

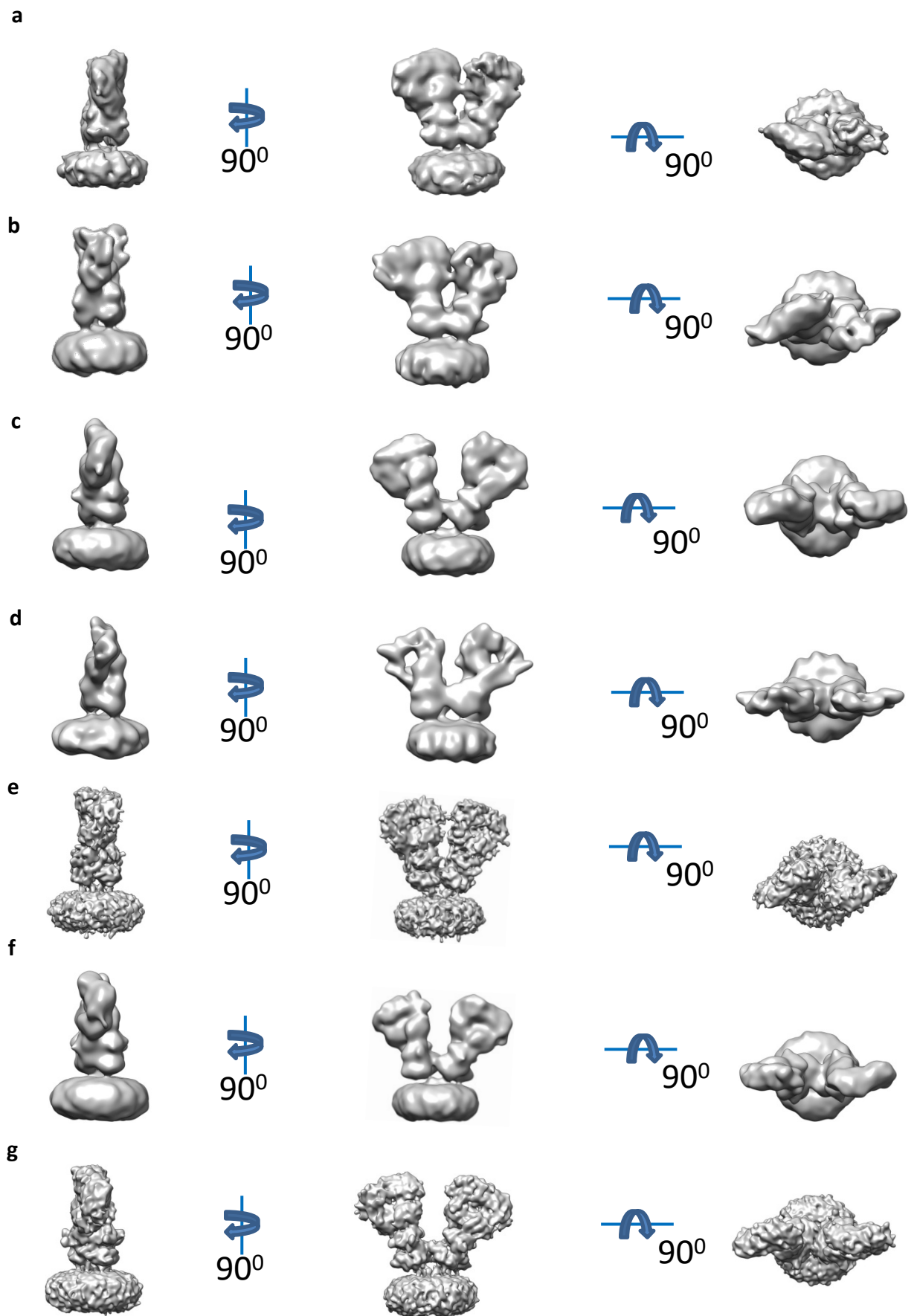
# **Cryo-EM Structures of the Ionotropic Glutamate Receptor GluD1 Reveal a Non-Swapped Architecture**

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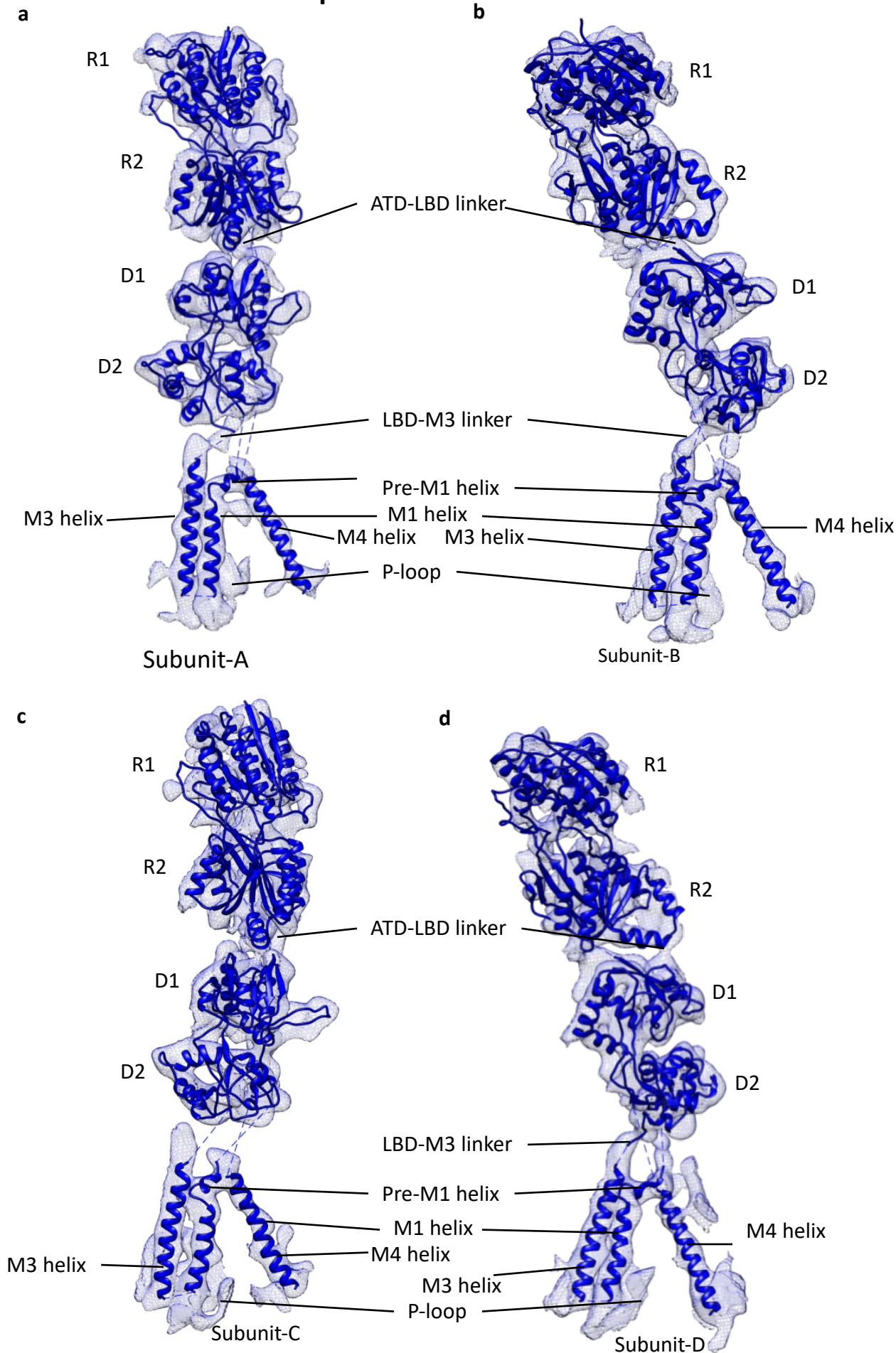
## **Supplementary Information**

