Protein Crystallography with a High-Brilliance Microfocus Sealed Tube

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Introduction

The increasing importance of macromolecular crystallography has lead to a rising demand for high performance multilayer mirrors and high brilliant X-ray sources enabling the analysis of small and weakly scattering samples. New microfocusing sealed tube X-ray sources, such as the Incoatec Microfocus Source IμS, are low-maintenance, high-brilliant sources that give a performance comparable to traditional 4 kW rotating anode systems. Here, we present results on the use of the IμS for protein screening and S-SAD phasing.

Experimental Set-up

Measurements were performed with a mar345dtb and a Bruker AXS Smart 6000 diffractometer, both equipped with an IμS and 2D Quazar multilayer optics (Quazar and Quazar MX). The source is air-cooled and operates at 30 W using Cu-K_α radiation.

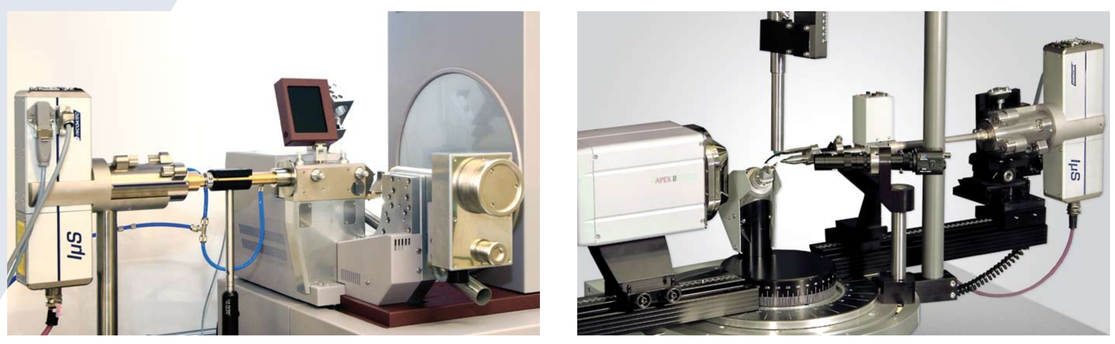

Fig. 1: Pictures of the mar345dtb and the Bruker AXS Smart diffractometer, both equipped with the microfocus sealed tube IμS.

SAD Phasing on Protein Crystals

Data sets were collected from measurements on several protein test crystals in order to solve their structures with SAD (single wavelength anomalous dispersion) phasing using the anomalous signal of S (thaumatin (Fig. 2), lysozyme) and Ca^{2+} (glucose isomerase, Fig. 3). In general, SAD phasing requires a strong and stable source and a highly redundant data set enabling the accurate determination of the weak Bijvoet differences. All data sets were obtained using a Bruker AXS Smart 6000 diffractometer equipped with an IμS (Quazar optics).

Thaumatin

crystal size [mm^3]	0.40x0.25x0.10
exposure time	40 s/°
total time	~ 18 h
diffraction limit [Å]	1.42
anom. signal limit [Å]	2.3 (17 S atoms)
$<I/\sigma>$	44.2 (4.4)*
<redundancy>	27.3 (12.7)*
R_{int}	0.0417 (0.4951)*
$R_{p.i.m.}$	0.0067 (0.1407)*
$R_1(I > 2\sigma(I)$; all)	0.1447; 0.1844

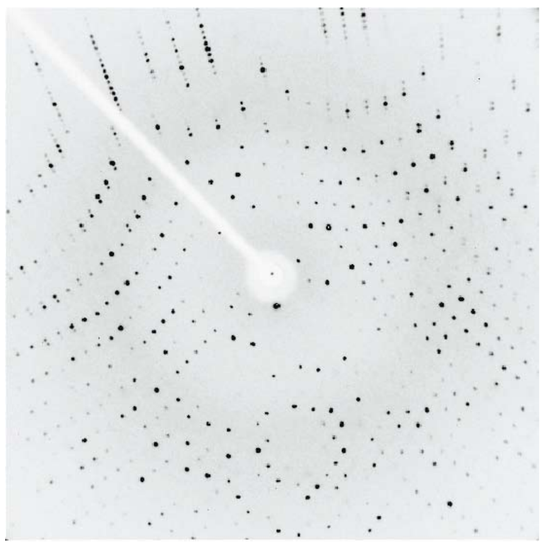

Fig. 2: Statistical data and a typical diffraction pattern of thaumatin (a = b = 57.86 Å; c = 149.55 Å; $P4_12_12$; T = 100 K; 208 amino acids / ASU). All 17 S atoms have been found in the initial phasing with SHELXD. (* values for outer resolution shell 1.52 – 1.42 Å).

