



TRAVEL GRANT REPORT

EMBO Workshop: Bacterial Network 2026

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Molecular microbiology

Author of report

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This conference was a great opportunity to present my work, through a poster, on a novel flagellin-binding protein in *caulobacter crescentus* that act on glycosylated flagellins.

I was very pleased to see that my work attracted a lot of interest. I received many relevant and stimulating questions from leading scientists in my research field, as well as from researchers working in other fields. These discussions allowed me to receive valuable feedback and to consider new perspectives on my research.

The conference also gave me the opportunity to attend very interesting keynote lectures and scientific talks on a wide range of topics. This allowed me to learn about the latest progress and discoveries in different fields and to have stimulating scientific exchanges with other PhD students, postdoctoral researchers, and PIs.

Finally, this conference was a great place to meet new people expand my scientific network, and establish potential collaborations.

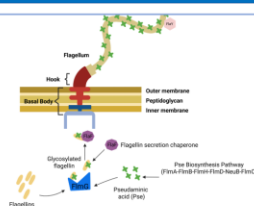
I would like also to thanks the SSM to supporting my participation in this conference. It was a great opportunity to developpe my scientific knowledge.

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Flagella are sophisticated biological nanomachines used by Gram-negative and Gram-positive bacteria for locomotion. The subunits that assemble into the extracellular flagellar filament are called flagellins. Many bacteria glycosylate flagellins in the cytoplasm, before secretion and assembly, by post-translational modification of serine or threonine residues with (derivatives of) pseudaminic (Pse) and/or its stereoisomer legionaminic (Leg) acid.

The α -proteobacterium *Caulobacter crescentus* synthesizes one flagellum per cell cycle. Its six different flagellin subunits are highly regulated at the level of synthesis, glycosylation and secretion. We recently identified FimG, the first identified member of a new class of cytoplasmic O-glycosyltransferases that transfers Pse to flagellins in the cytoplasm. The subsequent export and assembly of the glycosylated flagellin requires the FlaF secretion chaperone and the FlaCapping protein, respectively. These proteins must bind glycosylated flagellins. Are there other proteins that interact with flagellins?



To uncover proteins that interact with flagellins, we undertook an unbiased approach consisting in a pull-down of *C. crescentus* flagellin from lysates of WT and several mutant strains (affected in flagellin glycosylation, secretion or assembly) followed by LC-MS/MS analysis. We discovered a novel flagellin-binding protein, provisionally named here GfiA (GfiA^{Cc}).

[illegible]

GfiA^{Cc} over-expression decreases motility and affects flagellin steady-state levels in cells in both WT and $\Delta gfiA$ strains. Nonglycosylated flagellins ($\Delta neuB$ strain) are not affected.

GfiA function is conserved across different bacterial species as heterologous expression of *Campylobacter jejuni* GfiA (GfiA^{Cj}) affects flagellin steady-state levels and stability in both WT and Δ gfiA strains as GfiA^{Cc}.

Antibiotic chase experiments (chloramphenicol) show that upon GfiA over-expression, the stability of glycosylated flagellin is reduced. Lon protease seems to be involved in the degradation of flagellin promoted by GfiA.

GfIA^{Cj} has been shown to form a dimer by X-ray crystallography

AlphaFold prediction for GfIA dimer of *C. crescentus* (left) and *C. jejuni* (right) Top view

Alignment of GfIA^{Cj} and GfIA^{Cc} showing the dimerization interface (in purple) composed of 7 residues

AlphaFold prediction for GfIA dimer of *C. crescentus* (left) and *C. jejuni* (right) Front view

Co-immunoprecipitation experiments (IP with antibodies against flagellins) confirmed that GfIA binding to flagellins depends on glycosylation.

Conserved residues substitution at the putative GfIA^{Cc} dimerization interface do not disrupt dimerization (as previously shown for GfIA^{Cc}).

Conserved residues substitution at the putative GfiA^{Ci} dimerization interface do not disrupt dimerization (as previously shown for GfiA^{Cj}).

Co-immunoprecipitation experiments (IP with antibodies against flagellins) confirmed that GfiA binding to flagellins depends on glycosylation.

Although serine residues can also be glycosylated, replacing glycosylated threonine with serine abolished GfiA co-immunoprecipitation, suggesting that GfiA specifically recognizes glycosylated threonine rather than the sugar moiety alone.

Conclusions

- GfiA was identified as a new flagellin-binding protein by pull-down experiments.
- We suggest that GfiA interacts specifically with glycosylated flagellin.
- GfiA ectopic expression impairs motility and flagellin stability. The Lon protease seems to be involved in the degradation of flagellin promoted by GfiA.
- GfiA function is conserved across species.
- Interaction with GfiA requires glycosylated threonine residues.
- GfiA^{CC} seems to form a dimer as GfiA^Q. However, we have not clearly identified residues responsible for dimerization.

Perspectives:

- Does GfiA over-expression affect flagellar filament length?
- Does GfiA over-expression affect Pse on flagellins?
- Does GfiA be part of a complex with other proteins?
- Which residues are important for GfiA^{CC} dimerization?
- Does it need to be a dimer to be active?

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