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Molecular dynamics insight into mechanism of nucleotide incorporation specificity of human terminal deoxynucleotidyltransferase

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Terminal deoxynucleotidyltransferase (TdT), a unique eukaryotic X-family DNA polymerase, displays a clear deoxynucleotide triphosphate preference in its activity template-independent oligonucleotide primer elongation. The enzyme properties have been extensively studied by biochemical, mutational and structural approaches for decades. Multiple kinetic and crystal structure data are available, mostly for human or murine TdT orthologs. In order to complement experimental results known up today, molecular dynamics calculations of several complexes of human TdT with DNA and dNTPs have been carried out in the present study.

The MD simulations of two types of complexes were performed: i) pre-catalytic ternary protein/primer/dNTP complex and ii) post-catalytic binary protein/primer-dN oligonucleotide complex. Both types of complexes were constructed based on the available crystal structures of murine TdT with partially-resolved active site atom positions and homology modelling of human TdT investigated with classical MD approach.

Results of the simulations allow to elucidate the substrate preferences of TdT, with the numbers and ratios of hydrogen bonds between the base of incoming nucleotide and the protein in both pre-catalytic and post-catalytic models. Thus dGTP enjoys both experimentally the highest incorporation rate and theoretically the largest number of contacts in the models, with slight decrease in the post-catalytic state, while dCTP, the slowest to incorporate *in vitro*, also displays the minimal amount of contacts in the pre-catalytic state, with noticeable increase in the post-catalytic state *in silico*. Both dTTP and dATP share middling experimental elongation performance and similar pattern of bonds without drastic change in number between the two theoretically investigated states.

Obtained data shed light on the network contacts between dNTP and enzyme active site in the course nucleotide recognition and primer elongation. These findings expand the understanding of the mechanism behind specific interactions of human TdT with a DNA primer and dNTP.

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