



Experiment title:
MAD phasing of hemagglutinin-neuraminidase (HN) from Newcastle disease virus.

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Local contact(s):
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Report:

Crystals of hemagglutinin-neuraminidase (HN) from Newcastle disease virus suffer from severe non-isomorphism. Native data beyond 2.0Å have been collected, but after approximately 140 heavy atom derivative datasets, no useful phases have been obtained. We discovered, however, that [Pt₂I₂(en)₂](NO₃)₂ (PIP) gave a single, strong peak on an anomalous Patterson derived from data collected in-house.

A MAD experiment was therefore carried out at ESRF, with three datasets being collected to 3.1Å around the Pt absorption edge within 24 hours - unfortunately the first 24 hours were wasted looking for a good quality crystal. The crystals belong to space group P2₁2₁2₁ with a=71.7Å, b=80.2Å, c=198.5Å. One PIP per asymmetric unit of 100kDa theoretically gives a sufficient signal for MAD phasing, assuming full occupancy and high quality data. It is debatable whether our data were good enough. PIP was present, but anomalous Pattersons did not show as strong a peak as expected.

Dataset	Observed	Unique	Overall R ₁	Top shell R ₁	Completeness	φ	φ'
λ1	191062	23321	5.6	28.0	93.4	-16.7	14.0
λ2	177057	20336	6.2	27.1	98.1	-21.8	10.4
λ3	234341	24493	6.0	27.7	98.0	-8.2	9.3

These data were not of as high a quality as was hoped, as the PIP degrades the diffraction quality of the crystals with an increase in mosaicity. Nevertheless, phasing was attempted using both MLPHARE and SHARP. No interpretable maps have been produced, despite attempts at cross-cell averaging between phases derived from the MAD data and a poor MIR phase set.

We believe that the MAD data were not the best that could have been collected. The time of PIP soaking is critical, and our original dates for data collection had to be put back because of technical difficulties at ESRF. We were therefore left with crystals that had either been in PIP for too long or too short a time. In addition, we can now produce crystals of better quality.

We would like to thank Andy Thompson for all his expert help