38th Annual Conference of the MSBMB, 28th August 2013

Characterisation of F_{420} gamma-glutamyl ligase from *Mycobacterium tuberculosis*

Aisyah Rehan
Department of Biotechnology, Kulliyyah of Science
International Islamic University Malaysia

Acknowledgment

- Structural Biology Research Group,
  The University of Auckland
  - Prof. Ted Baker
  - Dr. Christopher Squire
  - Ghader Bashiri
  - Neil Patterson

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau




- International Islamic University Malaysia
  - Dean, Prof. Kamaruzzaman Yunus
  - Head of Biotechnology Department

الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

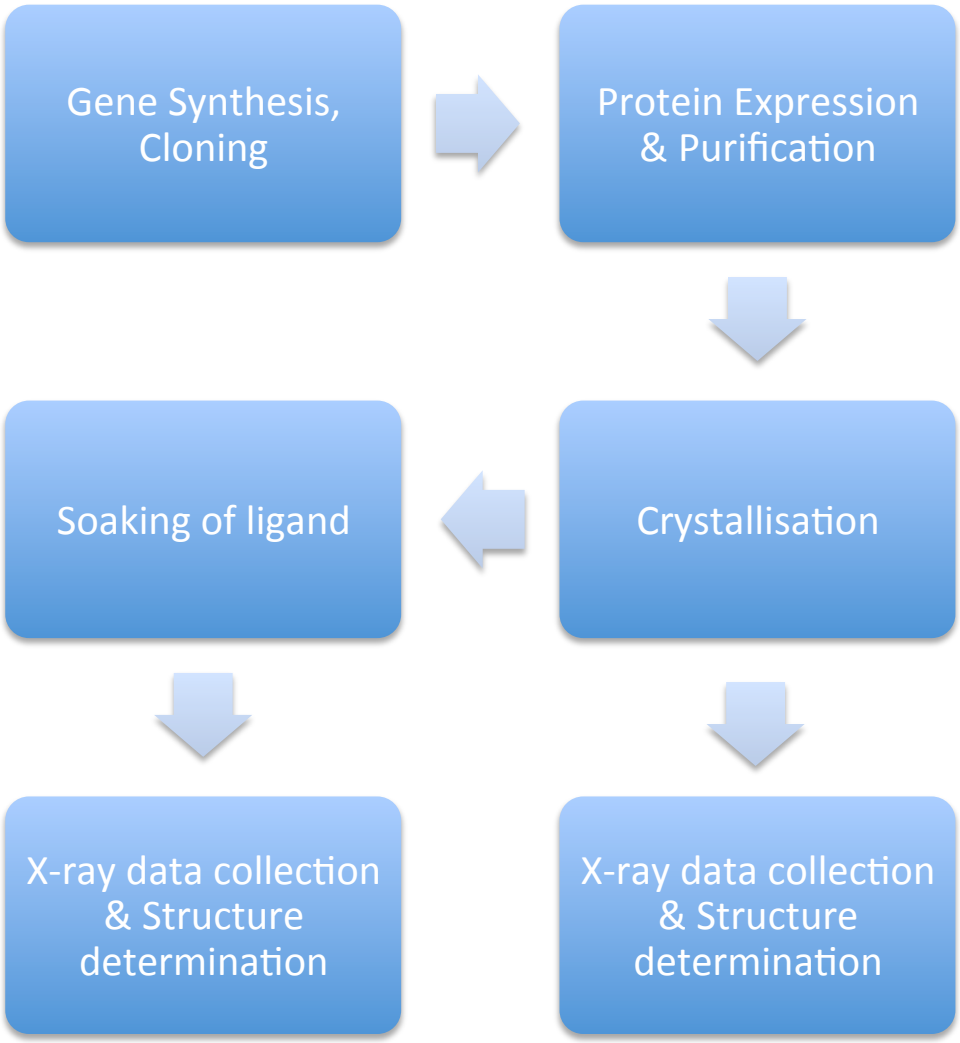
- MOHE / MOE

TB and *Mycobacterium tuberculosis*

- *M. tuberculosis* is the major aetiological agent causing human tuberculosis (TB)
- 2011 – 8.7 million new and relapsed TB infections
- 13% involved co-infection with HIV
- Ranks second only to HIV among infectious killers worldwide
- Transmitted by air – active bacilli in air droplets expelled by people affected with pulmonary TB
- Problems in combating TB: limitations in current TB therapies, persistence, multi-drug resistance, etc..

