Prediction of Ion Channel Activity Using Binary Kernel Discrimination

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Voltage-gated ion channels are a diverse family of pharmaceutically important membrane proteins for which limited 3D information is available. A number of virtual screening tools have been used to assist with the discovery of new leads and with the analysis of screening results. One such tool, and the subject of this paper, is binary kernel discrimination (BKD), a machine-learning approach that has recently been applied to applications in chemoinformatics. It uses a training set of compounds, for which both structural and qualitative activity data are known, to produce a model that can then be used to rank another set of compounds in order of likely activity. Here, we report the use of BKD to build models for the prediction of five different ion channel targets using two types of activity data. The results obtained suggest that the approach provides an effective way of prioritizing compounds for acquisition and testing.

INTRODUCTION

Ion channels represent a wide range of protein complexes that span the cell bilayer allowing the transport of ions through the otherwise ion-impermeable membrane.[1] Most significantly, channels are capable of regulating their ionic permeability in response to a diverse range of signals, including temperature, mechanical stress, pH, various modulating ligands, and membrane potential.[2−5] Different types of ion channels translate these varied stimuli, into opening or closing of the channel involved and subsequent modification of the action potential across the cell mebrane. Thus, ion channels are fundamental to the correct functioning of neuronal responses and constitute a major mechanism by which many different cell families interact with their rapidly changing environment. The subfamily of channels which respond to changes of electrical potential are called voltage-gated (hereafter, VG).[6,7]

VG ion channels have a simple role in the cell: to respond to changes in membrane potential by changing the ability of their chosen ion(s) to flow. Complexity arises when the results of this simple action are integrated into the behavior patterns of different cell types, wherein a change in intracellular calcium (for example) may elicit a chain of events culminating in the synthesis of new proteins and a change in the way the cell responds to further stimuli. Through this complexity of response, ion channels may be responsible for many diverse functions in cells, such as the regulation of the cardiac action potential,[8] control of neuronal excitability,[9] transmission of pain signals along the spinal cord to the brain,[10] and the release of neurotransmitters that orchestrate signaling between cells.[11] This plethora of actions means that VG ion channels have been targeted for drug development.[12] In the case of the human ether-a-go-go related gene, blockage of the channel as an action side effect is a common cause

for attrition in drug candidates in later stages of development.[13] It is now accepted that, despite many similarities, subtle differences in the mechanism of action or 3D structures can be exploited to identify selective compounds. Until very recently, the inability to obtain X-ray structural information for the pharmaceutically relevant VG channels has impeded the design of effective blockers or activators,[14] with pharmacophore development/searching, library design, and standard similarity searches being the only chemoinformatics methodologies that have been used in the field thus far.[15]

These difficulties render the search for potent and selective ion channel modulators an attractive application domain for the use of virtual screening methods. These seek to rank a database of previously untested molecules in order of decreasing probability of activity, so that compound acquisition and testing can be focused on those molecules that have high a priori probabilities of activity. Several different approaches to virtual screening have been described in the literature.[16,17] Here, we focus on the use of machine-learning methods, which require a training set of molecules for which the (in)activities have already been determined and which use the actives and inactives in the training set to develop a classification or ranking function that can then be applied to molecules of unknown activity in the test set. Specifically, we describe the use of the binary kernel discrimination (BKD) machine-learning method in simulated virtual screening experiments based on several Xention data sets. Ideally, a model should be able to discriminate, for example, not just between sodium and potassium channel blockers but also between members of the same family (for example, Kv1.3 compared to Kv1.5 or HERG).

METHODS

Ion Channel Models. Models for five different channels have been developed during the course of this study.

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The $K_v1.1$ subfamily is expressed in the embryonic nervous system, brain, and lymphoid thymocyte precursors. The $K_v1.1$ subunits can associate with $K_v1.2$ and $K_v1.4$ subunits, especially in the cerebellum. Point mutations in $K_v1.1$ result in the disruption of this association and episodic ataxia type I, a rare autosomal dominant neurological disorder characterized by brief episodes of ataxia.[18]

$K_v1.2$ channels are uniformly distributed in the heart and brain. They play diverse functional roles in several neuronal compartments, especially in the regulation of pre- and postsynaptic membrane excitability. $K_v1.2$ colocalizes with $K_v1.1$ in the nodes of Ranvier in myelinated axons, and in the brain, in particular, the axons and nerve terminals; $K_v1.2$ coassembles with $K_v1.4$ subunits.

