# **Supplementary Information**

# Inhibition of Amyloid Aβ Aggregation by High Pressures or Specific D-Enantiomeric Peptides

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# **Experimental Procedures**

# Materials

Uniformly <sup>15</sup>N-labelled, <sup>13</sup>C, <sup>15</sup>N-labelled and unlabelled Aβ(1-40) (4329.9 Da) was purchased from rPeptide (Bogard, Georgia, U.S.A.). Residues 1 - 40 of Aβ(1-40) correspond to residues 672 - 711 of βAPP770 (UniProtKB entry P05067). EDTA-d<sub>16</sub> and Tris-d<sub>11</sub> were purchased from Cambridge Isotope Laboratories (Andover, Massachusetts, U.S.A.). RD2 and RD2D3 are D-enantiomeric peptides with the amino acid sequences ptlht hnrrr rr-NH<sub>2</sub> (1598.86 Da) and ptlht hnrrr rrrpr trlht hrnr-NH<sub>2</sub> (3180.69 Da), respectively. The peptides were purchased as reversed phase high performance liquid chromatography purified products (P&E, Potsdam Germany).

#### Sample preparation

All NMR samples were pre-treated with hexafluoro-2-propanol (HFIP) for producing uniform, unaggregated A $\beta$ (1-40) peptide.<sup>[1]</sup> Details of the procedure are described by Munte et al..<sup>[2]</sup> For removing higher aggregates some samples were centrifuged at 50,000 g for 3 h at 277 K and only the supernatant was used for NMR spectroscopy. The samples used in Fig. 1 contained 750  $\mu$ M A $\beta$ (1-40) dissolved in buffer A in 99.5% <sup>2</sup>H<sub>2</sub>O or 60  $\mu$ M <sup>15</sup>N-labeled A $\beta$ (1-40) dissolved in buffer A in 92% <sup>1</sup>H<sub>2</sub>O / 8% <sup>2</sup>H<sub>2</sub>O. The sample used for the titration with RD2D3 (Fig. 2) contained 60  $\mu$ M <sup>15</sup>N-labeled A $\beta$ (1-40) dissolved in 50 mM Tris-d<sub>11</sub>, pH 7.00, 90 mM NaCl, 0.5 mM EDTA-d<sub>16</sub>, 0.2 mM dioxane and 1 mM NaN<sub>3</sub>, 50  $\mu$ M DSS) in 92% <sup>1</sup>H<sub>2</sub>O / 8% <sup>2</sup>H<sub>2</sub>O and 12  $\mu$ M RD2D3. The pH of samples was controlled by measuring the chemical shifts of Tris-d<sub>11</sub> signals. It was adjusted for the A $\beta$ -RD2D3 sample by addition of small amounts of DCl or NaOD until the chemical shift of the Tris-d<sub>11</sub> signal corresponded to the sample in absence of RD2D3. The A $\beta$ -concentration in both samples was identical.

The samples used in Fig. 3 contained 60  $\mu$ M (titration with RD2) and 65  $\mu$ M A $\beta$ (1-40) (titration with RD2D3), respectively, dissolved in 50 mM Tris-d<sub>11</sub>, pH 7.00, 90 mM NaCl, 0.5 mM EDTA-d<sub>16</sub>, 0.2 mM dioxane and 1 mM NaN<sub>3</sub>, 50  $\mu$ M DSS) in 92% <sup>1</sup>H<sub>2</sub>O / 8% <sup>2</sup>H<sub>2</sub>O. Concentration of the samples used for the titration study with the D-enantiomeric peptides contained 60  $\mu$ M <sup>15</sup>N-labeled A $\beta$ (1-40) dissolved in buffer A in 92% <sup>1</sup>H<sub>2</sub>O / 8% <sup>2</sup>H<sub>2</sub>O (sample C). A sample D was prepared by addition of a solution of 5 mM RD2D3 to sample C, resulting in a final concentration of 240  $\mu$ M RD2D3, respectively. The pH of samples was controlled by measuring the chemical shifts of Tris-d<sub>11</sub> signals. It was adjusted for sample D by addition of small amounts of DCl or NaOD till the chemical shift of the Tris-d<sub>11</sub> signal corresponded to that of sample C. Different peptide concentrations were obtained by mixing sample C with appropriate quantities of sample D.

