APPLICATION NOTE 2

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Epitope Identification and Affinity Determination of Aß-specific Antibodies by online SPR- MS

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The accumulation of extracellular plaques containing the neurotoxic ß-amyloid peptide fragment, Aß(1-42) of ß-amyloid precursor protein (ßAPP), is one of the characteristics of Alzheimer's disease (AD). Although ßAPP has been recognised as a key molecule for AD, its molecular (patho)physiological degradation, proteolytic pathways and cellular interactions of Aß are still unclear. Studies towards the development of immuno-therapeutic methods for AD have yielded initial success in transgenic mouse models of AD, in producing therapeutic antibodies by immunisation with Aß(1-42), that disaggregate Aß-plaques and fibrils. Using proteolytic excision of the immobilised Aß antigen-immune complex in combination with ESI mass spectrometry, the Aß-plaque specific epitope was identified as an N-terminal peptide Aß(4-10), accessible in Aß(1-42) as well as in oligomeric Aß-fibrils [1, 2].

Recently, we have identified the epitope recognised by Aß-autoantibodies in serum, capable of eliciting a neuroprotective effect to inhibit the formation of Aß-plaques, to be located in the carboxyterminal region of the Aß sequence. The differential epitope structures of Aß-specific antibodies from healthy individuals and AD patients provides a breakthrough and molecular basis for (i), the development of new immuno-therapeutic approaches by passive immunisation with Aß-specific antibodies, and (ii), the development of new diagnostic tools for AD with absolute specificity (Figures 1; 2) [2, 3]. The primary structures of polyclonal Aß-autoantibodies were elucidated by two-fold Aß- epitope specific affinity chromatography from human immunoglobulin G, using a combination of overlapping proteolytic digestion (trypsin, α-chymotrypsin), HPLC isolation, and high resolution MS, which provided sequence data for Fv domains, CDR motifs, and framework regions. The plaque-protective Aß-epitope of the Aß-autoantibody was identified by proteolytic extraction-MS using the online SPRMS- epitope analyser, to reside in the Aß(17-28) tryptic peptide sequence. To identify the epitope, the Aß- autoantibody was immobilized on a dextran-SPR

affinity chip, and a tryptic mixture of Aß-peptide fragments injected through the SPR autosampler followed by ESI-MS. Subsequent online desalting of analyte prior to MS was performed after elution of affinity captured Aß-peptide which provided identification of the Aß(17-28) epitope peptide (protonated molecular mass 1324,8). Following elution of undigested Aß(1-40) through the microfluidic interface, SPR affinity determination revealed high affinity with a K_D of ca. 3.5 nM (Figures 3 and 4). Based on this epitope peptide, interactions of the plaque-protective Aß-epitope with two fibril inhibiting peptides, cystatin-C and humanin, were evaluated at the molecular level to gain insight into the mode of action of Aß-autoantibodies [4].

In this study we show that the online SPR- ESIMS combination is a powerful tool to enable the simultaneous affinity isolation, structure identification and affinity quantification of an Aß- plaque protective epitope from the complex of Aß-autoantibodies immobilized on a gold chip. The high application potential of online-SPR-MS has become further evident in recent studies of the identification of an unusual mixed-disulfide antibody epitope of the rheumatoic target protein, HLA-B27; and the interaction site identification of chaperone complexes of lysosomal enzymes [5, 6]. Current applications confirm that interaction epitopes as diverse as antigen-antibody and lectin- carbohydrate complexes [7], and binding constants (K_D) from milli- to nanomolar ranges are amenable to SPR-MS analysis. These results indicate that applications of the online-SPR-MS epitope analyzer are well feasible to affinity-based biomarker evaluation; identification of protein and peptide epitopes; precise antibody affinity characterization; and direct label-free antigen quantification.

