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Structural analysis of acyltransferases involved in O-antigen modification

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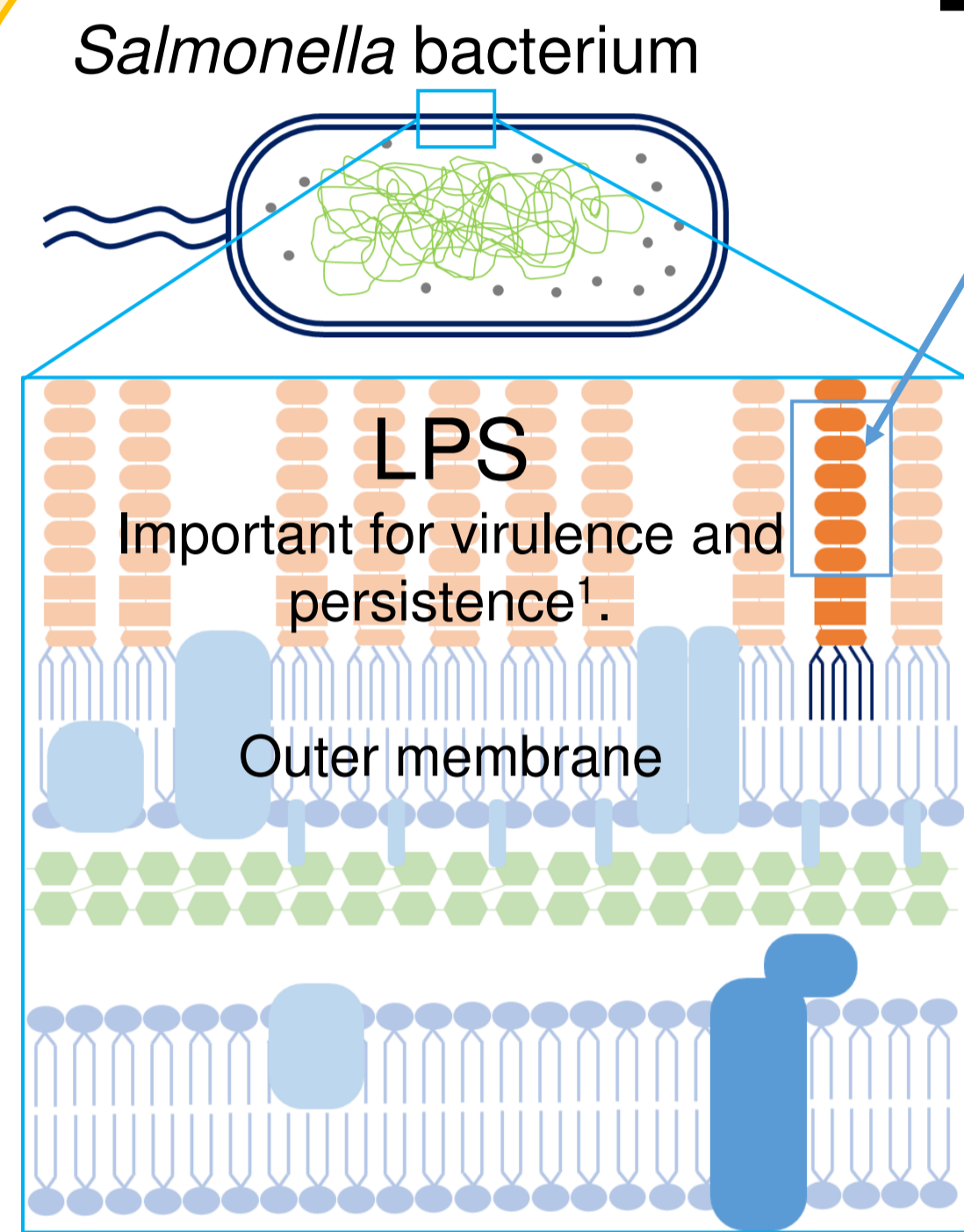
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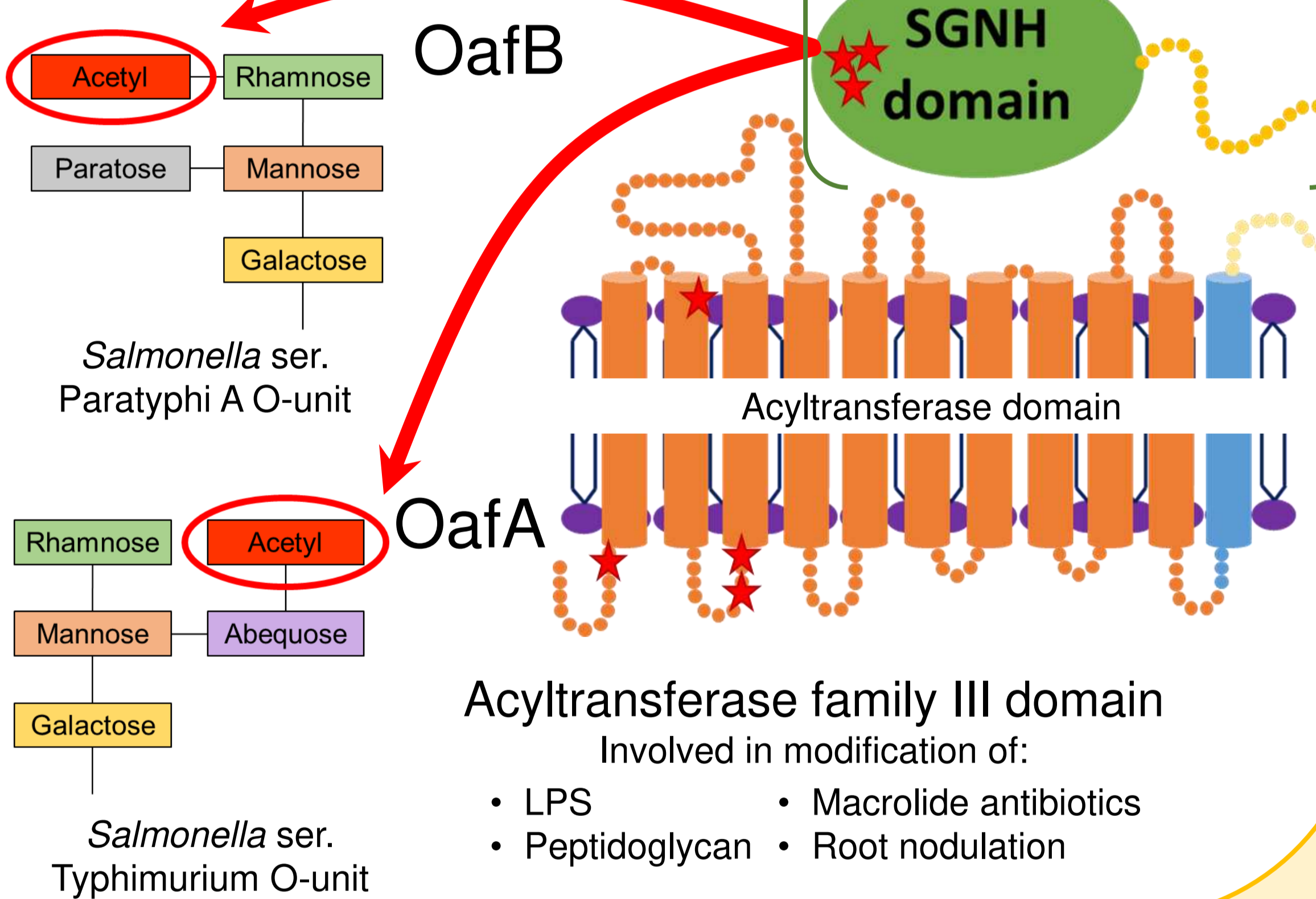