# NMR spectroscopy

All NMR spectra were recorded with Bruker Avance 800 NMR spectrometer operating at a <sup>1</sup>H frequency of 800.20 MHz. Measurements were performed in a 5 mm TCI cryo probe. The absolute temperature inside the probe heads was calibrated by measuring the chemical shift difference  $\Delta\delta$  between the methyl and hydroxyl resonance of 100% methanol.<sup>[3]</sup> For the assignment of the chemical shifts of A $\beta$ (1-40) 2D-TOCSY (55 ms mixing time), 2D-NOESY (200 ms mixing time) and [<sup>1</sup>H-<sup>15</sup>N]-HSQC spectra were recorded at 278 K, pH 7.1. <sup>1</sup>H-NMR shifts were referenced to DSS used as internal standard (0 ppm) or to perdeutero Tris-d<sub>11</sub> whose

pressure and temperature dependent shifts have been mapped before. <sup>15</sup>N and <sup>13</sup>C chemical shifts were indirectly referenced to DSS according to Wishart et al.<sup>[4]</sup>. More stable values are obtained using amino acid specific combined chemical shifts  $\Delta \delta_{comb}$ .<sup>[5]</sup> Because of the required additivity of the chemical shifts the Hamming distance has been used in these calculations.

#### **High pressure NMR experiments**

High pressure NMR experiments were performed in a home-built on-line pressure system as described earlier by Arnold et al. 2002.<sup>[6]</sup> Pressure was applied to the NMR sample via pressurized fluids (methylcyclohexane or water) contained in high pressure lines (High Pressure Equipment Company, Linden, PA, USA). For generating the pressure a manually operated piston compressor and an air-to-liquid pressure intensifier (Barocycler<sup>®</sup>, HUB440, Pressure BioSciences Inc., South Easton, MA, USA), which is controlled by the spectrometer, were used. For the polymerization experiments at high Aβ concentrations pressure produced by a homemade manually operated piston compressor was transmitted via a high pressure line by methyl cyclohexane to borosilicate or quartz capillaries with an outer diameter of 3.8 to 4.0 mm and an inner diameter of 1 mm. Alternatively, for the experiments at low Aβ concentrations, pressure was transmitted by water to the high pressure ceramic cell (with an outer diameter of 5 mm and an inner diameter of 3 mm) from Daedalus Innovations LLC (Aston. PA. USA). A PET (polyethylene terephthalate) membrane acts as a flexible separator between the pressure fluid and the aqueous sample. To reduce the volume of the ceramic cell, a cylindric PEEK (polyether ether ketone) displacement body was used. A titanium autoclave connects the ceramic cell with the closed pressure line.

#### **Determination of Stokes radii by NMR**

Translational diffusion measurements were acquired with a modified pulsed field gradient stimulated echo sequence (PFGSTE)<sup>[7]</sup> including bipolar pulses<sup>[8]</sup> as One-Shot PFGSTE<sup>[9]</sup> on the same samples used in the other experiments shown in figure 3. The intensity  $I_x$  of a signal or a group of signals of compound x is dependent on its translational diffusion coefficient  $D_x$ , the gradient strength *G* (in percent of the maximum field strength) and a parameter  $\alpha$  that describes the pulse sequence used, the length and absolute maximum strength of gradients and length of different delays (eq. S1a). Usually the signal  $I_x(0)$  at gradient strength 0 is perturbed by artifacts and the intensity value  $I_x(G = a)$  at a small gradient strength *a* (in this paper 2%) is taken for scaling and  $I_{rel} = I_x/I_x(a)$  is plotted as a function of *G*.  $I_{rel}$  can be fitted by eq. S1b with  $\alpha$  a global fit parameter and  $C_x = I_x(0)/I_x(a)$  and  $D_x$  parameters characteristic for compound x.

