Supporting Information

Nanopore sensing of botulinum toxin type B by discriminating an enzymatically cleaved peptide from a synaptic protein synaptobrevin 2 derivative

Yong Wang[1†*], Vedrana Montana[2,3†] Vladimir Grubišić[2], Randy F. Stout, Jr. [2,4],

Vladimir Parpura[2,3*], Li-Qun Gu[1*]

[1]Department of Bioengineering and Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65211, USA

[2]Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL 35294, USA;

[3]Department of Biotechnology, University of Rijeka, 51000 Rijeka, Croatia, EU

[4]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA;

[†] These authors contributed equally to this work

*Corresponding authors:

Dr. Yong Wang (wayong@missouri.edu), Dr. Vladimir Parpura (vlad@uab.edu) and Dr. Li-Qun Gu (gul@missouri.edu)

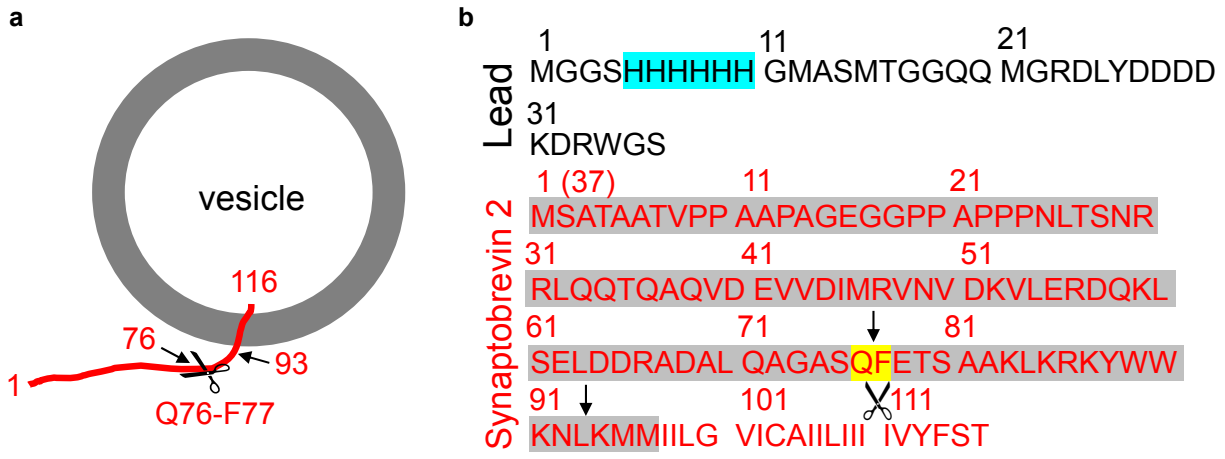

Figure S1. Design of a recombinant protein substrate for BoNT-B. **a)** The vesicular exocytotic protein synaptobrevin 2 (red), made of 116 amino acids (aa), spans the vesicular membrane. Its N-terminus is located in the cytosol, while the C-terminus is located intravesicularly. This protein is a target for proteolytic activity of BoNT-B which cleaves (indicated by scissors) the Q76-F77 peptide bond. The recombinant proteins we used, Lp-Sb2(1-93) and Lp-Sb2(1-76), contain truncated cytoplasmic domain (arrows), either aa 1-93 or aa 1-76, respectively, appended to C-terminus of the lead peptide (Lp). Drawing is not to scale. **b)** Protein sequences: the lead peptide (black letters) and synaptobrevin 2 (red letters; GeneBank accession number BC074003). Numbers denote the position of amino acids in the sequence. Six histidine residues in the lead peptide are highlighted in turquoise. The presence of consecutive histidines in the protein sequence eases the purification of the recombinant Sb2 derivatives. Arrows indicate truncation sites in Sb2 cytoplasmic domain (shaded in gray). Note that M1 of truncated Sb2 becomes M37 (indicated parenthetically) when fused to the lead peptide. Scissors indicate the proteolytic cleavage site by BoNT-B, which targets the peptide bond between amino acids highlighted in yellow.

