# Corpora non agunt nisi fixata : ligand receptor binding kinetics in G protein-coupled receptors

Xia, L.

## Summary

The present thesis focuses on the pharmacological concept of drug-target interaction, which dates back to the beginning of modern pharmacology. In **Chapter 1**, a general introduction provides evidence that a traditional equilibrium metrics-based rationale (i.e. optimization of drug affinity leads to better efficacy and safety) is unable to prevent current high attrition rates in the early phase of drug discovery. In the past decade drug-target binding kinetics (i.e. association and dissociation rate constants, residence time) has been gaining more and more attention, which constitutes a paradigm shift to better predict parameters of drug efficacy and safety. We decided to investigate binding kinetics of G protein-coupled receptors (GPCR), since GPCR are involved in various critical physiological and pharmacological functions, being the target of about 30% of all drugs on the market. Both the human cannabinoid receptor 1 ($hCB_1$) and the human adenosine $A_1$ and $A_3$ ($hA_1$ and $hA_3$) receptors were chosen as prototypical GPCR as well as potential drug targets. Historical aspects of drug-target binding kinetics is also summarized in **Chapter 1**.

In **Chapters 2** and **3**, our binding kinetic study was focused on the human cannabinoid $hCB_1$ receptor, which is one of the "main actors" in the endocannabinoid system (ECS). In the particular case of obesity, the ECS, including the $hCB_1$ receptor, is overactive with increased levels of endocannabinoids in plasma, and in central and peripheral tissues. Therefore, blockade of the $hCB_1$ receptor is a potential approach for the treatment of obesity. Rimonabant, a $hCB_1$ receptor inverse agonist, was developed by Sanofi-Aventis and introduced on the market in Europe in 2006. However, it was quickly withdrawn from the market due to the risk of unacceptable psychiatric side effects. Although researchers have experienced many setbacks on this drug target, the intensive drug discovery efforts have never been terminated. In **Chapter 2**, we performed an extensive structure- kinetics relationship (SKR) study, in addition to a traditional structure-affinity relationship (SAR) analysis, on a series of 1,2-diarylimidazol-4-carboxamide derivatives developed as $hCB_1$ receptor antagonists. The compounds show high affinities and a diverse range of kinetic profiles at $hCB_1$ receptor. In **Chapter 3**, another series of 1-

(4,5-diarylthiophene-2-carbonyl)-4-phenylpiperidine-4-carboxamide derivatives, and rimonabant as a comparison were selected for SKR analysis and further molecular pharmacological investigation. This study shows that $hCB_1$ receptor antagonists can have very divergent kinetics (the difference in residence time in **Chapter 3** is 159-fold, while 18.5-fold in **Chapter 2**), which are not correlated to their equilibrium affinities. Furthermore, their dissociation rates appear to define their (*in vitro*) pharmacological effect. For both **Chapters 2** and **3**, based on the recently resolved $hCB_1$ receptor crystal structures, we propose that the differences in dissociation can be explained by a different binding mode of long residence time antagonists compared to short residence time antagonists (i.e. rimonabant) in which "unfavorable" water molecules are displaced. We learned that, next to affinity, additional knowledge of binding kinetics is useful for selecting new $hCB_1$ receptor antagonists in the early phases of drug discovery for the treatment of obesity.

Next, we explored binding kinetics on the human adenosine $A_3$ ($hA_3$) receptor in **Chapters 4** and **5**. The $hA_3$ receptor has been suggested as a viable drug target in inflammatory diseases and in cancer. So far, a number of selective $hA_3$ receptor agonists (e.g. IB-MECA and 2-Cl-IB-MECA) inducing anti-inflammatory or anticancer effects are under clinical investigation. In **Chapter 4**, we expand on a series of pyrido[2,1-*f*]purine-2,4-dione derivatives as $hA_3$ receptor antagonists, and many compounds showed high affinities and a diverse range of kinetic profiles. From a $k_{on}$-$k_{off}$-$K_D$ kinetic map we divided the antagonists into three subgroups, providing a possible direction for the further development of $hA_3R$ antagonists. Additionally, we performed a computational modelling study that sheds light on the crucial receptor interactions dictating the compounds' binding kinetics. In **Chapter 5** $hA_3$ receptor agonists are under binding kinetics investigation. We first validated a kinetic assay for agonists. Then, two series of ribofurano and methanocarba ([3.1.0]bicyclohexane) adenosine derivatives were evaluated for both their affinity and kinetics. Afterwards, a retrospective evaluation linking residence times and *in vivo* efficacies was discussed. Last but not least, from a $k_{on}$-$k_{off}$-$K_D$ kinetic map we divided the agonists into three subgroups, providing a possible direction for the further development of $hA_3R$ agonists.

In **Chapter 6**, the application of a novel radio-isotopic technology in binding kinetics is described for the human adenosine $A_1$ receptor. Compared to the classic radioligand binding experiment, its robustness and potential for high-throughput screening may render this technology a preferred choice for further kinetics studies.

In the last **Chapter 7**, the binding kinetics investigations described in this thesis provide a better and multi-faceted understanding of drug-target interactions, and future perspectives are outlined. Hopefully, all findings from this thesis have brought new insights at a molecular understanding of ligand-receptor binding kinetics, and will offer suggestions for the design of better ligands with an appropriate kinetic profile, new technologies for rapid kinetic assessment, and ultimately suitable evaluation schemes for a better translation towards effective and safe drugs.