

# Homogeneous Receptor Ligand Binding Assay Based on TR-FRET

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# 1

## Introduction

Traditionally pharmacological receptor ligand binding assays for G-protein coupled receptors (GPCRs) have been performed using radioligands and filtration that is difficult to automate. Now we have developed a homogeneous receptor ligand binding assay based on time-resolved fluorescence resonance energy transfer technology (TR-FRET) also suitable for HTS applications and automation. As a model system we have used human galanin receptor subtype 2 (hGalR2). The endogenous agonist for hGalR2, galanin, is a 30-amino acid neuropeptide. In this model assay we have used Eu-labelled galanin as a donor component and various acceptor components; Alexa647-labelled lectins or biotinylated lectins in combination with APC (allophycocyanin) labelled streptavidin. Most lectins are multimeric and they are able to bind to their cognate oligosaccharides on the cell surface. This binding property of lectins enables us to bring acceptor molecule to the close proximity of the donor component, Eu-labelled galanin which is bound to the receptor binding cavity. As a result we have a sensitive, non-radioactive and user friendly receptor ligand binding assay.

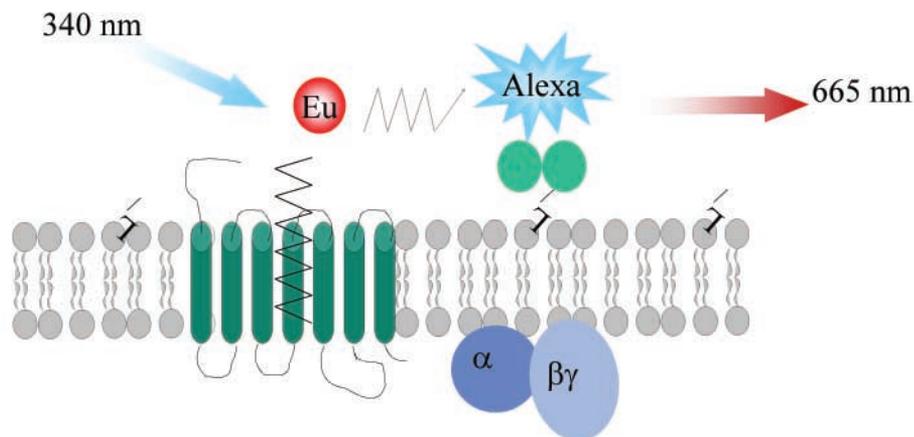
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## Methods

For the receptor ligand binding assay human galanin peptide (Bachem) was labelled with LANCE® Eu chelate W1024 (PerkinElmer) and purified with HPLC. Two different lectins with and without biotinylation were tested; WGA (wheat germ agglutinin) and DSL (datura stramonium lectin) are commercially available from Vector laboratories. Lectins were either labelled with Alexa647 (Molecular Probes) or biotinylated lectins was used in combination with streptavidin-APC (allophycocyanin) (PerkinElmer). WGA-APC was from Prozyme. Cell membrane preparation was done as described earlier (1) from the CHO cell line expressing hGalR2 ( $B_{max}$  3-6 pmol/mg).

### Assay principle:

- ▶ Pipet all assay components into the 96-well microtitration plate (tot vol. 90  $\mu$ l/well):
  - reaction buffer: 2.5 mM MgCl<sub>2</sub>, 25  $\mu$ M EDTA, 0.2% TSA-BSA, 0.2% Bacitracin, 1 mM PMSF in 50 mM Tris-HCl pH 7.4 (optimized for hGalR2)
  - cell membrane concentration: 1-10  $\mu$ g/well
  - WGA lectin labelled with Alexa (~500 ng/well) or biotinylated lectin: streptavidin-APC (1:1) (~500 ng/well)
- ▶ Incubation at +4°C or RT for 30 min - 4 h
- ▶ Measured in VICTOR<sup>2</sup>™V multilabel counter using factory-set LANCE protocol

