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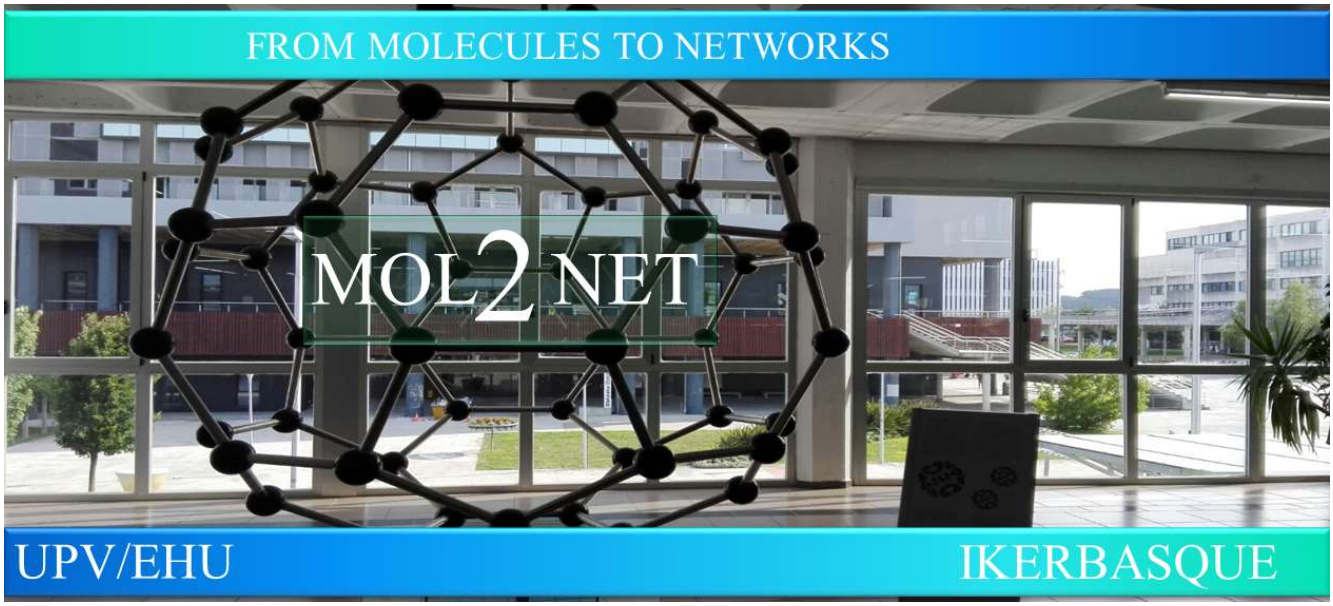

Bearing on drug-protein interaction network with structural features

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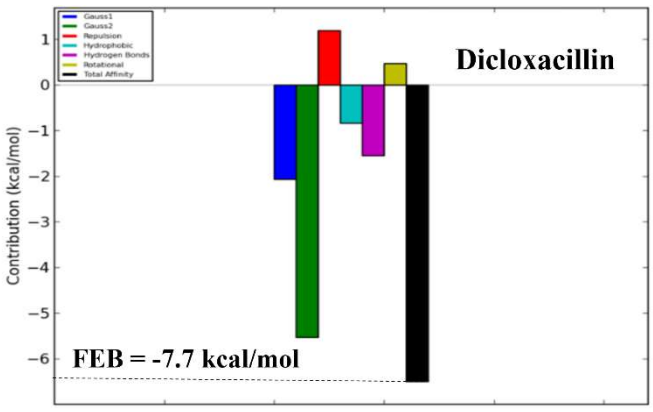
Graphical Abstract



Abstract.

