Imperial College London

MRC Centre for Molecular Bacteriology and Infection

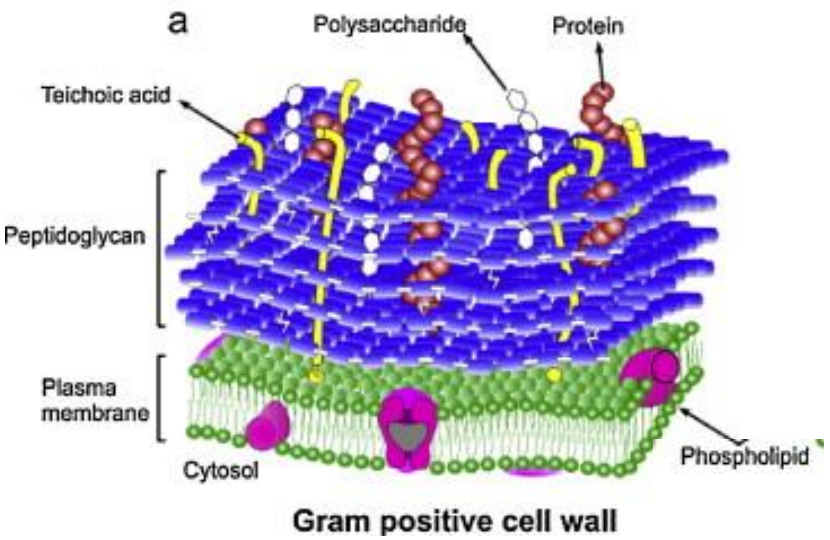
Alexandra Shaw

Sortase in *Clostridium difficile*

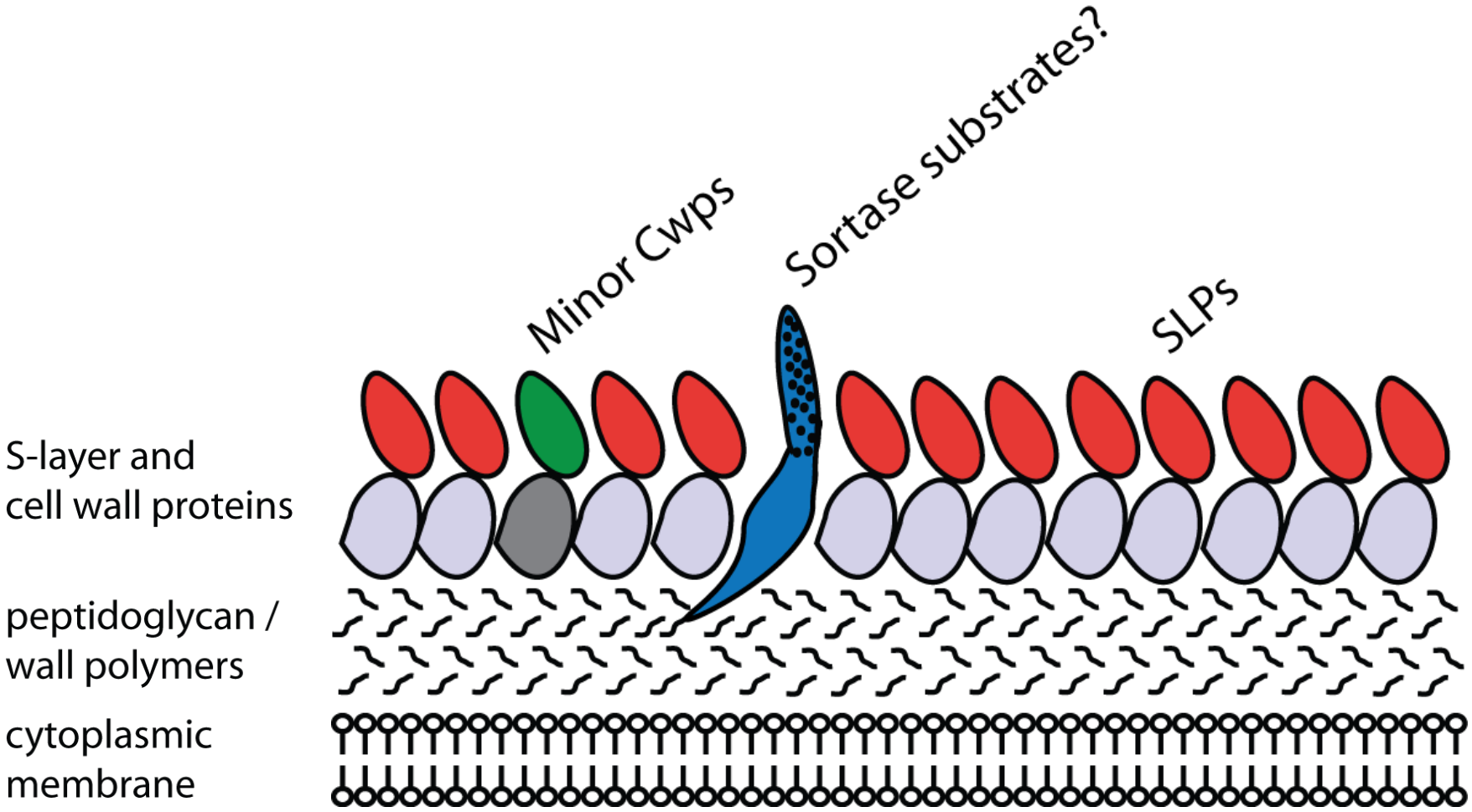
Is there more than the S-layer?!

