

Proximity Barcoding Assay Enables High-Plex Profiling of Surface Protein Co-Occurrence on Individual Mitochondria

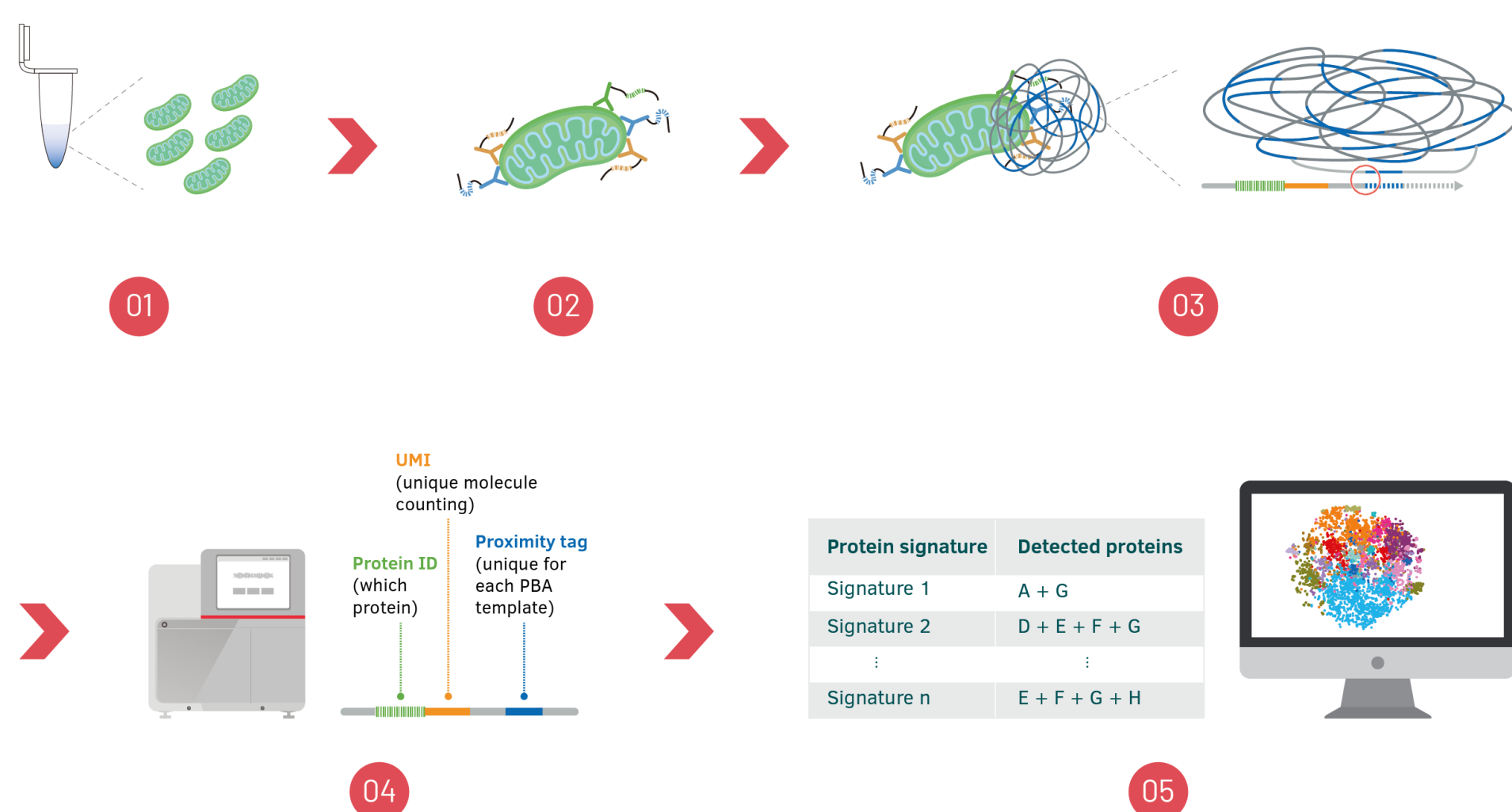
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Introduction

Bulk measurements cannot resolve multiplexed surface protein co-occurrence at the level of individual mitochondria. Single-mitochondrion approaches, including imaging and flow cytometry, remain limited in their ability to measure highly multiplexed protein co-occurrence on individual mitochondria.

Current approaches can measure mitochondrial features, but highly multiplexed protein co-occurrence on individual mitochondria remains challenging. Orgcode™ Mito applies the Proximity Barcoding Assay (PBA)¹ to profile 53 proteins on the mitochondrial outer membrane (MOM), revealing co-localized protein signatures on individual mitochondria.



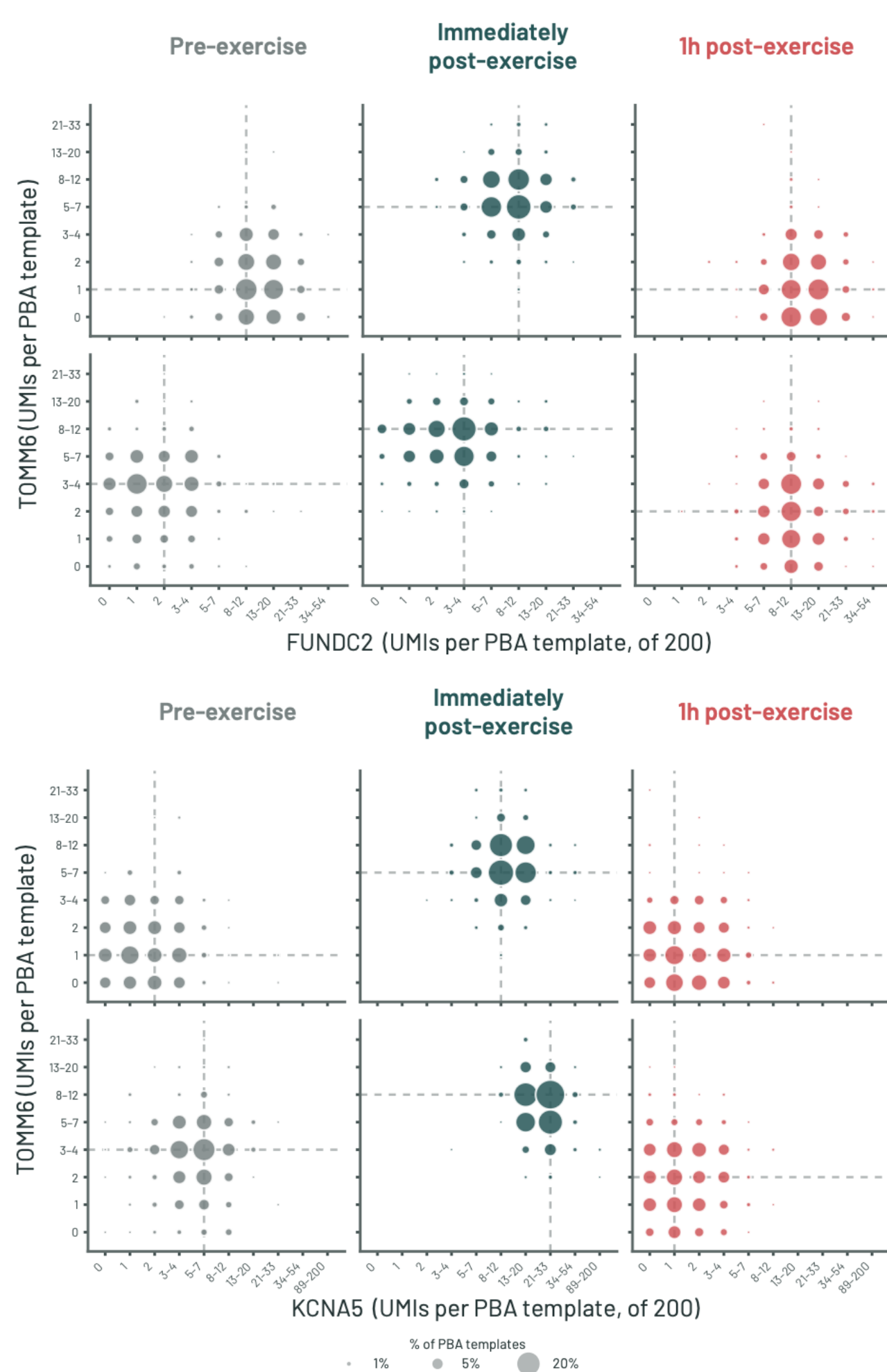
Orgcode™ Mito workflow

- Isolate mitochondria.** Prepare mitochondria from cultured cells, tissue lysates, or human plasma.
- Bind probes.** Incubate mitochondria with DNA-barcoded affinity probes.
- Assign proximity barcodes.** PBA links protein IDs detected in proximity to a barcode identifying their mitochondrial origin.
- Amplify and sequence.** Resulting DNA reads carry protein IDs, UMIs, and PBA barcodes.
- Profile protein signatures.** Generate a count matrix showing relative abundance and co-occurrence across individual mitochondria.

Applications

Exercise-associated changes in mitochondrial protein signatures in human plasma

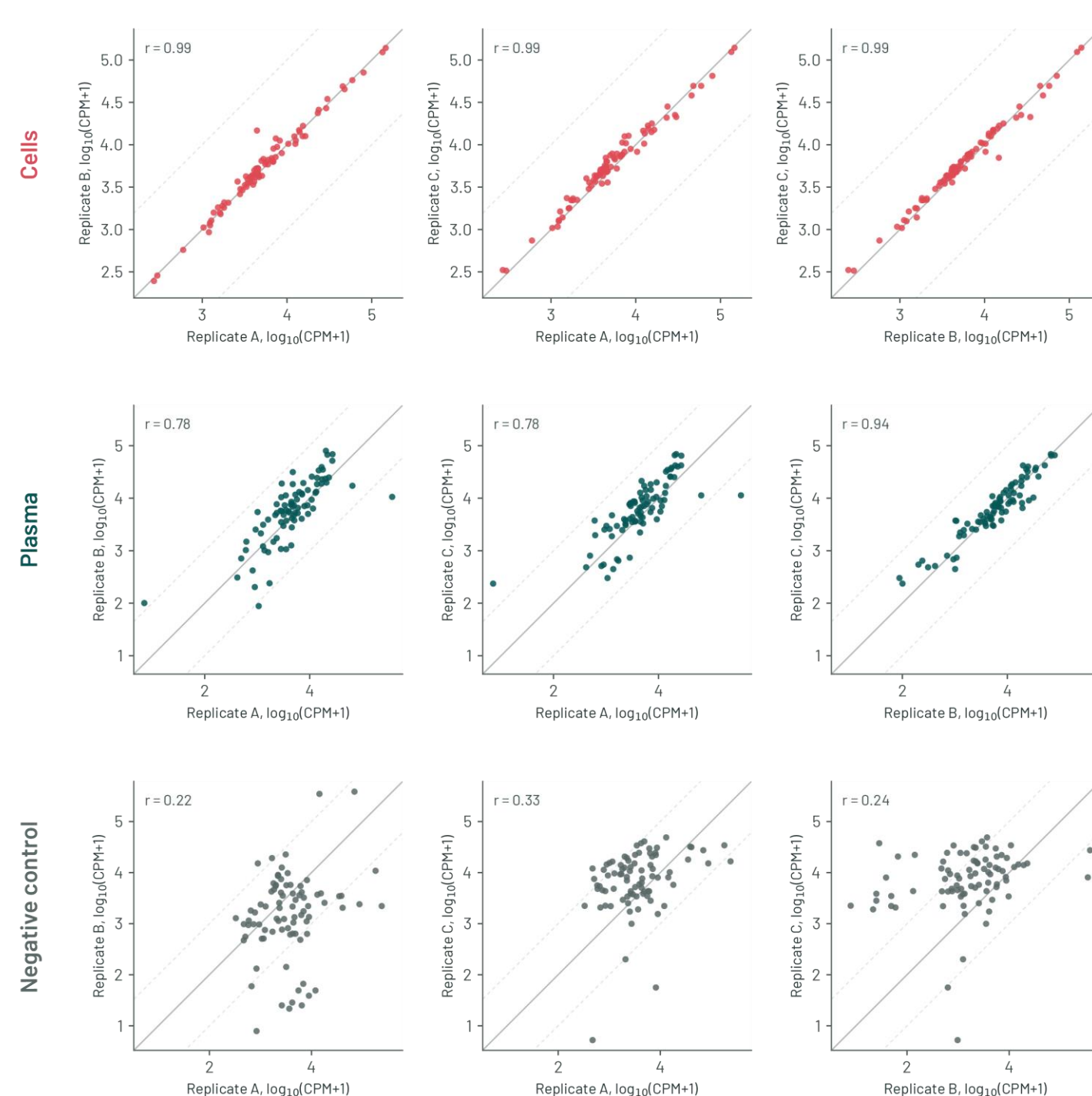
Plasma was collected from one healthy subject before moderate exercise, immediately after, and after 1 hour of rest. Isolated mitochondria were analyzed in duplicate using Orgcode Mito. PBA-template UMI counts for TOMM6/FUNDC2 and TOMM6/KCNA5 increased immediately after exercise, and returned to baseline after 1 hour of rest, suggesting time-dependent changes in mitochondrial protein signatures.



Replicates correlate within each sample type

Consistent profiles across sample types

Mitochondria isolated from HCT116 cells, plasma and negative control were tested with PBA in triplicate (A, B and C). Replicates showed higher correlation in cell-line and plasma samples than in the negative control, supporting reproducible Orgcode™ Mito profiling.



Three levels of PBA data analysis

Compare protein abundance • Detect co-localization • Explore heterogeneity

References:

1. Wu D, Yan J, Shen X, et al. Profiling surface proteins on individual exosomes using a proximity barcoding assay. *Nature Communications* 10, 3854 (2019). doi:10.1038/s41467-019-11486-1