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# Evidence that human P2Y1 and P2Y12 receptors form heterodimers

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**Introduction:** P2Y1 and P2Y12 receptors belong to the class A family of transmembrane GPCRs that are activated by endogenous nucleotides<sup>1</sup>. There is growing evidence that many GPCRs, including P2Y receptors, can exist as dimers or higher-order oligomers<sup>2</sup>. For example, P2Y12 and PAR4 receptors were recently reported to dimerise<sup>3</sup>. Our previous studies indicated that hP2Y1 and hP2Y12 receptors may form a functional heterodimer with novel pharmacological and signalling properties<sup>4</sup>. The aim of this project was, therefore, to characterise the physical interaction between hP2Y1 and hP2Y12 receptors.

**Method:** tSA201 cells were transfected or co-transfected with hP2Y1 and hP2Y12 receptors, tagged with HA or a fluorescent protein. Cellular localisation and co-localisation of the receptors were determined using confocal microscopy. Transfected cells were cultured in the absence or presence of the N-glycosylation inhibitor tunicamycin (2.0 µg/ml) for 16 hours to determine the role of N-glycosylation in receptors expression. Receptor cell surface expression was quantified using ELISA. To investigate physical interaction between the two P2Y subtypes, co-immunoprecipitation was performed using anti-HA-agarose beads followed by immunoblotting with anti-GFP, anti-HA then alpha-Tubulin antibodies.

**Result:** Following transfection on their own or together, both receptors were localised mainly at the cell membrane, and this was unaffected by tunicamycin. Co-immunoprecipitation confirmed that P2Y1 and P2Y12 receptors associate physically. Each subtype enhanced the other's surface expressions. In particular, expression of the P2Y12 receptor more than doubled that of P2Y1 receptors at the cell surface.

**Conclusion:** These results show that P2Y1 and P2Y12 receptors are physically associated at the cell membrane and that they enhance each other's cell surface expressions. These results are consistent with our previous data indicating that P2Y1 and P2Y12 receptors form a functional heteromer.

## References:

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4. Shakya-Shrestha S et al. (2010) *Mol. Cell Neurosci* 43: 363-369.