Unsupervised and supervised analysis of protein sequences

Computational Science (M. Weigt)

Master's Degree in Physics of Complex Systems

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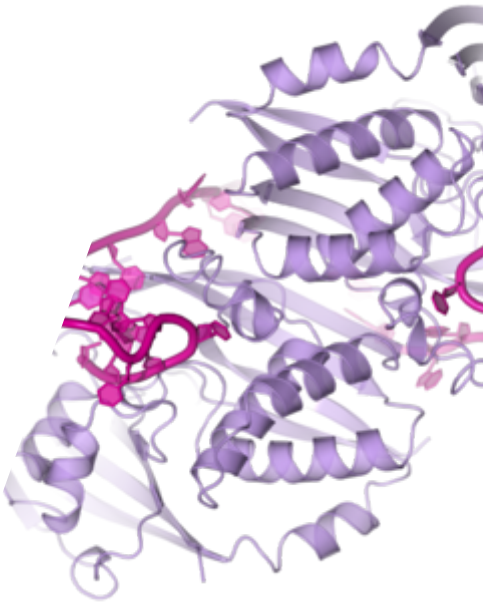

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Proteins

Proteins are large, highly complex and naturally occurring molecules can be found in all living organisms. A long chain of amino acids joined together by peptide bonds form proteins.

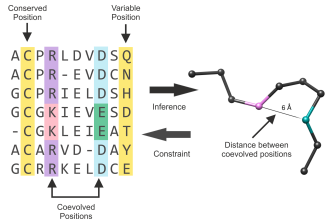

Figure: MSA

- We look for information in Multiple-sequence-Allignements (homologous sequences)
- It's difficult to get from a chain how the protein fold and function (limited data about secondary, tertiary etc. structure)

A lot of sequences of a protein are collected in a protein database. (ex: UniProt) stored in MSA.

MSA

Allignment of sequences that stores multicategorical variables (each element can assume 21 values)

Two datasets

- Natural sequences (1130)
- Artificial sequences (1003)

Data structure

- Data dimensionality: 96x20 dimensional data point
- Categories: **functional** or non-functional sequences

Figure: MSA

- **One-hot encoding** leads to use a 20-dimensional representation for each amminoacid, while the gap is mapped to the zero-vector.
- It blows up the feature vectors from L = 96 categorical variables to 20L = 1920 binary variables, but the numerical treatment is easier.

We construct a dictionary in order to rewrite the two matrices by changing the description of each amminoacid:

VARIABLE	CONTENT
seq_onehot_0	1130x1920 matrix of '0' and '1' for **natural** sequences
seq_onehot_1	1003x1920 matrix of '0' and '1' for **natural** sequences

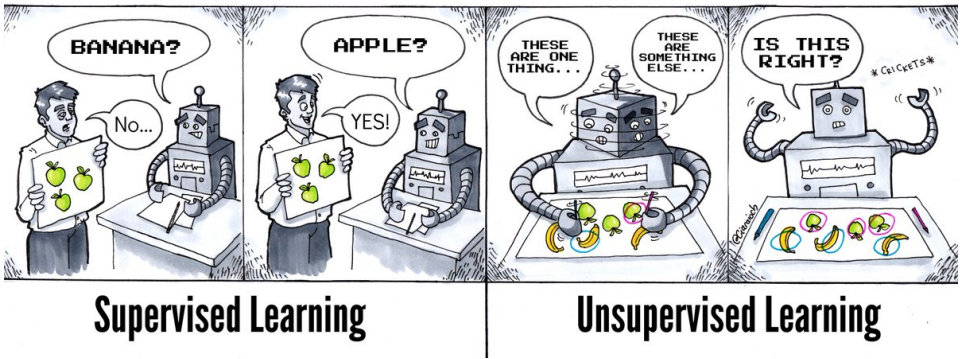

Figure: Supervised vs Unsupervised

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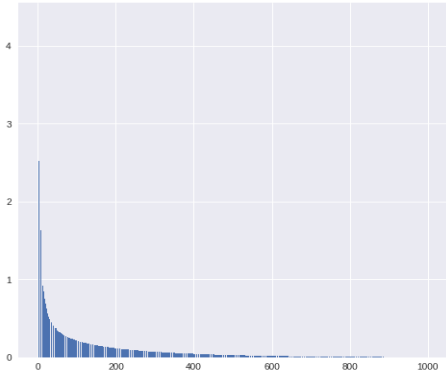
2 Dimensional reduction and data visualization

