Reviewer #1:

Akabori and coauthors present a comprehensive and largely confirmatory study of Tat peptide-membrane interactions. The negative finding of no pore formation is important, along with the characterization of bilayer thinning, interfacial peptide location and the increase in area per lipid. The point is well taken that a kinetically competent pathway for peptide translocation remains elusive. Other aspects of the Discussion are nevertheless somewhat confusing, particularly the statement that "the modeling result is supportive of the original simulation result of Herce and Garcia that Tat resides closer to the bilayer center than do the phosphocholine groups," where it is not entirely clear what the authors are trying to convey. A stronger summary at the end of Discussion could be helpful.

The simulations presented in this paper contribute clearly to figure 4 and somewhat to figure 7, although the steeper slopes in figure 7 are not addressed. At other times it is less obvious what is being learned from the simulations. For example, little is said about whether the simulations contribute to an understanding of the structure or dynamics of the bound peptides.

The following additional questions and comments come to mind:

The experimental form factors in figures 4A and 4C are confusing. This confusion could be removed if the same vertical scale would be employed in figures 4A and 4C.

I do not find SDP modeling in the list of abbreviations. The meaning of SDP also is not explained in the Methods.

The legend and labeling of figure 6 are unclear. It looks like the peptide-lipid ratio might be changing but this is not explained.

Figure 7 is quite confusing. The same vertical scale should be used in panels A, B, D. The identifying labels need only appear once (either one inset, or just put them in the legend, once). The trend for the MD simulations is always steeper, and then disagrees substantially in panel D. Some comments on the steepness and the disagreement would be appropriate.

I don't know what the points labeled "nuclear" are showing in figure 8. This figure is not well explained.

Figure 9 and the associated text are confusing, particularly with respect to whatever the authors intend to communicate about the guanidiniums. If the figure is meant to communicate that "more [guanidiniums] were pointing towards the bilayer center," this reader is unable to see how such a feature is conveyed in any way by figure 9. The peptide location, boundary lipids and lipid thinning are better represented, but it is quite mysterious as to why the peptide is shown as a cylinder.

Give the nature of the CD data, there are too many significant figures reported in table 1. The conclusions from the CD are nevertheless fine.

I would not change the manuscript based on what follows, but I make the comment anyway. There are small worries that the solvent removal, particularly removal of TFE or even TFA, could be incomplete using the methods described. No solvent controls are mentioned. The presence of residual solvent could be checked by using 19F NMR. The worry is that residual solvent can sometimes alter the results that are observed for some mixtures of lipids and peptides. Suitable controls would be useful in future experiments.

Reviewer #2:

This manuscript describe a study on the effects of the Tat peptide on lipid bilayers, using low angle and wide angle Xray scattering, MD simulations, CD spectroscopy, and a number of other techniques. The authors find that the membrane appears to thin, as indicated by the shift of phosphate groups toward the center of the bilayer upon Tat binding. The authors also indicate that the bending modulus has decreased and the area per lipid has increased as a result of Tat binding.

I appreciate the fact that the authors try to reconcile recent results, which I agree is much needed for this field. I find the results of this paper to be reasonable, as would many others. It can be argued that this is a selling point of the paper, and that it is likely right, but it can also be argued that it is because there is not too much new information in addition to our present knowledge if the main message is that the membrane thins when Tat binds to it. In this context, the analogy implicit in the 'carpet model' is remarkably prescient and powerful. Tat will thin membranes upon binding. Other peptides that adsorb on the surface also do this, and this idea has been well tested. Thinning is expected because putting extra excluded volume in the hydrophilic region will generally cause the effective area per lipid to increase, which can cause a corresponding membrane thinning for an incompressible membrane. Membrane softening also follows because thinner membranes are

easier to bend. It seems that if the goal is to advance the present understanding, then we need to go further. Perhaps I have missed something, and that there are other important conclusions, but then those should be emphasized more in the manuscript.

I have some questions and suggestions for improving the manuscript.

