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PAPER

Determination of the persistence length of helical and non-helical polypeptoids in solution†

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Control over the shape of a polymer chain is desirable from a materials perspective because polymer stiffness is directly related to chain characteristics such as liquid crystallinity and entanglement, which in turn are related to mechanical properties. However, the relationship between main chain helicity in novel biologically derived and inspired polymers and chain stiffness (persistence length) is relatively poorly understood. Polypeptoids, or poly(*N*-substituted glycines), constitute a modular, biomimetic system that enables precise tuning of chain sequence and are therefore a good model system for understanding the interrelationship between monomer structure, helicity, and persistence length. The incorporation of bulky chiral monomers is known to cause main chain helicity in polypeptoids. Here, we show that helical polypeptoid chains have a flexibility nearly identical to an analogous random coil polypeptoid as observed *via* small angle neutron scattering (SANS). Additionally, our findings show that polypeptoids with aromatic phenyl side chains are inherently flexible with persistence lengths ranging from 0.5 to 1 nm.

Introduction

The shape of a polymer chain is a reflection of its intramolecular interactions and can directly influence a large number of characteristics, including mechanical properties and self-assembly behavior. These intramolecular interactions include sterics, hydrogen bonding, hydrophobic interactions, and aromatic interactions, among others. The chain conformation in turn affects how the polymer can interact with the other chains around it, which influences both the mechanical properties of the polymer and its self-assembly into various structures. Classical polymers self-assemble *via* a balance of enthalpic interactions and entropic chain stretching penalties, which can both be complicated by the conformation of the polymer chain. In polymers with more complicated chemical interactions, such as polyelectrolytes and conjugated polymers, chains are often significantly stiffer (have a longer persistence length) than classical materials. The polymer backbone itself plays a large role in intramolecular interactions leading to chain shape, and polymers with highly conjugated or sterically hindered backbones, such as polyphenylene vinylene, have longer persistence lengths in solution (6–40 nm)[1] than those with backbones composed of more aliphatic linkages, such as polystyrene (1 nm).[2] Side chains can also play a significant role in the rigidity of a polymer chain. For example, a subtle difference of one carbon in a polysilylene side chain can increase the persistence length from 6.2 nm up to 85 nm.[3] Charged side chains introduce another level of complexity due to the ionic interactions between groups that can lead to chain stiffening.[4]

Secondary structure can also have a large impact on the persistence length of a chain. Helical secondary structure in particular correlates with increasing persistence lengths in polymers. Helical polyisocyanates[5] have a persistence length of 40 nm to 50 nm, and α-helical poly(γ-benzyl-L-glutamate)[6] (PBLG) has a persistence length up to approximately 200 nm at high molecular weights. Several design methods exist for the formation of a polymer helix; in particular, designing side chain interactions is an interesting route to controlling chain shape. Side chain interactions have a large influence on the formation of secondary structures in biological polymers and thus directly influence the persistence length of these polymers as well. For example, the addition of long stretches of prolines in a polypeptide induces the formation of a helix. Using FRET experiments, the persistence length of a polyproline type II helix was estimated to range from 9 nm to 13 nm[7] using a chain that was 20 monomers long in the *all-trans* form.

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† Electronic supplementary information (ESI) available: Fits of the persistence length using the wormlike chain model over a series of polypeptoid chain lengths. In addition, the persistence length analysis for a 36-mer polypeptoid consisting of a racemic mixture of the α-chiral side chain found in **2** is also available. Finally, the results of the semiflexible cylinder model fit allowing the contour lengths to fluctuate are reported. See DOI: 10.1039/c2sm07092h

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Here, we evaluate the effect of subtle changes in side chain size and chirality on the persistence length of N-substituted glycine polymers, also known as polypeptoids. Although polypeptoids are a relatively new material, they have recently attracted much attention in polymeric studies,[8–11] meaning there is a need to quantify their properties. Quantifying polymer properties, such as flexibility and persistence length, is important for modeling these systems and further understanding their self-assembly.[12]

Polypeptoids are sequence-specific, biomimetic polymers that have been shown to form stable secondary structures in solution depending upon the types of side chains incorporated into the polymer.[9,13–15] In particular, polypeptoids with bulky α-chiral side chains form a polyproline type I-like helix in solution. Unlike the α-helices of polypeptides, the polypeptoid helices are stabilized by steric interactions, rather than hydrogen bonding. Both theoretical[16] and experimental[17–20] studies have shown that the preferred conformation of the peptoid helix is entirely composed of *cis*-amide bonds with a periodicity of three residues per turn and a pitch of approximately 6 Å. These studies showed that a polypeptoid with α-chiral aromatic side chains prefers the all *cis* conformation to a *trans* conformation in the ratio of 2 : 1. In addition, the handedness of the helix is determined by the handedness of the α-chiral side chains, as the peptoid backbone is devoid of chirality. The presence of a helical fold would lead one to expect a stiffening of the polymer chain. However, our small angle neutron scattering (SANS) measurements have shown that helical polypeptoids have persistence lengths much smaller than expected. In fact, they are nearly as flexible as polypeptoids without any secondary structure.