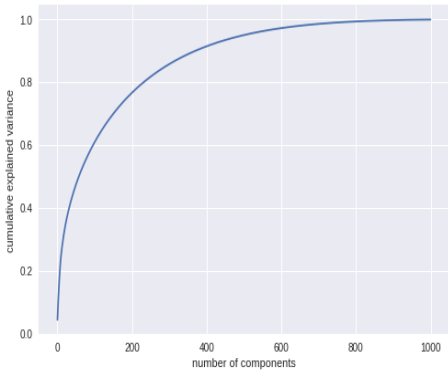
- **Curse of dimensionality:** large number of dimensions implies difficult in process the data and extract relevant information.
- **Dimensionality reduction:** reduce the complexity of the dataset via feature extraction

PCA

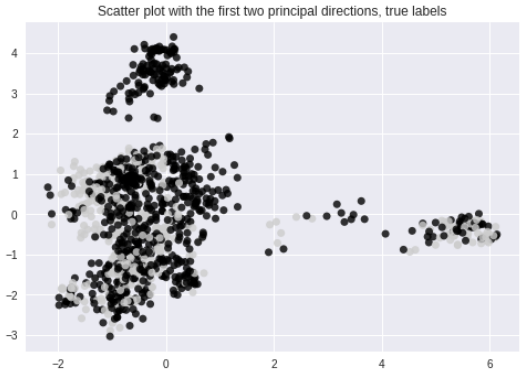
Principal Component Analysis aims to find the directions of maximum variance in high-dimensional data and projects it onto a new subspace with equal or fewer dimensions than the original one.

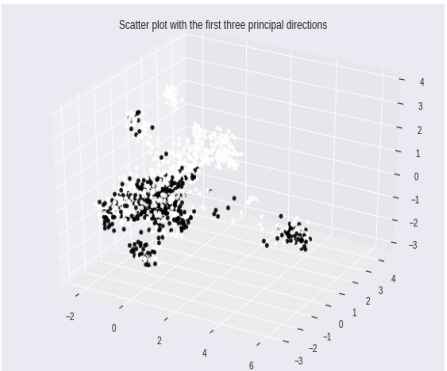
- **Goal:** to transform our dataset into one with a reduced dimensionality, without loosing too much information

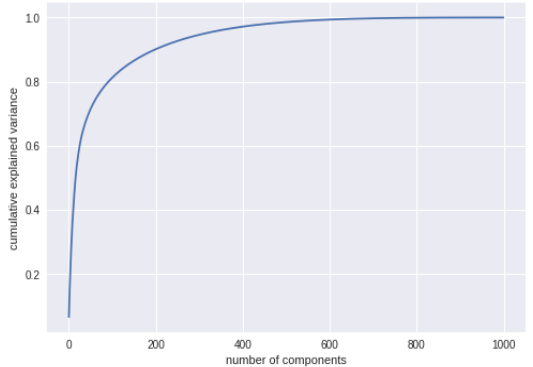




We just need around **370 features** in the new diagonalized and reduced sub-space instead of 1920 to keep almost all (90 percent) the information


Scatter plot with the first two principal directions, true labels
4
3
2
1
0
−1
−2
−3
−2
0
2
4
6

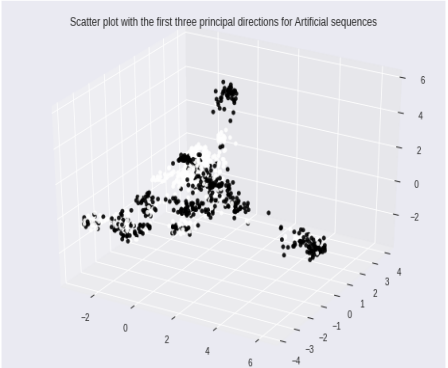

Scatter plot with the first three principal directions



In this case, one has to take around **200 dimensions** to keep 0.9 of the variance.

