



Isopeptide Ligation by Sortase-Mediated Ligation

M1 Xiaoyi Pan

240509

Content

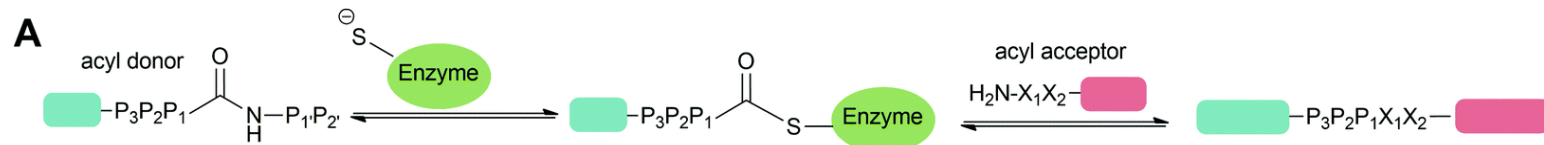
- Introduction
- Isopeptide ligation by SML
 - Enzymatic approach to isopeptide ligation -- CdSrtA
 - Acyl acceptor altering approach to isopeptide ligation -- Unnatural Amino Acid
- Summary

Enzymatic Protein Conjugation

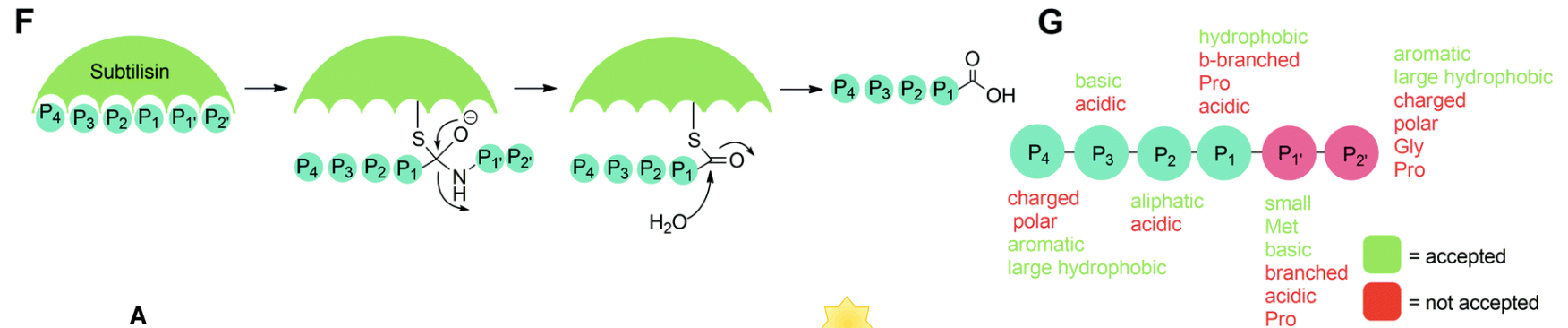
- ✓ Mild conditions
- ✓ High yield

- ✓ Fast kinetics

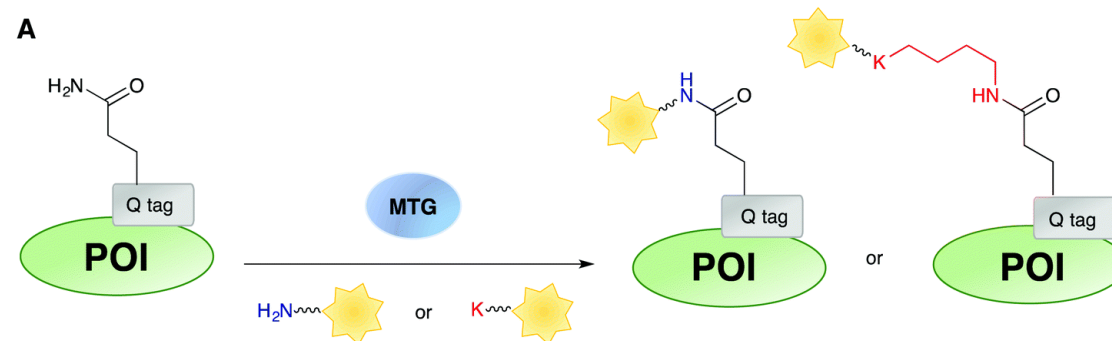
Sortase



Subtiligase



Microbial Transglutaminase (MTG)



Sortase-Mediated Ligation (SML)

- ✓ High Site-Specificity
- ✓ High level of overexpression efficiency

Improvement:

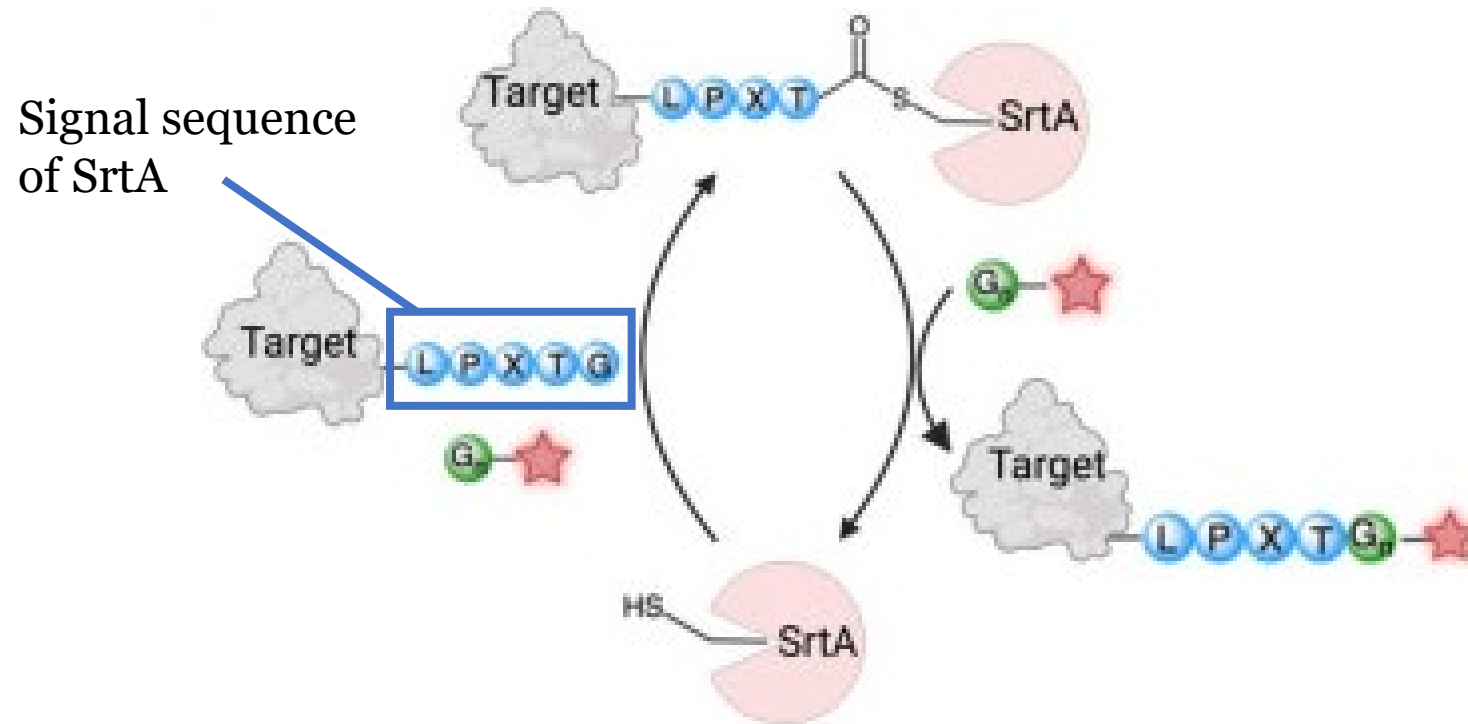
- Enhancing activity/robustness
- Reprogramming selectivity
- reducing reversibility
- etc.

→

A powerful tool for
peptide ligation

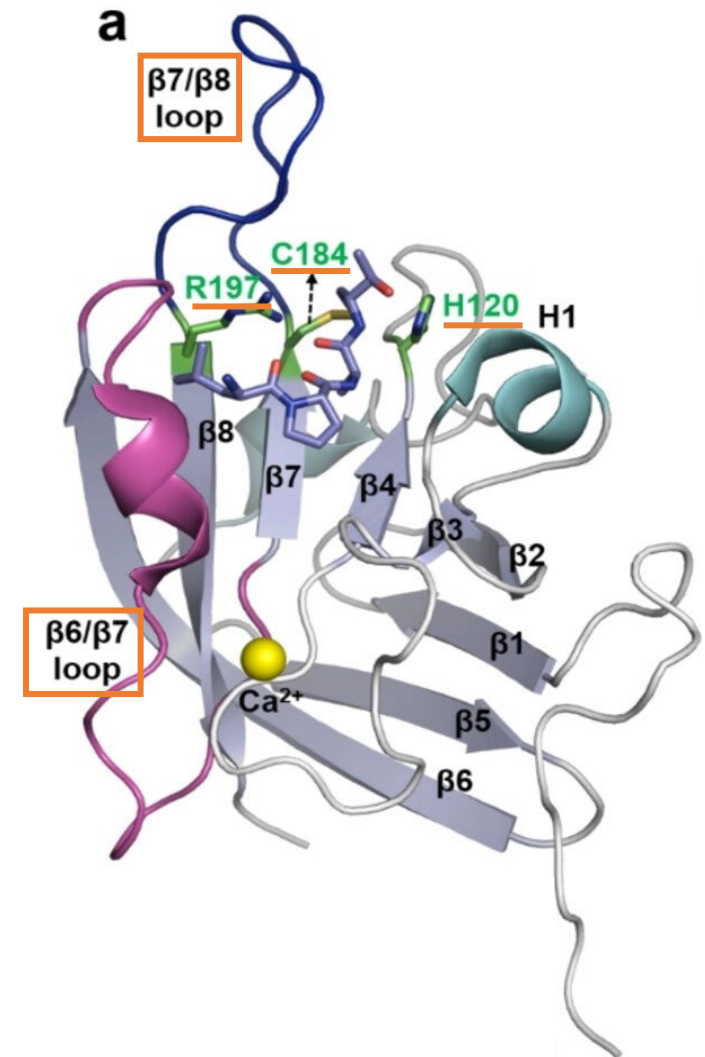
Sortase

- Generally found in Gram-positive bacteria.
- Sortase A (SrtA) is the most major class among 6 classes of sortase.

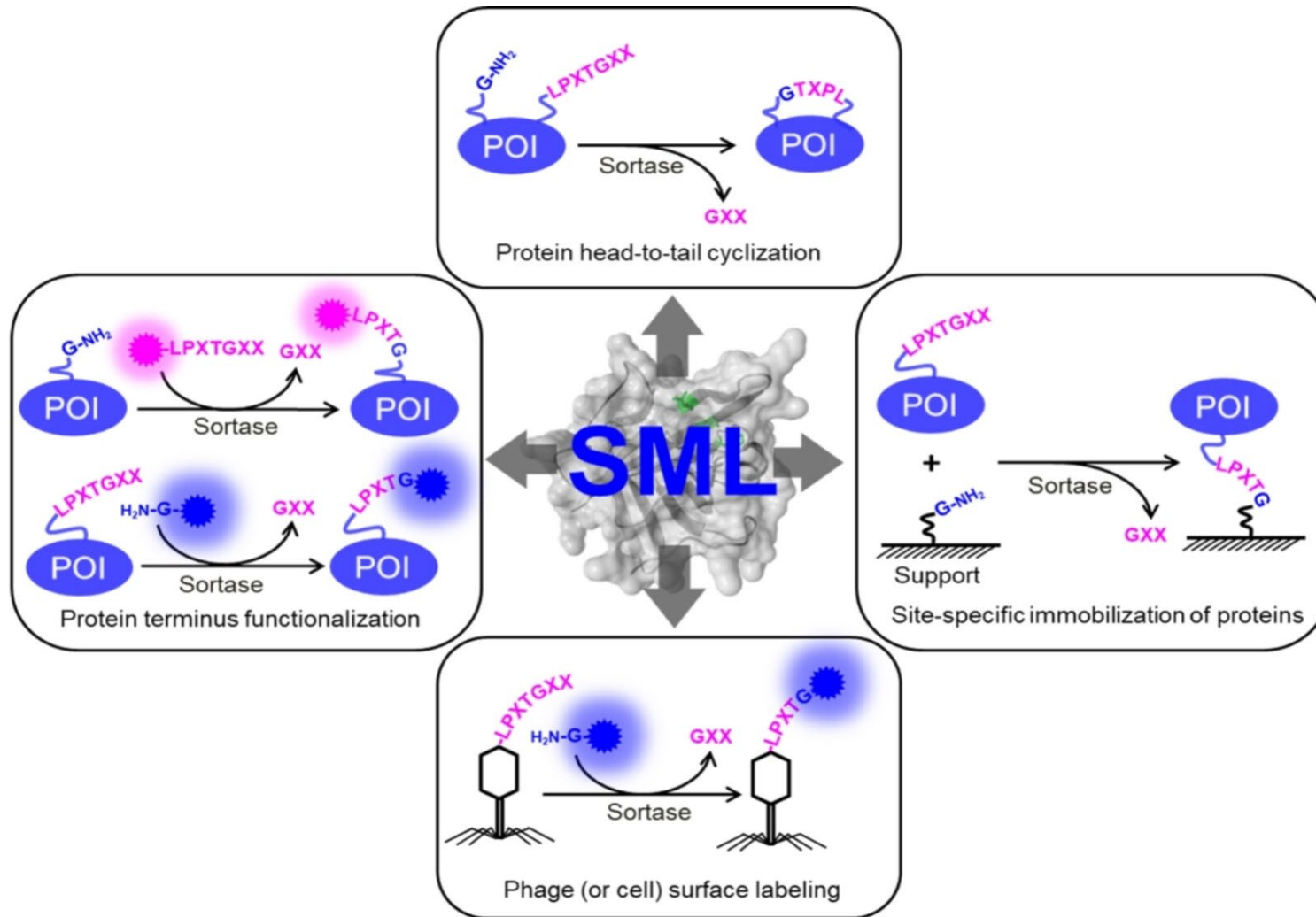


SaSrtA

- Found in *S. aureus* for the first time in 1999
- To anchor surface proteins to the bacterial cell wall
- ◆ 8-stranded β -barrel core shared across all sortase classes.
- His120, Cys184, and Arg197
- $\beta 6/\beta 7$ loop & $\beta 7/\beta 8$ loop are crucial for activity and sorting specificity



