

Revealing the Mechanism of Lysine-Directed Bioconjugation of Monoclonal Antibodies to Optimize Ligation Speed and Selectivity for Antibody-Drug Conjugates

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Antibody-drug conjugates (ADCs) are a powerful tool in modern oncology. They consist of a monoclonal antibody (mAb) chemically linked to a cytotoxic payload. These mAbs are engineered to recognize antigens that are unique to or overexpressed on cancer cells, delivering the payload to them. While stable and homogeneous ADCs have demonstrated remarkable efficacy in cancer treatment, their synthesis remains a significant challenge. Current methods often produce heterogeneous products with relatively low stability and lowered treatment effectiveness. We applied computational chemistry methods to study the mechanism of lysine-directed bioconjugation of ADCs. To this end, we investigated the unique reactivity of the K188 lysine residue in IgG1-class mAbs with pentafluorophenyl (PFP) esters using density functional theory (DFT) and molecular dynamics (MD). We used MD simulations to characterize the contributions of the local environment and nearby residues to K188 selectivity. We used DFT to elucidate the mechanism, including the role of the adjacent protonated histidine H189. We studied the reaction of K188 with modified PFP esters to optimize the ligation speed and selectivity of the reaction.

Our data showed that the reaction of PFP esters with K188 proceeds via a general-base-catalyzed addition-elimination. The presence and optimal positioning of histidine H189 and aspartic acid D151 within the K188 environment are crucial for lysine nucleophilic attack on a carbonyl carbon, while tyrosine Y192 is responsible for maintaining the structural integrity of the catalytic triad. Furthermore, the presence of the adjacent protonated histidine lowers all reaction energies, notably the reaction intermediate, which is required for a discrete intermediate to exist along either pathway. Modifying PFP esters with increasingly electron-withdrawing groups (EWGs) consistently lowers the reaction energy of the transition state, up to the point at which the tetrahedral intermediate no longer forms. Substitution at the α -carbon of the acyl group lowers the barrier considerably further, but without the contribution of the protonated histidine.