

Response to reviewers: Understanding the Nanoscale Structure of Inverted Hexagonal Phase Lyotropic Liquid Crystal Polymer Membranes

Benjamin J. Coscia Joseph Yelk Matthew A. Glaser Douglas L. Gin
Xunda Feng Michael R. Shirts

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We thank the reviewers for carefully reading over our manuscript and providing helpful comments. We have taken the suggestions into consideration and made appropriate revisions to the manuscript document. All changes to the text have been documented below.

1 Response to Reviewer 1

1. *This manuscript describes atomistic molecular dynamics simulations and diffraction analysis of hexagonally-arranged pores, each of which is made of many columns of the monomer Na-GA3C11. This physical system is of interest because such nanopores have potential to transform filtration applications. This work is of potential interest to the JPC readership because it provides atomistic insight into the origins of diffraction reflections observed in experiments, which could be used to resolve discrepancies about what the structure of these hexagonal arrays are, or used to engineer better filters. Overall, the manuscript provides comprehensive descriptions of metrics used to evaluate the simulated structures against experiments, but the number of things described and length of these descriptions obscures whether significant new physical insight into this system is made.*

Author reply: We thank the reviewer for their constructive feedback and aim to address all of their concerns.

2. *As written, it is not clear if the authors are presenting this study as a validation of GAFF and the present equilibration techniques, or whether this study is providing significant new physical insight into these lyotropic liquid crystals. The paper reads a bit more like the former, with a comprehensive discussion of the degree to which five scattering features expected in experiments are predicted in simulations. However, at the same time, the authors make arguments including (1) pores are made from five columns of monomers, (2) that water is present in the “dry” structures, and (3) that a scattering feature comes from tail packing, not tilt: These observations *could* be new physical insight into lyotropic liquid crystal structure, but as presented seem more like hypotheses than conclusions:*

Author reply: We thank the reviewer for offering their interpretation of the purpose of our study, as it is important that we properly convey our message. The main purpose of our work is to give a detailed description of the structure of a lyotropic liquid crystal membrane backed by experimental data. This work lays a foundation for future transport studies. By matching 2D-WAXS data, we have attempted to unveil the most experimentally-consistent structure possible. To clarify this purpose, we have modified and added text to the paragraph on page 6 of the introduction, in which we state our perceived importance of knowing the most experimentally-consistent structure:

Old text: “A molecular-level understanding of LLC polymer membrane structure, enabled by molecular dynamics (MD) simulations, can provide guidelines to reduce the large chemical space available to

design monomers for creation of separation-specific membranes. Useful molecular-level modeling should incorporate a detailed picture of the nanoscopic pore structure, which is crucial to understanding the role of monomer structure in solute transport and membrane design.

New text: “A molecular-level understanding of LLC polymer membrane structure, enabled by molecular dynamics (MD) simulations, will enhance our comprehension of the role of monomer structure in solute transport and provide guidelines to reduce the large chemical space available to design monomers for creation of separation-specific membranes. It is important that we model the system with a nanoscopic pore structure that is maximally consistent with experimental data in order to best approximate the chemical environment experienced by solutes in future simulated transport studies.”

We do not intend for this study to be a validation of GAFF for this particular system. We believe we would come to similar conclusions using other force fields, however validation of this hypothesis is left for a future study. We chose GAFF because it is widely used to simulate organic molecules and there are no catch-all force fields built for liquid crystal systems. We are interested in what information we can gain from a widely-used ‘out-of-the-box’ force field and in future work, we will validate whether GAFF is sufficient for reproducing trends in experimental separation properties. To clarify our use of GAFF, we have added the following text to Section 2.2 of the Methods on page 11.

New text: “We chose GAFF because it has been parameterized for use with organic molecules. Although researchers have optimized force fields, including GAFF, for use with liquid crystals, they are often tuned in order to reproduce one or more experimental observables for specific types of liquid crystal molecules.^{29,30} Since liquid crystals exist in a wide range of shapes and sizes, including candidate monomers which we may study, we chose to stick with a widely used force field. If we can reproduce experimental trends using a single ‘out-of-the-box’ force field, then there will be no need to complicate our studies with customized parameter sets.”

