Standardised and reproducible analysis of mass spectrometry-based single-cell proteomics data

Slides available at:

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Outline

State of the art in MS-SCP

MS-SCP: Mass spectrometry-based single-cell proteomics
MS-SCP consist of shotgun proteomics at single-cell level

- SCoPE2 quantifies thousands of proteins x thousands single-cells
- Full protocole available
- Full analysis script available

BUT

Lack of standardized analysis software

Our objectives

Provide a suite of software package dedicated to MS-SCP that fulfill:

- User-friendly
- Computationaly efficient
- Modularity: integrate other software packages
- Promote reproducibility
- Platform-independent
- Free of charge

R/Bioconductor is an ideal environment

Outline

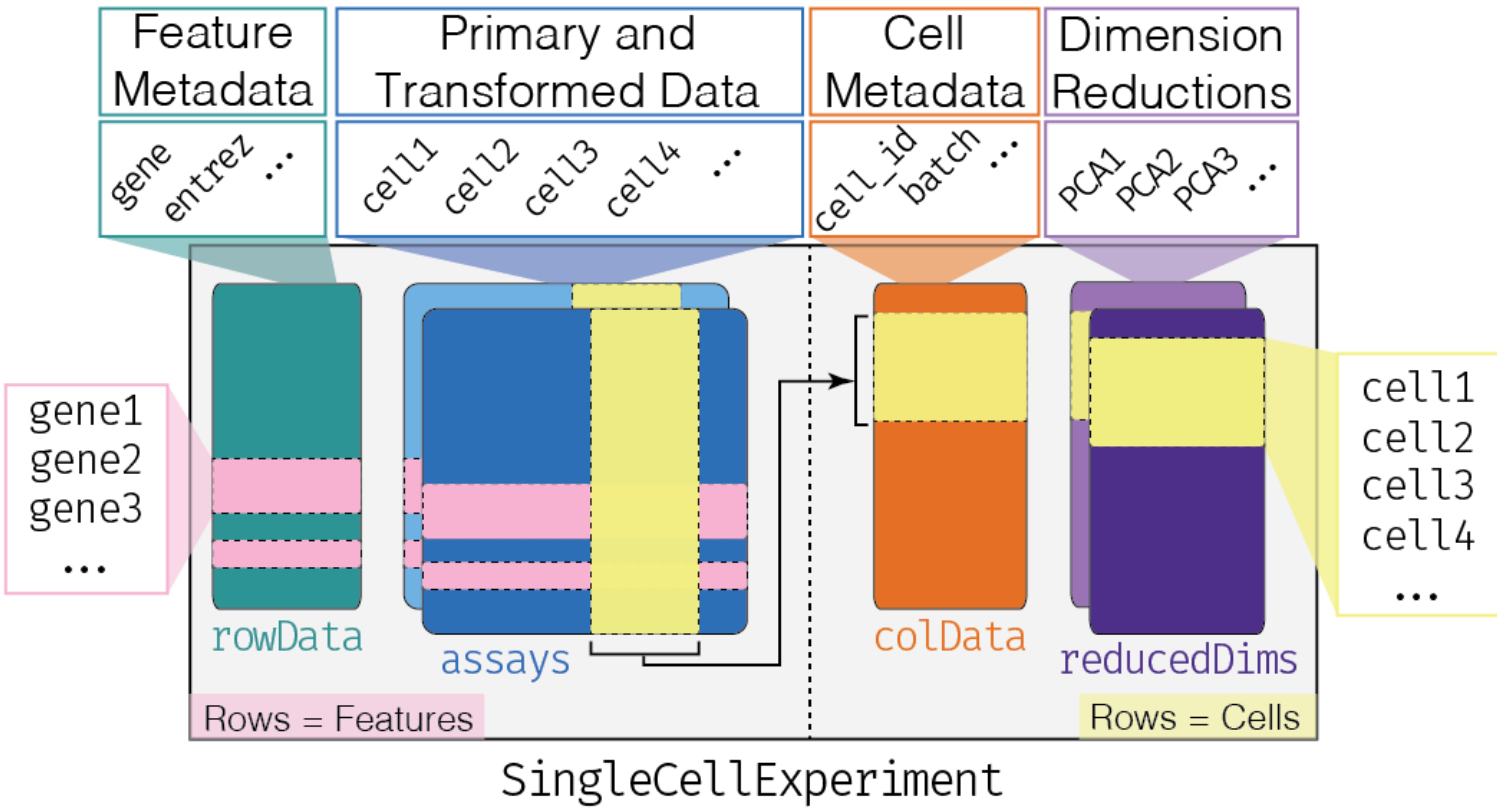
MS-SCP package suite

scpdata: distributes published MS-SCP datasets (e.g. SCoPE2 dataset)
scp: provides functionality for manipulating the MS-SCP data structure

Outline

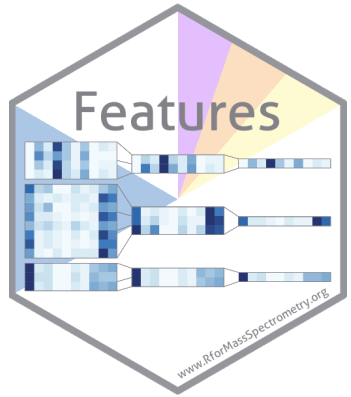
Data infrastructure (1)

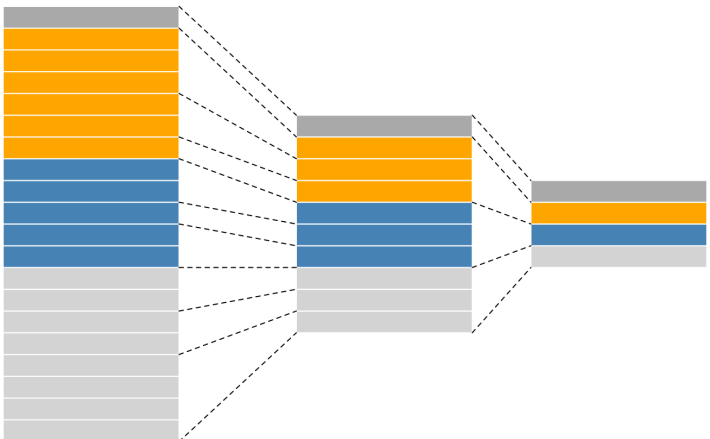
SingleCellExperiment: provides dedicated framework for single-cell data analysis.
Available on Bioconductor.

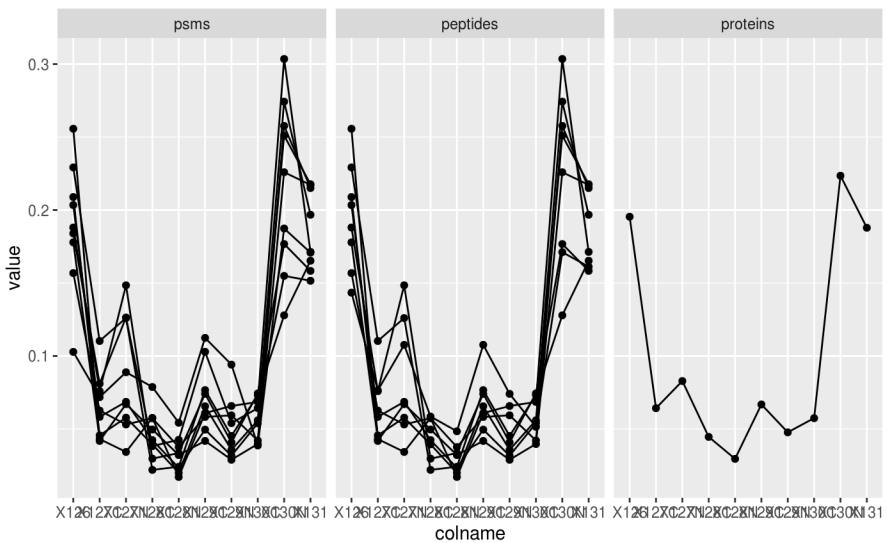


Data infrastructure (2)

QFeatures: data framework dedicated to manipulate and process MS-based quantitative data.
Submitted to Bioconductor.

