**Refining scRNAseq Analysis for Homogeneous Stem Cell Data: Exploring Clustering Approaches and Non-Negative Matrix Factorization to Uncover Dynamic Infection Responses**

**Kateřina Večerková1, 2, Irina Ribeiro Bas3, Michal Kolář1, 2, Meritxell Alberich Jordà3​**

1Laboratory of Genomics and Bioinformatics; Institute of Molecular Genetics of the Czech Academy of Sciences​

2Department of Informatics and Chemistry; University of Chemistry and Technology, Prague​

3Laboratory of Haematooncology, Institute of Molecular Genetics of the Czech Academy of Sciences​

Single-cell RNA sequencing (scRNAseq) is a powerful tool for exploring cellular heterogeneity, but standard analysis pipelines often fail when applied to homogeneous cell populations. In this study, we analyzed a time-series scRNAseq dataset from hematopoietic stem cells responding to infection across five timepoints and a control. Standard workflows predominantly captured variation due to cell cycle effects, obscuring relevant biological signals. To address this, we refined the clustering process through iterative exploration, aiming for exclusive and biologically meaningful marker expression. In parallel, we applied non-negative matrix factorization (NMF) to identify interpretable gene expression programs reflecting condition- and timepoint-specific responses. Our results demonstrate that adapting analysis workflows to data characteristics can reveal otherwise hidden transcriptional programs in homogeneous single-cell datasets.