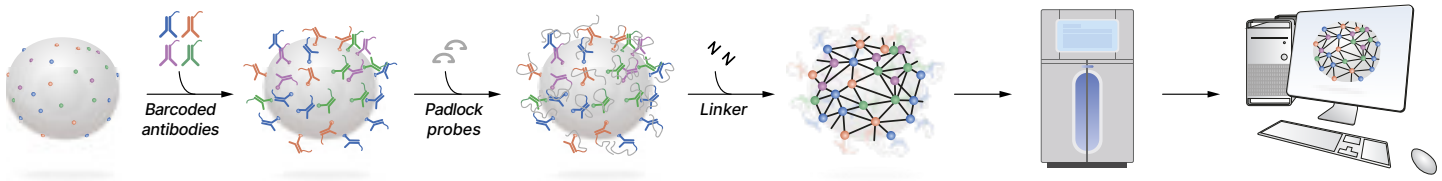
Discover the molecular basis of cell function through protein interactomics by Proximity Networks

Cell surface proteins work together by clustering or interacting with each other to prompt different cellular activities and functions. The interactome represents the network of functional interactions between proteins that drive essential biological processes such as signal transduction, migration, metabolism, gene regulation, and immune responses.

This dynamic interactome is vastly understudied in cell biology research, with massive potential for biomarker and drug target discovery, a better understanding of drug mechanisms, cell-to-cell interactions, and the discovery of new therapeutic avenues.

The Pixelgen Proxiome Kit, Immuno 155, uses a DNA based chemistry, the Proximity Network Assay, where each target protein and its proximal neighbors are assigned a unique spatial position across single cells at nanoscale resolution.

The Pixelgen Proxiome Kit enables researchers to study protein interactomics by Proximity Networks at an unprecedented scale. The validated protein panel consists of 155 immune cell surface protein targets and the output is a detailed map of up to 24 000 data points per single cell representing protein abundance, clustering and colocalization.



STEP 1
Cell fixation

STEP 2
Antibody binding

STEP 3
Rolling Circle Amplification

STEP 4
Proximity Network linking

STEP 5
Sequencing

STEP 6
Data analysis

Workflow of Proximity Networks Assay

The Pixelgen Proxiome Kit is based on the Proximity Network Assay, a technology for nanoscale spatial analysis of immune cell proteins. Barcoded antibodies are bound to cells in suspension and in situ amplified by rolling circle amplification (RCA). RCA is followed by addition of linker oligos and a gapfill-ligation reaction to form multiple connections between neighboring proteins. The data generated after sequencing these molecules create a nanoscale protein Proximity Network for each cell, which is then analyzed using spatial statistics to define the organization of each cell's protein interactome, as well as cell-cell interactions.

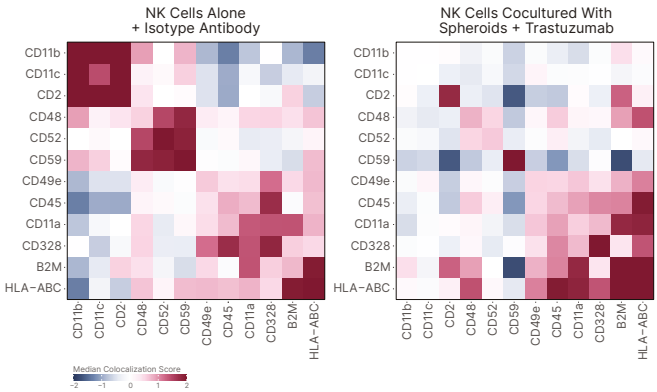
Why the Proximity Network Assay?

- Providing novel insights on the molecular basis of cellular behavior – at the functional protein cluster level
- Analyzing the protein interactome by Proximity Networks at high multiplex
- Discovery of complex and functional cell surface protein rearrangements
- Discover new biomarkers based on protein clustering for patient stratification
- Enhancing the understanding of therapy mode-of-action
- Assessing cell to cell interaction levels in suspensions of cells

Key features

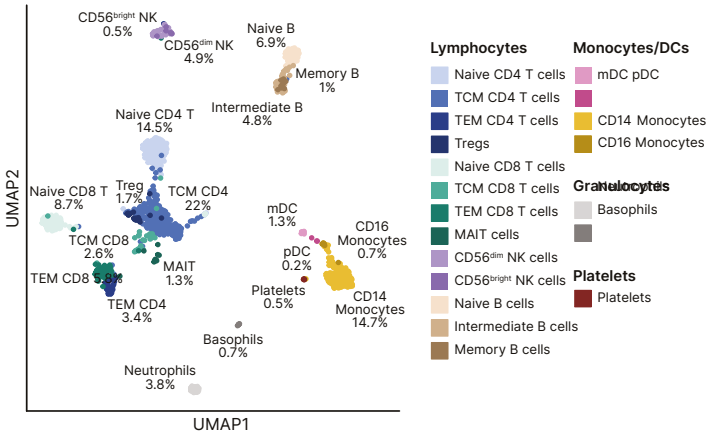
- Enabling nanoscale and high-multiplex protein interactomics studies (~50 nm resolution)
- 155 cell surface markers in parallel on single cells
- Providing protein abundance, clustering, and colocalization data
- Supported for fixed cells in suspension originating from PBMCs, BM, dissociated organoids, or fresh frozen tissue
- Instrument-free and easy to implement
- Open source analysis pipelines for data processing and analysis
- 8 reactions per kit and 1000 cells data output per reaction

Colocalization alterations in multiprotein domains detected by Proximity Networks



PBMCs from a healthy donor were cultured alone or cocultured with breast cancer spheroids (SKBR3) for 24 hours together with either an isotype control antibody or the anti-HER2 antibody, Trastuzumab. After incubation, tumors were collected and dissociated, and immune cells were enriched and processed through the Proximity Network Assay. Protein colocalization heatmaps reveal dynamic changes in the cell surface proteins of NK cells and identify alterations in multiprotein domains when compared between the two conditions.

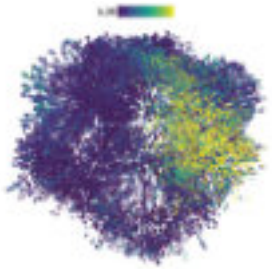
Cell detection – Detailed annotation using 155 markers



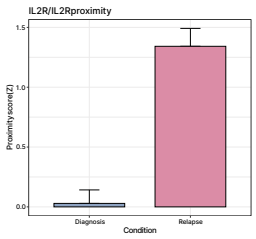
1000 Peripheral Blood Mononuclear Cells (PBMCs) from a healthy individual were analysed using the Pixelgen Proxiome kit. All major cell types of PBMC,as well as granulocytes and platelets, could be efficiently identified. Additionally, the kit contains markers for detection of other cell types including stem cells, AML and epithelial cells.

Proximity Network Analysis in Acute Myeloid Leukemia

After first line treatment, AML often recurs as more aggressive at relapse. Bone marrow cells from AML patients at first diagnosis was compared to at time of relapse by Proximity Networks. Cells were first annotated and myeloid blast cells further characterized. CD25 (IL2RA) was found to be differently clustered between these disease stages and variably so between patients. IL2RA signaling operates by clustering after IL2 binding to drive proliferation, which may indicate growth aggressiveness. Average of 3 patients shown below. Per cell abundance to clustering correlated moderately R^2=0.55.



Proximity Network of a single cancer cell



CD25 (IL2R) clustering at AML relapse

AML data produced in collaboration with Dr. Chris Mason at Weill Cornell Medicine.

Discover a new dimension in cell function by protein interactomics at scale

PIXELGEN PROXIOME KIT
Immuno 155
Part nr.: PROXIMM001

Scan for protein list