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A Universal Method for Detection of Amyloidogenic Misfolded Proteins

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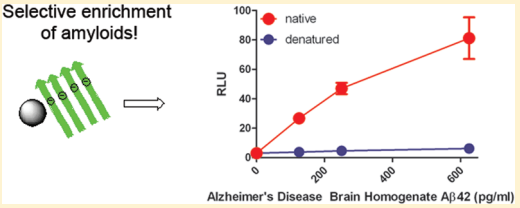
Supporting Information

ABSTRACT: Diseases associated with the misfolding of endogenous proteins, such as Alzheimer's disease and type II diabetes, are becoming increasingly prevalent. The pathophysiology of these diseases is not totally understood, but mounting evidence suggests that the misfolded protein aggregates themselves may be toxic to cells and serve as key mediators of cell death. As such, an assay that can detect aggregates in a sensitive and selective fashion could provide the basis for early detection of disease, before cellular damage occurs. Here we report the evolution of a reagent that can selectively capture diverse misfolded proteins by interacting with a common supramolecular feature of protein aggregates. By coupling this enrichment tool with protein specific immunoassays, diverse misfolded proteins and sub-femtomole amounts of oligomeric aggregates can be detected in complex biological matrices. We anticipate that this near-universal approach for quantitative misfolded protein detection will become a useful research tool for better understanding amyloidogenic protein pathology as well as serve as the basis for early detection of misfolded protein diseases.



Misfolded protein (MFP) diseases encompass a wide variety of disorders, including Alzheimer's and Parkinson's diseases, type II diabetes, prion diseases, and systemic amyloidosis. In each of these cases, the disease is associated with the misfolding of normal endogenous proteins into higher order aggregates containing increasing β sheet content. While the precise role of aggregation in the pathology of these disorders is not fully understood, the classic hallmark of MFP disease is the presence of large fibrillar protein deposits throughout the diseased tissue.[1,2] In addition to the insoluble deposits, soluble oligomeric MFPs may play a role in disease progression. Indeed, a wide range of small oligomeric MFPs have been reported to disrupt normal signal transduction processes and to be cytotoxic, and their formation may therefore be an early event in the pathology of the disease.[3−13]

Although there is widespread support for the theory that toxic aggregates are present at presymptomatic stages of disease,[3] developing new diagnostic tools based on detection of these species has been challenging. Common genomic and transcriptomic techniques are not useful for MFP detection because the aggregates are composed of endogenous proteins normally present in the body. Furthermore, because MFP species are often present in much lower levels than the normal monomeric isoforms, highly selective aggregate-specific reagents are required. While several compounds have been reported to bind large fibrils (e.g., thioflavin T, Congo red), these reagents do not capture the putatively toxic smaller oligomeric species that are of key interest.[14] Developing chemical tools for the enrichment of soluble oligomers has been further hindered by the lack of structural data, heterogeneity of oligomer preparations, and transient nature of these species.[3] As such, despite considerable research efforts,[15−21] there are no widely adopted approaches for the detection of aggregates in biological fluids.

To address the unmet medical need of early detection of MFP diseases, our lab has been developing reagents for the facile enrichment and sensitive detection of aggregated MFPs. Previously, we reported a reagent to capture and enrich misfolded prion fibrils and showed that it has utility for detecting beta amyloid.[22,23] Here, we describe the evolution of this tool into a selective capture reagent recognizing diverse aggregate species from multiple disease-associated proteins. In addition, we provide evidence that this universal capture stems from the highly conserved molecular features of amyloids.[24,25] The enrichment process is coupled to a novel assay format that amplifies the signal and provides quantitation of the individual MFPs from the sample. These misfolded protein assays (MPAs) not only provide a tool for better understanding of amyloid pathology but also may yield a new class of diagnostics that can detect MFP disease before irreversible cell damage occurs.[23]

EXPERIMENTAL PROCEDURES

Preparation of Peptoid/Peptide Bead Conjugates. Peptoids and peptides were prepared on Rink amide resin according to previously reported automated solid phase synthesis techniques.[26,27] Peptoid monomer incorporation was performed using cycles of (1) acetylation with bromoacetic acid and (2) bromine displacement with amines. Amino acid incorporation

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was accomplished using an Fmoc-protection strategy. Completed peptoids and peptides were cleaved from the resin using 95:2.5:2.5 trifluoroacetic acid:water:triisopropylsilane and purified by reverse phase HPLC. Biotinylated ligands were coated to streptavidin-displaying beads,[22] and thiolated ASR1 and glutathione were covalently bound to maleimide-displaying beads using Michael addition chemistry. Details of these syntheses are found in the Supporting Information.

