Systematic Determination of the Effects of Macrocyclization on Peptide Permeability into Mycobacteria

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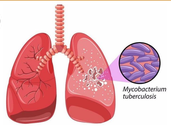
BACKGROUND

Tuberculosis (TB) is the World's Deadliest Infectious Disease!

Causative agent of TB is the bacterium *Mycobacterium tuberculosis (Mtb)*

10.6 million TB cases reported worldwide in 2022[1]

Current surge in U.S. incidence has reversed a decade-long decline[2]



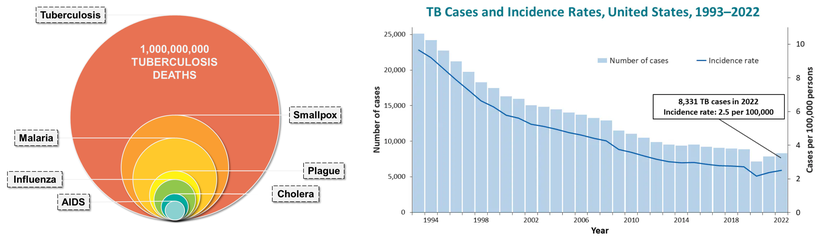
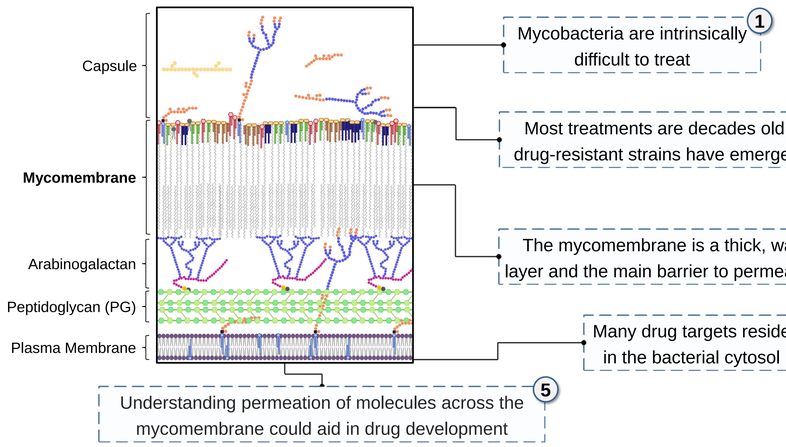


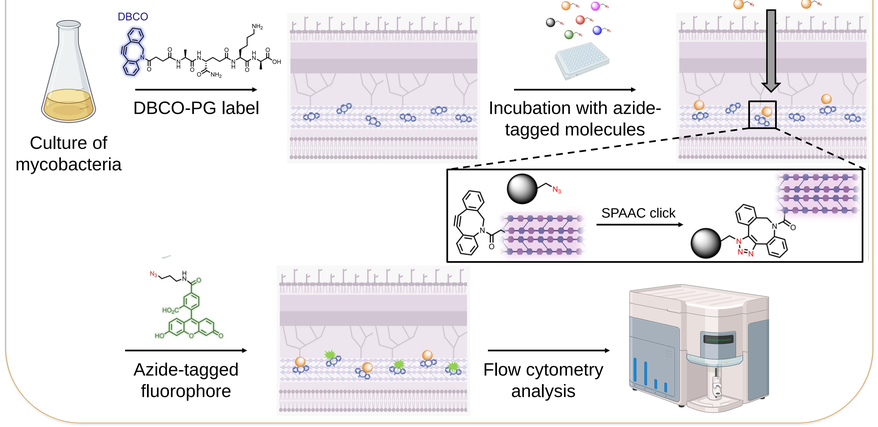
Figure: (Left) *Infectious-disease related deaths in the past 200 years.*[3] ***(Right)*** *TB cases in the U.S. per CDC.*[2]

A Major Bottleneck: The Mycobacterial Permeability Barrier[3]



Assessing Permeability Across the Mycomembrane

Our group previously reported a robust, high-throughput assay to assess the permeability of molecules modified with an azide tag across the mycomembrane[5]



APPROACH AND DESIGN

Peptide drugs have recently gained more attention as therapeutics

- Like small molecules, they are synthetically accessible
- Like biologics, they possess structural pre-organization and selectivity

Peptide drugs are disadvantaged by their lack of permeability across membranes

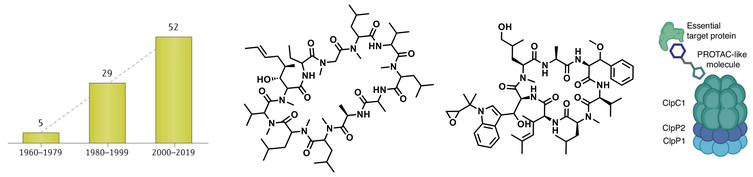


Figure: (Left) *Peptide drug approvals in the last six decades.*[6] ***(Center)*** Cyclosporin A *and* Cyclomarin A. ***(Right)*** *BacPROTAC.*[7]

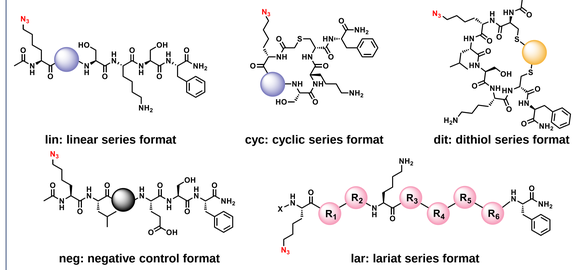
Cyclization is extensively used to enhance passive membrane permeability in mammalian cells

