Background of Nonlinear Dynamics

- We develop innovative high quality data analysis software for proteomics and metabolomics to help you:
  - Achieve breakthroughs and increase scientific understanding
  - Make real discoveries that you can pursue with confidence, leading to improvements in life quality for everyone
- Founded 1989 with head office in Newcastle upon Tyne, UK
  - US office based in Raleigh Durham, North Carolina
1. Based on a common concept, solving the key challenges in relative quantification
   - Progenesis LC-MS & Progenesis CoMet also benefit from a quantify-then-identify approach
2. Developed with key opinion leaders within proteomics and metabolomics
3. Adopted and proven across many labs around the world

Products:
   - Progenesis SameSpots for 2D gel based proteomics
   - Progenesis LC-MS for MS-based proteomics
   - Progenesis CoMet for MS-based metabolomics
   - Progenesis MALDI for MS-based proteomics

Progenesis™ Concepts

Goal of metabolomics discovery?

“To identify the **compounds** that warrant further investigation as **rapidly, objectively** and **reliably** as possible.”

- **Progenesis CoMet** developed with this in mind...
  - Produce a comprehensive **table of detected compounds**, which you can easily share or validate and put them in biological context
  - A complete analysis approach to combine quantification and identification of significantly changing compounds, in **one streamlined package**
  - An **objective approach** to analysis, using a complete matrix of data with no missing values, for results based on **reliable statistics**
Relative quantification of metabolites

- LC-MS (ESI) is one technique used for such quantification
- Examples of applications:
  - **Discovery** - relative changes in metabolite abundance related to experimental conditions
  - **Targeted** - monitoring the relative abundances of numerous known compounds simultaneously

Key steps in metabolomics discovery

- **Relative quantification of metabolites**
  - RT data alignment
  - Peak picking including **ion deconvolution**
  - Quality control, particularly of LC and quant
  - Data normalisation
  - Statistical analysis

- **Identification**
  - Several approaches (e.g. m/z, neutral mass, ± RT, ± MS/MS spectra)
  - No standardised search engine
  - Search against in-house or evolving public databases
  - Numerous putative ID's
Challenges of current analysis approaches

1. Many separate analysis tools and databases are used, with manual intervention to move data from one stage to the next
2. Little opportunity to visually explore the data
3. You can quantify something that hasn’t been identified, and identifications can be ambiguous
4. Discovery experiment needs not as well served as those of a more targeted metabolomics approach, which typically start with knowing what you are after
5. Missing values in the data reduce reliability of statistical tests
6. Not easy to provide a final list of putative compounds for validation and review by biologists

7. And yours?
How does it solve the challenges of metabolomics data analysis?

The challenge of missing values

- What are missing values?
  - Any feature that is not matched in every replicate in the experiment

- This is a problem because of the effect on various statistical tools which impact the conclusions drawn
  - Reduced power of the statistical tools
Automatic retention time alignment

Before

After

Sample 1

Sample 2

Sample 1 & 2 aligned

Retention time

m/z

Peak picking and co-detection

Mapping the detection to all runs avoiding missing data

Aggregate co-detection
Co-detection benefits

• Consistent peak picking across all runs
  – Vital for reliable relative quantification

No missing data!

Identification can be confirmed by any other file(s)

Compound ion identified in one, comparable across all groups

Compound ion quantification
Quantify co-eluting ions

Blue areas contributes to the abundance of the ion

Red areas contributes to the abundance of the co-eluting ion

Combining co-eluting compound ions

- Deconvolution of co-eluting ion forms
  - Enables the neutral mass to be determined
  - More accurate and reliable compound identifications
Measure expression in multiple groups

- Set up correct experiment design with valid statistics
  - Import experiment design files to **instantly create groups** from hundreds of runs

Quantifying compound abundances
Review compounds

- Quickly find the compounds that are **significantly changing** between any groups
  - e.g. Anova p-value, fold-change, power

Compound identification

- MetaScope, our search tool, is integrated into the software
  - Search your own data and return compound identifications, **including chemical structures** from SDF databases
Fragmentation support for identification

- **Search SDF databases** using neutral mass, m/z, adduct mass, fragment ion and retention time
- Return putative identifications with **fragment ion matches displayed**

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**Progenesis Stats**

Easy to use, multivariate statistical analysis
Univariate analysis (e.g. t-test / ANOVA)

Multivariate analysis
Statistical analysis of metabolomics data

- Find significant Metabolites using statistical tools with a complete matrix of quantitative data (no missing data) incl:
  - ANOVA p-values
  - Principal Components Analysis (PCA) unsupervised display of how samples group to best describe differences between them, a good QC check!
  - Correlation analysis of peptide ions i.e. see which peptides are highly correlated within and across groups
  - Power analysis per feature to reduce type II errors
  - Predictive power for predicting how many samples are needed to be confident you are not missing something
  - False discovery rate correction (q-values) to reduce type 1 errors

Put results into biological context

- Comprehensive data export options to complement your bioinformatics-based strategies
  - Compound measurements and identifications
  - Raw abundance of every isotope peak for every adduct
Capture publication-quality images

• Work through your analysis adding images and tables to a clip gallery
• Select high quality images for publications, posters and presentations
  – Saved as high resolution .png files (300 dpi)

Progenesis CoMet
Discover the significantly changing compounds in your samples…