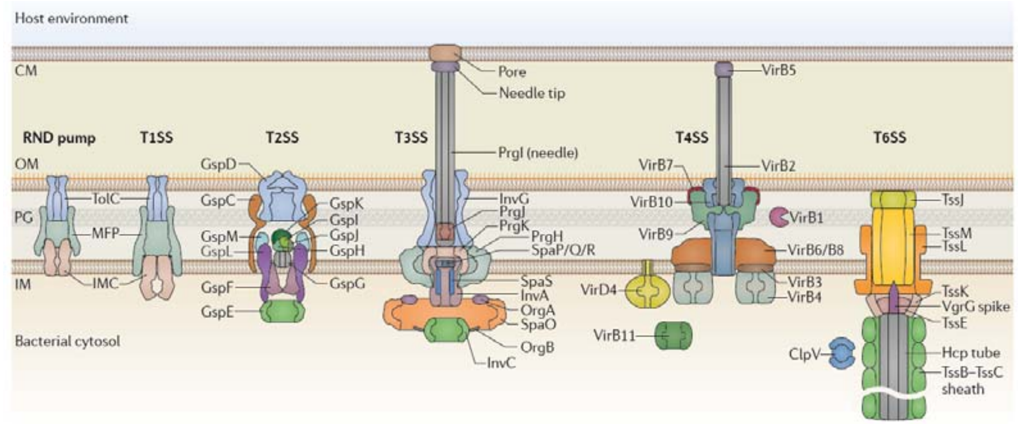
Structural studies of the membrane complex of the type 6 secretion system (T6SS)

An example of an integrated structural biology approach

Team « Host-Pathogen Interactions »

Alain Roussel

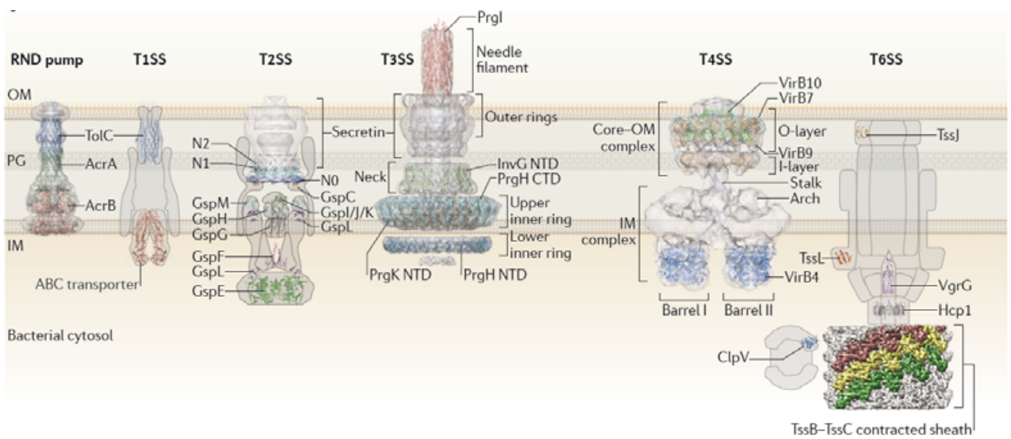
Secretion system of Gram-negative bacteria



Structural organization of the secretion systems that span the double membrane of Gram-negative bacteria

From Costa et al, Nat. Rev. Microb., 2015

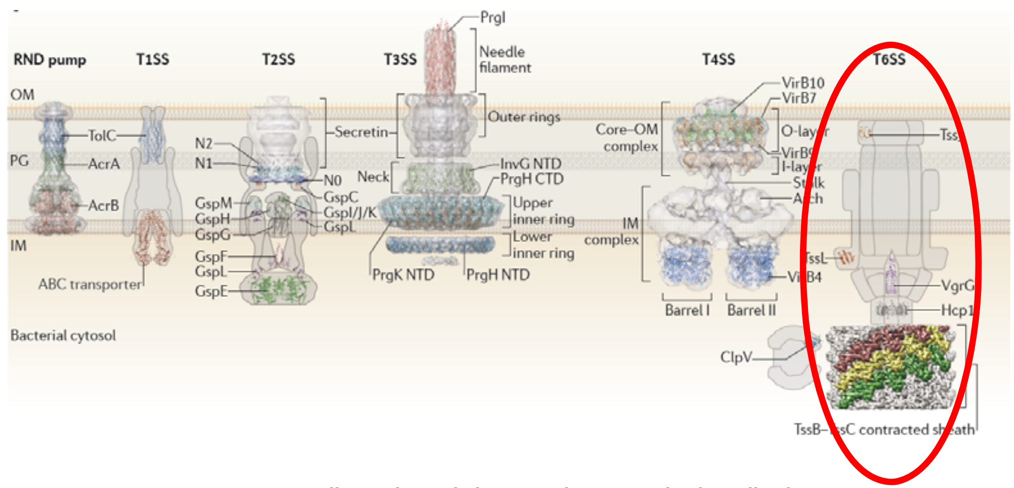
Secretion system of Gram-negative bacteria



X-ray crystallography and electron microscopy (EM) studies have provided near-atomic-resolution details of several systems

From Costa et al, Nat. Rev. Microb., 2015

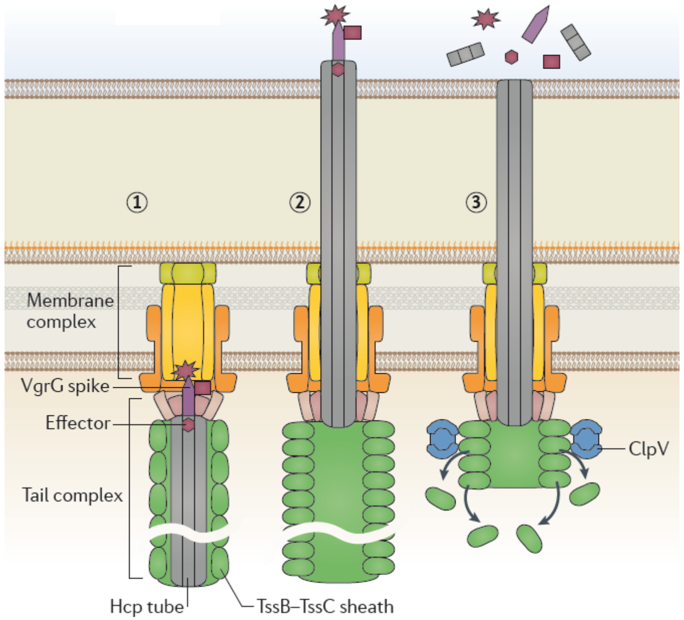
Secretion system of Gram-negative bacteria



X-ray crystallography and electron microscopy (EM) studies have provided near-atomic-resolution details of several systems

From Costa et al, Nat. Rev. Microb., 2015

Type VI secretion system (T6SS)



Step 1
The T6SS tail complex assembles onto the membrane complex. Effectors are recruited to the spike–tube complex.

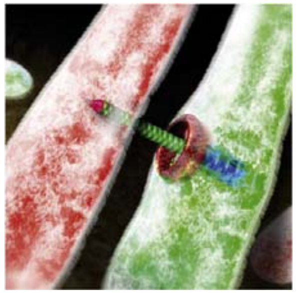
Step 2
An unknown extracellular signal triggers sheath contraction, which leads to the ejection of the spike–tube complex across the target membrane, thereby delivering effector proteins into the cell.

Step 3
The ATPase ClpV disassembles the contracted TssB–TssC sheath, which enables a new T6SS complex to be reassembled from the released subunits.

