High Resolution Mapping of Immune Cell Surface Protein Interactomics

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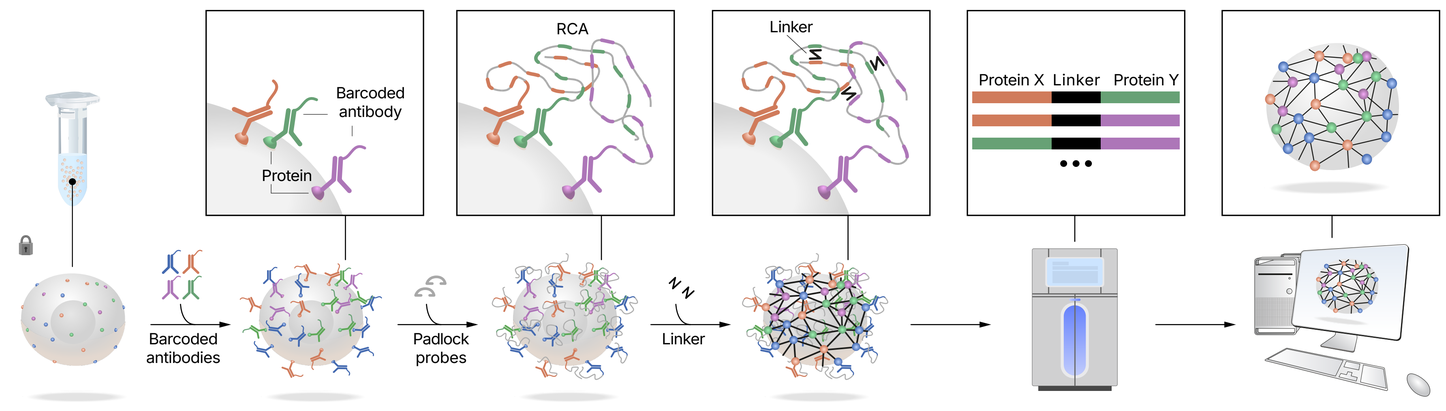
WHY PROTEIN INTERACTOMICS OF SINGLE CELLS?

Cellular function depends on dynamic interactions and nanoscale spatial organisation of proteins. While transcriptomic and proteomic methods have enabled single-cell profiling, scalable technologies allowing high-resolution analysis of protein organization at omics-scale are lacking. Here we present the Proximity Network Assay (PNA)[1], a DNA-microscopy-based[2,3] method for constructing three-dimensional nanoscale maps of 155 proteins in single cells without the use of optics. Protein interactomics by Proximity Networks delivers this new exciting omics dimension.



WHY USE PIXELGEN FOR YOUR RESEARCH?

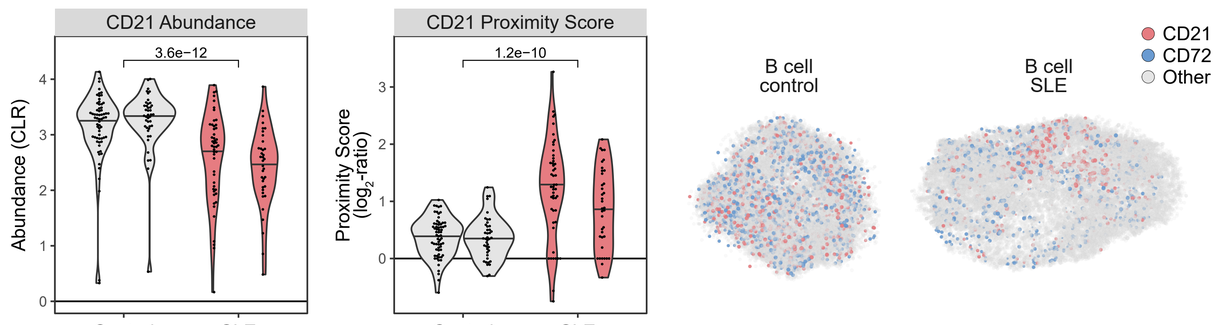
- IN-DEPTH DISEASE PROFILING
- UNRAVELLING MOLECULAR MECHANISMS
- DRUG TARGET IDENTIFICATION & MODE-OF-ACTION
- BIOMARKER DISCOVERY & PATIENT STRATIFICATION

WHAT DOES THE PIXELGEN PROXIOME KIT OFFER?

