

Peptides for the detection of amyloid fibril aggregates

Field of the invention

The present invention relates to the field of medicine and molecular diagnostics. In particular
5 it relates to molecules that specifically bind amyloid fibril aggregates. The invention further relates to method for the detection of the presence of amyloid fibril aggregates in a sample or subject and to methods of treating disease caused by amyloid fibril aggregates.

Background of the invention

10 Protein aggregation into fibrils characterizes the development of neurodegenerative diseases, such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Huntington's Disease (HD), which remain incurable due to lack of mechanistic understanding. In these diseases, aggregated proteins are hypothesised to have a causative relation with the disease onset. Therefore, it is crucial to understand the aggregation process. Current diagnosis relies on
15 phenotypical behaviour, family history, and genetic tests, and sometimes techniques such as PET tracing and brain imaging, which are based on large accumulations of the aggregation protein forming amyloid fibrils or brain degeneration, which happens late in the disease progression ("2024 Alzheimer's disease facts and figures," 2016; Thal et al., 2013; Jack et al., 2024).

A range of techniques can detect amyloid fibrils post-mortem or in vitro. Electron
20 microscopy and negative staining are useful to assess the morphology of the formed fibrils, as well as the branching, width, and length (Shi et al., 2021; Sunde & Blake, 1997) Dyes, as Thioflavin T (ThT) and Congo Red, are qualitative methods used for the identification of amyloid structures in vitro and histology staining (Elghetany & Saleem, 1988; Howie et al., 2008; Vassar & Culling, 1959). Antibodies, large biologics of 150 KDa, are capable to recognise the specific protein forming the
25 aggregate, but are typically not able to pass the blood brain barrier (Ko et al.; Ksiezak-Reding et al., 1987). There is a strong medical need for diagnostic tracer drugs for early identification of pathological protein fibrils in patients, such as Tau tracers. Ultimately, detection of protein fibrils would be desirable in body fluids, including plasma, CSF or tears.

Seed Amplification Assays (SAAs) have been developed to detect fibrils in fluids and
30 amplify their signal (Vaneyck et al. 2023; Concha-Marambio et al.; 2023). These assays rely on the prion-like properties of amyloid fibrils, which can multiply in the presence of available monomers. One such assay is RT-Quic (Okuzumi et al. 2023, Atarashi et al. 2023). If fibrils are available, these will seed for the formation of more fibrils, after an increase in signal can be monitored. These assays are limited to determining the presence of fibrillar seeds, and they cannot determine what species
35 is causative of the propagative aggregation nor provide information on fibril length. Also, the selection of the monomer is biasing the results towards specific diseases.

Fibril tracers require the development of molecules that specifically bind fibrils. Tau accumulation is linked to a variety of Tauopathies, among which AD. Tracers aim to recognise these abnormal accumulations and facilitate diagnosis of these Tauopathies. In 2020, imaging agent
40 Tauvid ([¹⁸F]-flortaucipir) was approved by the FDA (Commissioner, 2020; Jie et al., 2021; Xia et

al., 2013). Tauvid is currently the only Tau tracer approved for diagnosing living patients, with results that can discriminate disease as well as stage of the disease (Bejanin et al., 2017; Iaccarino et al., 2021; Jack et al., 2019; Merz et al., 2023; Ossenkoppele et al., 2018; Ossenkoppele et al., 2016; Leuzy et al., 2020; Jie et al., 2021; Mohammadi et al., 2023). Tracers for other aggregating proteins are also in development. For Huntingtin, the protein that aggregates in HD, tracers have passed tests on ex vivo samples (Delva et al., 2022; Herrmann et al., 2021; Liu et al., 2021; Liu et al., 2020; Matlahov et al., 2022). So far, tracer development has focussed on the recognition of aggregates of one protein. Fibril tracers currently available are typically limited to bind one specific fibril type. It is of great importance to develop techniques that specifically recognize fibril structures and early aggregated species, but not monomers to allow (early) diagnosis and monitoring of disease progression in patients.

Recently, diagnosis strategies in neurodegenerative diseases have shifted to fluid biomarkers in CSF and blood ((Ferreira, Theriault et al. 2023, Gonzalez-Ortiz, Kac et al. 2023, Johnson, Suárez-Calvet et al. 2023, Dubbelman, Hendriksen et al. 2024, Tijms, Vromen et al. 2024)). Those offer advantages compared to PET tracers, since they can predict the disease at pre-symptomatic stages. Moreover, they are less expensive and less invasive, making them more accessible. In CSF it has been shown that length of fibrils provides an indication of disease progression (Nirmalra et al. 2023). Therefore, studying the amyloid fibril present in body fluids of a patient may provide valuable insight into the progression of a patient, which may also be used for monitoring the effects of new therapeutics.

Summary of the invention

In a first aspect, the invention relates to a polypeptide comprising
i) the amino acid sequence:

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P-W-W-X¹-X²-P-W-W-P-W-H-H-P-X³

wherein,

X¹ is R or K, preferably R;

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X² is R or K, preferably R;

X³ is H or W, preferably H; or

ii) the amino acid sequence:

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P-W-W-R-R-P-W-W-P-W-H-H-P-H; or

W-W-H-P-E-P-P-H-V-R-S-W-S-W-W-R-H-G-R-G-E-D

wherein the amino acid sequence comprises no more than 4, 3, 2, or 1 amino acid substitutions, deletions and insertions,

and wherein the amino acid sequence of i) and ii) bind to aggregates or oligomeric precursors of amyloid fibrils.

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In a second aspect, the invention relates to a nucleic acid sequence encoding the polypeptide as described herein.

In a third aspect, the invention relates to a pharmaceutical composition comprising the polypeptide as defined in any one of the preceding claims, optionally further comprising a pharmaceutical excipient.

In a fourth aspect, the invention relates to a method of diagnosing and/or monitoring the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils or a predisposition to develop a condition caused by the aggregation of amyloid fibrils in a subject.

In one embodiment, the invention relates to a method of diagnosing a pathological condition caused by the aggregation of amyloid fibrils or a predisposition to develop a condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:

- a) providing a sample from the subject;
 - b) contacting the sample with the polypeptide as described in herein that specifically binds to aggregated amyloid fibrils; and
 - c) detecting the presence of the aggregated amyloid fibrils bound to the polypeptide,
- wherein detection of the aggregated amyloid fibrils bound to the polypeptide is indicative of the presence of aggregated amyloid fibrils.

In another embodiment, the invention relates to a method of monitoring the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:

- a) providing a sample from the subject;
 - b) contacting the sample with the polypeptide as described in herein that specifically binds to aggregated amyloid fibrils; and
 - c) determining at least one of fibril length, hydrodynamic radius of the amyloid fibril bound to the polypeptide and its location,
- wherein the fibril length, its hydrodynamic radius and/or its location provide an indication of the progression or identifying the disease state.

In a fifth aspect, the invention relates to a polypeptide as described herein, the nucleic acid as described herein or the pharmaceutical composition as described herein for use as a medicament. Preferably for use as a medicament in the treatment of a pathological condition caused by the aggregation of amyloid fibrils.

In a sixth aspect, the invention provides for use of the polypeptide as described in herein, the nucleic acid as described in herein or the pharmaceutical composition as described in herein for the detection of aggregated amyloid fibrils.

Description of the invention

Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. One

skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the method.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

As used herein, the term "and/or" indicates that one or more of the stated cases may occur, alone or in combination with at least one of the stated cases, up to with all of the stated cases.

As used herein, with "At least" a particular value means that particular value or more. For example, "at least 2" is understood to be the same as "2 or more" i.e., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, ... ,etc.

The word "about" or "approximately" when used in association with a numerical value (e.g. about 10) preferably means that the value may be the given value (of 10) more or less 0.1% of the value.

The terms "subject" and "patient" are used interchangeably throughout the specification and describe an animal, human or non-human, to whom treatment, diagnosis or monitoring of disease progression according to the methods of the present invention is provided.

The terms "protein" or "polypeptide" are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3-dimensional structure or origin, negatively charged polypeptides are also included within this definition.

"Amino acid" refers to one of the twenty biologically occurring amino acids encoded by the universal genetic code and to synthetic amino acids, including D/L optical isomers.

A "disease" or a "pathology" is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein if the disease is not ameliorated then the subject's health continues to deteriorate. In contrast, a "disorder" in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the subject's state of health.

A disease or disorder is "alleviated" or "ameliorated" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced. This also includes halting progression of the disease or disorder. A disease or disorder is "cured" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is eliminated.

The inventors have surprisingly developed peptides that specifically recognises protein fibrils, which will be described herein as "fibril paint". The peptides are characterised by a large number of

nitrogen-carbon containing delocalised side chains, such as W, H and R. These residues are able to engage in pi-stacking interactions and aromatic interactions with fibrils. The peptides can fluorescently stain amyloid fibrils, but not the non-aggregated monomeric form, of Tau and Huntingtin, which differ in structure and sequence. These peptides allow the possibility to measure the size of aggregates and fibrils during the whole aggregation process, for both recombinant and patient-derived material. These peptides are particularly useful because they provide for a method of diagnosing but also for monitoring the progression the disease caused by the aggregation of amyloid fibrils. Advantageously, these peptides can also be used to treat pathologies caused by amyloid fibrils.

Accordingly in a first aspect, the invention provides for a polypeptide comprising
i) the amino acid sequence:

P-W-W-X¹-X²-P-W-W-P-W-H-H-P-X³ (SEQ ID NO: 1)

wherein,

X¹ is R or K. In certain preferred embodiments X¹ is R;

X² is R or K, preferably R. In certain preferred embodiments X² is R.

X³ is H or W, preferably H. In certain preferred embodiments X³ is H

or ii) a polypeptide comprising the amino acid sequence:

P-W-W-R-R-P-W-W-P-W-H-H-P-H (SEQ ID NO: 2) or

W-W-H-P-E-P-P-H-V-R-S-W-S-W-W-R-H-G-R-G-E-D (SEQ ID NO: 3)

wherein the amino acid sequence comprises no more than 4, 3, 2, or 1 amino acid substitutions, deletions and insertions,
and wherein the amino acid sequence of i) or ii) bind to aggregates or oligomeric precursors of amyloid fibrils.

In one embodiment, the polypeptide of the invention comprises or consists of the sequence selected from the group consisting of SEQ ID NO: 2, 3, 8, 9 and 10.

The affinity of these peptides is sufficiently strong that they are not significantly released from fibrils during a detection run, such as in a run of at least 1 hour or longer, in a microfluidics device, such as the FIDA1 instrument.

The terms amyloids or amyloid aggregates may denote according to embodiments of the invention amyloid peptides as well as amyloid proteins. The term shall encompass tau, IAPP amyloid-beta, alpha-synuclein, huntingtin- Tau proteins and/or alpha-synucleins and/or other fibril-forming proteins. According to embodiments amyloids or amyloid aggregates may encompass or may be defined as aggregates of proteins and/or peptides that constitute aberrant association of two or more protein molecules, having a spherical and/or fibrillary morphology or fibrillar structure and comprising in particular oligomers from 3 nm-15 nm in diameter and fibrils of 6 nm-15 nm in

diameter and a β -sheet secondary structure or in other words cross- β structure. Furthermore, fibrils may have an ability to be stained by particular dyes, such as Congo red.

The polypeptides of the invention may be "altered" and contain deletions, insertions, or substitutions of amino acid residues which result in a functionally equivalent. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine, glycine and alanine, asparagine and glutamine, serine and threonine, and phenylalanine and tyrosine.

The term 'substitution' or 'substituted' in this context mean that another amino acid is present on the indicated position than in the corresponding parent molecule, which here is a polypeptide comprising the amino acid sequence of P-W-W-R-R-P-W-W-P-W-H-H-P-H (SEQ ID NO: 2) or W-W-H-P-E-P-P-H-V-R-S-W-S-W-W-R-H-G-R-G-E-D (SEQ ID NO: 3). Such parent molecule may exist physically as polypeptide or in the form of nucleic acid encoding such polypeptide, but may also merely exist in silico or on paper as amino acid sequence or a corresponding nucleic acid sequence encoding the amino acid sequence. A substitution in this context is therefore also considered present for instance if a protein is expressed from a nucleic acid that has been synthesized such that it encodes the mutation or substitution, even though the nucleic acid encoding the corresponding parent molecule was not initially actually prepared during the process, e.g. when the nucleic acid molecule has been prepared entirely by chemical synthesis.

