Bioinformatics, 33(22), 2017, 3685–3690
doi: 10.1093/bioinformatics/btx531
Advance Access Publication Date: 23 August 2017
Review

OXFORD

Sequence analysis

An introduction to deep learning on biological sequence data: examples and solutions

Vanessa Isabell Jurtz[1], Alexander Rosenberg Johansen[2], Morten Nielsen[1,3], Jose Juan Almagro Armenteros[1], Henrik Nielsen[1], Casper Kaae Sønderby[4], Ole Winther[2,4,*] and Søren Kaae Sønderby[4,*]

[1]Department of Bio and Health Informatics, [2]Department of Applied Mathematics and Computer Science, Technical University of Denmark, Lyngby, Denmark, [3]Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, Buenos Aires, Argentina and [4]Department of Biology, University of Copenhagen, Copenhagen, Denmark

*To whom correspondence should be addressed.
Associate Editor: Alfonso Valencia

Received on March 13, 2017; revised on July 27, 2017; editorial decision on August 14, 2017; accepted on August 22, 2017

Abstract

Motivation: Deep neural network architectures such as convolutional and long short-term memory networks have become increasingly popular as machine learning tools during the recent years. The availability of greater computational resources, more data, new algorithms for training deep models and easy to use libraries for implementation and training of neural networks are the drivers of this development. The use of deep learning has been especially successful in image recognition; and the development of tools, applications and code examples are in most cases centered within this field rather than within biology.

Results: Here, we aim to further the development of deep learning methods within biology by providing application examples and ready to apply and adapt code templates. Given such examples, we illustrate how architectures consisting of convolutional and long short-term memory neural networks can relatively easily be designed and trained to state-of-the-art performance on three biological sequence problems: prediction of subcellular localization, protein secondary structure and the binding of peptides to MHC Class II molecules.

Availability and implementation: All implementations and datasets are available online to the scientific community at https://github.com/vanessajurtz/lasagne4bio.

Contact: skaaesonderby@gmail.com

Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Machine learning is a specialization of computer science closely related to pattern recognition, data science, data mining and artificial intelligence (William, 2009). Within the field of machine learning, artificial neural networks, inspired by biological neural networks, have in recent years regained popularity (Schmidhuber, 2015). Their most recent success began with the development of effective methods to train deep neural networks (networks with multiple hidden layers), and the coining of the term deep learning around 2006 (Hinton and Salakhutdinov, 2006; Schmidhuber, 2015). Since then improvements have been made in part enabled by the access to greater computational resources, especially graphics processing units (GPU), enabling training of deep neural networks containing many parameters in reasonable time. Given this, specialized neural network architectures like convolutional neural networks (CNN) and recurrent neural networks (RNN) with long short-term memory cells (LSTM) can now be trained efficiently and have been successfully applied to many problems including image

recognition (Ciresan *et al.*, 2011; Krizhevsky *et al.*, 2012) and natural language processing tasks such as speech recognition (Geiger *et al.*, 2014) and language translation (Sutskever *et al.*, 2014).

The successes of neural networks have led to the development of various programming frameworks to build and train neural networks. Examples are PyTorch (http://pytorch.org/), Caffe (http://caffe.berkeleyvision.org) and TensorFlow (https://www.tensorflow.org). Our framework of choice here is Lasagne (Dieleman *et al.*, 2015), a well-established easy to use and extremely flexible lightweight Python library built on top of the Theano numerical computation library (Bastien *et al.*, 2016). While most other frameworks require the user to learn a dedicated programming language, Lasagne is Python-based and therefore relatively easy to use for bioinformaticians already programming in Python. Further Lasagne's active community ensures that the latest neural network training algorithms and architectures are available to the user.

Within bioinformatics, examples of deep learning applications include prediction of splicing patterns (Leung *et al.*, 2014), DNA and RNA targets of regulatory proteins (Alipanahi *et al.*, 2015), protein secondary structure (Wang *et al.*, 2016) and biomedical image analysis (Cha *et al.*, 2016; Moeskops *et al.*, 2016). However, the number of applications is still relatively small, and the application of deep learning methods within biology is in our view being held back due to a lack of examples and programming templates with a biological background facilitating a head start on the use of these libraries for non-experts.