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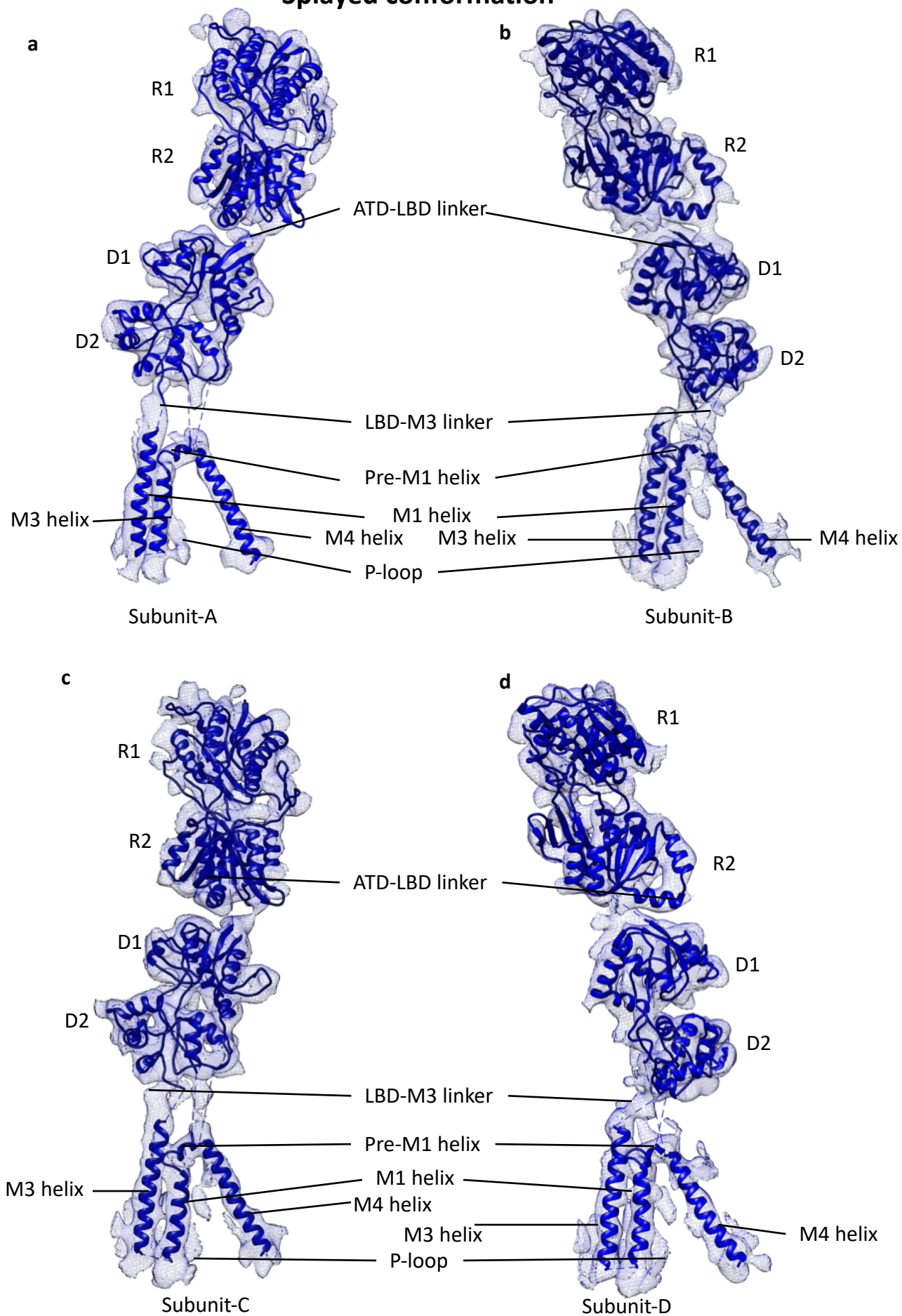
**Supplementary Fig. 1: Conformational heterogeneity of the extracellular domain.** a-g. The seven 3D classes obtained from the heterogeneous refinement in cryoSPARC. All the maps are shown in top and side views. Classes a, b and e show compact state of the receptor whereas c, d, f and g show the splayed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