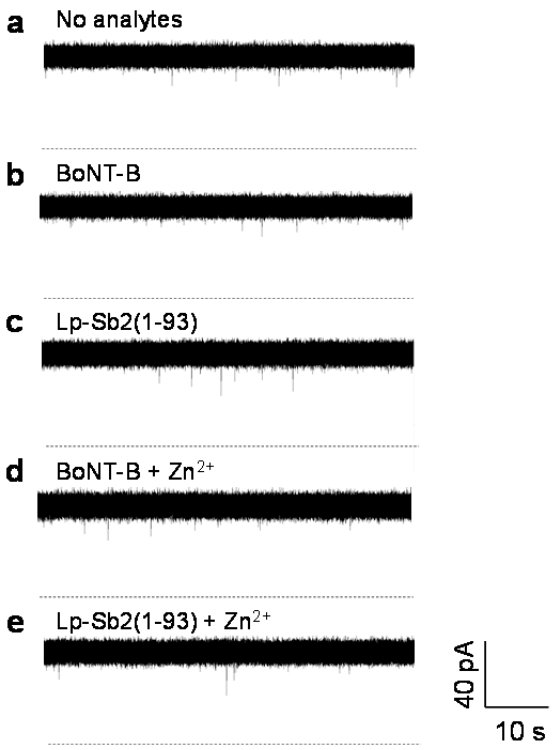

Figure S2. Control experiments for detection of BoNT-B activity using the aerolysin pore. **a-e)** Current traces for a) the empty pore without analytes, b) BoNT-B alone, c) Lp-Sb2(1-93) alone, d) BoNT-B with Zn^{2+}, and e) Lp-Sb2(1-93) with Zn^{2+}. These results demonstrate that the reactants do not substantially interact with the aerolysin pore.

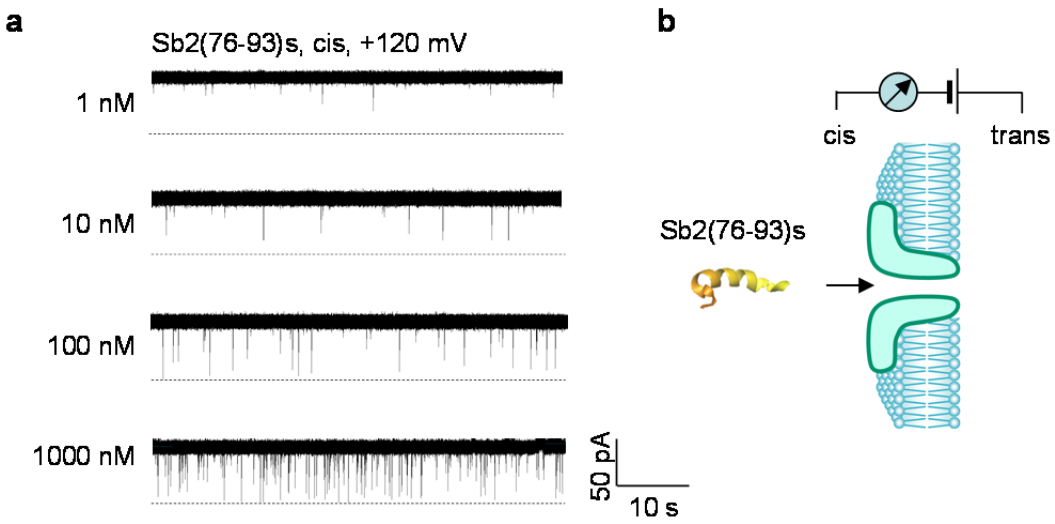

Figure S3. The transient aerolysin pore blocks generated by the synthetic peptide Sb2(76-93)s are concentration-dependent. **a)** The current traces across the aerolysin pore in the presence of Sb2(76-93)s (1 nM, 10 nM, 100 nM and 1000 nM) at +120 mV holding potential across the pore. **b)** Drawing of the experimental set-up. Arrow indicates the side of action by the peptide, i.e. direction of the pore block.

