

Protein synthesis is a complex process involving multiple steps. Errors at any stage can result in misfolded, fragmented or incorrectly localized proteins that are non-functional or even toxic, disrupting essential biological processes. Cells have, therefore, evolved protective mechanisms that ensure proteins are synthesized, folded and assembled efficiently, while clearing fragments and misfolded proteins that could cause harm. Gram-negative bacteria have outer and inner cell membranes enclosing a space called the periplasm, and protein quality control is important to maintain the heavy traffic of proteins moving through this compartment. This especially applies to proteins known as β -barrel outer membrane proteins (OMPs), which are exported from cytoplasm and must cross the periplasm as unfolded precursors (uOMPs) before being inserted into the outer membrane by a β -barrel assembly machine (BAM). OMP quality is maintained by periplasmic proteins known as chaperones, which prevent the folding and aggregation of uOMPs during their passage but keep them in a state ready for insertion into the membrane. They work in concert with protein-digesting enzymes (proteases) that catch any aberrant proteins that have escaped the chaperones. The most important protease is DegP, which is normally found as a mixture of hexamers and trimers in the periplasm. However, when the quantity of uOMPs increases, DegP assembles into larger superstructures that trap and recycle the uOMPs. We have studied the proteolytic enzymes produced by *Porphyromonas gingivalis*, an important pathogen responsible for gum disease (periodontitis), revealing a very large protein with a domain that resembles a protease known as toxilysin (the active component of toxins produced by strains of *Escherichia coli* and *Citrobacter freundii*, responsible for diarrhea). We have confirmed the activity of this protein and named it zuzalysin. By comparing the zuzalysin sequence to all known bacterial genomes, we found related genes in 12 of 41 major bacterial taxonomic groups (phyla), with a particular abundance in species belonging to the phylum Bacteroidota. The subsequent characterization of zuzalysin showed that it is located in the periplasm and is attached to the outer membrane. Structural analysis using cryogenic electron microscopy (cryo-EM) and X-ray diffraction of protein crystals revealed a novel pentameric structure of zuzalysin accompanied by bipentamers and dodecapentamers. The latter, which are huge oligomeric cages built of 12 pentamers, are the largest known metal-catalyst proteolytic machines discovered thus far. Using an artificial intelligence program called *AlphaFold-3*, we confirmed the zuzalysin's propensity to form pentamers, with the site that cleaves proteins facing the interior chamber of the pentamer. Based on these remarkable, fascinating results, we propose that zuzalysin superstructures in the periplasm control the quality of periplasmic proteins or degrade oligopeptides for import of short peptides into the cytoplasm and/or may be released as virulence factors that help the bacteria to initiate infections. The proposed project will therefore test the following hypotheses: *(i)* zuzalysin is exported to the periplasm, where it acts as an active homopentamer or an inactive bipentamer; *(ii)* if the cell membrane is breached, zuzalysin is exported to the extracellular space, where larger zuzalysin superstructures act preferentially on unfolded proteins; *(iii)* zuzalysin-like proteins from other members of the phylum Bacteroidota have the same propensity to form oligomeric structures that regulate their proteolytic activity; and *(vi)* zuzalysin contributes to extracellular virulence either indirectly (a requirement for the fitness of *P. gingivalis* in the harsh environment of inflamed periodontal pockets) or directly, by breaking down bactericidal peptides, as suggested by our preliminary results.