

Article

# A novel lid-covering peptide inhibitor of nicotinic acetylcholine receptors derived from $\alpha$ D-conotoxin GeXXA

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Supplementary Materials include:

Supplementary Table S1;

Supplementary Figure S1-S2.

**Table S1.** Molecular masses of peptides from NTD and sNTD preparation.

Step	Peptide	NTD		sNTD	
		Theoretical mass	Determined mass	Theoretical mass	Determined mass
1	N18	2352.65	2353.0	1547.75	1548.0
2	N6	2352.65	2353.0	1547.75	1548.0
3	N6*	2548.83	2549.0	1743.93	1744.0
4	N6-N18	4702.30	4702.0	3092.50	3093.0
5	NTD	4558.30	4558.0	2948.50	2948.0

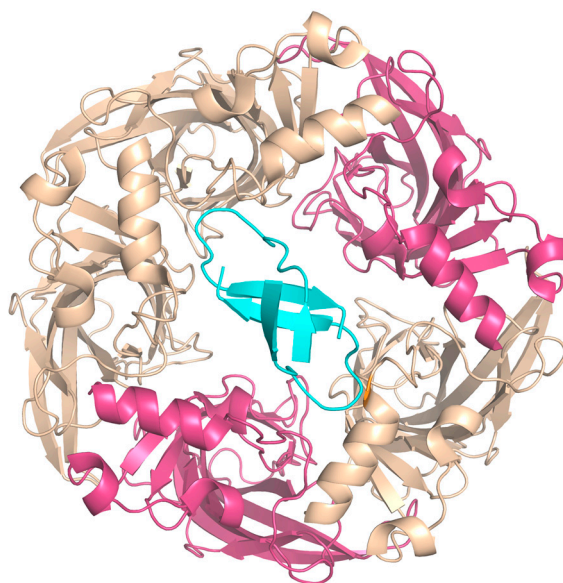


Figure S1: Top view of the  $\alpha D$ -GeXXA NTD (cyan) bound onto the top surface of the human  $\alpha 4$ (pink) $\beta 2$ (wheat) nAChR subtype (PDB 5KXI).

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rα1 1  --SEHETRLVAKLFKDYSSVVRPVDGHDREIVQVTVGLQLIQLINVDEVNQIVTTNVRKQ 58
hα3 1  --SEAEHRLFEERLEFDYNEIIRPVANVSDPVIHFEVMSQLVKVDEVNQIMETNLWLKQ 58
hα4 1  --AAHEERLLKKLFSGYNKWSRPVANISDVLVRFGLSIAQLIDVDEKNQMMTTNVVVKQ 58
hα7 1  --GEFQRKLYKELVKNYNPLERPVANDSQPLTVYFSLSLQIMDVDEKNQVLTNTNWLQM 58
hα9 1  ADGKYAQKLFNDLFEEDYSNALRPVEDTDKVLNVTLQITLSQIKDMDERNQILTAYLWIRQ 60
hα10 1 AEGRLALKLFRDLFANYTSALRPVADTDQTLNVTLEVTLSQIIDMDERNQVLTLYLWIRQ 60

rα1 59 QWVDYNLKWNPDYGGVKKIHIPSEKIWRPDVLYNNADGDFAIKFKTKVLLDYGHITW 118
hα3 59 IWNKYKLKWNPSDYGGAEFMRVPAQKIWKPDIVLYNNAVGDFQVDDKTKALKYTGEVTW 118
hα4 59 EWHDYKLRWDPADYENVTSIRIPSELIWRPDIVLYNNADGDFAVTHLTKAHLFHDGRVQW 118
hα7 59 SWTDHYLQWNVSEYPGVKTVRFDPGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQY 118
hα9 61 IWHDAYLTWDRDQYDGLDSIRIPSDLVWRPDIVLYNKADDESSEPVNTNVVLRYDGLITW 120
hα10 61 EWTDAYLRWDPNAYGGLDAIRIPSSLVWRPDIVLYNKADAQPPGSASTNVVLRHDGAVRW 120

rα1 119 TPPAIFKSYCEIIVTHFPFDEQNCSMKLGTWYDGSVVAINPESDQPDLSNFMESGEWVI 178
hα3 119 IPPAIFKSSCKIDVTYFPFDYQNCTMKFGSWSYDKAKIDLVLIGSSMNLKDYWESGEWAI 178
hα4 119 TPPAIYKSSCSIDVTFFPFDQNCTMKFGSWTYDKAKIDLVMHSRVDQLDFWESGEWVI 178
hα7 119 LPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQ--MQEADISGYIPNGEWDL 176
hα9 121 DAPAITKSSCVVDVTYFPFDNQCCNLTFGSWTYNGNQVDIFNALDSGDLSDFIEDVEWEV 180
hα10 121 DAPAITRSSCRVDVAAFPFDAQHCGLTFGSWTHGGHQLDVRPRGAAASLADFVENVEWRV 180

