SIFt-guided agonist/antagonist differentiation for beta2-AR ligands

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Introduction

While searching for new ligands for GPCR targets, the _in silico_ methods used in virtual screening campaigns enable fast and moderately efficient way of speeding up the process. Ligand docking protocols are considered the most accurate ways of determining the possibility of a compound to possess any affinity towards the target. However, this method cannot specify the type of it's agonist/antagonist function, especially when only docking scores are taken into consideration. To change that, a special docking protocol combined with post-docking analysis and docking constraint application was constructed. The results presented, expose several hot-spots within crystal structures of beta2-AR, by applying an extended set of agonist and antagonist ligands.

Methodology

The 8 antagonist-bound and 2 agonist-bound crystal structures of beta2-AR were extracted from PDB repository. Beta2-AR ligands were extracted from ChEMBL [1]. The compounds were initially filtered for their activity, that is compounds that had K_i below 1000nM. The resulting ligands were checked for agonist/antagonist data contained within their descriptions. The final set contained 38 active agonists and 21 antagonists.

From the 8 antagonist-bound crystal structures 2 were selected for further research, based on the completeness of their structures and their ability to dock active beta2-AR ligands.

All agonist and antagonist compounds were docked (using Glide software [2]) to the 4 beta2-AR crystal structures (agonist: 3P0G, 3PDS; antagonist: 2RH1, 3NY8) using a constraint forcing a positively charged amine to interact with D3.32 residue. Ten poses were returned for each compound, and Structural Interaction Fingerprint (SIFt) [3] profiles were calculated, as in previous research [4]. The SIFt profiles were then used in machine learning (ML) experiments using WEKA software, using 10-fold cross validation and different classification algorithms.

Results

The classification experiment using SMO (Sequential Minimal Optimization) support vector protocol returned a perfect classificator, that is all agonists and antagonists were properly classified. To extract the specific interactions that differentiate each type of ligands, the classificator buffer was extracted and the most important bits were found. After translation, the agonist and antagonist-specific hot-spots were indicated. What is worth noticing, the hot spots found would not be able to be highlighted using ligands co-crystalized in PDB records, since many of the ChEMBL antagonists are much bigger compounds, and therefore display different binding modes.

Conclusions

The SIFt-based interaction profiles led to a perfect discrimination of agonist and antagonist compounds for beta2-AR. The interactions responsible for the discrimination were highlighted and analyzed, which led to following conclusions:

