Transcriptomic data to support in vitro semi-mechanistic PK/PD modelling of Polymyxin B against Acinetobacter baumannii

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Institut Roche

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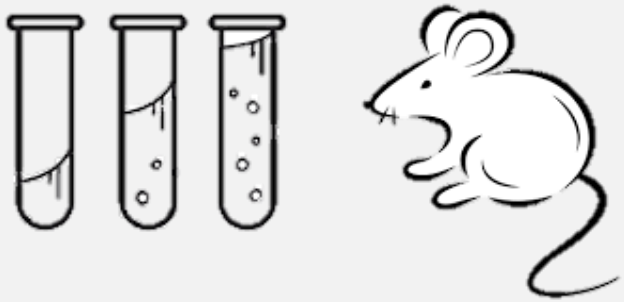
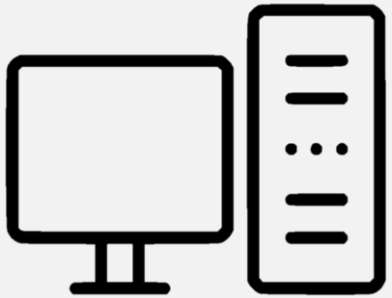
Work supported in part by F. Hoffmann-La Roche Ltd

PK/PD modelling



In vitro & *in vivo* studies



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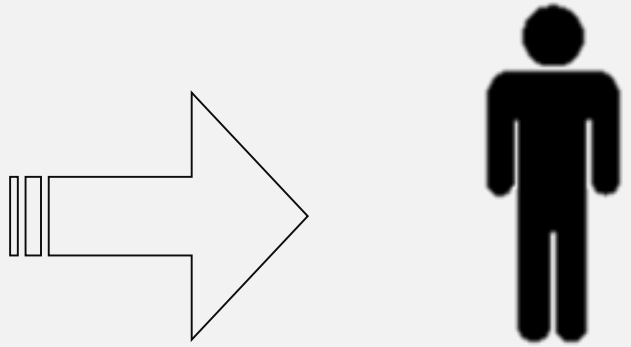
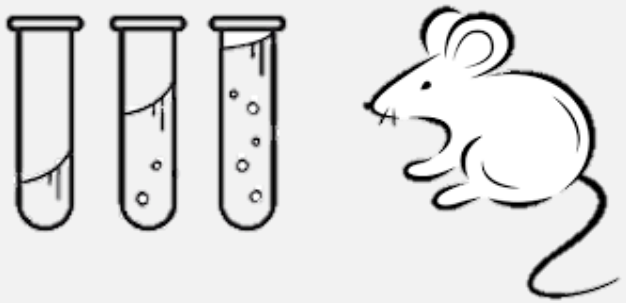


Prediction of human dosing regimens

PK/PD modelling



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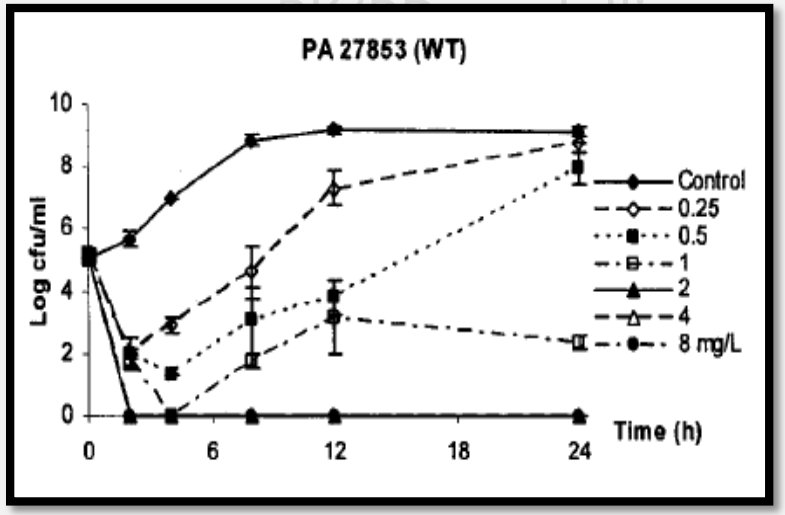
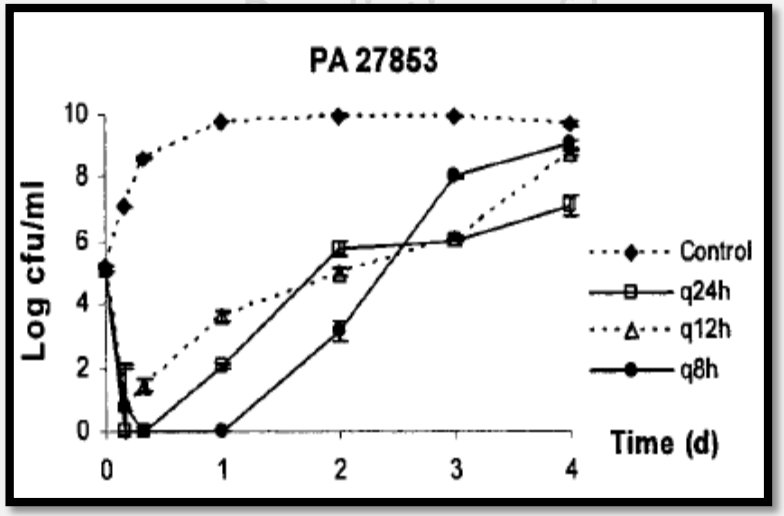


Time-kill

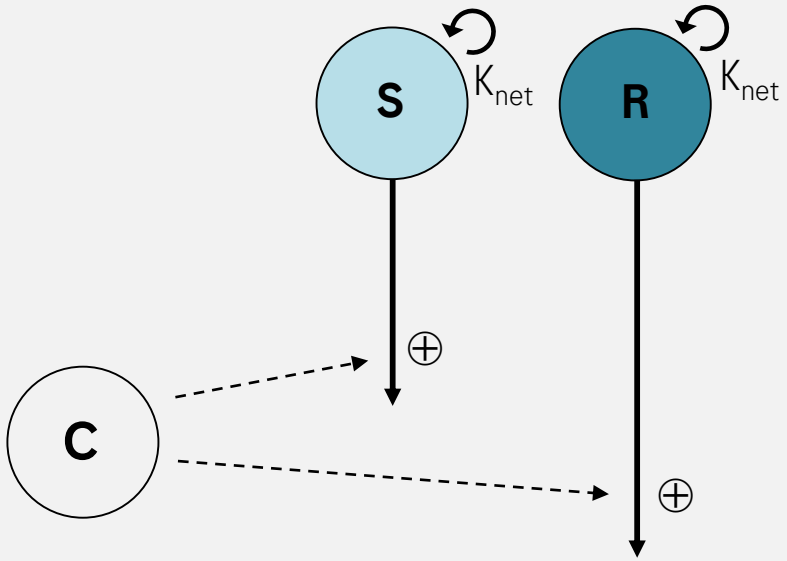


Hollow fiber

Tam *et al.*, 2005 Antimicrobial Agents and Chemotherapy

PK/PD model types

Hetero-resistance



Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
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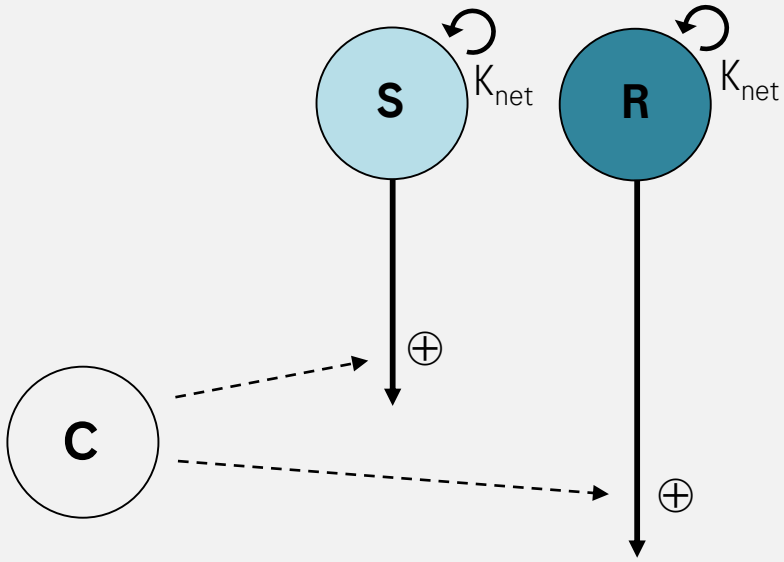

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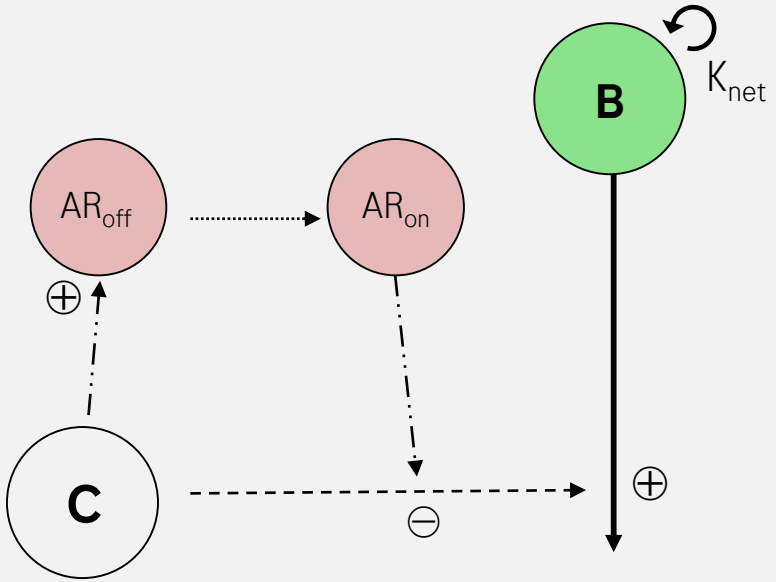
Adaptation



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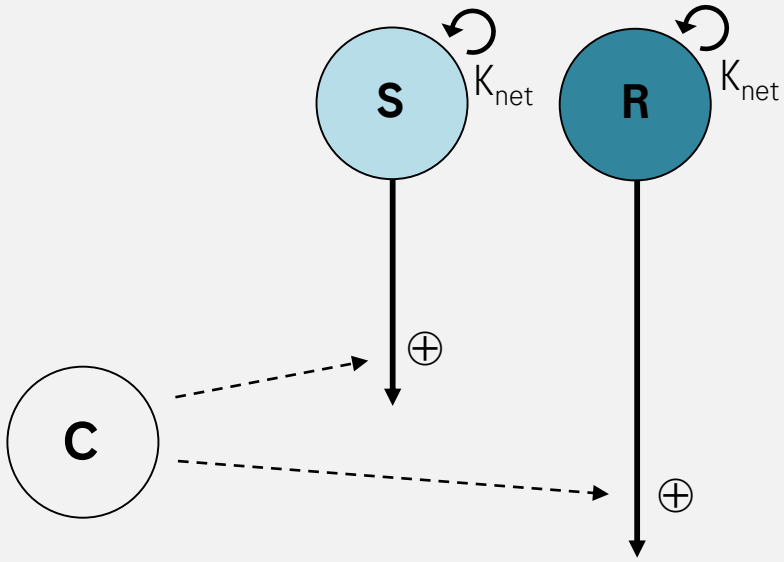

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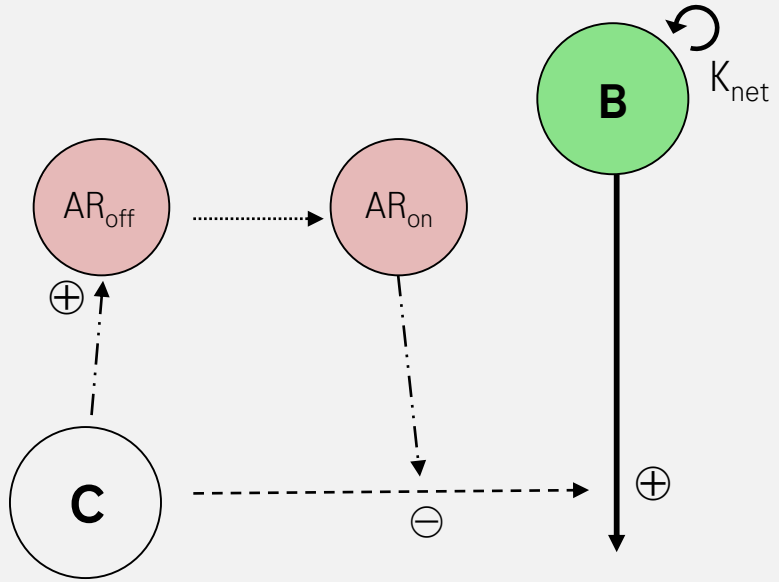
⇨ *Population analysis profiles (PAPs)*
⇨ *Sequencing*

