MOLECULAR SENSING

WHITE PAPER

The Role of Back-Scattering Interferometry in the Discovery and Development of Allosteric Ligands

DRUG DISCOVERY IS IN A "BIND"

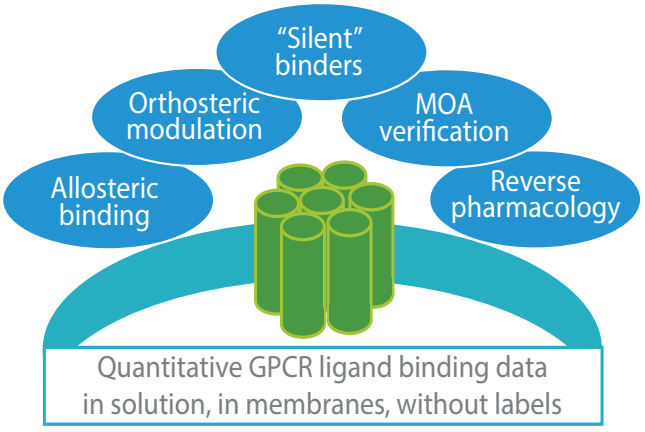
Back-Scattering Interferometry (BSI) is an emerging label-free conformation-sensitive technology that fills a vital need in discovery and development of pharmaceuticals in the highly valuable "allosteric" space. The drug development process is increasingly frustrated by the search for compounds that bind to the same sites as existing drugs or natural ligands for receptors such as GPCRs. Moreover, unwanted side effects tend to occur because these sites are shared among families of receptors with diverse functions. Development of effective drugs that bind to alternative, or allosteric, sites of receptors is incredibly attractive from efficacy, safety, and intellectual property perspectives. However, quantifying the binding of these allosteric drug candidates to receptors of interest is particularly difficult with established assay technology.

By enabling direct quantitative binding measurements of compounds to receptors in a native-like membrane matrix without the use of labels, surface tethering, or other complex assay set-up procedures, BSI is poised to greatly accelerate the pace of allosteric drug discovery.

Targeting GPCRs with Allosterics

G protein coupled receptors (GPCRs) represent important drug targets in all major therapeutic areas and are the target of roughly 40% of current drugs. The market for such therapeutics targeting GPCRs is projected to exceed $100 billion in annual sales by 2018[1]. Historically, the majority of drugs directed against GPCRs target the same binding site as endogenous ligands, the orthosteric site. There are numerous disadvantages to continued development along these lines, which include issues of direct competition with native signaling molecules, similarity of binding sites among closely related protein families hampering drug selectivity, and even intellectual property restrictions on further mining within the boundaries of well explored orthosteric chemical space[2].

Orthosteric site disadvantages can be overcome by targeting sites that are topologically distinct, yet produce downstream effects upon ligand binding: i.e. allosteric receptors. Allosteric modulators hold great promise as drug candidates by offering a broad 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. Drug development efforts in this space have been bolstered by the approval of a number of allosteric drugs by the FDA, with many more in the pipeline of various pharmaceutical companies[3].



THE PROBLEM

Despite the numerous advantages offered by allosteric modulators, there are surprisingly few assay technologies suited to their timely and efficient discovery and development.

Cell-based functional readouts are often employed for this task, but cannot address intermolecular binding directly, a property of drug candidates that is increasingly demanded by the development process and regulatory agencies to better inform drug mechanism of action (MOA)[4]. Thus, biophysical intermolecular binding data can be a crucial complement to cell based assays and even animal phenotypic studies.

A key challenge in assessing compound affinity for a GPCR target through direct binding studies *in vitro* is that GPCRs and other transmembrane proteins, when taken out of their native cell membrane environment, can be highly unstable and lose the organized structure that is critical to their activity. Therefore, any technique that can characterize the binding mechanisms of GPCRs in their native, or close to native, form will certainly impact characterization of these important drug targets, potentially identifying new promising therapeutic drug candidates and aid in elucidating their MOA.

