Proximity sequencing for single-cell measurement of protein complexes

The ability to measure protein complexes in single cells is currently limited to a very small number of targets. Combining a proximity ligation assay with single-cell sequencing creates the ability to measure hundreds of extracellular protein complexes and thousands of mRNAs in individual cells.

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The problem

Although the tools available to measure RNA and protein in single cells have developed over the past decade[1–3], few options are available for measuring proteins and their complexes at the single-cell level. Quantification of protein molecules is crucial to understanding and modeling biological processes, as proteins directly perform most actions in cells.

Measurement of protein complexes is a particularly difficult problem because the number of pairwise complexes scale quadratically: the dozens of proteins involved in a biological process could produce hundreds of different protein pairs that need to be measured in individual cells. Current technologies cannot measure more than a handful of protein complexes in single cells. These challenges have hindered the use of single-cell analysis in many applications in signaling, drug studies, pathogen–host interactions and cancer.

The solution

To address the challenge of measuring protein complexes, we developed a method called proximity sequencing (Prox-seq) that incorporates pairs of DNA-barcoded antibody probes into commonly used single-cell sequencing pipelines. Prox-seq produces a DNA signal if two antibodies are very close to one another and the barcoded oligos ligate, indicating the presence of a protein complex (Fig. 1a). Sequencing of the ligated DNA products allows quantification of proteins and their complexes.

Along with individual proteins and their complexes, Prox-seq measures the transcriptomes of single cells through mRNA sequencing. This provides multidimensional analysis of individual cells, using popular and practical sequencing platforms. As part of our validation, we applied Prox-seq to human cell lines and discovered that we could confidently identify several known protein complexes and immune cell types. We then applied Prox-seq to 8,700 individual human peripheral blood mononuclear cells (Fig. 1b) and measured 38 individual proteins, 741 protein dimers and thousands of transcripts from each cell. We found a novel interaction between surface proteins CD8 and CD9 in naïve CD8 T cells.

Finally, we studied human macrophages to observe receptor complex formation upon pathogen signaling, using Prox-seq data to train a logistic-regression classifier that identified the type of signal received by each macrophage.

The implications

An immediate application of Prox-seq is studying receptor and co-receptor interactions during immune signaling. Cells vary in receptor expression, resulting in heterogenous responses to signaling molecules. This phenomenon is understudied owing to a lack of effective tools. Prox-seq offers the opportunity to quantitatively couple variability in signaling molecules, receptor engagement and transcriptional response for individual immune cells. Prox-seq also has sufficiently high multiplexing to identify novel protein complexes. This defines an area for Prox-seq in cellular phenotyping, discovery of new cell types and functional states, and mapping of tissues and organs with single-cell resolution. Prox-seq is simple and does not require complicated experimental setups. Many existing bioinformatics software packages are readily applicable to Prox-seq, and we developed software for analysis of the unique aspects of Prox-seq data. We hope that these advantages will enable the widespread use Prox-seq by many laboratories.

There are two primary limitations of Prox-seq. First, the method requires antibodies, limiting its potential to available reagents and potentially creating false negatives owing to insufficient antibody–protein binding. However, antibodies have been used successfully for decades, and popular methods such as CITE-Seq[1] already use antibodies for single-cell protein measurements. Second, Prox-seq is currently limited to extracellular proteins, which prevents some applications in cell signaling.

Moving forward, the greatest untapped potential for Prox-seq lies in measuring intracellular proteins and complexes. No fundamental reason prevents this, and experiments to achieve intracellular Prox-seq are underway. Furthermore, increasing the number of Prox-seq probes targeting different proteins will result in truly multiplexed single-cell proteomic analysis, potentially measuring thousands of protein complexes in each cell.

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EXPERT OPINION

"The authors report Prox-seq, a method for analyzing pairwise protein interactions, and its application to a couple of model systems. The need for such a method is great, especially since this important aspect of biology cannot be analyzed at high throughput in small samples with existing methods." **Nikolai Slavov, Northeastern University, Boston, MA, USA.**

FIGURE

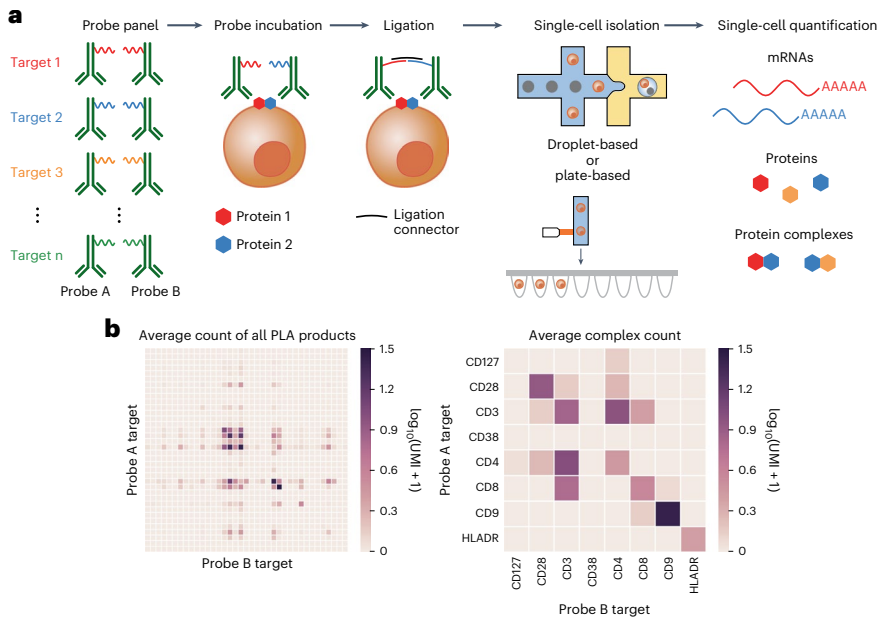

Fig. 1 | Proximity sequencing. a, Prox-seq features pairs of DNA-conjugated barcoded antibodies that undergo a proximity ligation assay (PLA). Ligated oligomers produce PLA products that are measured via single-cell sequencing, enabling the measurement of individual proteins and protein dimers on each cell. **b**, Total PLA product counts shown for peripheral blood mononuclear cells provide quantitative information on the abundance of individual proteins and pairwise protein complexes. © 2022, Vistain, L. et al.

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   This paper presents the foundational assay for Prox-seq: the proximity ligation assay.

BEHIND THE PAPER

When we set out to make highly multiplexed measurements of protein complexes in single cells, it was clear early on that a proximity ligation assay[4] could meet our design goals. In fact, we even wondered why it hadn't been used in this way before. After our first experiment, we found the likely answer. The data structure that Prox-seq produces is complicated. The quantities of protein pairs are coupled in subtle ways that at first make the data hard to interpret. The key insight was realizing that Prox-seq also produces the data we needed to make an estimate of how often protein pairs are created by random chance. Once we understood how often random pairs were formed, we could identify enriched pairs as protein complexes **L.V. & S.T.**

FROM THE EDITOR

"This work describes Prox-seq, which couples sequencing and barcoded proximity assays to profile proteins, protein–protein interactions and mRNAs in single cells. This single-cell multiomics tool will be useful for discovering new cell types and studying biological functions, which are mainly driven by changes in protein interactions." **Lei Tang, Senior Editor, *Nature Methods*.**