

4-1-2018

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Wang, Jasmine S-H; Whitehead, Shawn N; and Yeung, Ken K-C, "Detection of Amyloid Beta ($A\beta$) Oligomeric Composition Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS)." (2018). *Chemistry Publications*. 83.
<https://ir.lib.uwo.ca/chempub/83>

Detection of Amyloid Beta (A β) Oligomeric Composition using Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS)

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This is a pre-print of an article published in *Journal of The American Society of Mass Spectrometry*. The final authenticated version is available online at: <https://doi.org/10.1007/s13361-018-1896-z>

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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

Funding Sources

The University of Western Ontario, Natural Sciences and Engineering Research Council of Canada, Canadian Institutes for Health Research, Lawson Health and Research Institutes and the Canada Foundation for Innovation

ABSTRACT: The use of MALDI MS as a fast and direct method to detect the A β oligomers of different masses is examined in this paper. Experimental results suggest that A β oligomers are ionized and detected as singly charged ions, and thus the resulting mass spectrum directly reports the oligomer size distribution. Validation experiments were performed to verify the MS data against artifacts. Mass spectra collected from modified A β peptides with different propensities for aggregation were compared. Generally, the relative intensities of multimers were higher from samples where oligomerization was expected to be more favorable, and vice versa. MALDI MS was also able to detect the differences in oligomeric composition before and after the incubation/oligomerization step. Such differences in sample composition was also independently confirmed with an *in vitro* A β toxicity study on primary rat cortical neurons. An additional validation was accomplished through removal of oligomers from the sample using molecular weight cutoff filters, the resulting MS data correctly reflected the removal at the expected cutoff points. The results collectively validated the ability of MALDI MS to assess the monomeric/multimeric composition of A β samples.

KEYWORDS: Alzheimer's disease, Amyloid beta-derived diffusible ligand (ADDL), Oligomers, Non-covalent complexes, Protein aggregation, *In vitro* toxicity assay, Embryonic rat cortical neurons

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deterioration in cognition, memory, and behavior [1-2]. With more than 5 million North Americans suffering from this disease [3], AD is now considered the most common cause of dementia, and the 6th leading cause of death in the United States [4]. The adverse neuropsychiatric and behavioral symptoms patients suffer from pose as a significant strain on our health care system, and give rise to socioeconomic issues. Hallmarks of the disease include extracellular plaques characterized by the accumulation of protein-containing deposits primarily consisting of amyloid- β (A β) peptides and intracellular neurofibrillary tangles caused by aberrant phosphorylation of tau [5-7]. Previous work found these large fibrillary A β structures within neuritic plaques killed cultured neurons [8]. This and other evidence led to the amyloid cascade hypothesis which originally postulated that these insoluble fibrillary aggregates were the primary cause of neurodegeneration in AD patients [9]. Although extensive research has targeted these plaques, poor correlation between neurological deficits and plaque accumulation have been observed in AD patients [10-11].

Recent studies have shifted focus to examine the onset of A β aggregation from the monomeric peptides to the soluble form of A β multimeric complexes, and found evidence linking the accumulation of the A β oligomers to the progression and severity of AD, in particular, with long term hippocampal, age-onset memory failures [5-6, 12-15]. Due to these recent findings, modifications to the amyloid cascade hypothesis have been made to target the role of soluble A β oligomers in disease progression [16-18]. In healthy individuals, A β peptides are degraded within or cleared from the brain [19-20]. Reduction in the degradation or clearance efficiency results in accumulation of the soluble oligomers that correlate with disease onset and progression. Understanding the mechanisms and effects of A β oligomerization in the brain is therefore critical to the development of novel therapeutics for AD. A common experimental approach is the *in vitro* toxicity assay of A β oligomers on neuron cell cultures, where cellular viability is quantified to determine the effects of various forms of A β and/or potential therapeutics [21-25]. The A β samples for the *in vitro* experiments are typically obtained from commercial sources. Numerous protocols have been reported for *in vitro* oligomeric A β_{1-42} preparations with the largest variation residing in the monomerization and oligomerization solutions used [26-29]. Surprisingly, to our knowledge, comprehensive studies examining the effect of protocol conditions on the resulting oligomer size distribution are not available in the literature. In addition, it is not a common practice to verify the composition of the A β oligomers prior to use, partly due to the limitations in techniques available for fast and simple A β multimer characterization.

Currently, the most common means for oligomer quantification is gel electrophoresis [30]. The technique depends on the ability for SDS to bind to proteins of interest. This binding compromises the structural integrity of proteins resulting in dissociation of oligomer composition [31]. Low sample throughput and prevalence of gel smearing are also significant issues which act as limitations in resolution and possess accuracy issues [32]. Another prominent issue gel electrophoresis possesses is the inability to accommodate for a large mass range for multimer analysis. The percent composition of acrylamide within the gel is tailored for the target sample. The larger the peptide or protein under analysis, the lower the amount of acrylamide used in order to facilitate appropriate separation to obtain distinct bands. For samples containing a wide range of molecular weights, the set acrylamide concentration could not optimally accommodate for both extremes, resulting in either the smaller molecular weight peptides to run off the gel, or poor resolution of the larger peptides. Other techniques available to study A β aggregation include atomic force microscopy (AFM), surface plasmon resonance (SPR), absorbance and fluorescence [33-34]. AFM is generally suitable for detection of large A β aggregates such as fibrils, but not the soluble small oligomers. SPR is typically used to detect interactions in amyloid-ligand binding, but ligand interaction is not selective enough to differentiate oligomers by size. Likewise, absorbance and fluorescence can detect the bulk presence of oligomers, but lacks the ability to differentiate between small size differences of A β oligomers.

Chemical cross-linking could be used to stabilize protein-protein interactions. Several methods of cross-linking have been explored in the attempt to freeze A β oligomers in current oligomerization states. However, many of these methods require addition of cross-linkers or catalyst to induce cross-linking [35-36]. Introduction of these cross-linkers and catalysts at times can result in modifications of native amyloid composition and structure or induce further oligomerization leading to fibril formation. Complications also arise from non-specific interactions that occurs upon addition of these compounds. Due to the complexity introduction of these reactive moieties generate, photoinduced cross-linking of unmodified proteins (PICUP) was proposed as a means of cross-linking [32, 37]. PICUP induces cross-linking through rapid, visible light photolysis of tris-bipyridyl Ru(II). In the presence of electron acceptors, Ru(III) is produced. Ru(III) then acts as an electron abstraction agent forming carbon radicals which form new C-C bonds with nearby carbons. This form of crosslinking is attractive as it does not require insertion of a cross-linking group to the peptide and the m/z of the oligomers are not altered by the cross-linking. However, the carbon radical formation and C-C bond formation are non-specific, and can occur between preexisting oligomers with free monomers to form artificially larger oligomers [38]. Importantly, the observed crosslinked oligomer distribution is highly dependent on the crosslinking conditions of protein-to-Ru ratio and the power and duration of the photo illumination [39]. Finally, a separation may be required to remove the high concentration of quenching reagent, mercaptoethanol or dithiothreitol, prior to detection.

Electrospray ionization mass spectrometry (ESI MS) has been widely used as an alternative label free tool for amyloid beta analysis [40-42]. ESI is capable of transforming the multimeric forms of amyloidogenic peptides from solvated to gaseous ions as intact non-covalent complexes [43]. However, the resulting complex ions can possess a wide range of multiple charges; for example, $z = 2$ to 10. Oligomers of different sizes can therefore have identical m/z , making the peak assignment to oligomer sizes a perplexing task [44-45]. To overcome this, researchers turned to the use of ion mobility spectrometry (IMS), which can resolve the multimeric complex ions, not only by m/z , but also according to size based on their mobility in a drift tube [46-48]. In recent years, the combination of IMS with ESI MS has become an invaluable tool in deciphering the assembly mechanism of A β oligomers [31, 47, 49-53]. While ESI IMS-MS is extremely powerful, the interpretation of two-dimensional data of drift time and m/z is not a straight forward task for multiply-charged homo-multimers such as A β oligomers. The assignment of signals to oligomer of various sizes involves the consideration of monomer charge states, the ^{13}C isotope distributions with and without collision induced dissociation, and the drift time dependence of injection energy [50].

