Integrated interdisciplinary approaches to tackle antimicrobial drug resistance in tuberculosis

search for Achilles' heel in the TB-causing pathogen

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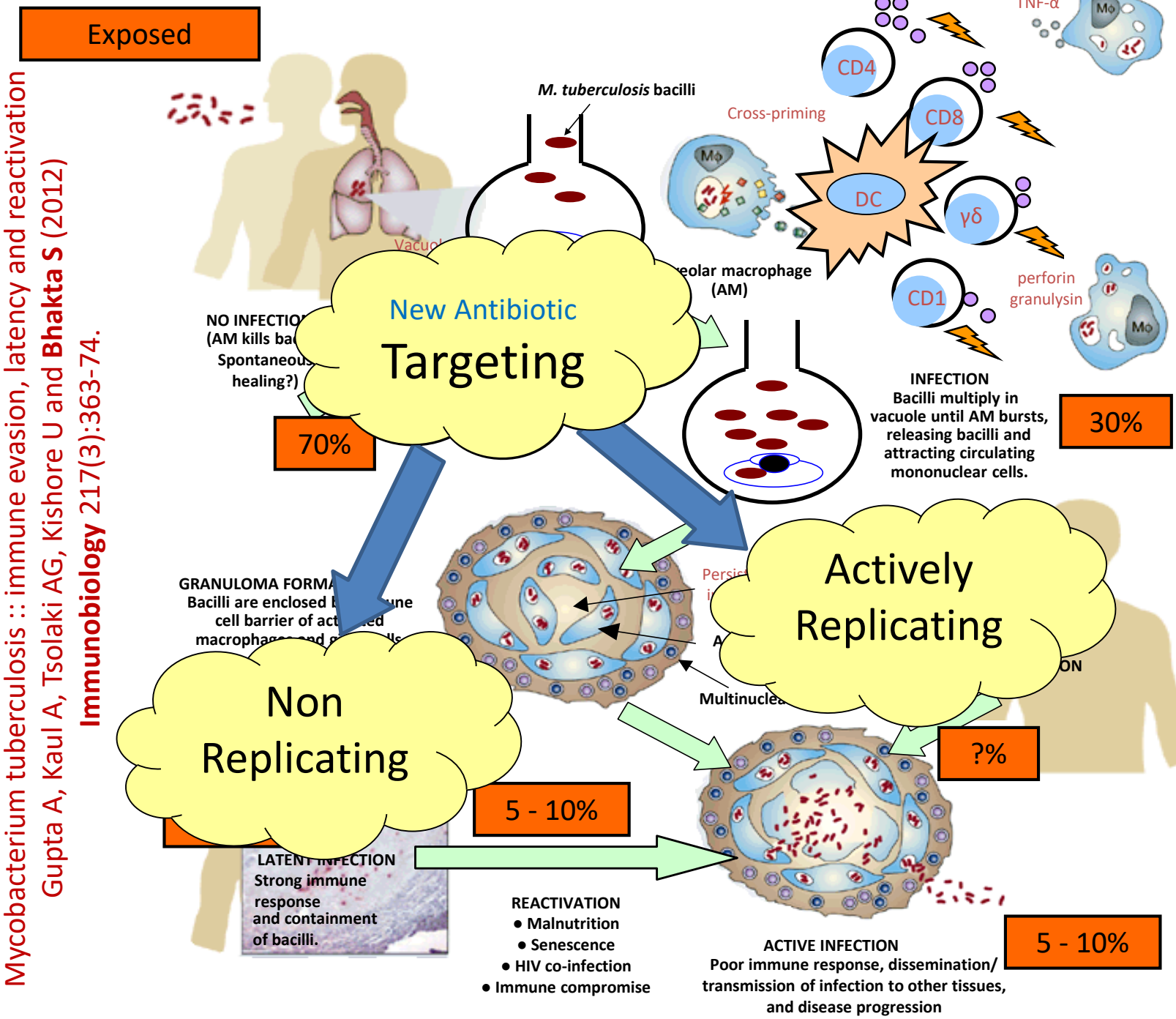
Institute of Structural and Molecular Biology ISMB

