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# COMPARISON BETWEEN HEURISTIC AND STATISTICAL ANALYSIS ON PROTEIN STRUCTURAL PROPERTIES

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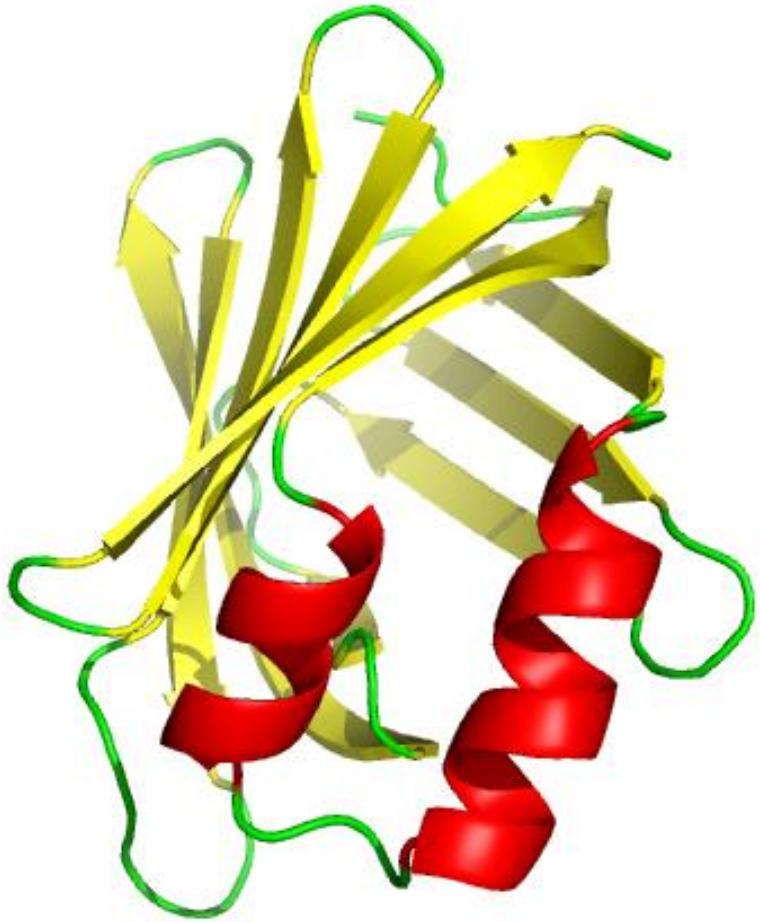
# Protein Structure

- **Biological macromolecules**
  - complex structural organization
  - balance of energetic factors
- **Homology among different organisms**
  - different sequence, same structure
  - amino acid substitution, different structure
- **Structure-function relationships**
  - some of them stronger than others
- **Examining protein structure**
  - analyzing conformational features



2CBR, A Chain, LCN, *Bos taurus*
(obtained with PyMol)

# Getting Data

- **Protein families with different architectural classification**
  1. Beta-lactamase (BLA)
  2. Cathepsin B (CTS)
  3. Ferritin (FTL)
  4. Glycosyltransferase (GTF)
  5. Hemoglobin (HGB)
  6. Lipocalin 2 (LCN)
  7. Lysozyme (LYS)
  8. P. Cell Nuclear Antigen (PCNA)
  9. P. Nucleoside Phosphorylase (PNP)
  10. Superoxide Dismutase (SOD)
- **153 Crystallographic structures**