We have added the following citations:

- Cook, M. J.; Wilson, M. R. Development of an All-Atom Force Field for the Simulation of Liquid Crystal Molecules in Condensed Phases (LCFF). *Mol. Cryst. Liq. Cryst.* 2001, 357, 149165.
- Boyd, N. J.; Wilson, M. R. Optimization of the Gaff Force Field to Describe Liquid Crystal Molecules: The Path to a Dramatic Improvement in Transition Temperature Predictions. *Phys. Chem. Chem. Phys.* 2015, 17, 2485124865.

Since our ultimate goal is to design new monomers for solute-specific separations, we will be employing the same techniques used in this work to unsynthesized monomers. In most cases, we will not have an experimental dataset to use for comparison. Despite all of the structural intricacies which we have revealed in this study, one of our main conclusions is that regardless of which system we study, the radial distribution of monomer components stays consistent. From this, we can hypothesize that we will extract similar transport results regardless of which configuration we use to conduct transport studies. This is an extremely important finding which may have been undersold in the original draft of the manuscript. We have added the following concluding paragraph to the end of Section 3.3 on page 45 in order to drive the point home:

New text: “Our observations suggest that the details of transport may be relatively independent of the structural differences between different possible structures studied here. Despite the structural intricacies that give rise to differences between the various metastable basins, all pores are characterized by dense, primarily hydrophilic cores with a gradient towards a primarily hydrophobic tail region far from the pore center. This implies that we might see the same trends in transport properties from any of the systems studied so far. Since the ultimate goal of our work is to design new monomers using high throughput simulations, achieving that goal becomes tractable without the need to optimize every system with an experimental dataset.”

We also modified a paragraph in our conclusion on page 51 to better emphasize our point:

Old text: “We characterized the environment centered around the membrane pores and learned that the pores are generally filled with monomer head groups and sodium ions. All dry systems studied showed a similar distribution of sodium, head groups and tails while the wet system shows evidence of

slight swelling, with minor changes in the distributions due to the presence of water molecules. We also observed that there is not a hard partition between hydrophilic and hydrophobic regions; instead, there is a gradient of chemical constituents. This finding raises questions about the nature of size-exclusion separations in systems without a well-defined pore size, which potentially could enable separations that vary with chemical identity as well as size.

New text: “Although exactly reproducing the experimental 2D-WAXS pattern required attention to minute structural details, we found that all systems showed similar radial distribution functions characterized by a gradual radial transition from a dense hydrophilic core to hydrophobic tails. This observation enables us to study new systems that have not been experimentally characterized with the expectation that any additional structural optimization will not greatly influence trends in transport property predictions. The compositional gradient itself raises questions about the nature of size-exclusion separations in systems without well-defined pore boundaries, which potentially could enable separations that vary with chemical identity as well as size.”

3. *1. For the simulations performed here, the authors do make a strong case that 5 columns per core gets the best pore-to-pore spacing (Figure 5). However, insufficient time is accessed to see whether the 4-, 5-, and 6-column initial conditions move towards or away from the expected pore spacing over many-microseconds trajectories. That said, for the quenches performed here, 5-columns per core seems more plausible than 4.*

Author reply: We agree with the reviewer that 5 columns-per-core is more plausible than 4 and that much longer simulations may provide further supporting evidence. However, there is very little motion on the hundreds of nanoseconds timescale that we simulated which suggests that we will not gain any significant new insight by extending our simulations into the microsecond regime. It is not feasible for us to carry out these simulations as it would consume our computational resources with little reward. Some confusion may have been generated by agreement of the disordered basin 6 column-per-pore system with experimental pore spacing. As mentioned in the text on page 23, the vertical stacking distance between monomers in that system is far too large. The columns are ‘stretched’ in the z -direction allowing the pores take up less space on the xy plane and thus pack closer together.

