Chemical engineering at the edge of life science. Selected cases of molecular simulations and free-energy calculations for biological and medical applications

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Noncovalent interactions are driving forces in numerous physiological processes. They are major determinants in many events which involve interactions between biomolecules. Noncovalent interactions play an important role in signal transduction, regulation, complexation, immune recognition, and adhesion. They are critical components of many processes, such as the folding of proteins and nucleic acids, biocatalysis, inhibition, activation, partition, and distribution, and are essential part of ligand-receptor and host-guest interaction. The challenge of molecular simulations in this field is at least twofold: to get insight into the mechanism underlining molecular interactions and recognitions, and to computationally determine the free energy for nonbonded interactions in complex biomolecular assemblies.

In this work we will present some recent results obtained by applying molecular simulations procedures and free energy calculation techniques to several cases concerning biological and medical applications.

The first case considered concerns the enantioselective hydrolysis of β-carboethoxy-γ-lactams by α-chymotrypsin (α-CT). Our experimental work [1] revealed that, at high conversion values, whilst three unreacted esters bringing different substituents ar the lactam nitrogen were recovered with a fairly good enantiomeric excess (i.e, e.e. from 95 to 99%), in the case of the unsubstituted lactam the hydrolyzed mixture was recovered in its racemic form. To rationalize these evidences, we performed a detailed molecular modeling/simulation study of the different α-CT/γ-lactam enantiomer complexes. The results obtained allowed us to probe the α-CT substrate specificity, and to explain its enantiomeric selectivity in terms of its three-dimensional structure and the interaction energy between the ligand and the amino acids forming the protein active-site. Figure 1a and b shows the resulting, optimized molecular models of the two docked α-CT/lactam enantiomer complexes. The different, more favorable orientation of the substrate in Figure 1a into the protein binding site is quite evident.

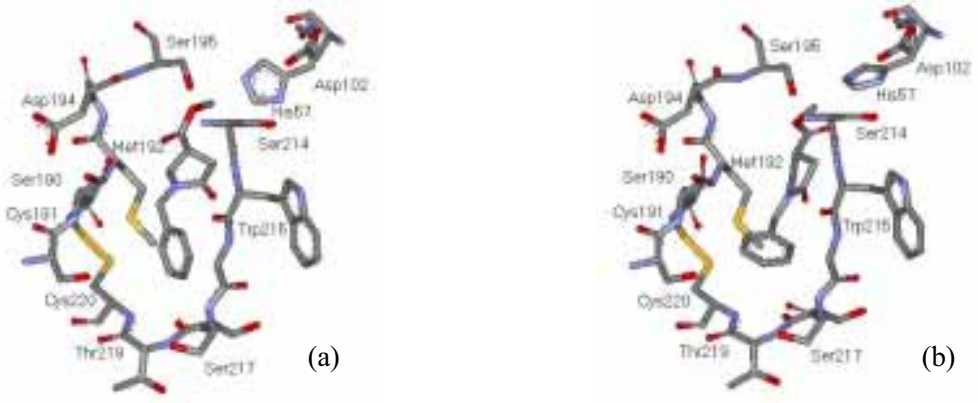

Fig.1. Optimized model of (*S*) (a) and (*R*)-methyl 1-(phenylmethyl)-5-oxo-3-pyrrolidinecarboxylate (b) bound to α-CT.

The second, selected case regards the design, synthesis and activity of new, efficient inhibitors of the HCV virus. A first study was conducted on the ribonucleotide reductase (RNR), and we used computer experiments to predict whether our newly designed nucleotide-based inhibitors could be employed as potential antiviral/antineoplastic agents. On the basis of detailed molecular modeling and free energy of binding calculations, we were able to discriminate between potentially active and inactive compounds, these results being confirmed by the relevant *in vivo* results [3,4]. The second investigation was focused on the design of new, potent peptidic and pseudopeptidic inhibitors with high specificity to the NS3 protease of the HVC virus by using structure-based molecular design and computer assisted combinatorial chemistry methods [5].

The third case deals with two, very actual topics of high medical interest: i) the interaction between tetracycline derivatives and the prion protein PrP^c, i.e. the pathogenic agent of human transmissible neurodegenerative disorders, such as the Creutzfeldt-Jakob disease [6], and the effects of some point mutations and their relations to the antitumor activity of p53 [7]. The product of the p53 tumor suppressor gene, TP53, has a central role in suppressing neoplastic transformation as it can respond to DNA damage by inducing cell cycle arrest or apoptosis. Loss of p53 function is present in most of human cancer; in about half of these tumors p53 is inactivated directly as a result of mutations. Figure 2 shows the peptide backbone atoms of the DNA-binding domain of the wild-type (yellow ribbon) and C238Y mutant (purple ribbon) of p53 monomer bound to a DNA double helix.

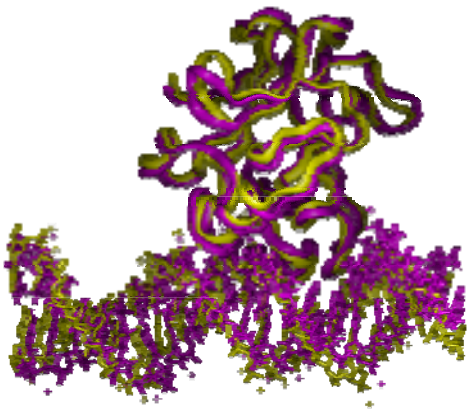

Fig. 2. Superposed structures of wild-type (yellow) and mutant (purple) p53 momomers bound to a DNA duplex.

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