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Supplementary Materials for

Structure of the saxiphilin:saxitoxin (STX) complex reveals a convergent molecular recognition strategy for paralytic toxins

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Movie S1 (.mp4 format). Sxph conformational changes upon STX binding.

	apo-Saxiphilin	Sxph:STX complex (soaked)	Sxph:STX complex (co-crystal
Data Collection			
Space group Unit cell	$P2_{1}2_{1}2_{1}$	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
a, b, c (Å) ^a	96.2, 111.3, 254.8	96.4, 110.8, 254.6	96.6, 111.7, 254.6
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	48.2-2.50 (2.54-2.50)	48.2-2.50 (2.54-2.50)	48.3- 2.12 (2.16-2.12)
Total reflections	1143210	1155387	1962129
Unique reflections	95268	94650	156355
Completeness (%)	99.8 (97.3)	99.6 (95.2)	99.9 (98.5)
Redundancy	12.0 (6.6)	12.2 (6.7)	12.5 (7.4)
Ι/σΙ	16.0 (1.1)	14.7 (0.5)	13.3 (0.3)
<u>CC_{1/2}</u>	1 (0.57)	1 (0.25)	1 (0.10)
Refinement ^D			
R _{work} (%)	22.4	23.7	23.8
R _{free} (%)	25.3	26.2	25.9
RMS deviations			
Bonds (Å)	0.003	0.003	0.002
Angles (°)	0.68	0.76	0.68
Average B factor	83.3	105.0	94.2
Protein	83.6	105.1	94.6
Water	59.5	69.7	69.4
Ligand	-	116.1	95.1
Ramachandran [%] Allowed/generous/disallowed		93.8/5.0/1.2	95.3/3.6/1.1
atistics for the highest resoluti nal refined models cover all re po-Saxiphilin			
Chain A, 171-178, 289, 571-573 Chain B, 1-4 and 169-178 xph:STX complex (soaked) Chain A : 1-3, 172-177,585-586		674, 701-711, and 717-718	
Chain B: 1-4, 169-179, and 572 xph:STX complex (co-crystal) Chain A : 1,2, 171-173, 585-580)	-674	

 Table S1. Crystallographic data collection and refinement statistics.

