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Soluble Prion Peptide 107–120 Protects Neuroblastoma SH-SY5Y Cells against Oligomers Associated with Alzheimer’s Disease

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Abstract: Alzheimer’s disease (AD) is the most prevalent form of dementia and soluble amyloid β (Aβ) oligomers are thought to play a critical role in AD pathogenesis. Cellular prion protein (PrPC) is a high-affinity receptor for Aβ oligomers and mediates some of their toxic effects. The N-terminal region of PrPC can interact with Aβ, particularly the region encompassing residues 95–110. In this study, we identified a soluble and unstructured prion-derived peptide (PrP107–120) that is external to this region of the sequence and was found to successfully reduce the mitochondrial impairment, intracellular ROS generation and cytosolic Ca2+ uptake induced by oligomeric Aβ42 ADDLs in neuroblastoma SH-SY5Y cells. PrP107–120 was also found to rescue SH-SY5Y cells from Aβ42 ADDL internalization. The peptide did not change the structure and aggregation pathway of Aβ42 ADDLs, did not show co-localization with Aβ42 ADDLs in the cells and showed a partial colocalization with the endogenous cellular PrPC. As a sequence region that is not involved in Aβ binding but in PrP self-recognition, the peptide was suggested to protect against the toxicity of Aβ42 oligomers by interfering with cellular PrPC and/or activating a signaling that protected the cells. These results strongly suggest that PrP107–120 has therapeutic potential for AD.

Keywords: Alzheimer’s disease; prion peptide; Aβ oligomers; ADDLs

1. Introduction

Alzheimer’s Disease International (ADI) and the American Alzheimer’s Association (AA) have estimated that over than 50 million people are living with dementia, and that Alzheimer’s disease (AD) is the most common cause and may account for 60–70% of dementia cases [1]. According to this, these numbers will increase dramatically, and this disease will considerably challenge the world healthcare system in the future.

Evidence indicates that AD is an aging-related disease, and frequency in individuals aged 85 or older is higher (one in three) compared to age 65 (one in nine) [1,2]. AD involves a loss of memory, cognitive impairment and behavioral instability [3]. Both extracellular neuritic plaques mainly composed of the amyloid beta (Aβ) peptide and intraneuronal neurofibrillary tangles containing the hyperphosphorylated tau protein are important histopathological hallmarks associated with AD [4,5].

Although accumulation of senile plaques formed by Aβ is a major histopathological trait of AD, it is widely accepted that soluble Aβ oligomers, forming as intermediate species in the process of neuritic plaque formation or released from the plaques, can effectively play a key role in neuronal
dysfunction and impair synaptic structure and function [6,7]. It is well-known that such oligomers can inhibit long-term potentiation (LTP)—a correlate of synaptic plasticity [8,9]—as well as activating expression of the complement system [10,11] and general neurotoxic [6,12].

Several studies have reported that the cell membrane protein PrP<sup>C</sup> mediates the abnormal effects of Aβ oligomers, particularly the oligomer-induced inhibition of LTP [13–15]. It was also reported that memory deficits in AD transgenic mice require the presence of PrP<sup>C</sup> [16], and that loss of synaptic markers, axonal degeneration and early death in transgenic mice are fully dependent on PrP<sup>C</sup> [17]. Other reports have shown that co-expression of PrP<sup>C</sup> and Aβ reduces longevity in Drosophila melanogaster, and that expression of Aβ individually cannot create pathogenic phenotypes [18]. In spite of the large body of evidence that PrP<sup>C</sup> mediates the aberrant effects of Aβ oligomers, other studies have indicated that it is not a necessary component for the toxicity cascade induced by Aβ oligomers, as Aβ-induced LTP inhibition or memory impairment has also been reported to occur independently of the overexpression or ablation of PrP<sup>C</sup> in the transgenic mice [19–21]. This has led to a controversy that remains unresolved [22].

PrP<sup>C</sup> is a membrane protein that is found in both neurons and glial cells [23] and has been proposed to have a role in cellular signaling, copper homeostasis, cell adhesion and even neuroprotection [24,25]. The mature form of human PrP<sup>C</sup> consists of a single polypeptide chain of 208 amino acid residues (residues 23–230). The N-terminal region of the protein (residue 23–120) is unstructured, while the C-terminal region (residues 121–230) is largely structured, consisting of three α-helices (residues 144–154, 173–194, 200–228) and two short anti-parallel β-strands (residues 128–131, 161–164) [26]. The protein is anchored to the cell membrane via a glycosyl-phosphatidyl-inositol (GPI) anchor encompassing residues 231–253, which is excised in the mature form [26–28]. PrP<sup>C</sup> function is poorly understood, although roles have been suggested in synaptic transmission, long-term memory, circadian rhythms, T cell function, hematopoietic stem cell renewal, copper-binding, apoptosis and oxidative stress homeostasis, among others [29]. The scrapie form (PrP<sup>Sc</sup>) is derived from PrP<sup>C</sup> [30–32]. The central event is the conversion from an α-helix rich structure to a form with a high β-sheet content. Accumulation of this infectious misfolded form of prion protein (PrP<sup>Sc</sup>) can cause a group of neurodegenerative diseases affecting both human and animals [30–32].

A large number of data have shown that PrP<sup>C</sup> has a high binding affinity for Aβ<sub>42</sub> oligomers [33,34]. Even studies showing that PrP<sup>C</sup>-expressing and PrP<sup>C</sup> knock-out mice were equally susceptible to Aβ<sub>42</sub> oligomer-induced cognitive impairment recognized that the oligomers interacted with PrP<sup>C</sup> with high affinity [19]. The first evidence of binding between Aβ<sub>42</sub> oligomers and PrP<sup>C</sup> dates back to 2009, when it was also found that the N-terminal region of PrP<sup>C</sup>, particularly the 95–110 residues, was involved in the binding [35]. One year later, Chen and co-workers showed that both N-terminal residues 23–27 and 92–110 were critically important for binding [36]. The importance of these two sequence regions was then confirmed by later reports [13,14,37–42].

Unlike the many reports showing a role of the N-terminal region of PrP<sup>C</sup> in the binding of Aβ oligomers, none of the studies reported so far have highlighted a role of the region encompassing residues 106–126 in Aβ<sub>42</sub> oligomer binding [34]. By contrast, this PrP segment was found to be mainly responsible for prion aggregation [43–49]. The AGAAAAGA palindromic sequence 113–120, in particular, is necessary for PrP<sup>C</sup>-PrP<sup>Sc</sup> interaction [44,45], and the two glycine residues at positions 114 and 119 have been suggested as particularly important for fibril formation [50].

