Conformations of peptoids in nanosheets result from the interplay of backbone energetics and intermolecular interactions

John R. Edison⁹, Ryan K. Spencer⁶, Glenn L. Butterfoss⁵, Benjamin C. Hudson⁶, Allon I. Hochbaum⁵, Anant K. Paravastu⁵, Ronald N. Zuckermann⁵, and Stephen Whitelam⁴,¹

⁹Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; ⁶Department of Chemical Engineering & Materials Science, University of California, Irvine, Irvine, CA 92697; ¹Center for Genomics and Systems Biology, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates; and ²School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332

Edited by Ken A. Dill, Stony Brook University, Stony Brook, NY, and approved April 13, 2018 (received for review January 8, 2018)

The conformations adopted by the molecular constituents of a supramolecular assembly influence its large-scale order. At the same time, the interactions made in assemblies by molecules can influence their conformations. Here we study this interplay in extended flat nanosheets made from nonnatural sequence-specific peptoid polymers. Nanosheets exist because individual polymer backbone elements can be linear and untwisted, by virtue of polymer backbone elements adopting alternating rotational states whose twists oppose and cancel. Using molecular dynamics and quantum mechanical simulations, together with experimental data, we explore the design space of flat nanostructures built from peptoids. We show that several sets of peptoid backbone conformations are consistent with their being linear, but the specific combination observed in experiment is determined by a combination of backbone energetics and the interactions made within the nanosheet. Our results provide a molecular model of the peptoid nanosheet consistent with all available experimental data and show that its structure results from a combination of intra- and intermolecular interactions.

The shape of a molecule influences how it packs and assembles, and the interactions made upon assembly can influence the shape of the molecule (1). This interplay is seen in simple molecules with few degrees of freedom (2) and in complex molecules, such as proteins, with many degrees of freedom (3). Thus, the conformations of molecules in self-assembled structures do not reflect the ensemble of conformations accessible in solution, and inspection of the conformations of a single molecule does not necessarily suggest which one it adopts, or what superstructure it makes, upon self-assembly. Here we use classical and quantum simulation methods and experimental data (4) to extend our understanding of the local conformations and molecular order of peptoid polymers within bilayer nanoscale assemblies called nanosheets. The residues of nanosheet-forming peptoids are arranged in an alternating sequence of hydrophobic and hydrophilic monomers, with the latter having opposite charge and showing that its structure results from a combination of intra- and intermolecular interactions.

Significance

Commonly observed secondary structures of proteins, such as α-helices and β-sheets, are built from a trans-amide backbone with residues sampling a single region of the Ramachandran plot. Here we report a secondary structure displayed by biomimetic peptoid polymers in which the backbone exhibits the cis-amide conformation and alternating residues display rotational states of opposed (pseudo)chirality. This structure is linear and untwisted and enables strands to pack densely into extended bilayer nanosheets.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Published under the PNAS license.

¹To whom correspondence should be addressed. Email: swhitelam@lbl.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1800397115/-/DCSupplemental.
successive residues spontaneously adopt an alternating pattern in which (on average) \((\phi_i, \psi_i) = (75°, -145°)\) and \((\psi_{i+1}, \phi_{i+1}) = (135°, -75°)\). These values are related by a reflection about the negative-sloping diagonal of the Ramachandran plot \((12, 13)\); i.e., they are twist-opposed states that satisfy \((\phi_{i+1}, \psi_{i+1}) = (-\psi_i, -\phi_i)\). Those simulations were initiated with residues in the \textit{trans} state \((\omega_i = \omega_{i+1} \approx 180°)\), a conformation that disposes the peptoid backbone toward linearity. For \textit{trans} residues, any choice of angle pairs of this nature causes the bonds N-C\(_{\alpha}\) of residue \(i\) and C\(_{\alpha}\)-C\(_{\mathrm{O}}\) of residue \(i + 1\) to point in the same direction (Fig. 1A) and so produces linear, untwisted strands (Fig. 1C). The same is not true of \textit{cis} residues (Fig. 1B), for which only a small subset of the Ramachandran diagram leads to linear strands (Fig. 1D). Moreover, the regions of the Ramachandran plot favored energetically by the isolated peptoid backbone (enclosed by the red contours in Fig. 1C and D) are consistent with linear strands for the \textit{trans} backbone but not for \textit{cis}. A similar conclusion is drawn by considering, in Fig. 1F, the linearity of \textit{cis} strands under a second design rule, in which adjacent rotational states possess opposing chirality. Such states satisfy
\((\phi_{i+1}, \psi_{i+1}) = (-\phi_i, -\psi_i)\) and are related by successive reflections in the two diagonals of the Ramachandran plot. This design rule was studied earlier in the context of polypeptides with alternating D and L amino acids \((10, 14)\). A small section of the energetically favored region of the Ramachandran plot corresponds to linear strands, but much less than trans under the previous rule (Fig. 1C).

