# SUPPORTING INFORMATION

## Agonist responses of (*R*)- and (*S*)-3-fluoro-*g*-aminobutyric acids suggest an enantiomeric fold for GABA binding to $GABA_C$ receptors

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## 1) Pharmacology: General Details

### Molecular biology

Pharmacological experiments were performed as previously described.[1-2]

Plasmids containing human $\rho_1$ and $\rho_2$ $GABA_C$ wild-type DNA (pcDNA3 and pKS respectively) inserts were linearized with *Xba*1 and *ECO*RI, respectively and T7 mMESSAGE mMACHINE kit (Ambion, Austin, TX) was used for mRNA synthesis.

### Expression of $\rho_1$ and $\rho_2$ receptors in *Xenopus* Oocytes

Oocytes from *Xenopus laevis* (South Africa clawed frogs) were harvested as described previously.[1] Stage V-VI oocytes were injected with 10-15 ng cRNA and then stored at 18 °C in ND 96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, pH 7.5) supplemented with 2.5 mM sodium pyruvate, 0.5 mM theophylline, 50 μg $ml^{-1}$ gentamycin.

**Electrophysiological Recordings**

Two to eight days after injections, the activity was measured by two-electrode voltage clamp recording using Geneclamp 500 amplifier (Axon Instruments, Foster City, CA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia), and Chart version 5.5.6 program as previously described.[2] Briefly, oocytes expression receptors were clamped at -60 mV with continuous flow of ND96 buffer. Compounds were screened for agonist activity by applying increasing concentrations of the compound to the cell bath until the maximal current was attained. Compounds were also screened for antagonist effects by testing the compound in the presence of a submaximal concentration of GABA (1 µM for $\rho_1$ and $\rho_2$ receptors).

**Data analysis**

The effects of agonists were evaluated for their agonist-concentration curves and all responses were normalised by the response activated by maximum concentration of GABA ($\rho_1$ and $\rho_2$, 100 µM). This data was fitted by least squares to the equation (1)

$$I = I_{max}[A]^{nH}/(EC_{50}{}^{nH} + [A]^{nH}) \qquad \text{Equation (1)}$$

Where $I$ is the peak current at a given concentration of agonist, $I_{max}$ is the maximal current generated by the concentration of agonist, [A] is the agonist concentration, $EC_{50}$ is the agonist concentration, which activates 50% of the maximum GABA response, and $n$H is the Hill coefficient.

## 2) Homology Modeling and Docking studies

Homology model of $\rho_1$ $GABA_C$ was generated by using the 'prime' suite in Maestro.[3] The crystal structure of the acetylcholine binding protein[4] (AChBP) from *L. stagnalis* (PDB code: 1I9B) was used as a template for generating the model. The sequence of $\rho_1$ $GABA_C$ (accession code: P24046) was aligned on the template in similar way to that of Adamian and Abdel-Halim *et al*.[5-6] Five subunits of the $\rho_1$ $GABA_C$ were individually made and merged to form a $\rho_1$ $GABA_C$ homopentmaric model. The OPLS_2005 all-atom force field was used for energy scoring of the protein and surface generalized Born (SGB) continuum solvation model for treating solvation energies and effects. The predicted model was then prepared for docking by using protein preparation wizard, wherein hydrogens were added, bond orders assigned and disulphide bonds created. Finally the corrected structure was optimized by restrained minimization using "impref minimization" by selecting hydrogens only so that heavy atoms were left untouched.

Docking studies were conducted using 'Glide' software as provided in Maestro.[7] A docking model was generated by forming a receptor grid around the active site amino acids of the two adjacent $GABA_C$ monomers. The centroid of Arg104, Ser168 of first chain and Tyr198 of adjacent chain was defined as the active site. The three ligands GABA **1**, (*S*)-3F-GABA **2**, and (*R*)-3F-GABA **3** were then docked in to the active site using extra-precision (XP) mode.

***References***

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