As a segment involved in PrP self-recognition, but not in Aβ-PrP<sup>C</sup> complex formation, we reasoned that a short peptide encompassing this region of the sequence might inhibit Aβ<sub>42</sub> oligomer toxicity. The hypothesis for this idea is that such a peptide might leave the Aβ oligomers unbound and unaltered, while engaging in interactions with PrP<sup>C</sup>. Our designed peptide encompassing residues 107–120 (PrP<sub>107–120</sub>) was found to be very soluble in physiological conditions, most probably because it lacks the highly hydrophobic region VVGGGLG (residues 121–126) of the PrP<sup>C</sup> fragment 106–126 responsible for prion aggregation [44,45,49]. Importantly, the peptide was found to prevent the generic toxic effects of Aβ<sub>42</sub> oligomers on neuroblastoma SH-SY5Y cells in absence of Aβ<sub>42</sub> oligomer binding.
and structural reorganization, but in presence of partial binding between PrP<sub>107–120</sub> peptide and endogenous cellular PrP<sup>C</sup>. Since this peptide is not aggregation-prone and has beneficial effects against Aβ-induced toxicity, it is of potentially great interest for rationalizing the pathogenesis of AD and routes to its prevention, as well as in setting up therapeutic strategies for the treatment of AD.

2. Results

2.1. Freshly Dissolved PrP<sub>107–120</sub> is Monomeric

We calculated the theoretical hydrodynamic radius (R<sub>h</sub>) for a peptide with the size of PrP<sub>107–120</sub> (14 amino acid residues) in an unfolded state, using the equation described in Section 4, and previously published in [51]. According to this equation, the R<sub>h</sub> value was found to be 0.99 ± 0.56 nm. Dynamic light scattering (DLS) shows that PrP<sub>107–120</sub> dissolved in water has a hydrodynamic diameter (D<sub>h</sub>) of 2.11 ± 0.68 nm, corresponding to a R<sub>h</sub> of 1.05 ± 0.34 nm (Figure 1), which is in good agreement with that estimated theoretically. The DLS distribution also showed very large PrP<sub>107–120</sub> aggregates at 70–7000 nm, but these are quantitatively irrelevant, as light scattering intensity is known to scale with the sixth power of the size. These results reveal that PrP<sub>107–120</sub> dissolved in water is unfolded and predominantly non-aggregated.

![Figure 1](image_url)

**Figure 1.** Size distribution of PrP<sub>107–120</sub>. Size distribution by light scattering intensity obtained with dynamic light scattering (DLS) for PrP<sub>107–120</sub> dissolved in plain water at 25 °C. Peptide concentration was 1 mg/mL. The large aggregates at 70–7000 nm are quantitatively irrelevant, as light scattering intensity scales with the sixth power of the size.

2.2. PrP<sub>107–120</sub> Remains Monomeric and Unstructured under Different Conditions

We then investigated whether different conditions that are generally favorable for protein aggregation have the ability to promote fibrillation for the designed peptide. The different conditions are listed in Section 4 and include various peptide concentrations, salt concentrations, pH values and co-solvents. As a representative example, we show the results obtained at 1.0 mg/mL peptide in 20 mM phosphate buffer, 200 mM Na<sub>2</sub>SO<sub>4</sub>, with a pH 7.0, at 37 °C (Figure 2). The DLS distribution showed a D<sub>h</sub> of ~1 nm immediately after incubation (0 h) under these conditions, which appears dominant in the population if we take into account that the intensity of scattered light scales with the sixth power of the diameter (Figure 2A). This peak is still clearly visible even after 5 days incubation under these conditions (Figure 2A). Aggregates increased in population after 5 days, yet they appear to be quantitatively irrelevant if we consider the relationship between scattered light intensity and diameter mentioned above. The Thioflavin T (ThT) fluorescence did not increase following the addition of the peptide after 5 days incubation under these conditions relative to the blank containing only ThT (Figure 2B), and the far-UV circular dichroism (CD) spectrum also remained unchanged with a single
negative peak at ~198 nm, which is typical of highly disordered states (Figure 2C). Hence, we did not observe a ThT-positive structure or significant changes in size and CD spectrum even after 5 days incubation. Similar results were obtained in all conditions tested (data not shown), indicating that PrP<sub>107–120</sub> is soluble and stable under all the conditions studied here that are, by contrast, potentially favorable for amyloid fibril formation.

![Figure 2](image-url)

**Figure 2.** Effect of 20 mM phosphate buffer, 200 mM Na<sub>2</sub>SO<sub>4</sub>, pH 7.0, 37 °C on PrP<sub>107–120</sub> (1.0 mg/mL) aggregation. (A) Size distributions by light scattering intensity of the peptide sample obtained with DLS at t = 0 h (left) and 120 h (right) under the conditions described above. (B) Thioflavin T (ThT) fluorescence spectra with buffer (blank) and PrP<sub>107–120</sub> sample after 0 and 120 h. Peptide sample was incubated as described above. ThT assay was carried out at 22 µM ThT, 0.12 mg/mL PrP<sub>107–120</sub> (final concentrations in the cuvette), pH 6.0, 37 °C. (C) Far-UV circular dichroism (CD) spectra of PrP<sub>107–120</sub> incubated for 0 and 120 h. Peptide sample was incubated as described above and diluted to 0.2 mg/mL in the same buffer before spectrum acquisition at 25 °C.

2.3. PrP<sub>107-120</sub> Reduces Aβ<sub>42</sub> Cytotoxicity on SH-SY5Y Cells

In order to analyze whether PrP<sub>107-120</sub> can rescue the cellular dysfunction induced by Aβ<sub>42</sub> oligomers, we used amyloid-derived diffusible ligands (ADDLs) formed from Aβ<sub>42</sub> peptide according to a well-established protocol [52]. ADDLs were chosen as representative Aβ<sub>42</sub> oligomers because they are widely used [52–55], and their morphology and purity are routinely verified [56,57]. These have been found to be toxic and increase intracellular Ca<sup>2+</sup> and reactive oxygen species (ROS) levels in cultured cells [53,58], and have been found in post-mortem AD brains using both polyclonal and monoclonal conformation-sensitive antibodies specific to ADDLs [54,59]. To this aim, we analyzed the effects of Aβ<sub>42</sub> ADDLs with a final concentration of 3-µM monomer equivalents (m.e.) on the metabolic activities of human SH-SY5Y cells. This immortalized neuronal cell model is mostly used for
AD research, as human cholinergic neurons are difficult to obtain and maintain and unsuitable for routine experiments.