Birkbeck UNIVERSITY OF LONDON

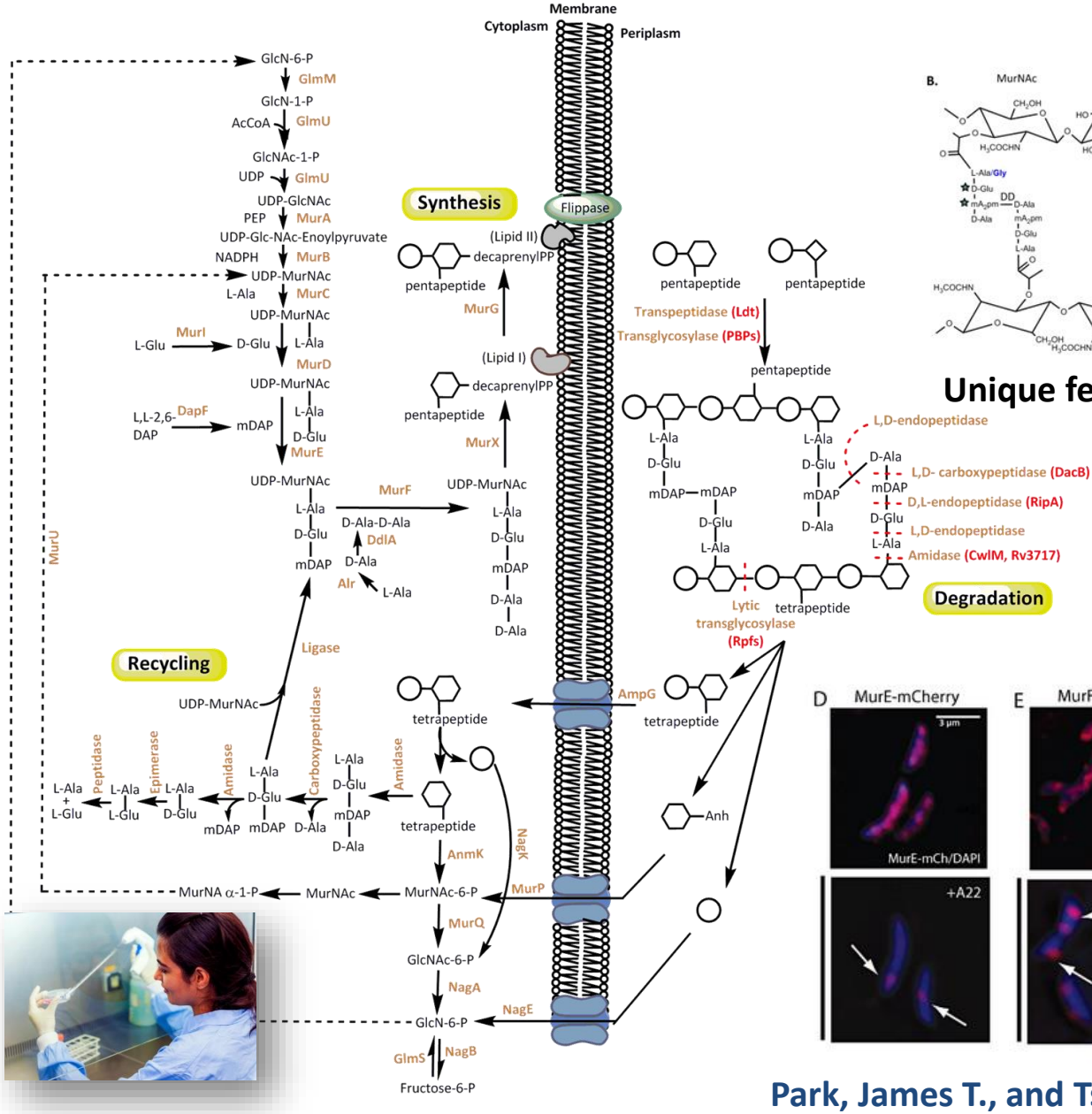
UCL

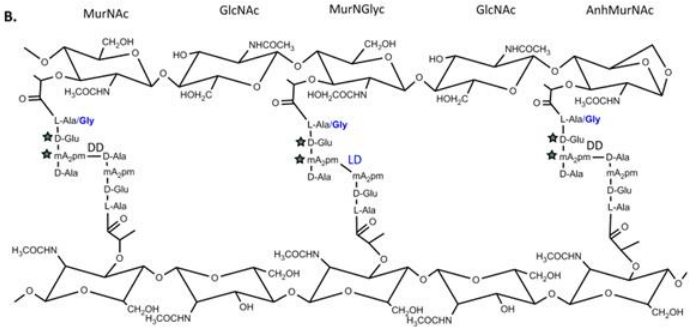
GCRF Global Challenges Research Fund

ANTIBIOTIC DISCOVERY ABX ACCELERATOR NETWORK

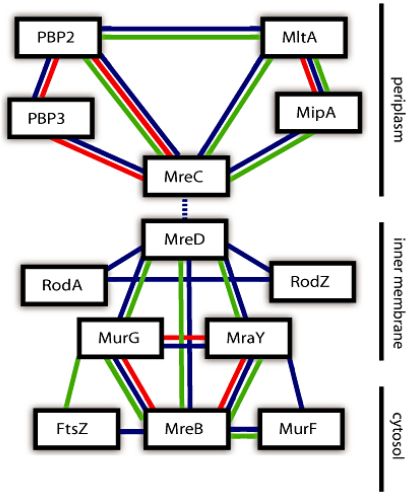

Exposed
Mycobacterium tuberculosis :: immune evasion, latency and reactivation
Gupta A, Kaul A, Tsolaki AG, Kishore U and Bhakta S (2012)
Immunobiology 217(3):363-74.
M. tuberculosis bacilli
IFN-γ
TNF-α
Mϕ
CD4
CD8
Cross-priming
DC
γδ
CD1
perforin
granulysin
NO INFECTIO
(AM kills ba
Spontaneous
healing?)
70%
New Antibiotic
Targeting
eolar macrophage
(AM)
INFECTION
Bacilli multiply in vacuole until AM bursts, releasing bacilli and attracting circulating mononuclear cells.
