

STUDIES OF QUANTUM MECHANICS/MOLECULAR MECHANICS OF SMALL METABOLITES FOR QUANTIFICATION OF CHEMICAL EXCHANGE SATURATION TRANSFER

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Introduction:

Chemical-exchange-saturation transfer (CEST) is an experimental technique which provides a new MRI imaging contrast for clinical diagnostics. This contrast is based on the chemical exchange between the metabolite water exchangeable group ($-NH_2$, $-NH$, $-OH$ groups) and bulk water. Therefore, small metabolites are studied which possess $-NH$, $-NH_2$, $-OH$ water exchangeable groups. In this project we made an attempt to quantify creatine-water chemical exchange rates using quantum mechanics/molecular mechanics (QM/MM) and molecular dynamics (MD) simulations. These are the first attempt to investigate CEST on different levels of complexity, bridging the gap between the experimental CEST technique on MR imaging systems and QM/MM simulations of small metabolites (e.g., creatine).

Materials & Methods:

In order to quantify a chemical exchange rate between water and a metabolite, multiple proton transfer reactions are simulated. One can determine a chemical exchange rate from the Arrhenius equation ($k_{ex} = A \exp(-E_a/k_b T)$), where A is pre-exponential factor which determines the frequency of collision and E_a is an activation energy characterizing the height of a barrier which protons need to overcome in order to transfer from solute to water and vice versa. The k_{ex} quantification in simulations can be divided in two parts. First: the pre-exponential factor (A) determination from a classical molecular dynamics simulation in Gromacs [1], second: Activation energy (E_a) calculation from the quantum mechanics/molecular mechanics simulations in CHARMM [2]. Taken together these two methods, k_{ex} can be calculated from Arrhenius equation. Due to the long time scales for the proton exchange, the QM/MM simulations are enhanced by applying umbrella sampling simulations. Umbrella sampling with a bias potential is used in order to force protons to exchange from water to a metabolite water exchangeable group and vice versa. The previous umbrella sampling simulation is the input for the next one. In umbrella sampling simulations the reaction path coordinate which characterizes a proton transfer should be determined. To describe a proton transfer in creatine the following reaction path coordinate (RC) was chosen:

$RC = (r_1 - r_2)/(r_1 + r_2) + (r_3 - r_4)/(r_3 + r_4)$, with r_1 - the distance between amine proton of creatine and nitrogen, r_2 - the distance between amine proton of creatine and water oxygen, r_3 - the distance between water proton and water oxygen, r_4 - the distance between water proton and $-NH_2$ creatine nitrogen. After series of umbrella simulations are performed, the free energy can be calculated using WHAM algorithm [3] and a free energy plot versus reaction coordinate can be extracted (fig. 2). The activation energy for proton transfer can be obtained by analyzing the free energy plot. One creatine molecule (in 0.44 nm water box) was simulated during QM/MM simulations. Each umbrella sampling window was simulated for 50 ps. One umbrella sampling simulation (umbrella sampling window) takes 24 hours. While, we need 10-15 umbrella sampling windows in order to obtain free energy plot (Fig. 2).

Results and Discussion:

Chemical exchange rates were determined from MD and QM/MM simulations for creatine $-NH_2$ group at pH = 6.5 and at three different temperatures T=309K, T=300K and T=293K (fig. 3). Multiple proton transfers between creatine and water were observed during QM/MM simulations (fig. 1). The chemical exchange rate temperature dependence can be seen on the figure 3. Figure 3 depicts the linear chemical exchange rate temperature dependence. At T=293K and pH=6.5 the chemical exchange for creatine $-NH_2$ group was calculated to be $k_{ex} = 102.9 \text{ s}^{-1}$ which is in agreement with experiment [4]. The activation energy was determined from the QM/MM umbrella sampling simulations is $E_a = 41.67 \text{ kJ/mol}^{-1}$ (fig. 2). The pre-exponential factor, computed from classical MD simulations is 29×10^8 . It is important to determine chemical-exchange rates experimentally and by simulations because the exchange rate is directly linked to tissue pH and many biological processes occur with a change in a pH value (e.g., apoptosis). Chemical-exchange-rate determination both in experiment and simulation may help to differentiate between normal and cancer tissue.

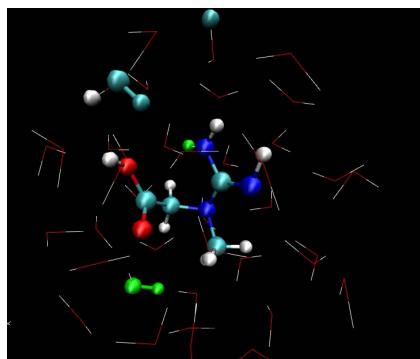


Fig. 1: Prospective view of multiple proton-proton transfer for creatine in QM/MM simulations for creatine. The "white" proton from creatine is transferred to a water molecule (blue). Blue proton from water molecule (blue) is transferred to another water molecule. A proton from "green" water molecule is transferred to creatine.

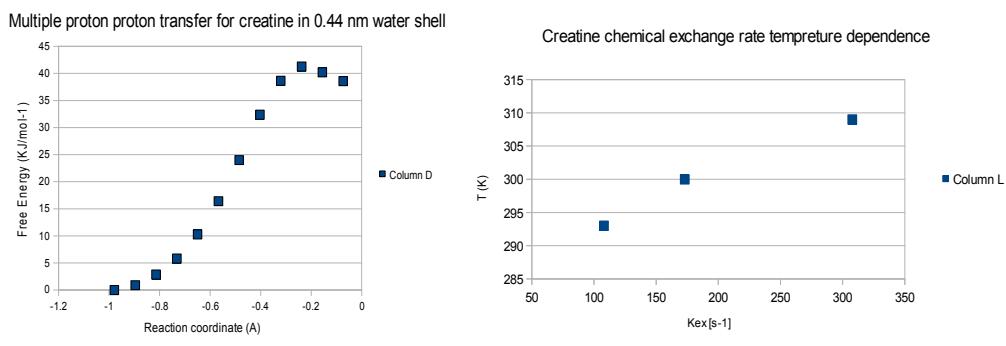


Fig. 2: Potential mean force versus reaction coordinate (obtained from QM/MM simulations in combination with umbrella sampling simulations). Activation energy can be extracted as from the height of the free energy barrier ($E_a = 41.67 \text{ kJ/mol}^{-1}$).

Fig. 3: Chemical exchange rate temperature dependence for $-NH_2$ group of creatine.

Conclusion:

The chemical exchange rate for creatine at pH=6.5 was determined, which is in good agreement with experimental values [4]. Based on these results, we propose a new methodology to access the chemical exchange rates on molecular level utilizing MD and QM/MM simulations.

References:

- [1] <http://www.gromacs.org/>
- [2] <http://www.charmm.org/>
- [3] <http://membrane.urmc.rochester.edu/sites/default/files/wham/doc/index.html>
- [4] Simplified quantification of labile proton concentration-weighted chemical exchange rate (k_{ws}) with RF saturation time dependent ratiometric analysis (QUESTRA): Normalization of relaxation and RF irradiation spillover effects for improved quantitative chemical exchange saturation transfer (CEST) MR, Phillip Zhe Sun, Magnetic Resonance in Medicine (2011).