Chapter

Glycodendrimers as Potential Multitalented Therapeutics in Alzheimer's Disease

Oxana Klementieva

Abstract

Finding successful therapies for the treatment of Alzheimer's disease (AD) is one of the most challenging tasks existing for human health. Several drugs have been found and validated in preclinical studies with some success, but not with the desired breakthroughs in the following clinical development phases. AD causes multiple brain dysfunctions that can be described as a brain organ failure, resulting in significant cognitive decline. Aggregation of amyloid proteins and neuronal loss are the hallmarks of AD. Thus, one of the strategies to treat AD is to find a multifunctional drug that may combine both anti-aggregation and neuroprotective properties. Such a candidate could be chemically modified dendrimers. Dendrimers are branched, nonlinear molecules with multiple reactive groups located on their surface. Chemical modification of reactive surface groups defines the property of the dendrimers. In this chapter, I will discuss poly(propylene imine) dendrimers with the surface functionalized with histidine and maltose as an example of a multifunctional therapeutic drug candidate able to protect the memory of AD transgenic model mice.

Keywords: dendrimer, histidine, aggregation, synapse, memory, Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is a complex neurological disease, which already in its earliest clinical phase is characterized by remarkable memory impairment. Multiple pieces of evidence suggest that in AD, memory impairment begins with dysfunction of synapses, a unique characteristic of nerve cells. Early neurochemical analyses of AD brain tissue revealed that the deficits in numerous neurotransmitters (including corticotropin-releasing factor, somatostatin, GABA, and serotonin) and the early symptoms correlate with dysfunction of cholinergic and glutamatergic synapses [1]. In addition to the deficits of the transmitters, many other biochemical and morphological indicators suggest that in early AD, synapses are under attack as reviewed in [2]. It has been shown that in biopsied AD cortex, there is a significant decrease in the numerical density of synapses in the brain and the number of synapses per cortical neuron [3]. The amyloid cascade hypothesis, one of the widely accepted theories, suggests that progressive accumulation and aggregation of amyloid-β proteins (Aβ) could be the main cause of AD, which triggers AD neuropathology. Aβ proteins are the proteolytic products of amyloid precursor protein (APP), a

type-I transmembrane protein which is highly expressed in neurons, known to regulate synaptic function and neurite outgrowth [4]. There are two main alternative enzymatic pathways to process APP [5]:

1. Non-amyloidogenic pathway, where APP is subjected to consecutive cleavage by α-and γ-secretases that cut APP within the Aβ fragment

2. Amyloidogenic APP pathway, where APP is subjected to cleavage by β-and γ-secretases generating Aβ, a mix of short peptides ranging from 38 to 43 amino acids in length able to form polymorphous aggregates, so-called oligomers, and fibrils [6]

APP processing is regulated by neuronal activity, and neuronal activity may favor β-secretase-mediated amyloidogenic cleavage of APP during which Aβ proteins are generated [7]. It was accepted that after APP cleavage, Aβ peptides are first secreted, and then, extracellularly, soluble Aβ peptides aggregate into amyloid plaques. This extracellular Aβ, which is the main constituent of amyloid plaques, is thought to be toxic to the neurons. More recently, the intraneuronal Aβ has been demonstrated and reported to be involved in neuronal damage [8, 9]. It has been demonstrated that Aβ attacks synapses, small membranous protrusions that permit one neuron to pass a signal (electrical or chemical) to another neuron.

It has been shown that synaptic activity may affect Aβ secretion [5], and it has been hypothesized that synaptic activity may stimulate the generation of Aβ although why this occurs and whether Aβ might have a normal function in neuronal synapse have not been understood well [10]. Strikingly, it has been shown that Aβ selectively binds to synapses when added to cultured neurons [11]. Further, the level of Aβ is shown to be increased in synaptosomes in early AD [12]. Immunoelectron microscopy and high-resolution immunofluorescence microscopy studies show that this early subcellular Aβ accumulation leads to progressive damage of neurites and synapses [13]. Thus, synapses could be sites of early accumulation of pathogenic Aβ. It is believed that soluble Aβ oligomers rather than monomeric or fibrillar Aβ are the main neurotoxic species. However, a structure of neurotoxic Aβ oligomers and the nature of their effects on synapses are not identified [14].

Despite advances, the efforts to target neurotoxic Aβ oligomers in the brain are confounded by high polymorphism of amyloid structures [15]. Oligomer specific antibodies may interact mainly with a specific type of Aβ conformers against which these antibodies were produced [16]. Therefore, to target polymorphic Aβ oligomers, a cocktail from several antibodies might be required. Another way to modulate Aβ aggregation could be via establishing H-bond interactions [17] to favor the formation of less toxic Aβ species [18].

To fight a brain disease such as AD pathology, both synapse protection and anti-amyloid modulation would be desired properties of a possible therapeutic drug. However, to protect synapses and to modulate Aβ aggregation, amyloid aggregation modulator and neuroprotective therapeutics have to be delivered to the synapse. One way to deliver both therapeutic molecules is to use a compound which may carry both molecules simultaneously. Such multifunctional compound could be a dendrimer.

Dendrimers are three-dimensionally branched, globular macromolecules built by a series of iterative steps from a small core molecule which defines the type of the dendrimer [19]. They were first synthesized and described in 1978 [20], and since then dendrimers are in focus, due to their outstanding complexation properties. The most important features of dendrimers are controlled molecular structure, nanoscopic size, and high tunable availability of multiple functional groups at the dendrimer surface. Dendrimers are composed of three elements: a core branched

