ESRF	**Experiment title:** Crystal structure of *T. thermophilus* aminoacyl-tRNA syntbetases and their substrate complexes	**Experiment number:** LS611
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Names and affiliations of applicants (* indicates experimentalists):

*Stephen CUSACK EMBL Grenoble Outstation
*Anya YAREMCHUK
Michael Tukalo

Report:

The aim of this experiment was to continue measurements on crystals of a number of aminoacyl-tRNA synthetase/substrate complexes in particular the prolyl- and histidyl-tRNA synthetases.

(1) *T. thermophilus* prolyl-tRNA synthetase (ProRSTT). As described in an accompanying report the crystal structure of ProRSTT, a class IIa aminoacyl-tRNA synthetase, was determined to 2.43Å resolution using data measured on BM14. In addition the ProRSTT-proline complex structure was determined using data to 2.9Å resolution measured on the Swiss-Norwegian beamline and shows how substrate binding induces very significant conformational changes. We have also crystallised the complex with cognate $tRNA^{pro}$ which offers the possibility to observe how the class IIa C-terminal RNA binding domain interacts with the tRNA anti-codon stem-loop.

A tetragonal crystal form was obtained with either $tRNA^{pro}$(GGG) or $tRNA^{pro}$(GGC). The bipyramidal crystals grow to a maximum size of 400x400x400 μm^3 and of are space-group $P4_32_12$ (#96). The crystals diffract to a maximum resolution of 3.1Å when exposed to the most intense undulator radiation on ID2 but even when frozen to l00K, using 28% glycerol as cryo-protectant, there is rapid radiation damage. A complete data set has been collected to 3.5Å resolution with $tRNA^{pro}$(GGC) and to 3.6Å resolution $tRNA^{pro}$(GGG). Details of the data collections on ID2 are given below.

	ProRS/tRNA^{pro}(GGG)	ProRS/tRNA^{pro}(GGC)
Beamline	ID2	ID2
Detector	300mm Mar	300mm Mar
Wavelength	0.99Å	0.99 Å
Exposure/image	36s/0.8 deg	70s/1deg
Space-group	$P4_32_12$	$P4_32_12$
Cell dimensions	a=b=142.7Å c= 230.9Å	a=b=143.1Å c= 228.6Å
# crystals	2	2
Resolution	15-3.6Å	13-3.5Å
Total reflections	67322	65451
Unique reflections	25930	26892
Average redundancy	2.7	2.4
Completeness	90.0	97.6
R-merge (highest bin)	0.101 (0.288)	0.094 (0.265)

The tRNA complex structure was solved by molecular replacement (programme AMORE) using the refined model of the ProRSTT dimer a search model and the resolution range 4.5-9A. The correct solution was the 15th highest peak in the rotation search (correlation = 0.11) but highest in the translation search (correlation=O.338 in space-group $P4_32_12$), the second highest translation solution being in the wrong space-group, $P4_12_12$ (correlation=0.211).

The tRNA complex structure had to be refined carefully because of the limited resolution (3.5Å), the free R-factor being an indispensable guide. The molecular replacement solution for the protein dimer was firstly improved by rigid-body refinement of domains. The free-correlation rose from 0.55 to 0.66 due to significant re-orientations of the anti-codon binding domains. A map then showed clear difference density for a single tRNA molecule bound to the synthetase dimer, there being no room in the crystal for a second tRNA. A model was built in stages for most of the tRNA except the acceptor stem (for which density is absent) making use of fragments of tRNA^{phe} and tRNA^{asp}. The density was good enough to discriminate Py:P from P:Py base-pairs in the anticodon stem, assignments which were confirmed when the sequences of *the T. thermophilus* tRNA^{pro} isoacceptors became known. Refinement was continued by a combination of manual adjustment and positional refinement while maintaining as far as possible tight non-crystallographic and geometrical restraints. The current R-free (R-factor) is 0.364 (0.344).

Despite the modest resolution, the mode of binding of the anti-codon stem-loop to the class IIa anti-codon binding domain is clear, the main interacting surface being the P-sheet and an a-helix which aproaches the anti-codon loop from the major groove side. The distortion introduced into the anti-codon loop is reminiscent of that occuring upon cognate tRNA binding to class IIb synthetases, with bases G-35, G-36 and G-37 splayed out, but differs in that base C-34 remains stacked under base U-33 and bases U-32 and A-38 form a base-pair. In common with the class IIa synthetases GlyRS and ThrRS, ProRS has to recognise cognate tRNAs in which the second and third anti-codon nucleotides uniquely define the amino acid, whereas the first (wobble) base is variable (i.e. NGG for tRNA^{pro} isoacceptors, NCC for tRNA^{gly} and NGU for tRNA^{thr}). The structure explains how this functional requirement is met by clearly showing specific recognition of bases G-35 and G-36 by side-chains from the synthetase, whereas base C-34 makes no interactions and is stacked under base U-33 in a pocket which could accommodate any nucleotide. More precise details of the interaction of tRNA^{pro} with ProRS awaits a higher resolution structure of the complex.