer was dissolved in methanol (7 mg/ml) and then passed through a 0.45 μ m filter. The solution (12 μ l) was applied to a 300-mesh lacey carbon grid. Excess liquid was blotted by filter papers for 2 seconds to form a thin film prior to vitrification in liquid ethane. The sample was then loaded to a cryo specimen holder and imaged on S1032_FEI G2 F30 Tecnai TEM operated at 100kV.

Dynamic light Scattering (DLS). DLS measurement was conducted on a Brookhaven Instrument (90 Plus PALS) whose laser wavelength is 640 nm. DLS data was collected by BIC particle solutions software (version 3.1) and analyzed using CONTIN method. Lognormal median diameters which were reported as effective diameters and averaged size distributions were obtained from triplicate measurements. *c/l*-PNMG₁₀₀ and *c/l*-P(NBG-*r*-NPgG)₁₀₀-*g*-PEG bottlebrush polymer were dissolved in MeOH (6.0-6.3 mg/mL) and then filtered through hydrophilic filters (0.1 μ m) into clean cuvettes before DLS measurement.

B. Computations

Computational studies were carried out on the linear and the cyclic counterpart of the PNMG₁₀₀ polypeptoid (see Figure 1b) in methanol solvent. In the cyclic case, the two ends are represented by oppositely charged groups, namely -O⁻ and -NH₃⁺ whereas in the linear case the end groups are -OH and -NH₃⁺. While these are not the end groups in the actual peptoids (Figure 1 a), they carry the appropriate charge and hence present a reasonable representation of the relevant end group interactions

Electronic structure calculations. The partial charges on the atomic sites of the polymer and methanol were determined by carrying out electronic structure calculations on two representative clusters: the first consisting of three monomeric units (Figure 1c) in order to represent the polymer and the second consisting of a single methanol molecule to determine the charges for the methanol. Specifically, the CHELPG method was used,⁴⁸ which determines the charges by fitting to the *ab initio* electrostatic potential at specific grid points around the cluster. The final charges on the polymer were obtained by averaging over the three monomeric units in the cluster. These calculations were carried out using Density Functional Theory approach with the B3LYP functional and the 6-31G basis set. These calculations were performed using the

GAUSSIAN09 suite of programs.⁴⁹ The final charges are tabulated in the supporting information section.

Molecular Dynamics (MD) simulations.

Single Polypeptoid chain. The initial configurations for each of the two types of polypeptoids were generated by connecting 100 monomers, each with a methyl side chain. In the case of the cyclic polypeptoid, the starting configuration had the two charged ends in proximity to each other, mimicking the compound obtained from synthesis while for the linear counterpart, the starting structure consisted of the monomeric units arranged in a line.⁴⁴ The OPLSAA force field parameters for the Lennard-Jones and intramolecular interactions were used along with the partial charges determined from electronic structure calculations for the peptoids.^{50, 51} The same dihedral parameters that are used in the peptide case for the planarity of the amide bond were used here. The OPLS based force-field has previously been used successfully to model peptoids.⁵² A short 50 ps simulation of the *l*-PNMG₁₀₀ was carried out in vacuum at a temperature of 300 K in the NVT ensemble in a cubic box of length 100 Å, and similarly for the *c*-PNMG₁₀₀ polymer. The resulting *l*-PNMG₁₀₀ with a chloride counter anion and *c*-PNMG₁₀₀ were each solvated, using the packmol program,⁵³ in a periodic cubic box of length 100 Å consisting of the same number (14500) of methanol (represented using the united atom OPLS force-field)⁵⁰ molecules in each case. Each system was equilibrated for 10 ns, with a 1 fs timestep, in the canonical (NVT) ensemble at a temperature of 300 K using a Nose-Hoover thermostat. Production runs of 50 ns each were then carried out for each peptoid system. A snapshot from the simulation of the cyclic polymer case is shown in Figure S1a in the Supporting Information (SI).

Two polypeptoid chains. The initial configuration for the linear and cyclic cases were generated by replicating the respective single chain boxes in the x-direction resulting in a periodic box with two polypeptoid chains, and 29000 methanol molecules = of length 200 Å × 100 Å × 100 Å. Each system was once again equilibrated for 10 ns, with a 1 fs time step in the NVT ensemble followed by a 50 ns production run. A snapshot from the simulation of the cyclic polymer case is shown in Figure S1b in the SI.

Umbrella Sampling (US) simulations for the two polypeptoid chain case. Umbrella sampling along with the weighted histogram analysis method (WHAM) was used to determine the free energy profile or potential of mean force (PMF) of bringing the center of mass of one *c*-PNMG₁₀₀ / *l*-PNMG₁₀₀ monomer to the center of mass of another *c*-PNMG₁₀₀ / *l*-PNMG₁₀₀ monomer in methanol solution.^{54, 55} A total of sixteen umbrella sampling windows ranging from an inter-polymer distance of 50 Å to 22 Å were carried out for the linear case, each with a ten nanosecond equilibration run followed by a 40 ns production run. A harmonic force constant of 3.0 kcal/mol was used for the 22 and 23 Å windows and 2.0 kcal/mol for the remaining windows. For the linear case a total of eighteen umbrella sampling windows were used with a harmonic force constant of 3.0 kcal/mol for the 20, 22 and 23 Å windows and 2.0 kcal/mol for the remaining windows. The errors bars for the PMFs were obtained using block averaging, with each block from a 10 ns run.

