

**PrP-grafted antibodies bind certain amyloid  $\beta$ -protein aggregates,  
but do not prevent toxicity**

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## **Abstract**

**Background** The prion protein (PrP) is known to bind certain soluble aggregates of the amyloid  $\beta$ -protein (A $\beta$ ), and two regions of PrP, one centered around residues 19-33, and the other around 87-112, are thought to be particularly important for this interaction. When either of these sequences are grafted into a human IgG the resulting antibodies react with disease-associated PrP conformers, whereas the parental b12 IgG does not.

**Methods** Human antibodies containing grafts of PrP 19-33 or 87-112 were prepared as before (Solforosi *et al.* 2007) and tested for their ability to recognize synthetic and Alzheimer's disease (AD) brain-derived A $\beta$ . Since aqueous extracts of AD brain contain a complex mixture of active and inactive A $\beta$  species, we also assessed whether PrP-grafted antibodies could protect against neurotoxicity mediated by AD brain-derived A $\beta$ . For these experiments, human iPSC-derived neurons were grown in 96-well plates at 5000 cells per well and on post-induction day 21, AD brain extracts were added +/- test antibodies. Neurons were imaged for 3 days using an IncuCyte live-cell imaging system, and neurite number and density quantified.

**Results** Grafted antibodies bound a significant portion of aggregated A $\beta$  in aqueous AD extracts, but when these antibodies were co-incubated with neurons treated with brain extracts they did not reduce toxicity. By contrast, PrP and the PrP fragment N1 did protect against A $\beta$ .

**Conclusions** These results further demonstrate that not all A $\beta$  oligomers are toxic and suggest that PrP derivatives may allow development of agents that differentially recognize toxic and innocuous A $\beta$  aggregates.

**Key words:** Alzheimer's Disease, prion protein, neurotoxicity

## 1. Introduction

Neurodegenerative diseases are an enormous public health problem, which affect tens of millions of sufferers. A hallmark of disorders such as Alzheimer's disease (AD), Parkinson's disease and transmissible spongiform encephalopathies (TSEs) is the presence of aggregates formed from neuronal proteins of unrelated sequences (Selkoe, 2003; Silveira et al., 2005; Soto, 2003; Walsh and Selkoe, 2016). Consequently, there is considerable interest in the therapeutic targeting of these aggregates and on their detection as diagnostic indicators. Two of the most widely studied polypeptides involved in neurodegeneration are the cellular prion protein (PrP<sup>C</sup>) and the amyloid  $\beta$ -protein (A $\beta$ ).

PrP is critical for the neurotoxicity that underlies the degenerative neurological disorders known as prion diseases or TSEs. Prions are infectious and propagate by converting  $\alpha$ -helix-rich PrP<sup>C</sup> into  $\beta$ -sheet-rich PrP<sup>Sc</sup>. Persuasive evidence indicates that oligomerization and fibrillization of A $\beta$  are key events in AD (Karran and De Strooper, 2016; Selkoe and Hardy, 2016), and over the past decade, data have emerged which suggest that PrP<sup>C</sup> may serve as a receptor that mediates certain aspects of A $\beta$  toxicity (Gunther and Strittmatter, 2010; Smith and Strittmatter, 2017). In addition to binding soluble aggregates of A $\beta$  (aka A $\beta$  oligomers), the N-terminal domain of PrP<sup>C</sup> is known to bind aggregated forms of PrP and PrP<sup>Sc</sup>, conformers of a yeast prion protein and certain designed  $\beta$ -sheet-rich peptides, leading some to speculate that PrP may be capable of binding a variety of aggregated, amyloid-prone proteins (Beland and Roucou, 2012; Resenberger et al., 2011). Special attention has focused on the interaction between PrP and A $\beta$  oligomers. Notably, while the pathological significance of A $\beta$  binding to PrP has been contentious, there is complete agreement that PrP binds A $\beta$  oligomers with high affinity and that PrP is a sub-stoichiometric inhibitor of A $\beta$  aggregation (Chen et al., 2010; Fluharty et al., 2013; Nieznanska et al., 2018; Nieznanski et

al., 2012; Younan et al., 2013). There is also general agreement that two sites within PrP (one around residues 23-33 and the other around 87-112) are important for binding A $\beta$  oligomers (Figure 1A) (Chen et al., 2010; Fluharty et al., 2013; Nieznanska et al., 2018; Younan et al., 2013). N1 (23-111) is a naturally occurring soluble form of PrP (Biasini et al., 2012; Mange et al., 2004) which retains all of the potential residues involved in *Site I* and most (if not all) of the residues implicated in *Site II*. Importantly, several groups have shown that N1 or similar fragments which retain the A $\beta$  binding sites exhibit binding and aggregation inhibition activity comparable to that of full-length PrP (Fluharty et al., 2013; Nieznanska et al., 2018; Nieznanski et al., 2012; Um et al., 2012). Thus, it may be possible to exploit the PrP-A $\beta$  oligomer binding sequences to develop tools to allow the measurement and/or targeting of toxic A $\beta$ . However, since full length PrP is itself a potential neurotoxin (Chesebro et al., 2005) other derivatives of PrP would be more desirable (Biasini et al., 2012). In prior studies, short overlapping sequences covering the entire PrP molecule were used to replace the extended heavy chain complementary-determining region 3 (CDR3) of a human IgG, and their ability to bind PrP<sup>Sc</sup> was investigated (Moroncini et al., 2004; Solfrosi et al., 2007). Analysis of 20 different grafted monoclonal antibodies (mAbs) revealed three high affinity PrP<sup>Sc</sup> recognition motifs, centered around 19-33, 89-112, and 136-158 (Solfrosi et al., 2007). Given that 2 of 3 PrP<sup>Sc</sup> binding sites have been implicated in A $\beta$  binding we investigated whether such antibodies could also bind A $\beta$ .

Here, we show that while recombinant PrP can bind synthetic A $\beta$  oligomers with high affinity, grafted PrP mAbs do not. Yet PrP-grafted antibodies can immunoprecipitate certain aggregates of A $\beta$  from the soluble phase of AD brain. These results and their implications for understanding and targeting toxic and innocuous forms of A $\beta$  are discussed.

## 2. Results

### 2.1 Recombinant PrP, but not PrP-motif grafted antibodies, bind oligomers of synthetic A $\beta$

The cellular prion protein (PrP) is known to specifically and tightly bind A $\beta$  oligomers and this interaction is believed to be mediated by 2 distinct binding sites within the N-terminus of PrP (*Site I* and *Site II*, Figure 1A). These two binding sites have also been implicated in the interaction between PrP<sup>C</sup> and PrP<sup>Sc</sup> (Biasini et al., 2008; Moroncini et al., 2004; Solforosi et al., 2007), and it has been shown that mAbs containing grafts of *Site I* and *Site II* can bind PrP<sup>Sc</sup> (Solforosi et al., 2007). Here, we investigated whether PrP-grafted mAbs known to bind PrP<sup>Sc</sup> could also bind A $\beta$  oligomers. Three distinct preparations of A $\beta$  (see Supplementary Figure 1 for detailed characterization) were tested for their ability to bind to plate-immobilized recombinant PrP (Figure 1B). As in prior studies A $\beta$ -derived diffusible ligands (ADDLs), a recipe-based preparation of A $\beta$ 1-42 which contains monomers, protofibrils, and globular oligomers, bound tightly to PrP with a mean EC<sub>50</sub> of 113 nM (95% CI 96 – 133 nM), based on 3 independent experiments. In contrast, A $\beta$  monomer (EC<sub>50</sub> not determinable) and fibrils (EC<sub>50</sub> not determinable) showed no or little affinity for PrP (Figure 1B). Next, we examined whether plate-immobilized PrP-grafted mAbs could bind A $\beta$ . None of the mAbs bound A $\beta$  monomers, but grafted mAbs 19-33 and 87-112 and the parental mAb, b12, each bound ADDLs (Figure 1C). However, the finding that b12 and the grafted mAbs bind ADDLs to similar extents indicates that binding is not PrP graft-specific, but rather a result of a generic interaction between the b12 backbone and some component of ADDLs.