The work described herein focuses on a simpler mode of MS based on matrix assisted laser desorption ionization (MALDI). In contrast to ESI, MALDI generates gaseous ions of peptides and proteins predominately in the singly charge state ($z = 1$). As a result, the measured m/z values of A β oligomers directly represent the mass of the oligomers. To our knowledge, Van Duyne and co-workers presented the first use of MALDI MS to detect oligomers of A β_{1-42} [54], but the use of MALDI MS was not the focus of their work, and only one mass spectrum of the oligomers was presented as a supplementary figure. Also, the adoption of this application has not been observed in the literature. Most importantly, the authors did not present validation experiments to confirm that the observed A β complex ion distribution indeed reflect that of original oligomeric A β sample solution. Instead, they claimed that "the observed ions are likely a combination of intact molecular ions, fragments from larger oligomers, and gas phase aggregates". In this paper, we will examine the validity of MALDI MS in measuring the composition of A β multimers. Particularly, we aim to investigate whether the oligomers detected by MALDI MS were indeed artificial A β complexes non-existent in the original sample, merely a result of the non-specific associations of A β peptides, either during sample preparation or during the MALDI process. To achieve this, we begin by reproducing the oligomer detection by MALDI MS with wildtype A β_{1-42} and A β_{1-40} , and comparing with the different results obtained from other variants of A β peptides with varying tendencies of oligomerization. Secondly, we manipulated the size distribution of A β_{1-40} oligomer samples using molecular weight cut off filters, and subsequently illustrated the expected changes in the resulting MS data. After validating MALDI MS in measuring A β multimeric composition, the method has been applied to examine the A β samples prepared using published protocols on monomerization and oligomerization for *in vitro* toxicity assays.

METHODS

Reagents. All A β peptides were purchased from Bachem (King of Prussia, PA). They include the wildtype A β_{1-42} and A β_{1-40} , a peptide with same amino acid composition but in scrambled sequence A β_{1-40S} (AEGDSHVLKE GAYMEIFDVQ GHVFGGKIFR VVDLGSHNVA), the reversed sequence peptide A β_{42-1} , and the mutant that has high tendency of oligomerization A $\beta_{1-40}(E22Q)$ (DAEFRHDSGY EVHHQKLVFF AQDVGSNKGA IIGLMVGGVV). Ethanol, methanol, and acetonitrile (ACN) were purchased from Caledon (Ontario, Canada), and hexafluoro-2-propanol (HFIP), dimethyl sulfoxide (DMSO), Sinapinic acid (SA), trypsin, amphotericin B solution, 7% poly-L-ornithine from Sigma Aldrich (St. Louis, MO). Ham's F-12 nutrients mixture, B27 supplement, N2 supplement, penicillin/streptomycin, glutamax, and 4'-6-diamidino-2-phenylindole (DAPI) were purchased from Life Technologies (Grand Island, NY). Formic acid, ammonium hydroxide was obtained from EM Science (Darmstadt, Germany), and trifluoroacetic acid (TFA) was purchased from Fischer Scientific (Ottawa, ON, Canada). Neurons were isolated from Wistar rats purchased from Charles River (Montreal, QC, Canada). Hank's balanced salt solution (HBSS) and neural basal media were from Wisent (St. Jean-Baptiste, QC, Canada). Trypsin inhibitor was obtained from Roche Life Sciences (Indianapolis, IN), and propidium iodide was purchased from Biotium (Hayward, CA).

Amyloid Beta Sample Preparation. To optimize the monomerization condition, A β peptides were suspended in one of the four monomerization solvents, NH₄OH, TFA, HCO₂H, or HFIP, at a concentration of 1 mM. Our preliminary MALDI MS results revealed that highest peak intensities from the monomer with minimum signals from multimers were obtained with NH₄OH (data not shown), and thus was chosen in our work. To prepare for short term storage, the sample was fractionated in 10 μ L aliquots and lyophilized for 1 hour at -100°C and stored at -80°C. To prepare for use, each aliquot of lyophilized powder was resuspended in 10 μ L of DMSO and sonicated in a 37°C water bath for 10 minutes. The sample was then brought to a concentration of 150 μ M with the F12 solvent and vortexed. The final sample solution was incubated at 4°C for 24 hours to allow for oligomerization.

Molecular Weight Cut-Off (MWCO) Filtration. MWCO Filters at 10 kDa and 30 kDa were purchased from Pall Corporation (Ann Arbor, MI). A β sample aliquots of 70 μ L were pipetted into each filter and centrifuged 4 times in 30 second intervals at 14 000 rpm with Centrifuge 5417c from Eppendorf (Westbury, NY). Typically, 60 - 65 μ L of filtered sample solution was collected. ThermoFisher Scientific NanoDropTM Spectrophotometer 2000c (Waltham, MA) was used to assess the final filtrate concentration in five replicates.

Primary Embryonic Rat Cortical Neuron Cultures. Studies were conducted using neurons isolated from embryonic rat brain. Pregnant Wistar rats were sacrificed for surgical removal of E18 embryos. Cortices from

each embryo were extracted and placed in 14 mL conical tubes containing 1.8 mL of HBSS and centrifuged at 4000 x g for 1 minute at room temperature. HBSS was aspirated and 1.8 mL of Solution A (71.4% HBSS, 0.086% MgSO₄ (1 M), and 28.6% trypsin) was added. The mixture was agitated to ensure neurons were free floating and the sample was placed in an automated rotator for 25 minutes at 37°C. After rotation, 3.6 mL of Solution B (96.0% HBSS, 0.109% MgSO₄ (1M), 2.40% 10 mg/mL DNase I, and 1.54% 100 µg/mL trypsin inhibitor) was added. The solution was mixed for 2 minutes followed by centrifugation at 4000 x g for 5 minutes at room temperature. The HBSS was then aspirated and the pellet was re-suspended in 6 mL of Solution C (89.5% HBSS, 0.215% MgSO₄ (1 M), 5.82% DNase I, and 4.47% trypsin). The cells were transferred to a 50 mL falcon tube and another 6 mL of Solution C was added. The cells were titrated and centrifuged at 4000 x g for 5 minutes and the supernatant aspirated. Neurobasal plating media was prepared with 96% neural basal media, 2% B27 supplement, 0.8% N2 supplement, 0.5% penicillin/streptomycin, 0.25% Glutamax, and 0.1% amphotericin B solution. The resulting pellet from centrifugation was then re-suspended in the prepared neurobasal plating media, and counted with a hemocytometer prior to plating in 35 mm wells coated with 7% poly-L-Ornithine at a density of 0.5 x 10⁶ cell/well. The cells were stored at 37°C with 5% CO₂ and the medium was changed on the third day of plating prior to use.

Neuron Cellular Viability in Presence of Amyloid Beta. Aβ₁₋₄₂ was prepared as previously described. NH₄OH was used as the lyophilization solvent and the lyophilized powder was suspended in DMSO and F12 medium to 150 µM. An Aβ₁₋₄₂ sample was prepared and incubated for 24 hours while a second sample was prepared at the time of use providing the oligomer and monomer conditions respectively. Three different environmental conditions were provided for each cell population (N = 4) with 10 µL additions of pre-incubated Aβ₁₋₄₂ (monomer), and post incubation Aβ₁₋₄₂ (oligomer). One set of cultures were left without any environmental alterations to provide a baseline (control) of the natural decrease in cellular viability over time. The cells were incubated for 6, 24, 36, and 48 hours. PI staining was applied through addition of 10 µL of PI 45 minutes prior to fixation. The cells were fixed with 500 µL of 4% formaldehyde followed by 400 µL of 2% formaldehyde. The fixing agent was removed with 3 consecutive PBS (1x) washes.