30%
GRANULOMA FORMA
Bacilli are enclosed b
une
cell barrier of act
ed
macrophage
Actively
Replicating
Non
Replicating
?%
5 - 10%
LATENT INFECTION
Strong immune response and containment of bacilli.
REACTIVATION
• Malnutrition
• Senescence
• HIV co-infection
• Immune compromise
ACTIVE INFECTION
Poor immune response, dissemination/ transmission of infection to other tissues, and disease progression
5 - 10%

Cell wall PG – a dynamic structure





Unique features of mycobacterial PG



Park, James T., and Tsuyoshi Uehara. MMBR (2008)

White, Courtney L., *et al*. Mol Micro (2010)

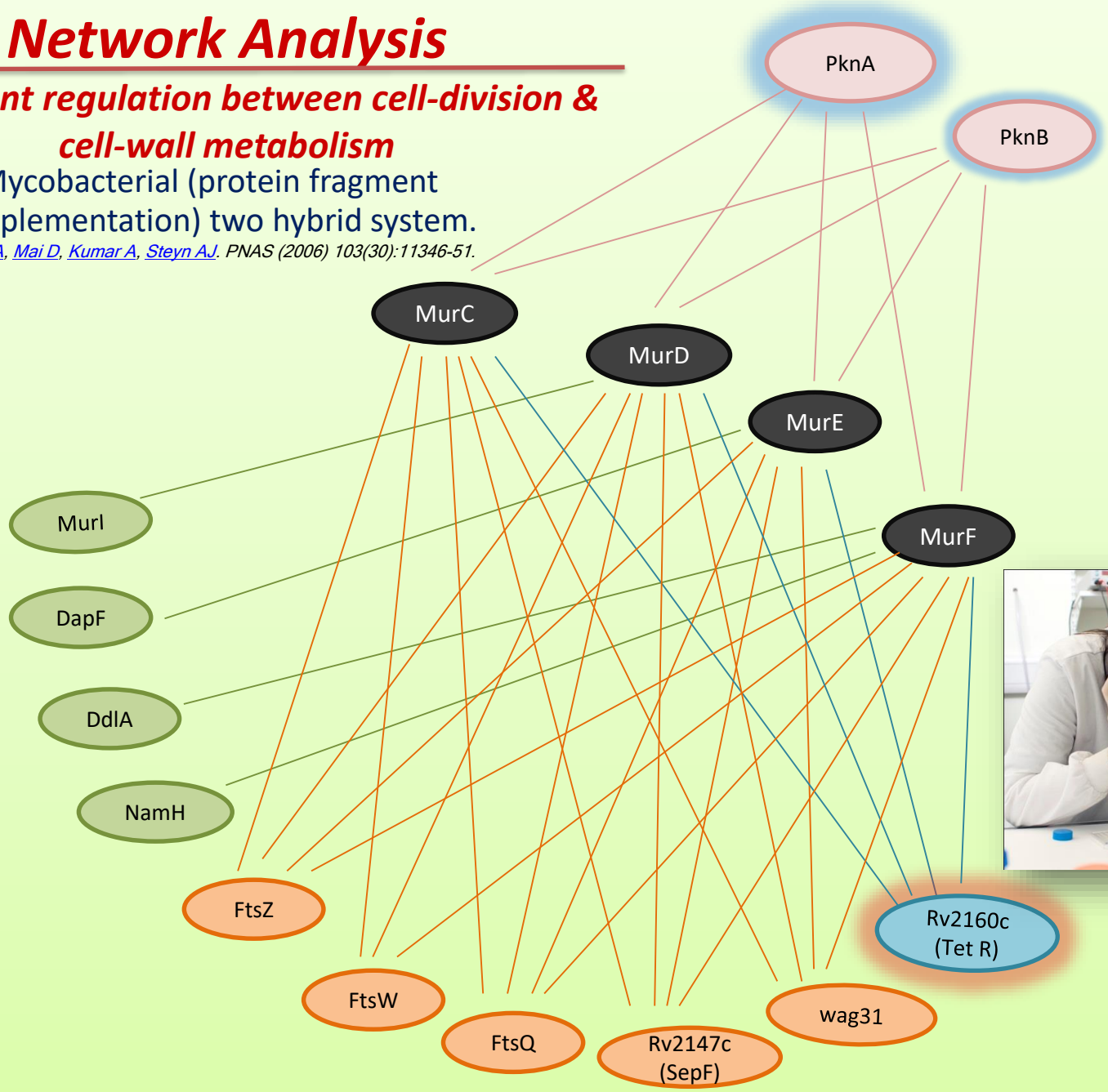
Maitra, A. *et al* (June 2019) FEMS Microbiology Reviews.

