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Description

The present invention relates to a peptide containing at least one amino acid sequence as shown in SEQ ID NR: 2 and its use, in particular in medicine.

Due to the demographic development in the coming decades, the number of people suffering from age-related diseases will increase. Particularly noteworthy here is so-called Alzheimer's disease (AD, Alzheimer's dementia, Latin=Morbus Alzheimer).

A feature of Alzheimer's disease are extracellular deposits of the amyloid beta peptide (A-beta peptide). This deposition of A-beta peptide in plaques is typically determined post mortem in the brains of AD patients. Therefore, various forms of A-beta peptide - such as e.g. fibrils- are blamed for the emergence and progression of the disease. In addition, for some years the small, freely diffusible A-beta oligomers have been recognized as the major cause of the emergence and progress of AD.

A-beta monomers, as building blocks of A-beta oligomers, form continuously in the human body and are probably not toxic per se. They may even have a neuroprotective function. A-beta monomers can be randomly stored together depending on their concentration. The concentration is dependent on their rate of formation and rate of degradation in the body. An increase in the concentration of A-beta monomers occurs in the body with increasing age, such that spontaneous aggregation of the monomers to form A-beta oligomers is more likely. The A-beta oligomers thus formed could proliferate in analogy to prions and eventually lead to Alzheimer's disease.

No active ingredient or medicament currently exists that has an effect on the causes of AD. The medicaments currently used and approved alleviate some of the symptoms that occur in AD. However, they are not capable of slowing the disease progression or of bringing about a cure. Some substances exist which have shown success in animal experiments in the prevention, but not (necessarily) in the treatment, of AD.

An important difference between prevention and treatment or even cure of AD lies in the fact that prevention can potentially be achieved by preventing the formation of the first A-beta oligomers. A few A-beta ligands are adequate for prevention which do not necessarily have high affinity or selectivity with regard to A-beta oligomers.

The formation of A-beta oligomers from numerous monomers is a higher order reaction and is therefore dependent to a high degree on the A-beta monomer concentration. Therefore, a small reduction in the active A-beta monomer concentration already leads to prevention of the formation of the first A-beta oligomers. The preventive

therapeutic concepts and substances currently in development are based on this mechanism. In the treatment of AD, however, a completely new situation could be initiated. Here, A-beta oligomers or possibly also even larger polymers or fibrils are present which proliferate by prion-like mechanisms. However, this proliferation is a lower
5 order reaction and is therefore scarcely dependent on the A-beta monomer concentration.

The substances known from the state of the art reduce the concentration of A-beta monomers and/or oligomers in a variety of ways. For example, gamma secretase modulators are known, which have been used for prevention in animal experiments.

10 Various sequences of D-amino acids which bind to A-beta peptides are known from WO 02/081505. These sequences of D-amino acids bind to amyloid beta peptides with a dissociation constant (K_D) of 4 μ mol.

Hybrid compounds, consisting of amino pyrazoles and peptides which prevent A-beta oligomerization, are known from WO 2011/147797.

15 For many substances which have shown positive results in animal experiments, this effect could not be confirmed in human clinical trials. In phase II and III clinical trials, only people who have been clearly diagnosed with AD are allowed to be treated. In this case, a small reduction in the A-beta monomer concentration is no longer adequate to prevent the formation of even more A-beta oligomers from those already present, e.g. by a prion-like mechanism. However, the proliferation of A-beta oligomers or even better their
20 destruction or neutralization is absolutely necessary in order to influence the course of the disease.

Currently, AD is mainly diagnosed by neuropsychological tests by examining people in whom symptoms of dementia have already occurred. It is known, however, that A-beta
25 oligomers and the fibrils and plaques formed therefrom over time in the course of the disease occur up to 20 years before the onset of symptoms in the brain of patients, and may have already caused irreversible damage. However, in practice there is currently no possibility of diagnosing AD before the outbreak of symptoms.

Thus, there still exists a requirement for novel compounds (active ingredients)
30 which bind very specifically and with high affinity to A-beta oligomers, and thus prevent their proliferation. Said compounds should have no undesirable side effects and in particular not provoke any immune response. The compounds should also recognize toxic A-beta oligomers and therefore also the small, freely diffusible oligomers at low

concentrations, and completely destroy and/or prevent the (prion-like) proliferation thereof.

