Technical note

Performance of Olink® Explore 3072 in Brain Tissue Lysates

Background

Proteomics technologies enable a broad understanding of the molecular regulation of the human brain (1). Thus, proteomic profiling of brain tissue is increasingly used to identify changes to the brain proteome that can reveal biomarkers and therapeutic targets for neurological disorders (2-3). Recent multi-omic studies revealed disease-related proteomic changes that were not identified at the nucleic acid level, thus, highlighting the utility of integrating proteomics approaches to dissect comprehensive co-expression network changes in neurological disorders (4). However, as brain tissue is a complex sample type (5-6), we sought to evaluate the compatibility between this matrix and Olink® Proximity Extension Assay (PEA™) technology.

Proof of Concept

Proteomic analysis of brain tissue provides critical insights into the pathobiology of neurological disorders. The aim of this technical study was to evaluate the compatibility and technical performance of Olink PEA technology with brain tissue lysates.

Study Design

This proof-of-concept study analyzed 15 brain tissue lysates using Olink® Explore 3072 platform, including the Oncology, Oncology II, Neurology, Neurology II, Inflammation, Inflammation II, Cardiometabolic, and Cardiometabolic II panels (8 Olink® Explore 384 panels total). Olink® Explore 3072 is a valuable tool for protein biomarker research, covering ~3,000 biomarkers from small sample volumes (6 µL). The proteomics platform leverages PEA technology (Figure 1) and combines the power of dual antibody recognition with DNA-based technologies such as next generation sequencing (NGS) to enable high throughput proteomic analysis at sensitive, specific, and scalable levels (7).

To capture the broadest dynamic range, samples were run neat (i.e., undiluted) and at different dilutions (1:10-1:10,000). NPX (see the Definitions and further description in the QC section, below) ranges were calculated for each assay and each dilution after removing NPX values below the limit of detection (LOD). Dilutions with the largest range of NPX values are preferred to dilutions that show small NPX ranges between samples within a dilution.

Definitions

NPX™: an arbitrary, normalized value expressed in Log2 scale units. NPX is used by Olink to intuitively reflect protein abundance in the sample. The use of NPX allows users to identify changes in the levels of each individual protein and compare those changes across samples within a project, enabling the identification of study-, disease-, or treatment-specific protein signatures.

Matrix effect: the effect on an analytical assay caused by all sample components except the specific compound (protein) being analyzed.