The formation of protein-drug complexes is a fundamental resource for the sustainability of life. These complexes must have specific and precise characteristics in order to maintain biological equilibrium. Despite the large number of studies carried out on this subject, the enormous complexity deriving from both the nanometer scale and the times involved make its study a complex challenge. Therefore, a multidisciplinary approach involving

	experimental and theoretical approaches is necessary. In this paper we present a detailed example of a routine for the analysis and characterization of the stability and structure of protein drug complexes

Introduction *(optional)*

Proteins are one of the pillars of life. Their involvement in transport, catalysis or structural functions make them major players in biological systems[1, 2]. However, their activity and efficiency depend on a delicate balance that is constantly threatened by both energetic factors, such as temperature, and mechanical factors due to the high crowding of molecules in the cellular environment.An effective study requires a multidisciplinary approach, both experimental and theoretical. Therefore, in this work we analyze the interactions between fibrinogen (a blood serum protein) and dicloxacillin (an antibiotic). Beyond the medical and pharmacological interest of such a system, we consider very relevant the methodological approach that allows us to discern the structural and atomic changes that both molecules undergo and their functional repercussions. To carry out this proposal we will combine experimental techniques such as Raman and fluorescence spectroscopy with computational methods such as docking and elastic network models[3, 4].

Results and Discussion *(optional)*

In the present study we address the prediction of druggable fibrinogen binding sites considering the fibrinogen whole structure (E-region, D-region 1 and D-region 2) in order to determine the highest concentration and patterns of distribution of active binding sites throughout the fibrinogen structure. The obtained results show that the fibrinogen protein has multiple cavities more densely located in the N-terminal central nodule E-region (Figure 1)

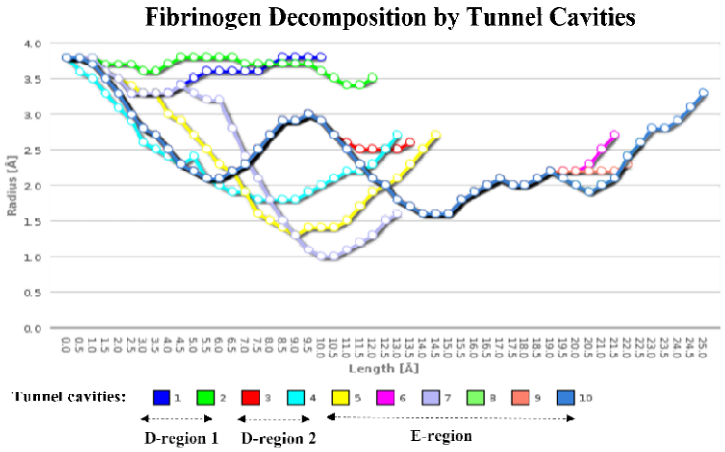


Figure 1. Graphical representation of length (Å) vs. radius (Å) obtained for the fibrinogen tunnel sub-cavities (10 entangled tunnels) belonging binding sites for each fibrinogen regions

With those results in mind, we performed the molecular docking simulations focus our attention on the potential docking interaction between the penicillin β-lactam antibioticswith the fibrinogen E-region. An overview of the results of 3D/2D-lig-plot diagrams (Figure 2) revealed that the most relevant antibiotic binding interactions with the fibrinogen E-region (pocket 1) are mainly based on hydrophobic (C···C)-backbone-side-chain non-covalent interactions. We suggest that the presence of two Cl atoms results in less quantity of hydrophobic (C···C)-backbone-side-chain non-covalent interactions for the dicloxacillin could promotes a decrease of the thermodynamic destabilizing effects on the residue side-chain packing of the E-region (pocket 1),potentially inducing a minor quantity of local perturbations over the interacting chains composing the E-region and without affect the flexibility properties and ligand-binding properties of the fibrinogen[5].

