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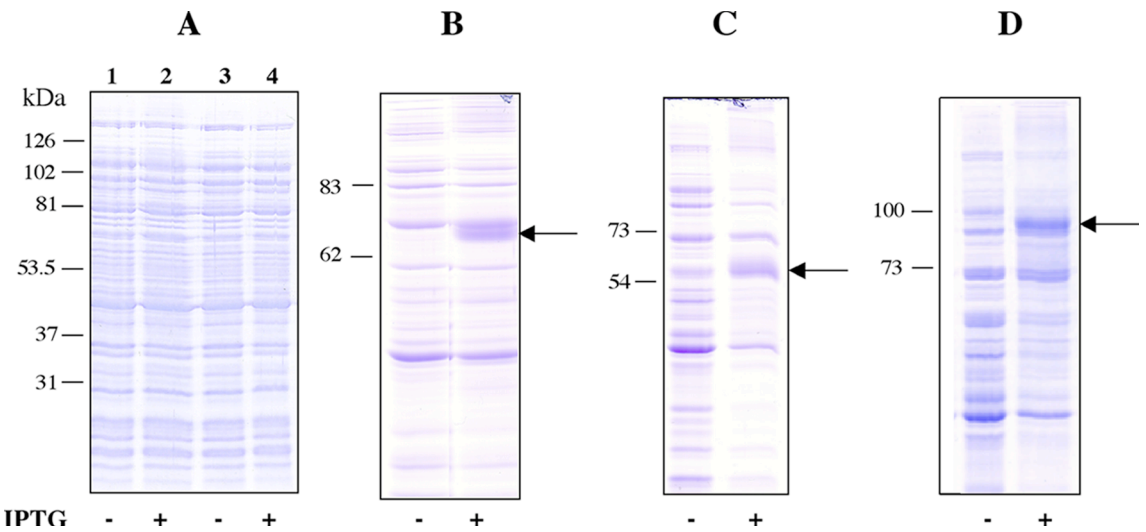
SUPPLEMENTAL FIGURES

Supplemental Figure 1: Overexpression of the human AVP V2 GPCR: comparison of the different fusions.

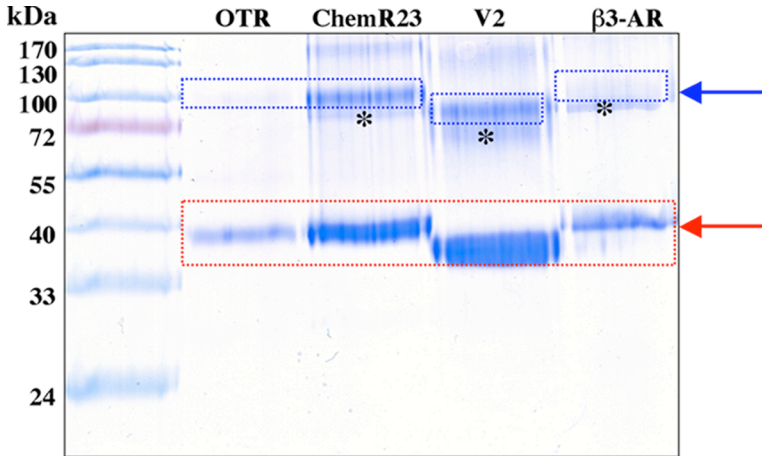
Total bacterial proteins were separated onto 12% SDS-polyacrylamide gels and stained with Coomassie Blue. Equivalent quantity of proteins was put into each well. In A, V2 was expressed as a single protein (lane 3 and 4). Lanes 1 and 2 correspond to negative controls: Rosetta^{TM}(DE3) were transformed with an empty pET21a. V2 was expressed as fusion with the β_5I (B), the PLC (C) or the PurF (D) partner, respectively. In B, C and D, arrows indicate recombinant fusions.

Supplemental Figure 2: Thrombin cleavage of β_5I fusions and purification of GPCRs.

Representative fusions were chosen for thrombin cleavage. Purification of the isolated OTR, ChemR23, V2 and β3AR was then done. Samples were loaded onto 12% SDS-polyacrylamide gels and proteins stained with Coomassie Blue. 80 μl of each GPCR were put into each well. GPCR monomers are shown with red arrows and dotted squares, SDS-resistant dimers indicated with blue arrows and dotted squares. Incompletely cleaved fusions were also detected in the purified samples, as shown by asterisks. Due to an additional gel filtration step, only OTR monomers are seen.



Supplemental Figure 1



Supplemental Figure 2