Aminergic GPCRs from a site-directed mutagenesis perspective – analysis and prediction

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Introduction

The group of aminergic G protein-coupled receptors (GPCRs) is one of the most important targets in drug discovery campaigns because they control the whole range of the physiological processes occurring in the living organism.[1] As the knowledge on those receptor is still limited, especially in terms of structural aspects, various tools are used for determination of structural drivers for ligand affinity, with extensive mutagenetic studies as a core methodology in this field.

In the presented study, the mutagenetic data on compounds affinities within the aminergic subfamily were collected and analyzed. Additionally, a protocol for the prediction of the consequence of particular amino acid substitution for various ligands was developed.

Mutagenetic data

The prepared data collections were analyzed from various points of view with the special focus put on the number of datapoints available for particular receptor subtype (Figure 1).

The muscarinic receptors were definitely the most populated in terms of the mutagenetic data (2333 datapoints) with M_1R providing the highest contribution to this result (1214 datapoints).

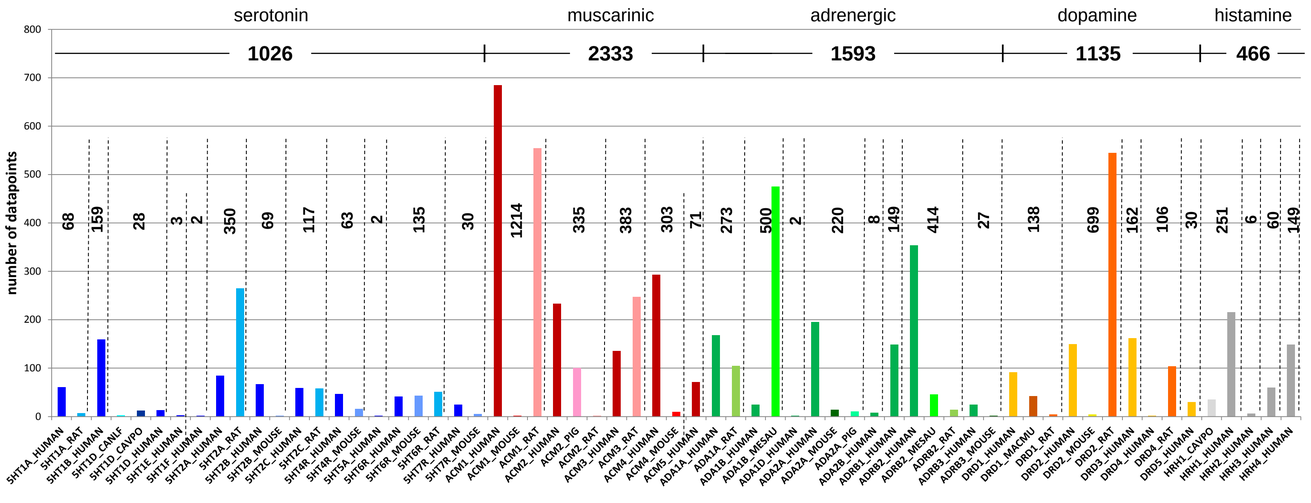


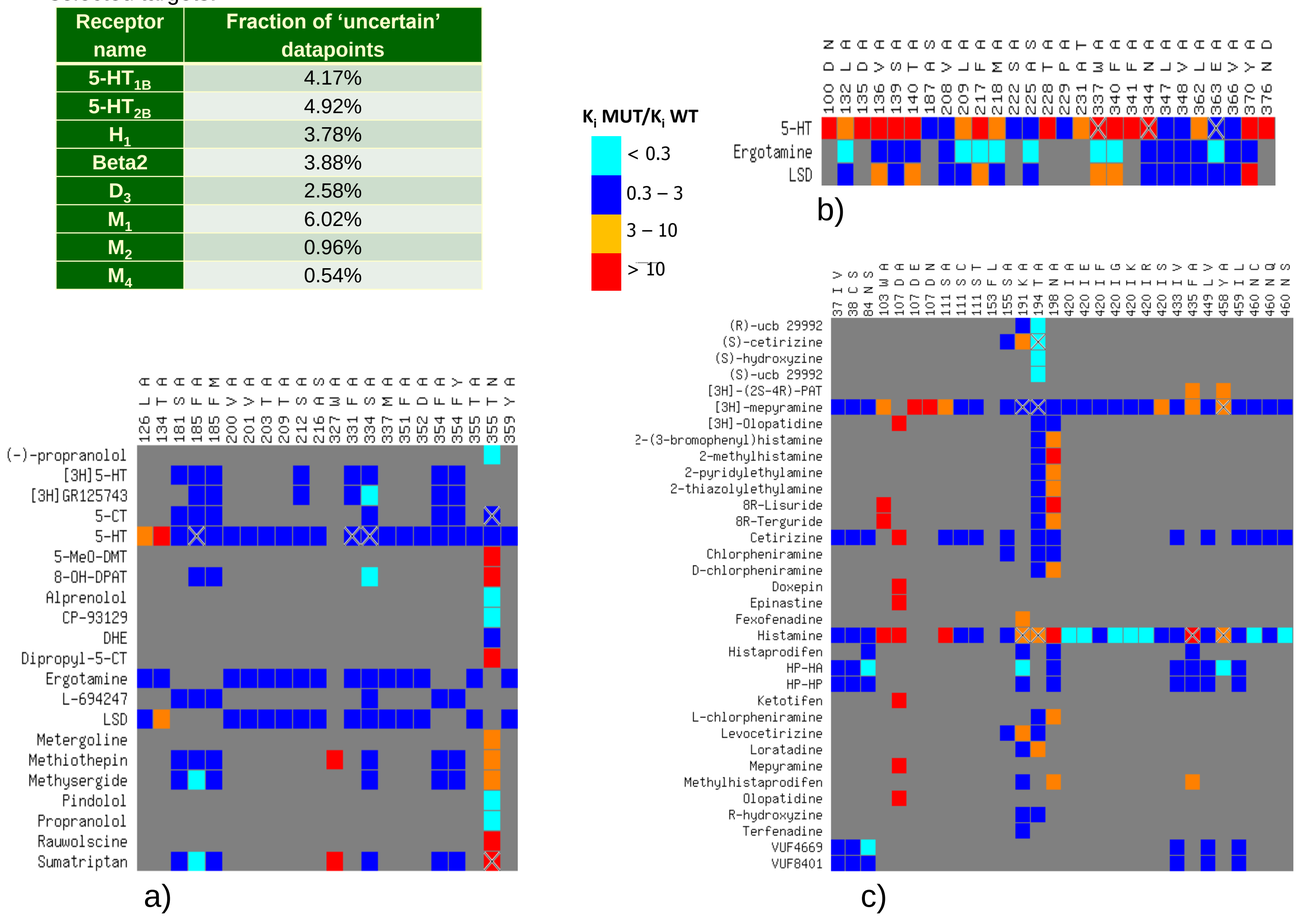
Figure 1. Number of mutagenetic datapoints available for particular receptor subtypes.

Mutational effect classes/data uncertainty

The mutational effects expressed as K_i MUT/K_i WT (or equivalent affinity parameter value) were grouped into four classes: < 0.3; 0.3 – 3; 3 – 10; > 10. The heat maps obtained for selected crystallized human aminergic GPCRs are presented in Figure 2. If one or more datapoints were available for a given pair point mutation/ligand, the median value was taken, and standard deviation (SD) was calculated; if SD values were higher than the distance to another class, the data point was considered as uncertain, which was marked with a cross. The fraction of 'uncertain' points for the targets for which crystal structures for human sequences are available was rather low and varied from 0.54% for M_4R, up to 6.02% for M_1R (Table 1).

Table 1. Fraction of uncertain datapoints for selected targets.

