Cryo-EM Structures of the Ionotropic Glutamate Receptor GluD1 Reveal a Non-Swapped Architecture

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Supplementary Information

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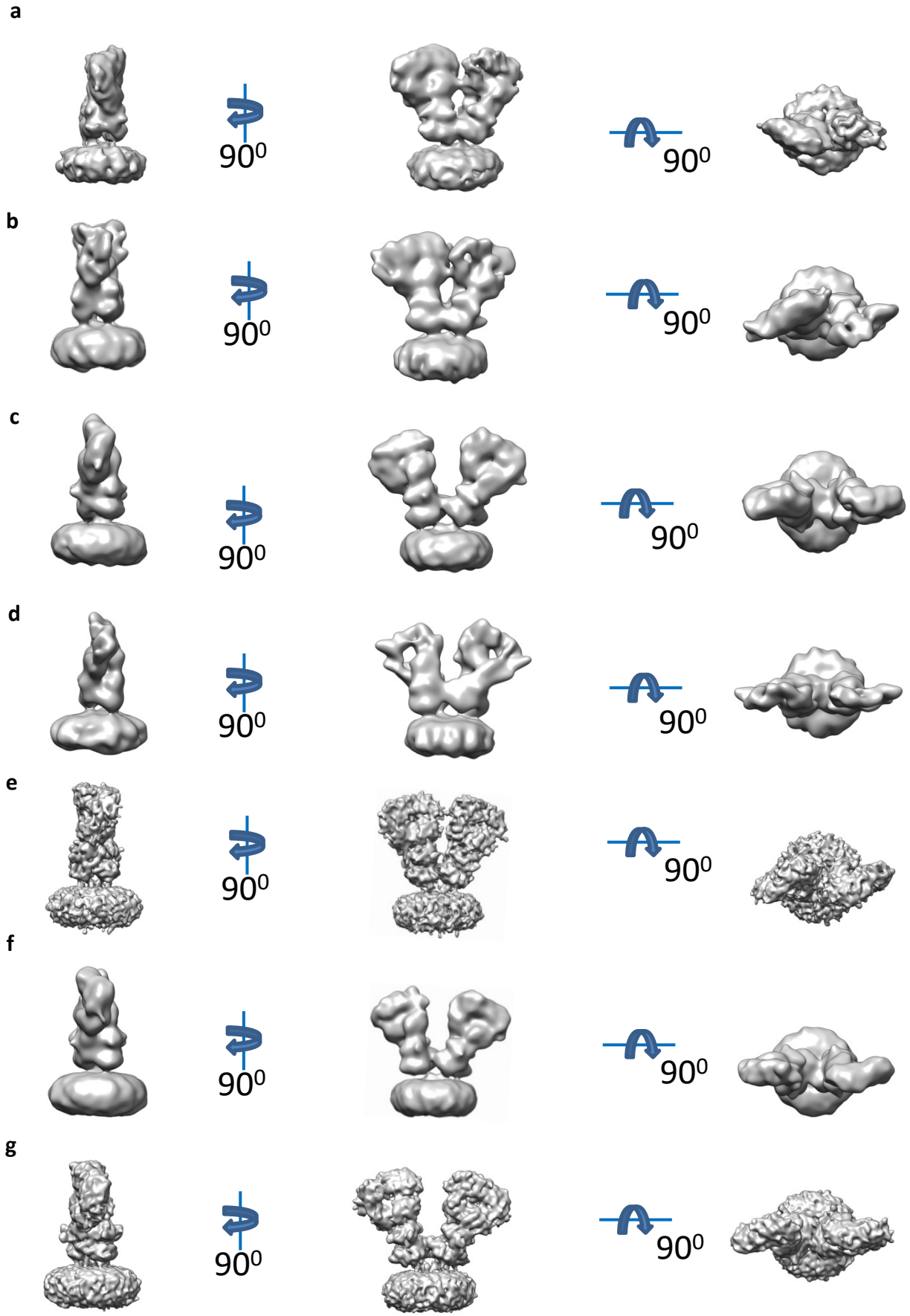
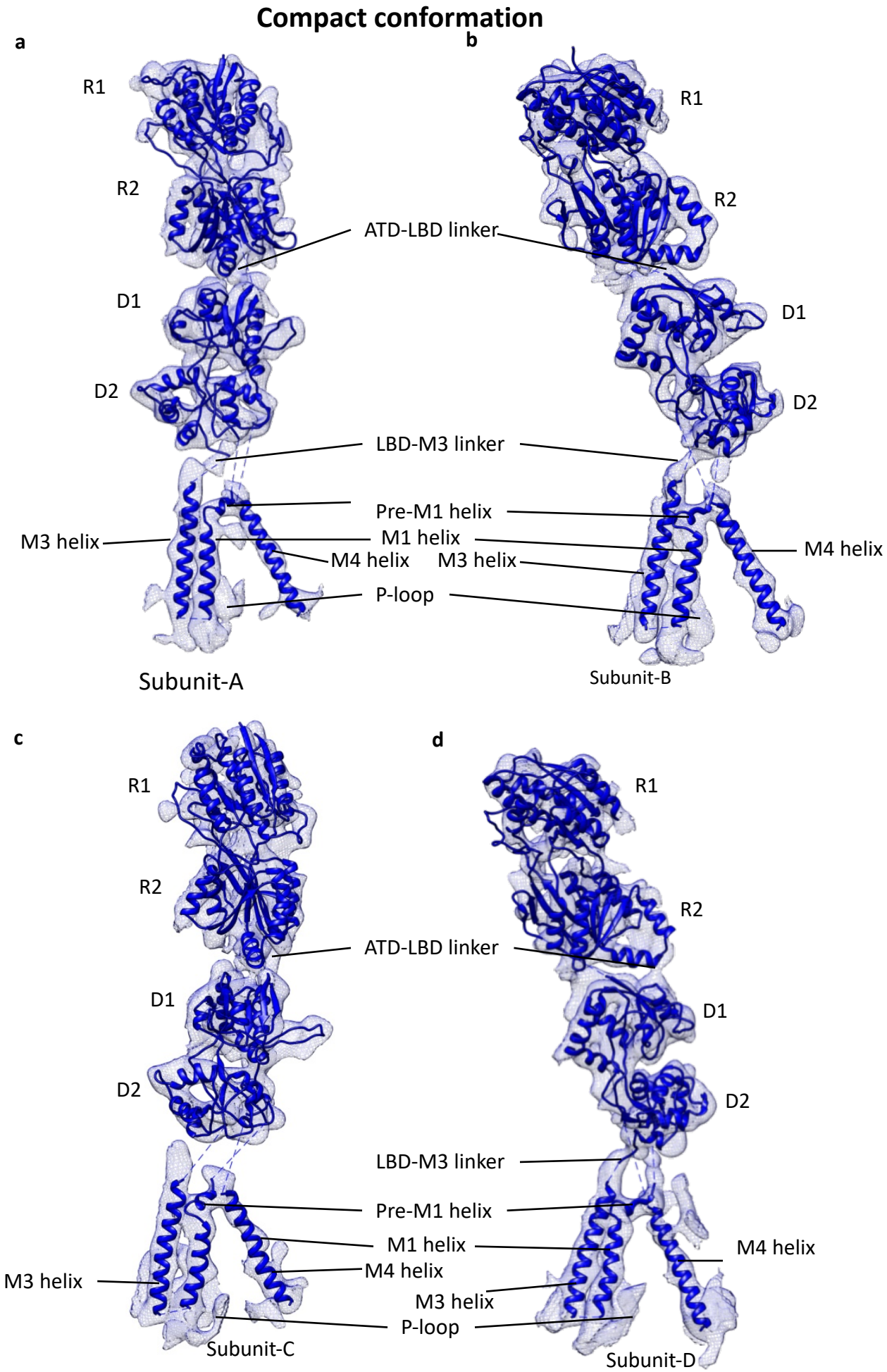
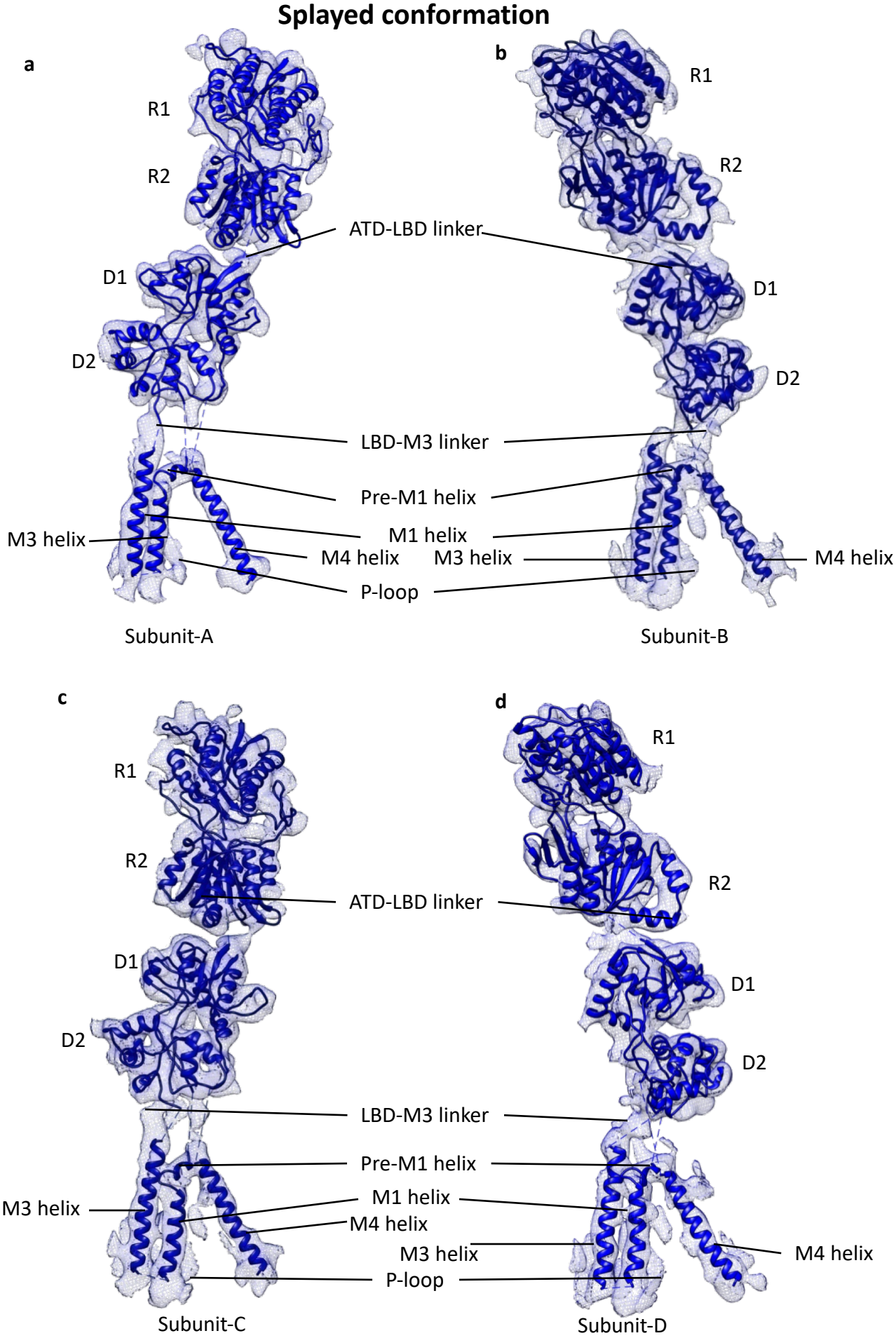


