A D-enantiomeric peptide interferes with hetero-association of amyloid-β oligomers and prion protein*

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ABSTRACT

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that affects millions of people worldwide. One AD hallmark is the aggregation of amyloid-β (Aβ) into soluble oligomers and insoluble fibrils. Several studies have reported that oligomers rather than fibrils are the most toxic species in AD progression. Aβ oligomers bind with high affinity to membrane-associated prion protein (PrP), leading to toxic signaling across the cell membrane, which makes the Aβ–PrP interaction an attractive therapeutic target. Here, probing this interaction in more detail, we found that both full-length, soluble human (hu) PrP(23–230) and huPrP(23–144), lacking the globular C-terminal domain, bind to Aβ oligomers to form large complexes above the megadalton size range. Following purification by sucrose density-gradient ultracentrifugation, the Aβ and huPrP contents in these hetero-assemblies were quantified by reversed-phase HPLC. The Aβ:PrP molar ratio in these assemblies exhibited some limited variation depending on the molar ratio of the initial mixture. Specifically, a molar ratio of about four Aβ to one huPrP in the presence of an excess of huPrP(23–230) or huPrP(23–144) suggested that four Aβ units are required to form one huPrP-binding site. Of note, an Aβ-binding all-D-enantiomeric peptide, RD2D3, competed with huPrP for Aβ oligomers and interfered with Aβ–PrP hetero-assembly in a concentration-dependent manner. Our results highlight the importance of multivalent epitopes on Aβ oligomers for Aβ–PrP interactions and have yielded a new all-D-peptide-based, therapeutically promising agent that competes with PrP for these interactions.

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly population. One of its hallmarks is the accumulation of extracellular neuritic plaques consisting mainly of fibrillar amyloid-β peptide (Aβ) (1). Initially, these plaques were thought to be the toxic species in AD, but several lines of evidence now indicate that the levels of soluble oligomeric forms of Aβ (Aβ oligo) correlate best with the neurotoxic effects observed during AD (2, 3).

Many Aβ receptors have been described to date (4). One of them is the cellular prion protein (PrPc), which binds Aβ oligo with nanomolar affinity (5–10). PrPc is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein highly expressed in the brain. PrPc itself can misfold into the scrapie isoform PrPsc sporadically or after infection, leading to neuronal damage and disease such as the transmissible spongiform encephalopathies (11). The interaction of Aβ oligo with PrPc bound to the metabotropic glutamate receptor 5 (mGluR5) leads
to toxic signalling across the cell membrane by activating intracellular Fyn kinase (12, 13). Fyn kinase phosphorylates N-methyl-D-aspartate (NMDA) receptors (14, 15) and alters the NMDA receptor localisation leading to destabilisation of dendritic spines (12). Furthermore, Fyn kinase hyperphosphorylates the tau protein, which assemblies into neurofibrillary tangles, a further hallmark of AD (16). Hyperphosphorylation of tau is dependent on the Aβ-PrP interaction (17). Therefore, understanding the Aβ-PrP pathway will open new therapeutic strategies by targeting the Aβ-PrP interaction (18).

The binding regions of Aβ_oligo have been mapped to residues 23-27 and ~95-110 in the N-terminal part of PrP (5, 19–22) (Fig. 1A). Soluble N-terminal PrP fragments inhibit the assembly of Aβ into amyloid fibrils and block neurotoxic effects of soluble oligomers (20, 23), presumably by competing with membrane-anchored PrP^C for Aβ_oligo. This competition might also explain the suggested neuroprotective function of the naturally produced soluble N1 fragment (amino acids 23-110/111) of PrP (24), which contains both Aβ_oligo-binding regions. The binding regions on Aβ, however, could not be identified so far and might constitute a specific conformational epitope of Aβ_oligo (21). All these data show that the Aβ-PrP interaction is a promising point of intervention to prevent the toxic signalling in AD.

In the past years, we could identify a number of D-enantiomeric peptides as promising drug candidates for direct elimination of Aβ(1-42)_oligo (25–30). The advantage of D-peptides over L-peptides is their higher protease resistance, resulting in slower degradation and longer half-life (31, 32). For Aβ(1-42)-directed D-peptides, high stability in media simulating the route of orally administered drugs (33) and enhanced proteolytic stability in murine plasma and organ homogenates was shown (34). The lead compound of these D-peptides, D3, had been selected by mirror-image phage display (26, 35). D3 and its tandem version, D3D3, convert toxic Aβ species into non-toxic species (25, 28). Treatment with D3 reduces the number of amyloid plaques (26) and improves cognition in transgenic AD mice (28). One derivative of D3 called RD2 shows enhanced binding to Aβ (36, 37), and both RD2 and D3 have demonstrated desirable pharmacokinetic properties (29, 38). A further promising derivative is the D-peptide RD2D3, a head-to-tail tandem combination of RD2 and D3 (30, 34, 39). RD2D3 binds Aβ(1-42) with a K_D of 486 ± 69 nM (39). All of these therapeutically promising D-peptides contain a high fraction of basic residues, which is reminiscent of the proposed binding sites for Aβ on PrP (5, 19–21). Therefore, the Aβ-binding D-peptides might be suitable compounds for interference with the Aβ-PrP interaction by competing with PrP for Aβ(1-42)_oligo.

Recently, we introduced the “quantitative determination of interference with Aβ aggregate size distribution” (QIAD) assay, which allows the determination of a compound’s efficacy to eliminate Aβ(1-42)_oligo (25). This assay enables the separation of Aβ assemblies by density gradient ultracentrifugation (DGC) and the quantification of these assemblies by UV-detected reversed-phase (RP)-HPLC. For the present study, we have refined the QIAD assay to achieve simultaneous quantification of Aβ(1-42), of recombinant anchorless human PrP (huPrP) constructs, and of D-peptides in a single RP-HPLC run in order to (i) characterise the Aβ-huPrP interaction in detail, and (ii) evaluate the influence of the tandem D-peptide RD2D3 on this interaction. We investigated four different huPrP protein constructs, namely huPrP(23-230), huPrP(23-144), huPrP(90-230), and huPrP(121-230) (Fig. 1A), and added them in different concentrations to preformed Aβ(1-42)_oligo-huPrP complexes. The separation of these complexes from Aβ(1-42) or huPrP monomers and Aβ(1-42)_oligo by sucrose DGC and subsequent RP-HPLC analytics (Fig. 1B) allowed the determination of molar ratios between Aβ(1-42) and huPrP within the complexes. We show that these ratios are dependent on the concentration of huPrP added. We imaged Aβ(1-42)_oligo-huPrP(23-144) complexes by atomic force microscopy (AFM) and observed a correlation between the applied huPrP(23-144) concentration and the size and morphology of the hetero-assemblies. We analysed the influence of the D-peptide RD2D3 on the Aβ(1-42)_oligo-huPrP(23-144) interaction by determining its effect on the Aβ(1-42)/huPrP ratio within the assemblies. We show that RD2D3 competes with the Aβ(1-42)_oligo-huPrP(23-144) interaction and might thus be a potential therapeutic agent.
RESULTS

\( A\beta(1-42)_{\text{oligo}} \) and all huPrP protein constructs are soluble when analysed separately - In addition to full-length huPrP (huPrP(23-230)) three huPrP deletion constructs were investigated: the N-terminal fragment huPrP(23-144), and the C-terminal fragments huPrP(90-230) and huPrP(121-230) (Fig. 1A). huPrP(23-230) and huPrP(23-144) contain both regions (residues 23-27 and ~95-110) described to be necessary for high-affinity binding of \( A\beta(1-42)_{\text{oligo}} \) (5, 19–22). In huPrP(90-230) the N-terminal binding region (residues 23-27) is missing, whereas in huPrP(121-230) the second binding region (residues ~95-110) is missing as well. All huPrP protein constructs were expressed in Escherichia coli (E. coli). Therefore they did not contain any posttranslational modifications (glycosylation, GPI anchor). They were purified either by immobilised metal ion affinity chromatography (IMAC) or by size exclusion chromatography (SEC) and subsequent RP-HPLC, yielding them as highly pure samples confirmed by SDS-PAGE and analytical RP-HPLC (Fig. S1). The purified huPrP(23-230), huPrP(90-230) and huPrP(121-230) proteins contain the disulfide bond between Cys-179 and Cys-214 as is confirmed by comparative RP-HPLC analyses of the purified vs. their TCEP reduced states (Fig. S2).

Before investigating the \( A\beta(1-42)_{\text{oligo}} \)-huPrP interaction, we checked the binding partners separately in their purified states to confirm that they remain soluble at the chosen buffer conditions, which were a compromise between neutral pH and conditions required for stability of \( A\beta \) oligomers and detergent-free solubility of huPrP constructs favouring absence of phosphate and low salt. This is of note as all huPrP protein constructs (40–43) as well as \( A\beta \) (44, 45) are able to convert into fibrils under certain conditions, and such a conversion may hamper the analysis of \( A\beta(1-42)_{\text{oligo}} \)-huPrP complexes. We performed CD spectroscopy analysis of huPrP(23-144), huPrP(23-230), and huPrP(90-230), solution NMR spectroscopy of huPrP(23-144) and huPrP(23-230), as well as AFM measurements of huPrP(23-144).

CD spectroscopy of huPrP(23-144) (Fig. 2A) indicated a disordered conformation at neutral pH consistent with reports in the literature (46). In addition, AFM measurements of huPrP(23-144) confirm the absence of fibrils or large aggregates. Here, huPrP(23-144) forms a thin film on the mica surface with a height of 1 nm to 2 nm. Very rarely, single amorphous particles were observed (Fig. 2B). CD spectroscopy of the C-terminal huPrP(90-230) construct and full-length huPrP(23-230) (Fig. 2A) both showed the predominantly \( \alpha \)-helical structures typical for the native prion protein fold (47) rather than the predominantly \( \beta \)-sheet conformation of amyloid fibrils.

