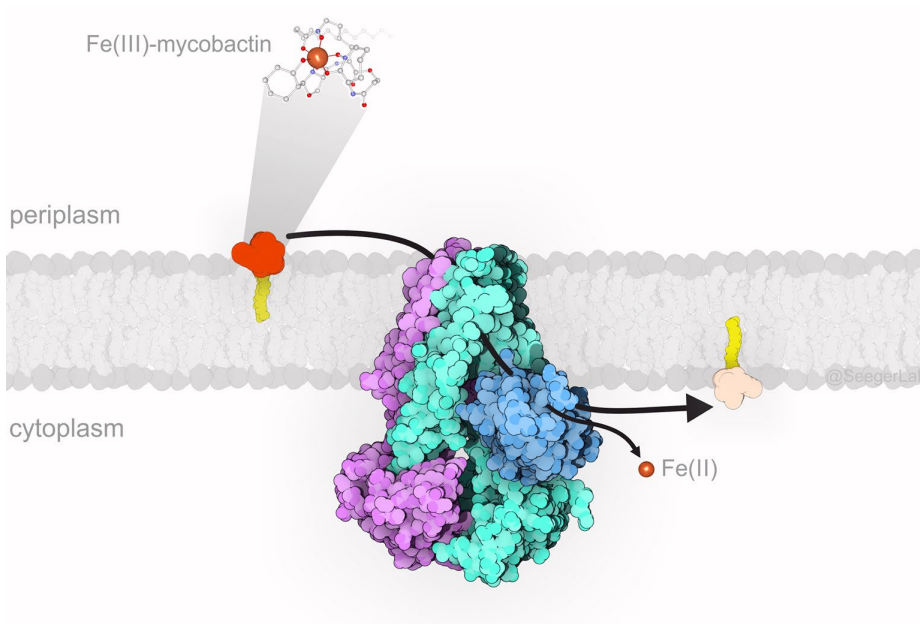
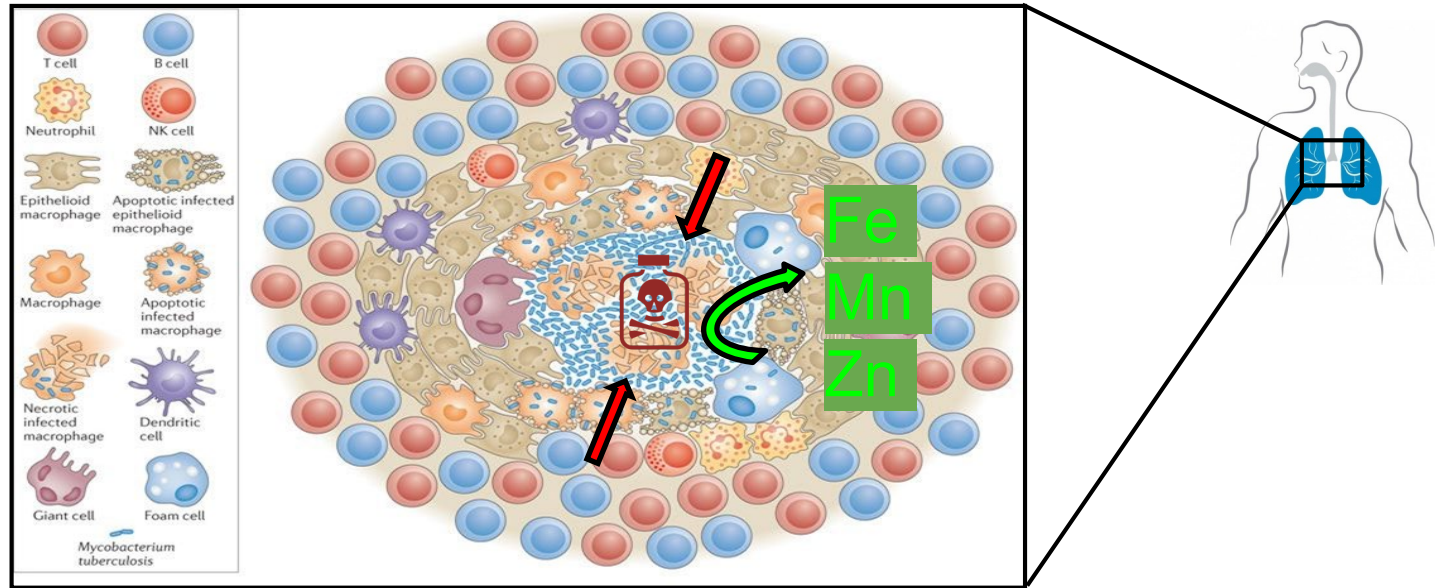


The ABC exporter IrtAB imports and reduces mycobacterial siderophores



Fabian Arnold, Miriam Weber, Imre Gonda
Tuberkulose Symposium
25. März 2021

Why does *M. tuberculosis* need siderophores?