WHO (2012). *Global Tuberculosis Report.* Geneva: World Health Organization (WHO).

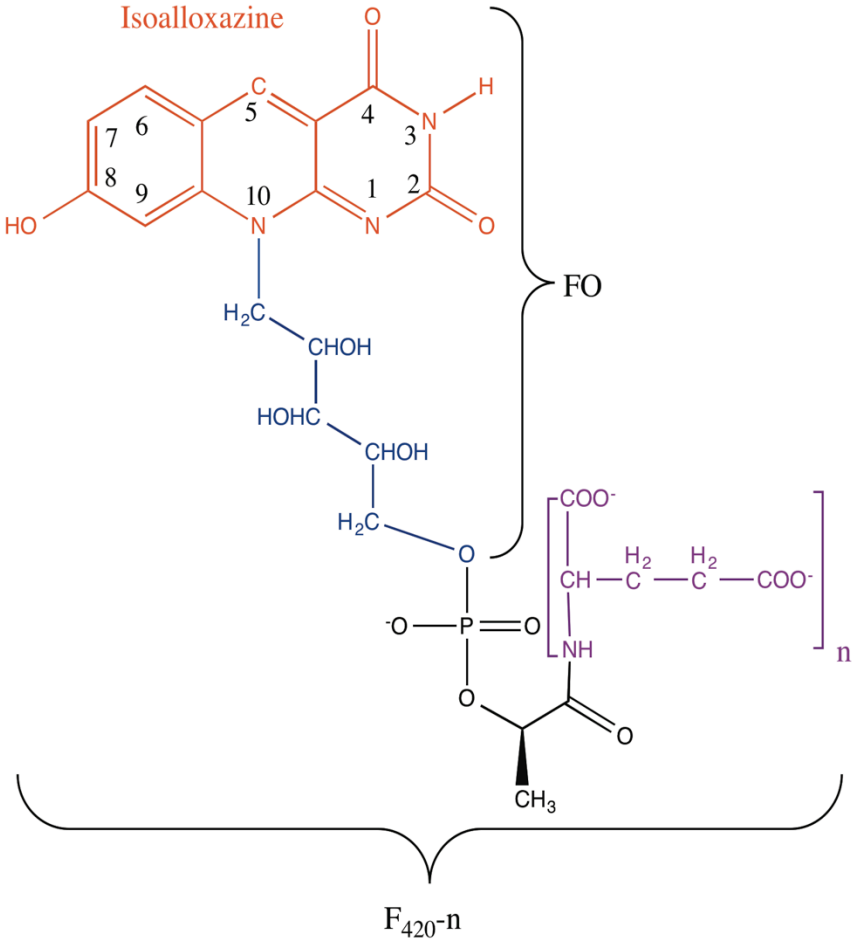
Structural biology to understand fundamental of *M. tuberculosis*



- To determine the 3-dimensional structure of protein at molecular level (atomic resolution)
- X-ray crystallography may provide hypotheses of functional activities of the protein of interest

Our research focuses on coenzyme F_{420}, a flavin cofactor

- 7,8-didemethyl-8-hydroxy-5-deazaflavin
- Archaea (methanogenesis) & bacteria
- At least 28 F_{420}-dependent enzymes identified in *M. tuberculosis*[1]
- Molecular structure very similar to FMN



[1]Selengut, J. D., & Haft, D. H. (2010) *Journal of Bacteriology*, 192(21), 5788-5798

F_{420} biosynthesis

HPP + Compound 6

FbiC (rv1173)

(FO synthase)

FO intermediate

FbiA (rv3261)

(phospholactate transferase)

F_{420}-0

FbiB

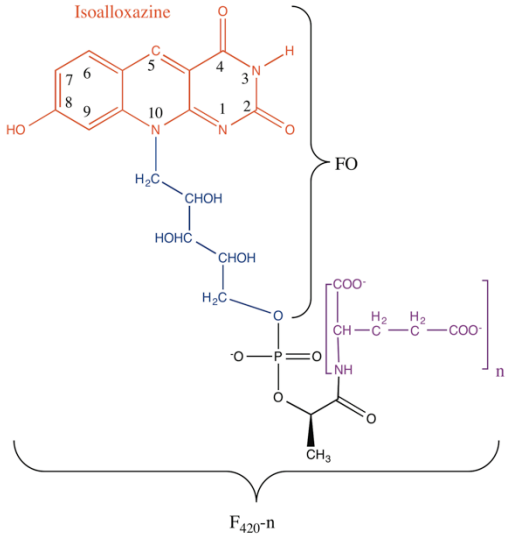
(glutamyl ligase)

