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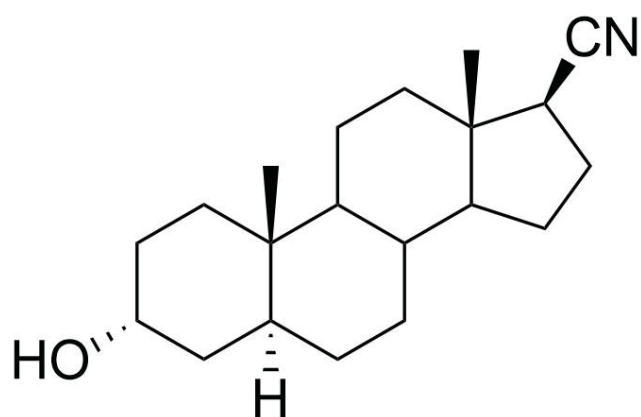
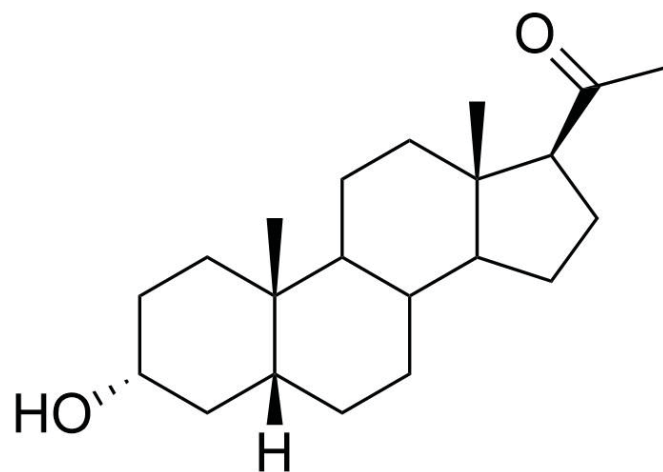
Supplemental Information

Inhibitory actions of potentiating neuroactive steroids in the human $\alpha 1\beta 3\gamma 2L$ GABA_A receptor

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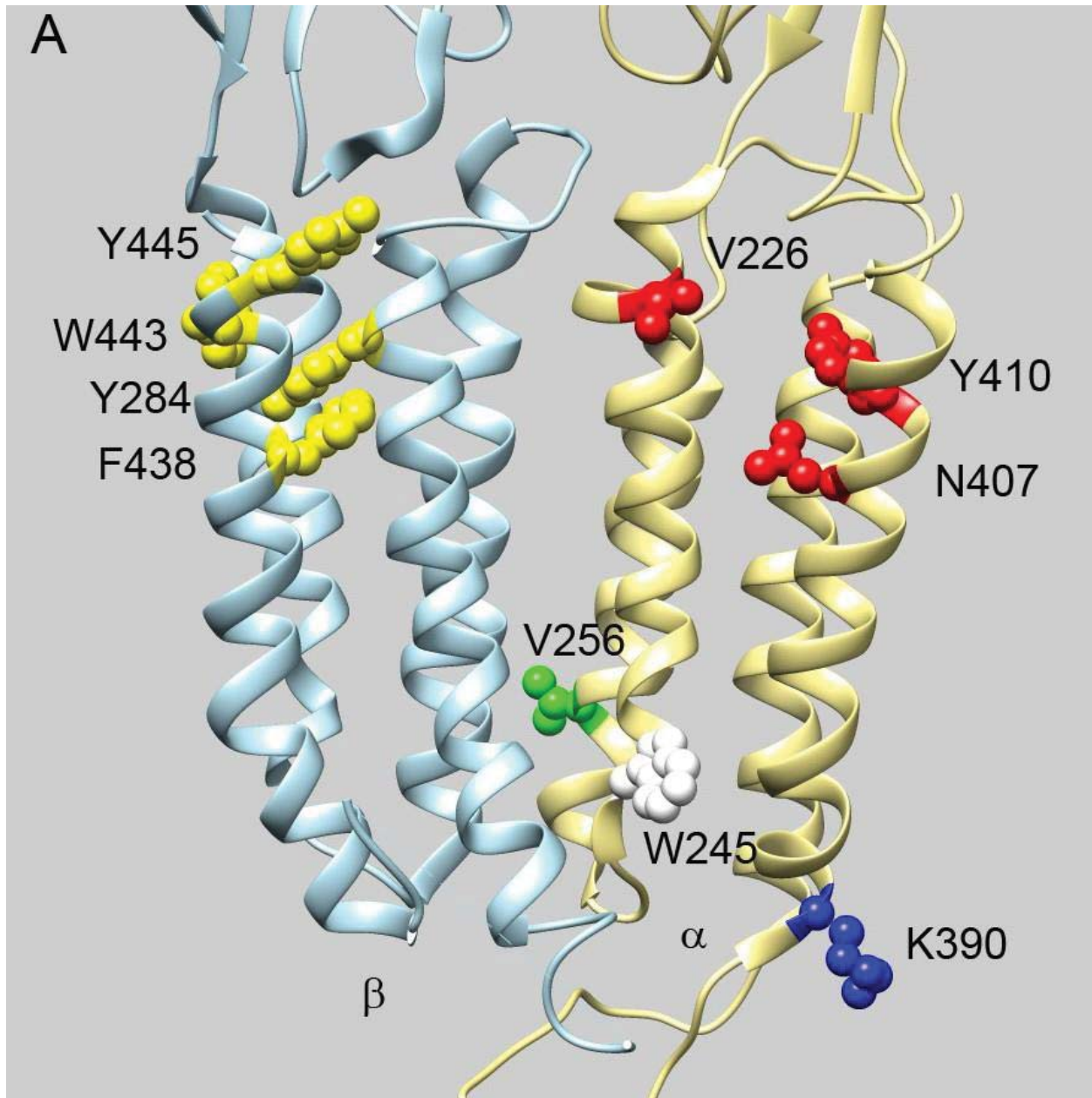
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Supplemental Figure 1



