Characterization and crystallization of MID962-1200: A domain of a trimeric autotransporter from *Moraxella catarrhalis*

Mahmudul Hasan[1], Karolina Förnvik[1], Marjolein Thunnissen[1] and Kristian Riesbeck[2]

Department of Biochemistry and Structural Biology, Kemicentrum, PO Box 124, Lund University, 22100 Lund, Sweden[1];

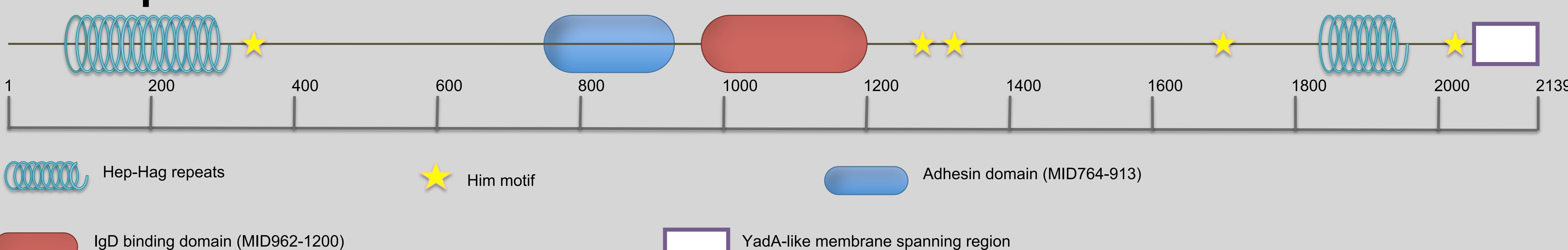
Medical Microbiology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, 20502 Malmö, Sweden[2]

Email: Mahmudul.Hasan@biochemistry.lu.se

Background

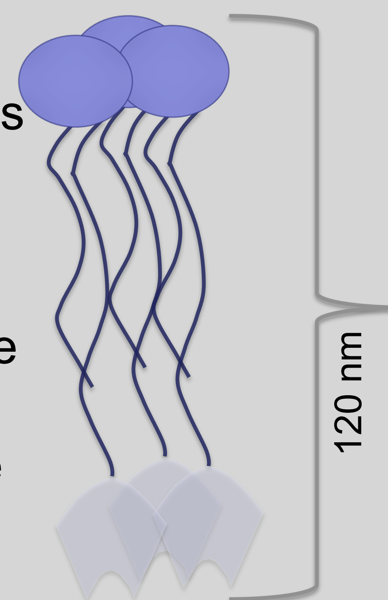
Moraxella catarrhalis is a new emerging pathogenic bacterium that is involved in otitis media and sinusitis in children as well as lower respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). Over the last 20 to 30 years, the bacterium has emerged as a genuine pathogen. 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 MID$^{764\text{-}913}$ is responsible for binding to type II alveolar epithelial cells as well as hemagglutination.

MID Properties:

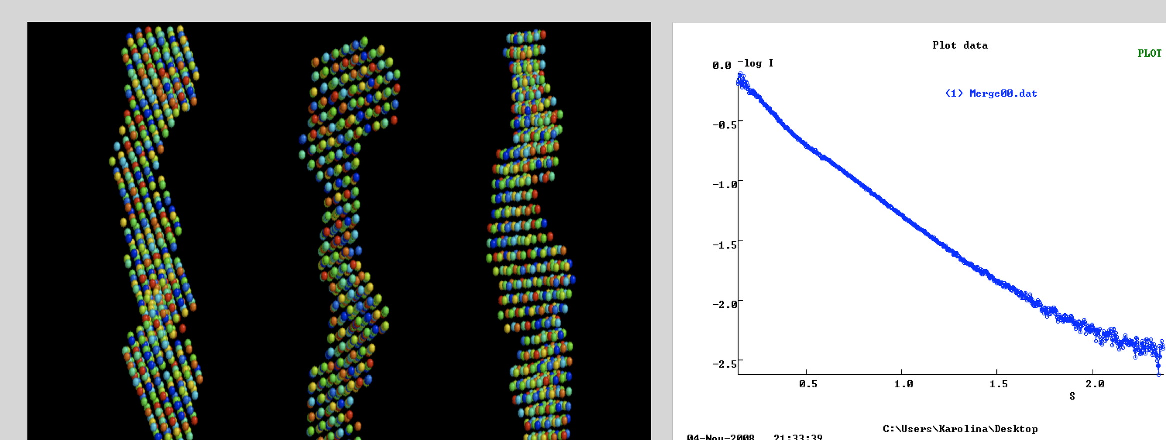


MID belongs to the oligomeric coiled-coil autotransporter group of proteins (OCA family), which are able to drive their own secretion across the bacterial outer membrane. It shares low sequence similarities with *Yersinia enterocolitica* OMP YadA and the *Haemophilus influenza* Hia adhesin.