Biological Samples. Alzheimer's disease (AD) and control brain samples were obtained from the tissue bank of the Swiss National Reference Centre of Prion Diseases (Zürich, Switzerland). CJD and normal brain homogenates used in the comparison between ASR1 and the prion peptide sequence were from the National Institute for Biological Standards and Control (NIBSC, United Kingdom). Normal and type II diabetic pancreatic tissue was acquired 3−6 h postmortem and immediately flash-frozen through the National Disease Research Interchange (NDRI, Philadelphia, PA). Prion infected hamster brains from terminally infected Syrian hamsters (strain 263K) were purchased as a 10% brain homogenate from the TSE Resource Centre (now located at the Roslin Centre, University of Edinburgh). Normal pooled cerebrospinal fluid (CSF) for oligomer spiking experiments was purchased from Analytical Biological Services Inc. (Wilmington, DE) and Bioreclamation (Liverpool, NY). 10% brain and pancreatic homogenates were prepared by homogenization of the tissues in 0.2 −0.25 M sucrose.

In Vitro Aggregate Preparation. The Aβ42 aggregates were prepared from synthetic Aβ42 (Anaspec, San Jose, CA) as previously described: fibrils were prepared per Stine et al.,[28] WT and mutant globulomers were prepared per Barghorn et al.,[29,30] ADDLs were prepared per Lambert et al.,[9,31] and ASPDs were prepared per Noguchi et al.[32] Alpha synuclein fibrils were prepared by shaking a 5 mg/mL solution of purified recombinant α-synuclein (rPeptide, Bogart, GA) for 5 days at 37 °C.[33] Amylin fibrils were prepared from synthetic peptide (Anaspec, San Jose, CA) as reported previously.[13,34] Serpin A1 fibrils were prepared from recombinant protein (Sigma-Aldrich, St. Louis, MO) as reported previously.[35]

Oligomer Characterization. Protein concentration was assessed using the Micro BCA or Better Bradford protein concentration kits (Pierce Thermo Fisher Scientific, Rockford, IL) using bovine serum albumin as a standard. Oligomers were separated by 4−20% Tris-Glycine SDS-PAGE (Invitrogen, Carlsbad, CA) for 1.5−2 h at 120 V and gels were stained with a Colloidal Blue Staining Kit (Invitrogen, Carlsbad, CA). Native gel analysis was performed on 4−20% tris-glycine gels for 5−6 h at 100 V and 4 °C. Size-exclusion chromatography was performed on a Superdex200 column (GE Healthcare, Piscataway, NJ) in PBS, running at a flow rate of 1 mL/min. To determine total Aβ protein in each fraction, a 2 μL aliquot of each fraction was denatured with GdnSCN (final concentration 4 M) for 30 min at rt and then diluted into TBST + 0.1% BSA. The samples were then added, along with 0.2 μg/mL 4G8-HRP (Covance, Princeton, NJ, in PBS + 0.1% BSA + 0.01% casein), to a plate coated with the 12F4 antibody (Covance, Princeton, NJ). The plate was incubated 1 h, washed, and treated with a luminescent substrate (Pierce SuperSignal ELISA Femto) before detecting on a luminometer. Cross-linking studies were performed by incubating the oligomer in 1 mM glutaraldehyde for 2 h at rt and quenching with 5 mM ethanolamine.

MPA Assay. Details for the MPA assays for the various aggregates can be found in the Supporting Information. Briefly, paramagnetic beads displaying the ligands of interest (0.09−1 mg) were added to the wells of a microtiter plate, and the sample of interest was added. The plate was incubated at 37 °C for 1 h, and the unbound material was removed by washing. The bound material was eluted, denatured, and reconditioned before placing on a protein-specific immunoassay.

Calculations. pIs were calculated using Expasy tools: http://www.expasy.ch/cgi-bin/protparam.

RESULTS

Development of Peptoid Capture Reagent. Our initial efforts in the MFP field focused on detecting the highly infectious misfolded forms of the prion protein in blood as a step toward the prevention of transfusion-acquired prion disease. This previous work showed that several peptides derived from the prion sequence bound to the pathogenic aggregated form of prion preferentially over the normal form.[22] Attachment of these peptides onto solid support to create peptide−bead conjugates thus allowed for a three-step misfolded protein assay (MPA): (1) selective enrichment of aggregates via the aggregate-specific peptide reagent, (2) denaturation and elution of the captured protein from the bead as monomers, and (3) detection of the eluted protein using a standard immunoassay. Using this MPA format, one of the identified sequences, prion residues 23−30, KKRPKPGG, captured low levels of aggregated prion from a variant Creutzfeldt−Jakob disease (vCJD) brain homogenate spiked into plasma with ~4000-fold selectivity for the misfolded, pathogenic form.[22]

