Galanin Receptor 3 (GALR3) is a G-protein-coupled receptor with a widespread distribution in the brain and plays a role in a variety of physiologic processes including cognition/memory, sensory/pain processing, hormone secretion, and feeding behavior. Therefore, GALR3 is considered an attractive CNS drug target (Freimann et al., 2015) [1]. This dataset contains GALR3 point mutants that improve recombinant protein expression and thermal stability of the receptor contained in virus-like particles (VLPs) or obtained by detergent-puriﬁcation of baculovirus-infected insect cells. The mutations listed can be grouped in those that improve the stability of the agonist-bound and the antagonist-bound form of the receptor. Protein characteristics in terms of protein expression and thermal stability were comparable between GPCR-VLP and GPCR overexpressing Sf9 cultures. The further analysis and detailed results of these mutants as well as their impact on biophysical assay development for drug discovery can be found in "Method for
Rapid Optimization of Recombinant GPCR Protein Expression and Stability using Virus-Like Particles" (Ho et al., 2017) [2].
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**Specifications Table**

| Subject area               | Biology                          |
|----------------------------|----------------------------------|
| More specific subject area | GPCR Protein Engineering and Stabilization |
| Type of data               | Table, figure                    |
| How data was acquired      | Western Blot, Radioligand binding assay, HPLC, thiol-specific fluorochrome N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM) assay [3], Electrospray ionization (ESI)-Mass Spectrometry (MS), hybrid quadrupole time-of-flight mass spectrometer (Q-ToF Ultima, Waters, Manchester, UK) |
| Data format                | Filtered and analyzed            |
| Experimental factors       | Does not apply                   |
| Experimental features      | GALR3 mutants were produced and screened in virus-like particles using label and label-free assay formats; recombinant receptor protein expression and stability was obtained in virus-like particles and after detergent-solubilization from recombinant Sf9 expressions. |
| Data source location       | Does not apply                   |
| Data accessibility         | The data are included in this article |

**Value of the data**

- The GALR3 mutants listed here aid in increasing the recombinant protein expression yield and stabilization of the receptor in either the agonist or antagonist-bound form.
- Mutational analysis of the GALR3 variants was performed in VLPs. The chemically stabilizing environment of the phospholipid bilayer in a VLP eliminates the need for recombinant overexpression, purification and detergent solubilization during the iterative protein engineering process.
- GALR3 protein quality from VLPs was comparable to detergent-purified receptors from over-expressing Sf9 cultures.

1. Data

The dataset of this article provides information on GALR3 mutants that stabilize the receptor in either an agonist-bound or antagonist-bound form. Table 1 shows how 23 of the 210 point mutants expressed on VLPs increase [125I]-galanin binding and thermal stability compared to WT. In addition, a direct correlation of the $B_{\text{max}}$ values with recombinant expression yields of the mutants in Sf9 cultures was shown (Table 1 and Fig. 1). Combinations of these mutants were made to find the best thermostabilizing GALR3 agonist-bound (Table 2) and GALR3 antagonist-bound (Table 3) variants.
2. Experimental design, materials and methods

2.1. Characteristics of GALR3 point mutants in VLP versus detergent purified receptors from over-expressing Sf9 cultures

Initial rank ordering of 210 GALR3-VLP point mutants, mostly located in GALR3 transmembrane helices, was based on expression yield obtained from a radiometric assay measuring the binding of [125I]-porcine galanin. Figure 1 shows a Western blot analysis of GALR3 WT and point mutants. The point mutants were selected out of 210 GALR3 mutants generated using the VLP platform and based on a \( \frac{B_{\text{max}}(\text{mutant})}{B_{\text{max}}(\text{WT})} \) value of \( \geq 1.0 \) measured using a [125I]-porcine galanin radiometric competition binding assay. The 19 mutants were then expressed in insect cells and lysates were run on an SDS-PAGE. The protein of interest was visualized by Western blot utilizing an anti-6xHis epitope antibody directed against the GALR3 C-terminal 6xHis-tag.