Here, we seek to alter this by providing a non-expert introduction to the field of deep neural networks with application examples and ready to apply and adapt code templates illustrating how convolutional and LSTM neural networks can be successfully designed and trained on biological data to achieve state-of-the-art performance in prediction of (i) protein subcellular localization, (ii) protein secondary structure and (iii) peptides binding to Major Histocompatibility Complex Class II molecules. Implementations of the methods are available online to be used by non-expert end-users as templates for developing models to describe a given problem of interest https://github.com/vanessajurtz/lasagne4bio. The code can run both on CPU and cuda-enabled GPU. GPU gives of the order of a 100-fold speed-up.

2 Deep learning background

The traditional neural network architecture is the feed forward neural network with one hidden layer, in which each input neuron is connected to each neuron in the hidden layer and each neuron in the hidden layer is in turn connected to each neuron in the output layer. A non-linear so-called activation function, most often tanh or sigmoid, is applied to give the output of a neuron given its input. For classification problems the number of units in the output layer is equal to the number of classes. The softmax function is often used to make the outputs probabilistic, that is given an input the network assigns a probability to each class. For the prediction of a continuous output, a single linear output neuron is typically used. All connections in the network are directed from input to output, and it is possible to build deep networks by adding more hidden layers where each neuron will be connected to each neuron in the following layer. This type of architecture is visualized in Figure 1A. The hidden layer of a feed forward neural network is also referred to as a dense layer, because is fully connected to the previous layer.

In convolutional neural networks (CNNs) information also flows only from the input to the output, layer by layer. They are however not fully connected, but instead slide a filter (a set of weights) over the input that feeds into a different neuron in the next layer each time it is moved as illustrated in Figure 1B. The filter will thereby identify features in input irrespectively of where they appear. This concept is visualized in Figure 1C using the example of a convolutional filter detecting a motif in an amino acid sequence. Pooling such as mean pooling (averaging of nearby positions) enables the network to become invariant to small local deformations in the input. Convolutional neural networks often consist of many convolutional filters and many convolutional and pooling layers to enable the network to integrate the information from the different filters and various levels of abstraction (LeCun *et al.*, 2015). When applied to biological problems, convolutional neural networks are ideally suited to locate motifs, for example in a protein sequence, independent of their position within the sequence.

Recurrent neural networks (RNNs) are neural network models for sequential data. In addition to the feed forward connections they have time-delayed connections between the neurons of a hidden layer (Graves, 2012). RNNs process the sequence one element at a time, in biological sequence context one residue after the other. The information therefore flows both from input to output and along the sequence. In this way memory is generated and the neural network gains the ability to store and integrate information from past inputs. Long short-term memory (LSTM) neural networks are a special type of RNNs in which the scalar-valued hidden neuron is replaced with the LSTM memory block. The LSTM memory block is inspired by a computer memory cell where context-dependent input, output and forget gates control what is written to, read from and kept in the cell in each time-step. In this way, it is easier for the network to store a given input over many time steps.

LSTMs (and in general RNNs) can by construction handle input sequences of varying length. This makes RNNs highly flexible for different types of tasks where either the input (many-to-one), the output (one-to-many) or both are sequences (many-to-many). Many-to-one is a model to process an entire sequence one amino acid at a time and then predict whether or not it has a certain biological property after having seen the entire sequence (here exemplified in the prediction of peptide binding to MHCII). The many-to-many approach, also known as tagging or sequence labeling, can for example be used to predict the secondary structure classification of each amino acid in a protein. Performance can be improved by changing to the many-to-many bidirectional approach (biLSTM) where the network processes the input sequence forwards and backwards and bases each prediction not just on what came before the current position but also on what comes after. The same approach can be used in a many-to-one approach here exemplified in protein subcellular localization prediction.

For a more detailed explanation of the CNN and LSTM architectures, including the mathematical formulas to calculate the output and update weights, we refer the reader to textbook 'Deep Learning' by Goodfellow *et al.* (2016).

2.1 Designing your model

In this section, we highlight some of the key issues to consider when constructing deep neural networks such as how to avoid overfitting when increasing network size and depth.

