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Topical Perspectives

Evaluation of the absolute affinity of neuraminidase inhibitor using steered molecular dynamics simulations

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ABSTRACT

The absolute free energy difference of binding (ΔG) between neuraminidase and its inhibitor was evaluated using fast pulling of ligand (FPL) method over steered molecular dynamics (SMD) simulations. The metric was computed through linear interaction approximation. Binding nature was described by free energy differences of electrostatic and van der Waals (vdW) interactions. The finding indicates that vdW metric is dominant over electrostatics in binding process. The computed values are in good agreement with experimental data with a correlation coefficient of $R = 0.82$ and error of $\sigma\Delta G_{exp} = 2.2$ kcal/mol. The results were observed using Amber99SB-ILDN force field in comparison with CHARMM27 and GROMOS96 43a1 force fields. Obtained results may stimulate the search for an Influenza therapy.

1. Introduction

Three influenza pandemics have killed several millions of people in the last century including the A/H1N1 in 1918, A/H2N2 in 1957 and A/H3N2 in 1968 [1,2]. Recently, the influenza virus including A/H5N1 [3,4], A/H1N1 [5,6], A/H5N8 [7], and A/H7N9 [8] have infected a large number of humans all over the world. The drug target for a treatment of influenza virus mainly is the neuraminidase enzyme because it is a vital factor in delivery progeny virus to human cell [9]. Although popular antiviral drugs were designed to inhibit the acting enzyme such as oseltamivir, zanamivir, and peramivir, etc, numerous resistances however still persist [10–12]. Therefore, the studies of potential inhibitors preventing influenza to operate through an efficient inhibition of neuraminidase remains a subject of great current interest. In such problems, the binding affinity of a ligand to a receptor turns out to be a decisive information that can be determined in simulations using various theoretical approaches.

Fast pulling of ligand (FPL) method is a highly appropriate protocol to determine the binding affinity of an inhibitor to neuraminidase, and the obtained results were correlated well to experiment with low CPU time consumption [13,14]. However, this paradigm is only able to provide the relative binding affinity of the inhibitor using the rupture force and/or pulling work measurement [15,16]. In computer-aided drug design and/or biophysical chemistry problems, the absolute value of binding free energy of a potential inhibitor is essential to be evaluated, due to the requirement of understanding of the essence of binding and prediction of inhibition constant.

The FPL approach has recently been improved in combining with the linear interaction energy (LIE) technique to assess the absolute free energy difference of binding between two *bound* and *unbound* states of the HIV1 protease-ligand complexes [17]. In the current scheme, the ligand unbinding process over the steered molecular dynamics (SMD) simulations is started from a *bound* state, where the ligand forms full non-bonded contact to the receptor, and then completed at an *unbound* state, where the ligand has zero non-bonded contact to the protein. There are thus two quasi-equilibrium states of the solvated complexes associating with their two equilibrium states, *bound* and *unbound*, in terms of molecular dynamics (MD) simulations. The free energy difference of binding between the inhibitor to neuraminidase can thus be evaluated through the difference of free non-bonded interaction energies of

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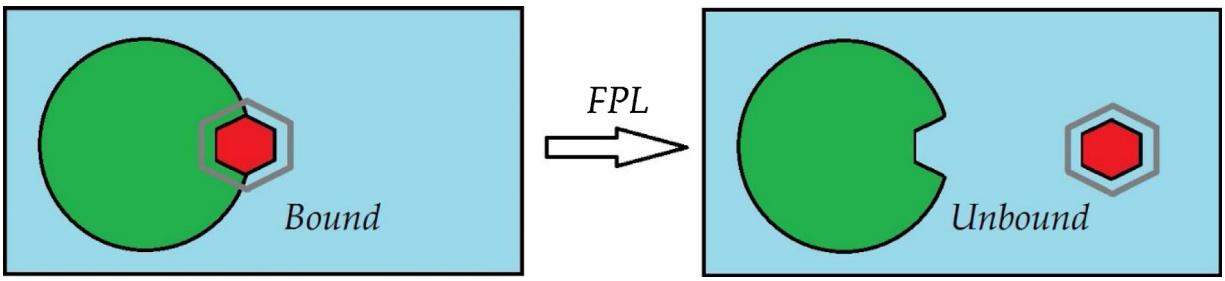

Fig. 1. Computational modeling of the binding free energy determination of the neuraminidase-inhibitor complex. The green unit is representative of the neuraminidase enzyme. The hole of neuraminidase is a binding cavity of the protein. The red unit represents the inhibitor. The grey line around the inhibitor corresponds to the non-bonded interaction region of the ligand to the surrounding molecules. At a *bound* state, the inhibitor forms non-bonded interaction to both receptor and solvation molecules. At a *unbound* state, the inhibitor forms full non-bonded interaction to solvation molecules. The interaction energy difference between two states characterizes the absolute binding free energy through the linear interaction energy (LIE) formula.

the ligand to enclosed molecules between two *bound* and *unbound* states. The estimation was carried out using the formula of the linear interaction energy (LIE) method [18].

