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This is the final version of the accepted manuscript. The original article has been published in final edited form in:

Molecular Cell
2022 JAN 20 ; 82(2): 234-236
doi: 10.1016/j.molcel.2021.12.028

Publisher: Cell Press

ATACing single cells with phages

Patrick Maschmeyer[1], Simon Haas[2]

[1] Berlin Institute of Health (BIH) at Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany; Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Institute for Medical Systems Biology (BIMSB), 10115 Berlin, Germany; Department of Hematology, Oncology and Cancer Immunology, Charité - Universitätsmedizin Berlin, 10115 Berlin, Germany.

[2] Berlin Institute of Health (BIH) at Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany; Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Institute for Medical Systems Biology (BIMSB), 10115 Berlin, Germany; Department of Hematology, Oncology and Cancer Immunology, Charité - Universitätsmedizin Berlin, 10115 Berlin, Germany; German Cancer Consortium (DKTK), 69120 Heidelberg, Germany; Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), 69120 Heidelberg, Germany; Division of Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ) and DKFZ-ZMBH Alliance, 69120 Heidelberg, Germany. Electronic address: simon.haas@bih-charite.de.

Abstract

Fiskin et al. (2021) developed a “multi-omics” approach that integrates phage-displayed single-domain antibodies (“nanobodies”) with the assay for transposase-accessible chromatin (PHAGE-ATAC) to simultaneously determine protein expression, chromatin accessibility, and mitochondrial DNA mutations (for clonal tracing) in single cells.

Main text

Single-cell genomic technologies have been invaluable to study cellular heterogeneity and to assess complex cell states and mechanisms of cell regulation (Regev et al., 2017). Extension of these tools into multimodal (“multi-omics”) approaches, provides the possibility to concomitantly characterize multiple aspects of cellular biology, such as the transcriptome, protein expression, and global (epigenomic) chromatin accessibility in thousands of single cells (Mimitou et al., 2021; Stuart and Satija, 2019; Triana et al., 2021). For inclusion of protein-profiling, usually antibodies that recognize surface proteins are conjugated with DNA oligonucleotides, which serve as barcodes and enable protein quantification (Shahi et al., 2017; Stoeckius et al., 2017). This approach takes advantage of a large reservoir of existing antibodies. However, raising antibodies against newly identified targets is comparatively expensive and the requirement for tagging each antibody of a library with a unique oligonucleotide barcode limits scalability and construction of pooled antibody libraries. To overcome these limitations, Fiskin et al. introduced phages to the toolbox of single-cell genomics in a recent study in *Nature Biotechnology* (Fiskin et al., 2021). With PHAGE-ATAC, the authors developed a multimodal single-cell genomics approach that combines protein quantification by phage-displayed single-domain antibodies (“nanobodies”) with epigenomic chromatin accessibility profiling and detection of mitochondrial DNA mutations that can be used for clonal tracing of native cells (Fiskin et al., 2021; Lareau et al., 2021; Ludwig et al., 2019) (Figure 1). Of note, extension of this workflow into PHAGE-ASAP enables simultaneous

quantification of proteins by phage-display and oligonucleotide-conjugated antibodies (Fiskin et al., 2021; Mimitou et al., 2021).

To integrate protein quantification by phage-displayed nanobodies into the 10x Genomics single-cell ATAC sequencing (scATAC-seq) platform, phages were engineered to encode 3 essential components in their genomes. Phagemids carried (1) DNA sequences encoding the complementary determining regions (CDRs) 1, 2, and 3 of an antigen-specific nanobody, (2) the Illumina Read 1 (RD1) sequence as a PHAGE-ATAC tag (PAC-tag) and (3), the p3 gene required for display of nanobodies on the surface of the phage. While the surface display of the nanobody facilitates specific binding of the phage to cells expressing the target antigen, the Illumina RD1 sequence enables the generation of ATAC fragment libraries and phage-derived tag (PDT) libraries with the 10x Genomics scATAC-seq pipeline. After sequencing of the PDT-libraries, the hypervariable CDR3 sequence of the phage nanobody (pNb) is used as a genetic barcode encoding the nanobody identity that initially bound its target antigen, thereby enabling quantification of the expressed antigen on the cell.