Background



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

Bacteria modify the O-antigen by acetylation to increase diversity².



OafA and OafB
add acetyl groups to abequose³ and rhamnose⁴ respectively. Both have attached SGNH domains.

Research Aims

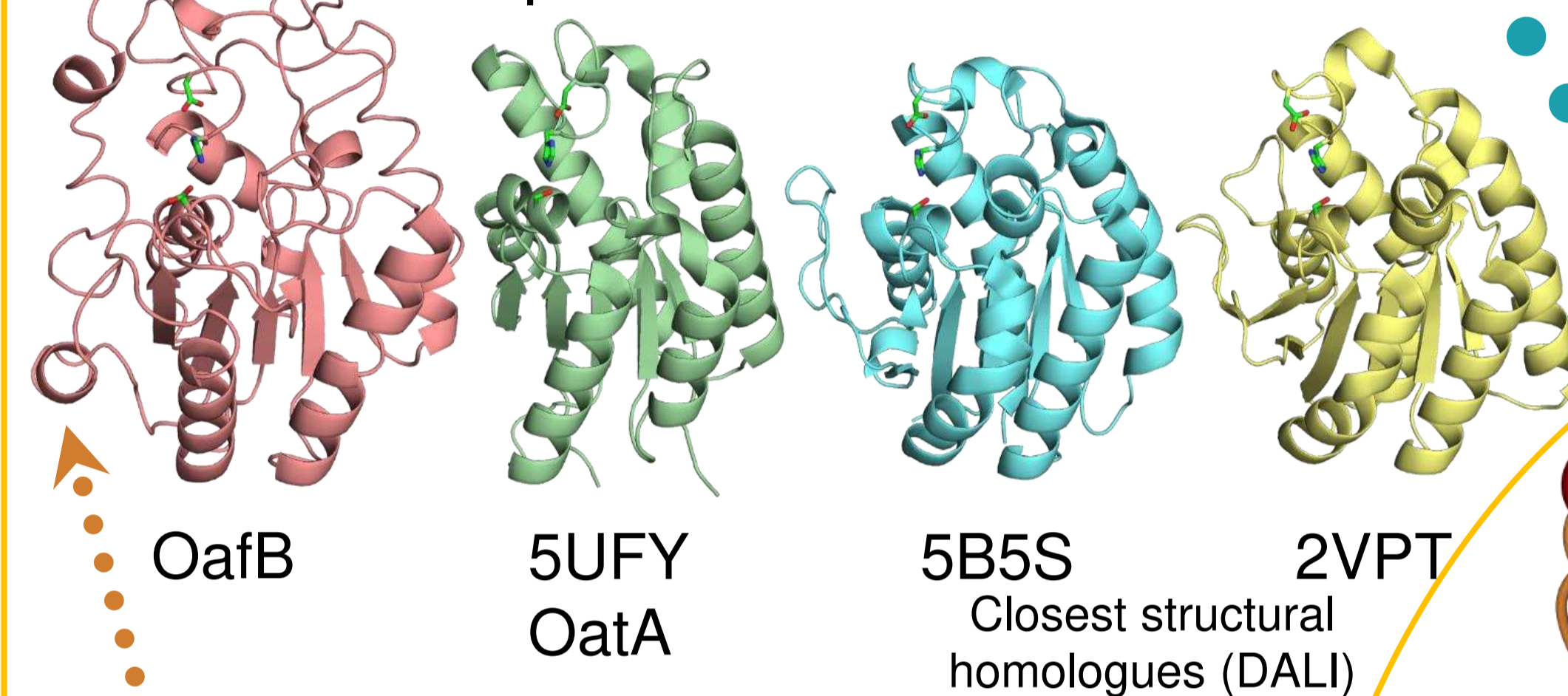
Structural characterisation of SGNH domains from OafA and OafB.
Understand mechanism of action of acyltransferases.

SGNH domain

Not always present, but required for function where present.