PCA - Artificial sequences

2 Dimensional reduction and data visualization



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3 Clustering

The aim of this task is now to perform **clustering** on the datasets. We want to see if the method is able to discriminate:

- Within the natural sequences, the functional from the non-functional ones.
- Within a concatenated dataset, the natural from the artificial ones.

Method: we are going to use K-Means clustering algorithm.

A **Cluster** is a collection of data points aggregated together because of certain similarities.

K-Means

The algorithm mainly performs two tasks:

- Determines the best value for K center points or centroids by an iterative process.
- Assigns each data point to its closest k-center. Those data points which are near to the particular k-center, create a cluster.

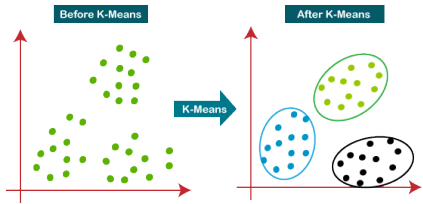
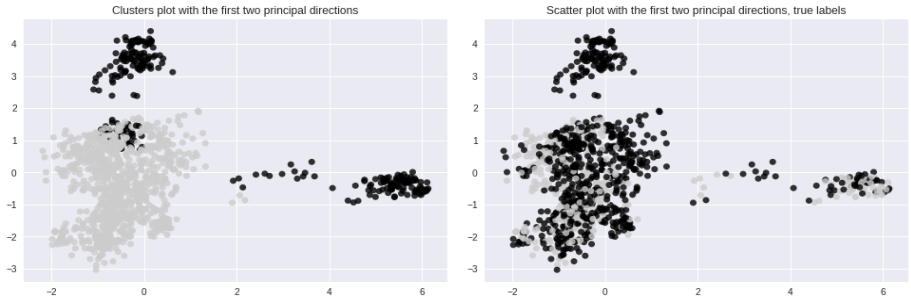


Figure: K-Means


Clusters plot with the first two principal directions
Scatter plot with the first two principal directions, true labels
4
3
2
1
0
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−2
−3
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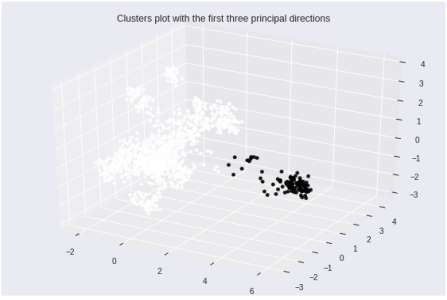
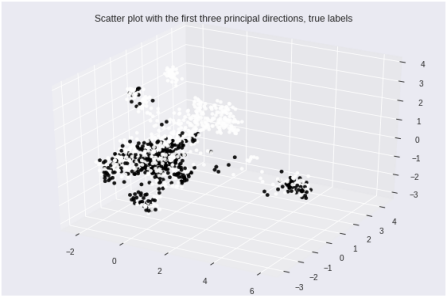
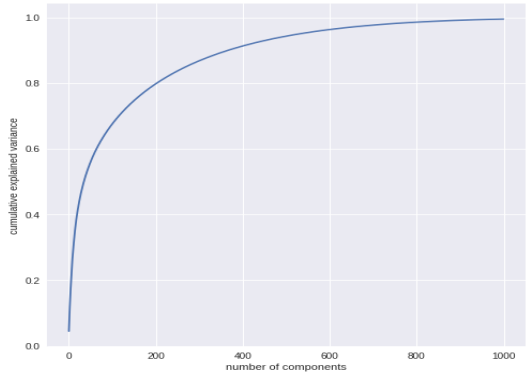
4
6





As we can see, the data don't seem in general to respect and follow the labels with two clusters,**functional and non functional sequences are not separated into different clusters**.