Furthermore, there exists a need for novel compounds which can be used as probes for the detection and labelling of A-beta oligomers, in particular if the disease is not well
5 advanced and the oligomers only occur at low concentrations.

A further object of the present invention was to provide substances which not only focus on extracellular A-beta peptides, like most compounds known from the state of the art, but specifically bind soluble A-beta oligomers. Furthermore, the novel compounds should inhibit or prevent formation of fibrils of A-beta peptides.

10 It was also an object to provide novel peptides, preferably derivatives of the D-enantiomeric D-peptide D3 (SEQ ID NR: 11), which have efficient properties compared to D3. The properties include, inter alia, binding affinity and specificity for A-beta species, inhibition of A-beta fibril formation, inhibition of A-beta cytotoxicity, precipitation of A-beta oligomers and conversion of A-beta fibrils to non-toxic, non-amyloidogenic species.

15 This object is achieved by a peptide containing an amino acid sequence according to SEQ ID NR: 2 (RD2).

Further disclosed are the peptides SEQ ID NR: 1 (D3-Delta-HTH), SEQ ID NR: 3 (RD1), SEQ ID NR: 4 (RD3), SEQ ID NR: 5 (DB3), SEQ ID NR: 6 (NT-D3), SEQ ID NR: 7 (DB1), SEQ ID NR: 8 (DB2), SEQ ID NR: 9 (DB4) and/or SEQ ID NR: 10 (DB5) and/or homologs, fragments
20 and parts thereof. This object is also achieved by polymers of the SEQ ID NR: 2 (RD2).

Further disclosed are the polymers SEQ ID NR: 1 (D3-Delta-HTH), SEQ ID NR: 3 (RD 1), SEQ ID NR: 4 (RD3), SEQ ID NR: 5 (DB3), SEQ ID NR: 6 (NT-D3), SEQ ID NR: 7 (DB1), SEQ ID NR: 8 (DB2), SEQ ID NR: 9 (DB4) and/or SEQ ID NR: 10 (DB5) and/or homologs thereof.

Fragments and parts may show a similar or identical effect to the peptides.

25 The peptides according to SEQ ID NR: 1, SEQ ID NR: 2, SEQ ID NR: 3, SEQ ID NR: 4, SEQ ID NR: 5, SEQ ID NR: 6, SEQ ID NR: 7, SEQ ID NR: 8, SEQ ID NR: 9 and/or SEQ ID NR: 10 and homologs thereof consist up to 100% of D-amino acids.

A polymer in the context of the disclosure is formed from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more monomers selected from the group consisting
30 of SEQ ID NR: 1, SEQ ID NR: 2, SEQ ID NR: 3, SEQ ID NR: 4, SEQ ID NR: 5, SEQ ID NR: 6, SEQ ID NR: 7, SEQ ID NR: 8, SEQ ID NR: 9 and/or SEQ ID NR: 10 and homologs thereof, which for already A-beta oligomer.

The polymers according to the invention are composed of identical monomers or a combination of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different monomers, different to the aforementioned monomers, as so-called combination polymers. The monomers may also be, in part, identical. The number of identical monomers in the combination polymers is arbitrary.

In an alternative, the combination polymers contain, apart from a peptide with the amino acid sequence according to SEQ ID NR: 2, of at least one monomer selected from the group consisting of SEQ ID NR: 1, SEQ ID NR: 3, SEQ ID NR: 4, SEQ ID NR: 5, SEQ ID NR: 6, SEQ ID NR: 7, SEQ ID NR: 8, SEQ ID NR: 9 and/or SEQ ID NR: 10.

Polymers may be prepared, for example, via chemical synthesis or peptide synthesis.

In an embodiment of the invention, the monomers are covalently linked to one another. In a further embodiment, the monomers are non-covalently bound to one another.

A covalent binding or linking of the monomer units is present in the context of the invention if the peptides are linearly linked head-to-head, tail-to-tail or head-to-head with one another without intervening linker or linker groups being used.

A non-covalent linking is present in the context of the invention if the monomers are linked to one another via biotin and streptavidin, particularly streptavidin tetramer.

In a variant of the present invention, the monomers may be linked together linearly, in particular as described above. In another variation, the monomers are linked together branched to give the polymer according to the invention.

