Supporting information

Toward the mode of action of the clinical stage all-D-enantiomeric peptide RD2 on Aβ42 aggregation

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Table S1. Buffer viscosities and densities at 20 °C.

Buffer viscosity (P) [a]	20 mM sodium phosphate, 50 mM NaCl	0.01015
	55 mM Tris-HCl, 50 mM NaCl	0.01024
	H_2O	0.01002
Buffer density (g/cm^3) [a]	20 mM sodium phosphate, 50 mM NaCl	1.003
	55 mM Tris-HCl, 50 mM NaCl	1.002
	H_2O	0.9982

[a] Values were calculated using the software Sednterp (Version 20130813 BETA).

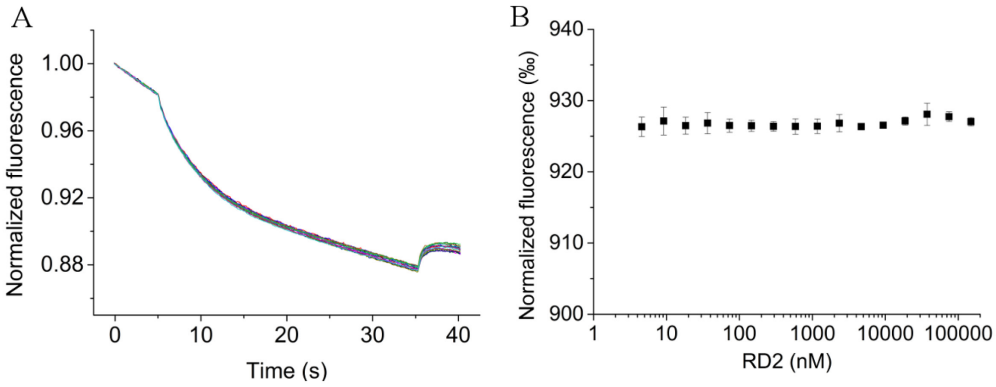

Figure S1. Microscale thermophoresis analysis of fluorescein in the presence of various concentrations of RD2. Fluorescein at 40 nM was titrated with RD2 in 20 mM sodium phosphate, 50 mM NaCl, 0.01% (v/v) Tw20 (pH 7.4) at 22 °C. Time traces from one measurement are shown in (A). The quantification of thermophoresis based on the time traces is displayed in (B). Experiments were performed in triplicate.

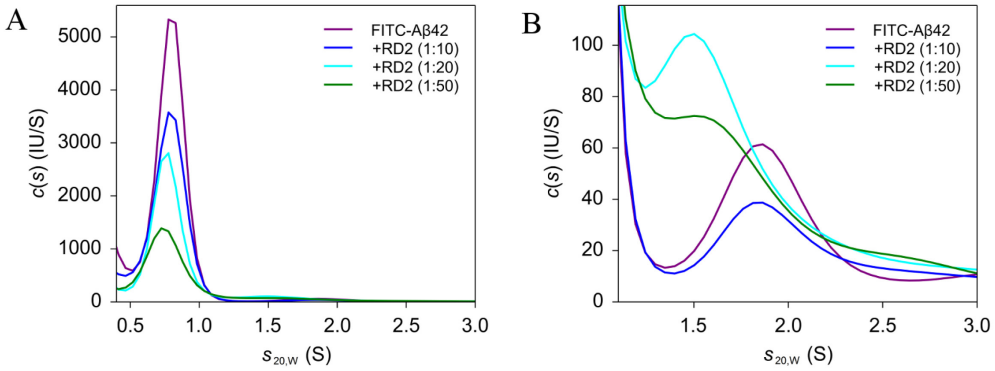

Figure S2. Sedimentation coefficient distribution analysis of 0.33 µM FITC-Aβ42 incubated with different concentrations of RD2 in 55 mM Tris-HCl, 50 mM NaCl (pH 7.4) in the presence of 0.01% Tw20 (v/v) at 20 °C (A). All samples were centrifuged at 60,000 rpm for 15 h. Sedimentation profiles were analyzed using the continuous distribution c(*s*) Lamm equation model. *s*-values were standardized to the *s*-value in pure water at 20 °C ($s_{20,w}$). The magnification of the size distribution within 1 and 3 S is displayed in (B).

