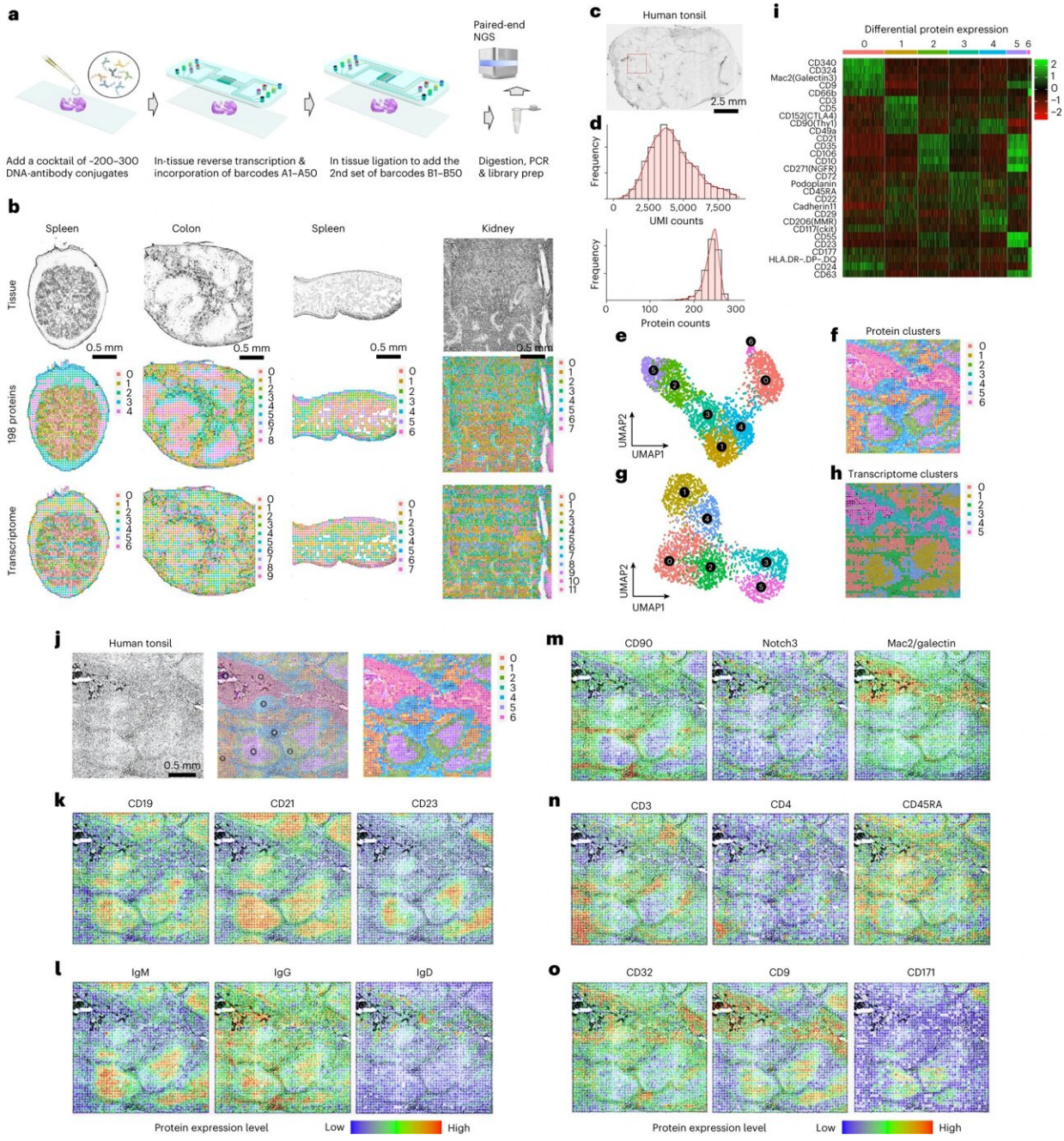
Team co-maps proteins and transcriptome in human tissues

March 16 2023, by Isabella Backman



Spatial-CITE-seq workflow design and application to diverse mouse tissue types and human tonsil for co-mapping of proteins and whole transcriptome. **a**, Scheme of spatial-CITE-seq. A cocktail of ADTs is applied to a PFA-fixed tissue section to label a panel of ~200–300 protein markers in situ. Next, a set of DNA barcodes A1–A50 is flowed over the tissue surface in a spatially defined manner via parallel microchannels, and reverse transcription is carried out inside each channel for in-tissue synthesis of cDNAs complementary to endogenous mRNAs and introduced ADTs. Then, a set of DNA barcodes B1–B50 is introduced using another microfluidic device with microchannels perpendicular to the first flow direction and subsequently ligated to barcodes A1–A50, creating a 2D grid of tissue pixels, each of which has a unique spatial address code AB. Finally, barcoded cDNA is collected, purified, amplified and prepared for paired-end NGS sequencing. **b**, Spatially resolved 189-plex protein and whole transcriptome co-mapping of mouse spleen, colon, intestine and kidney tissue with 25-μm pixel size. Upper row: bright-field optical images of the tissue sections. Middle row: unsupervised clustering of all pixels based on all 189 protein markers only and projection onto the tissue images. Lower row: unsupervised clustering of whole transcriptome of all pixels and projection to the tissue images. Colors correspond to different proteomic or transcriptomic clusters indicated on the right side of each panel. **c**, Image of a human tonsil tissue section. The region mapped by spatial-CITE-seq is indicated by a dashed box. **d**, Per-pixel UMI count and protein count histograms. **e**, UMAP plot of the clustering analysis of all pixels based on 273 proteins only. **f**, Spatial distribution of the clusters (0–6) indicated by the same colors as in **e**. **g**, UMAP plot of the clustering analysis of all pixels based on the mRNA transcriptome. **h**, Spatial distribution of the transcriptomic clusters (0–5) indicated by the same colors as in **g**. Pixel size: 25 μm. **i**, Differentially expressed proteins in the clusters shown in **c** and **d**. **j**, Tissue image of the mapped region (left), spatial proteomic clusters (right) and the overlay (middle). **k**, Individual surface protein markers related to B cells and follicular DCs. **l**, Functional protein markers such as immunoglobulins showing spatially distinct distribution of GC B cells (IgM), matured B cells (IgG) and naive B cells (IgD), in agreement with B cell maturation, class switch and migration. **m**, Individual protein markers enriched in the extracellular region (CD90, Notch3) and crypt (Mac2). **n**, Individual T cell

protein markers CD3, CD4 and CD45RA showing T cell zones and subtypes. **o**, Individual protein markers CD32, CD9 and CD171. CD32 identified a range of immune cells, including platelets, neutrophils, macrophages and DCs, trafficking from vasculature. CD9 identified plasma cell precursors in GCs and crypt. CD171, a neural cell adhesion molecule, is found highly distinct in the GC dark zone. Color key: protein expression from high to low. Credit: *Nature Biotechnology* (2023). DOI: 10.1038/s41587-023-01676-0

To understand how cells behave, researchers also need to understand the molecules that make them work. "If someone wants to know how the kidney functions, they have to know what's going on inside the kidney cells," says Yang Liu, Ph.D., assistant professor of pathology. "This is defined by the protein activity."

But most spatial transcriptome sequencing studies don't include the proteins, leaving out vital information about the mechanisms of disease progression. Now, in their latest study, a Yale team performed a high-plex protein and whole-transcriptome co-mapping that measured nearly 300 proteins and transcriptome in human tissues. They published their findings in *Nature Biotechnology* on February 23.

"This is a game changer—crossing the central dogma of molecular biology and looking at hundreds of proteins simultaneously," says Rong Fan, Ph.D., Harold Hodgkinson Professor of Biomedical Engineering and of Pathology and the study's senior author.

Protein data has been largely absent from studies because of the technical limitations of immunofluorescence and immunohistochemistry, which made it challenging to image proteins in large numbers. "About 10 years ago, if someone simultaneously imaged a handful of protein markers, it was stunning," says Fan.

But in 2020, the team published a study in *Cell* using a technique called deterministic barcoding in tissues. The work involved delivering DNA tags into tissues using antibodies that can target specific proteins, tagging 22 proteins in total, in addition to mRNA molecules. This was the first study to co-map the transcriptome and proteins.

In their latest study, the team built on their previous work by using these methods to profile the whole transcriptome and 189 proteins in several mouse tissues. They also measured the whole transcriptome and 273 proteins in human tissue. "Our study is unique for two reasons. First, we co-profile RNA and proteins at the same time in the same tissue sections. There are no other technologies that can do this," says Liu, who was the first author of the study. "Second, nearly 300 proteins imaged in the same tissue section is really a world record."

Among the human tissues collected were skin samples following vaccination by the COVID-19 Moderna vaccine. With the guidance and expertise of David Hafler, MD, chair and William S. and Lois Stiles Edgerly Professor of Neurology and professor of immunobiology, Mary Tomayko, MD, Ph.D., associate professor of dermatology and of pathology, and Marcello DiStasio, MD, Ph.D., assistant professor of pathology, the team performed human skin biopsies to better understand immune activation responses at the injection site. They discovered a unique subset of cells known as peripheral helper T cells aggregated at the site.

"The way we were able to identify the cells and visualize where they are highlights the power of our technology," says Fan. The team also used their technology to measure human lymphoid tissues such as tonsils and revealed distinct immune reactions in collaboration with Joseph Craft, MD, Paul B. Beeson Professor of Medicine (Rheumatology) and professor of immunobiology, Stephanie Halene, MD, Arthur H. and Isabel Bunker Associate Professor of Medicine and chief of hematology,

and Mina Xu, MD, associate professor of pathology and laboratory medicine, and director of hematopathology.

The study only observed surface proteins, or proteins on the cell membrane. The team hopes to apply its technology to also look at intracellular signaling proteins and extracellular matrix proteins. "In the future, we want to continue to make this technology more powerful and increase the number of proteins we study to thousands," says Fan.

The team is excited about the study's implications in terms of better understanding disease and aging. For instance, they are interested in using their technology to learn more about inflammation in aged or diseased tissues. They also are optimistic about using this technology to study tumors and the tumor microenvironment.

"Now we can study hundreds of proteins and define different cell types. Then, we can see how these different cell types interact with tumor cells," says Fan. "This is a powerful technology, and it's prime time to dive into these challenging human health and disease research questions."

More information: Yang Liu et al, High-plex protein and whole transcriptome co-mapping at cellular resolution with spatial CITE-seq, *Nature Biotechnology* (2023). DOI: 10.1038/s41587-023-01676-0

Provided by Yale University