A branched polymer according to the invention can be a dendrimer, in which the monomers are linked together covalently or non-covalently.

Alternatively, the monomers may also be linked to a platform molecule (such as, e.g. PEG or sugar) and thus form a branched polymer.

Alternatively, combinations of these options are also possible.

Monomers and polymers are hereinafter referred to as peptides according to the invention.

A variant, which is not according to the invention, is a peptide having the amino acid sequence according to SEQ ID NR: 1, SEQ ID NR: 2, SEQ ID NR: 3, SEQ ID NR: 4, SEQ ID NR: 5, SEQ ID NR: 6, SEQ ID NR: 7, SEQ ID NR: 8, SEQ ID NR: 9 and/or SEQ ID NR: 10 and/or homologs thereof with an identity of 50%.

“Homologous sequences” or “homologs” signifies that an amino acid sequence has an identity with any of the aforementioned amino acid sequences of the monomers of at least 50, 55, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%. In place of the word “identity”, the terms

5 “homologous” or “homology” are used synonymously in the present description. The identity between two nucleic acid sequences or polypeptide sequences is calculated by comparison with the BESTFIT program based on the algorithm of Smith, T. F. and Waterman, M. S (Adv. Appl. Math. 2: 482-489 (1981)) setting the following parameters for amino acids: Gap creation penalty: 8 and Gap extension penalty: 2; and the following

10 parameters for nucleic acids: Gap creation penalty: 50 and Gap extension penalty: 3. Preferably, the identity between two nucleic acid sequences or polypeptide sequences is defined by the identity of the nucleic acid sequence/polypeptide sequence respectively over the entire sequence length, which is calculated by comparison using the GAP program based on the algorithm of Needleman, S. B. and Wunsch, C.D. (J. Mol. Biol. 48:

15 443-453) setting the following parameters for amino acids: Gap creation penalty: 8 and Gap extension penalty: 2; and the following parameters for nucleic acids: Gap creation penalty: 50 and Gap extension penalty: 3.

In the context of the present invention, two amino acid sequences are identical if they have the same amino acid sequence.

20 In a variant, which is not according to the invention, homologs are understood to mean the corresponding retro-inverso sequences of the monomers mentioned above. The term “retro-inverso sequence”, in accordance with the invention, refers to an amino acid sequence which is composed of amino acids in the enantiomeric form (inverso: chirality of the alpha-carbon atom inverted) and in which the sequence order has also

25 been reversed compared to the original amino acid sequence (retro=backwards).

In a further variant, the peptides according to the invention bind to parts of the amyloid beta peptide.

In a further variant, which is not according to the invention, the peptides have sequences which differ from the specified sequences by up to three amino acids.

30 Furthermore, sequences are also used as peptides which contain the sequences mentioned above.

In a further variant, which is not according to the invention, the peptides have fragments of the aforementioned sequences or have sequences homologous to the aforementioned sequences.

5 The invention relates to a peptide for use in medicine, preferably for the treatment of Alzheimer's disease.

According to the present invention, the peptide consists of D-amino acids.

10 In a further variant, the invention relates to a peptide according to the invention for in vivo use for the inhibition of formation of fibrils of amyloid beta peptides. The polymers according to the invention detoxify the A-beta oligomers or polymers formed therefrom, and also fibrils, by binding thereto and thus converting them into non-toxic compounds. Accordingly, the present invention also provides a peptide for in vivo use for detoxifying A-beta oligomers, polymers formed therefrom or fibrils.

The invention also relates in an embodiment to peptides according to the invention which are linked to a further substance.

15 In the context of the invention, the linking takes the form of a chemical bond as defined in Rompp Chemie Lexikon, 9th Edition, Volume 1, page 650 ff, Georg Thieme Verlag Stuttgart, preferably a main valency bond, particularly a covalent bond.

20 In a variant, the substances are medicaments or active ingredients, defined according to the Medicines Act § 2 or § 4 (19), as of September 2012. In an alternative, active ingredients are therapeutically active substances which are used as medicinally active substances. Preferably, anti-inflammatories are used.

In a further variant, the substances are compounds which enhance the effect of the peptides.

