Supplementary materials for

Resolving the Morphology of Peptoid Vesicles at the One Nanometer Length-Scale Using Cryogenic Electron Microscopy

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This file includes:

**Figure S1.** Cryo-EM micrographs and CTF estimations of pNeh\textsubscript{18}-b-pNpm\textsubscript{18}. A. Cryo-EM micrographs of pNeh\textsubscript{18}-b-pNpm\textsubscript{18} vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.

**Figure S2.** Cryo-EM micrographs and CTF estimations of pNeh\textsubscript{26}-b-pNpm\textsubscript{10}. A. Cryo-EM micrographs of pNeh\textsubscript{26}-b-pNpm\textsubscript{10} vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.

**Figure S3.** Cryo-EM micrographs and CTF estimations of pNeh\textsubscript{30}-b-pNpm\textsubscript{6}. A. Cryo-EM micrographs of pNeh\textsubscript{30}-b-pNpm\textsubscript{6} vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.

**Figure S4.** Cryo-EM micrographs and CTF estimations of pNeh\textsubscript{32}-b-pNpm\textsubscript{4}. A. Cryo-EM micrographs of pNeh\textsubscript{32}-b-pNpm\textsubscript{4} vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.

**Table S1.** Summary of defocus values of micrographs measured in CTF

**Figure S5.** Three other classes that represent 2\% of the data in the classification of pNeh\textsubscript{26}-b-pNpm\textsubscript{10} vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm.

**Figure S6.** Views of y-z plan in relaxed monolayer atomic model (19 ns) and relaxed bilayer atomic model (95 ns). Scale bar represent 10 nm.

**Figure S7.** All 6 classes in the classification of pNeh\textsubscript{18}-b-pNpm\textsubscript{18} vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm.

**Figure S8.** All 6 classes in the classification of pNeh\textsubscript{30}-b-pNpm\textsubscript{6} vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm.

**Figure S9.** All 6 classes in the classification of pNeh\textsubscript{32}-b-pNpm\textsubscript{4} vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm.

**Figure S10.** Views of x-y plan in relaxed monolayer atomic models. A. pNeh\textsubscript{18}-b-pNpm\textsubscript{18} (48 ns). B. pNeh\textsubscript{30}-b-pNpm\textsubscript{6} (30 ns). C. pNeh\textsubscript{32}-b-pNpm\textsubscript{4} (3 ns).

**Figure S11.** Effect of membrane curvature on analysis.

Other supplementary materials for this manuscript include the following files:

Relaxed atomic models of monolayer and bilayer structures after MD simulation in PDB format
NEH18NPM18_monolayer_48ns.pdb
NEH26NPM10_monolayer_19ns.pdb
NEH26NPM10_bilayer_95ns.pdb
NEH30NPM6_monolayer_30ns.pdb
NEH32NPM4_monolayer_3ns.pdb
Figure S1. Cryo-EM micrographs and CTF estimations of pNeh₁₈-b-pNpm₁₈. A. Cryo-EM micrographs of pNeh₁₈-b-pNpm₁₈ vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.
Figure S2. Cryo-EM micrographs and CTF estimations of $\text{pNeh}_{26}$-$b$-$\text{pNpm}_{10}$. A. Cryo-EM micrographs of $\text{pNeh}_{26}$-$b$-$\text{pNpm}_{10}$ vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.
Figure S3. Cryo-EM micrographs and CTF estimations of pNeh$_{30}$-b-pNpm$_{6}$. A. Cryo-EM micrographs of pNeh$_{30}$-b-pNpm$_{6}$ vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrograph.
Figure S4. Cryo-EM micrographs and CTF estimations of pNeh$_{32}$-b-pNpm$_4$. A. Cryo-EM micrographs of pNeh$_{32}$-b-pNpm$_4$ vesicles. B. CTF estimations of corresponding micrographs. Left half panel is the simulated CTF and the right half panel is the FFT of micrographs.
<table>
<thead>
<tr>
<th></th>
<th>Micrograph (A)</th>
<th>Micrograph (B)</th>
<th>Micrograph (C)</th>
<th>Micrograph (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNeh$<em>{18}$-b-pNpm$</em>{18}$</td>
<td>0.8/0.8 μm</td>
<td>1.4/1.3 μm</td>
<td>1.4/1.3 μm</td>
<td>0.7/0.6 μm</td>
</tr>
<tr>
<td>pNeh$<em>{26}$-b-pNpm$</em>{10}$</td>
<td>1.7/1.7 μm</td>
<td>1.5/1.3 μm</td>
<td>1.9/1.1 μm</td>
<td>1.7/1.5 μm</td>
</tr>
<tr>
<td>pNeh$<em>{30}$-b-pNpm$</em>{6}$</td>
<td>1.9/1.9 μm</td>
<td>1.2/0.9 μm</td>
<td>1.3/1.1 μm</td>
<td>2.0/1.7 μm</td>
</tr>
<tr>
<td>pNeh$<em>{32}$-b-pNpm$</em>{4}$</td>
<td>1.9/1.7 μm</td>
<td>1.9/1.6 μm</td>
<td>1.6/0.8 μm</td>
<td>2.1/1.6 μm</td>
</tr>
</tbody>
</table>

