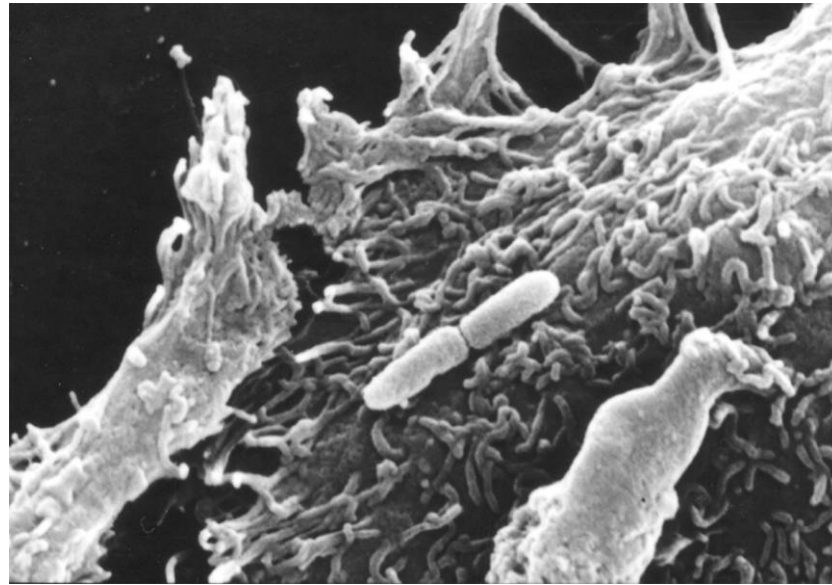


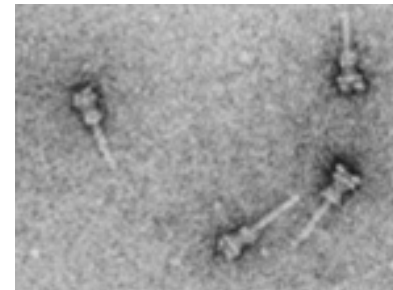


Seringues protéiques : quand les bactéries injectent des toxines



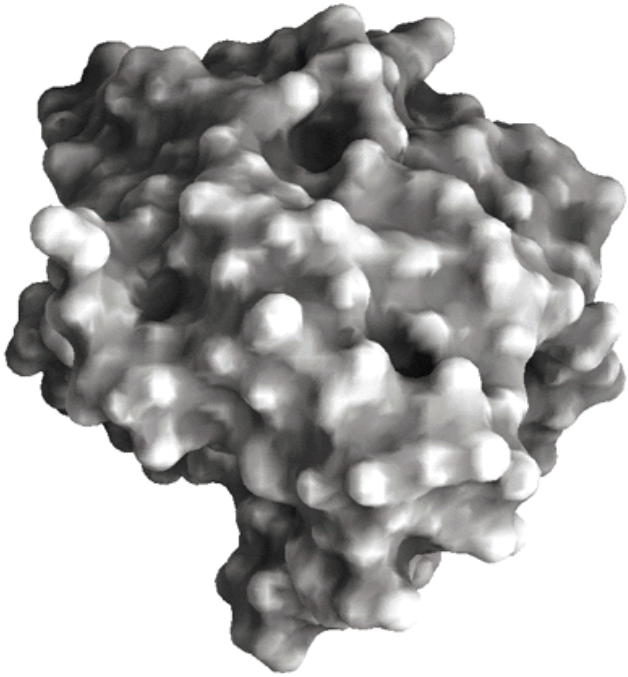
Eric Faudry
UMR 5092
CEA-Grenoble

<http://www-dsv.cea.fr/bbsi/pseudo>



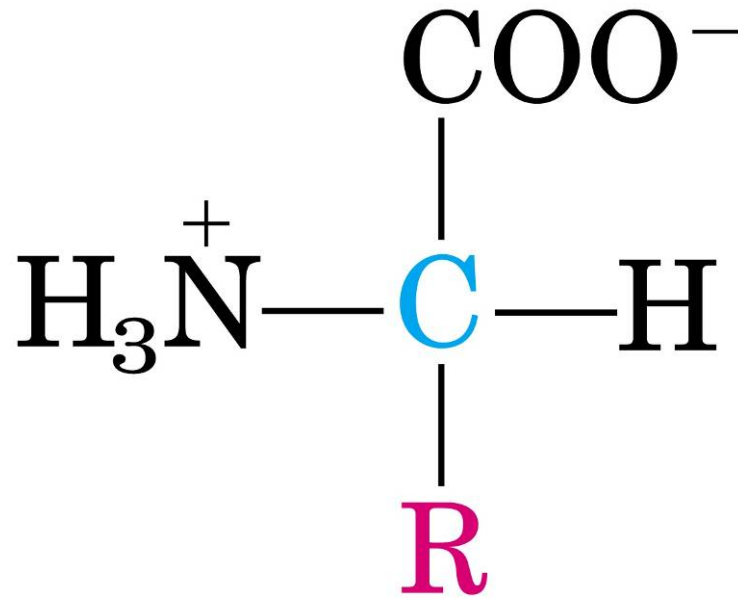
Les Protéines

Les protéines



- Les protéines sont des macromolécules
 - Polymères d'acides aminés
 - Importance fonctionnelle
 - Enzymes
 - Récepteurs
 - Régulateurs
 - Rôle structural
 - Moteurs
 - Acteurs du vivant
-

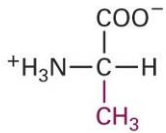
La structure des acides aminés



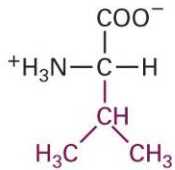
R = alcool, chaîne aliphatique, groupe aromatique...

Différents types d'acides aminés

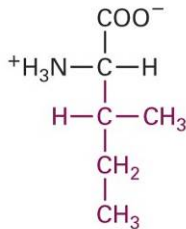
Acides aminés hydrophobes



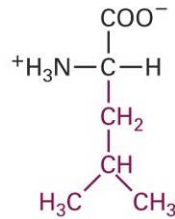
Alanine
(Ala or A)



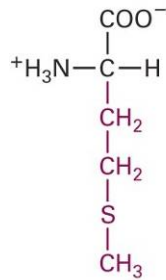
Valine
(Val or V)



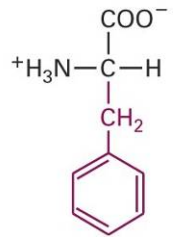
Isoleucine
(Ile or I)



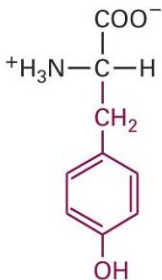
Leucine
(Leu or L)



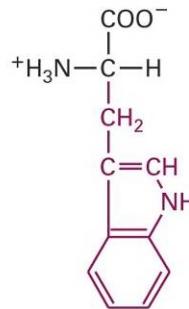
Methionine
(Met or M)



Phenylalanine
(Phe or F)

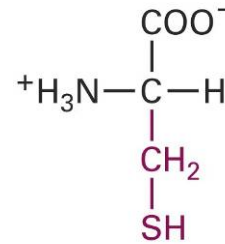


Tyrosine
(Tyr or Y)

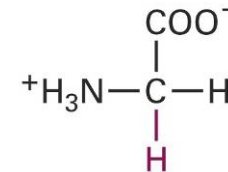


Tryptophan
(Trp or W)

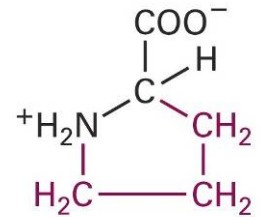
Acides aminés « spéciaux »



Cysteine
(Cys or C)



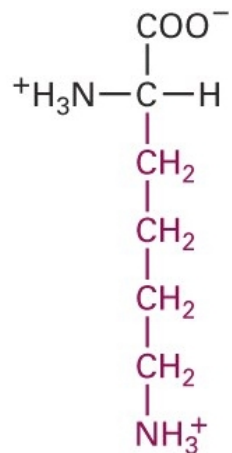
Glycine
(Gly or G)



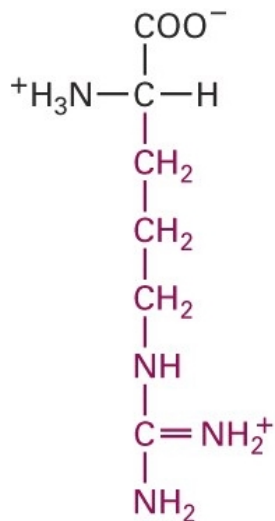
Proline
(Pro or P)

Les Acides aminés hydrophiles

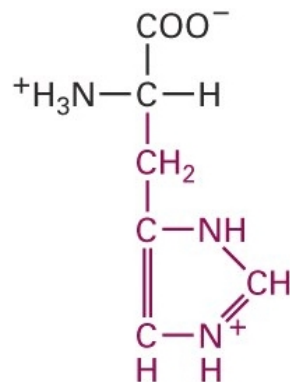
Acides aminés basiques



Lysine
(Lys or K)

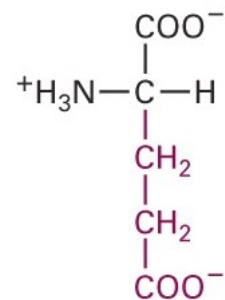
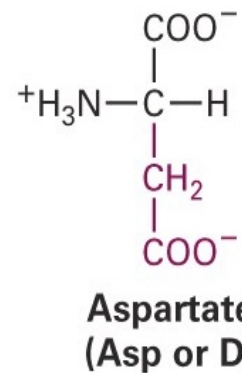


Arginine
(Arg or R)



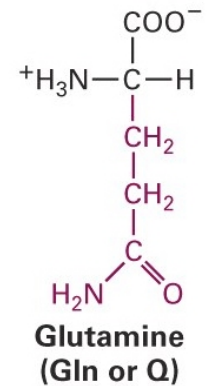
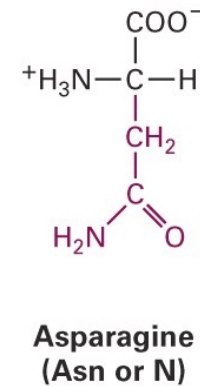
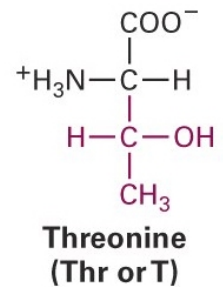
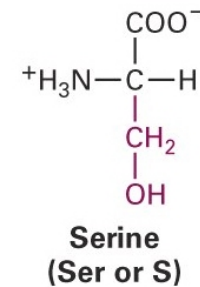
Histidine
(His or H)

Acides aminés acides

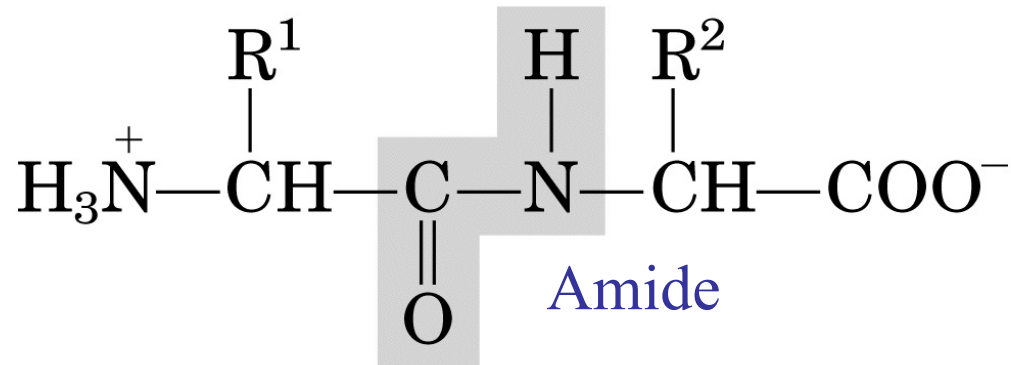
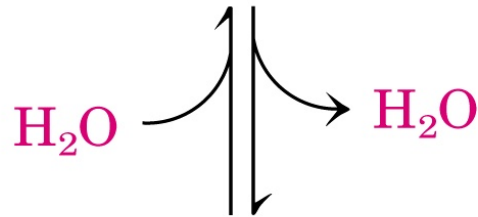
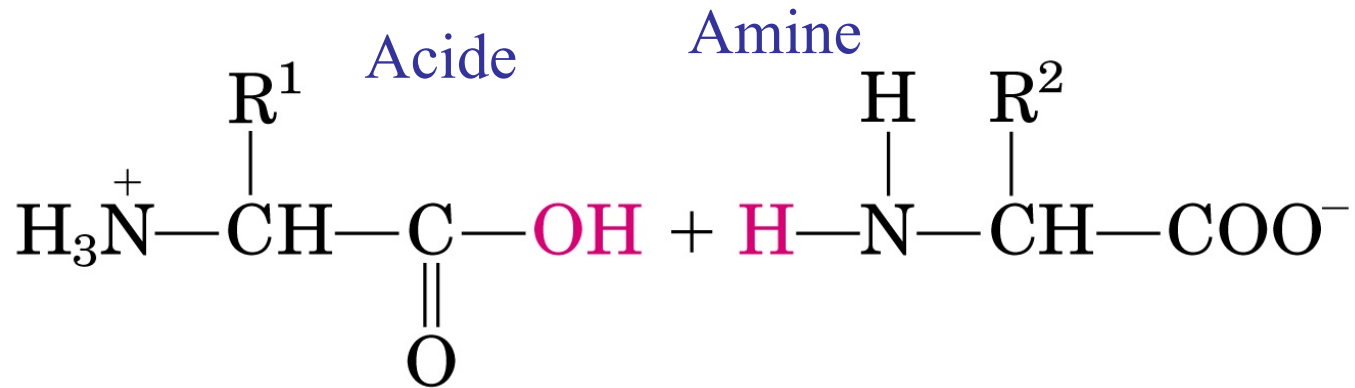


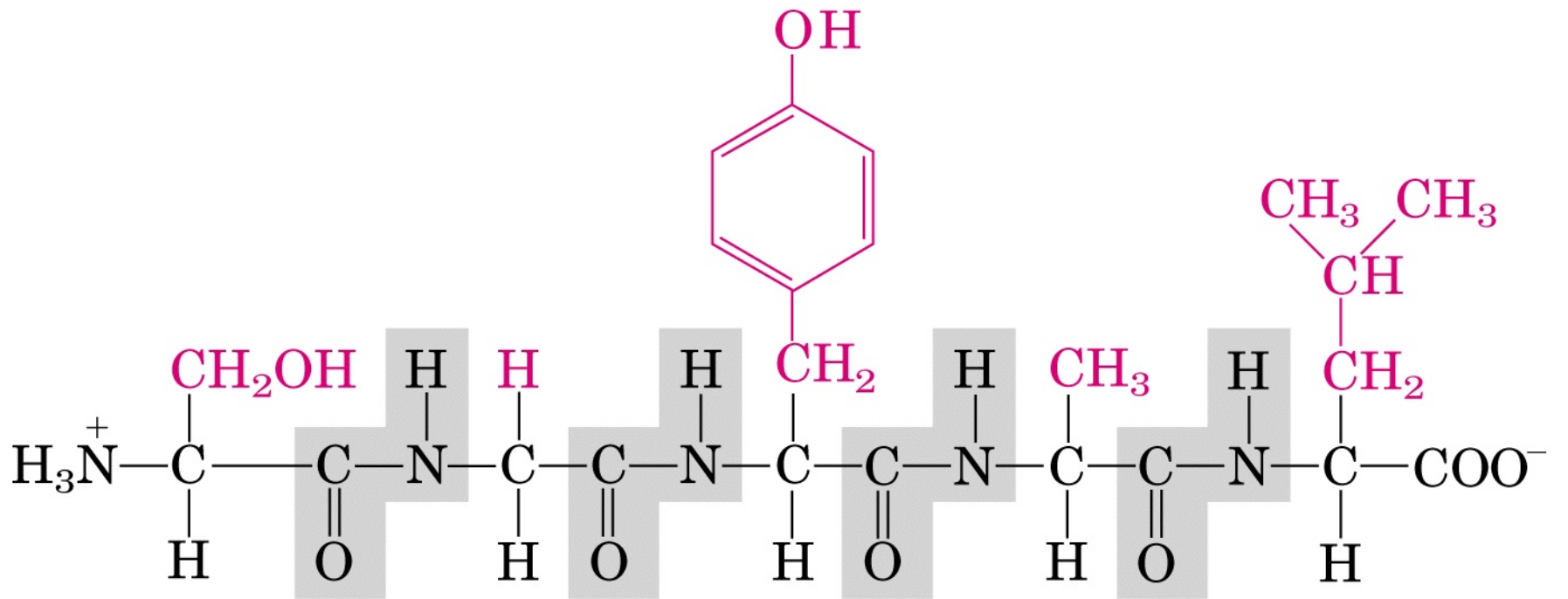
Glutamate
(Glu or E)

Acides aminés polaires non chargés



Les protéines sont formées par condensation

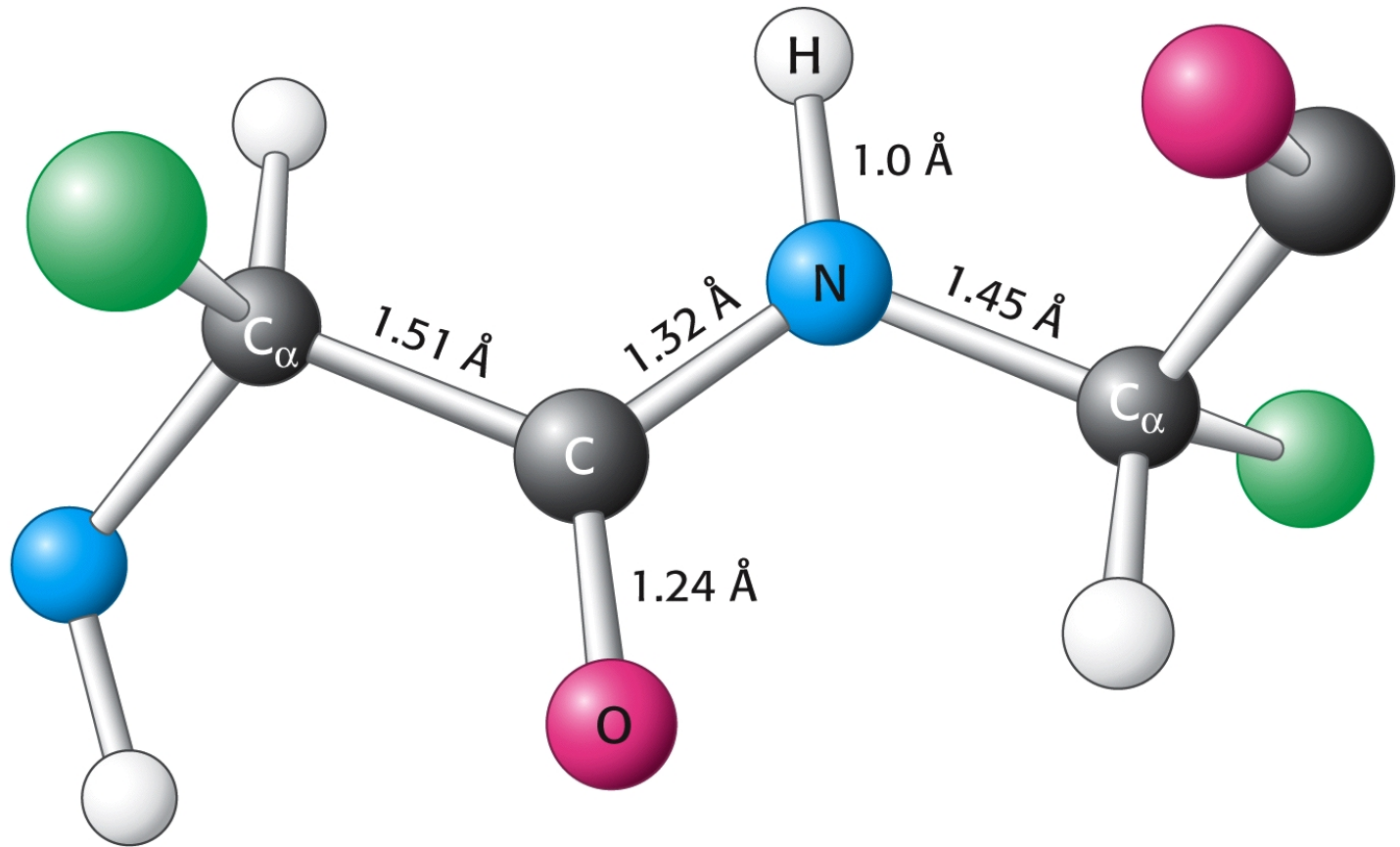




Amino-
terminal end

Carboxyl-
terminal end

Dimensions



Niveaux de structure

Structure primaire = séquence en acides aminés

Structure secondaire = arrangement entre acides aminés proches dans la séquence

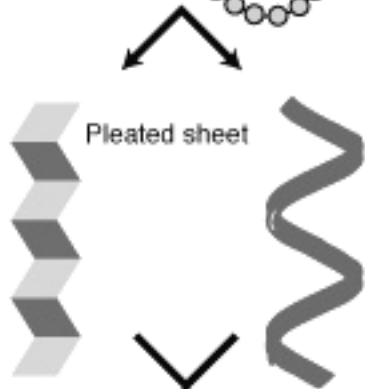
Structure tertiaire = repliement de la chaîne peptidique structure stabilisée par interaction entre acides aminés éloignés dans la séquence