4. *Is this significant physical insight? It is insight, but the authors do not make a compelling case for how 5 columns would change how we think about these crystals, or how it would impact membrane engineering (or more importantly, whether these 5-column structures assemble in the solution phase, or are only locally stable).*

Author reply: We thank the reviewer for pushing us to provide more significant physical insight based on the number of monomers per column. The number of columns-per-pore influences the pore radius which is important for determining the limits of size-exclusion-based separations. It also tells us the density of interacting sites within the pore which may play a role in small molecule transport. We have added the following text to the main document at the end of Section 3.1 on page 24:

“The number of columns per pore dictates the density of interaction sites within each pore and the pore radius. A higher number of interaction sites will surely play a role in transport of molecules that have an affinity for the monomer head groups. The pore radius increases with the number of columns per pore (See Figure S19). This radius is likely related to the size of the molecules which can be excluded by this type of membrane. One may be able to control LLC membrane pore size by selecting monomers that are stable in configurations with more columns-per-pore.”

We are unable to definitively answer the question of whether the 5-column structures are most likely to assemble in solution phase. The system does not self-assemble on a timescale reasonable for simulation. See Section S3 of the supporting information where we attempted self-assembly. These sorts of predictions are left for a separate effort that will use coarse grain models.

5. *2. The authors make a pretty compelling case that water causes the R-double scattering feature by showing its absence when water is added, but that the R-double appears and persists for 200ns after the water makes its way into the crystal. However, the authors also show that nonuniformly spaced monomers can give rise to the R-Double feature, a seeming counterexample to the necessity of water. Is the R-double feature with water because the monomers*

are now irregularly spaced? What happens when the waters are removed and the scattering re-performed in Fig 17c(bottom)?

Author reply: We thank the reviewer for exposing a potentially unclear explanation of the origin of R-double. R-double appears in a dry system with non-uniformly spaced monomers *only* if z -direction position restraints are applied to monomer head groups in order to maintain unevenly spaced stacking. As soon as the restraints are released, R-double fades and the monomer spacing becomes uniform. We modified the text on page 41 in order to more clearly convey this message:

Old text: “We can also produce R-double if the LLC monomers are not uniformly spaced in the z -direction, but instead form pairs that stack less than 3.7 Å apart, with COMs that are spaced 7.4 Å from neighboring pairs of monomers (Figure 13b). Our force field causes our system to tend towards uniformly spaced layers. Simulations of unevenly spaced systems are only stable if position restraints are applied to heavy atoms of the phenyl rings. Additionally, there is little evidence from QM studies of stacked $\pi - \pi$ systems that such uneven stacking could be energetically stable.⁵²

Modified text: “We can also produce R-double if the LLC monomers are not uniformly spaced in the z -direction, but instead are placed in pairs that stack less than 3.7 Å apart, with COMs that are spaced 7.4 Å from neighboring pairs of monomers (Figure 13b). Simulations of unevenly spaced systems are only stable while position restraints are applied to heavy atoms of the phenyl rings. There is little evidence from quantum mechanical studies of stacked $\pi - \pi$ systems that such uneven stacking could be energetically stable.⁵² As soon as we remove position restraints, our system tends towards uniformly spaced monomers.”

R-double is only long-term stable without position restraints if a small amount of water is present. We believe that water facilitates the appearance of R-double by stabilizing non-uniform spacing through hydrogen bonding, as the reviewer suggests. We clarified the text on page 42 in order to make this conclusion clear:

Old text: “When two vertically stacked monomer head groups hydrogen bond with a shared water molecule, the monomers are drawn closer together (as illustrated in Figure 14b), which creates an asymmetry that allows R-double to appear. If a monomer head group shares a hydrogen-bonded water molecule with a head group above itself, it will be less likely to share a water molecule with a head group below it due to geometric constraints. The monomer head group below can just as easily share a water molecule with a head group below itself. In this scenario, the COMs of each pair are 2 times the π -stacking distance apart which would lead to R-double (much like the configuration in Figure 13b). There are a modest number of occurrences of this scenario, which we quantify in further detail in the SI, Section S24.”