All simulations, both canonical and umbrella sampling, were carried using the LAMMPS package.⁵⁶

III. RESULTS AND DISCUSSION

SANS Experimental Results. All cyclic and linear PNMG and P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer with varying degree of polymerization were dissolved in deuterated methanol at 1 wt% and the dilute solutions were subjected to SANS measurement at room temperature. The measured SANS profiles were first fitted using the model of cyclic ideal chains,⁵⁷ to obtain the structural information such as Kuhn length and the cross sectional radius. This model describes the behaviors of polymers following Gaussian statistics in θ -solvent condition where the interaction between monomers can be eliminated. For clarity, two representative plots of cyclic PNMG homopolymer and P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer with targeted DP at 100 are shown in Figure 2, respectively, which is the same DP used in MD simulation afterwards. In all data fitting, each data point was weighted by taking the counting statistic into account. The inverse of the standard deviation of each data point was input as the weight function. Reduced chi-square was used in the minimization. The contour lengths were fixed based on the DP values determined by size-exclusion chromatography coupled with differential refractive index and multi-angle light scattering detectors (SEC-DRI-MALS) for the PNMG homopolymer (452.6 Å) and ¹H NMR spectroscopy for *c*-P(NBG-*r*-NPgG)-*g*-PEG

bottlebrush copolymer (511 Å) (Table 1). The fitted Kuhn length is 5.9 Å and the cross sectional radius 1.5 Å for the *c*-PNMG homopolymer, and 18.7 Å and 9.5 Å for *c*-P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer, respectively. However, it can be seen that for the PNMG homopolymer, the model fitting deviates significantly from the experimental data points, indicating that the system is not composed of cyclic ideal chains, which can be attributed to the non- θ -solvent condition or aggregation of molecules. One can also note that the discrepancy of cyclic P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer (Figure 2b) between the model fitting and experimental data is smaller than that for the cyclic PNMG homopolymer (Figure 2a).

Model-free Guinier analysis on the SANS profiles were also performed for all the samples, and their radii of gyration (R_g) were obtained. Representative Guinier plots are demonstrated in Figure 3. The Q range was selected with the criteria of $R_g Q_{\max} < 1$. We previously demonstrated that a cyclic polypeptoid synthesized with zwitterionic ring-opening polymerization method can be converted into the linear analog that bears one neutral chain-end and one positively charged chain-end with a chloride counter ion in the solution through reaction with acyl chloride (AcCl).⁴¹ The resulting linear analog has the same DP or contour length as the cyclic precursor. The size of a cyclic polymer is expected to be smaller than its linear analog having the same contour length, resulting in a characteristic ratio ($R_{g,c}/R_{g,l}$) in the 0.5 (θ condition) to 0.6 range, due to their intrinsic topological difference.^{58, 59} Hence, it is expected that the R_g of linear analogs obtained by AcCl treatment should be larger than the cyclic polypeptoid precursor. To our surprise, the radii of gyration of the cyclic PNMG homopolymer and the P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer are larger than their respective linear analogs by 44-74% (homopolymer) and 20-47% (bottlebrush copolymer) respectively, at the entire DP range (Figure 4). For example, the R_g of cyclic PNMG (DP=100) is 29.1 Å which is larger than that of the linear analog (19.0 Å) by 53%. The theoretical R_g for the linear PNMG (DP = 100) is calculated to be 19.6 Å by using $R_g = Nb^2/6$, which agrees well with the R_g determined by SANS. This indicates the linear PNMG chains present as individually solubilized random coils. By contrast, the cyclic polymers may not behave as individually solubilized molecules in solution and have formed small clusters, resulting in larger effective R_g s relative to the linear counterparts. It should also be noted that the SANS profiles of all cyclic and linear PNMG and P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymers do not show any notable increase in intensity at the low q region, indicating the absence of large aggregates in these dilute solutions.