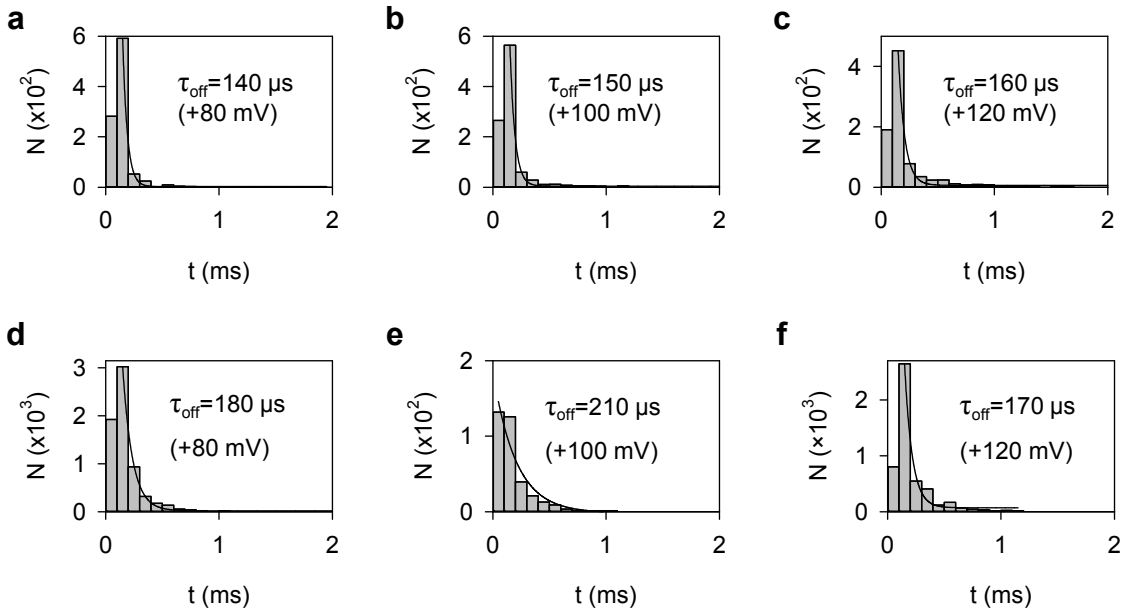

Figure S4. Time constant histograms for Sb2(77-93)d or Sb2(76-93)s aerolysin pore current blocks. **a-c,** Sb2(77-93)d, the C-terminal fragment/digest after Lp-Sb2(1-93) cleavage by BoNT-B/Zn^{2+} at voltages +80, +100 and +120 mV; **d-f)** Synthetic Sb2(76-93)s peptide at the same set potentials as in a-c. N, the number of events. Note that panels c and f here are borrowed from Fig 2 h and j, respectively, for comparison.