$$I_x(G) = I_x(0) e^{-\alpha D_x G^2}$$
 (S1a)

$$I_{x,rel} = C_x(a) e^{-\alpha D_x G^2}$$
(S1b)

The Stokes radius  $r_x$  of compound x is defined by

$$D_{\rm x} = \frac{k_{\rm B} \cdot T}{6\pi \cdot \eta \cdot r_{\rm x}} \tag{S2}$$

with  $k_{\rm B}$  the Boltzmann constant,  $\eta$  the viscosity of the solution and T is the absolute temperature. The Stokes radius  $r_{\rm X}$  for a compound x and can be calculated from the Stokes radius  $r_{\rm Y}$  of a compound y as

$$r_{\rm x} = r_{\rm y} \frac{D_{\rm y}}{D_{\rm x}}$$
(S3)

In the calculations we used a hydrodynamic radius  $r_{\rm H}$  of 0.307 nm for Tris<sup>[10]</sup> that was contained in the sample.

## **Quantification of data**

For quantitative experiments 90-degree pulses and a repetition time of 13 s was used that is larger than 5-times the  $T_1$ -values of the compounds under investigation. The  $T_1$ -times of the relevant components were determined by inversion recovery experiments. At 277 K the  $T_1$ -times of the methyl groups of DSS, Tris and the H<sup> $\delta$ </sup> resonances of the tyrosine and histidine residues of free A $\beta$  were 1.4 s, 1.1 s, and < 0.8 s, respectively. When the sample contains as reference DSS, the DSS signal intensity increases with pressure more strongly than expected from the compression of the solvent alone. This is in agreement with the observation that DSS interacts with A $\beta$ -aggregates.<sup>[11]</sup> Consequentially, it cannot be used as an internal standard for the concentration determination of A $\beta$ . Therefore quantification of NMR-visible resonances was performed by using the residual perdeuterated signal of Tris-d<sub>11</sub> added to the sample with a known concentration. The degree of perdeuteration of Tris-d<sub>11</sub> was determined as 99.31 ± 0.03% from the integrals of the corresponding methyl resonances.

#### Analysis of the polymerization reaction

The pressure dependence of the Gibbs free energy  $\Delta G_{1i}$  is given<sup>[12]</sup> as

$$\Delta G_{1i}(T,P) = \Delta G_{1i}^0(T_0,P_0) + \Delta V_{1i}^0(P-P_0) - \frac{\Delta \beta_{1i}^{0i}}{2}(P-P_0)^2$$
(54)

where  $\Delta V_{1i}^{0}$  and  $\Delta \beta_{1i}^{0'}$  are the differences of the partial molar volumes and of the partial molar compressibility factors between state 1 and state *i* at temperature  $T_0$  and pressure  $P_0$ , respectively. When the total concentration  $c_T$  of monomeric units is larger than the dissociation constant  $K_D$  of a monomeric unit M from the polymer P, the concentration of the free monomer  $c_1$  is given by<sup>[13]</sup>

$$c_1 = K_D = e^{-\frac{\Delta G}{RT}} \tag{S5}$$

If the condition  $c_1 < K_D$  holds, for a linear polymer with a maximum chain length N,  $c_1$  can be described by

$$c_1 = \frac{c_T (1 - c_1/K_D)^2}{N(c_1/K_D)^{N+1} - (N+1)(c_1/K_D)^{N+1}}$$
(S6)

For large chain lengths, *c*<sup>1</sup> can be approximated by

$$c_1 = c_T \left(1 - \frac{c_1}{\kappa_D}\right)^2 \tag{57}$$

Equation S7 has two solutions but only one solution is physically meaningful

$$c_{1} = K_{D} \left( 1 + \frac{K_{D}}{2 c_{T}} - \sqrt{\frac{K_{D}^{2}}{4 c_{T}^{2}} + \frac{K_{D}}{c_{T}}} \right)$$
(58)

Note that in a high pressure experiment the change of the total concentration with pressure has to be taken into account because of the compressibility of the solvent. The dependence of the dissociation constant of the monomer from the polymer on pressure can again be described by equation S4.

