Systematic and standardized comparison of reported Amyloid-β receptors for sufficiency, affinity, and Alzheimer’s disease relevance

Levi M. Smith1,2, Mikhail A. Kostylev1, Suho Lee1, and Stephen M. Strittmatter1,*

From the 1Program in Cellular Neuroscience, Neurodegeneration & Repair, Departments of Neurology and of Neuroscience, Yale University School of Medicine, New Haven, CT 06536, USA, and 2Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06536, USA

Running title: Standardized comparison of reported Aβ receptors

*To whom correspondence should be addressed: Stephen M. Strittmatter, CNNR Program, BCMM 436, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06536, USA

stephen.strittmatter@yale.edu

Keywords: Alzheimer’s disease, amyloid beta (Aβ) oligomers, Aβ receptor, neurodegeneration, prion, prion protein (PrPc), leukocyte immunoglobulin-like receptor subfamily B member 2 (LilrB2), nogo receptor 1 (NgR1), EphA1, FcγRIIb, SorLA, Sortilin, p75NTR, PGRMC1, NLGN1, RAGE, EphB2, FZD5, mGluR5, EphA4, nAchRa7, NR2B, NR1

Abstract

Oligomeric assemblies of amyloid-β peptide (Aβo) in the brains of individuals with Alzheimer’s disease (AD) are toxic to neuronal synapses. More than a dozen Aβ receptor candidates have been suggested to be responsible for various aspects of the molecular pathology and memory impairment in mouse models of AD. A lack of consistent experimental design among previous studies of different receptor candidates limits evaluation of the relative roles of these candidates, producing some controversy within the field. Here, using cell-based assays with several Aβ species, including Aβo from AD brains obtained by autopsy, we directly compared the Aβ-binding capacity of multiple receptor candidates while accounting for variation in expression and confirming cell-surface expression. In a survey of 15 reported Aβ receptors, only cellular prion protein (PrPc), nogo receptor 1 (NgR1), and leukocyte immunoglobulin-like receptor subfamily B member 2 (LilrB2) exhibited direct binding to synaptotoxic assemblies of synthetic Aβ. Both PrPc and NgR1 preferentially bound synaptotoxic oligomers rather than non-toxic monomers, and the method of oligomer preparation did not significantly alter our binding results. Hippocampal neurons lacking both NgR1 and LilrB2 exhibited a partial reduction of Aβo binding, but this reduction was lower than in neurons lacking PrPc under the same conditions. Finally, binding studies with soluble Aβo from human AD brains revealed a strong affinity for PrPc, weak affinity for NgR1 and no detectable affinity for LilrB2. These findings clarify the relative contributions of previously reported Aβ receptors under controlled conditions and highlight the prominence of PrPc as an Aβ-binding site.

The pathophysiology responsible for the clinical signs and symptoms of AD have been studied since Alois Alzheimer first described the characteristic senile plaques and neurofibrillary tangles in the brain of a patient who suffered deteriorating psychological condition that included sleep disturbances, memory impairment, and confusion (1). Today, AD is the leading cause of dementia worldwide and carries with it a tremendous economic burden projected to exceed two trillion U.S. dollars by the year 2030 (2). Despite more than a century of research, AD remains without a treatment that is capable of curing, preventing, or slowing the progression of disease. The role of senile plaques and neurofibrillary tangles in the etiology of Alzheimer’s disease continues to be investigated and contested within the field. In 1998, Lambert et al. described the self-assembly of synthetic amyloid beta monomers into soluble, multimeric, non-fibrillar, aggregates dubbed Aβo. These oligomers were potently neurotoxic, capable
of inducing cell death, and inhibited long-term potentiation (LTP) in organotypic hippocampal slices (3). Aβ0 are immunologically distinct from monomers or fibrils, induce synapse loss, and are correlated with disease progression (4-8). Similar species of Aβ0 were identified in brains from human AD patients in 2003 (9). The observations that synthetic and AD brain-derived Aβ0 bound to neurons in a trypsin-sensitive manner gave rise to the search for cell surface receptors capable of binding extracellular Aβ0 and transducing their neurotoxic signal intracellularly. More than a dozen proteins have been reported as responsible for mediating the deleterious effects of Aβ0 on neurons ((10-25); reviewed in (26)). These studies have been highly disparate in both the quality and nature of evidence used to qualify a candidate as a receptor for Aβ (26). Variation in Aβ preparations, experimental design, and model systems have led to a call for a sharing of materials and validation of results between laboratories (26-28).

To address these discrepancies and better understand the relative contributions of each putative receptor to Aβ0 neurotoxicity, we compared the potential of each receptor to confer Aβ binding capacity to heterologous cells and neurons, the ability of each candidate to discriminate between non-toxic monomers and toxic oligomers, and the effect of different oligomer preparations on the binding profile. To determine whether synthetic preparations of Aβ faithfully recapitulate the binding profile of Aβ found in the brains of patients with AD, we also compared the ability of candidate receptors to bind soluble Aβ extracted from the brains of patients diagnosed with AD. These insights are critical to clarifying the roles of these receptors in AD pathogenesis and their therapeutic value to drug development. Preventing the interaction of neurotoxic Aβ0 with its receptors is an attractive drug target and clinical trials targeting RAGE, PGRMC1, and p75NTR are underway (NCT00141661, NCT00566397, NCT02916056, NCT02080364, NCT03522129, NCT03507790, NCT03069014) (29-32).

Results

PrPc, LilrB2, and NgR1 bind oligomeric Aβ

Few descriptions of candidate receptors for Aβ have included a demonstration of sufficiency for conferring Aβ binding to live cells. To examine this attribute, we compiled a panel of putative receptors for Aβ and subcloned the cDNA of each into expression vectors encoding a Myc epitope at the cytoplasmic terminus of transmembrane proteins or at the mature amino terminus of glycosylphosphatidylinositol (GPI) anchored proteins which included PrPc and NgR1. These orientations were selected so as to leave the extracellular, Aβ binding domains undisturbed. The panel investigated here includes PrPc, LilrB2, NgR1, ephrin type-A receptor 1 (EphA1), low affinity immunoglobulin gamma Fc region receptor II-b (FcγRIIb), sortilin-related receptor (SorLA), sortilin, tumor necrosis factor receptor superfamily member 16 (p75NTR), membrane-associated progesterone receptor component 1 (PGRMC1), neureilin 1 (NLGN1), advanced glycosylation end product-specific receptor (RAGE), ephrin type-B receptor 2 (EphB2), frizzled-5 (FZD5), metabotropic glutamate receptor 5 (mGLuR5), and ephrin type-A receptor 4 (EphA4) (10-25). We also included Neuropilin-1 (NRP1) as a negative control (Figure S1). To our knowledge, this protein has not been implicated in binding Aβ.