While the bead−KKRPKPGG conjugate showed excellent sensitivity and selectivity (Figure 1), the proteolytic stability of the peptide in a blood-based assay was a concern. To mitigate the risk of proteolytic degradation, peptidomimetics which confer proteolytic resistance were tested.[36] Given that conversion of proline to the unnatural Phe analogue, (*S*)-*N*-(1-phenethyl)glycine, in some protein−protein interactions can reportedly increase binding affinity,[37] we prepared a panel of peptides containing N-substituted glycines (or peptoids). Peptide−peptoid hybrids and peptoids analogous to the original peptide sequence (Figure 1A) were assayed for their ability to capture misfolded prion spiked into plasma. The most efficient and synthetically tractable peptoid, termed aggregate-specific reagent, or ASR1 (Figure 1B), provided highly selective capture for the misfolded form of prion (Figure 1C), demonstrating improved selectivity relative to the original prion-derived peptide ($>10^3$ preference for the misfolded form).[22]

Capture of Misfolded Protein Aggregates of Diverse Size and Origin. Like prion, many other MFPs ultimately aggregate into fibrils consisting of ordered β strands running perpendicular to the fibril axis.[24,38] Though the relative orientation of the strands may vary, the resulting cross β sheet amyloids have similar properties: ~4.7 Å interstrand spacing and ~6−11 Å intersheet separation for all known amyloidogenic proteins.[24,25,39] To see if ASR1 recognized this general motif, rather than prion fibrils specifically, ASR1 was tested for its ability to capture amyloid fibrils composed of diverse MFPs (Table 1). Aggregation of amylin, or islet amyloid polypeptide (IAPP), a peptide secreted by pancreatic beta cells, has been linked to the development of type II diabetes.[4] Given the significantly different properties of prion and amylin (in terms of localization, predicted monomer structure, and length), aggregated amylin was chosen for proof of principle studies. Normal and diabetic pancreas

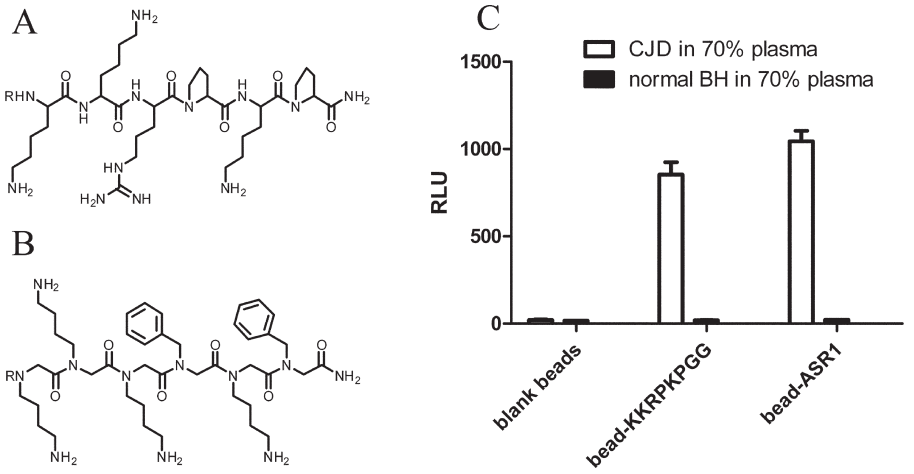

Figure 1. Development of ASR1. (A) Key fragment of the original prion sequence identified to specifically interact with aggregated prion. (B) Conversion of the peptide to a synthetically tractable protease-resistant peptoid scaffold. (C) Comparison of the ability of the two reagents to selectively capture misfolded prion from vCJD brain samples spiked into 70% plasma. 1 μL of 10% brain homogenate in 70% plasma was incubated with 0.1 mg of peptide- or ASR1-coated beads. The bound material was eluted, reconditioned, and quantitated by a prion-specific ELISA. White bars show MPA results with misfolded prion-containing vCJD brain samples, and black bars show MPA results with normal brain samples. Data shown are the average of triplicate samples ± SD.

