

# Analysis of Alzheimer's Disease Markers in Complex Biological Samples using Derivatized Arrays

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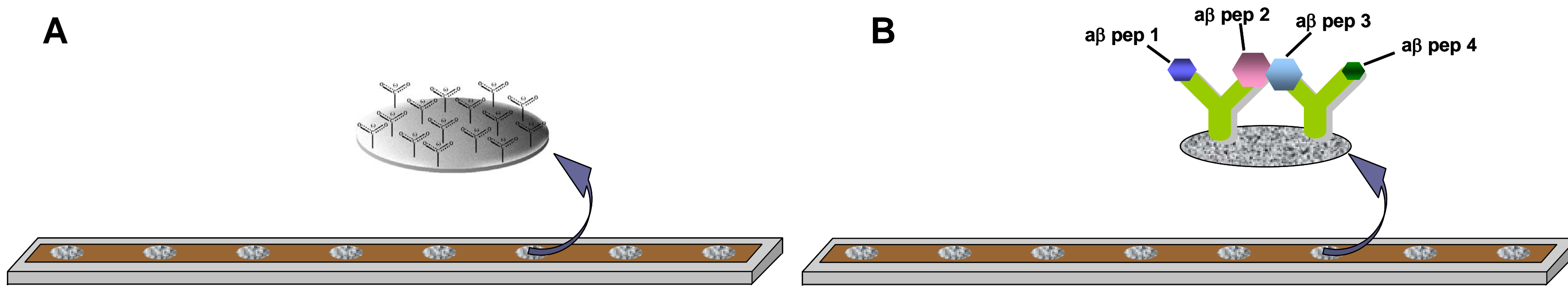
## Introduction

The use of derivatized arrays and time-of-flight mass spectrometry for the top-down profiling of intact proteins has previously yielded a large number of candidate biomarkers, biological indicators of a phenotypically altered state, from complex biological samples. We show here that this combination is also of value for the targeted assay of established biomarkers such as amyloid beta peptides. Amyloid beta peptides can be difficult to study using standard methods due to their multiplicity and small size. Numerous fragments potentially contained in samples creates issues of cross-reactivity and incomplete results with traditional methods such as ELISA. Detection by mass spectrometry has the advantage of enabling the clear distinction between fragments that may share the same antibody recognition sequence. Multiple amyloid beta peptides can be captured simultaneously from biological samples using a targeted proteomics approach combining immunological sample enrichment and MALDI: Mass Spectrometry In-Situ ImmunoAssay (MaS-ISIA).

