Otago Spotlight Series

Infectious Disease Research

Spotlight 2018: Infectious Diseases

Kurt L. Krause, MD, PhD, FIDSA

Biochemistry

University of Otago

Reflecting on what scientists can do now

- Study or determine genomes and proteomes
- Rapidly identify key genes and proteins
- Determine their sequence and likely structure
- Deduce mechanism of action
- Target them for diagnostics and treatments
- Possibility of new drugs, new vaccines and even “personalized medicine”

Targets for Modern Biochemistry

- Aging
- Regeneration
- Cancer
- Atherosclerosis
- Diabetes, Obesity
- Inflammation
- Dementia, Stroke, Mental Health
- Infectious Diseases

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Protein vs. Protein – my view

- In my lab we study protein structure and function
- In our view, infectious disease is a struggle between groups of proteins – because proteins do the bidding of the cell
- Host's proteins vs Pathogen's proteins
- The side whose proteins triumph, will prevail
- Inhibiting, or removing, or blocking proteins is critical; antibiotics are actually protein blockers

Key steps

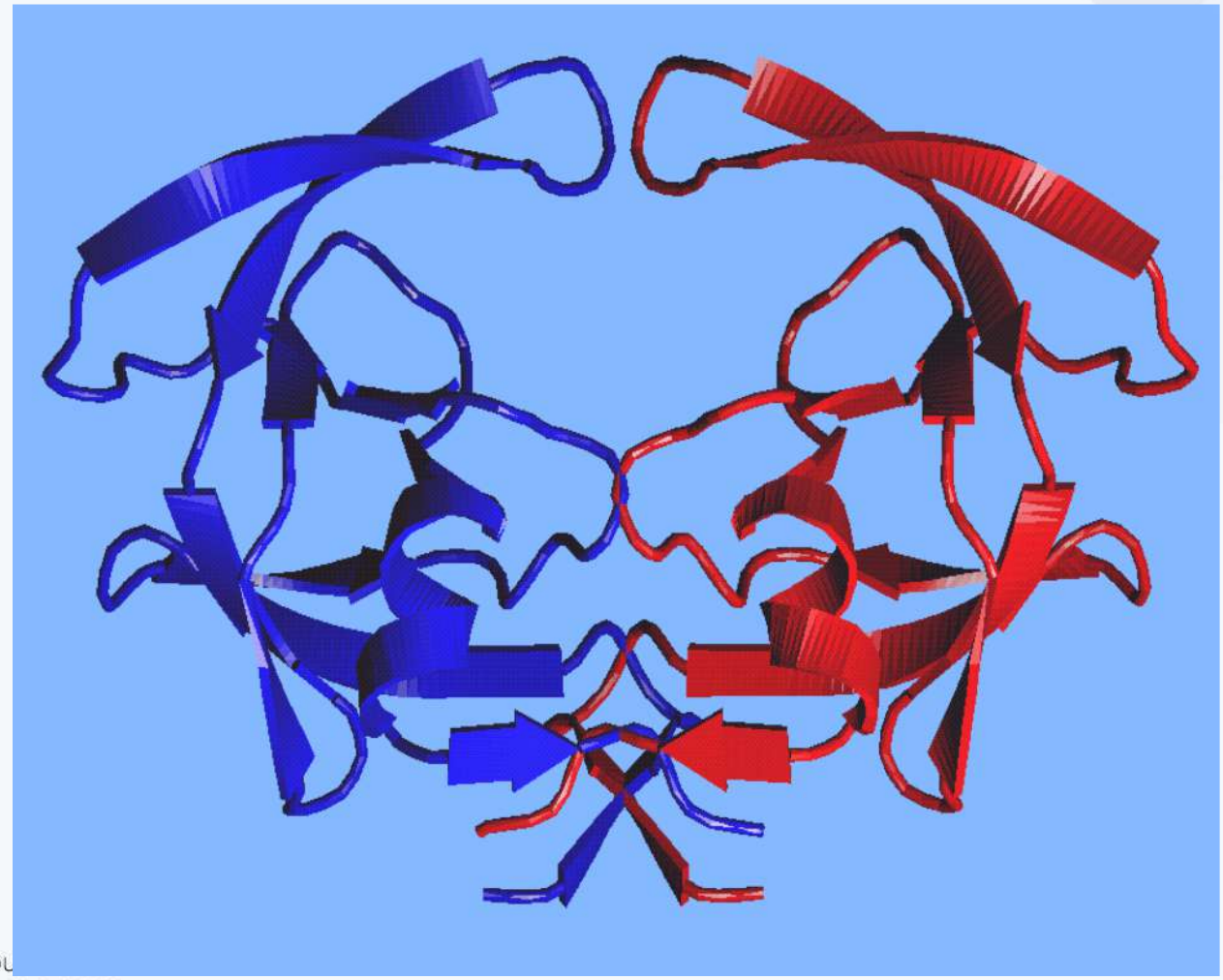
- Choose a disease to target - in my lab - TB, drug resistant bacteria
- Choosing the correct target, generally a protein or group of proteins
- Getting the basic science right – *Successful translational research is built on the results of basic science research*
- Can you find/design bioavailable, nontoxic, small molecules that bind to and inhibit your target(s) – hit.
- Testing and improving, repeat --> --> drug candidate –lead.
- Can you get it to market…?

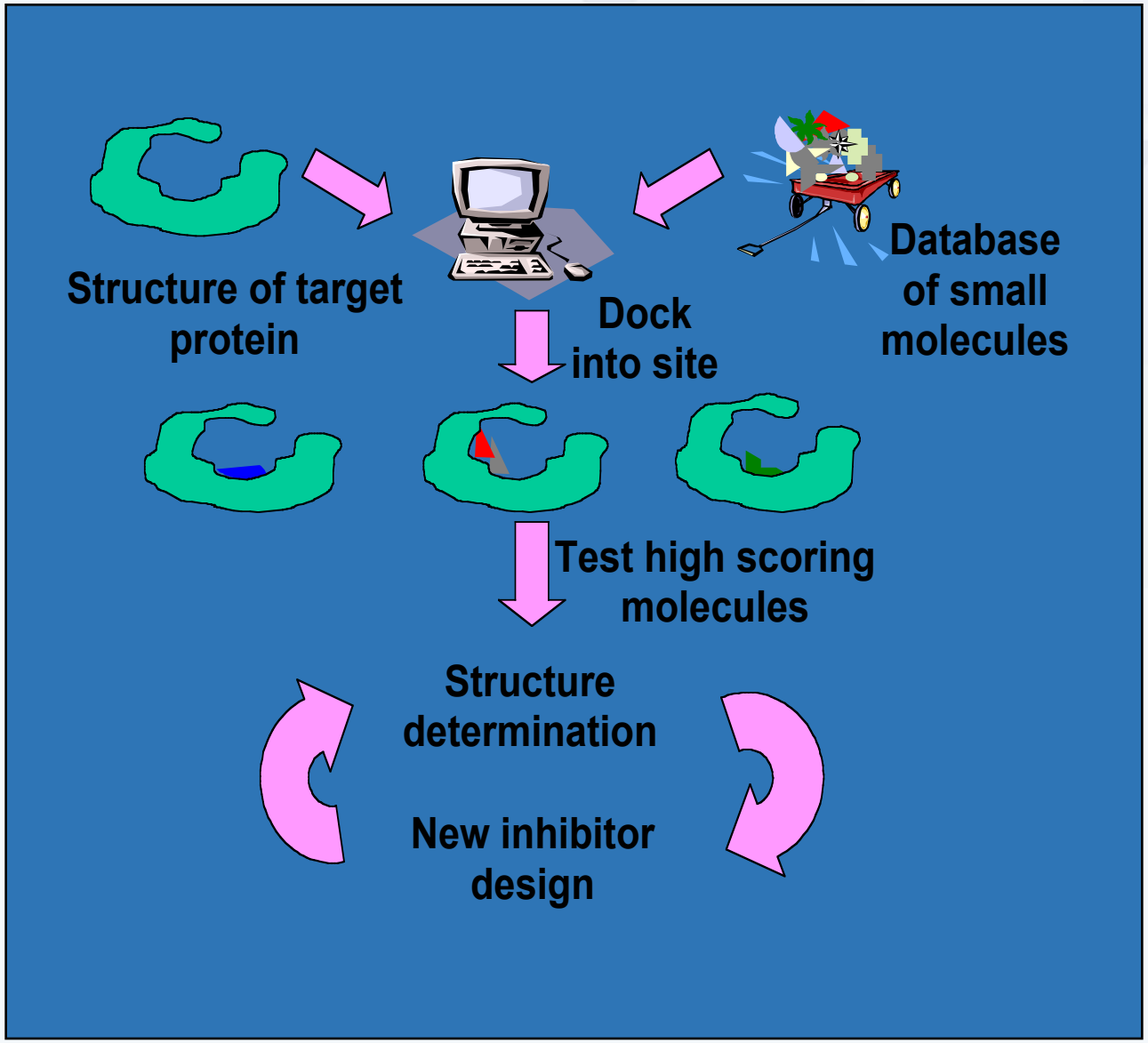
Getting a drug candidate to market

- Preclinical – $50M
  - Testing your compound in isolated organisms
  - Resistant and clinical organisms
  - Cellular toxicity
  - Testing in animals – at least 2 – good model needed
  - ADME – absorbance, distribution, metabolism, excretion

