

ImmunolMaging: Investigation of Tissue-less Tissue Imaging and Simplification of the Tissue Proteome Using Animal Models

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Introduction

Crude tissue imaging is the experimental equivalent of serum, with its 15 orders of magnitude of concentration, being restricted to neat investigation only, with the additional complication that the sample is inherently heterogeneous. In serum, it is straightforward to quantify a certain protein of interest using ELISAs, but specific detection by an untargeted method such as LC-MS may fail. Affinity based enrichment is a useful method for targeted analysis as it enriches the molecule of interest significantly and permits mass spectrometric detection even without further chromatographic workup.

MALDI tissue imaging enables correlation of molecular information with morphological and clinical features. However, peptide and protein imaging is an untargeted approach that enables discovery, but does not provide targeted information per se. The crude sample is extremely complex and lower-abundance/low-hydrophilicity species are difficult to detect. We describe a hyphenated targeted proteomics approach combining immunological sample enrichment and MALDI imaging: imaging Mass Spectrometry In-Situ ImmunoAssay (iMaS-ISIA). In this approach, MALDI imaging becomes a targeted analysis tool preserving spatial abundance patterns, providing molecular information about recognized proteins and the distribution of highly related antigens such as the set of truncation variants of the β -amyloid peptides – information that goes undetected with all current tissue analysis methods (e.g. FISH, IHC – similarly for serum, ELISA also detects the antigen *in toto*). Multiple peptides can be captured simultaneously from biological samples using a mass spectrometry based immunoassay as long as they contain the recognized epitope.

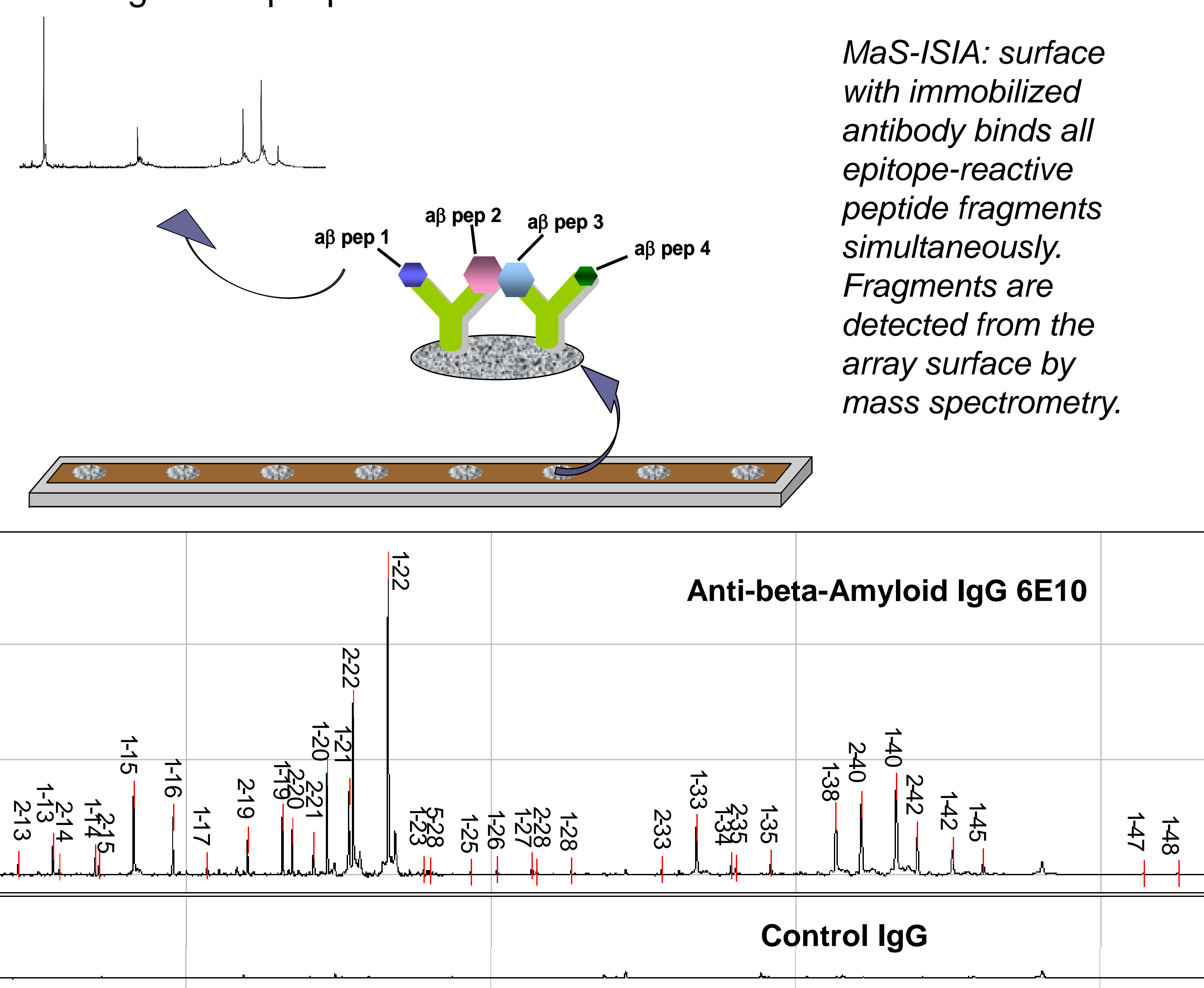
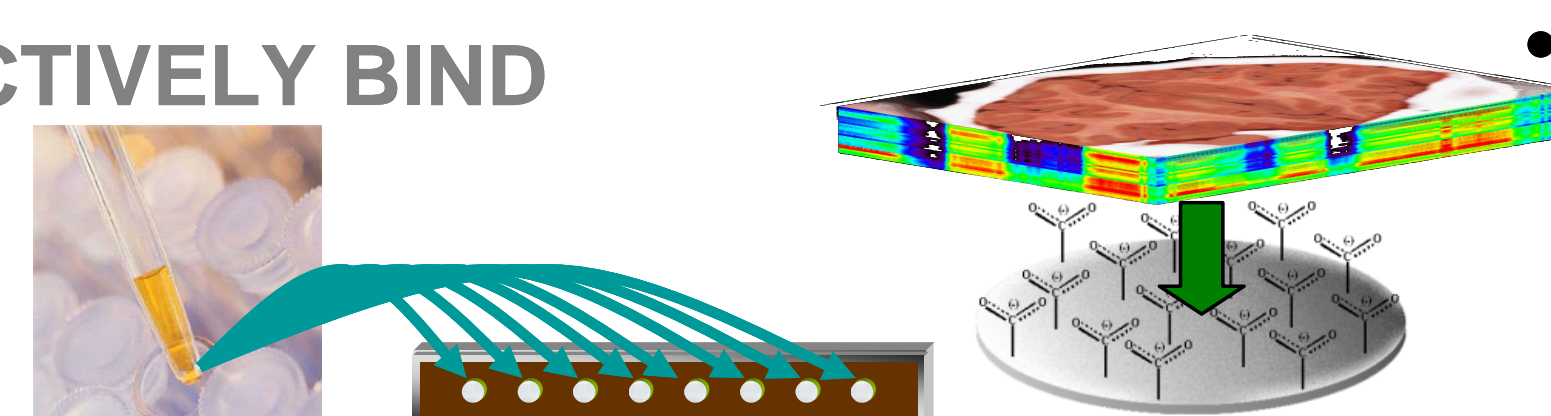


