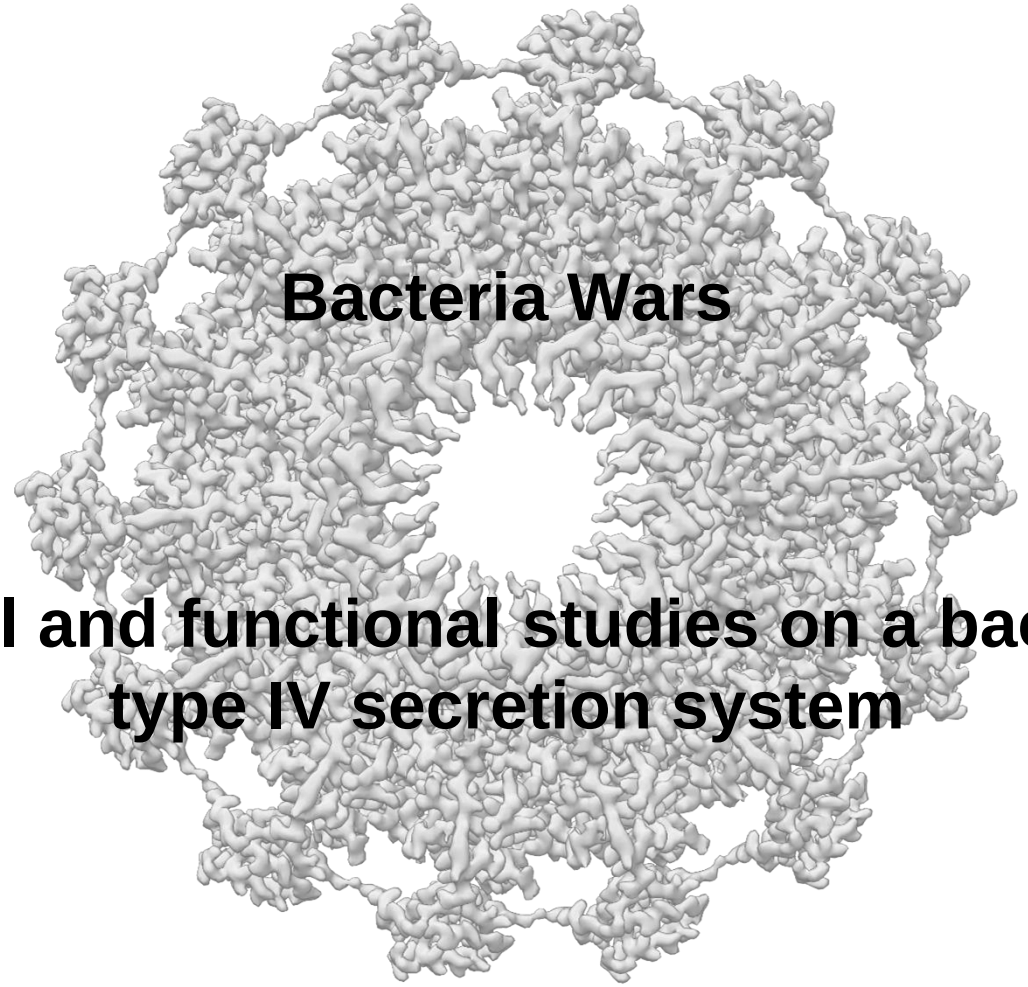
Universidade de São Paulo
Instituto de Química

International Centre for Theoretical Physics
South American Institute for Fundamental Research
ICTP SAIFR



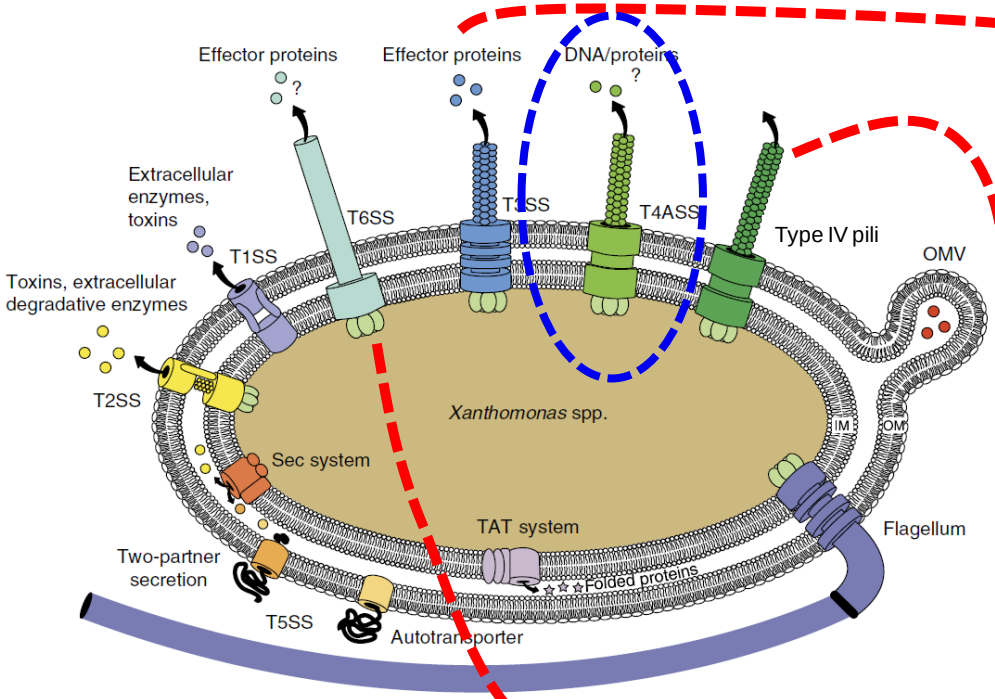
Bacteria Wars

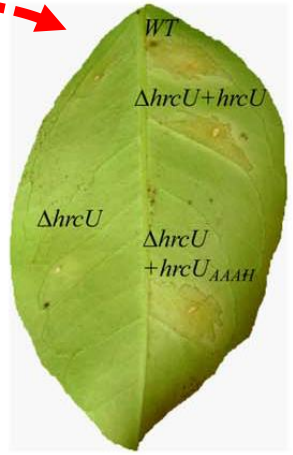
Structural and functional studies on a bactericidal type IV secretion system

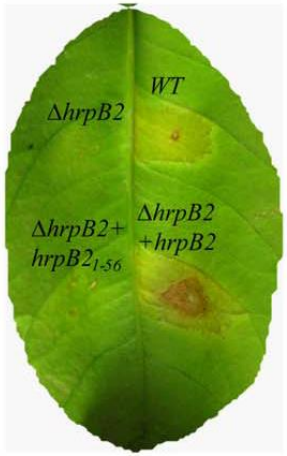
Shaker Chuck Farah

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brasil

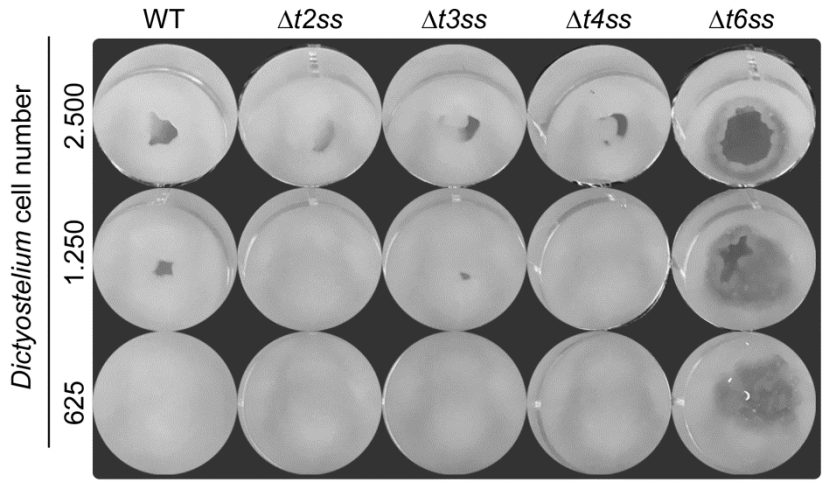
CEPID B3
Biologia de Bactérias e Bacteriófagos



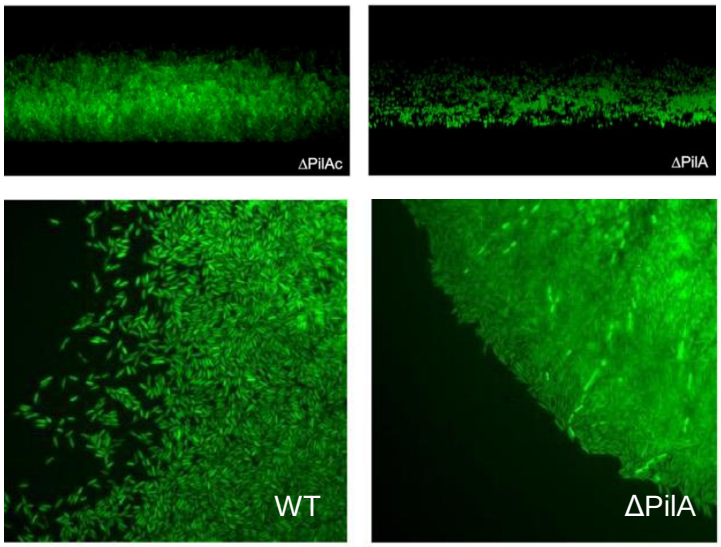




T3SS – canker symptoms in plant host
Cappelletti et al (2011)



T6SS – protection against predatory ameobas
Bayer-Santos et al (2018)



T4 pilus – twitching motility and biofilm
(Guzzo et al, 2009, 2013; Dunger et al, 2014, Llontop et al, 2022)

Article

Cryo-EM structure of a type IV secretion system

https://doi.org/10.1038/s41586-022-04859-y

Received: 19 April 2021

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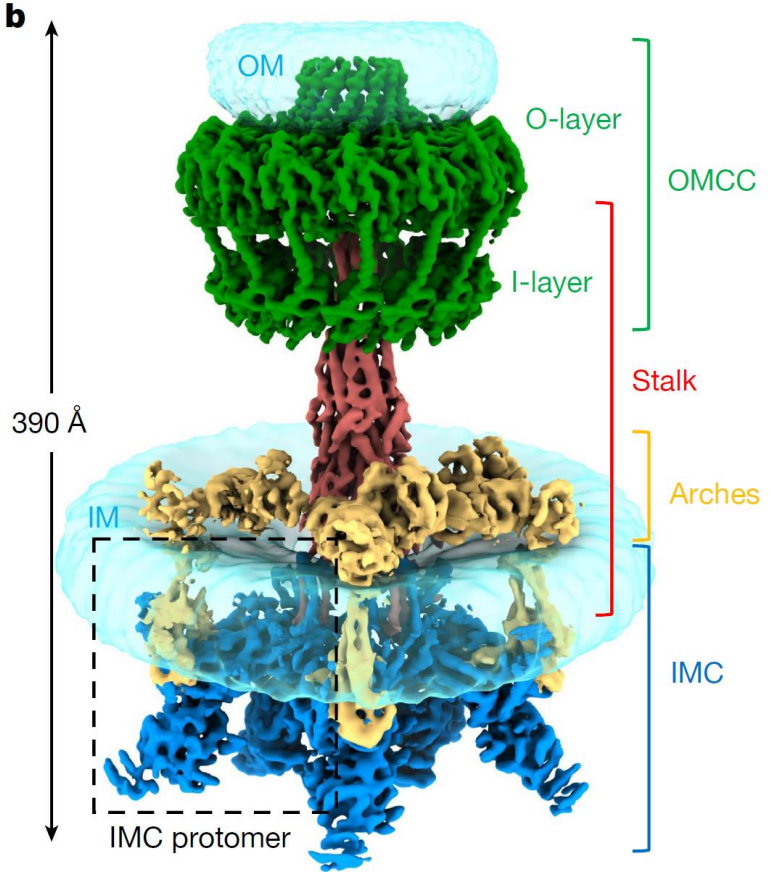
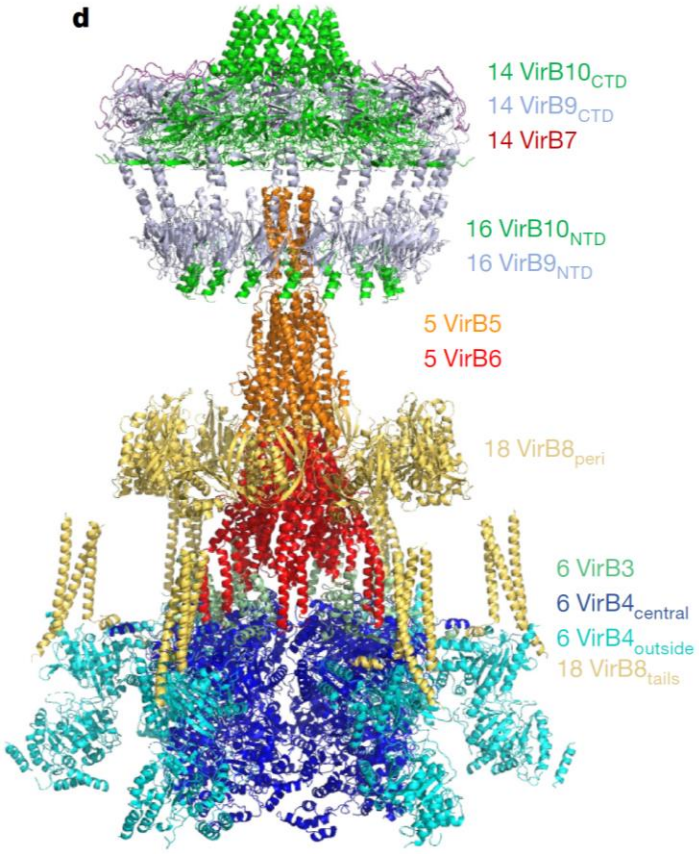
Kévin Macé[1,8]✉, Abhinav K. Vadakkepat[1,8], Adam Redzej[1], Natalya Lukoyanova[1], Clasien Oomen[1], Nathalie Braun[1,5], Marta Ukleja[1,6], Fang Lu[1], Tiago R. D. Costa[1,7], Elena V. Orlova[1], David Baker[2], Qian Cong[2,3]✉ & Gabriel Waksman[1,4]✉

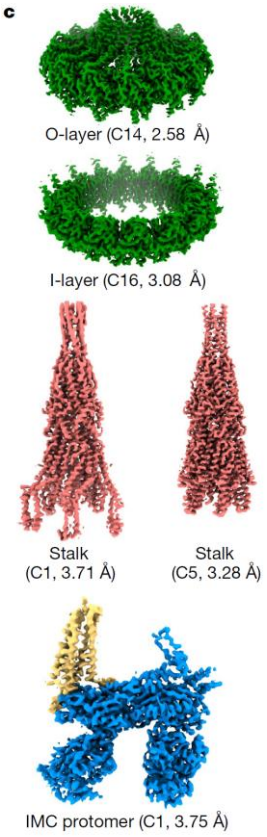
A spectacular example of a difficult structural biology project attacked by several techniques

- Membrane proteins
- Symmetry Mismatch
- Compositional heterogeneity
- Conformational heterogeneity
- Disordered regions
- CryoEM + Crystallography + AlphaFold
- Composite model construction
- VirD4 coupling ATPase not present

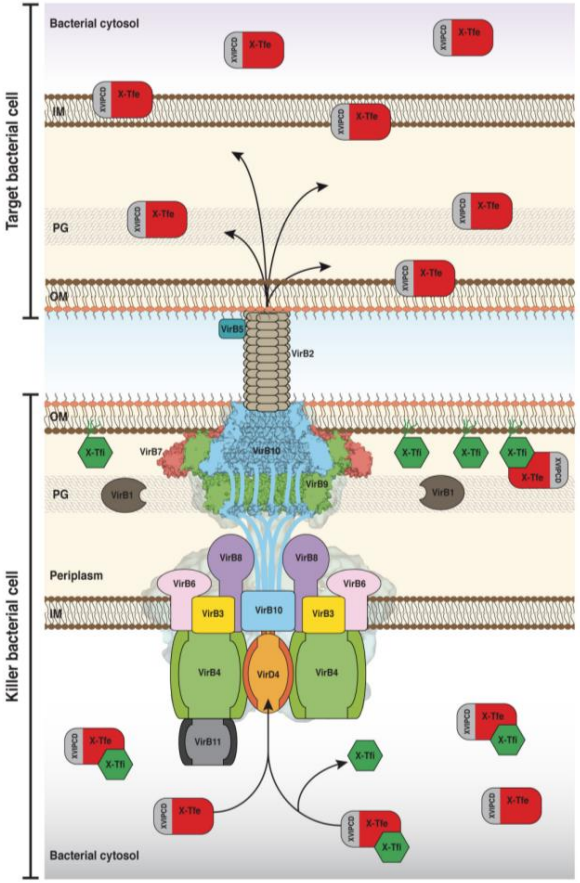
Prototypical conjugative T4SS

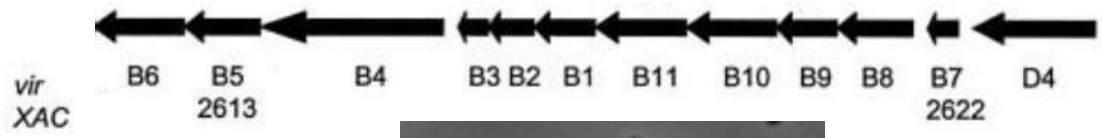


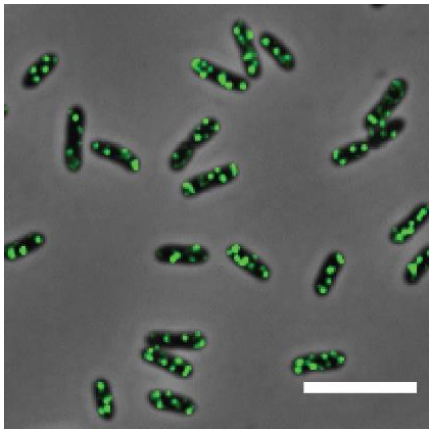




The Xanthomonas Type IV Secretion System







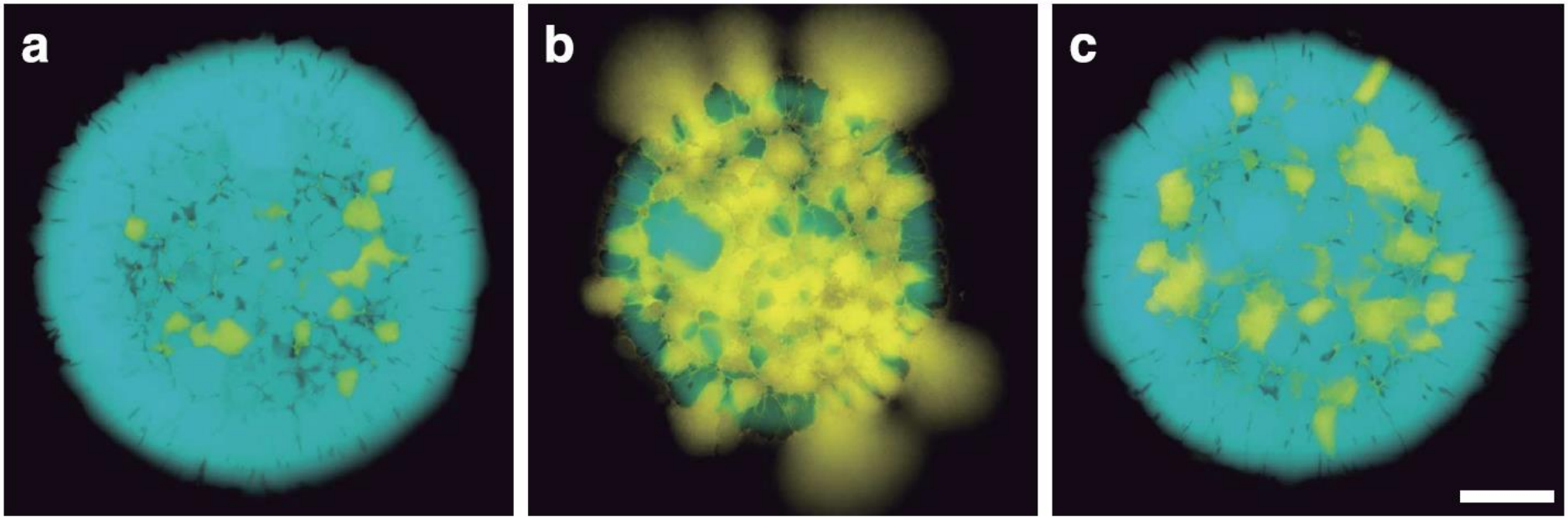
Physiological role(s) of the machine

- Identification and characterization of protein substrates
- Mutagenesis studies

How the machine works - Protein-protein interactions and structures of:

- the components of the pore
- substrate activity and secretion
- molecular motifs responsible for substrate recognition
- inhibitors/immunity proteins

The *X. citri* X-T4SS offers an advantage in growth competition experiments



***X. citri* WT CFP**

***X. citri* ΔvirB7 CFP**

+ *E. coli* DH5α YFP

***X. citri* ΔvirB7 + *virB7* CFP**

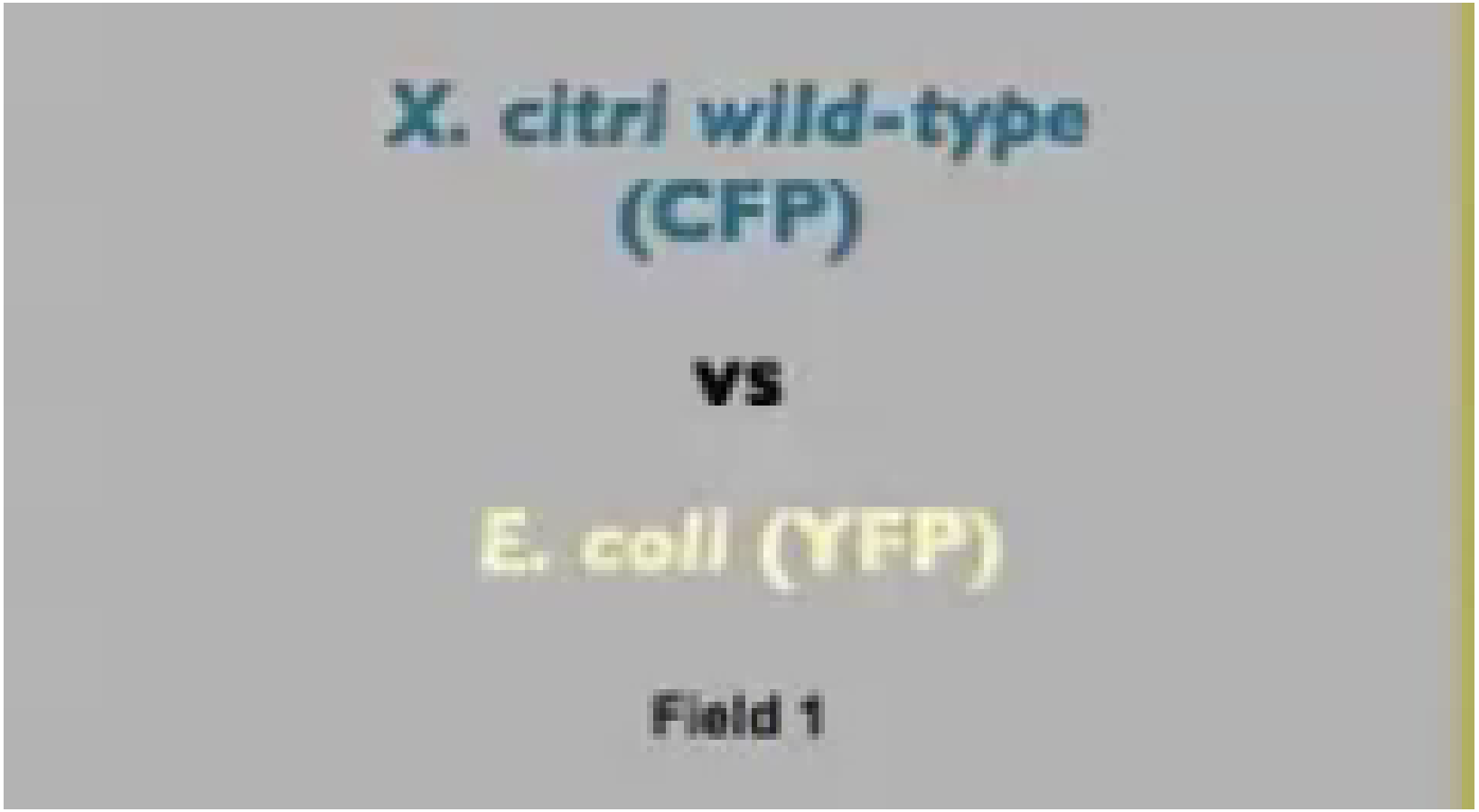
Mixed colonies; scale bar: 1 mm

Souza *et al*. (2015)

X. citri

vs

E. coli

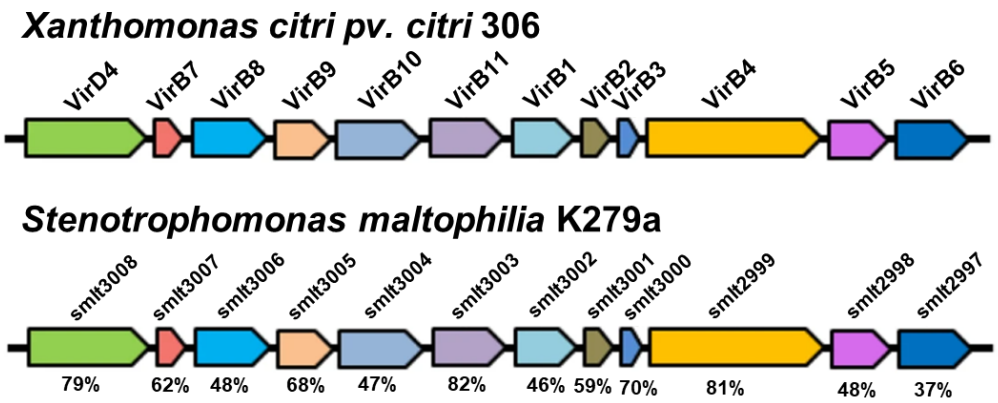


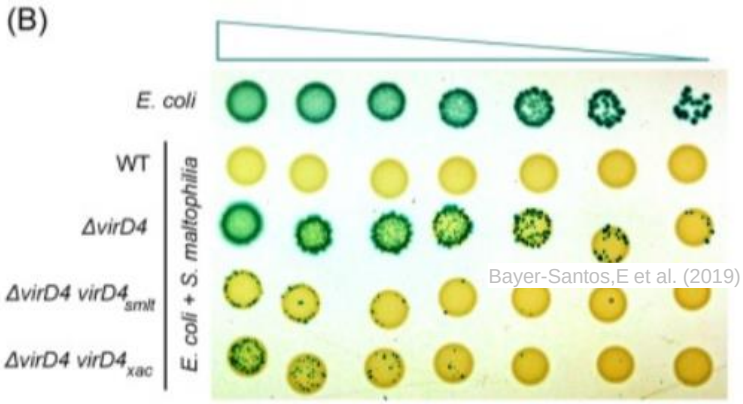
Souza *et al*. (2015)

RESEARCH ARTICLE

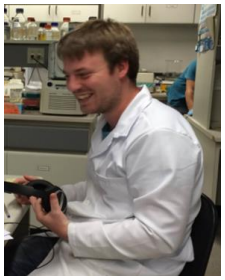
The opportunistic pathogen *Stenotrophomonas maltophilia* utilizes a type IV secretion system for interbacterial killing

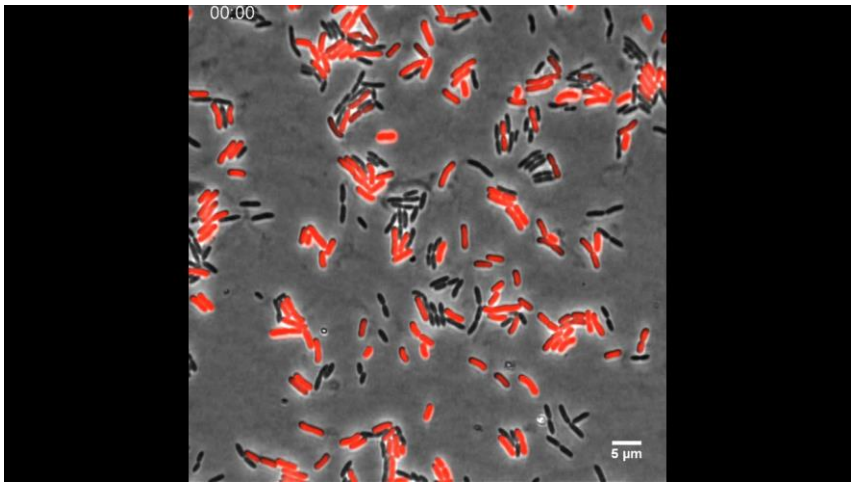
Ethel Bayer-Santos[1,2☯], William Cenens[1☯], Bruno Yasui Matsuyama[1☯], Gabriel Umaji Oka[1], Giancarlo Di Sessa[1], Izabel Del Valle Mininel[1], Tiago Lubiana Alves[1], Chuck Shaker Farah[1]*





Bayer-Santos,E et al. (2019)



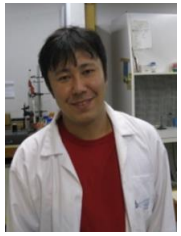


SOME STRUCTURAL STUDIES OF THE *XANTHOMONAS* TYPE IV SECRETION SYSTEM

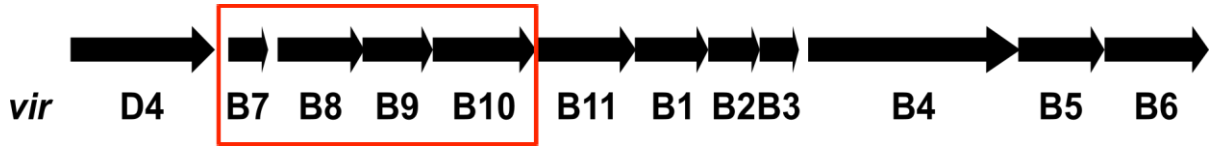
Roberto Salinas

Diorge Souza

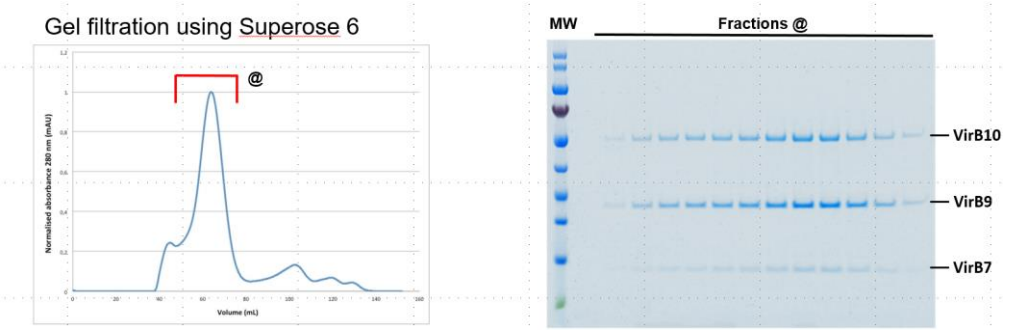
Germán Sgro



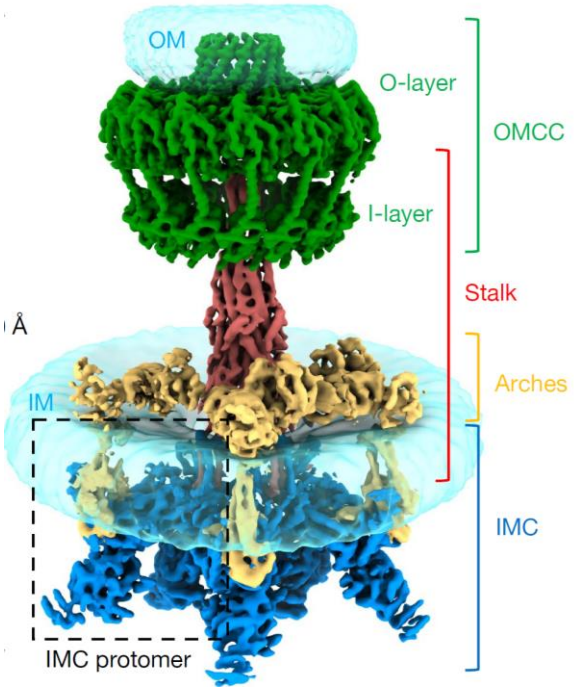
Gabriel Oka



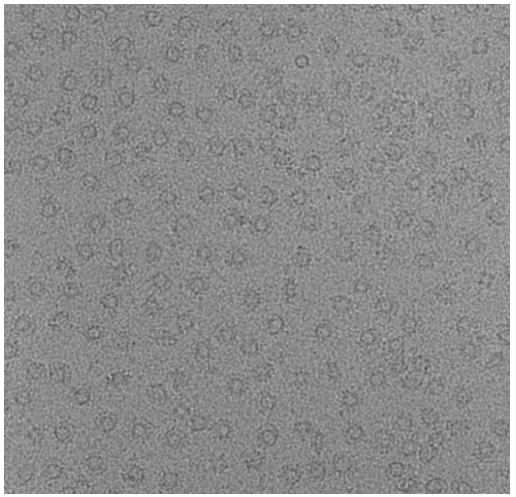
Outer membrane Core complex (OMCC) = VirB7-B9-B10

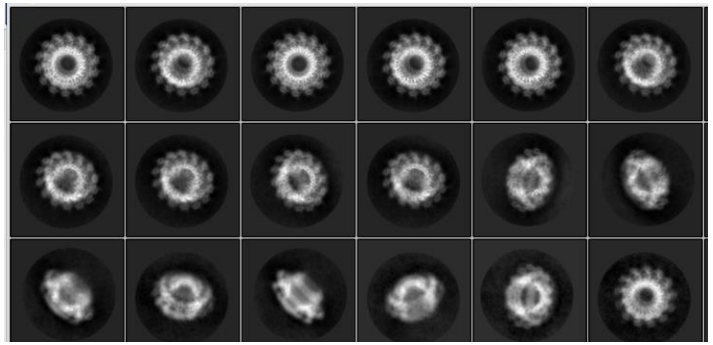


Macé et al (2022)

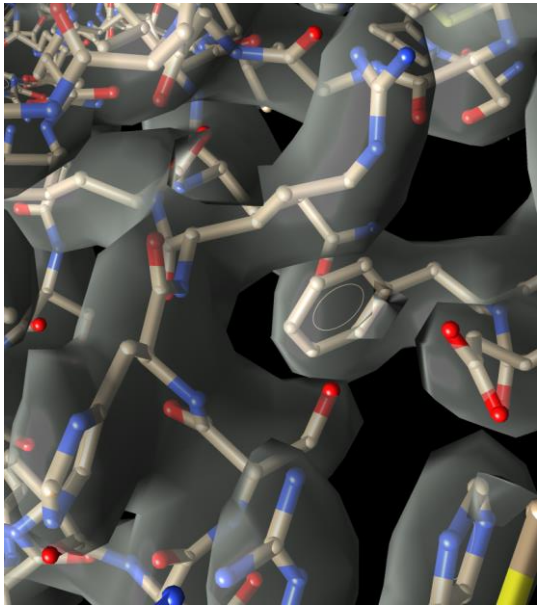


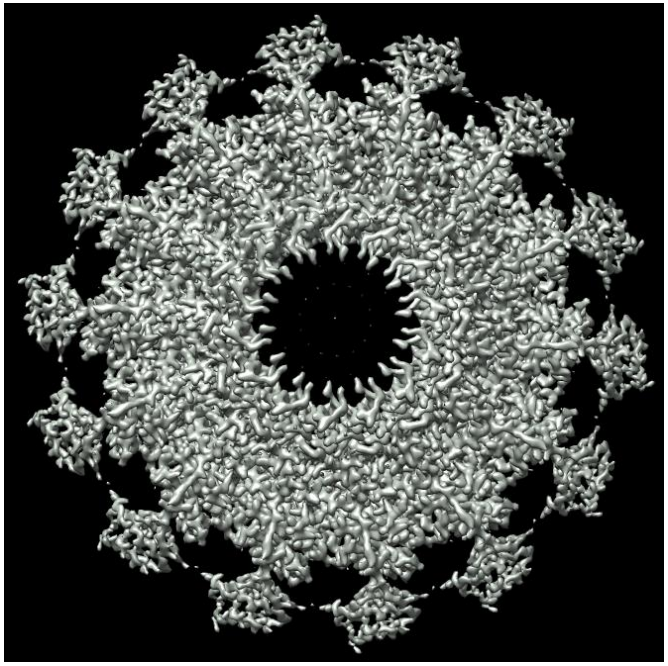
R388 plasmid
Conjugative T4SS



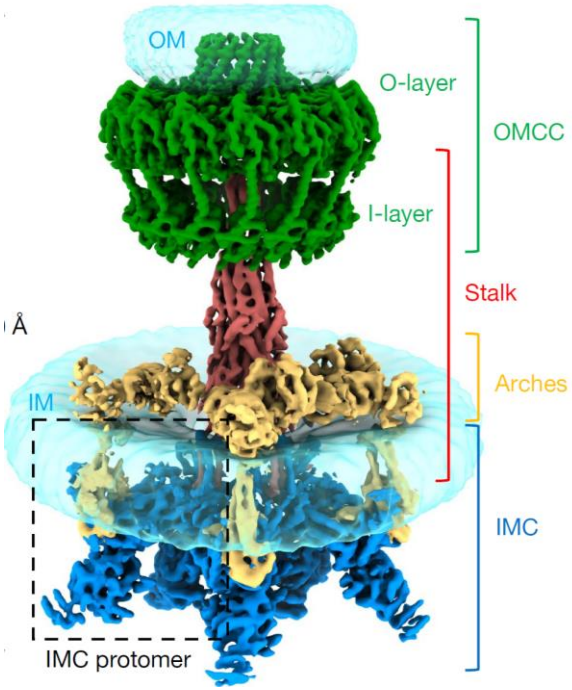


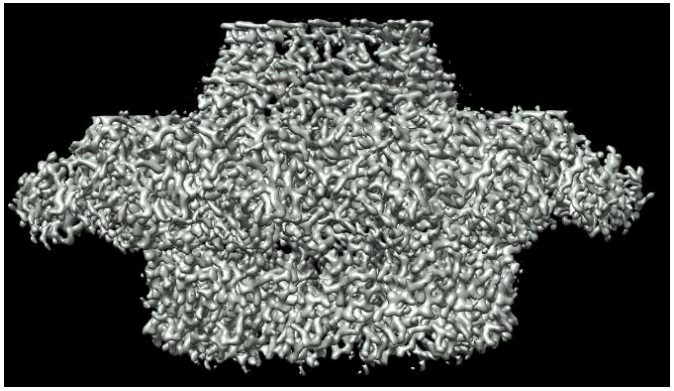
Sgro, Germán G., et al. (2018)



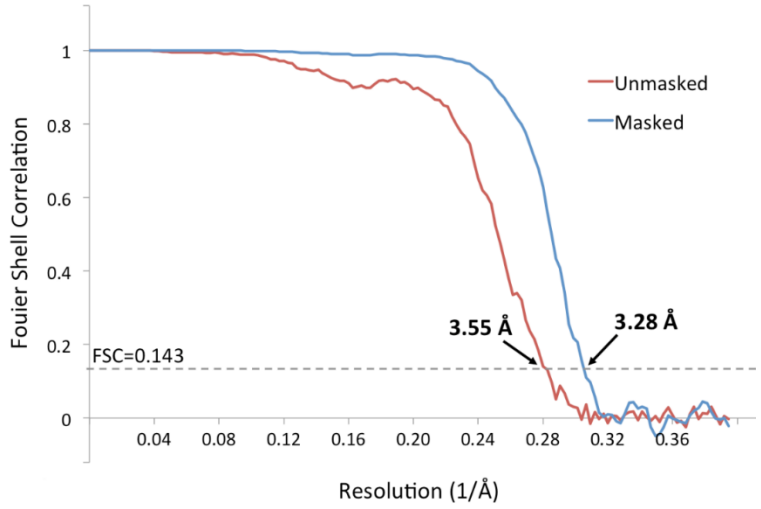


Macé et al (2022)

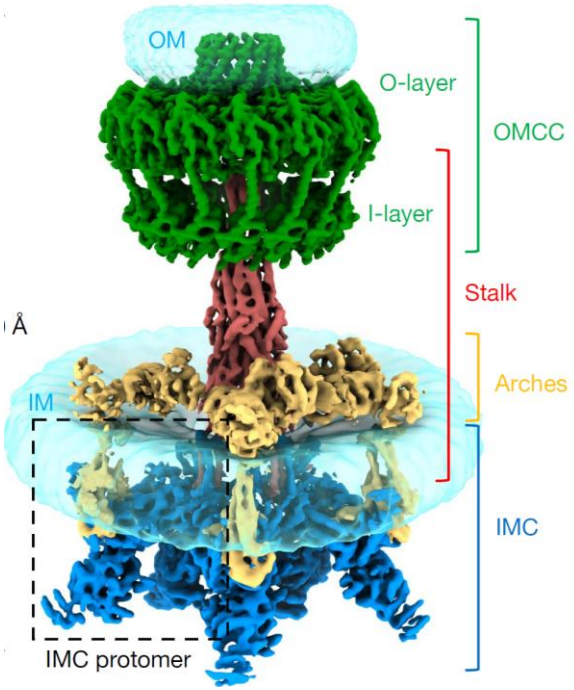


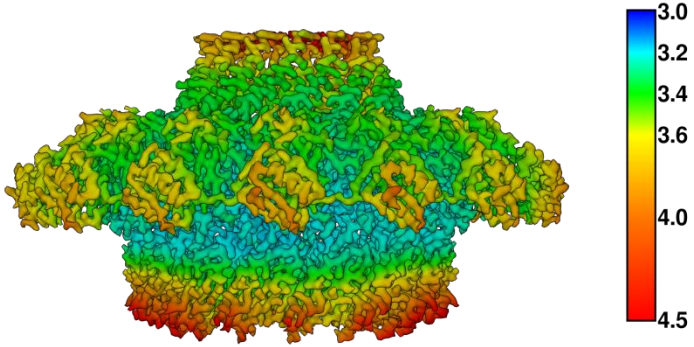


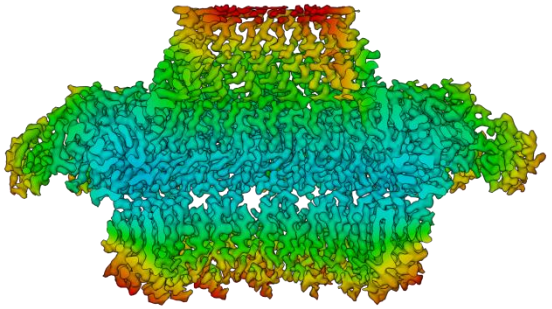
Sgro, Germán G., et al. (2018)

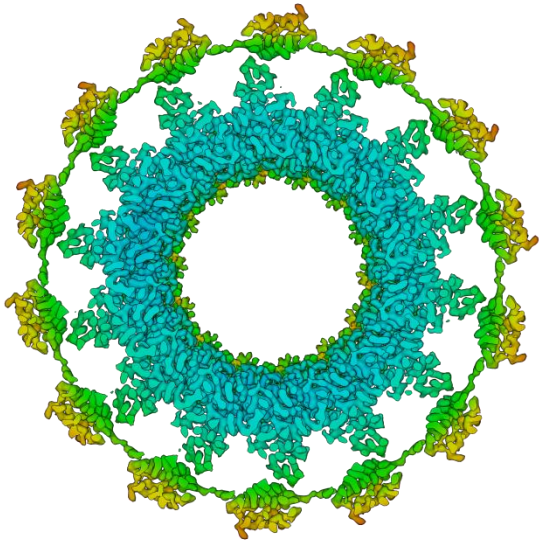


Macé et al (2022



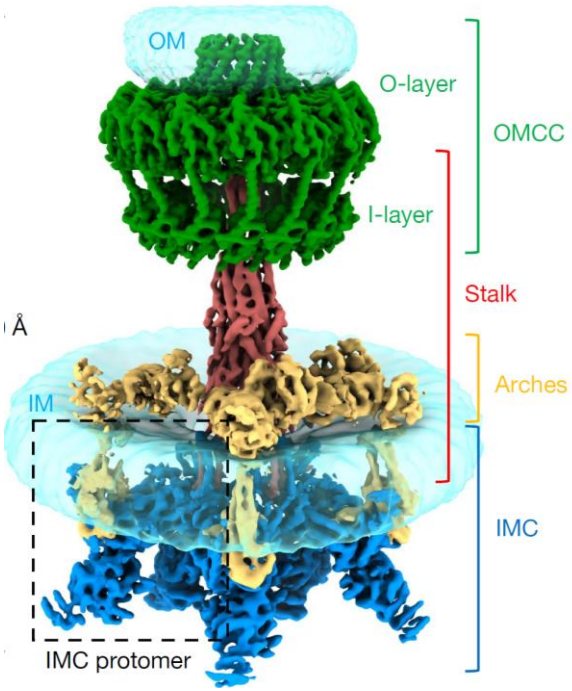


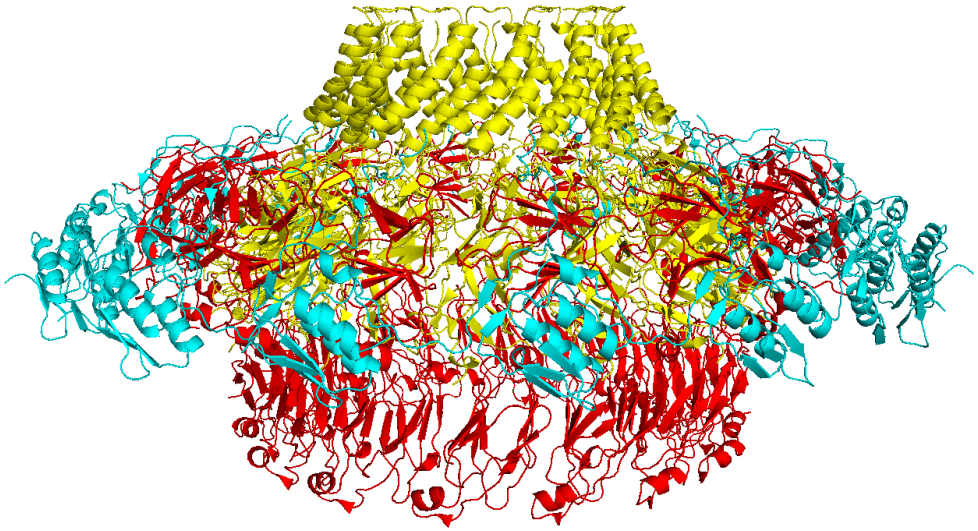




Sgro, Germán G., et al. (2018)

Macé et al (2022)





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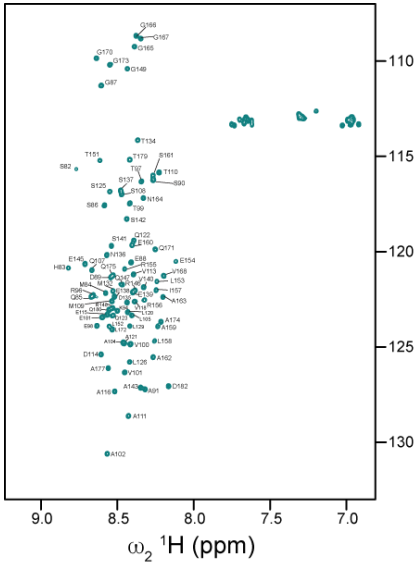

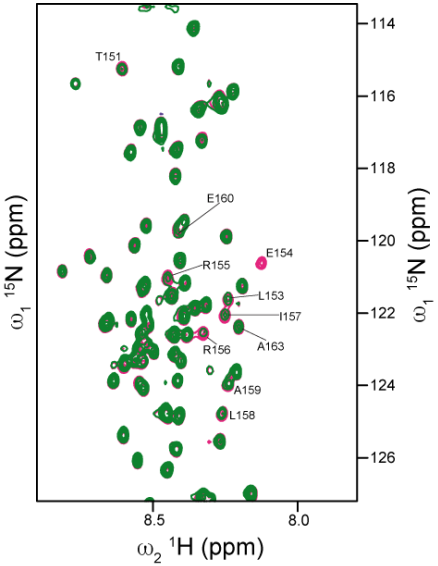

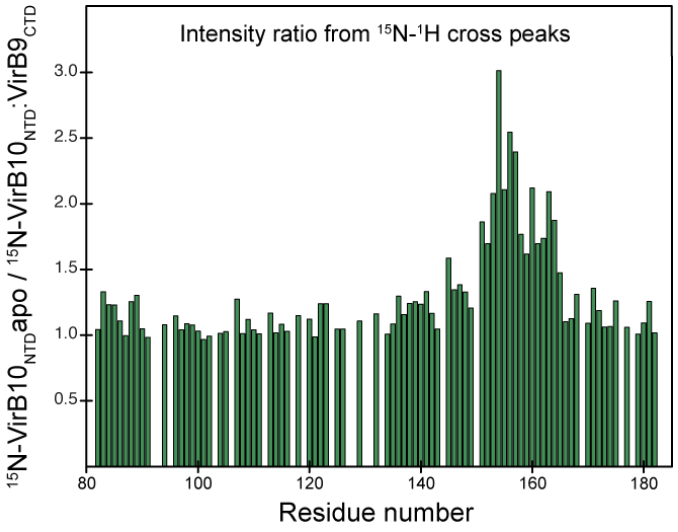

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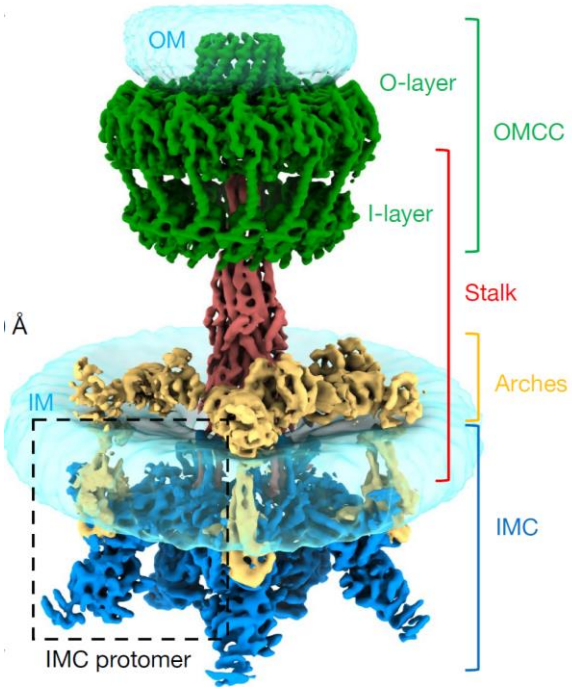
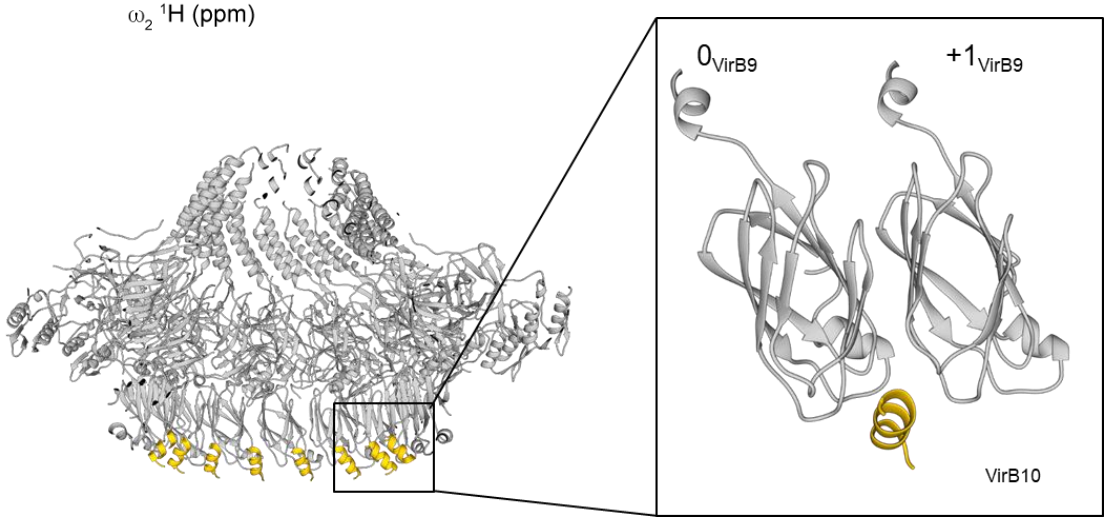
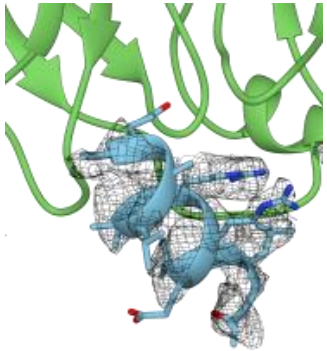
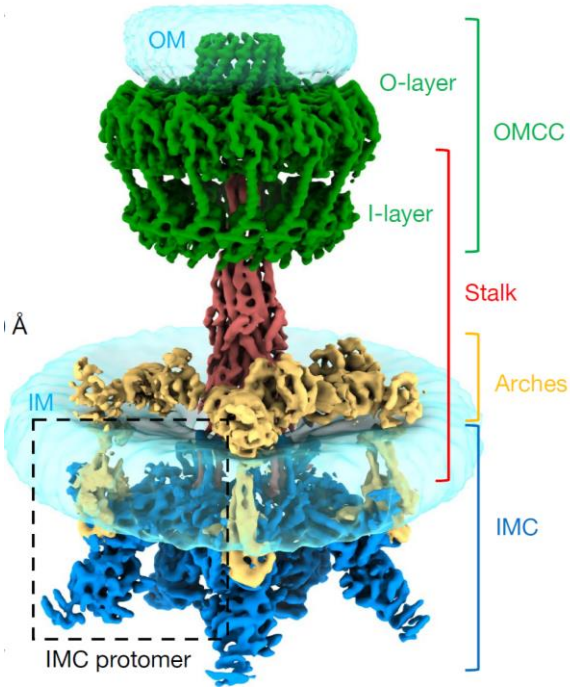




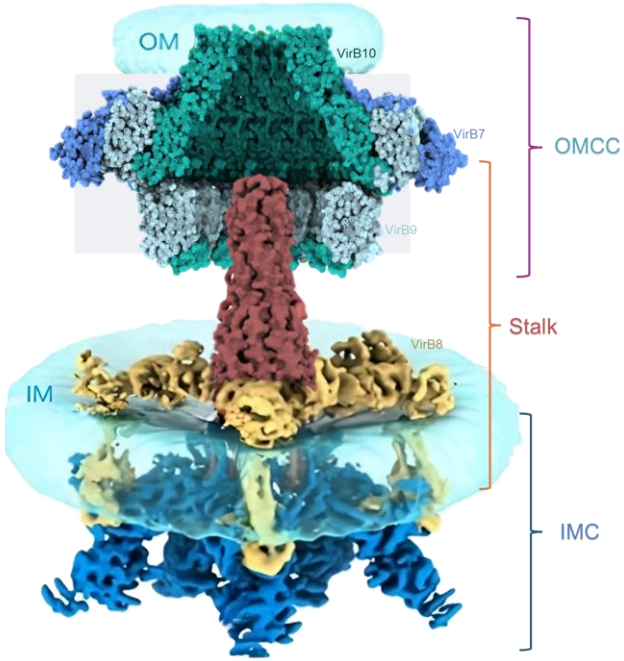


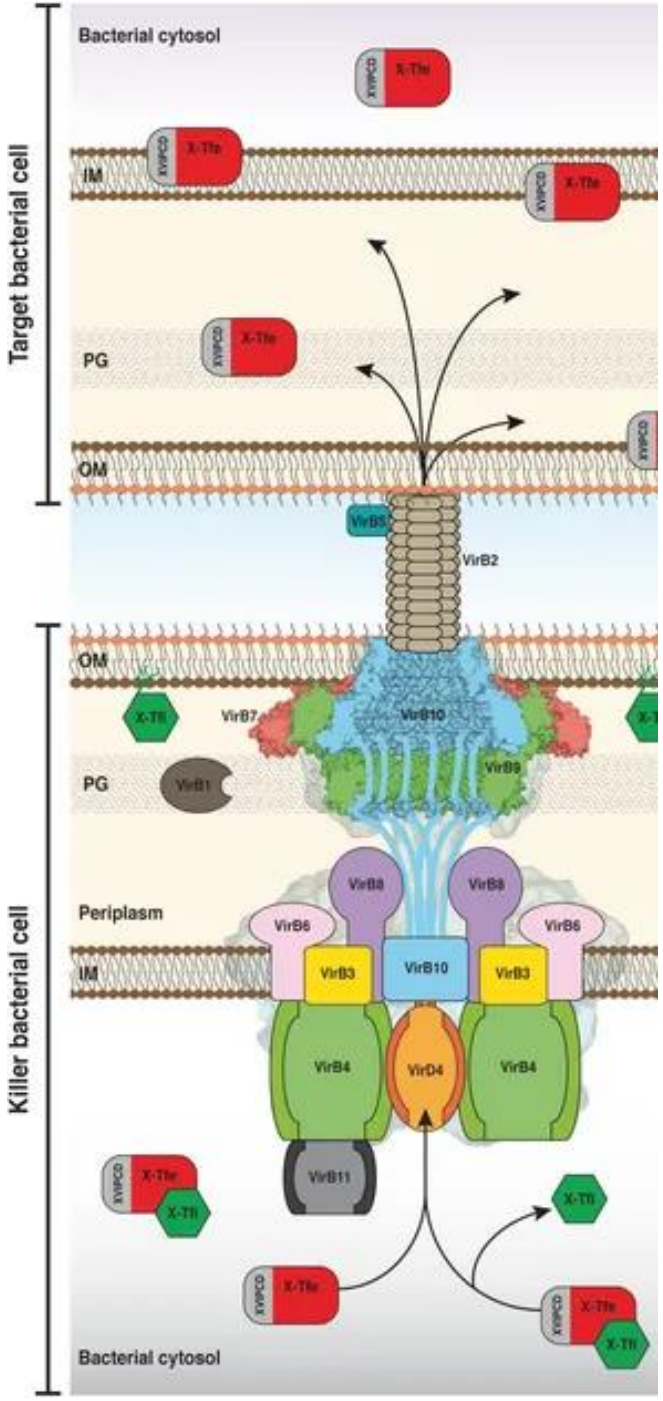
Sgro, Germán G., et al. (2019)

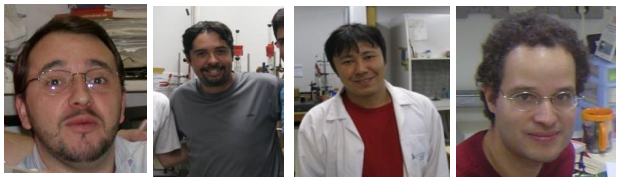
Macé et al (2022)



R388/*X. citri* composite

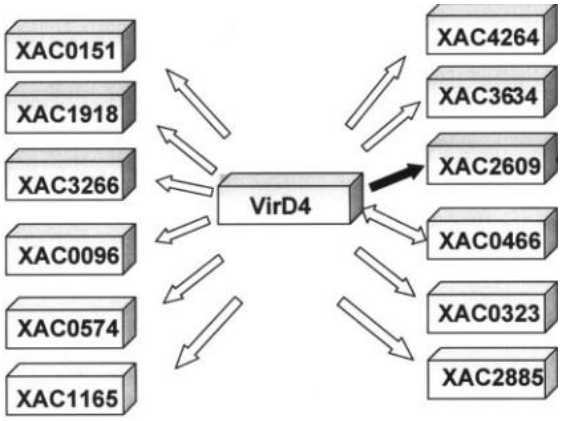


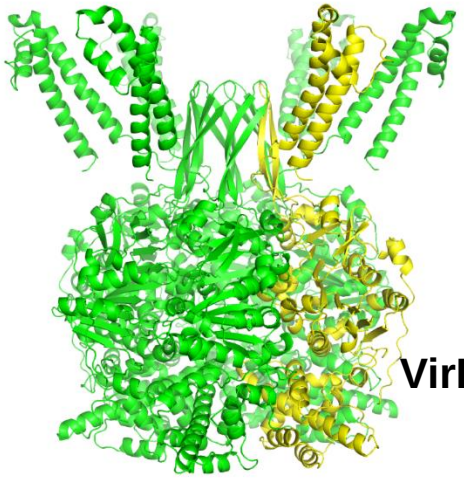




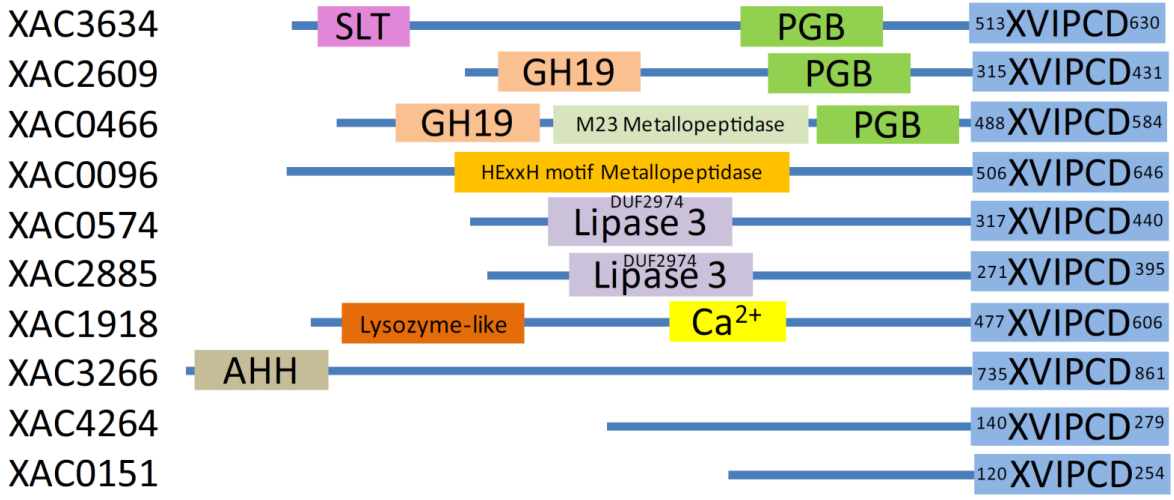
Alegria et al, 2005, J. Bacteriol.
Souza et al 2015, Nat. Commun.
Oka et al, 2022 PNAS

X. citri effectors X-Tfes

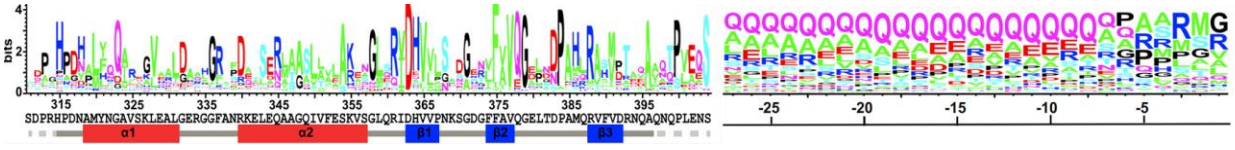


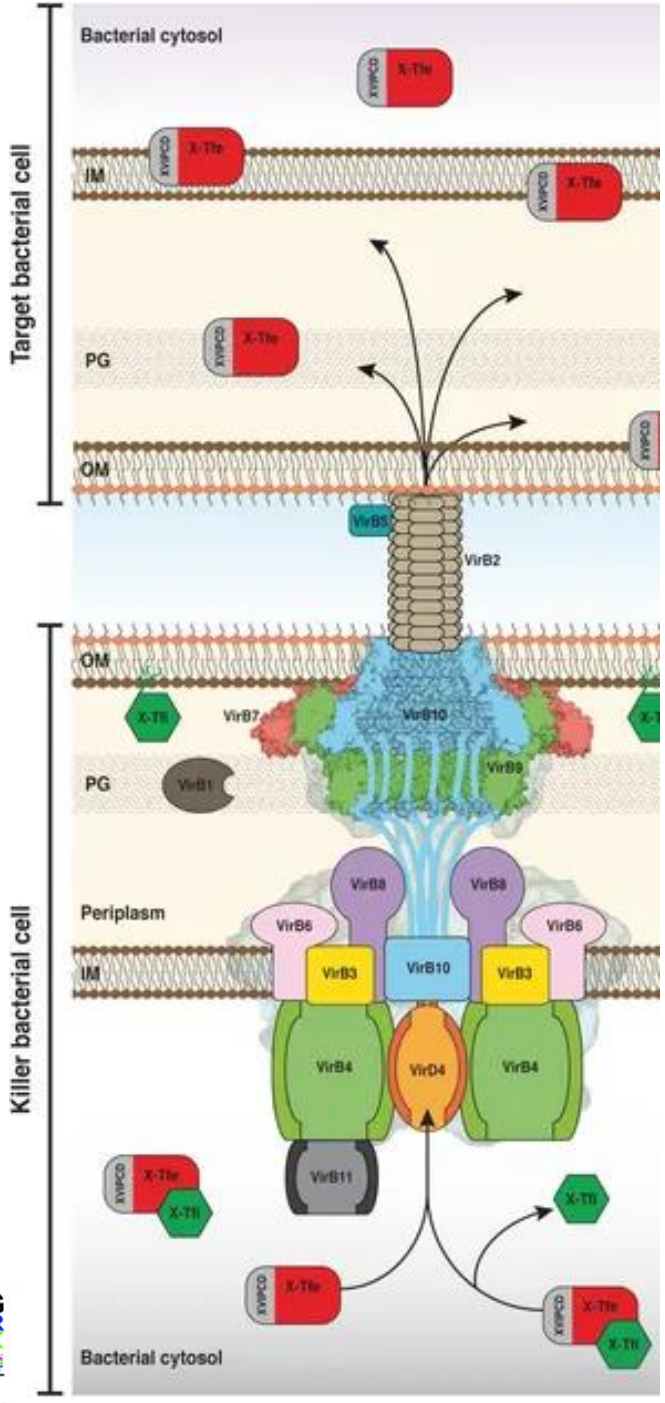


VirD4

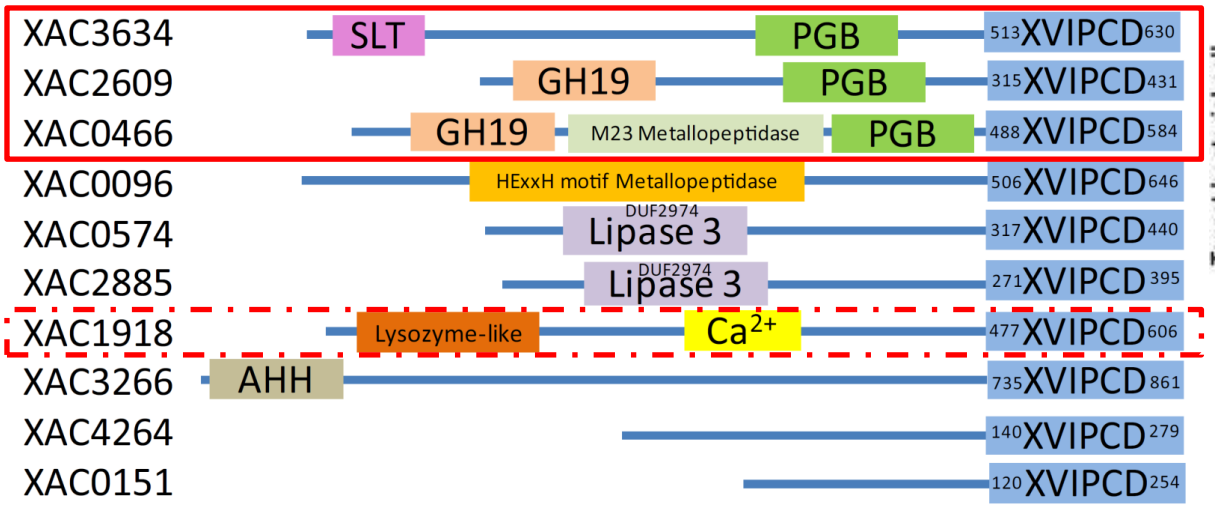


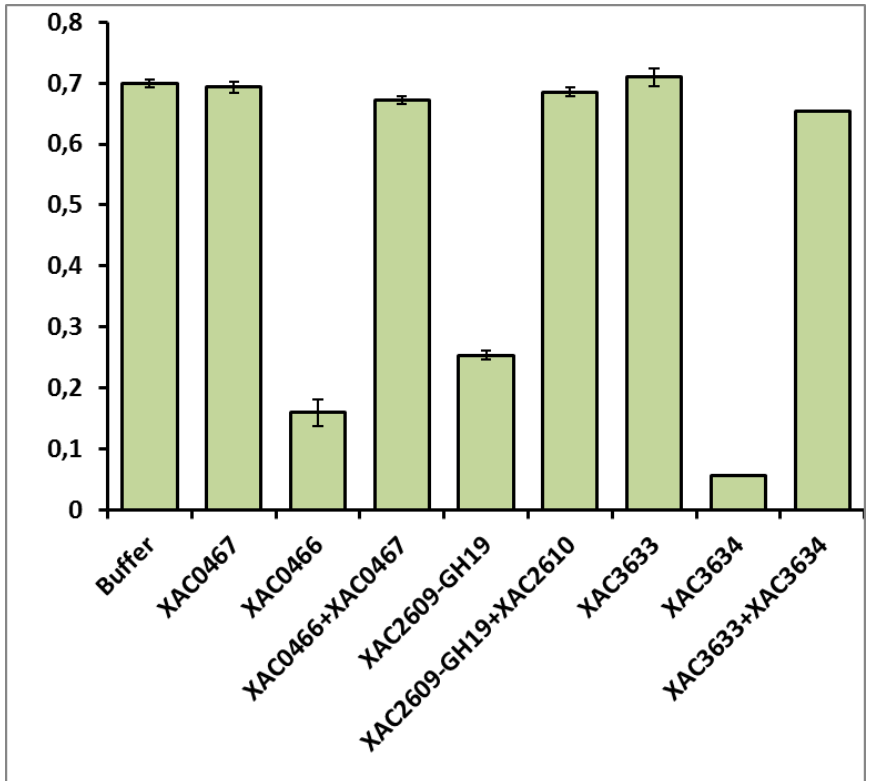
XVIP conserved **d**omain (**XVIPCD**)



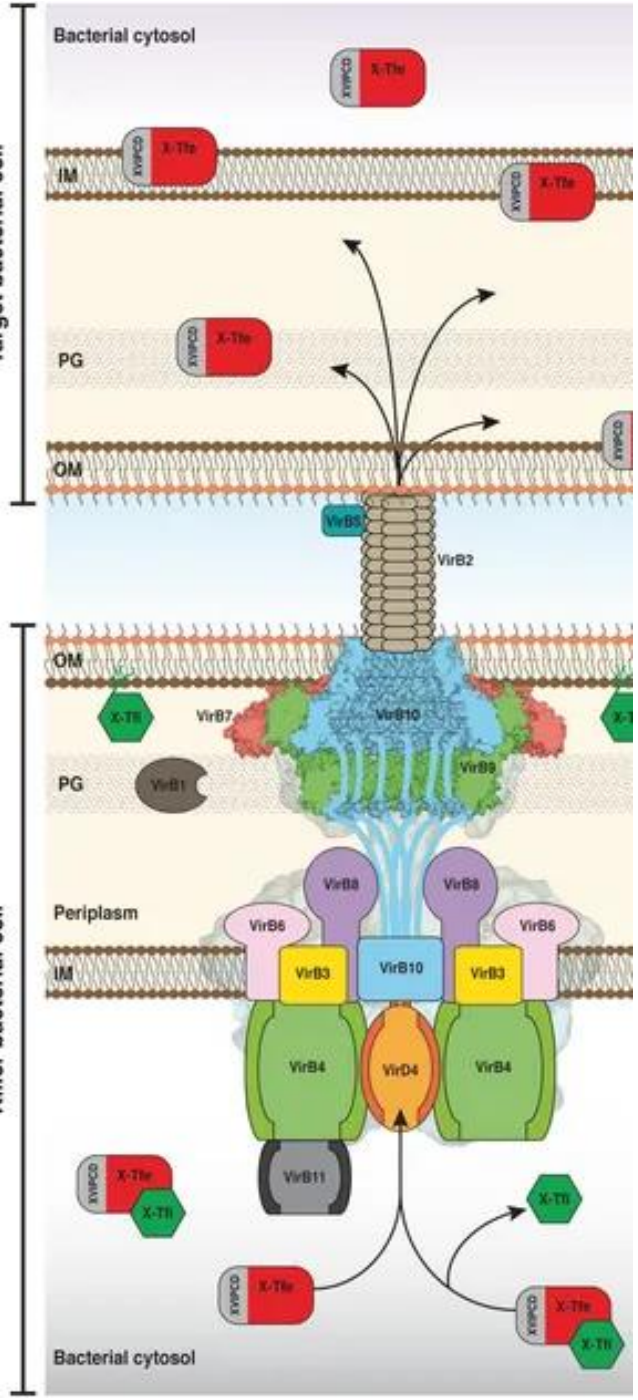


X. citri effectors (toxins) X-Tfes

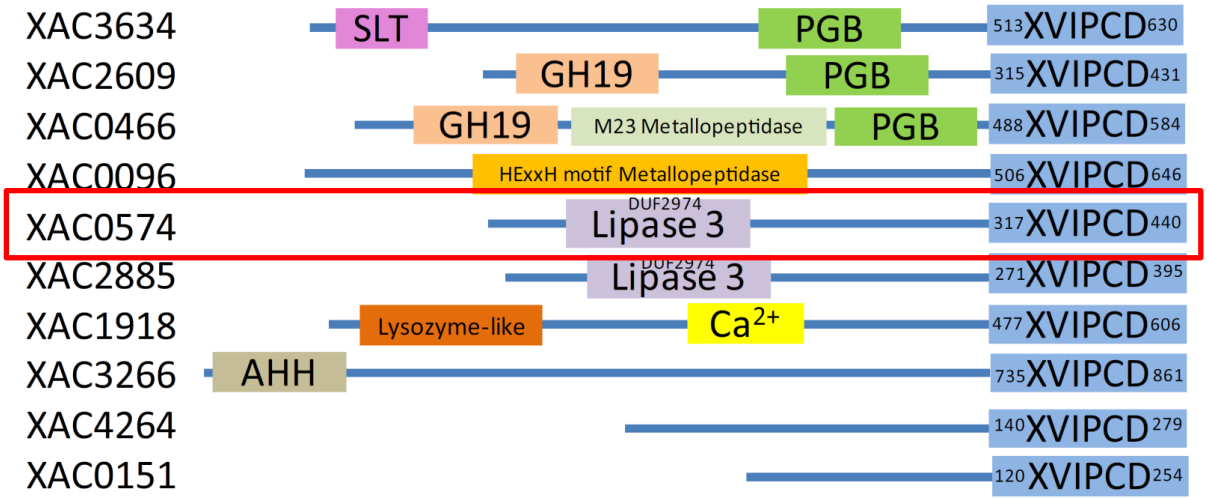




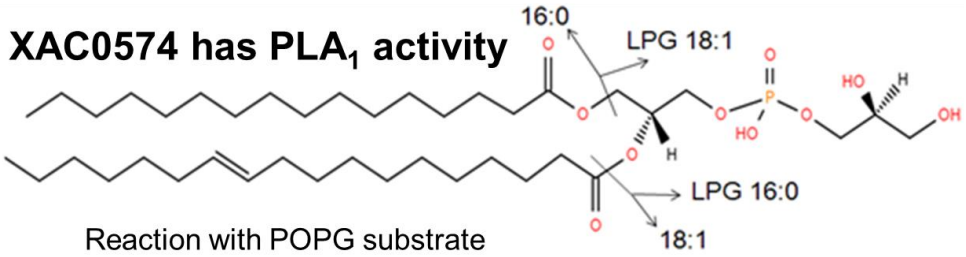
In vitro cell wall (*M. luteus*) lysis assay



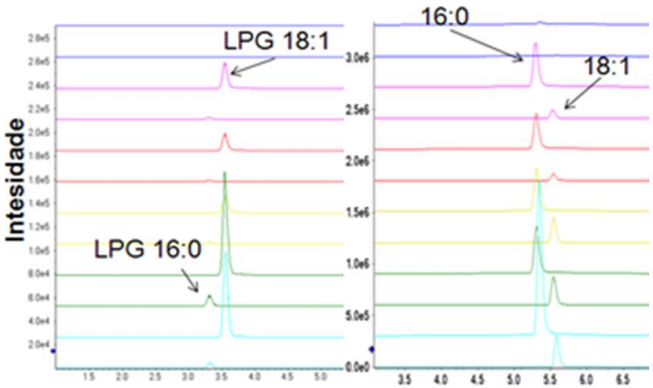
X. citri effectors (toxins) X-Tfes

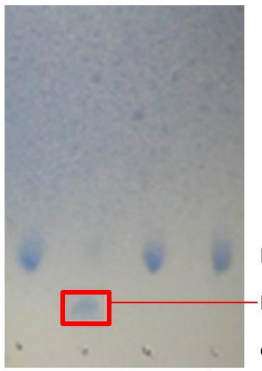


XAC0574 has PLA_1 activity

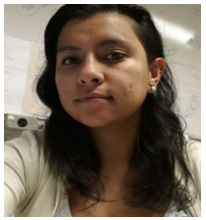


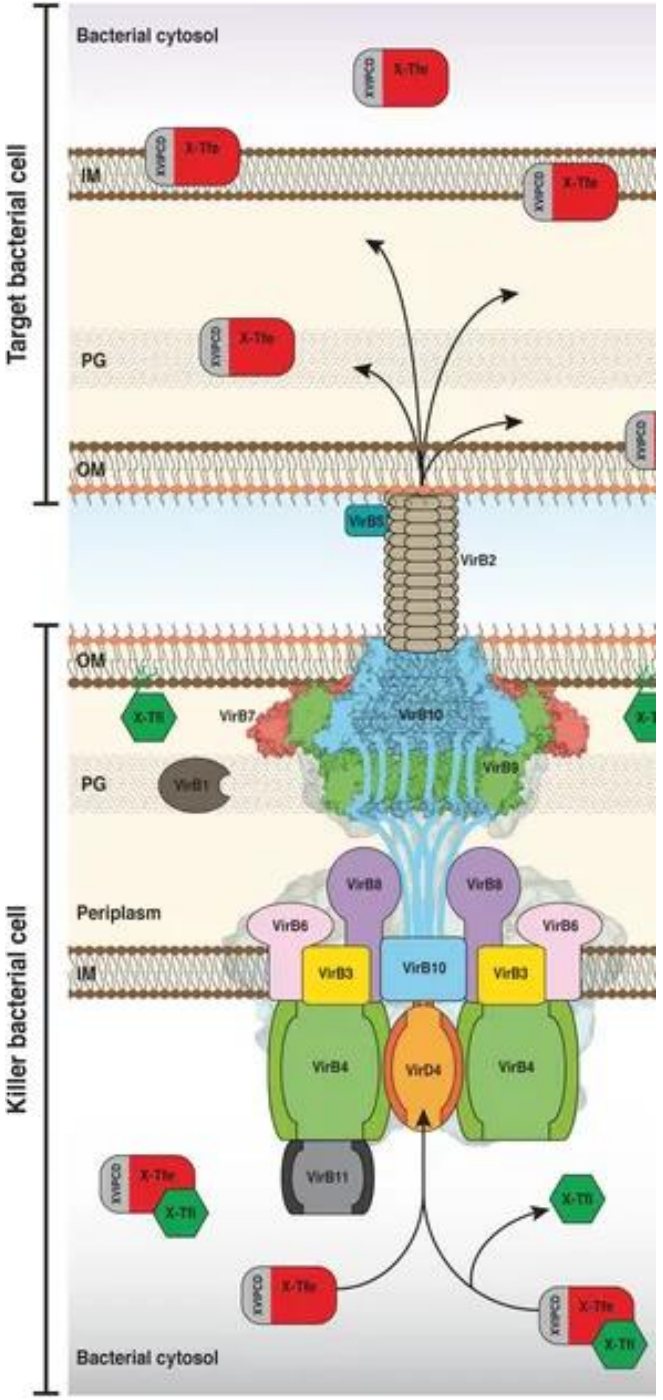
Reaction with POPG substrate





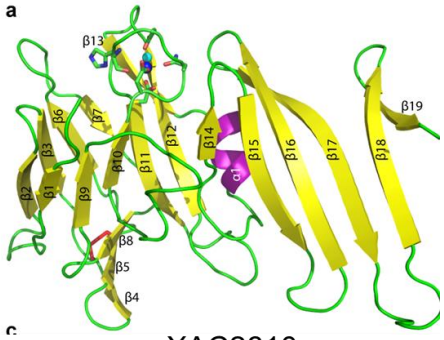
control
XAC0574
XAC0573
0574 + 0573



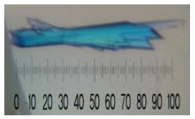


X. citri immunity proteins X-Tfis

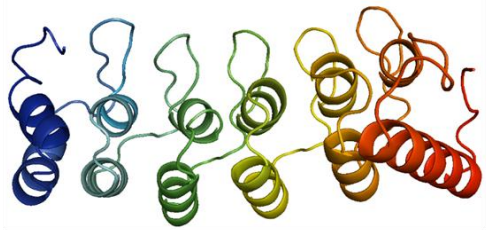
Souza et al., 2015



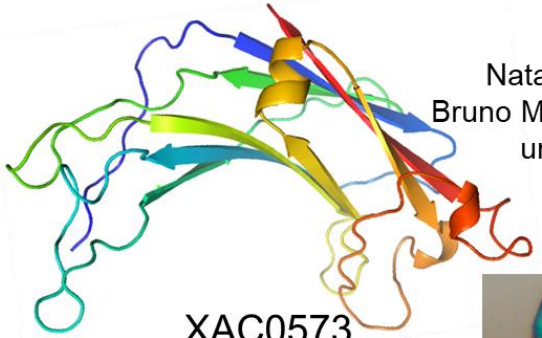
XAC2610
β-propeller fragment fold



Diorge Souza, unpublished

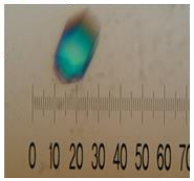


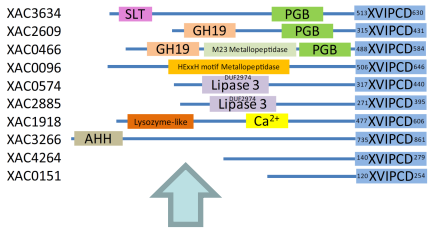
XAC2884
6X-ankyrin-repeat domain protein



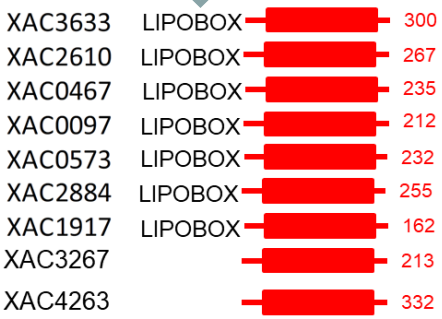
Natalia Bueno, Bruno Matsuyama, unpublished

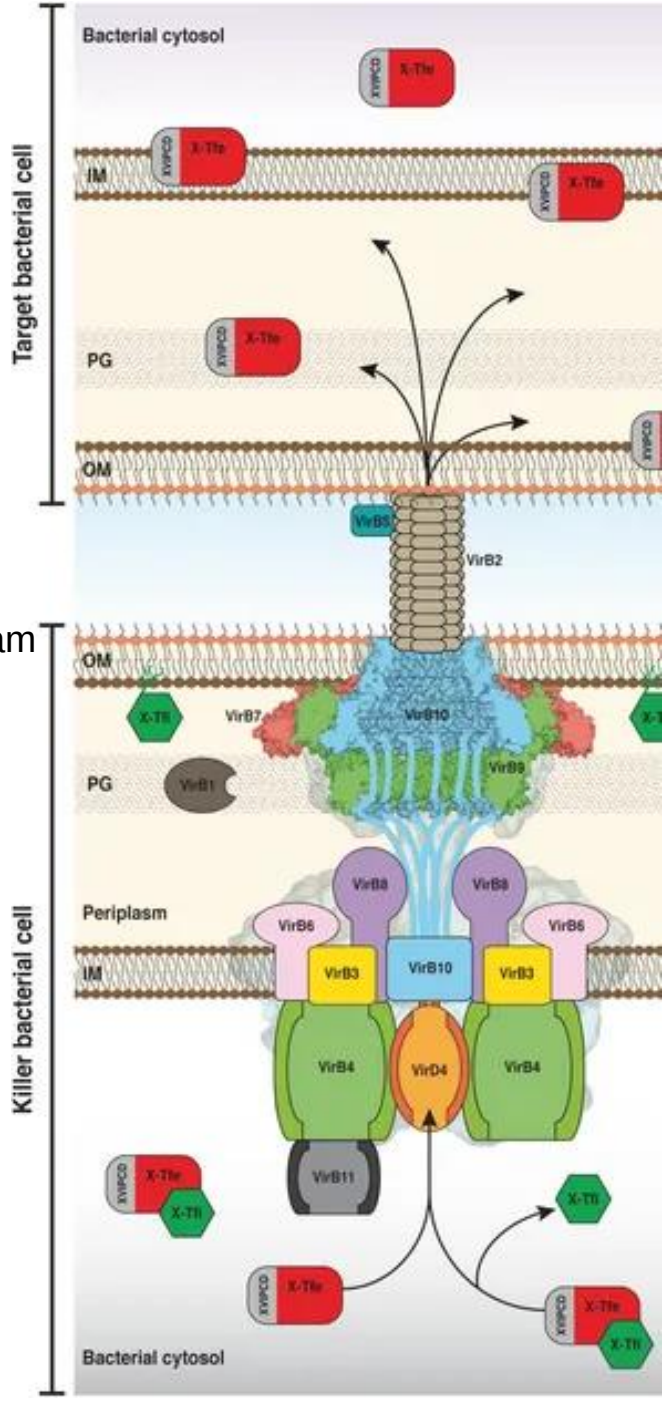
XAC0573
β-sandwich



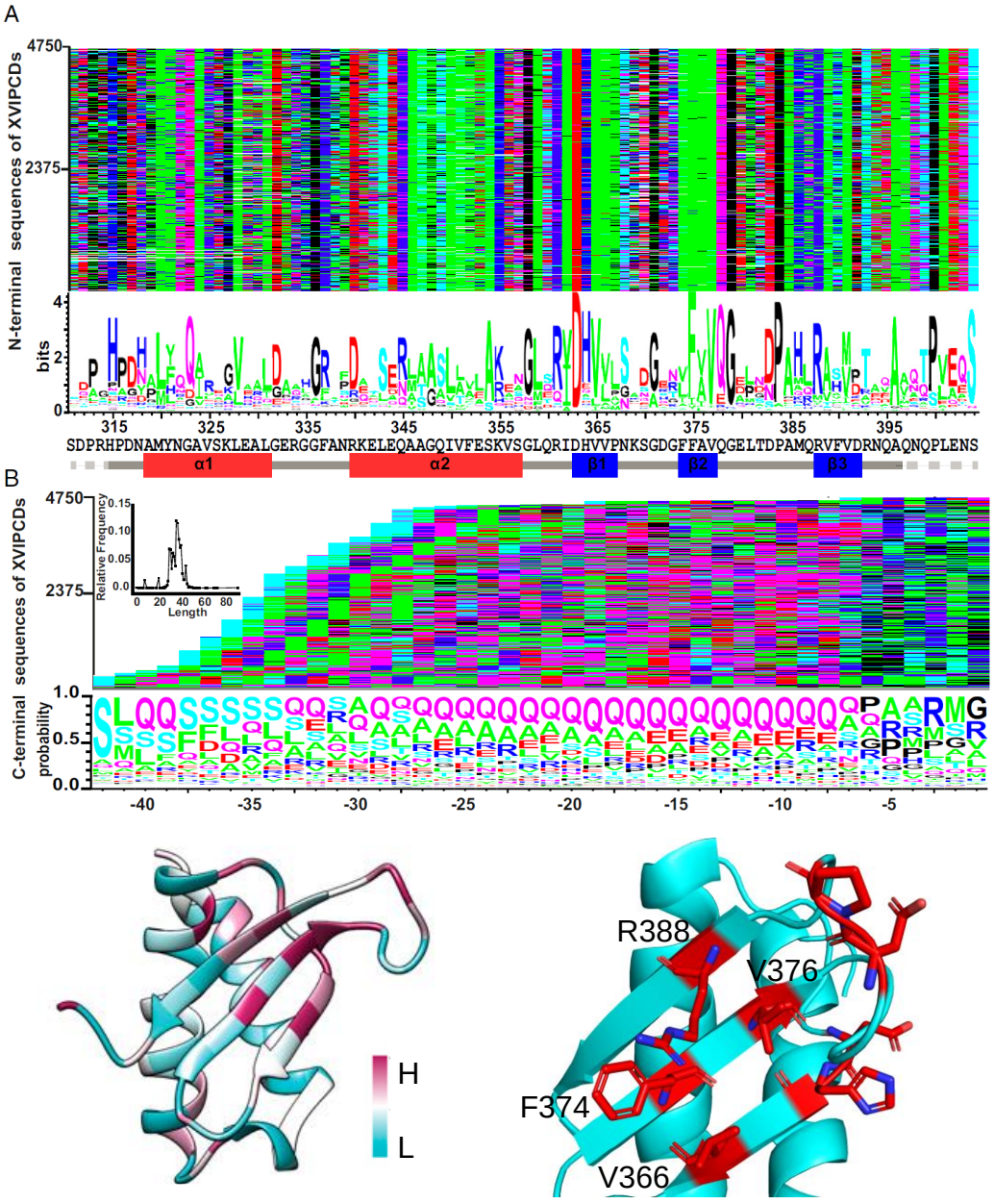


Each effector has its own cognate immunity protein coded immediately upstream

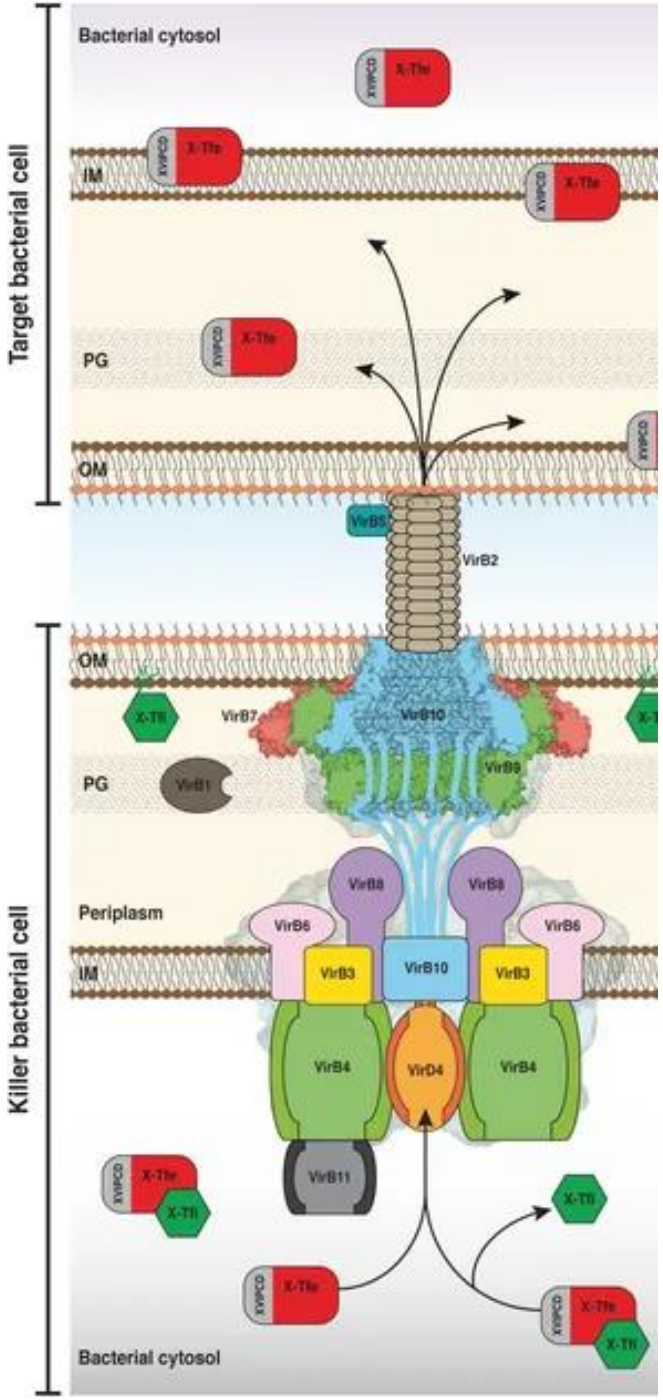




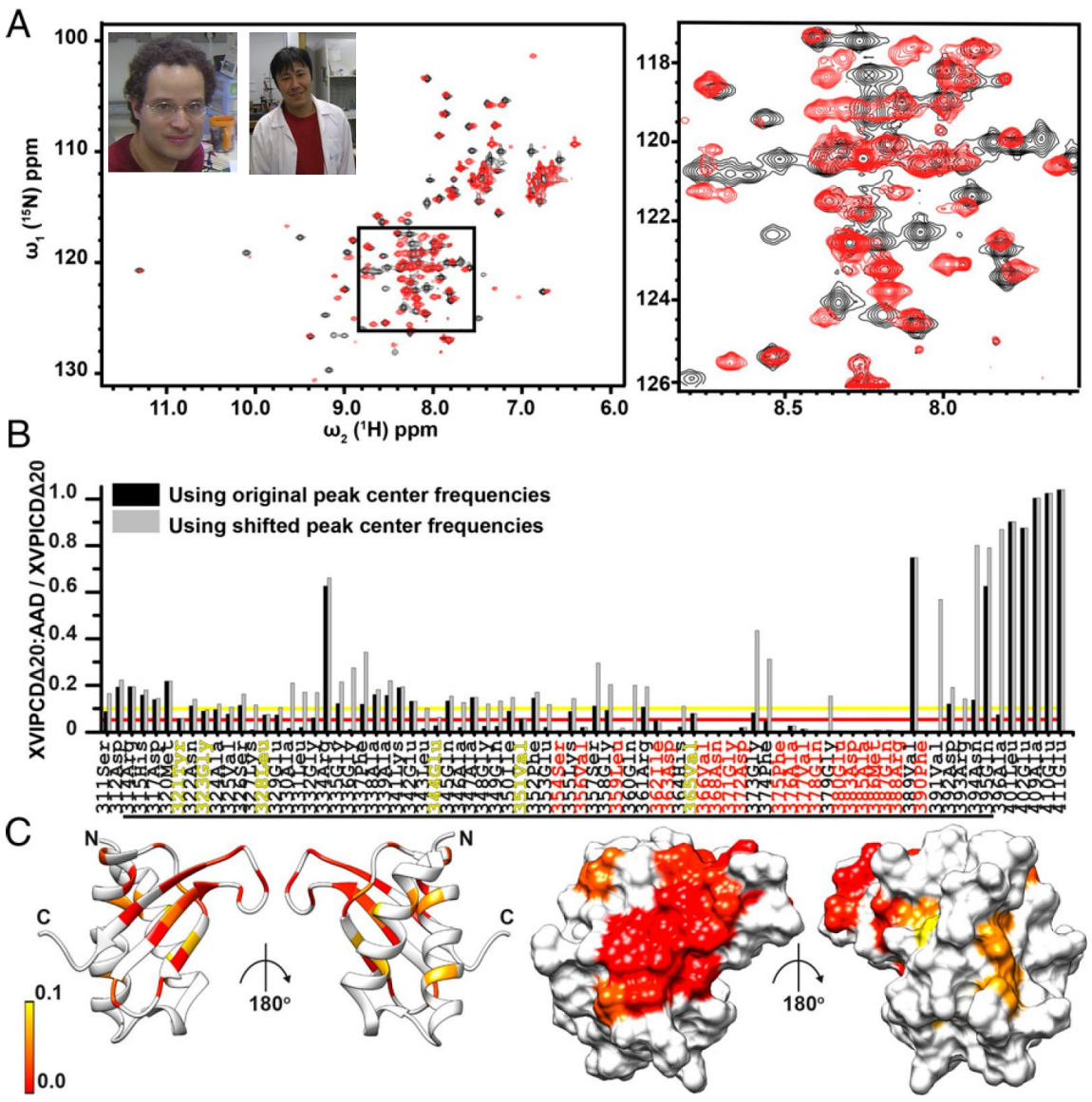
X. citri effector conserved domains (XVIPCD)



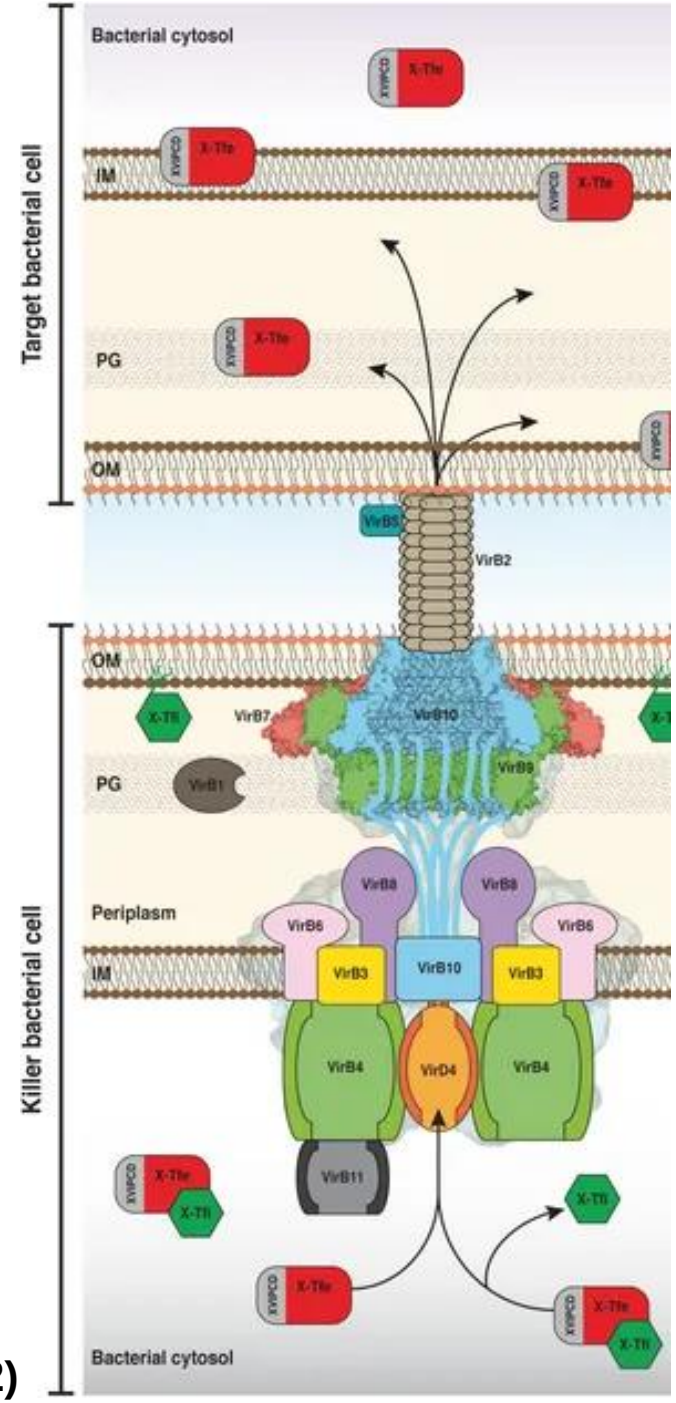
XVIPCD NMR structure (Oka et al, 2022)



X. citri effector conserved domains (XVIPCD)

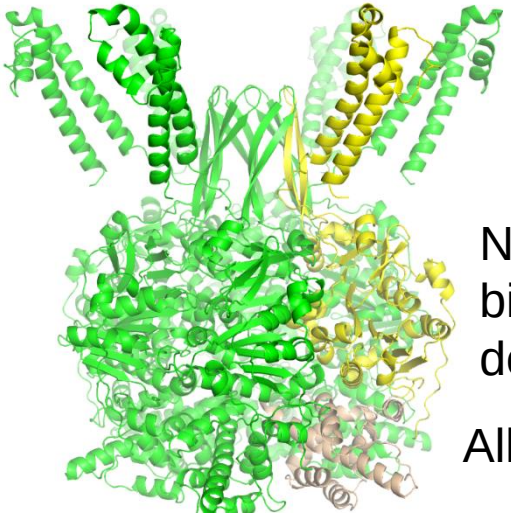


VirD4$_{AAD}$ induced perturbations in XVIPCD (Oka et al, 2022)



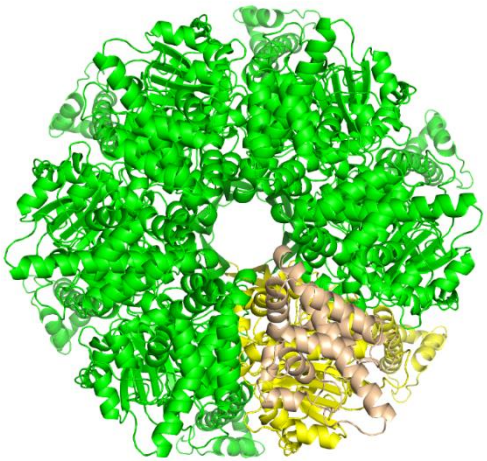
AlphaFold model of VirD4 hexamer

Trans-membrane helices



Nucleotide binding domian

All alpha domain



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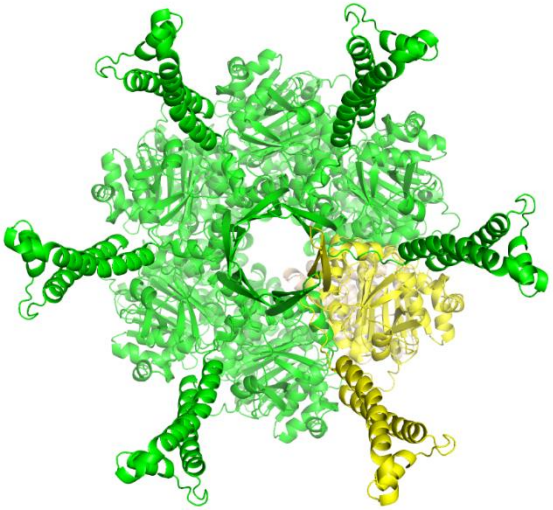

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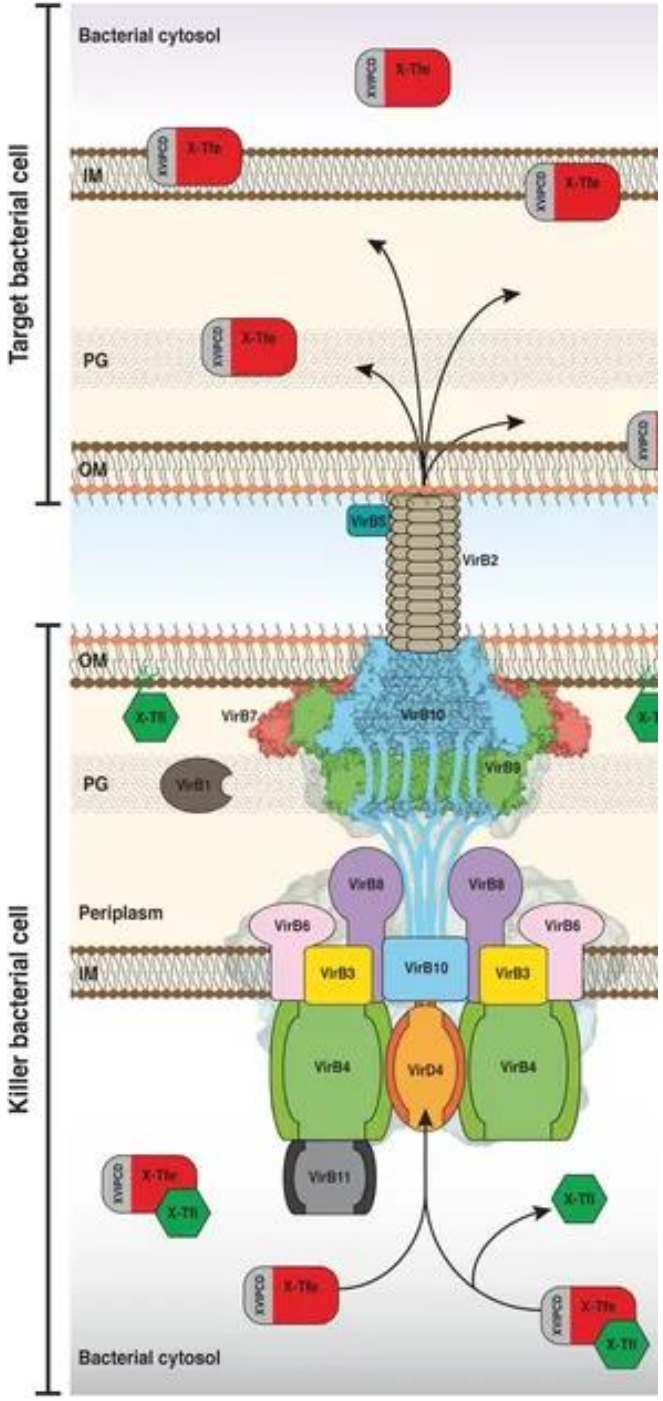
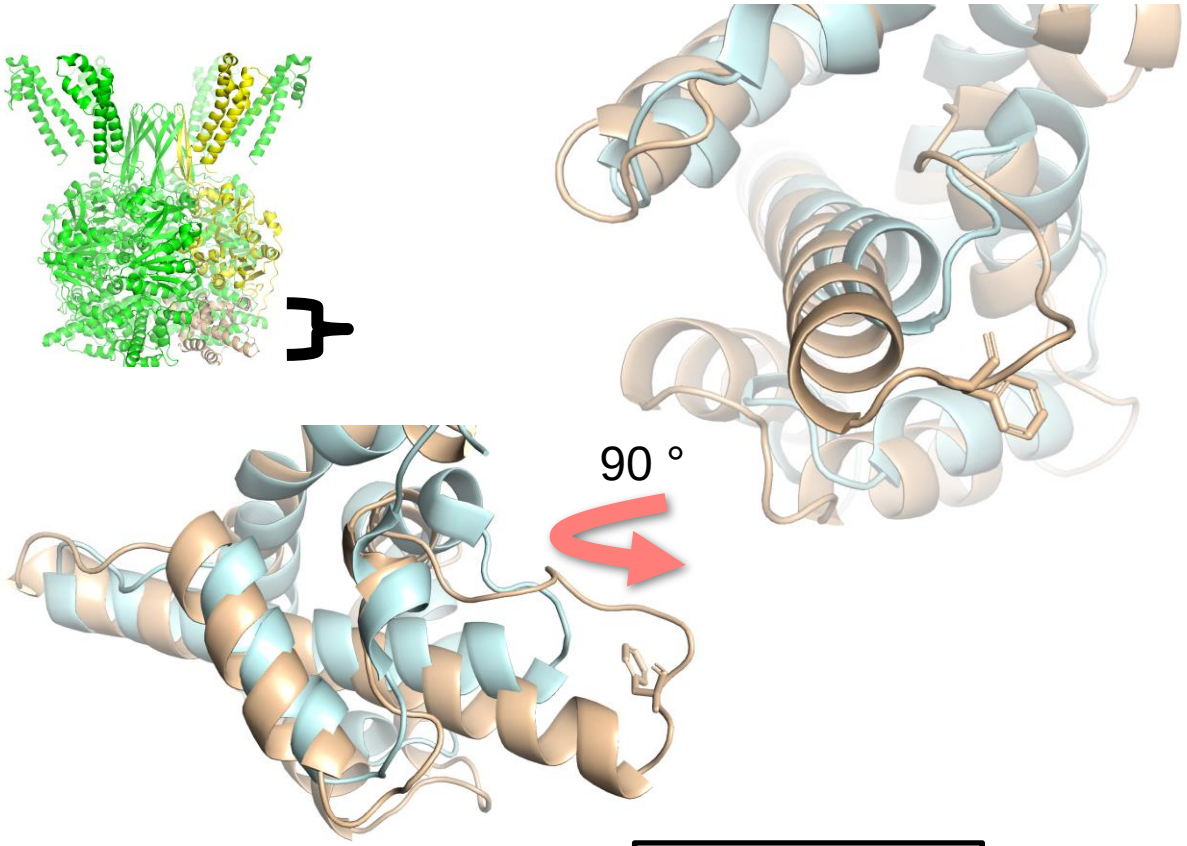
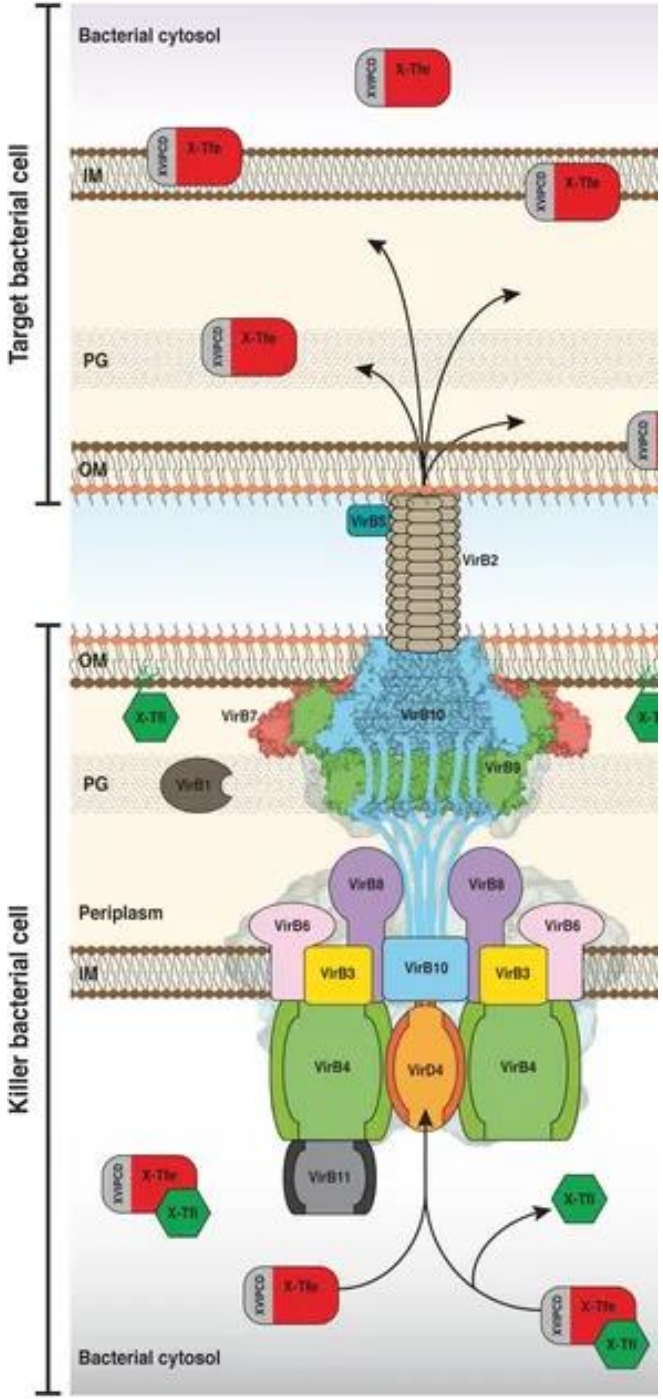


Extended loop in Xanthomonadaceae VirD4 all alpha domains

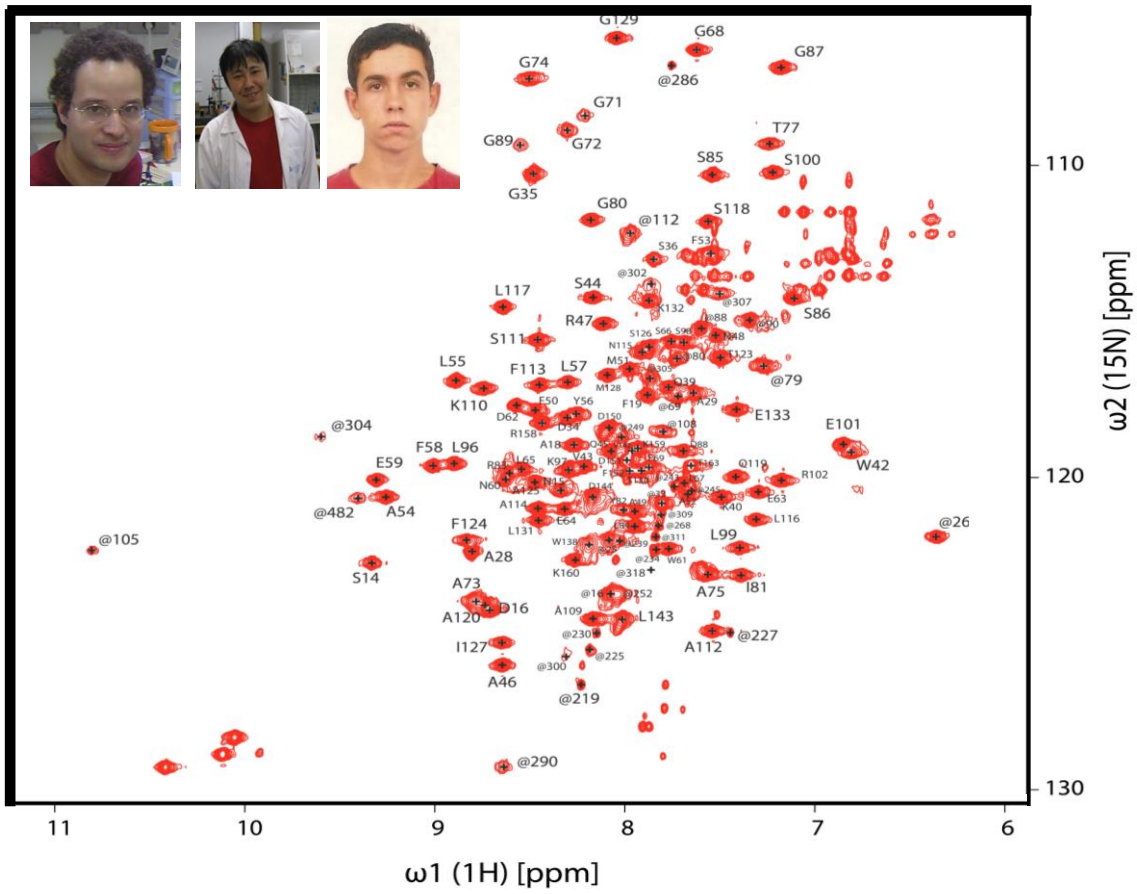



```
S_enterica      LSVVPRGKTDEAEEWASYGRLLLRETAKKL----------------ALIGTPSMRELFH   244
A_tum_D4        ANLITA-KGKGAEGFIDGARDLFVAGILTCI----------------ERGTPTIGAVYD   271
Dy_jiang_D4     AILYPD-EPGKDPFWTSQSRAAFTGFTGFMFEAWDRSWRMGLPSDPNTVEDFPSFERILR   282
Lut_rhiz_D4     SMLYPD-GSEDQKFWVSQARNAFMAFSLYLCEKWEDDERKNRPMA---VRSKPTLGMIYR   278
Lys_anti_D4     AMLYPD-GSDDQKFWVSQARNAFMAFTLYLFEAWEDAKKVGFPFG----T-IPTLGAVYR   275
Lys_enz_D4      AMLYPD-GSDDQKFWISQARNAFMAFTLYLFEAYDDAQKVGFPFA----T-QPTIGAVYR   274
X_citri_D4      AMLYPD-GSDDQKFWVSQARNAFMAFALYLFENWDEELSLGFPGG----AGAPTLGGIYR   275
S_malto_D4      AMLYPD-GAEDQKFWVSQARNAFMAFTLYLFENWDDERSSGFPGG----SGTPTLGSVYR   279
                  :          : . .*  :                          *:  :
```



Partial assignment of the VirD4 all alpha domain

Gabriel Oka, Luis Cezar, Roberto Salinas



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LMSIAAMLYPDGSDDQKFWVSQA

RNAFMAFALYLFENWDEELSLGFP

GGAGAPTLGGIYRLSSGDGTDLKK

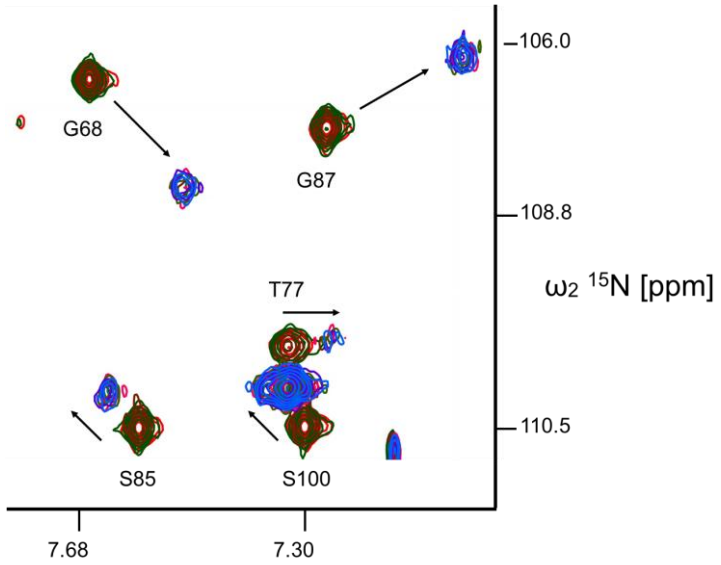
YLKSLSERRFLSSNAKSAFANLLS

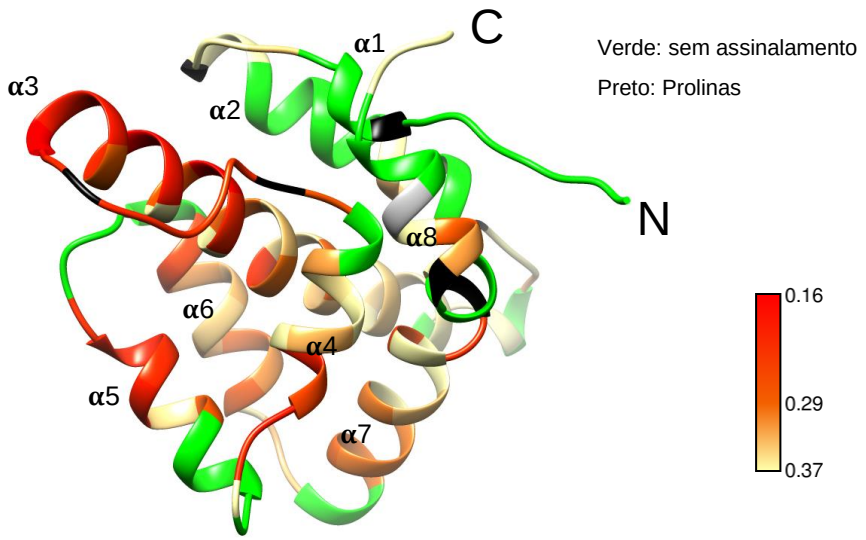
QADETFASIMGTLKEPLNAWINPVL

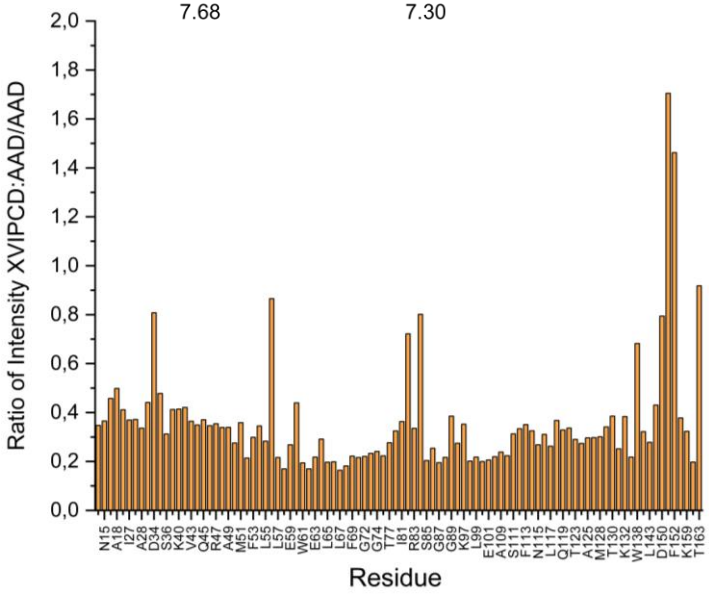
DAATSADDFVLTDLRKKKMT

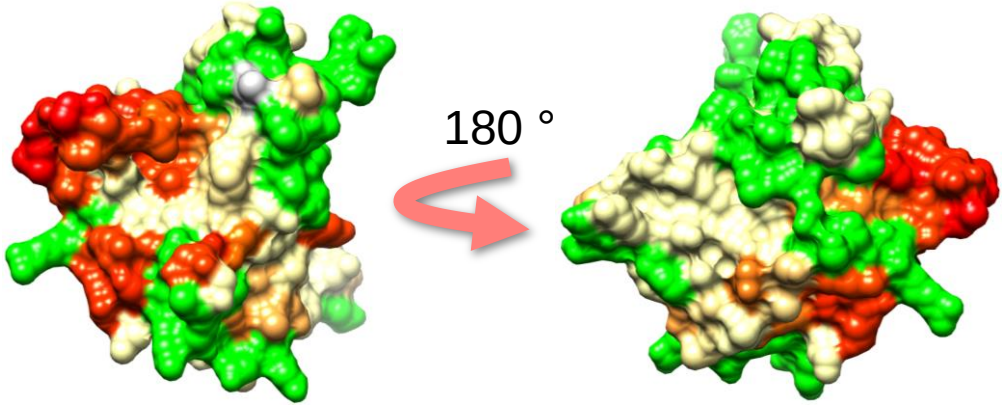
Amino-acid sequence of VirD4_AAD showing the assigned residues in the ^{1}H-^{15}N Trosy spectrum. The protein has **163** amino-acids. The residues in blue are those that are still assign. The ^{1}H-^{15}N Trosy spectrum of the VirD4_AAD contain **132** backbone amide cross peaks out of **155** peaks expected based on the amino acid sequence. Thus, signals for **24** ^{1}H-^{15}N spin pairs were absent in the spectrum. From the **132** observed signals, **98** could be assigned to a VirD4_AAD amino acid residue. **34** ^{1}H-^{15}N spin pairs are not yet assigned.

Identification of VirD4$_{AAD}$ residues involved in interactions with the X-Tfe$^{XAC2609}_{XVIPCD}$



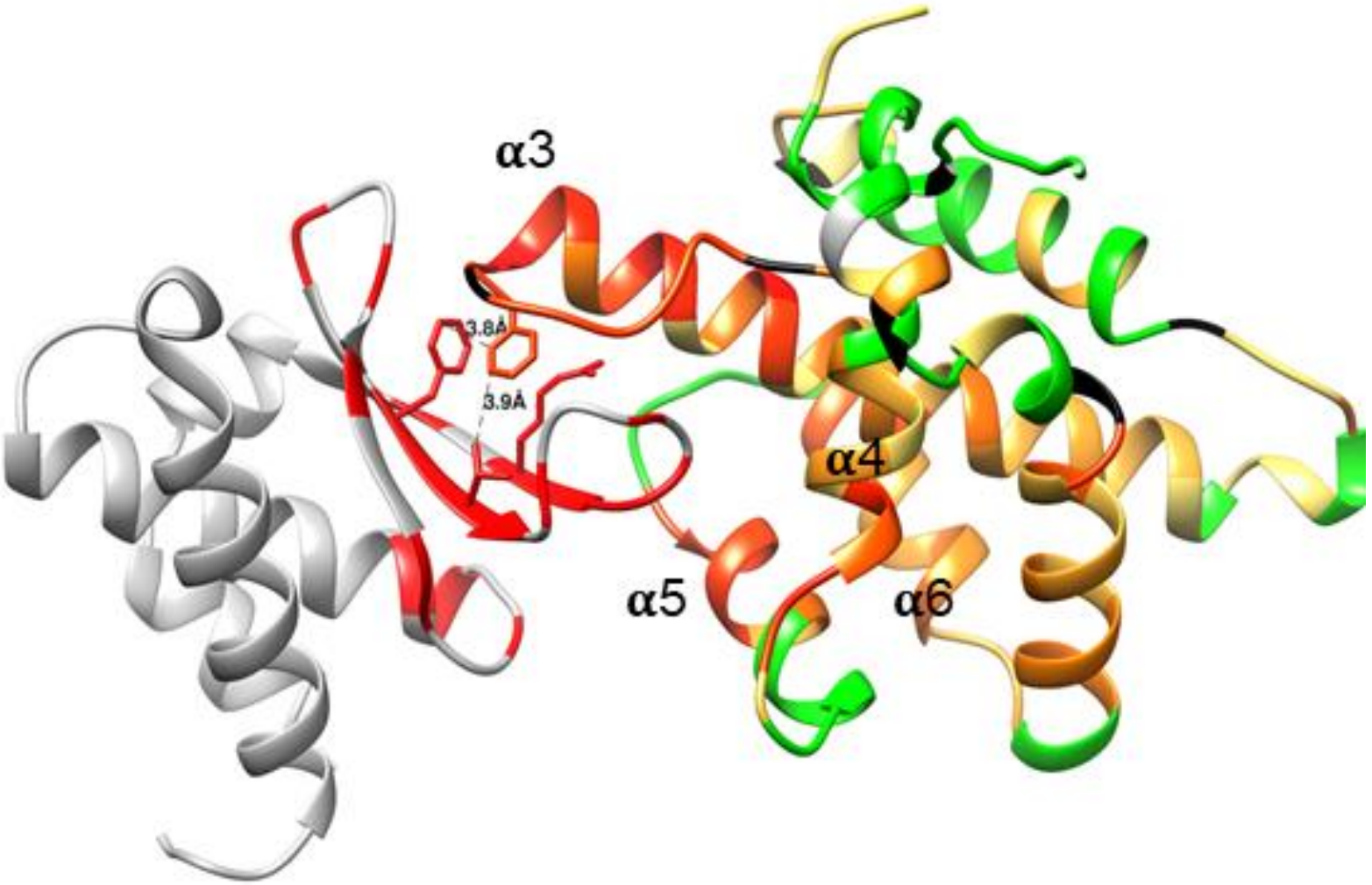




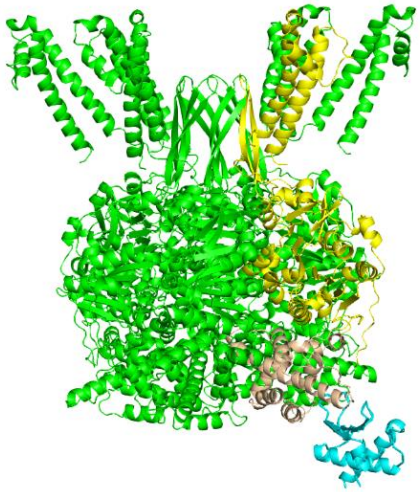


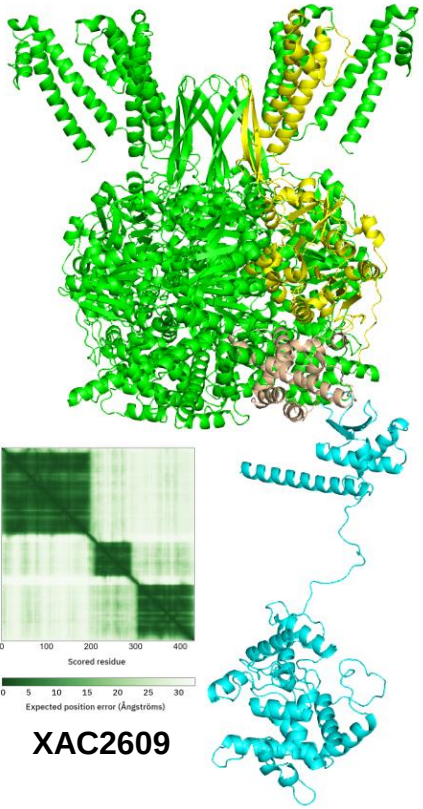
Gabriel Oka, Luis Cezar, Roberto Salinas

