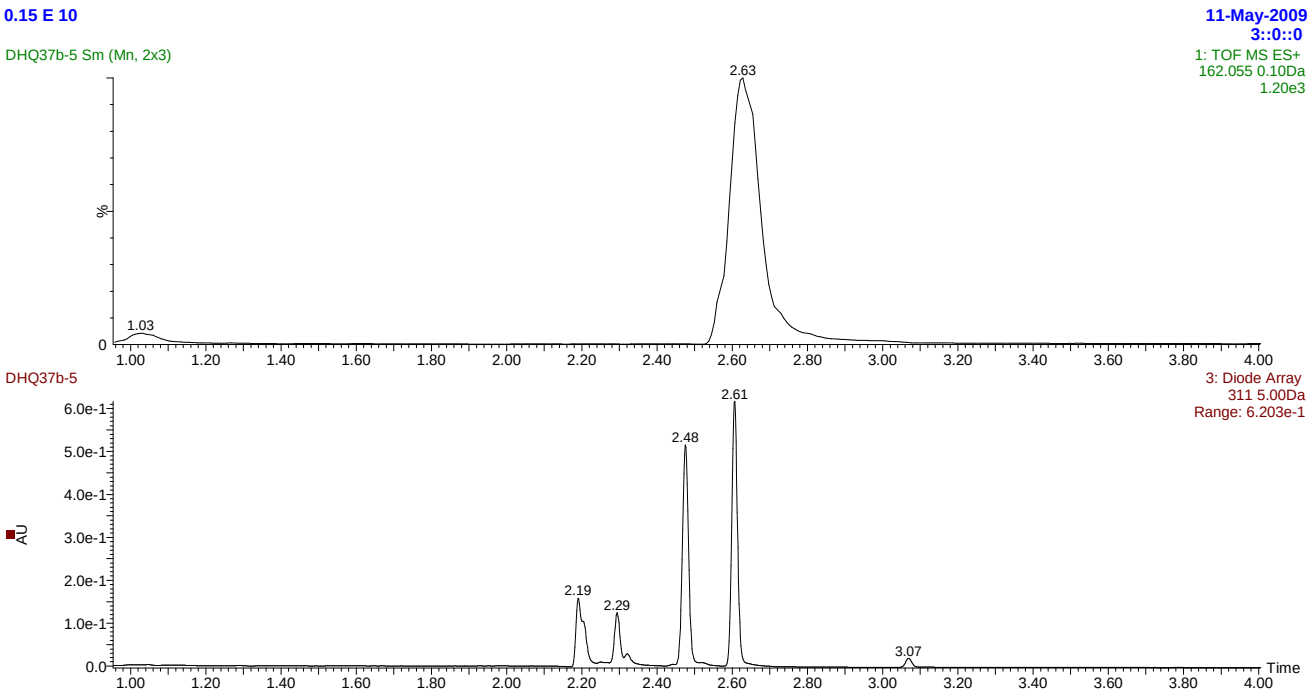
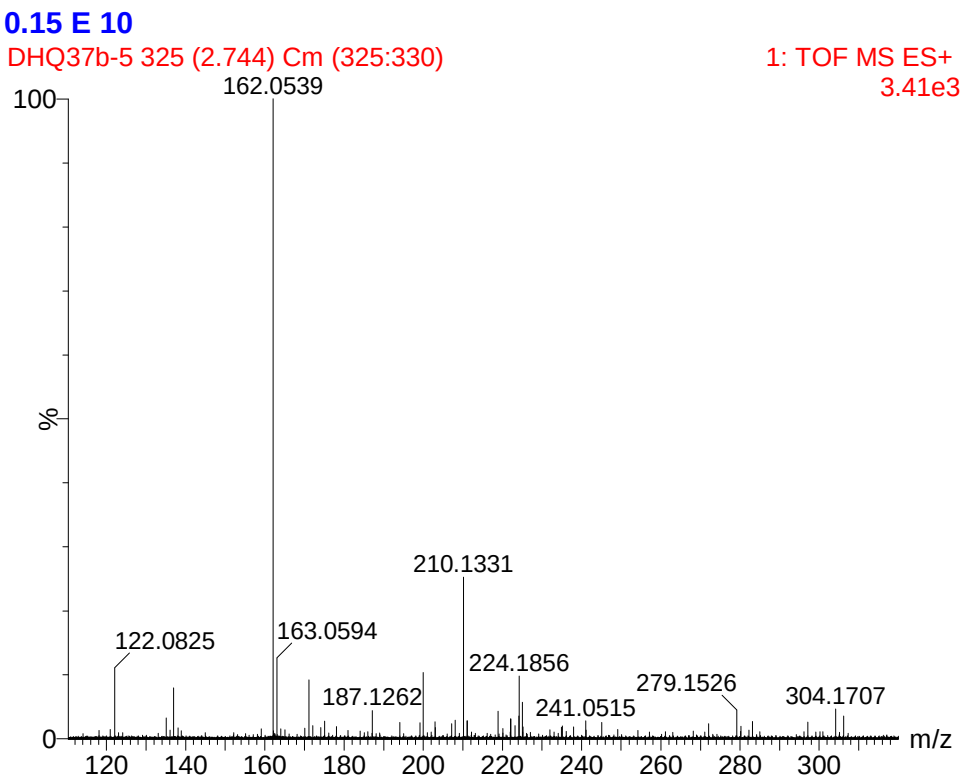
