# Physico-chemical characterization of beta-amyloid oligomers and study of their function in biological processes at molecular level

**Ph.D. thesis**

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# INTRODUCTION

Alzheimer's disease (AD), described by Alois Alzheimer in 1907, is one of the most common neurodegenerative disorders in the aged population. The main pathologyical hallmarks are senile plaques, composed of extracellularly accumulated amyloid-beta (Aβ) fibrills and intracellularly hyperphosphorylated-tau protein filaments, called neurofibrillar tangles (NFT).
A major advance in the study of AD came with the sequencing of the main peptide constituent of the senile plaque, the Aβ peptide. This observation led to the so-called 'amyloid cascade hypothesis', which states that the deposition of amyloid, made up principally of Aβ 1-42, would be the central event in the initial pathogenesis of AD.
Biochemical studies show that NFTs contain hyperphosphorylated tau proteins. Tau protein is a microtubular associated protein (MAP) that ensures the construction of the microtubular network. During neurodegeneration, tau is abnormally phosphorylated at proline-directed serine/ threonine phosphorylation sites.

## *1) Aggregation of Aβ 1-42*

Aβ peptides consist of 39-42 amino acids and generated after enzymatic cleavage by β- and γ-secretases from a membrane protein, called amyloid precursor protein (APP), encoded on chromosome 21. Initially these peptides possess α-helical/ random coil conformation, which turns readily into β-sheet in aqueous environment. This conformational transition induces aggregation producing Aβ- oligomers, protofibrils and fibrils. Fibrillar Aβ was suspected of being the primary agent that causes the AD-related histopathology and eventually clinical symptoms. However the accumulation of the predominantly fibrillar Aβ containing plaques does not correlate well with the clinical symptoms and plaque accumulation appears later than the observed histopathology. Nowadays it is evident, that extracellular plaques, including fibrillar form of Aβ, are the consequence of several processes and alterations that contribute to the development of the disease and Aβ 1-42 in soluble oligomeric form is considered to be the main toxic species and most likely responsible for neuronal disfunction in AD. The amyloid cascade hypothesis proposed that Aβ aggregation is the central event associated with neurotoxicity. The analysis of the aggregation pathway *in vitro* and biochemical characterization of Aβ deposits isolated from AD brains indicate that Aβ oligomerization occurs via distinct intermediates, including oligomers of 3 to 50 Aβ monomers, annular oligomers, protofibrils, fibrils and plaques. Nevertheless, knowledge about the detailed

mechanism underlying Aβ aggregation and β-amyloid fibril formation is still limited since the structure of soluble oligomers and different aggregated forms is very challenging to study, because they are generally difficult to obtain in large quantities for high resolution structural techniques, and they are temporally unstable, rapidly aggregating into more mature fibrillar forms, caused by the presence of high number of hydrophobic amino acids in the sequence. To overcome the problem of solubility and high aggregation propensity of Aβ, we used a precursor of human Aβ synthetized in our laboratory instead of the convenient synthetic form. The precursor peptide („iso-Aβ") has a low tendency for aggregation. It contains an ester bond in its backbone that decreases the propensity of the peptide to aggregate, thus it has an enhanced water solubility.

The most important factors in the toxicity of Aβ oligomers are the aggregation grade and the peptide conformation. Thus one of our aims was to characterize the assemblies of Aβ with a series of biochemical methods. These methods are: 1, fast protein liquid chromatography- size exclusion chromatography (FPLC-SEC), 2, sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE); 3, western blot (WB); 4, dinamic light scattering (DLS), 5, and transmission electron microscopy (TEM).

