# The Haemolysin System from *E. coli* – a Paradigm for Type I Secretion Systems?


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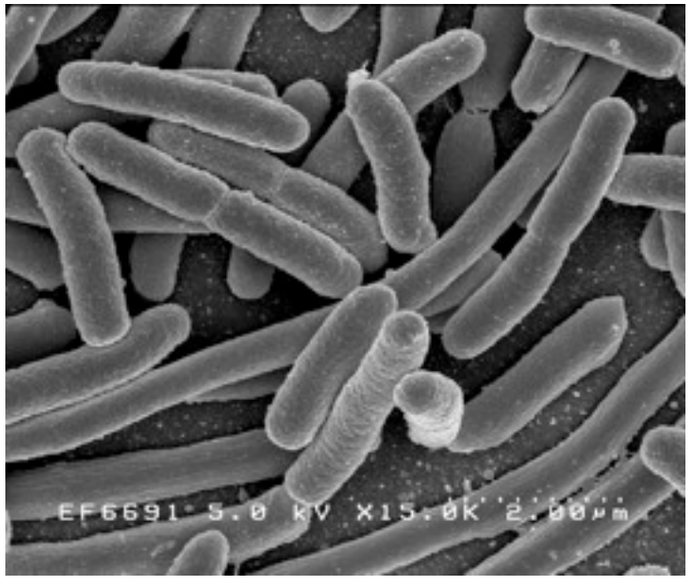
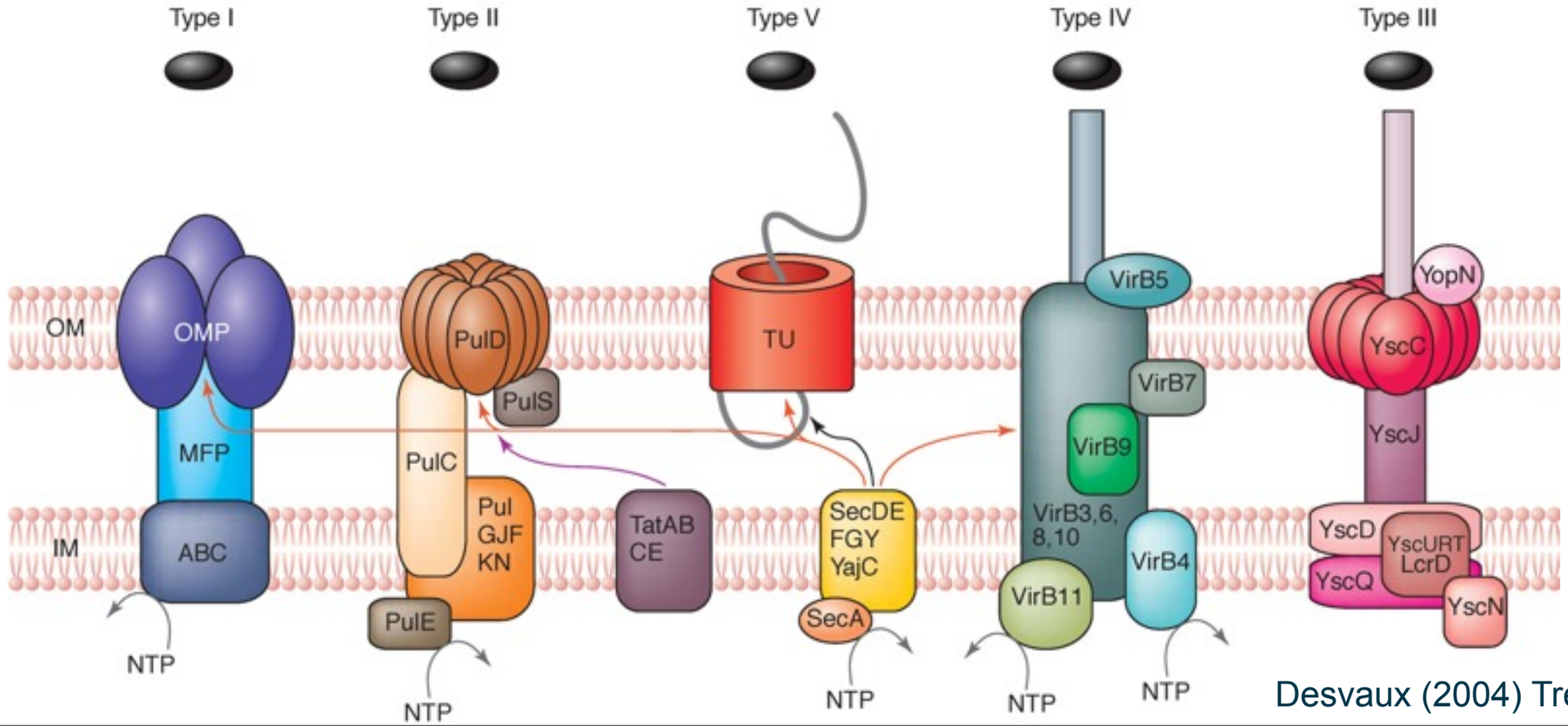
# Protein Secretion in Gram-negative Bacteria

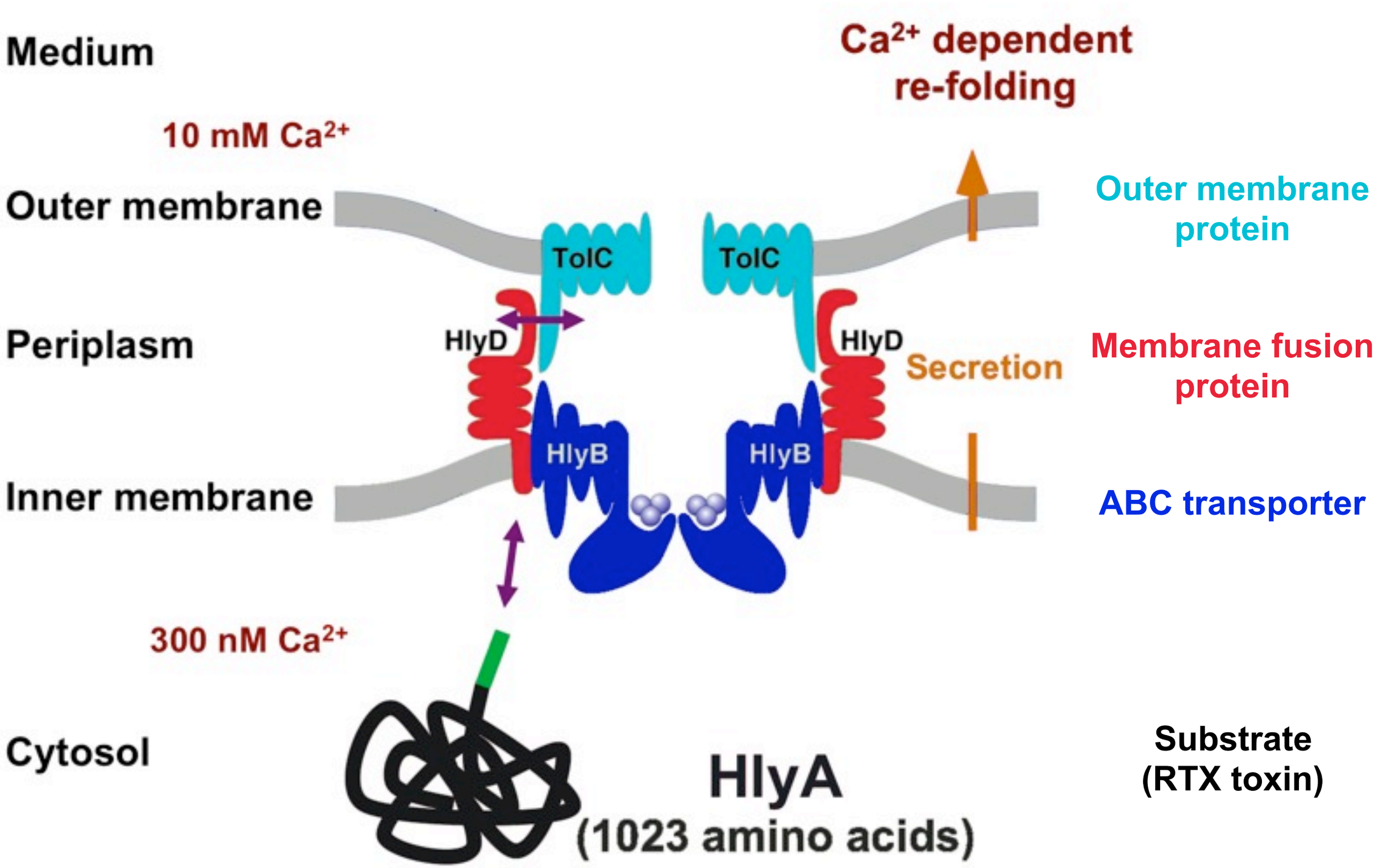



Cytosol
Inner membrane
Periplasmic space
Outer membrane
Extracellular space (**secretion**)