Sortase in Gram positive bacteria

- Membrane bound enzyme
- Covalently attaches its substrates to the peptidoglycan cell wall
- Sortase substrates are major surface proteins and virulence factors in Gram positive bacteria
- *C. difficile* has an S-layer and other surface proteins
  - are sortase substrates expressed?
  - does *C. difficile* have a functional sortase?
  - are sortase substrates important in virulence?

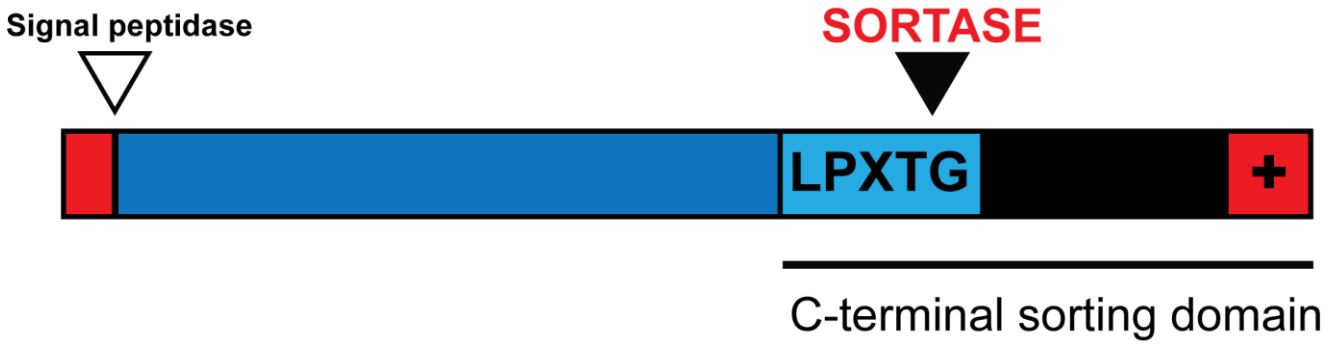


Surface of *C. difficile*



Sortase and its substrates in Gram positive bacteria