F_{420}-1

FbiB (rv3262)

(glutamyl ligase)

F_{420}-2



- In mycobacterium species, the predominant structures contain F_{420}-5 and F_{420}-6 glutamate groups.
- Polyglutamylated F_{420} is a conserved feature observed in reactions by F_{420}-dependent enzymes

FbiB

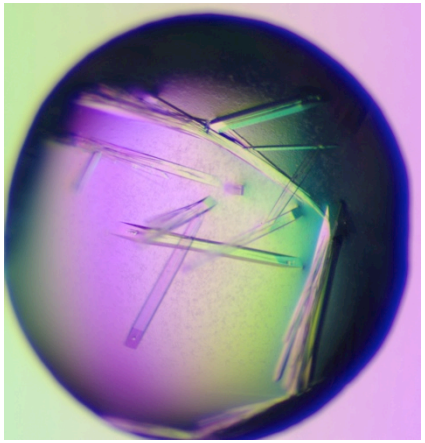
- BLASTp: Comprises 2 domains
- N-terminal domain:
  - annotated as a gamma-glutamyl ligase (Pfam PF01996)
  - 37% sequence identity with an archaeal γ-glutamyl ligase (PDB ID 2G9I)
- C-terminal domain:
  - sequence-similarity to FMN-dependent nitroreductase family (Pfam PF0081)
  - Has been hypothesized to facilitate elongation of polyglutamate tail of F_{420} in mycobacterial species (NCBI CDD TIGR03553)
- It is postulated that N-terminal domain would add up to 2 glutamate residues to the coenzyme as seen in methanogens, and the C-terminal domain would add more glutamate residues to the coenzyme

http://blast.ncbi.nlm.nih.gov/BLAST.cgi, http://www.uniprot.org

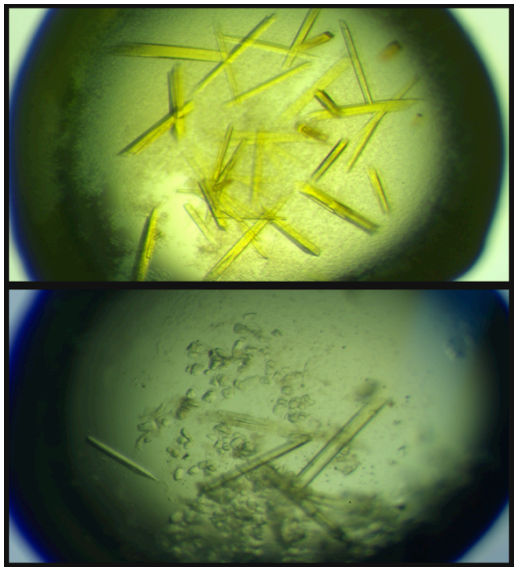
FbiB cloning, expression, purification, and crystallisation

- Gateway cloning (6XHis-tagged, GST-tagged)
- Readily expressed and purified as full length and individual domain constructs
- In both *E. coli* and *M. smegmatis* vectors
- However, only its C-terminal domain was successfully crystallized
- The 3-dimensional structure of FbiB C-terminal domain was determined to 1.75Å resolution
- Phased by multiwavelength anomalous dispersion (MAD) methods using bromide-soaked crystals