To validate this system, the authors engineered phages that express nanobodies recognizing enhanced green fluorescent protein (EGFP) and performed PHAGE-ATAC on a mixture of mouse $EGFP^-$ NIH 3T3 cells, human $EGFP^-$ HEK293T cells, and HEK293T cells that expressed (membrane-bound) EGFP. Computational analysis of the sequenced PDT library recovered the respective input ratios of cells, while quality metrics of the scATAC-seq library were comparable to those without additional protein detection. Moreover, PDT counts of phages binding EGFP were specifically found on cells that were identified as human by scATAC-seq. Importantly, phages were also able to detect intracellular proteins following fixation and cell lysis. Together, these results show that PHAGE-ATAC is capable of detecting and quantifying target proteins via the antigen-specific binding of nanobodies.

Next, the authors extended the modalities of their approach. In parallel to phage-mediated protein detection and chromatin accessibility, they recorded mitochondrial genotypes (PHAGE-ATAC) and proteins by oligonucleotide-conjugated antibodies (PHAGE-ASAP) on peripheral blood mononuclear cells (PBMCs) (Fiskin et al., 2021; Lareau et al., 2021; Ludwig et al., 2019; Mimitou et al., 2021). Phage nanobodies were antigen-specific and PHAGE-ATAC recovered accessible chromatin and mitochondrial genotypes, while PHAGE-ASAP also captured protein expression by oligonucleotide-labeled antibodies, demonstrating that PHAGE-ATAC/ASAP is able to detect proteins, chromatin accessibility, and mitochondrial genotypes in the same (single) cells and can be combined with antibody-derived tag library protocols.

Oligonucleotide-tagged antibodies may also be used for sample multiplexing and overloading, followed by computational demultiplexing via sample-specific oligonucleotide barcodes (cell hashing). Fiskin et al. tested whether pNbs were also suitable for cell hashing by generating four distinct anti-CD8 hashtag pNbs. Each of the four hashtag pNbs carried a unique silent mutation in their CDR3-sequence, which can be utilized as a barcode while it does not alter the structure and function of the anti-CD8 nanobody. Subsequent staining of $CD8^+$ T cells from four healthy donors with these hashtags allowed pooling and “overloading” of the sample for the 10x pipeline with ~20,000 cells. Subsequent cell hashing by PHAGE-ATAC allowed efficient doublet removal, re-assignment of cells to their original donors (with a classification accuracy

of 92.9%) and resulted in high quality data for >8,300 cells, exhibiting the possibility to use pNbs for sample multiplexing in scATAC-seq.

An advantage of phage-derived nanobodies over antibodies is their suitability for screening and selection from pooled phage libraries against a target antigen of interest in a short amount of time. Importantly, phage library generation and selection can be performed *in vitro* and does not require immunization of animals (Bradbury et al., 2011). To illustrate the utility of antigen-specific phages for PHAGE-ATAC, the authors generated a synthetic high-complexity library of 4.96×10^9 pNbs. Next, they selected pNbs recognizing $EGFP^+$HEK293T cells. After three rounds of selection, a high fraction of pNb clones recognized $EGFP^+$ cells, containing pNbs with binding affinities comparable to phages that previously have been reported to strongly bind to EGFP. These results show that phage-displayed nanobody libraries can be selected to rapidly generate new barcoded affinity reagents for PHAGE-ATAC applications (Figure 1).

Finally, the authors evaluated the possibility of multiplexing epitope detection and monitoring antigens with multiple target-specific pNBs simultaneously using PHAGE-ATAC. For this, the authors utilized 7 synthesized and library-selected phages that bind to the SARS-CoV-2-S spike protein, to EGFP and to the PBMC lineage markers CD4, CD8, and CD16. Afterward, they mixed PBMCs, SARS-CoV-2-S^+EGFP^-HEK293T cells, and SARS-CoV-2-S^-EGFP^+HEK293T cells and characterized them by PHAGE-ATAC. The filtered datasets recovered the input ratio of mixed cells and expected cell states. In addition, the authors observed mutually exclusive detection of anti-SARS-CoV-2-S PDTs, anti-EGFP PDTs, and anti-PBMC PDTs, while PDT levels of pNbs recognizing identical antigens correlated highly with each other. Together, these results show that PHAGE-ATAC facilitates detection of host and viral antigens and can be leveraged for multiplex single-cell characterization by synthesized as well as library-selected phages.