The $K_v1.3$ channel is found in lymphocytes and is involved in the immune system response. Effector memory (T_{EM}) cells play a specific role in the development and maintenance of autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, and psoriasis, and it has recently been shown that the ion channel $K_v1.3$ plays a major role in regulating responses in these T cells.[19] $K_v1.3$ channel blockers may be useful in the modulation of T-cell activation in the progression of an immune response.[20]

The $K_v1.5$ channel is responsible for the ultrarapid delayed rectifier K+ current in the cardiac atria. Atrial fibrillation, which affects around 2 million people in the U.S.A. alone, may be treated by delaying cardiac action potential repolarization, an outcome that can be achieved through selective blockade of the $K_v1.5$ channel.[21] Although the binding sites for several classes of $K_v1.5$-modulating drugs have been postulated, no conclusive evidence is available to justify the extensive/exclusive use of 3D structure-based rational design of new ligands.[22]

Finally, the $Na_v1.3$ channel plays a critical role in excitable cells underlying the rapid action potentials that are characteristic of neurons and muscle cells. There are at least nine distinct Na_v channels cloned from mammals. $Na_v1.3$ neuronal channels are primarily expressed in the early stages of development and are virtually undetectable in the adult nervous system. However, after various injuries that lead to the generation of neuropathic pain states in animals (such as ligation of the sciatic nerve), it has been found that $Na_v1.3$ expression is upregulated in the dorsal root ganglion, and this phenomenon is suggested to play a functional role in the ectopic activity that is a characteristic of neuropathic pain.[23,24]

The data sets used in this study were produced using mostly internal high-throughput screening (HTS) facilities. Flux activity data were collected by quantifying the influx and efflux of ions using atomic absorption spectroscopy and require the recruitment of approximately 50 000 cells. Flux-based screens are often affected by problems of increased noise or inadequate control of experimental conditions.[25] Electrophysiological data on the other hand are high-fidelity recordings of single cell measurements performed on the PatchPilot platform.[26] Electrophysiology data result from a direct measurement of a cell's electrochemical behavior and usually comply with strict quality control criteria.

Binary Kernel Discrimination. BKD was first applied to problems in chemoinformatics by Harper et al.[27] It assumes that molecules are represented by binary fingerprints encoding information about the two-dimensional (2D) structures

of training set and test set molecules.[28] A fingerprint of length N is a vector of N bits, ones and zeros, that correspond to the presence or absence of specific small, atom/bond fragments. Pairs of such fingerprints can be used to quantify the similarity or distance between a pair of compounds by counting the number of bits that are set to one in each compound and the numbers of bits that are set to one in just one or the other of the pair.[29,30]

BKD uses the squared Euclidean distance, d_{ij}, between a pair of compounds i and j represented by binary fingerprints (i.e., the number of bit positions in i that have a different value to the corresponding ones in j). The distance is used in a kernel function suggested by Aitchison and Aitkin,[31]

$$K_\lambda(i,j) = \lambda^{N-d_{ij}}(1 - \lambda)^{d_{ij}} \qquad (1)$$

where λ is a parameter between 0.5 and 1 whose precise value is to be determined. The kernel function is used to obtain a mathematical expression for the probability that a given compound is active, and the resulting probabilities can then be used to rank a set of compounds. It is assumed that there is a training set of molecules that have been previously tested and that have been determined as either active or inactive in the bioassay of interest.

The ranking is produced using a scoring function that is the ratio of the sum of the kernel functions computed with all the training set actives and the sum of the kernel functions computed with all the training set inactives. For some previously untested molecule, j, the scoring function is hence

$$S(j) = \frac{\displaystyle\sum_{i \in \text{Active}} K_\lambda(i,j)}{\displaystyle\sum_{i \in \text{Inactive}} K_\lambda(i,j)} \qquad (2)$$

An optimum value of λ is obtained by analysis of the training set. Different values of λ are used to compute scores for training set compounds using eq 2 above. The optimum λ is taken to be that which results in the lowest sum of ranks for the training set actives; other training criteria can be used, but this approach to optimization is both simple and effective in operation.[32] This value of λ is then used to compute scores for the test set compounds using eq 2. In practical applications, the top few percent of the ranked test set is expected to contain more actives than the lower-ranked compounds, and these few molecules may then be screened, rather then screening the entire test set.

BKD has previously been used to produce models for pesticide and drug activity,[27,32−35] where it has been found to be comparable with, or superior to, other, more established, ranking methods that are based on the use of binary fingerprints, and it can also be used with nonbinary chemical data sets.[36] Here, we apply BKD to the Xention ion-channel data sets described previously.