Glucose Isomerase

crystal size [mm^3]	0.24x0.24x0.15
exposure time	160 s/°
total time	~ 43 h
diffraction limit [Å]	1.50
anom. signal limit [Å]	2.7 (Ca^{2+}, 9 S)
$<I/\sigma>$	21.8 (3.5)*
<redundancy>	14.4 (4.5)*
R_{int}	0.0652 (0.4191)*
$R_{p.i.m.}$	0.0149 (0.1959)*
$R_1(I > 2\sigma(I)$; all)	0.1587; 0.2047

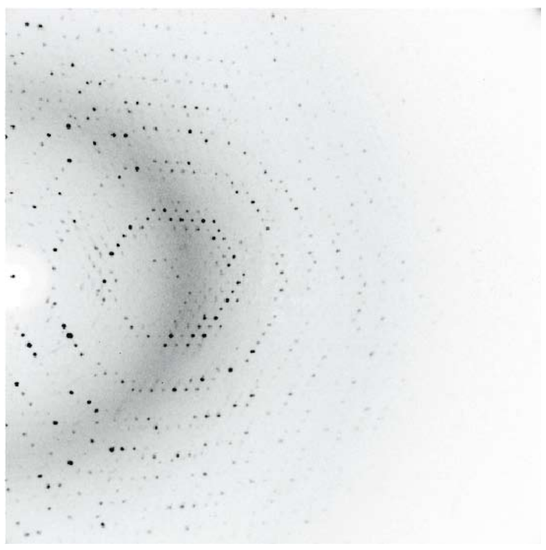

Fig. 3: Statistical data and a typical diffraction pattern of glucose isomerase (a = 93.88 Å, b = 99.68 Å, c = 102.90 Å; $I222$; T = 100 K; 388 amino acids / ASU). Ca^{2+} site has been found in the initial phasing with SHELXD (* values for outer resolution shell 1.60 – 1.50 Å).

Screening of Small Protein Crystals

In order to compare the performance of the IμS (Quazar optics) with a traditional rotating anode system, a small single crystal of the enzyme thrombin was measured on the mar345dtb. Beforehand, the crystal had been measured on a rotating anode system where it showed diffraction down to 2.0 Å (20 min/°, 4 kW). The results of the IμS measurement are summarized in Figure 4.

Thrombin

crystal size [mm^3]	0.10x0.10x0.05
exposure time	20 min/°
total time	~ 69 h
diffraction limit [Å]	1.95
$<I/\sigma>$	13.7 (2.8)*
<redundancy>	3.7 (3.6)*
R_{int}	0.0807 (0.4175)*
$R_{p.i.m.}$	0.0487 (0.2540)*
$R_1(I > 2\sigma(I)$; all)	0.2107; 0.2693

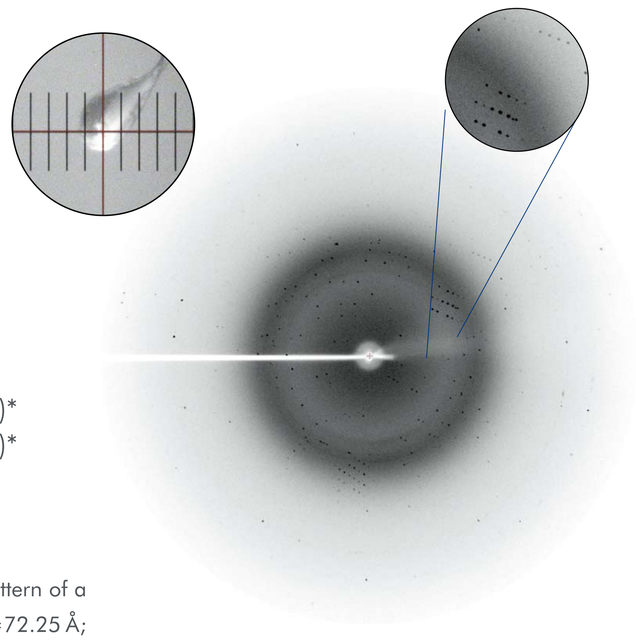

Fig. 4: Statistical data and a typical diffraction pattern of a small thrombin crystal (a=70.27Å; b=71.58Å; c=72.25 Å; β=100.21°; $C2$; T=100 K; 290 amino acids/ASU). (* values for outer resolution shell 2.05 – 1.95 Å).