OafB-SGNH domain has a unique structure

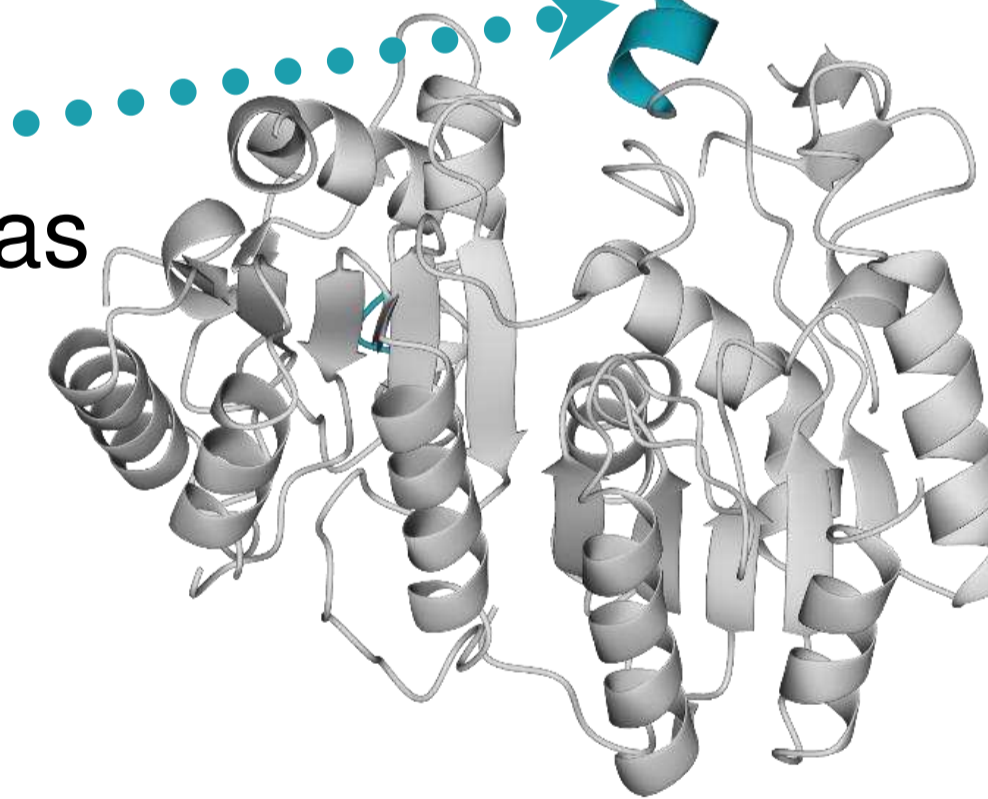
Additional helix of OafB gives more elongated shape: this is not seen in other SGNH domains.



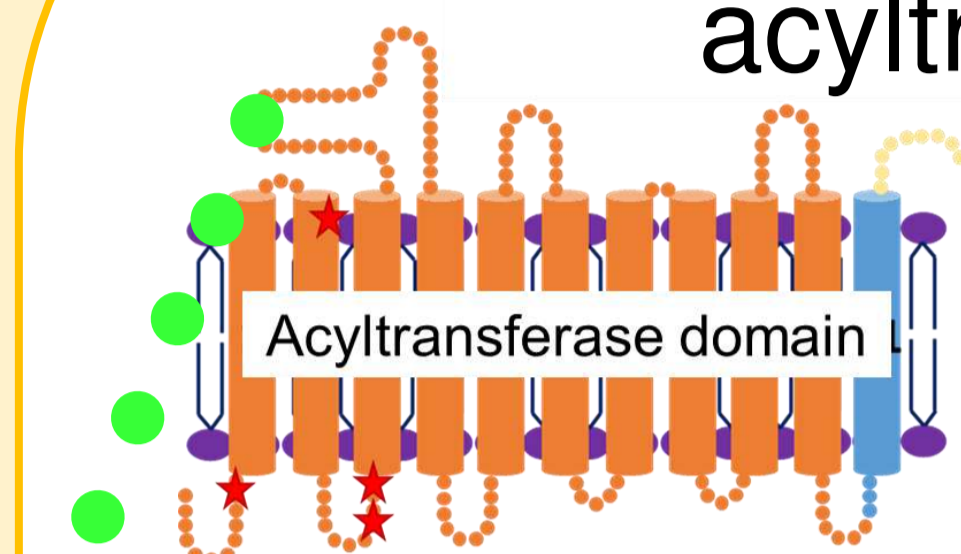
Periplasmic linker forms a structured extension of SGNH domain

