Towards a better understanding of the bacterial type II secretion pathway

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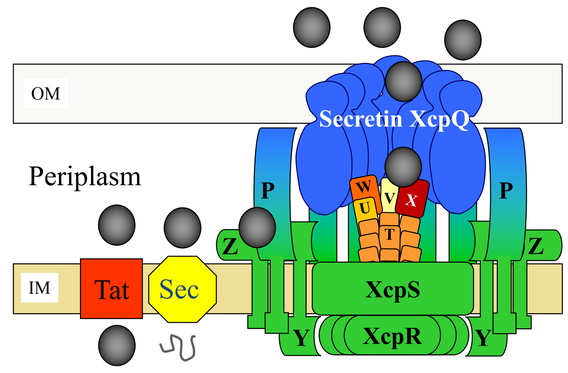
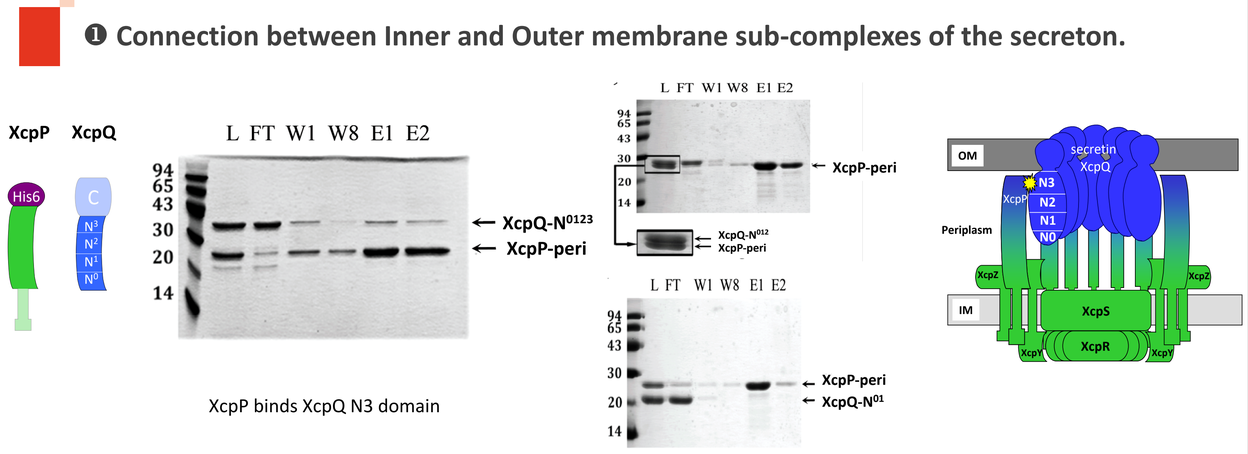
Introduction

The type II secretion pathway (T2SS) is unique in its ability to promote the transport of large, folded and sometimes multimeric proteins. In this secretion process, exoproteins are first exported into the periplasm by the Sec or the Tat export pathway (1). The final release into the medium is promoted by a multiprotein complex spanning the whole bacterial envelope called "Secreton". The mode of action of this machinery has never been clearly elucidated. However, due to its analogy with the type IV piliation system (T4P), the "piston" hypothesis involving a pilus-like structure, the pseudopilus, pushing out the substrate through an outer membrane pore remains the most probable model (2-4). We used biophysical and biochemical tools to establish the *in vitro* periplasmic interaction network between secreton components or between secreton components and secreted substrates. **All together our data allow us to propose a new model for the *Pseudomonas aeruginosa* Xcp type II secretion process including, for the first time, substrate recognition and transport** (5, 6).