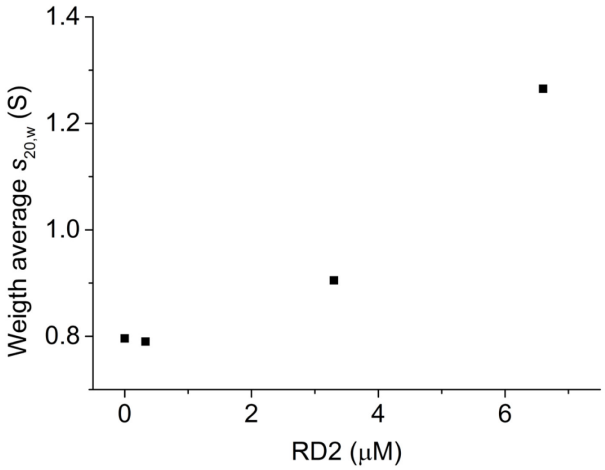

Figure S3. The weight average $s_{20,w}$ of FITC-Aβ42 samples incubated with different concentrations of RD2 as determined by peak integration of the size distribution in *c*(*s*) analyses shown in Figure 3 in the main text.

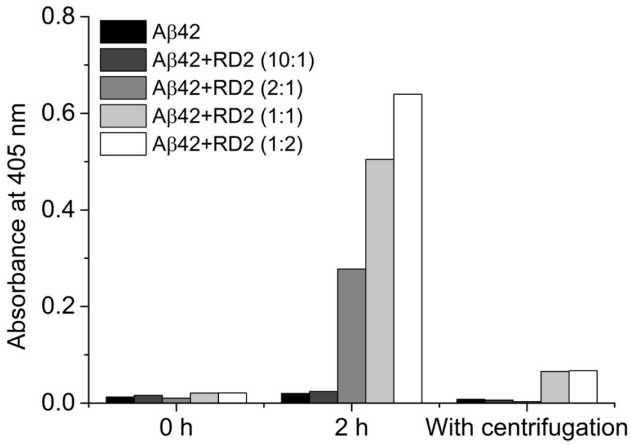

Figure S4. Turbidity assay of Aβ42 in the absence or presence of RD2. 40 μM Aβ42 was incubated with different concentrations of RD2 (molar ratios 10:1, 2:1, 1:1 and 1:2, Aβ42:RD2) in 20 mM sodium phosphate, 50 mM NaCl, 0.01% (v/v) Tw20 (pH 7.4) at ambient temperature. The turbidity was measured using the absorbance at 405 nm at 0 and 2 h of incubation. Afterwards all samples were centrifuged at 726 *g* for 15 min and the turbidity was measured again for the supernatants.