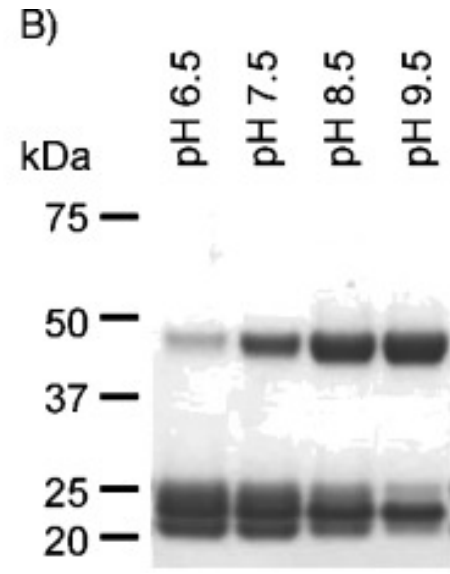
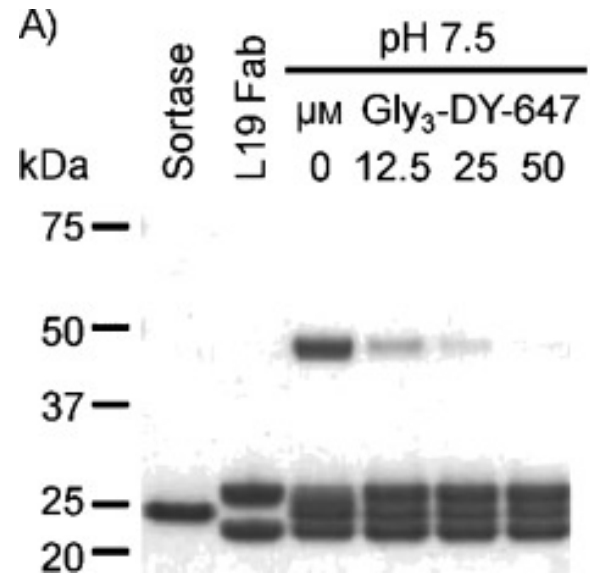
Previous application



- N-terminal
 - Oligoglycine as acyl acceptor
- How about apply to isopeptide ligation?

Isopeptide Ligation

- Side reaction



← Conjugate with Lys side chain

× Low selectivity

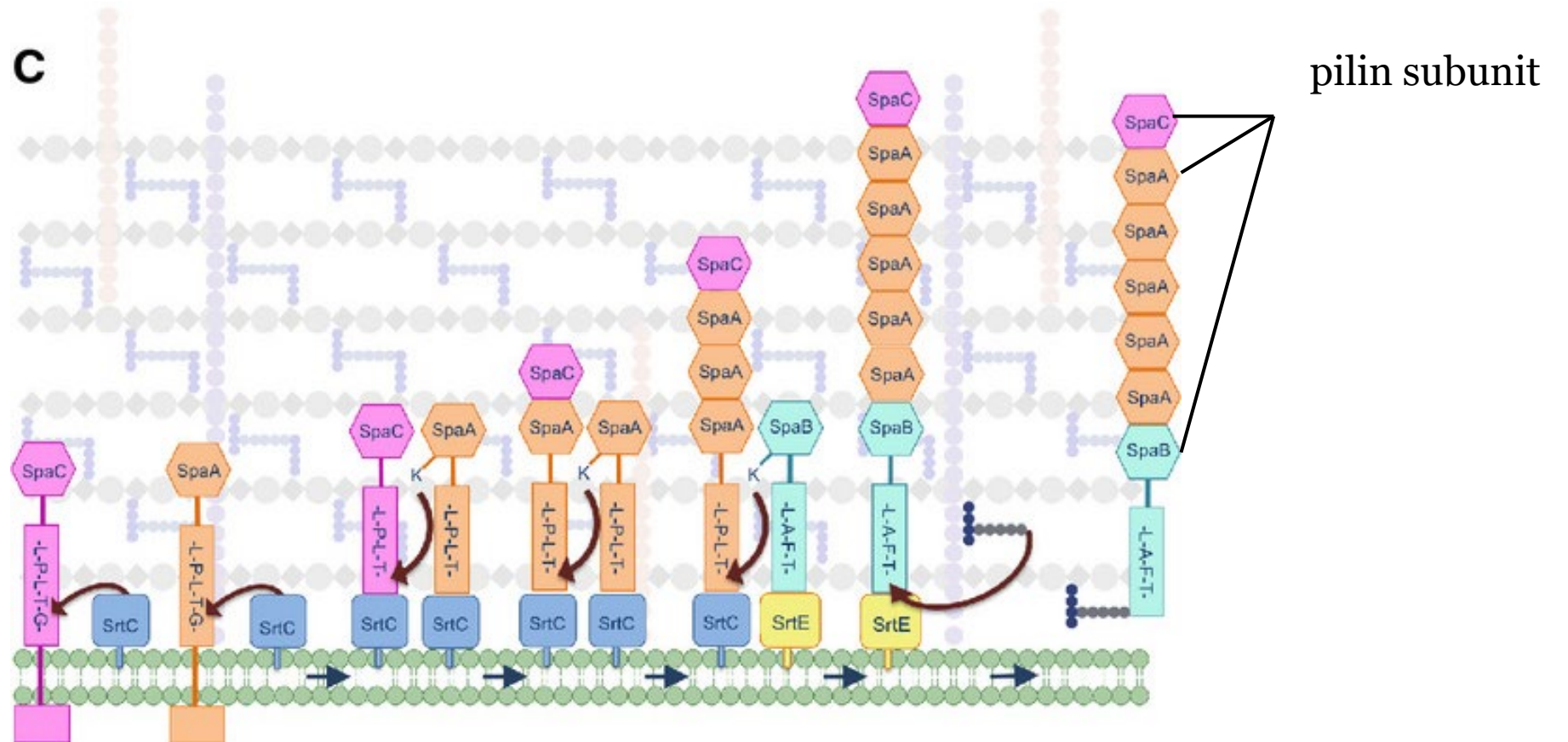
× Inefficient

Content

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 - Acyl acceptor altering approach to isopeptide ligation -- Unnatural Amino Acid
- Summary

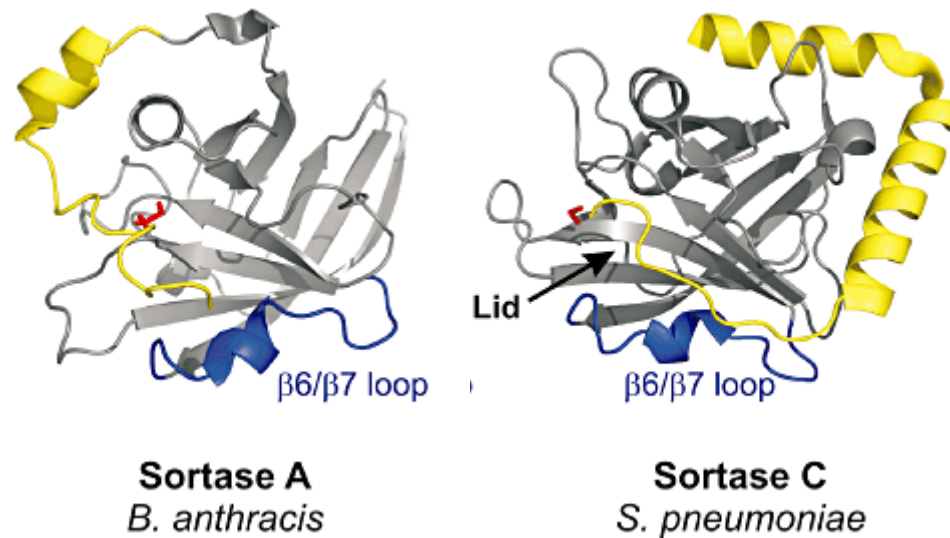
SrtC

- Class C: Pilins biosynthesis related
- Signal sequence: LPLTG



Structure of SrtC

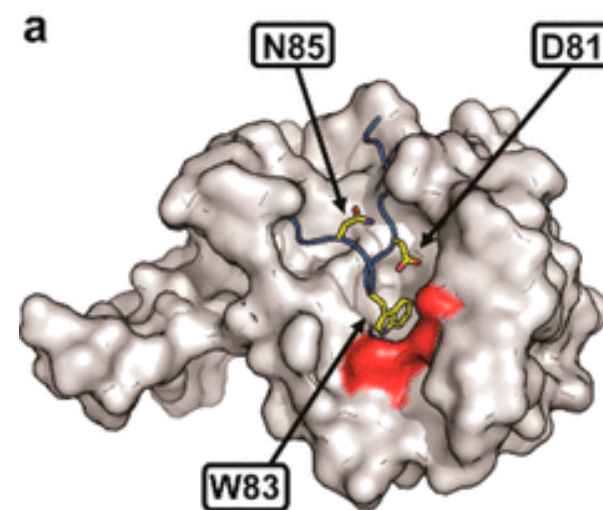
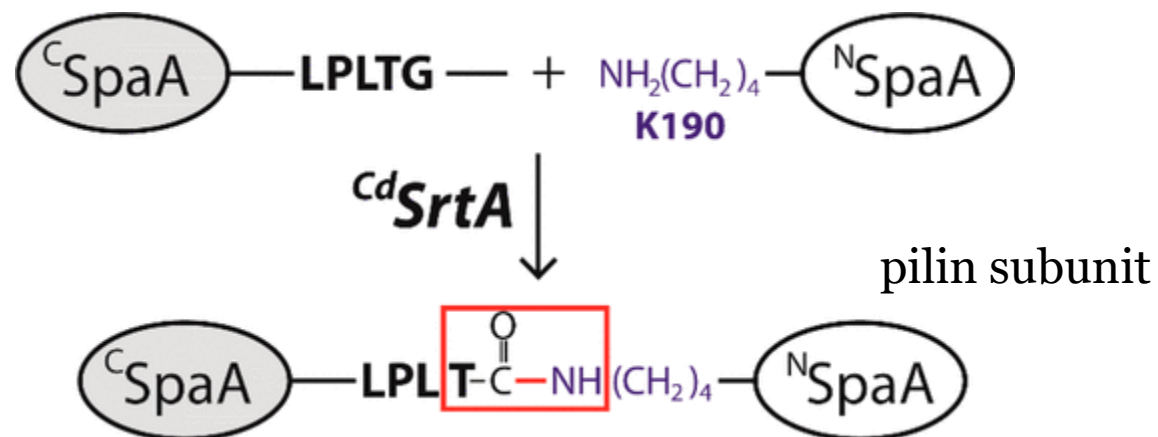
- Lid



- Responsible for recognizing signal sequence and pilin motif.

