Sarah McComas

Started March 15, 2016

**Introduction**:

Understanding protein structure and function is crucial to deepening our knowledge of how our bodies work and how diseases affect them. A large portion of these proteins involved in these processes exist inside the cell membrane and act among many things as cell gates, signals, etc. Of these membrane proteins, a majority of them are comprised of alpha helices. In fact, about one fourth of all genes transcribed are alpha transmembrane (TM) proteins \*\*CITE TOPCONS1 \*\*\* From understanding the protein topology, we can further work on things like drug delivery. To do this with traditional techniques would not be possible given time and financial constraints but an in silico approach would allow for fast and accurate prediction of the structure of these proteins.

Since the beginning of computational biology, there has been a lot of predictors formed in attempts to accurately and quickly determine protein topology. These include TMHMM, TOPCONS, DAS and many others \*\*\*CITE\*\*\* . They use a variety of algorithms and factors such as HMM’s to determine protein structure and now are very accurate and fast. My predictor here draws inspiration from these predictors, using BLAST, and SVM to attempt to accurately and with relative speed predict topology for protein sequences. Another useful feature of these protein predictors is that they are accessible on the web, which greatly increases the number of proteins we have predicted today \*\*\*MNETION NUMBER/?? FROM PDB??\* when everybody can collaborate and use open resourses. In addition, predictors can draw inspiration from each other and see how their models work together. Predictors can also be used in conjunction with each other for other reasons. Topology knowledge can for example help with other understandings like in fold recognition

Current limitations with predictors is accuracy and time. Many databases such as PDB will only confirm the protein in their database with experimental backup ie xray crystallography bl. a. \*\*\*\*is this true???\*\*\*\* . But consensus based predictions greatly improve the reliability of these models and allow for more accurate prediction than ever. There is still a lot of disagreement between models which is something we need to work on \*\*TOPCONSPAPER1\*\*\* but models such as TOPCONS have attempted to answer that problem because it uses consensus prediction.

This model presented is a basic model, which uses programs such as BLAST to increase the accuracy and sensitivity of the model, multiple sequence alignment is better than single sequence alignment.

My model takes inspiration from these models and although it is not nearly as good as TOPCONS or TMHMM it does use a basic SVM trained on known alpha membrane structures to predict with about a 90% accuracy where in the sequence one can expect that the protein in inside the membrane. A basic local webserver is also available in which one can post a membrane sequence and receive a prediction based on that sequence and a BLAST output within a few minutes. An SVM is used here, which takes a set of data who already have a sequence annotated for structure and uses information from this to make a model and further classify new examples based on single sequence or evolutionary information using PSIBLAST.

A support vector machine (SVM) was trained in this predictor. These are particularly useful in problems where one must classify data based on properties it does or does not have. In this predictor, training data was performed on sequences of known structure and positive and negative examples for each amino acid at a certain position were calculated from this. From here, the SVM was trained with both single sequence and later on multi sequence (ie evolutionary) information which provided a slower yet more accurate model. These models from the SVM were then later on the webserver. The models could be optimized with several parameters to achieve the most successful accuracy as well as the highest MCC, indicative of a low amount of false positives and negatives and can also be reflected in the ROC curve which shows the TP and FP rates against each other.

Although it is already better than random accuracy when one trains an SVM in a single sequence, adding evolutionary information from BLAST yields much more accurate results because a position specific scoring matrix (PSSM) \*\*\*CITE \*\*\* which calculates the likelihood of the amino acid being at that specific position. This is much more specific than single sequence reads, which simply indicate a binary-type data of the amino acid being at that position or not. This means the SVM training for the BLAST outputs is much slower but also much more accurate.

There are many good predictors out there, now the only thing is just improvement with time. To produce more accurate reads to the point that we don’t have to physically confirm them with research is the general goal, and of course to increase computational speed is always desirable.

Hydrophobicity is usually a good indicator of topology and is usually the basis for why a certain amino acid would be in the membrane. The biochemical properties of cell membranes require that these proteins are mainly hydrophobic in the membrane. This means that although the SVM is not trained to look at the hydrophobicity of each amino acid, automatically more hydrophobic ones will be much more present in the membrane and receive a higher ‘score’ for that. (valine for example)

**METHODS:**

As mentioned, the predictor was formed based on SVM learning algorithms. To do this, the SVM must be trained first. This was accomplished in slightly different methods, for the SVM was trained on both single sequence information as well as the evolutionary information from PSIBLAST. Data was extracted from a dataset whos structural information was already known, in order to dictate target values (showing what is a positive example, inside the membrane, and what is a negative example, outside the membrane). The amino acid letters were also transformed into a number, 0-20, assigned to that amino acid. This was held consistent throughout the entire program. For both sequences there was a value appended to each amino acid number, for single sequence this was a binary value indicating the presence of that amino acid at the indicated position, and for evolutionary information this was a PSSM number, indicating the likelihood of the amino acid being at that position as indicated by PSIBLAST. Cross validation sets were also created from the database, 6 sets of about 50 sequences in each set in order to give more accuracy and precision to the SVM. One dataset was excluded in order to complete the optimization, which tested different parameters of the SVM models in order to increase the accuracy and MCC values and therefore decrease the amount of false positives and negatives found in the data.

Homologs were also considered in this model preparation. It is important to not have homologs in separate cross validation sets because otherwise the accuracy will be falsely high since they are nearly the same sequence. For this, CD-HIT \*\*\*\*CITE\*\*\*\* was used to determine homologs in the dataset before proceeding.

This prediction algorithm was developed and is important because it is often amino acids properties and predictions that are the same or similar in forming certain structures. This is especially true for transmembrane proteins because inside the protein requires hydrophobicity whereas outside the cell surface often calls for hydrophilicy. This also plays a role in alpha helix formation, as amino acid sequences inside the helix can only be a certain size and the sequence can only be a certain length to form the helix. Although the SVM does not account for the information such as this, this is how the SVM machines can accurately say that these amino acids at these positions are the most likely to be a transmembrane helix.

Processing time for this