A principled approach to analyse and understand single-cell proteomics data. How to focus on the underlying biology?

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8 May 2025

Outline

Single-cell technologies unravel cellular heterogeneity

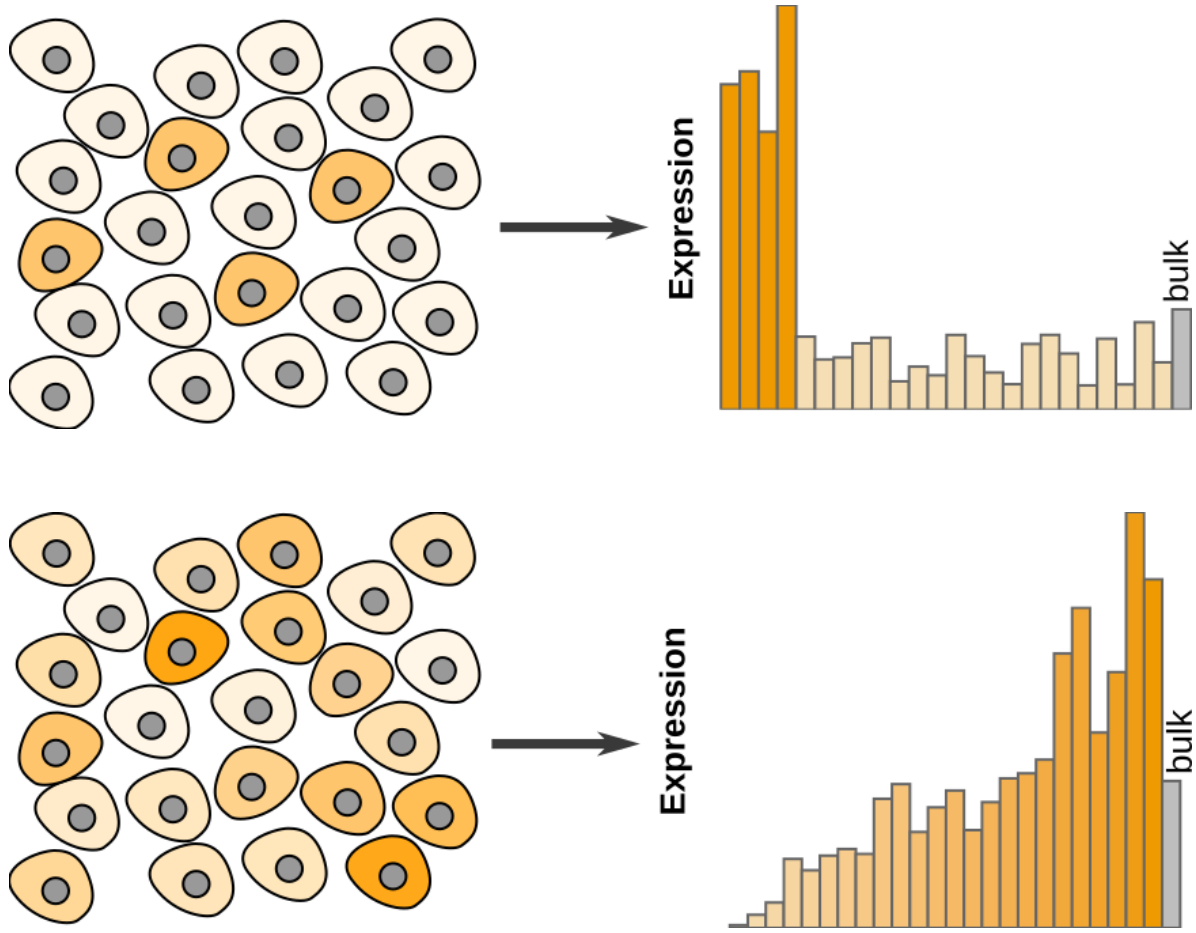
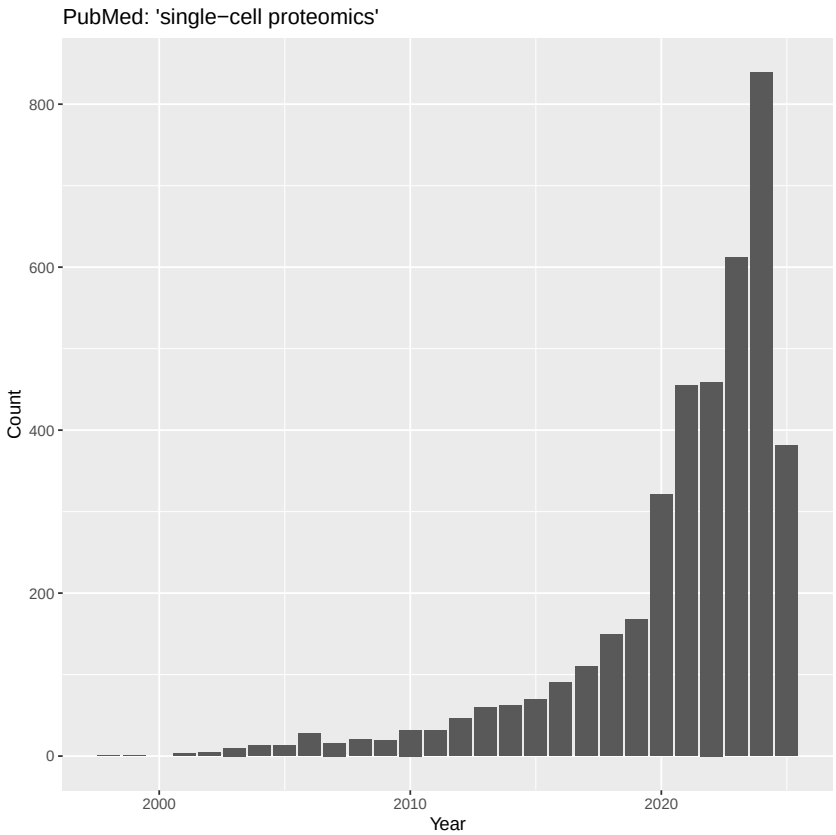


Figure: Cell types and cell states, subpopulation identification, differentiation trajectories (in the absence of known markers).

SCP: . . . – 2019 – 2023 – . . .



August 2019: in a Nature Methods Technology Feature[a], Vivien Marx *dreamt* of single-cell proteomics.

March 2023: Nature Methods published a special issue with a Focus on single-cell proteomics[b] and Initial recommendations for performing, benchmarking and reporting single-cell proteomics experiments[c].

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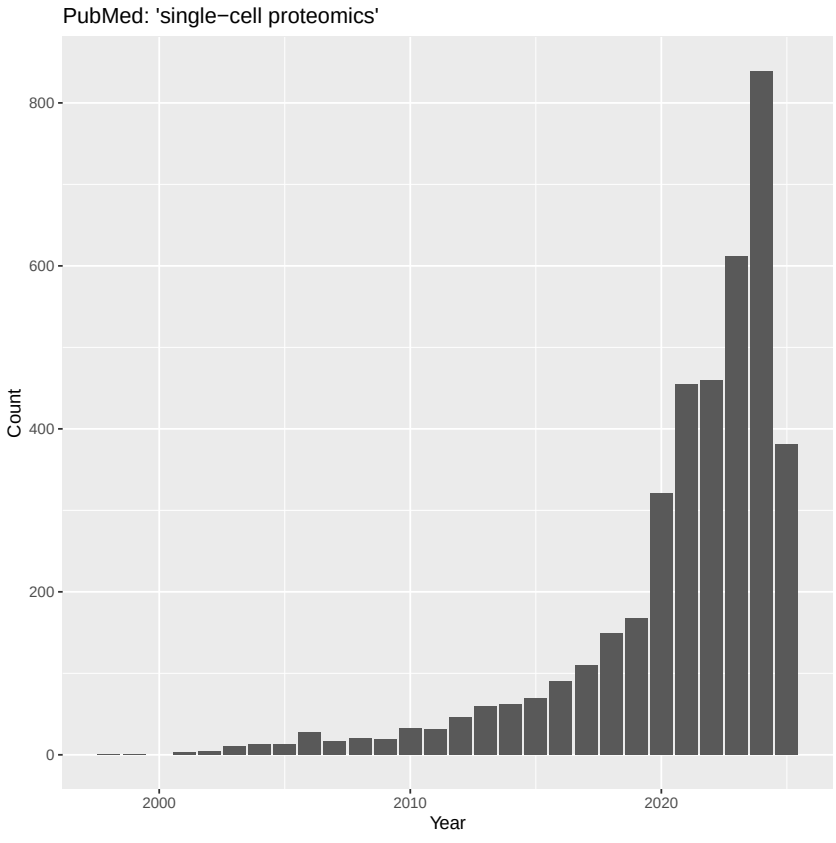
[a] 10.1038/s41592-019-0540-6

[b] www.nature.com/collections/bdfhafhdeb

[c] 10.1038/s41592-023-01785-3



Possible through better sample preparation, reduction of loss of material, miniaturisation, automation, better MS, greater sensitivity, DDA and DIA, LFQ and labelling, . . .

. . . and appropriate **experimental designs** and **computational approaches**.

