Characterization of the β-barrel Assembly Machinery in *Fusobacterium nucleatum*

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Fusobacterium nucleatum is a Gram-negative, anaerobic human oral microbiome constituent. Though a steady state commensal, this pathobiont plays a role in periodontal disease, endodontic infections, pre-term births, and colorectal cancer. *F. nucleatum* achieves its pathogenicity through a class of outer membrane proteins (OMPs) called adhesins. Several key adhesins are β-barrel OMPs, highlighting the importance of the β-Barrel Assembly Machinery complex (BAM). BAM plays a vital role in the biogenesis of β-barrel OMPs in Gram-negative bacteria. The most studied BAM complexes are found in Proteobacteria and are composed of an OMP BamA and several accessory lipoproteins. Notwithstanding diversity in numbers of BAM complex components among Gram-negative bacteria, BamA and BamD are consistently essential for viability. However, preliminary searches of the *F. nucleatum* genome have revealed only the presence of BamA, the integral membrane protein of the complex. The nature of auxiliary proteins in *F. nucleatum* and the structure of its BamA (*Fn*BamA) remain unresolved. Thus, the objective of this study is to determine both the protein structure of *Fn*BamA and to identify the accessory proteins with which it binds. We employ techniques including X-ray crystallography and cryo-electron microscopy (cryo-EM) to accomplish the structural aims of this proposal and have obtained the first-ever structure of *Fn*BamA using single-particle cryo-EM. Pull-down assays coupled with proteomics elucidate the identity and nature of proteins accessory to BamA. In vivo mutagenesis of *Fn*BamA will reveal effects on organismal viability and subsequent ability to infect mammalian cells. This research is innovative in its exploration of membrane protein biology in *F. nucleatum* through examining BAM, an intricate system unstudied in this challenging anaerobic organism. We expect to advance the mitigation of *F. nucleatum* pathogenesis in the oral microbiome and beyond by detecting the binding partners and solving the structure of *Fn*BamA.