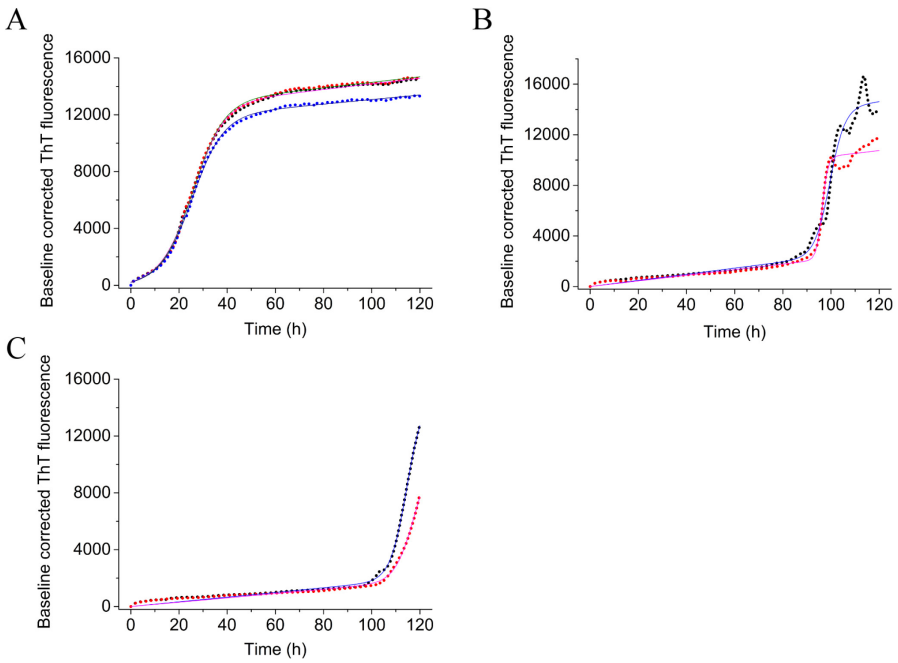

Figure S5. Amylofit analysis of the ThT kinetics for Aβ42 in the absence or presence of RD2. 20 μM Aβ42 proteins alone (A), 20 μM Aβ42 proteins with 1 μM RD2 (B) and 20 μM Aβ42 proteins with 2 μM RD2 (C) were incubated in 20 mM sodium phosphate, 50 mM NaCl (pH 7.4) at 20 °C for 120 h. All kinetics were fitted using the webserver Amylofit with the customized equation (eq.1). Raw data and fitted data are presented in short dot and solid curves, respectively. Samples were prepared in duplicate (red and blue).

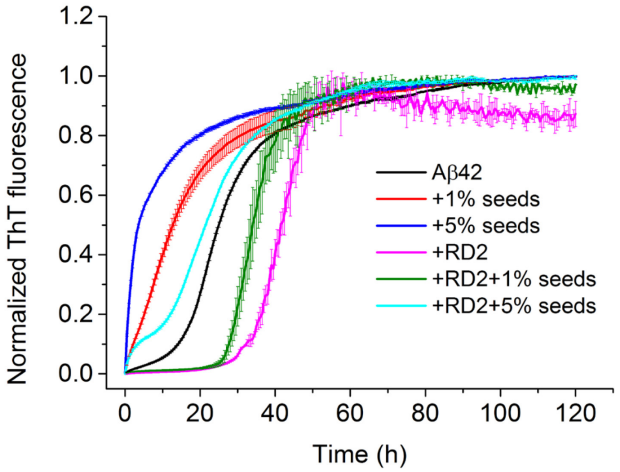

Figure S6. Seeding experiments of Aβ42 in the absence or presence of 0.1 fold RD2. Aβ42 at 10 µM was incubated with or without 1 µM RD2 in the presence of different concentrations of Aβ42 seeds in 20 mM sodium phosphate, 50 mM NaCl (pH 7.4) at 22 °C. The averaged data was normalized to maximum plateau height. Samples were prepared in duplicate.

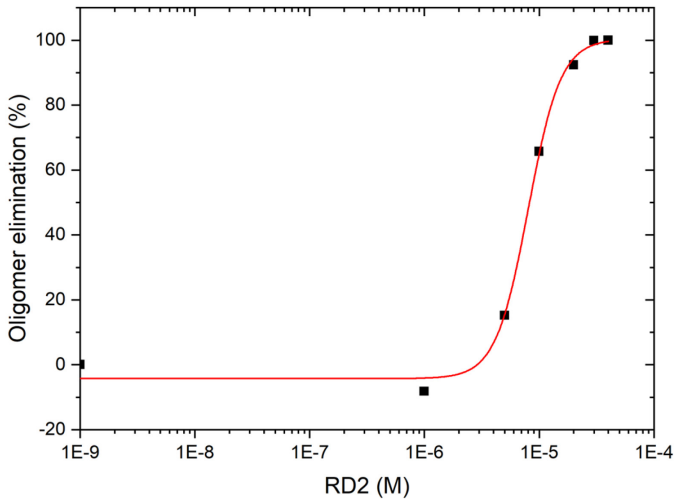

Figure S7. Samples of 80 µM Aβ42 were analyzed with substoichiometric concentrations of RD2 (1-15 µM) in the QIAD assay. Data taken from van Groen et al. 2017 [1] were reevaluated and fitted with a Hill equation; $y = START + (END - START) * x^n/(k^n + x^n)$. A concentration of 10^{-9} M for RD2 was assigned to Aβ42 samples alone for data evaluation. The obtained Hill coefficient (n) is 3.

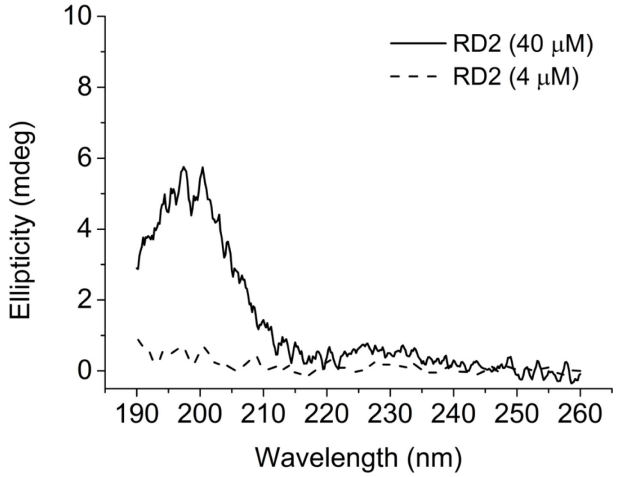

Figure S8. CD spectra of RD2 at different concentrations. RD2 samples at 40 μM and 4μM were prepared in 20 mM sodium phosphate, 50 mM NaF (pH 7.4) and were subjected to CD measurements.