- Human Clinical Testing – $900M or more
  - Phase 1 – healthy volunteers – dosage, toxicity
  - Phase 2 – patients with illness – efficacy, safety
  - Phase 3 – clinical trial – efficacy, safety, comparison
  - Approval to market drug
  - Phase 4 – post-marketing surveillance

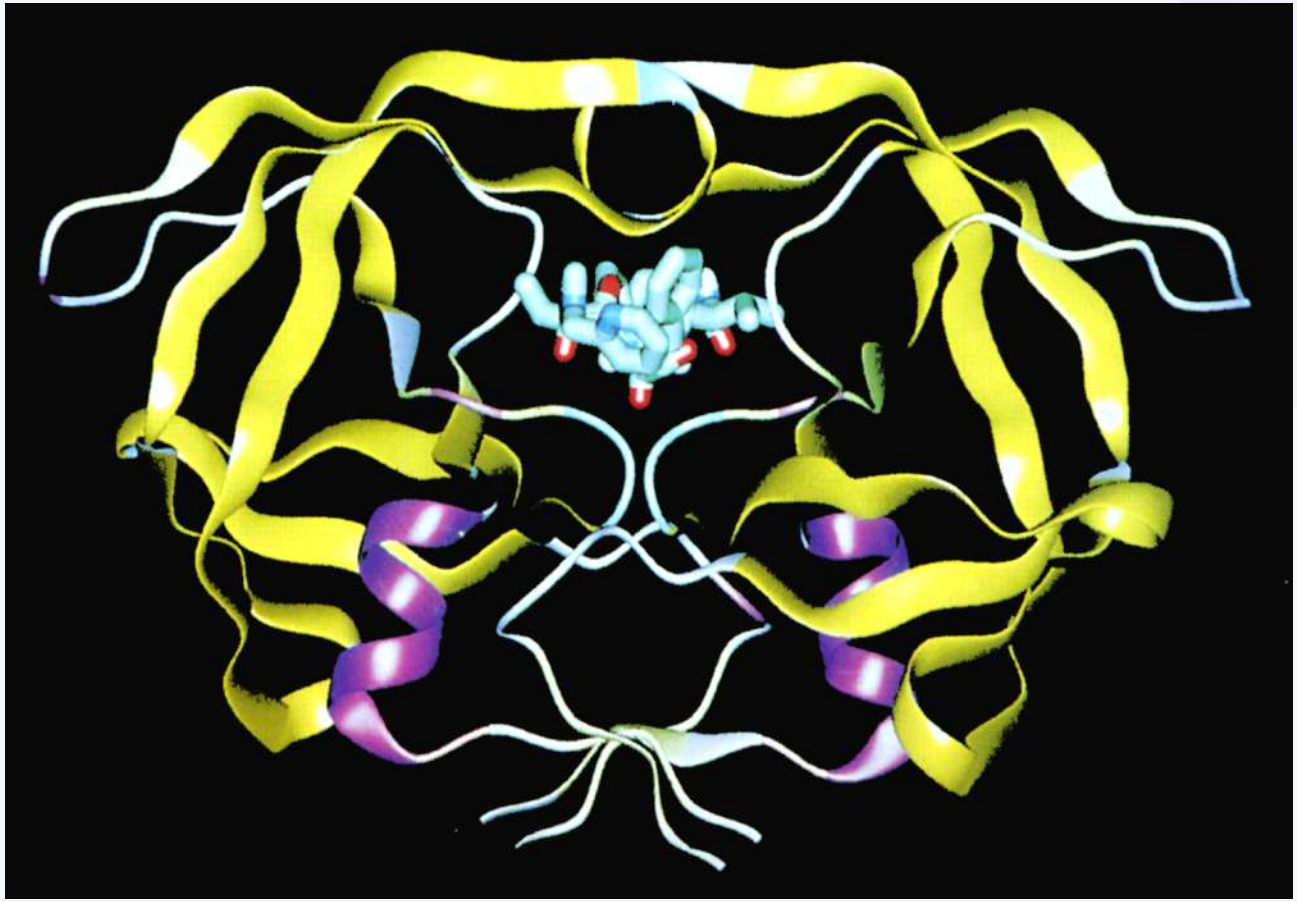
HIV Protease – success story





Irwin, J.J., et al., Nanotech 2002

HIV protease inhibitors



J. W. Erickson, 1995

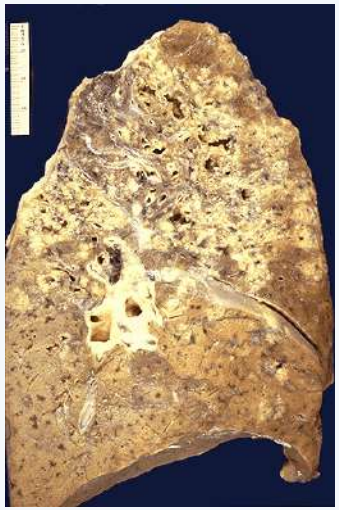
HIV Protease

- Over 200 PDB entries
- Over 35 groups
- Countless leads and inhibitors
- 12 - 14 years
- 9 marketed drugs -saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, lopinavir, atazanavir !
- Huge benefits to patients and society

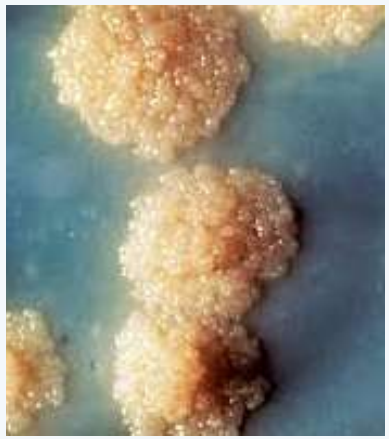
Targeting TB

- 1,000,000 - 2,000,000 annual deaths
- 2 billion latently infected people
- Complicated and lengthy treatment
- Antibiotic resistance is increasing
- More drugs are needed