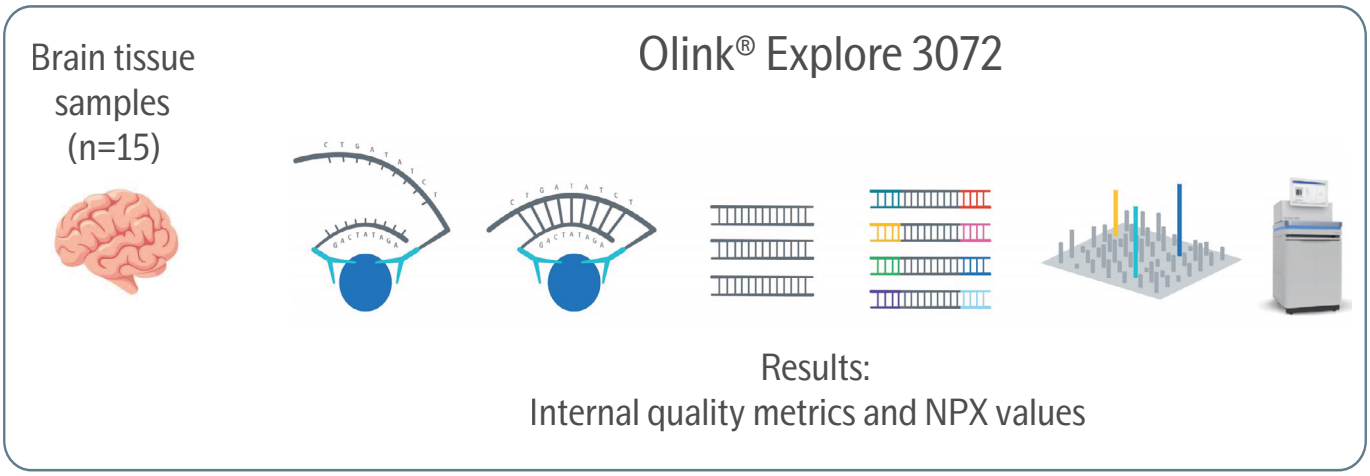

Figure 1. PEA with NGS readout leverages dual antibody recognition with sample- and protein-specific barcoding for high sensitivity and specificity. Antibody pairs with DNA oligonucleotides bind the target antigen in solution (Immunoreaction). Oligonucleotides in proximity hybridize and are extended by DNA polymerase. The resulting DNA template contains a unique barcode pair for each protein (Extension). Barcoded DNA templates are amplified by PCR, incorporating a unique index sequence to identify each sample (Amplification), and the amplicons are sequenced using NGS.