rα1 179 KEARGWKHWVFYSCCPNTPYLDITYHFVMQRLPLYFIVN 217
hα3 179 IKAPGYKHDIKYNCCE-EIYPDITYSLYIRRLPLFYTIN 216
hα4 179 VDAVGTYNTRYECCA-EIYPDITYAFVIRRLPLFYTIN 216
hα7 177 VGIPGKRSERFYECCK-EPYPDVTFTVMRRRTLYYGLN 214
hα9 181 HGMPAVKNVISYGCS-EPYPDVTFTLLLKRRSSEFYIVN 218
hα10 181 LGMPARRVLTYGCS-EPYPDVTFTLLLRRRAAAYVCN 218

rβ1 1  SEAEGQLLKKLFS--YDSSVRPAQEVGDRVGVSIGLTLAQLISLNEKDEEMSTKYVLDL 58
hβ2 1  TDEEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIMTTNVWLTQ 60
hβ4 1  ANAEEKLMDLLNKTRYNNLIRPATSSQLISIKLQLSLAQLISVNEREQIMTTNVWLKQ 60
mε 1  KNEELSLYHHLFDN--YDPECRPVRPEDTVITLKVITLNLISLNEKEETLTSVWIGI 58
rδ 1  LNEEQRLIQHLFEEKGYNKELRPVARKEDIVDVALSLTLSNLISLKEVEETLTNVWIDH 60

rβ1 59  EWTDYRLSWDPAEHDGIESLRVTAESVWLPDVLLNNDGNFDVALDINVVVSFEGSVRW 118
hβ2 61  EWEDYRLTWKPEEFDNMKKVRLPSKHIWLPDVLYNNADGMYEVSFYSNAVVSYDGSIFW 120
hβ4 61  EWTDYRLTWNSSRYEGVNILRIPAKRIWLPDIVLYNNADGTYEVSVYTNLIVRSNGSVLW 120
mε 59  DWHDYRLNYSKDDFAGVGILRVSEHVWLPEIVLENNIDGQFGVAYDSNVLVYEGGYVSW 118
rδ 61  AWIDSRLQWNAEEFGNITVLRLPSDMVWLPEIVLENNNDGSFQISYACNVLVSDSGHVTW 120

rβ1 119 QPPGLYRSSCSIQVTYFPFDWQNCTMVFSSYSDSSEVSLKTGPDPDGQ--ERQEIYIH 175
hβ2 121 LPPAIYKSACKIEVKHFPFDQNCTKFRSWTYDRTEIDLVLKSE-----VAS 168
hβ4 121 LPPAIYKSACKIEVKYFPFDQNCTLKFRSWTYDHTEIDMVLMTP-----TAS 168
mε 119 LPPAIYRSTCAVEVTYFPFDWQNCSLIFRSQTYNAEEVEFIFAVDDDGN--TINKIDID 175
rδ 121 LPPAIFRSSCPISVTYFPFDWQNCSLKFSSLKYTAKEIRLSLKQEEEDNRSYPIEWIID 180

rβ1 176 EGTFIENGQWEIIHKPSRLIHLPGDRRGKEGHREEVIFYLIIRRKPLFYLVN 228
hβ2 169 LDDFTPSGEWDIVALPGRRENPDDS-----TYVDITYDFIIRRKPLFYTIN 215
hβ4 169 MDDFTPSGEWDIVALPGRRTVNQDP-----SYVDVITYDFIIRRKPLFYTIN 215
mε 176 TAAFTENGEWAIDYCPGMIRRYEGGSTEGP--GETDVIYTLIIRRKPLFYVIN 226
rδ 181 PEGFTENGEWAIVHRAAKVNVDPSPVDST--NHQDVTFYLIIRRKPLFYIIN 231

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Figure S2: Sequence alignment of the extracellular domain of rat (r), human (h) and mouse (m) nAChR subunits studied in this work. Fully conserved sequences are highlighted in dark gray, and partly conserved in light gray. The sequence of the N-terminal  $\alpha$ -helix is underlined in  $\alpha$ 4 and  $\beta$ 2 subunits. Two additional acidic residues, Asp2 and Asp13 in the N-terminal  $\alpha$ -helix of  $\beta$ 2 in comparison to  $\beta$ 4 subunit, are colored red. Asp13 is more likely to be interacting with the Arg residues of NTD (see Figure 1b in the main text). The acidic residues in  $h\alpha$ 9,  $m\epsilon$ , and  $r\delta$  subunit corresponding to Asp13 of  $\beta$ 2 subunit are also colored red.