THE IRON FIST OF *H. INFLUENZAE*

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INTRODUCTION

Haemophilus influenzae was the first bacterial genome fully sequenced (1). It commonly lives harmlessly in the nasopharynx but can cause severe infections - especially in children and immunocompromised individuals - when it enters the bloodstream (2). The most dangerous strain, type b (Hib), can lead to sepsis, meningitis, or pneumonia (3). Fortunately, an Hib vaccine is already widely used in infants to battle the devastating consequences of the bacteria. A key factor in the pathogenicity of *H. influenzae* is its ability to acquire iron from the host, which is essential for its survival (4). The three proteins, that we studied for this research, are all involved in the infection rate of the bacteria. Understanding how these proteins function and interact can help identify strategies to disrupt iron acquisition and reduce the bacteria's ability to cause disease.

METHODS

Bioinformatics tools used:

- BLAST
- ORFfinder
- KBase
- InterPro
- AlphaFold
- TMHMM
- iTol
- Clustal Omega
- PyMol

OAPA



Figure 1: AlphaFold prediction of OpaA structure

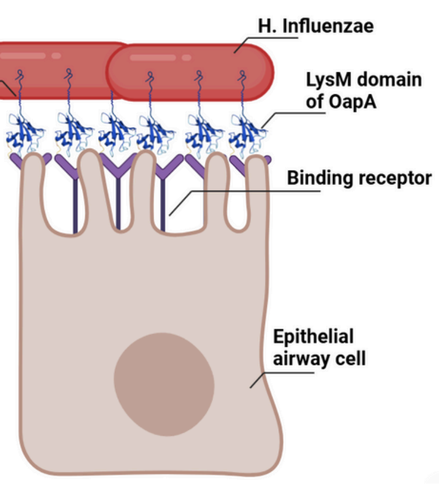


Figure 2: Schematic view of OapA mechanism with nametags. Not to scale.

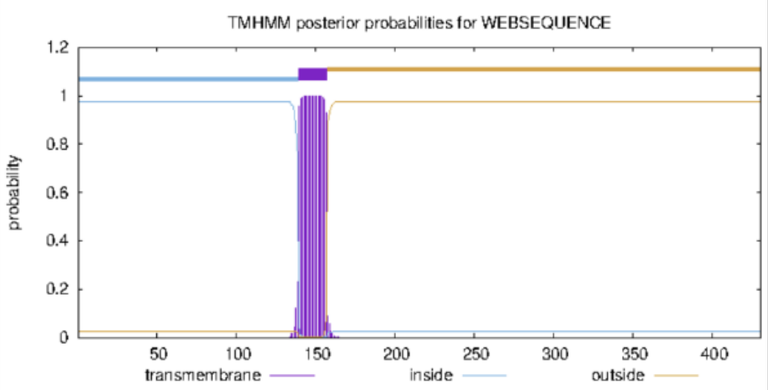


Figure 3: TMHMM result of OapA, where blue is intracellular, purple is trans-membrane and orange is extracellular

After inserting the sequence into TMHMM, OapA was found to be a trans-membrane protein. With a single TM-helix around aa-residues 140-160.

Aa residues	Domains
1-98	Intrinsically Disordered Regions (IDR)
130-160	TM Helix
351-431	LysM-like Domain

Figure 4: Interpro results of the OapA sequence put into a table. Aa residues indicates the amino acid position within the protein. "Domains" indicates the domain names.

Interpro shows an IDR at the N-terminal, which AlphaFold also shows. The first domain is around aa-residue 140-160, which is the TM-helix. The second domain is the C-terminal (LysM) domain. This C-terminal domain is also shown in AlphaFold as high confidence. The N-terminal is made up of an IDR, which can have various functions. It is believed to play a role in the phase regulation of *H. influenzae*, meaning it's useful in adapting to changing environments.

TDHA

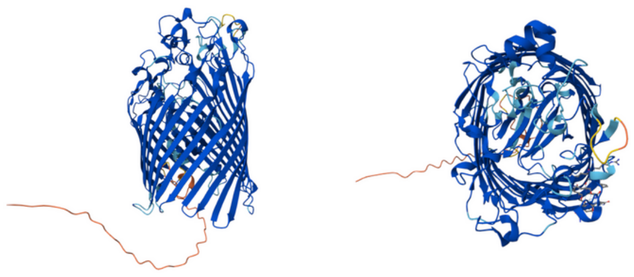


Figure 5: AlphaFold 'side' and 'top' view of TonB dependent heme receptor A

β-barrel domain: Forms the transmembrane channel through the outer membrane. It creates a cylindrical pore through which heme can be transported once the receptor is activated by the TonB system.

Extracellular loops: The loops on the top of the β-barrel face the extracellular environment. Involved in substrate recognition, binding and specifity of the receptor for heme.

Plug Domain: located inside the β-barrel. It blocks the β-barrel in the resting state, but opens through conformational change upon heme binding and TonB interaction.

N-terminal signal peptide: The signal peptide directs the protein to the outer membrane.