Single-cell technologies

	FC	scRNA-Seq	SCP
features	10	10^4	10^3
cells	10^6	10^4	10^3
samples	10 - 100	1 - 10	1 . . .
	sample/cell throughput	feature throughput	**functional**

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	sample/cell throughput	feature throughput	**functional**

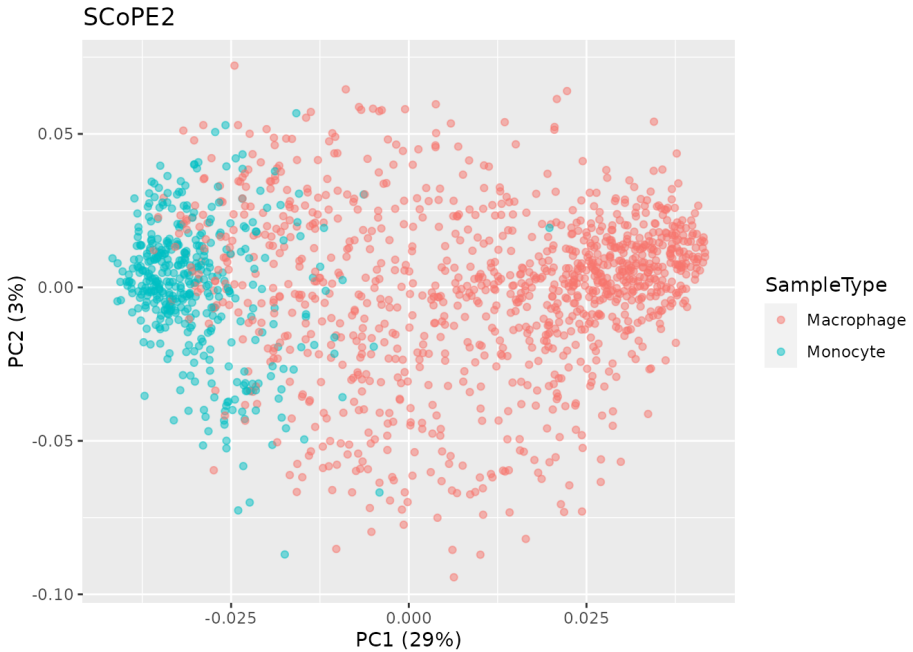
- FC vs. SCP $\rightarrow$ unsupervised.
- RNA $\rightarrow$ intention vs. protein $\rightarrow$ action.
- Inference of direct regulatory interactions with minimal assumptions (Slavov, 2022; Hu et al., 2023).
- Post-translational modifications.

Outline

Material (1)

The SCoPE2 dataset

- Seminal dataset published by Specht et al. (2021)
- 1096 macrophages, 394 monocytes (after QC)
- 9354 peptides, 3042 proteins
- **Pre-print, data and code available since 2019**



Reproducible research

First steps

- SCoPE2 (and other) repetition/reproduce/replication → **QFeatures** and **scp** packages
- SCoPE2 (and other) data curation → **scpdata** package

More details

- `https://bioconductor.org/packages/QFeatures`
- `https://bioconductor.org/packages/scp`
- `https://bioconductor.org/packages/scpdata`

Build expertise and improve current state-of-the-art

Methods

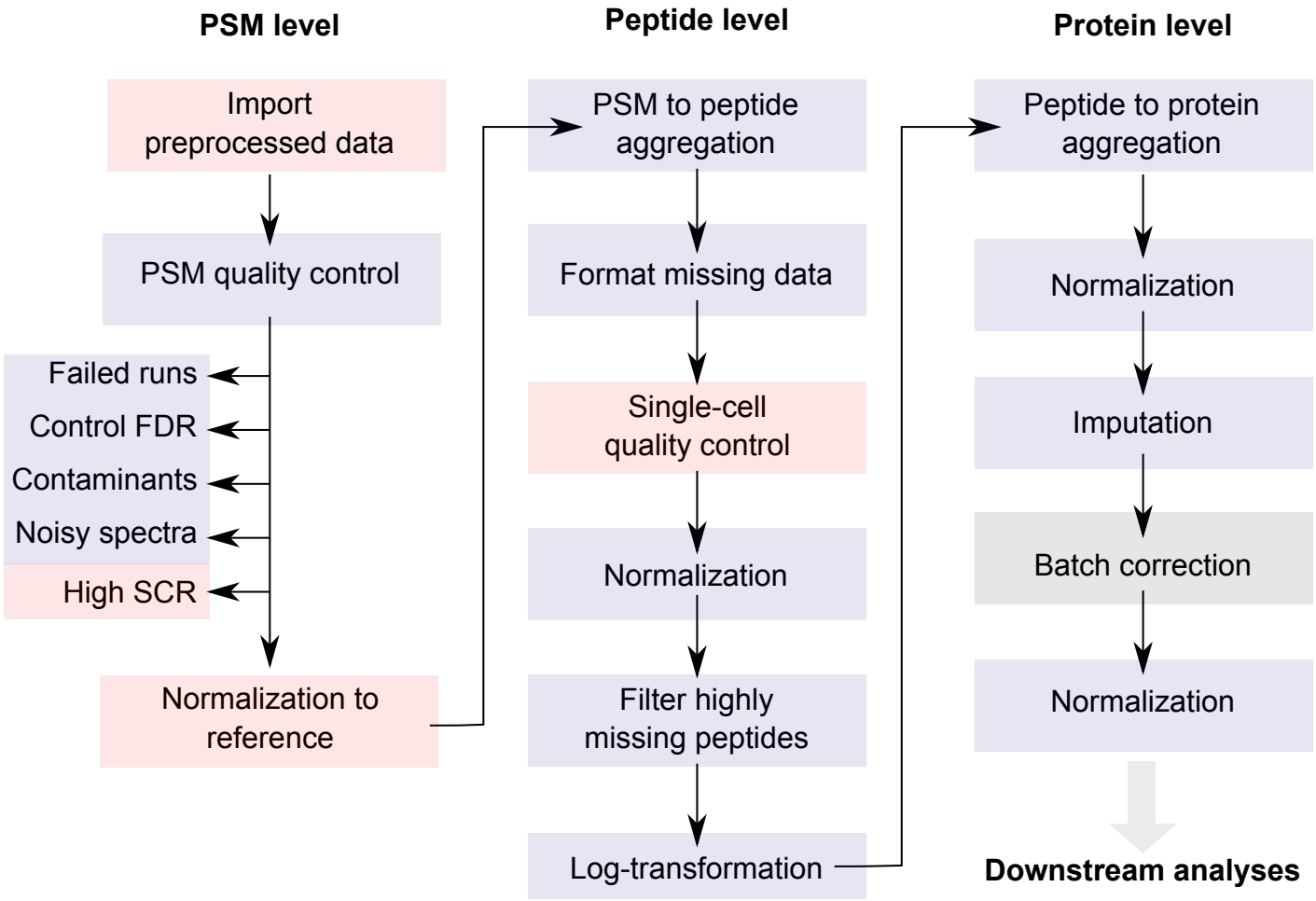


Figure: **Overview of the key steps performed in the SCoPE2 pipeline** (Vanderaa and Gatto, 2021). Blue boxes: **QFeatures**. Red boxes: **scp**. Gray box: **sva::ComBat**.

Outline

Challenge 1: batch effects

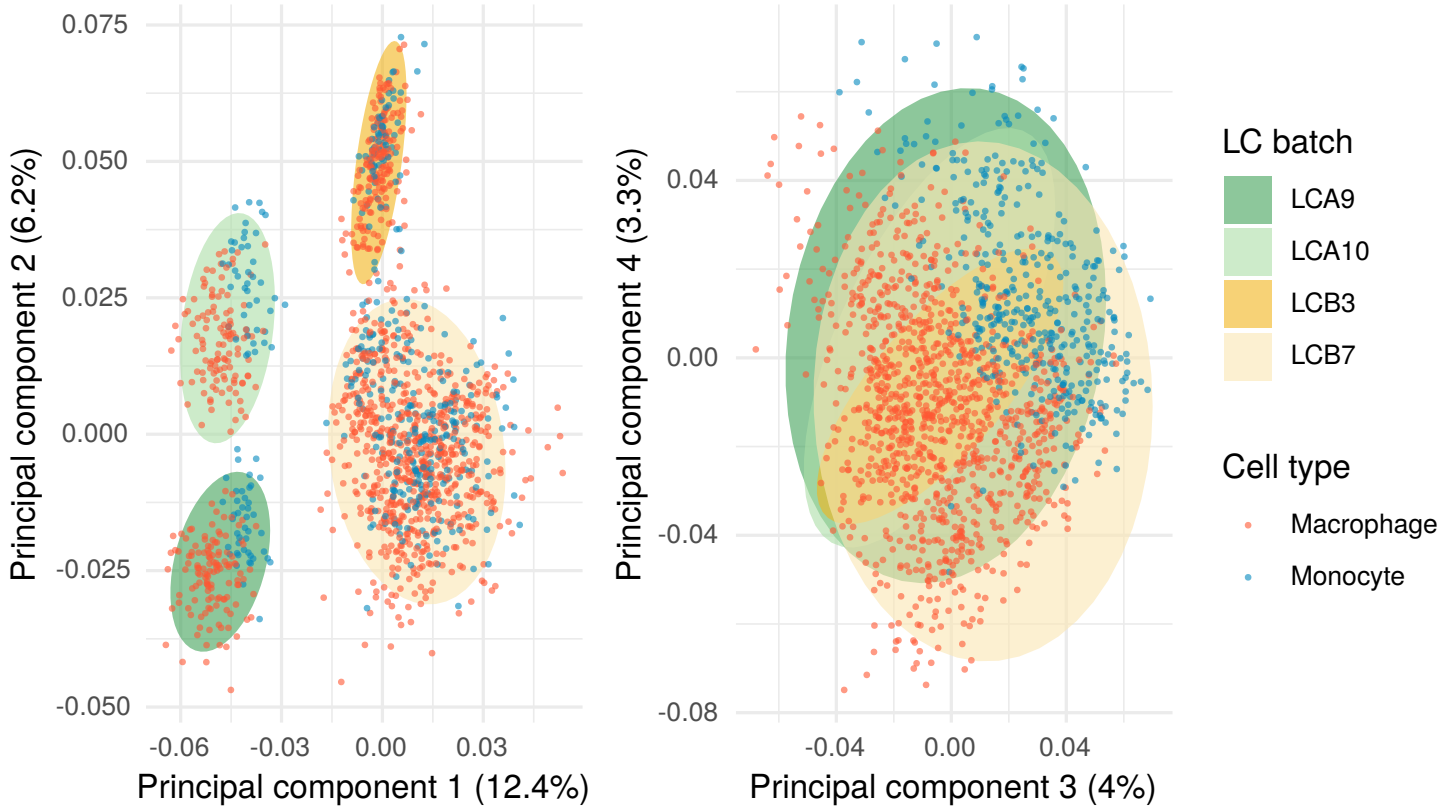


Figure: PCA for the first four components. Each point represents a single-cell and is colored according to the corresponding cell type (Vanderaa and Gatto, 2021).