EXPERIMENTAL DETAILS

A data set of 14 632 compounds was tested against five different VG ion channel targets. Each compound is classed as active, inactive, or ignored (untested) against any particular target. The criterion for this classification was 50% inhibition

PREDICTION OF ION CHANNEL ACTIVITY

J. Chem. Inf. Model., Vol. 47, No. 5, 2007 **1963**

Table 1. Number of Available Compounds for Each Target Type and Size of Test Sets

target	data type	compounds available		compounds in test set	
		actives	inactives	actives	inactives
Na$_v$1.3	Flux	143	14 100	14	1400
K$_v$1.1	Flux	232	116	23	1160
K$_v$1.2	Flux	70	14 135	7	1410
K$_v$1.3	Flux	188	14 122	18	1410
K$_v$1.5	Flux	28	11 528	5	1150
K$_v$1.1	EPhys	64	11 744	6	1170
K$_v$1.3	EPhys	118	14 187	11	1410
K$_v$1.5	EPhys	275	11 932	27	1190

Table 2. Mean Percentage of Actives Found in the Specified Percentage at the Top of the Ranked Test Set[a]

target	data type	percentage of actives in the specified percentage of the ranked test set		
		1%	5%	10%
Na$_v$1.3	Flux	28 ± 9	55 ± 8	66 ± 10
K$_v$1.1	Flux	15 ± 7	32 ± 11	42 ± 11
K$_v$1.2	Flux	47 ± 11	59 ± 16	63 ± 20
K$_v$1.3	Fl$_v$ux	48 ± 16	78 ± 11	83 ± 9
K$_v$1.5	Flux	6 ± 14	10 ± 20	14 ± 18
K$_v$1.1	EPhys	47 ± 25	68 ± 17	75 ± 13
K$_v$1.3	EPhys	46 ± 13	75 ± 10	82 ± 13
K$_v$1.5	EPhys	34 ± 4	87 ± 6	91 ± 5

[a] Results are the mean and standard deviation when averaged over 10 different test sets.

of the current measured after the application of the molecule at the HTS concentration. All structures in the corporate database were characterized by a Unity 2D molecular fingerprint[37] for the development of the BKD models.

About 10% of the actives and 10% of the inactives were randomly selected to be a test set, that is, a set of molecules for which the activity was assumed to be unknown and for which predictions were hence required. The remaining active and inactive compounds were used as a training set with which to construct a BKD model that was then used to rank the test set. The effectiveness of the ranking, and hence of the model that gave rise to it, was assessed by counting how many actives occurred in the top 1%, 5%, and 10% of the ranked test set. In this simulated virtual-screening environment, the actives are known; in practice, the top-ranked molecules from the ranked test set would then be assayed (as in the final validation experiment described in the next section).

This procedure was repeated with a different partition of the data into test and training sets. A total of 10 different partitions per target type were used, and in all but one case, each run used a completely different test set than the others. The exception was the K$_v$1.5 data, where there were only 28 actives so that a 10% selection would give a test set containing just two or three actives. This was felt to be too small a number, so five actives were used for five different partitions of the test/training data. The BKD program was then run a second time, with a different random number seed. There are thus 10 different BKD runs for this data set (as with the other experiments), but there was some degree of overlap in the test sets that were used. The numbers of active and inactive compounds available for each combination of target and data type, and the sizes of the test sets, are shown in Table 1.

It should be noted that an experiment such as this is at least as much a test of how good the structural descriptors are as it is a test of how good the machine-learning algorithm is: any such algorithm can only produce a good model for activity if the descriptors used to represent the training set compounds are able to encapsulate features that distinguish the active and inactive compounds. Tripos Unity 2D fingerprints[37] have been used previously in successful studies of virtual screening methods[33,34] and were employed for the experiments reported below. Experiments were also carried out with Scitegic Pipeline Pilot fingerprints;[38] the results obtained with the two types of fingerprint were very similar, and we have hence included only the results that were obtained using the Unity fingerprints.

RESULTS AND DISCUSSION

BKD models were developed using a training set and then applied to a test set 10 times for each combination of target and data type (as detailed in Table 1). The results of these experiments are shown in Table 2, which lists the mean percentage of actives (rounded to the nearest integer given the small numbers of actives involved) found in the specified percentage from the top of the ranking. If molecules were selected at random, then we would expect 1%, 5%, and 10% of the actives to occur in the top 1%, 5%, and 10%, respectively, of the ranked test sets. In fact, of course, we hope that the BKD model that is being used will have predictive ability so that many more actives will be observed at the top of the ranking than would be the case from using random selection.