New text: “The addition of a small amount of water to the pores stabilizes non-uniform stacking of head groups much like that shown in Figure 13b. When two vertically stacked monomer head groups hydrogen bond with a shared water molecule, the monomers are drawn closer together (as illustrated in Figure 14b), which creates the asymmetry that allows R-double to appear. If a monomer head group shares a hydrogen-bonded water molecule with a head group above itself, it will be less likely to share a water molecule with a head group below it due to geometric constraints. The monomer head group below can just as easily share a water molecule with a head group below itself. There are a modest number of occurrences of this scenario, which we quantify in further detail in the SI, Section S24.”

The reviewer asked us to re-perform the scattering analysis on the same system with water molecules removed. We would like to emphasize that we attempted to make a similar argument by removing just the head group atoms from the trajectory and re-performing the scattering analysis (Figure 14a). In the absence of head groups, R-double is not present, implying that their structure (which is influenced by the presence of water molecules) is responsible for the R-double reflection. For completeness, we performed the scattering analysis on the same system with water molecules removed, as suggested by the reviewer, and R-double is still present. This is the expected result since the structure of water itself does not give rise to R-double. We’ve added the resulting simulated XRD pattern to the Supporting Information, Figure S32.

6. *3. The authors make the case that the R-spots feature comes from the structure of the hexagonal tails, not their tilt. The authors should perform the scattering analysis to check*

if the R-spots are present on the tilted-tails they constrain: This quick experiment would nail down their interpretation here, or show that the tilted tails are also plausible (but not observed in this model).

Author reply: We thank the reviewer for their suggestion. We do not deny that the tilt of the monomer tails is a plausible explanation for the appearance of reflections that resemble R-spots in many liquid crystal systems. While the reviewer’s suggested experiment is a good idea, its results in this case are ambiguous for a couple reasons. First, the tails may still pack hexagonally in the tilted configuration which would independently give rise to R-spots. Second, it is necessary to impose high force constant ($> 1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$) position restraints in order to maintain a tilt angle close to that implied by the 2D WAXS data. The resulting high degree of order in the system gives way to reflections which are easily misinterpreted. We have added the following text highlighted in red to the text shown on page 28 in order to acknowledge that tail tilt is a valid explanation of R-spot-like reflections:

Modified text: “Previous literature has attributed the R-spots reflection in this particular WAXS dataset as the result of tilted alkane chains¹⁶. **This explanation is not unfounded as there are examples in literature of systems where tilted liquid crystals give rise to reflections that resemble R-spots.**^{45,46} We measured the tilt angle of the alkane chains ...”

We have added the following citations in support of the above statement:

- Percec, V.; Imam, M. R.; Peterca, M.; Wilson, D. A.; Graf, R.; Spiess, H. W.; Balagurusamy, V. S. K.; Heiney, P. A. Self-Assembly of Dendronized Triphenylenes into Helical Pyramidal Columns and Chiral Spheres. *J. Am. Chem. Soc.* 2009, 131, 76627677.
- Gearba, R. I.; Anokhin, D. V.; Bondar, A. I.; Bras, W.; Jahr, M.; Lehmann, M.; Ivanov, D. A. Homeotropic Alignment of Columnar Liquid Crystals in Open Films by Means of Surface Nanopatterning. *Adv. Mater.* 2007, 19, 815820.

7. *These arguments would be stronger if there weren’t so many qualifications about simulation timescales and GAFF, in particular its ability to predict the shortest-scale feature (pi-stacking) for this system. How does everything else change if the force-field were tuned to get that feature correct?*

Author reply: We thank the reviewer for their constructive criticism. Many of our findings may be relatively independent of the force fields used, but we feel that we should include these qualifications in order to not overstate our case. It is common for researchers to run simulations for an insufficient length of time and to make bold claims. While we feel that we have run our simulations sufficiently long, we still acknowledge the shortcomings of our techniques in order to inspire other researchers to build on our initial work.