Table 1. Characteristics of Select Misfolded Proteins and Their Associated Diseases[a]

	disease characteristics		MFP characteristics		
misfolded protein	common associated diseases	location of in vivo aggregates	protein size (amino acids)	pI (calculated)	common in vivo modifications
alpha synuclein	PD, synucleinopathies	brain	140	4.7	PO_4^{3-}, NO_2, Ac
amylin	diabetes type II	pancreas	37	8.9	amidation
beta amyloid (Aβ)	AD, CAA	brain	<43	5.4	truncation
prion	spongiform encephalopathies	brain	253	9.1	glycolipidation
serpin A1	preeclamspia	placenta	418[b]	5.4	glycosylation, truncation
tau	AD, PD, tauopathies	brain	351−776[c]	6.6	PO_4^{3-}, glycosylation

[a] PD = Parkinson's disease; AD = Alzheimer's disease; CAA = cerebral amyloid angiopathy. [b] Highly polymorphic, multiple variants. [c] Alternate splice variants observed.

tissue homogenates were put into an MPA coupled with an amylin-specific immunoassay (Figure 2A). ASR1 captured amylin from the diabetic tissue, but not the normal tissue, in a concentration-dependent manner, suggesting that ASR1 was indeed capable of interacting with aggregates composed of alternate proteins. Pancreatic tissue that was denatured prior to ASR1 treatment was not captured, highlighting the conformational sensitivity of ASR1 (Figure 2A). Similar results were seen with amyloid beta 42 (Aβ42) in Alzheimer's disease brain homogenates (AD BH) (Figure 2B) as well as in vitro prepared fibrillar alpha-synuclein and serpin A1 (also known as α1-antitrypsin), the MFPs associated with Parkinson's disease and pre-eclampsia,[40] respectively (Figure 2C,D). As with amylin fibrils, ASR1 selectively captured the fibrillized form of these misfolded proteins over the monomeric form. Collectively, these studies suggest that ASR1 is a powerful tool for capturing aggregates from diverse proteins.

In many MFP diseases, it is the smaller oligomeric species, rather than the large fibrillar aggregates, that are thought to be involved in the etiology of the disease.[3,41] For example, AD research suggests that it may be Aβ42 oligomers diffusing through the central nervous system (CNS) that disrupt synaptic structure and plasticity, leading to neuronal loss, inhibition of long-term potentiation, and ultimately dementia.[5,8,9,12] To investigate whether ASR1 could capture nonfibrillar aggregates, MPAs were performed with a panel of diverse oligomeric Aβ42 species. Globular, micellar, and spherical aggregates were prepared and serially diluted into cerebrospinal fluid (CSF), the relevant CNS matrix. While ASR1 captured even the smallest of the Aβ oligomer models in our panel, the globulomer (~12−16-mers),[29] with sub-femtomole sensitivity in the presence of excess endogenous CSF Aβ, an inverse correlation was observed between limit of detection (LoD) and aggregate size (Table 2 and Figure 2E). This suggests that avidity interactions between the repeating units of the aggregate may play a role in the highly sensitive aggregate capture.

Capture Reagent Net Charge Is a Critical Parameter for Fibril Capture. To understand the mechanism of ASR1−Aβ42 aggregate binding, the role of the individual residues in ASR1 was examined. A panel of peptoid−bead conjugates with varying sequence, charge, and hydrophobicity was prepared (Supporting Information, Figure S1) and an MPA with these diverse reagents was performed for AD BH spiked into CSF (Figure 3A). Peptoids with net positive charge provided efficient capture, independent of the precise sequence. Similar trends were

