The XcpV/GspI pseudopilin has a central role in the assembly of a quaternary complex within the T2SS pseudopilus

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The XcpV/GspI Pseudopilin has a central role in the assembly of a quaternary complex at the tip of the T2SS Pseudopilus

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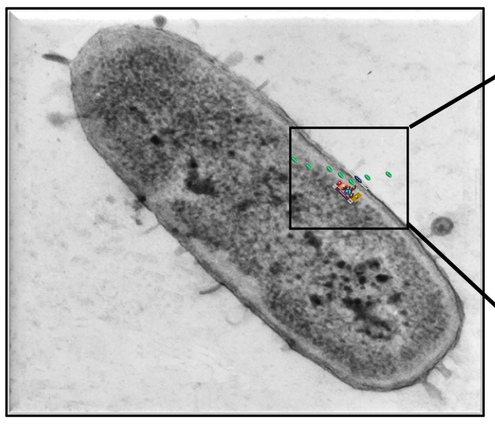
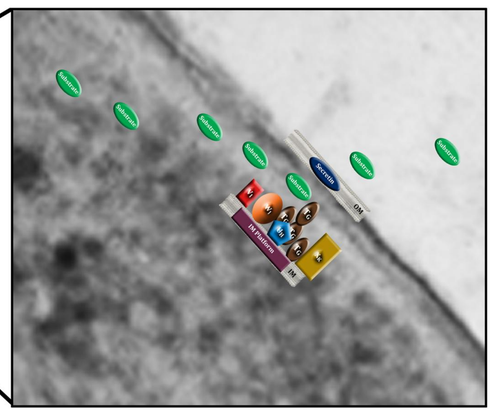
Gram-negative bacteria use the sophisticated type II secretion system (T2SS) to transport a large number of exoproteins from the periplasmic space into the extracellular environment. However, if individual T2SS components are well characterized, very little is known about their multimeric organization during the secretion process. Five proteins of the T2SS, the pseudopilins GspG-H-I-J-K, are thought to assemble into a pseudopilus involved in the extrusion of the substrate through the outer membrane pore. Recent structural data have revealed that three of them, GspI-J-K, are organized in a trimeric complex proposed to be located at the tip of the GspG constituted pseudopilus. In the present work we combined two different biochemical techniques to investigate protein protein interactions between the five Pseudomonas aeruginosa Xcp pseudopilins. The soluble domains of XcpT-U-V-W-X (respectively homologous to GspG-H-I-J-K) were purified and tested by surface plasmon resonance (SPR) and affinity purification for all possible interactions combinations. We found that $XcpX_K$ interacts with $XcpV_I$ which itself interacts with $XcpW_J$ confirming the crystallized trimeric complex. Interestingly, our systematic approach also revealed a new interaction between $XcpU_H$ and one member of the trimeric complex, $XcpW_J$, thus suggesting the existence of a quaternary complex involving $XcpU_H$-V_I-W_J-X_K at the tip of the pseudopilus. This quaternary complex was then successfully identified by affinity chromatography. Moreover, by testing various combinations of purified pseudopilins by SPR and affinity chromatography we were able to dissect the different successive steps involved in the development of the quaternary complex. We then propose a model for the quaternary pseudopilin complex formation centered around $XcpV_I$ which first recruits XcpXK on one side and $XcpW_J$ on another side on which $XcpU_H$ then binds. This central role of $XcpV_I$ in pseudopilus formation fully agrees with our previous physiological data on its proposed involvement in pseudopilus initiation.