Experimental Methods

Materials

Compounds **1** and **2** (Fig. 1) were synthesized using a step-wise solid-phase submonomer synthesis method[21] on a custom robotic synthesizer or a commercial Aapptec Apex 396 robotic synthesizer. All polypeptoids were acetylated on the resin, cleaved from the resin using 95% v/v trifluoroacetic acid in water, and purified using reverse-phase HPLC, as previously described.[8,10] The synthesis was confirmed by electrospray mass spectrometry on an Agilent 1100 series LC/MSD trap system (Agilent Technologies, Santa Clara, CA) and by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry on a 4800 series MALDI-TOF (Applied Biosystems, Carlsbad, CA) with a laser power of 5000. MALDI samples were prepared using a 1 : 1 ratio of polypeptoid in acetonitrile (1 mg mL^{-1}) and 1,8,9-anthracenetriol (10 mg mL^{-1} in tetrahydrofuran). The monomer sequences for the polypeptoids studied here are denoted in Fig. 1, with Nme = *N*-(2-methoxyethyl)glycine, Npe = *N*-(2-phenylethyl)glycine, and Nrpe = (*R*)-*N*-(1-phenylethyl)glycine. Several polymers were made with *n* (the number of repeat units as designated in Fig. 1) varying from 3 to 8 (Table 1). The majority of this publication will discuss the polymers where *n* is equal to 6, forming a polypeptoid with 36 total monomers.

Circular dichroism (CD)

CD measurements were performed on a J-185 CD spectrometer (Jasco Inc., Easton, MD). Stock solutions of the polypeptoids were made in tared vials using 5 mg mL^{-1} of peptoid powder in acetonitrile. The stock solutions were then diluted to a concentration of 0.08 mg mL^{-1} before acquiring CD spectra. CD spectra were acquired using a quartz cell (Hellma USA, Plainview, NY) with a path length of 1 mm. A scan rate of 50 nm min^{-1} was used, and 3 measurements were averaged for each compound.

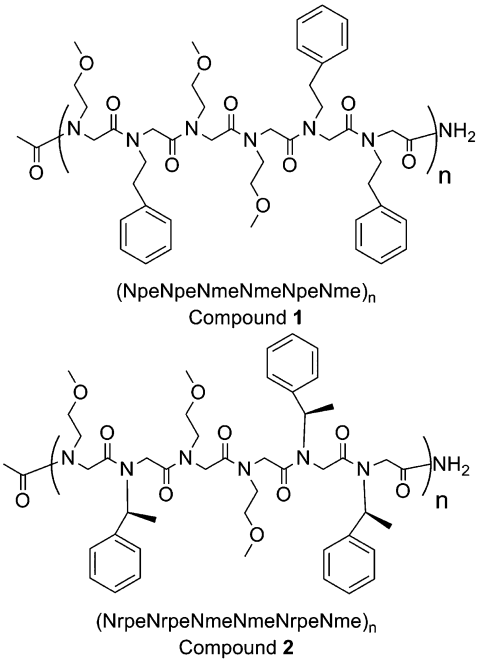


Fig. 1 **1** contains achiral aromatic side chains, while **2**, a helix-forming polypeptoid, contains alpha-chiral side chains. The value of *n* ranges from 3 to 8 for chains of varying lengths.

Small angle neutron scattering (SANS)

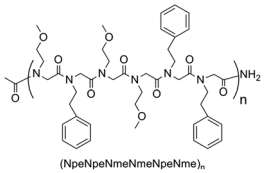
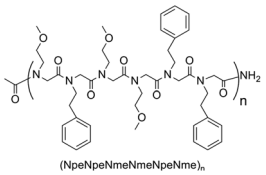
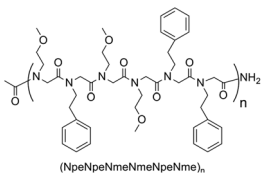
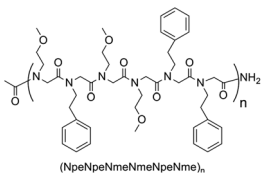
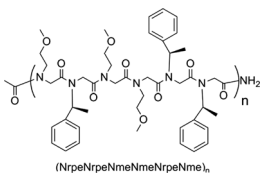
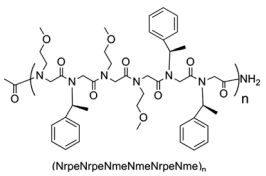
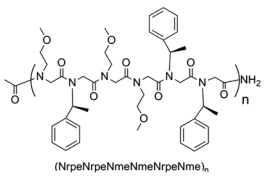
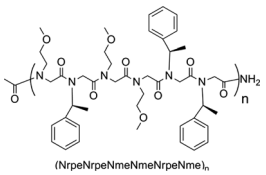
SANS studies were conducted at the NG3 SANS line at the National Institute of Standards and Technology (NIST) Center for Neutron Research in Gaithersburg, Maryland and at the CG-3 Bio-SANS line at the High Flux Isotope Reactor at Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee. Samples were prepared at a concentration of 10 mg mL^{-1} in deuterated acetonitrile to enhance the contrast between the polypeptoids and the solvent. Quartz banjo cells (Hellma USA, Plainview, NY) with a path length of 1 mm and 2 mm were used at NIST and ORNL, respectively, in a temperature controlled multiple position sample holder. A neutron wavelength of 6 Å was used at both beamlines, and data were collected at two different instrument configurations (1.3 m and 4 m at NIST, and 1.7 m and 14.5 m at ORNL). The data were reduced using the NCNR SANS reduction macros[22] and the Spice SANS reduction program in Igor Pro.

