Exploring bimodal equilibrium in MBD2 protein recognition of methylated CpG dinucleotides

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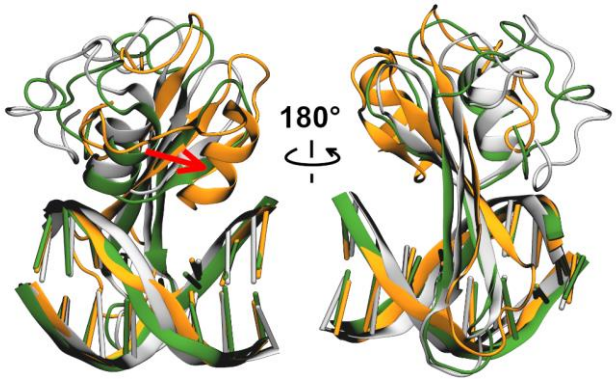
Transcription factors are proteins that regulate gene expression by binding to sequence-specific DNA target sites. The search mechanism for finding this target site can be explained by the model of facilitated diffusion, where the protein switches from three-dimensional diffusion in bulk solution to a one-dimensional sliding motion along the DNA surface [1]. While the sliding process has been studied computationally at atomistic and coarse-grained resolution, little is known about the search-to-recognition process at the atomistic level [2].

MBD2 is a transcription factor and member of the methyl-CpG binding domain (MBD) protein family that selectively recognises mCpG dinucleotides, i.e. a methylated cytosine followed by a guanine. DNA methylation is a mechanism for epigenetic regulation, meaning it affects gene expression.



We performed extensive classical MD simulations to investigate the recognition complex formation of MBD2 and mCpG DNA. When being placed one base-pair away from the specific site, MBD2 formed a stable complex at the target site on the order of microseconds. Surprisingly, RMSD clustering analysis revealed two stable conformations at the recognition site of which only one (green) resembles the original crystal structure (grey). Characterisation of these two states showed different DNA conformation as well as different hydrogen bond interactions between MBD2 and the DNA. Due to the absence of the hydrogen bond between S47 and the DNA backbone of a methylated cytosine in the secondary state, we performed additional simulations of the S47A mutant. These indeed resulted in structures that closer resemble the secondary state than the recognition state from the crystal structure. NMR experiments of our collaborators ultimately confirmed mCpG selectivity for the S47A mutant but showed also reduced binding affinity in agreement with our MD simulations. With these findings, our study illustrates that MBD2 explores a bimodal equilibrium in the process of mCpG recognition.

[1] P. H. von Hippel, O. G. Berg, *JBC*, **1989**, *264(2)*, 675-678.
[2] L. Dai, et al., *PNAS*, **2021**, *118(23)*, e2102621118.