In some embodiments, the polypeptides of the invention may be chemically modified. For example, a polypeptide can be mutated to modify peptide properties such as detectability, stability, biodistribution, pharmacokinetics, half-life, surface charge, hydrophobicity, conjugation sites, pH, function, specificity, and the like. N-methylation is one example of methylation that can occur in a peptide of the disclosure. In some embodiments, a peptide may be modified by methylation on free amines such as by reductive methylation with formaldehyde and sodium cyanoborohydride.

In one embodiment, the polypeptide according to the invention comprises at least one detectable tag. A detectable tag or detectable label as used herein is intended to mean an individual measurable moiety, such as a radioisotope, fluorochrome, dye, or other moiety known in the art that is measurable by analytical methods. A detectable tag or detectable label can be attached to a polypeptide using methods well known in the art.

In one embodiment, the polypeptide as described herein comprises a second tag. In one embodiment, the second tag is a negatively charged amino acid sequence. In certain embodiments the second tag is a protein recognition system. In one example, the second tag is a protein-quality control system such as the E3-ligase CHIP, such as for example the negatively charged amino acid sequence comprises SEQ ID NO: 5.

The polypeptide as defined herein can be used as a warhead in a bifunctional (Proteolysis targeting chimera) PROTAC molecule. PROTACs, are small molecules that inhibit the function of their target proteins by targeting them for degradation by the ubiquitin proteasome system. The

design of PROTAC and their ability to the selective and immediate degradation of target protein have been well described in the art such as in (Winter, G. E. et al. DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for in vivo target protein degradation. Science 348, 1376-1381 (2015), Bondeson, D. P. et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat. Chem. Biol. 11, 611-617 (2015)) In preferred, embodiments, when used in a
 5 PROTAC molecule, the polypeptide as define herein comprises the negatively charged amino acid sequence comprises SEQ ID NO: 5 for recognition by CHIP.

The polypeptide as defined herein can also be modified for use in other forms of targeted protein degradation, by at least one of proteasomal degradation, autophagy and other degradation
 10 pathways (see Figure 12). Examples of such modifications can be made to the second tag, signaling for autophagy (called, AUTAC, ATTEC, AUTOTAC, LYTAC among others). Examples of such tags target p62/SQSTM1 for example.

In one embodiment, least one of the detectable tag and second tag is linked to the amino acid sequence or to the polypeptide through a flexible linker.

15 The term "linker" as used in accordance with the present invention relates to a sequence of amino acids (i.e. peptide linkers) as well as to non-peptide linkers. Peptide linkers as envisaged by the present invention are peptide or polypeptide linkers of at least 1 amino acid in length. Preferably, the linkers are 1 to 100 amino acids in length. More preferably, the linkers are 5 to 50 amino acids in length and even more preferably, the linkers are 10 to 20 amino acids in length. It is well known
 20 to the skilled person that the nature, i.e. the length and/or amino acid sequence of the linker may modify or enhance the stability and/or solubility of the molecule. Thus, the length and sequence of a linker depends on the composition of the respective portions of the fusion protein. In some embodiments, the linker is a flexible amino acid linker. As used herein the term "flexible linker" refers to peptide comprising at least a portion composed of flexible amino acid residues that allow adjacent
 25 modules to move relative to one another. In one embodiment the flexible linker is GSGS (SEQ ID NO: 4).

In one embodiment, the detectable tag and the second tag are linked at opposite ends of the polypeptide.

In one embodiment, the detectable tag is selected from the group consisting of a radionuclide,
 30 an isotope, an optical label, a magnetic material, an affinity tag and any combination thereof.

In certain embodiment, the detectable labels is a radionuclide selected from the group consisting of of ^{110}In , ^{111}In , ^{177}Lu , ^{118}F , ^{52}Fe , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{90}Y , ^{89}Zr , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, ^{120}I , ^{123}I , ^{124}I , ^{125}I , ^{131}I , $^{154-158}\text{Gd}$, ^{32}P , ^{11}C , ^{13}N , ^{15}O , ^{186}Re , ^{188}Re , ^{51}Mn , $^{52\text{m}}\text{Mn}$, ^{55}Co , ^{72}As , ^{75}Br , ^{76}Br , $^{82\text{m}}\text{Rb}$, ^{83}Sr , or other gamma-, beta-, or positron-emitters.

35 Without limitations, examples of a detectable tag can include optical reporters or optical labels. Suitable optical reporters or optical labels include, but are not limited to, fluorescent reporters and chemiluminescent groups.

A wide variety of fluorescent dyes are known in the art. Typically, the fluorophore is an aromatic or heteroaromatic compound and can be a pyrene, anthracene, naphthalene, acridine,
 40 stilbene, indole, benzindole, oxazole, thiazole, benzothiazole, cyanine, carbocyanine, salicylate,

anthranilate, coumarin, fluorescein, rhodamine or other like compound. Suitable fluorescent reporters include xanthene dyes, such as fluorescein or rhodamine dyes, including, but not limited to, Alexa Fluor® dyes (Invitrogen Corp.; Carlsbad, Calif), fluorescein, fluorescein isothiocyanate (FITC), Oregon Green™, rhodamine, Texas red, tetra-rhodamine isothiocyanate (TRITC), 5-carboxyfluorescein (FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), tetrachlorofluorescein (TET), 6-carboxyrhodamine (R6G), N,N,N,N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX). Suitable fluorescent reporters also include the naphthylamine dyes that have an amino group in the alpha or beta position. For example, naphthylamino compounds include 1-dimethylaminonaphthyl-5-sulfonate, 1-anilino-8-naphthalene sulfonate, 2-p-toluidinyl-6-naphthalene sulfonate, and 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS). Other fluorescent reporter dyes include coumarins, such as 3-phenyl-7-isocyanatocoumarin; acridines, such as 9-isothiocyanatoacridine and acridine orange; N-(p(2-benzoxazolyl)phenyl)maleimide; cyanines, such as Cy2, indodicarbocyanine 3 (Cy3), indodicarbocyanine 5 (Cy5), indodicarbocyanine 5.5 (Cy5.5), 3-(-carboxy-pentyl)-3'-ethyl-5,5'-dimethyloxacarbocyanine (CyA); 1H,5H,11H, 15H-Xantheno[2,3,4-ij:5,6,7-i'j']diquinolizin-18-ium, 9-[2(or 4)-[[[6-[2,5-dioxo-1-pyrrolidinyl]oxy]-6-oxohexyl]amino]sulfonyl]-4(or 2)-sulfophenyl]-2,3,6,7,12,13,16,17-octahydro-inner salt (TR or Texas Red); BODIPY™ dyes; benzoxadiazoles; stilbenes; pyrenes; and the like. Many suitable forms of these fluorescent compounds are available and can be used.

Examples of fluorescent proteins suitable for use as imaging agents include, but are not limited to, green fluorescent protein, red fluorescent protein (e.g., DsRed), yellow fluorescent protein, cyan fluorescent protein, blue fluorescent protein, and variants thereof (see, e.g., U.S. Pat. Nos. 6,403,374, 6,800,733, and 7,157,566). Specific examples of GFP variants include, but are not limited to, enhanced GFP (EGFP), destabilized EGFP, the GFP variants described in Doan et al, *Mol. Microbiol.*, 55:1767-1781 (2005), the GFP variant described in Cramer et al, *Nat. Biotechnol.*, 14:315319 (1996), the cerulean fluorescent proteins described in Rizzo et al, *Nat. Biotechnol.*, 22:445 (2004) and Tsien, *Annu. Rev. Biochem.*, 67:509 (1998), and the yellow fluorescent protein described in Nagai et al, *Nat. Biotechnol.*, 20:87-90 (2002). DsRed variants are described in, e.g., Shaner et al, *Nat. Biotechnol.*, 22:1567-1572 (2004), and include mStrawberry, mCherry, morange, mBanana, mHoneydew, and mTangerine. Additional DsRed variants are described in, e.g., Wang et al, *Proc. Natl. Acad. Sci. U.S.A.*, 101:16745-16749 (2004) and include mRaspberry and mPlum. Further examples of DsRed variants include mRFPmars described in Fischer et al, *FEBS Lett.*, 577:227-232 (2004) and mRFPruby described in Fischer et al, *FEBS Lett.*, 580:2495-2502 (2006).

Very small particles, termed nanoparticles, also can be used as detectable tags to the polypeptides as described herein. These particles range from 1-1000 nm in size and include diverse chemical structures such as gold and silver particles and quantum dots.

Another type of nanoparticle that can be used as a detectable tags are quantum dots. Quantum dots are fluorescing crystals 1-5 nm in diameter that are excitable by a large range of wavelengths of light. These crystals emit light, such as monochromatic light, with a wavelength

dependent on their chemical composition and size. Quantum dots such as CdSe, ZnSe, InP, or InAs possess unique optical properties.

In certain embodiments, the magnetic material is selected from the group consisting of nanocomposite-containing nanoparticles, superparamagnetic iron oxide nanoparticles, and any
5 combination thereof.

In certain embodiments, the affinity tag is selected from the group consisting of: an epitope, streptavidin, or avidin binding peptide, biotin and an oligohistidine sequence.

In a second aspect the invention provides for a nucleic acid sequence encoding the polypeptide as described herein.

10 In a third aspect, the invention provides for a pharmaceutical composition comprising the polypeptide as described herein. In some embodiments the pharmaceutical composition further comprising a pharmaceutical excipient.

The pharmaceutically acceptable excipient such as an adjuvant, or vehicle, is for administration of the polypeptide to a subject. Said pharmaceutical composition can be used in the
15 methods of treatment described herein below by administration of an effective amount of the composition to a subject in need thereof.

The term "pharmaceutically acceptable excipient", as used herein, is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (see e.g.
20 "Handbook of Pharmaceutical Excipients", Rowe et al eds. 7th edition, 2012, www.pharmpress.com). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include
25 buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins,
30 such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter ions such as sodium; metal complexes (e.g. Zn²⁺-protein complexes); and/or
35 non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

In a fourth aspect, the invention relates to a method of diagnosing and/or monitoring the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils or a predisposition to develop a condition caused by the aggregation of amyloid fibrils in a subject.

In one embodiment, the invention relates to a method of diagnosing a pathological condition caused by the aggregation of amyloid fibrils or a predisposition to develop a condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:

- a) providing a sample from the subject;
 - 5 b) contacting the sample with the polypeptide as described in herein that specifically binds to aggregated amyloid fibrils; and
 - c) detecting the presence of the aggregated amyloid fibrils bound to the polypeptide,
- wherein detection of the aggregated amyloid fibrils bound to the polypeptide is indicative of the presence of aggregated amyloid fibrils.

10 In another embodiment, the invention relates to a method of monitoring the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:

- a) providing a sample from the subject;
- b) contacting the sample with the polypeptide as described in herein that specifically binds to
- 15 aggregated amyloid fibrils; and
- c) determining at least one of fibril length, hydrodynamic radius of the amyloid fibril bound to the polypeptide and its location,

wherein the fibril length, its hydrodynamic radius and/or its location provide an indication of the progression or identifying the disease state.

20 "Diagnosis" as used herein generally includes determination as to whether a subject is likely affected by a given disease, disorder or dysfunction. The skilled artisan often makes a diagnosis on the basis of one or more diagnostic indicators, i.e., a biomarker, the presence, absence, frequency, or amount of which is indicative of the presence or absence of the disease, disorder or dysfunction.

The subject can be symptomatic (e.g., the subject presents symptoms associated with

25 Tauopathies (e.g., AD, AGD, CBD, PiD, PSP), such as, for example changes in personality, behavior, sleep patterns, and executive function, memory loss, confusion, inability to learn new things, difficulty carrying out multistep tasks, problems coping with new situations, hallucinations, delusions, and paranoia, impulsive behavior, inability to communicate, weight loss, seizures, skin infections, difficulty swallowing, groaning, moaning, grunting, increased sleeping, lack of control of

30 bowel and bladder, disorders of word finding, disorders of reading and writing, disorientation, supranuclear palsy, a wide-eyed appearance, difficulty in swallowing, unwarranted anxiety, irrational fears, oniomania, impaired regulation of social conduct (e.g., breaches of etiquette, vulgar language, tactlessness, disinhibition, misperception), passivity, low motivation (aboulia), inertia, over-activity, pacing and wandering, etc. The subject can be asymptomatic (e.g., the subject does

35 not present symptoms associated with a Tauopathy, or the symptoms have not been recognized).

In some embodiments the subject is a prodromal patient a "prodromal dementia patient" is a person who does not suffer from a senile dementia as defined above, but has an increased likelihood to develop senile dementia. Likewise a "prodromal Alzheimer patient" is a person who does not suffer from AD, but has an increased likelihood to develop AD.

40 In some embodiments, the subject has preclinical dementia.

In one embodiment, the detected aggregate is a pathological aggregate. The term "pathological aggregates" refers to amyloid and non-amyloid aggregates. Amyloid aggregates can be further distinguished between amyloid fibrils and amyloid oligomers. In a preferred embodiment the pathological aggregates are intracellular aggregates. It is known that many diseases associated with pathological aggregates that are catalogued as forming extracellular pathological aggregates also form intracellular aggregates. Indeed, IAPP and amyloid-beta have intracellular and extracellular aggregates.

As used herein, "detecting", "detection", "determining", and the like are understood to refer to an assay performed for identification of pathological aggregates. Assays to detect such protein complexes are well known in the art and are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological or enzymatic tags or labels of standard use in the art. Examples of methods used to measure fluorescence intensity for each molecule in a sample solution include fluorescence correlation spectroscopy (FCS) and fluorescence intensity distribution analysis (FIDA). The "detection limits" are understood to be the lowest concentration of pathological aggregates, required for presence detection and are determined by the binding constant for the indicator-ligand interaction, the limit of detection for the indicator (typically in the picomolar range for many fluorophores using the current instrumentation), the difference in diffusivity between the unbound indicator and the indicator-analyte complex, and the standard deviation for determining the peak variance. In cases of a strong selective interaction, it should be feasible to achieve sensitivities in the sub-nano to picomolar range, in a FIDA method, as an example.

In one preferred embodiment, the detection method used in the method of the invention is by FIDA which detects fluctuations in fluorescence intensity of a molecule present in the focused region of an optical detector. Fitting the data reveals the averaged hydrodynamic radius of the particle as a measure of the size. In addition, detection of individual spikes passing the detector allows conclusions about the number of fibrils in the sample.

In yet a further aspect, the invention provided for a method for imaging the presence of pathological aggregates. With such a method a reliable test to confirm the presence or absence of nanostructured sized amyloid aggregates may be established. The method for imaging comprises:

- a) providing a sample from the subject;
- b) contacting the sample with the polypeptide as described herein that specifically binds to aggregated amyloid fibrils; and
- c) detecting the presence of the aggregated amyloid fibrils bound to the polypeptide; and,
- d) optionally, determining at least one of the state, conformation, length, concentration of the amyloid fibrils and proteins within the amyloid fibrils,

wherein detection of the aggregated amyloid fibrils bound to the polypeptide is indicative of the presence of aggregated amyloid fibrils, and wherein of the state, conformation, length, concentration of the amyloid fibrils and proteins within the amyloid fibrils provide an indication of the progression or identifying the disease state.

In one preferred embodiment, the polypeptide as described herein that specifically binds to aggregated amyloid fibrils, detects the presence of the aggregated amyloid fibrils at a concentration of fibrils that is less than 10 nM, 5, nM, 2 nM, 1 nM, 500 pM, 400 pM or 200 pM, wherein preferably, the aggregated amyloid fibrils are detected a FIDA method, preferably in (human) serum.

5 In some embodiments, the sample is a "biologic sample". As used herein, the term "biological sample" or "sample" refers to a sample obtained or derived from a subject. By way of example, the sample may be selected from the group consisting of body fluids, blood, whole blood, plasma, serum, mucus secretions, tears, urine or saliva. In some embodiments the sample is, or comprises a blood sample. In some embodiments, the sample is collected from the brain of a subject, e.g.,
10 brain tissue or pancreatic tissue. In some embodiments the biological source for detection of the biomarkers is a blood sample, a serum sample or a plasma sample. In some embodiments, the sample is cerebrospinal fluid (CSF) or urine. In some embodiments, the sample is pancreatic tissue or brain tissue.

In some embodiments, the sample is collected from a biopsy. A biopsy is a sample of tissue
15 taken from the body of a living subject. A biopsy sometimes also refers to the medical procedure that removes tissue from a living subject. In some embodiments, the sample can be collected through a punch biopsy. A punch biopsy is done with a circular blade ranging in size from 1 mm to 8 mm. In some embodiments, the sample can be collected from fine-needle aspiration biopsy (FNAB or FNA). Fine-needle aspiration biopsy is a procedure used to investigate superficial (just
20 under the skin) lumps or masses. In some embodiments, a thin, hollow needle is inserted into the body to collect samples.

In some embodiments, the sample is from a live subject. For example, the sample can be collected from a subject during a medical procedure, e.g., a surgery.

In some embodiments, samples are collected from post-mortem specimens, e.g., human
25 post-mortem brain specimens.

In one embodiment, the detected amyloid aggregates is from an amyloid forming protein such as Tau, IAPP amyloid-beta, alpha-synuclein, huntingtin- Tau proteins and/or alpha-synucleins. More examples of proteins forming pathological aggregates (particularly amyloid oligomers and amyloid fibrils) and associated pathologies, are outlined in Table 1 on pages 32, 33 and 34 of Chiti
30 F and Dobson C M (2017) Annu. Rev. Biochem. 86: 27-68).

In a further aspect, the invention provides for the polypeptide, the nucleic acid or the pharmaceutical composition as described herein for use as a medicament.

In a further aspect, the invention provides for a method of treating a pathological conditions caused by the aggregation of amyloid fibrils in a subject in need thereof, the method comprising the
35 step of administering the polypeptide, the nucleic acid or the pharmaceutical composition as described herein to the subject.

As used herein, "treating a pathological conditions caused by the aggregation of amyloid fibrils" means reducing the frequency or severity of at least one sign or symptom of a disease or disorder that occurs due to the aggregation of amyloid fibrils. "Treat," "treating" or "treatment" are
40 each used interchangeably herein to describe reversing, alleviating, or inhibiting the progress of a

disease and/or injury, or one or more symptoms of such disease, to which such term applies. Depending on the condition of the subject, the term also refers to preventing a disease, and includes preventing the onset of a disease, or preventing the symptoms associated with a disease. A treatment may be either performed in an acute or chronic way. The term also refers to reducing the severity of a disease or symptoms associated with such disease prior to affliction with the disease. Such prevention or reduction of the severity of a disease prior to affliction refers to administration of a pharmaceutical composition to a subject that is not at the time of administration afflicted with the disease. "Preventing" also refers to preventing the recurrence of a disease or of one or more symptoms associated with such disease. "Treatment" and "therapeutically," refer to the act of treating, as "treating" is defined above.

In one embodiment, the polypeptide, the nucleic acid or the pharmaceutical composition as described herein is for use as a medicament in the treatment of a pathological condition caused by the aggregation of amyloid fibrils. In one embodiments, the polypeptide for use as a medicament or for use in a methods of treatment as described herein comprises a negatively charged second tagged as described herein, such as for example the negatively charged amino acid sequence comprises SEQ ID NO: 5.

In one embodiment, the polypeptide for use as a medicament or for use in a methods of treatment as described herein is used in as a warhead in a bifunctional (Proteolysis targeting chimera) PROTAC molecule. In preferred, embodiments, when used in a PROTAC molecule, the polypeptide as define herein comprises the negatively charged amino acid sequence comprises SEQ ID NO: 5 for recognition by CHIP.

In one embodiment, the pathological condition is an amyloidosis such as systemic lysozyme, insulin, hemodialysis amyloidosis, or a neurodegenerative disease, including Tauopathies such as Alzheimer disease (AD), Corticobasal degeneration (CBD) and Pick's disease (PiD), and Synucleinopathies such as Parkinson disease (PD), and Lewy Body disease, or other neurodegenerative diseases such as prion diseases, Argyrophilic grain disease (AGD), Progressive supranuclear palsy (PSP), Huntington's Disease (HD) and Amyotrophic lateral sclerosis (ALS).

In yet a further aspect, the invention provides for use of the polypeptide, the nucleic acid or the pharmaceutical composition as described herein for the detection of aggregated amyloid fibrils. In certain embodiments, the use as described herein is for the staining of tissue samples such as for example postmortem tissue.

In yet a further aspect, the invention provides for the use of the polypeptide or the nucleic acid as described herein for inhibiting the aggregation of amyloid fibrils. The inhibition of aggregation may occur in vitro, for example in in vitro assays or in vivo as part as the treatment as described herein.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The present invention is further described by the following examples which should not be construed as limiting the scope of the invention.

Description of the figures

Figure 1: *FibrilPaint1 is a specific label for protein fibrils.* Inhibition of TauRD (A-D) and HttEx1Q44 (E-H) aggregation by FibrilPaint1 (A, E), FibrilPaint2 (B, F), FibrilPaint3 (C, G) and FibrilPaint4 (D, H). B) All peptides lower the end-plateau in a dose-dependent manner for both TauRD and HttEx1Q44 aggregation. Binding of fibril paint to monomeric or fibrillar TauRD (I) or HttEx1Q44 (J). Samples containing fibrils significantly increase Rh, corresponding to the fibril size.

Figure 2: *FibrilPaint1 is specific for amyloid aggregates.* A) Binding of 0.2 μM FibrilPaint1 to TauRD fibrils in presence of 50% cell lysate. In presence of cell lysate, FibrilPaint1 has a hydrodynamic radius Rh of 1.7 nm, incubated with 4h-TauRD fibrils has an average Rh of 14 nm. B) No binding of 0.2 μM FibrilPaint1 to amorphous aggregated Luciferase by heat shock. FibrilPaint1 has a size of 1.7 nm, incubated with heat shock luciferase the size remains the same.

Figure 3: *Aggregation of TauRD and HttEx1Q44 over time.* Hydrodynamic radius Rh of aggregates as determined with FIDA for TauRD (A) and HttEx1Q44 (D). In parallel experiments, aggregation was monitored with a ThT assay (B, E) for TauRD and HttEx1Q44. The end-product of TauRD was pictured with negative stain EM after 24 h (C) and for HttEx1Q44 after 6 h (F).

Figure 4: *Fibril inhibition over time by FibrilPaint.* Aggregation of TauRD (A) and HttEx1Q44 (B) in presence or absence of 2 μM FibrilPaint1. In presence of FibrilPaint1, Rh remains 3.5 nm for TauRD (A) and 28 nm for HttEx1Q44 (B). Both datasets are representative experiments for three independent performed experiments. The end product of TauRD with and without FibrilPaint1 was imaged by negative stain EM after 24 h (C), and after 6 h for HttEx1Q44 (D).

Figure 5: *Measurement of patient-derived material from various Tauopathies.* A) Hydrodynamic radius of fibrils extracted from patients diagnosed with Alzheimer's Diseases (AD), Frontotemporal Dementia (FTD) and Corticobasal Degeneration (CBD), measured with FibrilPaint1. B) Structural representation of the Tau fibrils of in different tauopathies, and recombinant Tau fibrils for comparison. The images were generated in PyMol software based on the coordinates of the cryo-EM structures. Functional groups are coloured according to the YRB script. PDB codes: AD paired helical filament, 5O3L; AD straight filament, 5O3T; FTD narrow filament, 6GX5; CBD type I 6TJO; CBD type II 6VH7; Snake recombinant Tau, 6QJH; twister recombinant Tau, 6QJM; Jagged recombinant Tau 6QJP. C) Negative stain EM of patient-derived fibrils from AD, CBD and FTD (FLTR). D) Example of AD fibrils designated in two groups: PHFs and fibrils that could not be clearly recognised as such. E) Structural representation of AD paired helical filament consisting of 342 layers (left). The image was generated in PyMol software base of coordinates of cryo-EM structures. Functional groups are coloured according to YRB script. F) Hydrodynamic radius prediction of different layers of AD paired helical filament (left) using FIDABio hydrodynamic radius prediction tool (right). Lines in prediction indicate the found average HR for AD fibrils, resembling 1100 layers.

