Hunter College Seminar
Friday, October 13th
3:00 PM
1403 Hunter North

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Enzyme conformational dynamics control HIV reverse transcriptase specificity and evolution of drug resistance

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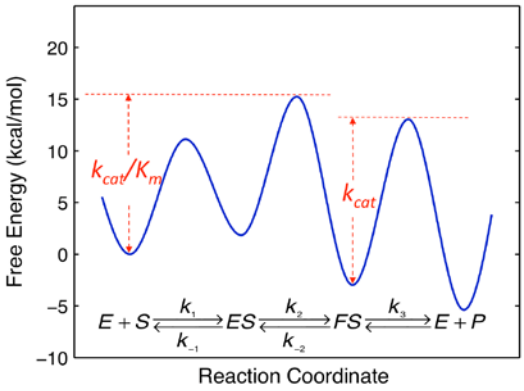
The role of induced-fit in enzyme specificity has been a controversial topic for the past 50 years (1-3). Structural studies often reveal a change in enzyme structure from an open to a closed state after substrate binding, which could be explained by the need for an open site to bind substrate, but a closed state to align residues for catalysis. Enzyme specificity is a kinetic phenomenon that cannot be addressed by measurements at equilibrium. Fersht argued that a two-step substrate binding reaction involving a change in enzyme structure after formation of an initial enzyme-substrate complex cannot contribute more to specificity than an equivalent one-step binding mechanism with the same net free energy difference (4). More recently, Warshel has argued that a pre-chemistry enzyme conformational change cannot contribute to specificity unless it is rate-limiting (5). In this lecture, I will present data and analysis to demonstrate how induced-fit contributes to enzyme specificity and explain the errors in logic leading to contrary conclusions. DNA polymerases present in ideal model system to study enzyme specificity because fidelity is important biologically, the alternative substrates are well known, and single turnover kinetic studies can be performed with ease to directly observe events leading up to catalysis. Kinetic measurements establish that the conformational change occurring after substrate binding is the major determinant of enzyme specificity for HIV reverse transcriptase, as revealed in the free energy profile shown at the right (6). This conclusion is supported by full MD simulations of the conformational change in the presence of the correct nucleotide in comparison to a mismatched base (7,8). Moreover, our analysis helps to explain the unusual behavior of some nucleoside analogs and the evolution of resistance to nucleoside analogs used to treat HIV infections (6,9).



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