

D-peptides targeting PHF6* and PHF6 of Tau as potential therapeutics for neurodegenerative diseases

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Alzheimer's disease (AD) is characterized by abnormal Tau protein aggregation, detectable e.g. intracellularly as neurofibrillary tangles. Tau pathology strongly correlates with clinical symptoms and neuronal death. Two motifs within Tau, PHF6* (275-VQIINK-280) and PHF6 (306-VQIVYK-311), are known to be essential for Tau aggregation [1]. Using (mirror image) phage display procedures against full-length Tau and PHF6*, respectively, we have selected Tau targeting D-peptides, binding to Tau PHF6* ("MMD3" [2]) and PHF6 ("ISAD1" [3]). The ability of the D-peptides to bind to Tau and inhibit its fibrillization were characterized by a variety of biochemical assays, *in silico* modeling and cell culture experiments. MMD3 and ISAD1, binding to motifs PHF6* or PHF6, respectively, inhibited Tau fibrillization to a similar extent. They induced the formation of high molecular weight Tau aggregates that lack fibrillar β -sheet conformation. Cell culture experiments demonstrated that the D-peptides were taken up by N2a cells, were not toxic and prevented cytotoxicity of Tau [2,3].

1. Two phage display based selections were performed

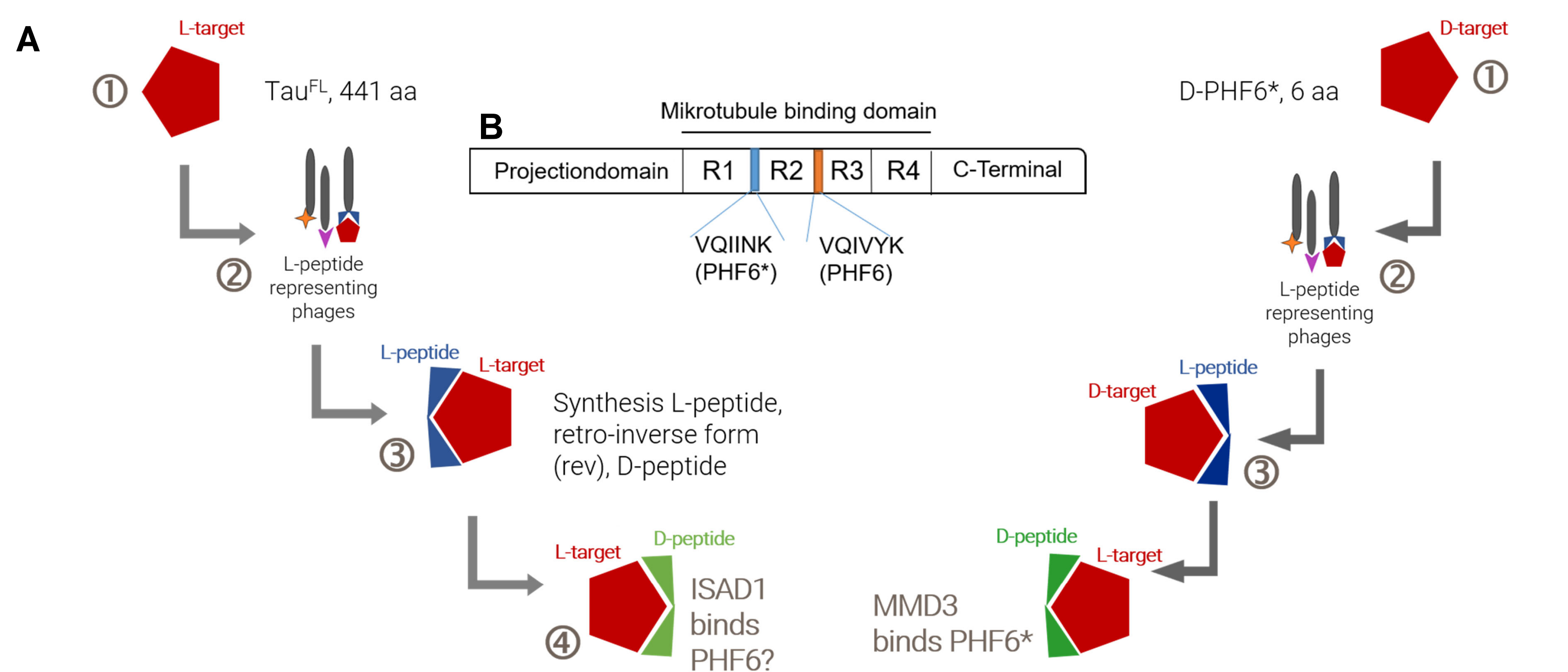


Fig. 1: A: Two hexapeptide motifs within Tau, PHF6* and PHF6, drive Tau aggregation [1]. Two phage display procedures were performed. B: D-peptide MMD3 was selected using mirror image phage display against D-PHF6* [2]. ISAD1 was selected using a phage display procedure using full length Tau as target. *In vitro* and *in silico* experiments indicated that ISAD1 binds to the PHF6 site of Tau [3].

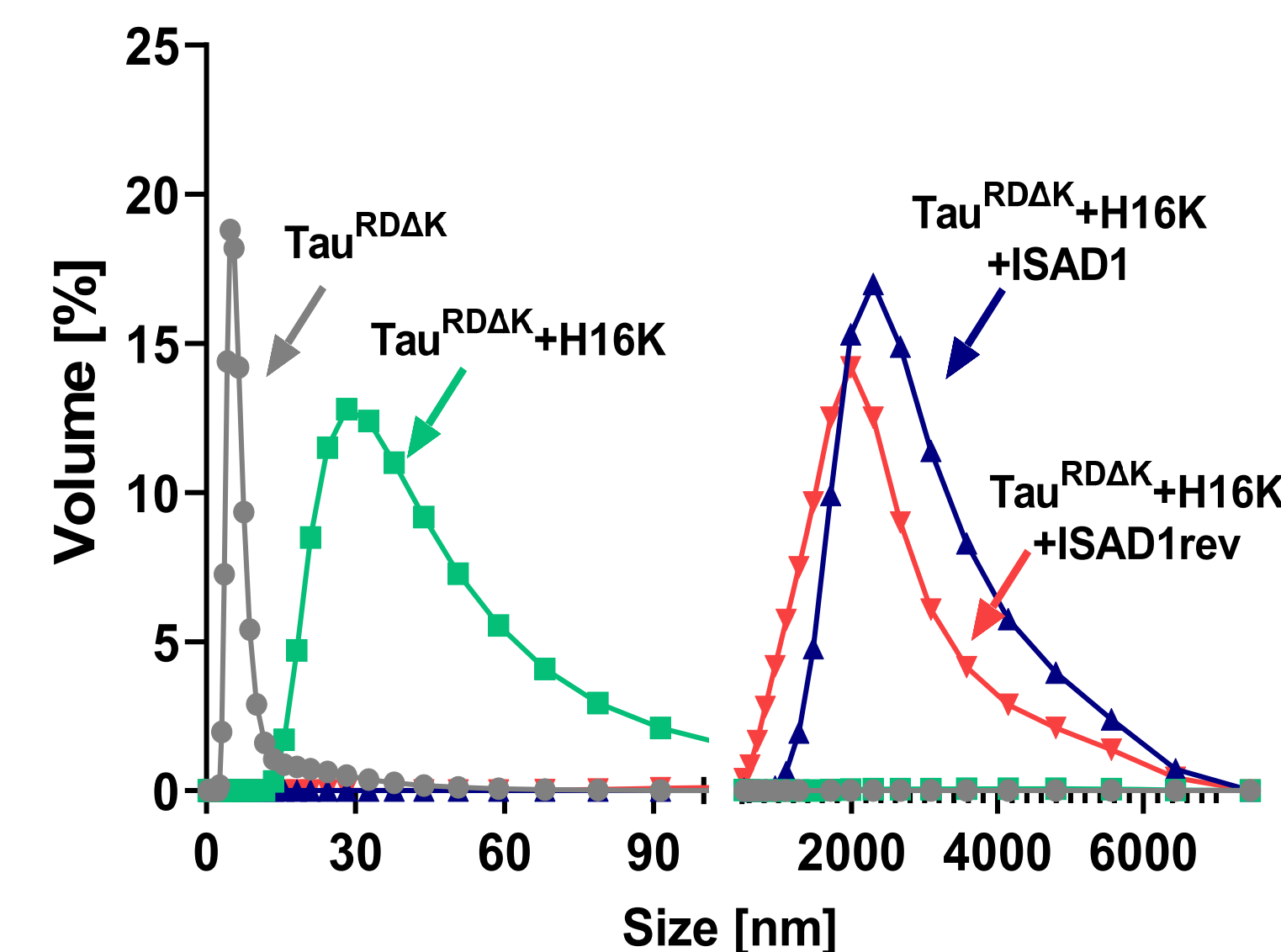
2. PHF6 and PHF6* binding D-peptides inhibited Tau fibrilization similarly well