**2.40.128.x β-β barrel**

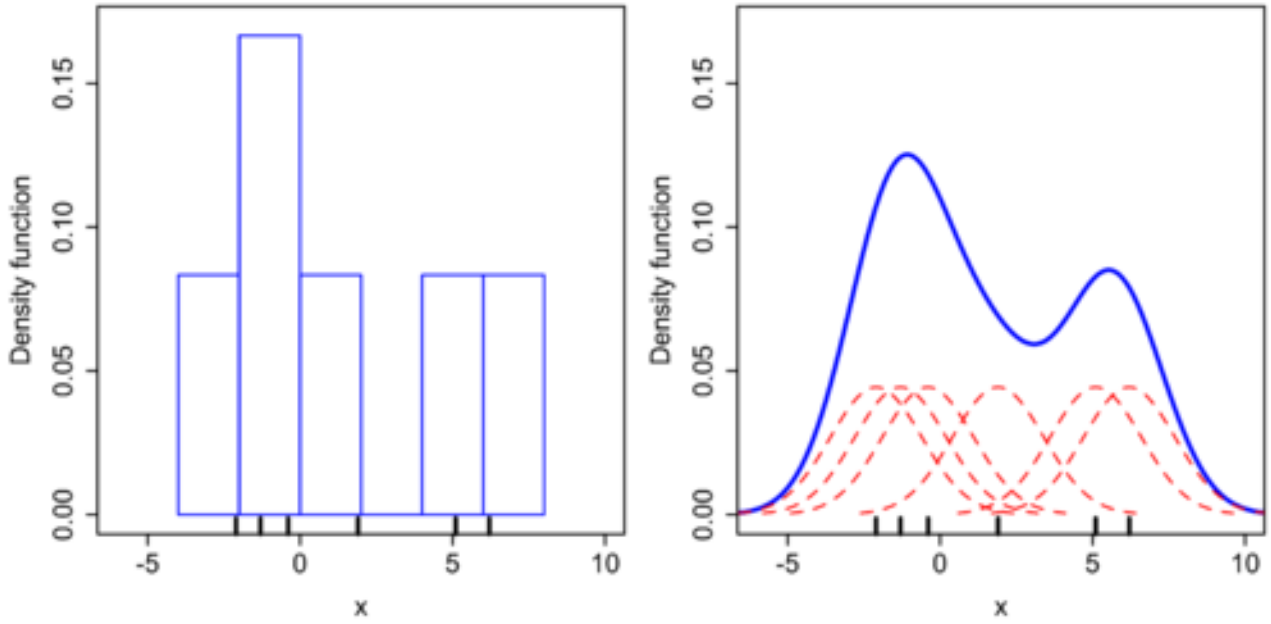
## Cleaning Data

1. Vadar
2. McVol
3. R-script

- **Similar number of structures, 13-19 for each family**
  - only wild-type, one for organism
  - less than 50 residues about length
- **Only one chain in homo-multimeric proteins**
  - chain A where available (chain E in 1M73 and chain X for 3CH2)
- **Structural-geometrical properties**
  - secondary structure, hydrogen bonds, accessible surface areas, torsion angles, packing defects, charged residues, free energy of folding, volume, salt bridges
- **Percentage features and standard score form**
  - better stability in evaluations

# EDA: Kernel Density Distribution

- **Non-parametric estimation of p.d.f.**
  - based on a finite data sample
- **Overcoming the histogram graph**
  - a more effective way to show the distribution of a variable
- **How variables are distributed**
  - for each protein family




$$\widehat{f_h}(x) = \frac{1}{nh}\sum_{i=1}^{n} K\left(\frac{x - x_i}{h}\right) \quad \text{with} \quad h \approx 1.06\,\frac{\hat{\sigma}}{\sqrt[5]{n}}$$

(if $K$ is a Gaussian distribution for univariate data)

# EDA: Correlation

- **Pearson's correlation coefficient**
  - graphical correlation matrix

$$\rho_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y}$$

- **Partial correlation coefficient**
  - able to avoid the collinearity

$$\rho_{yz \cdot x} = \frac{\rho_{yz} - \rho_{yx}\rho_{zx}}{\sqrt{1 - \rho_{yx}^2}\sqrt{1 - \rho_{zx}^2}}$$

- **Dissimilarity measurement**
  - Pearson's distance

$$d_{xy} = 1 - |\rho_{xy}|$$

- **T-Student test for significance**
  - confidence level of 0.95

$$t = \rho \sqrt{\frac{n-2}{1-\rho^2}}$$

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# EDA: Principal Component Analysis

- **Multivariate & unsupervised statistical method**
  - compressed data, new relationships
- **Summarizing initial variables into new ones**
  - semi-heuristic decision on variables number
- **Clusterization and outlier detection**
  - interpretation allowed to investigator
- **Sparse PCA, a hybrid technique with regression**
  - not all the variables are in the PCs

$$\begin{cases} PC_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1c}X_c \\ PC_2 = a_{21}X_1 + a_{22}X_2 + \cdots + a_{2c}X_c \\ \ldots \\ PC_l = a_{l1}X_1 + a_{l2}X_2 + \cdots + a_{lc}X_c \end{cases}$$

$$\max_{a_m} \left\{ \frac{1}{r} \sum_{i=1}^{r} \left( \sum_{j=1}^{c} a_{1j}x_{ij} \right)^2 \right\} \quad \text{with} \quad \sum_{j=1}^{c} a_{1j}^2 = 1$$

$$(\Sigma - \lambda_m I)a_m = 0$$

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# Classification: Variable Importance

- **Categorizing observations**
  - by means of predictive models for classification