- 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

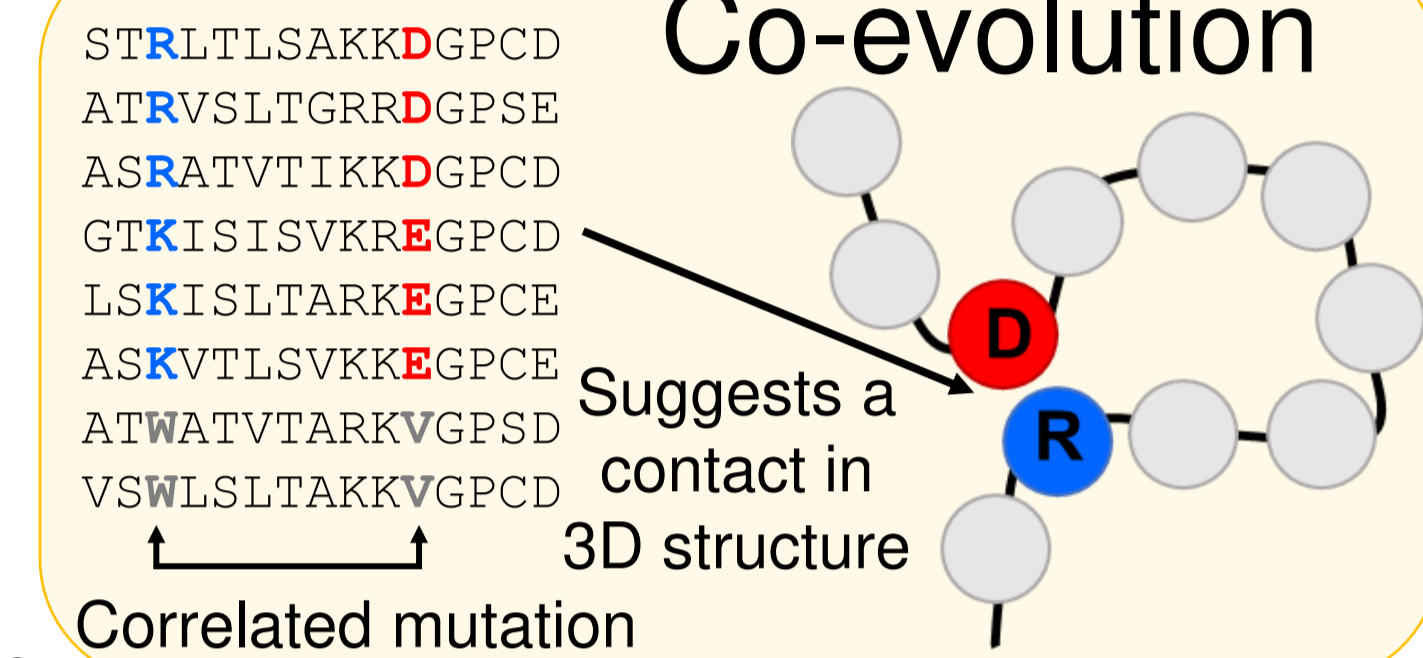


SGNH domain interacts with acyltransferase domain



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.