In conclusion, the study by Fiskin et al. demonstrates that PHAGE-ATAC is highly specific and sensitive in detecting cellular protein expression. Moreover, it showcases that PHAGE-ATAC and PHAGE-ASAP enable scalable profiling of single cells and can be embedded into a “multi-omic” framework that has the potential to advance and refine multimodal single-cell genomics approaches in the future.

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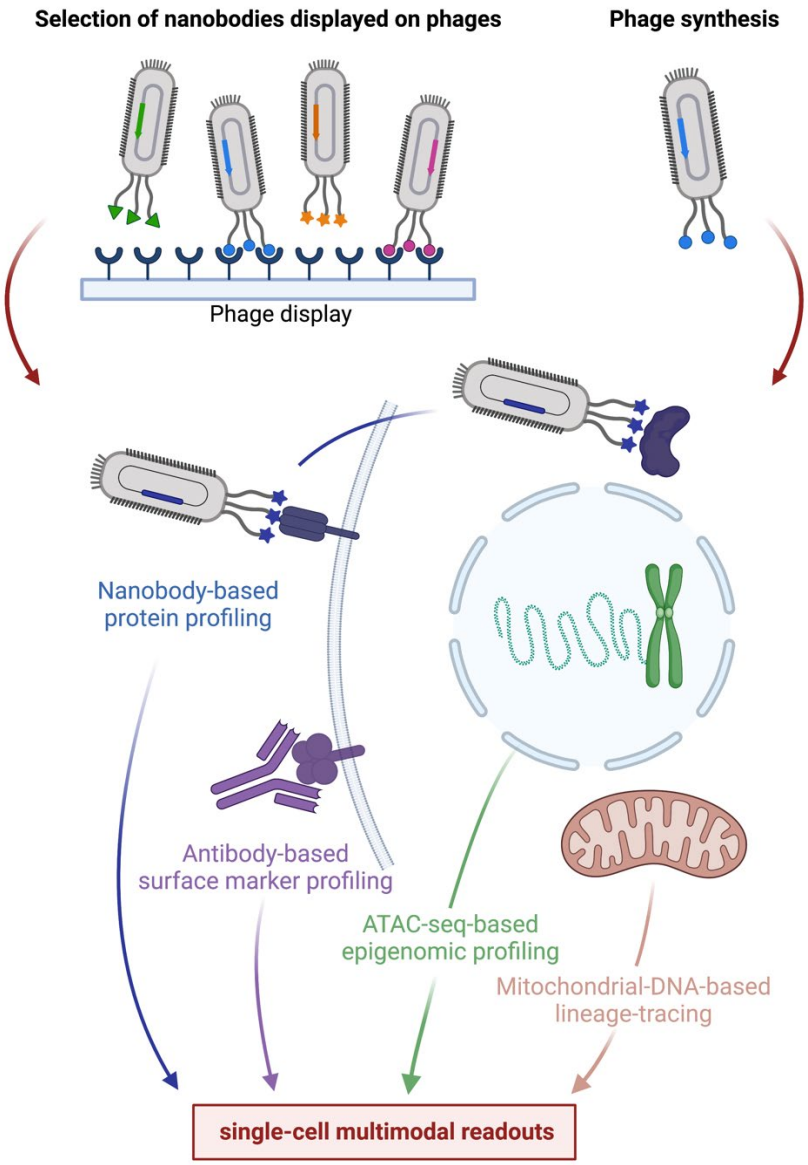

Figure 1: PHAGE-ATAC is a multimodal genomics approach that can be utilized for profiling of proteins, chromatin accessibility and mitochondrial genotypes in single cells.