Self-assembly of XcpQ secretin N domain into hexamer of dimers provides new molecular insights into architecture and dynamics of widespread outer membrane channels

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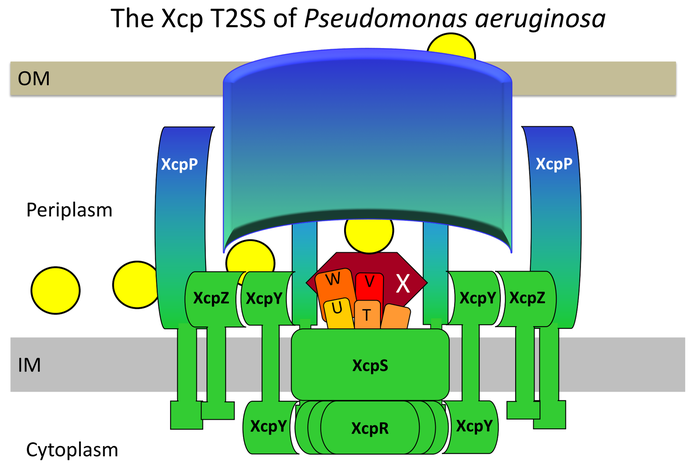
INTRODUCTION

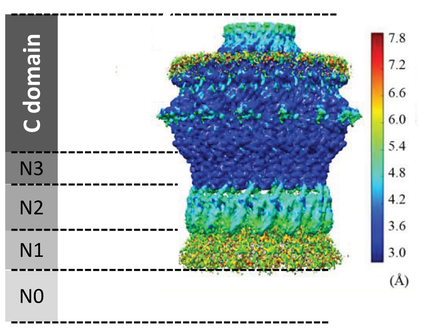
The type II secretion system (T2SS) releases **large folded virulence factors** across the outer membrane (OM) of many Gram-negative pathogens. This **trans-envelope nanomachine** is constituted by an inner membrane (IM) platform assembling a pilus-like structure and a **specific gating** operated by an outer membrane channel called **secretin**.

Secretins are giant homo-multimeric proteins composed of an outer membrane channel-forming C domain and a periplasmic N domain involved in substrate recruitment and connection with IM components. We have a good understanding of the OM-embedded, pore-forming C-terminal beta-barrel channel of secretin. In contrast, the high flexibility of the secretin periplasmic N-terminal domain has been an obstacle in obtaining the detailed structural information required to uncover its molecular function. **Here we elucidate the structural organization of the N domain of *Pseudomonas aeruginosa* secretin XcpQ and reveal new insights into its function.**

The Xcp T2SS of *Pseudomonas aeruginosa*



CryoEM envelope of Vc GspD

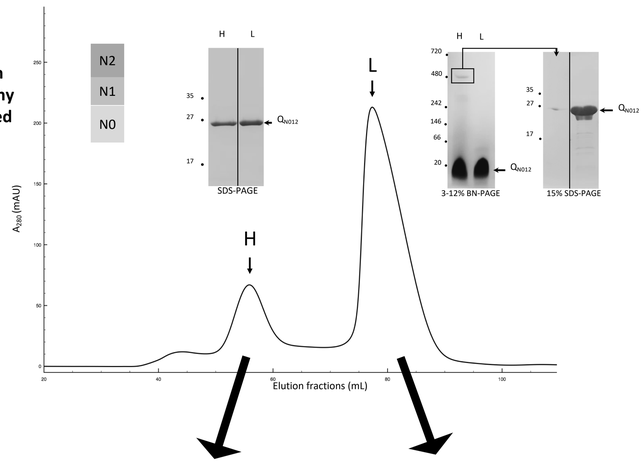


Adapted from Yan NSMB 2017

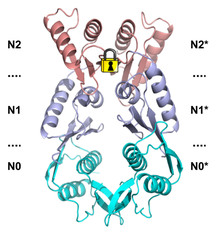
In vitro self-oligomerization of purified XcpQ$_{N012}$ into ring shaped hexamer of dimers

Purified XcpQ$_{N012}$ oligomerizes into dimers (L) and dodecamers (H)