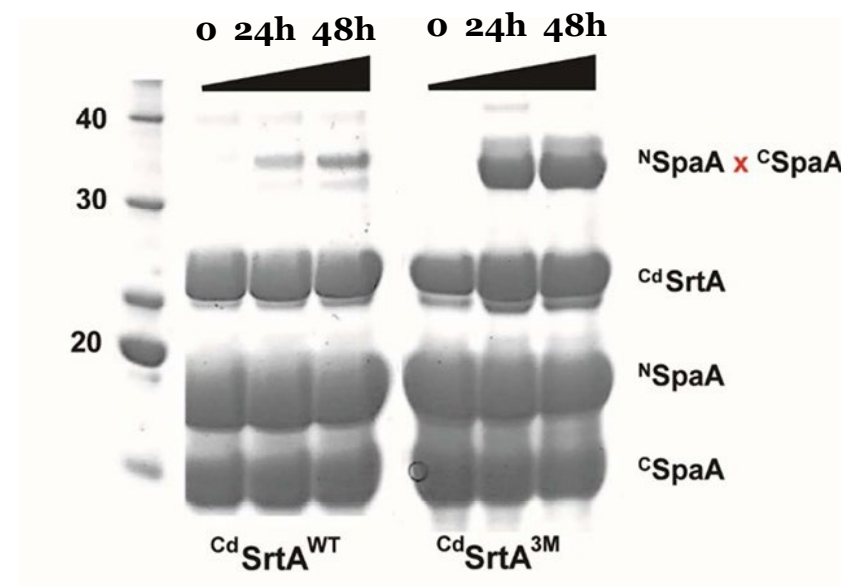
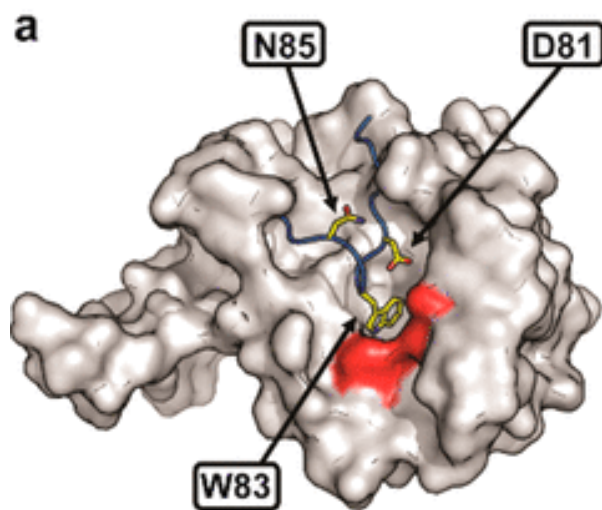
CdSrtA

- From *Corynebacterium diphtheriae*
- H160, C222, R231



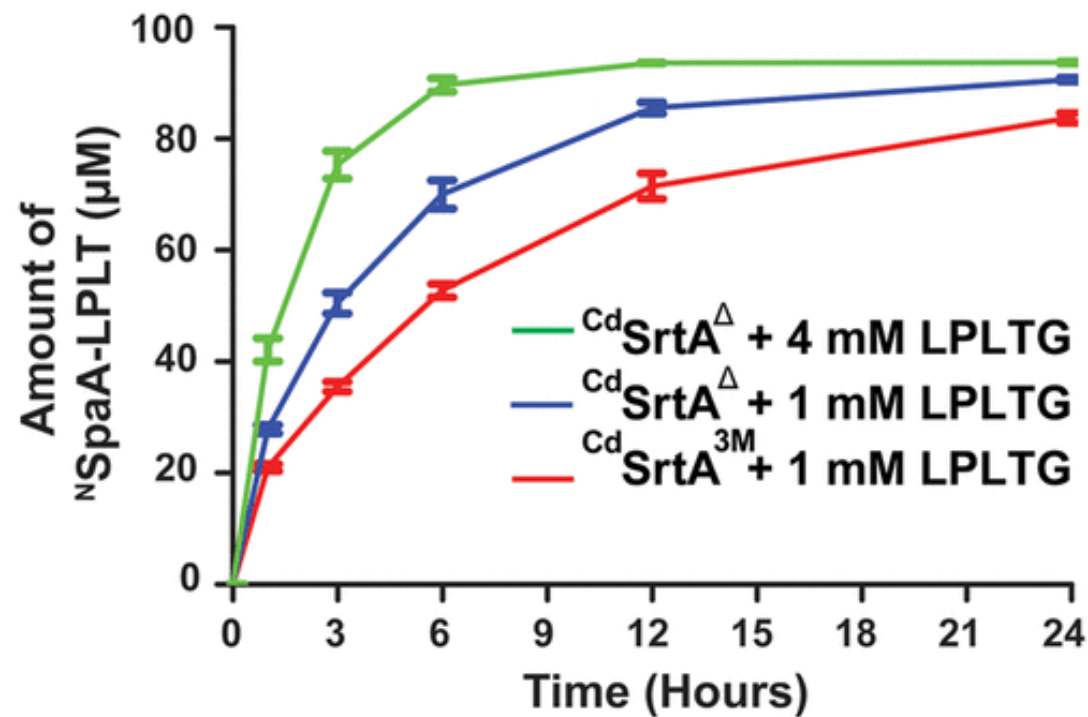
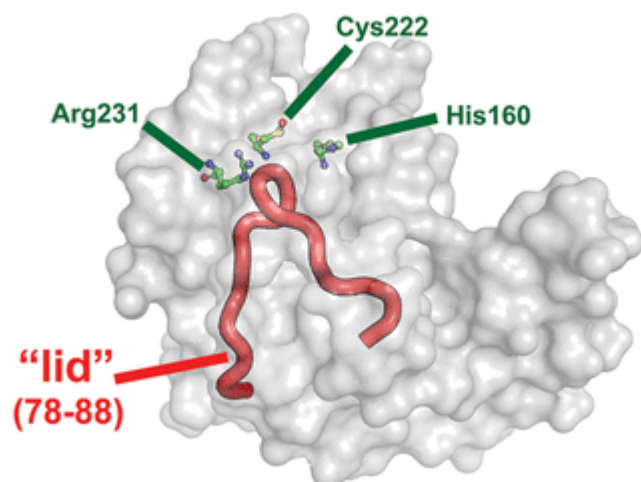
CdSrtA_{3M}