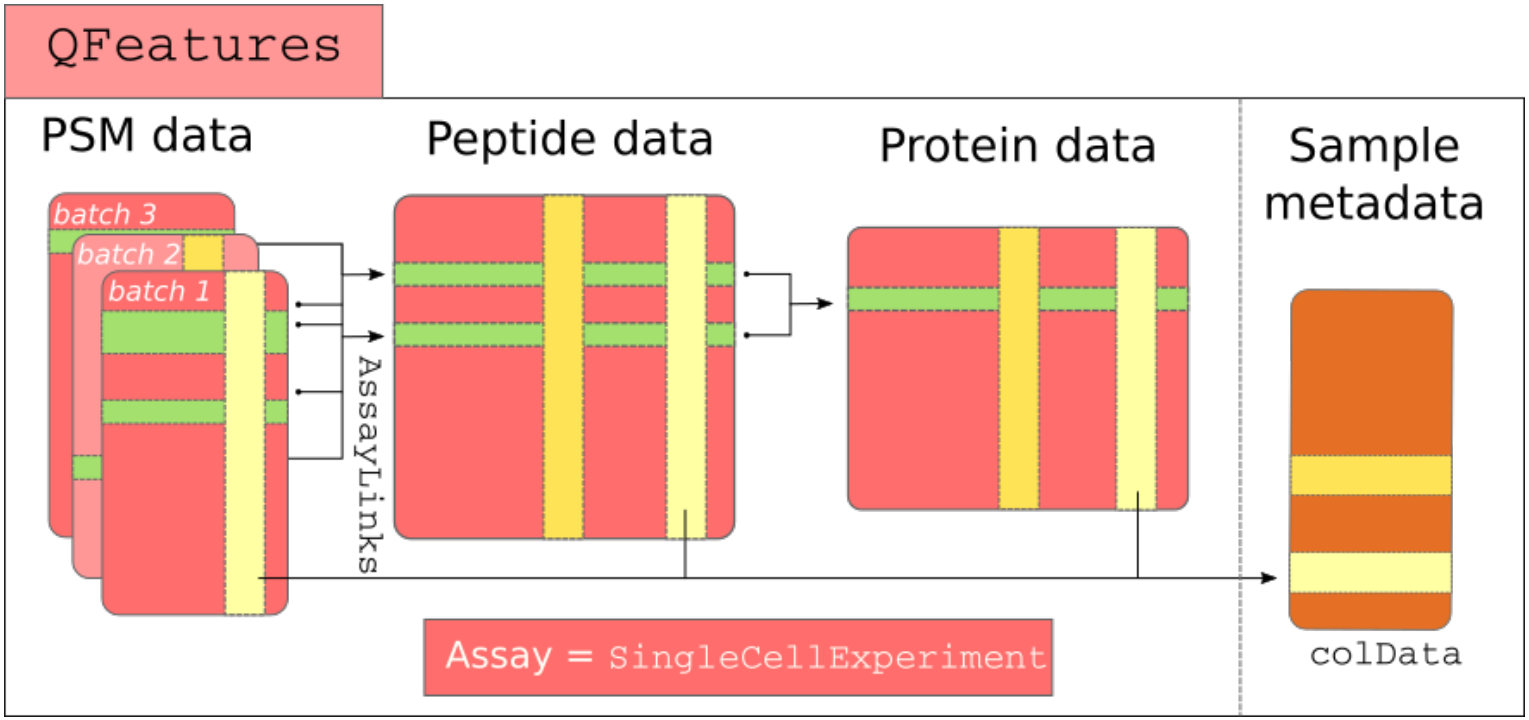






Data infrastructure (3)