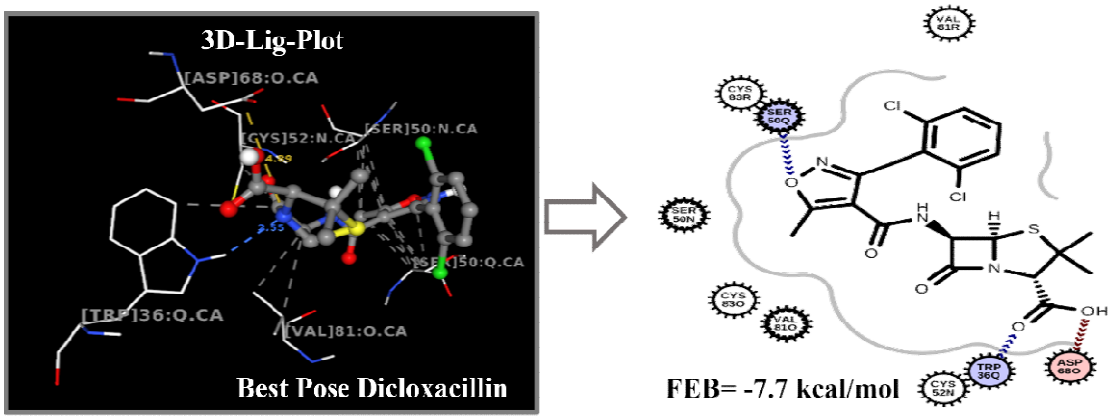


Figure 2. Representation of 3D and 2D lig-plots interaction diagrams for the best β-lactam antibioticbinding-poses obtained in the fibrinogen E-region

The results on LPRS (local perturbation response scanning maps) allowed us to investigate the collectivity parameters in low-frequency modes (k, from 1 to 7), and explain the observed larger-scale movements associated to long distance-based allosteric signal propagation affecting the inter-residue network communication (Figure 3). We observed thatdicloxacillin promote a significant increase in the degree of collectivity with a peak, and subsequently an abrupt decrease of the collectivity parameter (K_k-values) in the residue range from Phe 65:N to SER 75:N, with subtle conformational differences as can be observed in the end of the profiles of collectivity mode vs. residue index. We note the presence of greater number of long-distance-based local perturbations. Particularly, involving the signal transduction-based perturbations from the block of i-sensor residues (from regulatory non-target SER67:Q to PHE79:Q) triggering allosteric responses in j-effector residues (ranging from GLY35:Q to PHE79:Q).[6, 7]

In order to understand how dicloxacillin, affect the secondary structure of the protein, we have analyzed Raman spectra for fibrinogen in the presence of the penicillin at two different ratios (Figure 4, left). The amide I band, between 1600 cm^{-1} and 1700 cm^{-1} is stronger than the Amide III band and is composed by the contributions of α-helix at 1655 cm^{-1}, β-sheet at 1670 cm^{-1} and random coil at 1680 cm^{-1}. Other small peaks are attributed to: C_α-C stretch (895 cm^{-1}), PHE and TRP (1035 cm^{-1}), Amide II (1328 cm^{-1}), N-H bend indole ring (1414 cm^{-1}), C-H_2 and CH_3 deformation (1448 cm^{-1}) and TRP(1554

cm^{-1}). In the presence of penicillin, amide I band shifts to lower wavenumbers. These changes in the position (and intensity) of the amide I band indicate that under drug interactions the fibrinogen experiment changes in the secondary structure mainly in the α-helix content and with more intensity in the case of cloxacillin. Considering that the modifications in the spectrum occur mainly in this area, it can be postulated that the adsorption occurs in lateral chains of amino acids (N and Q chains, as suggested by the LPRS maps), altering the contents of secondary structure but with few changes in the general structure of the protein[8, 9].

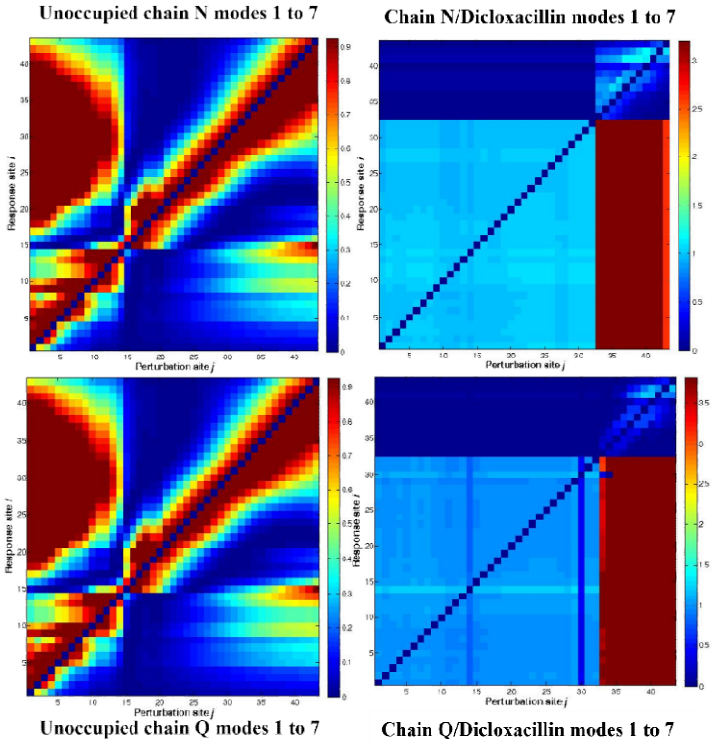


Figure 3. Graphical representation of the behavior of the parameter degree of collectivity K_k (a.u) for the first seven low frequency normal modes 1-7 vs. chain N and Q residue index