Many established assay technologies for examining the molecular interactions of GPCRs rely on removing the protein from its native membrane environment and/or subjecting it to substantial modification before characterization, such as attachment to a stationary support surface. Alternatively, fluorescent and radioisotope labeled assay techniques may be employed, but introduce well-known associated problems. A technique that has the ability to assay the molecular interactions of GPCRs in their native form or a native-like mimetic without labels or tethering to a surface will be of immense utility in the discovery and development of efficacious drugs with clear MOA.

Labeled vs. Label-Free Technologies

Classical techniques such as radioligand binding may be applied to the search for potent allosteric drugs, but in a standard configuration only measure changes in orthosteric binding of a radioactive tool ligand. Additionally, these studies bring regulatory as well as health and safety concerns associated with radioactive material stewardship.

An alternative is the use of fluorescent ligands, however these compounds tend to have lengthy development times, structural restrictions, and potentially high background interference, along with the same informational gaps as radioligands, such as lack of direct measurement of unlabeled allosteric compound interaction with the target[5].

These considerations make label-free assay technologies an attractive alternative. The two most popular platforms in this space are isothermal calorimetry (ITC) and surface plasmon resonance spectroscopy (SPR). ITC can present technical challenges for working with integral membrane proteins such as GPCRs, can

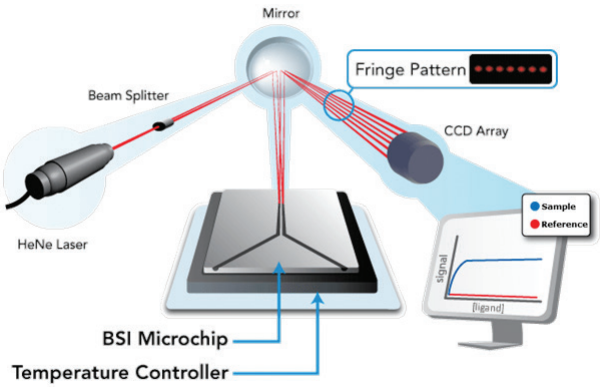
Drug or Candidate	Disease Area	Target
Cinacalcet (Amgen)	Chronic kidney failure	Calcium receptors
XY4083 (Xytis)	Alzheimer's disease	Nicotinic acetylcholine receptor (alpha7 subtype)
GSK729327 (GlaxoSmithKline)	Schizophrenia	AMPA-type ionotropic glutamate receptors
AADX10059 (Addex) AZD2066 (AstraZeneca) AFQ056 (Novartis)	Gastroesophageal reflux disease (GERD)	Metabotropic glutamate receptor 5
Maraviroc (Pfizer)	HIV	CCR5 receptor
Xen2174 (Xenome)	Pain	Norepinephrine transporter
ADX48621 (Addex) AFQ056 (Novartis)	Parkinson's disease	Metabotropic glutamate receptor 5

Adapted from Wenner, M, "A New Kind of Drug Target" Scientific American, August 2009

have high material consumption requirements, and is limited to detecting enthalpic, but not entropic, binding interactions. Application of SPR to membrane proteins has made great strides, but due to the diminutive ratio of the mass of a small molecule ligand to the large GPCR target, assay development can be incredibly challenging and potentially plagued by artifacts arising from the need to tether the target to a solid support.

THE BSI SOLUTION

Back-scattering interferometry (BSI) is an emerging label-free technology for mass- and matrix-independent biophysical characterization of small molecule interaction with complex integral membrane proteins in a native-like environment. BSI allows for the determination of compound affinity (K_d) directly for GPCR ligands without the use of labels or tethering the target to a surface, which is difficult for integral membrane targets and can result in low sensitivity and unwanted artifacts. By detecting minute changes in the refractive index of a solution, BSI can accurately quantify GPCR binding affinities for both orthosteric and allosteric ligands without the use of labels and free in-solution under physiological, native-like conditions in a variety of membrane and membrane-mimic environments.



Back-Scattering Interferometer schematic.
Light from a HeNe laser is split into two beams and then directed to strike a microfluidic chip that serves as an interferometric chamber. The resultant interference fringe pattern is picked up by a mirror and directed to strike a camera.