# Compact conformation

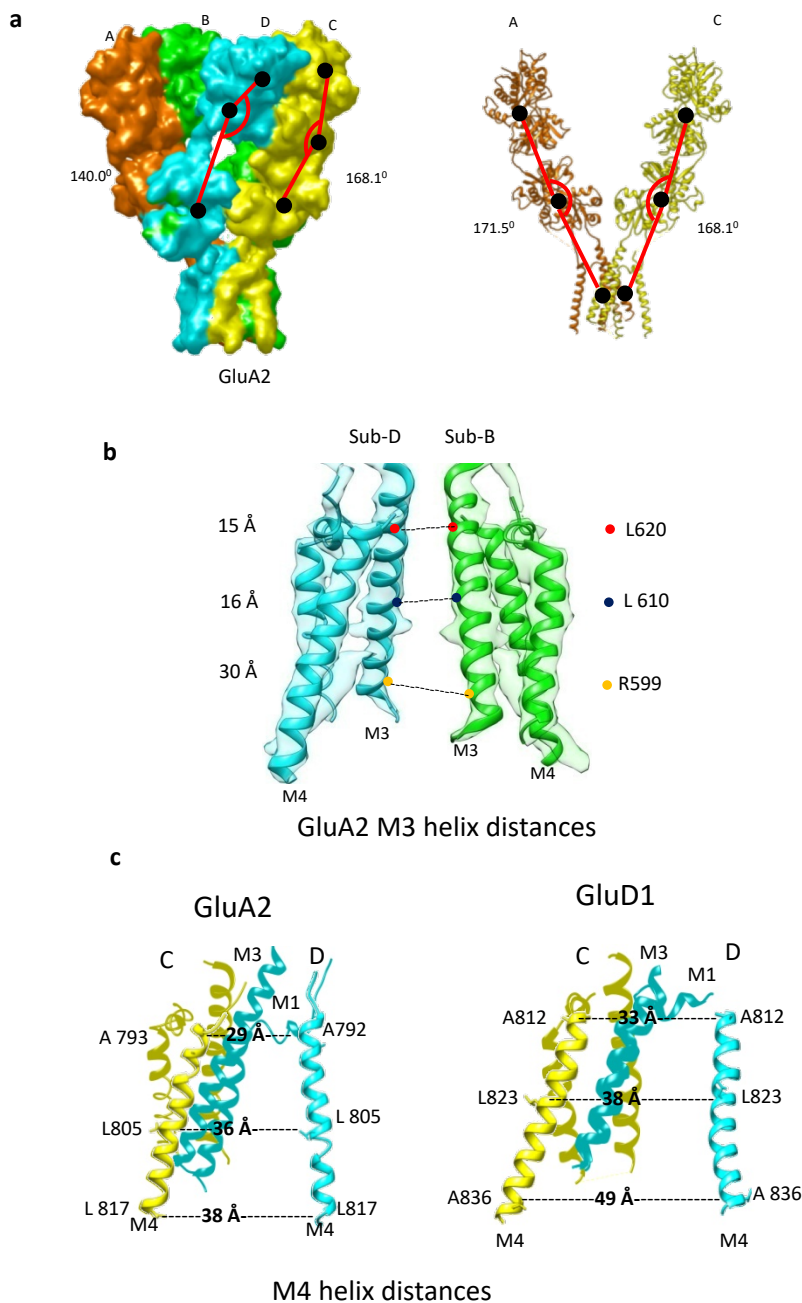


**Supplementary Fig. 2:** Panels **a-d** show segmented density maps fitted with atomic models of receptor subunits A, B, C and D respectively. Sub domains, linkers between domains and helices of TM are indicated.

### Splayed conformation



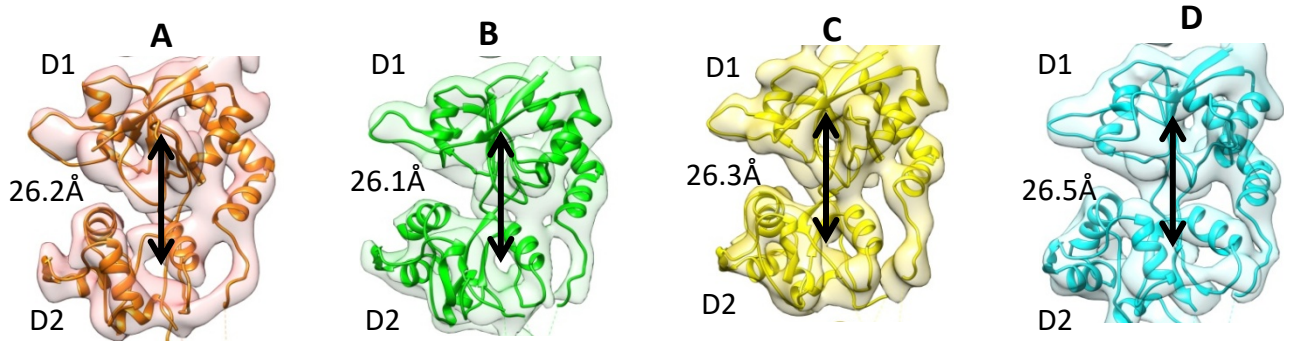
**Supplementary Fig. 3:** Panels **a-d** show segmented density maps fitted with atomic models of receptor subunits A, B, C and D respectively. Sub domains, linkers between domains and helices of TM are indicated.



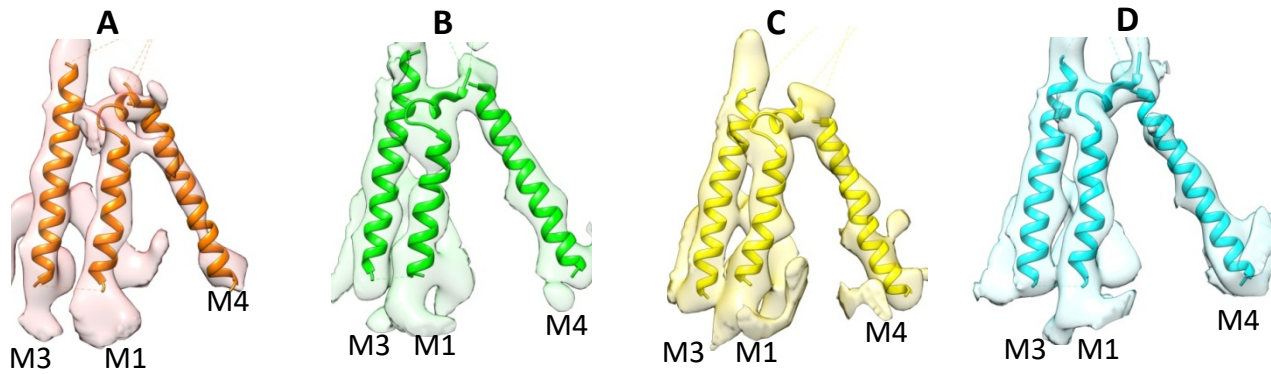
**Supplementary Fig. 4: Comparison of receptor architecture and domain arrangement of GluD1 with GluA2.** **a**, Volume generated in chimera for GluA2 cryst (PDB ID:3KG2) and chains A and C are shown along with angles subtended by COMs of ATD, LBD and TM layer. **b**, TM domains of GluA2 receptor along 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.

