Understanding the molecular basis of damaged DNA recognition by the protein MutS

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For any living organism it is of vital importance to safeguard the code stored in its DNA, where the instructions for making life possible are written. But damage occurs constitutively, as single point mutations appear naturally during DNA replication. At the molecular level the change produces a DNA mismatch (MM), a DNA defect occurring when two non-complementary bases are aligned in the same base pair of a duplex. MMs are quickly repaired in DNA by the highly conserved DNA MisMatch Repair mechanism (MMR), which ensures DNA integrity and genome stability. The first step in the MMR pathway is the recognition of the MM by the protein MutS. This first recognition step is crucial as it determine the repair efficiency of the whole pathway. MutS is able to recognize all possible mismatches using two key residues: PHE36, which stabilize the MM base on the Crick strand through stacking interaction; and GLU38, which is thought to form hydrogen bonds with the same MM base.

The interaction with PHE36 is non-specific regardless of the MM base. But according to the chemical nature of each bases, and the x-ray structures analyzed, GLU38 should exist in at least two different protoned states to always satisfy, regardless of the MM, the requirements for the formation of a hydrogen bond, acting as a “recognition switch”. In this work we used pKa calculations, Molecular Dynamics simulations, and Umbrella Sampling techniques to study the role of GLU38 with different MMs. Using high resolution x-ray structures we reconstructed by symmetry a complete MutS protein, which was used as a template to study 6 different MMs. Our initial results seem to suggest that GLU38 must always be protonated in order to maintain the hydrogen bond interaction with the MM base, rejecting the “recognition switch” hypothesis. Furthermore, our results challenge the validity of some of the model deposited in the PDB that were refined from diffraction data using the deprotonated state of Glu38.