

Supplemental Information

Analysis of modulation of the $\alpha 1$ GABA_A receptor by combinations of inhibitory and potentiating neurosteroids reveals shared and distinct binding sites

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Supplemental Table 1.

	X	Y	Z
SITE I	10.771	-30.716	228.666
SITE II	-5.189	-19.647	216.231
SITE III	6.259	-16.749	233.316

Supplemental Table 1. Coordinates of the docking box centroids. Site I is the intersubunit site (Hosie et al., 2006, Nature 444:486-489). Site II is the intrasubunit site near the interface between membrane-spanning and extracellular domains (Chen et al., 2019, PLoS Biol 17:e3000157). Site III is the intrasubunit site near the cytoplasmic end of TM3 and TM4 domains (Lavery et al., 2017, Nat Struct Mol Biol 24:977-985).

Supplemental Fig. 1

ρ1 PILRRSPDITKSPLTKSEQLLRIDDDHDFSMRPGFGGPAIPVGVQVESLDSISEVDMDFMTTLYLRHYWKDERLSFPSTNNLSMTFDGRLVKKIWVP
 β3 SFVKETVD-----KLLK--GYDIRLRPDFGGPPVCVGMNIDIASIDMVSEVNMDYTLTMYFQQYWRDKRLAYSGL-PLNLTLDNRVADQLWVP
 GLIC-α1 -----MVSPPPPIADEPLTVNTGIYLIIECYSLDDKAETFKVNAFLSLSWKDRRLAFDPV-RSGVRVKTYEPEAIWIP

ρ1 DMFFVHSKRFSFIHDTTDDNVMLRVQPDGKVLVSLRVTVTAMCNMDFSRFPLDTQTCLEIESYAYTEDDLMLYWKKGNDLSLKTDERISLSQFLIQEFH
 β3 DTYFLNDKKS FVHGVTVKNRMIRLHPDGTVLYGLRITTTAACMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYWRGGDKAVTGVERIELPQFSIVEHR
 GLIC-α1 EIRFVNVENA----RDADVVDISVSPDGTVQYLERFSARVLSPLDFRRYPFDSQ--TLHIYLIVRSVDTRNIVLAVDLEKVGKNDVFLTGWDIESFT

ρ1 TTKLAFYSSTGWY-NRLYINFTLRRHIFFFLLQTYFPATLMVMSWVSFWIDRRVAVPARVPLGITTTLTMSTIITGVNASMPRVSYIKAVDIYLWVS
 β3 LVSRNVVFA-TGAY-PRLSLSFRLKRNIGYFILQTYMPSILITILSWVSFWINYDASAARVALGITTTLTMTTINTHLRETLPKIPYVKAIDMYLMGC
 GLIC-α1 AVVKPANFALEDRLSKLDYQLRISRQYGYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFVVTTLTMTTSLISARNSLPKVAYATAMDWFIACV

TM1

TM2

TM3

ρ1 FVFVFLSVLEYYAAVNYLTT-----SQPARAA-IDKYSRIIFPAAYILFNLIYWSIFS
 β3 FVFVFLALLEYAFVNYIFF-----SQPARAAAIDRWSRIVFPFTFSLFNLVYWLYYV
 GLIC-α1 YAFVFSALIEFATVNYFTK-----SQPARAAKIDRLSRIAFLPLFGIFNLVYWATYL

TM3

TM4

Supplemental Fig. 1. Sequence alignment for human ρ1 (accession code P24046), human β3 (P28472), and the GLIC-α1 chimeric subunits (Lavery et al., 2017, Nat Struct Mol Biol 24:977-985).

Supplemental Fig. 2. The model of the human $\rho 1$ receptor (accession code P24046) was built based on homology with the human $\beta 3$ structure (PDB ID: 4COF) and the GLIC- $\alpha 1$ chimeric receptor (PDB ID: 5OSB). Insert link to rho1_pentamer.pdb

Supplemental Fig. 3. Docking of $3\alpha 5\beta P$, $3\alpha 5\beta PS$, β -estradiol, and $5\alpha THDOC$ to the intersubunit site located at the interface between Chains B and A (Site I; Hosie et al., 2006, Nature 444:486-489). Insert link to SiteI-poses.pdb

Supplemental Fig. 4. Docking of $3\alpha 5\beta P$, $3\alpha 5\beta PS$, β -estradiol, and $5\alpha THDOC$ to the intrasubunit site near the interface between membrane-spanning and extracellular domains on Chain A (Site II; Chen et al., 2019, PLoS Biol 17:e3000157). Insert link to SiteII-poses.pdb

Supplemental Fig. 5. Docking of $3\alpha 5\beta P$, $3\alpha 5\beta PS$, β -estradiol, and $5\alpha THDOC$ to the intrasubunit site near the cytoplasmic end of TM3 and TM4 domains on Chain A (Site III; Lavery et al., 2017, Nat Struct Mol Biol 24:977-985). Insert link to SiteIII-poses.pdb