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

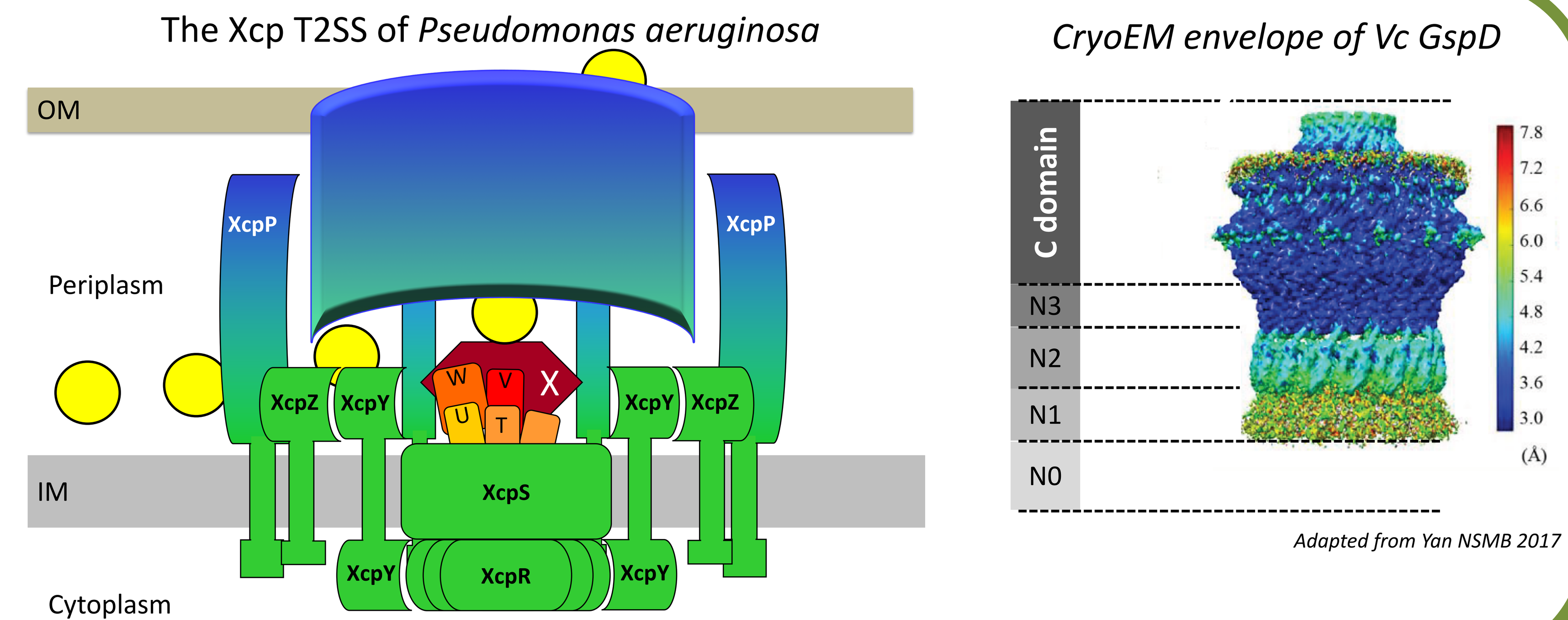
Badreddine Douzi*[1], Nhung. T. T. Trinh[1], Sandra Michel-Souzy[1], Aline Desmyter[1], Geneviève Ball[1], Pascale Barbier[2], Artemis Kosta[1], Eric Durand[1], Katrina T. Forest[3], Christian Cambillau[1], Alain Roussel[1] & Romé Voulhoux*[1].

[1]: Aix Marseille Univ- CNRS - Marseille, France [2]: Aix Marseille Univ. Inserm, CRO2, Marseille, France [3]: University Wisconsin-Madison, Madison, USA