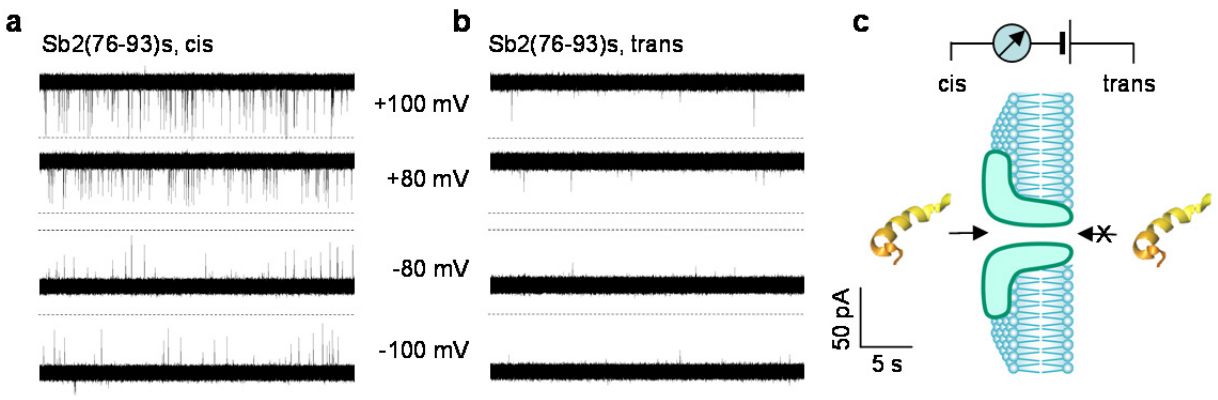

Figure S5. Detection directionality and voltage dependence of Sb2(76-93)s in the aerolysin nanopore assay. **a, b)** The nanopore current traces in the presence of synthetic Sb2(76-93)s peptide (1 μM) either in cis (a) or trans (b) side of the chamber at holding potentials ±80 mV and ±100 mV; **c)** Drawing shows a detection layout. Arrow indicates *cis*, but not *trans* (crossed arrow), specificity of the pore block by the peptide.

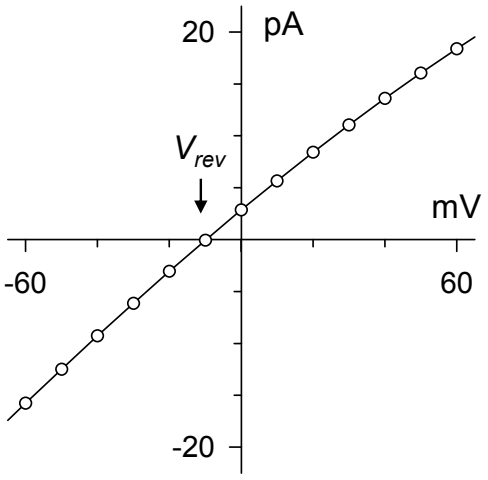

Figure S6. Current-voltage (I-V) curve for the aerolysin pore recorded in asymmetric 1 M/0.2 M (cis/trans) KCl solutions. The reversal potential V_{rev} was -10 mV. The calculated cation/anion permeability ratio P^+/P^-= 0.4 suggests that aerolysin forms a moderately anion selective pore.