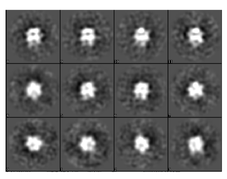
Size exclusion chromatography (SEC) of purified XcpQ$_{N012}$



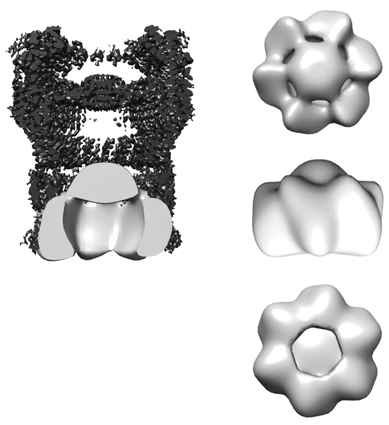
SEC, EM and SDS-PAGE analysis of the purified XcpQ$_{N012}$-S210C



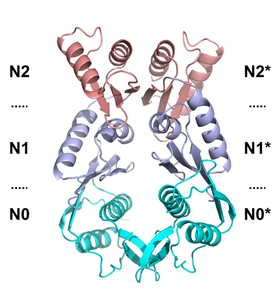
Negative staining EM of "H" fraction



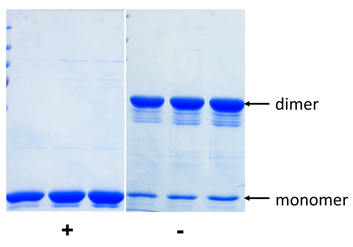
Negative staining EM of "H"



X-Ray 3D structure of "L"

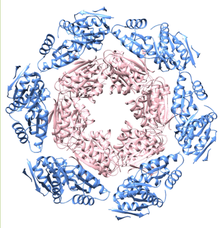


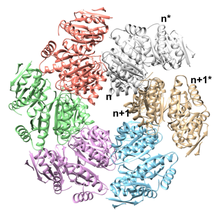
SEC dodecameric fractions



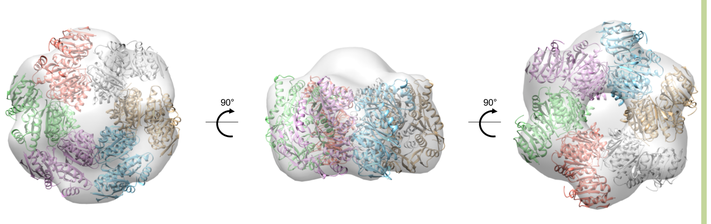
Atomic model of XcpQ$_{N012}$ dodecamers

Best "SymmDock" model for hexamer of dimers

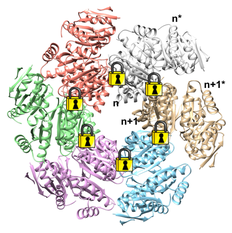




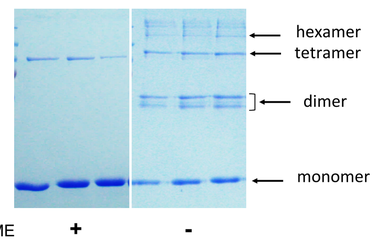
Fitting of the model into the EN envelope



SEC and SDS-PAGE analysis of the purified XcpQ$_{N012}$-T54C-Q86C

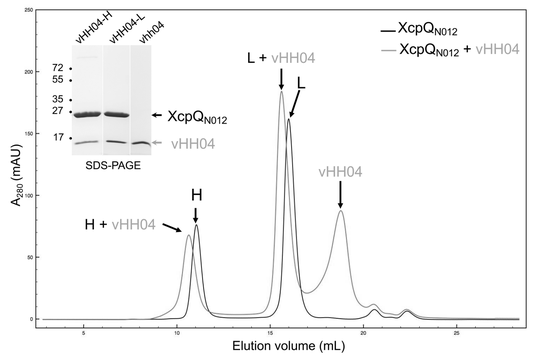


SEC dodecameric fractions

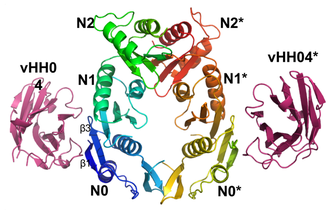


Identification of XcpQ$_{N012}$ inter-dimer interface by nanobody co-structure and interference