The Pixelgen Proxiome Kit, Immuno 155, enables nanoscale (~50 nm) single-cell protein interactomics. It supports processing of suspended cells from various source e.g. PBMCs, bone marrow, cell lines, organoids, or frozen tissue. The method is built on a simple NGS prep protocol and recuires no specialized instrumentation. Data is processed using open-source analysis pipelines and the output includes protein clustering, colocalization, and abundance for each single cell. One kit supports 8 reactions, generating data for 1,000 cells per reaction.

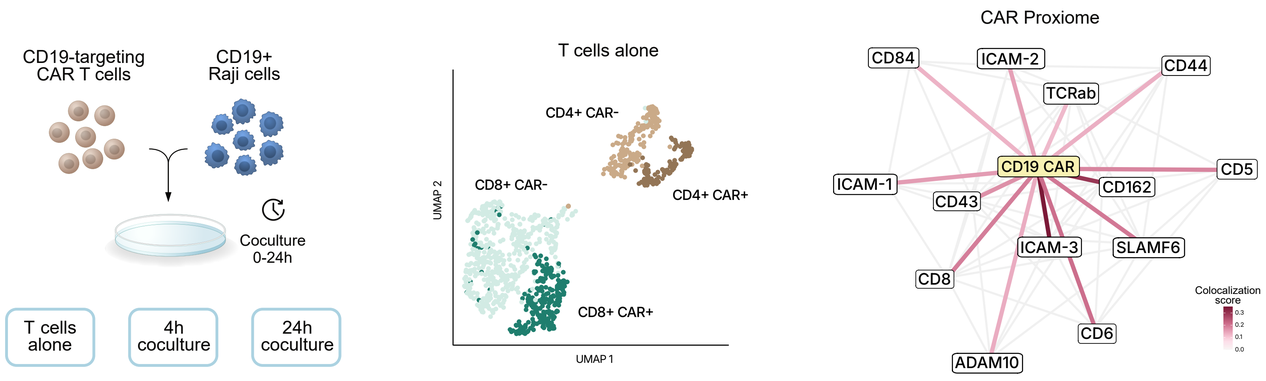
UNIQUE PROTEIN ORGANIZATION IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

The Proximity Network Assay (PNA) can be harnessed to provide insights into disease mechanisms of auto immune disorders like SLE. PNA was used to profile B cells from patients of systemic lupus erythematosus (SLE, n = 2) and healthy controls (Control, n=2).

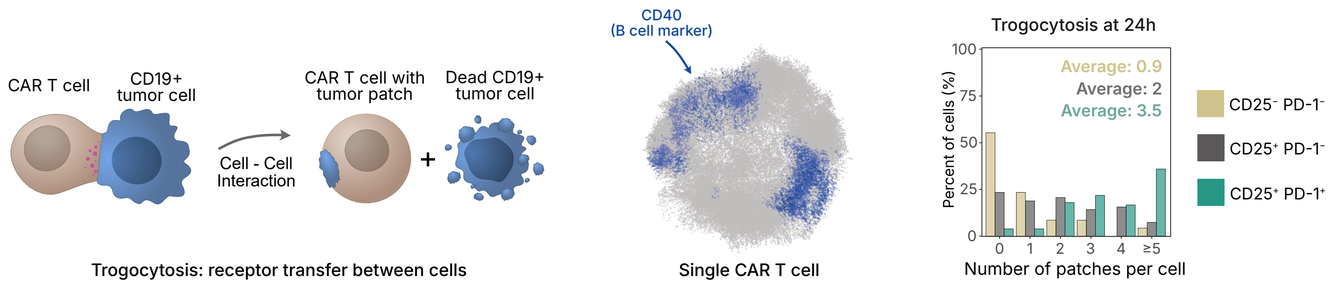


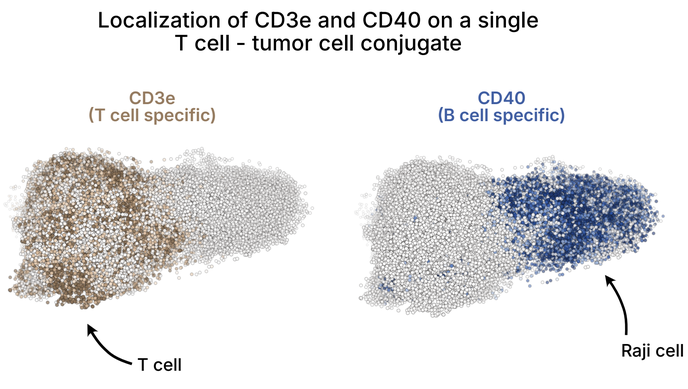
Despite being downmodulated in SLE patients, the BCR-regulating protein CD21 displayed a higher Proximity Score in lupus B cells, reflecting greater protein clustering and possible affects on autoreactive signaling.

DEEP CHARACTERIZATION OF CD19-TARGETING CAR T CELLS



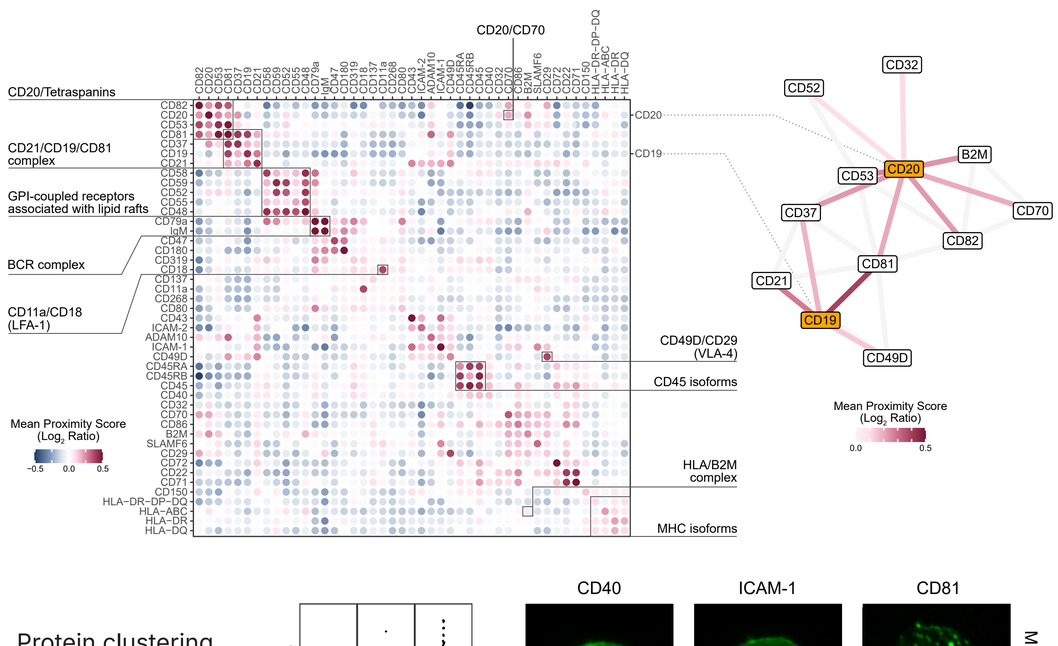
The Proximity Network Assay was used to profile a CD19-targeting CAR T cell product at rest and during tumor cell interaction. The CD19-targeting CAR, known for its low degree of tonic signalling, displayed a dispersed distribution and colocalization with proteins involved in T cell activation and immune synapse formation, including TCRαβ, CD5, CD6, CD44, and ICAM-1/2/3, suggesting its integration into signaling-competent regions.