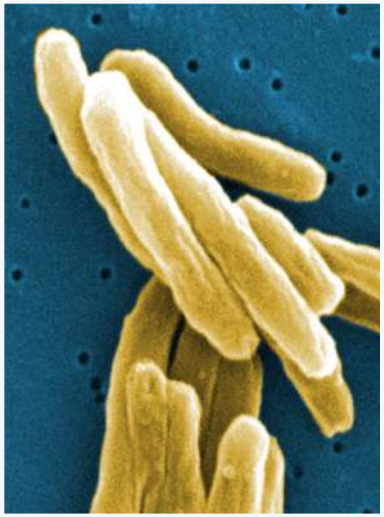
New Drugs for Targeting TB



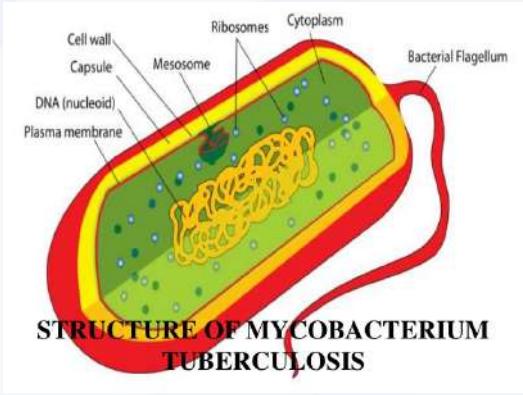
Slideshare.net



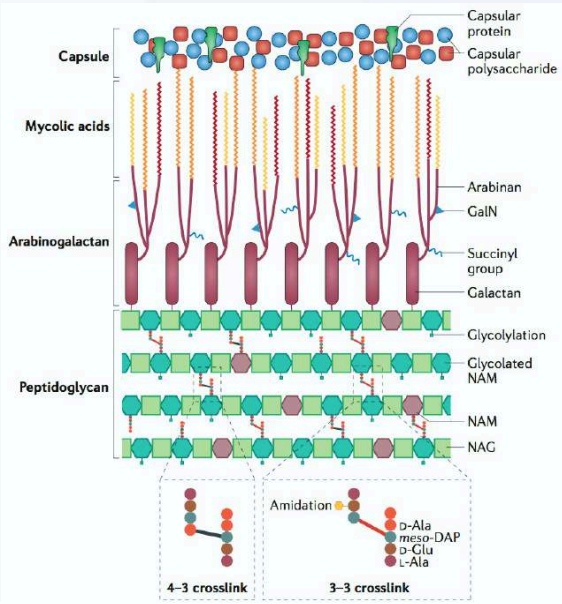
CDC, USA



CDC, USA



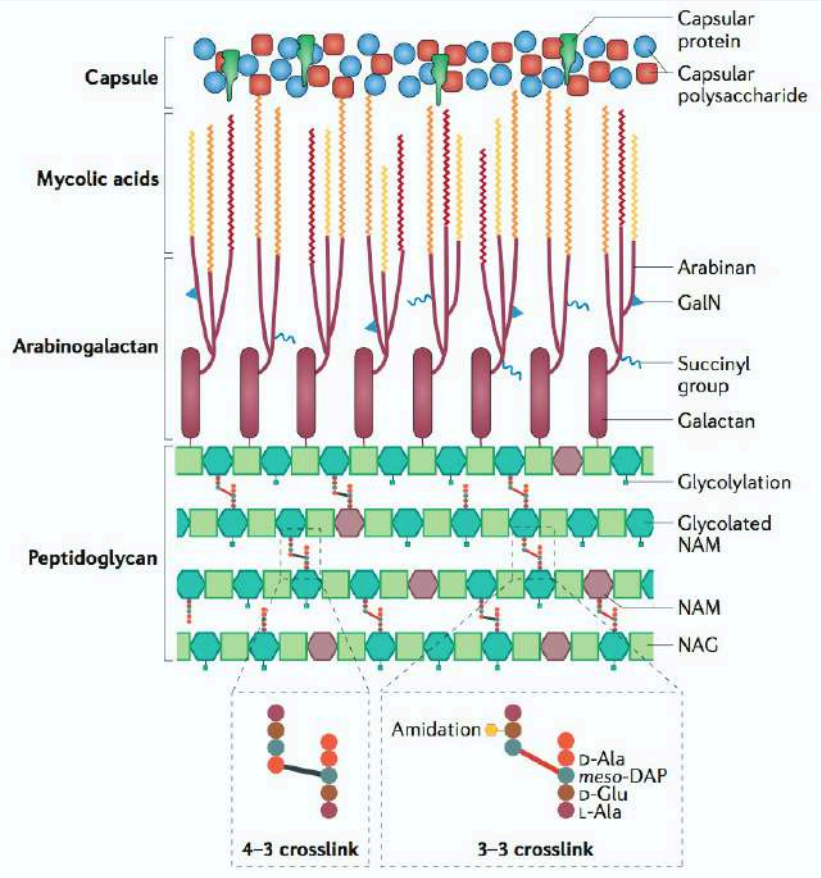
Austin TB



Kieser and Rubin, 2014

TB from the lung to the plate to its molecular structure

Targeting the Cell Wall in TB

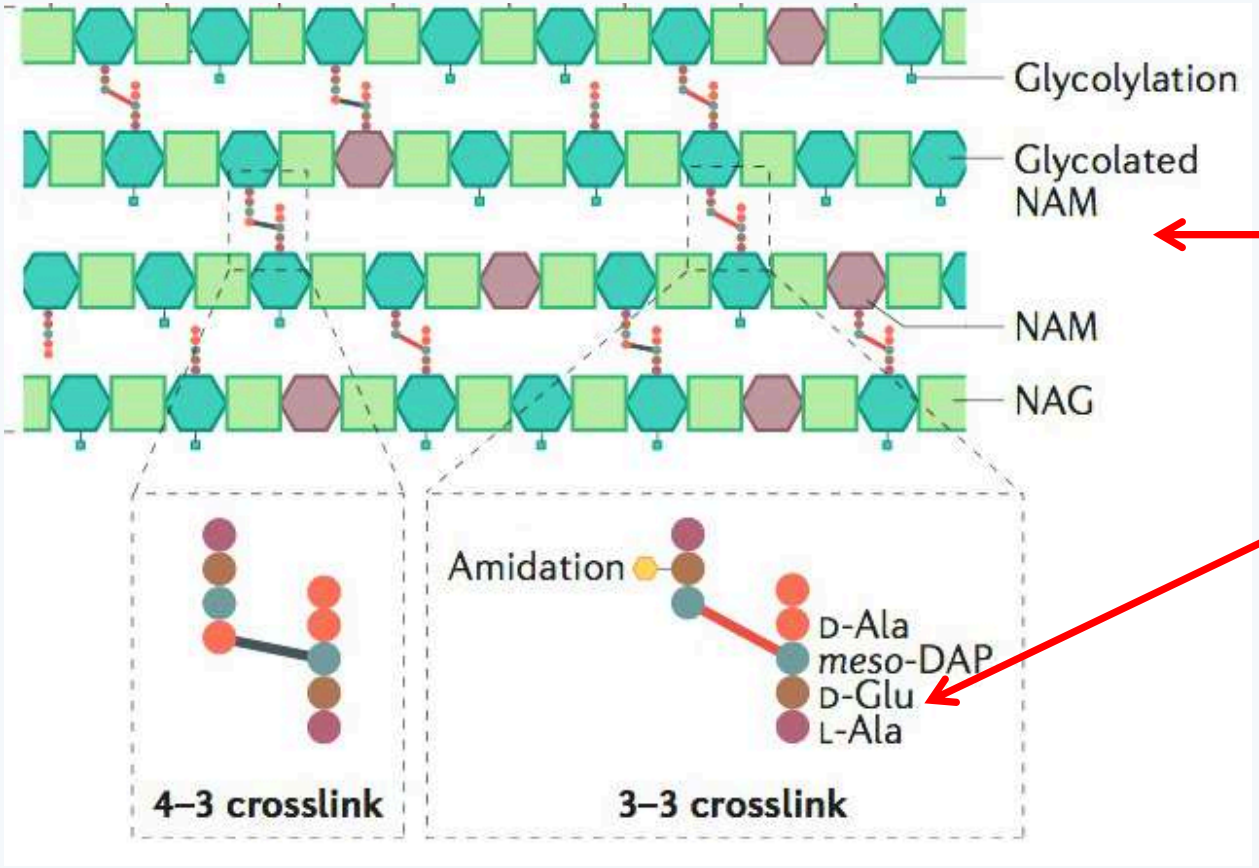


Kieser & Rubin, Nat. Rev. Mic. 2014

Kieser and Rubin, 2014

Historically, many of the most effective antibiotics target the cell wall (Penicillins, Cephalosporins, Carbapenams, Mono...)

Targeting the Cell Wall in TB



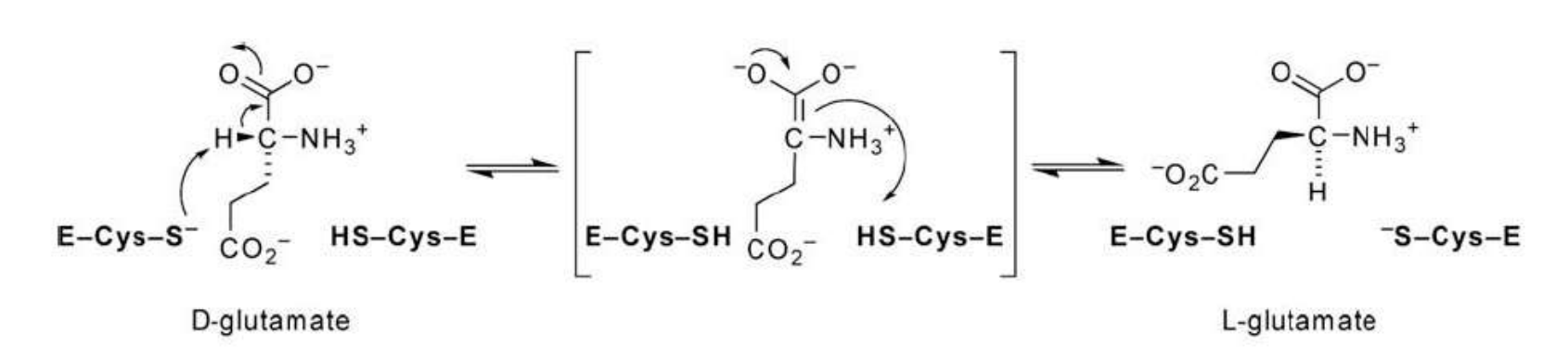
Peptidoglycan

Targeting D-glutamate

Targeting Glutamate Racemase

Kieser & Rubin, Nat. Rev. Mic. 2014

Glutamate Racemase (MurI)



Source: J. Potrykus et al. (2009) **ABB**

- MurI (Rv1338) is responsible for converting L-glutamate to D-glutamate.
- As D-glutamate is an essential component of the bacterial cell wall peptidoglycan, inhibition of MurI activity is lethal to most bacteria.
- MurI function is absent in humans, making the enzyme attractive as a target for drug discovery.
- Previous inhibitor design projects for *S.pneumo, B. anthracis, E. faecalis, H.pylori*; AstraZeneca & others

Overall Goal – can we develop Glutamate Racemase inhibitors as a new drug for TB?

- Explore the essentiality of glutamate racemase in mycobacteria
- Determine the three dimensional structures of glutamate racemase from mycobacteria
- Use the structure as a template for inhibitor design
- Synthesize or identify new inhibitors
- Aside: Project begun in Texas; moved to NZ

Essentiality of Glutamate Racmase

JB JOURNAL OF BACTERIOLOGY
Published Twice Monthly by the American Society for Microbiology
1916–present
SUBSCRIBE

Note: Performing your original search, **Is glutamate racemase essential?**, in PubMed Central will retrieve 29 citations.