The solubility and overall conformational properties of huPrP(23-230) and huPrP(23-144) were confirmed in more detail by solution NMR spectroscopy. The solution structure of huPrP had originally been determined in acetate buffer at pH 4.5 and 20 °C (47) and found to comprise a highly disordered N-terminal region (residues 23-124) followed by a globular C-terminal domain (residues 125-228) featuring three long \( \alpha \)-helices and a relatively small two-stranded antiparallel \( \beta \)-sheet. Under these buffer conditions we indeed obtained well-dispersed solution NMR spectra of excellent quality for huPrP(23-230) (Fig. S3A), with sharp narrow line shapes and chemical shifts similar to those reported in the literature (47), thereby demonstrating that the protein is soluble and natively folded. huPrP(23-144) also exhibits high-quality solution NMR spectra under these buffer conditions (Fig. S3A). As expected for huPrP(23-144), only backbone amide resonances in the random coil region (\( ^1H \) chemical shifts between about 8.0 ppm and 8.6 ppm; (48)) were observed (Fig. S3A), suggesting that not only the N-terminal region from residues 23 to 124 is highly disordered but that also residues 125 to 144 are disordered in huPrP(23-144). To build a bridge between the quality control of the huPrP samples done at pH 4.5 and the solution conditions used for the interaction studies done at pH 7, we investigated the pH dependence of the overall conformational properties of huPrP(23-144) by solution NMR spectroscopy in a series of pH steps from 4.5 to 7.0. Although the shift in protonation equilibrium of the seven histidine side chains upon increasing the pH was associated with readily identifiable chemical shift changes for several backbone amide resonances, the quality and overall appearance of the solution NMR spectra of huPrP(23-144), including the limited resonance dispersion indicative of a disordered conformation, remained
very similar over this pH range (Fig. S3B). Over the course of several days to weeks, the NMR samples did not show obvious signs of any significant formation of visible precipitate, nor of any deterioration, nor signal loss of the solution NMR spectra. To test for any sample degradation or aggregation in a more quantitative fashion, we monitored the intensity of 58 sufficiently well-resolved amide resonances of a sample of 89 µM [U-¹³⁵N] huPrP(23-144) in 50 mM Tris-HCl (pH 7.2) in 10 % D₂O in a series of [¹H,¹³⁵N] HSQC spectra recorded at 600 MHz, 5.0 °C, but no change in resonance intensity exceeding even a fraction of a percent was observed over the monitoring period of more than 48 hours (Fig. S3D). Taken together, these NMR-spectroscopic results show that huPrP(23-144) is readily soluble up to concentrations of about 0.3 mM, highly disordered in solution under mildly acidic to neutral buffer conditions, and remains soluble and disordered for at least several days at the conditions tested.

Aβ(1-42)ₜ₁₃₀ was prepared by incubating monomeric Aβ(1-42) in buffer at pH 7.4 for 2 h at 22 °C under agitation. AFM of the Aβ(1-42)ₜ₁₃₀ samples showed small spherical particles with heights of 1 nm to 6 nm, rarely up to 10 nm (Figs. 2C and S4), and confirmed that the chosen incubation conditions produce high amounts of Aβ(1-42)ₜ₁₃₀ without formation of Aβ(1-42) fibrils or larger aggregates.

Our assay for studying the Aβ(1-42)ₜ₁₃₀-huPrP interaction is based on the QIAD protocol (25). In the present work, this assay includes the separation of a sample containing Aβ(1-42) assemblies and/or huPrP by sucrose DGC, followed by qualitative and quantitative analysis of the interaction partners by SDS-PAGE and RP-HPLC (Fig. 1B). For calibration of the sucrose gradient standard proteins ranging from 43 kDa to 669 kDa were used (Fig. S5). Thyroglobulin, the reference protein with the highest molecular mass (669 kDa), was found in fractions 7 to 10 indicating that proteins, complexes, or aggregates that can be found in higher (and thus denser) fractions (11 to 14) must have molecular weights in the megadalton range or larger.

Initially, Aβ(1-42)ₜ₁₃₀ and huPrP were separately analysed by sucrose DGC. Either 80 µM of pre-incubated Aβ(1-42) or either 10 or 20 µM of the respective huPrP protein construct were applied on a sucrose gradient and centrifuged for 3 hours. Silver-stained Tris/Tricin SDS-PAGE gels as well as RP-HPLC quantification of all gradient fractions revealed the distribution of the proteins among the fractions and hence among different assembly states (Figs. 3 and S6A). The pre-incubated Aβ(1-42) sample showed a broad distribution of Aβ(1-42) within the sucrose gradient (Fig. 3, A and E), containing mainly Aβ(1-42)ₜ₁₃₀ (fractions 3 to 7) as well as residual monomeric Aβ(1-42) (fractions 1 and 2), as we have previously established (25). The highest concentrations of huPrP(23-230), huPrP(23-144), huPrP(90-230), as well as huPrP(121-230) were found in fractions 1 to 3 confirming that huPrP occurs predominantly in a soluble monomeric state. In addition, minor amounts of huPrP were present in fractions 4 to 6 and 11 to 14, the latter representing high molecular weight aggregates. AFM and CD spectroscopy (Fig. 2) suggest that these were nonfibrillar, amorphous aggregates.

Aβ(1-42)ₜ₁₃₀ forms high molecular weight hetero-assemblies with huPrP(23-230) as well as with huPrP(23-144) - After confirmation of the soluble, nonfibrillar state of all huPrP constructs as well as the size distribution of pre-incubated Aβ(1-42) we analysed the effect of huPrP binding on the size distribution of Aβ(1-42)ₜ₁₃₀. Aβ(1-42), pre-incubated to form Aβ(1-42)ₜ₁₃₀, was added to full-length huPrP(23-230) to yield samples with final concentrations of 80 µM Aβ(1-42) and 2 µM, 5 µM, 10 µM, or 20 µM huPrP(23-230) (concentrations refer to the samples before separation by sucrose DGC).

After DGC, each fraction was qualitatively analysed by SDS-PAGE as well as quantitatively analysed by RP-HPLC with respect to the Aβ(1-42) and huPrP(23-230) contents (Fig. 4). The increase of the applied concentration of huPrP(23-230) correlated with the decrease of the concentration of Aβ(1-42) in fractions 3 to 7 along with the increase of Aβ(1-42) concentration in fractions 11 to 14. For example, upon addition of 20 µM huPrP(23-230) the average Aβ(1-42) concentration fell from 6 µM to 0.3 µM in fractions 4 to 7 but rose from 0.1 µM to 15 µM in fractions 11 to 14 (Fig. 4). huPrP(23-230) was mainly detected in fractions 11 to 14 (see below). This clearly confirms direct interaction between huPrP(23-230) and Aβ(1-42)ₜ₁₃₀, huPrP(23-230) interaction was preferential with Aβ(1-42)ₜ₁₃₀, as the concentration of Aβ(1-42) monomers in fractions 1 and 2...
decreased only slightly with increasing huPrP(23-230) concentration. Moreover, instead of simply forming one-to-one complexes, which would be found not far away from fractions 3 to 7, huPrP(23-230) and Aβ(1-42)\textsubscript{oligo} form large supramolecular hetero-assemblies, which are located in fractions 11 to 14 and hence have molecular weights in or above the megadalton range (according to the calibration of the density gradient, Fig. S5).

In the absence of Aβ(1-42), huPrP(23-230) appeared in fractions 1 to 4 (Fig. 3, B and F). In contrast, in samples containing 10 µM or 20 µM huPrP(23-230) and 80 µM pre-incubated Aβ(1-42), the vast majority of huPrP(23-230) was observed in fractions 11 to 14 (Fig. 4, C, D and F), indicative of hetero-association with Aβ(1-42)\textsubscript{oligo}. Interestingly, at lower huPrP(23-230) concentrations of 2 µM and 5 µM, faint huPrP(23-230) bands were observed in fractions 4 to 10. This observation can be explained by an initial formation of smaller Aβ(1-42)\textsubscript{oligo}-huPrP(23-230) complexes, potentially of lower density, which convert to larger complexes when increasing the applied huPrP(23-230) concentration (see also Fig. 8).

The quantitative analysis of the DGC fractions by RP-HPLC enabled the determination of the molar ratios between Aβ(1-42) and huPrP(23-230) within the complexes (Table 1) calculated for every experiment from the total amount of Aβ(1-42) molecules and the total amount of huPrP(23-230) in fractions 11 to 14. Averaging over fractions 11 to 14 was necessary as the occurrence of the complexes in the individual fractions can slightly vary between experiments due to the manual fractionation of the gradients. This also explains the larger error bars of Aβ(1-42) and huPrP(23-230) in the complex-containing fractions 11 to 14 compared to the error bars in fractions 1 to 10.

Incubation of Aβ(1-42)\textsubscript{oligo} with 2 µM huPrP(23-230) led to an Aβ/PrP ratio of 12.1 ± 1.7 within the DGC purified complexes (Table 1). Application of higher huPrP(23-230) concentrations resulted in the decrease of the Aβ/PrP ratio due to higher huPrP(23-230) content within the hetero-assemblies. Incubation of Aβ(1-42)\textsubscript{oligo} with 20 µM huPrP(23-230) led to the Aβ/PrP ratio of 4.2 ± 0.9. At this applied concentration of huPrP(23-230), it can additionally be found in fractions 1 to 4 (Fig. 4, D and F), indicating that the PrP-binding capacity of Aβ(1-42)\textsubscript{oligo} is fully saturated such that an excess of huPrP(23-230) remains monomeric and unbound to Aβ(1-42)\textsubscript{oligo}. The saturaibility of the Aβ(1-42)\textsubscript{oligo}-huPrP(23-230) hetero-association indicates that it occurs by a defined binding mode and is not just an unspecified co-precipitation of both proteins.