C-terminal domain X-ray data and Structure determination

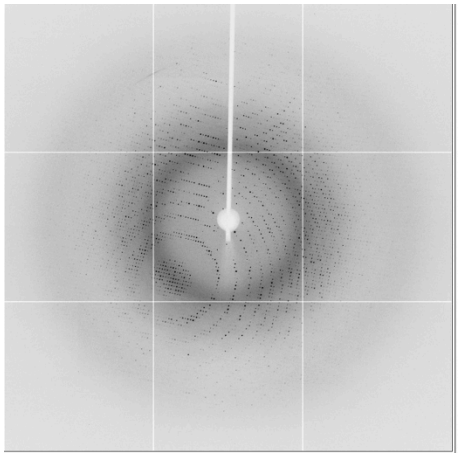


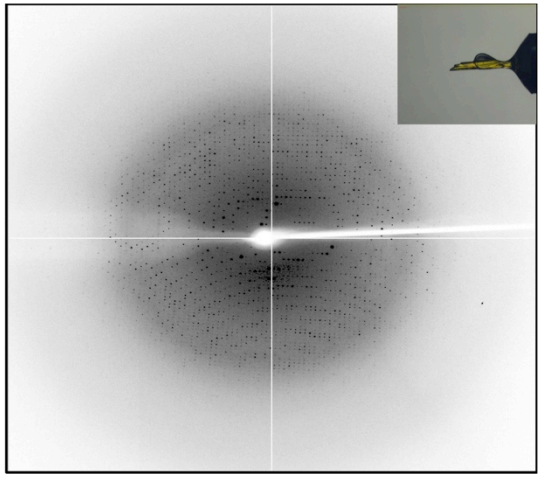
Top: Apo-crystals of FbiB C-terminal domain (refined to 1.75Å)



Top: FMN-soaked FbiB C-terminal domain crystals (refined to 1.9Å)

Bottom: F_{420}-0 soaked FbiB C-terminal domain (refined to 1.9Å)



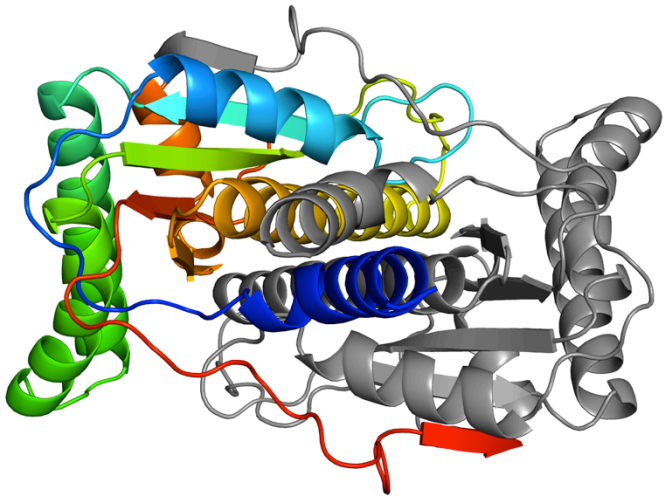


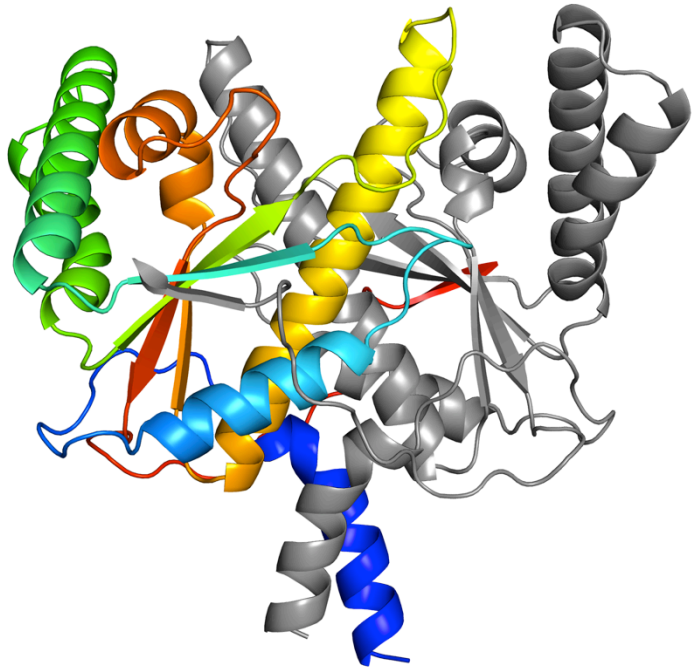
Diffraction image of
Left: apo-FbiB C-terminal domain crystals
Right: FMN-soaked FbiB C-domain crystals

Crystal data for different crystal forms of FbiB C-terminal domain

	Apo-FbiB C-domain	**FbiB C domain-F_{420}-0 complex**	**FbiB C-domain-FMN complex**
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$
Unit cell dimensions	a = b = 136.89 c = 102.09 Å α = β = γ = 90°	a = b = 136.62 c = 101.75 Å α = β = γ = 90°	a = b = 137.14 c = 101.40 Å α = β = γ = 90°
No. of molecules in the asymmetric unit	4	4	4
Solvent content (%)	54.50	54.23	54.38
Vm ($Å^3$/Da)	2.70	2.69	2.70

