

## Application Note 3

# Protein Screening with I $\mu$ S™ Measurements on Thermolysin and Carbonic Anhydrase II

### Introduction

Knowledge of the 3D structure of proteins is the key to understanding their functionality and is essential when developing new efficient drugs for many modern diseases. Proteins usually crystallize as very small crystals (< 0.15 mm) that only show weak diffraction. In order to determine an accurate 3D structural model with high, preferably atomic, resolution highly intense X-ray sources like rotating anodes or synchrotrons are required. In home laboratories, rotating anodes are usually used to determine the crystal quality in the first screening and to get a structure model. Despite the progress in the development of new and powerful microfocusing rotating anodes, many structural biology labs nowadays still use traditional rotating anode systems (0.3 mm focusing cup, multilayer optics of 1<sup>st</sup> generation).

In this application note, we will be comparing the performance of the Incoatec Microfocus Source I $\mu$ S™ with a traditional rotating anode by investigating small crystals of thermolysin (TLN) and carbonic anhydrase II (CA II).

### Experimental Set-up

Small crystals of TLN and CA II were first measured on a marresearch desktop beamline® equipped with a mar345 detector and the I $\mu$ S™ (Cu-K $\alpha$ ; 45 kV, 650  $\mu$ A) (Fig. 1). Afterwards, the same crystals were measured again using a well maintained Rigaku RU-H3R rotating anode combined with a Xenocs multilayer mirror (FOX 2D 12-38P) and a Rigaku R-Axis



Fig. 1: Incoatec Microfocus Source I $\mu$ S™ in combination with a mardtb.

IV goniometer. The rotating anode was operated at 44 kV and 80 mA. All measurements were carried out at 100 K.

The two proteins under investigation were metallo-enzymes which both contain Zn<sup>2+</sup> ions as cofactors in their structures. While TLN was used for crystallization in its native form, p-Chloromercuribenzoic acid was applied as an additive for CA II crystallization.

## Results and Discussion

At first, the X-ray flux of the two systems was measured with a Rigaku/MSC PIN diode. Both sources had virtually the same flux. Due to the smaller beam profile (FWHM 0.25 mm ( $\mu\text{S}^{\text{TM}}$ ) vs. 0.30 mm (RU-H3R)), the flux density of the  $\mu\text{S}^{\text{TM}}$  is slightly higher and the contribution to the background is, therefore, lower.

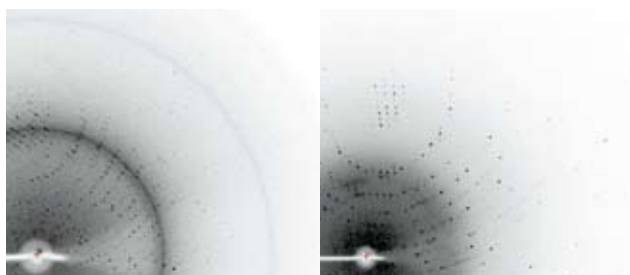


Fig.2: Diffraction pattern of the thermolysin crystal (left) and the carbonic anhydrase crystal (right) recorded with the  $\mu\text{S}^{\text{TM}}$ .

Figure 2 shows two typical diffraction patterns of the two proteins recorded with the  $\mu\text{S}^{\text{TM}}$  and the mardtb. Different software packages were used for data reduction to assure independence from different integration and scaling algorithms. In order to compare each sample on the different systems the same software was used. Tables 1 and 2 summarize the details of the data processing.

System	$\mu\text{S}^{\text{TM}}$ + mar345	RU-H3R + R-AXIS IV
Exposure time	10 min	10 min
$\Delta\varphi$	0.5°	0.5°
Software	HKL2000	HKL2000
Resolution	2.00 Å (2.03 - 2.00 Å)	2.10 Å (2.14 - 2.10 Å)
R(sym)	8.1 % (46.7 %)	9.7 % (45.1 %)
$\langle 1/\sigma \rangle$	17.1 (3.2)	20.2 (4.4)
Completeness	97.9 (94.6)	99.9 % (100 %)
Multiplicity	4.3 (4.5)	6.7 (6.6)

Tab.1: Thermolysin; values in brackets refer to the high resolution shells.

System	$\mu\text{S}^{\text{TM}}$ + mar345	RU-H3R + R-AXIS IV
Exposure time	5 min	5 min
$\Delta\varphi$	0.5°	0.5°
Software	Imosflm, Scala	Imosflm, Scala
Resolution	1.44 Å (1.52 - 1.44 Å)	1.50 Å (1.58 - 1.50 Å)
R(sym)	3.9 % (22.6 %)	4.0 % (21.4 %)
$\langle 1/\sigma \rangle$	22.1 (5.7)	28.8 (6.4)
Completeness	92.5 (87.8)	94.3 % (90.2 %)
Multiplicity	4.3 (4.2)	5.7 (5.7)

Tab.2: Carbonic anhydrase II; values in brackets refer to the high resolution shells; the resolution was limited by the different sizes of the active area of the detectors.

The comparative measurements of the two proteins TLN and CA II show that the data from the  $\mu\text{S}^{\text{TM}}$  have the same quality as the data from the rotating anode system. Notably, the two data sets measured with the  $\mu\text{S}^{\text{TM}}$  have a slightly better resolution. The anomalous signal in the CA II data from the  $\mu\text{S}^{\text{TM}}$  was used down to 2.4 Å when determining the heavy atom substructure (Hg, Zn, S) with SAD phasing. Whether radiation damage reduced the quality of the data from the rotating anode or not, was however, not further investigated.

## Conclusion

The measurements show that the data quality obtained with the  $\mu\text{S}^{\text{TM}}$  is comparable to that of the Rigaku RU-H3R rotating anode system, which is definitely sufficient for screening and structure modelling. In contrast to rotating anodes, the  $\mu\text{S}^{\text{TM}}$  contains a sealed X-ray tube with a stationary target and needs virtually no maintenance. It delivers an intense and stable flux, unlike rotating anode systems where the flux decreases significantly within months, caused by the degradation of the anode surface.

The  $\mu\text{S}^{\text{TM}}$  is, therefore, an effective and cost-saving replacement for older rotating anode systems giving the same performance but with significantly reduced maintenance.

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