Adaptation



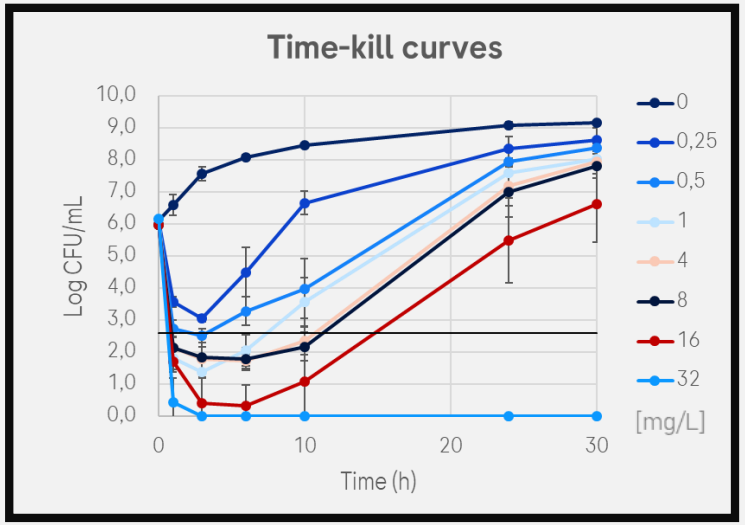
Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
Tam *et al.*, 2005 Journal of Antimicrobial Chemotherapy

⇨ *RT-qPCR*
⇨ *Transcriptomics*

Bacteria and Compound

→ *Acinetobacter baumannii: ESKAPE pathogen*

→ *Polymyxin B : last resort antibiotic for MDR Gram- bacteria*



Molecular Omics

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Cite this: *Mol. Omics*, 2020, 16, 327

Pan-transcriptomic analysis identified common differentially expressed genes of *Acinetobacter baumannii* in response to polymyxin treatments†

Mengyao Li,[a] Su Mon Aye,[a] Maizbha Uddin Ahmed,[b] Mei-Ling Han,[a] Chen Li,[c] Jiangning Song,[cd] John D. Boyce,[a] David R. Powell,[e] Mohammad A. K. Azad,[a] Tony Velkov,[f] Yan Zhu*[a] and Jian Li*[a]

MICROBIAL GENOMICS

RESEARCH ARTICLE

Boinett *et al.*, *Microbial Genomics* 2019;5
DOI 10.1099/mgen.0.000246

MICROBIOLOGY SOCIETY

OPEN DATA OPEN MICROBIOLOGY

Clinical and laboratory-induced colistin-resistance mechanisms in *Acinetobacter baumannii*

Christine J. Boinett,[1,2,3] Amy K. Cain,[1,4] Jane Hawkey,[5,6,7] Nhu Tran Do Hoang,[2] Nhu Nguyen Thi Khanh,[8] Duy Pham Thanh,[2] Janina Dordel,[1,9] James I. Campbell,[2,3] Nguyen Phu Huong Lan,[2,10] Matthew Mayho,[1] Gemma C. Langridge,[1,11] James Hadfield,[1] Nguyen Van Vinh Chau,[10] Guy E. Thwaites,[2,3] Julian Parkhill,[1] Nicholas R. Thomson,[1,12] Kathryn E. Holt[5,6] and Stephen Baker[2,3,13,*]

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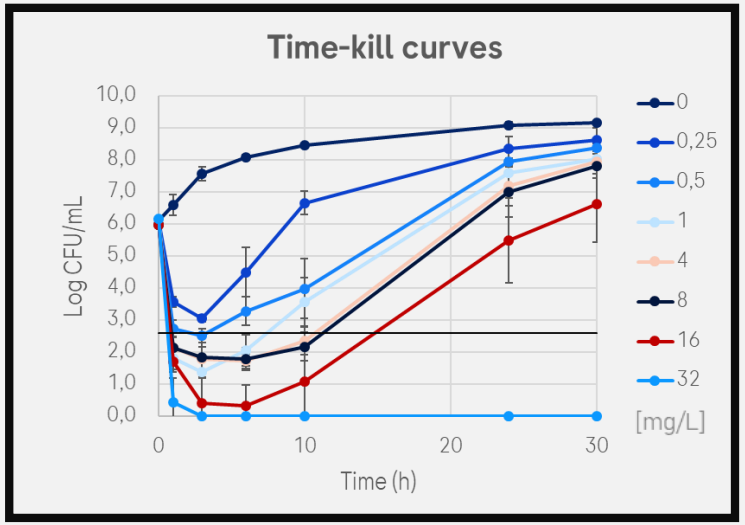

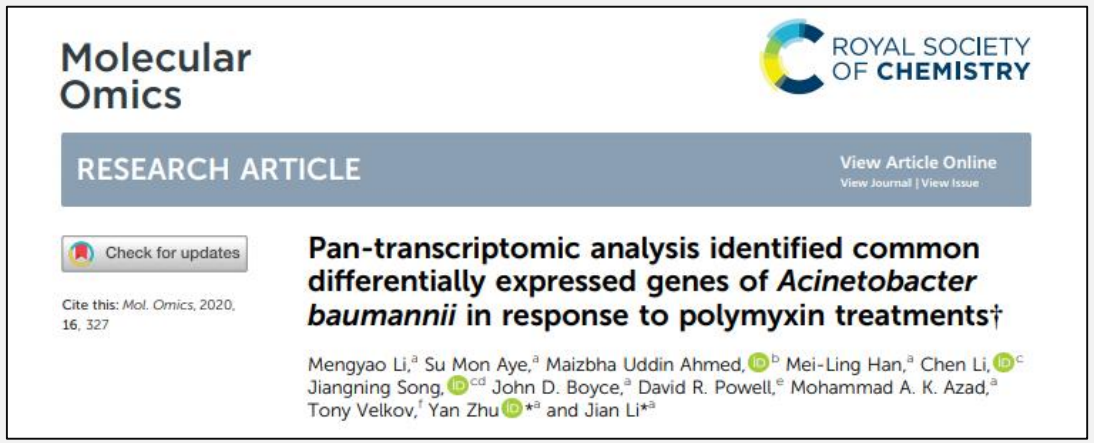
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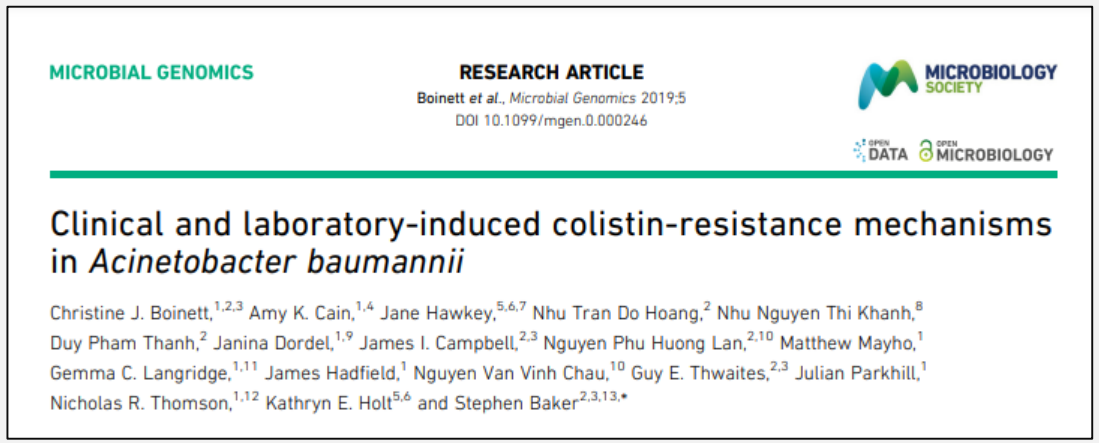
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Objective: Integrate transcriptomics data into *in vitro* PK/PD models

Strains

- Two clinical MDR *Acinetobacter baumannii* isolates obtained before and after colistin treatment [1]
  - **AB121** *pmbS* (MIC = 0.5 mg/L)
  - **AB122** *pmbR* (MIC = 64 mg/L)

[1] Jaidane *et al.*, 2018 International Journal of Antimicrobial Agents

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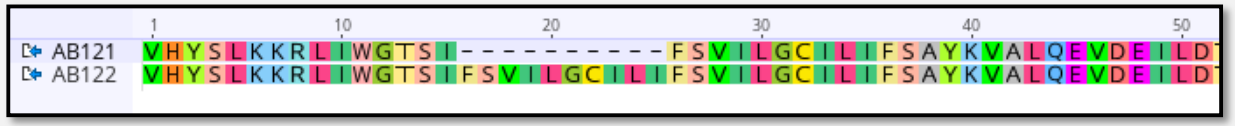

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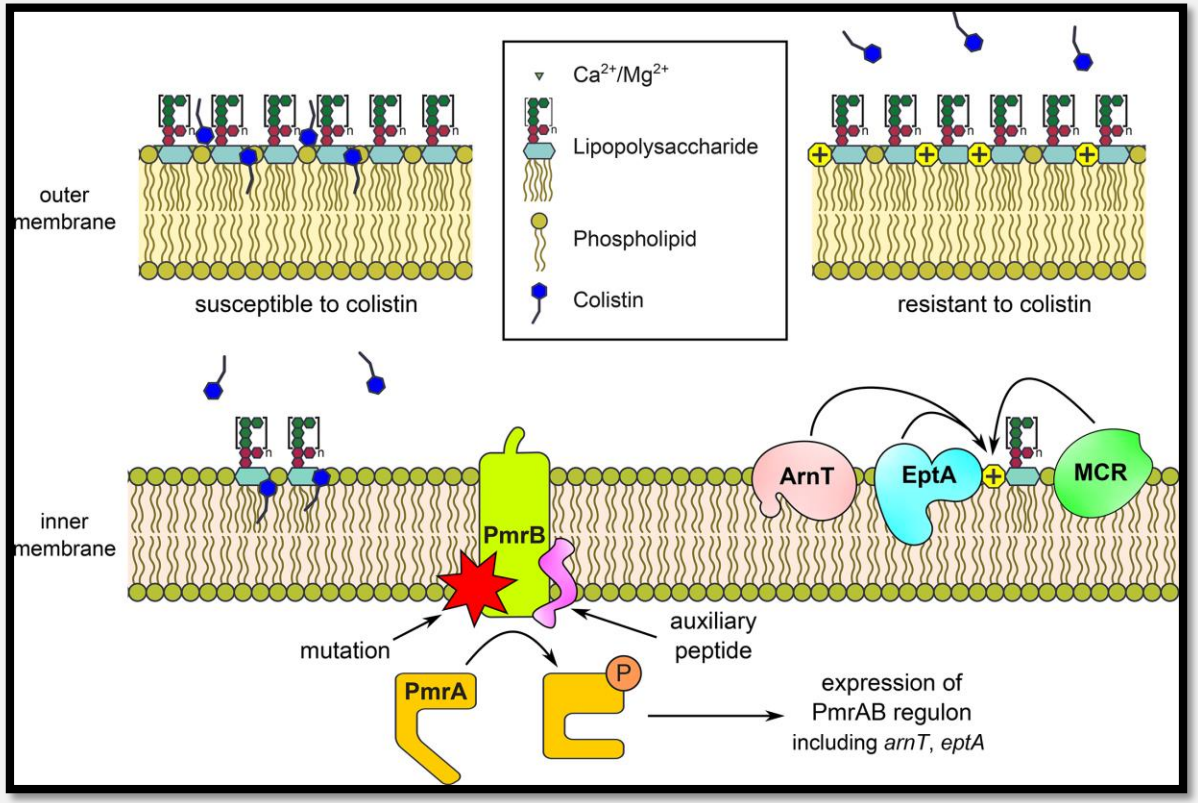
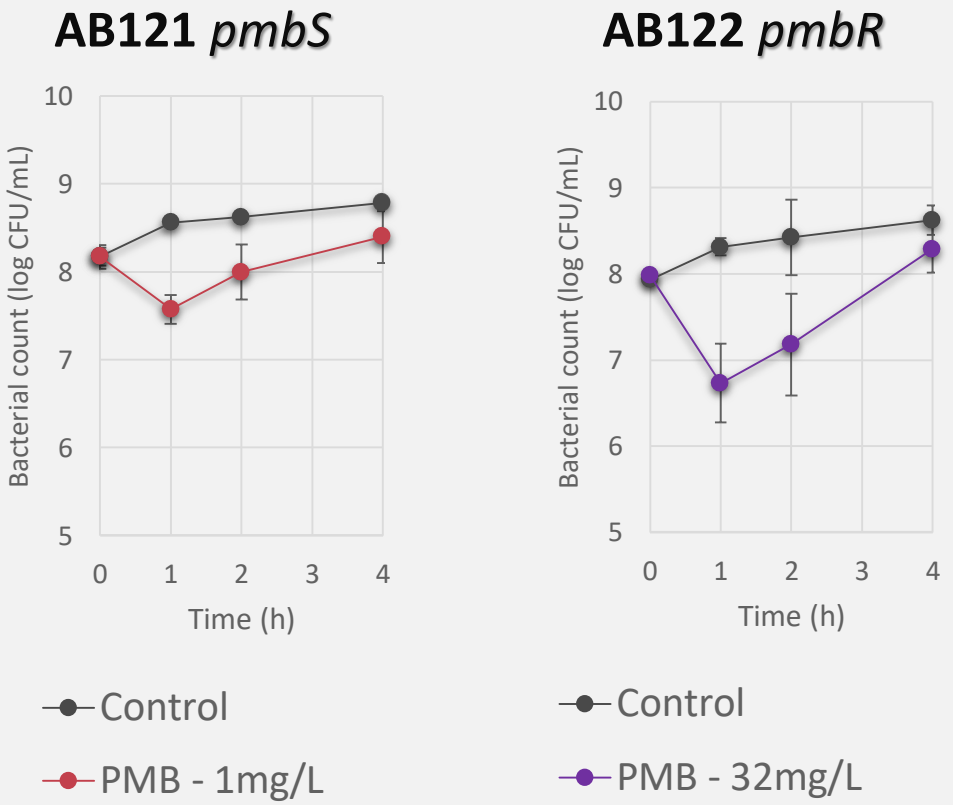


