ESRF	**Experiment title:** "Interaction of a tRNA synthetase with its tRNA under high hydrostatic pressure conditions".	**Experiment number**: LS-2405
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Shifts:	**Local contact(s)**: Dr. Johannes Möller	*Received at ESRF:*

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Report:

Aminoacylation of tRNA with its cognate amino acid by a tRNA synthetase (aaRS) is one of the key steps in translation and thus in protein synthesis. After investigating the structural changes of $tRNA^{Phe}$ using small-angle X-ray scattering under high hydrostatic pressure conditions (*p*-SAXS) at the ESRF (cf. Report LS-2377) the aim of this beam time was to analyze the conformational changes of an aaRS and their consequences upon its binding behaviour to the tRNA using *p*-SAXS. We have chosen the human mitochondrial phenylalanyl-tRNA synthetase (mtPheRS). It is the smallest known enzyme catalyzing aminoacylation, and is highly conserved in eukaryotic mitochondria. SAXS studies on $tRNA^{Phe}$, mtPheRS, and the RNA-protein complex under high pressure should help to gain a deeper understanding of the effect of pressure on the aminoacylation reaction, being of importance for understanding the physiology of organisms living in the deep sea, where pressures up to 1 kbar-level and beyond are encountered.

First, the pressure-dependent behavior of mtPheRS alone was investigated (Fig. 1). The pressure-stable buffer was 50 mM Tris-HCl, the same as used to study the stability of $^{t}RNA^{Phe}$ under pressure before (cf. LS-2377). After a slight decrease up to 1000 bar, the intensity increases drastically at low *q*-values upon further pressurization, indicating the formation of larger species, which can possibly be attributed to *in situ* multimerization / aggregation. *In situ* aggregation under pressure is a rather rare phenomenon, since pressure usually prevents aggregation. Hydrophobic interactions are weakened by pressure and water bound to a protein surface requires a lower volume. Thus, usually dissociation is favored by pressure, which makes new surfaces accessible to water. A similar behavior is shown in the presence of $^{t}RNA^{Phe}$ (1 eq.). The oscillation upcoming at higher pressures (indicated by astra (*) in Figs. 1 and 2), especially in the presence of tRNA, indicates that the larger structures formed under pressure are relatively monodisperse. A more detailed analysis of the data and modelling is in progress. To conclude, our measurements revealed a completely unexpected *in situ* multimerization of the protein complex under high pressure conditions, which might shed new light on the deteriorating effect pressure imposes on intracellular protein synthesis.

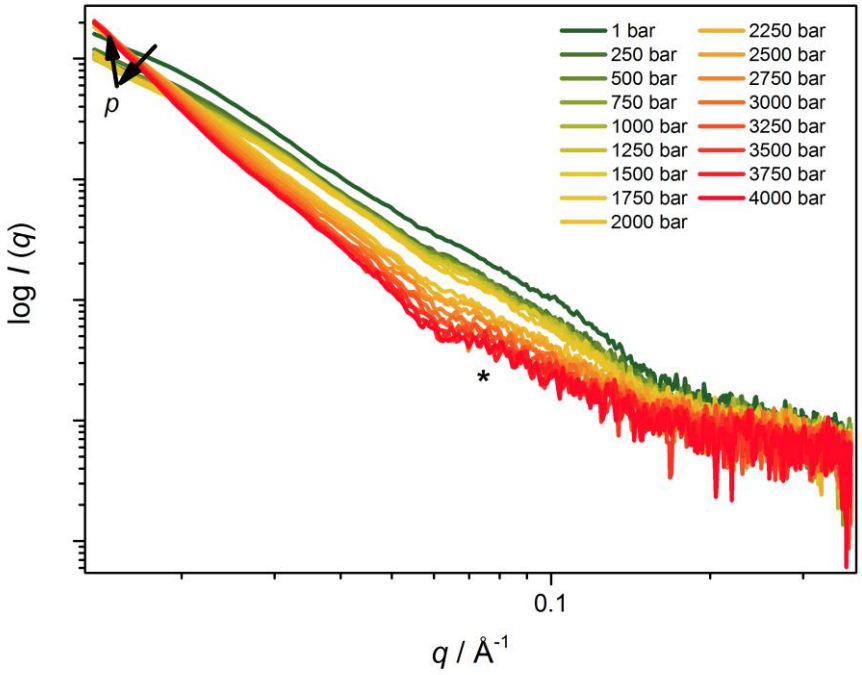

Figure 1: Pressure-dependent scattering intensity profiles of 1 wt% mtPheRS in 50 mM TrisHCl-buffer, pH 7.5.

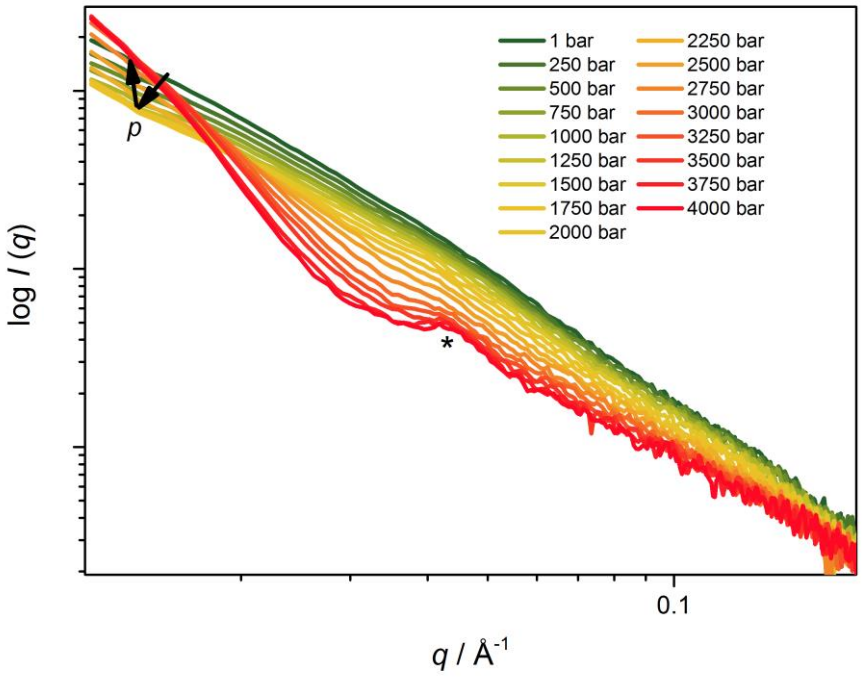

Figure 2: Pressure-dependent scattering intensity profiles of 0.5 wt% mtPheRS and 0.25 wt% (i.e. 1 eq.) $tRNA^{Phe}$ in 50 mM TrisHCl-buffer, pH 7.5.