Results and discussion

Circular dichroism

Compounds **1** and **2** were designed to be non-structured and helical, respectively, through the incorporation of aromatic side chains with tunable chirality. Compound **2** contains 50% α-chiral aromatic side chains while compound **1** contains 50% achiral

Table 1

Compound	Repeat unit	*n* (number of repeat units)	*N* (total number of monomers)	M_{obs}/M_{theo}	Structure
Compound **1a**	 (NpeNpeNmeNmeNpeNme)$_n$	3	18	2548.5/2546	Non-helical
Compound **1b**	 (NpeNpeNmeNmeNpeNme)$_n$	4	24	3379.4/3375	Non-helical
Compound **1c**	 (NpeNpeNmeNmeNpeNme)$_n$	6	36	5033.2/5033	Non-helical
Compound **1d**	 (NpeNpeNmeNmeNpeNme)$_n$	8	48	6701.6/6691.1	Non-helical
Compound **2a**	 (NrpeNrpeNmeNmeNrpeNme)$_n$	3	18	2549.2/2546	Helical
Compound **2b**	 (NrpeNrpeNmeNmeNrpeNme)$_n$	4	24	3377.5/3375	Helical
Compound **2c**	 (NrpeNrpeNmeNmeNrpeNme)$_n$	6	36	5028.2/5033	Helical
Compound **2d**	 (NrpeNrpeNmeNmeNrpeNme)$_n$	8	48	6604.4/6691.1	Helical

aromatic side chains instead. Previous literature has shown that **2** forms a polyproline type I-like helix in solution with all *cis* amide bonds[14] as described above. However, **1** was designed to be minimally structured by removing the α-chiral substituent that provides the steric influence for secondary structure formation. Indeed, circular dichroism (Fig. 2) in acetonitrile shows that there is helix formation of **2** as demonstrated by the characteristic peaks at 192 nm, 202 nm, and 218 nm. This helix formation is constant across several polymers of varying molecular weights (**2a** through **2d**) with little deviation in the per-residue molar

ellipticity. Previously, it was shown that as the chain length of a polypeptoid containing 100% Nrpe residues increases, the per-residue molar ellipticity also increases until a chain length of 13 residues is reached. After 13 residues, the ellipticity remains approximately constant for longer chain lengths, suggesting that the overall fraction of helical isomers is stabilized.[20] A similar trend was also observed for peptoid helices consisting of bulky *N*-1-naphthylethyl side chains, except in that case, the per-residue molar ellipticity reached a maximum after only 5 monomer units.[17]

It would be helpful to gain some quantitative insight about the helical content of these molecules using CD, as is often done for proteins. However, CD is a quantitative technique for proteins because there is a vast number of known protein structures, allowing the development of algorithms that can compute a reliable estimate of the fraction of α-helices, β-sheets, and random coils by comparing new CD data to that of the known structures.[23]There are few X-ray solved structures of polypeptoids, meaning that it is not possible to reliably calculate the fraction of helicity for polypeptoids from CD.

Because CD does not yield information about the population of conformers for polypeptoids, polypeptoid secondary structure has previously been established using a combination of 2D NMR, X-ray crystallography, and molecular modeling studies. These studies first confirmed the presence of a helical conformation in a very short polypeptoid (5 monomers in length) containing bulky chiral, aromatic residues ((*S*)-*N*-(1-phenylethyl)glycine, Nspe).[16] However, the 2D NMR studies for $Nspe_5$ also show the presence of other isomers and conformations in the amount of approximately 40% in methanol solution.[17,18] Thus, the α-helix-like CD signature observed for $(Nspe)_5$ and other peptoid helices is from an ensemble of closely related conformations in rapid equilibrium with one another.

The presence of these other polypeptoid conformers is most likely due to the *cis*/*trans* isomerization of the backbone amide bonds, which may enable the polypeptoid backbone to sample many conformations. To probe whether the fraction of helices can be controlled, the effect of temperature and solvent on the per-residue molar ellipticity was examined. As **2c** is heated from 20 °C to 70 °C in acetonitrile (Fig. 3a), there is no change in the spectrum shape and only a slight decrease in the peak intensity at 218 nm from 19 200 deg cm^2 dmol^{-1} to 15 300 deg cm^2 dmol^{-1}. This result is consistent with the observation by Wu *et al.* that $Nrpe_6$, $Nrpe_{12}$, and $Nrpe_{18}$ all retain their helical CD signature at increased temperature, suggesting that the peptoid helices are stable to thermal unfolding because they are sterically constrained rather then hydrogen bond-stabilized.[20] This is the case for all of the helical polypeptoids investigated here, as shown in the ESI.† In addition, changing the solvent from acetonitrile to methanol has a minimal effect on the spectrum shape and intensity at increased temperature (Fig. 3b). This result is also consistent with previous studies: Armand *et al.* previously observed a peptoid helix in methanol solution using 2D NMR.[18] These experiments indicate that the peptoid helices are stable to both temperature and solvent.

