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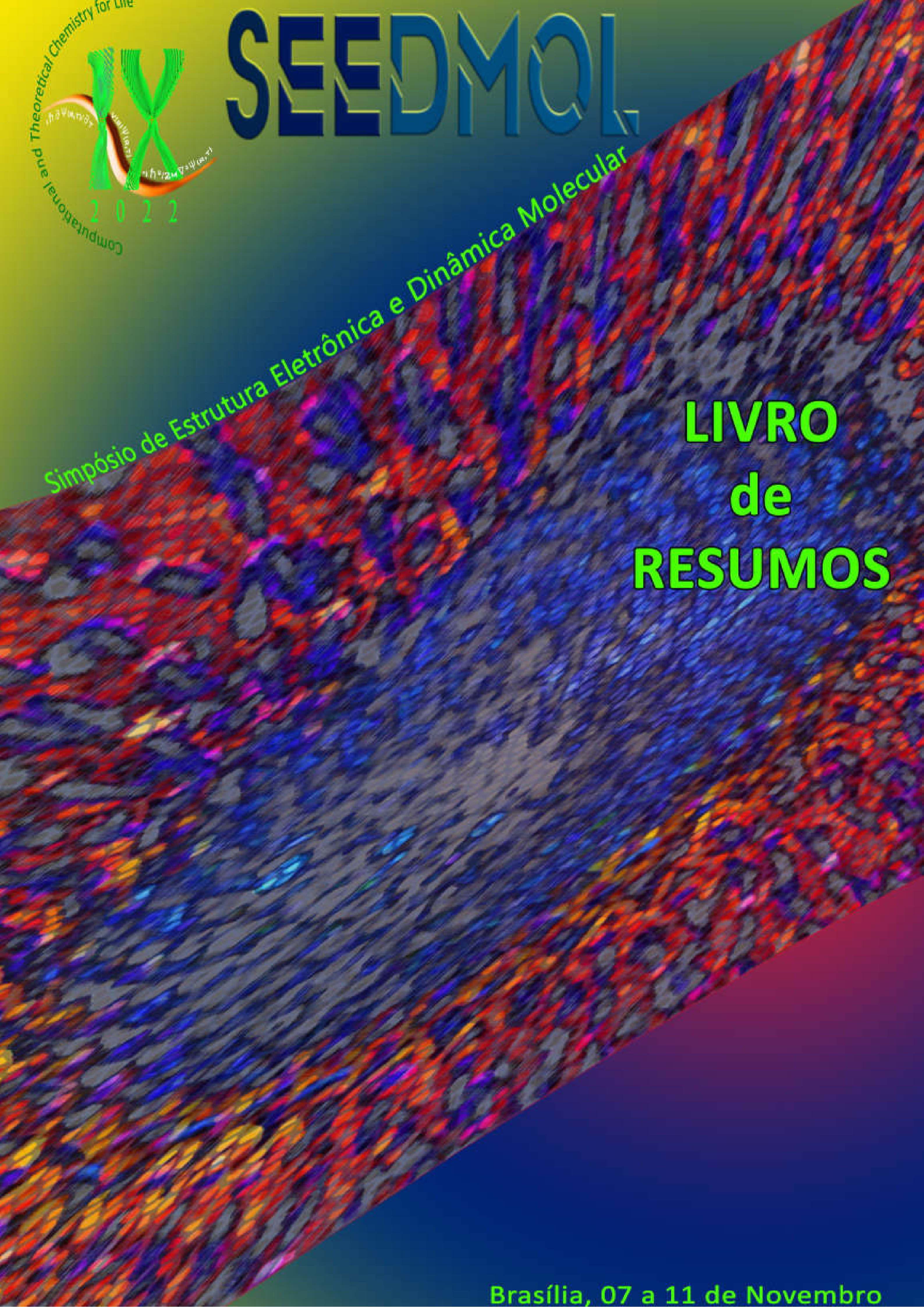

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A New Strategy to Model the Mechanism of Action of Covalent Inhibitors

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Abstract

Covalent inhibitors have received renewed interest with the success of many drugs belonging to this class of compounds. However, modeling the mechanism of action of covalent compounds is challenging since it is necessary to leverage both the noncovalent interactions between ligand and target protein, and the chemical reaction leading to the covalent adduct. In this work, we applied free energy perturbation (FEP) calculations to determine the relative binding free energy of a series of eight nitrile-based compounds against human cathepsin L, to treat the nonbonded interactions in both noncovalent and covalent states. The results showed that the covalent state presents a good correlation with experimental data, but the noncovalent state can be valuable to fine tune the inhibition activity. In order to treat the formation of the covalent bond, we applied the cluster approach methodology to modeling covalent inhibitors. The results show the efficiency of this approach to study the effect of different warheads in the formation of the covalent adduct.