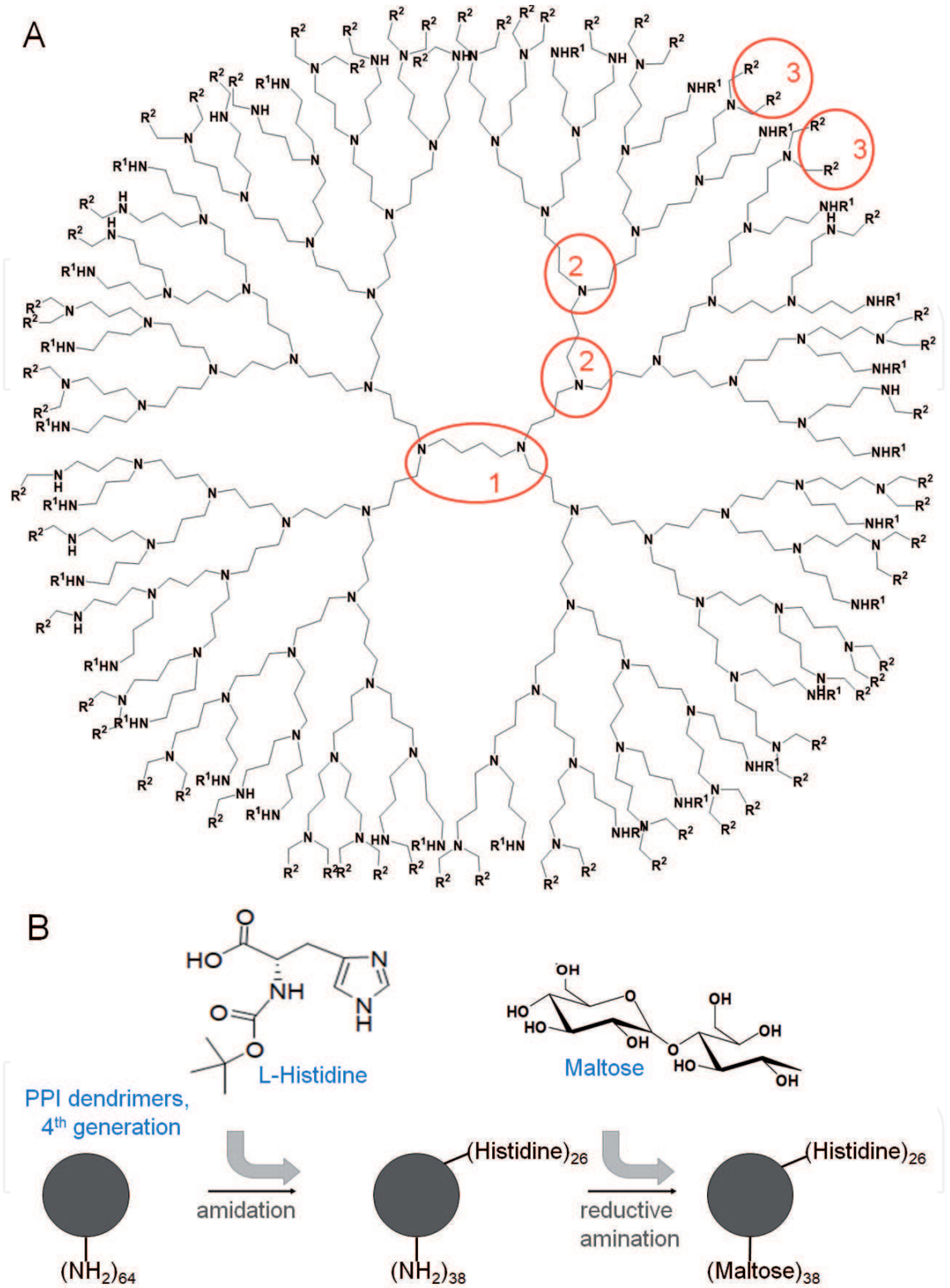

Figure 1.
Structure and chemical modification of dendrimers. (A) Molecular structure of poly(propylene imine) dendrimers of the fourth generation. Circle 1 shows the core; circle 2 indicates branching points of the dendrimers; circle 3 shows the terminal groups, R^1 and R^2. Fifth-generation PPI dendrimer (Eindhoven, the Netherlands) was renamed as fourth-generation (G4) PPI dendrimers following the uniform nomenclature [59]. (B) Example of surface modification of the PPI dendrimer. A reaction pathway shows the synthesis of G4 histidine-maltose PPI dendrimers first with histidine (R^1) and then with maltose (R^2). Conjugation with histidine and maltose neutralizes the positive charge of the primary amino groups [27].

dendron and terminal groups which could be used for dendrimer functionalization. The number of surface functional groups of the dendrimer depends on the degree of dendrimer branching (**Figure 1**). For example, PPI or PAMAM dendrimers of the

second generation have 16 functional groups on their surface, the third generation has 32, and the fourth dendrimer generation has 64 functional groups. Strikingly, the number of terminal groups increases exponentially, while the size increases linearly. The terminal groups on the dendrimer surface can be used for surface modification and dendrimer functionalization. Such modifications could change dendrimers' surface charge and, for example, reduce toxicity associated with a cationic surface charge as reviewed by Appelhans et al. [21]. Dendrimers are most commonly synthesized using divergent or convergent different synthetic pathways [22]. Importantly, the high tunability of dendrimers' surface allows endless possibilities for dendrimers' biomedical applications, for example, for pharmaceutical applications, the terminal groups can be functionalized with different active conjugates such as specifically targeting antibodies, drugs, metal ions or imaging agents, and more [23]. Moreover, several research groups demonstrated that some types of dendrimers are able to cross the BBB [24–27], showing their applicability for the research and possibly treatment of brain diseases.

In the present chapter, I summarize the experimental evidence showing that functionalized poly(propylene imine) dendrimers may provide multitargeting properties for dendrimers increasing their potential for the treatment of AD.

2. Amyloid aggregation and dendrimers

According to the amyloid cascade hypothesis, Aβ peptides are important players triggering the AD development. Multiple in vitro studies have demonstrated that the Aβ peptides can form fibrils and other aggregates called oligomers. The formation of insoluble Aβ fibril follows a nucleation-dependent polymerization mechanism (**Figure 2**) as described [28]. The formation of soluble Aβ oligomers in vivo is largely unknown; it is believed that soluble Aβ oligomers may precede fibril formation [29] and are more toxic than mature Aβ fibrils [30].

In the search for drugs that would inhibit neuronal death in Alzheimer's disease, one of the ways one can use is to find compounds that interfere with Aβ, cleaning the brain tissues from neurotoxic Aβ oligomers. It has been demonstrated that PPI dendrimers modified with maltose are capable of interfering with the amyloid formation in vitro [18, 26, 31, 32]. Amyloid fibril formation is usually monitored

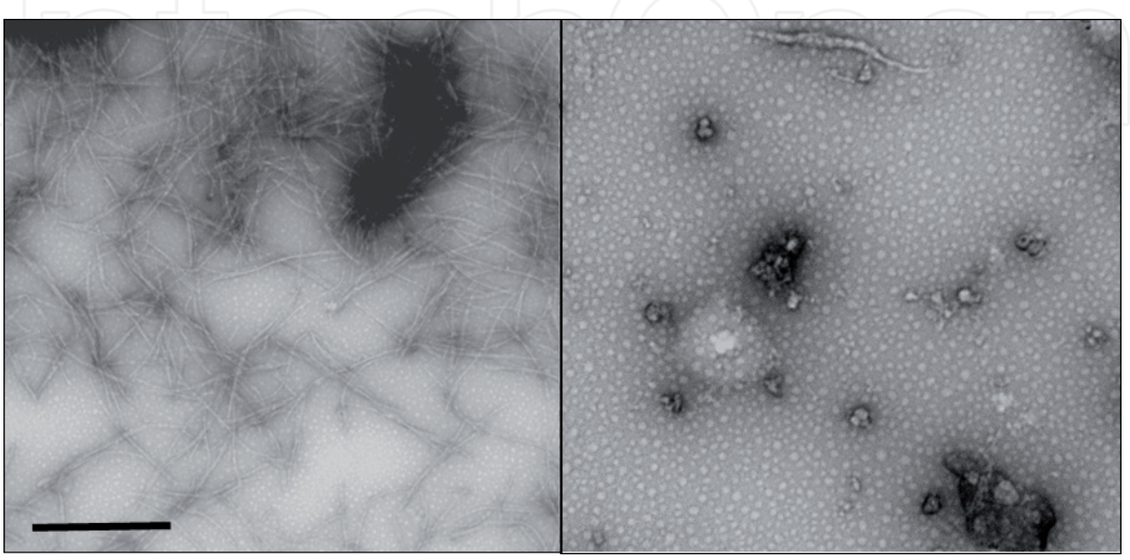

Figure 2.
Example of amyloid fibrils and amyloid oligomers. (A) Electron micrographs of the Aβ(1–40) fibrils (B) Aβ(1–40) oligomers prepared as described [9]. Scale bar is 200 nm.