The N-terminal PrP construct huPrP(23-144) shows a similar behavior as the full-length huPrP(23-230) upon interaction with Aβ(1-42)\textsubscript{oligo}. With increasing huPrP(23-144) concentrations ranging from 2 µM to 40 µM, Aβ(1-42)\textsubscript{oligo} in fractions 3 to 7 disappeared and higher Aβ(1-42) concentrations were detected in fractions 11 to 14 (Fig. 5) due to the formation of high molecular weight Aβ(1-42)\textsubscript{oligo}-huPrP(23-144) complexes. Formation of assemblies with molecular weights larger than the megadalton range was confirmed by dynamic light scattering (DLS), showing that the Aβ(1-42)\textsubscript{oligo}-huPrP(23-144) assemblies mainly have sizes from 0.6 to 6 µm (Fig. 6).

When huPrP(23-144) was added in final concentrations of 2 µM, 5 µM, 10 µM, and 20 µM, huPrP(23-144) was identified exclusively in fractions 11 to 14 after DGC. Upon application of 40 µM huPrP(23-144) to Aβ(1-42)\textsubscript{oligo} about 10 % of the detected huPrP(23-144) was found in fractions 1 to 4 (Fig. 5, F and H) indicating an excess of huPrP(23-144) and thus a saturation of Aβ(1-42)\textsubscript{oligo}-huPrP(23-144) complexes with huPrP(23-144). Although huPrP(23-144) was in excess at the applied concentration of 40 µM (Fig. 5, F and G), there was still some monomeric Aβ(1-42) left in fractions 1 and 2, again confirming that huPrP(23-144) forms complexes only with oligomeric but not with monomeric Aβ(1-42), an observation, which is in full accordance with previous studies (5, 19, 20).

Incubation of Aβ(1-42)\textsubscript{oligo} with 2 µM huPrP(23-144) resulted in the Aβ/PrP ratio of 10.1 ± 0.8 in the Aβ(1-42)\textsubscript{oligo}-huPrP(23-144) complexes (Table 1). Increasing the applied huPrP(23-144) concentration to 40 µM progressively lowered the Aβ/PrP ratio, down to a value of 3.93 ± 0.04. Further increase of the applied huPrP(23-144) concentration to 60 µM (Fig. S8) did not further decrease the Aβ/PrP ratio (4.04 ± 0.08, Table 1) in the high molecular weight complexes, in agreement with the Aβ/PrP ratio of ~4 observed when an excess of huPrP(23-230) was applied. This indicates that four Aβ units are
required to form one PrP binding site. The similar behaviour of huPrP(23-144) and huPrP(23-230) with respect to Aβ(1-42) 
oligo binding suggests that huPrP(23-144) contains all epitopes required for binding to Aβ(1-42) 
oligo in line with previous findings of other groups (5, 19–22).

We verified that the Aβ/PrP ratio of ~4 is constant when Aβ(1-42) 
oligo is saturated with huPrP, by adding different final Aβ(1-42) 
oligo concentrations of 20 µM, 40 µM, 60 µM, 80 µM to a saturating concentration (40 µM) of huPrP(23-144). At all Aβ(1-42) 
oligo concentrations the Aβ/PrP ratio in the hetero-associates was ~4 with deviations within the experimental error (Table 1).

Deletion of the huPrP N-terminal impairs Aβ(1-42) 
oligo-huPrP hetero-association - Besides the N-terminal huPrP(23-144) and the full-length huPrP(23-230), we further analysed the C-terminal PrP constructs huPrP(90-230) and huPrP(121-230), which lack the proposed Aβ binding site at position 23-27 and, in the case of huPrP(121-230), also the proposed binding site at position ~95-110 (Fig. 1A). At all applied concentrations of huPrP(90-230), ranging from 2 µM to 20 µM, the protein concentrations detected in fractions 11 to 14 were only slightly elevated (Fig. 7). Even in the presence of 20 µM huPrP(90-230) the majority of Aβ(1-42) 
oligo was still present in fractions 3 to 7. The majority of huPrP(90-230) was found in fractions 1 to 4 at all applied huPrP(90-230) concentrations, similar to the distribution of huPrP(90-230) without Aβ(1-42) 
oligo (Fig. 3, D and H). When 20 µM huPrP(90-230) was applied to Aβ(1-42) 
oligo only about 15 % of huPrP(90-230) was found in fractions 11 to 14. This is in contrast to the observations, when 20 µM huPrP(23-230) or huPrP(23-144) were applied to Aβ(1-42) 
oligo and about 96 % of huPrP(23-230) or 100 % of huPrP(23-144), respectively, were found in these fractions. Therefore, compared to huPrP(23-230) (Fig. 4) and huPrP(23-144) (Fig. 5), huPrP(90-230) (Fig. 7) is almost incapable of forming high molecular weight Aβ(1-42) 
oligo-huPrP complexes. This is in full agreement with previous studies that demonstrated the importance of both Aβ(1-42) 
oligo binding sites on PrP (19–22).

When 20 µM huPrP(121-230) was applied to Aβ(1-42) 
oligo even less Aβ(1-42) and huPrP were found in fractions 11 to 14 than in the case of huPrP(90-230) (Fig. S6, B and C). This demonstrates that the proposed binding site at position ~95-110, while on its own not sufficient for high-affinity interaction, does contribute to Aβ(1-42) 
oligo binding.

The morphology of Aβ(1-42) 
oligo-huPrP(23-144) hetero-assemblies is dependent on the huPrP concentration - For structural characterisation of the Aβ(1-42) 
oligo-huPrP complexes, we focused on the N-terminal huPrP(23-144) construct, as the PrP C-terminus is not required for the complex formation. Hetero-assemblies formed in the presence of different huPrP(23-144) concentrations ranging from 1 µM to 40 µM were analysed by AFM. Unbound Aβ(1-42) or huPrP(23-144) were removed by centrifugation and repeated washing steps, followed by imaging of the hetero-assemblies on mica in air using intermittent contact mode. Imaging was particularly challenging because the assemblies were several micrometres high with deep holes and high stickiness, which led to rapid contamination of cantilever tips. In order to display the different features of the samples in more detail, images were taken of the outer surfaces of the hetero-assemblies (Fig. 8), several hundred nanometres above the mica support.

For complexes prepared from 80 µM Aβ(1-42) and 1 µM huPrP(23-144) (Fig. 8B), we observed loose clusters of irregular spheres or globules, which again exhibited globular substructures. The clusters typically measured about 200 nm in height and up to 2.5 µm in width with substructures of 20 nm to 70 nm in height. Because of the curvature of the surfaces only a crude estimate of the size of these hetero-assemblies and their substructures was possible due to their clustering.

Hetero-assemblies prepared at an increased huPrP(23-144) concentration of 5 µM (Fig. 8C) were up to 500 nm high and had a more compact appearance, suggesting a tighter interaction between the sub-assemblies. The surface of the globular sub-assemblies seemed to be smoother in this case. When the huPrP(23-144) concentration for hetero-association was further raised to 40 µM (Fig. 8D) the resulting hetero-assemblies were up to 1 µm high, exhibiting globular sub-assemblies with smooth surface appearance and unresolved substructure. In all cases, Aβ(1-42) 
oligo-huPrP(23-144) hetero-assemblies (Figs. 8, B-D) were much larger than Aβ(1-42) 
oligo (Fig. 8A),
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Table 1

| Fraction | Aβ(1-42)/huPrP(23-144) Ratio |
|----------|-------------------------------|
| 1-3      | 4:1                           |
| 4-10     | 4:1                           |
| 11-14    | 1:4                           |

We have previously shown that an average Aβ(1-42) oligo consists of about 23 monomeric units (25). Combining this finding with the Aβ/PrP ratios determined here, the hetero-assemblies contain approximately six huPrP(23-230) or huPrP(23-144) molecules on average per Aβ(1-42) oligo in the presence of an excess of huPrP. Therefore, a simplified model of Aβ(1-42) oligo-huPrP assemblies can be drawn (Fig. 8, E and F). This model also considers the potential of huPrP to crosslink Aβ(1-42) oligo via its two basic N-terminal binding sites (residues 23-27 and ~95-110), which of course cannot be formed by huPrP(90-230) or huPrP(121-230). Such crosslinks may play a crucial role in Aβ(1-42) oligo-huPrP assembly due to the multivalent presentation of epitopes on Aβ(1-42) oligo.

At low concentrations of huPrP(23-144), the huPrP binding sites on Aβ(1-42) oligo are only partially occupied (Fig. 8E), leading to moderate assembly of Aβ(1-42) oligo, presumably promoted by charge neutralisation and huPrP-induced crosslinking. This is in line with the loose appearance of hetero-assemblies in AFM (Fig. 8B). At high concentrations of huPrP(23-144), the huPrP binding sites on Aβ(1-42) oligo are fully occupied (Fig. 8F), resulting in saturated Aβ(1-42) oligo assembly, in agreement with the compact appearance of hetero-assemblies in AFM (Fig. 8D).

The D-enantiomeric peptide RD2D3-FITC competes with huPrP for binding to Aβ(1-42) oligo - Soluble N-terminal PrP fragments, including the naturally produced neuroprotective N1 fragment, block neurotoxic effects of soluble oligomers (20, 23, 24), presumably by competing with membrane-anchored PrPC for Aβ oligo. In line with this, we find that huPrP(23-144) rescues the viability of PC-12 cells from Aβ(1-42) oligo–induced toxicity in a concentration-dependent manner, according to the MTT cell viability test (Fig. S9). Compounds that compete with membrane-anchored PrPC for Aβ oligo in a similar fashion might therefore be of therapeutic interest. Similarly to huPrP, our well-characterised Aβ-binding D-peptides form hetero-assemblies with Aβ(1-42) oligo (28). These specific D-peptides contain a high ratio of basic amino acid residues and are in that respect similar to the Aβ(1-42) oligo-binding sites within the PrP N-terminus (5, 19–22). Similar to the soluble N-terminal PrP fragment huPrP(23-144), the D-peptide RD2D3 shows rescue of PC-12 cell viability from Aβ(1-42) oligo–induced toxicity in the MTT test (Fig. S9 and (30)). Therefore, the D-peptides might act by a similar mechanism as N-terminal huPrP fragments, i.e., competition with membrane-anchored PrPC for Aβ oligo. To investigate this hypothesis, we analysed the effect of the D-peptide RD2D3, labelled with a fluorescence dye (FITC), on the Aβ(1-42) oligo-huPrP(23-144) interaction.

Determination of Aβ/PrP ratios within the hetero-assemblies might be a suitable approach to identify potential drug candidates that interfere with the Aβ-huPrP interaction. If a compound competes with huPrP(23-144) for Aβ(1-42) oligo, the Aβ(1-42)/huPrP(23-144) ratio within the complexes will change to higher values due to decreased huPrP(23-144) binding to Aβ(1-42) oligo.

First, we verified that huPrP(23-144) and RD2D3-FITC do not form high molecular weight complexes with each other (Fig. S10). For studying the effect of RD2D3-FITC constant final concentrations of 80 µM Aβ(1-42) and 40 µM huPrP(23-144) in the samples applied to DGC were chosen. Under these conditions, in the absence of RD2D3-FITC, the PrP-binding capacity of Aβ(1-42) oligo is fully exploited, resulting in an Aβ(1-42)/huPrP(23-144) ratio of 4 : 1 in the hetero-assemblies (Table 1) and a slight excess of huPrP(23-144) that remains monomeric (Fig. 5). The Aβ(1-42)/huPrP(23-144) ratio of 4 : 1 was set as reference for the analysis of potential effects of RD2D3-FITC on the Aβ-huPrP interaction. We compared the effect of different orders of application of RD2D3-FITC or huPrP(23-144) to the sample. Either RD2D3-FITC or huPrP(23-144) were pre-incubated with Aβ(1-42) and the other compound was applied after 2 h for further 30 min. Alternatively, Aβ(1-42) oligo was preformed and RD2D3-FITC and huPrP(23-144) were mixed and simultaneously applied to Aβ(1-42) oligo for further 30 min. When 40 µM RD2D3-FITC was applied after pre-incubation of huPrP(23-144) with Aβ(1-42) oligo (Fig. 9, C and D), the majority of RD2D3-FITC was located in fractions 1 to 3 after DGC. Although low concentrations of RD2D3-FITC were found in the fractions containing hetero-assemblies (fractions 11 to 14), the Aβ(1-42)/huPrP(23-144) ratio remained at 4 : 1 as in the reference (Fig. 10). The pre-incubation of
40 μM RD2D3-FITC with Aβ(1-42) before huPrP(23-144) application (Fig. 9, E and F), however, resulted in a drastic decrease of huPrP(23-144) bound in the hetero-assemblies and in an increase of RD2D3-FITC in fractions 11 to 14. At the same time, the Aβ(1-42)/huPrP(23-144) ratio changed to 14.3 ± 0.5 (Fig. 10). huPrP(23-144) was mainly found in fractions 1 to 3, in agreement with a monomeric, unbound state. Simultaneous application of huPrP(23-144) and RD2D3-FITC to Aβ(1-42)_b oligo resulted in an intermediate outcome (Fig. 9, A and B). Here, the Aβ(1-42)/huPrP(23-144) ratio was 10.4 ± 0.4, which is significantly increased compared to the ratio for early huPrP(23-144) application, but lower than that for early RD2D3-FITC application. Reduction of the RD2D3-FITC concentration from 40 μM to 20 μM (Figs. 10 and S11) resulted in the same tendency with respect to the ratios and the protein distributions within the gradient and reduced Aβ(1-42)/huPrP(23-144) ratios due to the decreased D-peptide concentration. These results demonstrate the competition between huPrP(23-144) and RD2D3-FITC for Aβ(1-42)_b oligo. The degree of competition of RD2D3-FITC was dependent on the concentration as well as on the order of RD2D3-FITC and huPrP(23-144) application.

**DISCUSSION**

In 2009, Laurén et al. reported that oligomeric Aβ binds to membrane-anchored PrP(C), leading to toxic signalling across the cell membrane (5). While subsequent studies questioned the role of PrP(C) in toxic signalling (7, 49–51), further evidence was gained in support of the original findings (5, 12, 13, 20). According to the current view of PrP(C)-Aβ_b oligo-induced signalling, metabotropic glutamate receptor 5 (mGluR5) interacts with PrP(C) and activates the intracellular Fyn kinase when Aβ oligomers are bound to membrane-anchored PrP(C) (12, 13). This activation leads to hyperphosphorylation of tau protein as well as to phosphorylation of N-methyl-D-aspartate (NMDA) receptors, two mechanisms which finally lead to neuronal damage (12, 14, 15, 17). The elucidation of these mechanisms has opened new strategies to prevent toxic signalling in AD by targeting one of these proteins or mediators.

The Aβ(1-42)_b oligo-huPrP interaction is at the core of the PrP-mediated toxic signalling cascade. Here, we have characterised the Aβ(1-42)_b oligo-huPrP interaction, by applying a set of soluble huPrP constructs and by taking advantage of the QIAD assay (25), which enables determination of the size distribution of Aβ assembly species and their complexes based on separation by DGC. We find that Aβ(1-42)_b oligo and huPrP readily associate to form hetero-assemblies above the megadalton range (Figs. 4-6 and 8). The hetero-assemblies are imaged by AFM as μm-sized clusters of nm-sized globular substructures (Fig. 8).

Hetero-association is greatly impaired for the huPrP variants devoid of the N-terminus, huPrP(90-230) and huPrP(121-230) (Figs. 7 and S6), in agreement with the notion that both Aβ-binding sites in the huPrP N-terminus (residues 23-27 and -95-110 (5, 19-22)) are required for high-affinity interaction. This is in line with recent reports showing that the effect of soluble, anchorless PrP(90-231) with respect to prevention of Aβ-mediated cytotoxicity was substantially weaker compared to full-length huPrP or N-terminal huPrP (52). In addition, Nieznanski et al. showed that about 10-fold higher concentrations of huPrP(90-231) than of huPrP(23-231) or huPrP(23-144) were required to achieve comparable inhibitory effects on Aβ(1-42) fibril formation (23). Similarly, a complete loss of binding capacity to Aβ(1-42)_b oligo after deletion of the N-terminal region 23-89 was observed (19). The Aβ-binding sites in huPrP constitute positively charged patches, suggesting that an electrostatic component may promote the interaction. In this context it is worth noting that the presence of negatively charged patches on Aβ(1-42)_b oligo has been inferred from engineering of Aβ(1-42)_b oligo-binding proteins (53).

Further distinctive features of the Aβ(1-42)_b oligo-huPrP hetero-association comprise (i) disordered conformation of the binding sites in free PrP, (ii) multivalency (an average Aβ(1-42)_b oligo can interact with six huPrP molecules), and (iii) a stoichiometry that is not fixed, but constrained to a relatively narrow window (the Aβ/PrP ratio is in the range of 4:1 to 12:1). We searched the literature for protein-protein interactions with similar characteristics and found two notable cases, the interaction of nucleophosmin with nucleophosmin-binding proteins (54), and heteroprotein coacervation of
β-lactoglobulin and lactoferrin (55, 56). The interaction of nucleophosmin with binding proteins containing arginine-rich linear motifs is involved in nucleolus formation by liquid-liquid phase separation. The interaction features an electrostatic component, intrinsic disorder in the free binding motifs, as well as multivalency: acidic oligomers of nucleophosmin interact with proteins that contain at least two basic linear motifs (54). Heteroprotein coacervation of β-lactoglobulin and lactoferrin features a constrained stoichiometry, with some variation depending on the molar ratio of the initial mixture (55, 56).

The molar ratios in the Aβ(1-42)_{oligo}-huPrP hetero-assemblies suggest that an average Aβ(1-42)_{oligo} can directly interact with up to six huPrP molecules. This multivalent interaction, established here for soluble huPrP constructs, may also have consequences for GPI-anchored PrP(C). For example, clustering of PrP(C) can promote the activation of Fyn kinase (57, 58). Moreover, the multivalency of Aβ(1-42)_{oligo} may contribute to the formation of ternary complexes with other membrane receptors (59). N1, a secreted, soluble N-terminal fragment resulting from α-cleavage of huPrP, comprises both Aβ(1-42)_{oligo}-binding sites and is therefore primed for hetero-association with Aβ(1-42)_{oligo}. Intriguingly, N1 is neuroprotective, inhibits Aβ(1-42)_{oligo}-mediated neurotoxicity (20), and forms co-aggregates with Aβ that have been detected in post mortem brain tissue (60).

As the Aβ-PrP interaction might be a possible therapeutic target in treating Alzheimer’s disease, there is great effort to identify either Aβ- or PrP-binding compounds that inhibit the Aβ-PrP interaction. For example, dextran sulfate sodium (61) and Chicago Sky Blue 6B (62) inhibit binding of Aβ(1-42)_{oligo} to PrP. Similarly, anti-PrP antibodies blocked oligomeric Aβ binding to PrP and prevented Aβ oligomer-induced neurotoxicity (5, 63–65). The QIAD assay in its version introduced here, permitting simultaneous quantification of Aβ(1-42), huPrP, and compound, allows identification and characterisation of a compound’s interference with the Aβ-PrP interaction. We find that the Aβ-PrP ratio in the hetero-associates (Fig. 10) is a suitable indicator of a compound’s competition with PrP for Aβ(1-42)_{oligo}.

We have previously identified a number of D-enantiomeric peptides as promising drugs that eliminate Aβ(1-42)_{oligo} and improve cognition in transgenic AD mice (25–27, 66). Here, we have observed that the D-peptide RD2D3 interferes with the binding of huPrP(23-144) to Aβ(1-42)_{oligo}. As a rescue of cell viability of Aβ-treated cells (Fig. S9 and (30)) and enhancement of cognition (39) was shown for RD2D3, our studies suggest that interference with the Aβ-PrP interaction might be one potential mechanism of action of this class of D-peptides.