All of the achiral polypeptoids studied here (**1a** to **1d**) show no net ellipticity because they do not contain chiral residues. Hence, little information about their structure can be gained using CD. Small angle neutron scattering (SANS) is therefore used to probe the difference in chain statistics for these two series of molecules.

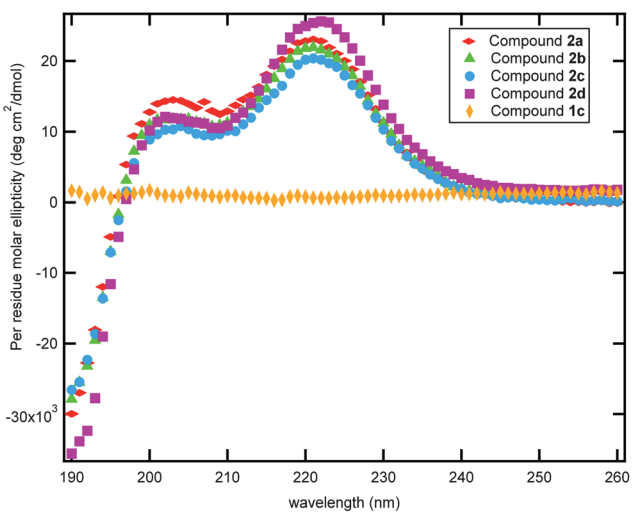


Fig. 2 CD spectra for different chain lengths of a helical polypeptoid as well as a non-helical polypeptoid.

Small angle neutron scattering (SANS)

Because **2** forms a helix in solution, it was anticipated that its chain would be stiffer than the corresponding analog **1**; however, the SANS experiments detailed here show that the difference in chain stiffness is not as large as expected. Plotting the scattering intensity, I, *versus* the scattering angle, q, (Fig. 4a) for the two 36-mer compounds yielded several insights about the polypeptoid chains. First, the two sequences both have typical scattering patterns for a single chain in dilute solution. It is expected that the intensity should scale with q as -2 for a random coil conformation and scale as -1 for a rod-like conformation. For a single chain, one should see the change in scaling behavior provided the appropriate q-range. For both polypeptoids, there was an exponential decrease over the q-range from 0.07 Å^{-1} to 0.22 Å^{-1} with a scaling of approximately -1.5. Around $q \approx 0.22$ Å^{-1}, the intensity scales as 0.6–0.8. The deviation from -2 scaling indicates that the polypeptoids are not forming Gaussian coils in solution, while the deviation from -1 scaling is most likely a result of noise in the data at high q.

To see the change in scaling better, a Kratky plot was used (Fig. 4b), which plots Iq^2 *vs.* q. This has the effect of making the intensity data in the Gaussian regime tend toward a horizontal asymptote. The q-value at which the data deviate from this asymptote and begin to increase linearly (with an intercept coinciding with the origin) is inversely related to the persistence length. Because the intensity does not quite scale as -2 with q, it is difficult to pinpoint the exact transition. However, a good approximation was made by selecting the point at which Iq^2 deviates from a straight line passing through the origin (the red line in Fig. 4b). A straight line passing through the origin on a Kratky plot corresponds to the scattering function for a rod; thus, the departure from this behavior indicates scattering from a molecule above its persistence length. The approximate q-value for this transition is marked by the dashed line in Fig. 4b, and it is clear that it is quite similar for both sequences. To determine the

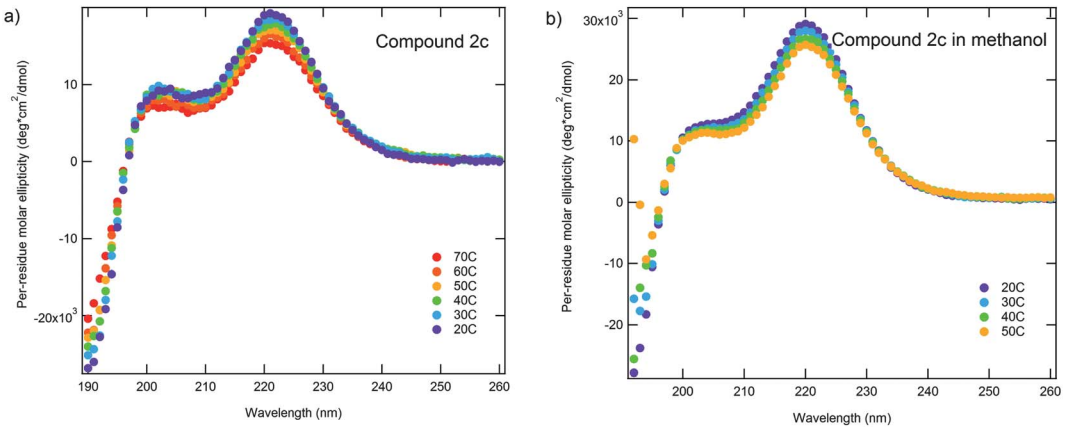


Fig. 3 Heating (a) and solvent (b) do not significantly affect the CD spectra of **2c**.

persistence length, the equation $L_p = k/q^*$ was used, where k is a proportionality constant and q^* is the q-value for the transition in the Kratky plot. The value of the constant k given in literature is $6/\pi$ by Kratky[24] and Koyama,[25] or 2.87, according to Burchard and Kajiwara.[26] The proportionality constants available indicate that the persistence length for both **1** and **2** is on the order of 0.8 nm to 1.3 nm. Thus, both **1** and **2** are more flexible than other helical polymers, including helical polypeptides. In fact, these polypeptoids have a flexibility very similar to that of polystyrene[2] (~1 nm).

