EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** The Architecture of a Mucus Binding Protein from a Gut Commensal Bacterium: A Multimodular Ig-Binding Adhesin	**Experiment number**: MX-1348
Beamline: ID14-3	**Date of experiment**: from: 3/12/11 to: 4/12/11	**Date of report**: 23/1/2013
Shifts:	**Local contact(s)**: Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Andrew Hemmings*

Miss Sabrina Etzold*

Mr Arthur Li*

Report:

Objectives of the study: *Lactobacillus reuteri* MUB is a cell-surface protein that is involved in bacterial interaction with mucus and colonization of the digestive tract. The 353 kDa mature protein is representative of a broadly important class of adhesins that have remained relatively poorly characterized due to their large size and highly modular nature. MUB contains two different types of Mub repeat (Mub1 and Mub2) present in six (R-I to R-VI) and eight (R-1 to R-8) copies, respectively (Fig. 1A) and shown to be responsible for the adherence to intestinal mucus. Mub1 and Mub2 repeats share 30-35% sequence identity (Fig. 1B) and are expected to have similar 3D structures. We have solved the X-ray crystal structures of both Mub1 (RV) and Mub2 (R5; Fig 1C) repeats each comprising two structurally-related domains [2]. In this experiment we sought to use small angle X-ray scattering (SAXS) methods to determine the solution structures of MUB fragments consisting of various combinations of Mub1 and Mub2 repeats by fitting X-ray crystal structures of individual repeat proteins to solution X-ray scattering envelopes.

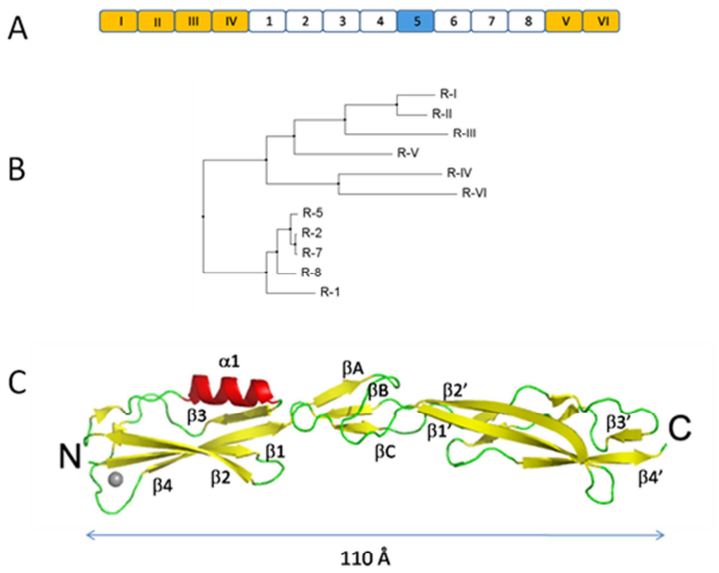

Fig 1. MUB domain organization and repeat structure

Experimental results: The recombinant single (R5, RI and RV) and multiple tandem repeat (R8V, RVVI and RI-II-III) proteins generated from the 14-repeat mucus binding protein (MUB) of *Lactobacillus reuteri*

ATCC 53608, were taken in PBS gel filtration buffer to the ESRF beamline ID14-EH3 for SAXS analysis. Scattering data was collected for all proteins in a 1:2 dilution series with concentrations of 21 to 0.2 mg/mL for RI and 10 to 0.3 mg/mL for all other constructs. Matching buffer samples were exposed before and after each sample as a control.

Ideal scattering curves were generated by merging curves of different concentration, analysed via PRIMUS and values for R_g and I(0) were determined from Guinier plot. A distance distribution function P(r) was computed manually using GNOM to describe particle shape and size by determining D_{max}, R_g and I(0) (see table). The P(r) functions of all Mub proteins show the characteristic curve shape of elongated proteins with a steep increase to an early peak at an R (=D_{max}) value of 2 nm and long linear fall (P(r) of R5 shown as example) [1].

