A new mode of action for the treatment of AD

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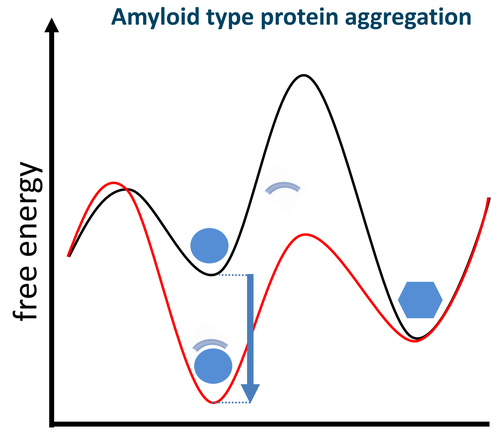
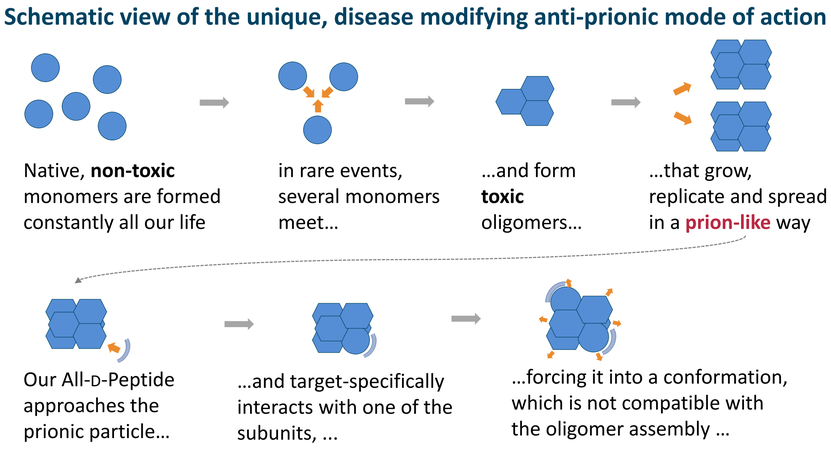
Objectives

Alzheimer´s Disease (AD) currently affects more than 30 million people worldwide, but to date, no curative or disease modifying treatment is available. We have developed a new mode of action (MoA) to disassemble toxic Aβ oligomers into functional monomeric Aβ building blocks. This mode of action is realized by an all-D-enantiomeric peptide ligand named RD2 that stabilizes Aβ monomers in their native intrinsically disordered conformation. This is a purely thermodynamic MoA, which does not require inhibition of enzymes or ion channels, and is therefore not prone to show side effects. The aim of this study was to prove this MoA.

Mode of Action of Anti-Prionic All-D-Peptide RD2





Methods

Sedimentation velocity analysis
20 µM Aβ42 was incubated with or without 0.1 fold RD2 in 20 mM sodium phosphate, 50 mM NaCl (pH 7.4) at 20 °C for 24 h. All samples were centrifuged at 45.000 rpm, 20 °C for 15.5 h. Data was analyzed using the c(s) model implemented in Sedfit to obtain the sedimentation coefficient distribution.

Density gradient centrifugation (DGC)
Brain homogenates 10 % (w/v) were fractionated by DGC on discontinuous gradients of iodixanol (prepared according to[2])

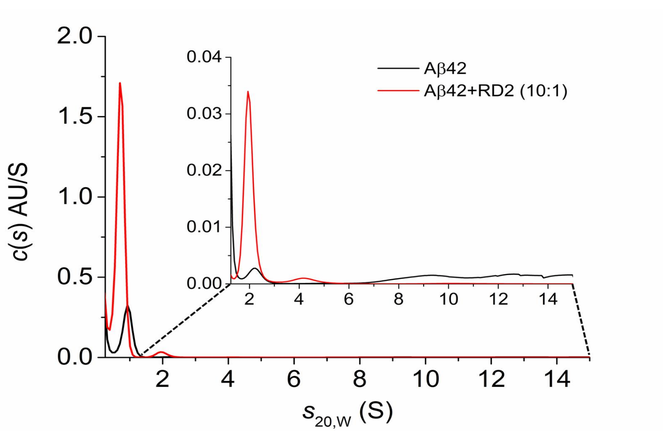
***Ex-vivo* target engagement pre-incubation with RD2**
Fraction 10 of human brain homogenate, containing a large portion of Aβ-assemblies, was pre-incubated with different concentrations of RD2. Non fractionated human brain homogenates were incubated with RD2 or control D-peptides. Samples were analyzed by sFIDA assay.