The lack of specific binding by PrP-grafted mAbs could have resulted due to inactivation of the mAbs which can occur during purification, or as a consequence of steric obstruction preventing 4G8 from detecting oligomers bound to grafted mAbs. We addressed these possibilities first by testing if grafted mAbs could bind PrP<sup>Sc</sup> (Supplementary Figure 2 A) and

second by using an alternative anti-A $\beta$  mAb, 6E10, for detection (Supplementary Figure 2 B and C). Grafted mAbs 19-33 and 87-112 specifically recognized PrP<sup>Sc</sup>, but not PrP<sup>C</sup>. The parent mAb b12 did not immunoprecipitate (IP) either PrP<sup>C</sup> or PrP<sup>Sc</sup> (Supplementary Figure 2 A). These results confirm that the grafted PrP mAbs used are active. Furthermore, when the N-terminal anti-A $\beta$  mAb, 6E10, was used in place of 4G8, PrP-grafted and b12 parent mAbs produced similar binding curves (compare Figure 1B and Supplementary Figure 2 B). Again, monomers showed no binding to PrP. However, unlike 4G8 use of 6E10 allowed appreciable detection of fibrils. Nonetheless, even with 6E10, the EC<sub>50</sub> for ADDLs (234 nM, 95% CI 218 – 251 nM) was an order of magnitude tighter than for A $\beta$  fibrils (1731 nM, 95% CI 1601 – 1870 nM). Thus, irrespectively of the anti-A $\beta$  mAb used, PrP preferentially bound ADDLs. Hence, it is apparent that the inability of PrP-grafted mAbs to bind ADDLs does not result due to trivial experimental artifacts.

## **2.2 PrP-grafted antibodies immunoprecipitate aggregates of A $\beta$ from AD brain extracts**

Given that PrP-grafted mAbs were capable of immunoprecipitating PrP<sup>Sc</sup> from infected mouse brains and that it is uncertain if synthetic A $\beta$  assemblies are relevant to A $\beta$  structures that occur *in vivo*, we asked whether PrP-grafted antibodies could IP aggregated A $\beta$  from human brain. Extensive data indicate that aqueous extracts of AD brain contain an array of A $\beta$  species of which only a portion has disease-relevant activity (Hong et al., 2018). Here, we examined whether PrP-grafted antibodies 19-33 and 87-112 could IP A $\beta$  from aqueous extracts of 4 end-stage AD brains. Grafted mAbs each IP'd a portion of A $\beta$  from all AD brain extracts examined, whereas the parent b12 did not bind any A $\beta$ . In all cases 19-33 bound more A $\beta$  than 87-112 (Figure 2A), whereas no A $\beta$  was detected in IPs of control brain extract. IP/WB detected 2 distinct A $\beta$ -immunoreactive bands, one consistent with A $\beta$  monomer (~4

kDa) and the other with A $\beta$  dimer (~7 kDa). Previous work has shown that the ~7 kDa species most likely represents a mixture of covalently cross-linked A $\beta$  dimers (Brinkmalm et al., 2018; Mc Donald et al., 2015; Vazquez de la Torre et al., 2018) and some aberrantly migrating monomer (Watt et al., 2013). Samples before and after IP were also analyzed using an A $\beta$ x-42 MSD-based immunoassay. Treatment of brain extracts with the chaotropic agent GuHCl dissociates A $\beta$ -aggregates to monomers, and enables their detection (Mc Donald et al., 2015). Thus, by measuring A $\beta$ x-42 in extracts +/- GuHCl treatment, it is possible to quantify the amount of native A $\beta$ 42 monomer (- GuHCl), and A $\beta$ 42 aggregates that can be broken down to monomer by incubation with GuHCl (+ GuHCl). Relative to the starting extract (Before IP), immunoprecipitation with S97, a polyclonal pan-A $\beta$  antibody, depleted A $\beta$  monomer from AD brain extracts by 90 – 96%, and effectively reduced aggregated A $\beta$  by 48 – 56% in AD1 – AD4 (Figure 2B). Treatment with PrP-motif grafted mAb 19-33 reduced aggregated A $\beta$  in AD1, AD2, and AD3 brains by 36 – 56%, and in AD4 by 21%. It is noteworthy that AD4 contained a much higher starting level of aggregated A $\beta$  (425.6 ng/mL) than the other three AD brain extracts (69.5 – 165.7 ng/mL). PrP-motif grafted antibody 87-112 reduced aggregated A $\beta$  by 16 – 38%. The finding that grafted antibody 87-112 is less effective at reducing the level of soluble aggregates than grafted antibody 19-33 is in keeping with IP/WB results which indicate that 19-33 IP'd approximately twice as much A $\beta$  as 87-112. In AD brain extracts A $\beta$  monomer levels were modestly decreased relative to starting extracts, but unchanged versus parent b12 treatment. Thus, grafted mAbs 19-33 and 87-112 can IP a pool of aggregates, but do not IP A $\beta$  monomer.

### **2.3 PrP and PrP N1, but not PrP-grafted antibodies protect against A $\beta$ -induced neuritotoxicity**

Having established that PrP-motif grafted antibodies can bind aggregated A $\beta$  in AD brain extracts, we next assessed whether these mAbs recognize bioactive A $\beta$  species. Recently, we described a live-cell imaging paradigm to measure the effect of AD brain extracts on human neurons (Hong et al., 2018; Jin et al., 2018). Differentiated iPSC-derived human neurons (iNs) (see Figure 3A and 3B for a details) were treated with AD brain extracts and imaged every two hours for 72 hours. Exposure to AD1 brain extract caused a time-dependent decrease in neurite length (Figure 4A and B) (Medium, black; AD1, blue;  $p < 0.001$ , medium vs. AD1; one-way ANOVA). Co-administration of the aggregate specific anti-A $\beta$  mAb, IC22 (Jin et al., 2018; Mably et al., 2015), was used as a positive control to rescue A $\beta$ -dependent neuritotoxicity. Co-administration of IC22 conferred near complete protection against neuritotoxicity induced by AD1 (AD1, blue; AD1 + IC22, grey;  $p < 0.001$ , AD1 vs. AD1 + IC22; one-way ANOVA). However, none of the PrP-motif grafted antibodies were protective (AD1, blue; b12, red; 19-33, light green; 87-112, pink;  $p > 0.05$ , AD1 vs. b12, AD1 vs. 19-33, AD1 vs. 87-112; one-way ANOVA). To test the generalizability of these results, we used a second AD brain extract. As with AD1, AD2 caused a time-dependent loss of neurite length, which was rescued with IC22 but not with PrP-grafted antibodies (Figure 4C and D). To test whether PrP can reduce neuritotoxicity induced by AD brain extract, we treated cells with AD2 brain extract in the presence and absence of PrP or the PrP fragment N1. At concentrations equal to or greater than used for IC22 (Figure 4, 0.02  $\mu$ M), PrP and the N1 fragment provided almost complete protection against AD2 induced neuritotoxicity (AD2, blue; AD1 + 0.02  $\mu$ M PrP, light green;  $p < 0.001$ , AD2 vs. AD2 + 0.02  $\mu$ M PrP; one-way ANOVA) (Figure 5 A and B); (AD2, blue; AD1 + 0.02  $\mu$ M N1, light red;  $p < 0.001$ , AD2 vs. AD2 +

0.02  $\mu$ M N1; one-way ANOVA) (Figure 5 C and D). Importantly, when tested alone, none of the mAbs, PrP, and N1 did not affect neurite integrity (Figure 3 C - F).

Collectively, our results indicate that while PrP-grafted mAbs show little affinity for synthetic A $\beta$  aggregates (ADDLs) they do bind certain A $\beta$  aggregates in extracts of AD brain, but they do not immuno-neutralize the neurotoxic forms therein. In contrast, forms of recombinant PrP and the N1 fragment of PrP, which contains A $\beta$  binding *Sites I and II*, do attenuate A $\beta$  neuritotoxicity.

### 3. Discussion

Compelling evidence indicates that PrP can bind certain soluble aggregates of A $\beta$  and that this interaction is mediated by two distinct sites within the flexible N-terminus of PrP. Intriguingly, the sites believed to be important for A $\beta$  binding have also been implicated in the interaction between PrP<sup>C</sup> and PrP<sup>Sc</sup> (Lau et al., 2007; Moroncini et al., 2004; Solforosi et al., 2007; Turnbaugh et al., 2012) and it is known that antibodies into which these sites have been grafted can bind PrP<sup>Sc</sup> (Biasini et al., 2008; Moroncini et al., 2004; Novitskaya et al., 2006; Solforosi et al., 2007). These observations prompted us to investigate whether PrP-grafted mAbs can bind the most disease relevant form of A $\beta$ , protein extracted from AD brain.