Receptor name	Fraction of 'uncertain' datapoints
5-HT_{1B}	4.17%
5-HT_{2B}	4.92%
H_1	3.78%
Beta2	3.88%
D_3	2.58%
M_1	6.02%
M_2	0.96%
M_4	0.54%



. **Figure 2**. Effect of a given point mutation expressed as K_i MUT/K_i WT for a) 5-HT_{1B}R; b) 5-HT_{2B}R; c) H_1R

Mutational effect prediction

The protocol for the prediction of the effect of mutation was based on docking of the considered ligands to the target binding site (PLANTS docking software)[2] and analysis of the similarity of its interaction to ligands for which the effect observed upon particular point mutation was known. At first, all docking poses obtained were filtered on the basis of the ionic interaction of ligand with D3.32 considered as indispensable for proper anchoring to the receptor.[3] The interactions of examined ligands with target proteins were characterized by interaction fingerprints (IFP),[4] and the docking poses were filtered in terms of their similarity (expressed as Tanimoto coefficient of two IFPs) to the reference ligands (the co-crystallized ones). The examined ligand was assigned to the mutational class of the compound to which it had the highest IFP similarity, unless the similarity coefficient was lower than the particular threshold (Figure 3).

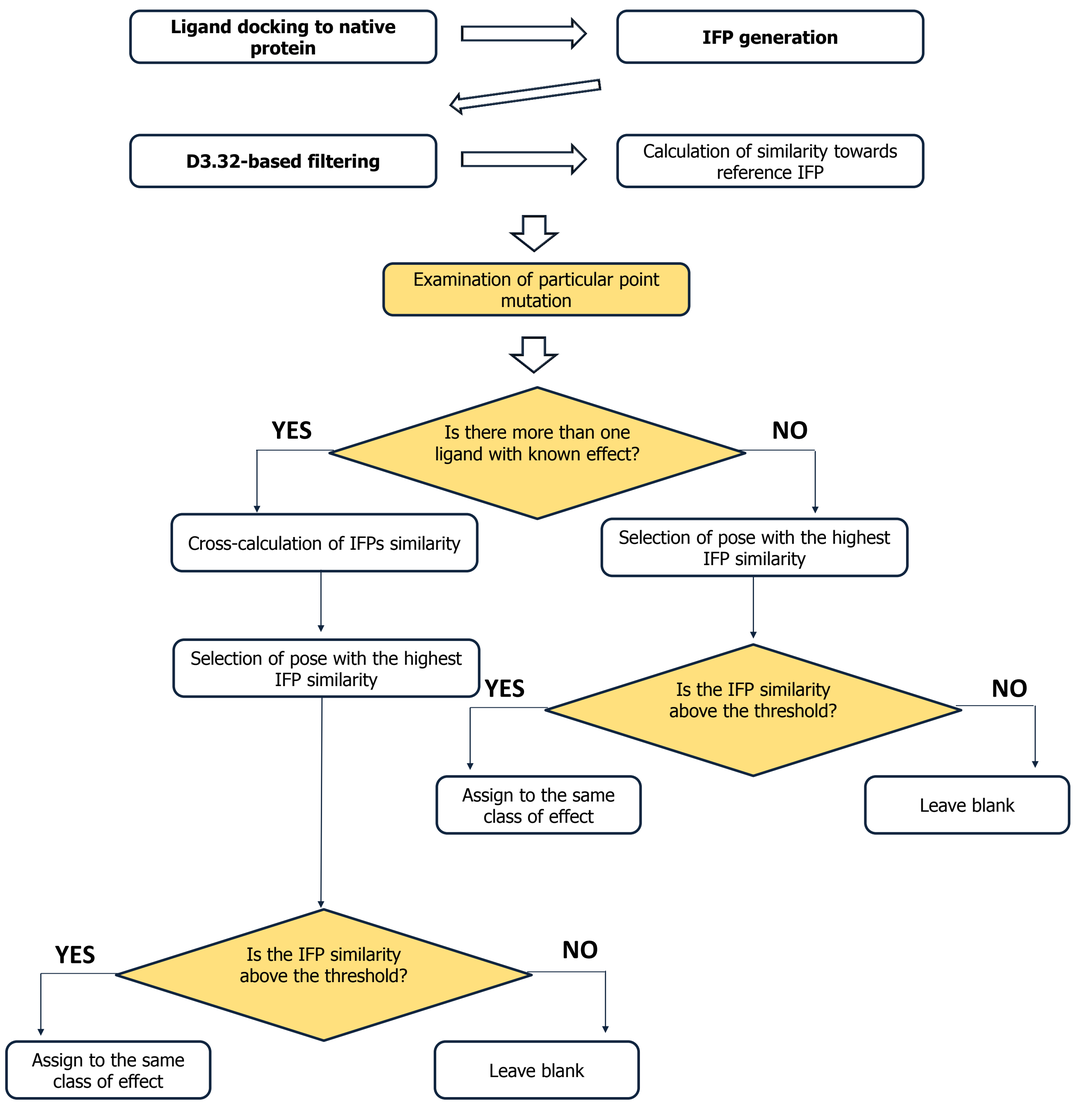


Figure 3. The protocol used for mutational effect prediction.

Mutational effect prediction - results

The results of the mutational data predictions are presented on the example of H_1 receptor. At first, the applicability of the developed protocol was verified in retrospective studies in leave-one-point out experiments, in which the datapoints were successively removed and underwent predictions (Figure 4). Various IFP similarity thresholds were tested, but the highest accuracy of predictions was obtained when it was set at 0.5. In the prospective study, all datapoints with known mutational effect were taken for making predictions for new points (Figure 5).

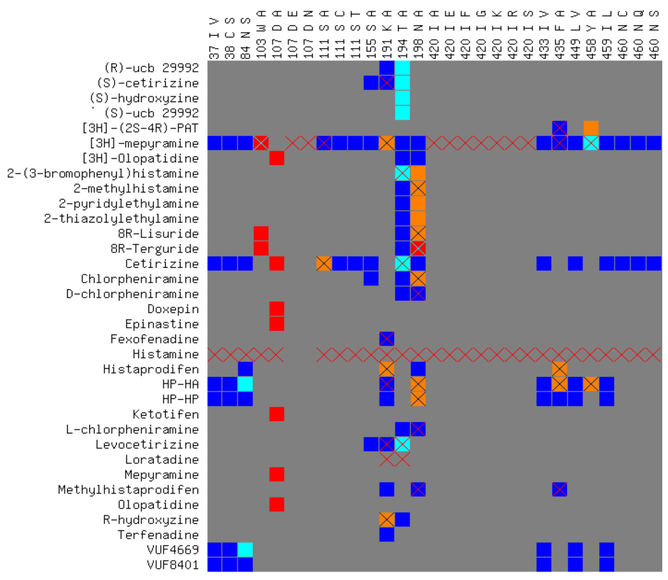


Figure 4. Retrospective evaluation of the protocol for mutational effect prediction for H_1R. Errors in predictions and 'blank' fields are indicated with crosses.

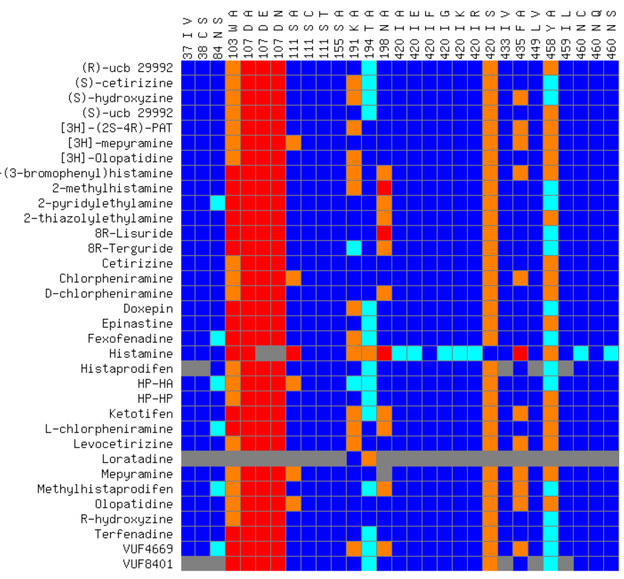


Figure 5. Mutational effect prediction for new datapoints.

References

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