Supporting Information

Development of a Fluorescence Internal Quenching Correction Factor to Correct BoNT/A Endopeptidase Kinetics using SNAPtide

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In this study, fluorescence internal quenching (FIQ) correction factors were derived using an unquenched SNAPtide peptide to quantify the signal quenching over a range of SNAPtide concentrations and temperatures. The FIQ Correction Factors developed provide a convenient method to allow for improved accuracy in determining and comparing BoNT/A LC activity and kinetics using SNAPtide over a broad range of concentrations and temperatures.

This Supporting Information contains:

Figure S-1: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 25^{o}C.

Figure S-2: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 30^{o}C.

Figure S-3: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 37^{o}C.

Figure S-4: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 40^{o}C.

Figure S-5: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 45^{o}C.

Figure S-6: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (μM) at 50^{o}C.

Figure S-7: Slope of trendline showing FIQ Correction Factor/μM SNAPtide calculated for each temperature.

Figure S-8: Comparison of Michaelis-Menten plots for uncalibrated and FIQ correction factor calibrated velocities for BoNT/A LC endopeptidase kinetics (100 nM LCA).

Figure S-9: Comparison of Lineweaver-Burk plots for uncalibrated and FIQ correction factor calibrated velocities for BoNT/A LC endopeptidase kinetics (100 nM LCA).

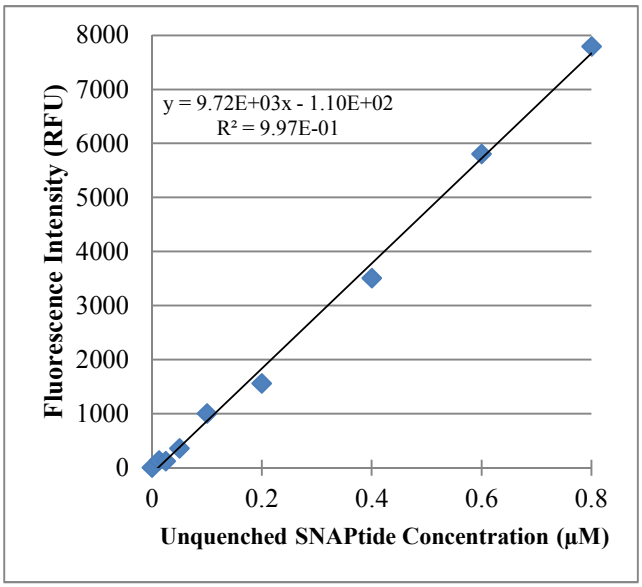

Figure S-1: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 25°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

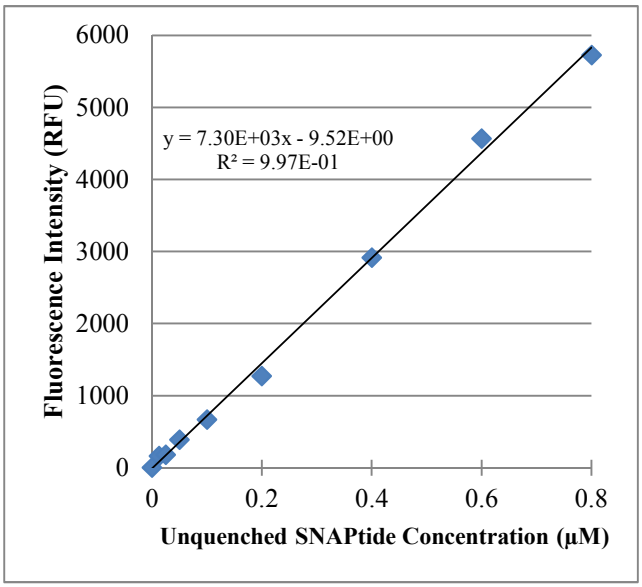

Figure S-2: Sample standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 30°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

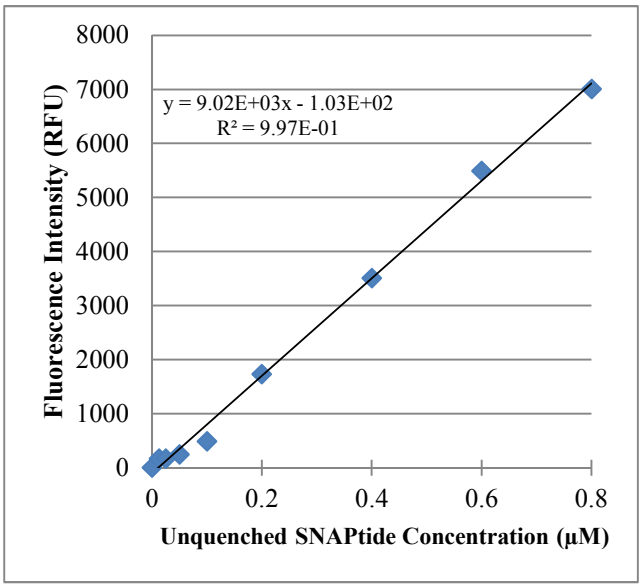

Figure S-3: Standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 37°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

