Standard Project

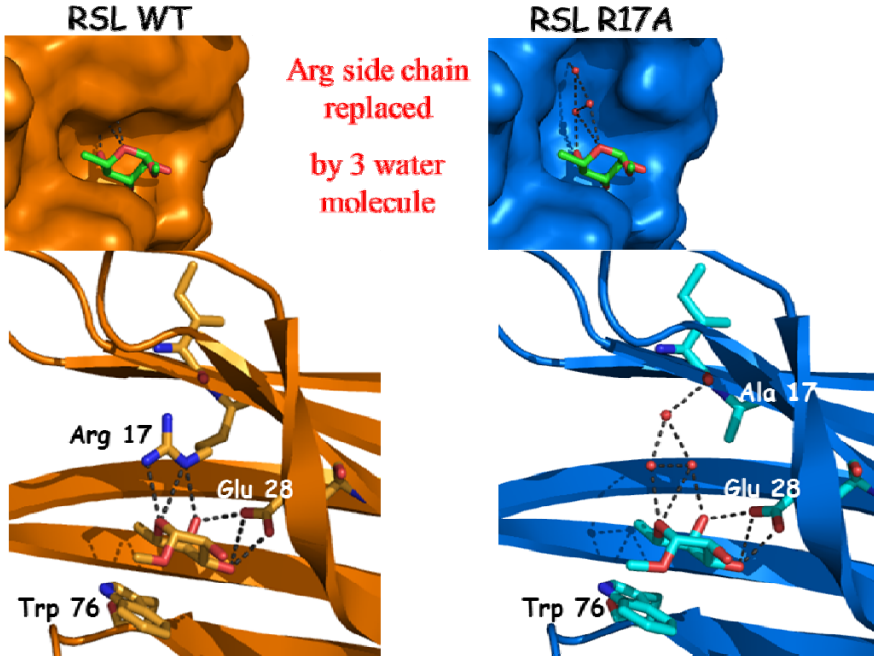
Experimental Report template

Studies of protein-sugar interactions at molecular level		Proposal number: 20101098
Beamline: BM30A	**Date(s) of experiment:** from: 22/04/2011 to: 23/04/2011	**Date of report:** 01/10/2011
Shifts: 3	**Local contact(s):** Dr. Franck Borel	*Date of submission:*

Objective & expected results (less than 10 lines):
We are looking at the interaction protein-sugar at a molecular level to determine the binding properties (lectins) and catalytic mechanism (glycosyl hydrolases). For that we determine the Xray structures of protein in complex with several ligand at high resolution (>2.2 Å). The analysis of the binding sites will give use clues for the design of sugar based inhibitors or for biotechnological applications.

Results and the conclusions of the study (main part):

Our trial to trap the covalent intermediate of the arabinofuranosidase Arb93A from *Fusarium oxyporum* by a short soaking with its substrate failed. Only the product could be seen in the electron density. Shorter soaking time or specific inhibitor will have to be tested.
The N-terminal domain of the discoidin II from *Dictyostelium discoideum* has been proposed to be a lectin binding fucose. Cocrystallisation of the discoidin II with 2mM fucose was performed but the data collected did not reveal any sugar could in the binding site. The domain has to be isolated and further characterise to determine its ligand before collecting new data.



The lectin from *Ralstonia solanacearum* (RSL) forms very stable trimer with two binding sites per monomer. It binds L-fucose with high affinity (µM) and all the binding sites are on the same surface making it a good model to study multivalency.We are looking at the effet s of mutation in the different binding sites on the lectin multivalency and specificity. We determine the structure of the R17A mutant who has been shown by calorimetry to have only three functionnal fucose binding sites. In the structure due to the local high concentration in substrate, we found a fucose moiety in all six binding sites. In the mutated binding sites, the interactions made by the arginine side chain are replaced by interaction with three water molecules (cf figure). New tests will be done using lower fucose concentration and on other mutants.

Justification and comments about the use of beam time (5 lines max.):

The beamline is appropriate for collection of Xray data on protein crystal at high resolution. There is a sample changer.
We tested about 30 crystals and collected three datasets in 17 hours.
The sample changer got stuck in the dewar and we could not use the beamline from 2:30 AM.

Publication(s):
-Arnaud J., Audfray A., Varrot A. and Imberty A. manuscript in preparation.
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