ADEPTRIX

Tech Note 2018-03-01

Targeted Proteomic Assays to Simultaneously Monitor Total Protein and Multiple Sites of Phosphorylation using Affinity Bead Assisted Mass Spectrometry (Affi-BAMS™) and MALDI MS

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INTRODUCTION

Proteomic studies often employ multi-dimensional analytical methods such as nano-LC-ESI-MS/MS to simplify sample complexity and reduce the wide dynamic range of proteins within the biological specimen. The time and expertise required to implement LC-MS methods can often be a barrier to adopting targeted proteomic methods for a particular translational research program. In this study, we present the use of a novel method that integrates immuno-affinity purification (IAP) with Matrix Assisted Laser Desorption Mass Spectrometry (MALDI-MS) for targeted proteomics of cultured cells to simultaneously monitor total protein and site specific phosphorylation to 4EBP1. The proprietary method, called BAMS™ (Bead Assisted Mass Spectrometry), can be used to monitor 1 – 100's of protein targets in a single assay. The assay is conducted in a microarray format, which can accommodate up to 2286 assays on a single MALDI slide, with as little as 10 µg of total protein, making it an ideal analytical platform for biomarker screening of cell cultures, tissues, liquid biopsies and primary cells.

METHODS

Human gastric cancer cells (MKN45) were homogenized in standard urea lysis buffer to extract soluble cellular proteins, which were then reduced and alkylated prior to protease digestion and subsequent C18 purification. Purified peptides were reconstituted in PBS for affinity capture of target peptides using Affi-BAMS beads. For each bead, the captured target peptides were eluted within a restricted 500 micron area onto an ITO coated glass slide in the presence of CHCA matrix for MALDI MS analysis using a Bruker Autoflex Speed. Spectra were acquired over the mass range of 1.0 to 7.0 kDa using 10000 laser shots (consuming less than 5% of the sample) in positive ion mode at a laser power of ~ 25%. Calibration of the mass spectra was achieved using a calibration solution comprised of C18 purified tryptic digest of bovine serum albumin.

CONCLUSION

The Affi-BAMS assay platform technology enables targeted proteomics of proteins, post-translational modifications and protein isoforms by integrating highly specific antibody capture on high capacity magnetic beads along with the sensitivity and resolution of MALDI mass spectrometry for rapid biomarker screening.

