A Tool for Quantitative Analysis of Proteomics Data

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◆ Introduction

- **Proteomics** is "the study of proteins, how they're modified, when and where they're expressed, how they're involved in metabolic pathways and how they interact with one another" – *Mark Wilkins*
- Quantitative proteomics has become increasingly effective in understanding the biology and biomarkers for diseases.
- Issues related to quantitative proteomics[1]:
  - Systematic variations among technical and biological replicate measurements
  - Inference of protein abundances from the observed peptide abundances
  - Undetected peptides leading to "missing values"
  - Statistical comparison of sample groups
- **Inferno** is designed to address these issues featuring:
  - Normalization methods
  - Missing value imputation algorithms
  - Peptide to protein rollup methods
  - Statistical plots
  - Hypothesis testing schemes (unbalanced data, random effects)

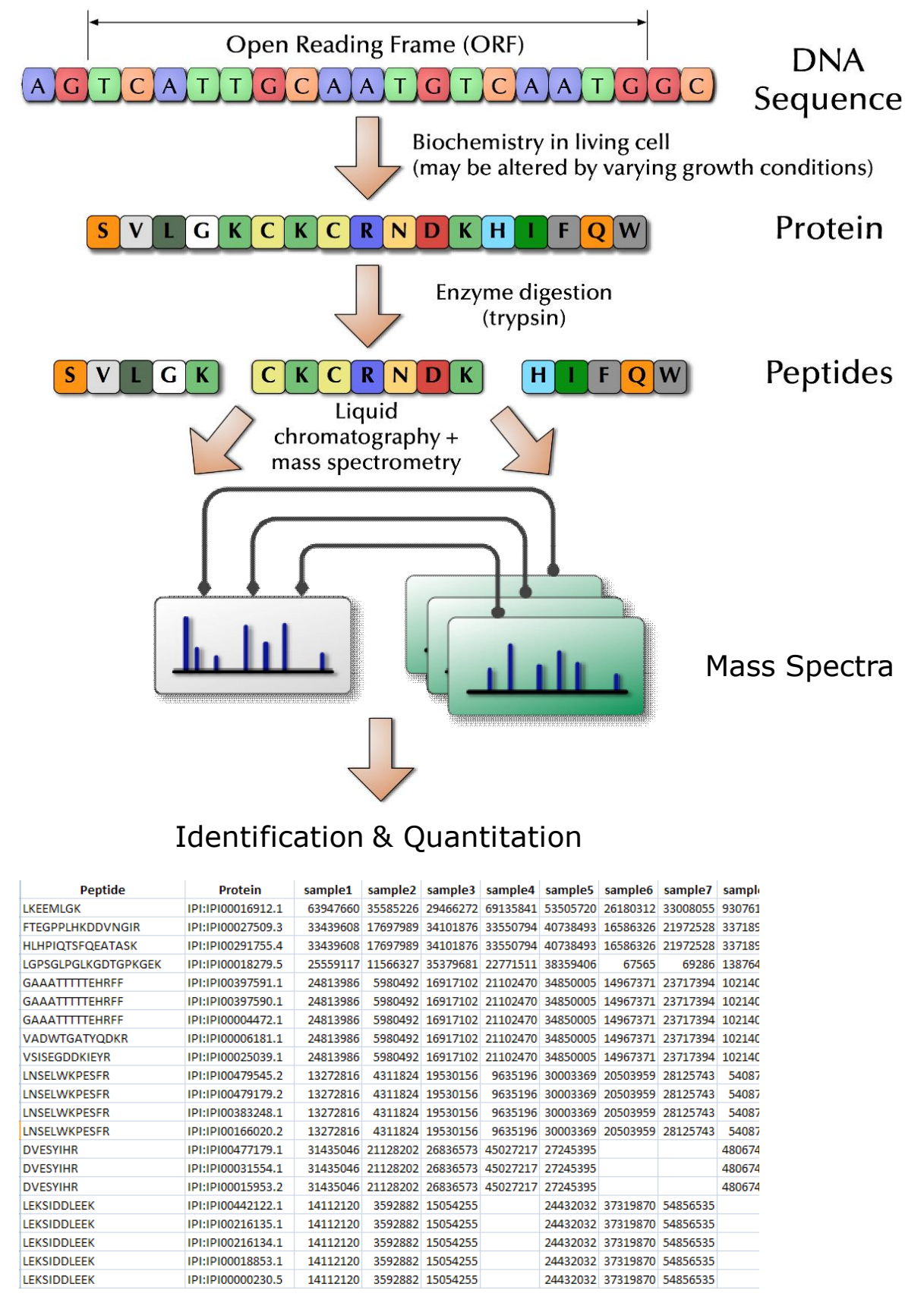


Figure 1. Proteomics Process

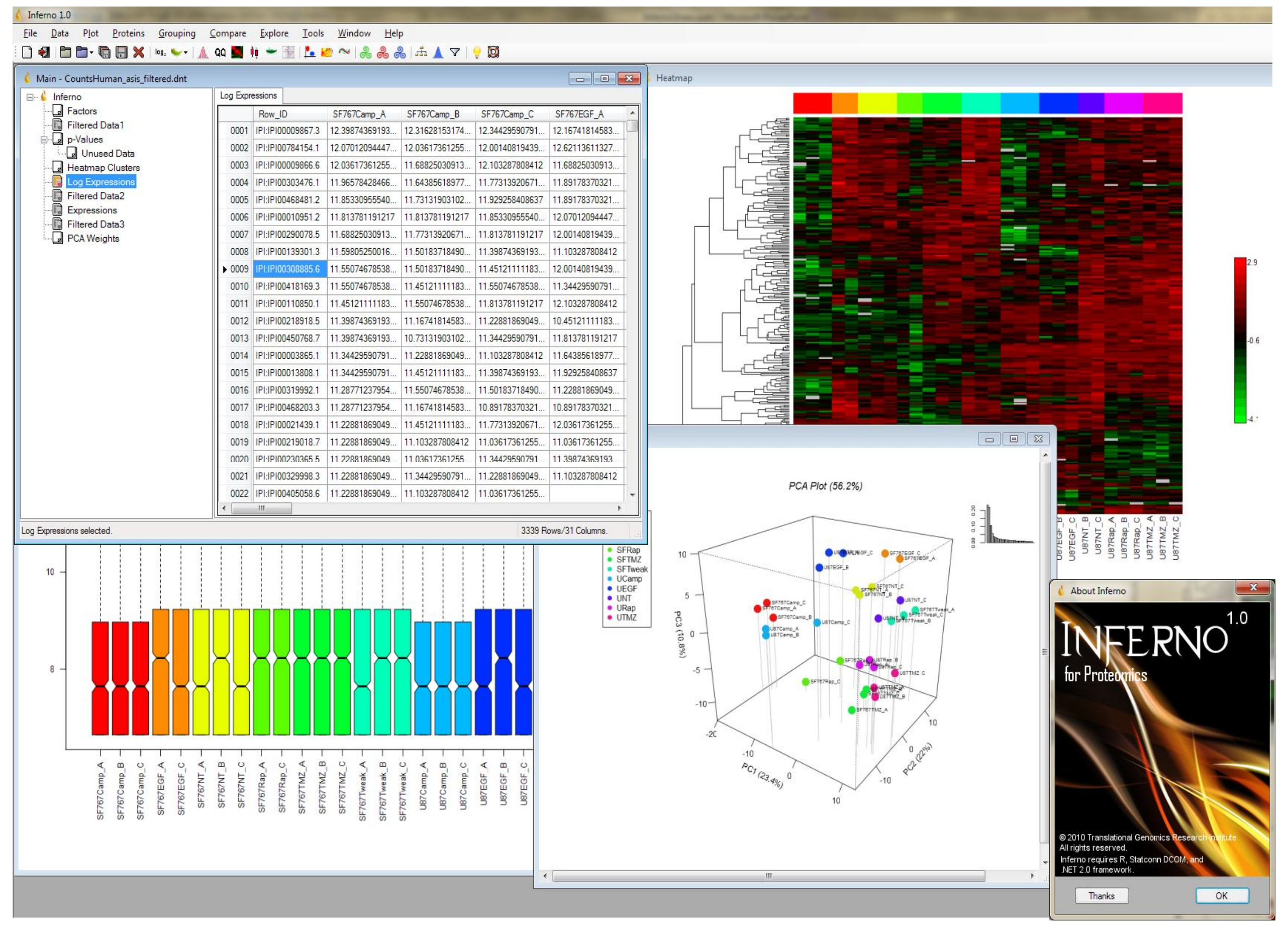
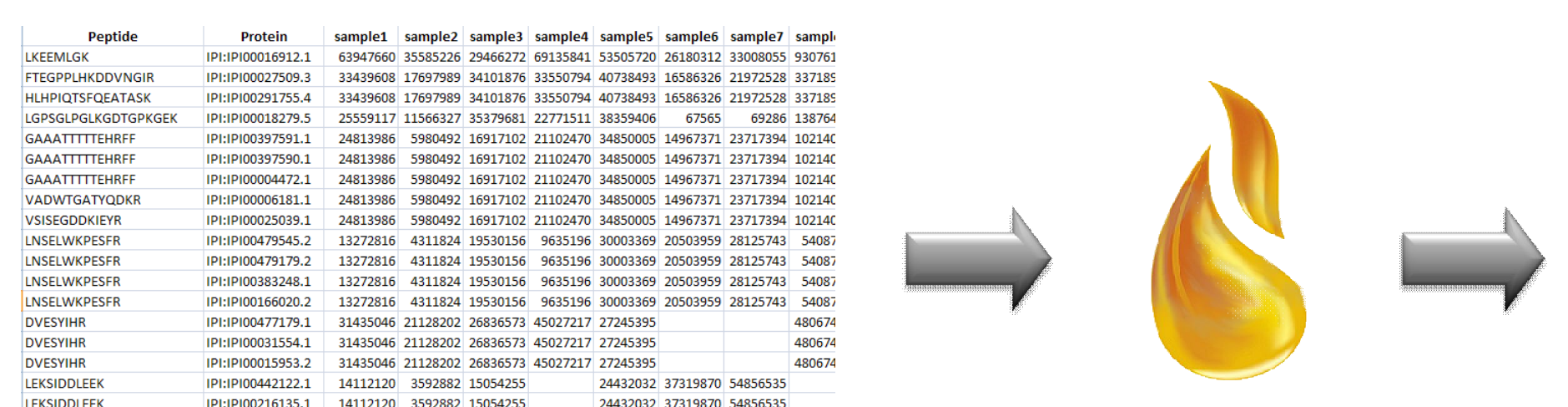