Name	Target	Reference	IC ₅₀ (μM)
ISAD1	PHF6	[3]	2.9
ISAD1rev	PHF6	[3]	20.9
MMD3	PHF6*	[2]	4.65
MMD3rev	PHF6*	[2]	5.23
APT	PHF6	[5]	5.95
MMD3-ISAD1	both	-	9.52
ISAD1-MMD3	both	-	8.60
MMD3-PEG-ISAD1	both	-	4.29
ISAD1-PEG-MMD3	both	-	1.83

We used ThT assays to determine IC₅₀ values for full length Tau fibrilization inhibition by different D-peptides, targeted against PHF6* or PHF6. In addition, tandem peptides of MMD3 and ISAD1 were tested with ISAD1-PEG-MMDA showing lowest IC₅₀ value.

3. Selected D-peptides modulated Tau toxicity by reformation to amorphous aggregates

Fig. 2: Effect of the selected D-peptides on Tau aggregation and particle size distribution, measured by DLS and pelleting assay. Samples for DLS measurement and pelleting assay were prepared with 10 μM Tau^{RDΔK280}, 2.5 μM heparin and 20 μM ThS. The D-peptides were added in a concentration of 100 μM. All samples were incubated for 24 hours. The average of the results are shown as volume graph. Particle size distribution of Tau^{RDΔK280} with ISAD1 and ISAD1rev show formation of larger aggregates in DLS (Figure 3A, black and green curve). Tau^{RDΔK280} monomer show hydrodynamic size of <10 nm diameter (grey curve). Similar results were obtained with MMD3 and MMD3rev.