Dynamic Light Scattering Results. DLS analysis of the dilute methanol solution containing the cyclic or the linear PNMG homopolymer (targeted DP = 100) at room temperature revealed a monomodal distribution of particle sizes with modest polydispersity index (0.12-0.14) (Figure 5). The cyclic PNMG was shown to have a hydrodynamic radius ($R_h=25.0\pm0.5$ Å, PDI = 0.14) which is slight larger than that of the linear counterpart (21.9 ± 1.0 Å, PDI = 0.12). Similarly, DLS analysis of the cyclic and linear P(NBG-*r*-NPgG)-*g*-PEG graft copolymers (targeted DP = 100) in dilute methanol solution also revealed the same trend of relative hydrodynamic size with the cyclic polymer ($R_h = 49.7\pm0.3$ Å, PDI = 0.11) being slightly larger than that of the linear analog ($R_h = 44.1\pm0.1$ Å, PDI = 0.14). The percentage difference in R_h between the cyclic and linear polymers (13-14%) is smaller than that in R_g determined by SANS. This could be due to the difference in hydrodynamic interaction for different chain architecture. Regardless, the DLS results also suggest the aggregate formation in the cyclic polypeptoids, consistent with the SANS results.

Cryo-Transmission Electron Microscopy Results. To verify the formation of small clusters of cyclic polymers, we measured the cyclic P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer (targeted DP=400) using cryo-TEM. Due to the resolution limit of cryo-TEM, we selected the largest cyclic polypeptoid bottlebrush copolymer for direct imaging in vitrified methanol. A representative cryoTEM image is shown in Figure 6a. It is evident from Figure 5 that a number of small clusters comprised of less than ten molecules are present in addition to individual molecules of cyclic bottlebrush copolymers. The presence of these small clusters can explain the abnormal variation of the particle sizes (R_g) in Figure 4. The mean diameter of these small clusters ($d = 12.2\pm2.8$ nm) (Figure 6b) are in the same length scale of a single polymer chain (Figure 4b). Thus, their features in SANS profiles cannot be separated from each other. The measured SANS profile will be the average of the profiles of all species including single molecules, dimers, trimers, and oligomers, weighted by their scattering power. Therefore, it is not surprising that there is large deviation between the model fitting using the cyclic Gaussian chain model and the experimental SANS data, as the model only describes the structure of a single polymer chain. The cluster structures observed in the dilute methanol solution of this cyclic bottlebrush copolymer sample (DP 400) are expected to present for those with lesser DP values and also for the PNMG homopolymer, since they have similar chemical composition and

the SANS measurements show the same trend in the R_g variation for all DP values and both types of cyclic polymers.

As the cyclic polypeptoids contain oppositely charged (zwitterionic) chain ends, it is plausible that the dipole-dipole interaction may provide the driving force for the cluster formation. By contrast, the linear counterparts only bear a positively charged chain end with a chloride counter-ion. The absence of dipole-dipole interaction may preclude the cluster formation in solution for the linear polymers. To further investigate the aggregation behavior of the cyclic and linear polypeptoids in dilute solution, molecular dynamic simulation has been conducted.

Molecular Dynamics Simulation Results. To further investigate the mechanism and driving force of the cluster formation, we performed MD simulations on the cyclic and linear PNMG molecules in methanol solution with both DP=100. First, as shown in the left panel of Figure 7, the SANS form factors of a cyclic (green line) and a linear (red line) PNMG molecule were calculated from the simulation trajectory, with each atom weighted by its neutron scattering length.⁶⁰ It can be seen that the Guinier region of the scattering profile for a cyclic molecule extends more towards high Q than a linear one, which indicates a smaller overall size than its linear analog, and it agrees with the theoretical expectation.⁵⁸ In the intermediate and high Q region ($Q > 0.1 \text{ \AA}^{-1}$), the scattering profile of a cyclic homopolymer decays faster than a linear one, which means the scatterers in a cyclic polymer are more densely packed like compact colloids rather than loose coils given by a linear polymer. The bump in the scattering profile of a cyclic molecule around $Q = 0.4 \text{ \AA}^{-1}$ suggests that the interface between the polymeric materials and the solvent is relatively well-defined and spherical, compared to the irregular interface for a linear one. The scattering function of a dimer of two cyclic polymers is also calculated, and its Guinier region shifts towards low Q , corresponding to a larger size as expected. Compared to the experimental data in the right panel of Figure 6, where the SANS profile of cyclic polymers has the Guinier region shifted towards low Q , it confirms that the solution of cyclic polymers may actually contain dimers or even oligomers. This then raises the question as to what the driving force is that causes the aggregation in the case of the cyclic peptoid and is addressed below.

The process to form a dimer in the cyclic case can be described using the free energy profile, also called the potential of mean force (PMF), between two interacting molecules as a

function of the center of mass separation. The use of the center of mass distance as the collective variable is appropriate as it allows for the study of the two polymers as they approach each other without biasing the polymers in any specific orientation. Using umbrella sampling, we obtained the PMF of two cyclic PNMG polymers with DP=100 in methanol solution, as shown in Figure 8 (a). At distances greater than 30 Å, the PMF is essentially constant, within the statistical error. At a separation of about 24 Å there is a free energy minimum of around 0.5 kcal/mol, indicating an attractive region, which may come from the dipole-dipole interaction or solvophobic interaction, and which in turn drives the formation of a dimer. Overall, the statistical error of the PMF is comparable to the energy barrier and the depth of the relative free energy and is of the order of $k_B T$, which means both the formation and dissociation of dimers can occur at a fast rate and there exists an equilibrium between the two. Therefore, in solution, monomer, dimers, and oligomers should exist, and indeed this agrees with our experimental measurements. On the other hand, the PMF or free energy profile for association of the linear counterpart as a function of the distance between the center of masses of the two polymers (see Figure 8 (b)) shows no minimum and in fact the free energy increases as the two come closer. This suggests that the linear polymer can at best form transient aggregate species in dilute solution.