Figure 6: *FibrilPaint1 is a specific label for protein fibrils.* Binding of FibrilPaint to fibrils of A) α -syn and A β (FibrilPaint peptide only, blue; α -syn fibrils light green; A β fibrils light red) and B) IAPP fibrils.

- 5 Figure 7: *FibrilPaint1 is specific for amyloid aggregates.* (A) Binding of 0.2 μ M FibrilPaint1 to TauRD fibrils in buffer or 50% cell lysate. In buffer, FibrilPaint1 has a size of 1.7 nm, incubated with TauRD fibrils has an average size of 54 nm. In 50% cell lysate, FibrilPaint1 has a size of 1.7 nm, incubated with TauRD fibrils has an average size of 39 nm. (B) Binding of 0.2 μ M FibrilPaint1 to TauRD fibrils in buffer or 50% human serum. In presence of buffer, FibrilPaint1 has a size of 1.7 nm, incubated with TauRD fibrils has an average size of 52 nm. In 50% human serum, FibrilPaint1 has a size of 1.7 nm, incubated with TauRD fibrils has an average size of 54 nm. (FibrilPaint1 in buffer, dark blue; folded luciferase, dark green; heat-soaked luciferase; light green; TauRD fibrils in buffer, light blue; FibrilPaint1 in cell lysate, dark purple; TauRD fibrils in cell lysate, light purple; FibrilPaint1 in human serum, light yellow; TauRD fibrils in human plasma, dark yellow). C) FibrilPaint1 binds fibrils in Htt brain supernatant.

- Figure 8: *FibrilPaint1 binds to amyloid fibrils and the E3 ligase CHIP.* FibrilPaint1 is designed as a PROTAC: the C-terminal side of the peptide binds to amyloid fibrils (A) whereas the EEVD motif binds to ubiquitin E3-ligase CHIP (B) schematic of FibrilPaint1 with its C-terminal amino acids targeting amyloid fibrils, and its EEVD motif recruiting the E3 ligase CHIP, after which a complex is formed and the ubiquitin is transferred to the amyloid fibril. (C). FibrilPaint1 binds to TauRD fibrils, but not to monomers. C) HttEx1Q44. FibrilPaint1 binds to CHIP with an affinity of 3 μ M. Without the EEVD motif, FP1 cannot bind CHIP.

- 25 Figure 9: *FibrilPaint1 acts as a PROTAC for the ubiquitination of fibrils.* Ubiquitination of TauRD (A), HttEX1Q44 (B), α -synuclein (C), and Amyloid β (D) in lilac in buffer, in purple after addition of urea. Without ATP in yellow, after which the size remains the size of ubiquitin only.

- Figure 10: FibrilPaint1 ubiquitinates patient-derived fibrils.

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Figure 11: FibrilPaint peptides binding to fibrils. FibrilPaint binding to TauRD (A) or HttEx1Q44 (B) fibrils. FibrilPaint peptide alone are shown in dark grey, in the presence of TauRD in light grey, in the presence of HttEx1Q44 in light grey (FP8: not determined). Bound species show an increased hydrodynamic radius, corresponding to the radius of the bound fibril.

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Figure 12: Schematic of the alterations of the FibrilPaint tags which may lead to targeted protein degradation of fibrils. Tags may be altered to mediate ubiquitination or other modifications of fibrils signaling for protein degradation, either thorough proteasomal degradation, autophagy or other cellular systems. Tags may also be altered to signal directly for the recruitment of degradation systems.

40

Table 1: Sequences

Name	Sequence	SEQ ID NO:
FibrilPaint peptide	P-W-W-X ¹ -X ² -P-W-W-P-W-H-H-P-X ³	1
FibrilPaint peptide	P-W-W-R-R-P-W-W-P-W-H-H-P-H	2
FibrilPaint peptide	W-W-H-P-E-P-P-H-V-R-S-W-S-W-W-R-H-G-R-G-E-D	3
Linker	GSGS	4
Neg. amino acid seq	EEVD	5
FLAG-tagged human Tau-RD	MGHHHHHHGSDSEVNQEAKPEVKPEVKPETHINL KVSDGSSEIFFKIKKTTPLRRLMEAFKRQGKEMD SLRFLYDGIQADQTPEDLDMEDNDIIEAHREQIG G	6
Tagged -HttEx1Q44	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKV TVEHPDKLEEKFPQVAATGDGPDIIIFWAHDRFGGY AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAY PIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKG KSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDI KDVGVNAGAKAGLTFLVDLIKHKHMNADTDYSIA EAAFNKGETAMTINGPWAWSNIDTSKVNYPVTVLP TFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYL LTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAT MENAQKGEIMPNIQMSAFWYAVRTAVINAASGR QTVDAALAAQAQTNAAAASEFSSNNNNNNNNNNLGI EGRMATLEKLMKAFESLSFQQQQQQQQQQQQQQ QQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ QPPPPPPPPPPQLPQPPPPQAQPLLPQPQPPPP PPPPPGPAVAEEPLHRPSGSHHHHHH	7
FibrilPaint1	PWWRRPWWPWHPH-GSGS-EEVD	8
FibrilPaint5	PWWRRPWWPWHPHR	9
FibrilPaint7	PWWRRPWWPWHPH-GSGS-RRVD	10
FibrilPaint2	PWIRRPWWPWTTPT-GSGS-EEVD	11
FibrilPaint3	PWWDDPWWPWTTPT-GSGS-EEVD	12
FibrilPaint4	PWWDDPWWPWHPH-GSGS-EEVD	13
FibrilPaint9	PWIRRPWWPWTTPTR	14
FibrilPaint10	PWWDDPWWPWTTPTD	15
FibrilPaint11	PWWDDPWWPWHPHD	16
FibrilPaint12	pwwrrpwwpwhhphr-GSGS-EEVD	17
FibrilPaint13	pWWrrpWWpWhhphr-GSGS-EEVD	18

Examples**Methods and Materials***Expression and purification of Tau and Huntingtin*

We produced N-terminally FLAG-tagged human Tau-RD (Q244-E372, with pro-aggregation mutation Δ K280) in *E. Coli* BL21 Rosetta 2 (Novagen), with an additional removable N-terminal His₆-Smt-tag (MGHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQ GKEMDSLRFlydGIRIQADQTPEDLDMEDNDIIEAHREQIGG – SEQ ID NO: 6). Expression was induced at OD₆₀₀ 0.8 by addition of 0.15 mM IPTG and incubation at 18 °C overnight. Cells were harvested by centrifugation, resuspended in 25 mM HEPES-KOH pH=8.5, 50 mM KCl, flash frozen in liquid nitrogen, and kept at – 80 °C until further usage. Pellets were thawed at 37°C, followed by the addition of ½ tablet/50 ml EDTA-free Protease Inhibitor and 5 mM β -mercaptoethanol. Cells were disrupted using an EmulsiFlex-C5 cell disruptor, and lysate was cleared by centrifugation. Supernatant was filtered using a 0.22 μ m polypropylene filtered and purified with an ÄKTA purifier chromatography System. Sample was loaded into a POROS 20MC affinity purification column with 50 mM HEPES-KOH pH 8.5, 50 mM KCl, eluted with a linear gradient 0-100%, 5 CV of 0.5 M imidazole. Fractions of interest were collected, concentrated to 2.5 ml using a buffer concentration column (vivaspin, MWCO 10 KDa), and desalted using PD-10 desalting column to HEPES pH 8.5, ½ tablet/50 ml Complete protease inhibitor, 5 mM β -mercaptoethanol. The His₆-Smt-tag was removed by treating the sample with Ulp1, 4 °C, shaking, overnight. The next day, sample was loaded into POROS 20HS column with HEPES pH 8.5, eluted with 0-100% linear gradient, 12 CV of 1M KCl. Fractions of interest were collected and loaded into a Superdex 26/60, 200 pg size exclusion column with 25 mM HEPES-KOH pH 7.5, 75 mM NaCl, 75 mM KCl. Fractions of interest were concentrated using a concentrator (vivaspin, MWCO 5 KDa) to desired concentration. Protein concentration was measured using a Nanodrop UV/Vis spectrophotometer and purity was assessed by SDS-PAGE. Protein was aliquoted and stored at – 80 °C.

Expression and purification of HttEx1Q44.

We produced HttEx1Q44 with in *E. Coli* BL21 Rosetta 2 (Novagen), with an additional N-terminal MBP- and C-terminal His₆-tag (MKIEEGKLVINGDKGYNGLAEVGGKFEKDTGKVTVEHPDKLEEKFPQVAATGDGPDIIFFWAH DRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWE EIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDNAGAKAGLTFL VDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVG VLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKKDKPLGAVALKSYYYEELAKDPRIAATMENAQK GEIMPNIPQMSAFWYAVRTAVINAASGRQTVDAALAAQTNAAAASEFSSNNNNNNNNNNNLGIE GRMATLEKLMKAFESLKSFQQ QQQQQPPPPPPPPPPPPQLPQPPPPQAQPLLPQPPPPPPPPPPPPGPAVAEEPLHRPSGSHHHH HH – SEQ ID NO: 7). Expression was induced at OD₆₀₀ 0.8 by addition of 0.15 mM IPTG and incubation at 18 °C overnight. Cells were harvested by centrifugation, resuspended in 50 mM HEPES-KOH pH=8.5, 50 mM KCl, flash frozen in liquid nitrogen, and kept at – 80 °C until further

usage. Pellets were thawed at 37°C, followed by the addition of ½ tablet/50 ml EDTA-free Protease Inhibitor and 5 mM β-mercaptoethanol. Cells were disrupted using an EmulsiFlex-C5 cell disruptor, and lysate was cleared by centrifugation. Supernatant was filtered using a 0.22 μm polypropylene filtered and purified with an ÄKTA purifier chromatography System. Sample was loaded into a
5 POROS 20MC affinity purification column with 50 mM HEPES-KOH pH 8.5, 100 mM KCl, eluted with a linear gradient 0-100%, 10 CV of 0.5 M imidazole, 30 mM NaCl. Fractions of interest were collected and concentrated to desired concentration using concentrating column (vivaspin, MWCO 30 KDa). Protein concentration was measured using a Nanodrop UV/Vis spectrophotometer and purity was assessed by SDS-PAGE. Protein was aliquoted and stored at – 80 °C.

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Expression and purification of α-synuclein

Human α-synuclein (α-Syn) wild-type was produced recombinantly as described previously (Stöckl et al., 2011). Briefly, protein was produced using the pT7 7 expression system in E. coli BL21 (DE3) cells. After IPTG induction, cells were harvested by centrifugation and resuspended in
15 10 mM Tris-HCl, PH 8.0, 1 mM EDTA and 1 mM Pefabloc protease inhibitor cocktail. Cells were disrupted by sonication and lysate was cleared by centrifugation. Nucleotides were removed by addition of 1% w/v streptomycin sulphate followed by centrifugation. α-Syn was precipitated by 0.3 g/ml ammonium sulfate and subsequent centrifugation. The pellet was dissolved in 10 mM Tris-HCl, pH 7.4, 1 mM Pefabloc and filtered through a 0.2 μm membrane. Sample was loaded onto a
20 Resource Q anion exchange column (GE) coupled to an ÄKTA purifier chromatography system. Sample was eluted with a salt gradient from 0-1 M. Fractions of interest were collected and dialyzed against 10 mM HEPES, 50 mM NaCl, pH 7.4. Concentration was measured using NanoDrop UV/Vis spectrophotometer and purity was assessed by SDS-PAGE. Purified protein was aliquoted, flash-frozen with liquid nitrogen and stored in 10 mM TRIS pH 7.4 at -80 °C until further use.

25

Amyloid-β (1-42) monomer and fibril preparation

Amyloid-beta peptide 1-42 (Aβ42) was purchased from Sigma-Aldrich (A9810). Monomers were solubilized in PBS pH 7.4, according to established protocols (Vandersteen et al., 2012). Aliquots of 10 μM peptide were stored at -20 °C. Monomers were freshly thawed prior to
30 experiments.