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Epitope identification and affinity determination of β-amyloid antibody

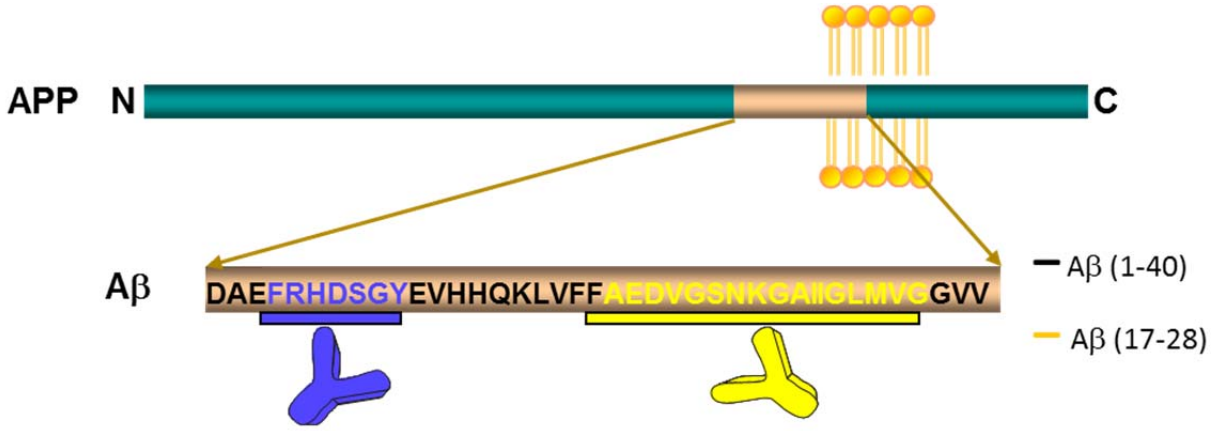

Fig 1: Abeta epitope peptides resulting from the cleavage of the molecule by APP. Abeta epitopes against antibodies are highlighted.

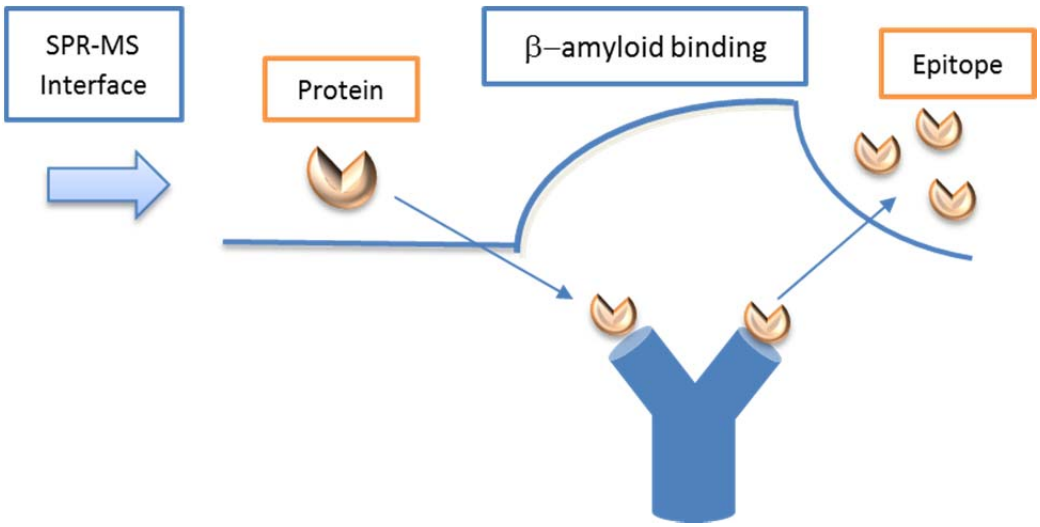

Fig. 2: Schematic representation of Amyloid protein binding to the Aß- specific antibody. The SPR response increases exponentially during the binding and decreases upon epitope elution.

