## Structure-based design of AChBP ligands, new insights and applications

Edink, E.S.

2011

***document version***
Publisher's PDF, also known as Version of record

# Chapter 2

## Aim and scope of the thesis

**Fragment-based Drug Discovery**

In comparison with traditional high-throughput screening (HTS), fragment-based drug discovery (FBDD) is characterized by the screening of smaller libraries of compounds (typically containing 1000-5000 fragments) with lower molecular weight (i.e., smaller than 300 Da).[1] The essence of FBDD is that small molecular fragments are ideally suited for probing protein binding sites for key binding interactions but small enough to minimize the chances of unfavorable interactions that prevent them from binding.[2] Because the size of chemical space increases exponentially with molecular size, fragment-based screening results in substantially better coverage of chemical space and typically results in higher hit rates than HTS.[3-5] Furthermore, fragment hits often provide better starting points in terms of physicochemical properties.[6] Due to its attractiveness in terms of a highly efficient drug discovery process, FBDD has transformed in less than a decade, from a niche area of research that was only applied by small biotech companies to a serious alternative for HTS that is currently being applied (often in parallel with HTS) by every major drug discovery firm. Illustrative of the impact that FBDD is making on the drug development process, is the increasing number of FBDD-derived compounds that are in phase I and II clinical trials.[5,7] Very recently, the first FBDD-derived drug, Zelboraf® (vemurafenib) has been approved by the FDA and may be considered a case study on how FDBB can enable a more efficient drug discovery as it took only 6 years from the start of the program to FDA approval whereas it has recently been estimated that this process takes 13 years on average.[8-10]

A crucial aspect of FBDD is the efficient optimization of the binding affinity of fragment hits towards high affinity clinical candidates. Structural biology has been shown to be extremely helpful to guide the optimization of fragment hits towards novel and potent lead compounds.[7,11-14] As AChBP provides relative easy access to X-ray co-crystal structures, we embarked on FBDD approaches to develop new ligands that bind to AChBP. Next to finding novel ligands with affinity for AChBP and to improve our understanding of ligand-protein interactions, the studies were also aimed at increasing our understanding of FBDD.

**Thermodynamic Analysis**

Although structure-activity relationship studies have almost exclusively been using affinity and activity data, there is also growing awareness that this experimental data is an indirect measure of measuring the complementarity between ligand and protein. A more direct way of describing intermolecular interactions is by considering the thermodynamic properties of these interactions. Thermodynamic analysis provides access to the constituents of the Gibbs energy of binding ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$). The dissection of binding energy into separate enthalpic and entropic contributions adds an additional dimension to binding affinity and can provide crucial insights into the physical forces that drive ligand-protein interactions. For example, favorable changes in enthalpy may result from direct binding forces such as formation of hydrogen bonds, van der Waals contacts and $\pi$-$\pi$ interactions. On the other hand, favorable changes in entropy often result from changes in the ligand or protein's conformational freedom and the displacement of water molecules as apolar regions of the binding site and the ligand are brought together.

Determination of thermodynamic binding profiles may be especially useful in a FBDD context. Typically, fragment hits bind with low affinity to their respective protein partners, and as a consequence, extensive increases in binding affinity need to be realized to obtain low nM clinical candidates. As will be discussed in a survey of scientific literature (**Chapter 3**), it is easier to improve binding affinity by optimizing entropy, with the addition of hydrophobic groups, than it is through enthalpy, which requires polar interactions to be optimized. Therefore, choosing a fragment hit in which binding is enthalpically driven as a starting point and adding hydrophobic groups during optimization, may provide an efficient route to high affinity compounds with both favorable changes in enthalpy and entropy and a reduced risk of attrition. In addition, thermodynamic analysis may be very useful in guiding and monitoring (structure-based) fragment optimization. In combination with structural data, the stepwise fragment growing process provides an ideal dataset with which to improve our understanding of the thermodynamics of binding. In **Chapter 3**, the techniques that enable thermodynamic analysis of fragment-protein complexes are discussed, the currently available thermodynamic data on fragment-protein complexes are summarized and several key studies that highlight the role of thermodynamics in FBDD are discussed in more detail.

