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

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References

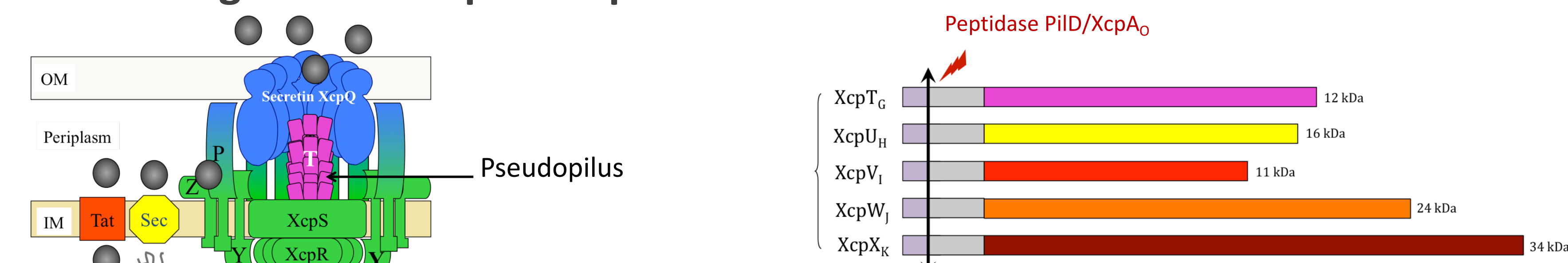
1. Douzi, B., et al., (2012) *Philosophical Transactions B* **367**,1059-72.
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Summary

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 pseudopilus constituted by the helicoidal assembly of the major pseudopilin XcpT. Four other minor 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 pseudopilin XcpU. In order to understand at a molecular level the role of the 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.

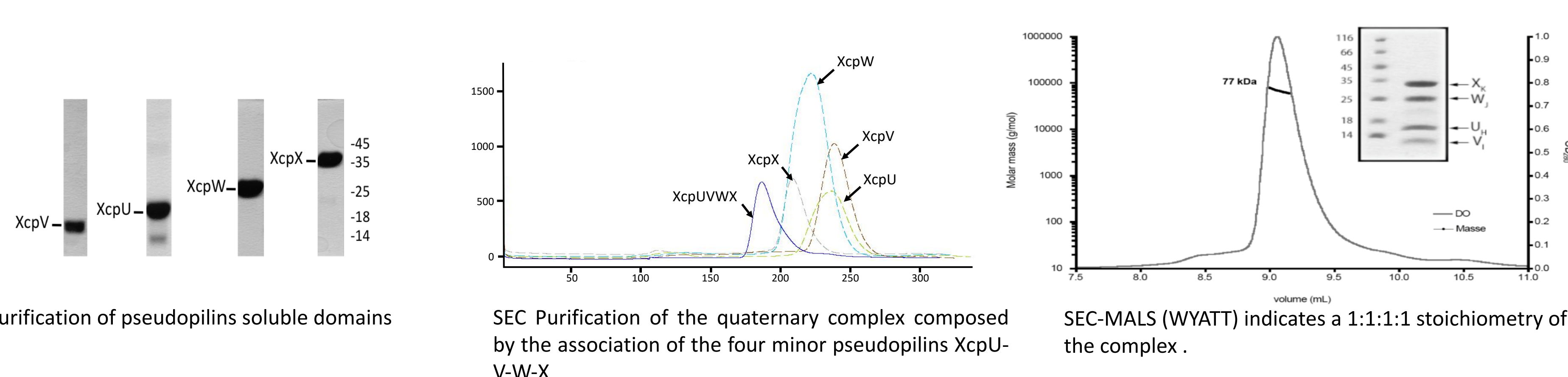
1- The enigmatic T2SS pseudopilus.



