

Unravelling the role of a tandem pair of glycoside transporters in the marine bacterium *Zobellia galactanivorans*

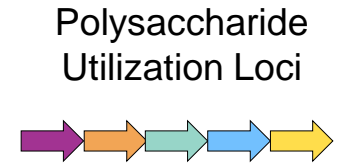
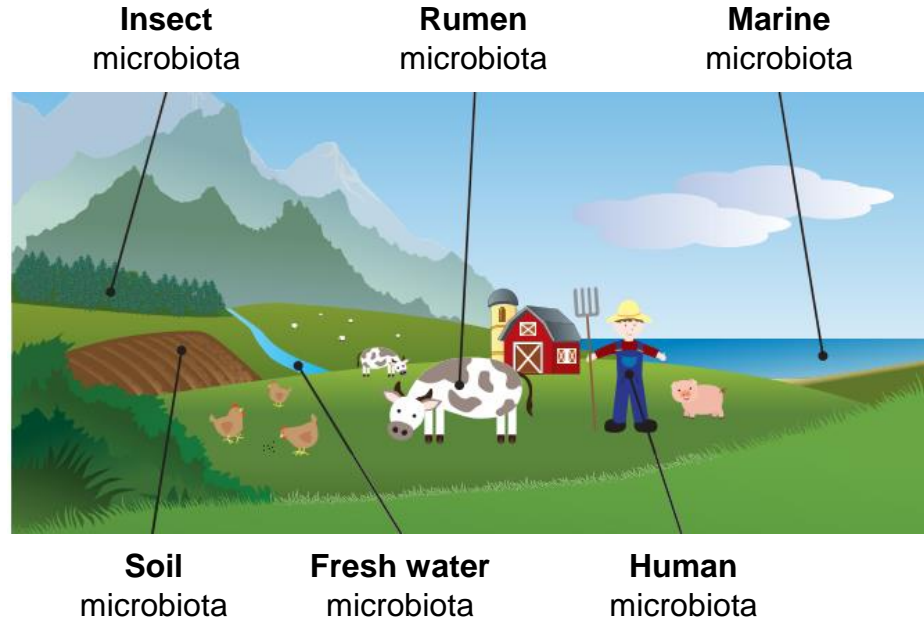
Aurore LABOUREL - INRAE researcher



Bacteroidota rule the world

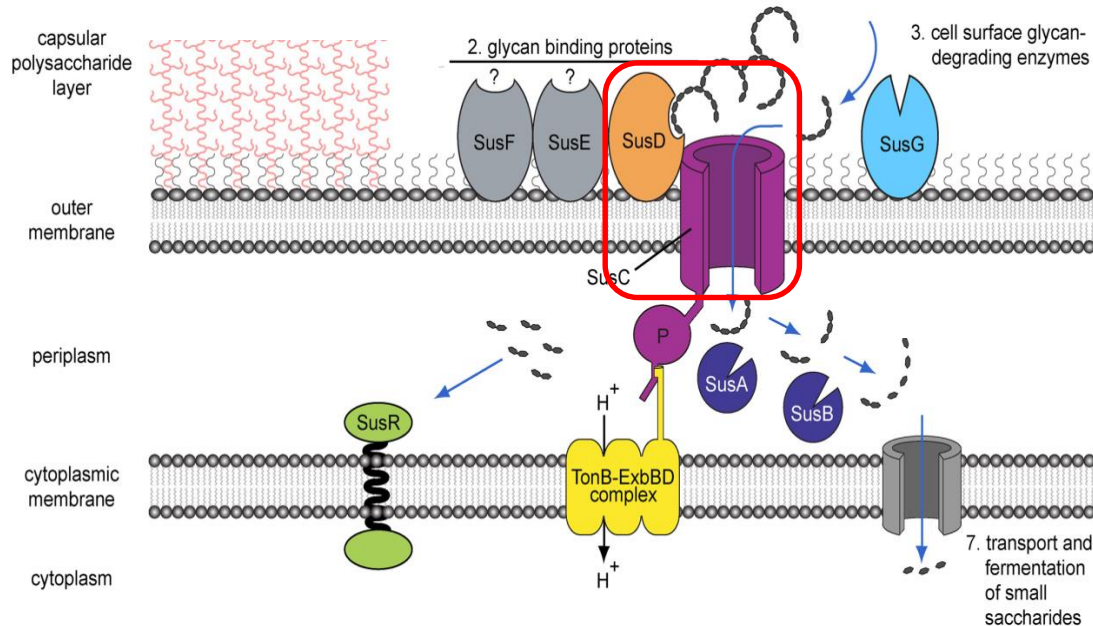
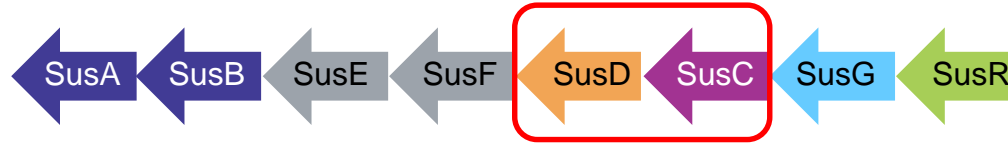