Journal List > J Bacteriol > v.175(10); May 1993

J Bacteriol. 1993 May; 175(10): 2970–2979.

Copyright notice

The murI gene of Escherichia coli is an essential gene that encodes a glutamate racemase activity.

P Doublet, J van Heijenoort, J P Bohin, and D Mengin-Lecreulx

URA 1131 du Centre National de la Recherche Scientifique (CNRS), Université Paris-Sud, Orsay, France.

▸ This article has been cited by other articles in PMC.

Abstract

The murI gene of Escherichia coli was recently identified on the basis of its ability to complement the only mutant requiring D-glutamic acid for growth that had been described to date: strain WM335 of E. coli B/r (P. Doublet, J. van Heijenoort, and D. Mengin-Lecreulx, J. Bacteriol. 174:5772-5779, 1992). We report experiments of insertional mutagenesis of the murI gene which demonstrate that this gene is essential for the biosynthesis of D-glutamic acid, one of the specific components of cell wall peptidoglycan. A special strategy was used for the construction of strains with a disrupted copy of murI, because of a limited capability of E. coli strains grown in rich medium to internalize D-glutamic acid. The murI gene product was overproduced and identified as a glutamate racemase activity. UDP-N-acetylmuramoyl-L-alanine (UDP-MurNAc-L-Ala), which is the nucleotide substrate of the D-glutamic-acid-adding enzyme (the murD gene product) catalyzing the subsequent step in the pathway for peptidoglycan synthesis, appears to be an effector of the racemase activity.

J Bacteriol

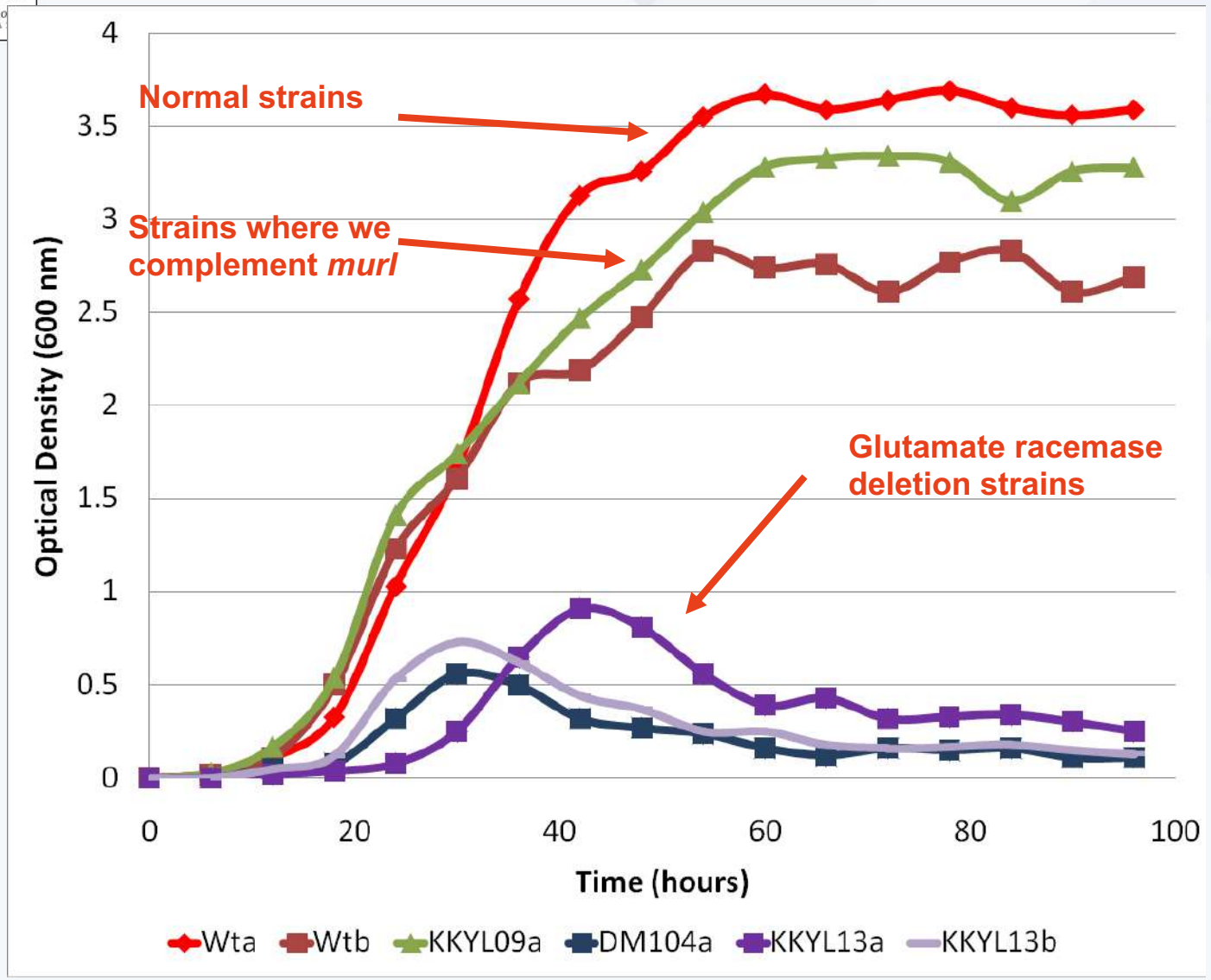
J Bacteriol

- Good evidence that MurI is essential in many pathogens e.g. *E. coli, S. pneumoniae*
- Mycobacteria – the literature was confusing

Our hypothesis

- Based on the results in other bacteria and on our review of the genome we felt glutamate racemase was essential
- Findings in *mycobacterial* studies were contradictory
- We decided to remove the gene for glutamate racemase in mycobacteria and see if the bacterium needed D-glutamate to grow

Growth characterization of gene deletion mutants



Investigation of the Essentiality of Glutamate Racemase in *Mycobacterium smegmatis*

Yang Li,[a] Roman Mortuza,[a] Daniel L. Milligan,[a] Sieu L. Tran,[b] Ulrich Strych,[c] Gregory M. Cook,[b] Kurt L. Krause[a]

Department of Biochemistry[a] and Department of Microbiology and Immunology,[b] Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand; Baylor College of Medicine, Section of Pediatric Tropical Medicine, Houston, Texas, USA[c]

The mycobacterial cell wall frequently has been used as a target for drug development, and D-glutamate, synthesized by glutamate racemase (MurI), is an important component of peptidoglycan. While the essentiality of the *murI* gene has been shown in several bacterial species, including *Escherichia coli*, *Bacillus anthracis*, and *Streptococcus pneumoniae*, studies in mycobacteria have not yet provided definitive results. This study aimed to determine whether *murI* is indeed essential and can serve as a possible target for structure-aided drug design. We have achieved this goal by creating a Δ*murI* strain of *Mycobacterium smegmatis*, a close relative of *Mycobacterium tuberculosis*. The deletion of the *murI* gene in *M. smegmatis* could be achieved only in minimal medium supplemented with D-glutamate, demonstrating that MurI is essential for growth and that glutamate racemase is the only source of D-glutamate for peptidoglycan synthesis in *M. smegmatis*.

Li et al, 2014, J Bact.