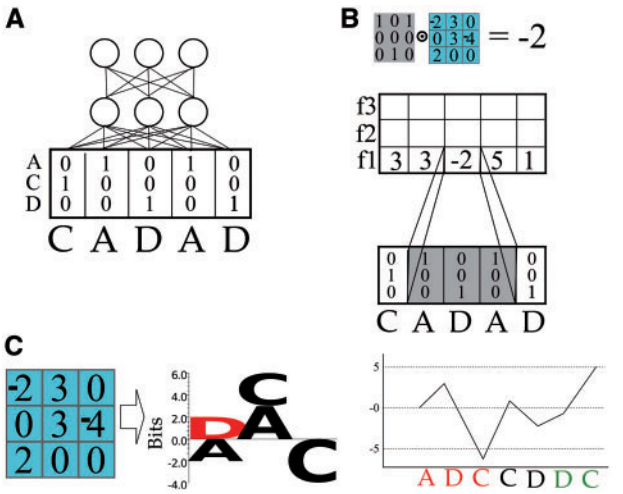


Fig. 1. (**A**) Feed forward network. Amino Acids C, A, D, A, D are encoded as 'one-hot' vectors with a 1 at the position corresponding to the amino acid type (A, C or D), and zero otherwise. (**B**) Convolutional neural network. A filter (blue) is slid over the input sequence. The filter here has a length of three amino acids. At each position the filter has a preference for different amino acid types. The filter output is calculated by taking the sum of the element-wise product of the input and the filter position-specific weights. Each time the filter is moved, it feeds into a different hidden neuron in the hidden layer, here visualized in the f1 row. Multiple filters will give multiple inputs to the next layer {f1, f2, f3, . . .}. (**C**) A filter can be visualized as a sequence motif. This helps to understand which amino acids the filter prefers at each sequence position. When the filter is slid over the input sequence, it functions as motif detector and becomes activated when the input matches its preference. For example, this filter has negative output for sub-sequences ADC and positive for DCD

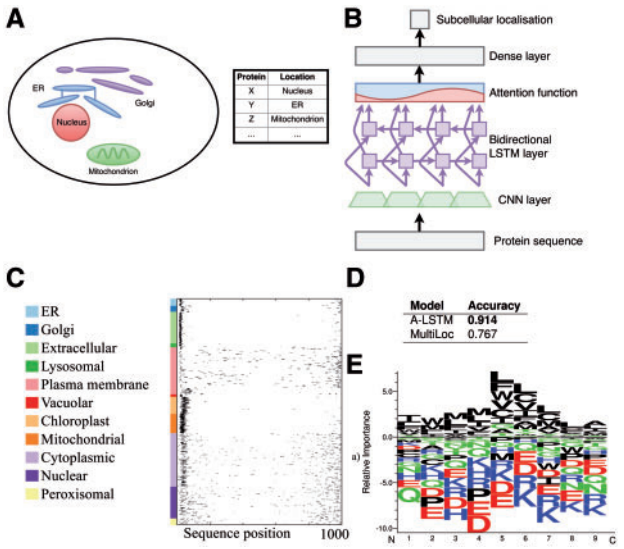


Fig. 2. (**A**) Schematic illustration of subcellular localization classification. (**B**) The neural network architecture used to predict the subcellular localization of proteins. (**C**) Visualization of the positions within the protein amino acid sequence that have high importance for the prediction of subcellular localization. Sequence position importance is determined by an attention function and the middle part of the protein sequences have been cut out in order to align N- and C-terminus. The different subcellular localization classes are shown on the y-axis. (**D**) Table of the A-LSTM performance compared to the state of the art sequence driven SVM prediction method MultiLoc. (**E**) Visualization of convolutional filter. For this filter charged amino acids will suppress the output (blue, red) while hydrophobic amino acids will increase the output (black). (C) and (D) are adapted from (Sønderby *et al.*, 2015)

2.1.1 Sequence encoding

When working with biological sequence data the numeric representation of the data influences model performance. An obvious solution is to use the so-called sparse or one-hot encoding (see Fig. 1A), where an amino acid is represented by a vector of the length 20, containing a single one and 19 zeros. The parameters connecting an amino acid input to the first hidden layer is often called the embedding for that amino acid. Another possibility is to use the BLOSUM matrix (Eddy, 2004) for encoding, representing each amino acid by its corresponding row in the BLOSUM matrix. The BLOSUM matrix captures information about which pairs of amino acids are easily interchangeable during evolution, but it does not capture information about the evolutionary constraints on a protein family (i.e. which amino acid positions are highly conserved and which are variable). This information can effectively be captured in a sequence profile. A sequence profile has the dimensions protein length times the number of amino acids and is conventionally generated by running PSI-BLAST (Altschul *et al.*, 1997) against a reference database. Encoding protein sequences as such profiles has demonstrated very helpful for prediction of for instance secondary structure (Jones, 1999).

