JOINT MEETING OF POLISH SYNCHROTRON RADIATION SOCIETY MEMBERS AND SOLARIS CENTRE USERS

ATP-DEPENDENT DNA DAMAGE RECOGNITION BY UVRA: THE USE OF CRYO-EM IN THE STUDY OF PROTEIN CONFORMATIONAL CHANGES

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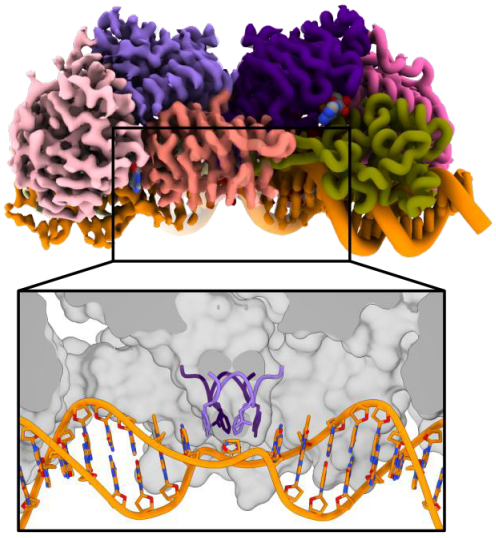

Nucleotide excision repair (NER) is one of the fundamental pathways involved in the detection and repair of various DNA distorting lesions. In bacteria, NER involves four key proteins: UvrA, UvrB, UvrC, and UvrD. UvrA is a dimeric ATPase that detects the DNA damage and hands it over to UvrB – a weak helicase that verifies the presence of damage. UvrA then dissociates, and UvrC endonuclease is recruited. UvrC incises the DNA on both sides of lesion, and the excised fragment is removed by UvrD helicase. Finally, the resulting gap is filled by DNA polymerase I.

We used cryoelectron microscopy (cryo-EM) to study the role of the ATPase activity of UvrA in damage recognition. We determined a series of cryo-EM structures of UvrA in the presence and absence of nucleotides and in complex with three different DNA substrates. These structures allowed us to visualize a major rearrangement of the UvrA dimer upon ATP binding. These conformational changes are used to mechanically probe the integrity and deformability of DNA and indirectly detect the presence of DNA lesion. We also solved the cryo-EM structure of the UvrA:UvrB:DNA complex that sheds a light on how UvrB is loaded on the DNA for the verification of the presence of the lesion. Our structural findings are supported by complementary biochemical studies.

In summary, our series of cryo-EM structures presents snapshots of the mechanism of ATP-dependent DNA damage recognition by UvrA. It shows that mechanical probing can indirectly detect a broad range of DNA lesions and provides a framework for a deeper understanding of the bacterial NER pathway.