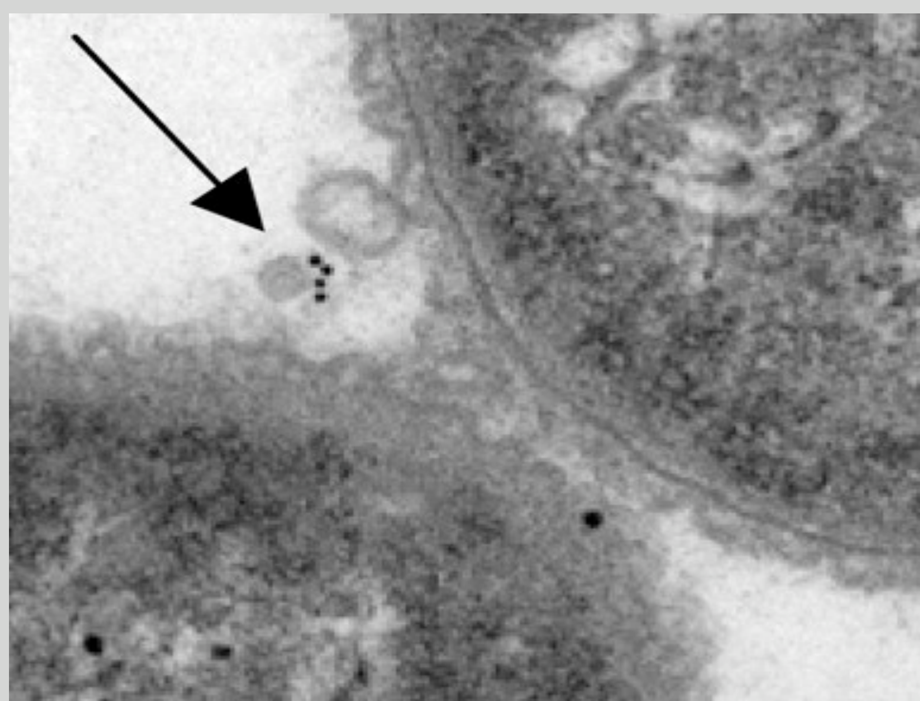
The signal peptide on the N-terminal directs export of the precursor protein across the inner membrane via the Sec machinery and type V pathway. Subsequently, the beta domain inserts into the outer membrane and forms a beta-barrel structure with a central channel, allowing extrusion of the passenger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



SAXS Studies:

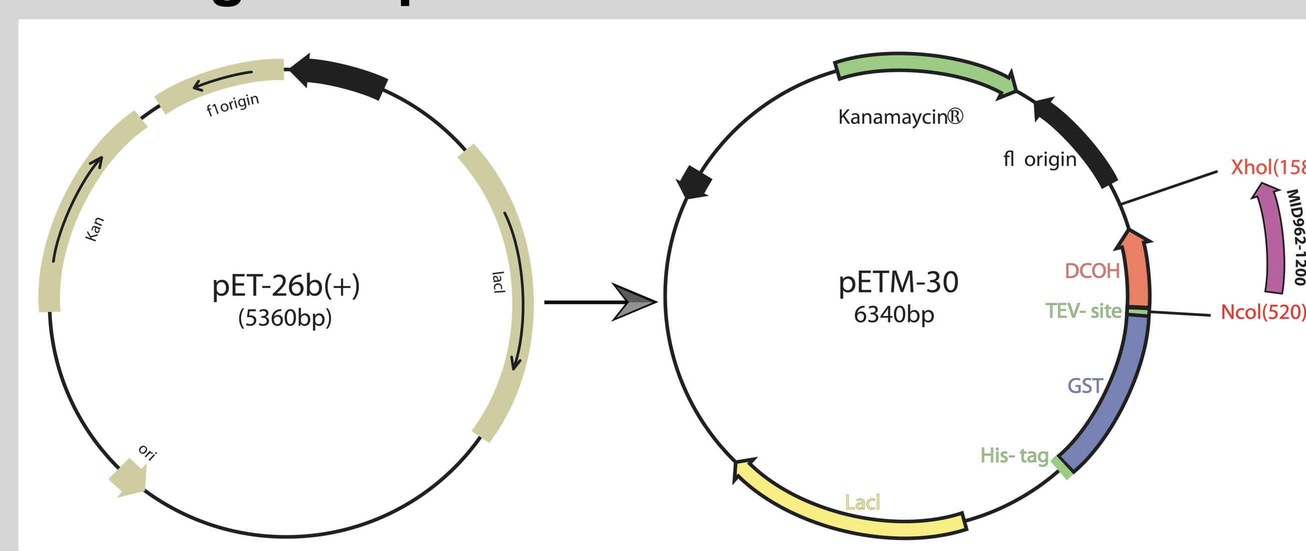


SAXS scattering curves of IgD binding domain is very clear: no dispersity. Very indicative of trimeric arrangement. *Ab initio* modelling suggests elongated fibrous shape.
(Data collected at beamline X31, EMBL-Hamburg)