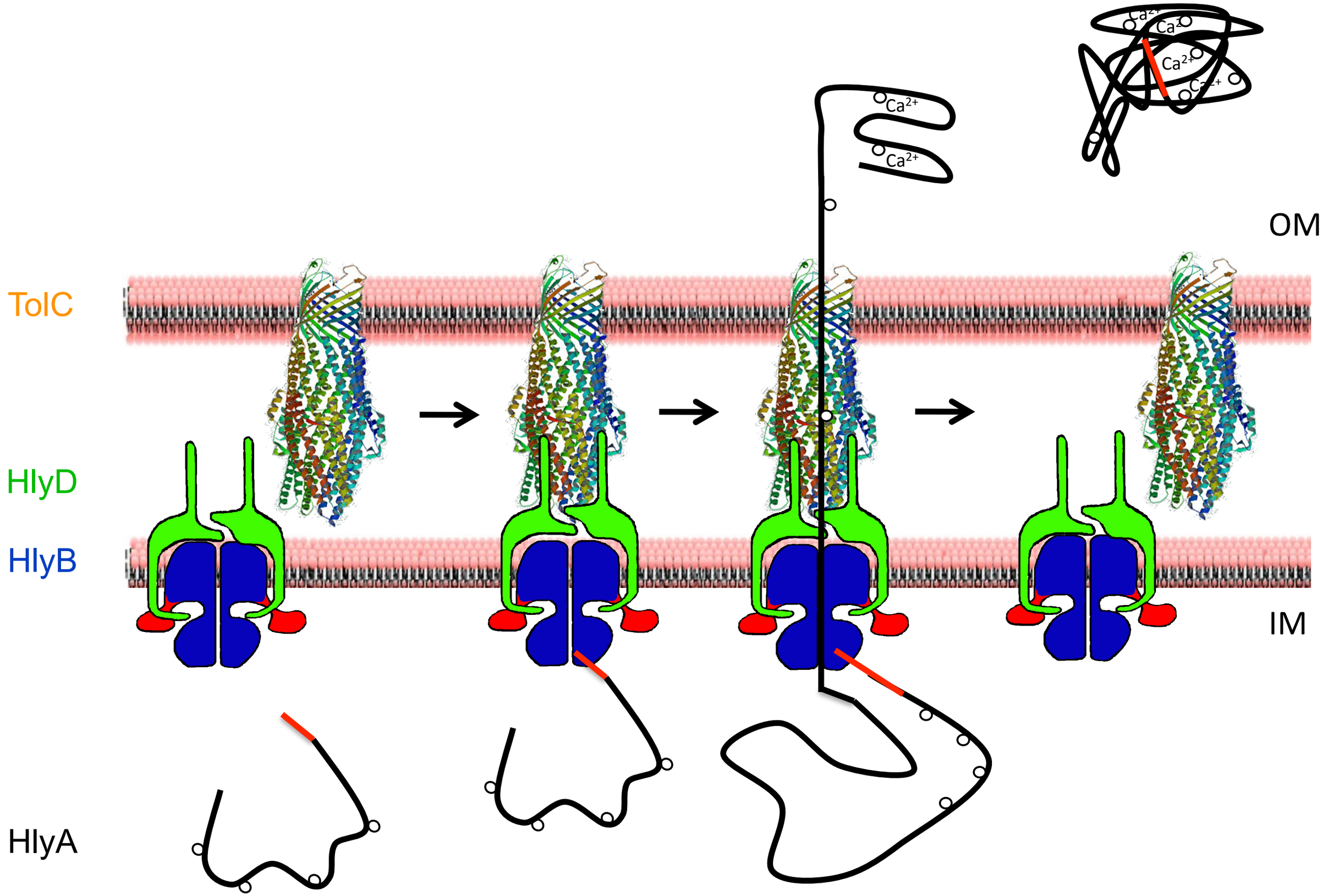



Desvaux (2004) Trends Microbiol

# The Haemolysin A Machinery



# What we think happens ...



# Bacteriocine ABC Transporters

```
NukT     LQNSDQDCLLACYSMILSYFGKNVSINSLYKREM-IPPDGLSISYLKELNIKYELNMKVY
CvaB     HQTETAECGLACLAMICGHFGKNIDLIYLRRKFN-LSARGATL--AGINGIAEQLGMATR
ClyB     AQGEHSECALACITMLLNYYGNQSTLVELREKYG-VPKGGLTIKN--IRTVFDEYGFDVS
LagD     YQQDEKDCGVACIAMILKHYGTEITIQRLRELSG-TDLDGTSA--FGIKKTFEKLGFDAP
LtnT     FQVAQTECGLCCVRTILDYFGYETTVTKLRILKE-PGRDGSSF--NDIRKLLERFGVDSK
ComA     PQVDQMDCGVASLAMVFGYYGSYYFLAHLRELAK-TTMDGTTA--LGLVKVAEEIGFETR
HlyB     -SCHKIDYGLYALEILAQYHNVSVNPEEIKHRFD-TDGTGLGL--TSWLLAAKSLELKVK
LktB     -SQKNTNLALQALEVLAQYHNISINPEEIKHKFD-IDGHGLNQ--TKWLLAAKSLGLKVR
PaxB     SFKQKNDYGLHALVILAQYHNIAVSPEEIKHKFD-PEGKGIDL--VAWLLAAKSFELKAK
RtxB     -K--TSQPALSALIILAHYHGIAANPADISHHFSGSLNNDLSE--TEWLLAAKKLELKAK
ApxIIIB  PFNEKIDYGLHALVILAQYHNVAVNPEEVKHKFD-LDGKGLDL--VAWLLAAKSLELKMK
AqxB     -FNQKEDYGLYALTILAQYHNIAVNPEELKHKFD-LEGKGLDL--TAWLLAAKSLELKAK

NukT     RIKDKEKTFR--VISKIKKPIIVH---W-DLNHPVIVKN---VKKKHIEIVNPEI--GKV…
CvaB     ALSL---ELD--ELRVLKTPCILH---W-DFSHPVVLVSV---KRNRYVLHDPAR--GIR…
ClyB     TFKS---SFS--NYLDLPTPVISY---W-NNQHPVVIEK---IKKKKVLILDPAS--NKR…
LagD     AFKA---GDETWQEKDIPLPLIAHIISEQKYQHYVVVYK---VKGDEIWIADPAK--GKI…
LtnT     LYKV---KDNR-IFSTLQLPIIIY---W-KNVHPVCVER---ISKKTVIIMDPSV--GRT…
ComA     AIKA---DMTLFDLPDLTFPFVAHVLKEGKLLHYYVVTG---QDKDSIHIADPDPGVKLT…
HlyB     QVKK---TID--RLNFISLPALVW---REDGRHPILTKVS--KEANRYLIFDPEQ-RNPR…
LktB     TANK---TVD--RLPFLHLPALAW---RDDGEHPILLKID--QETDRYLIFDPIQ-KNPI…
PaxB     KVKK---SID--RLPFIHLPALIW---RDDGQHPILTKID--TQTNRYLIFDPEE-RNPK…
RtxB     VVKQ---PIS--RLPMASLPALVW---REDGDHPLLAKIDGTGEATQYLIHDPSE-SRPI…
ApxIIIB  RVKK---SIE--RLPFIHLPALIW---RDDGQHVILMKID--TQTNRYLIFDPEE-RNPK…
AqxB     QVKK---SID--RLEFIALPALVW---RDDGQHPILTKID--SKAQKYLIFDPET-RNPR…
```

Dawson & Locher (2006) Nature

# C39 Peptidases

C39 peptidases are cysteine proteases

Specific for ABC transporters

Cleavage occurs after a GG motif

Catalytic dyad/triade (Cys-His-Asp)

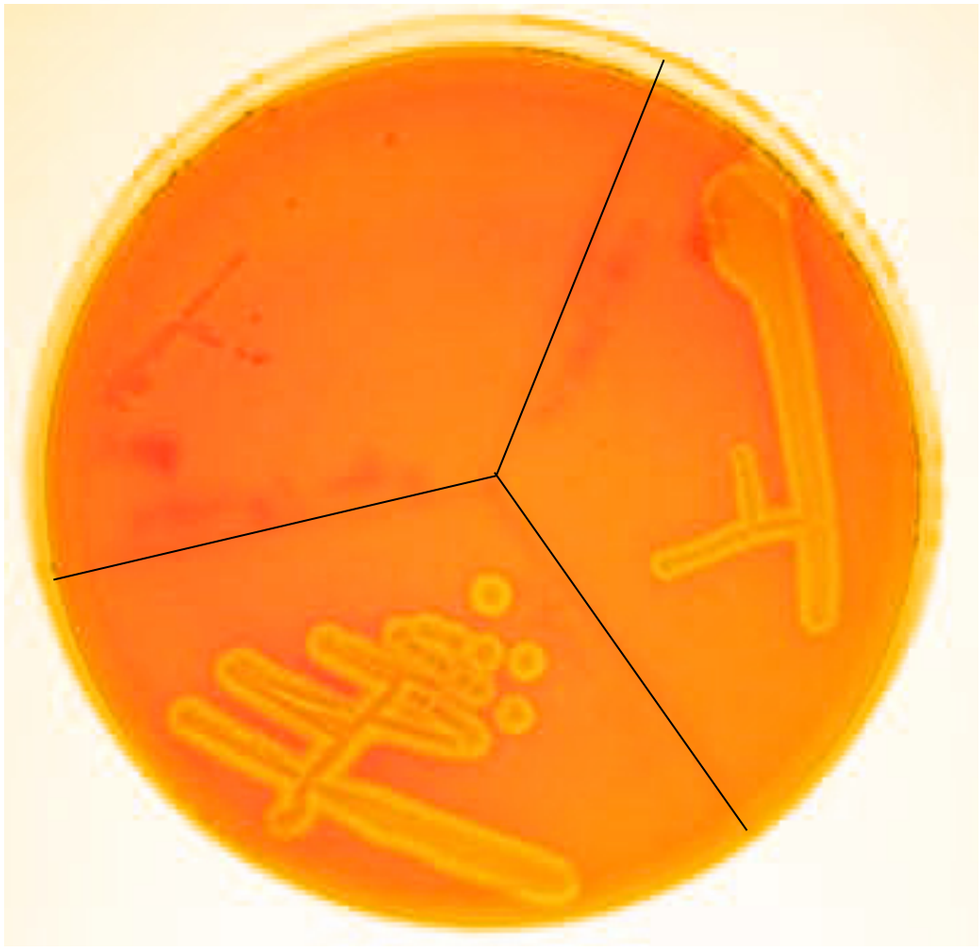
Consensus sequence – LSXXELXXIX**GG** (Substrate)

The C39-domain of HlyB:
Consensus sequence – **GG**XGXDUX (RTX)
Tyr instead of Cys