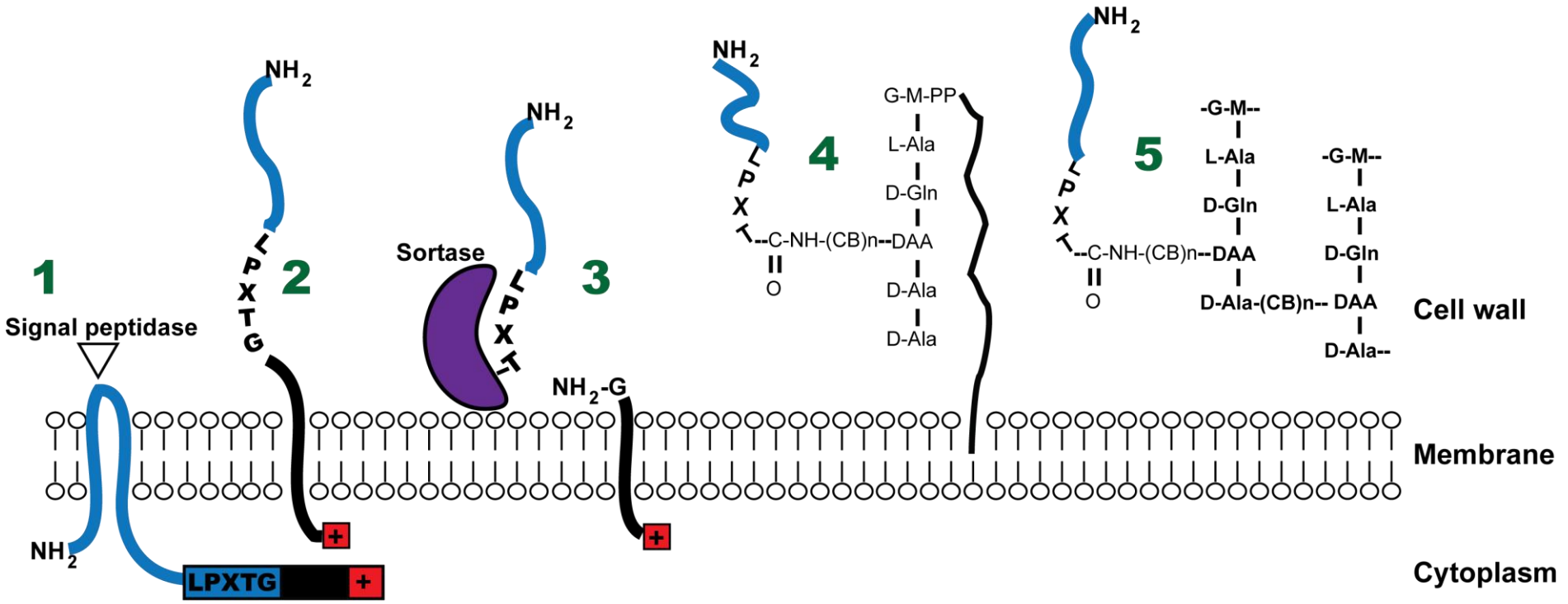
- Sortases mediate attachment of proteins to the peptidoglycan cell wall



- Recognise C-terminal sorting domain of substrates
  - "LPxTG"-like motif
  - Hydrophobic region
  - Charged tail

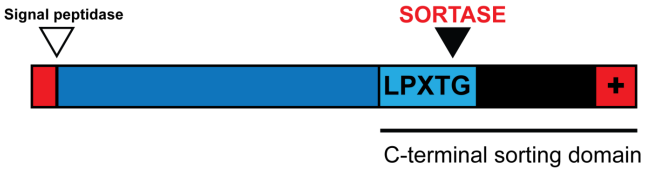
- 6 different classes of sortase; A - F

Action of sortase in *Staphylococcus aureus*



Adapted from Ton-That et al

Sortase and substrates in C. *difficile* 630



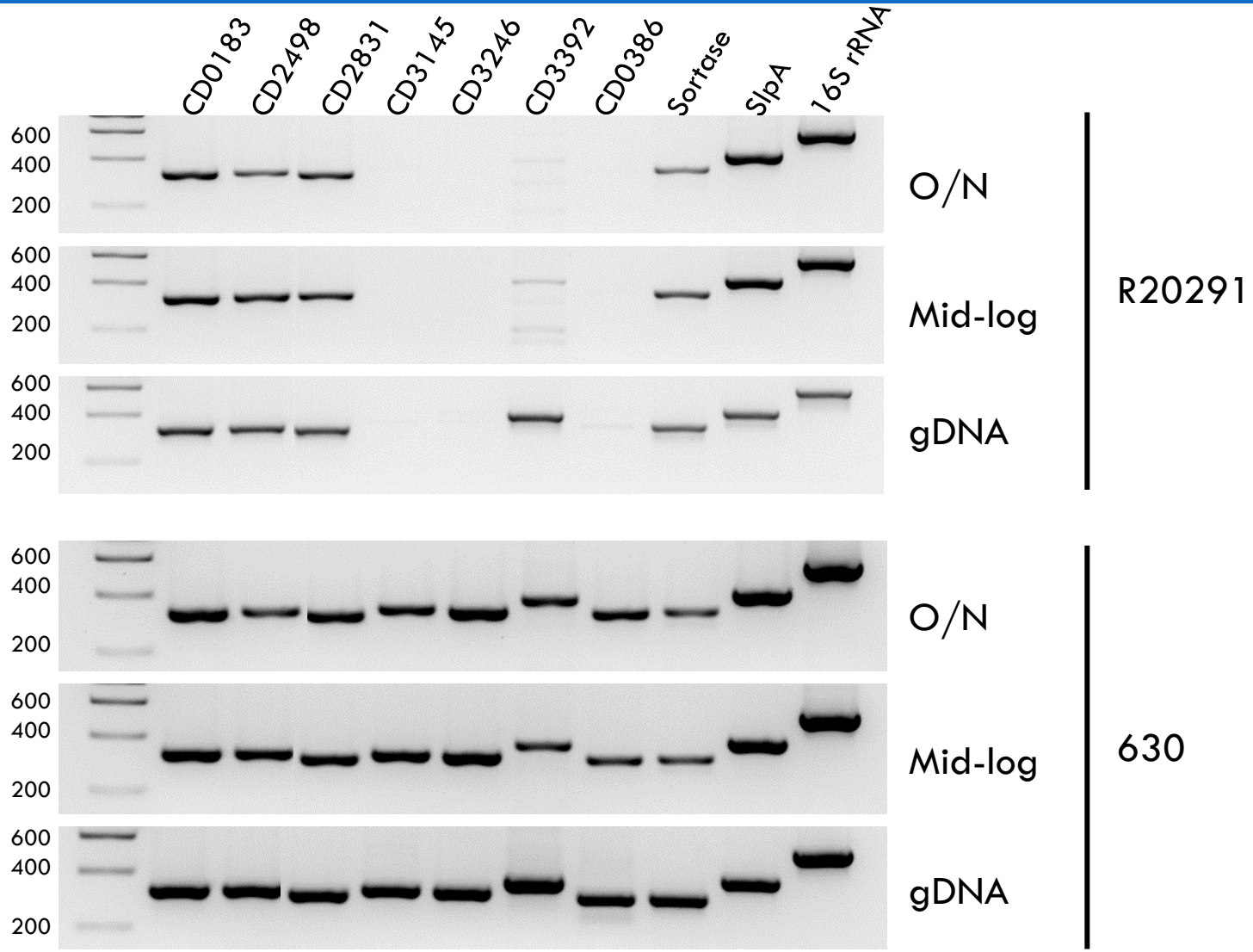
- 7 potential sortase substrates identified

