

Proximity Barcoding Assay (PBA)

Making molecular context measurable

PBA combines DNA-barcoded affinity probes and shared proximity barcodes to profile multiple molecular targets in parallel at high plex, while preserving molecular context through a high-throughput next-generation sequencing (NGS) readout.

Beyond bulk molecular analysis

Bulk measurements average signals across large populations, which can obscure differences between biological structures and the relationships between molecules. PBA reveals molecular composition, co-localization and heterogeneity across discrete biological structures.

Scientific foundation

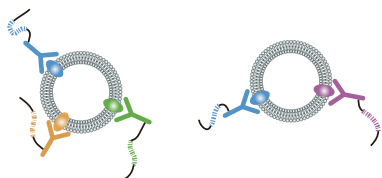
PBA was first described for highly multiplexed surface-protein profiling of individual extracellular vesicles:

Wu D. et al. Profiling surface proteins on individual exosomes using a proximity barcoding assay. *Nature Communications* 10, 3854 (2019).

How PBA works

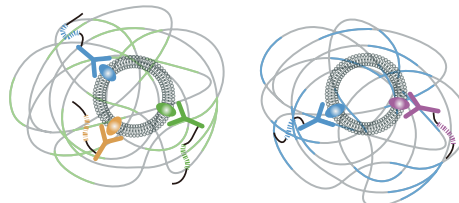
01 Label targets

DNA-barcoded PBA probes bind their molecular targets.



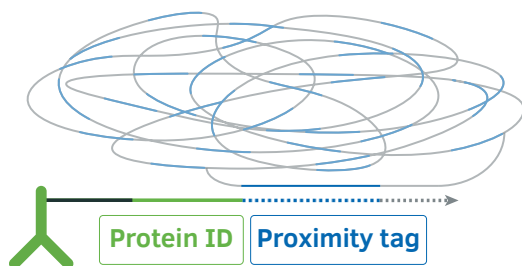
02 Capture proximity

PBA templates capture probes bound to nearby targets on the same biological structure.



03 Assign a shared proximity tag

Captured PBA probes are extended with a shared proximity tag, linking the identities of nearby proteins.



04 Next-generation sequencing

Barcoded DNA molecules are amplified and analyzed by next-generation sequencing (NGS).



05 Computational analysis

Sequencing data are decoded into molecular profiles, co-localization patterns and biological heterogeneity



Reveal molecular relationships hidden by bulk measurements

PBA provides complementary layers of molecular information - from overall target abundance to co-localization patterns and biological heterogeneity.

01

Molecular abundance

Measure overall target abundance

Quantify targeted molecules across a sample to identify differences in molecular composition.

What is present, and how much?

02

Molecular co-localization

Identify targets that occur together

Shared proximity barcodes reveal targets occurring together in close proximity, enabling analysis of molecular combinations.

Which targets occur together?

03

Biological heterogeneity

Resolve distinct molecular signatures

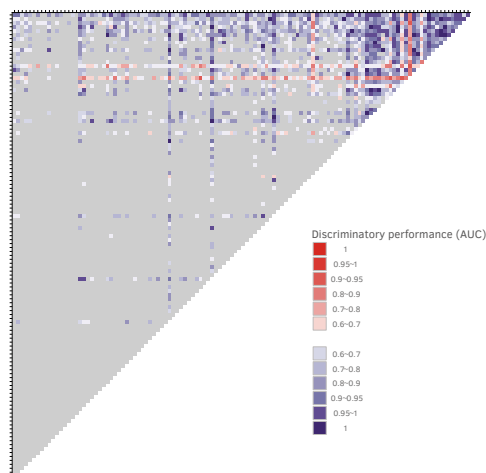
Compare molecular patterns across discrete biological structures to identify subpopulations and signatures obscured by bulk measurements.

How do biological structures differ?

PBA in practice: extracellular vesicle profiling

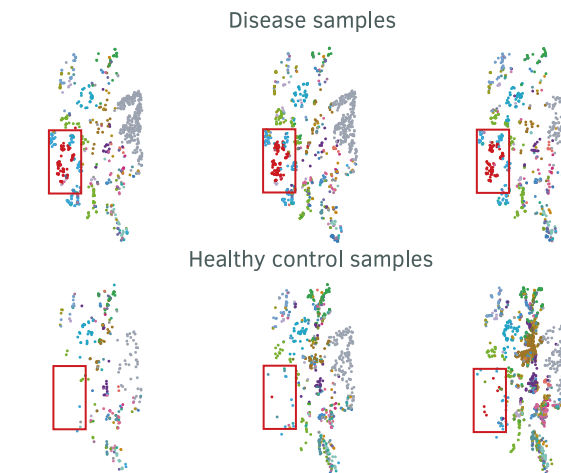
PBA can reveal EV surface-protein abundance, co-localization patterns and heterogeneous EV subpopulations that may be obscured by bulk measurements.

Protein co-localization on individual EVs



PBA identifies combinations of surface proteins that occur together on individual EVs and may distinguish biological sample groups.

EV subpopulations and biological heterogeneity



Clustering of single-EV surface-protein profiles reveals EV subpopulations that differ between disease samples and healthy controls.

Illustrative single-EV profiling data. Colours represent EV clusters.

One technology. Multiple biological structures.

Extracellular vesicles

Surface protein profiling and EV heterogeneity

Protein complexes

Protein composition, interactions and modifications

Organelles

Organelle-associated protein signatures and heterogeneity

Explore what PBA
can reveal in your research.

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