**C39-like domain (CLD)**

# A Potential Role of the CLD

*E. coli*
BL21/HlyB-C39+HlyD



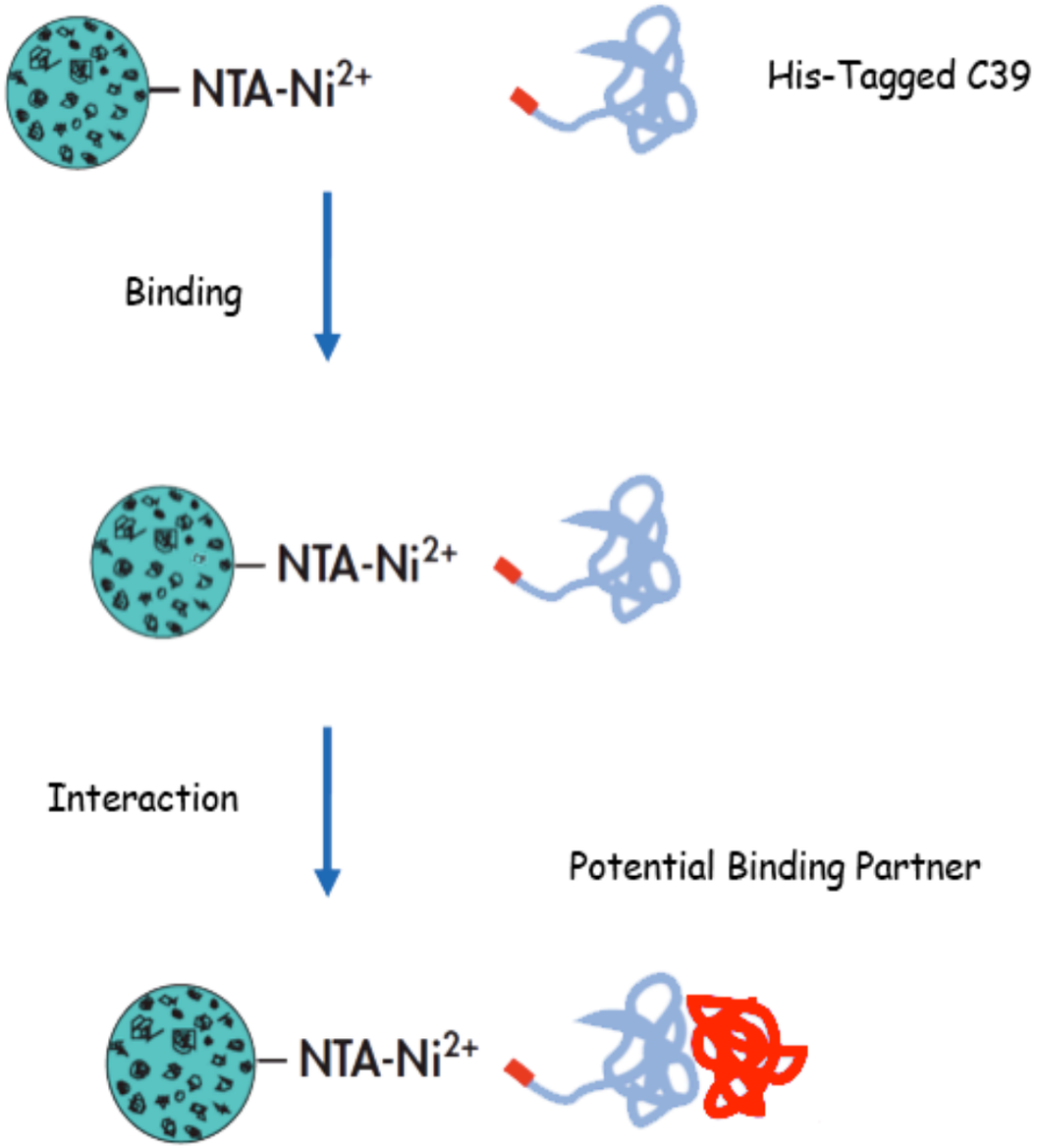
*E. coli*
SE 5000

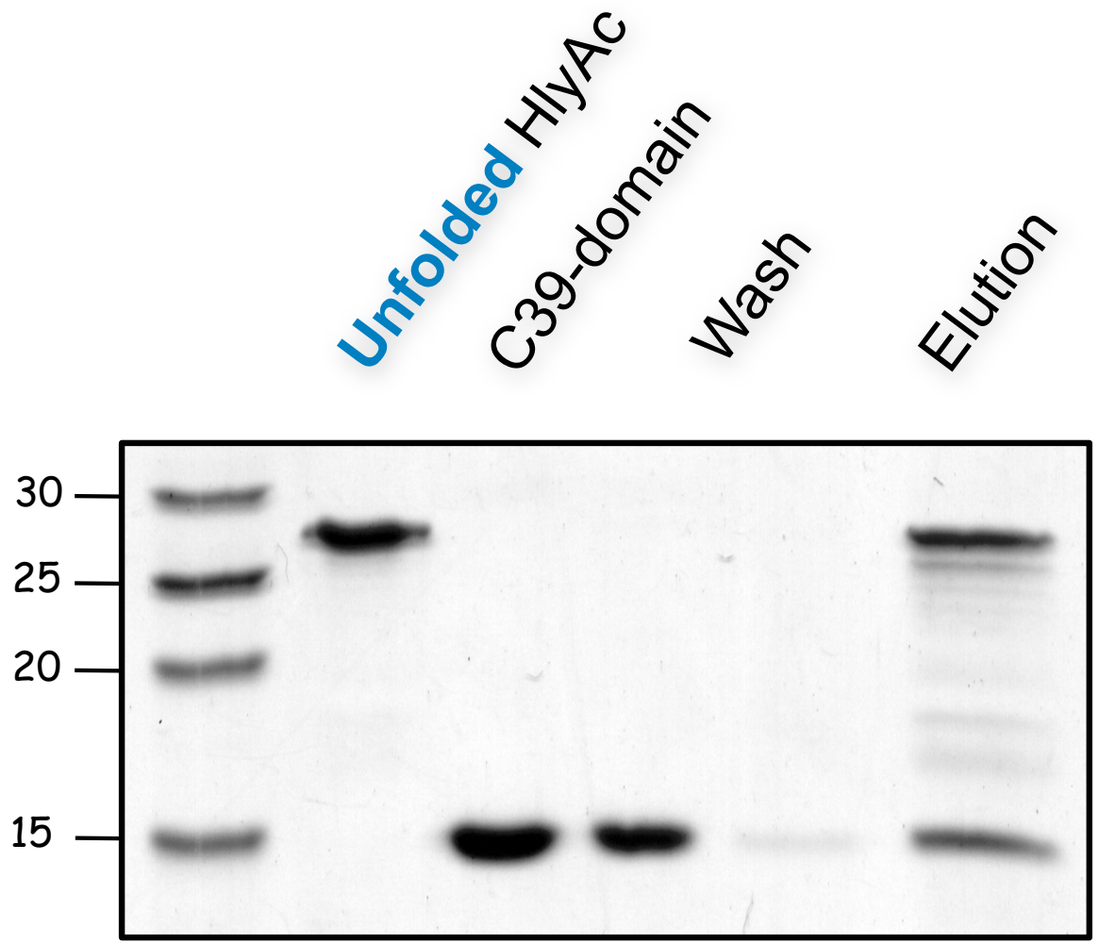
*E. coli*
BL21/HlyB+HlyD

# Interaction Partner(s)

## Protein–Protein Interaction

### "Interaction Assay Ni-NTA Magnetic Beads"



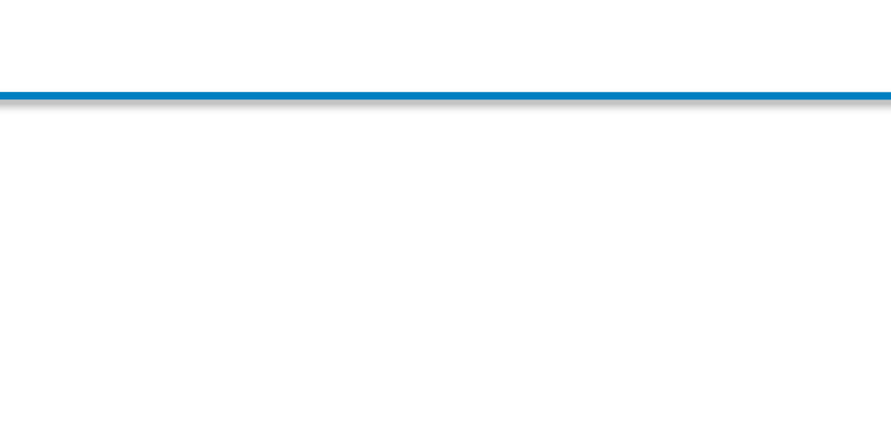
# Controls ....