- Contain two dimers in the asymmetric unit



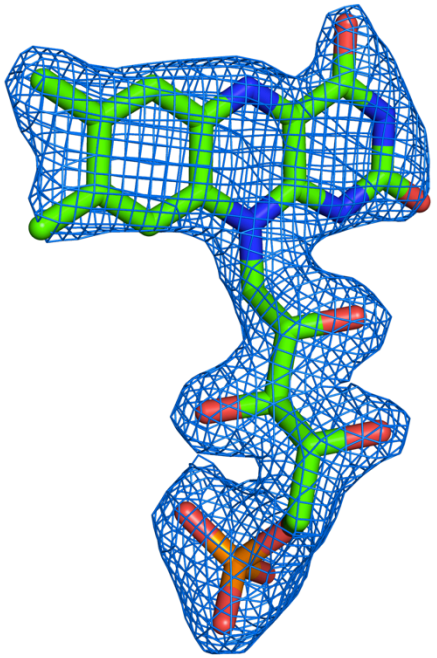


Structure of FbiB C-domain dimer

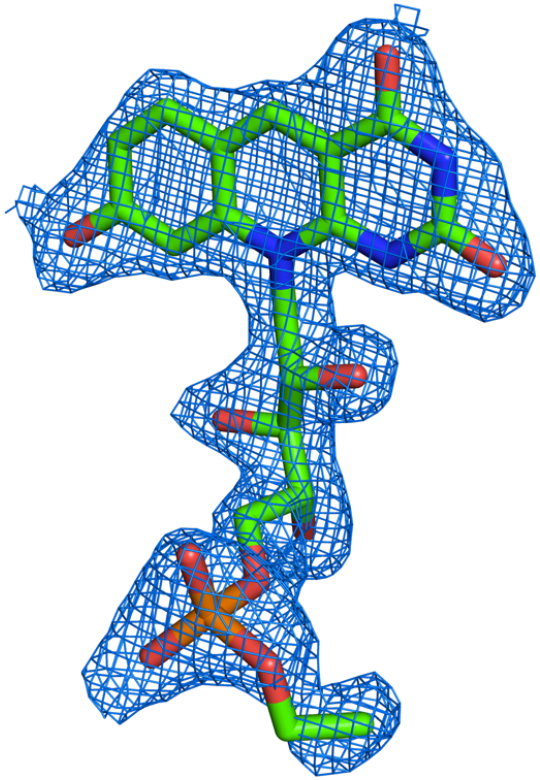
- **Top and side view of the FbiB C-domain dimer**
  - The antiparallel beta strands at the protein core are flanked by helices.
  - monomer A is rainbow-coloured from its N-terminus (blue) to C-terminus (red) whereas monomer B is in grey.
  - Displays a fold that is typical of FMN-dependent nitroreductases family

Refinement statistics: R_{factor}/R_{free} (%) = 22.3/25.7, rmsd = 0.010Å, 97.5% residues in most favoured regions, one ramachandran outlier

Electron density map around FMN and F_{420}-0 molecule (contoured at 1.5 sigma)