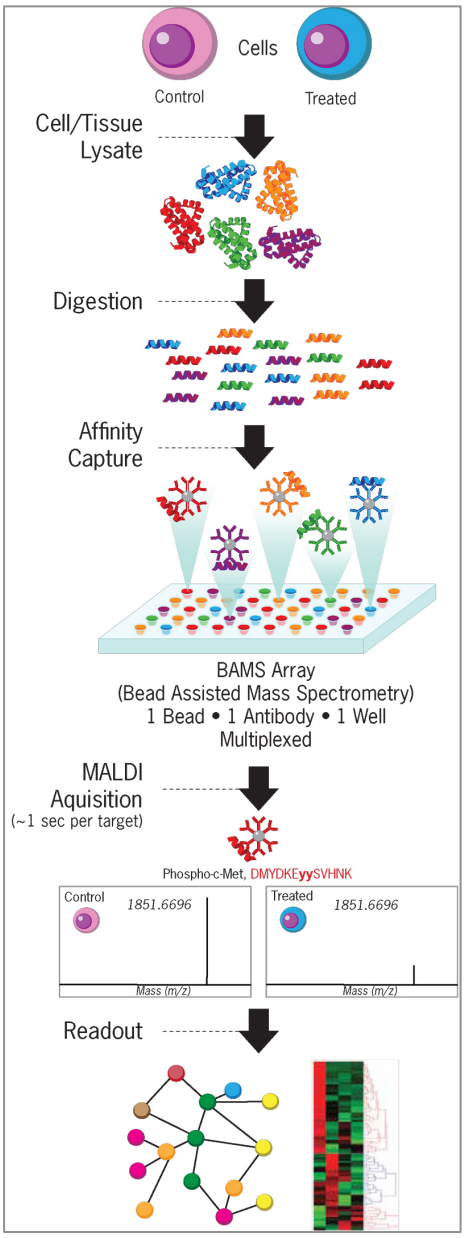

Figure 1. Overall Affi-BAMS workflow. A variety of samples types can be processed for an Affi-BAMS assay (tissue, cell lines, liquid biopsies). The assay consists of the following steps: 1) soluble protein is extracted from the biological material, 2) protein is digested with protease (i.e. trypsin or chymotrypsin), 3) BAMS affinity capture beads are used to enrich for corresponding target peptides to each target protein or post-translational modification, 4) affinity capture beads are washed and then assembled into an ordered array onto an ITO coated MALDI slide, 5) captured peptides are eluted from the beads within the microwell chamber and deposited onto the microarray slide for MALDI MS scanning, 6) the acquired MALDI MS mass spectrum is compared to a spectral library for identification of the target protein or PTM, 7) the MALDI MS intensity signal is used to determine relative quantitation based on an internal reference standard. Pathway profiling is conducted by assembling mixtures of Affi-BAMS beads targeted to proteins within specific signaling networks. BAMS assays are a novel immunoaffinity assay platform for targeted protein analysis. BAMS assays have been configured to monitor key proteins in a wide variety of cellular signaling networks (see www.adeptrix.com/products).

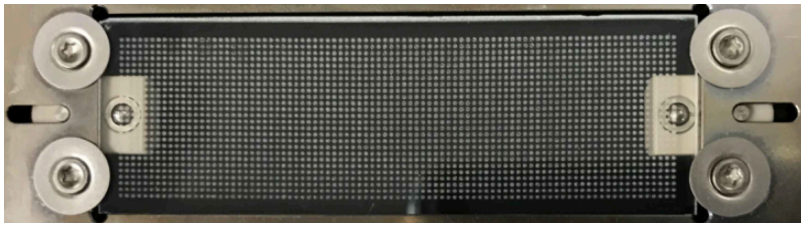

Figure 2. Eluted peptides deposited onto an ITO coated glass slide. Immuno-affinity captured peptides are eluted into an organized array on an ITO coated glass slide for MALDI MS data acquisition. The array contains 2286 spots. MS acquisition takes approximately 1 sec/spot (~ 1.5 hours for entire slide).

Protein Name	Site	Tryptic Peptide Sequence	MH+ (trypsin)
4EBP1	total	(K) RAGGEESQFEMDI (-)	1469.58
4EBP1	T37 & Y46	(R) RVVLGDGVQLPPGDYSTtPGGTLFSTtPGGTR (I)	3365.51
4EBP1	S65	(K) FLMECRNsPVTK (T)	1562.74
4EBP1	T70	(R) NSPVTKtPPR (D)	1177.23
Akt1	total	(R) RPHFPQFSYSASGTA (-)	1653.81
Akt1	T308	(K) DGATMKtFCGTPEYLAPEVLEDNDYGR (A)	3131.32
Akt1	S473	(R) RPHFPQFsYSASGTA (-)	1733.77
Akt2	total	(R) THFPQFSYSASIR (E)	1540.75
Akt2	T309	(K) EGISDGATMKtFCGTPEYLAPEVLEDNDYGR (A)	3517.73
Akt2	S474	(R) THFPQFsYSASIR (E)	1621.68
Akt3	total	(R) RPHFPQFSYSASGR (E)	1636.80
Akt3	T305	(K) EGITDAATMKtFCGTPEYLAPEVLEDNDYGR (A)	3545.78
Akt3	S472	(R) RPHFPQFsYSASGR (E)	1717.78
CTNNB1	total	(R) SFHSGGYGQDALGMDPMMEHEMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDTDL (-)	6868.93
CTNNB1	S552	(R) RTsMGGTQQQFVEGVR (M)	1861.97
CTNNB1	S675	(K) RLsVELTSSLFR (T)	1488.62

Table 1. Tryptic peptides for Affi-BAMS bead enrichment to simultaneously monitor both total protein and phosphopeptide abundance. Affi-BAMS assays have been configured for measurement of both total protein abundance and phosphorylation status for the following proteins: 4EBP1, AKT1, AKT2, AKT3 and CTNNB1. The ability to monitor the relative phosphorylation of key regulatory proteins is valuable for translational biology since post-translational modifications (i.e. STY phosphorylation, KR methylation, K acetylation) are responsible for modulation of protein activity, function, localization and half-life. These unique peptides can be monitored using mass spectrometry because they all have distinct masses that serve as a unique identifier. The MH+ values correspond to the calculated average mass of the phosphorylated peptide (amino acid residue in lower case bold red text). The site designation for each phosphorylated peptide is annotated in the Site column for each protein.