[1] Jaidane *et al.*, 2018 International Journal of Antimicrobial Agents
[2] *Janssen et al., 2021 PLoS Genetics*

Bacterial samples preparation

Time-kill curves for transcriptomics



→ Transcriptomics study for both strains on T0, T1h, T2h and T4h samples

Example for AB121

WGCNA

Weighted Gene Correlation Networks for Analysis [1,2]

- **Dataset preparation** *(with DESeq2* [3]*)*

Normalization and thresholding by variance (>90% quantile to reduce noise) - *from 3799 to 363 genes* -

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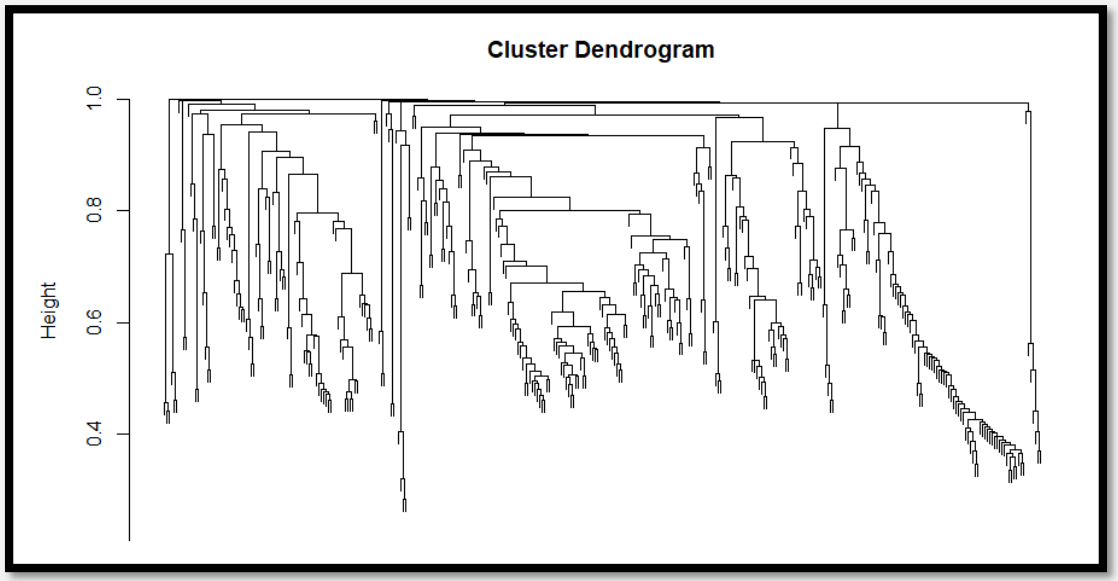
- **Construction of a gene co-expression network**

Use of interaction patterns among genes



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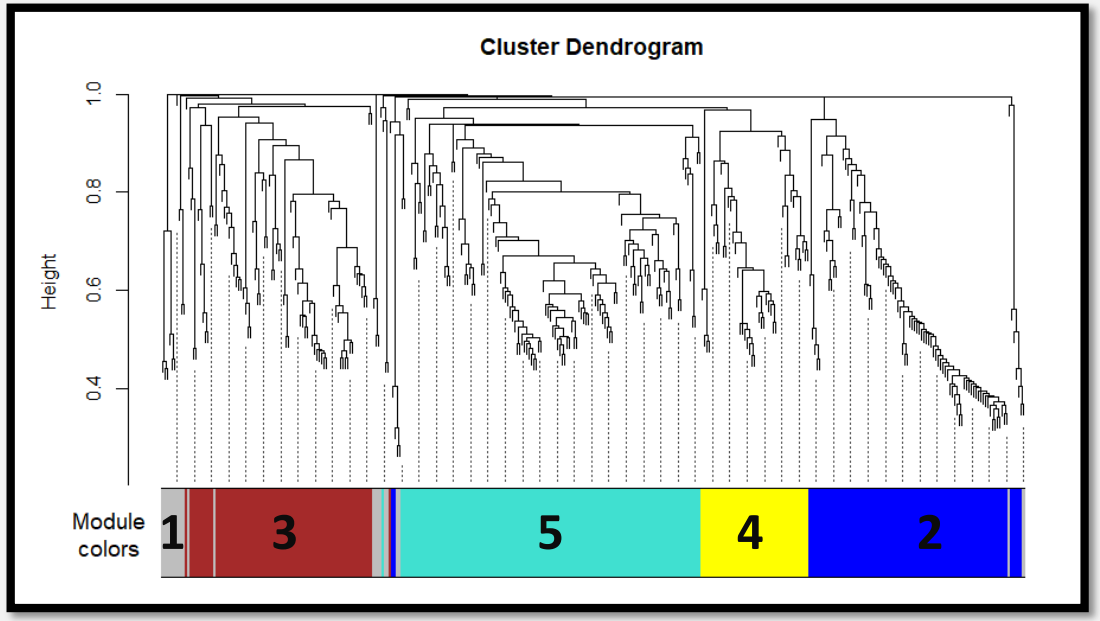
Use of interaction patterns among genes

- **Identification of modules**



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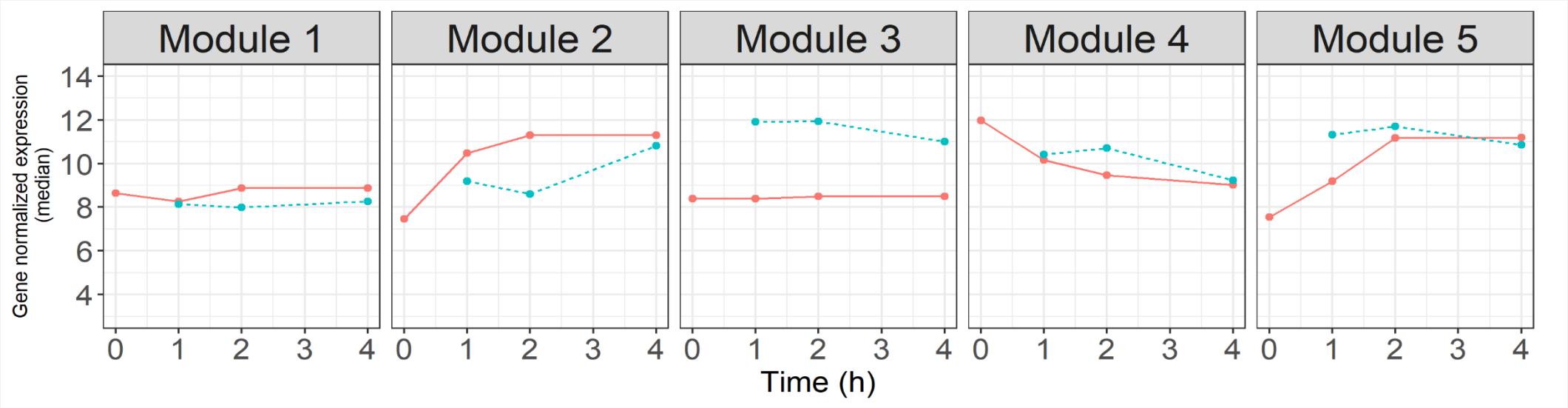
AB121 *pmbS*

Modules expression profiles



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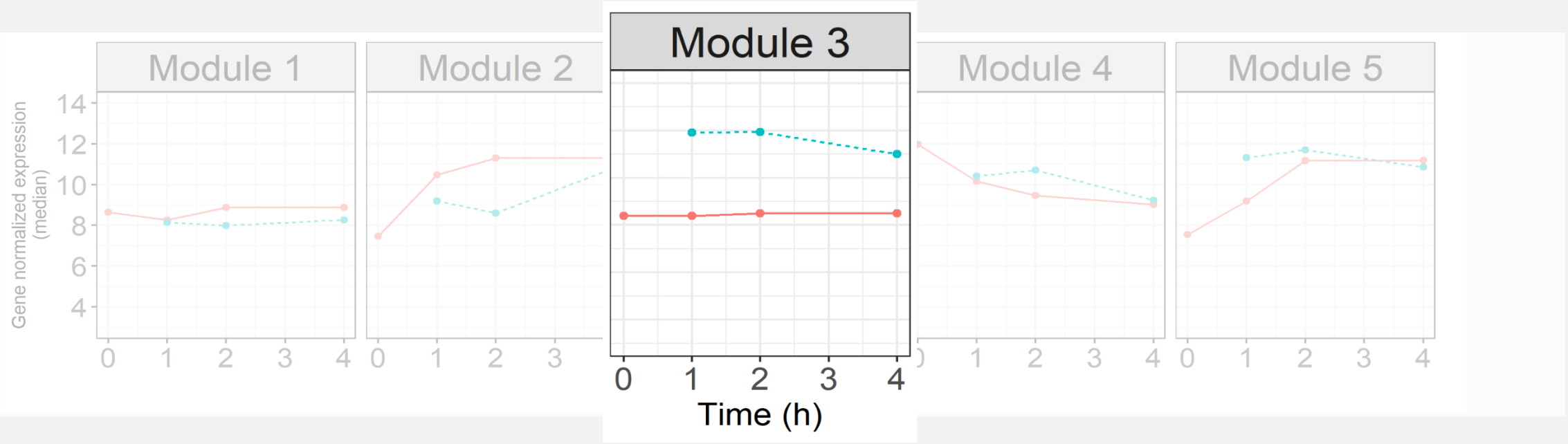
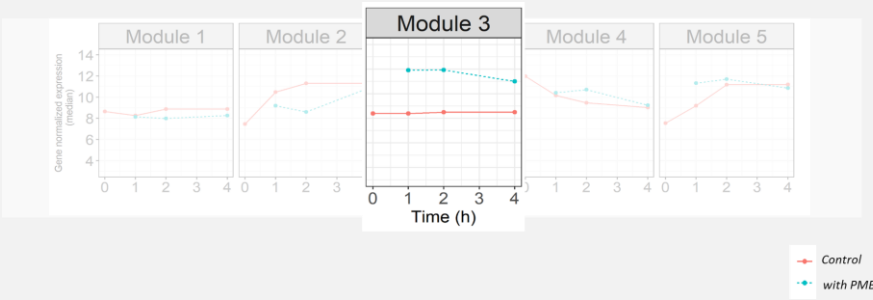


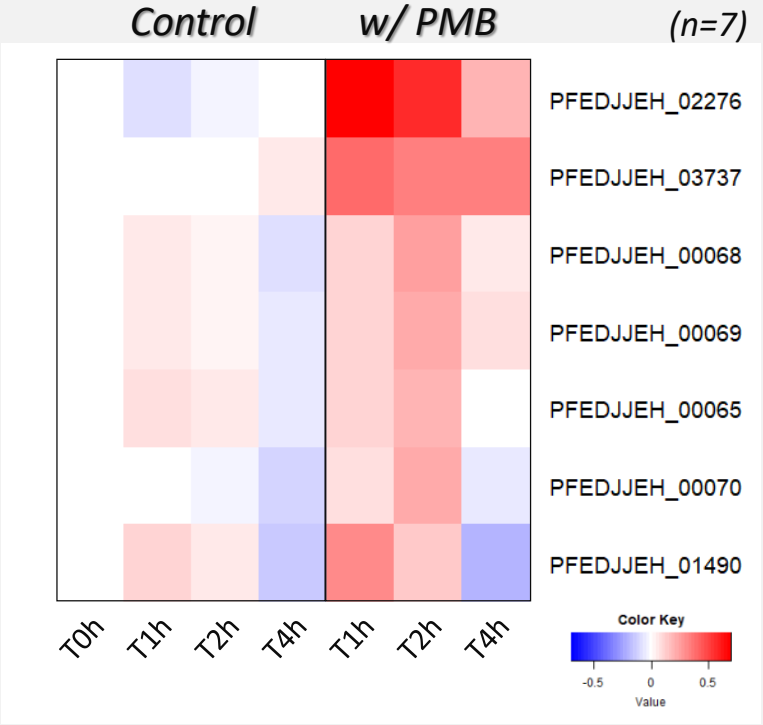
AB121 *pmbS*

→ ***91 genes allocated to this module***

Gene expressions per pathway



Amino-acids metabolism



- *35 genes associated with general metabolisms*
- *21 hypothetical proteins*

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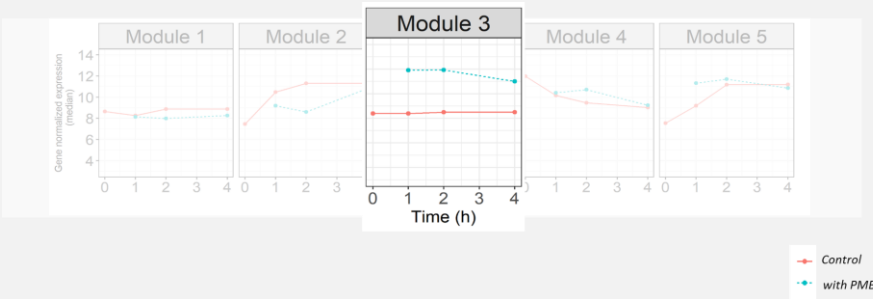

