

Fact Sheet – Xention’s Ion Channel Drug Discovery Capabilities

Xention has developed a highly effective suite of ion channel-focussed research and development capabilities that have allowed us to successfully target many important channels across a number of therapeutic areas. The key elements of these capabilities comprise:

- Extensive ion channel tissue culture experience and expertise, enabling the development of stable cell lines expressing single channels of interest in recombinant systems, and provision of suitable cells for automated electrophysiology screening
- Comprehensive manual and automated electrophysiology screening capabilities covering a wide range of throughput capacities
- Detailed in silico ion channel databases and efficacy prediction capabilities based on large datasets from proprietary sources and literature data
- Highly experienced medicinal chemistry team with ion channel-focussed experience and comprehensive discovery chemistry capabilities

Xention’s Tissue Culture Expertise

The tissue culture group at Xention has the role of creating, optimising and maintaining recombinant cell lines expressing the ion channels of interest in the company. Xention currently has a collection of approximately 70 mammalian ion channel cell lines, including many complex, heteromultimeric cell lines that have been constructed internally, hence the vast majority of ion channel target and selectivity screens are available internally for high quality electrophysiology examination. Individual cell lines are routinely optimised for expression and stability for each platform on which they will be tested. Low temperature and cell arrest techniques have been developed on a case by case basis to provide highly stable, robust assays that are carried out for weeks, months and years at a time supporting discovery and optimisation activities.

Xention’s Ion Channel Screening Capabilities

Electrophysiology is the gold standard for the study of ion channels and involves use of the patch-clamp technique in manual or automated format. This technique generates high-quality measurements of ion flow through the channel under investigation and an understanding of the nature and kinetics of blockade of the channel by putative drugs. In its traditional, manual form the technique is labour-intensive and requires highly skilled operators and its low throughput precludes its introduction early in the drug discovery process. By integrating electrophysiology early in discovery, our medicinal chemists are able to use genuinely high-content data to understand structure-activity relationships (SAR) i.e. the specific components of potential drug candidates that are responsible for the required activity on the target of interest, and we can thus develop compounds with preferred modes of action in a rational manner.

Xention has assembled a high quality collection of ion channel screening facilities to support a range of different targets and throughput requirements. HTS is provided for with an ion flux capability using atomic absorption spectroscopy for screening tens or hundreds of thousands

of small molecules. This platform is supported by automated electrophysiology devices that are used to confirm all screening hits and to support all medicinal chemistry lead optimisation and selectivity screening activities. Xention currently uses several Nanion PatchLiner and Sophion QPatch devices to support medicinal chemistry, and has accumulated very substantial experience of assay development and optimisation for calcium channels, sodium channels, potassium channels and TRP receptors on these platforms.

Further ion channel screening capabilities in regular use at Xention include the Molecular Devices FLEX platform for assays amenable to imaging technologies such as for channels that flux calcium ions, as well as a comprehensive suite of manual patch clamp devices with specific set ups to allow fast perfusion and recording from human tissue.

Computational Chemistry Resources at Xention

Xention research activities are supported by an extensive and fully integrated chemoinformatics/bioinformatics software platform. Xention uses commercially available software to collect, analyse and store screening results in a standard Oracle database format, but our scientists are able to access the results and inform drug design decisions through a customised web interface. Furthermore, we have painstakingly assembled an ion channel-focused database (“XENBASE”) that is proprietary and second to none in its value to ion channel drug development.

These computational tools, in conjunction with our “wet” biology activities, has enabled the company to develop:

- Enhanced and novel pharmacophore models;
- Privileged ion channel chemistry scaffolds; and
- Selectivity models for specific ion channels of interest.