Bacteroidota
managed to colonize
almost all environments



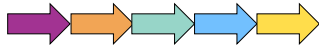
Polysaccharide Utilization Loci (PUL) ...the teeth and the throat of Bacteroidota

PUL = set of physically-linked genes organized around a *susCD* gene pair



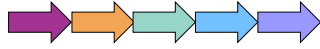
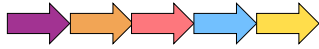
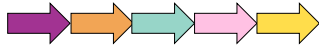
The PUL dogma

1 PUL



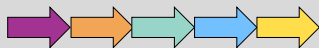
1 substrate

Several PULs



1 substrate

1 PUL



Several substrates

?

Characterization of the transporters is in the blind spot

Structural & functional characterization

Transporters



Transmembrane proteins

CAZymes

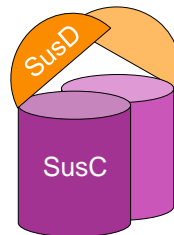


Terrapon *et al.*, 2018

Polysaccharide-Utilization Loci DataBase (PULDB)

68,500 PUL predictions in 2,065 Bacteroidota species

3 SusC/D structures



2017, Glenwright *et al.*, *Nature*

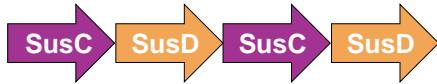
2021, Gray *et al.*, *Nature Com.*

2023, White *et al.*, *Nature*

SusCDs form
homodimers *in vivo*

My interest

The particular case of the **tandem repeat SusCD** (trSusCD)



- Approximately represent **3%** of the encoded PULs in the phylum Bacteroidota (2019, Lapébie *et al.*, Nature Communications)
- Provide a **selective advantage** in environment where diverse dominant species compete for similar metabolic niches (2020, Galvez *et al.*, Cell Host and Microbiome)
- The structural characterization of the trSusCD has never been investigated so far
 - homodimers? **Heterodimers?**
 - **versatility?** Can one PUL containing trSusCD target different substrates?

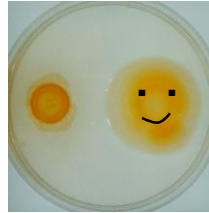
(The only example of a PUL displaying substrate versatility contains a tandem repeat SusCD)

2015, Mackenzie *et al.*, AEM

The model organism



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Station Biologique
de Roscoff



