Disruption of the posttranslational activation of the translation factor EF-P as a new target for antimicrobial agents.

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Since the discovery of penicillin, many previously deadly infections become easily curable. The development of antibiotics saved countless lives and contributed to significant elongation of the expected lifespan. Unfortunately, this "miracle" is not given once forever as bacteria do evolve and develop various defence mechanisms leading to their resistance to known therapies. Moreover, they tend to exchange once-developed resistance between each other and overuse of known antibiotics accelerates this process. This requires constant effort by scientists and medics to look for new drugs to fight pathogenic bacteria. In 2017 WHO published a list of high-risk bacteria, among them Gram-negative *Salmonella spp.*, *Acinetobacter spp.*, and *Shigella spp.*, against which no new therapies were approved in the past decade

A good starting point for the development of new drugs is the identification of processes that are indispensable for the survival of the pathogenic cell and are either absent or significantly different from the host. The translation is a process of decoding the genetic information contained in mRNA to assemble a series of amino acids in a defined sequence forming proteins, the latter being workhorses for virtually all cellular functions. This highly sophisticated process is essential for all life and is governed by Ribosomes. Unsurprisingly these marvelous molecular machines built as complexes of nucleic acids and proteins are already targets of several groups of antibiotics.

Not all amino acid combinations are equally easy to assemble. The translation process is more difficult for given amino acid sequences, for example, a stretch of two, three, or more consecutive prolines often results in ribosomal stalling. Interestingly polyproline stretches (polyPro) due to their specific, rigid nature usually play a significant role in proteins, therefore evolution instead of opting against them developed means to overcome the related difficulty. During the process of translation, ribosomes are assisted by a number of so-called elongation factors that regulate various aspects of protein synthesis and help them to work efficiently. One of them – elongation factor P (EF-P) is dedicated to alleviating polyPro induced stalling. EF-P is an ortholog of eucaryotic elongation factor eIF5A, but in spite of the similarity in function and partly in the structure their activity relies on completely different posttranslational modifications. In eIF5A activation always requires a polyamine-dependent hypusination of a conserved lysine. Activation of EF-P may vary between bacterial species and most frequently relies on β-lysinylation of lysine. In some species rhamnosylation or 5-amino-were also reported alternative posttranslational modifications activating EF-P. The said modifications are also introduced by a different set of enzymes therefore the risk of cross-reactivity of potential inhibitors is largely limited. Importantly it was demonstrated that activated (i.e. β-lysinylated) EF-P is crucial for both *Salmonella* and *Shigella* virulence and deletion of either EF-P or its modifying enzyme renders the bacterium extremely susceptible to stresses such as even low and otherwise harmless doses of antibiotics.

The aim of this project is the structural characterization of enzymes involved in the activation of EF-P in pathogenic Gram-negative bacteria and the development of selective inhibitors of the process. To tackle the later problem a structure-guided drug development will be initialized. A novel technique – crystallographic fragment screening will be used to screen hundreds of low-weight compounds and select the most promising binders. These will subsequently be subjected to hit-to-lead development leading to new chemical scaffolds with the potential to aid our battle against antibiotic resistance.