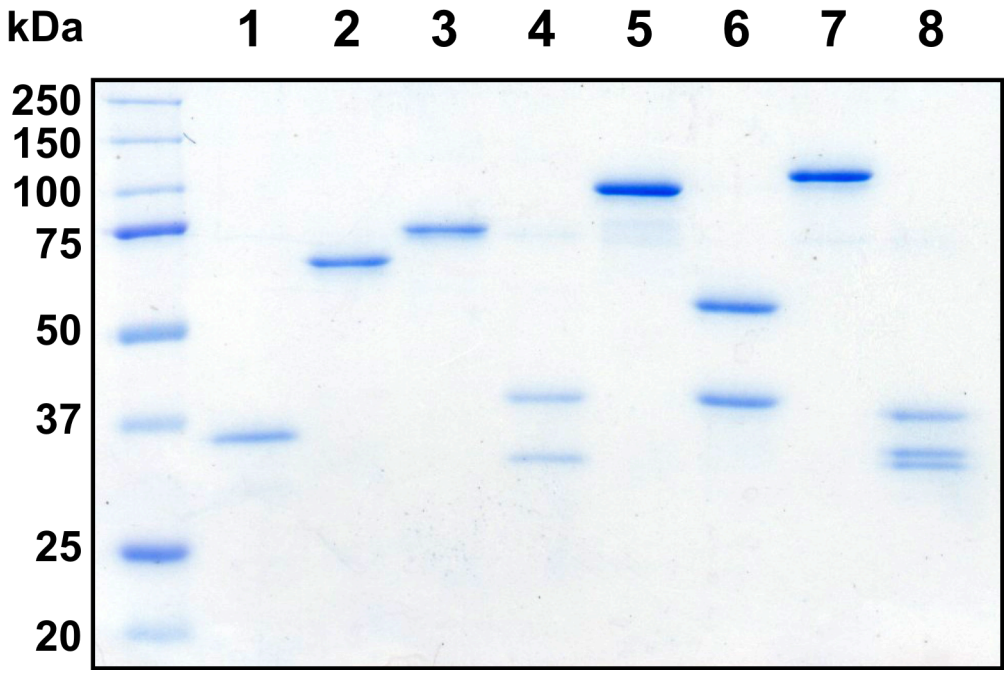
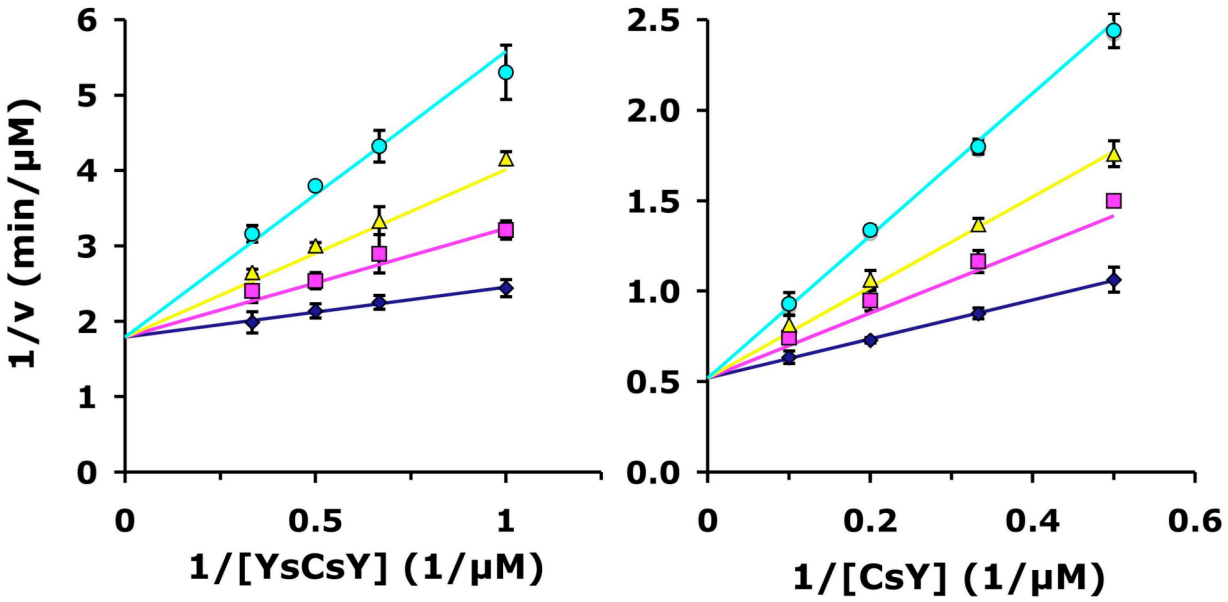
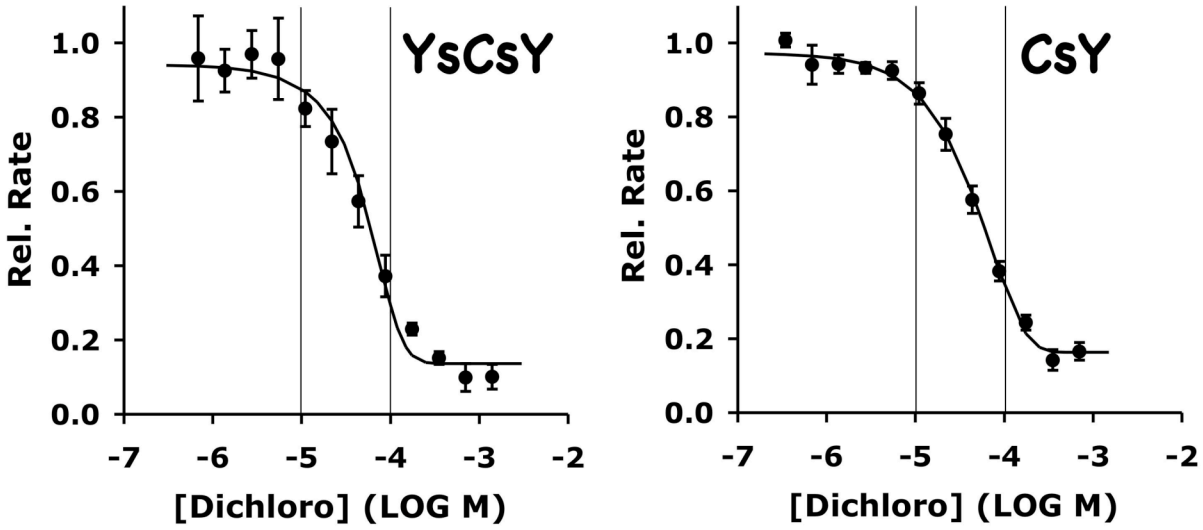