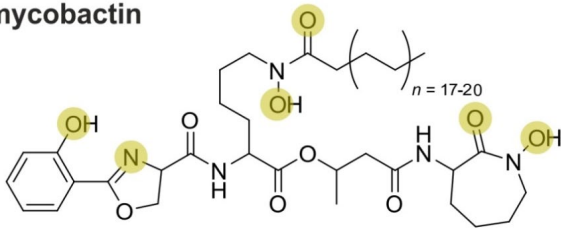


- «Nutritional Immunity» present in the human host
- Decreased virulence in *M. tuberculosis* strains with impaired siderophore synthesis/transport

The Mycobactins

- *M. tuberculosis* produces two types of siderophores, collectively called «mycobactins»
- Synthesized by a series of enzymes MbtA - MbtK

mycobactin

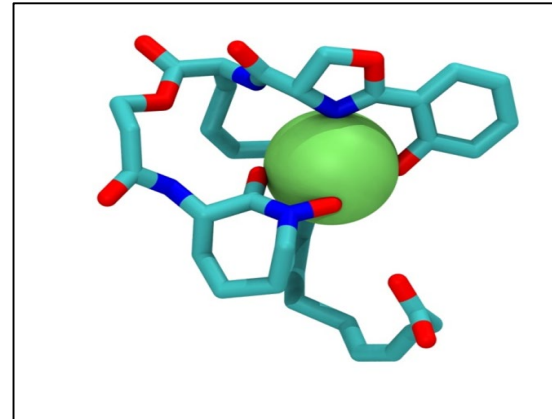
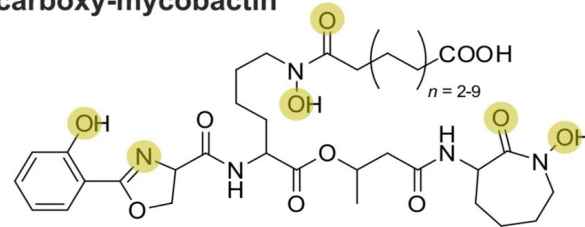


mycobactin

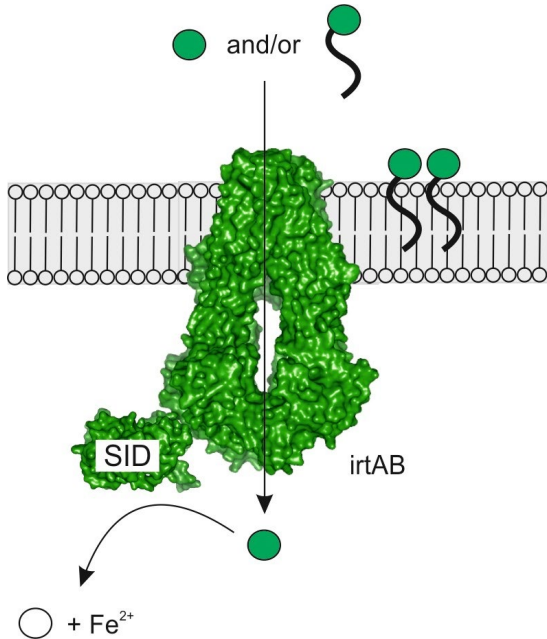
MW ~ 900 Da

(MBT)

carboxy-mycobactin



IrtAB working model

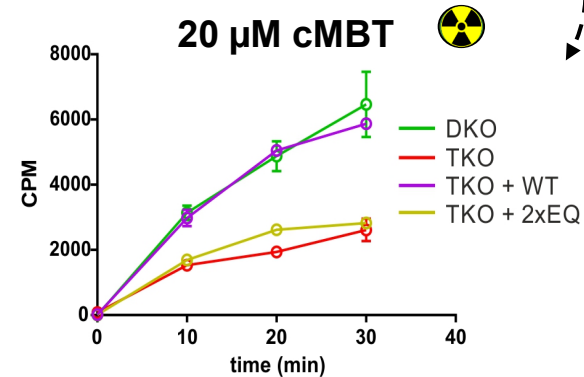
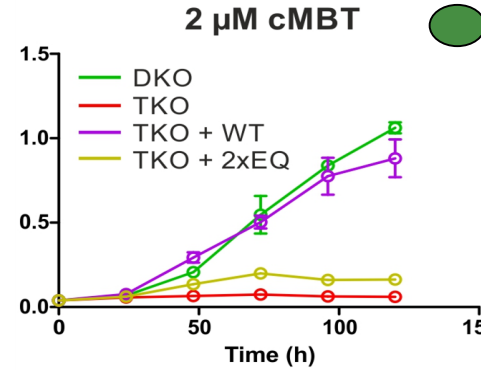
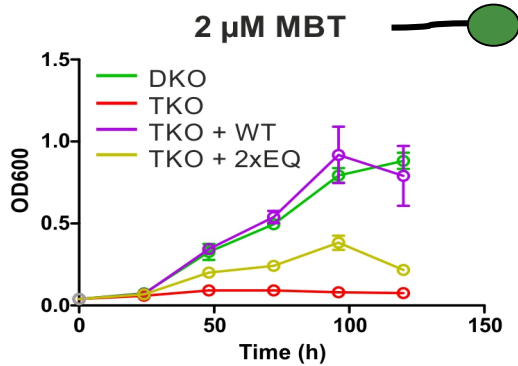


IrtAB was postulated (*in vivo* evidence) to be involved in siderophore trafficking and contribute to virulence in *M. tuberculosis*