Semi-flexible cylinder model. Because the determination of the inflection point on the Kratky plot can be somewhat subjective, a more precise determination of the persistence length can be obtained by modeling and comparison to experimental data. The NIST NCNR analysis macro has been used to model these molecules as semi-flexible cylinders with excluded volume,[27] according to the Kratky and Porod model of a wormlike chain. In this model, the cylinder of the chain is assumed to be composed of a series of connected locally stiff chain segments. The length of these segments is called the Kuhn length and is calculated by holding the scattering length densities and the contour length constant and fitting a Kuhn length and a radius to the semi-flexible cylinder. The equations for this model are described in the ESI.† Previously, 2D solution NMR was used to estimate a pitch of approximately 0.6 nm for a similar peptoid helix.[18] Based on this value for the pitch and the number of turns expected in **2c** (12, as there are 3 residues per turn), it is expected that the peptoid helix will have a contour length of approximately 7.2 nm. This value was used as the contour length for **2c**. For compound **1c**, the contour length was held at 13.0 nm, which corresponds to the distance along the peptoid backbone if all of the amide bonds are in the *trans* configuration. Table 2 shows the results of the fit. Interestingly, the persistence lengths are found to be quite similar: approximately 0.5 nm for **1c** and 1.0 nm for **2c**. Compound **2c** has a longer persistence length than **1c**, but it clearly is not stiff relative to other polymers containing secondary structure, such as PBLG molecules.[6] The persistence lengths calculated from this semiflexible cylinder model match relatively well with the range estimated from the Kratky plot. Additionally, if the contour lengths are allowed to be fit by the

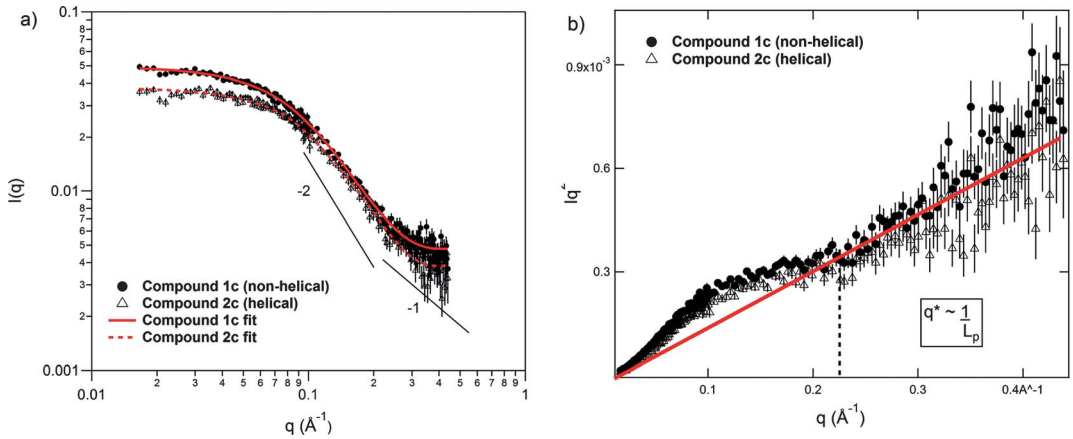


Fig. 4 Small angle neutron scattering (SANS) shows the relatively short persistence length of both polypeptoids. The model fits are shown in (a), demonstrating good fits for the data. In addition, lines with scalings of −1 (rigid rod) and −2 (Gaussian coil) have been added as references. The intensity presented here is absolute intensity in units of cm^{-1}. The Kratky plot in (b) emphasizes the change in scaling behavior. Incoherent background has been subtracted from the data before the fits.

Table 2 Fitted parameters for compounds **1** and **2** with **n** = **6**. Compound **2** has a shorter contour length and a longer persistence length than **1**, indicating its helical conformation imparts some stiffness to the chain

Polypeptoid	Contour length (nm)	Persistence length[a] (nm)	Persistence length[b] (nm)	Radius (nm)[a]
1c	13.0	0.51 ± 0.04	0.56	0.93 ± 0.2
2c	7.2	1.05 ± 0.08	1.12	0.99 ± 0.3

[a] As fitted by the flexible cylinder model. [b] As fitted by the wormlike chain model.

semiflexible cylinder model, the same trend holds; compound **2c** has a shorter contour length and a larger persistence length than **1c**. These results are presented in the ESI (Table S1).†

The model fit indicates that the helical conformation of **2c** most likely imparts some rigidity to the polymer. However, **2c** still has a relatively short persistence length when compared to other helical polymers. Although the circular dichroism implies that the helical conformation is favored, the short persistence length indicates that the polymer can sample many different conformations and only a portion of the polymer chains adopt a full helical conformation at any given point in time. In addition, the helix observed here may simply be quite flexible. Previous intrinsic viscosity measurements of polymeric helical (*S*)-*N*-(1-phenylethyl)glycines (from 4–40 kg mol^{-1}) in DMF were consistent with random coil behavior such that Guo *et al.* concluded the persistence length of these chains must be less than 9 nm.[11] Flexible helical polymers have also previously been seen, such as in the case of chiral poly(2-oxazoline)s[28] where the polymer showed CD signal, indicating helix formation, but scattering indicated a random coil chain conformation.

