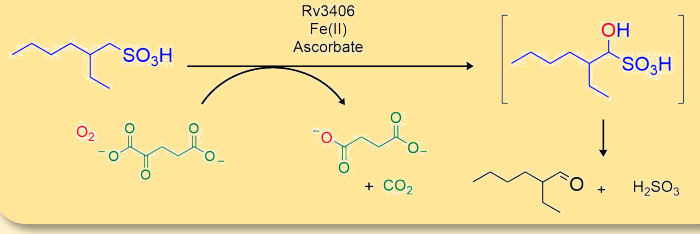
Structural, mechanistic and inhibition studies of *M. tb* Fe(II)/2OG-dependent dioxygenase Rv3406

Mycobacterium tuberculosis

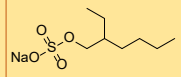
- *M. tb* is responsible for over 1.5m deaths per year worldwide.
- Multidrug therapy uses combinations of antibiotics.
- TB treatment is lengthy and expensive.
- *M. tb* are developing resistance against existing drugs.
- New inhibition target is needed to develop new treatments.

Fe(II)/2OG-dependent dioxygenase Rv3406

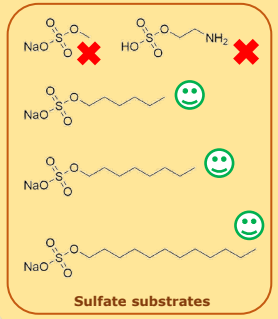
- Type II alkyl sulfatase provides sulfite for cell growth.
- Rv3406 shown to affect antibiotic resistant.
- Inhibition of Rv3406 might be an efficient way for new TB treatment.
- Structural study helps to understanding the binding.
- Current studies are incomplete, most data remains unknown.

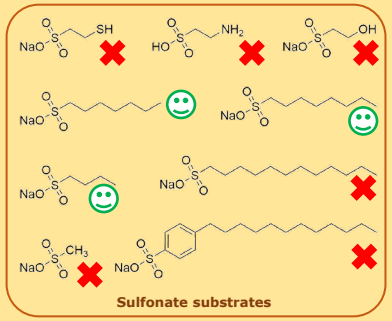


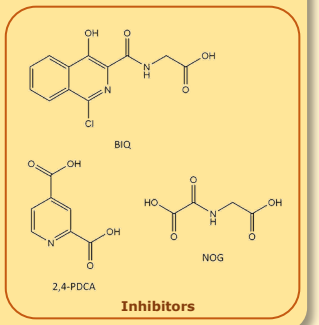
Substrate & inhibitor analogue studies

Substrate	K_M / μM	V_{max} / μM s^{-1}	k_{cat} / s^{-1}	k_{cat} / K_M / M^{-1} s^{-1}
 2-ethylhexyl sulfate	1.5	33.8	16.9	11.6

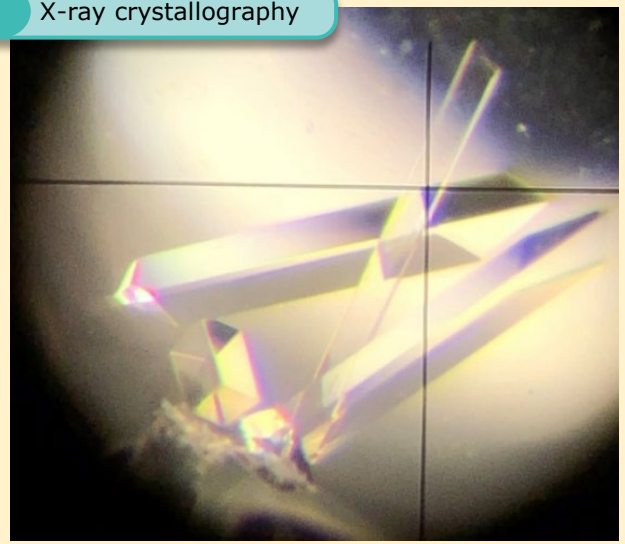
- Understand substrate selectivity might help with antibiotic design.
- Sulfates and sulfonates are both substrates.
- Optimal substrates chain length between 6 to 7 carbons.





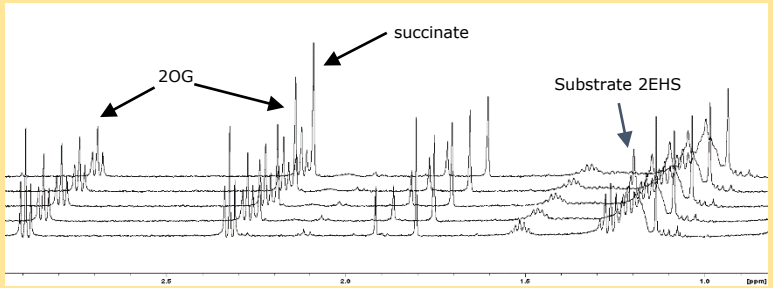


X-ray crystallography



Rv3406 NMR kinetics

- ^{1}H NMR-based assay was developed to measure Rv3406 activity by monitoring substrates and products.
- Rv3406 is highly active with supply of Fe(II), ascorbate, 2OG and 2-ethylhexyl sulfate.
- Several substrate analogues were screened for selectivity with Rv3406.
- Inhibition studies of Rv3406 by 2OG analogues.



Future work

- X-ray crystallography with substrates and inhibitors.
- Point mutation studies on the role of oligomeric states of Rv3406 to its catalytic activities.

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2. J. M. Bollinger Jr, W.-c. Chang, M. L. Matthews, R. J. Martinie, A. K. Boal and C. Krebs, Mechanisms of 2-oxoglutarate-dependent oxygenases: the hydroxylation paradigm and beyond, Royal Society of Chemistry London, 2015.
3. Sogi, K.M., et al., Mycobacterium tuberculosis Rv3406 is a type II alkyl sulfatase capable of sulfate scavenging. PloS one, 2013. 8(6): p. e65080.

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