Uncovering the Flexible Architecture of a Complex Macromolecular Machine in DNA Repair at 4-5 Å Using Cryo-EM

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Our group is dedicated to improve the mechanistic understanding of large macromolecular complexes important in DNA repair and DNA damage signalling. Many of these processes are regulated in the cell by multi-subunit macromolecular complexes. Elucidating the structure of these large complexes provides relevant information about how they function. Recent advances in cryo-electron microscopy (cryo-EM) methods allow approaching these complex machineries, but dealing with structural flexibility and heterogeneity is still a major issue in order to reach high resolution. In my talk, I will introduce recent advances in the cryo-EM methodology, and where this field is moving. I will describe examples from our current research in the laboratory to introduce the potential of this methodology, but also major areas of difficulty. I will describe several multi-subunit complexes that work in the DNA damage response, and which combine rigid and flexible regions. The structure of these flexible complexes is been analysed using a specific strategy for each region in the complex. Rigid interactions can be resolved at high resolution, whereas flexible regions could only be resolved at medium resolution after extensive classification of the data into sub-populations grouping molecules in a similar conformation. The work that I present is a joint effort of most members in our group, together with collaborators in the UK. This collaborative approach has been important to address the difficulties found when analysing these large and flexible macromolecular complexes.