Network Analysis

A stringent regulation between cell-division & cell-wall metabolism

Mycobacterial (protein fragment complementation) two hybrid system.

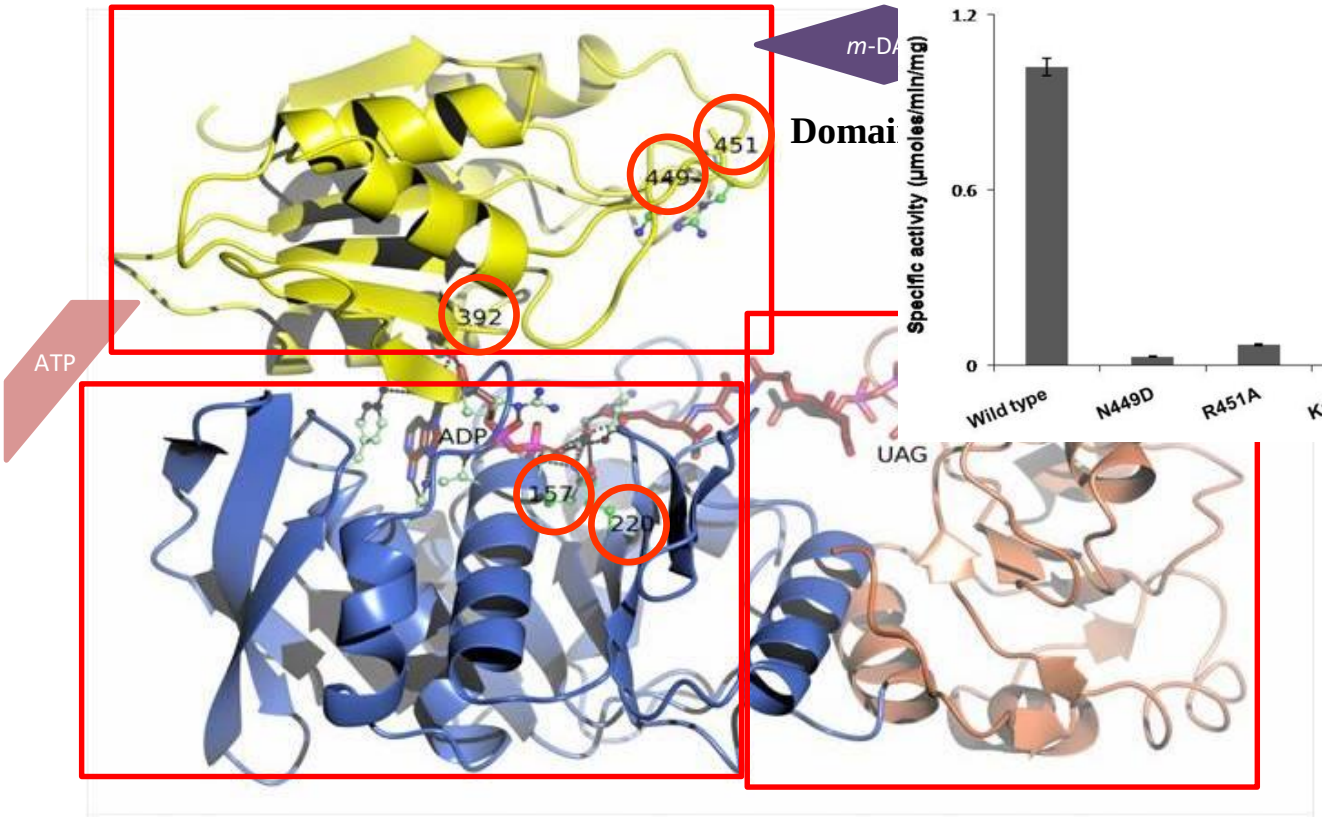
Singh A, Mai D, Kumar A, Steyn AJ. PNAS (2006) 103(30):11346-51.