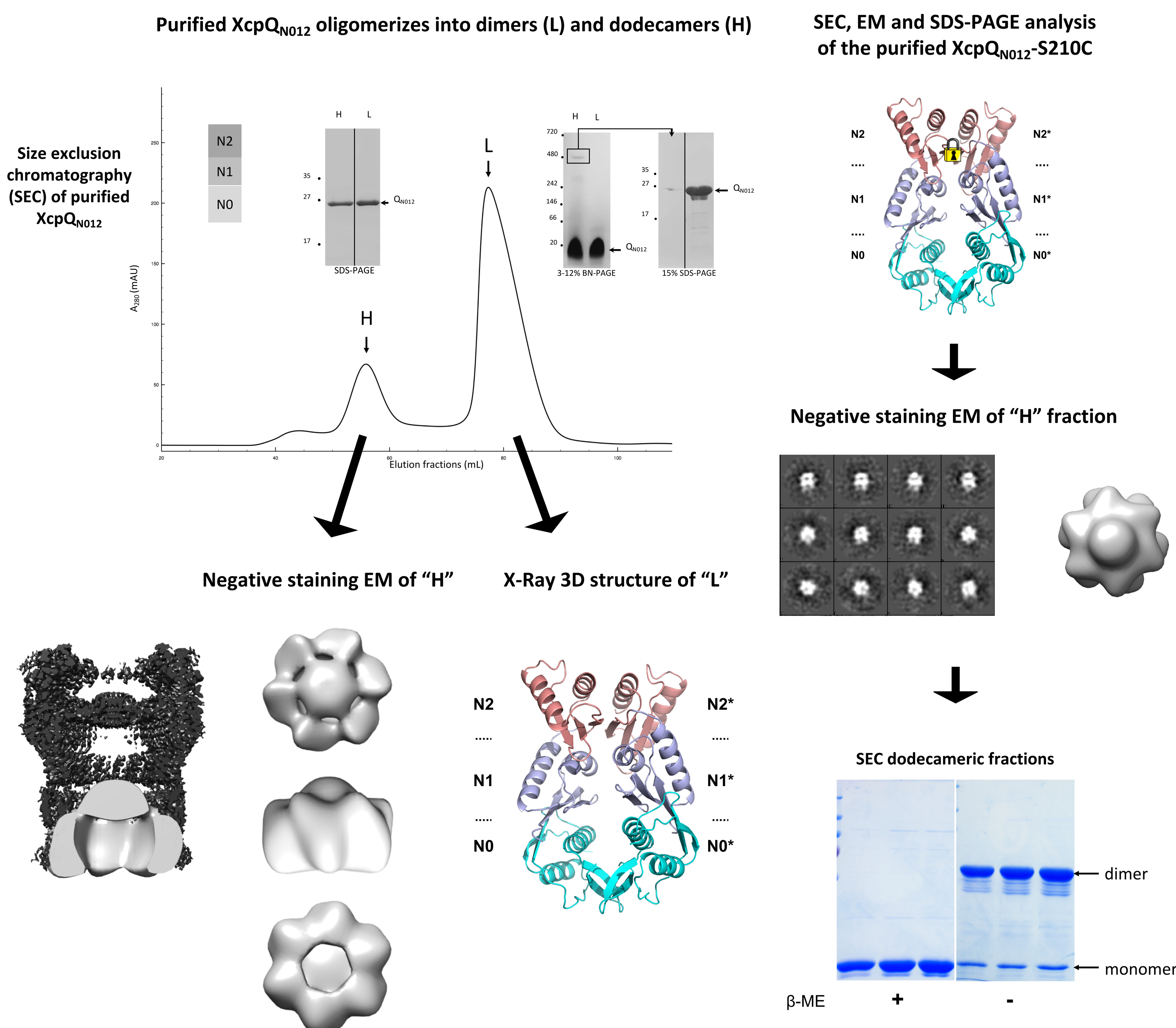
INTRODUCTION

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**.