**EXPERIMENTAL PROCEDURES**

**Purification of huPrP**

All huPrP constructs, huPrP(23-230), huPrP(23-144), huPrP(90-230) and huPrP(121-230), were generated recombinantly in E. coli. huPrP(23-230) and huPrP(90-230) were cloned in pET-11a vectors and transformed in Rosetta 2 (DE3) as described by Luers et al. (67). Both constructs contain the natural polymorphism M129. Before induction E. coli was grown in TB medium at 37 °C and 160 rpm shaking. At OD_{600} of 0.7 recombinant protein expression was induced by adding 0.5 mM IPTG, and growth temperature was lowered to 25 °C. Cells were harvested after overnight expression. For the preparation of isotope labelled [U-^{15}N] or [U-^{13}C,^{15}N] huPrP(23-230) M9 minimal medium containing the desired isotopes was used. The purification is based on the protocol of Mehlhorn et al. (68). Harvested cells were washed with 1x PBS buffer for 30 min, harvested again and resuspended in 3 ml digestion buffer (1x PBS, 20 mM MgCl_2, DNAse I, containing protease inhibitor cocktail (COMPLETE EDTA free, Roche, 1 tablet per 100 ml) per gram cells and stored at -20 °C. The cells were disrupted at 1.2 kbar in a cell disruption system (Constant Systems) and the lysate was centrifuged at 28,700 x g for 1 h at 4 °C. The insoluble inclusion bodies were dissolved in about 10 ml of 6 M Guanidinium-HCl, 5 mM DTT, 12.5 mM Tris-HCl, pH 8.0 and centrifuged again (see above). The supernatant was separated by SEC on a HiLoad 26/60 Superdex 200 preparative grade column. Analytical samples of every second elution fraction were precipitated with 20 % (w/v) TCA to remove the guanidinium-HCl and were investigated by SDS-PAGE. huPrP(23-230) or huPrP(90-230) containing fractions were pooled and purified by RP-HPLC. A semi-preparative C8 column (Zorbax
300 SB-C8 Semi-Preparative, 9.4 mm x 250 mm, Agilent) allowed the separation of huPrPs from impurities using a 20 - 30 % (v/v) gradient of acetonitrile + 0.1 % (v/v) TFA within 15 min followed by a 10 min step of isocratic 30 % (v/v) acetonitrile + 0.1 % (v/v) TFA. The purifications were performed at 80 °C and at a flow rate of 4 ml/min. The elution fractions containing huPrP were pooled, dried by lyophilisation and finally dissolved in MilliQ water and adjusted to concentrations ranging from 96 μM to 140 μM.

Stocks of 100 - 200 μl were frozen in liquid nitrogen and stored at -80 °C. We chose water for the preparation of the huPrP stock solutions as huPrP is highly soluble in water.

huPrP(23-144) was cloned in a pET302/NT-His vector and transformed in E. coli BL21(DE3). This huPrP construct also contains the natural polymorphism M129. Cells were grown in LB medium at 37 °C and 160 rpm shaking and incubated over night after induction at these conditions. For the preparation of isotope labelled [U-15N] or [U-13C,15N] huPrP(23-144) M9 minimal medium containing the desired isotopes was used. Resuspension and disruption of the cells were performed as described above. The insoluble inclusion bodies were dissolved in 10 ml of 6 M guanidinium-HCl, 100 mM NaCl, 30 mM Tris-HCl, pH 7.4 and centrifuged (see above). The supernatant was separated by IMAC with two serially connected 5 ml Protino Ni-NTA columns (Macherey-Nagel). A washing step with 30 mM Tris-HCl, pH 7.4 allowed the removal of the denaturing agent guanidinium-HCl. The elution occurred with a linear gradient from 0 mM to 500 mM imidazol, 30 mM Tris-HCl, pH 7.4. The huPrP(23-144) containing fractions (verified by SDS-PAGE) were pooled and the hexa-histidine tag was cleaved by TEV protease. RP-HPLC purification, lyophilisation and storage of the protein were performed as described above.

The expression plasmid for huPrP(121-230) was obtained from Dr. Werner Kremer, University of Regensburg. As described in (47) it was cloned in pRSET A vector with N-terminal histidine tail and thrombin cleavage site. The Plasmid was transformed in Rosetta 2 (DE3). Before induction E. coli was grown in 2YT medium at 37 °C and 160 rpm shaking. At OD600 of 0.6 recombinant protein expression was induced by adding 1 mM IPTG, and growth temperature was lowered to 22 °C for over-night expression. After harvesting and washing the cells twice with 5 mM EDTA, 25 mM Tris-HCl, pH 8.0 they were resuspended in 2 mM EDTA, 1 % Triton X-100, 0.1 mg/ml lysozyme, 50 mM Tris-HCl, pH 8.0 and incubated 30 min at 37 °C. After addition of 0.1 mg/ml DNAse, 15 mM MgCl2 and incubation for 30 min at 37 °C they were sonicated on ice five times for 1 min (Bandelin Sonopuls, sonotrode VS 70 T, 60 % amplitude).

The insoluble inclusion bodies were harvested by centrifugation (see above), washed with 5 mM EDTA, 12.5 mM Tris-HCl, pH 8.0 and dissolved in 8 M Guanidinium-HCl, 12.5 mM Tris-HCl, pH 8.0 at 4 °C. After 1 h centrifugation (see above), the supernatant was separated by IMAC with two serially connected 5 ml Protino Ni-NTA columns (Macherey-Nagel). The elution of the hexa-histidine tagged PrP(121-230) occurred with a linear gradient of 500 ml from 0 mM to 500 mM imidazol in 6 M Guanidinium-HCl, 12.5 mM Tris-HCl, pH 8.0.

huPrP(121-230) containing fractions were pooled and purified by RP-HPLC. A semi-preparative C8 column (Zorbax 300 SB-C8, 9.4 mm x 250 mm, Agilent) allowed the separation of huPrP(121-230) from impurities using a 20 - 48 % (v/v) gradient of acetonitrile + 0.1 % (v/v) TFA within 20 min followed by a 10 min step of isocratic 48 % (v/v) acetonitrile + 0.1 % (v/v) TFA. The purification was performed at 80 °C and at a flow rate of 4 ml/min. The elution fractions containing huPrP(121-230) were pooled, and dried by lyophilisation. Thrombin cleavage of the fusion protein was performed with 2.5 mg/ml fusion protein in 50 mM MES, pH 6.0 with finally 8U thrombin (Serva 36402.02)/mg protein for 7 d, when nearly 98 % of the protein was digested. RP-HPLC purification, lyophilisation and storage of the protein were performed as described above.

### Preparation of Aβ(1-42) stocks

1 mg of synthetic Aβ(1-42) (Bachem AG) was incubated with 700 μl hexafluoro-2-propanol (HFIP) over night. The solution was divided in 36 μg aliquots in LoBind reaction tubes (Eppendorf AG) and lyophilised in a rotational vacuum concentrator system connected to a cold trap (both Martin Christ Gefriertrocknungsanlagen GmbH). The lyophilisates were stored at room temperature.
Standard proteins for DGC calibration

40 µg of the standard proteins ovalbumin, conalbumin, aldolase, apoferritin and thyroglobulin in 30 mM Tris-HCl, pH 7.4 from a gel filtration high molecular weight calibration kit (GE) were analysed by sucrose DGC (see below) to calibrate the gradient.

Preparation of samples containing Aβ(1-42) and huPrP (any construct)

a) Pre-incubation of Aβ(1-42): For formation of Aβ(1-42)oligo, Aβ(1-42) was incubated at concentrations of typically 81 µM - 100 µM to achieve an identical final concentration of 80 µM Aβ after huPrP addition in all samples prepared for DGC. The incubation was performed in 30 mM Tris-HCl-buffer, pH 7.4 at 22 °C and 600 rpm shaking for 2 h without further agent. This particular incubation time ensures the production of high amounts of Aβ(1-42)oligo without formation of larger aggregates or fibrils that would appear in the bottom fractions of the density gradient and might affect analytics of Aβ-huPrP complexes.

b) Addition of huPrP: huPrP stock solutions in MilliQ water were centrifuged directly before use in a TL 100 ultracentrifuge with a TLA-55 rotor (Beckman) for 30 min at 4 °C and 100,000 x g in order to remove potential aggregates. Final concentrations of 2 µM to 60 µM of huPrP(23-144), 20 µM of huPrP(121-230) or 2 µM to 20 µM of either huPrP(23-230) or huPrP(90-230) were added to the pre-formed Aβ(1-42)oligo for further 30 min at 22 °C and 600 rpm shaking. The final volume of each sample was 100 µl.

In another setup of experiments the concentration of Aβ(1-42)oligo was varied (20 µM, 40 µM, and 60 µM) and the huPrP(23-144) concentration was set constant to 40 µM.

Preparation of samples containing Aβ(1-42), huPrP(23-144) and RD2D3-FITC

Three different orders of application of RD2D3-FITC and huPrP(23-144) were analysed.

a) Mix of huPrP(23-144) and RD2D3-FITC (Simultaneous): Aβ(1-42)oligo were generated as described in “pre-incubation of Aβ(1-42)” above. After 2 h pre-incubation, huPrP(23-144) and RD2D3-FITC were added simultaneously to yield samples with final concentrations of 40 µM huPrP(23-144), 80 µM Aβ(1-42) and 20 µM or 40 µM RD2D3-FITC. The samples were incubated for further 30 min at 22 °C and 600 rpm shaking.

b) Addition of huPrP(23-144) during Aβ incubation (First huPrP then RD2D3-FITC): Aβ pre-incubation was done as described before but in the presence of 0.5 molar equivalents of huPrP(23-144). After 2 h pre-incubation, RD2D3-FITC was added to yield samples with final concentrations of 40 µM huPrP(23-144), 80 µM Aβ(1-42) and 20 µM or 40 µM RD2D3-FITC. The samples were incubated for further 30 min at 22 °C and 600 rpm shaking.

c) Addition of RD2D3-FITC during Aβ incubation (First RD2D3-FITC then huPrP): Aβ pre-incubation was done as described before but in the presence of 0.5 or 0.25 molar equivalents of RD2D3-FITC. After 2 h pre-incubation, huPrP(23-144) was added to yield samples with final concentrations of 40 µM huPrP(23-144), 80 µM Aβ(1-42) and 20 µM or 40 µM RD2D3-FITC. The samples were incubated for further 30 min at 22 °C and 600 rpm shaking.

