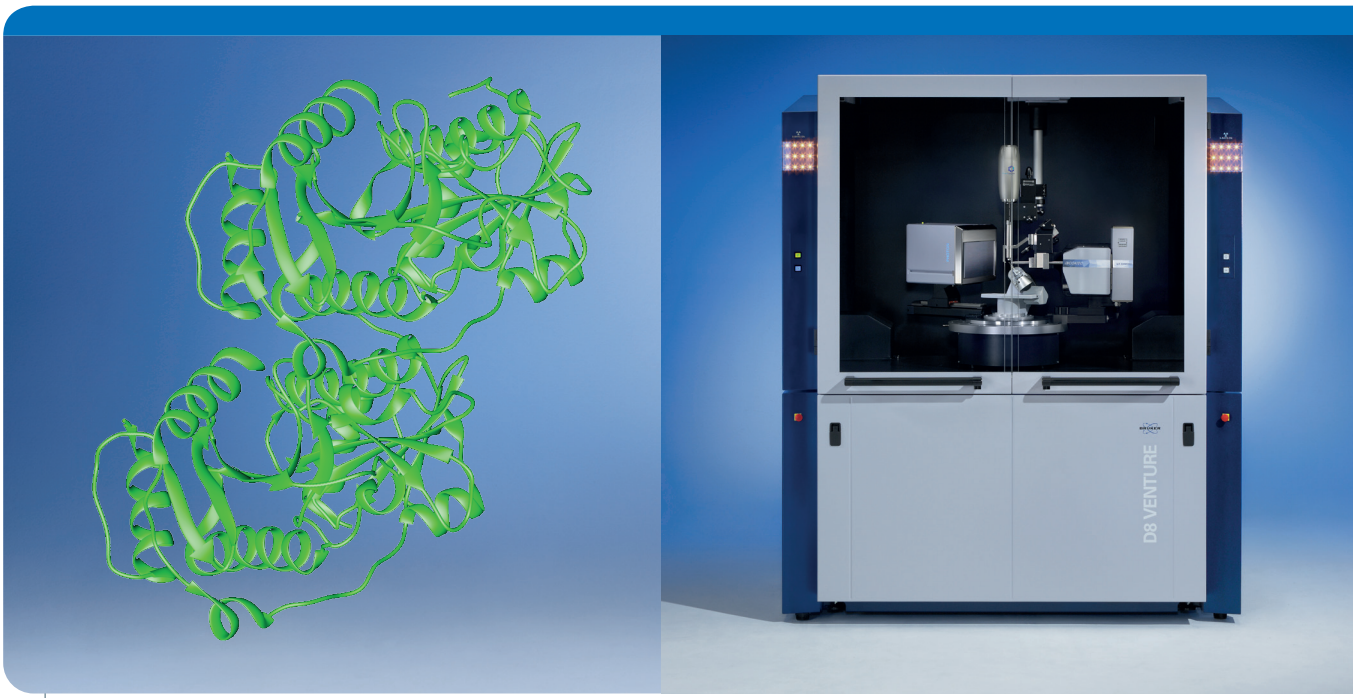

Application Note SC-XRD 525

Novel In-house Structure of a Cognate Chaperone-Toxin Complex

- High Resolution Data from D8 VENTURE with IμS DIAMOND and PHOTON III M28

Introduction

Pseudomonas aeruginosa is widely known to be an emerging opportunistic gram-negative pathogen that harbors multiple virulence factors for manipulating a host's cell signaling pathways and immune response[1]. This pathogen is also solely responsible for more than 10% of hospital acquired infections. Like other gram-negative pathogens, *Pseudomonas aeruginosa* uses a modular proteinaceous syringe-like apparatus, known as the Type III Secretion System (T3SS), to establish the host-pathogen interaction by injecting effector toxins or exotoxins into the host cell[1]. Anatomically, this complex nano-syringe possesses an architecture of two inner- and outer-membrane spanning channels, a hollow needle complex and a translocon pore on the host membrane. From a physiological perspective, in addition to the architectural proteins, the T3SS possesses stabilizing chaperones (SpcS, SpcU), regulatory proteins, and effector toxins (ExoT, ExoS, ExoU, ExoY)[1]. Prior literature states that before secretion of these toxins into the host cell, the toxins form a complex with their cognate chaperone (e.g. ExoT-SpcS), providing stability to the toxins. These stabilized toxin-chaperone complexes are essential for the pathogenicity of the bacteria.

In light of this, a high-resolution structure of the ExoT-SpcS complex would provide valuable insight into the toxin-chaperone interaction and enable the development of compounds that could specifically disrupt the interaction. This could be helpful in impairing the secretion of this toxin protein into the host cell.

Crystal Growth and Screening

The ExoT-SpcS protein complex was recombinantly overexpressed in E. coli BL21 (DE3) cell lines. Purification of the complex was done by Ni^{2+} affinity chromatography followed by size exclusion chromatography. High-throughput screening was performed with the purified protein using commercial crystallization screens, which provided initial small crystallites (Figure 1). After optimizing the crystallization conditions, well diffracting crystals, 50 µm in size, were obtained using 28% PEG3 350, 0.1 M Bis-Tris pH 6.3 precipitant solution.

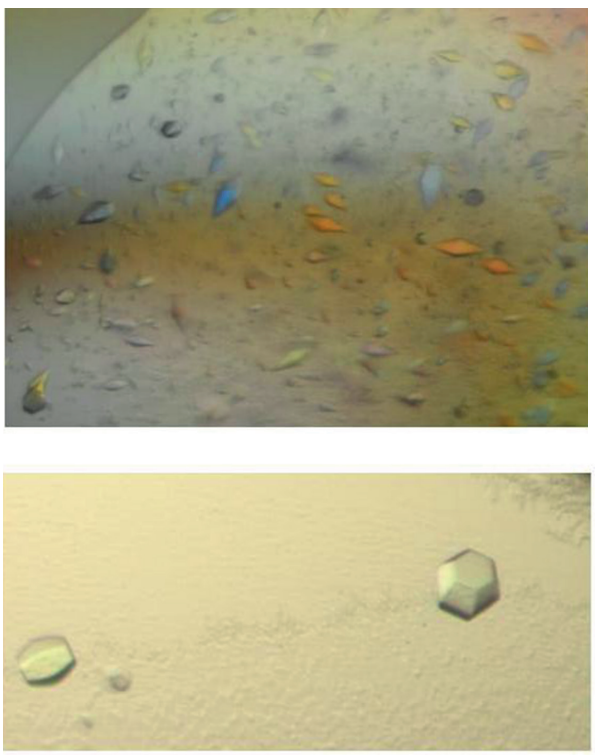

Figure 1: ExoT-SpcS protein complex crystals (top) as initially obtained from commercial screens and (bottom) as used for final data collection.

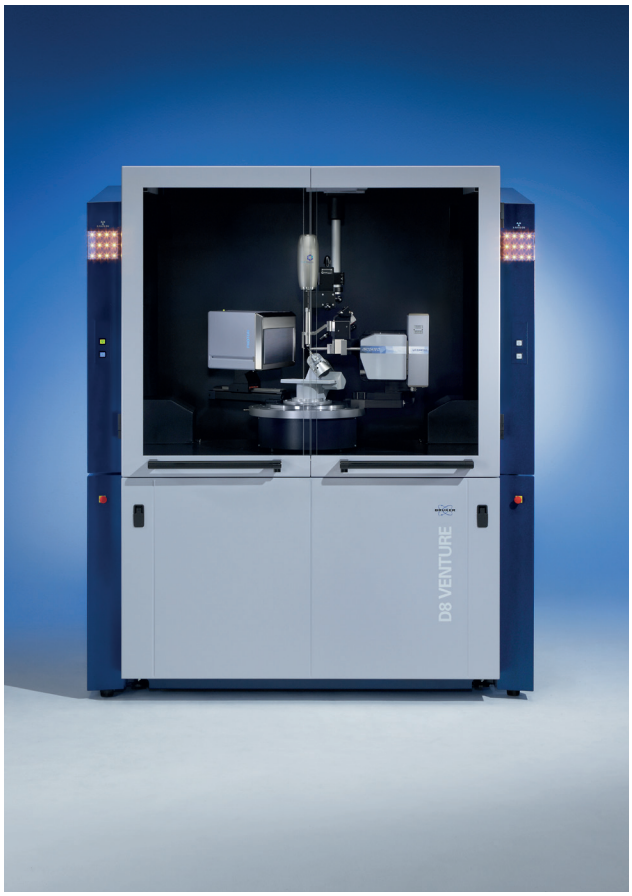

Figure 2: D8 VENTURE with PHOTON III 28 and IµS DIAMOND.

Data Collection and structure solution

Replacing an imaging plate system with rotating anode generator, a Bruker D8 VENTURE with IµS DIAMOND and PHOTON III detector (Figure 2) has been installed very recently. This brand new in-house system, including a low-temperature device and the PROTEUM3 software suite, proved extremely crucial for the success for both the screening during the optimization of the crystallization conditions and the final high quality data collection.

Crystals were cryo-protected with Paratone oil just before beam exposure P. The data collection strategy routine of the PROTEUM3 suite was applied to conveniently calculate the scans required for a resolution of 2.26 Å. The corresponding data set was collected at 100 K in just 4 hours with exposures of only 5 s/degree. Data indexing, integration and reduction were also performed with the PROTEUM3 suite (Table 1). The structure was solved by molecular replacement using PHASER. Manual model-building was performed in Coot 8.9, and final refinement cycles were carried out in PHENIX[4].

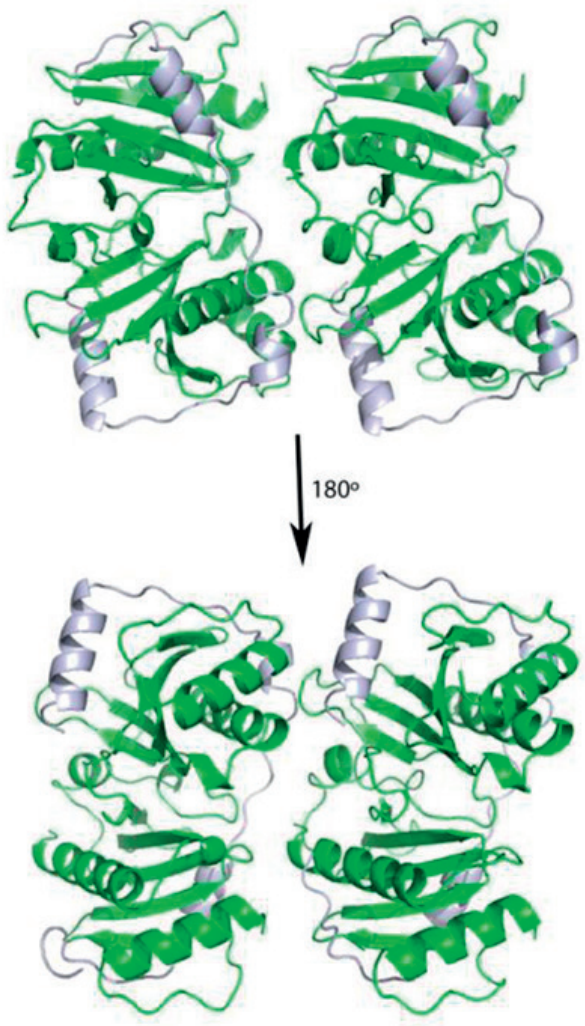

Figure 3: Crystal structure of ExoT(23-79)-SpcS complex from *P. aeruginosa* at 2.2 Å resolution. The figure shows a hexameric assembly of the proteins with each ExoT (sky-blue) bound with two SpcS (light green) molecules.

Diffraction system	D8 VENTURE with KAPPA goniometer
X-ray source	IμS DIAMOND, Cu-Kα
X-ray detector	PHOTON III 28
Software for data collection and integration	PROTEUM3
Space Group	$P12_11$ (No. 4)
Resolution Range [Å]	34.79 – 2.26 (2.34 – 2.26)
Unit Cell [Å] [°]	 $a=53$, $b=63$, $c=83$; $\alpha=90$, $\beta=98$, $\gamma=90$
Unique reflections	24989
Completeness (%)	90.28
Mean $I/\sigma(I)$	7.2
Wilson *B*-factor	28.33
R-work	0.21
R-free	0.25
CC(1/2)	0.991
Macromolecule atoms	4574
Ligand atoms	12
Solvent atoms	155
Protein residues	578

Table 1: Structural statistics of ExoT-SpcS complex (PDB ID: 6JNP).

Discussion

Analysis of the structure (Figure 3) showed that the complex is a dimer of trimers. Each ExoT binds with two SpcS molecules forming a large binding groove. From this structure (PDB ID: 6JNP) it can be hypothesized that developing any drug target capable of blocking the key residues of SpcS required for binding with ExoT, can completely disturb the stability of ExoT inside the bacterial cell. Hence, this could provide us a key to impair the ExoT secretion through T3SS.

Conclusion

Although the system was installed in our laboratory only a couple of months ago, the D8 VENTURE rapidly became the core instrument for our group. The diffraction instrument is extremely powerful as it can deal with ultimately small, weakly diffracting samples. The outstanding performance of the hardware goes along with the ease-of-use of the PROTEUM3 software suite. The system is very easy to install as it is fully air cooled, does not require any cooling water, and is connected to single phase power. Finally and maybe most important: our group rapidly familiarized with the operation of the instrument and the accompanying software, i.e. we lost no time when we switched from another supplier to Bruker. The introductory training we received from Bruker's extremely knowledgeable technicians and application scientists was outstanding. Today, we take benefit from the many clever features Bruker has built into its excellent hardware and software.

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