Expression and Purification of Bacterial Membrane Receptor Domains: The case of the Heme receptor HasR from Pseudomonas aeruginosa

Federico Bosetto*[1], Maria Zacharopoulou*[1], Mark Bycroft[1], Giovanna Zinzalla[1], Monika Kish[2], Pamela J. Rowling[1], Stephen H. McLaughlin[3], Jonathan J. Phillips[2], Laura S. Itzhaki[1], Ioanna Mela[1]

[1]Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1PD, UK
[2]University of Exeter, Living Systems Institute, Stocker Road, Exeter, EX4 4QD, UK
[3]Medical Research Council Laboratory of Molecular Biology, Francis Crick Ave, Trumpington, Cambridge, CB2 0QH, UK
E-mail: fb604@cam.ac.uk

HEME ASSIMILATION SYSTEM

One of the *Pseudomonas aeruginosa*'s iron acquisition methods involves the extracellular hemophore (HasA) which transfers heme to the receptor (HasR), forming the heme assimilation system[1] (*Figure 1*).

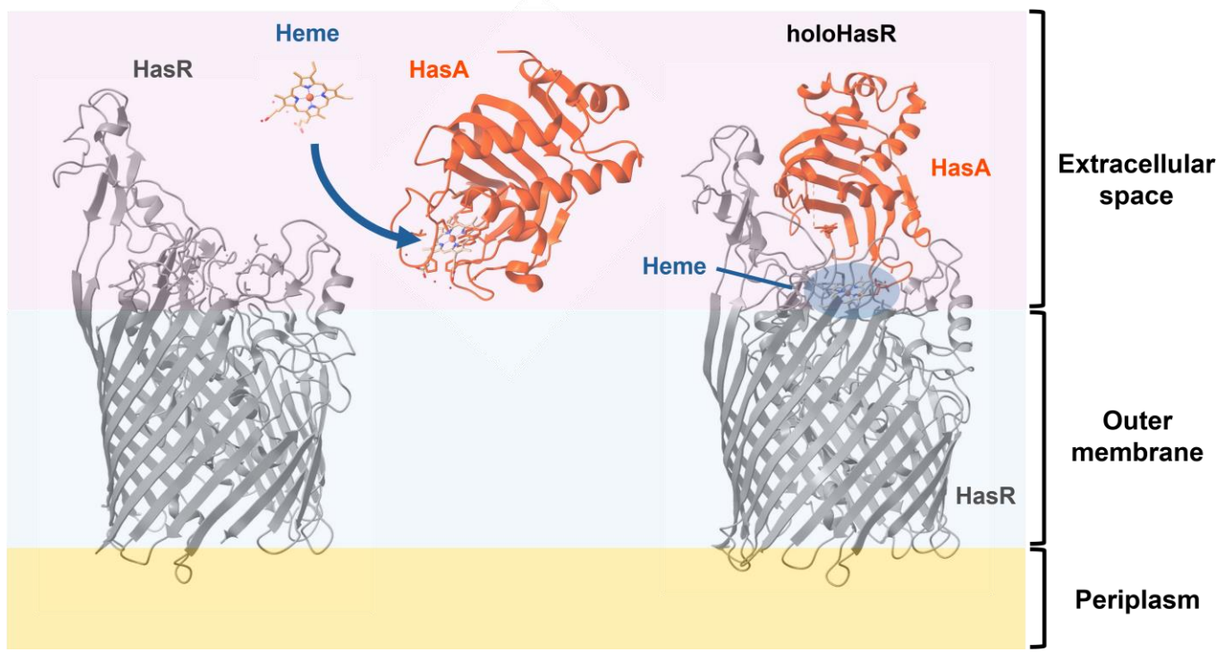


Figure 1. Heme assimilation system in *P. aeruginosa*, constituted by a receptor (HasR, in grey) and a hemophore (HasA, in orange), which binds to heme (blue arrow), and together form the holoHasR.

