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Pauw, Katrina Forest

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# Minor pseudopilins of the type II secretion system in *P. aeruginosa*: placing pieces in a large puzzle

Badreddine DOUZI<sup>1</sup>, Eric DURAND<sup>1</sup>, Loïc QUINTON<sup>2</sup>, Edwin De PAUW<sup>2</sup>, Katrina FOREST<sup>3</sup> and Romé VOULHOUX<sup>1</sup> <sup>1</sup>CNRS Aix-Marseille University, FRANCE <sup>2</sup> Liege University, BELGIUM <sup>3</sup>University of Wisconsin-Madison, USA

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### Summary

Introduction: The type II secretion system (T2SS) is the unique identified machinery able to secrete a wide range of folded proteins from the periplasm to the extracellular milieu (1). The secretion process is carry-out by multiprotein complexes sharing high similarities with the type IV piliation system. The T2SS assembles in the periplasm an enigmatic pilus-like structure called the pseudopilins XcpU, V, W and X, structurally compatible with the pseudopilus, are also member of the T2SS. Previous structural studies have shown that three of them (XcpV, W and X homologs) are organized in an helicoidal ternary complex presumably located at the tip of the pseudopilus (2). Results and discussion: We combined affinity chromatography, SPR, SEC MALS and NMR chemical shift perturbation to investigate the interaction network between the soluble domains of the five pseudopilins of the P. aeruginosa Xcp machinery. We revealed an unprecedented strictly ordered quaternary complex, including the so far not assigned minor pseudopilins and their organization within the pseudopilus, we investigated the quaternary complex using SAXS, crystallography and Mass-spectroscopy. We generated the SAXS envelop of the complex in which we were able to dock a model of the XcpV-W-X ternary complex. In our model, the fourth minor pseudopilin XcpU integrate the ternary complex by binding the lower part of XcpW following the helicoidal arrangement of the pseudopilus, thus suggesting for XcpU a linker function between the body and the tip of the pseudopilus.



**3- Orchestration of the four minor pseudopilins within the quaternary complex** .

- BIAcore (SPR)

- Co-purifcation:

- The Pseudopilus is composed by the multimerisation of the major pseudopilin XcpT.
- XcpU, XcpV, XcpW and XcpX are minor pseudipilins belonging to the pilin/pseudopilin family.
- The five Pseudopilins are composed by an hydrophobic N-terminal part and a variable soluble C-terminal domain.
- The Pseudopilins are maturated by the prepilin peptidase XcpA/PilD also involved in T4 pilins maturation.
- It is proposed that the T2SS pseudopilus acts as a piston to push exoproteins through the secretin.
- → How the minor pseudopilins integrates the pseudopilus and what is their role in the pseudopilus assembly and on the secretion process?

# 2- Identification of a minor pseudopilins quaternary complex.







--> This complex is in agreement with the previously published ternary complex between GspI-J-K from ETEC (2):

V-W-X







- SPR experiments identified three interaction between minor pseudpilins: U-W, V-W and V-X.
- No interaction was found between W&X or V&U and between the major pseudopilin XcpT and the 4 minor pseudopilins.
- Pull-down experiments confirm SPR results.
- $\rightarrow$  Our data reveal the existence of a strictly ordered quaternary complex from XcpU to XcpW through XcpW and V

## - NMR chemical shift perturbation:



- W\*+V+U  $W^* + V + U + X$ Superposition of HSQC of W in presence of U-V and HSQC of W in
  - NMR shift perturbation chemical SPR pull-down and confirms experiments.
  - No binding of X to W was detected even when W is in presence of U and V.

# 4- Structural study of the quaternary complex.

- Low resolution structure of the XcpUVWX complex by Small-Angel X-ray Scattering (SAXS):



#### presence of UVX. X have no effect on the complex UVW. observed. Combining our results In contrast to what is found in the ternary The 4 minor pseudopilins interact • Due to the helicoïdal nature of the and the structure of Gsplsequentially to form an ordered complex isolated from ETEC T2SS (2), no pseudopilus, we propose that U constitutes J-K, we propose that our quaternary complex where U binds interaction was detected between W and X in the lower component of the quaternary complex W, W binds V and V binds X. the Xcp quaternary complex. complex which confer to U an important forms the tip of the role in pseudopilus assembly. pseudopilus where XcpU integrates the ternary GspK (X) GspJ (W) $\longleftrightarrow$

### - Chemical cross-link coupled to Mass Spectrometry (XL-MS):

In order to understand how U integrates the quaternary complex, we identified the binding interfaces between U and W by XL-MS.



In vitro cross-linking of the purified U and W. U/W complexes were separated by SDS-PAGE "In gel" trypsin digestion of selected complexes Peptides identification by Mass spectrometry (MALDI)

Crystals of the quaternary

complex



Peptides identified in individual protein but never recovered in selected complexes (in green) may correspond to peptides involved in the W/U interaction. Involving the lower part of XcpW and the upper part of XcpU

Interestingly, when superimposed with the ternary complex, the protected part of W (in green) is outward facing the ternary complex (to welcome U).





→ Identification at molecular level by XL-MS and X-ray of the pseudopilin/substrate interface to define the secretion signal (motif recognized on secreted protein by T2SS).