`scp` = `SingleCellExperiment` + `QFeatures`



Load data

Load the SCoPE2 dataset called `specht2019v2`

```
library(scpdata)
data("specht2019v2")
```

Dataset overview

```
show(specht2019v2)
```

```
An instance of class QFeatures containing 179 assays:
 [1] 190222S_LCA9_X_FP94AA: SingleCellExperiment with 2823 rows and 11 col...
 [2] 190222S_LCA9_X_FP94AB: SingleCellExperiment with 4297 rows and 11 col...
 [3] 190222S_LCA9_X_FP94AC: SingleCellExperiment with 4956 rows and 11 col...
 ...
 [177] 191110S_LCB7_X_APNOV16plex2_Set_9: SingleCellExperiment with 4626 r...
 [178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
 [179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
```

Sample metadata

(test slide: to discuss)

The sample metadata can be retrieved in the `colData`

```
1 colData(specht2019v2)
```

```
DataFrame with 2517 rows and 6 columns
                                                      Set     Channel  SampleType     lcbatch
sortday      digest
                                                <character> <character> <character> <character> <character>
190222S_LCA9_X_FP94AA_RI1               190222S_LC...         RI1     Carrier        LCA9
s8           N
190222S_LCA9_X_FP94AA_RI2               190222S_LC...         RI2   Reference        LCA9
s8           N
190222S_LCA9_X_FP94AA_RI3               190222S_LC...         RI3      Unused        LCA9
s8           N
190222S_LCA9_X_FP94AA_RI4               190222S_LC...         RI4  Macrophage        LCA9
s8           N
190222S_LCA9_X_FP94AA_RI5               190222S_LC...         RI5  Macrophage        LCA9
s8           N
...                                               ...         ...         ...         ...
...          ...
191110S_LCB7_X_APNOV16plex2_Set_9_RI12 191110S_LC...        RI12  Macrophage        LCB7
s9           U
191110S_LCB7_X_APNOV16plex2_Set_9_RI13 191110S_LC...        RI13  Macrophage        LCB7
s9           U
191110S_LCB7_X_APNOV16plex2_Set_9_RI14 191110S_LC...        RI14  Macrophage        LCB7
s9           U
191110S_LCB7_X_APNOV16plex2_Set_9_RI15 191110S_LC...        RI15    Monocyte        LCB7
s9           U
191110S_LCB7_X_APNOV16plex2_Set_9_RI16 191110S_LC...        RI16  Macrophage        LCB7
s9           U
```

Metadata

The sample metadata can be retrieved in the `colData`

```
colData(specht2019v2)
```

- Batch name
- Channel name
- Sample info: sample type, treatment, ...
- Batch info: chromatographic batch, digestion batch, ...

Metadata

The sample metadata can be retrieved in the `colData`

```
colData(specht2019v2)
```

- Batch name
- Channel name
- Sample info: sample type, treatment, ...
- Batch info: chromatographic batch, digestion batch, ...

The feature metadata can be retrieved in the `rowData`, but assay specific

```
rowData(specht2019v2[[1]])
```

- PSM level: reverse hit, PEP, m/z value, charge, ...
- Peptide level: sequence, length, modification, mass, ...
- Protein level: name, sequence, gene name, ...

Analysis workflow

1. Load data

PSM data

```
[1] 190222S_LCA9_X_FP94AA: SingleCellExperiment with 2823 rows and 11 columns
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...
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```

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```

2. PSM filtering
3. Expression channel by reference channel division
4. PSM to peptides aggregating
5. Single cells filtering based on median CV
6. Normalization
7. Removal of highly missing peptides
8. Log-transformation

Peptide data

```
[178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
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Analysis workflow

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PSM data

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Peptide data

```
[178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
```