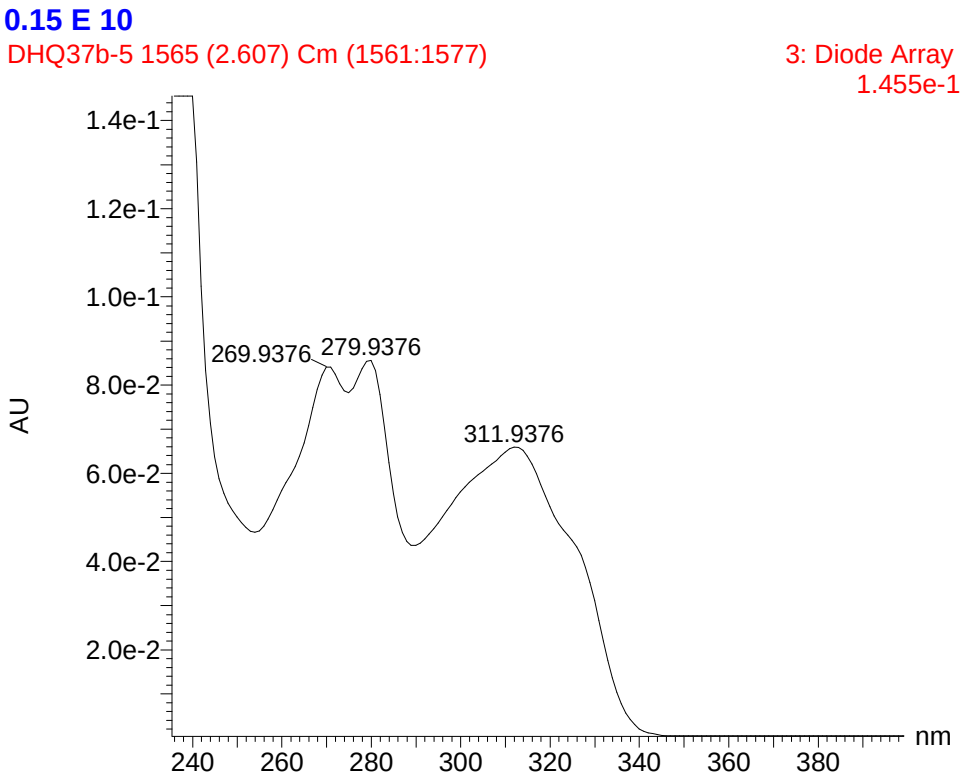