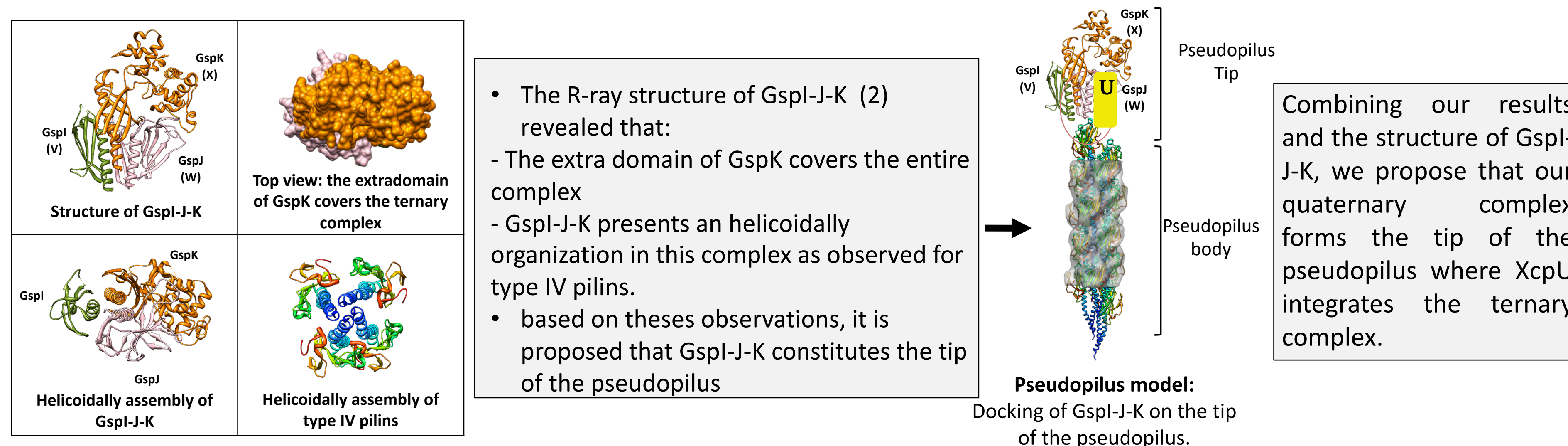
- The Pseudopilus is composed by the multimerisation of the major pseudopilin XcpT.
- XcpU, XcpV, XcpW and XcpX are minor pseudopilins belonging to the pilin/pseudopilin family.
- The five Pseudopilins are composed by an hydrophobic N-terminal part and a variable soluble C-terminal domain.
- The Pseudopilins are matured by the prepeptidase 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):

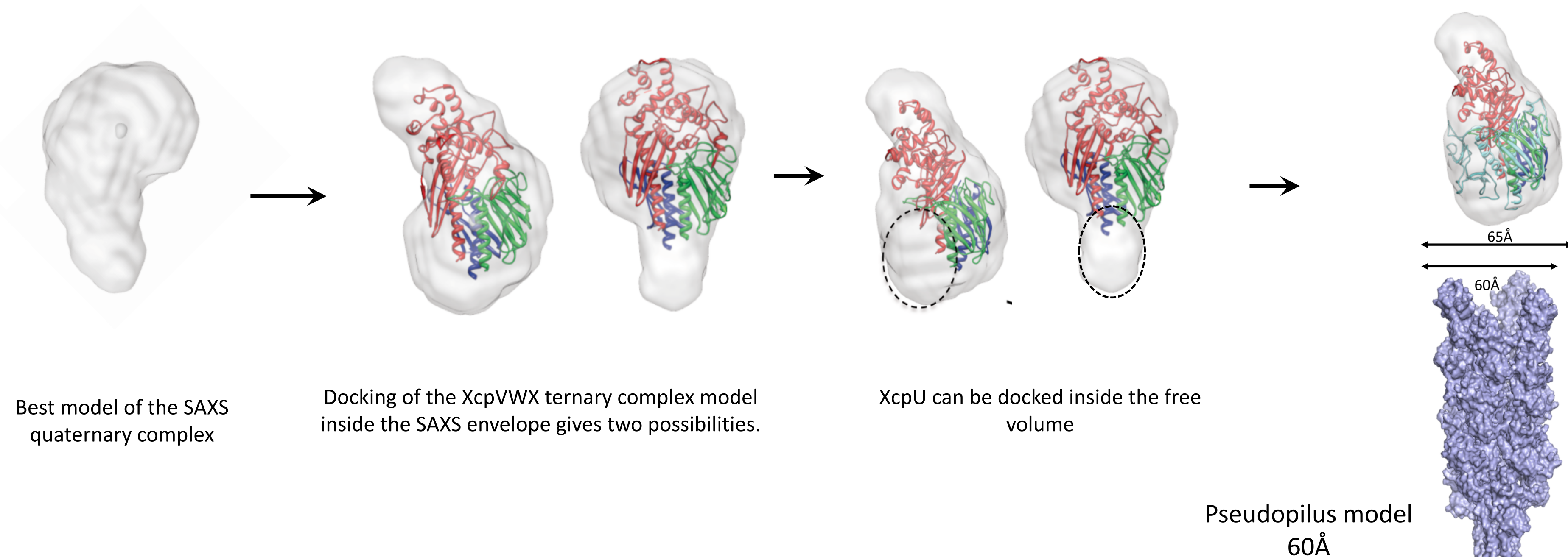


- The R-ray structure of GspI-J-K (2) revealed that:
 - The extra domain of GspK covers the entire complex
 - GspI-J-K presents an helicoidally organization in this complex as observed for type IV pilins.
 - based on these observations, it is proposed that GspI-J-K constitutes the tip of the pseudopilus

Pseudopilus model: Docking of GspI-J-K on the tip of the pseudopilus.