The metabolic activity of the cells was analyzed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is a widely used indicator of mitochondrial reduction capacity. The ability of SH-SY5Y cells to reduce MTT significantly decreased to 66.6 ± 4.5% following treatment for 24 h with Aβ42 ADDLs at 3 µM m.e. (Figure 3). By contrast, no detectable change was observed in cells treated with PrP107–120 and monomeric Aβ42 at final concentrations of 0.75 and 3 µM, respectively (Figure 3). The addition of the PrP107–120 (0.75 µM) to Aβ42 ADDLs (3 µM) significantly increased cell viability to 79.7 ± 4.3% compared with the cells treated with Aβ42 ADDLs (3 µM) in the absence of the peptide (Figure 3). In another experiment, PrP107–120 at a concentration of 0.75 µM was added to monomeric Aβ42 peptide at a concentration of 3 µM, and the resulting sample was maintained under conditions favorable for ADDL formation before addition to the cells (Aβ42 + PrP107–120). In this case, we found a cell viability of 86.4 ± 2.5%, again indicating protection by the PrP107–120 peptide. These results suggest that PrP107–120 can act as an inhibitor of Aβ42 ADDL oligomer toxicity.

2.4. PrP107-120 Reduces Ca2+ Influx Induced by Aβ42 Oligomers

An early cellular insult caused by Aβ42 ADDLs, and Aβ42 oligomers more generally, when added to the cellular medium, is the permeabilization of the plasma membrane with a rapid influx of calcium ions (Ca2+) from the extracellular space to the cytosol [53,58,60]. To investigate whether PrP107–120 can prevent the increase of Ca2+ levels mediated by the Aβ42 ADDL oligomers, we monitored the influx of Ca2+ in SH-SY5Y cells treated with Aβ42 ADDLs in presence and absence of PrP107–120.
same concentrations used for the MTT assay. The quantification of the intracellular Ca\(^{2+}\)-derived fluorescence in confocal microscopy images shows that the treatment of the cells with A\(\beta_{42}\) ADDLs caused a significant increase in intracellular Ca\(^{2+}\) up to 246 \(\pm\) 21\% compared with untreated cells, taken as 100\% (Figure 4). By contrast, the cellular exposure to A\(\beta_{42}\) + PrP\(_{107-120}\), A\(\beta_{42}\) ADDLs + PrP\(_{107-120}\) and PrP\(_{107-120}\) alone triggered a minor increase of intracellular Ca\(^{2+}\)-derived fluorescence (i.e., to 116 \(\pm\) 13\%, 132 \(\pm\) 7\% and 130 \(\pm\) 11\% respectively) that was significantly lower than that observed in cells treated with A\(\beta_{42}\) ADDLs (Figure 4). This result suggests a protective role of PrP\(_{107-120}\) in the Ca\(^{2+}\) influx mediated by the A\(\beta_{42}\) ADDLs in neuronal cells. Since the concentrations of Ca\(^{2+}\) and PrP\(_{107-120}\) in the cell medium were 2 mM and 0.75 \(\mu\)M, respectively, it can be ruled out that the inhibition of ADDL-induced Ca\(^{2+}\) influx mediated by the prion peptide arises from peptide-Ca\(^{2+}\) binding, as the peptide is over three orders of magnitude sub-stoichiometric.

Figure 4. Analysis of intracellular Ca\(^{2+}\) levels of SH-SY5Y cells treated with A\(\beta_{42}\) oligomers and PrP\(_{107-120}\). (A) Representative scanning confocal microscopy images of intracellular free Ca\(^{2+}\) levels in SH-SY5Y cells loaded with Fluo-4 AM probe. The cells were treated for 1 h with A\(\beta_{42}\) ADDLs, PrP\(_{107-120}\), A\(\beta_{42}\) ADDLs + PrP\(_{107-120}\) and A\(\beta_{42}\) + PrP\(_{107-120}\) pre-incubated under conditions promoting ADDL formation prior to addition to the cell medium. All samples were initially in 2\% (v/v) DMSO and F-12 Ham medium at concentrations of 100 and 25 \(\mu\)M (m.e.) for A\(\beta_{42}\) and PrP\(_{107-120}\), respectively, and were diluted 33-fold before each experiment into cellular medium without DMSO to final concentrations of 3 and 0.75 \(\mu\)M (m.e.) for A\(\beta_{42}\) and PrP\(_{107-120}\), respectively. Scale bar = 15 \(\mu\)m. (B) Semi-quantitative analysis of intracellular Ca\(^{2+}\) derived fluorescence. Experimental errors are S.E.M. (\(n = 4\)). The triple (***) asterisks refer to \(p\) values \(< 0.001\) relative to the untreated cells. The triple (###) symbols refer to \(p\) values \(< 0.001\) relative to A\(\beta_{42}\) ADDLs.
2.5. PrP<sub>107-120</sub> Reduces Intracellular Reactive Oxygen Species (ROS) Induced by Aβ<sub>42</sub> Oligomers

Another effect caused early by Aβ<sub>42</sub> ADDLs when added to the extracellular medium of cells is the increase of ROS levels in the cytosol [58,61]. We observed an increase of ROS-derived fluorescence up to 190 ± 19% in SH-SY5Y cells treated with Aβ<sub>42</sub> ADDLs compared with untreated cells, and no significant change in cells treated with PrP<sub>107-120</sub> alone (Figure 5). By contrast, the treatment with Aβ<sub>42</sub> ADDLs + PrP<sub>107-120</sub> and Aβ<sub>42</sub> + PrP<sub>107-120</sub> did not cause any significant change in ROS-derived fluorescence levels compared with untreated cells, and levels significantly lower than those observed after treatment with Aβ<sub>42</sub> ADDLs (Figure 5). Therefore, PrP<sub>107-120</sub> can effectively protect SH-SY5Y cells against the oxidative stress induced by Aβ<sub>42</sub> ADDLs.

![Figure 5](image_url)

**Figure 5.** Analysis of intracellular reactive oxygen species (ROS) levels of SH-SY5Y cells treated with Aβ<sub>42</sub> oligomers and PrP<sub>107-120</sub>. (A) Representative scanning confocal microscopy images of intracellular free ROS levels in SH-SY5Y cells loaded with CM-H<sub>2</sub>DCFDA. The cells were treated with the same samples described in the Figure 4 legend. Scale bar = 15 µm. (B) Semi-quantitative analysis of intracellular ROS-derived fluorescence. Experimental errors are S.E.M. (n = 3). The triple (***asterisks refer to p values < 0.001 relative to the untreated cells. The triple (###) symbols refer to p values < 0.001 relative to Aβ<sub>42</sub> ADDLs.

2.6. PrP<sub>107-120</sub> Reduces the Toxicity of Other Model Oligomers

In order to assess whether the protective role of PrP<sub>107-120</sub> observed with ADDLs was specific to this peptide and oligomer system or was more generally exerted against misfolded protein oligomers, we also tested whether PrP<sub>107-120</sub> has the ability to decrease the cytotoxicity of another type of misfolded oligomer, those formed by the model protein HypF-N named type A and found to have effects similar to those of Aβ<sub>42</sub> [62–65]. We observed an increase of ROS-derived fluorescence in SH-SY5Y cells upon treatment with type A HypF-N oligomers up to 214 ± 39% (Figure 6). Exposure to HypF-N oligomers
+ PrP{}^{107-120} and HypF-N preincubated with PrP{}^{107-120} under conditions promoting type A HypF-N oligomer formation showed non-significant changes in intracellular ROS-derived fluorescence (93 ± 2% and 124 ± 19%, respectively).

