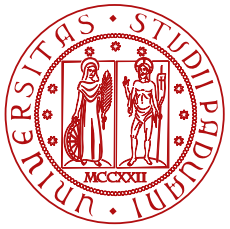


Università degli Studi di Padova

Dipartimento di Ingegneria dell'Informazione

Master Thesis in Computer Engineering

Protein Intrinsic Disorder Detection Based on Structural Features

Master Candidate

Alberto Crivellari

Student ID 2061934

Supervisor

Prof. Alexander Miguel Monzon

University of Padova

Co-supervisor

Prof. Damiano Piovesan

University of Padova

Academic Year
2022/2023

To my girlfriend,
my parents and friends.

Abstract

In this thesis we describe the work that I, Alberto Crivellari, along with the professors Damiano Piovesan and Alexander Monzon, carried out during my internship period. The work we have done can be summarized in analysing the existent software tool, developing a procedure to detect secondary structure in proteins, a procedure to compute relative solvent-accessibility (rsa) of amino acids and another procedure that allows to use a different file (*.fcz) as input file for the tool. Then we integrated all these procedures into the existent software tool. We finally analysed the results to evaluate their quality, by means of different plots and ML models.

More in detail, we studied and analysed the pre-existent software tool, AlphaFold-disorder, a Python application which makes predictions on protein structure using as input protein data files. As the name says, the main prediction regards disorder, but there is also a column of the table, which is the output, that contains statistics on binding between aminoacids. Actually under this software tools there are three predicting algorithms: AlphaFold-pLDDT, AlphaFold-rsa and AlphaFold-binding. The first two are algorithms that predict disorder, using respectively the statistics of pLDDT and the rsa, while the last one predicts binding.

For the first procedure, we developed an algorithm which detects the secondary structure of proteins by using distances and angles between aminoacids, calculated with protein atomic coordinates. The idea behind this method is explained in a scientific paper[3].

For the second procedure, we implemented a library to calculate aminoacids' solvent-accessibility using Shrake & Rupley algorithm. Then we obtained the relative measure by applying normalization factors.

For the last procedure, we implemented a library that allows to create, read, compress and decompress *.fcz files, which are compressed files for protein data. With this procedure we can use the compressed *.fcz files as input instead, allowing us to store large proteins' datasets with less space required.

Finally, we integrated these 3 procedures into the existing software tool and evaluated the results with various plots and ML models.

Contents

List of Figures

List of Tables

List of Algorithms

List of Code Snippets

List of Acronyms

NMR Nuclear Magnetic Resonance

DNA DeoxyriboNucleic Acid

RNA RiboNucleic Acid

ATP Adenosine TriPhospate

IDP Intrinsically Disordered Proteins

DSSP Define Secondary Structure Prediction

SASA Solvent Accessible Surface Areas

CLI Command Line Interface

CSV Comma Separated Values

TSV Tab Separated Values

PIP Package Installer for Python

CAID Critically Assessment of protein Intrinsic Disorder prediction

PDB Protein Data Bank

mmCIF MacroMolecular Crystallographic Information File

SS Secondary Structure

SSE Secondary Structure Elements

RSA Relative Solvent Accessibility

pLDDT predicted Local Distance Difference Test

P-SEA Protein Secondary Element Assignment

ML Machine Learning

LASSO Least Absolute Shrinkage and Selection Operator

SVM Support Vector Machines

ROC Receiver Operating Characteristic

1 Introduction

1.1 BioComputing UP

BioComputing UP is a research laboratory and is part of the Department of Biomedical Sciences of the University of Padua. The research there is focused on developing bioinformatic tools and high quality computational methods which are put to use to solve important biological issues. Its main fields are: structural biology, functional biology, genome wide analysis, genetic diseases and cancer studies.

1.2 Internship description

The internship project I carried on during the stage at BioComputing Up was mainly about expanding a pre-existent software tool, Alphafold-disorder, with newer libraries. In particular, my tasks were:

- Studying Alphafold-disorder code and related papers, to understand the scientific principles beneath;
- Implementing an algorithm which detects protein secondary structure, based on atomic distances and angles, calculated with protein atomic coordinates and the library BioPython. This algorithm was described in a paper [3];
- Integrating this secondary structure detection algorithm into the software tool Alphafold-disorder;

- Developing a procedure to compute the solvent-accessibility for each aminoacid in the protein, using the algorithm devised by Shrake and Rupley in 1973, by integrating a specific library;
- Integrating this procedure to compute solvent-accessibility into Alphafold-disorder;
- Integrating the library FoldComp and developing a quick procedure to allow input files of the type .fcz in Alphafold-disorder. FoldComp is a library that creates .fcz files as a compression of normal protein data files, .pdb files. FoldComp library is described in a scientific paper [2];
- Evaluating results through plots and machine learning models.

1.3 Chapters' description

This thesis is divided into chapters based on the 6 tasks I've accomplished in my internship.

This first chapter is just a brief introduction.

The second chapter is an overview on proteins, starting from the basics concepts of biology, with a focus on proteins with intrinsically disordered regions, to how computer science is applied in this field.

The third chapter is focused on the description of Alphafold-disorder and the relevant libraries involved, such as DSSP.

The fourth chapter describes the development and integration on the software tool of the procedures we worked on: a procedure to detect secondary structure detection, another procedure to compute solvent-accessibility and finally the implementation of the foldcomp library for reading .fcz files.

The fifth chapter describes the process of analysis of the results produced by the new software tool Alphafold-disorder (SASA) through plots and machine learning models, to evaluate the quality of the results produced.

In the sixth chapter, we will draw some conclusions based on the insights provided by the analysis with plots and ML models.

2

An Overview on Proteins

2.1 Basics of Molecular Biology

2.1.1 Cells

Life is made of cells, the fundamental working units of every living system. They are composed of :

- 70% of water;
- 23% of macromolecules, such as:
  - proteins;
  - polysaccharides.
- 7% of small molecules, such as:
  - lipids;
  - amino acids;
  - nucleotides.

Cells are the smallest structural unit of an organism capable of independent functioning.Each cell follows the same common cycle of birth, replication, protein synthesis and death.

Cells have common features in their structure, in the next page I will show a figure, representing the main components of a cell.

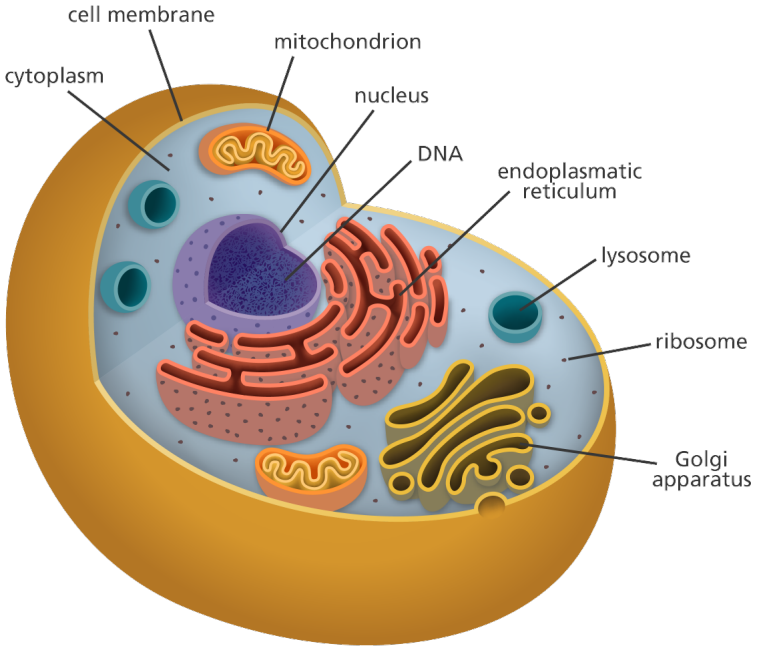


Figure 2.1: Cell structure

Let's analyse the main components among the ones we can see from the figure 2.1:

- **Nucleus**: it's the main component of the cell which contains the DNA, the genetic information of the individual. Around the nucleus there is the nucleus membrane, not annotated in the figure, that separates the nucleus from the cytoplasm;

- **Golgi apparatus**: or Golgi vesicles. Its purpose is to help processing and packaging the molecules, especially proteins that will be exported from the cell;

- **Mitochondria**: they are one of the most important cellular organelles, since they generate most of the chemical energy, the ATP, to power the cell's biochemical reactions. They generate the ATP through aerobic respiration;

- **Cytoplasm**: a gelatinous liquid that fills the inside of the cell, it's made of water, salts and various enzymes. It has many purposes, such as giving the cell a specific shape, protecting the cell organelles and assisting the cell's metabolic processes;

- **Cell membrane**: also called plasma membrane, is a semipermeable membrane that separates the interior of the cell from the outside environment. This membrane regulates the transport of molecules entering and exiting the cell, thanks to its semipermeability.

There are other elements we didn't describe in a cell, such as the endoplasmic reticulum and some cell organelles such as peroxisome, lysosome, ribosome, secretory vesicles and others.

2.1.2 DNA

The nucleus of the cell contains the DNA, which is the genetic information of the individual. It might slightly change between cells, especially among different types of cells, such as muscle cells and brain cells, since their functionalities are different.

Chromosomes

The genome is an organism's complete set of DNA, the human genome contains about 3 billion DNA base pairs and 24 distinct chromosomes.

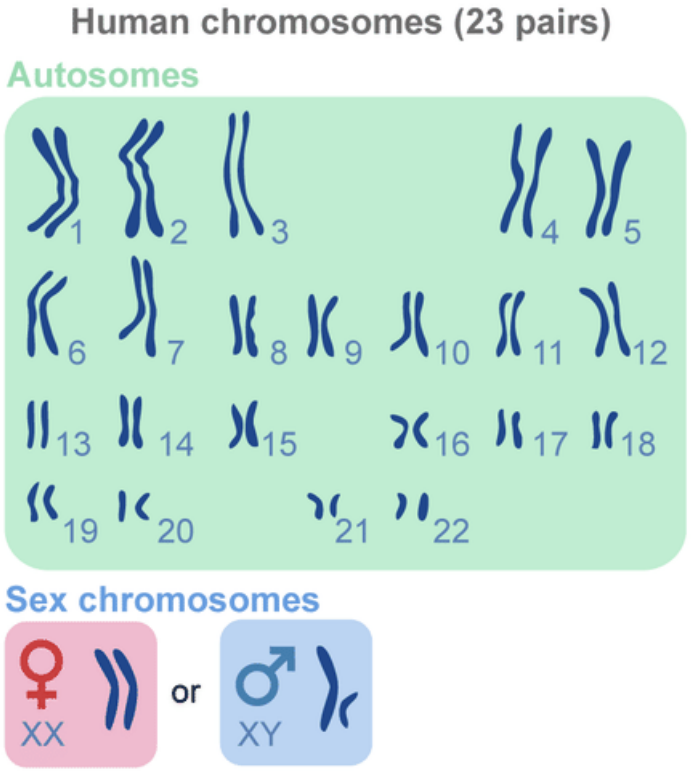


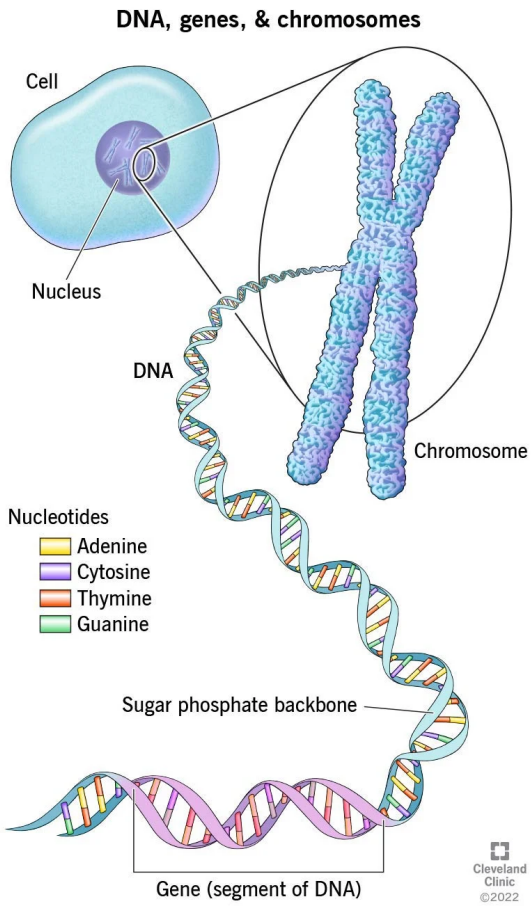
Figure 2.2: Chromosomes set in human genome

In figure 2.2 we can see the set of 24 chromosomes in the human genome. A cell normally contains 23 chromosomes, the 22 common ones, the autosomes,

plus one of the sex chromosomes, "XX" in females and "XY" in males.

Genes

Each chromosome contains many genes, the basic functional units of heredity. Genes are specific sequences of DNA that encode instructions on how to make proteins.



From the figure on the left we can see how genes, along with the DNA, fold into chromosomes, which will contain a really high density of DNA. Finally the chromosomes will be stored inside the nucleus of the cell.

Genes determine the principal hereditary stuff in the human being, from the height, to growth, muscular mass and appetite. Some of these elements are defined by a group of genes, others from just a singular gene. Mutations of some genes can modify the characteristics of a human and they can make alterations as harmless as a different eye color or they can result in more serious outcomes, like diseases. These mutations can also provide beneficial effects, such as immunity or protection for some diseases, for example a specific gene mutation is known for its protection against malaria.

Genes are vastly studied, especially their function in the DNA: some genes are highly related to diseases, such as:

- Huntington disease;
- Down syndrome;
- Cystic fibrosis;

- Albinism;
- various types of cancer.

DNA structure

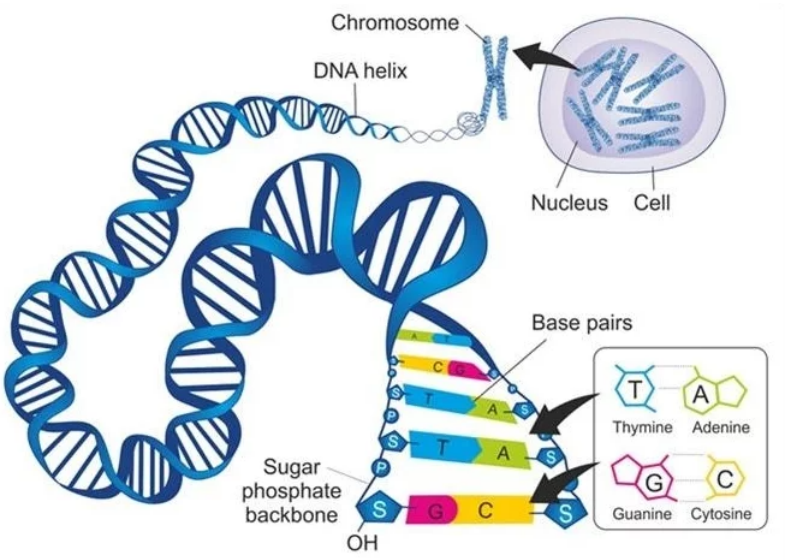


Figure 2.3: DNA structure

The figure 2.3 represents the DNA structure, a double helix macromolecule composed of nucleotides. A nucleotide is a molecule composed of:

- **Sugar phosphate backbone**: a molecule of sugar combined with a group phosphate;
- **Nitrogenous base**: there are four possible bases in DNA:
  - Adenine;
  - Thymine;
  - Guanine;
  - Cytosine.

The nucleotides pair up with the nucleotides of the opposite helix, via the nitrogenous base: the only possible pairs are Adenine-Thymine and Guanine-Cytosine.

The DNA can replicate itself or can be used to produce proteins, which are particular molecules that participate in most of the essential processes of the human body:

- building and repairing body structures;
- digesting nutrients;
- hormones: some hormones are proteins or protein-derived. Hormones are chemical messengers that flow through blood to coordinate different body's functions;
- executing various metabolic functions, or assisting the execution.

We will now see more in detail this process from DNA to proteins, called protein synthesis.

2.1.3 Central Dogma of Biology

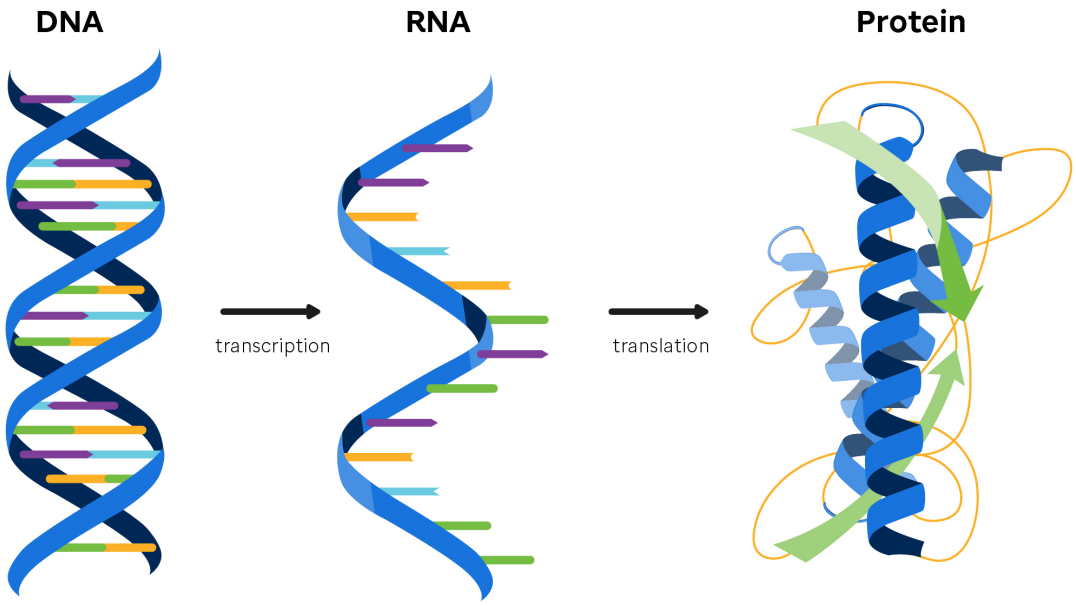


Figure 2.4: Central Dogma of Biology

The figure 2.4 represents the Central Dogma of Biology, which explains the flow of genetic information within a biological system: from DNA to RNA to protein.

Transcription

Transcription is the process of copying a segment of DNA into RNA. An enzyme called RNA polymerase produces an RNA strand, by passing through a DNA strand. The resulting RNA is the copy of the coding strand of the "input" DNA , while the strand used as template to create this copy is called template strand.