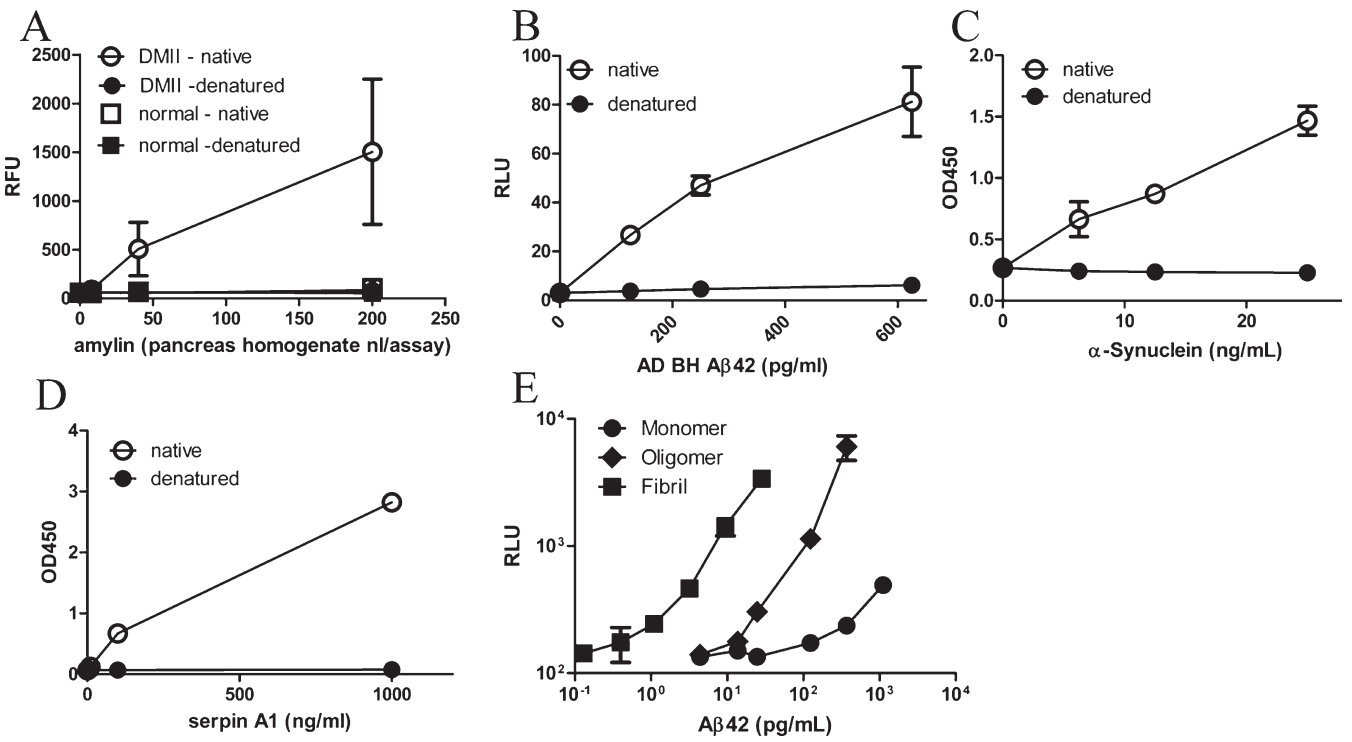

Figure 2. Conformational-dependent capture of a wide variety of aggregated proteins by ASR1. (A) Aggregated amylin from diabetes mellitus type II (DMII) pancreas tissue spiked into 80% plasma, (B) aggregated Aβ42 from AD brain tissue spiked into 80% plasma, (C) in vitro aggregated alpha-synuclein spiked into 80% CSF, and (D) in vitro aggregated serpin A1 spiked into PBS were incubated with ASR1 beads. Bound material was denatured, eluted, and quantitated on protein-specific ELISAs. The MPAs show concentration-dependent capture of aggregated species (open symbols), while denatured species show little capture (filled symbols). (E) Aβ fibrils, oligomers, and monomers were spiked into buffer and incubated with ASR1 beads. Bound material was denatured, eluted, and quantitated on an Aβ-specific ELISA. ASR1 captures larger, more ordered aggregates more efficiently than smaller species, consistent with a mechanism that relies on interaction with repeating monomer units present in the aggregate. Data shown are the average of triplicate samples ± SD.

Table 2. ASR1 Captures a Wide Variety of Aβ Aggregates[a]

model	reference	components	size	shape	LoD (pg/mL)	LoD (amol)
monomer	n/a	Aβ42	~4.5 kDa	unstructured	>1000	>2.2 × 10^4
globulomer	29, 30	Aβ42, DMSO, SDS	~60 kDa	globular	~75	~125
ADDL	9, 31	Aβ42, media?	kDa−MDa (1 MDa)	micellar, fibrillar	~200	~20
ASPD	32	Aβ42, media?	kDa−MDa (1 MDa)	spherical, fibrillar	~200	~20
fibrils	28	Aβ42	>1 MDa	fibrillar	~20	<2
ADBH	n/a	Aβ40, Aβ42, other?	>1 MDa	fibrillar	~0.6−4	<0.4

[a] CSF samples spiked with diverse aggregates were subjected to the MPA to determine the limit of detection (LoD) of the different species. LoDs in pg/mL are calculated as 2 times over background in duplicate assays. LoDs in amol are calculated based on reported/observed size (for heterogenous aggregates, a molecular weight (MW) from the middle of the MW range is used).

observed when prions from CJD brain homogenate were tested (Figure 3B). The ability of ASR1 and other positively charged peptoids to enrich MFPs of disparate properties suggests that the mechanism of binding relies not on a lock-and-key interaction, but rather takes advantage of more general characteristics of the aggregates, and, in particular, some negatively charged feature unique to the aggregated form of the protein.

Understanding the molecular basis for the ASR1−MFP interaction is challenging due to the limited number of high-resolution amyloid structures available.[24] However, both the Aβ42 fibril structure reported by Riek et al.[42] and the Aβ40 fibril model reported by the Tycko lab[43] exhibit negatively charged surface features formed from the repeating elements found in the aggregates. In both cases, the E22 of each Aβ peptide aligns on the outside of the β-sheet, creating a negative charge stripe on the protein surface parallel to the fibril axis (Figure 3C). Thus, a unique surface "epitope" present on the aggregate, but not its monomeric counterpart, is available to bind ASR1.