AIM

We expressed and characterized the N-terminal plug and the Secretin/TonB domains of HasR, both as a single construct and as individual domains (*Figure 2-A and -B*).

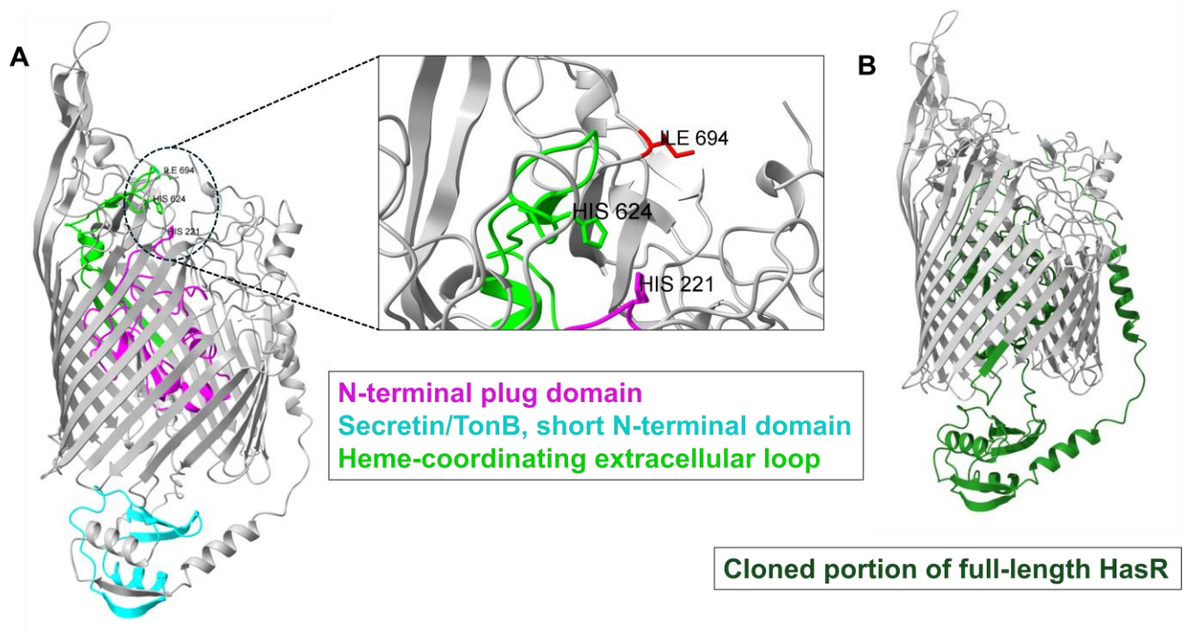
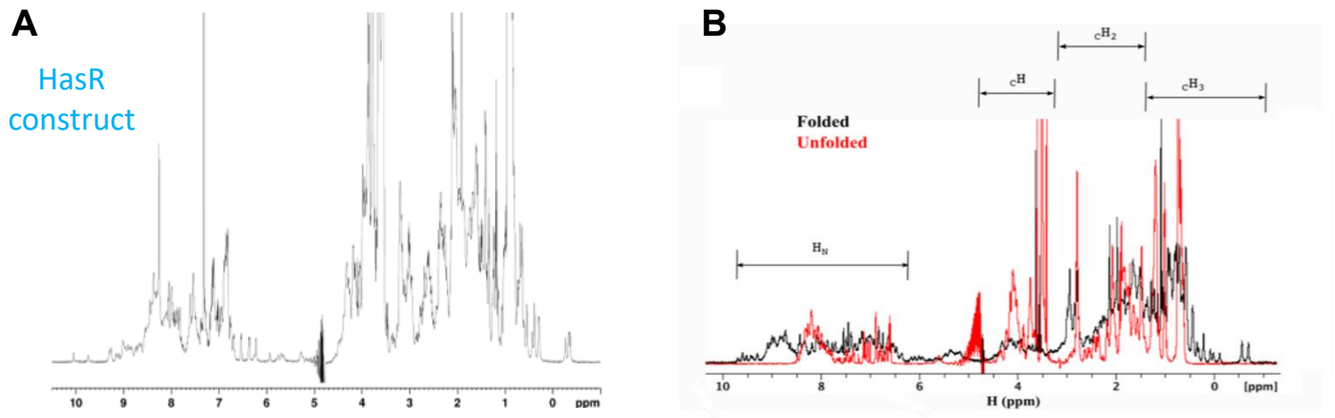