Structure quaternaire = arrangement de plusieurs chaînes peptidique entre elles



Primary protein structure
is sequence of a chain of amino acids

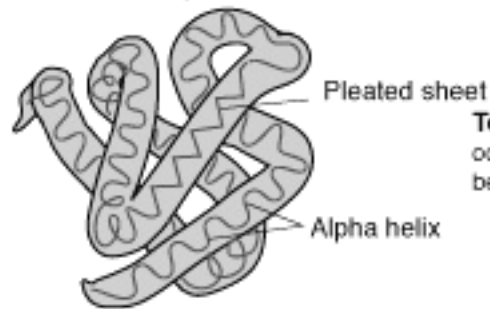
Amino Acids



Pleated sheet

Alpha helix

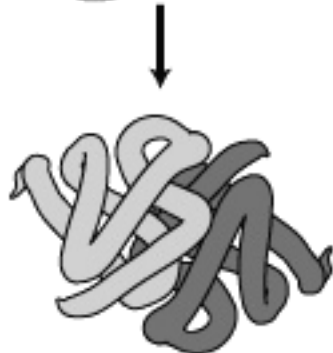
Secondary protein structure
occurs when the sequence of amino acids
are linked by hydrogen bonds



Pleated sheet

Alpha helix

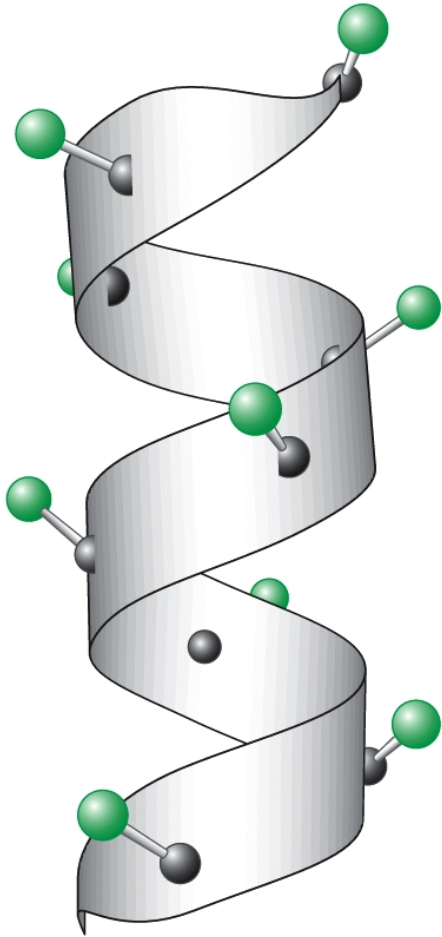
Tertiary protein structure
occurs when certain attractions are present
between alpha helices and pleated sheets.



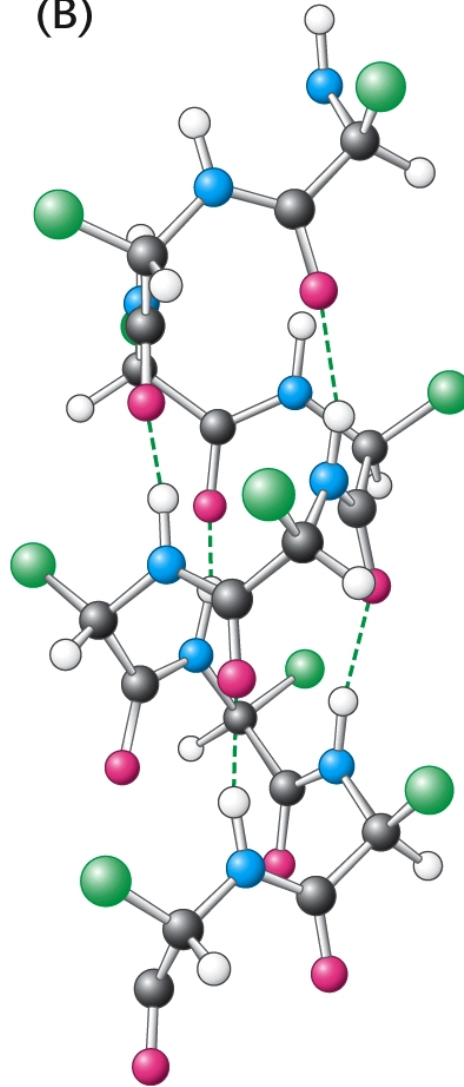
Quaternary protein structure
is a protein consisting of more than one
amino acid chain.

Structure secondaire: hélice α

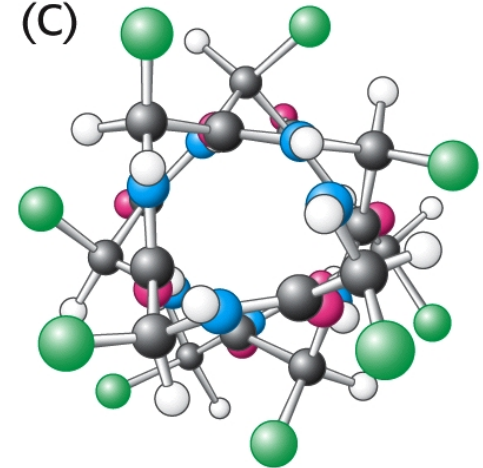
(A)



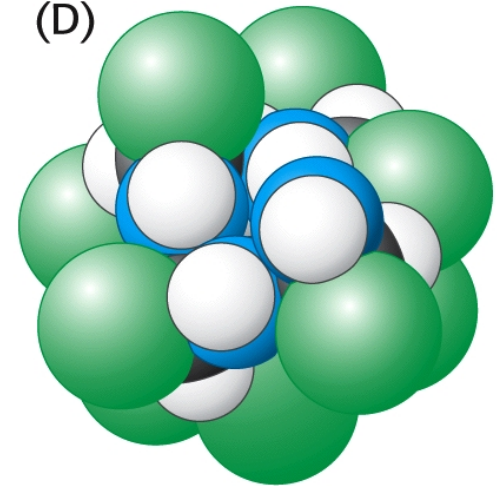
(B)



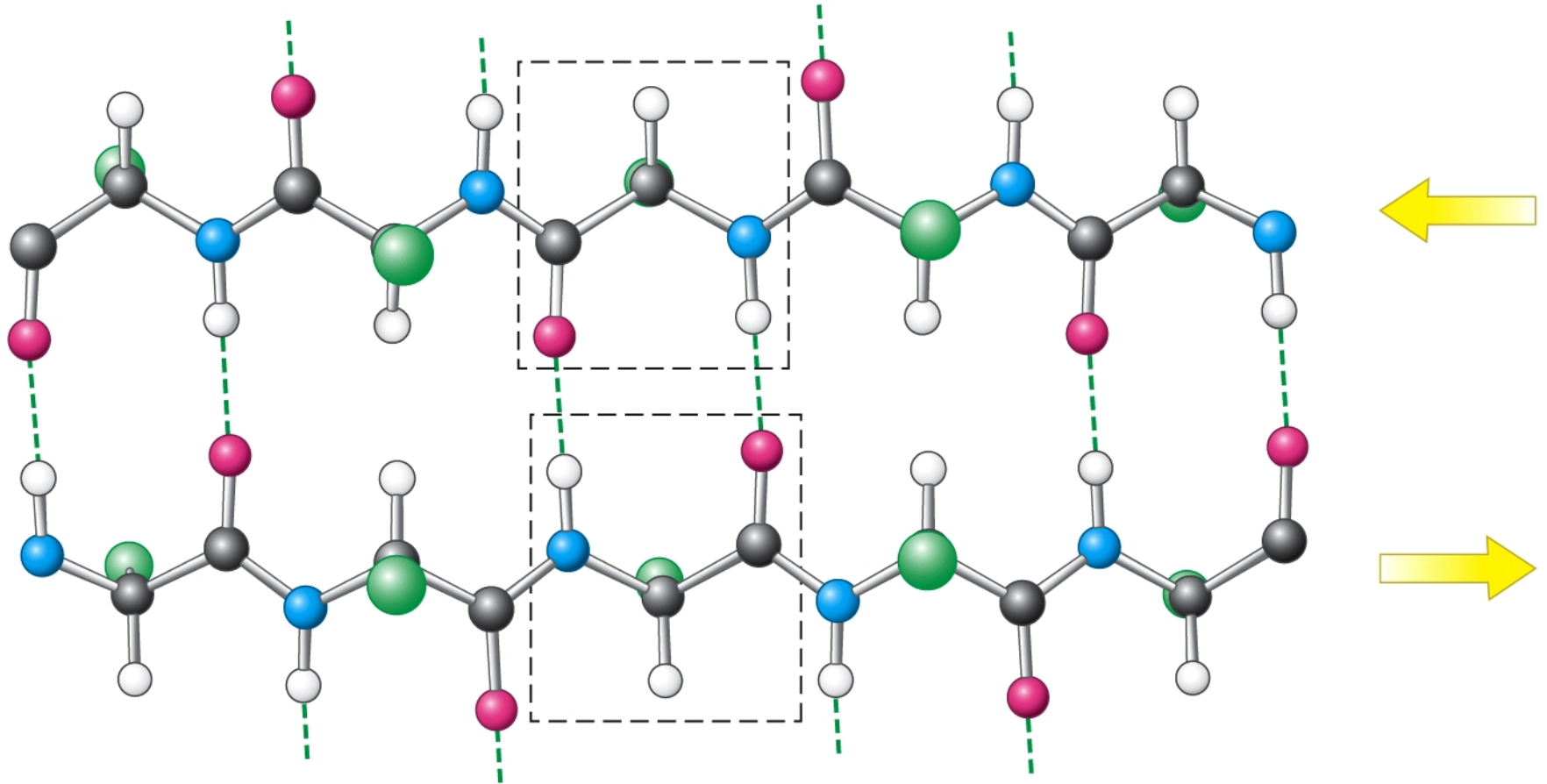
(C)



(D)



Structure secondaire: feuillet β



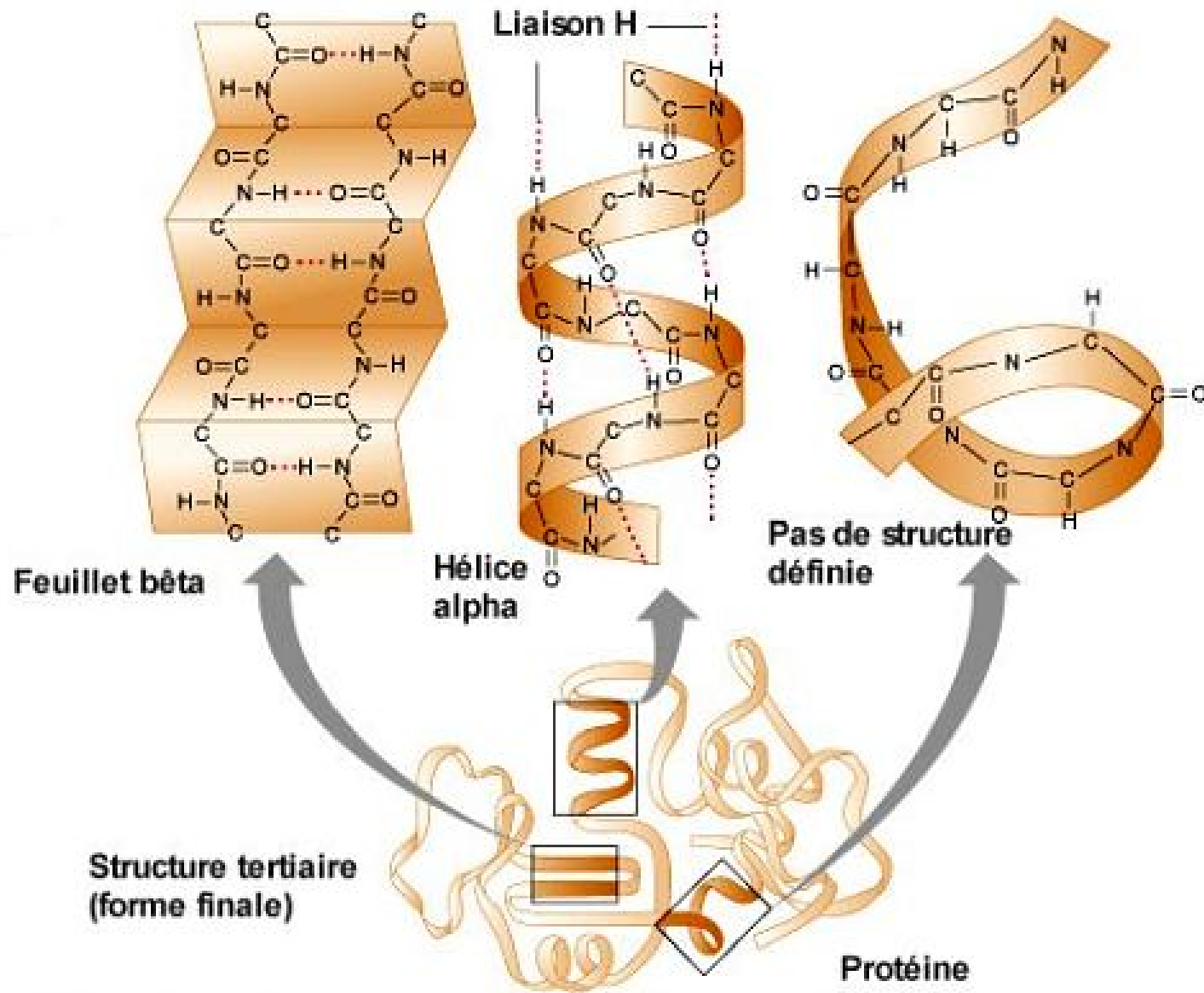
Structure tertiaire

β Conformation
 $2,000 \times 5 \text{ \AA}$

α Helix
 $900 \times 11 \text{ \AA}$

Native globular form
 $130 \times 30 \text{ \AA}$

Tailles théoriques correspondant à différentes conformations d'une même protéine

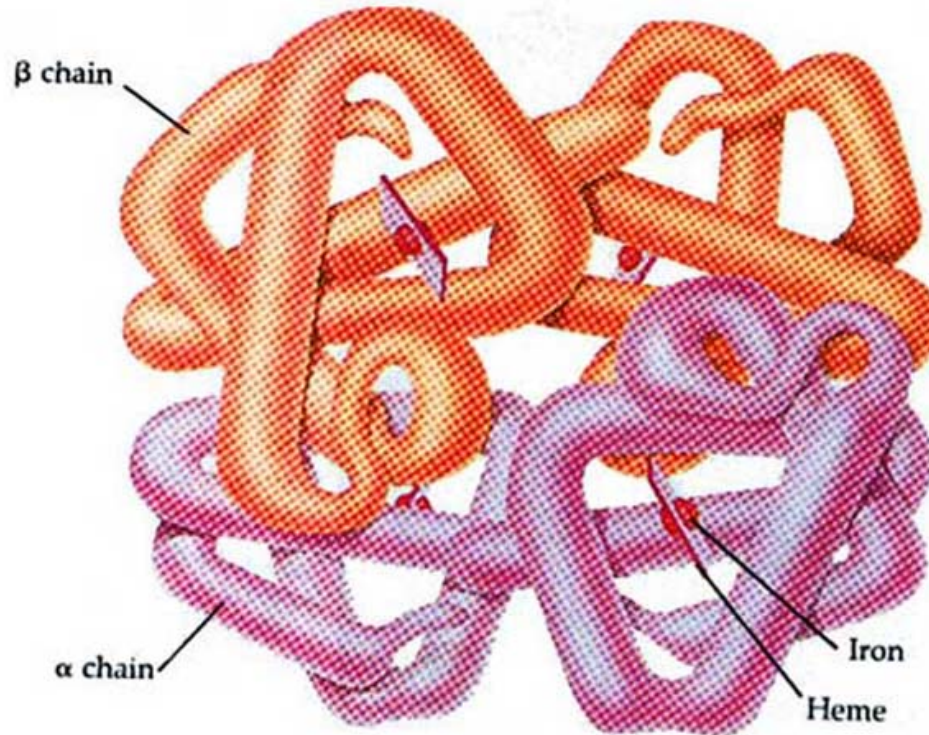


Différents types de structure quaternaire

- Fibre
- Globule
- Oligomères : petits polymères



(a) Collagen



(b) Hemoglobin

Détermination expérimentale de la structure

Résolution

Biochimie

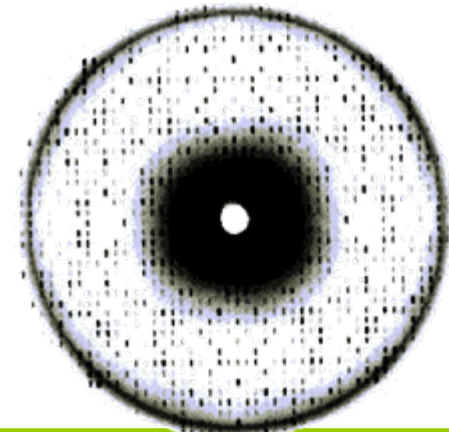
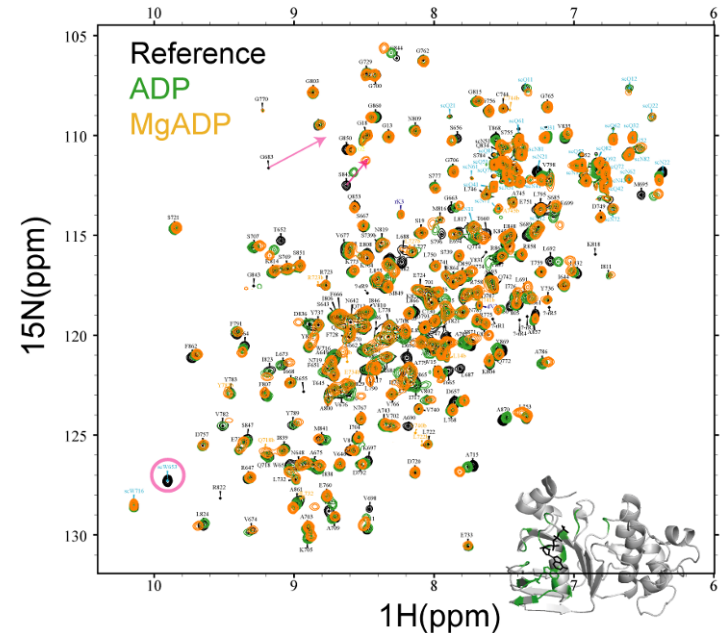
Spectrométrie de masse

Spectroscopie

Microscopie électronique

RMN

Diffraction des rayons X...

