Supplementary Information: Bead-Based Microfluidic Toxin Sensor Integrating Evaporative Signal Amplification

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1 Relevant data

1.1 BoNT/A

For our particular sensor, a significant signal as compared to background (BG) has been defined as:

$$\frac{signal - BG}{stdev_{BG}} > 3 \tag{1}$$

Using equation (1), all signals from toxin cleavage of *flu*-SNAP were $\geq$ 100X BG (data not shown).

All signals were compared to a series of blank values (n=12 for ALC, n=10 for holotoxin) to estimate the sensor's detection limits for each enzyme. Using equation 2, the smallest measurement x_L that could be detected with a 95% confidence level, where $\bar{x}_b$ and s_b are the blank measurement mean and standard deviation, respectively, and k is the confidence level (0.95):

$$x_L = \bar{x}_b + ks_b \tag{2}$$

Using equation 2 as a measure of signal reliability, experimental results indicate that the bead-based microfluidic sensor is capable of positively detecting low levels of ALC (20 pg/mL) (Table 1) and BoNT/A holotoxin (10 pg/mL) (Table 2). Initial sample sizes are given (n_i) as well as final sample sizes (n_f) reflecting discarded values due to high z scores.

Table 1: Normalized fluorescent signals for ALC

ALC (μg/mL)	Normalized signal	n_i/n_f
20	2.322	10/10
0.2	1.455	10/9
0.002	1.395	11/10
0.00002	1.123	9/8

1.2 BoNT/B

Experimental results indicate negligible cross-reactivity with BoNT/B holotoxin (1 μg/mL and 10 ng/mL) (Table 3). Initial sample sizes are given (n_i) as well as final sample sizes (n_f) reflecting discarded values due to high z scores.

Table 2: Normalized fluorescent signals for BoNT/A

BoNT/A (μg/mL)	Normalized signal	n_i/n_f
0.67	2.214	4/4
0.01	1.841	7/5
0.001	1.383	8/7
0.0001	1.271	8/6
0.00001	1.245	7/6

Table 3: Normalized fluorescent signals for BoNT/B

BoNT/B (μg/mL)	Normalized signal	n_i/n_f
1	0.680	3/3
0.01	0.681	3/3

2 Relevant images

2.1 COMSOL

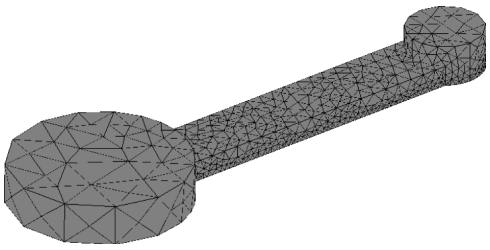

Figure 1: Mesh representation of COMSOL model used for traditional straight channel device.

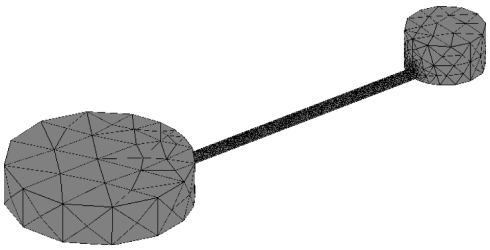

Figure 2: Mesh representation of COMSOL model used for the diffusion valve device.