ESRF	**Experiment title:** Structural properties of tRNA at harsh prebiotic conditions	**Experiment number**: LS-2377
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Shifts:	**Local contact(s)**: Dr. Johannes Möller	*Received at ESRF:*

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Report:

The small-angle x-ray scattering (SAXS) study was aimed at elucidating changes in shape and size of a representative tRNA molecule, $tRNA^{Phe}$, under extreme conditions of temperature and pressure. Moreover, the effect of cosolutes, such as Mg^{2+} and the osmolytes trimethylamine-N-oxide (TMAO) and urea, on the latter was studied. Complementary Fourier-transform infrared and fluorescence spectroscopic studies were carried out in our lab to reveal complementary changes in tertiary and secondary structure. The overall goal of the project was to evaluate the conformatonal stability of RNA at harsh deep-sea-like conditions, where prebiotic catalytic RNA reactions might have evolved.

The measurements of the tRNA 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 with a high flux. The short exposure times (0.1 s) at the high brilliance beamline ID02 allowed us to measure a whole pressure series (from 1 bar to 4000 bar, with step size of 200 bar) with a single sample of only 10 µL volume without detectable radiation damage of the RNA molecule. Owing to the short expose time, measurements could be carried out over a wide range of temperatures and cosolute concentrations as well. The data quality obtained was very good, allowing us to precisely calculate the pair distance distribution functions, $p(r)$, and Kratky plots, to reveal shape changes upon the addition of Mg^{2+} and the cosolutes as a function of temperature and pressure.

Our measurements revealed a surprisingly high stability of the $tRNA^{Phe}$ at pressures up to 4000 bar. As example, pressure-dependent SAXS data of $tRNA^{Phe}$ at 20°C in the absence (Figure 1) and presence (Figure 2) of Mg^{2+} ions are displayed. Even in the absence of stabilizing Mg^{2+} ions, no complete unfolding ot the $tRNA^{Phe}$, as monitored in the presence of urea and at elevated temperatures, is observed. According to the calculated $p(r)$ functions, the changes of the shape and size upon compression are very small. This is in good agreement with our FT-IR and fluorescence data, which reveal a maximum of only 15% of unpaired bases up to a pressure of 10,000 bar, indicating only small changes in the secondary and tertiary structure. The biological implications of the high stability of tRNA under high hydrostatic pressure conditions could be that it is possible that the step from an RNA world via tRNA to a protein world proceeded in the deep sea, where pressures up to the 1000 bar level are encountered. A more detailed analysis of all data including shape reconstructions is underway.

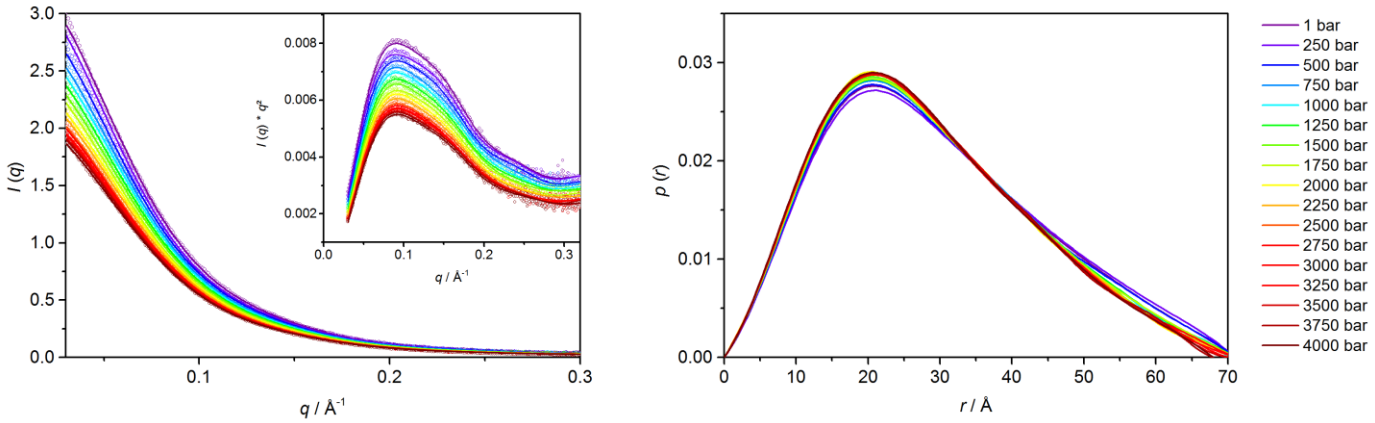

Figure 1: Pressure-dependent SAXS data of $tRNA^{Phe}$ at 20°C (3 wt% in 50 mM Tris-HCl buffer with 0.1 mM EDTA, pH 7.5). Intensity profiles at different pressures (left) with Kratky representation of the respective data (insert). Solid lines represent fits obtained by using the indirect Fourier-transformation method to calculate the pair-distance distribution functions (right).

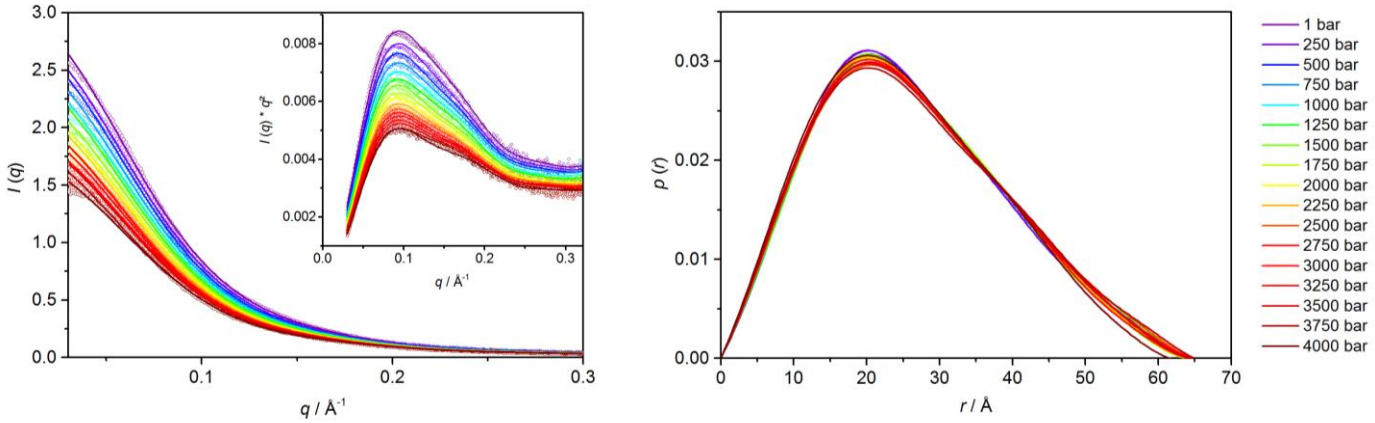

Figure 2: Pressure-dependent SAXS data of $tRNA^{Phe}$ at 20 °C (3 wt% in 50 mM Tris-HCl buffer, pH 7.5, with 0.1 mM EDTA and 15 mM $MgCl_2$). Intensity profiles at different pressures (left) with Kratky representation of the respective data (insert). Solid lines represent fits obtained by using the indirect Fourier-transformation method to calculate the pair-distance distribution functions (right).