Follow-up study in *M. tuberculosis* appeared later in 2015 – MurI essential

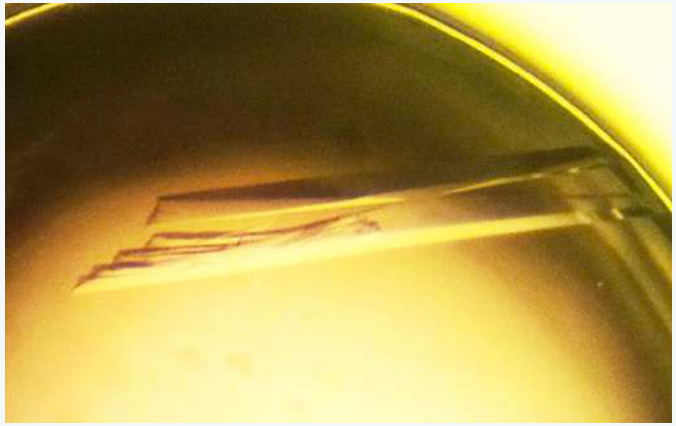
Morayya et al, 2015, Gene

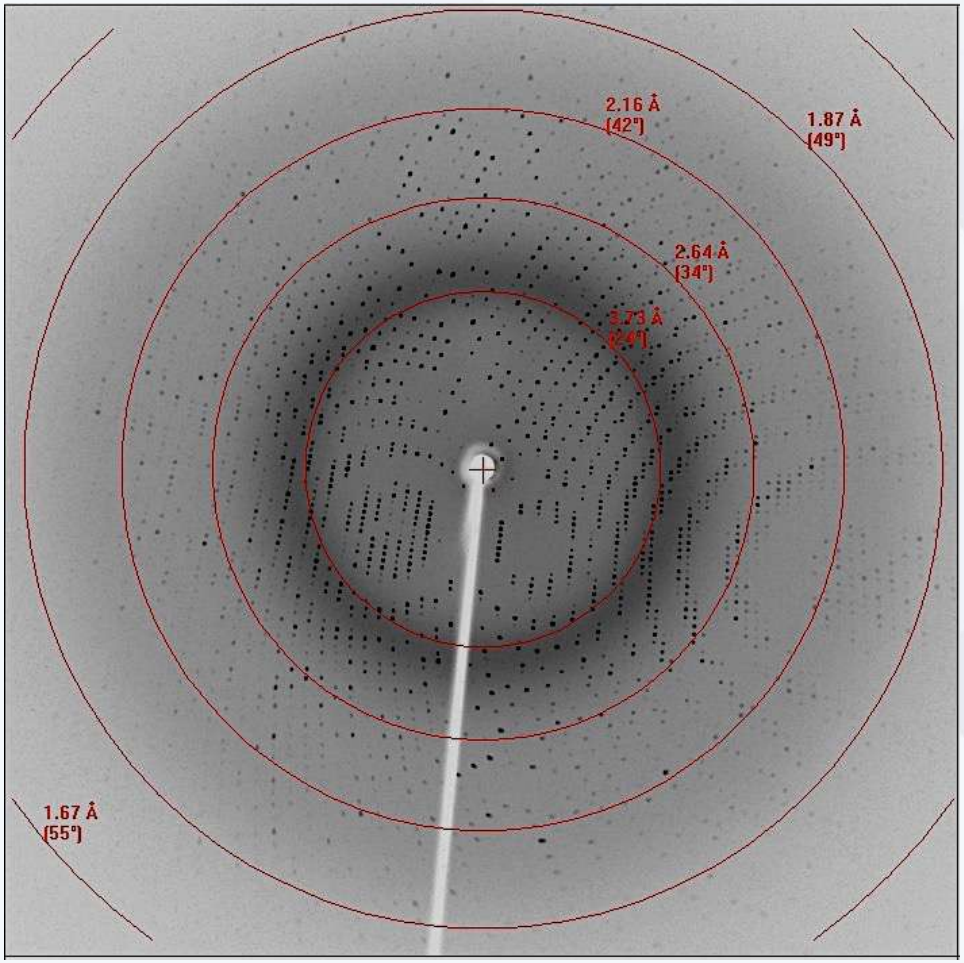
…encouraged to continue our protein based studies

Structural Biology

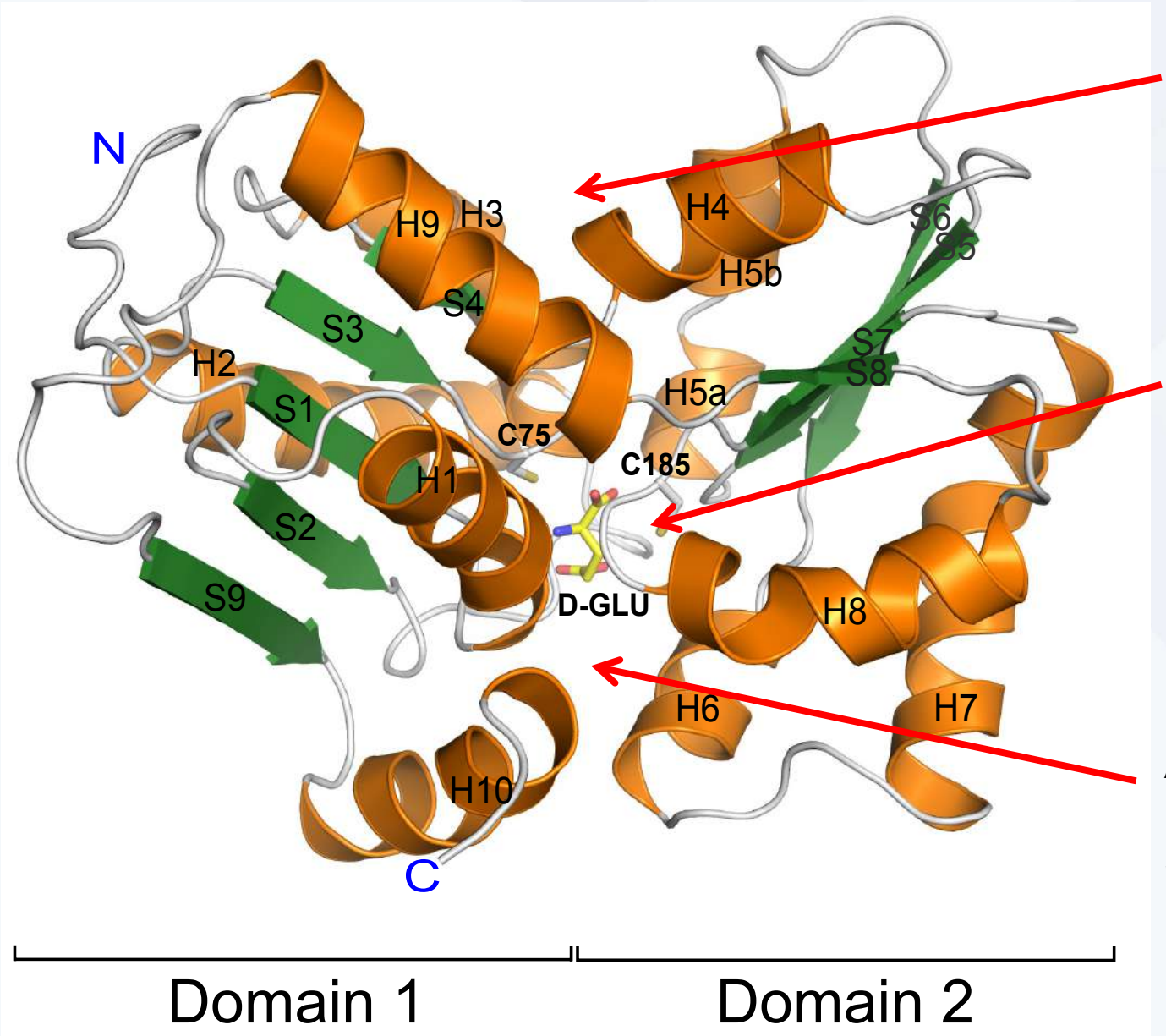
- TB glutamate racemase very difficult protein
- Almost insoluble under all conditions, < 100µg/L
- Review of the other TB researchers showed many started, but then gave up
- We continued protein work over time
- Some success in our group with MurI from both *M. tuberculosis* and *M. smegmatis*

Crystallography Studies



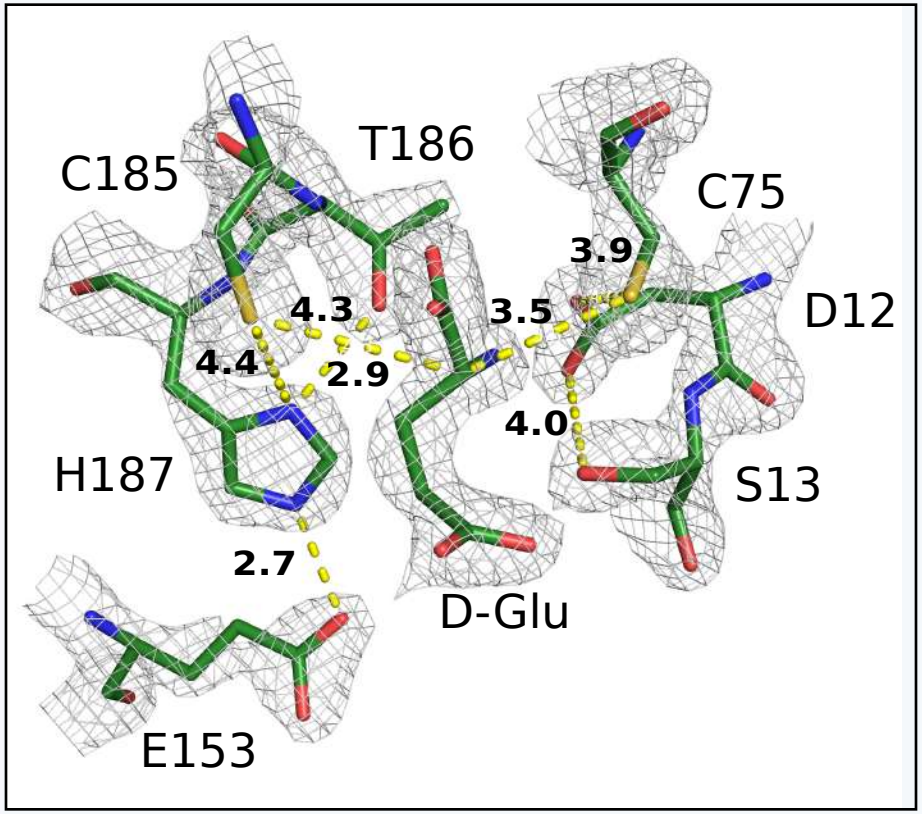


Success with MurI from *M. tuberculosis (*2.3 Å) and *M. smegmatis (*1.8 Å)