Introduction

Covalent inhibitors are a significant class of small molecule drugs and they have been used for over a century ago since the registration of Aspirin. Historically, the suitability of covalent inhibitors has been challenged due to toxicity issues. However, the interest has been recently renewed with the success of many antidiabetics and antineoplastics drugs in the past decade.[1] As the mechanism of covalent ligand inhibition involves noncovalent interactions and the formation of a covalent adduct through a chemical reaction, designing new inhibitors of this class is an arduous task.[2] Thus, this work has the goal to explore and discuss how to model reversible covalent inhibitors of human cathepsin L (hCatL), employing free energy perturbation (FEP) to treat the noncovalent interactions in the covalent and noncovalent states, and the quantum mechanics (QM) cluster approach methodology to investigate covalent adduct formation.

Methodology

For the noncovalent interactions, FEP was used to evaluate the relative binding affinity of eight nitrile-based inhibitors (Fig. 1).

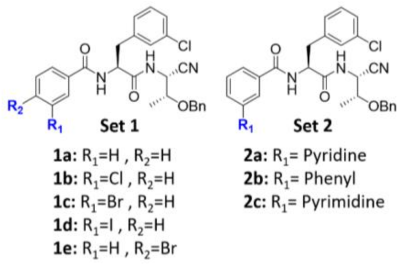

Figure 1: 2D representation of hCatL reversible covalent inhibitors. They were divided into two groups according to their chemical features.

All the molecular dynamics (MD) simulations and relative binding free energy

(RBFE) calculations were carried out using AMBER18. The strategy used here was a stepwise decharge-vdW-recharge process taking compounds 1a and 2a as reference. The alchemical transformations were performed in the protein and water environments for both covalent and noncovalent states. For the P3-halogenated compounds at meta position, an extra-point (EP) of positive charge was applied in the recharge step in order to account for the sigma hole effect.

To examine the formation of the covalent adduct, a cluster model with 309 atoms was built (Fig. 2; PDB 3HHA). The model consists of residues in the active site (Gln19, Gly23, Ser24, Trp26, Ala27, Ser29, Gly67, Gly68, Leu69, Met70, Ile136, Ala138, Phe143, Met161, Asp162, His163, Asn187, Ser188, Trp189, Trp193) and the core of dipeptidyl nitrile-based compound. Calculations were performed at the B3LYP/6-31G(d,p) level with Gaussian16.

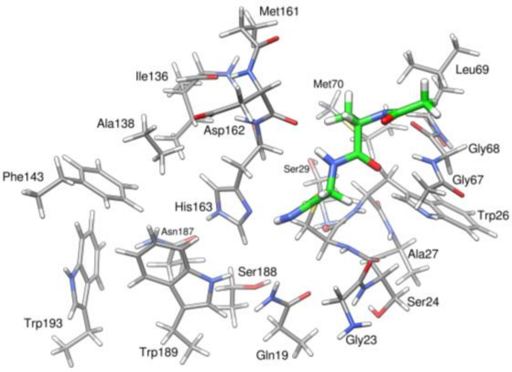

Figure 2: Optimized structure of the active site model in the covalent state. The nitrile-based ligand is shown in green. The Cα and most of the hydrogens were kept fixed in the geometry optimization.

Results and Discussion

The RBFE values were estimated using the Zwanzig relationship and the results for $\Delta\Delta G_{noncov}$ and $\Delta\Delta G_{cov}$ are shown in Table 1. FEP results reveal a strong correlation (r = 0.86, Fig. 3a) between the experimental data and RBFE values for the covalent state. In contrast, a modest correlation is found with the free energy changes determined for the noncovalent state (r = 0.69; Fig. 3b). Furthermore, the mean unsigned error (MUE) is reduced from 1.3 to 0.9 kcal mol^{-1} from noncovalent to covalent state.

Table 1: Experimental (Exp) and calculated (FEP) RBFE values (kcal mol^{-1}) for the noncovalent and covalent states. Absolute errors are given in parenthesis.