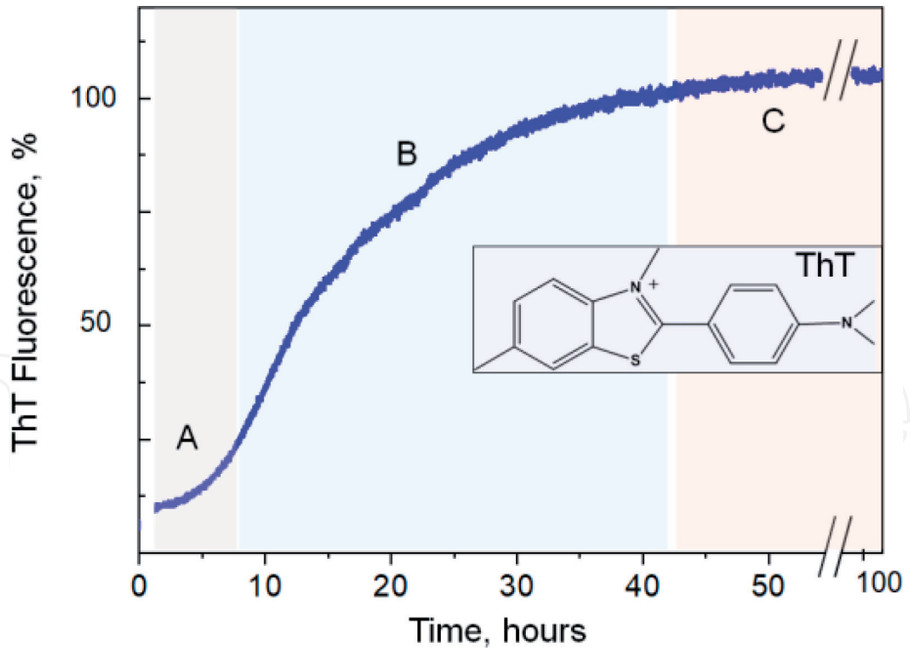

Figure 3.
Characteristic aggregation curve for amyloid fibril formation. Sigmoid-shaped curve 5 μM recombinant Aβ(1–42) kinetics as detected by ThT fluorescence over time and displayed as % of total ThT binding. Area (A) corresponds to the lag phase (nucleation), area (B) corresponds to the growing phase, and area (C) corresponds to final ThT fluorescence plateau. Inset: molecular structure of ThT.

using the fluorescence dye thioflavin T (ThT). The dye becomes fluorescent when interacting with the ordered β-sheet structures characteristic for amyloid fibrils. With the fibril growth, the ThT fluorescence increases until its value reaches a plateau. **Figure 3** demonstrates the sigmoid-shaped line corresponding to the ThT kinetics corresponding to the fibril growth of Aβ(1–40), where the lag (nucleation) phase is followed by the elongation phase and plateau; when all ThT molecules have intercalated into β-sheets of the amyloid fibrils, the aggregation kinetics of amyloids is reviewed [33].

PPI dendrimers modified with maltose may, in the case of Aβ(1–40) or Aβ(1–42), interfere with amyloid fibril formation in a concentration-dependent manner, indicating that maltose PPI dendrimers bind amyloid proteins [18]. **Figure 4** demonstrates the ThT fluorescent kinetics of Aβ(1–40) and Aβ(1–42) in the presence of maltose PPI dendrimers. As expected, Aβ alone forms the typical amyloid fibrils [29]. However, when the maltose PPI dendrimers are present, the morphology of amyloid fibrils is altered, demonstrating binding of the dendrimers to Aβ [18, 26, 34, 35]. The electron micrograph shows the morphology of amyloid fibril in the presence of maltose PPI dendrimers. Fibril clumps were generated by incubating maltose PPI dendrimers with Aβ(1–40). As it has been suggested that dendrimers interact with Aβ thus, fibrils seem to be varnished by maltose dendrimers and clumped together, and importantly, no Aβ oligomers were observed in the presence of maltose PPI dendrimers [18]. Thus it is reasonable to think that maltose dendrimers interacting with Aβ may form hybrid fibrils, shifting the balance between oligomeric and fibrillar forms of Aβ toward less toxic hybrid products.

Dendrimers' intrinsic toxicity is an important issue in relation to their potential biological applications [36]. It was observed that unmodified PPI dendrimers have high intrinsic toxicity for cells [37, 38]. It was hypothesized that this toxicity could be related to the dendrimer capacity of establishing strong interactions of electrostatic nature [39]. It has been demonstrated that dendrimers with a surface decorated by polysaccharides, such as maltose or maltotriose, confer less toxicity [40, 41]. The charge of the dendrimer covered by polysaccharides is close to neutral;

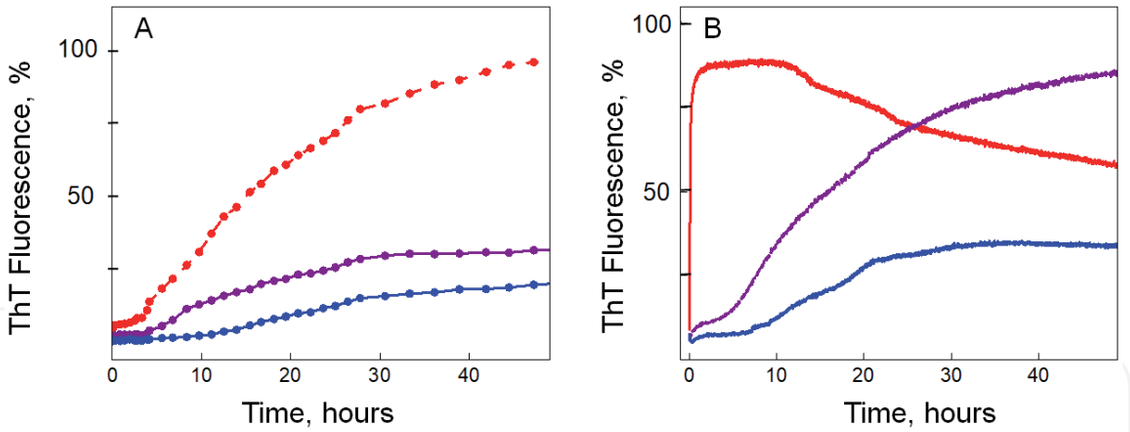

Figure 4.
Effect of G4 histidine-maltose PPI dendrimers on the fibrillization of Aβ. (A) Aggregation of 20 μM Aβ(1–40) in the absence (red) and the presence of histidine-maltose PPI dendrimers. (Magenta) 20 μM Aβ(1–40) in the presence of dendrimers at dendrimer/peptide ratio = 0.1, (blue) 20 μM Aβ(1–40) in the presence of dendrimers at dendrimer/peptide ratio = 1. (B) Aggregation of 25 μm Aβ(1–42) in the absence (red) and in the presence of histidine-maltose PPI dendrimers. (Magenta) 25 μM Aβ(1–42) in the presence of dendrimers at dendrimer/peptide ratio = 0.1, (blue) 25 μM Aβ(1–42) in the presence of dendrimers at dendrimer/peptide ratio = 1. The temperature was 37°C, the pH was set to 7.4, and the concentration of ThT was 6 μM (adapted with permission from [27]).

thus the interaction of dendrimer with other biomolecules is driven by hydrogen bonds, which is less strong; therefore, dendrimers covered by polysaccharides are less toxic [37, 38, 40].