Assuming (in agreement with the literature) that in a good approximation only monomeric A $\beta$  is visible in the solution NMR spectra, the concentration  $c_1$  of the monomers can be obtained from the integrals of the resonance lines when an internal standard with known concentration is available (in our case Tris). The concentration  $c_1$  is then given by

$$c_1 = c_R \frac{V_i}{V_R} \tag{S9}$$

with  $c_{R}$  the concentration of the reference (that has to be corrected in high pressure NMR spectroscopy for the compression of the solvent),  $V_{R}$  the integral of the corresponding signal in the 1D or 2D spectrum, and  $V_{i}$  the integral of a given atom in amino acid *i* in the 1D spectrum or a cross peak of the amino acid *i* in the HSQC spectrum. The quantification of the NMR visible peptide was performed by comparing the integrals of non-exchangeable protons the H<sup>ε</sup>-resonance line of Tyr10 and the H<sup>ε1</sup>-resonances of His6, His13, and His14 with the signal of the Tris buffer with known concentration.

#### Sedimentation velocity analysis

Sedimentation velocity (SV) analysis was carried out to determine the size distributions of A $\beta$ (1-40) peptides in the presence or absence of RD2D3. All experiments were performed using a Beckman Optima XL-A ultracentrifuge (Beckman-Coulter, Brea, CA, USA), equipped with a fluorescence detection system<sup>[14]</sup> (Aviv, Lakewood, NJ, USA) and a four-hole rotor. Fluorescein isothiocyanate (FITC) labelled A $\beta$ (1-40) peptides were commercially available (Catalog No. H-6326, Bachem, Bubendorf, Switzerland) and the fluorophore was connected to the additional alanine at the N-terminus of A $\beta$ (1-40) (hereafter referred to as FITC-A $\beta$ (1-40)). Peptide purity determined by HPLC was 92.4% according to the manufacturer. RD2D3 was synthesized and purchased from peptides & elephants GmbH (Potsdam, Germany). FITC-A $\beta$ (1-40) was first dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol and divided into small aliquots. All aliquots were then lyophilized and stored at -80 °C before use. For improved dissolution FITC-A $\beta$ (1-40) was predissolved in 6  $\mu$ l 10 mM NaOH before adding buffer containing 50 mM Tris, 90 mM NaCl, 0.5 mM EDTA and 0.01% Tween-20. The working concentration of FITC-A $\beta$ (1-40) was 1  $\mu$ M. RD2D3 stock solution was added into FITC-A $\beta$ (1-40) solutions

accordingly to get final concentrations of 0  $\mu$ M, 1  $\mu$ M, 5  $\mu$ M and 20  $\mu$ M. The final volume for each sample was 110  $\mu$ l. For AUC measurement 100  $\mu$ l samples were loaded into 3 mm titanium double sector cells with quartz glass windows. After 3.5 h incubation at 10 °C in the machine all samples were centrifuged at 60,000 rpm (~257,000g) at 10 °C for 15 h. Note that pH values for all samples were maintained at 7.0 during sample preparation and ultracentrifugation. The software package Sedfit (Version 15.01b) was used to analyze all the datasets<sup>[15]</sup>. In detail, continuous distribution *c*(*s*) Lamm equation model<sup>[16]</sup> was applied to evaluate size distributions of samples treated with or without RD2D3. Fitting parameters including buffer density and viscosity were calculated using Sednterp (Version 20130813 BETA).<sup>[17]</sup> The partial specific volume of FITC-A $\beta$ (1-40) was determined according to the method of Durchschlag and Zipper.<sup>[18]</sup> The graphical outputs were generated by GUSSI (Version 1.2.1)<sup>[19]</sup> and the final *s*-values were corrected to *s*-values in water at 20 °C (*s*<sub>20,w</sub> - values).

