ESRF	**Experiment title:** "Elucidation of conformational changes in ribozymes under the influence of Mg^{2+}, temperature and pressure by SAXS"	**Experiment number**: LS-2506
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Shifts: 9	**Local contact(s)**: Dr. Johannes Möller	*Received at ESRF:*

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Report:

Ribozymes are RNA molecules with catalytic activity, which are regarded as precursors of modern enzymes. The conformational changes, which are also highly relevant for performing catalysis, are still not well understood. This is especially the case for the conformations they adopt under extreme conditions where ribozymes might have evolved. We conducted temperature, pressure and time dependent experiments on two different model ribozymes, the hairpin and the hammerhead ribozyme, including the influence of divalent ions (Mg^{2+}), which are required to for their self-cleavage activity. The experiments were performed in order to evaluate the conformational stability of these RNA molecules under environmental conditions where ribozymes and protocells might have evolved, such as hydrothermal vent environments in the deep sea.

As an example, we report here the pressure-dependend structural changes of the hairpin ribozyme in buffer without magnesium (Figure 1) as well as time-dependend measurements at 1 bar employing different magnesium concentrations (Figure 2). The radius of gyration, R_g, decreases already at relatively low pressures such as 500 bar and 1000 bar. In the regime between 1500 an 2500 bar, the R_g remains constant, before it further decreases. The Kratky plots reflect this behavior. The maximum shifts towards higher *q*-values until 1500 bar, and remains almost constant until 2500 bar. Above this pressure, the logarithmic shape of the scattering curves reflects unfolding of the ribozyme. These findings are in excellent agreement with our FRET studies revealing that low pressures favor the active docked-state of the ribozyme, which is more compact, and thus favor the self-cleavage reaction, while higher pressures disfavor the latter by denaturation of the ribozyme. Thus, pressures of up to 1000 bar, as can be found in the deep sea, would favor the enzymatic activity of the ribozyme.

Time-dependent studies show only very slight changes in the scattering profiles for all conditions displayed here. Higher magnesium concentrations might speed up the cleavage reaction, but concominantly favor the oligomer formation of the ribozymes at the concentrations needed for this study here, making unbiased

interpretation of the data difficult. The minor changes observed might be explained by observing an ensemble of docked and undocked states being in equilibrium with each other and partial product dissociation only owing to the cleavage reaction. A more detailed analysis of the data is in progress, which will be complemented by single-molecule FRET studies to be able to disentangle the different steps involved in the cleavage reaction.

The measurements of the two model ribozymes under high hydrostatic pressure conditions in our home-built high pressure cell with strongly absorbing diamond windows was only enabled by having access to a Synchrotron source with a sufficicently high flux. The short exposure time allowed us recording high quality time dependent data. The results, complemented with kinetic single-molecule fluorescence spectroscopic data, will provide new insights into the stability and reactivity of ribozymes under extreme environmental conditions of temperature and pressure.

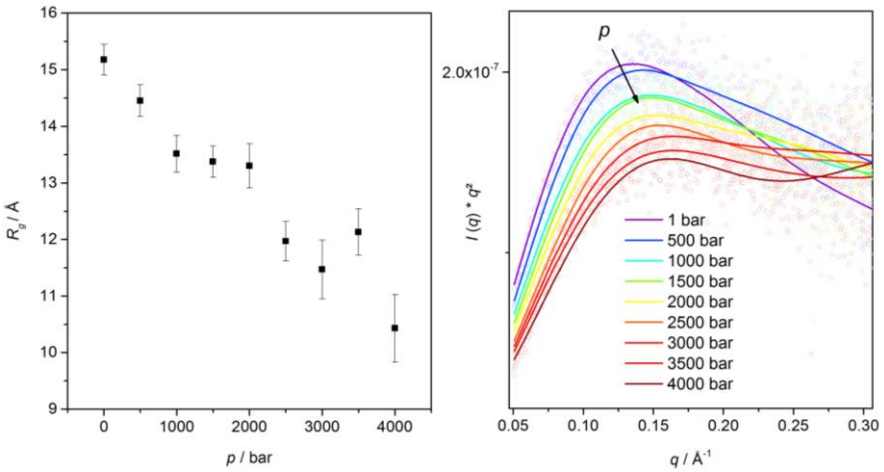

Figure 1: Pressure dependentd changes in the radius of gyration (left) and the Kratky plots (right) of the 0.5 wt% hairpin ribozyme in 50 mM Tris-HCl buffer without magnesium.

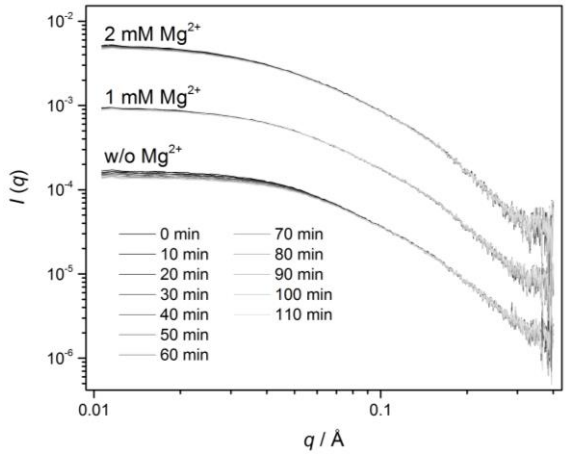

Figure 2: Time-dependent scattering profiles of 0.5 wt% hairpin ribozyme in 50 mM Tris-HCl with different magnesium concentrations.