Robichaud et al. present all-atom MD simulations of the SP-B dimer in the presence of different lipid bilayer configurations. This study is an extension of their previous work on the SP-B monomer (ref 36). Overall, the MS is well presented and interesting behaviours of the protein dimer were observed.

Main comments:

1) The study uses four starting orientations that were based on the earlier monomer work. It is not clear to me that the end state of the monomer runs are relevant starting points for constructions the dimers. Please include a justification in the MS.

   We have added a paragraph to the results (lines 144-146) and a sentence to the methods (lines 226-234) to more fully explain the justification for the starting structures. The new paragraph is copied below:

   “In addition to representing well energy-minimized structures from previous monomeric simulations, the chosen starting configurations also represent classes of SP-B structures that have been proposed to account for SP-B’s activity, as follows. The closed configuration was included to match early homology models of SP-B that were based on closed saposin superfamily structures determined in water [30]. The open configurations are inspired by the structure envisioned by many experimentalists whose work probes the functional mechanisms of SP-B (e.g. 3,5]). The bent configurations represent an intermediate topology between these open and closed configurations that seemed particularly active in promoting bilayer structure transitions in the earlier simulations of the SP-B monomers [36].”

2) The starting points for the four simulations have a big effect on each run. Notably, none of them converge to similar configurations indicating that the results are highly influenced by the choices of the initial states. As a result, the four starting points produce four different “stories”. If additional starting configurations would have been included, presumably more behaviours would have been observed. Please include a discussion of these points in the MS. Can some aspects of the four runs be merged into a more cohesive story?
The four runs do suggest parts of a unified story if viewed as potential structures along the standard stages of membrane fusion. Two sentences have been added (lines 613-617) outlining this vision along with a reference to the earlier paper where we expand on this in detail, copied below:

“We and others [14,30,36,40,45] have envisioned the various SP-B-promoted lipid structures as potentially unified by SP-B promotion of lipid structures analogous to the standard structural steps in membrane fusion. As detailed in [36], the key SP-B-promoted lipid structures would be 1) close contact of the two membranes (Figure 10b), 2) thinning (Figure 7a, 8c) and hemifusion stalk formation, and 3) fusion pore formation (Figure 6b).”

3) Please include a section that compares the new results with previously published CG studies (refs 43-45). The time scales are different, but are some of the observed states (qualitatively) related? Even if there is no apparent correspondence, it would be helpful to point this out.

A paragraph has been added to the end of the discussion highlighting the similarities and differences between the course-grained work and our all atom work. And is copied below:

“In considering how these all-atom results compare with earlier coarse-grained simulations [43-45], it is good to bear in mind the longer timescales of the coarse-grained work, which of course come at the cost of reduced structural detail. Nonetheless, the close bilayer to bilayer contacts encouraged by SP-B in the O12 system (e.g. Figure 10b,c) do appear to resemble the early stages SP-B-promoted vesicle and bilayer/monolayer fusion activities observed in the coarse-grained simulations, where bent SP-B forms a “scaffold” that promotes the formation of a lipid bridge. Curiously, the SP-B-promoted bilayer crease observed in the bilayers here (Figure 8b) entails development of the opposite lipid curvature than observed for the coarse-grained simulations of SP-B interacting with monolayers. i.e. in the all-atom simulations, SP-B promoted negative curvature, with headgroups bending inwards, while in the coarse-grained simulations the curvature is positive. Also worth noting is that the kinetics of fold formation in the coarse-grained systems were seen to be faster with dimeric SP-B than with monomeric SP-B which is in keeping with how early in the all-atom simulation dimeric SP-B induced crease formation (Figure 8b).”

Minor comments/corrections:
3) Ref 43 is incomplete.

Fixed

4) Fig 3: The range on the Y-axes for panels a, b and c are 0.008, 0.015 and 0.0125 x10^6 kJ/mol, respectively. Also, simulations BI1 and O11 are combined in one panel, presumably
because they start at the same absolute potential value. This makes it difficult to compare the four simulations. I suggest presenting four panels (one for each run), all with the same Y range.

The ranges of the y-axes are now all the same. BI1 and OI1 were placed on the same plot because they have the exact same system composition (as detailed in Table 1). This makes it easier to directly compare these two simulations, whereas the other systems have different compositions and thus quantitatively comparing their energies is not as useful.

5) Fig 5: It would be better to show a single residue range on the X-axis of panels a-d (1-79; and not 1-17,1-79 side by side). Maybe with solid/dashed lines for the two subunits? (unaveraged in all cases).

This figure has been updated following your recommendation.