We agree with the reviewer that we could tune our force field in order to exactly reproduce the $\pi - \pi$ stacking distance seen experimentally. Surely there will be some structural differences. There are many ways to tune a force field in order to achieve this. As stated earlier, we prefer to work with an ‘out-of-the-box’ force field since they are widely used and can be applied to vast number of systems. The ultimate goal of our work is to create new monomers that can achieve specific separations. If we can gain experimentally-consistent insights using our model simulated with GAFF, then we can have increased confidence in future predictions generated from systems built with unsynthesized monomers. With that said, we think that it would be interesting to repeat our analysis using different and/or optimized force fields in a future study in order to comment on the significance of any differences.

We have modified the paragraph of our conclusion which describes future work (page 51) in order to acknowledge the possibility that a more tuned force field could influence our results:

Old text: “Future work, based on what has been learned in this study, may help further improve the structural agreement between experiment and simulation”

New text: “Future work, based on what has been learned in this study, may help further improve the structural agreement between experiment and simulation and test the sensitivity of our current conclusions to a more accurate structure.”

8. *The MD-Structure-Factor repository is not available at the provided github link, nor does it appear to be online anywhere. So, the correctness of that code cannot be checked.*

Author reply: We thank the reviewer for pointing this out and apologize for our mistake. The referenced GitHub repository was set to private due to miscommunication with our collaborators but it has now been made public. Please feel free to read through the code. We have performed checks to ensure that the code works properly, especially when simulating monoclinic unit cells. For example, we generated test trajectories containing thousands of frames made up of configurations of randomly placed point scatterers in a monoclinic unit cell. We expect that the simulated diffraction pattern will have a gaussian shape. This exact result is shown in the Figure S20 of the SI. With improper treatment of the monoclinic geometry, the gaussian shape is skewed into an elliptical shape.

9. *One might want to check that code based on Figure 7f, where presumably spherical scatterers generate reflections with anisotropy (diamonds instead of squares or circles). This kind of an artefact is plausible if there are errors handling the non-cubic box, or with scattering angles that aren't parallel to a box axis. llcsim/analysis/correlation.py looks like it has the machinery for doing the scattering analysis.*

Author reply: We thank the reviewer for their astute observation and the resulting improvements to our manuscript. We generated Figure 7f by calculating the structure factor of a 2-dimensional array of hexagonally-packed scatterers. It is meant to be a simplified representation of the scattering exhibited by hexagonally packed alkane tails so that we can qualitatively understand where we would expect R-spots to appear if tails indeed pack in that way. Since it is possible to generate a rectangular unit cell which contains hexagonally packed point scatterers, there are no artifacts due to improper treatment of periodicity. Although we believe our calculations are correct, we originally used only eight total scatterers in the 2D unit cell and then plotted the structure factor with a contour plot (`matplotlib.pyplot.contourf`) which does not interpolate the data. We re-created Figure 7f by simulating the structure factor of 100 hexagonally packed scatterers, in order to get higher fourier space resolution, and then plotting the result with `matplotlib.pyplot.imshow` and Gaussian interpolation. We have added the code used to generate this figure to the `llcsim` repository which can be accessed at `llcsim/analysis/hexagonal_packing.py`. Additionally, we have added an entry to Table S1 in order to make it clear where the code can be found.

10. *Regarding the ion conductivities, how do the numbers presented here compare against ion conductivities predicted with GAFF and other polarizable and nonpolarizable force fields? If GAFF is systematically too high in other cases, it could help explain the order-of-magnitude discrepancy observed here.*

Author reply: We thank the reviewer for bringing up a good point. There are few studies with experimental comparisons of sodium ion conduction. Conduction of potassium in ion channels is quite common. The ionic conductivity is directly related to the diffusivity of the ion of interest, so if the dynamics of sodium are overpredicted in our model, this could give rise to the discrepancy between experiment and simulation. The diffusivity of sodium ions has been overpredicted with multiple models, including AMBER where the diffusivity is about 2x too high. We have modified the text on page 48 to include additional reasoning for the discrepancy between the simulated and experimental ionic conductivity.

