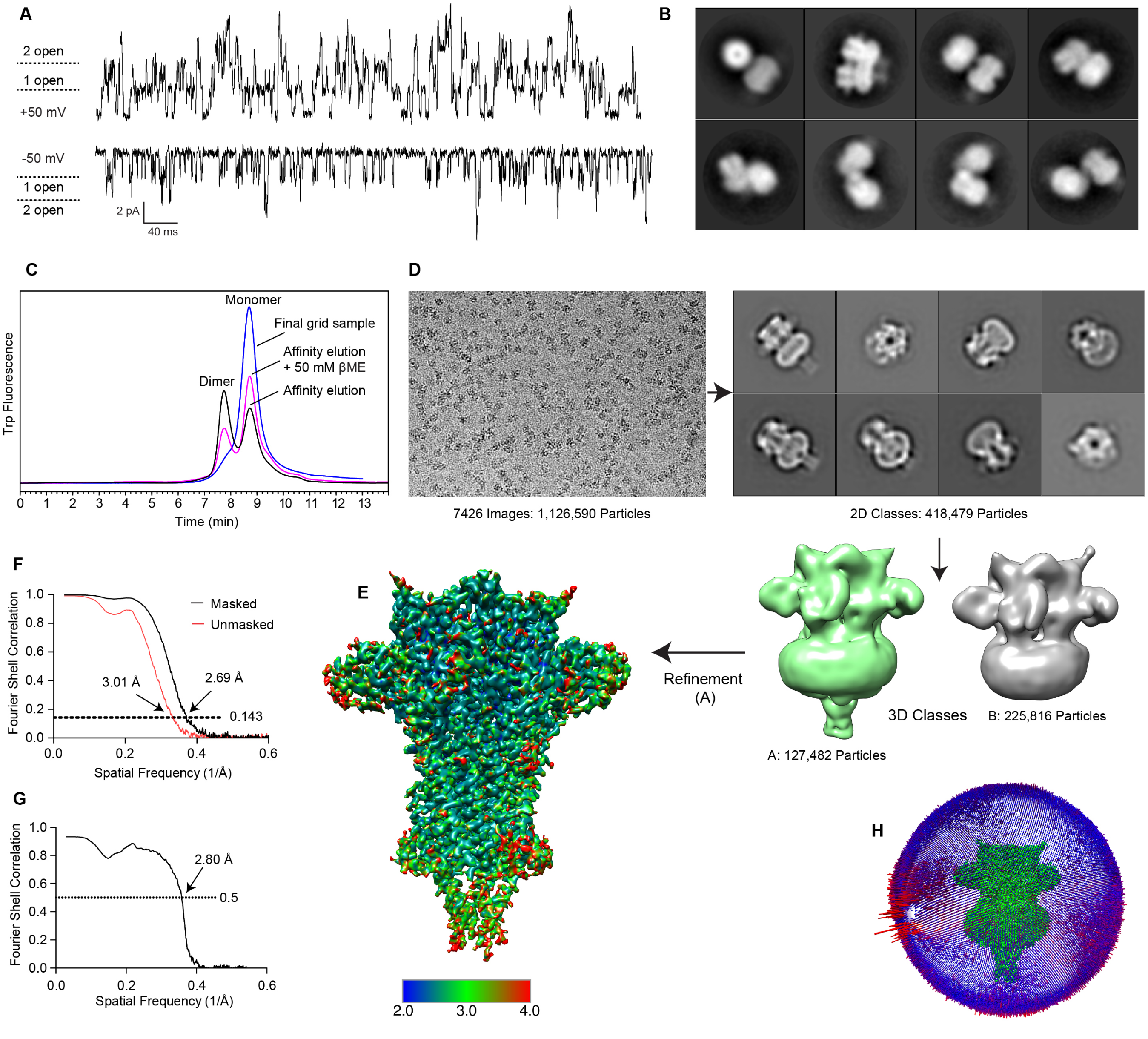


Figure S1. Biochemistry, Cryo-EM image processing and map-model parameters

Related to Figure 1 and STAR Methods

(A) Single channel recordings from excised patches from *Torpedo* receptor reconstituted into soy lipid liposomes. Dashed lines indicate approximate current levels for 1 and 2 channels open. Single channel current amplitudes are non-rectifying in the range of +50 to -50 mV with a mean single channel amplitude of 2.3 pA, equivalent to γ = 46 pS. (B) 2D classification from a small cryo-EM dataset in which dimeric receptors were not treated with reducing reagent reveals heterogeneity in relative orientations between receptors. (C) Analytical size-exclusion chromatography of preparation used to generate high resolution reconstruction at different steps. (D) Relion workflow. (E) Local resolution map. Angular distribution histogram. (F) FSC curve. (G) Map-model FSC. (H) Angular distribution histogram.

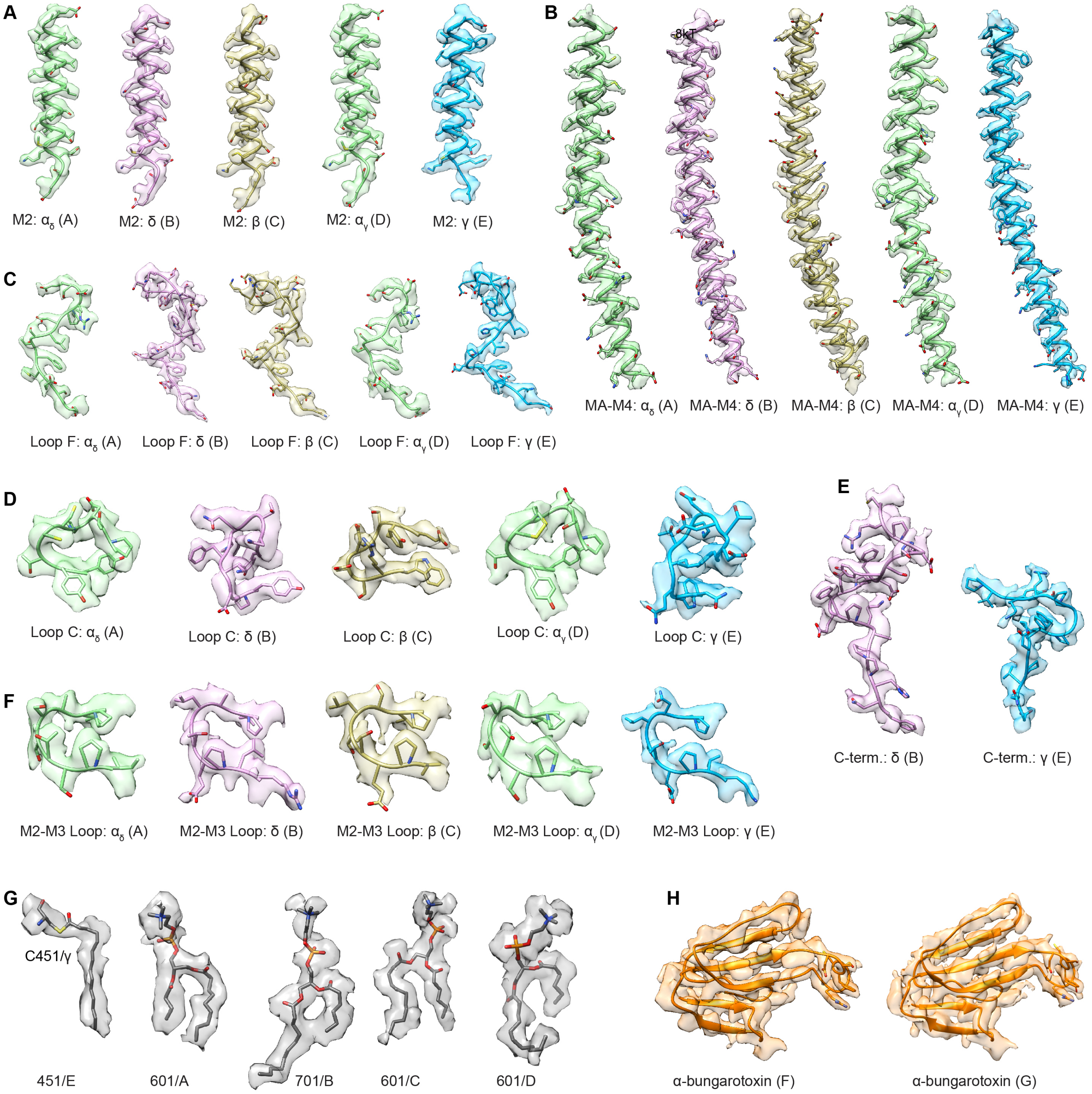
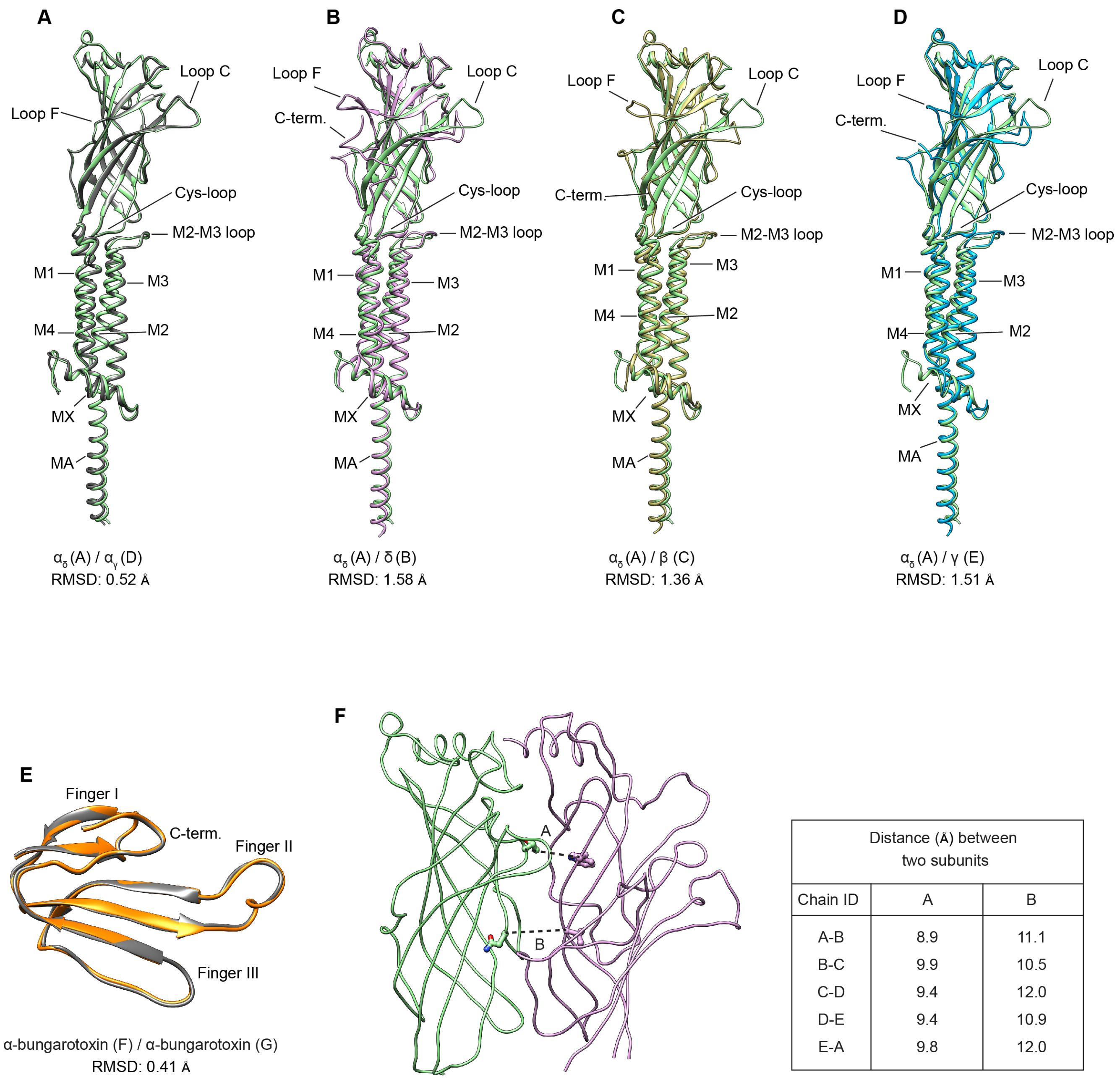


Figure S2. Representative map quality

Related to Figure 1

(A) M2 α-helices. (B) MA-M4 α-helices. (C) Loop F. (D) Loop C. (E) Extended C-termini of δ and γ subunits. (F) M2-M3 loop. (G) Presumptive palmitoylation at C451/γ and bound PC lipids. (H) α-bungarotoxins. Density map of protein region was contoured at a threshold of 0.006 and for lipid 0.003 in UCSF Chimera.