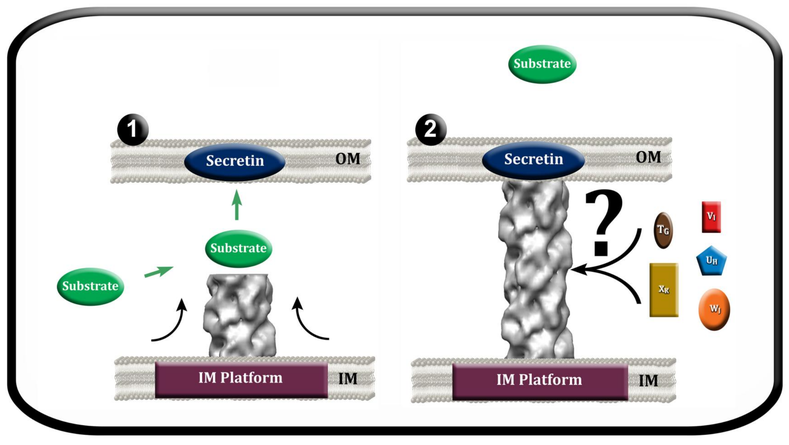
Pseudomonas aeruginosa



T2SS

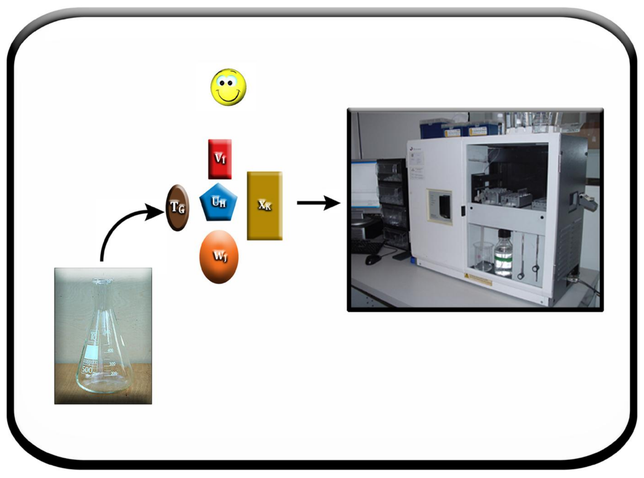


The T2SS Piston Model



1 The secretion is performed by a Pseudopilus assembly

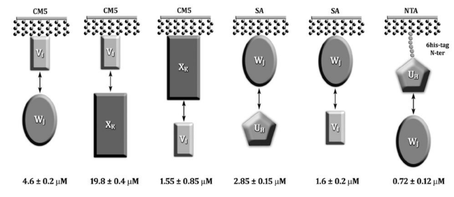
1 Expression of Xcp pseudopilin soluble domains Use of BIAcore 1000 for interaction study



2 Pseudopilin interaction network using Surface Plasmon Resonance (SPR)

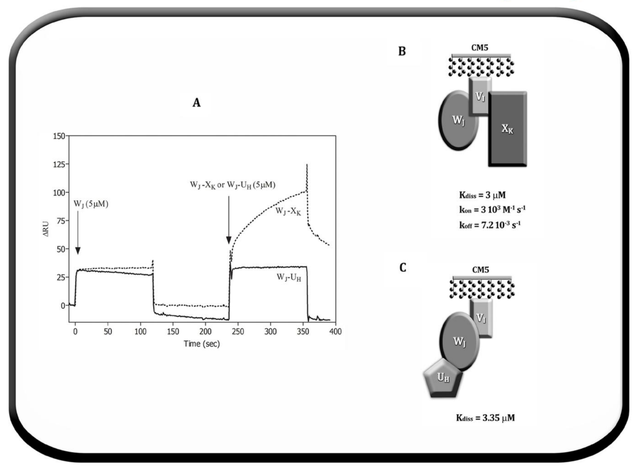
		Analyte				
		T_G (12 kDa)	U_H (16 kDa)	V_I (11 kDa)	W_J (24 kDa)	X_K (34 kDa)
Bound Ligand	T_G (SA)	–	–	–	–	–
	$^{his}U_H$ (NTA)	–	–	–	0.72 ± 0.12	–
	V_I (CM5)	–	–		4.6 ± 0.2	19.8 ± 0.24
	W_J (SA)	–	2.85 ± 0.15	1.6 ± 0.2	–	–
	X_K (CM5)	–	–	1.55 ± 0.85	–	–

B



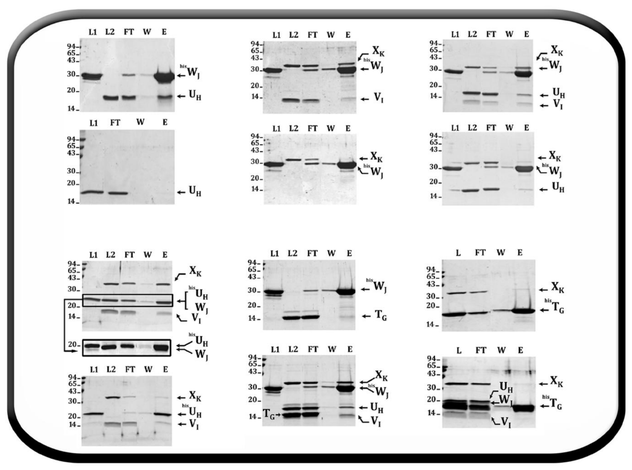
(A) Interactions tested : each ligand with the 5 analytes. The K_{diss} values (µM) for the interaction detected. In bracket: the chip used for each ligand and the molecular weight of each analyte.
(B) Schemes for all positive interactions, with the K_{diss} values indicated.

3 Epitope mapping of binary mixes using Surface Plasmon Resonance (SPR)



(A) W_J and binary mixes (W_J-U_H and W_J-X_K, 5 µM) were passed on V_I.
(B, C) Schemes of the ternary complexes proposed, with K_{diss} of the interaction and k_{on}, k_{off} values when calculable.