The conformational changes that occur in a target protein upon ligand engagement cause a miniscule shift in refractive index to which BSI is sensitive. Since orthosteric and allosteric sites are conformationally linked, orthosteric binding can affect binding affinity of allosteric ligands and vice versa, thus BSI can readily distinguish orthosteric, postitive allosteric, and negative allosteric binding in complex membrane protein systems. Additionally, BSI is capable of detecting "silent" binders to allosteric sites that have no apparent functional effect, but may serve as chemical scaffolds for further development of potent positive and negative allosteric modulators.

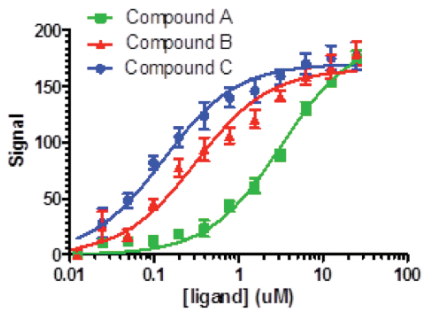
The Importance of Direct Binding Determination in Pharmacology

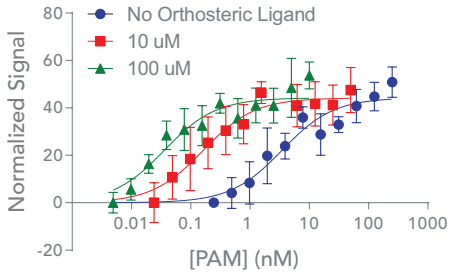
It is critical in any modern drug development effort to demonstrate that a pharmaceutical compound engages with its target. Confirmation of direct binding of a candidate small molecule to the receptor of interest is a defining component to verification of a suspected MOA for that compound. Establishing clear MOA is crucial not only to successful drug development efforts, but is increasingly demanded by regulatory entities such as the FDA and international equivalents. BSI assays provide this information, constituting a significant value proposition to the drug development process.

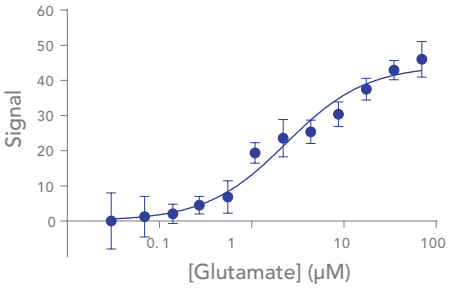
Both cell-based and cell-free functional assays provide invaluable data for screening and characterization of drug candidates. These types of assays are particularly useful in identifying putative allosteric ligands for targets such as GPCRs. However, these assays have some "black box"aspects in that binding and signal generation are several steps removed and can be affected by functional characteristics of many biomolecular interactions.

Confirmation of allosteric potentiation/inhibition from functional assays, and further, quantitative information on allosteric compound binding in a minimal system is a strength of BSI technology that cannot be supplied by functional assays alone. As allosteric drug development projects gain prominence in pharmaceutical pipelines, label-free assay technologies such as BSI are poised to make a significant impact.

Applications of BSI to GPCR Binding Systems







Allosteric Drug Candidate Profiling

BSI allows direct binding measurements of libraries of allosteric compounds without the need for labeling. In this role, BSI serves as an efficient secondary screening platform and complement to functional activity assays. Measurement of binding competition with radiolabeled or fluorescent compounds is only an indirect assessment of affinity and can be highly probe dependent.

Characterization of Allosteric Binding Mechanisms

Binding of an allosteric ligand to a GPCR in a crude membrane preparation. BSI allows discrimination between affinity vs. efficacy-driven modes of action for allosteric modulators. The increase in ligand affinity (decrease in K_d) as a function of increasing orthosteric ligand (agonist) concentration characterized the ligand as a positive allosteric modulator that acts through conformational binding cooperativity. Such assays are difficult or impossible using standard technologies.

Small Molecule Endogenous Ligands

Binding of glutamate to metabotropic glutamate receptor 5 expressed in crude membrane fractions. A K_d of 2.4 Œ°M can be computed from the binding curve, which is compatible data gleaned from other assay types. BSI is able to measure such low affinity interactions due to its sensitivity to receptor conformational changes in free solution. Available SPR techniques for similar measurements are hampered by the unfavorable size ratio of such a small ligand to the large receptor (and associated lipid or detergent molecules), while downstream steps in radioligand assays allow dissociation of low affinity ligands before readout is achieved.

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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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