Insight into the active site conformation of DNA repair enzyme MBD4 from molecular simulations

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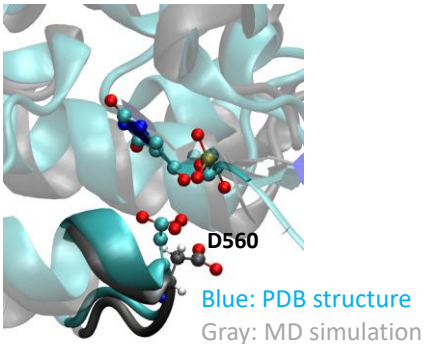
Methyl-CpG Binding Domain 4 (MBD4) is an enzyme that belongs to a family of epigenetic regulator proteins (the MBD family), which all possess a domain that specifically binds to methyl-cytosine. However, only MBD4 has a C-terminus domain that has a glycosylase function, hence giving it the ability to participate in DNA repair [1].

It has been demonstrated that D560 is a key residue for MBD4's activity, and it has been suggested that it plays a role in nucleophile positioning. Interestingly, the D560N mutation is completely inactive, even though it could fulfill said role without any issue, indicating that D560 might have additional interactions that impact the activity of the glycosylase [2].

Through atomistic MD simulations, we observed that D560 deviates from the conformation that is shown in the crystal structure. This behavior was observed for different nucleotides, including Thymine, Uracyl (both are known susbtrates of MBD4) and Pseudo-Uridine (not a substrate, but was used to replicate the crystal structure) inside the active site. However, by changing the protonation state of D560, or by making the D560N mutation, said residue now displays the exact same conformation as in the crystal structure. This leads us to believe that the free protein displays more flexibility in the region where D560 is located than what is shown in the crystal structure. Our data suggest that D560 in the crystal is more likely neutral than in the originally proposed charged form.



[1] A. Bellacosa, *Journal of Cellular Physiology*, **2001**, *187(2)*, 137-144

[2] L. S. Pidugu, et. al., *Journal of Molecular Biology*, **2021**, *433(15)*, 167097