IMPACT OF PROTEIN FLEXIBILITY IN THE SUBSTRATE CLEAVAGE OF HIV-PR EVALUATED USING NORMAL MODES AND DENSITY FUNCTIONAL THEORY

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HIV protease is a major drug target used in highly active anti-retroviral therapy (HAART), where protease inhibitors are commonly used. Most inhibitors are designed having a fixed protein framework in mind, an approach that might not be ideal. In another study we propose a methodology to generate and select relevant configurations from an ensemble of states generated using NMA (Normal Mode Analysis) in order to understand the relationship between substrate/drug binding with protein flexibility and function.

The role of protein flexibility on catalytic activity is a controversial subject. To shed light into this topic we propose the use of previously generated configurations using NMA of the substrate bound HIV-PR to investigate the cleavage reaction using pure QM (Quantum Mechanics) and QM/MM (QM/Molecular Mechanics) methods. Our aim is to understand how large-scale protein motion could possibly affect HIV-PR catalytic activity.

Starting from a substrate bound crystal structure of HIV-PR (PDB assertion code 1KJF) we generate an ensemble of structures using NMA. Emphasis is placed on the lowest 20 states with largest displacements. Selected relevant structures were used to investigate the barrier for the substrate cleavage reaction using PM6 (a semi-empirical method) and Density Functional Theory (B3LYP-D3 functional and def2-SVP basis set). We used for the QM calculations an active site made of 18 amino acids in addition to 5 water molecules.

Results indicate a strong dependence of the barrier with the initial configuration being selected. Changes in the low frequency mode 8, that produces a twist of the whole active site, leads to very low barriers when compared to the equilibrium structure. Our result indicates a strong dependency between the initial configuration used and the resulting cleavage barrier. Calculations using QM/MM are underway and should produce a more accurate description for the cleavage reaction.

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