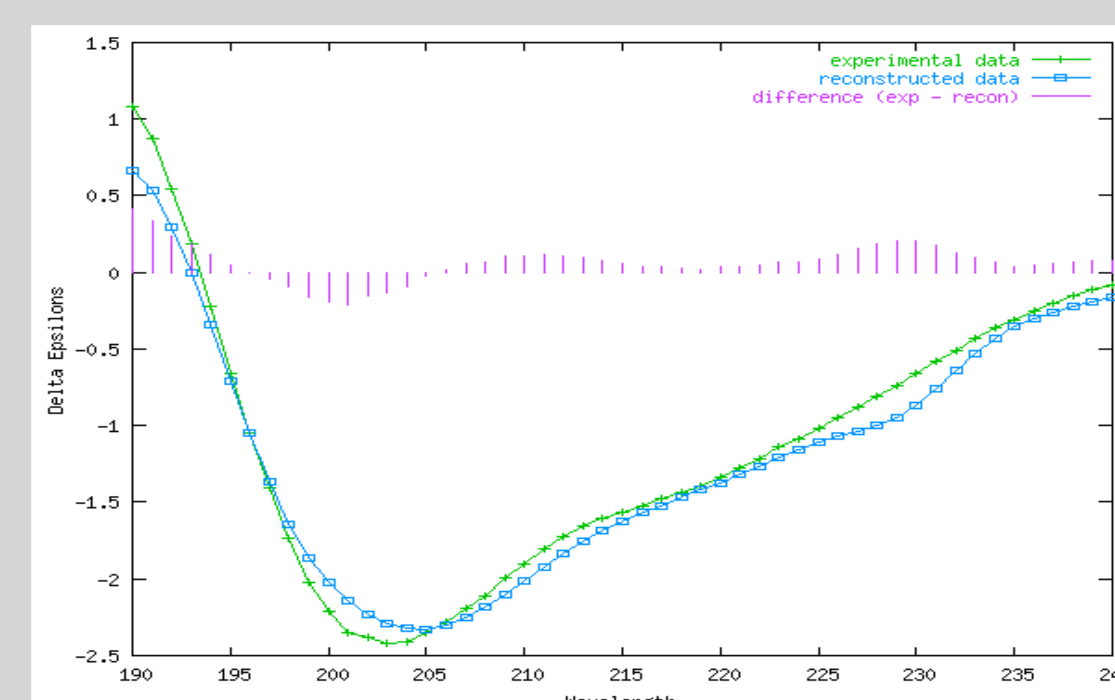
M. catarrhalis release outer membrane vesicles (OMVs) or blebs (pointed by arrow) and it is believed that these blebs play a pathogenic role. OMVs are released from the surface of the cell during growth and occur in various environments, including liquid culture, solid culture and biofilms.

Cloning & Expression:



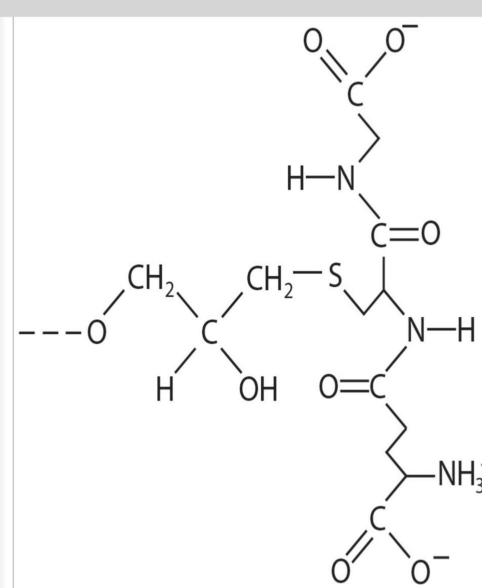
The MID$^{962\text{-}1200}$ sequence was previously cloned in pET-26b(+) vector which carries an N-terminal *pelB* signal sequence for potential periplasmic localization. The protein was expressed in *E. coli,* but instead of being located only in the periplasmic space the protein also get secreted in the media. Now we have cloned it in pETM-30 (from EMBL). SDS-PAGE shows the expression of the protein is in the cell. Expression of the gene from this plasmid allows to have GST as well as His tag with the protein. The GST and His-tag allows purification on glutathione sepharose and Ni-NTA column respectively.

Circular Dichroism Spectroscopy:



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 aproximately 11% alpha helices, 32% beta 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.

Crystallization Trials and Microseeding:

The protein in 30 mM Tris buffer (pH 8.5) at concentration of 8 mg/ml was used and initial hits were found in PACT screen. Microcrystals from the hits crushed using beads from Hampton Research and were used as seeds. Sitting drop with vapor diffusion method were set up with 300 nl protein, 200 nl reservoir and 100 nl seed solution in eight different dilutions using Mosquito. A comparison between grid screen and seeding indicates that the later method can only produce large enough crystal to test in the beam.

Future Plans:

We got crystal from previous crystallization trial and dataset collected at MAX-lab (beamline I711) 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:

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