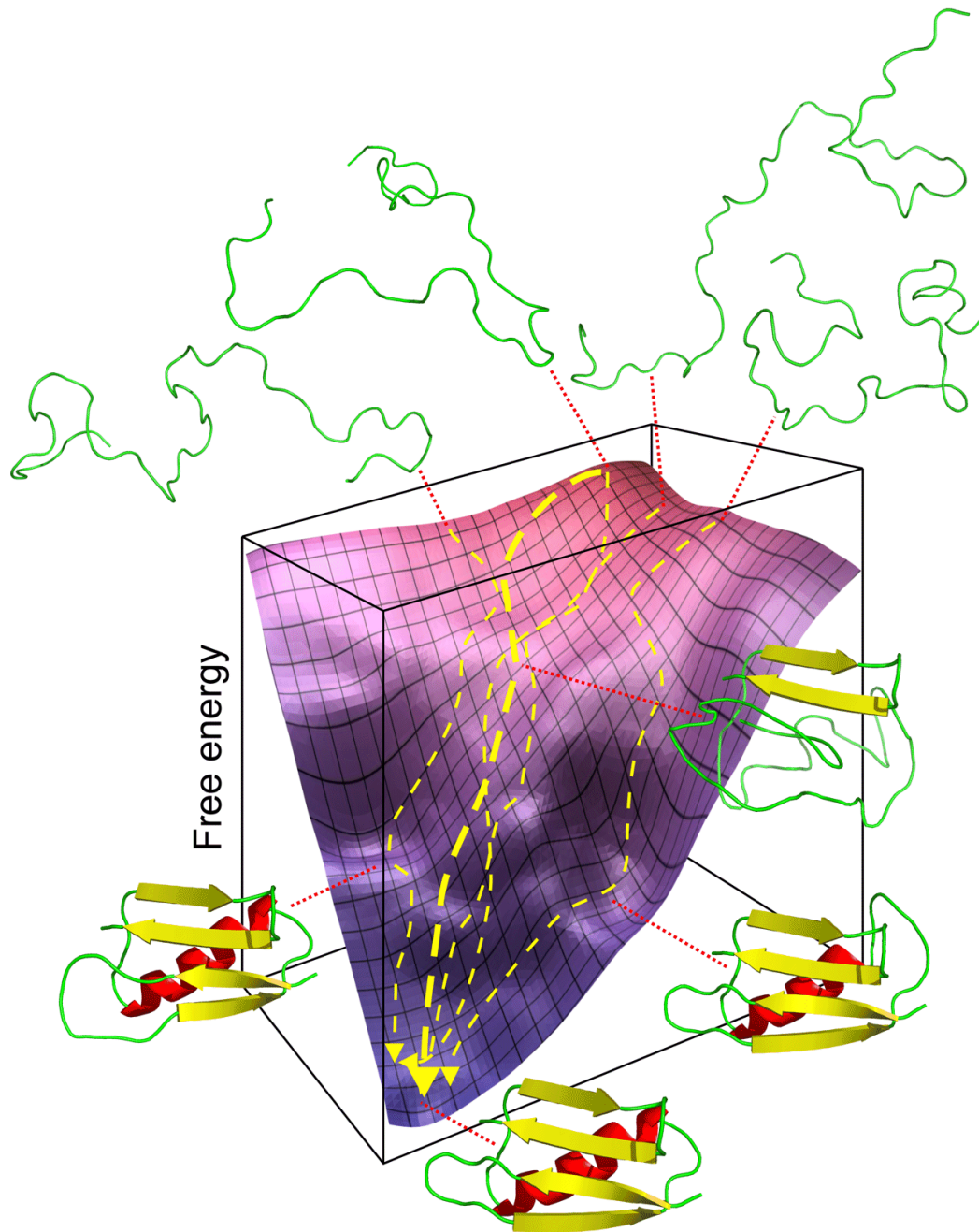


Le paradoxe de Levinthal

Chaque AA existe dans deux conformations
 $2^{100} \sim 1.3 \times 10^{30}$ conformations

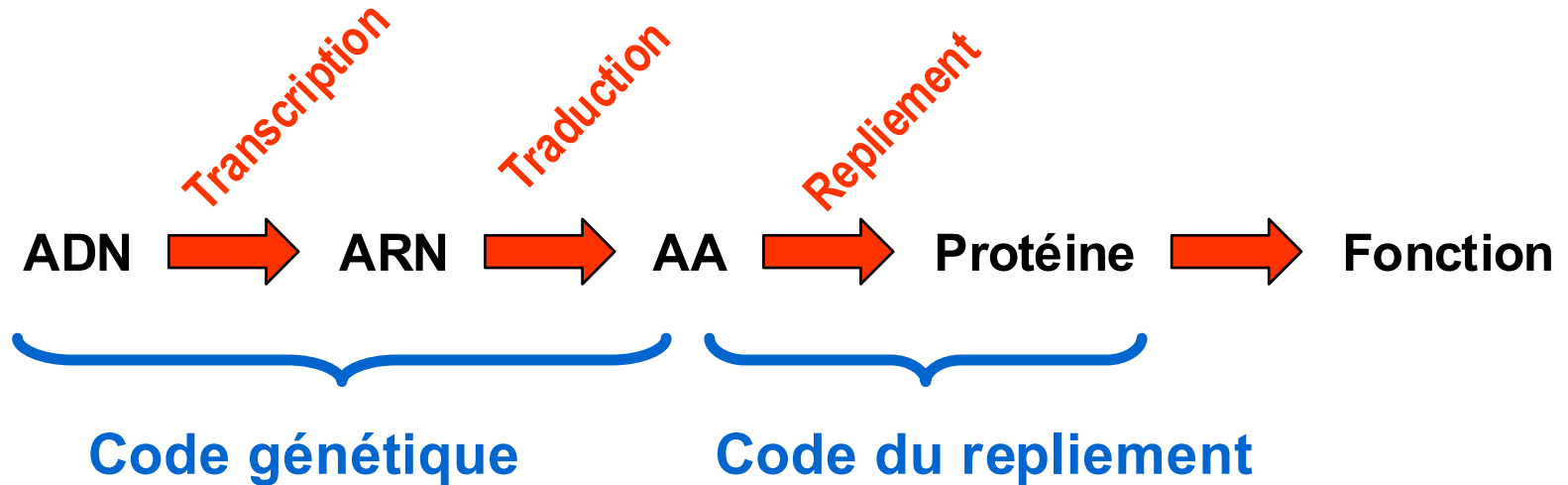
Vitesse de conversion entre 2 conformations
 $< 10^{13} \text{ s}^{-1}$

Une recherche au hasard à travers l'ensemble de
conformations nécessiterait
 $1.3 \times 10^{17} \text{ s} \sim 4$ milliards d'années !!!!



Paysage énergétique

De l'ADN aux protéines et aux fonctions



Relation structure / fonction des protéines

- Contraction, mouvement (muscle)
- Prise en charge et modification des substrats par les enzymes
- Reconnaissance : protéines complémentaires
- Assemblage, oligomérisation, polymérisation
- Structure dynamique

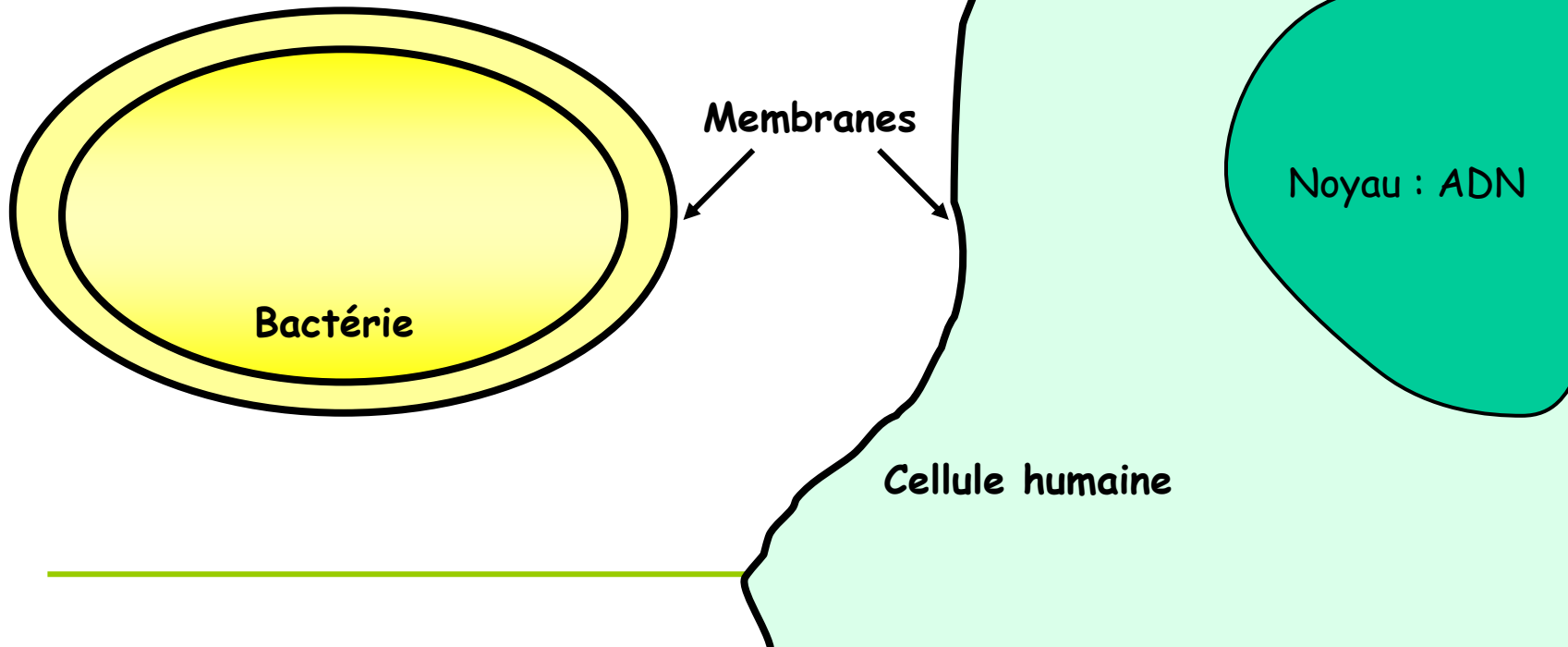
Les bactéries

Les bactéries

Taille : 0,5 à 3 μm

Pas de noyau : ADN dans le cytoplasme

Membranes différentes



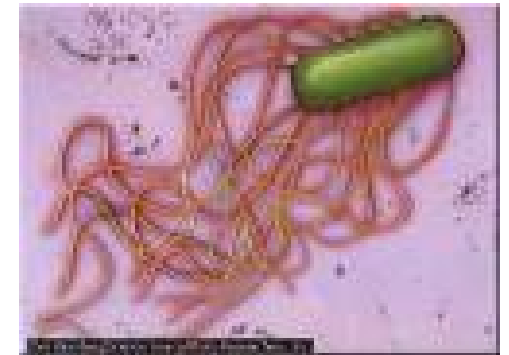
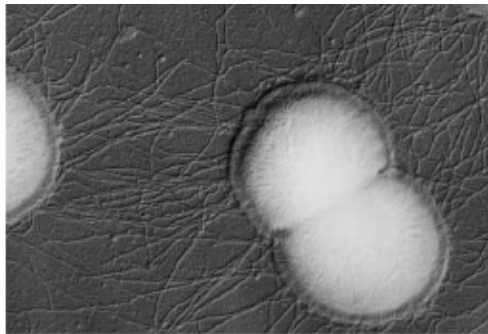
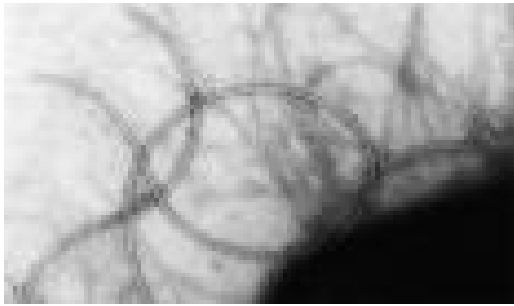
Pseudomonas aeruginosa



- Bacille motile : un flagelle
 - Bacille pyocyanique : sécrète des pigments
 - Habitat naturel : sol, lacs, plantes, surfaces humides
 - **Pathogène opportuniste**
 - 30% des infections nosocomiales
 - patients immunodéprimés (cancer, SIDA, brûlés...)
 - mortalité moyenne de 35% (50% dans les septicémies)
 - résistance aux antibiotiques en augmentation
 - **Mucoviscidose**
 - 75% des décès des patients
-

Appendices bactériens

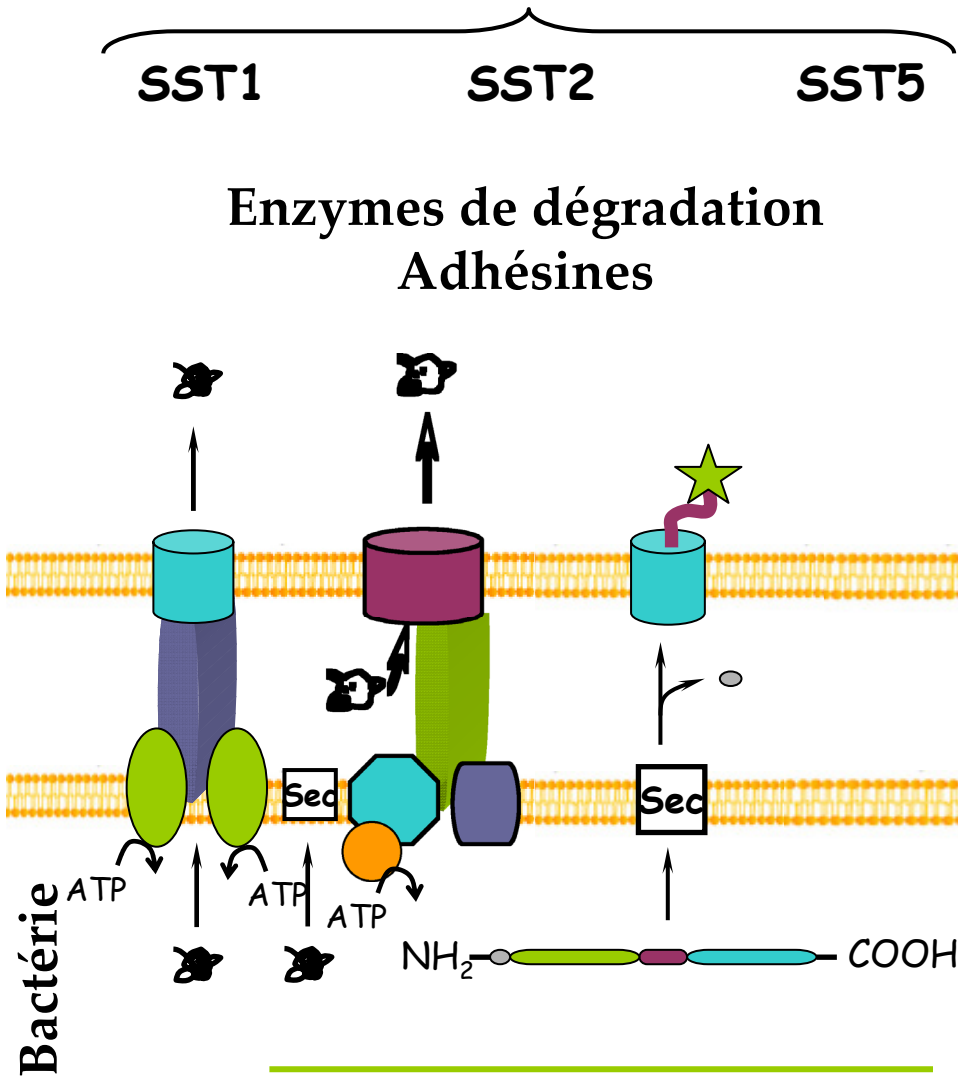
pili, fibres, fimbriae, flagelle, injectisomes...



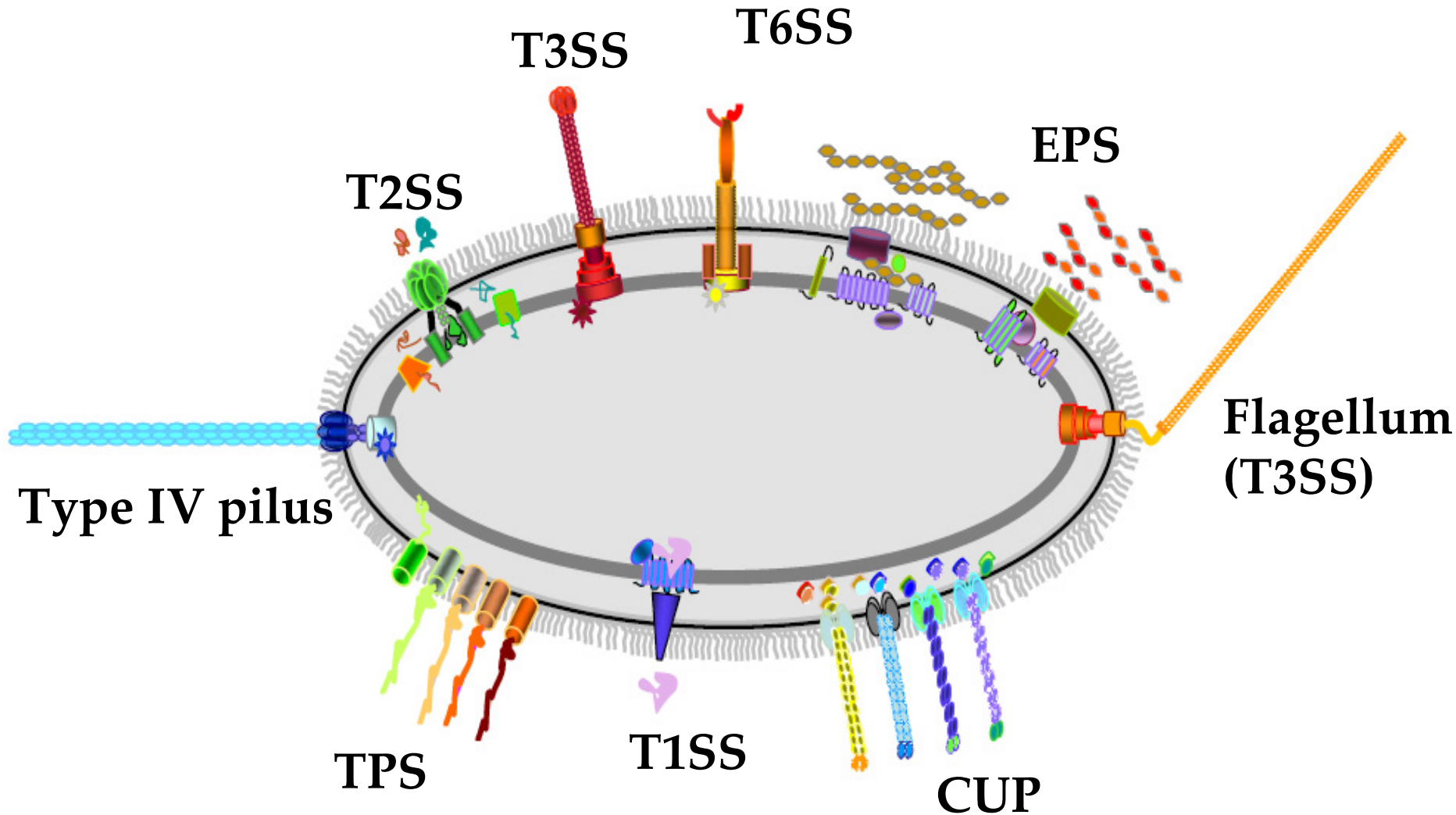
- structures « poilues »
 - composées souvent d'une protéine assemblée en « fibre »
-