- To properly distinguish agonists and antagonists, the compound sets must be docked to both agonist and antagonist bound crystal structures.
- Agonist and antagonist crystals have diverse differentiation capabilities and different amino acids taking part in the process
- A proper implementation of docking constraints may result in an effective virtual screening model for agonist/antagonist differentiation
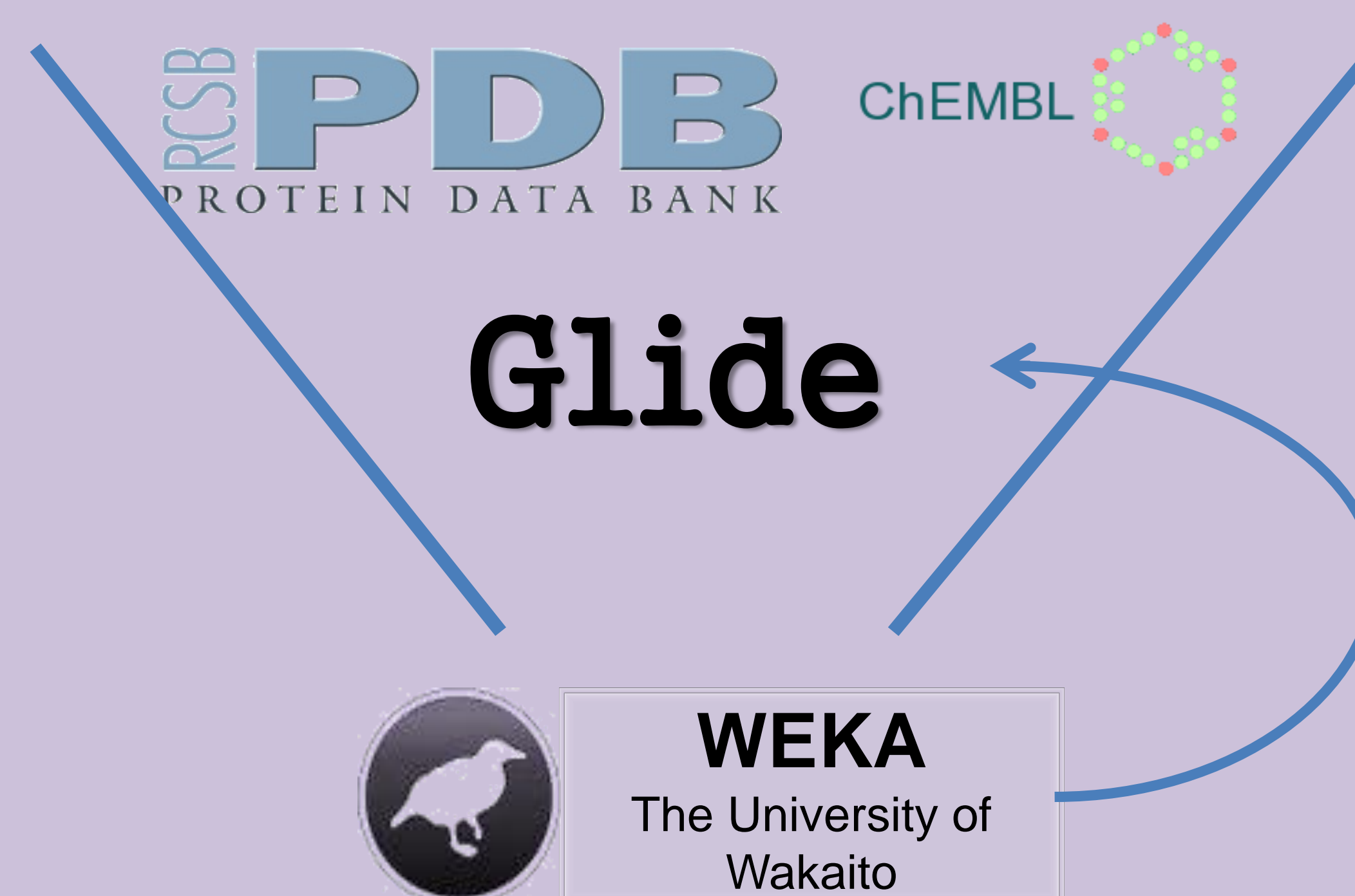