- **Different algorithms used:**
  - random forest (RFO)
  - recursive partitioning (RPA)
  - stochastic gradient boosting (GBM)
  - boosting model (C50)
  - flexible discriminant analysis (FDA)
  - nearest shrunken centroid (NSC)

- **Different scores for variable importance estimation**
  - percentage of variables occurence (ranking)

**Resampling:**

a) Training set of 70% of data
b) Testing set of 30% of data
c) 10-fold cross validation
d) Repeating 10 times

# Classification: Performance

- **Accuracy**
  - proportion of true results among the total number of cases examined

$$ACC = \frac{TP + TN}{P + N}$$

- **Sensitivity**
  - proportion of positives that are correctly identified as such

$$TPR = \frac{TP}{P}$$

- **Specificity**
  - proportion of negatives that are correctly identified as such

$$TNR = \frac{TN}{N}$$

- **Kappa coefficient**
  - reliability of a statistical classification, related to the possible best classification

$$K = \frac{Pr(o) - Pr(e)}{1 - Pr(e)}$$

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# R Tools

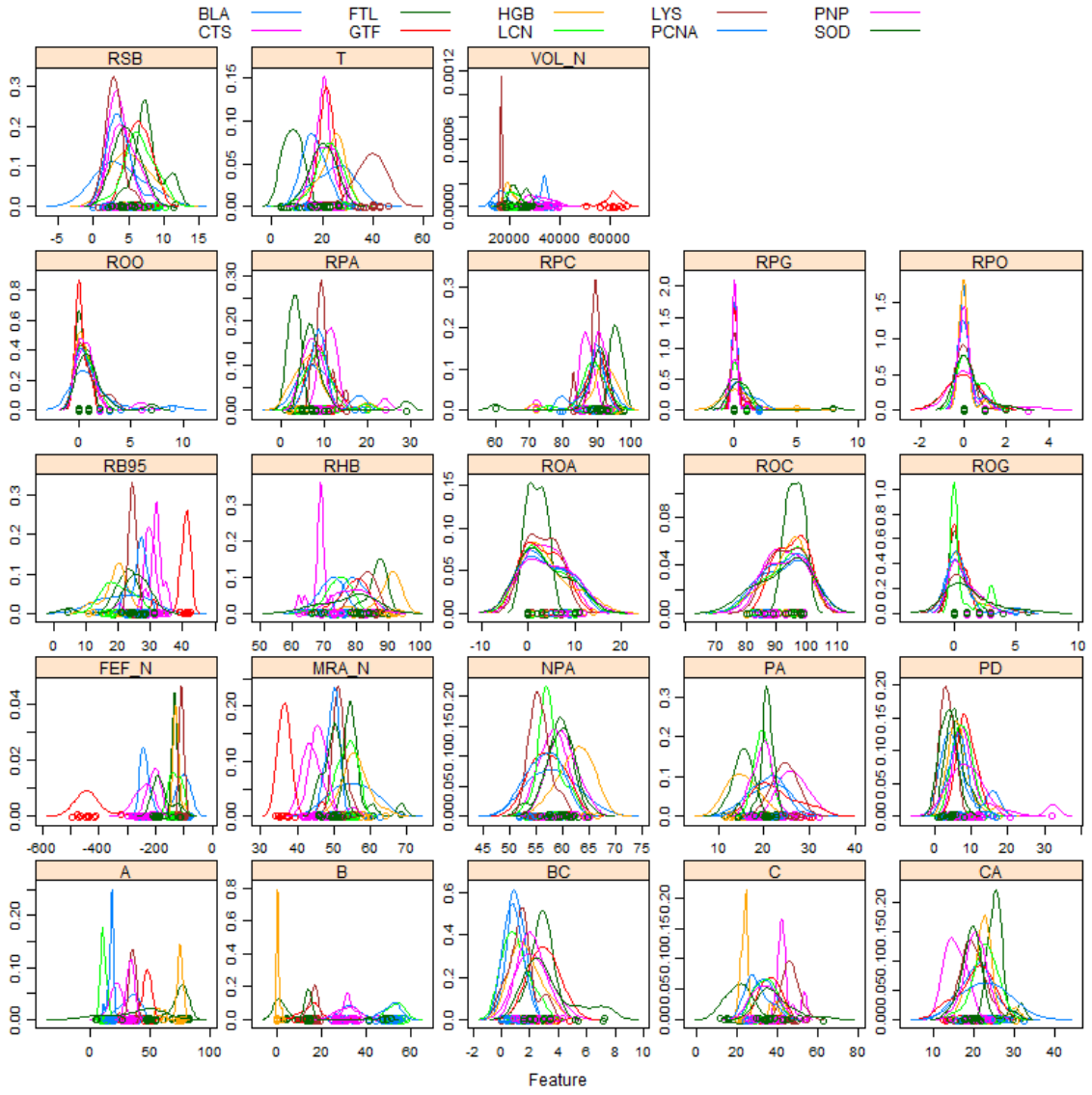
- ***R* environment in *Rstudio* IDE**
  - user and developer
  - Comprehensive R Archive Network (CRAN) & Bioconductor
- ***corrplot, Hmisc, ppcor***
- ***sparcl, GeneNet, caret***
- ***lattice, ggplot2, directlabels***