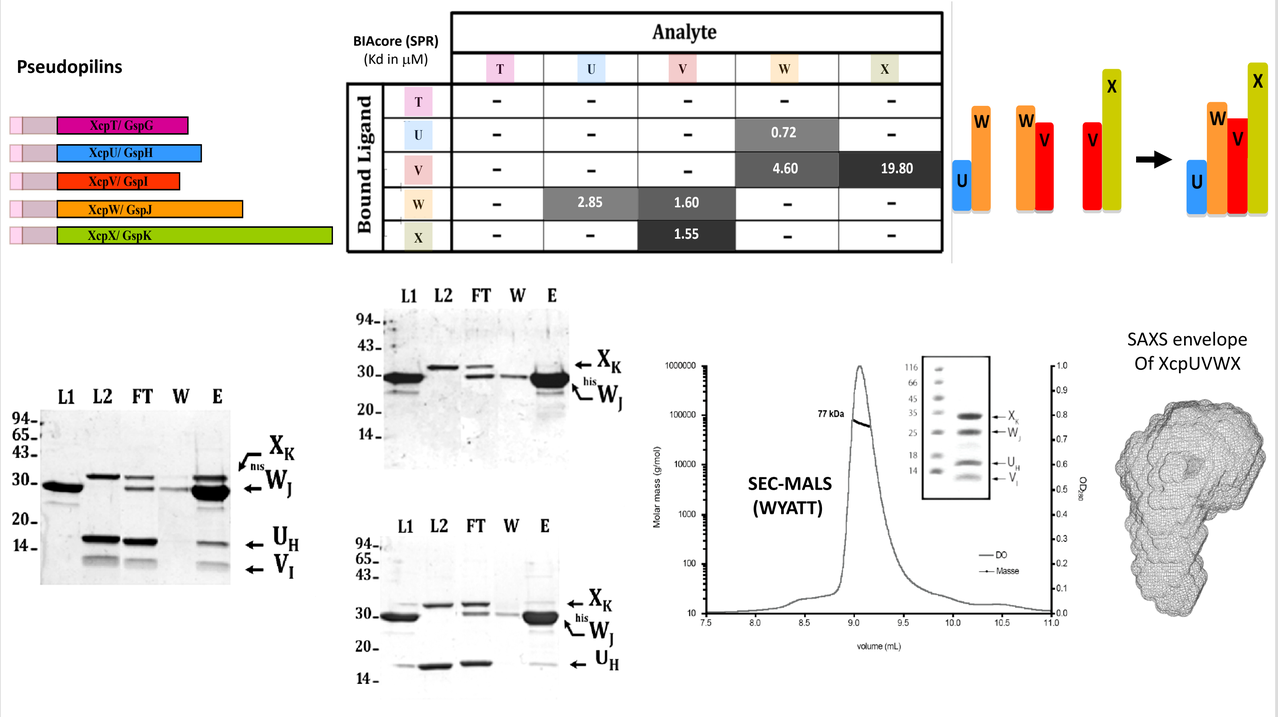
❶ Connection between Inner and Outer membrane sub-complexes of the secreton.



- The N3 domain of XcpQ interacts with XcpP-peri (Pull down and Biacore)
- 1:1 stœchiometry between XcpP-peri and XcpQ-N^{0123} (gel filtration & SEC-MALS (WYATT))

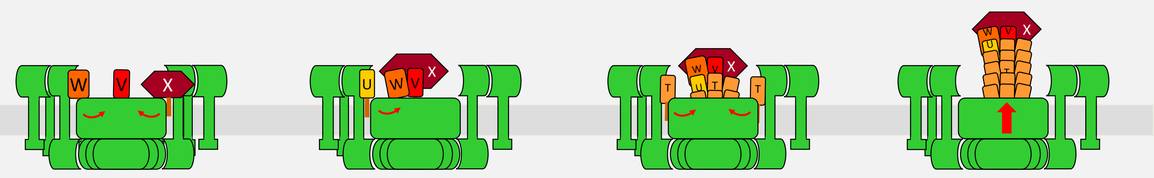
→ 12 units of XcpP shield the secretin dodecamer forming a periplasmic cage (6).

❷ Identification of a minor pseudopilin quaternary complex.