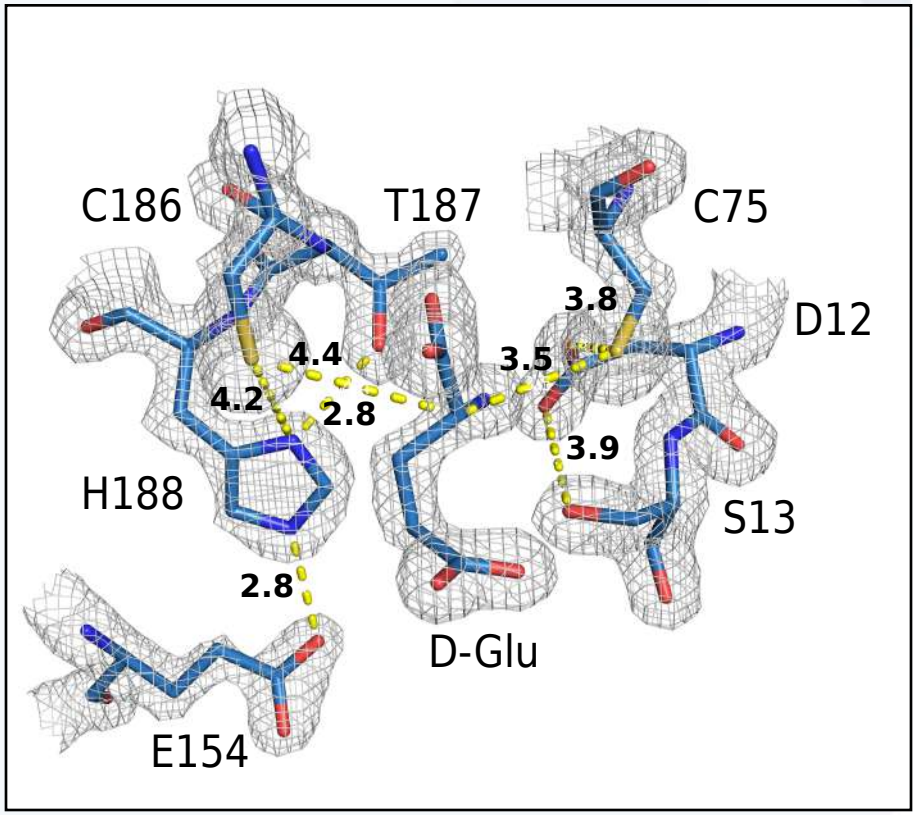

N
C
H9
H3
H4
H5b
S6
S5
S4
S3
S7
S8
H2
S1
H5a
C75
C185
H1
S2
S9
D-GLU
H8
H6
H7
H10
Hinge
Active Site
Allosteric Binding Site
Domain 1
Domain 2

TBMurI and SMMurI active sites

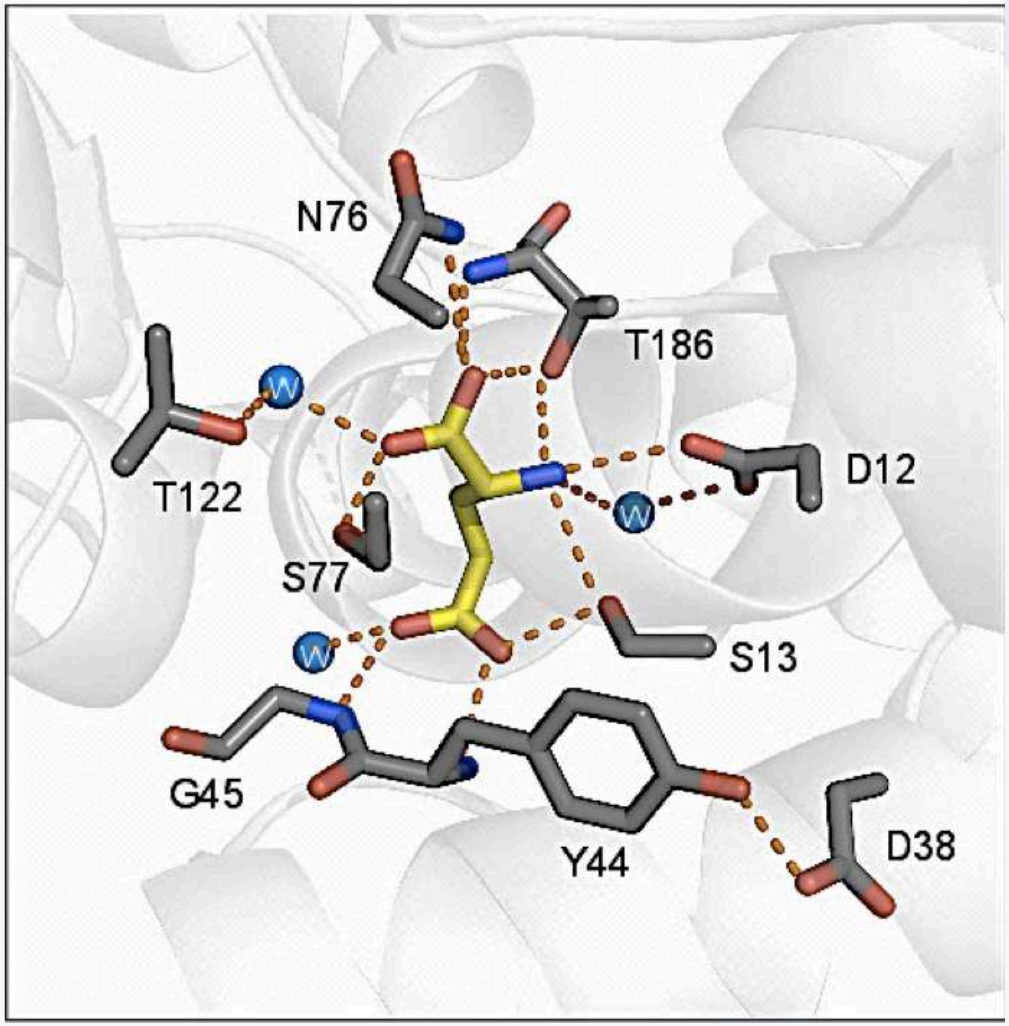
TBMurI (2.3 Å)



SMMurI (1.8 Å)