**Research Aims**

AChBP can be considered an excellent research tool to study FBDD approaches. Due to its water soluble nature, this nAChR ligand binding domain homolog provides relative facile access to X-ray co-crystal structures enabling structure-based optimization. Moreover, compared to membrane-bound proteins, water-soluble proteins are easier to incorporate in biological assays and better applicable in biophysical techniques such as SPR biosensor analysis and isothermal titration calorimetry (ITC). For these reasons, we have embarked on a fundamental study in which AChBP is used to increase our knowledge on how to efficiently optimize fragment hits. In addition, we have studied which techniques are suitable to monitor the fragment-optimization process in terms of efficiency and information content. In the process, we kept an eye on translating our findings with AChBP to the homologous but therapeutically relevant nicotinic receptors. Thus, at the beginning of this project we set ourselves with the following research aims:

- Increase our knowledge on how to efficiently optimize fragments towards high affinity binders using AChBP as a model protein.
- Investigate if thermodynamic analysis can contribute to a more efficient fragment-optimization process using AChBP as a model protein.
- Investigate if our fragment-optimization studies on AChBP can contribute to our understanding on how to design subtype-selective ligands for the therapeutically relevant human nAChRs.

**Outline of the thesis**

In **Chapter 3**, the technologies that enable thermodynamic analysis of fragment-protein complexes are discussed. In addition, the available thermodynamic data of fragment-protein complexes is summarized and several key studies that highlight the role of thermodynamics in FBDD are discussed in more detail. **Chapter 4** describes the successful optimization of a fragment hit by growing into AChBP's

lobeline-pocket. The fragment optimization was monitored with X-ray co-crystal structures and thermodynamic analysis using SPR biosensor analysis and ITC. **Chapter 5**, focuses on the structure-based design, synthesis and structure-activity relationships of dibenzosuberyl- and benzoate substituted tropines as ligands for acetylcholine binding protein. These studies resulted in the identification of an $\alpha 7$ nAChR selective fragment hit. The optimization of this fragment towards a potential dual action anti-inflammatory agent is described in **chapter 6**. The final **chapter 7** summarizes the most important conclusions of this thesis and evaluates the outcome of our research aims.

**References**

1. Congreve, M., Carr, R., Murray, C. & Jhoti, H. A 'rule of three' for fragment-based lead discovery? *Drug Discov Today* **8**, 876-7 (2003).
2. Hann, M.M., Leach, A.R. & Harper, G. Molecular complexity and its impact on the probability of finding leads for drug discovery. *J Chem Inf Comput Sci* **41**, 856-64 (2001).
3. Bohacek, R.S., McMartin, C. & Guida, W.C. The art and practice of structure-based drug design: a molecular modeling perspective. *Med Res Rev* **16**, 3-50 (1996).
4. Fink, T., Bruggesser, H. & Reymond, J.L. Virtual exploration of the small-molecule chemical universe below 160 Daltons. *Angew Chem Int Ed Engl* **44**, 1504-8 (2005).
5. Chessari, G. & Woodhead, A.J. From fragment to clinical candidate--a historical perspective. *Drug Discov Today* **14**, 668-75 (2009).
6. Keseru, G.M. & Makara, G.M. The influence of lead discovery strategies on the properties of drug candidates. *Nat Rev Drug Discov* **8**, 203-12 (2009).
7. de Kloe, G.E., Bailey, D., Leurs, R. & de Esch, I.J. Transforming fragments into candidates: small becomes big in medicinal chemistry. *Drug Discov Today* **14**, 630-46 (2009).
8. Bollag, G. et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* **467**, 596-9 (2010).
9. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm268241.htm.
10. Bunnage, M.E. Getting pharmaceutical R&D back on target. *Nat Chem Biol* **7**, 335-9 (2011).
11. Hajduk, P.J. & Greer, J. A decade of fragment-based drug design: strategic advances and lessons learned. *Nat Rev Drug Discov* **6**, 211-9 (2007).
12. Sledz, P. et al. Optimization of the interligand overhauser effect for fragment linking: application to inhibitor discovery against Mycobacterium tuberculosis pantothenate synthetase. *J Am Chem Soc* **132**, 4544-5 (2010).
13. Wang, Y.S. et al. Application of fragment-based NMR screening, X-ray crystallography, structure-based design, and focused chemical library design to identify novel microM leads for the development of nM BACE-1 (beta-site APP cleaving enzyme 1) inhibitors. *J Med Chem* **53**, 942-50 (2010).
14. Zhu, Z. et al. Discovery of cyclic acylguanidines as highly potent and selective beta-site amyloid cleaving enzyme (BACE) inhibitors: Part I--inhibitor design and validation. *J Med Chem* **53**, 951-65 (2010).