To further study the dissociation of solvent molecules from the cyclic polymers, we calculated the radial distribution function of the oxygen atom of methanol to the oxygen of the carbonyl group on the PNMG backbone, in Figure 9. The very sharp peak at $r=3.3$ Å corresponds to the first solvation shell and the small peak at $r=7$ Å to the second solvation shell. Hence the first solvation shell coordination number is defined as the number of solvent molecules with the O atom of its hydroxyl group within this cutoff distance of $r=3.3$ Å from the O atom of the corresponding carbonyl group of the polymer. On the basis of the spatial distribution of solvent molecules around the polymer, we can estimate the solvation level of cyclic PNMG molecules during the dimer formation, which is later used to demonstrate the solvophobic interaction. We define a monomer in the PNMG polymer as “coordinated” to solvent molecules if there is at least one oxygen atom from the hydroxyl group in methanol located within 3.3 Å from the oxygen in the carbonyl group of the monomer. The percentage of the coordinated monomers in the whole polymer chain indicates the level of solvation of the PNMG. This coordination percentage is plotted as a function of the center of mass separation distance during dimer formation, as shown in Figure 10. When two PNMG molecules are approaching each other but still separated ($r>35$

Å), the coordination percentage gradually and smoothly decreases, which may be due to the rearrangement of the first solvation shell influenced by the hydrodynamic interaction through the excluded solvent molecules. In the range of $22 \text{ Å} < r < 35 \text{ Å}$, the coordination percentage shows a plateau between 25 Å and 35 Å , and then a drop from $r=25 \text{ Å}$ to 22 Å . Combined with the PMF, a clear picture emerges regarding the aggregation process. When two cyclic PNMG molecules approach each other within 35 Å , the orientation of methanol molecules located in the first solvation shell between the two polymers are rearranged, to minimize the free energy, which give the constant coordination percentage. Until their separation distance reaches $r=25 \text{ Å}$, the first solvation shell between the two PNMG polymers starts to get fully excluded and the coordination percentage approaches its minimum value. It is worth noting that, even at the shortest separation distance, more than half of the monomers in PNMG located in the outer region of the dimer are still exposed to the surrounding solvent molecules, and only the monomers in the middle of the two PNMG molecules are solvent-excluded. Even in the form of separated molecules, a fraction of the monomers may be shielded by other ones from being solvated. Therefore, it is reasonable that the coordination percentage is reducing from 80.5% to 67.5%.

In our zwitterionic cyclic polymer system, solvophobic interactions, demonstrated in the coordination percentage curve (Figure 10), plays an important role in the final structure of the aggregate, in addition to the dipole-dipole interaction, with the dipole created by the oppositely charged ends of the cyclic polymer. To further reveal the mechanism of dimer formation and the interplay between these forces, we calculated the probability distribution of the dipole-dipole distance at different separation distances between the two PNMG molecules, presented in Figure 11. When the dipole-dipole interaction is stronger than the solvophobic interaction, the dipoles prefer to minimize their distance to reduce the free energy, and as a result, the two molecules will rotate with the dipoles towards each other. However, the dipole shows much greater preference to be solvated than the backbone. When the dipoles are rotated close to each other, they will also tend to bring solvent molecules between the two PNMG molecules. This will counteract the solvophobic interaction, resulting in an increase of the free energy due to the excluded volume effect. Therefore, the conformation of the dimer is determined by the competition between the attractive dipole-dipole and solvophobic interaction and the effectively repulsive interaction arising from the solvation of the dipoles, and it is apparent in the dipole-