Mutation		Exp	Noncov	Cov
1a→1b	H→Cl	-0.9	-2.5 (1.6)	+1.3 (2.2)
1a→1c	H→Br	-1.7	-2.0 (0.3)	-0.4 (1.2)
1a→1d	H→I	-1.9	-1.9 (0.02)	-0.9 (1)
1a→1e	H→*p*-Br	-0.1	-0.9 (0.7)	-0.3 (0.1)
2a→1b	N→CH	+2.4	-1.3 (3.6)	+3.1 (0.7)
2a→1c	CH→N	+1.7	0.0 (1.7)	+ 1.5 (0.2)

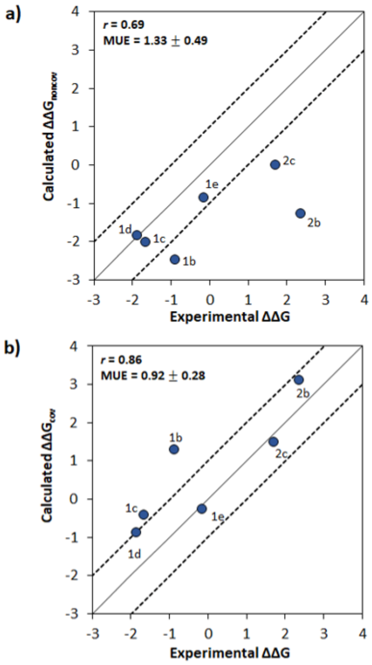

Figure 3: Comparison of the free energy changes (kcal mol^{-1}) obtained for alchemical transformations in the (a) noncovalent and (b) covalent states with the experimental relative binding affinities.

The RBFE values were justified based on the proposed binding mode, including additional calculations of the hydration free energy for the set 2 of compounds.[3]

To estimate the contribution of the covalent bond formation, QM/MM calculations can be generally used, but they are expensive and it is not trivial to prepare the system and obtain a path for the reaction. On the other hand, QM calculations can be performed for simplified systems (i.e, the ligand and the catalytic residues) that enable the use of higher levels of theory, but they omit the effect due to the anisotropic environment in the binding pocket.[4] Alternatively, the Fukui function can be employed to evaluate the intrinsic reactivity of the warhead,[5] but a direct translation of these values to the free energy needs further studies. In this way, the QM cluster approach can be valuable to obtain the free energy regarding the formation of the covalent adduct and can circumvent the problems aforementioned for the other methods.

Here, we used cluster models for the covalent adduct (Fig. 2; bound to the reactive Cys) and also a cluster model for the unbound ligand in the presence of the ionic pair Cys^-/His^+. Thus, one can estimate the contribution of the covalent bond formation by combining these values. In the case of the nitrile warhead in a dipeptidyl scaffold, we obtained a value of -6.5 kcal/mol, which is reasonable considering that the nitrile is not a very reactive warhead and for reversible inhibitors, a value of up to -23.5 kcal/mol can be expected for the barrier of the reverse reaction.[7]

This value can be used to compare other warheads/ligands and determine the contribution of this moiety for a specific target. On account of this, more calculations are being executed for an aldehyde-based inhibitor to compare the results with the nitrile warhead and also with experimental data. Also, we will perform calculations at a higher level of theory, simulating the effect of protein surrounding ($\varepsilon \sim 4$) and including zero-point energy (ZPE) corrections.[6]

Ultimately, with a proper design of the alchemical pathway in FEP, it will be possible to combine the results of the cluster approach methodology and is expected to obtain an accurate absolute binding free energy (ABFE) value for reversible covalent inhibitors.

Conclusions

The results obtained from FEP calculations indicate that the covalent state presents a good correlation with experimental data, and the ligands can be ranked with high accuracy. Hence, it is assumed that the covalent complex is more relevant for the binding free energy than the noncovalent complex and FEP can be employed to study the noncovalent interactions in covalent ligands. The cluster approach seems to be a practical procedure to obtain the energy of the formation of the covalent bond between the ligand and the hCatL and can be of great value to modeling different warheads, though more calculations are ongoing to confirm this issue. Finally, combining the results from FEP calculations and the cluster approach may allow us to estimate the ABFE of the reversible covalent inhibitors, which will be an outstanding contribution to the area.

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