# Structural characterisation of the catalytic domain of botulinum neurotoxin X - high activity and unique substrate specificity

Geoffrey Masuyer, Sicai Zhang, Sulyman Barkho, Yi Shen, Linda Henriksson, Sara Kosenina, Min Dong and Pål Stenmark

#### Supplementary information:

BoNT/X	1 1	TT	→ <u>3</u> 0	20 <u>40</u>	<b>→</b> TT <b>&gt;</b> 50	000 60	<b>TT</b> 70
BoNT/B BoNT/B BoNT/D BoNT/F BoNT/G TeNT	MKLEINKEN . PVTINNEN MTWPVKDEN MPVAINSEN . PVNIKXEN . PITINNER	YNDPIDGINV YNDPIDNNNI YSDPVNDNDI YSDPVNDNDI YNDPVNDDTI YNDPINNDDI YSDPVNNDTI	TMRPPRHSDKI MMEPPFAR. YLRIPQNK. YMQIPYEE. MMEPFNDP. MMEPFVCK.	INKGKGP <mark>FKAFQ</mark> GTGRYYKAFK LITTPVKAFM KSKKYYKAFE GPGTYYKAFR GLDIYYKAFK	VIKNIWIVPERY ITDRIWIIPERY ITQNIWVIPERF IMRNVWIIPERN IIDRIWIVPERF ITDRIWIVPERY	NFTNNTNDLNIP TFGYKPEDFNKS SSDTNPSLSKPP TIGTNPSDFDPP TYGFQPDQFNAS EFGTKPEDFNPP	SEPI.MEADATYNP SGIFNRDVCEYYDP RPT.SKYQSYYDP ASLK.NGSSAYYDP TGVFSKDVYEYYDP SSLI.EGASEYYDP
BoNT/X	80	00000000000 90 10		120	<b>→</b> TT <b></b>	▶ TT —	→TT> 140 150
BoNT/B BoNT/B BoNT/D BoNT/F BoNT/G TeNT	NYLNTPSEK DYINTNDKK SYISTDEOK NYLTTDAEK TYIKTDAEK NYIRTDSDK	DEFIQGVIKVJ NIFLQTMIKLJ DTFLKGIIKLJ DRYLKTTIKLJ DRYLKTTIKLJ DRFLQTMVKLJ	ERIKSKPEGEH NRIKSKPIGEH KRINERDIGK KRINSNPAGK NRINSKPSGOF NRIKNNVAGEJ	UTELTSSSIPL ULEMIINGIPY UINYLVVGSPF ULQEISYAKPY ULDMIVDAIPY ULDKIINAIPY	PLVSNGAL LGDRRVPLEEFN MGDSSTPEDTFD LGNDHTPIDEFS LGNASTPPDKFA LGNSYSLLDKFD	TLSDNETI TNIASVTVNKLI FTRHTTNIAVEK PVTRTTSVNIKI ANVANVSINKKI TNSNSVSFNLLE	AYQENNNIVSNLQA SNPGEVERKKGIFA FENGSWKVTNIITP STNVESSMLL IQPGAEDQIKGLMT QDPSGATTKSAMLT
BoNT/X	<b>-</b>	TT		180	▶ <b>→</b>	→ TT 200 21	o 220
BoNT/X BoNT/B BoNT/D BoNT/F BoNT/G TeNT	NLVIYGPGP NLIIFGPGP SVLIFGP <b>I</b> P NLLVLC <mark>A</mark> GP NLIIFGPGP NLIIFGPGP	DIANNATYGI VLNENETIDIC NILDYTAS.L DIFESCCYPVI VLSDNFTDSM VLNKNEVRGI	ZSTI GIQNHFJ FLQGQQSNI RKLIDPDVVYDI IMNGHSI VLRV.DNKNYFI	ISNCECTLSEV ASRECTCCIMOM SFECTCTLSIL SNYCTCSINIV ISECTCARMMI CRDCTCSIMOM	SFSPFYLKPFDE KFCPEYVSVFNN KVAPEFLLTFSD TFSPEYEYTFND RFCPSCLNVFNN AFCPEYVPTFDN	SYGNYRSLVNIV VQENKGAS VTSNQSSA ISGGHNSS VQENKDTS VIENITSL	NKFVKREFAPDPAS .IFNRRGYFSDPAL .VLGKSIFCMDPVI TESFIADPAI .IFSRRAYFADPAL .TIGKSKYFQDPAL
BoNT/X	20000000 230	00000 T' 240	<b>· →→</b>	→ TT→2 260	20000 2002 270	00000000 280 2	00000000000 0 90 300
BoNT/X BoNT/B BoNT/D BoNT/F BoNT/G TeNT	TIMHEIVHV IIMHEIIHV AIMHEITHS SIAHEIIHA TIMHEIIHV LIMHEIIHV	THN <mark>LYGI</mark> SNRI LHGLYGIKVDI LHQLYGINIP: LHGLYGARGV LHGLYGARGV LHGLYGIKISI LHGLYGMQVS:	YFYYNFDTG.KI DL.PIVPNE.KI DKRIRPQVSEC YEETIEVK.QA NL.PITPNT.KI SH.EIIPSK.QA	IETSRQQNSLIF KFFMQSTDAIQA SFFSQDGPNVQF APLMIAEKPIRL EFFMQHSDPVQA IIYMQHTYPISA	EELLTFGGIDSK EELYTFGGQDPS EELYTFGGLDVE EEFLTFGGODLN EELYTFGGHDPS EELFTFGGQDAN	AISSLIIKKIIE IITPSTDKSIYD IIPQIERSQLRE IITSAMKEKIYN VISPSTDMNIYN LISIDIKNDLYE	TAKNNYTTLISERI KVLQNFRGI.VDRI KALGHYKDI.AKRI NLLANYEKI.ATRI KALQNFQDI.ANRI KTLNDYKAI.ANKI
BoNT/X	22	ورور ، 310	·20000 <b>→</b> 320	тт → 202 ззо	340 340	1111111 350 3	60 370 <b>-</b>
BoNT/X BoNT/B BoNT/D BoNT/F BoNT/G TeNT	NTVTVE NKVLVCISD NNINKTIPS SEVNSAP NIVS.SAQG SQVTS.CND	NDLLK P.NININIYKI SWISNIDKYKI P.EYDINEYKI S.GIDISLYKO P.NIDIDSYKO	YIKN <mark>K</mark> .IPVQ( NKFKDKYKFVEI KIFSEKYNFDKI DYFQWKYGLDKI QIYKNKYDFVEI QIYQQKYQFDKI	GRICNFKLDTAE DSEGKYSIDVES DNTCNFVVNIDK NADCSYTVNENK DPNCKYSVDKDK DSNCQYIVNEDK	FEKKLNTILFVL FDKLYKSLMFGF FNSLYSDLTNVM FNEIYKK.LYSF FDKLYKALMFGF FQILYNSIMYGF	NESNLAQRFSII TETNIAENYKIK SEVVYSSQYNVK TESDLANKFKVK TETNLAGEYGIK TEIELGKKFNIK	VRKHYLKERPIDPI TRASYFSDSLPPVK NRTHYFSRHYLPV. CRNTYFIKY.EFLK TRYSYFSEYLPPIK TRLSYFSMNHDPVK
BoNT/X	▶ 380	<b>390</b>	20 <u>000</u> 400	TT TT → 410	420	430	
BoNT/B BoNT/B BoNT/D BoNT/F BoNT/G TeNT	YVNILDDNS IKNLLDNEI FANILDDNI VPNLLDDDI TEKLLDNTI IPNLLDDTI	YSTLEGFNIS YTIEEGFNIS YTIRDGFNLT YTVSEGFNIG YTQNEGFNIA YNDTEGFNIS	SQGSN.DFQGQI DKDMEKEYRGQI NKGFNIENSGQI LAVNNRGQ SKNLKTEFNGQI SKDLKSEYKGQI	LESSYFEKI NKAINKQAYEEI NIERNPAL.QKL SIKLNPKIIDSI NKAVNKEAYEEI MRVNTNAFRNV	ESNAL, RAFIKI SKEHLAVYKIQM SSESVVDLFTKV PDKGLVEKIVKF SLEHLVIYRIAM DGSGLVSKLIGL	CPRNGLLYN CKSVK CLRLTK CKSVIPRK CKPVMYK CKKIIPPTNIRE	AIYRNSKN