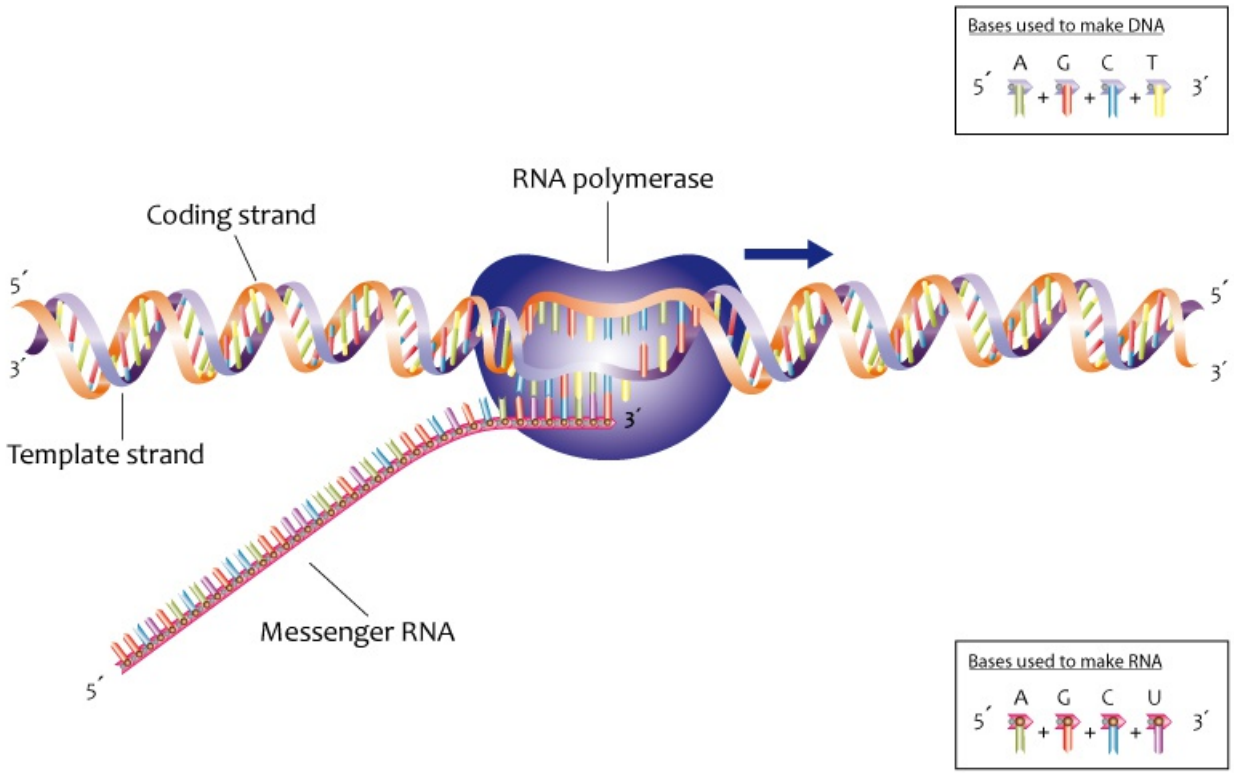


Figure 2.5: Central Dogma of Biology

In the figure above, we can see how RNA polymerase creates the RNA. We can note that in the figure the RNA is called messenger RNA, also known as **mRNA**.

There are other two types of RNA, tRNA and rRNA. Let's see the differences:

- **mRNA**: messenger RNA, contains the genetic information from the DNA. mRNA specifies ;

- **tRNA**: transfer RNA, transfer the correct amino acid to the ribosome. It acts as a bridge between the mRNA and the ribosome;
- **rRNA**: ribosomal RNA, combined with ribosomal proteins creates the ribosome.

Translation

Translation is the process by which the genetic information from the DNA is converted into a functional protein. It's also known as protein synthesis.

The ribosome reads the following three nucleotides of the mRNA, this triplet is the **codon**, then the ribosome brings in the correct tRNA. The correct tRNA is the tRNA, whose triplet, the **anticodon**, matches the actual triplet of the mRNA. Once we have a matched tRNA, the tRNA transfers the amino acid to the growing protein chain of the ribosome.

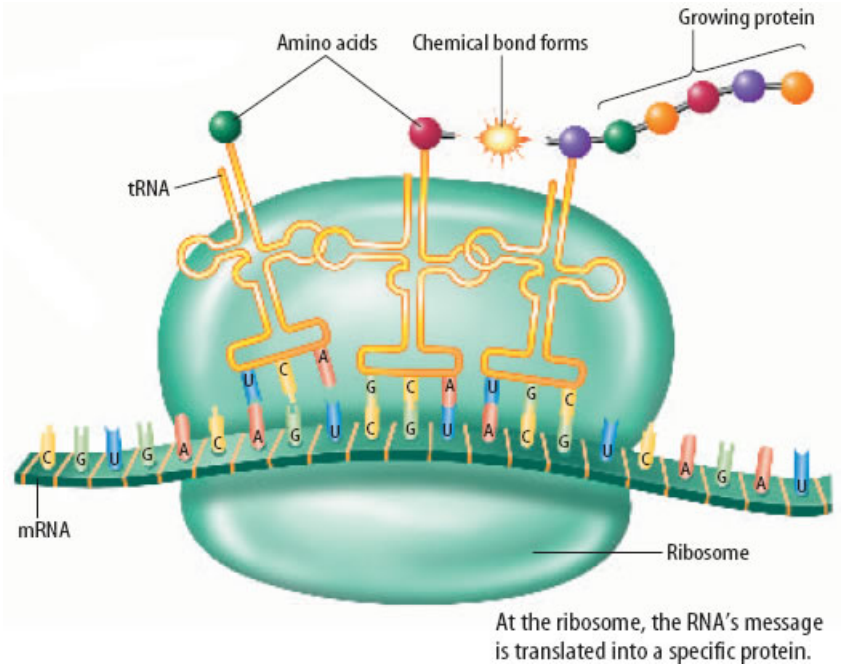


Figure 2.6: Protein Synthesization

In figure 2.6 we can see how the ribosome, along with tRNAs, identifies the correct amino acid and transfers it into the growing protein, through a chemical bond.

In the figure 2.7 above, we can observe the Genetic Code: the set of triplets of nucleotides, codons and the corresponding amino acid.

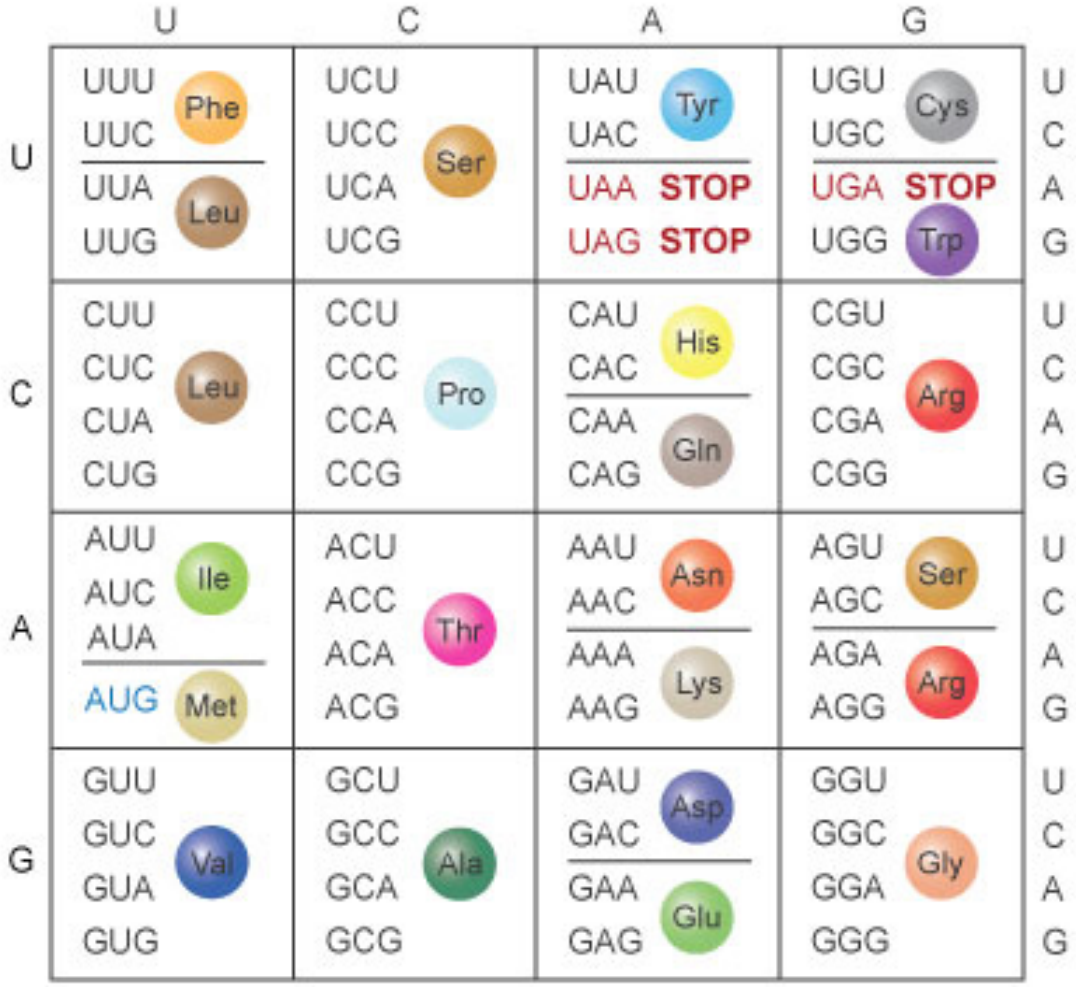


Figure 2.7: Genetic code

We can observe 4 particular combinations of nucleotides that correspond to signal of start and stop the protein synthesization:

- The codon **AUG** identifies the **START** signal for the protein translation;
- The codons **UAA, UAG, UGA** identify the **STOP** signal, which ends the translation process producing the final protein.

2.1.4 Proteins

Proteins are large, complex molecules made up of amino acids, smaller subunits also referred to as residues. The residues are the building blocks of a macromolecule, so in this case the residues are the incorporated amino acids. Proteins are linear chains of different combinations of 20 different amino acids.

Proteins' Functions

- Make up the cellular structure;
- Form body's major components, such as skin and hairs;
- Form hormones to communicate with other cells;
- Form enzymes to regulate gene activity.

The functions of proteins depend both on the amino acids' order and types and on the three-dimensional structure the protein folds into.

Protein Folding

Proteins tend to fold into lowest energy three-dimensional conformation. They already begin to fold already when the amino acid chain is being formed during translation.

Different amino acids have different chemical properties and by interacting with each other the protein starts to fold adopting its functional structure.

The structure of the protein is really important for the protein's function:

- Determines which amino acids are exposed outside the protein;
- Determines which substrates the protein can react with.

Substrates are molecules or compounds that participate in a chemical reaction, they are starting materials or reactants which are acted upon by enzymes or catalysts.

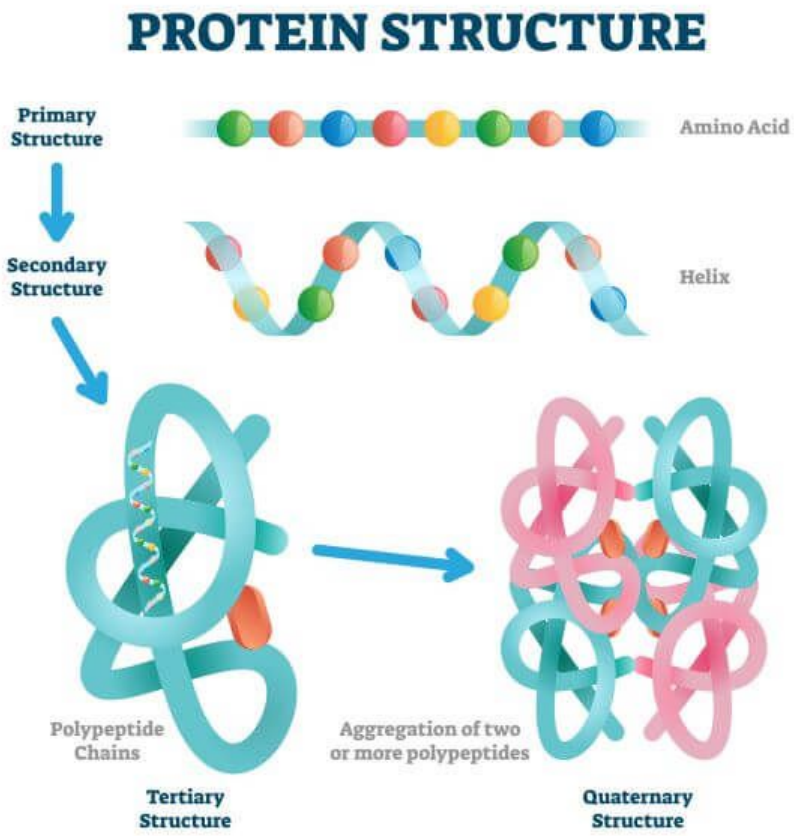


Figure 2.8: Structure of proteins

From figure 2.8 we can see the four types of structures in a protein:

- **Primary structure**: simply the sequence of amino acids;
- **Secondary structure**: local structural patterns formed by residues;
- **Tertiary structure**: the global structure of the peptide chain;
- **Quaternary structure**: the global structure and aggregation of the various peptide chains in the protein.

The main two types of secondary structures are :

- **Alpha-helix**: proteins bury most of their hydrophobic residues in the interior core, forming a spiral structure resembling an helical spring;
- **Beta-sheets**: segments of the protein are stretched out and aligned in a sheet-like arrangement.

Amino Acids

Lastly, let's talk about the amino acids. Amino acids are monomers[1] of proteins, each amino acid has a specific chemical behavior. All amino acids in the human genetic code have a carboxyl group (-COOH) and an amino group (-NHH) bound to the central carbon atom.

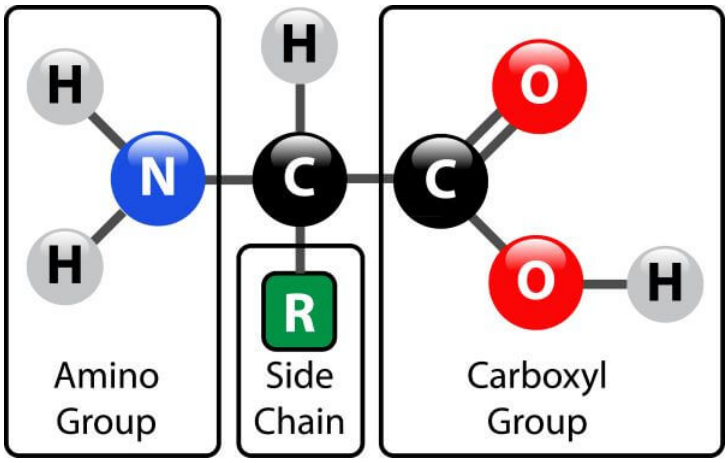


Figure 2.9: Structure of amino acids

In the figure above, we can observe the general structure of amino acids in the human genetic code.

The carboxyl group and the amino group are the same for each one of the 20 amino acids, they differ for the side chain. Side chains differ in these 3 features :

- three-dimensional structure;
- electric charge;
- hydrophobicity.

Amino acids are mainly classified by hydrophobicity :

- hydrophobic amino acids repel water, they are also called non-polar amino acids.
- hydrophilic amino acids are attracted to water, they are also called polar amino acids.

[1]A monomer in molecular biology is a molecule that can bond with other monomers to create a macromolecule.

Other classifications take into account the structure, functionality or electrical charge of the amino acid. The latter can be either uncharged, positively charged or negatively charged. Some other classifications are based on the particularity of the side chains, such as Sulfur-Containing. Sulfur-Containing amino acids, as the name suggests, contain sulfur.

Full Name	**Abbreviation (3 Letters)**	**Abbreviation (1 Letter)**	**Polarity**
Glycine	Gly	G	Non-Polar
Alanine	Ala	A	
Valine	Val	V	
Leucine	Leu	L	
Isoleucine	Ile	I	
Methionine	Met	M	
Phenylalanine	Phe	F	
Tryptophan	Trp	W	
Proline	Pro	P	
Serine	Ser	S	Polar
Threonine	Thr	T	
Cysteine	Cys	C	
Tyrosine	Tyr	Y	
Asparagine	Asn	N	
Glutamine	Gln	Q	
Aspartic Acid	Asp	D	
Glutamic Acid	Glu	E	
Lysine	Lys	K	
Arginine	Arg	R	
Histidine	His	H	

Table 2.1: List of Amino Acids

In the table 2.1, we can see the list of the 20 amino acids in the Genetic Code with :

- Polarity;
- Three letters abbreviation;
- One letter abbreviation.

To check which triplet of nucleotides corresponds to each amino acid in the Genetic Code, we can go back to figure 2.7.

2.1.5 Tandem Repeat Proteins

Tandem repeat proteins are the product of minimal folds from the repetition of simpler units. Their buried residues are more conserved, with a large surface and a high sequence variability.

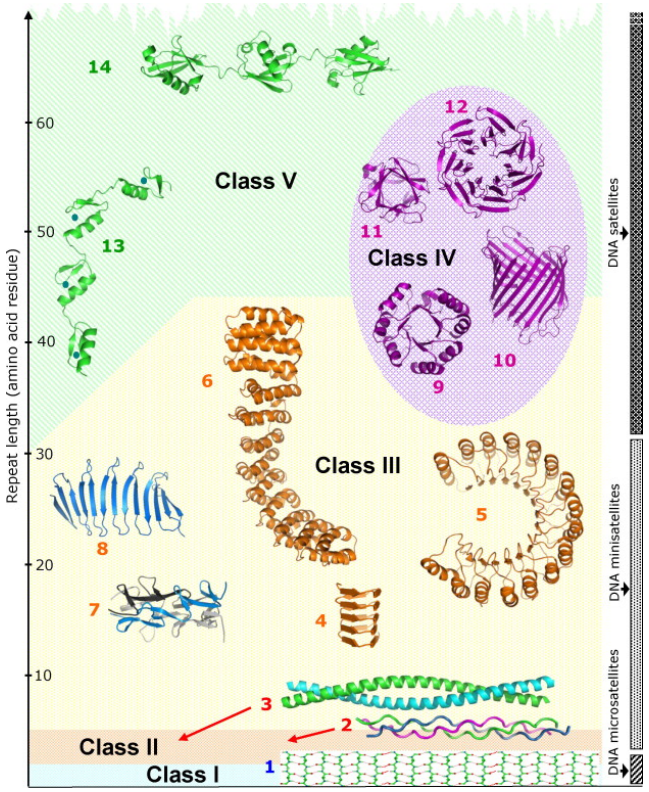


Figure 2.10: Tandem Repeat Proteins

From the figure 2.11 we can see what tandem repeat proteins look like. We can also observe how they are divided into classes; tandem repeat proteins are classified according to periodicity in 5 classes:

- Class I: Aggregates;
- Class II: Collagen and Coiled-coils;
- Class III: Solenoids;
- Class IV: Toroids;
- Class V: Beads on a string.

2.1.6 Intrinsically Disordered Proteins

A polypeptide chain can be classified as different types of proteins, such as:

- Membrane proteins;
- Globular proteins;
- Tandem repeat proteins;
- Intrinsically disordered proteins.

We already saw Tandem Repeat Proteins, now we will explore Intrinsically Disordered Proteins.

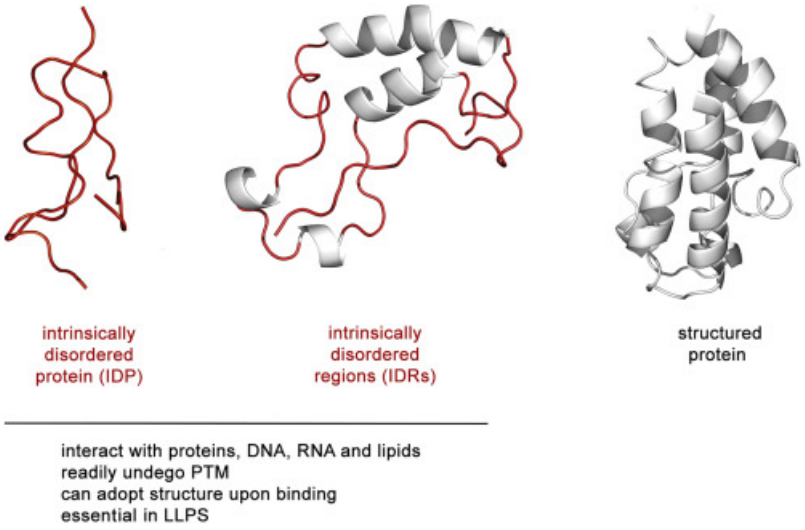


Figure 2.11: Intrinsically Disordered Proteins

In disordered proteins the cost of folding is higher and so the protein has a lesser degree of freedom in folding.

It has complex surfaces which means high specificity with low versatility. For specificity we mean that when 2 proteins fit together, we need 2 highly specific protein structures to fit together, since their shape is so complex, an average protein can't be a proper fit.