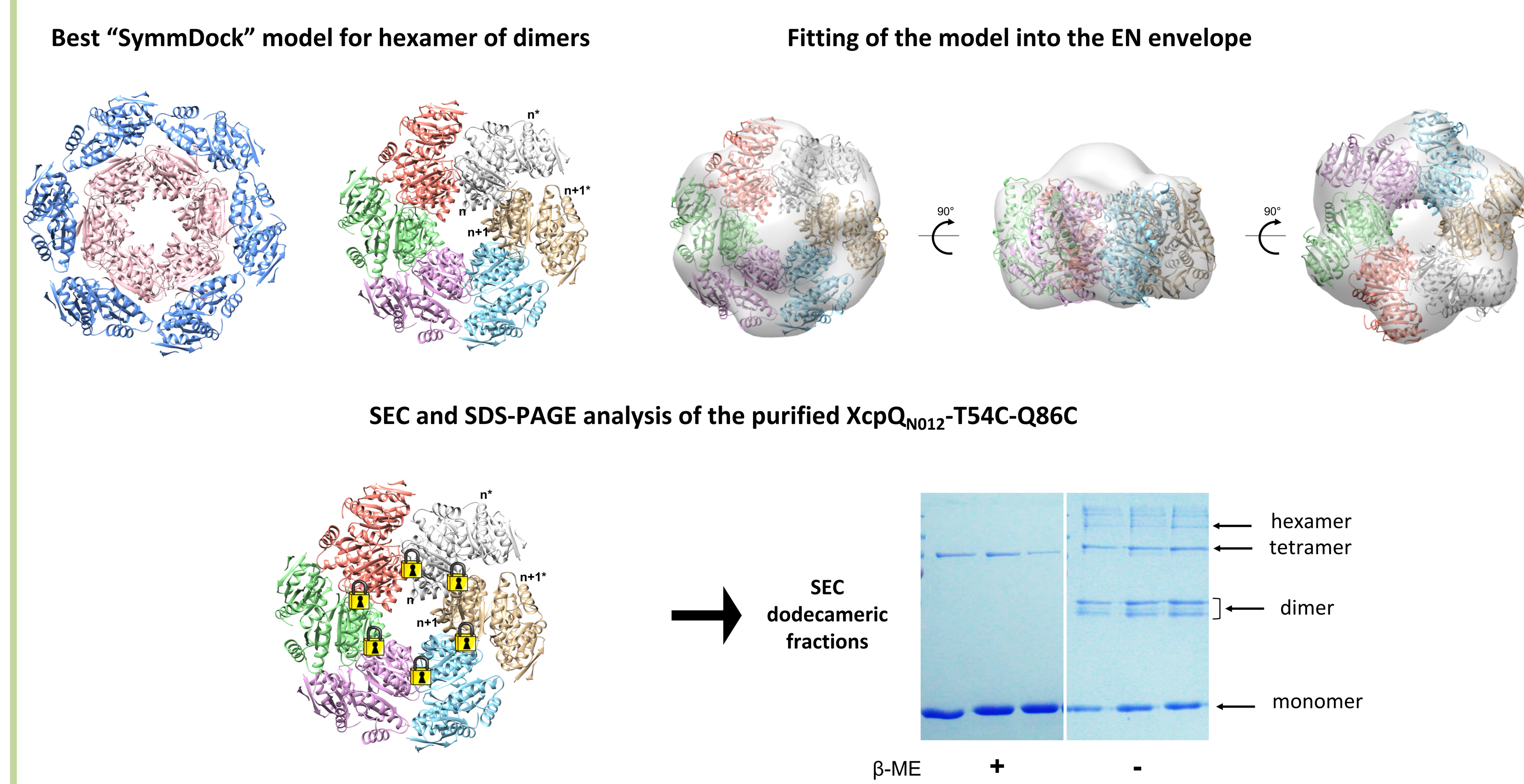
Secretins are giant homo-multimeric proteins composed of an outer membrane channel-forming C domain and a periplasmic N domain involved in substrate recruitment and connection with IM components. We have a good understanding of the OM-embedded, pore-forming C-terminal beta-barrel channel of secretin. 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.**



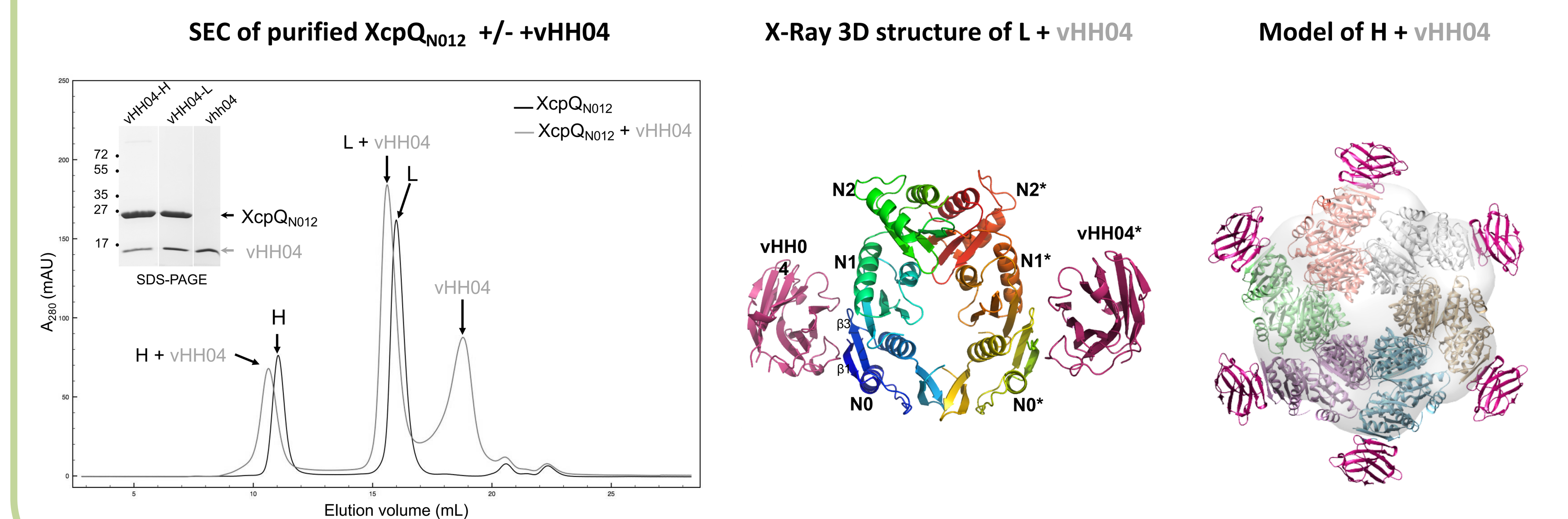
In vitro self-oligomerization of purified XcpQ$_{N012}$ into ring shaped hexamer of dimers