**Folded** HlyAc

CLD

Wash

Elution




**Unfolded** HlyAc **–SecSeq**

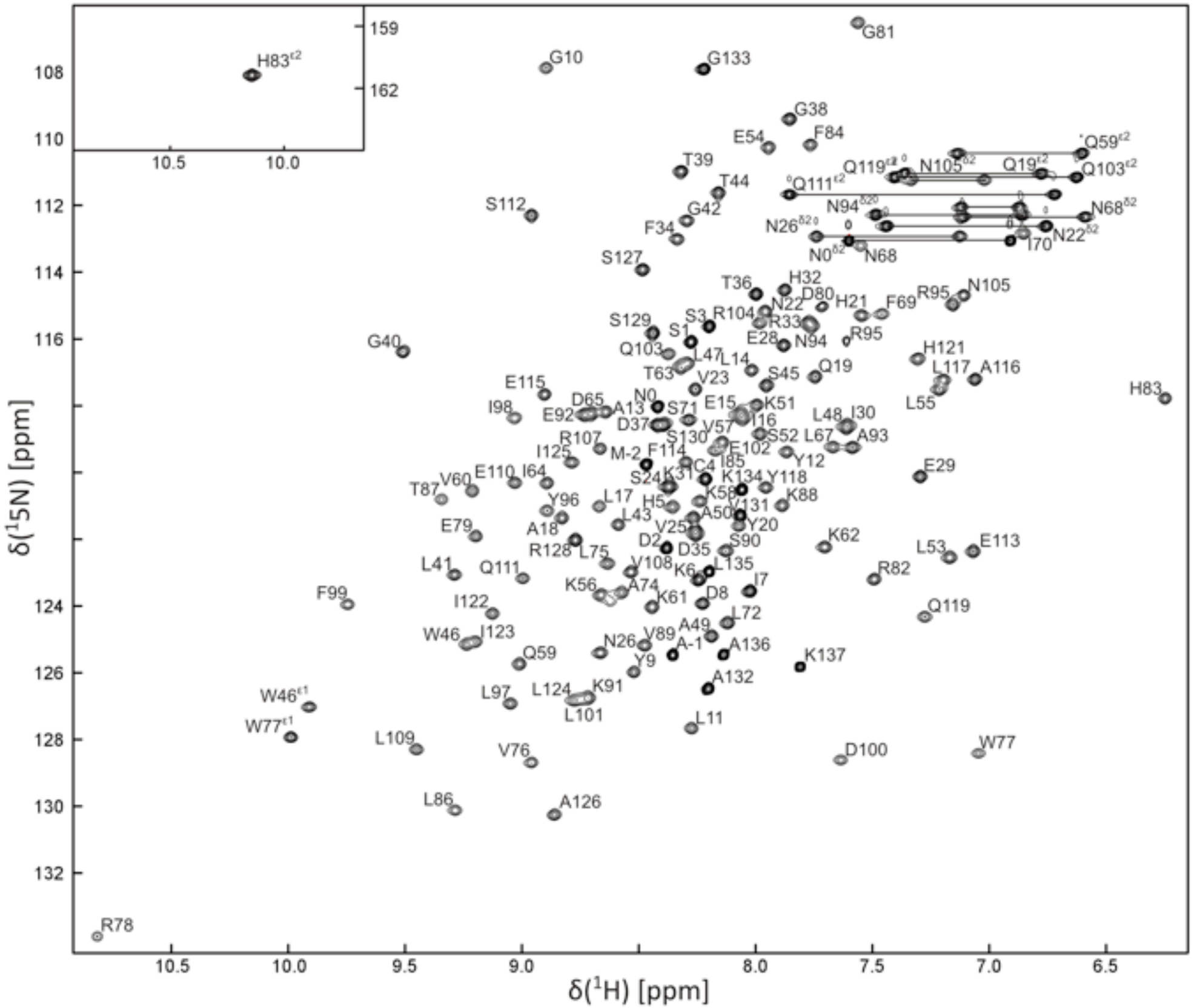
CLD

Wash

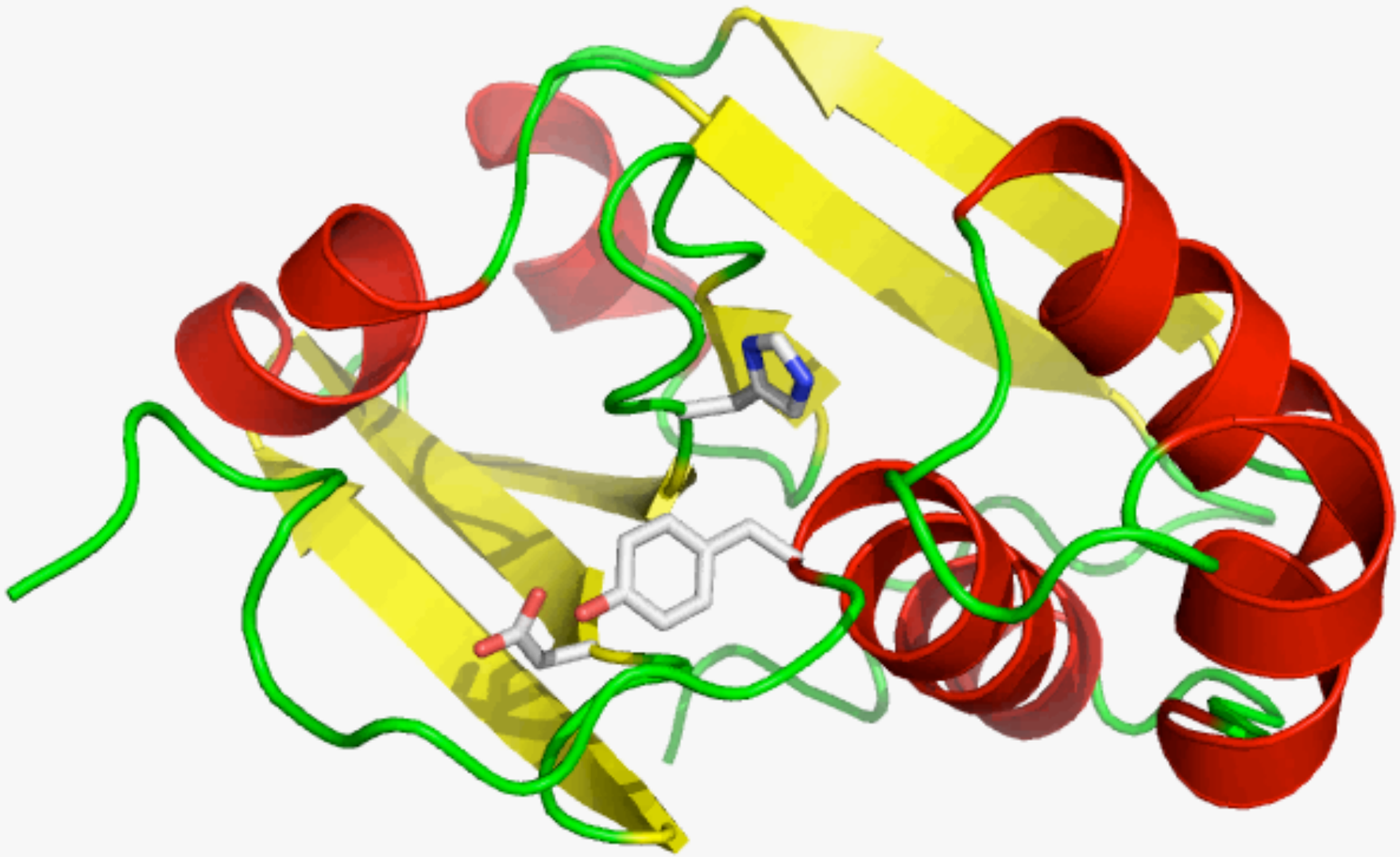
Elution


The unfolded state without the SecSeq is recognized

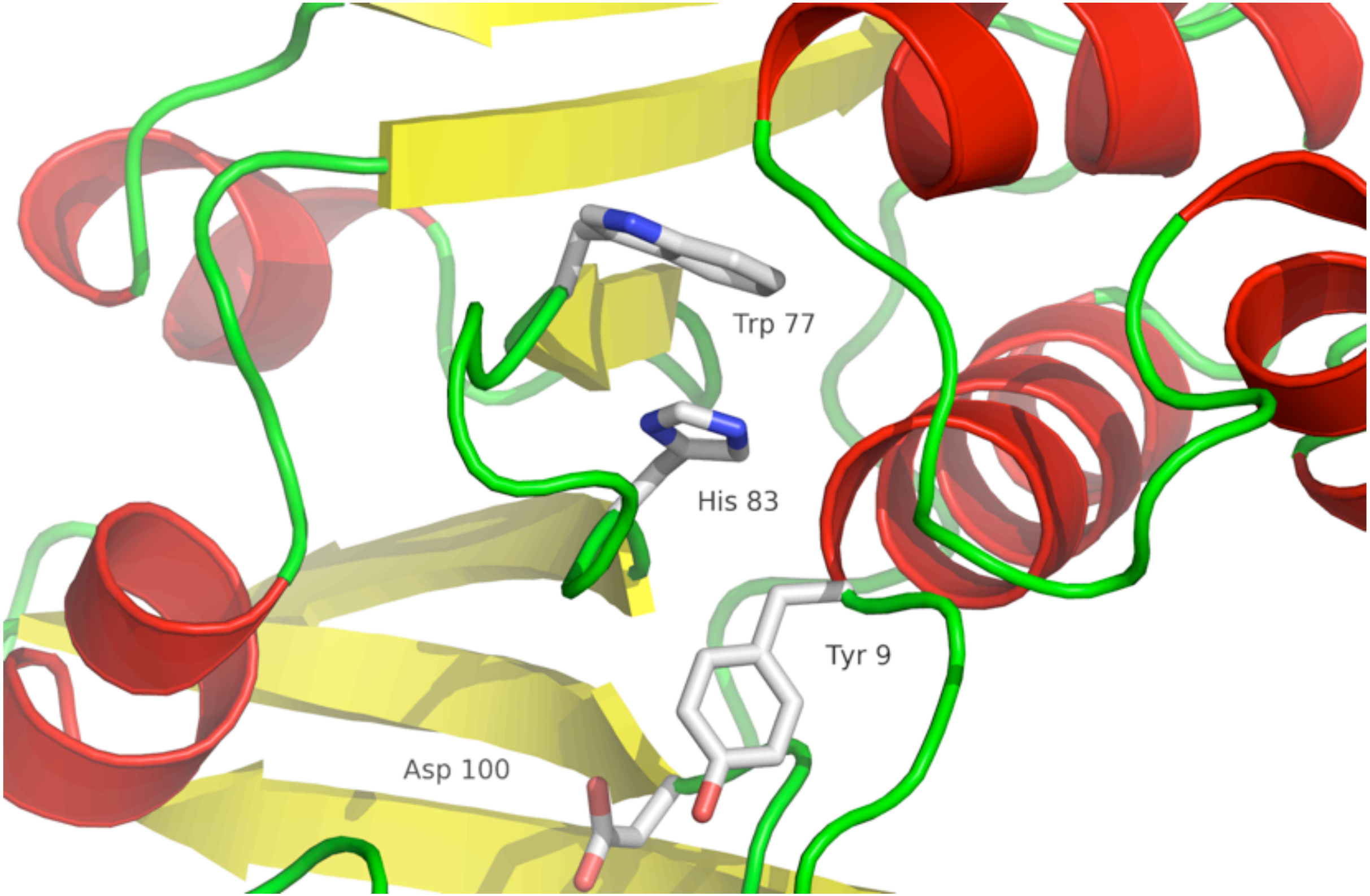