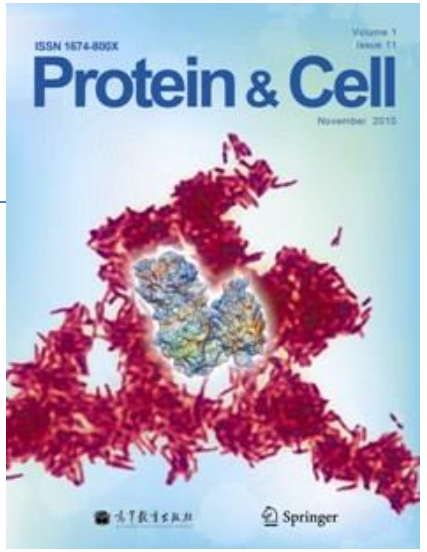
Munshi, T. …Bhakta, S. (2013) *PLOSone* 8(3)

Structure of Mtb-MurE

(PDB: 2xja & 2wtz)



Domain 2



Three domains:

1: N1-139: Rossmann fold typical of nucleotide binding proteins, binding to UDP

2: 140-378: Central domain binding ATP and activating the dipeptide

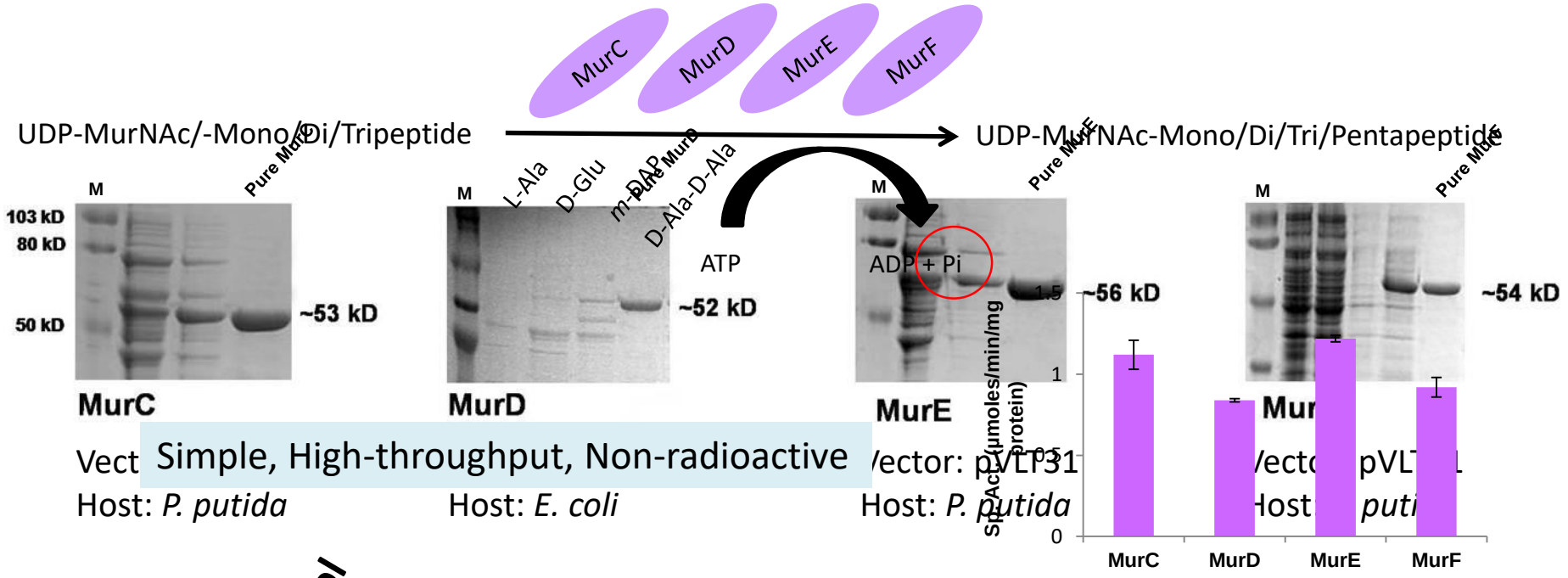
3: 379-535C: C-terminal domain recognizing and binding to m-DAP