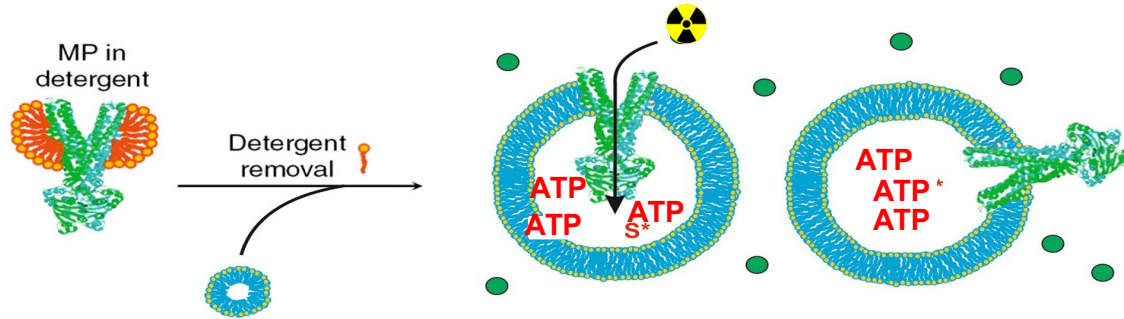
Open questions...

- What is the substrate of IrtAB?
- Is IrtAB importing or exporting siderophores? (ABC Type I exporter fold)
- Is the siderophore interacting domain (SID) involved in siderophore reduction?

Siderophore-dependant growth assay in *M. smegmatis*

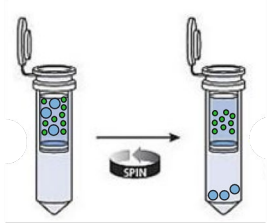


Siderophore import in proteoliposomes

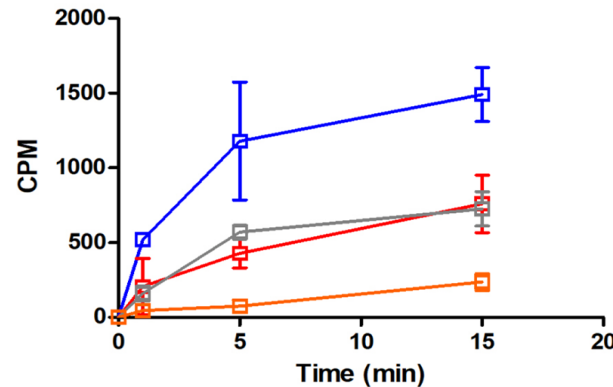


- Reconstitution in proteliposomes with ARS
- Monitor the transport directionality

At given timepoints:

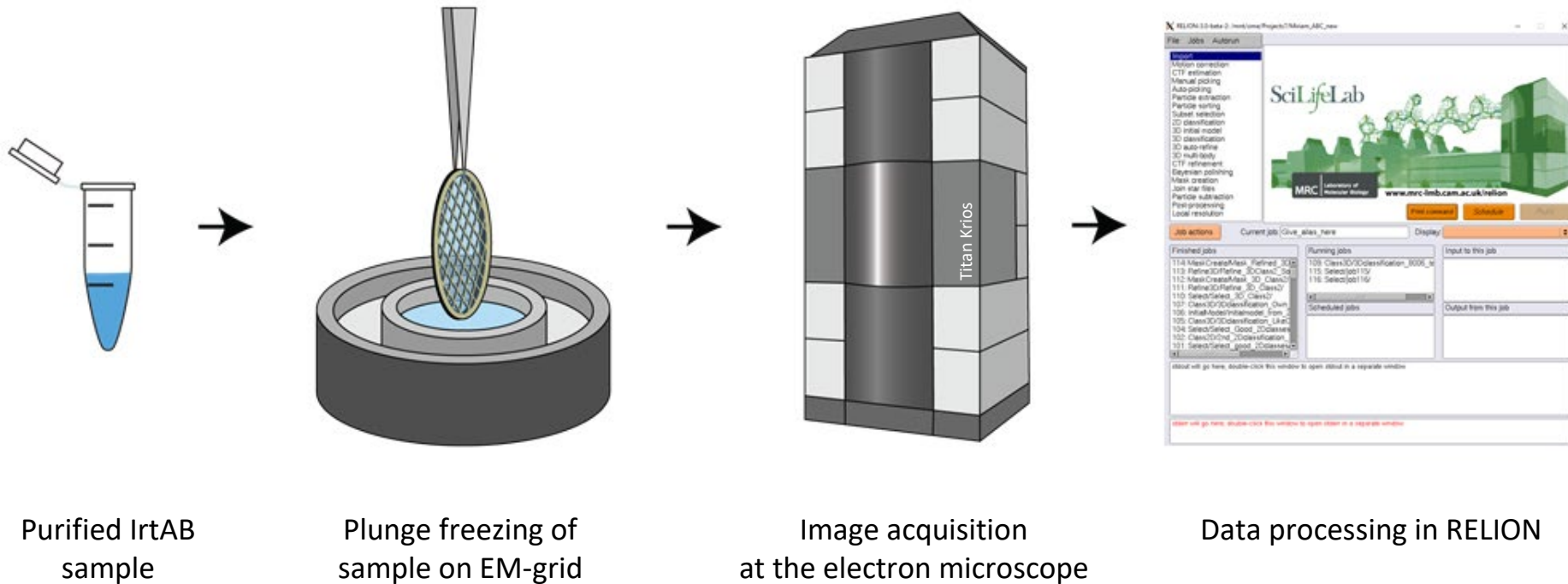


⁵⁵Fe-cMBT uptake



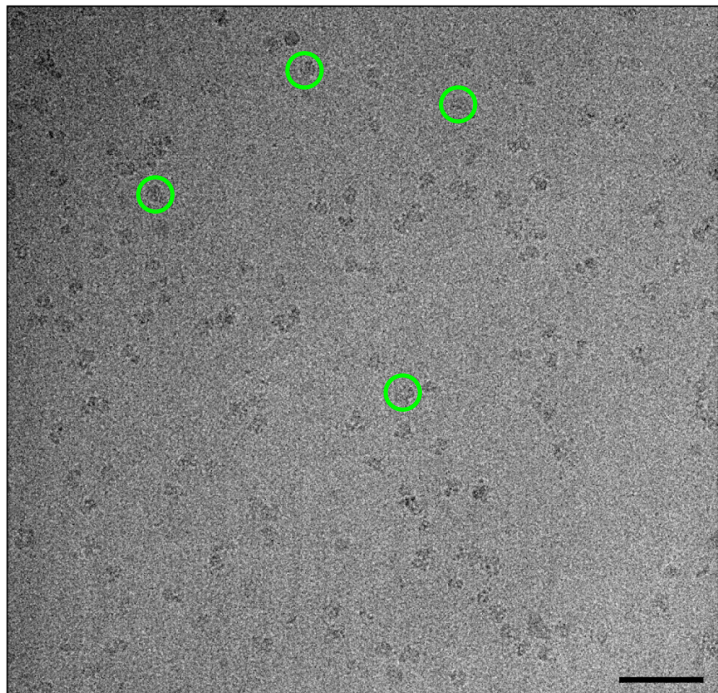
- IrAB PL + ATP (inside)
- IrAB PL without ATP
- Empty PL
- IrAB PL + ATP (outside)

Cryo-EM sample preparation and data collection

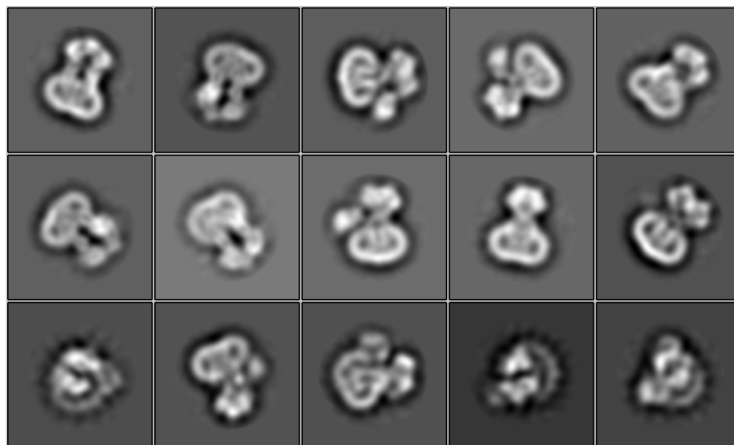


Structure determination with Cryo-EM

2 168 micrographs → Picked 335 045 particles



Classified particles in 2D to cover different orientations & remove false-positives