**Classification and Regression Training:**

a) data splitting
b) pre-processing
c) feature selection
d) model tuning using resampling
e) variable importance estimation

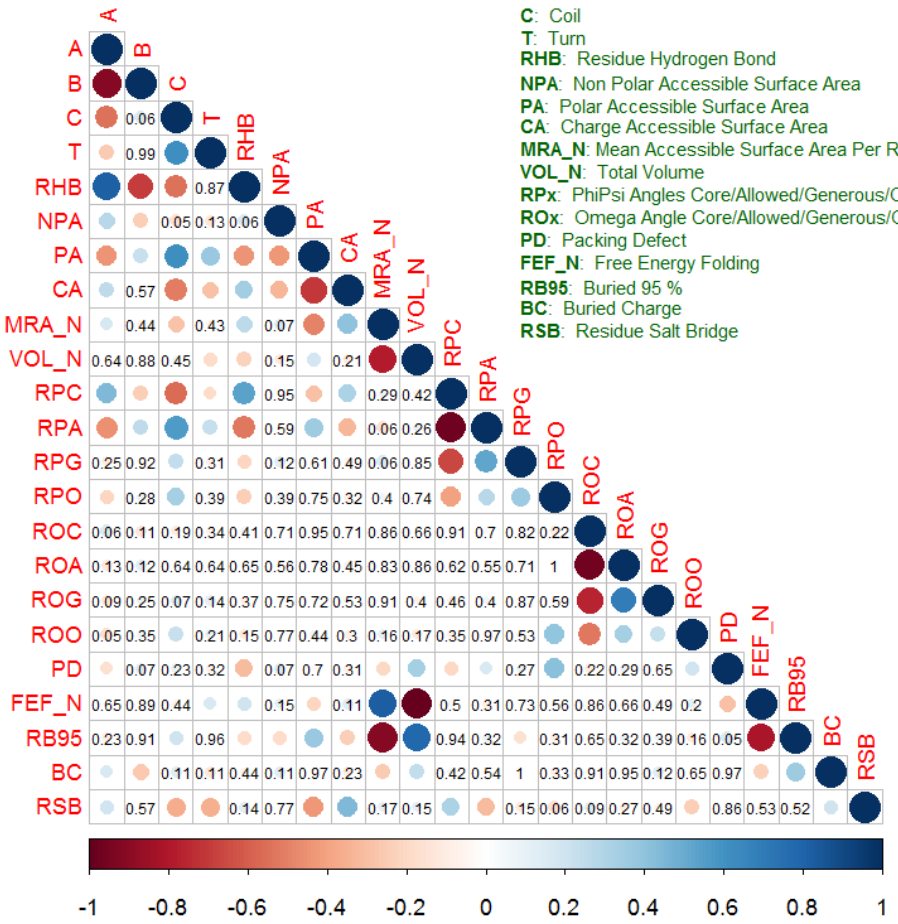
# Density Panels

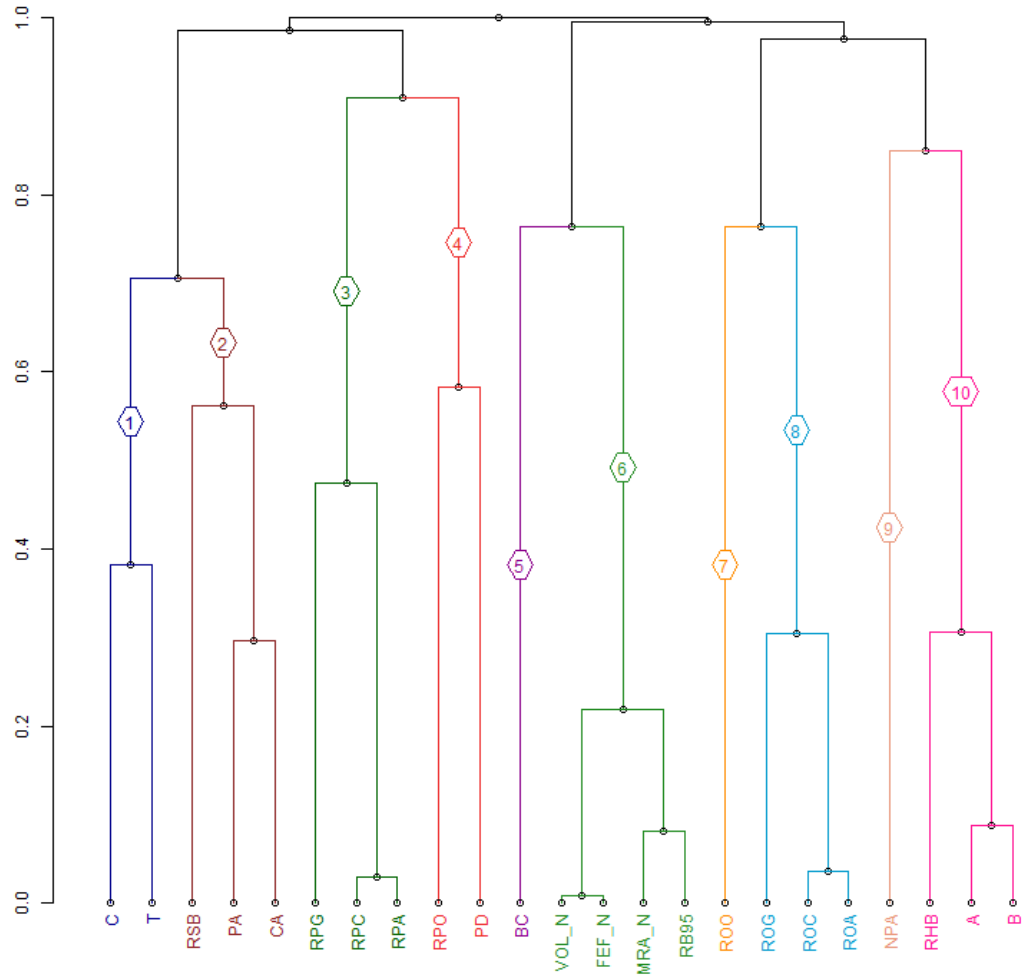
- **Features distributions regularity**
  - unimodal and centered
- **Some valuable results**
  - ROC, ROA in FTL
  - T in PNP and GTF
  - RB95 in all the families
- **A good overview**
  - on protein families
  - on protein structural features



# Dissimilarity Dendrogram

**A**: Alpha Helix
**B**: Beta Sheet
**C**: Coil
**T**: Turn
**RHB**: Residue Hydrogen Bond
**NPA**: Non Polar Accessible Surface Area
**PA**: Polar Accessible Surface Area
**CA**: Charge Accessible Surface Area
**MRA_N**: Mean Accessible Surface Area Per Residue
**VOL_N**: Total Volume
**RPx**: PhiPsi Angles Core/Allowed/Generous/Outside
**ROx**: Omega Angle Core/Allowed/Generous/Outside
**PD**: Packing Defect
**FEF_N**: Free Energy Folding
**RB95**: Buried 95 %
**BC**: Buried Charge
**RSB**: Residue Salt Bridge



# Features Network

- **Two kind of relationships**
  - continuous line: partial correlation
  - dotted line: partial anticorrelation
- **Pruning excessive features**
  - peripheral ones