α_δ (A) / α_γ (D)
RMSD: 0.52 Å

α_δ (A) / δ (B)
RMSD: 1.58 Å

α_δ (A) / β (C)
RMSD: 1.36 Å

α_δ (A) / γ (E)
RMSD: 1.51 Å

α-bungarotoxin (F) / α-bungarotoxin (G)
RMSD: 0.41 Å

Distance (Å) between two subunits		
Chain ID	A	B
A-B	8.9	11.1
B-C	9.9	10.5
C-D	9.4	12.0
D-E	9.4	10.9
E-A	9.8	12.0

Figure S3. Comparison between subunits

Related to Figure 1

(A-D) Pairwise superposition of all five subunits; α_δ (chain A, green), α_γ (chain D, gray), β (chain C, yellow), γ (chain E, blue) and δ (chain B, violet) and their corresponding RMSDs are written underneath. (E) Superposition of the two α-bungarotoxins bound to two different interfaces; α_δ/δ (chain F) and α_γ/γ (chain G). (F) Assessments of ECD asymmetry by measuring distances between corresponding Cα positions. Noteworthy is that while loop C is propped open by the toxin, this local asymmetry does not propagate to a more globally asymmetric ECD.

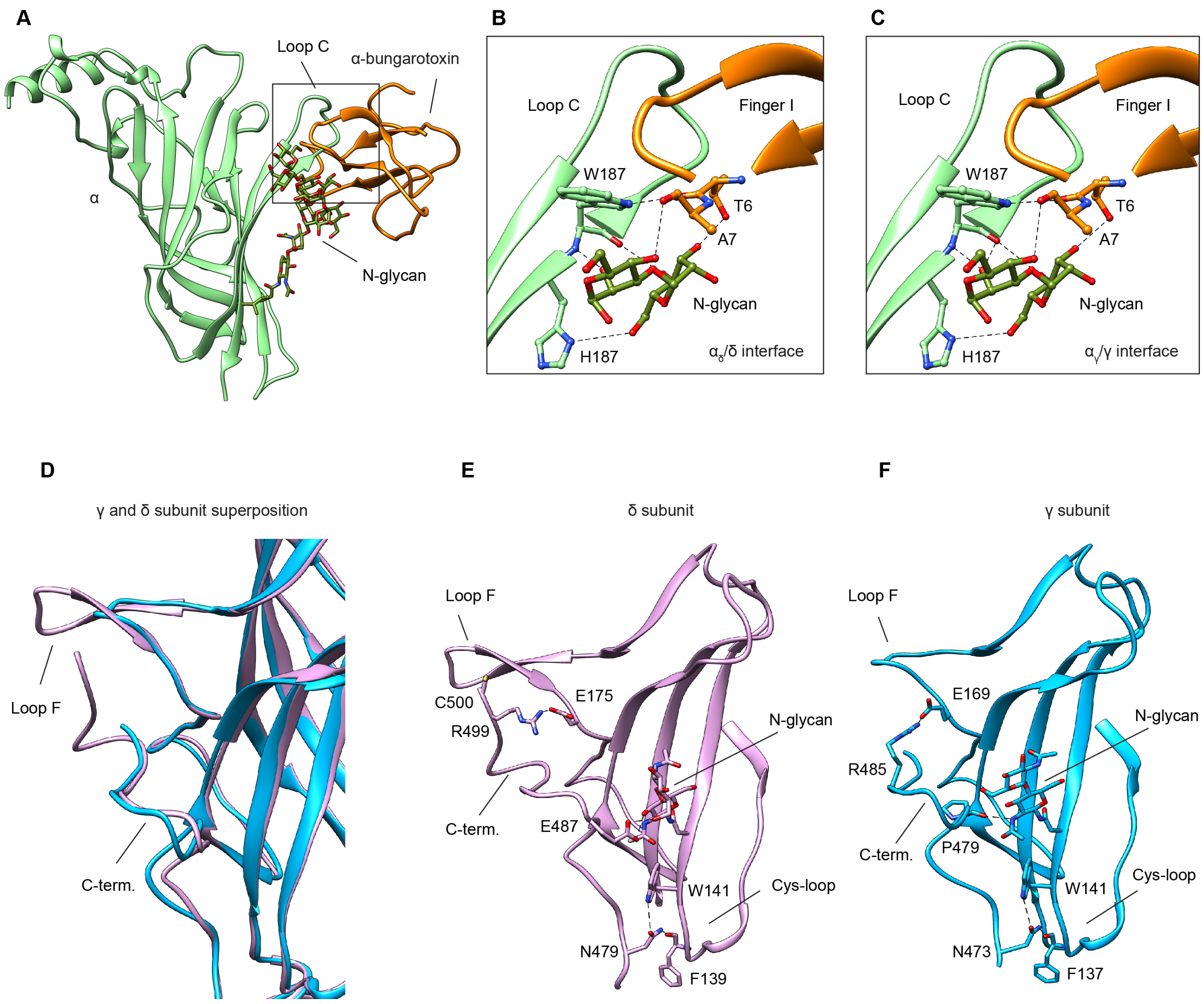


Figure S4. Glycosylation site at primary binding site and extended loop F and C-terminus from complementary binding site

Related to Figure 1 and Figure 2

(A) Glycosylation site between α-subunit and toxin. (B, C) Interaction between N-glycan, loop C of receptor and toxin at α_δ and α_γ subunit interfaces respectively. (D) Superposition of γ and δ subunit showing the difference in their loop F and C-terminus. (E, F) Interaction of C-terminus with Cys-loop and loop F at δ and γ subunit respectively. Interacting residues are shown as stick model. Residue C500 in the δ subunit makes the disulfide bond between two monomers of nicotinic receptor in native *Torpedo* membrane.

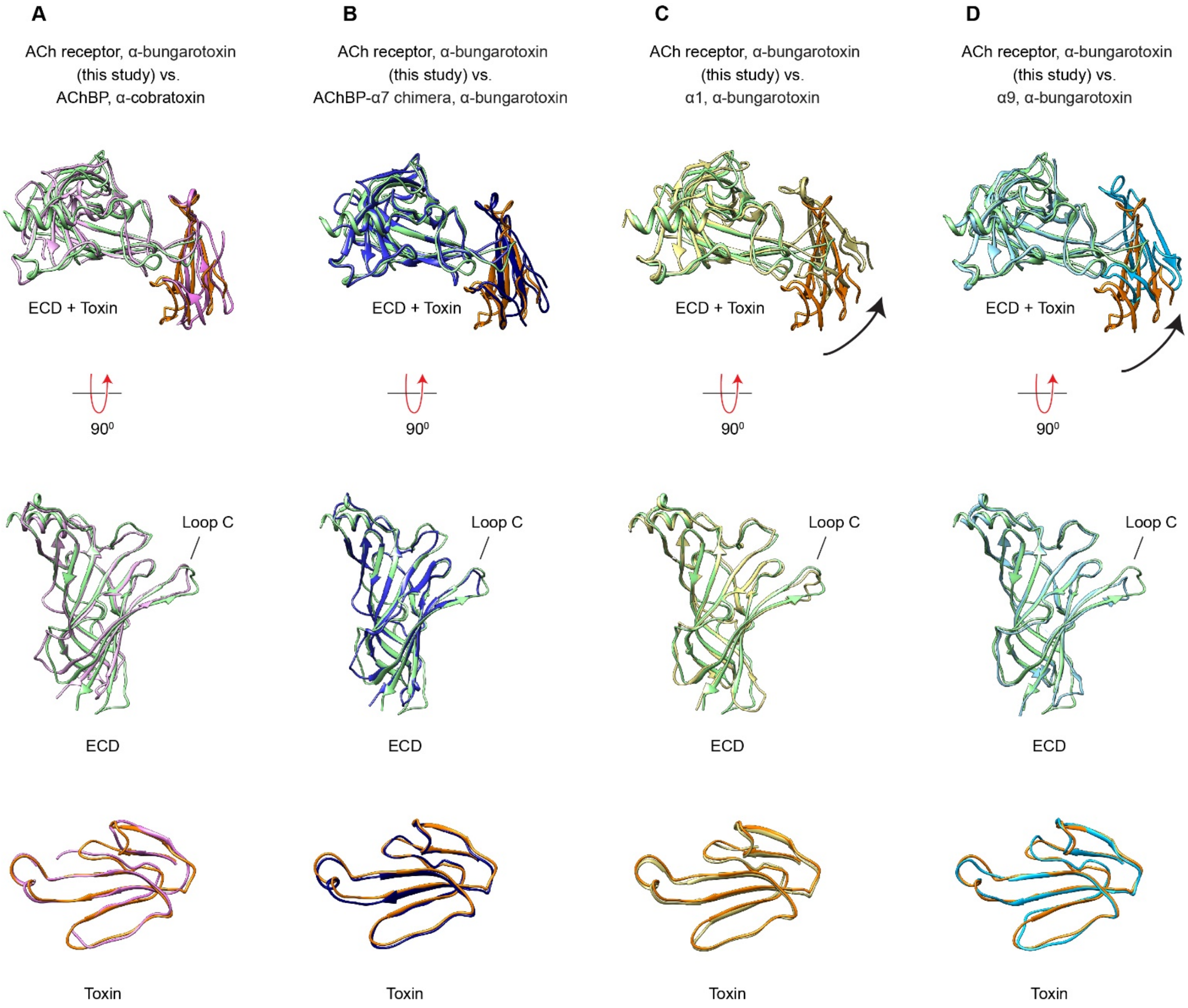
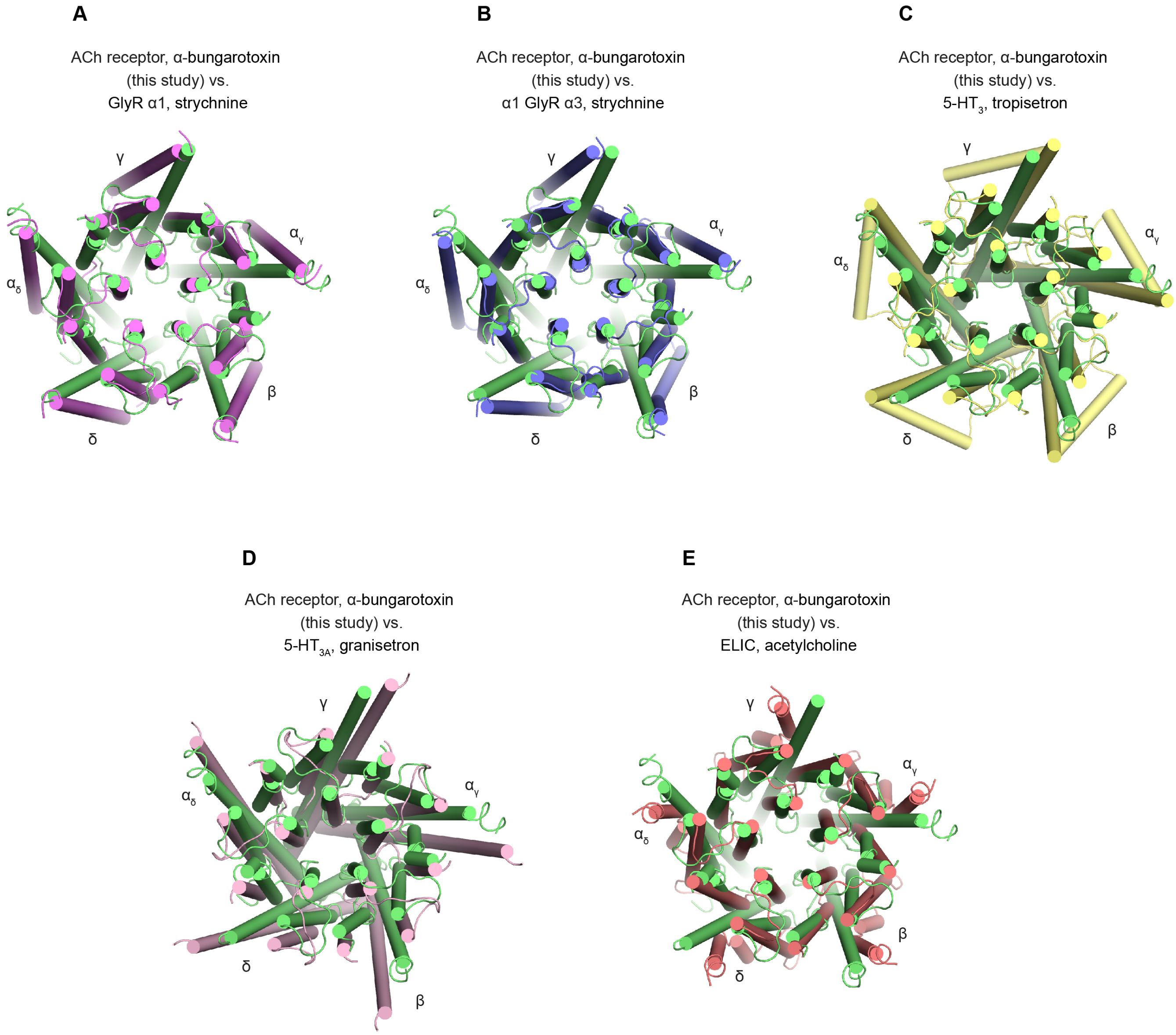


Figure S5. Comparison of the ECD and toxin conformations

Related to Figure 1 and Figure 2

Comparison of *Torpedo* receptor – α-bungarotoxin complex (receptor - green, toxin - orange) with (A) AChBP complex with α-cobratoxin (PDB ID: 1YI5; violet); (B) AChBP-α7 chimera with α-bungarotoxin (PDB ID: 4HQP; blue); (C) α1-ECD complex with α-bungarotoxin (PDB ID: 2QC1; yellow). (D) α9-ECD complex with α-bungarotoxin (PDB ID: 4UY2; cyan).



