# Lessons learned from docking with the presence of water molecules – is it worth it?

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## Introduction

Water molecules play an important role in many aspects of ligands binding and can at least be considered to be the next dimension in an understanding of effects triggered by the creation of a ligand-receptor complex. Ideally, computational approaches should create and incorporate water networks in ligand docking and binding mode analysis. With the releasing of appropriate software, approaches incorporating water molecules were applied for, e.g. explanation of SAR studies, justification of in vitro results or evaluation if a presence of water molecules improves docking studies. Nevertheless, the presence of water molecules in GPCR's binding site and their influence on different aspects of ligand binding have not yet been explored [1].

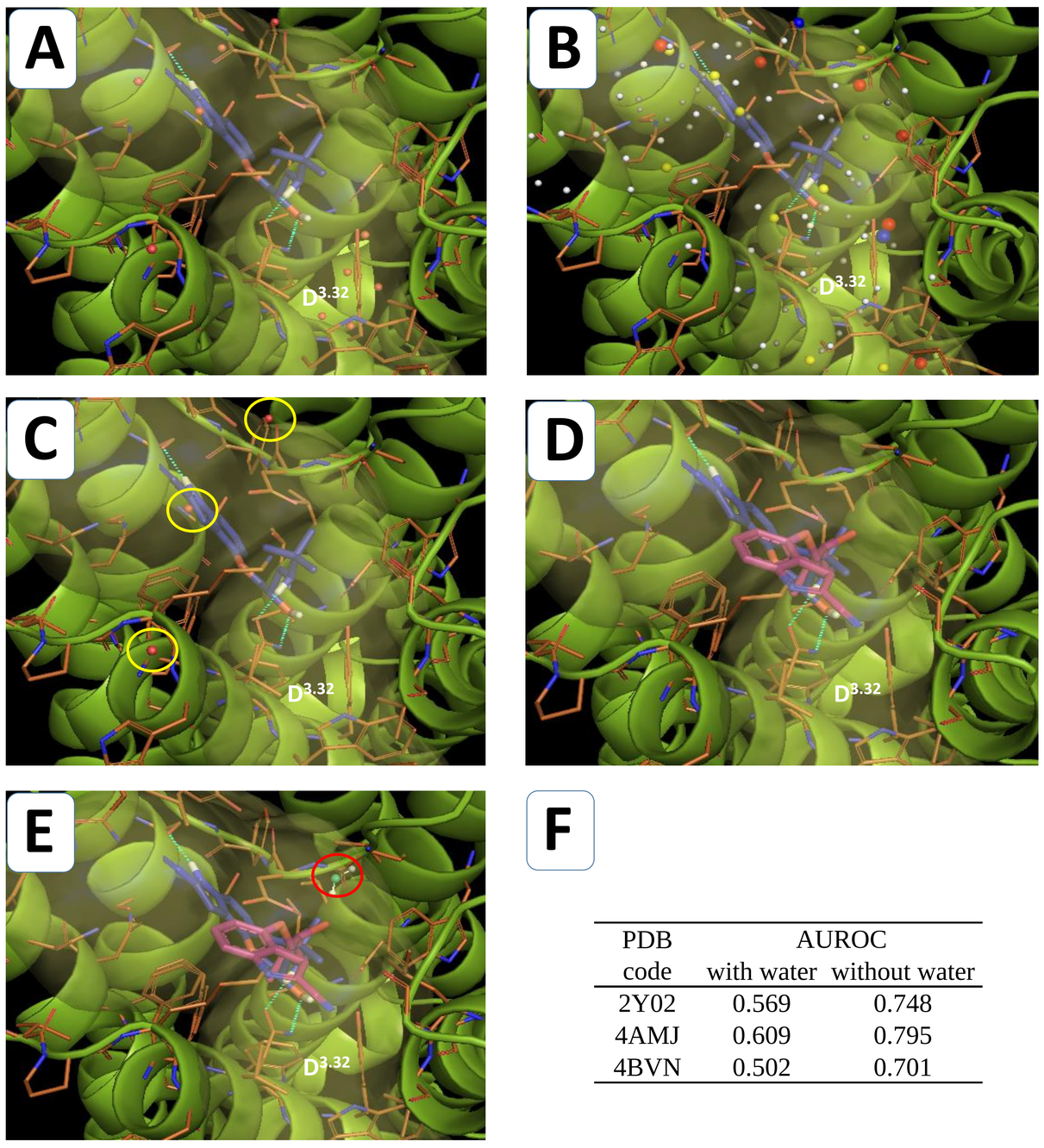
In this study, for every single crystallized target from GPCR's class A aminergic and peptide families, water networks were generated in WaterFLAP [2]. Several issues related to the application of water molecules for docking studies were considered: (i) energy and location of the water molecules within the binding site and the correspondence to the water molecules present in the crystal structures, (ii) recreation of binding mode observed in crystal complexes by docking with a presence of predicted water, (iii) how the results of screening experiments depend on the incorporation of water molecules.