DGC and RP-HPLC analysis of the fractions

This method is an adjusted assay based on the QIAD assay described in Brener et al. (25). In our case not only Aβ(1-42) but also the prion protein (either huPrP(23-230), huPrP(23-144) or huPrP(90-230)) and the D-peptide RD2D3-FITC are quantified. This assay contains the following steps:

a) DGC: Each 100 µl sample (see “Preparation of samples containing Aβ(1-42) and huPrP” or “Preparation of samples containing Aβ(1-42), huPrP(23-144) and RD2D3-FITC”) was applied on a discontinuous 30 mM Tris-HCl (pH 7.4) buffered sucrose gradient containing the following volumes and sucrose concentrations (from bottom to top): 300 µl of 60 % (w/w), 200 µl of 50 % (w/w), 200 µl of 25 % (w/w), 400 µl of 20 % (w/w), 400 µl of 15 % (w/w), 150 µl of 10 % (w/w) and 150 µl of 5 % (w/w). Each gradient was stepwise layered in a 11 x 34 mm polyallomer centrifuge tube. Gradients were centrifuged in a TL 100 ultracentrifuge using a TLS-55 rotor (Beckman) for 3 h at 4 °C and 259,000 x g. The centrifuged gradients were manually fractionated from top to bottom into 13 - 142 µl fractions. The remaining volume (arithmetically 54 µl) was mixed with 80 µl of 30 mM Tris-HCl, pH 7.4 forming fraction 14. For
all calculations, a dilution factor of 2.48 was included for fraction 14.

b)  **RP-HPLC analysis:** Each fraction was analysed by Tris/Tricine SDS-PAGE (see below) and RP-HPLC. For the quantification of Aβ(1-42), huPrP (all constructs) and RD2D3-FITC, 20 µl of each fraction was applied on a Zorbax 300 SB-C8 Stable Bond Analytical column, 4.6 mm x 250 mm (Agilent) and was measured with an Agilent 1260 infinity system. Each compound was separated by a 10 - 40 % (v/v) Acetonitrile gradient + 0.1 % (v/v) TFA within 25 min at 80 °C and a flow rate of 1 ml/min. These harsh conditions are necessary to ensure the dissociation of the formed complexes, especially in the density gradient fractions 11 to 14. For detection of the substances the UV absorbance at 214 nm was used. Known concentrations of Aβ(1-42), huPrP (all constructs) as well as RD2D3-FITC and their corresponding plot of peak area versus protein concentration enabled the calibration and finally the calculation of the protein concentrations present in the fractions. The programme package ChemStation by Agilent allowed data recording and peak area integration. All histograms were illustrated with OriginPro 9.0.

c)  **Determination of Aβ/huPrP ratios:** All generated complexes containing Aβ(1-42), huPrP (and RD2D3-FITC) were verified in the gradient fractions 11 to 14. For the calculation of Aβ/huPrP ratios, Aβ(1-42) and huPrP amounts in fractions 11 to 14 were summed up. Averaging over fractions 11 to 14 was necessary as the appearance of the complexes can shift a little within the different fractions due to manual fractionation of the gradients. Then, Aβ(1-42) amounts were divided by huPrP amounts to get a ratio for each experiment. The mean ± SD of the ratios was calculated over all performed experiments.

**Verification of the disulfide bond in huPrPs between Cys-179 and Cys-214 by RP-HPLC**

To analyse whether purified huPrP(23-230), huPrP(90-230) and huPrP(121-230) under study contain a disulfide bond between Cys-179 and Cys-214 in the fully oxidised state, purified samples of 5 uM protein were reduced overnight with 25 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in 6 M Guanidinium-HCl, 100 mM Tris-HCl, pH 7.4 and analysed by RP-HPLC as described above. Samples treated only with 6 M Guanidinium-HCl, 50 mM Tris-HCl, pH 7.4 were used as controls. The reductive opening of the disulfide bond results in a characteristic elongation of the retention time for the reduced state when compared to the oxidised states.

**SDS-PAGE and silver staining**

Qualitative analysis of the DGC fractions was done by Tris/Tricine-SDS-PAGE followed by silver-staining. To this end, 15 µl of each fraction was diluted 1:1 in sample buffer (12 % glycerol, 4 % SDS, 50 mM Tris-HCl pH 7.4, 2 % β-mercaptoethanol), applied onto 20 % Tris/Tricine gels, and subjected to gel electrophoresis at 40 mA per gel. The preparation of the Tris/Tricine gels is based on the protocol by Schägger and von Jagow (69). Gels containing samples with RD2D3-FITC were analysed by fluorescence detection (excitation: 470 nm, emission: 530 nm) before silver staining. Silver staining of the gels based on the protocol by Heukeshoven et al. (70) allowed the visualisation of protein bands.

**Dot blot analysis**

For further qualitative verification of the Aβ(1-42) and huPrP(23-144) contents within DGC fractions a dot blot was performed. 2 µl of all 14 denatured sucrose DGC fractions were pipetted onto two pieces of Biotrace NT nitrocellulose membrane (Pall) and allowed to air dry. After blocking with 5 % milk powder in 30 mM Tris-HCl, pH 7.6 for 30 min the membranes were incubated for 15 min with 0.2 µg/ml prion protein monoclonal antibody Saf32 (bertin bioreagent) or 0.6 µg/ml cell culture supernatants of Aβ(1-8) recognising IC16 antibody in 5 % milk powder, 30 mM Tris-HCl, pH 7.6. After three 5 min washes with 30 mM Tris-HCl, pH 7.6 the membranes were incubated 15 min with 0.1 µg/ml peroxydase-conjugated AffiniPure Goat Anti-Mouse IgG(H+L) (Jackson ImmunoResearch) in 5 % milk powder, 30 mM Tris-HCl, pH 7.6. After three 5 min washes with 30 mM Tris-HCl, pH 7.6 the immunoreactivity was visualised with super signal west pico chemiluminescence substrate (Pierce).

**Dynamic light scattering**

Dynamic light scattering (DLS) was performed on a Submicron Particle Sizer Nicomp 380 (PSS Nicomp, Santa Barbara, USA). Data were analysed with the Nicomp algorithm using the volume-weighted Nicomp distribution analysis. The DLS
sample of Aβ(1-42)_ oligo-huPrP(23-144) complexes derived from 80 µM Aβ(1-42) and 40 µM huPrP(23-144) was prepared by pooling fractions 11 to 14 after sucrose DGC. For data analysis a measured refractive index in the sample of 1.409 corresponding to 44.8 % sucrose and a viscosity of 9.2 cp was taken into account (71).

**MTT cell viability assay**

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based cell viability assays (37) were performed to investigate the cytotoxicity of 1 µM Aβ(1-42)_ oligo in the absence or after either mixing and further incubation of Aβ(1-42)_ oligo with 0.02, 0.1 or 0.5 µM huPrP(23-144) or with 0.2, or 1 µM RD2D3, respectively.

Rat pheochromocytoma PC12 cells (Leibniz Institute DSMZ, Braunschweig, Germany) were cultivated in DMEM medium supplemented with 10 % fetal bovine serum and 5 % horse serum. 10,000 cells per well were seeded on collagen-coated 96 well plates (Gibco, Life technology, Carlsberg, USA) and incubated in a 95 % humidified atmosphere with 5 % CO2 at 37 °C for 24 h.

Added Aβ(1-42)_ oligo alone or either after mixing and further incubation with huPrP(23-144) or with RD2D3 were prepared as described in the “Preparation of samples containing Aβ(1-42) and huPrP (any construct)” section. The prepared stock solutions contained either 80 µM pre-incubated Aβ(1-42) alone or 80 µM pre-incubated Aβ(1-42) mixed and further incubated with 1.6 µM, 8 µM or 40 µM huPrP(23-144) or 80 µM pre-incubated Aβ(1-42) mixed and further incubated with 16 µM or 80 µM RD2D3.

After further incubation for 24 h in 95 % humidified atmosphere with 5 % CO2 at 37 °C cell viability was measured using the Cell Proliferation Kit I (MTT) (Roche, Basel, Switzerland) according to the manufacturer’s instruction. The absorbance of the formazan product was determined by measuring at 570 nm after subtracting the absorption at 660 nm in a Polarstar Optima plate reader (BMG, Offenburg, Germany). All results were normalised to cells that were treated with buffer only. The arithmetic mean of all 15 measurements per approach was calculated.

**AFM**

AFM was done using a Nanowizard 3 system (JPK Instruments AG). All samples were prepared as described in “preparation of samples containing Aβ(1-42) and huPrP”. 50 µl of Aβ(1-42)_ oligo or 25 µl of huPrP(23-144) were incubated on freshly cleaved mica for 3 min or 30 min, respectively. Aβ(1-42)_ oligo-huPrP(23-144) hetero-assemblies had to be cleared from unbound Aβ(1-42) or huPrP(23-144) and were therefore centrifuged at 16,100 x g and 4 °C for 30 min and washed twice with 100 µl 30 mM Tris-HCl, pH 7.4, respectively. The complexes were then resuspended in 100 µl of 30 mM Tris-HCl, pH 7.4. Then 50 µl of the complexes were incubated for 30 min on freshly cleaved mica. All samples were washed three times with MilliQ water and dried in a gentle stream of N2.

The samples were measured using intermittent contact mode with a resolution of 512 pixel or 1024 pixel and line rates of 0.5 to 1 Hz in ambient conditions with a silicon cantilever with nominal spring constant of 26 N/m and average tip radius of 7 nm (Olympus OMCL-AC160TS). Due to the differing composition of the megadalton sized aggregates concerning adhesion, stiffness and perforation, the imaging parameters (amplitude, setpoint and gain) had to be adapted slightly and the cantilever had to be changed often.