Chain B: 1-4, 169-178, and 572-573

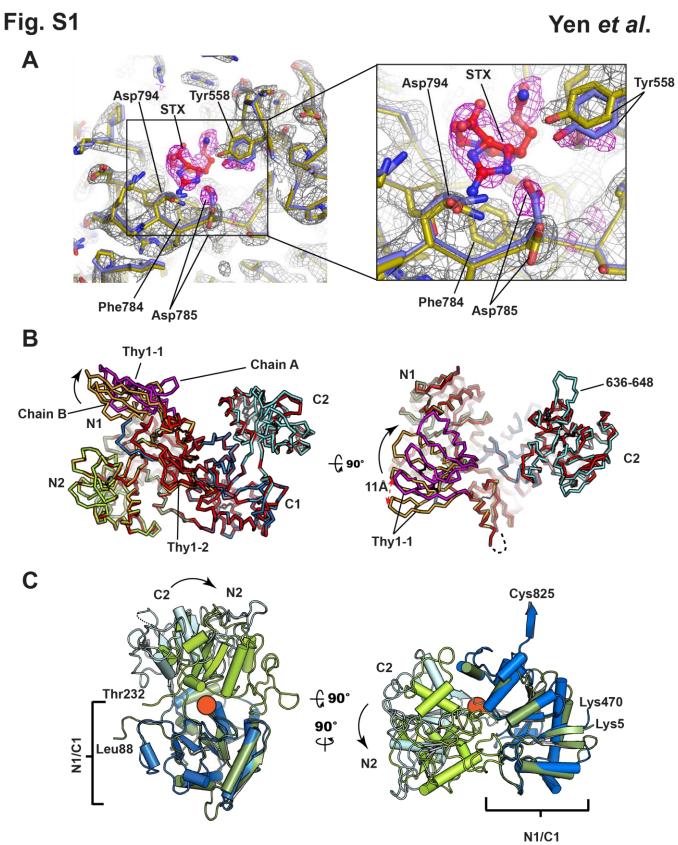


Fig. S1. Sxph structural analysis. (**A**) Exemplar 2Fo-Fc electron density (1.5σ) (grey) and Fo-Fo (5σ) (magenta) for Sxph and STX, respectively. Sxph (olive) and Sxph:STX (marine) are shown and select residues are labeled. STX is red. (**B**) Ribbon diagram superposition of apo-Sxph Chain A (red and magenta) and Chain B (RMSD_{Ca} = 0.61 Å over 663 residues). Chain A Thy1-1 is colored magenta. Chain B subdomains are colored: N1 (smudge), N2 (Limon), Thy (bright orange), C1 (marine), and C2 (cyan). Relative displacement of the Thy1-1 is indicated. (**C**) Superposition of apo-Sxph N-lobe (N1, smudge; N2, limon) and C-lobe (C1, marine; C2, cyan). Relative motions of N2 and C2 subdomains are indicated. As a point of reference, the orange sphere marks position that corresponds to the transferrin Fe³⁺ binding site. The N-lobe and C1 cores have lowest B-factors (average B factor of 74.0 Å² for N-lobe and 63.4 Å² for C1 domain), whereas the majority of C2 is more mobile (average B-factor of 108.4 Å²).

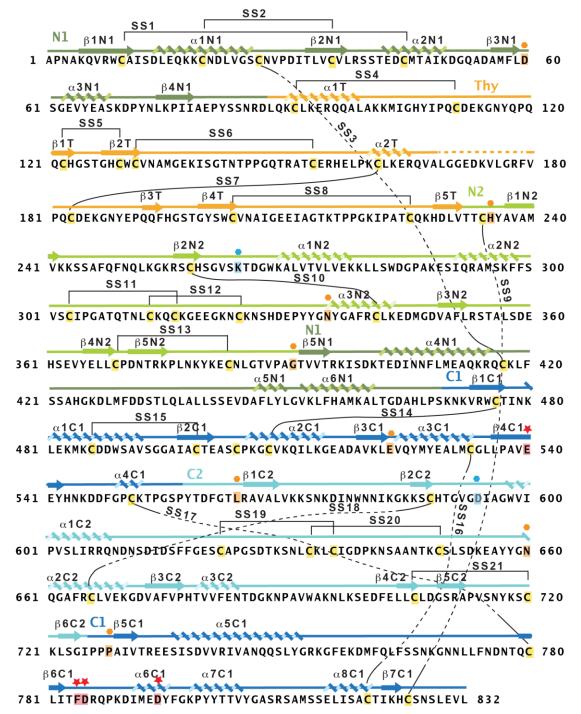


Fig. S2. Sxph sequence, secondary structure, and disulfide map. *Rana catesbeiana* Sxph sequence. Secondary structures are indicated. Domains are labeled and colored as in fig. S1A. Cysteine residues (yellow) and disulfide bonds (SS#) are indicated. Residues corresponding to transferrin Fe³⁺ and carbonate ligands are indicated by orange and blue hexagons, respectively and highlighted. Residues corresponding to STX-interacting residues are indicated by the red star and are highlighted.

Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMe Pig_ICA

Sxph Human_ST Rabbit_ST

Human LT

Pig_ICA

Chicken OT

Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMelanotransferrin Pig_ICA

Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMelanotran Pig_ICA

Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMelano Pig_ICA

Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMelanotransferrir Pig ICA

β**1N1** α **1N1** β**2N1** α 2 N 1 β**3N1** α 3 N 1 β**4N1** α **1**T β**1T** β**2**T $\alpha 2T$ β**3**Τ β**4**Τ β**5**Τ β**1N2** 123 HGSTGHCWCVNAMGEK I SGTNTPPGQTRATCERHELPKCLKERQVALGGDEKVLGRFVPQCDEKGNYEPQQFHGSTGYSWCVNA I GEE I AGTKTPPGK I PATCQKHDLVTTCHYAVAMVKKSSAFQ 248 - QTFYYAVAVVKKDSGFQ 108 - KTFYYAVALVKKGSNFQ 108 ----RTHYYAVAVVKKGGS FQ 105 TTSYYAVAVVKKGTEFT 105 - GTSYYAVAVVRRSSHVT 101 - QTHYYAVAVVKKGSDFQ 108 🧛 α 3 Ν 2 β**2 N 2** α **1N2** $\alpha 2 N 2$ β3N2 • β4N2 β5N2 **65N1** α 4 N 1 α 5 N 1 α 6 N 1 β**3C1** α3C1 _β1C2 β**2C1** α**4C1** β**1Ç1** α 1C1 α2C1 α**3C1** β**4C1**¥ ***** **Are Are Are**