25 In an alternative, such compounds are amino pyrazole and/or amino pyrazole derivatives. Amino pyrazole derivatives in the context of the invention are 3-aminopyrazole-5-carboxylic acid or 3-nitropyrazole-5-carboxylic acid and also all derivatives thereof in which the heterocyclic CH group has been replaced by -CR- or -N- or -O- or -S- and also all peptidic dimers, trimers or tetramers, preferably amino pyrazole trimer, derived therefrom.

30 In a further alternative, said compounds are compounds which improve the solubility of the peptides and/or passage of the blood-brain barrier.

In an alternative, the peptides according to the invention have any desired combination of at least two or more features of the variants, embodiments and/or alternatives described above.

5 The invention also relates to a peptide according to the invention for in vivo use for binding to aggregated A-beta peptides.

The invention further relates to a method for preparing the peptide according to the invention by peptide synthesis, as is known to the skilled person, for example, organic synthetic methods for any compounds with low molecular weights (low-molecular weight compounds) and/or mutagenesis and recombinant preparation.

10 The invention further relates to a composition containing the peptide according to the invention, particularly for the treatment of Alzheimer's disease.

The present invention further relates to a composition containing the peptide according to the invention, for in vivo use for preventing toxic A-beta oligomers, or for destroying polymers or fibrils formed therefrom.

15 The "composition" according to the invention may be, e.g., a vaccine, a medicament (e.g. in tablet form), an injectable solution, a food or food supplement, containing the peptide according to the invention in a formulation being prepared due to technical expertise.

The invention also relates to a kit containing the peptide according to the invention.

20 In such a kit, the peptides according to the invention may be packaged in containers, optionally with/in buffers or solutions. All components of the kit can be packaged in the same container or separately. Furthermore, the kit may contain instructions for use thereof. Such a kit may contain, for example, in accordance with the invention, in an injection vial with a stopper and/or septum. Furthermore, a disposable
25 syringe can also be contained therein.

The present invention further relates to the use of the peptide according to the invention as a probe for the identification and qualitative and/or quantitative determination of amyloid beta oligomers or fibrils.

30 Further disclosed is also a probe, which is not according to the invention, containing the peptide according to the invention for the identification and qualitative and/or quantitative determination of amyloid beta oligomers.

Such probes are of major significance, since an early diagnosis of AD is thereby made possible. With early diagnosis, the disease may already be counteracted at a very early stage.

Such molecular probes may contain the polymer according to the invention and optionally dyes, fluorescent dyes, radioactive isotopes, (PET etc.), gadolinium (MRI), and also alternative substances suitable for imaging the probes and the patients, e.g. may be injected intravenously. After passage through the blood-brain barrier, the probes can bind to A-beta oligomers and/or plaques. The A-beta oligomers and/or plaques thus labelled can be visualized by imaging methods such as, e.g. SPECT, PET, CT, MRT, proton MR spectroscopy etc.

Furthermore, the invention also relates to the use of the peptide for the prevention of amyloid beta oligomers and/or amyloid beta peptide aggregates and/or amyloid beta fibrils.

The peptide according to the invention is also used for the detoxification of toxic amyloid beta oligomers and/or aggregates. It is particularly used to bind to amyloid beta oligomers and/or aggregates and thus to form amorphous, non-toxic aggregates.

The peptides according to the invention bind particularly effectively to A-beta oligomers, particularly to soluble A-beta oligomers.

A particularly strong binding of the peptides according to the invention to the target molecules is caused by high specificity and/or affinity of the inventive peptides for the target molecule. The complexes formed have a low dissociation constant (KD).

By means of the thioflavin T-test, it could be shown that the peptides according to the invention very efficiently inhibit the formation of fibrils of A-beta peptides, particularly SEQ ID NR: 1-5, in particular SEQ ID NR: 1 and/or SEQ ID NR: 5.

Examples

1.

The D3 variants listed in Table 1 were chemically synthesized.