- Can promote intramolecular hydrogen bonding
- Decreases solvent –accessible surface area

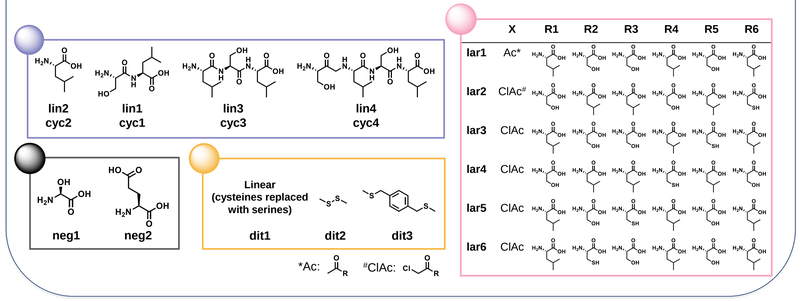
Cyclization as a permeability strategy has not yet been conclusively validated in mycobacteria

The unique composition of the mycobacterial cell envelope necessitates empirical testing of cyclization as a passive permeability strategy in mycobacteria

We set out to systematically isolate the impact of cyclization on peptide permeability across the mycomembrane in live cells

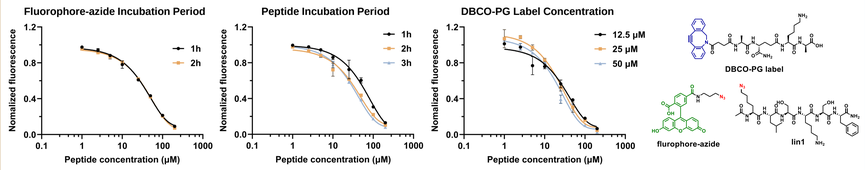


- Majority thioether-cyclized
- *C*-term amidated
- *N*-term acetylated or used as cyclization handle
- Lys modified to have azide
- Phe for UV characterization
- Cys thiol used as cyclization handle
- Leu, Ser, Lys residues to modulate hydrophobicity and solubility



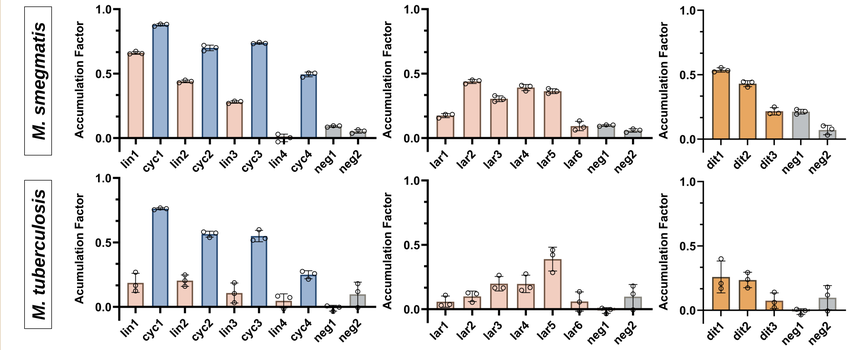
RESULTS

Assay Development and Benchmarking in *M. smegmatis* (*Msm*)



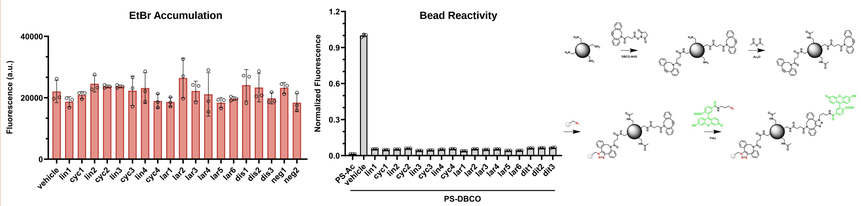
- 2-hour peptide incubation, 1-hour fluorophore-azide incubation, and 25 μM DBCO-PG label were chosen
- Permeability profile was largely unaffected by the number of DBCO moieties and fluorophore-azide incubation period

Effect of Cyclization on Peptide Permeability into Live *Mtb* and *Msm*



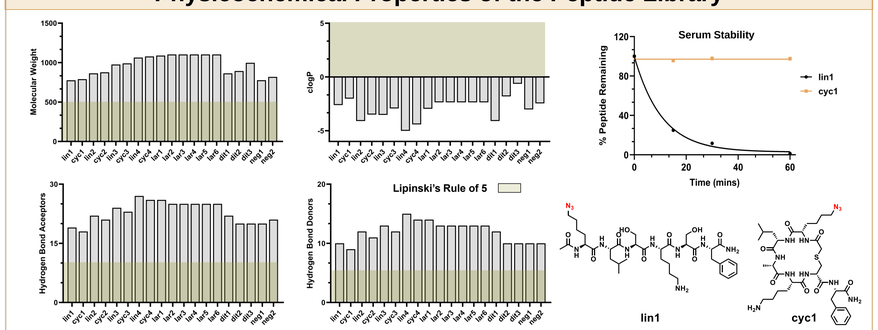
- Overall, peptides were less permeable across the *Mtb* mycomembrane when compared to *Msm*
- Thioether-cyclized peptides generally permeate the mycomembrane better than their linear counterparts

Peptide Reactivity and Membrane Integrity



- EtBr accumulation unaffected by peptide incubation, confirming membrane integrity and cellular viability
- All peptides were found to readily react with beads displaying DBCO epitopes

Physicochemical Properties of the Peptide Library



- The library breaks Lipinski's rule of 5
- cyc1 was found to be considerably more serum-stable than lin1

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Pires research lab

NIH National Institutes of Health