EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

# 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*

## Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

### Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

## Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

### Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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 regulated degradation system of the transcription factor ComK and the Phospholipase C PLC #1 system | **Experiment number**: MX-2255 |
|---|---|---|
| **Beamline**: BM29 | **Date of experiment**: from: 4th Nov. 2020 to: 5th Nov. 2020 | **Date of report**: 27/11/2020 |
| **Shifts:** 3 | **Local contact(s)**: Dr. Mark Tully | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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**Report:** Samples from the proposal number MX-2255 were measured on 4th and 5th of November 2020. There were no technical problems with the beamline during the measurements.

**Project 1:** The regulated degradation system of the transcription factor ComK:

A set of proteins encoded by late competence genes are responsible for the binding, processing and internalization of transforming DNA, and are present only in the competent subpopulation[1]. The transcription factor ComK is needed for the transcription of these genes, and is active and expressed only in the cells fated to become competent[2]. ComK also activates the transcription of genes needed for recombination and DNA repair[3].

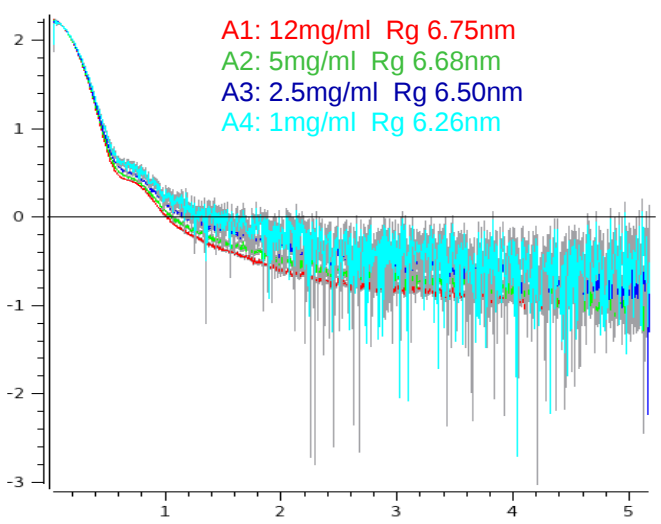
ComK control is embedded in a complex signal transduction network: In exponentially growing B. subtilis cells, ComK is constantly antagonized by the adaptor protein MecA. MecA not only targets ComK for degradation by ClpCP, but also directly inhibits ComK activity. At higher cell density in post-exponential cells, signaling via a quorum sensing system causes the stable phosphorylation of the response regulator ComA, which results in the synthesis of the small protein ComS. ComS competes with ComK for binding to MecA, which results in the release of ComK from MecA mediated inhibition and degradation. Since ComK is a positive autoregulatory transcription factor, this release results in the exponential synthesis of ComK in the subpopulation of competence-developing B. subtilis cells. The MecA-dependent retargeting of the abundant ComK protein for ClpCP degradation is essential for the escape from competence[4].

Thus, We would like to probe the structures of Comk alone, ComK_DNA complex and ComK_MecA_ClpCP complex to elucidate the structual information of this regulated proteolysis system. ComK as a transcription factor, not only induces the transcription of competence genes which encode the proteins necessary to form the DNA receptors but also interacts with MecA to the ClpCP protease for its degradation through a signal-transduction pathway, thereby regulating a key developmental process in Bacillus subtilis. Here we aim to understand how the transcription factor ComK interact with MecA and ClpCP and how this interaction

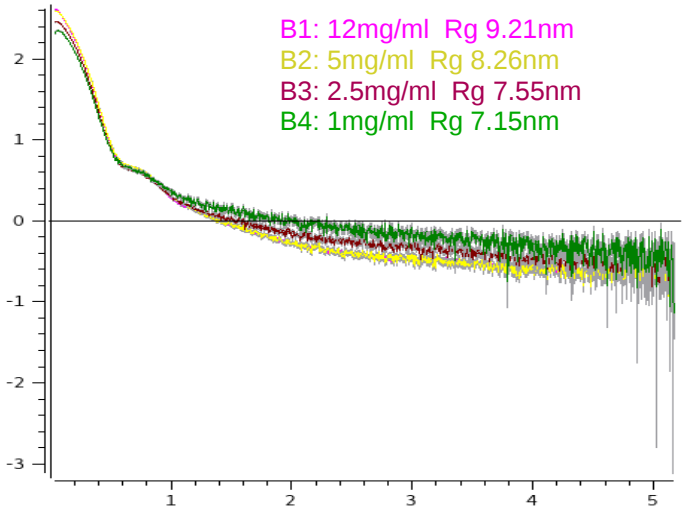
results in ComK degradation. The data obtained by SAXS would then be combined with information obtained by NMR, SANS and EPR to generate a mechanistic information of the ComK degradation system.

