Purification and Crystallization of *Porphyromonas gingivalis* NFeoAB

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Abstract

Iron is involved in crucial biological processes and is essential for nearly all living organisms. Because different oxidation states of iron are dominant within various biological niches, bacteria necessitate oxidation-state specific iron uptake systems to meet their iron requirements. In anaerobic and/or acidic environments such as those in subgingival biofilms, the more soluble ferrous (Fe^{2+}) form of iron is typically pervasive. Ferrous iron is transported via the Feo system, which contributes to the virulence of several pathogenic bacteria, including the common human pathogen *Porphyromonas gingivalis*. However, little is known about the mechanism of ferrous iron transport, which could be targeted as a potential way to combat antibiotic resistance. Within the Feo system there may be as many as three proteins: FeoA, FeoB, and FeoC (**Fig. 1 & 2**). FeoA and FeoC are soluble accessory proteins, whereas FeoB is the main transporter and is a membrane protein. Previous experiments in our lab have shown that there is an interaction between FeoA and FeoB. Our current work aims to understand the atomic-level details of this interaction within the *P. gingivalis* pathogen. To do so, we are optimizing purification conditions of *P. gingivalis* NFeoAB, a naturally occurring fusion protein. Once optimized, we plan to crystallize this fusion in order to gain three-dimensional insight into how FeoA and FeoB interact. In parallel, we are actively pursuing expression and functional characterization of the intact close homolog *Porphyromonas gulae* FeoAB. We have determined that purifying the protein using immobilized metal-affinity chromatography (IMAC) followed by anion-exchange (AIX) chromatography finished by size-exclusion chromatography (SEC) leads to highly pure *Porphyromonas gingivalis* NFeoAB. We are currently determining optimal expression conditions of *Porphyromonas gulae* FeoAB. These studies will provide insight into how FeoA and FeoB interact in pathogenic, one-component systems.

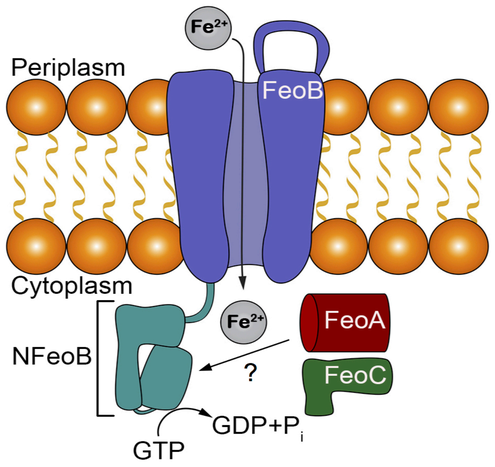


Figure 1. Cartoon depiction of the Feo system. How ferrous iron is transported via the Feo system remains unknown, including how FeoA and FeoC may contribute to the process.