Surface-based fluorescence intensity distribution analysis (sFIDA) assay
Anti-Aβ antibodies (Nab 228) are covalently immobilized on a glass surface. Captured Aβ species are labeled by two different fluorophore coupled antibodies. Since the antibodies epitopes overlap monomers cannot be captured and labeled by two further antibodies at the same time, making the assay specific for aggregates of Aβ.

Results

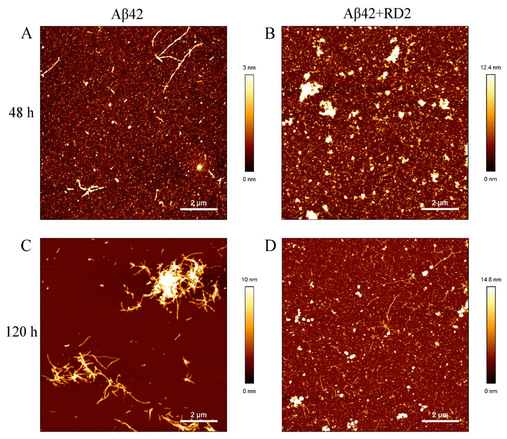
RD2 remodels the aggregation of Aβ42 by stabilizing monomeric species

Sedimentation velocity analysis of Aβ42 in the absence or presence of substoichiometric RD2



Overall distributions and an amplification of 1.25 to 14 S

Morphologies of Aβ42 in the absence or presence of RD2 acquired by AFM

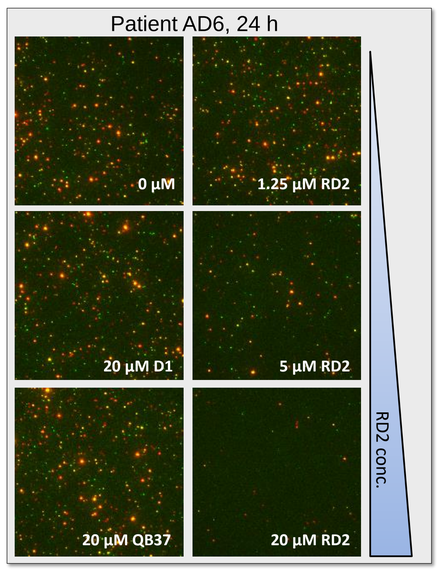


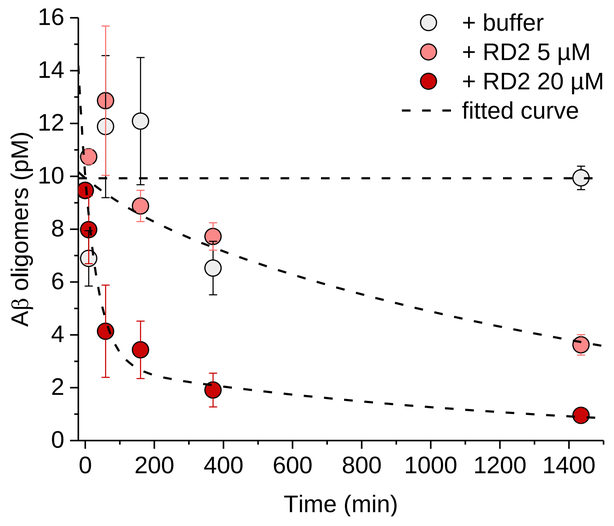
[1] Zhang et al., ACS Chem Neurosci 2019