Les systèmes de sécrétion des bactéries à gram-

Sécrétion milieu extracellulaire



machineries d'export de *P. aeruginosa*



Pathogénie bactérienne

Sécrétion de Type III :

- Injectisome
- Aiguille (sécrétion)
- Translocon
- Toxines (effecteurs)
- Chaperonnes (convoyage et repliement)

Thèmes de recherche :

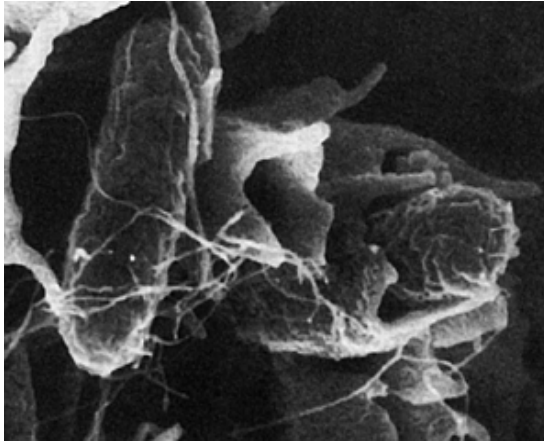
- 1/ composition et structure de l'injectisome
 - 2/ assemblage des éléments
 - 3/ sécrétion & injection
 - 4/ effets du SST3 sur la cellule hôte
 - 5/ drogues anti-infectieuses ?
-

Maladies infectieuses causées par les bactéries à gram négatif

BACTERIES	MALADIE	VECTEUR	CHARACTERISTIQUES
<i>Pseudomonas aeruginosa</i>	maladies nosocomiales / mucoviscidose	faible dans les défenses de l'hôte	infections difficiles à traiter, récurrentes
<i>Yersinia pestis</i>	peste	présence de rats/puces	bactériémie/fièvre importante delirium/décès en 3-5 jours
<i>Salmonella typhi</i> <i>Shigella dysenteriae</i>	Fièvre typhoïde dysenterie à bacille	eau contaminée, mauvaises conditions sanitaires	Malaise/fièvre/décès, Mortalité infantile
<i>E. coli</i> (enteroinvasive)	diarrhées		crampes/diarrhées Douleurs abdominales sévères

Effets induits par le SST3

*entrée de *Salmonella*

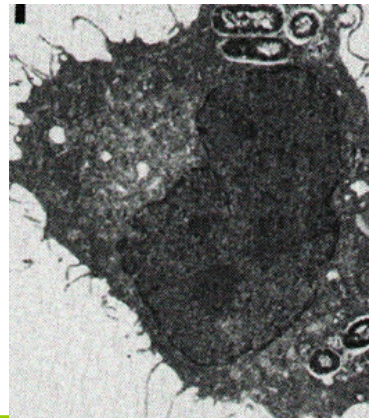
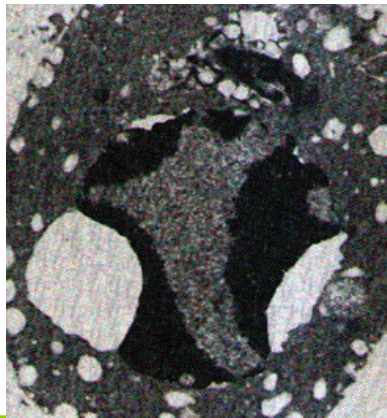


*lésions induites par *E. coli*



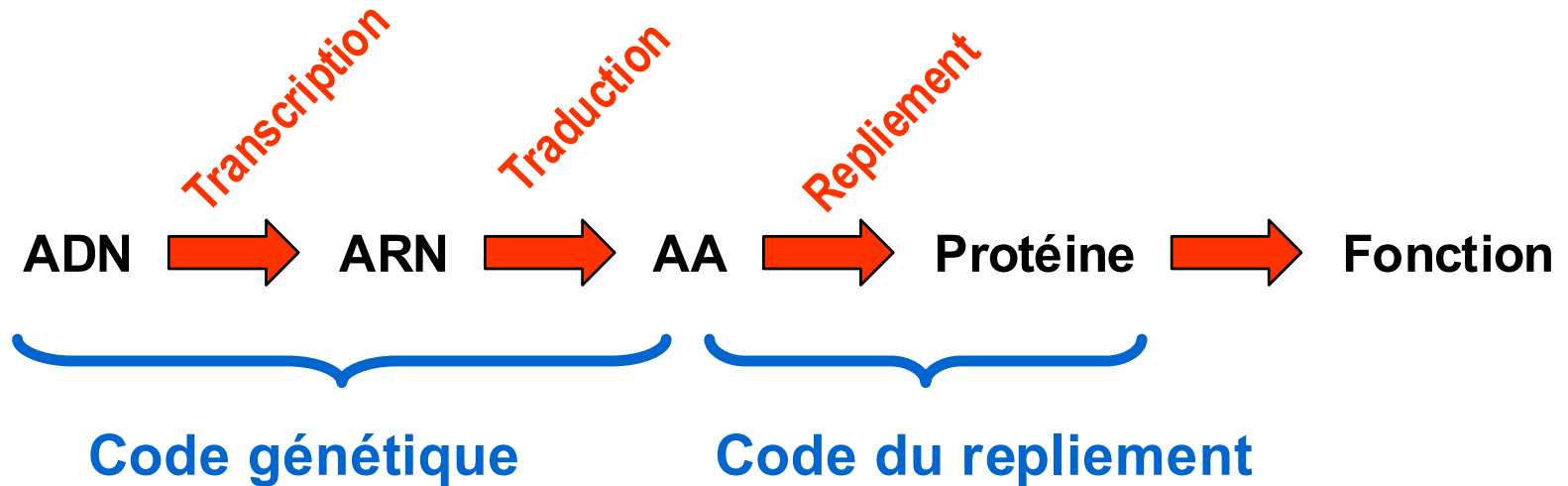
*induction de la mort cellulaire : *Yersinia*, *Salmonella*, *Shigella*...

Macrophage infecté
par *Shigella*



mutant ne possédant
pas le SST3

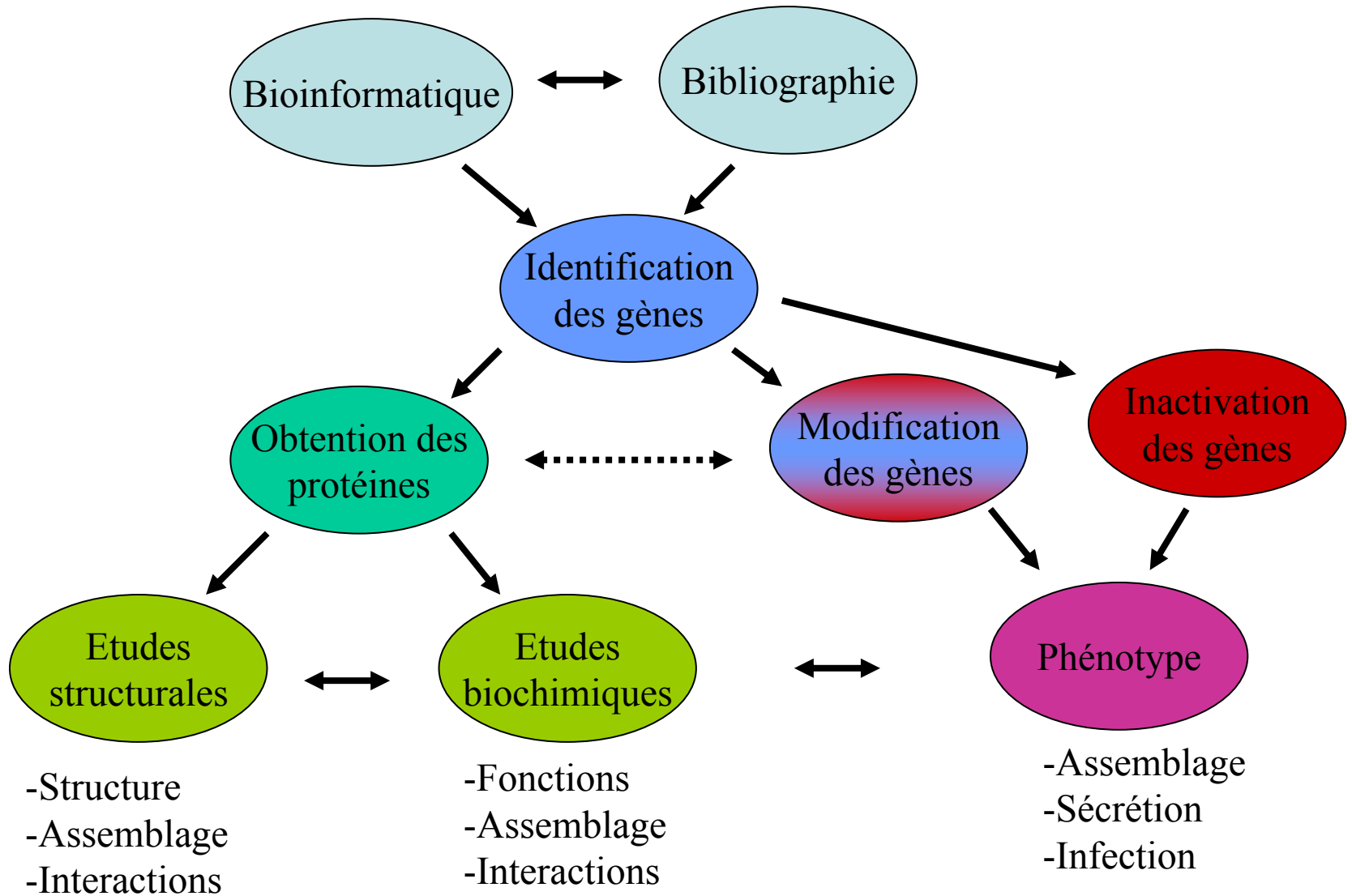
Nos outils



Il est possible de :

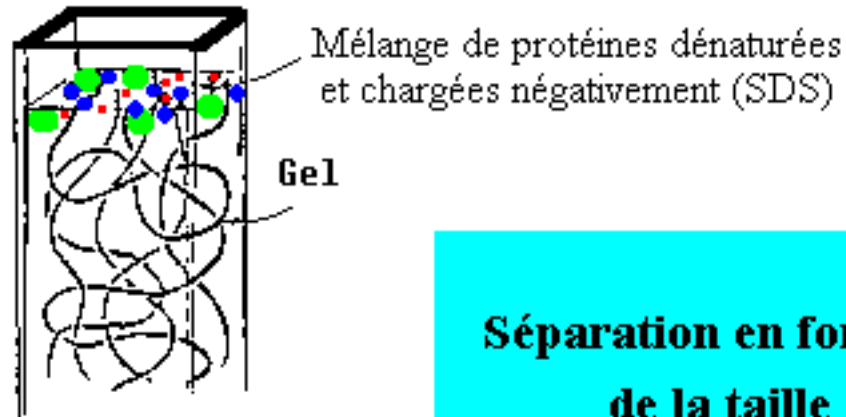
- Modifier ou inactiver un gène dans les bactéries : souches mutantes
 - Utiliser un gène pour faire produire la protéine correspondante : protéine recombinante
-

Stratégies d'étude

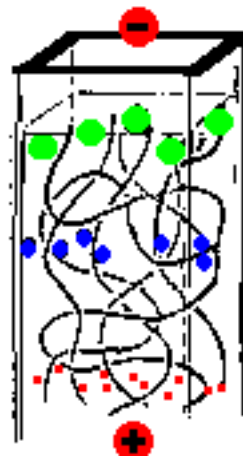


Analyse des protéines par Gel d'acrylamide

- PAGE (Polyacrylamide gel electrophoresis) : SDS-PAGE



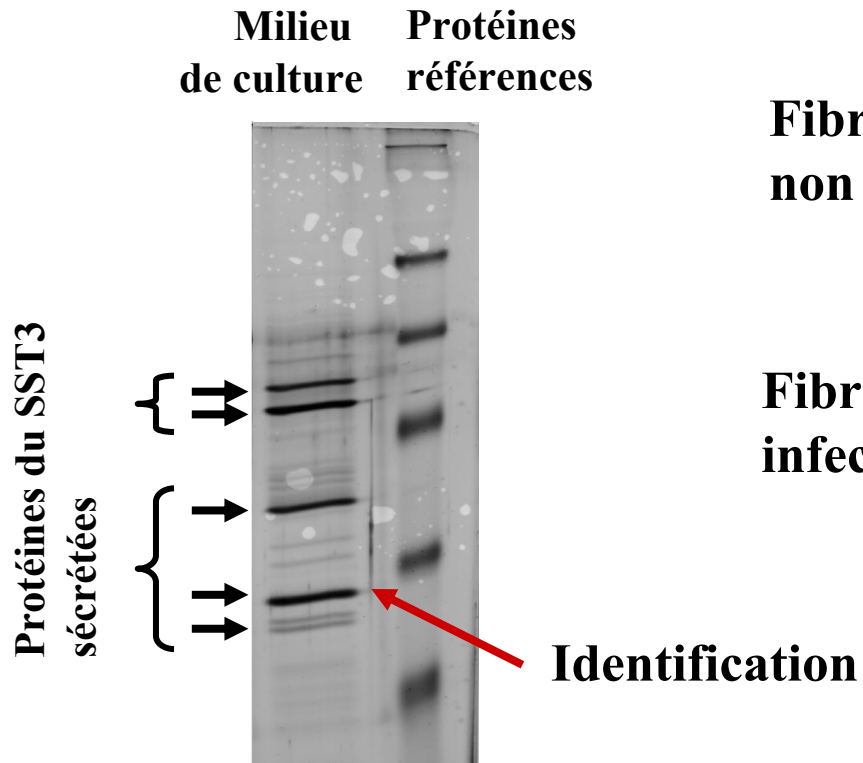
Séparation en fonction de la taille



Electrophorèse

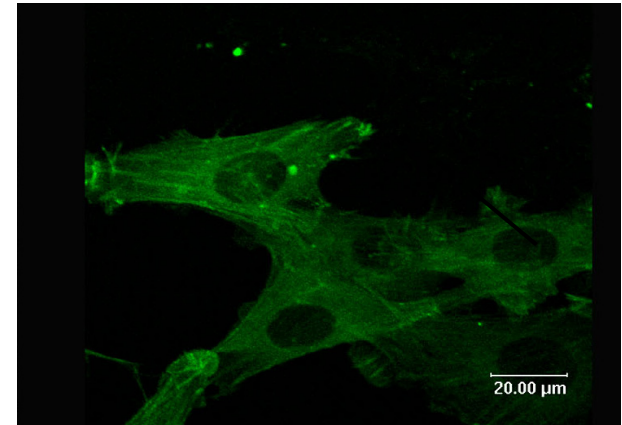
Nos outils

Induction *in vitro* du SST3 Sécrétion de protéines

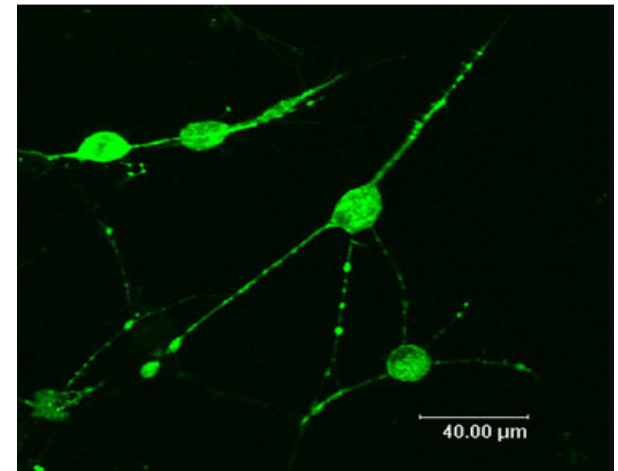


Fibroblastes
non infectés

Effet du SST3 sur cellules



Fibroblastes
infectés (3h)



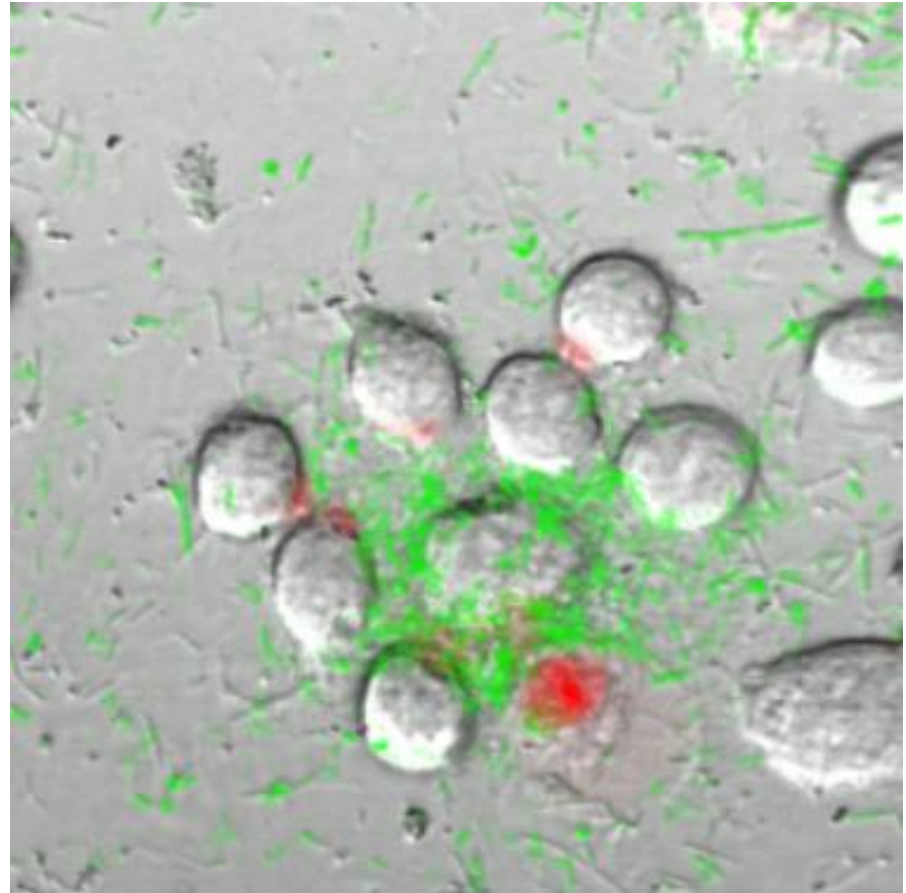
Nos outils

Cellules de l'immunité attaquée

par un essaim de

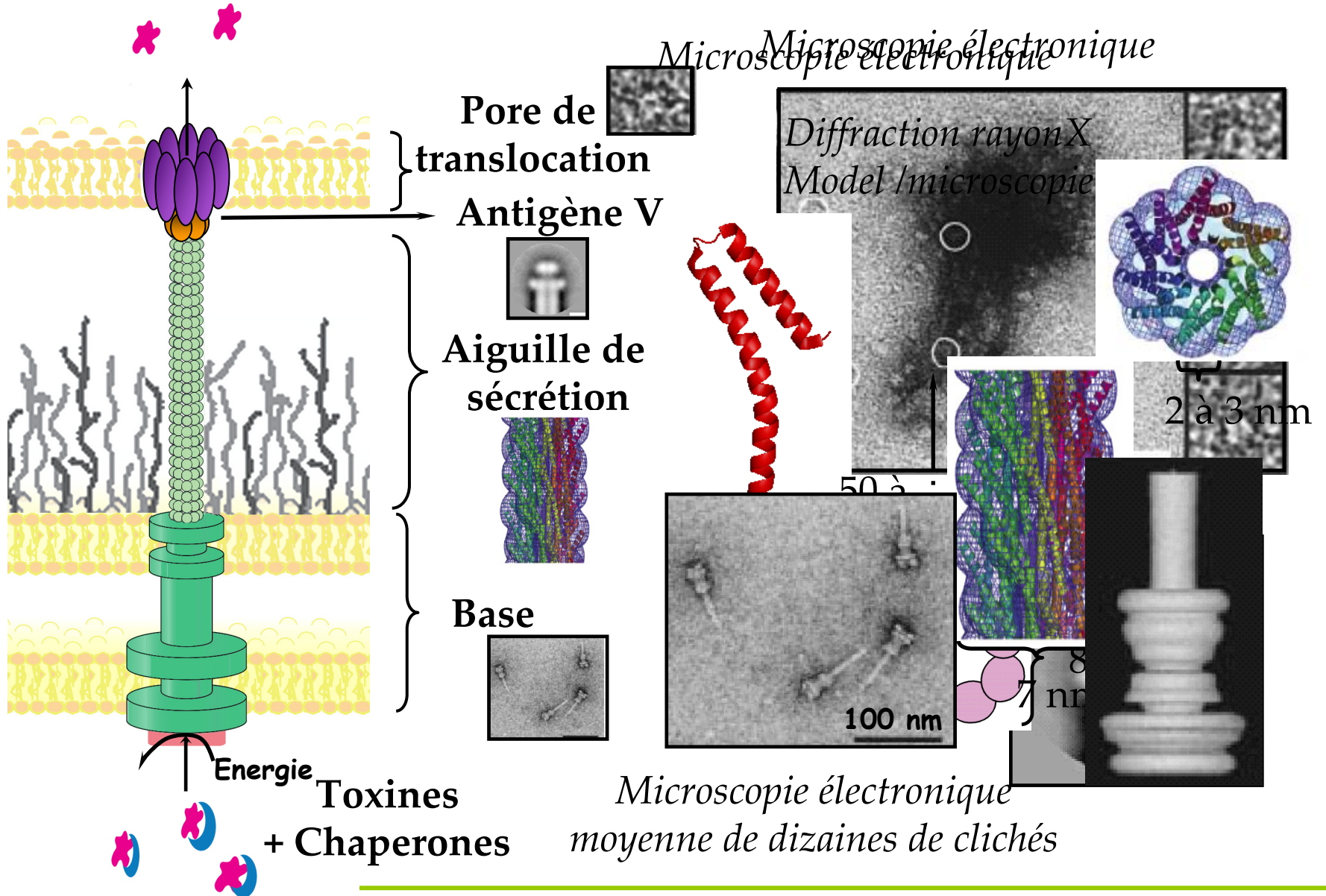
*P. aeruginosa (vert grâce à
protéine fluorescente, GFP)*

=> Mort de la cellule (lyse)

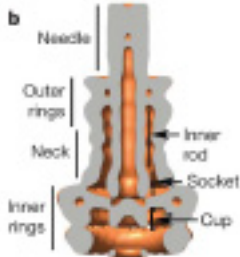
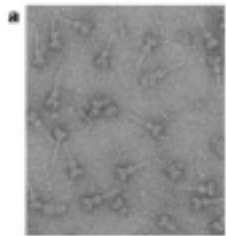
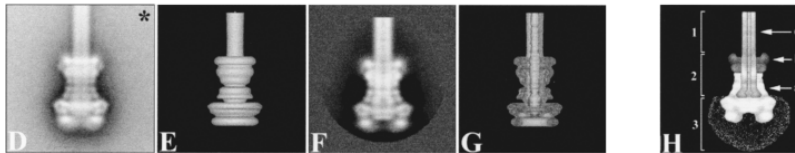
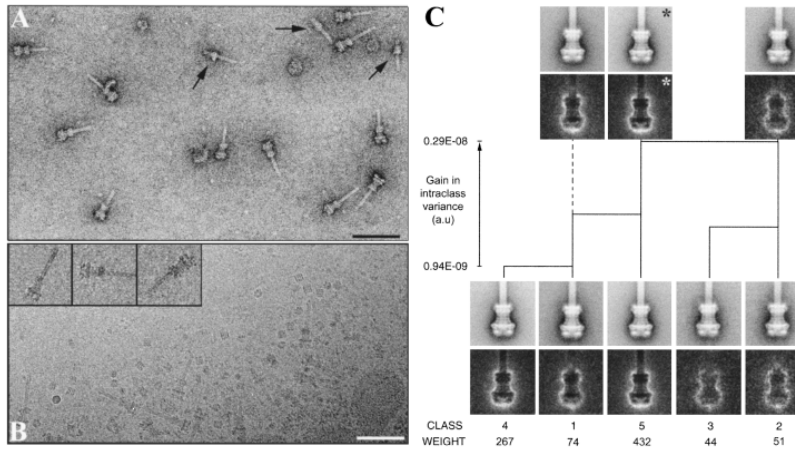


Structure macro-moléculaire du SST3

Cellule hôte
Extracellule
Bactérie

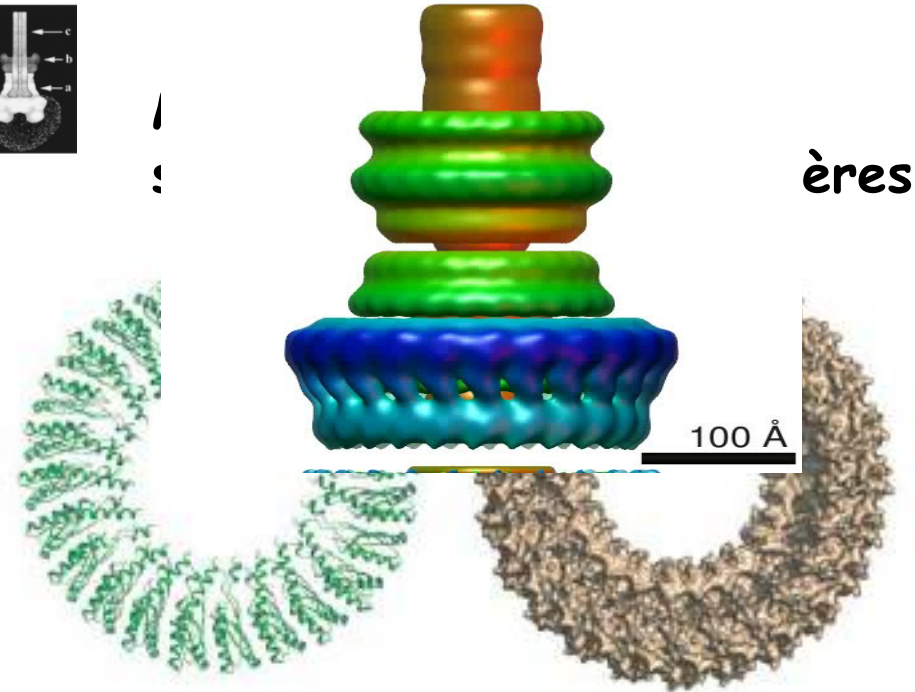


Structure de la base

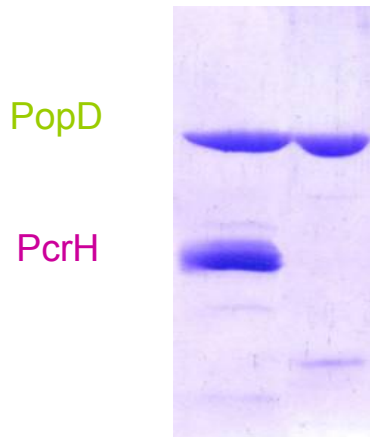
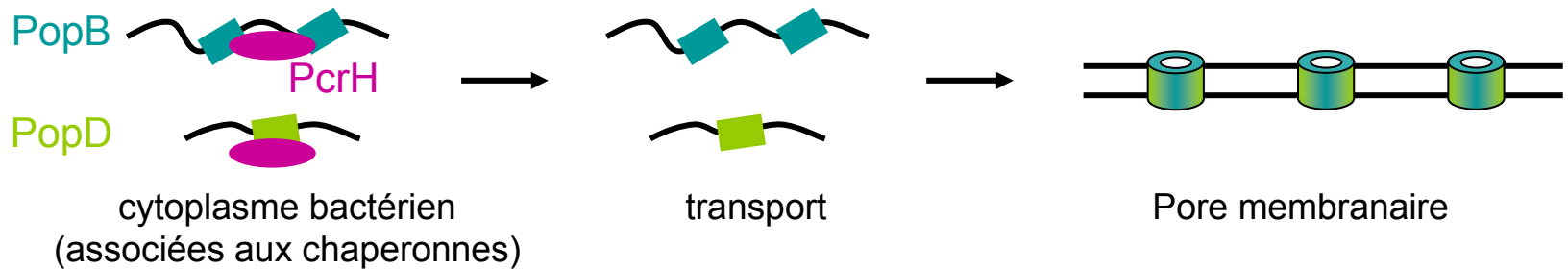


Microscopie électronique :
Oligomères (assemblages)

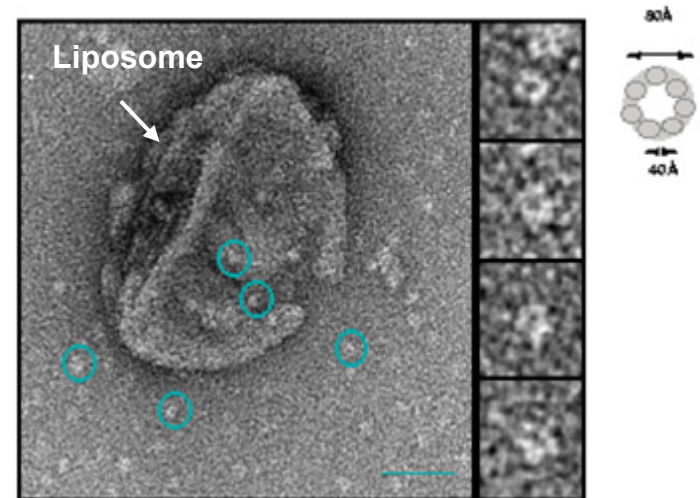
Rayon X : structure
atomique des monomères



Les translocateurs : PopB et PopD



Aspects structuraux ?
Fonctionnalité ?



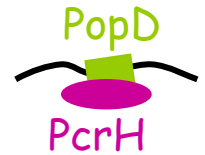
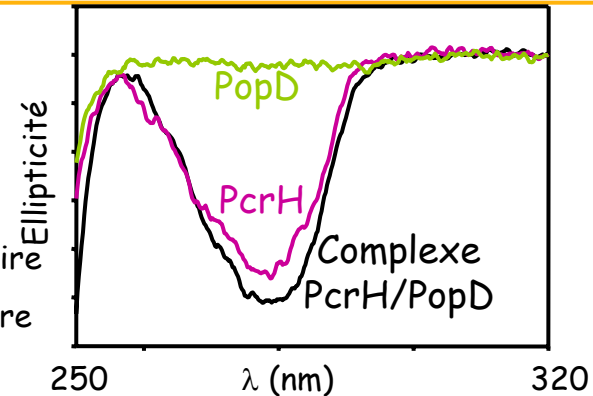
Biophysique : spectroscopie

Schoehn et al, EMBO J 2004

Assemblage du translocon Pop

Molten globule

- Dichroïsme circulaire
 - UV lointains : structure 2^aire
 - UV proches : structure 3^aire



Dichroïsme circulaire :

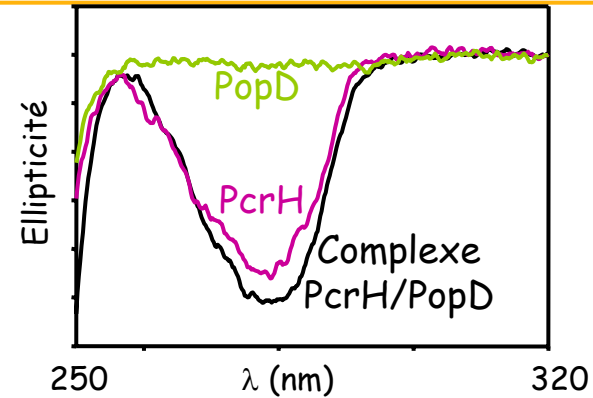
- Absorption polarisée de la lumière
- Propriétés optiques des acides aminés
- Dépend de leur environnement

Collaboration : Vincent Forge (CEA-Grenoble)

Assemblage du translocon Pop

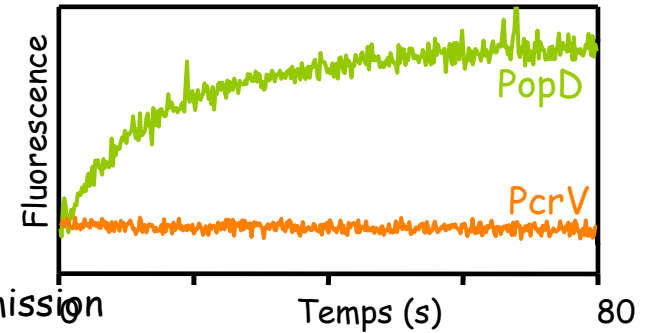
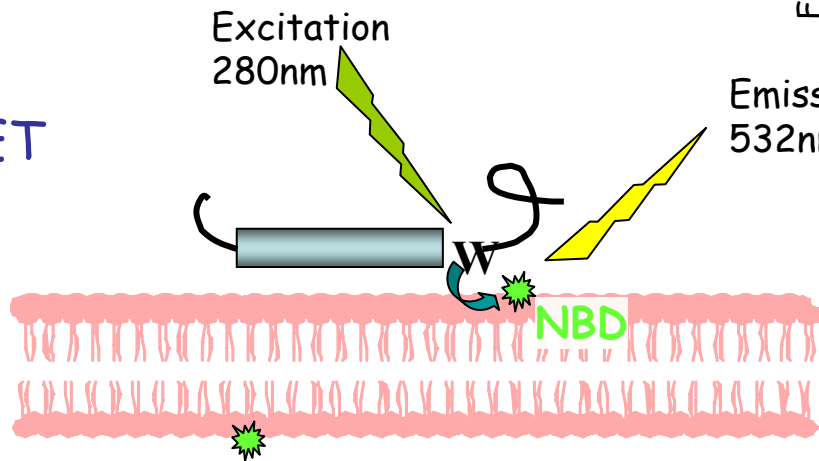
Molten globule

- Energétiquement favorable



Liaison à la membrane

FRET

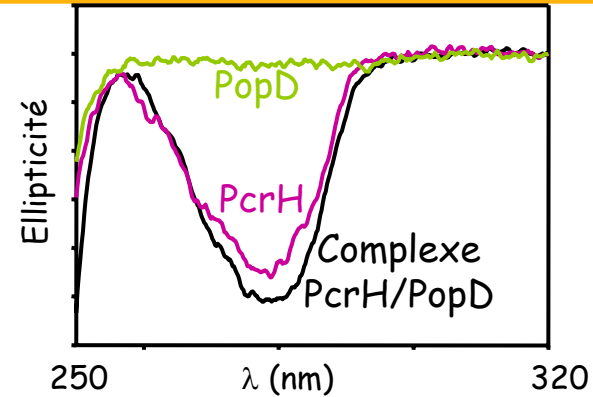


Collaboration : Vincent Forge (CEA-Grenoble)

Assemblage du translocon Pop

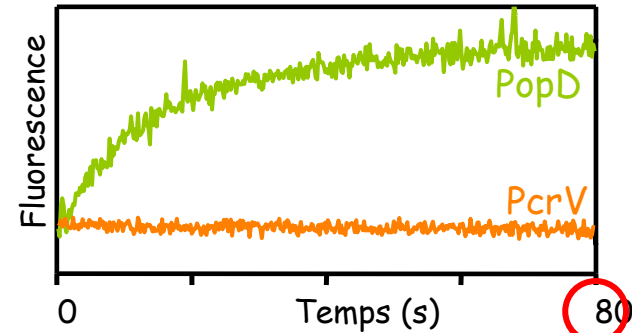
Molten globule

- Energétiquement favorable



Liaison à la membrane

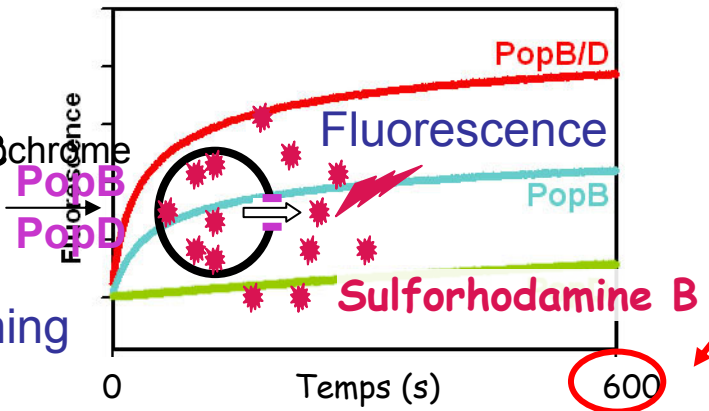
étapes distinctes



Formation de pores : synergie

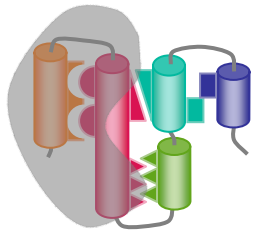


Self-quenching

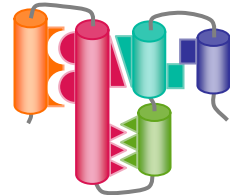


Assemblage du translocon Pop

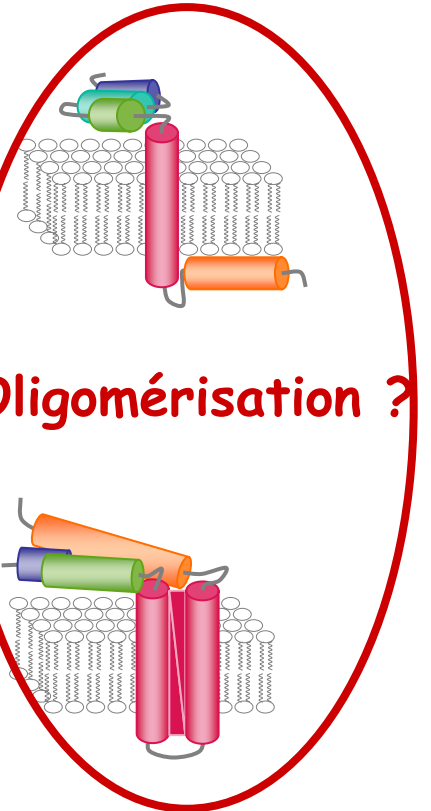
Translocateur du Type III



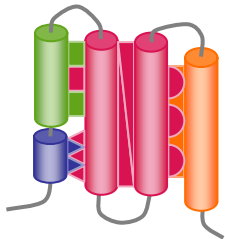
complex
dissociation



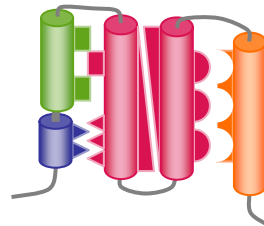
insertion



Toxine (toxine diphtérique)

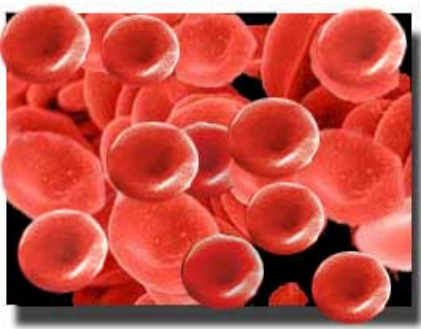


déplie
pH...



insertion

Insertion du translocon dans les membranes



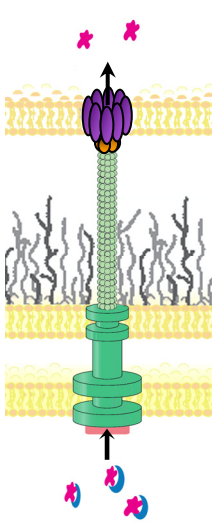
Red Blood Cells

**Infection de globules rouges - Hémolyse -
Identification des protéines dans les membranes**

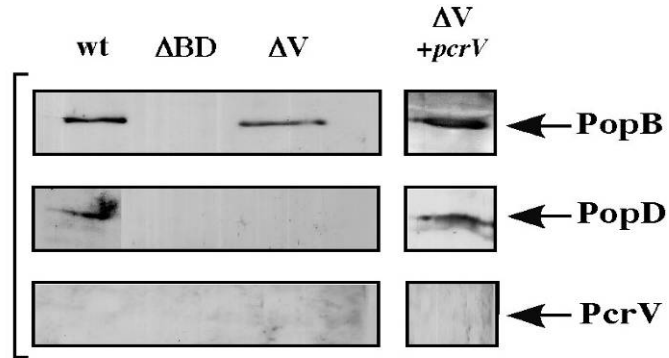
Utilisation de bactéries mutantes possédant ou non
les protéines du translocon PopB et PopD et
l'adaptateur PcrV



L'antigène V et pore de translocation



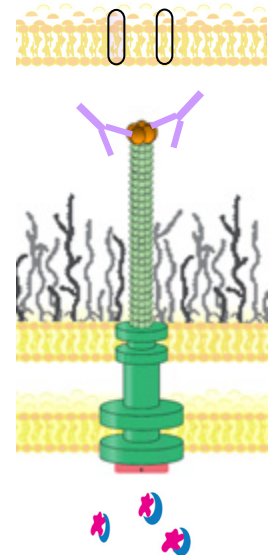
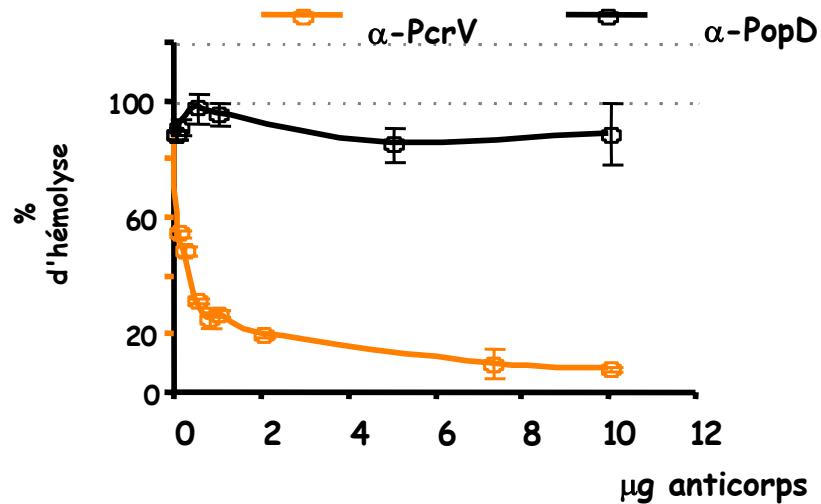
Protéine
dans les
membranes



Hémolyse

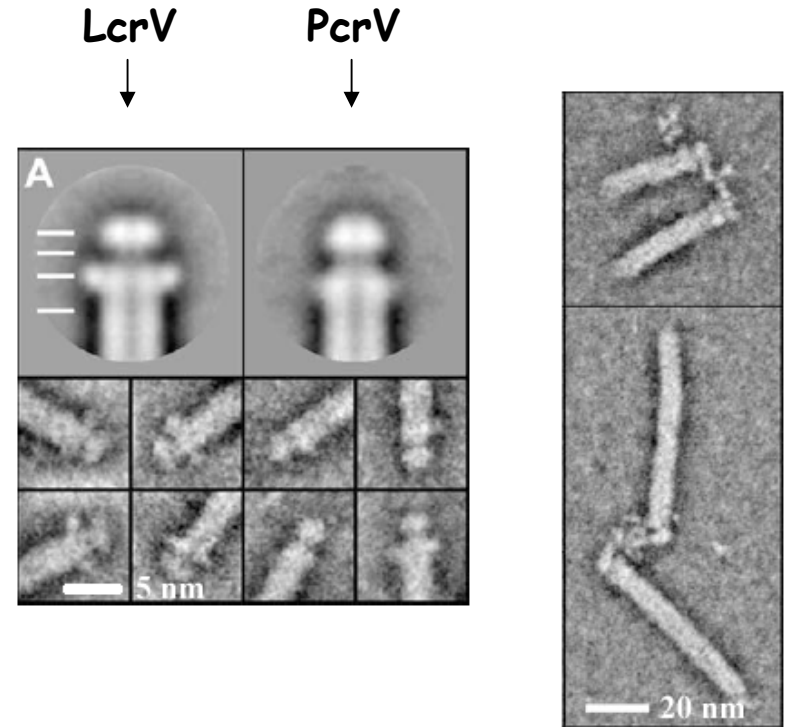
+ - - +

L'antigène V est nécessaire à l'insertion du pore



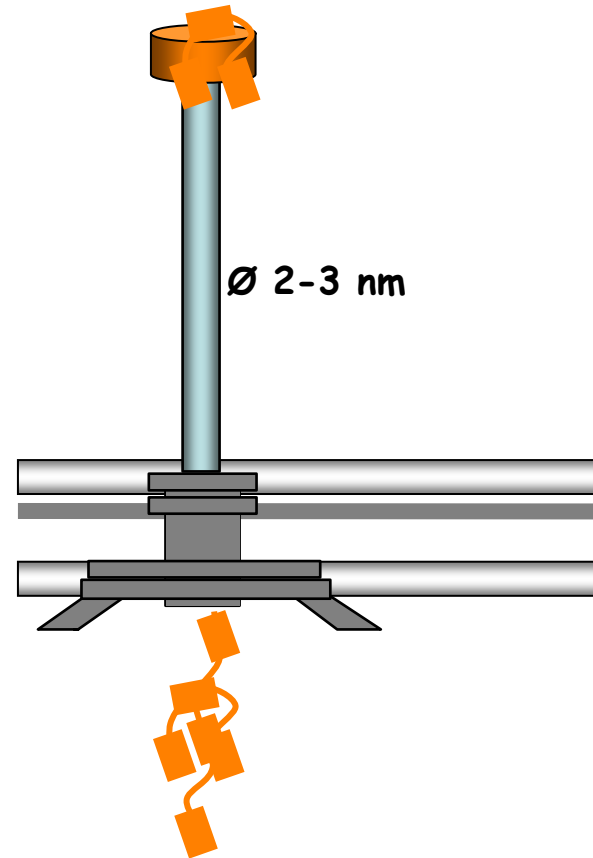
Localisation de PcrV au sommet

L'antigène V se situe au
sommet de l'aiguille
Oligomérisation ?

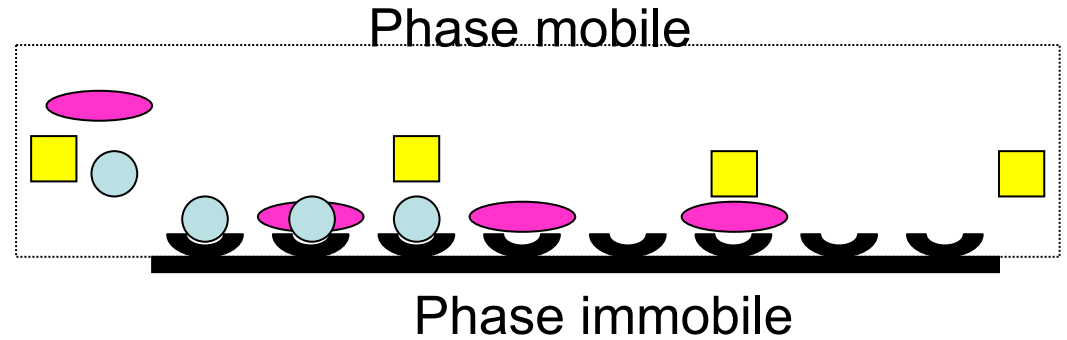
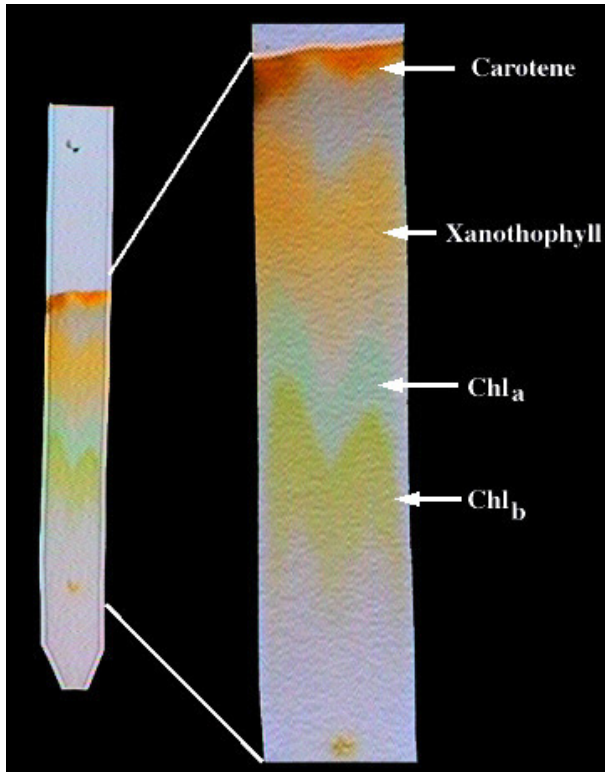


Oligomérisation de l'antigène V

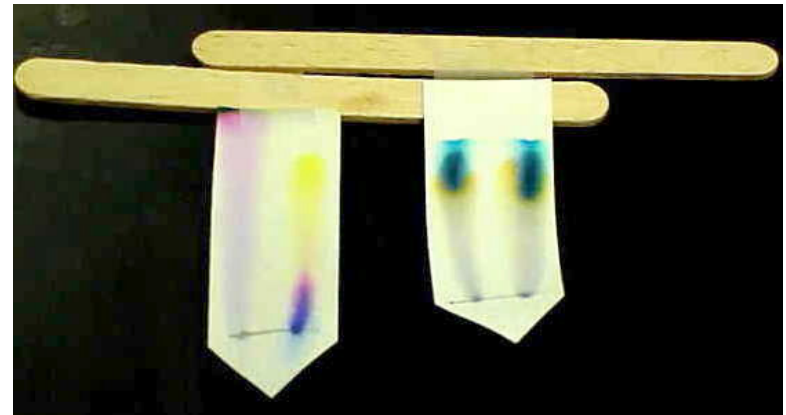
Des changements
de conformation lors
de la sécrétion