- The 4 minor pseudopilin periplasmic domains interact sequentially to form an ordered quaternary complex
- 1:1:1:1 stœchiometry between minor pseudopilins in the quaternary complex.
- *The high resolution 3D structure of XcpVWX homologs revealed a localization at the tip of the pseudopilus (7)*
- *XcpV is the only minor pseudopilin essential for pseudopilus assembly suggesting an initiating function (8)*

→ Model for pseudopilus assembly in type II secretion (5):



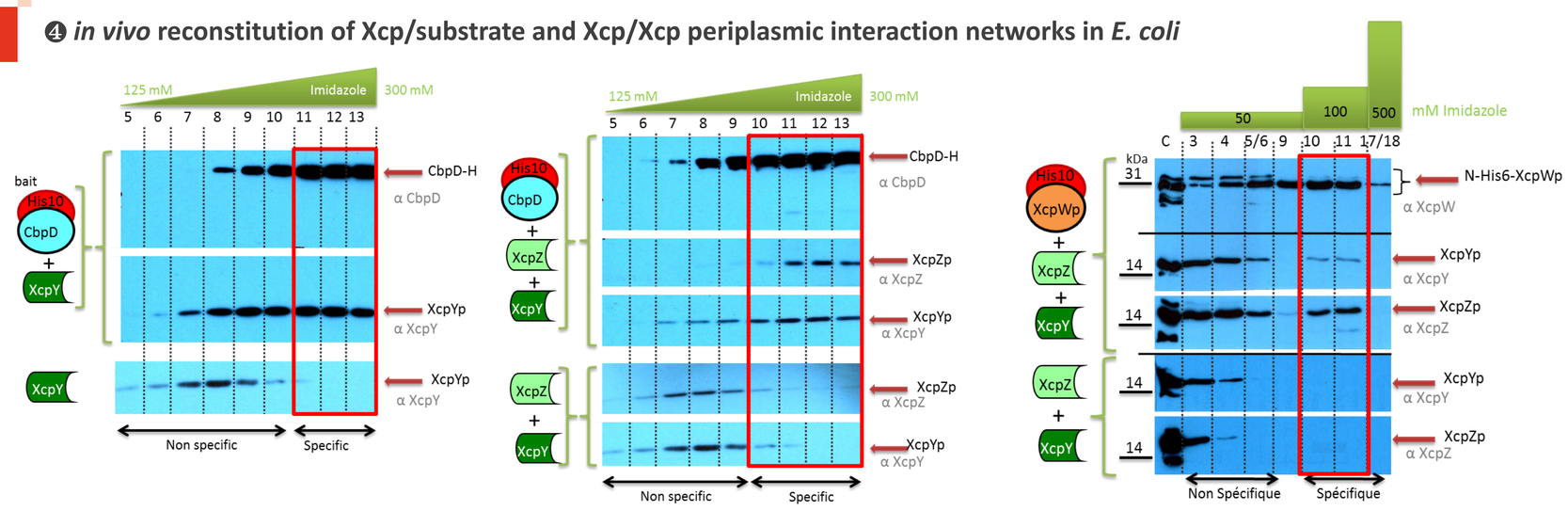
❸ Interaction between Xcp and Hxc substrates and Xcp secreton components.

		Analyte (periplasmic domains)										Analyte	
		XcpP	Secretin (XcpQ)			Major Pseudopilin	Minor Pseudopilins					LasB (Xcp)	LipA (Xcp)
	BIAcore (SPR) (Kd in µM)		N3 N2 N1 N0	N2 N1 N0	N1 N0	T	U V W X	U	V	W	X	LasB	LipA
ligand	XcpP-peri	–	3.6	–	–	–	–	–	–	–	–	3.6	6.8
	LasB (Xcp)	2.0	12.5	21.6	27.8	–	0.9	12.5	24.6	–	12.7	ND	
	LapA (Hxc)	–	–	–	–	–	–	–	–	–	–		

- The Xcp substrate (LasB) directly binds XcpP, XcpQ (N0-1 domains) and minor pseudopilins (BIAcore).
- No interaction found between the Hxc T2SS substrate (LapA) and Xcp secreton components

→ T2SS substrates are specifically interacting with several secreton components (6).

