# Atomic-resolution diffraction data of S1 nuclease

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**Abstract**

S1 nuclease is a metalloenzyme containing three zinc ions in its active site, widely used in the biotechnological industry. The possibility of extraction of these ions by a chelating agent ethylenediaminetetraacetic acid was studied. The modified nuclease was crystallized and diffraction experiments were performed on the obtained crystals. The highest diffraction limit was 1.0 Å with the space group being determined as $P1$. The data set was integrated using *DIALS* software and scaled using *AIMLESS*. Key statistics arising from the data processing were evaluated.

**Keywords:** nuclease; protein crystallography; X-ray structure analysis; data processing, atomic resolution.

## Introduction

The S1 nuclease belongs to a broad S1-P1 family of nucleases. Our S1 nuclease originates from *Aspergillus Oryzae*, a mold widely used mainly in biotechnology research but also in the food industry [1]. The S1 nuclease is an endonuclease (*i.e.*, capable of cleavage of bonds inside the nucleic acid chain) with preference towards single-stranded DNA. It can also be classified as a metalloenzyme, since it contains three $Zn^{2+}$ ions in the active site [2]. One of the applications in biochemical research is determination of the secondary structure of nucleic acids [3].

According to the results published previously [4], removal of one zinc ion using ethylenediaminetetraacetic acid (EDTA) results in a significant loss of the S1 nuclease activity (about 50%). Removal of two or all three zinc ions results in complete and irreversible inactivity together with the disruption of the secondary structure of the S1 nuclease.

In this work, experiments aimed at the chelation of zinc ions using EDTA followed by crystal structure determination were conducted. Crystallization attempts with the EDTA-treated enzyme were successful and the obtained crystals were subjected to diffraction experiments at the Bessy II synchrotron radiation source, Helmholtz Zentrum Berlin. Diffraction data at atomic resolution were collected and processed.

## Materials and Methods

Enzyme provided by Novozymes A/S was deglycosylated using Endo-F1 and transferred to a storage buffer (50 mM NaCl, 25 mM Bis-Tris, pH 6.0). The protein concentration was 12 mg/ml. Consequently, a solution of EDTA-treated S1 nuclease (1:1 molar ratio) was prepared. This solution was incubated for 30 minutes at room temperature before the crystallization was initiated. The protein concentration at this stage was set at 10 mg $ml^{-1}$.

For crystallization, the vapor diffusion method with a hanging drop configuration at 20 °C was used. The duration of crystal growth was mostly about 7-10 days. Protein crystals grew in a crystallization condition containing 200 mM NaCl, 50 mM $CaCl_2$, 25% (w/v) PEG 3350, 100 mM Bis-Tris, pH 5.5. The crystal used for further diffraction experiments is shown in Figure 1.

Diffraction experiments were performed at the Bessy II synchrotron facility, Helmholtz Zentrum Berlin, Germany. The data collection parameters are shown in Table 1. The diffraction pattern of the crystal of EDTA-treated S1 nuclease including presence of ice rings (originating from either an insufficiently cryo-protected crystal or powder diffraction of the ice covering the protein crystal) is shown in Figure 2.

Table 1: Data collection parameters

| | |
|---|---|
| X-ray wavelength [Å] | 0.918 |
| Temperature [K] | 100 |
| Detector type | Pilatus 6M |
| Crystal-to-detector distance [mm] | 150 |
| Exposure time per frame [s] | 0.1 |
| Oscillation angle/total range [°] | 0.1/360 |

Table 2: Statistics after data integration (*DIALS*) and scaling (*AIMLESS*). Values in parentheses are for the low-resolution shell, values in braces are for the high resolution.

| | | | |
|---|---|---|---|
| Resolution range [Å] | $44.71 - 1.00$ | $(44.75 - 2.71)$ | $\{1.02 - 1.00\}$ |
| No. reflections (total) | 794 555 | (43 672) | {36 998} |
| No. reflections (unique) | 222 064 | (11 836) | {10 656} |
| Completeness [%] | 83.8 | (89.5) | {80.1} |
| Average multiplicity | 3.6 | (3.7) | {3.5} |
| Space group | | $P1$ | |
| $CC_{1/2}$ | 0.985 | (0.982) | {0.554} |
| Average I/$\sigma$ | 6.8 | (14.1) | {1.4} |
| $R_{merge}$ | 0.087 | (0.058) | {0.669} |
| $R_{meas}$ | 0.103 | (0.067) | {0.794} |
| $R_{pim}$ | 0.053 | (0.035) | {0.422} |
| Wilson $B$ [Å$^2$] | 8.3 | | |

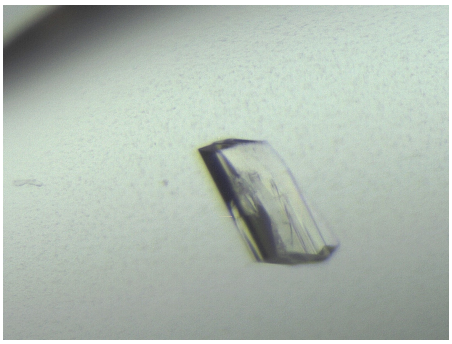

Figure 1: Crystal of S1 nuclease modified by chelating agent EDTA (1:1 molar ratio) selected for the diffraction experiment.