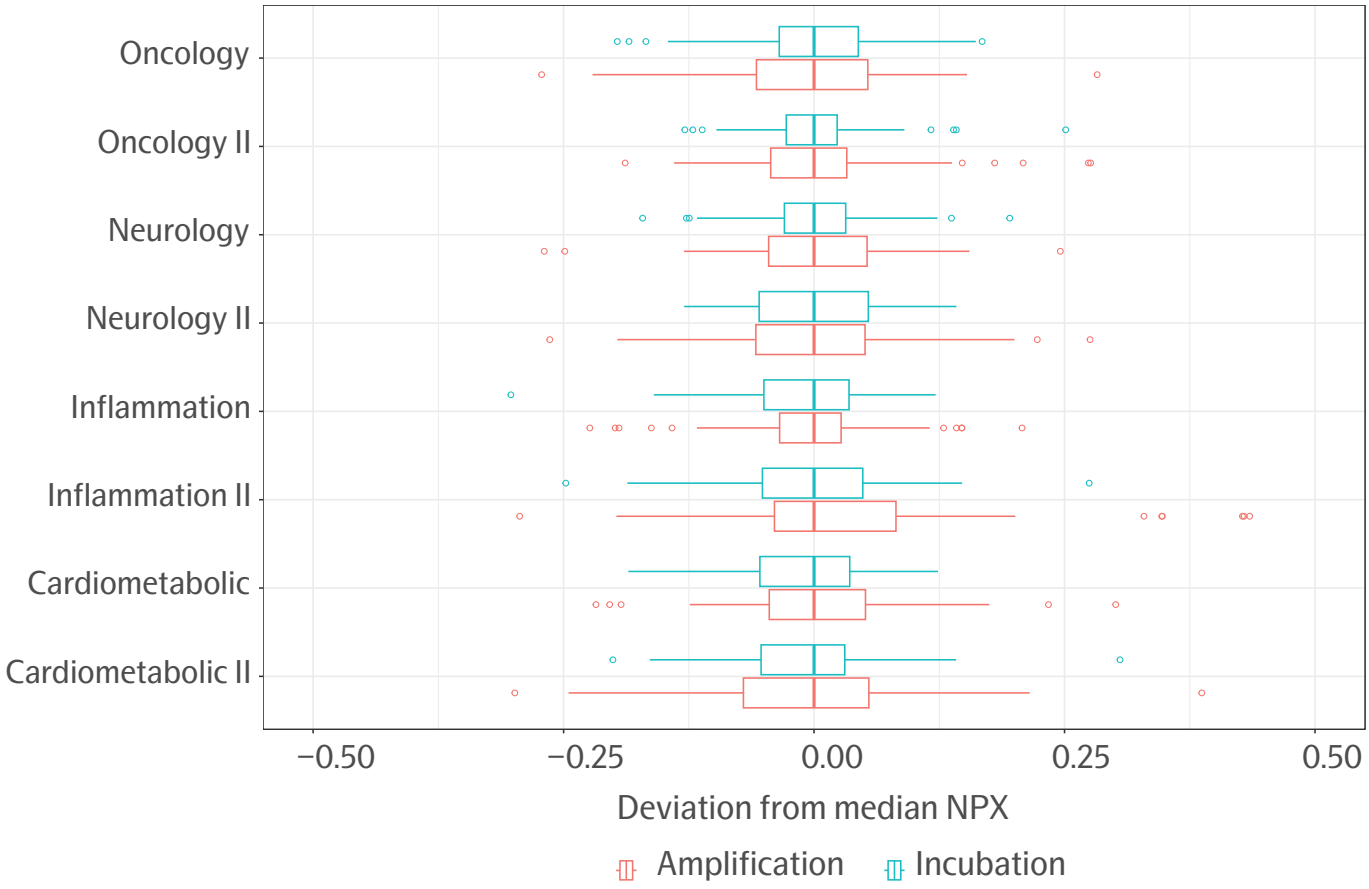

Figure 2. The protein biomarkers targeted by the Olink® Explore platform are distributed over eight panels. The boxplot shows the deviation of NPX values for the internal controls (amplification (red) and incubation (blue)) from the median NPX of all brain tissue samples (n=15) in each of the Olink Explore panels.

Results

Quality Control (QC)

The built-in Olink Explore QC system uses internal controls designed to monitor the three main steps of the Olink protocol: the Immunoreaction step, the Extension step, and the Amplification/Detection step. The incubation control (for the immunoreaction) and the amplification control (for the amplification/detection) are specifically used to monitor assay performance, as well as the quality of individual samples. Such controls are spiked into each sample at the same concentration, and thus, their signal in the form of barcoded NGS reads is expected to be the same throughout the plate. The reads are then translated into relative quantification units, referred to as NPX. As an additional QC assessment of technical reproducibility, the deviation of individual NPX values from the median NPX of all samples can be performed using the analytical software (MyData Olink® Cloud software and Olink® NPX Explore software) to indicate potential pipetting errors or pre-analytical variation(s) in the samples affecting performance (e.g., matrix effects; see **Definitions**).

The data generated from the incubation and amplification controls were first examined in each sample as a standard QC assessment. The NPX deviations from the corresponding medians of the incubation and amplification controls were then compared across each of the brain tissue samples (Figure 2). The data presented in Figure 2 indicate that the number of samples deviating from the median NPX was unbiased and not higher in any specific panel. Those data highlight the technical consistency in the sample analysis workflow and the absence of interference in brain tissue lysates.

Detectability of proteins in brain tissue lysates on Olink® Explore 3072

The detection of proteins in brain tissue lysates was assessed across each panel of the Olink Explore platform. The average detectability across each panel (i.e., the number of samples above the limit of detection (LOD)) ranged from 66.1% in the Oncology II panel to 86.6% in the Cardiometabolic panel (Table 1).

Table 1. Comparison of panel detectability in brain tissue lysates analyzed on Olink® Explore 3072

Explore 384 Panel	Assays per panel	Detectability (% of samples above the LOD)
Oncology	368	74.9%
Oncology II	368	66.1%
Neurology	367	83.3%
Neurology II	367	71.3%
Inflammation	368	67.8%
Inflammation II	369	81.9%
Cardiometabolic	369	86.6%
Cardiometabolic II	367	74.9%

Figure 3 details the absolute number of assays in each detection interval for each Olink Explore 384 panel. Using a detectability cutoff of 80 - 100%, protein detectability across the Olink Explore platform ranged from 189 proteins in the Oncology II panel to 301 proteins in the Cardiometabolic I panel. Using the same detectability cutoff of 80-100%, protein detection in all other panels ranged from 220 - 290 proteins (Figure 3). Thus, brain tissue lysates exhibited a

high level of protein detectability across the complete Olink Explore platform, with 1,988 proteins out of 2,944 assayed (68%) being detected in >80% of the samples. For comparison, 68-86% of the Olink Explore 3072 proteins were identified in human brain tissue in the RNA-seq expression data available from v 22.0.proteinatlas.org, the Human Protein Atlas (8).