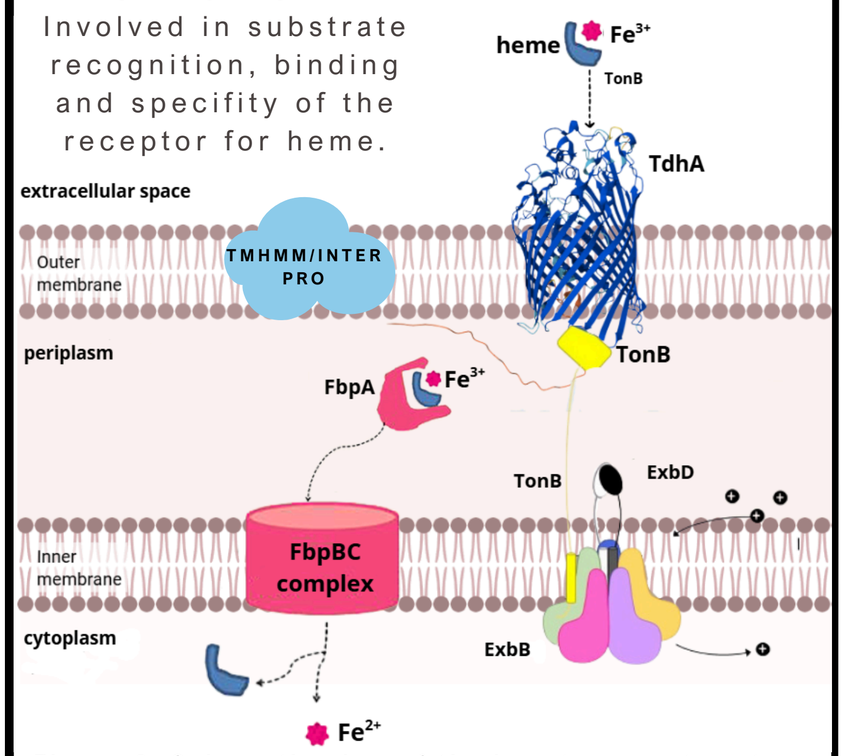


Figure 6: Schematic view of the iron transport system.

FBPC2

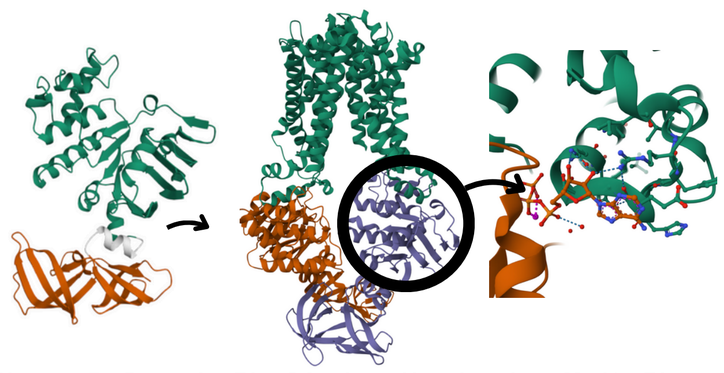


Figure 7: Protein FbpC2 visualized using Mol*. First image shows a single FbpC2 protein, the second one shows it in a complex with FbpB, and the third one shows FbpC2 with ATP bound.

After TdhA transfers Fe^{3+} to the periplasm, the FbpABC transporter takes over the ferric iron to transfer it over the inner membrane. The role of FbpC2 is the ATP hydrolysis to generate the energy needed to perform this transfer. FbpC2 is found in a complex together with FbpB, and binds ATP with residues of its alpha helices (Figure 7).

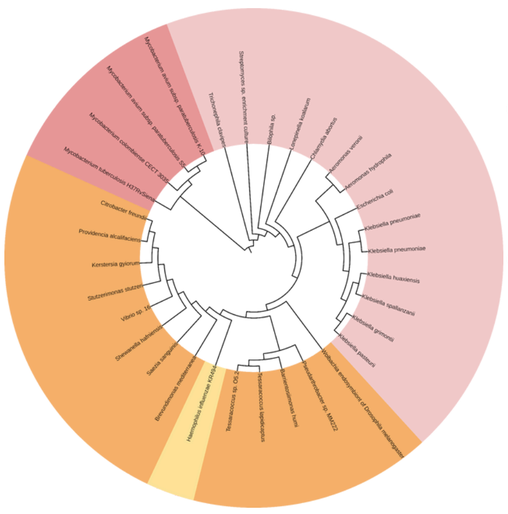


Figure 8: Phylogenetic tree of proteins similar to FbpC2 in other organisms. Closely related organisms are coloured alike and positioned in clades. *H. influenzae* is coloured in yellow. Produced using NCBI database and iTol.

Figure 8 shows the phylogenetic tree. Visible is that there are multiple organisms in which a protein is highly alike to FbpC2. Before the phylogenetic tree was made, a multiple sequence alignment was performed. This showed that the sequences in different organisms are quite alike. We thus consider the sequences to be conserved amongst organisms.

CONCLUSION

By using the mentioned bioinformatics tools, we were able to shed light on the pathogenic mechanisms of *H. influenzae* by analyzing the chosen fragments. We were able to identify three proteins of interest in the fragments; OapA, TdhA and FbpC2. These proteins all have their own function in the pathogenicity of *H. influenzae*. While OapA is important for the creation of colonies in the airway, TdhA and FbpC2 are important for the acquisition of Fe^{3+} which is necessary for the function of the bacterium.

Existing vaccines, like the Hib vaccine, target polysaccharides. Future therapies could instead focus on blocking heme acquisition (5). This approach could serve as a preventive strategy, offering new vaccine possibilities against *H. influenzae* and helping to reduce child mortality rates.

REFERENCES