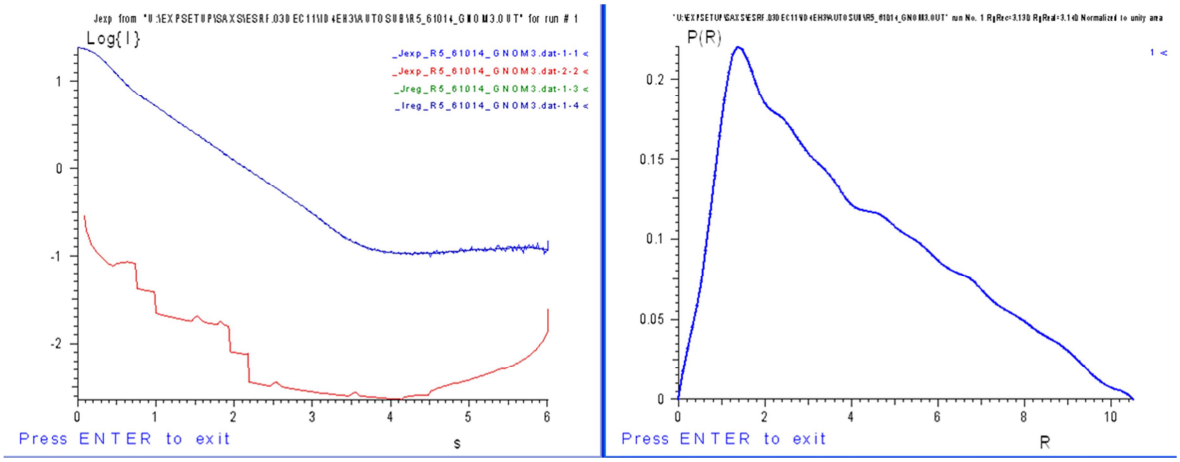

Fig 2.:P(r) function for Mub-R5 computed from merged ideal scattering curve.

Estimated Molecular Dimensions: The estimated D_{max} for the solution envelope of all tested single and double repeat proteins is in line with an expected molecule length of 11.0 nm observed in the R5 [2] and RV [unpublished data] crystal structures.

Table 1: Parameters estimated from computed P(r) function

Protein sample	**Rg [nm]**	**I(0)**	**D_{max} [nm]**	**Chi^{2} ***
Mub-R5	3.13	23.9	10.5	0.92
Mub-RI	3.28	16.5	10.5	4.62
Mub-RV	3.22	26.3	11.5	1.19
Mub-R8V	5.96	41.9	20.5	3.26
Mub-RVVI	5.92	48.8	21.5	4.43
Mub-RI-II-III	6.21	50.5	23.5	2.18

* fit of P(r) model to scattering data

However, the D_{max} of 23.5 nm for the triple RI-II-III repeat is smaller than the theoretically expected value of 33.0 nm based on X-ray structure data of a single protein repeat, when manually computing a P(r) function with a linear fall especially close to the intersection of the curve with the x-axis [see figure below].

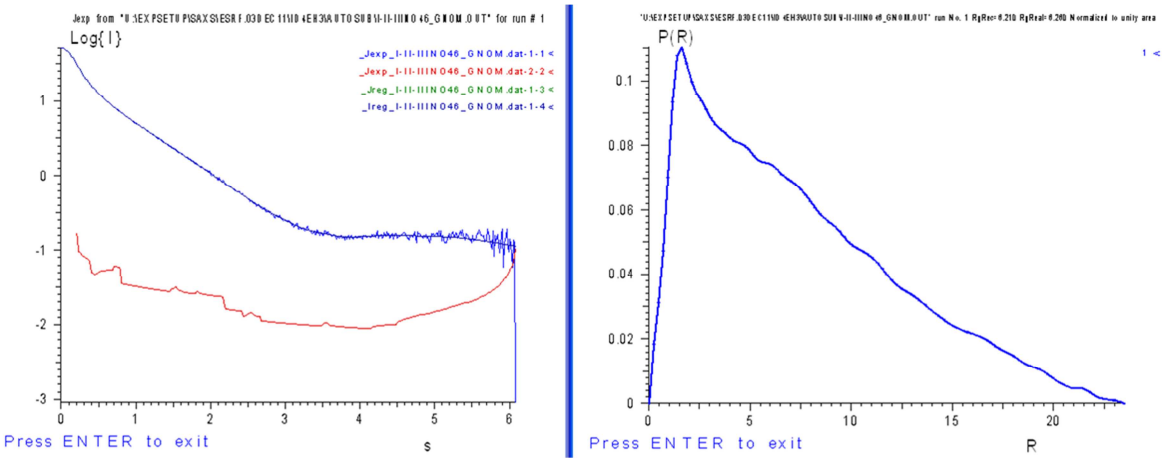

Fig. 3: P(r) function for Mub-RI-II-III computed from merged ideal scattering curve with R (=D_{max}) of 23.5 nm.

GNOM analysis for the triple repeat allowing a D_{max} of 33 nm was not possible. Hence, the collected data can not describe the theoretical size for the triple repeat proteoin RI-II-III of 33 nm. Therefore, the P(r) function with a D_{max} of 23.5 nm was used for subsequent solution envelope reconstruction.