From Costa et al, Nat. Rev. Microb., 2015

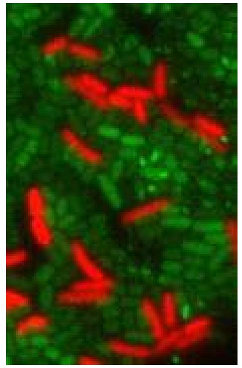
Model for the mechanism of substrate secretion

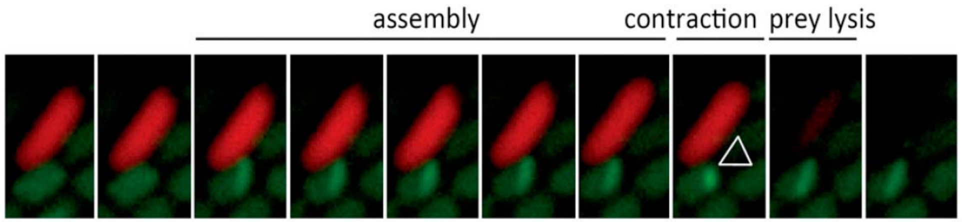
Type VI secretion system (T6SS)



Enteroaggregative E. coli (EAEC) cells producing TssB2-sfGFP (green)

prey cells (E. coli W3110) producing mCherry (red)



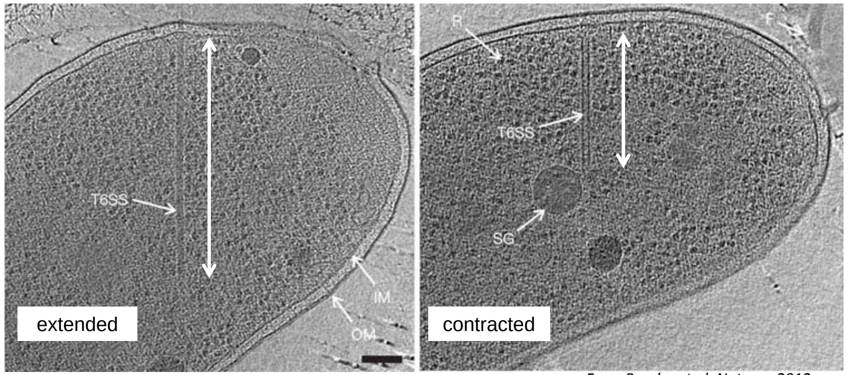


The scale bar is 1 μm. Individual images were taken every 7.5 min.

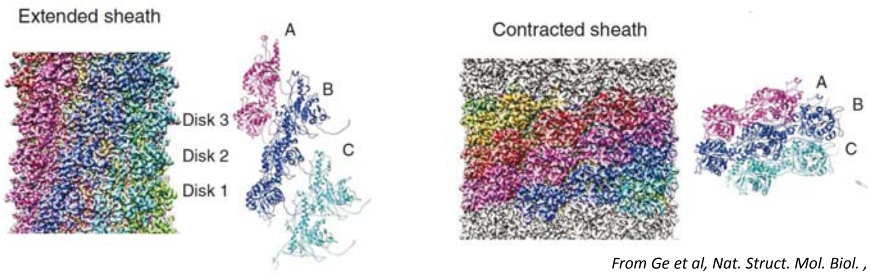
Imaging type VI secretion-mediated bacterial killing

From Brunet et al, Cell report, 2013

Type VI secretion system (T6SS)



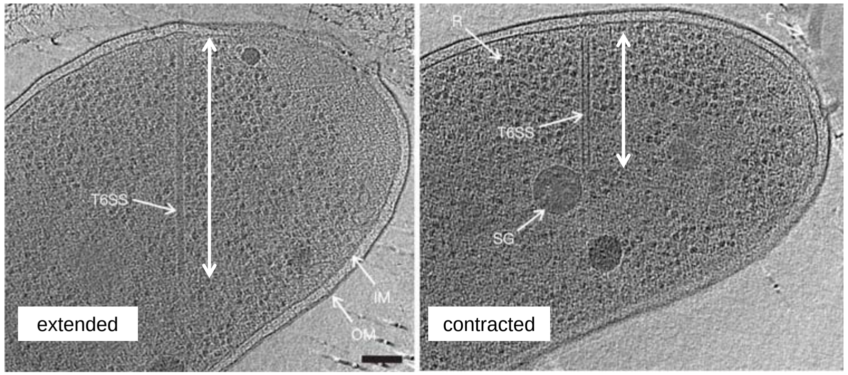
From Bassler et al, Nature , 2012



From Ge et al, Nat. Struct. Mol. Biol. , 2015

Type VI secretion system: secretion by a contractile nanomachine

Type VI secretion system (T6SS)



Energy per subunit : 12 kcal mol^{-1}; 1 μm sheath : 1500 subunits
Total energy during contraction : 18000 kcal mol^{-1}
(equivalent to hydrolysis of 1600 molecules of ATP)

Contraction happens in less than 5 ms
Speed of the VgrG spike : 100 μm s^{-1}
(kinesin moves at a rate of around 0.5 μm s^{-1})

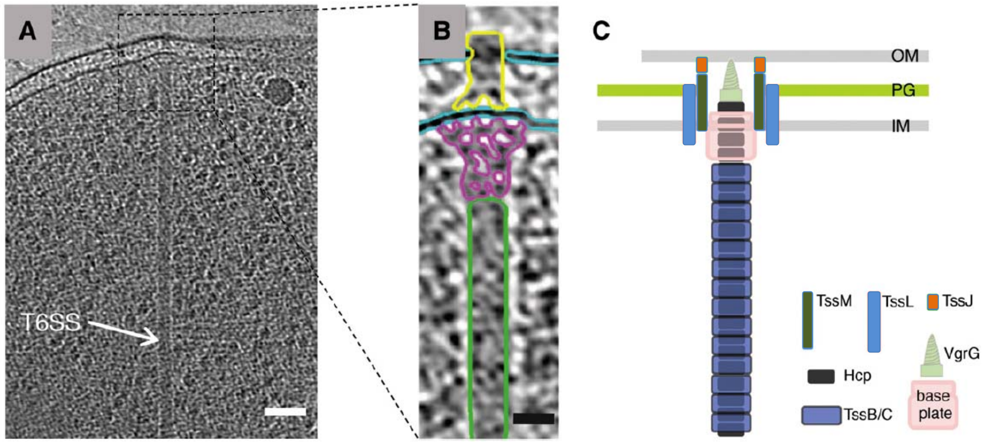
Rotation (phage T4) : one turn per 100 nm
For T6SS : 10 turns in less than 5 ms
Rotation : 120000 rpm

From Bassler, Phil. Trans. Royal Soc. , 2015

Type VI secretion system: secretion by a contractile nanomachine

T6SS membrane complex

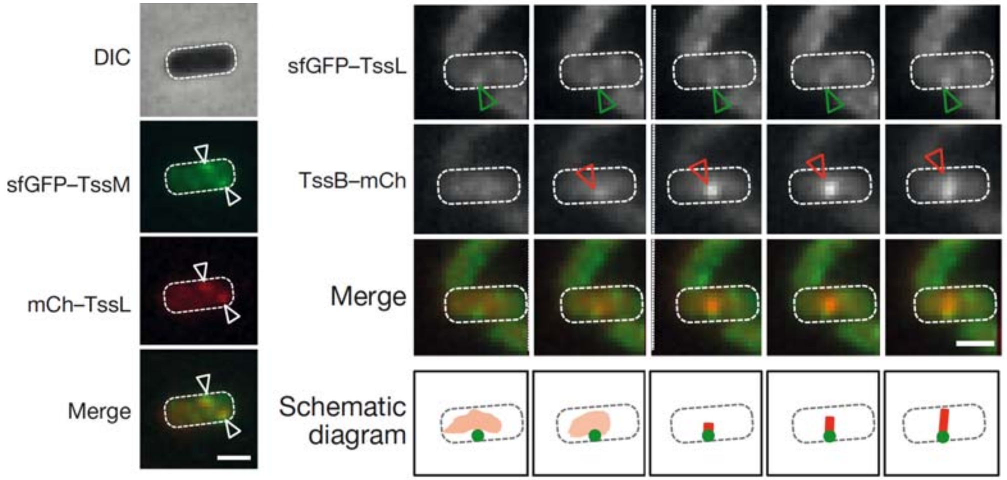
Type VI secretion system consists of 13 core component proteins, from TssA to TssM