The height images of Aβ(1-42)_ oligo and huPrP(23-144) were flattened with the JPK Data Processing software 5.0.69. The statistics of particle dimensions of Aβ(1-42)_ oligo were done with the Gwyddion 2.44 grain analysis. After plane levelling, grains were marked with a threshold of 13 %. The maximum height of the individual grain was corrected with subtraction of the grain minimum.

The lateral size is affected by tip convolution effects (Δ) in AFM images. Considering the nominal radius of rtip = 7 nm of the AFM tip, we corrected the size of the lateral dimension according to equation (1) for objects below the tip round end as shown in Canet-Ferrer et al. (72). h describes the height of the object.

\[ Δ = r_{tip} \times \cos\left[\arcsin\left(\frac{r_{tip} - h}{r_{tip}}\right)\right] \]  

**CD spectroscopy**

6 µM huPrP(23-230), huPrP(23-144) or huPrP(90-230) in 10 mM Tris-HCl, pH 7.4 were
analysed by CD spectroscopy. Each sample was transferred into a cuvette (110-QS, 1 mm, Hellma Analytics) and spectra from 186 nm to 280 nm were recorded at a scan speed of 50 nm/min in a Jasco J-815 spectropolarimeter. Spectra of 10 mM Tris-HCl, pH 7.4 were used as reference and subtracted from the protein spectra. Ten spectra of each huPrP sample were recorded and accumulated to improve the signal-to-noise ratio.

**Solution NMR spectroscopy**

NMR samples of 0.2 mM [U-\text{\textsuperscript{15}}N] huPrP(23-230) with 10 mM sodium acetate (pH 4.5) in 10 % (v/v) D\textsubscript{2}O and of between 0.11 mM and 0.36 mM [U-\text{\textsuperscript{15}}N] or [U-\text{\textsuperscript{13}}C,\text{\textsuperscript{15}}N] huPrP(23-144) with different buffers (50 mM, pH ranging from 4.5 to 7.2) in 10 % (v/v) D\textsubscript{2}O were investigated by recording 2D [\textsuperscript{1}H,\text{\textsuperscript{15}}N] HSQC spectra (73) at different temperatures ranging from 5.0 °C to 20.0 °C on Bruker 600 MHz, Varian 800 MHz, or Varian 900 MHz NMR spectrometers equipped with cryogenically cooled triple or quadruple resonance probes with z axis pulsed field gradient capabilities. The sample temperature was calibrated using methanol-\textsubscript{d\textsubscript{4}} (99.8 %) (74). The \textsuperscript{1}H\textsubscript{2}O resonance was suppressed by gradient coherence selection, with quadrature detection in the indirect \textsuperscript{15}N dimension achieved by the echo-anticrcho method (75, 76). A WALTZ-16 sequence (77) with a field strength of at least 1.1 kHz was employed for \textsuperscript{15}N decoupling during acquisition. At least 927 (128) complex data points were acquired with a spectral width of 16 ppm (26.0 ppm) in the \textsuperscript{1}H (\textsuperscript{15}N) dimension. All NMR spectra were processed using NMRPipe and NMRDraw (78) and analysed with NMRViewJ (79). \textsuperscript{1}H chemical shifts were referenced with respect to external DSS in D\textsubscript{2}O and \textsuperscript{15}N chemical shifts were referenced indirectly (80).

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FOOTNOTES

The abbreviations used are: AD, Alzheimer’s disease; Aβ, amyloid-β; PrP, prion protein; Aβ oligo, soluble oligomeric forms of Aβ; PrPc, cellular prion protein; GPI, glycosylphosphatidylinositol; PrPSc, scrapie isoform of PrP; mGluR5, metabotropic glutamate receptor 5; NMDA, N-methyl-D-aspartate; QIAD, quantitative determination of interference with Aβ aggregate size distribution; DGC, density gradient ultracentrifugation; RP, reversed-phase; huPrP, human PrP; AFM, atomic force microscopy; IMAC, immobilised metal ion affinity chromatography; SEC, size exclusion chromatography; HFIP, 1,1,1,3,3,3-Hexafluoro-2-propanol
TABLE 1. Aβ/huPrP ratios within Aβ(1-42)_{oligo}-huPrP hetero-assemblies. The ratios were calculated for every experiment as the quotient of the total amount of Aβ(1-42) molecules and the total amount of huPrP in fractions 11 to 14 after sucrose DGC. For the calculation of Aβ(1-42)/huPrP(23-230) ratios the huPrP(23-230) concentrations in fractions 11 to 14 found with huPrP(23-230) alone (Fig. 3, B and F) were not considered as they were negligibly small. For concentrations labelled with ‘n.d.’ molar ratios were not determined for huPrP(23-230). Comparing the same (huPrP) concentration, similar ratios were obtained for both huPrP(23-144) and huPrP(23-230). Full saturation of the PrP-binding capacity of Aβ(1-42)_{oligo} as evident in the case of 40 µM and 60 µM huPrP(23-144) by the presence of free monomeric huPrP(23-144) (Figs. 5F and S8), resulted in a ratio of about 4 Aβ : 1 huPrP(23-144). Experiments are done in replicates of n = 3 ± SD for all huPrP(23-230) concentrations applied, for 5 µM, 20 µM, 40 µM, 60 µM huPrP(23-144) in the presence of 80 µM Aβ(1-42), and for 20 µM and 60 µM Aβ(1-42) in the presence of 40 µM huPrP(23-144); n = 4 ± SD for 40 µM Aβ(1-42) in the presence of 40 µM huPrP(23-144); and n = 5 ± SD for 2 µM and 10 µM huPrP(23-144) in the presence of 80 µM Aβ(1-42).

| Aβ [µM] | huPrP [µM] | Aβ/huPrP(23-144) | Aβ/huPrP(23-230) |
|---------|------------|-------------------|------------------|
| 80      | 2          | 10.1 ± 1.7        | 12.1 ± 1.7       |
| 80      | 5          | 11.3 ± 0.6        | 9.6 ± 2.3        |
| 80      | 10         | 8.3 ± 1.1         | 6.3 ± 1.7        |
| 80      | 20         | 4.9 ± 0.3         | 4.2 ± 0.9        |
| 80      | 40         | 3.93 ± 0.04       | n.d.             |
| 80      | 60         | 4.04 ± 0.08       | n.d.             |
| 60      | 40         | 3.6 ± 0.2         | n.d.             |
| 40      | 40         | 4.1 ± 0.8         | n.d.             |
| 40      | 60         | 4.2 ± 0.6         | n.d.             |

FIGURE LEGENDS

FIGURE 1. Schematics of the investigated huPrP constructs huPrP(23-230), huPrP(23-144), huPrP(90-230) and huPrP(121-230) (A) and of the assay to quantify the composition of hetero-assemblies (B). (A) The binding sites for Aβ(1-42)_{oligo} (5, 19–22) are marked in blue and the corresponding sequence is shown in the huPrP(23-230) construct with basic amino acid residues highlighted in red. OR marks the octarepeat region from residues 51 to 91. huPrP(23-230), huPrP(90-230) and huPrP(121-230) contain a disulfide bond between Cys^{179} and Cys^{214} in the structured C-terminal part of the protein. (B) 80 µM of Aβ(1-42) were incubated for 2 h to obtain Aβ(1-42)_{oligo} before different quantities of the respective prion protein were added to the sample. After 30 min co-incubation the sample was separated by sucrose DGC and fractionated. Each fraction was analysed by SDS-PAGE as well as by quantitative RP-HPLC analysis.

FIGURE 2. Analysis of purified huPrP by CD spectroscopy (A) and AFM (B), and of Aβ(1-42)_{oligo} by AFM (C). huPrP(23-230) (A, black) and huPrP(90-230) (A, blue) show predominantly α-helical CD spectra, while the N-terminal huPrP(23-144) (A, red) is present in random-coil conformation (MRE: mean residue ellipticity). 1 µm^{2} AFM images of 200 nM huPrP(23-144) (B) or 800 nM Aβ(1-42)_{oligo} (C), scale bars: 200 nm. huPrP(23-144) forms a thin film on the mica surface, not higher than 1 nm to 2 nm (B). The generated Aβ(1-42)_{oligo} species are seen as spherical particles with heights ranging from 1 nm to 6 nm (C).

FIGURE 3. Distribution of incubated Aβ(1-42) (A, E), huPrP(23-230) (B, F), huPrP(23-144) (C, G) and huPrP(90-230) (D, H) after sucrose DGC. (A–D) Silver-stained Tris/Tricine SDS-PAGE gels and (E–H) corresponding histograms after RP-HPLC analysis. In every image, fractions are shown from left to right: 2 µM, 10 µM, 40 µM, and 80 µM huPrP(23-144).
right corresponding to the fractions from top to bottom of the gradient. Aβ(1-42) can be found in fractions 1 to 10 (A, E) with fractions 3 to 7 being the main Aβ(1-42)_{d oligo}-containing fractions. All huPrP constructs are mainly detected in fractions 1 to 3, but low concentrations can also be found in fractions 11 to 14, especially in the case of huPrP(23-230). Experiments are done in replicates of n = 5 (E), n = 4 (F), n = 6 (G), n = 4 (H) ± SD (standard deviation).

FIGURE 4. Formation of hetero-assemblies of Aβ(1-42)_{d oligo} and huPrP(23-230). (A-D) Silver-stained Tris/Tricine SDS-PAGE gels after application of 80 µM of pre-incubated Aβ(1-42) with varying huPrP(23-230) concentrations onto a sucrose gradient and (E, F) corresponding histograms after RP-HPLC analysis show the distribution of Aβ(1-42) and huPrP(23-230). With increasing applied huPrP(23-230) concentrations, Aβ(1-42)_{d oligo} (fractions 3 to 7 in E) decreases. Moreover, both Aβ(1-42) and huPrP(23-230) appear in fractions 11 to 14 (bottom of the gradient). Concentrations of Aβ(1-42) in each gradient fraction according to the applied huPrP(23-230) concentration are shown in E and concentrations of huPrP(23-230) are shown in F. Experiments are done in replicates of n = 3 ± SD for all huPrP(23-230) concentrations applied.