Table 1: Listing of the D3 derivatives investigated

Description of the peptide (with explanation)	Amino acid sequence (D-enantiomer)
D3 (SEQ ID NR: 11)*	rprrlrhthnrr
NT-D3 (D3-N-Terminus) (SEQ ID NR: 6)*	rprrlr
RD1 (rational D3) (SEQ ID NR: 3)*	pnhhrrrrrttl
RD2 (rational D3) (SEQ ID NR: 2)	ptlrhthnrrrr
RD3 (rational D3) (SEQ ID NR: 4)*	rrptlrhthnrr
D3Δhth (D3 with deletion hth) (SEQ ID NR: 1)*	rprrlrnrr

* Comparative examples not according to the invention

The effects of the peptides described in the table on A-beta aggregation were investigated by means of density gradient centrifugation. Thus, A-beta was mixed with the respective peptide and the particles formed are separated according to size. The sample to be investigated was placed on the surface of the centrifuge tube in which a density gradient was charged, which consisted of layers of different iodixanol concentrations in this example. During the several hours of separation, the molecules sediment at different rates in the solvent, namely, more rapidly the larger the particles. The centrifugation is terminated at a suitable time point and different constituents of the sample are obtained in the various layers which are analysed by SDS-PAGE. A-beta without addition of peptide served as control. For example, the evaluation of runs with A-beta and A-beta with RD2 is shown in Fig. 1.

As can be seen in Fig. 1, A-beta could be detected in all fractions in the A-beta sample. This changes on addition of RD2. Here, fewer A-beta oligomers could be detected in the fractions 3-9 (A-beta oligomer fraction). Large aggregates are formed which are detectable in fractions 10-15. D3 showed no effect when the concentration of peptide used was 20 μM. In earlier studies, it was found that D3 in higher concentrations precipitates A-beta oligomers and converts them into large, amorphous and non-toxic aggregates (Funke et al., ACS Chem. Neurosci. 2010). All D-enantiomeric peptides which were characterized with A-beta using the method mentioned above were labelled with FITC. The amount of dye detectable in fraction 12 was used to estimate the binding strength of the respective D3 derivative to A-beta oligomers or the oligomer precipitating

effects thereof *in vitro*. The results for all peptides are shown in Table 2. It was shown that the peptide RD2 especially has a significant effect on A-beta aggregation compared to D3.

Table 2: Summary of results of the modulation of the A-beta1-42 aggregation behaviour of various D3 derivatives

Peptide	Peptide bound in fraction 12 [%]
A-beta control without peptide	0
D3*	4
NT-D3*	0
RD1*	3
RD2	20
RD3*	4
D3Δhth*	2

* Comparative examples not according to the invention

2.

The peptides were also tested with respect to their *in vitro* binding to various A-beta conformers (A-beta monomers, oligomers, fibrils) using ELISA. All peptides showed weak binding to A-beta monomers but relatively high affinity to oligomers and fibrils (Fig. 2). Interestingly, biotinylated monomers were not detected at the amino terminus, whereas the “seedless” monomers (aggregation seed-free preparation) were weakly bound to the carboxy terminus biotinylation. This may be interpreted as a potential indication of an epitope binding site of the D3 derivatives localized to the amino terminus.

3.

Some peptides were used in the thioflavin T (ThT) aggregation test. ThT is a fluorescent dye which binds to β -sheet-rich structures of various amyloid proteins and fluoresces at 440 nm on excitation. The emission may be correlated in this manner with the relative fibril content in the sample. ThT tests are used for measuring the fibrillation of A-beta and are used especially in ligands to detect potential inhibitory effects of these on A-beta aggregation. The peptides were used in a ratio of 1:10 (A-beta: peptide) with a 10 μ M A-beta solution. Fifteen hours later, after reaching the saturation phase in the A-beta control with no addition of ligand, the ThT fluorescence of the co-incubation of D-

peptides and A-beta was evaluated and presented as a percentage of the A-beta control incubation. This shows that all peptides used significantly reduce formation of A-beta fibrils (Fig. 3).

5 4.

Furthermore, D3 variants were identified which have an increased binding strength for A-beta, particularly for A-beta oligomers, with at least the same effect on the aggregation thereof, compared to D3. Stronger binding may suggest a more efficient therapeutic effect.

10 By means of a PepSpot analysis, using a membrane from JPT (Berlin) on which more than 300 D3 variants had been immobilized, variants were investigated in order to identify which bind with more affinity to A-beta (1-42). D3, D3 variants with varying amino acid sequences (so-called saturation mutagenesis—each amino acid was exchanged with their D-enantiomeric form from all 19 other naturally occurring amino acids) and controls were
15 immobilized on a trioxa membrane carboxy terminal. After the incubation with 5 μ M A-Beta(1-42) oligomers for 5 min and washing steps, the signals from bound A-Beta were detected via an anti-A-Beta antibody (6E10). The binding signals were evaluated as signal intensity/area. For the analysis, the signal intensity of the original D3 (mean value) was compared with that of the derivatives. The amino acid exchanges which resulted in
20 stronger binding of A-beta oligomers are summarized in Table 3.