Initial experiments involved synthetic A $\beta$  and recombinant PrP. As in numerous prior studies PrP tightly bound soluble A $\beta$  aggregates (ADDLs), but showed little affinity for A $\beta$  monomers (Chen et al., 2010; Fluharty et al., 2013; Freir et al., 2011; Lauren et al., 2009; Nicoll et al., 2013; Nieznanski et al., 2012; Resenberger et al., 2011; Um et al., 2012). In parallel experiments, PrP-grafted mAbs bearing A $\beta$  binding *Sites I* (19-33) and *II* (87-112) only very weakly bound ADDLs, and this binding was indistinguishable from that produced by the parental b12 mAb that lacked any PrP sequence. Since it is not clear whether structures such as ADDLs are ever present in the human cerebrum, we went on to test if PrP-grafted mAbs could bind disease relevant forms of A $\beta$  extracted from AD brain. Prior studies used an IP/WB paradigm to demonstrate that *Site I* and *II*-containing grafted mAbs do not bind PrP monomer (i.e. PrP<sup>C</sup>) PrP<sup>Sc</sup>, but bind both infectious and (Moroncini et al., 2004; Solforosi et al., 2007), non-infectious pathological aggregates of PrP (Biasini et al., 2008). We adapted a similar approach using aqueous extracts of human brain alongside extracts of scrapie-infected mouse brain. As in earlier reports, b12 19-33 and b12 87-112 specifically IP'd PrP<sup>Sc</sup>

from infected brain but did not IP PrP<sup>C</sup>. In two separate experiments b12 87-112 IP'd noticeably more PrP<sup>Sc</sup> than b12 19-33. Both grafted mAbs also IP'd a portion of soluble A $\beta$  from the bioactive extracts of two AD brains, but in these samples b12 19-33 IP'd more A $\beta$  than did b12 87-112. Differences in the relative affinity of b12 19-33 and b12 87-112 for PrP and A $\beta$  aggregates while interesting should be interpreted with caution due to the small number of brain samples tested and limited quantitative precision of IP/WB paradigms. On the other hand, the finding that neither of the PrP-grafted mAbs IP'd native A $\beta$  monomer in 4 different AD brain extracts is clear cut, and demonstrates that like recognition of PrP<sup>Sc</sup>, binding of A $\beta$ , is aggregation specific. Interestingly, mAb 15B3 which selectively recognizes aggregates of PrP can also bind aggregated but not monomeric forms of A $\beta$  (Stravalaci et al., 2016). These and our results indicate that certain A $\beta$  and PrP aggregates have a common structure. Future studies should explore whether the common recognition of A $\beta$  and PrP aggregates extends to other disease-related proteins (Corbett et al., 2018).

While A $\beta$  aggregation is widely acknowledged to be central to AD pathogenesis, an increasing body of evidence indicates that only a sub-population of A $\beta$  aggregates are bioactive (Brinkmalm et al., 2018; Hong et al., 2018; Lesne, 2013). As with PrP and prion disease (Chiesa et al., 2008; Sandberg et al., 2011; Sandberg et al., 2014), the form or forms of A $\beta$  which mediate toxicity are not yet defined. Hence, rather than relying on simple binding assays to understand and target the small portion of A $\beta$  that is toxic, it is essential to utilize bioactivity assays. To address whether PrP-grafted mAbs recognize toxic A $\beta$  we utilized a very recently developed medium-throughput video-microscopy paradigm to monitor disruption of neurite integrity by AD brain derived A $\beta$  (Hong et al., 2018; Jin et al., 2018). In prior studies we demonstrated that certain anti-A $\beta$  mAbs could attenuate AD brain A $\beta$  mediated neuritotoxicity (Jin et al., 2018). Here, we show that even though a single

round of IP with b12 19-33 and b12 87-112 can pull down an appreciable portion of A $\beta$  in bioactive AD brain extracts (~36% and ~23% of respectively), neither grafted mAb altered the time-course nor the extent of neuritotoxicity. By contrast, when used at the same concentration as PrP-grafted mAbs (0.02  $\mu$ M), our in-house anti-A $\beta$  aggregate-preferring mAb, 1C22 (Mably et al., 2015), nearly fully protected against AD brain A $\beta$ . Thus, it appears that b12 19-33 and b12 87-112 cannot effectively immunoneutralize neuritotoxic A $\beta$ .

PrP and a PrP-fragment (N1) both of which contain A $\beta$  binding *Sites I and II* conferred protection against AD brain A $\beta$ . This is in line with prior studies which reported that N1 attenuated A $\beta$ -mediated toxicity (Fluharty et al., 2013; Nieznanski et al., 2012). Why PrP-grafted mAbs should be able to bind at least some soluble aggregates of A $\beta$ , but not impact A $\beta$  toxicity is not yet clear, but it is tempting to speculate that the lack of reactivity with bioactive A $\beta$  is because the grafted mAbs used are monospecific. That is, b12 19-33 contains two 19-33 grafts and no 87-112, and b12 87-112 contains two 87-112 grafts and no 19-33. Binding of bioactive A $\beta$  by PrP, and by extension PrP-grafted mAbs, may require both binding *Sites I and II*, and it is plausible that the presence of two of the same binding sites on the same grafted mAb may restrict the forms and sizes of A $\beta$  assemblies that can be bound. In future studies it will be interesting to generate and test whether bispecific mAbs containing both *Sites I and II* can protect against AD brain A $\beta$ , and to employ our all-human video-microscopy paradigm to compare the protection afforded by full length PrP, N1, and PrP-grafted bispecific mAbs.

## 4. Material and Methods

### 4.1 Reagents and antibodies

Size exclusion standards were obtained from Bio-Rad (Hercules, CA, USA). All other chemicals and reagents were from Millipore-Sigma (Burlington, MA) unless otherwise noted. Antibodies are described in Table 1.

### 4.2 Preparation of Recombinant Prion Protein 23-231 and 23-109

*Escherichia coli* strain BL21(DE3)*pLysS* (New England BioLabs, Ipswich, MA) was transformed with the pTrcHis B vector expressing murine PrP<sub>23-231</sub> (Jackson et al., 1999; Nicoll et al., 2013; Zahn et al., 1997), with minor modifications. Ampicillin resistant colonies were selected, expanded and expression was induced by the addition of 1 mM IPTG for 16 hours at 37 °C and 225 RPM. Cultures were harvested by centrifugation at 6,000 × *g* for 15 minutes, washed in PBS and lysed by sonication (2 × 120 seconds bursts at 30% output) in extraction buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.1% Tween-20, 50 U/mL Benzonase, 10 µg/mL lysozyme). Suspensions were sedimented at 10,000 × *g* for 30 min and the inclusion body-enriched pellets were extracted by sonication (2 × 120 seconds bursts at 30% output) in solubilization buffer (6 M GuHCl / 50 mM Tris-HCl pH 8.0 / 0.8% β-mercaptoethanol). Suspensions were sedimented at 21,000 × *g* for 45 minutes and the PrP-enriched supernatant was clarified with 5 µm and 0.45 µm syringe filters prior to on-column oxidative refolding. Filtered supernatants were loaded onto 5 mL HisTrap HP columns (GE Life Sciences, Marlborough, MA) at 1 mL/minute using a BioLogic DuoFlow FPLC system (Bio-Rad, Hercules, CA) and washed with 10 column volumes (CV) of Buffer A (6 M GuHCl, 10mM Tris-HCl, 100 mM Na<sub>2</sub>HPO<sub>4</sub>, 10mM Glutathione pH 8.0) at 1 mL/minute. Bound PrP was refolded in a linear gradient of Buffer A to Buffer B (10mM Tris-HCl, 100 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 8.0) for 30 CV at 0.213 mL/minute (11 ¾ hours). The following day, the column was eluted in a linear

gradient of Buffer B to Buffer C (10mM Tris-HCl, 100 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 M imidazole, pH 5.8) for 3 CV at 0.5 mL/minute and the fractions containing PrP were buffer exchanged with 2 kDa dialysis cassettes (ThermoFisher, Waltham, MA) overnight at 4 °C against 1000 volumes of Bis-Tris HCl, pH 6.5. The poly-histidine tag was cleaved from PrP using 50 U restriction grade thrombin (Novagen, Madison, WI) overnight with agitation, and cleaved PrP was separated from the free histidine tag using a 5 mL HisTrap HP column. The fractions containing purified PrP were dialyzed overnight at 4 °C against 1000 volumes of Bis-Tris HCl, pH 6.5. Protein purity was determined by SDS-PAGE/silver staining and mass spectrometry, and secondary structure was analysed by circular dichroism (Jasco J-815, Jasco, Easton, MD). Concentration was determined by measuring absorbance at 280 nm and using the predicted extinction coefficient (63370,  $\epsilon_{280} = \text{M}^{-1}\text{cm}^{-1}$ ).