CD0183	Putative cell wall hydrolase
CD2498	Putative D-alanyl-D-alanine carboxypeptidase. Putative sporulation-specific penicillin-binding protein
CD2831	Putative collagen-binding protein
CD3145	Putative serine-aspartate-rich surface anchored fibrinogen-binding protein
CD3246	Putative surface protein
CD0386	Putative collagen-binding protein, Cna protein B-type domain protein
CD3392	Putative collagen-binding protein, Cna protein B-type domain protein

SPA	...LPETG...EEN....PFIGTTVFGGLSLALGAALLAG.....RRREL
BcpA	...LPVTG...GIGT...TLFTVIGLTLMLTAAFVFF.....RKKFARN

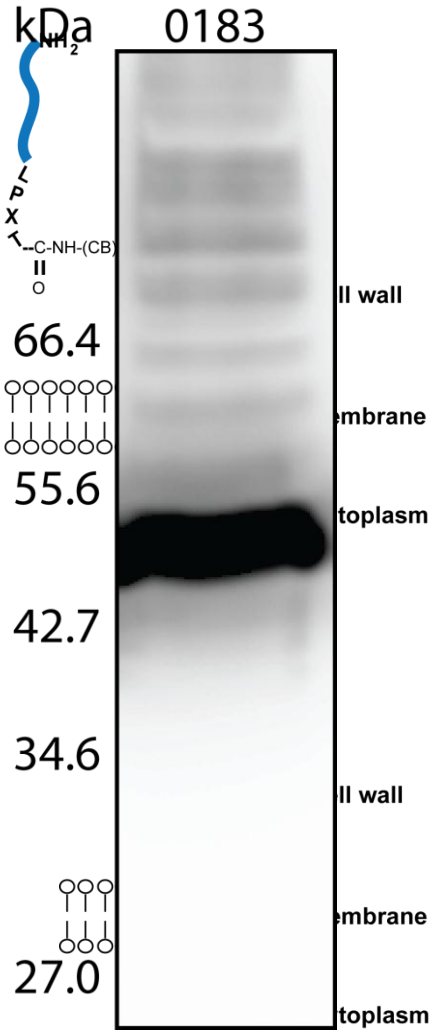
- Two copies of sortase B
  - Sortase genes
    - CD2718 – conserved in all strains; full length
    - CD3146 – contains an in frame stop codon; truncated