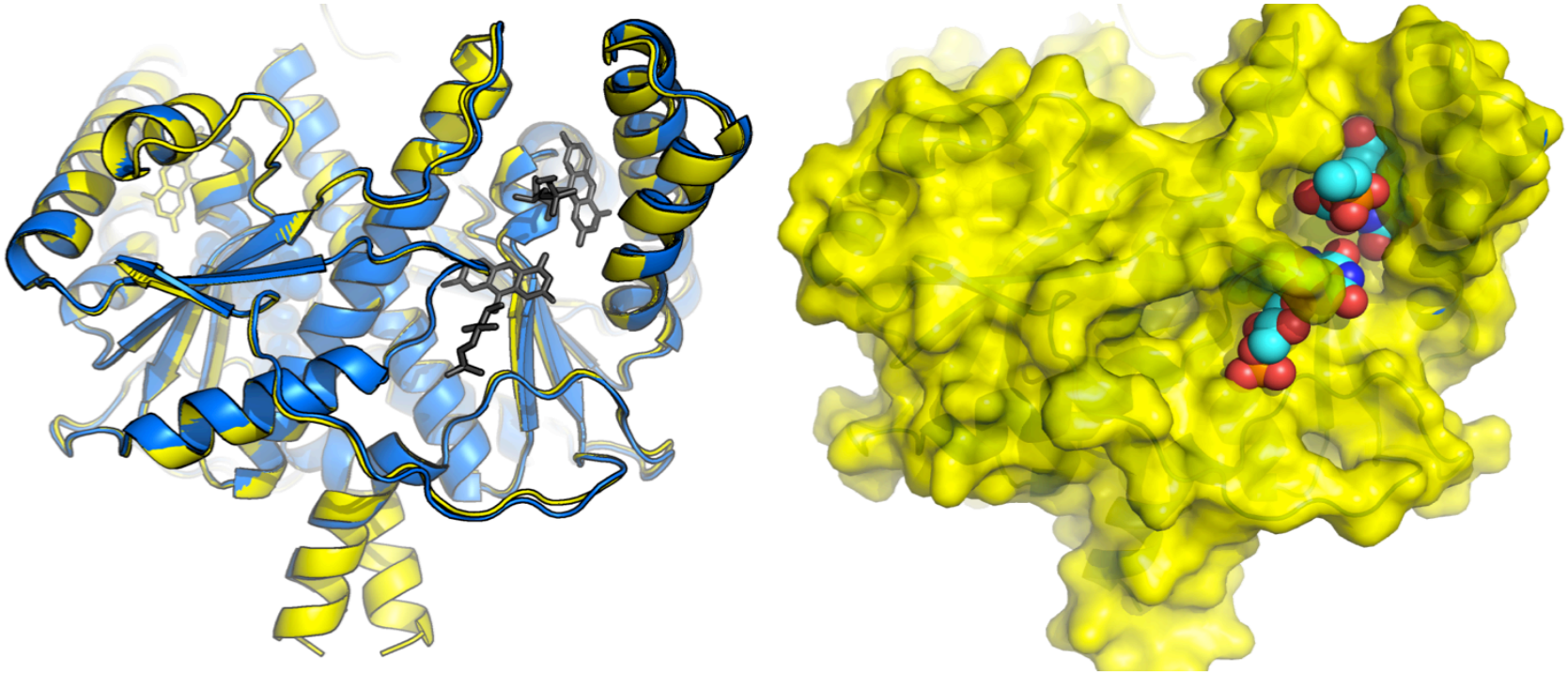


FMN



F_{420}-0

Different binding sites for FMN and F_{420}-0 molecule



Overlay of FMN-bound and F_{420} bound structures:
Left: The protein dimer is represented as a ribbon diagram and ligands as stick models
Right: The protein dimer as a surface representation with ligands as spheres on the right

Summary

- Full length FbiB and its individual domain constructs readily expressed and purified in both *E. coli* and *M. smegmatis*
- Only C-terminal domain of FbiB has been successfully crystallised
- The structure of apo-FbiB C-terminal domain, as well as FMN-bound and F_{420}-0-bound FbiB C-terminal domain has been solved
- Closer inspection revealed different conserved binding sites for FMN and F_{420}-0 ligand
- As FMN is not a natural ligand for FbiB enzyme, its role will need to be further investigated

Thank you for your attention

TB and *M. tuberculosis*

- can remain dormant for a long time before becoming infectious when the immune system of the host is compromised
- a non-sporulating bacterium, yet can remain dormant within the human host for years
- can survive in hypoxic (oxygen-depleted) and nutrient-depleted media

Russell, D. G. (2007) *Nature Reviews. Microbiology,* 5(1), 39-47.
Hett, E. G., & Rubin, E. J. (2008). *Microbiology and molecular biology reviews: MMBR,* 72 (1), 126-156.

Relevance of studying coenzyme F_{420} in mycobacteria

- it plays important roles in particular condition such as hypoxia – may be relevant to mycobacterial persistence
- Low-cross activation by mammalian enzymes – make F_{420}-related proteins promising drug targets
- Structural information on enzymes from the F_{420} biosynthesis pathway might provide information on how this coenzyme is regulated in mycobacteria, and how they might be developed as therapeutic targets

Solution of apo-FbiB C-terminal domain structure

- Solved using multi-wavelength anomalous dispersion (MAD) methods for phase determination
- Halide (bromide) soaked FbiB C-terminal domain crystals – display significant anomalous signal that can be used as a reference to locate the positions of these atoms