Model for the mechanism of substrate secretion

From Zoued et al, B.B.A.., 2014

Biogenesis of the T6SS membrane-associated core complex

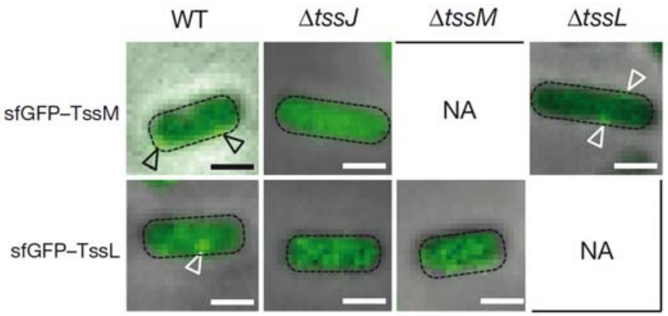


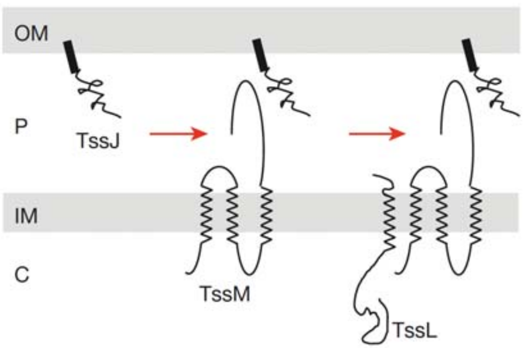
TssM and TssL
co-localize

The membrane complex serves as a docking site
for tail sheath polymerization.

From Durand et al, Nature, 2015

Biogenesis of the T6SS membrane-associated core complex

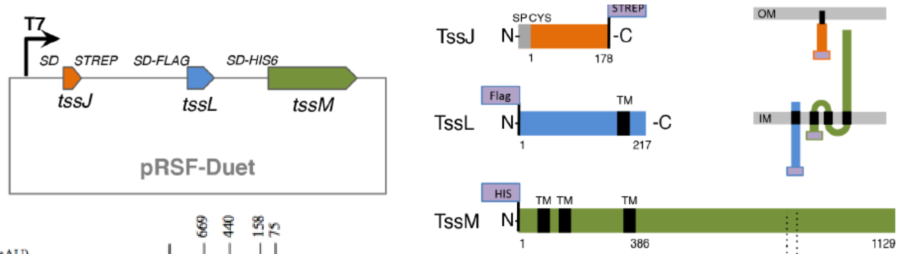


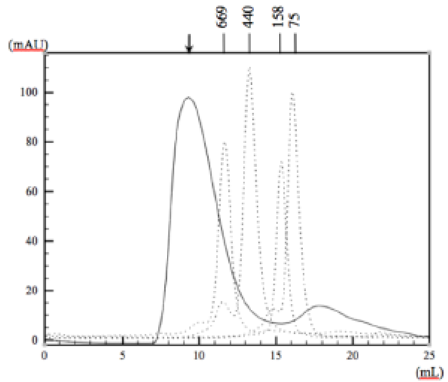


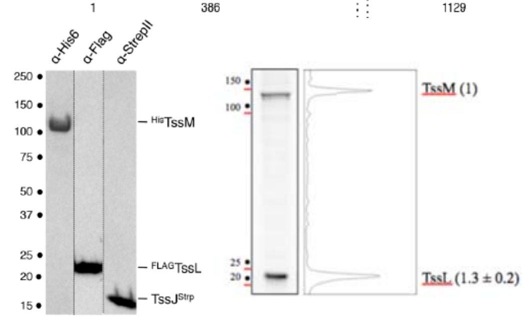
TssJ is used as a nucleation factor.
The biogenesis of the T6SS membrane
core complex is pursued by the inward
sequential addition of TssM and TssL

From Durand et al, Nature, 2015

Expression and purification of the T6SS membrane core complex

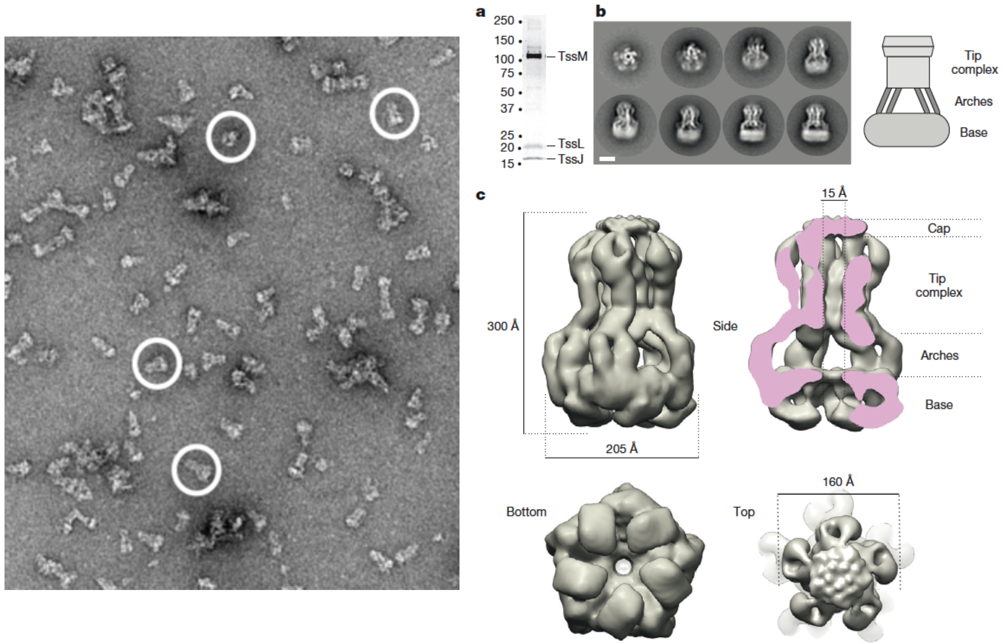






From Durand et al, Nature, 2015

Structure of the T6SS membrane complex determined by negative stain EM



From Durand et al, Nature, 2015

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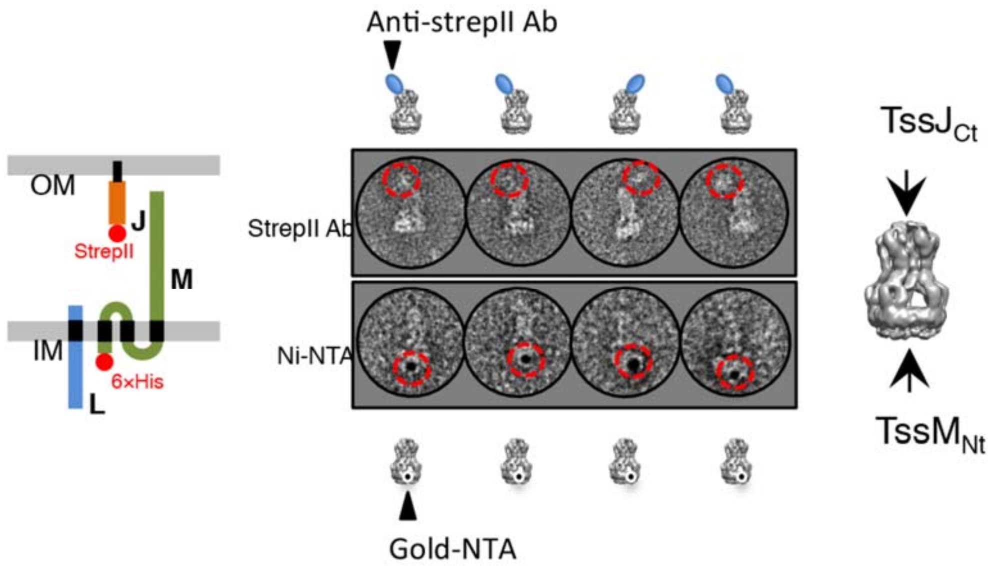
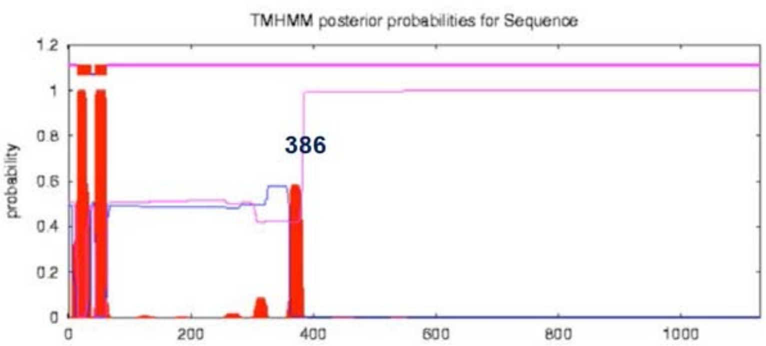
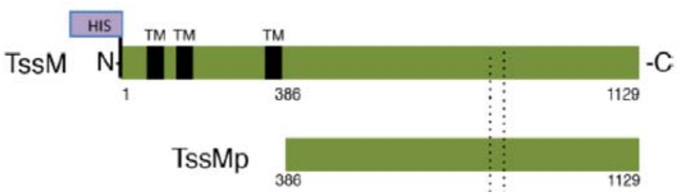
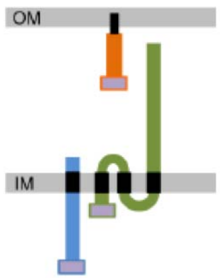