## Compact

a

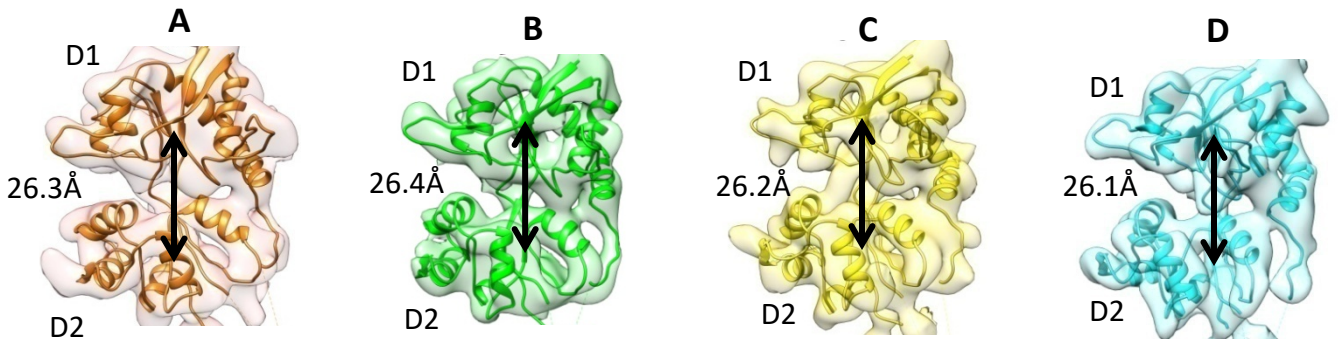


b

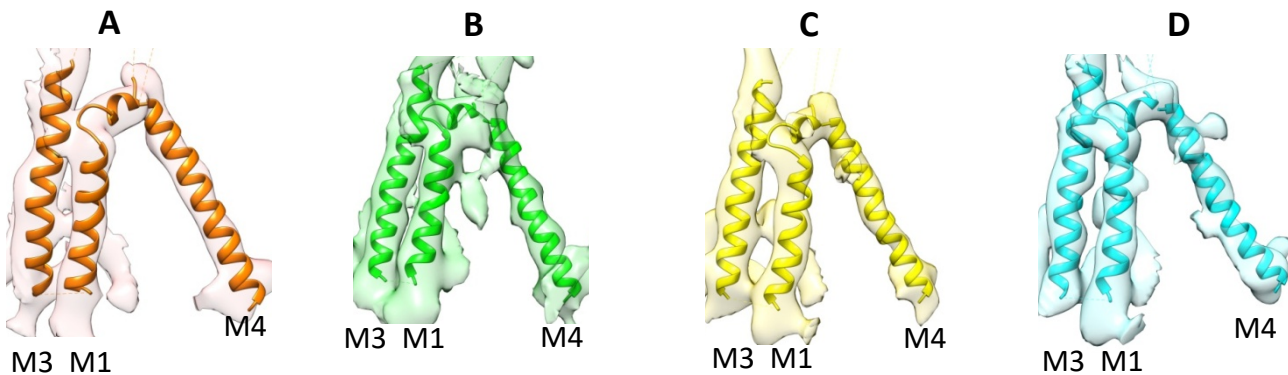


## Splayed

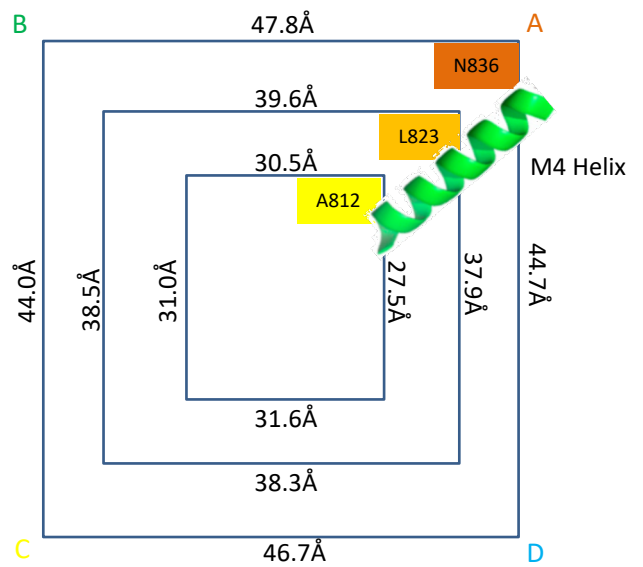
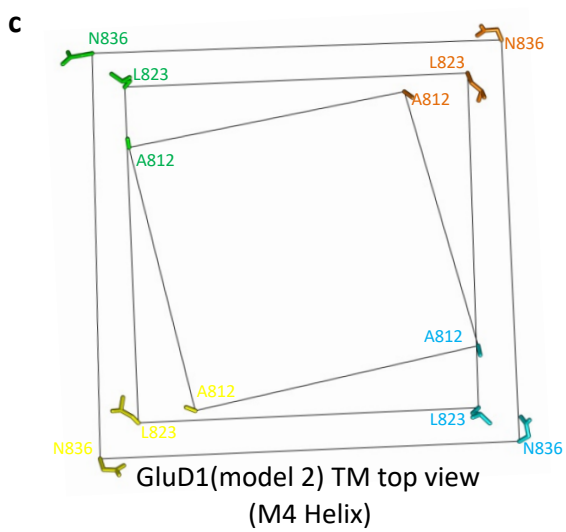
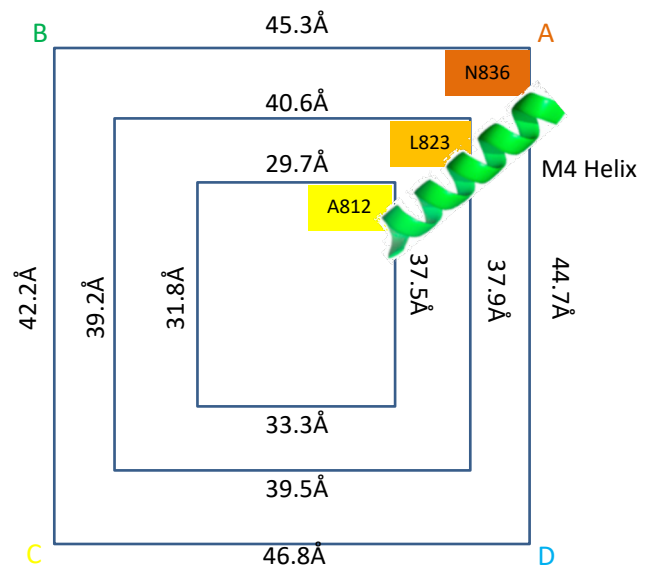
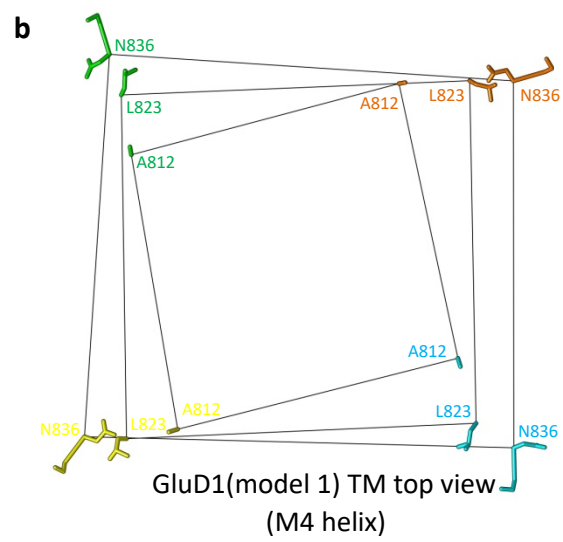
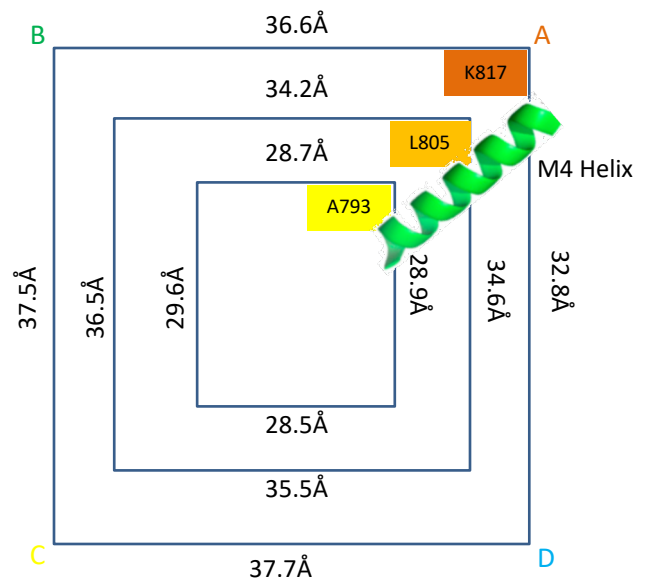
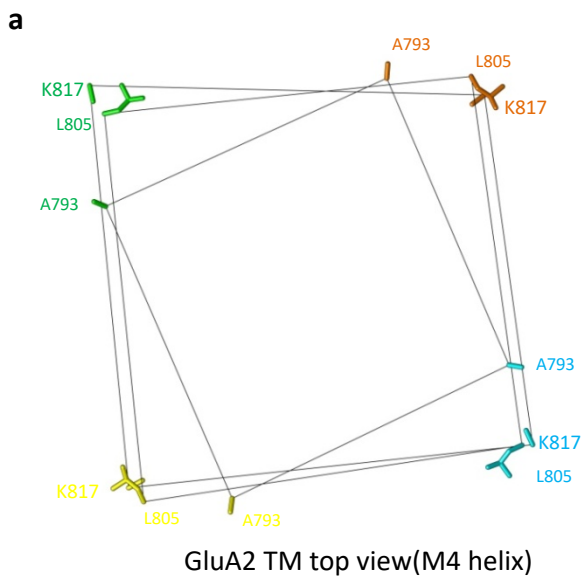
c



d