dipole distance distribution. A configuration with the dipole-dipole distance smaller than the center of mass separation distance indicates that the dipole-dipole interaction dominates in this conformation. On the other hand, when the dipole-dipole distance is larger than the center of mass distance, the solvophobic interaction coupled with dipole solvation is the dominant force in determining the conformation of the dimer. The dipole-dipole distance probability distributions at four representative center of mass separation distances are presented in Figure 11. Representative snapshots of the dimer conformation, from umbrella sampling simulations, corresponding to each probability peak in Figure 11 are shown in Figure 12, with the dipoles highlighted. It can be seen that for the snapshot from the umbrella sampling window with $r_0=36$ Å (cyan curve) a majority rests in the range above r_0 and only a small part appears in the range below r_0 , which indicates that at this separation distance dipole-dipole interactions do not play an important role. However, at $r_0=32$ Å (red curve), the majority is below r_0 , which means that dipole-dipole interactions dominate in determining the conformation. At $r_0=28$ Å (green curve), the conclusion is similar. At $r_0=24$ Å (blue curve), we see that all events are located in the region above r_0 , which implies that the solvophobic interaction dominates and the methanol between the PNMG molecules has been fully excluded, with the result that the dipoles are rotated opposite to each other in order to achieve a better solvation environment. The dipoles, open to the solvent at this inter-polymer distance, along with the hydrophobic and dipole-dipole interactions will all contribute to further clustering to form trimers and other oligomers. From the inter-polymer distance dependence of these probability distribution functions, we can directly observe the competition between these driving forces during dimer formation and their effect on the conformation. Lastly, the need for the dipoles to be solvated suggests a reason for the formation of only small oligomers. With increasing cluster size, some of the dipoles will inevitably be buried in the interior region of the cluster, thus losing their solvation shell, which is unfavorable and will thereby restrict the aggregation to fairly small cluster sizes. To reiterate, the formation and structure of the cyclic dimer is driven by both the solvophobic effect and the dipole-dipole interactions and opposed by the solvation of the dipoles, with the different interactions dominating at different inter-polymer distances. In the case of the linear counterpart there is no dipole-dipole interaction to form a stable dimer and furthermore the charged ends prefer to be solvated, resulting in the free energy increasing continuously with decreasing distance. Interestingly a different set of collective variables, the distance between the dipoles for the cyclic

case and the distance between the charged and uncharged end groups in the linear case, show the similar trends in the free energy and are discussed in greater detail in the SI (Figure S2 and S3).

At this point it is worth noting that the experimental data for the cyclic polypeptoids can be thought of as arising from association of several macro zwitterions i.e. the positive end of one polymer aggregates with the negative end of another and so on. The simulations were carried out with a starting configuration with the two charged ends of the polypeptoid in proximity to each other, since this mimics the polypeptoid architecture that was obtained from the synthesis. The center of mass coordinate based collective variable does not bias the simulations against the opening of the cyclic polypeptoid chain, However, this is not seen in the simulations. This behavior of the cyclic polypeptoids in methanol arises because the comparatively low dielectric constant of methanol does not allow for the opening of the dipole in the cyclic zwitterionic polypeptoids. This, in turn, prevents the charged ends of each zwitterionic polypeptoid from aggregating with the oppositely charged ends of other zwitterionic polypeptoids in solution.

IV. CONCLUSIONS

A combination of experimental and computational methods have been used to study the aggregation of cyclic and linear polypeptoids in methanol. SANS and cryo-TEM experiments revealed that the cyclic polymers formed clusters which were not observed for the linear counterpart. The free energy profile as a function of inter-polymer distances suggests an equilibrium between the cyclic chain monomer and dimer forms. Analyses of the trajectories from the simulations indicate that the dipole-dipole interaction along with the attractive solvophobic effect (expulsion of the solvating molecules between the two cyclic polypeptoid chains) favor the dimerization whereas the effective repulsive interaction arising due to the solvation of the dipoles greatly reduces the attractive component in aggregation. The resulting cluster now consists of two dipoles that are exposed to the solvent allowing for further aggregation. However, the repulsive forces due to the solvation of the dipoles suggests that the aggregation is limited to small clusters as larger clusters would necessitate the burial, to some extent, of the dipoles in the interior. This is corroborated by the experimental data that shows the presence of monomeric to small oligomeric clusters.

ACKNOWLEDGEMENTS

This work is supported by the U.S. Department of Energy under EPSCoR Grant No. DE-SC0012432 with additional support from the Louisiana Board of Regents. The Research at Oak Ridge National Laboratory's High Flux Isotope Reactor and Spallation Neutron Source was sponsored by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy. We thank Dr. Yun Liu for his help on the SANS measurements performed on NGB30m SANS at NCNR NIST, and informative discussions. This work utilized facilities supported in part by the National Science Foundation under Agreement No. DMR-1508249. We acknowledge the support of the National Institute of Standards and Technology, U.S. Department of Commerce, in providing the neutron research facilities used in this work. We acknowledge the LSU High Performance Computing Center and the Louisiana Optical Network Initiative (LONI) for computer time.

Table 1. The number average degree of polymerization (DP) of the cyclic PNMG homopolymer and cyclic P(NBG-*r*-NPgG)-*g*-PEG copolymer samples used in the SANS measurements

Targeted DP	<i>c</i> -PNMG homopolymer		<i>c</i> -P(NBG- <i>r</i> -NPgG)- <i>g</i> -PEG bottlebrush copolymer	
	DP (SEC) ^a	PDI (SEC) ^c	DP (NMR) ^b	PDI (SEC) ^c
100	124	1.01	140	1.07
200	188	1.01	224	1.15
300	241	1.02	375	1.12
400	412	1.01	480	1.13

^a. DP was calculated from M_n/M_o where M_n was determined by SEC-DRI-MALS analysis using $dn/dc = 0.0991(8) \text{ ml} \cdot \text{g}^{-1}$ in 0.1 LiBr/DMF at 25 °C and M_o is the repeating unit mass (71.08 g·mol⁻¹). ^b. DP was determined from end-group analysis using ¹H NMR spectroscopy. ^c. PDI is determined from SEC-DRI-MALS analysis.