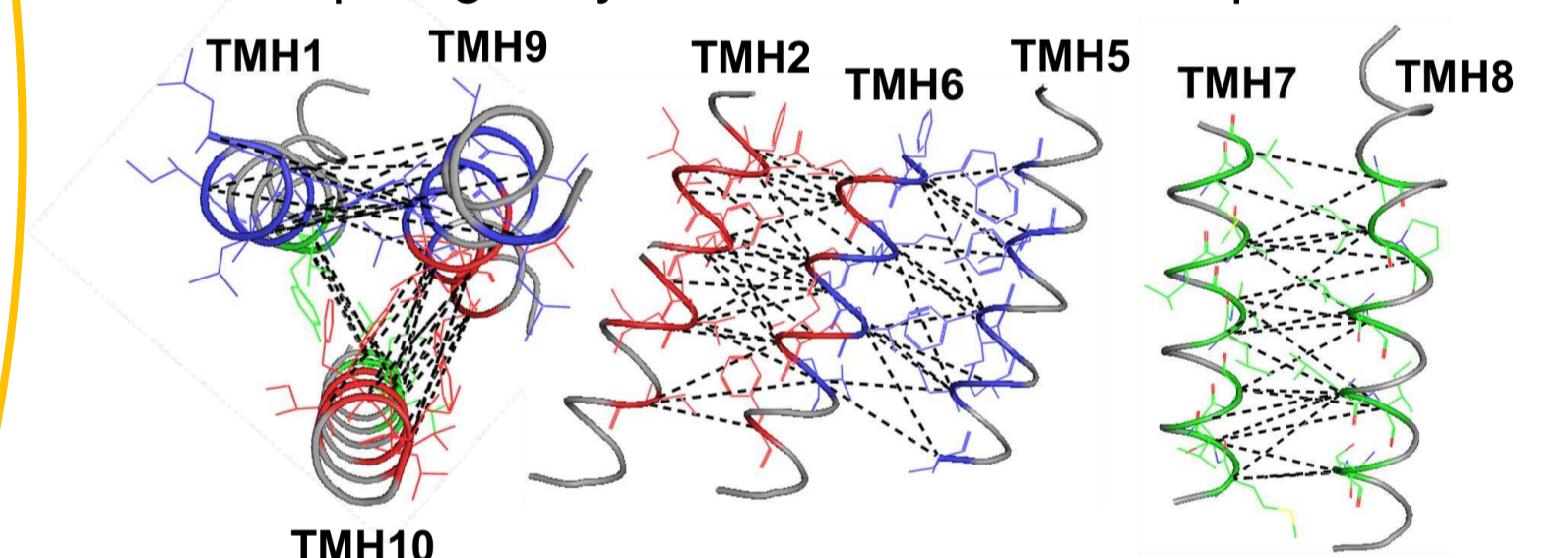
Co-evolution



STRLTLSAKKDGPCD
ATRVSLTGRRDGPSE
ASRATVTIKKDGPCD
GTKISISVVKREGPCD
LSKISLTKARKEGPCE
ASKVTLVSKKREGPCE
ATWATVTARKVGPSPD
VSWLSLTKAKKVGPCD

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.