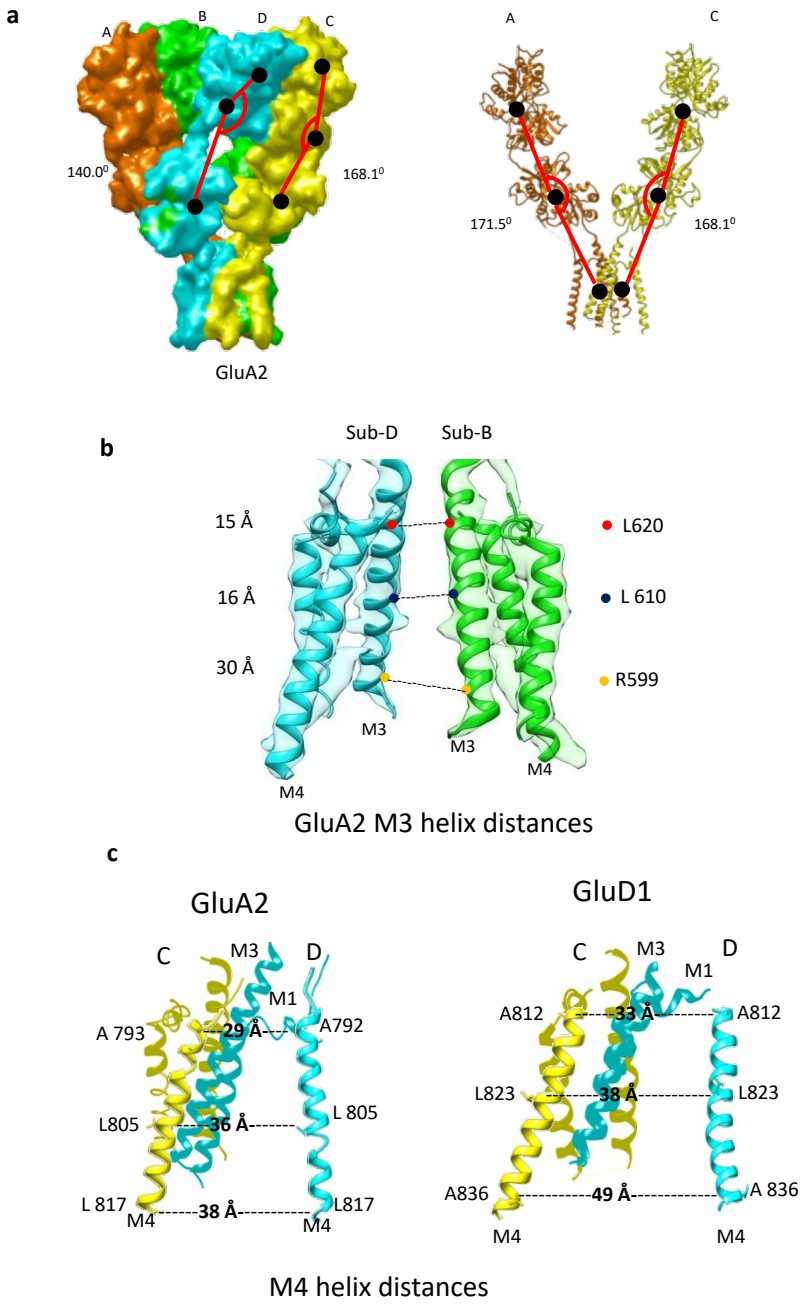
Supplementary Fig. 1: Conformational heterogeneity of the extracellular domain. **a-g**, The seven 3D classes obtained from the heterogeneous refinement in cryoSPARC. All the maps are shown in top and side views. Classes a, b and e show compact state of the receptor whereas c, d, f and g show the splayed conformations due to variable movements of the extracellular dimer arms.



