

# Supplementary Materials: Structural and Biochemical Characterization of Botulinum Neurotoxin Subtype B2 Binding to Its Receptors

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Complete sequences of protein constructs used in this study:

BONT binding domain (HC) used for all experiments

>His-FLAG-TEV-HC/B1

```
MGHHHHHHSVDYKDDDDKEDLYFQSHMNSEILNNIILNLRKYKDNLLIDLSGYGAKVEVYDGV  
LNDKNQFKLTSSANSKIRVTQNNIIFNSVFLDFSVFWIRIPKYKNDGIQNYIHNEYTIINC  
MKNNSG  
WKISIRGNRIIWTLLIDINGKTKSVFFEYNIREISEYINRWFFVTITNLLNNAKIYINGK  
LESNTDI  
KDIREVIANGEIIFKLDGDDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDF  
WGNPLMYNK  
EYYMFNAGNKNSYIKLKKDSSVGEILTRSKYNQNSKYINRDLIYGEKFIIRKSNSSQ  
SINDDIVRKE  
DYIYLDFFNLNQEWVRVYTYKYFKKEEKLFLAPISDSDEFYNTIQIKEYDEQPTYSQ  
LLFFKKDEEST  
DEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKKRKPYNLKLGCNWQFIPKDEGWTE
```

>His-FLAG-TEV-HC/B2

```
MGHHHHHHSVDYKDDDDKEDLYFQSHMNSEILNNIILNLRDNNLIDLSGYGANVEVYDGV  
E  
LNDKNQFKLTSSNSTSEIRVTQNNIIFNSMFLDFSVFWIRIPKYKNDGIQNYIHNEYTIIN  
CIKNN  
S  
GWKISIRGNRIIWTLLDINGKTKSVFFEYSIREDISYINRWFFVTITNNSDNAKIYINGK  
LESNIDIK  
DIGEVIANGEIIFKLDGDDIDRTQFIWMKYFSIFNTELSQSNIEEYKIQSYSEYLKDF  
WGNPLMYNKE  
EYYMFNAGNKNSYIKLKKDSSVGEILTRSKYNQNSNYINRNLVYGEKFIIRKSNSSQ  
SINDDIVRKE  
DYIYLDFFNSNREWRVYAYKDFKEEKLFLANIYDSNEFYKTIQIKEYDEQPTYSQ  
LLFFKKDEES  
TDEIGLIGIHRFYESGIVLKDYNFCISKWYLKEVKKRKPYNPNLGCNWQFIPKDEGWIE
```

Protein receptors used in the synaptotagmin-binding assays

>GST-hSytI

```
MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDG  
DVKLT  
QSMIIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRVIAYSKDFETLKVDFL  
SKLPEMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDK  
YLKSSK  
YIAWPLQGWQATFGGGDHPKSDLIEGRGIPGHHHHHHHGMVSESHHEALAAPVTTVAT  
V  
PSNATEPASPGEKEDAFSKLKEKFMNELHK
```

>GST-hSytII

```
MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDG  
DVKLT  
QSMIIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRVIAYSKDFETLKVDFL  
SKLPEMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDK  
YLKSSK  
YIAWPLQGWQATFGGGDHPKSDLIEGRGIPGHHHHHHHGMRNIFKRNQEPVAPATTTAT  
M  
PIGPVDNSTESGGAGESQEDMFAKLKEKLFNEINK
```

>GST-rSytII

```
MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDG  
DVKLT  
QSMIIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRVIAYSKDFETLKVDFL  
SKLPEMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDK  
YLKSSK
```

YIAWPLQGWAATFGGGDHPPKSDLIEGRGIPGHHHHHHHHGMRNIFKRNQEPIVAPATTTATM  
PLAPAAPADNSTESTGTGESQEDMFAKLKDKFFNEINK