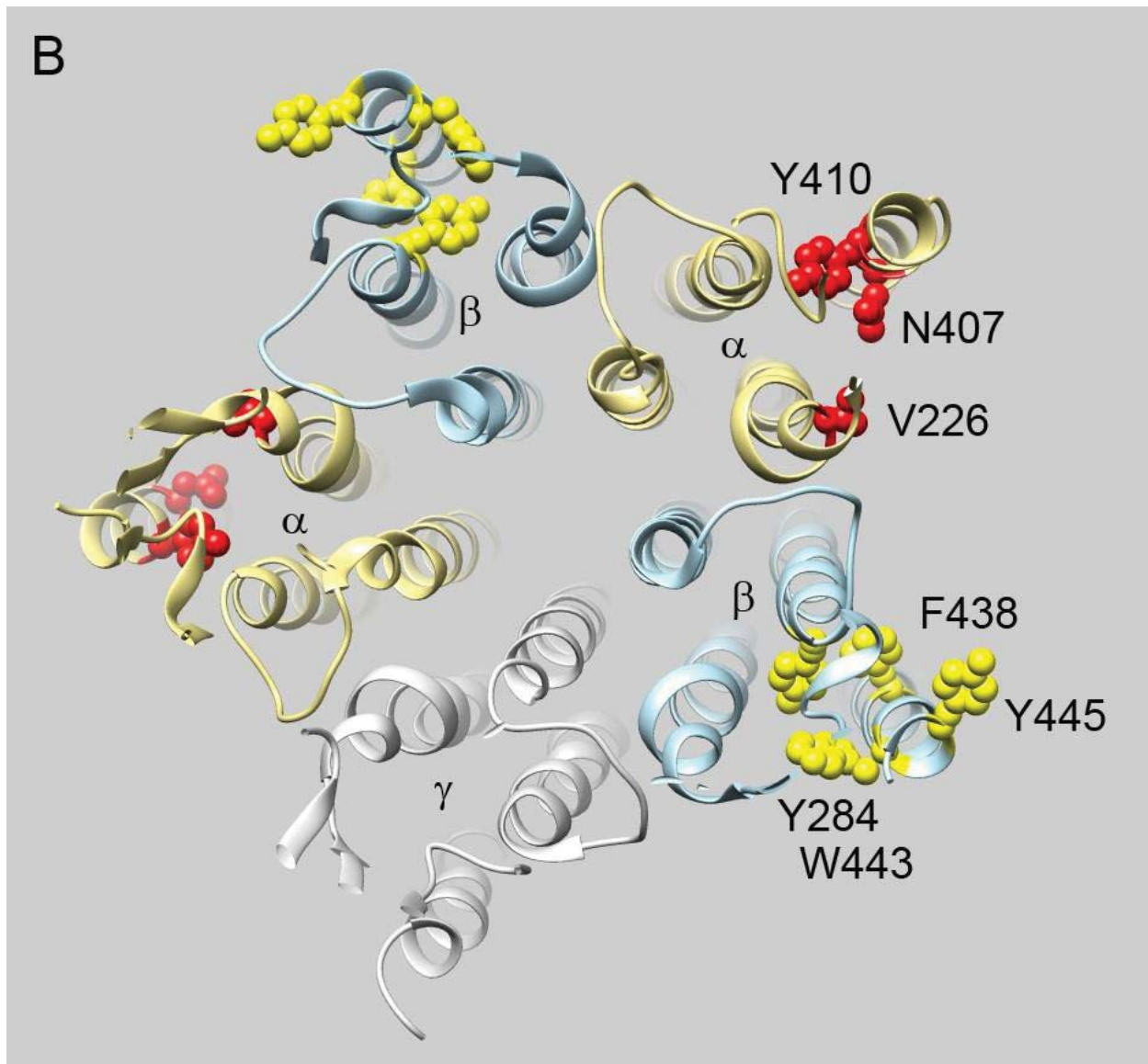
Structures of the endogenous neurosteroid 3 α -hydroxy-5 β -pregnan-20-one (3 α 5 β P, top) and the synthetic neuroactive steroid 3 α -hydroxy-5 α -androstane-17 β -carbonitrile (ACN, bottom).