# The NMR Structure of the CLD



# The Overall Fold

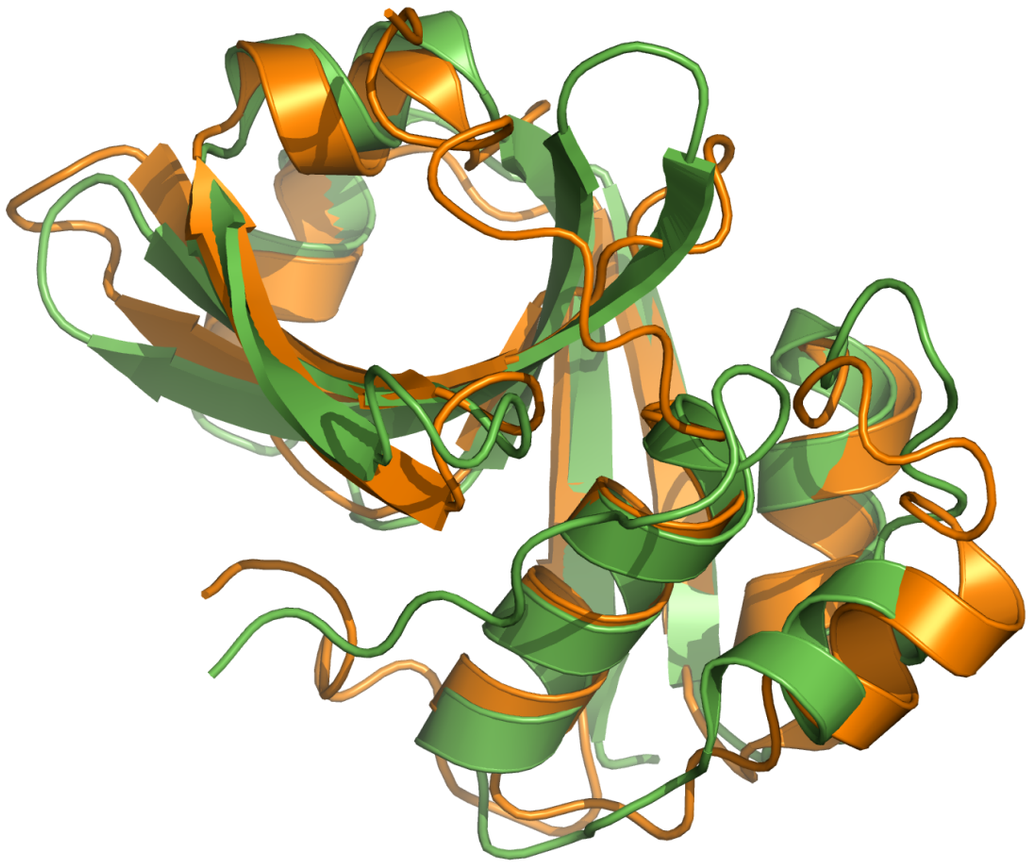


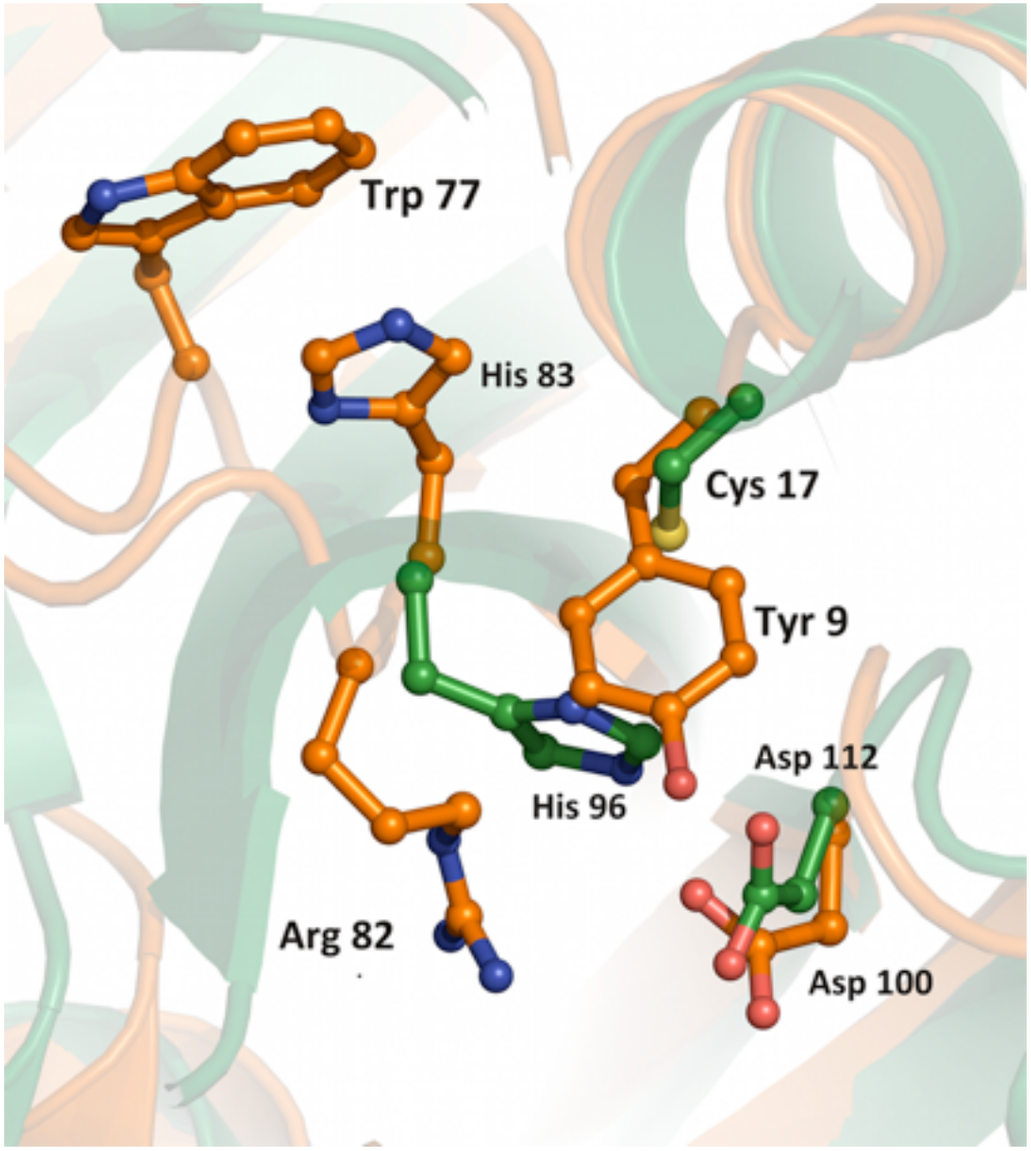
# The Active Site



# CLD – C39: A Comparison

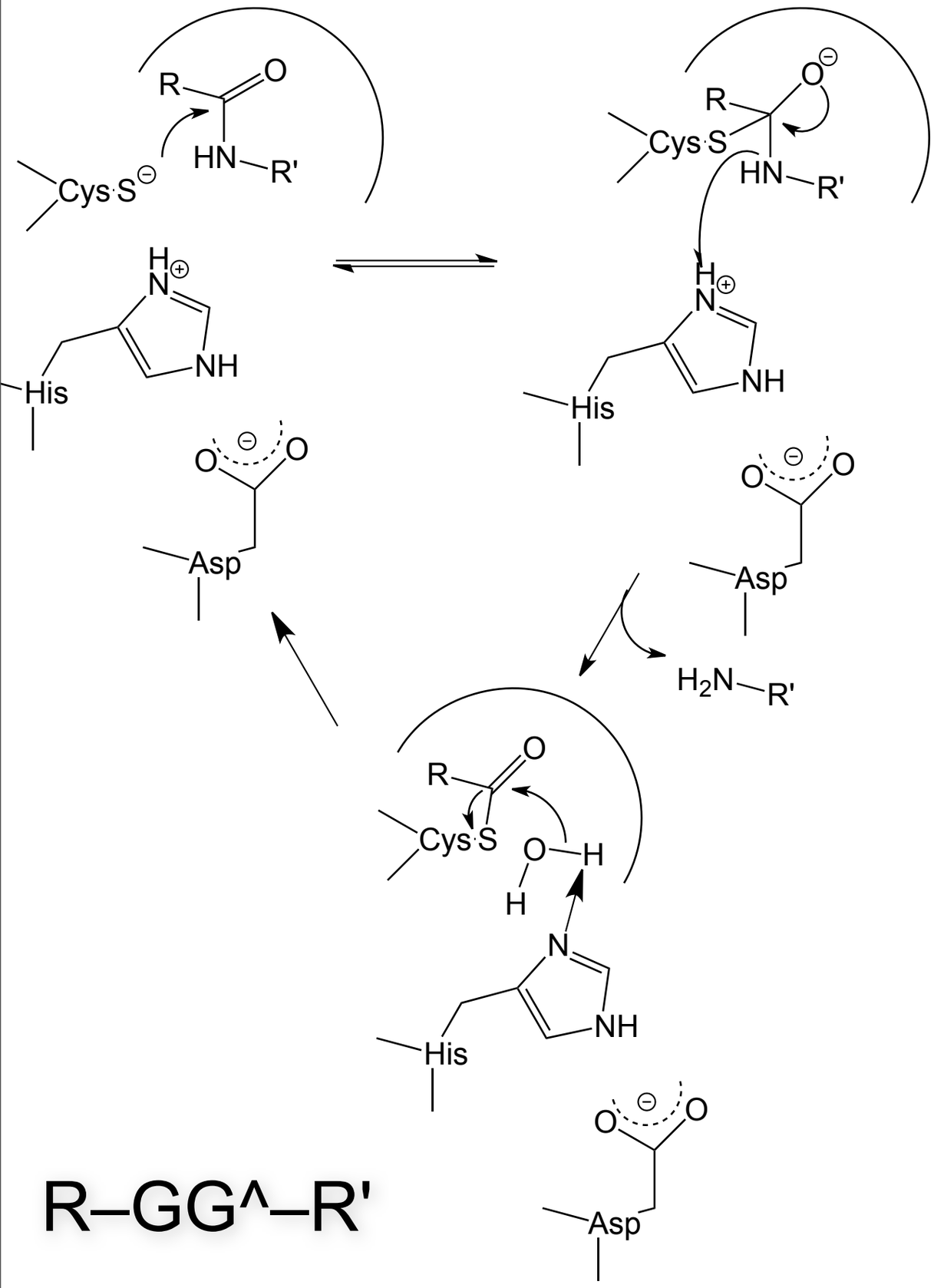
ComA-PEP* / CLD






*Ishii et al. (2010) JBC **285** 10777- 10885

# Mechanistic Implications




R–GG^–R'