2.1.2 Regularization and training

Regularization techniques aim to prevent overfitting and are especially relevant for large models containing many parameters. Before applying regularization techniques it is important to partition the dataset carefully dealing with data redundancy and set up the neural network training using cross-validation (further detailed in the Supplementary Material). Dropout (Hinton *et al.*, 2012) reduces overfitting by introducing discrete noise during training. For every training input the output of each hidden unit is set to zero with a certain preselected probability. Batch normalization (Ioffe and Szegedy, 2015) re-parameterizes the hidden unit activation in order to increase convergence speed but also makes the output stochastic, creating a regularizing effect. Appropriate weight initialization (Glorot and Bengio, 2010) and gradient optimization techniques [e.g. Adam (Kingma and Ba, 2015)] also influence the overall performance. Combining ensembles of 5–10 models initialized with different random seeds usually leads to a substantial increase in performance.

2.1.3 Attention

In standard many-to-one RNNs, the last hidden state is most often used as input to a downstream network. However, when certain subparts of the input sequence contain most of the predictive information, this approach is suboptimal. Here, it will be beneficial to add attention weighting (Bahdanau *et al.*, 2015; Jaderberg *et al.*, 2015) (as illustrated in Fig. 2B). Attention is implemented by learning a context dependent normalized weight for each hidden state of the input sequence. The input to the downstream network is then the weighted average over all hidden states. This normalized weight is computed in two steps: (i) a one hidden layer dense network takes each hidden state of the RNN as input and (ii) passes these network outputs through a softmax function. In one of the examples shown later, we use such attention to visualize and identify parts of a protein sequence that are important for subcellular localization prediction.

2.1.4 Pooling

Max-pooling provides a way of reducing the input or hidden layer size by selecting only the maximally activated neuron from a

number of neighboring neurons. Alternatively mean pooling can be performed where the mean activation is calculated among neighboring neurons. Pooling is often used in convolutional neural networks (LeCun *et al.*, 2015). To make a convolutional neural network independent of the input sequence length, global max-pooling can be applied where only the maximally activated neuron of the input or hidden layer is selected for each of the convolutional filters. In this way, the number of hidden neurons generated by the convolutional filters is equal to the number of filters and not influenced by the input sequence length.

3 Example biological applications

To demonstrate the usefulness of the CNN and LSTM neural network architectures for machine learning on biological data, we have applied the framework to three important biological problems: subcellular localization, protein secondary structure and peptide binding to MHC Class II (MHCII) molecules. The details of the data, models and training and testing procedure are described in the Supplementary Material. Note, however that not all problems are equally suited for analyses using deep neural network methods (the limiting factor in most cases being the amount of data available), and that definition of optimal network architectures (guided by biological insights) and training protocols is as important for a successful application as the choice of deep learning method. An in-depth coverage of these themes is however beyond the scope of this manuscript. However, a generally good starting architecture for machine learning on biological sequence data has an input-to-output structure as shown in Figure 2B: Encoded seq-to-CNN-to-biLSTM-to-Attention-to-Dense-to-Output. Dependent upon the problem at hand, one may omit architecture elements or introduce skip connections as shown in Figure 3B. In the online material, we have included Jupyter Notebook implementations of different models starting from a simple dense feed forward model up to a full model including CNN, LSTM and attention to illustrate the contribution of each architectural element. This is discussed in more detail below.

3.1 Subcellular localization

In eukaryotes, proteins are either secreted from the cell or sorted into numerous different cellular sub-compartments such as e.g. nucleus, cytoplasm and Golgi (see Fig. 2A). For some locations, the proteins are known to contain specific sequence motifs, where the best understood signal is the N-terminal signal peptide in proteins belonging to the secretory pathway. However, other subcellular compartments are also known to contain weaker signals such as the nuclear localization signal in nuclear proteins, membrane crossing alpha-helices in membrane proteins or KDEL-type retention signals for endoplasmic reticulum proteins (Lodish *et al.*, 2016).

The task of predicting protein sorting or subcellular localization has attracted large interest in the bioinformatics field (Emanuelsson *et al.*, 2007) and can be generally stated as a sequence to multi-class prediction problem. The state of the art data driven approach for prediction of subcellular location of proteins is MultiLoc (Höglund *et al.*, 2006), which is based on support vector machines (SVM) taking into account N-terminal targeting regions, amino acid composition and protein sequence motifs. The SherLoc2 method (Briesemeister *et al.*, 2009) combines the sequence-based MultiLoc method with text mining approaches and the inclusion of Gene Ontology (GO) terms to improve performance.