Supplemental Figure 2



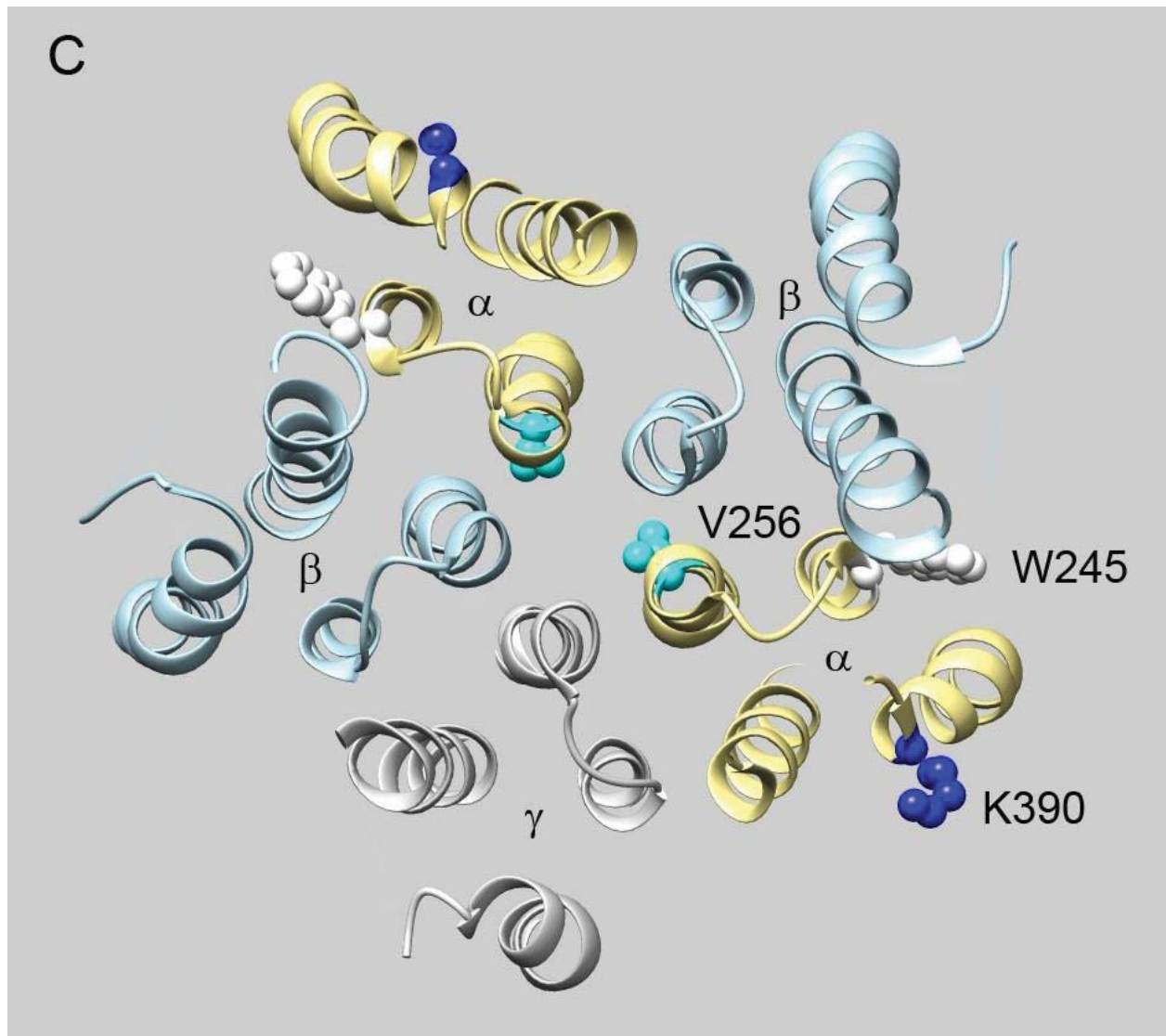
The figure shows a side view of the transmembrane domains (TMD) of one α (khaki) and one β (light blue) subunit, viewed from the lipid membrane. The mutated residues are shown as spheres. In the $\alpha 1$ subunit residues in the $\alpha 1$ intrasubunit site are shown in red ($\alpha 1(V2260)$, $\alpha 1(N4070)$, $\alpha 1(Y410)$), in the β - α intersubunit site in white ($\alpha 1(W245)$), as well as the residue identified by Lavery et al (Lavery et al., 2017) (blue, $\alpha 1(K390)$) and the 2' residue in the TM2 domain (green, $\alpha 1(V256)$). In the $\beta 3$ subunit residues in the β intrasubunit site are shown in yellow ($\beta 3(Y284)$, $\beta 3(F438)$, $\beta 3(W443)$, $\beta 3(Y445)$). The figure was produced in Chimera 1.17.3 (Pettersen et al., 2004) on the structure of the human $\alpha 1\beta 3\gamma 2$ GABA_A receptor (accession code 6i53; (Lavery et al., 2019)).

Supplemental Figure 3



The figure shows the external end of the TMDs of all 5 subunits (α khaki, β light blue and γ cyan) viewed from the extracellular fluid. The intracellular half of the TMDs has been omitted for clarity. Residues are identified as in Supplemental Figure 2. The figure was produced in Chimera 1.17.3 (Pettersen et al., 2004) on the structure of the human $\alpha 1\beta 3\gamma 2$ GABA_A receptor (accession code 6i53; (Lavery et al., 2019)).

Supplemental Figure 4



The figure shows the internal end of the TMDs of all 5 subunits (α khaki, β light blue and γ cyan) viewed from the cytoplasm. The extracellular half of the TMDs has been omitted for clarity. Residues are identified as in Supplemental Figure 2. Note that no residues in the shown region of the β subunit were mutated. The figure was produced in Chimera 1.17.3 (Pettersen et al., 2004) on the structure of the human α1β3γ2 GABA_A receptor (accession code 6i53; (Lavery et al., 2019)).

References

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- Lavery D, Thomas P, Field M, Andersen OJ, Gold MG, Biggin PC, Gielen M, and Smart TG (2017) Crystal structures of a GABAA-receptor chimera reveal new endogenous neurosteroid-binding sites. *Nat Struct Mol Biol* **24**: 977-985.
- Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, and Ferrin TE (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**: 1605-1612.