In collaborations between the research groups of Dietmar Appelhans (Leibniz Institute of Polymer Research, Dresden, Germany), Josep Cladera (Autonomous University of Barcelona, Spain), and Isidro Ferrer in Barcelona (University of Barcelona, Spain), it has been shown that distinct PPI dendrimers with electroneutral maltose shell, with cationic maltose or maltotriose shell, were tested against amyloid toxicity in vivo and in vitro. The evaluation of the toxicity of Aβ in the presence of PPI maltose dendrimers showed that the dendrimers could significantly reduce the Aβ toxicity compared to Aβ alone [26].

Interestingly, only the electroneutral maltose dendrimers were able to reduce the toxicity of Alzheimer's disease brain extracts in cultured SH-SY5Y neuroblastoma cells [26]. Moreover, maltose PPI dendrimers with electroneutral or cationic surface penetrated the cytoplasm of cultured cells. Additionally, they penetrated inside the brain when administered to AD transgenic mice intranasally [26]. These PPI maltose dendrimers were able to modify amyloid plaque load in the brains of AD transgenic animals, showing anti-amyloid potential for in vivo applications. However, the studied maltose PPI dendrimers could not reverse memory impairment in APP/PS1 mice following chronic administration. Strikingly, cationic maltose dendrimers were neurotoxic in vivo and caused cognitive decline in non-transgenic mice [26]. Taken together, these results suggest that maltose PPI dendrimers require further optimization of biocompatibility.

3. Modified PPI dendrimers as potential multifunctional therapeutics for Alzheimer's disease

As it has been mentioned at the beginning of the chapter, Alzheimer's disease is a fatal neurodegenerative disorder. AD is characterized by a decade-long presymptomatic phase, and it is during the presymptomatic phase, before synaptic damage and neuronal loss, that therapies are most likely to be effective [42]. Thus, a preventive treatment which could protect synapses and reduce the neurotoxicity of Aβ

oligomers is one such strategy. Such successful drug candidates for AD treatment have to possess both anti-amyloidogenic and neuroprotective properties. Therefore, a modification of maltose dendrimers with a molecule with neuroprotective characteristics was the next logical step in search of the new drug candidate for the treatment of AD.

To further improve the pharmacological properties of maltose PPI dendrimers, it was decided to modify PPI dendrimers of the fourth generation with maltose and histidine. Maltose was used due to anti-amyloidogenic properties; histidine was added due to several reasons: it is selectively transported through the BBB [43]. Histidine has chelating properties for Cu^{2+} ions [44]. Thus these properties were considered to be important since Cu ion dyshomeostasis may play a detrimental role in AD progression [45], and importantly, histidine has been shown to have some neuroprotective capacity [46]. After the modification, G4 PPI dendrimers modified with maltose and histidine were supposed to possess both anti-amyloid and neuroprotective properties simultaneously.

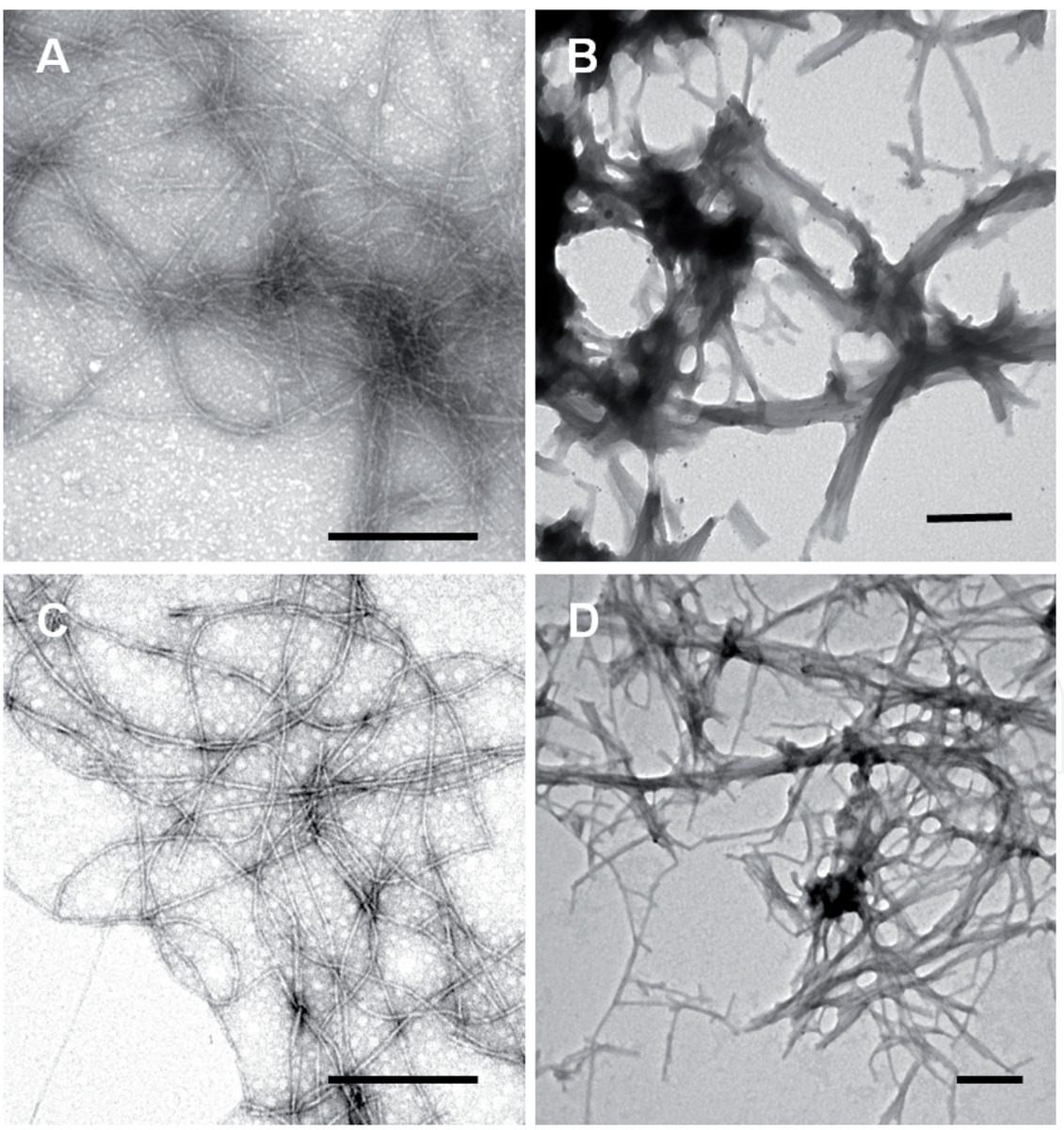

Figure 5.
Effect of G4 histidine-maltose PPI dendrimers on Aβ morphology. (A) Electron microscopy micrographs of 25 μM Aβ(1–40) incubated at pH 7.4 for 24 h. (B) 25 μM Aβ(1–40) incubated at pH 7.4 in the presence of G4 histidine-maltose PPI dendrimers at the ratio 1 to 1. (C) Aβ(1–42) incubated at pH 7.4 for 24 h. (D) Aβ(1–42) incubated in the presence of G4 histidine-maltose PPI dendrimers (clumped fibrils). Scale bar is 200 nm.