585 KKSCH TGVGD I AGWV I PVSL I RRQN --- DN --- SD I DS FFGESCAPGSD TK -- SNLCKLC I GDPKNSAANTKCSLSDKEAYYGNQGAFRCLVE - KGDVA FVPH TVV FEN TDGKNPAVWAKNLK 688 447 KKSCH TAVGRTAGWN I PMGLL YNKI --- NH --- CRFDE FFSEGCAPGSKD -- SSLCKLC MGS --- SULADEPNNKEGYYGY TGAFRCLVE - KGDVA FVKHOTVPON TGGKNPDPWAKNL 552 446 KKSCH TAVDRTAGWN I PMGLL YNKI --- NH --- CRFDE FFSEGCAPGSKD -- SSLCKLC MGS --- SVCAPNNEGYYGY TGAFRCLVE - KGDVA FVKHOTVPON TGGKNPDPWAKNL 552 456 KKSCH TAVDRTAGWN I PMGLL YNT --- NH --- CRFDE FFSQSCAPGSDFR -- SNLCALC I GDEQ -- GENKOVPNSNERYYGY TGAFRCL 24 - KGDVA FVKDVT LON TGGRNSEPWAKDLK 552 456 KKSCH TAVDRTAGWN I PMGLI HNRT --- GS --- CKFDE Y FSQSCAPGSDPR -- SNLCALC I GDEQ -- GENKOVPNSNERYYGY TGAFRCL 24 - KGDVAFVKDVT LON TGGKNNEAWAKDLK 557 451 KKSCH TAVGRTAGWN I PMGLI HNRT --- GS --- CKFDE Y FSQSCAPGSDPR -- SNLCALC I GDEQ -- GENKOVPNSNERYYGY TGAFRCL 24 - KGDVAFVKDVT LON TGGKNNEAWAKDLK 567 456 KKSCH TAVGRTAGWO I PMGLI HNRT --- GS --- CKFDE Y FSQSCAPGSDPR -- SNLCALC I GDEQ -- GENKOVPNSNERYYGY TGAFRCL 24 - KGDVAFVKDVT LON TGGKNNEAWAKDLK 567 456 KKSCH TAVGRTAGWO I PMGLI HNRT -- GS --- CKFDE Y FSQSCAPGSDPR -- SNLCALC I GDEQ -- GENKOVPNSNERYYGY TGAFRCL 24 - KGDVAFVKDVT VD TD TGKNNEAWAKDLK 562 456 KKSCH TAVGRTAGWO I PMGLI HNRT -- GS --- CKFDE Y FSQSCAPGSDPE -- SRLCALC VGG -- AFNON YGYGAFRCL 24 - KGDVAFVKDVT VD TD TGKNNEAWAKDLK 562 450 KKSCH TAVGRTAGWO I PMGI I HNRT -- GS --- CKFDE Y FSQSCAPGSDPE -- SRLCALC VGG SQS PSG - PAHTCAPNSHEGYHGFSGALRCL 24 - KGDVAFVKHTT VD TN TGHNSEPWAFL 562 450 KKSCH TAVGRTAGWO I PMGI I PMGFI YNQT --- GS --- CKLDE FFSQSCAPGSDPE --- SRLCALC SG I SGQ - PAHTCAPNSHEGYHGFSGALRCL 24 - KGDVAFVKHTT VD TN TGHNSEPWAFL 562