2.7. PrP{}^{107-120} Reduces Aβ_{42} ADDLs Internalization in SH-SY5Y Cells

In order to study the effects of PrP{}^{107-120} on the cellular internalization of Aβ_{42} ADDLs in SH-SY5Y cells, we performed immunostaining experiments using the 6E10 specific antibody against Aβ_{42} and wheat germ agglutinin to stain Aβ_{42} and the cell membrane, respectively. Cells were exposed for 1 h to the various protein samples described above and added to the cell medium. Thus, for the intracellular Aβ_{42}-derived fluorescence (green) in the SH-SY5Y cells, we observed more than 78% reduction in cells treated with Aβ_{42} ADDLs + PrP{}^{107-120}, and more than 74% reduction in cells treated with Aβ_{42} + PrP{}^{107-120} in terms of intracellular ADDLs levels with respect to cells treated with Aβ_{42} ADDLs taken as 100% (Figure 7). These results indicate that PrP{}^{107-120} is able to significantly reduce Aβ_{42} ADDL internalization in SH-SY5Y cells when added either before or after the Aβ_{42} oligomerization process.
Figure 7. Effect of PrP\textsubscript{107–120} on A\textsubscript{β}\textsubscript{42} ADDL internalization in SH-SY5Y cells. (A) Representative scanning confocal microscopy images of SH-SY5Y cells treated with the indicated samples and showing A\textsubscript{β}\textsubscript{42} ADDLs. The cells were treated with the same samples described in the Figure 4 legend. The cellular membrane was stained with wheat germ agglutinin (red fluorescence) and A\textsubscript{β}\textsubscript{42} ADDLs were labelled with mouse monoclonal primary antibody 6E10 and anti-mouse secondary antibody (green fluorescence). Scale bar = 10 µm. (B) Semi-quantitative analysis of intracellular A\textsubscript{β}\textsubscript{42} ADDL-derived fluorescence. Experimental errors are S.E.M. (n = 3). The triple (*** ) asterisks refer to p values < 0.001 relative to the untreated cells. The triple (### ) symbols refer to p values < 0.001 relative to A\textsubscript{β}\textsubscript{42} ADDLs.

2.8. PrP\textsubscript{107–120} Does Not Change the Structure or Aggregation State of A\textsubscript{β}\textsubscript{42} ADDLs

To determine whether PrP\textsubscript{107–120} can modify the structure of A\textsubscript{β}\textsubscript{42} ADDLs into non-toxic A\textsubscript{β}\textsubscript{42} oligomers or cause a change in their aggregation state (either fibrils, large aggregates or monomers),
we carried out a number of tests using dot-blot, ThT fluorescence, far-UV CD and ANS fluorescence on ADDLs in the presence and absence of PrP<sub>107–120</sub>, using the same samples used for cell toxicity.

The presence of Aβ<sub>42</sub> ADDLs was monitored by a dot-blot immunoassay using the conformation-sensitive antibody 19.3 specific for Aβ<sub>42</sub> ADDLs [66] and the monoclonal antibody 6E10, which is able to bind all types of Aβ<sub>42</sub> species. For Aβ<sub>42</sub> ADDLs, Aβ<sub>42</sub> + PrP<sub>107–120</sub> and Aβ<sub>42</sub> ADDLs + PrP<sub>107–120</sub> we observed a recognition for both antibodies, whereas the PrP<sub>107–120</sub> spot did not show any cross-reaction (Figure 8A), suggesting that PrP<sub>107–120</sub> cannot change the structure or oligomerization state of ADDLs.

**Figure 8.** Effect of PrP<sub>107–120</sub> on Aβ<sub>42</sub> ADDL structure. (A) Dot-blot immunoassay for Aβ<sub>42</sub> ADDLs, Aβ<sub>42</sub> + PrP<sub>107–120</sub>, Aβ<sub>42</sub> ADDLs + PrP<sub>107–120</sub> and PrP<sub>107–120</sub> samples. All samples were initially in 2% (v/v) DMSO and F-12 Ham medium at concentrations of 100 and 25 µM (m.e.) for Aβ<sub>42</sub> and PrP<sub>107–120</sub>, respectively. They were then spotted in two different nitrocellulose membranes, probed with the conformation-sensitive anti-ADDL antibody 19.3 (first line) and with conformation-insensitive anti-Aβ<sub>42</sub> antibody 6E10 (second line). (B) ThT fluorescence assay for free ThT (blue), Aβ<sub>42</sub> ADDLs (black), Aβ<sub>42</sub> + PrP<sub>107–120</sub> (red) and Aβ<sub>42</sub> ADDLs + PrP<sub>107–120</sub> (green). Samples were incubated as described above. ThT assay was carried out at 22 µM ThT (final concentration in the cuvette), pH 6.0, 37 °C. (C) Far-UV CD spectra for Aβ<sub>42</sub> ADDLs (black), Aβ<sub>42</sub> + PrP<sub>107–120</sub> (red) and Aβ<sub>42</sub> ADDLs + PrP<sub>107–120</sub> (green) and PrP<sub>107–120</sub> (purple). Samples were incubated as described above and diluted before spectrum acquisition to final concentrations of 22.2 µM Aβ<sub>42</sub> and 5.55 µM PrP<sub>107–120</sub>, 25 °C. (D) ANS fluorescence spectra for free ANS (55 µM final concentration) and the same samples indicated in panel C. Fluorescence spectra were recorded at 25 °C.

Aβ<sub>42</sub> ADDLs did not bind ThT and did not increase its fluorescence, behavior that was not found to be affected by the PrP<sub>107–120</sub> peptide (Figure 8B) and suggests that the peptide was not able to change
the structure of the ADDLs into a stable and ThT-positive β-sheet structure. Moreover, the CD spectra observed for the Aβ_{42} + PrP_{107–120} and Aβ_{42} ADDLs + PrP_{107–120} were found to be similar to those resulting from the sum of the spectra of Aβ_{42} ADDLs alone and PrP_{107–120} alone, indicating that the PrP_{107–120} peptide did not significantly change the secondary structure of Aβ_{42} ADDLs (Figure 8C). Finally, the spectrum of ANS in the presence of Aβ_{42} ADDLs did not change if the ADDLs were formed in the presence of the peptide (Aβ_{42} + PrP_{107–120}) or pre-incubated in the presence of the peptide after their formation (Aβ_{42} ADDLs + PrP_{107–120}), suggesting again that the PrP_{107–120} peptide was not able to change the Aβ_{42} ADDL structure (Figure 8D).