A: ACh receptor, α-bungarotoxin (this study) vs. GlyR α1, strychnine

B: ACh receptor, α-bungarotoxin (this study) vs. α1 GlyR α3, strychnine

C: ACh receptor, α-bungarotoxin (this study) vs. 5-HT_3, tropisetron

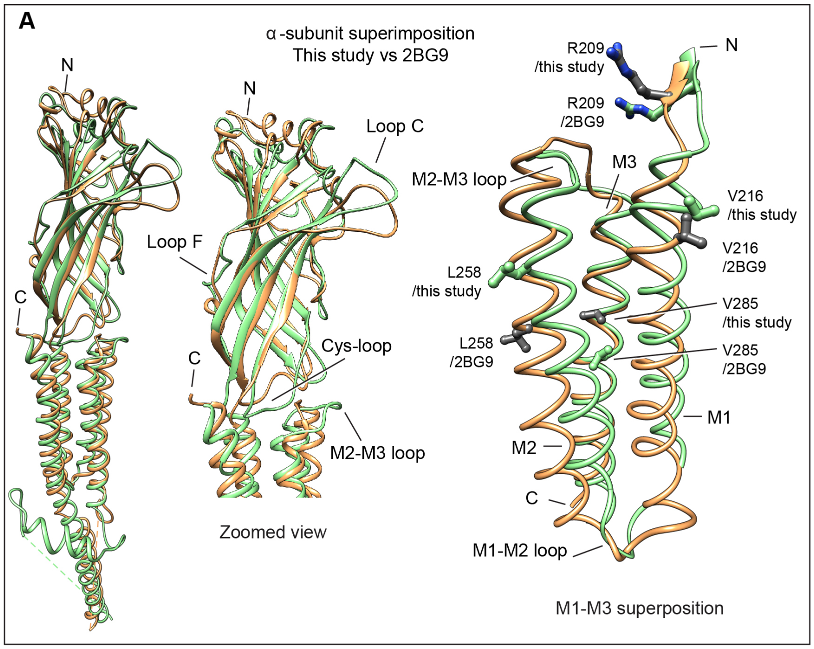
D: ACh receptor, α-bungarotoxin (this study) vs. 5-HT_{3A}, granisetron

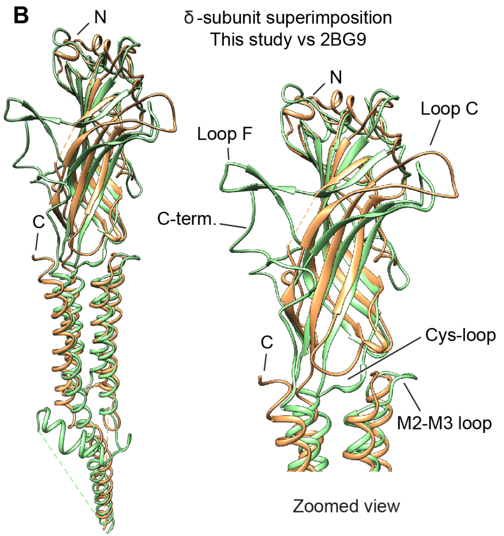
E: ACh receptor, α-bungarotoxin (this study) vs. ELIC, acetylcholine

Figure S6. Comparison of the transmembrane domain conformations

Related to Figure 3

Superposition of toxin-bound *Torpedo* receptor TMD (green) with antagonist-bound Cys-loop receptor structures: (A) GlyR α1, strychnine (PDB ID: 3JAD, violet); (B) GlyR α3, strychnine (PDB ID: 5CFB, blue); (C) 5-HT_3, tropisetron (PDB ID: 6HIS, yellow); (D) 5-HT_{3A}, granisetron (PDB ID: 6NP0, pink); (E) ELIC, acetylcholine (PDB ID: 3RQW, red).

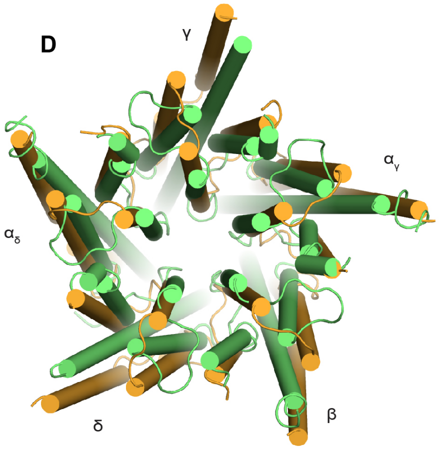




C

RMSD (Å)			
α	β	γ	δ
3.62	2.76	2.71	3.12

Torpedo ACh receptor
resting state (this study) vs Resting, 2005 (2BG9)



E

Interface area (Å^2)		
Subunits	This study	2BG9
α_δ - δ	3177	1304
δ - β	3282	1850
β - α_γ	3201	1413
α_γ - γ	3045	1661
γ - α_δ	3202	1699

Interface area (Å^2) at ligand-binding subunits	
GlyR α1, strychnine - 3JAD	2130
GlyR α3, strychnine - 5CFB	2243
5-HT3, Tropisetron - 6HIS	3172
5-HT3, granisetron - 6NP0	3034
5-HT3, apo - 6BE1	3190
GABA$_A$, bicuculline - 6HUK	3033

Torpedo ACh receptor
resting state (this study) vs Open, 2012 (4AQ9)

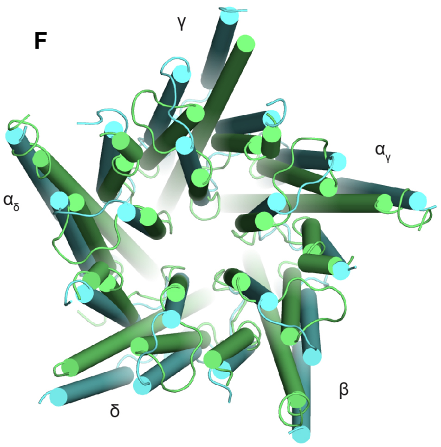
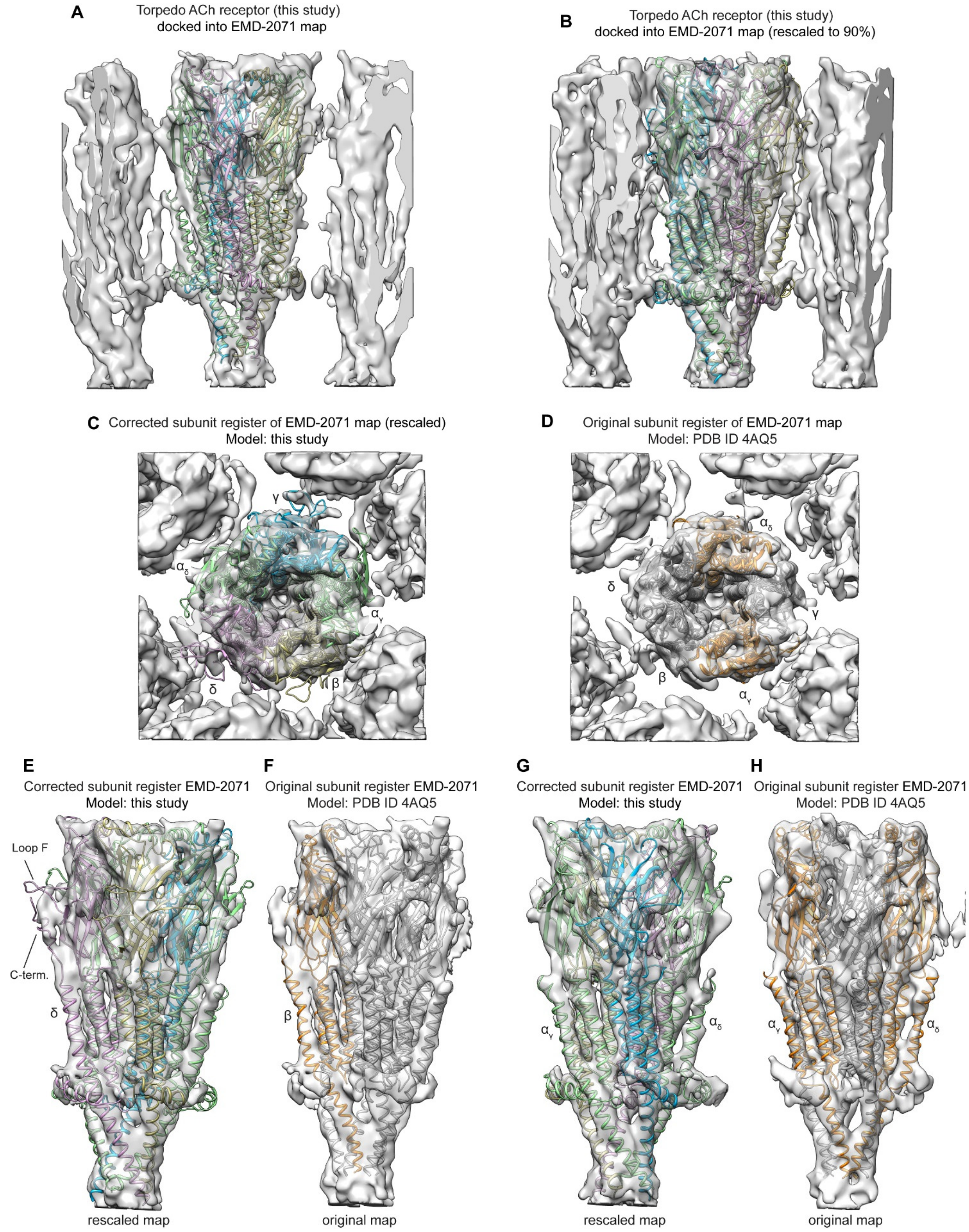


Figure S7. Comparison with earlier structures of the *Torpedo* nicotinic receptor

Related to Figure 1 and Figure 3

(A) Superposition of α subunits from our structure (green) and the *Torpedo* resting state structure (PDB ID: 2BG9, orange). The ECD in the earlier models was derived from crystal structures of the acetylcholine binding protein and agrees well with our model. Notable differences include the N-terminal α1 helix, which is roughly planar to the membrane in our model and other eukaryotic superfamily structures; loop C, which in α subunits likely relates to toxin binding in our model absent from the 2BG9 condition, but is modeled differently in other subunit as well (c.f. panel B); and the Cys-loop, which buckles up toward the ECD core in the 2BG9 and 4AQ9 models, while in our toxin-bound model the Cys-loop rests in the ECD surface of the TMD in a manner similar to other recent superfamily structures. Throughout much of the TMD we confirm a register error in the 2BG9 and 4AQ9 models (right side of panel A, with the same residues from both structures shown as sticks, 2BG9 sticks in grey). The M1-M2 and M2-M3 loops adopt divergent conformations, which is likely the source of the TMD register discrepancy. (B) Superposition of δ subunits between our structure and 2BG9. Several elements we observe were not modeled in 2BG9 or 4AQ9: loop F insertions, long C-termini, MX-helices from all subunits. (C) RMSD of α-carbons between corresponding subunits of the toxin-bound structure and 2BG9. (D) Comparison of the TMD conformations between our structure and 2BG9. (E) Interface area between subunits for the toxin-bound *Torpedo* receptor structure and 2BG9 (upper table) and, for comparison, the surface area buried at antagonist-bound and apo (for 5-HT$_{3A}$) interfaces of other Cys-loop receptors (lower table). Glycine and GABA$_A$ receptors lack an ordered ICD that would contribute to the larger surface area buried in 5-HT$_{3A}$ and *Torpedo*. As such, the GABA$_A$ receptor + bicuculline has remarkably extensive interfaces while the earlier *Torpedo* receptor model is very loosely packed. The relatively weak interface contacts in the earlier *Torpedo* model led us to make the model-map comparisons shown in Figure S8, where we observed that the maps used for building the earlier models likely used an incorrect magnification factor during data processing. The increased pixel size would result in an artifactually larger map and would explain why the molecular interfaces are so loose and the pores are so large in earlier models 2BG9, 4AQ5 and 4AQ9. Surface area analysis was generated by PDBePISA. (F) Comparison of the TMD conformations between our structure and *Torpedo* open state structure (PDB ID: 4AQ9, cyan).



A Torpedo ACh receptor (this study) docked into EMD-2071 map

B Torpedo ACh receptor (this study) docked into EMD-2071 map (rescaled to 90%)

C Corrected subunit register of EMD-2071 map (rescaled) Model: this study

D Original subunit register of EMD-2071 map Model: PDB ID 4AQ5

E Corrected subunit register EMD-2071 Model: this study

F Original subunit register EMD-2071 Model: PDB ID 4AQ5

G Corrected subunit register EMD-2071 Model: this study

H Original subunit register EMD-2071 Model: PDB ID 4AQ5

rescaled map

original map

rescaled map

original map

Figure S8. Putative magnification and subunit register errors in earlier structures of the *Torpedo* ACh receptor

Related to Figure 1

(A) The model from this study (with toxin removed) docked into the earlier closed-state *Torpedo* map (EMD-2071, related to 4AQ5 resting-state model) illustrates the expanded nature of the earlier *Torpedo* maps. (B) We found that rescaling this earlier *Torpedo* map to 90% of its original size resulted in a good fit of the model from the current study. Docking of the current study's model into the EMD-2071 map suggested that in addition to the pixel size discrepancy, the subunit register assigned for the 2BG9, 4AQ5 and 4AQ9 models may also be incorrect. We make this suggestion based on large density blobs in the EMD-2017 map, in the ECD, that align well with the large C-terminal extension and large F-loop insertions present in the δ and γ subunits but absent in the α subunit sequence. (C) What we suggest is the correct subunit register of the model from this study docked into the rescaled EMD-2017 map. (D) PDB 4AQ5 model in its original position in the EMD-2017 map, where the largest blobs of density are assigned to the α and β subunits, in what we suggest is the incorrect subunit register relative to the map. (E) Model and map as in panel C, illustrating fit of C-terminal and loop F substructures in the EMD-2017 map with what we suggest is the correct register. In the earlier model and map, shown in panel (F) what we suggest should be the δ subunit was assigned as the β subunit. Panels (G, H) provide rotated views of the same model-map combinations shown in E and F, wherein what we suggest is the correct subunit register in the map leaves no density extending from the C-termini of the α subunits or at the position of the F loops in the ECD.