The results in Table 2 show that, in most cases, the recall of actives is much greater than would be expected by randomly sampling the test set. The results are worst for the K$_v$1.5 Flux data set, but even here the recall of actives is still better than random. However, this is the data set with by far the fewest number of actives with which to perform the experiment, and there is probably insufficient data to make a reliable model. There is a great deal of variation between the 10 different tests set for each target/data type, but this is hardly surprising given the small numbers of actives on which each model is based. Overall, the results show that the BKD models rank significant proportions of the test set actives toward the top of the ranking. For example, it can be seen that, with the exception of K$_v$1.5 Flux, anything between 32% and 87% (mean values) of the training set actives is found in the top 5% of the total ranked test set: these percentages represent enrichments of 6.4−17.4 fold when compared to random selection. It is noticeable that the models based on the more accurate electrophysiology data give better average performance than do those based on the flux data.

A check was carried out to confirm that the BKD models were characterizing specific types of activity, rather than ion-channel activity in general, using the K$_v$1.3 Flux data. The inactives ranked in the top 5% of the test sets (71 compounds) were checked to see which of them were active against another target. The average number of inactives that were active against Na$_v$13., K$_v$1.1, K$_v$1.2, and K$_v$1.5 were 0 (exactly), 3.4, 0.7, and 0.1, respectively, from which we conclude that the model used is one that is specific to the prediction of K$_v$1.3 Flux activity.

In any virtual screening method such as BKD, there is always the possibility that the model simply finds actives that are very similar to an active in the training set and, as such, will not be able to provide much assistance in the search for novel active compounds. One basic form of virtual screening that does this by definition is simply to rank a test set by the similarity of each compound to a single known active, a technique known as similarity searching.[29,30] An improved version of this approach can be used when multiple actives are available: this approach, called group fusion, involves computing the similarities between a test set molecule and each of the known actives, taking the maximum such similarity and then ranking the test set molecules in decreasing order of their associated maximum similarities.[33,36] We have generated group-fusion rankings for the targets with electrophysiology data, with the similarities being calculated using Unity fingerprints and the Tanimoto coefficient. We then compared the actives found in the top 5% of the test set for the BKD and group-fusion rankings. If an active compound is found by BKD but not by group fusion, then we refer to it as a *BKD-bonus* active, as this is a compound that is not sufficiently similar to any one of the training set actives for it to be found by the group-fusion searches. Conversely, an active compound found by group fusion but not by BKD is referred to as a *BKD-miss* active.

We found that, for $K_v 1.1$, $K_v 1.3$, and $K_v 1.5$ electrophysiology data sets, there were, on average, 0.4, 0.3, and 0.9 BKD-bonus actives, respectively. In all but one case, the test sets contained 1 or 0 BKD-bonus actives, the exception being one $K_v 1.5$ test set where there were three such actives. We also found that there were, on average, 0.0, 0.2, and 0.3 BKD-miss actives for $K_v 1.1$, $K_v 1.3$, and $K_v 1.5$, respectively. There is hence a net gain of BKD-bonus over BKD-miss actives, although the difference varies across the three targets. The advantage is not large but is sufficient to show that at least some proportion of the compounds correctly predicted to be active by the BKD models have only a low level of 2D similarity to the known actives in the training set.

The effect of molecular similarity on predictive performance was further studied in a second set of experiments that used just the electrophysiology data. Here, rather than randomly dividing the available data into training sets and test sets, we deliberately selected training set actives to be quite similar to each other, while randomly selecting training set inactives. The remaining compounds were then used as a test set and thus contained actives that were relatively dissimilar to the training set actives. This procedure made it more difficult for the BKD models to identify the test set actives and thus provided a stiffer test for the method. The training set actives were selected by a simple algorithm as follows. An initial active was picked at random and added to the training set, which was then built up by adding the currently unselected active with the highest mean similarity to those already in the training set. This procedure was repeated until the required number of actives had been reached, and the required number of training set inactives was then selected randomly. The BKD models were produced from the training sets as before; the test sets were ranked, and then a note was made of the number of actives in the top 5% of the ranking.

Results obtained using a range of similarity values for the three data sets are shown in Tables 3–5. Each row in any

Table 3. Similarity Experiments Using $K_v 1.1$ Electrophysiology Data[a]

mean similarity between training set and test set actives	percentage of actives in top 5% of ranked test set
0.26	0
0.26	0
0.32	19
0.32	19
0.33	22
0.36	56
0.36	56
0.36	59
0.37	53

[a] Training set of 32 actives and 3200 inactives; test set of 32 actives and 8539 inactives.

