Proteomics Core
Tel Aviv University

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**Instrumentation**

**Q-Exactive Mass Spectrometer**
Mass analyzer: Orbitrap
Resolution: up to 140,000
Speed: 12 Hz
Intra-scan dynamic range >5000:1
Cost: $0.5M

**EASY-nLC1000 UHPLC**
Nano-UHPLC
Max pressure: 1000 bar
Cost: $60,000

**Dionex-ultimate 3000**
Standard UHPLC
Max pressure: 3000 bar
Cost: $80,000
Instrumentation

EASY-nLC1000 UHPLC
Nano-UHPLC
Max pressure: 1000 bar
Cost- $60,000

EASY-spray
Cost- $15,000
Proteomics in the lab
Instrumentation

EASY-nLC1000 UHPLC
Nano-UHPLC
Max pressure: 1000 bar
Cost- $60,000

EASY-spray
Cost- $15,000

2013
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Proteomics technologies in ICORE

- Proteome analysis
- Phosphoproteomics
- Protein interactions
- Quantitative analysis with SILAC or label-free
- Acetylome
Global Proteomic Profiling

Aim:
• Complete coverage of the biological system

Challenges:
• Limited dynamic range- very abundant proteins mask lowly expressed ones.
• Sensitivity- limited protein amount does not enable deep analysis.
• Measurement time- more measurement time leads to deeper coverage.
What is complete transcriptome?
Filtering RNA-seq data

Nagaraj et al. MSB 2011
Aim:
• Identify the binding partners of proteins of interest.

Challenges:
• Requires large initial protein amounts.
• Depends on protein expression levels.
• Interactions in unique cellular compartments might be masked.

Experimental approaches:
• Immunoprecipitation of endogenous protein.
• Pull down of tagged proteins with crosslinked anti-tag antibody.
• Sepharose/magnetic beads.
Phosphoproteomics

Aim:
• Deep coverage of the phosphorylation sites in a biological system.

Challenges:
• Very low abundance of phosphorylated proteins.
• Limited by instrument sensitivity.
• Requires large initial protein amounts.
• Unknown number of phosphorylation sites- no estimation of coverage.
• Reduced quantitative accuracy.
• The role of most phosphorylation sites is still unknown.