ο α 2 C 2

β6C1 ¥¥ α6C1 ¥

β**3C2** α**3C2**

α7C1

α8C1

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Sxph Human_ST Rabbit_ST Human_LT Chicken_OT hMelanotransferrin Pig_ICA	556 EKDYELLCLDGTRKPVEEYANC 553 EEDFELLCLDGTRKPVSEAHNC 568 LADFALLCLDGKRKPVTEARSC 563 MDDFELLCTDGRRANVMDYREC 577 SEDYELLCPNGARAEVSQFAAC	HLARAPNHAVVTRKDKEAC-VHKILROODHLFGSNVTDCSGNFCLFRS HLAKAPNHAVVSRKDKAAC-VKOKLLDLOVYEVGNTVADCSSKFCMFHS HLAMAPNHAVVSRKDKVER-LKOVLLHQOAKFGRNGSDCPDKFCLFQS VLAEVPTHAVVVRPKANK-IRDLLERQEKRFGVNGSEK-SKFMMFES LAGIPHAVVVRPKINIFTVYGLLOKAODLFGODHNKNGFMFDSSN	NKGNNLLFNDNTQCLITFDRQPKDIMEDYFGKPYYTTVYGASRSAMSSELISAC 820 ETKDLLFRDDTVCLAKLH-DRNTYEKYLGEEYVKAVG-NLRKCSTSSLLEAG 671 KTKDLFRDDTKCLVDR-GKNTYEKYLGPQYVAGIT-NLKKCSTSPLLEAG 681 ETKNLLFNDNTECLARLH-GKTTYEKYLGPQYVAGIT-NLKKCSTSPLLEAG 680 (HGQDLLFKDATVRAVPVG-EKTTYRGNLGLDYVAALEGMSSQACSGAAAPAP 698 STEDLLFSDDTECLANLQ-DKITYQKYLGPEYLQAIA-NVRQCFPSELLDAG 680
	β 7C1		
Sxph	821 TIKHC	825	
Human_ST	675 T <mark>F</mark> RRP	679	
Rabbit_ST	672 T <mark>F</mark> HKH	676	
Human_LT	687 E FLRK	691	
Chicken_OT	681SFLEGK	686	
hMelanotransferrin		719	
Pig_ICA	681 T <mark>F</mark> HGN	685	

β4C2 β5C2

β**2C2**

 α 1C2

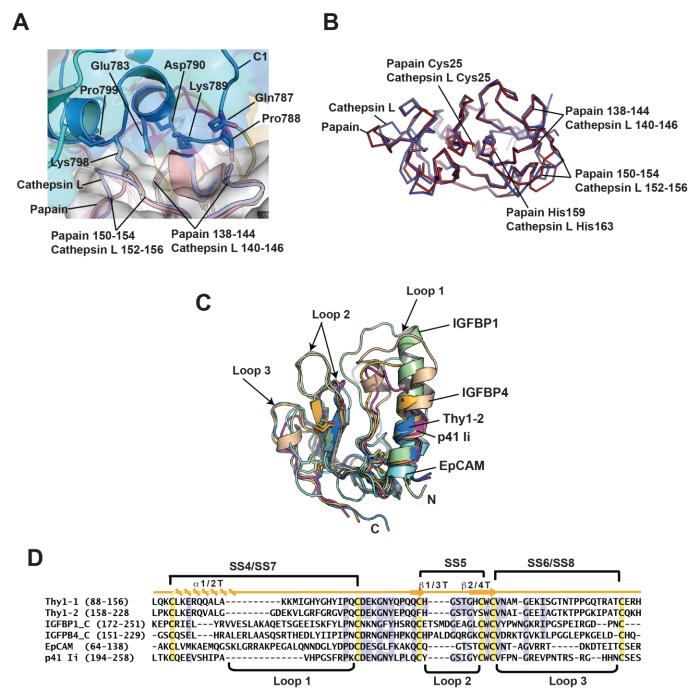
β6C2 _β5C1

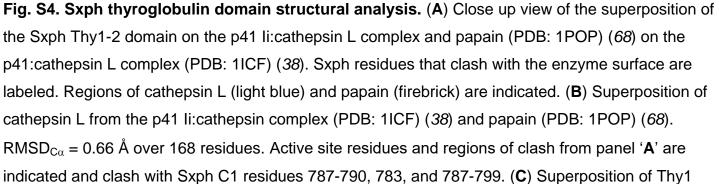
Fig. S3. Comparison of Sxph and representative transferrin family member sequences.

α5C1

Sequence alignment of *Rana catesbeiana* Sxph with representative members of the transferrin family. Human serum transferrin (Human ST) (UniProtKB: P02787), rabbit serum transferrin (Rabbit ST) (UniProtKB:P19134), human lactotransferrin (Human_LT) (UniProtKB:P02788), chicken ovotransferrin (Chicken OT) (UniProtKB:P02789), human melanotransferrin (Melanotransferrin) (UniProtKB:P08582), and pig inhibitor of carbonic anhydrase (Pig_ICA) (UniProtKB:Q29545). Sxph

domain and secondary structures are indicated and colored as in fig. S1B. Residues corresponding to transferrin Fe^{3+} and carbonate ligands are indicated by orange and blue hexagons, respectively and highlighted (*38*).





domains from Sxph (Thy1-1, bright orange, Thy1-2, marine), p41 (1ICF, magenta) (38), IGFBP1 (1ZT3, green) (69), IGPBP4 (PDB: 2DSR, wheat)(70), and EpCAM (PDB: 4MZV, aquamarine)(71).
(D) Sequence comparison of Thy1 domains from 'C'. Secondary structure elements and disulfide bond labels are from the Sxph Thy1 domains.