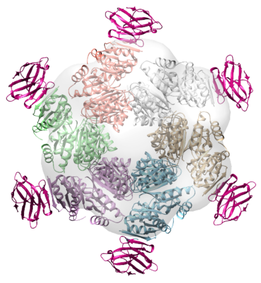
SEC of purified XcpQ$_{N012}$ +/- +vHH04



X-Ray 3D structure of L + vHH04

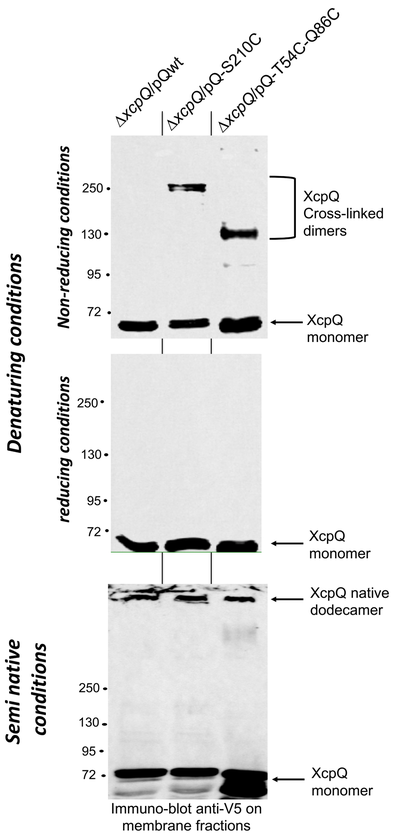


Model of H + vHH04

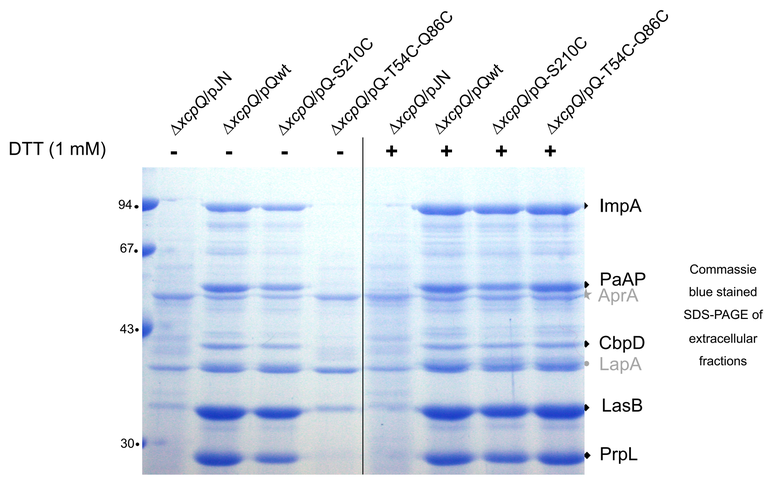


in vivo secretin dynamic revealed by cysteine cross linking

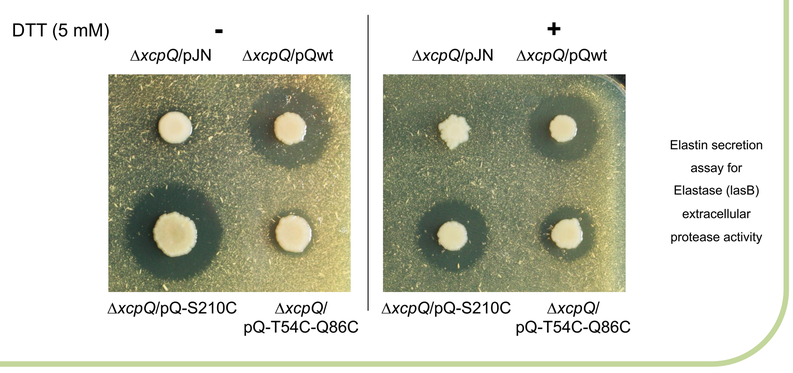
Oligomerization properties of XcpQ cysteine variants



