HIV-1 protease: a good system to evaluate protein-ligand interactions, water role and protonation state, using an empirical approach

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Introduction

A set of 23 protein-ligand complexes of HIV-1 protease and inhibitors has been used as a validation test of an empirical approach, to study protein ligand interactions, considering the role of water molecules involved and the protonation state of protein and ligand ionizable groups [1].
It is well known that the protein ligand binding process is a concerted sum of single events, so many aspects have to be considered [2]. We have demonstrated that an empirical approach based on experimental LogP values and structural information could be used to design new ligands and to understand biomolecular association from several points of view [3]. It is also known that water presence and behaviour can affect the binding [4]. Furthermore, modeling the exact protonation state of several ionizable groups leads to a more realistic in silico model design [5].
HIV-1 protease-ligand complexes represent a good system to experiment the empirical approach of the HINT scoring software, because of the good resolution of crystal data, the well known behaviour of the most important water molecule, WAT301, the presence of a set of water molecules in the cavity surrounding the ligands and, moreover, because a more exact treatment of protein and inhibitor ionizable groups could affect the correctness of the models.
We have first analysed the role played by five water molecules placed into the active site and well determined both by X-ray crystallographic analyses and GRID simulations. In addition, we have considered the contributions of another twelve waters surrounding the binding cavity. The different values of the HINT scores, calculated for ligand-water and protein-water interactions, could thus be used to define a water importance scale and to understand the role played by each molecule in the binding stabilisation. We have pointed out, in agreement with data reported in literature, the significance of water 301, whose presence is necessary for the complex formation, and the less relevance of water 313, 313', 313bis and 313bis', which don't really affect the binding process but contribute to define the cavity shape. Finally, analyses of the environment surrounding the external ligand extremities, performed for one single HIV-1 protease-inhibitor complex, confirmed our supposition that protein and ligand solvation waters could make strong interactions with one of the two entities or with both but, nevertheless, are not essential for the binding process.
Again, the exactly setting of the protonation state was analysed on a protein ligand complex (pdb code 1A30) where experimental Ki at different pH values was carried out [6].

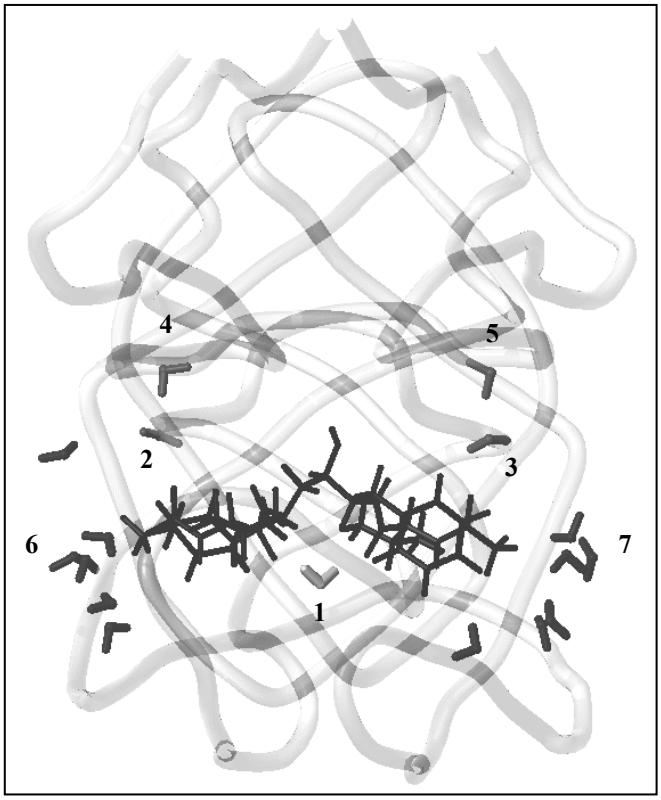

Fig. 1 Schematic representation of HIV-1 protease-inhibitor CGP 53820 complex. The numbers identify the different water molecules considered in the computational analyses: (1) water 301, (2) water 313, (3) water 313', (4) water 313bis and (5) water 313bis', (6 and 7) group of twelve water molecules (six on one side and other six on the other) placed at the borders of the binding cavity

References

[1] M. Fornabaio, F. Spyrakis, A. Mozzarelli, P. Cozzini, D.-J. Abraham, G.-E. Kellogg, Simple, intuitive calculations of free energy of binding for protein-ligand complexes. 3. Including the free energy contribution of structural water molecules in HIV-1 Protease. *J. Med. Chem*., accepted.

[2] G.-E. Kellogg, M. Fornabaio, F. Spyrakis, A. Lodola, P. Cozzini, A. Mozzarelli, D.-J. Abraham, Getting it right. Modeling of pH, Solvent and "Nearly" Everything Else in Virtual Screening of Biological Targets. *J. Mol. Graphics & Modelling*, in press.

[3] P.Cozzini, M.Fornabaio, A.Marabotti, D.-J.Abraham, G.-E.Kellogg and A.Mozzarelli, Simple, intuitive calculations of free energy of binding for protein-ligand complexes: 1. Models without explicit constrained water. *J. Med. Chem*., 45:2469-2483, 2002.

[4] P. Cozzini, M. Fornabaio, A. Marabotti, D.-J. Abraham, G.-E. Kellogg, A. Mozzarelli, Free energy of ligand binding to protein: evaluation of the contribution of water molecules by computational methods. *Review. Curr. Med. Chem*., in press.

[5] M. Fornabaio, P. Cozzini, A. Mozzarelli, D.-J. Abraham, G.-E. Kellogg, Simple, intuitive calculations of free energy of binding for protein-ligand complexes. 2. Computational Titration and pH Effects in Molecular Models of Neuraminidase-Inhibitor Complexes. *J. Med. Chem*., 46:4487-4500, 2003.

[6] J.-M. Louis, F. Djda, N.-T. Nasced, A.-R. Kimmel, D.-R Davies, Idrophilic peptides derived from the transframe region of Gag-Pole inhibit the HIV-1 protease. *Biochemistry*, 37:2105-2110, 1998.