DEePNOvOV2: Better De novo Peptide Sequencing With Deep Learning

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ABSTRACT

We introduce DeepNovoV2, the state-of-the-art neural networks based model for de novo peptide sequencing. Contrary to existing models like DeepNovo or DeepMatch which represents each spectrum as a long sparse vector, in DeepNovoV2, we propose to directly represent a spectrum as a set of \((m/z, \text{intensity})\) pairs. Then we use an order invariant network structure (T-Net) to extract features from the spectrum. By representing spectrums as sets of peaks, we argue that our method is more straightforward and do not have the accuracy-speed/memory trade off problem. Our experiments show that comparing to the original DeepNovo model, DeepNovoV2 has at least 15\% relative improvement on peptide accuracy.

Keywords de novo peptide sequencing · Deep Learning · order invariant model

1 Introduction

In proteomics, De novo peptide sequencing from tandem Mass Spectrometry (MS) data is the key technology for finding new peptide or protein sequences. It has successful applications in assemble monoclonal antibody sequences (mAbs)\(^1\) and great potentials in identifying neoantigens for personalized cancer vaccines\(^2\). Given the importance of the de novo peptide sequencing technology, massive research have been done in this area and different tools have been proposed\(^3\)\(^4\)\(^5\)\(^6\)\(^7\). In 2017, Tran et al. first introduced deep learning to de novo peptide sequencing and proposed DeepNovo, a neural network based de novo peptide sequencing model\(^8\) for Data Dependent Acquisition (DDA) MS data. Inspired by the success of image captioning model\(^9\), DeepNovo integrated two fundamental types of neural networks, CNNs and LSTM, in order to extract features from both the spectrum and the “language model of peptides”. In DeepNovo, each spectrum is represented as a long intensity vector and CNNs are applied on segments of this vector to extract features and make predictions of the next amino acid. CNNs have been proved as effective tools for pattern recognition in different applications like image classification, object detection and sentiment analysis\(^10\)\(^11\)\(^12\). By applying CNNs to the intensity vector, DeepNovo could learn from noisy spectrum. It is reported that DeepNovo
outperformed the the decade long-standing state of the art records of de novo sequencing algorithms by a large margin of 38.1–64.0% at the peptide level[8].

In 2019, Tran et al. further extended DeepNovo on Data Independent Acquisition (DIA) MS data and proposed DeepNovo-DIA[2], the first de novo peptide sequencing algorithm for DIA MS/MS spectrums. Comparing to DDA, DIA data are in general harder to interpret because the spectrum generated by DIA often contains multiple peptides’ fragment ions. On the other hand, the multiplexity and noise in the DIA spectrums make deep neural networks a more reasonable choice. In DeepNovo-DIA, each detected feature is represented by 5 spectrums, where each spectrum is discretized into a intensity vector as in DeepNovo. Thus the input to DeepNovo-DIA is a matrix of shape 5 by the length of intensity vector. Then DeepNovo-DIA applies 2D convolution on the input to utilize the information provided by the extra dimension of retention time and hopefully to learn the coeluting patterns. It is also worth noting that Tran et al. reported by changing cross entropy loss to focal loss[13], they observed a significant improvement on peptide accuracy[2].

In this paper, we propose various improvements to DeepNovo. We named the improved model DeepNovoV2. Our experiment results on several DDA MS datasets show that DeepNovoV2 has beaten DeepNovo by a significant margin of at least 15% on peptide level.