4. D-peptides reduced toxic effects of intracellularly expressed Tau^{RDΔK} aggregates

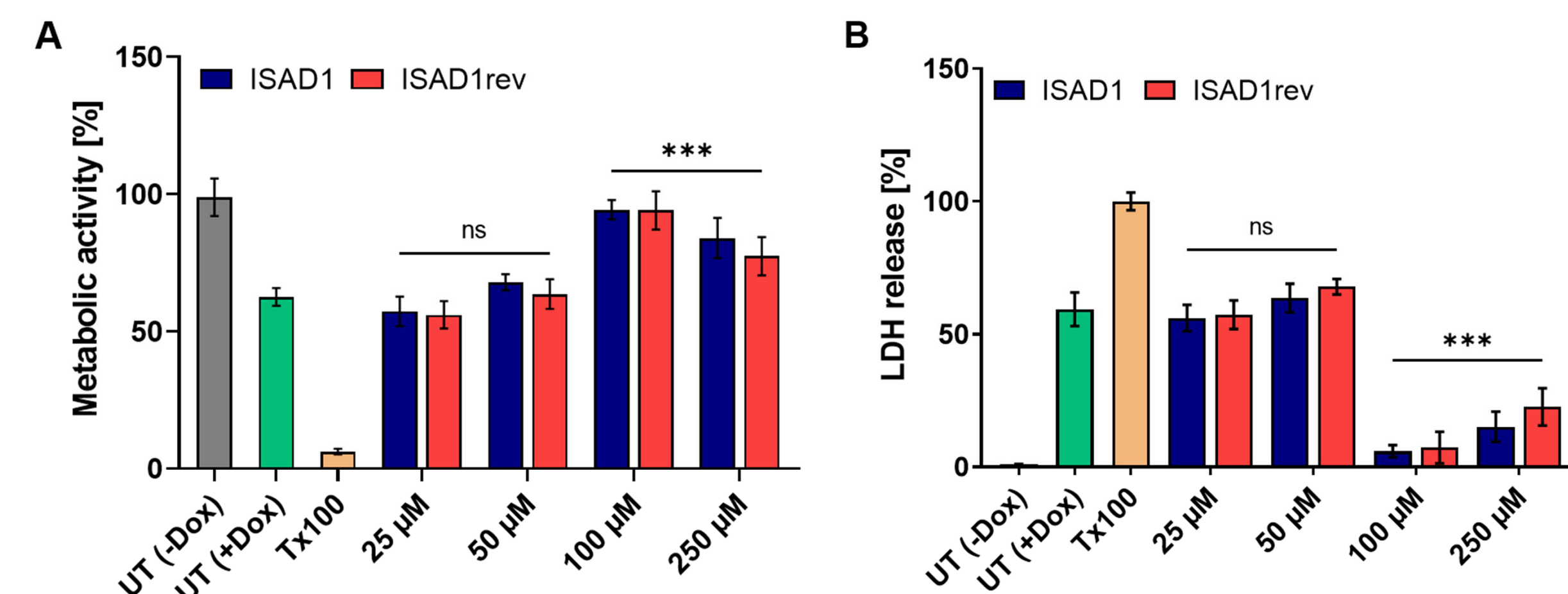


Fig. 3: Expression of Tau^{RDΔK} in N2a cells was induced by adding 1 μg/ml doxycycline. The cells were incubated with different concentrations of ISAD1 and ISAD1rev (25, 50, 100, 250 μM) for 24 hours at 37 °C followed by determination of MTT and LDH intensities. (A) Peptides prevent intracellular toxic Tau effects by preserving metabolic activity from 100 μM concentration (blue and red bars) similar to untreated cells (grey bar, UT -Dox) [n = 2; one-way ANOVA with Tukey's post hoc test; F(11, 60) = 126; ***p ≤ 0.001]. (B) Protection of cell membrane integrity by D-peptides (blue and red bars) determined by LDH release [n = 2; one-way ANOVA with Tukey's post hoc test; F(11, 60) = 256; ***p ≤ 0.001] [1].

Conclusion

Based on these results we conclude that our D-amino acid peptides binding to PHF6* and PHF6, respectively, may be interesting for therapy of AD by promoting off-pathway assembly of Tau, thus preventing its toxicity. It has been previously demonstrated that D-amino acid peptides are protease stable and less immunogenic than L-peptides, and these characteristics may render them suitable for *in vivo* applications. Cell and blood brain barrier permeability are possible advantages of peptides over therapeutic antibodies, which might have more affinity towards their target molecules, but cannot cross membranes. If therapeutic peptides are compared to small chemical molecules, their larger binding surface might of advantage, promising a more successful inhibition of protein-protein interactions [6].

References: [1] van Bergen et al. (2001): J. Biol. Chem., doi:10.1074/jbc.M105196200; van Bergen et al. (2000): PNAS, doi:10.1073/pnas.97.10.5129 [2] Malhis et al. (2020): ChemBioChem, doi:10.1002/cbic.202100287 [3] Aillaud et al. (2022): Alz Res Ther, DOI: 10.1186/s13195-022-00959-z [4] Drechsel et al. (1992): Mol Biol Cell, doi:10.1091/mbc.3.10.1141 [5] Dammers et al. (2016): PLoSOne, doi: 10.1371/journal.pone.0167432 [6] Liu. et al. (2016): Chem Rec, 16: 1772–1786; Matsson et al. (2016): Adv Drug Deliv Rev, 101: 42–61; Petta et al. (2016): Mol Ther 2016; 24: 707–718.