Challenge 2: missing data

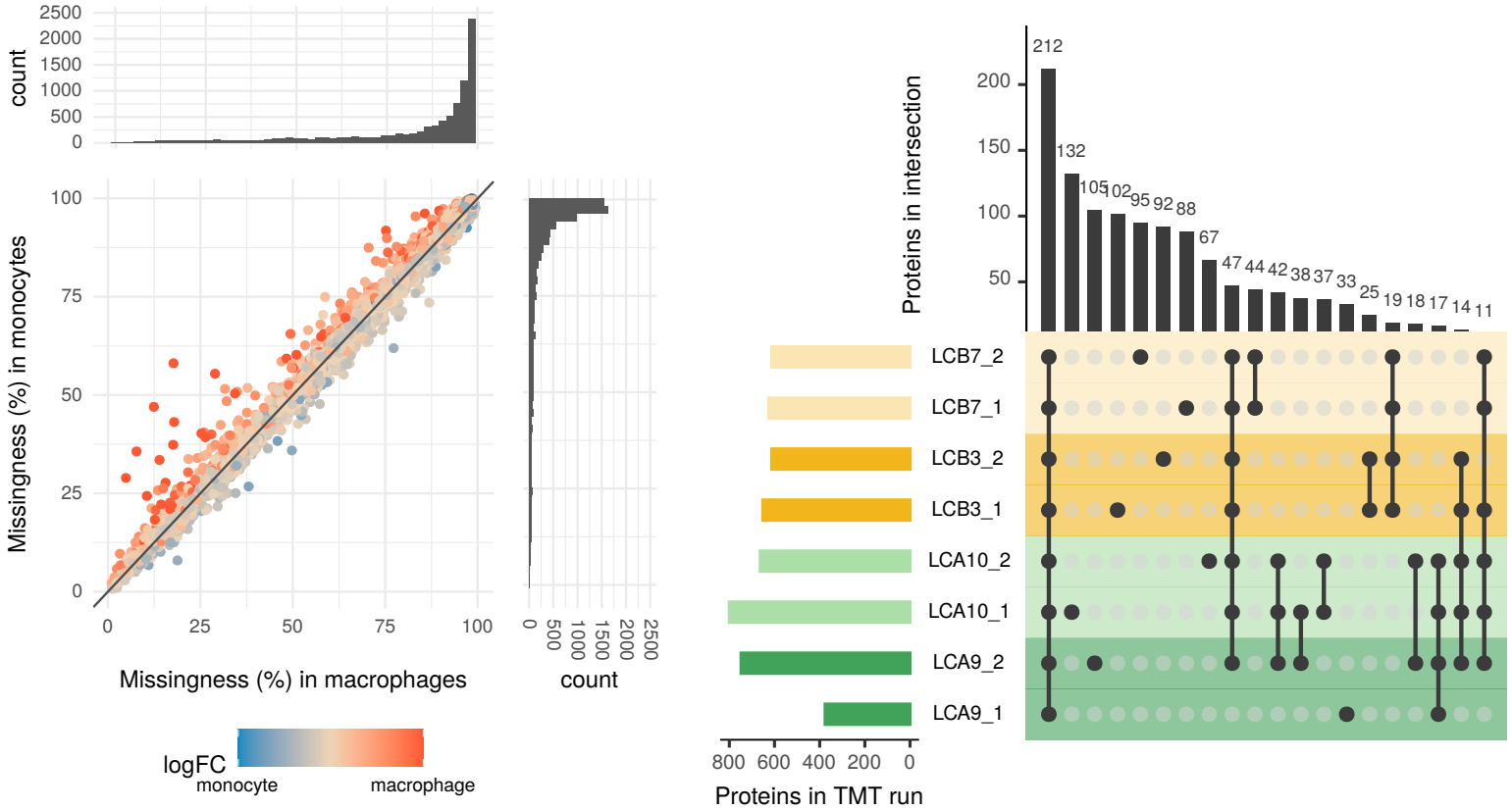


Figure: Missing data is the consequence of biological and technical components (Vanderaa and Gatto, 2021, 2023b).

Challenge 3: 1 + 2

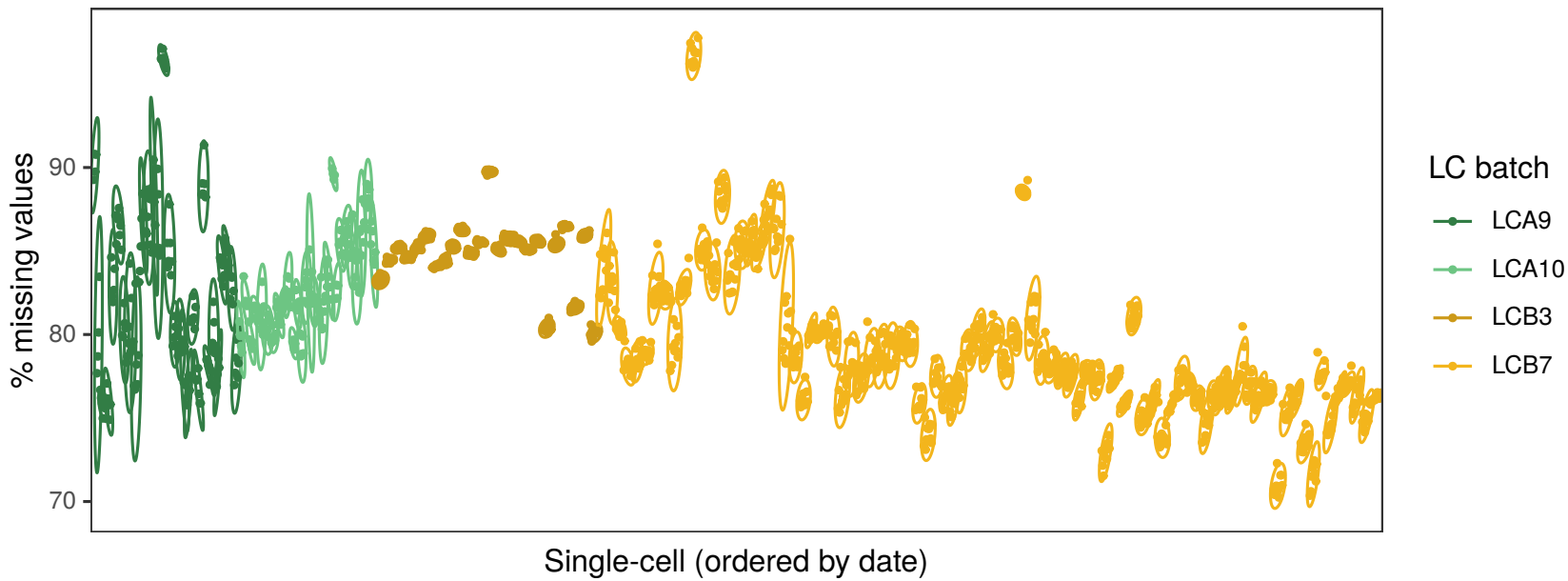
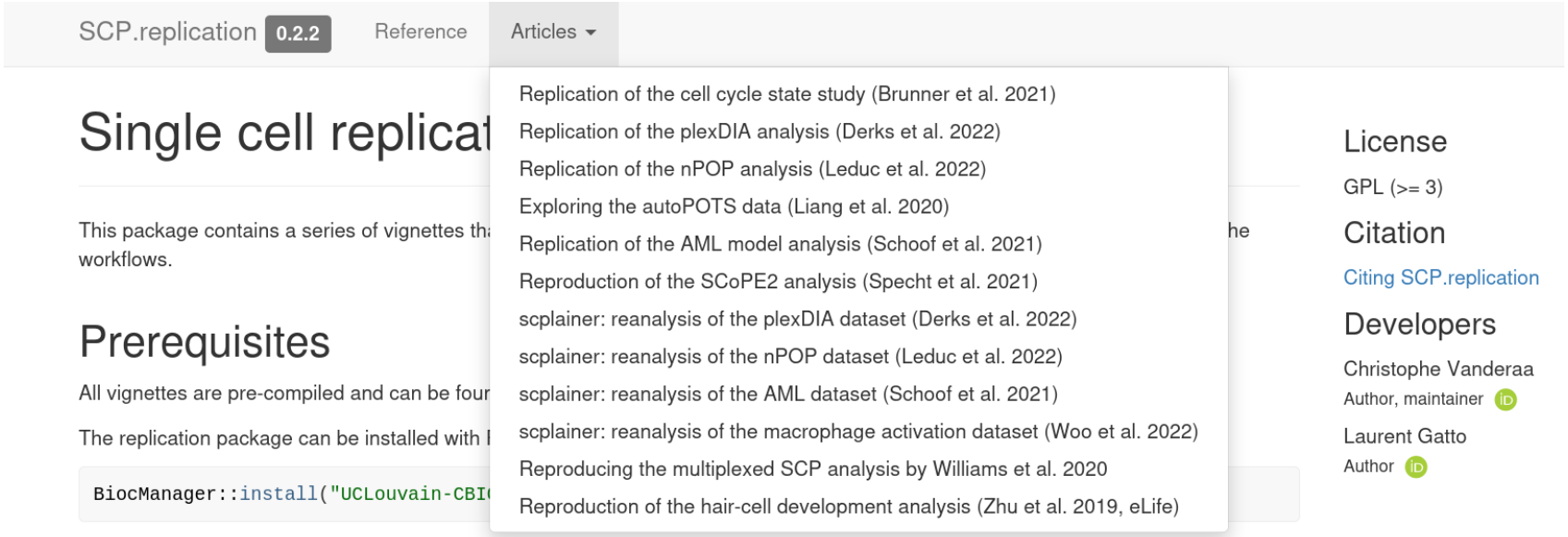


Figure: Influence of batch on data missingness (Vanderaa and Gatto, 2021).

Data analyses review

- How do researches process their data?
- How do they deal with batch effects?
- How do they deal with missing data?

Replication



https://uclouvain-cbio.github.io/SCP.replication

Figure: **SCP.replication**: **systematic reproduction/replication** of published SCP studies using the **scp** package (Vanderaa and Gatto, 2023a).

Systematic review

Figure: Single-cell data processing: **one workflow per paper/lab**. (Vanderaa and Gatto, 2023a)

Problem

- Complex data, many alternative pipelines.
- **Different pipelines produce different results** (see Vanderaa and Gatto (2023a)).
- Little control/understanding of the implications of what is done to the data.