## Materials and Methods

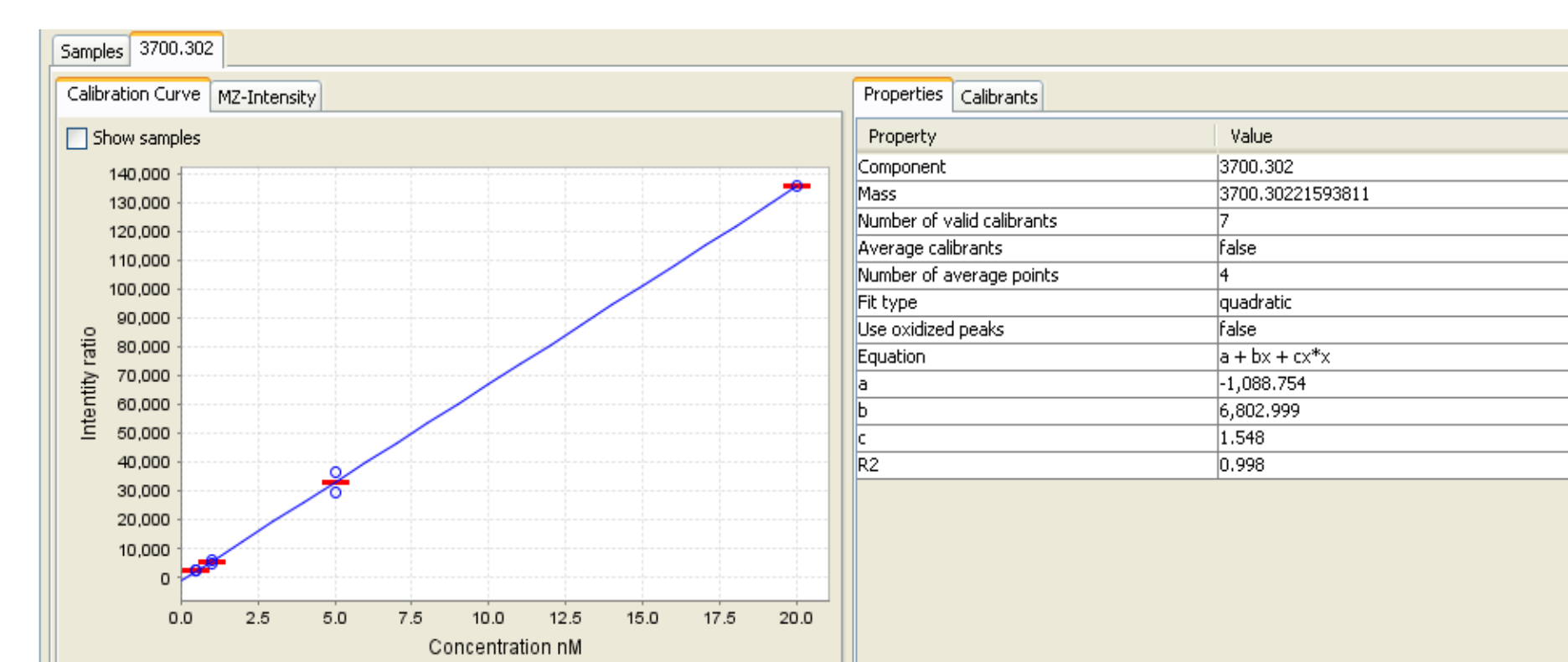
Microliter volumes of sample (artificial CSF or E.coli lysate [Bio-Rad Laboratories]) were separately applied to chromatographic ProteinChip arrays or ProteinChip arrays covalently functionalized with Bovine IgG or 6E10 antibody [Covance] specific to N-terminal residues 1-17 of the human amyloid beta protein. Select samples were spiked with decreasing concentrations of amyloid beta peptides (synthetic human fragments Pyr 3 (3-42), 1-28, 1-33, 1-38, 1-40, 1-42 and/or 1-43 [Signet]) After sample binding, the array surface was washed to remove non-specifically bound proteins, and 25% saturated alpha-cyano-4-hydroxycinnamic acid [Bio-Rad] was applied.

ProteinChip Arrays were analyzed using an ultrafleXtreme mass spectrometer [Bruker Daltonics] in linear mode. Specialized geometry files and rasterization patterns were used for automatic data collection on Partition 1 of 4 across each sample spot. Data was analyzed using Lucid™ Proteomics Software 2.0 [Bio-Rad].

