Targeting GPCRs with Small Molecule Allosterics

A snapshot of TruBind Assay work completed by researchers in MSI Drug Discovery Services laboratories worldwide

SUMMARY

The Importance of GPCRs

Allosteric modulators hold great promise as drug candidates by offering a braod range of advantageous effects on GPCR function. By exerting their influence at less structurally conserved sites, these compounds can achieve higher levels of selectivity over orthosteric binding compounds. As they do not directly compete for endogenous ligand binding, they offer a more fine-tuned modulatory control that goes beyond simple activation/inhibition and have saturable limits on their activity that can be attractive from a pharmacological perspective.

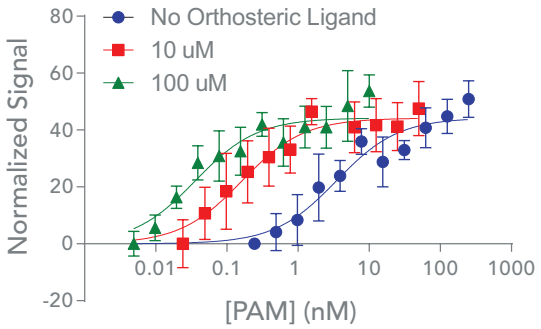
Challenges in Discovery of GPCR Allosterics

GPCRs, when removed from their native cell membrane environment, can be highly unstable and lose the organized structure that is critical to their activity. Many established technologies require removal of GPCRs from the membrane and cause substantial modification through attachment to a surface before characterization. In addition, high material consumption, limits on detecting enthalpic but not entropic binding interactions, and large target:compound mass ratio issues further complicate matters.

SUPPORTING DATA

GPCR Allosteric Discovery – The BSI Advantage

Back-Scattering Interferometry is a label-free, free-solution molecular interaction technology that has demonstrated ability to characterize small molecule GPCR interactions, detect target conformational change and characterize distinct modes of allosterism such as affinity- versus efficacy-driven mechanisms, all while using intact membrane proteins.

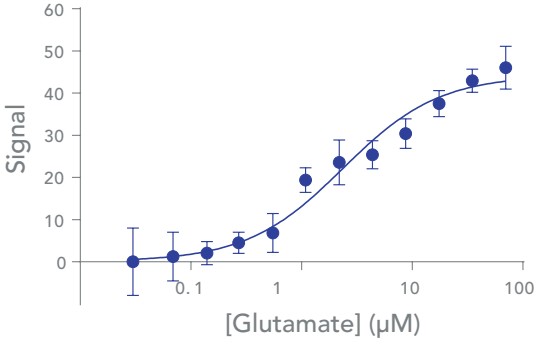


Class A GPCR. *Increase in allosteric ligand affinity (decrease in K_d) as orthosteric ligand concentration is increased identifies the titrated compound as a positive allosteric modulator.*

Characterization of Allosteric Mechanism

A key advantage of BSI is its applicability to mechanistic studies, being able to directly determine allosteric small molecule compound affinity for large, complex targets. Additionally, demonstration of conformational coupling between orthosteric and allosteric sites can bridge medicinal chemistry and pharmacology goals for refinement of drug profiles.

BSI is able to measure low affinity interactions, when other techniques fail, due to its sensitivity to receptor conformational changes in free solution.



The affinity of glutamate for the mGluR5 receptor. *A K_d of 2.4 µM can be computed from the binding curve, which is comparable with data from other techniques.*

MOLECULAR SENSING

TruBind™ Assay Services

Back-Scattering Interferometry at Work

IDENTIFICATION OF NOVEL Zn^{2+} MODULATED GPR39 AGONISTS[1]

BSI has been used in the characterization of small molecule ligand binding to human GPR39 overexpressed in crude membrane fractions in free solution. GPR39 is a Zn^{2+} responsive GPCR under investigation as a therapeutic target for type-2 diabetes. The ability to measure the affinity of small molecule agonists such as Zn^{2+} is especially novel, given the unfavorable mass ratio and fast off rate that complicates the use of more established binding assays. Representatives from multiple novel GPR39 agonist series have been evaluated and BSI-derived affinity and functional assay-derived potency correlate for compounds of varying scaffolds.

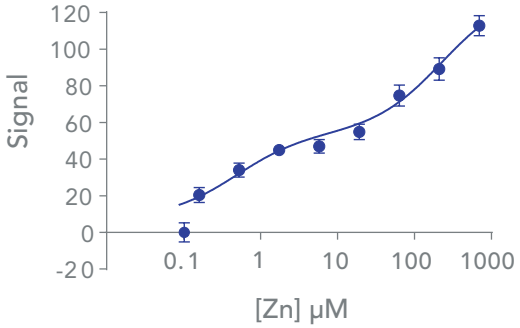


Figure 3: *BSI was used to quantitate the binding of Zn^{2+} to GPR39. The data fits best to a two-site binding model with a high-affinity K_D of 0.54 ± 0.06 µM and low-affinity K_D of 215 ± 34 µM.*

KEY BENEFITS

TruBind BSI Technology delivers key benefits in the investigation of GPCR inhibitors, with no tags, no surface attachments for true binding characterization.

- Uniquely informs medicinal chemistry for complex and difficult to address targets
- Maintains target integrity: label- and tether-free, free-in-solution; target in native, or native-like, environment
- Target conformation sensitive detection
- Sensitivity to directly detect small molecule binding to large, complex targets
- Determination of mode of allosteric modulation
- Rapid assay development
- Discovery of high-value 'next-generation' therapeutic candidates

1. NOVEL GPR39 AGONISTS: CORRELATION OF BINDING AFFINITY USING LABEL-FREE BACK-SCATTERING INTERFEROMETRY WITH POTENCY IN FUNCTIONAL ASSAYS

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Company

Molecular Sensing, Inc. (MSI), is a commercial stage drug discovery tools and contract research services company with headquarters and drug discovery services laboratories in Nashville, Tennessee and an R&D center in Los Gatos, California, along with a European operations center near Frankfurt, Germany.

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