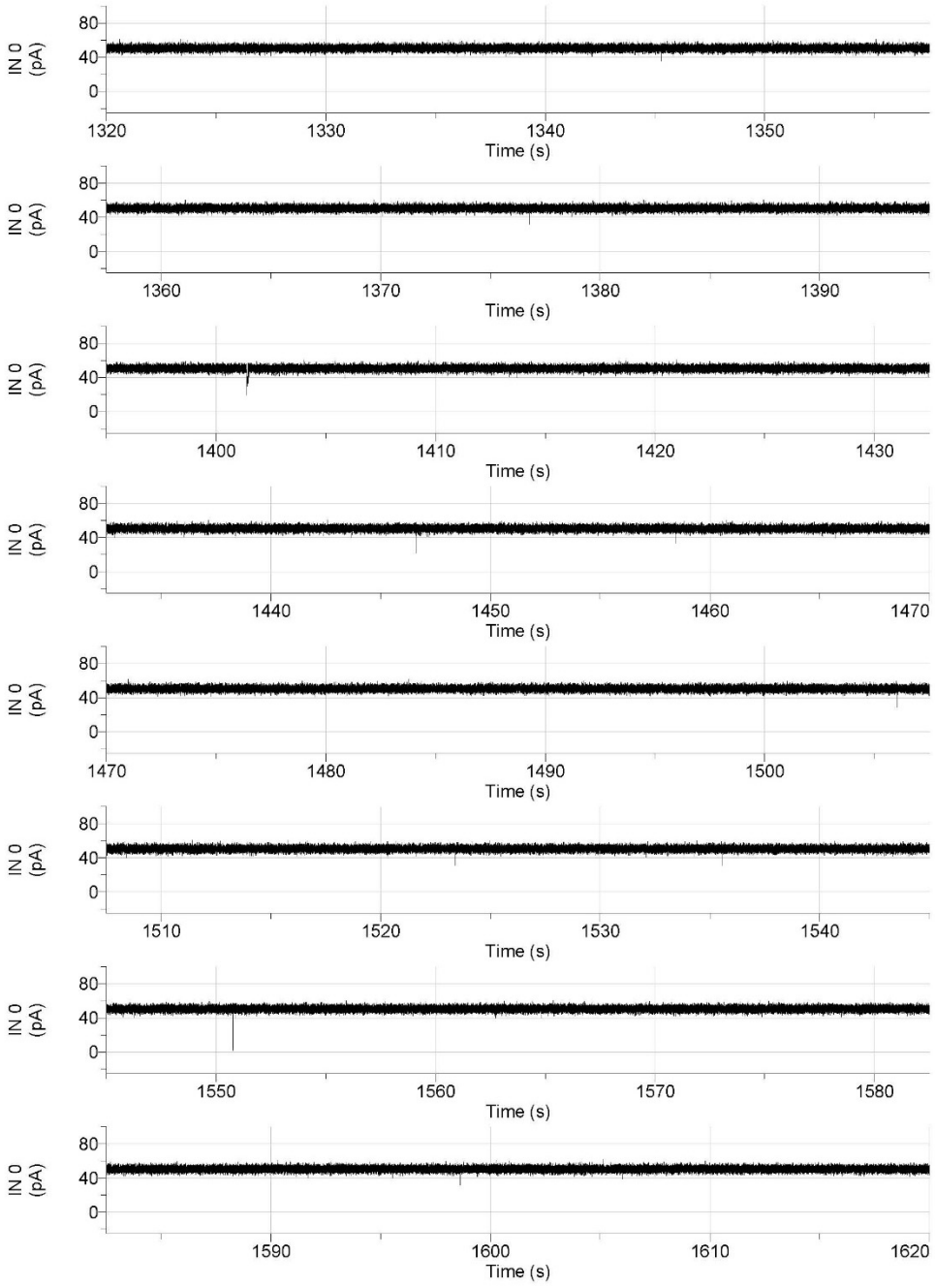

Figure S7. Long current trace (5 minutes) obtained with the empty aerolysin pore at +120 mV applied from the trans side (the cis side was grounded). Note the absence of blocking currents.

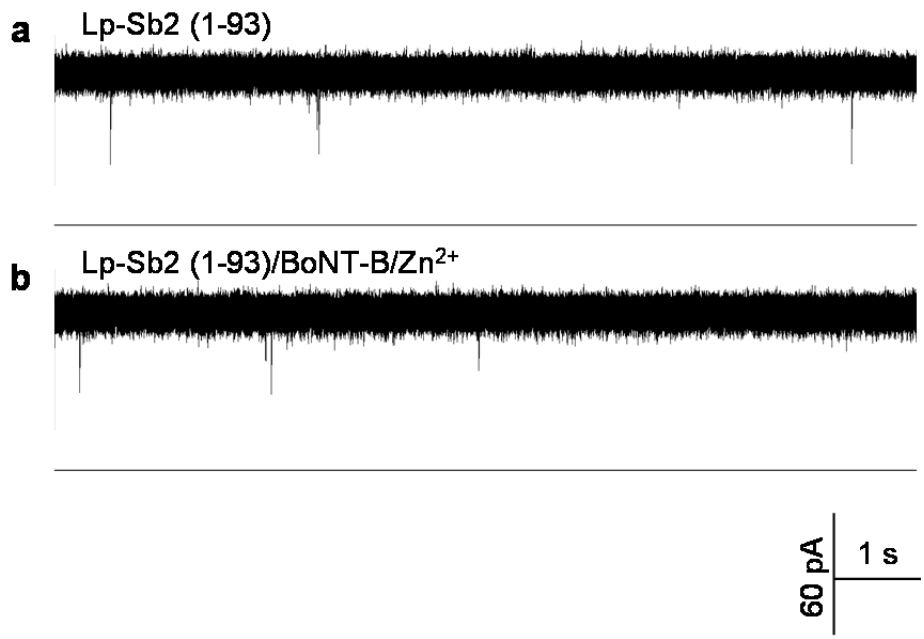

Figure S8. Inability to detect BoNT-B activity by using the α-hemolysin pore. Current traces were recorded at +160 mV using 1 M/0.2 M (cis/trans) KCl asymmetric solutions buffered with 10 mM Tris (pH7.5). Reactants, Lp-Sb2(1-93) alone or with BoNT-B/Zn^{2+} (after 3-hour pre-incubation), were presented at the cis side. **a-b)** Current traces in the presence of (a) 20 nM Lp-Sb2(1-93) alone or in (b) the mixture with 500 pM BoNT-B/ 100 nM Zn^{2+}. The α-hemolysin pore does not offer peptide/protein specificity suitable for detection of the BoNT-B itself or its cleavage product(s).