Figure 1. (a) Sketch of the experimental cyclic polypeptoid structure *c*-PNMG (DP=100) (left) and the linear polypeptoid structure *l*-PNMG (DP=100) (right). (b) Sketch of the simplified cyclic and linear peptoids simulated in this work. The cyclic polypeptoid is a zwitterion with a negatively charged -O group at one end and a positively charged -NH₃⁺ at the other that are very close together forming a cyclic peptoid in methanol solution, while the linear case has a single positively charged -NH₃⁺ group. (c) The representative peptoid cluster used to determine the partial charges. Red spheres are used for oxygen atoms, blue for nitrogen, grey for carbon and white for hydrogen.

Figure 2. Representative SANS profiles of (a) the cyclic PNMG homopolymer and (b) the cyclic P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer, both with targeted DP at 100. The black solid line is the data fitting using cyclic Gaussian chain model. (Note: In all data fitting, each data point was weighted by taking the counting statistic into account. The inverse of the standard deviation of each data point was input as the weight function. Reduced chi-square was used in the minimization).

Figure 3. Guinier plots of (a) the cyclic PNMG homopolymer, (b) the linear PNMG homopolymer analog, (c) the cyclic P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer and (d) the linear P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer analog. All samples have targeted DP at 100. The linear analogs were obtained by AcCl treatment of the corresponding cyclic polymer precursors. The obtained radii of gyration (R_g) values are shown in each panel.

Figure 4. The plot of radius of gyration (R_g) versus experimentally determined degree of polymerization for (a) the cyclic PNMG homopolymers (open red circle) and the linear analogs (open black circle) as well as (b) the cyclic P(NBG-*r*-NPgG)-g-PEG bottlebrush copolymers (solid red circle) and their linear analogs (solid black square). The linear polymeric analogs were obtained by AcCl treatment of the corresponding cyclic polymer precursors. All cyclic polymers have larger R_g than their respective linear analogs, indicating the possibility of aggregate formation for the cyclic polymers.

Figure 5. The DLS plot of normalized scattering intensity versus the hydrodynamic diameter for (a) the cyclic PNMG homopolymers (black bar) and the linear analogs (red bar) as well as (b) the cyclic P(NBG-*r*-NPgG)-g-PEG bottlebrush copolymers (black bar) and their linear analogs (red bar). The targeted DP is 100 for both the cyclic and linear PNMG and P(NBG-*r*-NPgG)-g-PEG backbone.

Figure 6. (a) Representative cryo-TEM image of cyclic P(NBG-*r*-NPgG)-*g*-PEG bottlebrush copolymer with a targeted DP at 400 in vitrified methanol. Dimers, trimers, and oligomers are indicated by the red arrows. (b) The histogram analysis of the aggregated particle sizes seen in the cryo-TEM micrograph. (Note: a total of 124 measurements of aggregated particle size are used in the analysis). The mean diameter of the aggregated particle is 12.2 ± 2.8 nm, consistent with the particle dimension determined from the SANS analysis (Figure 4b).

Figure 7. Form factors of the PNMG polypeptoids (targeted DP=100) from simulation (left) compared to the experimental small-angle neutron scattering (SANS) data (right). In the left panel (simulation) the linear PNMG polypeptoid is shown in red, the cyclic monomeric version is shown in green and the cyclic dimer in blue. In the right panel (experimental data) the linear PNMG is shown in red and the cyclic PNMG in blue. Note that the curves in the right panel were obtained from the measured intensity in experiments from which the incoherent scattering has been subtracted, and the coherent scattering is normalized by its extrapolated zero-angle scattering, to make it easier to compare with the calculated form factor from MD simulation.

Figure 8. The free energy profile as a function of the center of mass separation between (a) two *c*-PNMG polymer chains (DP=100) and (b) two *l*-PNMG polymer chains (DP=100).

Figure 9. The radial distribution function, $g(r)$, where r is the distance between the oxygen atoms of the polymer backbone and the oxygen atoms of the methanol solvent from MD simulation at $T=300\text{K}$. The first minimum is at $3.3 \text{\AA}$.

Figure 10. The percentage of O atoms on the polymer backbones that is coordinated to methanol as a function of the distance between the center of mass of the two *c*-PNMG monomers (DP=100).

Figure 11. Dipole-dipole distance distribution as a function of the distance between the center of mass of the two *c*-PNMG monomers (DP=100). The curves are normalized to have unit area.