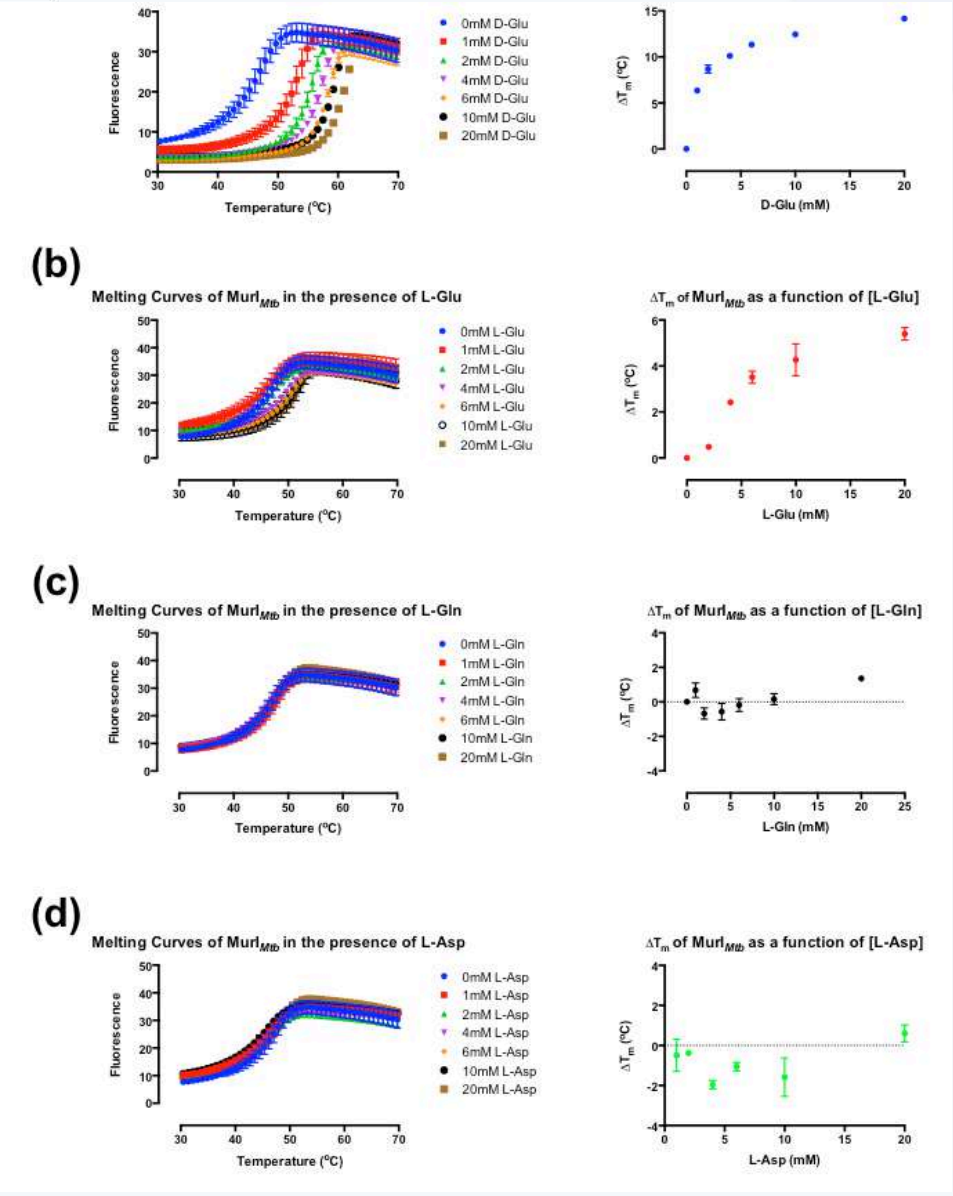


Glutamate binds within the active-site of MurI

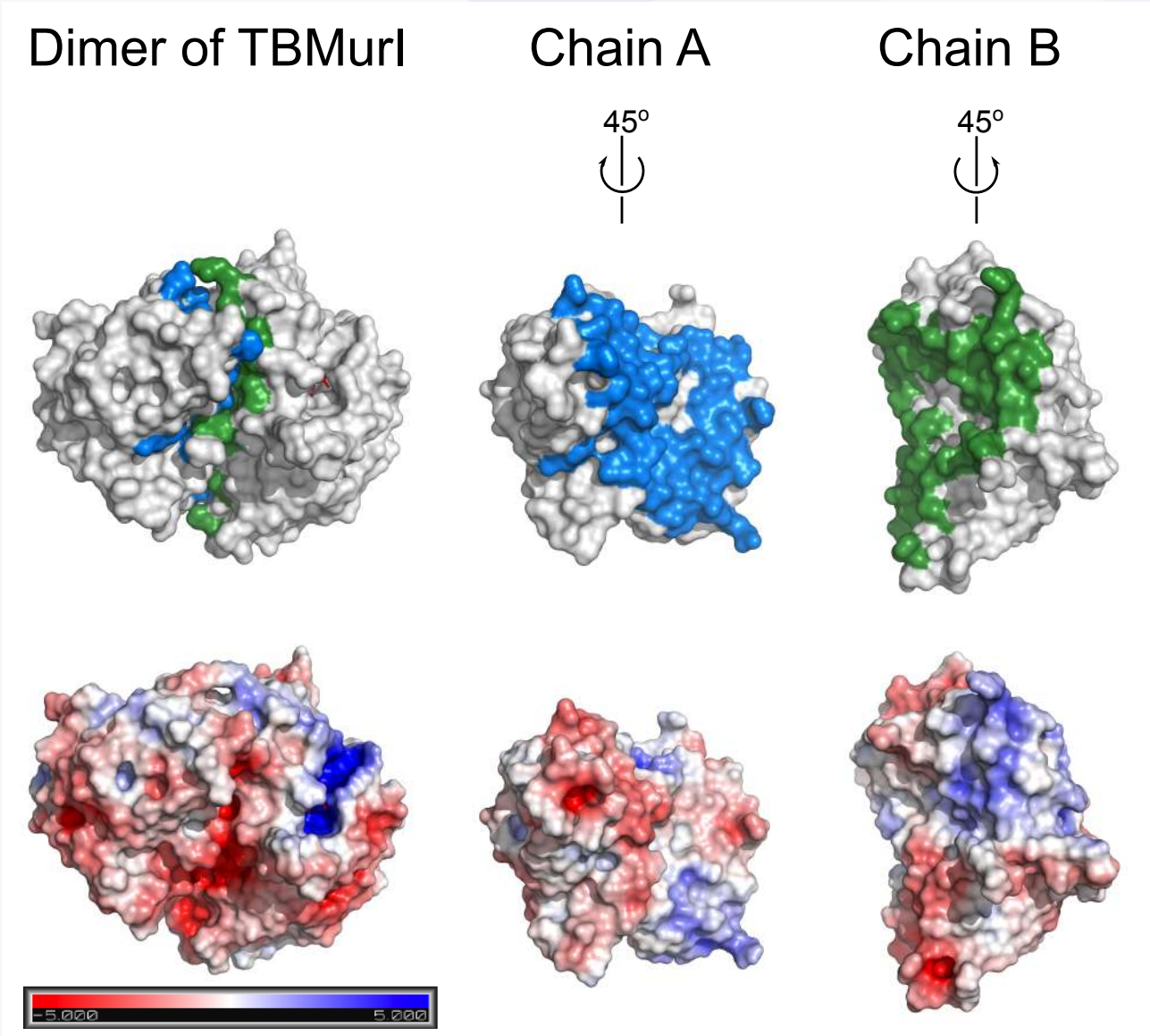


Poen et al, 2016

Binding of glutamate stabilizes MurI

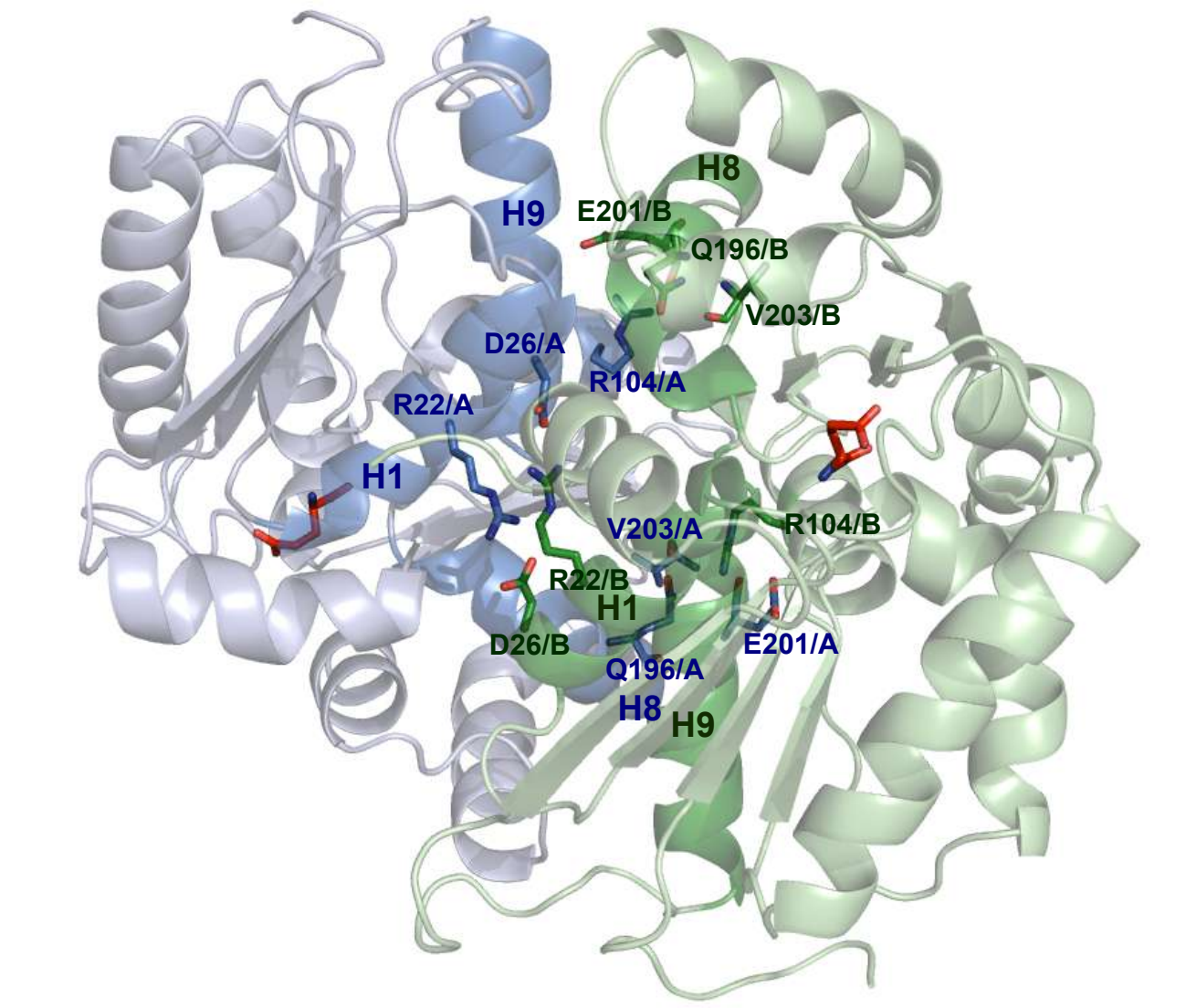


- Very Strong
- Very Specific

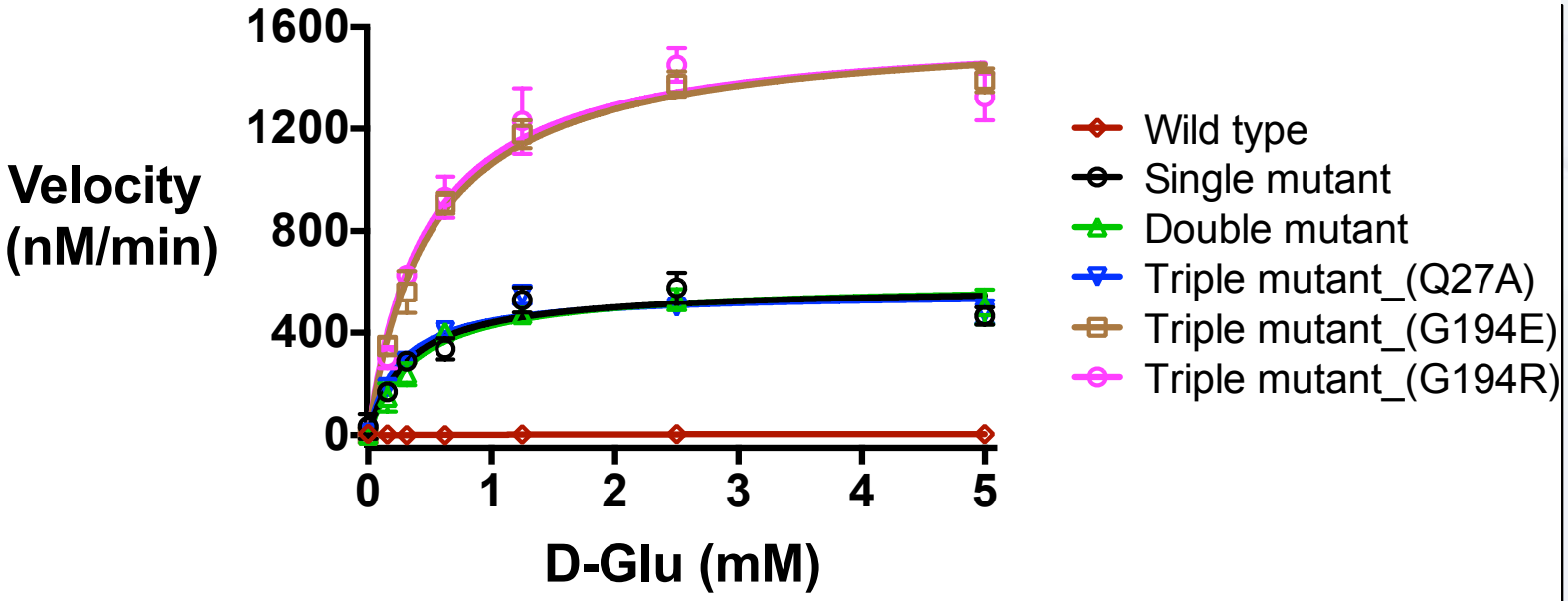


Dimer interface often found to be important in MurI

Molecular Details of Dimer Interaction



Kinetic engineering of the dimer



Glutamate Racemase	K_m (mM)	k_{cat} (min^{-1})
Wild type	nd	nd
Single mutant(D26R)	0.32±0.09	0.058±0.004
Double mutant (D26R/R105A)	0.39±0.06	0.059±0.003
Triple mutant (D26R/Q27A/R105A)	0.26±0.04	0.056±0.002
Triple mutant (D26R/R105A/G194E)	0.51±0.04	0.160±0.004
Triple mutant (D26R/R105A/G194R)	0.46±0.06	0.159±0.006
$MurI2_{Ba}$	0.42±0.02	48.4±0.7

Biochem. J. (2016) **473**, 1267–1280 doi:10.1042/BCJ20160186

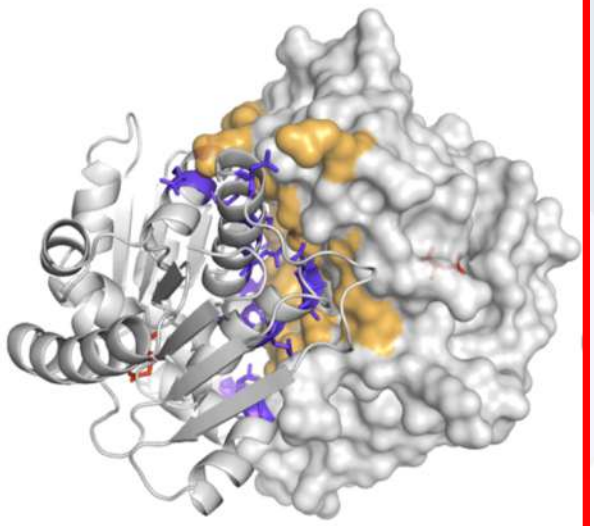
Exploring the structure of glutamate racemase from *Mycobacterium tuberculosis* as a template for anti-mycobacterial drug discovery

Sinothai Poen*, Yoshio Nakatani*†, Helen K. Opel-Reading*, Moritz Lassé‡, Renwick C.J. Dobson‡§ and Kurt L. Krause*[1]

*Department of Biochemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand
†Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin 9054, New Zealand
‡Biomolecular Interaction Centre, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
§Department of Biochemistry and Molecular Biology, Bio21 Institute, University of Melbourne, Parkville, VIC 3010, Australia

Glutamate racemase (MurI) is responsible for providing D-glutamate for peptidoglycan biosynthesis in bacteria and has been a favoured target in pharmaceutical drug design efforts. It has recently been proven to be essential in *Mycobacterium tuberculosis*, the causative organism of tuberculosis, a disease for which new medications are urgently needed. In the present study, we have determined the protein crystal structures of MurI from both *M. tuberculosis* and *Mycobacterium smegmatis* in complex with D-glutamate to 2.3 Å and 1.8 Å resolution respectively. These structures are conserved, but reveal differences in their active site architecture compared with that of other MurI structures. Furthermore, compounds designed to target other glutamate racemases have been screened but do not inhibit mycobacterial MurI, suggesting that a new drug design effort will be needed to develop inhibitors. A new type of MurI dimer arrangement has been observed in both structures, and this arrangement becomes the third biological dimer geometry for M