We assessed the expression of each protein at the extracellular surface by cell surface biotinylation. Anti-Myc immunoprecipitation and immunoblot confirmed that each protein was trafficked to the cell surface and of the expected molecular size (Figure 1A). Furthermore, similar amounts of each receptor protein reached the plasma membrane and were subject to biotinylation by extracellular reagent. The Myc-tagged cytoplasmic protein collapsin response mediator protein 2 (CRMP2) was included as a control, and demonstrated that only proteins localized to the cell surface were biotinylated.

For heterologous cell Aβ binding experiments we chose COS-7 cells, as they exhibit minimal basal Aβ binding. By staining transfected cells with an anti-Myc antibody and quantifying the average intensity, we found that receptor expression was similar across the panel (Figure 1 B&C). Utilizing a common epitope tag for expression-normalization across the panel is critical to making direct comparisons of Aβ binding. This allows us to account for any expression differences between constructs and experiments, without introducing variability from differences in the avidity of
receptor-specific antibodies or potential interference with binding in the case of receptors whose Aβ binding domains have not been identified.

The concentration of oligomeric Aβ in AD patient brains has been reported to be ~2.6 nM monomer equivalent (7). To examine the ability of candidate receptors to bind neurotoxic oligomers of Aβ, transfected cells were incubated with 1 µM monomer equivalent (~5 nM oligomer) biotinylated Aβ oligomers (BAβo) at 4°C to allow binding but minimize receptor internalization following binding. A high concentration was utilized so as to allow detection of Aβ binding even to low affinity receptors. Following fixation and staining, the receptor expression-normalized Aβ binding was calculated and normalized to that of human PrPc (hPrPc). Figure 2 A&B show that PrPc, LilrB2, and NgR1 exhibit Aβo binding capacity. Among these, PrPc binds Aβo more than either LilrB2 or NgR1 (Figure 2B). None of the other receptors demonstrated detectable Aβo binding with a lower limit of detection equal to 3% of hPrPc binding.

**PrPc and NgR1 preferentially bind synaptotoxic Aβ species**

An important characteristic of a receptor capable of transducing pathological signaling in response to Aβo is an ability to discriminate between neurotoxic oligomers and non-toxic monomeric Aβ. Aβ monomers spontaneously and rapidly assemble into high molecular weight species in physiological buffers. Samples incubated overnight yield preparations enriched for oligomeric assemblies which can be resolved using size exclusion chromatography (SEC) (Figure 3A). Analysis of the relative abundance of oligomeric Aβ in different preparations demonstrates that Aβ freshly prepared in Ham’s F12 medium is 7% high molecular weight (HMW) Aβo. Oligomeric preparations are 21% Aβo. Oligomerization can be inhibited by the use of non-physiological buffers. When Aβ is dissolved in 0.1N NaOH, only 3% of the preparation is HMW Aβo (Figure 3B). Incubating transfected cells with freshly prepared Aβ in F12 is intended to provide a context in which monomeric Aβ is more readily available for binding, however the preparation cannot be considered purely monomeric. Figure 3C shows the expression-normalized monomeric Aβ signal for each receptor compared to oligomeric Aβ binding to hPrPc. None of the receptors bind this monomeric preparation of Aβ to a great extent (Figure 3C). By comparing oligomeric and monomeric binding we examined each receptor’s ability to discriminate Aβ species. PrPc and NgR1 demonstrate an ability to distinguish between oligomeric Aβ and monomeric Aβ. LilrB2 binds oligomeric and monomeric Aβ to similar extents (Figure 3D).

**Neither temperature nor Aβ oligomerization method alters the binding profile of the receptor panel**

Conducting binding assays at 4°C affords convenience but decreases the fluidity of the cell membrane and impacts the thermodynamics of receptor-ligand interactions. To determine if temperature influences the binding profile of the receptor panel, we incubated transfected cells with 1 µM monomer equivalent BAβo at 37°C. As with the 4°C incubation, only PrPc, LilrB2, and NgR1 bind BAβo at 37°C (Figure 4A). In comparing binding at both temperatures, we found no effect of temperature on BAβo binding (Figure 4B).

Multiple methods for the generation of oligomeric Aβ assemblies from monomeric synthetic peptides have been reported (10,33,34). Differences in preparation lead to unique profiles with regard to the types and relative abundance of multimeric Aβ assemblies as assessed under native conditions using SEC. Oligomers prepared in F12 culture media, incubated overnight, and separated from insoluble fibrillar species by centrifugation, generate larger species of oligomers with masses in the range of 100 kDa and larger (7,10). To account for differences in oligomeric species generated, we utilized a second preparation of soluble multimeric Aβ termed globulomers (Aβg) that generates primarily a 60 kDa species by SEC (33). Atomic force microscopy confirms differences in sizes of soluble oligomers generated by the different preparations (Figure 4C). Incubation of transfected cells with Aβg demonstrate a binding profile similar to that observed with the oligomeric preparations (Figure 4D). When comparing binding of BAβo and Aβg to the binding competent receptors, there is no effect of preparation on the binding (Figure 4E).
NgR1 and LilrB2 are minor contributors to Aβo binding to neurons
Our survey of putative receptors has revealed that of those reported, only PrPc, LilrB2, and NgR1 are sufficient for binding to neurotoxic preparations of synthetic Aβ. PrPc has been demonstrated to be responsible for 50% of Aβo binding to hippocampal neurons (10). To examine the contribution of the two additional Aβo receptors confirmed here (11,12), we examined the role of NgR1 and the murine homolog of LilrB2, Pirb, in double knockout (DKO) neurons under the same conditions used for PrPc (10). Loss of NgR1 and Pirb results in a 20% decrease in BAβo binding to DKO neurons (Figure 5A&B).

PrPc is the highest affinity receptor for Aβo
To better understand the relative affinities of the positive receptors, we implemented plate-based assays of Aβ binding to purified protein. This assay is more sensitive and quantitative than the cell-based assays and allows for measurement of binding kinetics. We again utilized oligomeric, globulomeric, and monomeric Aβ. Figures 6A-F show the binding curves and scatchard plots for Aβo, Aβg, and Aβm, respectively. As measured in the cell-based assays, hPrPc, LilrB2, and NgR1 bound neurotoxic oligomers and globulomers (Figure 6). The plate assay faithfully recapitulated effects of Aβ preparations on binding profiles. We observed highly similar binding of Aβo and Aβg to hPrPc and NgR1 (Figure 6G,H) and a preference of LilrB2 for Aβo over Aβg (Figure 6I) as was also observed in Figure 4E. Binding of Aβm to the receptors was minimal as was observed in the cell-based assay (Figure 6E,F). The dissociation constants and Bmax extracted from Figure 6 are reported in Table 1. PrPc is the highest affinity receptor for neurotoxic Aβo and Aβg with affinities of 1.4 nM and 1.5 nM, respectively. The affinity of NgR1 for these preparations was ~3 to 4 fold lower than hPrPc. LilrB2 demonstrated the lowest affinity with KD of 42.3 nM and 207.2 nM. To quantify each receptor’s ability to discriminate between neurotoxic and non-toxic preparations of Aβ, we calculated a discrimination factor by dividing each receptor’s KD for Aβm by that of the indicated species. hPrPc was highly discriminative in its preference for oligomeric and globulomeric Aβ with discrimination factors of 68.5 and 63.9, respectively. NgR1 also discriminated in binding, although to a lesser degree than hPrPc. LilrB2 exhibited a modest preference for Aβo compared to monomers and bound Aβg with same affinity as for Aβm (Table 1).

