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# Crystal structure analysis of ligand bound form of autoimmune disease related receptor CD72

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## 1 Introduction

B cells play a key role in the immune system by making antibodies. CD72 is an inhibitory co-receptor that negatively regulates B cell antigen receptor (BCR) signaling. The ligand-binding domain of CD72 at the extracellular region belongs to the C-type lectin-like domain (CTLD) superfamily. We have demonstrated that it recognizes the nuclear autoantigen Sm/RNP composed of proteins and RNA, and suppresses autoimmune diseases such as systemic lupus erythematosus [1]. The crystal structure of the ligand-binding domain of mouse CD72$^{a}$, a lupus-resistant allele, has been determined at 1.2 Å resolution. Electrostatic potential analysis of the molecular surface of CD72$^{a}$-CTLD suggest that positively charged patch at the putative ligand-binding site will be suitable for binding Sm/RNP that contain negatively charged region derived from phosphate back bone of RNA.

It has been also reported that mouse CD72$^{c}$, a lupus-susceptible allele, has several amino acid substitutions on CTLD, and CD72$^{c}$-CLTD shows a reduced affinity to Sm/RNP. Since many of the basic amino acids at the putative ligand binding site were replaced with other acidic or neutral amino acids, it was suggested that loss of the positively charged patch will cause the reduced affinity to Sm/RNP. However, the detailed molecular mechanism of the reduced affinity still unclear because there has been no report of the structure of CD72$^{c}$-CLTD. To obtain the structural basis of the interaction mode between CD72$^{c}$-CLTD and Sm/RNP, we have initiated the crystallographic study of CD72$^{c}$-CLTD.

## 2 Experiment

Recombinant mouse CD72$^{c}$-CTLD was overexpressed using BL21(DE3) cells and refolded via on-column refolding method using cOmplete His-Tag Purification Resin (Merck). Proteins were treated with thrombin protease (GE Healthcare) overnight to remove the hexa-histidine tag. The cleaved protein was applied onto a Superdex 200 prep grade column (GE Healthcare). The fraction of the monomer CD72$^{c}$-CTLD was concentrated to 5–10 mg/ml. Crystallization conditions were screened using commercially available screening kits (Hampton Research) with the sitting-drop vapor diffusion method. The obtained crystals were large enough for X-ray diffraction experiments of about 200 μm cubic, but clusters of hundreds or thousands of microcrystals despite many efforts to optimize the crystallization conditions (Fig. 1).

Prior to data collection, the crystals were soaked into the solution containing 20% glycerol as a cryoprotectant, and flash frozen using liquid nitrogen. X-ray diffraction experiments were performed at beameline 17A at PF, KEK. The wavelength, transmittance, beam size, exposure time and oscillation width were 0.9800 Å, 50%, 40 × 20 μm$^2$, 0.5 sec and 0.5 degree, respectively. The diffraction images were collected using Eiger X16M (Dectris) with camera distance of 253.0 mm.

## 3 Results and Discussion

The crystals diffracted up to 8 Å resolution. Obtained diffraction images strongly indicated that the crystals were crystal cluster because the diffraction pattern appeared to be strong concentric rings (Fig. 2). However, the diffraction rings were observed at even low resolution such as 20-30 Å. Therefore, it was strongly suggested that the diffraction was from protein crystals. Further crystallization condition optimization can promise to succeed in determining the crystal structure of CD72$^{c}$-CLTD.

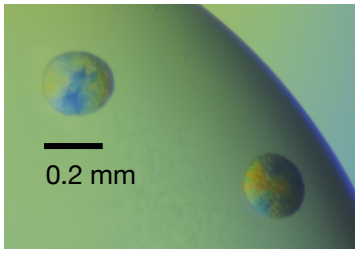


Fig. 1: Crystal clusters of CD72$^{c}$-CLTD.

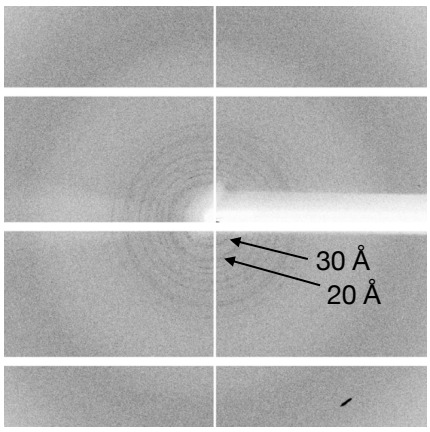


Fig. 2: Diffraction image of CD72$^{c}$-CLTD.

## References

[1] C. Akatsu *et al.*, *J. Exp. Med.*, **213**, 2691 (2016).

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