Aβ42 fibrils were prepared by quiescent incubation at 37 °C for 20 hours in presence of 0.02 w/v% NaN3. After 4 hours at room temperature (21 °C), we verified the presence of Aβ42 aggregates using AFM. AFM samples were prepared by adsorbing 10 μl of 5 times diluted sample onto mica (Muscovite mica, V 1 quality, EMS) for 45 minutes, after which the mica sheets were carefully
35 washed with milliQ 4 times, covered and left to dry overnight. The Aβ42 fibril samples were stored at room temperature (21 °C).

IAPP preparation and aggregation

Human Islet amyloid polypeptide (hIAPP) (Amylin (human) trifluoroacetate salt, product no.
40 4030200) was purchased from Bachem. Peptides were monomerized by dissolving in hexafluoro-

2-propanol at a final concentration of 1 mg/ml. The solution was incubated for 15 minutes at room temperature with occasional agitation. Monomerized hIAPP was aliquoted in glass vials containing 50 µg of peptide per vial. The solvent was evaporated by gently blowing a stream of nitrogen gas while swirling the vial around to generate a peptide film around the glass vial. The aliquots with dry peptide film were stored at -20°C. Prior to use, peptide vials were allowed to reach room temperature before an aliquot of ice-cold aggregation buffer of 20 mM ammonium acetate buffer, pH6.8, filtered through a 0.2-µm filter immediately before use, was added to reach stock concentrations of around 100 µM. The stock concentration was estimated by absorbance at 280 nm using the calculated molar extinction coefficient of 1,615 M⁻¹ cm⁻¹. To aggregate hIAPP, the concentration was adjusted to 10 µM, 30 µM or 50 µM, by diluting it in aggregation buffer. The samples were incubated quiescently at 25°C for 24 hours.

Peptide synthesis and purification

The peptides were synthesized using a Liberty Blue Microwave-Assisted Peptide Synthesizer (CEM) with standard Fmoc chemistry and Oxyma/DIC as coupling reagents. The peptide concentrations were measured by UV spectroscopy. The peptides were labeled with 5(6)-carboxyfluorescein at their N' termini. The peptides were cleaved from the resin with a mixture of 95% (v/v) trifluoroacetic acid (TFA), 2.5% (v/v) triisopropylsilane (TIS), 2.5% (v/v) triple distilled water (TDW) agitating vigorously for 3 hours at room temperature. The volume was decreased by N₂ flux and the peptides precipitated by addition of 4 volumes of diethylether at -20 °C. The peptides were sedimented at -20 °C for 30 minutes, then centrifuged and the diethylether discarded. The peptides were washed three times with diethylether and dried by gentle N₂ flux. The solid was dissolved in 1:2 volume ratio of acetonitrile ACN:TDW, frozen in liquid Nitrogen and lyophilized. The peptides were purified on a WATERS HPLC using a reverse-phase C18 preparative column with a gradient of ACN/TDW. The identity and purity of the peptides was verified by ESI mass spectrometry and Merck Hitachi analytical HPLC using a reverse-phase C8 analytical column.

Fibril extraction

Brain material of FTD and CBD were obtained from the Dutch Brain Bank, project number 1369. Brain material for AD was donated by prof. J. J. M. Hoozemans from the VU Medical Centra Amsterdam.

PHFs and SFs were extracted from grey matter of prefrontal cortex from patients diagnosed with AD. Tissue was homogenized using a Polytron(PT 2500E, Kinematica AG) on max speed in 20 % (w/v) A68 buffer, consisting of 20 mM TRIS-HCl pH 7.4, 10 mM EDTA, 1.6 M NaCl, 10% sucrose, 1 tablet/10 ml Pierce protease inhibitor, 1 tablet/10 ml phosphatase inhibitor. The homogenized sample was spun from 20 minutes, at 14000 rpm at 4 °C. Supernatant was collected, and the pellet was homogenized in 10% (w/v) A68 buffer. The homogenized was spun once more. The supernatants of both centrifugations were combined and supplied with 10% w/v Sarkosyl, and incubated for 1 hour on a rocker at room temperature. The sample was ultracentrifuged for 1 hour at 100000xg and 4 °C. The supernatant was discarded, and the pellet was incubated overnight at

4 °C in 20 µl/0.2 g starting material of 50 mM TRIS-HCl pH 7.4. The next day, the pellet was diluted up to 1 ml of A68 buffer, and resuspended. To get rid of contamination, the sample was spun for 30 minutes at 14000 rpm, 4 °C. Supernatant was collected and spun once more for 1 hour at 100000xg, 4 °C. The pellet was resuspended in 30 µl 25 mM HEPES-KOH pH 7.4, 75 mM KCl, 75 mM NaCl, and stored at 4 °C up to a month.

Narrow filaments were extracted from the grey matter of the middle frontal gyrus from patients diagnosed with FTD. Fibrils were extracted following the protocol for AD fibrils. After the first ultracentrifugation step, the pellet was resuspended in 250 µl/1g of starting material of 50 mM Tris pH 7.5, 150 mM NaCl, 0.02% amphipol A8-35. The sample was centrifuged for 30 minutes at 3000xg and 4 °C. Pellet was discarded, and the supernatant was ultracentrifuged for 1 hour at 100000xg and 4 °C. The pellet was resuspended in 30 µl of 50 mM TRIS-HCl pH 7.4, 150 mM NaCl, and stored at 4 °C up to a month.

CBD fibrils were extracted from the grey matter of the superior parietal gyrus of patients diagnosed with CBD. Tissue was homogenized using a Polytron (PT 2500E, Kinematica AG) on max speed in 20 % w/v 10 mM TRIS-HCl pH 7.5, 1 mM EGTA, 0.8 M NaCl, 10% sucrose. The homogenate was supplied with 2% w/v of sarkosyl and incubated for 20 minutes at 37 °C. The sample was centrifuged for 10 minutes at 20000xg, and 25 °C. The supernatant was ultracentrifuged for 20 minutes at 100000xg and 25 °C. The pellet was resuspended in 750 µl/1g starting material of 10 mM TRIS-HCl pH 7.5, 1 mM EGTA, 0.8 M NaCl, 10% sucrose, and centrifuged at 9800xg for 20 minutes. The supernatant was ultracentrifuged for 1 hour at 100000xg. The pellet was resuspended in 25 µl/g starting material of 20 mM TRIS-HCl pH 7.4, 100 mM NaCl, and stored at 4 °C up to a month.

For Htt measurements, brain material was obtained from the Dutch Brain Bank, under project number 1504. Tissue was homogenized with A68 buffer, consisting of 20 mM TRIS-HCl pH 7.4, 10 mM EDTA, 1.6 M NaCl, 10% sucrose, 1 tablet/10 ml Pierce protease inhibitor, 1 tablet/10 ml phosphatase inhibitor. The homogenized sample was spun from 20 minutes, at 14000 rpm at 4 °C. Supernatant was collected, and the pellet was homogenized in 10% (w/v) A68 buffer. The homogenized was spun once more. The supernatants of both centrifugations were combined and diluted to 25% supernatant, 200 nM FibrilPaint1 and a final concentration of 0.5% pluronic to prepare the sample for FIDA investigation.

ThioflavinT aggregation assay

Aggregation of 20 µM Tau-RD in 25 mM HEPES-KOH pH 7.4, 75 mM KCl, 75 mM NaCl, ½ tablet/50 ml Protease Inhibitor, was induced by the addition 5 µM of heparin low molecular weight in presence of 45 µM ThioflavinT. Impact of peptides was assessed by adding 0.02, 0.2 or 2 µM of peptide. Fluorescent spectra was recorder every 5 minutes, at 37 °C and 600 rpm during 24 hours in CLARIOstar® Plus.

Aggregation of 20 µM HttEx1Q44 in 25 mM HEPES-KOH pH 7.4, 75 mM KCl, 75 mM NaCl, ½ tablet/50 ml Protease Inhibitor, was induced by the cleavage of C-terminal MBP-tag with Factor Xa, in presence of 45 µM ThioflavinT. Impact of peptides was assessed by adding 0.02, 0.2 or 2

µM of peptide. Fluorescent spectra was recorder every 5 minutes, at 37 °C and 600 rpm during 24 hours in CLARIOstar® Plus.

Negative Staining Electron Microscopy (nsEM)

5 Carbon-coated copper grids (200 or 300 mesh) were glow discharged for 20 s. Fibrils were diluted in buffer if necessary (25 mM HEPES-KOH pH 7.4, 75 mM KCl, 75mM NaCl) and 1 µl of samples were loaded onto the grid surface and incubated for 60 s. Excess sample was blotted off with filter paper while holding the grid vertically. Grids were incubated for 30 s on a 2% uranyl acetate drop of 100 uL to stain the grids. Processed grids were analysed with a transmission
10 electron microscope at 120 kV (Talos L120C, Thermoscientific, Utrecht, Netherlands). Images were analysed with ImageJ.

Size measurement with Flow Induced Dispersion Analysis (FIDA)

Flow Induced Dispersion Analysis (FIDA) was used to determine fibril size, using the
15 hydrodynamic radius as parameter. The FIDA experiments were performed using a FIDA 1 with a 480 nm excitation source.

Different timepoint preformed Tau-RD or HttEx1Q44 fibrils were diluted to a final concentration of 2 µM in 25 mM HEPES-KOH pH 7.5, 75 mM KCl, 75 mM NaCl, 0.5% pluronic (for Tau-RD) or 50 mM HEPES-KOH pH 7.5, 150 mM KCl, 0.5% pluronic (for HttExQ4), together with
20 200 nM of FibrilPaint1. For patient derived Tau filaments, AD, CBD or FTD fibrils were diluted to a final concentration of 2 µM in 20 mM TRIS-HCl pH 7.4, 100 mM NaCl, 0.5 % pluronic (for AD and CBD), or 50 mM TRIS-HCl pH 7.4, 150 mM NaCl, 0.5 % pluronic (for FTD). The mode of operation use is capillary dissociation (Capdis). In this, the capillary is equilibrated with buffer followed by injection of the sample. Only the indicator sample contains fibrils, in order to minimize stickiness to
25 the capillary.

Ubiquitination of amyloid fibrils

For the ubiquitination reaction, enzymes were diluted into ubiquitination buffer (40mM TRIS pH = 8, 5 mM MgCl₂, 0.05% TWEEN-20 and 1 mM DTT) to a final concentration of 500 nM E1, 500
30 nM E2, 500 nM E3, 10 nM Fluorescein-Ubiquitin, 500 nM FibrilPaint, 5 µM fibrils, and in presence or absence of 5 mM ATP. The reaction was then incubated for 1 h at 30 °C. To determine ubiquitination, sample was loaded onto FIDA1 with a 480 nm excitation source. The mode of operation use is capillary dissociation (Capdis). In this, the capillary is equilibrated with buffer followed by injection of the sample (Table 2).

Table 2. Experimental parameters for Capdis analysis of protein fibril size up to a R_h of 75 nm.

<u>Tray</u>	<u>Vial</u>	<u>Pressure (mbar)</u>	<u>Time (s)</u>	<u>Outlet</u>	<u>Measure</u>	<u>Comments</u>
<u>2</u>	<u>1</u>	<u>3500</u>	<u>45</u>	<u>Variable</u>	<u>No</u>	<u>1 M NaOH</u>
<u>2</u>	<u>2</u>	<u>3500</u>	<u>75</u>	<u>Variable</u>	<u>No</u>	<u>Buffer equilibration</u>
<u>1</u>	<u>Analyte</u>	<u>3500</u>	<u>20</u>	<u>Variable</u>	<u>No</u>	<u>Buffer</u>
<u>1</u>	<u>Indicator</u>	<u>50</u>	<u>10</u>	<u>Variable</u>	<u>No</u>	<u>Sample application</u>
<u>1</u>	<u>Analyte</u>	<u>75</u>	<u>1800</u>	<u>Variable</u>	<u>Yes</u>	<u>Buffer</u>

Tray 1 was maintained at 37 °C and tray 2 and capillary chamber at 25 °C

Microscale thermophoresis binding assay

5 Binding between FibrilPaint1 or FibrilPaint6 and CHIP was analyzed using Monolith (NanoTemper Technologies) Microscale thermophoresis system (MST). Thermophoresis was monitored at a concentration of 50 nM of FibrilPaint and a dilution series of CHIP, in 25 mM HEPES-KOH pH 7.5, 75 mM KCl, 75 mM NaCl. Samples were transferred to premium capillaries (NanoTemper Technologies) and measurements were performed at 37 °C, with medium blue LED power and at MST infrared laser power of 50% to induce thermophoretic motion. The infrared laser was switched on 1 second after the start of the measurement for a 20 seconds period. Datapoints used were the MST response at 5 seconds.