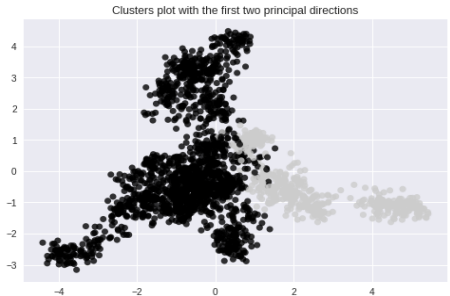
Doing PCA on the concatened set:

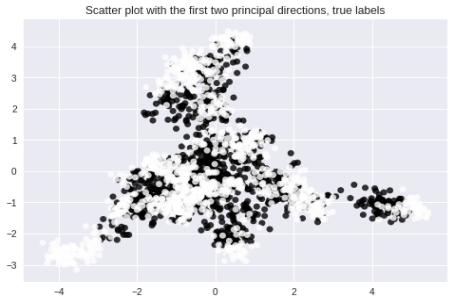


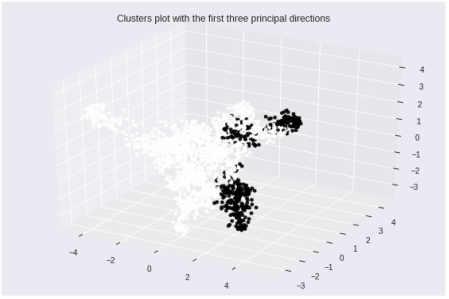
Hence, we need to keep around **360 dimensions** for this total set.

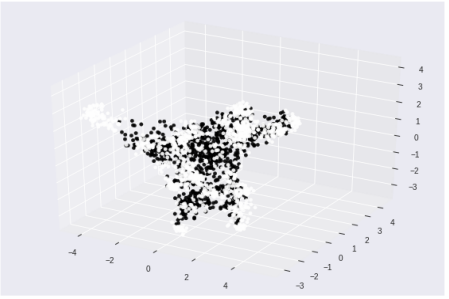
K-means on concatenated dataset

3 Clustering









Again, as we can see, the clustering algorithm was not able to catch the different features between **natural and artificial** thus, the two are not well discriminated.

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4 Predicting protein functionality

Idea: we focus now on a Supervised Learning task, for which we want to use a **classifier** in order to select as precisely as possible the correct label for our datapoints.
We are going to test several models:

- **Logistic Regression**
- Ensambling models
- K-NN

Splitting Data

- Training Set: 80 percent of the data
- Test Set: 20 percent of the data

Goals

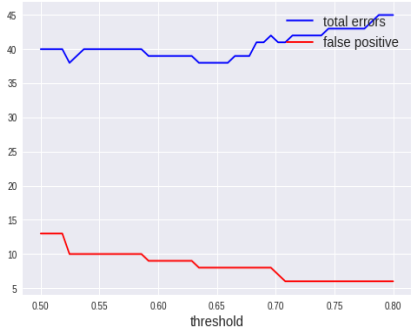
- Maximize Test score
- Avoid overfitting
- Keep the model as simple as possible

The score of the model results in:

- 99% on training set
- 73% on test set

With the following confusion matrix

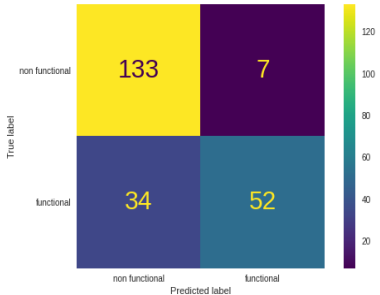
One can optimize the number of false positive and the total error changing the **treshold probability** for which the soft classifier gives a binary result.



The perfect trade-off seems to be around a treshold of **0.70**

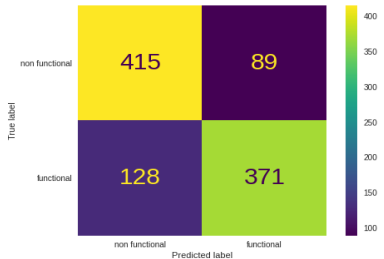
Setting this new treshold, we can see that the model will predict less false positive, increasing the average score of the model:

- Average score on test set 78.3%
- Std of the score 2.3%



One can now try to use the entire set of **natural** sequences as the **training** one, while the **artificial** sequences will be used as **test** set for the logistic model:
The score of the model results in:

- 99% on training set
- 78% on test set



One can ask how the performance depend on the **variability of the random state**, introduced necessarily everytime logistic regression is done, due to the splitting of the dataset. Making 100 iterations one gets:

- Average score 78.71%
- Std 2.44%

Higher than what we got without averaging over randomness.