Next, to assess the technical reproducibility of the assay, we evaluated the coefficients of variation (CVs) of the overall Olink Explore platform in biological and technical brain tissue replicates. We compared those data to the reference CVs of pooled plasma calculated in previous internal analyses. As can be seen in Figure 4, CVs in brain tissue samples were similar to those in pooled plasma, especially for technical replicates. A total of 73% of Olink Explore proteins were under a standard CV cutoff of 15% in biological brain tissue replicates. However, the technical reproducibility of the platform was higher, with 90% of proteins displaying a CV ≤15% across technical replicates. Thus, the Olink Explore platform collectively exhibited high technical precision across biological and technical brain tissue replicates.

As the study of brain tissue lysates can contribute unique insights into disease biology of neurological disorders, we assessed the detectability of specific proteins of neurological importance. Figure 5 shows a representative set of selected neurologically-relevant proteins measured in the Olink Explore 3072 platform. All such proteins exhibited good detectability and reproducibility across replicate samples and were detected above the LOD (Figure 5).

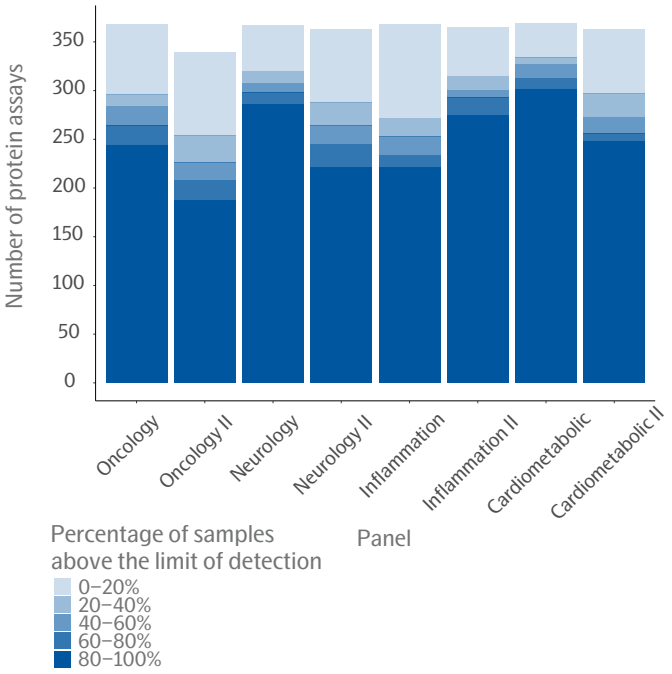

Figure 3. Protein detectability per Olink Explore 384 panel.

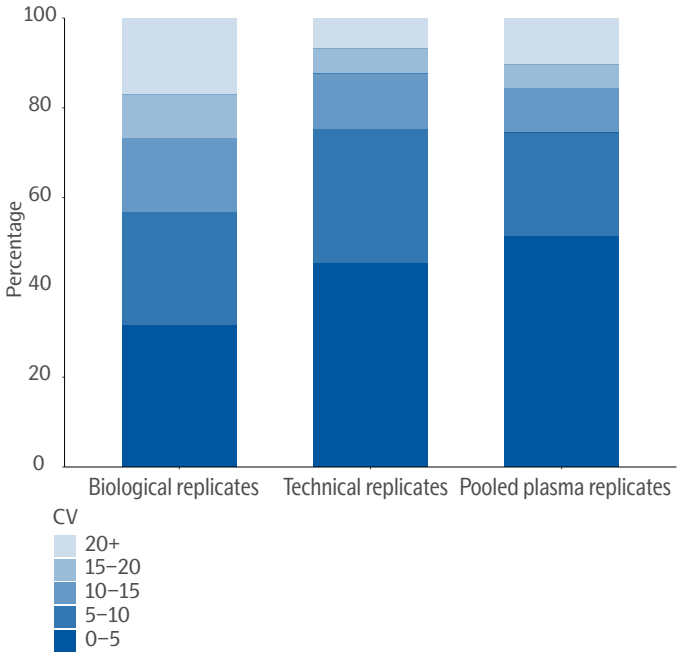

Figure 4. Protein detectability across different CV ranges in biological and technical brain tissue replicates, and pooled plasma.