Results

15 *Development of a multi-targeting method to determine amyloid size.*

We set out to develop a method to determine the size of multiple amyloid fibrils. Our strategy is established using Tau and Huntingtin (Htt), two aggregating proteins which are unrelated in sequence and structure. Htt fibrillation is directly correlated to the onset and progression of Huntington's disease (HD)(Andrew et al., 1993). HD is an autosomal inherited disorder, caused by the expansion of a glutamine stretch in the exon 1 of the Htt protein. Tau is an intrinsically disordered protein that undergoes amyloid aggregation in different pathologies. Tau fibril formation is highly related to the progression of tauopathies, including Alzheimer's disease (AD), frontotemporal dementia (FTD), or corticobasal degeneration (CBD), amongst others.

Our previous work (Garfagnini et al., 2023) described the development of a family of peptides that inhibit aggregation of several unrelated proteins at early stages. We used these peptides as a starting point to develop fibril paint. To enhance the peptide binding to aggregates, we increased the density of π -stacking and H-bonding residues (Ferrari et al., 2020) and varied the net charge and the number of aromatics in the peptides. A fluorescein group (Fl-) was added at the N-terminus to facilitate detection. In some of the peptides a negatively EEVD sequence C-terminally with a GSGS spacer was added to the peptides, creating oppositely charged sections, with a positively charged N-terminal region and a negatively charged C-terminus. A fluorescein group (Fl-) was

added to the N-terminus to facilitate detection. The net charge and the number of aromatics in the peptides were varied.

- This resulted in a set of peptides with different charge, number of aromatics and uneven distribution of the different types of residues (Table 3). Designed to make fibrils visible, we call this class of peptides fibril paint.

Table 3: List of FibrilPaints

Name	Sequence*	SEQ ID NO	Binding	
			Tau	Htt
FibrilPaint1	(FI)-PWWRRPWWPWHHPH-GSGS-EEVD	8	Yes	Yes
FibrilPaint5	(FI)-PWWRRPWWPWHHPHR	9	Yes	Yes
FibrilPaint6	(FI)-PWWRRPWWPWHHPH	2	Yes	Yes
FibrilPaint7	(FI)-PWWRRPWWPWHHPH-GSGS-RRVD	10	Yes	Yes
FibrilPaint8	(FI)-VWHPEPPHVRWSWWRHGRGED	3	Yes	n.d.
FibrilPaint2	(FI)-PWIRRPWWPWTTPT-GSGS-EEVD	11	No	No
FibrilPaint3	(FI)-PWWDDPWWPWTTPT-GSGS-EEVD	12	No	No
FibrilPaint4	(FI)-PWWDDPWWPWHHPH-GSGS-EEVD	13	No	No
FibrilPaint9	(FI)-PWIRRPWWPWTTPTR	14	No	No
FibrilPaint10	(FI)-PWWDDPWWPWTTPTD	15	No	No
FibrilPaint11	(FI)-PWWDDPWWPWHHPHD	16	No	No
FibrilPaint12	(FI)-pwwrrpwwpwwhhphr-GSGS-EEVD	17	No	No
FibrilPaint13	(FI)-pWWrrpWWpWhhphr-GSGS-EEVD	18	No	No

*“(FI)” represents a fluorescein

10 The four peptides inhibit Tau and Htt amyloid aggregation

- We assessed whether the designed fibril paint peptides could interact with multiple species of aggregated proteins. To do so, we tested the inhibitory capacity of the peptides on amyloid aggregation of two unrelated proteins, Tau Repeat Domain (Q244-E372, TauRD) with the pro-aggregation mutation Δ K280, and Huntingtin Exon 1 comprising a 44 residues-long polyglutamine stretch (HttEx1Q44) with a ThT assay. This is an established method to monitor protein fibrils by a fluorescence dye, thioflavin-T (ThT), that emits fluorescence upon binding to fibrils. The data of four of the fibril paints is shown herein. The four fibril paint peptides strongly inhibited Tau-RD aggregation at substoichiometric concentration in a dose-dependent manner (Fig. 1A-D). FibrilPaint1 was most effective, lowering the fluorescent intensity in the plateau by 92%, followed by FibrilPaint4 (89%), FibrilPaint2 (82%) and FibrilPaint3 (73%).

All fibril paints also inhibited HttEx1Q44 aggregation in a dose-dependent manner, although less potently than for Tau (Fig. 1E-H). Here, FibrilPaint4 had the strongest effect, lowering the fluorescent intensity of the plateau with 74%. FibrilPaint2 (55%), FibrilPaint3 (54%) and FibrilPaint1 (30%) inhibited aggregation of the polyglutamine protein to some extent, too. Overall, the tested

peptides inhibit amyloid aggregation of both Tau-RD and HttEx1Q44 at substoichiometric concentrations. This is remarkable as both proteins are entirely unrelated in sequence and the only common property is the formation of fibrils.

5 *FibrilPaint1 binds to Tau and Htt fibrils*

Next, we screened our peptides for binding to TauRD and HttEx1Q44 fibrils (Fig. 1I-J). To do so, we developed an application of FIDA to visualize the size of amyloid aggregates. With FIDA, fluorescently labelled sample of interest are passed through a long capillary by pressure and pass the detector in the end, resulting in a fluorescent signal. Smaller species diffuse faster and bigger ones slower, resulting in a narrow or broad dispersion fluorescent signal, respectively. Since the FIDA relies on the physical diffusion properties of the sample, we can calculate the hydrodynamic radius (Rh) of labelled species from the fluorescent signal. The averaged Rh values are an ensemble value, representing an average value of all labelled particles.

15 The Rh is a parameter corresponding to a size of a molecule or a complex in solution. It is the radius of the sphere created by the tumbling particle. Rh values can be estimated from the structural coordinates, either by experimental methods or by predictions such as AlphaFold (Table 4).

20 **Table 4.** Protein structures and their predicted hydrodynamic radii. The number of residues and the molecular weight are given for the total structure as available in the PDB. If structures are available, the included 1 layer Rh is the Rh a structure would have if it could fold in its fibrillar conformation with just one layer

Protein structure	PDB code	Nr. residues	MW (kDa)	Predicted R _h (nm)	
				1 layer	5 layers
Snake recombinant Tau	6QJH	177	18.67	2.29	3.49
Twister recombinant Tau	6QJM	144	15.57	1.73	2.68
Jagged recombinant Tau	6QJP	144	15.57	1.75	2.70
TauRD monomer		128	14.21	4.16	
Tau AD Paired helical filament	5O3L	730	79.40	3.33	4.33
Tau AD straight filament	5O3T	730	79.40	3.29	4.34
FTD narrow filament	6GX5	282	30.41	2.84	4.32
CBD type I	6TJO	321	137.76	2.67	4.07
CBD type II	6VH7	642	69.65	4.12	6.25
HttEx1Q44 monomer		120	13.82	3.4	
Luciferase	1LCI	550	60.82	3.35	
Recombinant alpha-synuclein fibril	6CU7	140	144.76	2.66	3.5349
Recombinant amyloid-β(1 -42) fibril	5OQV	42	40.68	2.41	3.15

Determining the Rh for aggregating species requires a compound that labels the aggregate without altering its properties. Aggregated proteins flow through the capillary at 37°C to increase the speed of the flow without exceeding physiological temperatures. We used low pressure (75 bar) and long flow times (35 min) to sustain Taylor's conditions for larger species. To minimize the volume of entered fibrils to sub-microliters, as well as reducing possible interactions inside the capillary, we used a capillary dissociation set-up. Here we premixed the fibril paint peptides with fibrils and let the mixture diffuse in buffer only. Binding of the fluorescently labelled fibril paint peptide to protein fibrils will increase the average Rh value, as the peptide now tumbles together with the larger fibril.

Testing the fibril paint peptides for binding to TauRD fibrils that aggregated for 4 h showed that FibrilPaint 1 binds effectively increasing the average Rh value from 1.2 nm to 45 nm (Fig. 1I). Importantly, Incubation of fibril paint 1, with TauRD monomer did not lead to an increased Rh value, indicating that it does bind specifically to the fibril but not to the monomer. The binding of FibrilPaint 2 to 4, and 9-13 was not sufficiently stable bind to TauRD fibrils to effectively fluorescently label them. It is interesting that not all act as non-covalent label, given that all fibril paint peptides inhibit aggregation of TauRD. It indicates that labelling and aggregation prevention are two different properties.

Testing the fibril paint peptides for labelling of HttEx1Q44 fibrils resulted in remarkably similar results. The average Rh of FibrilPaint1, increased in the presence of HttEx1Q44 fibrils while it did not bind to the monomer (Fig. 1J). The fibril paint peptides 2 to 4, and 9-13 2-4 are also ineffective in binding to HttEx1Q44 (data shown for FibrilPaint 2-4 Fig. 1J).

Next, to demonstrate the ability of FibrilPaint1 to recognize various amyloids, we tested its capacity to bind α -Syn, A β 42 fibrils and polypeptide (IAPP, amylin). While α -Syn fibrils are associated with synucleinopathies like Parkinson's Disease (Goedert et al., 2017; Spillantini et al., 1997), the deposition the A β peptide forming plaques is linked to the development of AD (Hardy & Higgins, 1992; Long & Holtzman, 2019), IAPP forms amyloid fibrils in type II diabetes mellitus (T1DM) disease. Remarkably, FibrilPaint1 bound to α -Syn, A β 42 and IAPP fibrils, revealing an average size of 33 nm, 50 nm (Fig. 6A) and 10nm, respectively (Fig. 6B). This demonstrates that FibrilPaint1 detects multiple protein fibrils.

FibrilPaint1 is amyloid-specific

We investigated whether FibrilPaint1 is specific to fibrillar aggregates. First, we tested its ability to specifically recognise TauRD fibrils in presence of an abundance of other cellular proteins, by adding E. coli cell lysate. We incubated pre-formed TauRD fibrils together with FibrilPaint1 in a 1:100 ratio in 50% cell lysate. Under these conditions, FibrilPaint1 shows an increased size of 14 nm, which is not the case when incubating FibrilPaint1 alone in cell lysate (Fig. 2A). Thus, FibrilPaint1 specifically recognises fibrils in a complex cellular mixture.

Having confirmed the ability of FibrilPaint1 to bind protein fibrils and not monomers, we determined the specificity of FibrilPaint1 to amyloid structures. We used Luciferase as an established paradigm for non-amyloid aggregates (Parsell et al., 1994). Luciferase is a globular 61

KDa protein with a Rh of 3.4 nm that forms amorphous aggregates when denatured by heat shock. We incubated FibrilPaint1 together with heat-shocked Luciferase in a 1:100 ratio. The Rh of FibrilPaint1 in the FIDA measurement is not affected by the presence of luciferase aggregates (Fig. 2B). This indicates that FibrilPaint1 does not bind to amorphous luciferase aggregates. Together, these data demonstrate that FibrilPaint1 binds specifically to amyloid fibrils, undisturbed by the presence of other biomolecules.

To test FibrilPaint1 in a more physiological and disease-relevant environment, we incubated FibrilPaint1 in absence or presence of pre-formed TauRD fibrils in 50% human serum. FibrilPaint1 alone in 50% human serum appeared to be 1.5 nm, which correlates to the 1.4 nm observed in buffer (Fig. 7B). In presence of pre-formed TauRD fibrils, the size increases up to 34 nm in 50% human serum, and 42 nm in buffer (Fig. 7B). These findings enhance the significance of FibrilPaint1 within a clinically relevant environment.

