

Molecular Dynamics Studies of the Ubiquitin Conjugation Mechanism

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One of the main functions for ubiquitin is to signal proteins for cellular degradation. Ubiquitination of proteins occurs in three enzymatic steps. In the second step, ubiquitin transfers to a conjugating enzyme, called E2, which then transfers ubiquitin to a lysine in the target protein. However, the mechanistic details for this final transfer remain unclear. Although it is clear that ubiquitin does bind, there are no studies that show exactly how this happens. The most favored proposal involves a step-wise mechanism with a tetrahedral oxyanion intermediate. This work probes the accuracy of the oxyanion hypothesis. In particular, the oxyanion is hypothesized to be stabilized by forming hydrogen-bonds with a nearby asparagine residue. By using molecular dynamics (MD), combined with umbrella sampling, a free energy profile of asparagine rotation can be constructed to see if it is possible for it to stabilize the oxyanion hole. Furthermore, information about the hydrogen-bonding environment in the active site can be gleaned.