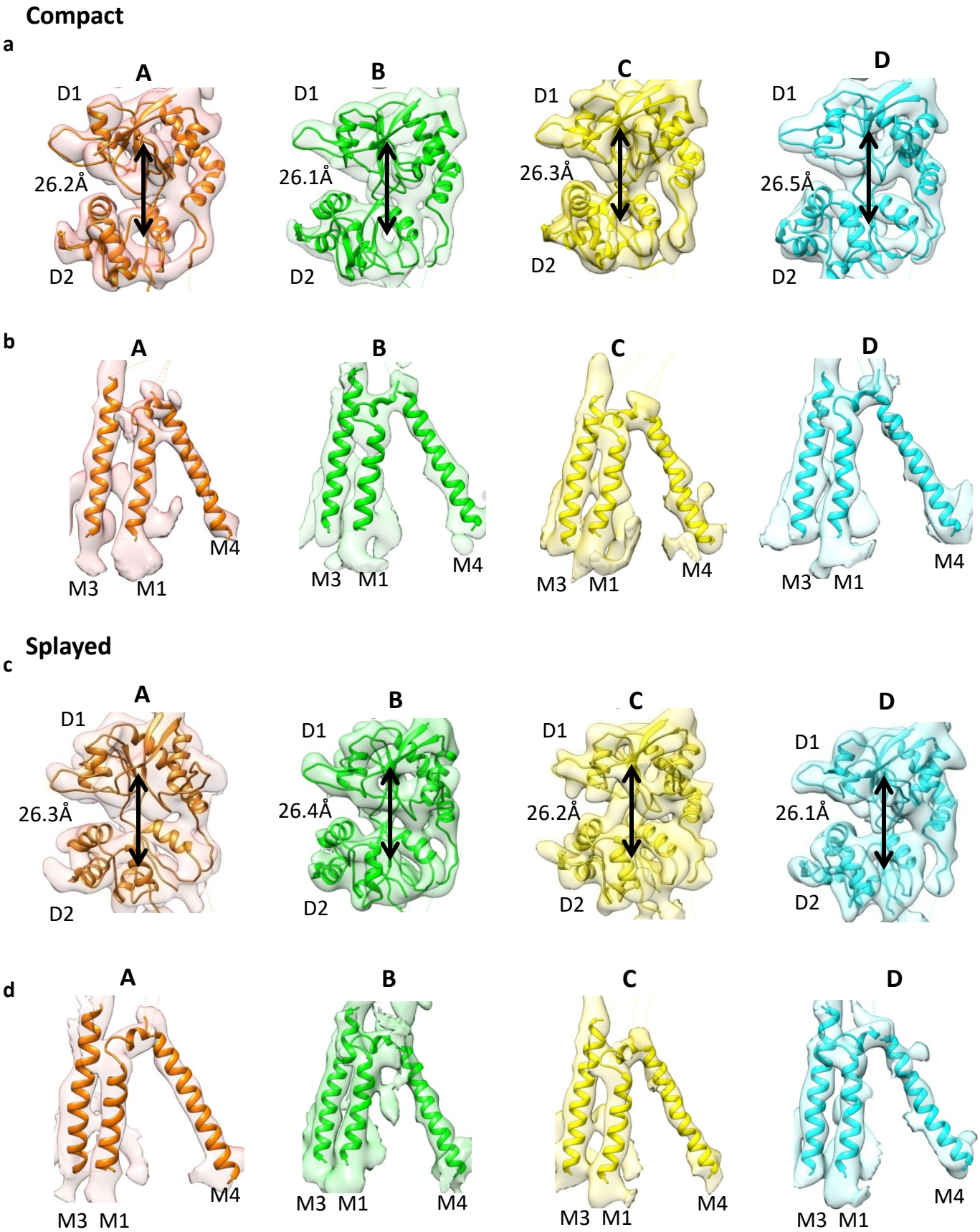
Supplementary Fig. 2: Panels **a-d** show segmented density maps fitted with atomic models of receptor subunits A, B, C and D respectively. Sub domains, linkers between domains and helices of TM are indicated.



Supplementary Fig. 3: Panels **a-d** show segmented density maps fitted with atomic models of receptor subunits A, B, C and D respectively. Sub domains, linkers between domains and helices of TM are indicated.

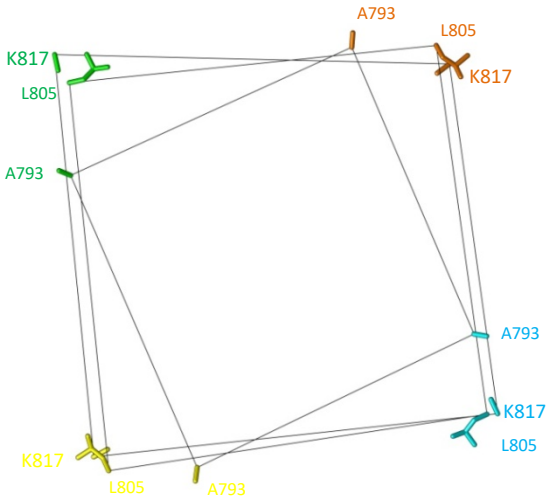


Supplementary Fig. 4: Comparison of receptor architecture and domain arrangement of GluD1 with GluA2. a, Volume generated in chimera for GluA2 cryst (PDB ID:3KG2) and chains A and C are shown along with angles subtended by COMs of ATD, LBD and TM layer. **b**, TM domains of GluA2 receptor along with segmented density map is shown. The distances between 2-fold symmetric residues R599, L610 and L620 on M3 helix for the two subunits are shown. Panel **d** shows distances between residues of M4 helix for GluA2 residues A793 (top), L805 (middle) and L817 (bottom) and corresponding residues in GluD1 highlighting the much broader and splayed arrangement of GluD1 TM domains.