Figure 2. Inferno for Proteomics Software

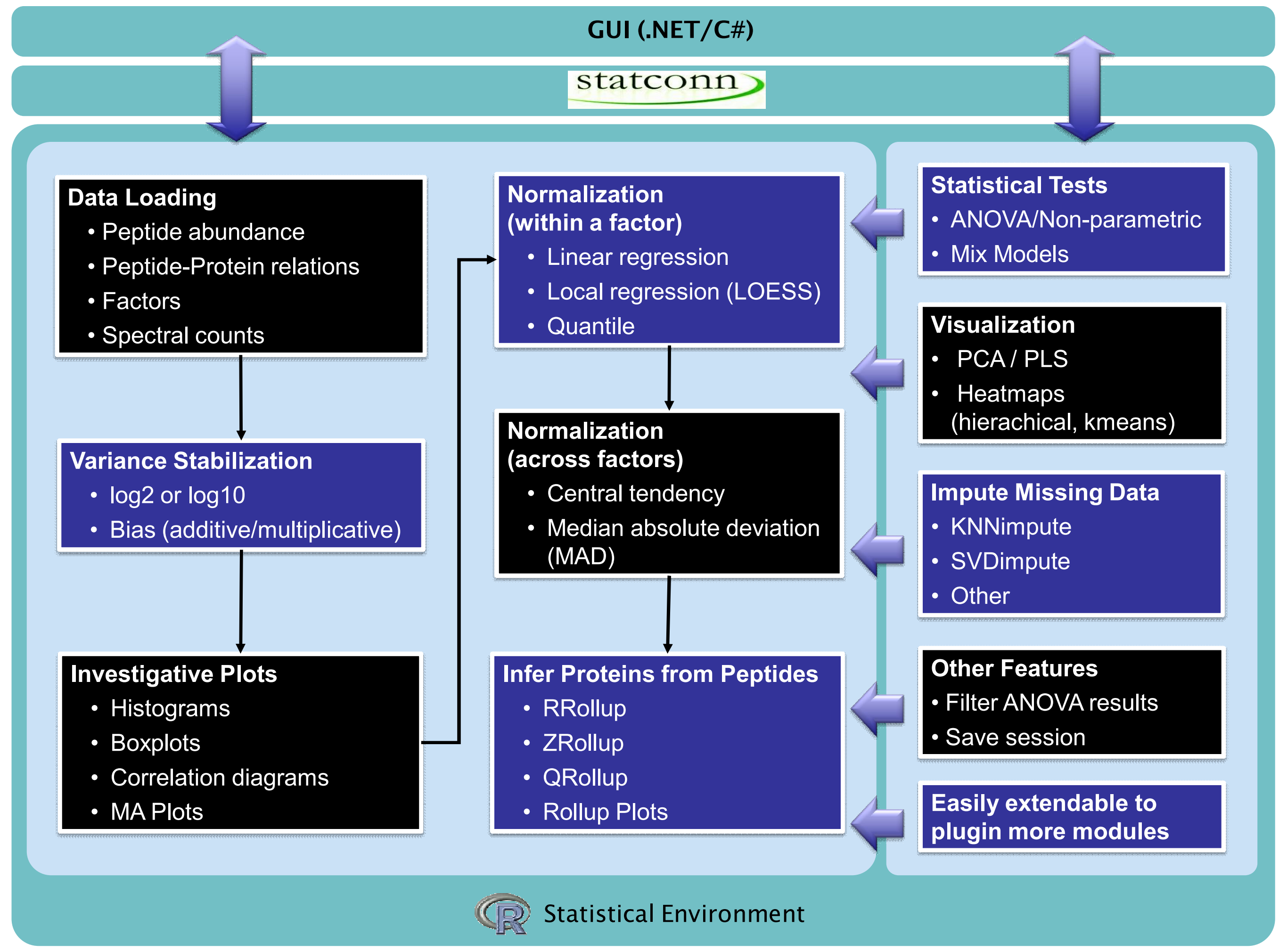
◆ Inferno Design and Features

Multiple samples grouped using factors

- Biological conditions
- Biological replicates
- Technical replicates



Factors capture the experimental design via fixed and random effects. This information is later used in normalization, imputation, and hypothesis testing methods in Inferno.



GUI (.NET/C#)

statconn

Data Loading
- Peptide abundance
- Peptide-Protein relations
- Factors
- Spectral counts

Variance Stabilization
- log2 or log10
- Bias (additive/multiplicative)

Investigative Plots
- Histograms
- Boxplots
- Correlation diagrams
- MA Plots

Normalization (within a factor)
- Linear regression
- Local regression (LOESS)
- Quantile

Normalization (across factors)
- Central tendency
- Median absolute deviation (MAD)

Infer Proteins from Peptides
- RRollup
- ZRollup
- QRollup
- Rollup Plots

Statistical Tests
- ANOVA/Non-parametric
- Mix Models

Visualization
- PCA / PLS
- Heatmaps (hierachical, kmeans)

Impute Missing Data
- KNNimpute
- SVDimpute
- Other

Other Features
- Filter ANOVA results
- Save session

Easily extendable to plugin more modules

Statistical Environment

Figure 3. Inferno Design and Analysis Flow

◆ Normalization Methods[2]

Normalization is done to minimize systematic variation.