9. Peptides to proteins aggregation
10. Normalization
11. Imputation
12. Batch correction

Peptide data

```
[179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
```

Outline

PSM filtering

Filter out features based on the feature metadata

Example: filter out reverse hits. The filter is applied to the `Reverse` field in the feature metadata

```
filterFeatures(specht2019v2,
               ~ Reverse != "+")
```

Source code in `QFeatures`

QC metrics (1)

Interesting metrics for MS-SCP quality control:

- Sample to carrier ratio: ratio of the carrier channel intensity signal over the sample channel intensity
- Peptide FDR[1]: expected rate of wrongly assigned features to a given peptide
- Cell median CV[2]: reliability of the protein quantification summarized over each cell.

Example:

```
computeMedianCV(specht2019v2,
                i = "peptides",
                proteinCol = "protein",
                peptideCol = "peptide",
                batchCol = "Set")
```

Source code in `scp`

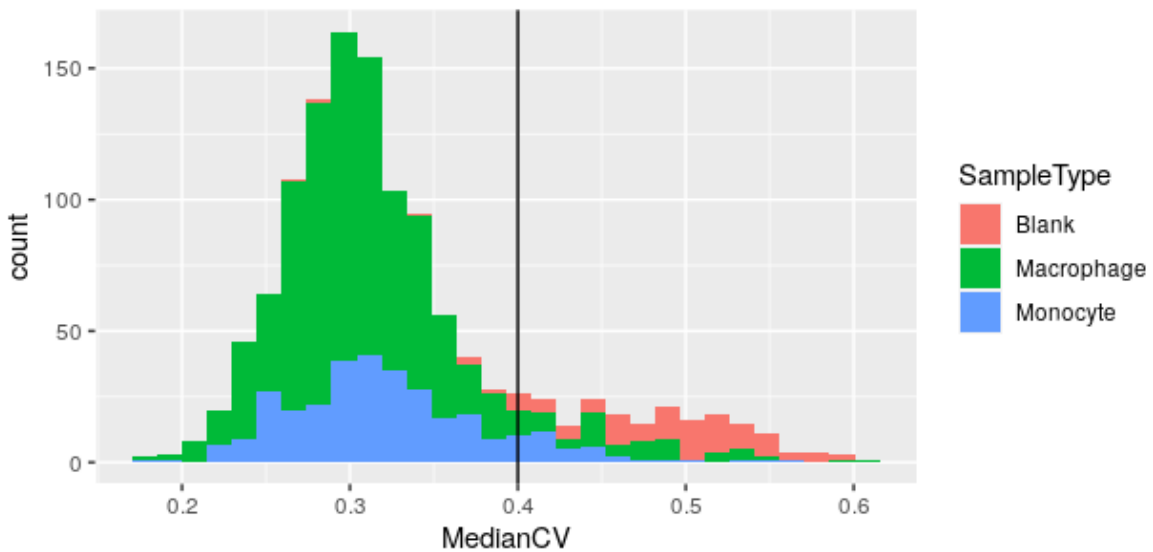
[1] false discovery rate

[2] coefficient of variation

QC metrics (2)

QC metrics are stored in the data set for plotting or subsetting

```
library(tidyverse)
specht2019v2[["peptides"]] %>%
  colData %>%
  data.frame %>%
  ggplot(aes(x = MedianCV,
             fill = SampleType)) +
  geom_histogram() +
  geom_vline(xintercept = 0.4)
```



Feature aggregation

Feature aggregation includes 2 steps:

- Combine the quantiative data from multiple features to a single aggregated features
- Store the relationship between the parent features and the aggregated features

Example: aggregate peptides to proteins

```
aggregateFeatures(specht2019v2,
                  i = "peptides",
                  name = "proteins",
                  fcol = "protein",
                  fun = colMedians, na.rm = TRUE)
```

Source code in `QFeatures`

Managing missingness

`0`'s can be either **biological** or **technical** zero. They are better relaced by `NA`'s.

```
zeroIsNA(specht2019v2,
         i = "peptides")
```