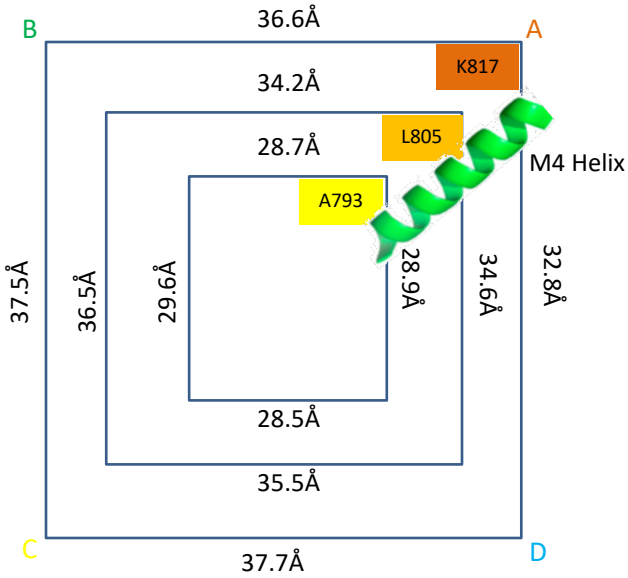


Supplementary Fig. 5: LBD domains have an extended cleft. Figure show the atomic models fitted into segmented density for LBD and TM domains from compact (**a and b**) and splayed (**c and d**) models. The distance between the COM of D1 (upper lobe) and D2 (lower lobe) of LBD are shown and are indicative of an extended cleft consistent with the bound ligand 7-CKA.

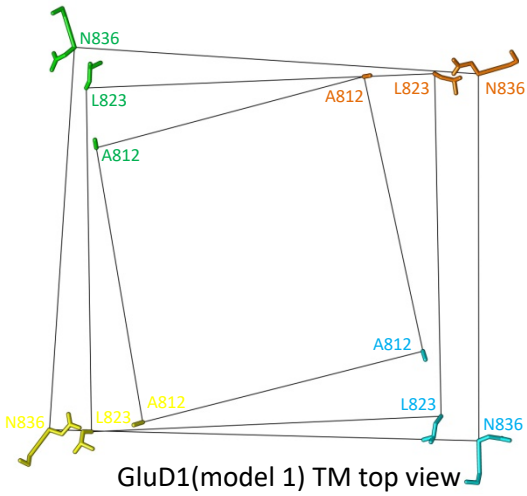
a



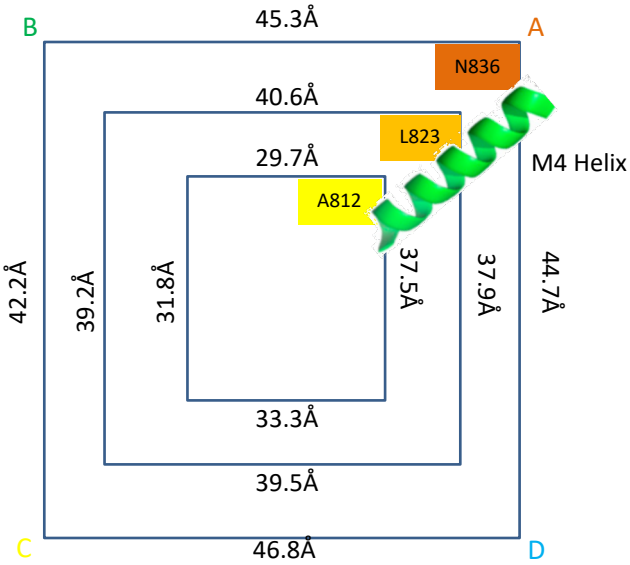
GluA2 TM top view(M4 helix)



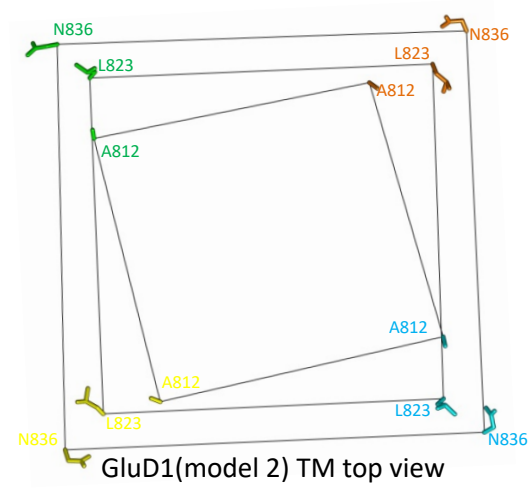
b



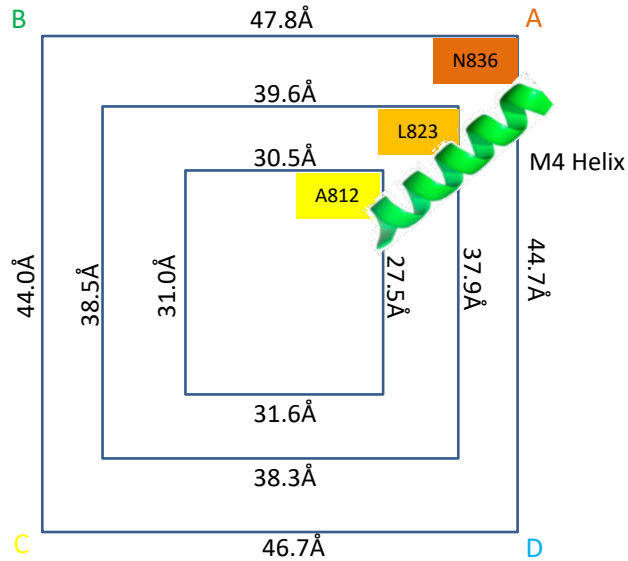
GluD1(model 1) TM top view
(M4 helix)