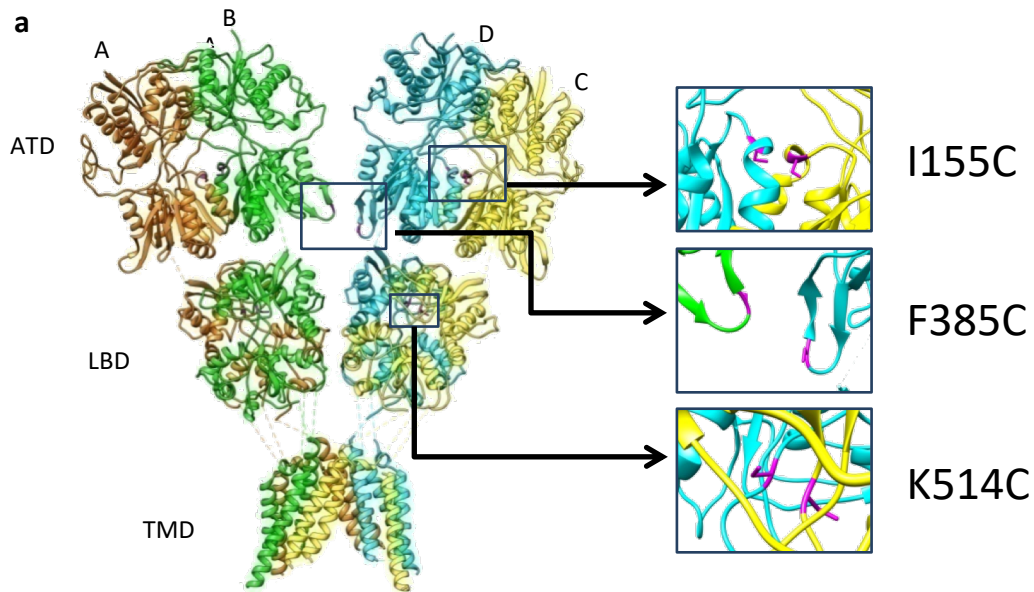


**Supplementary Fig. 5: LBD domains have an extended cleft.** Figure show the atomic models fitted into segmented density for LBD and TM domains from compact (**a and b**) and splayed (**c and d**) models. The distance between the COM of D1 (upper lobe) and D2 (lower lobe) of LBD are shown and are indicative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