2.9. PrP_{107–120} Does Not Colocalise with Aβ_{42} ADDLs but Partially Colocalises with Cellular PrP^C in SH-SY5Y Cells

The inability of PrP_{107–120} to change the structure of Aβ_{42} ADDLs, yet its ability to decrease ADDL toxicity, raised two possible hypotheses: (i) PrP_{107–120} binds to ADDLs and shields their hydrophobic patches that are responsible for toxicity, or (ii) it interacts with the cells and renders them less vulnerable to ADDL toxicity. To verify which hypothesis was correct, the interaction of PrP_{107–120} and Aβ_{42} was studied in cell cultures. Confocal microscopy images showed an absence of co-localization between Aβ_{42} ADDLs (3 µM m.e.) and PrP_{107–120}, both with ratios of 4:1 (0.75 µM PrP_{107–120}) and 1:1 (3 µM PrP_{107–120}), suggesting an absence of interaction between the two peptides (Figure 9). By contrast, PrP_{107–120} (0.75 µM) was found to partially colocalize with endogenous cellular PrP^C (Figure 10, white arrows). These results also showed a significant, albeit weak, expression of PrP^C in our SH-SY5Y cell system, which is in agreement with the levels of expression of the Human Protein Atlas (Figure 10).

![Figure 9. Absence of co-localization of Aβ_{42} ADDLs with PrP_{107–120}. Cells were treated for 1 h with the same samples described in the Figure 4 legend and indicated on top of the images (added to the cell medium). PrP_{107–120} was labelled with BODIPY TMR-X NHS Ester (red fluorescence), Aβ_{42} ADDLs with mouse monoclonal primary antibody 6E10 and a secondary antibody (green fluorescence) and the nuclei with the Hoechst dye (blue fluorescence). Scale bar = 10 µm.](image-url)
Figure 10. Partial co-localization of PrP_{107-120} with endogenous cellular PrP\textsuperscript{C}. Cells were treated for 1 h with the PrP\textsubscript{107–120} sample described in the Figure 9 legend and indicated on top of the images (added to the cell medium). PrP\textsubscript{107-120} was labelled with BODIPY TMR-X NHS Ester (red fluorescence) and cellular PrP\textsuperscript{C} was labelled with mouse monoclonal primary antibody PrP (5B2) and Alexa Fluor 488-conjugated anti-mouse secondary (green fluorescence). The white arrows indicate the co-localization spots in the merged images. Scale bar = 10 µm.

3. Discussion

The results obtained here show that the synthetic peptide PrP\textsubscript{107–120} is soluble and unstructured in solution and can significantly protect neuroblastoma SH-SY5Y cells against a representative form of oligomeric A\textsubscript{β42}, namely, ADDLs. In particular, in the presence of the peptide, A\textsubscript{β42} oligomers caused a remarkably lower \textsuperscript{Ca}^{2+} influx from the cellular medium to the cytosol, a remarkably lower increase of cellular ROS and a significantly lower alteration of mitochondrial metabolic activity. Furthermore, they had a remarkably lower ability to enter the cytosol across the cell membrane. The absence of interaction of the PrP\textsubscript{107–120} peptide with ADDLs, and the partial colocalization of the peptide with endogenous cellular PrP\textsuperscript{C}, indicates that the peptide protects the cells against A\textsubscript{β42} oligomer toxicity, at least in part, by interfering with cellular PrP\textsuperscript{C}.

Previous studies have suggested that the cell surface protein PrP\textsuperscript{C} mediates the toxicity of A\textsubscript{β42} oligomers by binding to them and affecting synaptic plasticity and other neuronal functions [13–18], although a general consensus on this point has not yet been found [19–21]. One PrP\textsuperscript{C} region of the sequence thought to be involved in A\textsubscript{β42} binding encompasses approximately residues 95–110 [13,14,33–35,37–42]. An antibody raised against residues 93–109 of PrP\textsuperscript{C} (anti-PrP\textsuperscript{C93–109} GD11) and a synthetic peptide corresponding to residues 98–107 (PrP\textsubscript{98–107}) were found to inhibit the toxicity of the A\textsubscript{β42} ADDLs to organotypic hippocampal slices, whereas a control PrP\textsubscript{213–230} peptide did not have any such effects [37]. It was proposed that the antibody and the PrP\textsubscript{98–107} peptide exert their effect by binding to PrP\textsuperscript{C} and the diffusible A\textsubscript{β42} oligomers, respectively, thus preventing their interaction in both cases [37].

In this study we have used a different perspective: Rather than using a synthetic PrP\textsuperscript{C} peptide binding to A\textsubscript{β42}, we have used a synthetic PrP\textsuperscript{C} peptide involved in PrP self-recognition (PrP\textsubscript{107–120}), with the goal of achieving a similar biological result with a different mechanism, namely, binding to membrane-anchored PrP\textsuperscript{C} and impeding the binding of the latter to A\textsubscript{β42} oligomers. We found that PrP\textsubscript{107–120} significantly reduced the toxicity of A\textsubscript{β42} oligomers with or without pre-incubation of the peptide with A\textsubscript{β42} during the process of ADDL oligomer formation. Biophysical analyses and a dot-blot immunoassay excluded an alteration of the A\textsubscript{β42} ADDL structure or aggregation state in the
presence of the peptide, ruling out the hypothesis that the reduction of Aβ42 oligomer toxicity and internalization mediated by PrP107-120 is due to this reason. Moreover, no PrP107-120 colocalization with Aβ42 was found in the cell cultures using confocal microscopy even at high concentrations of PrP107-120, indicating a lack of any significant interaction between the two peptide species. In contrast, a partial colocalization between PrP107-120 and endogenous PrP C was found, indicating that the peptide can bind to the cellular prion protein. Furthermore, akin to previous observations, the role of PrP C in mediating oligomer toxicity is not restricted to Aβ42 oligomers, but also to other β-sheet rich proteins [67], and our results indicate a similar effect of PrP107-120 on Aβ42 oligomers and type-A HypF-N oligomers, used here as a positive control of toxic oligomeric species. However, we cannot completely exclude that PrP107-120 stimulates a well-defined intracellular signaling [25] and causes less vulnerability and greater resistance against Aβ42 oligomer toxicity independently of its interaction with membrane-anchored PrP C.

Overall, although the results obtained here in vitro on a cell culture line need to be validated in vivo on animal models, the present study shows the potential therapeutic value of peptides corresponding to the region of the sequence of PrP C involved in self-recognition, or other molecules potentially mimicking the same sequence trait, for the treatment of AD. This could open new avenues to the identification of the mechanism of interaction of soluble prion-derived peptides and Aβ42 oligomers, as well as to the mechanism through which PrP C mediates the toxic effects of Aβ42 oligomers.