In vitro evaluations demonstrated that histidine-maltose PPI dendrimers could interact with Aβ. As maltose PPI dendrimers, G4 histidine-maltose PPI dendrimers did not prevent fibril formation but clump Aβ fibrils (**Figure 5**). Importantly, small oligomeric aggregates were not present in the studied suspensions in the presence of the dendrimers. Interestingly, the intensity of ThT was significantly decreased following the aggregation of Aβ probably due to the competition of the dendrimers with ThT for binding to Aβ(1–40) or due to change of structure, resulting in lower ThT fluorescence quantum yield [47, 48]. To test if G4 histidine-maltose PPI dendrimers could reduce the neurotoxicity of Aβ, primary neurons derived from wild-type mouse were treated with 1 μM Aβ(1–42) in the presence of the dendrimers at the ratio 1 to 1. As it was demonstrated by cell viability assay, histidine-maltose PPI dendrimers significantly reduced the neurotoxicity of soluble Aβ oligomers [27]. **Figure 6** shows the neuronal viability in the presence of the dendrimers and Aβ(1–42) oligomers as assessed by a lactate dehydrogenase (LDH) activity assay. 1 μM G4 histidine-maltose PPI dendrimers were added to primary neurons and incubated 24 h before the assay; as it was documented, the dendrimers alone were not toxic to the neurons. 1 μM recombinant Aβ(1–42) monomers, oligomers, and fibrils were added to primary neurons and incubated 1 h at 37°C in the presence and the absence of dendrimers. The results demonstrate that G4 histidine-maltose PPI dendrimers significantly reduced the toxicity of Aβ(1–42) for primary neurons.

In vivo evaluations demonstrated that chronic treatment with histidine-maltose PPI dendrimers of APP/PS1 mice prevented AD-related memory impairment [27]. **Figure 7** shows the results of the memory test after the treatment. APP/PS1 mice harbor two human genes: APP with the KM670/671NL, the Swedish mutation, and PSEN1 with the L166P mutation [49]. In APP/PS1 mice, human Aβ increases with age, but Aβ42 is preferentially generated over Aβ40, and the expression of the human APP transgene is approximately 3-fold higher than the endogenous murine APP [50]. For the treatment, APP/PS1 and wild-type mice were randomly divided into four groups, two groups (transgenic and wild type) were treated intranasally with histidine-maltose PPI dendrimers, and two groups (transgenic and wild type) were given intranasally phosphate saline. Administration lasted 3 months until animals reached the age of 6 months, the age when the first cognitive decline is detected [51]. Memory evaluation tests were performed at the end of treatment using two object recognition tests in a Vmaze^{R} as described [51].

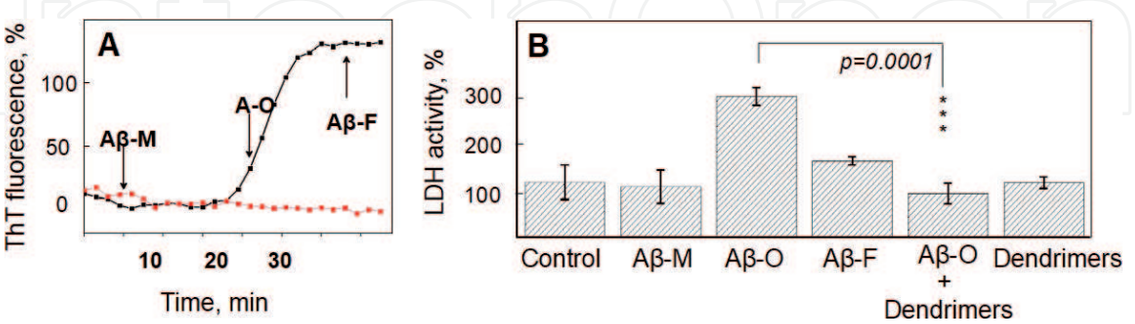

Figure 6.
G4 histidine-maltose PPI dendrimers reduce the toxicity of Aβ oligomers for cultured primary neurons. (A) ThT fluorescence variation was used to monitor aggregation of 10 μM Aβ(1–42) in PBS at 37°C (black line); red line corresponds to ThT alone. The arrows indicate the time when aliquots of Aβ(1–42) were taken for neuronal viability assay. Aβ-M, a monomeric form of Aβ(1–42); Aβ-O, an oligomeric form of Aβ(1–42); Aβ-F, mature fibrils of Aβ(1–42); (B) 1 μM of G4 histidine-maltose PPI dendrimers were added to primary neurons and incubated 24 h before a cell viability assay. Cell viability was assessed by a lactate dehydrogenase activity assay. For the assay, 1 μM Aβ(1–42) of monomers, oligomers, and fibrils were added to wild-type primary neurons and incubated 1 h at 37°C. statistics: one-way ANOVA followed by Tukey's post hoc test; data are expressed as mean ± SD. Primary neurons were derived from the brains of wild-type mouse embryos and cultured for 19 days. The experiment was performed in triplicate, one embryo per replica (adapted with permission from [27]).

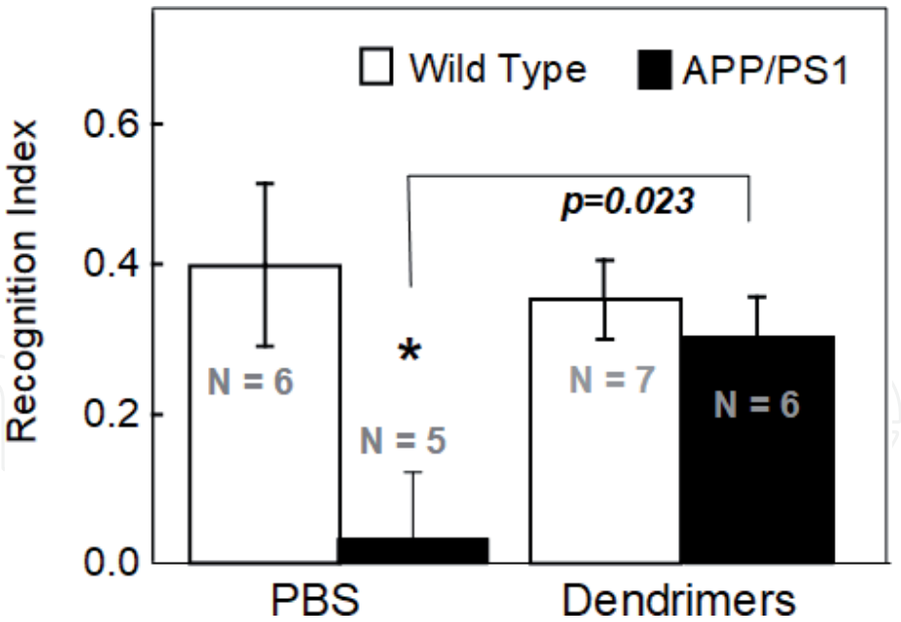

Figure 7.
G4 histidine-maltose PPI dendrimers can protect memory in vivo. Memory performance in the V-maze shows significant improvement after preventive treatment with histidine-maltose PPI dendrimers. Treatment procedure: at the age of 3 months, animals were randomly divided into four groups; two groups control and APP/PS1 mice were given intranasally 5 μL of PBS, and two groups received intranasally 5 μg/day of G4 histidine-maltose PPI dendrimers (dendrimers). Treatment lasted 3 months until animals reached the age of 6 months when APP/PS1 mice display cognitive impairment [51]. Statistics: two-way ANOVA with genotype and treatment as between factors followed by Tukey's post hoc test; data are expressed as mean ± SEM (adapted with permission from [27]).