5 Co-migration of pseudopilin soluble domains, batch-co-purified on affinity column

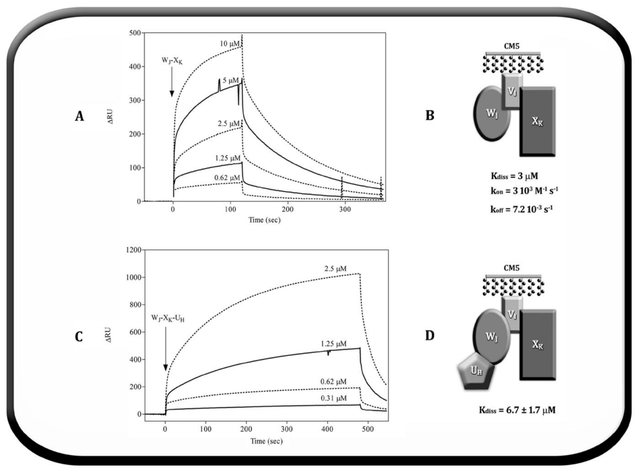


Each of the his6-tagged protein was mixed with different untagged protein partners . After affinity co-purification of proteins bound to the Ni^{2+}-NTA-magnetic-beads, the different fractions were analyzed on a 15% SDS-PAGE. L1, containing the his6-tagged protein; L2, containing the untagged protein partners; L, containing tagged and untagged proteins. FT, flow-through; W, final wash; and E, elution.

Team

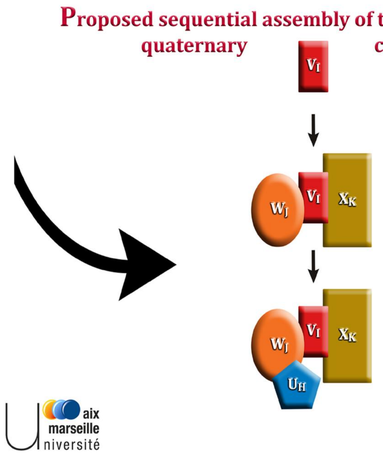
How does the pseudopilus form ?

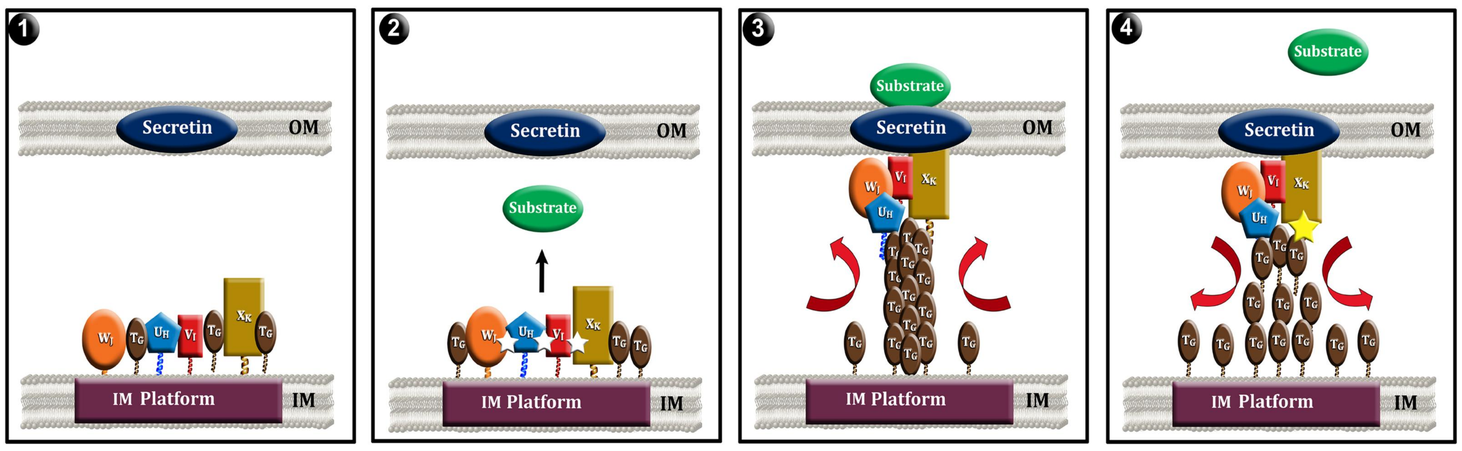
4 Affinity determination of W_J-X_K and W_J-X_K-U_H mixes on V_I immobilized



(A) Binding pattern of W_J-X_K mixture at concentrations between 10 and 0.62 µM.
(C) Binding pattern of W_J-X_K-U_H mixture at concentrations between 2.5 and 0.31 µM.
(B, D) Schemes of the corresponding ternary complexes with K_{diss} of the interaction, and k_{on}, k_{off} values when calculable.

Proposed sequential assembly of the pseudopilins quaternary complex





1 Is time zero of the pseudopilus formation.

2 The presence of the substrate to be translocated activates (white stars) the formation of the complex.

3 The pseudopilus formed by the assembled quaternary complex and the polymerization of $XcpT_G$, pushes the substrate through the secretion pore.

4 After secretion, the interaction between $XcpX_K$ and secretin activates (yellow star), the pseudopilus desassembly, and the system goes back to the resting state of time zero.

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