TWO-DAY SHORT COURSE, Saturday and Sunday
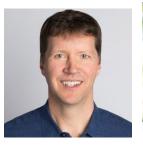**13 Quantitative Proteomics: Case Studies**

Instructors

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Preliminary Agenda

Time	Day1: 06/03/23 - Introduction	Day2: 06/04/23 - Advanced
9:00am-10:00am	Lecture : Fundamental concepts of statistical experimental design	**Case study 2: Refining targets with DIA data in Skyline.** *Highlights*: Preparing targets for a DIA experiment with many replicates
10:00am-10:30am	break	break
10:30am-12:00pm	**Case study 1 : Two-group group comparison design; SRM data in Skyline.** *Highlights*: (1) Importance of experimental design, distinction between bio and tech replicates, and randomization. (2) Data exploration with chromatograms, signal interference, types of missing values). (3) Normalization with global standards.	**Case study 2: Multi-group comparison design with many bio replicates; DIA data in Skyline.** *Highlights*: (1) Batch analysis of subsets of replicates. (2) Use of controls. (3) Between- and within-batch normalization. (3) Types of missing values. (4) Filtering with respect to quality of quantitative signals
12:00pm-1:00pm	Lunch	Lunch
1pm-2pm	**Case study 1**, continued.	**Case study 2**, continued.
2pm-2:30pm	break	break
2:30pm-3:15pm	Lecture : Statistical inference, multiple testing, MSstats package	**Case study 2 : Statistical inference for multi-group experimental design in MSstatsShiny.** *Highlights:* (1) Annotations of unbalanced experimental design with multiple groups and age confounder. (2) Impact of adjustment for age on statistical conclusions. (3) Impact of various choices of normalization, filtering, and handling missing values on statistical conclusions.
3:15 - 4 pm	**Case study 1 : Statistical inference for two-group comparison designs in MSstatsShiny.** *Highlights:* (1) Annotations of experimental design in experiment with bio and tech reps. (2) Use and interpretation of statistical models. (3) Visualization of the results.	
Extra time	Extra practice and Q&A: Skyline and MSstats based on participants' feedback	Extra practice and Q&A: Skyline and MSstats based on participants' feedback

In Case study 1, we will analyze an SRM proteomics dataset from a case-control study of heart failure using a salt-sensitive rat model, which includes biological and technical replicates. Participants will explore the importance of study design (in particular of normalization and randomization), the importance of visualizing chromatograms (and signal interferences), and setting up, conducting and interpreting the results of statistical analyses in MSstats.

In Case study 2, we will analyze a modern Orbitrap DIA dataset from the MacCoss lab proteomics dataset of cerebrospinal fluid from a Parkinson's and Alzheimer's cohort with healthy age-matched controls. Participants will learn how to handle and analyze DIA data in Skyline and in MSstats, and explore how data collection and processing can influence our statistical analysis; including batching, filtering, normalization, and missing values.

Additional course materials will be available at this link.