| GALR3 mutant | \( \frac{B_{\text{max}}(\text{mutant})}{B_{\text{max}}(\text{WT})} \) VLP samples | Tm(mutant)-Tm (WT) VLP samples | Density (mutant)/Density (WT) Sf9 cell lysate |
|--------------|-------------------------------------------------|---------------------------------|----------------------------------|
| WT           | 1.0                                             | 0                               | 1.0                              |
| A115L        | 7.0                                             | 2.9                             | 6.7                              |
| S110A        | 5.3                                             | 5.6                             | 6.4                              |
| A141L        | 3.8                                             | 3.3                             | 4.4                              |
| R125A        | 3.2                                             | 3.7                             | 3.1                              |
| L100A        | 3.1                                             | 3.7                             | 4.3                              |
| L208A        | 3.0                                             | 3.5                             | 3.7                              |
| L211A        | 2.9                                             | 3.3                             | 3.6                              |
| S117A        | 2.8                                             | 3.3                             | 3.7                              |
| C272A        | 2.3                                             | 3.8                             | 3.6                              |
| P21A         | 2.0                                             | 2.5                             | N/A                              |
| A19L         | 1.8                                             | 2.2                             | 2.4                              |
| A97L         | 1.8                                             | 3.7                             | 3.1                              |
| A236L        | 1.7                                             | 1.5                             | N/A                              |
| R120A        | 1.7                                             | 3.3                             | 2.7                              |
| C70A         | 1.7                                             | 2.0                             | 3.2                              |
| R235A        | 1.6                                             | 3.4                             | N/A                              |
| W50A         | 1.5                                             | 4.0                             | 2.0                              |
| A268L        | 1.5                                             | 2.0                             | 2.4                              |
| G144A        | 1.5                                             | 1.7                             | 1.8                              |
| A49L         | 1.2                                             | 4.4                             | 1.1                              |
| L93A         | 1.1                                             | 4.7                             | 1.4                              |
| V116A        | 1.1                                             | 6.1                             | 0.8                              |
| A198L        | 1.0                                             | 2.3                             | N/A                              |

Summary of 23 (out of a total of 210) GALR3 mutants that exhibited a \( \frac{B_{\text{max}}(\text{mutant})}{B_{\text{max}}(\text{WT})} \) value of \( \geq 1.0 \) with their concomitant thermal stability relative to WT. Both values were obtained from a [125I]-porcine galanin radiometric competition binding assay using VLP-GALR3 samples. VLP samples were prepared in a small scale, only sufficient for one thermal stability experiment, performed in duplicate. Protein expression yield improvement relative to WT was obtained consequently of the same mutants when recombinantly expressed using baculovirus-infected Sf9 insect cells. These values are presented in the table as relative band intensities measured from a Western blot analysis utilizing an anti-6xHis epitope antibody directed against the GALR3 C-terminal 6xHis-tag. Small-scale expression samples were used and only sufficient for one Western-blot analysis. The intensity of the protein bands corresponding to the GPCR of interest was measured by the software ImageJ (https://imagej.nih.gov). N/A means not available.

Fig. 1. Western blot analysis of GALR3 WT and point mutants. The point mutants were selected out of 210 GALR3 mutants generated using the VLP platform and based on a \( \frac{B_{\text{max}}(\text{mutant})}{B_{\text{max}}(\text{WT})} \) value of \( \geq 1.0 \) measured using a [125I]-porcine galanin radiometric competition binding assay. The 19 mutants were then expressed in insect cells and lysates were run on an SDS-PAGE. The protein of interest was visualized by Western blot utilizing an anti-6xHis epitope antibody directed against the GALR3 C-terminal 6xHis-tag.
[125I]-galanin to the receptor and comparing $B_{\text{max}}$ values of the mutants relative to the $B_{\text{max}}$ value of WT GALR3. 23 of the 210 mutants showed $B_{\text{max}}$ (mutant)/$B_{\text{max}}$ (WT) values of ≥ 1.0 and an increase in thermal stability relative to WT GALR3 (Table 1). 19 GALR3 point mutants were then recombinantly expressed in Sf9 insect cells to measure protein expression yields as determined by SDS-PAGE/Western blot band intensities (Fig. 1). Expression levels of the 19 single mutants followed the same rank-order as the $B_{\text{max}}$ values measured by the radiometric assay on the corresponding VLP samples (Fig. 1 and Table 1).

Table 2
Protein characteristics of GALR3 agonist-bound mutant combinations.