*2) Factors, that influence the aggregation of Aβ 1-42: metals in Alzheimer's disease*

There are several scientists who think, that self-aggregating properties of Aβ are insufficient to explain the accumulation of the peptide in different brain regions and assume the presence of other neurochemical factors that have to play an important role in the aggregation process. One of these factors might be the pathological interaction between Aβ and cerebral metal ions e.g. $Zn^{2+}$ and $Cu^{2+}$. There is an extensive literature demonstrating their potential involvement and interaction with biochemical substrates in cognitive impairment and other processes that may contribute to neurodegeneration, including AD. $Zn^{2+}$ has a well documented interaction with Aβ pepide and its modulation with age may initiate AD related pathology. In AD due to the abundantly expressed $Zn^{2+}$ transporter proteins (ZnTs) that induce an abnormal uptake and distribution of $Zn^{2+}$, high local $Zn^{2+}$ release is observed from the presynaptic vesicles to the postsynaptic membrane in depolarization-dependent manner in several parts of the brain, including neocortex, hippocampus and amygdala. It is well known that high affinity bindig is observed between $Zn^{2+}$ and Aβ. The $Zn^{2+}$-Aβ binding prevents the formation of the regular amyloid fibrils, it induces the accumulation of large unorganized aggregates of smaller non-fibrillar forms of Aβ. Since the Aβ- $Zn^{2+}$ complex is proven to be toxic metal chelators may act as potential blockers of Aβ fibrillization, Aβ-dependent oxidative stress induced

neurotoxicity, in this way act as drugs or therapeutic ways in AD. There is a potent $Zn^{2+}$ chelator molecule, *N*1,*N*2-bis(pyridine-2-yl-methyl)ethane-1,2-diamine (ENDIP), the effect of which was extensively studied in *in vitro* experiments in our laboratory.

*3) Interactions of Aβ 1-42 oligomers*

Protein–protein interactions provide the molecular basis for the structural and functional organization within cells. How intra- and/or extracellular Aβ oligomers form and take effect on cells remains unclear. Multiple Aβ oligomer conformations are produced via different pathways, indicating that the mechanisms of formation may also differ for extracellular and intracellular oligomers. The liberation of Aβ could potentially occur wherever APP and β- and γ- secretases are localized for example in several cellular compartments. If this cleavage takes place within the confines of the cell, Aβ will be intracellular. If liberation occurs at the plasma membrane or in the secretory pathway, it will be released extracellularly.

Perhaps there is no single molecular pathological mechanism behind AD and Aβ modulates cellular functions by binding to a large group of functionally important proteins in the cellular protein network and also to a variety of biomolecules. Mapping the Aβ interaction partners can help to discover novel pathways in disease pathogenesis. The identification of new binding partners is important, because these interactors might function as a physiological ligand and hence could be the molecular target of novel therapeutic strategies. Biological systems are very complicate, hence they are consist of molecular networks. To understand the basic cellular events which mediate complex cellular processes, like the interactions between proteins/ protein networks and Aβ oligomers, microarray- based analysis was applied. It is an effective tool, as it is a rapid and economic way to interpret molecular processes, which occur in AD. These protein mycroarrays, also known as protein chips, are miniaturized, parallel assay systems that contain small amounts of purified proteins in a high-density format. The arrays are prepared by immobilizing purified proteins onto a microscope slide in an active state at high densities in such a fashion that the proteins remain in a moisturized environment. After proteins are immobilized on the slides, they can be probed for a variety of functions. The reaction signals are usually measured and recorded by fluorescent or radoisotope labeling.

*AIMS OF THE STUDY*

It is very important to know what kind of Aβ aggregates we have during our biological experiments, therefore we characterized our Aβ solutions versatilely. There are several unanswered questions on the pathological role of Aβ oligomers in the central nervous system, e.g. which aggregation form of the peptide has deleterious effect on neurons, which factors influence the aggregation, which receptors, membrane proteins or intracellular systems are involved in these processes, etc. There are certain unclearified issues regarding in this subject, since AD is a very complex disease. The main concept of my work is to study these questions.