**Measured samples and results**

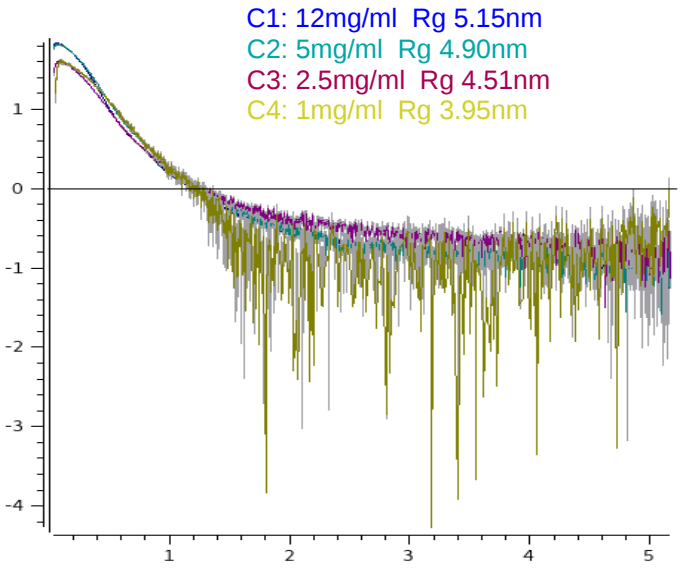
For protein complexes, we measured in total four different kinds of complex: sample A: MecA121_ClpC-DWB, sample B: ComK_MecAFL_ClpC-DWB, sample C: MecA121_ClpC-$\Delta D_2$ and sample D: ComK_MecAFL_ClpC-$\Delta D_2$. Each protein complex, we measured in batch mode with four dilution points (12mg/ml, 5mg/ml, 2.5mg/ml, 1mg/ml). We also proceeded with the sample E: ComK_DNA complex at 2 different concentrations (0.9mg/ml, 0.45mg/ml) in batch mode. All measurements were performed at room temperature to make the data compatible with NMR data.
The data was analyzed using software from the ATSAS suite. Model free structural parameters such as I(0) and Rg have been extracted from the scattering intensity curves using PRIMUS software (Fig. 1). These parameters were used to build the distance distribution function P(r) using D max that satisfy smooth tailing in the large distances area. Ab initio modeling has been performed using as a first step DAMMIF software to find 10 best scoring models and then the average of these models was used as a searching volume for DAMMIF modeling to find 10 best scoring models.(Fig. 2).



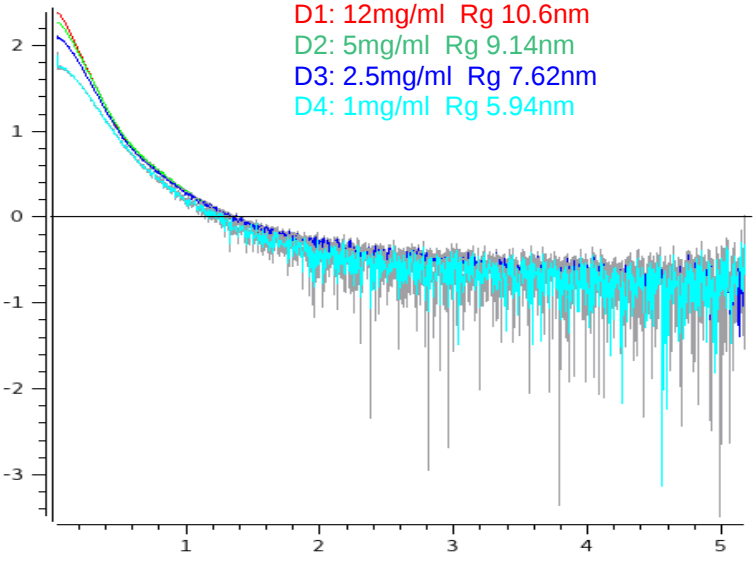

Sample A (A1, A2, A3, A4)