Finally, we tested if FibrilPaint1 could also target patient fibrils in the heterogeneous environment of the brain. We liquified brain-material derived from Htt patients. We diluted the Htt brain supernatant to 25% and added FibrilPaint1. The size increased from 1.5 nm FibrilPaint1 only, to 4.5 nm, indicating that FibrilPaint1 binding to amyloid fibrils (Fig. 7C).

After confirming the ability of FibrilPaint1 to determine fibril size in human serum, the inventors aimed to estimate the concentration threshold for such measurements. Recombinant TauRD fibrils were titrated into serum and the size was measured with FibrilPaint1 /FIDA for decreasing concentrations. The TauRD fibrils in this experiment had an Rh value of 21 nM. The inventors observed a consistent Rh value for fibril samples formed from monomers down to 200 nM. An Rh value of 21 nm corresponds to 260 layers for a PHF-shaped fibril (Fig. 7D). Taking into account the relationship between Rh and mean fibril length for an PHF-shaped fibril (Fig. 7D) the inventors estimated the lowest observed concentration of fibrils corresponded to 400 pM (Fig. 7D). These findings indicate that FibrilPaint1 determines fibril length at subnanomolar amyloid concentrations within a clinically relevant environment.

Monitoring fibril kinetics

We now set out to monitor aggregation kinetics by increase in Rh using FibrilPaint1 and FIDA, for both TauRD and HttEx1Q44 (Fig. 3A, D). After 0.5 h, the aggregation reaction of TauRD resulted in an increase of the averaged Rh from 1.7 nm to 2 nm. All species measured at t=0.5h have the same size, indicating full binding of FibrilPaint1, without any dissociation taking place, and no larger species are available at that timepoint. Full-length recombinant Tau has an Rh between 2.11 nm and 2.78 nm for three layers of aggregate (Zhang et al., 2019). Our TauRD has an unknown Rh as a fibril, but we expect it to have an Rh between 2-2.5 nm. Depending on the binding site of FibrilPaint1, the Rh of FibrilPaint may affect the size of this various extends. However, this species with a bound size of 2 nm could be the earliest aggregate measurable by FibrilPaint1.

TauRD aggregation continued, and rapidly increased to a size of 10 nm after 2h (Fig. 3A). Fibril sizes measured were also more heterogeneous, indicating the presence of multiple aggregates with varying lengths. Only the largest species were plotted in (Fig. 3A). From this point

on, fibrils were formed with extended fibril lengths, meaning the Rh cannot be directly converted to the radius anymore, but must be converted to fibril length through the molecular weight and radius of gyration (He & Niemeyer, 2003; Yoshizaki & Yamakawa, 1980). This would mean that the fibril size reached a length of 58-64 nm for a radius between 2 and 2.5 nm. TauRD aggregation reached
5 a plateau at an Rh value of 30 nm after 8 h, corresponding to a length of 260-280 nm (Fig. 3A).

For HttEx1Q44, Rh increased to 5 nm after 1 h. This already exceeds the predicted Rh of the unfolded monomeric structure of HttEx1Q44, which is estimated to be 3.4 nm. Therefore, this structure consists of multiple monomers. Aggregation then exponentially increased up to an average Rh of 500 nm after 7 h (Fig. 3E). As the particle size reached the upper limit of the FIDA
10 measurement, we could not determine if and when it reaches a plateau value.

We monitored the aggregation process in parallel with the established ThT assay, for both TauRD and HttExon1Q44. (Fig. 3B, E). The ThT fluorescent signal rises immediately after addition of heparin to TauRD and increases rapidly reaching a plateau already after 4 h (Fig. 3B). This is faster than what we observed in the FIDA measurements. It suggests that formed fibrils may already
15 be saturated by ThT but are still growing in length. We obtained similar results for HttEx1Q44. The ThT revealed a lag-phase of approximately 2 hours and a plateau after 5 h of aggregation (Fig. 3E), while the size of the fibrils still increased (Fig. 3D). The aggregation of HttEx1Q44 seems more aggressive than aggregation of TauRD, resulting in much larger sizes. This may indicate that another process is going on which let the fibrils stick together. We conclude that both methods are
20 complementary, in which the ThT assay providing a signal representing the presence of fibrils while the FIDA measurements provide a measurement for the length of the fibrils that goes beyond the early aggregation phase.

Characterising fibril shape

25 We used negative stain EM imaging to characterise the shape of the TauRD fibrils after 24 h aggregation and HttEx1Q44 fibrils after 6 h (Fig. 3C, F). TauRD fibrils appeared as long, single, fibrillar structures (Fig. 3C). The length of the Tau fibrils varied strongly and was on average 500 to 600 nm (data not shown), bigger than our FIDA observations. This is as expected, as smaller fibrils are excluded due to the lower detection limits of the technique.

30 On the contrary, HttEx1Q44 fibrils stucked together to form larger clusters of fibrils (Fig. 3F) (Boatz et al., 2020; Isas et al., 2021; Matlahov et al., 2022; Nazarov et al., 2022). We noted a large variation between the sizes of these clusters: from just a few fibrils of 50-100 nm to enormous clusters of several μm in width. A representative shape formed by these clusters is around 800 nm long and 200 nm wide (Fig. 3F). Since this shape is more spherical than singular fibrils, it would result in an
35 Rh of 400 nm in FIDA. We measured sizes with an Rh 300-500 nm in FIDA experiments. These clusters clarify the rapid increase in size detected with FIDA and reaching the upper limit of the FIDA measurement.

FibrilPaint1 determines the size of inhibited species

With the FIDA application for size determination, we set out to determine at which size FibrilPaint is inhibiting amyloid aggregation as seen in ThT assays (Fig. 1A, E). ThT assays showed almost complete suppression of TauRD aggregation in presence of FibrilPaint1 (Fig. 1A) and a 30% inhibition of HttEx1Q44 aggregation (Fig. 1E) at 1:10 peptide : monomer. We repeated the same experimental settings and left TauRD and HttEx1Q44 under aggregating conditions in presence of FibrilPaint1, to investigate its inhibiting capabilities. Next, we took samples at various timepoints and measured them in FIDA (Fig. 4). In presence of FibrilPaint1, TauRD aggregation reached a Rh of 3.5 nm after 2 h incubation. This species of 3.5 nm remained stable for 12 h (Fig. 4A). Negative staining EM images confirmed that TauRD inhibited by FibrilPaint1 resulted in fibrils so small they were not detectable (Fig. 4C). Under the same inhibiting conditions, HttEx1Q44 aggregated to an average size of 28 nm in presence of FibrilPaint1, remaining stable over 24 h (Fig. 4B). Here, Htt fibrils had much more space between them, showing more singular fibril shapes (Fig. 4D).

With the sizes acquired for FibrilPaint1 inhibition, we can determine the stage where inhibition takes places. This size would be expected for a dimeric PHF for this species, this Rh correspond to a fibril of 2 layers, thus consisting of four Tau molecules (Fig. 5F). Alternatively, an Rh of 3.5 nm would also correspond to a stack of five layers of the snake-shaped recombinant fibril (Table 4). Interestingly, the fibrils were too small to be visible in negative staining EM images (Fig. 4C). HttEx1Q44 has a molecular weight of 13.8 kDa, resulting in an estimated Rh of 2.1 nm, if the protein is folded into a perfect spherical shape, to 3.5 nm, if the protein is completely unfolded. Measured size of the inhibited HttEx1Q44 aggregate is approximately 28 nm when bound to FibrilPaint1. Assuming all fibrils are completely singular now, we can estimate the fibril length of Htt to be between 240 nm and 280 nm. In the ThT assays (Fig. 1A, E), we see almost complete suppression of the ThT signal for TauRD, but not for HttEx1Q44, although there is a lowering of the plateau. It is evident that the inhibited state of Htt includes more monomers than Tau. It may be that in presence of Fibrilpaint1, TauRD cannot assemble the amyloid structure that ThT recognises, where HttEx1Q44 can.

FibrilPaint1 for monitoring patient-derived fibrils

FibrilPaints 1, 5, 6, 7 and 8 could have potential as a lead compound for diagnostic tracing of protein fibrils in patients. This would require that the FibrilPaints would recognise patient-derived fibrils. Interestingly, cryo-EM structures of Tau fibrils show that their shape differs for the various tauopathies, and heparin-induced recombinant fibrils (Fig. 5B). For its potential as Tau tracer, it is important to assess the FibrilPaints ability to recognise patient derived fibrils of several tauopathies.

Tau aggregation characterises the progression of different tauopathies. Monomeric Tau can undergo post-translational alternative splicing leading to six different isoforms, with either four (4R) or three (3R) repeats of the microtubule-binding domain. Depending on the isoform incorporated in the fibril, tauopathies can be classified as 4R (corticobasal degeneration, CBD), 3R (frontotemporal disorder, FTD) or 4R/3R (AD). Both size and morphology can reveal information about fibril

formation, the interface and the stability of the fibrils. It is therefore of importance to characterise the size of various patient-derived fibrils.

We set out to measure the size of patient-derived Tau fibrils from three different tauopathies. To test whether FibrilPaint1 could also be applicable for patient-derived fibrils, we purified fibrils from patients diagnosed with CBD, FTD and AD. We imaged the purified fibrils with negative stain EM to confirm the typical disease-specific fibril shape (Fig. 5C). Both Paired Helical Filaments (PHFs), with their typical twist, as well as Straight Filaments (SFs) can be seen.

Next, we performed FIDA analysis to determine the size of the patient-derived Tau fibrils (Fig. 5A). Our in parallel synthesised recombinant TauRD fibrils had an average Rh of 54 nm. Fibrils measured from a patient diagnosed with AD had an apparent average Rh of 49 nm, with little variation between the measurements (Fig 5A). For CBD, the observed Rh was 95 nm (Fig 5A). Fibrils from FTD appeared as an homogeneous population, with an average Rh of 69 nm (Fig 5A). These data reveal that FibrilPaint1 is suitable to interact with and characterise protein fibrils derived from primary material.

Finally, we generated a model to relate Rh to fibril length. In silico, we stacked different layers of fibrils on top each other and calculated the predicted size with FIDABio Rh prediction tool. One full turn of the twist of a PHF is made by stacking 342 layers (Fig. 5D). One layer has a length of 4.7 Å, giving the full twist a length of approximately 160 nm (Fitzpatrick et al., 2017). For AD fibrils, we measured an Rh of 49 nm, which corresponds to a fibril of 1100 layers (Fig. 5F), which would give a fibril length of approximately 510 nm. In nsEM data derived from fibrils purified from an Alzheimer patient, we assessed in total 103 fibrils, of which 48 show a typical PHF structure. We used the repetitive element in PHF fibrils to determine their average length. The average PHF in our sample had 2.7 turns, which corresponds to 430 nm (Fig. 5E). This very well matches the average length calculated from the Rh value determined by FIDA measurements of FibrilPaint1-bound Tau fibrils.

Various Fibril Paints target amyloids

Next, we set out to search for FP1 derivatives that can maintain fibril-binding properties. The modifications include shortening of the sequence to exclude linker and E3 binding motif (FP5 and FP6), change of the net charge (FP5-FP13), randomisation of the sequence (FP8) and alteration of the 3D structure using D-amino acids (FP12 and FP13).

We used TauRD and HttEx1Q44 fibrils, and measured the size in FIDA. Five FP peptides increase size in presence of TauRD and HttEx1Q44 fibrils, grown for 20 hours and 5 hours, respectively (Fig 11). Specifically, the Rh measured for TauRD fibril together for five active FP (FP1, FP5, FP6, FP7 and FP8) increased to an average size of 37 nm. In case of HttEx1Q44, we observed an average Rh of 188 nm (FP1, FP5, FP6 and FP7; the value for FP8 was not determined). The fibril size variation within the measurement using different FPs is minimal and does not depend on the FP. Given that the aggregation process is a seeded event, it is expected to observe some variation in the measured Rh. Together, these results indicate that FP1, FP5, FP6, FP7 and FP8 are effective fibril binders.

The identification of four new FP peptides binding amyloid fibrils (FP5, FP6, FP7 and FP8) demonstrates that the sequence of FP1 can be varied while maintaining specific recognition of amyloid fibrils. First, the change in charge does not compromise binding ability. FP1 has a net charge of -1, and FP5, FP6, FP7 and FP8 have a net charge of +3, +2, +2, and 0, respectively. FP5, FP6 lack the GSGS and the EEVD motif, and in FP7 EEVD has been replaced by RRVD, indicating that those motifs are not required for detection of amyloid fibrils. In FP8, the order of the amino acid sequence is altered, indicating a potential to vary the sequential order of the residues.