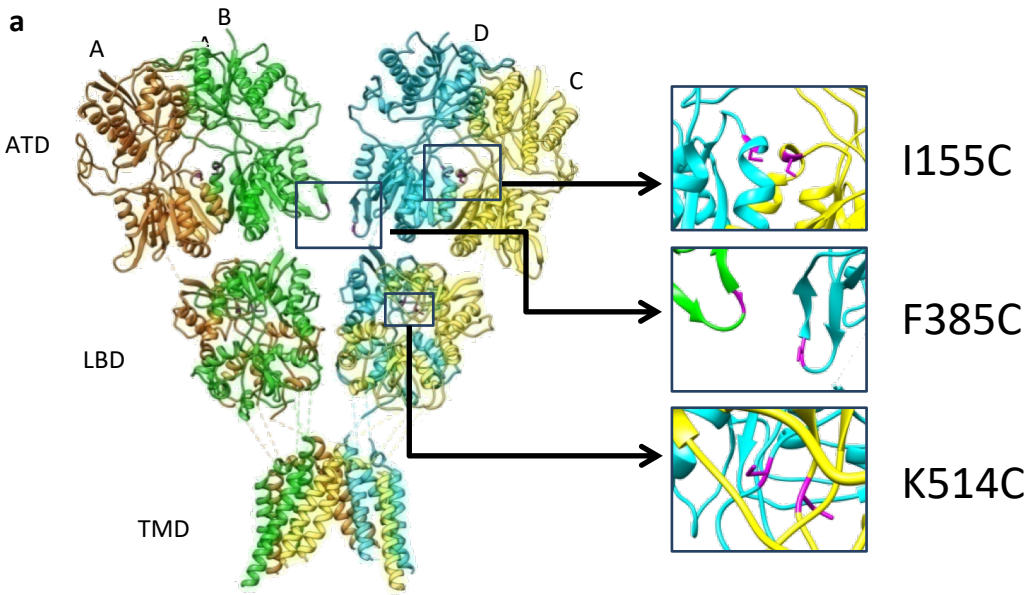
c



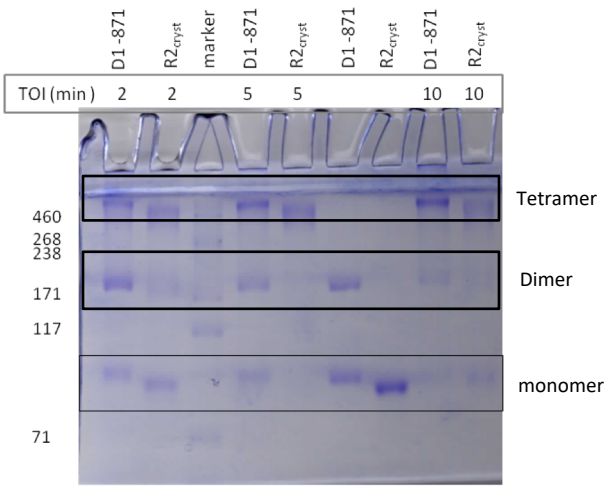
GluD1(model 2) TM top view
(M4 Helix)



Supplementary Fig. 6: Comparison of GluD1 and GluA2 TM domains. GluD1 M4 helix amino acids A812 and L823 and corresponding residues A793 and L805 of GluA2 were used to measure and compare inter subunit distances at TM layer. N836 of GluD1 and corresponding residue K817 from GluA2 was used for the distance measurement at the bottom of the M4 helix. The quadrilaterals formed by joining the C alpha atoms of the selected amino acids on M4 helix of GluA2 homotetramer in closed state (**a**), GluD1 receptor in compact (**b**) and splayed conformation (**c**) are shown. The three quadrilaterals represent the three layers along the height of M4 helix. The inter subunit distances between the corresponding amino acids are depicted with concentric squares and labeled residues along the length of M4 helix for one subunit is shown.

