ESRF	**Experiment title:** Vibrio cholerae neuraminidase Newcastle disease hemagglutinin-neuraminidase (HN)	**Experiment number**: LS-2087
Beamline: ID14-2	**Date of experiment**: from: 26/11/01 to: 27/11/01	**Date of report**:
Shifts: 3	**Local contact(s)**: Joanne McCarthy	*Received at ESRF:*

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Report:

***Vibrio cholerae* secretes a neuraminidase (VCNA), or sialidase, which removes sialic acid from higher order gangliosides to produce GM1, the binding site for cholera toxin. The enzyme therefore plays a significant role in the pathogenesis of the bacterium. We had previously determined (in 1994) the structure of the 83kDa VCNA to 2.4Å which showed a catalytic sialidase domain flanked by two lectin-like domains. In this study, we were unable to show inhibitor binding nor were we able to determine the carbohydrates recognised by the lectin domains.**

In recent experiments we were able to co-crystallise VCNA with inhibitors and a cocktail of mono and disaccharides. Cryo conditions were also discovered, as the orginal data had been collected at room temperature.

5 datasets were collected, all of which gave Rsyms of ~ 7%:

VCNA + DANA	**30s per frame**	**2.7Å**
VCNA + DANA – new crystal	**10s per frame**	**1.8Å**
VCNA + DANA- same as above, translated	**10s per frame**	**1.8Å**
VCNA + monosaccharides	**10s per frame**	**1.8Å**
VCNA + monosaccharides – new crystal	**10s per frame**	**1.8Å**
VCNA + disaccharides	**10s per frame**	**3.0Å**

The VCNA + DANA data has provided an unexpected result: as well as clear density for the inhibitor in the active site, there is clear density for sialic acid in one of the lectin domains. Mass spec of the DANA solution used for the co-crystallisation revealed a small percentage of sialic acid as a contaminant. This exciting result shows that VCNA carries a lectin domain to target it to the very receptors it is seeking to hydrolyse. No sialic acid was observed in the second lectin domain, and the nature of carbohydrate recognised by the second domain remains a mystery. The mode of sialic acid recognition is quite different to other proteins that recognise this sugar, e.g. influenza virus hemagglutinin, sialoahdesin, wheat germ agglutinin.

Several attempts were made to collect datasets from NDV HN crystals soaked in a non-hydrolyseable substrate analogue. All crystals suffered from freezing, and despite multiple attempts, only one dataset was collected to 2.5Å resolution – 240 images of 0.5 degrees, 10s per image. Unfortunately, no ligand density was observed in the difference maps.