Charge Features on Oligomers Impact Capture Efficiency. In the putative binding model where aligned charges on the surface of the MFP interact with ASR1, the strength of the interaction would be dependent on the length and degree of order of the β sheets in the MFP. While lower detection limits were observed for the most strongly ordered and largest fibrils (Table 2), we asked if this model could be extended to less ordered oligomers. As such, the ~12−16-mer globulomer[29] was

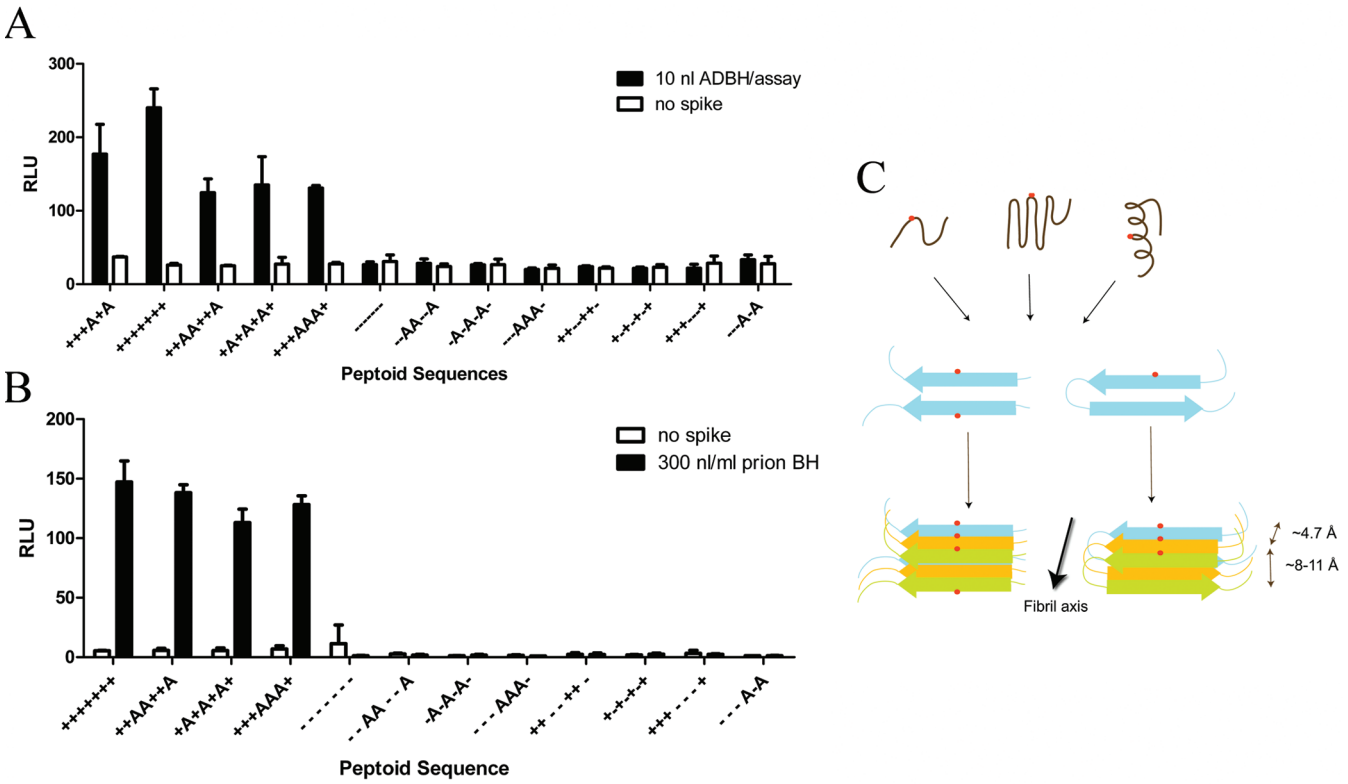

Figure 3. Key role of electrostatics in selective aggregate capture. A panel of diverse peptoids of the format biotin-Ahx-Ahx-peptoid-NH_2 was prepared, coated to magnetic beads, and tested for their ability to capture MFPs spiked into CSF. The ligands are shown in shorthand to indicate the peptoid sequence from amine to carboxy terminus: A = aromatic (the phenylalanine mimic *N*-benzylglycine), + = positively charged (the lysine mimic *N*-(4-aminobutyl)glycine), − = negatively charged (the glutamic acid mimic *N*-(2-carboxyethyl)glycine). CSF was spiked with 10 nL/well ADBH (A) or 300 nL/mL hamster prion BH (B) and incubated with the peptoid−bead conjugates indicated. Bound material was denatured, eluted, and quantitated on protein-specific ELISAs. Data shown are the average of replicate samples ± SD (duplicate for A, triplicate for B). (C) Surface-exposed acidic residues (shown as red dots) align into repeating structures during aggregation of MFPs, contributing to the acidic charged "epitope" proposed to mediate the interaction with ASR1.