Table 3: The D-amino acid sequence of D3 is shown in the first row in bold. In the rows below are listed the type and location of the amino acid exchanges which resulted in an increase in binding strength to A-beta oligomers.

R	P	R	T	R	L	H	T	H	R	N	R
		I		T		P		D	Q	Q	
						Q			E	D	
						R					
						S					

25

5.

In order to confirm the results and to obtain novel variants with even stronger affinity for A-beta by combination with the amino acid exchanges described in table 4,

further D3 variants were coupled to PepChip arrays from Pepscan and their A-beta binding strength was investigated. The selection of the variants were based on the results of the Pepspot membrane evaluation already described. In 6 independent experiments, the A-beta binding to the D3 derivatives was detected via coupled FITC labelling. In this case, 5 μ M A-Beta FITC was used. The detection was performed in a FLA8000 microarray scanner from Fujifilm and the binding signals were measured with the AIDA Array Metrix Software and evaluated with the aid of Mathlab Version 7.10.0.449. Fig. 4 shows the binding signals of a selected PepChip as example.

Five of the peptides immobilized on the PepChips exceeded the limit in at least four of six experiments and thus showed considerably higher binding affinity to A-beta compared to D3. These peptide sequences are summarized in Tab. 4. Among these, sequences with combined exchanges can be found from Tab. 3.

Table 4: Sequences of the D-peptides which have, compared to D3, in immobilised form, a stronger A-beta monomer binding strength

Sequence	Name
RPITRLHTDRNR (SEQ ID NR: 7)	DB1
RPITTLQTHQNR (SEQ ID NR: 8)	DB2
RPITRLRTHQNR (SEQ ID NR: 5)	DB3
RPRTLRLRTHQNR (SEQ ID NR: 9)	DB4
RPITRLQTHEQR (SEQ ID NR: 10)	DB5

Description of the figures

Fig. 1: Modulation of the A-beta1-42 aggregation behaviour by RD2, analysed by an iodixanol density gradient. The iodixanol gradient was overlayed with 100 μ l of a 80 μ M A-beta1-42 solution and 100 μ l of a 80 μ M A-beta and 20 μ M RD2 mixture. The mixture was then centrifuged at 259 000 $\times$ g for 3 h at 4° C. The 15 fractions (fraction 15 is the pellet boiled with the loading buffer) were harvested manually immediately after the centrifugation and analysed by Tris-Tricin-SDS-PAGE followed by silver staining. If the run was carried out under identical conditions with 20 μ M D3, no change was apparent compared to the run with only A-beta.

Fig. 2: ELISA for the relative quantification of the binding of the peptides to various A-beta1-42 conformers. Seedless monomers of carboxy terminal biotinylated A-beta (light grey bars) and oligomers (dark grey bars) and fibrils (black bars) of amino terminal biotinylated A-beta monomers were each immobilized at 5 µg/ml and the D-peptide applied at a concentration of 10 µg/ml. The relative quantification of the binding of the peptides in a duplicate determination is shown as absorption at 450 nm after subtraction of the background absorption.

Fig. 3: ThT aggregation test of A-beta1-42 for the quantification of the relative fibril content in the presence of various peptides. The concentration of A-beta was 10 µM and the peptides were added in a ratio of 1:10 (A-beta:peptide). The fluorescence of 10 µM A-beta was set to 100% and the values and standard deviations of the other incubations are given as percentages of this maximum value. None of the peptides showed a significant ThT fluorescence with no A-beta.

Fig. 4: Results of a PepChip experiment. Different D3 variants were immobilized on the PepChip. The PepChip was incubated with FITC labelled A-beta (5 µM). The FITC fluorescence intensity was read as a measure of the binding strength of the respective D3 derivatives. Values of the mean and standard deviation were calculated from the 11 values obtained for the D3 controls likewise applied at 11 different positions on the chip. Mean and standard deviation were added and the result was defined as the limit which a D3 derivative must attain in order to clearly have higher affinity for A-beta compared to D3. The integrals of the binding signals of all peptides are shown which exceed this limit. The individual bars are based on the means of three exactly identical peptide spots. a.u.: arbitrary units, relative fluorescence units. Due to the observed variance in the results, this entire experiment was carried out six times.