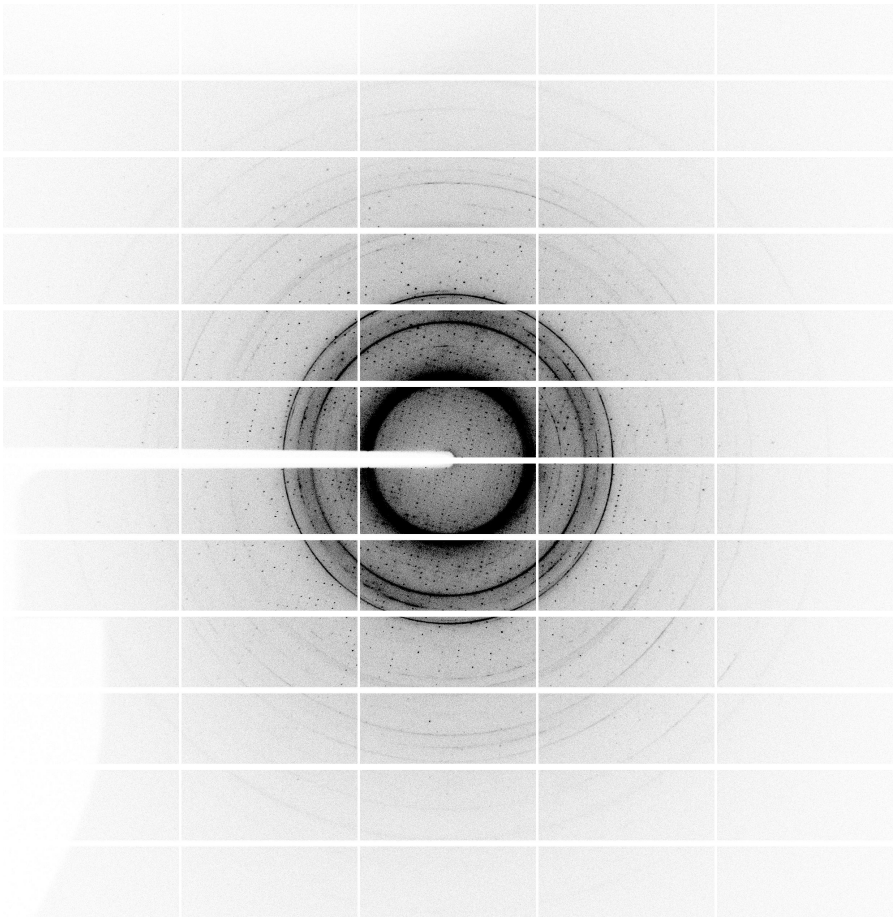

Figure 2: Diffraction pattern of the crystal of EDTA-treated S1 nuclease (1:1 molar ratio) measured at Bessy II synchrotron, HZB.

The data collected during the experiment was integrated using *DIALS* [5] software with a high-resolution diffraction limit of 1.0 Å. Consequently, scaling of the measured data was carried out using *AIMLESS* [6] software with the results listed in Table 2.

## Results and Discussion

The diffraction data from the crystal of the EDTA-treated S1 nuclease were processed at the atomic resolution of 1.0 Å. Such high resolution of diffraction on S1-nuclease crystals has not been published yet [7]. Since zinc ions play also a role of structure stabilizers, a deterioration in resolution was expected. Hence, obtaining the atomic resolution was rather surprising.

Low completeness (below 90 %) both in the overall resolution range and in the outer shell (high resolution) is caused by $P1$ space group, which has no symmetry elements other than identity (Table 2). However, the data presented in this work were obtained using only the angle $\kappa = 0°$. Merging this data set with another data set with a different crystal orientation (*e. g.*, $\kappa = 100°$) would lead to an increase in data completeness.

Higher multiplicity could be obtained by measuring in a wider total range, *i. e.*, in more oscillations. However, possible radiation damage must be considered in this case, since the exact properties of crystals containing EDTA-treated S1 nuclease are not known.

In spite of the inferior values of multiplicity and completeness, we consider the results of the key statistics ($CC_{1/2}$, $I/\sigma$, and $R$-values, Tab. 2) to be satisfactory. The overall values seem to be very promising for further model building. Despite the deterioration of the statistics in the outer shell, the values show that our data is well applicable.

It is possible to push the 1.0 Å diffraction limit used in this work to an even better resolution using the paired refinement [8] with a use of a reasonably modified model of S1 nuclease after several cycles of refinement. However, this validation requiring the structural model is beyond the scope of this work.

## Conclusions

We succeeded to measure the diffraction data of EDTA-treated S1 nuclease. The presence of EDTA in 1:1 molar ratio did not significantly affect the diffraction quality of the crystal. The high-resolution diffraction limit of the data was set to 1.00 Å. The structure determination process is currently ongoing to reveal the structural changes caused by the EDTA treatment.

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