Features containing too many missing data (e.g. $>= 99$ %) should be removed

```
filterNA(specht2019v2,
         i = "peptides",
         pNA = 0.99)
```

Source code in `QFeatures`

Data transformation

Common data transformation can easily be applied:

- Normalization
- Log-transformation
- Imputation

Example: log_2-transformation:

```
logTransform(specht2019v2,
             i = "peptides",
             base = 2,
             name = "peptides_log")
```

Source code in `QFeatures`

Custom functions

Some custom function can be applied to the data set too. Example: batch correction using `sva::ComBat`. First, extract the data to correct

```
sce <- specht2019v2[["proteins"]]
```

Build the correction matrix and apply the ComBat algorithm

```
batch <- colData(sce)$Set
model <- model.matrix(~ SampleType, data = colData(sce))
assay(sce) <- ComBat(dat = assay(sce),
                     batch = batch,
                     mod = model)
```

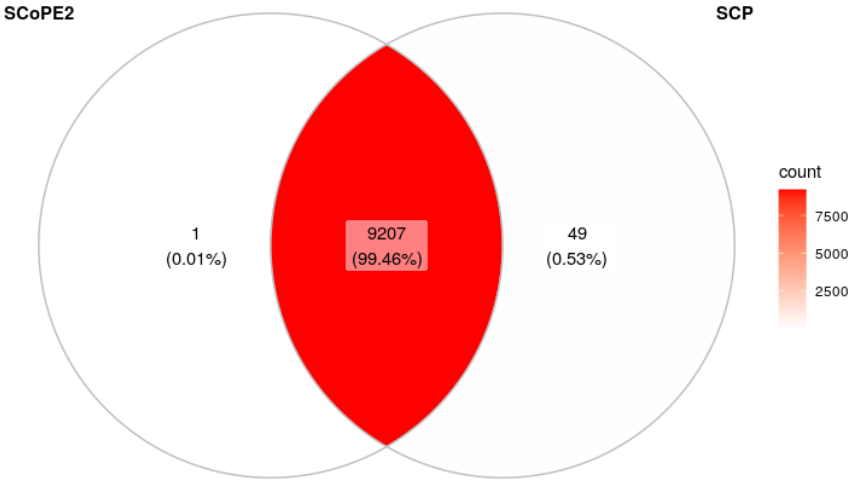
Add the corrected protein to the dataset and keep feature relationships

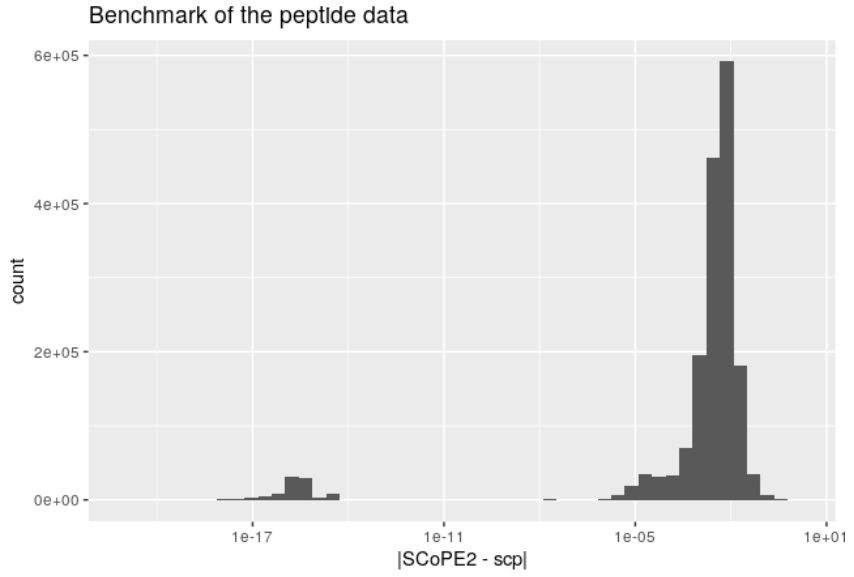
```
addAssay(specht2019v2,
         sce,
         name = "proteins_batchC") %>%
addAssayLinkOneToOne(from = "proteins",
                     to = "proteins_batchC")
```