PrPc and NgR1 bind Aβ present in the brains of patients with AD
Despite the ability of preparations of synthetic Aβ to generate neurotoxic species with masses similar to those found in the brains of patients with AD and to cause neurotoxicity in vitro, ex vivo, and in vivo, it is critical examine each receptor’s disease relevance using pathological Aβ present in disease (10,33,35-37). Having validated the concordance of our plate-based assay with the observations made in living cells, we quantified binding of Aβ present in TBS-soluble extracts of brains from 10 patients with autopsy-confirmed AD and compared it with signal from brains of 11 cognitively normal patients. The mean anti-Aβ signal from wells coated with hPrPc was highest and significantly greater than that from cognitively normal patients. LilrB2 exhibited no binding to Aβ present in brains of patients with AD and no difference between AD and control brains. NgR1 modestly bound Aβ present in AD patient brains and this signal was significantly greater than that in brains from cognitively normal patients.

Discussion
Here, a panel of putative Aβ receptors was expressed on the surface of non-neuronal cells and examined for sufficiency to mediate Aβ binding. By accounting for differences in receptor expression using a common epitope tag across the receptors we made direct comparisons of Aβ affinity. Importantly, binding took place in living cells rather than cell-free systems. This allows for potential binding events to take place at the cell surface and in a physiological context with respect to the lipid and protein environment. Our experiments demonstrate that most of the reported receptors for Aβ are not sufficient to confer Aβ binding capacity to cells using three different preparations of synthetic Aβ. It is possible that those receptors that failed to bind Aβ in our experiments require a coreceptor that is not present in COS-7 cells. While our cell surface biotinylation experiments demonstrate successful transport.
through the secretory system, which is typically associated with proper protein folding, it does not definitively exclude misfolding which could impact ligand binding. While these negative proteins may have Aβ affinity under some experimental conditions, in a cellular context their binding affinity is clearly much less than the three positive receptors. By including an analysis of binding to monomeric Aβ we revealed that PrPC and NgR1 discriminate between pathological and non-toxic forms of Aβ. It should be noted that neither oligomeric nor monomeric preparations are exclusive to a single species, but that these designations are meant to reflect the assemblies for which the preparation is enriched. It is possible that the reduced but persistent binding of monomer preparations to PrPC and NgR1 may reflect the decrease in the abundance of Aβo. It is clear, however that upon enrichment for monomeric Aβ, none of the candidates which failed to bind Aβo showed an increase in binding. We also found that LilrB2 does not differentiate in binding these oligomeric and monomeric Aβ preparations. The promiscuity of LilrB2 may indicate that an epitope present in both species of synthetic Aβ mediates this interaction. Recently, Cao et al. demonstrated that LilrB2 can bind minimal peptides comprising Aβ amino acids 1-21, 15-35, and a tandem repeat of 16-21 without a specific oligomerization procedure (38). In contrast, PrPC has been shown to undergo structural changes specifically in the presence of oligomeric Aβo (39).

Neuronal acetylcholine receptor subunit alpha-7 (nAchRa7) and NMDA receptor subunits 1 and 2B have also been reported to bind Aβ (40-42). We sought to include these in our investigation however, efficient transport to the cell surface as determined by cell surface biotinylation experiments was insufficient to make comparisons with the rest of the panel. Despite these data, and because we have observed that even very low expression levels of the high affinity receptor PrPC can result in robust Aβo binding, nArchRa7 with and without the chaperone RIC-3, and GluN2B with and without GluN1 were included in our cell binding experiments. No detectable Aβ binding signal was observed in nArchRa7 or GluN2B experiments (data not shown).

While the ability to bind Aβo is a crucial characteristic for a receptor, we found it important to interrogate the necessity of confirmed receptors for Aβo binding to neurons. Pirb is the mouse homolog of Lilrb2 and was shown to bind Aβo (11). By generating double knockout mice deficient in NgR1 and Pirb, we found that up to 20% of binding of synthetic Aβo to neurons is mediated by these two receptors.

Preparations of synthetic Aβ peptide provide a convenient source of neurotoxic Aβ with species representative of those found in the brains of AD patients. When investigating the relevance of a potential receptor to human disease, the gold standard should be an ability to bind Aβ present in the brains of patients diagnosed with AD. We first validated our plate-based assay by replicating the experiments done in mammalian cells and found the two to be in close agreement. We determined that hPrPC is the highest affinity receptor for Aβo (K_d=1.4), followed by NgR1 (K_d=3.9), and LilrB2 (K_d=42.3). We then utilized this assay for the detection of binding to soluble Aβ found in extracts of brains from patients diagnosed with AD. We found that hPrPC and NgR1 bind Aβ present in AD patient brains and that hPrPC did so to the greatest extent. Finally, we found that despite binding synthetic Aβ from multiple preparations and in cell-based and purified protein assays, LilrB2 did not detectibly bind the Aβ species present in AD patient brains.

The experiments described here provide much needed clarity to the field regarding the nature of the interaction of neurotoxic Aβo in the extracellular space with the cell surface. The observation made here that NgR1 and LilrB2 account for up to 20% of Aβo binding to hippocampal neurons, coupled with previous observations that PrPC is responsible for 50% of Aβo binding to neurons, and the knowledge that Aβo binds neurons in a trypsin-sensitive manner, suggests that at least one additional receptor for Aβo remains to be identified (3,10). Future characterizations of novel Aβo receptors should include experiments testing the necessity and sufficiency for cellular Aβo binding. It will also be necessary to examine the disease relevance of observations made with synthetic peptides and
transgenic animals by using materials obtained from patients with AD.

**Experimental procedures**

*Organ culture* COS-7 or HEK-293T cells were grown at 37°C with 5% CO₂ on 100 mm tissue culture dishes (Fisher Scientific 08-772E) in DMEM (Gibco 11965) supplemented with 10% FBS and 1% Penicillin-Streptomycin (Gibco 15140). For Aβ binding experiments, Cos-7 cells were seeded into 8-well chamber slides (Lab-tek 154941) and grown under the same conditions. Transfections were performed using Lipofectamine 3000 (ThermoFisher L3000015) diluted in OptimEM (ThermoFisher 31980). 