From Durand et al, Nature, 2015

The TssM periplasmic domain

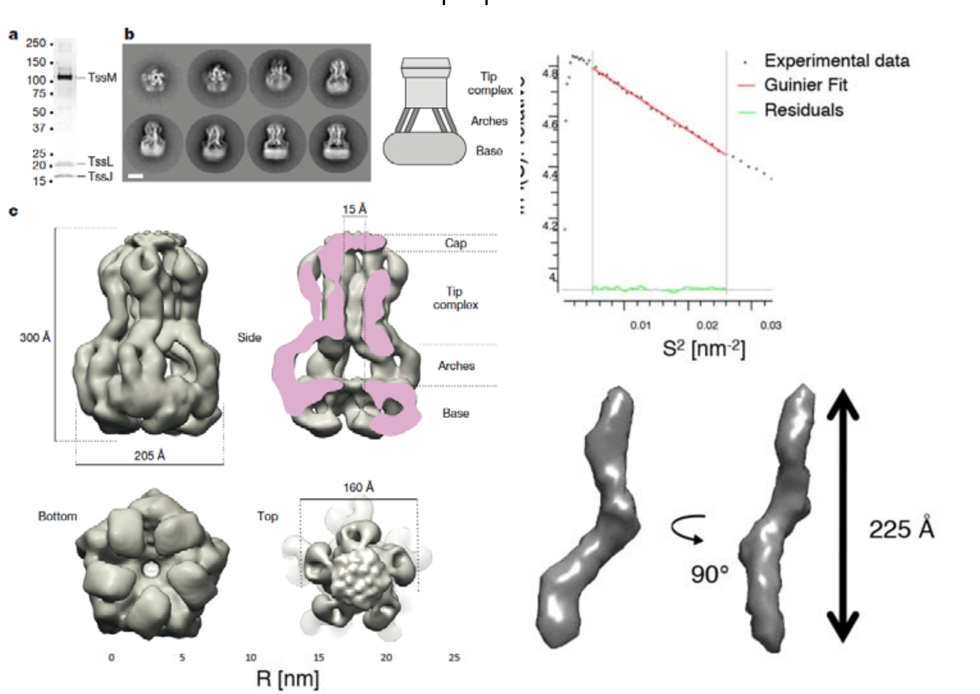




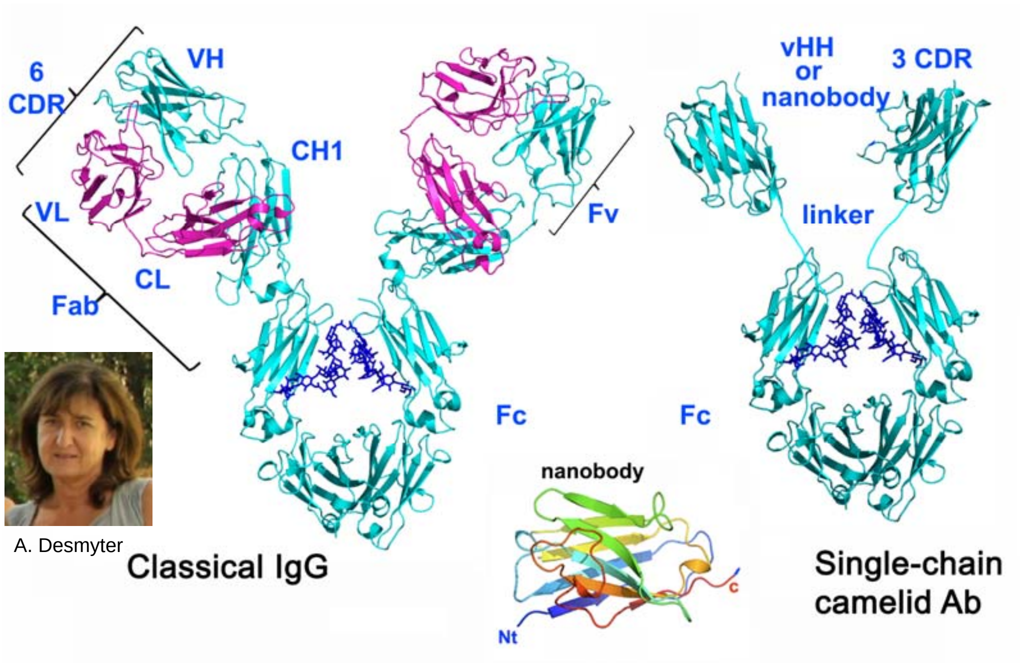


Expression OK but No Crystal !

The TssM periplasmic domain



Selection and production of camelid VHH/nanobodies @AFMB



A. Desmyter

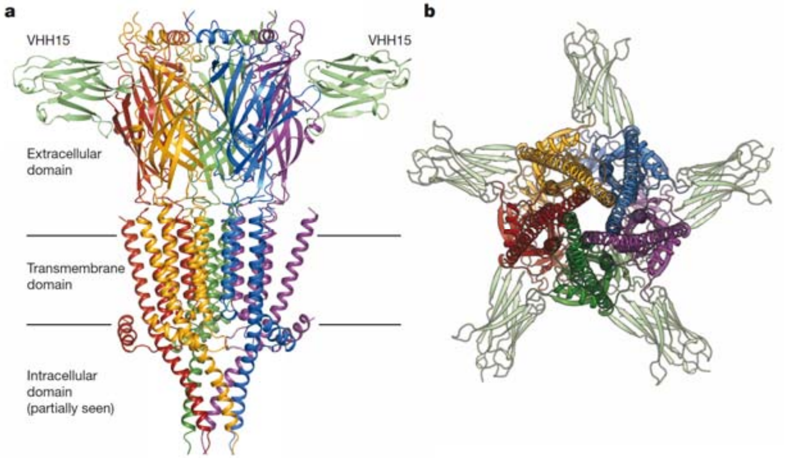
From Desmyter et al, COSB, 2015

ARTICLE 276 | NATURE | VOL 512 | 21 AUGUST 2014

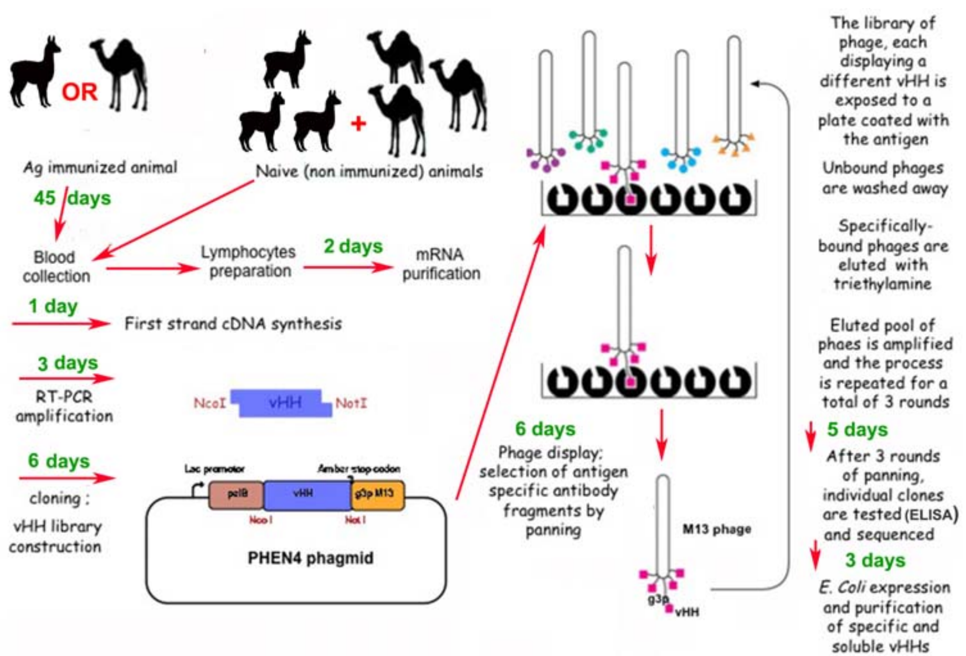
X-ray structure of the mouse serotonin 5-HT$_3$ receptor

Ghérici Hassaine[1]*†, Cédric Deluz[1]*, Luigino Grasso[1], Romain Wyss[1], Menno B. Tol[1], Ruud Hovius[1], Alexandra Graff[2], Henning Stahlberg[2], Takashi Tomizaki[3], Aline Desmyter[4], Christophe Moreau[5,6,7], Xiao-Dan Li[8], Frédéric Poitevin[9], Horst Vogel[1] & Hugues Nury[1,5,6,7]


A. Desmyter

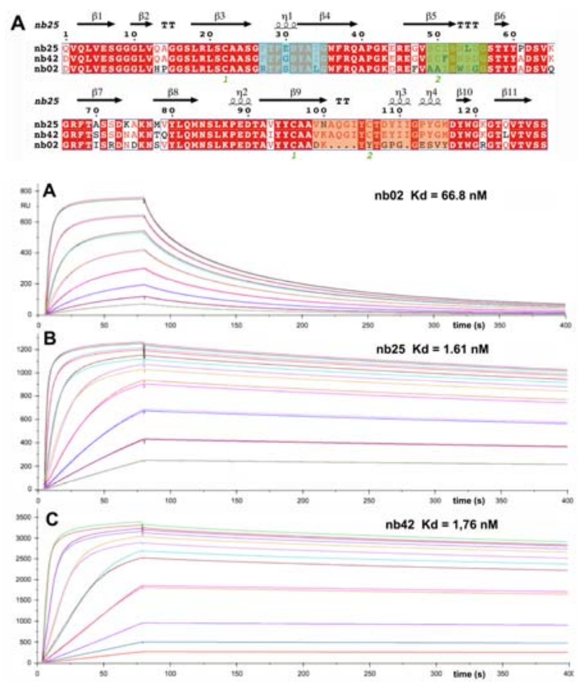


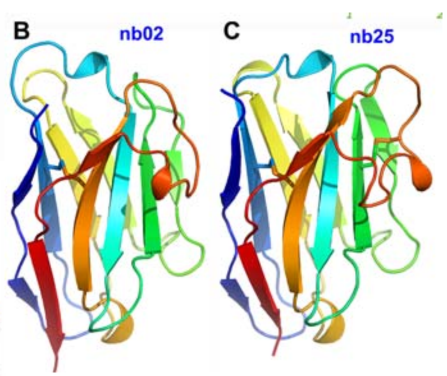
Selection and production of camelid VHH/nanobodies @AFMB

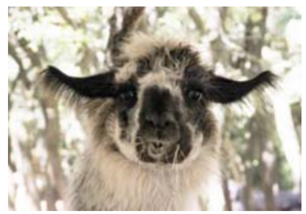


From Desmyter et al, COSB, 2015

Nanobodies nb02 and nb25 bind TssMp with nanomolar affinity

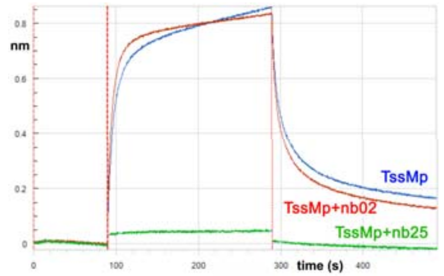


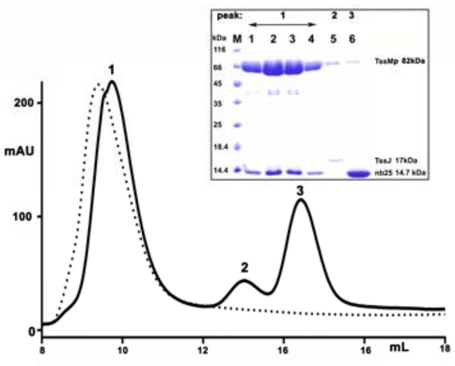




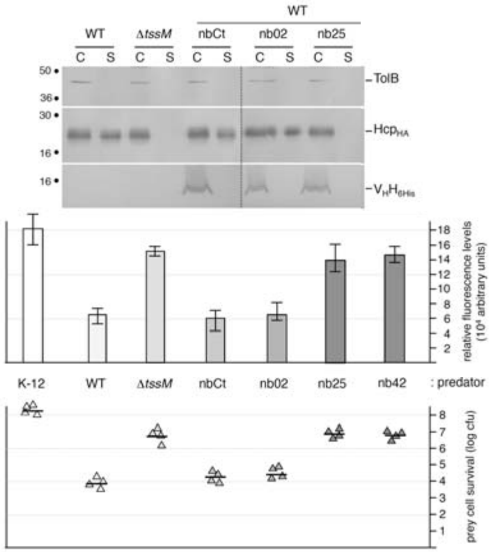
From Nguyen et al, Plos One, 2015

nb25 disrupts the TssMp:TssJ complex



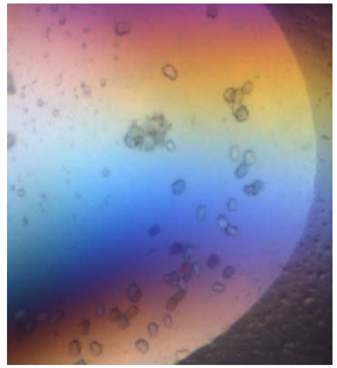


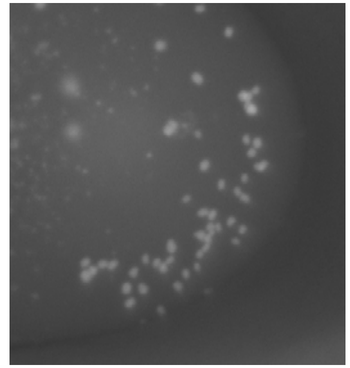
nb25 specifically affects T6SS function



From Nguyen et al, Plos One, 2015

Crystals of TssMp in complex with nb02 and nb25



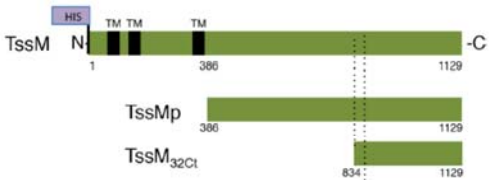


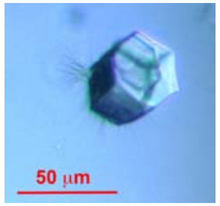
Data collection Soleil PX2

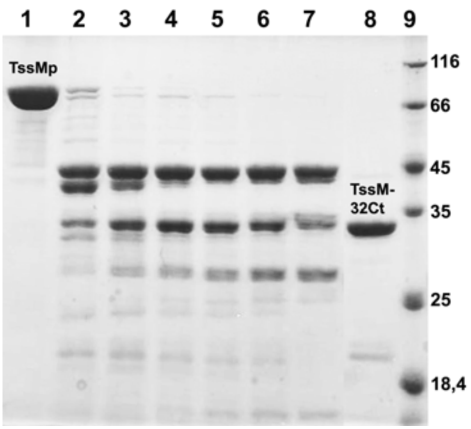
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas
24.66	937	242	371	65.2%	4.0%	4.0%	932	21.74	4.7%
18.63	1679	364	450	80.9%	5.9%	5.8%	1664	20.24	6.7%
15.58	2133	451	547	82.4%	8.3%	7.8%	2110	15.93	9.3%
13.66	2374	508	620	81.9%	12.4%	12.2%	2341	10.29	14.0%
12.32	2804	597	716	83.4%	19.3%	19.1%	2769	7.48	21.8%
11.30	3024	633	761	83.2%	25.1%	25.4%	2976	5.72	28.1%
10.50	3363	695	828	83.9%	43.8%	45.5%	3309	3.43	48.9%
9.85	3607	742	877	84.6%	61.6%	64.9%	3550	2.47	68.6%
9.31	3750	775	930	83.3%	83.5%	86.8%	3683	1.74	92.9%
total	23671	5007	6100	82.1%	14.6%	14.8%	23334	7.73	16.5%