Old text: "The calculated value of ionic conductivity is 5 times higher than experiment likely because we simulated infinitely long, aligned pores. The ionic conductivity measurement to which we are comparing was done with an 80 μm -thick film, nearly 10,000 times thicker than our simulated system. The thick film is likely imperfectly aligned and has defects leading to non-contiguous pores. It has been shown that there is a large dependence of ionic conductivity on the alignment of the pores. The ionic conductivity of an isotropically aligned film is ca. 85 times lower than that of the nearly aligned film to which we are comparing.¹⁶ We hypothesize that a thin, perfectly aligned film would have a value of ionic conductivity in closer agreement with our model."

Revised text: "The calculated value of ionic conductivity is 5 times higher than experiment likely because we simulated infinitely long, aligned pores **and because our model over-predicts the diffusivity of**

sodium. The ionic conductivity measurement to which we are comparing was done with an 80 μm -thick film, nearly 10,000 times thicker than our simulated system. The thick film is likely imperfectly aligned and has defects leading to non-contiguous pores. It has been shown that there is a large dependence of ionic conductivity on the alignment of the pores. The ionic conductivity of an isotropically aligned film is ca. 85 times lower than that of the nearly aligned film to which we are comparing¹⁶. **Additionally, sodium ions parameterized with AMBER parameters exhibit bulk water diffusion coefficients that are two times greater than experiment.**⁵⁶ We cannot definitively say how this ratio changes in the confined LLC pore environment, but it is likely that sodium diffuses faster in our system which contributes to a higher ionic conductivity than experiment.”

11. *If the authors either (a) strengthen the cases for their proposed structural insights being highly likely (not just plausible), or (b) make a stronger case for why these particular insights have important ramifications for our understanding of LLC’s (their significance), then it would be easier to publish this immediately. This reviewer’s opinion is that this work would find a broader interested audience with a more concise focus on these physical insights, which could be accomplished by moving more content (e.g., dihedral correlations) to the SI.*

Author reply: We feel that the additions and modifications to the sections cited above have made the intent of our work clearer and helped communicate the significance of our learned insights for future work on this topic. We have clarified the purpose of work and what we expect to learn from it. We have commented on the significance of knowing the number of columns-per-pore. We have emphasized the observation that the radial distribution of monomer components is relatively independent of the system’s initial configuration which is important for future high-throughput work. We have clarified and strengthened our reasoning for the existence of the R-double feature.

We sympathize with the reviewer’s desire for a more concise manuscript but feel that most of the information presented is necessary in order to come to a full understanding of the work we’ve performed (an opinion shared by Reviewer 2). As a compromise, we’ve made efforts to shorten the manuscript by moving figures and discussions which draw the reader’s attention from the important physical insights to the SI. Excluding additional citations (new citations take up about a page in the new draft), we have reduced the total length of the manuscript by 3 pages (including any additions documented above). The following changes made this possible:

- We moved the plots of the 1D Correlation functions, $g(z)$, used to calculate correlation lengths from Section 3.2.4 to the SI, now Figure S24. The most important information, the correlation lengths, is already presented in Table 2.
- We moved the discussion of the influence of system size on $g(z)$ from Section 3.2.4 to the Supporting Information, Section S19. We supplemented that discussion in the main text with the following text:
“We show that the system size in the z -direction does not significantly alter $g(z)$ in Section S19 of the SI.”
- We removed the discussion of a system that we annealed from 500 K to 300 K from Section 3.2.4 because it is described again (with better context) in Section 3.4.
- We moved the discussion of a correlation function, $g(z)$, that averages all slices of the 3D correlation function and the associated figure from Section 3.2.4 to Section S19.
- We’ve supplemented the discussion of the influence of increased noise in the z -direction and in the xy plane on the shape and intensity of R- π with the following paragraph:
“We also used our simplified systems in order to study the influence of increased disorder in each dimension. Increasing z -directional disorder reduces the intensity of R- π without changing the shape of its q_r cross-section. Increasing disorder on the xy plane, somewhat counterintuitively, reduces the FWHM of the q_r cross-section of R- π with an insignificant effect on its intensity. We explain these points in more depth in Section S20.”