4. Materials and Methods

4.1. PrP107–120 Preparation

The synthetic human prion protein fragment spanning residues 107–120 (PrP107–120) with the sequence Ac-TNMTHAGAAAAGA (purity by HPLC > 95%) was purchased from Biomatik (Wilmington, DE, USA). The <5% impurities are mainly peptides with similar sequences, as are often found in solid-state peptide synthesis [68]. The counterions of peptide preparations (trifluoroacetate and guanidinium) are not supposed to be cell protectors and did not interfere with our analysis. Metal ions or other agents were not present. The lyophilized peptide was stored at −20 °C, and for each experiment 1 mg of peptide was dissolved in 1 mL of water.

4.2. Preparation of Aβ42 ADDLs, Aβ42 ADDLs + PrP107–120 and Aβ42 + PrP107–120 Samples

Lyophilized synthetic Aβ42 in a trifluoroacetate salt (Bachem, Bubendorf, Switzerland) was dissolved in pure hexafluoro-2-isopropanol (HFIP) to 1 mM. For each experiment, the solvent was evaporated using gentle nitrogen flow and the peptide was reconstituted in 2% (v/v) dimethyl sulfoxide (DMSO) and F12 Ham medium to a final concentration of 100 µM. Aβ-derived diffusible ligands (ADDLs) were prepared after 24 h incubation at 4 °C, as previously reported [52], and checked for their distinctive characteristics using atomic force microscopy, Western blotting and dot-blot, as previously described [56,57]. In this study, we used the following samples: (A) 100 µM m.e. of Aβ42 ADDLs; (B) 25 µM PrP107–120 (final concentration) added to preformed 100 µM m.e. of Aβ42 ADDLs at a 1:4 molar ratio, incubated at 4 °C for 2 h (Aβ42 ADDLs + PrP107–120); (C) 25 µM PrP107–120 (final concentration) added to 100 µM Aβ42 at a 1:4 molar ratio before Aβ42 aggregation, maintained at 4 °C for 24 h under the same conditions used to form Aβ42 ADDLs (Aβ42 + PrP107–120); and (D) 25 µM PrP107–120. All four samples were in 2% (v/v) DMSO and F-12 Ham medium and were diluted 33-fold before each experiment into cellular medium without DMSO to final concentrations of 3 and 0.75 µM (m.e.) for Aβ42 and PrP107–120, respectively.

4.3. Preparation of HypF-N Oligomers

HypF-N was purified as described previously [62] and stored at −80 °C. Before each experiment, the protein sample was thawed, centrifuged at 13,000 rpm (17950× g) for 10 min, and the concentration was measured at 280 nm. The sample was then diluted to 48 µM in 12% (v/v) trifluoroethanol, 50 mM
acetate buffer and 2 mM dithiothreitol, at pH 5.5, as this condition is known to promote aggregation of HypF-N and proteins of the same structural family [62,69]. After 4 h at 25 °C, the sample was centrifuged at 12,000 rpm (15,300 × g) for 15 min and the pellet was dried with a gentle nitrogen flow and resuspended to 12 µM m.e. in cellular medium with or without PrP_{107–120}, to a final concentration of 3 µM (4:1 molar ratio).

4.4. Bicinchoninic Acid (BCA) Assay

Stock bovine serum albumin (BSA) standard (Sigma-Aldrich, Saint Louis, MO, USA) was prepared at a 2-mg/mL final concentration in water. Eight BSA samples with concentrations ranging from 0 to 2000 µg/mL were prepared by dilution for the standard curve. The bicinchoninic acid (BCA) working reagent was prepared by mixing 10 mL of solution A (Bicinchoninic Acid solution, Sigma-Aldrich) with 200 µL of solution B (4% w/v CuSO_{4}·5H_{2}O in water). The blank and protein samples were mixed with the resulting BCA working reagent at a 1:8 ratio; thus, 25 µL of each peptide or BSA or blank samples were mixed with 200 µL of BCA working reagent and were added to 96-microplate wells. The plate was incubated at 60 °C for 15 min and, after 5 min cooling to room temperature, the absorbance of all wells was measured at 562 nm using an ultrafast BioTek Synergy H1 plate reader (Winooski, VT, USA). All absorbance values were blank subtracted. Peptide concentration was calculated by interpolation using a standard curve (absorbance versus protein concentration) obtained with the eight BSA samples.

4.5. Fibrillation of PrP_{107–120}

The aggregation kinetics of PrP_{107–120} was investigated under different solution conditions at 37 °C: (A) 0.5 mg/mL peptide in 20 mM phosphate buffer, 200 mM NaCl, pH 7.0; (B) 0.5 mg/mL peptide in 20 mM HCO_{3}−, 200 mM NaCl, pH 10.5; (C) 0.5 mg/mL peptide in 20 mM phosphate buffer, 200 mM NaCl, 10% (v/v) trifluoroethanol, pH 7.0; (D) 1.0 mg/mL peptide in 20 mM acetate buffer, 200 mM NaCl, pH 4.0; (E) 1.0 mg/mL peptide in 20 mM HCO_{3}−, 200 mM NaCl, pH 10.5; (F) 1.0 mg/mL peptide in 20 mM phosphate buffer, 500 mM NaCl, pH 7.0; (G) 1.0 mg/mL peptide in 20 mM phosphate buffer, 15% (v/v) methanol, pH 7.0; (H) 1.0 mg/mL peptide in 20 mM phosphate buffer, 200 mM Na_{2}SO_{4}, pH 7.0. Fibril formation was monitored by the ThT fluorescence assay, DLS and CD spectroscopy for all conditions.

4.6. Dynamic Light Scattering

In a first experiment, to determine the size of monomeric PrP_{107–120}, 1 mg of peptide was dissolved in 1 mL of water. The sample was filtered through a 0.22-µm filter and size distribution analysis was performed at 25 °C by a Zetasizer Nano S DLS device from Malvern Panalytical (Malvern, Worcestershire, UK) thermostated with a Peltier system and checked for its reliability with polystyrene latex beads with known hydrodynamic diameter. A 10-mm plastic cell with a reduced volume was used. The refractive index and viscosity were 1.333 and 0.88 cP. The cell position and attenuator index were set automatically. The theoretical R_{h} for a peptide was calculated by the following equation:

\[
R_{h} (\text{Å}) = (2.21 \pm 1.07) N^{0.57} \pm 0.02
\]

where N is the number of amino acid residues [46]. The theoretical D_{h} was twice the R_{h} value. In a second experiment, to investigate the effect of different conditions on peptide aggregation, measurements were carried out using 1 mg of peptide dissolved in 1 mL of selected buffers and following the size distribution over time for several days at 37 °C, using the same technical apparatus and cell described above. The refractive index and viscosity were set according to the various conditions.