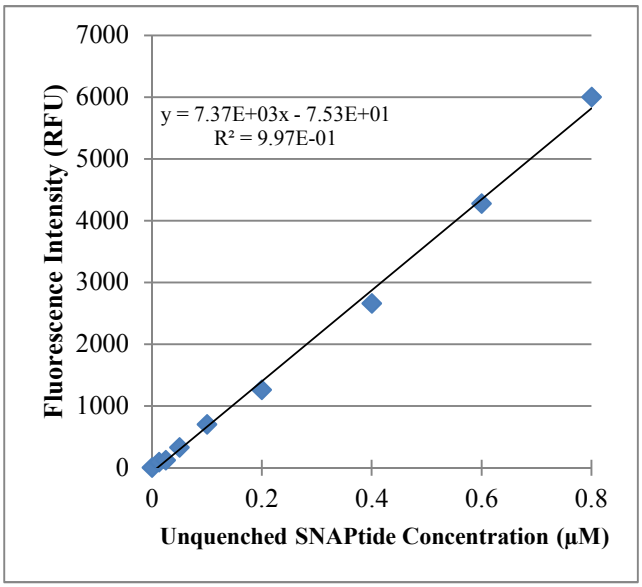

Figure S-4: Standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 40°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

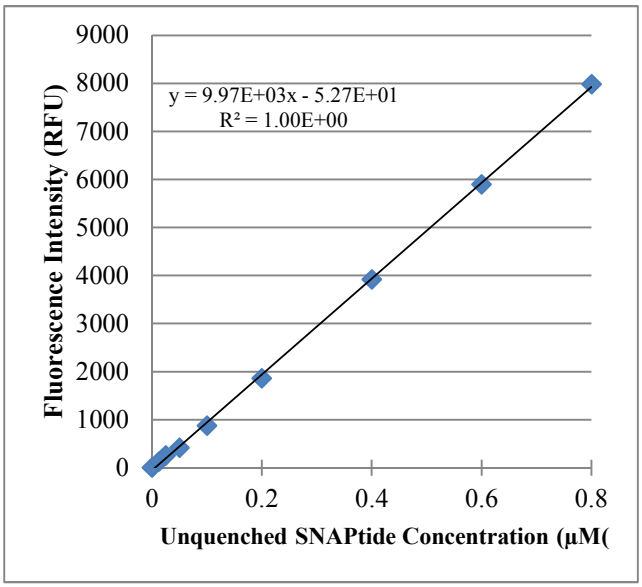

Figure S-5: Standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 45°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

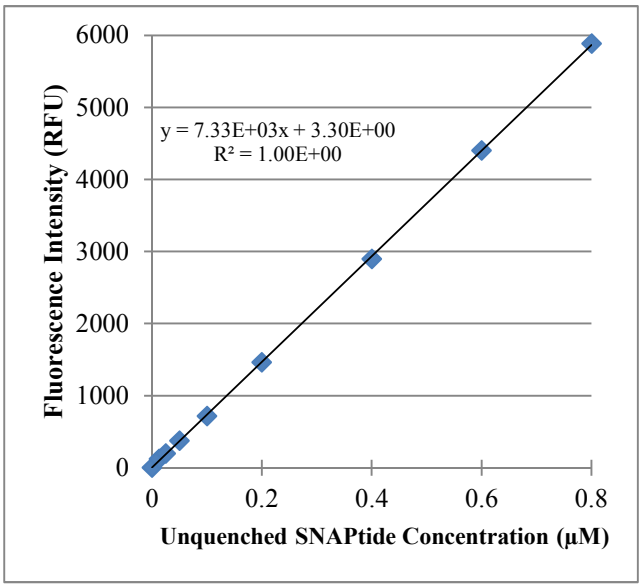

Figure S-6: Standard curve of fluorescence signal (RFU) vs. unquenched SNAPtide concentration (µM) at 50°C. Signals were normalized for the blank (0.00 µM unquenched SNAPtide).