Z. galactanivorans



Delesseria sanguinea

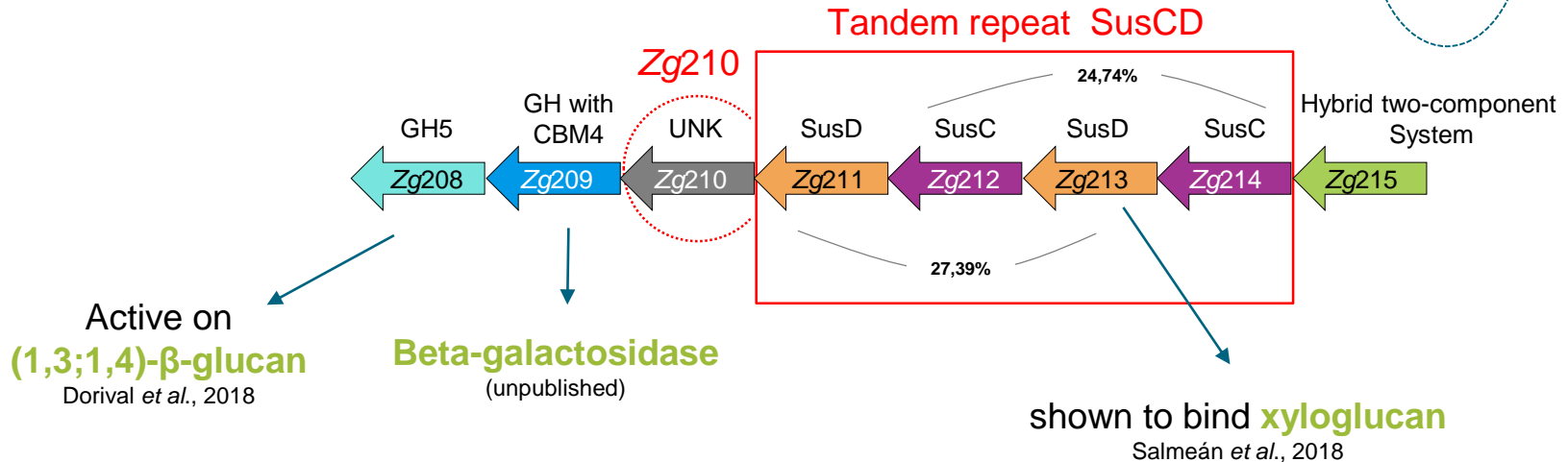
A marine bacterium called *Zobellia galactanivorans* Dsij^T

- Belongs to Bacteroidota
- Genome sequenced and annotated (rich in CAZymes and PULs)
- Aerobic, non-pathogenic
- Model organism for the degradation of algal polysaccharides
- Genetic tool available (recombinations)

The precious PUL

PUL upregulated in:
laminarin, (1,3;1,4)- β -glucan and xylan conditions

Thomas *et al.*, 2017; Brunet *et al.*, 2022



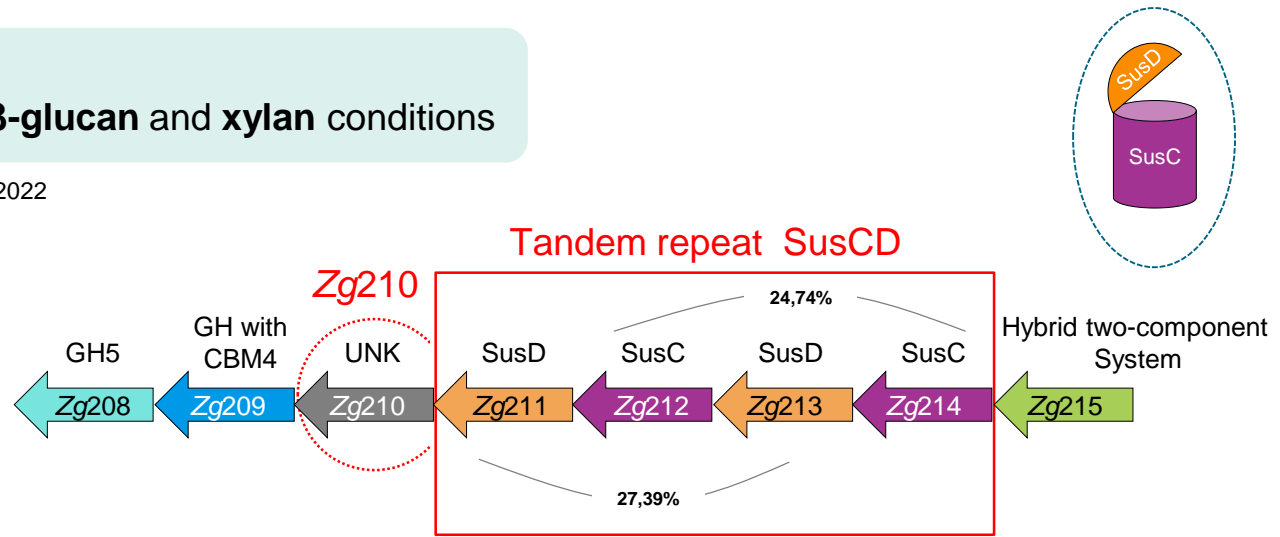
- « Small » PUL
- Presence of a tandem repeat SusCD
- Presence of Zg210, a protein of unknown function
- **Preliminary data available**

Z. galactanivorans does not grow on xyloglucan as sole carbon source !

The main questions

PUL upregulated in:
laminarin, (1,3;1,4)- β -glucan and xylan conditions

Thomas *et al.*, 2017; Brunet *et al.*, 2022



- Is $Zg210_{UNK}$ a new CAZyme? Is it a Surface Glycan Binding Protein?
- Do $Zg211_{SusD}$ and $Zg213_{SusD}$ bind to the same substrate?
- What is (are) the substrate(s) imported by these SusCDs?
- What is the quaternary structure of the trSusCD? Homodimers? Heterodimers? Others?