We have implemented four models (see online Jupyter Notebooks) starting from a simple feed forward (FNN) dense model up to the full model including CNN, LSTM and attention architecture visualized in Figure 2B and called A-LSTM in the following. We apply these models to a reduced version of the subcellular localization dataset to illustrate the effect of hyper parameter choices and network architecture on predictive performance and running times. In this example, the model with the lowest performance was FFN, with a classification accuracy fraction (Acc) of 0.757. The CNN and CNN-LSTM models followed with an accuracy of 0.783 and 0.814, respectively. Finally, the model with the highest accuracy was the A-LSTM with Acc = 0.830. If we evaluate the performance for the different prediction classes for the four models, we do not see large differences for the classes characterized with many proteins (data not shown). However, the A-LSTM demonstrates improved performance for classes characterized with very few examples, such as ER, Golgi apparatus, lysosome and vacuole. The main drawback of the models using LSTM layers compare to the FFN and CNN is the training time. On average, LSTM models take 10 to 12 s per epoch, whereas FFN takes 0.3 s and CNN takes 2.5 s. This could be a limiting factor with more data or a more complex architecture. In the remainder of the section, we discuss the findings for A-LSTM trained on the entire MultiLoc dataset.

In the subcellular localization problem, the position of the sorting signal is often of interest to the biologists. LSTM neural networks combined with an attention function are ideally suited to solve this task. The model presented here is a modified and improved version of the one presented by Sønderby *et al.* (2015). The architecture of our A-LSTM neural network is visualized in Figure 2B and is based on a convolutional layer followed by a bi-directional LSTM. Additionally, we applied an attention function after the LSTM allowing us to assign an importance to each position in a protein sequence with regards to subcellular location. Here, we calculate the activations of the LSTM memory blocks (hidden state) for each position in the protein sequence, and apply the attention function to determine the importance of each hidden state (giving an indication of the importance of the underlying position in the input sequence). Next, we calculate the attention weighted sum of all hidden states that are used as input to a dense feed forward neural network with one hidden layer and softmax output to obtain the final prediction of subcellular localization. All models were trained on the same dataset as the MultiLoc method described above.

The reasoning behind this choice of architecture is based upon the following idealized flow of information from discriminative subsequences to classification: The CNN filters deal with the issue of sequence alignment, and provide a learned filter-bank sensitive to specific sequence motives. When such a motif is present somewhere in the sequence, it will lead to an increased absolute input to the LSTM at that position. The biLSTM layers will integrate this information locally both forward and backward in the sequence and the attention function uses its learned context awareness to pass the discriminatory information from these RNN hidden states on to the dense layer and then to the softmax classification.

A-LSTM achieved an accuracy of Acc = 0.914, outperforming the MultiLoc method which had an Acc = 0.767. SherLoc2 outperformed our models with an Acc = 0.93. However, SherLoc2 is not purely sequence data driven but also includes text-mining approaches, and we hypothesize extending our A-LSTM ensemble in that way would improve performance further.

In Figure 2C, we illustrate where in the sequence the A-LSTM model assigns weight for proteins belonging to different subcellular compartments. Sequences from the compartments ER, extracellular, lysosomal and vacuolar have clearly marked N-terminal signal peptides. Chloroplast and mitochondrial proteins also have N-terminal

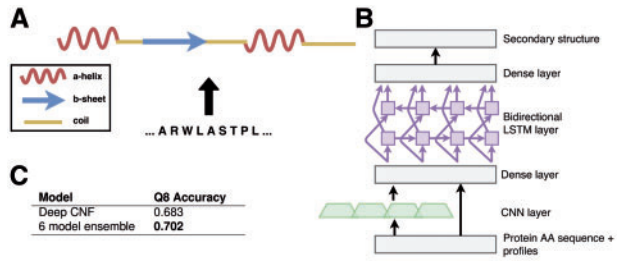
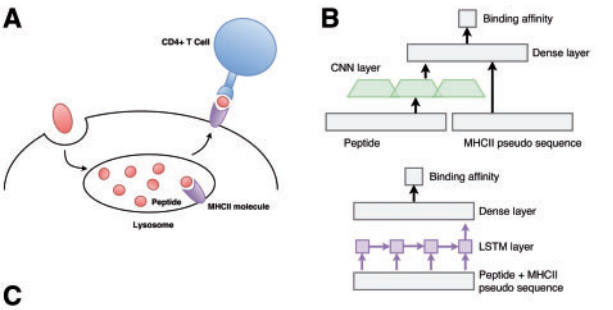


Fig. 3. (**A**) Visualization of the task of secondary structure prediction based on the protein amino acid sequence. (**B**) A flowchart showing the succession of different layers in our neural network model to predict protein secondary structure. The skip connection is implemented by concatenating the output of the CNN layer with amino acid input. (**C**) Performance of our model compared to the state of the art DeepCNF (Wang *et al.*, 2016) method



