The ornithine-based siderophore as a potential carrier of peptide nucleic acid into *Escherichia coli* cells

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Research goal

The rapid spread of antimicrobial resistance, especially in the case of gram-negative bacteria, encourages searching for new alternative delivery ways through the restrictive outer membrane, which is an outer layer of the bacterial envelope [1].

Iron chelators, known as **siderophores**, are secreted, recognized and later transported through the outer membrane by the TonB-dependent transport system (**TBDT**) [2].

Peptide nucleic acid (PNA) is a nucleic acid mimic with high affinity towards natural nucleic acids. PNA can be used as an antisense oligonucleotide, which binds to the target mRNA in a complementary manner regulating its expression. Gene-targeting is a promising approach against various diseases, including bacterial infections. However, due to poor cellular permeability, PNA cannot act as a gene silencing agent on its own [3].

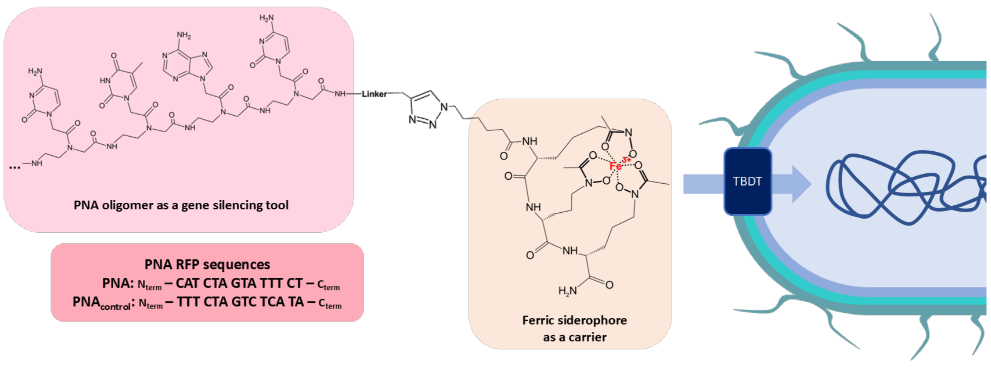

Figure 1: The Trojan horse strategy which uses the bifunctional conjugate of the synthetic siderophore and a PNA oligomer enables the passage through the *E. coli* membrane.

Our goal is to synthesize an ornithine-based siderophore, which upon conjugation with a PNA oligonucleotide will ensure non-invasive transport of PNA into *E. coli* cells. To confirm that, PNA anti-rfp sequence was used to target the reporter gene *mrfp1* expressing red fluorescent protein (RFP) inside *E. coli* cells (Figure 1).

Synthesis

Modified ornithine derivative (N^{δ}-hydroxy-N^{δ}-acetyl-ornithine) was used as a building block, because it provides hydroxamate groups capable of binding ferric iron.

S_L siderophore was synthesized using the solid-phase peptide synthesis (SPPS) technique with appropriate Fmoc strategy (Figure 2).

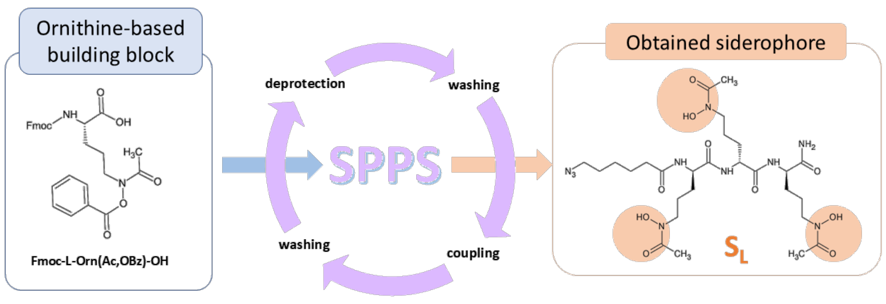

Figure 2: Chemical structures of the Fmoc-protected modified ornithine derivative used for the S_L siderophore synthesis. Hydroxamate groups are highlighted.

In order to verify its carrier potential S_L was later used for conjugation with PNA oligomers via copper-catalyzed azide-alkyne cycloaddition (also known as "click reaction"), which resulted in S_L–PNA conjugates connected via un-cleavable triazole ring (Figure 1).

PNA oligomers (Figure 1) used for conjugation were synthesized manually using appropriate SPPS protocol.

Circular Dichroism (CD) Spectroscopy

CD spectra of the synthesized siderophore mimic - S_L were recorded to verify iron(III)-coordination properties and later were compared to CD spectra of two natural siderophores - coprogen and ferrichrome (Figure 3). Upon binding iron(III) siderophores obtain structure, which can be assigned to Δ or Λ optical isomer structure using CD analysis. Ferrichrome complex represents Λ configuration and coprogen obtains structure for Δ isomer. [4]

