

Report for BM14U28 (Nov 19, 2001)

The green algae *Dunaliella salina* can grow in a wide range of salt concentrations, which classifies it as "halotolerant", as opposed to halophilic (requiring high salt) or mesophilic (requiring low salt). It is one of the few organisms that grows in the Dead Sea. Since *D. salina* is photosynthetic, it requires a mechanism to take up CO_2 from the environment. A key player in CO_2 uptake in any organism is the enzyme carbonic anhydrase, which converts bicarbonate ion (HCO_3^-) to CO_2 and vice versa. One of the carbonic anhydrases located on the surface of the organism was cloned and isolated. It shows the unusual property of having activity in both high and low salt conditions, in contrast to halophilic enzymes which are inactive in low salt conditions, and mesophilic enzymes which are inactive at high salt concentrations. Since molecular replacement with known carbonic anhydrase structures did not work, we wanted to use the zinc ion bound in the active site for a MAD experiment. The protein is a 30KD chain which forms a dimer. The space group was $P2_1$ with 2 molecules per asymmetric unit. Thus we had 2 zinc atoms per 60KD ASU. Since technical failure at a different beam-line prevented us from collecting useful data, we applied to BM-14 for fast access in the autumn of 2001, collecting data on the 19th of November, 2001.

We were able to get a very clear anomalous signal from the active site zinc ions, giving very sharp Patterson maps after 270° of data, although we collected 360° for better accuracy (Figure 1). For data collection statistics, see Table 1. The data gave very clear MAD maps using CNS (Figure 2), which allowed tracing of $\sim 85\%$ of both chains in the dimer **without** using NCS. Refinement is essentially complete and analysis of the structure under way.

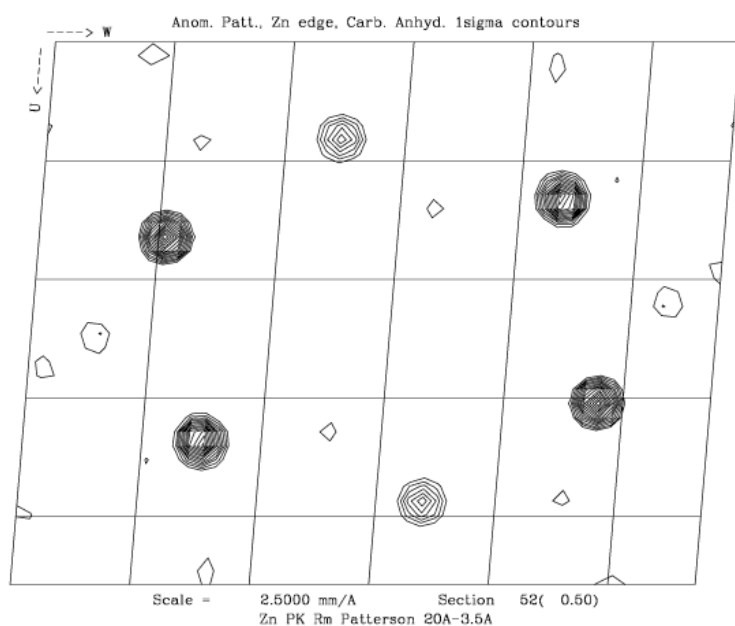


Figure 1: Harker section of anomalous Patterson map from 360° of data. The two zinc atoms of the non-crystallographic dimer are clearly visible. The third peak is a zinc atom bound at the dimer interface.

Table 1: Data collection statistics

	Peak	Inflection	Remote
λ (Å)	1.28255	1.28295	0.9840
D (mm)	88.4	108	120
2θ swing	0°	0°	0°
Date sweep	360°	125°	225°
Resolution (Å)	30 – 2.08	30 – 2.4	30 – 2.0
Measured Refl. (rejected)	926,353 (1204)	21,1984	740,576 (2008)
Uniq. Refl.	38,190	23,542	43,789
Overall Redundancy	6.8	2.6	2.3
$\langle I/\sigma \rangle$	22	26.2	14
% Refl. < 3 σ in highest resolution shell	15 (2.15 - 2.08Å)	13.2 (2.29 – 2.4Å)	31.2(2.07 – 2.0Å)
R_{merge} (highest shell)	0.045 (0.088)	0.026 (0.054)	0.048 (0.132)
% Completeness (highest shell)	98.4 (96.7)	92.3 (85.8)	98.5 (93.1)

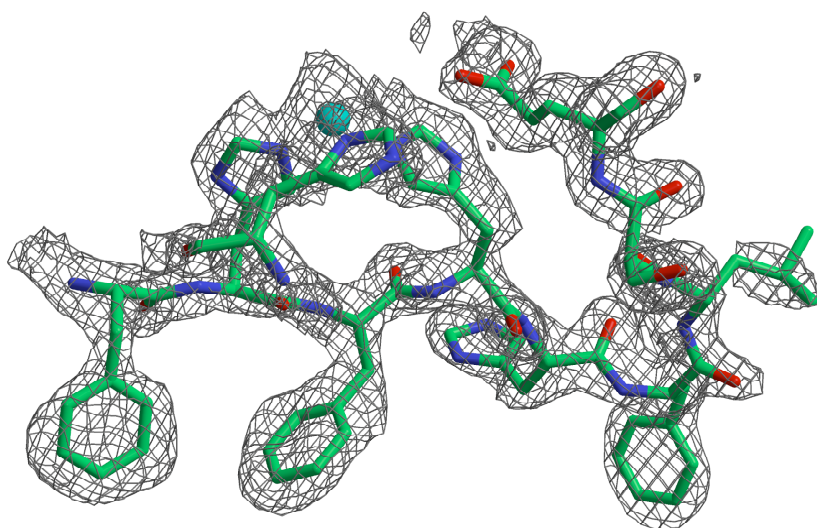


Figure 2: Initial MAD-phased map of active site region (1.5Å). The catalytic zinc ion is shown in cyan. Picture from XtalView and Raster3D.