## Supplementary Figure S1: Sequence alignment of LC/X with other VAMP-cleaving LCs

#### Supplementary Figure S2: Loop 250: Comparison of the loop between LCs

Ribbon representation of LC/X (Red), compared to the LC of serotypes A, (cyan, PDB 3BON), B (sand, PDB 1EPW), D (yellow, PDB 2FPQ), F (gold, PDB 2A97), and Te (light orange, PDB 1Z7H). The zinc ion is shown as a grey sphere.



### Supplementary Figure S3: Catalytic site: conserved Zn<sup>2+</sup> coordination

LC/X (Red) was superposed with the LC of serotypes A, (cyan, PDB 3BON), B (sand, PDB 1EPW), D (yellow, PDB 2FPQ), F (gold, PDB 2A97), and Te (light orange, PDB 1Z7H). The zinc ion is shown as a grey sphere and the zinc-coordinating residues as sticks.



## **Supplementary Figure S4: LC/X cleavage sites on its VAMP substrates.** Cleavage site is highlighted in red.

VAMP1	35.	QQTQAQVEEVVDIIRVNVDKVLERDQKLSELDD	RA <mark>DALQAGASQFESSAAKLKR</mark>	.88
VAMP2	33.	QQTQAQVDEVVDIMRVNVDKVLERDQKLSELDD	RA <mark>DALQAGASQFETSAAKLKR</mark>	.86
VAMP3	20.	QQTQNQVDEVVDIMRVNVDKVLERDQKLSELDD	RA <mark>DALQAGASQFETSAAKLKR</mark>	.73
VAMP4	54.	KHVQNQVDEVIDVMQENITKVIERGERLDELQD	KS <mark>ESLSDNATAFSNRSKQLRR</mark>	.107
VAMP5	7.	ERCQQQANEVTEIMRNNFGKVLERGVKLAELQQ	RS <mark>DQLLDMSSTFNKTTQNLAQ</mark>	.60
Ykt6	140.	TKVQAELDETKIILHNTMESLLERGEKLDDLVS	KS <mark>EVLGTQSKAFYKTAR</mark>	.189

## Supplementary Table S1: Exponential fit parameters (see Fig. 6A)

	Α	k (min <sup>-1</sup> )	С	R <sup>2</sup>
LC/X	0.19	0.07	0.11	0.95
LC/B	0.15	0.02	0.14	0.94