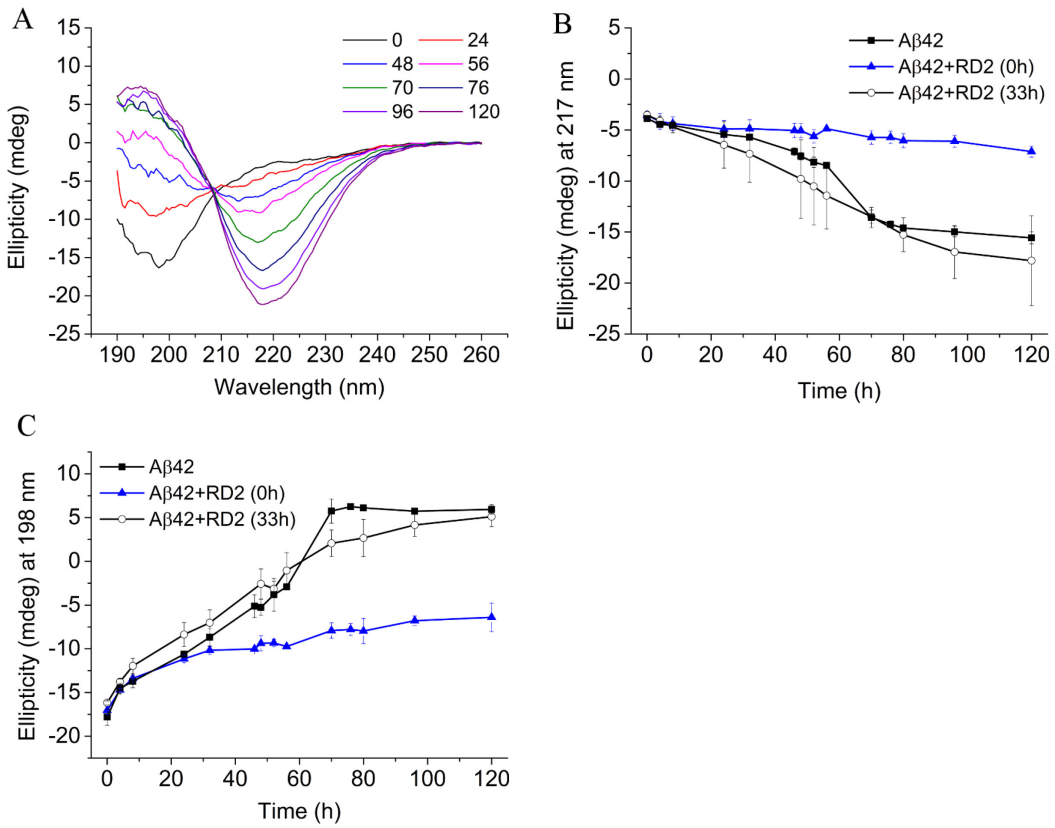

Figure S9. CD analysis of 40 μM Aβ42 with 4 μM RD2 addition after Aβ42 was incubated for 33 h in 20 mM sodium phosphate, 50 mM NaF (pH 7.4) at 20 °C. (A) CD spectra were obtained using the same experimental condition as measurements shown in Figure 5. Transition kinetics by plotting ellipticity values at 217 nm (B) and 198 nm (C) against the incubation time are displayed and compared with kinetics for 40 μM Aβ42 alone and 40 μM Aβ42 with RD2 incubation from the beginning (0 h).

Reference

1. van Groen, T., Schemmert, S., Brener, O., Gremer, L., Ziehm, T., Tusche, M., Nagel-Steger, L., Kadish, I., Schartmann, E., Elfgen, A., Jurgens, D., Willuweit, A., Kutzsche, J., and Willbold, D. (2017) The Abeta oligomer eliminating D-enantiomeric peptide RD2 improves cognition without changing plaque pathology, *Scientific reports 7*, 16275.