C

			NetMHCIIpan-3.0		CNN+LSTM		Consensus	
Allele	#	# bind	PCC	AUC	PCC	AUC	PCC	AUC
DRB1-0101	717	550	0.809	0.898	0.801	0.902	**0.821**	**0.909**
DRB1-0301	703	408	0.702	0.851	0.680	0.846	**0.708**	**0.859**
DRB1-0701	682	375	0.755	0.872	0.744	0.873	**0.761**	**0.879**
DRB1-0801	838	363	0.718	0.87	0.745	0.880	**0.752**	**0.883**
DRB1-1101	813	426	0.788	0.903	0.793	0.901	**0.8**	**0.905**
DRB1-1301	803	462	0.563	0.782	**0.609**	0.800	**0.609**	**0.804**
DRB1-1302	765	404	0.38	0.704	**0.431**	**0.733**	0.424	0.729
DRB1-1501	758	218	0.49	0.762	0.500	0.764	**0.506**	**0.767**
DRB3-0202	726	288	0.489	0.755	0.489	0.758	**0.499**	**0.76**
DRB3-0301	782	449	**0.556**	**0.773**	0.45	0.722	0.515	0.754
DRB4-0101	778	235	**0.275**	0.232	0.632	0.64	0.267	**0.646**
DRB4-0103	764	474	0.538	0.799	0.539	0.793	**0.55**	**0.802**
DRB5-0101	731	461	0.691	0.835	0.695	0.842	**0.706**	**0.845**
mean			0.596	0.803	0.593	0.803	**0.609**	**0.811**

Fig. 4. (**A**) MHCII molecules present peptides derived from the extracellular environment to T-helper cells. Here we predict which peptides are able to bind a given MHCII molecule, which is an important step on the way to identifying T-cell epitopes. (**B**) The CNN (left side) and LSTM (right side) architectures used to predict peptide binding to MHCII molecules. (**C**) Performance per MHCII allele of NetMHCIIpan-3.0, CNN + LSTM and the consensus method (NetMHCIIpan-3.0 and CNN + LSTM) on the evaluation set

sorting signals. For the plasma membrane category, we observe that some proteins have signal peptides, while the model generally focuses on sections, presumably transmembrane helices, scattered across the rest of the protein sequence with some overabundance close to the C-terminus. Cytoplasmic and nuclear proteins do not have N-terminal sorting signals, and we see that the attention is scattered over a broader region of the sequences. Figure 2D gives an example of a convolutional filter that prefers hydrophobic amino acids.

3.2 Secondary structure prediction

After translation, proteins fold into a 3-dimensional structure, known as the tertiary structure (Fig. 3A). Knowledge of a protein's structure can help to understand its function. Therefore *de novo* prediction of protein structure from sequence is a problem of great biological interest (Dill and MacCallum, 2012). An important step in the prediction of tertiary protein structure is the prediction of the secondary structure, the local conformations of the peptide backbone. There are three main classes of secondary structure: alpha-helix, beta-strand and coil. These can be further divided into 8 classes (Kabsch and Sander, 1983) (3 sub-classes of helix, 2 sub-classes of strands and 3 sub-classes of coil).

Secondary structure prediction is a many-to-many problem or sequence-tagging problem because we predict the secondary structure class for each amino acid in the protein sequence. The state of the art sequence-based method to solve this problem is DeepCNF (Wang *et al.*, 2016) which is based on a combination of deep convolutional neural networks and conditional neural fields.

The architecture of our neural network to predict secondary is shown in Figure 3B and is a combination of convolutional and LSTM neural networks (Sønderby and Winther, 2014). The attention step is omitted here because the information in input and output to a high degree is co-located. Using either a CNN layer and/or a skip layer makes no large difference for this application. Here, we report the CNN plus skip results. An ensemble of neural networks was trained on the same data as DeepCNF. The performance of our neural network ensemble is given in Figure 3C and reaches a Q8 accuracy of 0.702, thus outperforming DeepCNF.