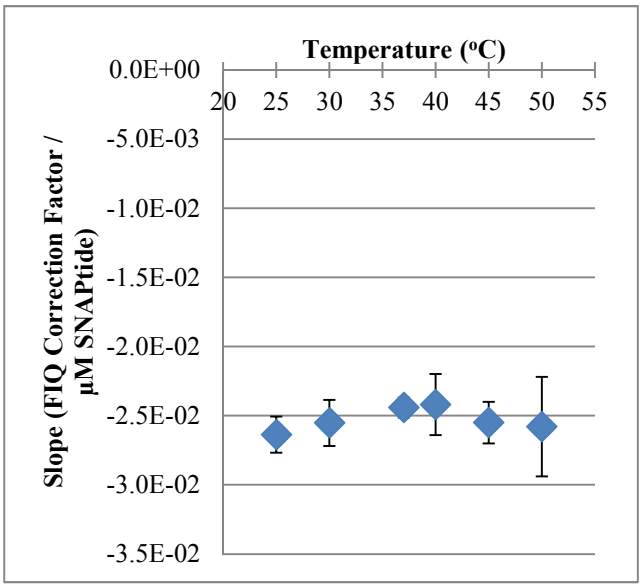

Figure S-7: Slope of trendline showing FIQ Correction Factor/µM SNAPtide calculated for each temperature. These slopes can be used to calculate FIQ Correction Factors at SNAPtide concentrations between 0.00-10.0 µM and at reaction temperatures between 25-50°C if the 0.0 µM SNAPtide sample is assumed to have an FIQ Correction Factor of 1.00. Error bars shown are standard deviations of the mean for triplicate samples.

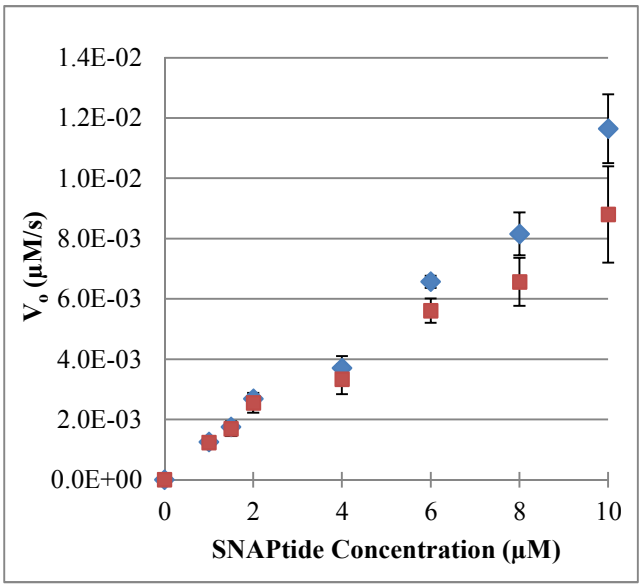

Figure S-8: Comparison of Michaelis-Menten plots for uncalibrated (■) and FIQ correction factor calibrated (◆) velocities for BoNT/A LC endopeptidase kinetics (100 nM LCA). Error bars shown are standard deviations of triplicate samples.

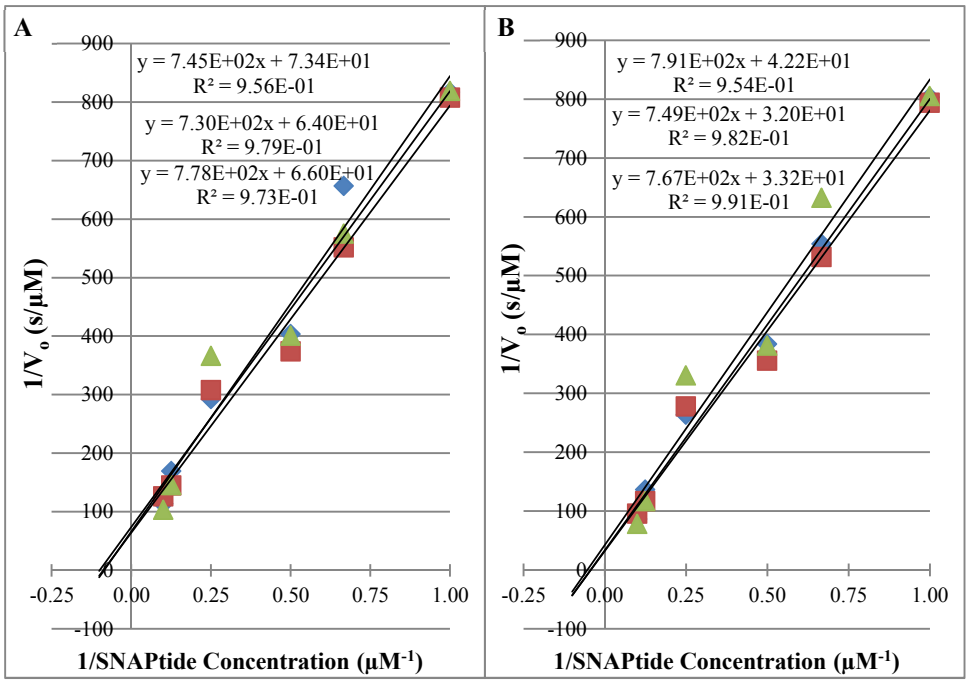

Figure S-9: Comparison of Lineweaver-Burk plots for uncalibrated (A) and FIQ correction factor calibrated (B) velocities for BoNT/A LC endopeptidase kinetics (100 nM LCA). Each plot shows the trendline for each of the triplicate runs. Run 1: ◆ Run 2: ■ Run 3: ▲