Crystal Structures of the Rv3066 Transcriptional Regulator from *Mycobacterium tuberculosis*

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Abstract

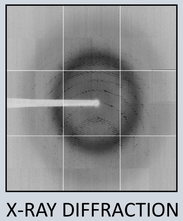
The *Mycobacterium tuberculosis* Rv3066 protein is a proposed transcriptional regulator controlling the expression of the multi-drug efflux pump Mmr (Rv3065). We determined the crystal structures of both the apo and ethidium-bound forms of this regulator at 2.3 Å resolution. The structures suggest that Rv3066 is an all α-helical two-domain protein similar to other members of the TetR-family. The N-terminal domains contain a conserved helix-turn-helix DNA binding motif with significant conformational variations, suggesting conformational flexibility. The C-terminal domains display a multi-drug binding cavity containing anionic charged and aromatic amino acids necessary for the binding of cationic drugs. *In vitro* studies reveal that the dimeric Rv3066 regulator binds to a 14-bp palindromic inverted repeat (IR) sequence at the promoter region to repress the expression of Mmr.

Introduction

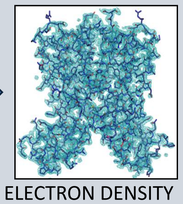
Tuberculosis (TB) is an infectious disease caused by the bacterium *M. tuberculosis* that has resulted in millions of deaths annually throughout the world. Cases of TB have been on the rise mainly due to the appearance of drug resistant strains. Drug resistance can be attributed to multiple factors including the inability of taking up a drug, alteration and inactivation of the drug inside the cell, and drug efflux through drug efflux pumps. The Rv3066 protein regulates the expression of the multi-drug efflux pump Mmr. The ultimate goal of this research is to elucidate the structural and functional relationship of this transcriptional regulator.

X-Ray Crystallography

CRYSTAL



X-RAY DIFFRACTION

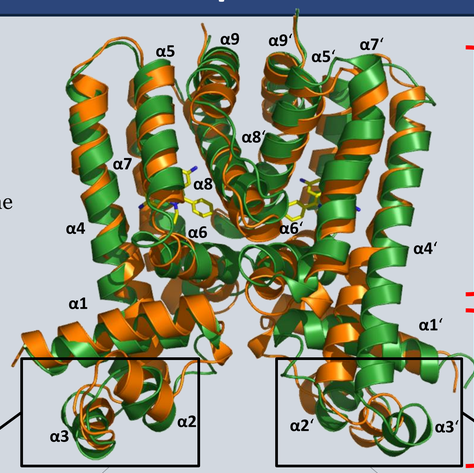


ELECTRON DENSITY AND STRUCTURE

Crystallization Conditions:
Apo-Rv3066: 24% PEG 4000, 0.1 M Na-acetate (pH 5.0), 0.2 M $MgCl_2$
ET-bound: Apo-Rv3066 crystals soaked in 0.5mM ethidium bromide solution

	apo-form	ET-bound
Data Collection		
Wavelength (Å)	0.978	0.978
Space group	$P2_12_12$	$P\ 3_121$
Cell constraints(Å)	a=78.7 b=118.9 c=42.1	a=99.1 b=99.1 c=66.5
Resolution(Å)	2.32 (40.00-2.32)	2.30 (40.0-2.30)
Completeness (%)	99.9 (99.9)	100.0 (99.6)

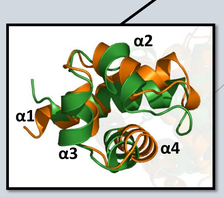
Differences between Apo and ET-bound Rv3066



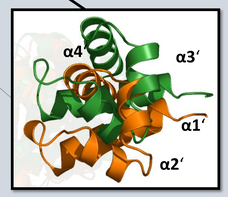
Green: ET-bound
Orange: apo-form
Yellow: ET within the binding pocket

C-terminal multi-drug binding domain

N-terminal DNA binding domain



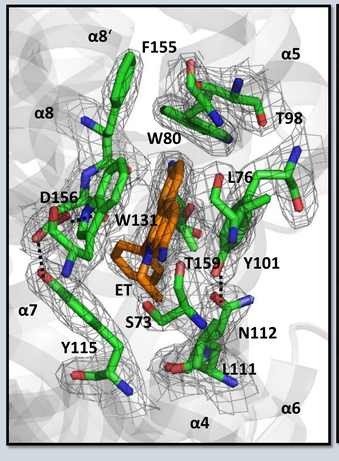
Ligand binding at the C-terminal domain induces conformational change at the N-terminal DNA-binding domain. Conformational changes are primarily due to a rigid body rotational motion within the dimer interface of the regulator.