1. The physico-chemical and biological characterization of Aβ 1-42 oligomers.

It is very important to obtain well characterized, relatively stable oligomer solutions for reproducible results during biological experiments. In order to achieve this, synthetic Aβ 1-42 was prepared in our lab from its precursor peptide, called „isopeptide". By using the precursor and elaborating a standardized sample preparation protocol, we are able to prepare well characterized peptide solution in a reproducible manner, which could be therefore succesfully used for several chemical and biological experiments. For the characterization, we used numerous physico-chemical methods, such as FPLC-SEC, SDS-PAGE, WB, DLS and TEM, to follow the aggregational processes during different incubation times.

2. Studies on the influence of $Zn^{2+}$ and a metal ion chelator molecule, ENDIP on the aggregation and the hydrodinamic diameter of the aggregates, pretreated with $Zn^{2+}$.

3. In order to understand the pathomechanism of AD in molecular level, studies of the interactions between Aβ and proteins/protein networks needed. Our aim was to study Aβ 1-42 oligomer-protein interactions *in vitro* by protein array technology (as a novel postgenomic method), in a high throughput manner. These arrays contain 8163 human proteins covering a significant portion of the human proteome. Thus the results may show the most important interactions between human proteins with Aβ oligomers.

MATERIALS AND METHODS

*1) Synthesis of the Aβ peptides*

Peptide synthesis was carried out in our Alzheimers's research group.

*2) Preparation of iso-Aβ peptide-derived amyloid oligomers*

For study the aggregation of Aβ oligomers, iso Aβ 1-42 was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and incubated for 24 h at room temperature. HFIP was removed in vacuo, followed by dissolution of the oligomeric peptide in Milli- Q ultrapure water (pH 5.0) to 500 μM and sonicated for 5 min. For the different experiments the peptide stock solutions were diluted in the required solutions.

*3) Transmission Electron Microscopy*

For TEM experiments ten microliter droplets of iso-Aβ oligomer or purified ribosome solutions were placed on 400 mesh Formvar-carboncoated copper grids. Specimens were studied with a Philips CM 10 transmission electron microscope (FEI Company, Hillsboro, OR) operating at 100 kV. Images were taken by a Megaview II Soft Imaging System and analyzed by an AnalySis 3.2 software package (Soft Imaging System, Munster, Germany).

*4) Dynamic Light Scattering*

This technique uses light to hit small particles thus light scatters in all directions. These molecules undergo Brownian motion, while scattered light undergoes either constructive or destructive interference by the surrounding particles and within this intensity fluctuation, information is contained about the time scale of movement of the scatters. Assuming the scattering particles to be hard spheres, their apparent hydrodynamic radius can be calculated from the diffusion parameters by using the Stokes-Einstein equation.

*5) FPLC-SEC experiments*

For the experiment, iso Aβ was dissolved as discussed above and aggregated for different time intervals: 0 min, 3 hours, overnight and 3 days at 37 $^{0}$C. These samples were loaded onto a Superose 6 10 300 FPLC column and the aggregates were separated at 0.5 ml/min flow in HCBS as elution buffer at 25 °C. Compared with protein calibration standards, exact size of the aggregates could be calculated.

*6) SDS-PAGE, Western Blot*

For the Aβ western blot, 50 uM samples were loaded onto 15% SDS-polyacrylamide gel (2,5 ug/ lane). The gel was then transferred to a nitrocellulose membrane using an electroblotting apparatus.

For preparation and western-blot analysis of purified ribosomes: Protein concentrations of purified ribosomes were determined by the BCA protein assay kit. To assess the purity of the purified ribosomes, a Western-blot was performed with the ribosomal protein specific anti-RPL36A antibody.

*7) Labeling of the monoclonal antibody with Alexa fluor-647 succinimidyl ester*

Alexa Fluor 647 succinimidyl ester was used for labeling monoclonal anti-Aβ antibody, BAM10. A 5-fold excess of the fluorescein dye was added to the gently stirred solution of the protein in an ice-bath. To neutralize the excess of the succinimidyl ester, 0.1 M of aqueous hydroxylamine solution was applied. The labeled antibody was separated from the free dye in the Microcon tube. and stored in the original buffer under the same conditions as that used for the unlabeled antibody.