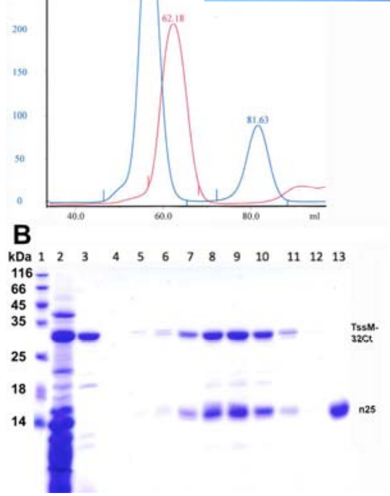
The $TssM_{32Ct}$/nb25 complex





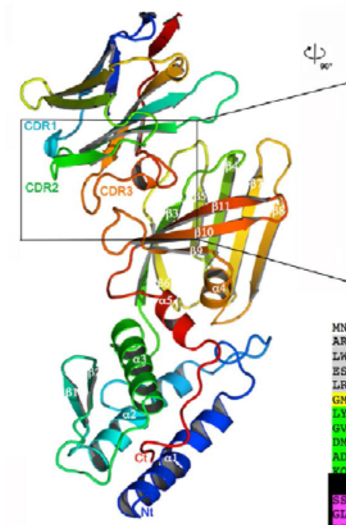


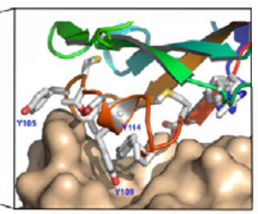
Limited proteolysis

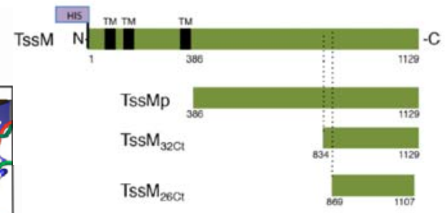


From Nguyen et al, Acta Cryst F, 2015

The $TssM_{32Ct}$/nb25 complex





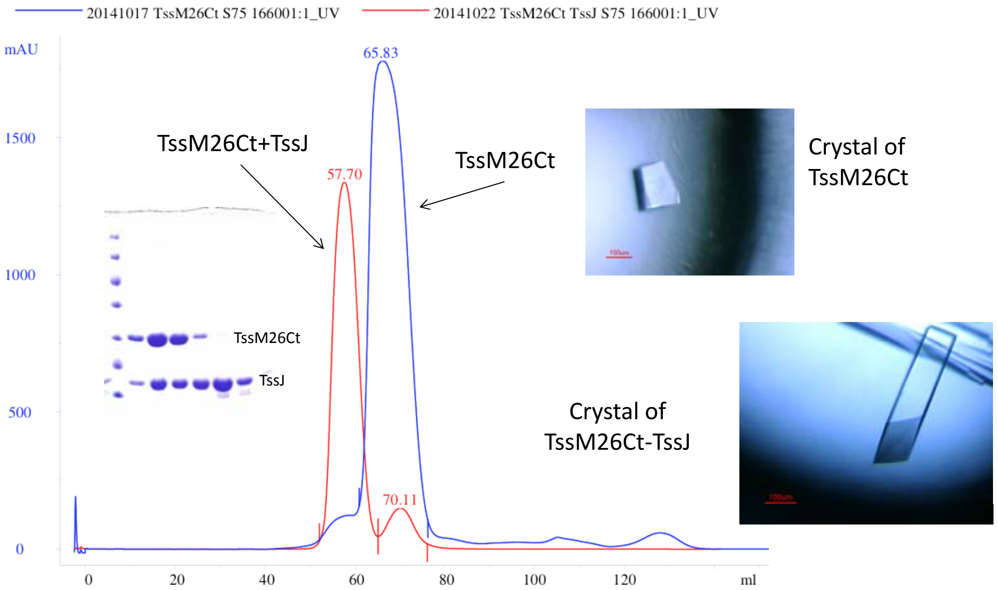


TssM26ct

TssM32ct

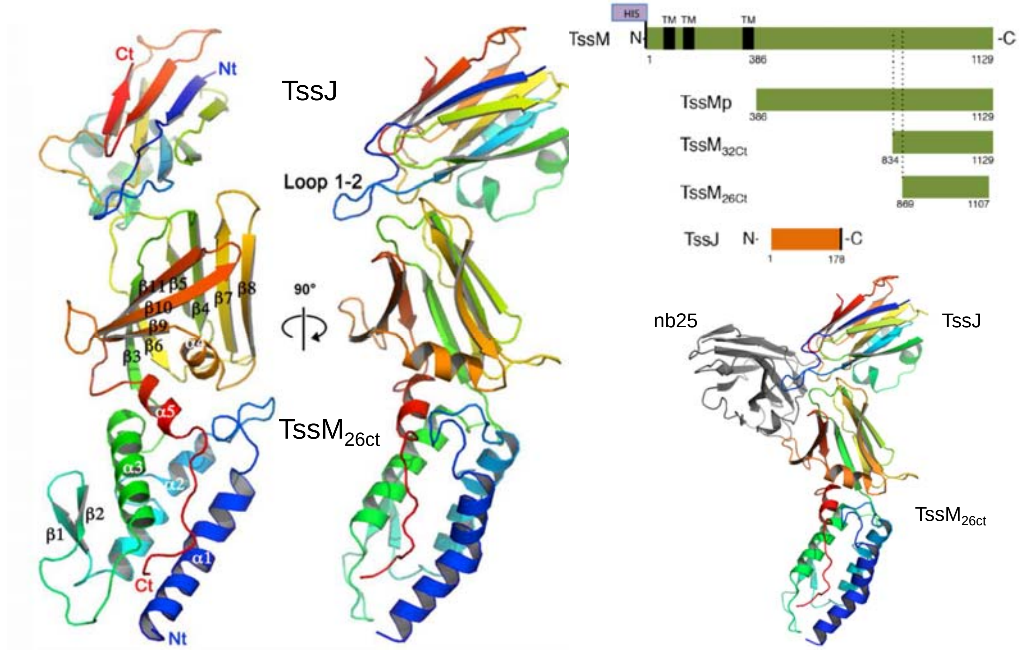
From Nguyen et al, Acta Cryst F, 2015

The TssM$_{26ct}$/TssJ periplasmic complex



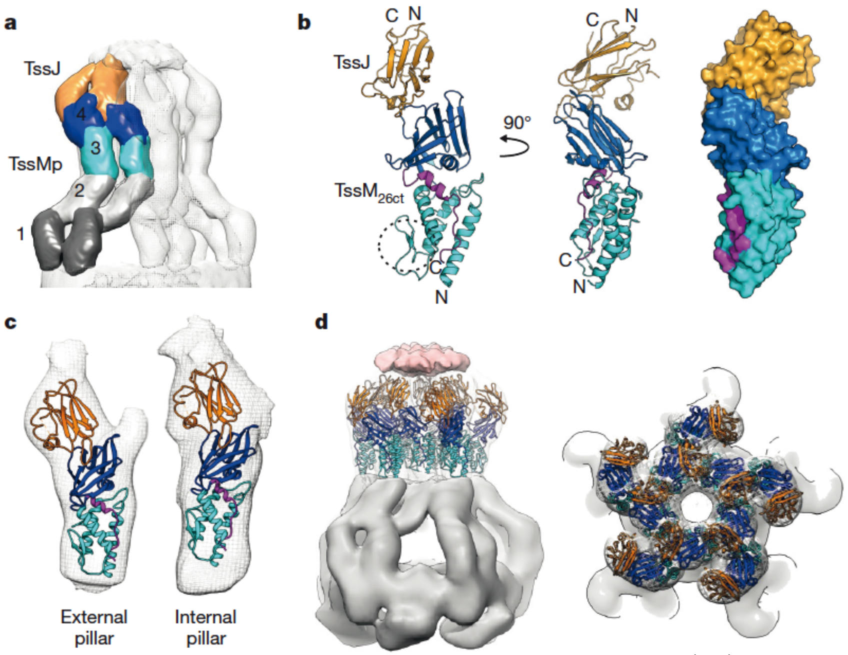
From Durand et al, Nature, 2015

The TssM$_{26ct}$/TssJ periplasmic complex



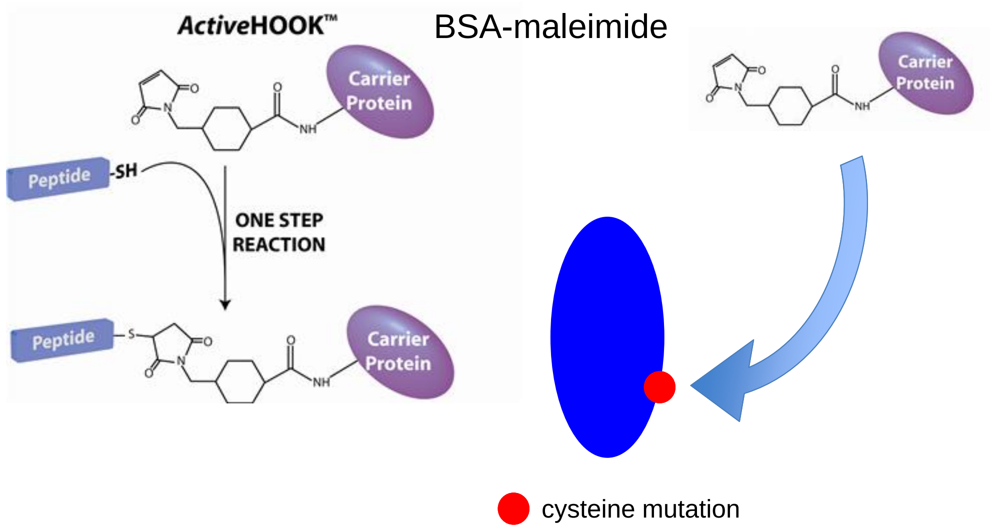
From Durand et al, Nature, 2015