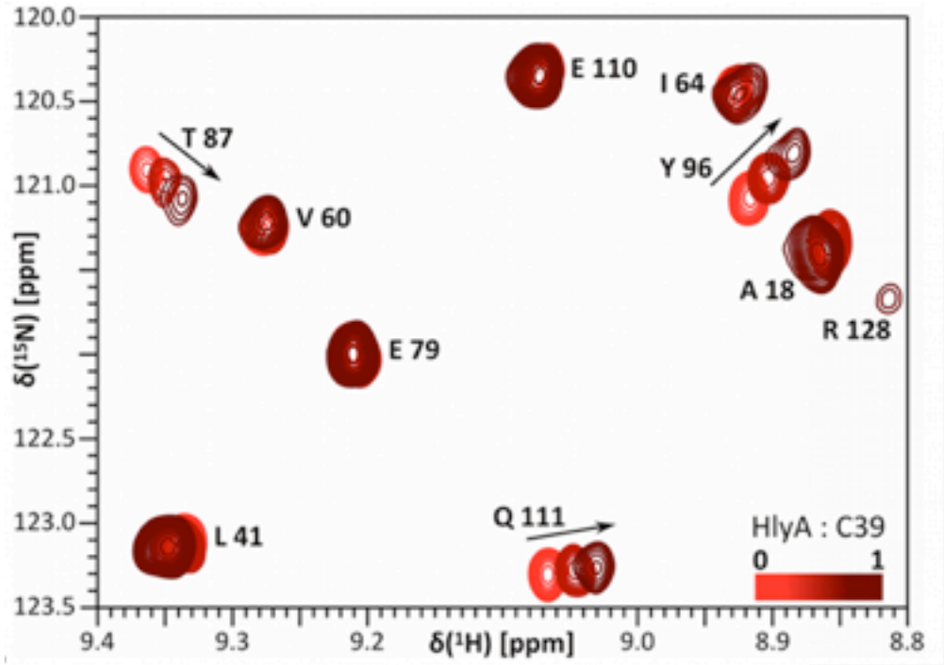
No thiolate ($Tyr^{9}$)

