Supporting Information

A Bacteriophage-Based Platform for Trace Detection of Proteases

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1. Phagemid Construction

DNA encoding the AGT reactive domain (amplified from pSNAP-tag®(T7) Vector, NEB N9174S), a BamHI restriction site, SNAP-25 (fragment corresponding to amino acids 141 – 206 of synaptosomal associated protein of 25kDa, amplified from Ultimate™ ORF Clone, Invitrogen, IOH9748), and a C-terminal glycine was assembled and inserted between *Sfi*I and *Ava* I restriction sites of pCGMT vector[1]. Phagemids coding for alternative substrates were constructed by substituting the SNAP-25 coding fragment with a Syb fragment (amino acids 33 – 94 of synaptobrevin 2, amplified from ATCC**,** MGC-45137) or a sequence coding for MAPKK (mitogen-activated protein kinase kinases) derived peptide (RRKKVYPYPMEATIA) using the *BamH*I and *Ava* I restriction sites.

2. Phage Preparation

The phagemids were transformed into *E. coli* XL1 blue. The cells were grown in 200 ml of 2 × YT media containing tetracycline and carbenicillin at 37 °C until the OD_{600} reached 0.6. The culture was inoculated with 500 µl of 5×10^{12} cfu/ml of helper phage (Strategene, 200251) and incubated at 37 °C for 1 h while gently rocking. The infected cells were collected by centifugation and resuspended in 200 ml of fresh 2 × YT with carbenicillin, kanamycin, and isopropyl β-D-thiogalactoside. The resuspended culture was grown at 30 °C overnight, spun down, and phage isolated from the supernatant by PEG precipitation (mixing supernatant with ¼ of the volume of 30% PEG 8000 and 15% NaCl). The phage was stored in PBS containing 1 mM DTT at –80 °C.

3. Phage Attachment

The phages displaying the AGT reactive tag and toxin substrates were attached to SNAP-Capture magnetic beads (NEB, S9145S) according to the manufacturer's manual. A ratio of 10 µl of beads per 1 ml of phage solution at a concentration of 1×10^{12} cfu/ml was used. The phage in 2 mM DTT and beads were mixed on a rotator at room temperature for 1 h. The beads were then washed to remove unbound and non-specifically bound phage. The beads were washed with 1 mM DTT in PBS, followed by two washes with 1 mM DTT and 0.5% Tween-20 in PBS. The beads were incubated at 55 °C for 20 min, after which the liquid was discarded. The beads were washed three more times with the same buffer, once with 1 mM DTT in PBS, and once with the appropriate cleavage buffer before being used in the cleavage reaction.

4. Phage Cleavage

The beads were resuspended in the cleavage buffer and distributed into wells of a flat-bottom untreated 96-well plate (Corning, #3370). Dilutions of the toxin in cleavage buffer were added to the beads and incubated for 3 h at room temperature. After 3 h, a magnetic particle separator was used to separate the beads, and the supernatant was collected to quantify the phage.

5. Phage Quantification

Colony count titer

The post-cleavage phage was initially diluted 1/10 into PBS, followed by 6 fold serial dilutions into PBS. *E. coli* XL1 blue grown to OD_{600} ~0.6 in 2 × YT with tetracycline were added to the phage dilutions in a ratio of 1:1 and incubated with the phage for 45 min at 37 °C without agitation. The cells were then plated on agar plates containing tetracycline and carbenicillin to select for infected bacteria and incubated at 37 °C overnight, followed by colony counting to quantify the phage.

qPCR

Platinum SYBR Green qPCR SuperMix-UDG kit (Intvitrogen 11733038) was used with phage dilutions as the template according to the manufacturer's procedure. 5 μL of initial phage dilution into water (1:11) was added into each reaction (40 μL total volume). The cycling program went as follows: 95 °C for 2 min; 45 cycles of: 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s.

The following primers were used:

SNAP-25 phage quantification

Forward primer: 5′- GAGCCCGAGAAAATGAAATG

Reverse primer: 5′-GCGATTCTGGGTGTCAATCT;

Syb phage quantification

Forward primer: 5′- ACATCATGAGGGTGAACGTG

Reverse primer: 5′- GTATTTGCGCTTGAGCTTGG;

MAPKK phage quantification

Forward primers: 5′- CGGTTATGAAGGTGGTCTGG

Reverse primer: 5′- GCTTCCATCGGATACGGATA.