3.3 Predicting peptides binding to MHC class II molecules

MHC class II molecules (MHCII) are an essential part of the adaptive immune system and involved in the detection of extracellular pathogens. They present peptides derived from proteins of the extracellular environment to T-helper cells (see Fig. 4A). A peptide can only be recognized by a T cell, i.e. be a T cell epitope, if it is able to bind an MHCII

molecule expressed by the host (Roche and Furuta, 2015). When interested in predicting an immune response to pathogens, the prediction of peptides binding to MHCII molecules is an important step, since only a small fraction of all peptides are able to bind to MHCII molecules (Castellino *et al.*, 1997). Peptides binding to MHCII molecules can differ greatly in length greatly since the binding cleft of the MHCII molecules is open on both sides, allowing the peptide to extend beyond the binding cleft. Further, the binding core of a peptide, which has a length of approximately nine amino acids, can be located anywhere within the peptide (Nielsen *et al.*, 2010).

The state of the art method for predicting peptide binding to MHCII molecules is NetMHCIIpan-3.0 (Karosiene *et al.*, 2013), which relies on an ensemble of feed forward neural networks trained by an algorithm tailored to the problem (Andreatta *et al.*, 2011; Nielsen and Lund, 2009).

Here, we trained an ensemble of CNNs and LSTMs to predict peptide binding to MHCII molecules. The LSTM is uni-directional and attention is omitted because the peptide sequences are so short. The neural network architectures are visualized in Figure 4B. All models were trained on the dataset and dataset partitions used to develop the NetMHCIIpan method. An ensemble of CNNs and LSTMs achieved performance on par with NetMHCIIpan-3.0 on both testing and evaluation data. Further, combining feed forward nets trained in the NetMHCIIpan fashion with CNN and LSTM networks to form a consensus method significantly improved performance. Figure 4C shows the performance of the consensus method for individual MHCII molecules in the evaluation set.

4 Discussion

The regained interest in deep neural network architectures within machine learning fields such as image and speech recognition is only

slowly spreading into biology and bioinformatics. One potential cause of this is the lack of examples or code templates tailored to bioinformatics problems combined with the notion that the implementation and training of deep learning methods is complicated and computationally challenging. Here, we have aimed to help overcome these barriers by demonstrating how simple code can easily and effectively be developed to train CNN and LSTM neural network models to predict properties of important biological problems. We have done this for three distinct biological problems: predictions of subcellular localization, secondary structure and peptide binding to MHCII molecules, and in all cases demonstrated state of the art performance.

We have performed all model development and evaluation using the Lasagne library. However, our findings are general and we expect comparable results could have been obtained using any of the currently available frameworks for deep learning.

The applications we have chosen are all described by protein data. It is however clear that the framework is equally suited to work on any type of sequence data, for example nucleotide sequences (Leung *et al.*, 2014). Others have also applied similar models to images of biological or medical relevance (Cha *et al.*, 2016; Moeskops *et al.*, 2016).

Funding

This work has been supported by the the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under Contracts HHSN272201200010C and Novo Nordisk Foundation. We thank the NVIDIA Corporation for the donation of Titan X GPUs.

Conflict of Interest: none declared.

References

Alipanahi,B. *et al.* (2015) Predicting the sequence specificities of DNA- and RNA-binding proteins by deep learning. *Nat. Biotechnol.*, **33**, 831–838.

Altschul,S.F. *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.

Andreatta,M. *et al.* (2011) NNAlign: a web-based prediction method allowing non-expert end-user discovery of sequence motifs in quantitative peptide data. *PLoS One*, **6**, e26781.

Bahdanau,D. *et al.* (2015) Neural Machine Translation by Jointly Learning to Align and Translate. *Proceedings of International Conference on Learning Representations (ICLR)*, arXiv preprint arXiv:1312.6077.

Bastien,F. *et al.* (2016) Theano: A Python framework for fast computation of mathematical expressions. *arXiv e-prints*.

Briesemeister,S. *et al.* (2009) SherLoc2: a high-accuracy hybrid method for predicting subcellular localization of proteins. *J. Proteome Res.*, **8**, 5363–5366.

Castellino,F. *et al.* (1997) Antigen presentation by MHC class II molecules: invariant chain function, protein trafficking, and the molecular basis of diverse determinant capture. *Hum. Immunol.*, **54**, 159–169.

Cha,K.H. *et al.* (2016) Urinary bladder segmentation in CT urography using deep-learning convolutional neural network and level sets. *Med. Phys.*, **43**, 1882.

Ciresan,D. *et al.* (2011) A committee of neural networks for traffic sign classification. In: *The 2011 International Joint Conference on Neural Networks*. IEEE, pp. 1918–1921.

Dieleman,S. *et al.* (2015) Lasagne: First release. doi: 10.5281/zenodo.27878.