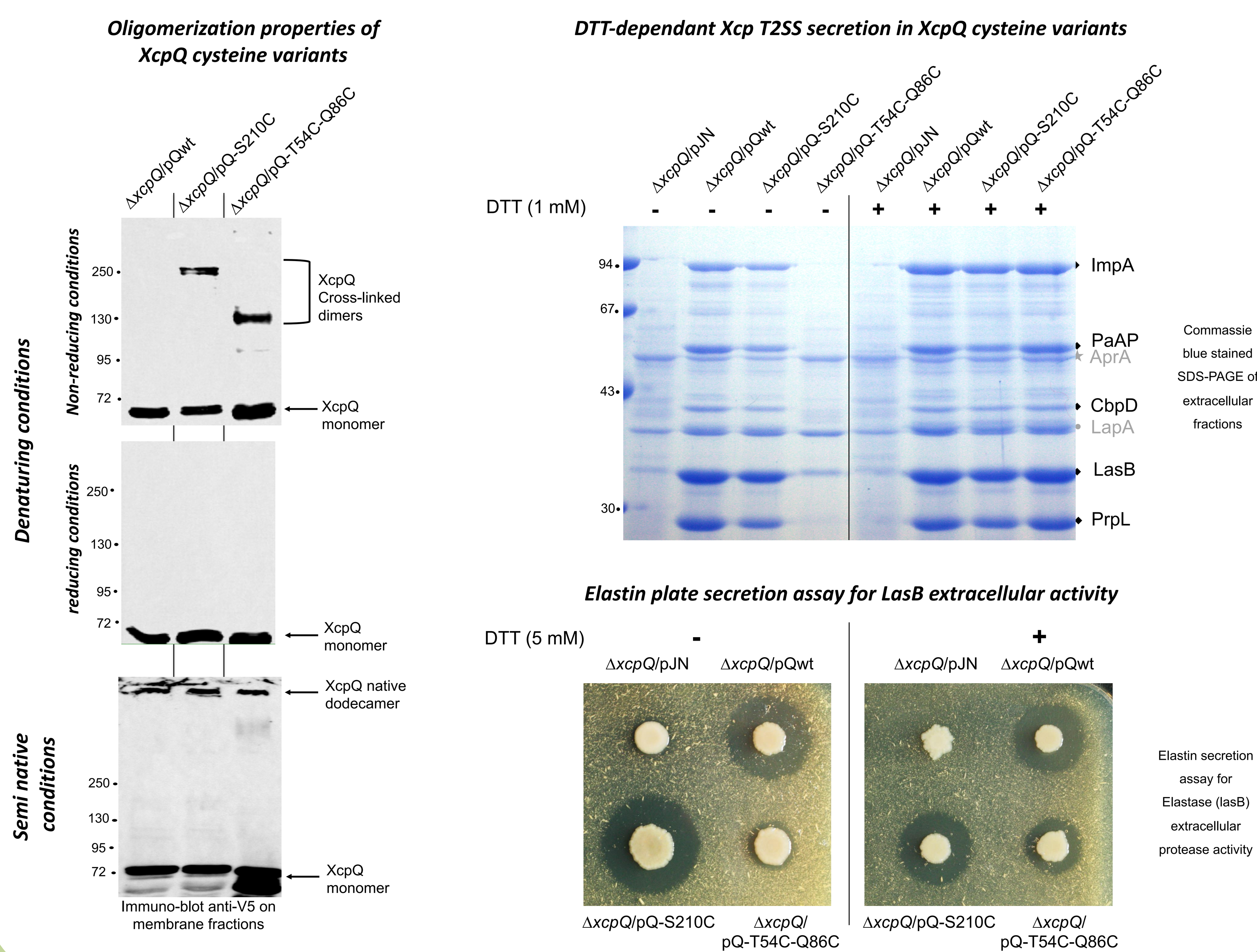
Atomic model of XcpQ$_{N012}$ dodecamers