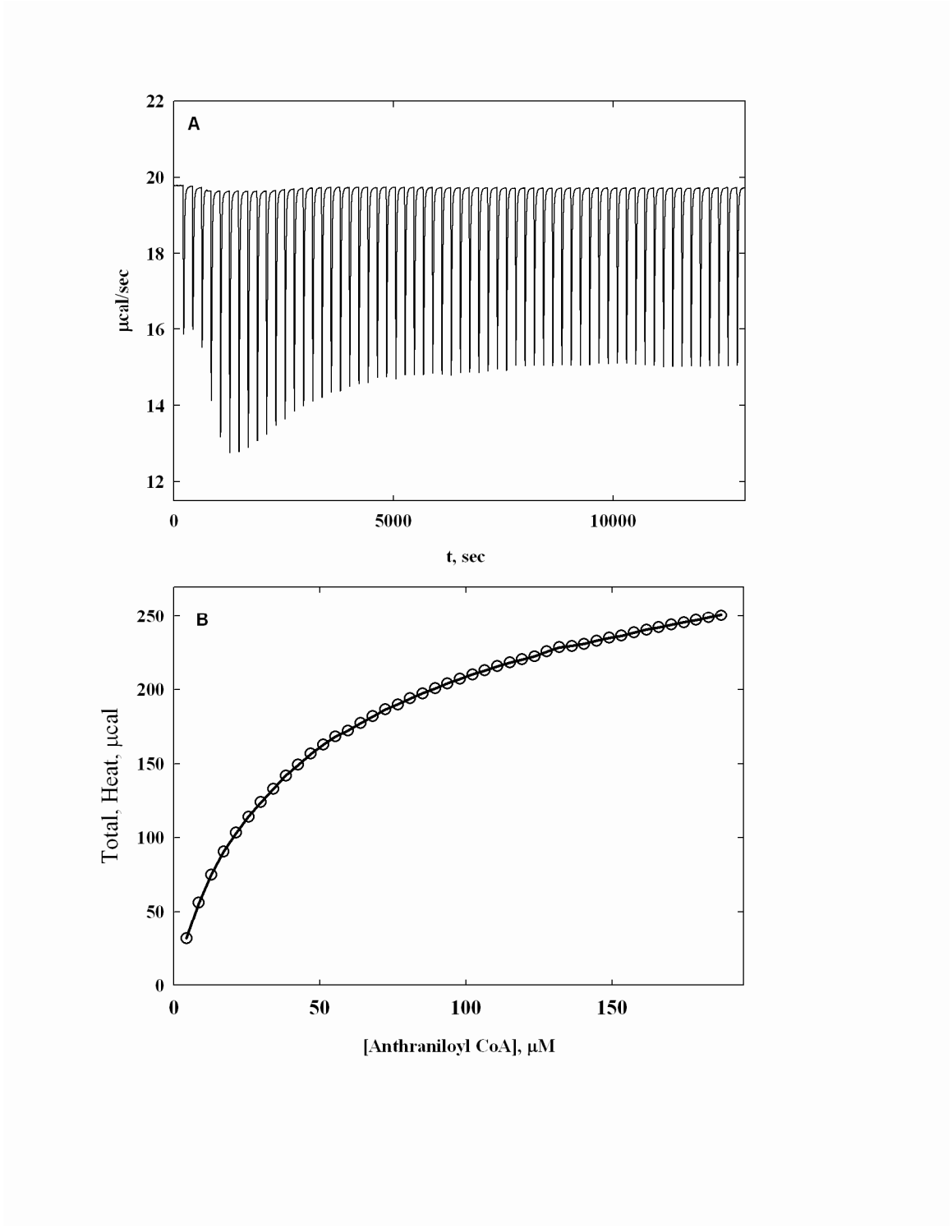
Supplementary Figure 1A. HPLC analysis of PqsD activity. Upper panel, extracted ion chromatogram of DHQ (*m/z* = 162.055; retention time, 2.63 min) identifies the peak at 2.62 minutes in the lower panel as DHQ.



Supplementary Figure 1B. Electrospray ionization mass spectrum of the material eluting at 2.62 min in *Supplementary Figure 1B* above confirms that it is DHQ (m/z_{obsd} = 162.0539; m/z_{calcd} = 162.0555).



Supplementary Figure 1C. UV spectrum of the material eluting at 2.62 min in *Supplementary Figure 1B* above is consistent with the UV spectrum of authentic DHQ.



Supplementary Figure 2. Analysis of the interaction between Cys112Ala PqsD and ACoA by isothermal titration calorimetry. (A) Raw ITC data. A considerable heat of dilution is apparent and was noted in similar injections into buffer only. (B) Data were analyzed by plotting total heat evolved vs [ACoA] and fitting to the following hyperbolic function where y is cumulative heat evolved, y_o is an offset term for a nonzero baseline, a

is the amplitude of the curve, and *b* is the apparent k_d. The first four injections were excluded from the fit due to noise.

$$y = y_0 + \frac{ax}{b + x} \quad \text{(Supplementary Equation 1)}$$