Dill,K.A. and MacCallum, J.L. (2012) The protein-folding problem, 50 years on. *Science*, **338**, 1042–1046.

Eddy,S.R. (2004) Where did the BLOSUM62 alignment score matrix come from? *Nat. Biotechnol.*, **22**, 1035–1036.

Emanuelsson,O. *et al.* (2007) Locating proteins in the cell using TargetP, SignalP and related tools. *Nat. Protoc.*, **2**, 953–971.

Geiger,J.T. *et al.* (2014) Robust speech recognition using long short-term memory recurrent neural networks for hybrid acoustic modelling. In: *Interspeech ISCA Singapore*.

Glorot,X. and Bengio,Y. (2010) Understanding the difficulty of training deep feedforward neural networks. In: *Proceedings of the International Conference on Artificial Intelligence and Statistics (AISTATS'10). Society for Artificial Intelligence and Statistics*.

Goodfellow,I. *et al.* (2016) Deep Learning. http://www.deeplearningbook.org.

Graves,A. (2012) *Supervised Sequence Labelling with Recurrent Neural Networks*, Springer, Berlin, Heidelberg.

Hinton,G.E. *et al.* (2012) Improving neural networks by preventing co-adaptation of feature detectors.

Hinton,G.E. and Salakhutdinov, R.R. (2006) Reducing the dimensionality of data with neural networks. *Science*, **313**, 504–507.

Höglund,A. *et al.* (2006) MultiLoc: prediction of protein subcellular localization using N-terminal targeting sequences, sequence motifs and amino acid composition. *Bioinformatics*, **22**, 1158–1165.

Ioffe,S. and Szegedy,C. (2015) Batch Normalization: Accelerating Deep Network Training by Reducing Internal Covariate Shift. *Proceedings of the 32nd International Conference on Machine Learning*, pp. 448–456.

Jaderberg,M. *et al.* (2015) Spatial Transformer Networks.

Jones,D.T. (1999) Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.*, **292**, 195–202.

Kabsch,W. and Sander,C. (1983) Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, **22**, 2577–2637.

Karosiene,E. *et al.* (2013) NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. *Immunogenetics*, **65**, 711–724.

Kingma,D. and Ba,J. (2015) Adam: A Method for Stochastic Optimization, *Proceedings of International Conference on Learning Representations (ICLR)*, arXiv preprint arXiv:1412.6980.

Krizhevsky,A. *et al.* (2012) ImageNet Classification with Deep Convolutional Neural Networks. In: Pereira, F. *et al.* (eds) *Advances in Neural Information Processing Systems 25*. Curran Associates, Inc., pp. 1097–1105.

LeCun,Y. *et al.* (2015) Deep learning. *Nature*, **521**, 436–444.

Leung,M.K.K. *et al.* (2014) Deep learning of the tissue-regulated splicing code. *Bioinformatics*, **30**, i121–i129.

Lodish,H. *et al.* (2016) *Molecular Cell Biology*. 8th ed. W. H. Freeman, New York.

Moeskops,P. *et al.* (2016) Automatic segmentation of MR brain images with a convolutional neural network. *IEEE Trans. Med. Imaging.*, **35**, 1252–1261.

Nielsen,M. *et al.* (2010) MHC class II epitope predictive algorithms. *Immunology*, **130**, 319–328.

Nielsen,M. and Lund,O. (2009) NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. *BMC Bioinformatics*, **10**, 296.

Roche,P.A. and Furuta,K. (2015) The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat. Rev. Immunol.*, **15**, 203–216.

Schmidhuber,J. (2015) Deep learning in neural networks: An overview. *Neural Netw.*, **61**, 85–117.

Sønderby,S.K. *et al.* (2015) Convolutional LSTM networks for subcellular localization of proteins. In: Dediu, A.-H. *et al.* (eds) *Algorithms for Computational Biology*. Springer International Publishing, New York, pp. 68–80.

Sønderby,S.K. and Winther,O. (2014) Protein Secondary Structure Prediction with Long Short Term Memory Networks. arXiv: 1412.7828

Sutskever,I. *et al.* (2014) Sequence to Sequence Learning with Neural Networks. In: Ghahramani, Z. *et al.* (eds) *Advances in Neural Information Processing Systems*. Curran Associates, Inc., New York, pp. 3104–3112.

Wang,S. *et al.* (2016) Protein secondary structure prediction using deep convolutional neural fields. *Sci. Rep.*, **6**, 18962.

William,L.H. (2009) Machine Learning-Encyclopedia Britannica. Encyclopædia Britannica, Inc.