Computational insights into the central role of *Xeroderma pigmentosum* group A (XPA) protein in nucleotide excision repair (NER)

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CHAPTER 7

Summary and future prospects

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7.1. Overview of the thesis

The main theme of the current thesis involves a structural understanding of the role of XPA in NER in-depth, which acts as a scaffold protein unifying all the NER core proteins to the damaged site and making sure that the damage is properly repaired.

In the first part of this thesis, we investigated the structural properties of XPA in monomer and dimer forms. we further determined the better representative model to represent the DBD region of XPA. The MD trajectory and PPI profile investigations revealed that the XPA_{98-239} homodimer is significantly more stable than the other XPA dimer variation. The XPA homodimers' monomers had an inter-monomeric spacing of ~20-22 Å, which might be exploited to bind DNA during the NER process. Hydrogen bonding, salt bridges, and a large number of hydrophobic contacts were all observed to have a significant role in stabilizing both homodimers, with XPA_{98-239} homodimer surpassing its other variant in all aspects.

The DPI between XPA and DNA was next investigated in both monomer and homodimer stages, and we found that XPA monomer in the presence of DNA exhibited higher structural alterations but maintained its stability throughout the simulation. Investigating XPA homodimer in the presence of DNA also revealed alterations in DPC's backbone structure and interface region, as well as an increase in the ASA of the interface residues. It was discovered that the ASA of XPA homodimer in B and U states was caused by the partner attraction effect of XPA homodimer with the DNA after further study. Our DPC has a strong propensity for binding, according to BFE research. The C-terminal XPA homodimer residues, primarily lysine residues, were found to be implicated in DPI by PRED experiments.

In the third part of this work, we studied the effect of XPA's binding with XPE, another DNA damage verifying protein seen during GG-NER. $XPA_{185-226}$ was bound to the cleft region of XPE. The BFE analysis with the MM-PBSA method demonstrated the high binding affinity between the two proteins. Residues L191, V193, W194, E198, E202, E205, R207, and F219 of XPA and R20, R47, and L57 of XPE were found to be involved in the formation of the XPA-XPE complex, respectively, according to PRED analysis and PPI profile investigation. We found the

PPI between the PPC of XPA-XPE to be transient in nature. We further noticed that XPA upon R207G mutation disassociates completely with XPE *in silico*. In comparison to WT XPA, it was discovered that the atomic fluctuations in R207G XPA were significantly higher, indicating that this mutation in complex with XPE is not stable, and may result in NER failure. BFE analyses between XPA and XPE revealed that WT XPA had a better binding affinity with XPE in contrast to mutant XPA.

In the last part of the thesis, we attempted to understand the PPIs between PPCs of PIC members by determining the full-length XPA computationally. XPA monomer in monomer showed that its DNA binding domain (DBD) was associated with RPA70AB and ERCC1 on either side. The C-terminal portion of XPA was attached by the P52 and P8 subunits of the TFIIH complex. The N-terminal portion of XPA was associated with RPA32C. Additionally, PPIs between ERCC1 and RPA70AB as well as p8 of the TFIIH complex and RPA70AB were found. PIC's nature in the case of XPA homodimer showed a similar result to that of XPA monomer. Particularly, XPA2 was found to bind to the p5 protein of TFIIH, indicating that it helps TFIIH function as a helicase to start the NER process.

7.2. Future prospects

This thesis provides an atomistic insight into the structural features of XPA and its DPI with the DNA in both monomer and dimer forms. It also gives a comprehensive understanding of XPA's nature with various other members of the NER pathway. Furthermore, computational perception regarding the positioning of the XPA about the PIC members was understood.

This work has further possibilities that include:

I. DPC between XPA homodimer and the members of PInC can be studied.
II. Understanding the mechanism of NER can be used in developing the strategies to compensate/elevate the DNA repair activities of patients with *XP* diseases.

III. Drug screening can be done against XPA to decrease the chemotherapeutic resistance by designing the SMIs.

IV. XPA's relation with novel XPA binding (XAB) proteins: XAB1, XAB2, XAB3, XAB4, and XAB5, can be explored in-depth.

V. The PPI between XPA and PCNA can be studied to find out whether XPA is indeed involved in ligation activity with PCNA or not.

VI. Novel mutations of XPA are being reported every year, these can be investigated *in silico*.