EUROPEAN SYNCHROTRON RADIATION FACILITY

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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

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:** Probing the Free Energy Landscape of Holliday Junctions By X-Ray Scattering Interferometry	**Experiment number**: MX-1911
Beamline:	**Date of experiment**: from: 05.03.2017 to: 06.03.2017	**Date of report**: 22.05.2017
Shifts:	**Local contact(s)**: Gabriele Giachin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Dr. Jan Lipfert, LMU Munich, Chair of Biophysics and Applied Materials

* Thomas Zettl, LMU Munich, Chair of Biophysics and Applied Materials

* Steffen Sedlak, LMU Munich, Chair of Biophysics and Applied Materials

Report:

The beam time allocated to proposal MX 1911 was used to perform solution bioSAXS measurements on the basic dynamic nucleic acid motive the Holliday junction in order to probe the conformational states under varying salt conditions. Therefore, we used an emerging synchrotron based structural technique, X-ray Scattering Interferometry (XSI), to resolve the conformational ensemble of the Holliday junction under a wide range of solution conditions and to provide precise structural and dynamical information for individual Holliday junction states. XSI measures the interference pattern between a pair of site-specifically attached gold nanocrystals and thus provides absolute distance distributions that directly report on the underlying macromolecule conformational ensemble. Holliday junctions are a fundamental nucleic acid structure motif that plays a central role in genetic recombination and other cellular processes and has wide applications in DNA nanotechnology. Table 1. gives an overview of the tested gold label pairs and Table 2. gives an overview on the various salt conditions. We performed 10 runs in 'flow' mode using the automated sample robot installed at BM29. Sample profiles were analyzed for radiation damage and matching profiles were averaged. Appropriate buffer profiles were averaged and subtracted from the sample profiles. Figure 1 shows an example of the attachment positions of the three gold label pairs XB, HB and HX and the corresponding SAXS data recorded at high salt (10 mM $MgCl^2$). As predicted from literature the junction favors the *isoII* conformation at high and intermediate salt. This can be seen by looking at the high distance peak (89.1 Å,

Fig. 1b, blue trace) which is in good agreement with the theoretical value of 93Å calculated by using a rise of 3.32 Å per base and a linker offset of 20 Å and the low distance peak for gold pair XB (52.2 Å, **Fig. 1b**, red trace). Moreover, the low distance peak for the HB gold pair centers at 57.8 Å in *isoI* whereas the XB pair centers at 52.2 Å in *isoII*. The distance distributions obtain at low salt (30 mM Tris-HCl + 10 mM Sodium Ascorbate, **Fig. 1c**) match with the model in literature that the junction does not adopt either of the stacked conformations but is forced to stay in an open conformation (**Fig. 1c**) due to electrostatic repulsion and insufficient screening by the low salt buffer. However, two important features can be seen comparing the distributions (**Fig. 1c**). Firstly, there is a small shift between the center of the HB pair distribution and the RH distribution and a difference in variance which can be caused by the different central bases between the pairs. Potentially, another reason can be the difference between the overall sequences of the stems. Secondly, the center of the HX distance distribution centers at 93.3 Å whereas a theoretical prediction using the planar model, 3.32 Å helical rise and 22 Å axial displacement for the labels sums up to 95 Å. However, this calculation is missing the gap in the center of the junction which should be close to the diameter of a double helix. For the lower bound of the estimation and using the diameter for the minor groove (12 Å) the total distance sums up to 107 Å whereas the upper bound using the diameter for the major groove (22 Å) is 117 Å. These two findings strongly disagree with the current model of a square planar conformation used in many publications and give rise to a conformation which is rather pyramidal shaped. We started to collaborate with a group doing molecular dynamic simulations on the Holliday junction refined with our distance distribution in order to obtain more structural information on the motive.

Gold label pairs:
HB
XB
HX
RH

Table 1: Measured gold label pairs for XSI experiments.

Buffer conditions:
10 mM $MgCl_2$, 30 mM Tris-HCl, 10 mM Sodium Ascorbate
150 µM $MgCl_2$, 30 mM Tris-HCl, 10 mM Sodium Ascorbate
30 mM Tris-HCl, 10 mM Sodium Ascorbate
1M NaCl, 30 mM Tris-HCl, 10 mM Sodium Ascorbate
150 mM NaCl, 30 mM Tris-HCl, 10 mM Sodium Ascorbate

Table 2: Buffer conditions used for the XSI experiments.

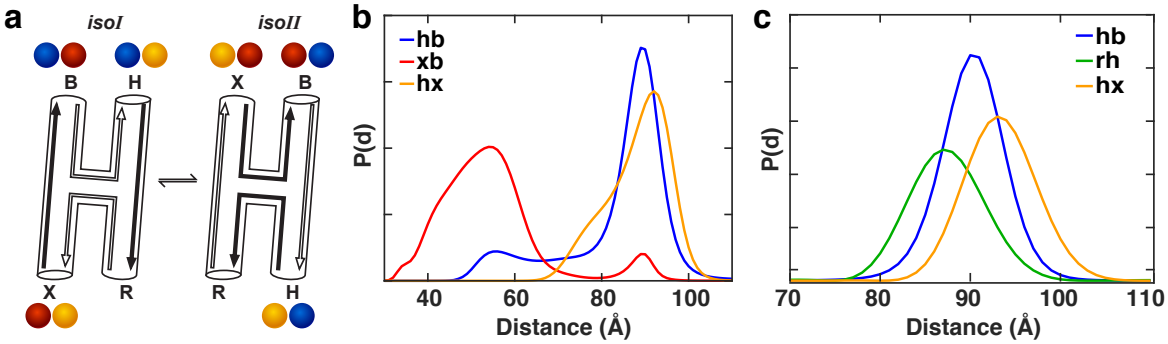

Figure 1: Sample design and traces recorded at intermediate and high salt for the Holliday junction. a) Model of the two different stacked conformations with the measured gold label pairs HB (blue), XB (red) and HX (yellow). b) Distance distribution recorded at 10 mM $MgCl^2$ + 30 mM Tris-HCl + 10 mM Sodium Ascorbate with the colors corresponding the pairs shown in a). c) Distance distribution recorded at 30 mM Tris-HCl + 10 mM Sodium Ascorbate.