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Eschatological scrutiny of unprofessional usage of molecular docking; how unreliability in computational methods arises from amateurish mistakes

A B S T R A C T

The impact of computational methods in drug design and discovery is prevailing in both academic and industrial scales. Molecular docking is one of the favorite tools for assessment of the interactions between a ligand and its congener macromolecule. *In silico* approaches and especially molecular docking are gaining much attention in recent years due to their cost-effective nature. In this letter to editor, we want to briefly describe how an undisciplined and unorganized research with molecular docking can result in highly equivocal results. This discussion can be useful for other scientist to avoid these pitfalls. This paper addresses the article by V. Suganya and V. Anuradha entitled "In silico molecular docking of astaxanthin and sorafenib with different apoptotic proteins involved in hepatocellular carcinoma" published in 6th of March 2019 on Biocatalysis and Agricultural Biotechnology.

Molecular docking is reckoned as a very instructive and informative instrument in drug design and discovery (de Ruyck et al., 2016), protein-protein interactions (Safavi et al., 2019), bioremediation (Liu et al., 2018) and also studying the catalytic mechanism of enzymes (Bhattacharjee et al., 2017). Nowadays there are a growing number of publications using this popular approach to identify the hit ligand molecules for a specific macromolecular target; that can be a protein, DNA and even RNA.

Recently a new term has been applied in the process of drug discovery called "reverse docking" or "inverse docking" (Xu et al., 2018) which is practically the reverse process of molecular docking; in which a ligand molecule (or a few) will be docked against a large number of proteins in order to identify the plausible targets which exhibit the best computational free energy. SePreSA server can be easily used for this reverse docking and drug repurposing purposes (Yang et al., 2009). This process is especially useful for organic chemists which are not dealing with the biological activity of molecules and the novelty of the synthetic process or the new compounds they have obtained, is the main point. So, as long as reverse docking verified the possible target molecule, they embark on assessing the pharmacological activity of those compounds to catch new hit molecules as drug candidates. Reverse docking is not the only way to detect the potential other targets of a specific compound. To identify the off-label targets of a drug (or ligand) molecule, a database search is also very auspicious. For instance, ChEMBL, Reaxys and SciFinder can be used for detection of similar ligand molecules with different biological activities than the "mother ligand". Normal molecular docking can be used thereafter to determine the computational binding evaluation and the possibility of inhibition/activation of that receptor.

In this letter to editor we want to address a few issues raised by Suganya and Anuradha (2019) paper. First to emphasize is that promoting apoptosis by a certain compound does not necessarily signify that it interacts with apoptotic proteins. For instance, the compound might interact with a battery of other proteins like transcription factors e.g. NF- κB (Dolcet et al., 2005) or proteasome proteins (Gupta et al., 2018) or many other pathways. Hence, suggesting a specific ligand molecule for a specific protein molecule without underlying literature evidence or without further experimental (or computational methods like reverse docking) techniques which justify it, would be reprehensible.

In the paper by Suganya and Anuradha, a series of molecular docking were performed with sorafenib and astaxanthin on growth factor receptors EGFR and VEGFR and three apoptotic proteins (BCL2, caspase 3 and caspase 9) to "prove" the mechanism of these molecules as anti-cancer agents. Even if all of the component and methodology used in this study were accurate, we would arguably declare that these approaches are far enough to provide a clear insight about the mechanism of these anti-cancer molecules. The word "prove" which was used in this article, could be applied when there were more appropriate methods to assess the binding interactions. Today, it is generally accepted that molecular docking cannot say anything about the agonistic or antagonistic nature of a certain ligand (Chen, 2015). A molecule can demonstrate a highly negative free energy values in docking studies and it only binds to a certain site on the receptor. It may be an agonist, an antagonist, a partial or reverse agonist, an allosteric modulator or even none of them. The pharmacological activity for a compound is closely related to the conformational change it induces on its congruent receptor. Of course there are some computational methods in which you can determine the nature of behavior (Miao et al., 2018) but molecular docking is too preliminary to disclose any information like this.

The next point about this article, is that the proteins used, were not satisfactorily prepared before starting the docking operation (no details in the method section). It is plainly corroborated in previous surveys that many structures embedded in protein data bank (PDB database) are not ready for a molecular docking procedure (Bietz et al., 2016). In order to have a legitimate structure for docking, certain parameters should be checked. A reliable resolution is considered to be crucial for this purpose. A typical C–C bond length is about 1.5 Å and as the "numerical" resolution of X-ray crystallography rises, the ability for distinguishing between different residues and their interconnection is gradually lost. Many hydrogen-bond networks are irresolute and with this cloudy binding pocket, no precise docking calculations can be performed. In order to get rid of these problems, one might use molecular dynamics (MD) to prepare the structure and additionally, many other softwares provide in-built preparation tools. The other problem with PDB proteins is that they sometimes lack some amino acid residues