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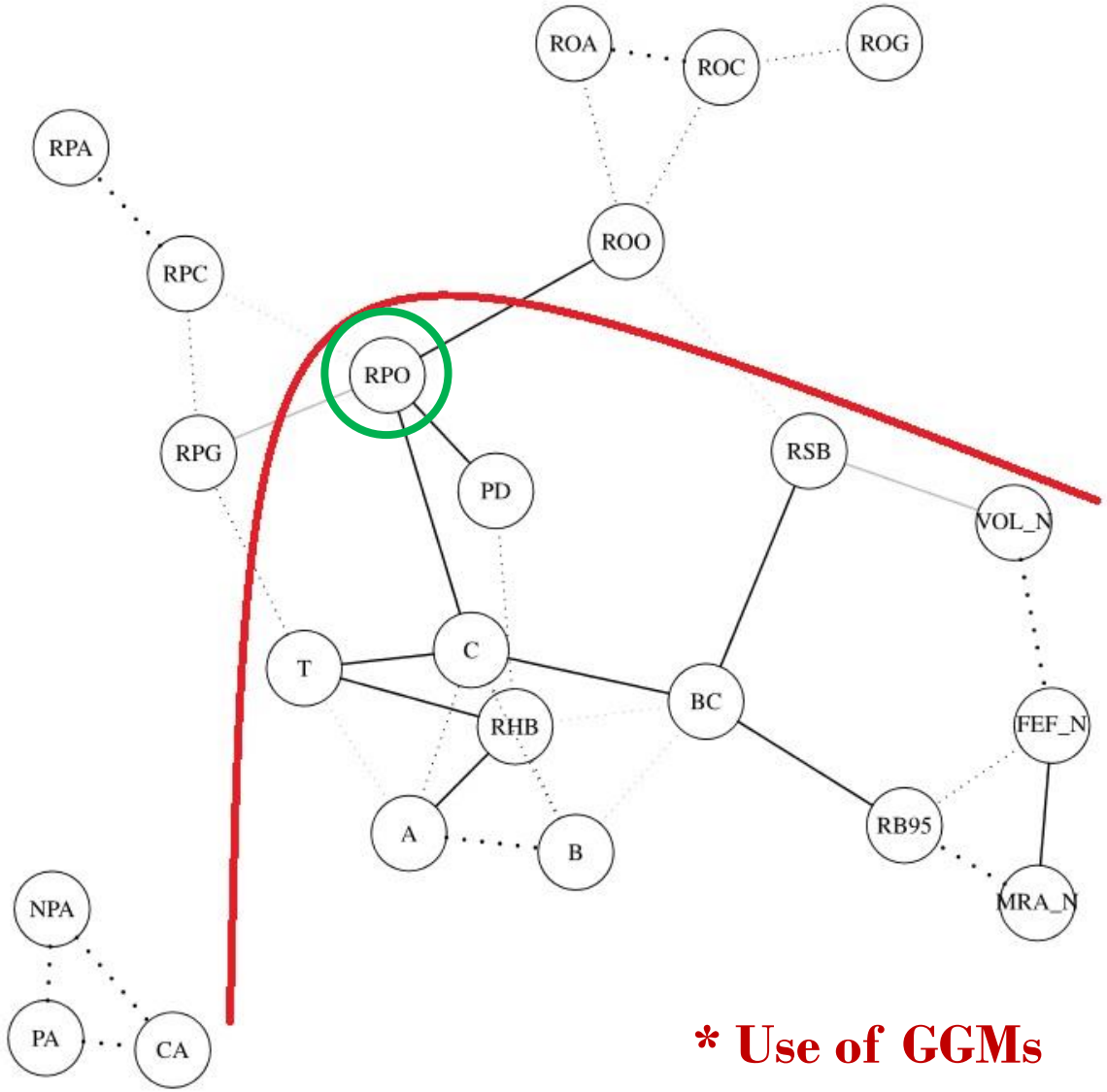
**a) Phi-Psi Angles**
**b) Omega Angle**
**c) ASA information**