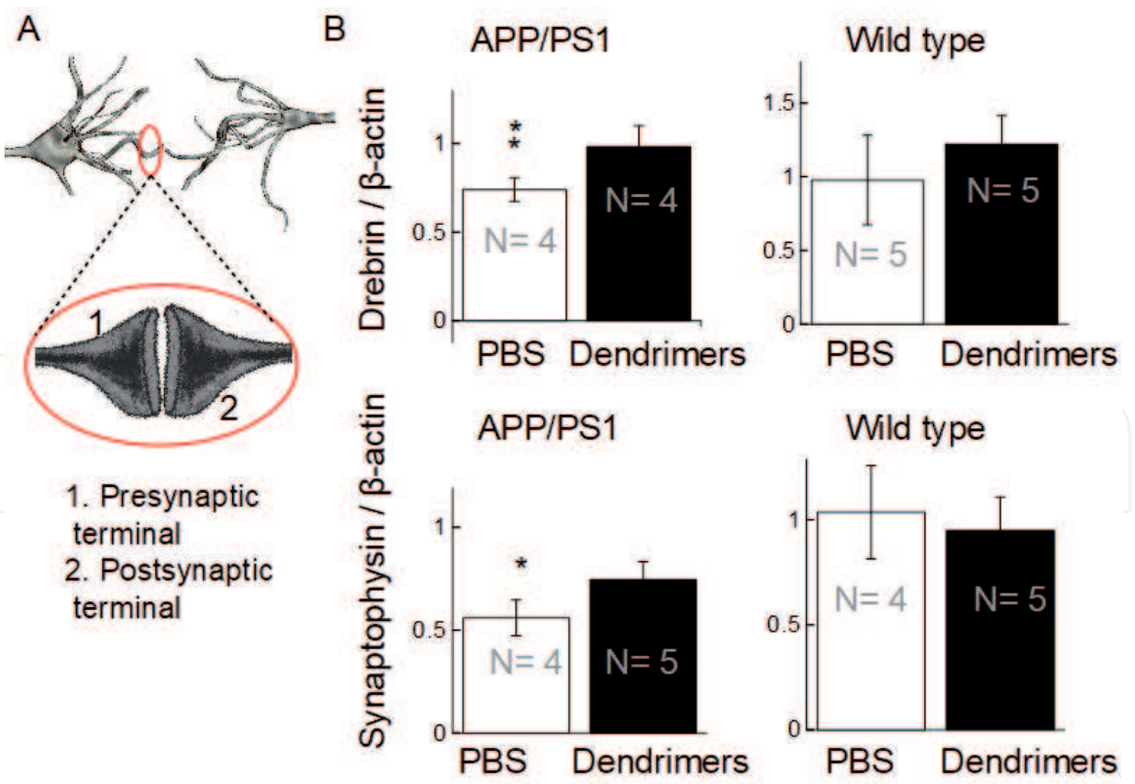

Figure 8.
G4 histidine-maltose PPI dendrimers protect synapses in vivo. (A) Synapse is a junction between two neurons, which consist of pre- and postsynaptic terminals characterized by specific pre- and postsynaptic proteins. Synaptophysin was used to assess presynapse, while drebrin was used to evaluate postsynapse. Brain tissue homogenates of control mice and mice treated with G4 histidine-maltose PPI dendrimers (dendrimers) were analyzed using Western blotting; β-actin was used for protein normalization. Statistics: Student's t-test (N is the number of animals per group, Western blotting was done in triplicate). Data are expressed as mean ± SD.

To understand a possible mechanism behind the memory rescue, the levels of pre- and postsynaptic markers in the brain of treated APP/PS1 mice were evaluated by Western blotting. Pre- and postsynaptic markers, such as drebrin and synaptophysin, play a crucial role in the synaptic plasticity and are downregulated in AD [52, 53]. Loss of synaptophysin correlates with cognitive impairments in AD patients and AD transgenic models [53, 54]; Psd95 knockout animals have impaired basal synaptic transmission and learning deficit [55]; transgenic animals lacking synaptophysin have reduced novel object recognition [56]. Importantly, it has been shown that loss of synaptophysin immunoreactivity precedes amyloid plaque formation [57, 58]. Preventive treatment of AD transgenic mice with G4 histidine-maltose PPI dendrimers prevented a decrease in synaptic proteins compared to PBS-treated mice [27].

In contrast, G4 histidine-maltose PPI dendrimers did not change the level of these synaptic proteins in WT mice, indicating that, most likely, the level of their mRNA expression was not affected [27]. Thus it is reasonable to think that the increased levels of pre- and postsynaptic proteins are more likely an effect of reduced synaptic loss in the treated AD transgenic animals (**Figure 8**). Thus a possible mechanism of memory protection in APP/PS1 could be the synapses were shielded by the dendrimers from toxic Aβ oligomers or the toxicity of Aβ oligomers were inactivated in the presence of the dendrimers.

4. Conclusions and perspectives

Dendrimers, which represent a type of 3D polymers, have been in the spotlight for three decades in biomedical and pharmaceutical research, and their chemistry and synthesis are continuously progressing by efforts from many research groups and companies. Although there are still many unclear problems in AD, in this chapter, functionalization of dendrimers dedicated to the prevention of memory decline in AD pathogenesis has been discussed. Based on the reviewed literature, PPI dendrimers have been shown to be useful in the way of the surface functionalization, which tuned their biochemical properties. Strikingly, the effect of the surface functionalization with histidine and maltose magnified exponentially neuroprotective properties of PPI dendrimers, resulting in an unprecedented outcome, such as memory protection in AD transgenic animals.

In this chapter, I have analyzed the functionalization of PPI dendrimers, which tuned the intrinsic properties of PPI dendrimers and converted them into a multifunctional drug candidate against Alzheimer's disease. Modification of the dendrimer surface with maltose allowed dendrimers successfully to interfere with Aβ(1–42) by forming nontoxic hybrid glycofibrils. Modification of the dendrimer surface with histidine improved the ability of the dendrimers to cross the blood–brain barrier and resulted in synaptic protection. By reducing the level of soluble amyloid oligomers, on the one hand, and conferring synapse protection, on the other hand, the dendrimers were given multifunctionality against main features of AD, synaptic loss, and aggregation of Aβ. These observations, coming out of the studies on the interaction of dendrimers with amyloid peptides [18, 26, 27, 31, 41], carried out in vitro and in vivo, point toward a possible use of dendrimers (in particular functionalization of PPI dendrimers with histidine and maltose) as a multifunctional drug candidate against Alzheimer's disease.