Structure of the TssJLM tip complex (*in vitro*)



From Durand et al, Nature, 2015

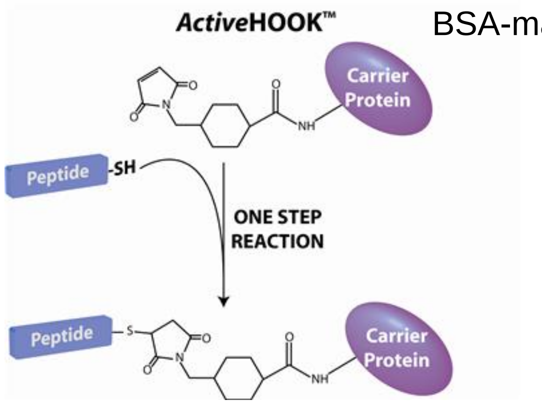
Cell surface accessibility of the T6SS membrane core complex during secretion



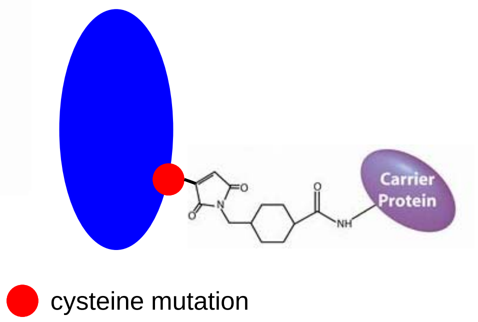
Treatment with a cysteine-reactive, membrane-impermeant BSA-maleimide

From Durand et al, Nature, 2015

Cell surface accessibility of the T6SS membrane core complex during secretion



BSA-maleimide



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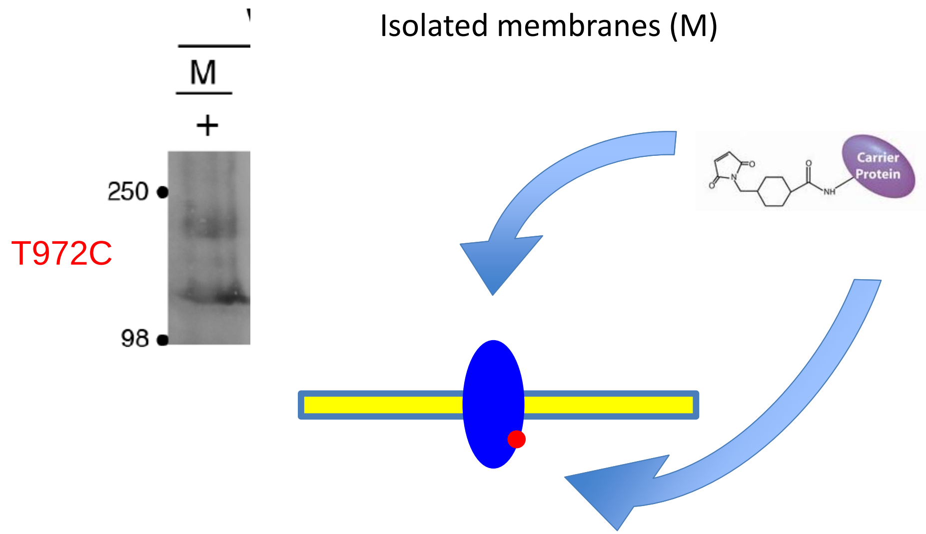

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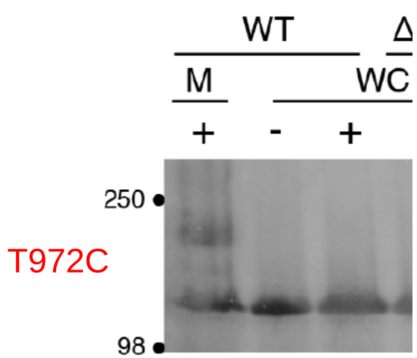
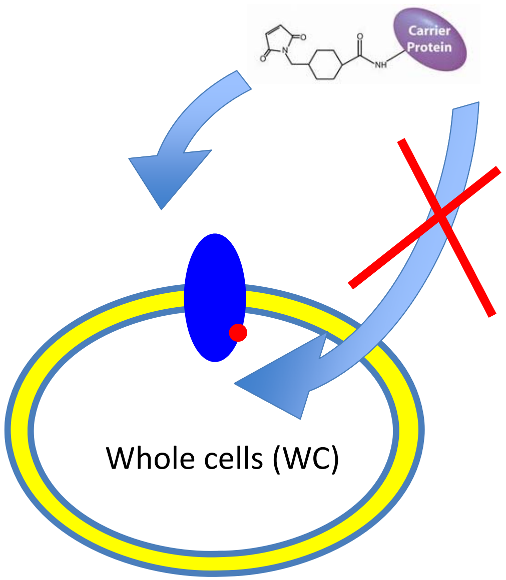


