	Experiment title: *Crystallography of deoxy-gramicidin S and avian reovirus sigmaC protein*	**Experiment number**: MX-570
Beamline: ID23-1	**Date of experiment**: from: 04-SEP-2006 at 16:00 to: 05-SEP-2006 at 8:00	**Date of report**: 15/01/2007
Shifts: 2	**Local contact(s)**: Dr. Gordon Leonard	*Received at ESRF:*

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Report:

Gramicidins are non-ribosomally synthesized peptides. Gramicidin S (GramS), produced by Bacillus brevis, is a cationic cyclic deca-peptide antibiotic with twofold symmetry: cyclo-[Val-Orn-Leu-^{D}Phe-Pro]$_2$, in Orn stands for the amino acid ornithine. It has antimicrobial activity against bacteria and fungi. Gijs Grotenbreg and Mark Overhand of the Bio-Organic Synthesis group, Leiden University have synthesised native GramS and various analogues in order to study the influence of sugar amino acid residues on the peptide structure and antibiotic activity. Two analogues have already been crystallised and their structure solved (Grotenbreg et al., 2004; 2006). In the first of these structures, six GramS analogue molecules form a beta-barrel with a hydrophobic exterior and hydrophilic interior, indicating GramS may function as a membrane channel. In the second analogue, with improved antibiotic activity, peptide molecules form dimers related by a binary axis perpendicular to the pore channel. Six dimers (twelve molecules) give rise to one complete helical turn in a putative pore. For an F-phenyl-derivative of GramS synthesised in the group of Anne Ulrich (Salgado et al., 2001), it was shown by 19F-solid state NMR studies that gramicidin S tilts at high concentration. This would bring the charges into the middle of the bilayer and it was proposed that oligomerisation into a beta-barrel is a way of avoiding charges in the middle of bilayer. We have now crystallised another analogue, deoxy-GramS. Rotating anode data collected to 1.2 Å resolution did not allow structure solution, we hoped higher resolution data (to 1.0-0.8 Å) will allow us to solve the structure. **However, the data collected at ID23-1 and processed afterwards did not lead to a dataset of higher resolution, suggesting the limit of diffraction is due to the crystals and cannot be improved by irradiating with higher flux x-ray beams.**

Avian reovirus causes serious disease in birds, leading to important losses in the poultry industry. Ten structural proteins have been identified in strain S1133 (Martinez-Costas et al., 1997): core components lambdaA, lambdaB, lambdaC, muA and sigmaA and external proteins muB, muBC, muBN, sigmaB and sigmaC. Four non-structural proteins are also encoded: p10, p17, sigmaNS and muNS. The avian reovirus

cell-attachment sigmaC protein is a parallel homo-trimer (Grande et al., 2002), but the receptor to which it binds is unknown. We have previously solved a C-terminal fragment of sigmaC (amino acids 157-326; van Raaij et al., 2005; Guardado-Calvo et al., 2005) and have now obtained crystals of a larger fragment (amino acids 117-326). Using rotating anode data to 2.8 Å resolution, we have shown the additional domain forms a triple alpha-helical coiled coil, but some regions were poorly resolved, especially a putative zinc-binding site between the alpha-helical and C-terminal domains. Higher resolution data should allow us to finalise the structure. **Due to an unfortunate incident with the beam backstop just before the second shift and consequent loss of beamtime, it was not possible to measure data on these crystals.**