However, to find a successful drug against AD, other modifications of histidine-maltose PPI dendrimers might be required. For example, the ability to cross the blood-brain barrier, cell wall penetration, distribution in the specific tissue, and biodegradation could be tuned for a particular dendrimer application.

Acknowledgements

I acknowledge Dr. Dietmar Appelhans (Leibniz Institute of Polymer Research Dresden, Dresden, Germany) for the generation of histidine-maltose PPI dendrimers, collaboration, and valuable discussions.

The work is supported by MultiPark (Lund University), Vinnova, Swedish Research Council grants.

Thanks

I thank Dr. Stefan Broselid for fruitful discussions and editorial help.

Author details

Oxana Klementieva
Department of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden

*Address all correspondence to: oxana.klementieva@med.lu.se

References

[1] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002;**298**(5594):789-791

[2] Small DH, Mok SS, Bornstein JC. Alzheimer's disease and Aβ toxicity: From top to bottom. Nature Reviews. Neuroscience. 2001;**2**(8):595-598

[3] Davies CA, Mann DM, Sumpter PQ, Yates PO. A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. Journal of the Neurological Sciences. 1987;**78**(2):151-164

[4] Martinsson I, Capetillo-Zarate E, Faideau M, Willén K, Esteras N, Frykman S, et al. APP depletion alters selective pre- and post-synaptic proteins. Molecular and Cellular Neurosciences. 2019;**95**:86-95

[5] Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, et al. APP processing and synaptic function. Neuron. 2003;**37**(6):925-937

[6] Xue C, Tran J, Wang H, Park G, Hsu F, Guo Z. Aβ42 fibril formation from predominantly oligomeric samples suggests a link between oligomer heterogeneity and fibril polymorphism. Royal Society Open Science. 2019;**6**(7):190179

[7] Li X, Uemura K, Hashimoto T, Nasser-Ghodsi N, Arimon M, Lill CM, et al. Neuronal activity and secreted amyloid β lead to altered amyloid β precursor protein and presenilin 1 interactions. Neurobiology of Disease. 2013;**50**:127-134

[8] Takahashi RH, Nagao T, Gouras GK. Plaque formation and the intraneuronal accumulation of β-amyloid in Alzheimer's disease: Intraneuronal accumulation of β-amyloid. Pathology International. 2017;**67**(4):185-193

[9] Klementieva O, Willén K, Martinsson I, Israelsson B, Engdahl A, Cladera J, et al. Pre-plaque conformational changes in Alzheimer's disease-linked Aβ and APP. Nature Communications. 2017;**8**:14726

[10] Spires-Jones TL, Hyman BT. The intersection of amyloid Beta and tau at synapses in Alzheimer's disease. Neuron. 2014;**82**(4):756-771

[11] Lacor PN. Synaptic targeting by Alzheimer's-related amyloid oligomers. The Journal of Neuroscience. 2004;**24**(45):10191-10200

[12] Bilousova T, Miller CA, Poon WW, Vinters HV, Corrada M, Kawas C, et al. Synaptic amyloid-β oligomers precede p-tau and differentiate high pathology control cases. The American Journal of Pathology. 2016;**186**(1):185-198

[13] Takahashi RH. Oligomerization of Alzheimer's-amyloid within processes and synapses of cultured neurons and brain. The Journal of Neuroscience. 2004;**24**(14):3592-3599

[14] Bayer TA. Intracellular accumulation of amyloid-beta—A predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. Frontiers in Aging Neuroscience. 2010. Available from: http://journal.frontiersin.org/article/10.3389/fnagi.2010.00008/abstract. [Accessed: 1 August 2019]

[15] Close W, Neumann M, Schmidt A, Hora M, Annamalai K, Schmidt M, et al. Physical basis of amyloid fibril polymorphism. Nature Communications. 2018;**9**(1):699

[16] Baghallab I, Reyes-Ruiz JM, Abulnaja K, Huwait E, Glabe C. Epitomic characterization of the specificity of the anti-amyloid Aβ monoclonal antibodies 6E10 and 4G8. Journal of Alzheimer's Disease. 2018;**66**(3):1235-1244

[17] Dhanavade MJ, Sonawane KD. Insights into the molecular interactions between aminopeptidase and amyloid beta peptide using molecular modeling techniques. Amino Acids. 2014;**46**(8):1853-1866

[18] Klementieva O, Benseny-Cases N, Gella A, Appelhans D, Voit B, Cladera J. Dense shell glycodendrimers as potential nontoxic anti-amyloidogenic agents in Alzheimer's disease. Amyloid–dendrimer aggregates morphology and cell toxicity. Biomacromolecules. 2011;**12**(11):3903-3909

[19] Svenson S, Tomalia DA. Dendrimers in biomedical applications—Reflections on the field. Advanced Drug Delivery Reviews. 2012;**64**:102-115

[20] Buhleier E, Wehner W, Vögtle F. "Cascade"- and "nonskid-chain-like" syntheses of molecular cavity topologies. Synthesis. 1978;**1978**(02):155-158

[21] Appelhans D, Klajnert-Maculewicz B, Janaszewska A, Lazniewska J, Voit B. Dendritic glycopolymers based on dendritic polyamine scaffolds: View on their synthetic approaches, characteristics and potential for biomedical applications. Chemical Society Reviews. 2015;**44**(12):3968-3996

[22] Mlynarczyk DT, Kocki T, Goslinski T. Dendrimer structure diversity and tailorability as a way to fight infectious diseases. In: Seehra MS, editor. Nanostructured Materials—Fabrication to Applications. London, United Kingdom: IntechOpen Limited; 2017

[23] Najlah M, D'Emanuele A. Synthesis of dendrimers and drug-dendrimer conjugates for drug delivery. Current Opinion in Drug Discovery and Development. 2007;**10**(6):756-767

[24] Wu G. Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates. Molecular Cancer Therapeutics. 2006;**5**(1):52-59

[25] Huang R-Q, Qu Y-H, Ke W-L, Zhu J-H, Pei Y-Y, Jiang C. Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. The FASEB Journal. 2007;**21**(4):1117-1125

[26] Klementieva O, Aso E, Filippini D, Benseny-Cases N, Carmona M, Juvés S, et al. Effect of poly(propylene imine) glycodendrimers on β-amyloid aggregation in vitro and in APP/PS1 transgenic mice, as a model of brain amyloid deposition and Alzheimer's disease. Biomacromolecules. 2013;**14**(10):3570-3580

[27] Aso E, Martinsson I, Appelhans D, Effenberg C, Benseny-Cases N, Cladera J, et al. Poly(propylene imine) dendrimers with histidine-maltose shell as novel type of nanoparticles for synapse and memory protection. Nanomedicine: Nanotechnology, Biology and Medicine. 2019;**17**:198-209