**Plasmids and cloning** cDNA encoding proteins of interest were obtained from Genecopoeia, Dharmacon, and Origene. cDNAs were subcloned into Myc-tagged expression vectors pcDNA3.1 (+/-myc-His A (Thermo V80020) or pSecTag2A (Thermo V90020). Accession numbers for cDNA sequences are: hPrP (NM_000311.3), mPrP (NM_011170.3), LibrB2 (XM_006726139.1), NgR1 (NM_023004.5), EphA1 (BC_130291.1), FcYRIib (BC_026327.1), SorLA (NM_003105.5), Sortilin (NM_002959.5), PGRMC1 (NM_006667.4), NLGN1 (NM_014932.3), RAGE (AB036432.1), EphB2 (BC146296.1), FZD5 (BC172518.1), mGluR5 (NM_017012.1), EphA4 (BC026327.1), CRMP2 (NM_001386). The construct encoding NRP1 has been previously described (43). Constructs were verified by DNA sequencing.

**Cell surface biotinylation and immunoprecipitation**

Dishes of transfected HEK293T cells were washed three times with ice-cold PBS pH 8 and biotinylated with 10 mL of 0.48 mg/mL Sulfo-NHS-LC-LC-Biotin (Thermo Fisher 21338) in PBS pH 8, and incubated at room temperature for 30 minutes. Cells were washed once with 50 mM Tris pH 8 and then twice with ice-cold PBS pH 8. Cells were harvested in 1 mL of ice-cold PBS and gently scraped to collect. Collected cells were transferred to a new tube and centrifuged at 500 x g for 3 minutes at 4°C to pellet cells. The cell pellet was resuspended in 1 mL of RIPA lysis buffer (Millipore 20-188) with PhosSTOP (Roche 04906837001) and Complete Mini protease inhibitors (Roche 11836170001) and sonicated. After sonication, samples were centrifuged at 100,000 x g, 4°C for 30 minutes. Supernatants were transferred to c-Myc beads (Pierce 20169) and incubated at 4°C with end-over-end mixing for 1 hour. Beads were washed three times with RIPA buffer. Proteins were eluted with 4X Laemmli sample buffer (Bio-Rad 161-0747) and analyzed by immunoblotting using anti-Myc (Cell Signalling #2276, 1:1000), donkey anti-mouse IRDye 680LT (Li-Cor 926-68022, 1:20,000) and streptavidin IRDye 800CW (Li-Cor 926-32230, 1:20,000), and imaged with the Odyssey infrared imaging system (Li-cor Biosciences).

**Synthetic Aβ Preparations** Lyophilized synthetic amyloid beta (1-42) was purchased from The ERI Amyloid Laboratory, LLC. Vials of peptide were reconstituted in 1,1,1,3,3,3-hexafluoropropanol at 10 mg/mL, boiled at 70°C for 1 hour, aliquoted into microcentrifuge tubes at 0.5 mg/tube and allowed to dry overnight at room temperature. The next day, the aliquots were further dehydrated in a SpeedVac for 1 hour. Oligomers were prepared by dissolving an aliquot of Aβ in 40 μL of DMSO, separating into two aliquots and diluted in 1 mL of F12 medium (Atlanta Biologicals M15350) to 55 μM and incubated at room temperature overnight to allow oligomerization. The next day, samples were centrifuged in a tabletop centrifuge (Eppendorf 5430) at 20,817 x g for 15 minutes to precipitate fibrillar protein. The supernatant was treated as 55 μM and diluted to working concentrations in F12 media. Monomeric Aβ was prepared by diluting the DMSO-dissolved peptide in F12 to working concentration as quickly as possible. Globulomer Aβ was generated according to the previously published protocol (33). HFIP-processed synthetic Aβ peptide films were reconstituted in DMSO at 5 mM (22 μl DMSO/0.5 mg Aβ). Then, 250 μl of 1X PBS, pH 7.4 was added to each tube immediately followed by 31 μl of 2% sodium dodecyl sulfate (SDS). The resulting mix was incubated in the sealed tubes at 37°C for 4-6 h and then diluted 4-fold with deionized water (resulting in 1.2 ml/tube final volume) and incubated at 37°C overnight to yield mature globulomer Aβ. The preparations were then centrifuged for 10 min at 10,000 x g and the supernatants were concentrated using 30 kDa cutoff Amicon filters. Concentrated Aβ (1 mM Aβ monomer) were then dialyzed against 1000-fold excess of PBS diluted 4-fold with water (0.25X PBS, pH 7.4) using 100 μl 20 kDa cutoff Slide-A-Lyzer mini cassettes. Dialysis buffer was changed 3 times over a course of 36 hours. Dialyzed Aβ was then centrifuged at 100,000 x g for 1 h at 4°C.
and supernatants were aliquoted and flash-frozen in liquid nitrogen. Typically, 70% of initial Aβ (dried HFIP film) was converted into Aβg as determined by absorbance at 280 nm using ProtParam-derived extinction coefficient (1490 M⁻¹ cm⁻¹). For atomic force microscopy and SEC, NaOH monomer samples were generated by dissolving HFIP-treated Aβ in 440 µl of 0.1 M NaOH, resulting in a 250 µM stock, and processed for atomic force microscopy or SEC immediately.

**Immunocytochemistry and heterologous cell binding assays** COS-7 cells were cultured in 8-well chamber slides as described above. For Aβ binding experiments, the culture media was removed from wells, leaving a volume sufficient to cover the cells, washed with F12, and the indicated Aβ preparation was added. Slides were then incubated for two hours at the indicated study temperature. Wells were washed three times with PBS, and fixed with 3.7% formaldehyde (J.T. Baker 2106) in PBS with 6% sucrose (AmericanBio AB01900) for 30 minutes at room temperature. The fixative was then removed and cells were permeabilized by incubation in 0.2% Triton X-100 (Fisher Scientific 42235-5000) in PBS for 30 minutes. After permeabilization, cells were blocked by incubation with 5% normal donkey serum (Jackson ImmunoResearch 017-000-121) for 1 hour at room temperature. After blocking, cells were incubated with the indicated primary antibodies for anti-Myc (Cell Signalling #2276, 1:5000), or anti-Aβ (Cell Signalling #8243, 1:1000), diluted in 1% BSA (Sigma # A9647) at 4°C overnight. The next day, cells were washed three times with PBS with incubation periods of 0, 3, and 5 minutes. The indicated appropriate secondary antibodies diluted in 1% BSA in PBS (Donkey anti-mouse Alexa Fluor 488 Life Technologies #A21202, 1:500; Donkey anti-rabbit Alexa Fluor 568, Life Technologies #A10042, 1:500; Streptavidin Alexa Fluor 568, Life Technologies S-11226, 1:500) were then added to wells and incubated for 1 hour at room temperature. Wells were then washed four times with 0.02% Triton X-100 in PBS with incubation times of 0, 3, 5 and 15 minutes. After washing, slides were mounted using Vectashield with DAPI (Vector laboratories H-1200) and imaged on an Axio Imager M2. CellProfiler was used for quantification of Aβ binding to transfected cells (44). DAPI and anti-Myc signal were used for the identification of transfected cells and the intensity of Aβ and Myc inside of cell areas as well as background were measured in Aβ treated and non-treated wells. Background signal was subtracted from cellular signal, and from that value, mean Aβ signal in cells transfected with cytoplasmic EGFP was subtracted. The transfection-specific, background-subtracted Aβ signal was normalized by background-subtracted Myc signal and expressed as a percent of that signal in hPrP² transfected cells which were included in every experiment for comparison. Limit of detection (LOD) was calculated as the mean background-subtracted Aβ signal in cells transfected with cytoplasmic EGFP, expressed as a percent of that in hPrP transfected cells, plus two standard deviations.