Quazar MX Optics for Very Small Crystals

Next generation multilayer technology enables the fabrication of new high-reflective multilayer optics dedicated to the analysis of very small crystals (≤ 0.1 mm), the so called Quazar MX optics. The Quazar MX optics delivers twice the flux density of the regular Quazar mirrors at the price of a higher divergence (β=7.6 mrad) and a smaller beam cross-section (FWHM=0.12 mm). This results in higher integrated intensities and a lower background. The IμS together with a Quazar MX mirror is, therefore, ideal for determining the structure of small protein crystals (≤ 0.1 mm). Figure 5 shows a comparison of the performance of the two optics measured on a small thaumatin crystal (crystal size 0.15 x 0.10 x 0.05 mm^3, exposure time 120 s/°, total time ~ 24 h).

FWHM [mm]	0.25
β [mrad]	5.1
diffraction limit [Å]	2.05
<%>	95.8 (98.4)*
$<I/\sigma>$	12.2 (2.7)*
R_{int}	0.0968 (0.4569)*
R_σ	0.0785 (0.3800)*

FWHM [mm]	0.12
β [mrad]	7.6
diffraction limit [Å]	1.92
<%>	96.4 (98.8)**
$<I/\sigma>$	13.2 (2.9)**
R_{int}	0.0775 (0.4162)**
R_σ	0.0640 (0.3570)**

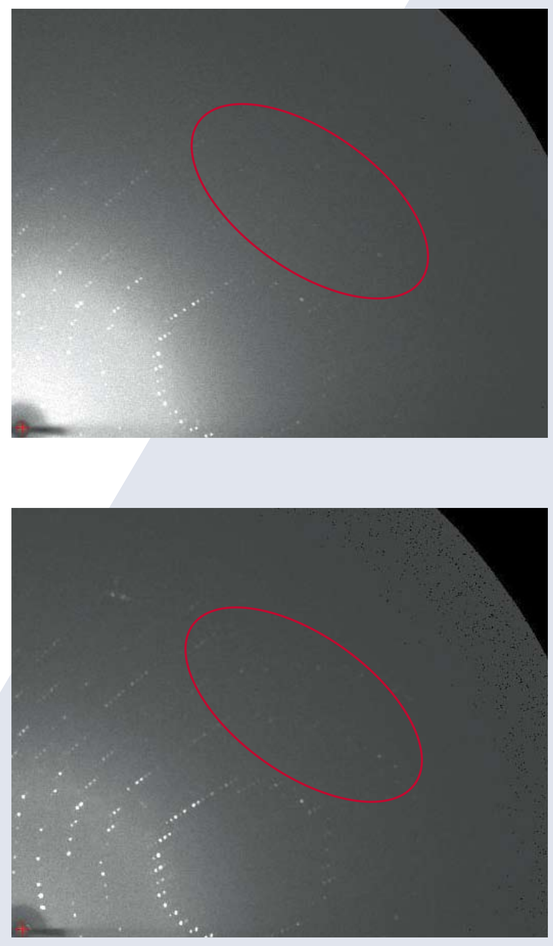

Fig. 5: Statistical data and a typical diffraction pattern of a small thaumatin crystal, measured with a regular Quazar mirror (above) and a Quazar MX mirror (below) (values for outer resolution shells: * 2.15 – 2.05 Å; ** 2.02-1.92 Å).

Conclusion The IμS has all the advantages of a sealed tube system and a flux density exceeding that of traditional home-lab X-ray sources. It offers a high performance at only 30 W together with low maintenance and low operating costs. The IμS was successfully used for in-house screening and SAD phasing experiments on several protein crystals. With the IμS and a Quazar mirror we have measured at least 1.5 times the flux density of a traditional 4 kW rotating anode system with Montel200 or Osmic blue mirrors. The new Quazar MX optics delivers an increase in flux density by a factor of 2 and a smaller beam, compared to the regular Quazar optics. The IμS together with a Quazar MX mirror is, therefore, ideal for small protein crystals (≤ 0.1 mm).

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