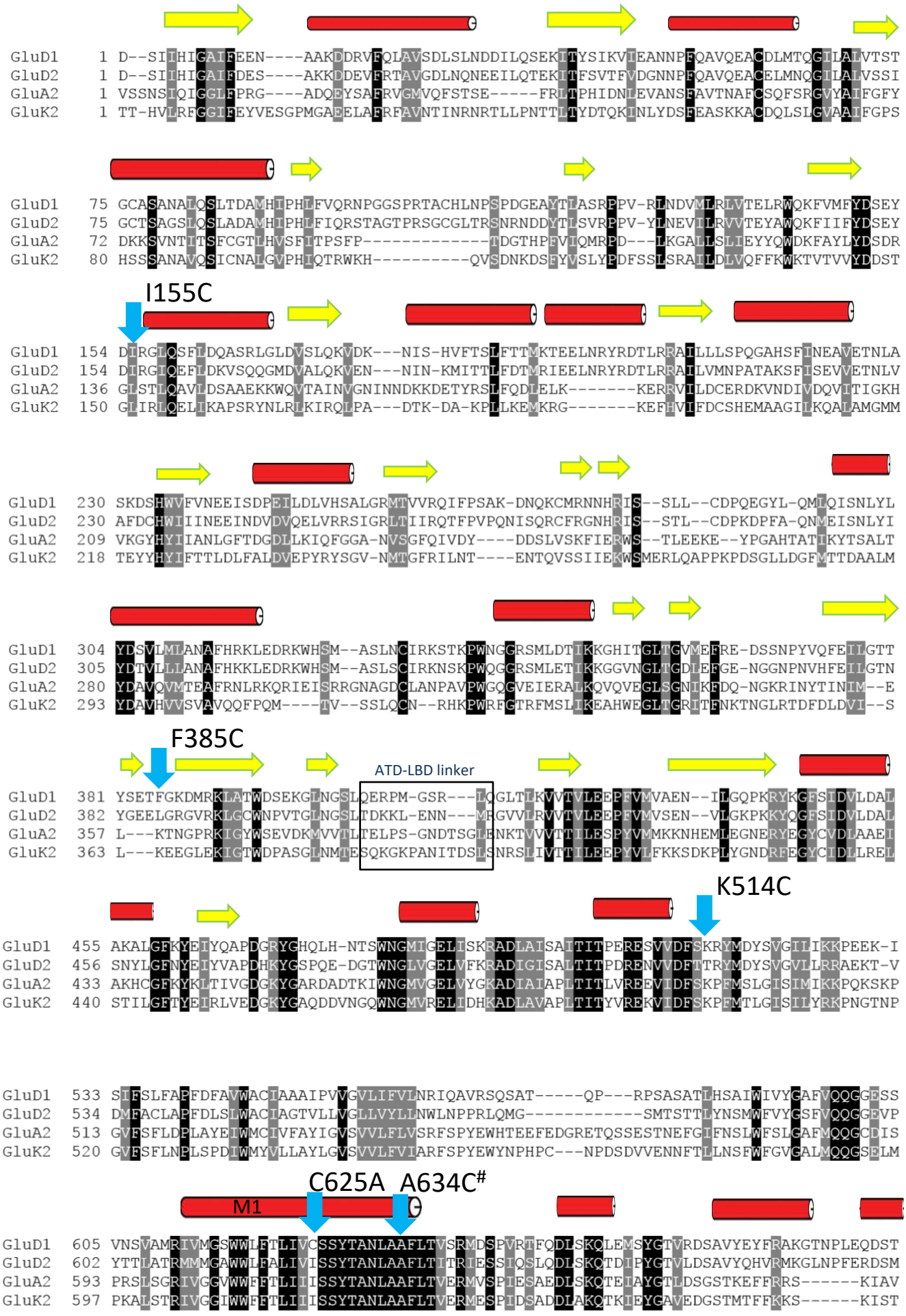


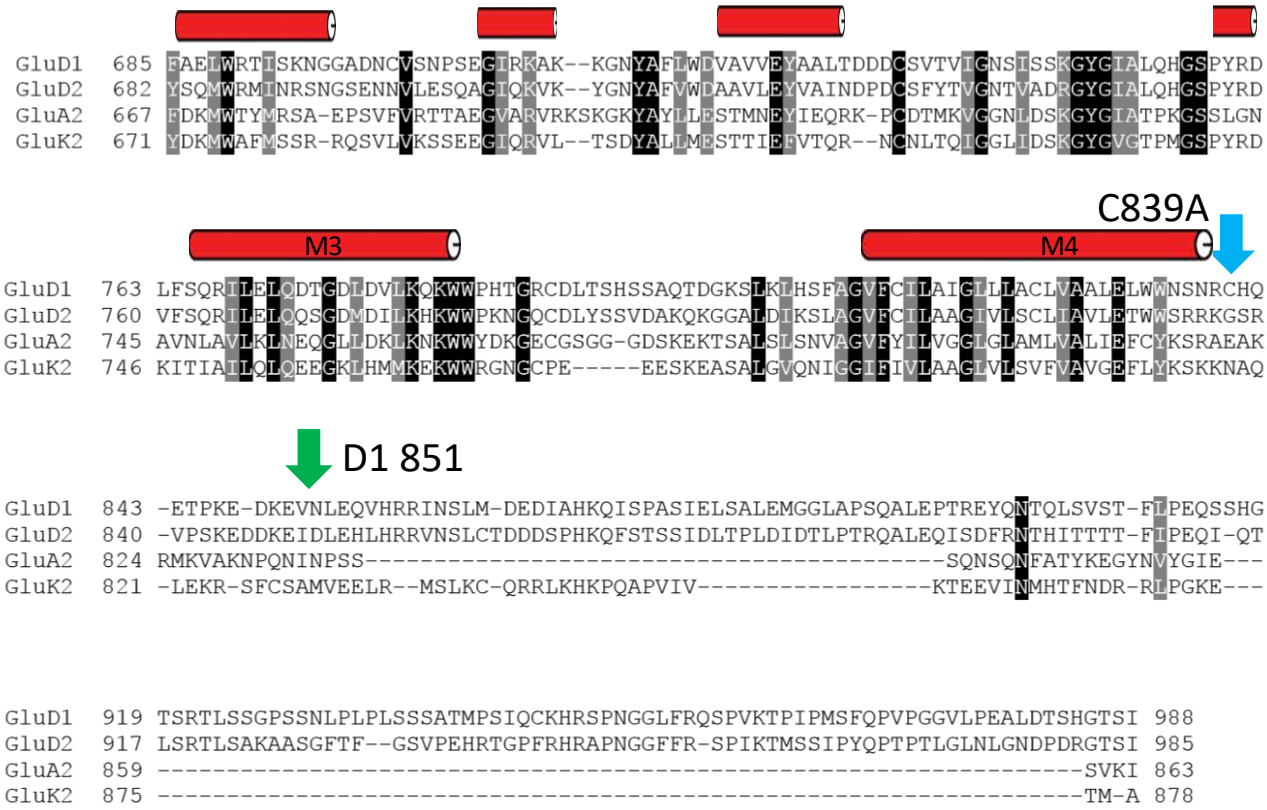
Supplementary Fig. 7: Cysteine cross-linking based validation of receptor interfaces. a, Atomic model representing compact GluD1 homotetramer. The selected amino acids for mutation to cysteine at the 2-fold symmetric dimer interface for ATD (I155), LBD (K514), and ATD dimer-of-dimer (F385) interfaces are shown.



Supplementary Fig. 8: Glutaraldehyde cross-linking of purified GluD1 and GluA2 receptors. Purified GluD1 Δ851 and GluA2cryst (PDB ID: 3KGC) proteins in 20 mM HEPES, 150 NaCl, 0.75 mM DDM were crosslinked with 3mM glutaraldehyde for 2, 5, or 10 mins. While GluA2cryst showed bands primarily at monomer and tetramer position consistent with swapped architecture, GluD1 showed significant fraction of protein in dimeric population even after 10 min incubation.