Electron micrographs

Particle picking

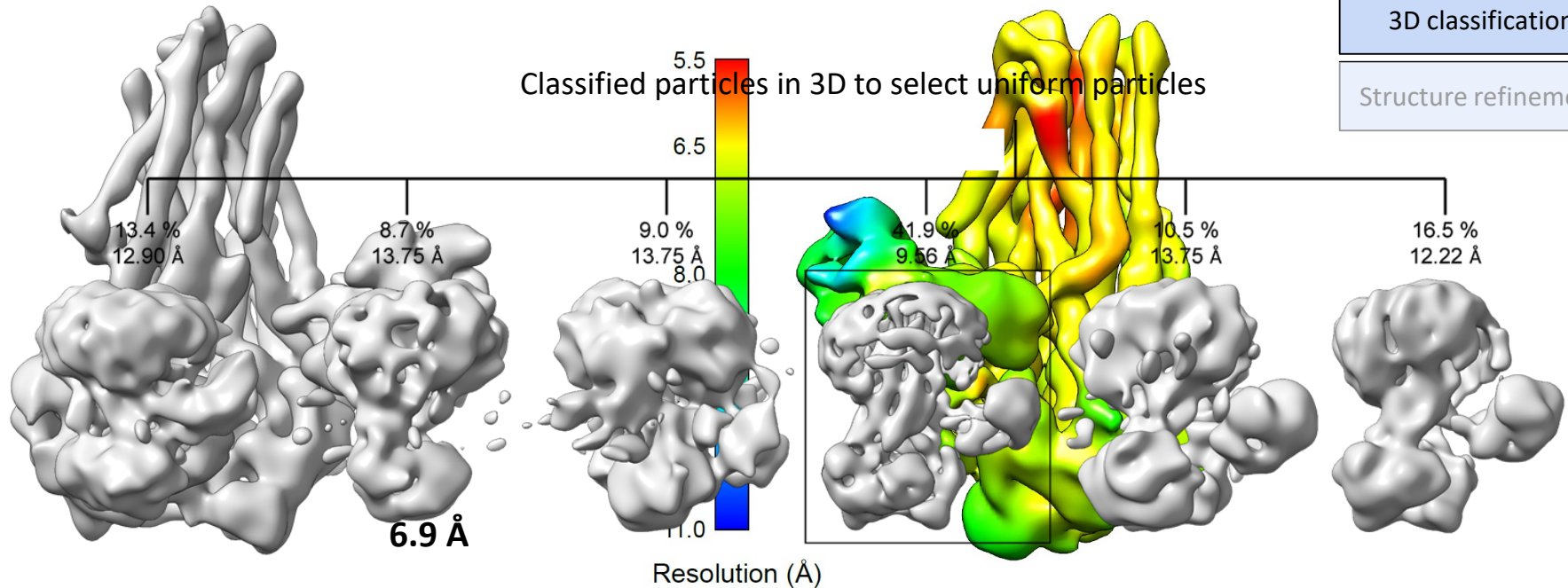
2D classification

3D classification

Structure refinement

Structure determination with Cryo-EM

Refine 3D structure to highest possible
resolution



Electron micrographs