*8) Probing the ProtoArray human protein microarray*

The ProtoArray Human Protein microarray 4.0 was blocked and iso-Aβ to a final concentration of 10 μM was added on the top of the array After incubation, the array was probed with Alexa-647 labeled anti-Aβ mAb. Scanning was carried out using a GenePix Personal 4100A microarray scanner.

*9) ProtoArray data processing*

The signal intensity of each protein spot was calculated by subtracting the median background value from the median spot value. Protein signal intensities were normalized to concentration so that the signal intensities normalized to median were divided by the protein concentration values of the spots.

*10) Aurora A kinase activity assay*

To study the effect of Aβ oligomers on the activity of Aurora A kinase we used an HTScan® Aurora A Kinase Assay Kit. 0 min and 24 h Aβ aggregates or staurosporine were incubated with Aurora A kinase and ATP for 5 min, and biotinilated substrate for 30 min was added. After incubation with the primary antibody phospho-PLK for 2h at RT, HRP-labelled

secondary antibody was incubated for 30 min with the samples. Tetra-methylbenzidine (TMB) solution and stop solution was added and absorbance was read at 450 nm with FLUOstar OPTIMA Multidetection Microplate Reader

*11) Ribosome purification*

The frozen tissue was homogenized and after centrifugation at 8000g for 10 min the supernatant layered over a sucrose gradient of 2M and 1M sucrose. The sample was centrifuged and the pellet was resuspended in storage buffer

*12) Enzyme-Linked Immunosorbent Assay (ELISA): measurement of oligomeric Aβ ribosome binding*

0.1 μg of purified ribosome was coated in each well of a 96 well plate and Iso-Aβ oligomer was added. After 1 h of incubation a monoclonal Aβ antibody was added and after the wells were incubated with HRP-conjugated anti-mouse secondary antibody the absorbance at 370 nm was constantly monitored using a FLUOstar OPTIMA Multidetection Microplate Reader

*13) In Vitro translation assay*

To permit ribosomal binding, iso-Aβ oligomer was preincubated with the Rabbit Reticulocyte Lysate System. After the preincubation step Luciferase control RNA template was added to the reactions. The luminescence produced by the translated luciferase was measured on a LumiStar Optima luminometer. Expressed luciferase control protein was also investigated by Western-blot.

RESULTS

*1) Characterization of Aβ 1-42 oligomers*

Aβ aggregates, incubated for different time intervals, fractionated on a Superose 6 10 300 column. In the freshly dissolved sample (0 min) there is an intensive peak at the smaller molecular masses between ~ 2300 and ~ 12000 Da. This means, that the great majority of the aggregates consist of approximately monomers, dimers and trimers. In case of the sample incubated for 3 hours, there is a significant difference, namely this time seemed to be enough for the developement of larger aggregates. There is a broad distribution of aggregates between ~ 83000 and ~ 480000 Da. Compared to the third sample, which was incubated overnight, the amount of low- n aggregates decreased for the benefit of the high n- aggregates. In case of the

sample incubated for 3 days, the tendency is the same as in case of the overnight sample, but the intensities are lower, because in this case we applied the half of the protein amount of the other three samples.

For the Western Blot experiments, we loaded the same Aβ samples as for the FPLC-SEC measurements. We used two types of antibodies, a sequence specific monoclonal antibody: Bam-10, and a conformation (fibril) specific polyclonal antibody: OC. On the membrane incubated with Bam-10, the freshly dissolved samples are mostly low- n oligomers. It can be seen that, with progress of time high-n aggregates appear and possess a strong signal compared to that of the low-n oligomers. On the second membrane, incubated with OC antibody, a broad range of aggregates are present, but they are not distinct as much as on the first membrane.