- Robust linear regression
- Lowess method
- Quantile method
- Global intensity adjustment using Median Absolute Deviation (MAD)
- Central tendency adjustment

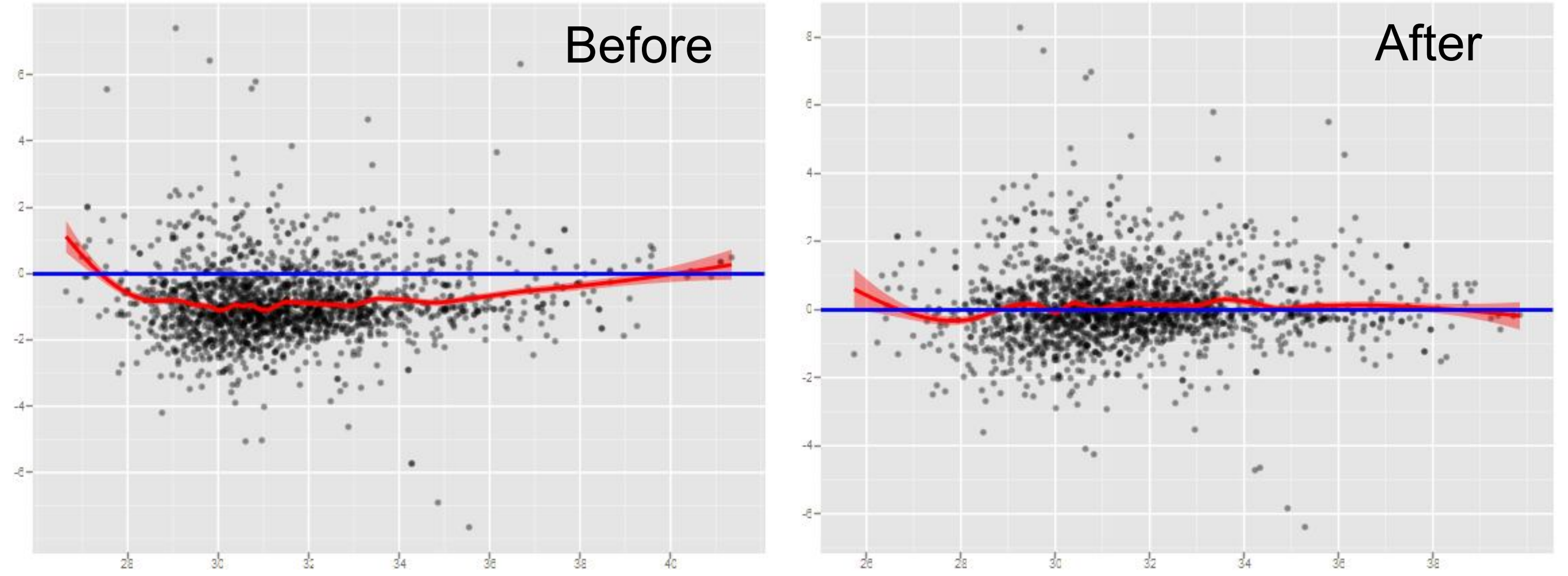


Figure 4. Normalization using LOWESS

◆ Protein Quantitation

- 156 proteins (ANOVA with an FDR of 5%)
- Hierarchical clustering groups related proteins