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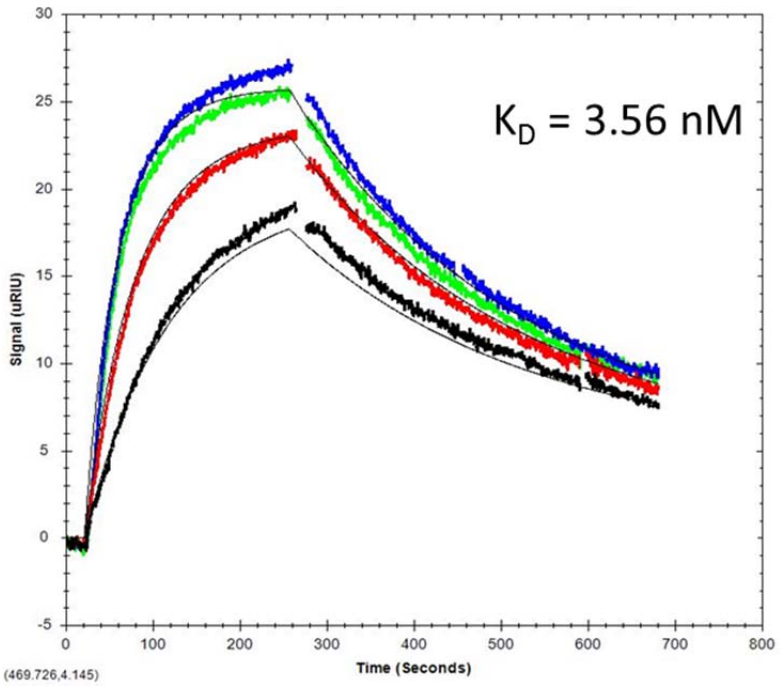

Fig. 3: SPR determination of a dilutions series of Aß(1 - 40) upon processing via the SPR-MS interface. Kinetic evaluation resulted in a calculated KD of 3.56 nM.

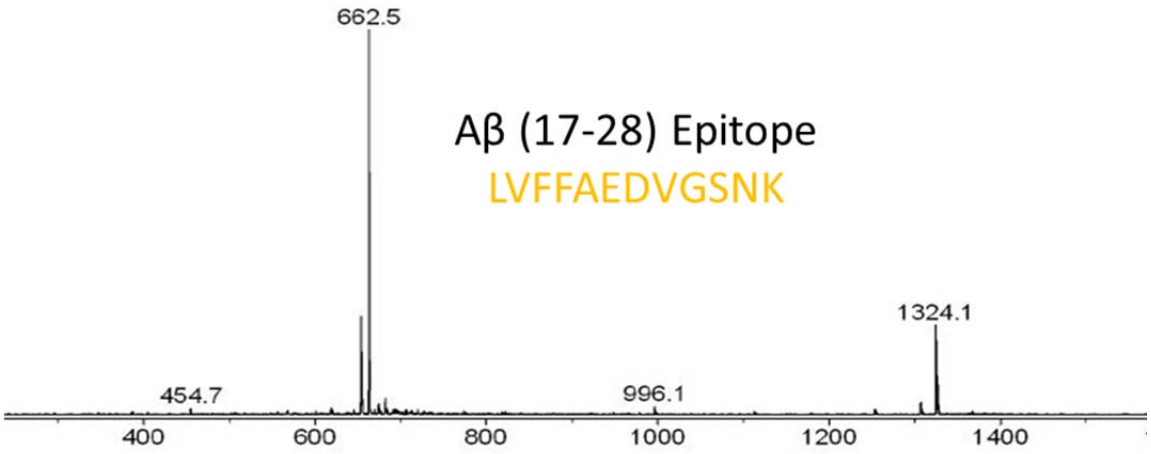

Fig. 4: ESI-MS identification by online SPR-MS of the epitope Aß(17-28) eluted from the Aß-antibody upon proteolytic extraction.