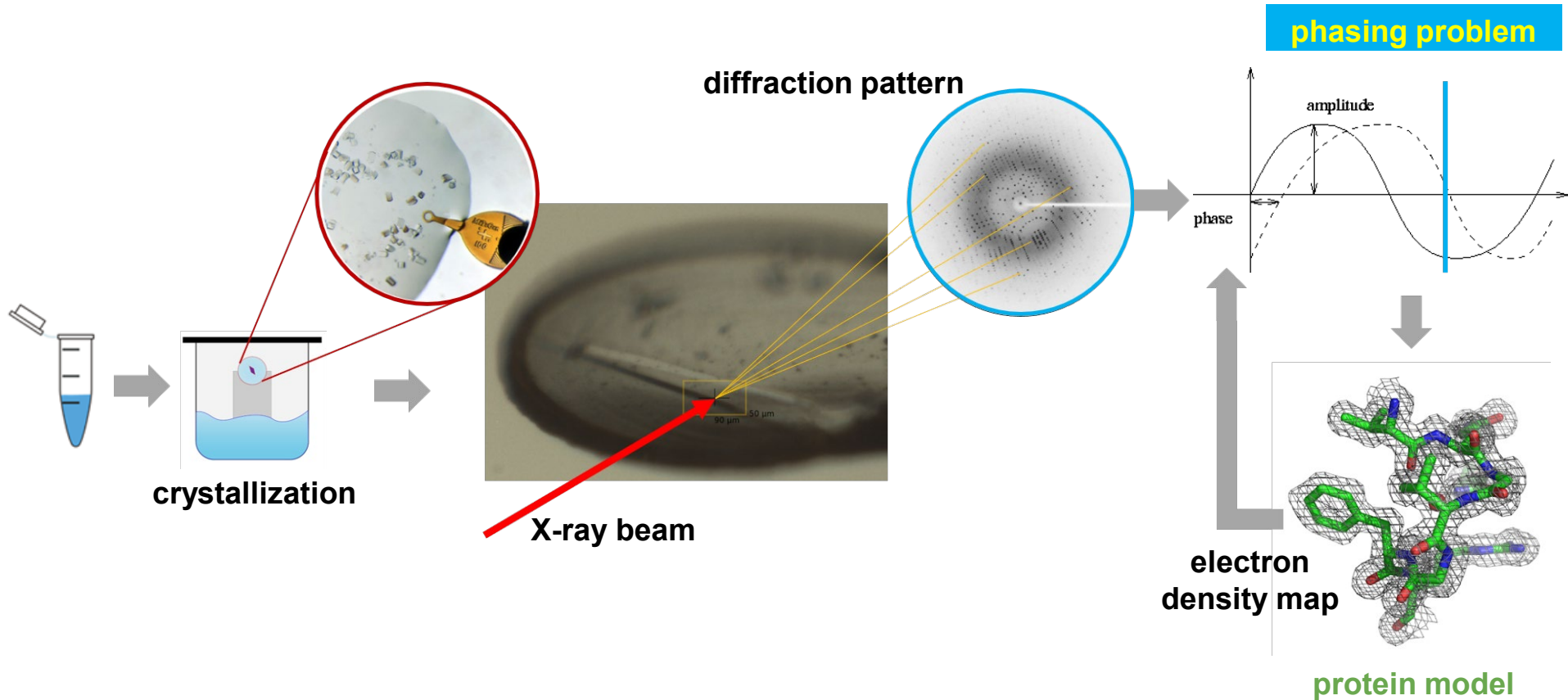
Particle picking

2D classification

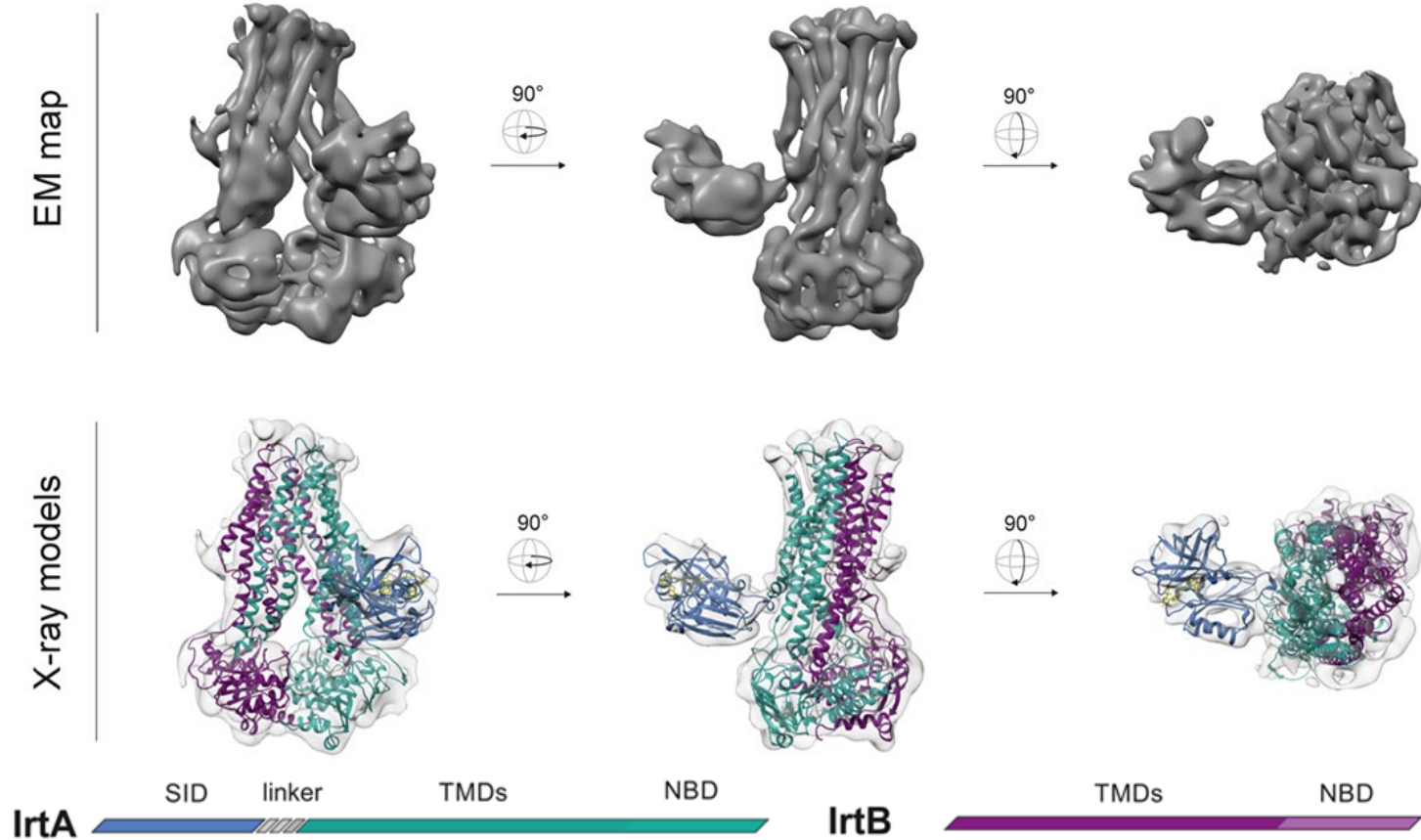
3D classification

Structure refinement

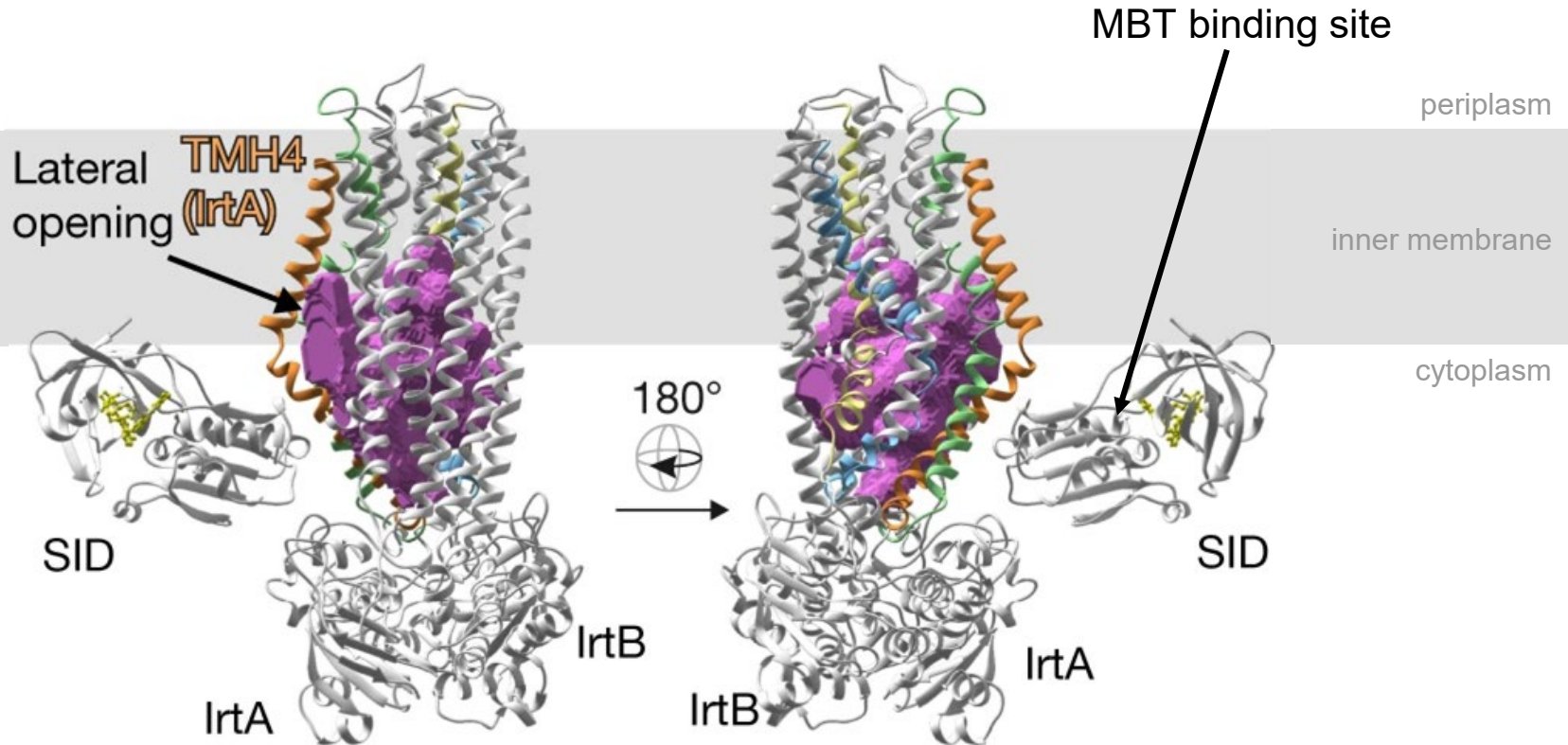
High resolution structures with X-ray crystallography