*** Use of GGMs**

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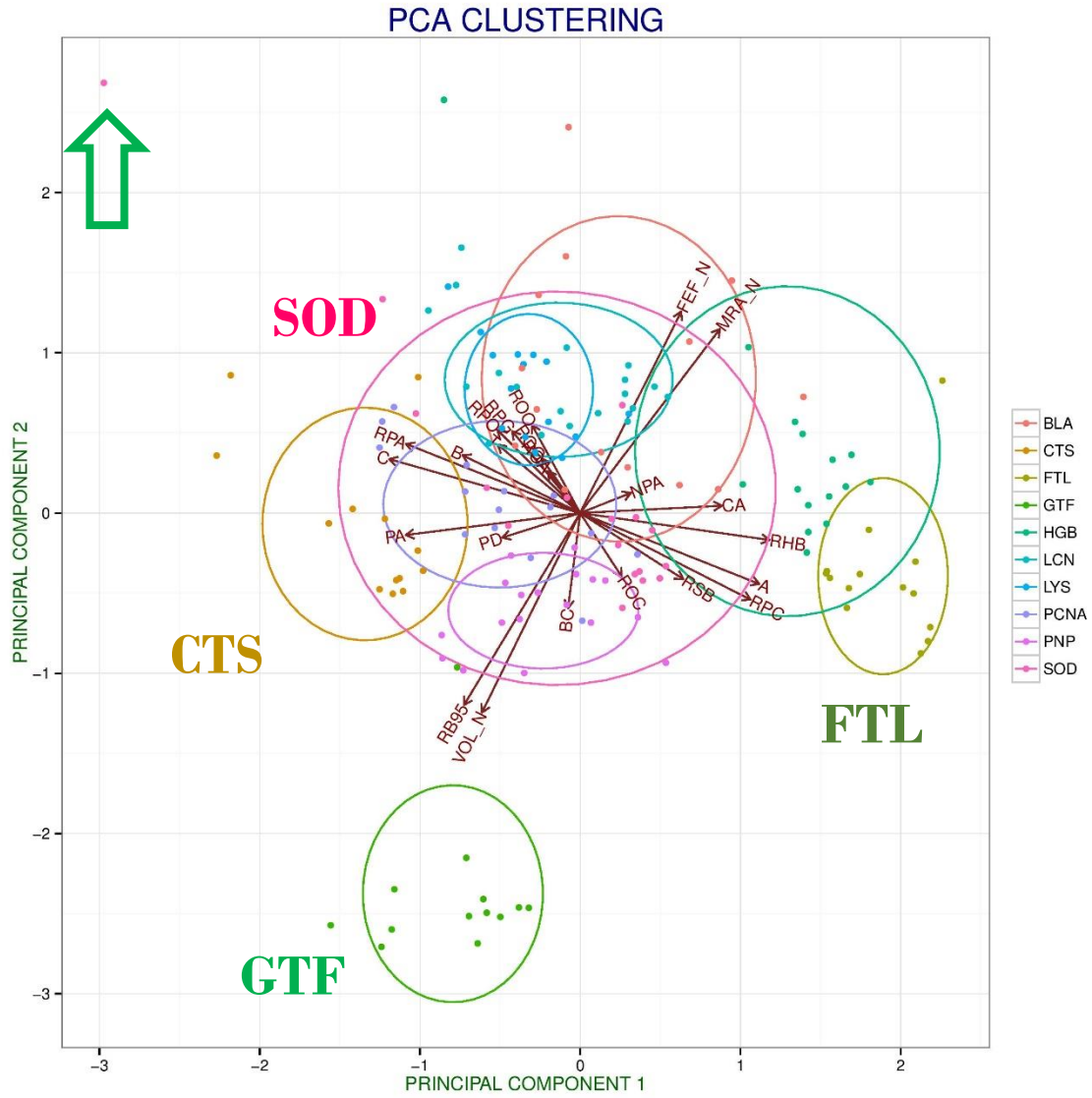
# PCA & sPCA

- **Clustering for protein families**
  - GTF, FTL, CTS, …
- **SOD is in a wide open position**
  - multi-structural architecture
- **(Possible) outliers detection**
  - *Pseudomonas putida* SOD
- **sPCA is coherent with the dendrogram**
  - 60% of explained variance
  - sPCs are clusterized



# Features Classification

| | RFO | RFO_S | RPA | RPA_S | GBM | GBM_S | C50 | C50_S | FDA | FDA_S | NSC | NSC_S | PERCENTAGE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 8 | 8 | 4 | 4 | 1,00 |
| B | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 1,00 |
| C | 10 | 9 | 8 | 8 | 6 | 8 | | | | | 7 | 10 | 0,67 |
| T | 7 | 8 | 5 | 5 | 10 | 7 | 6 | 6 | 4 | 4 | 9 | 8 | 1,00 |
| RHB | 9 | 6 | | | 9 | 9 | 5 | 5 | 9 | 9 | 8 | 7 | 0,83 |
| NPA | | | | | | | | | | | | | 0,00 |
| PA | 8 | 10 | | | 12 | 10 | | | 5 | 5 | 10 | 9 | 0,67 |
| CA | 11 | 12 | 9 | 9 | 8 | 12 | | | | | 11 | 11 | 0,67 |
| MRA_N | 6 | 5 | 6 | 6 | 7 | 6 | | | 7 | 7 | 5 | 5 | 0,83 |
| VOL_N | 2 | 1 | 3 | 3 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1,00 |
| RPC | | | | | | | | | | | | | 0,00 |
| RPA | | | | | | | | | | | 12 | | 0,08 |
| RPG | | | | | | | | | | | | | 0,00 |
| RPO | | | | | | | | | | | | | 0,00 |
| ROC | | | | | | | | | | | | | 0,00 |
| ROA | | | | | | | | | | | | | 0,00 |
| ROG | | | | | | | | | | | | | 0,00 |
| ROO | | | | | | | | | | | | | 0,00 |
| PD | | | | | 11 | | | | | | | | 0,08 |
| FEF_N | 3 | 4 | 4 | 4 | 5 | 5 | 4 | 4 | 3 | 3 | 2 | 2 | 1,00 |
| RB95 | 5 | 7 | 7 | 7 | 4 | 4 | | | | | 6 | 6 | 0,67 |
| BC | 12 | 11 | | | | 11 | 7 | 7 | 6 | 6 | | | 0,58 |
| RSB | | | | | | | | | | | | 12 | 0,08 |