AlphaFold model of VirD4-XVIPCD complex

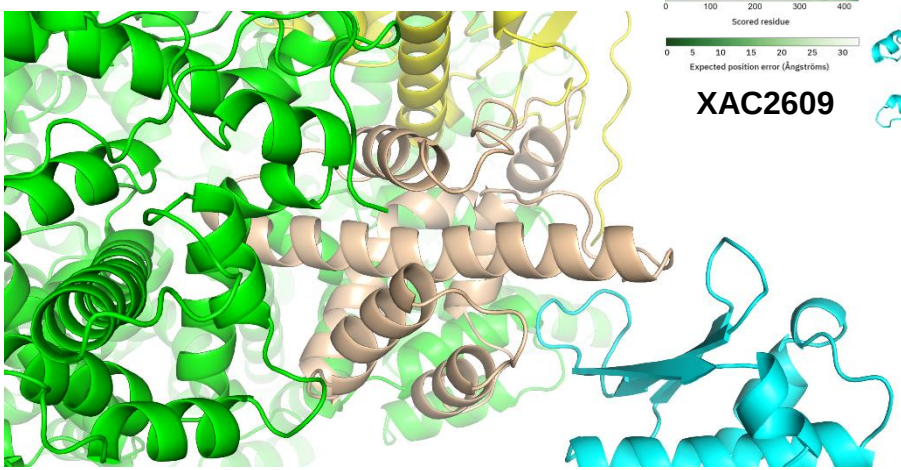


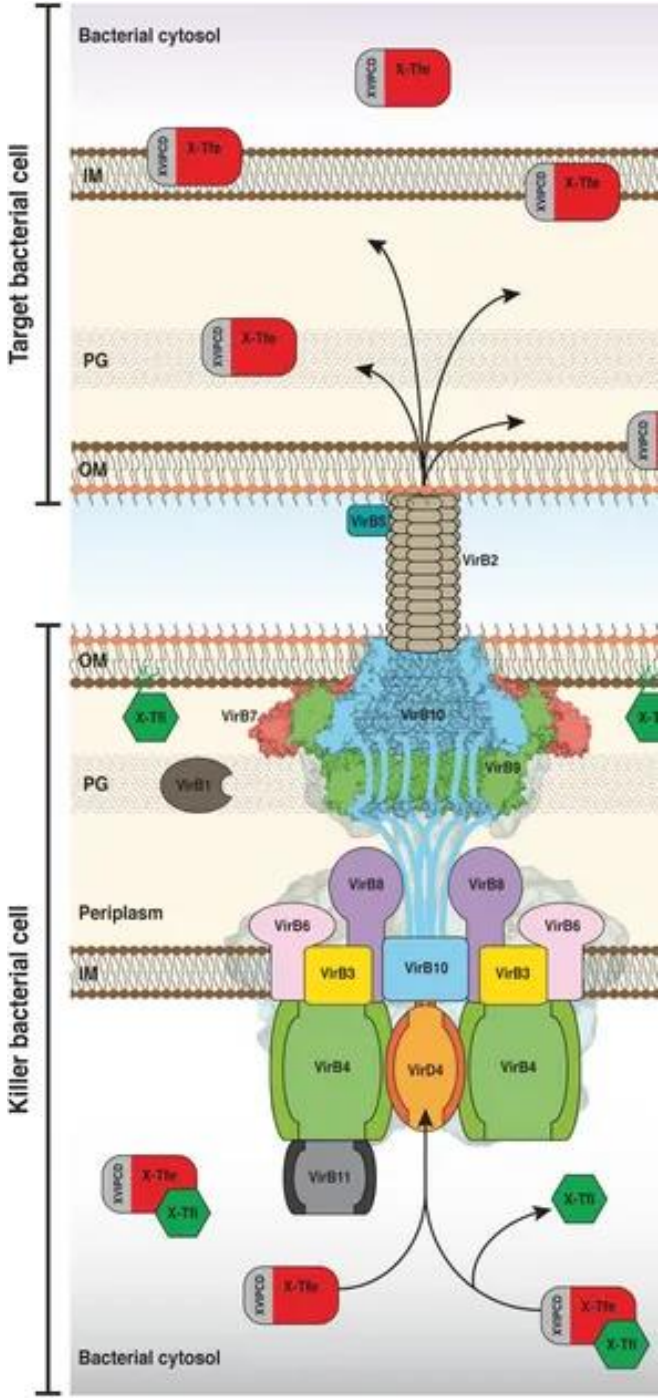
AlphaFold model of VirD4-XVIPCD complex



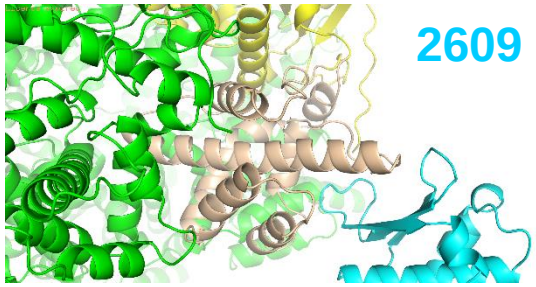


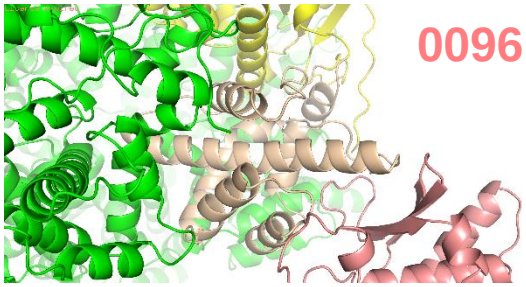
XAC2609

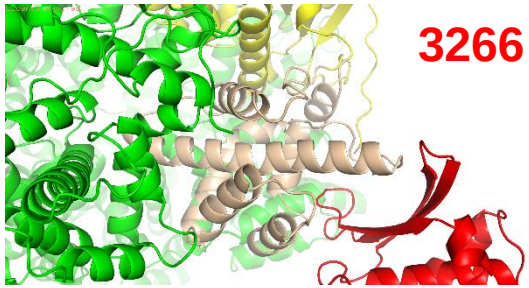


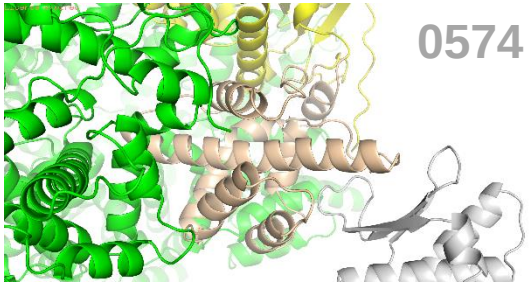


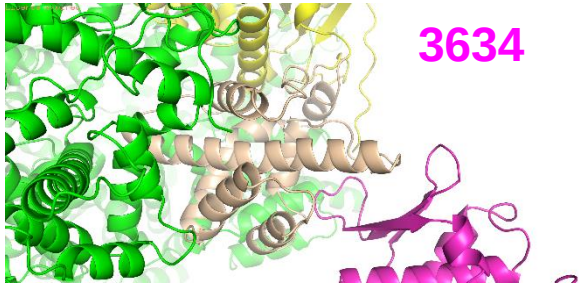
Models of other VirD4-XVIPCD complexes

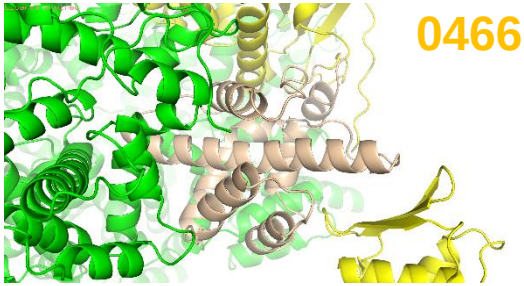


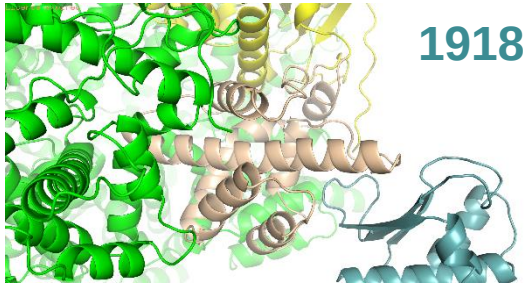


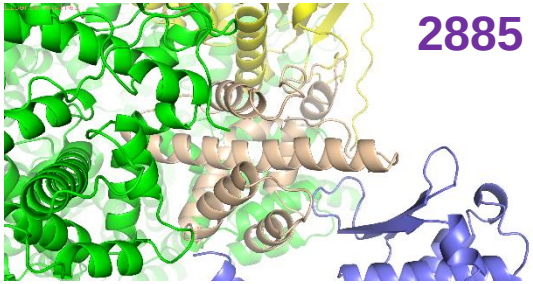


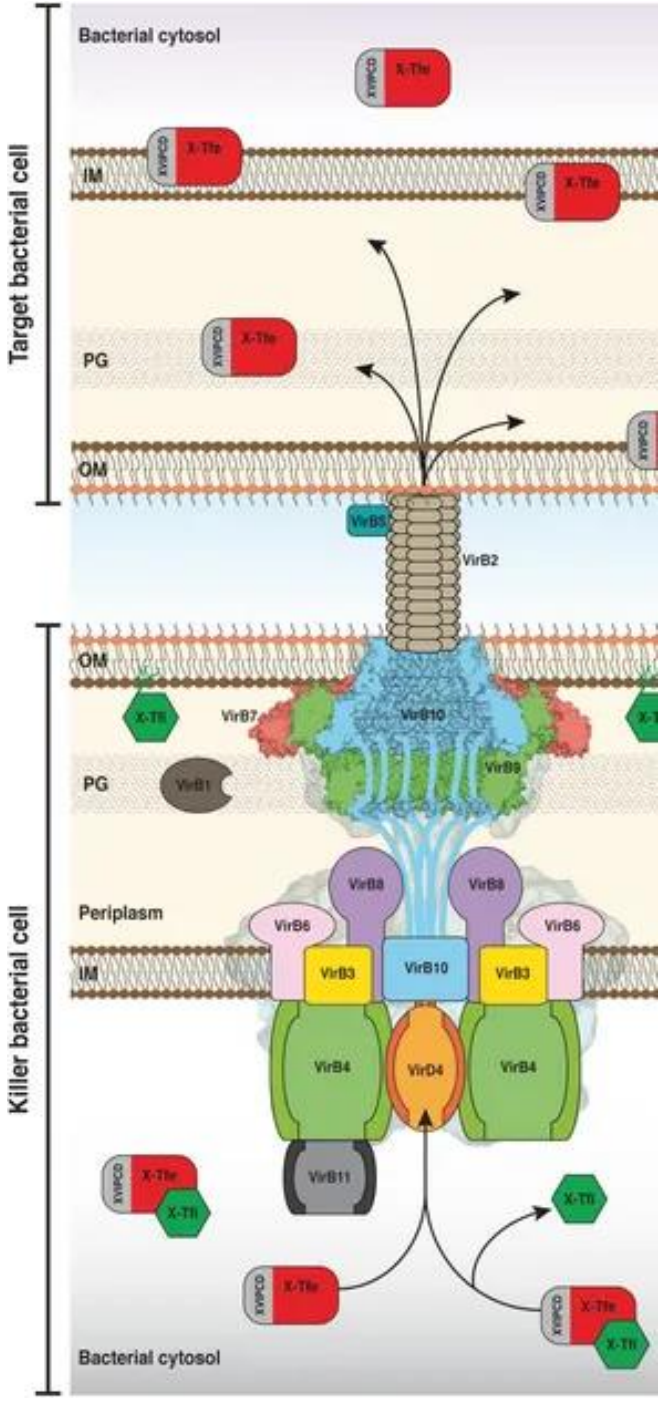




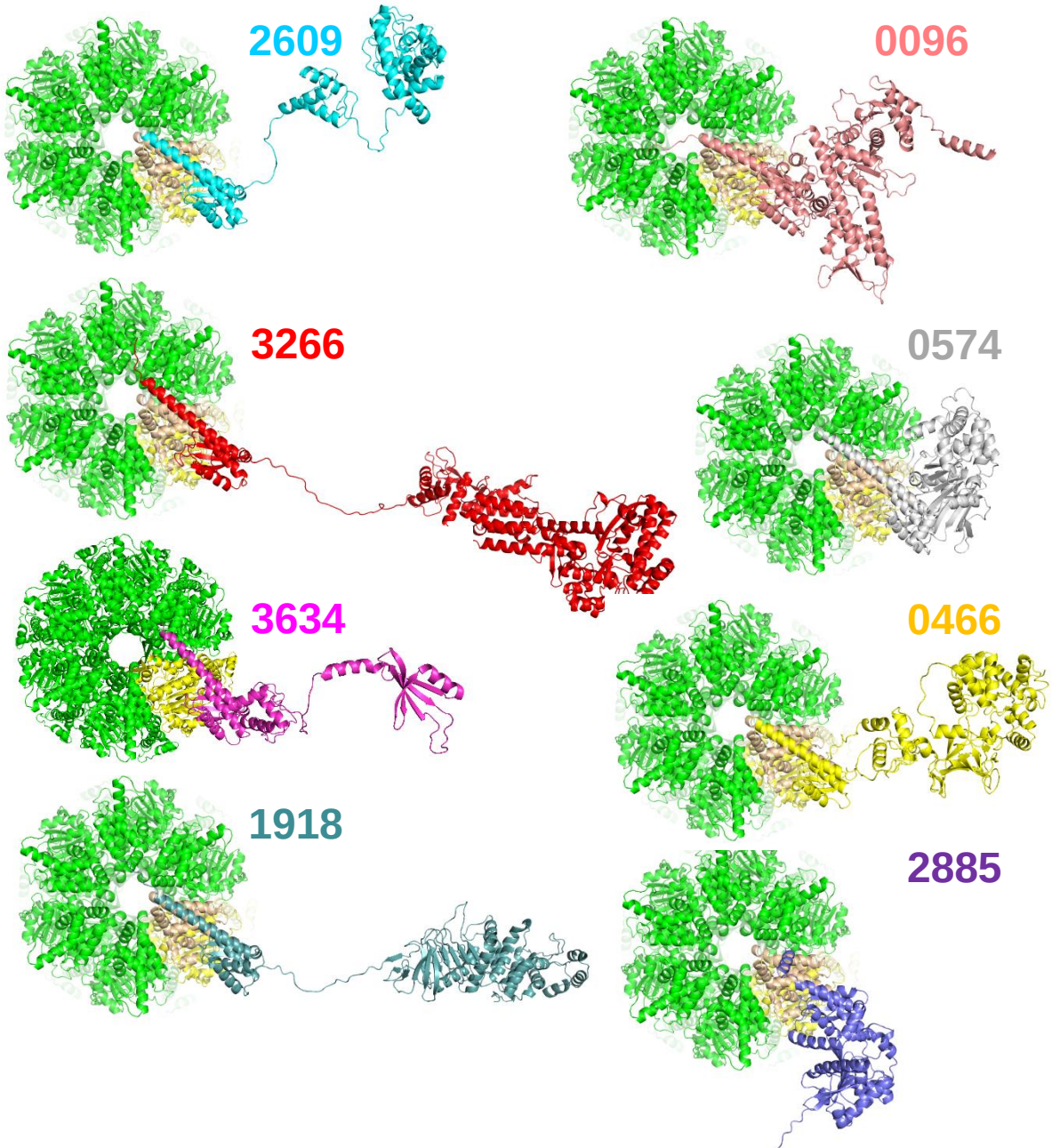


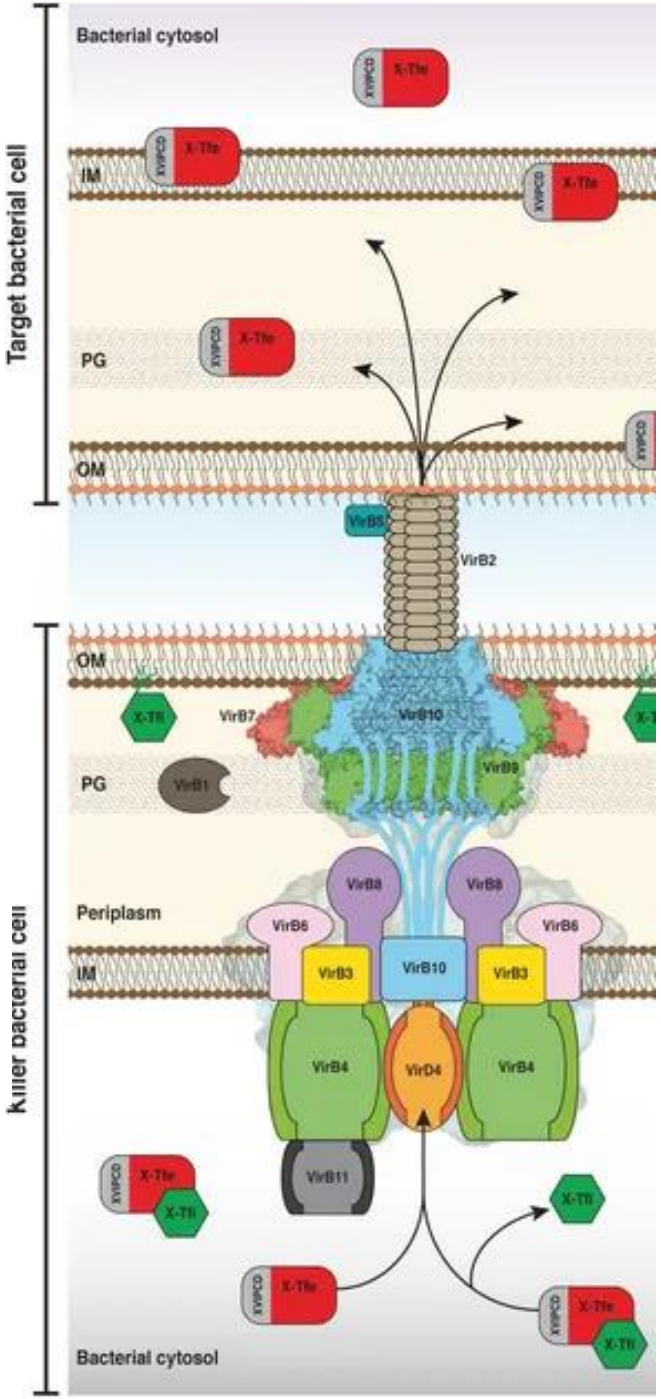




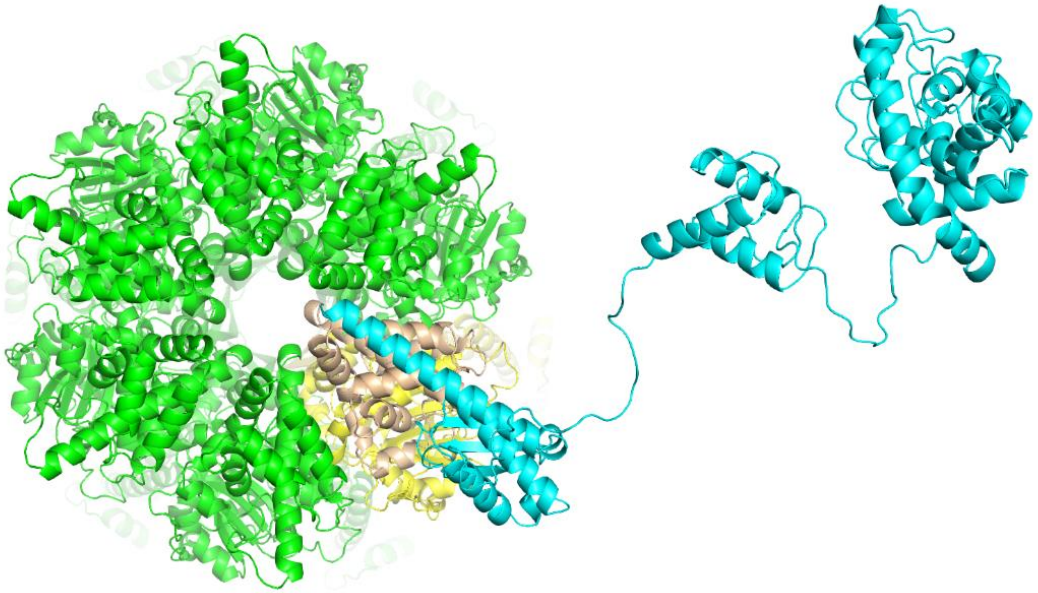


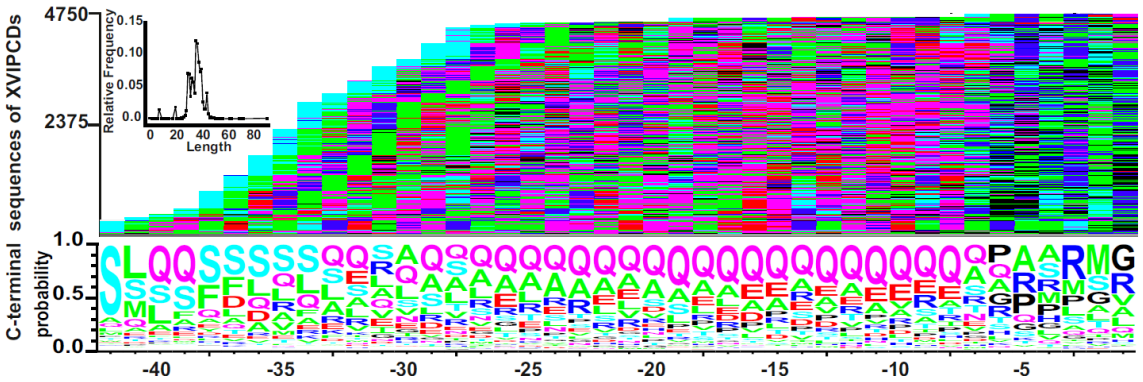
Models of other VirD4-XVIPCD complexes





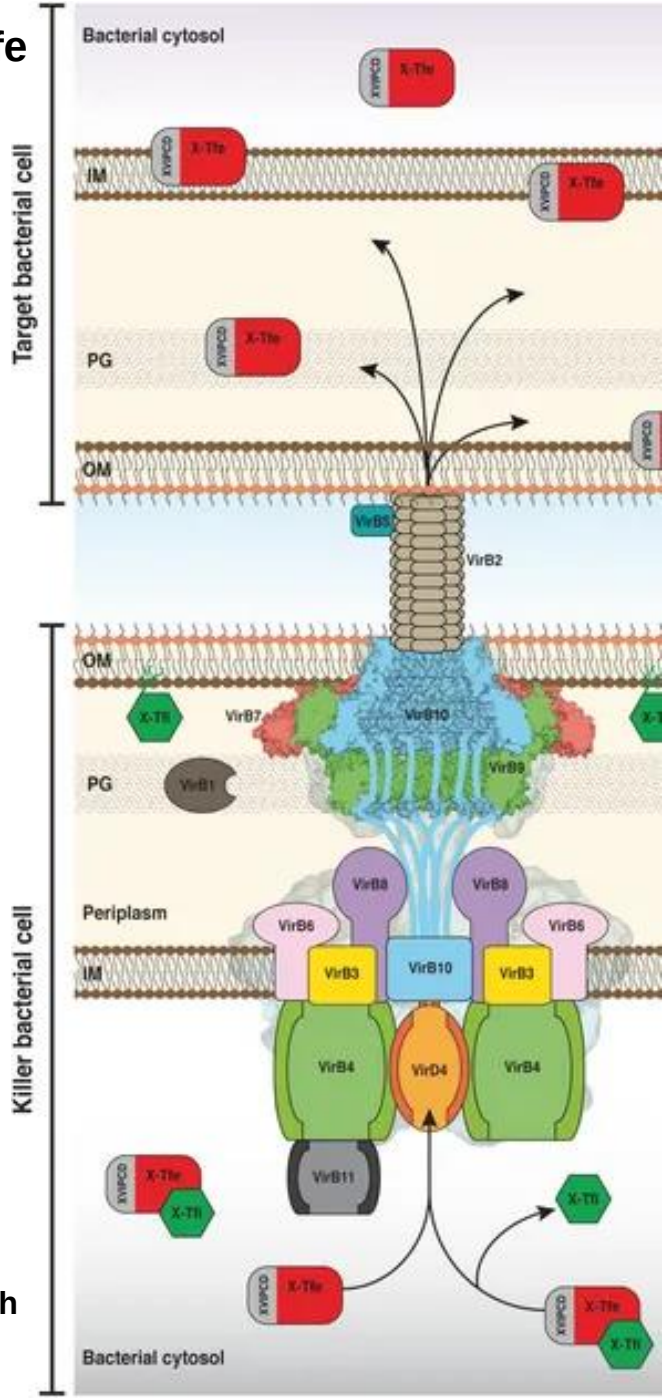
The VirD4$_{AAD}$-XVIPCD interaction may help to poise the X-Tfe extreme C-terminus for insertion into the hexamer channel



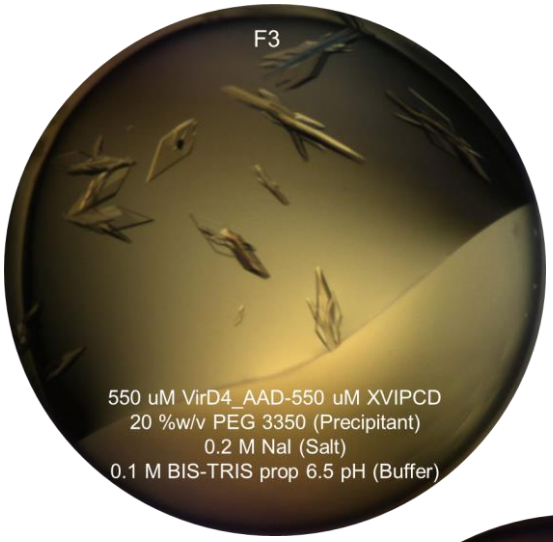


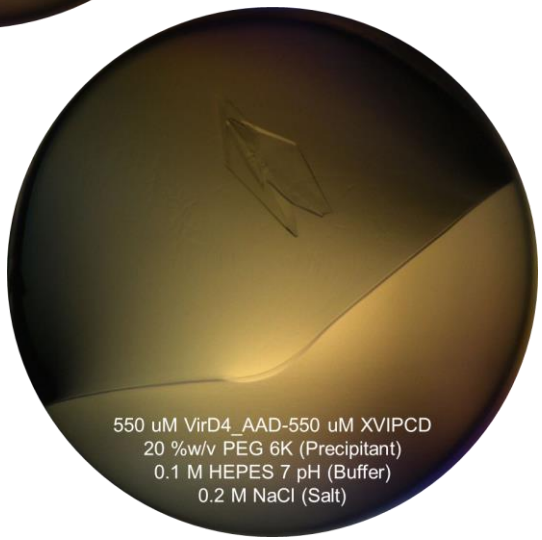
Oka et al (2022): All of the mutations that significantly impaired XVIPCD binding to VirD4$_{AAD}$ also compromised the ability of X-TfeXAC2609 to be transferred into and lyse target *E. coli cells*.

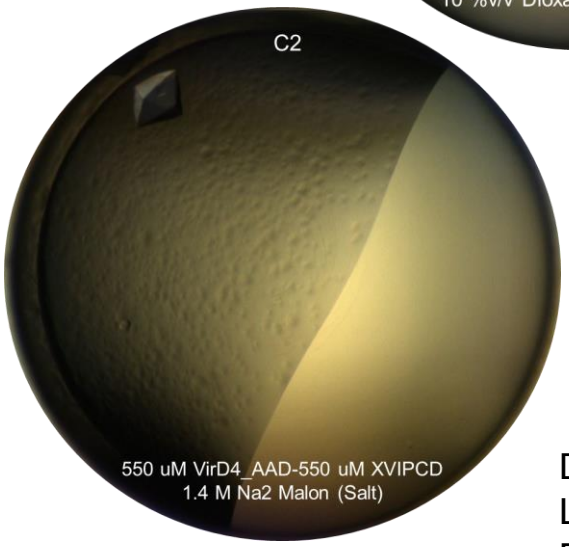
Of particular interest is the six- and nine residue deletions in the carboxyl-terminal tail of XAC2609 and XAC3634 that impaired *E. coli* killing even though it did not significantly affect binding to VirD4$_{AAD}$. Thus, the extreme carboxyl terminus of the XVIPCD is apparently involved in an essential step in the secretion pathway that may be distinct from binding to VirD4$_{AAD}$.



Future crystal structures of the $VirD4_{AAD}$-X-$Tfe^{XAC2609}_{XVIPCD}$ complex may provide evidence for or against these hypotheses







Daniela Sifuentes
Luis Cezar
Roberto Salinas
Chuck Farah

Main points covered

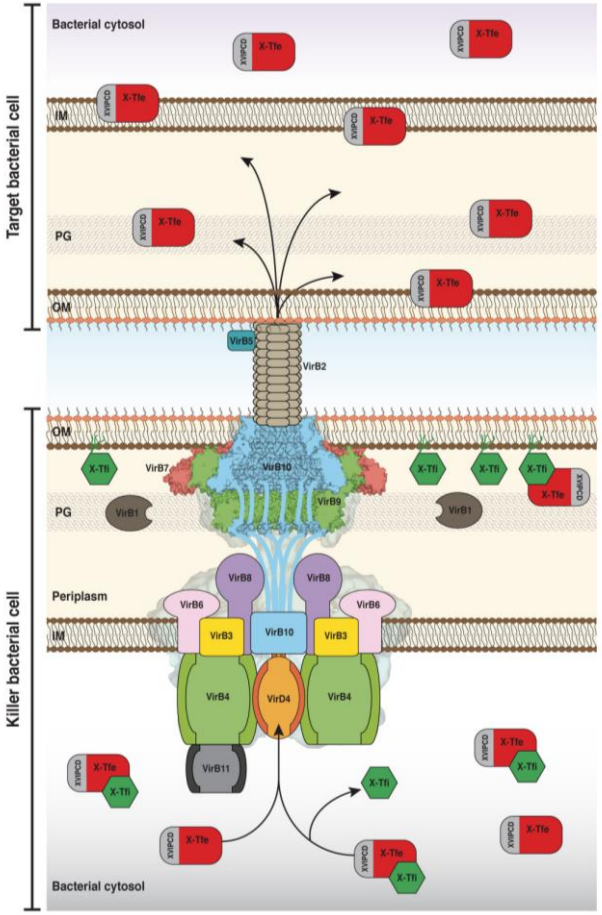
Many species from the Xanthomonadales order carry a chromosomally encoded T4SS capable of transferring toxic effectors into other Gram-negative bacterial species.

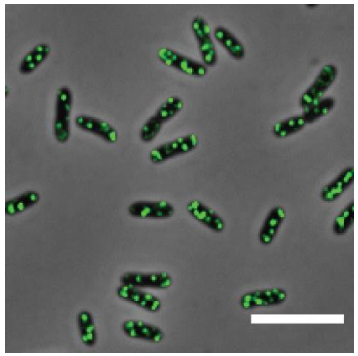
Effectors/toxins are recognized by way of an interaction between a conserved C-terminal and the All-Alpha-Domain of the VirD4 coupling protein.

Effectors carry N-terminal domains with different catalytic activities

The $VirD4_{AAD}$-XVIPCD interaction may help to poise the X-Tfe extreme C-terminus for insertion into the hexamer channel

Some Outstanding Questions and Future Directions





- T4SS structural biology
- incorporation of other components into larger complexes
- recognition of toxins by the X-T4SS
- Can we identify distinct conformational states of the core complex?
- Tomography of X-T4SS in cell

- Coordination of ATPase activities with toxin transport.

For example: what happens after VirD4-XVIPCD recognition?

- Can we directly observe toxin transfer from a *Xanthomonas* cell to a target cell?

- How do toxins that act in the target cell cytosol get there from the periplasm?

- Structural basis of X-T4SS self/non-self discernment

- X-T4SS effectors/toxins – diversity and evolution

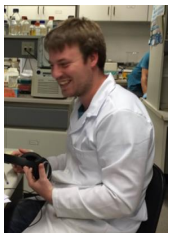
VERY SPECIAL AND TALENTED PEOPLE

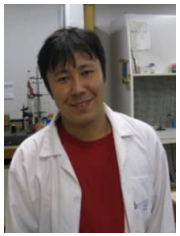

Roberto Salinas


Germán Sgro

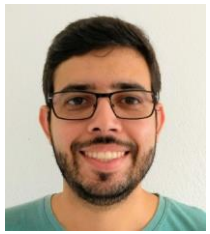

Diorge Souza


German Dunger


William Cenens


Gabriel Oka


Natalia Bueno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Cristiane Guzzo


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Tiago L. Alves


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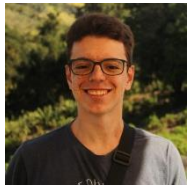
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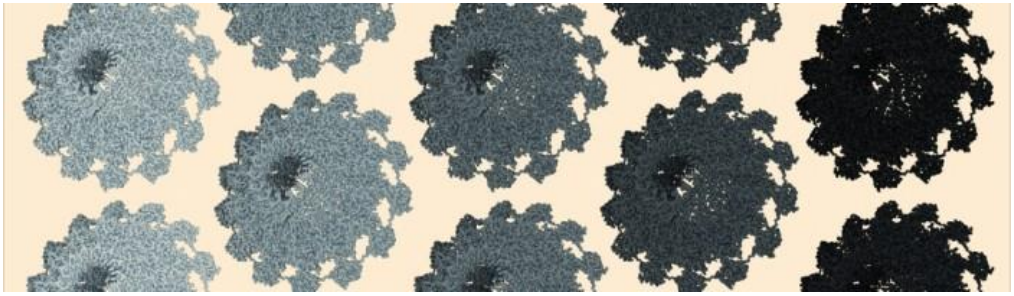

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