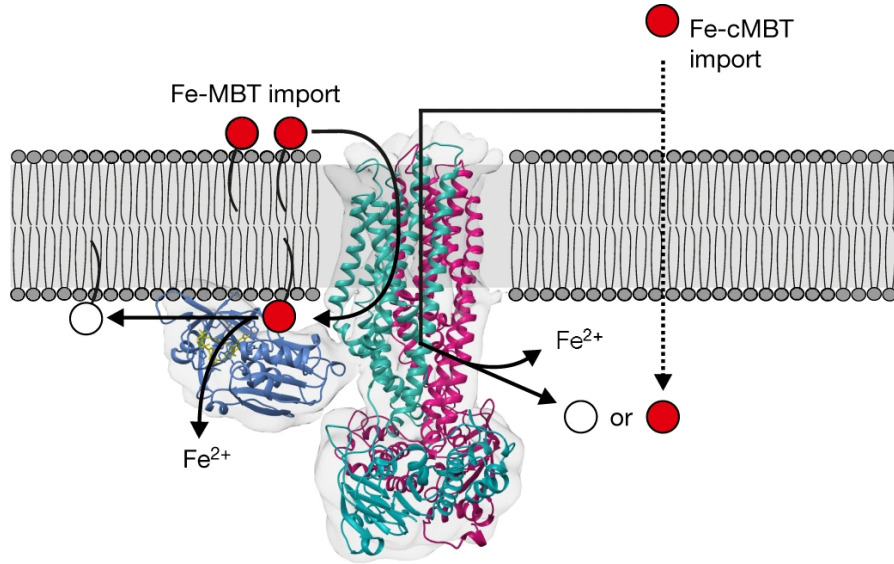
Crystallography and cryo-EM meets



Structural peculiarities of IrtAB



Taking it all together...



- LrtAB is responsible for the **import** of cMBT and MBT
- The main role of LrtAB is the import and reduction of MBT

Open Questions...

- How does LrtAB **recognize** and **transport** mycobactins?
- How can we **exploit** LrtAB for antituberculous drug development?

Acknowledgements

Article

The ABC exporter IrtAB imports and reduces mycobacterial siderophores

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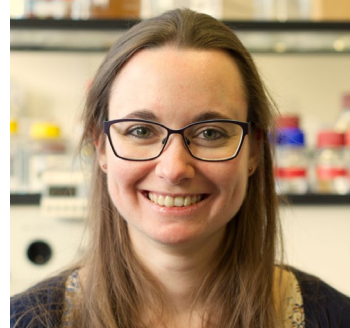
Prof. Ohad Medalia (UZH)

Prof. Jörn Piel (ETH)

Prof. Gabriele Meloni (UT Dallas)