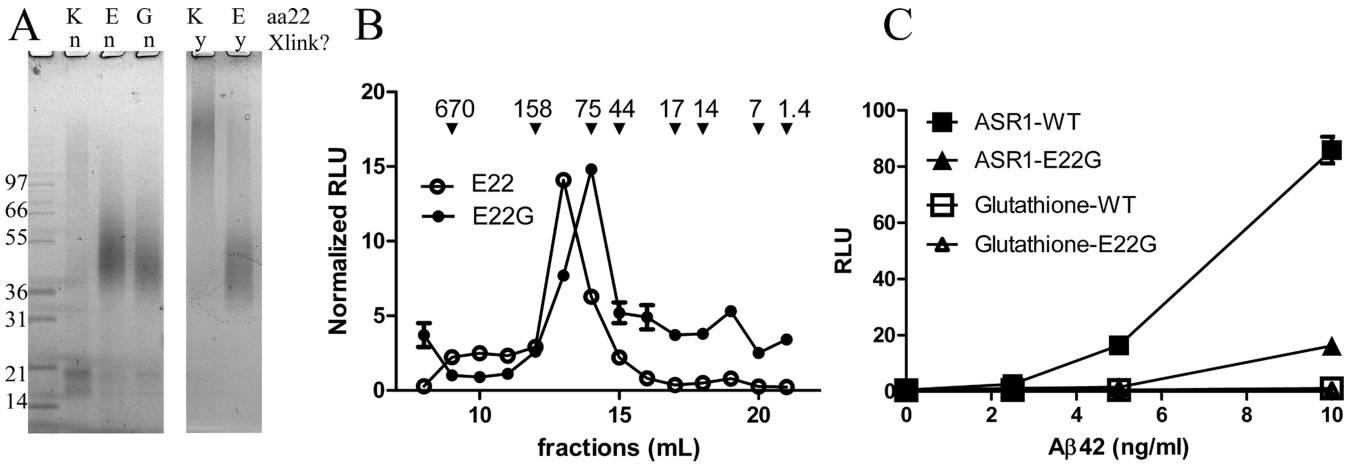

Figure 4. Properties and capture of globulomer mutants. E22G and WT globulomers had similar size (and SDS stability) by SDS-PAGE (A) and size-exclusion chromatography (B). E22K mutant oligomers were not SDS stable but, after cross-linking, showed slower migration than wt on SDS PAGE (A). (C) Wild type and mutant oligomers were spiked into CSF and incubated with ASR1 beads. Bound material was denatured, eluted, and quantitated on Aβ-specific ELISAs. Wild type oligomers were captured by ASR1 in a concentration-dependent manner. E22G, which has similar properties to the wt but lacks the key E22 residue, was only weakly captured. Negative control beads bearing glutathione beads did not yield any signal.

more carefully examined. Epitope mapping demonstrated that the key E22 residue was present on the surface of the oligomer (Figure S2A), consistent with the reported fast H−D exchange of the E22 amide bond in globulomers.[30] To assess the role of this glutamate residue in ASR1−oligomer binding, we tested whether globulomers lacking this negatively charged residue on

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their surface would be effectively captured by ASR1. To this end, Aβ peptides containing the Italian (E22K) or Arctic (E22G) mutations were subjected to the globulomer oligomerization protocol. Given that these mutations impact aggregation propensity and amyloid structure,[44] the resulting products were examined using biochemical techniques to ascertain whether the structural features were consistent with the E22 WT globulomer. Both E22G and E22K peptides formed aggregated species by native gel (Figure S2b), but only E22G aggregates showed similar stability and size to the WT by SDS-PAGE (Figure 4A). Size exclusion chromatography further demonstrated the similarity in size and homogeneity of the WT and E22G oligomers (Figure 4B). The WT and E22G peptides were subjected to the MPA. While the WT globulomer was captured by ASR1 in a concentration-dependent manner, the E22G mutation greatly diminished capture (Figure 4C). These results are consistent with the model that exposed E22 residues mediate the interaction of Aβ oligomers with ASR1 and suggest that even small oligomers may maintain the repetitive features that define amyloid fibrils.