FibrilPaint as a PROTAC strategy to Ubiquitinate amyloid fibrils

PROTACs, are small molecules that inhibit the function of their target proteins by targeting them for degradation by the ubiquitin proteasome system. We set out to apply the FibrilPaints as PROTAC for the ubiquitination of amyloid fibrils (see exemplary mode of action in Fig. 8A). First we established that we can use the FIDA system to follow the ubiquitin transfer by tracking the Rh (data not shown). If ubiquitination takes place, the size of Rh increases substantially due to the formation of large complexes (Fig. 8B). We designed FibrilPaint1 as a PROTAC, using the c-terminal part of the Protac to recognize the amyloids and the EEVD motif comprised in FibrilPaint1 to form a ternary complex with the target amyloid and the E3 ligase CHIP. CHIP then was titrated to FibrilPaint1 and the Rh was measured. As can be shown in Fig. 8C, FibrilPaint1 shows an increase in Rh depending on the concentration of CHIP, indicating that FibrilPaint1 is indeed capable of ubiquitination of amyloid fibrils.

To employ FibrilPaint1 as a PROTAC strategy, the transfer of ubiquitin to the amyloid fibrils was further validated. In order to do so, we added four different recombinant fibrils (TauRD, HttEX1Q44, asyn, Abeta fibrils) to the ubiquitination reaction in the presence or absence of ATP and measured the reaction with the FIDA system. ATP is required to form a high-energy thioester bond between Ubiquitin and E1, so if ATP is absent, no ubiquitination should take place.

The results are presented in Fig. 9A-D, In the presence of ATP all Rh values exceeded the largest measured sizes of ubiquitin binding by the ubiquitin transfer system (Fig. 9B-E), thereby confirming ubiquitin is bound by fibrils. In the absence of ATP, Rh size indicates that no ubiquitination takes place.

Next, we investigated whether the fibrils were covalently ubiquitinated. For that we added Urea in the mixture. Urea denatures the ubiquitination machinery. So if the ubiquitin has been covalently bound to the fibrils, the measured complex will remain intact. However if the machinery has not transferred the ubiquitin, only the ubiquitin complex will remain. The results are presented in Fig. 9A-D where it can be seen that the Rh values did not change after the addition of urea, indicating that the fibrils are covalently ubiquitinated.

FibrilPaint can ubiquitinate patient-derived fibrils.

We wanted to test if FibrilPaint was also able to ubiquitinate patient-derived fibrils. To this end, we extracted fibrils from patients diagnosed with AD, FTD and CBD (Falcon et al., 2018; Fitzpatrick et al., 2017; Zhang et al., 2020) and repeated the ubiquitination reaction with these

patient-derived fibrils. The results confirm that FibrilPaint1 also allows the ubiquitination of patient-derived fibrils (Fig. 10).

Discussion

5 We have designed fibril paint peptides that recognise and bind to amyloid fibrils. The selected FibrilPaints can label fibrils from patients diagnosed with three different tauopathies, which have different folds (Falcon et al., 2018; Fitzpatrick et al., 2017; Goedert et al., 2019; Scheres et al., 2020; Zhang et al., 2020). In combination with FIDA, these FibrilPaints proves a valuable tool for the study of the aggregation pathway of TauRD and HttEx1Q44, two aggregating proteins which are unrelated
10 in sequence and structure. Importantly, the FibrilPaints1, 5, 6, 7 and 8 are specific for amyloid fibrils, and not for monomeric precursors or amorphous aggregates. FibrilPaints1, 5, 6, 7 and 8 is therefore a specific tool accessible to determine the presence of fibrils, in all stages of the aggregation process.

The FibrilPaints bypasses the need to label protein fibrils for modern fluorescence-based
15 detection methods. As shown, FibrilPaint1 binds non-covalently to fibrils, without altering the structure of the fibrils (Fig. 1, 4). This proves useful when handling precious, patient-derived material, avoiding material loss or artefacts due to labelling procedures. It also allows the detection of amyloid fibrils in presence of cell lysate (Fig. 2A). FibrilPaint1 therefore proves to be a selective compound for detection of amyloids and can potentially be used as a tracer for detecting multiple
20 aggregating diseases. With amyloidosis also present in other diseases such as diabetes and Parkinson's Disease, FibrilPaint1 could be interesting tool for detecting pathological fibrils outside the brain (Brundin et al., 2017; Cao et al., 2020).

The development of tracer drugs for early diagnosis of neurodegenerative diseases remains crucial. To date, Tauvid is the only FDA approved Tau tracer for AD (Commissioner, 2020; Jie et al., 2021; Mohammadi et al., 2023). Cryo EM data reveals Tauvid does not bind in a regular
25 stoichiometric manner to AD fibrils, but it does to CTE Type I filaments (Shi et al., 2022). Regarding tracers for Htt, those have only been tested in ex vivo samples (Delva et al., 2022; Herrmann et al., 2021). We have demonstrated the ability of FibrilPaints to specifically bind to fibrils of patients diagnosed with AD, CBD and FTD (Fig. 5A), as well as recombinant HttEx1Q44 fibrils and Htt brain
30 supernatant (Fig. 1J, Fig 7C), at substoichiometric concentrations (up to 1:1000; calculated on the monomer). This makes FibrilPaints1, 5, 6, 7 and 8 a potential tracer specific to amyloid fibrils of varying nature, able to recognise the repetitive structure of the fibrils (Fig. 11). The peptide-nature of FibrilPaints 1, 5, 6, 7 and 8 is biocompatible and can be further functionalised as lead compound to develop a tracer for neurodegenerative diseases at early stages.

35 The combination of FibrilPaints1, 5, 6, 7 and 8 with FIDA provides a valuable research tool. FibrilPaint1 with FIDA does not only establishes the presence of amyloids, but gives a structural parameter; the Rh (Jensen & Østergaard, 2010). The average Rh of FibrilPaint1-stained fibrils is determined without bias towards larger or smaller particles, which could be useful to assess the stage of the disease (Lobanova, 2022; Nirmalraj et al., 2023). With recent advances in cryo-EM,
40 many fibril structures are available as PDB files (Scheres et al., 2020). The average Rh can then

directly be compared to the structure of the fibril. Therefore, combining FibrilPaints1, 5, 6, 7 and 8 with FIDA measurements allows to monitor the average length of the fibrils. This is specifically important for monitoring disease progression, as fibril length is an indication of disease stage (Nirmalra et al. 2023).

5

FibrilPaints1, 5, 6, 7 and 8 are a suitable tool for further development into a tracer for neurodegenerative and other fibrillar diseases, as well as disease-modifying compounds such as targeted protein degradation (Fig 12). The ability to specifically bind multiple amyloid fibrils opens the door for the diagnosis of varying neurodegenerative diseases. Its ability to recognize early aggregates provides the opportunity to recognize first stages of the diseases. This may allow intervention before severe damage in the brain has occurred.

10

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Claims

1. A polypeptide comprising
i) the amino acid sequence:

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$$P-W-W-X^1-X^2-P-W-W-P-W-H-H-P-X^3$$

wherein, X^1 is R or K, preferably R, X^2 is R or K, preferably R, and X^3 is H or W, preferably H; or

- 10 ii) the amino acid sequence:

$$P-W-W-R-R-P-W-W-P-W-H-H-P-H$$

- 15 wherein the amino acid sequence comprises no more than 4, 3, 2, or 1 amino acid substitutions, deletions and insertions,

wherein the amino acid sequences of i) and ii):

- bind to aggregates or oligomeric precursors of amyloid fibrils; and
- do not bind to the non-aggregated monomeric form of amyloid fibrils.

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2. A polypeptide according to claim 1, wherein the polypeptide comprises at least one detectable tag.

3. A polypeptide according to claim 1 or 2, wherein the polypeptide comprises a second tag.

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4. A polypeptide according to any one claims 1 – 3, wherein at least one of the detectable tag and second tag is linked to the amino acid sequence or to the polypeptide through a flexible linker, preferably a flexible amino acid sequence.

- 30 5. A polypeptide according to any one claims 1 – 4, wherein the detectable tag and second tag are linked at opposite ends of the polypeptide.

6. A polypeptide according to any one claims 1 – 5, wherein the detectable tag is selected from the group consisting of a radionuclide, an isotope, an optical label, a magnetic material, an affinity tag and any combination thereof.

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7. A polypeptide according to claim 6, wherein at least one of:
a) the radionuclide is selected from the group consisting of ^{124}I , ^{18}F , ^{11}C , $^{99\text{m}}\text{Tc}$, ^{123}I and any combination thereof;

- b) the optical label is selected from the group consisting of a fluorescent dye, a fluorescent protein, a chemiluminescent dye, a quantum dot, and any combination thereof;
- c) the magnetic material is selected from the group consisting of nanocomposite-containing nanoparticles, superparamagnetic iron oxide nanoparticles, and any combination thereof;
- d) the affinity tag is an epitope, streptavidin, or avidin binding peptide, biotin, or an oligohistidine sequence.
8. A polypeptide according to any one of claims 3 – 6, wherein second tag is a negatively charged amino acid sequence, preferably wherein the negatively charged amino acid sequence comprises SEQ ID NO: 5.
9. A nucleic acid sequence encoding the polypeptide according to any one of the preceding claims.
10. A pharmaceutical composition comprising the polypeptide as defined in any one of the preceding claims, optionally further comprising a pharmaceutical excipient.
11. A method of diagnosing the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils or a predisposition to develop a condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:
- a) providing a sample from the subject
 - b) contacting the sample with the polypeptide as described in any one of claims 1 to 8 that specifically binds to aggregated amyloid fibrils; and
 - c) detecting the presence of the aggregated amyloid fibrils bound to the polypeptide, wherein detection of the aggregated amyloid fibrils bound to the polypeptide is indicative of the presence of aggregated amyloid fibrils.
12. A method of monitoring the progression or identifying the disease state of a pathological condition caused by the aggregation of amyloid fibrils in a subject, the method comprising:
- a) providing a sample from the subject;
 - b) contacting the sample with the polypeptide as described in herein that specifically binds to aggregated amyloid fibrils; and
 - c) determining at least one of fibril length, hydrodynamic radius of the amyloid fibril bound to the polypeptide and its location,
- wherein the fibril length, its hydrodynamic radius and/or its location provide an indication of the progression or identifying the disease state.

13. The method according to claim 11 or 12, wherein the sample is at least one of brain, or other tissue, plasma, cerebrospinal fluid (CSF), urine or tears.
- 5 14. The method according to any one of claims 11 – 13, wherein the detected amyloid aggregates is from an amyloid forming protein such as Tau, IAPP amyloid-beta, alpha-synuclein, huntingtin- tau proteins and/or alpha-synucleins.
- 10 15. The polypeptide as described in any one of claims 1 to 8, the nucleic acid according to claim 9 or the pharmaceutical composition according to claim 10 for use as a medicament.
- 15 16. The polypeptide as described in any one of claims 1 to 8, the nucleic acid according to claim 9 or the pharmaceutical composition according to claim 10 for use as a medicament in the treatment of a pathological condition caused by the aggregation of amyloid fibrils.
- 20 17. The method according to any one of claim 11 – 14 or the use according to claim 15 or 16, wherein the pathological condition is an amyloidosis such as systemic lysozyme, insulin, hemodialysis amyloidosis, or a neurodegenerative disease, including Tauopathies such as Alzheimer disease (AD), Corticobasal degeneration (CBD) and Pick's disease (PiD), and Synucleinopathies such as Parkinson disease (PD), and Lewy Body disease, or other neurodegenerative diseases such as prion diseases, Argyrophilic grain disease (AGD), Progressive supranuclear palsy (PSP) Huntington's Disease (HD) and Amyotrophic lateral sclerosis (ALS).
- 25 18. Use of the polypeptide as described in any one of claims 1 to 8, the nucleic acid according to claim 9 or the pharmaceutical composition according to claim 10 for the detection of aggregated amyloid fibrils.