Polyproline helices have also been observed to be flexible; in this case, the proline group creates a tertiary amide similar to those in the polypeptoid backbone. For tertiary amides, the energy barrier to rotation about the C–N bond is much lower than for the secondary amides that dominate proteins.[38] In addition, the *cis*/*trans* configurations are much closer in energy for tertiary amides,[29–31] and thus the isomerization can occur much more readily. Previously, the activation energy for the *cis*/*trans* isomerization in dimer peptoids was measured to be on the order of 17–20 kcal mol^{-1},[32] which is similar to the energies measured for prolyl peptide bonds.[29,33,34] It is well known that prolyl peptide bonds are expected to have much higher *cis* : *trans* ratios (1 : 3) compared to planar peptide bonds (~1 : 1000)[35,36] and that the polyproline I helix, which consists of all *cis* bonds, has a much shorter persistence length than that of a traditional peptide α-helix such as PBLG.[7] Because the peptoid helices studied here were also shown to have a relatively high *cis* : *trans* ratio (~2–3 : 1),[16–18] it should not be entirely unexpected that they are quite flexible. Recently, the introduction of much bulkier side groups (*N*-1-naphthylethyl) has been shown to raise the *cis* : *trans* ratio to >19 : 1,[17] suggesting that even larger substituents can increase the energy barrier of backbone rotation and therefore increase chain stiffness.

Wormlike chain model. Further information about the chain conformation and the persistence length can be obtained by evaluating the radius of gyration over a series of chain lengths and fitting the wormlike chain formula. To obtain the radius of gyration (R_g), a line was fit to the scattering data in a Guinier plot ($\ln I(q)$ *vs.* q^2). For compounds **1c** and **2c**, the R_g values differ slightly, yielding a value of 14.7 ± 0.1 Å and 13.3 ± 0.1 Å, respectively. This small decrease in R_g is most likely due to the more compact packing of the helical compound that stems from its secondary structure.

The wormlike chain formula[37] relates R_g to L_p:

$$R_g^2 = \frac{LL_p}{3} - L_p^2 + \frac{2L_p^3}{L}\left[1 - \frac{L_p}{L}\left(1 - e^{-L/L_p}\right)\right] \quad (1)$$

where L is the contour length as calculated using the geometry of the molecule and the previous 2D NMR studies. Using this value and the measured R_g, it is possible to solve the equation for the persistence length. Table 2 shows the values for the persistence length as determined by this method. This analysis supports the conclusions drawn from the semiflexible cylinder model. The persistence length for **2c** is slightly longer than that of **1c** (1.12 nm *vs.* 0.56 nm), but they are both short on an absolute scale.

As calculated by the wormlike chain model, the persistence length is plotted against the number of monomers for chains of 18, 24, 36, and 48 residues (Fig. 5). The first conclusion from this plot is that **2** consistently has a higher persistence length than **1**. Additionally, the shorter molecules have higher fitted persistence lengths, especially in the case of the helical molecule. The helical chain of 18 monomers could not fit the wormlike chain model with any reasonable value of the persistence length, indicating that the 18-mers are too short to be treated using this analysis. This is probably not due to actual differences in the number of residues present in the helical conformation as CD has shown that all of the different length polymers have very similar per-residue molar ellipticities. It is more likely that the wormlike chain model is not valid for short chains where the persistence length is not sufficiently shorter than the contour length. However, the chains of 36 and 48 monomers have reached an asymptote in their persistence length, suggesting that these polymers are of sufficient length to treat using the wormlike chain model. It is possible to fit the wormlike chain model

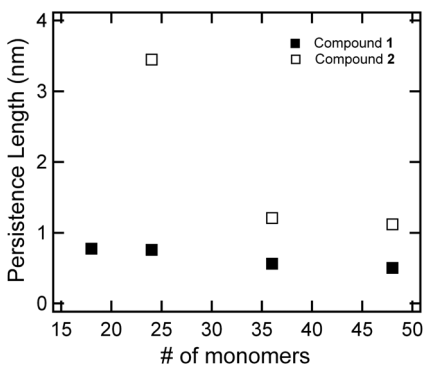


Fig. 5 The persistence lengths of polypeptoids ranging in length from 18 to 48 monomers (Compound 1 series and Compound 2 series) as determined from the wormlike chain model are plotted *versus* the number of monomers. The shorter chains have significantly higher persistence lengths, indicating that the molecules are too short to be treated using this method.

simultaneously to the polymers of different lengths and obtain a persistence length for each type of polymer. This analysis is presented in the ESI (Fig. S1) and yields very similar values for the persistence lengths.†