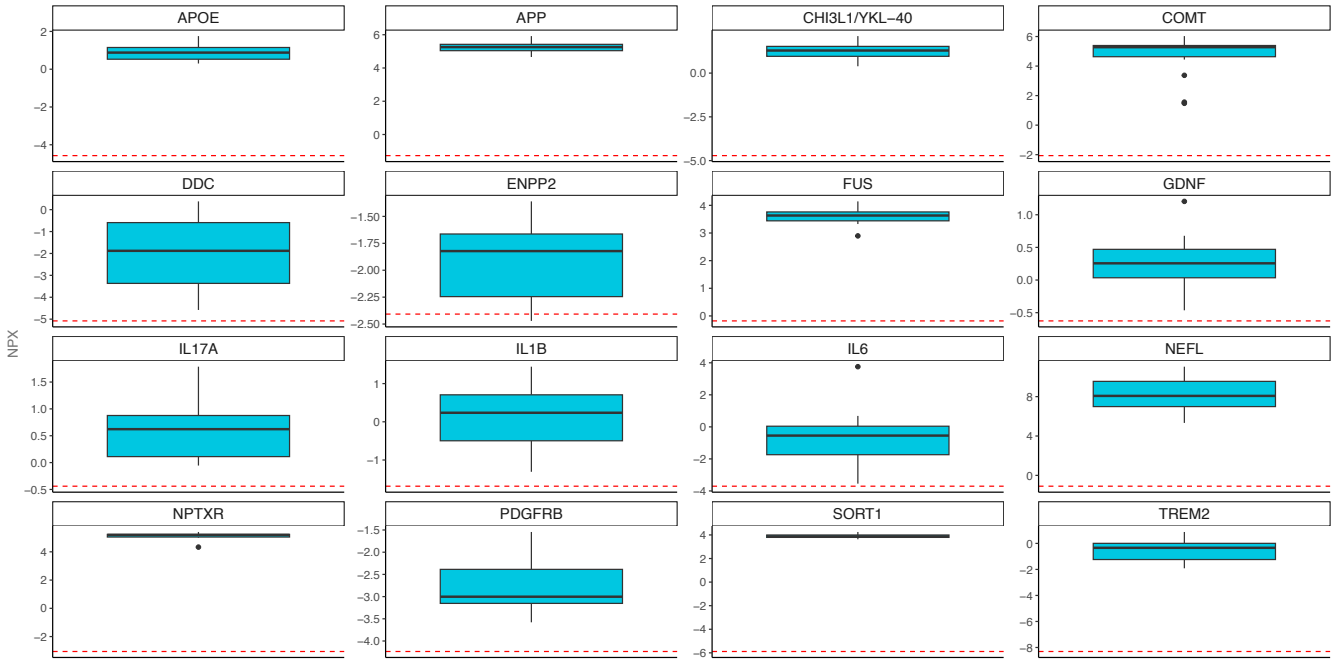

Figure 5. Detectability of selected proteins of interest in neurodegenerative and neuroinflammatory conditions in brain tissue lysates using the Olink Explore platform. Boxes represent the median and interquartile range of the NPX values across replicates, while the bars represent the maximum and minimum NPX values across replicates. Dashed lines indicate the assay lower limit of detection.

Summary

In this study, we analyzed brain tissue lysates on the Olink Explore 3072 platform to assess their compatibility with Olink PEA technology. Quality metrics derived directly from brain tissue lysates, as well as those derived from comparisons to pooled plasma indicated that matrix effects from brain tissues were not present. High levels of protein detectability were observed across all Olink Explore panels, and the technical precision observed in brain tissue lysates was similar to that observed in plasma. The collective data indicate that brain tissue lysates are compatible with the Olink Explore 3072 platform and are also likely to be compatible with other Olink products (e.g., Olink® Target, Olink® Flex, Olink® Focus panels) due to the consistent use of PEA technology across all product lines (9-10; for more information please contact support@olink.com).

References

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