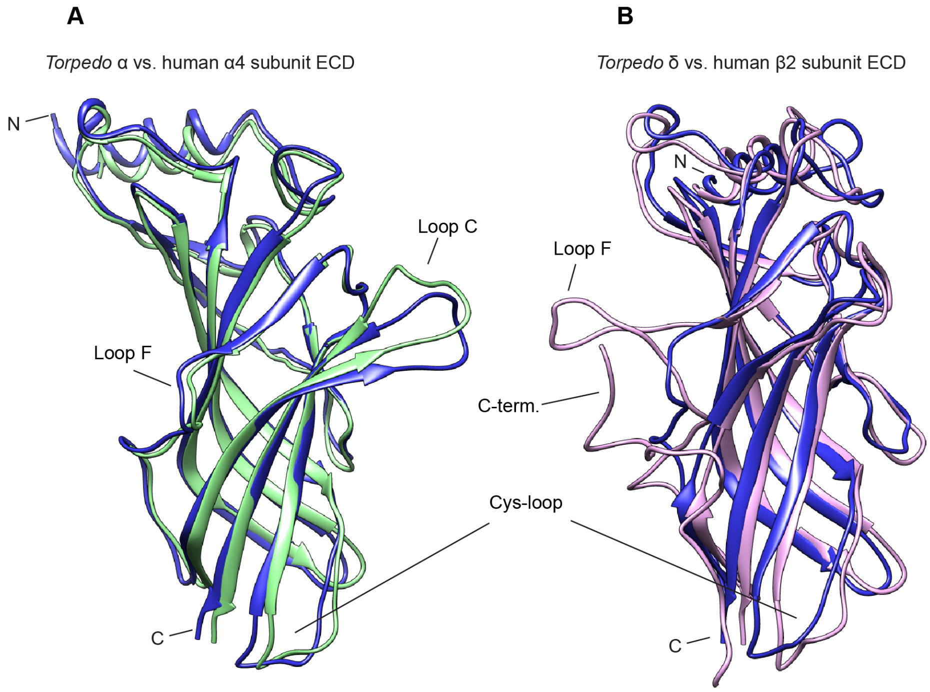
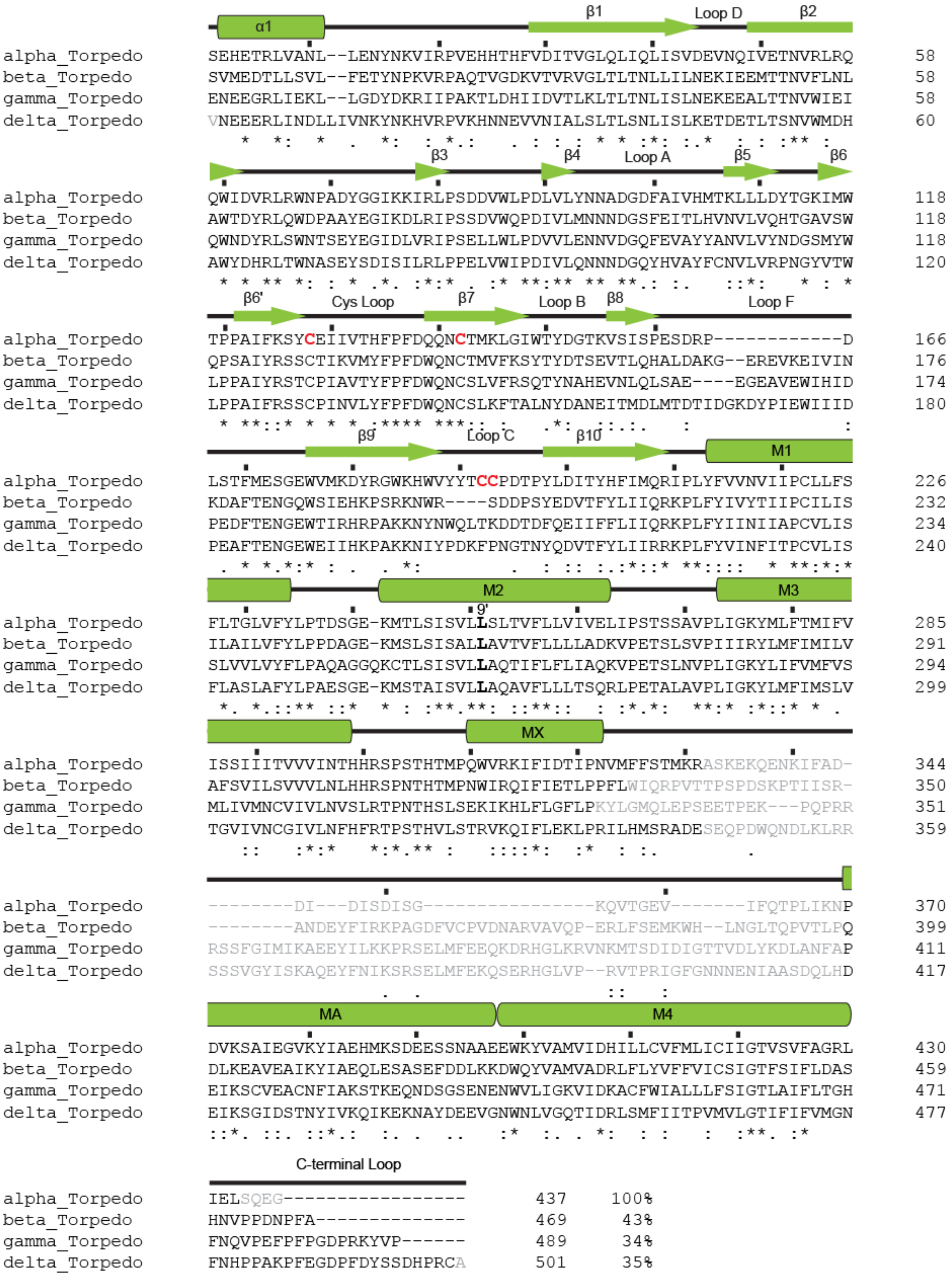


Figure S9. Comparison of the *Torpedo* ECD conformation with human nicotinic receptor, α4β2 (PDB ID: 6CNJ)

Related to Figure 1

(A) Superposition of *Torpedo* α (green) and human α4 (blue) subunits. (B) Superposition of *Torpedo* δ (violet) and human β2 (blue) subunits.



```
alpha_Torpedo      SEHETRLVANL--LENYNKVIRPVEHHTHFVDITVGLQLIQLISVDEVNQIVETNVRLRQ      58
beta_Torpedo       SVMEDTLLSVL--FETYNPKVRPAQTVGDKVTVRVGLTLTNLLILNEKIEEMTTNVFLNL      58
gamma_Torpedo      ENEEGRLIEKL--LGDYDKRIIPAKTLDHIIDVTLKLTLTNLISLNEKEEALTTNVWIEI      58
delta_Torpedo      VNEEERLINDLLIVNKYNKHVRPVKHNNEVVNIALSLTLSNLISLKETDETLTSNVWMDH      60
                      *  *:  *  .  *:  : *.:   . : : : * * :*: :.*  : : :** :

alpha_Torpedo      QWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTKLLLDYTGKIMW      118
beta_Torpedo       AWTDYRLQWDPAAYEGIKDLRIPSSDVWQPDIVLMNNNDGSFEITLHVNVLVQHTGAVSW      118
gamma_Torpedo      QWNDYRLSWNTSEYEGIDLVRIPSELLWLPDVVLENNVDGQFEVAYYANVLVYNDGSMYW      118
delta_Torpedo      AWYDHRLTWNASEYSDISILRLPPELVWIPDIVLQNNNDGQYHVAYFCNVLVRPNGYVTW      120
                    * * ** *: : * .*. :*:* . :* **:** ** **.: :.   ::*:   * : *

alpha_Torpedo      TPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSISPESDRP------------D      166
beta_Torpedo       QPSAIYRSSCTIKVMYFPFDWQNCTMVFKSYTYDTSEVTLQHALDAKG--EREVKEIVIN      176
gamma_Torpedo      LPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQLSAE----EGEAVEWIHID      174
delta_Torpedo      LPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYDANEITMDLMTDTIDGKDYPIEWIIID      180
                    * **::* * * * .**** ***:: :   .*:  :.:.    :              :

alpha_Torpedo      LSTFMESGEWVMKDYRGWKHWVYYTCCPDTPYLDITYHFIMQRIPLYFVVNVIIPCLLFS      226
beta_Torpedo       KDAFTENGQWSIEHKPSRKNWR----SDDPSYEDVTFYLIIQRKPLFYIVYTIIPCILIS      232
gamma_Torpedo      PEDFTENGEWTIRHRPAKKNYNWQLTKDDTDFQEIIFFLIIQRKPLFYIINIIAPCVLIS      234
delta_Torpedo      PEAFTENGEWEIIHKPAKKNIYPDKFPNGTNYQDVTFYLIIRRKPLFYVINFITPCVLIS      240
                    . * *.*:* : .  . *:        .  : :: :.:*::* **::::  * **:*:*

alpha_Torpedo      FLTGLVFYLPTDSGE-KMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFV      285
beta_Torpedo       ILAILVFYLPPDAGE-KMSLSISALLAVTVFLLLLADKVPETSLSVPIIIRYLMFIMILV      291
gamma_Torpedo      SLVVLVYFLPAQAGGQKCTLSISVLLAQTIFLFLIAQKVPETSLNVPLIGKYLIFVMFVS      294
delta_Torpedo      FLASLAFYLPAESGE-KMSTAISVLLAQAVFLLLTSQRLPETALAVPLIGKYLMFIMSLV      299
                    *. *.::** ::*  * : :**.**: ::**::  : :*.*:  **:* :*::* * .

alpha_Torpedo      ISSIIITVVVINTHHRSPSTHTMPQWVRKIFIDTIPNVMFFSTMKRASKEKQENKIFAD-      344
beta_Torpedo       AFSVILSVVVLNLHHRSPNTHTMPNWIRQIFIETLPPFLWIQRPVTTPSPDSKPTIISR-      350
gamma_Torpedo      MLIVMNCVIVLNVSLRTPNTHSLSEKIKHLFLGFLPKYLGMQLEPSEETPEK---PQPRR      351
delta_Torpedo      TGVIVNCGIVLNFHFRTPSTHVLSTRVKQIFLEKLPRILHMSRADESEQPDWQNDLKLRR      359
                      ::    :*:*   *:*.** :   ::::*:  :*  : :.        .

alpha_Torpedo      --------DI---DISDISG----------------KQVTGEV-------IFQTPLIKNP      370
beta_Torpedo       --------ANDEYFIRKPAGDFVCPVDNARVAVQP-ERLFSEMKWH--LNGLTQPVTLPQ      399
gamma_Torpedo      RSSFGIMIKAEEYILKKPRSELMFEEQKDRHGLKRVNKMTSDIDIGTTVDLYKDLANFAP      411
delta_Torpedo      SSSVGYISKAQEYFNIKSRSELMFEKQSERHGLVP--RVTPRIGFGNNNENIAASDQLHD      417
                                   .  .                 ::   :

alpha_Torpedo      DVKSAIEGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFMLICIIGTVSVFAGRL      430
beta_Torpedo       DLKEAVEAIKYIAEQLESASEFDDLKKDWQYVAMVADRLFLYVFFVICSIGTFSIFLDAS      459
gamma_Torpedo      EIKSCVEACNFIAKSTKEQNDSGSENENWVLIGKVIDKACFWIALLLFSIGTLAIFLTGH      471
delta_Torpedo      EIKSGIDSTNYIVKQIKEKNAYDEEVGNWNLVGQTIDRLSMFIITPVMVLGTIFIFVMGN      477
                   ::*. ::. ::*.:  :. .  ..   :*  :. . *:  : :   :  :**. :*

alpha_Torpedo      IELSQEG-----------------       437    100%
beta_Torpedo       HNVPPDNPFA--------------       469     43%
gamma_Torpedo      FNQVPEFPFPGDPRKYVP------       489     34%
delta_Torpedo      FNHPPAKPFEGDPFDYSSDHPRCA       501     35%
```

Figure SI1: Sequence alignment of *Torpedo* nicotinic receptor subunits.