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Solution: a principled approach

- KISS (*Keep it simple stupid!*), as simple as possible.
- Use what we know to **model** our data.
- Control what we do, **quantify** effects.

Outline

Given that we aren't sure about the effect of data processing...

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Let's start with **minimally processed data**

- Remove low quality precursors and cells
- Aggregate from precursors into peptides
- $\log_2$-transform
- Remove features with *too many* NAs
- No imputation

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And use ANOVA–simultaneous component analysis (ASCA)-like methods (Thiel et al., 2017), implemented as the **scplainer** approach (Vanderaa and Gatto, 2024) in the **scp** package (Vanderaa and Gatto, 2023a).

(1) Linear modelling

$$y = \beta_0 + \beta_1 \times group + \epsilon$$

$$y = \beta_0 + \beta_1 \times group + \beta_i \times batch_i + \epsilon$$

(2) Quantify the effects' contributions

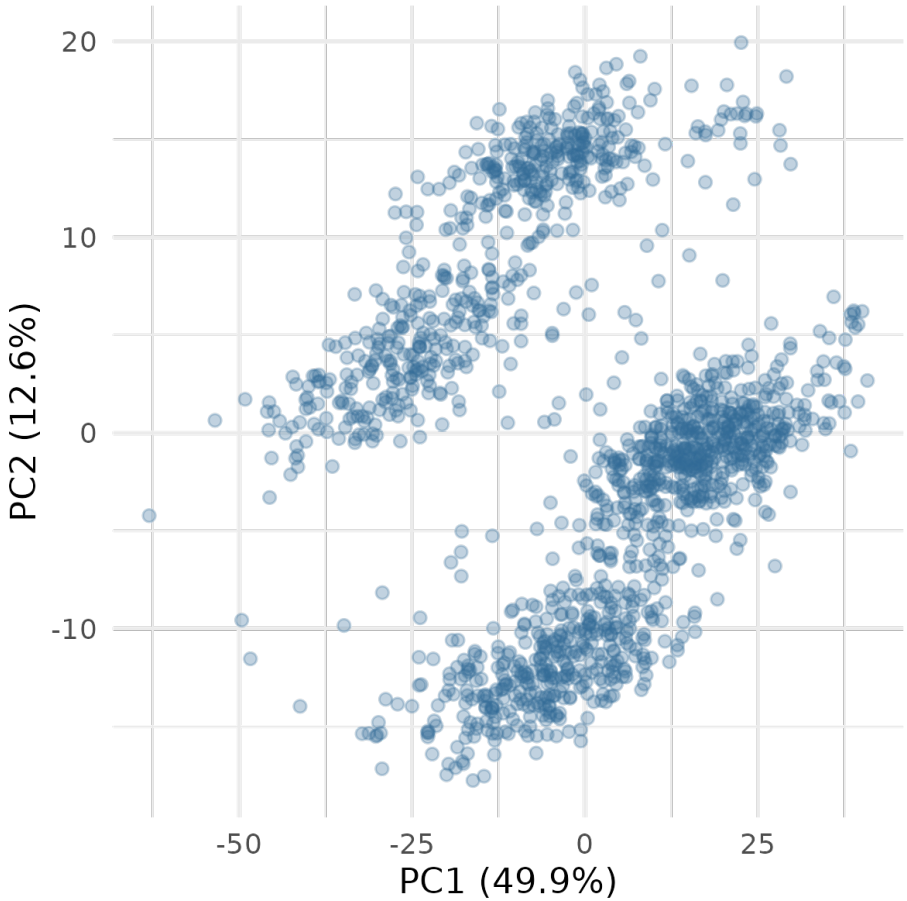
(3) Principal Component Analysis

On effect + residual matrices (of dimensions *features* $\times$ *samples*).

Material (2)

The nPOP dataset

- Data from Leduc et al. (2022)
- nano-ProteOmic sample Preparation
- 877 monocytes, 878 melanoma cells
- 19374 peptides, 3348 proteins
- **Availability of data and code**



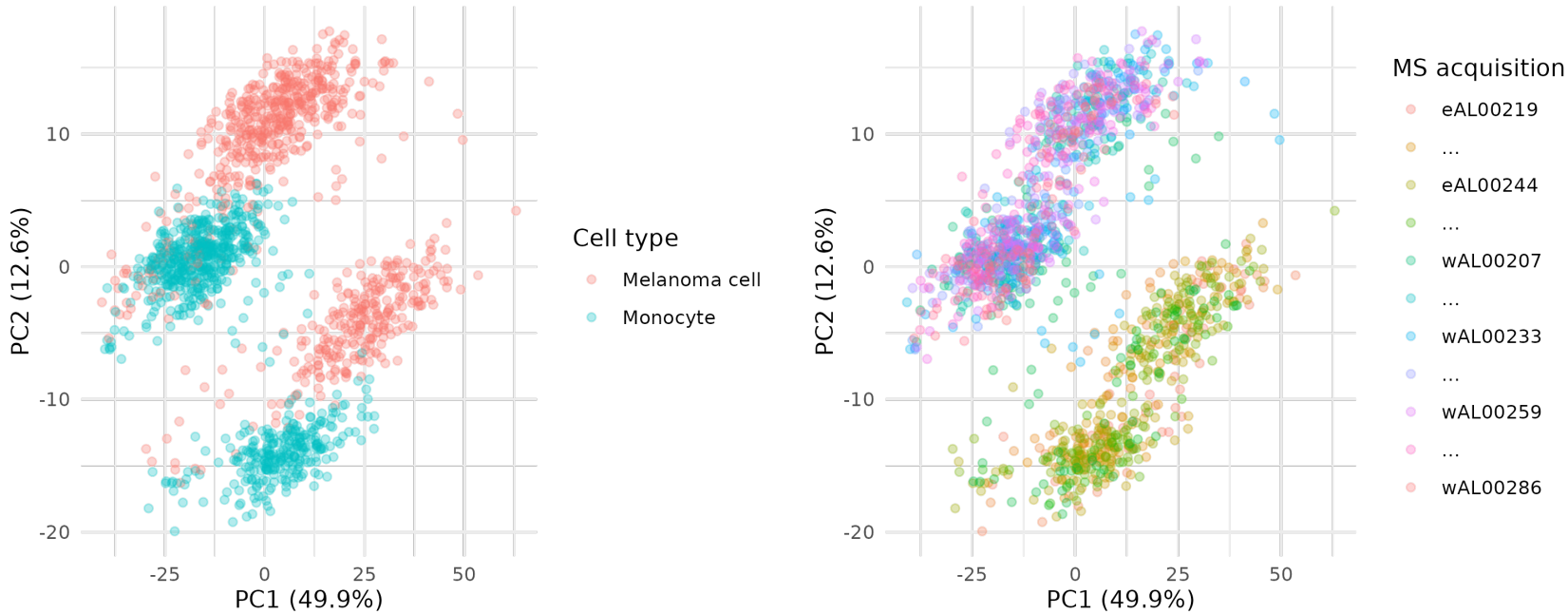


Figure: Melanoma cells and monocytes (left) acquired across multiple acquisition batches (right) (Leduc et al., 2022).

$$y = MS\ \ acquisition + TMT\ \ channel + Cell\ \ type + \epsilon$$

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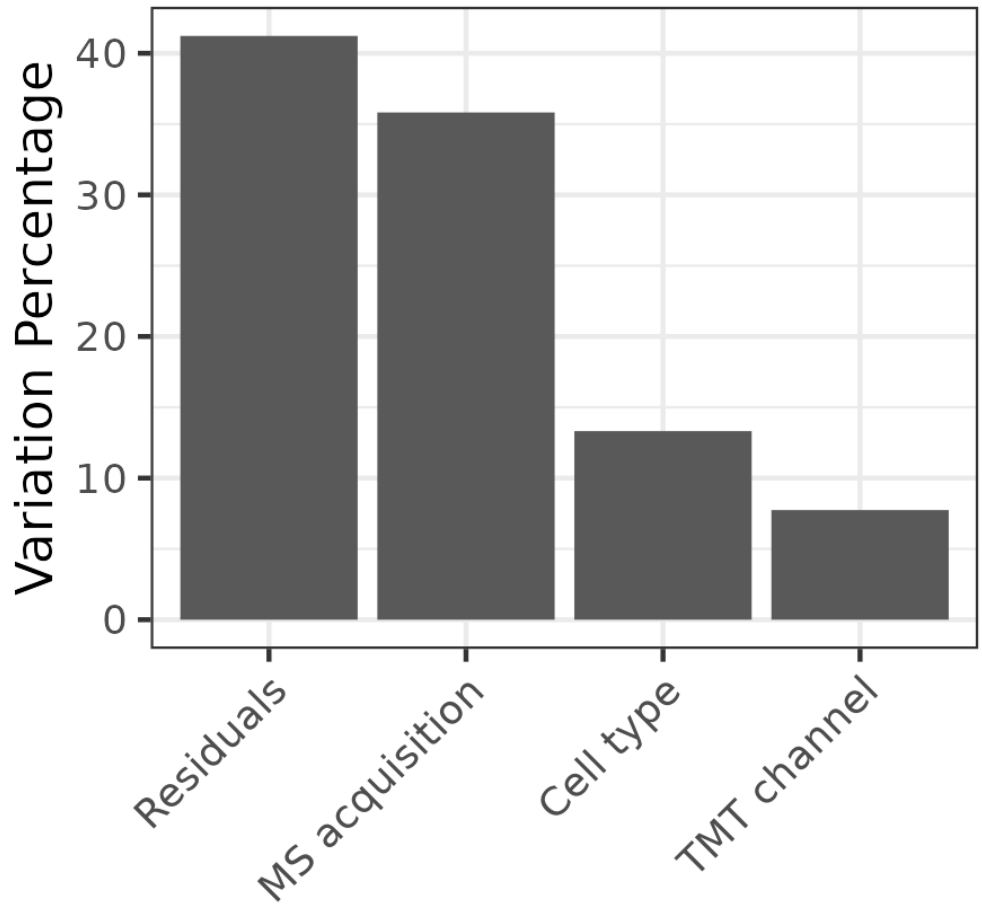
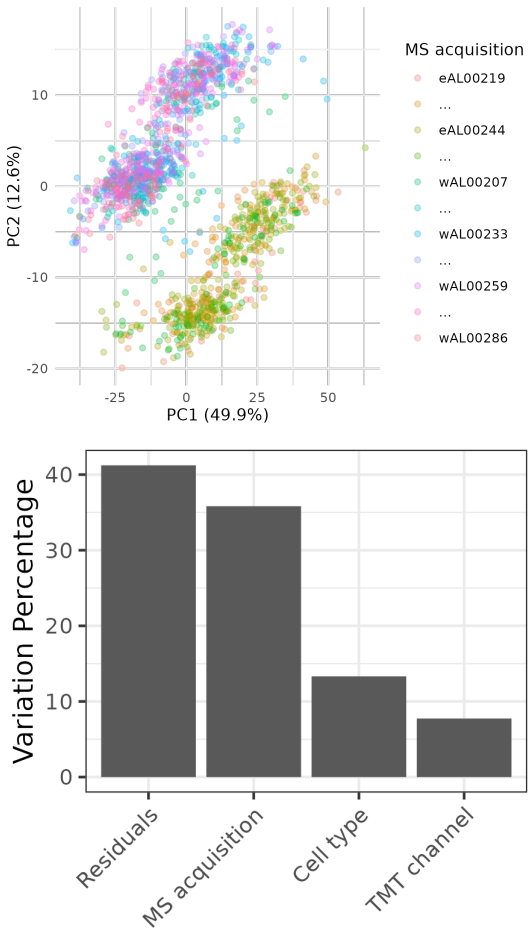