Transcription of sortase substrates by RT PCR

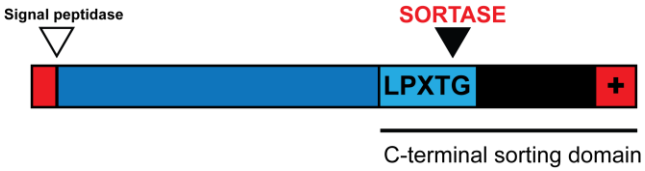


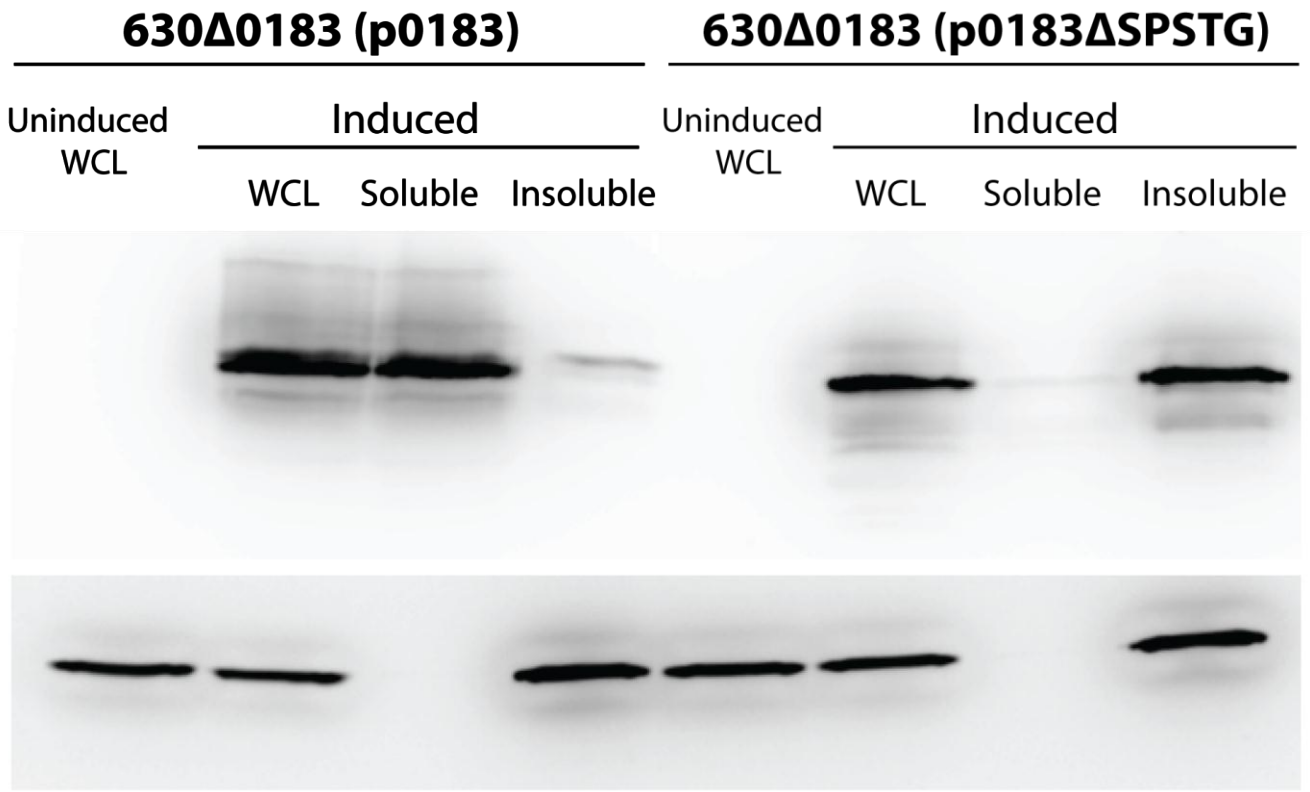
Does C. *difficile* have a functioning sortase?

- Sortase substrates:
  - are covalently attached to the peptidoglycan cell wall using "LPxTG" motif
  - are present in the soluble fraction of cell lysates if cell wall hydrolysis occurs
  - are present in the insoluble fraction of cell lysates if retained in the membrane (i.e. in LPxTG mutations or sortase not active)
- This was investigated using the putative sortase substrate CD0183 in strain 630::erm0183



