Supporting Material

The 5-HT_3AB receptor shows an A_3B_2 stoichiometry at the plasma membrane

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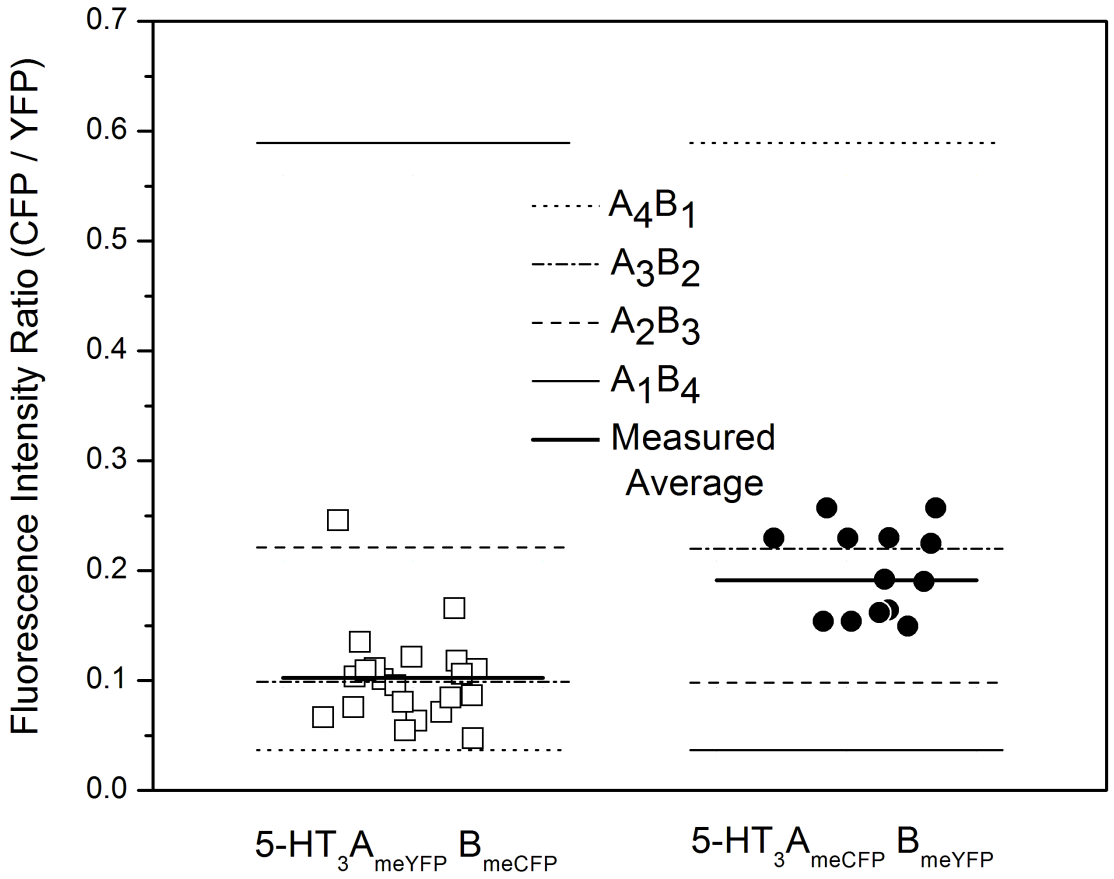

Figure S1. Fluorescence intensity ratio (FIR) on 5-HT_3AB membrane sheets: support for the A_3B_2 subunit ratio. Heteromers were expressed from a 1:9 ratio of A subunit to B subunit cDNA. Each point corresponds to a single membrane sheet's FRET corrected FIR. Expected average values for various stoichiometries were calculated using C as determined by Eq. 5. These data provide one of the data points in Figure 3 and Table 2.