Supplementary Notes


GluD1 1 D--SIIHIGAIFEEN----AAKDDRVFQLAVSDLSLNDDILQSEKITYSIKVIEANNPFQAVQEACDLMTQGILALVTST
GluD2 1 D--SIIHIGAIFDES----AKKDDEVFRTAVGDLNQNEEILQTEKITFSVTFVDGNNPFQAVQEACELMNQGILALVSSI
GluA2 1 VSSNSIQIGGLFPRG----ADQEYSAFRVGMVQFSTSE-----FRLTPHIDNLEVANSFAVTNAFCSQFSRGVYAIFGFY
GluK2 1 TT-HVLRFGGIFEYVESGPMGAEELAFRFAVNTINRNRTLLPNTTLTYDTQKINLYDSFEASKKACDQLSLGVAAIFGPS
GluD1 75 GCASANALQSLTDAMHIPHLFVQRNPGGSPRTACHLNPSPDGEAYTLASRPPV-RLNDVMLRLVTELRWQKFVMFYDSEY
GluD2 75 GCTSAGSLQSLADAMHIPHLFIQRSTAGTPRSGCGLTRSNRNDDYTLSVRPPV-YLNEVILRVVTEYAWQKFIIFYDSEY
GluA2 72 DKKSVNTITSFCGTLHVSFITPSFP----------TDGTHPFVIQMRPD---LKGALLSLIEYYQWDKFAYLYDSDR
GluK2 80 HSSSANAVQSICNALGVPHIQTRWKH----------QVSDNKDSFYVSLYPDFSSLSRAILDLVQFFKWKTVTVVYDDST
I155C
GluD1 154 DIRGLQSFLDQASRLGLDVSLQKVDK---NIS-HVFTSLFTTVKTEELNRYRDTLRRAILLLSPQGAHSFINEAVETNLA
GluD2 154 DIRGIQEFLDKVSQQGMDVALQKVEN---NIN-KMITTLFDTMRIEELNRYRDTLRRAILVMNPATAKSFISEVVETNLV
GluA2 136 GLSTLQAVLDSAAEKKWQVTAINVGNINNDKKDETYRSLFQDLELK-------KERRVILDCERDKVNDIVDQVITIGKH
GluK2 150 GLIRLQELIKAPSRYNLRLKIRQLPA---DTK-DA-KPLLKEMKRG-------KEFHVIFDCSHEMAAGILKQALAMGMM
GluD1 230 SKDSHWVFVNEEISDPEILDLVHSALGRMTVVRQIFPSAK-DNQKCMRNNHRISS--SLL--CDPQEGYL-QMLQISNLYL
GluD2 230 AFDCHWIIINEEINDVDVQELVRRSIGRLTIIRQTFPVPQNISQRCFRGNHRISS--STL--CDPKDPFA-QNMEISNLYI
GluA2 209 VKGYHYIIANLGFTDGDLLKIQFGGA-NVSGFQIVDY----DDSLVSKFIERWSS--TLEEKE--YPGAHTATIKYTSALT
GluK2 218 TEYYHYIFTTLDLFALDVEPYRYSGV-NMTGFRILNT----ENTQVSSIIEKWSMERLQAPPKPDSGLLDGFMTTDAALM
GluD1 304 YDSVLMLANAFHRKLEDRKWHSM--ASLNCIRKSTKPWNGGRSMLDTIKKGHITGLIGVMEFRE-DSSNPYVQFEILGTT
GluD2 305 YDTVLLLANAFHKKLEDRKWHSM--ASLSCIRKNSKPWQGGRSMLETIKKGGVNGLIGDIEFGE-NGGNPNVHFEILGTN
GluA2 280 YDAVQVMTEAFRNLRKQRIEISRRGNAGDCLANPAVPWGQGVEIERALKQVQVEGLSGNIKFDQ-NGKRINYTINIM--E
GluK2 293 YDAVHVVSVAVQQFPQM----TV--SSLQCN--RHKPWRFGTRFMSLIKEAHWEGLTGRITFNKTNGLRTDFDLDVI--S
F385C
ATD-LBD linker
GluD1 381 YSETFGKDMRKLATWDSEKGLNGSLQERPM-GSR---LQGLTLKVVTVLEEPFVMVAEN--ILGQPKRYKGFSIDVLDAL
GluD2 382 YGEELGRGVRKLGCWNPVTGLNGSLTDKKL-ENN---MRGVVLRVVTVLEEPFVMVSEN--VLGKPKKYQGFSIDVLDAL
GluA2 357 L---KTNGPRKIGYWSEVDKMVVTLTELPS-GNDTSGLENKTVVVTTILESPYVMMKKNHEMLEGNERYEGYCVDLAAEI
GluK2 363 L---KEEGLEKIGTWDPASGLNMTESQKGKPANITDSLSNRSLIVTTILEEPYVLFKKSDKPLYGNDRFEGYCIDLLREL
K514C
GluD1 455 AKALGFKYEIYQAPDGRYGHQLH-NTSWNGMIGELISKRADLAISAITITPERESVVDFSKRYMDYSVGILIKKPEEK-I
GluD2 456 SNYLGFNYEIYVAPDHKYGSPQE-DGTWNGLVGELVFKRADIGISALTITPDRENVVDFTTRYMDYSVGVLLRRAEKT-V
GluA2 433 AKHCGFKYKLTIVGDGKYGARDADTKIWNGMVGELVYGKADIAIAPLTITLVREEVIDFSKPFMSLGISIMIKKPQKSKP
GluK2 440 STILGFTYEIRLVEDGKYGAQDDVNGQWNGMVRELIDHKADLAVAPLTITYVREKVIDFSKPFMTLGISILYRKPNGTNP
GluD1 533 SIFSLFAPFDFAVWACIAAAIPVVGVLIFVLNRIQAVRSQSAT-----QP---RPSASATLHSAIWIVYGAFVQQGGESS
GluD2 534 DMFACLAPFDLSLWACIAGTVLLVGLLVYLLNWLNPPRLQMG----------SMTSTTLYNSMWFVYGSFVQQGGEVP
GluA2 513 GVFSFLDPLAYEIWMCIVFAYIGVSVVLFLVSRFSPYEWHTEEFEDGRETQSSESTNEFGIFNSLWFSLGAFMQQGCDIS
GluK2 520 GVFSFLNPLSPDIWMYVLLAYLGVSVVLFVIARFSPYEWYNPHPC---NPDSDVVENNFTLLNSFWFGVGALMQQGSELM
C625A A634C#
M1
GluD1 605 VNSVAMRIVMGSWWLFTLIVCSSYTANLAAFLTVSRMDSPVRTFQDLSKQLEMSYGTVRDSAVYEYFRAKGTNPLEQDST
GluD2 602 YTTLATRMMMGAWWLFALIVISSYTANLAAFLTITRIESSIQSLQDLSKQTDIPYGTVLDSAVYQHVRMKGLNPFERDSM
GluA2 593 PRSLSGRIVGGVWWFFTLIIISSYTANLAAFLTVERMVSPIESAEDLSKQTEIAYGTLDSGSTKEFFRRS------KIAV
GluK2 597 PKALSTRIVGGIWWFFTLIIISSYTANLAAFLTVERMESPIDSADDLAKQTKIEYGAVEDGSTMTFFKKS------KIST



Sequence alignment and construct design. Rat GluD1, GluD2, GluA2 and GluK2 sequences were aligned. Identical residues are shaded in black while homologous amino acids are shaded in grey. The C-terminal truncation site at residue 851 is marked with green arrow. The various cysteine mutants that were made for cross-linking experiments are shown with cyan arrows. A634C mutant in SYTANLAAF motif to generate constitutively active receptors is marked with a #. Secondary structure for Delta1 is annotated above the sequence alignment and is shown as red cylinder for an helix and yellow arrow for a beta strand.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GluD1_851       DSIIHIGAIFEENAAKDDRVFQLAVSDLSLNDDILQSEKITYSIKVIEANNPFQAVQEAC  60
Modelled        -SIIHIGAIFEENAAKDDRVFQLAVSDLSLNDDILQSEKITYSIKVIEANNPFQAVQEAC  59
                 S2

GluD1_851       DLMTQGILALVTSTGCASANALQSLTDAMHIPHLFVQRNPGGSPRTACHLNPSPDGEAYT  120
Modelled        DLMTQGILALVTSTGCASANALQSLTDAMHIPHLFVQRNPGGSPRTACHLNPSPDGEAYT  119

GluD1_851       LASRPPVRLNDVMLRLVTELRWQKFVMFYDSEYDIRGLQSFLDQASRLGLDVSLQKVDKN  180
Modelled        LASRPPVRLNDVMLRLVTELRWQKFVMFYDSEYDIRGLQSFLDQASRLGLDVSLQKVDKN  179

GluD1_851       ISHVFTSLFTTMKTEELNRYRDTLRRAILLLSPQGAHSFINEAVETNLASKDSHWVFVNE  240
Modelled        ISHVFTSLFTTMKTEELNRYRDTLRRAILLLSPQGAHSFINEAVETNLASKDSHWVFVNE  239

GluD1_851       EISDPEILDLVHSALGRMTVVRQIFPSAKDNQKCMRNNHRISSLLCDPQEGYLQMLQISN  300
Modelled        EISDPEILDLVHSALGRMTVVRQIFPSAKDNQKCMRNNHRISSLLCDPQEGYLQMLQISN  299

GluD1_851       LYLYDSVLMLANAFHRKLEDRKWHSMASLNCIRKSTKPWNGGRSMLDTIKKGHITGLTGV  360
Modelled        LYLYDSVLMLANAFHRKLEDRKWHSMASLNCIRKSTKPWNGGRSMLDTIKKGHITGLTGV  359

GluD1_851       MEFREDSSNPYVQFEILGTTYSETFGKDMRKLATWDSEKGLNGSLQERPMGSRLQGLTLK  420
Modelled        MEFREDSSNPYVQFEILGTTYSETFGKDMRKLATWDSEKGLNGSL----------LTLK  408
                                                ATD
                                                L405  ATD- LBD linker  L417