❹ *in vivo* reconstitution of Xcp/substrate and Xcp/Xcp periplasmic interaction networks in *E. coli*

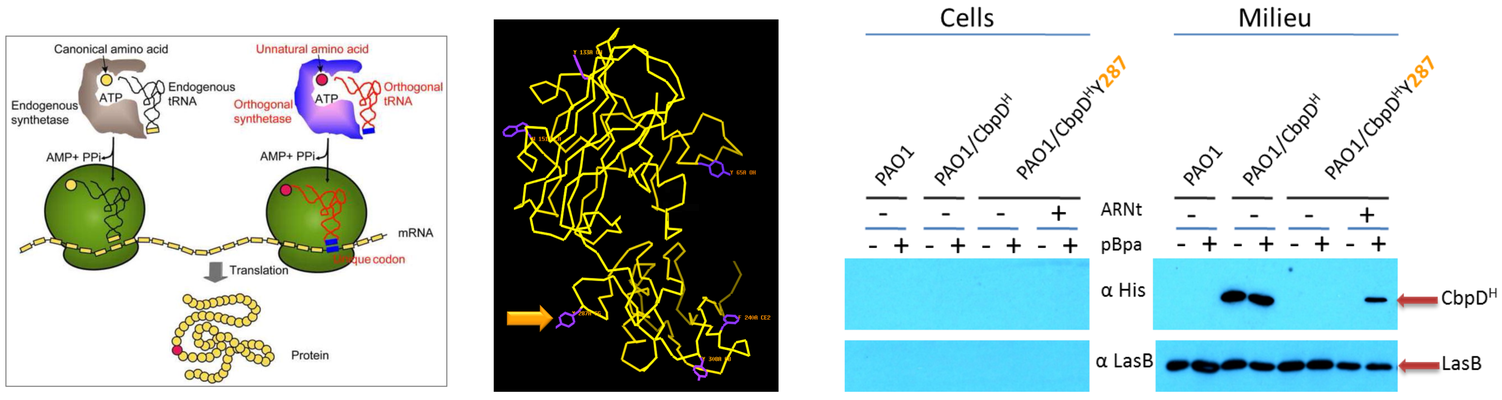


- Co-production of up to 7 Xcp domains together with the substrate thanks to the 4 compatible pDUET vectors.
- Identification of a CbpD/XcpY periplasmic complex.
- Identification of a CbpD/XcpY/XcpZ periplasmic complex
- Identification of a XcpW/XcpY/XcpZ periplasmic complex

→ Identification of new Xcp/Xcp and Xcp/substrate complexes

- **Alternative way to explore the T2SS interactome**
- **Preparative assay for 3D structure determination**

❺ Exploration of *in vivo* interactions in *P.aeruginosa* thanks to the photo-crosslinking.

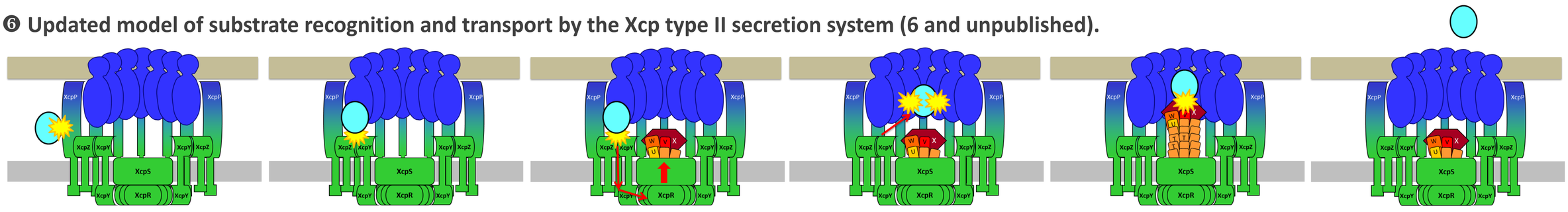


- Setting up of the photo-crosslinking assay in *P. aeruginosa*
- Targeted amino acids: the 6 aromatic residues exposed at the surface of CbpD
- CbpDY287 production and secretion is tRNA & pBpa-dependent

→ Proof of principle that photo-crosslinking is functional in *P. aeruginosa*

→ A very promising tool to follow *in situ* the substrate during the secretion process

❻ Updated model of substrate recognition and transport by the Xcp type II secretion system (6 and unpublished).



1. Specific recognition of the substrate in the periplasm by the peripheral component of the Secreton XcpP

2. Transition of the substrate to XcpY/Z

3. Trigger ATPase activation and pseudopilus assembly

4. Transition of the substrate into the vestibule of the secretin.

5. Contact between the pseudopilus tip and the substrate in the vestibule of the secretin

6. Expulsion of the substrate and pseudopilus retraction.