**Response to Reviewers:**

We would like to thank the reviewers for a careful and thorough reading of our Tat manuscript and for many critical comments that have improved our paper. We now respond to each reviewer separately:

**Reviewer #1:**

In order to avoid confusion, a new Conclusions section has been added at the end of the Discussion section to summarize the important findings of this work.

The MD simulations were vital in our understanding of the lack of water pore in the membrane. Please see **Fig. 4** and the accompanying text.

The experimental form factors in **Fig. 4A** and **4C** have been placed on the same vertical scale to avoid confusion.

Scattering Density Profile (SDP) modeling program has been added to the list of abbreviations, and is also defined the first time it is used in the Materials and Methods.

The legend to **Fig. 6** has been amended to include: Increasing mole ratios (P/P+L) are shown in the figure legends.

**Fig. 7** has been amended so that the same vertical axis, 26 to 39, is used in **A**, **B** and **D**. Please see the comments in response to Reviewer #3 regarding these trends.

**Fig. 8** has been amended so that the legend reads “Nuclear mimic”. The nuclear mimic was described in Materials and Methods. It is a mimic of the lipids in the nuclear membrane. An additional sentence has been added: This indicates that the chains are more disordered as the Tat concentration in the membrane increases.

**Fig. 9**. The use of a cylinder to depict Tat’s membrane association allows using volume information and half-width results from the MD simulations. It is purely a subjective choice; we could also have used a rectangular [parallelepiped](http://www.ask.com/allabout?q=Parallelepiped&qsrc=470). We would like to defend our choice of a cylinder for Fig. 9 because it was convenient to show the precise molecular dimensions that resulted from the volume measurements and the MD simulations. This is a to-scale drawing where the dimensions in Fig. S8 and Table S1 are shown pictorially. Although Fig. 9 is a simple scheme, the distances are explained in section 3.7, Summary of Results.

CD spectroscopy: Excess significant figures, as well as standard deviations obtained by averaging results obtained from different basis sets, have been removed from **Table 1.**

Solvent removal. This is a continuing question for our lab’s work, since our samples are always prepared in organic solvent. As mentioned in the Materials and Methods, solvents were removed by evaporation in the fume hood (usually for one day), followed by two hours under vacuum (at room temperature). We have previously used gravimetric analysis to determine that no further weight loss occurs after two hours under vacuum. We also used deuterium NMR and deuterated solvents to test for solvent removal. Our NMR results yielded ~1% residual organic solvent in our samples. We have now added the results of this test to Materials and Methods.

**Reviewer #2:**

Regarding the main conclusions of this work, that Tat thins and softens membranes, and disorders chains; these are basically our results. We did not see evidence for a pore formation, even though Tat is known to translocate through membranes. While our result did not dramatically show a pore, it supports models where a membrane perturbation is required, such as the carpet model, or even the toroidal pore model, if it is short-lived. By performing MD simulations in collaboration with Garcia’s group, we compared our X-ray data directly to their outputted form factors to confirm the headgroup location of Tat. We feel that our X-ray data combined with precise MD simulations is a powerful contribution to this field of membrane-translocation peptides.

“It is not clear what the relation is between simulations and experiments, and what is the goal of the comparison.” By using MD simulations to provide a form factor, we can directly compare our experimental form factor to the simulated form factor. This is a valuable tool to check our model against the MD simulation. Our modeling program, SDP, relies on Gaussians and error functions to fit the positions of components in the membrane, where the positions of the peaks are allowed to move. The SDP program was described in great detail in Ref. 44. Using the SDP program we found two results: Tat in the headgroup region and Tat in the hydrocarbon interior. These results were displayed in **Fig. S5**, where Chi-Square, or goodness of fit, is plotted vs. Tat Z position across the bilayer. After we obtained this result we asked our collaborators to start their atomistic simulation at the same two Tat locations. The comparison of form factors is shown in **Fig. 4**, where only the Tat headgroup position produced a form factor from the MD simulation that agreed with our experimental result. The poor agreement shown in **Fig. 4C** caused us to rule out the Tat hydrocarbon position. We wrote above **Fig. 4**, “However, as shown in **Fig. 4C** the corresponding form factor calculated from MD simulations does not match well with experiments.” Therefore, the MD simulation helped us to rule out a spurious result from our simple SDP modeling program. By contrast, this comparison has also shown that MD simulations that find a water pore in the center of the bilayer caused by Tat are incorrect, at least with this system and couterions. We have now added the following sentence close to **Fig. 4**:

Thus, by comparing the experimental and simulated form factors, Tat’s headgroup position was validated, while the hydrocarbon position was ruled out.

Regarding the contributions of Dr. Rieko Ishima, we were appreciative of her efforts to help us to determine the secondary structure of Tat in water. Rieko preferred not to be a coauthor on this work since her contribution was minor. She was acknowledged at the end of the paper in the submitted version.

Thank you for the heads-up concerning the new simulations from Wisconsin. We found an article by Wu, Cui and Yethiraj, “Why do arginine and lysine organize lipids differently? Insights from coarse-grained and atomistic simulations”, published in J. Phys. Chem. B., 2013. This is indeed an interesting work, using both Martini and atomistic simulations. These authors have *in silico* evidence that arginine binds to lipid headgroups differently from lysine, in that they bind not only to the phosphate group but also to the carbonyls. We now cite this paper in our Discussion section since its results compare favorably with our results shown in **Fig. S8**, that shows the arginine electron density at both the phosphate group and the glycerol-carbonyl and the lysine group mostly outside of the phosphate group.

About the range of D-spacings in diffuse scattering data, we have published many papers on our use of diffuse scattering, and we felt that it was not necessary to go in great detail regarding the methods again. As the sample hydrates through the vapor to within 5 angstroms of full hydration, the layers start to fluctuate, producing the diffuse scattering data that obtains both elastic properties and structure. The form factor doesn’t change as the sample hydrates, which allows us to sample several D-spacings, close to full hydration.

For an explanation of our methods and the dependence of the scattering on Qr, please see references #42, 43 and 44.

Reviewer #2 comments that the silicon wafer can have the effect of quenching induced curvature. By contrast, we have published several papers on the ripple phase, formed in these thick layers, where there is a permanent corrugation. Since our layers are so thick, there is negligible effect of the silicon wafer on the membrane’s ability to curve. Indeed, we would not observe changes in bending modulus if this were the case.

**Reviewer #3.**

Reviewer #3 had only a few comments, but suggested two papers of interest. One has already been included due to Reviewer #2’s comments. The second paper, Jungwirth et al., used a combined experiments and simulations to study the interaction of free arginine or free lysine, and also decapeptides of arginine or lysine with neutral and charged membrane mimics. They observed aggregation of peptides, which we did not observe in our experiments, although that could have taken place in our experiments. We cannot distinguish aggregated Tats from homogeneously spaced Tats on the surface of the membrane. Jungwirth et al did observe membrane thinning, as we did, and we now add this citation to our Discussion.

In the final comment of Reviewer #3, there is concern that the MD simulation results shown in **Fig. 7** vary more swiftly than the experimental results. **Fig. 7** has now been redrawn to place **A**, **B** and **D** on the same vertical scale at the suggestion of Reviewer #1, which has the effect of minimizing the apparent differences between simulation and experiment. In **Fig. 7C** the area/lipid does increase more dramatically in the simulation compared to experiment. The MD simulations were performed at several fixed areas and Tat positions, since in the atomistic simulation there is not sufficient time for a rearrangement of Tats in the bilayer. The results in **Fig. 7** are averaging the best fits to the X-ray data for each parameter, with standard deviations shown. We have no explanation for why the area/lipid increases with Tat concentration in the simulations more so than in our modeling. Force fields are still not perfect, although by comparing to experiment, there is more confidence in the simulated result.