Figure: We are now in a position to **quantify known and unknown effects**: percentages of explained variances of our explained (known) and unexplained (residuals) effects. NB: low biological variance $\neq$ low quality!

PCA on effect matrices

$$y = \textit{MS acquisition} + \textit{TMT channel} + \textit{Cell type} + \epsilon$$



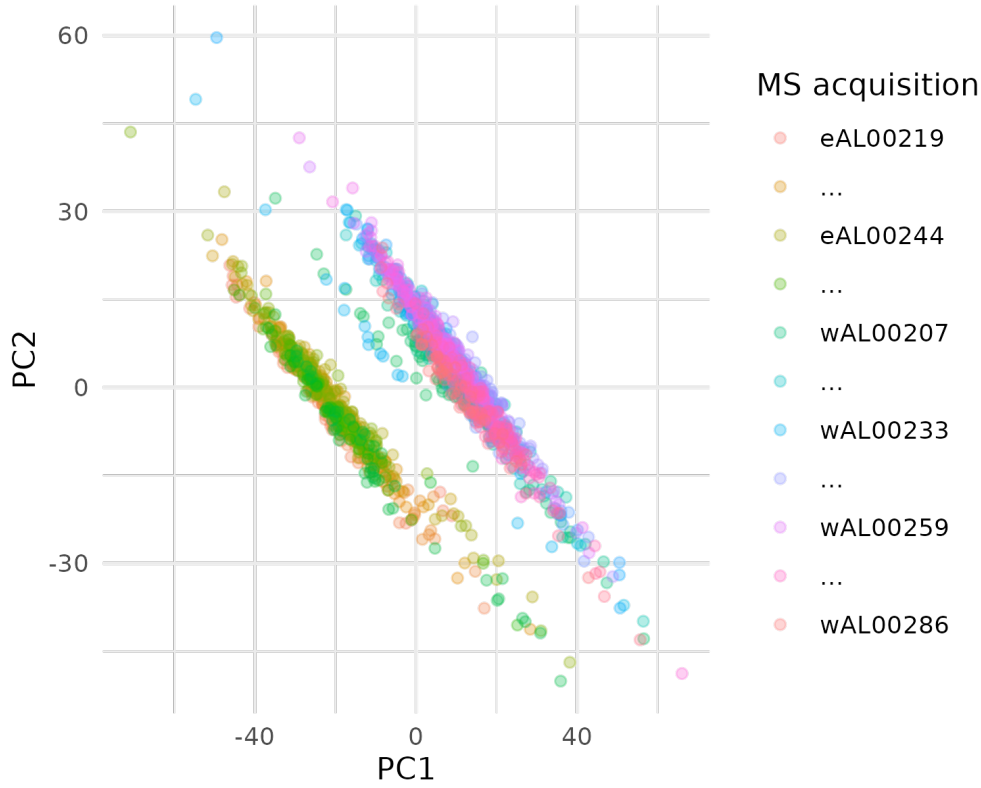
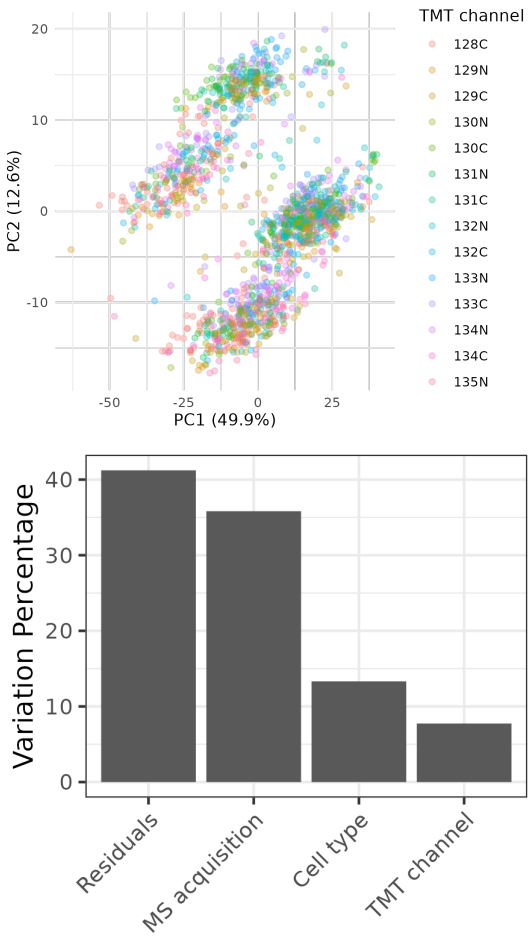


Figure: PCA on the **MS acquisition** effect matrix.

PCA on effect matrices

$$y = MS\ acquisition + TMT\ channel + Cell\ type + \epsilon$$



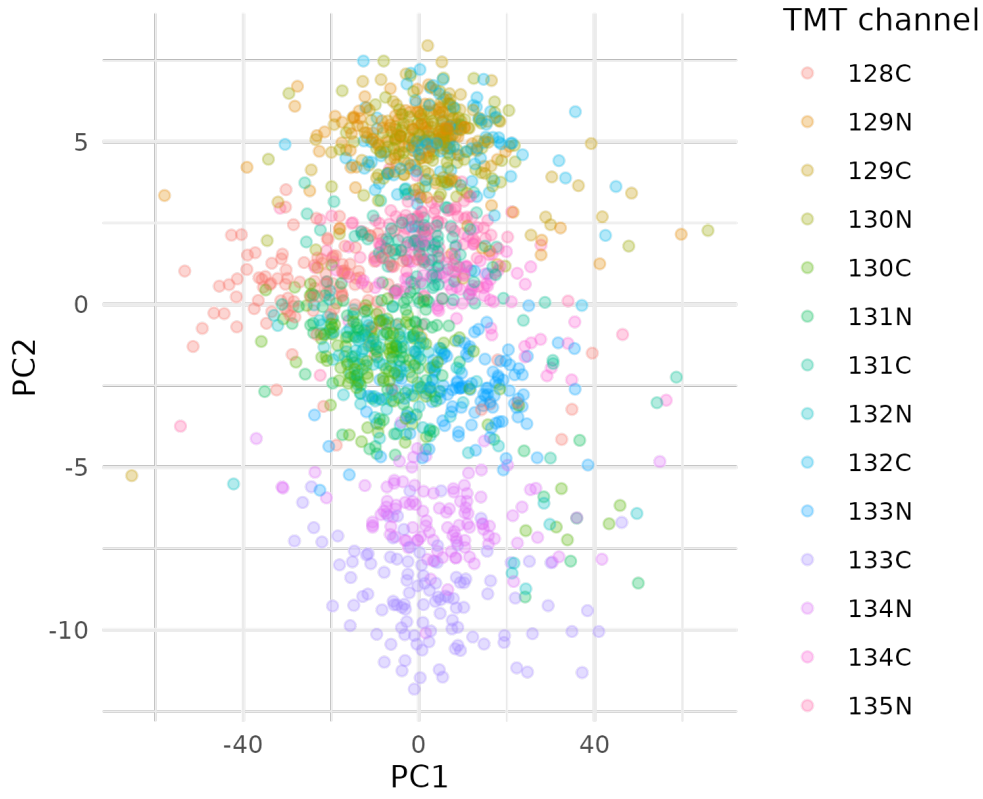
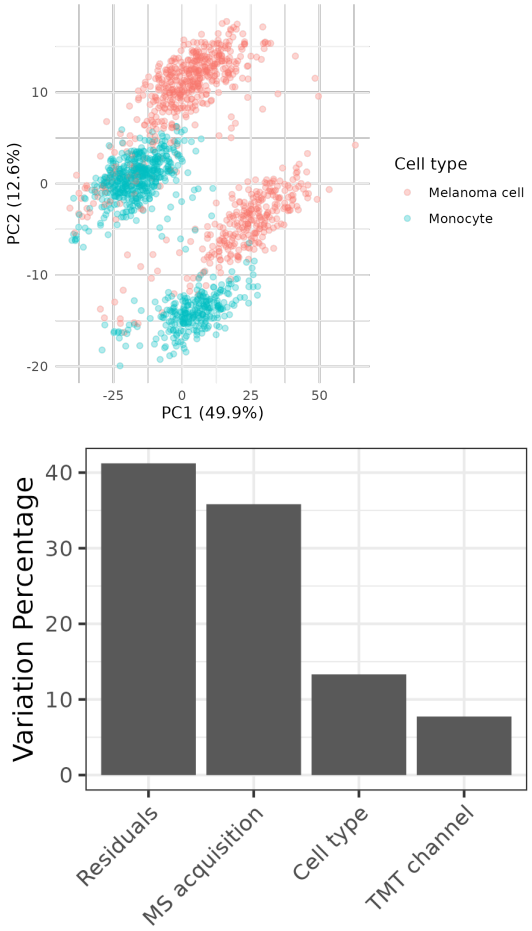


Figure: PCA on the **TMT channel** effect matrix.

