

# High resolution crystal structure analyses of the complexes of CD28 family molecules and signaling proteins

Nobutaka NUMOTO<sup>1,\*</sup>

<sup>1</sup> Medical Research Institute, Tokyo Medical and Dental University (TMDU),  
1-5-45 Yushima Bunkyo-ku, Tokyo, 113-8510, Japan

## 1 Introduction

T cell regulation plays an important role in the human immune system and requires two independent signals. One is an antigen-specific signal via the T cell receptor (TCR) upon binding to the major histocompatibility complex (MHC), and the other is an antigen non-specific signal via the CD28 family molecule upon binding to the B7 molecule group. The intracellular region of CD28 consists of about 40 residues and transmits a signal into the cell by recruiting other molecules. The tyrosine residue in the amino acid sequence of YMNM in the intracellular region of CD28 is phosphorylated by the Tyr kinase, and the phosphorylated Tyr (pY) containing pYMNM sequence is recognized by the signaling molecules such as Growth factor receptor-bound protein 2 (Grb2). The SH2 domain of Grb2 specifically binds to the consensus sequence of pYXNX, which matches the pYMNM sequence in the CD28. Grb2 exhibits a monomer - dimer equilibrium that is thought to regulate normal and abnormal functions. We have previously reported the crystal structure of Grb2 SH2 domain in complex with the CD28 phosphopeptide, SDpYMNMTP, and elucidated that the recognition mechanism of pY and the Asn residue in the consensus sequence.

In this study, we have determined the crystal structures of the SH2 domain of Grb2 (Grb2-SH2) without the phosphopeptide at 1.15 Å resolution and a domain swapped dimer of Grb2-SH2 at 2.0 Å resolution [1]. Obtained structures reveal that Trp121 is the key residue at the hinge region of C-terminal helix swapping, and the dihedral angle in the main-chain of Trp121 is energetically preferred in the domain swapped dimer structure than that in the monomer structure.

## 2 Experiment

Grb2-SH2 was overexpressed in *Escherichia coli* and purified as described previously. Crystallization was performed by the hanging-drop vapor-diffusion method at 20°C. Prior to data collection, the crystals were soaked in cryo-protectant solutions containing 20% (v/v) glycerol along with their respective reservoir buffers and flash-frozen using nitrogen gas stream at 95 K.

X-ray diffraction experiments were performed at the beamlines BL-1A and NW12A at PF and PF-AR, KEK. All data were processed and scaled using XDS [2]. The initial phases were determined by the molecular replacement method using the program PHASER [3]. Several cycles of

manual model rebuilding by using COOT [4] and refinement by using PHENIX [5] were performed.

## 3 Results and Discussion

Crystals of the monomeric Grb2-SH2 were obtained in the buffer containing 0.1 M sodium chloride, 0.1 M HEPES (pH 7.5), 1.6 M ammonium sulfate. The structure was determined at 1.15 Å resolution, which is the highest resolution analysis among over 30 structures of the peptide bound/unbound Grb2-SH2.

Crystals of the domain-swapped dimer of Grb2-SH2 were unexpectedly obtained from the monomer fraction of the purified protein, using the reservoir solution containing 0.1 M MES (pH 6.5), 12% (w/v) polyethylene glycol 20,000. The structure was determined at 2.00 Å resolution.

Trp121 was the hinge residue of the extended, domain swapped form compared to the monomeric globular structure (Fig. 1). The dihedral angles in the main-chain of Trp121 in the monomer structure,  $\phi = -133^\circ$  and  $\psi = -82^\circ$ , are energetically disadvantageous, while those in the dimer structure,  $\phi = -153^\circ$  and  $\psi = 147^\circ$ , are energetically preferred. The peptide plane flipping at this residue seemed to cause the large conformational change for the C-terminal extended form.

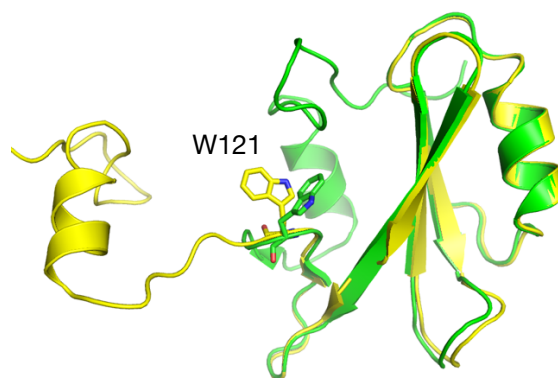


Fig. 1: Structures of monomeric (green) and domain-swapped (yellow, one protomer) form of Grb2-SH2.

## References

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\* numoto.str@mri.tmd.ac.jp