In Silico Ion Channel Screening

In silico screening describes the process of prioritizing large number of potential screening compounds through the judicious use of the information available for the specific target. This process allows us to ‘scan’ the vast quantity of commercially available chemistry to highlight those compounds that are worthy of inclusion in our discovery process for the generation of ‘real’ screening data. The principal sources of information directing this selection process are the two-dimensional and three-dimensional descriptors of preferred ion channel blockers that we generate using XENBASE. The recent availability of crystal structure information (Figure 1) has allowed us to generate good quality homology models of our targets (Figure 2) and to investigate possible binding modes of ligands to help with their further optimisation.

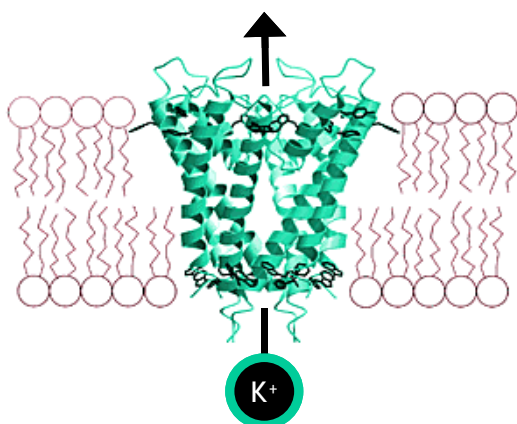


Figure 1. A schematic picture of an ion channel showing the passage of (in this case) a potassium ion.

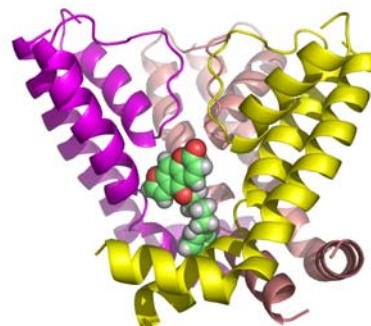


Figure 2. An image of a small molecule docked into its hypothetical binding site in an homology model of one of our targets picture of an ion channel showing the passage of (in this case) a potassium ion.

Our Ion Channel Specific Compound Library

Compound libraries generally available in the industry have not been developed with ion channel targets in mind, and most scaffolds in pharma libraries are heavily biased towards targets such as GPCRs and enzymes. Thus, the starting material likely to be relevant for ion channel drug discovery is in low abundance from these libraries and hit rates are typically low.

To circumvent this problem, Xention has developed a compound library which is focused on ion channel targets. The library has been constructed from a number of components, each complementary and adding value, including the following:

- Pharmacophores developed from ion channel targets we have worked from which we generate compounds that correspond to these pharmacophore structures;
- Two-dimensional descriptors devised from our electrophysiology data which can be used to identify compounds to enrich our library;
- Compounds generated through our ongoing medicinal chemistry efforts most of which have novel scaffolds.

Xention has integrated these key components of a high quality ion channel drug discovery capability to generate a unique platform that is able to successfully drive drug discovery programmes in this target class by applying the following advantages:

- Use of electrophysiology early in the discovery process. We have found that it is absolutely essential to use electrophysiology in all stages of the medicinal chemistry process to support the identification and development of high-affinity ion channel ligands with appropriate selectivity and to optimise those ligands for

the preferred mode of action. This offers a very real advantage over alternative assay techniques that do not provide a direct read-out of the effect of a small molecule on the ion channel of interest.

- In silico ion channel screening. Computational approaches to in silico screening have been validated by Xention for several of our discovery targets and found to offer a real, faster and cheaper, alternative to high-throughput screening.
- The XENBASE chemoinformatics database. Having an ion-channel focussed informatics platform means that we can rapidly generate hypotheses of 2D and 3D descriptors for ligand interactions with the target protein, generate SAR relationships across internal and literature data and inform all activities within our research programmes.
- Xention's ion channel-focused library. Our compound collection is supported by focused computational chemistry expertise to enable further enrichment so that our screening hit rates are very high and the majority of screening hits are very 'lead-like' in nature.
- Extensive ion channel knowledge base. Comprehensive knowledge of target expression profile, electrophysiological characteristics and pharmacological characteristics of ion channels enables accelerated target selection, assay development, screening, and the development of clinically interesting modulators for selected targets.