4- Structural study of the quaternary complex.

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

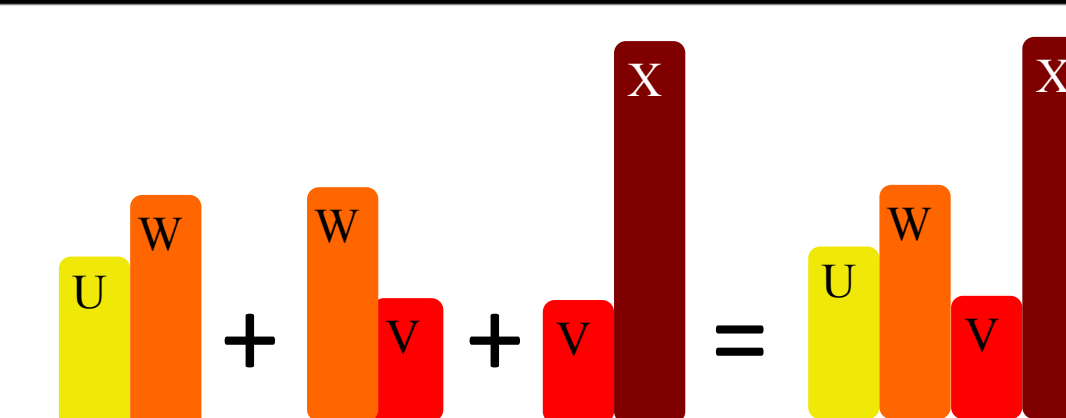


- SAXS model validates the helicoidal association of the minor pseudopilins inside the quaternary complex.
- The tightness of the SAXS envelop is incompatible with a peripheral integration of XcpU to the ternary complex.
- XcpU therefore probably integrate the ternary complex vertically this lead us to integrate vertically in agreement with XC-MS data.
- Interestingly, the width of the SAXS envelope is compatible with the width of the pseudopilus body.

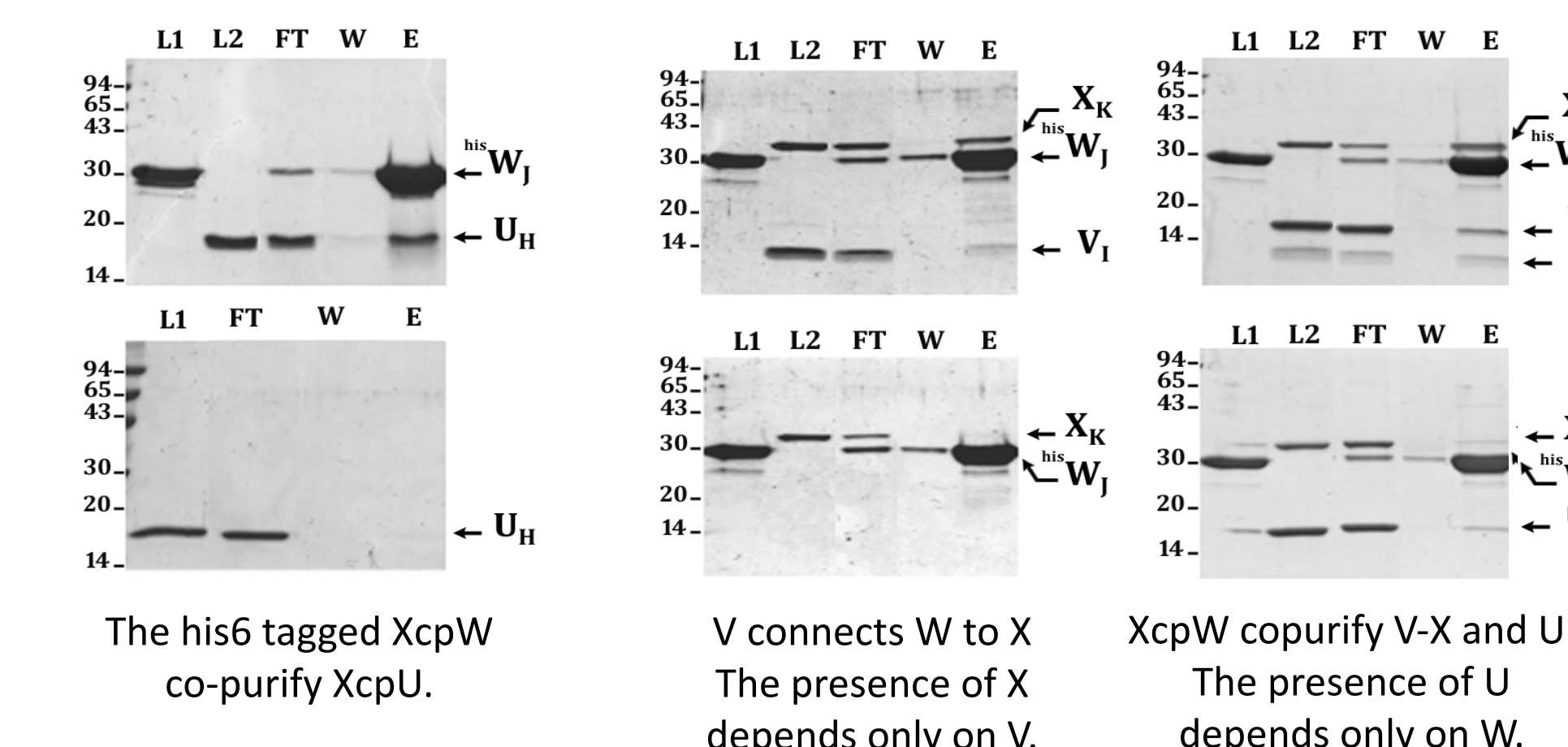
3- Orchestration of the four minor pseudopilins within the quaternary complex.

