

**Functional Study 3: Elucidate the role and function of the Mycobacterium tuberculosis virulence-associated protein Rv0577.**

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**Status:** Project completed

**Time-line:**

ORIGINAL TIMELINE	ORIGINAL MILESTONE	REVISED MILESTONE	ACHIEVED
<b>Quarter 1</b> 09.2012- 11.2012	<ul style="list-style-type: none"> <li>ITC experiments with wild type Rv0577 (PNNL)</li> <li>clone four Rv0577 point mutations (LANL)</li> </ul>	<ul style="list-style-type: none"> <li>ITC experiments with wild type protein (PNNL)</li> <li>clone two Rv0577 point mutations (LANL)</li> </ul>	X
<b>Quarter 2</b> 12.2012- 02.2013	<ul style="list-style-type: none"> <li>collect HSQC spectra of <sup>15</sup>N-labeled mutant protein (PNNL)</li> <li>collect relaxation data for wild-type protein with and without ligand (PNNL)</li> <li>chromatography (LANL) and chemical shift mapping perturbation (PNNL) of mutant proteins</li> <li>collect NOE data on <sup>13</sup>C/<sup>15</sup>N-labelled ligand-bound complex (PNNL)</li> </ul>	<ul style="list-style-type: none"> <li>Complete ITC experiments (PNNL)</li> <li>collect relaxation data for wild-type protein with and without ligand (PNNL)</li> <li>chromatography (LANL) and chemical shift mapping perturbation (PNNL) of mutant proteins</li> <li>collect NOE data on <sup>13</sup>C/<sup>15</sup>N-labelled ligand-bound complex (PNNL)</li> </ul>	X
<b>Quarter 3</b> 03.2013- 05.2013	<ul style="list-style-type: none"> <li>collect ITC data on mutant proteins (PNNL)</li> <li>analyze relaxation data (PNNL)</li> <li>structure calculations from NOE data (PNNL)</li> </ul>	<ul style="list-style-type: none"> <li>collect ITC data on mutant proteins (PNNL)</li> <li>structure calculations from NOE data (PNNL)</li> </ul>	X
<b>Quarter 4</b> 06.2013- 08.2013	<ul style="list-style-type: none"> <li>Pursue interesting leads (PNNL)</li> <li>prepare and submit manuscript(s) (PNNL)</li> </ul>	<ul style="list-style-type: none"> <li>analyze relaxation data (PNNL)</li> <li>prepare manuscript(s) (PNNL)</li> </ul>	X

Quarter 1 (September-November, 2012): Work performed during this period was described in detail in the First Quarterly\_report (2012\_24). Briefly, we obtained two single-substitution point mutants (Q<sub>128</sub>A and X<sub>15</sub>A) from Dr. Chang Kim at LANL and prepared fresh protein from these and the wild type. The ITC instrument was calibrated in preparation for experiments with WT protein.

Quarter 2 (December 2012-February, 2013): Work performed during this period was described in detail in the previous Semi-Annual Report (2013\_09). Briefly, Isothermal titration calorimetry (ITC) experiments were started. Relaxation data for the WT protein was collected. Assembly of a manuscript describing available results began.

Quarter 3 (March-May, 2013): Most of the ITC data collection has been completed and the analysis of the relaxation data (T1, T2, and heteronuclear NOEs) is continuing.

Analysis of NOE data with the ligands (2-MeA and 6-MeA) failed to identify unambiguous NOEs between the ligand and MytuD.17269.a (Rv0577c). Consequently, it will not be possible to determine a structure for a ligand-bound complex.

Quarter 4 (June-August, 2013): ITC data collected for the mutants did not show any significant difference in binding relative to the WT protein. This suggests these residues may play little, in any, direct role in ligand binding. A manuscript describing the structure and the ligand binding surface of MytuD.17269.a is in progress with plans to submit in the First Quarter of Year 7.

In late 2012, protein from MytuD.17269.a was sent to Dr. Ron Quinn at Griffith University in Australia to use mass spectrometry to screen for identify additional small molecule ligands in the 526-fragment Eskitis Nature Bank library and the ~200 molecule GSK-TB library. Ten very good "hits" were identified, and chemical shift perturbation studies were performed using one of the fragments, ricinine. Dr. Quinn is currently exploring options to obtain additional funding (from GlaxoSmithKline and/or the Bill and Melinda Gates Foundation-funded TB Alliance) to continue this work.