Low versatility is the opposite: since the shape is quite complex, it doesn't fit together with most proteins, hence it's not versatile.

Due to their complex structures, disordered proteins are less prone to environmental stress: they can preserve their function in unstable conditions, such as high temperature.

In cell regulation around 25% of proteins are disordered, those are involved in highly dynamic and complex processes that require proteins with high specificity.

IDPs (Intrinsically Disordered Proteins) are really interesting to study, because they are implicated in several pathologies and due to their different functions they are involved in:

- Regulatory functions;
- Central role in the assembly of macromolecular machines, such as ribosomes;
- Transport of molecules through nuclear pore;
- Binding: IDPs can participate in one-to-many and many-to-many interactions, where one IDP region binds to multiple molecules, potentially gaining very different structures in the bound state.

They are also implicated in several pathological conditions, like cancer, cardiovascular diseases, and neurodegenerative diseases.

Some of the first classes of IDPs were notable due to their pathological roles in neurodegenerative diseases.

2.2 Computational Biology: Computer Science Applied to Biology

The growing amount of data in the field of Molecular Biology brought the scientists to exploit Computer Science. Computers can process data way faster than human beings, and especially in this field, where we have so much information on DNA, genes and proteins, it's really useful to compute and process these pieces of informations faster and possibly with less errors.

This new field takes the name of Bioinformatics, the union of Biology, Computer Science and Statistics. Statistics is needed because most of the information isn't 100% reliable, hence statistics methods are used. The main fields of bioinformatics are:

- **Structural data analysis**: its purpose is to predict the three-dimensional structures of proteins, nucleic acids and other biological molecules. Understanding the structure of these molecules can provide insights into their functions and interactions;

- **Omics data analysis**: for example genomics, proteomics, metagenomics, epigenomics and so on. Omics data is a broad term that refers to large-scale datasets generated from various biological technologies. These large-scale datasets enable researchers to study different aspects of biological systems, often on a global or comprehensive scale. For example, in genomics analysis, we have large-scale datasets focused on genome data, so we can analyze DNA sequences and genes organization and functions.

In this thesis the focus will be on structural data analysis, since the software tool I talk about analyzes the three-dimensional structure to obtain information on the disorder or binding propensity.

2.2.1 Structural Data

Let's start off with the basic macromolecular structure data, down below we can see a figure that represents structure in the different macromolecules.

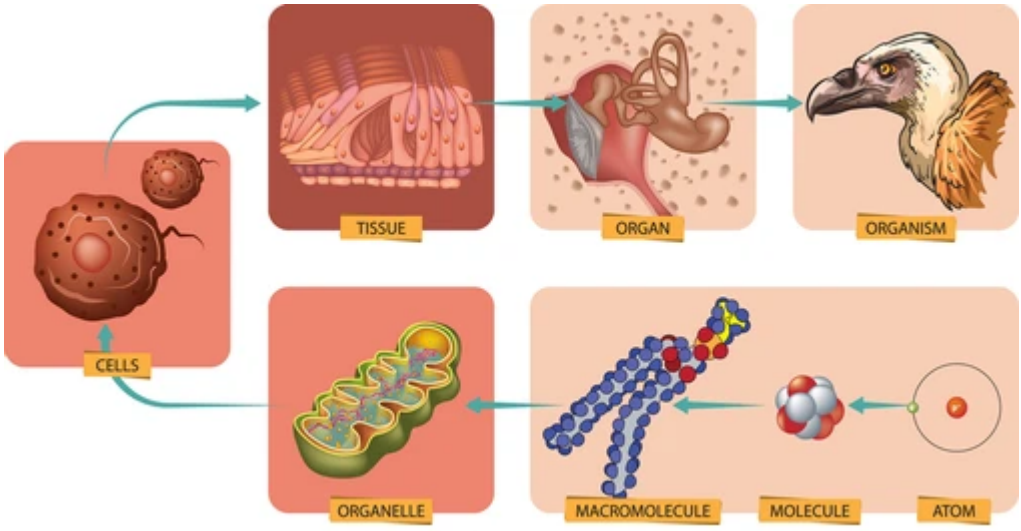


Figure 2.12: Atom to macromolecules to organism

In figure 2.12 we can see the various steps from molecules to macromolecules to bigger complexes up to the final organism.

The passage between molecules to macromolecules, can be observed more in detail in the following figure.

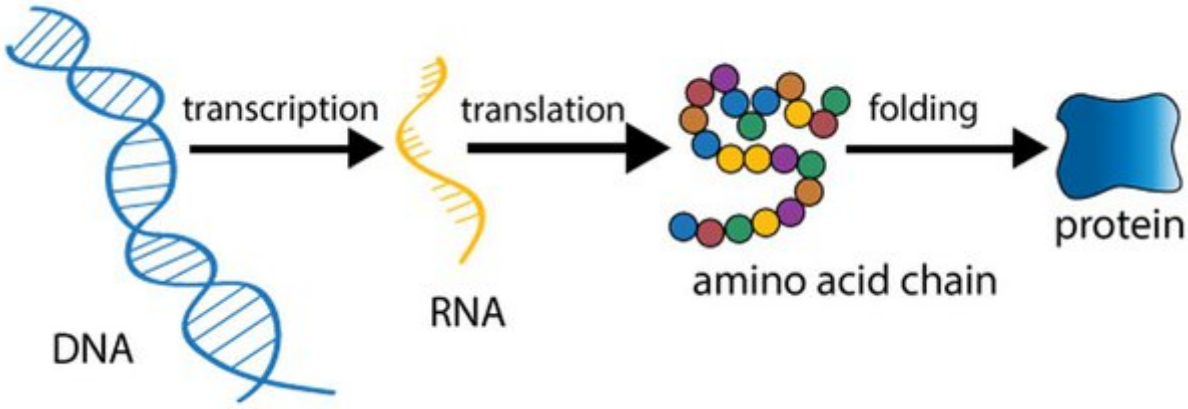


Figure 2.13: Atom to macromolecules to organism

In figure 2.13 we can see the passages and we can observe each structure from DNA to RNA to peptide chains to protein. We already explained earlier the phases of transcription, translation and folding in chapter 2.1.3.

We can use structural data for:

- Structure prediction;

- Predicting protein interactions: protein folding, binding, protein assemblies;
- Structure comparison: by comparing the moolecules' structure we can determine to which species it belongs to and where in the evolutionary scale it stands;
- Exploring mechanisms of interaction with ligands: metabolites, drug compounds, DNA and others.

From molecular data we get information on coordinates and then we want to obtain some kind of knowledge, for example:

- Mutation X disrupts the function of enzyme Y which causes disease Z.

"Coordinates by themselves just specify shape and are not necessarily of intrinsic biological value, unless they can be related to other information"
Integrative database analysis in structural genomics, Mark Gerstein, Nature Structural Biology

There are many databases that store information on structural data for many proteins or other macromolecules, some of them also contains sequence data or metabolic pathways data. Sequence data are just data on DNA so the sequences, while metabolic pathways are related to which pathway a given gene activate.

Down below we can see a picture of most databases for bioinformatics data.

Figure 2.14: PDB-derived databases

These are all PDB-derived databases, since PDB (Protein Data Bank) was one of the first to try and build up an archive on protein structural data, and so all these databases are derived from the PDB archive.

Lastly, we can see a figure representing the most used biological technologies for obtaining structural data.

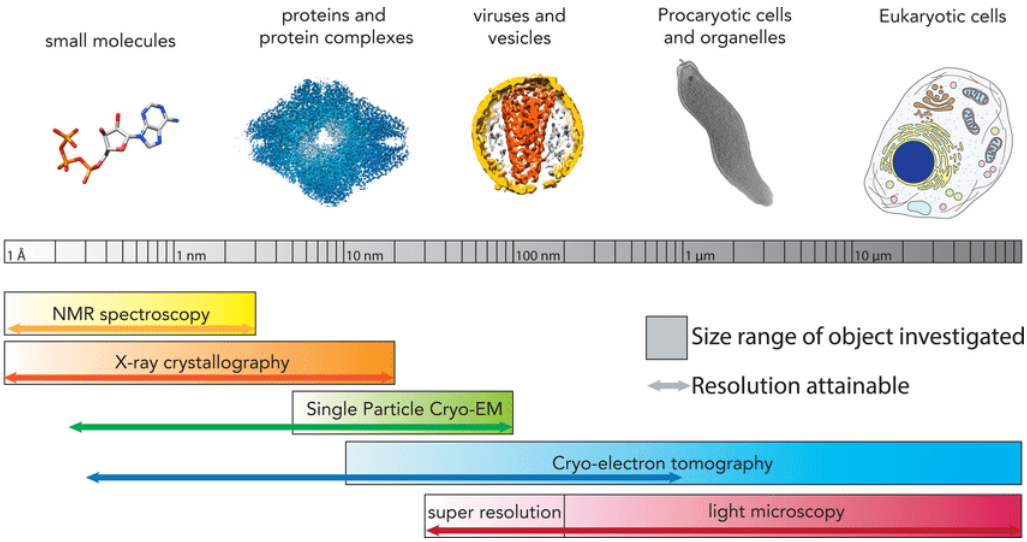


Figure 2.15: Biological technologies for obtaining structural data

In our case, for protein chains and residues, the most interesting biological technologies are the first two from left:

- X-ray crystallography;
- NMR spectroscopy.

To close up this chapter I will talk about one of the most important libraries in bioinformatics: BioPython.

2.2.2 BioPython

Regarding Computer Science the main languages used for bioinformatics are:

- R;
- Matlab;
- Python.

For python there is the library BioPython, which is a library containing classes, functions and modules for the various necessities in bioinformatics.

There are a lot of subpackages under the package Bio of BioPython, in our case we are just interested in 3 of them:

- **Bio.PDB**: contains classes that deal with macromolecular crystal structures. In particular it includes PDB and mmCIF parsers, the DSSP wrapper[2], the SASA module and the Structure class;
- **Bio.Data**: collection of useful biological data, for example in our case we use it for the normalization factors for RSA;
- **Bio.SeqUtils**: contains mhelper functions to deal with sequences.

In particular from Bio.PDB we use the classes:

- *PDBParser*: as the name suggests, it's a class which reads a .pdb file and saves it into a variable of the type Structure class;
- *MMCIFParser*: similar to PDBParser, but for .mmCIF files;
- *DSSP*: the python wrapper for the software tool DSSP;
- *SASA*: a class for calculating solvent accessibility;
- *Polypeptide*: a class with helper functions to deal with protein chains, in particular we use the function **is_aa()** to see if a string is an identifier for an amino acid.

On the other hand the Bio.Data subpackage has been used just for the normalization factors for SASA, the dictionary *residue_sasa_scales*.

[2]A wrapper is a class or procedure that translates a library's existing interface into a compatible interface, it "wraps" the underlying library. It's often used to enable cross-language, in this case the wrapper enables the c++ library "DSSP" to be used in Python.

And Bio.SeqUtils has been used for the helper function *Seq1*, which converts a sequence of amino acids with three-letters code into a sequence of amino acids with one-letter code.

For more information on BioPython and its subpackages and submodules, here is the link to the documentation.

3

Analysis of AlphaFold-disorder

3.1 Introduction

AlphaFold-disorder is a software tool developed by my co-supervisor Damiano Piovesan, my co-supervisor Alexander Miguel Monzon and other members of the BioComputing UP laboratory. The objective of the software tool is to predict disorder and binding of the amino acids with high accuracy.

The web application CAID (Critical Assessment of Protein Intrinsic Disorder), also developed by members of the BioComputing UP laboratory, was used to measure the quality of the software tool prediction.

In particular 3 algorithms within the tool were measured:

- **AlphaFold-pLDDT**: uses pLDDT to predict disorder values;
- **AlphaFold-rsa**: uses pLDDT and RSA statistics to predict disorder values;
- **AlphaFold-binding**: uses pLDDT and RSA statistics to predict binding values.

Each of these algorithms produces a table containing binding, disorder and other statistics for every amino acid, or residue[1].

The professors and researchers of the laboratory published a scientific paper[4] regarding this software tool: Intrinsic protein disorder and conditional folding in AlphaFoldDB.

[1]In molecular biology, a residue refers to a monomer within a polymeric chain. In the case of a protein, a residue refers to an aminoacid.

In this chapter we will see more in detail the CAID web application and the 3 algorithms within AlphaFold-disorder.

3.1.1 Disorder

Disorder, in the context of Molecular Biology of proteins, refers to how "out-of-order" is the structure of a part of the protein, usually a region. Two of the three algorithms inside the software tool predict the disorder propensity of a certain residue, amino acid.

This disorder propensity is a probability for that residue of being part of a disordered region. Disordered regions are quite important to study, because, as we talked about in the previous chapter, proteins with intrinsically disordered regions are involved in many functions and pathologies.

3.1.2 Binding

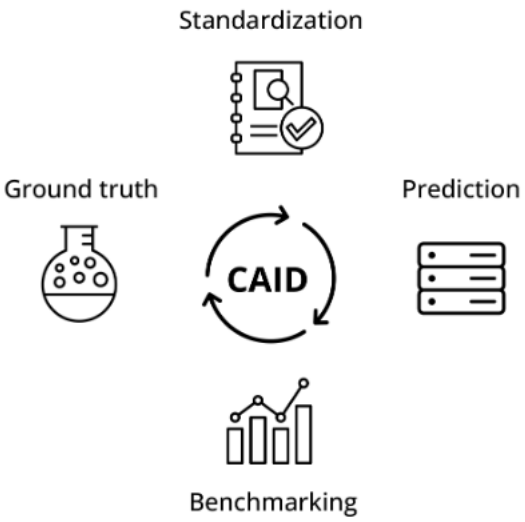
The other algorithm within the software tool is a predictor for binding, which means it predicts a binding propensity for each residue, namely the probability for that residue to bind with some ligand.

This binding propensity can overlap with disorder propensity, since many disordered regions tend to have higher binding activity.

3.2 CAID

CAID, Critical Assessment of Protein Intrinsic Disorder prediction, is a web application established in 2018 to determine the state-of-art for predicting disorder and binding on IDRs, Intrinsic Disordered Regions.

The idea is to compare different softwares for this kind of prediction: each of them assigns to every residue of the protein the propensity for that residue to be intrinsically disordered. Accuracy of predictions are evaluated by means of ground truths, obtained by a reference, in this case the reference is the DisProt database. On CAID both software runtimes and accuracy of predictions are compared.



Here we can see a picture representing the CAID cycle: Ground Truth, Standardization, Prediction and Benchmarking. Let's analyze them more in detail :

- **Ground Truth**: DisProt database was selected as reference because it contains a large number of manually curated disorder and binding annotations at a protein level. DisProt annotates association between intrinsically disordered regions and a biological function.
- **Standardization**: Participants submit their softwares, and the CAID organizers encapsulate code into containers, providing standardization across different machines. This also makes it easier to deploy them and to package softwares with dependencies.
- **Prediction**: Running the containers implemented in the Standardization step on the Ground Truth targets generates predictions. The cluster used by CAID can execute all methods in parallel within some resource constraints (90 GB RAM, 48 threads, 4 Hours per sequence).
- **Benchmarking**: The performances of the various methods are evaluated by a number of metrics. CAID identifies the confidence threshold to optimize the performance for a specific metric. In benchmarking and runtimes pages are reported some of the assessment results.

The 3 algorithms we mentioned earlier, AlphaFold-pLDDT, AlphaFold-rsa and AlphaFold-binding, are already present in their containers on the CAID application.

3.3 AlphaFold-pLDDT

This predictor is provided by the software tool AlphaFold-disorder. It's a predictor for disorder, which means that it predicts the propensity for each

residue to be intrinsically disordered.

Its predictions are based on the pLDDT, predicted Local Distance Difference Test, which is already present in .pdb files from AlphaFold predictions as the B-factor. This algorithm just obtains a statistic representing disorder propensity for that residue, out of the B-factor.

AlphaFold-pLDDT uses "1 - pLDDT" as disorder propensity.

The optimal classification threshold found with CAID is 0.312, representing pLDDT <68.8 %, this threshold was selected by maximizing the F1-Score performance on the CAID DisProt dataset.

3.4 AlphaFold-rsa

This predictor is provided by the software tool AlphaFold-disorder. This tool, as the last one we talked about, is also a predictor for disorder.

On this predictor, the disorder propensity is calculated with the relative solvent accessibility, RSA, over a local window centered on the residue whose disorder propensity we want to predict. DSSP was used to obtain the relative solvent accessibility and the optimal local window, of 25 residues, was chosen with a grid search in the range of 1 to 50 residues.

The optimal classification threshold for AlphaFold-rsa is 0.581, which means RSA < 41.9 %. Again the threshold was selected by maximizing F1-Score performance on the CAID DisProt dataset.

3.5 AlphaFold-binding

Finally we have the last predictor in the software tool AlphaFold-disorder. This is a binding predictor, which means that it predicts a statistic that represents the propensity of a residue to bind.

This predictor makes use of both the pLDDT and the RSA calculated for the other 2 predictors: in the scientific paper [4] that describes the software tool AlphaFold-disorder, we can see the relation between RSA, pLDDT and this binding propensity.

$$AlphaFold\_Bind(T) = \begin{cases} AlphaFold\_RSA, \\ \quad AlphaFold\_RSA \leq T \\ T + \rho LDDT(1-T), \\ \quad AlphaFold\_RSA > T \end{cases}$$

That T represents a threshold, obtained again from maximizing the F1-Score on the CAID DisProt dataset. The optimal threshold found with CAID is 0.773.

Below 0.773 the binding propensity is the value from AlphaFold-rsa(T); above 0.773 we use the pLDDT score to compute the binding propensity.

3.6 Analysis of the Code

Let's quickly see the most important points in the code of this software tool. The main points are:

1. Parsing of input parameters;
2. Parsing of input file(s);
3. Extraction of pLDDT and RSA for each residue;
4. Computation of predictions;
5. Creation of output files.

3.6.1 Parsing Input Parameters

To parse input they used the library parse_args along with Path and PurePath of the library pathlib.

```
    import argparse
    from pathlib import Path, PurePath
```

Code 3.1: import libraries parsing

Parse args it's used to parse parameters given in terminal, when using the software tool. These are the main parameters:

- **--in-struct (-i)**: the input file(s). Either a single file, folder or file listing containing the relative paths of .PDB or .mmCIF files;
- **--out (-o)**: name for the output file(s).
- **--format (-f)**: format for the output file, it can be either "tsv" or "caid";
- **-dssp**: the path for the mkdssp executable, by default is just the alias "mkdssp" which is needed to be setted in the environment variables;
- **-ll**: log level, it can be one of the following: "notset", "debug", "info", "warning", "error", "critical".

```
def parse_args():
    parent_parser = argparse.ArgumentParser(add_help=False)

    group = parent_parser.add_mutually_exclusive_group(required=True)
    group.add_argument('-i', '--in_struct', type=str,
            help='A single file, folder or file listing
            containing (gzipped) PDB or mmCIF files (relative paths)'
            )
[...]
    parent_parser.add_argument('-ll', type=str, choices=['notset',
        'debug','info','warning','error','critical'],
        default='info', help='Log level')
    main_parser = argparse.ArgumentParser(parents=[parent_parser])
    return main_parser.parse_args()
```

Code 3.2: Command-line arguments parsing.