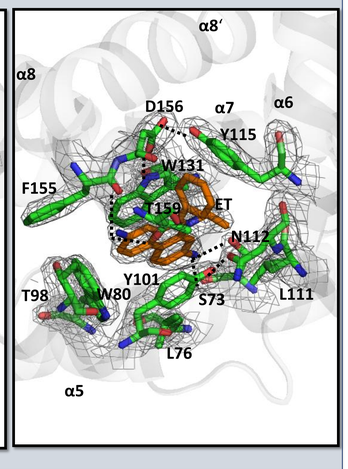


Interactions within the Ligand Binding Pocket

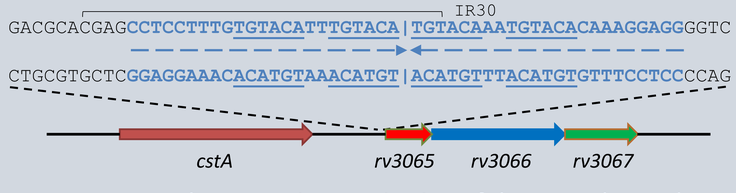
Rv3066 residues	Distance to ET (Å) A chain	B chain
S73†	2.64	2.67
S73† backbone	2.87	2.91
L76	3.25	3.42
W80	3.63	3.49
T98	3.50	3.54
Y101	4.10	3.24
L111	4.60	4.68
N112	3.29	3.18
Y115	3.30	3.55
W131	3.02	3.14
F155†	2.86	2.72
D156	3.38	3.49
T159†	2.90	2.66

† H-bond to ET



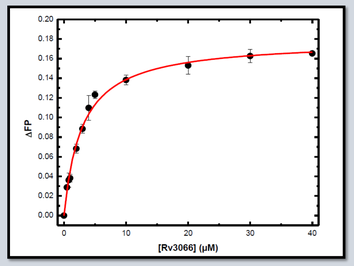


Inverted Repeat (IR)

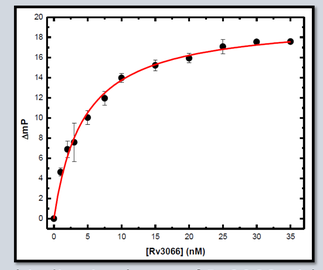


Boxed arrows represent the genomic organization of the genes located around the IR. The sequence forming the IR is highlighted in blue and indicated by the dashed arrows. The 30 base pair IR used in our experiments is indicated.

Fluorescence Polarization

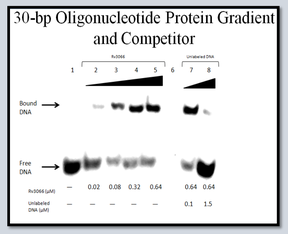


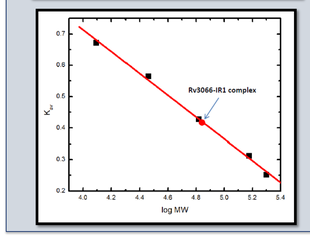
The binding isotherm of Rv3066 with ethidium shows a K_D of 2.9 ± 0.2 μM.



The binding isotherm of Rv3066 with the 30-bp IR shows a K_D of 4.4 ± 0.3 nM.

Rv3066 Binds Specifically to its IR

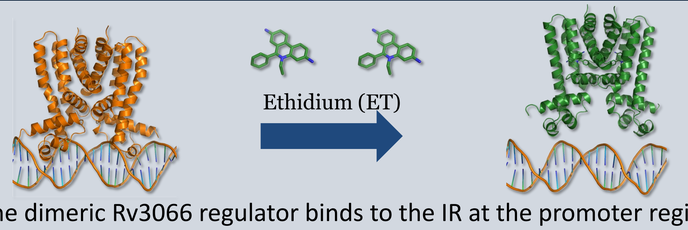




Top: Gel shift studies were done to test if Rv3066 was binding specifically to its predicted IR. Lane 1: Reference DNA; 2-5 : increasing concentrations of Rv3066; 6: blank; 7 and 8:unlabeled 30-bp IR for specific competition. Lane 8 shows the DNA shifting back to the free position indicating that binding of Rv3066 is specific.

Bottom: Gel filtration experiment showing average molecular weight of 67.3 ± 3.8 kDa for the Rv3066-IR complex, which indicates two Rv3066 molecules are bound one 30-bp IR.

Conclusions



- The dimeric Rv3066 regulator binds to the IR at the promoter region, preventing the expression of the Mmr multi-drug efflux pump.
- Ethidium binding at the C-terminal multi-drug binding domain induces conformational change at the N-terminal DNA-binding region.
- This net result is the release of the regulator from the DNA promoter region, allowing for the production of the efflux pump.

Citations and Acknowledgements

1. Jani Reddy Bolla, Sylvia V. Do, Xiao Chen, Chih-Chia Su, Feng Long, Hsiang-Ting Lei, Kanagalaghatta R. Rajashankar, and Edward W. Yu. *Sequence Structural and functional analysis of the transcriptional regulator Rv3066 of Mycobacterium tuberculosis* (Submitted)
2. Wadsworth Center, New York State Department of Health. "Disease Carriers. Bacteria: Mycobacterium tuberculosis." http://www.wadsworth.org/databank/mycotubr.htm (Accessed 3/15/2012
3. webTB. http://webtb.org/ (Accessed 3/14/20112

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