Reaction Ct values, defined as the number of cycles it takes for the fluorescence to reach a defined threshold, were converted into phage concentrations using a calibration curve obtained from phage dilutions of known concentration.

6. References

(1) Gao, C.; Lin, C. H.; Lo, C. H.; Mao, S.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Proc Natl Acad Sci U S A* **1997**, *94*, 11777.

Days stored	Storage conditions	LOD [pM]
0	+ 4 °C	10
2	+ 4 °C	12
7	+ 4 °C	12
14	+ 4 °C	7
14	– 80 °C	8

Table S1. Limits of detection (LODs) of BoNT/A light chain using SNAP25-phage pre-attached to magnetic beads. Cleavage reactions were carried out at 25 °C for 3 h in HZTD buffer (40 mM HEPES pH 7.4, 20 μM $ZnCl_2$, 0.1% Tween 20, 1 mM DTT). The phage was attached to the beads as described and stored at +4 °C or -80 °C in PBS with 1 mM DTT for given periods of time prior to use ("0" indicates that the phage was used immediately).

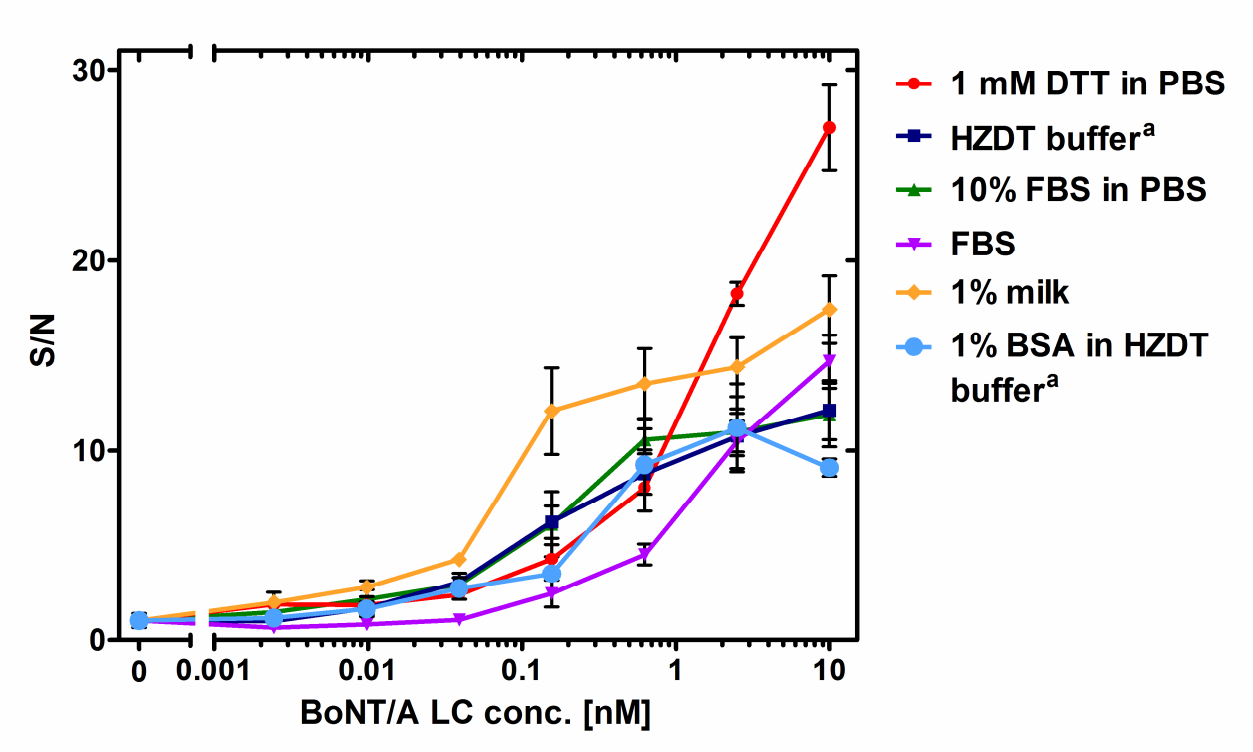

Figure S1. Relative signal intensities in different cleavage buffers and various BoNT/A light chain (LC) concentrations. (a) HZTD buffer: 40 mM HEPES pH 7.4, 20 μM $ZnCl_2$, 0.1% Tween 20, 1 mM DTT.