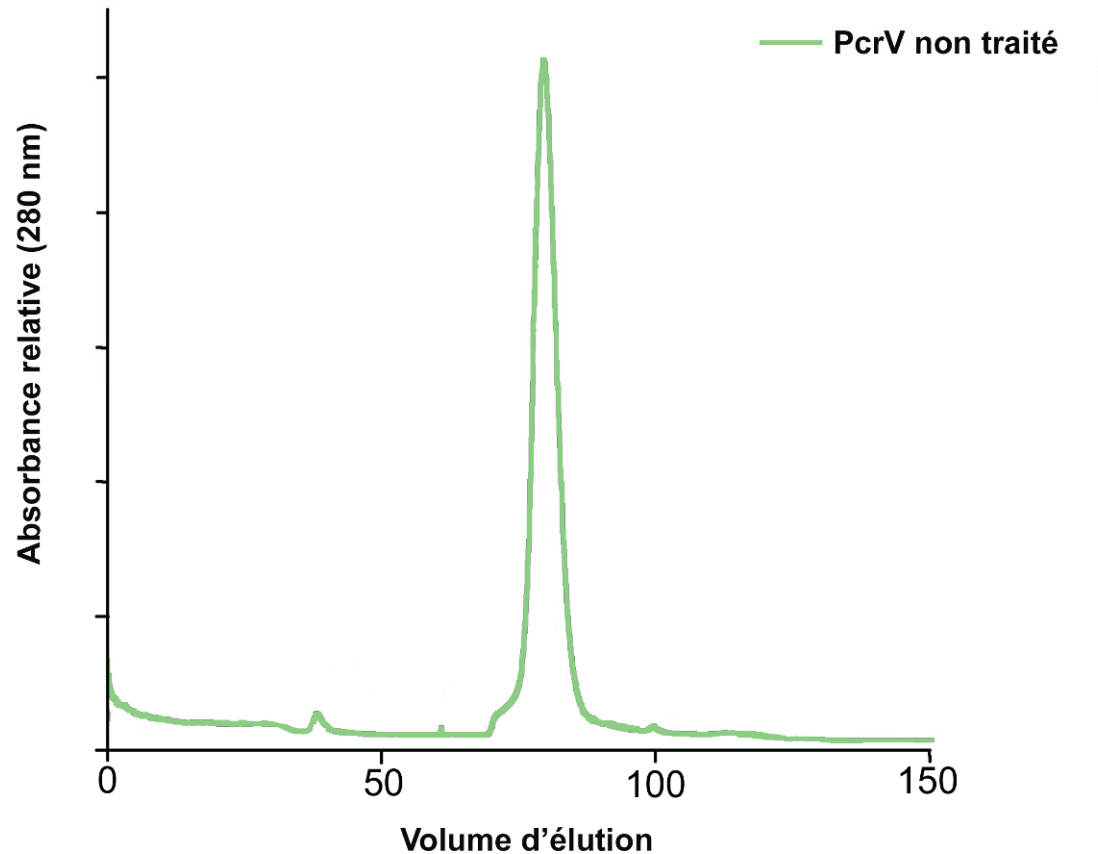
Chromatographie



Séparation selon la taille ou d'autres propriétés physico-chimiques



Oligomérisation de l'antigène V



Chromatographie d'exclusion

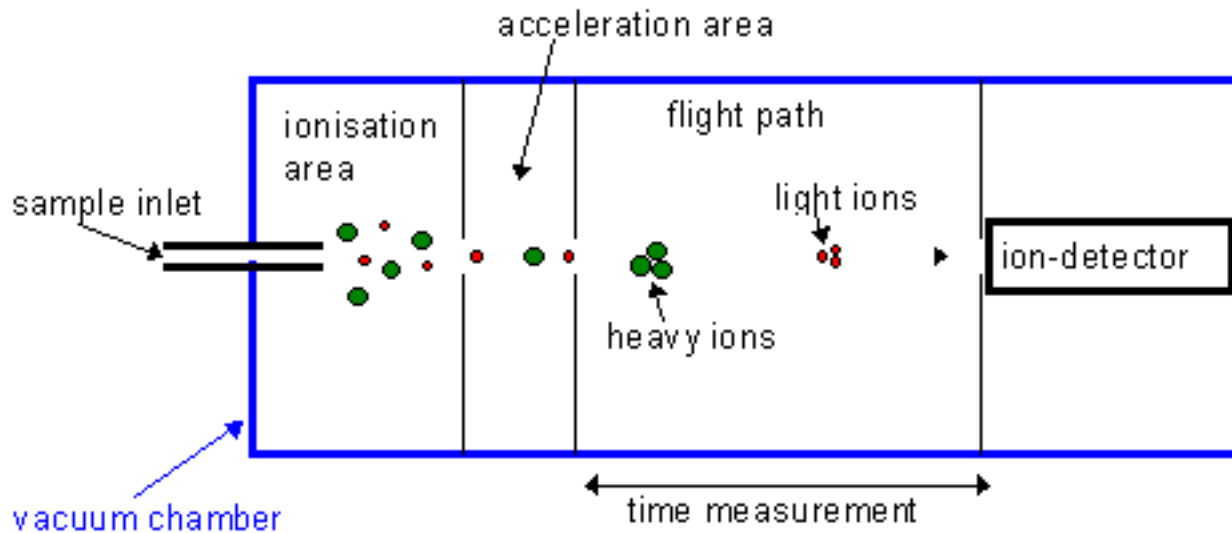
Séparation selon la taille

Grandes protéines

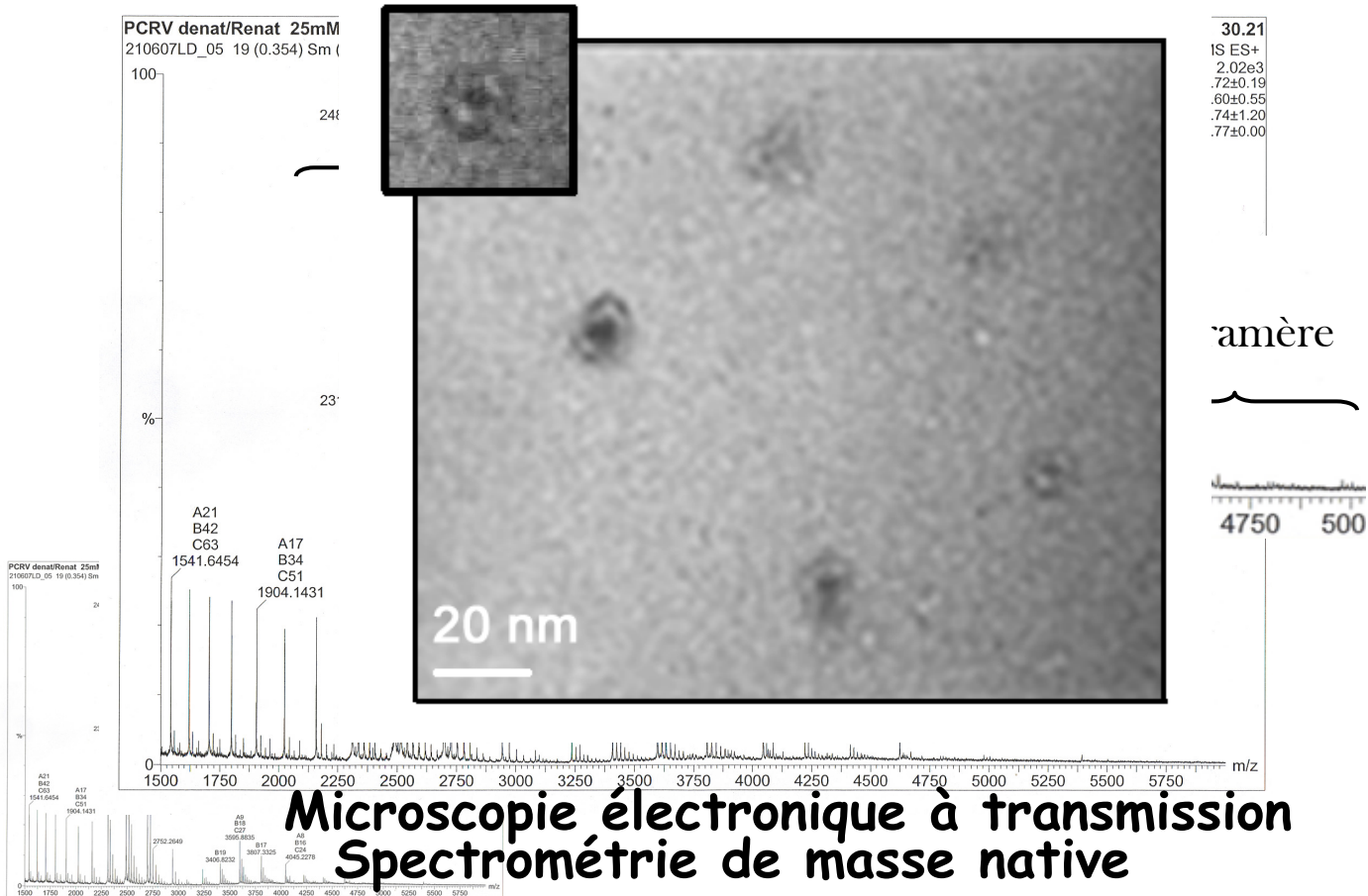
petites protéines

Spéctrométrie de masse

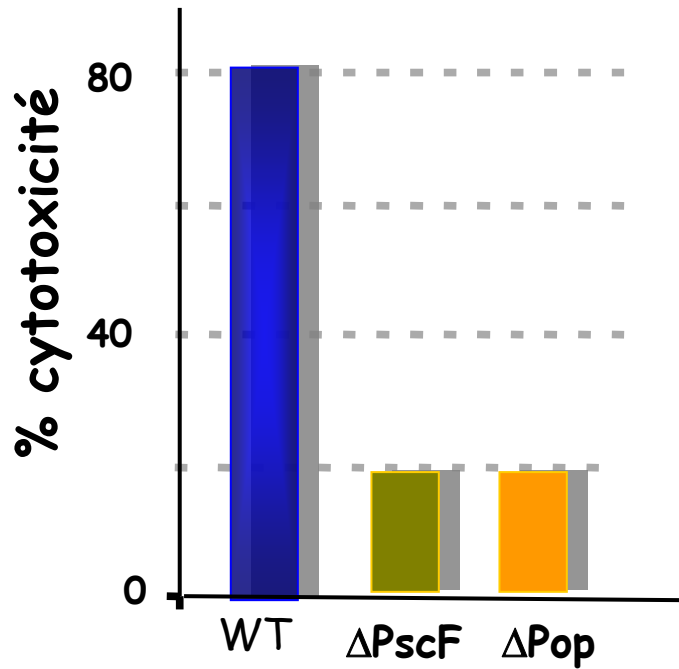
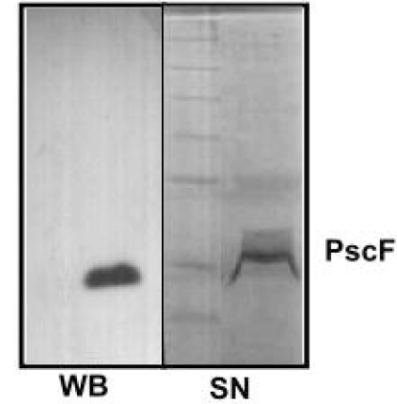
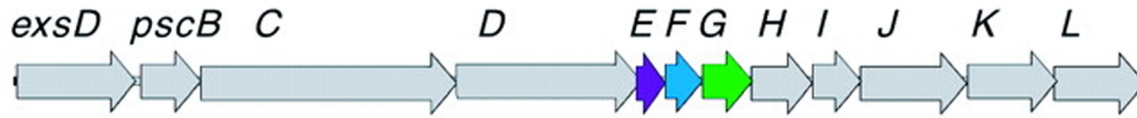
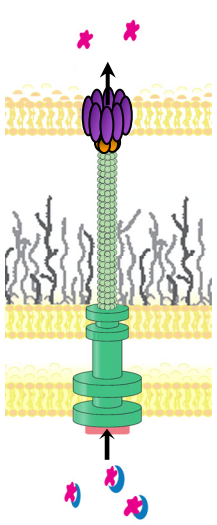
Identification de la masse de composés ionisables



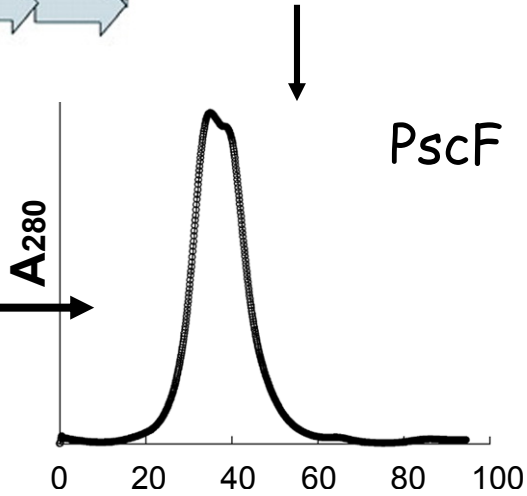
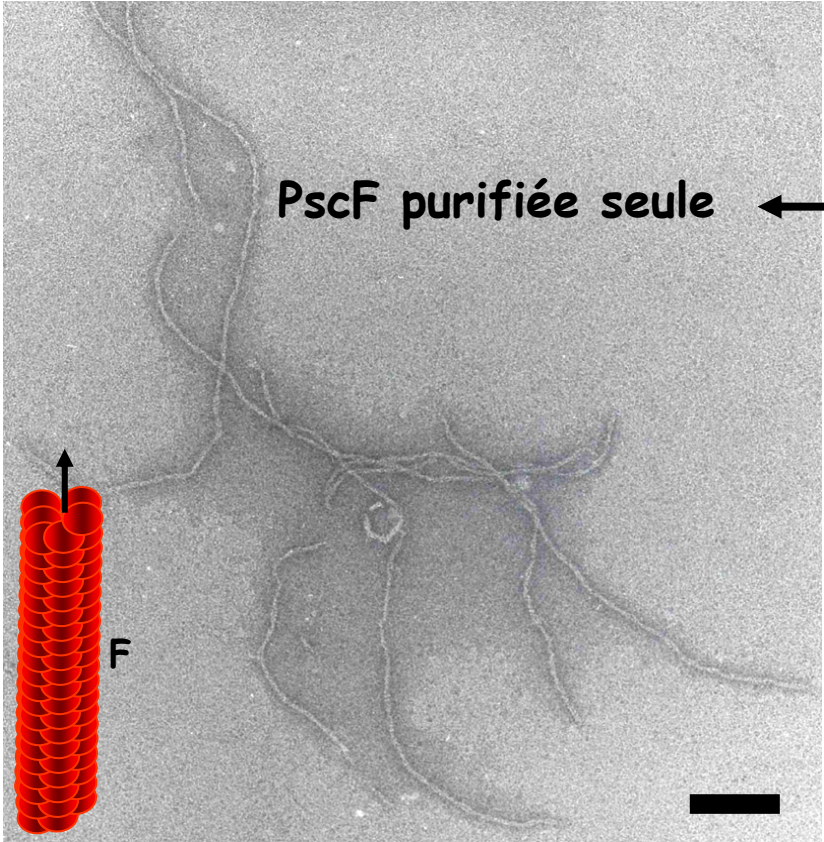
Oligomerisation de l'antigène V



L'aiguille du SST3 est formée par PscF

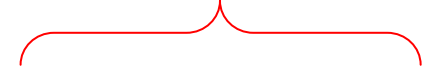


PscF est complexée à deux régulateurs de polymérisation

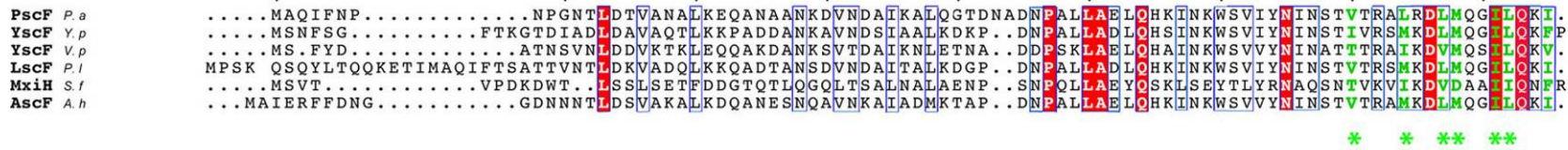


L'heterotrimère est conservé dans plusieurs pathogenes

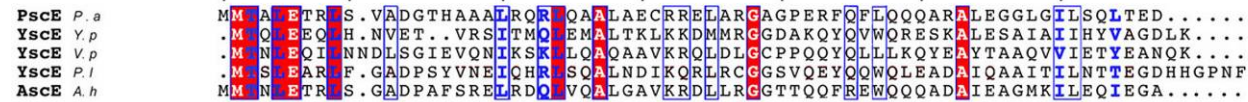
Sans cette partie =
non polymérisation et non cytotoxique



PscF



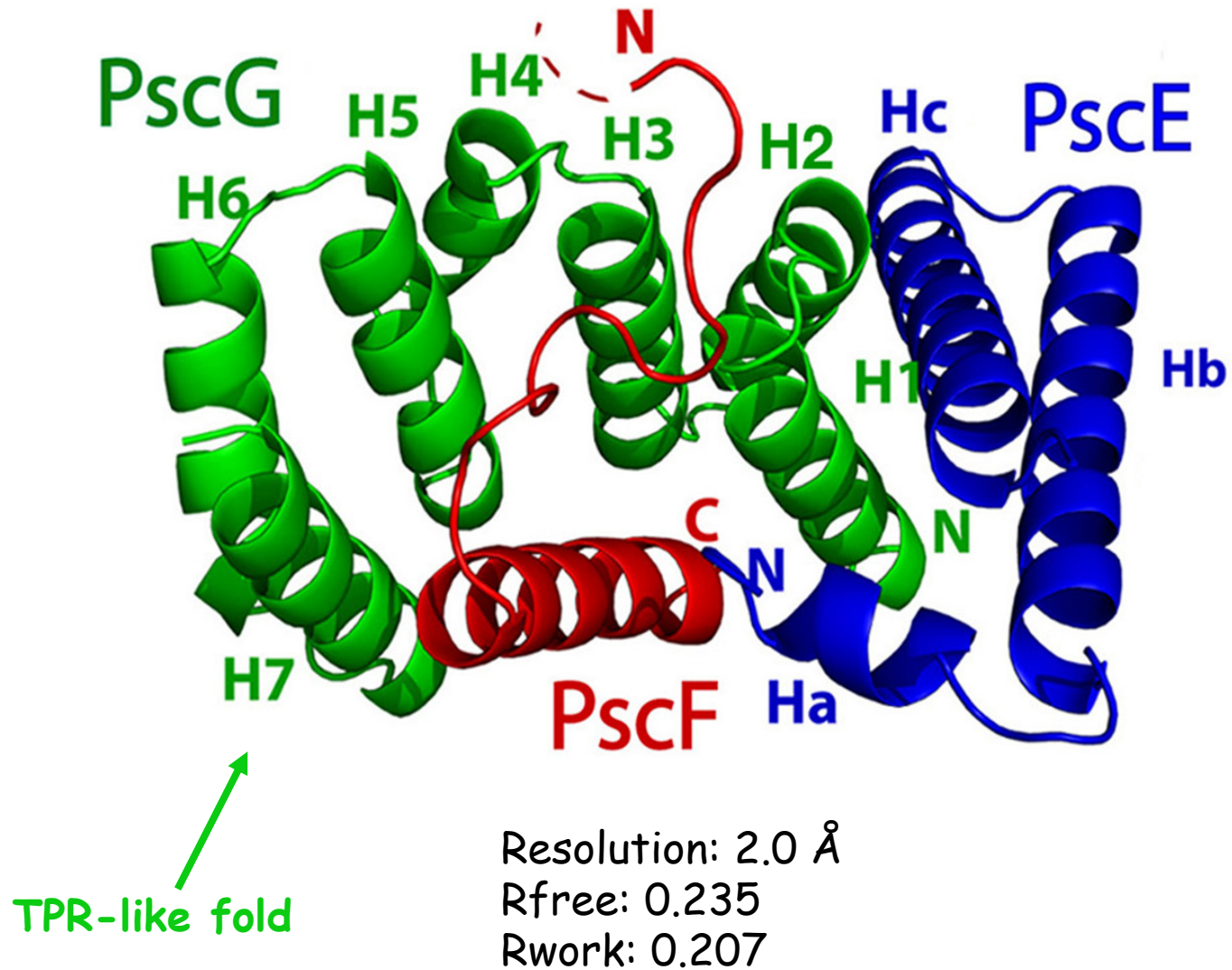
PscE



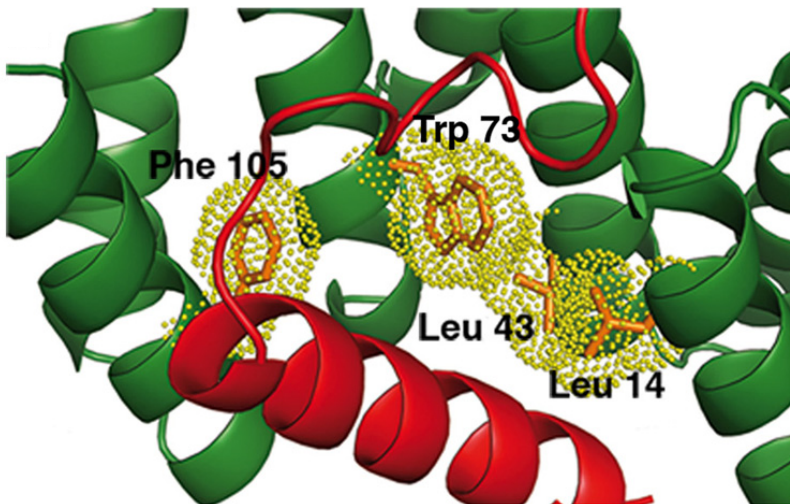
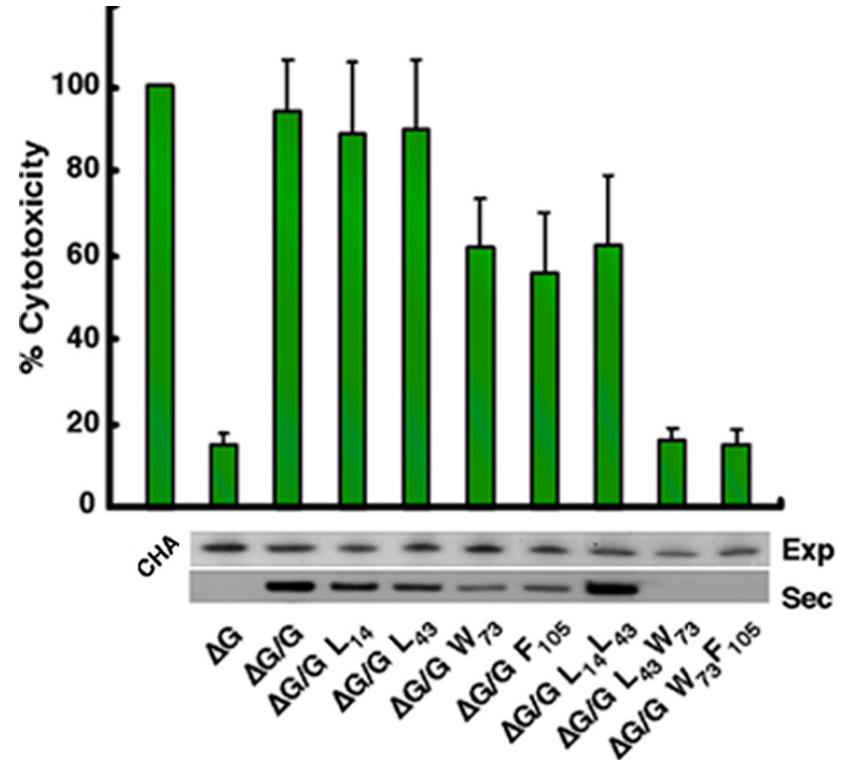
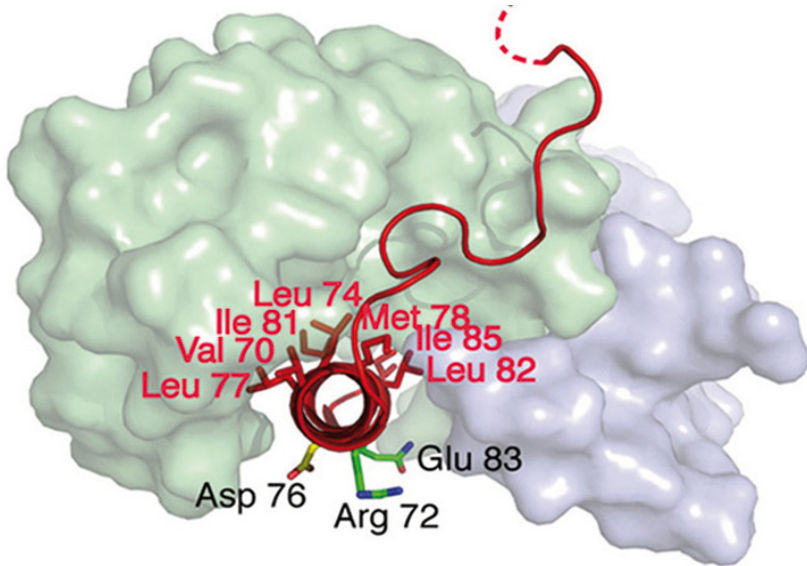
PscG



Structure du complexe ternaire



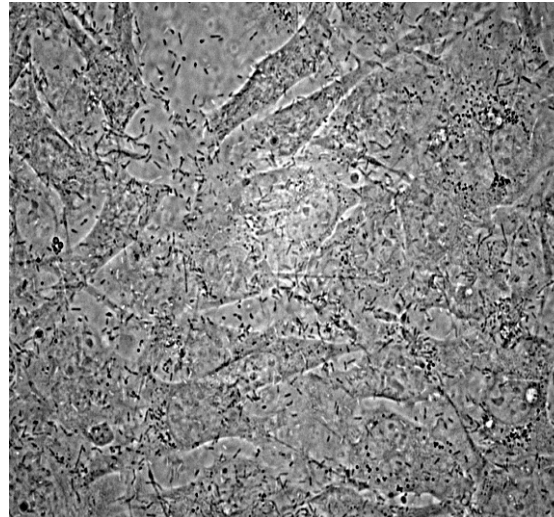
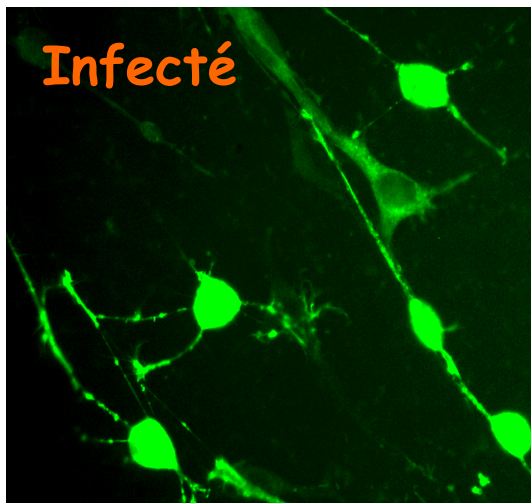
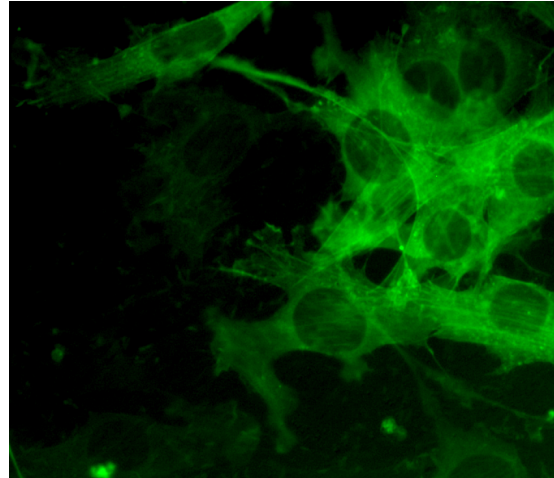
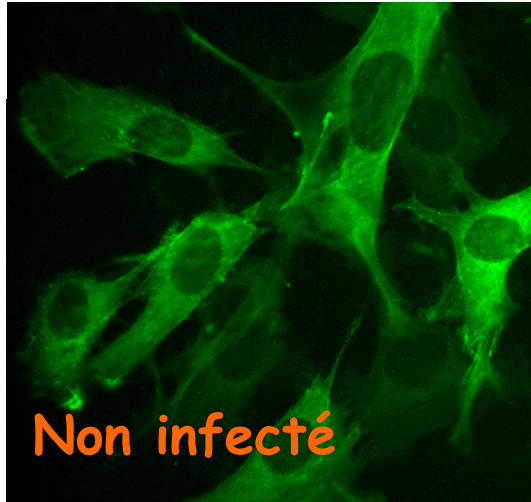
L'interface entre PscF et PscG est essentielle pour la cytotoxicité