FIGURE 5. Formation of hetero-assemblies of Aβ(1-42)_{d oligo} and huPrP(23-144). (A-F) Silver-stained Tris/Tricine SDS-PAGE gels after application of 80 µM of pre-incubated Aβ(1-42) with varying huPrP(23-144) concentrations on a sucrose gradient and (G, H) corresponding histograms after RP-HPLC analysis show the distribution of Aβ(1-42) and huPrP(23-144). With increasing applied huPrP(23-144) concentrations, Aβ(1-42)_{d oligo} (fractions 3 to 7 in G) decreases. Moreover, both Aβ(1-42) and huPrP(23-144) appear in fractions 11 to 14 (bottom of the gradient). When 40 µM huPrP(23-144) are added, the Aβ(1-42)_{d oligo}-huPrP(23-144) interaction gets saturated, reflected by the presence of huPrP(23-144) in fractions 1 to 3 (F, H). Concentrations of Aβ(1-42) according to the applied huPrP(23-144) concentration are shown in G and concentrations of huPrP(23-144) are shown in H. In addition to silver staining of Tris/Tricine SDS-PAGE gels, dot blot analysis detecting either Aβ or huPrP of the DGC fractions after application of 80 µM Aβ(1-42) and 40 µM huPrP(23-144) was performed (Fig. S7), confirming qualitative analyses by silver staining of Tris/Tricine SDS-PAGE gels as well as quantitative RP-HPLC analyses. Experiments are done in replicates of n = 5 (for 2 µM and 10 µM huPrP(23-144) applied ± SD) and n = 3 (for 5 µM, 20 µM, and 40 µM huPrP(23-144) applied ± SD).

FIGURE 6. Size distribution of Aβ(1-42)_{d oligo}-huPrP(23-144) complexes measured by dynamic light scattering. The Aβ(1-42)_{d oligo}-huPrP(23-144) assemblies mainly have sizes in the range from 0.6 to 6 µm.

FIGURE 7. Impaired formation of hetero-assemblies of Aβ(1-42)_{d oligo} and huPrP(90-230). (A-D) Silver-stained Tris/Tricine SDS-PAGE gels after application of 80 µM of pre-incubated Aβ(1-42) with varying huPrP(90-230) concentrations on a sucrose gradient and (E, F) corresponding histograms after RP-HPLC analysis show the distribution of Aβ(1-42) and huPrP(90-230). With increasing applied huPrP(90-230) concentrations, just slightly increased protein concentrations of both Aβ(1-42) and huPrP(90-230) are found in the bottom fractions 11 to 14. Concentrations of Aβ(1-42) according to the applied huPrP(90-230) concentration are shown in E and concentrations of huPrP(90-230) are shown in F. Experiments are done in replicates of n = 3 ± SD for all huPrP(90-230) concentrations applied.

FIGURE 8. AFM images of Aβ(1-42)_{d oligo}-huPrP(23-144) complexes (A-D) and model of the complexes (E, F). (A-D) 1 µm² AFM images of Aβ oligomers (A) and Aβ(1-42)_{d oligo}-huPrP(23-144) complexes generated with 80 µM of pre-incubated Aβ(1-42) and either 1 µM (B), 5 µM (C) or 40 µM huPrP(23-144) (D), scale bars: 200 nm. (E, F) Model of Aβ(1-42)_{d oligo}-huPrP complexes with low (E) and high (F) huPrP content. For clarity complexes are shown two-dimensional. Aβ(1-42)_{d oligo} is shown in blue, huPrP in yellow, binding sites on huPrP in green (E, bottom right corner). One Aβ(1-42)_{d oligo} is composed of 23 monomers on average (25) (E, top right corner). Based on the ratio of 4 Aβ (monomer equivalent) to 1 huPrP in case of saturation with huPrP, the hetero-assemblies contain six huPrP molecules per Aβ(1-42)_{d oligo}. The hetero-assemblies show many detailed substructures at low huPrP concentrations (E),
symbolised by the grey background in the model. After saturation with huPrP all binding sites on Aβ(1-42)_oligo are occupied leading to compact complexes with smooth surface (D, F).

**FIGURE 9. Interference of the Aβ(1-42)_oligo-huPrP(23-144) interaction by RD2D3-FITC.** Distribution of 80 µM Aβ(1-42), 40 µM huPrP(23-144), and 40 µM RD2D3-FITC, after sucrose DGC for different orders of RD2D3-FITC and huPrP(23-144) addition. (A, C and E) Aβ(1-42) and huPrP(23-144) distributions are shown in silver-stained Tris/Tricine SDS-PAGE gels and the distribution of RD2D3-FITC after fluorescence detection on the same gels. (B, D and F) Quantification by RP-HPLC of each component. huPrP(23-144) and RD2D3-FITC were either (A, B) simultaneously added to Aβ(1-42)_oligo, (C, D) huPrP(23-144) was pre-incubated with Aβ before RD2D3-FITC addition, or (E, F) RD2D3-FITC was pre-incubated with Aβ before huPrP(23-144) addition. Dependent on the order of application of RD2D3-FITC or huPrP(23-144) to the sample, the distributions of huPrP(23-144) and RD2D3-FITC change. Experiments are done in replicates of n = 3 ± SD for all orders of application of RD2D3-FITC or huPrP(23-144) to the sample.

**FIGURE 10. Altered Aβ/huPrP(23-144) ratios within Aβ(1-42)_oligo-huPrP(23-144) assemblies show the interference by RD2D3-FITC.** For all experiments constant concentrations of 80 µM Aβ(1-42) and 40 µM huPrP(23-144) were used. The reference of 3.93 ± 0.04 Aβ/huPrP(23-144) results from data obtained in the absence of RD2D3-FITC (Table 1). The strongest interference of RD2D3-FITC with the Aβ(1-42)_oligo-huPrP(23-144) interaction was observed at the higher RD2D3-FITC concentration (40 µM), when RD2D3-FITC was pre-incubated with Aβ before addition of huPrP(23-144). Experiments are done in replicates of n = 3 ± SD for all orders of application of RD2D3-FITC or huPrP(23-144) to the sample.
FIGURE 1

A

huPrP(23-230)

huPrP(23-144)

huPrP(90-230)

huPrP(121-230)

B

incubation

Aβ

Aβ complexes

DGC

fractionation

SOD-PAGE

HPLC analysis

abscissa

Aβ time

huPrP

Interference with Aβ-PrP complex formation
Interference with Aβ-PrP complex formation

FIGURE 2

A  huPrP  

B  huPrP(23-144)  

C  Aβ(1-42) oligo  

MRE [deg/s cm²] 

Wavelength [nm] 

0 nm  10 nm  10 nm 

0 nm  10 nm  10 nm 

huPrP(23-230)  

huPrP(90-230)  

huPrP(23-144)
Interference with Aβ-PrP complex formation

FIGURE 3

A  Aβ(1-42)  B  huPrP(23-230)  C  huPrP(23-144)  D  huPrP(90-230)

E  F  G  H

Interference with Aβ-PrP complex formation
Interference with Aβ-PrP complex formation

FIGURE 4

A + 2 μM huPrP(23-230)  B + 5 μM huPrP(23-230)  C + 10 μM huPrP(23-230)  D + 20 μM huPrP(23-230)

E

F

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Interference with Aβ-PrP complex formation

FIGURE 5

A + 0 µM huPrP(23-144)  B + 2 µM huPrP(23-144)  C + 5 µM huPrP(23-144)

D + 10 µM huPrP(23-144)  E + 20 µM huPrP(23-144)  F + 40 µM huPrP(23-144)

G

H
Interference with Aβ-PrP complex formation

FIGURE 6

Diameter [nm]

Relative volume

0.0 0.2 0.4 0.6 0.8 1.0 1.2

10 100 1000 10000 100000
Interference with Aβ-PrP complex formation

FIGURE 7

A + 2 μM huPrP(90-230)

B + 5 μM huPrP(90-230)

C + 10 μM huPrP(90-230)

D + 20 μM huPrP(90-230)

E

F

Fraction

Fraction

Aβ(1-42) [μM]

huPrP(90-230) [μM]

80 μM Aβ(1-42)

80 μM Aβ(1-42) [+ 2 μM huPrP(90-230)]

80 μM Aβ(1-42) [+ 5 μM huPrP(90-230)]

80 μM Aβ(1-42) [+ 10 μM huPrP(90-230)]

80 μM Aβ(1-42) [+ 20 μM huPrP(90-230)]

2 μM huPrP(90-230) [+ 80 μM Aβ(1-42)]

5 μM huPrP(90-230) [+ 80 μM Aβ(1-42)]

10 μM huPrP(90-230) [+ 80 μM Aβ(1-42)]

20 μM huPrP(90-230) [+ 80 μM Aβ(1-42)]
FIGURE 8

Interference with Aβ-PrP complex formation

A. Aβ(1-42) oligo
B. + 1 μM huPrP(23-144)
C. + 5 μM huPrP(23-144)
D. + 40 μM huPrP(23-144)

E. Diagram showing Aβ oligomer and PrP interaction
F. Enlarged view of Aβ oligomer and PrP interaction

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Interference with Aβ-PrP complex formation

FIGURE 9

A Simultaneous

B

C First huPrP then RD2D3-F

D

E First RD2D3-F then huPrP

F

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Interference with Aβ-PrP complex formation

FIGURE 10

![Bar chart showing the ratio Aβ/huPrP(23-144) for different conditions and concentrations of RD2D3-FITC. The categories include Reference, Simultaneous, First huPrP then RD2D3-F, First RD2D3-F then huPrP, and First RD2D3-F then huPrP added with 20 µM RD2D3-FITC or 40 µM RD2D3-FITC.](http://www.jbc.org/Downloaded from)
A D-enantiomeric peptide interferes with hetero-association of amyloid-β oligomers and prion protein
Nadine S. Rösener, Lothar Gremer, Elke Reinartz, Anna König, Oleksandr Brener, Henrike Heise, Wolfgang Hoyer, Philipp Neudecker and Dieter Willbold

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