Table S1. In silico protein/peptide analysis

Analysis	Recombinant Lp-Sb2(1-93)	Recomb; N-frag Lp-Sb2(1-76)/d	Recomb; C-frag Sb2(77-93)d	Synthetic Sb2(76-93)s
Length (aa)	129	112	17	18
Molecular Weight ($g \cdot mol^{-1}$)	14156.88	12005.52	2169.38	2297.51
1 μg=	70.637 pmol	83.295 pmol	460.961 pmol	435.254 pmol
*Molar Extinction Coefficient, A[280] ($M^{-1} \cdot cm^{-1}$)	19630	6970	12660	12660
*1 A[280] corr. to	0.72 mg/ml	1.72 mg/ml	0.17 mg/ml	0.18 mg/ml
*A[280] of 1 mg/ml	1.39 AU	0.58 AU	5.84 AU	5.51 AU
Isoelectric Point	6.04	5.12	10.17	10.17
Charge at pH 7 (*e*)	-2.70	-6.70	3.76	3.76

Numbers in parentheses associated with proteins/peptides indicate amino acids (aa). Lp-Sb2(1-76) here is either a recombinant protein or is obtained as the N-terminal digest [/d; referred to in the text as Lp-Sb2(1-76)d] after Lp-Sb2(1-93) cleavage by BoNT-B/Zn^{2+}. The C-terminal fragment of the latter cleavage is Sb2(77-93)d, which is synthetically emulated by Sb2(76-93)s. Abbreviations: frag, fragment; Lp, 36-aa lead sequence containing a six histidine tag for purification of recombinant (Recomb) proteins; Sb2, synaptobrevin2; A[280], absorbance at 280 nm in water; 1 A[280] corr. to, absorbance of 1 corresponds to listed mass concentration. * The path length is 1 cm.

Table S2. Biochemical analysis of the sterile filtered fetal bovine serum, as per the manufacturer (HyClone™, Thermo Fisher Scientific Inc., Waltham, MA).

HyClone®

Product: **FETAL BOVINE SERUM**
Central American Sourced,
Processed in the USA
Catalog#: **SH30910**
Lot#: **AWB98615**
Filtration: **0.1 µm Sterile Filtered**

Manufacture Date: **FEB/2011**
Expiration Date: **FEB/2016**
Total Batch Volume: **1935.834 L**

BIOCHEMICAL ASSAY

Test	Lot Value	
PROTEINS/OTHER		
Albumin	1.9	gm/dL
Alkaline Phosphatase	277	mU/mL
Blood Urea Nitrogen	16	mg/dL
Creatinine	2.93	mg/dL
Gamma Globulin Electrophoresis	2.49	% tp
IgG	0.104	mg/mL
Glucose	42	mg/dL
Glutamic Oxaloacetic Transaminase (SGOT)	66	mU/mL
Glutamic Pyruvic Transaminase (SGPT)	34	mg/mL
Lactate Dehydrogenase	1939	mU/mL
Osmolality	310	mOsm/Kg
pH	7.02	
Total Bilirubin	0.5	mg/dL
Total Protein	3.5	gm/dL
TRACE METALS/IRON		
Calcium	13.2	mg/dL
Chloride	103	mEq/L
Inorganic Phosphorus	8.4	mg/dL
Iron	188	ug/dL
Percent Saturation (Iron)	74	
Sodium	140	mmol/L
Potassium	10.4	mmol/L
Total Iron Binding Capacity (TIBC)	254	ug/dL