**Introduction**

Proteomic studies that examine protein and PTM abundance often employ multi-dimensional techniques that can provide a wide dynamic range of multiplexed assays within a single biological specimen. The multiplexing of these methodologies can offer the ability of conducting target and non-target discovery programs for drug development or biomarker screening due to streamlined workflows, data management, and increased throughput compared to sequentially targeted approaches. 

**Methods**

Cell Culture and Away: BEKs (EBV) and BMSCs were passed in RPMI media with 10% FBS and penicillin/streptomycin. BMSCs were passaged to sub-confluency (~90% confluence) and split at a ratio of 1:3. Live cell numbers were counted to determine cell density. After cell density determination, cells were seeded into 12-well plates (2 × 10^6) in RPMI with 10% FBS and 1% penicillin/streptomycin. The next day, the media was changed to RPMI with 10% FBS, 1% penicillin/streptomycin, and the cells were cultured until 80% confluence. 

**Results**

**Figure 1**. Single bead and RPLS assay. MALDI-TOF MS peptide (30 µM) was detected using the Bead-assoxted Mass Spectrometry (BAMS) bead reagent and separated by MALDI-TOF MS. The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

**Discussion**

The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

**Figure 2**. Multiplex peptide-protein quantitation exemplified. Multiple peptide-protein quantitation exemplified for BAMS assay. The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

**Figure 3**. Multiplex peptide-protein quantitation exemplified. Multiple peptide-protein quantitation exemplified for BAMS assay. The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

**Figure 4**. Multiplex peptide-protein quantitation exemplified. Multiple peptide-protein quantitation exemplified for BAMS assay. The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

**Figure 5**. Multiplex peptide-protein quantitation exemplified. Multiple peptide-protein quantitation exemplified for BAMS assay. The ability to multiplex multiple protein targets onto a single MALDI plate and peptide concentration data from the same analyte by assessing MS/MS fragment ions that are phosphorylated, acetylated, or glycosylated is shown for BAMS assay. (A) Bead-assisted MALDI-TOF MS signal for a single bead. (B) MALDI-TOF MS signal for a single bead. (C) MALDI-TOF MS signal for a single bead. (D) MALDI-TOF MS signal for a single bead.

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