DTT-dependant Xcp T2SS secretion in XcpQ cysteine variants

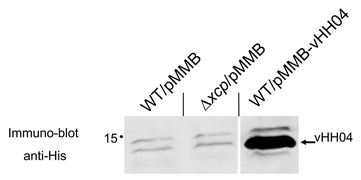


Elastin plate secretion assay for LasB extracellular activity

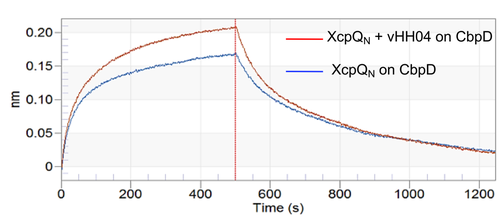


in vivo secretin interactome refined by vHH interference

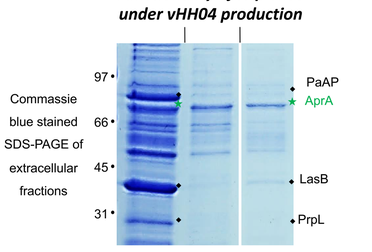
vHH04 production in P. aeruginosa



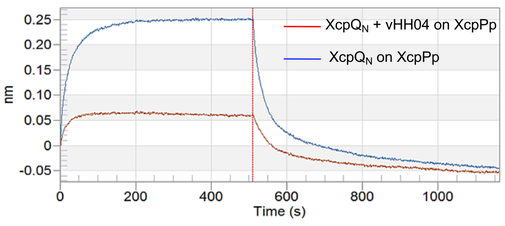
vHH04 interference on XcpQ$_N$/CbpD interaction by Bli (Biolayer interferometry)



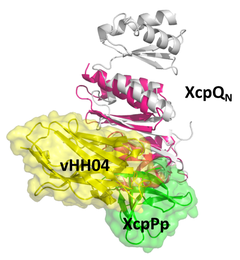
Functionality of Xcp T2SS under vHH04 production



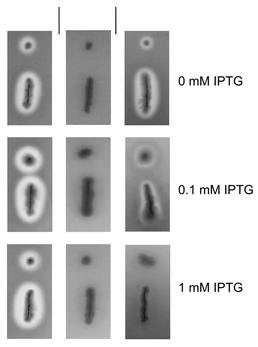
vHH04 interference on XcpQ$_N$/XcpPp interaction by Bli



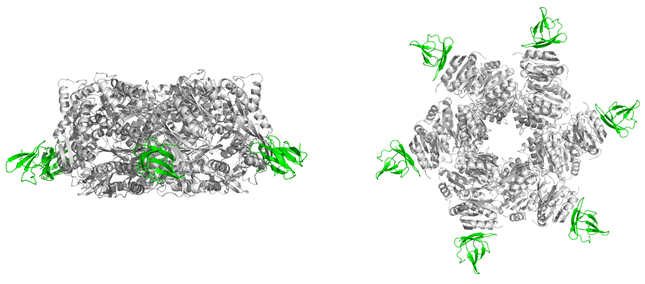
3D Model of vHH04 and XcpPp binding on XcpQ$_N$



Xcp T2SS inhibition upon vHH04 production



3D Model of XcpPp binding (X6) on XcpQ$_N$ dodecamer (X12)



CONCLUDING REMARKS

In the present work, we used **electron microscopy and X-ray crystallography** to unravel the structural organization of XcpQ T2SS secretin N domain into **hexamer of dimers**. Additional ***in vivo* cysteine cross-linking**, **nanobody interference** and **secretion experiments** revealed: (i) the **physiological relevance** of the structure; (ii) a **flexibility requirement** of the secretin N domain during the secretion process; (iii) the **stoichiometric organization** between secretin and its inner membrane partner, (iv) a possible **entry door** for the secreted effectors through the secretin periplasmic gate. Taken together our data provide significant insights into the **understanding of secretin organization, functioning and interactome** in the context of type II secretion. They moreover provide new tools and open new directions for further investigations, necessary to fully understand the dynamics and the assembly process of T2SS secretins, the role of the interacting partners on secretin dynamics as well as the effect of IM partners on secretin function.