Oral presentation

An integrated structural approach to eukaryotic FeS helicases

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Helicases are essential and ubiquitous enzymes, playing a key role in a variety of cellular processes, from DNA replication to repair, recombination, as well as RNA translation and transport. Due to their important role in viruses, bacteria and eukaryotic cells, they are *emerging as a new class of antibacterial, antiviral and ant-cancer drug targets* . A subset of helicases play specialised and specific functions by resolving/remodelling a variety of *atypical DNA structures*, such as G-quadruplexes, triplexes, Holliday junctions, as well as displacement loops (D-loops and R-loops): among those a major role is played by two families of helicases, the RecQ and FeS family. *Helicases containing FeS-clusters* are ubiquitous but their exact mechanism of action is poorly understood; in particular, no structural information are available for some medically-relevant members of the family *, like FANCJ, DDX11 and RTEL1.* The combination of the intrinsic conformational flexibility, FeS cluster lability and size makes them challenging targets for structural biology.

DDX11 plays an important role in sister-chromatid cohesion, associates with the replisome and is involved in processing non-canonical nucleic acid structures [2]. We have demonstrated its ability to unwinding physiologically-relevant unimolecular G4 and collected *Cryo-EM data for the protein in complex with a DNA fork* : a preliminary 5 Å structure has been determined (Figure 1A). Despite the relatively low resolution, we can clearly see the path of the DNA fork bound to the helicase including the double helix, and the 5' single strand across the motor domains. Interestingly, in same regions the structure differs significantly from the AlphaFold model. We are currently refining the structure towards atomic resolution.

RTEL1 is involved in the stability and elongation of telomeres, DNA replication and repair, stability of fragile sites and removal of G4-associated. We have obtained a *preliminary structure of the catalytic domain of RTEL1*, in a complex with a DNA fork (Figure 1B); further data collection and refinement are in progress. In addition to the catalytic core, the protein includes a C-terminus, which has a modular structure with folded domains interspersed by disordered sequences. Using a *combination of crystallography, SAXS, NMR and biochemistry*, we obtained a comprehensive picture of the architecture and possible function of the C-terminal region of human RTEL1 (Figure 1C).

These structures provide an essential framework to better understand the role of these enzymes, to elucidate their mechanism of action in the processing of non-canonical nucleic acid structures, (such as G-quadruplexes, D-loops and R-loops), and to shed light on their involvement in genetic diseases and cancer development.

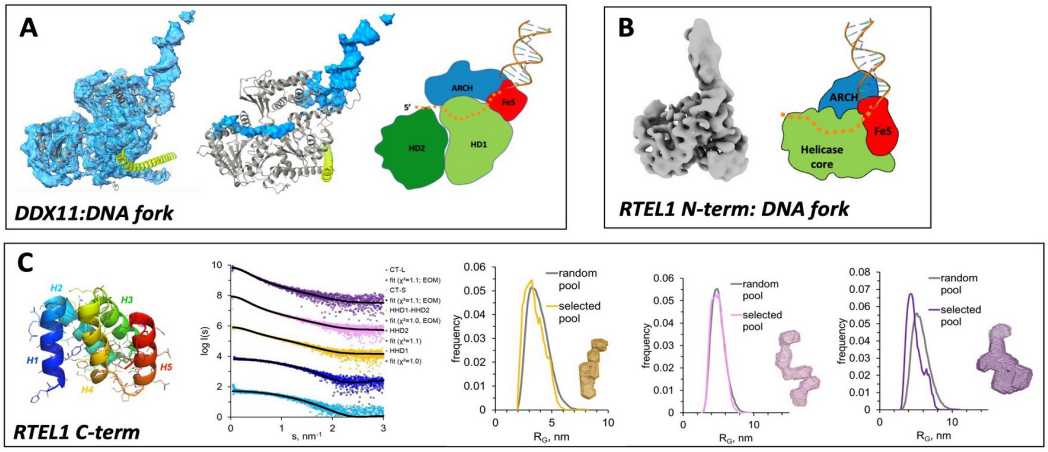

Figure 1. **A)** Cryo-EM reconstruction of the DDX11 helicase in complex with a DNA fork substrate, showing the dsDNA helix and the path of the 5' ssDNA. **B)** Cryo-EM reconstruction of the catalytic N-terminal domain of RTEL1 with a DNA fork. **C)** Crystallographic and SAXS analysis of the C-terminal domain of human RTEL.