	Experiment title: BAG-LEBS-2008-2	**Experiment number**: MX-781
Beamline: ID14-2	**Date of experiment**: from: 09 february 2009 at 17:30 to: 10 february 2009 at 8:00	**Date of report**: 25/2/09
Shifts: 6	**Local contact(s)**: Dr. A. Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Pierre Roblin (PostDoc), Louis Renault (CNRS Research Assistant),
Laboratoire d'Enzymologie et Biochimie Structurales (LEBS)
Bat. 34, CNRS,
1, avenue de la Terrasse
91 198 Gif-sur-Yvette cedex, FRANCE

Report:

Characterizing Spire-stabilized actin-nuclei by SAXS and crystallographic studies to understand the multi-functional activities of Spire on actin self-assembly (1.3 shift)

BACKGROUND : The highly dynamic polymerization of monomeric globular ATP-actin (G-actin) in filament (F-actin) is essential for a variety of cellular processes, including formation of membrane protrusions required for cell migration, neurite extension, cell adhesion or endocytosis. Spire is a protein containing four consecutive actin binding motifs called WH2 domains, involved in early stage of developpmental processes and has been identified in 2005 as new actin-nucleator inducing new actin filament in vitro [1]. We have recently shown that the regulation of actin assembly by Spire is more complex and multi-functional [2,3]. Spire regulates both sequestration and nucleation on monomeric G-actin, and severing and capping on actin filaments [2].

GOALS : We target the molecular basis in WH2 domain arrangements to regulate these sequestering, nucleating, capping and severing activities. We are trying to determine SAXS and crystallographic structures of different actin-nuclei stabilized by Spire that we expect to correspond to different conformations. We use now constructs of Spire in complex with 2 or 4 actin. As the complexes with Actin-ATP or Actin-ADP regulate different activities, we target theses complexes in different actin nucleotide states.

SAXS EXPERIMENTS AND RESULTS OBTAINED FOR THE 11 HOURS ALLOCATED ON ID14-3

At the end of january 2009, we have pursued a SAXS experiment on the beamline SWING with an online HPLC system to collect SAXS data on the different species in solution separated by a size exclusion chromatography. It helped us to get rid of unsaturated species, aggregation and polymerized actin (F-actin). We have thus obtained the first suitable SAXS data without aggregations but we need to increase the resolution of the data. We have indeed a good signal to noise ratio to rather smalll momentum transfers of about 0.15 Å-1 due to the dilution in the size exclution chromatography elution. This small resolution does not allow to discriminate between several models very different in term of functions.

During the 11h allocated on ID14-3 on 9th february 2009 evening , we have try to obtain SAXS data to a maximum momentum transfers of 0.35 Å-1 for complexes of Spire with four Actin-ATP or Actin-ADP in the presence or absence of drugs inhibiting actin polymerization but not complex formation. We have try to optimize bufffer conditions, concentrations, degradations under X-ray exposure to obtain good and reproducible SAXS data at high concentrations (up to 15mg/ml) of Spire-actin complexes without aggregations/actin polymerization. We collected 10 frames of 30 seconds each at different concentrations and tested as well 10 frames of 3 seconds for degradation effects on the more sensitive complexe. As our complexes are sensitive to the temperature and the cooling system was not yet implemented on the beamline, high concentrations with actin-ATP and most of the concentrations with actin-ADP were difficult to merge. Additionnally we most likely moved slightly the capillary along the different manual injections and we faced strong problems to obtain stable buffer baselines between the different concentrations of each complexes. We thus could not merge the different concentrations to obtain full SAXS data curves. We will use these first optimizations for the next allocations.

We are really thankful to Dr. Adam Round for his strong support during the collect, especially for debugging the interface in collecting data (BsxCube closed alone with shutter remaining closed when restarting) and the network for 1.5h in the middle of the night.

REFERENCES

1. Quinlan ME, Heuser JE, Kerkhoff E, Mullins RD. (2005). Drosophila Spire is an actin nucleation factor. *Nature 433*, 382-8.
2. Bosch M, Le KH, Bugyi, Correia JJ, Renault L, Carlier MF. (2007) Analysis of the function of Spire in actin assembly and its synergy with formin and profilin. *Molecular Cell* 28(4), 555-68;
3. Renault L., Bugyi B. and Carlier MF. (2008) Spire and Cordon-bleu: multifunctional regulators of actin dynamics. *Trends in Cell Biology* 18(10):494-504. review