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Report: Our goal was to determine the resolution limit of crystals of the integral plasma membrane H^+-ATPase from N. *crassa,* to obtain a complete dataset at the resolution limit and to determine lattice reproducibility for potential MIR studies. These crystals diffract to 8Å on laboratory sources and 2D crystals obtained under similar conditions also show a lack of electron diffraction beyond 8Å (M. Auer and W. Kühlbrandt, pers. comm.).

78 crystals, hexagonal plates ranging in diameter from 170 to 500 μm and in thickness from 30 to 150 μm, were frozen in loops at UNC and characterized at the MP1 for diffraction quality. 7 crystals showed diffraction to 8-9Å resolution. Previously, two different unit cells had been observed, 167 x 720Å and 171 x 720Å. Of the 7 best-diffracting crystals, only one showed the larger unit cell and it had extremely high mosaicity. Separate experiments showed that the larger unit cell could be induced by unfrozen transport. This lattice was not judged a priority for data collection, leaving 6 diffraction candidates. Separately, a crystal of similar habit was found that grew under conditions which are compatible with enzymatic activity. Although it diffracted to only 15Å in the lab, it was included to determine diffraction quality and lattice parameters.

Datasets were collected from 5 of the 6 best diffraction candidates. In every case, the crystals diffracted well beyond 8Å. Using a 30% R_{symm} cut-off, the highest resolution obtained was 5.8Å (see Table I). Spots were clearly visible to 4Å on several images, but could not be reliably integrated due to low signal-to-noise ratio. The mosaic spread was

<=1°, so that optimal data were collected from oscillation ranges <1° (lower background). However, the long read-out time of the detector required the final datasets to be collected using 2° oscillations. Data were integrated using XDS. There is clear evidence of radiation damage during the course of data collection: using DENZO, the overall I/σ dropped by 10-40% percent after 600 s.

Table I: Data collection statistics: (image plate distance, 370mm; λ=0.9903Å).

crystal	Δφ	t	d_{max}	R_{symm}	Compl.	≥3σ	α
109	2°	30s	6.8Å	0.091	0.92	0.88	0.08-0.34
110	0.5°	30s	6.3Å	0.067	0.68	0.82	0.04-0.26
137	0.5°	10s	5.8Å	0.058	0.53	0.84	0.06-0.28
138	0.5°	30s/5s	6.2Å	0.072	0.74	0.81	0.04-0.21
151	2°	20s	6.6Å	0.089	0.95	0.87	0.04-0.36

The crystal grown under conditions supporting enzyme activity diffracted to better than 7.5Å with similar lattice parameters, although it showed a split lattice. This potentially opens the way to studies of conformational changes associated with the proton-transport cycle.

The higher resolution and completeness of the data relative to that collected from lab sources revealed that the crystals are twinned about an axis of non-crystallographic symmetry, so that the underlying symmetry is R3, while the crystals show apparent R32 symmetry. Due to NCS, the twin-related intensities are correlated, so that the Rees test (1) yields only a lower bound for the twin fraction a. Untwinning by the method of Britton (2), the smallest a that does not produce a negative correlation between the twinned halves of each R3 dataset yields an overestimate of the twin fraction. The limits are shown in Table I. It is likely that most crystals have a true twin fraction of 0.15-0.2.

Crystal lattice reproducibility was good, with maximum deviations of 0.1% in a and 0.3 % in c above the error in indexing, corresponding to 0.03 and 0.13 lattice points in the reduced lattice, respectively at 6Å resolution.

Summary: We have demonstrated that these crystals of the N. *crassa* H^+-ATPase diffract to at least 5.8Å, and that crystalline order is present to 4Å. This is the highest resolution currently available for any P-type ATPase. Isomorphism of the frozen crystals is adequate for heavy atom work. We have obtained two lower-resolution datasets that are nearly complete. When merged following untwinning, the higher resolution datasets will also exceed 90% completeness.

Perspectives: These crystals are grown under similar conditions to those yielding 2d arrays. Tilt-series data from the 8Å 2d electron microscopic images are being used for molecular replacement calculations and as a reference "untwinned" dataset to calculate a for each crystal, permitting data to be merged. The greater completeness, higher resolution and availability of non-crystallographic symmetry averaging relative to the EM data should provide a more detailed view of the molecular structure of the H^+-ATPase. Resolution extension to 4Å may be possible if we can reduce background scatter and radiation damage. Further goals includes structures revealing the active site and conformational changes associated with ion pumping.

(1) Rees, D.C. 1980 Acta Cryst. A36:578. (2) Britton, D. 1972 Acta Cryst. A28:296.