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Patentkrav

1. Peptid, indeholdende mindst en aminosyresekvens ifølge SEQ ID NO: 2 (RD2:ptlhthnrrrrr).
- 5 2. Peptid ifølge krav 1, **kendetegnet ved, at** peptidet er forbundet med et yderligere stof.
3. Peptid ifølge et af de foregående krav indeholdende en monomer valgt fra gruppen bestående af SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5,
10 SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 og/eller SEQ ID NO 10.
4. Peptid ifølge et af de foregående krav til anvendelse in vivo til binding af et aggregeret amyloid-beta-peptid.
15
5. Peptid ifølge et af kravene 1 til 3 til anvendelse in vivo til hæmning af fibrildannelse af amyloid-beta-peptider.
6. Peptid ifølge et af kravene 1 til 3 til anvendelse in vivo til at forhindre amyloid-
20 beta-oligomerer og/eller amyloid-beta-peptidaggregater.
7. Peptid ifølge et af kravene 1-3 til anvendelse in vivo til afgiftning af toksiske amyloid-beta-oligomerer og/eller aggregater.
- 25 8. Peptid ifølge et af kravene 1 til 3 til anvendelse i medicin.
9. Peptid ifølge et af kravene 1 til 3 til anvendelse i behandlingen og/eller forebyggelsen af Alzheimers sygdom.
- 30 10. S sammensætning indeholdende et peptid ifølge et af kravene 1-3.
11. Kit, indeholdende et peptid ifølge et af kravene 1 - 3 eventuelt med/i buffere eller opløsninger.

12. Anvendelse af et peptid ifølge et af kravene 1-3 in vitro som en sonde til identificering, kvantitativ og/eller kvalitativ bestemmelse af amyloid-beta-fibriller og/eller amyloid-beta-oligomerer.