[28] Arosio P, Cukalevski R, Frohm B, Knowles TPJ, Linse S. Quantification of the concentration of Aβ42 propagons during the lag phase by an amyloid chain reaction assay. Journal of the American Chemical Society. 2014;**136**(1):219-225

[29] Benseny-Cases N, Cócera M, Cladera J. Conversion of non-fibrillar β-sheet oligomers into amyloid fibrils in Alzheimer's disease amyloid peptide aggregation. Biochemical and Biophysical Research Communications. 2007;**361**(4):916-921

[30] Taneja V, Verma M, Vats A. Toxic species in amyloid disorders: Oligomers or mature fibrils. Annals of Indian Academy of Neurology. 2015;**18**(2):138

[31] McCarthy JM, Franke M, Resenberger UK, Waldron S, Simpson JC, Tatzelt J, et al. Anti-prion drug mPPIg5

Inhibits PrPC conversion to PrPSc. PLoS One. 2013;**8**(1):e55282

[32] McCarthy JM, Rasines Moreno B, Filippini D, Komber H, Maly M, Cernescu M, et al. Influence of surface groups on poly(propylene imine) dendrimers antiprion activity. Biomacromolecules. 2013;**14**(1):27-37

[33] Arosio P, Knowles TPJ, Linse S. On the lag phase in amyloid fibril formation. Physical Chemistry Chemical Physics. 2015;**17**(12):7606-7618

[34] Klajnert B, Peng L, Ceña V. Dendrimers in Biomedical Applications. Cambridge: European Cooperation in the Field of Scientific and Technical Research (Organization), Royal Society of Chemistry (Great Britain), RSC Publishing; 2013. 204 p

[35] Ottaviani FM, Cangiotti M, Fiorani L, Fattori A, Wasiak T, Appelhans D, et al. Kinetics of amyloid and prion fibril formation in the absence and presence of dense Shell sugar-decorated dendrimers. Current Medicinal Chemistry. 2012;**19**(34):5907-5921

[36] Jain K, Kesharwani P, Gupta U, Jain NK. A review of glycosylated carriers for drug delivery. Biomaterials. 2012;**33**(16):4166-4186

[37] Ziemba B, Janaszewska A, Ciepluch K, Krotewicz M, Fogel WA, Appelhans D, et al. In vivo toxicity of poly(propyleneimine) dendrimers. Journal of Biomedical Materials Research. Part A. 2011;**99A**(2):261-268

[38] Ciepluch K, Ziemba B, Janaszewska A, Appelhans D, Klajnert B, Bryszewska M, et al. Modulation of biogenic amines content by poly(propylene imine) dendrimers in rats. Journal of Physiology and Biochemistry. 2012;**68**(3):447-454

[39] Palmerston Mendes L, Pan J, Torchilin V. Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. Molecules. 2017;**22**(9):1401

[40] Klajnert B, Appelhans D, Komber H, Morgner N, Schwarz S, Richter S, et al. The influence of densely organized maltose shells on the biological properties of poly(propylene imine) dendrimers: New effects dependent on hydrogen bonding. Chemistry: A European Journal. 2008;**14**(23):7030-7041

[41] Fischer M, Appelhans D, Schwarz S, Klajnert B, Bryszewska M, Voit B, et al. Influence of surface functionality of poly(propylene imine) dendrimers on protease resistance and propagation of the scrapie prion protein. Biomacromolecules. 2010;**11**(5):1314-1325

[42] Sperling R, Mormino E, Johnson K. The evolution of preclinical Alzheimer's disease: Implications for prevention trials. Neuron. 2014;**84**(3):608-622

[43] Yamakami J, Sakurai E, Sakurada T, Maeda K, Hikichi N. Stereoselective blood-brain barrier transport of histidine in rats. Brain Research. 1998;**812**(1-2):105-112

[44] Oshima T, Oshima C, Baba Y. Selective extraction of histidine derivatives by metal affinity with a copper(II)-chelating ligand complex in an aqueous two-phase system. Journal of Chromatography B. 2015;**990**:73-79

[45] Wang H, Wang M, Wang B, Li M, Chen H, Yu X, et al. The distribution profile and oxidation states of biometals in APP transgenic mouse brain: Dyshomeostasis with age and as a function of the development of Alzheimer's disease. Metallomics. 2012;**4**(3):289

[46] Tang S-C, Arumugam TV, Cutler RG, Jo D-G, Magnus T, Chan SL, et al. Neuroprotective actions of a histidine analogue in models of ischemic stroke: Histidine analogue in models

of ischemic stroke. Journal of Neurochemistry. 2007;**101**(3):729-736

[47] Jimenez JL, Nettleton EJ, Bouchard M, Robinson CV, Dobson CM, Saibil HR. The protofilament structure of insulin amyloid fibrils. Proceedings of the National Academy of Sciences. 2002;**99**(14):9196-9201

[48] Sulatskaya AI, Kuznetsova IM, Turoverov KK. Interaction of thioflavin T with amyloid fibrils: Fluorescence quantum yield of bound dye. The Journal of Physical Chemistry. B. 2012;**116**(8):2538-2544

[49] APPPS1. ALZFORUM [Internet]. Available from: https://www.alzforum.org/research-models/appps1 [Accessed: 1 August 2019]

[50] Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, et al. Aβ42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO Reports. 2006;**7**(9):940-946

[51] Aso E, Lomoio S, López-González I, Joda L, Carmona M, Fernández-Yagüe N, et al. Amyloid generation and dysfunctional immunoproteasome activation with disease progression in animal model of familial Alzheimer's disease: Amyloid generation and UPS in FAD mice. Brain Pathology. 2012;**22**(5):636-653

[52] Shim KS, Lubec G. Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and down syndrome. Neuroscience Letters. 2002;**324**(3):209-212

[53] Poirel O, Mella S, Videau C, Ramet L, Davoli MA, Herzog E, et al. Moderate decline in select synaptic markers in the prefrontal cortex (BA9) of patients with Alzheimer's disease at various cognitive stages. Scientific Reports. 2018;**8**(938):1-14

[54] Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al. Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Annals of Neurology. 1991;**30**(4):572-580

[55] Nagura H, Ishikawa Y, Kobayashi K, Takao K, Tanaka T, Nishikawa K, et al. Impaired synaptic clustering of postsynaptic density proteins and altered signal transmission in hippocampal neurons, and disrupted learning behavior in PDZ1 and PDZ2 ligand binding-deficient PSD-95 knockin mice. Molecular Brain. 2012;**5**(1):43

[56] Schmitt U, Tanimoto N, Seeliger M, Schaeffel F, Leube RE. Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience. 2009;**162**(2):234-243

[57] Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, et al. High-level neuronal expression of a beta 1-42 in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2000;**20**(11):4050-4058

[58] Tampellini D, Capetillo-Zarate E, Dumont M, Huang Z, Yu F, Lin MT, et al. Effects of synaptic modulation on -amyloid, synaptophysin, and memory performance in Alzheimer's disease transgenic mice. The Journal of Neuroscience. 2010;**30**(43):14299-14304

[59] Mark JE. Polymer Data Handbook. 2nd ed. Oxford, New York: Oxford University Press; 2009. p. 1250