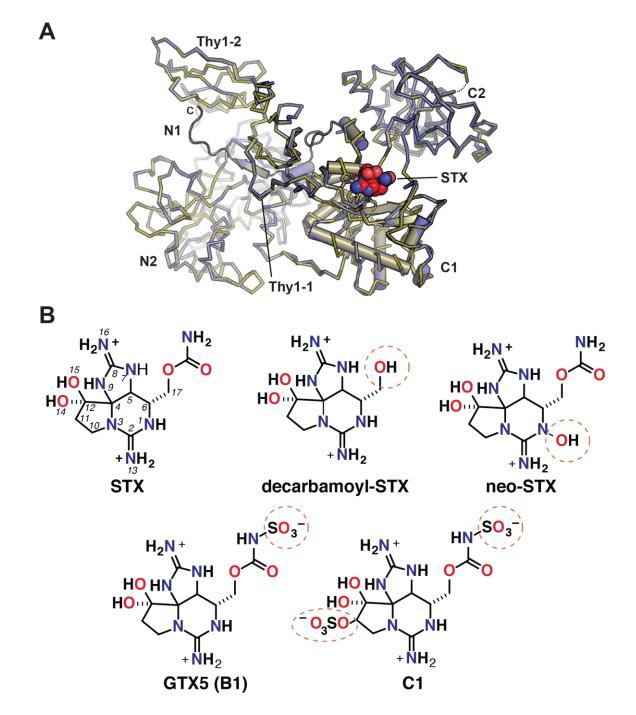
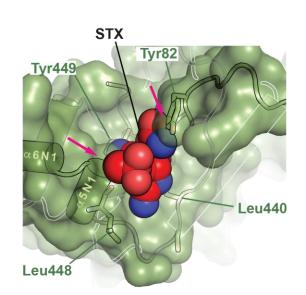


Fig. S5. STX-binding site and STX derivatives. (A) Superposition of apo-Sxph (olive) and STX bound STX (slate) shown as ribbons (RMSD_{C α} = 0.21 Å over 671 residues). C1 domain is also shown in cartoon representation. STX (red) is shown as space filling. Residues in the STX binding site are shown as sticks. (B) STX and derivatives. STX atom numbering is shown in italics. Dashed circles indicate sites of modification in STX derivatives. Gonyautoxin 5 is also known as 'B1'.

Β Α Tyr82 Carbamate STX Glu540 Tyr449 Tyr795 a70 ∝6 /ν Ala79 HK Leu440 Asp785 Ser441 Asp794 Leu448 Phe784 С **STX** B5T α**8C1**. BTC **N1** C1



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Fig. S6. Structural comparison of Sxph N1 and C1 domains. (**A**) Superposition of the Sxph N1 (green), and C1 (slate) domains (RMSD_{Ca} = 0.90 Å over 86 residues). STX is shown in red. N1 domain residues corresponding to C1 domain STX binding residues are shown as sticks and are labeled. (**B**) Surface rendering of the Sxph N1 proto-pocket (green). STX is shown in space-filling representation. Magenta arrows indicate clash sites. (**C**) Superposition Sxph N1 (green), and C1 (slate) domains. Elements from Sxph that occlude the N1 domain proto-pocket are labeled with black letters. STX (red) is shown in space filling representation.