For production of PrP<sub>23-109</sub> (N1), *Escherichia coli* strain BL21 Star was transformed with the pET101 vector expressing murine PrP<sub>23-109</sub>. This vector was expressed and purified as described previously (McDonald et al., 2014), with minor modifications. Cells were lysed, then purified with an ÄKTA purification system (GE Healthcare, Marlborough, MA, USA) using a Ni<sup>2+</sup>-immobilized metal ion affinity column. Protein was eluted with 5 M guanidine HCl, 0.1 M Tris acetate, 0.1 M potassium phosphate (pH 4.5). A<sub>280</sub> positive fractions spanning the elution peak were combined, and the pH was raised to 8 by addition of potassium acetate. The neutralized solution was desalted into 20 mM potassium acetate, pH 5.5 using a HiPrep 26/10 desalting column (GE Healthcare, Marlborough, MA, USA). This material was then purified using a C4 reverse-phase HPLC column (Grace/Vydac, Columbia, MD, USA). Fractions containing the purified protein were pooled, lyophilized, and stored at -80 °C. Protein stocks were reconstituted in 0.22 µm filtered water. Protein purity was determined by SDS-PAGE/Coomassie staining. N1 was further characterized by Western Blot

using anti-PrP mAbs directed against A $\beta$  binding *Sites I and II* (Supplementary Figure 3 A and B). N1 and PrP bind ADDLs with similar affinity, and show little affinity for fibrils and monomers in an ELISA like assay (Supplementary Figure 3 C).

#### **4.3 Preparation of A $\beta$ conformers**

A $\beta$ 1-40 monomers and A $\beta$ 1-42 fibrils were prepared as previously described (O'Malley et al., 2016). To remove pre-existing aggregates peptides were incubated in 50 mM Tris-HCl, pH 8.5, containing 5 mM EDTA and 7 M guanidine HCl for at least 16 hours. A $\beta$ 1-40 monomers were isolated by chromatographing disaggregated peptide on a Superdex 75 10/300 SEC column (GE Life Sciences, Marlborough, MA, USA) eluted with 50 mM ammonium bicarbonate, pH 8.5 (Supplementary Figure 1A). Peak fractions were collected and concentration determined by measuring UV absorbance at 275 nm (O'Malley et al., 2014). Aliquots were flash-frozen in liquid nitrogen and stored at -80 °C. To generate fibrils A $\beta$ 1-42 monomer was isolated using the same column as for A $\beta$ 1-40, but this time eluted in 20 mM sodium phosphate, pH 8.0. Peak fractions were pooled, the concentration measured, and the solution diluted to 20  $\mu$ M in 20 mM sodium phosphate, pH 8.0. A portion of the sample was mixed with Thioflavin T (20  $\mu$ M) to monitor formation of fibrils (Supplementary Figure 1B). The remainder was incubated without Thioflavin T. When fibril formation had reached its maximum (Supplementary Figure 1B) fibrils were harvested by centrifugation at 100,000 x *g* for 60 minutes. Fibrils were resuspended in an equal volume of phosphate-buffered saline, pH 7.4, and washed 2 times. A $\beta$  fibrils were flash-frozen in liquid nitrogen and stored in small aliquots at -80 °C. A $\beta$ -derived diffusible ligands (ADDLs) were prepared as described (Freir et al., 2011). In brief, 1 mg A $\beta$ 1-42 was dissolved in HFIP, and incubated at 37 °C for 1 hour in a 2 mL lobind protein Eppendorf tube (Eppendorf, Hamburg, Germany). The HFIP was evaporated under a gentle stream of Helium to produce a clear film, and then

stored over desiccant at -20 °C. The peptide film was dissolved in anhydrous DMSO and diluted to 100 µM in phenol red-free DMEM/F12 media (Thermo Scientific, Waltham, MA, USA). Samples were incubated at 4 °C for 48 hours, centrifuged at 14,000 x *g* for 10 minutes, and the supernatant flash-frozen in liquid nitrogen and stored at -80 °C. ADDLs contained a mixture of Aβ monomer and soluble aggregates which appeared on EM as imperfect spheres of ~5 nm diameter and protofibrils up to 200 nm in length (Supplementary Figure 1C).

#### **4.4 Electron microscopy**

Sample or buffer (10 µL) was loaded onto 200 mesh formvar/carbon-coated copper grids and left for 60 seconds, and fixed with 0.5% glutaraldehyde for 60 seconds. Grids were wicked dry and washed with 0.22 µm filtered ultra-pure water. Finally, samples were stained with 2% uranyl acetate for 120 seconds, wicked dry and then allowed to air dry.

#### **4.5 Expression and purification of PrP-grafted antibodies**

PDR12 vector containing the b12 light chain and the b12 human γ1 heavy chain gene for the parental b12 antibody, and the b12 antibody with PrP 19-33 and 87-112 grafts were described previously (Biasini et al., 2008; Moroncini et al., 2004; Solfrosi et al., 2007). The cysteine residue 22 in the PrP sequence 19-33 was mutated to alanine to avoid unwanted dimerization. Expi293 cells were transfected at a ratio of 90 µg of plasmid to  $1.75 \times 10^8$  cells using ExpiFectamin 293 reagent (Gibco, Grand Island, NY, USA) and 15 µg pAdVantage vector (Promega, Madison, WI, USA). Cells were grown in vented 500 mL Erlenmeyer flasks with shaking (122 rpm) at 37 °C and 5% CO<sub>2</sub> for 96 hours. Cell medium was collected and clarified by sequential centrifugation at 150 x *g* for 6 minutes and 3,500 x *g* for 10 minutes, followed by filtration through a 0.45 µm filter. Antibodies were purified using a Hitrap Protein A column (GE, Boston, MA, USA) and eluted with 0.1 M sodium citrate buffer, pH 3, into 1 M

Tris, pH 9.0. Immediately after purification, antibodies were buffer exchanged into phosphate-buffered saline, pH 7.4 using a 40 k cut-off Zeba column (Thermo Scientific, Waltham, MA, USA), and then passed through a 0.22  $\mu\text{m}$  filter. Purity was assessed by SDS-PAGE/Coomassie staining.

#### **4.6 Mice and preparation of brain lysates**

Intracerebral inoculations of C57BL/6 were performed at Boston University with the Rocky Mountain Laboratory strain of scrapie (RML) as described previously (Fang et al., 2016). Mice were inoculated intracerebrally with 1% brain homogenate (30  $\mu\text{L}$ ) from the brain of a terminally ill RML infected mouse. The inoculated mice were monitored until the appearance of clinical signs (~170 days after the inoculation). At that time animals were euthanized. Brains were collected, and stored at  $-80\text{ }^{\circ}\text{C}$ . All animal procedures were performed in accordance with the National Institutes of Health Policy on the Use of Animals in Research and were approved by the Boston University medical centre institutional animal care and use committee. Ten percentage (w/v) homogenates of mouse hemibrains were prepared in phosphate buffer saline, pH 7.2, containing 1% Triton X-100 and EDTA-free Complete protease inhibitors (Roche, Indianapolis, IN, USA) using 1 mm glass beads (Benchmark Scientific, Edison, NJ, USA) and a Beadbug microtube homogenizer (Benchmark Scientific). Brain homogenates were passed once through a 0.5 cc insulin syringe with a 28-gauge needle (Becton Dickinson, Franklin Lakes, NJ, USA) and cleared by sequential centrifugation at  $1,000 \times g$  for 30 seconds and  $16,000 \times g$  for 5 minutes at  $4\text{ }^{\circ}\text{C}$ . Protein concentration was measured with a BCA protein kit (Pierce, Rockford, IL, USA) and adjusted to 1 mg/mL.