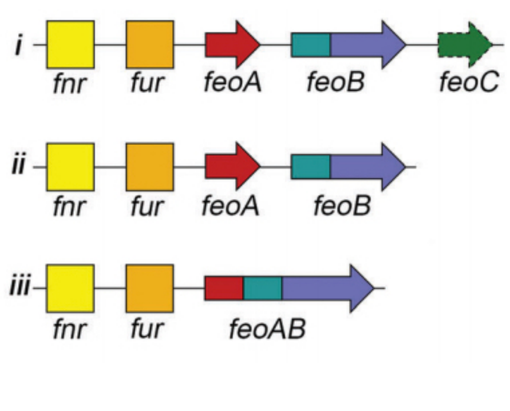


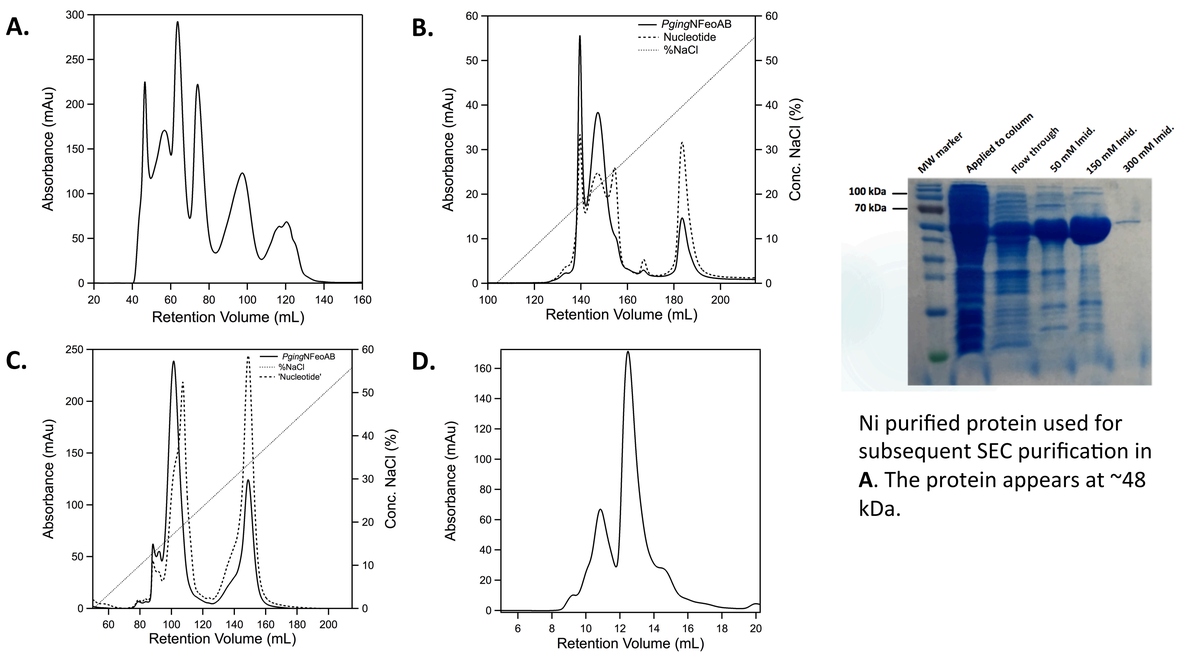
Figure 2. Several arrangements of the *feo* operons have been identified and demonstrate that FeoB is the main component of the Feo system.

Objectives

To elucidate critical, atomic-level details of the interaction between FeoA and FeoB in *P. gingivalis*, I planned to:

1. Optimize purification of *Porphyromonas gingivalis* NFeoAB using AIX and SEC
2. Optimize expression of intact *Porphyromonas gulae* FeoAB and intact *Porphyromonas gingivalis* FeoAB for subsequent purification
3. Crystallize and determine the structure of *P. gingivalis* NFeoAB to understand how FeoA and FeoB interact

Purification of *P. gingivalis* NFeoAB

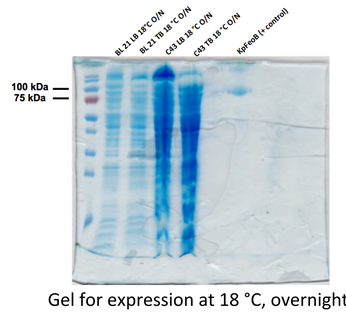


Ni purified protein used for subsequent SEC purification in **A**. The protein appears at ~48 kDa.

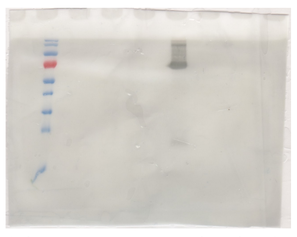
Figure 3. Purification of *P. gingivalis* NFeoAB. **(A)** Preliminary SEC purification of *P. gingivalis* NFeoAB demonstrates that the oligomeric states cannot be resolved. **(B)** Preliminary AIX purification using a 0-2M NaCl gradient shows nucleotide-bound protein and unresolved peaks. **(C)** A modified AIX purification using a 0-1M NaCl gradient. Separation of the nucleotide is still achieved and resolution of the main peak is improved. **(D)** Subsequent SEC purification of protein purified in **C**, demonstrating that the modified AIX purification protocol improves oligomeric homogeneity.