3.6.2 Parsing Input Files

First of all the library pathlib is employed to obtain the path of the input file(s) and then we have 4 possibilities, identified through if-else 's :

- **one protein file**: so either a ".pdb" file, a ".pdb.gz", ".cif" or ".cif.gz" file;
- **list of files**;
- **a directory**: each protein file within this directory is processed;
- **non-protein files** are recognized and not processed.

```
if __name__ == '__main__':

[...]

    p = Path(args.in_struct)
    # args.in_struct is the CLI parameter for input files
    if p.is_file():
        # input is a single struct file or file with list
        if ''.join(PurePath(p).suffixes) in ['.pdb', '.pdb.gz',
        '.cif', '.cif.gz'] :
            #process single file as input
            processed_data = process_file(p)
[...]
        else:
            # process list of files as input
            with open(p, 'r') as list_file:
                for file in list_file:
                    real_file = Path(p.parent, Path(file.strip()))
                    processed_data = process_file(real_file)
[...]
    else:
        # input is a directory
        start_T = time.time()
        for file in p.iterdir():
            if ''.join(PurePath(p).suffixes) in ['.pdb', '.pdb.gz',
            '.cif', '.cif.gz'] :
                #process every file in the directory
                processed_data = process_file(p)
```

Code 3.3: Input files parsing

3.6.3 Extraction of Residues' Statistics

This part is implemented under the main procedure "process_pdb". To implement this procedure a few libraries have been used: the main ones are BioPython and pandas. The input parameters for the procedure are three:

- **pdb_file**: the actual protein file, so the path to that file;
- **pdb_name**: the name of the file;
- **dssp_path**: the path to the mkdssp executable.

Description of the procedure

The procedure can be divided in 4 steps :

1. **Load structure**: the protein file is loaded and parsed into a proper data structure: "Bio.PDB.Structure", a class in BioPython that represents a macromolecular structure. This parsing is done through "Bio.PDB.PDBParser" if it's a .pdb file or with "Bio.PDB.FastMMCIFParser" if it's a .mmCIF file.

2. **DSSP invocation**: DSSP is a software tool used to obtain interesting statistics for each residue. The software tool is invoked through a Python wrapper[2] class: "Bio.PDB.DSSP" provided by BioPython.

3. **Extraction of statistics**: Finally the statistics of interests are extracted by iterating the residues. The statistics of interests are 3:

    1. **pLDDT**: obtainable without DSSP, from the B-factor value already present in the .pdb file;
    2. **RSA**: it represents how much the solvent can access this residue. It's provided by DSSP;
    3. **SS**: secondary structure of the residue. Also provided by DSSP.

4. **Save statistics in a DataFrame**: Finally the statistics of interest are stored in a DataFrame, a class provided by the library "pandas". Dataframe is the most popular way to manage a table and easily write it in a ".csv" or ".tsv" file.

[2]A wrapper is a class or procedure that translates a library's existing interface into a compatible interface, it "wraps" the underlying library. It's often used to enable cross-language, in this case the wrapper enables the c++ library "DSSP" to be used in Python.

Code snippet of the procedure

For more information on DSSP there is the original scientific paper [1] and the updated repository of github: DSSP. Down below we can see an adjusted code snippet to illustrate how this extraction of statistics for each residue works. It's adjusted for the purpose of showing it in this thesis.

```
import pandas as pd
from Bio.PDB import PDBParser, DSSP
from Bio.PDB.MMCIFParser import FastMMCIFParser
from Bio.SeqUtils import seq1
[...]
def process_pdb(pdb_file, pdb_name, dssp_path='mkdssp'):
[...]
    # Load the structure
    file_ext = Path(rel_file).suffix
    if file_ext in ['.pdb']:
        structure = PDBParser().get_structure('', real_file)
    else:
        # assume mmCIF
        structure = FastMMCIFParser().get_structure(''. real_file)
[...]
    # Calculate DSSP. WARNING Check the path of mkdssp
    dssp_dict = dict(DSSP(structure[0], real_file, dssp=dssp_path))
[...]
    # Parse b-factor (pLDDT) and DSSP statistics of interest
    df = []
    for i, residue in enumerate(structure.get_residues()):
        lddt = residue['CA'].get_bfactor() / 100.0
        rsa = float(dssp_dict.get((residue.get_full_id()[2],
residue.id))[3])
        ss = dssp_dict.get((residue.get_full_id()[2], residue.id))[2]
        df.append((pdb_name, i + 1, seq1(residue.get_resname()),
lddt, 1 - lddt, rsa, ss))
    df = pd.DataFrame(df, columns=['name', 'pos', 'aa', 'lddt',
'disorder', 'rsa', 'ss'])
    return df
```

Code 3.4: Extraction residues' statistics

3.6.4 Computation of Predictions

We already have the predictions of AlphaFold-pLDDT, here we will describe the procedure for predictions of the other 2 algorithms: AlphaFold-RSA and AlphaFold-Binding.

The procedure is called *make_prediction* and it asks as input three parameters:

- **df**: dataframe containing the data on residue from DSSP;
- **window_rsa**: the window of residues to consider when calculating rsa;
- **thresholds_rsa**: the threshold of rsa that represents a high solvent-accessibility.

The last 2 parameters have a default value, based on empirical tests: 25 residues for the window_rsa and 0.581 for the thresholds_rsa.

The default value for window_rsa was found with grid search, with various numbers for the parameter, 25 was the best one. While for thresholds_rsa, the default value was obtained by maximizing the F1 score, on CAID.

Both in window_rsa and in thresholds_rsa we can have multiple values, for an easier comparison, the parameters are in fact lists of values.

By default this procedure adds to the dataframe 2 columns, one for AlphaFold-RSA disorder prediction and one for AlphaFold-Binding binding prediction. With more than elements in the parameter lists of window_rsa and thresholds_rsa we will have more columns.

Let's now analyze the code of the procedure.

```
def make_prediction(df, window_rsa=[25], thresholds_rsa=[0.581]):
    for w in window_rsa:
        column_rsa_window='disorder-{}'.format(w)
        half_w = int((w-1)/2)
        tmp_pad = np.pad(df['rsa'], (half_w, half_w), 'reflect')
        # running mean of array tmp_pad, with window: half_w*2 +1
        df[column_rsa_window] = moving_average(tmp_pad, half_w*2 +1)

        for th_rsa in thresholds_rsa:
            column_rsa_binding = 'binding-{}-{}'.format(w, th_rsa)
            df[column_rsa_binding] = df[column_rsa_window].copy()
            df.loc[df[column_rsa_window]>th_rsa,column_rsa_binding] =
df.loc[df[column_rsa_window]>th_rsa,'lddt'] * ((1-th_rsa)+th_rsa)
```

Code 3.5: Procedure make_prediction()

At line number 6 a procedure moving_average() is invoked: the procedure uses the NumPy library to compute the moving average of an array, using convolution.

While on lines 11 and 12 we have some complex computation that uses the thresholds_rsa to transform the scores.

3.6.5 Creation of Output Files

Finally the data obtained from DSSP and the predictions computed are saved in 2 .tsv files. Actually the first .tsv file is done before the procedure make_prediction, since it doesn't include the predictions' columns. For simplicity of explanation I preferred to talk about it in the same part as for the 2nd output file.

This creation of output files is incorporated in the main procedure, using the function "to_csv" provided by the Pandas library.

Let's explore the code.

```
if __name__ == '__main__':
    fout_path = Path(args.out)
[...]
    data = data.append(processed_data)
[...]
    fout_name = '{}/{}_data.tsv'.format(fout_path.parent, fout_path.
   stem)
    # First .tsv file
    data.to_csv(fout_name, sep='\t', quoting=csv.QUOTE_NONE, index=
   False, float_format='%.3f')
[...]
    for name, pdb_data in data.groupby('name'):
        pred = pred.append(make_prediction(pdb_data.copy(),
   window_rsa=args.rsa_window,
            thresholds_rsa=args.rsa_threshold))
[...]
    fout_name = '{}/{}_pred.tsv'.format(fout_path.parent, fout_path.
   stem)
    pred.to_csv(fout_name, sep='\t', quoting=csv.QUOTE_NONE, index=
   False, float_format='%.3f')
```

Code 3.6: Creation of output files

fout_path is the information of name and path provided as parameter in the invocation of the software tool from shell, obtained form the parameter .out of

the result from the output data structure of parse_args procedure, seen in Code Snippet 3.1. data is the DataFrame obtained from the procedure process_pdb, seen in Code Snippet 3.4. It contains the data from the protein files and their statistics obtained with DSSP. pred is the DataFrame obtained from the procedure make_prediction, seen in Code Snippet 3.5. It contains the predictions computed in that procedure and the data from protein files and DSSP.

The two files obtained are .tsv files, they can be opened with every text editor, but to be able to see the table correctly we should open it with Microsoft Excel or the alternatives provided by Google or LibreOffice.

Here we can see a screenshot representing the file of predictions in .tsv opened with LibreOffice Calc.

	A	B	C	D	E	F	G	H	I
1	name	pos	aa	lddt	disorder	rsa	ss	disorder-25	binding-25-0.581
2	109m	1	M	0.299	0.701	0.198	C	0.098	0.098
3	109m	2	V	0.254	0.746	0.362	C	0.089	0.089
4	109m	3	L	0.196	0.803	0.021	C	0.088	0.088
5	109m	4	S	0.171	0.829	0.086	C	0.09	0.09
6	109m	5	E	0.163	0.837	0.149	H	0.099	0.099
7	109m	6	G	0.159	0.841	0.031	H	0.092	0.092
8	109m	7	E	0.134	0.866	0.016	H	0.099	0.099
9	109m	8	W	0.127	0.873	0	H	0.109	0.109
10	109m	9	Q	0.123	0.877	0.169	H	0.109	0.109
11	109m	10	L	0.12	0.88	0.038	H	0.109	0.109
12	109m	11	V	0.11	0.89	0	H	0.109	0.109
13	109m	12	L	0.12	0.88	0.019	H	0.108	0.108
14	109m	13	H	0.12	0.88	0.239	H	0.093	0.093

Figure 3.1: An example of .tsv output file of AlphaFold-disorder

3.7 Usage of AlphaFold-disorder software tool

3.7.1 Dependencies

In order to use AlphaFold-disorder, we first need :

- the actual Python script **alphafold_disorder.py**;
- at least one protein file, in format **.pdb**, **.mmCIF**, **.pdb.gz** or **.mmCIF.gz**;
- the DSSP executable: **mkdssp**;
- the following libraries: **BioPython**, **Numpy** and **Pandas**.

3.7.2 Usage

To execute the software tool we can write the following command in the CLI:

```
python3 AlphaFold-disorder -i pdbs/ -o output
```

Instead of "output" we can write the name for the output file that we desire, and instead of "pdbs/" we can give as input a protein file, a directory containing protein files or a file with the relative paths of protein files.

3.7.3 DSSP Installation

There are two ways to install DSSP:

1. Download the pre-compiled file from the latest release in GitHub;
2. Build the the source file from the GitHub repository.

To build the source file we have to install and build a few libraries, but it's all well-document in the readme.md file of the DSSP repository.

3.7.4 Python Libraries Installation

To install the 2 Python libraries required we need **Python3** and **pip3**, Package Installer for Python. Then to install the 2 Python libraries we just have to execute the following commands from the CLI:

- **BioPython**: pip3 install biopython;
- **Numpy**: pip3 install numpy;
- **Pandas**: pip3 install pandas.

4

AlphaFold-disorder (SASA): Development of Procedures

In this chapter we will talk about the actual development part of the thesis. We worked on three procedures:

- **Detection of secondary structure using protein atomic coordinates**: development of a procedure that assigns to each residue its secondary structure. It's based on P-SEA algorithm, described in this scientific paper[3];
- **Computation of RSA, using Shrake-Rupley algorithm**: We calculated SASA using Shrake-Rupley algorithm and then computed RSA with normalization factors;
- **Implementation of FoldComp library**: for reading protein files in .fcz, which is a compression of .pdb files.

Let's analyse the process of development for each of these procedure.

4.1 Secondary Structure Detection with Protein Atomic Coordinates

4.1.1 Introduction

In the scientific paper of P-SEA, the researchers suggest an algorithm based on protein atomic coordinates of the central carbon atom of the residue, the α-Carbon. This algorithm assign the secondary structure to a residue r, based

on distances and angles between the α-carbon of the residue and the α-carbon of its neighbor residues.

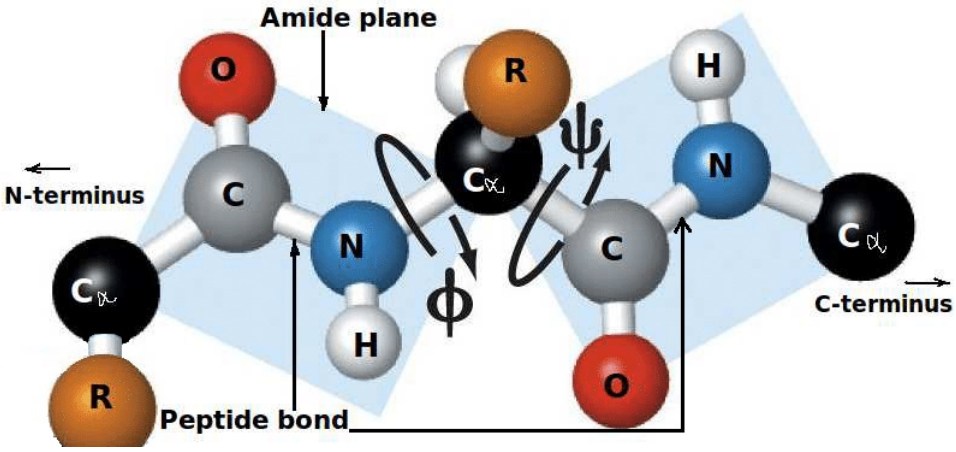


Figure 4.1: α-Carbon atoms in a protein

α-Carbon atoms are the core of residues, in the figure 4.1 we can see them in a piece of a protein chain.

Now back to the P-SEA algorithm: for each residue i, we want to calculate these distance measures:

- d2i: it's the distance between the residue (i-1) and the residue (i+1);
- d3i: it's the distance between the residue (i-1) and the residue (i+2);
- d4i: it's the distance between the residue (i-1) and the residue (i+3);

And these angle measures:

- τi: the angle formed by the residues (i-1), i and (i+1);
- αi: the dihedral angle formed by the residues (i-1), i, (i+1) and (i+2).

Distances and angles are computed between the α-carbons of bonded residues. Specific range of values in d2, d3, d4, τi, αi determine in which secondary structure that residue falls into, among the main three categories:

- **Helices**;
- **Strands**: such as beta-sheets parallel and anti-parallel;
- **Coils**: turns and loops;

The thresholds for belonging in one of the two non coil secondary structure are shown in the table 4.1.

Let's see how we implemented the idea of algorithm discussed on the paper[3].

Parameters	Helix	Strand
Angle τ (°)	89 ± 12	124 ± 14
Dihedral angle α (°)	50 ± 20	-170 ± 45
Distance d2 (Å)	5.5 ± 0.5	6.7 ± 0.6
Distance d3 (Å)	5.3 ± 0.5	9.9 ± 0.9
Distance d4 (Å)	6.4 ± 0.6	12.4 ± 1.1

Table 4.1: Parameters for P-SEA assignment of secondary structure.

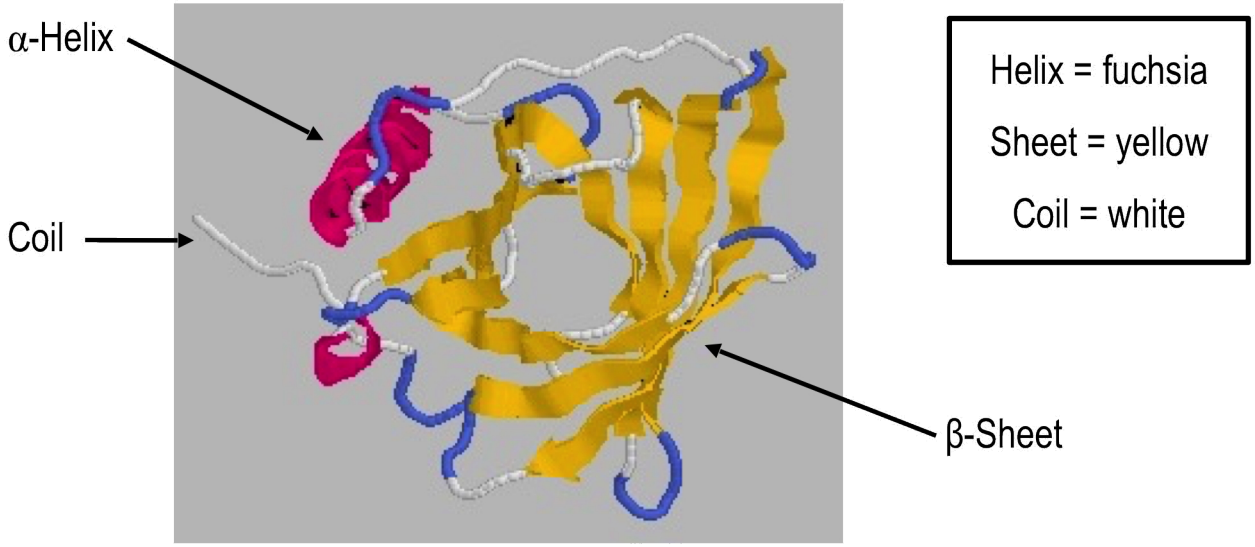


Figure 4.2: The threee secondary structure P-SEA can identify

4.1.2 Implementation of PSEA procedure

Let's explore how we implemented the PSEA algorithm in a procedure. Let's first analyze the various steps required from the algorithm:

1. Get the coordinates of the residue's α-carbon atom, for each residue in the protein;
2. Compute distances and angles for each residue, as described in the paper;
3. Assign to each residue it's secondary structure, based on computed distances and angles.

We will analyze these steps one by one in this section.

Get α-carbon atom's coordinates for each residue

In this first step we want to obtain the list of residues in the protein and then for each residue we want the coordinates of its α-carbon.

We store them in a matrix with 3 columns and as many rows as the number of residues. In each row we store the 3 coordinates of the α-carbon atom.

The basic idea would be to:

- Obtain the list of atoms from the protein chain;
- Obtain the list of α-carbon atoms from the whole list of atoms;
- For each α-carbon atom, obtain its residue with the BioPython function "get_parent()";
- Store the coordinates of that α-carbon atom in the row of that residue, in the matrix.

Algorithm 1 Pseudocode for extracting α-carbons coordinates

```
Require: struct, a protein structure
  list_atoms = struct.get_atoms()
  res_start_ids = get_res_start()
  list_ca = empty list of size "len(res_start_ids)"
  for atom in list_atoms do
    if atom.name = "CA" then
      list_ca.append(atom)
    end if
  end for
  matrix_coordinates = empty matrix of size "len(res_start_ids) x 3" {Res start
  ids are long the number of residues in the protein chain, and 3 because we
  have 3 coordinates: x,y,z.}
  for ca in list_ca do
    matrix_coordinates.append(ca.coordinates)
  end for
```

This is the basic pseudo-code for this step. Although internally, the code is more optimized for computational purposes: we used NumPy methods to use the residues starting indexes more efficiently to create the α-carbon coordinates' matrix.

Compute distances and angles between α-carbon atoms

Now we have a list of α-carbon atoms, with their coordinates. The next step described in the paper is computing distances and angles between these atoms, to assign the correct secondary structure.

For each row of the matrix, which represent the residue's α-carbon atom, compute the 5 measures:

- **d2**: distance between atom at row i and atom at row i+2;
- **d3**: distance between atom at row i and atom at row i+3;
- **d4**: distance between atom at row i and atom at row i+4;
- angle τ: angle between three consecutive atoms: at rows i, i+1 and i+2;
- angle α: angle between four consecutive atoms: at rows i, i+1, i+2 and i+3.

I will show with a few pictures what distances and those 2 angles looks like, in the case of 5 consecutive α-carbons.

For simplicity the α-carbon are directly linked, but in reality they have a lot of atoms between them, atoms that aren't α-carbon.

Let's start from the angle τ.