- Mutation: D81G, W83G, N85A

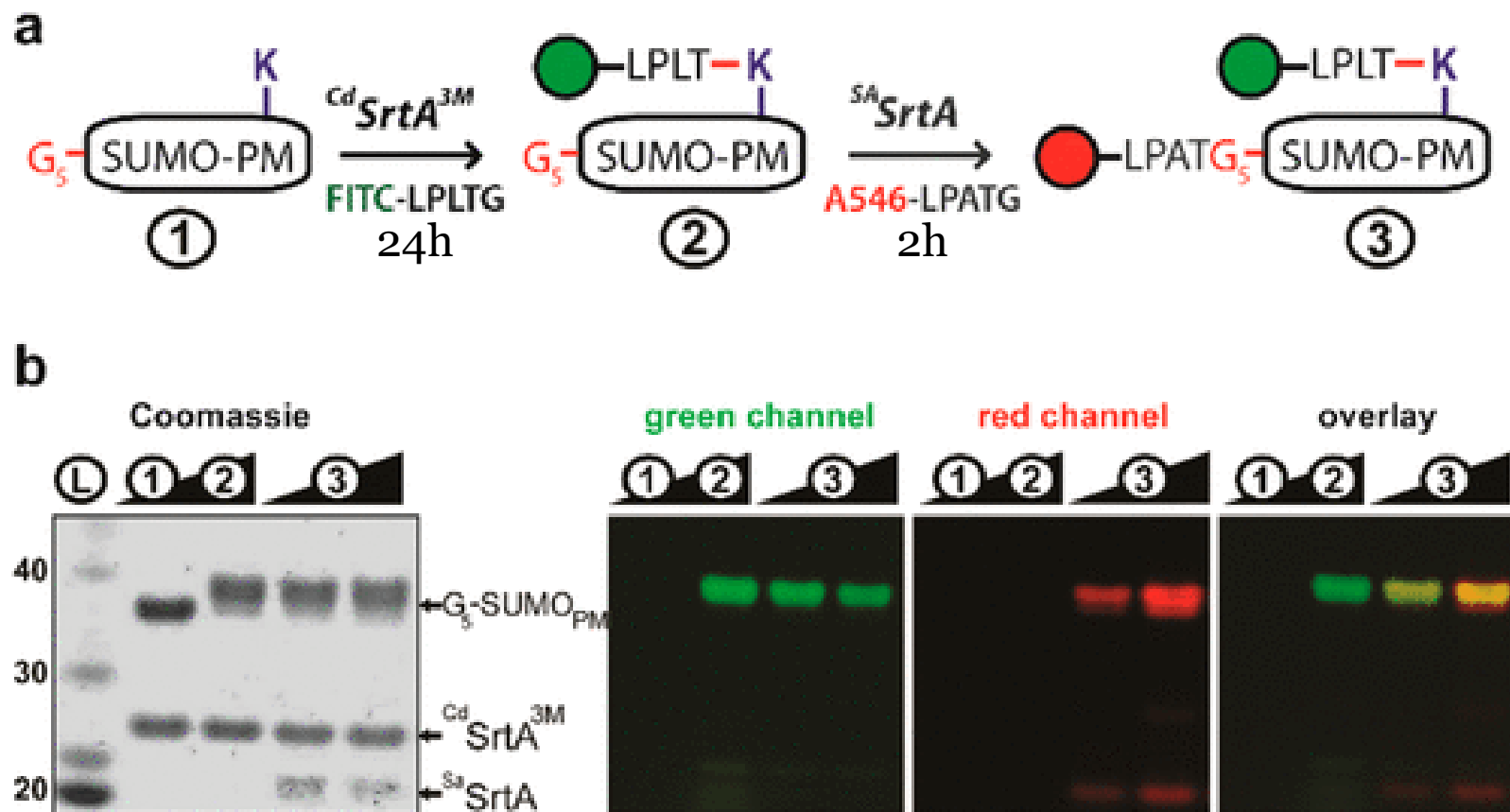


✓ In vitro

CdSrtA Δ



Dual labeling



Short Summary

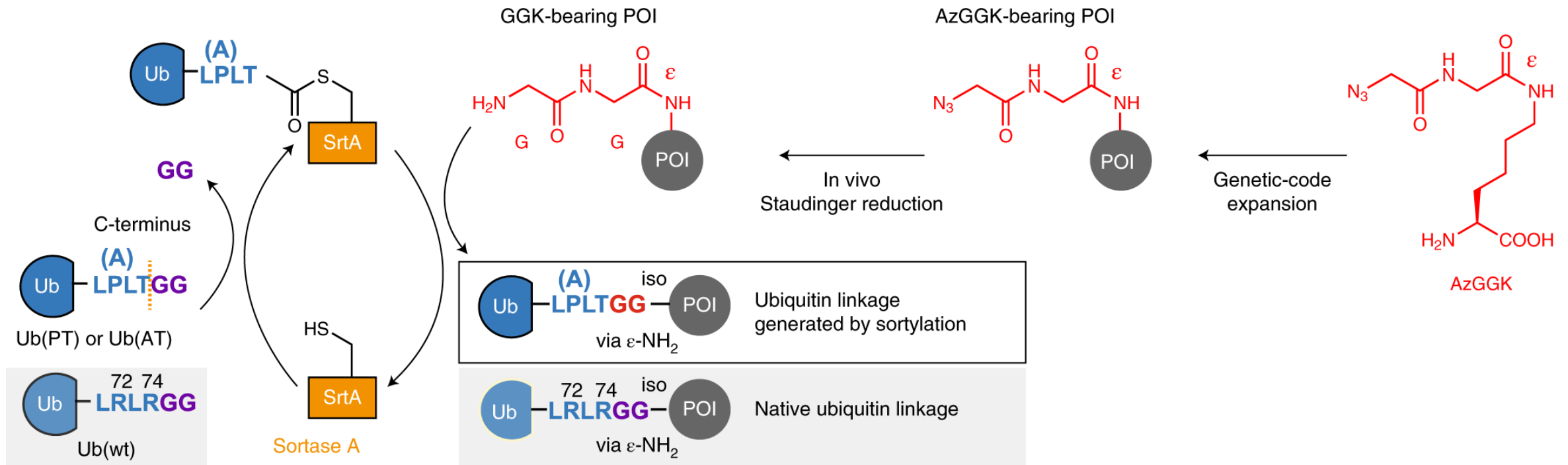
- ✓ Mild condition
- ✓ High selectivity

Kinetics of ^{Cd}SrtA Catalyzed Lysine–Isopeptide Formation

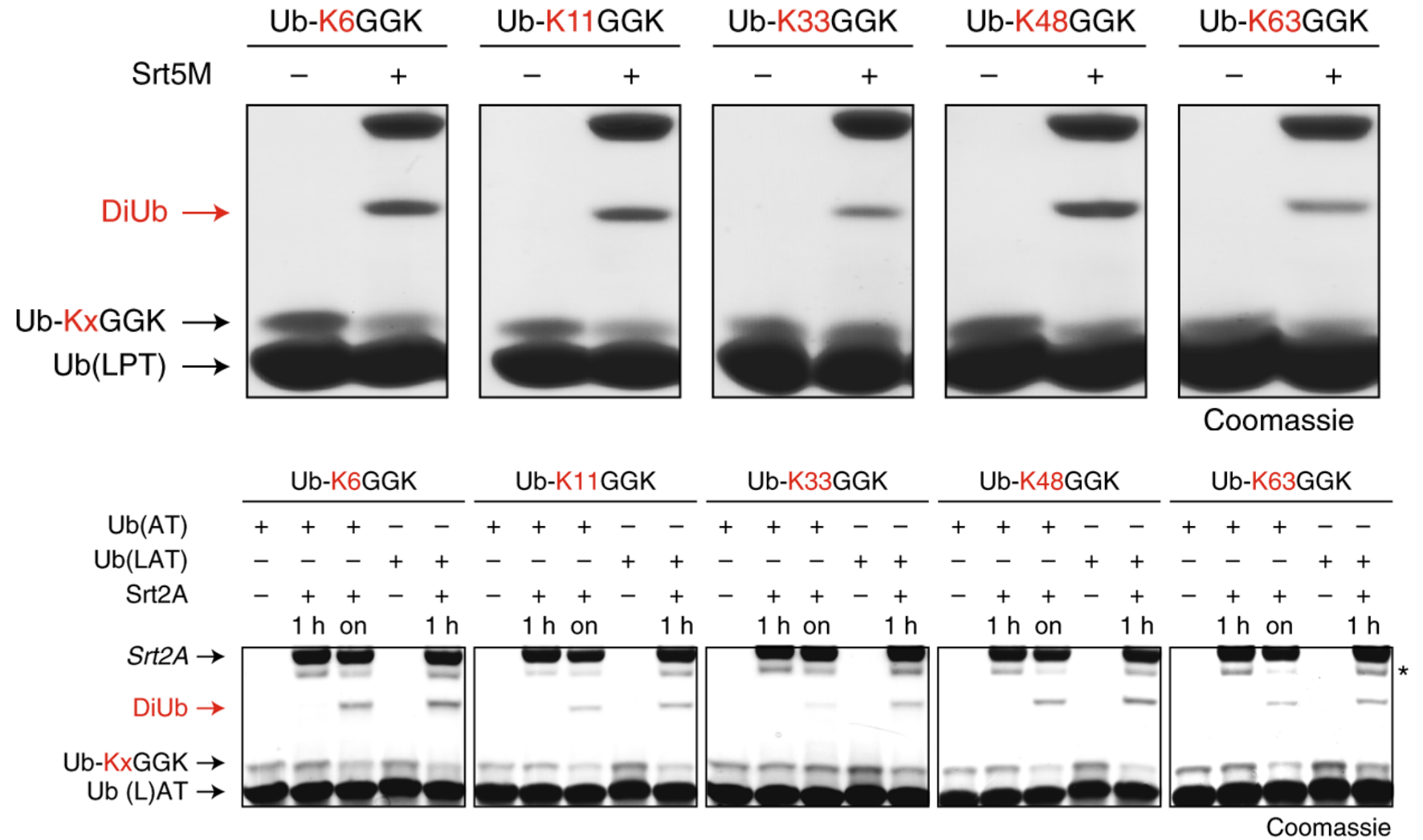
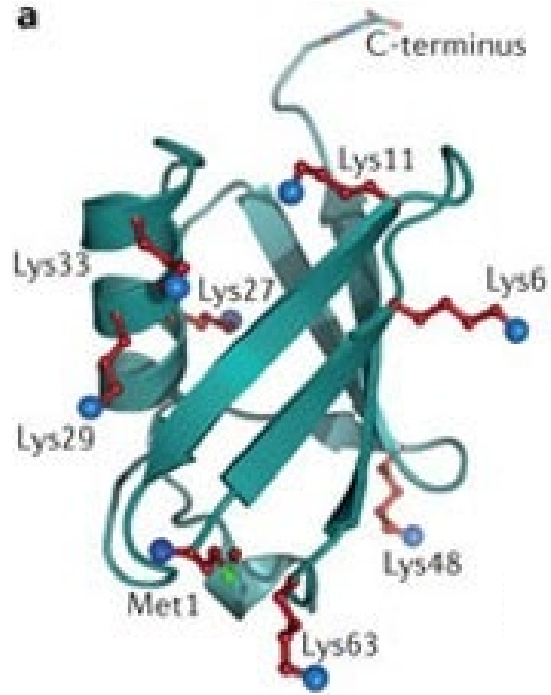
	$k_{\text{cat}} \times 10^{-5} \text{ (s}^{-1}\text{)}$	${}^{\text{N}}K_{\text{M}} \times 10^{-4} \text{ (M)}$	${}^{\text{S}}K_{\text{M}} \times 10^{-4} \text{ (M)}$	$k_{\text{cat}}/{}^{\text{N}}K_{\text{M}} \text{ (s}^{-1} \text{ M}^{-1}\text{)}$
^{Cd} SrtA	n.d.	n.d.	n.d.	n.d.
^{Cd} SrtA ^{3M}	5.6 ± 0.8	0.7 ± 0.1	20 ± 10	0.7 ± 0.1
^{Cd} SrtA ^Δ	40 ± 0.1	1.6 ± 0.4	16 ± 3	2.5 ± 0.6
^{Sa} SrtA	1600 ± 100	1.8 ± 0.1	73.3 ± 10.1	86 ± 5

- × Slow
- × Pilin Motif required

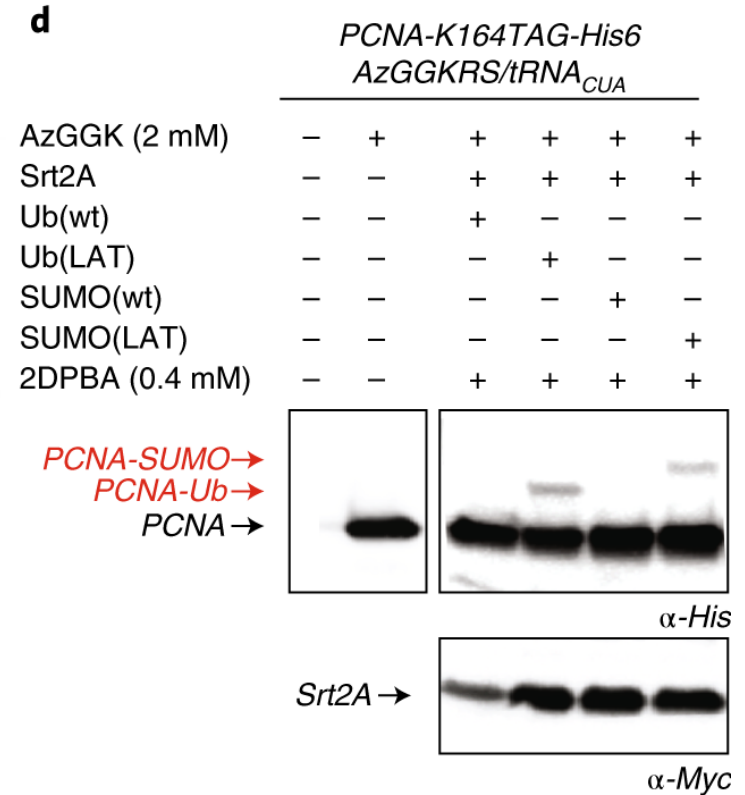
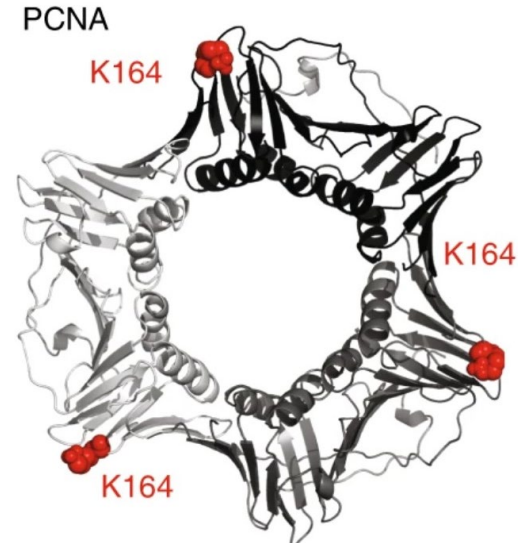
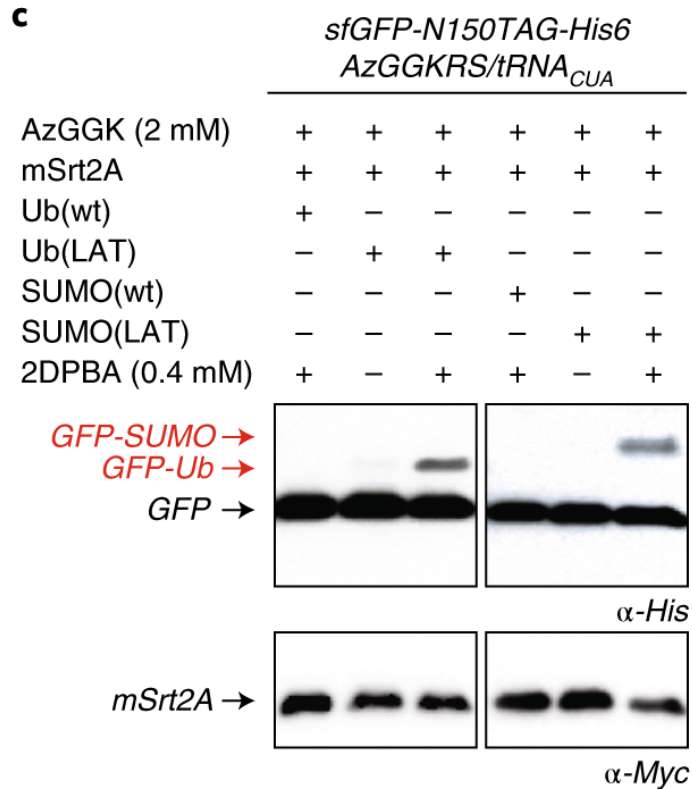
Unnatural Amino Acid



In vitro

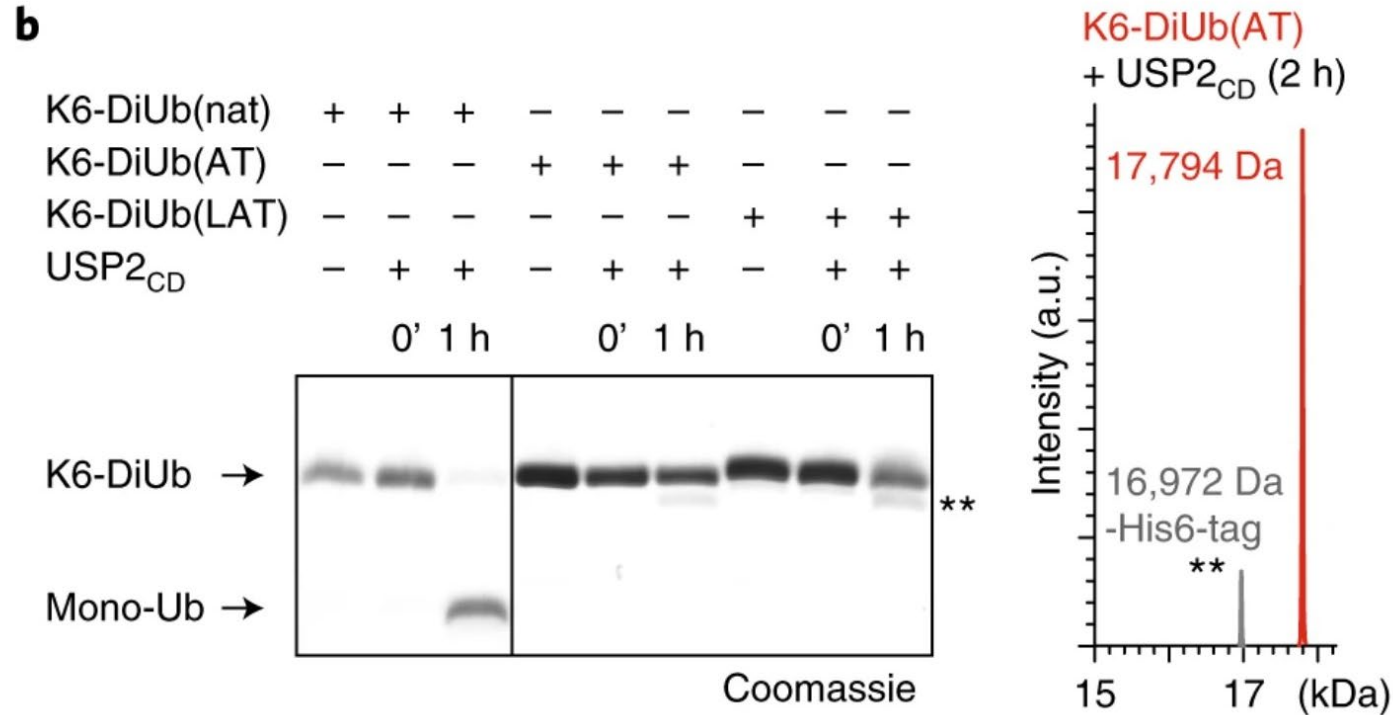


in cellulo



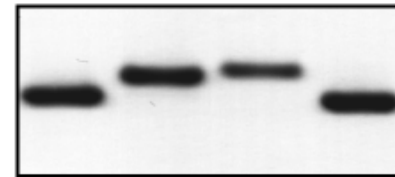
- Applicable for complex and multidomain

Resistant to DUB (deubiquitylase)

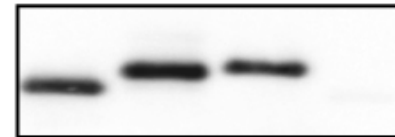


Structurally Equivalent to WT

K63-DiUb(wt)	+	-	-	-
K63-DiUb(AT)	-	+	-	-
K63-DiUb(LAT)	-	-	+	-
K48-DiUb(wt)	-	-	-	+