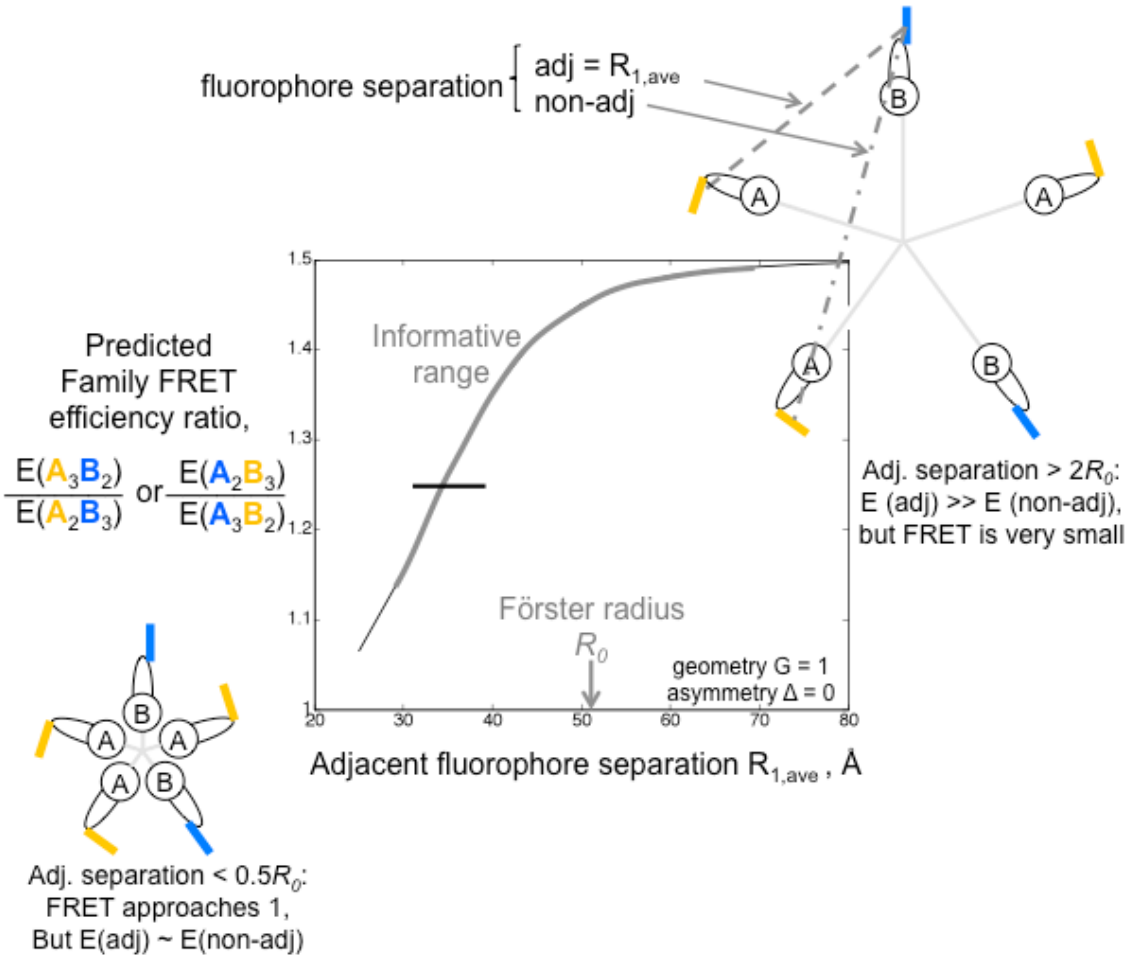

Figure S2. A schematic interpretation of family FRET measurements, based on the theory and simulations of Ref (1). The 5-HT_3AB receptor is depicted, with A_3B_2 stoichiometry; the A_2B_3 stoichiometry is not depicted. Each A subunit has a **YFP** acceptor, and each B subunit has a **CFP** donor, fused into the M3-M4 cytoplasmic loops. The average separation between adjacent (adj) fluorophores is termed $R_{1,ave}$ in Ref (1); this parameter varies along the X-axis. The Y-axis shows that family FRET efficiency, $E(A_{YFP}B_{CFP})$ for the A_3B_2 stoichiometry, always exceeds $E(A_{CFP}B_{YFP})$ for the A_3B_2 stoichiometry. The Förster radius R_0 is ~ 52 Å for this fluorophore pair.

In its simplest form, the theory simulates a regular planar pentagon that has radially disposed fluorophore dipoles. Departures from this structure are described by parameters termed geometry/coplanarity G, and asymmetry Δ. Ref (1) presents simulations over ranges of G and Δ; for the simulations presented here, G and Δ are set to 1 and 0, respectively, to represent a regular planar pentagon.

For all reasonable values of $R_{1,ave}$, G and Δ, FRET efficiency for the A_3B_2 stoichiometry, $E(A_{YFP}B_{CFP})$, exceeds that for A_2B_3, $E(A_{YFP}B_{CFP})$, by a factor of 1.1 to 1.4. For fluorophore separations < $0.5R_0$, E approaches 1, but E for an adjacent pair only slightly exceeds E for a non-adjacent pair; this vitiates accurate measurements. For fluorophore separations > $2R_0$, E for an adjacent pair >> E for a non-adjacent pair, but the E values

become unmeasurably small. This again vitiates accurate data. Therefore FRET measurements can distinguish the two stoichiometries, if $0.6R_0$ < the fluorophore separation < $1.4R_0$. The structures of cytoplasmic loops are not known. Structural data for the extracellular binding domains and transmembrane domains of Cys-loop receptor shows that homologous residues on adjacent subunits are separated by 5 to 40 Å. Fluorescent proteins have a diameter of 25 Å, precluding a closer approach of the fluorophores. Because we do not know G and Δ, more specific inferences cannot be made about the size / shape of the M3-M4 loops, or about the fluorophore separation $R_{1,ave}$.

The heavy horizontal line shows the average ratio determined in this study, 1.24 (Figure 4). Because this value exceeds 1, the data are compatible with a stoichiometry of A_3B_2. This compatibility, not a value for the fluorophore separation, is the major conclusion of this section.

1. Srinivasan, R., C. I. Richards, C. Dilworth, F. J. Moss, D. A. Dougherty, and H. A. Lester. 2012. Forster Resonance Energy Transfer (FRET) Correlates of Altered Subunit Stoichiometry in Cys-Loop Receptors, Exemplified by Nicotinic α4β2. Int J Mol Sci 13:10022-10040.