PCA on effect matrices

$$y = MS\ acquisition + TMT\ channel + Cell\ type + \epsilon$$



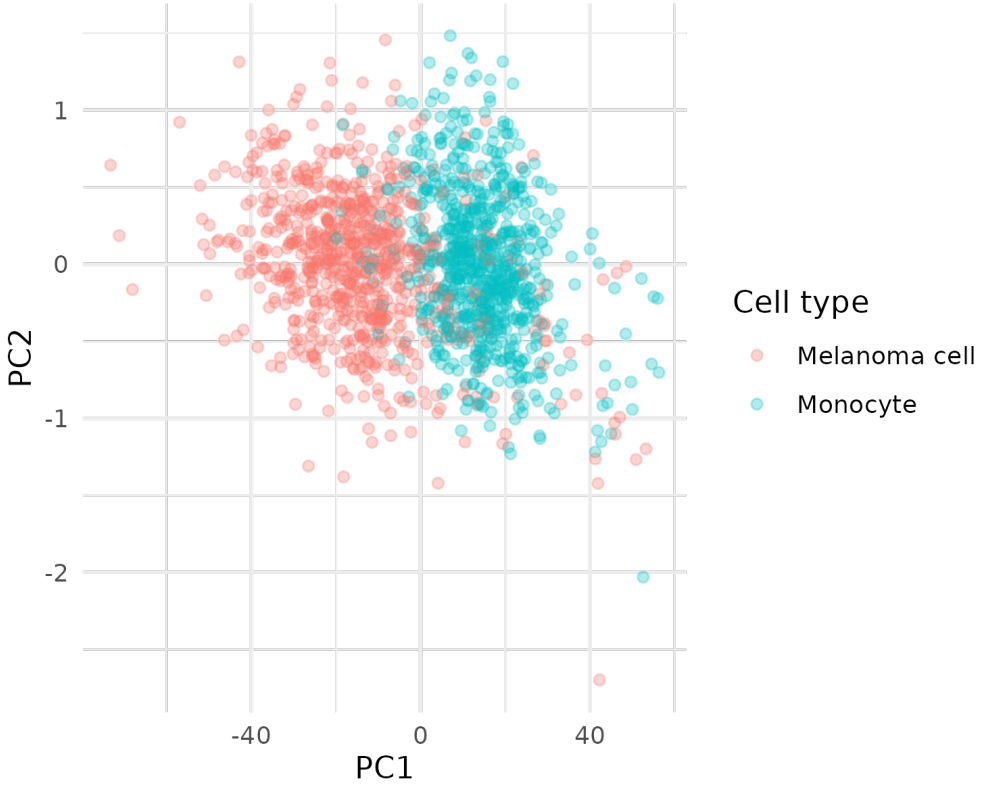
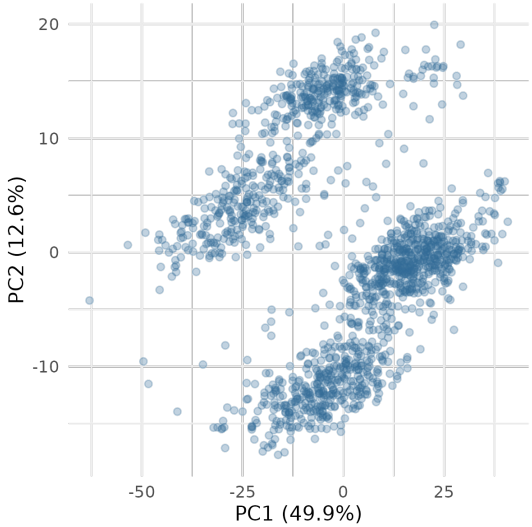
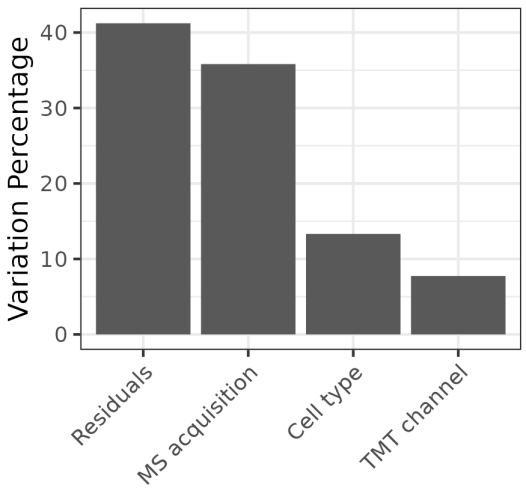


Figure: PCA on the **Cell type** effect matrix.

PCA on effect matrices

$$y = MS\ acquisition + TMT\ channel + Cell\ type + \epsilon$$





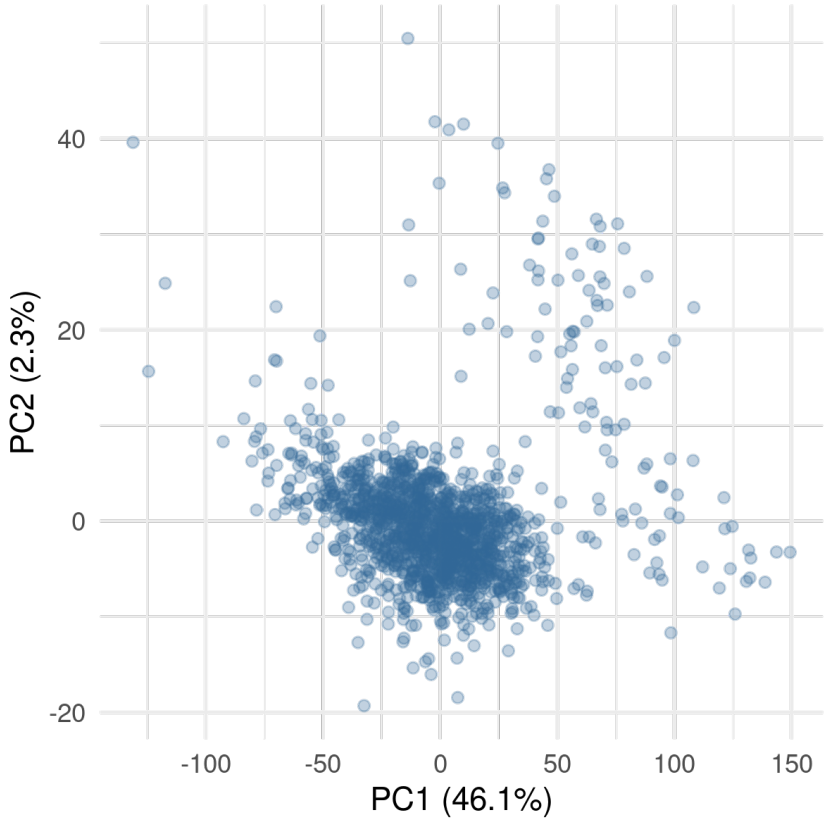
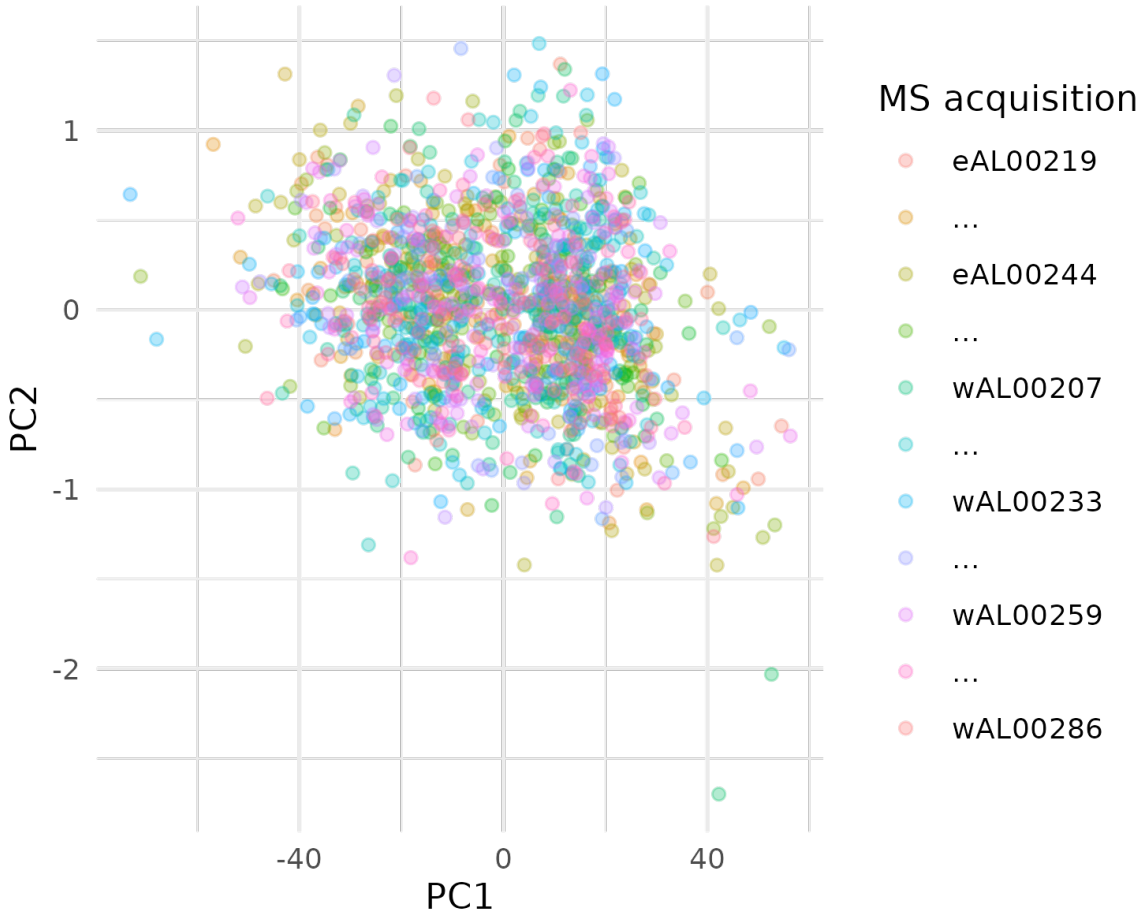


Figure: PCA on the **residuals** effect matrix.