Thus, a geometrical analysis of the peptoid backbone indicates that the trans conformation achieves linearity of the backbone in a way that is natural in a geometric sense and preferred in an energetic sense. However, new solid-state NMR experiments indicate that peptoid backbones in nanosheets are predominantly cis \((4)\). The NMR study focused on backbone residues 7, 8, and 15, because our simulations predict that these residues experience different environments (Fig. 2). The residues of nanosheet-forming peptoids are alternately hydrophobic and hydrophilic, with the latter having opposite charge and being segregated into blocks by charge type (Fig. 2A and B). As a

---

**Fig. 2.** The cis nanosheet (B, D, F, J, and L) is more ordered and more densely packed than the trans nanosheet (A, C, E, G, I, and K). (A and B) A single peptoid taken from a nanosheet, with atoms colored by residue name. In A–J positively charged N-(2-aminoethyl) glycine residues are shown in red, negatively charged N-(2-carboxyethyl) glycine residues are shown in black, and N-(2-phenylethyl) glycine residues are shown in tan. (C and D) Top view of the backbone atoms of the top layer of nanosheets. (E and F) Phenyl rings belonging to the top layer of nanosheets. Insets show the orientation distribution of the phenyl groups plotted on a unit sphere. (I and J) Side views of the nanosheets. All renderings are done from MD configurations using visual molecular dynamics (VMD) \((15)\) and Persistence of Vision Raytracer. (K and L) Top view of alternating sarcosine, N-(2-phenylethyl) glycine monolayers optimized using ab initio simulations at the M06-2X/6-31G\(^\ast\) level of theory under 2D periodic boundary conditions (unit cell shown in tan and replicates in maroon). We also show the side view of one of the strands to highlight the backbone structure (compare with Fig. 1 G and H).
result, the electrostatic energy of the nanosheet is minimized when polymers adopt a “brick” configuration in which certain residues, including residues 7 and 8, sit adjacent to residues in neighboring peptoids, while residue 15 sits adjacent to gaps in the structure (7, 9). NMR shows that residues 7 and 8 in peptoid backbones adopt the cis conformation with high probability and that residue 15 is equally likely to be cis or trans, thereby indicating that these residues indeed experience different environments in the nanosheets. The high cis propensity of residues 7 and 8, however, is not consistent with the existing nanosheet model (9) and therefore warrants another modeling study.

**Nanosheet Design**

To perform this study we built two versions of the nanosheet, using either all-trans or all-cis backbones; details can be found in *SI Appendix, 1. Simulation setup*. We used a staged scheme to obtain ordered nanosheets. First, we built peptoid polymers into brick nanosheet structures (7, 9), solvated them, and simulated them using Nanoscale Molecular Dynamics (NAMD) (16) in the NPT (fixed number of particles N, pressure P, temperature T) ensemble (simulations done using the constant-tension ensemble yielded similar results). Upon initial relaxation some parts of these nanosheets became disordered, but a majority of backbones adopted the ordered forms shown in Fig. 1 G and H. These are \( \Sigma \) (“sigma”) strands (9) whose residues alternate between the rotational states shown in Fig. 1 I and J, respectively [the cis-\( \Sigma \) strand’s rotational states are similar to those of the “\( \omega \)-strand” motif found in crystalline peptoid trimers (17)]. Second, we built structures in which all polymers were initialized with these rotational states (*SI Appendix, Fig. S1*); these nanosheets remained stable and ordered upon 200 ns of simulation.

In Fig. 2 we show that the cis nanosheet is more densely packed (\( \geq 31\% \) more chains per unit area) and has a higher degree of order than the trans nanosheet. The backbones of the cis nanosheet are linear, with the exception of a kink at the middle residue (which sits adjacent to gaps in the brick pattern), and the phenyl rings in the interior of the sheet exhibit both positional and orientational order. As a result, the interpeptoid interactions made in the cis nanosheet are more favorable than those made in the trans nanosheet, and the sum of the intra- and interpeptoid energies per molecule of the cis nanosheet is lower, by about 9 \( \kappa_b T \) (5.3 kcal/mol) per residue, than that of the trans nanosheet. In other words, the interactions made by peptoids within the cis nanosheet compensate for the penalty incurred to keep the cis backbone in a linear configuration.

Our quantum mechanical calculations are consistent with structures obtained from molecular-dynamics simulations. Model chains of alternating sarcosine, \( N \)-(2-phenylethyl) glycine residues optimized at the M06-2X/6-31G* level of theory (18) under 2D periodic boundary conditions with a unit cell of two four-residue chains yield all-trans and all-cis strands consistent with the \( \Sigma \) motifs shown in Fig. 2 K and L (compare with Fig. 1 G and H). The aromatic rings in the phenyl layer of the all-cis system are linear, with the exception of a kink at the middle residue, which sits adjacent to gaps in the brick pattern.

**Table 1. Experimental and simulation measurements**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value, Å</th>
<th>Experimental technique</th>
<th>Simulation trans, Å</th>
<th>Simulation cis, Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of nanosheets</td>
<td>30</td>
<td>AFM</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td>Spacing between strands</td>
<td>4.5</td>
<td>XRD</td>
<td>4.8</td>
<td>4.45</td>
</tr>
<tr>
<td>Lamellar spacing between sheets</td>
<td>28</td>
<td>XRD</td>
<td>23</td>
<td>30</td>
</tr>
</tbody>
</table>

AFM, atomic force microscopy; XRD, X-ray diffraction.

Fig. 3. The cis nanosheet’s properties agree with experimental measurements. (A and B) X-ray diffraction scattering spectra \( I^*(q) \) vs. \( q \) of the simulated cis and trans nanosheets computed using the Debye scattering equation (for details see *SI Appendix, 4. Simulation of X-ray Scattering*). (C) Height distribution of the simulated cis and trans nanosheets plotted together with Gaussian curves of mean \( \mu \) and SD \( \sigma \) that best fit the data. (D) Free-energy profiles for rotation of the amide bond (\( \omega \)) in residues 6/7 and 14/15 in a randomly chosen chain in an all-cis nanosheet. (E) Snapshot from our biased simulations (using softened dihedral angle potential terms) that reveal an increased propensity to form trans amides at the hinge region (residue 15) of the nanosheet. Backbone atoms are shown in blue if they are cis and in red if they are trans. The two shaded gray circles highlight residues 6/7 and 14/15 of a strand. *Inset* shows the fraction of trans-like residues as a function of residue number after 20 ns of our biased simulation.
show an edge-face interstrand packing pattern consistent with the molecular dynamics (MD) simulations (Fig. 2L).

As shown in Table 1 and Fig. 3A and B, the dimensions of the cis nanosheet are in better agreement with experiment than those of the trans nanosheet. We note that NMR data (and indeed all experimental data summarized in this paper) are obtained from dry nanosheets. We do not have a direct probe of the charge distribution of the hydrophilic residues in the dry state. However, the consistency between the results from the dry experiments and the solvated and dry cis-nanosheet simulations indicates that the hydrophilic residues are likely to remain maximally charged when dried (simulation methods for dry and wet nanosheets are described in SI Appendix, 1. Simulation setup and 4. Simulation of X-ray Scattering).

The models presented can be regarded as idealized all-cis and all-trans versions of the nanosheet, because no cis-trans isomerization events were observed during simulation [as expected from our estimates of the associated free-energy barriers (19) and experimental estimates (20)]. It is therefore not possible, using direct simulation, to sample the dihedral angle $\omega$. NMR experiments indicate that $\approx 90\%$ of residues 7 and 8 are cis and $\approx 40\%$ of residue 15 are cis. Using enhanced sampling methods and modified potentials we found that residue 15, which sits adjacent to pockets (gaps) in the brick structure and so is less constrained than the other residues, appears to possess a propensity to adopt a near-equal mixture of cis and trans. Taking the all-cis model we used umbrella sampling (Fig. 3D) to show that the free-energy barrier for conversion of residue 15 from cis to trans is indeed less than that for residue 7. Further, an ad hoc “softening” (21) of the dihedral angle potential energy terms of the peptoid force field speeds cis-trans isomerization events and causes residue 15 to adopt cis or trans with roughly equal probability (Fig. 3E). Such features are consistent with experiment and suggest that the all-cis nanosheet model is a good approximation of the structure observed in experiment.

Conclusions

Our simulation results and experimental data (4) indicate that the conformations adopted by peptoids in nanosheets result from an interplay of interpeptoid contacts and the conformational preference of the backbone. Specifically, the high degree of order accessible to cis backbones and the resulting favorable interpeptoid interactions compensate the slight energetic strain required to maintain backbone linearity. The resulting conformation is a cis-2 strand (9) in which the backbone alternates between two rotational states and remains linear and untwisted (with a kink at residue 15). Nanosheets are flat and extended—potentially useful features for sensing and catalysis—because they are built from linear and untwisted peptoid polymers. The building motif that allows peptoid linearity is their adoption of twist-opposed rotational states (9). A growing body of work shows that proteins can form similar motifs (22–25); here we have shown that experimental data and molecular modeling can inform the design of nanostructures of this type.

ACKNOWLEDGMENTS.

This work was done as part of a User project at the Molecular Foundry at Lawrence Berkeley National Laboratory, supported by the Office of Science, Office of Basic Energy Sciences, of the US Department of Energy under Contract DE-AC02-05CH11231. J.R.E. and R.Z. were supported by Defense Threat Reduction Agency Contract/Grant DTRA010027-1587. R.Z. was supported by the DARPA Folded Non-Natural Polymers with Biological Function (Fold Fx) program. R.K.S. and A.I.H. were supported by the Air Force Office of Scientific Research Award FA9550-14-1-0350. QM calculations were carried out on the High-Performance Computing resources at New York University Abu Dhabi. This work used resources of the National Energy Research Scientific Computing Center, which is supported by the Office of Science of the US Department of Energy under Contract DE-AC02-05CH11231.