SYNTHETIC BIOLOGY OF MODULAR ENZYMES: FROM ENZYMES TO ENZYBIOTICS

Hans Gerstmans, Department of Applied Biosciences, Ghent University
Hans.gerstmans@ugent.be
Rob Lavigne, Department of Biosystems, Ghent University
Yves Briers, Department of Applied Biosciences, Ghent University

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Over the past few years antimicrobial resistance has evolved from a rare event to an everyday occurring problem in health care. The future looks even more grim due to the increase of antimicrobial resistance against antibiotics and the unprecedented discovery void of new antibiotic classes. Enzyme-based antimicrobials or enzybiotics represent a novel class of antibacterials. Specifically, endolysins encoded by bacterial viruses (bacteriophages) that degrade the peptidoglycan layer, have gained tremendous interest with many proof-of-concept studies up to clinical phase studies.

Initially, native endolysins were only considered for Gram-positive bacteria as the peptidoglycan layer of Gram-negative bacteria is protected by the outer membrane. However, Gram-negative pathogens constitute the largest threat for health care given the higher extent of multi- and pan-drug resistance and lower number of recently developed antibiotics or antibiotics in the pipeline. Using enzyme engineering, we have expanded the spectrum to Gram-negative bacteria. This is achieved by fusing outer membrane permeabilizing peptides via a linker to endolysins. The peptide locally destabilizes the outer membrane and transfers the endolysin moiety across the outer membrane, followed by active peptidoglycan degradation. Exposure of Gram-negative bacteria to these engineered enzybiotics (Artilysin®s) results in a prompt, highly bactericidal effect, which has been confirmed in *in vitro* keratinocyte cultures and nematodes. Case studies in wound care treatment of dogs have shown a successful outcome.

Enzymes are an unusual source for the development of antibacterials, but we have shown that exactly this enzymatic nature provides these engineered enzybiotics with novel features for antibacterials. First, they are rapid and act immediately upon contact. Real-time time-lapse microscopy shows that cells are killed within seconds. Second, they show no cross-resistance with existing antibiotics due to the novel mode-of-action and do not provoke resistance development. Third, they actively degrade all bacterial cells regardless if they are metabolically active or not, whereas traditional antibiotics require an active metabolism. Therefore, engineered enzybiotics are able to kill metabolically dormant persisters that cause recurrent, chronic infections (Briers et al., 2014; Gerstmans et al., 2016).

A unique feature of this class of enzybiotics is the engineering potential. They are modular proteins, comprising different domains: an outer membrane permeabilizing peptide, a linker sequence and an endolysin, which in turn comprises a cell wall binding domain and an enzymatically active domain. Depending on the modular composition and the specific order of modules, its enzymatic and antibacterial properties, expression level and stability can be modulated. Using combinatorial shuffling in a synthetic biology approach, we show how targeted antibacterials with diverse properties can be constructed.

In sum, engineered enzybiotics provide a platform approach for customized development of antibacterials with unique features based on their enzymatic nature.

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