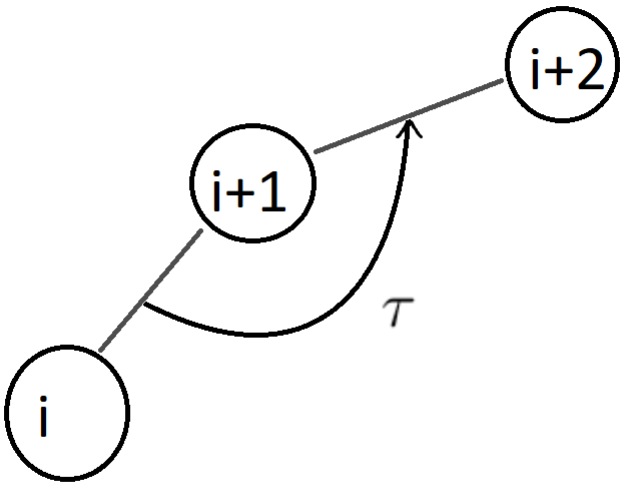


Figure 4.3: Angle τ

And then we have the 3 distances: d2, d3 and d4.

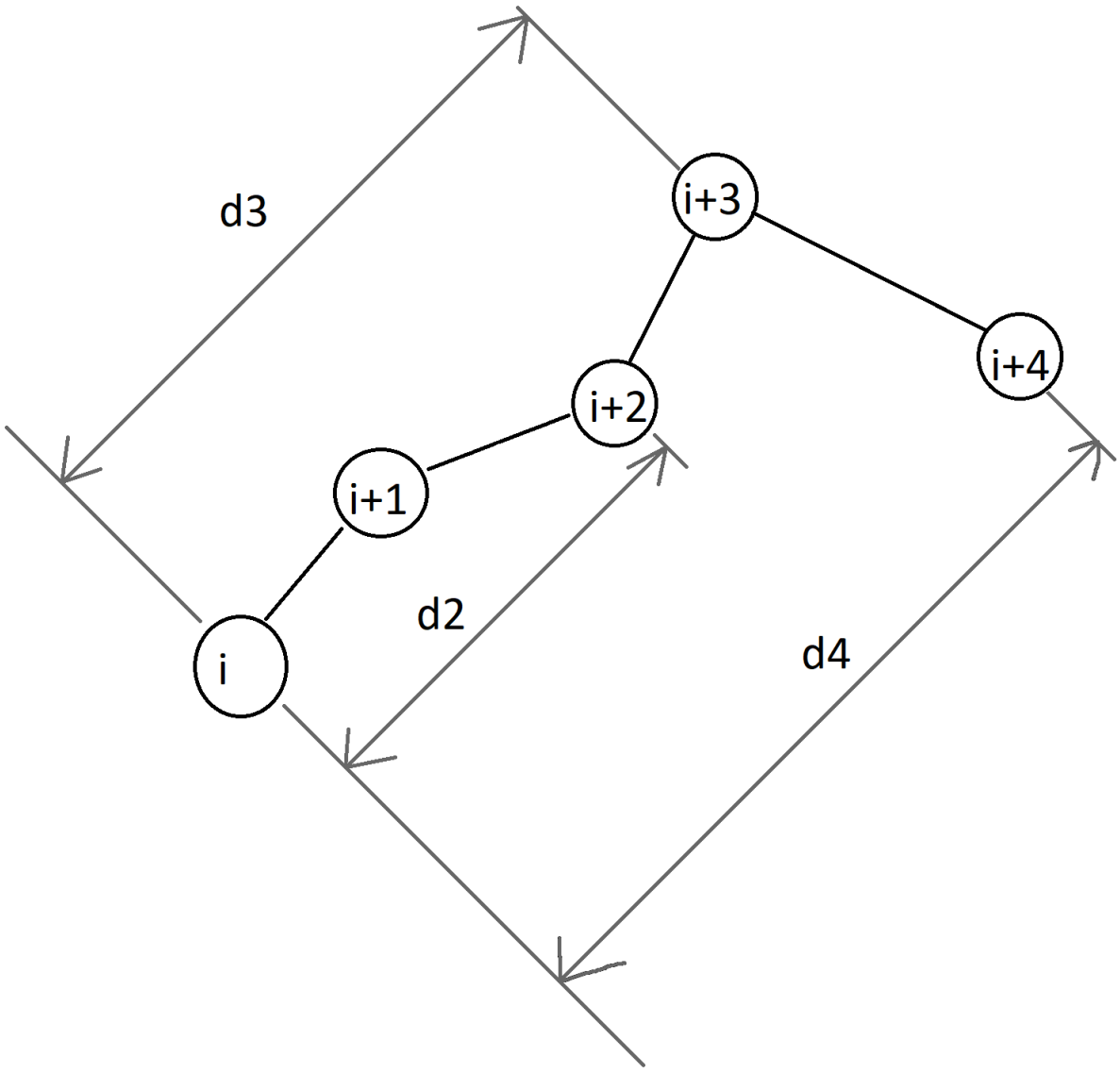


Figure 4.4: Distances d2, d3 and d4

And finally the dihedral angle, the angle α.

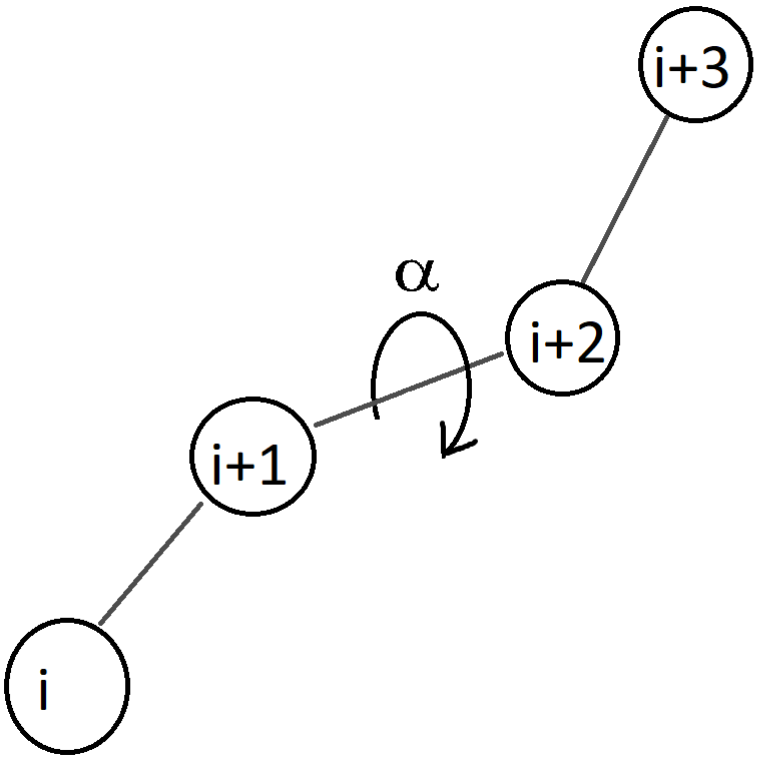


Figure 4.5: Angle α

Now let's explore how we computed these measures:

- **Distances**: for the distances we just use the euclidean norm, which is $||x||_2 = \sqrt{x_1^2 + ... + x_n^2}$

- **Angle** τ: for this angle we used the following formula $\tau = arccos(\frac{a \cdot b}{||a|| \cdot ||b||})$. With $a = (v_{i+1} - v_i)$ and $b = (v_{i+1} - v_{i+2})$.

- **Angle** α: to calculate the dihedral angle between the 4 data points we have to calculate the angle between the two half-planes defined by three consecutive points. The first semi-plane n_1 is defined by the points v_i, v_{i+1} and v_{i+2}. The second semi-plane is defined by the points v_{i+1}, v_{i+2} and v_{i+3}.

1. We computed the vectors u,v and w by vector subtraction: $a = \frac{v_{i+1}-v_i}{||v_{i+1}-v_i||}$, $b = \frac{v_{i+2}-v_{i+1}}{||v_{i+2}-v_{i+1}||}$ and $c = \frac{v_{i+3}-v_{i+2}}{||v_{i+3}-v_{i+2}||}$;
2. We computed the two half-planes $n_1 = a \times b$ and $n_2 = b \times c$ [1];
3. Finally we obtain the angle as $\alpha = arctan2((n_1 \times n_2) \cdot b,\ n_1 \cdot n_2)$.

Here we can see the pseudo-code to compute distances and angles.

Algorithm 2 Pseudocode for computing angles and distances between α-carbons coordinates

Require: ca_coord, a matrix containing the coordinates of the various α-carbon atoms
d2 = **distance_atoms**(ca_coords[:-2], ca_coords[2:])
d3 = **distance_atoms**(ca_coords[:-3], ca_coords[3:])
d4 = **distance_atoms**(ca_coords[:-4], ca_coords[4:])
tau = **angle**(ca_coords[:-2], ca_coords[1:-1], ca_coords[2:])
alpha = **dihedral**(ca_coords[:-3],ca_coords[1:-2],ca_coords[2:-1],ca_coords[3:-2])

As we can see from the pseudo-code we used the python language built-in functions to compute all distances and angles in oneshot, but it's possible to implement it with of for loops.

Assign secondary structure to each residue

For this last step, we want to compute which residue belongs to a helix structure and which residue belongs to a strand structure. The paper describes the procedure to assign to a residue the secondary structure, based on distances and angles, using the table for criteria: table 4.1.

For assigning residues to helical structure:

- Helices are first assigned to any segment long at least five residues, satisfying either one of the following helical criteria:
    1. Each residue's α-carbon in the segment satisfies both the helical criteria for d3i and for d4i distances;
    2. Each residue's α-carbon in the segment satisfies both the helical criteria for αi and τi angles.

[1] This $\times$ symbol is to indicate the cross product between two vectors, while the $\cdot$ symbol is for dot product. More information on the cross product here.

- Then each of this segment is lengthened by one residue at each end and if those 2 residues satisfies some criteria we assign them to helix structure too. The criteria is that the residue satisfies either:

    1. d3i distance helical criteria;
    2. τi angle helical criteria.

Regarding the assigment of strand structure to residues, the procedure is similar:

- Strand structure is assigned to any segment long at least three residues, satisfying either one of the following strand criteria:

    1. Each residue's α-carbon in the segment satisfies both the d2i, d3i and d4i distances criteria;
    2. Each residues's α-carbon in the segment satisfies both the τi and αi angles criteria.

- Then lengthening by one residue at each end if it satisfies the d3i distance strand criteria;

- Finally short β-strand (<4 residues) are kept only if they have enough contacts, which means they are included in a β-sheet.

Algorithm 3 Pseudocode for assigning secondary structure to residues

Require: ca_coords, d2, d3, d4, α, τ
basic_helix = (d3 $\in$ (4.8, 5.8) AND d4 $\in$ (4.8, 7)) OR ($\tau \in$ (75, 101) AND $\alpha \in$ (30, 70))
basic_helix = **mask_consecutive**(basic_helix, 5)
extended_helix = **extend_region**(basic_helix)
basic_strand = (d3 $\in$ (4.8, 5.8) AND d4 $\in$ (4.8, 7)) OR ($\tau \in$ (75, 101) AND $\alpha \in$ (30, 70))
basic_strand = **mask_consecutive**(basic_strand, 3)
extended_strand = **extend_region**(basic_helix)
extended_strand = **mask_regions_with_low_contacts**(extended_strand)
ss_prediction = list long len(ca_coords) initialized with all "C"
ss_prediction[extended_helix] = "H"
ss_prediction[extended_strand] = "E"

The procedures used in this pseudocode will be described later on in their section 4.1.3:

- **extend_region**;
- **mask_consecutive**;
- **mask_regions_with_low_contacts**.

After this step every residue will have its secondary structure assigned:

- **H** for helix structure residues;
- **E** for strand structure residues;
- **C** for coils;
- **"** for residues without the α-carbon.

4.1.3 Helper procedures

In this section we will describe more in detail the helper procedures used for the PSEA algorithm:

- **get_res_start()**: procedure that creates a list of atoms out of the protein structure and then computes the start of each residues, by changes of residue name, id or chain id. It's possible to pass as parameter to the procedure "move_hhem_to_end = True", this puts those atoms between chains to the end of the atom array, resulting in an higher accuracy of prediction of secondary structure in multi-chain proteins.
- **distance_atoms()**: computes the distance between two atoms;
- **angle()**: computes the angle between three atoms;
- **dihedral()**: computes the dihedral angle between four atoms, between their semiplanes;
- **mask_consecutive()**: this procedure is used to create the first step of helix and strand mask, where we want at least a segment of 3/5 residues satisfying a certain condition. It uses binary masks along with the library NumPy;
- **extend_region()**: this procedure is for the second step of finding if a residue belong to that structure, which is lengthening. This procedure lengthen the segment if the residues satisfy the necessary criteria;
- **mask_regions_with_low_contacts()**: finally this procedure is used in the strand structure mask, short strands are kept only if they have enough contacts. This procedure "removes" from the strand masks, short strands with low contacts.

I decided to not show the code snippets for these procedures because they are complex and not too useful to understand the code, but they can be found in the github repository in the file "utils_alphafold_disorder.py".

4.1.4 Integration

For the integration with the existing AlphaFold-disorder software tool, we encapsulated this psea algorithm in a procedure called get_sse_psea() which takes as input the protein structure and return as output an array of characters, with the secondary structure of each residue. And the procedure called from the software-tool for each protein file is called "process_pdb_psea()". Based on which method we want to use there is a "process_pdb_psea()" procedure and a "procedure_dssp()" one.

So the existing code isn't too much touched, since the only difference is that it has to call this procedure instead of the DSSP wrapper procedure.

Down below a code snippet of this procedure encapsulating the whole PSEA algorithm.

```
def get_sse_psea(structure, add_short_contacts = True, move_end_hhem
    = True) :
    res_start_id, atom_array = get_res_start(structure,
        move_end_hhem)
    ca_coord, novirtual_mask = get_ca_coord(res_start_id, atom_array)
    length, [d2i, d3i, d4i, ri, ai] = calc_dist_angles(ca_coord)
    helix_mask, strand_mask = calc_struct_mask(ca_coord, length,
        [d2i, d3i, d4i], [ri, ai], add_short_contacts)

    return finalize_sse(ca_coord, length, novirtual_mask,
        helix_mask, strand_mask)
```

Code 4.1: Procedure get_sse_psea()

We already talked about the procedures **get_res_start**, while **get_ca_coord**, like the name suggests creates the matrix of α-carbon coordinates, **calc_dist_angles** is the procedure for computing the distances and angles. **calc_struct_mask** is the procedure to compute the helix mask and strand mask, this procedure takes as parameter "add_short_contacts", which if set to true removes short strands with low contacts.

And finally the **finalize_sse** procedure uses the helix and strand masks to assign the secondary structure to each residue and returns an array of characters,

each character representing the secondary structure for the residue at that array position.

We compared the secondary structure predicted with this method and the secondary structure predicted with DSSP for a few hundred proteins and as the paper[3] of PSEA said we got an accuracy of around 80%.

To compare that we used a procedure called "compare_sses" which compares the secondary structure elements predicted from DSSP with the secondary structure predicted with PSEA algorithm.

```
def compare_sses(sse, dssp) :
    sse_dssp = [dssp[i][2] for i in dssp]

    counter1 = 0
    counter2 = 0
    countersize = 0
    for (i,j) in zip(sse, sse_dssp) :
        if j in ['H','G','I'] :
            j = 'a'
        elif j in ['B', 'E'] :
            j = 'b'
        else :
            j = 'c'
        if i == '': i = 'c'

        if i == j :
            counter1 += 1
        if i in ['a','b'] : i = 'p'
        if j in ['a','b'] : j = 'p'
        if i == j :
            counter2 += 1
        #else :
            #print(i,j)
        countersize+=1
    counter1 = counter1/countersize
    counter2 = counter2/countersize
    if counter1 == counter2 :
        return round(counter1,4)
    else :
        return [counter1/countersize, counter2/countersize]
```

Code 4.2: Procedure to compare SSEs

4.2 Computation of RSA with Shrake-Rupley Algorithm

Let's go on with the next algorithm we implemented: since we do not use DSSP for the secondary structure, we wanted to not use DSSP at all. The other reason why DSSP is used in AlphaFold-disorder is the RSA, relative solvent accessibility.

So we implemented an alternative way to compute the RSA, without the DSSP. This method is uses the Shrake-Rupley algorithm, to compute SASA, solvent accessible surface areas. This value is then transformed into the relative solvent accessibility with two rounds of normalization factors: first by using Sander's normalization factors, like in DSSP, and then by using an ad-hoc normalization factors we made to not have rsa values bigger than 1, obtained from a sample of two thousands proteins.

We can summarize the steps for implementing this procedure in 2 steps:

- Implement Shrake-Rupley algorithm to obtain the SASA statistics, for each residue;
- Normalization to obtain RSA statistics: both with Sander and Rost's normalization factors, explained in their scientific paper [5] and with our ad-hoc normalization factors.

4.2.1 Implement Shrake-Rupley algorithm

The Shrake & Rupley algorithm consists in a "rolling ball" of radius equal to a solvent molecule, which estimates the surface of the target molecule. This algorithm allows us to compute the SASA statistic (Solvent Accessible Surface Areas), more details can be found in the relative scientific paper[6].

To implement this algorithm in our code we just imported the library "Bio.PDB.SASA" and used the provided class "ShrakeRupley", with its function "compute()". We used the class to create an object of type "ShrakeRupley" and then applied the function "compute()" passing as parameters the protein structure and the level "R", R stands for residue, so that we compute the SASA statistic for each residue. We used the default paramters for the class ShrakeRupley since they are the right parameters for water as solvent, but in case of a different solvent we might want to change them.

We will see a snippet of the code for this procedure in the section 4.2.3.

4.2.2 Normalization

These SASA values aren't relative at all, they can have really high values, up to 200 or more. We want to obtain a statistic that represents the relative surface accessibility, so we have to perform some kind of normalization. We applied two rounds of normalization factors:

- **Sander and Rost's normalization factors**: these are factors for normalization developed by Sander and Rost, with the maximum values of ASA values (Accessible Surface Areas). To use them we import the library "Bio.Data.PDBData" which contains the dictionary "residue_sasa_scales", this dictionary provide the normalization factor for each residue, so we apply these normalization factors to the SASA value we obtained for each residue;

- **Ad-hoc normalization factors**: since after applying the Sander and Rost's normalization factors we still had RSA values bigger than 1, we designed another round of normalization factors. We took all the proteins from the DisProt database, which are 2547 proteins, computed the SASA values for each residue and divided them by the Sander and Rost's normalization factors obtaining the norm_SASA values. With all these norm_SASA values we built up a dictionary of ad-hoc normalization factors. Finally we divided the norm_SASA values by these ad-hoc normalization factors and obtained the final RSA values.

After these 2 rounds of division by normalization factors we obtain the RSA values, within a range of [0,1], representing the percentage of surface accessibility for that residue.

Now for reference I will show the values of the 2 rounds of normalization factors as tables.

3 Letters AA	1 Letter AA	Normalization Factor
Ala	A	106
Arg	R	248
Asn	N	157
Asp	D	163
Cys	C	135
Gln	Q	198
Glu	E	194
Gly	G	84
His	H	184
Ile	I	169
Leu	L	164
Lys	K	205
Met	M	188
Phe	F	197
Pro	P	136
Ser	S	130
Thr	T	142
Trp	W	227
Tyr	Y	222
Val	V	142

Table 4.2: Sander and Rost's normalization factors

3 Letters AA	1 Letter AA	Normalization Factor
Ala	A	1.713
Arg	R	1.280
Asn	N	1.476
Asp	D	1.435
Cys	C	1.551
Gln	Q	1.311
Glu	E	1.360
Gly	G	1.804
His	H	1.404
Ile	I	1.413
Leu	L	1.532
Lys	K	1.357
Met	M	1.420
Phe	F	1.434
Pro	P	1.490
Ser	S	1.515
Thr	T	1.507
Trp	W	1.423
Tyr	Y	1.346
Val	V	1.549

Table 4.3: Ad-hoc normalization factors

4.2.3 Integration

Regarding the integration into the exisiting software-tool AlphaFold-disorder, we implented this algorithm for RSA inside the procedure "process_pdb_psea" since it's an alternative way to compute rsa compared to the one in "process_pdb_dssp".