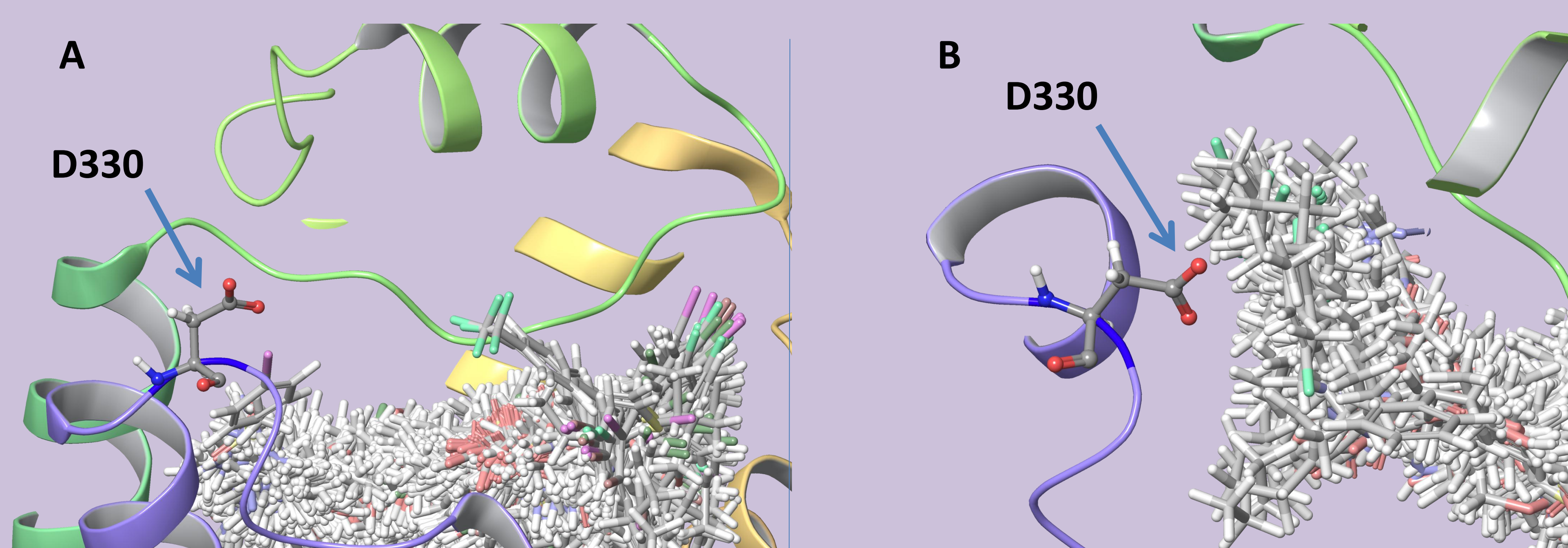**Figure 1.:** Symbolic workflow of the study.

Figure 2.: Visualization of all poses of agonists (A) and antagonists (B) docked to beta2-AR. It is visible how antagonists are docked in the vicinity of the D330 residue, while no agonists express this interaction.
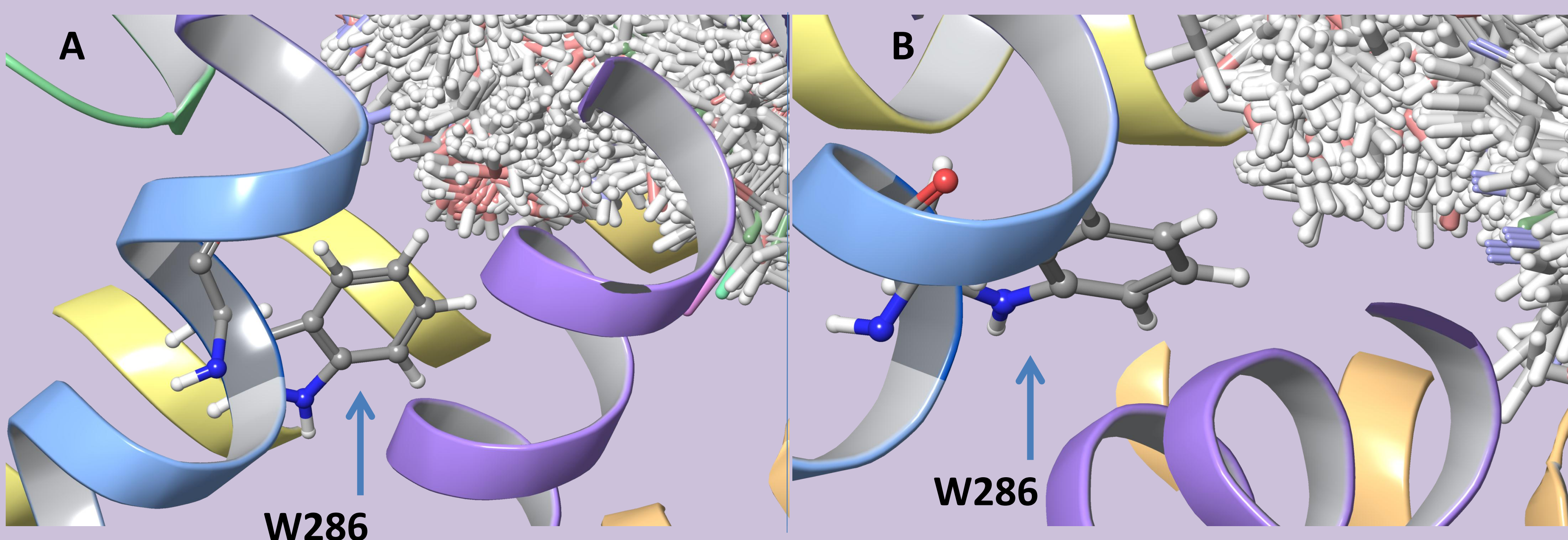