#### Surface plasmon resonance

Surface plasmon resonance (SPR) spectroscopy was performed on a Biacore T200 instrument (GE Healthcare, Uppsala, Sweden) at 298 K. N-terminally biotinylated A $\beta$ (1-42) was immobilized on a streptavidin coated sensor chip (GE Healthcare, Uppsala, Sweden) and a concentration series of RD2D3 ranging from 0.16 to 5  $\mu$ M was injected over the surface. Between each cycle, the surface was regenerated using 2 M guanidine hydrochloride. All measurements were performed in 20 mM phosphate buffer pH 7.4 including 100 mM sodium chloride. For evaluation, the response levels at the end of the association phase were plotted against the applied concentrations and fitted using the Langmuir steady state fit model implemented in the Biacore Evaluation Software 2.0 with RI = 0.

#### **Animal experiments**

In the present study 10 eight months old female homozygous tg-SwDI mice (human APP with Swedish K670N/M671L, Dutch E693Q and Iowa D694N mutations on a C57BL/6 background<sup>[20]</sup>) were used. The original mice were purchased from JAX (The Jackson Laboratory, USA) and maintain our own colony at the University of Alabama in Birmingham. Before treatment, the mice were housed 4/cage in our facility in a controlled environment (temperature 22 °C, humidity 50-60%, light from 06:00 a.m. -6:00 p.m.) with food and water available ad libitum. Following the implantation of the Alzet minipumps the mice were housed individually. The experiments were conducted according to the local Institutional Animal Care and Use Committee (IACUC) guidelines.

#### **Behavioral tests**

The mice were tested at the end of the treatment period in the following behavioral tests (open field, zero maze and Morris water maze tests).

# Open field test

The open field test was performed to evaluate the on anxiety-related behavior of the treated mice. The arena (42 cm  $\times$  42 cm surrounded with clear Plexiglass sides (20 cm high)) was subdivided into two areas:

border and center. The mice were monitored with a camera driven tracker system (Ethovision XT10, Noldus, The Netherlands) for 4 min. Time spent in the border and center was analyzed. After each testing day, and in between the mice, the apparatus is wiped out with chlorhexidine and 70% ethanol and allowed to air-dry.

#### Zero maze

Additionally to the open field test, the zero maze was accomplished to assess the anxiety-related behavior of the mice. The maze consisted of a circular arena (65 cm diameter) that is raised 40 cm above the table. The maze was separated into four equal parts by two 15 cm high walls of opaque material and two only 0.5 cm high walls. Therefore, it consisted of two open and two closed areas. The mice were put into the circle and monitored for 4 min with a camera driven tracker system (Ethovision XT10, Noldus, The Netherlands). Analyzed was the time mice spent in the open and closed arms. After each testing day, and in between mice, the apparatus is wiped out with chlorhexidine and 70% ethanol and allowed to air-dry.

#### Morris Water Maze

The mice were tested for 5 days in a Morris Water Maze (MWM). The maze consisted of a blue circular tank of clear water ( $23 \pm 1^{\circ}$ C). The mice were placed in the water at the edge of the pool and allowed to swim in order to locate a hidden, but fixed escape platform, using extra maze cues. On day 1, the mice were placed in the pool and allowed to swim freely for 60 s to find the hidden platform (or until they find the hidden platform); each animal was tested for four trials per day. A maximum swim time per trial of 60 s was allowed; if the animal did not locate the platform in that time, it was placed upon it by the experimenter and left there for 10 s. The inter-trial interval was 120 s. Each start position (east, north, south, and west) was used equally in a pseudo random order and the animals were always placed in the water facing the wall. The platform was placed in the middle of one of the quadrants of the pool (approximately 30 cm from the side of the pool). The mouse's task throughout the experiment was to find, and escape onto the platform. The animal was monitored by Ethovision 7.1.