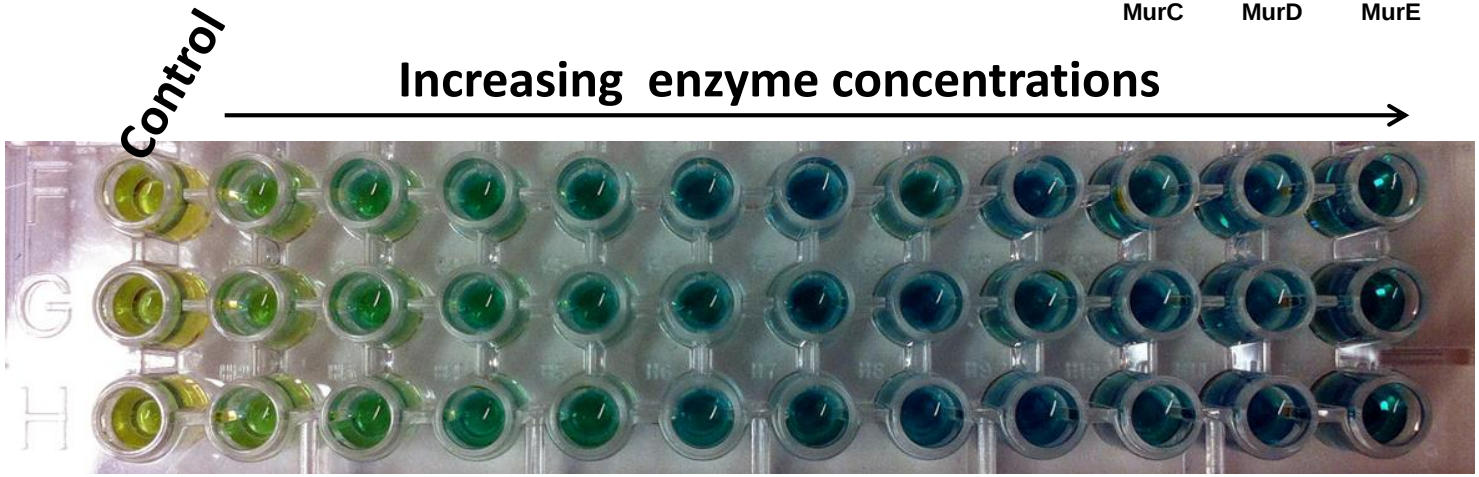
- **Mtb-MurE – 20.3% β strand, 29.8% α-helix**
- **Structurally similar to other Mur ligases**
- **N-terminus significantly different**
- **Enzyme mechanism conserved**

Basavannacharya, C. …Bhakta, S. (2010a) ***Tuberculosis*** 90: 16-24.

Basavannacharya, C. …Bhakta, S. (2010b) ***Protein & Cell*** 1: 1011-1022.