#### **4.7 Preparation of human brain extracts**

Human specimens were obtained from the Massachusetts ADRC Neuropathology core at the Massachusetts General Hospital in accordance with the Partners Institutional Review Board (Walsh BWH2011). Frozen temporal cortical tissue was obtained from 5 cases, 4 end-stage AD cases, and 1 subject who died free of AD (Table 2). All cases met current post-mortem and clinical diagnosis criteria. The post-mortem interval was 36 hours or less. Approximately 4 g of cortical tissue was dissected from each case. Extracts were prepared by homogenizing 1 g of tissue in 5 volumes of ice-cold artificial cerebrospinal fluid (aCSF) (124 mM NaCl, 2.8 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 26 mM NaHCO<sub>3</sub>, pH 7.4) containing 5 mM EDTA, 1 mM EGTA, 5 µg/mL leupeptin, 5 µg/mL aprotinin, 2 µg/mL pepstatin, 120 µg/mL Pefabloc and 5 mM NaF with 25 strokes of a motorized Teflon-glass Dounce homogenizer (Fisher, Ottawa, Canada) (Jin et al., 2018; Wang et al., 2017). Homogenates were centrifuged at 200,000 x *g* and 4 °C for 110 minutes. The upper 80% of the supernatant was removed and dialyzed against a 100-fold excess of aCSF using a 2000 MWCO Slide-A-Lyzer G2 dialysis cassettes (Fisher Scientific, Waltham, MA, USA) at 4 °C. Samples were dialyzed for at least 72 hours with three buffer changes.

#### **4.8 Recombinant PrP and PrP-grafted mAbs immunoassay**

Black half-area high binding 96-well plates (Greiner Bio-One, Frickenhausen, Germany) were coated with PrP (0.5 µM) or mAbs (13.33 nM) in 50 mM carbonate coating buffer, pH 9.6 at 30 µL/well and incubated at 37°C for 1 hour on an orbital shaker (Woodbridge, NJ, USA) at 300 rpm. Wells were washed 4 times with PBS containing 0.05% Tween 20 (TBS-T) and then blocked with SuperBlock (Thermo Scientific, Waltham, MA, USA) for 2 hours at 37°C at 300 rpm. All remaining steps were conducted at room temperature. Wells were washed 4 times with PBS-T. Samples were analyzed in duplicate (30 µL/well). Standards and samples were

diluted with PBS-T containing 0.1% BSA (w/v). All samples were incubated for 50 minutes at 37 °C on an orbital shaker at 300 rpm. Thereafter, wells were washed 4 times with PBS-T and 30 µL of biotinylated 4G8 (b4G8) or biotinylated 6E10 (b6E10) in PBS-T containing 0.5% (w/v) BSA was added to each well and incubated for 1 hour with shaking on an orbital shaker. Wells were washed four times and 30 µL of HRP-conjugated streptavidin (R&D, Minneapolis, MN, USA) in PBS-T containing 0.5% (w/v) BSA was added and incubated on an orbital shaker for 20 minutes. Subsequently, wells were washed 4 times with PBS-T and incubated with 50 µL of Pico Super Signal Chemiluminescence substrate (Thermo Scientific, City, USA). Chemiluminescence was measured using a Synergy H1 plate reader (Biotek, Winooski, VT, USA). Standard curves were fitted using non-linear regression using least squares (ordinary) fit with Graph Pad Prism (LaJolla, CA, USA).

#### **4.9 MSD Aβ<sub>x-42</sub> immunoassay**

This assay was performed as described before (Mably et al., 2015). All incubation steps were conducted at room temperature using an orbital shaker (Woodbridge, NJ, USA) at 300 rpm. MULTI-ARRAY 96 well black microplates (MSD, Rockville, MD, USA) were coated with 3 µg/mL of the monoclonal anti-Aβ antibody 266 in PBS, pH7.4 (40 µl per well). Thereafter, wells were blocked with 150 µL of 5% (w/v) blocker A (MSD, Rockville, MD, USA) in TBS for 1 hour. Plates were washed 3 times with TBS containing 0.05% Tween 20 (TBS-T). Incubation with guanidine HCl dissociates soluble Aβ aggregates allowing detection of Aβ<sub>x-42</sub> monomer (Mc Donald et al., 2015). Therefore, samples were analyzed with and without pre-treatment with 5 M GuHCl. In brief, 20 µL of sample was incubated with 50 µL of 7 M guanidine HCl overnight at 4 °C. Samples containing 5 M guanidine HCl were diluted 20-fold in TBS-T containing 1% (w/v) blocker A on the day of analysis. Consequently, blanks and Aβ<sub>1-42</sub> standards were also prepared in TBS-T containing 1% (w/v) blocker A and 0.25 M guanidine

HCl (5 M divided by the dilution factor of 20). After capture, wells were washed 3 times in TBS-T and incubated with biotinylated 21F12 antibody in TBS-T containing 1% (w/v) blocker A. Simultaneously, 1 µg/mL of the reporter reagent (SULFO-TAG labeled streptavidin) (MSD, Rockville, MD, USA) was added and incubated for 2 hours. Finally, wells were washed for 3 times with TBS-T and 150 µL 2 x MSD reading buffer was added to allow for electrochemiluminescence detection with a SECTOR imager (MSD, Rockville, MD, USA). The lower limit of quantification (LLOQ) was defined as the lowest standard with a signal higher than the average signal for the blank plus 9 SDs and allows a percent recovery  $\geq 100 \pm 20\%$  and a coefficient of variance (CV)  $\leq 20\%$ . In this study, LLOQ of the assay was either 9.8 or 19.5 pg/mL.

#### **4.10 Immunoprecipitation/Western Blot analysis**

PrP-grafted antibodies were used to immunoprecipitate (IP) PrP<sup>Sc</sup> from mouse brain extracts as described previously (Moroncini et al., 2004). Polyclonal goat anti-human or goat anti-mouse IgG (Fab')<sup>2</sup> (Thermo Scientific, Waltham, MA, USA) was conjugated to M280 Tosyl-activated paramagnetic Dynabeads (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Beads (5 mg) were washed and resuspended in 250 µL 0.1 M sodium phosphate, pH 7.4 containing 1.2 M ammonium sulphate and 100 µg IgG. Beads were then incubated with IgG for 16 hours at 37 °C on a rotating mixer. For all IPs, half ml aliquots of brain extract were diluted 1:1 in IP buffer (phosphate buffered saline, pH 7.2 containing 1% (v/v) Triton X-100 and 1 x Complete Protease inhibitor). Each aliquot was incubated with 5 µg of PrP-grafted antibody or ICSM35 for 2 hours at 25 °C on a rotating wheel. Then, 10 µL of IgG-conjugated Dynabeads (anti-human or anti-mouse, as appropriate) was added, and incubated for another 2 hours at 25 °C. Beads were collected and washed 10 times with 1 mL of IP buffer for 10 minutes. Finally, pellets were resuspended in 20 µL of

non-reducing 2X Laemmli loading buffer (150 mM Tris, pH 6.8 containing 6% SDS (w/v), 0.3% bromophenol blue (w/v), 30% glycerol (v/v)), and heated to 96 °C for 5 minutes. Samples were centrifuged for 15 seconds at 16,000 x *g* and electrophoresed on 4-12% pre-cast NuPAGE MES Bis-Tris gels (Thermo Scientific, Waltham, MA, USA). Proteins were transferred onto 0.2 µm PVDF (100 V for 30 minutes). PrP was detected with 6D11 (1 µg/mL) and bands visualized employing ECL.

IPs of 0.5 mL aliquots of human brain lysates were performed as described above with minor modifications. Human brain extracts were incubated with 5 µg PrP-grafted antibodies or 20 µL S97 antiserum for 2 hours at 4 °C on a rotating wheel. Then, 10 µL of IgG-conjugated paramagnetic beads (for PrP-grafted antibodies) or 30 µL of Protein A agarose beads (Merck Millipore, Burlington, MA, USA) (for S97) was added, and incubated overnight at 4 °C on a rotating wheel. All subsequent steps were carried out as described above, but proteins were transferred to 0.2 µm nitrocellulose membranes at 400 mA and 4 °C for 2 hours. Blots were microwaved in PBS, Aβ was detected using the anti-Aβ40 and anti-Aβ42 antibodies, HJ2 (1 µg/mL) and 21F12 (1 µg/mL) and visualized using the Li-COR Odyssey infrared imaging system (LiCor, Lincoln, NE, USA).

#### **4.11 Western Blot analysis of recombinant prion protein**

Proteins were electrophoresed on pre-cast 4-12% NuPAGE MES Bis-Tris gels (Thermo Scientific, Waltham, MA, USA) and transferred onto 0.2 µm nitrocellulose membranes at 400 mA for 2 hours. Blots were incubated in Odyssey blocking buffer (Li-COR, Lincoln, NE, USA) for 1 hour at room temperature, and then incubated with primary antibody in Odyssey blocking buffer (1 µg/mL MI0131 or 1 µg/mL ICSM35) overnight at 4 °C. Next day, blots were washed with phosphate buffered saline with 0.05% Tween-20 (PBS-T) 4 x 10 minutes.

