Slide 1
In this topic, you will see how a phage protein, called endolysin, has been transformed towards antimicrobial treatment that is capable of killing multi-drug resistant bacteria. First of all, it will be highlighted why we need new antimicrobial treatments. Next, I will explain how we can engineer endolyisins to become an antimicrobial treatment.

Slide 2
A very recent report of the World Bank tried to quantify what the current rate of antimicrobial resistance development could mean to the world. In total, this could lead to a global loss of 100 trillion dollar by 2050 if no serious action is undertaken. As such, we need a new, innovative vision to develop new antibiotics to tackle these multidrug resistant bugs and designer enzybiotics or enzyme-based antibiotics could provide the solution.

Slide 3
As seen in section 1.1, the bacterial virus or bacteriophage preys on bacteria in order to replicates itself. After attachment on a viable bacterial host, the phage will inject its genetic material, which will transform the bacterium in a virus producing machine. However, in the final stage of replication, the bacterium is completely filled with new phages, but they cannot escape the bacterium as the bacterial cell wall is still intact. Therefore, other phage proteins, called holins and endolysins come into play. Endolysins digest the bacterial peptidoglycan layer, which results in lysis of the host and release of the viral progeny.

Slide 4
So, at the end the new phages are produced, but cannot escape from the inside of the bacterium. Also, the endolysins are already produced but cannot reach the peptidoglycan as the cytoplasmic membrane blocks the access towards it. Therefore, a phage protein, called holin, makes holes into the cytoplasmic membrane by which the endolysins can pass. Upon reaching the peptidoglycan layer, the endolysins start degrading it until it is so weakened that the cell spontaneously lysis and thereby releases the new phages. Generally, endolysins consist of 2 domains: a cell-wall binding domain or CBD, which recognizes a specific target in the peptidoglycan and is represented by a hand. If the CBD is unbound, the hand will be open. When the specific target is recognized by the CBD it will bind to it and the hand will be closed. The second domain is the enzymatic active domain or EAD that will degrade the peptidoglycan and is represented by the “pac-man” figure. In the last figure, you can see the different enzymatic activities of endolysins.

Slide 5
As stated before, bacteria are becoming more and more resistant towards antibiotics. For this reason, people are searching for new antibacterial treatments and the use of endolysins is a very promising one. At the beginning of the century, it was discovered that exogenously added endolysins could lyse Gram-positive bacteria as easy as if they acted from the inside. Also, the principle remains the same, if the peptidoglycan layer is degraded sufficiently, the bacterium will lyse. Several different architectures have been observed in nature: several EADs or CBDs can be present, EADs or CBDs can be differently oriented.

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Unfortunately, native endolysins have only a limited antibacterial activity against Gram-negative bacteria when they are added exogenously. This is due to the outer membrane that forms an impermeable barrier to almost everything. Recently, this barrier was tackled by modifying the endolysins using protein-engineering to combine the self-promoted uptake mechanism of outer membrane permeabilizing peptides and peptidoglycan-degrading activity of endolysins to broaden the use of endolysins. These designer endolysins were coined Artilysins. In this movie, you can see an Artilysin at work against a multi drug resistant Pseudomonas strain. The outer membrane permeabilizing peptide will interfere with the stabilizing forces of the outer membrane and will form a wedge through the OM. Doing this, it will pull along the endolysin part, consisting of an EAD and CBD, through the outer membrane. Upon reaching the thin peptidoglycan layer of Gram-negative bacteria, the endolysin part will start degrading it, resulting in lysis and dead of the bacterium.

Slide 7

Endolysins proved to have additional benefits: the most important one is that bacteria cannot become resistant to them; endolysins work very fast, within minutes and they only kill the bad bugs, leaving the beneficial once unharmed. Also, as endolysins exist of several modules, we can exchange these modules and engineer endolysins to have another spectrum, increased thermostability or a longer shelf-live.