

Supplementary Materials: Engineering an effective human SNAP-23 cleaving botulinum neurotoxin A variant

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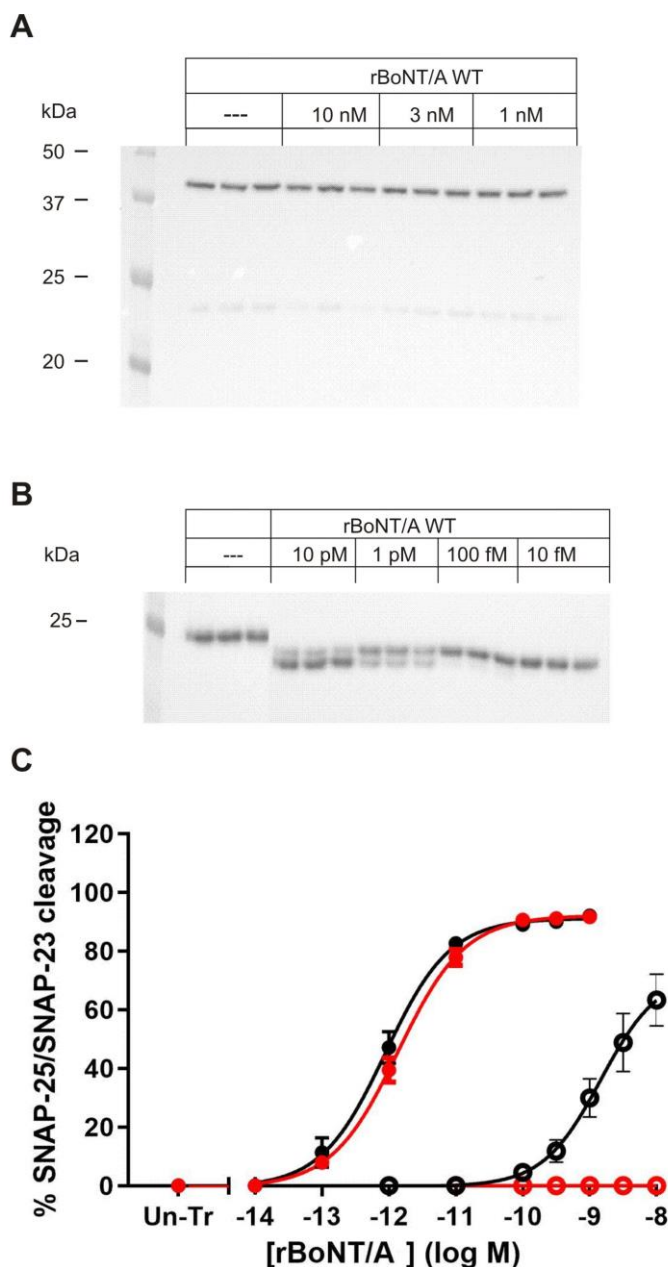


Figure S1. Activity of purified recombinant BoNT/A (E148Y, K166F, S254A, G305D) and BoNT/A wild-type in cortical neurons expressing hSNAP-23-GFP. (A, B) Three analyses conducted like those shown in Figure 2A and B were quantified. (C) Quantification depicted as a function of BoNT concentration. SNARE cleavage was determined for wild-type (red) and quadruple mutant (black) with SNAP-25 cleavage shown by filled circles and SNAP-23 shown by open circles. Calculated EC₅₀ values are shown in Table S5.

Table S1. Amino acid sequence of LC/A mutants of the Pro-182 binding pocket obtained by yeast-based screening.

						[hSNAP-23] 20 μ M			
						[LC/A] 6 μ M	10 μ M	% increase	
						% cleavage		vs wt	
	wild-type	E-148	T-307	A-308	Y-312	6.9	10.2		
	1	G	V	N	S				
	2	N	H	D	I				
	3	S	F	Y	E				
	4	M	M	T	L				
	5	H	W	V	K				
	6	I	T	I	I				
	7	M	W	D	V				
	8	H	N	N	L				
	9	E	V	N	K				
	10	L	L	S	S				
mutants obtained by screening	11	I	Y	H	L				
	12	N	I	P	V	19.0	n.a.	173	
	13	Y	F	N	L	14.0	n.a.	101	
	14	V	F	G	K				
	15	G	I	L	T				
	16	C	A	I	K				
	17	V	V	L	G				
	18	F	I	I	P				
	19	F	R	M	V				
	20	N	F	Y	V				
	21	Y	L	T	M	n.a.	22.8	123	
	22	Y	Y	H	S				
	23	V	C	T	I				
	24	N	F	I	S				
	consensus	13/24	19/24	13/24	13/24				
		arithmetical		actual					
	hydrophobic	25	42	58	33	50			
	hydrophobic	15	13	21	21	4			
	G, A, C	15	13	8	4	4	frequency (%)		
	P	5	0	0	4	4			
	N, Q, S	15	21	4	21	17			
	acidic	10	4	0	8	4			
	basic	15	8	8	8	17			
	12a	N					8.2	n.a.	18
	13a	Y					57.5	n.a.	728
mutants subcloned	12b		I	P	V		12.1	n.a.	74
	13b		F	N	L		10.4	n.a.	49
	21 a		L	T	M		n.a.	10.0	-2
	21.2		Y	L	I	M		n.a.	14.1