We also measured the effect of dicloxacillin on fluorescence emission spectra of fibrinogen at two temperatures (Figure 4, right). The interaction of the penicillin β-lactam antibioticwith fibrinogen causes the raise in the polarity of fibrinogen fluorophore surrounding. The fluorophore involves the presence of aromatic residues such as tyrosine and tryptophan since they emit fluorescence at the excitation wavelength of 280 nm. The reduction of the maximum peak suggest that the aromatic residues present on the samples had its surrounding environment modified, eventually being more hydrophilic with the increase of the drug in the solution[9].

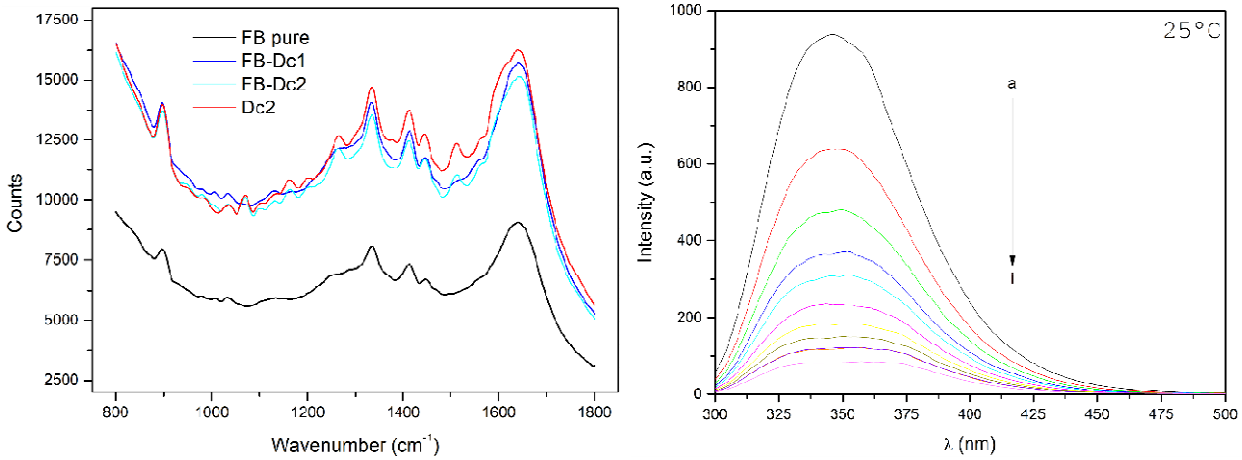


Figure 4. Left: Raman spectra of fibrinogen in presence of different penicillin ratios, 100 and 400 for samples 1 and 2 respectively. Right: Fluorescence emission spectra of Fibrinogen in the absence and presence of dicloxacillin. Dicloxacillin concentrations: (a-i) = (0.08, 0.17, 0.25, 0.33, 0.42, 0.5, 0.58, 0.66, 0.75, 0.83 µM).

Conclusions *(optional)*

Here we combined computational and experimental approaches to evaluates the conformational binding mechanisms of fibrinogen under interaction with dicloxacillin. The 3D/2D-lig-plot diagrams revealed that the most relevant dicloxacillin interactions with the E-region are mainly based on hydrophobic(C···C)-backbone-side-chain non-covalent and acceptor/donor interactions with critical regulatory E-region residues (SER50:Q> SER50:N) with high H-bond atom energy contribution. Collective low-frequency normal modes and LPRS maps revealed that conformational binding mechanism involve the allosteric modulation, long-distance perturbations, and remarkable conformational rigidification of the regulatory chains from E-region (N and Q). The experimental results excellently corroborated the computational.

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