Membranes were then incubated with infra-red labeled anti-human or anti-mouse secondary antibodies (Li-COR, Lincoln, NE, USA) for 1 hour at room-temperature, followed by 4 x 10 minutes washing with PBS-T and a final washing step in PBS. Immunoreactive bands were visualized using the Li-COR Odyssey infrared imaging system (LiCor, Lincoln, NE, USA).

#### **4.12 Production of induced neurons (iNs) from human induced pluripotent stem cells (iPSCs)**

Neurogenin 2 (Ngn2)-induced human neurons (Zhang et al., 2013) were prepared as described in Figure 1A (Hong et al., 2018; Jin et al., 2018). In brief, YZ1 iPSCs were maintained in medium containing DMEM/F12, Knockout Serum Replacement, penicillin/streptomycin, L-glutamine, MEM-NEAA, and  $\beta$ -mercaptoethanol (all from Invitrogen, Carlsbad, CA, USA) with addition of 10  $\mu$ g/mL basic fibroblast growth factor (bFGF; Millipore-Sigma, Burlington, MA, USA). iPSCs were plated at a density of 95,000 cells/cm<sup>2</sup> for viral infection. Ultrapure lentiviral titers were obtained from Alstem (Richmond, CA, USA) and used at the following concentrations: Tet-O-Ngn2-puro: 0.1  $\mu$ L/50,000 cells; Tet-O-FUW-EGFP: 0.05  $\mu$ L/50,000 cells; FUDeltaGW-rtTA: 0.11  $\mu$ L/50,000 cells. To induce Ngn-2 expression, doxycycline was added on iN day 1 at a concentration of 2  $\mu$ g/mL. On iN day 2, puromycin was added at 20  $\mu$ g/mL and was maintained in medium thereafter. On iN day 4, cells were plated on Matrigel (Corning, NY)-coated 96-well plates (5,000 cells/well) and maintained in Neurobasal media (Gibco, Carlsbad, CA, USA) containing Glutamax, 20% dextrose, MEM-NEAA, B27 and 10 ng/mL BDNF, CNTF, GDNF (PeproTech, Rocky Hill, NY, USA). On iN day 21, cells were used to investigate effects of AD brain extracts +/- mAbs.

#### **4.13 Treatment of iNs with AD brain extracts and mAbs and live-cell imaging**

Treatment of iNs and live-cell imaging was performed as previously described (Hong et al., 2018; Jin et al., 2018). Aliquots (2, 0.5 mL) of AD brain extracts were thawed on ice for 30-60 minutes, centrifuged at 16,000 x *g* for 2 minutes, and buffer-exchanged into iN medium using a 5 ml HiTrap desalting column (GE, Boston, MA, USA). Immediately prior to treatment, cells were placed in an IncuCyte Zoom live-cell imaging instrument (Essen Bioscience, Ann Arbor, MI, USA), and imaged every 2 hours for a total of 6 hours. After baseline imaging 100  $\mu$ L of medium was removed from each well of iN cells leaving a total volume of  $\sim$  100  $\mu$ L. Cells were then treated with 50  $\mu$ L of medium  $\pm$  AD extract and a further 50  $\mu$ L of medium  $\pm$  test mAbs. Antibodies were tested at 0.02  $\mu$ M (3  $\mu$ g/mL). PrP and N1 were each tested at 0.2 and 0.02  $\mu$ M, i.e., 4.6 and 0.46  $\mu$ g/mL for PrP, and 1.82 and 0.18  $\mu$ g/mL for N1. Cells were then returned to the IncuCyte instrument and imaged every 2 hours for a total of 72 hours. Images (Figure 3B) were analyzed using the Neural Track algorithm of IncuCyte Zoom 2016A software (Essen Bioscience, Ann Arbor, MI, USA). Neurite length were normalized to the average value measured in the 6 hours prior to addition of samples.

#### **4.14 Statistical analysis**

Statistical analyses were carried out using GraphPad Prism, version 5 (LaJolla, CA, USA). One-way ANOVA followed by Bonferroni's *post-hoc* test was performed to compare means. The significance threshold was set to a two-sided  $p \leq 0.05$ .

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## **Disclosures**

None of the authors have biomedical financial interests or potential conflicts of interest.

## **Author contributions**

DMW conceived the project, designed and supervised the research. DMW and DM wrote the manuscript. DM produced PrP-motif grafted antibodies and carried out the majority of experiments, analyzed data, prepared the Figures and conducted statistical comparisons. WH performed and analyzed IncuCyte experiments. GTC expressed, purified, and characterized recombinant PrP. WL carried out MSD assays and certain IP experiments. ADS prepared and characterized A $\beta$  monomers and fibrils. CF provided Scrapie infected mice brains. JC provided ICSM35 antibody. DH provided N1 and expert assistance for IP experiments from Scrapie infected mice brains. All the authors critically assessed and contributed to writing the manuscript.

## Figure Legends

**Figure 1. Recombinant murine PrP, but not PrP-motif grafted antibodies bind ADDLs with high affinity.** **(A)** Schematic of PrP and N1. The precise amino acid sequences constituting oligomer binding *Site I* and *Site II* are not yet known but appear to be centered around residues 19-33 and 87-112, respectively. The octapeptide repeat domain,  $\beta$ -sheets ( $\beta$ )1 and 2, and  $\alpha$ -helices (H)1-3 are indicated. PrP can undergo endoproteolytic cleavage yielding N-terminal secreted products referred to as N1 (23-111 or 23-112). We used a PrP fragment comprising the amino acid residues 23-109 for our study. **(B)** Direct binding of ADDLs, A $\beta$  fibrils, and A $\beta$  monomers was assessed using an ELISA-like microtiter plate assay. Preparations of A $\beta$  were incubated for 1 hour on PrP-coated plates and bound A $\beta$  was detected with biotinylated anti-A $\beta$  mAb 4G8. ADDLs bind PrP in a dose-dependent manner, whereas A $\beta$  monomers and fibrils show little affinity for PrP. Each data point is the average  $\pm$  SEM of three independent experiments. **(C)** Antibodies grafted with and without PrP residues 19-33 and 87-112 were coated onto microtiter plate wells and incubated with ADDLs (filled circles) or A $\beta$  monomers (open circles) for 1 hour, and then detected with the mAb 4G8. Red circles denote b12 mAb. Antibodies grafted with PrP motifs 19-33 and 87-112 are colored in light green and pink, respectively. Each data point is the average  $\pm$  SEM of two technical replicates. Where the error bars are not visible, the SEM is smaller than the size of the symbol. Results are representative of at least 2 individual experiments.

**Figure 2. PrP-motif grafted antibodies immunoprecipitate aggregated A $\beta$  from AD, but not control brain.** **(A)** Water-soluble extract from four AD brains (AD1 to AD4) and one control brain were incubated with PrP-motif grafted antibodies. The b12 antibody was used as negative control and the polyclonal antiserum, S97, as a positive control. Anti-human IgG

coupled to paramagnetic beads was used to capture b12 and b12-PrP grafted mAbs. S97 was captured with Protein A agarose beads. Precipitated A $\beta$  was detected via Western blot using anti-A $\beta$  mAbs, HJ2 and 21F12. Molecular weight markers are indicated on left, and the antibodies used for IP are indicated at the bottom of the gel. M (single arrow) denotes A $\beta$  monomer and double arrow refers to SDS-stable ~7kDa A $\beta$ . **(B)** Starting brain extracts and IP supernates were analyzed using an A $\beta$ x-42 MSD immunoassay with (right panel) and without (left panel) preincubation with 5 M GuHCl. ND denotes non-detected.

**Figure 3. Live-cell imaging and analysis of neurite length in iPSC-derived human neurons.**

**(A)** Schematic shows the timeline to generate, mature, and image iPSC-derived human neurons (iNs). **(B)** Upper panels: phase contrast images of iNs at the start (T=0 hours) and end of the imaging period (T=72 hours). Middle panels: images were analyzed using IncuCyte NeuroTrack algorithm for the identification of neurites (pink). Lower panels: NeuroTrack identified neurites are superimposed on the phase contrast image. The scale bar is 100  $\mu$ m. To test whether mAbs, PrP, and N1 protein are compatible with our assay, iNs were treated with mAbs, PrP, or N1 in the indicated concentrations, and cells imaged for 72 hours. **(C + D)** Time-course plots are similar for iN cells treated +/- mAbs, PrP or N1 protein. Each data point is the average of 3 wells  $\pm$  SD. **(D + E)** Neuritic length was averaged over the last 6 h of imaging, and values normalized to pre-treatment measurements.