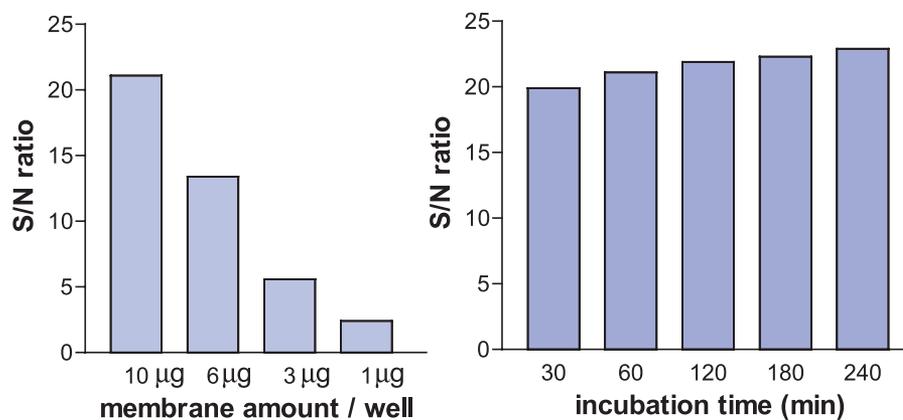


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## Results

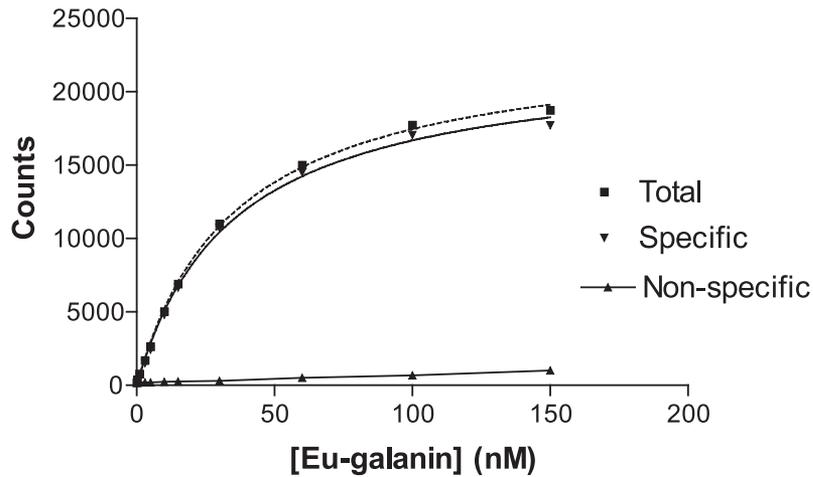
Acceptor component	RT (S/N ratio)	+4°C (S/N ratio)
WGA-Alexa647	19.8	21.9
DSL-Alexa647	5.5	5.7
WGA-APC	3.1	3.2
Bio-DSL + SA-APC	2.0	1.9
Bio-WGA + SA-APC	1.6	1.1

**Figure 1.** Comparison of different acceptor compounds and incubation temperatures in homogenous ligand binding assay.

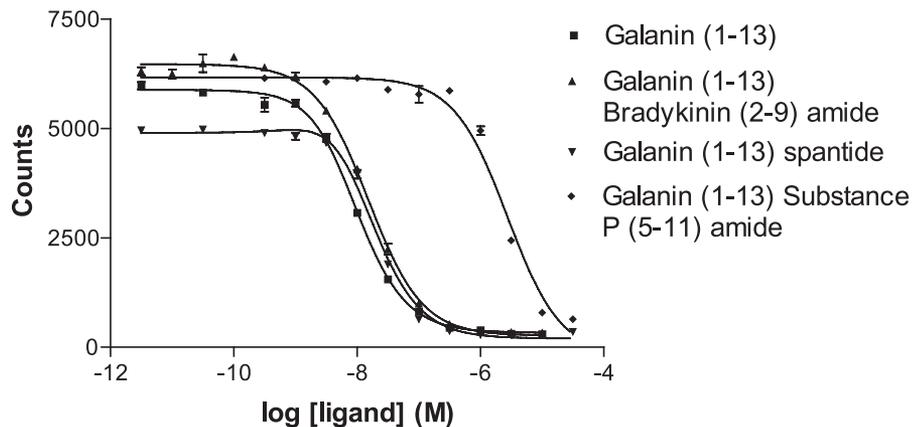


**Figure 2.**

- A) The effect of different membrane amounts / well using WGA-Alexa647 as an acceptor compound. Incubation was done at +4°C for 2 h.
- B) The effect of different incubation times on S/N ratio using 10 µg of membrane protein / well and WGA-Alexa647 as an acceptor compound.



**Figure 3.** Saturation curve for hGalR2 using Eu-labelled galanin and WGA-Alexa647. Reaction conditions for saturation and competition assays were: 10  $\mu$ g of membrane preparation and 500 ng/well WGA-Alexa647 in reaction buffer mentioned above. Plate was incubated 2 h at +4°C. Non-specific binding was measured in the presence of 5  $\mu$ M galanin.  $K_d$  for Eu-galanin is 34 nM.



**Figure 4.** Competition binding assay with galanin and three different antagonists.  $K_i$  values for these compounds were; galanin 6.5 nM, galanin (1-3) bradykinin (2-9) amide 9.6 nM, galanin (1-13) spantide 13.4 nM and galanin (1-13) substance P (5-11) amide 1.7  $\mu$ M. The results are means $\pm$ SEM of three replicates.

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## Conclusions

- ▶ We have developed a non-radioactive, homogeneous receptor ligand binding assay which is easy to perform and suitable for HTS applications.
- ▶ Assay can be performed at RT without any evidence of receptor protein degradation; not even with long incubation times (detected up to 4 hours).
- ▶ As low as 6  $\mu\text{g}$  of membrane protein / assay gives reasonable S/N ratios (S/N > 10).
- ▶ This homogenous LANCE receptor ligand binding assay gives  $K_i$  values which are slightly shifted to the right compared to the traditional radiolabel binding assay (2).

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## References

- 1- Janson *et al.*: Eur.J.Pharmacol. 374;137-146, 1999
- 2- Branchek *et al.*: TiPS 21;109-117, 2000



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