Conformational landscape of small ligands: A Multilevel strategy to determine the conformational penalty of bioactive ligands

Antonio Viayna, Jordi Juárez-Jiménez, Xavier Barril, F. Javier Luque
Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació,
Avgda. Prat de la Riba, 171, 08921, Santa Coloma de Gramanet
toni.viayna.89@gmail.com

Abstract- *Determining the conformational penalty required for adopting the bioactive conformation is still a challenging question in drug design, because a small uncertainty in this free energy component can lead to significant errors in the predicted activities. Herein, we use the Multilevel strategy, a methodology recently developed by our group, to explore the conformational preferences of ligands in solution, and to estimate the conformational cost of selecting the bioactive conformation.*

I. INTRODUCTION AND OBJECTIVES

In order to enhance the binding affinity, complementarity between the functional groups present in the ligand and the residues of the receptor's binding pocket is essential [1]. To achieve it, some conformational changes are required both in the ligand and the receptor. In the receptor case, those changes typically involve the rearrangement of side chains in the binding cavity and structural modifications in secondary structural elements. With regard to the ligand, conformational changes are associated with the adoption or selection of the bioactive conformation in the bound state. These conformational changes contribute to the binding free energy (ΔG_{bind}), which can be expressed as the addition of the free energy contribution due to the recognition between ligand and receptor in the bioactive conformation (ΔG_{int}) and the cost associated with the structural reorganization of both the ligand and receptor in solution (ΔG^{L}_{conf} and ΔG^{R}_{conf}). (Fig.1)

$$\Delta G_{bind} = \Delta G_{int} + \Delta G^{R}_{conf} + \Delta G^{L}_{conf}$$

Fig. 1. Sum of different contributions to the binding free energy during the ligand-receptor interaction

Focusing on the ligand, the bioactive conformation is just one of the many possible conformations in the physiological media. Intituively, one can expect that a good binder will be recognized by the receptor in a low energy conformation, but this is not always the case. Many times, the bound conformation might not correspond to the global minimum of the free ligand, and then a conformational penalty must be paid to adopt the bioactive conformation. If we consider that biological activity and binding free energy are directly related, then the existence of a high conformational penalty may lead to a significant error in the binding affinity and consequently in the predicted activity [2].

Different research groups have attempted to find computational strategies well suited to estimate the conformational cost needed for the selection of the bioactive conformation of ligands. [3] [4] [5]

Recently, our research group has developed the Multilevel strategy in order to explore the conformational preferences of drug-like compounds in solution and estimate the relative stability of the most populated conformations. [6][7]. The Multilevel strategy relies in two main approximations. First, it relies on the "predominant state approximation" [8], which states that the conformational space can be divided into different wells and the total configurational integral is equal to the sum of the configurational integrals of all the wells. Therefore, the free energy of a flexible molecule can be expressed as the addition of the contributions of the separate conformational wells. Second, the "Multilevel approach" assumes the combination of Low-Level methods to carry out the conformational sampling of flexible molecules to find the conformational minima, and then, High-Level methods are utilized to refine the relative stability of the wells (Fig. 2).

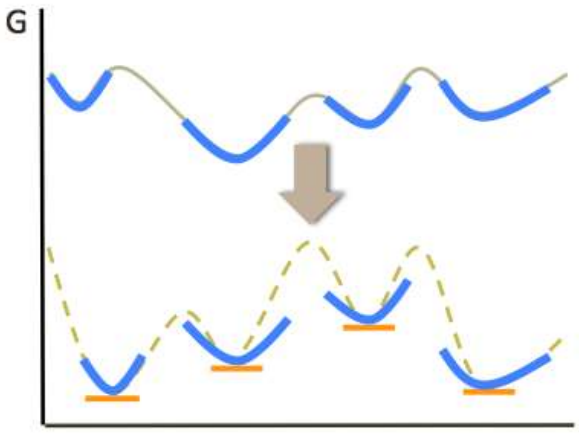

Fig. 2. Schematic representation of the Multilevel strategy. (Upper curve) The free energy surface is first explored at a Low-Level of theory, which permits to identify the major conformational wells. (Bottom curve) The relative stability of the minima is refined at a High-Level of theory, while including the local entropy of the conformaitional wells.

In this work, our interest is to use the Multilevel method to explore the conformational preferences of a diverse set of bioactive ligands in solution and to predict the conformational penalty of selecting the bioactive species, in order to validate this methodology.

II. METHODS

For each ligand, we perform the Low Level part, with classical Molecular Dynamics, using AMBER14 program and gaff force field. Prior to the production runs, we minimized the system, and then the system was equilibrated by rising the temperature from 50 to 298 K at constant volume. Finally, the density of the system was equilibrated at constant pressure, and finally production runs were performed at constant volume. The conformations sampled by the ligand from the trajectories were clusterized by considering the set of active torsions of the ligand, and finally to obtain the different wells.

The High Level refinement was developed taking the representative structure chosen as the minimum energy conformer of the different wells, and submitting it to a IEF-MST/B3LYP/6-31G(d) geometry optimization. The energy of the optimized structure was refined at the MP2/aug-cc-pVDZ level, including the zero-point energy correction, the solvation free energy, and the local conformational entropy of the well.

