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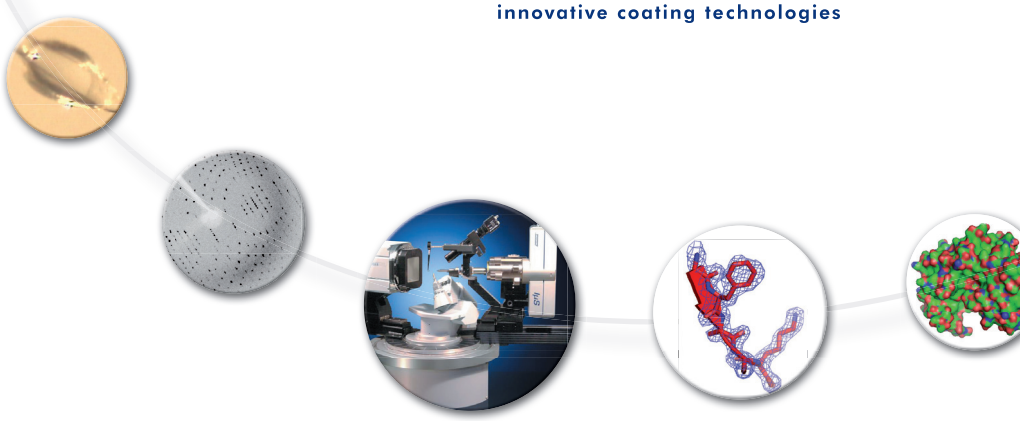

Application Note 6

Fragment Screening Using a Twinned Protein Crystal

Introduction

Fragment-based screening using X-ray crystallography has become one of the major techniques in contemporary drug design. The aim of fragment screening is to run a rapid data collection of a protein crystal in complex with a small fragment molecule, in order to find out whether the fragment has bound to an active site of the protein or not. The increasing popularity benefits from recent developments of highly brilliant X-ray sources as well as fast and sensitive area detectors. This allows high quality data to be collected within a reasonable period of time using a large number of different fragment chemotypes. This is especially beneficial if one wants to screen a fragment library.
Here, we present results that were obtained by collecting data from a twinned endothiapepsin crystal in complex with a small fragment molecule. The influence of the data completeness on the quality of the obtained electron density maps was analyzed, in order to find the minimum data collection time that is needed to detect the inhibitor in the active site.

Experimental Details

A data set of the endothiapepsin complex (P21, a = 45.40 Å, b = 72.82 Å, c = 52.87 Å, b = 110.0°, 0.45 x 0.07 x 0.04 mm3) was collected in 3.5 hours to a resolution of 2.6 Å. The data was processed to 60%, 80% and 96% completeness, resulting in data sets with total collection times of 1 h, 1 h 20 min and 3 h 30 min. The electron density map around the active site was inspected to see whether the fragment was bound. The data collection was performed using a Bruker AXS X8 PROSPECTOR equipped with an Incoatec Microfocus Source IμS with QUAZAR MX optics and an APEX II detector (Fig. 1). Data collection and processing were performed with the PROTEUM2 suite [1].

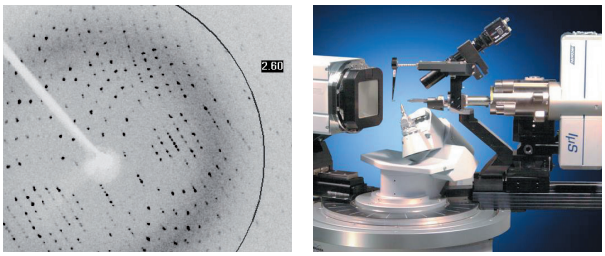

Figure 1: Diffraction pattern of he endothiapepsin crystal (left; 15 s/0.5°); X8 PROSPECTOR with a Cu-IμS MX microfocus X-ray source equipped with a QUAZAR MX optics and APEXII CCD detector (right).

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From the first few images it was found that the crystal under investigation was twinned. CELL_NOW was used to find the orientation matrices of the two domains, the twin matrix and the twin fraction [2]. After integration to a resolution of 2.6 Å, the twinned data was scaled with TWINABS [3]. Statistics for the 3.5 h data set and the two data subsets with 60% and 80% completeness are shown in Table 1 and Table 2.

Structure solution and refinement

The merged *hkl* file (HKLF 4 format) created by TWINABS was converted to a mtz format using the CCP4 suite [4]. Molecular replacement using the model (3PLD [5]) without waters and inhibitor fragment was performed with PHASER [6]. An initial rigid body refinement was performed with REFMAC [7]. The electron density maps obtained were visually inspected with COOT [8] (Fig. 2). The final refinement using the HKLF 5 format and the refined model with the inhibitor was performed with SHELXL [9] resulting in a final R1 = 13.31% with a twin ratio of 0.48 and an occupancy of the fragment of 100%.

Space group	$P2_1$
Resolution [Å]	41.00 - 2.59
Completeness [%]	96.9
Multiplicity	3.10
$<I/\sigma>$	9.1
R_{int} [%]	8.20
R1 [%]	13.31

Table 1: Data statistics of the complete data set

Completen. [%]	Collection time	# Frames	Multiplicity	$<I/\sigma>$*	R_{int} [%]#
60	1 h	160	1.43	12.1	7.56
80	1 h 20´	250	1.61	10.6	5.90
100	3h 30´	652	3.10	9.1	8.20

*Table 2: Data statistics at different stages of data completeness (HKLF 5 format; * for all composite reflections (belonging to both twin domains); # for all single (belonging to only one domain) and composite reflections).*

After molecular replacement

After rigid body refinement

After refinement (occupancy = 0.98)

Figure 2: Electron density map (F_o-F_c in green, 3σ; $2F_o$-mF_c in blue, 1.5σ) after molecular replacement (up left), rigid body refinement (up right) and final refinement (bottom left), calculated with the complete data set.

In order to determine the minimum time that is needed to see whether the fragment is present in the active site, the data was divided into subsets of 60% and 80% completeness (Tab. 2). Again, the merged hkl file (HKLF 4 format) was converted to a mtz file. After molecular replacement and a rigid body refinement, significant positive electron density in the active site was already visible using the 1 h data subset indicating the presence of the fragment (60% completeness, Fig. 3).

60% completeness, 1h *80% completeness, 1h 20´*

Figure 3: Electron density map after rigid body refinement (F_o-F_c in green, 3σ; $2F_o$-mF_c in blue, 1.5σ) with 60% (left) and 80% (right) completeness.

Conclusion

The combination of the high brilliance Cu-IμS MX and the very sensitive APEX II detector enabled us to see positive residual electron density at the fragment position after only one hour of data collection time. Complete data collected to a resolution of 2.6 Å was obtained after 3.5 hours with a quality suitable for a twin refinement of the structure including the inhibitor.
This experiment shows that such fragment binding studies can be performed in the home laboratory within a few hours using a high brilliance X-ray source and a very sensitive CCD detector. Sophisticated data processing software, such as the PROTEUM2 suite, successfully tackles common hurdles in protein crystallography, such as twinning.

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