



NMR assignments of the N-terminal domain of *Staphylococcus aureus* hibernation promoting factor (SaHPF)

Konstantin S. Usachev^{1,2} · Rustam Kh. Ayupov² · Shamil Z. Validov² · Iskander Sh. Khusainov^{2,3} · Marat M. Yusupov^{2,3}

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Abstract *Staphylococcus aureus*: hibernation-promoting factor (SaHPF) is a 22.2 kDa stationary-phase protein that binds to the ribosome and turns it to the inactive form favoring survival under stress. Sequence analysis has shown that this protein is combination of two homolog proteins obtained in *Escherichia coli*—ribosome hibernation promoting factor (HPF) (11,000 Da) and ribosome modulation factor RMF (6500 Da). Binding site of *E. coli* HPF on the ribosome have been shown by X-ray study of *Thermus thermophilus* ribosome complex. Hence, recent studies reported that the interface is markedly different between 100S from *S. aureus* and *E. coli*. Cryo-electron microscopy structure of 100S *S. aureus* ribosomes reveal that the SaHPF-NTD binds to the 30S subunit as observed for shorter variants of HPF in other species and the C-terminal domain (CTD) protrudes out of each ribosome in order to mediate dimerization. SaHPF-NTD binds to the small subunit similarly to its homologs EcHPF, EcYfiA, and a plastid-specific YfiA. Furthermore, upon binding to the small subunit, the SaHPF-NTD occludes several antibiotic binding sites at the A site (hygromycin B, tetracycline), P site (edeine) and E site (pactamycin, kasugamycin). In order to elucidate the structure, dynamics and

function of SaHPF-NTD from *S. aureus*, here we report the backbone and side chain resonance assignments for SaHPF-NTD. Analysis of the backbone chemical shifts by TALOS+ suggests that SaHPF-NTD contains two α -helices and four β -strands (β 1- α 1- β 2- β 3- β 4- α 2 topology). Investigating the long-term survival of *S. aureus* and other bacteria under antibiotic pressure could lead to advances in antibiotherapy.

Keywords HPF · Hibernation · Pathogen · Ribosome · Protein NMR · Resonance assignment

Abbreviations

IPTG	Isopropyl-thio- β -D-galactoside
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
RMF	Ribosome modulation factor
SaHPF	<i>Staphylococcus aureus</i> hibernation promoting factor
SaHPF-NTD	N-terminal domain of SaHPF
SaHPF-CTD	C-terminal domain of SaHPF
Cryo-EM	Cryo-electron microscopy
PIC	Protease inhibitor cocktail
PMSF	Phenylmethane sulfonyl fluoride

✉ Konstantin S. Usachev
k.usachev@kpfu.ru

¹ NMR laboratory, Medical Physics Department, Institute of Physics, Kazan Federal University, 18 Kremlevskaya, Kazan, Russian Federation 420008

² Laboratory of Structural Biology, Institute of Fundamental Medicine and Biology, Kazan Federal University, 18 Kremlevskaya, Kazan, Russian Federation 420008

³ Département de Biologie et de Génomique Structurales, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, Université de Strasbourg, 1 rue Laurent Fries, 67400 Illkirch, France

Biological context

Staphylococcus aureus (*S. aureus*) is one of the most dangerous pathogens for humans, it causes variety of community associated and nosocomial infections. Constantly increasing antimicrobial resistance of *S. aureus* indicates the strong requirement in development of the new drugs against this pathogen. Most of antibiotics efficiently used in clinic (more than 40%) target bacterial ribosome, a ribonucleoprotein particle synthesizing proteins in a cell. Most of them selectively inhibit translation machinery of