CD0183 is a sortase substrate

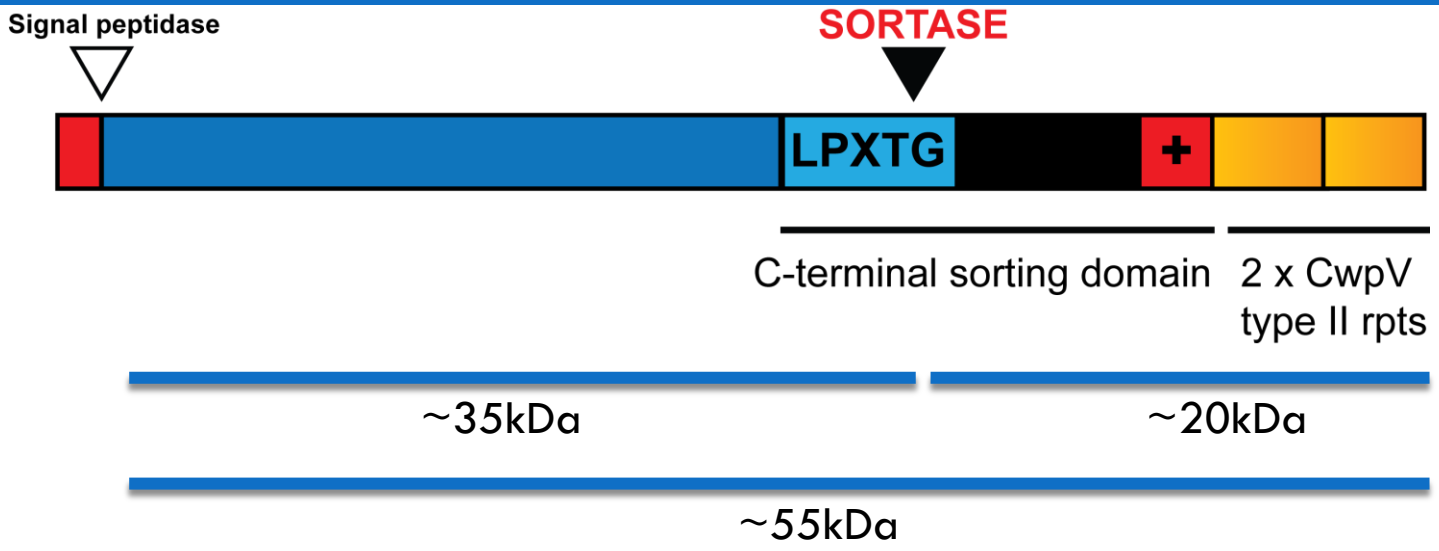


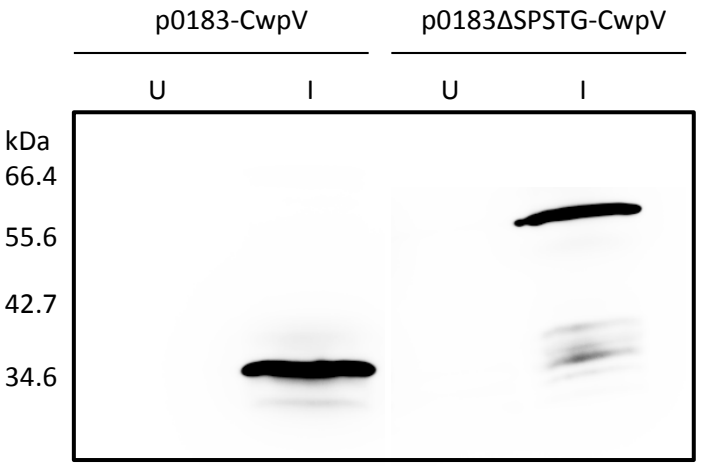


Confirms CD0183 as a sortase substrate

Sortase-mediated cleavage not directly demonstrated

Sortase cleavage of CD0183



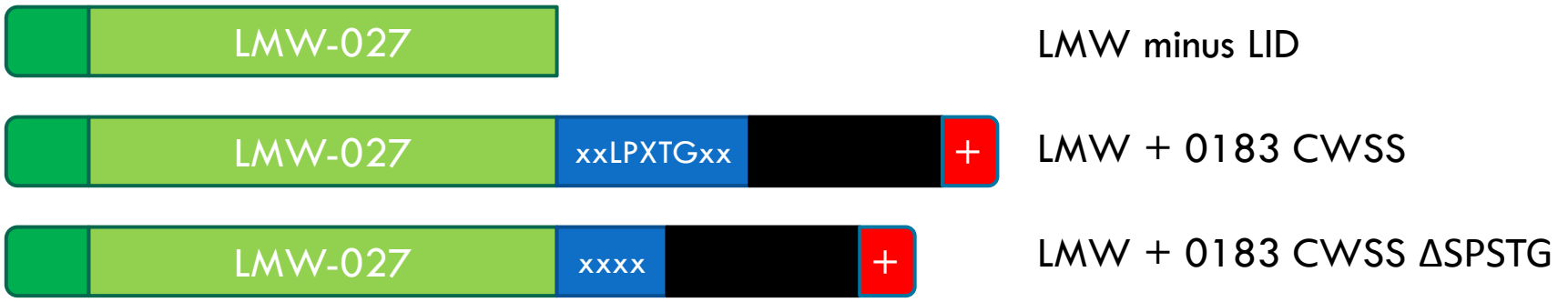


α-CD0183

LMW covalent attachment to cell wall in C. *difficile* 630

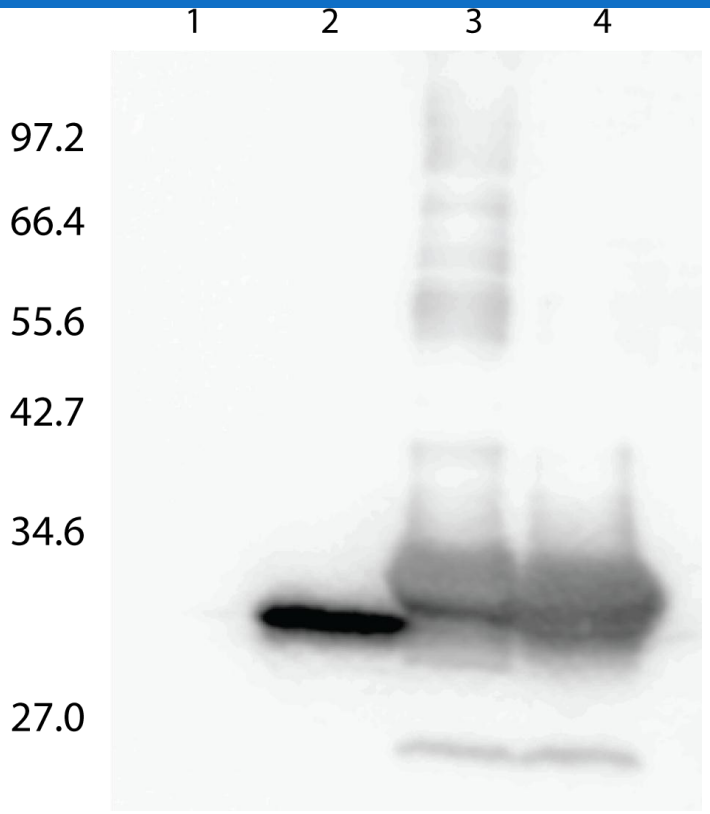
- If this system is active, one should be able to covalently attach other proteins to the cell wall

- Fusion protein created:
  - LMW SLP from 027 without LID domain