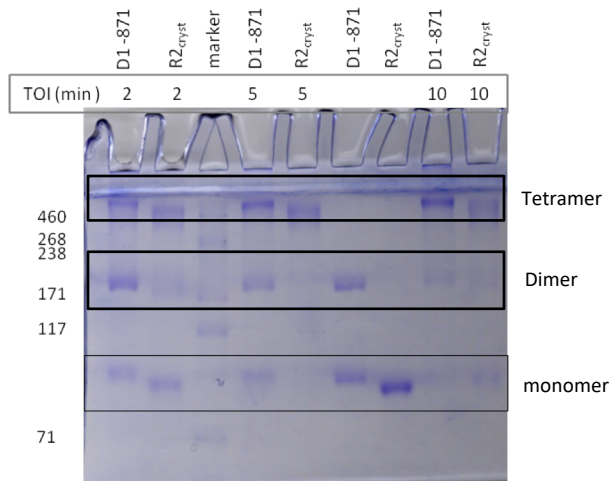


**Supplementary Fig. 6: Comparison of GluD1 and GluA2 TM domains.** GluD1 M4 helix amino acids A812 and L823 and corresponding residues A793 and L805 of GluA2 were used to measure and compare inter subunit distances at TM layer. N836 of GluD1 and corresponding residue K817 from GluA2 was used for the 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  $\Delta 851$  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--SIIHIGATFEEN----AAKDRVFLAVSDLSLNDLILQSEKITYSIKVIEANNPFOAVQVEACDLMTQGILALVTSI  
 GluD2 1 D--SIIHIGATFDES----AKKDDVEFRITAVGDLNQNNEELIQTEKITFSVTFVDGNPNPFOAVQVEACELMNQGILALVSSI  
 GluA2 1 VSSNSIQIGGLFPRG----ADQEYSAFRVGMVQFSTSE-----FRLTPHIDNLEVANSFAVTNAFCQFSRGRVYAFGFY  
 GluK2 1 TT-HVLRFGGIEEYVESGPMGAEELAFREAVNTINRNRLLPNTTLLTYDTQKINLYDSFEASKKACDQLSLGVAALFGPS




GluD1 75 GCASANALQSLTDAMHHPHFLVQRNPGGSPRTACHLNPSPDGEAYTLASRPPV-RLNDVMLRLVTELRWQKFMVFIYDSEY  
 GluD2 75 GCTSAGSLQSLADAMHHPHFLFIQRSTAGTPRSGCGLTRSNRNDYTLISVRPPV-YLNEVILRVVTEYAWQKFIIFYDSEY  
 GluA2 72 DKKSVNTITISFCGLTHVSFTIPSPF-----TDGTHPFVIQMRPD---LKGALLSLIEYYQWDFKAYLYDSDR  
 GluK2 80 HSSSANAVQSIICNALGVPHIQTRWKH-----QVSDNKDSFYVSLYPDFSSLSRAILLDLVQFFKWKTVTIVYDST

**I155C**



GluD1 154 DTRGIQSFLDQASRLGLDVSLLQKVDK---NIS-HVFTSLFTTMKTEELNRYRDTLRRALLLSLPQGAHSFINEAVETNLA  
 GluD2 154 DTRGIQEFLDKVSQQGMDVALQKVEN---NIN-KMITTLFDTMRIEELNRYRDTLRRALLVMNPATAKSFISEVVEETNLV  
 GluA2 136 GLSTLQAVLDSAAEKKQVTAIVNIGNINNDKDETYRSLFQDLELK-----KERRVILDCERDKVNDIVDQVITIGKH  
 GluK2 150 GLIRLQELIKAPSRYNLRKIRQLPA---DTK-DA-KPLLKEMKRG-----KEFHVIFDCSHEMAAGIILKQALAMGMM




GluD1 230 SKDSHWVFNVEEISDPEILLDLVHSALGRMTVVRQIFPSAK-DNQKCMRNNHRIS--SLL--CDPQEGYL-QMIIQISNLYL  
 GluD2 230 AFDCHWIIINEEINDVDVQELVRRSIRGLTIIIRQTFVPVQNISQRCFRGNHRIS--STL--CDPKDFFA-QNMEISNLYI  
 GluA2 209 VKGYHYIIANLGFDTGDLKIQFGGA-NVSGFQIVDY----DDSLVSKFIERWS--TLEEKE--YPGAHTATIKYTSALT  
 GluK2 218 TEYHYIIFTTLDLFDLVEPYRYSVGNMTGFRIILNT---ENTQVSSIIEKWSMERLQAPPKPDGGLDGFMTTDAALM



GluD1 304 YDSVLMLANAFHRKLEDRKWHSM--ASLNCIRKSTKPNWGGRSMLDTIKKGHITGLTGVMEFRE-DSSNPVYQFEILGTT  
 GluD2 305 YDVTLLLANAFHKKLEDRKWHSM--ASLSCIRKNSKPWQGGRSMLDTIKKGGVNGLTGDLIEFGE-NGGNPNVHFEILGTN  
 GluA2 280 YDAVQVMTFAFRNLRKQRIEISRRGNAGDCLANPAVWPWQGGVEIERALKQVQVEGLSGNIKFDQ-NGKRINYNTINIM--E  
 GluK2 293 YDAVHVVSVAVQQFPQM---TV--SSLQCN--RHKPWRFGTRFMSLKEAHWEGLTGRITENKTNGLRDTDFDLVLI--S


**F385C**



GluD1 381 YSETFGKDMRKLATWDSEKGLNGSLQERPM-GSR---IQGLTLKVVTVLEEPFVMVAEN--ILGQPKRYKGFSDVLDLAL  
 GluD2 382 YGEELGRGVRKLGCVNPTVGLNGSLTDKKL-ENN---MFGVVLRVTVLEEPFVMVSEN--VLGKPKKYQGFSDVLDLAL  
 GluA2 357 L---KTNGPRKIGYWEVDKVVVTLTELPS-GNDTSGLENKTVVVTILESPYVMKKNHEMLEGNERYEGYCVDLAAEI  
 GluK2 363 L---KEEGLEKIGTWDPASGLNMTESQKGPANITDSLSNRSLIIVTILEEPVILFKKSDKPLYGNDRFEGYCIDLREL