Single Repeat Solution Structures: Ab-initio shapes were calculated by GASPOR (Atsas online tool) with P1 point group symmetry and averaged using DAMAVER. Model calculations with two fold point group symmetry did not result in a sensible shape reconstruction. The resulting 3D envelopes without symmetry for all single repeat proteins showed an elongated shape of Mub proteins in solution with a length of about 10.5 nm. The superposition of low resolution shape reconstruction with high resolution crystal structures of R5 and RV was performed by SUBCOMP20. The crystal structures agree well with the solution envelopes for all single repeat proteins tested (Fig. 4).

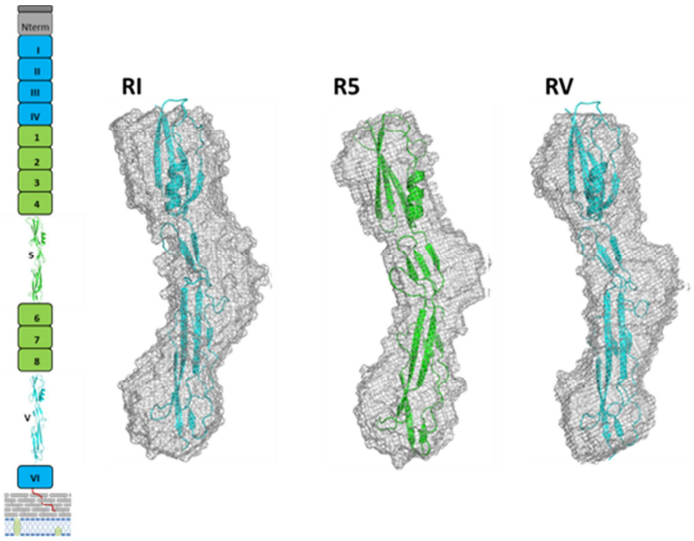

Fig 4. Single Mub repeat solution structures

Tandem Repeat Solution Structures: The resulting 3D envelopes (P1 symmetry) for tandem repeat proteins showed an elongated shape of Mub proteins in solution with a length of about 19.5 and 24.1 nm for double and the triple repeat proteins, respectively. The superposition of low resolution shape reconstruction with high resolution crystal structures of R5 and RV was again performed by SUBCOMP20. The agreement with crystal structure data fror the R8V and RVVI tandem repeat proteins was good (Fig 5). However, as expected, the solution envelope of RI-II-III does not allow an overlay with three single RV molecules due to the absence of suitable data to describe the expected particle size of 33 nm. This may be due to insufficient protein sample quality and presence of aggregation in PBS buffer.

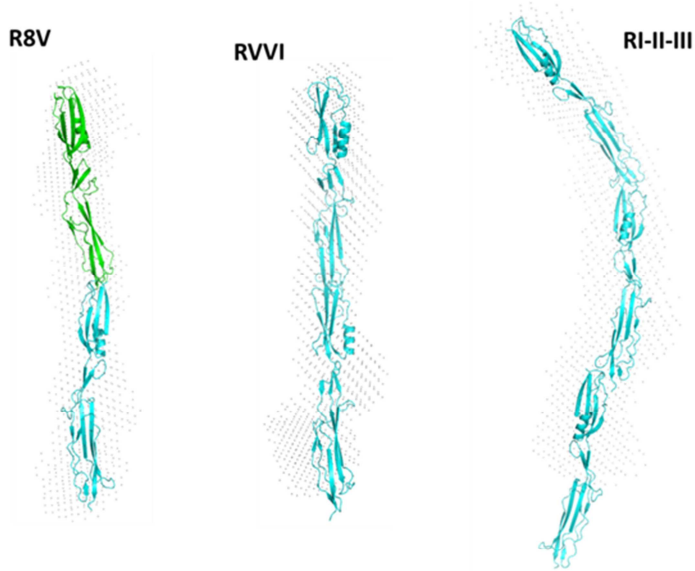

Fig 5. Multiple tandem Mub repeat solution structures

Conclusions:

1. Good agreement has been observed between crystal and solution structures of single Mub repeat proteins;
2. These individual repeats are arranged in an extended fashion in solution structures of tandem repeats;
3. In order to obtain suitable SAXS data for the triple domain (RI-II-III) protein (expected D_{max} 33.3 nm) the sample quality must be improved through use of alternative purification methods and sample buffers.

1. Putnam, C.D., et al., *X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution.* Q Rev Biophys, 2007. **40**(3): p. 191-285.
2. MacKenzie, D.A., et al., *Crystal structure of a mucus-binding protein repeat reveals an unexpected functional immunoglobulin binding activity.* J Biol Chem, 2009. **284**(47): p. 32444-53.