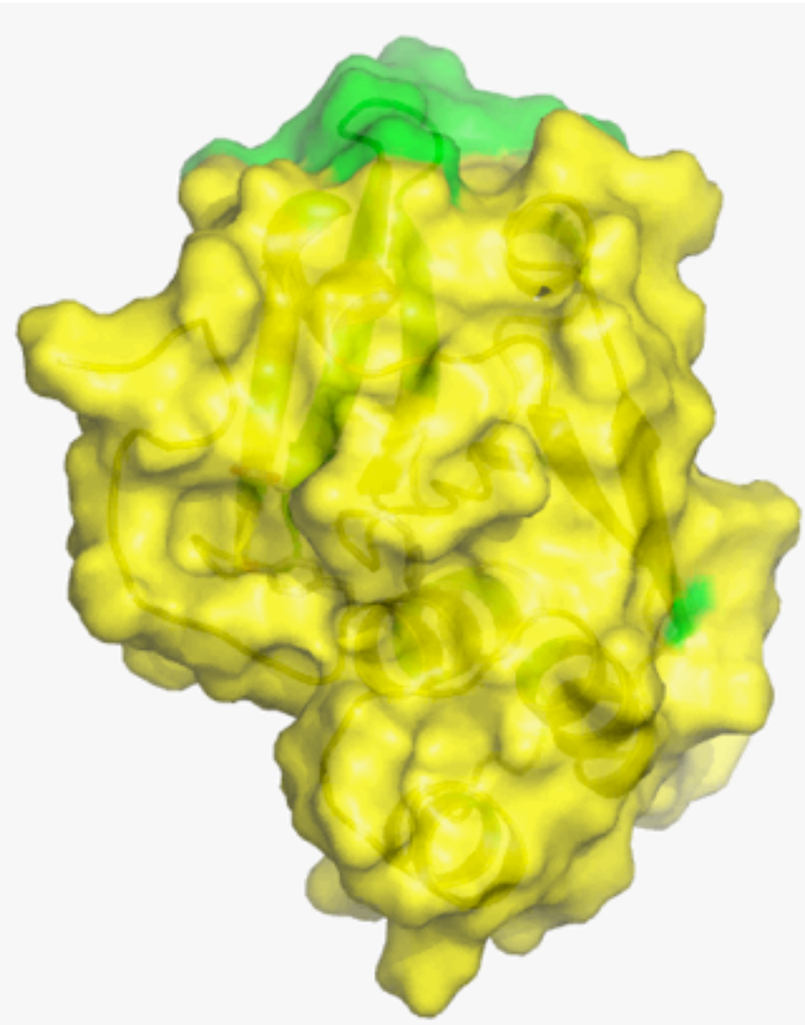
$His^{83}$ – $Trp^{77}$ interaction

$Tyr^{9}Cys$ is still inactive

# Interactions by NMR

Titration of HlyAc* (no SecSeq) / CLD

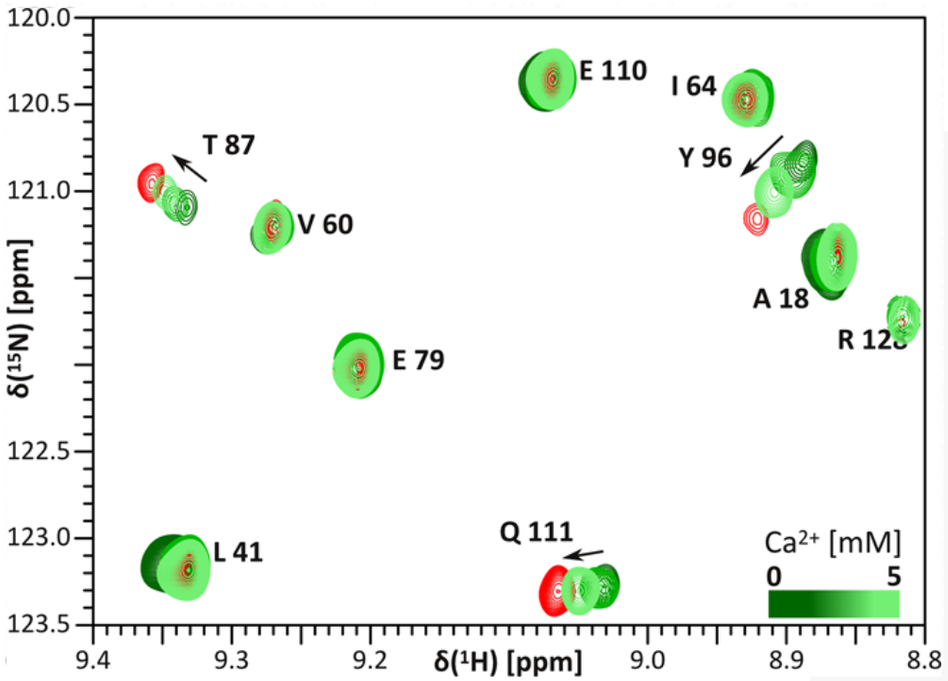


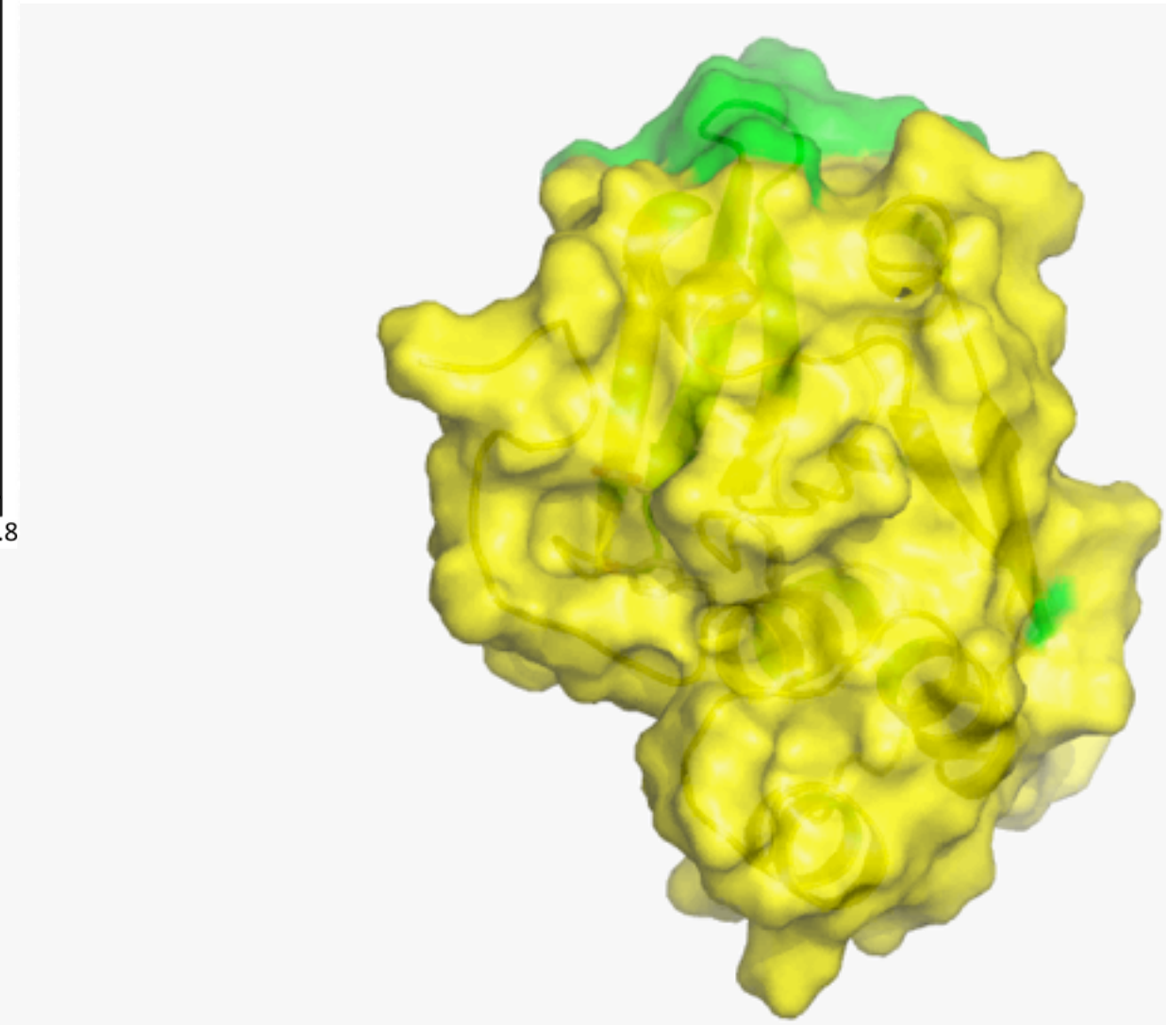



CLD harbors a 'continous' binding site

# Interactions by NMR

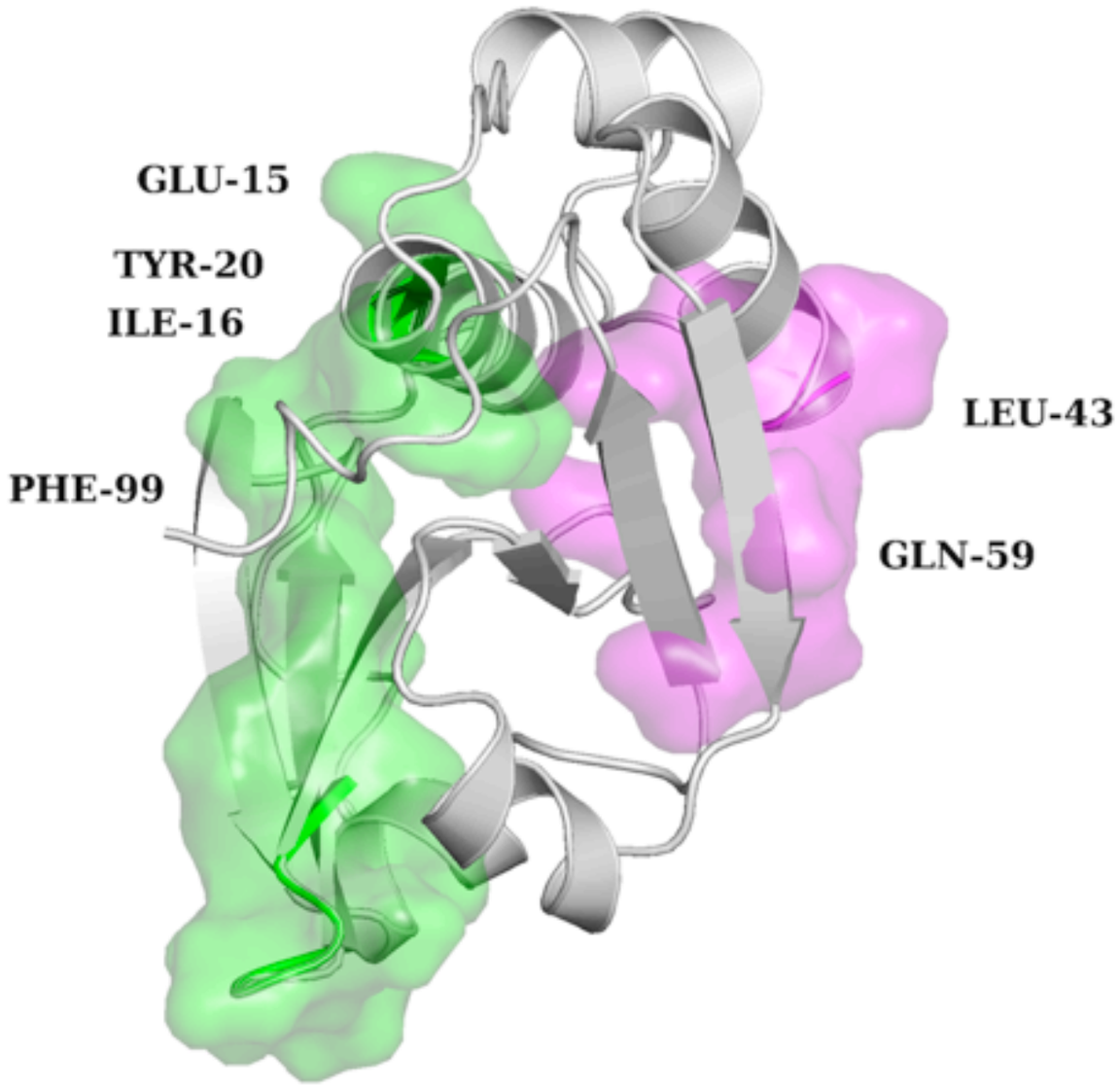
## Titration of HlyAc* (no SecSeq) / CLD