ATD-LBD linker

**K514C**



GluD1 455 AKALGFKYEITQAPDGRYGHQLH-NTSWNGMIGELISKRADLAISATITIPERESVVDVDFSKRYMDYSVGLIKKPEEK-I  
 GluD2 456 SNYLGFNYEIIYVAPDHRKYGSPQE-DGTWNLVGLVFKRADIGISATITIPDRENVVDFTRYMDYSVGLLRRRAEKT-V  
 GluA2 433 AKHCGFKYKLTIVGDKYKARDATKIWNMGVGLVYKADIAIAPITITLVREEVVDVDFSKPFMSLGLSISIMIKKPKQSKP  
 GluK2 440 STILGFTYEIRLVEDGKYGAQDDVNGQWNGMVRELIIDHKADLAVAPITITYVREKVIDVDFSKPFMTLGLSILYRKPNGTNP

GluD1 533 SIFSLFAFPDFFAVWACIAAAIPVGVLLFVLNRIQAVRSQSAT-----QP---RPSASATLHSAIIVIVYGAFFVQQGGS  
 GluD2 534 DMFACLAPFDLSLWACIAGTVLLVGLLVYLLNWLNPRLQMG-----SMTSTTLYNSMWFVYGSFVQQGGEVP  
 GluA2 513 GVFSFLDPLAYEIMCVFAYIGVSVVFLVSRFSPYEWHTTEEFEDGRETQSSESTNEFGIFNSLWFSLGAFFMQGCDIS  
 GluK2 520 GVFSFLNPLSPDIWVYLLAYLGVSVVLFVIARFSPYEWYNPHPC---NPDSDVVENNFTLLNSFWFGVGMALMQGGSILM

**C625A A634C#**



GluD1 605 VNSVAMRIVMGSWWLETLIVCSSYTANLAAFLTVSRMDSVPVTFODLSKQLEMSYGLTVRDSAVYVEYFRAKGTNPLEQDST  
 GluD2 602 YTTLATRMMMGAWWLEALIVISSYTANLAAFLTITRIEISSIQSLQDLSKQTDIPYGTVLDASVYQHVVMKGLNPFERDSM  
 GluA2 593 PRSLSGRIVGGVWVWFTLIISSYTANLAAFLTVERMVSPIESAEDLSKQTEIAYGLTDSGSTKEFFRS-----KIAV  
 GluK2 597 PKALSTRIVGGIWWFTLIISSYTANLAAFLTVERMSPIDSADDLAKQTKIEYCAVEDGSTMTFFKKS-----KIST


M1



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GluD1 685 FAELWRTTSKNGGADNCVSNPSEGIRKAK--KGNVAFVWVAVVEYAALTDDDCSVTVIIGNSTSSKGYGIALQHGSPYRD
GluD2 682 YSQMWRMLNRSNGSENNVLESQAGIQKVK--YGNVAFVWDAAVLEYVAINDPDCSFYTVGNTVADRGYGIALQHGSPYRD
GluA2 667 EDKMWTYMRSA-EPSVVFVRTTAEQGVARVRKSKGKYAYLLESTMNEYIEQRK-PCDTMKVGGNLDKSGYGIATPKGSSLGN
GluK2 671 YDKMWFVSSR-RQSVLVKSSEEGIQRVL--TSDYALIMESTTIEFVTQR--NCNLTQIGGLTDSKGYGVCTPMGSPYRD


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GluD1 763 LFSQRILELQDTGDLVVLKQKWWPHTGRCDLTSHSSAQTGKSLKLSHFAGVFCTLAIGILLACLVAALELWVNSNRCHQ
GluD2 760 VFSQRILELQSQGDMDIILKHKWWPKNCQCDLYSSVDAKQKGGALDIKSLAGVFCTLAAGIVLSCLIAVLETWWSRRKGSR
GluA2 745 AVNLAVLKLNEQGLLDKLNKWWYDKGECGSGG-GDSKEKTSALSLSNVAGVFYITLVGGLGLAMLVALIEFCYKSRAEAK
GluK2 746 KITIAILQLOEEGKLHMKWKWWRGNCP-----EESKEASALGVQNIIGGIFIVLAAGIVLSVVFVAVGEFLYKSKNAQ

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GluD1 843 -ETPKE-DKEVNLEQVHRRINSLM-DEDIAHKQISPASIELSALEMGGGLAPSQALEPTREYQNTQLSVST-FIPEQSSHG
GluD2 840 -VPSKEDDKIEDLEHLHRRVNSLCTDDDSPHKQFSTSSIDLTPLDIDTLPTRQALEQISDFRNTHITTTT-FIPEQI-QT
GluA2 824 RMKVAKNPQINPSS-----SQQNSQNFATYKEGYNVYIE---
GluK2 821 -LEKR-SFCSAMVEELR--MSLKC-QRRLLKHKPQAPVIV-----KTEEVINMHTFNDR-RLPGKE---

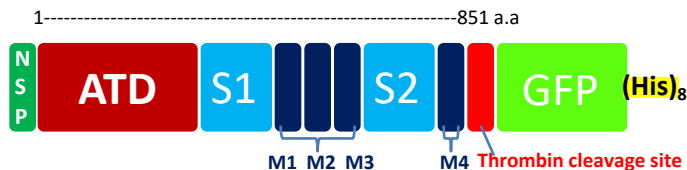
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GluD1 919 TSRTLSSGPSSNLPLPLSSSATMPSIQCKHRSPNGGLFRQSPVKTPIPMSFQVPVGGVLPEALDTSHGTSI 988
GluD2 917 LSRTLAKAASGFTF--GSVPEHRTGPFRRHRAPNGGFFR-SPIKTMSSIPYQPTPTLGLNLGNDFDRGTSI 985
GluA2 859 -----SVKI 863
GluK2 875 -----TM-A 878

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**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.