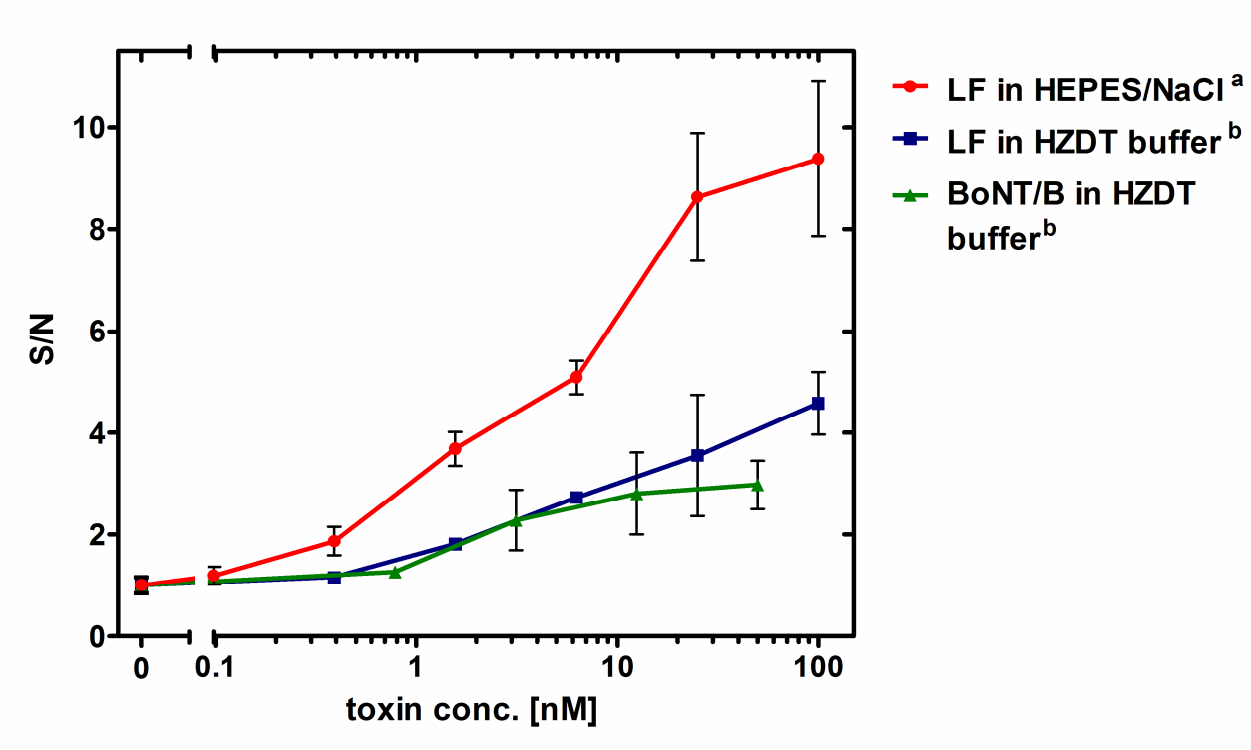

Figure S2. Relative intensities of assay response for MAKKP phage treated with LF and Syb phage treated with BoNT/B. (a) 40 mM HEPES pH 7.4 with 100 mM NaCl; (b) HZTD buffer: 40 mM HEPES pH 7.4, 20 μM $ZnCl_2$, 0.1% Tween 20, 1 mM DTT.

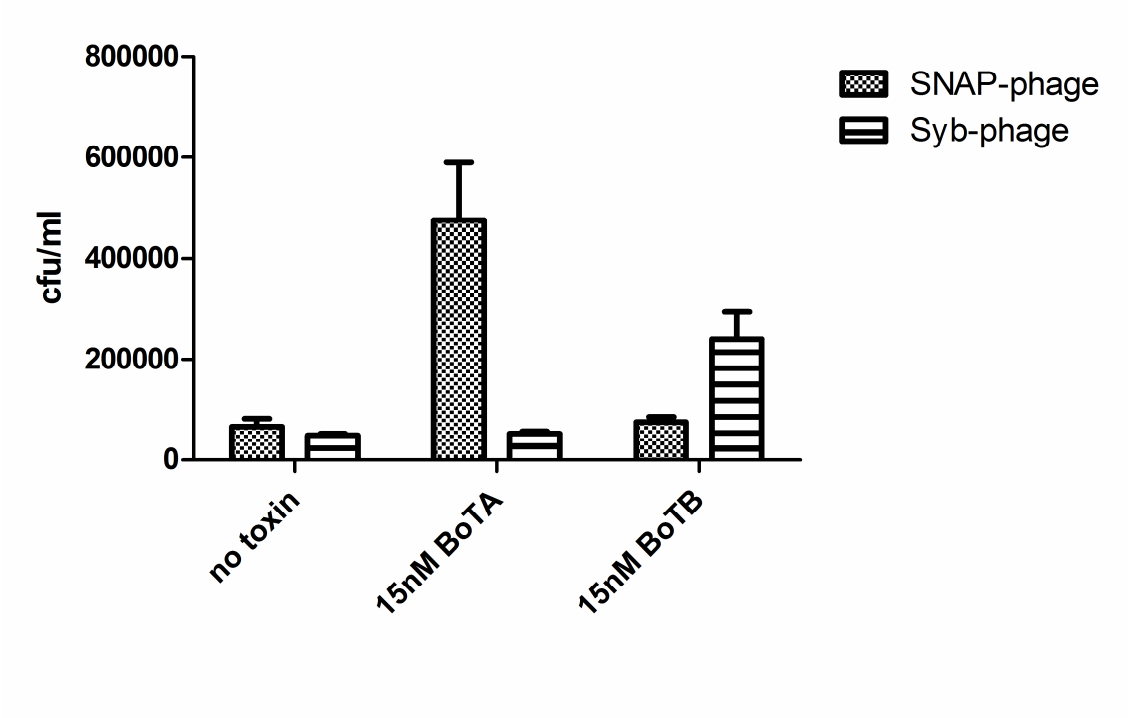

Figure S3. SNAP-phage and Syb-phage were treated in separate reactions with either BoNT/A or /B. Signals from substrates treated with non-native toxin are not different from those of untreated substrates.

