

Diffraction Analysis of a CD46 4-Domain Crystal Form

M. Larvie, A. Kumar, and T. Stehle (Massachusetts General Hospital/Harvard Medical School)

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Beamline(s): X12C, X25

Introduction: CD46, also known as membrane cofactor protein, is the cellular receptor for measles virus. The protein ectodomain consists of four concatenated "short consensus repeats" or SCR domains. Measles virus binds to domains SCR1 and SCR2, the natural ligands of CD46 (complement proteins C3b and C4b) bind to domains SCR3 and SCR4. We have recently determined the crystal structure of the SCR1-SCR2 fragment [1,2]. The purpose of this investigation was to (1) screen crystals of the complete CD46 ectodomain in order to identify the crystal most suitable for data collection, and (2) to collect a complete native data set off of this crystal form.

Methods and Materials: The CD46 ectodomain protein was expressed in CHO cells and purified via affinity chromatography and gel filtration. Crystals were grown from sodium citrate. The crystals diffract to about 13 Å and belong to space group P432 with $a=458$ Å. They have most likely 24 molecules in the asymmetric unit.

Results and Conclusions: We were able to screen about 30 crystals at X12C, and we identified the crystal that diffracted to the highest resolution. The crystals are quite variable, and because they diffract so poorly we cannot easily do screening on our home X-ray source. We used the "best" crystal to collect a complete (97% completeness) native data set on X25. The data extend to about 14 Å. We are presently engaged in trying to solve the structure of the four-domain fragment at low resolution using these data. Such a low-resolution structure should shed light on the overall conformation of the molecule, and also provide information about the association state of CD46, which remains unclear [1]. We are also engaged in producing another crystal form of this fragment that diffracts to higher resolution.

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References: [1] Casasnovas, J., Larvie, M. and Stehle, T. "Crystal structure of two CD46 domains reveals an extended measles virus binding surface". *The EMBO Journal* 18:2911-2922 (1999). [2] Manchester, M., Nanche, D. & Stehle, T. "CD46 as the measles virus receptor: form follows function." *Virology* 274:5-10 (2000).