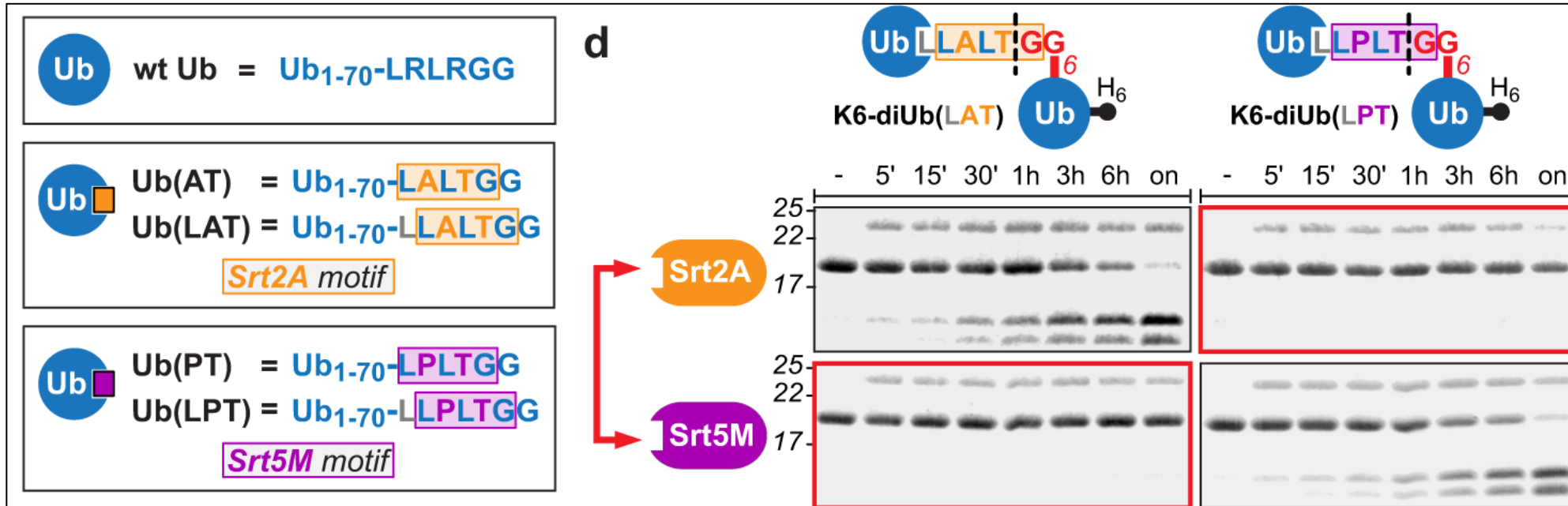
Coomassie



α -K63

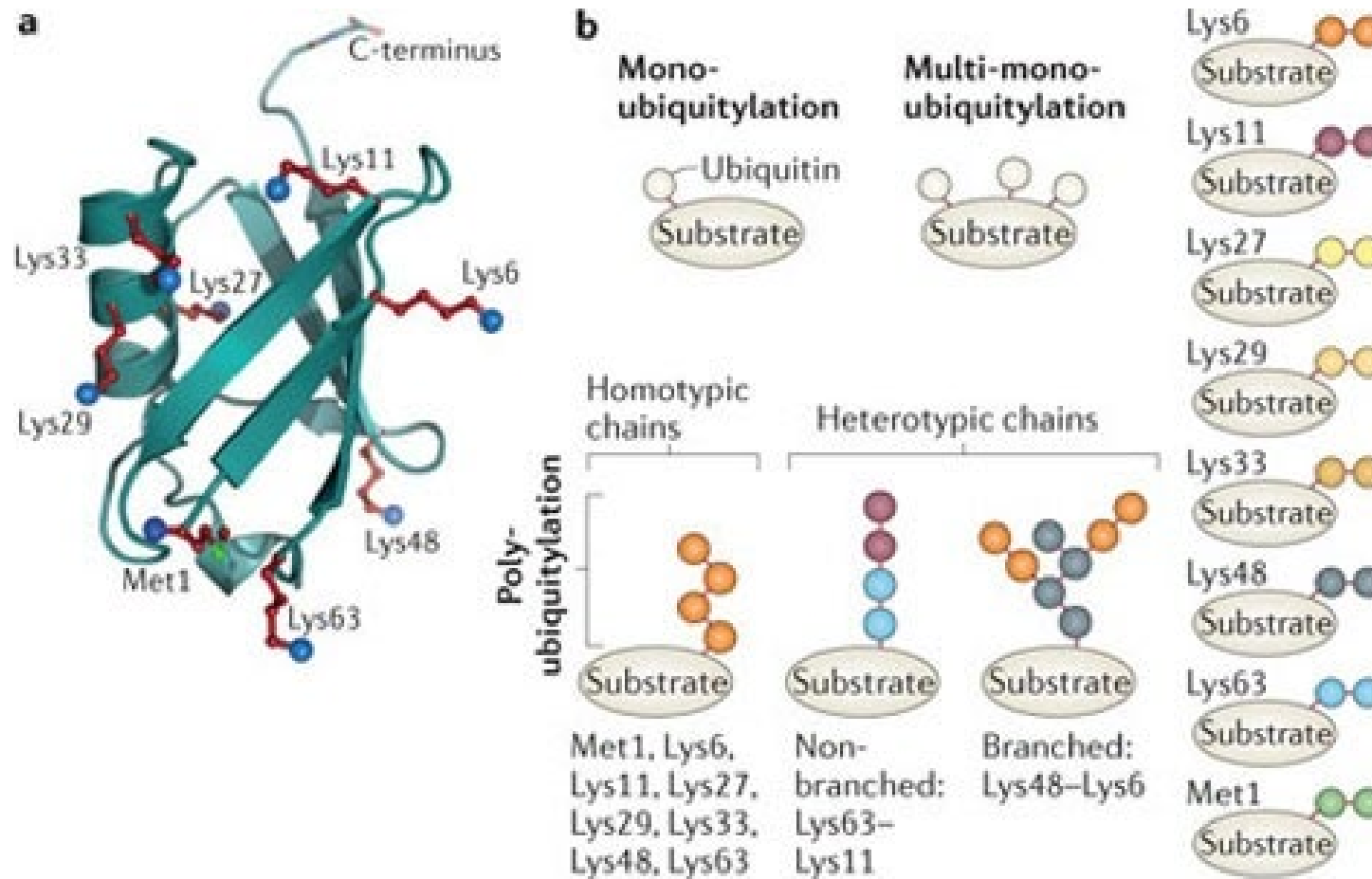
- Able to be recognized by antibody of WT

Type of SrtA

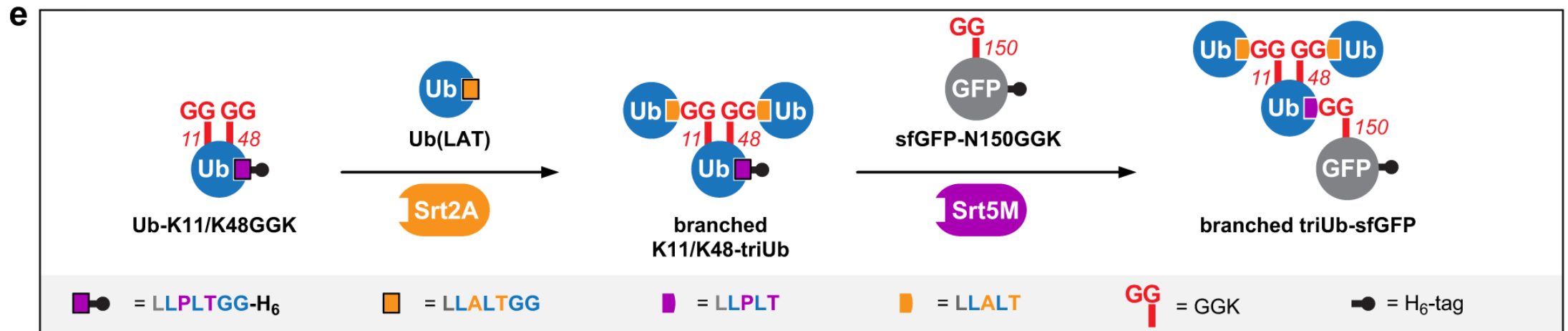
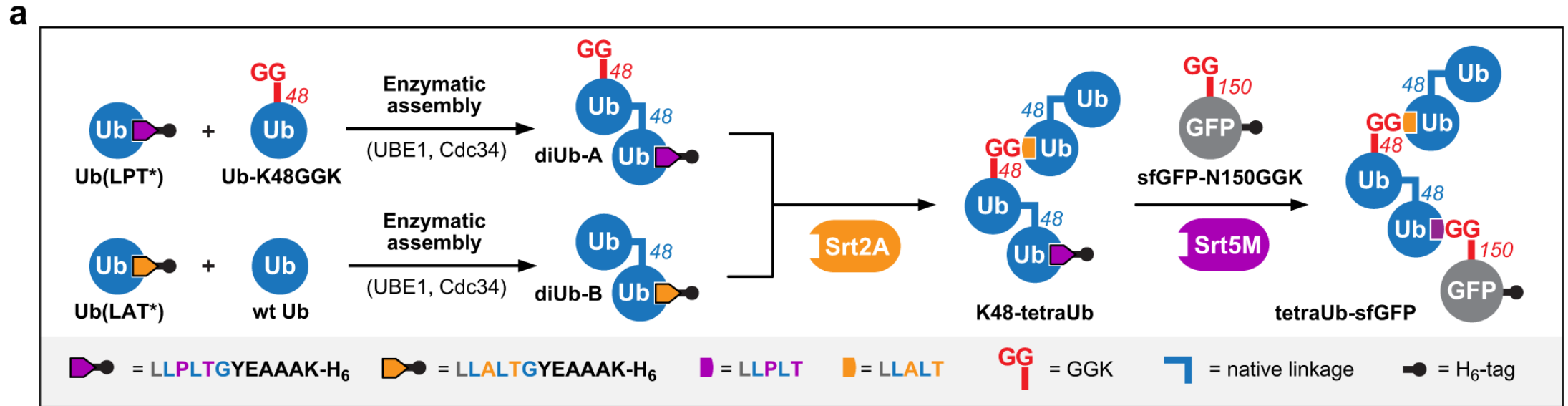


✓ Orthogonality of 2 types Sortase

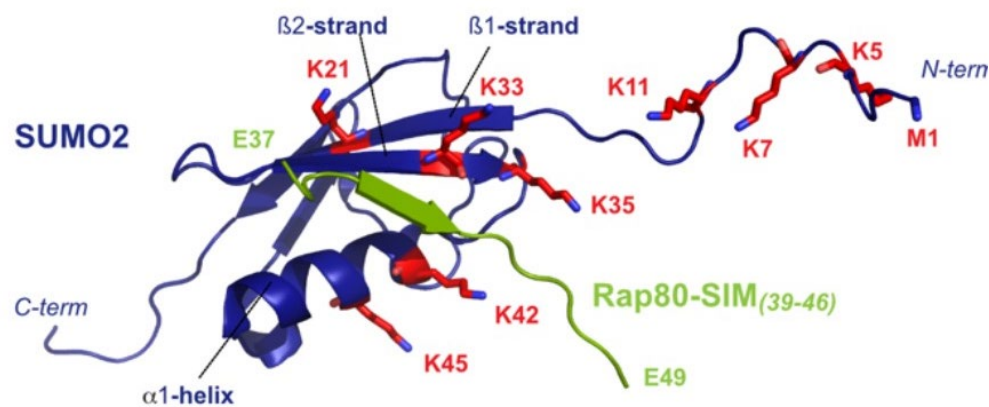
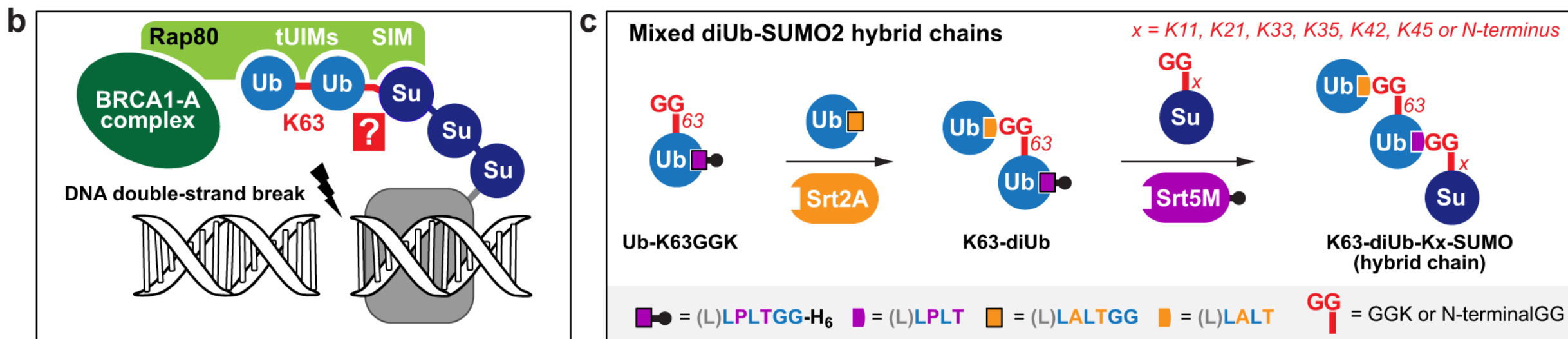
Structure of ubiquitylation