#### Statistics

All statistical calculations were performed using SigmaPlot Version 11 (Systat Software, Germany) and OriginPro8.5G. Data is represented as mean  $\pm$  SEM (behavioral tests), p > 0.05 was considered as not significant (n.s.). Mann-Whitney Rank Sum Test was used to analyze the results of the open field test and zero maze. Escape latency to the platform within the MWM was considered as not normal distributed and therefore analyzed by Friedman Repeated Measures Analysis of Variance.

# **Results and Discussion**

#### Dynamics of A<sub>β</sub>-polymerisation

Starting with monomeric  $A\beta$  a dynamic equilibrium is obtained that leads to virtually reversible population changes as response to external perturbations such as pressure and temperature. After a rapid

pressure jump of 40 MPa an equilibrium state is typically obtained after two to three hours at 283 K (Fig. S1). The repolymerisation at low temperatures after depolymerisation is a slow process of the order of 12 hours when enough seeds are still present in the sample. In the absence of seeds after complete depolymerization the time scale for polymerization is of the order of one week at 283 K.



**Figure S1.** *Time dependent depolymerization after pressure jump.* The concentration of Aβ-monomers  $c_1$  is plotted as a function of the time *t* after a pressure jump from 3 MPa to 40 MPa at 283 K. The sample contained 750  $\mu$ M Aβ(1-40), 50 mM Tris-d<sub>11</sub>, 90 mM NaCl, 0.5 mM EDTA-d<sub>16</sub>, 1 mM NaN<sub>3</sub> in D<sub>2</sub>O, pH 7.0. The data were fit by  $c_1(t) = c_1(\infty) - (c_1(\infty) - c_1(O))e^{-k_t}$  with *k* the apparent rate constant for dissociation. k is 0.38 10<sup>-3</sup> s<sup>-1</sup> with  $c_1(0) = 157 \,\mu$ M and  $c_1(\infty) = 280 \,\mu$ M.

#### Affinity of RD2D3 to A<sub>β</sub> monomers

Surface plasmon resonance (SPR) spectroscopy was used to determine the affinity of RD2D3 to A $\beta$ . Biotinylated A $\beta$ (1-42) monomers were on a streptavidin coated sensor chip (Fig. S2) at 298 K. The data can be well explained by assuming a homogeneous interaction. With this assumption the peptide binds with high affinity to A $\beta$  with a  $K_D$  value smaller than 500 nM.



**Figure S2.** Affinity determination of RD2D3 to A $\beta$  monomers. Surface plasmon resonance (SPR) spectroscopy was used to determine the affinity of RD2D3 to A $\beta$ . Biotinylated A $\beta$ (1-42) monomers were coupled on a streptavidin coated sensor chip and binding of the indicated RD2D3 concentrations was recorded for 180 s. (A) For evaluation, the binding responses at the end of the association phase were plotted over the RD2D3 concentrations and fitted with a steady state binding model. (B) The shown sensorgrams and fit are exemplary for four independent measurements. The  $K_D$  value is presented as mean  $\pm \sigma$ .

# Mechanism of heteropolymer formation

At higher relative concentrations of RD2 or RD2D3 the sharp NMR visible signals of Aβ become weaker and are strongly quenched. This indicates the formation of large mixed polymers with line widths too large to be observable by solution NMR spectroscopy as, in fact, they were observed by electron microscopy.<sup>[21]</sup> The reduction of the peak intensities in 1D- or 2D-spectra provides a quantitative measure for formation of these large polymers (expressed in concentrations of monomer units). In principle, monomeric free Aβ and Dpeptides should be observable as well as their small complexes.

