Dear Prof. Pedro Silva,

I thank you for your careful reading of our manuscript and your constructive feedback.

The statistics and text have been corrected to include the correct values. The overall conclusions of the study are not affected. I have also uploaded the homology model coordinates as supplementary materials as requested.

Ho Leung Ng

8/28/2021

Editor comments (Pedro Silva)

MINOR REVISIONS

Editor comments in red. My response in blue.

A) the loops in the beta2 model (171-196) are quite different from the one in the crystal structures (3p0g, 4ldo), and (more importantly) they keep the entrance to the inner channel more open than in those crystal structure). Also, the beta2 model provided contains carazolol, but the pose is quite different (RMSD=5.9 angstrom) from the one in structure 2rh1, incorntrast to the very good fit claimed in lines 257-258. Since your method does not prevent it from finding good docking poses in beta2, the disappointing behavior you found in H1, M1 and 5HT2B models cannot simply be attributed to a bad loop model. For this reason, I would suggest rephrasing the text in lines 313-317 ("In all the failed cases (histamine H1, muscarinic M1, and serotonin 5HT2B), while the homology models were sometime accurate at the level of backbone atoms in the 7tm region, the loops were modeled poorly and disrupted the modeled ligand binding pocket. In these cases, the homology models are not accurate enough for docking or ConDockSite") .

Lines 257-258 refer to the comparison of the cross-docking results rather than homology models.

We agree that our descriptions of loop differences were too simplistic. We have changed lines 313-317 to read, “In some of the failed cases (histamine H1, muscarinic M1, and serotonin 5HT2B), while the homology models were sometime accurate at the level of backbone atoms in the 7tm region, the loops were modeled poorly and disrupted the modeled ligand binding pocket. In other cases, the homology model backbones were modeled well but differences in the side chain conformations disrupted the integrity of the ligand binding sites.”

B) The loops in the A2A model (143-166) are also quite different from the ones in the crystal structures (2ydo, 4ug2,5iub,5k2, for A2A) : in this case, the modelled structure is more open than the crystal structures, and similar to that in structure PDB:5c1m of the mu-opioid receptor which leads me to believe that the differences in loop structure you find may suimply reflect different

physiological conformations of the GPCR. Regardless of the origin of those differences, neither the A2A (or the beta2 models) deposited as SI contain adenosine (or adrenaline), but a different molecule in quite a different pose. This makes it impossible to reproduce Fig. 1A and 1C.

Lines 143-166 and Figs. 1A and 1C refer to results from ConDockSite on cross-docked crystal structures rather than homology models. I have changed the Figure 1 heading to “Predicted and experimental ligand binding sites in A2A adenosine and beta2 adrenergic receptors from cross-docked crystal structures” to make this clearer. I have also added the cross-docked models to the SI.

C) Furthermore, in the 5HT2 model, the modelled loops (corresponding to aa 194-206 in DRZ) that are different from the ones in the 6drz structure are away from the binding site entrance, and in the H1 model, the loop actually leaves the entrance to the binding site more unencumbered than in the 3rze model. It therefore does not seem at all appropriate to attribute the observed poor performance of ConDurfDock in these instances to this loop (especially in comparison to the good behavior in spite of poor loops described above).

In the 5ht2b model, the problem loop in the homology model is actually aa 167-170, which extends Leu 169 into the correct ligand binding site. But there are also problems in the modeled side chain conformations which we described in lines 309-311: “Serious errors in homology modeling of the 5HT2B receptor side chains make it difficult or impossible to dock the ligand into the deep, restricted binding site.“ For the H1 model, loop 162-165 closes the top of the ligand binding site. There are also errors in the modeled side chain conformations of the TM helices that intrude into the binding site. We have added more details in lines 293-295: “The poor performance was due to the inaccurate modeling of a loop containing residues 162-165 over the top of the ligand binding site as well as multiple errors in the conformation of side chains in the transmembrane helixes.”