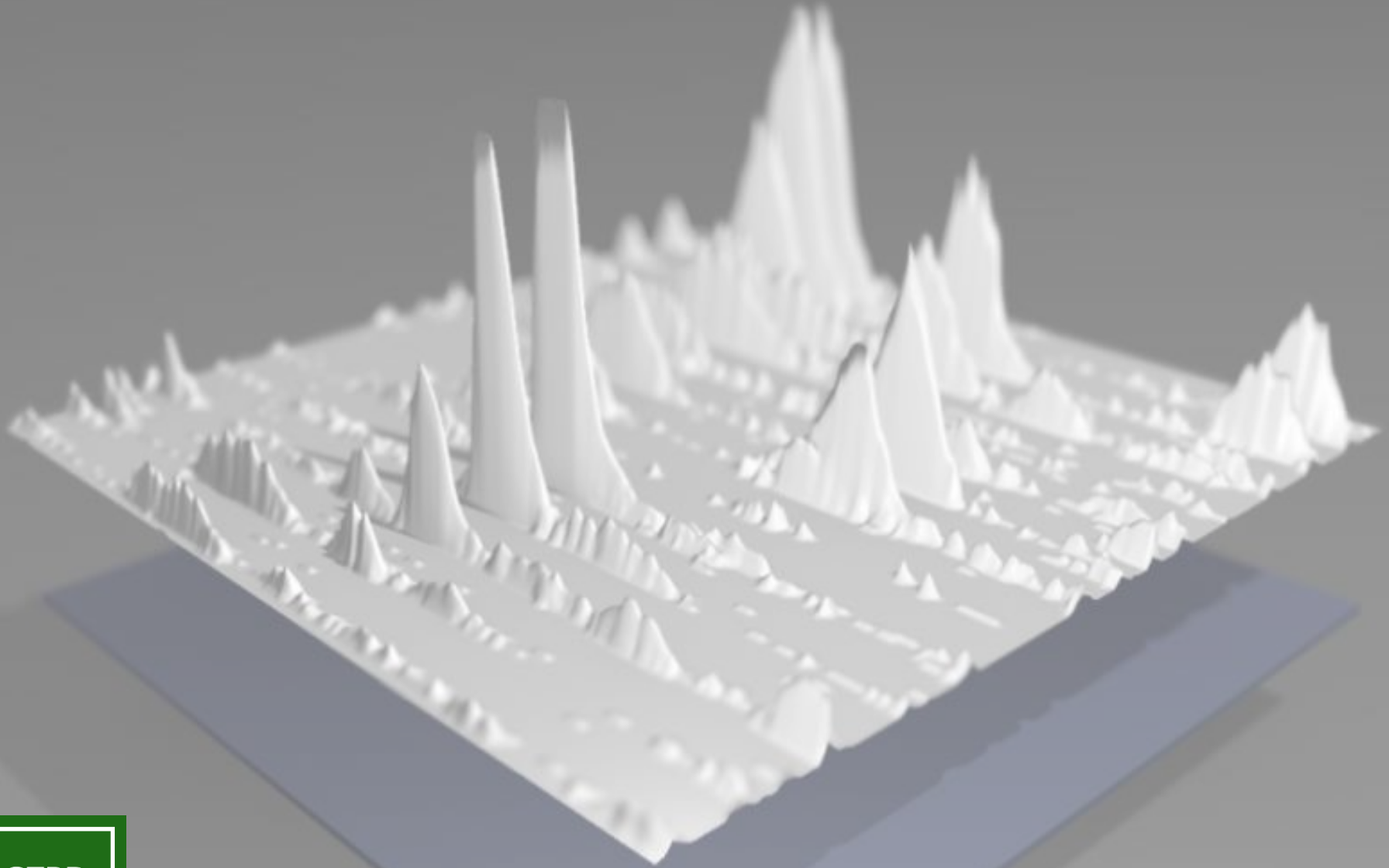
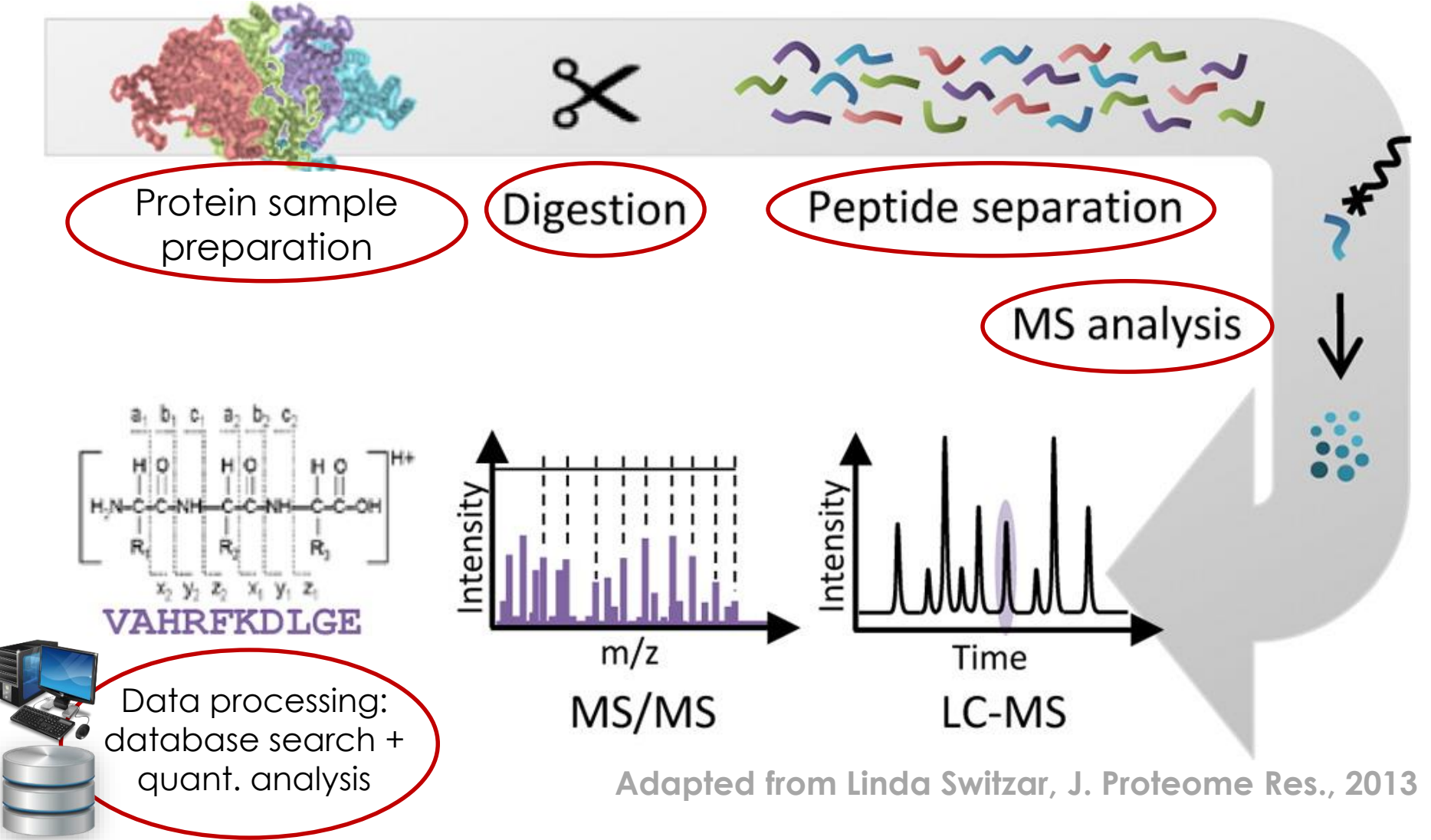