Figure 12. Snapshots corresponding to the peaks in the dipole-dipole distribution (Figure 9) from different umbrella sampling windows (r_0 = 24, 28, 32, 36 Å) of the two *c*-PNMG monomers (DP=100) (one shown in gray and the other in magenta). In the case of r_0 = 32 Å, three distinct peaks are seen and hence three snapshots are shown, c1 corresponding to the largest peak, c2 to the second largest and c3 to the third peak. Similarly for r_0 = 36 Å, two peaks are seen and d1 corresponds to the largest peak and d2 to the second largest peak. The positive and negative ends of each polymer are represented by a red and green sphere, respectively. The methanol solvent molecules are not shown for clarity.

REFERENCES

1. F. Sciortino, P. Tartaglia and E. Zaccarelli, *J. Phys. Chem. B*, 2005, **109**, 21942-21953.
2. P. D. Godfrin, N. E. Valadez-Perez, R. Castaneda-Priego, N. J. Wagner and Y. Liu, *Soft Matter*, 2014, **10**, 5061-5071.
3. A. Stradner, H. Sedgwick, F. Cardinaux, W. C. K. Poon, S. U. Egelhaaf and P. Schurtenberger, *Nature*, 2004, **432**, 492-495.
4. B. Y. Ha and A. J. Liu, *EPL (Europhysics Letters)*, 1999, **46**, 624.
5. H. Sedgwick, K. Kroy, A. Salonen, B. M. Robertson, U. S. Egelhaaf and C. K. W. Poon, *The European Physical Journal E*, 2005, **16**, 77-80.
6. C. J. Dibble, M. Kogan and M. J. Solomon, *Physical Review E*, 2006, **74**, 041403.
7. A. I. Campbell, V. J. Anderson, J. S. van Duijneveldt and P. Bartlett, *Phys. Rev. Lett.*, 2005, **94**, 208301.
8. G. Meng, N. Arkus, M. P. Brenner and V. N. Manoharan, *Science*, 2010, **327**, 560-563.
9. L. Hong, A. Cacciuto, E. Luijten and S. Granick, *Nano Lett.*, 2006, **6**, 2510-2514.
10. F. Sciortino, S. Mossa, E. Zaccarelli and P. Tartaglia, *Phys. Rev. Lett.*, 2004, **93**, 055701.
11. P. J. Lu, J. C. Conrad, H. M. Wyss, A. B. Schofield and D. A. Weitz, *Phys. Rev. Lett.*, 2006, **96**, 028306.
12. B. M. Mladek, D. Gottwald, G. Kahl, M. Neumann and C. N. Likos, *Phys. Rev. Lett.*, 2006, **96**, 045701.
13. P. Schurtenberger, R. A. Chamberlin, G. M. Thurston, J. A. Thomson and G. B. Benedek, *Phys. Rev. Lett.*, 1989, **63**, 2064-2067.
14. A. A. Louis, P. G. Bolhuis, J. P. Hansen and E. J. Meijer, *Phys. Rev. Lett.*, 2000, **85**, 2522-2525.
15. M. Konieczny, C. N. Likos and H. Löwen, *J. Chem. Phys.*, 2004, **121**, 4913-4924.
16. C. N. Likos, M. Schmidt, H. Löwen, M. Ballauff, D. Pötschke and P. Lindner, *Macromolecules*, 2001, **34**, 2914-2920.
17. A. Jusufi, M. Watzlawek and H. Löwen, *Macromolecules*, 1999, **32**, 4470-4473.
18. C. N. Likos, H. Löwen, M. Watzlawek, B. Abbas, O. Jucknischke, J. Allgaier and D. Richter, *Phys. Rev. Lett.*, 1998, **80**, 4450-4453.
19. C. N. Likos, S. Rosenfeldt, N. Dingenouts, M. Ballauff, P. Lindner, N. Werner and F. Vögtle, *J. Chem. Phys.*, 2002, **117**, 1869-1877.
20. M. Bernabei, P. Bacova, A. J. Moreno, A. Narros and C. N. Likos, *Soft Matter*, 2013, **9**, 1287-1300.
21. M. Z. Slimani, P. Bacova, M. Bernabei, A. Narros, C. N. Likos and A. J. Moreno, *ACS Macro Letters*, 2014, **3**, 611-616.
22. B. Hammouda, *Polymer*, 2009, **50**, 5293-5297.
23. B. Hammouda, D. L. Ho and S. Kline, *Macromolecules*, 2004, **37**, 6932-6937.
24. B. Y. Ha and A. J. Liu, *Physical Review E*, 1998, **58**, 6281-6286.
25. M. L. Henle and P. A. Pincus, *Physical Review E*, 2005, **71**, 060801.
26. P. É. A. Melchy and M. H. Eikerling, *Physical Review E*, 2014, **89**, 032603.
27. A. Shukla, E. Mylonas, E. Di Cola, S. Finet, P. Timmins, T. Narayanan and D. I. Svergun, *Proceedings of the National Academy of Sciences*, 2008, **105**, 5075-5080.
28. I. O. Götze, H. M. Harreis and C. N. Likos, *J. Chem. Phys.*, 2004, **120**, 7761-7771.
29. A. J. Archer and N. B. Wilding, *Physical Review E*, 2007, **76**, 031501.
30. D. Zhang, S. H. Lahasky, L. Guo, C.-U. Lee and M. Lavan, *Macromolecules*, 2012, **45**, 5833-5841.
31. N. Gangloff, J. Ulbricht, T. Lorson, H. Schlaad and R. Luxenhofer, *Chem. Rev. (Washington, DC, U. S.)*, 2016, **116**, 1753-1802.
32. H. K. Murnen, A. M. Rosales, A. V. Dobrynin, R. N. Zuckermann and R. A. Segalman, *Soft Matter*, 2013, **9**, 90-98.