For the normalization factors:

- Sander's normalization factors: it's enough to import the dictionary "residue_sasa_scales" from "Bio.Data.PDBData", which contains the normalization factors of each residue;

- Ad-hoc normalization factors: we created a file .json called "dict_max_rsa.json", which contains the normalization factors of each residue.

Once we have the dictionary of the normalization factors, we can obtain the factor of our residue simply by obtaining the value paired with that residue in the dictionary.

Down below a code snippet of the implementation of this Shrake & Rupley algorithm to obtain the RSA values.

```
def process_pdb_psea(pdb_file, pdb_name) :
    structure, real_file = extract_pdb(pdb_file)
    with open('dict_max_rsa.json','r') as f :
        dict_adhoc_factors = json.load(f)
[...]
    sse = get_sse_psea(structure)
[...]
    ShrakeRupley().compute(structure, level="R")
    for i, residue in enumerate(structe.get_residues()):
[...]
        rsa = residue.sasa / residue_sasa_scales['Sander'][residue.
   resname]
        rsa = rsa / dict_adhoc_factors[protein_letters_3to1[residue.
   resname]]
[...]
```

Code 4.3: Integration of rsa with SASA on process_pdb_psea procedure

4.3 Implementation of FoldComp Library

We used the library FoldComp, described in more detail in their scientific paper [2], to allow to use a new input file in the software tool.

With FoldComp we can read and write .fcz file, which are compressed protein files, this enable us to store more protein files in the same storage space.

Regarding the implementation, we have:

- "**decompress**" procedure of FoldComp;
- we made a procedure for checking if a file is a .fcz file "**is_fcz_file**": if the file starts with *b'FCMP:'*, then it's a .fcz file.

Since in our case we are interested just in reading files .fcz we just need the procedure "decompress" from the library, which decompress the .fcz file into a .pdb file.

We also used the library **StringIO** as a workaround to avoid saving the protein file after decompressing the .fcz file.

Let's see the integration of this library into the software-tool AlphaFold-disorder.

```
def is_fcz_file(filepath) :
    with open(filepath, 'rb') as test_f:
        return test_f.read(5) == b'FCMP:'

def extract_pdb(pdb_file) :
[...]
    elif is_fcz_file(real_file):
        with open(real_file, 'rb') as f :
            (name, pdb_filevalue) = foldcomp.decompress(f)
        structure = PDBParser(QUIET=True).get_structure('', StringIO(
   pdb_filevalue))
[...]
```

Code 4.4: Integration of FoldComp

5

Analysis of results' quality of AlphaFold-disorder (SASA)

AlphaFold/disorder (SASA) is the variation we developed of the software-tool AlphaFold-disorder, using PSEA and SASA instead of DSSP to compute the predictions, in particular we have the SASA variation for each of the three algorithms within the software-tool:

- AlphaFold-pLDDT (SASA);
- AlphaFold-rsa (SASA);
- AlphaFold-binding (SASA).

Now we have to understand if this new software-tool produces good predictions: we need to evaluate the results' quality. To do so, we will analyse the output datasets, which contain the predictions, of AlphaFold-disorder and AlphaFold-disorder (SASA) and compare them to a ground truth:

- Analyse the columns of the output datasets;
- Create plots or visualizations, to gain insights on the results and patterns between features (statistics and predictions);
- Finally create ML models and analyse the statistics and main plots of the best models.

	A	B	C	D	E	F	G	H	I
1	name	pos	aa	lddt	disorder	rsa	ss	disorder-25	binding-25-0.581
2	109m	1	M	0.299	0.701	0.198	C	0.098	0.098
3	109m	2	V	0.254	0.746	0.362	C	0.089	0.089

Figure 5.1: Columns of an AlphaFold-disorder (SASA) output file

5.1 Output dataset

The software-tool produces two output files:

- output_data.tsv: contains the statistics before computing the predictions;
- output_pred.tsv: contains both the statistics and the predictions.

We will consider only the latter one, since it includes the first one inside. The name of the files depends on the parameter given by command line when using the software-tool.

Both in AlphaFold-disorder dataset and AlphaFold-disorder (SASA) dataset we have the same columns, down below a screenshot of an output file opened with Excel.

Let's continue the analysis by creating a few plots, to visualize any pattern or relationship between columns and gain some insight.

5.2 Plots

We obtained the ground truth from the CAID web application, and we considered the structural features of the proteins present in these ground truths. These ground truths are simply lists of "0", "-" and "1" for each residue in some proteins: 0 when that characteristic is absent, 1 when is present and - when it's uncertain. We described this procedure to obtain and process the CAID ground truths in 5.5.

We tried a few different plots, to compare the relationships between the most important features of the datasets:

- lddt;
- rsa;
- disorder-25;
- binding-25.

Disorder-25 and Binding-25 are respectively the predictions of AlphaFold-rsa and AlphaFold-binding.

We can categorize the plot we made in two:

1. By ground truth used:

   - Binding: ground truth on binding, which is basically an array with an annotation for every residue, 1 if it's a residue that binds with other residues or molecules, 0 otherwise;
   - Disorder: same but on disorder.

2. By type of plot:

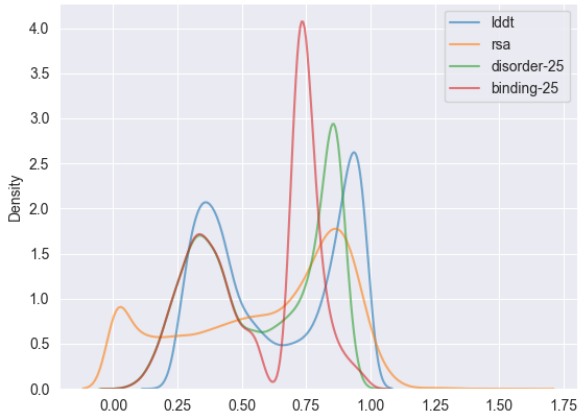
   - Density plot;
   - Histograms;
   - Jointplot;
   - Scatterplot.

We made the plots using the Python library **seaborn**, in particular we used the procedures scatterplot, kdeplot, jointplot and histplot. While the other are obvious, to create density plots we used the kdeplot procedures.

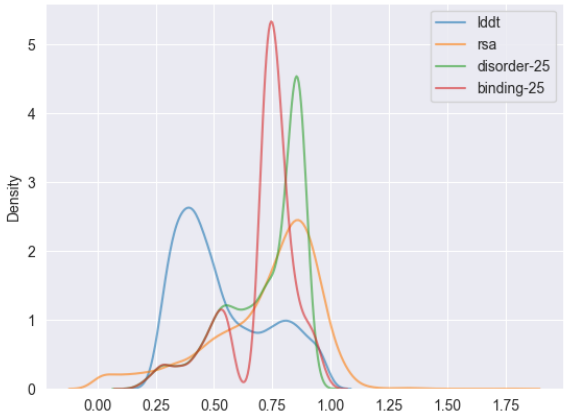
More information on seaborn, on the website.

5.2.1 Density plots

For density plots we have two setups per ground truth: for example for the binding ground truth we created the plot for every feature for residues where ground truth is 0 and another plot with every feature for residues with ground truth 1. We made equivalent plots for the disorder ground truth. Down below a few pictures to illustrate these two types of density plots.



(a) Density plot: every feature for residues binding absent



(b) Density plot: every feature for residues binding present

5.2.2 Histogram plots

For histogram plots we made three different types per ground truth:

- Every feature where the ground truth is 0;
- Every features when ground truth 1;
- For every feature we made a plot comparing values of that feature for residues in the two classes (ground truth=0 and ground truth=1)

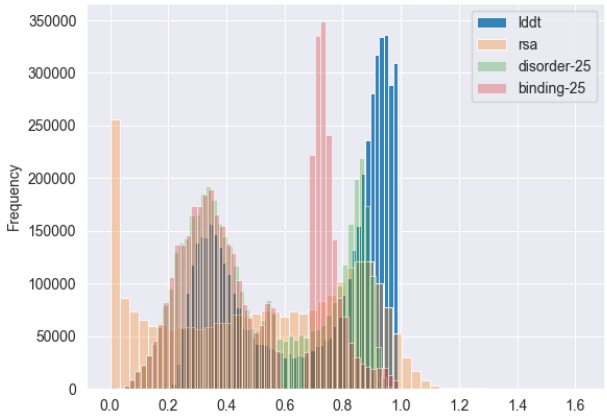
I will show the histogram plots we made for the disorder ground truth. We made equivalent plots for the binding ground truth too.

These were histogram plots for disorder ground truth, but as for density plots we made equivalent plots for the other ground truth. For the third type of histogram plot, we showed only for 2 features: rsa and lddt, but we made them for the other features considered too.

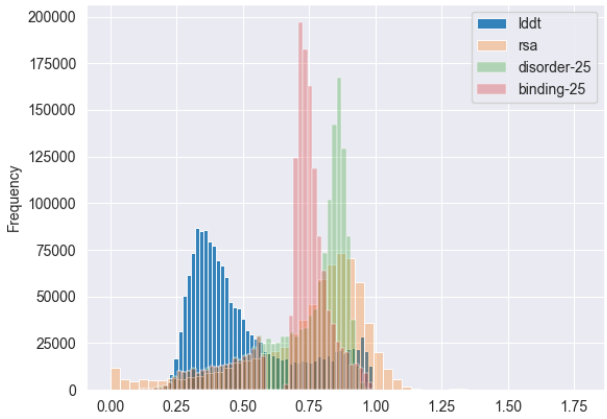
5.2.3 Scatter plots

We made scatterplots for every possible pair of the four features we considered: (rsa, lddt), (rsa, binding-25), (rsa, disorder-25), (lddt, binding-25), (lddt, disorder-25), (disorder-25, binding-25).

For every pair we made a plot against the positive ground truth (=1) and another against the negative ground truth. So in total we made fourteen scatter plots per ground truth. We will show the scatter plots made for one of the pairs: (rsa, lddt).



(a) Histogram plot: every feature - disorder absent

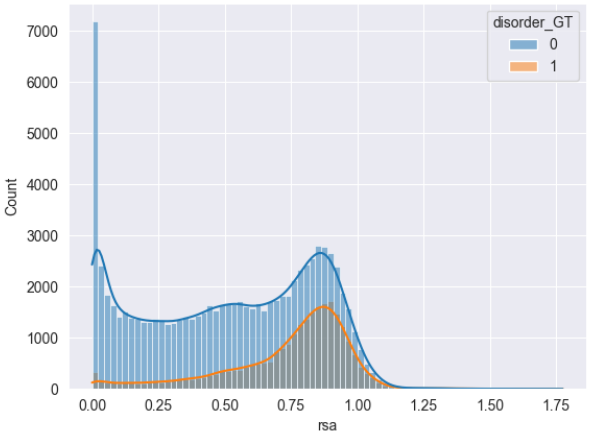


(b) Histogram plot: every feature - disorder present

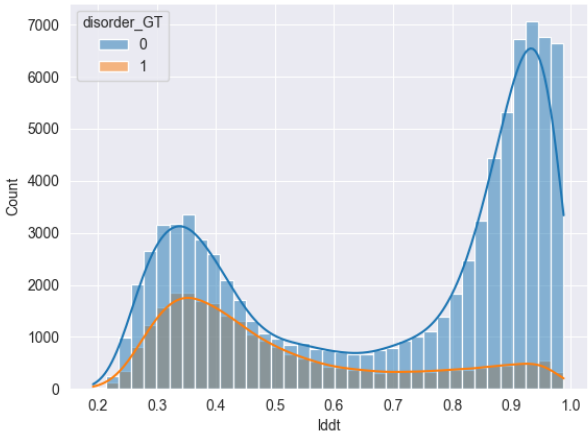
5.2.4 Joint plots

Finally, the last type of plots: joint plots. Similarly as with scatter plots, we made plots for every possible pair of features, both against the positive ground truth and against the negative ground truth.

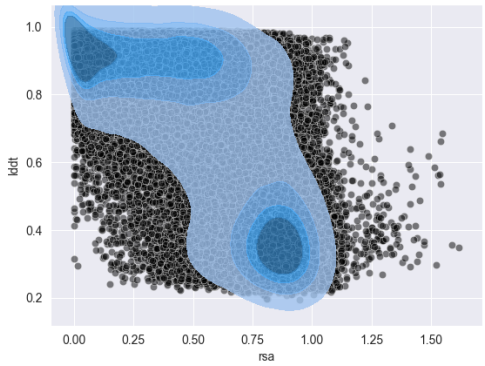
Let's see the joint plots for the same pair (rsa, lddt), to compare them with the scatter plots.



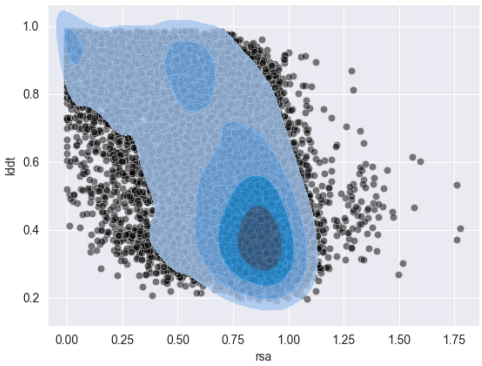
(a) Histogram plot: RSA feature - disorder present



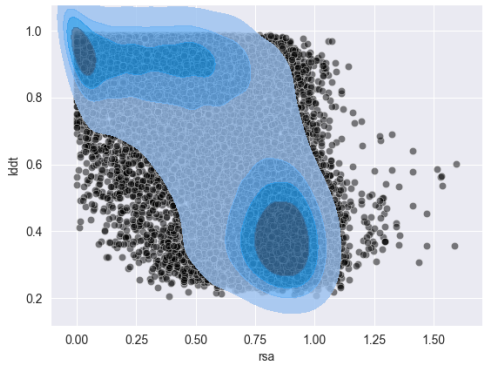
(b) Histogram plot: LDDT feature - disorder pres



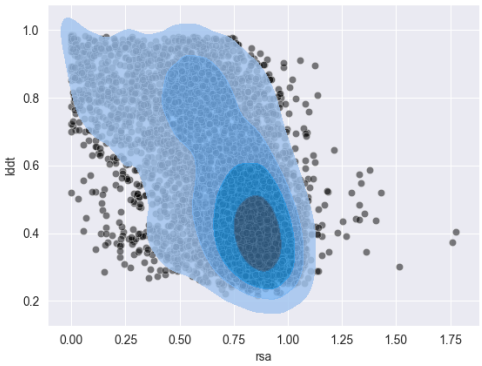
(a) Scatterplot (rsa, lddt) disorder absent



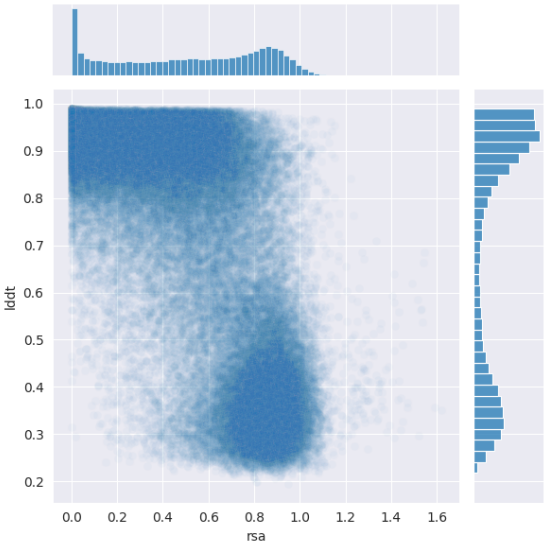
(b) Scatterplot (rsa, lddt) disorder present



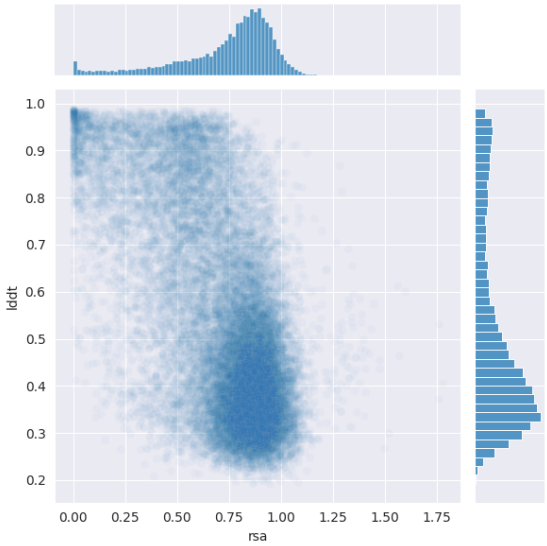
(a) Scatterplot (rsa, lddt) binding absent



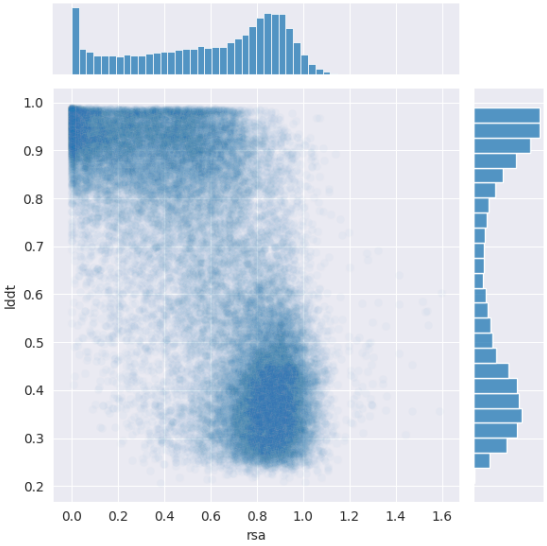
(b) Scatterplot (rsa, lddt) binding present



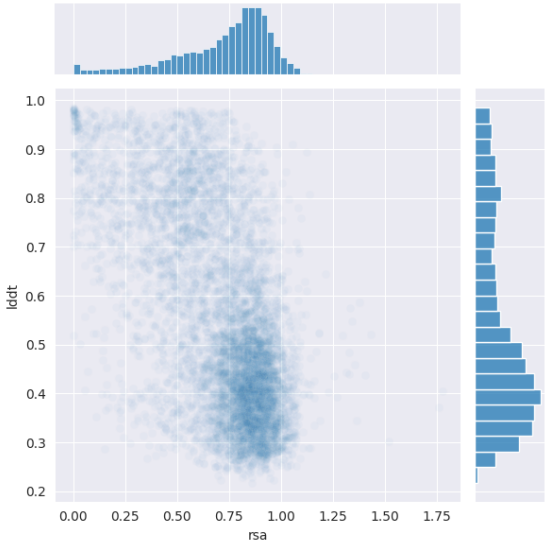
(a) Jointplot (rsa, lddt) disorder absent



(b) Jointplot (rsa, lddt) disorder present



(a) Jointplot (rsa, lddt) binding absent



(b) Jointplot (rsa, lddt) binding present

5.2.5 Interpretation of Plots

We made thirty-eight plots compared to the binding ground truth and another thrirty-eight compared to the disorder one.

We can clearly see that there is some correlation between the features, we can confirm that disorder and lddt are strictly correlated, and that rsa is correlated

both with disorder and binding.

To further explore and better evaluate the results, we decided to develop a few ML model and compare the precision-recall and ROC plots to the benchmark ones (AlphaFold-plddt, AlphaFold-rsa and AlphaFold-binding).

5.3 ML models

For the machine learning models we considered two different ground truths for disorder and one for binding:

- disorder-pdb;
- disorder-nox;
- binding.

these three ground truths are from the CAID web application. We described the procedure to obtain and process the CAID ground truths in 5.5.

We developed machine learning models using the three ground truths as target features for the test datasets: so we will have three test datasets, based on the ground truth used and one train dataset, where the target feature will be either binding or disorder, based on the test dataset used.

Let's analyse the preprocessing steps we made.