How good the model perform under **k-cross validation**?
We will split the dataset into k subsets to see many possible train-validation combinations.

Using a **2-cross validation** one obtains:

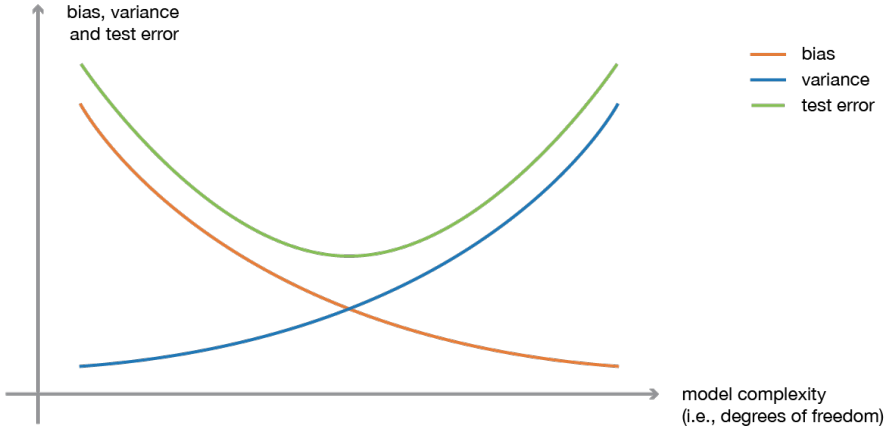
- score 0.756
- score 0.763

Whereas with a **10-cross validation** one obtains an average of 0.578, way lower than what expected!

Ensembling

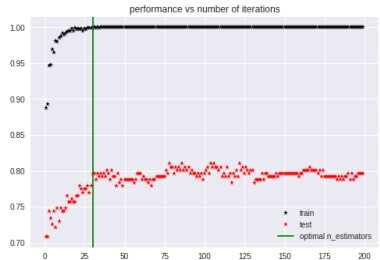
Ensembling strategies are ways to combine a certain number of weak classifier into a strong classifier.

- **What is a weak classifier?** A classifier whose prediction are weakly correlated to the real label (high-variance)
- **What is a strong classifier?** A good classifier (good bias-variance tradeoff)


bias, variance
and test error
bias
variance
test error
model complexity
(i.e., degrees of freedom)
underfitting
(high bias)
optimal
balance
overfitting
(high variance)

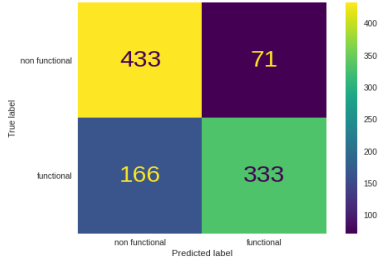
A **Random Forest** classifier is an ensemble method that gives as a result the majority vote between different decision trees

- We can measure the optimal number of trees to get the highest train and test score:



Giving the highest score of 79.6% when the number of estimators gets equal to **30**.

One can train the Random Forest classifier on a training set of the natural sequences, to then test it on the **total artificial dataset**. The confusion matrix looks like:



Evaluating the score now on the **test artificial set** this will be of **78.5**%

- Using **AdaBoost** as a strong classifier instead, the score has been evaluated to be of **77%**.
- Using **K-nn** with 20 neighbours and after PCA, the score has been evaluated to be of **73%**.

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5 Generating artificial sequences

Generative energy-based models are a class of models that are able to generate new data samples similar to a given training set. They work by learning an energy function that assigns a scalar value, called energy, to each possible configuration of the model's parameters and the input data.

Why?