Bioinformatic analysis → genomic context of *Zg210*_{UNK}?

Zobellia genus



- PUL conserved in 15 of the 18 strains
- ~1 *Zg210*_{UNK} homologue/genome

Tara Ocean (metagenomes)



- < 10 hits (pelagic ocean)
- Found **directly downstream trSusCD**,
- xylanases, sulfatases

... try other metagenomic data



→ Algae blooms trigger some Bacteroidota blooms

Telling *et al.*, 2012, Science
Telling *et al.*, 2016, eLIFE

Human gut microbiota



- ~175 hits
- Found **directly downstream trSusCD**
- Mainly with xylanases

Structural analysis of *Zg210*_{UNK}



Synchrotron Grenoble
~2 Å resolution
39 kDa, 39% id with BACOVA_03430

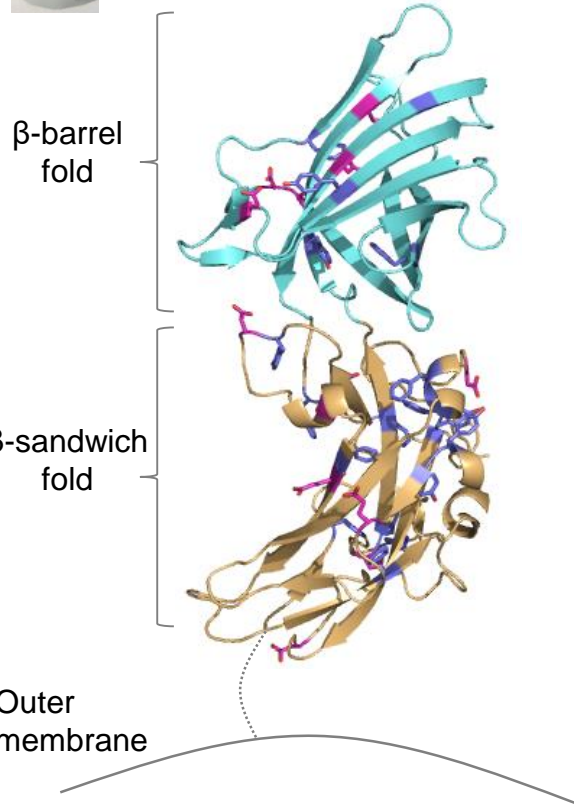
What is *Zg210*_{UNK}?

Surface glycan
binding protein?

CAZyme?

Sequences alignment of homologues:

- Conserved **E** and **D**
(catalytic residues known for the GHs)
→ No obvious active site
- Conserved aromatic residues **Y**, **W**, **F**, **H**
(for substrate/ligand stacking)
→ Most of them face the inside of the protein



β-barrel
fold

β-sandwich
fold

Outer
membrane

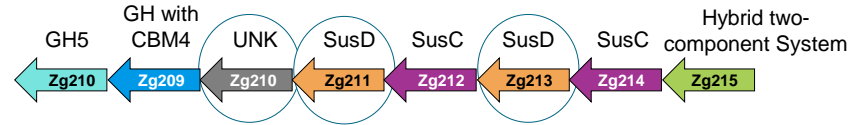
Binding test: which substrate(s) is targeted?

Zg210_{UNK}, Zg211_{SusD} and Zg213_{SusD} were produced in *E. coli* and purified

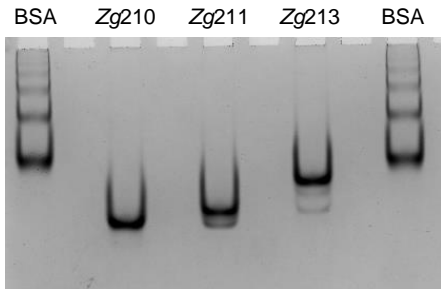
Affinity Gel Electrophoresis with 0.2% (w/v) substrates

PUL upregulated with:

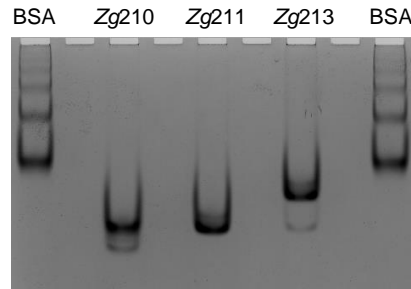
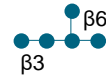
- laminarin
- (1,3;1,4)- β -glucan
- xylan



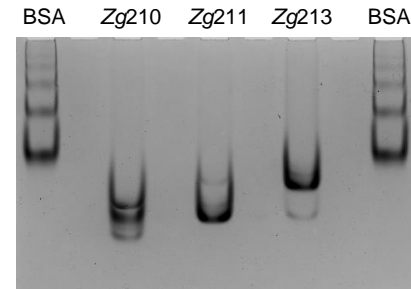
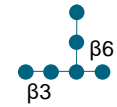
Control (H₂O)



Laminarin
(from *Laminaria digitata*)



Laminarin
(from *Eisenia bicyclis*)



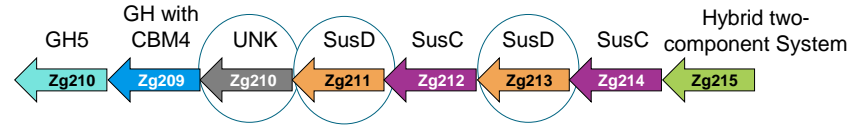
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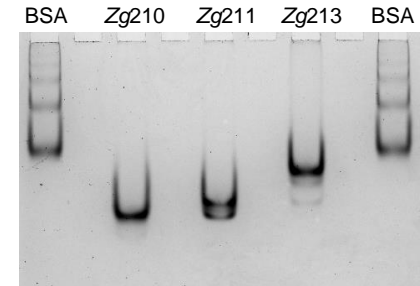
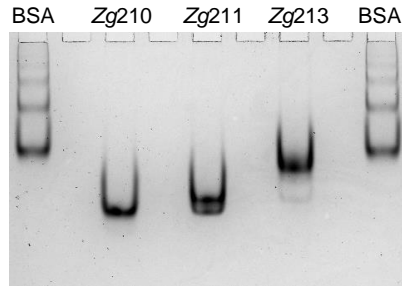
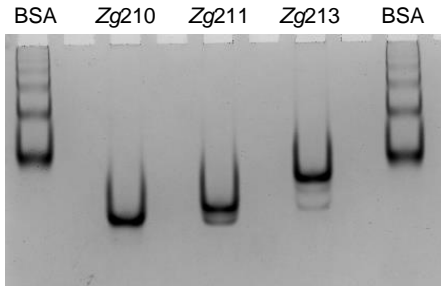
- laminarin
- (1,3;1,4)- β -glucan
- xylan



Control (H₂O)

Barley β -glucan

Lichenan
(contains more β 1,3 compared to barley)



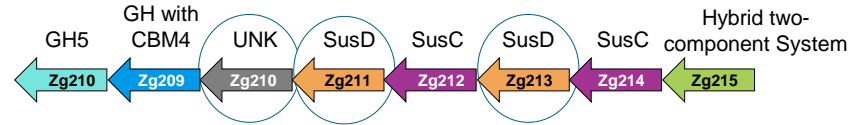
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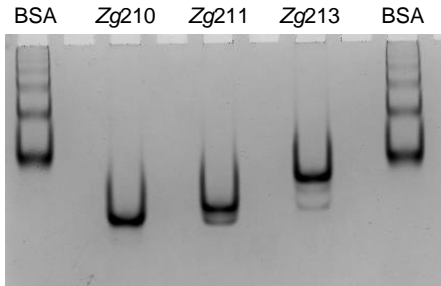
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- (1,3;1,4)- β -glucan
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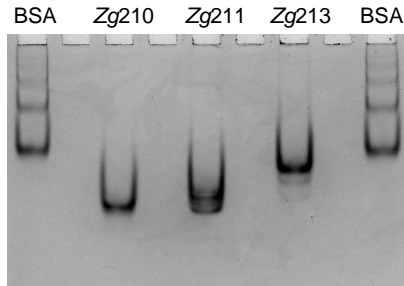


Control (H₂O)