Conclusions

In conclusion, the SANS study presented here yields two main insights into the chain conformation of polypeptoids. First, the polypeptoids studied here are inherently flexible in solution with persistence lengths ranging from 0.5 nm to 1.0 nm. Second, both the semiflexible cylinder model and the wormlike chain model indicate that inducing helicity by introducing bulky α-chiral 1-phenylethyl side chains into a 36-mer polypeptoid results in a small increase in the persistence length or rigidity of the molecule. However, the fitted persistence length is still quite short in comparison to other helical polymers, suggesting that the polymer retains considerable conformational freedom. In agreement with previous 2D solution NMR studies and intrinsic viscosity measurements, the SANS data presented here indicates that helical polypeptoids with α-chiral, bulky phenyl side chains prefer an all *cis*-amide bond configuration in solution but can readily isomerize to sample other conformations as well.

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References

1 C. L. Gettinger, A. J. Heeger, J. M. Drake and D. J. Pine, A photoluminescence study of poly(phenylene vinylene) derivatives - the effect of intrinsic persistence length, *J. Chem. Phys.*, 1994, **101** (2), 1673–1678.
2 A. Brulet, F. Boue and J. P. Cotton, About the experimental determination of the persistence length of wormlike chains of polystyrene, *J. Phys. II*, 1996, **6**(6), 885–891.
3 K. Terao, Y. Terao, A. Teramoto, N. Nakamura, I. Terakawa and T. Sato, Stiffness of polysilylenes depending remarkably on a subtle difference in chiral side chain structure: Poly{*n*-hexyl-[(*S*)-2-methylbutyl]silylene)} and poly{*n*-hexyl-[(*S*)-3-methylpentyl] silylene}, *Macromolecules*, 2001, **34**(8), 2682–2685.
4 S. Muller-Spath, A. Soranno, V. Hirschfeld, H. Hofmann, S. Ruegger, L. Reymond, D. Nettels and B. Schuler, Charge interactions can dominate the dimensions of intrinsically disordered proteins, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**(33), 14609–14614.
5 H. Gu, Y. Nakamura, T. Sato, A. Teramoto, M. M. Green and C. Andreola, Global conformations of chiral polyisocyanates in dilute solution, *Polymer*, 1999, **40**(4), 849–856.
6 E. Temyanko, P. S. Russo and H. Ricks, Study of rodlike homopolypeptides by gel permeation chromatography with light scattering detection: Validity of universal calibration and stiffness assessment, *Macromolecules*, 2001, **34**(3), 582–586.
7 R. B. Best, K. A. Merchant, I. V. Gopich, B. Schuler, A. Bax and W. A. Eaton, Effect of flexibility and *cis* residues in single-molecule FRET studies of polyproline, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**(48), 18964–18969.
8 H. K. Murnen, A. M. Rosales, J. N. Jaworsk, R. A. Segalman and R. N. Zuckermann, Hierarchical Self-Assembly of a Biomimetic Diblock Copolypeptoid into Homochiral Superhelices, *J. Am. Chem. Soc.*, 2010, **132**(45), 16112–16119.
9 K. T. Nam, S. A. Shelby, P. H. Choi, A. B. Marciel, R. Chen, L. Tan, T. K. Chu, R. A. Mesch, B. C. Lee, M. D. Connolly, C. Kisielowski and R. N. Zuckermann, Free-floating ultrathin two-dimensional crystals from sequence-specific peptoid polymers, *Nat. Mater.*, 2010, **9**(5), 454–460.
10 A. M. Rosales, H. K. Murnen, R. N. Zuckermann and R. A. Segalman, Control of Crystallization and Melting Behavior in Sequence Specific Polypeptoids, *Macromolecules*, 2010, **43**(13), 5627–5636.
11 L. Guo, J. H. Li, Z. Brown, K. Ghale and D. H. Zhang, Synthesis and Characterization of Cyclic and Linear Helical Poly(alpha-peptoid)s by *N*-Heterocyclic Carbene-Mediated Ring-Opening Polymerizations of N-Substituted *N*-Carboxyanhydrides, *Biopolymers*, 2011, **96**(5), 596–603.
12 N.P.B., H. B. Eitouni, Thermodynamics of Polymer Blends, *Physical Properties of Polymers Handbook*, ed. J. E. Mark, 2nd edn, 2007, ch. 19, pp. 339–356, Springer, New York.
13 S. A. Fowler and H. E. Blackwell, Structure-function relationships in peptoids: Recent advances toward deciphering the structural requirements for biological function, *Org. Biomol. Chem.*, 2009, **7** (8), 1508–1524.
14 K. Kirshenbaum, A. E. Barron, R. A. Goldsmith, P. Armand, E. K. Bradley, K. T. V. Truong, K. A. Dill, F. E. Cohen and R. N. Zuckermann, Sequence-specific polypeptoids: A diverse family of heteropolymers with stable secondary structure, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**(8), 4303–4308.
15 B. Yoo and K. Kirshenbaum, Peptoid architectures: elaboration, actuation, and application, *Curr. Opin. Chem. Biol.*, 2008, **12**(6), 714–721.
16 P. Armand, K. Kirshenbaum, A. Falicov, R. L. Dunbrack, K. A. Dill, R. N. Zuckermann and F. E. Cohen, Chiral N-substituted glycines can form stable helical conformations, *Folding Des.*, 1997, **2**(6), 369–375.
17 J. R. Stringer, J. A. Crapster, I. A. Guzei and H. E. Blackwell, Extraordinarily Robust Polyproline Type I Peptoid Helices Generated *via* the Incorporation of Î±-Chiral Aromatic *N*-1-Naphthylethyl Side Chains, *J. Am. Chem. Soc.*, 2011.
18 P. Armand, K. Kirshenbaum, R. A. Goldsmith, S. Farr-Jones, A. E. Barron, K. T. V. Truong, K. A. Dill, D. F. Mierke, F. E. Cohen, R. N. Zuckermann and E. K. Bradley, NMR determination of the major solution conformation of a peptoid pentamer with chiral side chains, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**(8), 4309–4314.
19 C. W. Wu, T. J. Sanborn, K. Huang, R. N. Zuckermann and A. E. Barron, Peptoid oligomers with alpha-chiral, aromatic side chains: Sequence requirements for the formation of stable peptoid helices, *J. Am. Chem. Soc.*, 2001, **123**(28), 6778–6784.