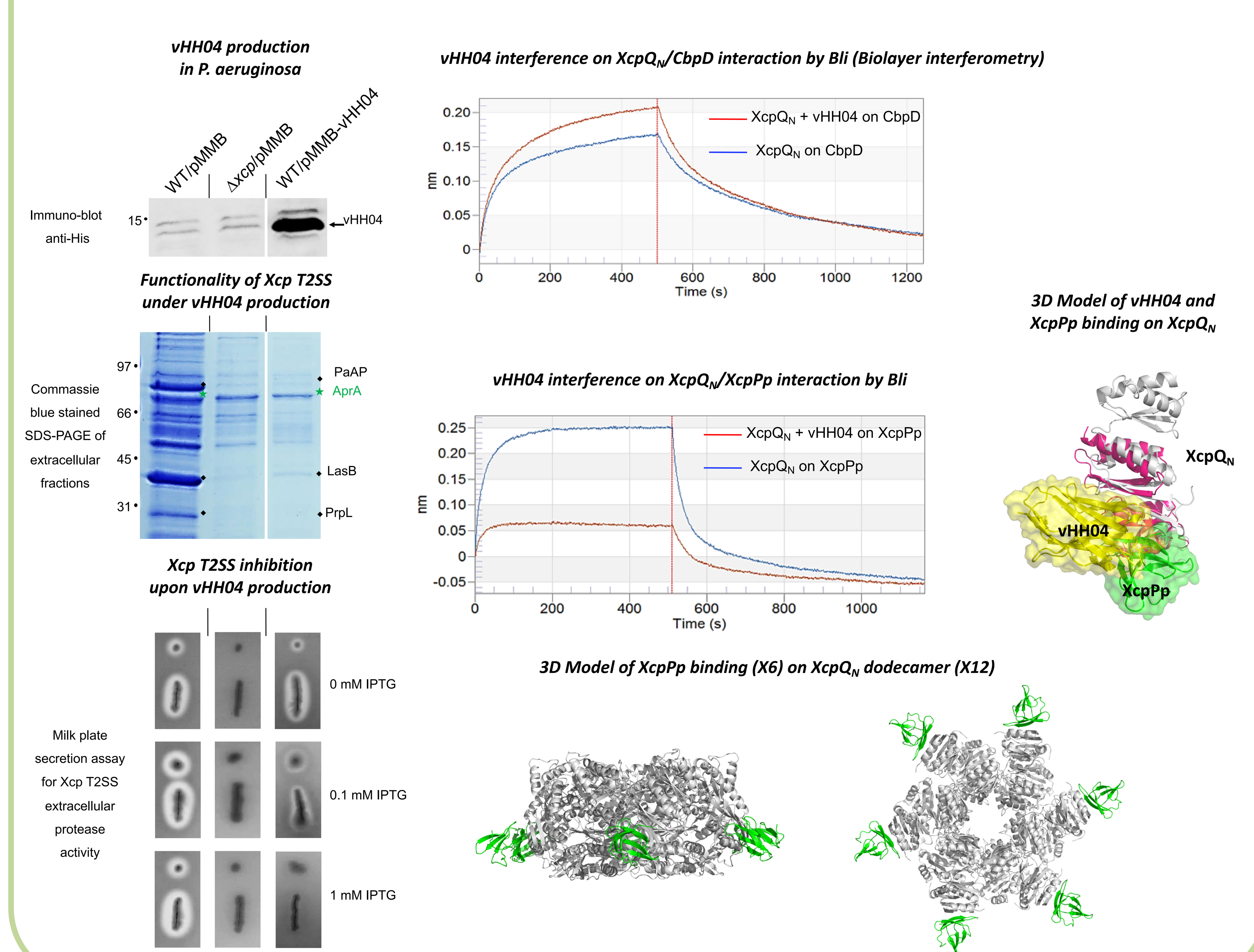
Identification of XcpQ$_{N012}$ inter-dimer interface by nanobody co-structure and interference



in vivo secretin dynamic revealed by cysteine cross linking



in vivo secretin interactome refined by vHH interference



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.