GluD1_851	DSIIHIGAI FEENA AKD DRVFQLAVSDLSLND DILQSEKITYSIKVIEANNPFQAVQEAC	60
Modelled	-SIIHIGAI FEENA AKD DRVFQLAVSDLSLND DILQSEKITYSIKVIEANNPFQAVQEAC	59
	▲ S2	
GluD1_851	DLMTQGILALVTSTGCASANALQSLT DAMHI PHLFVQRNPGGSPRTACHLNPS PDGEAYT	120
Modelled	DLMTQGILALVTSTGCASANALQSLT DAMHI PHLFVQRNPGGSPRTACHLNPS PDGEAYT	119
GluD1_851	LASRPPVRLNDVMLRRLVTELRWQK FVMFYDSEYDIRGLQSF LDQASRLGLD VSLQKVDKN	180
Modelled	LASRPPVRLNDVMLRRLVTELRWQK FVMFYDSEYDIRGLQSF LDQASRLGLD VSLQKVDKN	179
GluD1_851	ISHVFTSLFTTMKTEELNRYRDTLRRAILLLSPQGAHSFINEAVETNLASKD SHWVFVNE	240
Modelled	ISHVFTSLFTTMKTEELNRYRDTLRRAILLLSPQGAHSFINEAVETNLASKD SHWVFVNE	239
GluD1_851	EISDPEILDVLSALGRMTVVRQIFPSAKDNQKCMRNNHRISLLCDPQEGYLQMLQISN	300
Modelled	EISDPEILDVLSALGRMTVVRQIFPSAKDNQKCMRNNHRISLLCDPQEGYLQMLQISN	299
GluD1_851	LYLYDSVLMLANAFHRKLEDRKWHSMASLNCIRKSTK PWNNGRSMLDTIKKGHITGLTGV	360
Modelled	LYLYDSVLMLANAFHRKLEDRKWHSMASLNCIRKSTK PWNNGRSMLDTIKKGHITGLTGV	359
GluD1_851	MEFREDSSNPYVQFEILGTTYSETFGKDMRKLATWDSEKGLNGSLQERP MGSRLQGLTLK	420
Modelled	MEFREDSSNPYVQFEILGTTYSETFGKDMRKLATWDSEKGLNGSL-----LTLK	408
	ATD	
	▲ L405      ↓ ATD- LBD linker      ▲ L417	
GluD1_851	VVTVLEEPFVMVAENILGQPKRYKGF SIDVLDALAKALGFKYEIYQAPDGRYGHQLHNTS	480
Modelled	VVTVLEEPFVMVAENILGQPKRYKGF SIDVLDALAKALGFKYEIYQAPDGRYGHQLHNTS	468
GluD1_851	WNGMIGELISKRADLAISAITITPERESVVD FSKRYMDYSVGILIKKPEEKISIFSLFAP	540
Modelled	WNGMIGELISKRADLAISAITITPERESVVD FSKRYMDYSVGILIKK-----ISIFSLFAP	524
	S1	
	▲ K527      ↓ S1- M1 linker region      ▲ I532	
GluD1_851	FDFAVWACIAAAI PVVGVLI FVLNRIQAVRSQSATQPRPSASATLHSAIWIVYGAFVQQG	600
Modelled	FDFAVWACIAAAI PVVGVLI F-----	545
	M1	
	▲ F561	
	↓ Cytosolic region along with M2 loop	
GluD1_851	GESSVNSVAMRIVMGSWWLFTLIVC SSYTANLAAFLT VSRMDS PVRTFQDLSKQLEMSYG	660
Modelled	-----AMRIVMGSWWLFTLIVC SSYTANLAAFLT-----PVRTFQDLSKQLEMSYG	591
	▲ A609	
	M3	
	▲ T637      ↓ M3- S2 linker region      ▲ P644	
GluD1_851	TVRDSAVY EYFRAKGTNPLEQDSTFAELWRTISKNGGADNCVSNPSEGIRKAKKGN YAF L	720
Modelled	TVRDSAVY EYFRAKGTNPLEQDSTFAELWRTISKNGGADNCVSNPSEGIRKAKKGN YAF L	651
GluD1_851	WDVAVVEYAAL TDDDCSVTVIGNS ISSKGYGIALQH GSPYRDLFSQRILELQDTGDL DV L	780
Modelled	WDVAVVEYAAL TDDDCSVTVIGNS ISSKGYGIALQH GSPYRDLFSQRILELQDTGDL DV L	711
	S2	
GluD1_851	KQKWWPHTGRCDLTSHSSAQTDGKSLKLH SFAGVFCILAI GLLLA CLVA AELW WNSNRC	840
Modelled	KQKWWPHTGRCDL-----SFAGVFCILAI GLLLA CLVA AELW WNSN-----	753
	▲ L793      ↓ S2-M4 Linker      ▲ S810	
	M4	
	▲ N838	
GluD1_851	HQETPKEDKEV 851	
Modelled	-----	

Sequences alignment between GluD1  $\Delta$  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.

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      |---S1 start
GluD1_LBD 418 ---TLKVVTVLEEPFVMVAE--NILGQPKRYKGFSDVLDALAKALGFKYEIYQAPDGRY
GluD2_LBD 419 ---VLRVVTVLEEPFVMVSE--NVLGKPKRYCGFSDVLDALSNYLGFNIEIYVAPDHKY
GluK2_LBD 398 SNRSLIVTTILEEPYVLFKKS DKPLIYGNDRFE GYCIDLLRELSTILGFTYEIRLVEDGKY

GluD1_LBD 473 GHQL-HNTSWNGMIGELISKRADLAISAITITPERESVVDVFSKRYMDYSVGLILIKKP- 528
GluD2_LBD 474 GSPQ-EDGTWNGLVGELVFKRADIGISAITITPDRENVDFTTRVMDYSVGVLLRRS- 529
GluK2_LBD 458 GAQDDVNGQWNGMVRELI DHKADLAVAPTAITYVREKVIDESKPEMTLGISILYRKP 515
                                     S1 end-----|

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      M1 M2 M3 |---S1 start
GluD1_LBD 644 -----PVRTFQDLSKOLEMSYGTVRDSAVYEYFRAKGTNPLEQDSTFAELWRTIS
GluD2_LBD 641 -----SIQSLQDLSKQTDIPYGTVLD SAVYQHVRMKG LNPFERDSMYSQMWRMIN
GluK2_LBD 636 -----PIDSADDLAKQTKIEYGAVEDGATMTFFKKS-----KISTYDKMWA FMS

GluD1_LBD 695 KNGGADNCVSNPSEGIRKAKKGN YAFVWVAVVEYAALTD DDCSVTVIGNSISSKGYGI
GluD2_LBD 691 RSNGENNVLESQAGIQKVKYGN YAFVWDAAVLEYVAINDPDCSFYTVGNTVADRGYGI
GluK2_LBD 680 SRRQ-SVLVKSNEEGIQRVLTSDYAFVLMESTTIEFV--TQRNCNLTOIGGLIDSKGYGV

GluD1_LBD 753 ALQH GSPYRDLFSQRILELQDTGDL DVLKQKWWPHTGRC 792
GluD2_LBD 750 ALQH GSPYRDVFSQRILELQSGDM DILKHKWWPKNGQCD 789
GluK2_LBD 736 GTPM GSPYRDKITIAILOLQEEGKLHMMREKWWRGNGCPE 775
                                     S2 end----|

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**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).