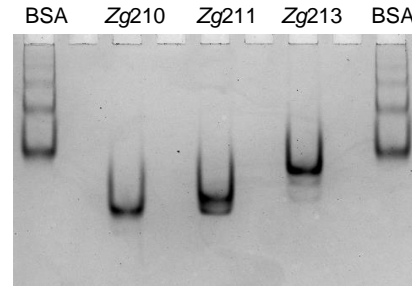
Birchwood xylan

(linear, partially acetylated ~24%)



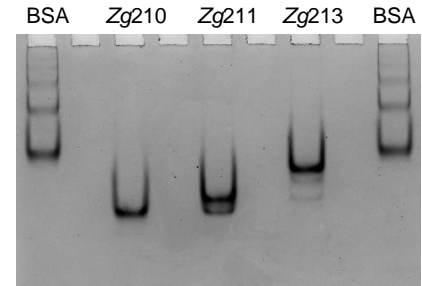
Beechwood xylan

(~13% GlcAOMe substitution)



Wheat arabinoxylan

(~38% Ara substitution)



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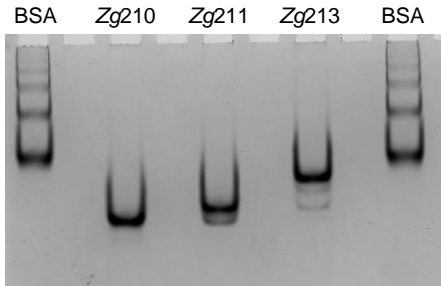
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- (1,3;1,4)- β -glucan
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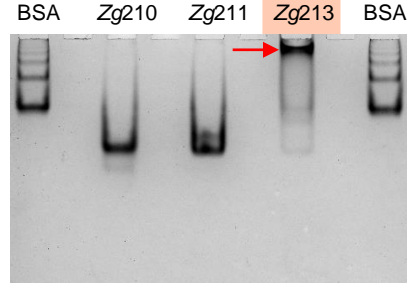


Previously shown to bind xyloglucan
Salmeán *et al.*, 2018

Control (H₂O)









Xyloglucan (tamarind seed)



Z. galactanivorans does not grow on xyloglucan as sole carbon source !

What's next?

Structure/function of the recombinant proteins

-  **Activity test** on plant and **algal** polysaccharides/oligos
-    **Binding assays** on **algal** polysaccharides/oligos
-   **3D structure** of $Zg211_{\text{SusD}}$ and $Zg213_{\text{SusD}}$ (X-ray, AlphaFold3)

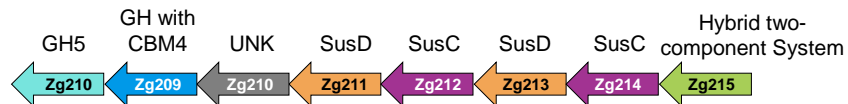
What if **Zg210_{UNK}** is something else than an Surface Glycan Binding Protein or an enzyme?

- Protein-protein interaction?
- Is it involved in the quaternary structure of the trSusCD?
 - Molecular dynamic

Catching several fish with a single worm...

Zg210_{UNK}:
protein-protein
interaction?

TrSusCD:
Homodimer?
Heterodimer?



Does this PUL form
an utilizome?

Genetic tool available

(François Thomas)

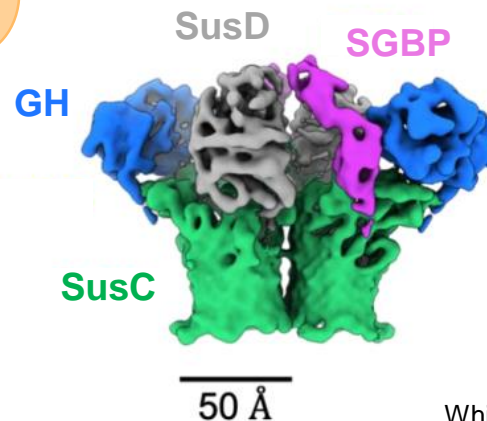
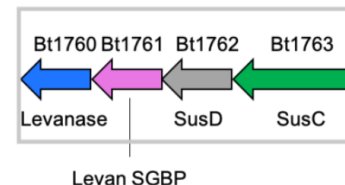
Purification of the SusCDs
directly from the membrane of *Z. galactanivorans*
→ X-ray/cryo-EM



With a little help
of my friend

The utilizome

PUL from *B. thetaiotaomicron*
(human gut microbiota)



Thank you 😊

Questions?



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