| | RFO | RFO_S | RPA | RPA_S | GBM | GBM_S | C50 | C50_S | FDA | FDA_S | NSC | NSC_S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ACCURACY | 0,95 | 0,95 | 0,59 | 0,60 | 0,94 | 0,95 | 0,89 | 0,88 | 0,95 | 0,94 | 0,89 | 0,83 |
| KAPPA | 0,95 | 0,94 | 0,54 | 0,56 | 0,93 | 0,94 | 0,88 | 0,86 | 0,94 | 0,93 | 0,88 | 0,81 |
| SENSITIVITY | 0,97 | 0,97 | 0,50 | 0,50 | 0,93 | 0,93 | 0,86 | 0,86 | 0,93 | 0,93 | 0,91 | 0,82 |
| SPECIFICITY | 0,99 | 0,99 | 0,95 | 0,95 | 0,99 | 0,99 | 0,99 | 0,99 | 0,99 | 0,99 | 0,99 | 0,98 |
| PERFORMANCE | 0,97 | 0,96 | 0,65 | 0,65 | 0,95 | 0,95 | 0,91 | 0,90 | 0,95 | 0,95 | 0,92 | 0,86 |
| P-VALUE | 1,00E-29 | 1,00E-29 | 1,83E-10 | 1,83E-10 | 1,46E-27 | 1,46E-27 | 5,05E-22 | 5,05E-22 | 1,46E-27 | 1,46E-27 | 1,37E-26 | 2,44E-17 |

# Features «Selection»

- **Comparing all the used techniques**

- **Variables subset as typical for the protein families dataset**
  - A, B, C, T, RHB, PA, CA, MRA_N, VOL_N, RB95

- **Free energy of folding (FEF_N) strictly related to volume**
  - because of the prediction formula…

- **Structural defects seem to influence the present study**
  - not so strong in all the methods

# Conclusions and Future Works

- **Graphical multivariate procedures are good tools**
  - characterization
  - fingerprints

- **Predictive models for classification to perform feature selection**
  - knowledge < information < data

- **How to improve the work?**
  - multivariate regression models
  - protein families number

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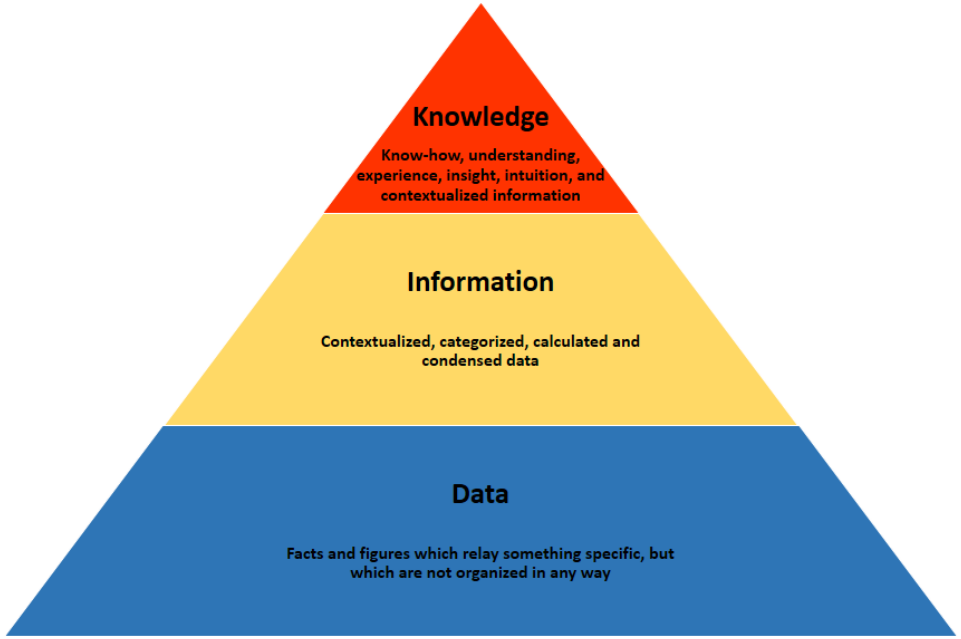
a) Transglutaminase
b) Superoxide Dismutase
c) Glycosyl Hydrolase



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