2 Methods

2.1 spectrum representation

Previously in DeepNovo, spectrums are represented as intensity vectors, where each index of the vector represents a small m/z bin and the value represents the sum of intensities of all peaks fall into that bin. This representation of spectrum naturally has the problem of accuracy and speed/memory trade off. For example, by default DeepNovo use a spectrum resolution of 10, which means every peak within a 0.1 Da m/z bin will be merged together and represented as an element of the intensity vector. However, we lose a lot of useful information about the exact location of each peak during the merging. On the other hand, if we need to build more accurate model and increase the resolution, we will end up with a significantly longer intensity vector and the model will needs more memory and time to train. In DeepNovoV2, to solve the accuracy-speed/memory trade off problem we propose to directly represent spectrum as a set of (m/z, intensity) pairs. For each spectrum we select the top N most intense peaks (by default we choose N = 500), and represent the spectrum as \( \{(m/z_1, I_1), \ldots, (m/z_N, I_N)\} \). Further, we denote \( M_{\text{observed}} = \{m/z_1, \ldots, m/z_N\} \) as the observed m/z vector and \( I = (I_1, \ldots, I_N) \).

2.2 feature extraction

We denote the number of tokens as \( n_{\text{vocab}} \) and number of ion types as \( n_{\text{ion}} \). To make a fair comparison with DeepNovo, we keep \( n_{\text{vocab}} = 26 \) (20 amino acid residues, 3 variable modifications and three special tokens: "start", "end" and "pad") and use the same eight ion types (b, y, b(2+), y(2+), b-H2O, y-H2O, b-NH3, and y-NH3) as in the original implementation of DeepNovo[8]. Similar to DeepNovo, at each step, we compute the theoretical m/z location for each token and ion type pair. The result is a matrix of shape \( (n_{\text{vocab}}, n_{\text{ion}}) \) and denoted as \( M_{\text{theoretical}} \). Next we expand the dimension of \( M_{\text{observed}} \) to make it a 3-dimensional tensor of shape \( (N, 1, 1) \), and then repeat \( M_{\text{observed}} \) on second dimension for \( n_{\text{vocab}} \) times and on third dimension for \( n_{\text{ion}} \) times. The result is denoted as \( M'_{\text{observed}} \) and it is a tensor of shape \( (N, n_{\text{vocab}}, n_{\text{ion}}) \). Similarly we expand \( M_{\text{theoretical}} \) to the shape of \( (1, n_{\text{vocab}}, n_{\text{ion}}) \), repeat on first dimension for \( N \) times and denote the result as \( M'_{\text{theoretical}} \). Then we can compute the m/z difference tensor (denoted as \( D \)) in which each element represents the difference between the m/z value for an observed peak and the theoretical m/z for a token and ion type pair.

\[
D = M'_{\text{observed}} - M'_{\text{theoretical}}
\]

It is worth noting that Equation 1 could be computed efficiently by using the "broadcast" behaviour in popular frameworks like Tensorflow[14] and PyTorch[15].

\[
\sigma(D) = \exp\{-|D| * c\}
\]

Based on expert knowledge of de novo peptide sequencing, we design an activation function \( \sigma \), shown in Equation 2. Here the exponential and absolute operations are all element wise operations. The intuition for \( \sigma \) is that an observed peak could only be considered matching a theoretical m/z location if the absolute m/z difference is small. For example, if we set \( c = 100 \), then an observed peak that is 0.02 Da away from a theoretical location would generate a signal of
\[ e^{-2} \approx 0.135, \text{ which is only one seventh of the signal of a perfect match. In our experiments, we tried setting } c \text{ to be a trainable parameter and updating it through backpropagation. It shows similar performance with setting } c = 100, \text{ thus for the simplicity of the model, we choose to fix } c \text{ to be 100.} \]

\[ F = \sigma(D)' \oplus I' \]

Finally, the feature matrix \( F \) for prediction is simply the concatenation of \( \sigma(D) \) and \( I \), as shown in Equation (3). Here \( \sigma(D)' \) is a \( N \) by \( n_{vocab} \times n_{ion} \) matrix reshaped from \( \sigma(D) \), \( I' \) is a \( N \) by 1 matrix reshaped from \( I \) and \( \oplus \) represents concatenation along the second dimension.