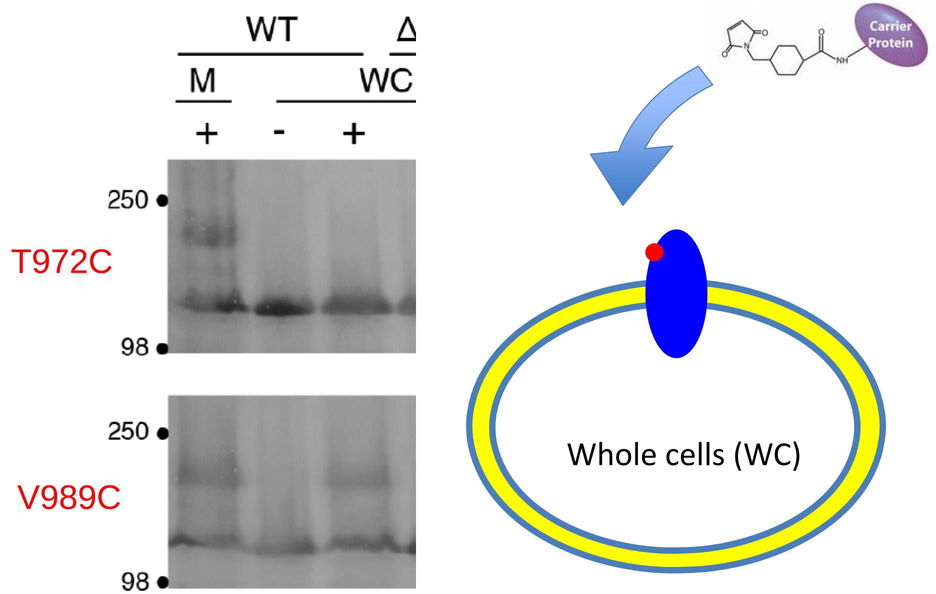


Position 972 is not accessible

Treatment with a cysteine-reactive, membrane-impermeant BSA-maleimide

From Durand et al, Nature, 2015

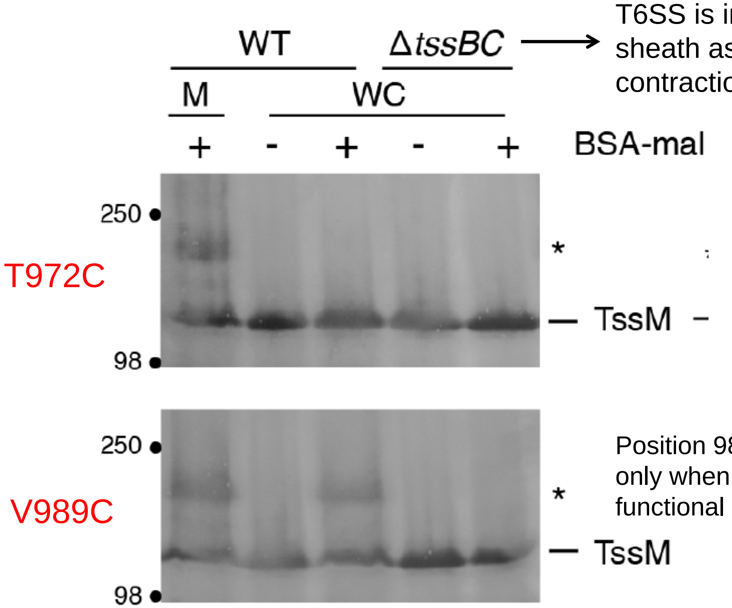
Cell surface accessibility of the T6SS membrane core complex during secretion



Treatment with a cysteine-reactive, membrane-impermeant BSA-maleimide

From Durand et al, Nature, 2015

Cell surface accessibility of the T6SS membrane core complex during secretion



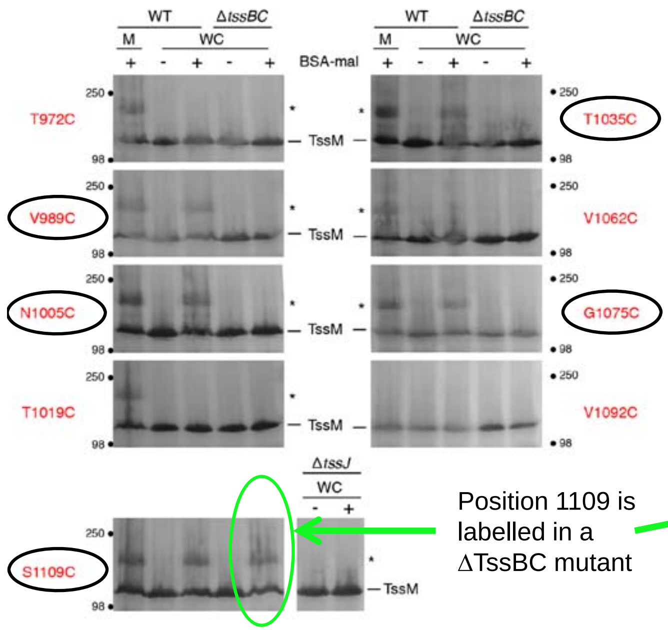
T6SS is inactive as no sheath assembly or contraction could occur.

Position 989 is accessible only when T6SS is functional

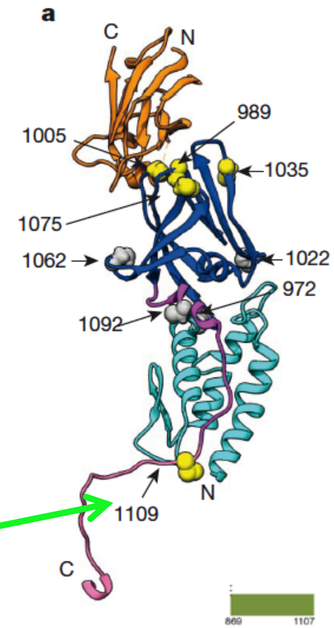
Treatment with a cysteine-reactive, membrane-impermeant BSA-maleimide

From Durand et al, Nature, 2015

Cell surface accessibility of the T6SS membrane core complex during secretion



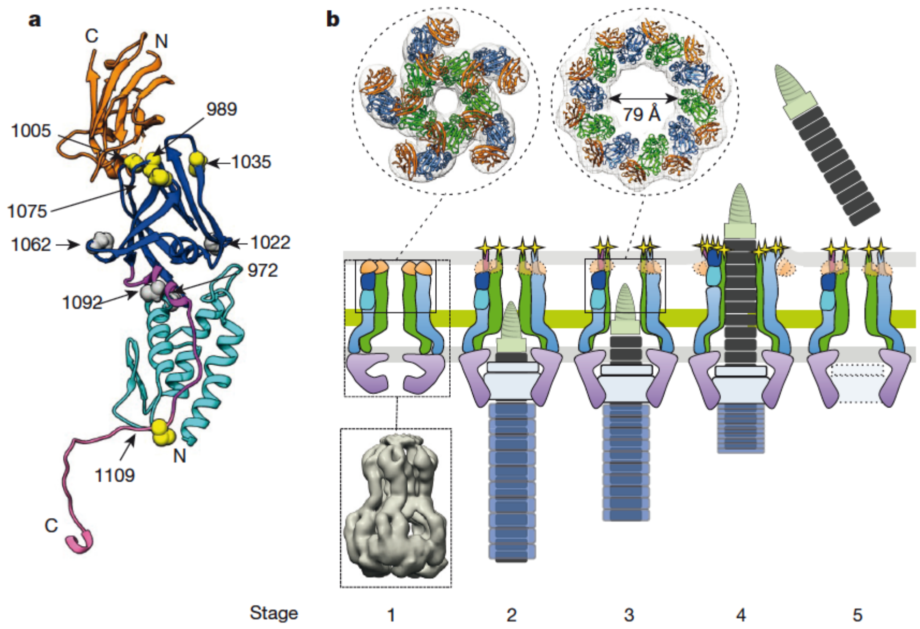
Position 1109 is labelled in a ΔTssBC mutant



Treatment with a cysteine-reactive, membrane-impermeant BSA-maleimide

From Durand et al, Nature, 2015

Cell surface accessibility and mechanism of action of the T6SS membrane core complex during secretion



From Durand et al, Nature, 2015


C. Cambillau


S. Nguyen


E. Cascales


A. Zoued


R. Fronzes

afmb architecture et fonction des macromolécules biologiques

LISM Laboratoire d'Ingénierie des Systèmes Macromoléculaires

Institut Pasteur


A. Desmyter

cnrs


E. Durand

erc European Research Council

Aix*Marseille université

ANR AGENCE NATIONALE DE LA RECHERCHE