

Report on MX- 1222 experiment

Structural studies of RuvC - Holliday junction

Holliday junctions (HJs) are four-way DNA structures that are intermediates in homologous recombination and certain DNA repair pathways. The DNA duplexes in HJs have to be separated in a process called resolution, which is carried out by specific nucleases called resolvases. In Gram-negative bacteria this role is fulfilled by RuvC— a dimeric nuclease related to RNases H. Our aim was to elucidate the details of the substrate recognition and catalysis by RuvC. We obtained crystals of RuvC in complex with short synthetic Holliday junctions and we collected a 3.8 Å dataset from these crystals using a microfocus beamline 23-2 at ESRF (experiment MX-998). The structure was solved by molecular replacement and revealed that the junction bound by RuvC adopts a two-fold symmetric unfolded conformation. In order to be able to publish these results in a high-impact journal, our aim was to either collect a better resolution dataset or to use experimental phasing and other methods to corroborate the correctness of our low-resolution model.

We used several approaches to improve the crystal quality. First, we used the modified versions of the synthetic Holliday junctions used to solve the original structure. The modifications included shortening and lengthening of the HJ arms, different positioning of the cognate sequence, introduction of sticky overhangs in the ends of the arms, and the removal of the central homology region to prevent the exchange point migration. We obtained numerous RuvC co-crystals with these new oligonucleotides, including several new crystal forms. 6 types of these crystals (a total of 120 crystals) were tested at beamline 23-2 but, unfortunately, none of them diffracted X-rays to a resolution better than 5 Å.

Our second approach to corroborate our structure was to obtain experimental phases to exclude the possibility of model bias, which is a concern at limited resolution. We produced crystals using selenomethionine-labeled RuvC protein and we also soaked the native crystals with tantalum bromide cluster. 26 Se-Met crystals and 16 soaked crystals were tested during MX-1222 experiment but again we did not obtain X-ray diffraction to a sufficient resolution.

The third approach was the crystallization of RuvC with HJ-forming oligonucleotides containing several bases modified with bromine. Diffraction data from such crystals would not only provide us with experimental phases but a strong signal coming from bromine atoms would also help us corroborate the placement of DNA bases in the model. Using brominated oligonucleotides we obtained multiple forms of regular and large crystals. 2 different kinds of these crystals were tested during MX-1222 experiments (a total of 22 crystals) but unfortunately, these crystals did not diffract X-rays.

Even though MX-1222 experiment did not provide us with new diffraction data, it allowed us to exclude several possibilities of improving the crystal quality and obtaining experimental phases. For now the most promising approach we plan to adopt is crystal dehydration using an on-line dehumidifying device. We are also testing RuvC orthologues from different species in crystallization trials. Clearly, our ultimate goal is to obtain a crystal structure of RuvC-HJ complex at a resolution of at least 3 Å. Such a structure would allow us to define the details of metal ion binding at the active site and the mechanism of cognate sequence recognition.