### 2.3 T Net

A spectrum is set of \((m/z, intensity)\) pairs, which means the order of peaks should be irrelevant. Therefore, the prediction network should have order invariant property with respect to the first dimension of \( F \). To the best of our knowledge, T Net (the building block of Point Net[16]) is the first model designed for this kind of order invariant data, thus we choose to use it to process \( F \). In essence, T Net is composed of 3 1d convolutional layers with kernel size 1, followed by a global max pooling layer and 3 fully connected layers. And similar with DeepNovo[8], we could add an LSTM aside from the T net to make use of the language model information of peptides. The structure for T Net is shown in Figure 1 and the full model structure of DeepNovoV2 is shown in Figure 2. And as suggested by Tran et al.[2], we used focal loss[13] instead cross entropy loss when training the model.

### 2.4 Initial state for LSTM

Similar to DeepNovo[8], we could include a LSTM module to capture the "language model" for peptides. To make good predictions about the next amino acid, it is important for the LSTM to be initialized with information about the original spectrum. DeepNovo used a spectrum CNN to extract features from intensity vectors and then used the extracted features to initialize the LSTM. In DeepNovoV2, we replace the spectrum CNN structure with a simple embedding matrix. Specifically, we create a sinusoidal m/z positional embedding, as suggested by Vaswani et al[17]:

\[
PE_{loc,2i} = \sin(loc/10000\pi_{2i}) \\
PE_{loc,2i+1} = \cos(loc/10000\pi_{2i})
\]

Here \( loc \) represents the m/z location after discretization, and \( d_{lstm} \) represents the dimension of the LSTM module. The sinusoidal embedding has a desired property that for any distance \( k \), \( PE_{loc+k} \) could be represented as a linear function of \( PE_{loc} \). This property is important because in mass spectrums the m/z differences between observed peaks contain useful information that indicates which amino acids possibly exist. By using the sinusoidal positional embeddings, we hope the model could easily learn to extract information from the m/z difference between peaks.
For each spectrum \( \{(m/z_i, I_i)\}_{i=1}^N \), we denote the positional embedding for \( m/z_i \) as \( E_i \). The spectrum representation \( S \) is computed by:

\[
S = \sum_{i=1}^N I_i E_i
\]  

(4)

Then the initial hidden states \( h_0 \) and \( c_0 \) will be both initialized to \( S \). By replacing spectrum CNN to a fixed positional embedding matrix, we reduce the number of parameters and computation needed in the model. And our experiment result shows that initializing with \( S \) gives similar results comparing to initialize with the result of spectrum CNN.

2.5 training parameters

We train DeepNovoV2 with Adam algorithm\(^{[18]}\) with an initial learning rate of \( 10^{-3} \). After every 300 training steps, the loss on validation set is computed. If the validation loss has not achieved a new low in the recent ten evaluations, then the learning rate would be dropped by half.
### Results and analysis

We test our model on a DDA dataset of Hela samples\[^1\]. The train-valid-test split is done in the same way suggested by DeepNovo\[^8\], i.e. train, valid and test set do not share any common peptides. Both DeepNovo and DeepNovoV2 models are trained for 20 epochs. The weights with the smallest validation error are then selected to do beam search. The metrics that we compare are amino acid tag mass accuracy and peptide accuracy. The result on ABRF DDA data is shown in Table 1.

We also tested DeepNovoV2 with the cross species data published by Tran et al.\[^8\] (MSV000081382). This data set contains DDA mass spectrums from nine species. For each species, we train a model using spectrums from the other 8 species, and test the model on it. In these experiments we set $N = 1000$.

As we can see from Figure 3, DeepNovoV2 outperforms DeepNovo consistently on peptide recall rate, by a large margin of 13.01–23.95%.

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\[^1\]PRG 2018: Evaluation of Data-Independent Acquisition (DIA) for Protein Quantification in Academic and Core Facility Settings. https://abrf.org/research-group/proteomics-research-group-prg
Figure 3: Amino acid recall, amino acid precision and peptide recall of DeepNovo and DeepNovoV2

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