**Size exclusion chromatography** SEC was performed on the described preparations of biotinylated Aβ using a Superdex 200 increase 10/300 GL column (GE Lifesciences 28990944) and Acta Pure 25 M1 (GE Lifesciences) with F9-C fraction collector. SEC was performed at 4°C. 1.5 mL fractions were collected in 2 mL deep 96-well plates (USA Scientific 1896-2110). For the representative trace, 50 µM BAβo in Ham’s F12 medium was loaded into a 500 µL sample loop. For dot-blot analysis 1 µM samples were loaded. Samples prepared in Ham’s F12 were ran using Ham’s F12 as the mobile phase. NaOH monomer samples were ran in a mobile phase of 0.25X PBS pH 7.4. Gel filtration standards used were blue dextran (Sigma D4772), thyroglobulin, bovine γ-globulin, chicken ovalbumin, equine myoglobin, and vitamin B12 (Bio Rad 1511901). Fractions were concentrated using centrifugal filters with a 3,000 nominal molecular weight limit (Amicon UFC500324). Dot-blot analysis was performed by applying 80% of concentrate volume to a nitrocellulose membrane using a Bio Rad Bio-Dot apparatus. Samples were incubated on the membrane for 4 hours at room temperature, washed twice with TBS, blocked for 1 hour at room temperature with Rockland blocking buffer for fluorescent Western blotting (Rockland MB070010TF) and probed overnight at 4°C with streptavidin IRDye 800CW (Li-Cor 926-32230, 1:1,000).

**Atomic force microscopy** AFM samples were prepared by placing 10 µl of 0.25 mg/ml Aβ preparations on freshly cleaved mica and allowing it to adsorb for 2 minutes. The sample was washed twice with 200 µl of Milli-Q water and dried carefully. Images where taken in a Bruker
Dimension Fastscan AFM in a tapping mode using Silicon Nitride Cantilevers.

Mouse breeding and care Mice were cared for by the Yale Animal Resource Center and all experiments were approved by Yale's Institutional Animal Care and Use Committee. NgR<sup>−/−</sup> (Rtn4r) and Pirb<sup>−/−</sup> mice have been previously described and maintained on a C57/B6 background (45,46). Pirb<sup>−/−</sup> mice were a generous gift of Dr. Toshiyuki Takai. NgR<sup>−/−</sup> and Pirb<sup>−/−</sup> were bred together to generate double heterozygous and eventually double knockout mice.

Neuron cultures and neuronal binding assays Mouse hippocampal neurons from WT and RTN4R<sup>−/−</sup>, Pirb<sup>−/−</sup> double knockout animals were isolated from E17 to P0 pups and cultured on MatTek dishes (P35G-1.5-14-C) coated with poly-d-lysine (MP Bio 0215017580) in Neurobasal media (Gibco 10888-022) supplemented with B-27 (Gibco 17504-044), Sodium Pyruvate (Gibco 11360-070), and Glutamax (Gibco 35050-061), with 1% Penicillin and Streptomycin (Gibco 15140). Binding experiments were performed at DIV 18-20. After washing, neurons were incubated with 500 nM monomer equivalent (~2.5 nM oligomer) biotinylated Aβ for 1 hour at 4°C. Following fixation, permeabilization, and blocking, dendrites were visualized using anti-MAP2 (1:2000 Millipore AB5622) and Alexa Fluor 488 donkey anti-rabbit IgG (1:1000 Life technologies A21206), and biotinylated Aβ was visualized with streptavidin Alexa Fluor 568 (1:500 Life technologies S11226). Z-stacks were collected at 40X using a spinning disc confocal microscope. Z-stacks were then max-projected using NIH ImageJ (47). Quantification was performed using CellProfiler (44). Integrated punctate Aβ signal was calculated after background subtraction and thresholding. Integrated signal was then normalized to the area positive for MAP2. When representing these data graphically, each data point represents a single region of interest from 5 to 7 dishes. The experiment was repeated four times.

Plate-based assay 384 well maxi-sorp plates (Thermo 460372) were coated overnight with 20 µl/well of the indicated protein at 250 nM in 100 mM BupH carbonate-bicarbonate coating buffer (Thermo 28382) at 4°C. Plates were washed twice with PBST (PBS 0.05% Tween 20), and blocked with 25 µl/ of Protein-Free T20 PBS Blocking buffer (Pierce 37573) for 4 hours at room temperature. After washing three times with PBST, 20 µl of either TBS fraction of human brain samples or synthetic Aβ was applied to wells and incubated overnight at 4°C. Aβ binding was detected using D54D2 anti-Aβ antibody (Cell Signaling #8243, 1:2000) in PBSTB (PBS, 0.05% tween 20, 0.5% bovine serum albumin) for 2 hours. After 4 washes with PBST, 20 µl of Eu-N1 Goat anti-rabbit IgG (Perkin Elmer AD105, 1:4000) diluted in DELFIA assay buffer (Perkin Elmer) and incubated for 1 hour at room temperature. After 4 washes with PBST, 20 µl of DELFIA Enhancement Solution (Perkin Elmer) was applied and time-resolved europium fluorescence was measured with a Victor 3V plate reader (Perkin Elmer).

Recombinant hexa-histadine tagged hPrP<sup>C</sup> was generated using the method previously described (7). The extracellular domain of LilrB2 is encoded by amino acids 22-461. We subcloned cDNA encoding this region into pSecTag2A (Invitrogen) to include a carboxy-terminal hexa-histidine tag. HEK293T cells were transfected with this ecto-LilrB2 construct and 24 hours later the medium was replaced with serum free medium. The next day the conditioned medium was collected and purified using the same method described for hPrP<sup>C</sup>. Human NgR-Fc decoy protein encodes amino acid residues 1-310 of human NgR1 with the Cys266Ala and Cys309Ala substitutions fused to the Fc domain of human IgG1. NgR(310)-Fc protein was produced as previously described and supplied by ReNetX Bio (48).

Human brain samples Post-mortem human tissue was collected in accordance with IRB protocols approved by Yale University. Samples of brain tissue were microscopically analyzed to confirm the clinical diagnosis of AD (Braak Stage V or higher). Samples from neurologically healthy controls were required to have no or minimal histopathological signs of AD (Braak 0 – II). Frozen prefrontal cortex was stored at -80°C until used. Human brain was weighed and Dounce-homogenized in 3 volumes of TBS, pH 7.4 (BioRad #170-6435) supplemented with Complete Mini Protease Inhibitors (Roche Diagnostics 1183617001) and PhosStop phosphatase inhibitors (Roche Diagnostics 04906837001). Samples were centrifuged at 100,000 xg at 4°C for 1 hour, the supernatant was collected, flash frozen in liquid nitrogen and stored at -80°C until assayed. The supernatant was referred to as the TBS soluble fraction. Patient
demographics for brains used are described in Table 2.