Figure 2-A and -B. (A) N-terminal plug (in purple), Secretin/TonB short N-terminal (in cyan) and heme-coordinating extracellular loop (in light green) domains of HasR in *P. aeruginosa*. ***(B)*** Highlight of the cloned portion of HasR used in this work (in dark green).

Folding of HasR CONSTRUCT and HasR INDIVIDUAL DOMAINS

NMR



HDX-MS

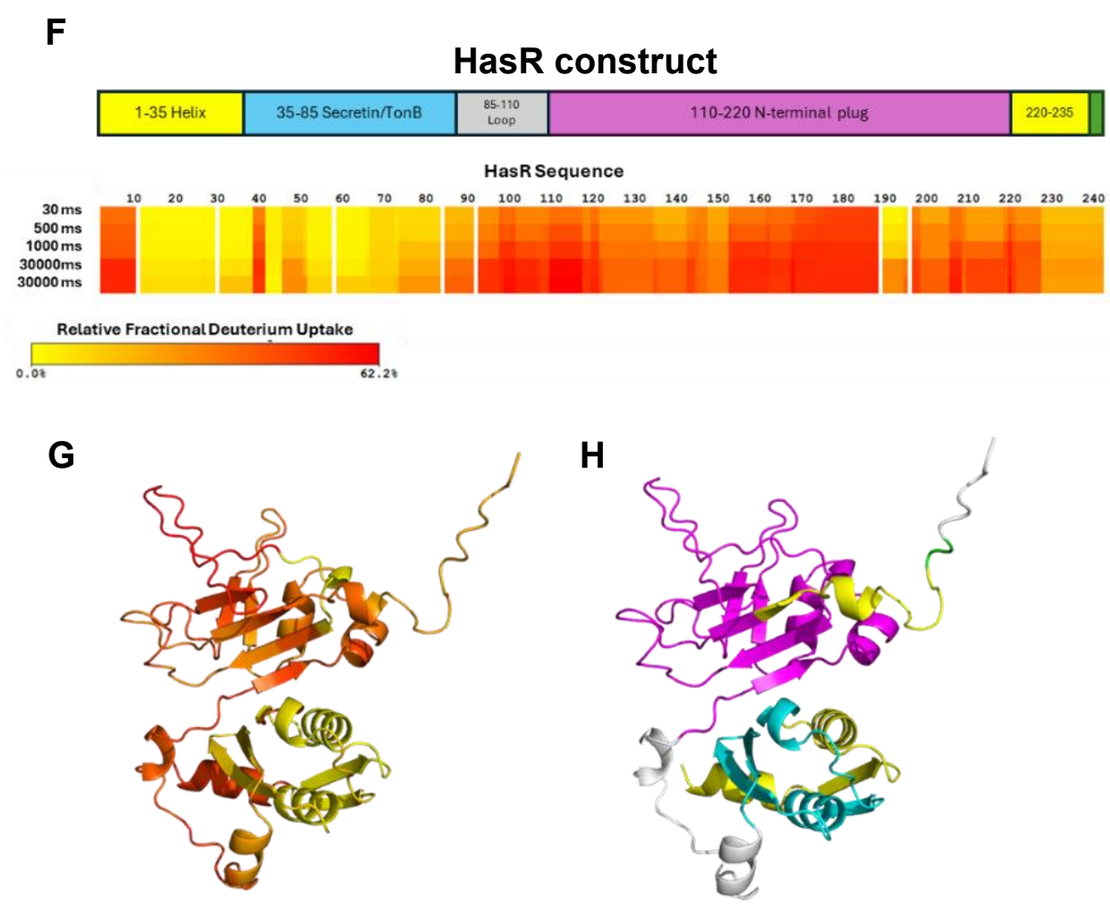


Figure 3-A, -B, -C, -D, -E, -F and -G. Folding characterization of HasR construct and HasR individual domains by NMR and HDX-MS. 1D ^{1}H spectrum of ***(A and B)*** HasR construct, ***(C)*** Secretin/TonB short N-terminal ***(D)*** and N-terminal plug domains. ***(E)*** Superimposition of the three spectra (N-terminal plug in green, Secretin/TonB short N-terminal in red and HasR construct in blue). HDX-MS of the HasR construct indicates a folded structure. ***(F)*** Deuterium uptake heatmap across the sequence of the HasR construct. Areas in yellow indicate folded and stabler regions of the protein, while areas in red indicate more dynamic or unfolded regions of the sequence (e.g. loops). ***(G)*** Superimposition of the deuterium uptake data on the HasR construct 3D structure. ***(H)*** Highlighted sequence domains on the HasR construct 3D structure (corresponding to F), to aid comparison.

The 1D ^{1}H spectra of the HasR construct *(Figure 3-A and -B)*, Secretin/TonB short N-terminal *(Figure 3-C)* and N-terminal plug domains *(Figure 3-D)* revealed good NMR signal dispersion, indicating the presence of a folded structure. In terms of HasR construct, the data showed that the protein contained a folded domain whereas the rest regions were unstructured *(Figure 3-A and -B).