In the present work, we aim to demonstrate that the improved FPL method is an appropriate way to evaluate the absolute binding free energy of a potential inhibitor to the neuraminidase enzyme. The binding mechanism is subsequently detected through a comparison of the free energy difference of electrostatic and van der Waals (vdW) interactions. The obtained results are in good comparison to experiment, and the calculated free energy is smaller than the experimental value by an amount of ~3.3 kcal/mol. The required computing resource is remarkably low since the unbinding process is short when employing a high pulling speed. These results may stimulate a search for new influenza therapy, since rapid and accurate determination of the absolute binding free energy between a candidate inhibitor and the neuraminidase enzyme is now available.

2. Materials and methods

2.1. Starting structures of the neuraminidase-inhibitor complexes

The crystal structures of the neuraminidase-inhibitor complexes were taken from the database of proteins (Protein DataBank – PDB) including 1XOG [19], 1XOE [19], 3CL2 [20], 3CL0 [20], 4B7J [21], 1BJI [22], 3CKZ [20], 3TI3 [23], 3TI5 [23], and 4B7Q [21]. These complexes were used as initial conformations of subsequent molecular dynamics simulations. Details of computational modeling were described in Fig. 1.

2.2. Unbinding pathway

It is known that the Caver 2.0 [24] has successfully detected the proper pulling pathway of the neuraminidase-inhibitor systems [13,14]. This package was employed here to predict the unbinding pathway of the investigated complex. The proper unbinding pathway is shown in Fig. 2.

2.3. Molecular dynamics (MD) simulations

The simulations were carried out using the GROMACS 5.1.3 package [25]. The neuraminidase and inhibitor were parameterized using the Amber99SB-ILDN force field [26] and general Amber force field 2 (GAFF2) [27], respectively. The charged and bonded information of the inhibitor were determined through combination of RESP method [28] and quantum chemical calculations at the Hartree-Fock HF/6-311G + (d,p) level. The neuraminidase-inhibitor complex was then solvated using the TIP3P water model [29] with periodic boundary condition (PBC) box, whose dimension is $7.33 \times 6.81 \times 9.77$ nm. In addition, the parameters of inhibitors were generated using PRODRG [30] and SwissParam [31] servers, when the neuraminidases were parameterized by GROMOS96 43a1 [32] and CHARMM [33] force fields, respectively, in which, the water models were used as SPC [34] and TIP4P [29] models. The ionization states of inhibitors were provided by PRODRG server [30]. The solvated box was then optimized before MD simulations. In the present work, we used the steered molecular dynamics (SMD) approach which will be describe in a following section. There were approximately 15,000 water molecules included inside the box. The counter ions, Cl^- and Na^+, were inserted into the solvated box to neutralize the soluble system. The energy minimization with steepest descent method was first applied to the solvated complex. A NVT simulation was subsequently carried out to restrain the minimized systems. Finally, the complex was relaxed in NPT simulations. All of MD parameters employed in the present work have been defined and given in previous studies [35–37].

2.4. Steered-molecular dynamics simulations

The equilibrated snapshot of the neuraminidase-inhibitor complex was used for starting conformation of SMD or force probe simulations. The neuraminidase C_α atoms were restrained over SMD simulations using a weak harmonic potential. The inhibitor was unbinding through an external force, which was assigned on the ligand center of mass. The orientation of the pulling force was properly rotated onward the Z-axis, which assimilates to the predicted unbinding pathway. The pulling parameters were chosen as described in previous works [13–15] including a unbinding speed of 0.005 nm/ps and a spring constant of 600 kJ/mol/nm^2. The slower pulling speed and different cantilever spring constant were not considered in this work since they do not much enhance the accuracy of the approach [13,15], and the computational modeling is only required to investigate two *bound* and *unbound* states. The evaluated modeling was described in Figs. 1 and 2.

2.5. Absolute binding free energy over SMD simulations

The free energy difference of binding between inhibitor and neuraminidase is computed making use of the LIE approximation [18]:

$$\Delta G_{cal} = \frac{1}{2}\Delta E^{cou} + \frac{1}{2}\Delta E^{vdW} \quad (1)$$

where ΔG_{cal} is the calculated absolute binding free energy, $\Delta E^{cou} = E^{cou}_{unboud} - E^{cou}_{bound}$ and $\Delta E^{vdW} = E^{vdW}_{unboud} - E^{vdW}_{bound}$ are the difference of electrostatic and vdW interaction energies of the inhibitor to surrounding molecules between two *bound* and *unbound* states, respectively.