Ubiquitylation by SrtA

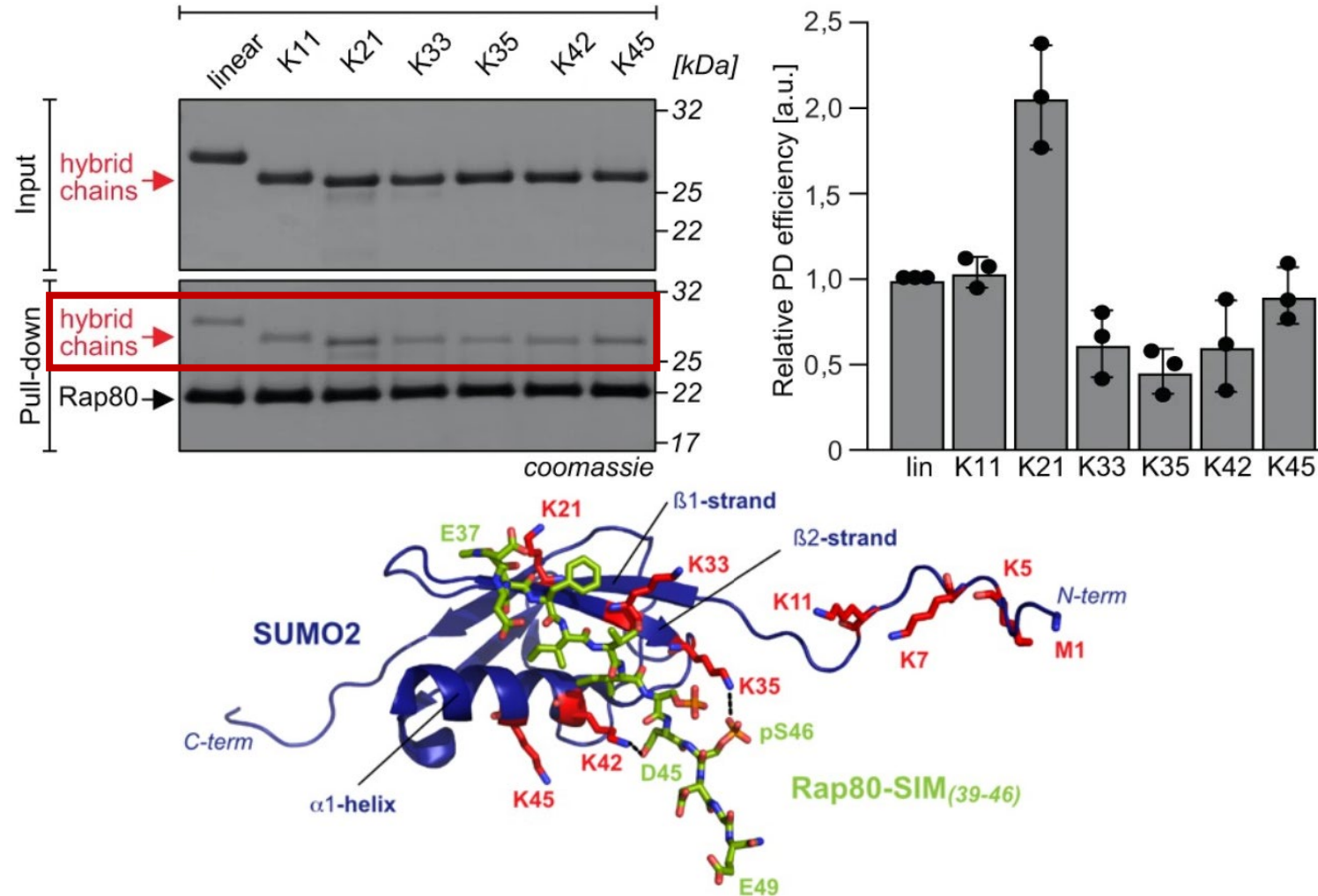


Study of SUMO binding site

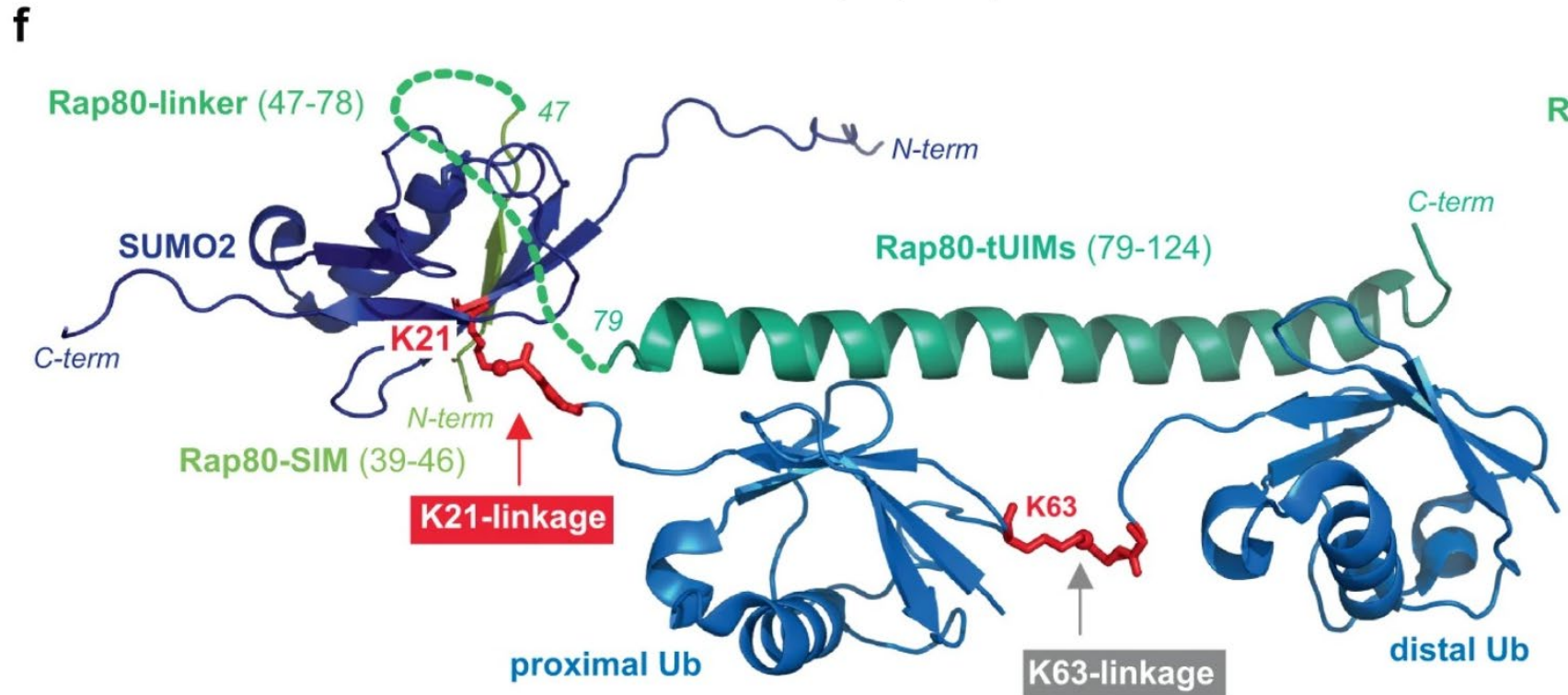


Study of SUMO binding site

b



Study of SUMO binding site

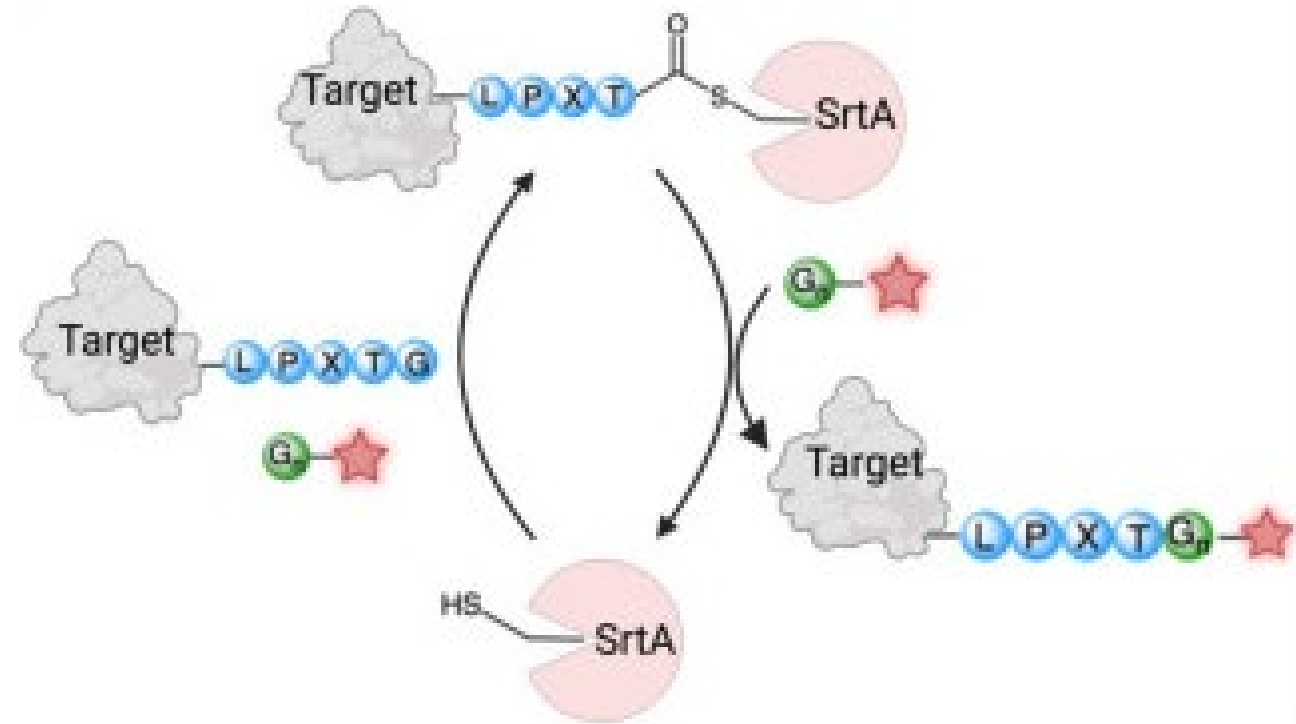


- K21-linked diUb-SUMO2 hybrid chain

Summary

- Sortase

- ✓ Mild Condition
- ✓ High Site Specificity
- ✓ Fast Kinetics
- ✓ High yield
- ✓ High Overexpression efficiency



- Generally, linking N-terminal to oligoglycine

Summary

Enzymatic Approach

- Using Sortase C as enzyme
 - ✓ In vitro
 - ✓ Dual labeling
- × Pilin motif required
- × Slow kinetics