* The three NMR spectra were superimposed *(Figure 3-E).* The resonances corresponding to the folded N-terminal plug domain (green trace) are absent from the spectrum of the original construct, indicating that this domain is not folded in the same way when expressed alongside the Secretin/TonB short N-terminal domain (red trace), which instead remains stably folded in any case.

HDX-MS analysis identified the Secretin/TonB short N-terminal domain as fully folded and stable, whilst the N-terminal plug domain was found to be weakly stable due to the loop regions, which are presumed to be highly flexible, and β-strands that conferred a certain degree of stability upon it *(Figure 3-F, -G and -H).*

CONCLUSIONS and FUTURE PERSPECTIVES

We describe a toolkit for the purification of extracellular parts of bacterial outer membrane proteins and establish such proteins as targets for DNA aptamer selection (*Figure 4*), with the aim to develop innovative therapeutic tools to efficiently target antibiotic resistant bacteria.

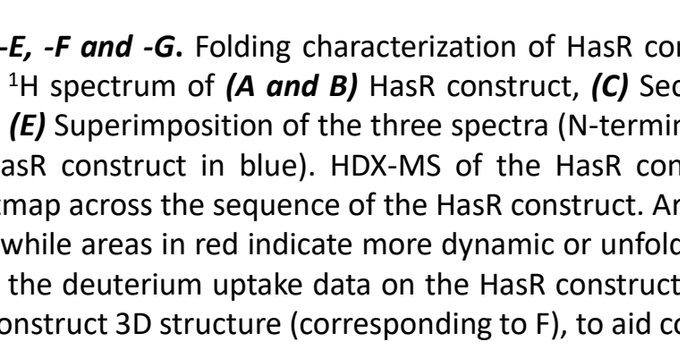


Figure 4. Representation of how an aptamer can interfere with the heme assimilation process by binding to HasR.

REFERENCES:
[1] Dent AT and Wilks A. 2020. Contributions of the heme coordinating ligands of the *Pseudomonas aeruginosa* outer membrane receptor HasR to extracellular heme sensing and transport. *J Biol Chem*. 295(30):10456-10467.

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