20 C. W. Wu, T. J. Sanborn, R. N. Zuckermann and A. E. Barron, Peptoid oligomers with alpha-chiral, aromatic side chains: Effects of chain length on secondary structure, *J. Am. Chem. Soc.*, 2001, **123** (13), 2958–2963.

21 G. M. Figliozzi, R. Goldsmith, S. C. Ng, S. C. Banville and R. N. Zuckermann, Synthesis of N-substituted glycine peptoid libraries, *Comb. Chem.*, 1996, **267**, 437–447.

22 S. R. Kline, Reduction and analysis of SANS and USANS data using IGOR Pro, *J. Appl. Crystallogr.*, 2006, **39**, 895–900.

23 S. M. Kelly, T. J. Jess and N. C. Price, How to study proteins by circular dichroism, *Biochim. Biophys. Acta, Proteins Proteomics*, 2005, **1751**(2), 119–139.

24 O. Kratky, Das Studium Geloster Fadenmolekule Mittels Der Rontgenkleinwinkelmethode, *Kolloid-Zeitschrift and Zeitschrift Fur Polymere*, **182**(1–2), 7.

25 R. Koyama, Light-scattering of stiff chain polymers, *J. Phys. Soc. Jpn.*, 1973, **34**(4), 1029–1038.

26 W. Burchard and K. Kajiwara, Statistics Of Stiff Chain Molecules.1. Particle Scattering Factor, *Proc. R. Soc. London, Ser. A*, 1970, **316** (1525), 185.

27 J. S. Pedersen and P. Schurtenberger, Scattering functions of semiflexible polymers with and without excluded volume effects, *Macromolecules*, 1996, **29**(23), 7602–7612.

28 M. M. Bloksma, S. Rogers, U. S. Schubert and R. Hoogenboom, Secondary structure formation of main-chain chiral poly(2-oxazoline)s in solution, *Soft Matter*, 2010, **6**(5), 994–1003.

29 W. J. Wedemeyer, E. Welker and H. A. Scheraga, Proline cis-trans isomerization and protein folding, *Biochemistry*, 2002, **41**(50), 14637–14644.

30 S. S. Zimmerman and H. A. Scheraga, Stability Oof *Cis*, *Trans*, And Nonplanar Peptide Groups, *Macromolecules*, 1976, **9**(3), 408–416.

31 B. Maigret, D. Perahia and B. Pullman, Molecular Orbital Calculations On Conformation Of Polypeptides And Proteins.4. Conformation Of Prolyl And Hydroxylprolyl Residues, *J. Theor. Biol.*, 1970, **29**(2), 275.

32 Q. Sui, D. Borchardt and D. L. Rabenstein, Kinetics and equilibria of *cis*/*trans* isomerization of backbone amide bonds in peptoids, *J. Am. Chem. Soc.*, 2007, **129**(39), 12042–12048.

33 S. Fischer, R. L. Dunbrack and M. Karplus, *Cis-Trans* Imide Isomerization Of The Proline Dipeptide, *J. Am. Chem. Soc.*, 1994, **116**(26), 11931–11937.

34 I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela and E. Katchalski, The Configurational Changes of Poly-L-proline in Solution, *J. Am. Chem. Soc.*, 1960, **82**(20), 5263–5279.

35 M. S. Weiss, A. Jabs and R. Hilgenfeld, Peptide bonds revisited, *Nat. Struct. Biol.*, 1998, **5**(8), 676–676.

36 P. Chakrabarti and D. Pal, The interrelationships of side-chain and main-chain conformations in proteins, *Prog. Biophys. Mol. Biol.*, 2001, **76**(1–2), 1–102.

37 H. Benoit and P. Doty, Light Scattering from Non-Gaussian Chains, *J. Phys. Chem.*, 1953, **57**(9), 958–963.

38 C. Dugave, *Cis-trans isomerization in Biochemistry,* Wiley-VCH, Freiburg, Germany, 2006.