Table 4. Similarity Experiments Using $K_v 1.3$ Electrophysiology Data[a]

mean similarity between training set and test set actives	percentage of actives in top 5% of ranked test set
0.42	39
0.42	39
0.43	41
0.43	39
0.46	54
0.47	56
0.47	59
0.47	61
0.49	75
0.49	73

[a] Training set of 59 actives and 5900 inactives; test set of 59 actives and 8287 inactives.

Table 5. Similarity Experiments Using $K_v 1.5$ Electrophysiology Data[a]

mean similarity between training set and test set actives	percentage of actives in top 5% of ranked test set
0.44	44
0.44	49
0.45	49
0.45	48
0.47	57
0.47	60
0.48	62
0.49	68
0.49	67
0.50	68
0.50	72

[a] Training set of 138 actives and 6000 inactives; test set of 137 actives and 5933 inactives.

one of these tables lists the mean Tanimoto similarity between the actives in the training set and the actives in the test set, and the percentage of the test set actives that are found to be in the top 5% of the test set after the appropriate BKD model has been used to generate a ranking of it. Note that similarities returned by the Tanimoto coefficient are in the range 0–1, and generally a value greater than around 0.7 is taken to mean that two molecules are topologically related. The mean similarities between test set and training set actives are all 0.5 or less, corresponding to a low average level of structural similarity. It can be clearly seen that, as would be expected, the percentage of test set actives found in the top 5% increases in line with the mean similarity between test set and training set actives. Importantly,

PREDICTION OF ION CHANNEL ACTIVITY

J. Chem. Inf. Model., Vol. 47, No. 5, 2007 **1965**

Table 6. Percentage of Actives Found in the Specified Percentage at the Top of the Ranked Test Set for Additional Na$_v$1.3 Data

data source	percentage of actives in the specified percentage of the ranked test set		
	1%	5%	10%
external actives	21	59	64
Xention actives	31	82	85

however, with the exception of the first two K$_v$1.1 test sets, the BKD models provide a good level of enrichment even when the test set actives are, on average, only mildly similar to the training set actives. That said, any machine-learning method will start to have problems if there is not some measure of agreement between the test set and the training set that is used to form the model. This was noted by Bender et al. as a serious problem in the recent McMaster comparison of virtual-screening methods[39] and was also evident here when additional experiments were carried out using Na$_v$1.3 data. These experiments used all of the existing data to form the training set and then a set of 914 newly tested molecules as the test set. The models that were produced were consistently poor; however, inspection of the intermolecular similarities showed that many of the new inactives were more similar (using Unity fingerprints and the Tanimoto coefficient) to the training set actives than were the new actives. In such circumstances, no machine-learning method is likely to be able to provide an acceptable level of classification performance.

Two more validation experiments were conducted. The first involved the flux screening of 100 000 compounds from an external library against a Na$_v$1.3 channel screen. About 1000 of the molecules showed significant activity. These hits, together with ca. 2000 structurally related structures retrieved in a series of 2D substructure searches of the library, were retested both externally and internally: the external assay confirmed 520 active molecules, and the internal assay a further 169 active molecules. There was little overlap between the two sets of molecules, pointing to the difficulties of comparing results from different flux screens. These two sets of compounds were mixed with 13 000 randomly selected inactives, and the resulting test set (13.7K) was ranked using our existing Na$_v$1.3 model. The results are shown in Table 6, where it can be seen that a substantial level of enrichment is obtained for both sets of actives. It is, perhaps, hardly surprising that there is a greater level of enrichment for the "Xention actives" molecules; that is, the BKD model that was originally developed using Xention internal data does a much better job of predicting the activity of molecules identified as active internally than externally.

The second validation experiment focused on the analysis of corporate compounds highly ranked according to the BKD Na$_v$1.3 model. The model was used to score all of the compounds in WDI.[40] The top-ranked molecules were checked for availability in the Xention corporate database and the entities identified (80) and cross-referenced with recent screening data. No less than 55% of the tested structures proved to be active (44 molecules) in the screen, demonstrating very clearly the predictive power of the model and hence validating the use of BKD as an aid to the in silico screening of VG ion channel modulators.

CONCLUSIONS

The experiments performed here demonstrate that BKD models derived from 2D structural data can be used to predict likely actives against ion channel targets. High ranking of the active compounds can be detected across a range of screening platforms of varied quality. The models have an advantage over similarity searches using known actives as queries. It can be seen that, as would be expected with any virtual screening method, the effectiveness of the model increases as the similarity between the active compounds in the training set and actives in the test set increases. Even when the average similarly between the training set actives and test set actives is not large, the models are still able to identify many test set actives; however, the models will fail if this similarity is too low.

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1966 *J. Chem. Inf. Model., Vol. 47, No. 5, 2007*

WILLET ET AL.

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