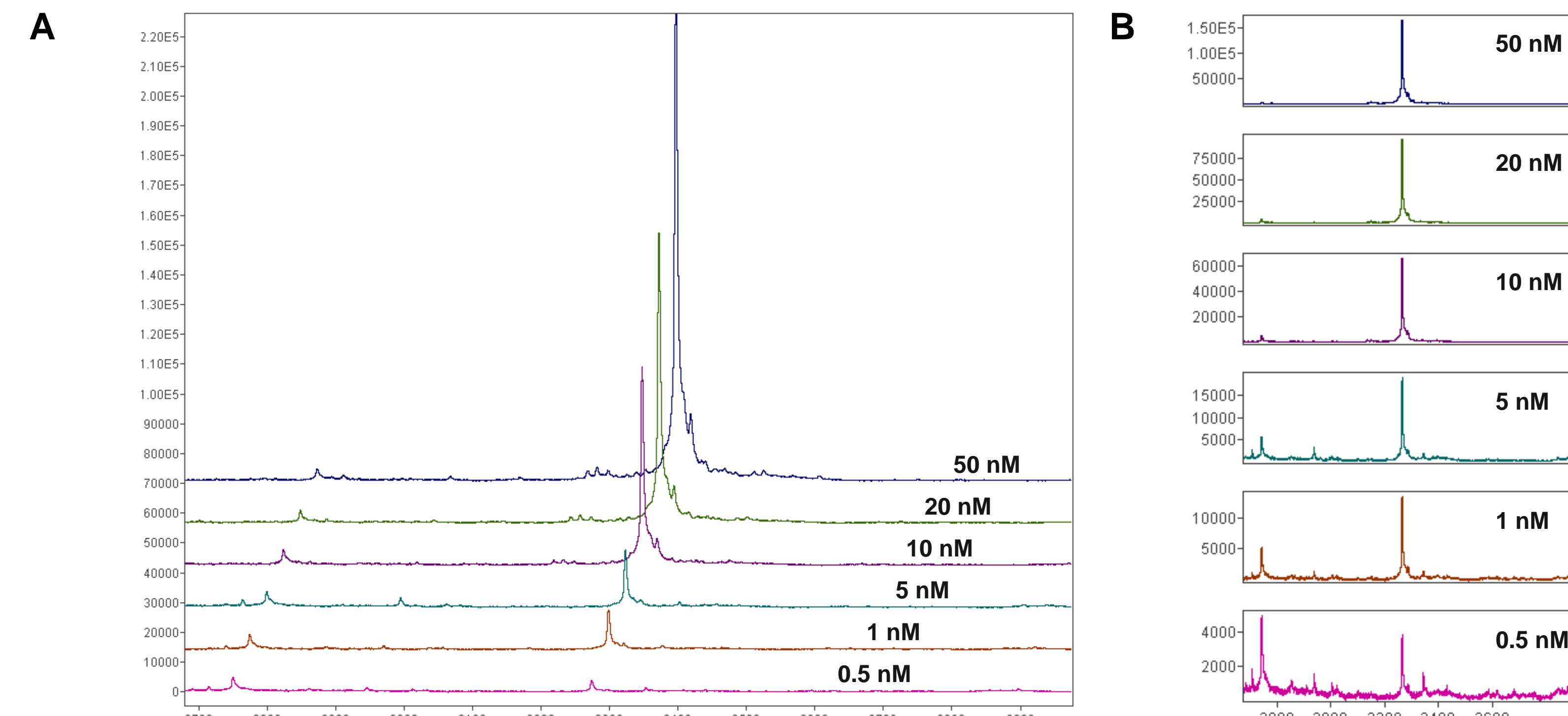


**Figure 1. Capture of proteins on ProteinChip arrays.** A) ProteinChip arrays with chromatographic surfaces bind subsets of peptides and proteins; B) ProteinChip arrays with immobilized antibody binds multiple peptide fragments simultaneously.