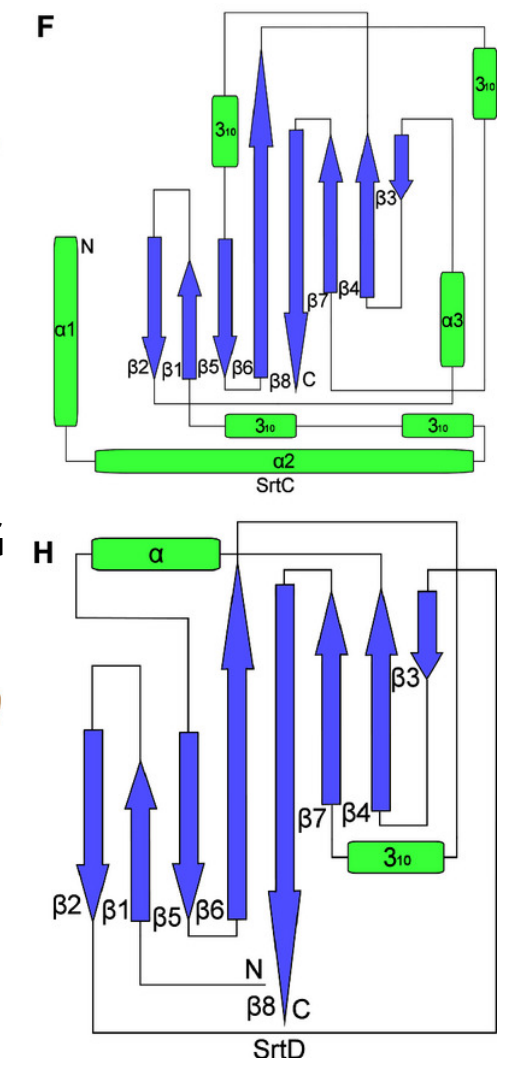
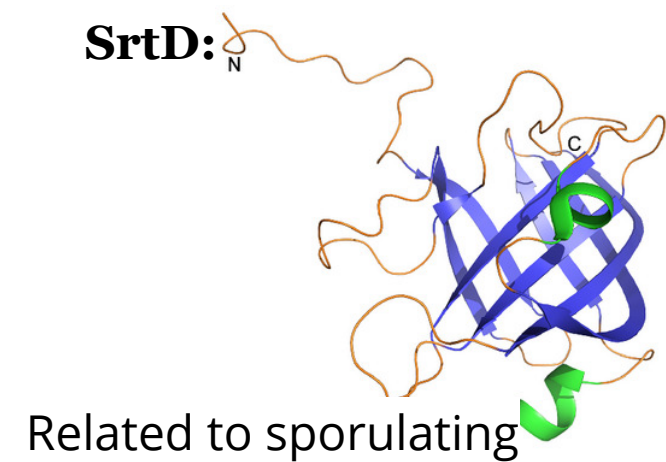
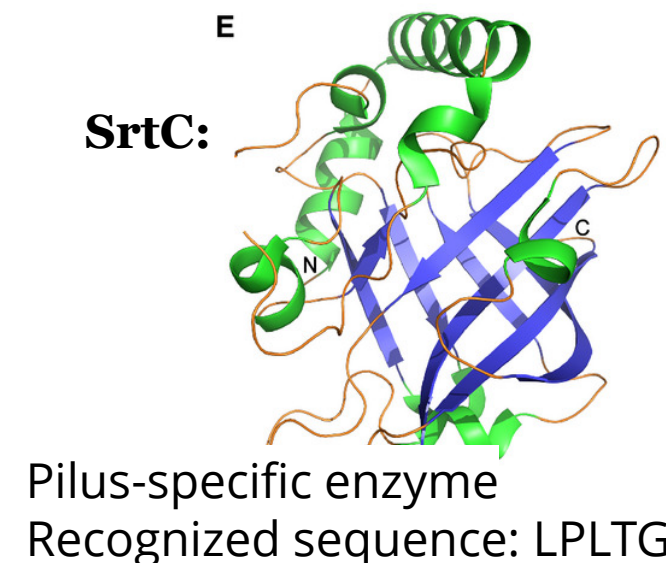
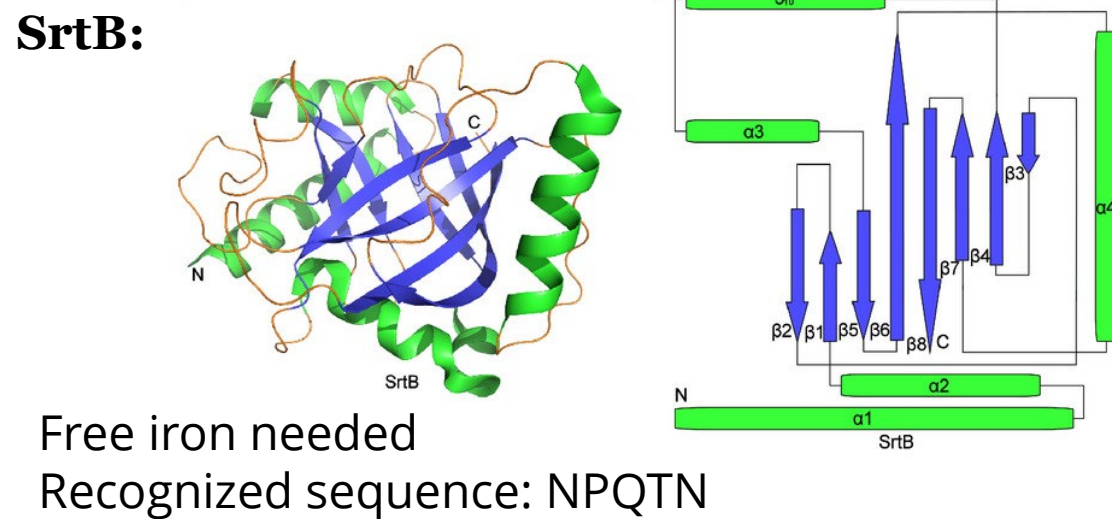
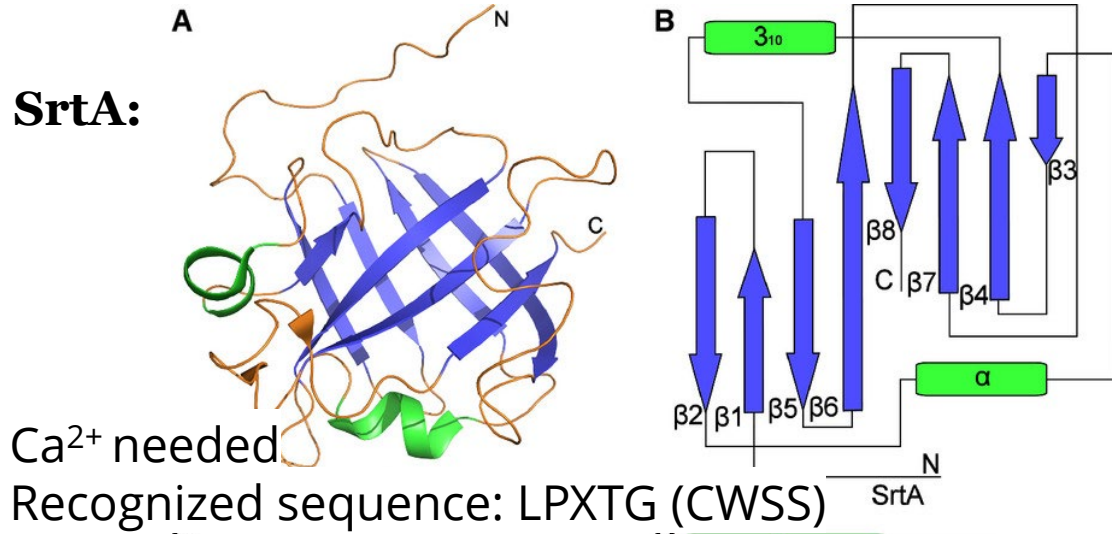
Acceptor altering approach

- Using unnatural amino acid as acyl acceptor
 - ✓ in vitro ✓ in cellulo
 - ✓ Dual labeling
- ✓ Applicable for complex and multidomain
- ✓ Resistibility to DUB
- ✓ Structurally Equivalent to WT