Related to Figure 1

The residues of unmodeled regions are in light grey. Sequence identity percent is listed at the end and is compared to the α subunit from *Torpedo*.

```
alpha_Torpedo      SEHETRLVANLLE--NYNKVIRPVEHHTHFVDITVGLQLIQLISVDEVNQIVETNVRLRQ   58
alpha1_Human       SEHETRLVAKLFK--DYSSVVRPVEDHRQVVEVTVGLQLIQLINVDEVNQIVTTNVRLKQ   58
alpha3_Human       SEAEHRLFERLFE--DYNEIIRPVANVSDPVIIHFEVSMSQLVKVDEVNQIMETNLWLKQ   58
alpha4_Human       AHAEERLLKKLFS--GYNKWSRPVANISDVVLVRFGLSIAQLIDVDEKNQMMTTNVWVKQ   58
alpha7_Human       GEFQRKLYKELVK--NYNPLERPVANDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQM   58
alpha9_Human       GKYAQKLFNDLFE--DYSNALRPVEDTDKVLNVTLQITLSQIKDMDERNQILTAYLWIRQ   58
beta_Torpedo       SVMEDTLLSVLFE--TYNPKVRPAQTVGDKVTVRVGLTLTNLLILNEKIEEMTTNVFLNL   58
beta1_Human        SEAEGRLRGKLFS--GYDSSVRPAREVGDRVRVSVGLILAQLISLNEKDEEMSTKVYLDL   58
gamma_Torpedo      ENEEGRLIEKLLG--DYDKRIIPAKTLDHIIDVTLKLTLTNLISLNEKEEALTTNVWIEI   58
gamma_Human        RNQEERLLADLMQ--NYDPNLRPAERDSDVVNVSLKLTLTNLISLNEREEALTTNVWIEM   58
epsilon_Human      KNEELRLYHHLFN--NYDPGSRPVREPEDTVTISLKVTLTNLISLNEKEETLTTSVWIGI   58
delta_Torpedo      VNEEERLINDLLIVNKYNKHVRPVKHNNEVVNIALSLTLSNLISLKETDETLTSNVWMDH   60
delta_Human        LNEEERLIRHLFQEKGYNKELRPVAHKEESVDVALALTLSNLISLKEVEETLTTNVWIEH   60
                         *   *.    *.    *.    . : : . : : ::  :.*  : : : : :
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```
alpha_Torpedo      QWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTKLLLDYTGKIMW   118
alpha1_Human       QWVDYNLKWNPDDYGGVKKIHIPSEKIWRPDLVLYNNADGDFAIVKFTKVLLQYTGHITW   118
alpha3_Human       IWNDYKLKWNPSDYGGAEFMRVPAQKIWKPDIVLYNNAVGDFQVDDKTKALLKYTGEVTW   118
alpha4_Human       EWHDYKLRWDPADYENVTSIRIPSELIWRPDIVLYNNADGDFAVTHLTKAHLFHDGRVQW   118
alpha7_Human       SWTDHYLQWNVSEYPGVKTVRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQY   118
alpha9_Human       IWHDAYLTWDRDQYDGLDSIRIPSDLVWRPDIVLYNKADDESSEPVNTNVVLRYDGLITW   118
beta_Torpedo       AWTDYRLQWDPAAYEGIKDLRIPSSDVWQPDIVLMNNNDGSFEITLHVNVLVQHTGAVSW   118
beta1_Human        EWTDYRLSWDPAEHDGIDSLRITAESVWLPDVVLLNNNDGNFYVALDISVVVSSDGSVRW   118
gamma_Torpedo      QWNDYRLSWNTSEYEGIDLVRIPSELLWLPDVVLENNVDGQFEVAYYANVLVYNDGSMYW   118
gamma_Human        QWCDYRLRWDPRDYEGLWVLRVPSTMVWRPDIVLENNVDGVFEVALYCNVLVSPDGCIYW   118
epsilon_Human      DWQDYRLNYSKDDFGGIETLRVPSELVWLPEIVLENNIDGQFGVAYDANVLVYEGGSVTW   118
delta_Torpedo      AWYDHRLTWNASEYSDISILRLPPELVWIPDIVLQNNNDGQYHVAYFCNVLVRPNGYVTW   120
delta_Human        GWTDNRLKWNAEEFGNISVLRLPPDMVWLPEIVLENNNDGSFQISYSCNVLVYHYGFVYW   120
                    * *  * :.   . .   ::.    :* *:::* *.           .  :   *   :
```

```
alpha_Torpedo      TPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSISPESDR------------PD   166
alpha1_Human       TPPAIFKSYCEIIVTHFPFDEQNCSMKLGTWTYDGSVVAINPESDQ------------PD   166
alpha3_Human       IPPAIFKSSCKIDVTYFPFDYQNCTMKFGSWSYDKAKIDLVLIGSS------------MN   166
alpha4_Human       TPPAIYKSSCSIDVTFFPFDQQNCTMKFGSWTYDKAKIDLVNMHSR------------VD   166
alpha7_Human       LPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQM--QE------------AD   164
alpha9_Human       DAPAITKSSCVVDVTYFPFDNQQCNLTFGSWTYNGNQVDIFNALDS------------GD   166
beta_Torpedo       QPSAIYRSSCTIKVMYFPFDWQNCTMVFKSYTYDTSEVTLQHALDAKGER--EVKEIVIN   176
beta1_Human        QPPGIYRSSCSIQVTYFPFDWQNCTMVFSSYSYDSSEVSLQTGLGPDGQG---HQEIHIH   175
gamma_Torpedo      LPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQLSA----EEGEAVEWIHID   174
gamma_Human        LPPAIFRSACSISVTYFPFDWQNCSLIFQSQTYSTNEIDLQLSQ----EDGQTIEWIFID   174
epsilon_Human      LPPAIYRSVCAVEVTYFPFDWQNCSLIFRSQTYNAEEVEFTFAVD---NDGKTINKIDID   175
delta_Torpedo      LPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYDANEITMDLMTDTIDGKDYPIEWIIID   180
delta_Human        LPPAIFRSSCPISVTYFPFDWQNCSLKFSSLKYTAKEITLSLKQDAKENRTYPVEWIIID   180
                      .* :* * : *  **** *:*.: :   .*    : :                   .
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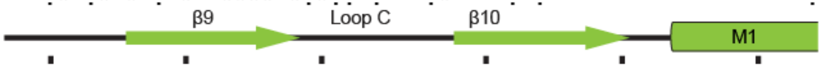


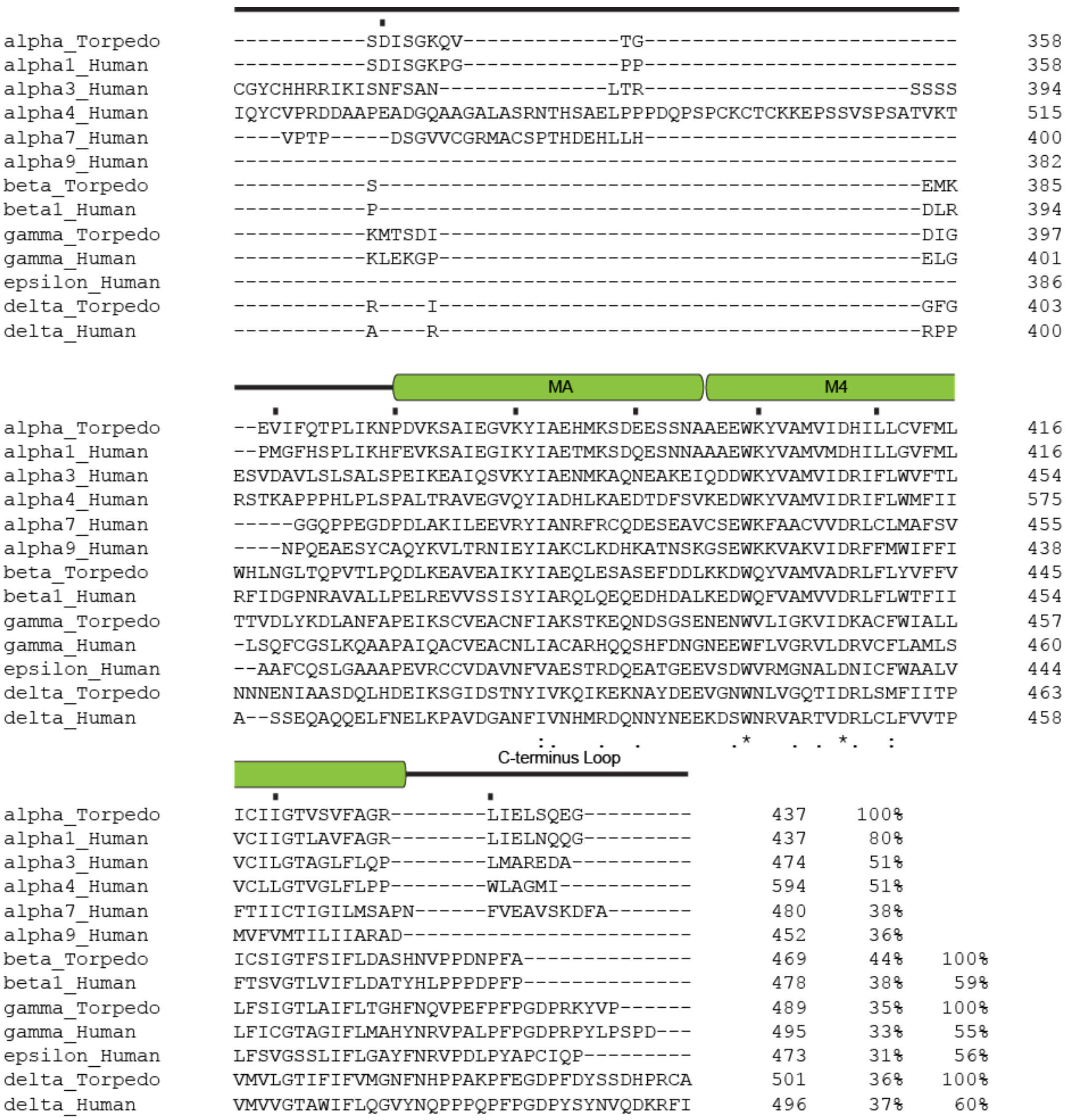
```
alpha_Torpedo      LSTFMESGEWVMKDYRGWKHWVYYTCCPDT--PYLDITYHFIMQRIPLYFVVNVIIPCLL   224
alpha1_Human       LSNFMESGEWVIKESRGWKHSVTYSCCPDT--PYLDITYHFVMQRLPLYFIVNVIIPCLL   224
alpha3_Human       LKDYWESGEWAIIKAPGYKHDIKYNCCEE---IYPDITYSLYIRRLPLFYTINLIIPCLL   223
alpha4_Human       QLDFWESGEWVIVDAVGTYNTRKYECCAE---IYPDITYAFVIRRLPLFYTINLIIPCLL   223
alpha7_Human       ISGYIPNGEWDLVGIPGKRSERFYECCKE---PYPDVTFTVTMRRRTLYYGLNLLIPCVL   221
alpha9_Human       LSDFIEDVEWEVHGMPAVKNVISYGCCSE---PYPDVTFTLLLKRRSSFYIVNLLIPCVL   223
beta_Torpedo       KDAFTENGQWSIEHKPSRKNWRSDDP------SYEDVTFYLIIQRKPLFYIVYTIIPCIL   230
beta1_Human        EGTFIENGQWEIIHKPSRLIQPPGDPRGGREGQRQEVIFYLIIRRKPLFYLVNVIAPCIL   235
gamma_Torpedo      PEDFTENGEWTIRHRPAKKNYNWQLTKDDT--DFQEIIFFLIIQRKPLFYIINIIAPCVL   232
gamma_Human        PEAFTENGEWAIQHRPAKMLLDPAAPAQEA--GHQKVVFYLLIQRKPLFYVINIIAPCVL   232
epsilon_Human      TEAYTENGEWAIDFCPGVIRRHHGGATDGP--GETDVIYSLIIRRKPLFYVINIIVPCVL   233
delta_Torpedo      PEAFTENGEWEIIHKPAKKNIYPDKFPNGT--NYQDVTFYLIIRRKPLFYVINFITPCVL   238
delta_Human        PEGFTENGEWEIVHRPARVNVDPRAPLDSP--SRQDITFYLIIRRKPLFYIINILVPCVL   238
                      :  . :* :    .                  .: : . ::*   :: :  : **:*
```

```
alpha_Torpedo       FSFLTGLVFYLPTDSG-EKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMI       283
alpha1_Human        FSFLTGLVFYLPTDSG-EKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMV       283
alpha3_Human        ISFLTVLVFYLPSDCG-EKVTLCISVLLSLTVFLLVITETIPSTSLVIPLIGEYLLFTMI       282
alpha4_Human        ISCLTVLVFYLPSECG-EKITLCISVLLSLTVFLLLITEIIPSTSLVIPLIGEYLLFTMI       282
alpha7_Human        ISALALLVFLLPADSG-EKISLGITVLLSLTVFMLLVAEIMPATSDSVPLIAQYFASTMI       280
alpha9_Human        ISFLAPLSFYLPAASG-EKVSLGVTILLAMTVFQLMVAEIMPAS-ENVPLIGKYYIATMA       281
beta_Torpedo        ISILAILVFYLPPDAG-EKMSLSISALLAVTVFLLLLADKVPETSLSVPIIIRYLMFIMI       289
beta1_Human         ITLLAIFVFYLPPDAG-EKMGLSIFALLTLTVFLLLLADKVPETSLSVPIIIKYLMFTMV       294
gamma_Torpedo       ISSLVVLVYFLPAQAGGQKCTLSISVLLAQTIFLFLIAQKVPETSLNVPLIGKYLIFVMF       292
gamma_Human         ISSVAILIHFLPAKAGGQKCTVAINVLLAQTVFLFLVAKKVPETSQAVPLISKYLTFLLV       292
epsilon_Human       ISGLVLLAYFLPAQAGGQKCTVSINVLLAQTVFLFLIAQKIPETSLSVPLLGRFLIFVMV       293
delta_Torpedo       ISFLASLAFYLPAESG-EKMSTAISVLLAQAVFLLLTSQRLPETALAVPLIGKYLMFIMS       297
delta_Human         ISFMVNLVFYLPADSG-EKTSVAISVLLAQSVFLLLISKRLPATSMAIPLIGKFLLFGMV       297
                    :: :. : . **  .* :*    :  **: ::* ::  . :* :   :*:: .:    :
```