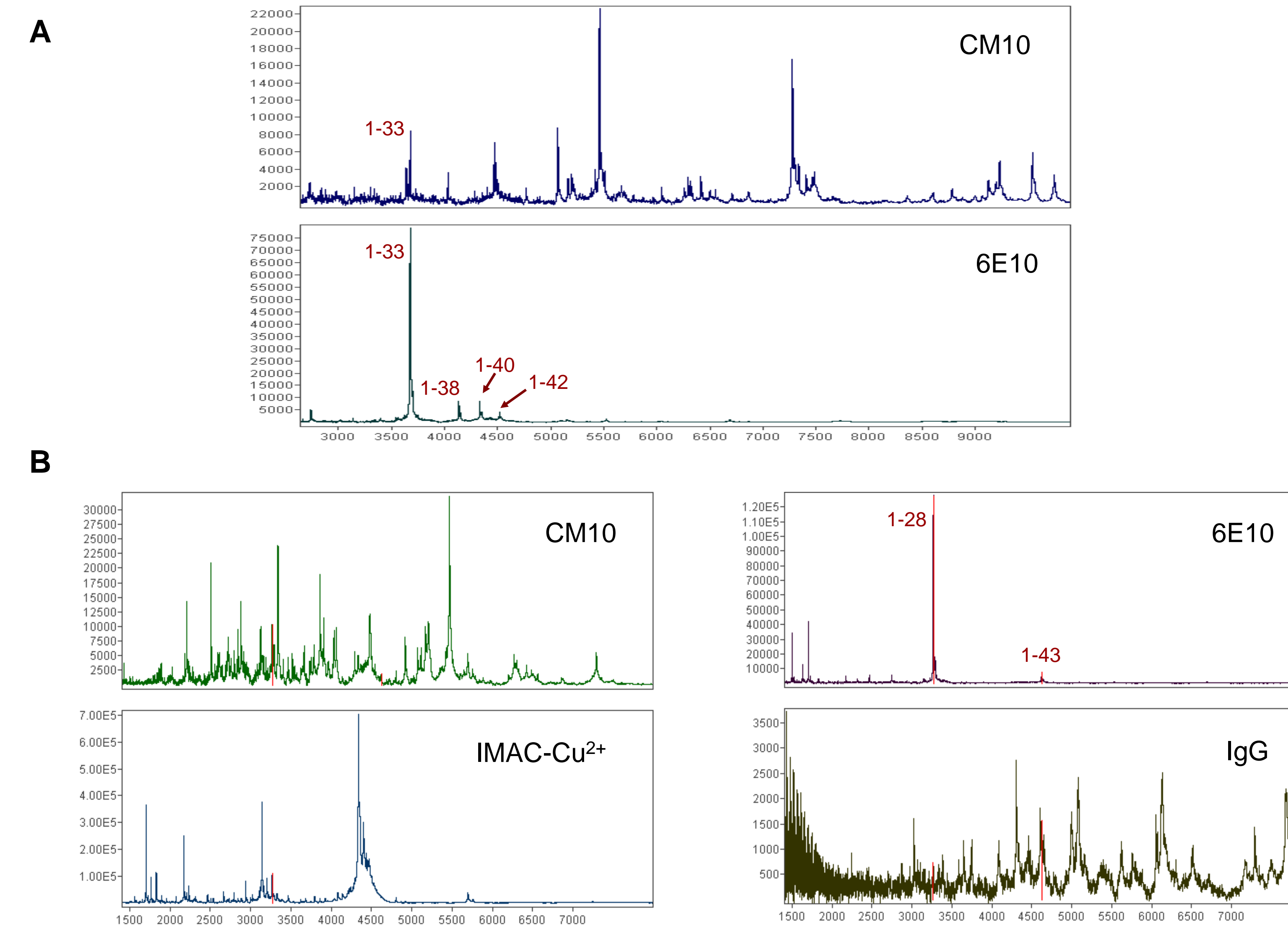
Fragment	aCSF	ecoli
1-28	0.2 nM	<0.2 nM
1-33	0.05 nM	0.05 nM
1-38	0.5 nM	0.5 nM
1-40	1 nM	0.5 nM
1-42	1 nM	0.5 nM
[Pyr 3] 2-42	2 nM	12.5 nM
1-43	2 nM	1 nM



**Table 1. LLOD of synthetic amyloid beta fragments on 6E10 arrays from aCSF or E.coli lysate.** Generation of calibration curve for amyloid beta 1-33 in Lucid Proteomics Software.



