X-ray crystallography of *Pyrococcus horikoshii* tRNA-guanine transglicosylase

[1,2]Osamu Nureki (0003440), [1]Shuya Fukai (0003519), [1]Ryu-ichiro Ishitani (0004145)

[1]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; [2]Cellular Signaling Lab., RIKEN

Archae bacteria in common have a characteristic post-transcriptionally-modified base, archaeosine, at the position of 15 in the D-loop of all the tRNAs. tRNA guanine transglycosylase (TGT) catalyzes the base exchange reaction between guanosine and preQ0, a preproduct of the archaeosine. TGT widely exists from prokaryote to eukaryote. Prokaryptic and eukaryotic TGTs post-transcriptionally modify the anticodon loop of specific tRNAs, while archaeal TGT modify the D-loop of all the tRNA. Archaeal enzyme has 300 a.a. extra domain as compared to prokaryptic and eukaryotic enzymes, which is suggested to function in the recognition of the tRNA L-shape.

To elucidate how archaeal TGT recognizes tRNA and how it catalyzes the base exchange reaction, we crystallized TGT from hyperthermophile, *Pyrococcus horikoshii*. The crystals belong to a tetragonal space group $P4i2_12$ with unit-cell parameters $a = b = 100.687$ Å, $c = 365.028$ Å. The crystals diffract X-ray upto 3.0 Å resolution in the BL41XU. The Rmerge and completeness were 0.108 (0.233) and 99.7% (49.8%), respectively (the values at the outer shell are shown in parentheless). Since the diffraction is weak, data collection at BL41XU is quite effective. In this beamtime, we collected MAD data with 4 wave lenghths, and successfully picked up 20 out of 32 Se sites by direct methods. Phasing by MAD is now in progress.

X-ray crystallography of *archaeal* tRNA methyltransferase (SpoU)

[1,2]Osamu Nureki (0003440), [1]Shuya Fukai (0003519), [1]Ryu-ichiro Ishitani (0004145)

[1]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo; [2]Cellular Signaling Lab., RIKEN

All the eubacterial and archaeal tRNA have methylation at position 18. The Gm18 methylation is catalyzed by Gm18 methlase or SpoU. The post-transcriptional modification is thought to be responsible for the structural stability of L-shaped tRNA at the core involving many tertiary base pairs. SpoU methyltransferase gives rise to a SpoU family, which is characterized by three conservative motifs. Previously, we determined crystal stricture of RrmA, a function-unknown SpoU family methyltransferase, which has a clear and deep trefoil knot. The two of the three conservative motifs are on the knotting loop and knotted C-terminus, which suggests that the active site is constructed on the very knot. To elucidate the structure•function relationship of the knotted SpoU methyltransferase, we crystallized archaeal SpoU enzyme (P4(1)2(1)2; $a=b=43.8$Å, $c=194.8$Å). The crystals diffracted X-ray beyond 2.2 Å resolution. In this beamtime, we collected the MAd data of the SeMet derivative crystals. However, the occupancy of Se is too low to determined the phase because of some failure of the sample preparation. Incooporation of SeMet into the SpoU enzyme by cell-free protein synthesis system is now in progress.