```
alpha_Torpedo       FVISSIIITVVVINTHHRSPSTHTMPQWVRKIFIDTIPNVMFFSTMKRASKEKQE----N       339
alpha1_Human        FVIASIIITVIVINTHHRSPSTHVMPNWVRKVFIDTIPNIMFFSTMKRPSREKQD----K       339
alpha3_Human        FVTLSIVITVFVLNVHYRTPTTHTMPSWVKTVFLNLLPRVMFMTR---PTSNEGN----A       335
alpha4_Human        FVTLSIVITVFVLNVHHRSPRTHTMPTWVRRVFLDIVPRLLLMKR---PSVVKDN----C       335
alpha7_Human        IVGLSVVVTVIVLQYHHHDPDGGKMPKWTRVILLNWCAWFLR---MKRPGEDKVRPA--C       335
alpha9_Human        LITASTALTIMVMNIHFCGAEARPVPHWARVVILKYMSRVLFVYDVGESCLSPH-----H       336
beta_Torpedo        LVAFSVILSVVVLNLHHRSPNTHTMPNWIRQIFIETLPPFLWIQR---PVTTPSP-----       341
beta1_Human         LVTFSVILSVVVLNLHHRSPHTHQMPLWVRQIFIHKLPLYLRLKR---PKPERDLMP--E       349
gamma_Torpedo       VSMLIVMNCVIVLNVSLRTPNTHSLSEKIKHLFLGFLPKYLGMQL---EPSEETPEK---       346
gamma_Human         VTILIVVNAVVVLNVSLRSPHTHSMARGVRKVFLRLLPQLLRMHV---RPLAPAAVQDTQ       349
epsilon_Human       VATLIVMNCVIVLNVSQRTPTTHAMSPRLRHVLLELLPRLLGSPP---PPEA-PRAA---       346
delta_Torpedo       LVTGVIVNCGIVLNFHFRTPSTHVLSTRVKQIFLEKLPRILHMSR---ADESEQPDWQND       354
delta_Human         LVTMVVVICVIVLNIHFRTPSTHVLSEGVKKLFLETLPELLHMSR---PAEDGPS---PG       351
                    .         .*::          :    : :::      :
```

```
alpha_Torpedo       K-------------------------------------IFADDIDI--------------       348
alpha1_Human        K-------------------------------------IFTEDIDI--------------       348
alpha3_Human        QKPR---------------------------------PLYGAEL-----SNLNCFSR---       354
alpha4_Human        RRLIESMHKMASAPRFWPEPEGEPPATSGTQSLHPPSPSFCVPLDVPAEPGPSCKSPSDQ       395
alpha7_Human        QHKQRR----CSLASVEMSAVAPPPASNGNL------------LY---------------       364
alpha9_Human        SRERDHLTKVYSK-----LPE----------------------------S----------       353
beta_Torpedo        ----DS-------KPT--------------------------IISRANDEYFIRKPAGDF       364
beta1_Human         PPHCSS-------PGS--------------------------GWGRGTDEYFIRKPPSDF       376
gamma_Torpedo       PQPRRR-------SSF--------------------------GIMIKAEEYILKKPRSEL       373
gamma_Human         SRLQNG-------SSG--------------------------WSITTGEEVALCLPRSEL       376
epsilon_Human       SPPRRA-------SSV--------------------------GLLLRAEELILKKPRSEL       373
delta_Torpedo       LKLRRS-------SSV--------------------------GYISKAQEYFNIKSRSEL       381
delta_Human         ALVRRS-------SSL--------------------------GYISKAEEYFLLKSRSDL       378
```

```
alpha_Torpedo       ------------------------------------------------------------       348
alpha1_Human        ------------------------------------------------------------       348
alpha3_Human        --------------------------------------------AESKGCKEGYPCQDGM       370
alpha4_Human        LPPQQPLEAEKASPHPSPGPCRPPHGTQAPGLAKARSLSVQHMSSPGEAVEGGVRCRSRS       455
alpha7_Human        ---------------------------------------------IGFRGLDGVHC----       375
alpha9_Human        ------------------------------NLKAARNKDLSRKKDMNKRLKNDLGCQGK-       382
beta_Torpedo        VCPVDNAR----------------V-----AVQPERLF----------------------       381
beta1_Human         LFPKPNR------------------------FQPELSA----------------------       390
gamma_Torpedo       MFEEQKDR----------------H-----GLKR--VN----------------------       388
gamma_Human         LFQQWQ-R----------------Q-----GLVAAALE----------------------       392
epsilon_Human       VFEGQRHR----------------Q-----GTWT--------------------------       386
delta_Torpedo       MFEKQSER----------------H-----GLVPRVTP----------------------       398
delta_Human         MFEKQSER----------------H-----GLARRLTT----------------------       395
```



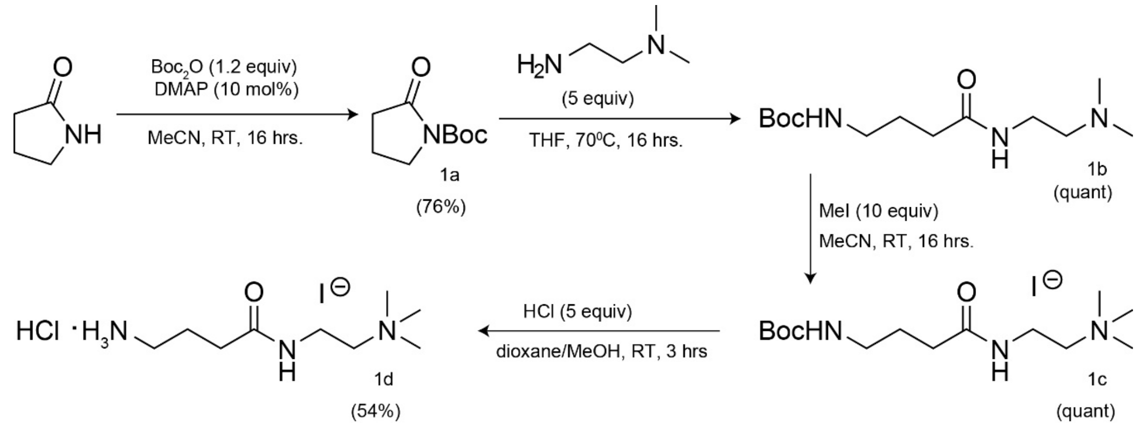
```
alpha_Torpedo      -----------SDISGKQV-------------TG--------------------------      358
alpha1_Human       -----------SDISGKPG-------------PP--------------------------      358
alpha3_Human       CGYCHHRRIKISNFSAN--------------LTR----------------------SSSS      394
alpha4_Human       IQYCVPRDDAAPEADGQAAGALASRNTHSAELPPPDQPSPCKCTCKKEPSSVSPSATVKT      515
alpha7_Human       ----VPTP-----DSGVVCGRMACSPTHDEHLLH--------------------------      400
alpha9_Human       ------------------------------------------------------------      382
beta_Torpedo       -----------S---------------------------------------------EMK      385
beta1_Human        -----------P---------------------------------------------DLR      394
gamma_Torpedo      -----------KMTSDI----------------------------------------DIG      397
gamma_Human        -----------KLEKGP----------------------------------------ELG      401
epsilon_Human      ------------------------------------------------------------      386
delta_Torpedo      -----------R----I----------------------------------------GFG      403
delta_Human        -----------A----R----------------------------------------RPP      400

alpha_Torpedo      --EVIFQTPLIKNPDVKSAIEGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFML      416
alpha1_Human       --PMGFHSPLIKHFEVKSAIEGIKYIAETMKSDQESNNAAAEWKYVAMVMDHILLGVFML      416
alpha3_Human       ESVDAVLSLSALSPEIKEAIQSVKYIAENMKAQNEAKEIQDDWKYVAMVIDRIFLWVFTL      454
alpha4_Human       RSTKAPPPHLPLSPALTRAVEGVQYIADHLKAEDTDFSVKEDWKYVAMVIDRIFLWMFII      575
alpha7_Human       -----GGQPPEGDPDLAKILEEVRYIANRFRCQDESEAVCSEWKFAACVVDRLCLMAFSV      455
alpha9_Human       ----NPQEAESYCAQYKVLTRNIEYIAKCLKDHKATNSKGSEWKKVAKVIDRFFMWIFFI      438
beta_Torpedo       WHLNGLTQPVTLPQDLKEAVEAIKYIAEQLESASEFDDLKKDWQYVAMVADRLFLYVFFV      445
beta1_Human        RFIDGPNRAVALLPELREVVSSISYIARQLQEQEDHDALKEDWQFVAMVVDRLFLWTFII      454
gamma_Torpedo      TTVDLYKDLANFAPEIKSCVEACNFIAKSTKEQNDSGSENENWVLIGKVIDKACFWIALL      457
gamma_Human        -LSQFCGSLKQAAPAIQACVEACNLIACARHQQSHFDNGNEEWFLVGRVLDRVCFLAMLS      460
epsilon_Human      --AAFCQSLGAAAPEVRCCVDAVNFVAESTRDQEATGEEVSDWVRMGNALDNICFWAALV      444
delta_Torpedo      NNNENIAASDQLHDEIKSGIDSTNYIVKQIKEKNAYDEEVGNWNLVGQTIDRLSMFIITP      463
delta_Human        A--SSEQAQQELFNELKPAVDGANFIVNHMRDQNNYNEEKDSWNRVARTVDRLCLFVVTP      458
                                            :.   .  .       .*   . . *.  :