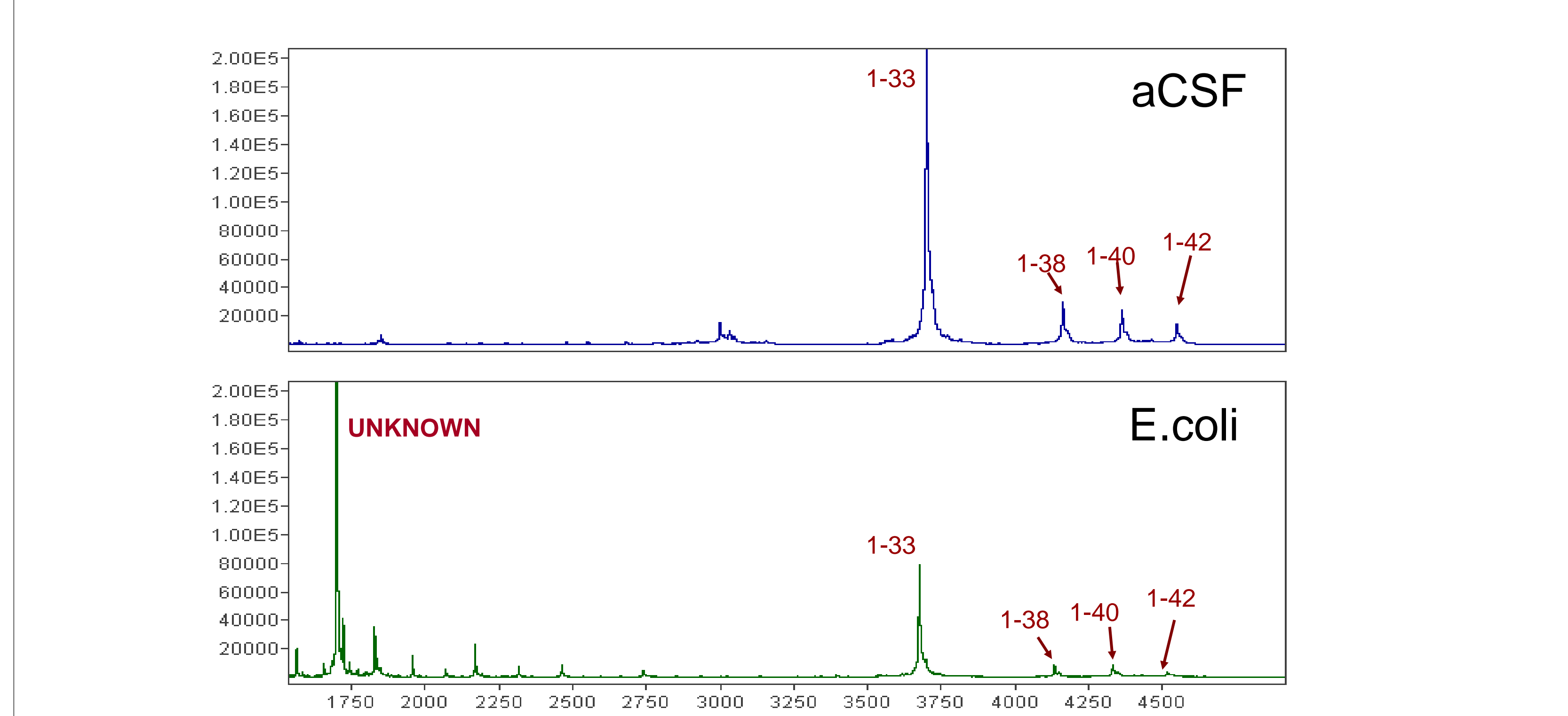
**Figure 2. Specific capture of amyloid beta 1-28 from E.coli lysate.** A) Spectral overlay plot, on same intensity scale; B) Stacked plot view, autoscaled intensity. Dose-responsive behavior is clearly demonstrated, allowing quantitation out of complex biological fluids.