- BIAcore (SPR):

Bound Ligand	Analyte				
	U	V	W	X	
U	-	-	-	0.72	-
V	-	-	-	4.60	19.80
W	2.85	1.60	-	-	-
X	-	1.55	-	-	-

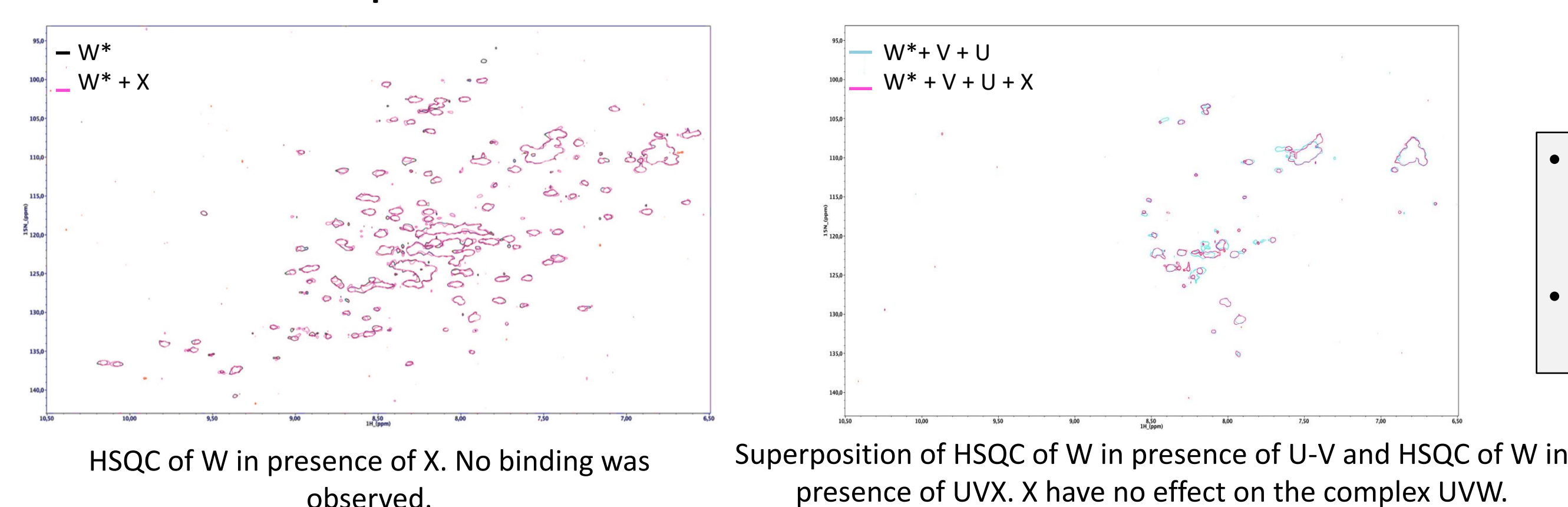


- Co-purification:



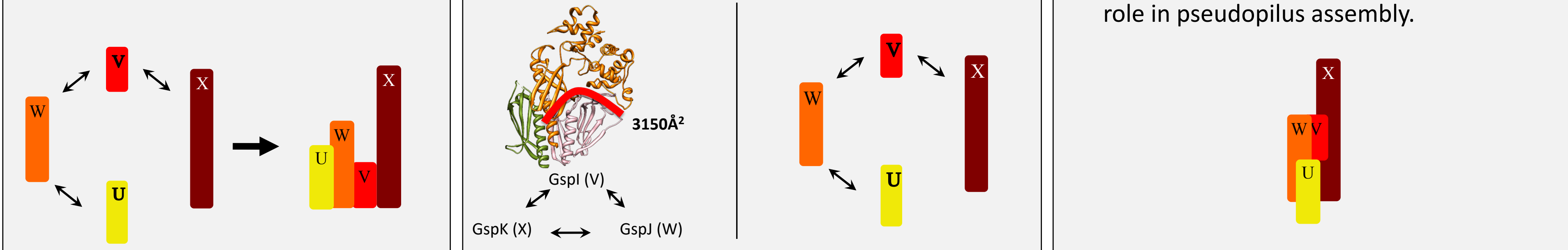
- SPR experiments identified three interaction between minor pseudopilins: U-W, V-W and V-X.
 - No interaction was found between W&X or V&U and between the major pseudopilin XcpT and the 4 minor pseudopilins.
 - Pull-down experiments confirm SPR results.
- Our data reveal the existence of a strictly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

- NMR chemical shift perturbation:



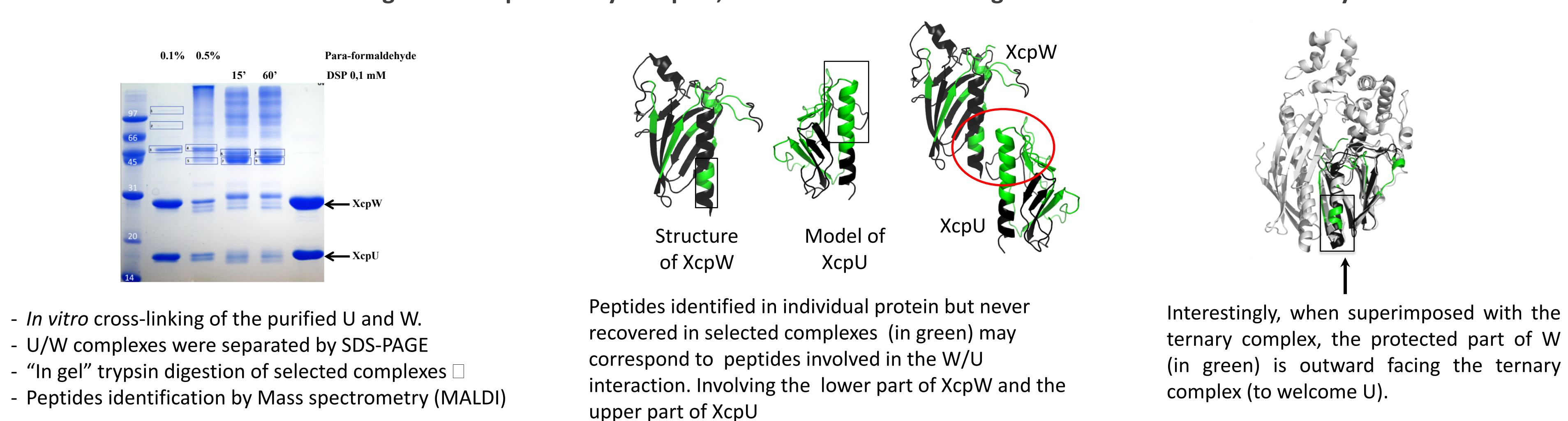
- NMR chemical shift perturbation confirms SPR and pull-down experiments.
- No binding of X to W was detected even when W is in presence of U and V.

- The 4 minor pseudopilins interact sequentially to form an ordered quaternary complex where U binds W, W binds V and V binds X.
- In contrast to what is found in the ternary complex isolated from ETEC T2SS (2), no interaction was detected between W and X in the Xcp quaternary complex.
- Due to the helicoidal nature of the pseudopilus, we propose that U constitutes the lower component of the quaternary complex which confer to U an important role in pseudopilus assembly.



- 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.



Future work

- Understand the pseudopilus tip atomic organization
 - XL-MS on the quaternary complex to reveal pseudopilin interfaces
 - X-ray 3D structure determination of the quaternary complex
- Understanding the role of the pseudopilus tip in the Type II secretion process
 - we have already identified a direct interaction between the pseudopilus tip and a secreted substrate (3).
 - 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).