POST-DOCKING DERIVATIZATION REVEALS PYRAZOLOQUINOLINONE BINDING MODE AT THE α+/ γ- $GABA_A$ RECEPTOR INTERFACE

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INTRODUCTION

gamma – Aminobutyric acid (GABA) is a wide-spread transmitter which binds to two pharmacologically diverging GABA receptors, $GABA_A$ and $GABA_B$. $GABA_A$ receptors ($GABA_A$Rs) are transmembrane pentameric ligand-gated chloride ion channels and represent an important target of many clinically relevant drugs (e.g. benzodiazepines, barbiturates, etc.). These receptors consist of different subunits (e.g. α,β,γ, etc.), which are drawn from nineteen subunit isoforms that are grouped into classes (e.g. α1-α6, β1-β3, etc.).[1-4] Each $GABA_A$ receptor possesses several extracellular and transmembrane small molecule binding sites – such as the high affinity benzodiazepine binding site (BZ site) at the α+/γ- interface.[5] This interface represents the allosteric binding site of the eponymous compound class of the benzodiazepines which exert anxiolytic, muscle-relaxant, sedative- hypnotic and anticonvulsant effects. Until today, benzodiazepines are widely used for medical treatments as well as in pharmaceutical research. To antagonize their effects various chemotypes have been introduced, e.g. the pyrazoloquinolinones (PQs).[6] However, all chemotypes possess partly overlapping and partly distinct *in vitro* and *in vivo* effects. This ambiguous behaviour might be based on the lack of knowledge regarding their binding sites and modes respectively.

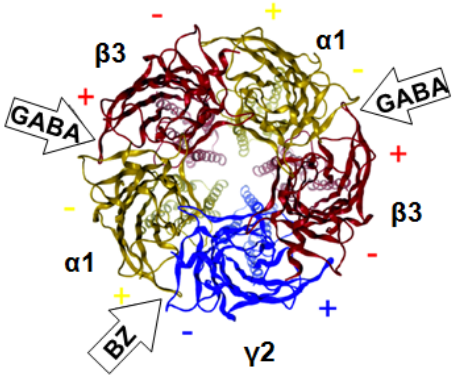

Figure 1: Homology model of a hetero-pentameric α1β3γ2 $GABA_A$ receptor (top view).

EXPERIMENTS

In order to determine the binding mode of pyrazoloquinolinones at the extracellular α+/γ- interface (BZ site) a homology model based on the crystal structure of the human $GABA_A$ β3 homopentamer was created consisting of two α1, two β3 and one γ2 subunits.[7] Flexible Molecular Docking was then performed using the PQ CGS8216 to get 100 different binding poses. For each pose an array of seven selected derivatives were built using the initial coordinates of CGS8216 to expand the dataset up to 800 poses in total (Figure 2). From the 7 analogues, four were classified as active (pKi > 8) while three were classified as inactive (pKi < 7). To validate the poses, the

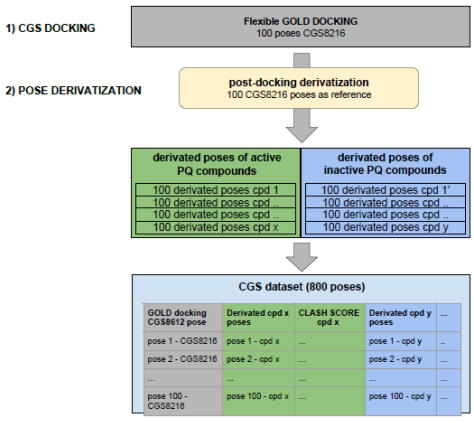

Figure 2: First part of the workflow outlining the Flexible Molecular Docking and the Post-docking Derivatization.

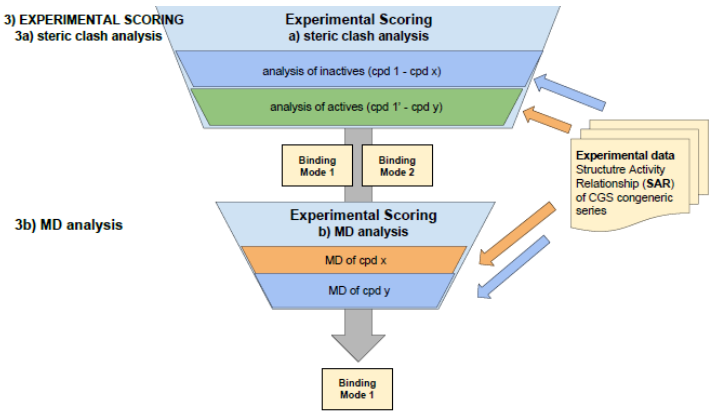

Figure 3: Second part of the workflow outlining the experimental/clash analysis and the MD analysis.

derivative analogue placements were analyzed for steric clashes and their compliance with Structure Activity Relationships (Experimental Scoring). The evaluation led to two reasonable binding modes which were further differentiated by Molecular Dynamic Simulations. Analysis of the variation of RMSD (root-mean-square deviation) and the occurring protein - ligand interactions resulted in one consistent binding hypothesis (Figure 3).

RESULTS AND DISCUSSION

We were able to identify a binding hypothesis for the pyrazoloquinolinones at the benzodiazepine binding site (α+/γ- interface) that is in great accordance with known SAR data (Figure 4). The filtering of potential binding poses was undertaken with a novel methodology termed "post-docking derivatization". This methodology makes use of SAR information for validation of docking poses to narrow down reasonable binding modes. On top of this analysis we performed Molecular Dynamic Simulations that corroborated the binding hypothesis with the greatest SAR accordance.

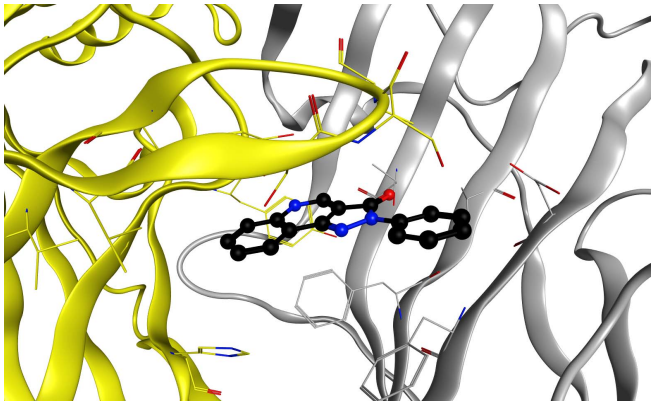

Figure 4: Final binding hypothesis of pyrazoloquinolinones at the the extracellular α+/γ- interface.

CONCLUSION

The binding hypothesis of the pyrazoloquinolinones will simplify the structure guided optimisation in the retrieval of required antagonists for the BZ site of the $GABA_A$ receptor. Furthermore, the binding hypothesis will positively influence ongoing research at homologous binding site of the $GABA_A$ receptor, e.g. the α+/β- interface (low affinity allosteric binding site of PQs).[8] In addition, the post-docking derivatization methodology represents a useful tool to easily cluster reasonable docking poses including important biological information.

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