5.3.1 Preprocessing

As test datasets we used the dataframes containing the proteins present in each of the ground truths, with target the ground truths.

While as train dataset we used the dataframe of all the proteins in the DisProt database, using as target either the disorder or binding functional annotations present in DisProt, based on the test dataset used. We described the procedure to obtain these proteins and these functional annotations from DisProt in 5.5.

We computed a one-hot encoding of the ss column: **ss_onehot**, so we have a number instead of characters "H", "E", "C". We did this both for test datasets and for train datasets.

We also added new features to improve the accuracy of ML models: we incorporated the Atchley scale in the datasets. This Atchley scale consists in five features that are defined for each residue.

Amino acid	Factor I	Factor II	Factor III	Factor IV	Factor V
A	−0.591	−1.302	−0.733	1.570	−0.146
C	−1.343	0.465	−0.862	−1.020	−0.255
D	1.050	0.302	−3.656	−0.259	−3.242
E	1.357	−1.453	1.477	0.113	−0.837
F	−1.006	−0.590	1.891	−0.397	0.412
G	−0.384	1.652	1.330	1.045	2.064
H	0.336	−0.417	−1.673	−1.474	−0.078
I	−1.239	−0.547	2.131	0.393	0.816
K	1.831	−0.561	0.533	−0.277	1.648
L	−1.019	−0.987	−1.505	1.266	−0.912
M	−0.663	−1.524	2.219	−1.005	1.212
N	0.945	0.828	1.299	−0.169	0.933
P	0.189	2.081	−1.628	0.421	−1.392
Q	0.931	−0.179	−3.005	−0.503	−1.853
R	1.538	−0.055	1.502	0.440	2.897
S	−0.228	1.399	−4.760	0.670	−2.647
T	−0.032	0.326	2.213	0.908	1.313
V	−1.337	−0.279	−0.544	1.242	−1.262
W	−0.595	0.009	0.672	−2.128	−0.184
Y	0.260	0.830	3.097	−0.838	1.512

Figure 5.9: Atchley scale

As the last step of preprocessing, we defined which features do we want to consider in the learning process and which one is the target.

For both the train and test datasets we consider as input features:

- lddt;
- rsa;
- ss_onehot;
- the five factors from the Atchley scale.

For the train datasets we consider as target feature the DisProt functional annotation, either binding or disorder annotation. In case we used as test dataset the one with target **binding ground truth**, we will use as target feature for the train set the binding functional annotations of DisProt, otherwise we will use as target feature the disorder functional annotations.

For the test datasets we consider as target feature either of the three ground truths.

5.3.2 Development of Machine Learning Models

For the development of the ML models we implemented the library **sklearn**, further details on the website.

The ML task we considered is regression, because we want to obtain the propensity of disorder or binding of the residues.

We implemented eight different ML models: svm, ridge, lasso, poisson-glm, tweedie, k-nearest neighbors, decision tree and extra tree.

In general to implement the libraries and compute the ML models we used the procedures provided by the library, in particular:

- fit(): this procedure allows us to fit the model with our train dataset;
- predict(): this procedure allows us to predict a value, given the input data of the test dataset. Then we will compare this value with the target value of the test dataset and evaluate the ML model performances.

We also have a class contructor to initialize each model, always provided by the library.

In the code snippets of the various ML models we will see the variables:

- **X_train**: input features of the train dataset;
- **y_train**: target feature of the train dataset;
- **X_test**: input features of the test dataset;
- **y_pred**: predicted values for output feature, given X_test.

Now I will show the different ML models and how we implemented them with the **sklearn** library.

SVM

Support Vector Machines are one of the most simple yet powerful machine learning models. The objective is find an N-dimensional hyperplane that clearly separates data points of different classes.

Let's see now the code snippet of its implementation on a Python notebook.

```
from sklearn.linear_model import *
[...]
clf = SGDRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.1: SVM implementation

The class SGDRegressor is defined in the module **linear_model** of sklearn, SGDRegressor stands for Stochastic Gradient Descent because the SVM hyperplane is computed with stochastic gradient descent. This is the most basic linear model for regression.

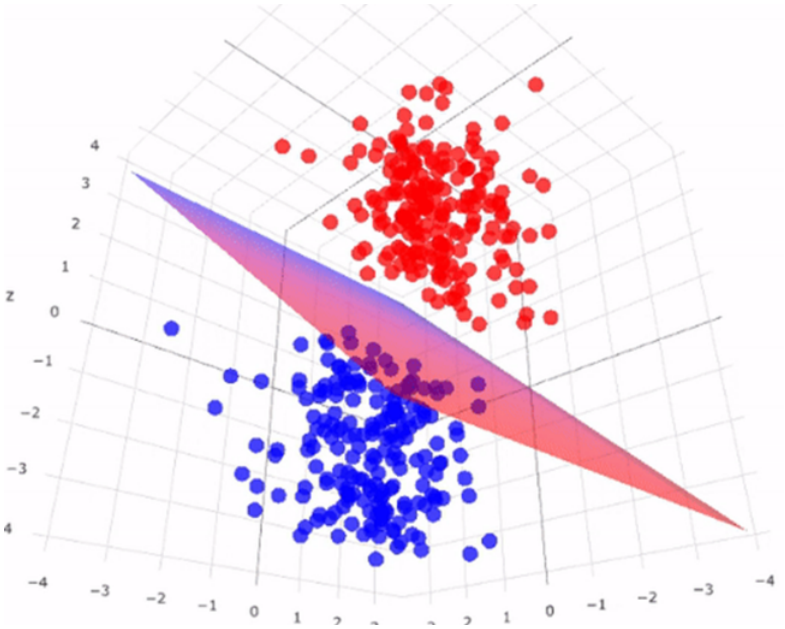


Figure 5.10: Picture of SVM hyperplan

5.3.3 Ridge

Ridge Regression, also known as Tikhonov regularization or L2 regularization, is a variation of linear regression: it adds a regularization term to make the model more robust.

The L2 regularization discourages large coefficient values: the larger the coefficient the more the regularization term penalizes the model. This makes the model more simple and stable, penalizing complex models.

```
from sklearn.linear_model import *
[...]
clf = RidgeCVRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.2: Ridge implementation

Here we used the **RidgeCV** class instead of simply **Ridge**, because we have cross-validation included too, which might increase performances. RidgeCV is also inside the module linear_model of sklearn.

5.3.4 LASSO

LASSO, Least Absolute Shrinkage and Selection Operator, is a linear regression model which uses L1 regularization. L1 regularization has the effect of setting some of the coefficients to exactly zero. This has the interesting property of performing feature selection, meaning that LASSO can be used to automatically select a subset of the most relevant features.

This is the ML model with better performances with our datasets, probably this automatic feature selection is very effective in our case.

Let's move on to the implementation.

```
from sklearn.linear_model import *
[...]
clf = LassoCV()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.3: LASSO implementation

LassoCV is also inside the module linear_model of sklearn. Even here the cross-validation is included.

5.3.5 Poisson GLM

Poisson Generalized Linear Model is a statistical model used for analyzing count data, where the data follows a Poisson distribution.

```
from sklearn.linear_model import *
[...]
clf = PoissonRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.4: Poisson-GLM implementation

PoissonRegressor is also inside the module linear_model of sklearn.

5.3.6 Tweedie GLM

Tweedie Generalized Linear Model is a statistical model used modeling data that follows a Tweedie distribution.

```
from sklearn.linear_model import *
[...]
```

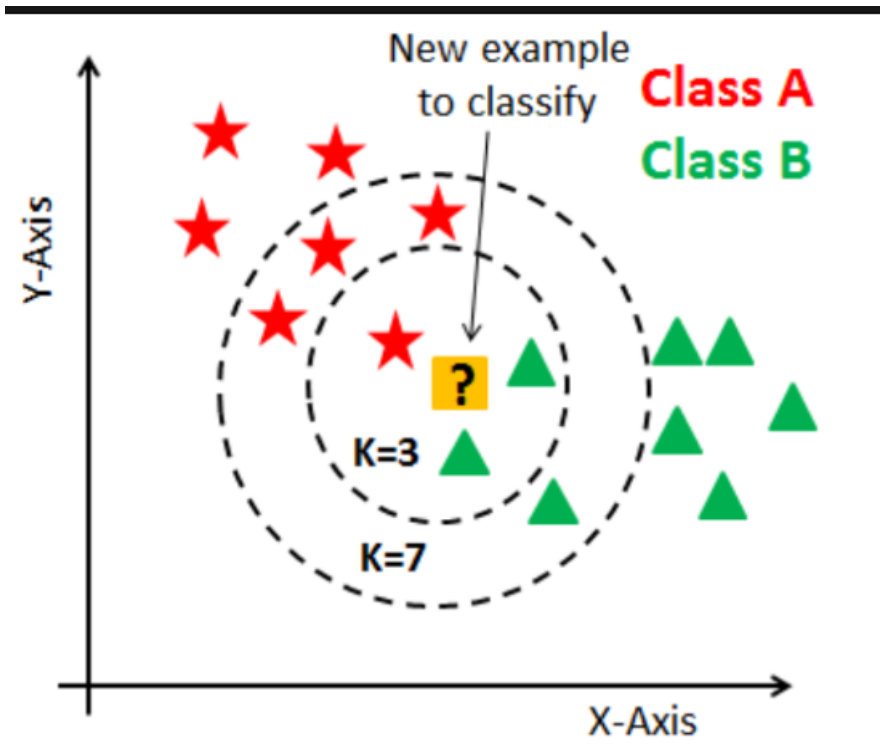


Figure 5.11: How KNN algorithm works

```
clf = TweedieRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.5: Tweedie-GLM implementation

TweedieRegressor is also inside the module linear_model of sklearn.

5.3.7 K-Nearest Neighbors

K Nearest Neighbors is a regression model that makes predictions based on the average of nearby data points.

```
from sklearn import neighbors
[...]
clf = neighbors.KNeighborsRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.6: K Nearest Neighbors implementation

KNeighborsRegressor is defined in the module **neighbors** of the sklearn library.

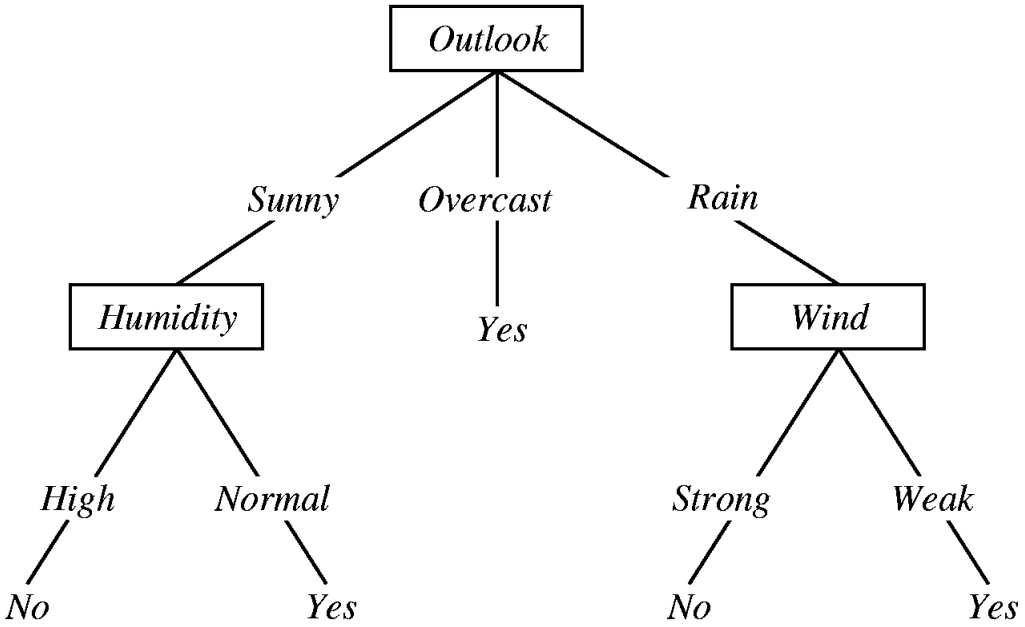


Figure 5.12: Example of a decision tree

5.3.8 Decision Tree

The decision tree splits the dataset into subsets based on the most significant feature, the split points are chosen to minimize the variance for regression. The process is then repeated for each subset, creating a tree-like structure.

To make predictions, the decision tree is traversed from the root to a leaf node, and the value associated with that leaf node is used as the final prediction. During the traversal, the algorithm evaluates the feature at each internal node based on the input features.

```
from sklearn import tree
[...]
clf = tree.DecisionTreeRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.7: Decision Tree implementation

DecisionTreeRegressor is defined in the module **tree** of the sklearn library.

5.3.9 Extra Tree

The extra tree is a combination of multiple decision trees, extremely randomized, to improve the performances and robustness of decision trees.