Quality control of proteomics data



Quality control of proteomics data

Bottom-up strategy: where can we have reproducibility issues?



Adapted from Linda Switzer, J. Proteome Res., 2013

➔ Each step of the workflow is a potential source of error

Quality control of proteomics data

I don't find what I was expecting, what could have gone wrong?

➤ **I have very few identifications...**

Can be anything from sample preparation (protein extraction for instance) to database search (wrong database used or wrong parameters)

➤ **I performed immunoprecipitation and I have identified too many proteins**

Might be improper cleaning of the sample, redo the experiment or use appropriate control

➤ **I have a lot of missing values in my quantitative data...**

If you compare very different proteomes then try a different strategy

If proteomes are supposed to be similar, you may have issues in the LC-MS setup

If you are doing label-free experiments maybe your software didn't aligned the runs correctly

➤ **My ID/QUANT data seem to be good but I don't find any variant proteins...**

1. Maybe your experiment was not inducing a change in your proteome

2. You may have a high biological variability => increase the number of replicates

➤ **USE STANDARD SAMPLES: A GOOD WAY TO MONITOR YOUR INSTRUMENT**

➤ **COMPLEX MIXTURES**

LC gradient optimization, test of instrument MS and MS/MS throughput performance

➤ **SINGLE PROTEIN SAMPLES (e.g. BSA, beta-gal, cytochrome C, myoglobin)**

Inter-runs quality control: LC issues (RT shifts, wider peaks), m/z calibration and sensitivity

➤ **SPIKED-IN SAMPLES (e.g. UPS1/UPS2)**

Benchmarking of both LC-MS instrument setup and data processing methods (requires a sufficient number of proteins)

➤ **SAMPLES OF INTEREST: TRY TO AVOID ADDITIONAL PROBLEMS**

- Define appropriate experimental design (e.g. minimum number of replicates)
- Optimize sample preparation
- Tune data processing parameters



Hands-on session



<https://github.com/GTPB/IBIP19/blob/master/pages/qc/>