    - Antigenically distinct from 630 LMW
    - Won't interact with 630 S-layer
  - CWSS from 0183 attached to C-terminus



LMW covalent attachment to cell wall

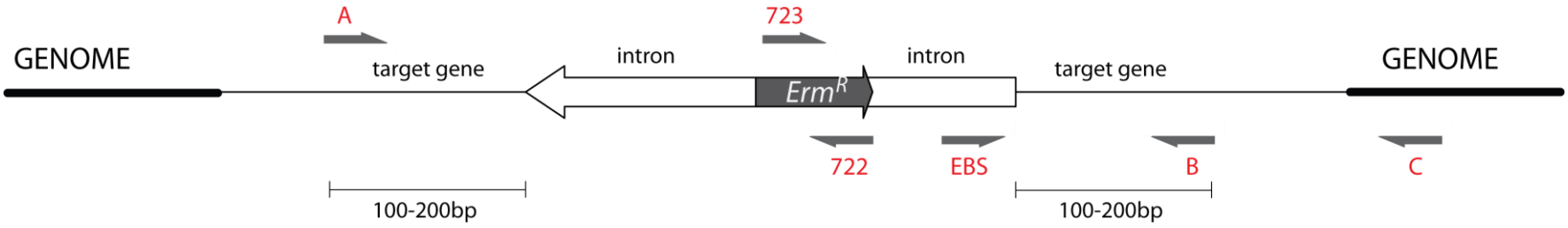
- LMW, LMW-0183CWSS and LMW-0183ΔSPSTG expressed on plasmids under the inducible pTet promoter (Fagan 2011)
- Whole cell lysates confirm expression of all plasmids
- Addition of 0183 CWSS produces a banding pattern
  - Suggestive of covalent attachment to the cell wall
  - Absent for LMW alone
  - Largely absent for LMW with SPSTG removed



