

## Summary

In this project we investigated several biophysical issues in epigenetics, in particular, those associated with the high fidelity of maintaining DNA methylation patterns. Cancer cells commonly exhibit aberrant methylation patterns and molecular level understanding of the phenomena will advance the search for targets of anticancer drugs. A crucial step in the process of maintaining methylation patterns of the DNA is the ability to distinguish hemi-methylated from either unmethylated or symmetrically di-methylated CpG sequences. We performed extensive molecular dynamics simulations and find that the binding mode of hemi-methylated DNA to the SRA domain of UHRF1 is very similar to that of unmethylated DNA. However, at the same time a very large difference in the binding constants exists. The difference in the binding free energy to hemi-methylated DNA is, approximately, 50 kJ/mol more favorable compared with the binding to unmethylated DNA. Although, this large difference can readily explain the high fidelity of discriminating between hemi-methylated and unmethylated DNA strands, it is, nevertheless, surprising given the high similarity of the binding modes. We find that the difference in the binding constants predominantly arises not due to hydrophobic interactions (as is normally attributed to) but due to a change in the electronic structure (partial charges) of the cytosine base upon methylation of its carbon at position 5 of the pyrimidine ring. Other contributions to the difference in binding constant is attributed to the observation that in the binding to an unmethylated CpG site, a water molecule is filling up the space between the DNA and the protein. This pushes the residues of the protein surrounding C5 away from the DNA and triggers larger fluctuations of nearby structured water from their average position. Furthermore, calculations of the difference in the binding free energies between hemi-methylated and fully-methylated CpG sites to UHRF1 indicates that the difference is much smaller, 18 kJ/mol (compared with the case of unmethylated site). Although, this value is somewhat larger than that observed experimentally, the trend of a significantly smaller difference is reproduced. The results from the first part of the project is now being prepared/submitted for publications in international peer-reviewed journals. In order to determine the role of UHRF1 in the flip-out mechanism of the target cytosine to be methylated by Dnmt1, we are calculating the free energy barrier for flipping this base, on the complementary strand, out of the helix. This is performed while UHRF1 is bound to the methylated-cytosine (thus, also in a flipped-out conformation) and for comparison for the same DNA strand free in solution when the methyl-cytosine is in the flipped-in conformation.