*2) Analysis of the effect of ENDIP on the aggregation and the size of the aggregates of Aβ after adding $Zn^{2+}$*

In order to determine the changes in the size of the aggregates, we used DLS measurement, a well established technique for measuring the size of small particles in solution, typically in the submicron region. The population of the iso Aβ-derived oligomers is proven to be outstandingly stable without the disturbance of extrinsic effects, such as administration of chelating ions, change of ionic strength of the medium, or application of preformed fibrils as seeds. The change in the Aβ size ($d$H) over time was monitored applying DLS measurements in 50 μM iso Aβ 1–42 solutions with and without addition of metal ion, $Zn^{2+}$ and chelator, ENDIP. The population of the iso Aβ-derived oligomers is proven to be outstandingly stable without the disturbance of extrinsic effects. When metal ion was added, a significant increase in $d_H$ could be observed, proving that $Zn^{2+}$ could promote Aβ precipitation. ENDIP applied in five fold excess after the fourth measurement, it lowered $d_H$ back to its initial value.

*3) Mapping of the binding partners of Aβ oligomers: Protein array experiments*

- *Oligomerization of Aβ*

The stability of the Aβ 1-42 oligomers was examined by dynamic light scattering. Therefore, we conducted TEM investigations as well. In parallel, the actual shape of the oligomers has to be characterized. The stability of Aβ 1-42 oligomers was monitored at $4\ ^0$C for the time interval typically applied in the microarray experiments. The oligomers possessed an average hydrodynamical diameter of 55.1 ± 3.9 nm and were proven to retain their size distribution during the time examined. . At the end of the incubation period, oligomers were visualized by

TEM. The shape of the oligomers could be considered to be spherical, as no sign of anisotropic forms could be observed on the TEM images.

- *Protein array based oligomeric Aβ interactome screen*

We applied two parallel Protoarray 4.0 protein arrays for the Aβ interactome analysis. These arrays contain duplicate spots for 8163 unique recombinantly expressed human proteins. Analysis of the raw images and signal intensities revealed that Aβ could bind to numerous proteins on the array, such as proteins that take part in regulation of transcription, translation, gene expression, metabolic processes, in nucleosome organisation, in processes of mitosis, etc. In a consequent study one of these potential interactors of Aβ, was chosen for further analysis.

We used another analysis method: to gain functional information about the Aβ interactors, we performed a Gene Ontology (GO) analysis, using the DAVID Web-based knowledgebase. The DAVID analysis revealed that the most highly impacted GO Biological Function category was the protein translation.

- *Results of the first analysis*

By using the first analysis method, among others we identified several binding partners, such as Aurora A kinase (AURKA), fibroblast growth factor (FGF), γ- tubulin, beta- secretase 1 (BACE1), mitogen- activated protein kinase 7 (MAPK7) and TAO kinase 3 (TAOK3) with relatively high binding intensities. All these proteins can be related to AD. We assumed that AURKA can be a target of our further experiments, because it is a serine/ threonine-protein kinase and it has regulatory role in tau phosphorylation through the activation of Gsk-3β protein. Therefore we decided that we try to confirm the binding to Aβ by other in vitro techniques. We utilized HTScan® Aurora A Kinase Assay Kit. In this assay we aimed to study if Aβ, in different incubation time (0 min, 24h) and in different concentrations, is able to inhibit the function of AURKA. The results show that, our Aβ preparations were unable to inhibit the phosphorylation of the substrate of the enzyme.

- *Result of the second analysis*

The “translation” functional category contained 24 proteins, including several mitochondrial and nonmitochondrial ribosome components along with other proteins required for translation, such as translation initiation factors.

- *Aβ binding to purified ribosomal complexes*

To support the protein array binding data, we performed an ELISA binding assay on purified rat hippocampal ribosomes. Aβ bound to immobilized ribosomes in a dose-dependent manner. Aβ binding to the hippocampus homogenates supported the protein array data showing that Aβ probably had multiple targets in the homogenate. On the other hand, binding of Aβ to purified ribosomes was stronger, indicating that the binding strength to ribosomal proteins was significantly higher than the binding to the "background" protein population in the hippocampus homogenate.