It is not clear what is the relation is between simulations and experiments, and what is the goal of the comparisons. For example, the authors write:

Although the SDP modeling of the X-ray data obtains excellent fits to the experimental form factors for a model with Tat deep in the hydrocarbon interior (see Fig. S5), the corresponding MD simulation (shown in Fig. 4.C) eliminates this spurious result.

Why does this follow? Why do simulations correct experiments and not the other around?

There are other examples of mutually contradicting results from different measurements that are presented in a nonproductive way:

In an effort to better determine the secondary structure of Tat, our collaborator, Dr. Rieko Ishima, performed 1D and 2D-NMR of Tat in solution at 10, 20 and 30oC. Her results showed no evidence for backbone hydrogen bond formation, indicating that the peptide does not have a stable <beta> conformation, at least on the time scale of the NMR measurement. Additionally, we analyzed the secondary structures of Tats from MD simulations using the Define Secondary Structure of Proteins (DSSP) program [69]. Data from the MD simulation which has the best fit to experimental X-ray form factors show that Tat contains neither <beta> nor <alpha>-helix structures. Therefore, both our solution NMR and MD simulation results find primarily random coil, with no significant <beta> structure, which contrasts with our CD findings of >50% <beta> conformation.

Rieko Ishima is not a co-author in this paper. Are these results being presented here or is this a kind of quotation?

The authors mention in that the main role of increased PE is to impact the amount of negative curvature. This is one consequence of increased PE of course, but an increase in PE will generally make the Gaussian modulus less negative also, which can also be important. (There are papers both theoretical and experimental on this.)

There exist recent multi-scale simulations from Wisconsin that test the idea of curvature generation by different peptides. Perhaps that will also be a good point of comparison besides the previous nice work of Angel Garcia. I am sure the authors know better, but the arguments are sloppy in a few places.

For example, the authors write:

One concern with diffraction experiments on samples consisting of adjacent bilayers in a stack or in a multilamellar vesicle is that the samples have to be partially dried to obtain conventional diffraction data. But then there is no pure water layer between adjacent bilayers, so a hydrophilic peptide is forced into the interfacial, partially hydrophilic region of the lipid bilayer. In contrast, by using diffuse scattering, we obtained structure from experimental samples that had a range of lamellar D spacings. How is this a consequence of diffuse scattering? How does diffuse scattering help one find a range of D spacings?

The authors also present results on the dependence of scattering on Qr, and therefore indicate that this shows a the bending modulus decrease, but there is little explanation. Here I think details of the analysis are especially important.

The authors write that they do not see significant membrane curvature induced by Tat binding. What is the expected readout if there were induced membrane curvature and how sensitive are these measurements to curvature generation? It seems that stacked multilayers are optimized for thickness measurements of membrane thinning. However, the deformation field induced by the silicon flat can have the effect of quenching induced curvature.

Reviewer #3: This is an interesting study where the authors combined a battery of experimental and simulation techniques to characterize the binding mode and impact of Tat on lipid bilayer properties. The authors have explored a fairly broad set of lipid compositions and peptide/lipid ratios. The key findings are that Tat binds at the interfacial region between the lipid and water, even with zwitterionic lipids, and that Tat binding locally thins the bilayer. Considering the great interest in cell penetration peptides/antimicrobial peptides, the current study makes a very valuable contribution. I have only a few minor questions, not necessarily in the order of importance:

1. A recent study by P. Jungwith on the binding of poly-arg appears to be relevant and could be discussed (JPC, B, 117 (2013) 11530).

2. It would be useful to characterize in a bit more detail the interaction between the Arg/Lys sidechains and lipid groups based on the extensive MD simulations. For example, a recent study by Wu et al. (JPC, B B 117, (2013) 12145) discussed possible differences between Arg/Lys in terms of their interactions with different lipid groups - are these also seen by the current studies?

3. In Fig. 7, the authors compared the findings from experiments and MD simulations. The MD results appear to be vary much more quickly than the experimental data. Is this due to the force field or other simulation details (e.g., finite size effect)? Are there ions in the simulation?