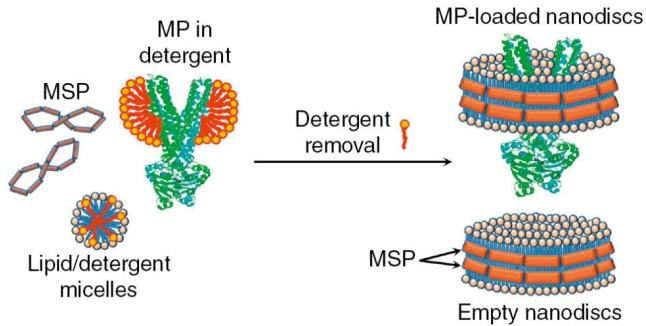


Universität Zürich

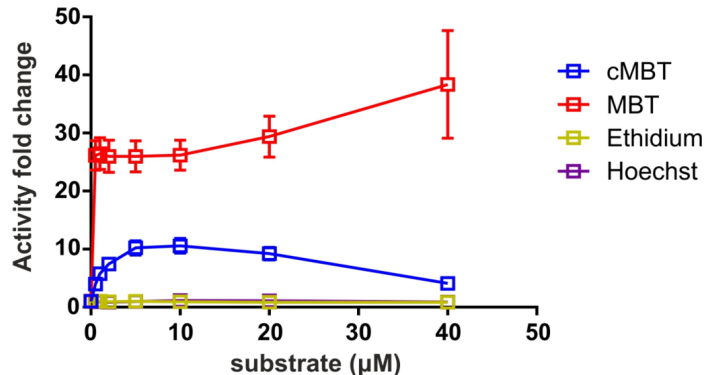


Appendix

ATPase activity is stimulated by siderophores

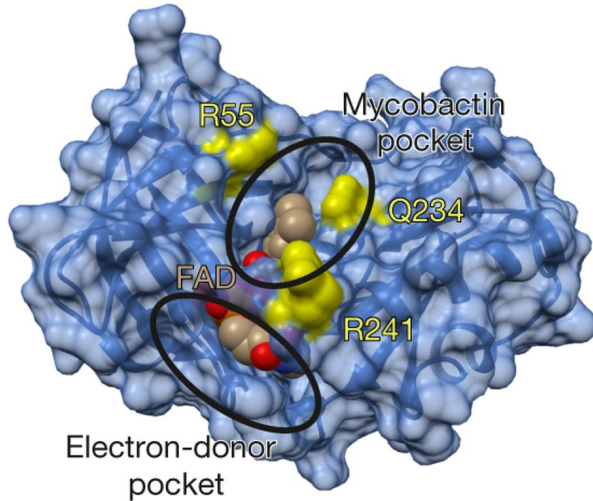


- No ATPase activity with detergent purified IrtAB
- ATPase activity restored after reconstitution in nanodiscs with *E. coli* polar lipids

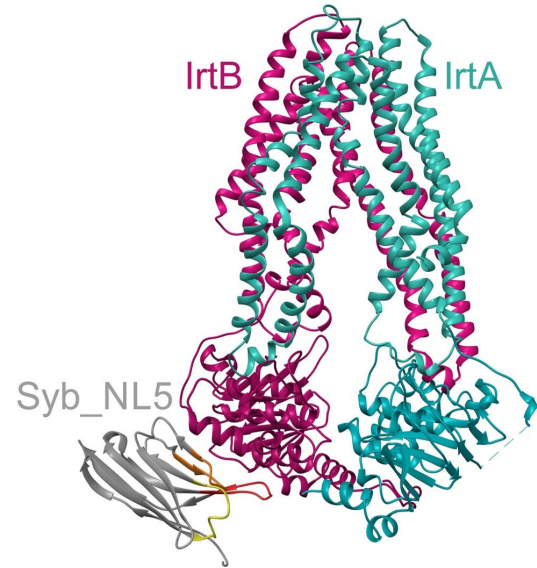


- Strong ATPase stimulation by the siderophores
- Stimulation by MBT significantly stronger
- Stimulation specific to siderophores

High resolution structures with X-ray crystallography



+

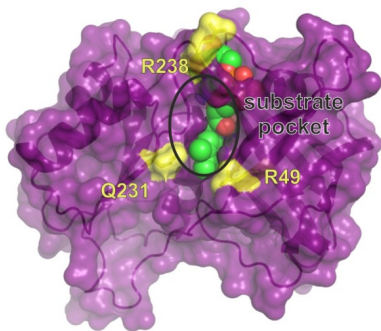


1.8Å resolution crystallographic structure
of the SID

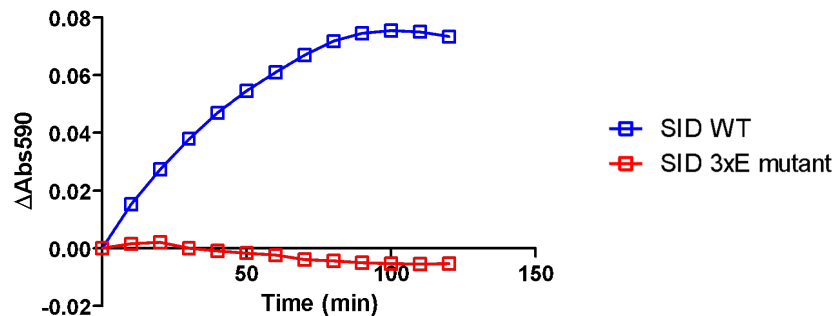
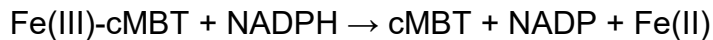
2.7Å resolution crystallographic structure
of IrtAB without the SID



Confirming the SID binding pocket



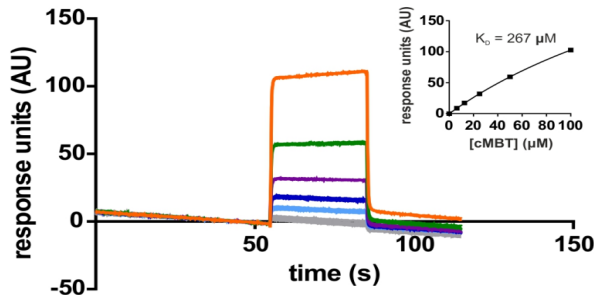
Fe(II)-Ferene



150 μM cMBT, 10 μM SID

SPR

Msm SID WT



Msm SID 3xE mutant

