# Crystallographic Study of G-CSF Receptor Complexed with G-CSF

**Masaharu ARITOMI (0003662), Naoki KUNISHIMA (0003650), *Kosuke MORIKAWA (0003651)**

Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

G-CSF (granulocyte colony-stimulating factor) is a cytokine which regulate the growth of premature leukocytes and their differentiation to neutrophiles. G-CSF transduce a signal from the outside to the inside of the cell through a specific binding to a membrane protein, GCSF receptor. It is known that two extracellular domains of the receptor, BN and BC, are essential for GCSF binding. In order to clarify the molecular recognition of the GCSF receptor and GCSF, we tried the X-ray crystallographic analysis of the complex of the two domains of the receptor (BN and BC; 25 kDa) and G-CSF (20 kDa). Using this beamtime, we collected the diffraction data of the native crystals to 2.8 Å resolution and the two heavy atom derivatives.

The complex of the G-CSF receptor and G-CSF was prepared by mixing equimolar of both proteins and subsequent purification using a gel filtration. A selenomethionyl GCSF was prepared using a conventional *E. coli.* overexpression system with a minimum culture medium containing selenomethionine. The native crystals were obtained by the hanging-drop vapor diffusion method using ammonium sulfate as a precipitant. The crystals belonged to the space group $I4_122$ with a cell dimension of a=b= 125.4 Å, c= 372.8 Å. The two complex molecules were present with a pseudo non-crystallographic dyad axis in an asymmetric unit. The selenomethionine derivative crystals were obtained by the same procedure as the native crystals except for using selenomethionyl GCSF as a part of the complex. The mercury derivative crystals were prepared by soaking the native crystals in a mother liquor containing 1 mM EMTS for 8 hours. The data collection was performed at 100 K using the R-AXIS IV detector. A typical size of the crystals used for data collection was 50 μm * 50 μm * 400 μm. The raw data were digitized and marged using the programs *DENZO* and *SCALEPACK*. The initial phase was estimated according to the method of multiple isomorphous replacement (MIR), through a procedure comprising a determination of heavy atom sites with the program *RSPS* and a subsequent refinement with the program *MLPHARE*. The overall figure-of-merit with the all reflection between the resolution of 15 Å and 3 Å was 0.299. The MIR phase was then improved by several density modification techniques with the program *DM*; which contained solvent flattening, histogram matching, and 2-fold molecular averaging. Using the density-modified map, the model building was performed with the program *QUANTA*, where the published atomic coordinates of GCSF and the BC domain of the GCSF receptor were used as a reference. The calculated phase from the partial structure comprising a complete model of GCSF and a polyalanine model of the GCSF receptor was combined with the MIR phase using the program *SIGMAA*. Orientation matrices and molecular envelopes used for the 2-fold averaging were further refined on the basis of the partial structure. The final phase-combined and density-modified map had a sufficient quality for unambiguous assignment of all the side chains of the GCSF receptor. The crystallographic refinement at 2.8 Å resolution is now in progress.