**Figure 4. PrP-grafted antibodies do not protect against A $\beta$ -induced neuritotoxicity.** Live-cell imaging was used to monitor the effects of A $\beta$ -containing AD brain extracts on iPSC-derived neurons (iNs) and whether or not PrP-grafted mAbs could protect neurons. On post-induction date 21, iNs were treated with medium alone (black circles), AD extract (blue), or

AD extract plus b12 (red), 19-33 (green), 87-112 (pink), or IC22 (gray). Each antibody was used at a final concentration of 0.02  $\mu$ M. **(A)** Time-course plot shows that AD1 extract caused neuritotoxicity and this could be attenuated by IC22, but not PrP-grafted mAbs. Each data point is the average of 3 wells  $\pm$  SD. **(C)** Similar results were obtained using extracts from AD2. **(B and D)** Neuritic length was averaged over the last 6 h of imaging, and values normalized to pre-treatment measurements. The results are representative of two independent experiments. Differences in means were assessed with ANOVA followed by Bonferoni's *post-hoc* test. n.s., non-significant;  $p>0.05$ ; \*\*\*  $p<0.001$ .

**Figure 5. PrP and N1 protect against A $\beta$ -induced neuritotoxicity.** The same paradigm as in Figure 4 was employed to monitor the effects of the A $\beta$ -containing AD 2 brain extract on iNs and whether or not PrP and N1 protein could protect neurons. On post-induction date 21, iNs were treated with medium alone (black circles), AD 2 extract (blue), or AD 2 extract plus 0.02  $\mu$ M PrP (light green), 0.2  $\mu$ M PrP (dark green), or IC22 (gray). **(A)** Time-course plot shows that AD2 extract caused neuritotoxicity and this could be attenuated by treatment with either PrP or IC22. Each data point is the average of 3 wells  $\pm$  SD. **(C)** Similar results could be obtained by treatment with N1 protein. Coincubation of iNs with AD 2 extract plus 0.02  $\mu$ M N1 protein (light red) and 0.2  $\mu$ M N1 protein (dark red) reduced AD brain extract induced neuritotoxicity. **(B and D)** Neuritic length was averaged over the last 6 h of imaging, and values normalized to pre-treatment measurements. Differences in means were assessed with ANOVA followed by Bonferoni's *post-hoc* test. The results shown are from a single experiment. \*\*\*  $p<0.001$ .

## References

- Beland, M., Roucou, X., 2012. The prion protein unstructured N-terminal region is a broad-spectrum molecular sensor with diverse and contrasting potential functions. *J Neurochem.* 120, 853-68.
- Biasini, E., Seegulam, M.E., Patti, B.N., Solforosi, L., Medrano, A.Z., Christensen, H.M., Senatore, A., Chiesa, R., Williamson, R.A., Harris, D.A., 2008. Non-infectious aggregates of the prion protein react with several PrPSc-directed antibodies. *J Neurochem.* 105, 2190-204.
- Biasini, E., Turnbaugh, J.A., Unterberger, U., Harris, D.A., 2012. Prion protein at the crossroads of physiology and disease. *Trends Neurosci.* 35, 92-103.
- Brinkmalm, G., Hong, W., Wang, Z., Liu, W., O'Malley, T., Sun, X., Frosch, M.P., Selkoe, D.J., Portelius, E., Zetterberg, H., Blennow, K., Walsh, D.M., 2018. Identification of neurotoxic cross-linked A $\beta$  dimers in Alzheimer brain. *in review*.
- Chen, S., Yadav, S.P., Surewicz, W.K., 2010. Interaction between human prion protein and amyloid-b (Ab) oligomers: role of N-terminal residues. *Journal of Biological Chemistry.* 285, 26377-83.
- Chesebro, B., Trifilo, M., Race, R., Meade-White, K., Teng, C., LaCasse, R., Raymond, L., Favara, C., Baron, G., Priola, S., Caughey, B., Masliah, E., Oldstone, M., 2005. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science.* 308, 1435-9.
- Chiesa, R., Piccardo, P., Biasini, E., Ghetti, B., Harris, D.A., 2008. Aggregated, wild-type prion protein causes neurological dysfunction and synaptic abnormalities. *J Neurosci.* 28, 13258-67.

- Corbett, G.T., Asfaw, A., Hall, T.C., Wang, Z., Liu, W., Collinge, J., Perikinton, M., Billinton, A., Walsh, D.M., 2018. A recipe for neurotoxicity: the prion protein, promiscuous binding, and soluble protein aggregates? Society for Neuroscience. Abstract #: 713.02, San Diego.
- Fang, C., Imberdis, T., Garza, M.C., Wille, H., Harris, D.A., 2016. A Neuronal Culture System to Detect Prion Synaptotoxicity. *PLoS Pathog.* 12, e1005623.
- Fluharty, B.R., Biasini, E., Stravalaci, M., Sclip, A., Diomede, L., Balducci, C., La Vitola, P., Messa, M., Colombo, L., Forloni, G., Borsello, T., Gobbi, M., Harris, D.A., 2013. An N-terminal fragment of the prion protein binds to amyloid- $\beta$  oligomers and inhibits their neurotoxicity in vivo. *Journal of Biological Chemistry.* 288, 7857-66.
- Freir, D., Nicoll, A.J., Klyubin, I., Panico, S., Mc Donald, J.M., Risse, E., Asante, E.A., Farrow, M.A., Sessions, R.B., Saibil, H.R., Clarke, A.R., Rowan, M.J., Walsh, D.M., Collinge, J., 2011. Interaction between prion protein and toxic amyloid  $\beta$  assemblies can be therapeutically targeted at multiple sites. *Nature Communications.* 2, 1341-51.
- Gunther, E.C., Strittmatter, S.M., 2010. Beta-amyloid oligomers and cellular prion protein in Alzheimer's disease. *J Mol Med (Berl).* 88, 331-8.
- Hong, W., Wang, Z., Liu, W., O'Malley, T.T., Jin, M., Willem, M., Haass, C., Frosch, M.P., Walsh, D.M., 2018. Diffusible, highly bioactive oligomers represent a critical minority of soluble A $\beta$  in Alzheimer's disease brain. *Acta Neuropathol.* 136, 19-40.
- Jackson, G.S., Hill, A.F., Joseph, C., Hosszu, L., Power, A., Waltho, J.P., Clarke, A.R., Collinge, J., 1999. Multiple folding pathways for heterologously expressed human prion protein. *Biochim Biophys Acta.* 1431, 1-13.
- Jin, M., O'Nuallain, B., Hong, W., Boyd, J., Lagomarsino, V.N., O'Malley, T.T., Liu, W., Vanderburg, C.R., Frosch, M.P., Young-Pearse, T., Selkoe, D.J., Walsh, D.M., 2018. An

- in vitro paradigm to assess potential anti-Abeta antibodies for Alzheimer's disease. Nat Commun. 9, 2676.
- Karran, E., De Strooper, B., 2016. The amyloid cascade hypothesis: are we poised for success or failure? J Neurochem. 139 Suppl 2, 237-252.
- Lau, A.L., Yam, A.Y., Michelitsch, M.M., Wang, X., Gao, C., Goodson, R.J., Shimizu, R., Timoteo, G., Hall, J., Medina-Selby, A., Coit, D., McCoin, C., Phelps, B., Wu, P., Hu, C., Chien, D., Peretz, D., 2007. Characterization of prion protein (PrP)-derived peptides that discriminate full-length PrP<sup>Sc</sup> from PrP<sup>C</sup>. Proc Natl Acad Sci U S A. 104, 11551-6.
- Lauren, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W., Strittmatter, S.M., 2009. Cellular prion protein mediates impairment of synaptic plasticity by amyloid b oligomers. Nature. 457, 1128-1132.
- Lesne, S.E., 2013. Breaking the Code of Amyloid-beta Oligomers. Int J Cell Biol. 2013, 950783.
- Mably, A.J., Liu, W., Mc Donald, J.M., Dodart, J.C., Bard, F., Lemere, C.A., O'Nuallain, B., Walsh, D.M., 2015. Anti-Abeta antibodies incapable of reducing cerebral Abeta oligomers fail to attenuate spatial reference memory deficits in J20 mice. Neurobiol Dis. 82, 372-384.
- Mange, A., Beranger, F., Peoc'h, K., Onodera, T., Frobert, Y., Lehmann, S., 2004. Alpha- and beta- cleavages of the amino-terminus of the cellular prion protein. Biol Cell. 96, 125-32.
- Mc Donald, J.M., O'Malley, T.T., Liu, W., Mably, A.J., Brinkmalm, G., Portelius, E., Wittbold, W.M., 3rd, Frosch, M.P., Walsh, D.M., 2015. The aqueous phase of Alzheimer's disease brain contains assemblies built from approximately 4 and approximately 7 kDa Abeta species. Alzheimers Dement. 11, 1286-305.