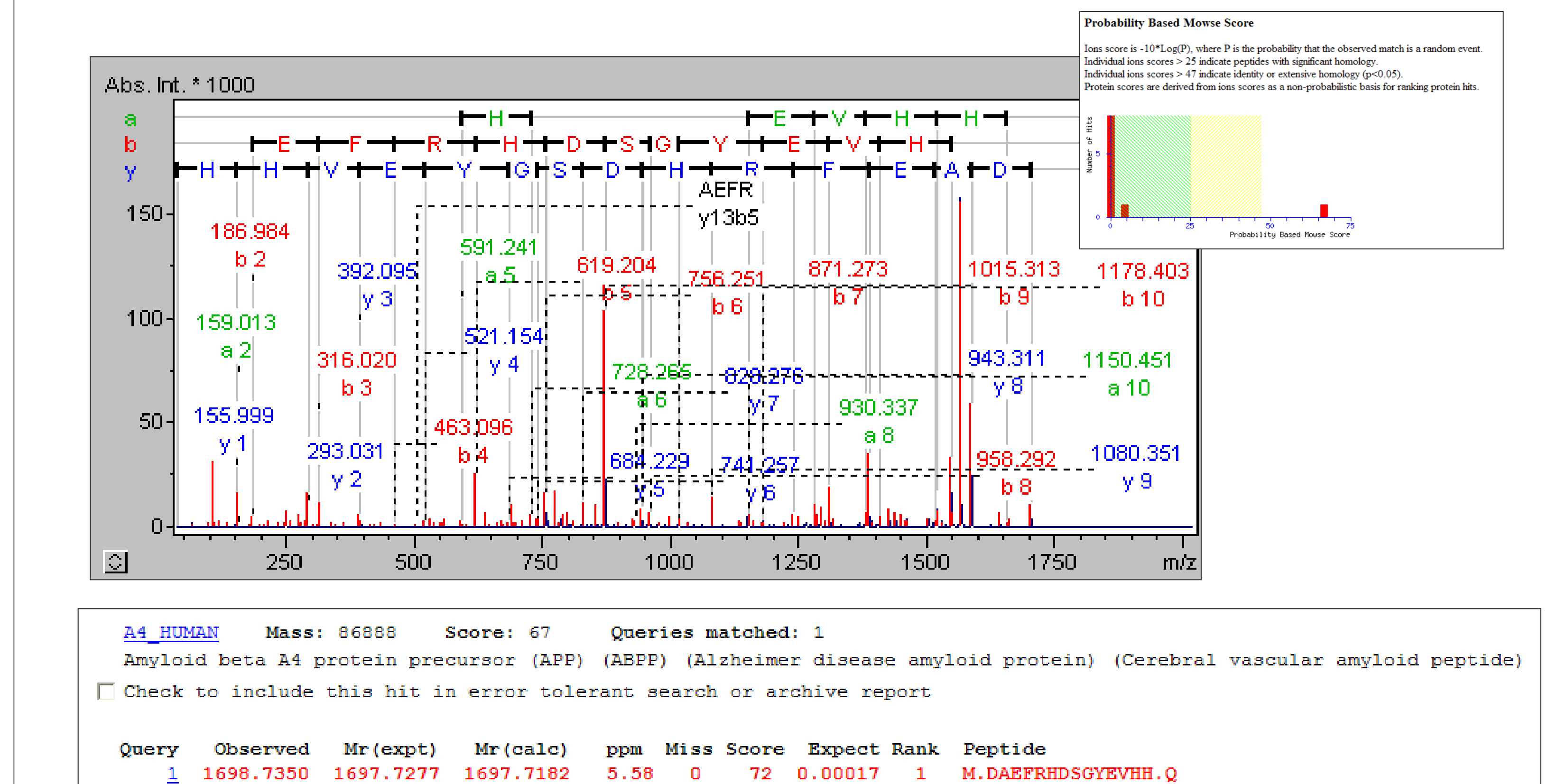
**Figure 3. Amyloid beta peptides spiked into E.coli lysate.** A) Spectral profile of a mix of fragments 1-33, 1-38, 1-40, 1-42 (5 nM) on cation exchange (CM10) and 6E10 antibody array; B) Spectral profile of a mix of fragments 1-28 and 1-43 (25 nM) on cation exchange (CM10) and metal affinity (IMAC) arrays [left panels], and 6E10 and IgG (negative control) antibody arrays [right panels]. All spectra are autoscaled. While the chromatographic arrays capture many diverse proteins from the lysate, enhancement and clean-up of the beta-amyloid signal may clearly be seen in the MaS-ISIA spectrum.

## Identification of a target peptide

A mix of amyloid beta peptides (1-33, 1-38, 1-40, and 1-42) were spiked into aCSF and compared to matching spikes into E.coli lysate. A prominent peak at 1699 Da was detected in the E.coli samples (Figure 6). In an attempt to determine if the peptide was a result of non-specific binding to the antibody array, or specific binding of an additional amyloid beta peptide (potentially resulting from exposure to the lysate or sample handling), the array was analyzed in reflectron and LIFT modes. The peptide was identified as amyloid beta 1-14 (Figure 7).



**Figure 4. Amyloid beta peptide mix spiked into aCSF and E.coli lysate.** An unexpected peptide is detected in the E.coli lysate



**Figure 5. LIFT analysis of peptide at 1699 Da yielding ID of amyloid beta 1-14.** This peak therefore did not occur from nonspecific binding, and was instead generated from native sample processing of the spiked peptides.

## Discussion

- The array-based, top-down approach of MaS-ISIA facilitates straightforward capture of peptides and proteins from complex biological samples and may be used for biomarker discovery or assay of known biomarker targets. Capture of amyloid beta peptides has been previously demonstrated by this method from spiked serum, human CSF and transgenic brain samples.
- While most amyloid beta assays require *a priori* knowledge of the expected peptides and/or multiple antibodies to monitor multiple fragments, the combination of array-based antibody capture with mass spectrometry used in these studies yields a simple assay that can reliably and simultaneously monitor multiple amyloid beta fragments. The ability to interface the arrays with a MALDI-TOF/TOF mass spectrometer permits positive identification of fragments or non-specifically bound proteins using TOF/TOF sequence analysis.