

## Targeted Proteomics Strategies – Skyline, DDA and DIA/SWATH

PROUDLY SUPPORTED BY SCIEX AND the AUSTRALASIAN  
PROTEOMICS SOCIETY



**MONDAY JANUARY 29 – WEDNESDAY JANUARY 31, 2018**

**LOCATION: UNIVERSITY OF MELBOURNE (PARKVILLE) – LECTORIAL  
ROOM 556, ARTS WEST NORTH WING ([see map](#))**

**REGISTRATION DESK:** Kenneth Myer Building (building 144) | 30 Royal  
Parade, Parkville, VIC 3052| ([see map](#))

### **Instructors:**

Brendan MacLean – University of Washington

Sandi Spencer – University of Washington

Brian Searle – Proteome Software / University of Washington

### **Closing Lecture:**

Professor Ruedi Aebersold – ETH Zurich, Switzerland

### **About this Workshop:**

This Pre-Lorne Workshop will introduce theoretical and practical concepts in targeted proteomics. Topics that will be covered include targeted acquisition focusing on selected reaction monitoring (SRM), data dependent acquisition strategies, statistical considerations, label-free quantitation and data independent acquisition (DIA) mass spectrometry (including SWATH-MS). We will introduce the targeted proteomic software environment Skyline and provide hands-on tutorials that demonstrate its functionality for the analysis of SRM, DDA and DIA/SWATH-MS data. Participants will be required to bring their own laptop with Skyline installed (requires Windows, or a Windows virtual machine for Mac/Linux users). Basic knowledge of mass spectrometry-based proteomics is assumed.

## FINAL PROGRAM

---

### MONDAY 29 JANUARY:

#### 09:30 REGISTRATION OPENS

*The registration and all catered sessions are taking place in the foyer of the Kenneth Myer Building, next to "Dr DAX".*

**Kenneth Myer Building (building 144)** | 30 Royal Parade, Parkville, VIC 3052 | ([see map](#))

*From 9:30-10:30 SETUP and MORNING TEA*

10:30-10:40 **Brendan MacLean**: Opening Remarks

10:40-11:40 Lecture: Moving from discovery to targeted proteomics (**Sandi Spencer**)

11:40-12:40 Lecture: Introduction to Skyline(**Brendan MacLean**)

*12:40-1:30 Lunch*

1:30-2:45 Hands-on: Targeted method editing in Skyline (**Brendan MacLean**)

2:45-4:00 Hands-on: Targeted method refinement in Skyline (**Brendan MacLean**)

*4:00-4:30 Break (Afternoon Tea)*

4:30-5:30 Lecture: Building prior knowledge for SRM assays (**Sandi Spencer**)

### TUESDAY 30 JANUARY:

8:30-9:45 Hands-on: Data analysis in Skyline (**Brendan MacLean**)

9:45-10:45 Lecture: Protein quantification (**Sandi Spencer**)

*10:45-11:15 Break (Morning Tea)*

11:15-12:15 Hands-on: Group comparison statistics and reports in Skyline (**Brendan MacLean**)

12:15-1:15 Hands-on: Calibrated quantification with Skyline (**Brendan MacLean**)

*1:15-2:00 Lunch*

2:00-2:30 Lecture: Overview and keys to success in processing DDA data with Skyline (**Brendan MacLean**)

2:30-4:00 Hands-on: Label-free quantitative analysis of DDA data with Skyline (**Brendan MacLean**)

*4:00-4:30 Break (Afternoon Tea)*

4:30-6:00 Hands-on: Parallel reaction monitoring (PRM) with Skyline (**Sandi Spencer**)

## WEDNESDAY 31 JANUARY:

9:00-9:30 Lecture: Introduction to DIA (**Brian Searle**)

9:30-10:30 Hands-on: Extracting peptides from DIA in Skyline (**Brian Searle**)

*10:30-11:00 Break (Morning Tea)*

11:00-11:30 Lecture: Details of DIA acquisition (**Brian Searle**)

11:30-12:30 Hands-on: Large-scale DIA with DDA libraries in Skyline (**Brian Searle**)

*12:30-1:30 Lunch*

1:30-3:30 Hands-on: Analysing DIA with Walnut/Skyline (**Brian Searle**)

*3:30-4:00 Break (Afternoon Tea)*

4:00-5:00 Lecture: Targeting proteomics at high throughput (**Ruedi Aebersold, ETH, Zurich**)

*5:00 WORKSHOP CLOSE*

## INSTRUCTORS

---

### **BRENDAN MACLEAN**



Brendan is well-known to the Australasian Proteomics community, having visited Lorne on a number of occasions previously and having conducted one-day pre-Lorne workshops in 2012/2013. Brendan worked at Microsoft for 8 years in the 1990s where he was a lead developer and development manager for the Visual C++/Developer Studio Project. Since leaving Microsoft, Brendan has been the Vice President of Engineering for Westside Corporation, Director of Engineering for BEA Systems, Inc., Sr. Software Engineer at the Fred Hutchinson Cancer Research Center and a founding partner of LakKey Software. In this last position he was one of the key programmers responsible for the Computational Proteomics Analysis System (CPAS), made significant contributions to the development of X!Tandem and the Trans Proteomic Pipeline, and created the LabKey Enterprise Pipeline. Since August, 2008 he has worked as a Sr. Software Engineer within the MacCoss lab and been responsible for all aspects of design, development and support in creating the Skyline Targeted Proteomics Environment and its growing worldwide user community.

### **BRIAN SEARLE**



Brian is the co-founder and chairman of the board of Proteome Software, Incorporated. Following an undergraduate degree in chemistry from Reed College, Brian was mentored in MS/MS-based proteomics by Ashley McCormack and software development by Mark Turner in Srinivasa Nagalla's lab at Oregon Health and Science University. In 2004 Brian co-founded Proteome Software with Mark and Ashley to produce and distribute cutting-edge data analysis software for proteomicists. As the owner of Proteome Software, Brian has produced numerous innovations in the analysis of MS/MS-based proteomics data. Brian is a member of the American Society for Mass Spectrometry (ASMS) and served on the board of directors for that organization. Brian also co-founded the Proteome Informatics Research Group of the Association of Biomolecular Resource Facilities (ABRF) society and is active in ABRF committees. In addition to furthering the goals of Proteome Software, Brian is concurrently pursuing a PhD at University of Washington.

### **SANDI SPENCER**



Sandi earned her Ph.D. from the University of North Carolina at Chapel Hill working in the Glish lab on real-time analysis of compounds in organic aerosol particles. She is currently a postdoctoral researcher in the MacCoss lab at the University of Washington working clinical proteomic assay development and quantitative method improvement. Her current projects include development of a kinase activity assay for prediction and monitoring of targeted therapeutic efficacy in cancer, developing an assay to monitor immune checkpoint proteins in formalin fixed paraffin embedded tissues to type solid tumors, and using data independent acquisition to empirically derive targeted assays and molecular signatures for neurodegenerative disease and cognitive decline.