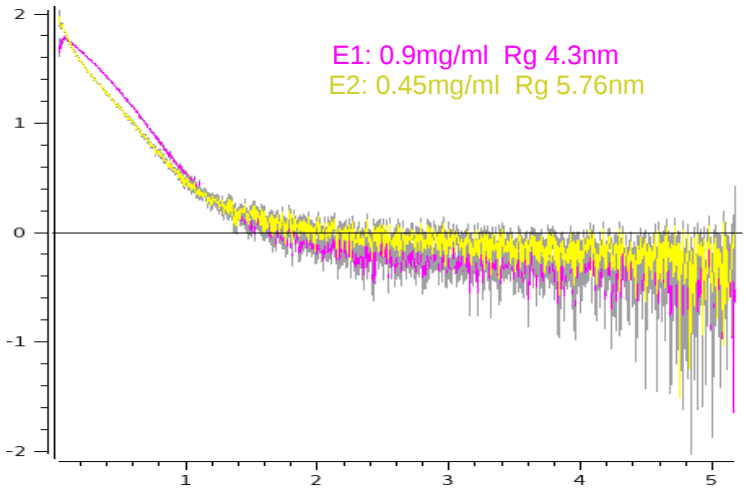

Sample B (B1, B2, B3, B4)




Sample C (C1, C2, C3, C4)



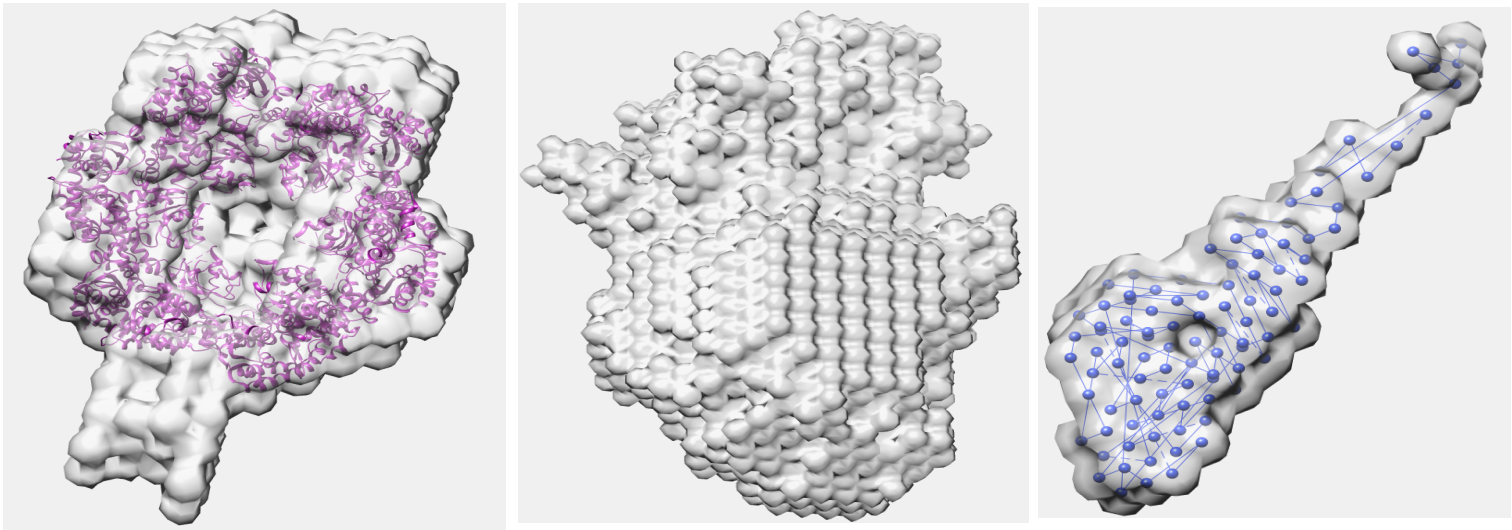

Sample D (D1, D2, D3, D4)




Sample E (E1, E2)

Figure 1: Shown here are the scattering curves of protein complexes (including dilution points) of sample A: MecA121_ClpC-DWB, sample B: ComK_MecAFL_ClpC-DWB, sample C: MecA121_ClpC-$\Delta D_2$ and sample D: ComK_MecAFL_ClpC-$\Delta D_2$. Also, sample E: ComK_DNA complex.

According to the experiment results we got above, we find out that protein complex of sample C and sample D are concentration dependent as the Rg values changed along with the sample concentration. So we still cannot build models for them based on what we get here. We need to further optimize their measuring conditions. So here we only shows the protein complex models we built for sample A, sample B and sample E (below).



Sample A (MecA121_ClpC-DWB) Sample B (ComK_MecAFL_ClpC-DWB) Sample E (ComK_DNA)

Figure 2: Shown here are the Dammif models built for sample A:MecA121_ClpC-DWB, sample B: ComK_MecAFL_ClpC-DWB, and sample E: ComK_DNA. The average of models for each sample is shown in gray and the availble PDB stucture (3pxg.pdb)is shown in magenta for comparison with sample A.