In the 1D-spectra the signals of free A $\beta$  are characterized by relatively sharp lines that show a strong reduction of signal intensity in the presence of D-peptides. The methyl signals of Val12 and Val18 can be observed without overlap with lines of the D-peptides (Fig. 3). The concentration of the visible D-peptides can be estimated from the signals of the methylene protons H<sup>e</sup> of arginines at 3.20 ppm are best suited since A $\beta$  contains only one arginine residue but RD2 and RD2D3 contain five and ten arginine residues, respectively. In the range between 0.8 and 1.0 ppm only the signals of methyl groups of valine, leucine and isoleucine residues are to be expected. It is dominated by the signals of A $\beta$ (1-40) that contains six valines, two leucines and two isoleucines. RD2 and RD2D3 contain only one and two leucine residues, respectively, with methyl resonances at 0.89 ppm and 0.83 ppm (the methyl groups of two leucines in RD2D3 have nearly identical chemical shifts). At higher concentrations of the D-peptide only a broad peak remains with weak sharp signals on the top corresponding to the resonance frequencies of the free peptides (Fig. 3A, B). The broad peak presumably represents the A $\beta$ -D-peptide complex which is also supposedly seen in [<sup>1</sup>H-<sup>15</sup>N]-spectra (Fig. S3). Its concentration at intermediate D-peptide concentrations can be determined by integrating this signal after subtracting the sharp signals from free A $\beta$  and free D-peptides in the 1D-spectra.

The titration experiments were performed by mixing different quantities of two samples, a sample containing A $\beta$ -only and a sample containing A $\beta$  in the same concentration and a D-peptide in high concentration. This method ensured that the total A $\beta$  concentration was constant in all experiments.



**Figure S3.** Formation of  $A\beta$  heterodimers. Superposition of [<sup>1</sup>H, <sup>15</sup>N] HSQC spectra of 65  $\mu$ M uniformly <sup>15</sup>N enriched A $\beta$  in absence (black) and presence of RD2D3 with a concentration ratio 1:0.4 (red), 1:0.8 (blue) and 1:2 (green). Inserts show only 1:0 and 1:2 ratios. Temperature 283 K.

# **Biological effects of RD2D3**

The positive biological effects of the D-enantiomeric peptides D3 and RD2 on the development of Alzheimer's disease in mice has been shown already.<sup>[21-24]</sup> For the variant RD2D3 they were not studied yet but are to be expected. We treated transgenic APPSwDI mice intraperitoneal (i.p.) for 4 weeks with 8 mg/kg/day of RD2D3 using Alzet micropumps. The Morris water maze (MWM) test (Fig. S4) is the most frequently used behavioral test of spatial learning and memory performance for rodents that relies on distal clues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent.



**Figure S4.** Morris water maze test in transgenic mice with and without treatment with RD2D3. Transgenic (APPSwDI) mice were treated with 8 mg/kg/day RD2D3 or saline i.p. for 4 weeks. Data is represented as mean  $\pm$  standard error of mean (SEM), \*p ≤ 0.05.

The intraperitoneal (i.p.) treatment of transgenic Alzheimer mice (tg-APPSwDI) with RD2D3 significantly improved the cognitive performance of the animals. Long-term memory evaluation in the Morris water maze did show significant differences between RD2D3 treated animals compared to vehicle treated tg control in escape latency (Fig. S4). The open field test provides data for the assessment of novel environment exploration and for the effects of drugs on anxiety-related behavior of mice, which can also be evaluated by zero maze. Changes in the behavior are hints for hypo- or hyperactivity. Importantly, RD2D3 had no influence on the general behavior of tg-APPSwDI mice as demonstrated by no differences in time spend in the center or open arena between saline and RD2D3 treated animals in the open field and the zero maze experiments (Fig. S5). Therefore, APPSwDI mice show no changes in general activity and fear upon RD2D3 treatment.



Figure S5. Influence of RD2D3 on the behaviour of mice. (Left) open field test, (right) zero maze test.

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# **Author Contributions**

I.A.C., C.E.M., M.B.E., W.K. and H.R.K. performed and evaluated the NMR experiments on Aβ. T.Za. carried out and evaluated with L.N.-S. the ultracentrifugation experiments. T.vG., I.K. and D.W. planned the animal experiments. T.vG. and I.K. performed, supervised and evaluated with J.K. the in vivo and ex vivo experiments. T.Zi. performed and evaluated the SPR experiments. H.R.K. devised the study together with D.W.. All authors read the manuscript carefully and discussed the results.