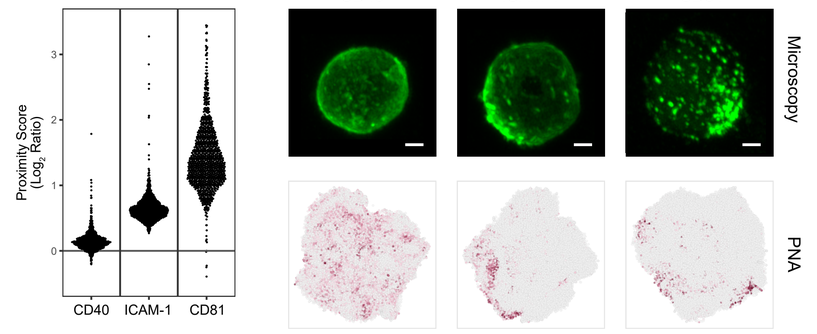


CAR cells, especially CD25+ PD-1+ cells, displayed large degrees of trogocytosis following 24h of coculture. A large number of markers were found to be trogocytosed including CD40, CD22 and ICAM-1. The PNA assay can efficiently differentiate between trogocytosis and conjugate formation enabeling correct characterization of cellular function.

RESOLVING THE RAJI CELL SURFACE INTERACTOME

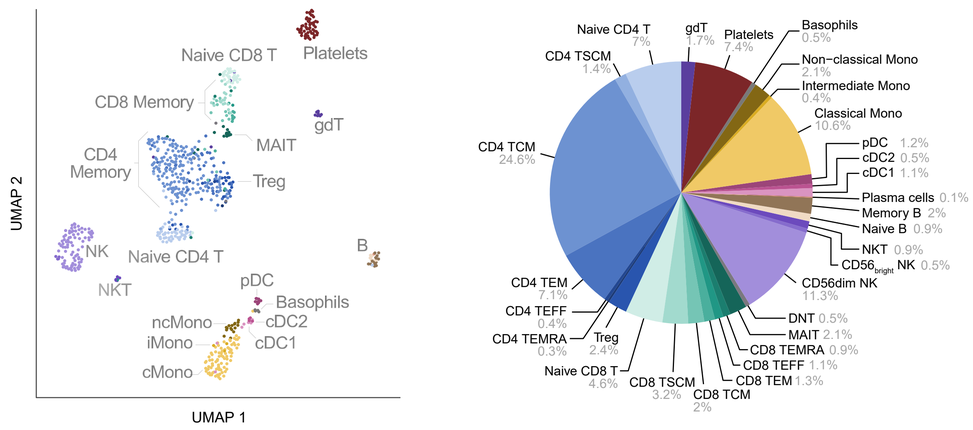


Protein clustering, as measured by the Proximity Score, correlates well with protein apperance in IF microscopy.



CELL IDENTIFICATION IN HETEROGENOUS CELL POPULATIONS

The 155-plex panel of the Proxiome kit was developed to cover the most important surface markers to characterize the function and identity of key human immune cell types. It enables the identification of ~30 major PBMC lineages.



SUMMARY

- **The Proximity Network Assay provides a new dimension of immune cell profiling by enabeling single-cell protein interactomics.**
- **It uncovers biomarkers, enables patient stratification, and sheds light on disease mechanisms and drug-mode-of-actions.**
- **Designed for ease of use, it requires no specialized instrumentation, offering a streamlined kit-and-software workflow for accessible, high-resolution analysis.**

The SLE study was performed in collaboration with Petter Brodin, Karolinska Institutet.

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References

1. Filip Karlsson et al. Single-Cell Protein Interactomes by the Proximity Network Assay. bioRxiv, (2025)

2. Boulgakov, A. A et al, Bringing microscopy-by-sequencing into view. Trends Biotechnol. 38, 154–162 (2020).

3. Filip Karlsson et al, Molecular pixelation: spatial proteomics of single cells by sequencing, Nature Methods, (2024)