Gene expressions per pathway

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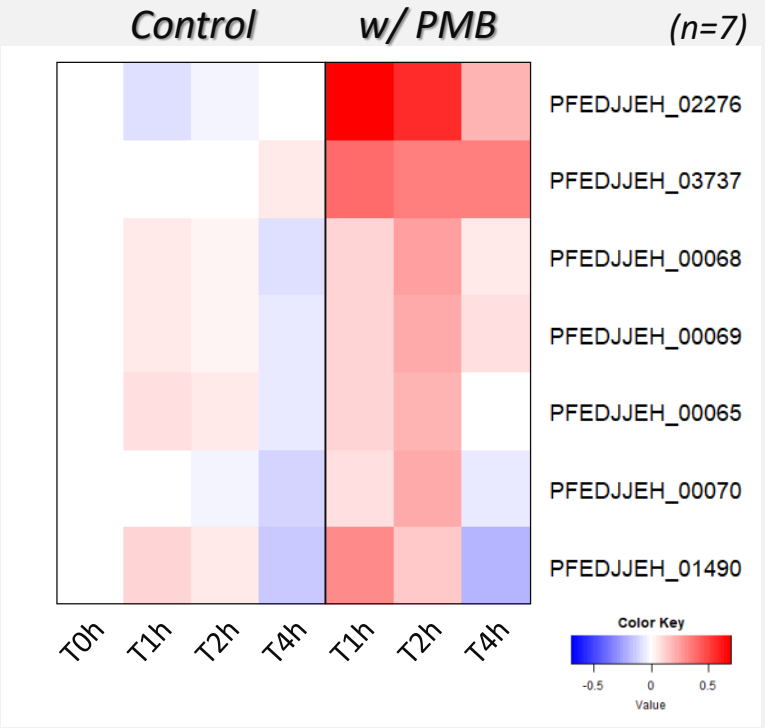
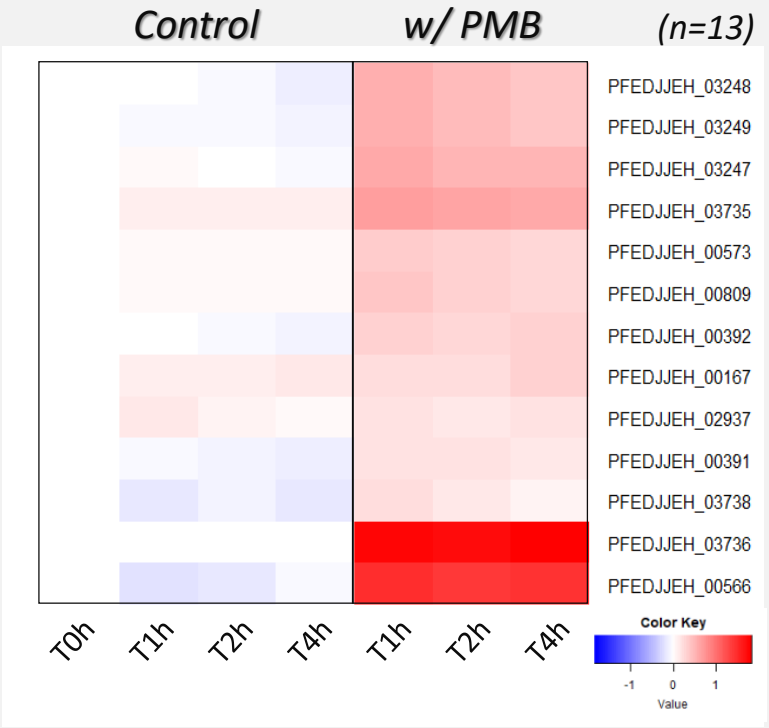


Efflux systems



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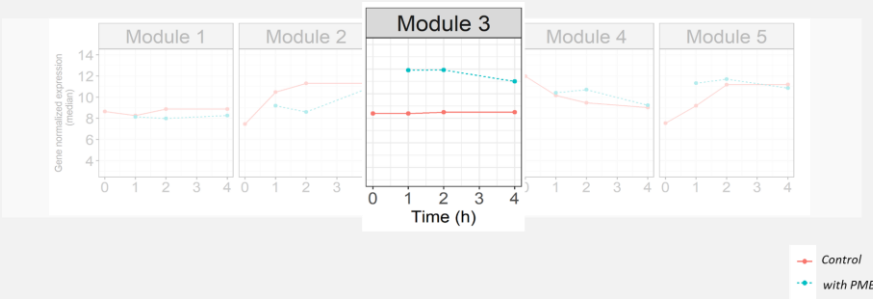

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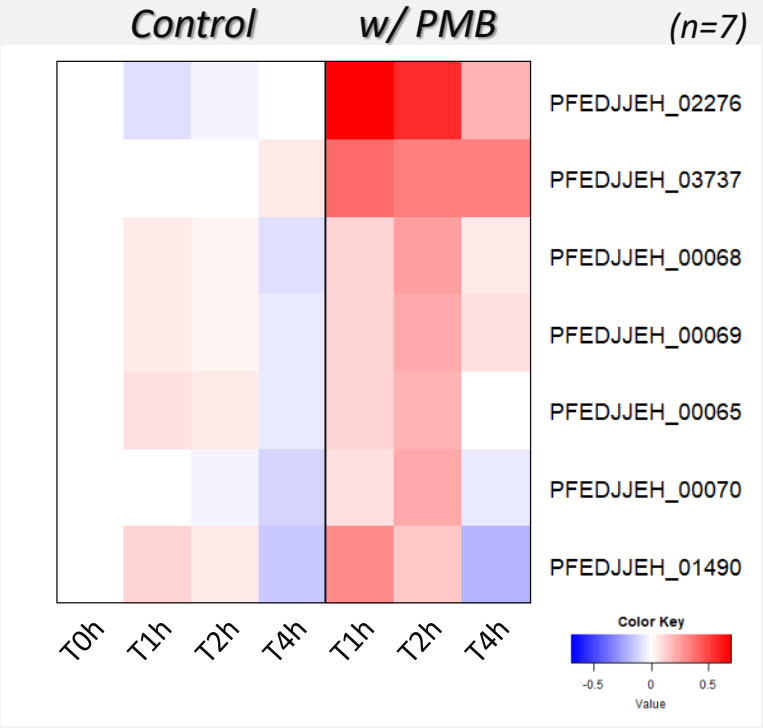

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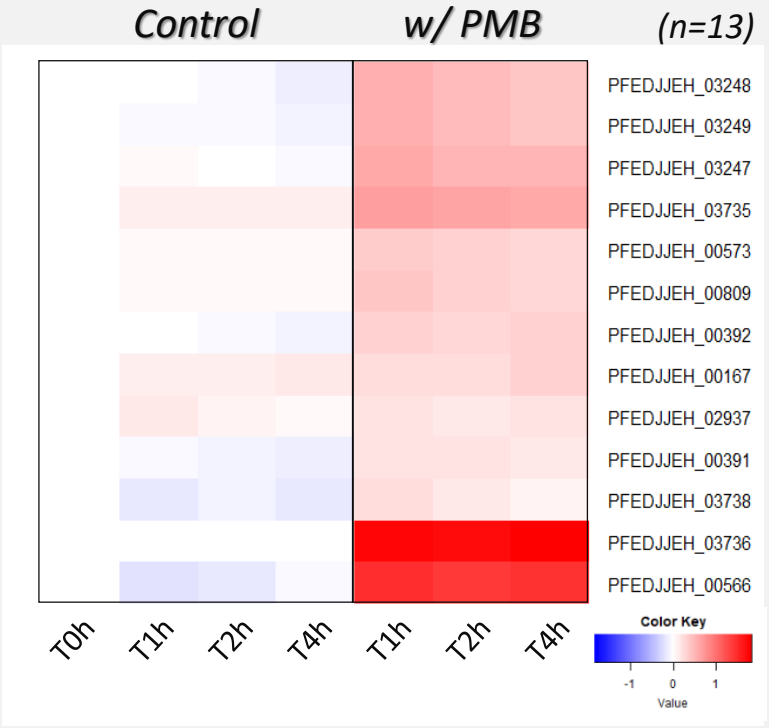
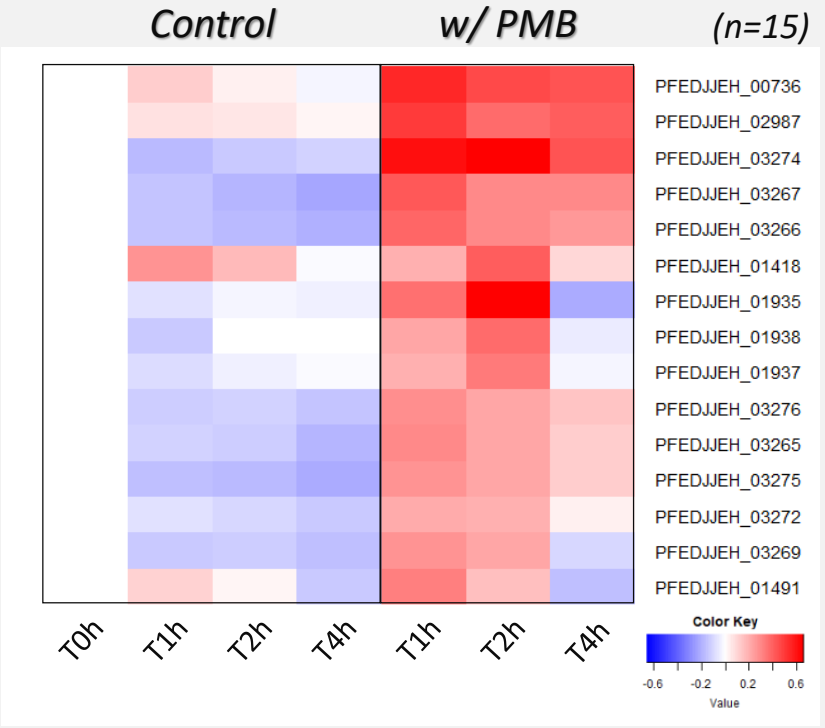


Membrane integrity



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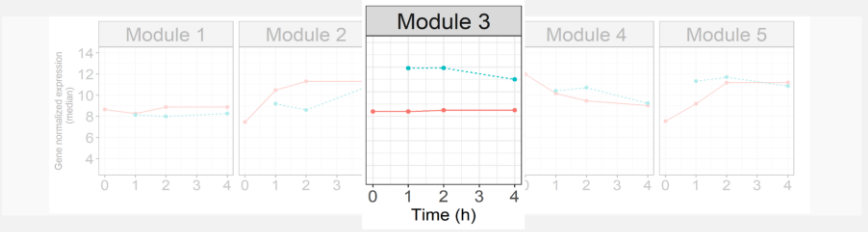

Amino-acids metabolism

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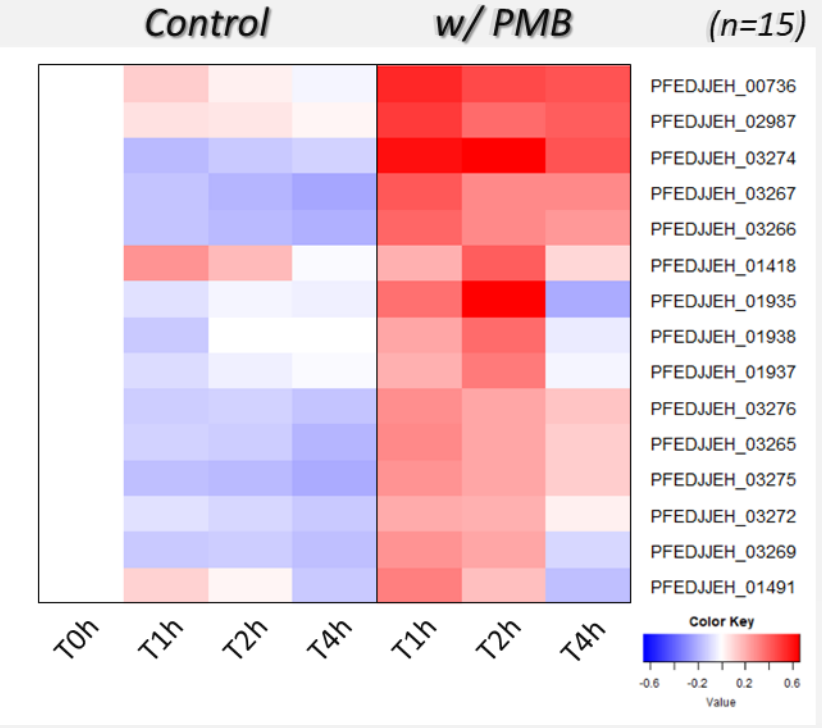

→ **Compensation of PMB membrane disruption activity by a over-production of membrane components ?**

Confirming literature results

Henry et al., 2012 Antimicrobial Agents and Chemotherapy
Park et al., 2015 Clinical Microbiology and Infection
Henry et al., 2015 Journal of Antimicrobial Chemotherapy
Cheah et al., 2016 Scientific Reports
Hua et al., 2017 Front. Cell. Infect. Microbiol.
Boll et al., 2020 PNAS
Chamoun et al., 2021 Int. Journal of Molecular Science