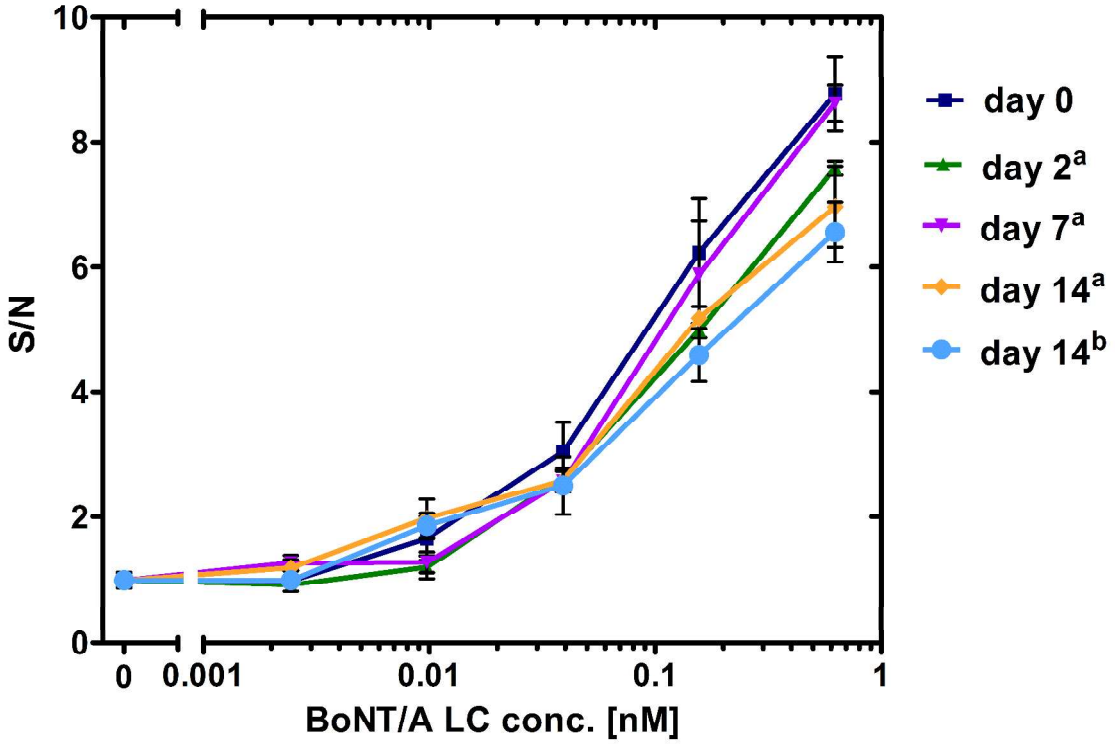

Figure S4. Relative signal intensities of assay performed with BoNT/A light chain and SNAP25-phage pre-attached to magnetic beads. Cleavage reactions were carried out at 25 °C for 3h in HZTD buffer (40 mM HEPES pH 7.4, 20 μM $ZnCl_2$, 0.1% Tween 20, 1 mM DTT). The phage was attached to the beads as described and stored at (a) +4 °C or (b) -80 °C in PBS with 1 mM DTT for given periods of time prior to use ("day 0" indicates that the phage was used immediately).

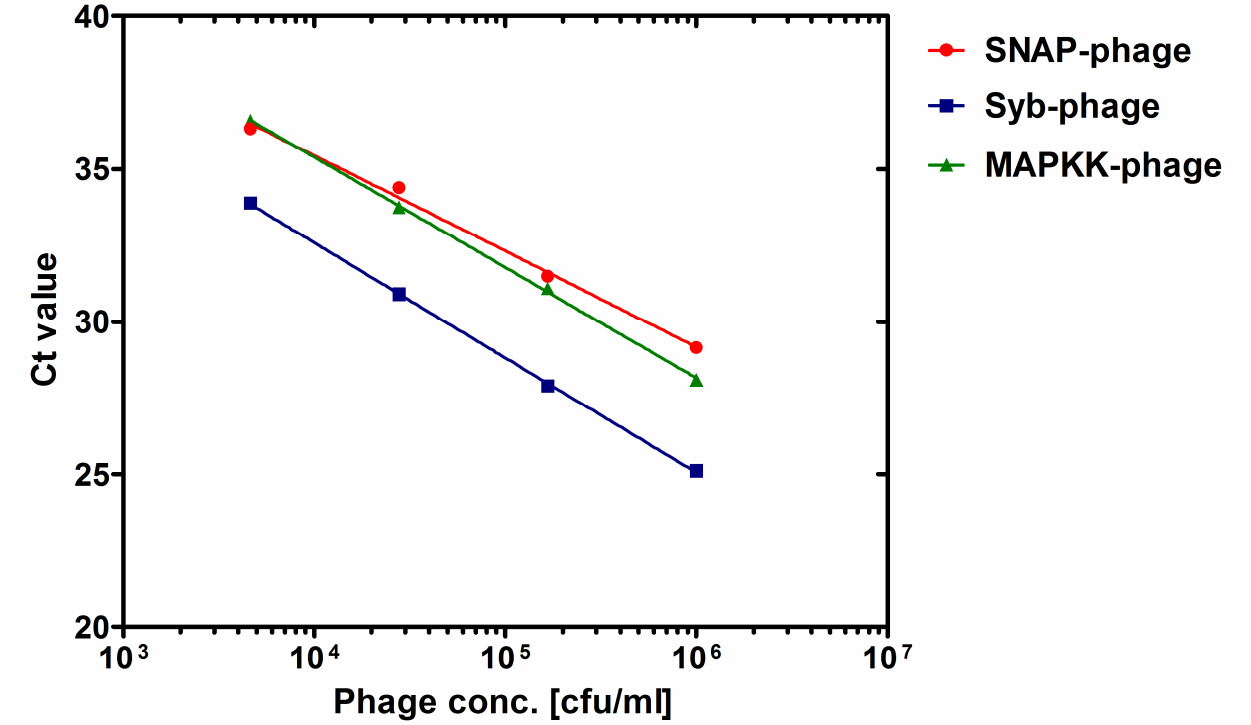

Figure S5. qPCR calibration curves for three substrate phages. Ct value corresponds to the cycle number when the fluorescence crosses the fluorescence threshold.