Supplementary Figure 1. SDS-PAGE analysis of BoNT/A-LC catalyzed cleavage of FRET substrates. Shown is an image of a Coomassie Blue-stained, 10% SDS-PAGE gel. Lane 1: CFP (325 aa); Lane 2: CFP-YFP; Lane 3: CsY; Lane 4: CsY after cleavage (345, 288 aa); Lane 5: CsYY; Lane 6: CsYY after cleavage (534, 345 aa); Lane 7: YsCsY; Lane 6: YsCsY after cleavage (345, 313, 288 aa).



Supplementary Figure 2. Inhibition of BoNT/A-LC catalyzed cleavage of YsCsY or CsY by GST-SNAP(141-206). Reactions were carried out at 30°C and at 10 µM of Zn^{2+} with 0, 5, 10, or 20 µM of GST-SNAP(141-206), 1 nM of BoNT/A-LC and 1, 1.5, 2 or 3 µM of YsCsY (left panel), or with 2 nM of BoNT/A-LC and 2, 3, 5, or 10 µM of CsY (right panel). The initial rates were determined from data recorded in filter mode at 528/20 nm and were analyzed by reciprocal plot for competitive inhibition. Kinetic parameters were obtained by fitting the data according to the equation:

$$\frac{1}{v} = \frac{1}{V_{\max}}\left(1 + \frac{K_m}{[S]}\left(1 + \frac{[I]}{K_i}\right)\right)$$



Supplementary Figure 3. Inhibition of BoNT/A-LC catalyzed cleavage of YsCsY and CsY by 2,4-dichlorocinnamic acid hydroxamate (DCCH). A solution of DCCH (7 mM) in DMSO was serially diluted in reaction buffer before addition of 3 μM of YsCsY (left panel) or CsY (right panel), followed by addition of 2 nM of BoNT/A-LC. Similar dilutions of DMSO without DCCH were made for the control reactions. Relative rates were calculated from the ratios of initial rate between corresponding wells with or without the inhibitor. The triplicate data were fitted according to a logistic function:

$y = \frac{a}{\left(1 - e^{b(x-c)}\right)} + d$, where *y* is the relative rate and *x* is the inhibitor concentration. IC_{50} values were calculated from the fitted curve at *y* = *0.5*.