alpha_Torpedo      ICIIGTVSVFAGR--------LIELSQEG---------       437    100%
alpha1_Human       VCIIGTLAVFAGR--------LIELNQQG---------       437     80%
alpha3_Human       VCILGTAGLFLQP--------LMAREDA----------       474     51%
alpha4_Human       VCLLGTVGLFLPP--------WLAGMI-----------       594     51%
alpha7_Human       FTIICTIGILMSAPN------FVEAVSKDFA-------       480     38%
alpha9_Human       MVFVMTILIIARAD------------------------       452     36%
beta_Torpedo       ICSIGTFSIFLDASHNVPPDNPFA--------------       469     44%   100%
beta1_Human        FTSVGTLVIFLDATYHLPPPDPFP--------------       478     38%    59%
gamma_Torpedo      LFSIGTLAIFLTGHFNQVPEFPFPGDPRKYVP------       489     35%   100%
gamma_Human        LFICGTAGIFLMAHYNRVPALPFPGDPRPYLPSPD---       495     33%    55%
epsilon_Human      LFSVGSSLIFLGAYFNRVPDLPYAPCIQP---------       473     31%    56%
delta_Torpedo      VMVLGTIFIFVMGNFNHPPAKPFEGDPFDYSSDHPRCA       501     36%   100%
delta_Human        VMVVGTAWIFLQGVYNQPPPQPFPGDPYSYNVQDKRFI       496     37%    60%
```

Figure SI2. Sequence alignment of *Torpedo* and human muscle nicotinic receptor subunits with human α subunits

Related to Figure 1

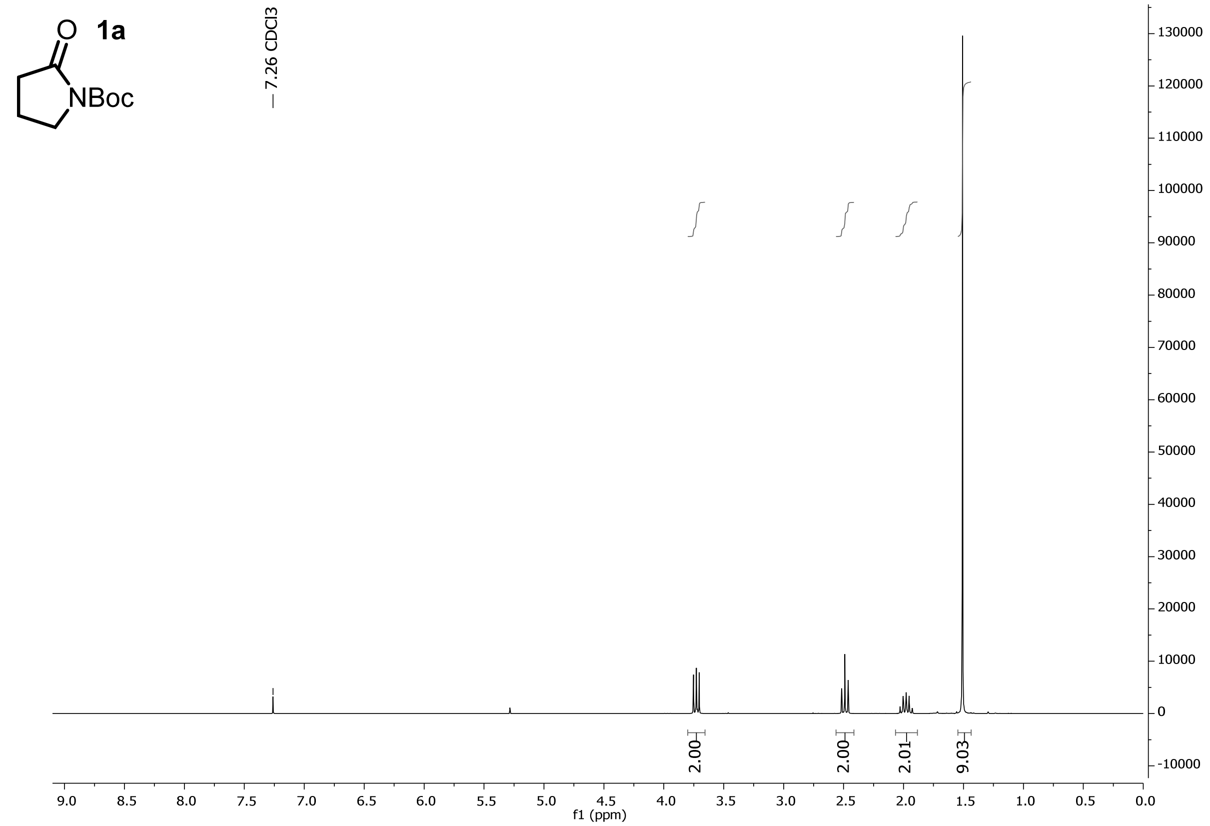
Sequence identity percent is listed at the end. The first column is compared to the α subunit from *Torpedo*. The second column is % sequence identity compared to the corresponding subunit from *Torpedo*.

A

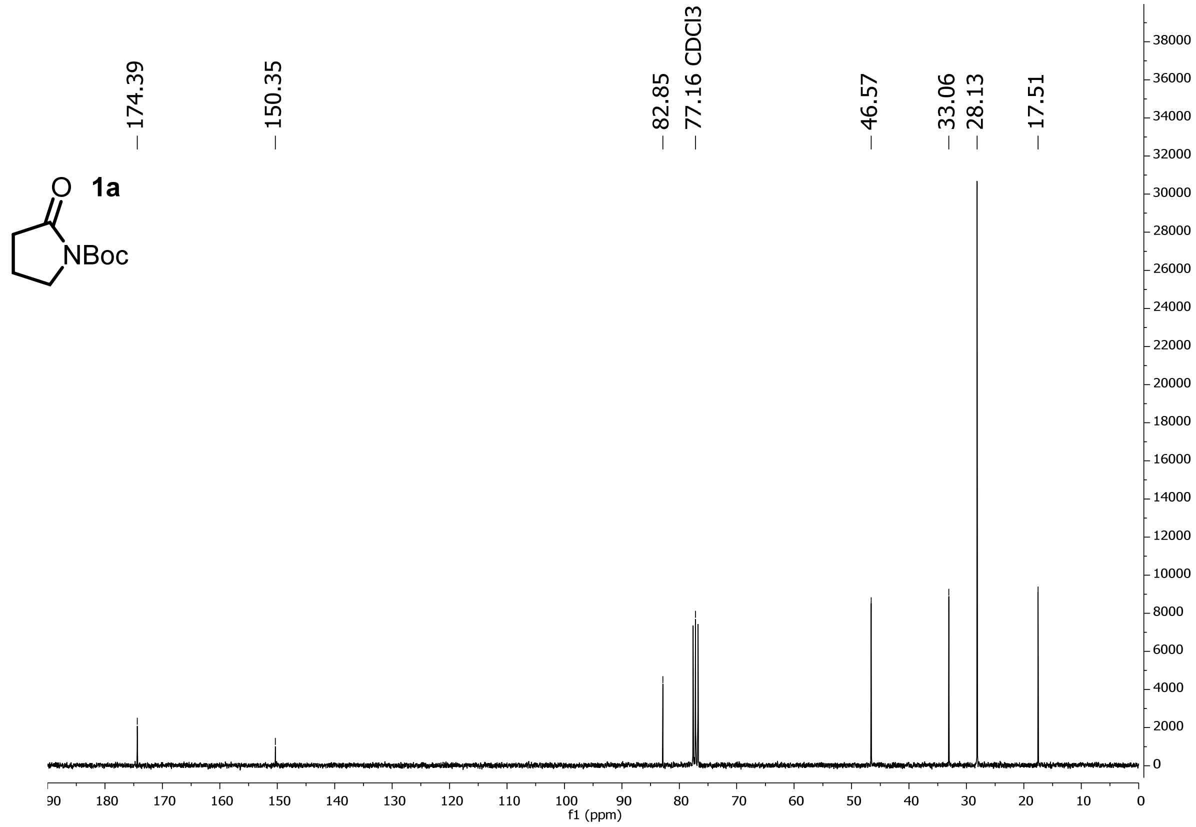


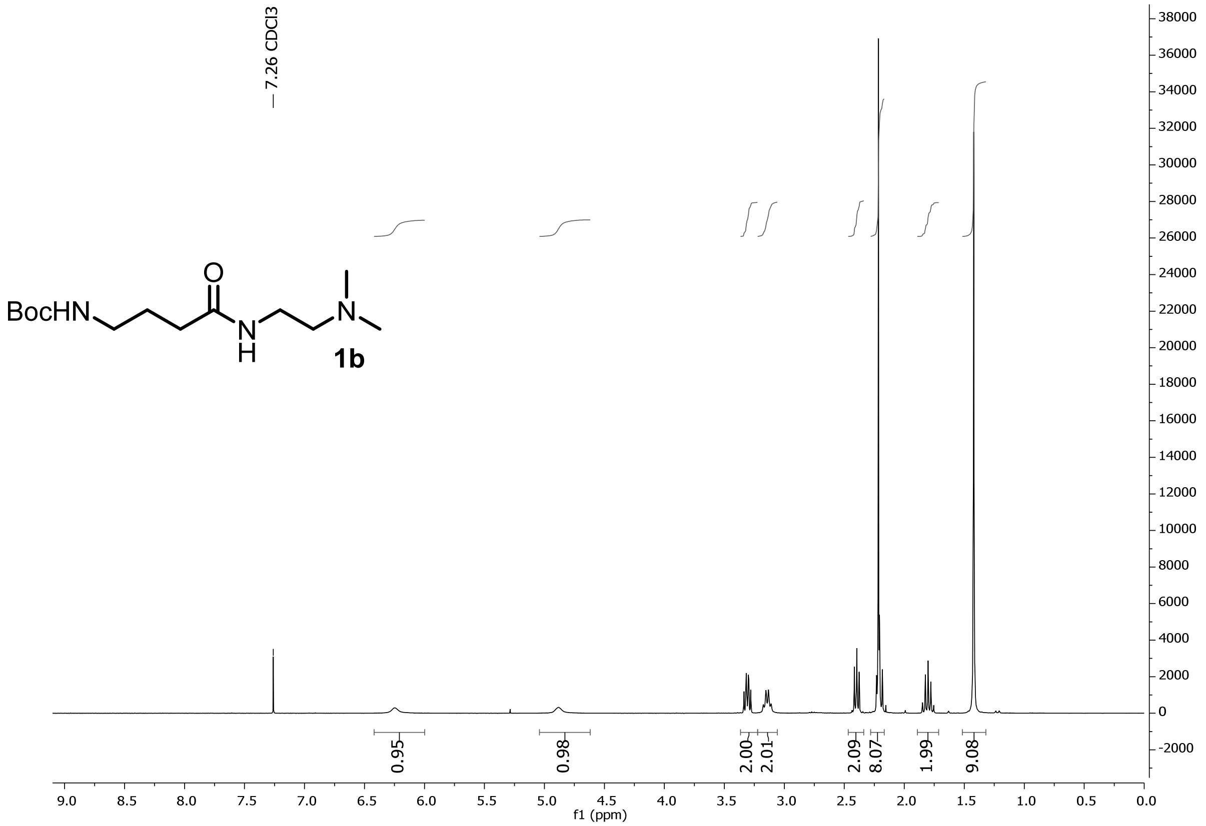
Boc_2O (1.2 equiv)
DMAP (10 mol%)
MeCN, RT, 16 hrs.
1a
(76%)
(5 equiv)
THF, 70⁰C, 16 hrs.
BocHN
1b
(quant)
MeI (10 equiv)
MeCN, RT, 16 hrs.
1c
(quant)
HCl (5 equiv)
dioxane/MeOH, RT, 3 hrs
HCl · H_3N
1d
(54%)

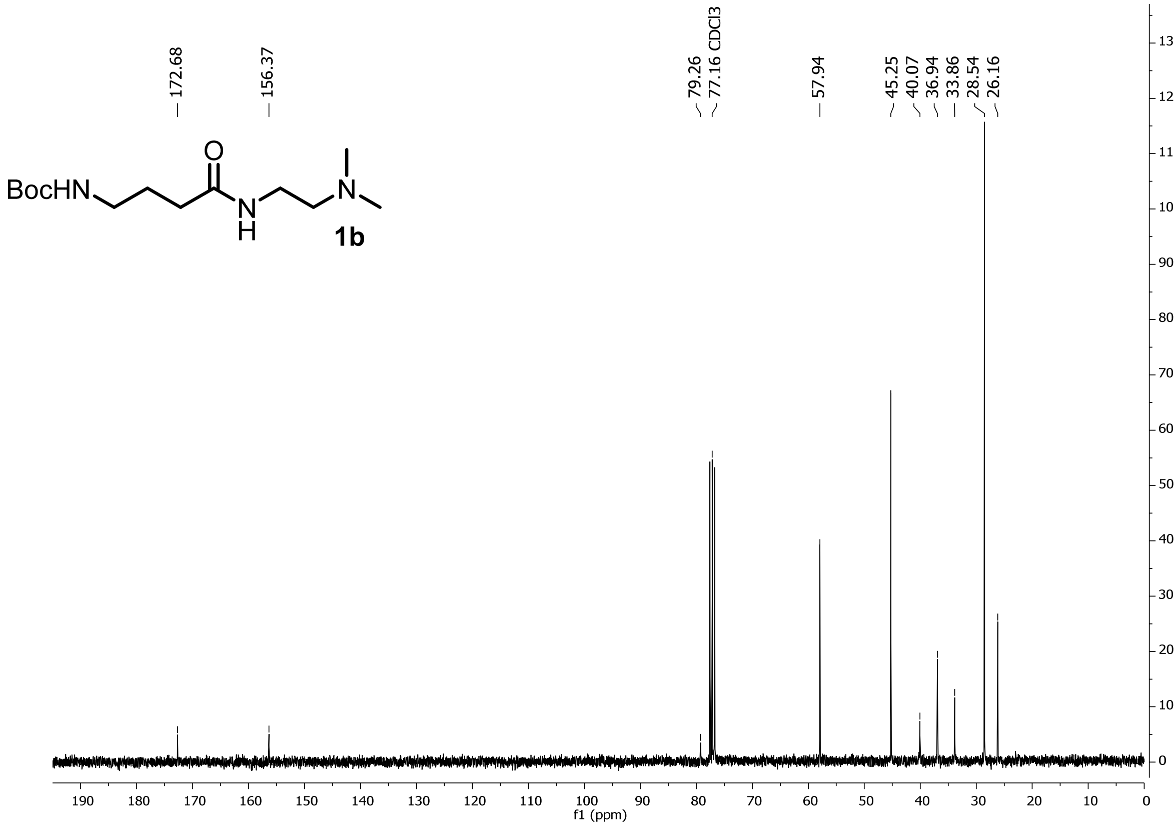
B

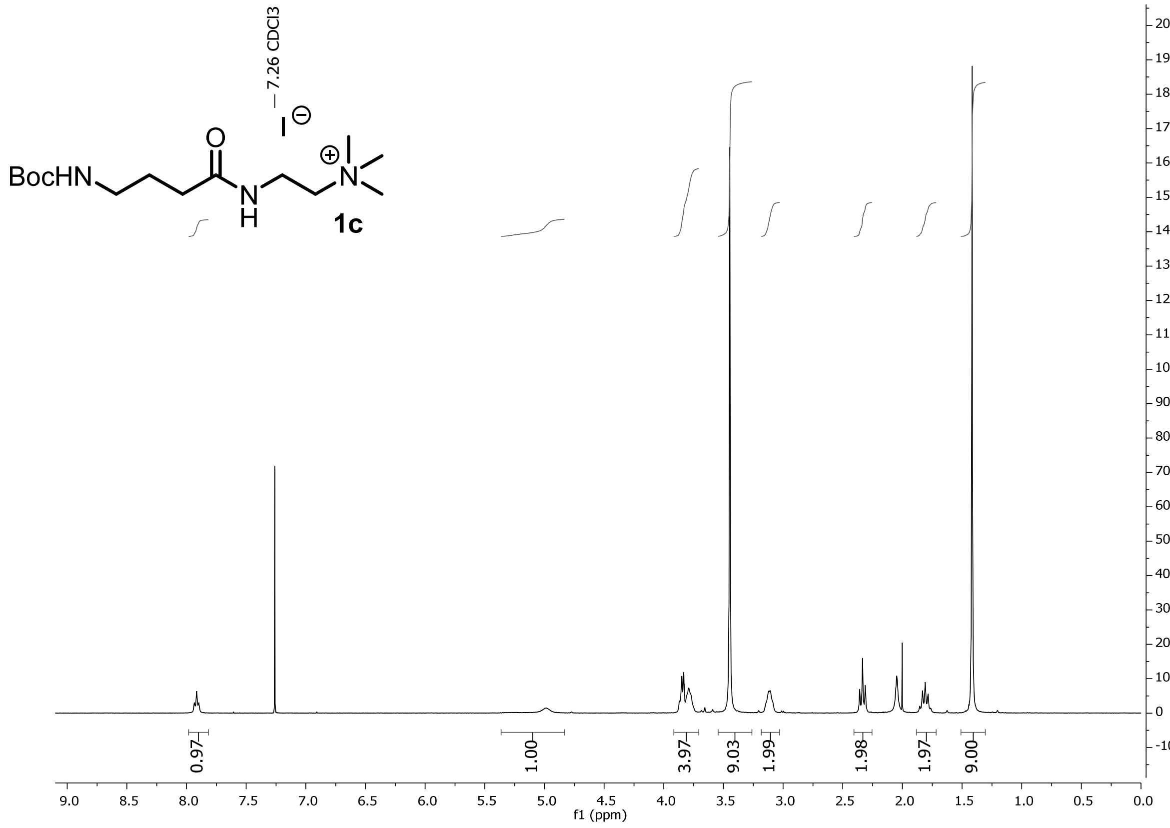


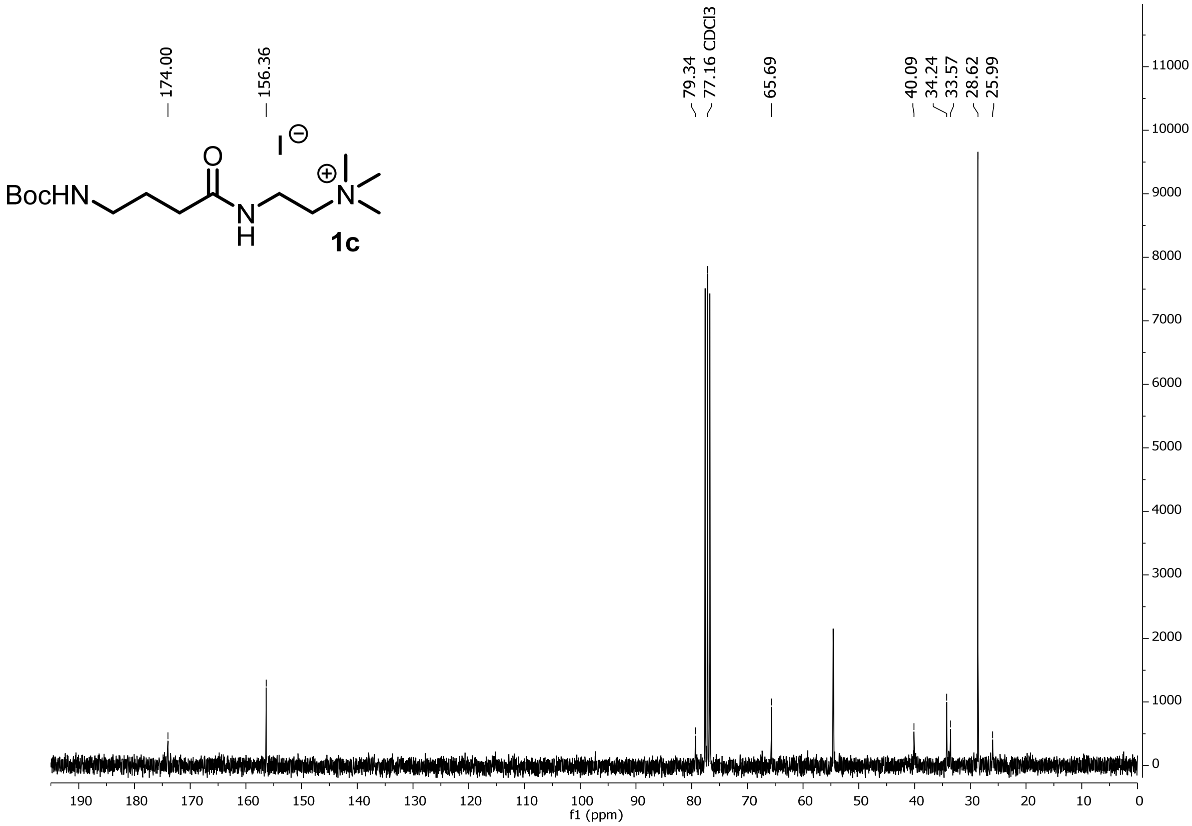
1a
NBoc
7.26 CDCl3
2.00
2.00
2.01
9.03
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000


1a
174.39
150.35
82.85
77.16 CDCl3
46.57
33.06
28.13
17.51
NBoc
O
f1 (ppm)
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
38000 36000 34000 32000 30000 28000 26000 24000 22000 20000 18000 16000 14000 12000 10000 8000 6000 4000 2000 0

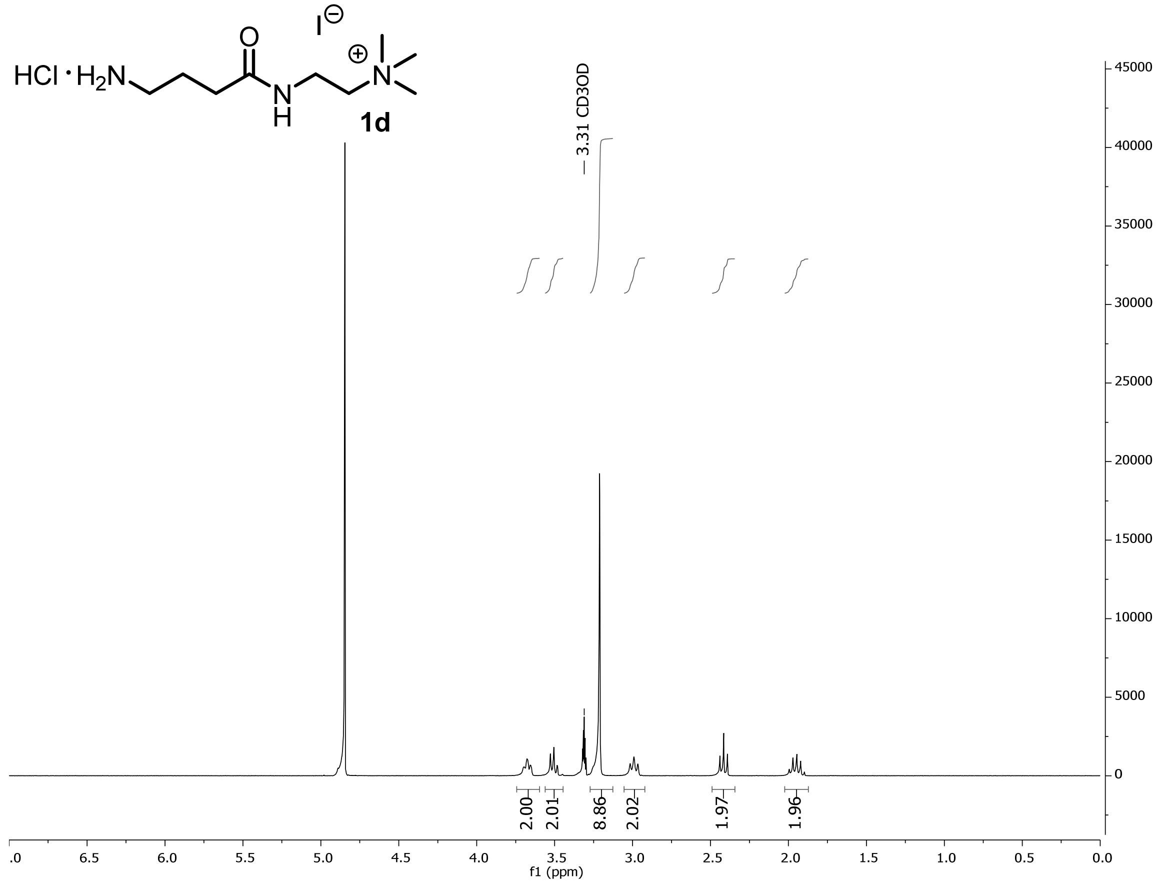

1b
7.26 CDCl3
BocHN
O
N
H
N
0.95
0.98
2.00
2.01
2.09
8.07
1.99
9.08
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
38000 36000 34000 32000 30000 28000 26000 24000 22000 20000 18000 16000 14000 12000 10000 8000 6000 4000 2000 0 -2000


172.68
156.37
79.26
77.16 CDCl3
57.94
45.25
40.07
36.94
33.86
28.54
26.16
BocHN
O
N
H
N
1b
f1 (ppm)


7.26 CDCl3
I ⊖
BocHN
O
N
H
⊕ N
1c
0.97
1.00
3.97
9.03
1.99
1.98
1.97
9.00
f1 (ppm)


1c

BocHN
O
N
H
I⊖
⊕N
174.00
156.36
79.34
77.16 CDCl3
65.69
40.09
34.24
33.57
28.62
25.99
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


1d

HCl · H2N
O
N
H
I⊖
⊕N
3.31 CD3OD
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
2.00
2.01
8.86
2.02
1.97
1.96