Does it work: negative control

Do we have any MS acquitison batch *leftovers* in the cell type effect?



Does it work: positive control

$$y = MS\ acquisition + TMT\ channel + \epsilon$$

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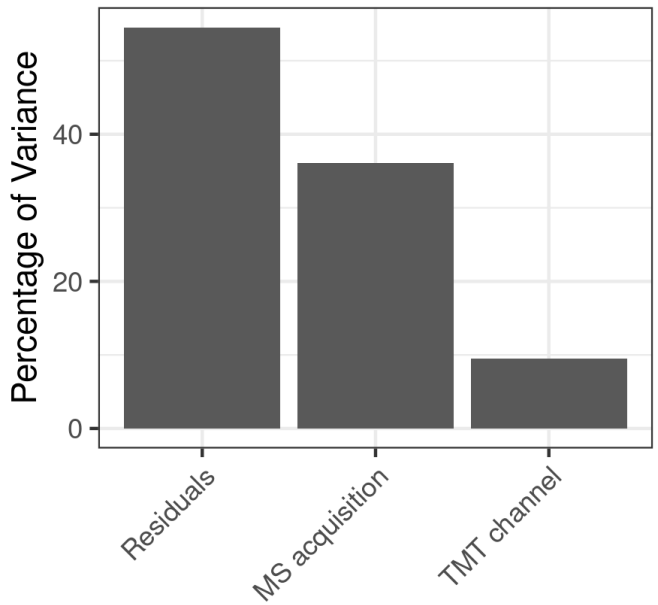

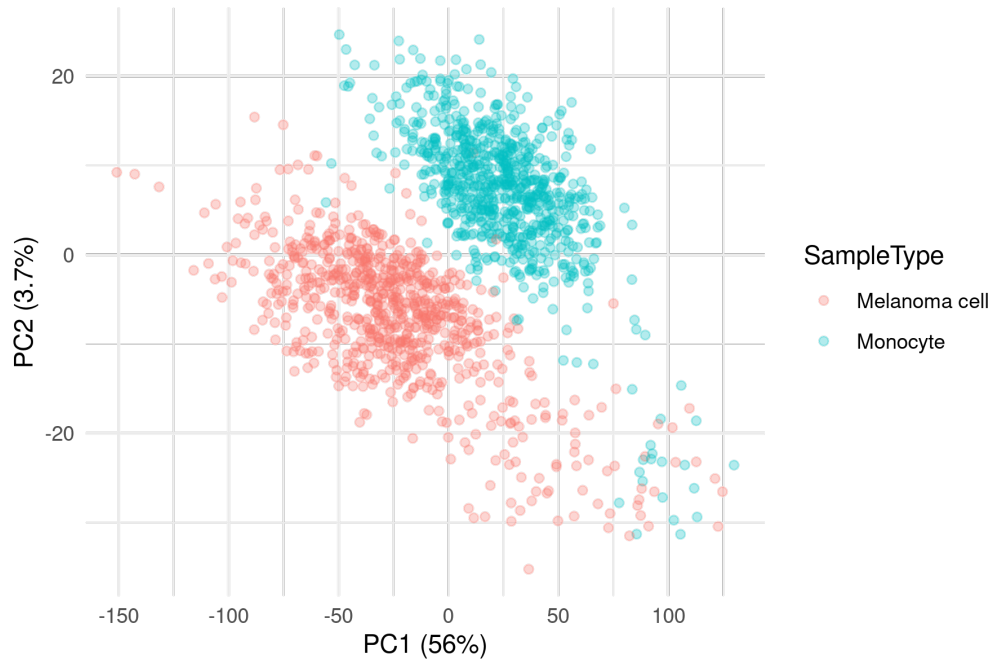

Does it work: new biology in the residuals

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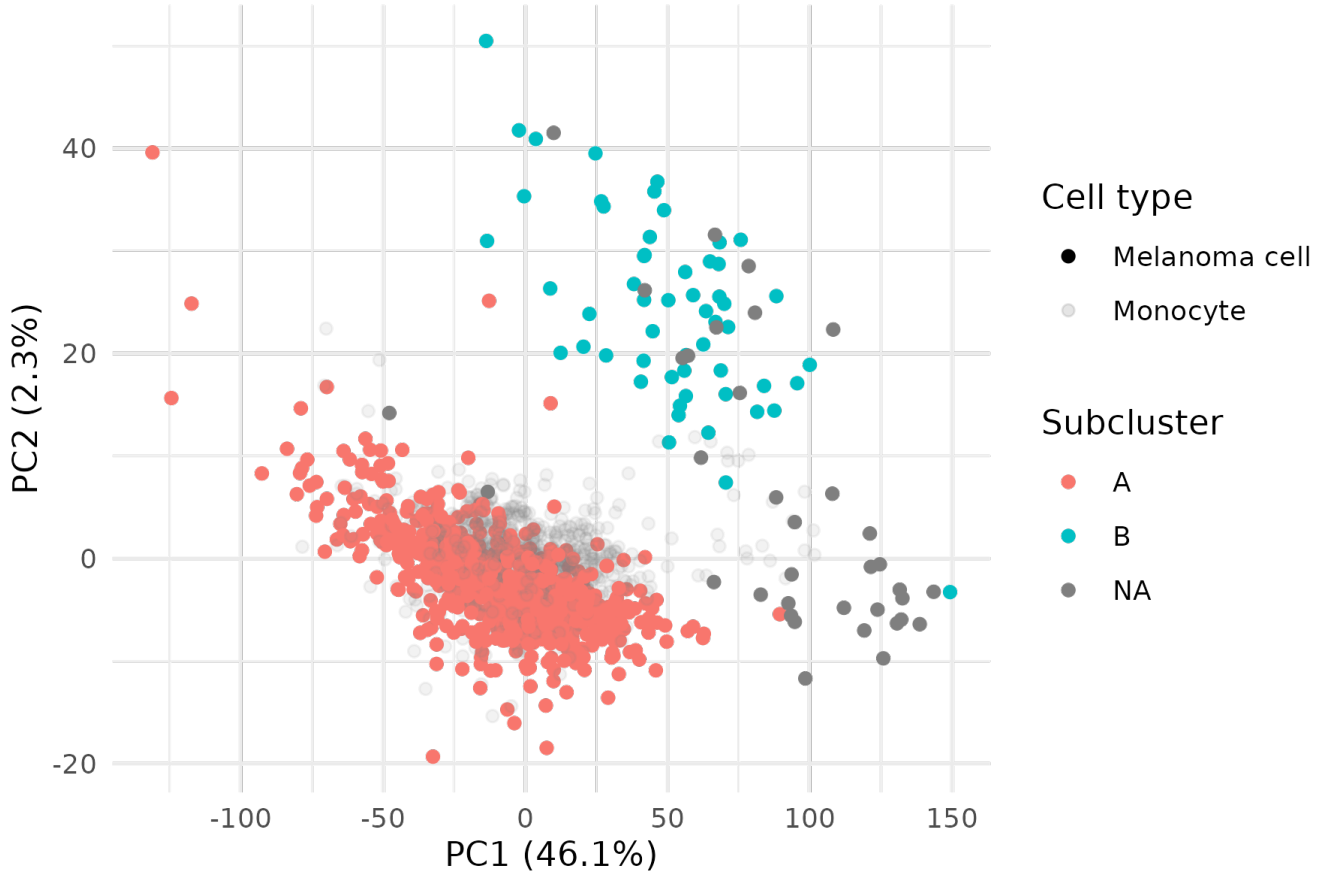

Figure: **Melanoma subpopulations**: transcriptomic signature associated with a cell state that is more likely to resist treatment by the cancer drug vemurafenib (clusters A and B from Leduc et al. (2022)).

Outline

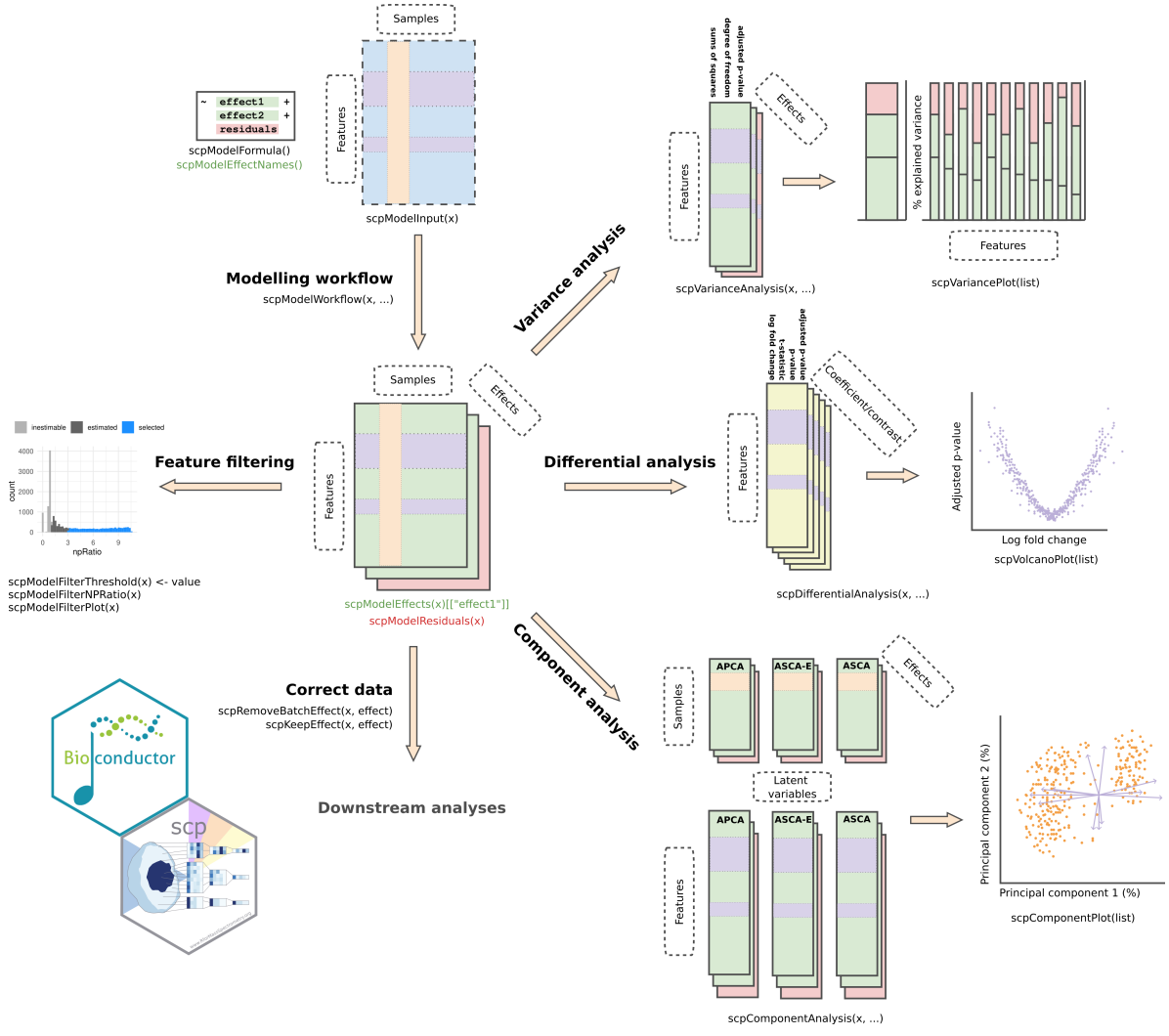

Figure: scp package - `scplainer`: using linear models to understand mass spectrometry-based single-cell proteomics data (Vanderaa and Gatto, 2024). Part of and integrates with **Bioconductor** (Huber et al., 2015) tools.

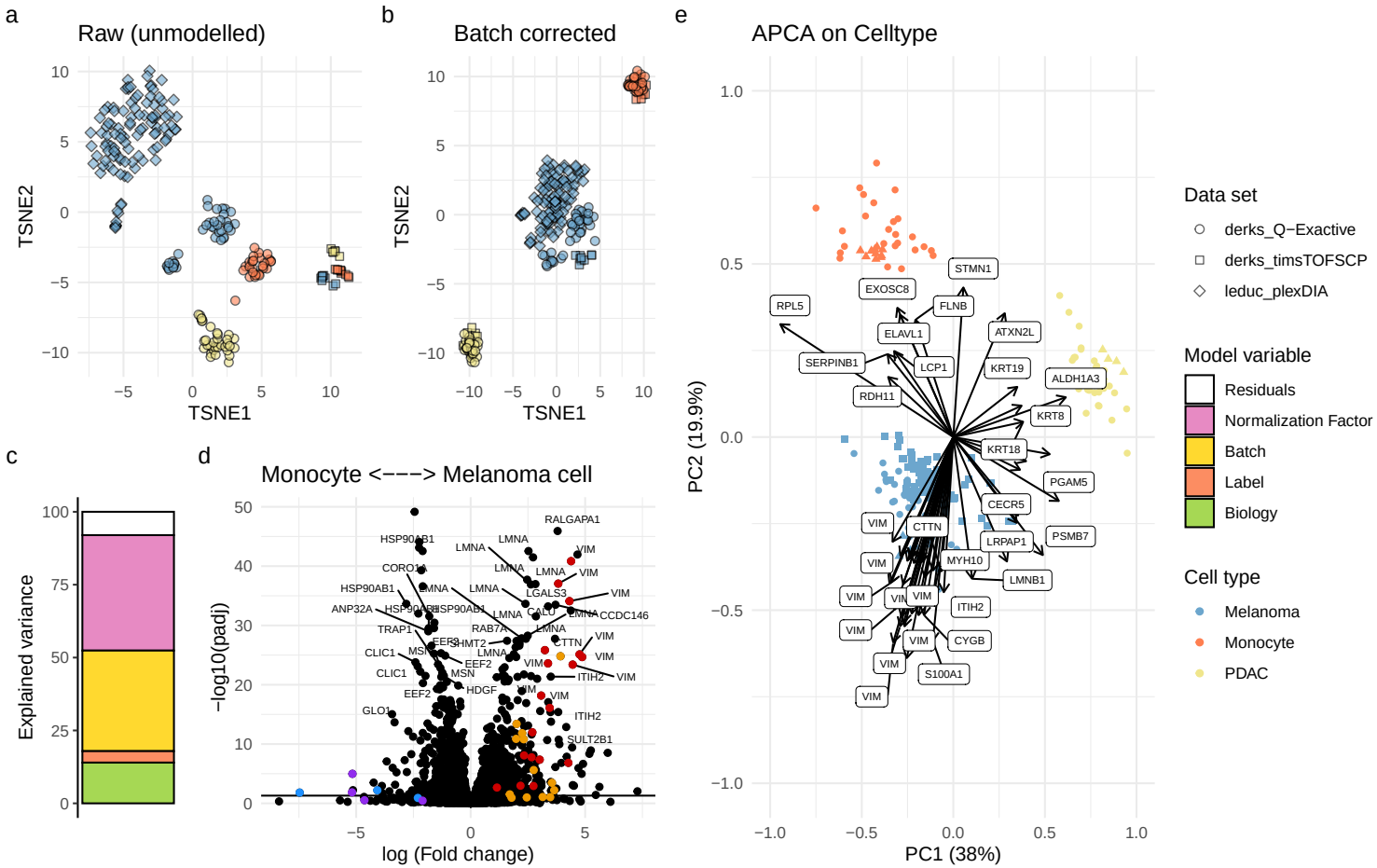

Figure: scplainer – variance (c), differential (d) and component (e) analysis, integration (a, b)

Outline

Conclusions

How to analyse ...

- We need a flexible and **principled computational approach** $\rightarrow$ control what we do, to guarantee the validity or our results.
- **Residuals** – what we don't know (yet), generally what we are most interested in.
- Importance of the **experimental design** (Gatto et al., 2023).
- Showed component analysis, differential abundance, analysis of variance. Also clustering, trajectory analysis, ... based on the batch-corrected/normalised effect matrices.
  Bioconductor *tool kit*.

How to analyse ...

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Simpler is better

- Data analysis should be as simple as possible, but no simpler.
- Data analysis should be as complex as needed, but not more complex.

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Software for data analysis

- Compose simple pipelines when possible.
- Compose more complex pipelines when necessary.
- Enable transparency and reproducibility (Markowetz, 2015).

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Users

- Knowledgeable in MS-based (single-cell) proteomics.
- Have basic knowledge of data analysis.
- Some R/Python/... experience.

References I

Laurent Gatto, Ruedi Aebersold, Juergen Cox, Vadim Demichev, Jason Derks, Edward Emmott, Alexander M Franks, Alexander R Ivanov, Ryan T Kelly, Luke Khoury, Andrew Leduc, Michael J MacCoss, Peter Nemes, David H Perlman, Aleksandra A Petelski, Christopher M Rose, Erwin M Schoof, Jennifer Van Eyk, Christophe Vanderaa, John R Yates, and Nikolai Slavov. Initial recommendations for performing, benchmarking and reporting single-cell proteomics experiments. *Nat. Methods*, pages 1–12, March 2023.

Mo Hu, Yutong Zhang, Yuan Yuan, Wenping Ma, Yinghui Zheng, Qingqing Gu, and X Sunney Xie. Correlated protein modules revealing functional coordination of interacting proteins are detected by Single-Cell proteomics. *J. Phys. Chem. B*, 127 (27):6006 – 6014, July 2023.

W Huber, V J Carey, R Gentleman, S Anders, M Carlson, B S Carvalho, H C Bravo, S Davis, L Gatto, T Girke, R Gottardo, F Hahne, K D Hansen, R A Irizarry, M Lawrence, M I Love, J MacDonald, V Obenchain, A K Oleś, H Pagès, A Reyes, P Shannon, G K Smyth, D Tenenbaum, L Waldron, and M Morgan. Orchestrating high-throughput genomic analysis with Bioconductor. *Nat Methods*, 12(2):115–21, Jan 2015. doi: 10.1038/nmeth.3252.

Andrew Leduc, R Gray Huffman, Joshua Cantlon, Saad Khan, and Nikolai Slavov. Exploring functional protein covariation across single cells using nPOP. *Genome Biol.*, 23(1):1–31, December 2022.

Florian Markowetz. Five selfish reasons to work reproducibly. *Genome Biol.*, 16:274, December 2015.

References II

Nikolai Slavov. Learning from natural variation across the proteomes of single cells. *PLoS Biol.*, 20(1):e3001512, January 2022.

Harrison Specht, Edward Emmott, Aleksandra A Petelski, R Gray Huffman, David H Perlman, Marco Serra, Peter Kharchenko, Antonius Koller, and Nikolai Slavov. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2. *Genome Biol.*, 22(1):50, January 2021.

Michel Thiel, Baptiste Féraud, and Bernadette Govaerts. ASCA+ and APCA+: Extensions of ASCA and APCA in the analysis of unbalanced multifactorial designs. *J. Chemom.*, 31(6):e2895, June 2017.

Christophe Vanderaa and Laurent Gatto. Replication of single-cell proteomics data reveals important computational challenges. *Expert Rev. Proteomics*, October 2021.

Christophe Vanderaa and Laurent Gatto. The current state of Single-Cell proteomics data analysis. *Curr Protoc*, 3(1):e658, January 2023a.

Christophe Vanderaa and Laurent Gatto. Revisiting the thorny issue of missing values in single-cell proteomics. *J. Proteome Res.*, 22(9):2775–2784, Aug 2023b. doi: 10.1021/acs.jproteome.3c00227.

Christophe Vanderaa and Laurent Gatto. scplainer: using linear models to understand mass spectrometry-based single-cell proteomics data. *bioRxiv*, 2024. doi: 10.1101/2023.12.14.571792.

UCLouvain

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