Proposal Report

Proposal Code: MX- 1496

Proposal Title: Structural studies of HsdR subunit of Type I Restriction Enzyme EcoR124I in complex with ATP and ADP.

The main goal of the experiment was to obtain diffraction and solve the structures of the ADP bound form of the restriction subunit (HsdR) of the Type I Restriction Modification system from *E.coli*, with subsequent comparison to the HsdR-ATP complex. The crystal structure of the WT HsdR with ATP was solved by us in 2008 (Lapkouski et.,al. 2009). HsdR is a dsDNA translocase, which uses the energy of ATP hydrolysis to do a mechanical work on DNA translocation in steps of 1ATP/1base pair translocation. Two helicase domains are responsible for the translocation and supposedly alternate their conformation as DNA is translocated and ATP is hydrolyzed.

We were able to grow large ~300x100x50 um crystals of ADP bound HsdR (**Fig. 1a**). Unfortunately, despite of the perfect crystals look the diffraction was not good. Images were either highly mosaic (**Fig. 1b**), anisotropic (**Fig. 1c**) or with badly split spots 90 degrees apart (**Fig. 1d**). After screening of ~50 crystals we collected 2 datasets. We were able to reach 3.20 Å resolution with 64% completeness for the best data set (**Table.1**). We were able to solve the structure by molecular replacement and see some changes of the loops in the ATP binding pocket. We are currently analyzing the data and the model. But due to the poor completeness, we will try to get a better dataset for publication.

We will improve the crystals. We already obtained crystals under different crystallization conditions and hope that this may diffract better. We are planning to submit a new proposal as a continuation of the current in order to collect better data sets.

Figure 1. Crystallyzation (1a) and selected frames of the HsdR-ADP crystals showing problems with high mosaicity (1b), anisotropic diffraction (1c) and split spots (1d).

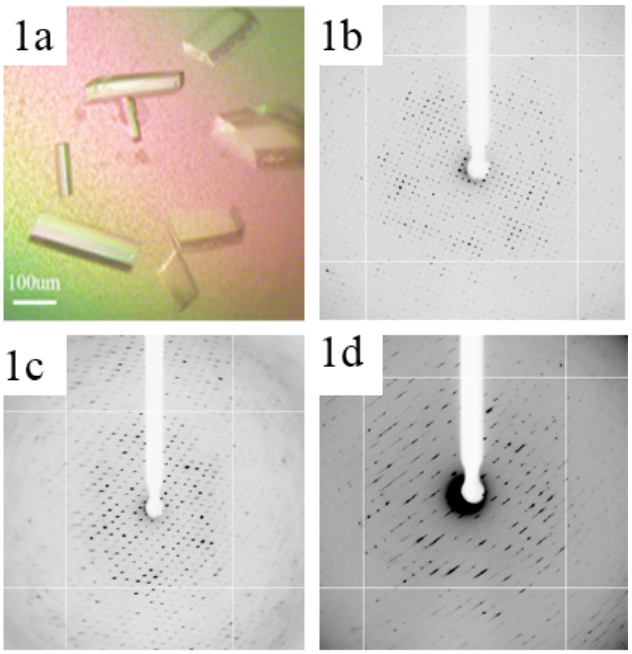

Table 1. Data collection statistics

Complex	**HsdR-ADP**
Space group	P21
Average unit cell, Å	a=131.40,b=126.33 c=165.39, β=110.99°
Resolution, Å	20.0-3.20(3.37-3.20)*
Rmerge, %	13.9(40.7)
Total reflections	118871(17585)
Unique reflections	53540(8138)
Mean((I)/sd(I))	4.1(2.2)
Average mosaicity	1.07
Multiplicity	2.2(2.2)
Completeness, %	64.7(67.4)

*Values in parenthesis are for the highest resolution shell