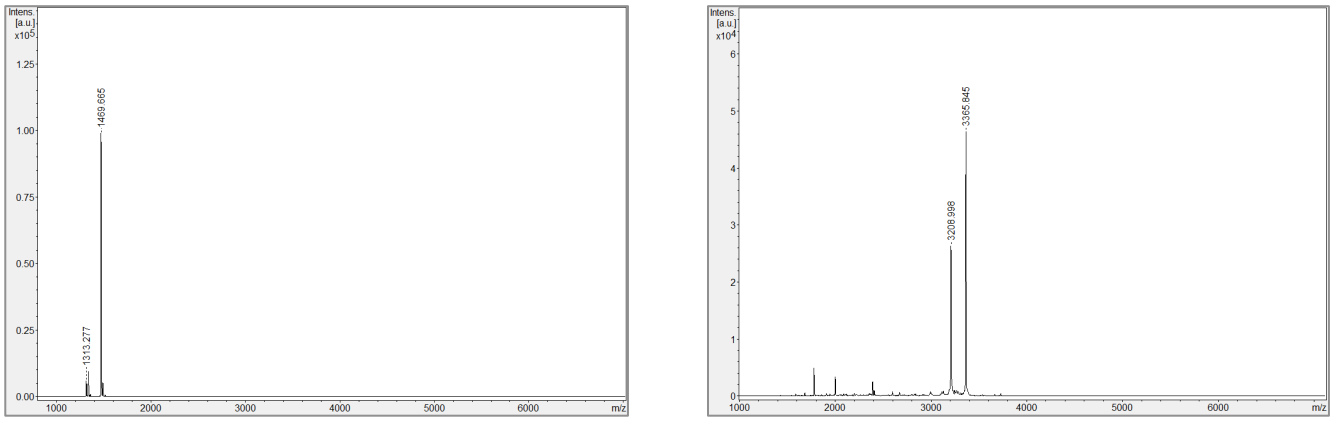

Figure 3. MALDI MS spectra of total and phosphorylated forms of 4EBP1. The MALDI MS spectra (linear mode MS) are shown from a BAMS assay using Affi-BAMS affinity capture beads for 4EBP1 (total) and 4EBP1 (T37/T46). Target peptides were captured using 200 µg of total protein (trypsin digested) from human gastric cancer cells (MKN45). Tryptic peptides for each protein target is captured onto an individual Affi-BAMS bead and the eluted peptide is independently measured by MALDI MS within the BAMS spot array. The mass measurements observed for each of the Affi-BAMS assays are unique for each of the target peptides. The 4EBP1 (total) tryptic peptide sequence, RAGGEESQFEMDI, has a calculated average mass of 1469.58 m/z (measured mass 1469.67 m/z). The 4EBP1 (T37/T46) tryptic peptide sequence, RVVLGDGVQLPPGDYSTtPGGTLFSTtPGGTR, has a calculated average mass of 3365.51 m/z (measured mass 3365.85 m/z). The other major peaks are the corresponding zero missed cleavage products. The Affi-BAMS method can be used to perform targeted proteomic assays to simultaneously monitor total and PTM sites (i.e. phosphorylation, acetylation, methylation) of proteins.

OVERVIEW

The Affi-BAMS assay platform has been engineered to enable targeted proteomics on existing MALDI MS instrumentation by providing efficient enrichment of target analytes that are spatially localized for rapid screening. The multiplexing capability of the assay allows for analysis of specific signaling networks to advance translational research efforts. Affi-BAMS assays have been configured to monitor many different signaling pathways and areas of disease biology to facilitate basic research and drug development (see www.adeptrix.com/products).