bacteria is a rational approach to improve their specific activity and gives rise to promising antibacterials.

The improved activity of modular endolysins over the globular ones raises a question: Why only some endolysins from a Gram-negative origin evolved to a modular design, if it provides such a benefit (Briers et al. 2009)? The presence of an OM on Gram-negative bacteria that impairs lysis of new potential host cells by the released endolysins from previous burst phage-infected cells and the much less thick peptidoglycan of Gram-negative bacteria (which does not require such a high muralytic activity as the thicker peptidoglycan of Gram-positive bacteria) do not seem to imprint enough selective pressure to maintain a CBD (and thus a larger modular enzyme) on endolysins from a Gram-negative background.

15.3.3.2.4 Protein Engineering of Gram-Negative Targeting Endolysins

The use of endolysins against Gram-negative in a clinical and therapeutic context requires a product that presents no toxicity against eukaryotic cells, most likely protein based. Consequently, protein engineering is a promising strategy to build new endolysin-based chimeras that can be used as antibacterials on Gram-negative pathogens. With this in mind, a new class of antibacterials called Artilysin® was created. These chimeras are created through the fusion of an LPS-destabilizing peptide to either the N- or C-terminus of endolysins, without affecting the endolysin secondary and tertiary structures. The ability of these peptides to destabilize the LPS is based on their amphipathic or polycationic properties, allowing the endolysin to get access to the peptidoglycan and kill the bacterial cells (Gerstmans et al. 2016).

This concept was tested with the most active endolysins, those presenting a modular structure, to assess if Artilysins could further optimize their activity. Therefore, different peptides were fused to the endolysins OBPgp279 and PVP-SE1gp146 and challenged against multidrug-resistant strains of *P. aeruginosa*, *E. coli*, and *S.* Typhimurium, which resulted in an increased antibacterial activity of the parent endolysins, reaching a reduction of 2.61 log units in just 30 minutes on the cell counts of *P. aeruginosa*. However, no significant reduction was observed on *E. coli* and *S. enterica*, showing that the sensitivity to Artilysins may be dependent on the LPS structure. By optimizing the length of the peptide linker between the peptide and the endolysin, the reduction was increased to 3.41 log units (Briers et al. 2014).

The mechanism by which Artilysin destabilizes the OM is different from that of EDTA since the synergy with EDTA is not compromised. The addition of EDTA further improved the chimera activity up to more than 5 log unit reduction on the cell counts of *P. aeruginosa* (Briers et al. 2014).

A new Artilysin created through the fusion of SMAP-29 (the 29-amino-acid sheep myeloid antimicrobial peptide) to the N-terminus of the modular endolysin KZ144 was shown to have a high and fast bactericidal effect on