Figure 1: Multiple β amyloid peptide detection from CSF by MaS-ISIA. Example spectrum for human CSF spiked with 20 nMolar each of β fragments 1-16, 1-33, 1-38, 1-40, 1-42, 1-45, 1-47, 1-48, 2-40, and 2-42. Spiked CSF was incubated at ambient temperature for 1 hour prior to assaying on antibody (6E10) coated-arrays, which resulted in additional processing of the β fragments. Control IgG shows the result when 6E10 was replaced with Bovine IgG (nonspecific binding control). The intensity axes for the two spectra are the same.

Methods: MaS-ISIA and iMaS-ISIA

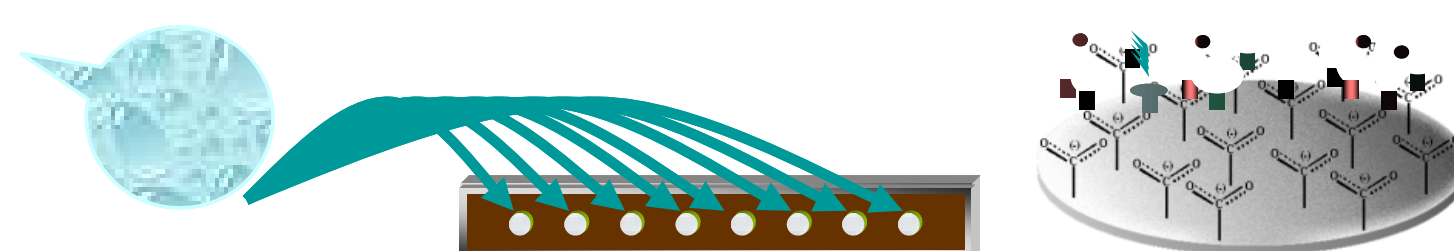
SELECTIVELY BIND



- **Step 1: Sample (fluid, tissue) is placed on a ProteinChip® Array**

- Affinity Capture – Proteins bind to chemical or biological sites on the array

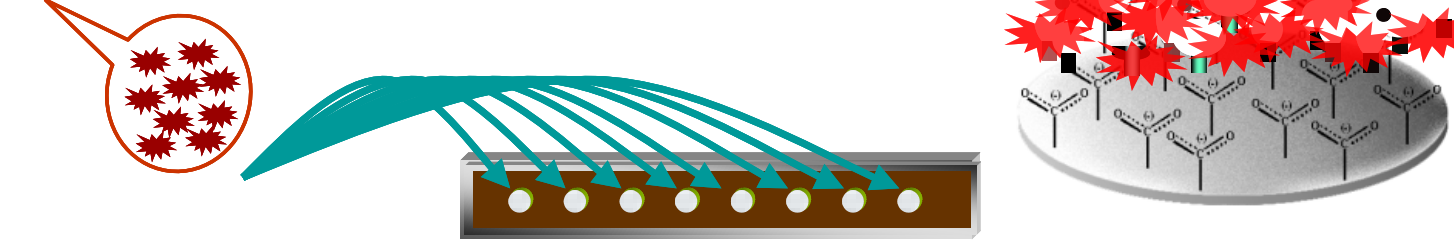
WASH



- **Step 2: Remove sample and unbound proteins**

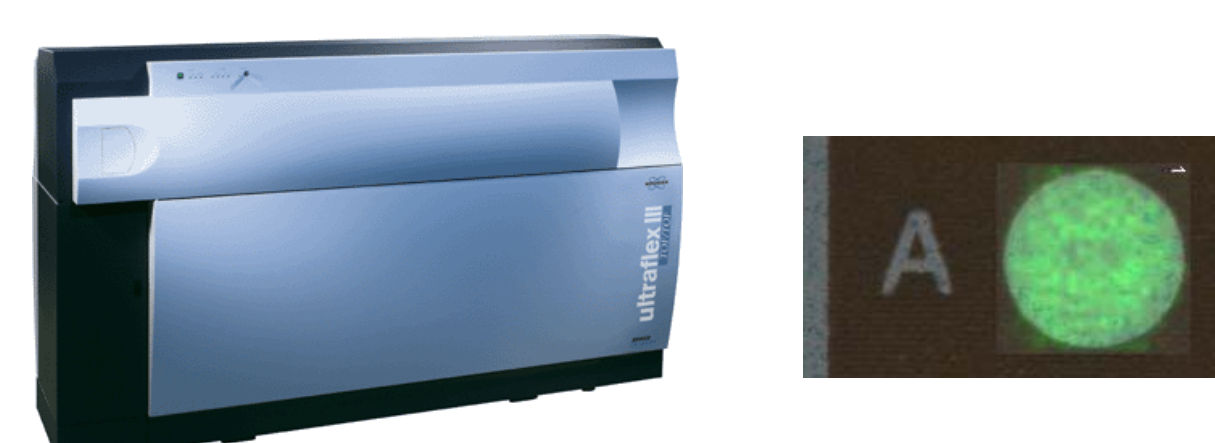
- Remove sample
- Wash the array with an appropriate stringency buffer
- Removes non-specifically bound proteins and interfering compounds (salts, detergents, etc.)
- Specifically bound proteins are retained in spatial fashion

MATRIX



- **Step 3: Add Matrix**

MS



- **Step 4: Profiling (MaS-ISIA) or Imaging (iMaS-ISIA)**

Figure 2: Methodology. Sample is applied to ProteinChip arrays. In the case of the tissue samples, molecules that have been transferred may be retained in spatial fashion and may be investigated either nonspatially for *in situ* profiling (MaS-ISIA) or spatially for imaging (iMaS-ISIA).

Profiling is applicable to different surface types: chromatographic surfaces for discovery and antibody-coupled surfaces for targeted assay (MaS-ISIA). With MaS-ISIA, cross-reactive antibodies may be exploited to simultaneously monitor and/or locate different forms of a species of interest. Peptides captured in this manner were both profiled and directly sequenced from the arrays using an ultraflex III MALDI-TOF/TOF (Bruker Daltonics). Quantitation was ensured via standardization of instrument response.

Conclusions

Coupling affinity capture from tissue with mass spectrometry is here shown to provide similar advantages to those provided by solution-based immunoassays for serum.

ImmunolMaging using retentate arrays with MALDI TOF/TOF analysis is shown to give sensitive molecular detection with retention of spatial orientation of biological species transferred from a fresh tissue onto a selective binding surface. In investigations of Alzheimer's Disease model samples for β -amyloid peptides, ImmunolMaging allowed targeting of the β -amyloid material within a sample – simultaneous concentration and clean-up of the peptides along with investigation of the spatial information. Spiking investigations, although not necessarily matching the transfer performance of native β -amyloid, give an initial insight into the quantitative potential of the technique.

Use of an epitope which targeted multiple peptide species simultaneously, allowed the investigation of the total amount of β -amyloid in a given location and also simultaneous investigation into the location and nature of all reacting β -amyloid peptides. This allows sensitive investigation into whether β -amyloid peptides co-occur, or occur in different locations throughout the brain. This is a tool that may lead to further understanding of the time and spatial distributions of different peptide species at different locations within the brain.