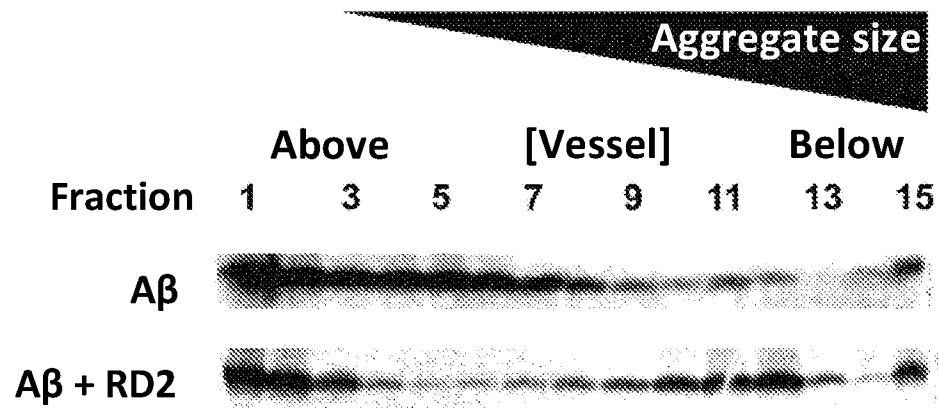


Fig. 1

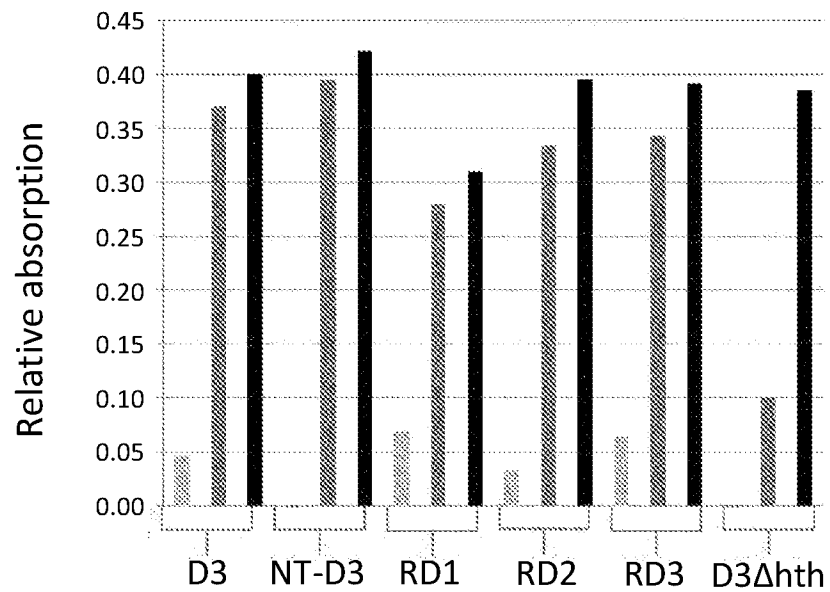


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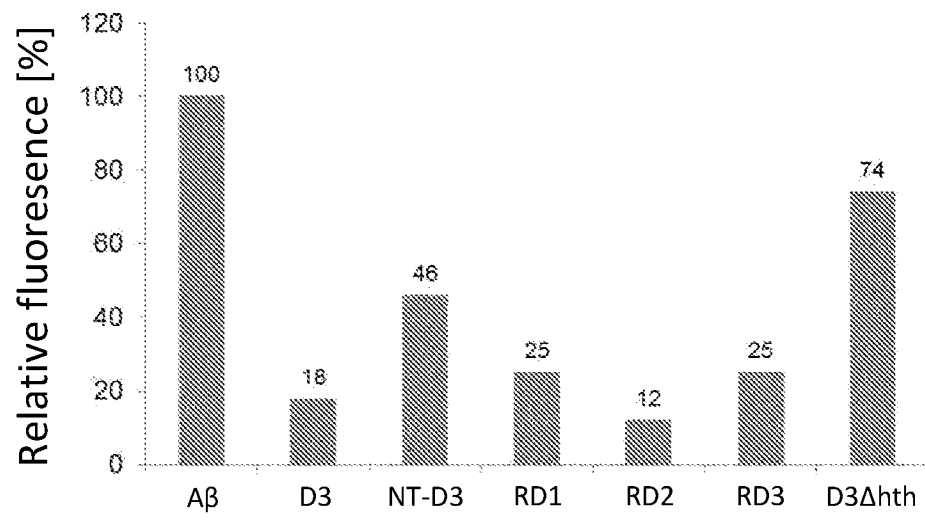


Fig. 3

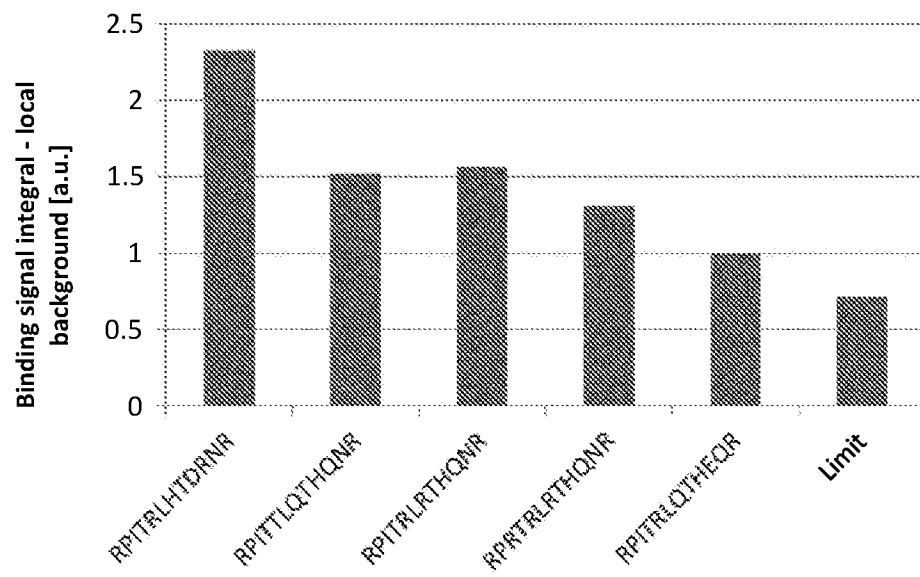


Fig. 4

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