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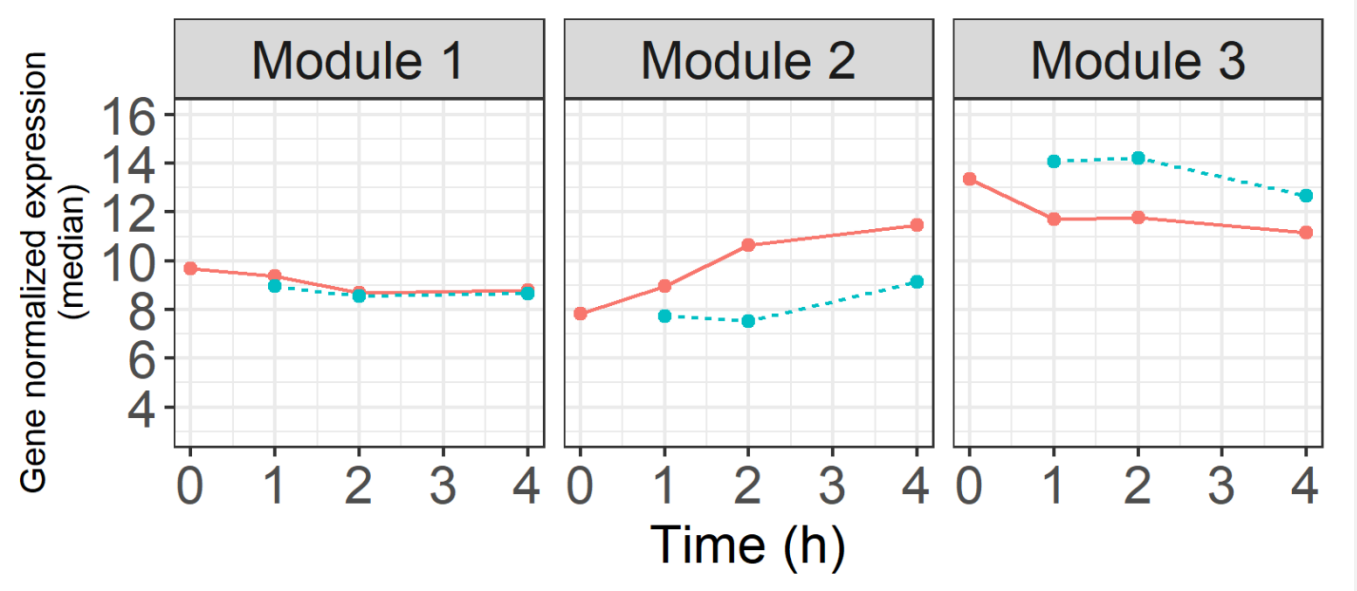
- *21 hypothetical proteins*

AB122 *pmbR*

Modules expression profiles



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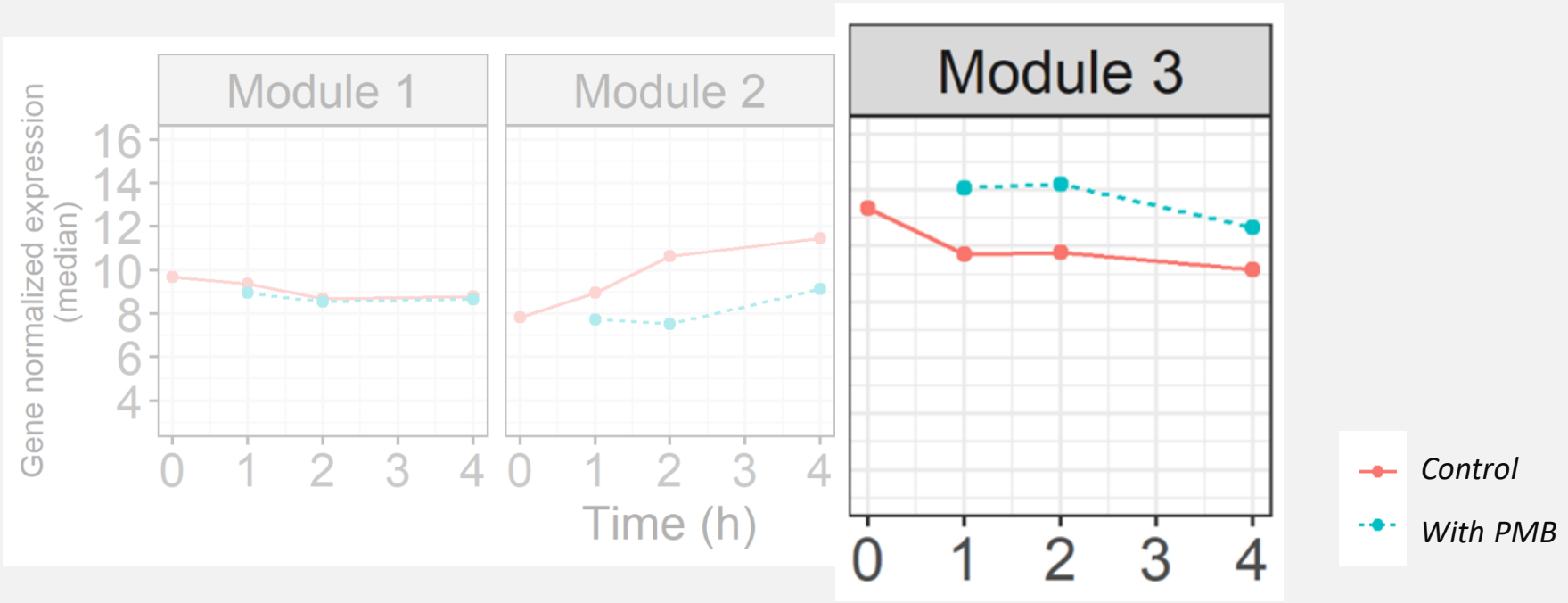
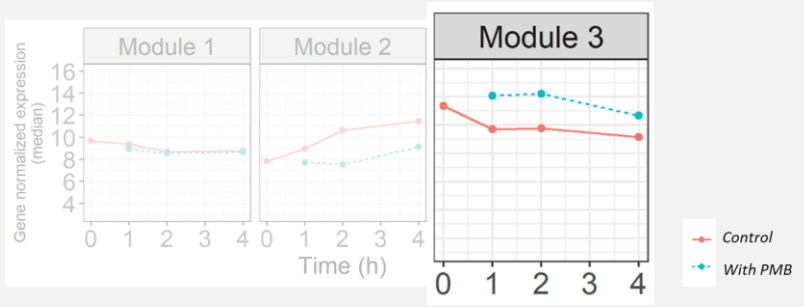


AB122 *pmbR*

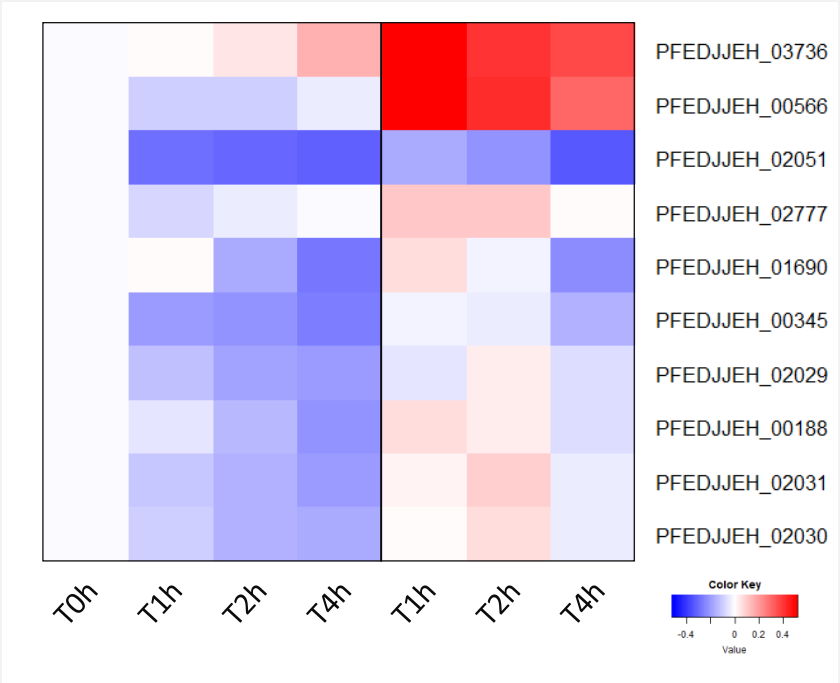
→ ***105 genes allocated to this module***

Gene expressions per pathway



Efflux systems

Control *w/ PMB* *(n=10)*



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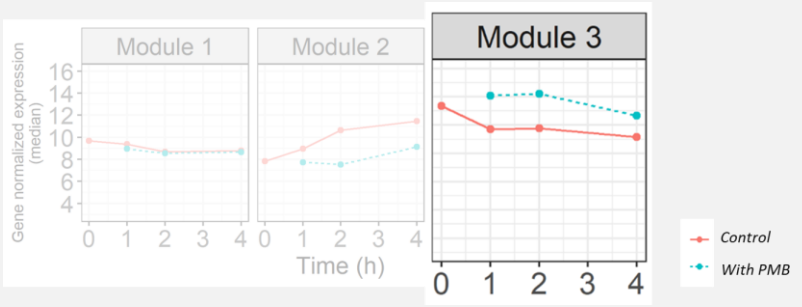

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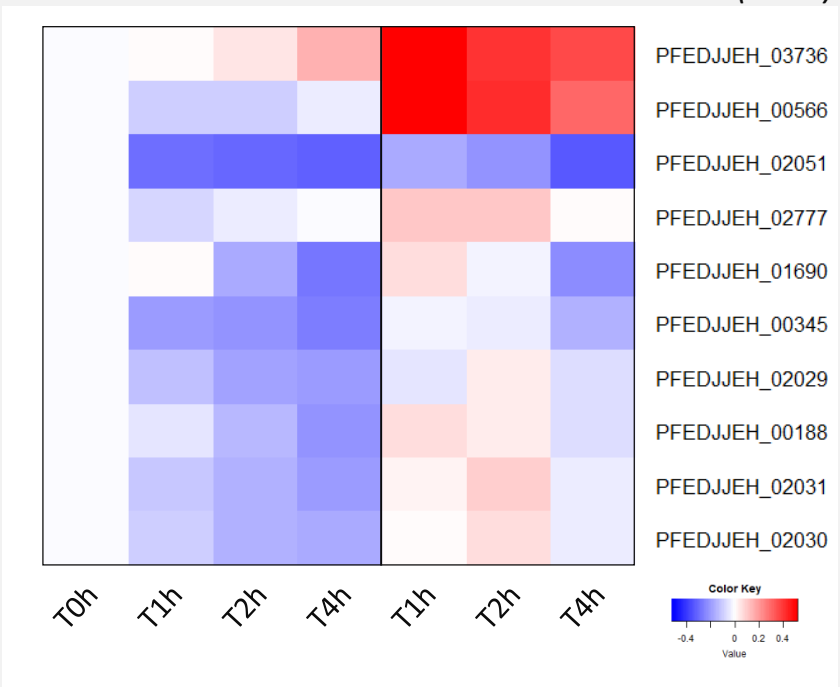
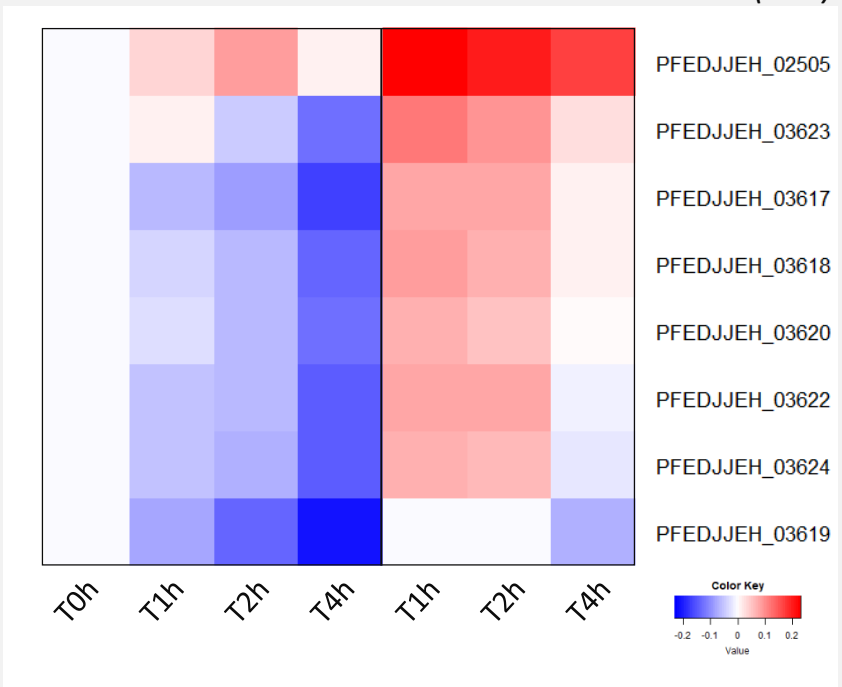