DISCUSSION

Since Alois Alzheimer first described AD, reagents for the detection of MFPs have been keenly sought after for clinical and research purposes.[1] The classic reagents used to detect amyloid, like Congo red and thioflavin T, are widely employed by pathologists but have several limitations. First, detection by these reagents is based on color or birefringence changes, limiting their sensitivity and potential for quantitation. Second, these reagents do not specify the type of protein found in the amyloid. Perhaps most importantly, they only allow for detection of a subset of fibrillar structures, rather than the smaller oligomers implicated as the primary pathological species. In response to these limitations, a variety of other amyloid-detecting reagents and approaches have been reported, including conjugated polyelectrolyes,[45] oligomer-specific antibodies[46,47] and ELISAs,[15,17,48] and RNA aptamers.[49,50] While these reagents generally allow for detection of a wider variety of aggregate conformations, they do not provide information on protein identity and may have limited specificity[51−53] and/or sensitivity.[17]

To address these issues, we have developed a new type of MFP assay that allows for sensitive and selective detection of specific proteins. The molecular recognition tool employed, a peptoid called ASR1, takes advantage of the common molecular features of aggregates to bind diverse MFP species, despite their inherent morphological heterogeneity. The proposed binding model suggests that the positively charged ASR1 binds to a negative charge patch comprised of electronegative atoms or exposed acidic residues from the component monomers in the aggregate (Figure 3C). Given the ubiquity of aspartate and glutamate residues in proteins, and the commonality of cross-beta sheets in MFPs, this charge patch "epitope" is likely a near-universal feature of natural protein amyloids. Indeed, there is evidence that endogenous amyloid binders, like apolipoprotein E and serum amyloid P, may bind MFPs through negative charge patches on amyloid surfaces.[54,55] Consistent with the hypothesis that ASR1 interacts through a charge patch commonly present on MFPs, ASR1 interacts with aggregates from amyloidogenic proteins with sizes ranging from <50 to >400 amino acids and pIs ranging from <5 to >9 (Table 1). Indeed, of the multiple amyloidogenic proteins and aggregate conformations tested, only one, a small oligomer of E22G mutant Aβ, failed to be effectively captured by ASR1, perhaps suggesting an absence of negatively charged repeating surface features on this mutant species. Importantly, while ASR1 effectively captures aggregates, it binds monomeric proteins with significantly less efficacy, presumably due to the relative weakness of a single ionic bond compared to the avidity-based capture proposed for aggregates. The preference for aggregates allows for sub-femtomole detection of Aβ aggregates in CSF that typically has 0.1−1 nM total Aβ as well as ∼4000-fold selectivity for aggregated prion over monomeric prion in plasma.

While ASR1 captures MFPs from diverse sources, the captured proteins themselves can be independently and sensitively measured using the MPA approach. MPAs provide sensitivity by virtue of two signal amplification steps: the denaturation of the oligomer into its component parts (i.e., a 100-mer oligomer yields 100 individual monomers) and the amplification of the resultant monomers in the final immunoassay. In addition to amplification, the use of protein specific immunoassays in the MPA allows for independent quantitation of component monomers, a key requirement for dissecting the molecular pathogenesis of MFP diseases.

The combination of near-universal enrichment, sensitivity, and protein-specific quantitation should allow ASR1 and MPAs to become powerful tools in both the diagnostic and research realms. ASR1 offers a means to separate amyloid from nonamyloid and thus may serve as a starting point for proteomic studies to better understand the amylome.[38] One limitation of ASR1 is that it may suffer from polyanion interference such as in heparinized plasma, where capture may be less efficient. Nonetheless, when placed in the context of the MPA assay, ASR1 provides one of the first opportunities for quantification of aggregates and putatively toxic species from biological matrices. The exquisite sensitivity of MPAs should continue to increase with improvements to the capture reagents and the downstream immunoassays, ultimately allowing for increased insight into the role of oligomers in MFP disease and the identification of disease-associated biomarkers. Indeed, we recently used this technique to demonstrate that endogenous Aβ40 oligomers in CSF are a putative biomarker for AD.[24] In addition to improved understanding of disease etiology, we hope MPAs can be used as the basis for early stage diagnostics and a tool for the development of effective therapeutics for misfolded protein diseases.

ASSOCIATED CONTENT

Supporting Information. Detailed protocols for the peptoid synthesis, ELISA conditions, and supplementary figures as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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DISCLOSURE

The authors of this work are or were employed by Novartis Vaccines and Diagnostics.

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ABBREVIATIONS

Aβ, beta amyloid; AD, Alzheimer's disease; ADDL, amyloid beta derived diffusible ligands; ASPD, amylospheroids; ASR1, aggregate specific reagent 1; BH, brain homogenate; CJD, Creutzfeldt–Jakob disease; CNS, central nervous system; CSF, cerebrospinal fluid; LoD, limit of detection; MFP, misfolded protein; MPA, misfolded protein assay; rt, room temperature; WT, wild-type.

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