III. RESULTS AND DISCUSSION

Preliminary results of 12 compounds of the set of drug-like ligands show that the conformational penalty is generally low, corresponding to around 80% of the set. (Fig. 3) This general tendency is not surprising, because most of the molecules are drug-like compounds, and we expect that the conformational cost has been minimized during their design.

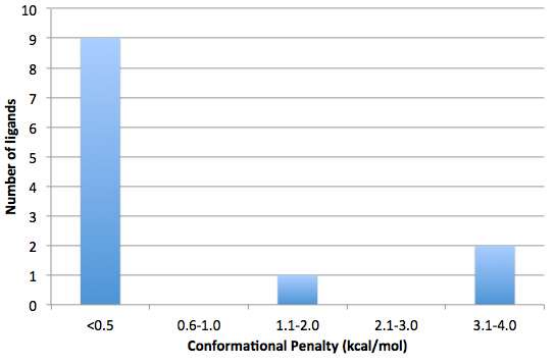

Fig. 3. Histogram confronting the number of ligands of the set and the different intervals of conformational penalty

Nevertheless, we have also detected that some ligands have a high conformational cost (> 2kcal/mol). In all cases, we were able to explain this cost, which was due to either steric hindrance, or breaking of intramolecular interactions.

As an illustrative example, the case of IQP ligand (PDB code 1YDR) (penalty = 3.8 kcal/mol) is a good example. This case is explained in terms of steric hindrance of the methylpiperazine moiety promoted upon filling the protein cavity (Fig. 4).

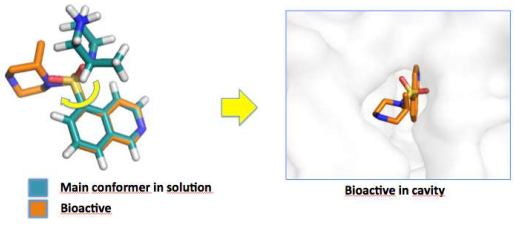

Fig. 4. Left: Superposition of the main conformer in solution according to Multilevel strategy (blue) and bioactive structure (orange) of the IQP ligand. Right: Bioactive structure inside the protein cavity.

Another illustrative example is BMU ligand (PDB code 1KV1) (penalty = 3.3 kcal/mol), which is explained in terms of the forced twisting of the bond between the urea and pyrazol groups. (Fig. 5)

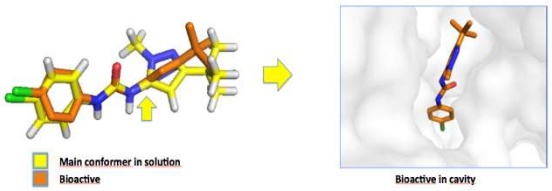

Fig. 5. Left: Superposition of the main conformer in solution according to Multilevel strategy (yellow) and bioactive structure (orange) of the BMU ligand. Right: Bioactive structure inside the protein cavity.

IV. CONCLUSIONS AND FURTHER WORK

As indicated in the results part, the drug-like compounds tend to have low conformational penalties. Only in few cases the cost is larger than 2 kcal/mol, which reflects the curated procedure required for the development of drugs.

Future work will be focused in completing the analysis of the whole set of compounds, to identify the factors that lead increase the conformational stress upon ligand binding, and to assess the possibility of introducing improvements in the Low-Level sampling methods.

ACNOWLEDGEMENTS

We thank Universitat de Barcelona (UB) for the financial support (APIF Fellowship). We thank the Centre de Serveis Científics i Académics de Cataluntya (CESCA) and the Barcelona Supercomputing Center (BSC) for computational facilities.

REFERENCES

[1] C. Bissantz, B. Kuhn and M. Stahl. "A Medicinal Chemsit's Guide to Molecular Interactions" J. Med. Chem. 2010, 53, 5061-5084

[2] S. Chung, J.B. Parker, M. Bianchet, L.M. Amzel and J.T. Stivers. "Impact of linker strain and flexibility in the design of a fragment-based inhibitor" Nat. Chem. Biol. 2009, 5, 407-413

[3] E. Perola and P.S. Charifson. "Conformational Analysis of Drug-like Molecules Bound to Proteins: An Extensive Study of Ligand Reorganitzation upon Binding" J. Med. Chem. 2004, 47, 2499-2510

[4] J. Tirado-Rives and W.L. Jorgensen. "Contribution of Conformer Focusing to the Uncertainity in Predicting Free Energies for Protein-Ligand Binding". J. Med. Chem. 2006, 49, 5880-5884

[5] K.T. Butler, F.J. Luque and X. Barril. "Toward Accurate Relative Energy Predictions of the Bioactive Conformation of Drugs" J. Comput. Chem. 2008. 30, 601-610

[6] F. Forti, C. Cavasotto, M. Orozco, X. Barril and F.J. Luque. "A Multilevel Strategy for the Exploration of the Conformational Flexibility of Small Molecules" J. Chem. Theory Comput. 2012, 8, 1808-1819

[7] J. Juárez-Jiménez, X. Barril, M. Orozco, R. Pouplana and F.J. Luque. "Assessing the Suitability of the Multilevel Strategy for the Conformational Analysis of Small Ligands" J. Phys. Chem. B. 2015, 119, 1164-1172

[8] M.S. Head, J.A. Given and M.K. Gilson. "Mining Minima: Direct Computation of Conformational Free Energy" J. Phys. Chem. A. 1997, 101, 1609-1618