Two Defocus values in each micrograph from A to D in Figure S1-S4 represents the maximum and minimum defocus due to the presence of astigmatism. All estimations were carried out using gCTF.
Figure S5. Three other classes that represent 2% of the data in the classification of pNeh$_{26}$-b-pNpm$_{10}$ vesicles. Classes 1a, 1b, and 2 are shown in the main text (Figure X). The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm.
Figure S6. Views of the y-z plan of pNeh$_{26}$-b-pNm$_{16}$ in relaxed monolayer atomic model (19 ns) and relaxed bilayer atomic model (95 ns). Scale bar represent 10 nm.
Figure S7. All 6 classes in the classification of pNeh$_{18}$-b-pNpm$_{18}$ vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm. Classes with the same nominal membrane thickness (within 8.5±0.4nm) are labeled 1a through e.
Figure S8. All 6 classes in the classification of pNeh$_{30}$-b-pNpm$_{16}$ vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm. Classes with the same nominal membrane thickness (within 11.7±0.2 nm) are labeled 1a and b.
Figure S9, All 6 classes in the classification of pNeh$_{32}$-b-pNm$_{4}$ vesicles. The number on top left corner indicates the proportion of images sorted into this class. Scale bar represent 10 nm. Classes with the same nominal membrane thickness (within 13.5±0.5 nm) are labeled 1a through d.
Figure S10. Views of x-y plan in relaxed monolayer atomic models. A. pNeh\(_{18}\)-b-pNpm\(_{18}\) (48 ns). B. pNeh\(_{30}\)-b-pNpm\(_{6}\) (30 ns). C. pNeh\(_{32}\)-b-pNpm\(_{4}\) (3 ns).
Application of the analysis presented in Figure 4 on the largest pNpm\textsubscript{26}-pNeh\textsubscript{10} vesicle in Figure S2 is shown in Figure S11 A; this large vesicle has radius of 227 nm (The radius of the vesicle in Figure 4 is 129 nm). As expected, the image in Figure S11 A is dominated by class 1 and class 2 boxes. We averaged the images of boxes in this particular vesicle, the averaged class 1 and class 2 images thus obtained are shown in Figure S11 B. In Figure S11 C, we show averaged class 1 and class 2 images obtained from all 4 vesicles. It is evident that class average from single vesicle are similar to those obtained from by averaging many different vesicles. This implies that differences in vesicle curvature do not affect our analysis.

**Figure S11.** Effect of membrane curvature on analysis.  
**A.** The locations of the boxes along the largest pNpm\textsubscript{26}-pNeh\textsubscript{10} vesicle in Figure S2, blue is classes 1a and 1b while light green is class 2. Pink represents an unsorted box.  
**B.** Classes and averaged images obtained from the largest pNpm\textsubscript{26}-pNeh\textsubscript{10} vesicle.  
**C.** Classes and averaged images obtained from all four pNpm\textsubscript{26}-pNeh\textsubscript{10} vesicles in Figure S2. Similarity of Figures B and C indicates that membrane curvature differences do not affect our analysis.