The full discussion, with figures, has been moved from Section 3.2.4 to Section S20. The subsection of Section 3.2.4 starting on page 33 of the main text now has a primary focus on the relationship between R- π and inter-column correlation.

- We moved the plots of dihedral autocorrelation functions to the SI, Figure S35.
- We moved the figure associated with physical property changes after cross-linking to the SI, Figure S36, since its main findings are summarized sufficiently in the text.

2 Response to Reviewer 2

1. *The authors report a computer simulation study of the detailed structure of several systems, which are similar to the cross-linked inverted hexagonal phase of self-assembled lyotropic liquid crystals (LLCs) used to develop porous polymer membranes for separations. Specifically, the authors studied the Col-h thermotropic (solvent-free) phase formed by the assembly of the Na-GA3C11 monomer, which is similar to the LLC phases used in the development of polymeric membranes. The Col-h phase has been characterized experimentally using wide-angle X-ray scattering (WAXS), but nonetheless important gaps in its structure still remains. Using all-atom classical molecular dynamics simulations, the authors performed a very careful and very detailed study of the structure of the Col-h phase formed by Na-GA3C11. The authors compared their simulated X-ray diffraction (XRD) patterns with existing 2D-WAXS experimental data, carefully discussed and explained the observed differences between simulations and experiments, and provided molecular-level details of the structure of this system that cannot be obtained from experiments. This study is very detailed; all relevant information is provided for interested readers to replicate results from this study (including links to Python scripts used to set up the simulations, analyze MD trajectories, and obtain simulated XRD patterns). The simulation results has been analyzed and discussed comprehensively (perhaps too comprehensively, as this paper is 63 pages long, has 21 figures and its Supporting Information file has 19 additional pages; however, I dont think the authors should make any efforts to shorten the paper, as I feel every presented component is required to fully understand the contributions of this study) Overall this is an extremely solid manuscript*

Author reply: We thank the reviewer for the kind assessment of our work. We also acknowledge that this is a long article

2. *I have no suggestions to further improve this paper, other than fixing a few small typos:*
 (1) Page 10, first paragraph: remove the page 58 associated with reference 25
 (2) Page 54: reference to the paper of Feng et al is missing

Author reply: We thank the reviewer for pointing out these minor errors in our main text. These issues have been corrected in the revised main text file.

3. (3) Page 54: mention examples of force fields that explicitly include π - π interactions

Author reply: We have adjusted our language in order to specifically mention and cite polarizable force fields as follows:

“Polarizable force fields such as AMOEBA which explicitly including $\pi - \pi$ interactions between aromatic functional groups may be able to draw stacked monomers closer together.”^{57–59}

We have added citations with relevant force field examples, including AMOEBA:

- Shi, Y.; Xia, Z.; Zhang, J.; Best, R.; Wu, C.; Ponder, J. W.; Ren, P. Polarizable Atomic Multipole-Based AMOEBA Force Field for Proteins. *J. Chem. Theory Comput.* 2013, 9, 40464063.
- Kaminski, G. A.; Stern, H. A.; Berne, B. J.; Friesner, R. A. Development of an Accurate and Robust Polarizable Molecular Mechanics Force Field from ab Initio Quantum Chemistry. *J. Phys. Chem. A* 2004, 108, 621627.
- Lopes, P. E. M.; Lamoureux, G.; Roux, B.; MacKerell, A. D. Polarizable Empirical Force Field for Aromatic Compounds Based on the Classical Drude Oscillator. *J. Phys. Chem. B* 2007, 111, 28732885.