4.7. ThT Fluorescence

ThT (Sigma-Aldrich) was dissolved to 25 µM, in 25 mM phosphate buffer, with a pH of 6.0, then filtered by a 0.45-µm filter. For a given peptide sample, 60 µL aliquots were added to 440 µL of resulting
solution. A 2 × 10 mm optical path-length cuvette was used, and fluorescence spectra were recorded at 37 °C using a PerkinElmer LS 55 fluorimeter (Waltham, MA, USA) equipped with a thermostated cell holder attached to a Thermo Haake C25P water bath (Karlsruhe, Germany) with an excitation wavelength of 440 nm and an emission wavelength range of 460–600 nm.

4.8. Circular Dichroism (CD) Spectroscopy

To determine the PrP107-120 polymerization kinetics in the presence of different conditions, 0.2 mg/mL of PrP107-120 peptide was used. To investigate the effect of the PrP107–120 peptide on Aβ42 oligomeric ADDLs, three samples were prepared, each 300 µL, including two separate samples of 22.2 µM Aβ42 ADDLs and one sample containing both 22.2 µM Aβ42 and 5.55 µM PrP107–120 at a 4:1 molar ratio. For removing DMSO, dialysis was performed using Spectra/Por 3 dialysis kits (MWCO 3.5 kDa, Spectrum Labs/Thermo Fisher Scientific, Waltham, MA, USA) and 10 mM phosphate buffer, with a pH of 6.0, for 3 h at 4 °C. For the preparation of Aβ42 ADDLs + PrP107–120 sample, PrP107–120 was added at a final concentration of 5.55 µM after dialysis and was incubated for 2 h at 4 °C. Far-UV CD spectra were measured at 25 °C between 190–260 nm with a 1-nm spectral step size and 50-nm/min scanning rate on a JASCO J-810 spectropolarimeter (Tokyo, Japan) equipped with thermostated cell-holder attached to a Thermo Haake C25P water bath (Karlsruhe, Germany). A 1-mm thermostated quartz cuvette was used for all CD spectra. Spectra were blank-subtracted and normalized to mean residue ellipticity.

4.9. Cell Culture

Adherent human neuroblastoma SH-SY5Y cells (A.T.C.C, Manassas, VA, USA) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Sigma-Aldrich), F-12 Ham with 25 mM N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic) acid (HEPES) and NaHCO3, supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and 1% penicillin/streptomycin and maintained at 37 °C and 5% CO2. When the cells reached 90% confluence, they were split using 0.25% trypsin-EDTA to a maximum of 20 passages.

4.10. MTT Assay

SH-SY5Y cells were plated at a density of 15 × 10^3 cells per well in a 96-well plate. After 24 h at 37 °C in a 5% CO2 atmosphere, cells were incubated for 24 h with different samples (Aβ42 ADDLs, Aβ42 + PrP107-120, Aβ42 ADDLs + PrP107-120 and PrP107-120, all pre-dissolved and pre-incubated in 2% (v/v) DMSO and F-12 Ham medium, as described above). Final concentrations of Aβ42 and PrP107-120 in DMEM were 3 and 0.75 µM (m.e.), respectively, with a 4:1 molar ratio. Cells were incubated with 0.5 mg/mL of MTT solution in Roswell Park Memorial Institute (RPMI) medium for 3 h at 37 °C, then with lysis buffer (20% SDS, 50% N,N-dimethylformamide, pH 4.7) for 1 h at 37 °C. The optical density was measured at 590 nm by a microplate reader (BioTek, Winooski, VT, USA).

4.11. Measurement of Intracellular Ca^{2+} Levels

SH-SY5Y cells were seeded on a glass coverslip in a six-well plate at a density of 40 × 10^3 cells per well. After 24 h, 600 µL of various samples (Aβ42 ADDLs, Aβ42 + PrP107-120, Aβ42 ADDLs + PrP107-120 and PrP107-120, all pre-dissolved and pre-incubated in 2% (v/v) DMSO and F-12 Ham medium, as described above) were added to cells for 1 h at 37 °C. Final concentrations of Aβ42 and PrP107-120 in DMEM were 3 and 0.75 µM (m.e.), respectively. After washing with phosphate buffered saline (PBS), cells were loaded with a 4-µM Fluo-4 AM probe (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 37 °C. Imaging was performed after excitation at 488 nm with a TCS SP8 confocal scanning microscope system (Leica Microsystems, Mannheim, Germany), using a Leica Plan Apo 63x oil immersion objective, taking a series of 1-µm-thick optical sections (1024 × 1024) through the cell depth for each sample and projecting them as a single composite image by superimposition.
A minimum of four images were captured for each sample and four replicates were used for each condition. Images were analyzed using Image J software (NIH, Bethesda, MD, USA).

4.12. Measurement of Intracellular Reactive Oxygen Species (ROS)

To detect intracellular accumulations of ROS, SH-SY5Y cells were cultured for 24 h on glass coverslips in a 6-well plate at a density of $40 \times 10^3$ cells per well. The medium was then replaced with 600 µL of various samples (Aβ42 ADDLs, Aβ42 + PrP<sub>107–120</sub>, Aβ42 ADDLs + PrP<sub>107–120</sub> and PrP<sub>107–120</sub>, all pre-dissolved and pre-incubated in 2% (v/v) DMSO and F-12 Ham medium, as described above) with final concentrations of 3 and 0.75 µM (m.e.) for Aβ42 and PrP<sub>107–120</sub> in DMEM, respectively. After 45 min, 5 µM of 2,7-dichlorodihydrofluorecein diacetate probe (CM-H2FDA, Thermo Fisher Scientific, Waltham, MA, USA) was added for 15 min at 37 °C. Finally, cells were washed twice in PBS and then fixed in 2% (v/v) paraformaldehyde for 10 min at room temperature. Cell image acquisition was performed using the TCS SP8 confocal system described in Section 4.11. All measurements were performed in triplicates and ROS levels were calculated with Image J software (NIH). The ROS detected with this probe included mainly hydrogen peroxide [70].

4.13. Immunofluorescence Staining

SH-SY5Y cells were seeded in glass coverslips for 24 h in a 6-well plate at a density of $40 \times 10^3$ cells per well, then exposed to 600 µL of various samples (Aβ42 ADDLs, Aβ42 + PrP<sub>107–120</sub>, Aβ42 ADDLs + PrP<sub>107–120</sub> and PrP<sub>107–120</sub>, all pre-dissolved and pre-incubated in 2% (v/v) DMSO and F-12 Ham medium, as described above) with final concentrations of 3 and 0.75 µM (m.e.) for Aβ42 and PrP<sub>107–120</sub> in DMEM, respectively. After 1 h at 37 °C, cells were washed with PBS and stained with 1:1000 diluted Alexa Fluor 633-conjugated wheat germ agglutinin (Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA) for 15 min. Cells were rinsed again and fixed with 2% (v/v) paraformaldehyde for 10 min at room temperature. After cell membrane permeabilization with 0.5% BSA in PBS + 0.5% Triton X-100, cells were incubated with 1:800 diluted mouse monoclonal 6E10 antibody (BioLegend, San Diego, CA, USA) for 1 h in 37 °C. After washing three times with PBS, cells were incubated with 1:1000 diluted Alexa Fluor 488-conjugated anti-mouse secondary antibody (Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA) for 1 h. All experiments were repeated three times and representative images of confocal microscopy are presented. Fluorescence was quantified with Image J software (NIH).