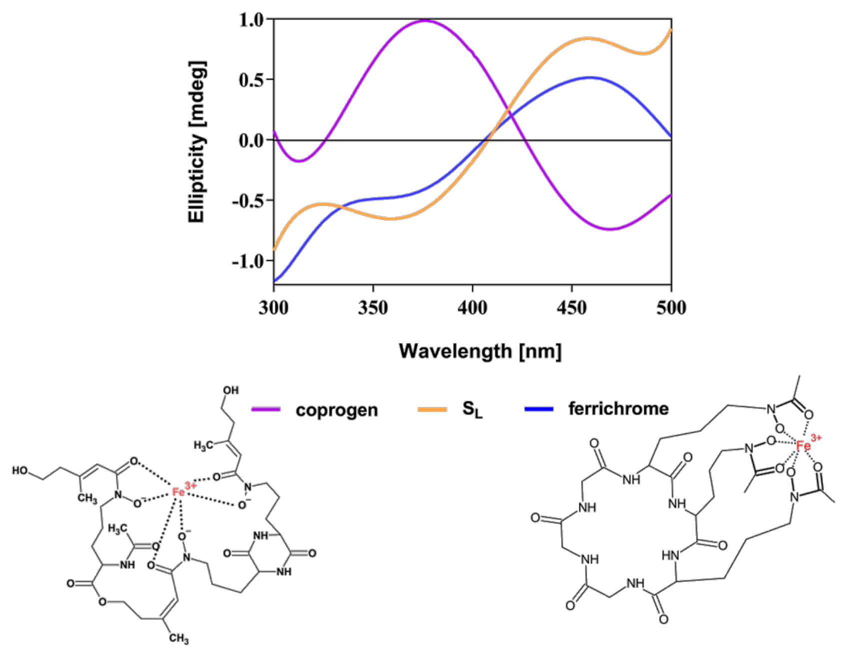

Figure 3: CD spectra of the natural and S_L siderophores in the presence of Fe^{3+} salt. Ferric complex structure of coprogen (left) and ferrichrome (right) are included. CD spectra of a natural cyclic siderophore – ferrichrome (and its ferric complex structure included on the right) is detected as Λ optical isomer (blue line).

Based on the CD spectra obtained for S_L, this siderophore chelates ferric iron adopting the structure of the isomer (Figure 3). Similar positions of the minima and maxima in the Fe(III)-S_L complex and ferrichrome spectra suggest that, while capturing iron, S_L adopts a structure comparable to the cyclic ferrichrome.

Microbial assays

After confirming the iron coordination properties of the S_L siderophore, uptake of PNA conjugates with S_L was tested on *E. coli* Δ*fur* mutant with continuous iron uptake (Figure 4, left). Bacteria were cultured in iron limiting conditions. RFP fluorescence was measured using the flow cytometry with two control cultures: bacteria carrying plasmids expressing RFP (with RFP) and bacteria lacking the RFP gene (without RFP).

Additionally, unconjugated S_L was used for the growth recovery assays. Several *E. coli* mutants lacking various TBDT receptors, proteins or reductases were used in order to investigate the S_L pathway through outer membrane receptors.

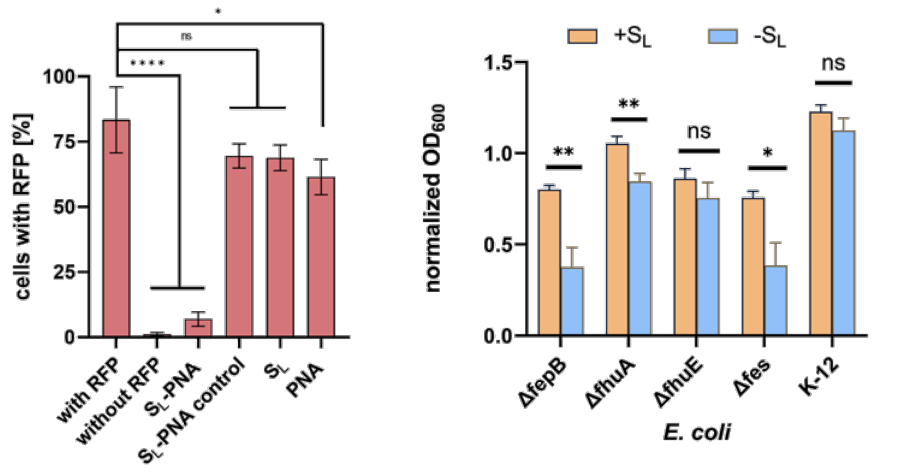

Figure 4: RFP fluorescence silencing in the *E. coli* Δ*fur* mutant cultured in iron limiting conditions (left). Growth recovery assay with various *E. coli* mutants with or without 16 μM S_L in iron limiting conditions (right). Statistical significance was determined by the two-way ANOVA test (****: $p<0.0001$, *: $p<0.05$, ns: non-significant)

Conclusions

- As shown by CD analysis, S_L efficiently binds ferric iron achieving the structure (Λ configuration) similar to natural cyclic siderophore - ferrichrome (Figure 3, orange and purple lines).
- The growth recovery assays on various *E. coli* mutants confirms the uptake of the linear siderophore mimic via the *E. coli* receptors recognizing the hydroxamate-type siderophores.
- Conjugates with S_L siderophore are recognized by *E. coli* iron uptake system and allow to transport PNA inside the bacterial cell.

Acknowledgements

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References

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Full study here:

Iron uptake pathway of *Escherichia coli* as an entry route for peptide nucleic acids conjugated with a siderophore mimic, U. Tsylents, M. Burmistrz, M. Wojciechowska, J. Stepień, P.Maj, J. Trylska, DOI: https://doi.org/10.3389/fmicb.2024.1331021