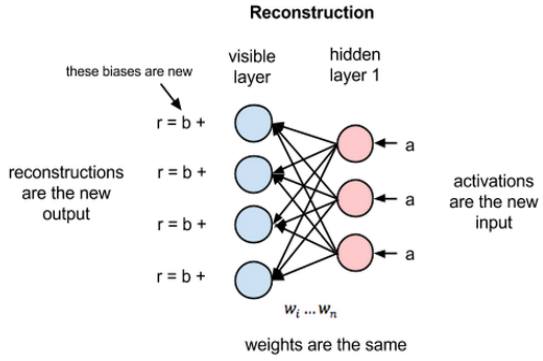
- By leveraging large datasets of known proteins, generative models can learn the patterns and rules governing protein folding
- to generate novel sequences that have never been seen before.

RBM

A Restricted Boltzmann Machine (RBM) is a popular choice for creating a generative model for protein sequences. RBMs are a type of generative stochastic artificial neural network that can learn to model the probability distribution of the input data.

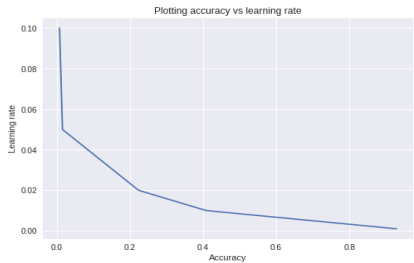
The RBM consists of two layers:

- **visible layer**, which represents the input sequences
- **hidden layer**, which captures the underlying patterns in the data.



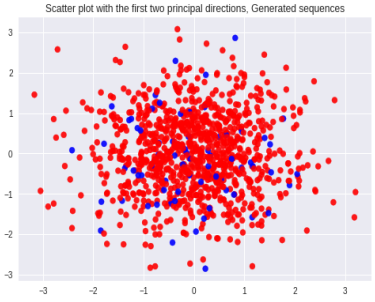
The two layers' variables are conditionally dependent on each other; in each iteration of **Gibbs sampling**, a sample from the hidden layer is generated given the current sample of the visible layer, and then a sample from the visible layer is generated given the updated sample of the hidden layer.

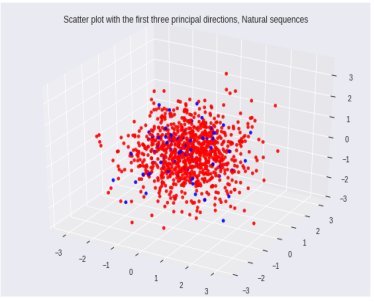
The velocity of convergence through gradient descent is led by the **learning rate**. One can play both with this and the **accuracy** (amount of proteins correctly predicted)



As we can see, an high learning rate leads to a small accuracy and viceversa, so we are going to use 21 hidden layers (as the number of important features should reflect the description of an aminoacid) and a learning rate of 0.001 to have a good accuracy.

The average prediction of the RBM on new proteins training the net with the natural sequences is 92.8%, which is an amazing result thanks to the parameters we chose. The prediction looks:





An amazing result... Let's discuss it

The model we chose is simple... only an hidden layer.
Q: Why this result then?

- Overfitting? Model has memorized the training data, and not generalizing well to new examples.
- Non variability? Maybe it is really good to produce just samples really close to a certain class without generalizing.

We could implement:

- Deep Belief Networks
- VAE
- GAN[1]

We could try to visualize data differently: (so far PCA... clustering)

- t-SNE (t-distributed Stochastic Neighbor Embedding)
- Kernel PCA

[1] ProteinGAN: A generative adversarial network that generates functional protein sequences

clearbox.ai

Me: I need more data

My generative model: Say no more

- “A fast learning algorithm for deep belief nets” G. Hinton, S. Osindero, Y.-W. Teh, 2006
- “Training Restricted Boltzmann Machines using Approximations to the Likelihood Gradient” T. Tieleman, 2008
- ”Inverse statistical physics of protein sequences: a key issues review” Simona Cocco, Christoph Feinauer, Matteo Figliuzzi, Rémi Monasson and Martin Weigt

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Thank you for listening!

Any questions?