4.14. Dot Blot

To determine the effect of PrP<sub>107–120</sub> on ADDLs structure, 2 µL of each sample (Aβ42 ADDLs, Aβ42 + PrP<sub>107–120</sub>, Aβ42 ADDLs + PrP<sub>107–120</sub> and PrP<sub>107–120</sub>, all dissolved in 2% (v/v) DMSO and F-12 Ham medium, as described above) were transferred onto a nitrocellulose membrane and allowed to dry for 15 min. For blocking the membrane, 1% (w/v) BSA in Tris-buffered saline and 0.1% Tween 20 (TBST) was used. After 1 h, the membrane was probed with 1:800 diluted mouse monoclonal antibody 6E10 (BioLegend, San Diego, CA, USA) or with 1:500 human antibody specific to ADDLs (clone 19.3, Creative Biolabs, Shirley, NY, USA) at 4 °C overnight. The day after, the membrane was washed three times and subsequently incubated for 1 h at room temperature with 1:3000 peroxidase-conjugated anti-mouse secondary antibody (Abcam, Cambridge, MA, USA) or 1:1000 peroxidase-conjugated anti-human secondary antibody (EMD Millipore, Temecula, CA, USA). Imaging was performed using an Amersham imager 600 (Cytiva, Washington DC, MD, USA).

4.15. ANS Binding Assay

The ANS solution was prepared by dissolving 30 mg of 8-anilino-1-naphthalenesulfonic acid (ANS, Sigma-Aldrich) in 10 mL of 25-µM phosphate buffer, with a pH of 6.0. After 20 min shaking, the solution was filtered by a 0.45-µm filter. ANS concentration was measured by optical absorbance at 375 nm and diluted in the same buffer to 55 µM. For each experiment, 450 µL of ANS solution was mixed with 50 µL of each sample (Aβ42 ADDLs, Aβ42 + PrP<sub>107–120</sub>, Aβ42 ADDLs + PrP<sub>107–120</sub> and
PrP$_{107-120}$, all dissolved in 2% (v/v) DMSO and F-12 Ham medium, as described above) or buffer as a blank. A 2 × 10-mm optical path-length was used and fluorescence spectra were recorded at 25 °C using the same PerkinElmer LS 55 fluorimeter described above, with excitation at 380 nm and emission at 400–650 nm.

4.16. Analysis of ADDLs Co-Localization with PrP$_{107-120}$

BODIPY TMR-X NHS Ester (Thermo Fisher Scientific, Waltham, MA, USA) was dissolved in DMSO. PrP$_{107-120}$ was dissolved in 0.1 M NaHCO$_3$ buffer, with a pH of 7.0. The two solutions were diluted at room temperature with continuous shaking for 1 h in the latter buffer at final concentrations of 3 mM peptide and 0.3 mM dye. Cells were cultured on glass coverslips in a 6-well plate at a density of 40 × 10$^3$ cells per well. After 24 h, cells were treated for 1 h with 600 µL of various samples (Aβ$_{42}$ ADDLs, Aβ$_{42}$ + labelled PrP$_{107-120}$, Aβ$_{42}$ ADDLs + labelled PrP$_{107-120}$ and labelled PrP$_{107-120}$, all pre-dissolved and pre-incubated in 2% (v/v) DMSO and F-12 Ham medium, as described above) with final concentrations of 3 and 0.75 µM (m.e.) for Aβ$_{42}$ and labelled PrP$_{107-120}$ in DMEM, respectively. After rinsing with PBS twice, 1:100 diluted fluorescent dye Hoechest (Immunochemistry Technologies, Bloomington, MN, USA) was added for 10 min at 37 °C. Following four washing steps with PBS, cells were fixed using 2% paraformaldehyde. Permeabilization of cells and Aβ$_{42}$ staining were performed as described above. In another experiment, the concentration of labelled PrP$_{107-120}$ was increased to 100 µM, and a 1:1 ratio for labelled PrP$_{107-120}$ to Aβ$_{42}$ was used in the initial pre-treatment in 2% (v/v) DMSO and F-12 Ham medium to prepare the Aβ$_{42}$ + labelled PrP$_{107-120}$ sample.

4.17. Analysis of PrP$_{107-120}$ Co-Localization with PrP$_{C}$

PrP$_{107-120}$ was labelled with BODIPY TMR-X NHS Ester, as described in Section 4.16. SH-SY5Y cells were cultured on a glass coverslip in a 6-well plate for 24 h at a density of 40 × 10$^3$ cells per well. After washing with PBS, cells were treated with 600 µL of labelled peptide at a final concentration of 0.75 µM for 1 h. After washing with PBS twice, 2% paraformaldehyde was added for 10 min. After fixation, cells were incubated with 1:250 diluted mouse monoclonal PrP (5B2) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at 37 °C. After washing with PBS, cells were incubated with 1:1000 diluted Alexa Fluor 488-conjugated anti-mouse secondary antibody (Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA). Imaging was performed after excitation at 488 and 561 nm to detect the cellular prion protein and the PrP$_{107-120}$ peptide, respectively, using the TCS SP8 confocal system described in Section 4.11.

4.18. Statistical Analysis

All data are presented as means ± S.E.M. (standard error of the mean). The difference between groups was analyzed using a Student’s t-test. The single (*#), double (**/##) and triple (**/*##) symbols refer to $p$ values < 0.05, < 0.01 and < 0.001, respectively.

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Abbreviations

- AD: Alzheimer’s disease
- PrPC: Cellular prion protein
- Aβ: Amyloid beta
- LTP: Long-term potentiation
- GPI: Glycosyl-phosphatidyl-inositol
- R_h: Hydrodynamic radius
- D_h: Hydrodynamic diameter
- ADDLs: Amyloid-derived diffusible ligands
- DMSO: Dimethyl sulfoxide
- HFIP: Hexafluoro-2-isopropanol
- ThT: Thioflavin T
- CD: Circular dichroism
- DLS: Dynamic light scattering
- DMEM: Dulbecco’s Modified Eagle’s Medium
- FBS: Fetal bovine serum
- MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
- ROS: Reactive oxygen species
- RPMI: Roswell Park Memorial Institute
- BSA: Bovine serum albumin
- BCA: Bicinchoninic acid

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