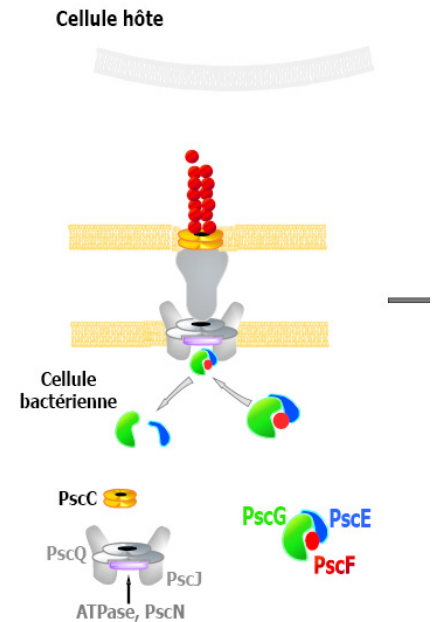
H1 >> L82, I85
 H3 >> I81, I85
 H5 >> L74, L77, M78, I81
 H7 >> V70, L77

Un mutant de PscG est incapable d'injecter les toxines

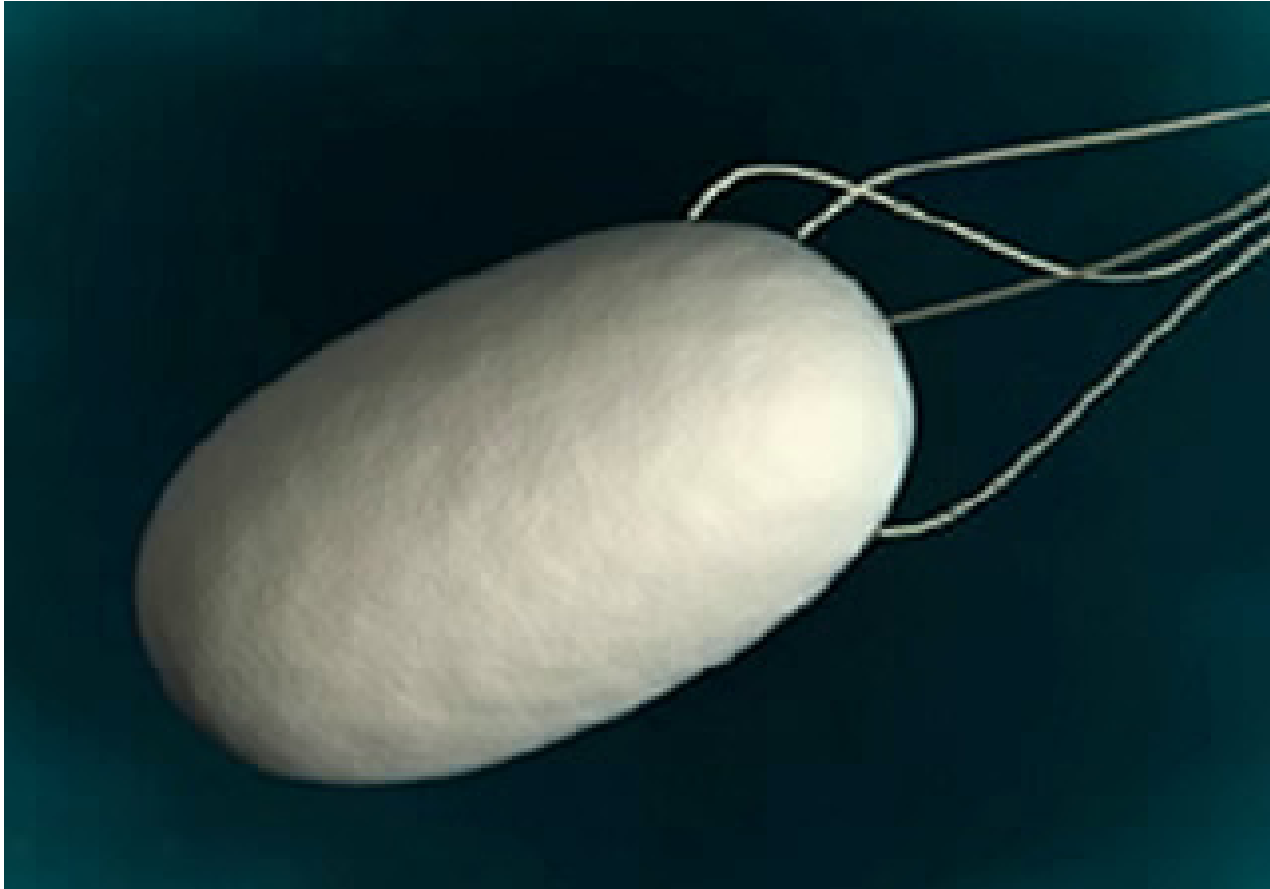
Fibroblasts GFP-actine



Mutant
 $PscG^{*}W_{73}F_{105}$



Assemblage du flagelle par le T3SS





BBSI, iRTSV, CEA/Grenoble

Dr François Boulay

Ina Attrée, CNRS
Sylvie Elsen, CNRS
Michel Ragno, CNRS
Tammy Chang, post-doc
Sophie Plé, post-doc
Julie Thibault, thèse
Caroline Gébus, thèse
Elodie et Emilie, stages BTS

IBS : Andréa Dessen

LEDyP

Jérôme Garin
Mathilde Louwagie

LCBM

Vincent Forge

+ **Groupe réponse cellulaire**



Structure de PcrV

Derewenda *et al.*, Structure, 2004



- * domaines Nter et Cter globulaires

- * coiled-coil intramoléculaire

($\alpha 7$ et $\alpha 12$)

Leu Zipper ($L_{X3}L_{X2}L_{X3}L$)

- * interaction avec LcrG : Coiled-coil intermoléculaire ($\alpha 7$ V et Nter de G)

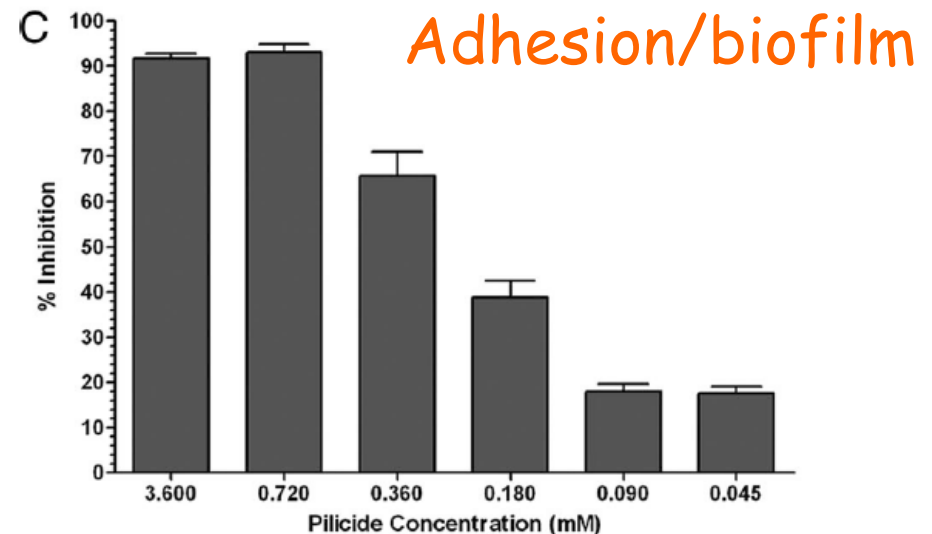
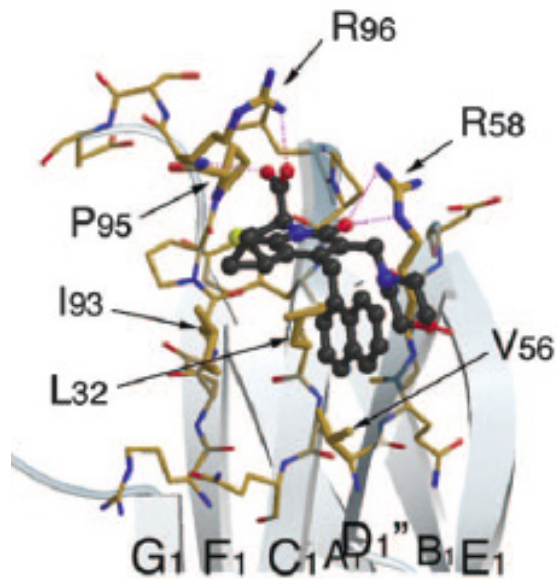
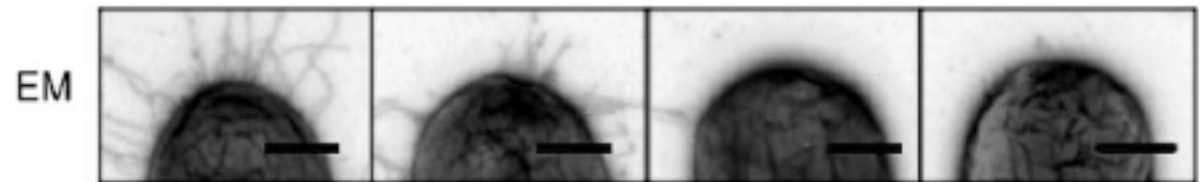
Ouverture LcrV possible

Hypothèse : formation dimères têtes bêtes par échange de domaines

Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria

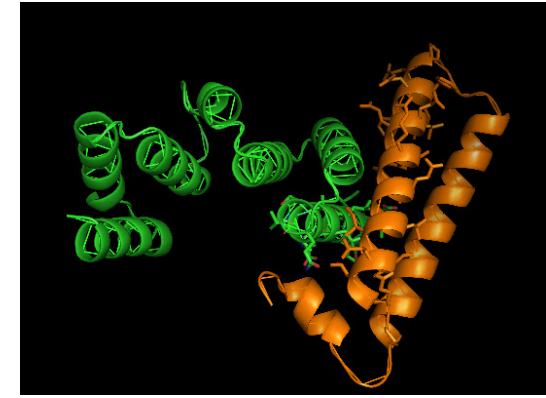
Jerome S. Pinkner^{*}, Han Remaut[†], Floris Buelens[†], Eric Miller^{*}, Veronica Åberg[‡], Nils Pemberton[‡], Mattias Hedenström[‡], Andreas Larsson[‡], Patrick Seed^{*}, Gabriel Waksman^{†§}, Scott J. Hultgren^{*§}, and Fredrik Almqvist^{*§}

Pilicides target chaperon/pilus complexes

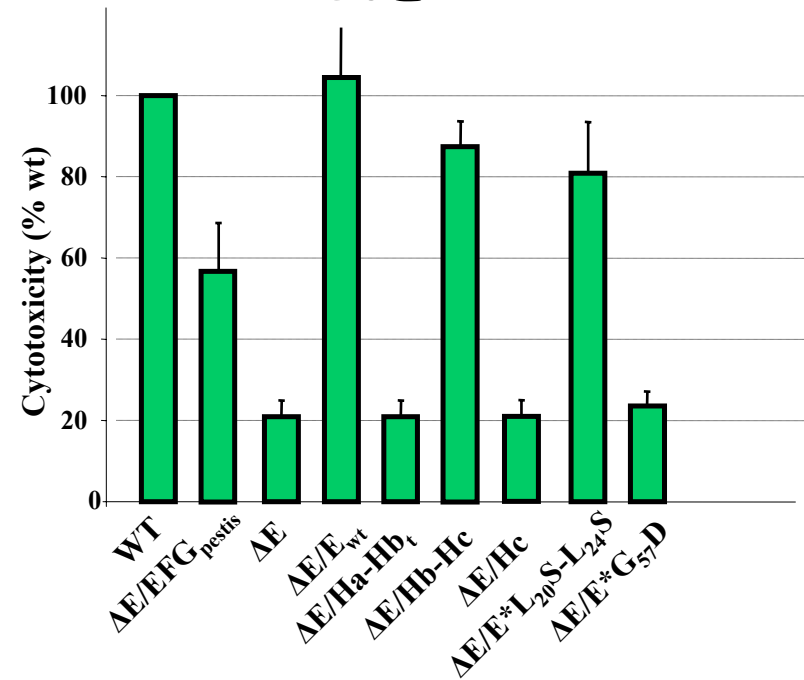


Complexe PscE-PscF-PscG comme cible thérapeutique

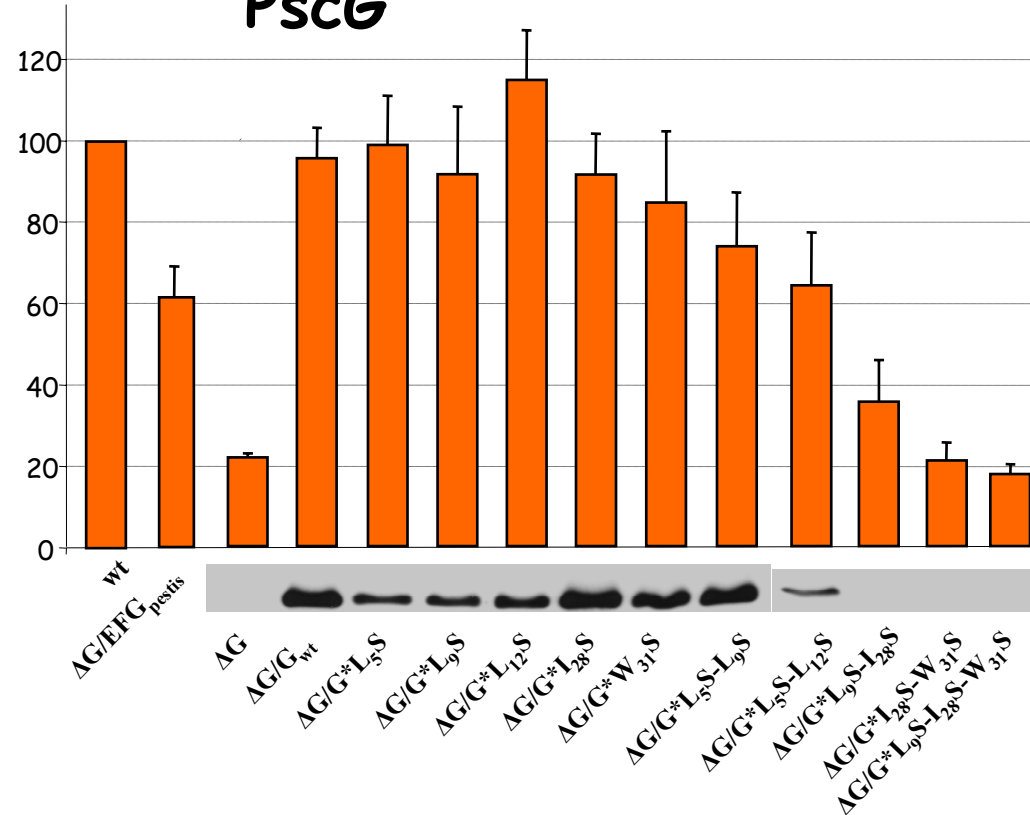
Infections / test de cytotoxicité



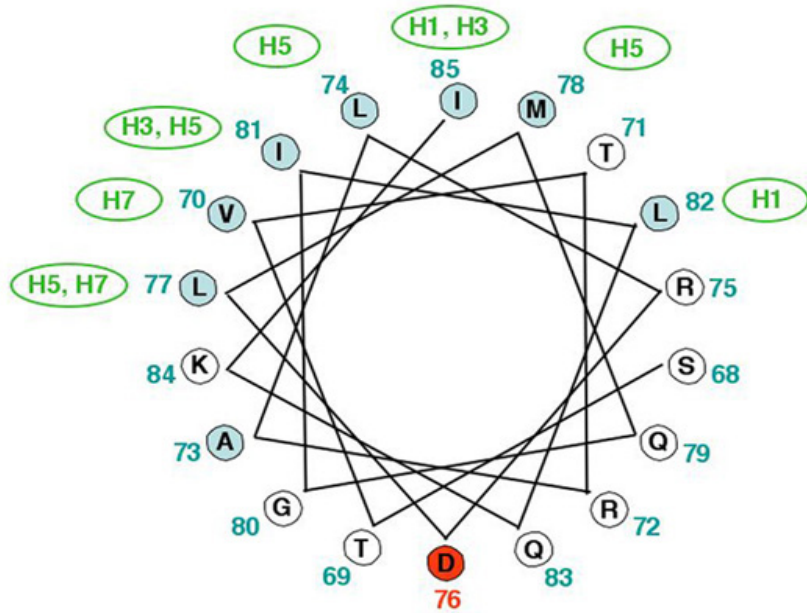
PscE



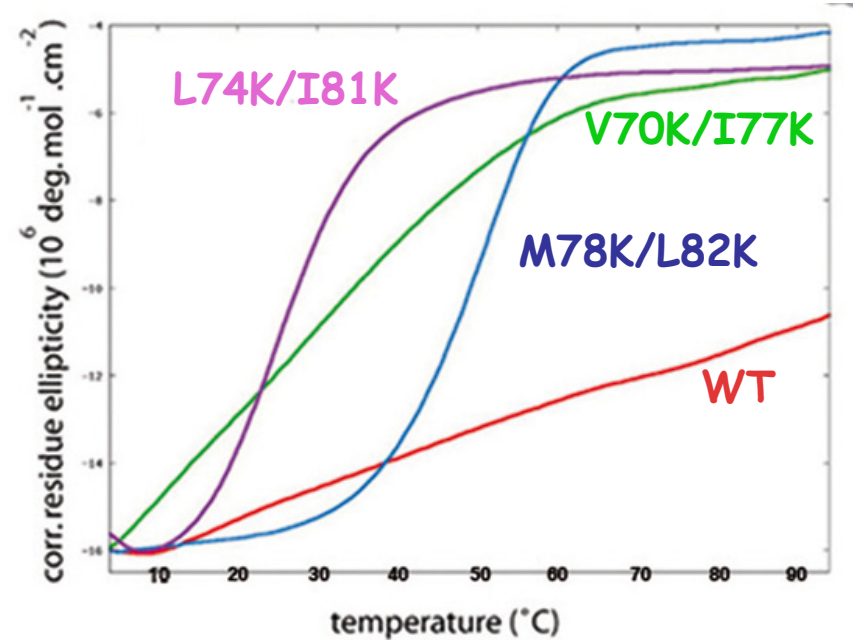
PscG



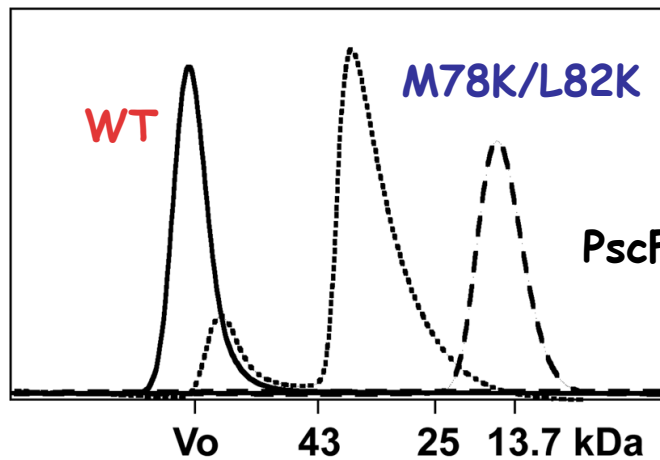
La face hydrophobe de PscF stabilise l'aiguille



Temperature scanning circular dichroism



Absorbance 280nm



PscF1-67: no polymers