Statistics All results are presented are mean ± standard deviation (S.D.) unless otherwise stated.

Acknowledgements: We thank Stefano Sodi for help with mouse husbandry and Dr. Toshiyuki Takai for providing Pirb knockout mice. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of interest: SMS is the founder of ReNetX Bio which seeks to develop therapeutics targeting NgR1, and is an Inventor on a patent regarding PrPc antagonism for Alzheimer therapy.
Standardized comparison of reported Aβ receptors

References

1. Alzheimer, A. (1906) Über einen eigenartigen schweren Erkrankungsprozeb der Hirrinde. Neurol Zentralbl 23, 1129-1136
2. Martin, P. (2015) World Alzheimer report 2015: the global impact of dementia. Alzheimer’s Disease International (ADI), London
3. Lambert, M., Barlow, A., Chorny, B., Edwards, C., Freed, R., Liosatos, M., Morgan, T., Rozovsky, I., Trommer, B., and Viola, K. (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proceedings of the National Academy of Sciences 95, 6448-6453
4. Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., and Glabe, C. G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300, 486-489
5. Shankar, G. M., Bloodgood, B. L., Townsend, M., Walsh, D. M., Selkoe, D. J., and Sabatini, B. L. (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. The Journal of neuroscience 27, 2866-2875
6. Lesně, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M., and Ashe, K. H. (2006) A specific amyloid-β protein assembly in the brain impairs memory. Nature 440, 352-357
7. Kostylev, M. A., Kaufman, A. C., Nygaard, H. B., Patel, P., Haas, L. T., Gunther, E. C., Vortmeyer, A., and Strittmatter, S. M. (2015) Prion-Protein-interacting Amyloid-β Oligomers of High Molecular Weight Are Tightly Correlated with Memory Impairment in Multiple Alzheimer Mouse Models. Journal of Biological Chemistry 290, 17415-17438
8. Kayed, R., Head, E., Sarsoza, F., Saing, T., Cotman, C. W., Necula, M., Margol, L., Wu, J., Breydo, L., and Thompson, J. L. (2007) Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. Molecular neurodegeneration 2, 18
9. Gong, Y., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M. P., Finch, C. E., Krafft, G. A., and Klein, W. L. (2003) Alzheimer's disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proceedings of the National Academy of Sciences 100, 10417-10422
10. Laurén, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature 457, 1128-1132
11. Kim, T., Vidal, G. S., Djurisic, M., William, C. M., Birnbaum, M. E., Garcia, K. C., Hyman, B. T., and Shatz, C. J. (2013) Human LirRB2 is a β-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer’s model. Science 341, 1399-1404
12. Park, J. H., Gimbel, D. A., GrandPre, T., Lee, J.-K., Kim, J.-E., Li, W., Lee, D. H., and Strittmatter, S. M. (2006) Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-β plaque deposition. The Journal of neuroscience 26, 1386-1395
13. Kam, T.-I., Song, S., Gwon, Y., Park, H., Yan, J.-J., Im, I., Choi, J.-W., Choi, T.-Y., Kim, J., and Song, D.-K. (2013) FcγRIIB mediates amyloid-β neurotoxicity and memory impairment in Alzheimer’s disease. The Journal of clinical investigation 123, 2791-2802
14. Carlo, A.-S., Gustafsen, C., Mastrobuoni, G., Nielsen, M. S., Burgert, T., Hartl, D., Rohe, M., Nykjaer, A., Herz, J., and Heeren, J. (2013) The pro-neurotrophin receptor sortilin is a major neuronal apolipoprotein E receptor for catabolism of amyloid-β peptide in the brain. The Journal of Neuroscience 33, 358-370
15. Andersen, O. M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., Von Arnim, C. A., Breiderhoff, T., Jansen, P., and Wu, X. (2005) Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proceedings of the National Academy of Sciences of the United States of America 102, 13461-13466
16. Andersen, O. M., Schmidt, V., Spoelgen, R., Gliemann, J., Behlke, J., Galatis, D., McKinstry, W. J., Parker, M. W., Masters, C. L., and Hyman, B. T. (2006) Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. *Biochemistry* **45**, 2618-2628

17. Spoelgen, R., Von Arnim, C. A., Thomas, A. V., Peltan, I. D., Koker, M., Deng, A., Irizarry, M. C., Andersen, O. M., Willnow, T. E., and Hyman, B. T. (2006) Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and β-secretase β-site APP-cleaving enzyme. *The Journal of Neuroscience* **26**, 418-428

18. Yaar, M., Zhai, S., Pilch, P. F., Doyle, S. M., Eisenhauer, P. B., Fine, R. E., and Gilchrest, B. A. (1997) Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *Journal of Clinical Investigation* **100**, 2333

19. Izzo, N. J., Xu, J., Zeng, C., Kirk, M. J., Mozzoni, K., Silky, C., Rehak, C., Yurko, R., Look, G., and Rishton, G. (2014) Alzheimer's therapeutics targeting amyloid beta 1–42 oligomers II: sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. *PloS one* **9**, e111899

20. Dinamarcia, M. C., Weinstein, D., Monasterio, O., and Inestrosa, N. C. (2011) The synaptic protein neuroligin-1 interacts with the amyloid β-peptide. Is there a role in Alzheimer’s disease? *Biochemistry* **50**, 8127-8137

21. Magdesian, M. H., Carvalho, M. M., Mendes, F. A., Saraiva, L. M., Juliano, M. A., Juliano, L., Garcia-Abreu, J., and Ferreira, S. T. (2008) Amyloid-β binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/β-catenin signaling. *Journal of Biological Chemistry* **283**, 9359-9368

22. Du Yan, S., Chen, X., Fu, J., Chen, M., Zhu, H., Roher, A., Slattery, T., Zhao, L., Nagashima, M., and Morser, J. (1996) RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature* **382**, 685-691

23. Cissé, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D. B., Sun, B., Orr, A., Lotz, G., Kim, D. H., and Hamto, P. (2011) Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature* **469**, 47-52

24. Renner, M., Lacor, P. N., Velasco, P. T., Xu, J., Contractor, A., Klein, W. L., and Triller, A. (2010) Deleterious effects of amyloid β oligomers acting as an extracellular scaffold for mGlur5. *Neuron* **66**, 739-754

25. Fu, A. K., Hung, K.-W., Huang, H., Gu, S., Shen, Y., Cheng, E. Y., Ip, F. C., Huang, X., Fu, W.-Y., and Ip, N. Y. (2014) Blockade of EphA4 signaling ameliorates hippocampal synaptic dysfunctions in mouse models of Alzheimer’s disease. *Proceedings of the National Academy of Sciences* **111**, 9959-9964

26. Smith, L. M., and Strittmatter, S. M. (2017) Binding Sites for Amyloid-β Oligomers and Synaptic Toxicity. *Cold Spring Harbor perspectives in medicine* **7**

