Supplemental Figure 1

Title: The unique α4(+)/(-)α4 agonist binding site in $(\alpha4)_3(\beta2)_2$ subtype nicotinic acetylcholine receptors permits differential agonist desensitization pharmacology vs. the $(\alpha4)_2(\beta2)_3$ subtype.

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A

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α4  HVETRAHAEERLLKKLF--SGYNKWSRPVANISDVVLVRFGLSIAQLIDVDEKNQMMTTN 86
β2  -----TDTEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIMTTN 83

                                          LoopA
α4  VWVKQEWHDYKLRWDPADYENVTSIRIPSELIWRPDIVLYNNADGDFAVTHLTKAHLFHD 146
β2  VWLTQEWEDYRLTWKPEEFDNMKKVRLPSKHIWLPDVVLYNNADGMYEVSFYSNAVVSYD 143
     LoopD                                                LoopE
                                       LoopB
α4  GRVQWTPPAIYKSSCSIDVTFFPFDQQNCTMKFGSWTYDKAKIDLVNMHSRVDQLDFWES 206
β2  GSIFWLPPAIYKSACKIEVKHFPFDQQNCTMKFRSWTYDRTEIDLVLKSEVASLDDFTPS 203
                                                        LoopF

                    LoopC
α4  GEWVIVDAVGTYNTRKYECCAEIYPDITYAFVIRRLP 243
β2  GEWDIVALPGRRNENPDDS---TYVDITYDFIIRRKP 237
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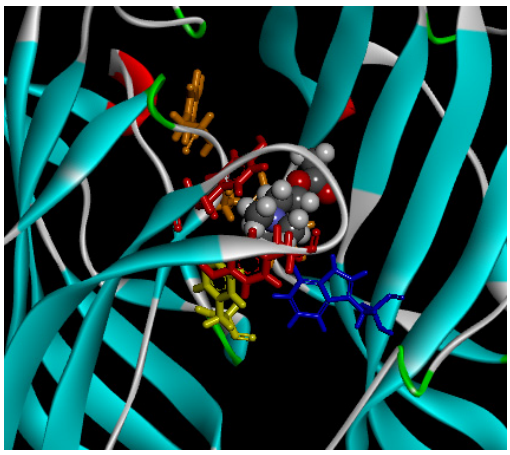
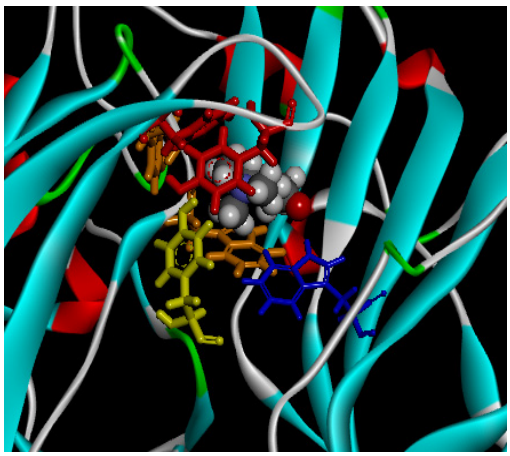
B



C



Mutation of the putative α4(+)/(-)α4 agonist binding pocket. A: Conserved structural loops line agonist binding pockets formed by the nAChR α4(+) face (Loops A-C) and neighboring α4(-) face (Loops D-F; see Methods for fuller description). The aromatic residues mutated in this study are color-highlighted according to their location within Loops A-D. Note that for this figure the conventional numbering scheme, beginning at the start methionine of each wild-type subunit, is used. B: Model of the α4(+)/(-)α4 agonist binding site; based on the 3SQ6 PDB file. There is considerable amino acid conservation between α4 and β2-nAChR subunits, including within the binding pocket loops. This allows formation of an additional agonist binding pocket at the α4(+)/(-)α4 interface only present in the LS α4β2-nAChR isoform (Harpsøe et al., 2011; Mazzaferro et al., 2011). Principal (+) face Loops A-C are contributed by the α4 subunit in LSP position 5 (left), and complementary (-) face Loops D-F are contributed by the α4 subunit in LSP position 4 (right). Residues mutated in this study are color-coded as for panel A with docked ACh shown in the binding center. The binding pocket is viewed facing directly towards Loop C. C: Same model as in Panel B, but viewing the binding pocket from below Loop C.