Target Engagement - *ex vivo*

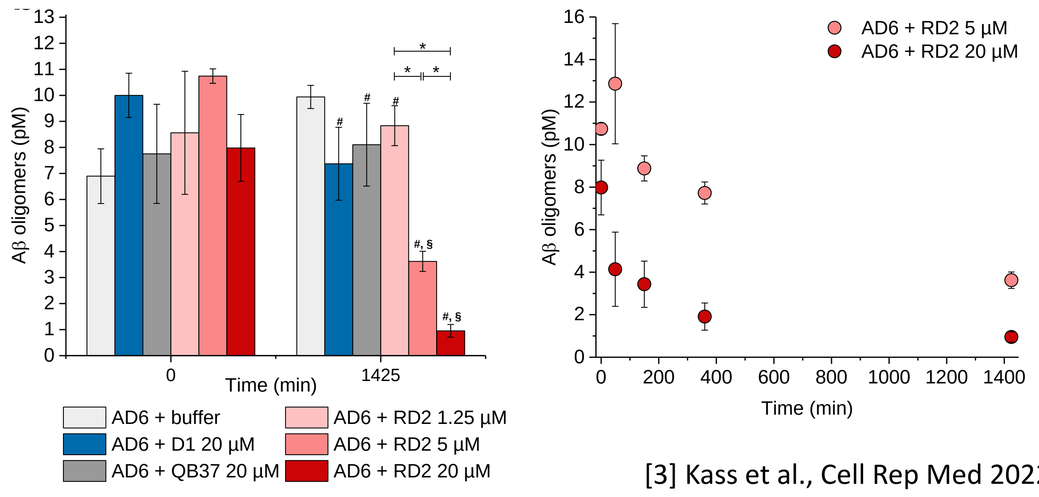
Ex vivo RD2 treatment reduces Aβ oligomer concentration in human brain tissue

Aβ oligomer elimination kinetics: dose- and time-dependence





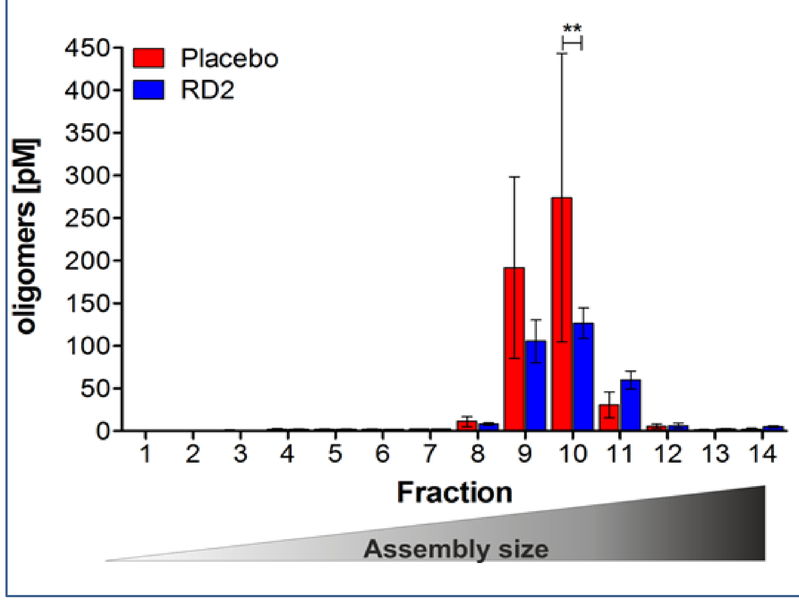
Time- and dose-dependent reduction of Aβ oligomers in human AD brain homogenate by RD2, but not by control D-peptides D1 and QB37



[3] Kass et al., Cell Rep Med 2022

Target Engagement - *in vivo*

RD2 treatment reduces Aβ oligomer concentration in the brain of APPswe/PS1ΔE9 mice



sFIDA assays on oligomeric Aβ levels in density gradient ultra-centrifuged mouse brain homogenate fractions. Oligomeric Aβ levels were investigated by sFIDA assay on the fractions of 18 months old APP/PS1 and non-transgenic mice.

[2] Schemmert et al., Mol Neurobiol 2019

Conclusion

We were able to demonstrate *in vitro* by analytical ultracentrifugation that RD2 eliminates toxic Aβ assemblies by stabilizing Aβ monomers in their native intrinsically disordered conformation. Furthermore, we could show that RD2 disassembled Aβ oligomers from brain tissue of former AD patients into Aβ monomers by *ex vivo* treatment. *In vivo* we could prove target engagement by showing a significant reduction of Aβ oligomers in the brains of APPswe/PS1ΔE9 mice, which were treated orally for 12 weeks with RD2, compared to placebo-treated mice.

In conclusion, we were able to prove *in vitro*, *ex vivo* and *in vivo* the new anti-prionic mode of action of RD2.