Microscopy. Fine tweezers were used to plate the cells fixed on 12 mm round cover slips. The cells were counter-stained with DAPI for nuclear visualization and imaged using a Nikon Eclipse Ni-E with fluorescent filters and a DS-Qi2 monochrome microscope camera. 20x magnification was used with appropriate filters for DAPI and PI stained cell images. Five images were acquired for each cell culture in order to obtain the average cell deaths vs. total cells per culture. ImageJ software was used to process and analyze the cell images obtained.

Statistical Analysis. All values were presented as the mean \pm standard error of the mean (S.E.M.) of four independent experiments (n=4). Statistical comparisons between experimental groups were performed using the GraphPad Prism software. A two-way ANOVA with Tukey's HSD test was applied. $P < 0.05$ was considered to have statistical significance.

Mass Spectrometry. Sinapinic acid (SA) was selected as the MALDI matrix on this work based on preliminary experiments comparing it with the two other most commonly used matrices, alpha-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid. SA matrix was prepared to 10 mg/mL in 50% ACN and 0.05% TFA. The A β sample and SA matrix were deposited as sandwiched layers, where 0.75 μ L of the A β solution was spotted between two layers of SA (0.75 μ L). The spots were washed twice with 1 μ L water prior to analysis. MALDI-MS sample analyses were performed on an Sciex TOF/TOF 5800 MALDI mass spectrometer. TOF-TOF Series Explorer in positive ion linear mode and Data Explorer were used for data acquisition and processing, respectively (Sciex). Total sum of 200 shots/spot were acquired with a 1 kHz OptiBeam on-axis Nd:YAG laser system. Lowest possible laser intensity was used to minimize dissociation and favor the detection of A β oligomers. Other instrument parameters were generally optimized for sensitivity while compromising resolution to an acceptable extent.

RESULTS AND DISCUSSION

MALDI MS Results from Wildtype A β Oligomers. We began our studies by performing the detection of wildtype A β_{1-40} and A β_{1-42} oligomers with MALDI MS based on the conditions reported by Van Duyne [54] (Figure 1). Briefly, samples were subject to monomerization and then oligomerization conditions. Sinapinic acid (SA) was the MALDI matrix, and the same spotting was used with a modification of including a washing step. Effects of different TFA concentrations in the matrix solution was assessed, and we concluded that a reduction in TFA concentrations was most beneficial in the detection of oligomers within the sample. This coincides with previous studies indicating low pH disruptions in the formation of non-covalent complexes [55]. The instrument's acquisition parameters were set to optimize for detection sensitivity. Our MALDI MS instrument appeared to be susceptible to ionization suppression of higher MW species by low MW ones. In other words, the detection of high MW oligomers was hindered by the presence of the monomers, as shown in Panels A and E of Figure 1. To alleviate this, mass spectra were acquired starting at higher m/z values to enhance the sensitivity for the large MW species.

The monomeric A β_{1-40} and A β_{1-42} peptides have a molecular weight of 4329.86 Da and 4514.10 Da respectively, and therefore only the singly charged ions the A β monomer and multimers were observed in all cases. Between the A β_{1-40} and A β_{1-42} , our spectra revealed that signals of high MW complexes were more prominent for

$A\beta_{1-40}$ than $A\beta_{1-42}$. Importantly, when comparing our data to those previously reported [54], the same general trend in oligomer size distribution was detected. That is, the signal intensity was the strongest with small MW species, and dropped off in a decaying fashion for larger complexes. There were also differences in the peak intensities, as expected, attributed to the different MALDI mass spectrometers used. The MALDI MS results also generally agree with those reported by gel electrophoresis, where substantial quantities of multimers relative to the monomer were typically detected, as bands of similar size and darkness. When compared to ESI MS, rather different looking mass spectra were reported with similar samples. The dominant two peaks in the ESI mass spectra of $A\beta_{1-42}$ correspond to the monomers with -3 and -4 charge states, and the sum of peak height from other peaks accounts for less than 10% of the total [50].

MALDI MS Results from $A\beta$ Variants. The preparation of $A\beta$ samples for MALDI MS analysis involve mixing it with UV absorbing matrices at acidic pH, which is then deposited on target plates and allowed to dry. Hence, a common concern is the possibility of artificial non-specific associations that could occur either during sample spot drying or within the mass spectrometer in gas phase. In the literature, numerous examples of MALDI MS application on complexes of biomolecules were reported and reviewed [55-57]. The consensus is that the technique is capable of detecting certain non-covalent multimeric complexes under optimized conditions. However, there is not one universally optimal protocol. Therefore, it is important to experimentally determine if the $A\beta$ non-covalent complexes detected by MALDI MS herein were real or merely an artifact. To achieve this, $A\beta$ variants with varying propensities in oligomerization, used by others in toxicity experiments, were acquired and analyzed.

We first selected the $A\beta_{1-40}$ variant with the same amino acid composition as wildtype, but in a random sequence, as a negative control. This variant, $A\beta_{1-40}S$, has been applied in Alzheimer's disease research as an inactive controls, and verified to exhibit minimal toxic effects compared to the wildtype [58]. Using the same experimental conditions for the wildtype $A\beta$ samples, mass spectra were collected for $A\beta_{1-40}S$, and the results revealed only a minimal level of dimerization (Figure 2). Importantly, the results presented a very strong evidence that gas phase aggregates and non-specific association of the dried $A\beta$ samples did not occur, at least not to a significant extent. Consequently, it argues that the multimeric signals observed by MALDI MS, such as those in Figure 1, in fact came from $A\beta$ complexes assembled in solution.

Another negative or inactive control of $A\beta$ used in Alzheimer's disease research is the reversed sequence variant, $A\beta_{42-1}$. Contrary to our expectation, [oligomerization was evident with the reversed peptide, and](#) the oligomeric composition observed from $A\beta_{42-1}$ (Figure 3) was very similar to that of wildtype $A\beta_{1-42}$ (Figures 1E to 1H). However, controversy on the effectiveness of this variant as an inactive control has been reported. For example,

Vadukul et al. presented experimental data that the reversed A β peptides self-assembled to form fibres within 24 h, even though they exhibited a reduced effect on cellular health compared to wildtype A β [59].

Finally, we completed this set of experiments by examining a third A β variant, A β_{1-40} (E22Q). This A β mutant was intended to aggregate more readily in comparison to the wildtype peptide [60-63]. To capture the more favorable oligomerization of A β_{1-40} (E22Q), we performed MALDI MS of the A β samples without the 24-hour incubation step. It essentially allowed us to capture the onset of oligomerization, immediately after the monomerized A β samples were put in oligomerizing conditions. Figure 4 present the mass spectra, comparing the A β_{1-40} (E22Q) with wildtype A β_{1-40} . The results between Figures 4B and 4D indicate a higher level of multimeric signals from the mutant, in agreement with the expected behavior of the mutant.

***In vitro* A β Toxicity Assay.** Another noteworthy point from the results in Figure 4 is the ability of our technique in detecting differences in the A β multimeric compositions before and after the incubation/oligomerization step; namely, the differences observed between Figures 1A-1B with Figures 4C-4D for A β_{1-40} . Very similar results were also observed for A β_{1-42} (data not shown). To verify that the incubation step indeed resulted in the intended oligomerization of A β peptides, we conducted an *in vitro* toxicity assay on embryonic rat cortical neuron cell cultures. The average cell death percentage was determined in 48 hours under the treatment of using our A β samples with and without the incubation step, which we refer to as the “oligomerized” and “monomerized” A β samples (Figure 5). The results obtained from the monomer treatment were indistinguishable from the control in all time points measured. In contrast, an accelerated rate of cell death was observed upon treatment with A β oligomers. At 24 hours, the toxicity of the oligomer sample treatment began to display a notable difference when compared to the monomer sample ($P = 0.07$). As the treatment was extended for 36 hours, the toxicity of the oligomer treatment was significantly higher than that of the control ($P = 0.002$). Once the treatment was carried out for 48 hours, statistically significant differences in toxicity between the oligomeric sample and that of the control and monomeric samples were observed ($p = 0.03$ and 0.01 respectively). In the literature, reports on the effect of A β oligomers varied depending on conditions such as cell culture preparation methods, stains, and dosage. For example, one reported a decrease in cellular viability from 90% to 55% upon exposure to A β oligomers while another reported 20% cellular viability after 48 hours [64-65]. Importantly, our results reaffirmed the significant toxicity of the oligomerized A β samples are in close agreement with the MALDI MS peak distribution observed.