Outline

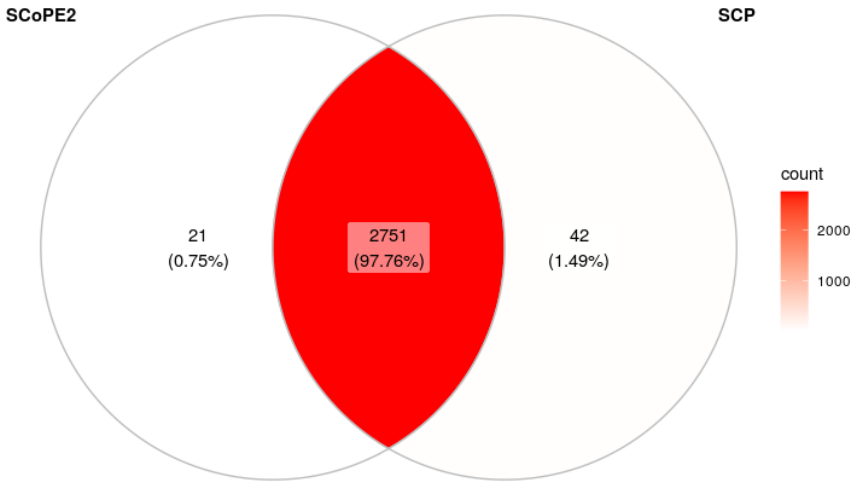
Benchmarking data matrices

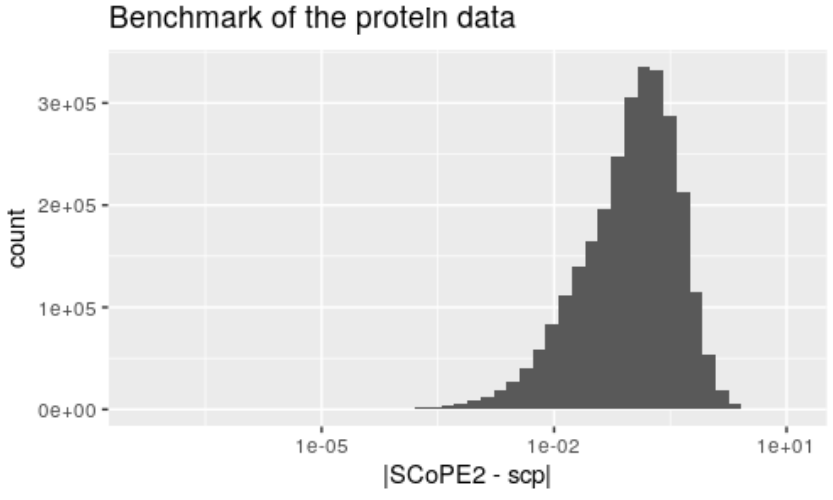
Peptides





Proteins





Replicate figures from SCoPE2 (1)

Replicate figures from SCoPE2 (2)

Missingness

Outline

Take home message

- `scp` package suite provides a standardized environment for performing MS-SCP data analysis
- Flexibly reproduce existing analyses from different groups or protocoles (multiplex vs label free)

Advantages:

- Allow automation of the analysis
- Facilitate new computational developments
- Promotes reproducibility
- Increases field visibility
- Include other modalities: scRNA-Seq, ATAC-Seq, etc

Resources

Packages

- `scp`: GitHub repository `UClouvain-CBIO/scp`
- `scpdata`: coming soon
- `QFeatures`: GitHub repository `rformassspectrometry/QFeatures`
- `SingleCellExperiment`: Bioconductor

SCoPE2 reproduction vignette

Available at...

Slides and source code

Available at...

Acknowledgements