27. Benilova, I., and De Strooper, B. (2013) Promiscuous Alzheimer's amyloid: yet another partner. *Science* **341**, 1354-1355

28. Purro, S. A., Nicoll, A. J., and Collinge, J. (2017) Prion protein as a toxic acceptor of amyloid-β oligomers. *Biological psychiatry* **83**, 358-368

29. Izzo, N. J., Staniszewski, A., To, L., Fa, M., Teich, A. F., Saeed, F., Wostein, H., Walko III, T., Vaswani, A., and Wardius, M. (2014) Alzheimer's therapeutics targeting Amyloid beta 1–42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. *PloS one* **9**, e111898

30. Galasko, D., Bell, J., Mancuso, J. Y., Kupiec, J. W., Sabbagh, M. N., van Dyck, C., Thomas, R. G., and Aisen, P. S. (2014) Clinical trial of an inhibitor of RAGE-Ab interactions in Alzheimer disease. *Neurology* **82**, 1536-1542

31. Burstein, A., Sabbagh, M., Andrews, R., Valcarce, C., Dunn, I., and Altstiel, L. (2018) Development of Azeliragon, an Oral Small Molecule Antagonist of the Receptor for Advanced Glycation Endproducts, for the Potential Slowing of Loss of Cognition in Mild Alzheimer’s Disease. *J Prev Alz Dis* **5**, 149-154
32. Yang, T., Knowles, J. K., Lu, Q., Zhang, H., Arancio, O., Moore, L. A., Chang, T., Wang, Q., Andreasson, K., and Rajadas, J. (2008) Small molecule, non-peptide p75NTR ligands inhibit Aβ-induced neurodegeneration and synaptic impairment. *PloS one* **3**, e3604

33. Barghorn, S., Nimmrich, V., Striebinger, A., Krantz, C., Keller, P., Janson, B., Bahr, M., Schmidt, M., Bitner, R. S., and Harlan, J. (2005) Globular amyloid β-peptide1– 42 oligomer—a homogenous and stable neuropathological protein in Alzheimer's disease. *Journal of neurochemistry* **95**, 834-847

34. Barghorn, S., Nimmrich, V., Striebinger, A., Krantz, C., Keller, P., Janson, B., Bahr, M., Schmid, M., Bitner, R. S., and Harlan, J. (2005) Globular amyloid β-peptide1– 42 oligomer—a homogenous and stable neuropathological protein in Alzheimer's disease. *Journal of neurochemistry* **95**, 834-847

35. Chromy, B. A., Nowak, R. J., Lambert, M. P., Viola, K. L., Chang, L., Velasco, P. T., Jones, B. W., Fernandez, S. J., Lacer, P. N., and Horowitz, P. (2003) Self-assembly of Aβ1-42 into globular neurotoxins. *Biochemistry* **42**, 12749-12760

36. Cleary, J. P., Walsh, D. M., Hofmeister, J. J., Shankar, G. M., Kuskowski, M. A., Selkoe, D. J., and Ashe, K. H. (2004) Natural oligomers of the amyloid-β protein specifically disrupt cognitive function. *Nature neuroscience* **8**, 79-84

37. Li, S., Hong, S., Shepardson, N. E., Walsh, D. M., Shankar, G. M., and Selkoe, D. (2009) Soluble Oligomers of Amyloid β Protein Facilitate Hippocampal Long-Term Depression by Disrupting Neuronal Glutamate Uptake. *Neuron* **62**, 788-801

38. Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., and Selkoe, D. J. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**, 535-539

39. Lamprecht, M. R., Sabatini, D. M., and Carpenter, A. E. (2007) CellProfiler™: free, versatile software for automated biological image analysis. *Biotechniques* **42**, 71-75

40. Kim, J.-E., Liu, B. P., Park, J. H., and Strittmatter, S. M. (2004) Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. *Neuron* **44**, 439-451

41. Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature methods* **9**, 671

42. Wang, X., Yigitkanli, K., Kim, C.-Y., Sekine-Konno, T., Wirak, D., Frieden, E., Bhargava, A., Maynard, G., Cafferty, W. B., and Strittmatter, S. M. (2014) Human NgR-Fc decoy protein via lumbar intrathecal bolus administration enhances recovery from rat spinal cord contusion. *Journal of neurotrauma* **31**, 1955-1966
FOOTNOTES

Funding was provided by National Institutes of Health grants R01AG034924, P50AG047270, RF1AG05300 and R35NS097283, and the Falk Medical Research Trust to S.M.S.

The abbreviations used are Aβo, amyloid beta oligomers; AD, Alzheimer’s disease; PrPC, cellular prion protein; NgR1, nogo receptor 1; LilrB2, leukocyte immunoglobulin-like receptor subfamily B member 2; LTP, long-term potentiation; RAGE, advanced glycosylation end product-specific receptor; PGRMC1, membrane-associated progesterone receptor component 1; EphA1, ephrin type-A receptor 1; FcγRIIb, low affinity immunoglobulin gamma Fc region receptor II-b; SorLA, sortilin-related receptor; p75NTR, tumor necrosis factor receptor superfamily member 16; NLGN1, neuroligin 1; EphB2, ephrin type-B receptor 2; FZD5, frizzled-5; mGluR5, metabotropic glutamate receptor 5; EphA4, ephrin type-A receptor 4; CRMP2, collapsin response mediator protein 2; NRP-1, neuropilin-1; BAβ, biotin amyloid beta; Aβg amyloid beta globulomers; Aβm amyloid beta monomers; Pirb, paired immunoglobulin-like receptor b, nArchRα7, neuronal acetylcholine receptor subunit alpha-7; RIC-3, resistance to inhibitors of cholinesterase 3; GluN2B, glutamate receptor ionotrophic NMDA 2B; GluN1, glutamate receptor ionotrophic NMDA 1

Table 1. Binding affinities for positive receptor candidates and preparations of synthetic Aβ

The K_D and Bmax of each of the receptors and the indicated Aβ preparation are shown. Concentrations are expressed as monomer equivalents. Discrimination factor is the quotient of a receptor’s K_D for monomeric Aβ divided by that of the indicated preparation. Greater values indicate a preference for binding to neurotoxic preparations.

|          | Aβo       | Aβg       | Aβm       |
|----------|-----------|-----------|-----------|
| PrPC     | K_D (nM)  | 1.4 ± 0.2 | 1.5 ± 0.2 | 95.9 ± 6  |
|          | Bmax (AU) | 6.27 x 10^6 | 5.9 x 10^6 | 6.46 x 10^6 |
|          | Discrimination Factor | 68.5 | 63.9 |
| LilrB2   | K_D (nM)  | 42.3 ± 4.4 | 207.2 ± 18.2 | 192.4 ± 12 |
|          | Bmax (AU) | 5.84 x 10^6 | 5.9 x 10^6 | 4.45 x 10^6 |
|          | Discrimination Factor | 4.5 | 0.9 |
| NgR1     | K_D (nM)  | 3.9 ± 0.4 | 6.6 ± 0.5 | 61.6 ± 5.3 |
|          | Bmax (AU) | 5.53 x 10^6 | 5.85 x 10^6 | 5.46 x 10^6 |
|          | Discrimination Factor | 15.8 | 9.3 |

