Life Sciences 225 (2019) 132

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Comment on: Triazoles bind the C-terminal domain of SMO: Illustration by docking and molecular dynamics simulations the binding between SMO and triazoles

Dear Prof Wold,

This letter is written to comment on the article: Triazoles bind the C-terminal domain of SMO: Illustration by docking and molecular dynamics simulations the binding between SMO and triazoles by Liu et al., which has recently been published in Life Sciences - 271 (2019) 222–228.

The presented study deals with an interesting topic: potential interactions between triazoles and Smoothened (SMO). The authors use a previously published crystal structure of SMO in complex with cholesterol and fused with the crystallization scaffold BRIL in the intracellular loop 3 (PDB ID: 5L7D [1]) in order to perform *in silico* docking of the ligands to the receptor and subsequently run the molecular dynamics (MD) simulations on the ligand-protein complexes. Since the authors employ solely a computational approach in their work, the quality of the structures and the interpretations of the receptor models they use are crucial. It is unfortunate, but the manuscript suffers from two major problems which should have been noticed during the peer review process.

Firstly, the authors define the N-terminal domain (NTD in the study) of SMO to be the crystallization scaffold BRIL, which is inserted in the intracellular loop 3. Further, the C-terminal domain (CTD in the study) of SMO is defined as the cysteine-rich domain (CRD). This concept is illustrated in the Fig. 4(a) of the paper. This understanding of the SMO structure is intrinsically wrong. SMO is a seven transmembrane domain spanning receptor, which has its N-terminus, including the CRD, located extracellularly, and its C-terminus located intracellularly. The 5L7D structure presents SMO recombinantly fused with BRIL between the transmembrane domain 5 and transmembrane domain 6 of SMO, in the place of the intracellular loop 3 of SMO. BRIL is not a natural part of the SMO protein - it is a scaffold protein used to stabilize the receptor and thus to facilitate its crystallization. Moreover, in stark contrast to the authors' claims, the long C-terminal domain of SMO has not been resolved in this, and in fact, in any of the published SMO structures to date.

Secondly, the authors run the MD simulations using SMO-BRIL. Inclusion of the crystallization scaffold BRIL in the MD poses in itself already a significant problem, because an artificial protein construct is simulated to draw conclusions about biologically relevant processes. Remarkably, the authors report the movement of BRIL as a conformational change of the NTD (Lines 1–5 in the 3.3 Conformational change and dPCA results). Since BRIL is not a natural part of the SMO protein, these experiments are not valid and their interpretation is false. In addition, the transmembrane protein is solvated only in water with NaCl. Since SMO is a G protein-coupled receptor embedded in the cell membrane, embedding this receptor in a lipid bilayer is required to perform simulations that would somehow attempt to reflect physiology and allow collecting meaningful data. In order to obtain biologically sound data, the scientifically-justified approach would be to:

1. Remove the BRIL from the 5L7D SMO structure,
2. Model the missing residues in accordance with another solved SMO structure,
3. Use the resulting model to run the ligand docking experiments.
4. Complexes of a ligand with a membrane protein must be solvated in water with ions and embedded in a lipid bilayer. Provided that this or a similar setup was employed, the system could then be used in the MD simulations. Should the authors want to proceed with the MD studies on SMO in the future, they are advised to refer to the literature on the subject (for the recent molecular dynamics methodology review and for the very recent works on SMO, please see [2–4]) In addition to the above-mentioned major flaws, the *in silico* results should be preferably complemented with *in vitro* data, especially since it remains unclear if the tested compounds at all interact with SMO. Furthermore, the references in the text are often not corresponding with the reference list, which makes it difficult to control the authors' claims (Ref [2] and [4] in the original paper are mentioned twice in the text referring to unrelated issues).

Taking all the above into consideration, the article suffers from the clear misconceptions in the basic understanding of receptor structure and biology.

References

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https://doi.org/10.1016/j.lfs.2019.03.075

Available online 06 April 2019
0024-3205/ © 2019 Published by Elsevier Inc.