| GALR3 mutant | Total protein yield (µg/L Sf9 culture) | SEC peak ratio (%monodisperse/aggregated) | $T_m$ (°C) + galanin |
|--------------|----------------------------------------|-------------------------------------------|---------------------|
| WT (reference) | 37                                     | 10                                        | 48°                 |
| S110A + V116A | 91                                     | 90                                        | 56°                 |
| A115L + A198L + C272A | 120                                | 83                                        | 56°                 |
| S110A + A115L + L208A | 96                              | 76                                        | 55°                 |
| S110A + A115L + R120A | 48                             | 50                                        | 50°                 |
| S110A + A115L + L211A | 79                              | 62                                        | 55°                 |
| S110A + A115L + A198L | 53                              | 62                                        | 53°                 |
| S110A + A115L + A236L | 38                             | 50                                        | 56°                 |
| S110A + V116A + A236L | 120                            | 93                                        | 62°                 |
| S110A + V116A + R125A | 160                            | 90                                        | 61°                 |
| S110A + V116A + L100A | 244                            | 86                                        | 58°                 |
| S110A + V116A + S117A | 201                         | 87                                        | 61°                 |
| S110A + V116A + L211A | 152                          | 81                                        | 60°                 |
| S110A + V116A + C272A | 188                      | 76                                        | 59°                 |
| S110A + V116A + L208A | 228                          | 93                                        | 59°                 |
| S110A + V116A + A141L | 95                           | 95                                        | 59°                 |
| S110A + R120A + A141L + A236L | 196                                      | 74                                        | 53°                 |
| S110A + V116A + L208A + A236L | 183                                  | 86                                        | 62°                 |
| S110A + R120A + L208A + A236L | 49                               | 92                                        | 55°                 |
| S110A + R120A + A141L + L208A + A236L | 169                                  | 96                                        | 57°                 |

Mutants are shown for which ≥ 50% monodispersity could be achieved after mid-scale (300 ml) recombinant expression in insect cells followed by detergent solubilization and purification. Expression was done once and provided enough material for one $T_m$ measurement. Receptor purity and monodispersity was analyzed using SDS-PAGE and analytical size-exclusion chromatography (SEC). Thermal stability was measured by the thiol-specific fluorochrome N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide (CPM) assay [3] *Note that WT GALR3 protein was mostly aggregated and its $T_m$ value cannot be accurately measured. However, GALR3WT was added to the table as a reference.

Table 3
Protein characteristics of GALR3 antagonist-bound mutant combinations.

| GALR3 mutant | Total protein yield (µg/L Sf9 culture) | %Mono-dispersion | $T_m$ (°C) (+DNS001131702) |
|--------------|----------------------------------------|------------------|-----------------------------|
| WT (reference) | 44                                     | 54               | 36 (±DNS001131702)          |
| R120A + A223L | 62                                     | 89               | 58 (±DNS001131702)          |
| V116A + R120A | 79                                     | 87               | 51 (±DNS001355175)          |
| V116 + A223L  | 82                                     | 68               | 55 (±DNS001355175)          |
| V116A + R120A + A223L | 72                                         | 90               | 58 (±DNS001355175)          |

Mutants are shown for which ≥ 50% monodispersity could be achieved after mid-scale (300 ml) recombinant expression in insect cells followed by detergent solubilization and purification in the presence of antagonist DNS001131702 or DNS001355175. Expression was done once and provided enough material for one $T_m$ measurement. Thermal stability was measured by the CPM assay [3].
2.2. GALR3 agonist-bound thermostabilizing variants

The best GALR3 single point mutants were combined for mid-scale (300 ml) recombinant expression in Sf9 insect cells and protein parameters such as expression yield, monodispersity and thermal stability in the presence of galanin are listed in Table 2, summarizing the best mutant combinations that improved the agonist-bound protein characteristics of the receptor.

2.3. GALR3 antagonist-bound thermostabilizing variants

In order to find the stabilizing GALR3 antagonist-bound mutants, we used the data from the GALR3-VLP [\(^{125}\text{I}\)]-galanin binding assay described above to select 88 single point mutants with \(B_{\text{max}}\) values \(\geq 80\%\) of WT, assuming that these displayed enough receptor density to be further evaluated in a size-exclusion chromatography/liquid chromatography mass spectroscopy (SEC/LC-MS) based binding assay. Prior to SEC/LC-MS, the GALR3-VLP samples were incubated with GALR3 antagonist, DNS000135175 (\(K_i = 55\) nM) or DNS001131702 (\(K_i = 127\) nM) and subjected to a temperature gradient ranging from 15 °C to 55 °C. Before the acquisition of the LC-MS spectra of the ligand, unbound ligand was removed by SEC. A total of 5 single point mutants were found to stabilize the antagonist form of GALR3 by \(\geq 4\) °C when compared to the WT form, and rank-ordered from most to least stabilizing as: A223L (\(\Delta T = 8.3\) °C), A218L (\(\Delta T = 7.2\) °C), R120A (\(\Delta T = 5.4\) °C), V116A (\(\Delta T = 4.6\) °C), E224A (\(\Delta T = 4.2\) °C).

Similar to the GALR3 agonist-bound studies, the best GALR3 single point mutants were combined for recombinant expression in Sf9 insect cells and protein parameters such as expression yield, monodispersity and thermal stability in the presence of a GALR3 antagonist-bound form are listed in Table 3.

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Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2017.04.057.

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