



Production, crystallization and X-ray diffraction analysis of a complex between a fragment of the TssM T6SS protein and a camelid nanobody

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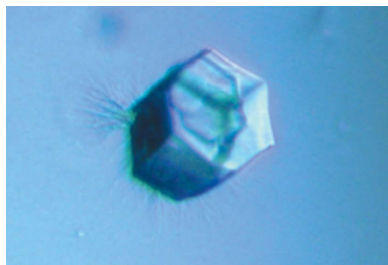
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The type VI secretion system (T6SS) is a machine evolved by Gram-negative bacteria to deliver toxin effectors into target bacterial or eukaryotic cells. The T6SS is functionally and structurally similar to the contractile tail of the *Myoviridae* family of bacteriophages and can be viewed as a syringe anchored to the bacterial membrane by a transenvelope complex. The membrane complex is composed of three proteins: the TssM and TssL inner membrane components and the TssJ outer membrane lipoprotein. The TssM protein is central as it interacts with both TssL and TssJ, therefore linking the membranes. Using controlled trypsinolysis, a 32.4 kDa C-terminal fragment of enteroaggregative *Escherichia coli* TssM (TssM_{32Ct}) was purified. A nanobody obtained from llama immunization, nb25, exhibited subnanomolar affinity for TssM_{32Ct}. Crystals of the TssM_{32Ct}-nb25 complex were obtained and diffracted to 1.9 Å resolution. The crystals belonged to space group $P6_4$, with unit-cell parameters $a = b = 95.23$, $c = 172.95$ Å. Molecular replacement with a model nanobody indicated the presence of a dimer of TssM_{32Ct}-nb25 in the asymmetric unit.

1. Introduction

The type VI secretion system (T6SS) is one of the key players during the intense warfare for nutrients that bacteria encounter in their ecological niche (Russell *et al.*, 2014). This large multi-protein complex is widespread in Gram-negative bacteria and is dedicated to the delivery of enzymatic effectors directly into bacterial or eukaryotic prey cells (Russell *et al.*, 2014; Durand *et al.*, 2014). Among the effectors that have already been identified and characterized, peptidoglycan hydrolases, DNases and phospholipases are the most common (Russell *et al.*, 2014; Durand *et al.*, 2014). Although T6SS phospholipase and DNase effectors might also affect the integrity of eukaryotic cells, eukaryotic specific effectors have been described such as the VgrG-borne actin cross-linking domain in *Vibrio cholerae* (Pukatzki *et al.*, 2007; Satchell, 2009; Durand, Derrez *et al.*, 2012).

The T6SS comprises a set of 13 conserved structural components that participate in the architecture and dynamics of the secretion system (Cascales & Cambillau, 2012; Coulthurst, 2013; Ho *et al.*, 2014; Zoued *et al.*, 2014). Architecturally, the T6SS can be seen as a syringe-like module tethered to the cell envelope through contacts with a membrane-associated complex composed of the TssJ, TssL and TssM proteins



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