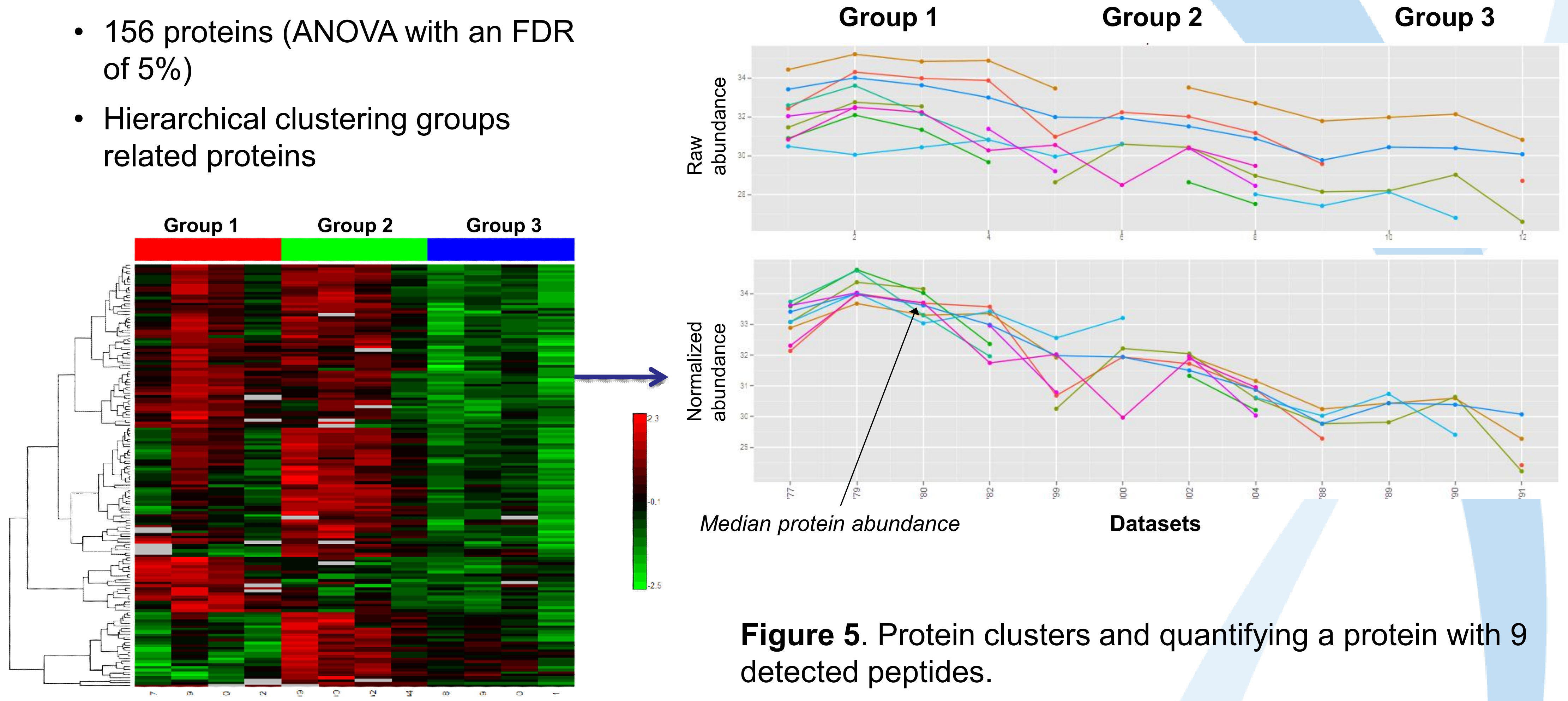
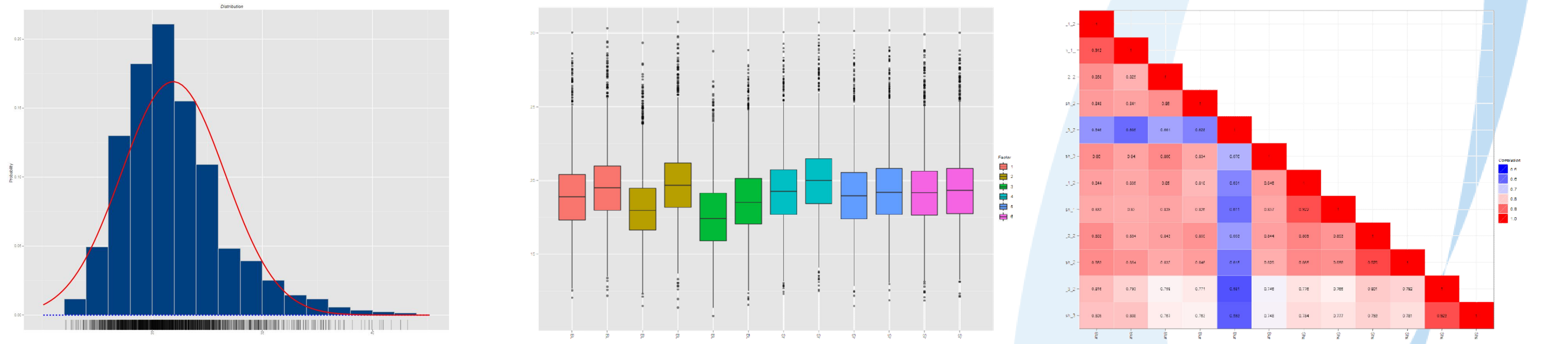
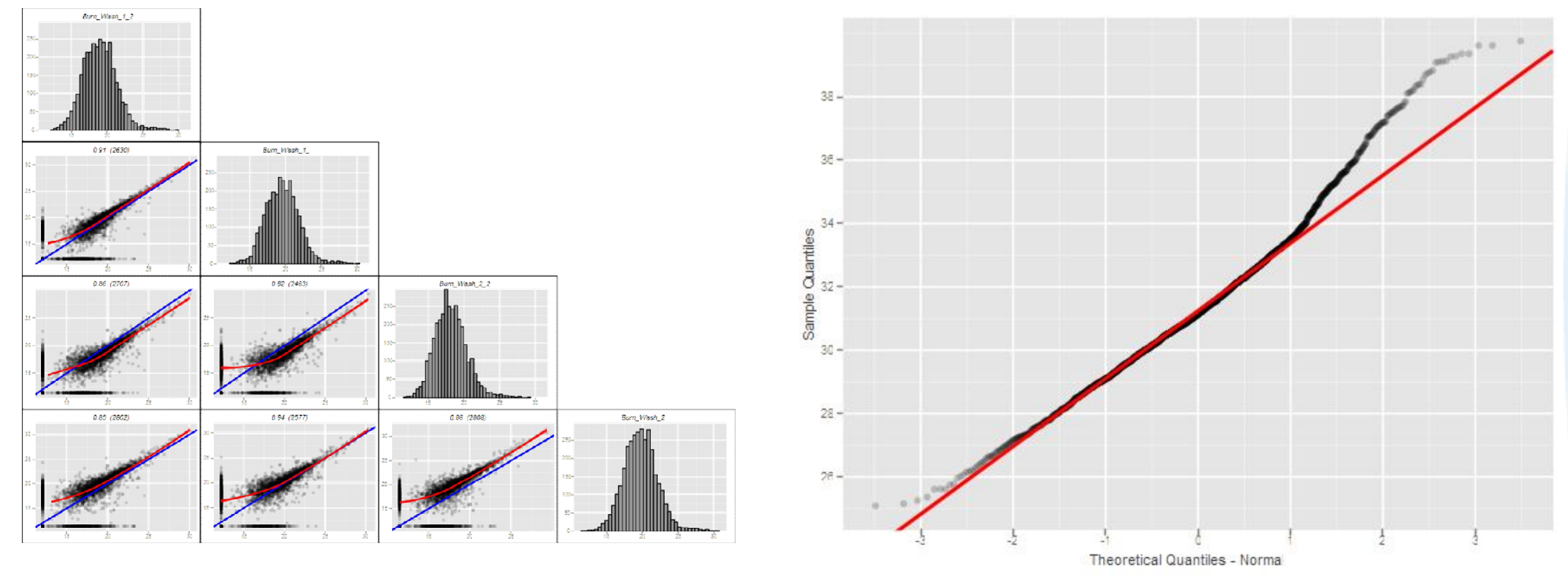


Figure 5. Protein clusters and quantifying a protein with 9 detected peptides.

◆ Statistical Plots





- Histograms
- BoxPlots
- Correlation diagrams
- QQ Plots
- …

◆ References

1. AD Polpitiya, et al. *Bioinformatics* 2008, 24: 1556-1558.
2. SJ Callister, et al. *J Proteome Res* 2006, 5: 277-286.

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Inferno is available at http://inferno4proteomics.googlecode.com