Table 2. Characteristics of human brain tissue samples

Patient characteristics for human brain tissue used are described. Values are ± standard deviation

|          | Sample size | Age (years) | Sex (% female) | Post-mortem interval (days) |
|----------|-------------|-------------|----------------|-----------------------------|
| AD       | 10          | 78 ± 12     | 50             | 13 ± 9                     |
| Ctrl     | 11          | 71 ± 14     | 64             | 20 ± 7                     |
Figure 1. Myc-tagged candidates are expressed at similar levels and traffic to the cell surface.
A. Immunoblots of RIPA-soluble cell lysates following cell-surface biotinylation and immunoprecipitation using anti-Myc agarose beads. Candidate receptors were immunoblotted with an anti-Myc antibody (left) and fluorescein-conjugated streptavidin (right) empty = empty vector control. B. Images of COS-7 cells transfected with the indicated candidate receptor and stained with anti-Myc antibody. Scale bar = 200 µm C. Quantification of B. All samples are normalized to hPrP^c Myc signal. N = 32 experiments for hPrP^c and...
8-14 for all others. Individual data points indicate different experiments. Error bars are standard deviation (S.D.).
Figure 2. Only PrP<sup>C</sup>, LilrB2, and NgR1 confer Aβ<sub>40</sub> binding capacity to cells.
A. COS-7 cells expressing the Myc-tagged candidate receptor of interest and incubated with 1 μM biotin Aβo at 4°C. Scale bar = 200 μm. B. Quantification of biotin Aβo binding to cells in A, normalized to candidate receptor Myc signal. For each experiment, Myc-normalized biotin Aβo binding was expressed as a percent of hPrPc in that experiment. One-sided T test comparing to an expected value of 100 (% hPrPc binding) are denoted by *. n.s.=not significant, *p<0.05, **p<0.01, ***p<0.001. Significance of a one-sided T test comparing to an expected value of 0 (no binding) are denoted by # below the Y axis. #p<0.05, ##p<0.01. N = 4-7 experiments. Individual data points indicate different experiments. Error bars are S.D.
Figure 3. PrP<sup>C</sup> and NgR1 preferentially bind neurotoxic Aβ<sub>o</sub>.  
A. SEC trace of 50 µM BAβ<sub>o</sub> showing absorbance at 280 nm. Alternate vertical columns indicate fractions collected for analysis. Yellow fraction indicates high molecular weight (HMW) Aβ. Elution time of various standards (kDa) are indicated by arrows. V<sub>o</sub> = void volume as determined by blue dextran MW=2,000 kDa.  
B. Detection and quantification of the effect of preparative method on the distribution of BAβ in different SEC fractions by dot blot analysis.  
C. Quantification of expression-normalized Aβ signal from COS-7 cells transfected with the indicated candidate receptor and incubated with 1 µM monomeric biotin Aβ at 4°C. Values are normalized to oligomeric biotin Aβ binding to hPrP<sup>C</sup>. One-sided T test comparing to an expected value of 100. N = 3-5 experiments.  
D. Binding of receptors to monomeric Aβ compared to Aβ<sub>o</sub> from Fig. 2B. Multiple T test with Holm-Sidak correction for multiple comparisons. Individual data points indicate different experiments. Error bars are S.D. n.s.=not significant, *p<0.05, **p<0.01, ***p<0.001.
Standardized comparison of reported Aβ receptors
Figure 4. Neither temperature nor oligomer preparation changes binding profile of candidate receptors.

A. Quantification of 1 μM biotin Aβ0 binding to cells expressing candidate receptors at 37°C. Binding is normalized to that of hPrPc at 4°C. One-sided T test comparing to an expected value of 100 (% hPrPc binding at 4°C). N = 3 - 6 experiments. B. Comparison of the capacity of a candidate receptor to bind biotin Aβ0 at 4°C (Fig. 2B) versus 37°C. Multiple T test with Holm-Sidak correction for multiple comparisons. C. Atomic force microscopy images showing differences in Aβ generated by different preparations. Images are 200 nm x 200 nm. D. Myc-normalized binding of 1 μM Aβ prepared using the globulomer protocol. Data are expressed relative to oligomerized Aβ binding to hPrPc. One-sided T test comparing to an expected value of 100 (% hPrPc binding) N = 3 experiments, 2 for PGRMC1. E. Comparison of positive receptors’ binding of oligomer (Fig. 2B) and globulomer Aβ. Multiple T test with Holm-Sidak correction for multiple comparisons. Individual data points indicate different experiments. Error bars are S.D. n.s.=not significant, *p<0.05, **p<0.01, ***p<0.001.
Figure 5. Loss of NgR1 and the mouse LilrB2 homolog Pirb decrease Aβ0 binding to neurons.
A. Images showing punctate biotin Aβ0 binding to dendrites of hippocampal neurons. Scale bar = 50 µm.
B. Quantification of mean Aβ0 signal in A. N = 43-94 images from 5-7 dishes in 4 experiments. Unpaired T-test. Error bars are S.D. *p<0.05, **p<0.01, ***p<0.001
Figure 6. hPrP<sup>C</sup>, NgR1, and LilrB2 coated plates recapitulate cell-based assay
A. Binding of Aβo to wells coated with hPrP⁶, NgR1, LilrB2, or Fc control protein. B. Scatchard plot of data in A. C. Binding of Aβ globulomers to wells coated with hPrP⁶, NgR1, LilrB2, or Fc control protein. D. Scatchard plot of data in C. E. Binding of Aβ monomers to wells coated with hPrP⁶, NgR1, LilrB2, or Fc control protein. F. Scatchard plot of data in E. G. Comparison of the binding of Aβo, Aβg, and Aβm to hPrP⁶. H. Comparison of the binding of Aβo, Aβg, and Aβm to NgR1. I. Comparison of the binding of Aβo, Aβg, and Aβm to LilrB2.
Figure 7. hPrP<sup>C</sup> and NgR1 bind soluble Aβ present in brains of AD patients.

Binding of Aβ in TBS soluble fraction of brains of AD patients or control patients (Ctrl) to wells coated with hPrP<sup>C</sup>, NgR1, or LilrB2. Each dot represents an individual patient brain. Multiple T tests with Holm-Sidak correction for multiple comparisons. Error bars are S.D. *p<0.05, **p<0.01, ***p<0.001
Systematic and standardized comparison of reported Amyloid-β receptors for sufficiency, affinity, and Alzheimer's disease relevance
Levi M. Smith, Mikhail A. Kostylev, Suho Lee and Stephen M. Strittmatter

J. Biol. Chem. published online February 20, 2019

Access the most updated version of this article at doi: 10.1074/jbc.RA118.006252

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts