MS07-P07

Understanding native state of Cpf1 protein from *Francisella novicida* by small-angle X-ray scattering

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Clustered regularly interspaced short palindromic repeats (CRISPRs) from *Prevotella* and *Francisella 1* (Cpf1) are RNA-guided endonucleases that produce cohesive double-stranded breaks in DNA by specifically recognizing thymidine-rich protospacer-adjacent motif (PAM) sequences. Even though, Cpf1 is emerging as a powerful genome-editing tool and numerous structures of various Cpf1 proteins have been solved, the apo-structure of Cpf1 remains elusive. In this study, to understand native state of Cpf1 protein from *Francisella novicida* (FnCpf1), we determined two solution structure of FnCpf1 with and without CRISPR RNA (crRNA) using small-angle X-ray scattering. Also, we visualized apo-structure of FnCpf1 using negative staining electron microcopy. By comparing between the apo-structure of FnCpf1 and with crRNA-bound structure, we realized that their overall shapes were similar as a closed form, suggesting that conformational change upon crRNA binding to FnCpf1 is not drastic, but a local induced fit might occur to recognize PAM sequences. However, the apo Cpf1 from *Moraxella bovoculi 237* (MbCpf1) was examined as an open form, indicating that MbCpf1 may have a large conformational change for crRNA binding changing from an open to a closed form. These results suggested that the crRNA-induced conformational changes in Cpf1 differ among species.

Keywords: Cpf1, SAXS, electron microscopy

MS07-P08

Structural characterisation of diruthenium paddlewheel compounds with cytosinato and adeninato ligands

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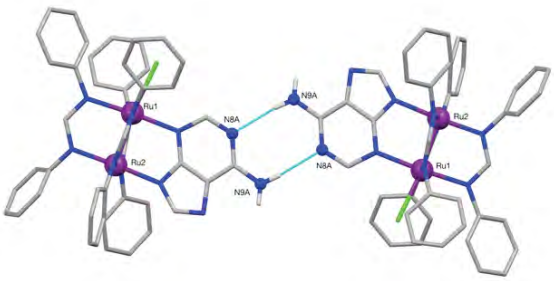
The interactions of dimetallic coordination compounds with biological molecules, in particular dirhodium and diruthenium metal-metal bonded complexes with paddlewheel geometry, are currently being invesigated as there is evidence indicating that dirhodium compounds covalently link with DNA, tRNA and proteins. In fact, paddlewheel diruthenium compounds can form metal-protein adducts, acting as inhibitors of C6 rat glioma cell proliferation or as inhibitors of glioma tumour growth *in vivo*. Open-padlewheel compounds have also been proved helpful for fingerprinting the junctions of RNA structure [1].

Recent works exploring the interactions of diruthenium moieties with biological species have focused on the elucidation of the coordination mode of the ruthenium atoms to the active sites of the potential ligands, as open-paddlewheel species have the tendency to include another bidentate ligand as a fourth bridge yielding a paddlewheel structure and thus gaining stability,

In this work, compounds $[Ru_2Cl(DPhF)_3(cyto)]$ and $[Ru_2Cl(DPhF)_3(ade)]$ have been synthesized and characterized by several techniques, including single crystal X-ray diffraction. Supramolecular interactions between these molecules have also been studied, paying particular attention to the intermolecular hydrogen bonds similar to the ones found in nucleic acids. Interestingly, it was found that compound $[Ru_2Cl(DPhF)_3(cyto)]$ is the first crystallographycally characterised ruthenium cytosinate, and the coordination mode found in $[Ru_2Cl(DPhF)_3(ade)]$ had not been previously described for adeninate (or adenine) in the crystal structure of a ruthenium complex.



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Keywords: Diruthenium paddlewheel compounds, antitumoral activity, supramolecular interactions