- McDonald, A.J., Dibble, J.P., Evans, E.G., Millhauser, G.L., 2014. A new paradigm for enzymatic control of alpha-cleavage and beta-cleavage of the prion protein. *J Biol Chem.* 289, 803-13.
- Moroncini, G., Kanu, N., Solforosi, L., Abalos, G., Telling, G.C., Head, M., Ironside, J., Brockes, J.P., Burton, D.R., Williamson, R.A., 2004. Motif-grafted antibodies containing the replicative interface of cellular PrP are specific for PrP<sup>Sc</sup>. *Proc Natl Acad Sci U S A.* 101, 10404-9.
- Nicoll, A.J., Panico, S., Freir, D.B., Wright, D., Terry, C., Risse, E., Herron, C.E., O'Malley, T., Wadsworth, J.D., Farrow, M.A., Walsh, D.M., Saibil, H.R., Collinge, J., 2013. Amyloid-beta nanotubes are associated with prion protein-dependent synaptotoxicity. *Nature Communications.* 4, 2416.
- Nieznanska, H., Bandyszewska, M., Surewicz, K., Zajkowski, T., Surewicz, W.K., Nieznanski, K., 2018. Identification of prion protein-derived peptides of potential use in Alzheimer's disease therapy. *Biochim Biophys Acta.* 1864, 2143-2153.
- Nieznanski, K., Choi, J.K., Chen, S., Surewicz, K., Surewicz, W.K., 2012. Soluble prion protein inhibits amyloid- $\beta$  (Ab) fibrillization and toxicity. *Journal of Biological Chemistry.* 287, 33104-8.
- Novitskaya, V., Makarava, N., Bellon, A., Bocharova, O.V., Bronstein, I.B., Williamson, R.A., Baskakov, I.V., 2006. Probing the conformation of the prion protein within a single amyloid fibril using a novel immunoconformational assay. *J Biol Chem.* 281, 15536-45.
- O'Malley, T.T., Oktaviani, N.A., Zhang, D., Lomakin, A., O'Nuallain, B., Linse, S., Benedek, G.B., Rowan, M.J., Mulder, F.A., Walsh, D.M., 2014. Abeta dimers differ from monomers in

- structural propensity, aggregation paths and population of synaptotoxic assemblies. *Biochem J.* 461, 413-26.
- O'Malley, T.T., Witbold, W.M., 3rd, Linse, S., Walsh, D.M., 2016. The Aggregation Paths and Products of Abeta42 Dimers Are Distinct from Those of the Abeta42 Monomer. *Biochemistry.* 55, 6150-6161.
- Resenberger, U., Harmeier, A., Woerner, A., Goodman, J., Muller, V., Krishnan, R., RM, V., Kretzschmar, H., Lindquist, S., Hartl, F., Multhaup, G., Winklhofer, K.a.T.J., 2011. The cellular prion protein mediates neurotoxic signalling of b-sheet-rich conformers independent of prion replication. *EMBO Journal.* 30, 2057-2070.
- Sandberg, M.K., Al-Doujaily, H., Sharps, B., Clarke, A.R., Collinge, J., 2011. Prion propagation and toxicity in vivo occur in two distinct mechanistic phases. *Nature.* 470, 540-2.
- Sandberg, M.K., Al-Doujaily, H., Sharps, B., De Oliveira, M.W., Schmidt, C., Richard-Londt, A., Lyall, S., Linehan, J.M., Brandner, S., Wadsworth, J.D., Clarke, A.R., Collinge, J., 2014. Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. *Nat Commun.* 5, 4347.
- Selkoe, D.J., 2003. Folding proteins in fatal ways. *Nature.* 426, 900-4.
- Selkoe, D.J., Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med.* 8, 595-608.
- Silveira, J.R., Raymond, G.J., Hughson, A.G., Race, R.E., Sim, V.L., Hayes, S.F., Caughey, B., 2005. The most infectious prion protein particles. *Nature.* 437, 257-61.
- Smith, L.M., Strittmatter, S.M., 2017. Binding Sites for Amyloid-beta Oligomers and Synaptic Toxicity. *Cold Spring Harb Perspect Med.* 7.
- Solfrosi, L., Bellon, A., Schaller, M., Cruite, J.T., Abalos, G.C., Williamson, R.A., 2007. Toward molecular dissection of PrPC-PrPSc interactions. *J Biol Chem.* 282, 7465-71.

- Soto, C., 2003. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci.* 4, 49-60.
- Stravalaci, M., Tapella, L., Beeg, M., Rossi, A., Joshi, P., Pizzi, E., Mazzanti, M., Balducci, C., Forloni, G., Biasini, E., Salmona, M., Diomedede, L., Chiesa, R., Gobbi, M., 2016. The Anti-Prion Antibody 15B3 Detects Toxic Amyloid-beta Oligomers. *J Alzheimers Dis.* 53, 1485-97.
- Turnbaugh, J.A., Unterberger, U., Saa, P., Massignan, T., Fluharty, B.R., Bowman, F.P., Miller, M.B., Supattapone, S., Biasini, E., Harris, D.A., 2012. The N-terminal, polybasic region of PrP(C) dictates the efficiency of prion propagation by binding to PrP(Sc). *J Neurosci.* 32, 8817-30.
- Um, J.W., Nygaard, H.B., Heiss, J.K., Kostylev, M.A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E.C., Strittmatter, S.M., 2012. Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat Neurosci.* 15, 1227-35.
- Vazquez de la Torre, A., Gay, M., Vilapriño-Pascual, S., Mazzucato, R., Serra-Batiste, M., Vilaseca, M., Carulla, N., 2018. Direct Evidence of the Presence of Cross-Linked Abeta Dimers in the Brains of Alzheimer's Disease Patients. *Anal Chem.* 90, 4552-4560.
- Walsh, D.M., Selkoe, D.J., 2016. A critical appraisal of the pathogenic protein spread hypothesis of neurodegeneration. *Nat Rev Neurosci.* 17, 251-60.
- Wang, Z., Jackson, R.J., Hong, W., Taylor, W.M., Corbett, G.T., Moreno, A., Liu, W., Li, S., Frosch, M.P., Slutsky, I., Young-Pearse, T.L., Spires-Jones, T.L., Walsh, D.M., 2017. Human Brain-Derived Abeta Oligomers Bind to Synapses and Disrupt Synaptic Activity in a Manner That Requires APP. *J Neurosci.* 37, 11947-11966.

Watt, A.D., Perez, K.A., Rembach, A., Sherrat, N.A., Hung, L.W., Johanssen, T., McLean, C.A., Kok, W.M., Hutton, C.A., Fodero-Tavoletti, M., Masters, C.L., Villemagne, V.L., Barnham, K.J., 2013. Oligomers, fact or artefact? SDS-PAGE induces dimerization of beta-amyloid in human brain samples. *Acta Neuropathol.* 125, 549-64.

Younan, N.D., Sarell, C.J., Davies, P., Brown, D.R., Viles, J.H., 2013. The cellular prion protein traps Alzheimer's Ab in an oligomeric form and disassembles amyloid fibers. *Federation of American Societies for Experimental Biology Journal.* 27, 1847-58.

Zahn, R., von Schroetter, C., Wuthrich, K., 1997. Human prion proteins expressed in *Escherichia coli* and purified by high-affinity column refolding. *FEBS Lett.* 417, 400-4.

Zhang, Y., Pak, C., Han, Y., Ahlenius, H., Zhang, Z., Chanda, S., Marro, S., Patzke, C., Acuna, C., Covy, J., Xu, W., Yang, N., Danko, T., Chen, L., Wernig, M., Sudhof, T.C., 2013. Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron.* 78, 785-98.