Manipulation of A β Oligomeric Composition with MWCO Filters. Further to examining the various A β variants, we performed sample filtration using MWCO filters with the intention of removing the higher MW complexes at two different MWCO points, 10 kDa and 30 kDa. Based on the discoveries presented, MALDI

MS should be able to detect differences in oligomeric composition after being manipulated by MWCO filtration. Specifically, a 10 kDa cut-off filter should in theory remove complexes larger than dimers (8659.72 Da). The resulting mass spectrum indeed revealed the expected reduction in signals of the large oligomers. The result is presented in Figure 6A, where a significant reduction the dimer signal intensity was observed. In comparison, when the A β ₁₋₄₀ oligomers were filtered at the 30 kDa cut-off point, significantly stronger dimer and trimer signals were observed (Figure 6B). The extent of sample loss due to non-specific adsorption during the MWCO filtration was determined by nano-drop spectrophotometer, which measured the filtrate concentration to be approximately three times lower than stock concentrations. For comparison, the mass spectrum from a 3-fold diluted A β sample, without MWCO filtration, is shown in Figure 6C. The results collectively allowed us to draw the conclusion that, despite the harsh, non-native, nature of MALDI MS, it is capable of revealing the oligomeric composition of A β complexes in solution. The A β complexes detected by MALDI agreed with expected oligomeric compositions of the solution samples. Finally, the intensity of the MALDI MS signals of these oligomers corresponded to their concentration at least in a semi-quantitative fashion. It is however noteworthy that the detection of very large A β oligomers, if present, is limited by the sensitivity of the MS.

CONCLUSION

In this paper, the validity of MALDI MS in the detection of A β monomeric/oligomeric composition was investigated. Our results verified the ability of MALDI MS to differentiate variants of A β peptides with different propensities of oligomerization, and the A β samples obtained before and after oligomerization incubation. In contrary to previous speculations, our work provided the evidence that the MALDI MS signals of A β oligomers did not result from random gas phase aggregation. Interestingly, we observed a significant extent of oligomerization from the reversed peptide, A β ₄₂₋₁, which supported the controversial report of reversed A β self-assembly reported in the literature. Even though MALDI MS is not a quantitative measurement of the A β oligomeric composition, the peak distribution broadly reflects the oligomeric composition in a qualitative manner. In terms of potential applications, MALDI MS will allow researchers to confirm the multimeric composition of their A β samples prior to their use in neurodegeneration research, or to determine the oligomerization affinity of novel A β mutants. The detection of A β oligomers with MALDI MS also presents an exciting opportunity of future work in the direct MS imaging of multimeric A β in brain tissues, although further research and development will be required to overcome background interferences and signal suppression.

ACKNOWLEDGEMENT

This work was funded by the University of Western Ontario, the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes for Health Research, Lawson Health and Research Institutes and

the Canada Foundation for Innovation. The authors thank Mrs. Lynn Wang for her help with cell culture preparations. The authors also acknowledge Mrs. Kristina Jurcic, the University of Western Ontario MALDI MS Facility and the Schulich School of Medicine and Dentistry for their support.

ABBREVIATIONS

AD, Alzheimer's disease; AFM, atomic force microscopy, A β , amyloid beta; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; F12, Ham's F12 media; IMS, ion mobility spectrometry; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MWCO, molecular weight cut-off; NH₄OH, ammonium hydroxide; SA, sinapinic acid; SPR, surface plasmon resonance; TFA, trifluoroacetic acid; TOF, time of flight; DAPI, 4'-6-diamidino-2-phenylindole.

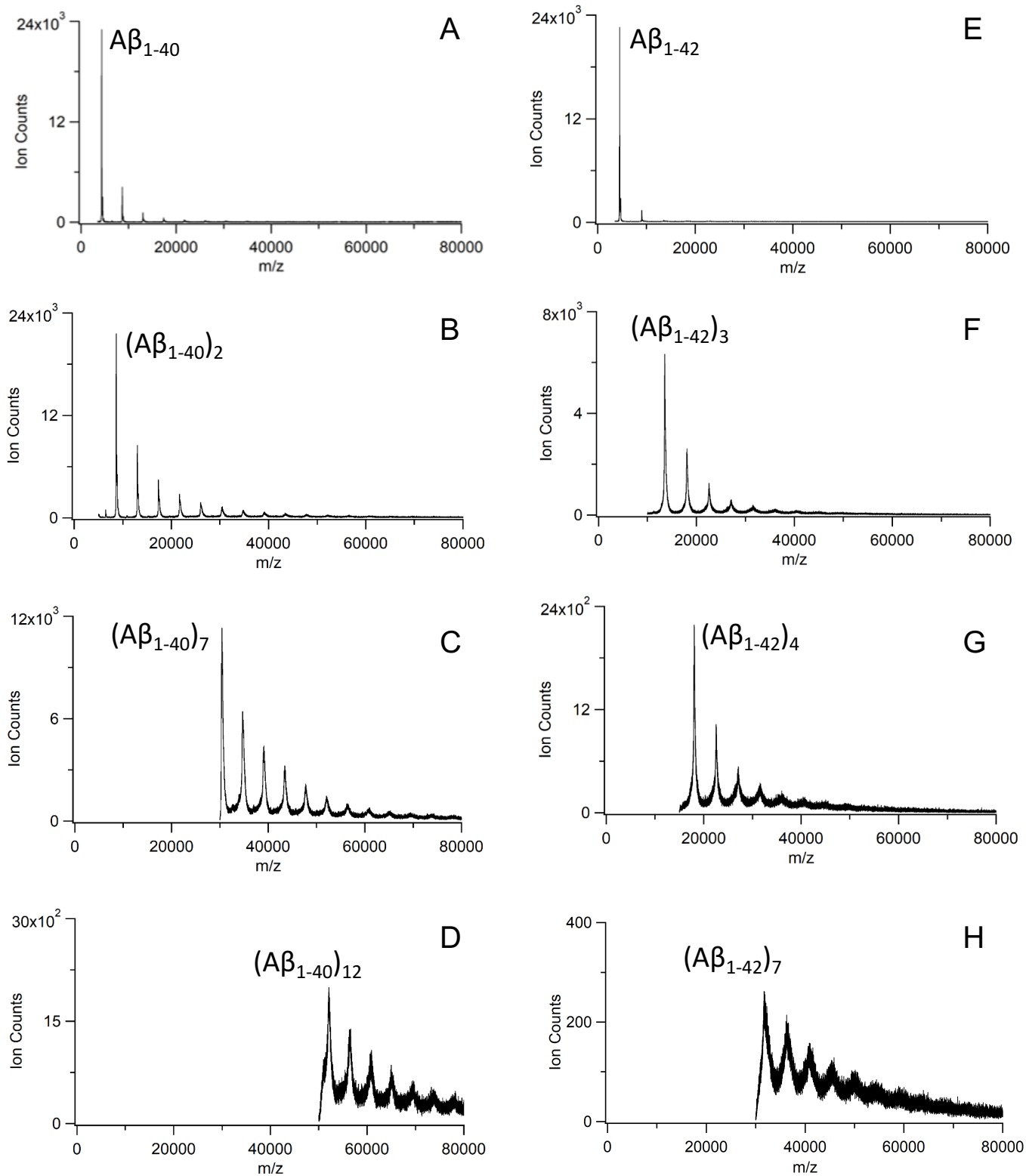


Figure 1. MALDI mass spectra of wildtype $A\beta_{1-40}$ (A to D) and $A\beta_{1-42}$ (E to H) samples prepared under conditions that promote oligomerization. Data acquisition was shifted to higher m/z starting values to enhance the detection of larger molecules.