Table S2. Amino acid sequence of LC/A mutants of the Lys-206 binding pocket obtained by yeast-based screening.

wild-type	L-256	V-258	L-367	F-369		
1	W	H	T	T		
2	V	H	N	G		
3	S	D	V	P		
4	R	C	S	R		
5	P	T	L	W		
6	R	V	G	Y	GAL promoter	
7	V	A	S	A		
8	I	A	G	W		
9	D	S	A	S		
10	D	A	L	G		
11	E	K	S	R		CYC1 promoter
12	R	G	A	F		
13	V	E	V	G		
14	R	E	L	L		
15	R	E	G	L		
16	W	V	L	R		
17	V	M	L	G		
18	I	H	A	S		
19	L	G	S	L		
20	I	D	A	Y		
consensus	8 + 2/20	6 + 1/20	7 + 4/20			
		5/20	7/20			
	arithmetical	actual				
hydrophobic	25	40	15	35	20	frequency (%)
hydrophobic	15	10	5	5	25	
G, A, C	15	0	30	35	25	
P	5	5	0	0	5	
N, Q, S	15	5	5	25	10	
acidic	10	15	25	0	0	
basic	15	25	20	0	15	

Table S3. Activity of various LC/A mutants on SNAP-25 in standard in vitro cleavage assays.

LC/A	% Cleavage ^a	SD	No. of Experiments	Relative Activity
wild-type	60.0	10.4	20	1
E148Y	29.1	4.8	12	0.48
K166F	62.1	7.7	4	1.03
S254A	96.2	1.0	4	1.59
E148Y/G305D	37.7	2.9	4	0.62
E148Y/K166F	27.8	10.1	4	0.46
K166F/G305D	66.2	14.2	10	1.09
E148Y/K166F/G305D	60.1	3.9	8	0.99

^a Assays were conducted as specified in Materials and Methods using a final concentration of 0.5 nM of LC/A.

Table S4. EC₅₀ values for SNAP-25 and hSNAP-23 cleavage by BoNT/A quadruple (QM) and BoNT/A wild-type (WT) in cortical neurons expressing hSNAP-23-mCherry. Data are mean ± SEM of n = 4–5 independent experiments performed in triplicate. Non-parametric t-test revealed no statistical significant difference between SNAP-25 cleavage by recombinant BoNT/A wild-type and SNAP-25 cleavage by recombinant BoNT/A-quadruple mutant (p = 0.06).

Experiment	SNAP-25		SNAP-25		hSNAP-23	
	WT		QM		QM	
	logEC ₅₀	pM	logEC ₅₀	pM	logEC ₅₀	pM
NBK68-37 ^a	-11.76	1.74	-11.89	1.29	-8.11	7762.5
NBK68-240 ^a	-11.82	1.51	-11.57	2.69	-9.92	120.23
NBK68-260 ^a	-12.02	0.955	-11.34	4.57	-9.28	524.8
NBK83-41	-11.75	1.78	-11.75	1.78	-9.04	912.0
NBK83-111	-11.71	1.95	-11.61	2.45	-9.31	489.8
mean ± s.e. mean	-11.81 ± 0.05	1.59 ± 0.17	-11.57 ± 0.09	2.87 ± 0.60	-9.39 ± 0.19	511.7 ± 161.80

Values obtained in experiment NBK68-37 were disregarded when calculating average values. Values are outliers due to use of a different batch of Lentivirus. ^aNBK numbers refer to experiments with QM

Table S5. EC₅₀ values for SNAP-25 and hSNAP-23 cleavage by BoNT/A quadruple (QM) and BoNT/A wild-type (WT) in cortical neurons expressing hSNAP-23-GFP. Data are mean ± SEM of n = 3–5 independent experiments performed in triplicate. Non-parametric t-test revealed no statistical significant difference between SNAP-25 cleavage by recombinant BoNT/A wild-type and SNAP-25 cleavage by recombinant BoNT/A-quadruple mutant (p = 0.07)

Experiment	SNAP-25				hSNAP-23	
	WT		QM		QM	
	logEC ₅₀	pM	logEC ₅₀	pM	logEC ₅₀	pM
NBK83-198	-11.76	1.74	-12.1	0.79	-8.95	1120
NBK83-235	-11.82	1.51	-11.84	1.45	-8.87	1350
NBK83-258	-12.02	0.955	-12.31	0.49	-8.52	3020
NBK83-41	-11.75	1.78				
NBK83-111	-11.71	1.95				
mean ± s.e. mean	-11.81 ± 0.05	1.59 ± 0.17	-12.08 ± 0.14	0.91 ± 0.28	-8.78 ± 0.13	1830 ± 598.70