# Structural studies of *Staphylococcus aureus* ribosome hibernation

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Bacteria and especially pathogenic species often cope with stress and nutrients limitations, and their survival strongly depends on the ability to preserve energy. One of the most beneficial mechanism to save energy for bacterium is deactivation of its own translation machinery, namely hibernation of ribosomes. In *S. aureus* this process is prompted by binding of stress protein SaHPF (*S.aureus* hibernation promoting factor) to 70S ribosomes, which triggers their association into inactive 100S ribosome dimers (disomes). In such stand-by mode ribosomes are preserved from degradation and from binding of several antibiotics, at the same time, they can rapidly (within 1-2 min) be transformed back into active ribosomes upon energy recovery. Unlike other bacteria, in *S.aureus* SaHPF is constitutively expressed in the cell, and part of ribosomes are converted into hibernating 100S even in rich media. Finding the way to prevent dimerization may significantly decrease survival ability of *S.aureus*.

Our recent cryo-EM studies demonstrated that integrity of giant 100S disomes (5 MDa) is maintained primarily by interactions between C-terminal domains (CTD) of SaHPF with each other [1]. N-terminal domain (NTD) of SaHPF binds to the 30S subunit at the P-site and A-site and may inhibit protein synthesis as observed for shorter variants of HPF in other species [2]. We have determined the solution structure of SaHPF-NTD using NMR spectroscopy and analyzed its binding with *S. aureus* 70S ribosome by HET-SOFAST NMR experiments and determined several protein regions which are close to the ribosome RNAs. Based on NMR and crystal structures of SaHFP-CTD we predicted the key amino acids and point mutations which may distort SaHPF dimers. Predicted mutants were purified and analyzed using several biophysical methods (i.e. AUC, DLS, gel filtration coupled MALS, native PAGE). According to our data, hydrophobic residues F160, Ile173, Tyr175 are the key players in SaHPF dimerization. Selected mutations will be next introduced into *S.aureus* cells in order to analyze their survival rate as well as their infectivity in mice. Our work paves the path for exploring of complementary approaches to combat this severe pathogen.

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1. Khusainov I., Vicens Q., et al., The EMBO journal. 36 (2017).
2. Usachev K., Ayupov R., et al., Biomolecular NMR Assignment. 12 (2018)