The mycobacterial MurI dimer is tightly as
in the nanomolar range. The enzyme binds
specifically, but is inactive in solution unles
is mutated. We created triple mutants of
M. smegmatis glutamate racemase (D26R/
that have appreciable activity (k_{cat} = 0.05
K_M = 0.26–0.51 mM) and can be utilized
antimicrobial candidates for inhibition.

Key words: analytical ultracentrifugation
glutamate racemase, monomer engineeri
tuberculosis, thermofluor.



Glutamate Racemase Results

- 1) We *have shown that glutamate racemase is essential for growth in mycobacteria-* (collaboration, Prof. G. Cook, Microbiology)
- 2) *We have learned to express and purify this enzyme*
- 3) *We have determined the crystal structure of both the M. tuberculosis and M. smegmatis enzymes* to 2.3Å and 1.8Å respectively
- 4) We have created a form that can be easily assayed to locate inhibitors
- 5) We are now screening to find new drugs for TB
- 6) Expanding to new bacteria: Pseudomonas – with Iain Lamont and Neisseria gonorrhoeae – with Joanna Hicks

Other Projects in our Lab

- Bacterial
  - Alanine racemase
  - Proline metabolic enzymes
  - Terminal oxidases – with G. Cook
- Viral
  - Neuraminidase – influenza A
  - Viral anti-inflammatory proteins – with Lyn Wise
- Ribosomes from pathogens - with Gerwald Jogl
- Crispr-Cas – with Peter Fineran
- Bioluminescence – with Nigel Perry

TB Glutamate Racemase Research Collaborators

Mycobacterial Physiology & Microbial Genetics

- Htin Aung
- Gregory Cook
- Sieu Tran
- Daniel Milligan
- Jenny Robson
- Li Yang
- Roman Mortuza
- Ulrich Strych

Medicinal Chemistry

- Bill Denny, UOA
- Jack Flanaghan, UOA
- Andrew Thompson, UOA

Protein Chemistry and Crystallography

- Sinothai Poen
- Julian Dzeck
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- Li Yang

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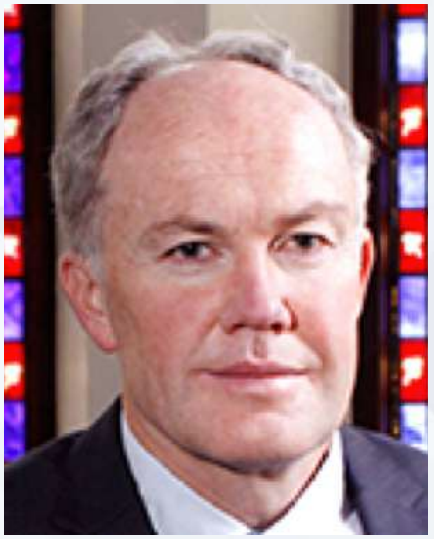

Greg Cook

Daniel Milligan

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Roman Mortuza

Uli Strych

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SAPERE AUDE

Te Whare Wānanga o Otāgo

NEW ZEALAND

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Infectious Disease Research

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