## Materials and Methods

- 21 different crystal structures for 16 GPCR targets were fetched from the GPCRdb (Table 1, Figure 1A).
- WaterFLAP software was used for generation of water molecules network within binding pockets (Figure 1B).
- The free energy of each water molecule was calculated.
- The coordinates of the predicted waters were compared to the coordinates of the crystal waters (Figure 1C).
- Co-crystalized ligands were redocked into the corresponding crystal structure with and without the presence of water molecules (Figures 1D and 1E).
- Positions from both dockings were evaluated by RMSD calculation and by comparing scoring function values.
- Retrospective screening for three $\beta_1$-adrenoceptor structures was conducted with 196 active compounds (ChEMBL, $K_i$ or equivalent less than 100 nM) and ~61k DUD-E decoys [3].
- Area under Receiver Operating Characteristics (AUROC) was used as the measurement of screening efficiency (Figure 1F).

**Table 1.** GPCR of class A and C used in the study.

| Receptor | PDB code | Res [Å] | X-ray ligand | Ligand function |
|---|---|---|---|---|
| 5-$HT_{1B}$R | 4IAR | 2.7 | Ergotamine | Agonist |
| 5-$HT_{2B}$R | 4IB4 | 2.7 | Ergotamine | Agonist |
| $\alpha_{2A}$R | 4EIY | 1.8 | ZM 241385 | Antagonist |
| CCR5 | 4MBS | 2.7 | Maraviroc | Antagonist |
| CXCR4 | 3ODU | 2.5 | IT1t | Antagonist |
| $D_3$R | 3PBL | 2.9 | Eticlopride | Antagonist |
| $H_1$R | 3RZE | 3.1 | Doxepin | Antagonist |
| $M_1$R | 5CXV | 2.7 | ZM 241385 | Antagonist |
| $M_2$R | 3UON | 3 | 3-Quinuclidinyl benzilate | Antagonist |
| | 4MQS | 3.5 | Iperoxo | Agonist |
| $M_3$R | 4U15 | 2.8 | Tiotropium | Antagonist |
| $M_4$R | 5DSG | 2.6 | Tiotropium | Antagonist |
| $mGlu_1$R | 4OR2 | 2.8 | FITM | NAM |
| $mGlu_5$R | 4OO9 | 2.6 | Mavoglurant | NAM |
| $\beta_1$-adrenoceptor | 4AMJ | 2.3 | (S)-Carvedilol | Inverse agonist |
| | 2Y02 | 2.6 | Carmoterol | Agonist |
| | 4BVN | 2.1 | DB08347 | Antagonist |
| | 3NYA | 3.2 | (-)-Alprenolol | Antagonist |
| $\beta_2$-adrenoceptor | 4LDE | 2.8 | BI167107 | Agonist |
| | 2RH1 | 2.4 | Carazolol | Inverse agonist |
| δ oppioid | 4EJ4 | 3.4 | Naltrindole | Antagonist |




| PDB code | AUROC with water | AUROC without water |
|---|---|---|
| 2Y02 | 0.569 | 0.748 |
| 4AMJ | 0.609 | 0.795 |
| 4BVN | 0.502 | 0.701 |

**Figure 1.** Influence of water molecules on binding mode. A – crystal structure of $\beta_1$-adrenoceptor (PDBid: 4BVN) with the ligand (cyanopindolol) and water molecules. B – water network predicted by the WaterFLAP software. C – common water structures (yellow circles) from crystal structures and the predicted water network. D – comparison of binding mode of the cyanopindolol from the crystal structure and docking pose of this ligand (docking was performed with the water network). E – crystal and docking pose of cyanopindolol with one bridging water molecule (red circle). F – comparison of the efficiency of retrospective screening experiments conducted with and without water molecules.

## Results and Conclusions

The results confirm that the lower resolution of the crystal structure, the less number of water molecules was predicted by WaterFLAP. Only 2.5% of predicted water molecules has ΔG less that -2.0 kcal/mol and has the potential for turning into bridging water. The software was able to predict only 37% of water molecules co-crystalized with the ligand (in comparison to 80% declared by the developers, who however tested the method on non-GPCR set of proteins) [2]. Redocking of co-crystalized ligands showed that the procedure without water, in 21 out of 23 cases, gave back better values of scoring functions and RMSD in comparison with the procedure with water. Only for 4 complexes, the best scored pose included bridging water.

The results show a rather surprisingly insignificant role of the water molecules for enhancement of the performed GPCR ligand docking studies. Moreover, comparison of docking and crystal complexes shows some trends, e.g. that a favorable value of ΔG of a water molecule does not guarantee the capability of the molecule to bridge interactions. The results indicate that application of water molecules for GPCR ligands docking studies is still challenging and have to be carefully supervised and should not be routinely used as a black-box procedure.

### References

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### Acknowledgements

The work was supported by the National Science Centre (Poland) grant no. 2016/21/N/NZ2/01725.

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