GluD1_851       VVTVLEEPFVMVAENILGQPKRYKGFSIDVLDALAKALGFKYEIYQAPDGRYGHQLHNTS  480
Modelled        VVTVLEEPFVMVAENILGQPKRYKGFSIDVLDALAKALGFKYEIYQAPDGRYGHQLHNTS  468

GluD1_851       WNGMIGELISKRADLAISAITITPERESVVDFSKRYMDYSVGILIKKPEEKISIFSLFAP  540
Modelled        WNGMIGELISKRADLAISAITITPERESVVDFSKRYMDYSVGILIKK----ISIFSLFAP  524
                                        S1
                                                K527  I532
                                                S1- M1 linker region

GluD1_851       FDFAVWACIAAAIPVVGVLIFVLNRIQAVRSQSATQPRPSASATLHSAIWIVYGAFVQQG  600
Modelled        FDFAVWACIAAAIPVVGVLIF-------------------------------------  545
                     M1
                                F561
                                                Cytosolic region  along with M2 loop

GluD1_851       GESSVNSVAMRIVMGSWWLFTLIVCSSYTANLAAFLTVSRMDSPVRTFQDLSKQLEMSYG  660
Modelled        --------AMRIVMGSWWLFTLIVCSSYTANLAAFLT------PVRTFQDLSKQLEMSYG  591
                         A609          M3                T637  P644
                                                M3- S2 linker region

GluD1_851       TVRDSAVYEYFRAKGTNPLEQDSTFAELWRTISKNGGADNCVSNPSEGIRKAKKGNYAFL  720
Modelled        TVRDSAVYEYFRAKGTNPLEQDSTFAELWRTISKNGGADNCVSNPSEGIRKAKKGNYAFL  651

GluD1_851       WDVAVVEYAALTDDDCSVTVIGNSISSKGYGIALQHGSPYRDLFSQRILELQDTGDLDVL  780
Modelled        WDVAVVEYAALTDDDCSVTVIGNSISSKGYGIALQHGSPYRDLFSQRILELQDTGDLDVL  711
                                        S2
GluD1_851       KQKWWPHTGRCDLTSHSSAQTDGKSLKLHSFAGVFCILAIGLLLACLVAALELWWNSNRC  840
Modelled        KQKWWPHTGRCDL----------------SFAGVFCILAIGLLLACLVAALELWWNSN--  753
                          L793  S2-M4 Linker  S810          M4          N838

GluD1_851       HQETPKEDKEV  851
Modelled        -----------
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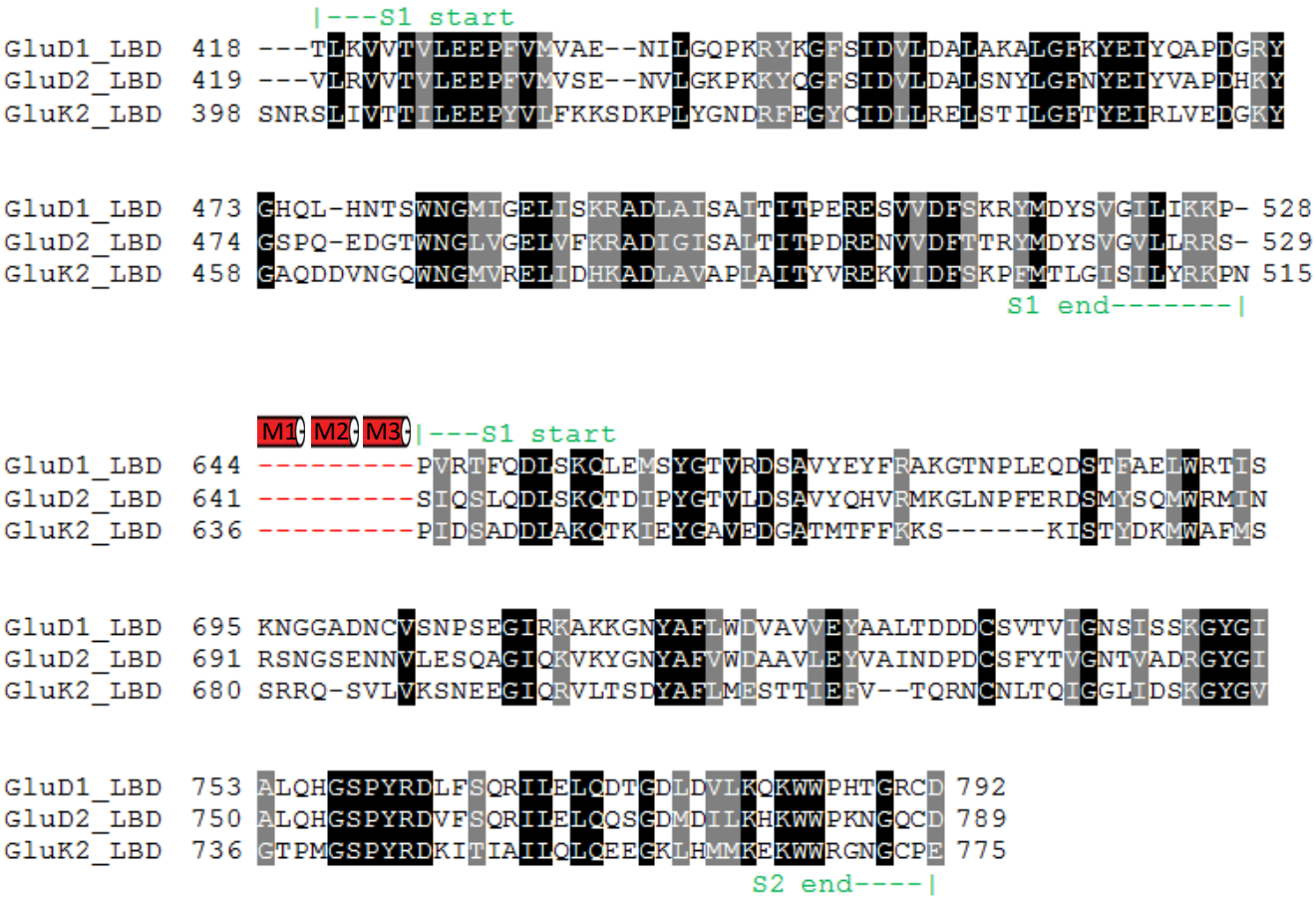
Sequences alignment between GluD1 Δ 851 construct and the actual fragments modelled into EM map is shown. Missing residues in the model that were not built due to limited resolution are indicated by dashed lines. The starting and ending amino acids of each modelled fragment/domain is also shown.



GluK2 LBD chimera in GluD1 receptor. Rat GluD1, GluD2 and GluK2 LBD sequences are aligned and shaded to show sequence identity and similarity. S1 and S2 fragments used for generating GluD1(GluK2LBD) receptor chimera is indicated. Red dashed lines indicated in alignment represent the position where TM helices M1, M2 and M3 are inserted (shown as red cylinders).