.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure SI 3: (A) Overview of ATM (2-[(4-aminobutanoyl)amino]-*N*,*N*,*N*-trimethylethanaminium) affinity reagent synthesis and their representative NMR Spectra.

Related to STAR Methods

Table SI1. Data collection and refinement statistics

Related to Figure 1 and STAR Methods

Data collection	
Microscope	FEI Titan Krios, OHSU
Magnification	130KX
Voltage (ke$^-$V)	300
Exposure time (s)	1
Number of frames	50
Electron exposure (e$^-$/Å^2)	35
Detector	Gatan K3
Pixel size (Å/pixel)	0.648
Micrographs	7426
Reconstruction	
Initial number of particles	1,126,590
Final number of particles for reconstruction	127,482
Symmetry imposed	C1
Box size (pixels)	384
Map Resolution (Å)	2.69
FSC threshold	0.143
Map-sharpening B-factor (Å^2)	-60
Refinement	
Number of non-H atoms	18,330
Protein residues	2178
N-glycan	34
Water	138
Lipid	6
Molprobity score	1.83 (99th percentile)
Clashscore	8.22 (98th percentile)
Poor rotamers (%)	0
R.M.S.D. values	
Bond lengths (Å)	0.011
Bond angles (°)	0.701
Ramachandran analysis	
Favored (%)	94.42
Outliers (%)	0

Table SI2. Mutations linked to congenital myasthenic syndromes (CMS)

Related to Figure 5

Red highlights indicate residues that are not conserved between human muscle and *Torpedo*.

From: Engel, A. G., Shen, X. M., Selcen, D. & Sine, S. M. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol* 14, 420-434, doi:10.1016/S1474-4422(14)70201-7 (2015)

Slow channel CMS mutants

Residue	**Our structure residue/number**
Ligand binding domain	
α-V156M	α-same
α-G153S	α-same
Coupling domain	
α-S269I	α-same
Pore domain	
α-N217K	α-same (M1)
α-V249F	α-same (M2/7′)
α-T254I	α-same (M2/12′)
α-C418W	α-same (M4)
β-V229F	β-T224
β-L262M	β-L257 (M2/9′)
β-T265S	β-T260 (M2/12′)
β-V266M	β-V261 (M2/13′)
δ-S268Y	δ-A268
δ-S268F	
ε-L221F	γ-L220 (M1)
ε-V259L	γ-V258 (M2/7′)
ε-T264P	γ-T263 (M2/12′)
ε-V265A	γ-I264
ε-L269F	γ-L268 (M2/17′)

Fast channel CMS mutants

Residue	**Our structure residue/number**
Ligand binding domain	
ε-T38K	γ-same
δ-L42P	δ-same
ε-W55R	γ-same
δ-E59K	δ-D59
α-V188M	α-same
ε-P121L	γ-same
ε-D175N	γ-D174
ε-N182Y	γ-N181
ε-E184K	γ-E183
Coupling domain	
α-V132L	same
Pore domain	
δ-P250Q	δ-same (M1)
α-F256L	α-same (M2/14′)
α-V285I	α-same (M3)

SI video legends:

Video SI1: Superposition of α subunits from α-bungarotoxin complex structure (green, this study) and apo receptor structure (orange, PDB ID: 2BG9). *Related to Figure 1*

Video SI2: Superposition of β subunits from α-bungarotoxin complex structure (this study) and apo receptor structure (PDB ID: 2BG9). Colors as in Video S1. *Related to Figure 1*

Video SI3: Superposition of γ subunits from α-bungarotoxin complex structure (this study) and apo receptor structure (PDB ID: 2BG9). Colors as in Video S1. *Related to Figure 1*

Video SI4: Superposition of δ subunits from α-bungarotoxin complex structure (this study) and apo receptor structure (PDB ID: 2BG9). Colors as in Video S1. *Related to Figure 1*

Video SI5: α-Bungarotoxin (chain F) with model shown as sticks and density map contoured at a threshold of 0.006 in UCSF Chimera. *Related to Figure 1*