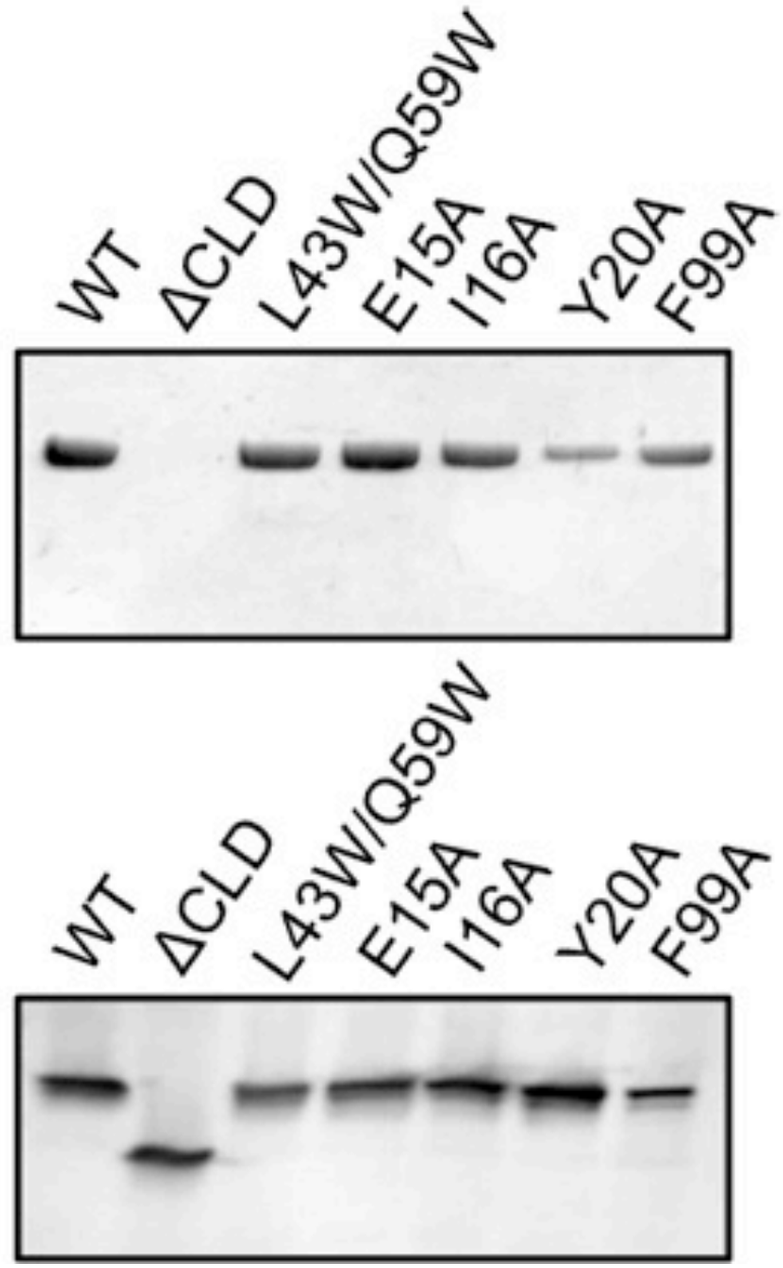


CLD harbors a 'continous' binding site

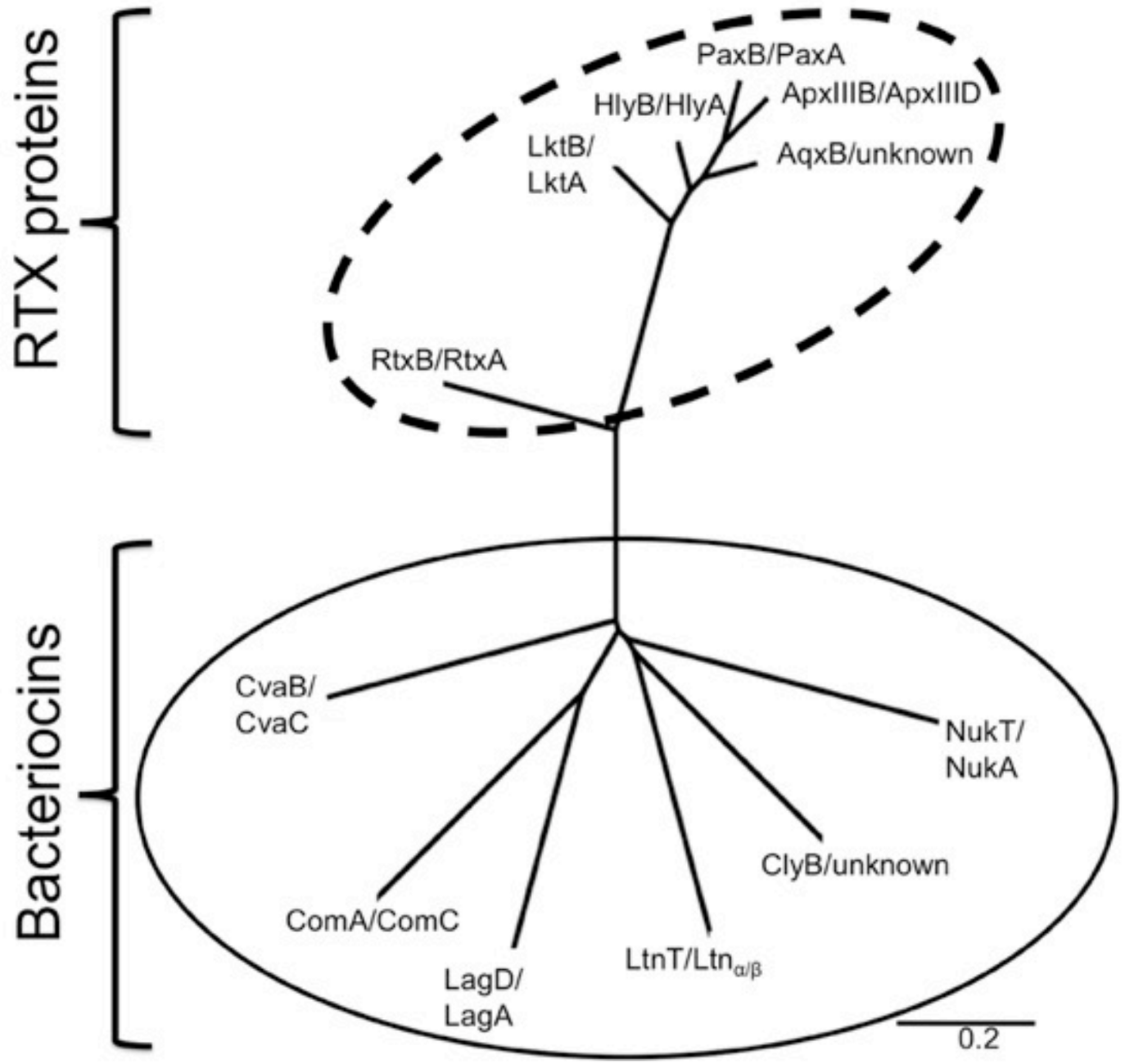
# Mutations – CLD versus ComA



# Phylogenetic Consequences

'Trp$^{77}$' – 'His$^{83}$' as an indicator

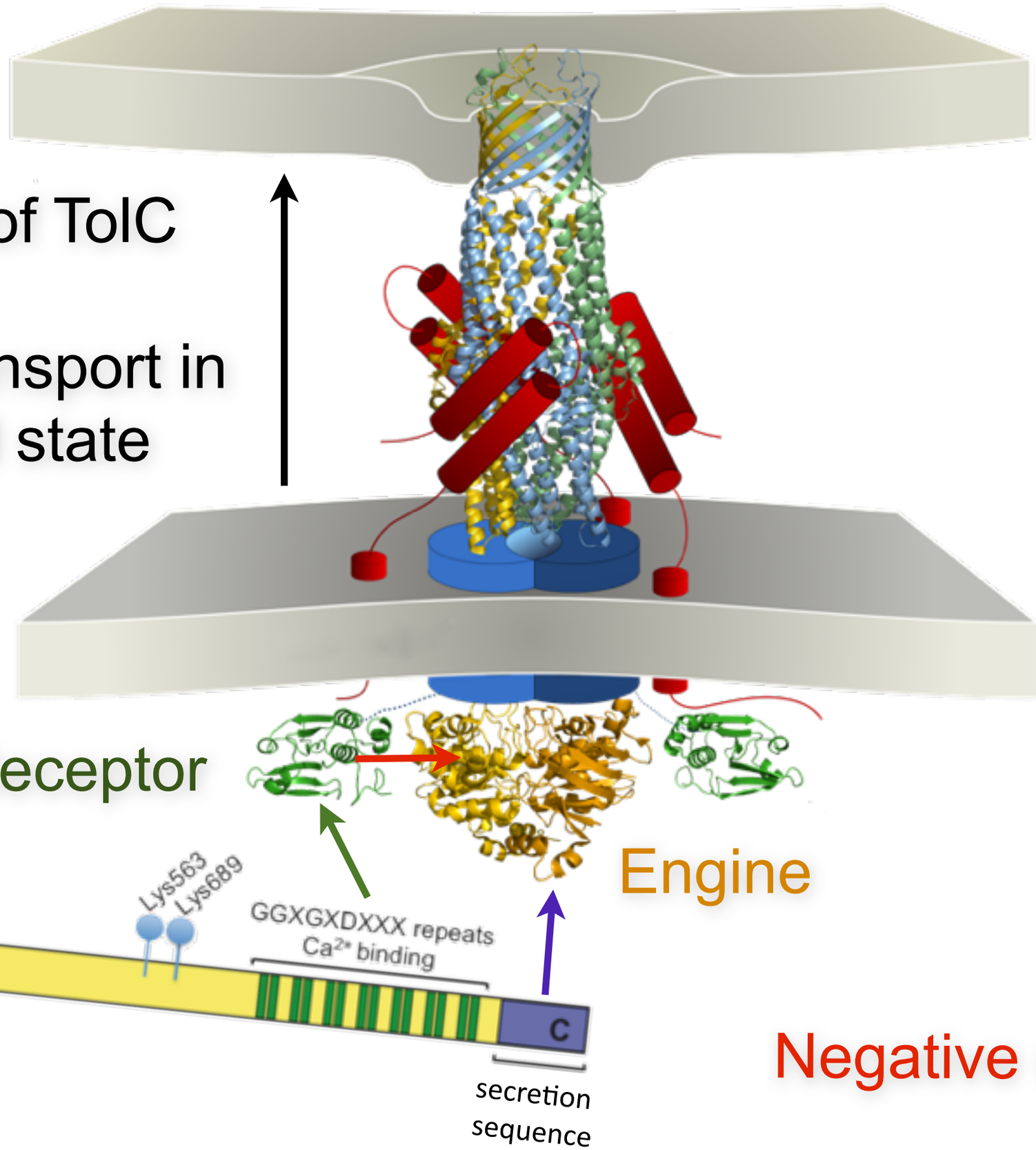



HasA/HasD systems

# A Model of the Early Steps

Recruitment of TolC

Substrate transport in the **unfolded** state




Negative regulation

André Abts
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Rakesh Gupta
Nils Hanekop
Nacera Infed
Miro Kirov

Marianne Kluth
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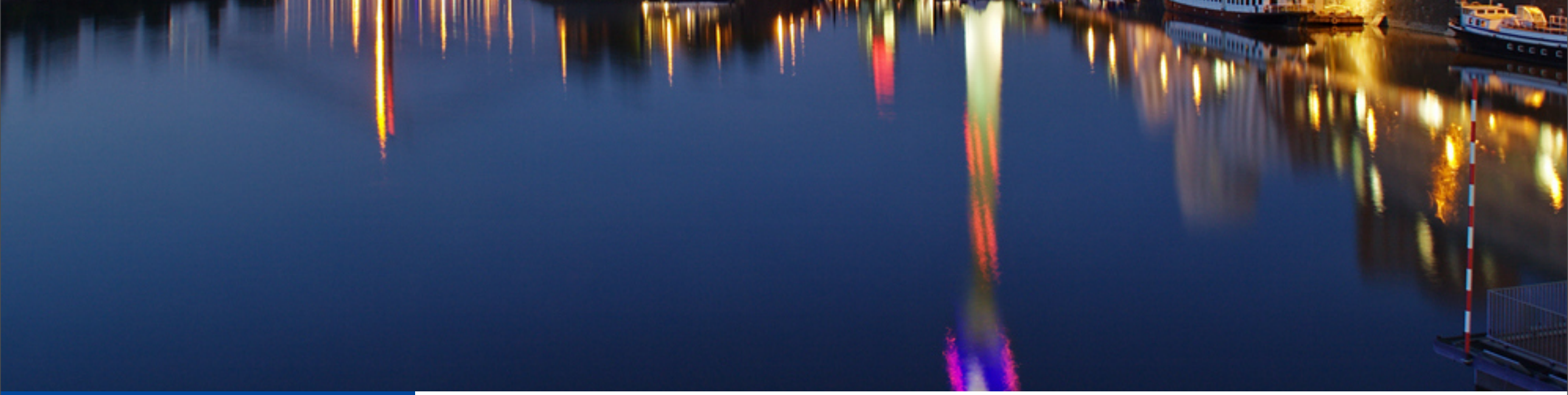
Ralf Kubitz (Düsseldorf)
Karl Kuchler (Vienna)
**Barry Holland (Paris)**
Bert Poolman (Groningen)
**Dieter Willbold (Düsseldorf)**

Text



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