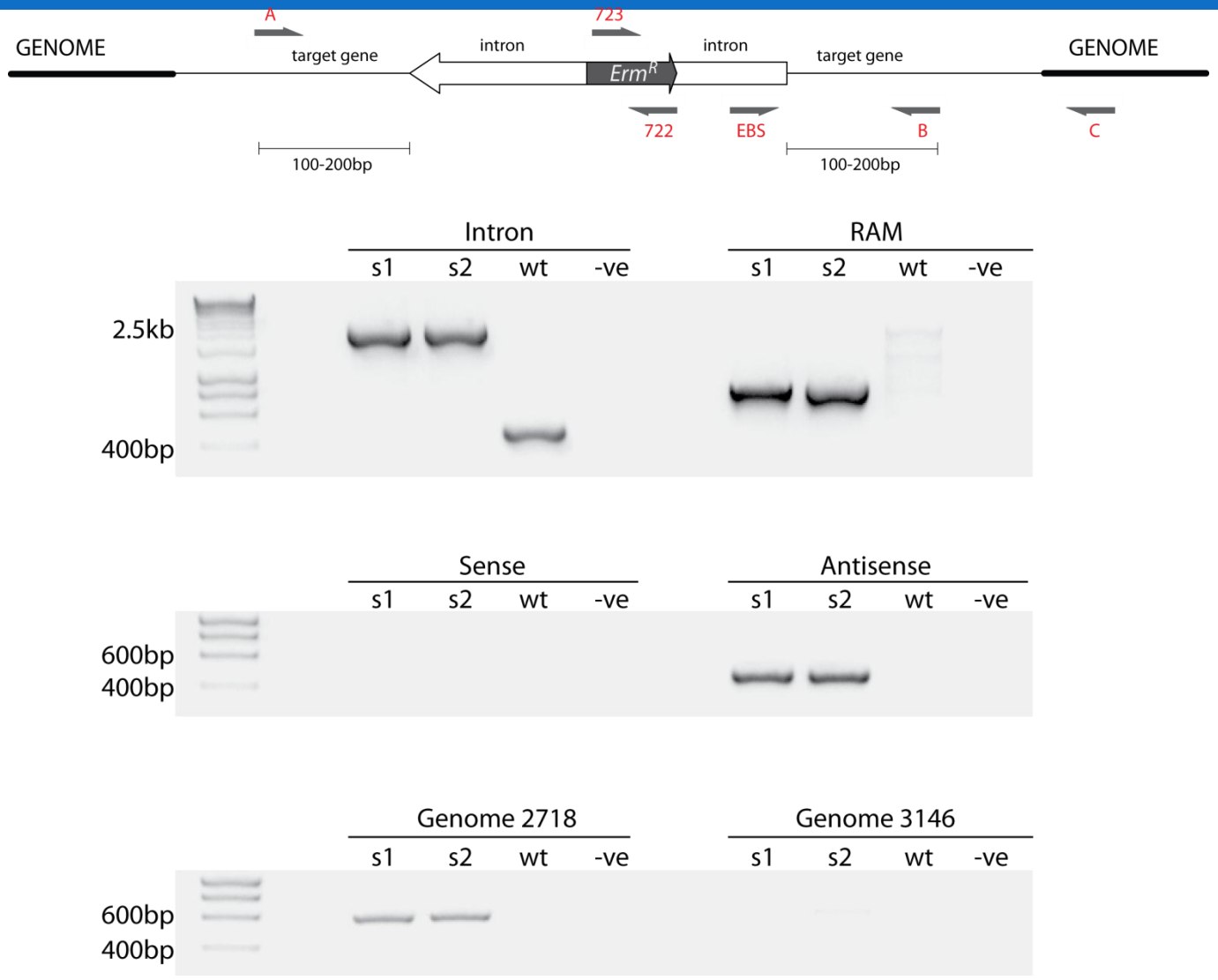
1 = wild type 630Δerm
2 = LMW
3 = LMW-0183 CWSS
4 = LMW-0183 CWSS ΔSPSTG

Creation of a sortase insertional mutant

- Further studies would be enhanced by use of a sortase mutant
- CD2718 targeted at position 354
- Erm resistant colonies screened for intron insertion
  - Primers A + B
- Screened to determine insertion into CD2718 rather than CD3146 (pseudo-gene)
  - Primers EBS + C



Creation of a sortase insertional mutant



Conclusions and further work

- Identified 7 potential sortase substrates
- Shown that the sortase anchoring system is active in C. *difficile*
- Created a knockout in CD2718 sortase
  - Further investigate 0183 cleavage
  - Characterise sortase mutant *in vitro* and *in vivo*
  - Investigate anchor of sortase substrates in C. *difficile*
  - Determine whether the other putative substrates are true substrates

Imperial College London

Thank you!

MRC Centre for Molecular Bacteriology and Infection

- Fairweather Group
  - Neil Fairweather
  - Robert Fagan

ESCMID

BBSRC bioscience for the future

NOVARTIS