- *Aβ-mediated inhibition of in vitro protein translation*

To test the effect of Aβ on the efficiency of translation, an *in vitro* translation reaction was performed. The results clearly showed a concentration dependent inhibitory effect on the luminescent activity of the luciferase enzyme. To exclude the possibility that the reduction of the luminescent signal intensity was due to the Aβ-mediated inhibition of luciferase enzyme function and not to the decreased luciferase translation, a Western-blot experiment was performed. The Western- blot data clearly showed that the observed reduction of luciferase function was the result of the reduction of luciferase protein expression.

## DISCUSSION

### *1) Physico-chemical characterization of beta-amyloid 1-42 oligomers, study of the aggregation processes*

TEM analysis identified 5-10 nm spherical particles, that appear at early times of incubation and disappear or their amount decreases as time goes by as shown in our WB and FPLC-SEC measurements. For our experiments we used isopeptide (iso Aβ 1-42), in which the incorporated ester bond decreases the propensity of the peptide to aggregate, thus it has an enhanced water solubility. To monitor the changes in the aggregation process of Aβ 1-42, we standardized our experiments according to the incubation time and the buffers. FPLC-SEC and WB studies (using sequence specific monoclonal antibody) show that Aβ 1-42 solutions that we used, contained a wide range of species, from low-molecular-weight to high-molecular-weight assemblies, the size of which increases with time (0 min, 3h, overnight, 3 days). WB analysis with OC antibody confirms that our preparations contained oligomers from low-n to high-n aggregates. These oligomers are present shortly after sample preparation and also after 3 days incubation.

The results have great importance, because amiloidogenic proteins and peptides can adopt a number of distinct conformations and a key issue is which of them are closely associated with the pathogenesis.

*2) Aggregation studies of Aβ 1-42 after adding $Zn^{2+}$ and ENDIP*

In neurodegenerative diseases, especially in AD there are several studies, which emphasize the role of metal ions and become an important research subject. These ions can interact with several proteins (α-synuclein, prion protein), contributing to changes in their function, conformation or in their aggregation state. In this work I only detail the role of $Zn^{2+}$ because it is well known that this ion influences Aβ aggregation. One therapeutic approach is to develop new chelating agents to inhibit or turn back the effect of the metal ions on the aggregation of Aβ, because Aβ- $Zn^{2+}$-complexes are toxic. We studied the effect of TPEN derivative ENDIP on the aggregation and the size of the assemblies of Aβ after adding $Zn^{2+}$ using DLS technology. In the sample containing only 50 uM Aβ 1-42, the size of the aggregates did not change during the time of the experiment (~ 2h), which means that no considerable aggregation occured. When we added equal amount of $Zn^{2+}$ to the Aβ solution after the first measurement, we observed a rapid increase of the hidrodynamic diameter ($d_H$) of these particles, proving that $Zn^{2+}$ could promote Aβ precipitation. The addition of ENDIP, in five fold excess, lowered $d_H$ back to its initial value. From these data we can conclude that ENDIP is able to prevent the metal-induced aggregation and to resolubilise Aβ.

*3) Interactome sreening of Aβ 1-42 oligomers*

To explore the Aβ cellular interactome, a protein array method was applied. Our protein array analysis revealed that the Aβ-related protein interaction is not specific; the peptide could interact with at least 324 cellular proteins. In the first analysis we categorized the binding partners according to the signal intensities. In this way we found AURKA protein, a serine/ threonine-protein kinase that has a potential role in Alzheimer's disease possessed a high binding intensity. We assumed that our Aβ oligomers would be able to affect the function of the AURKA protein, hereby influence tau phosphorylation indirectly. To determine the effect of our oligomers on the enzyme using an Aurora kinase assay kit, wherein we applied Aβ in different concentrations and with differnt incubation times (0 min, 24h.). Based on our results, we can conclude that, no effect of our Aβ preparations on the enzyme activity could be observed compared to staurosporine, a protein kinase inhibitor. The explanation for the controversy between the observed binding and the lack of the enzyme modulating effect can

be that Aβ may interact with AURKA, but the binding side may not influence the activity of the enzyme.

We also tried another analysis method, in which we grouped bound proteins in functional groups. The GO-based functional analysis showed that the most highly impacted cellular process was the translation. Aβ binding to several translation-related proteins, a simple interaction detection by protein array does not necessarily mean that the proteins bind *in vivo* or the binding has functional consequence. There could be several reasons for that. First the Aβ and its interactors might not be expressed at the same time or in the same cellular compartment. Second the Aβ binding site of the interactors can face to another molecule in a protein or other complex. Therefore, additional binding and functional studies are needed to validate the protein array results. Therefore, we decided to investigate the binding of Aβ to native ribosomes purified from rat hippocampus. The experiments proved that Aβ is able to bind to the correctly folded ribosomal complexes. Although we clearly showed that Aβ can bind to ribosomal proteins and also to native ribosomal complexes, this binding can occur on ribosomal regions that are not important in translation. Therefore, we functionally tested the Aβ inhibitory effect in an *in vitro* translation system. We also have to underline that although we confirmed the binding of Aβ to several proteins involved in translation *in vitro*, and we prove that it was able to decrease the de novo protein expression in an *in vitro* translation system, these findings do not mechanistically mean that this inhibition occurs *in vivo* and contributes to the pathogenesis of AD. It is possible that *in vivo* the increased oxidation of rRNA and mRNA and the direct binding of Aβ to various translation-related proteins are parallel mechanisms that might result a decreased protein synthesis in the diseased tissues.

PUBLICATIONS RELATED TO THE THESIS

**I.**

**Two pyridine derivatives as potential Cu(II) and Zn(II) chelators in therapy for Alzheimer's disease.**
Lakatos A, Zsigó E, Hollender D, Nagy NV, Fülöp L, Simon D, Bozsó Z, Kiss T.
Dalton Trans. 2010. Feb. 7; 39 (5):1302-15. **IF: 3.647**

**II.**

**Controlled in situ preparation of A beta(1-42) oligomers from the isopeptide "iso-A beta(1-42)", physicochemical and biological characterization.**
Bozso Z, Penke B, Simon D, Laczkó I, Juhász G, Szegedi V, Kasza A, Soós K, Hetényi A, Wéber E, Tóháti H, Csete M, Zarándi M, Fülöp L.
Peptides. 2010. Feb; 31 (2):248-56. **IF: 2.654**

**III.**

**Protein array based interactome analysis of amyloid-β indicates an inhibition of protein translation.**
Virok DP[#], Simon D[#], Bozsó Z, Rajkó R, Datki Z, Bálint É, Szegedi V, Janáky T, Penke B, Fülöp L.
J. Proteome Res. 2011. Apr. 1;10 (4):1538-47. **IF: 5.46**

**IV.**

**Interactions of pathological hallmark proteins: Tubulin polymerization promoting protein/p25, {beta}-amyloid and {alpha}-synuclein.**
Olah J, Vincze O, Virok D, Simon D, Bozso Z, Tokesi N, Horvath I, Hlavanda E, Kovacs J, Magyar A, Szucs M, Orosz F, Penke B, Ovadi J.
J. Biol. Chem. 2011. Aug. 8. **IF: 5.328**

INTERNATIONAL CONFERENCES:

*Posters:*

1. AD/PD 2009 – Prague: **Protective effect of putative drug candidates on the amyloid-beta oligomer-induced astrocyte activation in rat entorhinal cortex**

2. "Chemistry towards Biology" 2010 – Primosten: **Protein chip based interactome analysis of Aβ indicates an inhibition of the cellular translation machinery**

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