| ESRF | **Experiment title:** The structural basis for infectivity-neutralization escape of influenza virus mutants. | **Experiment number:** LS 523 |
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| **Beamline:** BL4/ID2 | **Date of experiment:** from: 2 October 1996 to: 4 October | **Date of report:** 10 Feb. 1997 |
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**Report:**

Experiment LS 523 was undertaken to determine the structures of complexes between point mutants of X31 strain influenza virus hemagglutinin (X31 HA) and HC19 antibody. Whereas HC19 recognises with high affinity (Kd = 1 nM) X31 hemagglutinin, it recognises with a lower affinity (Kd = 5 nM) HA with the single mutation 155 Thr->Ile and with a drastically lowered affinity (Kd = 4 μM) HA with mutation 13 1 Thr->Ile. Also the two mutations take place at very close locations in the interface of the HA X31 - HC19 complex (ref. 1), their consequences are very different and we have undertaken to determine what structural features are responsible for this difference.

X31 HA - HC19 complex structure was determined previously (ref. 1 ) with data collected in part at station ID2/BL4. However, because of the occurrence of a very long unit cell axis (515 A), data collection was limited to 3.25 Å resolution, although the diffraction limit extended to approx. 2.5 Å resolution. On 2-4 October 1996, we used the available instrumentation at ID2/BL4 to offset the detector from the beam and to collect data of the wild-type complex to the highest resolution: this was unsuccessful, because of the very quick decay of crystal diffraction at high resolution (even at -10°C). Moreover, cryo-cooling was not feasible, because, in our hands, it always resulted in a slight and redhibitory increase of crystal mosaic spread.

As for the two point mutant complexes, data collection for the 1 3 1 mutant was performed in September 1996 at DESY (Hamburg, Germany). During experiment LS 523, five crystals of the 155 mutant were used to collect a complete dataset to 3.25 Å resolution. Data were reduced with Denzo and Scalepack (ref. 2) to yield a 97% complete dataset with a Rsym on intensities of 0.107. The dataset proved highly isomorphous to wild-type complex data (Riso=11.5%): this allowed us to compute difference-fourier maps and to locate the mutation and its consequences in the complex interface. A thorough interpretation of relationships between affinity figures and structures is now in progress.

## REFERENCES

1 - Bizebard T., Gigant B., Rigolet P., Rasmussen B., Diat O., Bosecke P., Wharton S., Skehel J.J. & Knossow M., Nature, 376, 92 (1995).

2 - Minor W. & Otwinowski Z., personal communication.