Targeting Small Molecule Ion Channel Inhibitors

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

SUMMARY

Ion Channels in Drug Discovery

Ion channels are important targets for drug discovery in multiple therapeutic areas, including cardiovascular and neurology, with over 13% of known drugs acting through these critical Integral Membrane Proteins (IMP).

Ion Channel Inhibitor Discovery Challenges

Although there is significant interest in ion channel modulators as therapeutics, they are challenging drug discovery targets due to the difficulty in obtaining purified proteins that retain their biochemical and biophysical properties.

Conventional radio-ligand binding methods based on crude membrane preparations are limited in that they can only detect compounds that bind either to the same site as the tracer or to allosterically-coupled sites. In addition, these methods suffer from artifacts relating to the presence of multiple other proteins in the crude membrane preparation; and the general environmental and handling issues associated with radio-isotope usage.

Recently, nanodisc technology has developed into an important approach for studying the biophysical properties of many different classes of ion channels and integrated membrane proteins (IMPs). However, small molecule direct binding measurement remains a challenge, with Surface Plasmon Resonance (SPR) limited in its effectiveness due to the large mass differential between the ion channel/nanodisc complex and the small molecule ligands of interest.

BSI: Ion Channel Solutions

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

In addition, BSI is applicable to mechanistic studies, being able to directly determine small molecule compound affinity for ion channels, with binding K_ds consistent with compound functional potency.

SUPPORTING DATA

Characterization of Ion Channel

A chimeric ion channel (KcsA-Kv1.3) was studied as presented in a nanodisc. The binding of two small molecules (compound 1 and 2) to the ion channel was monitored using BSI. The interaction of these compounds with the ion channel could not be followed using SPR. This work was jointly done with Dr. Han Xu of Amgen[1].

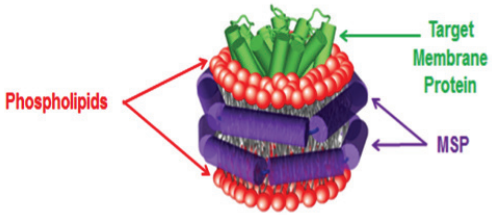

Figure 1: *Schematic of ion channel nanodisc construct.*

BSI is able to measure small molecule low affinity interactions with nanodiscs when other technique fail, due to its sensitivity to receptor conformational changes in free solution, as highlighted in Figure 2.

1. Assembly and Characterization of a Chimeric Potassium Ion Channel KcsA-Kv1.3 Nanodisc

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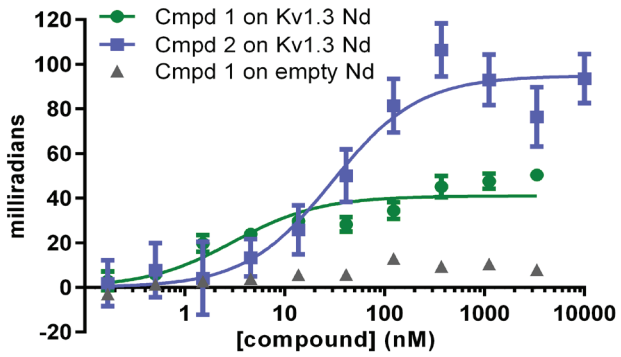
MOLECULAR SENSING

TruBind™ Assay Services

Back-Scattering Interferometry at Work



Compound 1 (350 Da) K_d 3.1 +/- 0.6nM
Compound 2 (360 Da) K_d 29.8 +/- 4.5nM

Figure 2: *Small molecule direct interaction data for two compounds binding KcsA-Kv1.3*

Binding K_ds of peptide blockers were determined by kinetic mode on Biacore™ and equilibrium mode by BSI. BSI determination shows almost 10-fold weaker affinity for both peptides compared to Biacore, which can be explained by the requirement of high Nanodisc concentration (300-500 pM) to generate reliable and robust signal in BSI. Pico-molar affinity of both peptides has demonstrated correct folding of the chimeric ion channel in the Nanodisc.

Although kinetic determination of small molecule binding isn't achievable on Biacore due to the low molecular weight of these ligands, binding K_ds have been determined by BSI and were consistent with their functional potency.

Ligand	K_d (nM)	MW (kDa)
SHK	0.20 ± 0.04	4
PEG-SHK	0.10 ± 0.02	24
Cmpd 1	3.1 ± 0.6	0.35
Cmpd 2	29.8 ± 4.5	0.36

Table 1: *Summary of BSI derived affinities for peptides and 2 small molecules.*

KEY BENEFITS

TruBind BSI Technology delivers key benefits in the investigation of ion channel 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

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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