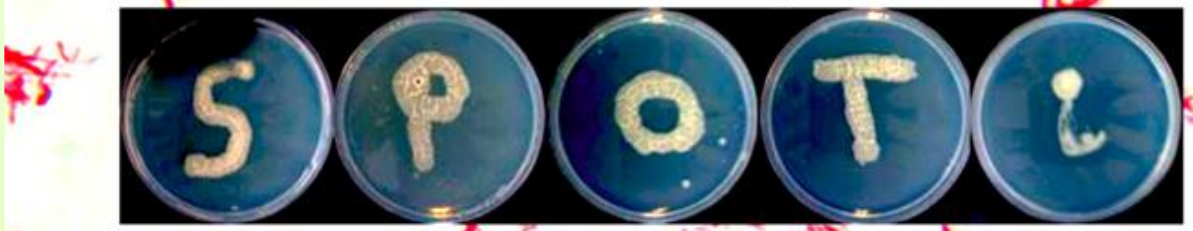
HT-assay for ATP-dependent Mur ligases





Whole-cell evaluation of Inhibitors and Drug Susceptibility Testing

ISMB Mycobacteria Research Laboratory
Birkbeck, University of London

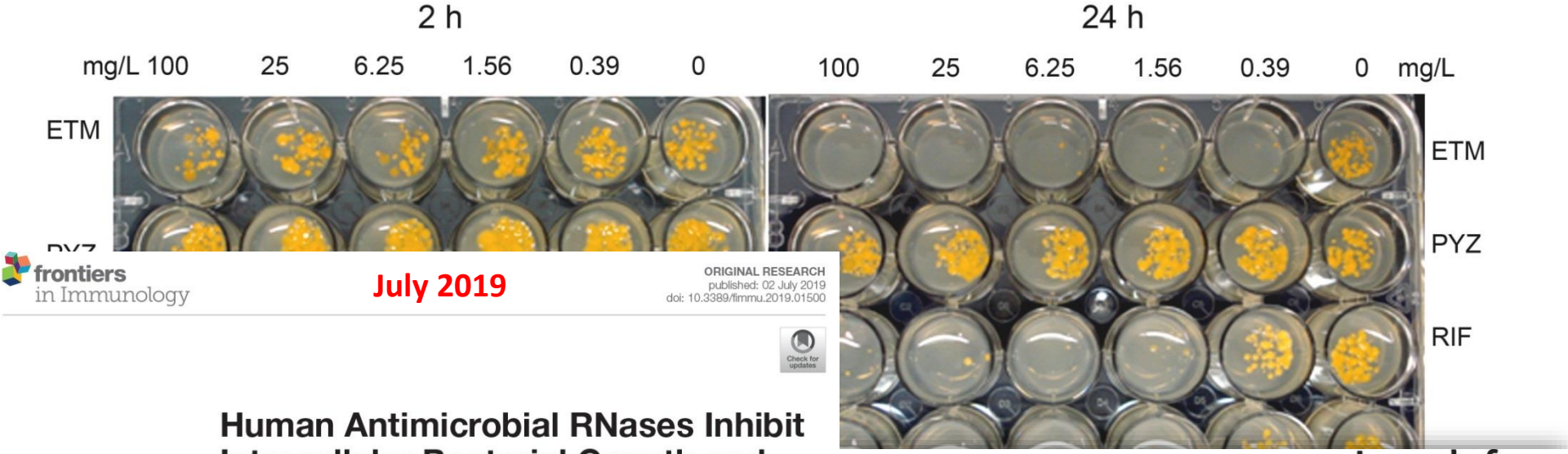


Institute of Structural and Molecular Biology
ISMB

2002 – 2019

MRC Medical Research Council Technology

Mycobacteria-infected macrophage model



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Human Antimicrobial RNases Inhibit Intracellular Bacterial Growth and Induce Autophagy in Mycobacteria-Infected Macrophages

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The development of novel treatment against tuberculosis is a priority global health challenge. Antimicrobial proteins and peptides offer a multifaceted mechanism suitable to fight bacterial resistance. Within the RNaseA superfamily there is a group of highly cationic proteins secreted by innate immune cells with anti-infective and immune-regulatory properties. In this work, we have tested the human canonical members of the RNase family using a spot-culture growth inhibition assay based mycobacteria-infected macrophage model for evaluating their anti-tubercular properties. Out of the seven tested recombinant human RNases, we have identified two members, RNase3 and RNase6, which were highly effective against *Mycobacterium aurum* extra- and intracellularly and induced an autophagy process. We observed the proteins internalization within macrophages and their capacity to eradicate the intracellular mycobacterial infection at a low micro-molar range. Contribution of the enzymatic activity was discarded by site-directed mutagenesis at the RNase catalytic site. The protein induction of autophagy was analyzed by RT-qPCR, western blot, immunofluorescence, and electron microscopy. Specific blockage of auto-phagosome formation and maturation reduced the protein's ability to eradicate the infection. In addition, we found that the *M. aurum* infection of human THP1 macrophages