Oxidative phosphorylation

Control *w/ PMB* *(n=8)*



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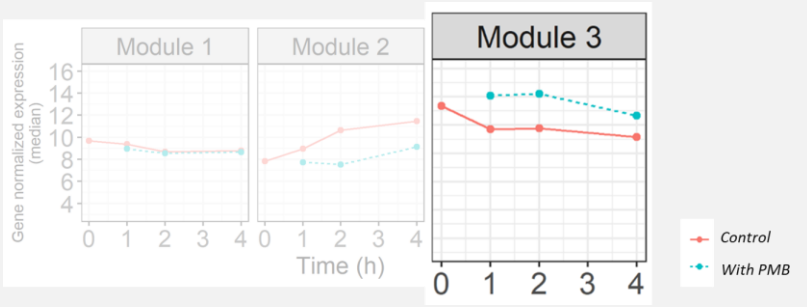
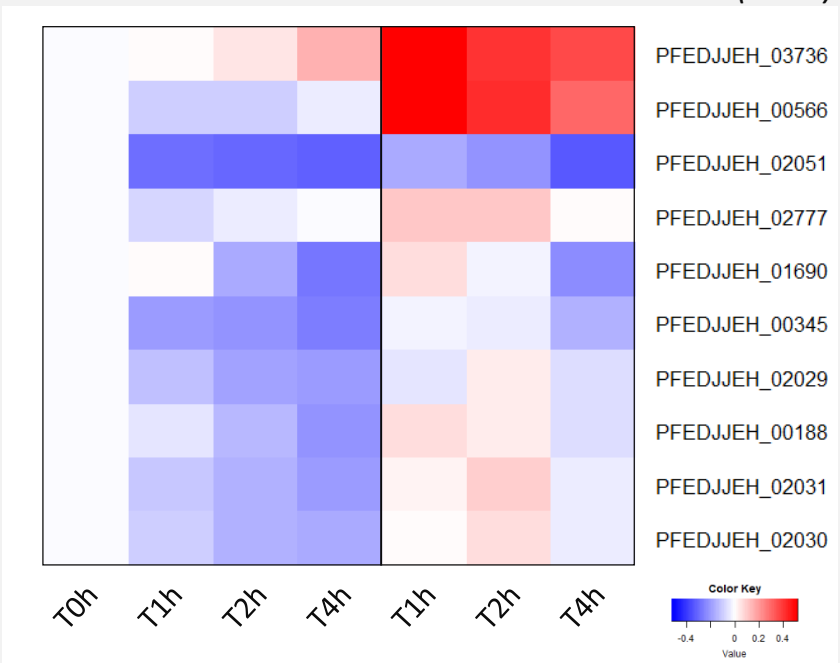
→ ***105 genes allocated to this module***

Gene expressions per pathway



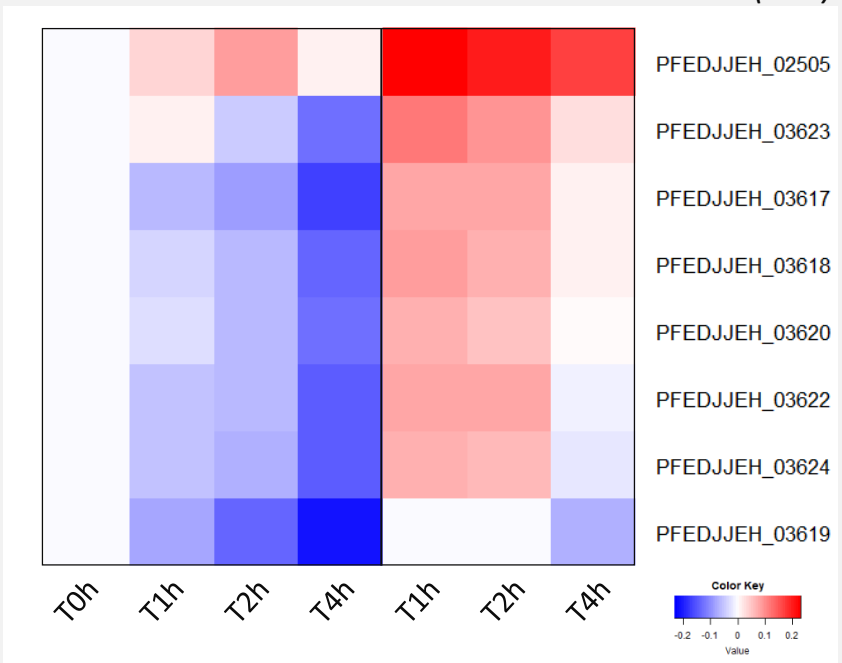
Efflux systems

Control *w/ PMB* *(n=10)*



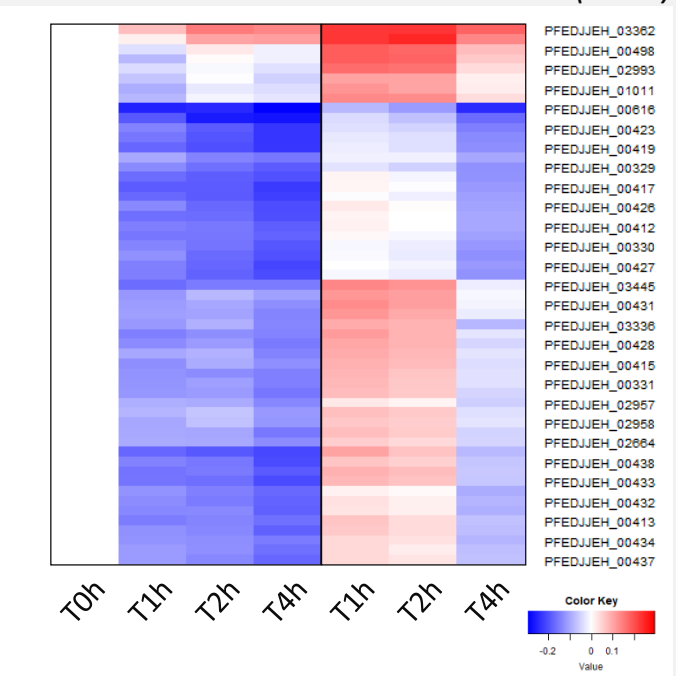
Oxidative phosphorylation

Control *w/ PMB* *(n=8)*



Protein synthesis

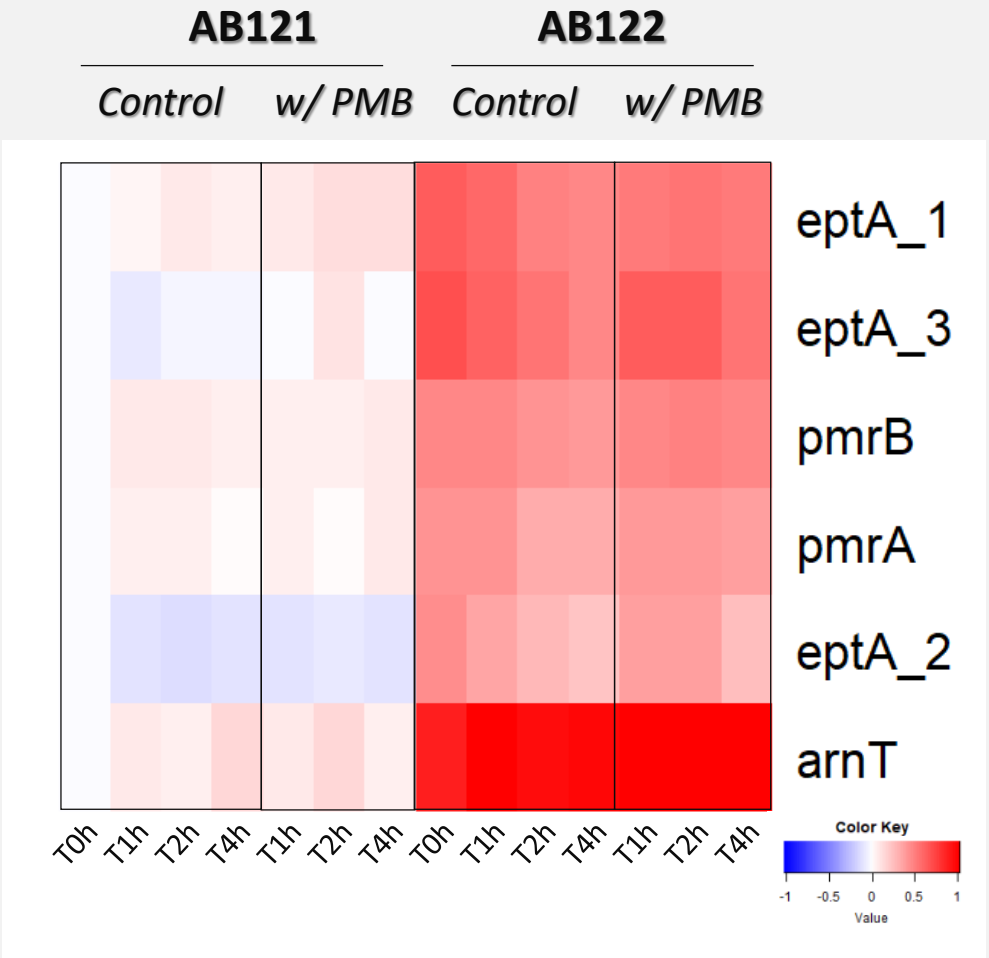
Control *w/ PMB* *(n=55)*

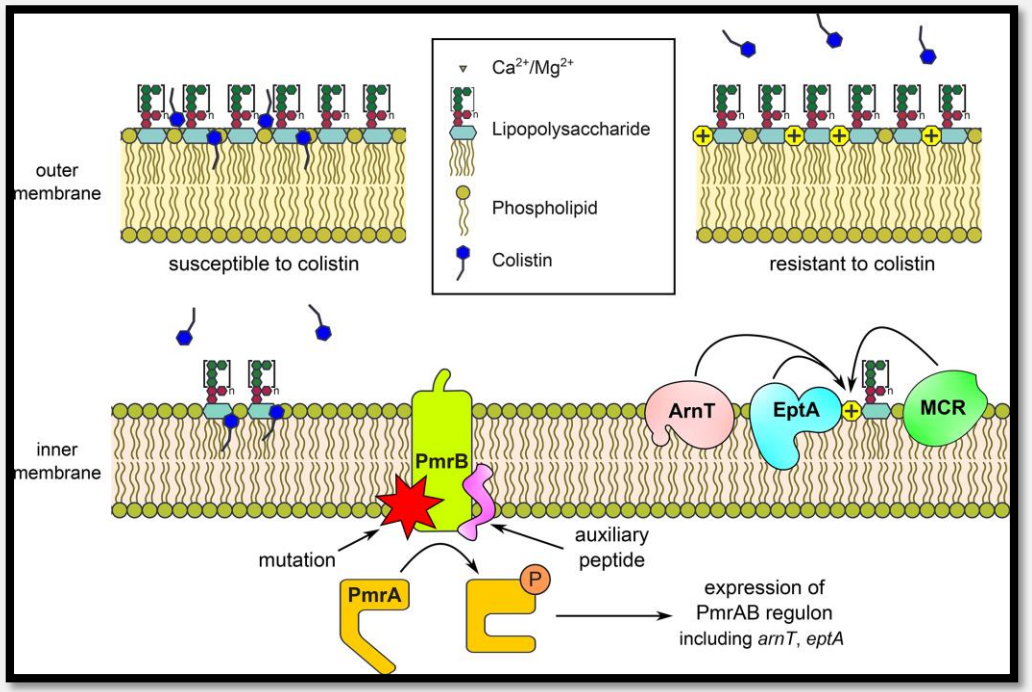


- *21 genes associated with general metabolisms*
- *11 hypothetical proteins*

AB122 *pmbR*

Resistance genes





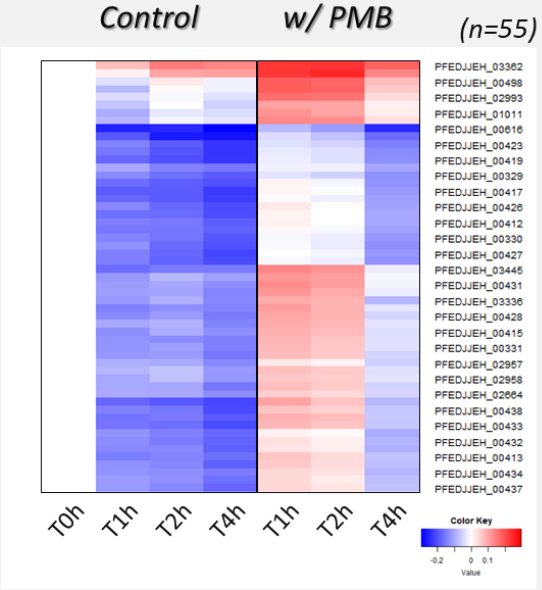
No expression change of genes involved in resistance with PMB addition

Janssen et al., 2021 PLoS Genetics

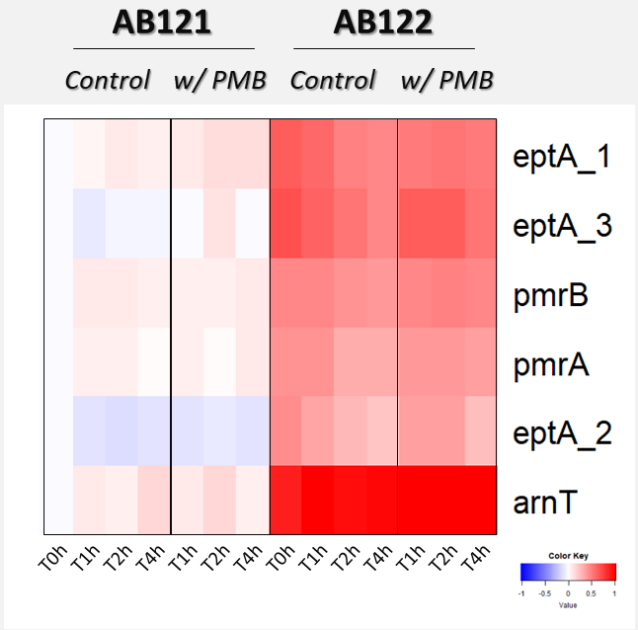
AB122 *pmbR*

Potential resistance mechanism

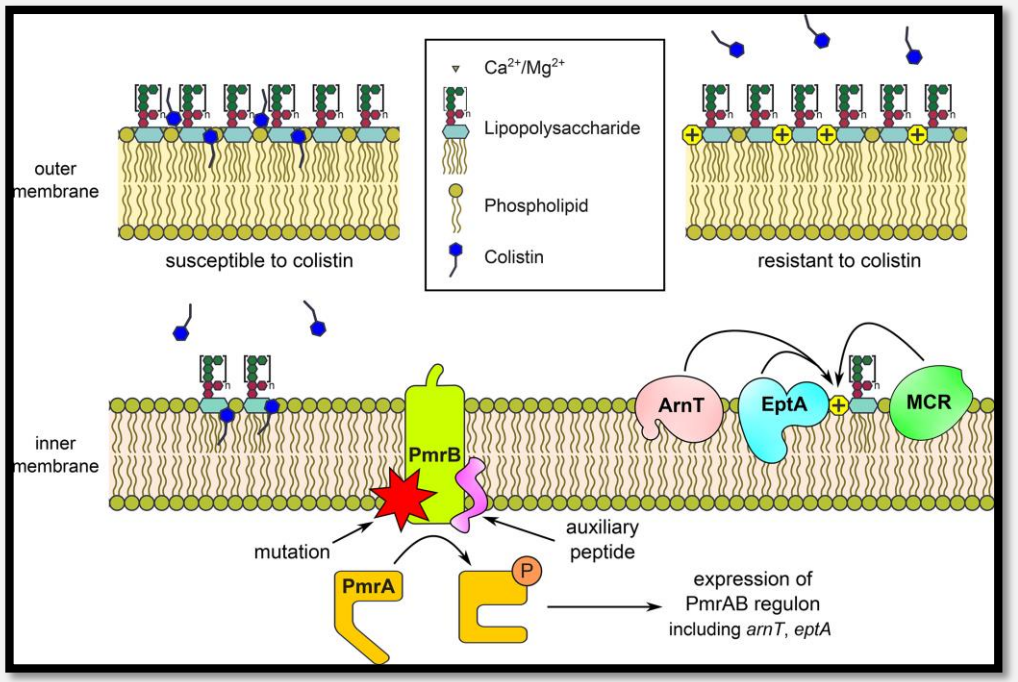
Protein synthesis



Increase of protein synthesis induced by PMB



No expression change with PMB addition of genes involved in resistance



[1]

Synthesis increase of eptA and arnT to change lipid A charge and prevent PMB binding ?