```
from sklearn import tree
[...]
clf = tree.ExtraTreeRegressor()
clf.fit(X_train,y_train)
y_pred = clf.predict(X_test)
```

Code 5.8: Extra Tree implementation

ExtraTreeRegressor is defined in the module **tree** of the sklearn library.

5.4 Evaluation of ML models' predictions

To evaluate the predictions of the ML models, we computed their ROC and Precision-Recall curves and compared them with the ROC and Precision-Recall curves of the software-tools AlphaFold-disorder and AlphaFold-disorder (SASA).

We will see the ROC curves and the Precision-Recall curves divided by ground truth used in the ML models training process.

5.4.1 Disorder PDB

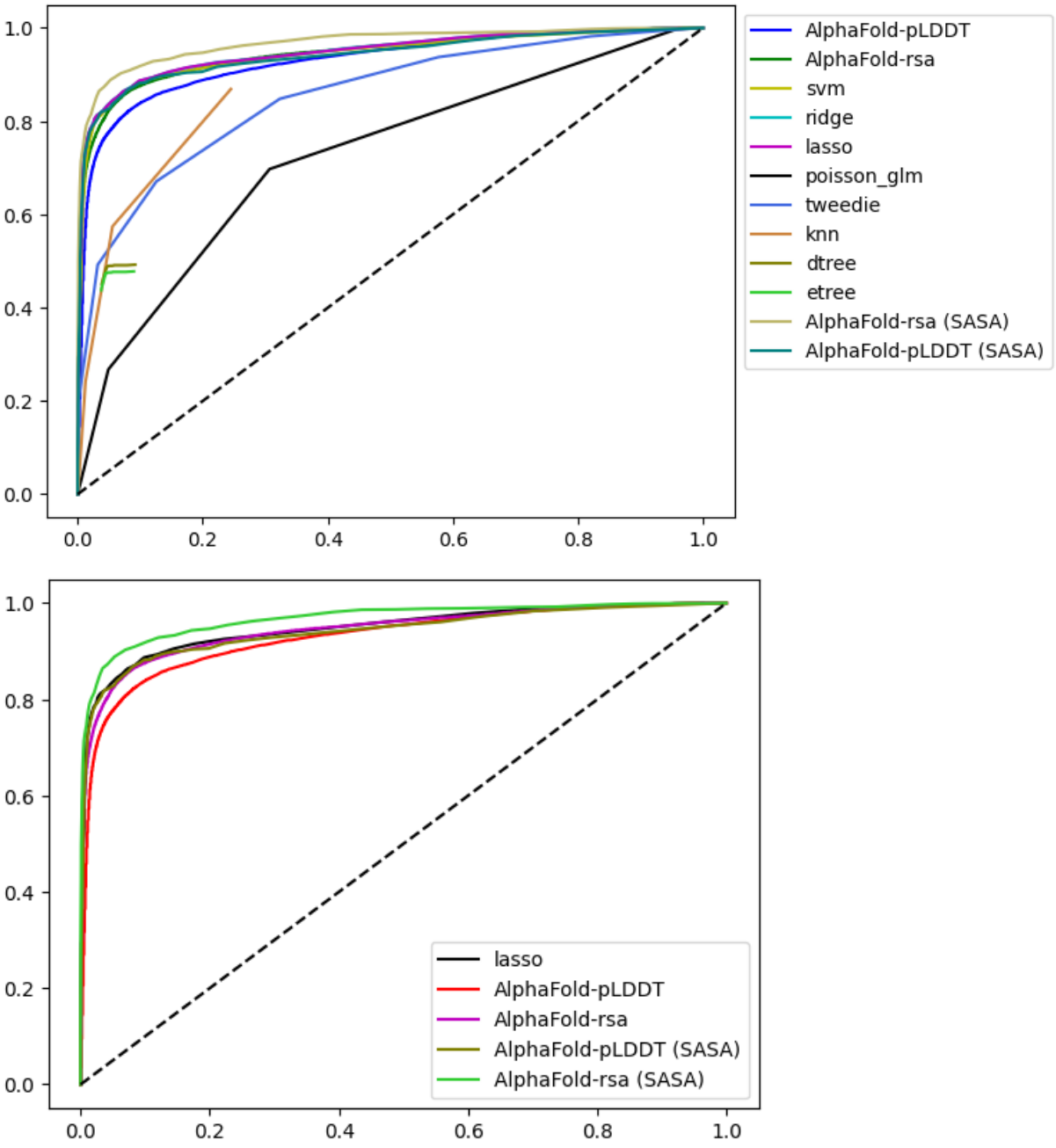


Figure 5.13: ROC curves - DisorderPDB ground truth

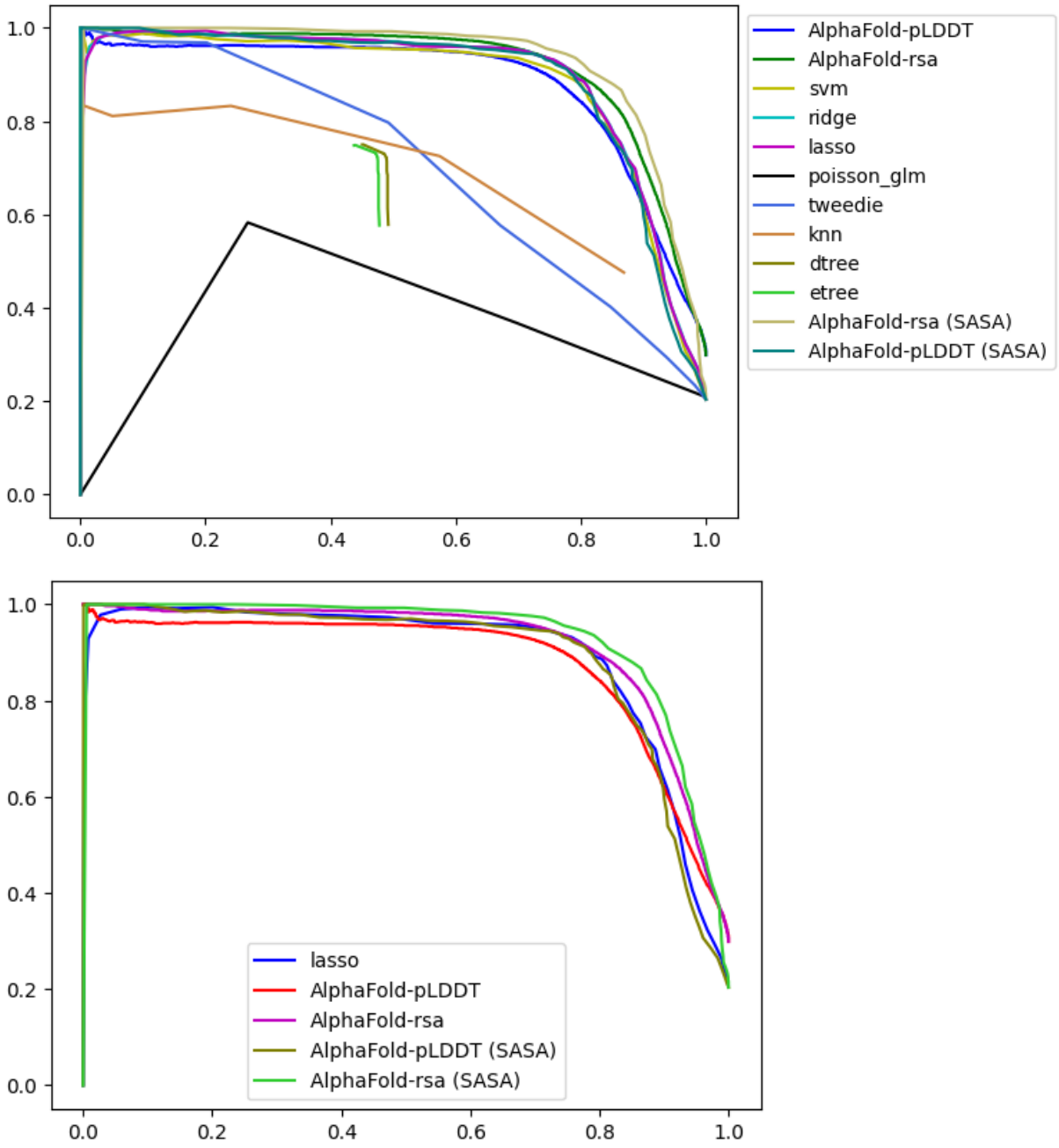


Figure 5.14: Precision-Recall curves - DisorderPDB ground truth

5.4.2 Disorder NOX

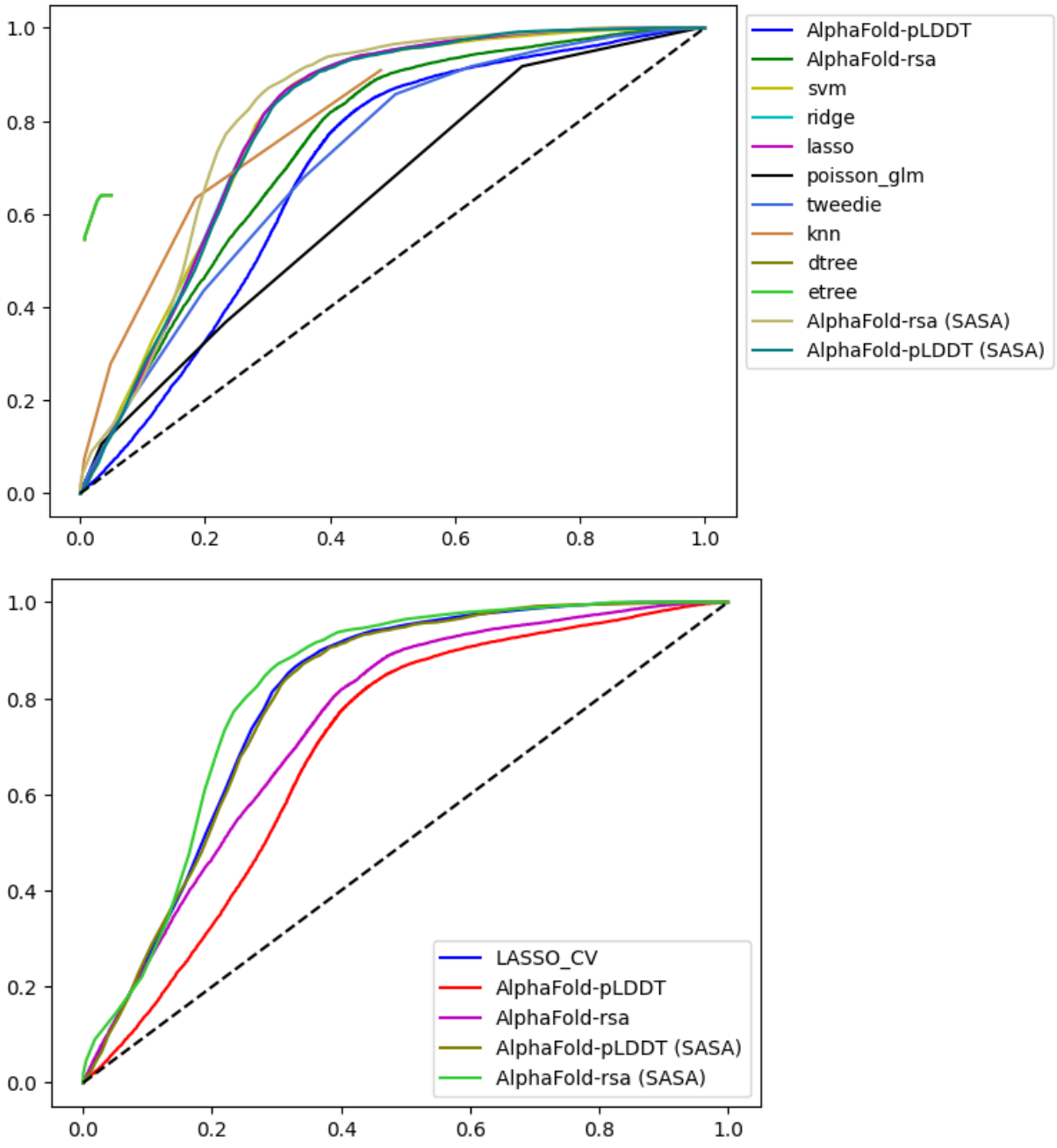


Figure 5.15: ROC curves - DisorderNOX ground truth

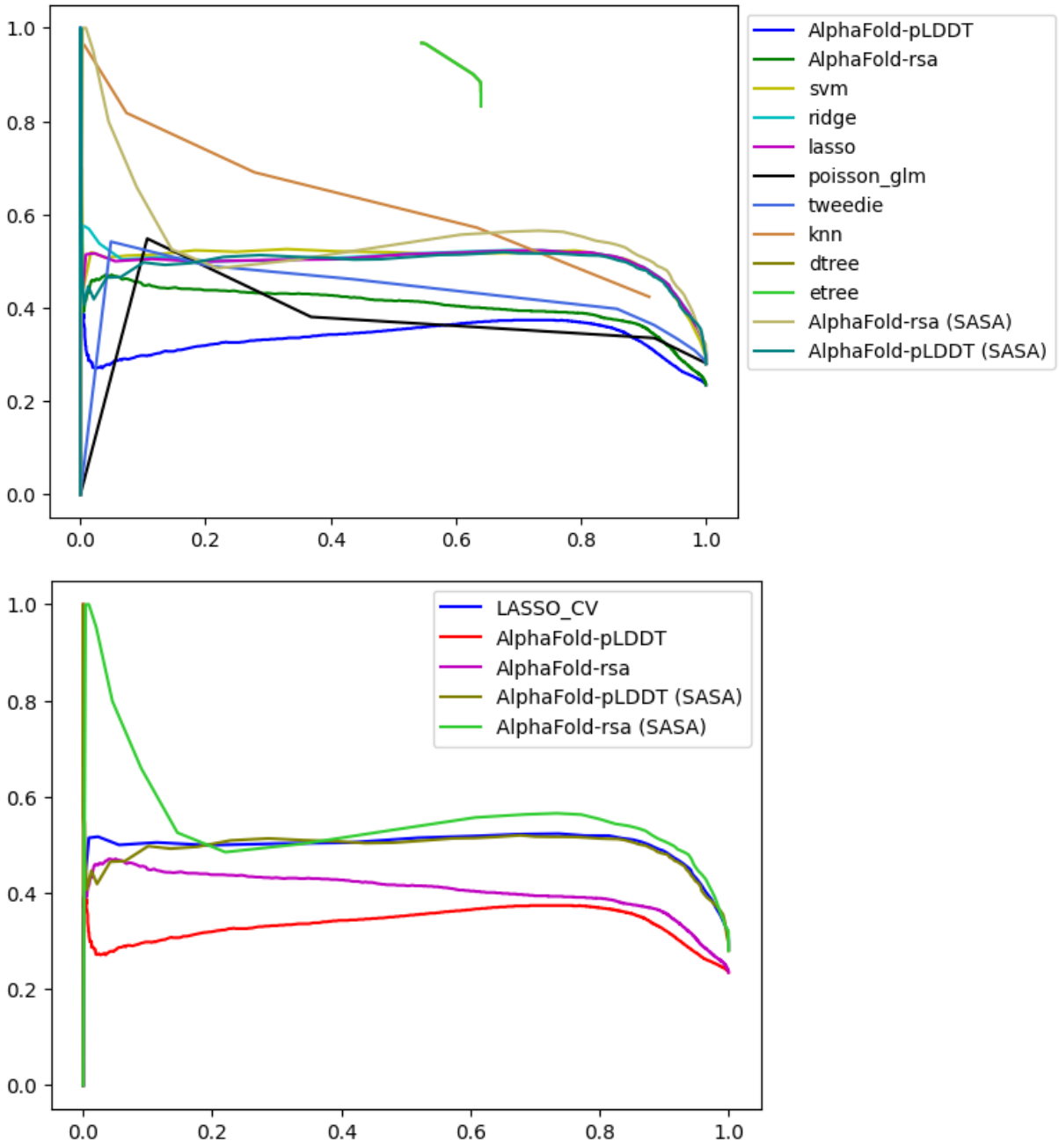


Figure 5.16: Precision-Recall curves - DisorderNOX ground truth

5.4.3 Binding

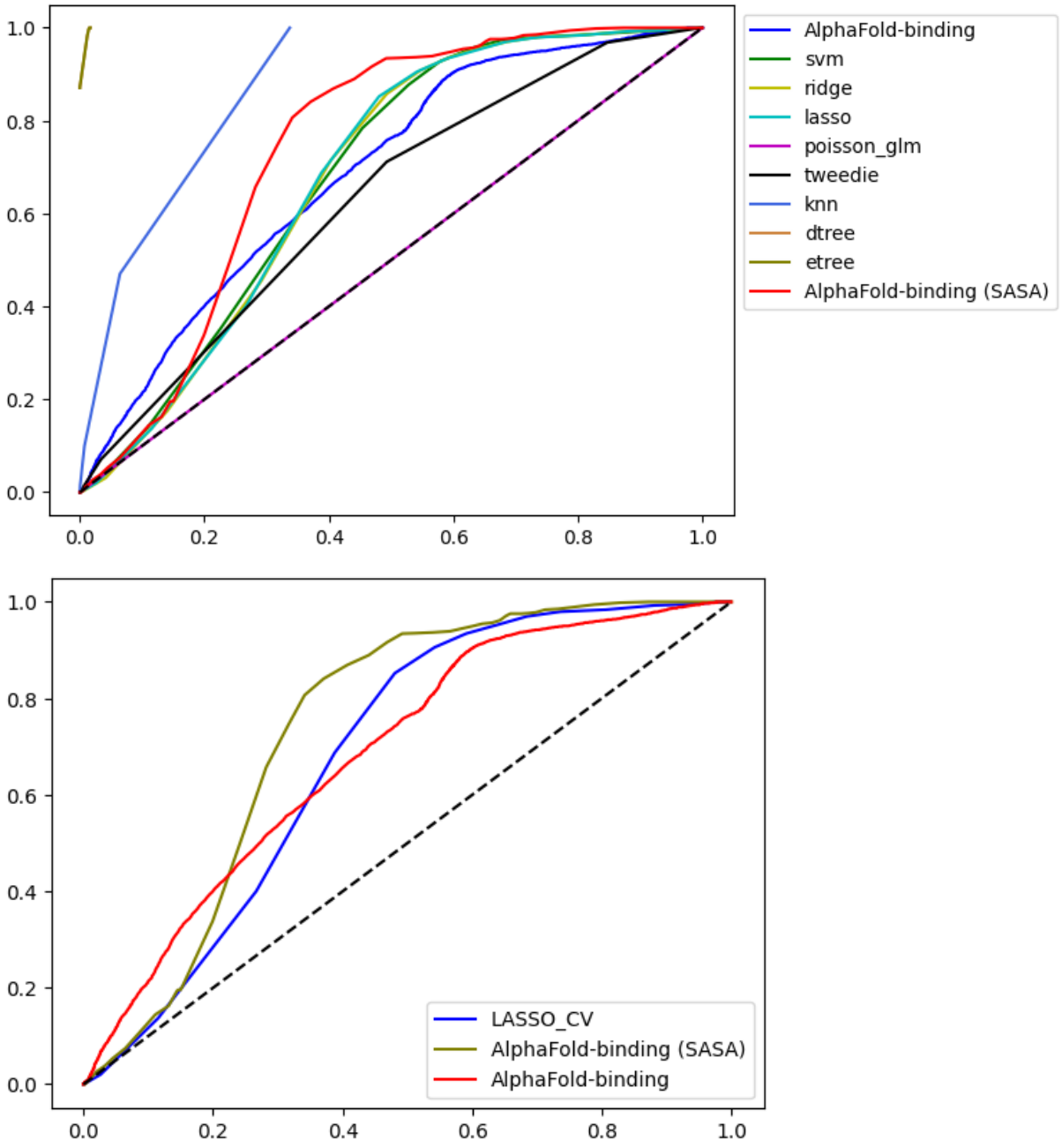


Figure 5.17: ROC curves - Binding ground truth

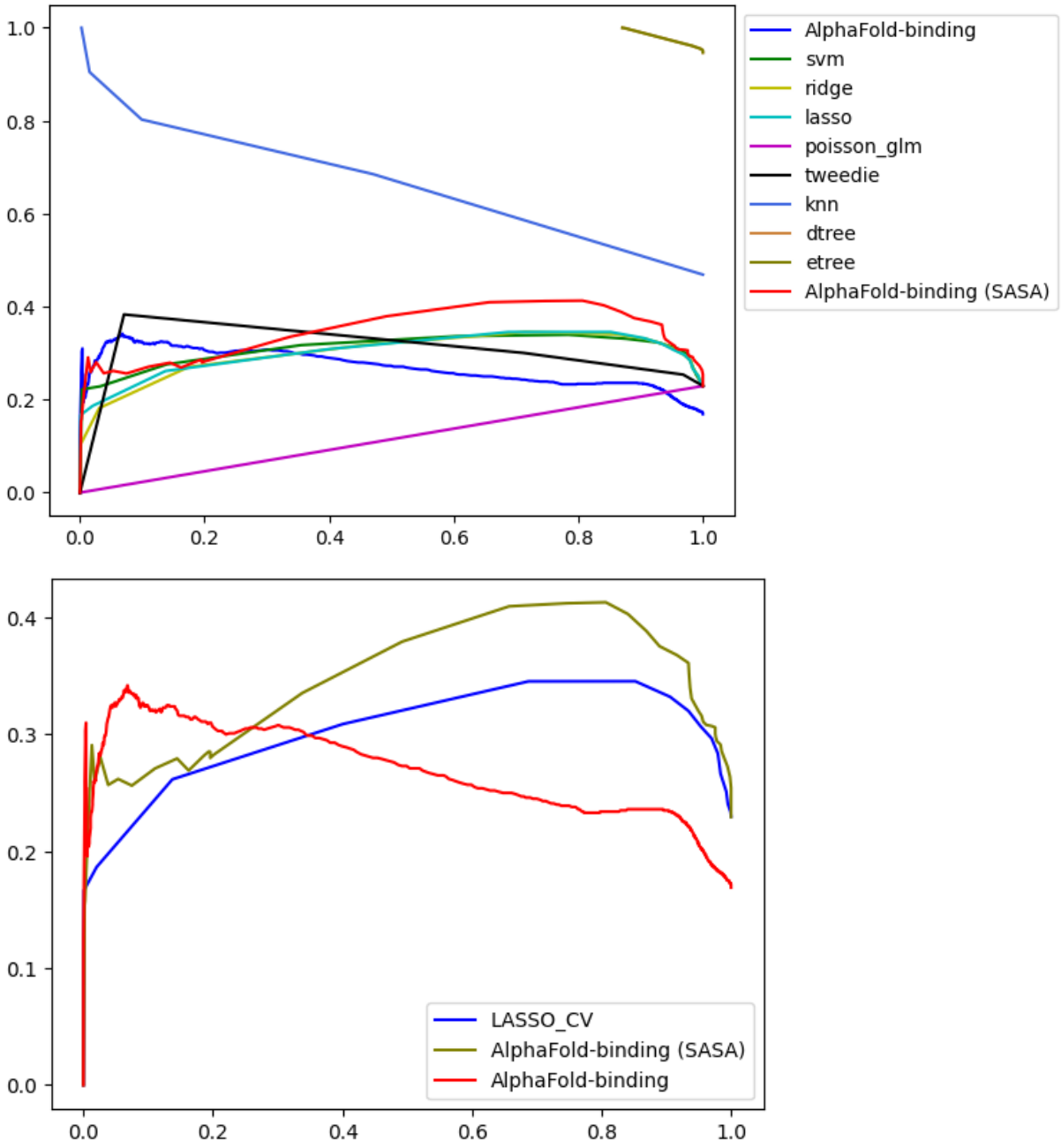


Figure 5.18: Precision-Recall curves - Binding ground truth

5.5 Helper Scripts for Analysis

5.5.1 Fetch proteins and functional annotations from DisProt database

First of all we downloaded the file .json from the DisProt website, in the download page. Then we used the library **json** of Python to extract from the json file, inside the json file we find all the information we want about DisProt proteins.

Let's quickly analyse the structure of this json: from the root of the json we have two keys, **data** and **size**. Size contains just the number of proteins in this json, and data contains an array of DisProt IDs. Each DisProt IDs have several key-value pairs, we are interested in **acc**, **disprot_id** and **regions**.

We are interested in the key **'acc'** of every DisProt protein because it is the UniProt ID: with these UniProt IDs we can fetch very quickly all the proteins structure of proteins in DisProt with the AlphaFold database API.

```
import requests
[...]
list_absent = []
for i in uniprot_ids :
    url = 'https://alphafold.ebi.ac.uk/files/AF-'+i+'-F1-model_v4.pdb
    '
    r = requests.get(url, allow_redirects=True)
    if r.reason == 'OK' :
        filename = '' + i + '.pdb'
        open(filename,'wb').write(r.content)
    else :
        list_absent.append(i)
        print("Error "+str(r.status_code)+", "+r.reason+": "+i)
```

Code 5.9: Script to use AlphaFold database API

Then we are interested in the functional annotations of every DisProt protein, contained inside the key **'regions'**: an array of annotations for that protein.

	uniprot	disprot	amino_id	amino_name	disorder-pdb_GT
0	P06837	DP02342	1	M	1
1	P06837	DP02342	2	L	1
2	P06837	DP02342	3	C	1
3	P06837	DP02342	4	C	1
4	P06837	DP02342	5	M	1
5	P06837	DP02342	6	R	1
6	P06837	DP02342	7	R	1
7	P06837	DP02342	8	T	1
8	P06837	DP02342	9	K	1
9	P06837	DP02342	10	Q	1

Figure 5.19: DataFrame for Disorder-PDB ground truth

5.5.2 Fetch ground truths arrays from CAID

We downloaded the ground truths data from the CAID web site, in the Data page. These ground truth files are in .FASTA, a file format for bioinformatic sequences.

In these files we have the ground truth for several DisProt ID, and for each of them to each residue is assigned a value between 0, 1 and '-'. '-' means no information, 1 is presence of disorder/binding and 0 is absence.

Finally we parsed these .FASTA files in pandas DataFrames and used them in our analysis. Down below an example of the first 10 rows of the DataFrame for disorder-PDB ground truth.

6

Conclusions

We developed a variation of the software-tool AlphaFold-disorder, replacing DSSP with PSEA and SASA. This was done mainly to avoid the dependency with DSSP and to explore an alternative way to obtain similar predictions, reproducing papers for this development as well. Then we integrated FoldComp too, so in the future we can store thousands of proteins with less storage required.

Once the development was finished we started to evaluate the results, we started with explorative plots to gain some visual insight on the features. Then we moved on to Machine Learning models to better evaluate the results. First of all we can see that AlphaFold-disorder (SASA) is on par to the AlphaFold-disorder with DSSP, which is a very good result. Then our ML models were good enough, with performance similar to the software-tools. Probably perfomances weren't better than the software-tools because we didn't have a big enough dataset for the ML models. Overall the results were quite satisfying but there is room for improvement.

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