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ited surrogate model for screening of drugs against *Mycobacterium tuberculosis*

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ntracellularly surviving and slow-growing pathogen, *Mycobacterium tuberculosis*, adapts the ment for its active and dormant life cycle. It is evident that the lack of appropriate high- ning of inhibitors within host cells is an impediment for the early stages of anti-tubercular We aimed to develop an integrated surrogate model that enhances the screening of large

nt mycobacterial species were compared for their growth, drug susceptibility and intracellular plate solid agar-based spot culture growth inhibition (SPOTi) assay was developed into a higher at. The uptake and intracellular survival of *Mycobacterium aurum* within mouse macrophage) were optimized using 24/96-well plate formats.

wing, non-pathogenic *M. aurum* was found to have an antibiotic-susceptibility profile similar to

Nature is an amazingly creative chemist!

Antimycobacterials from natural sources: ancient times, antibiotic era and novel scaffolds

Juan D. Guzman[1,2], Antima Gupta[1], Franz Bucar[3], Simon Gibbons[2], Sanjib Bhakta[1]

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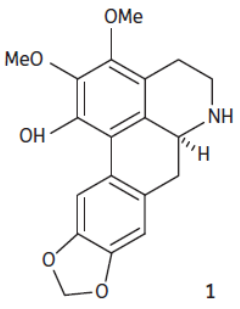
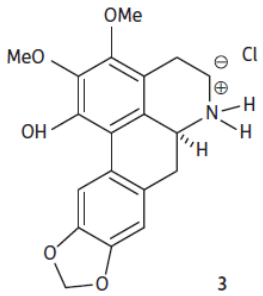
Frontiers in Biosciences 2012

Mtb-MurE inhibitory compounds first identified!

***Ocotea macrophylla* (Lauraceae)**





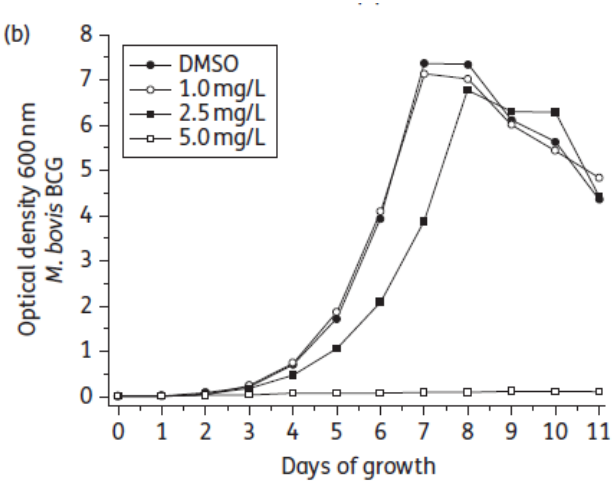


Table 1. MICs, GIC_{50}, SI and MurE IC_{50} of natural compounds **1**–**7**

Natural products	*M. tuberculosis* $H_{37}Rv$, MTT microtitre assay[21]	IC_{50} for *M. tuberculosis* MurE (μM) [mg/L]	SI[a]	IC_{50} for *M. tuberculosis* MurE (μM) [mg/L]
1	64	67 ± 11 [22.9]	0.97	67 ± 11 [22.9]
2	128	75 ± 15 [30.0]	0.52	75 ± 15 [30.0]
3	4	57 ± 14 [19.5]	12	57 ± 14 [19.5]
4	>128		<0.34	>1000 [>330]
5	128		0.35	286 ± 33 [93.9]
6	>128		<0.078	>1000 [>300]
7	128		0.28	184 ± 16 [42.8]
Isoniazid	0.125		>4000	>1000 [>137]
Rifampicin	0.25		ND	>500 [>411]

HT-assays Target vs Cell

Guzman JD…and Bhakta S.* (2015) *J Antimicrob Chemother.* 70(6):1691-703.
Munshi T,…and Bhakta S.* (2013) *PLoS One.* 2013;8(3):e60143.
Pesnot, T. …Hailes H.C. (2011) *Chem Commun (Camb).* 47(11): 3242.
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International Research Capacity-Building Workshop to Tackle AMR in TB

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