DNA-Repair Mechanisms: Molecular Simulations and Computational Alchemy

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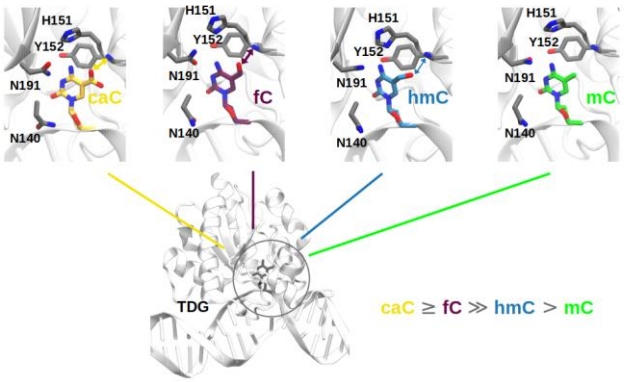

The DNA repair protein thymine DNA glycosylase (TDG) removes mispaired or damaged bases, such as oxidized methylcytosine, from DNA by cleavage of the glycosidic bond between the sugar and the target base flipped into the enzyme's active site. The enzyme is active against formyl-cytosine and carboxyl-cytosine, whereas the lower oxidized hydroxymethyl-cytosine and methyl-cytosine itself are not processed by the enzyme. To investigate the substrate specifity of TDG, we used extensive molecular dynamics simulations and thermodynamic integration of TDG complexed to DNA carrying one of four different (oxidized) methyl-cytosine bases methyl-cytosine (mC), hydroxymethyl-cytosine (hmC), formyl-cytosine (fC), or carboxyl-cytosine (caC), in extra- and intrahelical conformation, and in their amino- and imino-tautomeric forms. Our results indicate that discrimination of the oxidized methyl-cytosines does not take place in the initial complex formation before the base has been flipped out into the active site, and that imino-tautomers do not play a role in substrate recognition at this stage. For the extrahelical complexes, we observe a more favorable binding affinity of the higher oxidized forms, fC and caC, compared to the nonsubstrate bases hmC and mC. Despite rather comparable, reaction-competent conformations of the flipped bases in the active site of the enzyme, more and stronger interactions with active site residues account for the preferred binding of the higher oxidized bases. Overall, our computational results indicate that the enzyme discriminates the different oxidation forms of methyl-cytosine at the formation of the extrahelical complexes, and possibly also at a later chemical step.

[1] F. Beierlein, S. Volkenandt, P. Imhof, *J. Phys. Chem. B* **2022**, *126,* 1188. (DOI: 10.1021/acs.jpcb.1c09896)
[2] S. Volkenandt, F. Beierlein, P. Imhof, *Molecules* **2021**, *26,* 5728. (DOI: 10.3390/molecules26195728)