APPENDICES
APPENDIX A. EXPRESSION AND PURIFICATION OF YOP51*Δ162

The PTP Yop51*Δ162 was prepared by a modified procedure based on the work of Dixon and coworkers. The expression vector pT7-7 containing the deletion mutant of the Yersinia PTP was furnished by Prof. Jack Dixon of the University of Michigan. This plasmid was transformed via electroporation (Bio-RAD) into the BL21 (DE3) strain of E. coli (Novagen), and grown on selective media containing ampicillin. Positive transformants were selected and overexpressed at 37°C in 2XYT media containing IPTG (at a final concentration of 0.4 mM) which was added at OD_{600} = 0.6 - 0.7 absorbance units. After approximately 4 - 6 hours of expression, the culture was chilled to 4°C for 30 minutes. The culture was then centrifuged at 4°C at 5000x g for 20 minutes, and the pellets were resuspended in 20 ml of buffer A (100 mM NaOAc, 100 mM NaCl, 1 mM EDTA @ pH=5.7) per liter of culture. The cells were lysed with two passages through a French Pressure Cell at 4°C, maintaining pressures between 2000 and 3000 psi. The crude lysate was centrifuged at 5000x g for 20 minutes to remove cellular debris and resuspended in 4°C buffer A. The cell free extract was added to a solution of CM Sephadex C-25 resin which was pre-equilibrated in buffer A (15 ml resin per liter of culture) at 4°C, and gently shaken for one hour. The resulting mixture was packed into a column and washed with buffer A until the flow through of A_{280} < 0.01. The adhered proteins were then eluted using a linear salt gradient ranging from 100-400 mM NaCl. Fractions containing pure protein (as determined by coomasie stained acrylamide gel electrophoresis) were directly used in analyses.
APPENDIX B. $m_1$ EQUATION FOR ANALYZING NAP DATA

In Section 6.3.4.1, data was presented for the inactivation of Yop51*Δ162 by vanadate and NAP that was consistent with two inhibitory species being present, one with two vanadates and one ligand and one with two vanadates and two ligands. This section will present the required steps to analyze $m_1$ for this system. From Section 3.4.2, $m_1$ is

$$m_1 = \frac{k_0[S][E_0]}{K_m \sum_j \frac{k_{inact_j}[I_j]}{K_{i_j}}}$$

Taking the reciprocal and summing over two inhibitors leads to

$$\frac{1}{m_1} = \frac{K_m}{k_0[S][E_0]} \left( \frac{k_{inact_1}[I_1]}{K_{i_1}} + \frac{k_{inact_2}[I_2]}{K_{i_2}} \right) \text{ eq B-1}$$

If $I_1$ is designated as the two vanadate one ligand species, and $I_2$ as the two vanadate, two ligand species then

$$[I_1] = K_{V_1}[V]^1[L]^1 \quad \text{and} \quad [I_2] = K_{V_2}[V]^2[L]^2$$

Substituting these into eq B-1 and factoring out the vanadium leads to

$$\frac{1}{m_1} = \frac{K_m[V]^1}{k_0[S][E_0]} \left( \frac{k_{inact_1}K_{V_1}[L]^1}{K_{i_1}} + \frac{k_{inact_2}K_{V_2}[L]^2}{K_{i_2}} \right)$$

This is the equation used in Section 6.3.4.1 to analyze the data.
APPENDIX C. $m_1$ EQUATION FOR ANALYZING NC DATA

The NC data discussed in Section 6.3.4.2 could be modeled to eq 3-26 (given again below)

$$
\frac{d[P]}{dt} = \left( \frac{k_o[S][E_0]}{K_m \left( 1 + \frac{[S]}{K_m} + \frac{[V]}{K_v} + \frac{[V]^b[L]^b}{K_v'K_{VL}} \right)} \right)^* e^{-\left( \frac{k_{\text{max}}[V][L]^b}{2K_v'} \right) t} + \left( \frac{k_{\text{max}}[V][L]^b}{K_v'K_{VL}} \right)
$$

When fit to eq 3-27 (given again below)

$$
\nu = \frac{d[P]}{dt} = m_1 * e^{-m_1 t - m_1 t} \quad \text{eq 3-27}
$$

the value of $m_1$ will be

$$
\begin{align*}
\frac{k_o[S][E_0]}{K_m \left( 1 + \frac{[S]}{K_m} + \frac{[V]}{K_v} + \frac{[V]^b[L]^b}{K_v'K_{VL}} \right)}
\end{align*}
$$

If $m_1$ does not change with [L] this implies

$$
1 + \frac{[S]}{K_m} + \frac{[V]}{K_v} \gg \frac{[V]^b[L]^b}{K_v'K_{VL}}
$$

And thus

$$
\begin{align*}
\frac{k_o[S][E_0]}{K_m \left( 1 + \frac{[S]}{K_m} + \frac{[V]}{K_v} \right)}
\end{align*}
$$

Taking the reciprocal of this yields

$$
\begin{align*}
\frac{1}{m_1} = \frac{K_m \left( 1 + \frac{[S]}{K_m} + \frac{[V]}{K_v} \right)}{k_o[S][E_0]} = K_m \left( 1 + \frac{[S]}{K_m} \right) + K_m \left( \frac{[V]}{K_v} \right)
\end{align*}
$$

Therefore a graph of $m_1^{-1}$ versus [vanadate] will be linear with slope $\frac{K_m}{k_o[S][E_0]K_v}$.
APPENDIX D. USING A TAYLOR SERIES TO ANALYZE TC-o-BQ DATA.

In Section 3-7, it was shown that for a quinone (Q) reacting with a mercaptan (M), with $M_0 > Q_0$, $[Q]$ as a function of time is

$$[Q] = [Q_0] e^{-k_{QM}[M]t}$$

For small values of $x$, (Taylor series)

$$e^{-x} = 1 - x$$

So if $k_{QM}[M]$ is small then

$$[Q] = [Q_0] (1 - k_{QM}[M]t)$$

Now the rate of enzyme deactivation can be expressed by

$$\frac{d[E^*]}{dt} = k_{inact} \left\{ \frac{[E_0] - [E^*]}{1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q} - \frac{[Q_0]}{K_Q} (k_{QM}[M]t)} \right\} \approx [Q_0] (1 - k_{QM}[M]t) \quad \text{eq 3-28}$$

If

$$1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q} \gg \frac{[Q_0]}{K_Q} (k_{QM}[M]t)$$

then

$$\frac{d[E^*]}{dt} = k_{inact} \frac{[E_0] - [E^*]}{1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q}} \approx \frac{[Q_0]}{K_Q} (1 - k_{QM}[M]t)$$

Separating variables and integrating leads to

$$-\ln \frac{[E_0] - [E^*]}{[E_0]} = k_{inact} \frac{\left( t - \frac{k_{QM}[M]t^2}{2} \right)}{1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q}} \left( 1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q} \right)$$

Converting this to velocity
\[
\frac{d[P]}{dt} = \left( \frac{k_0[S][E_0]}{K_m \left( 1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q} \right)} \right) \ast e^{\frac{1}{2} \left( \frac{[Q_0]\left(1 - \frac{k_{Q0} [M]}{k_{Q0}} \right)}{1 + \frac{[S]}{K_m} + \frac{[Q_0]}{K_Q}} \right)}
\]

This can be fit to

\[
\nu = m_i \ast e^{-m_i s + m_i t^2}
\]

Which is eq 7-3.