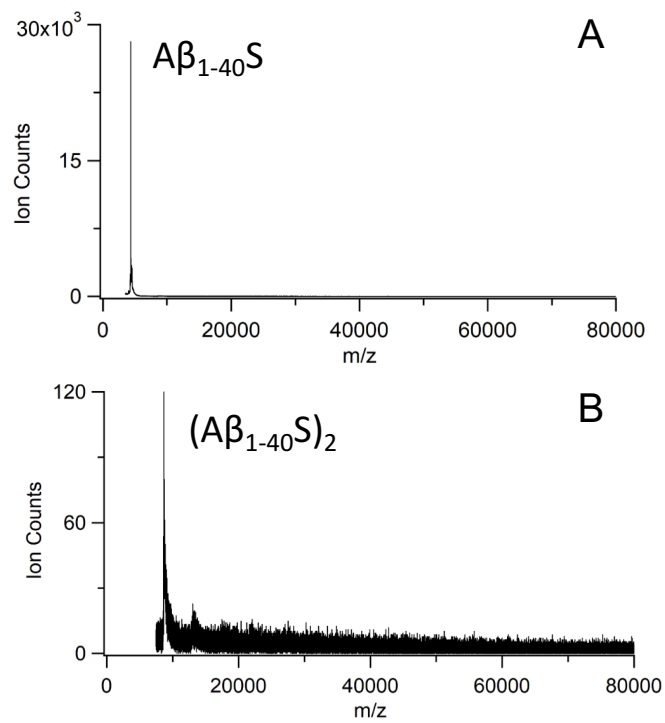


Figure 2. MALDI mass spectra of $A\beta_{1-40}S$, a variant with scrambled sequence acquired: at full range (A) or at a higher m/z to enhance the detection of larger complexes (B).

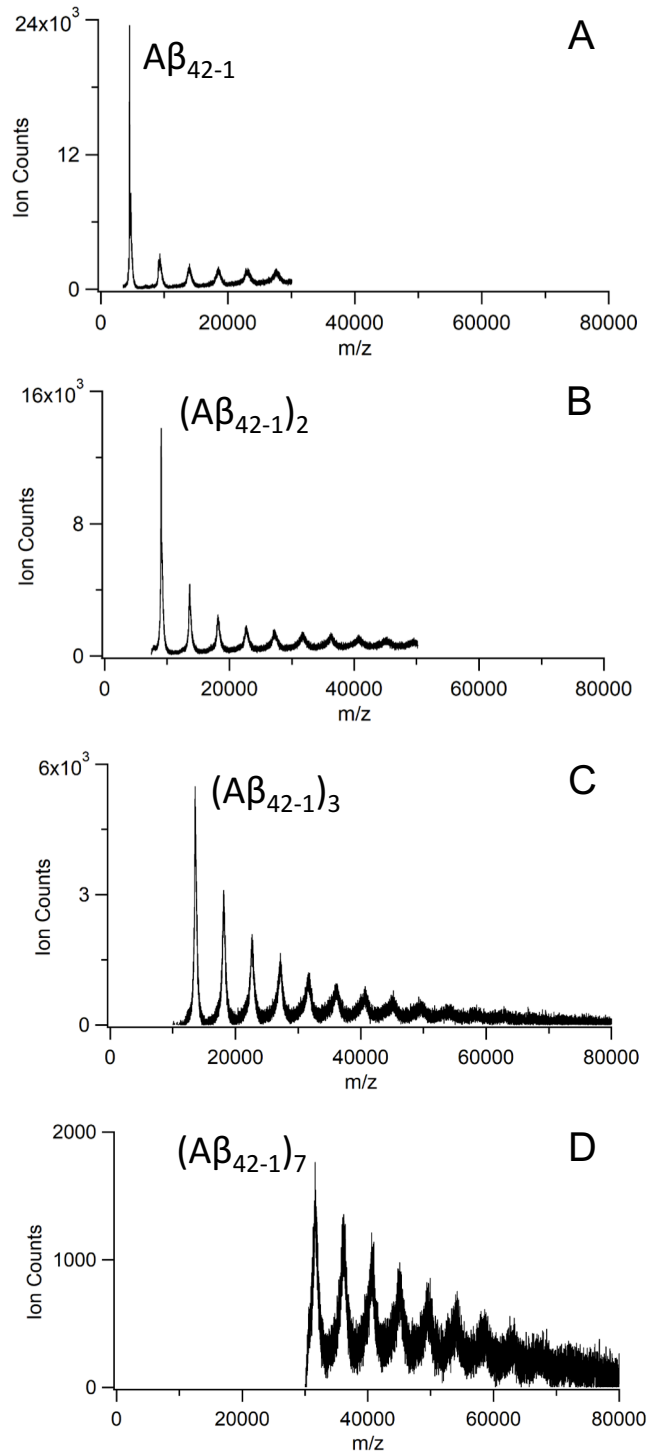


Figure 3. MALDI mass spectra of reversed sequence peptide $A\beta_{42-1}$ acquired at various starting m/z values to enhance the detection of larger molecules.

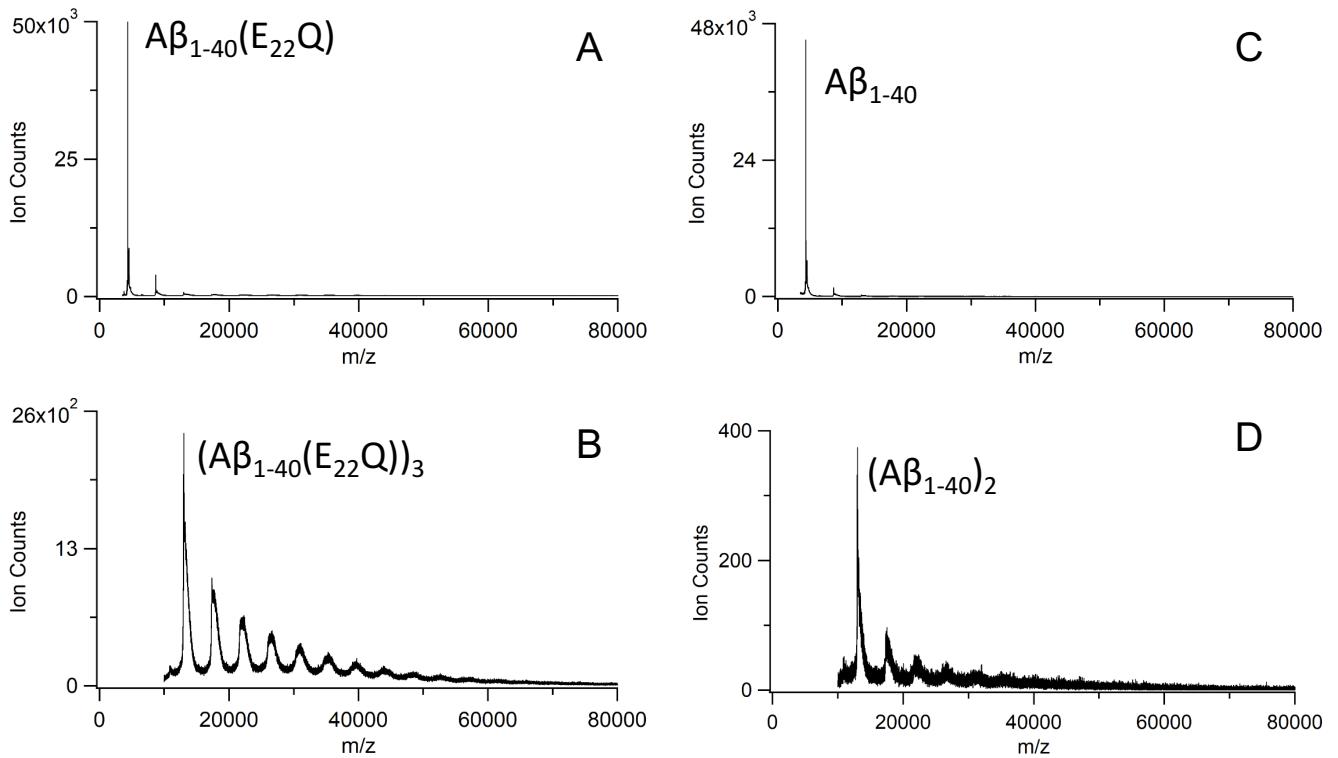


Figure 4. MALDI mass spectra of samples acquired without the 24-hour incubation to capture the onset of oligomerization for: the Aβ₁₋₄₀(E₂₂Q) mutant (A to B) and the wildtype Aβ₁₋₄₀ (C to D) acquired at two starting m/z values to enhance the detection of larger molecules.

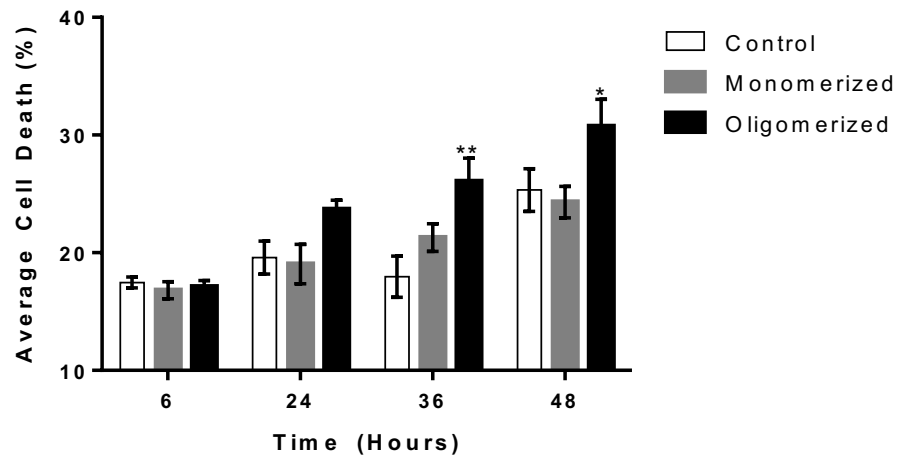


Figure 5. Rat cortical neuron cell death determined under the treatment of A β monomers and oligomers. Results are presented as the mean with S.E.M. (n=4). *: $P \leq 0.05$, **: $P \leq 0.01$ (two-way ANOVA with Tukey's HSD test).

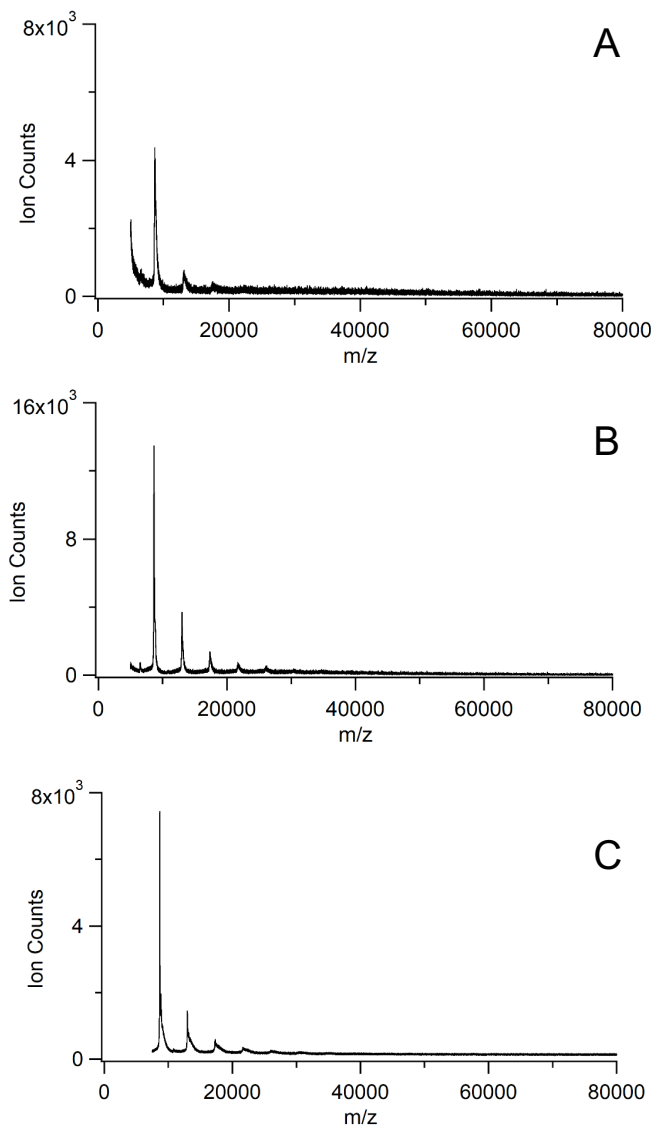


Figure 6. MALDI mass spectra of wildtype A β ₁₋₄₀ after passing through 10 kDa (A) and 30 kDa (B) MWCO filters, and spectra of wildtype A β ₁₋₄₀ diluted from 150 to 50 μ M to match the approximate concentration of the filtered samples (C). Spectra were acquired at the starting m/z to capture the dimeric and larger complexes.

1. Murphy, M. P.; LeVine, H.: Alzheimer's Disease and the Amyloid-beta Peptide. *Journal of Alzheimers Disease* 19 (1), 311-323 (2010)
2. Sakono, M.; Zako, T.: Amyloid oligomers: formation and toxicity of Abeta oligomers. *Febs J* 277 (6), 1348-58 (2010)
3. Hebert, L. E.; Weuve, J.; Scherr, P. A., Evans D. A.: Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology* 80 (19), 1778-83 (2013)
4. Murphy, S. L.; Xu, J.; Kochanek, K. D.: Deaths: final data for 2010. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System* 61 (4), 1-117 (2013)
5. Laurén, J.; Gimbel, D. A.; Nygaard, H. B., Gilbert, J. W.; Strittmatter, S.M.: Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457 (7233), 1128-32 (2009)
6. Wilcox, K. C.; Lacor, P. N.; Pitt, J.; Klein, W. L.: A beta Oligomer-Induced Synapse Degeneration in Alzheimer's Disease. *Cell Mol Neurobiol* 31 (6), 939-948 (2011)
7. Crescenzi, O.; Tomaselli, S.; Guerrini, R.; Salvadori, S.; D'Ursi, A. M.; Temussi, P. A.; Picone, D.: Solution structure of the Alzheimer amyloid beta-peptide (1-42) in an apolar microenvironment - Similarity with a virus fusion domain. *European Journal of Biochemistry* 269 (22), 5642-5648 (2002)
8. Gong, Y.; Chang, L.; Viola, K. L.; Lacor, P. N.; Lambert, M. P.; Finch, C. E.; Krafft, G. A.; Klein, W. L.: Alzheimer's disease-affected brain: Presence of oligomeric Abeta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proceedings of the National Academy of Sciences* 100 (18), 10417-10422 (2002)
9. Harman, D.: Alzheimer's disease: A hypothesis on pathogenesis. *Journal of the American Aging Association* 23 (3), 147-161 (2000)
10. Klein, W. L.; Krafft, G. A.; Finch, C. E.: Targeting small A beta oligomers: the solution to an Alzheimer's disease conundrum? *Trends in Neurosciences* 24 (4), 219-224 (2001)
11. Viola, K. L.; Klein, W. L.: Amyloid beta oligomers in alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathologica* 129, 183-206 (2015)
12. Kotilinek, L. A.; Bacskai, B.; Westerman, M.; Kawarabayashi, T.; Younkin, L.; Hyman, B. T.; Younkin, S.; Ashe, K. H.: Reversible memory loss in a mouse transgenic model of alzheimer's disease. *Journal of Neuroscience* 22 (15), 6331-6335 (2002)
13. Giordano, C. R.; Terlecky, L. J.; Bollig-Fischer, A.; Walton, P. A.; Terlecky, S. R.: Amyloid-beta neuroprotection mediated by a targeted antioxidant. *Scientific Reports* 4, Article number 4983 (2014)

14. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D.J.: Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416 (6880), 535-539 (2002)
15. Ferreira, S. T.; Klein, W. L.: The A beta oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. *Neurobiol Learn Mem* 96 (4), 529-543 (2011)
16. Hung, L. W.; Ciccotosto, G. D.; Giannakis, E.; Tew, D. J.; Perez, K.; Masters, C. L.; Cappai, R.; Wade J. D.; Barnham, K. J.: Amyloid-beta Peptide (A beta) Neurotoxicity Is Modulated by the Rate of Peptide Aggregation: A beta Dimers and Trimers Correlate with Neurotoxicity. *Journal of Neuroscience* 28 (46), 11950-11958 (2008)
17. Dahlgren, K. N.; Manelli, A. M.; Stine, W. B., Jr.; Baker, L. K.; Krafft, G. A.; LaDu, M.J.: Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 277 (35), 32046-53 (2002)
18. Stroud, J. C.; Liu, C.; Teng, P. K.; Eisenberg, D.: Toxic fibrillar oligomers of amyloid-beta have cross-beta structure. *Proc Natl Acad Sci U S A* 109 (20), 7717-22 (2012)
19. Hardy, J.; Selkoe, D. J.: Medicine - The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 297 (5580), 353-356 (2002)
20. Trimpin, S.; Deinzer, M. L.: Solvent-free MALDI-MS for the analysis of beta-amyloid peptides via the mini-ball mill approach: qualitative and quantitative advances. *J Am Soc Mass Spectrom* 18 (8), 1533-43 (2007)
21. Ebenezer, P. J.; Weidner, A. M.; LeVine, H. 3rd; Markesbery, W. R.; Murphy, M. P.; Zhang, L.; Dasuri, K.; Fernandez-Kim, S. O.; Bruce-Keller, A. J.; Gavilán, E.; Keller, J. N.: Neuron Specific Toxicity of Oligomeric Amyloid-beta: Role for JUN-Kinase and Oxidative Stress. *Journal of Alzheimers Disease* 22 (3), 839-848 (2010)
22. Ai, Z. B.; Li, C. Y.; Li, L. T.; He, G.: Resveratrol inhibits beta-amyloid-induced neuronal apoptosis via regulation of p53 acetylation in PC12 cells. *Molecular Medicine Reports* 11 (4), 2429-2434 (2015)
23. Li, J.; Ding, X.; Zhang, R.; Jiang, W.; Sun, X.; Xia, Z.; Wang, X.; Wu, E.; Zhang, Y.; Hu, Y.: Harpagoside ameliorates the amyloid-beta-induced cognitive impairment in rats via up-regulating BDNF expression and MAPK/PI3K pathways. *Neuroscience* 303, 103-114 (2015)
24. Cižas, P.; Jekabsonė, A.; Borutaitė, V.; Morkūnienė, R.: Prevention of Amyloid-Beta Oligomer-Induced Neuronal Death by EGTA, Estradiol, and Endocytosis Inhibitor. *Medicina-Lithuania* 47 (2), 107-112 (2011)

25. Kelley, A. R.; Perry, G.; Castellani, R. J.; Bach, S. B. H.: Laser-Induced In-Source Decay Applied to the Determination of Amyloid-Beta in Alzheimer's Brains. *ACS Chemical Neuroscience* 7 (3), 261-268 (2016)
26. Jan, A.; Hartley, D. M.; Lashuel, H. A.: Preparation and characterization of toxic A beta aggregates for structural and functional studies in Alzheimer's disease research. *Nat Protoc* 5 (6), 1186-1209 (2010)
27. Ryan, T. M.; Caine, J.; Mertens, H. D.; Kirby, N.; Nigro, J.; Breheny, K.; Waddington, L. J.; Streltsov, V. A.; Curtain, C.; Masters, C. L.; Roberts, B. R.: Ammonium hydroxide treatment of A β produces an aggregate free solution suitable for biophysical and cell culture characterization. *PeerJ* 1, e73 (2013)
28. Teplow, D. B.: Preparation of amyloid beta-protein for structural and functional studies. *Amyloid, Prions, and Other Protein Aggregates, Pt C* 413, 20-33 (2006)
29. Broersen, K.; Jonckheere, W.; Rozenski, J.; Vandersteen, A.; Vandersteen, A.; Pauwels, K.; Pastore, A.; Rousseau, F.; Schymkowitz, J.: A standardized and biocompatible preparation of aggregate-free amyloid beta peptide for biophysical and biological studies of Alzheimers disease. *Protein Engineering Design & Selection* 24 (9), 743-750 (2011)
30. Bitan, G.; Fradinger, E. A.; Spring, S. M.; Teplow, D. B.: Neurotoxic protein oligomers - what you see is not always what you get. *Amyloid-Journal of Protein Folding Disorders* 12 (2), 88-95 (2005)
31. Pryor, N. E.; Moss, M. A.; Hestekin, C. N.: Unraveling the Early Events of Amyloid-beta Protein (A beta) Aggregation: Techniques for the Determination of A beta Aggregate Size. *International Journal of Molecular Sciences* 13 (3), 3038-3072 (2012)
32. Bitan, G.; Lomakin, A.; Teplow, D. B.: Amyloid beta protein oligomerization: Prenucleation interactions revealed by photo-induced cross-linking of unmodified proteins. *Journal of Biological Chemistry* 276 (37), 35176-35184 (2001)
33. Amaro, M.; Kubiak-Ossowska, K.; Birch, D. J.; Rolinski, O. J.: Initial stages of beta-amyloid A β (1-40) and A β (1-42) oligomerization observed using fluorescence decay and molecular dynamics analysis of tyrosine. *Methods and Applications in Fluorescence* 1 (1), 1-13 (2013)
34. Lindberg, D. J.; Wranne, M. S.; Gatty, M. G.; Westerlund, F.; Esbjörner, E. K.: Steady-state and time-resolved Thioflavin-T fluorescence can report on morphological differences in amyloid fibrils formed by A beta(1-40) and A beta(1-42). *Biochem Biophys Res Commun* 458 (2), 418-423 (2015)
35. Moore, B. D.; Rangachari, V.; Tay, W. M.; Milkovic, N. M.; Rosenberry, T. L.: Biophysical Analyses of Synthetic Amyloid-beta(1-42) Aggregates before and after

Covalent Cross-Linking. Implications for Deducing the Structure of Endogenous Amyloid-beta Oligomers. *Biochemistry* 48 (49), 11796-11806 (2009)

36. Al-Hilaly, Y. K.; Williams, T. L.; Stewart-Parker, M.; Ford, L.; Skaria, E.; Cole, M.; Bucher, W. G.; Morris, K. L.; Sada, A. A.; Thorpe, J. R.; Serpell, L. C.: A central role for dityrosine crosslinking of Amyloid-beta in Alzheimer's disease. *Acta Neuropathol Commun* 1, 83 (2013)
37. Rahimi, F.; Maiti, P.; Bitan, G.: Photo-induced cross-linking of unmodified proteins (PICUP) applied to amyloidogenic peptides. *J Vis Exp* (23), (2009)
38. Bitan, G.; Teplow, D. B.: Rapid photochemical cross-linking--a new tool for studies of metastable, amyloidogenic protein assemblies. *Acc Chem Res* 37 (6), 357-64 (2004)
39. Bitan, G.: Structural study of metastable amyloidogenic protein oligomers by photo-induced cross-linking of unmodified proteins. *Methods Enzymol* 413, 217-36 (2006)
40. Bleiholder, C.; Dupuis, N. F.; Wytttenbach, T.; Bowers, M. T.: Ion mobility-mass spectrometry reveals a conformational conversion from random assembly to beta-sheet in amyloid fibril formation. *Nature Chemistry* 3 (2), 172-177 (2011)
41. Grasso, G.; Mineo, P.; Rizzarelli, E.; Spoto, G.: MALDI, AP/MALDI and ESI techniques for the MS detection of amyloid beta-peptides. *Int J Mass Spectrom* 282 (1-2), 50-55 (2009)
42. Martineau, E.; de Guzman, J. M.; Rodionova, L.; Kong, X.; Mayer, P. M.: Investigation of the Noncovalent Interactions Between Anti-Amyloid Agents and Amyloid beta Peptides by ESI-MS. *J Am Soc Mass Spectrom* 21 (9), 1506-1514 (2010)
43. Vrana, J. A.; Theis, J. D.; Dasari, S.; Mereuta, O. M.; Dispenzieri, A.; Zeldenrust, S. R.; Gertz, M. A.; Kurtin, P. J.; Grogg, K. L.; Dogan, A.: Clinical diagnosis and typing of systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics. *Haematologica* 99 (7), 1239-1247 (2014)
44. Smith, A. M.; Jahn, T. R.; Ashcroft, A. E., Radford, S. E.: Direct observation of oligomeric species formed in the early stages of amyloid fibril formation using electrospray ionisation mass spectrometry. *J Mol Biol* 364 (1), 9-19 (2006)
45. Ashcroft, A. E.: Mass Spectrometry and the Amyloid Problem-How Far Can We Go in the Gas Phase? *J Am Soc Mass Spectrom* 21 (7), 1087-1096 (2010)
46. Henderson, S. C.; Valentine, S. J.; Counterman, A. E.; Clemmer, D. E.: ESI/ion trap/ion mobility/time-of-flight mass spectrometry for rapid and sensitive analysis of biomolecular mixtures. *Anal Chem* 71 (2), 291-301 (1999)

47. Hoaglund, C. S.; Valentine, S. J.; Sporleder, C. R.; Reilly, J. P.; Clemmer, D. E.: Three-dimensional ion mobility TOFMS analysis of electrosprayed biomolecules. *Anal Chem* 70 (11), 2236-2242 (1998)
48. Ionuț Iurascu, M.; Cozma, C.; Tomczyk, N.; Rontree, J.; Desor, M.; Drescher, M.; Przybylski, M.: Structural characterization of beta-amyloid oligomer-aggregates by ion mobility mass spectrometry and electron spin resonance spectroscopy. *Anal Bioanal Chem* 395 (8), 2509-19 (2009)
49. Bernstein, S. L.; Dupuis, N. F.; Lazo, N. D.; Wytttenbach, T.; Condron, M. M.; Bitan, G.; Teplow, D. B.; Shea, J. E.; Ruotolo, B. T.; Robinson, C. V.; Bowers, M. T.: Amyloid-beta protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nature Chemistry* 1 (4), 326-331 (2009)
50. Bernstein, S. L.; Wytttenbach, T.; Baumketner, A.; Shea, J. E.; Bitan, G.; Teplow, D. B.; Bowers, M. T.: Amyloid beta-protein: Monomer structure and early aggregation states of A beta 42 and its Pro(19) alloform. *J Am Chem Soc* 127 (7), 2075-2084 (2005)
51. Baumketner, A.; Bernstein, S. L.; Wytttenbach, T.; Bitan, G.; Teplow, D. B.; Bowers, M. T.; Shea, J. E.: Amyloid beta-protein monomer structure: A computational and experimental study. *Protein Science* 15 (3), 420-428 (2006)
52. Zheng, X. Y.; Liu, D.; Klärner, F.-G.; Schraer, T.; Bitan, G.; Bowers, M. T.: Amyloid beta-Protein Assembly: The Effect of Molecular Tweezers CLR01 and CLR03. *Journal of Physical Chemistry B* 119 (14), 4831-4841 (2015)
53. Illes-Toth, E.; Smith, D. P.: Conformations and Assembly of Amyloid Oligomers by Electrospray Ionisation - Ion Mobility Spectrometry - Mass Spectrometry. *Current Analytical Chemistry* 9 (2), 165-180 (2013)
54. Anker, J. N.; Hall, W. P.; Lambert, M. P.; Velasco, P. T.; Mrksich, M.; Klein, W. L.; Van Duyne, R. P.: Detection and Identification of Bioanalytes with High Resolution LSPR Spectroscopy and MALDI Mass Spectrometry. *Journal of Physical Chemistry C* 113 (15), 5891-5894 (2009)
55. Song, F. H.: A study of noncovalent protein complexes by matrix-assisted laser desorption/ionization. *J Am Soc Mass Spectrom* 18 (7), 1286-1290 (2007)
56. Madler, S.; Erba, E. B.; Zenobi, R.: MALDI-ToF Mass Spectrometry for Studying Noncovalent Complexes of Biomolecules. *Applications of Maldi-Tof Spectroscopy* 331, 1-36 (2013)
57. Farmer, T. B.; Caprioli, R. M.: Determination of protein-protein interactions by matrix-assisted laser desorption/ionization mass spectrometry. *Journal of Mass Spectrometry* 33 (8), 697-704 (1998)

58. Ji, Y.; Permanne, B.; Sigurdsson, E. M.; Holtzman, D. M.; Wisniewski, T.: Amyloid beta40/42 clearance across the blood-brain barrier following intra-ventricular injections in wild-type, apoE knock-out and human apoE3 or E4 expressing transgenic mice. *Journal of Alzheimer's disease* : JAD 3 (1), 23-30 (2001)
59. Vadukul, D. M.; Gbajumo, O.; Marshall, K. E.; Serpell, L. C.: Amyloidogenicity and toxicity of the reverse and scrambled variants of amyloid-beta 1-42. *FEBS Lett* 591 (5), 822-830 (2017)
60. Solito, R.; Corti, F.; Fossati, S.; Mezhericher, E.; Donnini, S.; Ghiso, J.; Giachetti, A.; Rostagno, A.; Ziche, M.: Dutch and arctic mutant peptides of beta amyloid(1-40) differentially affect the FGF-2 pathway in brain endothelium. *Exp Cell Res* 315 (3), 385-395 (2009)
61. Kumar-Singh, S.; Julliams, A.; Nuydens, R.; Ceuterick, C.; Labeur, C.; Serneels, S.; Vennekens, K.; Van Osta, P.; Geerts, H.; De Strooper, B.; Van Broeckhoven, C.: In vitro studies of Flemish, Dutch, and wild-type beta-amyloid provide evidence for two-staged neurotoxicity. *Neurobiol Dis* 11 (2), 330-340 (2002)
62. Munoz, F. J.; Opazo, C.; Gil-Gómez, G.; Tapia, G.; Fernández, V.; Valverde, M. A.; Inestrosa, N. C.: Vitamin E but not 17 beta-estradiol protects against vascular toxicity induced by beta-amyloid wild type and the Dutch amyloid variant. *Journal of Neuroscience* 22 (8), 3081-3089 (2002)
63. Wang, Z. Z.; Natté, R.; Berliner, J. A.; van Duinen, S. G.; Vinters, H. V.: Toxicity of Dutch (E22Q) and Flemish (A21G) mutant amyloid beta proteins to human cerebral microvessel and aortic smooth muscle cells. *Stroke* 31 (2), 534-538 (2000)
64. Morkuniene, R.; Cizas, P.; Jankeviciute, S.; Petrolis, R.; Arandarcikaite, O.; Krisciukaitis, A.; Borutaite, V.: Small A beta(1-42) Oligomer-Induced Membrane Depolarization of Neuronal and Microglial Cells: Role of N-Methyl-D-Aspartate Receptors. *J Neurosci Res* 93 (3), 475-486 (2015)
65. Zou, K.; Kim, D.; Kakio, A.; Byun, K.; Gong, J. S.; Kim, J.; Kim, M.; Sawamura, N.; Nishimoto, S.; Matsuzaki, K.; Lee, B.; Yanagisawa, K.; Michikawa, M.: Amyloid beta-protein (A beta)1-40 protects neurons from damage induced by A beta 1-42 in culture and in rat brain. *J Neurochem* 87 (3), 609-619 (2003)