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X-ray crystallography of the complex of SeqA, a negative regulator of replication, and a hemi-methylated DNA

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Escherichia coli chromosomal DNA has approximately 1900 GATC sequences, which are methylated by Dam methyltransferase. In the DNA replication step, until the synthesizing dauter strand is methylated by Dam methyltransferase, the chromosomal DNA is kept in the hemimethylated state. *Escherichia coli* SeqA protein binds to the hemimethylated sequence in the *ori* region to restrict one cycle of replication per one cycle of cell division. To elucidate the mechanism how SeqA strictly recognizes the hemimethylated DNA, we crystallized the complex of SeqA and 10 bp or 16 bp DNA including hemimethylated adenosine. Crystals of the complex with 16 bp hemimethylated DNA belong to the mnoclinic spacegroup *C*2 (a=84.19 Å, b =67.98 Å, c = 88.21 Å, β =110.6°), and diffract X-ray up to 2.65 Å resolution. The Rmerge and the completeness were 0.038 (0.114) and 91.1% (57.1%), respectively (the values at the outer shell are shown in parentheless). However, the Wilson plot implies that the crystals are twined, and we could not determined the phase by Se-MAD phasing. On the other hand, crystals of the complex with 10 bp DNA belong to the hexagonal spacegroup *P*622 (a=b =152.96 Å, c = 119.1 Å), and diffracts X-ray beyond 3.2 Å resolution. By MAD method using SeMet derivative, we determined the phase and building the atomic model is now in progress. The structure reveals that two invariant residues play crucial role in the recognition of hemi-methylated double-stranded DNA.

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X-ray crystallography of the complex of epidermal growth factor (EGF) and EGF receptor

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EGF receptor is a 170-kDa membrane glycoprotein, which functions as a tyrosine kinase. Upon binding with growth factors such as EGF, EGF receptor activates a series of a kinase cascade to regulate the transcription of numbers of genes, which leads to the growth and differentiation of cells. We successfully crystallized the activated form of EGF receptor complexed with EGF (space group $P3_221$ with unit-cell parameters of a = b = 219.3 Å, c = 110.9 Å). In this beamtime, we collected the MAD data of the SeMet derivative crystals. We picked up 13 Se sites with the program SnB, and calculated the phase with the program MLPHARE. Density modification with the program RESOLVE significantly improved the phase, and we could make a mask by ourselves. Using the mask, density modification based on solvent flattening and NCS averaging with the program DM further improved the phase upto 3.8Å resolution. In the current map, we construct an polualanine model. Model improvement and molecular replacement against the native data upto 3.2Å resolution is now in progress.