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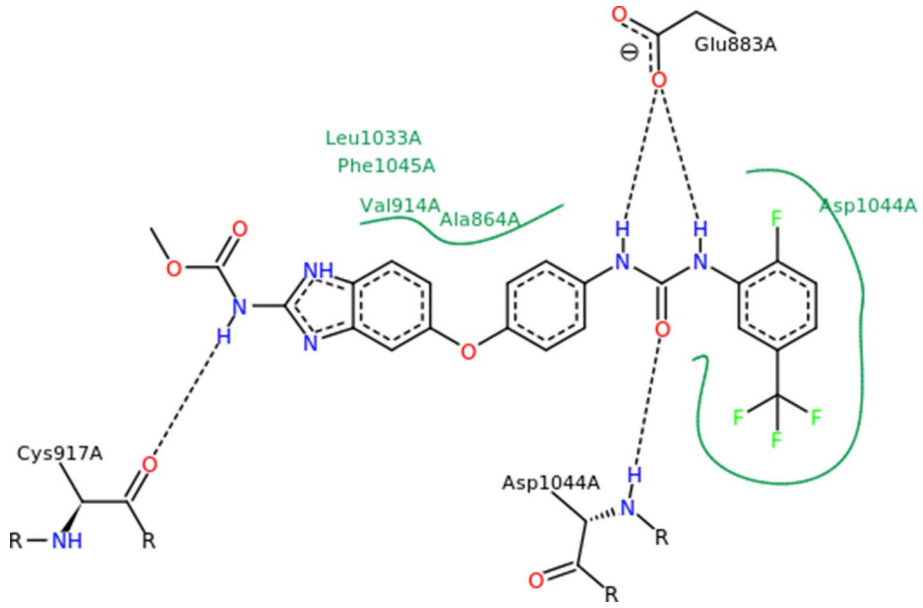

Fig. 1. Interactions between the ligand and VEGFR according to the PDB database (2OH4) (Hasegawa et al., 2007).

(which are lost during the crystallography process) and ought to be added to the structure prior to docking. This procedure which is termed loop modeling or loop building was not performed for the proteins of this article. In the case of caspase 9 (PDB ID: 2AR9, which was used in Suganya and Anuradha paper) the first 140 residues of all chains are missed in the pdb structure and this can profoundly impinge the whole result of docking operation. Of course, this part of the protein might be irrelevant to the binding site but no description about the nature of this region of the protein and its potential activity was addressed. To make it even more complicated, we should add that 2AR9 is the dimeric structure of caspase 9. It is well established that proteins shape dissimilarly in their different morpheein structures (Wasserman and Saphire, 2016). For an enzyme like caspase 9, in which dimer and monomer can both exist in cellular environment there should be a biochemical comparison between these two structures (which can be done by surveying the literature or computationally by running a molecular dynamics simulation on each structure). To make it succinct, the structure used in the dimeric state might have a distinctive structure and/or function from the monomeric state.

One of the most conspicuous and irrefutable errors of the Suganya and Anuradha study, is that the proteins used in the docking procedures are the mutated forms. 2AR9 has six mutation and is not the native structure of caspase 9 (Chao et al., 2005). Even if the function of the protein remains unaffected by these mutations, the binding of a ligand molecule can be influenced remarkably. This concept is fully investigated in a branch of pharmacology dubbed “pharmacogenetics” (Ma and Lu, 2011). 4LQM (EGFR) another protein used in this study, also displays one mutation according to the protein data bank. The title of the related article to this structure also demonstrates that the role of a mutation was assessed in lung cancer in that study (Yasuda et al., 2013).

There is a term called “hydrophobic bond” which was used in the Suganya and Anuradha research article and needs clarification. Although it is more common to state “hydrophobic interactions” due to the chemical nature of these interactions, there should be a transparent viewpoint about the nature of them. Many interactions which have been previously characterized as hydrophobic interactions are now more precisely announced as “π-cation”, “π- π” as well as “π-anion” interactions (Daze and Hof, 2016). The authors stated that His143 creates a hydrophobic interaction with astaxanthin. Because the imidazole ring of histidine can take part in π-cation interaction (Liao et al., 2013), this so-called hydrophobic interaction could be actually a π-cation interaction.

Another unacceptable flaw in the aforementioned paper, is the incorrect finding of the binding poses. According to the PDB database (2OH4) and Hasegawa et al. article (Hasegawa et al., 2007), the residues which interact with the designed ligand are completely different from those reported by Suganya and Anuradha (Fig. 1). Neither Leu834, Pro837, Met867, Leu868, His874, Pro909 for astaxanthin nor Lys824, Asp855 and Leu900 for sorafenib (Tables 5 and 6 from Suganya and Anuradha paper) are not involved in interactions with ligand in the previously examined paper by Hasegawa et al. Since their paper is accompanied by the crystallography, the docking process should be assessed by this more accurate experimental assay. It would be obvious also that molecular dynamics study reject these “bad binding poses” of sorafenib and astaxanthin and repell the ligands out from the binding site after a short nanoscale time of simulation.

Moreover the molecular docking study of Astaxanthin with 2W3L and 4LQM resulted in a very superficial binding site. There should be a comprehensive discussion in the article as why the binding pocket is so near the surface of these proteins. Usually, a right binding pose should be completely immersed deep in the structure of the receptor. Of course, there can be some exceptions to this rule but we think that based on other inaccuracies observed in the paper, this one is also unknowingly overlooked.

Although molecular docking is a very effective and highly proficient computational method, in the case where improper inputs are used, the results would be definitely questionable. Lack of literature surveys along with inexperienced application of *in silico* approaches, culminates in unreliability of computational sciences like bioinformatics and cheminformatics in academic society which should be strictly prohibited. The future of *in silico* methods are under attack by these preposterous and dubious findings. In today science *in silico* approaches play the indispensable part for biomedical researchers. The design of many drugs we use nowadays, have been started by these computer-aided drug design (CADD) procedures (Sliwoski et al., 2013) but the correctness of methodology is of the essence for cultivating decent results.

Conflicts of interest

There is no conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101224.

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