Figure 3.: W286 (W6.48) hot-spot in beta2-AR binding agonists (A) and antagonists (B). Agonists expose hydroxyl groups towards the W286 residue, while antagonists interact through hydrogen atoms.
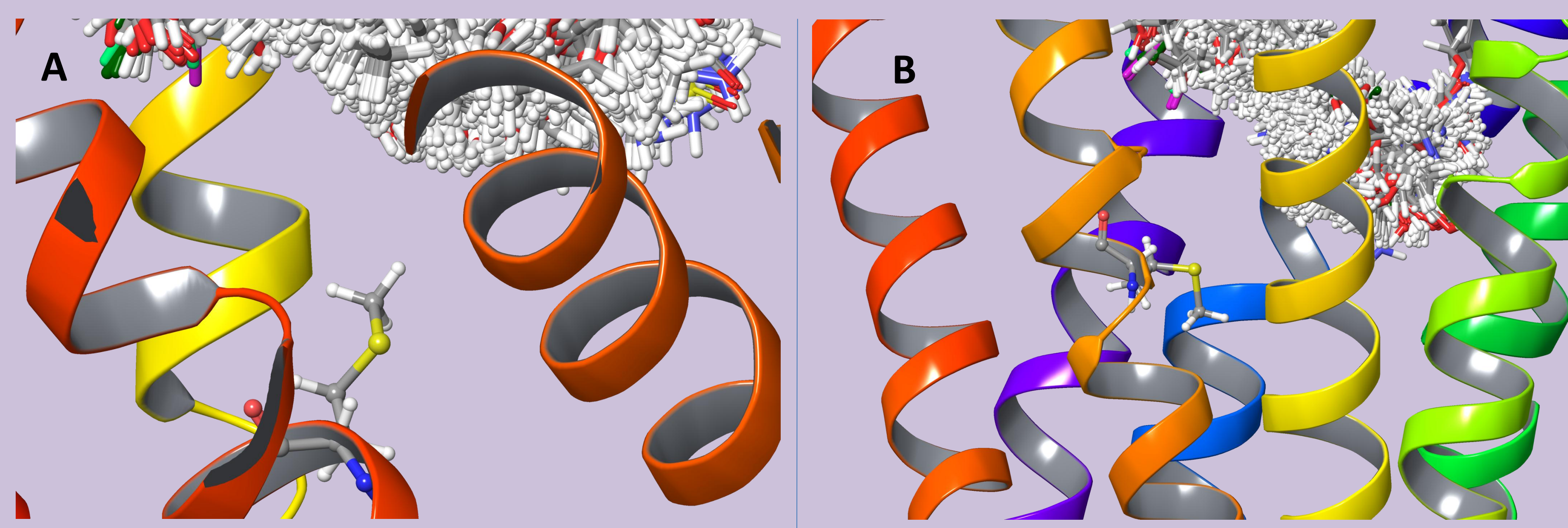

Figure 4.: Different conformation of M82 (M2.53) methyl group in 3NY8 (A) compared to 2RH1 (B). Inclusion of the bits responding to this residue leads to acquiring type-insensitive constraints and lowering the specificity of the experiment.

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