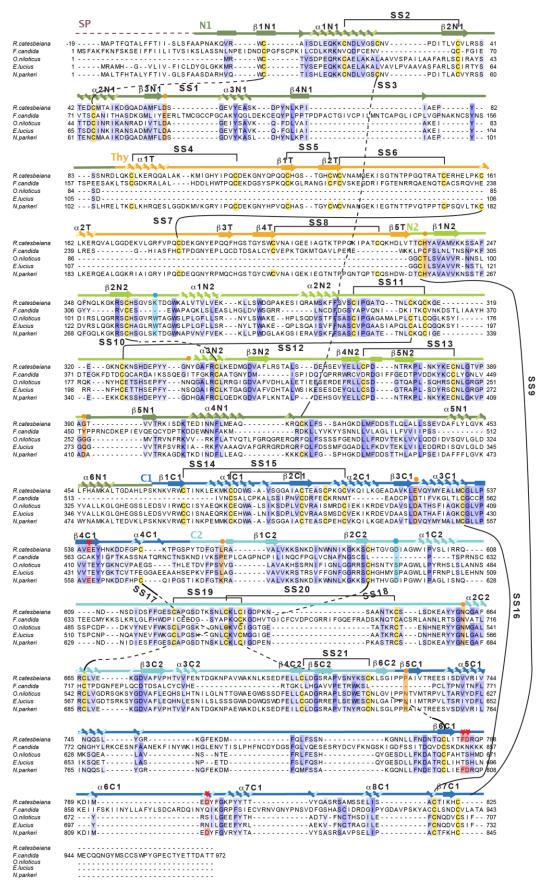


Fig. S7. Sequence comparison of Sxph and putative Sxph homologs. Sequence alignment of *Rana catesbeiana* Sxph with putative Sxph homologs from springtail (*Folsomia candida*) (NCBI: OXA56246.1), Nile tilapia (*Oreochromis niloticus*) (NCBI:XP_019214738.1), Northern pike (*Esox lucius*) (NCBI:XP_010879337.1), and High Himalaya frog (*Nanorana parkeri*) (NCBI: XP_018410833.1).



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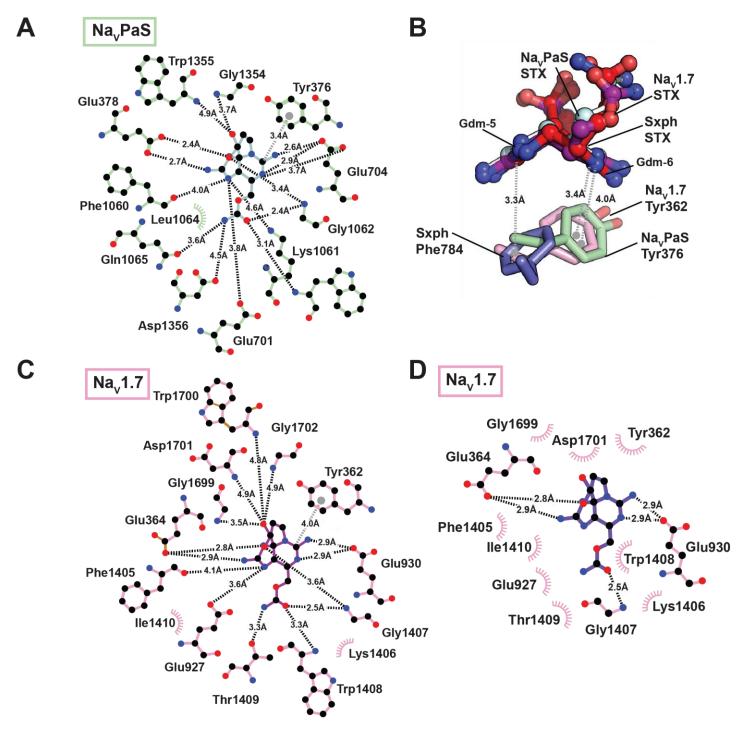


Fig. S8. Na_VPaS:STX and Na_V1.7:STX interactions. (A) Na_VPaS:STX interactions (PDB 6a91)(29) determined with LIGPLOT (67) and a cutoff of 5.0 Å. (B) Comparison of STX cation- π interactions with Sxph (blue), Na_VPaS (green), and Na_V1.7 (magenta). STX from the Sxph:STX complex (red), Na_VPaS:STX complex (cyan), and Na_V1.7:STX complex (violet) are indicated. (**C** and **D**) Na_V1.7:STX

interactions (PDB:6J8G) (47) determined with LIGPLOT (67) a cutoffs of 5.0 Å and 3.35Å, respectively.

Movie S1. Sxph conformational changes upon STX binding. Morph between the apo-Sxph and Sxph:STX structures showing the STX binding pocket. Select sidechain and backbone atoms are shown as sticks. STX is shown as red sticks.