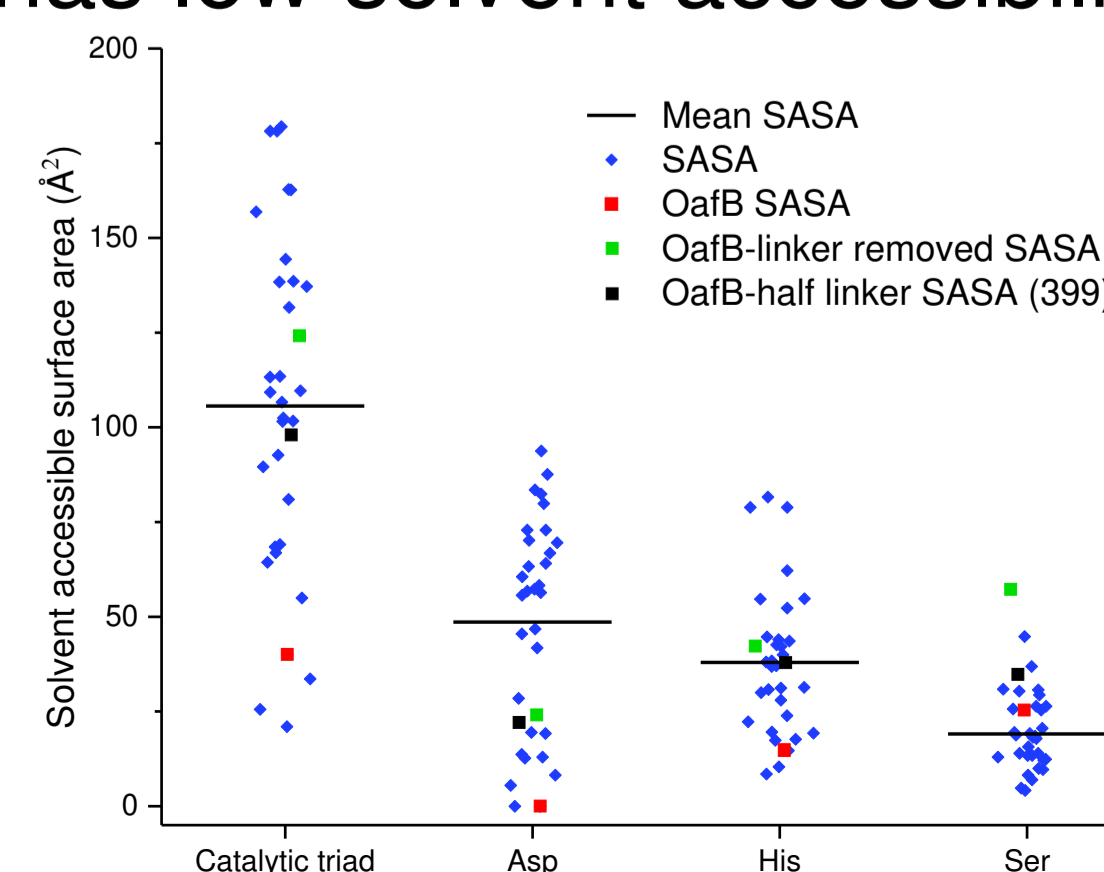
Conclusions

- 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

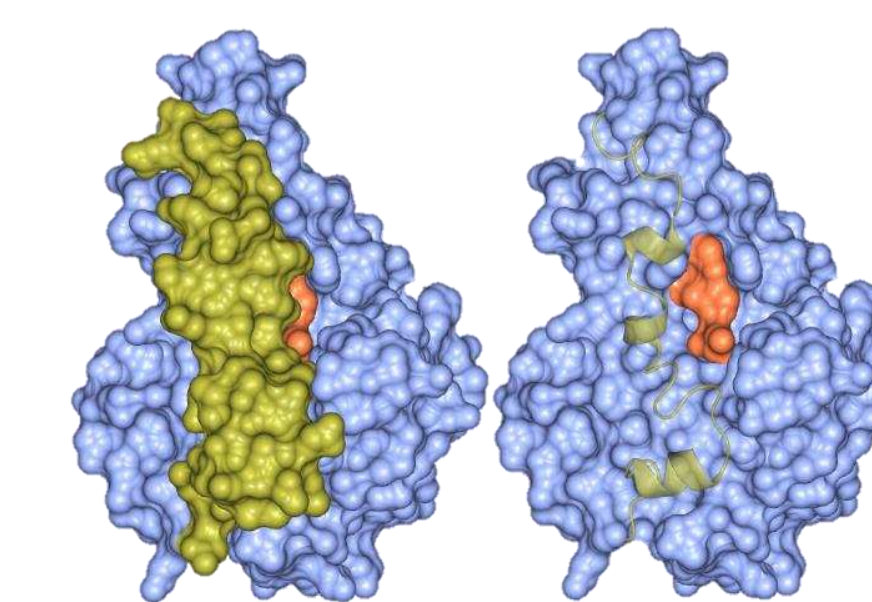
SGNH-extension occludes active site

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.