Study of PMB fixation to bacterial membrane by confocal microscopy

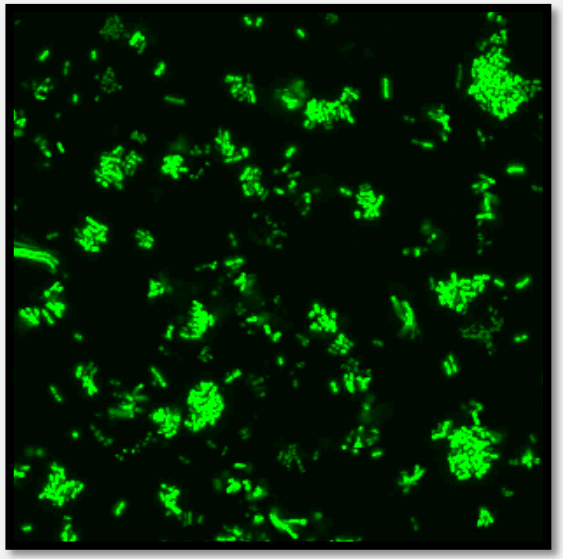
[1] *Janssen et al., 2021 PLoS Genetics*

AB121 & AB122 *Confocal microcopy*

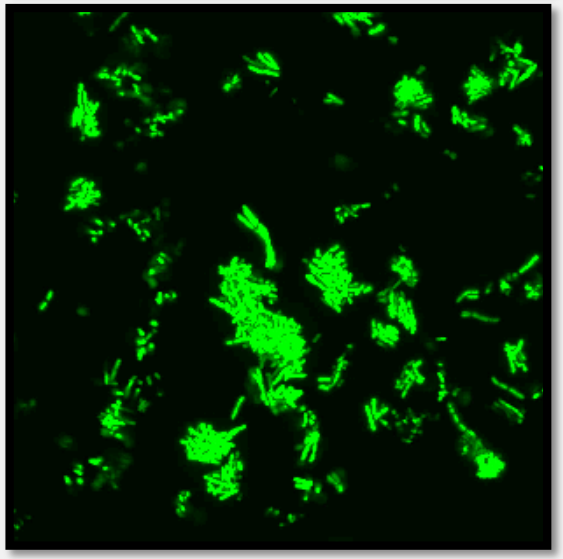
Green -> Syto 9

All bacteria

AB121 pmbS



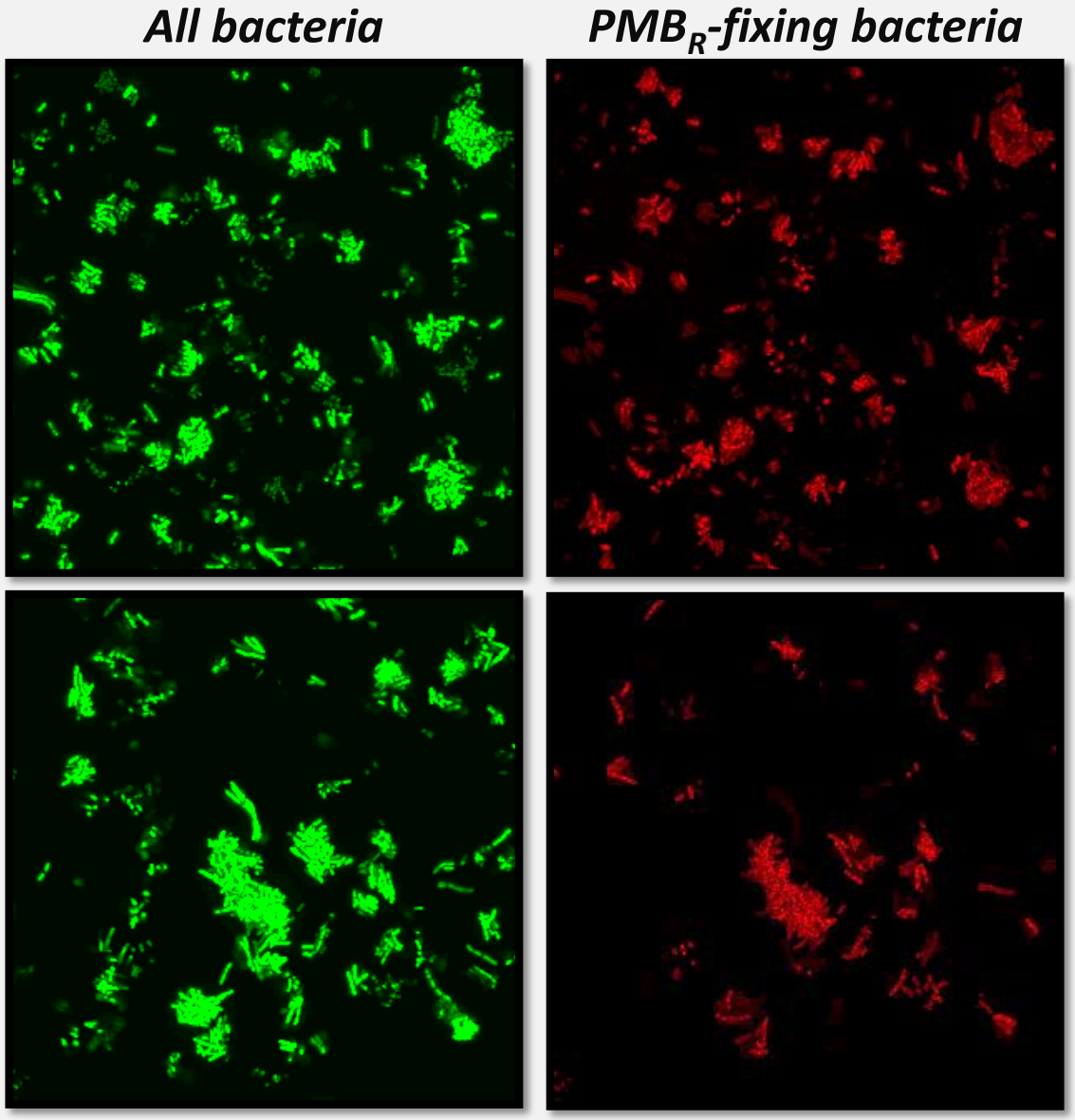
AB122 pmbR



T30min

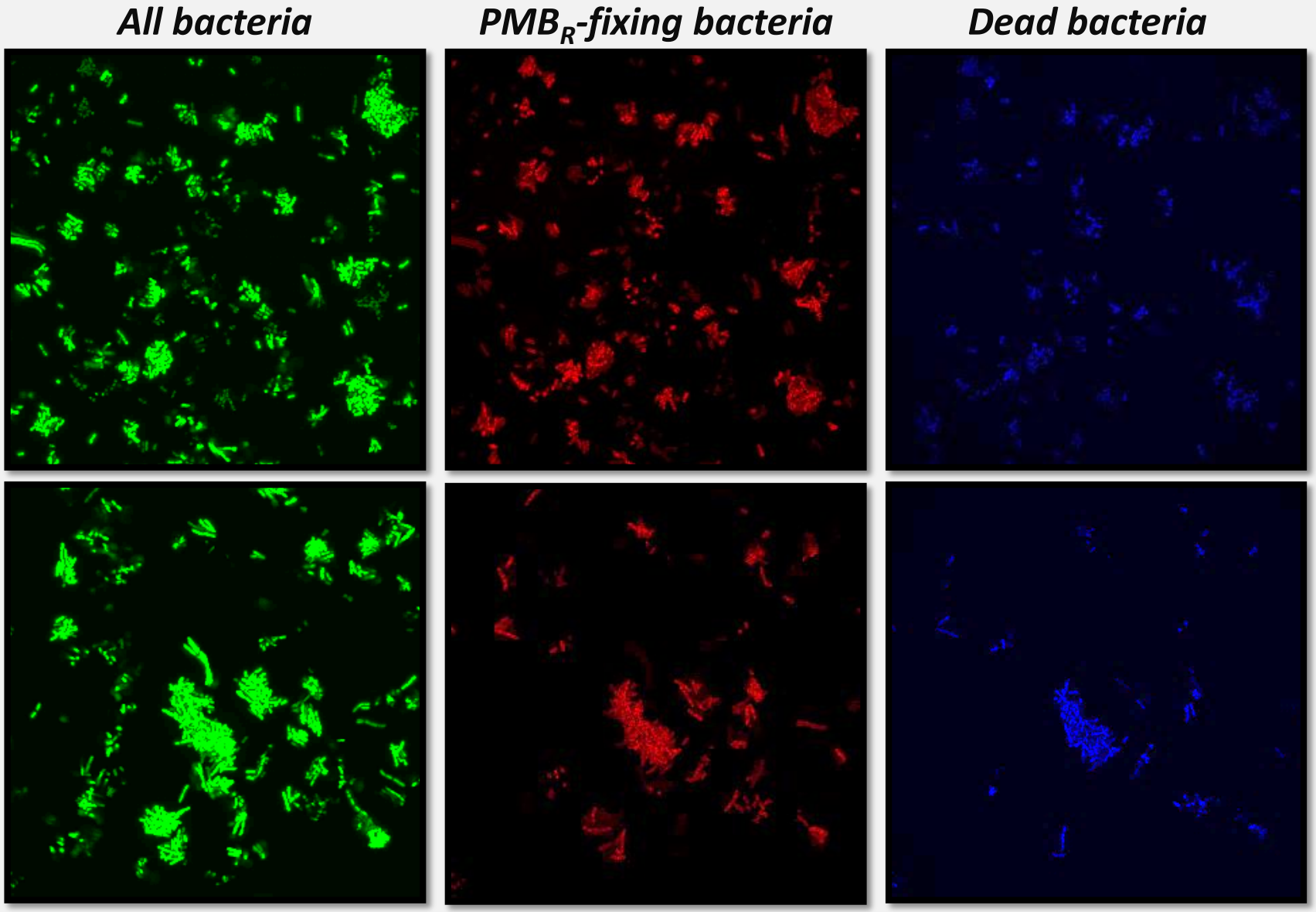
AB121 & AB122 *Confocal microcopy*

Green -> Syto 9
PMB_R -> PMB-Rhodamine



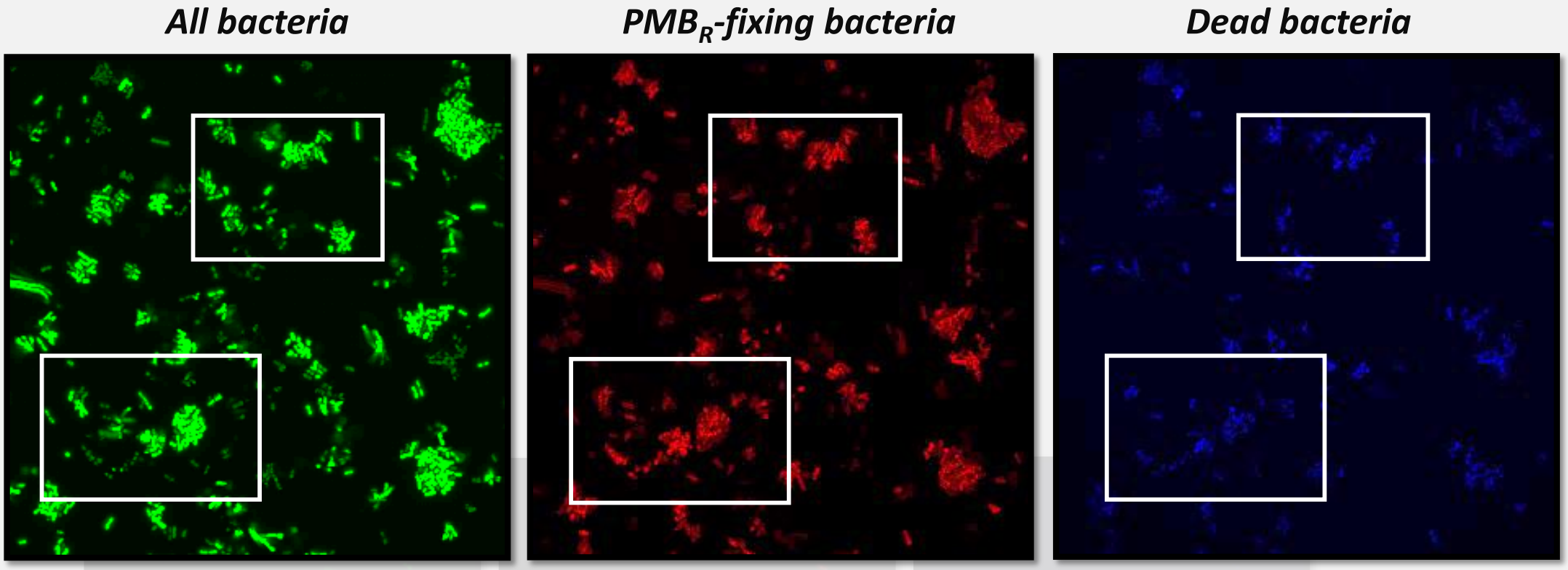
AB121 & AB122 *Confocal microcopy*

Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue



AB121 & AB122 *Confocal microcopy*

Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue



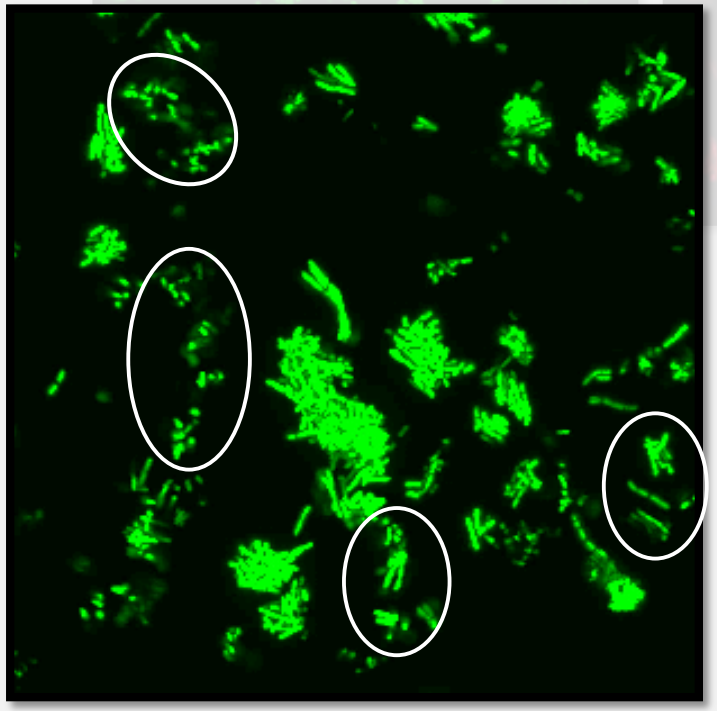
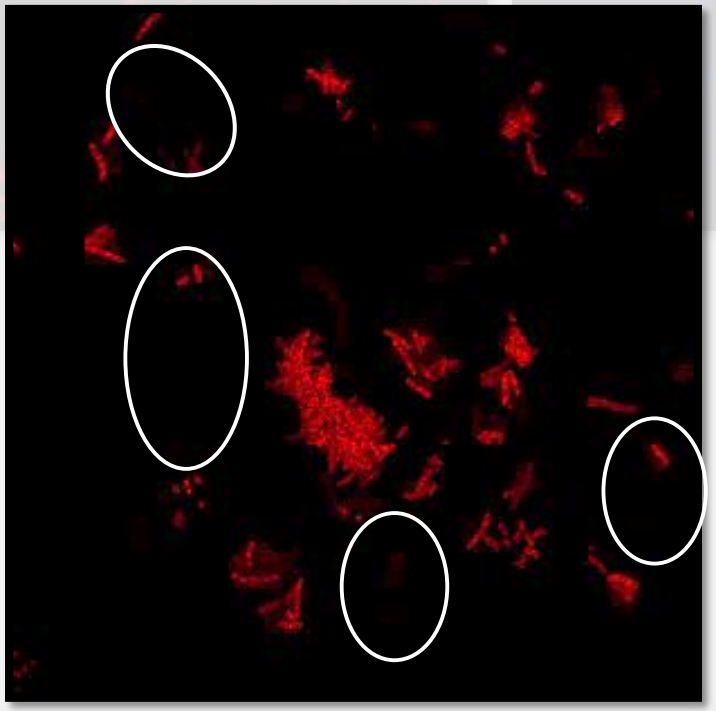
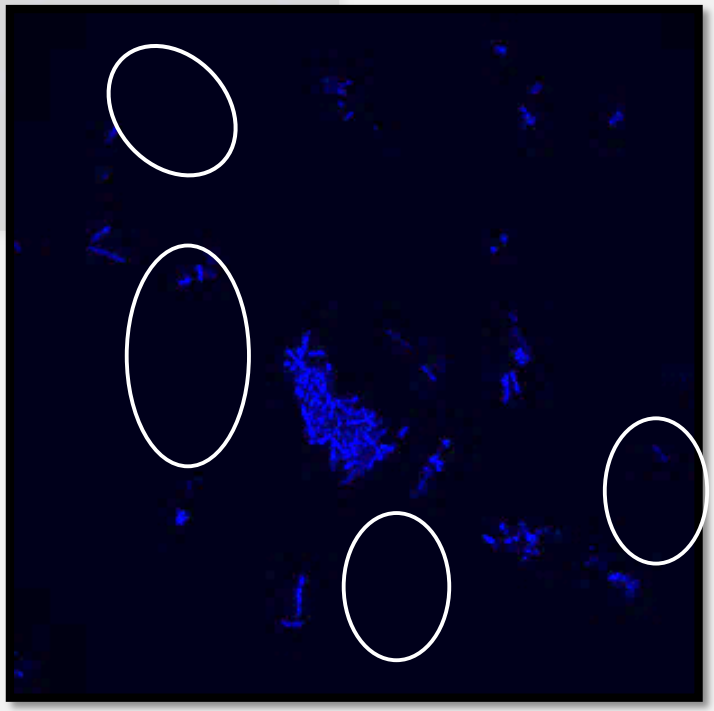
→ **For AB121: all bacteria are labelled with PMB_R**

T30min

AB121 & AB122 *Confocal microcopy*

Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue

→ **For AB122: presence of bacteria subpopulations not fixing PMB on their membranes**

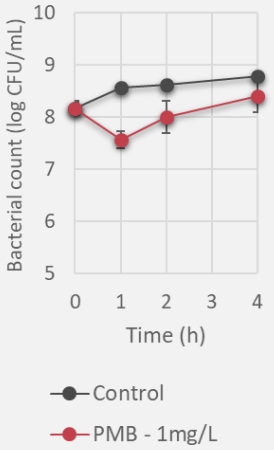
	All bacteria	*PMB_R-fixing bacteria*	*Dead bacteria*
AB122 pmbR			

T30min

Conclusion – Resistance mechanisms

