$$\frac{1}{v}=\frac{1}{k_{cat}[E]_0}\left(1+\frac{K_N\left(1+\frac{1}{K_{eq,1}\sum_{i=0}^{n-1}[D_i]}\right)}{[\mathrm{N}]}\right), \qquad (6)$$

the slope of the double reciprocal plot is $\frac{K_N}{k_{cat}[E]_0}\left(1+\frac{1}{K_{eq,1}\sum_{i=0}^{n-1}[D_i]}\right)$ and the intercept is $\frac{1}{k_{cat}[E]_0}$. In the current work, K_{eq} from Datta and Licata [ref] was used in this expression. The effect of uncertainties in K_{eq} on the resulting estimate of K_N were mitigated through the use of a large excess of [SP]=[D_0].

kcat and Keq,1 can be determined given an estimate of K_N by plotting 1/v against 1/[S_1]=$\sum_{i=0}^{n-1}[D_i]\approx[SP]_0$ at constant [N]. We find that according to the relationship

Comment [RC9]: This can be omitted

Above it says we ~~are~~ using Datta & Licata's Keq.1. Now here we also use our own expt. data to calculate Keq.1. If our calculated Keq.1 does not agree with Datta & Licatta's results, how would we explain?

$$\frac{1}{v}=\frac{1}{k_{cat}[E]_0}\left(1+\frac{K_N}{[N]}+\frac{\frac{K_N}{K_{eq,1}}}{\sum_{i=0}^{n-1}[D_i][N]}\right),$$

the slope of the double reciprocal plot is $\frac{\frac{K_N}{K_{eq,1}}}{k_{cat}[E]_0[N]}$ and the intercept is $\frac{1}{k_{cat}[E]_0}(1+\frac{K_N}{[\mathrm{N}]})$.

Of course, it is possible to simultaneously estimate both Keq and Kn if, for example, an accurate value of Keq,1 is not available. Since the enzyme binding rate constants are much smaller than those for nucleotide binding, it is essential to provide sufficient time

Comment [SC10]: Not used, not essential due to reduction of error above