Background

*Moraxella catarrhalis* is a new emerging pathogenic bacterium that is involved in otitis media and sinusitis in children as well as lower respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). Over the last 20 to 30 years, the bacterium has emerged as a genuine pathogen. In immuno compromised hosts, the bacterium can cause a variety of severe infections including pneumonia, endocarditis, septicemia, and meningitis. More troublesome, today more than 90% of all clinical isolates are β-lactam resistant. One of the most important virulence factors of *M. catarrhalis* is a 200 kDa outer membrane protein namely the Moraxella IgD-binding (MID) protein. The two different functional domains, MID962-1200 has capacity for IgD binding and MID764-913 is responsible for binding to type II alveolar epithelial cells as well as hemagglutination.

*MID* Properties:

MID belongs to the oligomeric coiled-coil autotransporter group of proteins (OCA family), which are able to drive their own secretion across the bacterial outer membrane. It shares low sequence similarities with *Yersinia enterocolitica* OMP-YadA and the *Haemophilus influenza* Hia adhesin.

The signal peptide on the N-terminal directs export of the precursor protein across the inner membrane via the Sec machinery and type V pathway. Subsequently, the beta domain inserts into the outer membrane and forms a beta-barrel structure with a central channel, allowing extrusion of the passenger domain across the membrane. Once on the surface of the organism, the passenger domain is usually cleaved from the translocator domain and released extracellularly.

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Cloning & Expression:

The MID962-1200 sequence was previously cloned in pET-26b(+) vector which carries an N-terminal pET signal sequence for potential periplasmic localization. The protein was expressed in E. coli, but instead of being located only in the periplasmic space the protein also get secreted in the media. Now we have cloned it in pETM-30 (from EMBL). SDS-PAGE shows the expression of the protein and it is believed that these blebs play a pathogenic role. OMVs are released from the surface of the cell during growth and occur in various environments, including liquid culture, solid culture and biofilms.

Circular Dichroism Spectroscopy:

Circular dichroism spectrum of MID962-1200 showed approximately 11% α-helices, 32% β-sheets, 30% unordered and rest are other structures.

SAXS Studies:

SAXS scattering curves of IgD binding domain is very clear: no dispersity. Very indicative of trimeric arrangement. Ab initio modelling suggests elongated fibrous shape.

(Data collected at beamline X31, EMBL-Hamburg)

Crystallization Trials and Microseeding:

The protein was gel filtrated in phosphate buffer (pH 8.0). CD data in millidegrees were collected using a Jasco-815 spectrometer. These data were then deconvoluted using the DichroWeb server. The analyzed data with a good NRMSD value showed approximately 11% α-helices, 32% β-sheets, 30% unordered and rest are other structures.

Purification:

Glutathione is attached to Sepharose by coupling to the oxirane group using epoxy-activation. The structure of glutathione is complementary to the glutathione S-transferase binding site. The GST tagged protein in bacterial cell lysate could be applied to the glutathione containing column and purified by affinity chromatography. Fusion proteins are then eluted from the glutathione sepharose under mild, non-denaturing conditions. The fusion protein contains a TEV protease site and this site is digested using in house made TEV protease to cleave the tag. The GST can be separated from the protein using the attached His-tag to it.

Future Plans:

We got crystal from previous crystallization trial and dataset collected at MAX-lab (beamline I17) but there was problem with reproducibility. This kind of behavior led to difficulty to produce good crystal for phasing experiments. Since the sequence of this part of protein does not contain any Cys or Met residues, we have to rely on heavy atom derivative for phasing. We are hoping that the new clone will produce the protein with more stability! After solving the apo structure our goal is to solve the structure in complex with IgD.

References: