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How do bacteria change their coats?

Structural analysis of acyltransferases involved in O-antigen modification

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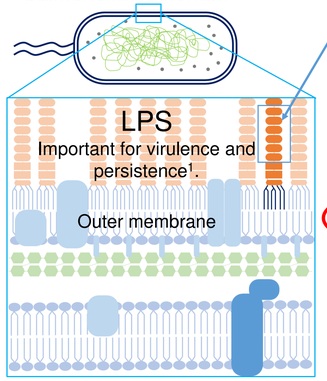
Research Aims

Structural characterisation of SGNH domains from OafA and OafB.

Understand mechanism of action of acyltransferases.

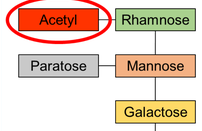
Background

Salmonella bacterium

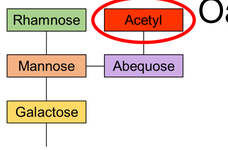


O-antigen made up of repeating units called **O-units.**

Bacteria modify the O-antigen by acetylation to increase diversity[2].



Salmonella ser. Paratyphi A O-unit

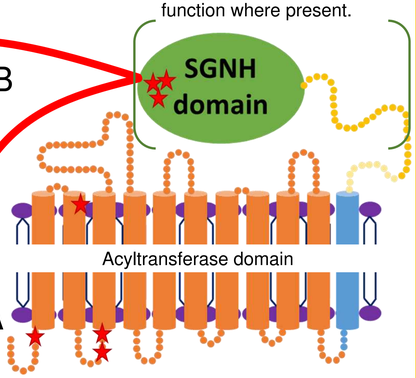


Salmonella ser. Typhimurium O-unit

OafB

OafA

SGNH domain
Not always present, but required for function where present.



OafA and OafB

add acetyl groups to abequose[3] and rhamnose[4] respectively. Both have attached SGNH domains.

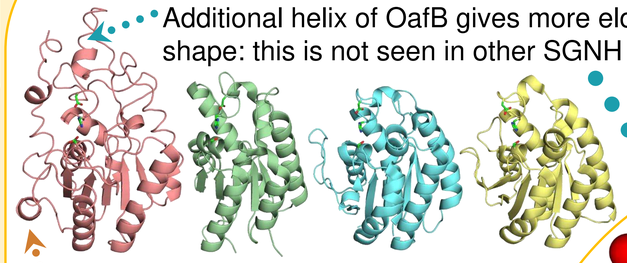
Acyltransferase family III domain

Involved in modification of:

- LPS
- Peptidoglycan
- Macrolide antibiotics
- Root nodulation

OafB-SGNH domain has a unique structure

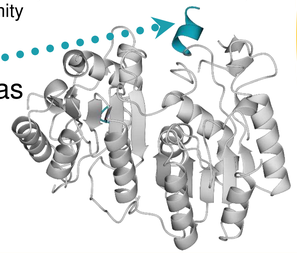
Additional helix of OafB gives more elongated shape: this is not seen in other SGNH domains.

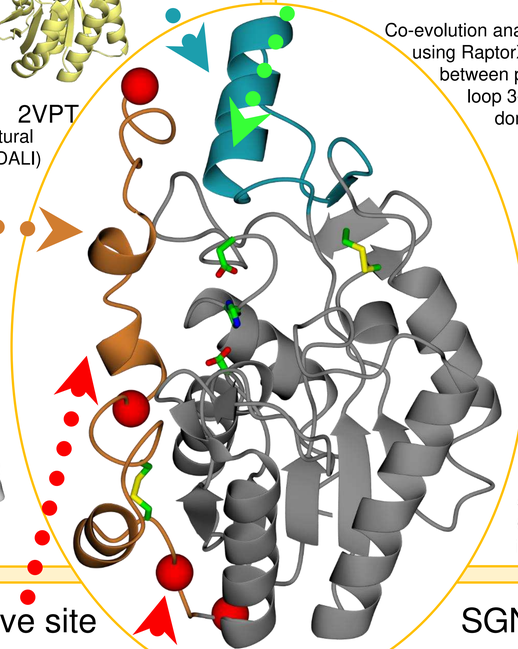


Periplasmic linker forms a structured extension of SGNH domain

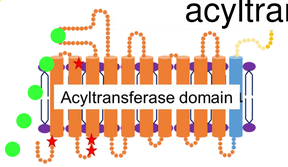
- Few residues between end of extension and transmembrane helix
- SGNH domain and acyltransferase domain likely to be in close proximity

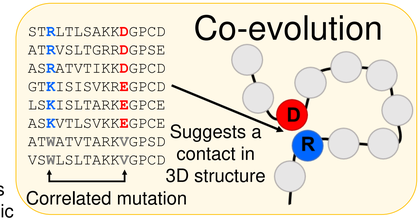
OafA-SGNH domain also has the additional helix seen in OafB-SGNH





SGNH domain interacts with acyltransferase domain

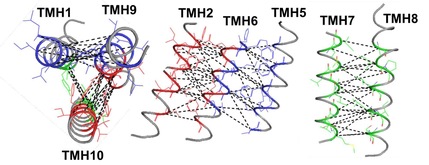




Co-evolution analysis using RaptorX predicts between periplasmic loop 3-4 in acyltransferase domain and additional helix in SGNH domain. Suggesting the two domains are likely to interact.

Structural predictions of acyltransferase domain

Co-evolution analysis of OafB acyltransferase domain predicts interaction between transmembrane helices. Modelling suggests these interactions make sense topologically and as helical wheel plots.



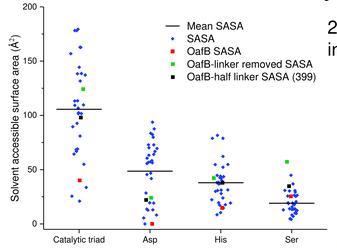
AT3 domain has a novel fold?

Other proteins with 10 TMH do not have same helices interacting as seen in the AT3 domain.

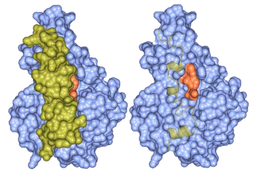
SGNH-extension occludes active site

Active site has low solvent accessibility

1. Solvent accessible surface area, measured using Pymol, of catalytic triad is lower for OafB than for other SGNH domains.



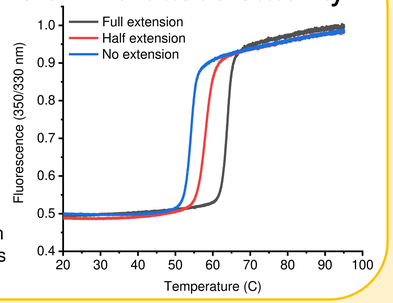
2. Solvent accessible surface area increases when extension removed:



- Removal of the extension decreases the melting temperature by 10 °C
- Removal of half of the extension decreases the melting temperature by 5 °C

In silico analysis combined with melting temperature and NMR data has then been used to predict if other AT3-SGNH proteins have structured extensions.

SGNH-extension increases stability



Conclusions

- Structure of OafB-SGNH domain has novel structural features – an additional helix and structured extension
- Active site of OafB-SGNH domain has low solvent accessibility due to occlusion from structured extension – removal of the extension increases accessibility but decreases stability
- Co-evolution analysis suggests the acyltransferase and SGNH domains are likely to interact
- Structural predictions from co-evolution analysis suggests acyltransferase domain may have a novel structure

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