Supporting Information: Self-Assembled Peptide Monolayers as Toxin Sensing Mechanism within Arrayed Microchannels

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1 Experimental

1.1 Channel fabrication

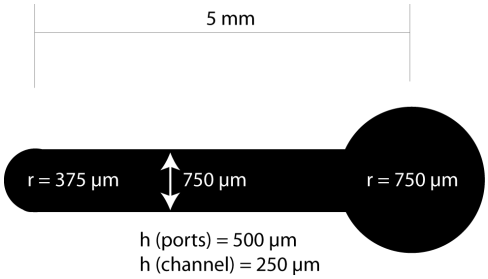

Figure 1: Microchannel dimensions

1.2 Recombinant SNAP-25

TOPO TA PCR cloning kit, Gateway pDest14 cloning kit, Gateway pDONOR 221 cloning kit, IPTG, NuPage SDS-PAGE gel system, S.N.A.P MINI prep kit and Probond protein purification system were purchased from Invitrogen. Oligers were purchased from IDT. Codon/plus BL21 competent cells were purchased from Stratagene.

Expression and purification of tagged partial SNAP-25. Positive recombinant pDest14SNAP25 DNA was transformed into codon/plus BL21 competent cells. One colony was inoculated into 2 mL LB medium containing 100 μg/mL ampicillin and shaken overnight at 37°C. 500 μL of the overnight culture was inoculated into 50 mL LB medium with 100 μg/mL ampicillin and shaken at 37°C until OD/550 reached between 0.5-0.6. IPTG was then added to a final concentration of 1 mM to induce the expression for 3 h. Cell pellets were collected by centrifugation at 5000 rpm for 15 min. Resulting protein was purified by using Probond protein purification system as described by manufacturer. Purified recombinant SNAP-25 was verified by SDS-PAGE.

1.3 SNAPtide® FRET assay

Lyophilized SNAPtide® (#521, FITC/DABCYL) was purchased from List Biological Laboratories (Campbell, CA) and immediately dissolved in DMSO to make a 2.5 mM stock solution. For all

FRET assays with BoNT/A, stock solution was diluted to 7.75 μM in the assay buffer (20 mM HEPES with 0.1% Tween-20, pH 8.0). Toxin incubation with the SNAPtide® took place for 3 h at 35°C.

1.4 Data processing

To remove experimental error from all data sets, the sample median was used as a robust estimator.[1] A sample median (T) was found for all intensity values within a particular data set from which a median of all absolute deviations (MAD) was determined as a scale estimator (S). Data points with a z score greater than 2.5 were removed from sample sets (equation 1):

$$z = \frac{x_i - T}{S} \tag{1}$$

2 Relevant data

2.1 BoNT/A

The BoNT/A holotoxin and light chain were incubated with the FRET SNAPtide® in both buffer and soup to verify toxin activity in the latter medium. As shown in 2, toxin activity was, in fact, heightened in soup ($^*P$ <0.01, $^{**}P$ <0.001). The same concentration of holotoxin (500 ng/mL) in buffer did not produce a distinguishable signal as compared to the respective control (NS). While previously undetectable using the SAM-based assay, 2 μg/mL ALC was detected using the SNAPtide®. The 20-fold greater activity of the 50 kDa light chain as compared to the 150 kDa holotoxin is expected with this FRET substrate.[2]

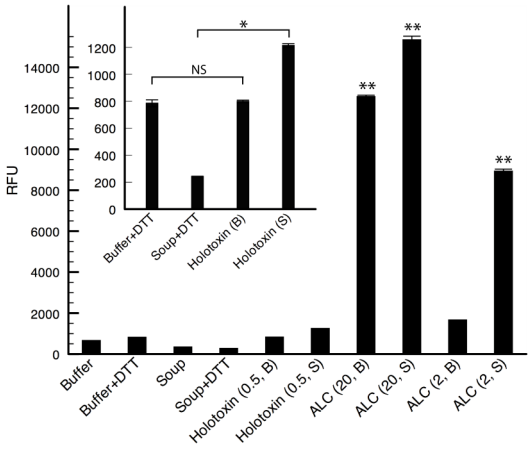

Figure 2: SNAPtide® shows increase in toxin activity in soup as compared to buffer for both the holotoxin (500 ng/mL) and ALC (2 and 20 μg/mL).

3 References

1. P. Rousseeuw, in *Handbook of Statistical Methods for Engineers and Scientists*, ed. H. Wadsworth, McGraw-Hill Publishing Company, New York, 1990, ch. Robust Estimation and Identifying Outliers, pp. 16.2-16.5.

2. T. Christian, N. Shine, L. Eaton and K. Crawford, List Biological Laboratories, Inc. Poster presented at 5th International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins, 2005.