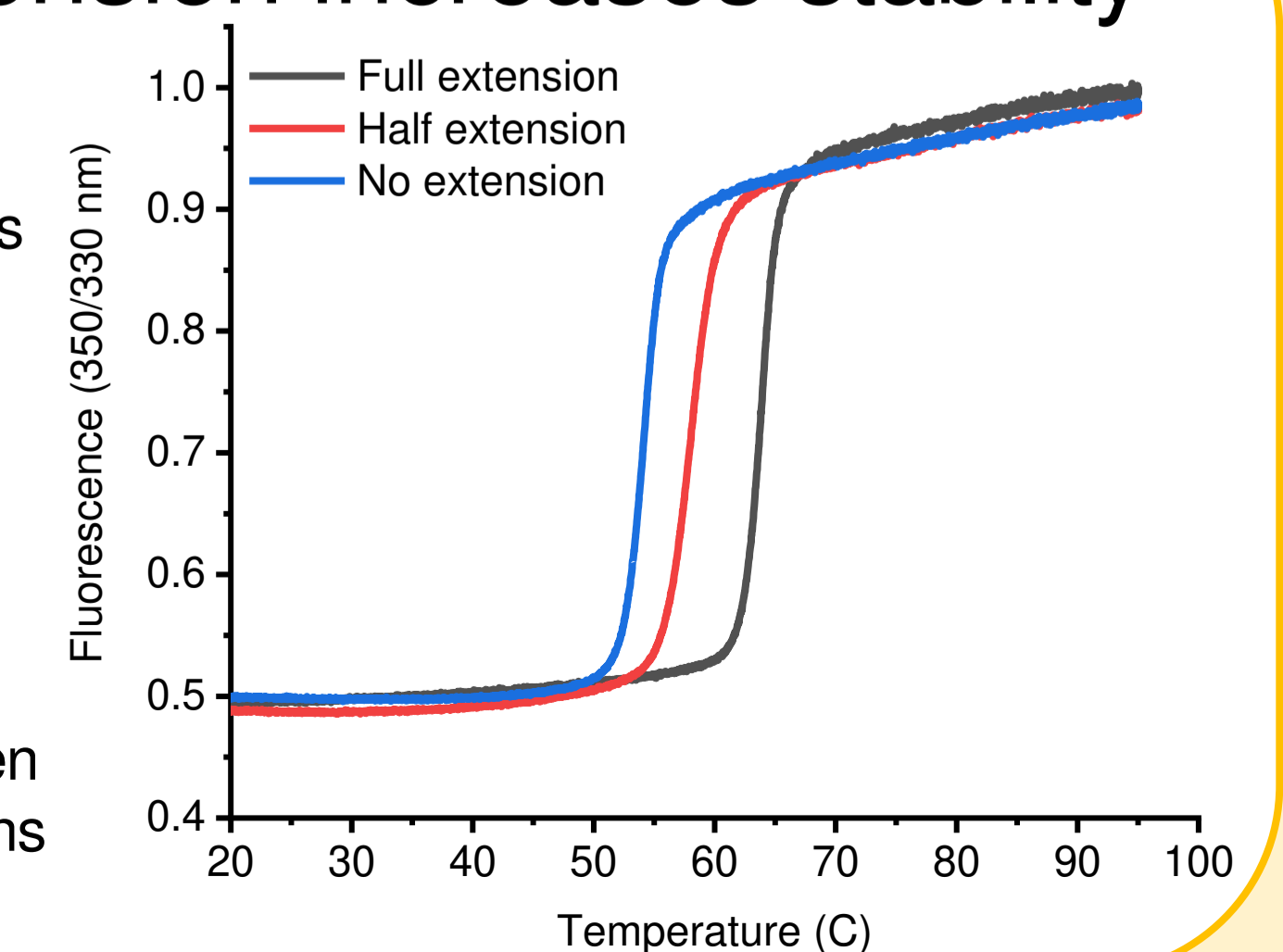
2. Solvent accessible surface area increases when extension removed:



SGNH-extension increases stability

- 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.



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