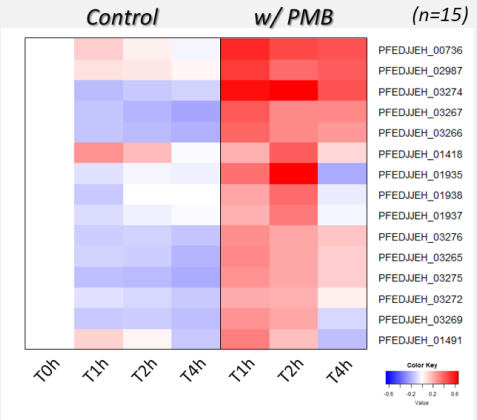
Time-kill curves

AB121 *pmbS*



Transcriptomic and confoncal microscopy results

Membrane remodeling



Potential resistance mechanism

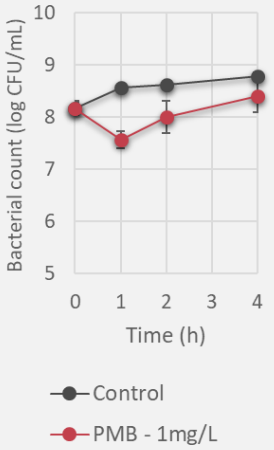
Over-production of membrane components to prevent PMB membrane disruption ?

Adaptation ?

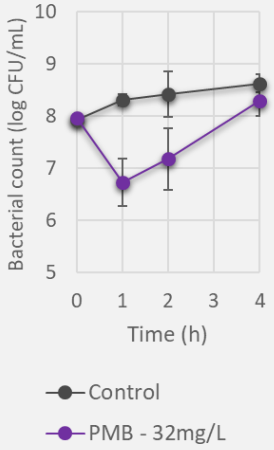
Conclusion – Resistance mechanisms

Time-kill curves

AB121 *pmbS*

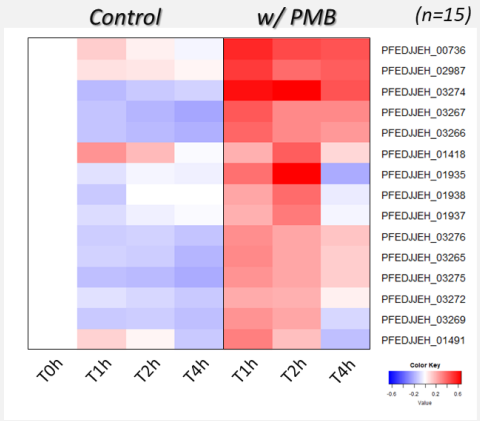


AB122 *pmbR*

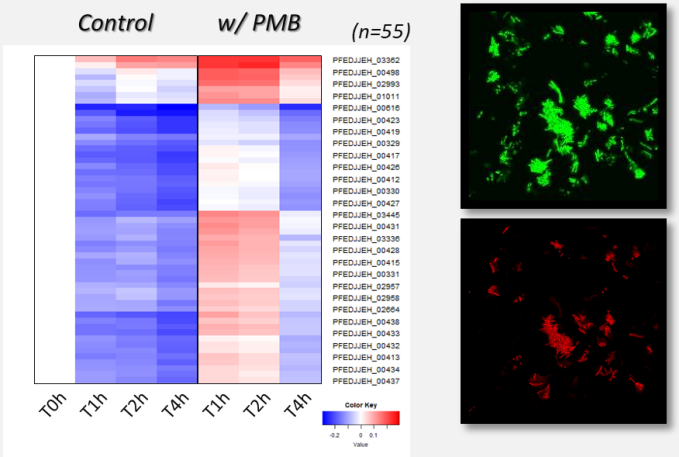


Transcriptomic and confoncal microscopy results

Membrane remodeling



Protein synthesis



Potential resistance mechanism

Over-production of membrane components to prevent PMB membrane disruption ?

⇩

Adaptation ?

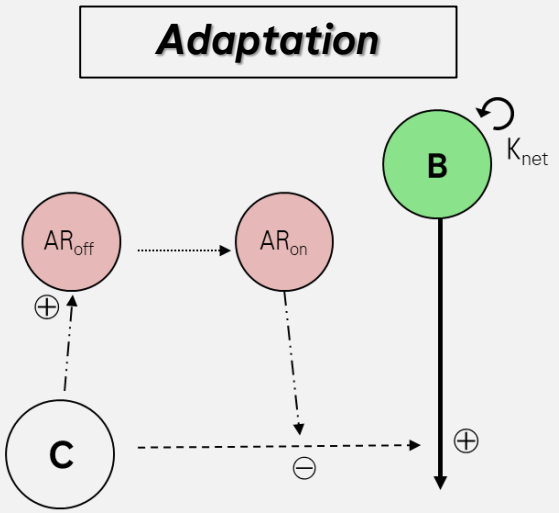
Synthesis increase of eptA to change lipid A charge and prevent PMB binding ?

⇩

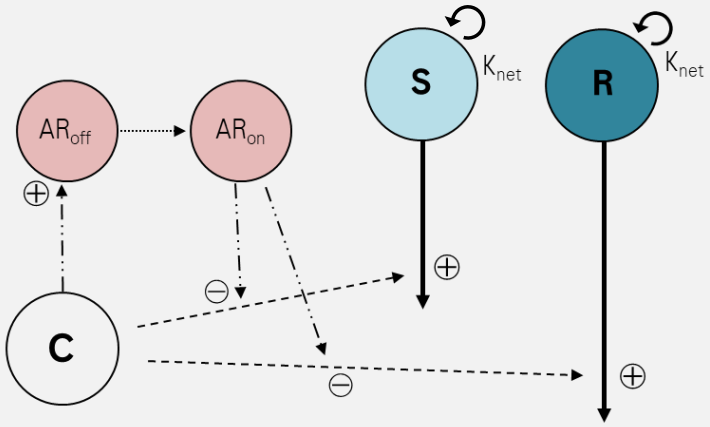
Hetero-resistance + Adaptation ?

Conclusion – Modelling

AB121 pmbS



AB122 pmbR



- ***PK/PD model choice***

✓ Transcriptomics may orient the selection of model structure

✓ Results need to be expanded (Proteomics)

Thanks for your attention

INSERM U1070

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