Thanks for Listening

Appendix

6 classes of sortase

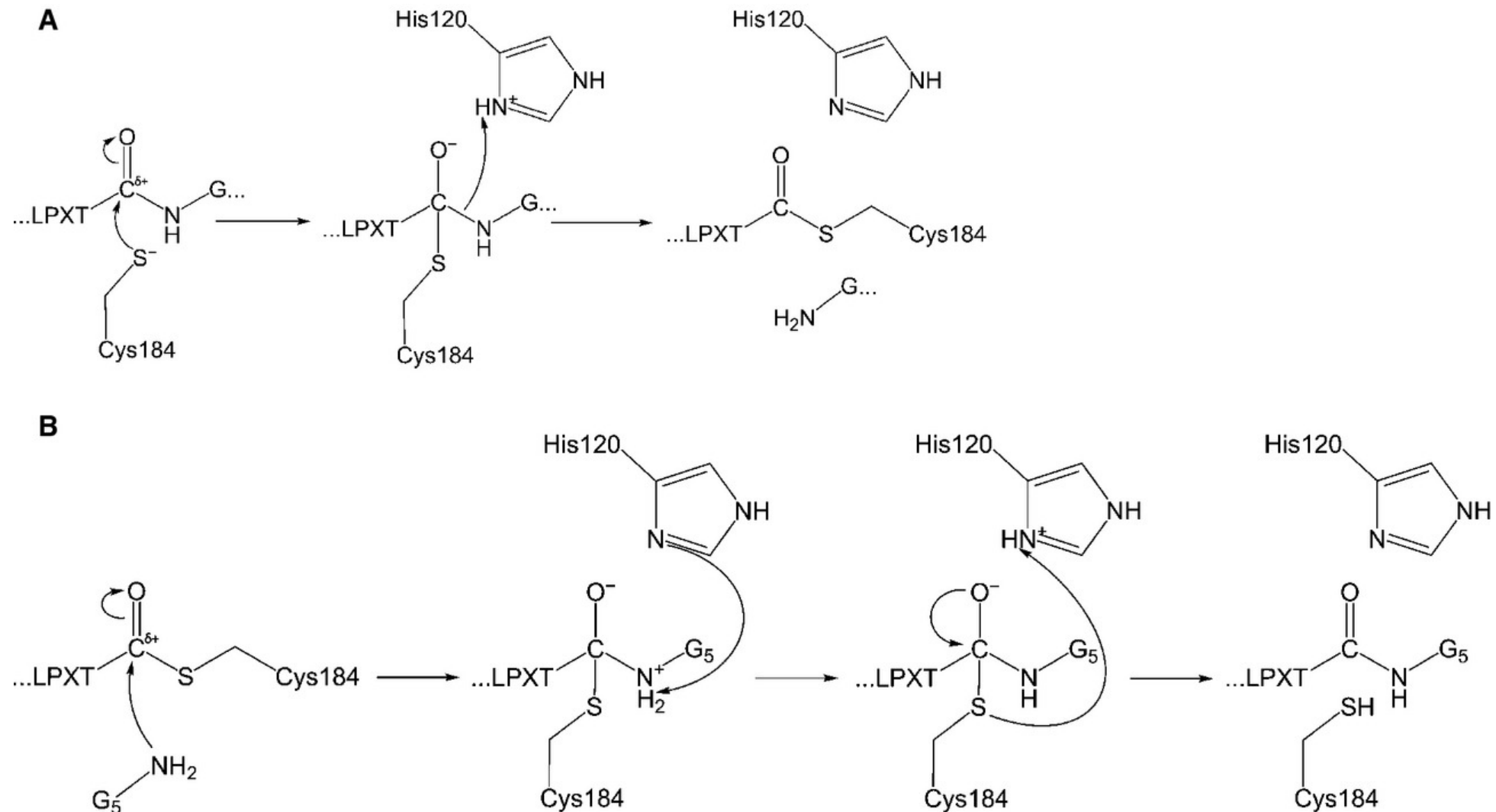


6 classes of sortase

SrtE: Housekeeping Sortase
Recognized sequence: LAXTG

SrtF: From *P. acnes*
No need of Ca
Recognized sequence: LPXTG

Reaction mechanism of SaSrtA



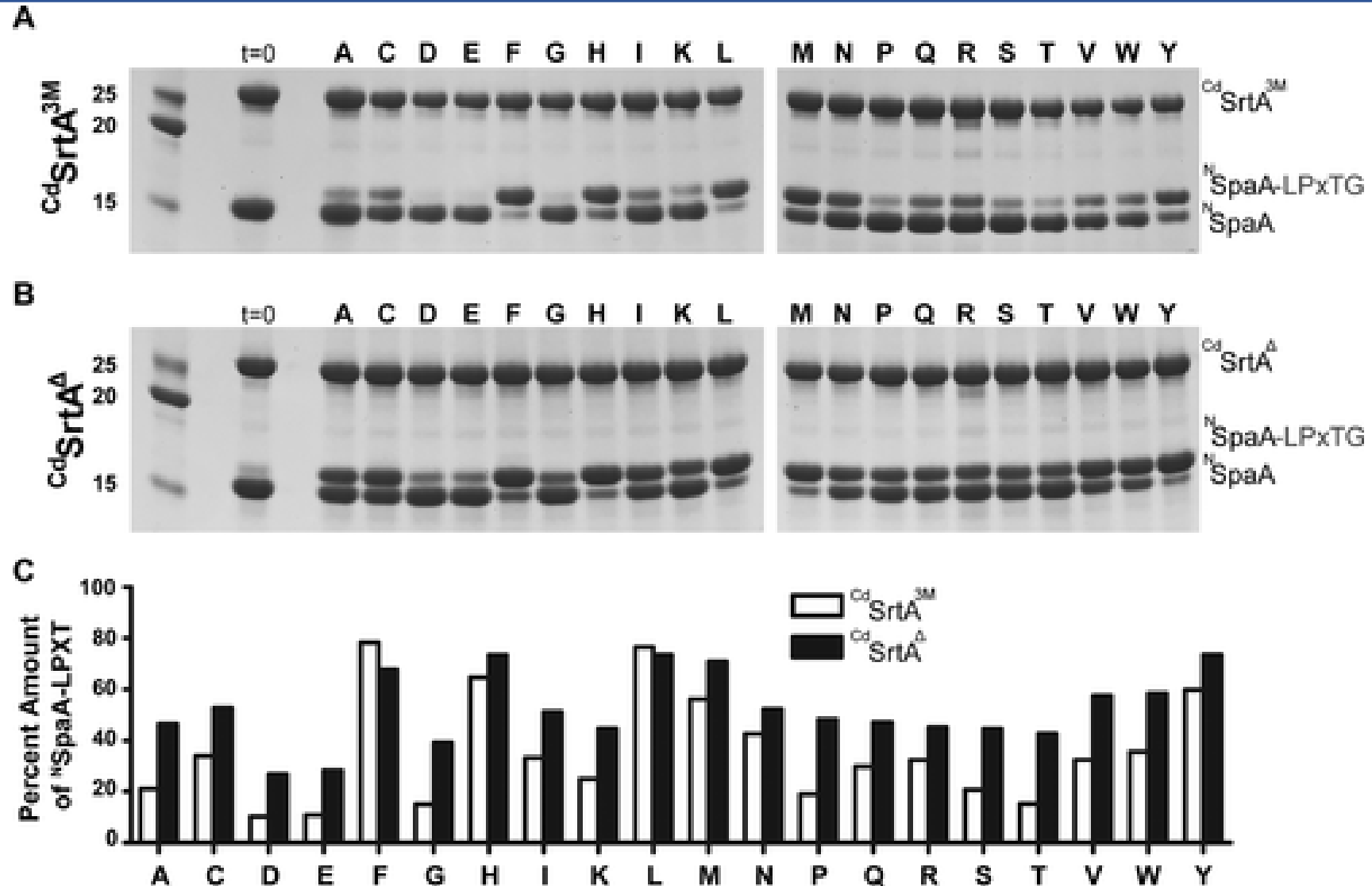
GG-motif

- Diglycine is necessary for nucleophile of SaSrtA

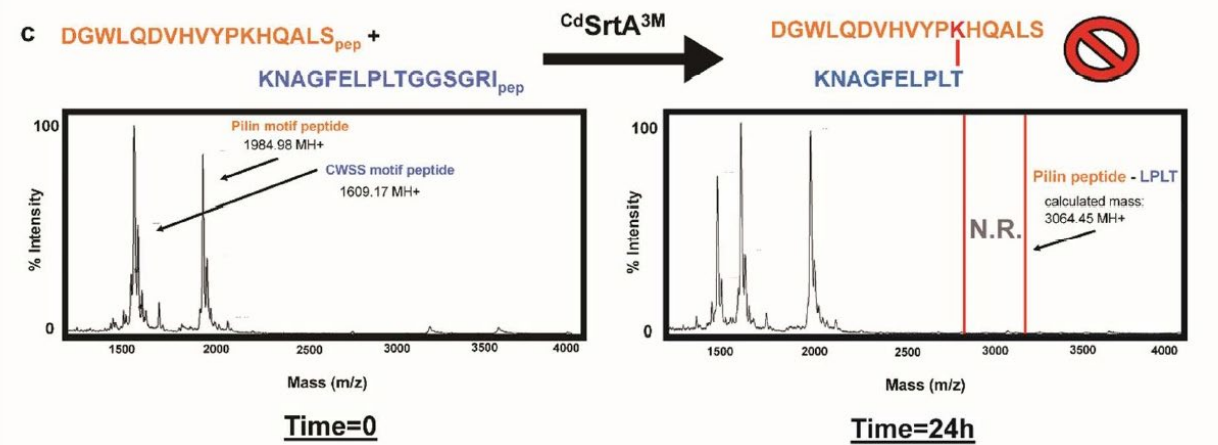
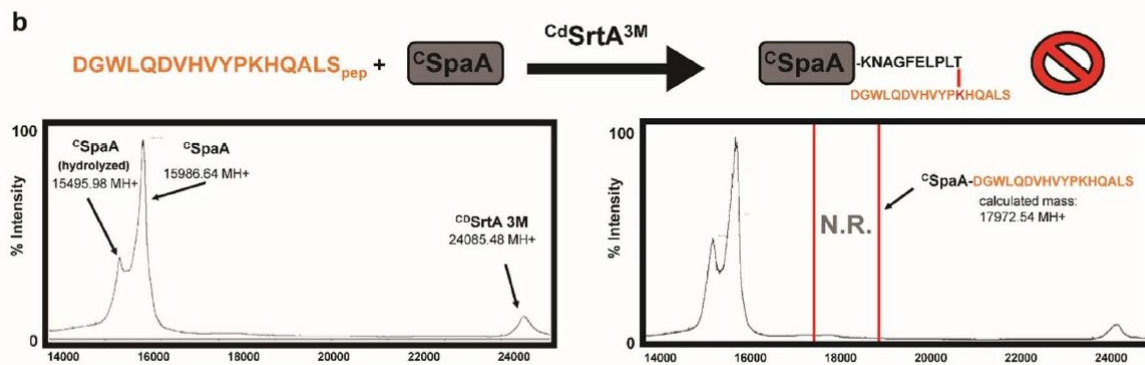
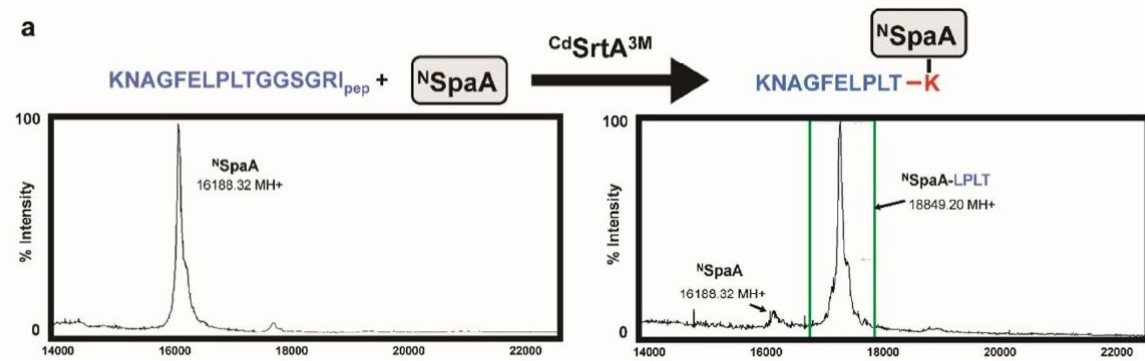
Table 1: Possible Nucleophiles for the Sortase Transpeptidation Reaction^a

nucleophile	$K_{m,app}$ (μ M)	V_m (μ M/min)
GlyGlyGlyGlyGly	not tested	
GlyGlyGlyGly	16 ± 2	0.11 ± 0.01
GlyGlyGly	24 ± 4	0.09 ± 0.01
GlyGlyHis	20 ± 2	0.11 ± 0.01
GlyGlyLeu	35 ± 5	0.10 ± 0.01
GlyGly	30 ± 4	0.10 ± 0.01
GlyAla	127 ± 22	0.10 ± 0.01
GlyVal	208 ± 28	0.09 ± 0.01
AlaGly	NS ^b	
ValGly	NS ^b	
AlaAla	NS ^b	
Gly	NS ^b	
Ac-Gly	NS ^b	
Gly-NH ₂	23 ± 2	0.12 ± 0.01
DTT	NS ^c	
BME	NS ^c	

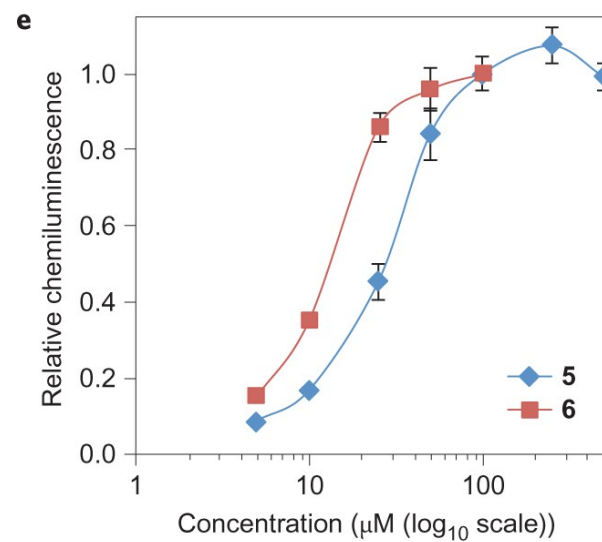
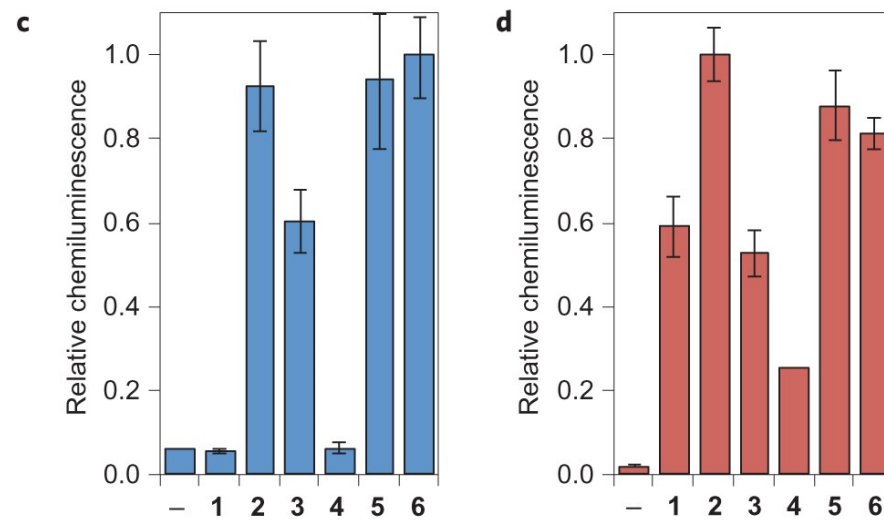
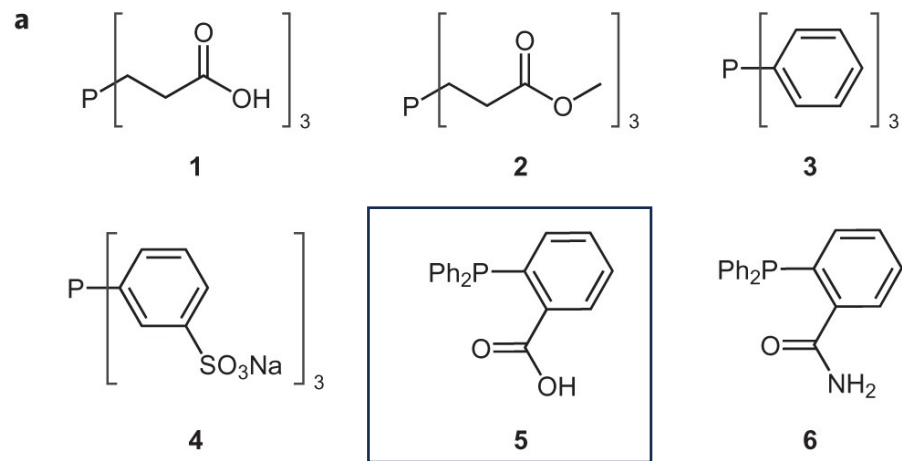
Signal sequence of CdSrtA^{3M} & CdSrtA^Δ



Pilin motif



2DPBA



Srt5M

- P94R/D160N/D165A/K190E/K196T

	$k_{\text{cat}}, \text{ s}^{-1}$	$K_m \text{LPETG}, \text{ mM}$	$k_{\text{cat}} / K_m \text{LPETG}, \text{ M}^{-1} \text{ s}^{-1}$	$K_m \text{GGG-COOH}, \mu\text{M}$
WT(SaSrtA)	1.5 ± 0.2	7.6 ± 0.5	200 ± 30	140 ± 30
P94R/D160N/D165A/K190E/K196T (5M)	5.4 ± 0.4	0.23 ± 0.02	$23,000 \pm 3,000$	$2,900 \pm 200$

Srt5M & Srt2A

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Srt2A      MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVCFH105DENESLDD108Q112ONISIA
mSrt2A    MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVCFH105KENQSLDD108Q112ONISIA
wtSrtA    MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATPEQLNRGVSF105AENESLDD108Q112ONISIA
Srt5M     MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVSF105AENESLDD108Q112ONISIA
Srt7M     MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVSF105AKENQSLDD108Q112ONISIA
*****
Srt2A      GHTFIDRPNYQFTNLKAAKPGSMVYFKVGN171ETRIYKMTSIRKVHPNAVEVLDEQEGKDKQ
mSrt2A    GHTFIDRPNYQFTNLKAAKPGSMVYFKVGN171ETRIYKMTSIRKVHPNAVEVLDEQEGKDKQ
wtSrtA    GHTFIDRPNYQFTNLKAAKPGSMVYFKVGN171ETRYKMTSIRDVKPTDVGVLDEQEGKDKQ
Srt5M     GHTFIDRPNYQFTNLKAAKPGSMVYFKVGN171ETRYKMTSIRNVKPTAVEVLDEQEGKDKQ
Srt7M     GHTFIDRPNYQFTNLKAAKPGSMVYFKVGN171ETRYKMTSIRNVKPTAVEVLDEQEGKDKQ
*****
Srt2A      LTLVTCDDYNEETGVWESRKIFVAT  KGS      50
mSrt2A    LTLVTCDDYNEETGVWESRKIFVAT  KGS      50
wtSrtA    LTLITCDDYNEKTGVWEKRKIFVAT  KLE      50
Srt5M     LTLITCDDYNEETGVWETRKIFVATEVKLE  150
Srt7M     LTLITCDDYNEETGVWETRKIFVATEVKLE  150
***:*****:*****_*****

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NMR of K21