33. A. M. Rosales, H. K. Murnen, S. R. Kline, R. N. Zuckermann and R. A. Segalman, *Soft Matter*, 2012, **8**, 3673-3680.
34. S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr and W. H. Moos, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2657-2662.
35. L. Guo, J. Li, Z. Brown, K. Ghale and D. Zhang, *Peptide Science*, 2011, **96**, 596-603.
36. P. Armand, K. Kirshenbaum, R. A. Goldsmith, S. Farr-Jones, A. E. Barron, K. T. Truong, K. A. Dill, D. F. Mierke, F. E. Cohen, R. N. Zuckermann and E. K. Bradley, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 4309-4314.
37. K. Kirshenbaum, A. E. Barron, R. A. Goldsmith, P. Armand, E. K. Bradley, K. T. Truong, K. A. Dill, F. E. Cohen and R. N. Zuckermann, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 4303-4308.
38. C.-U. Lee, A. Li, K. Ghale and D. Zhang, *Macromolecules*, 2013, **46**, 8213-8223.
39. A. M. Rosales, H. K. Murnen, R. N. Zuckermann and R. A. Segalman, *Macromolecules*, 2010, **43**, 5627-5636.
40. C. Fetsch and R. Luxenhofer, *Polymers*, 2013, **5**, 112.
41. L. Guo, S. H. Lahasky, K. Ghale and D. Zhang, *J. Am. Chem. Soc.*, 2012, **134**, 9163-9171.
42. S. H. Lahasky, W. K. Serem, L. Guo, J. C. Garno and D. Zhang, *Macromolecules*, 2011, **44**, 9063-9074.
43. L. Guo and D. Zhang, *J. Am. Chem. Soc.*, 2009, **131**, 18072-18074.
44. A. Li, L. Lu, X. Li, L. He, C. Do, J. C. Garno and D. Zhang, *Macromolecules*, 2016, **49**, 1163-1171.
45. G. D. Wignall, K. C. Littrell, W. T. Heller, Y. B. Melnichenko, K. M. Bailey, G. W. Lynn, D. A. Myles, V. S. Urban, M. V. Buchanan, D. L. Selby and P. D. Butler, *J. Appl. Crystallogr.*, 2012, **45**, 990-998.
46. C. J. Glinka, J. G. Barker, B. Hammouda, S. Krueger, J. J. Moyer and W. J. Orts, *J. Appl. Crystallogr.*, 1998, **31**, 430-445.
47. S. Kline, *J. Appl. Crystallogr.*, 2006, **39**, 895-900.
48. C. M. Breneman and K. B. Wiberg, *J. Comput. Chem.*, 1990, **11**, 361-373.
49. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Journal*, 2009.
50. W. L. Jorgensen and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1988, **110**, 1657-1666.
51. W. L. Jorgensen, D. S. Maxwell and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1996, **118**, 11225-11236.
52. S. H. Park and I. Szleifer, *J. Phys. Chem. B*, 2011, **115**, 10967-10975.
53. L. Martínez, R. Andrade, E. G. Birgin and J. M. Martínez, *J. Comput. Chem.*, 2009, **30**, 2157-2164.
54. B. Roux, *Comput. Phys. Commun.*, 1995, **91**, 275-282.
55. M. Souaille and B. Roux, *Comput. Phys. Commun.*, 2001, **135**, 40-57.
56. S. Plimpton, *J. Comput. Phys.*, 1995, **117**, 1-19.
57. B. Hammouda, in *Polymer Characteristics*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1993, DOI: 10.1007/BFb0025862, pp. 87-133.
58. J. Roovers, in *Cyclic Polymers*, ed. J. A. Semlyen, Kluwer Academic, Dordrecht, The Netherlands, 2nd edn., 2000, pp. 347-384.
59. S. S. Jang, T. Çağın and W. A. Goddard, *J. Chem. Phys.*, 2003, **119**, 1843-1854.

60. Y. Liu, V. S. Bryantsev, M. S. Diallo and W. A. Goddard III, *J. Am. Chem. Soc.*, 2009, **131**, 2798-2799.