Expression Testing of *P. gulae* FeoAB

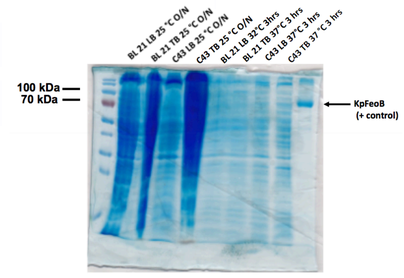
The most common arrangements of the Feo system are the three- and two-component systems in which FeoA (**Fig. 5A**), FeoB (**Fig. 4**) and/or FeoC are encoded as standalone proteins (**Fig. 2 i & ii**). These arrangements occur in ~13% and ~54% of all sequenced bacterial genomes, respectively, and are by far the most studied. Collectively, *feoA* is present alongside *feoB* in ~80% of all sequenced bacterial genomes, suggesting that FeoA is an essential component of the Feo system. Additionally, FeoA contains an SRC homology 3 (SH3) fold, which typically mediates protein-protein interactions by recognizing an xPxxP epitope on its partner protein. Intriguingly, NFeoB contains an xPxxP epitope (**Fig. 5B**) Thus, we hypothesize that the SH3 fold of FeoA recognizes the xPxxP epitope on FeoB and may contribute to the ferrous iron transport process. Further evidence suggesting that FeoA and FeoB interact comes from the existence of one-component systems in which bacteria naturally encode for FeoA tethered to NFeoB (**Fig. 2 iii**). However, this arrangement only occurs in ~3% of bacterial genomes, are poorly understood, and no crystal structures exist for neither the soluble domain nor the intact protein. By optimizing the expression of *P. gulae* FeoAB and *P. gingivalis* FeoAB, we will be able to study the intact, one-component systems and gain insight into how FeoA and FeoB interact.



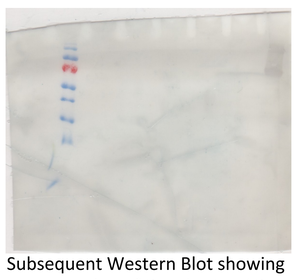
Gel for expression at 18 °C, overnight



Subsequent Western Blot showing no expression of *P. gulae* FeoAB



Gel for expression at 25 °C, overnight and 37 °C, 3 hours



Subsequent Western Blot showing no expression of *P. gulae* FeoAB

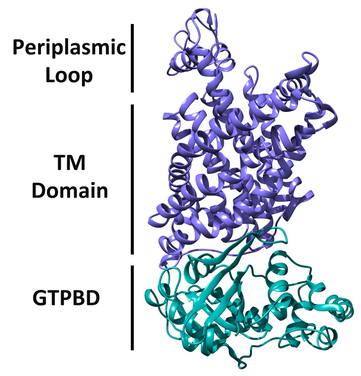


Figure 4. *De novo* model of *Ec*FeoB. The periplasmic loop, the transmembrane domain, and the GTP binding domain are labeled. The GTPBD contains the xPxxP epitope.

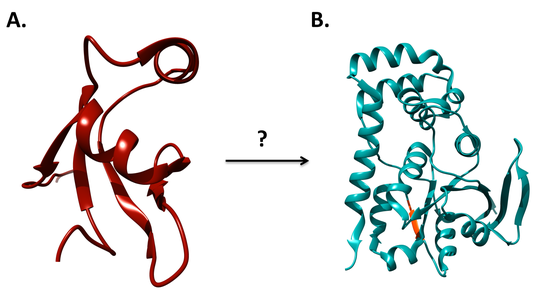


Figure 5. NMR structure of *Ec*FeoA and crystal structure of *Ec*NFeoB. **(A)** SH3 folds, found in FeoA, commonly mediate protein-protein interactions. **(B)** An xPxxP epitope, recognized by SH3 folds, is present in the GTPBD of FeoB, colored orange.

Conclusion

1. **Using a 0-1M NaCl gradient during AIX purification of *P. gingivalis* NFeoAB, instead of a 0-2M NaCl gradient, improves separation of protein from nucleotide and improves the resolution of the main peaks.**
2. **Subsequent SEC purification of *P. gingivalis* NFeoAB demonstrates that oligomeric homogeneity of the protein can be improved and sufficient resolution can be achieved for crystallization.**
3. **Expression testing of full length *P. gulae* FeoAB shows that the protein does not express.**

Future Directions

1. **Further optimize the purification of *P. gingivalis* NFeoAB for crystallization**
2. **Express and purify intact *P. gulae* FeoAB for subsequent structural studies**
3. **Optimize the expression of other FeoAB constructs**
4. **Determine how FeoA affects Fe^{2+} transport and GTPase activity**

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