Designed peptide increases clearance of Aβ toxic oligomers and inhibits plaque formation

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Background

The recent approval of Alzheimer's Disease (AD) therapies targeting amyloid-β (Aβ) pathology, including Lecanemab and Donanemab, has renewed enthusiasm for this target and paved the way for new advances targeting even earlier in disease progression.

While AD is associated with the eventual accumulation of large, insoluble Aβ plaques in the brain, earlier low molecular weight soluble oligomers are considered to be the toxic element, causing disruption of synaptic transmission, neuroinflammation, and tau phosphorylation (1). These oligomers form a nonstandard protein structure, termed α-sheet, which is a defining feature of these toxic oligomers (2). We have designed peptides that selectively target α-sheet structure for diagnostic and therapeutic purposes.

Here we describe the specificity of our lead compound, Soluble Oligomer Binding INhibitor (SOBIN), for α-sheet oligomers over other forms of Aβ. We next explored SOBIN's effects on phagocytosis by microglia *in vitro*. Finally, we measured the *in vivo* effects of SOBIN on learning and memory and on plaque burden in the Tg2576 AD mouse model.

Methods

SOBIN was produced using conventional solid-phase peptide synthesis using previously described methods (3). α-sheet enriched Aβ oligomers were generated as described previously (2).

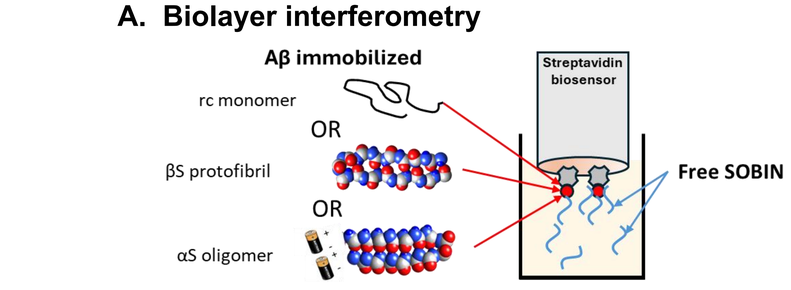
The affinity of SOBIN to random coil monomers, α-sheet oligomers, and β-sheet protofibrils was measured using biolayer interferometry (BLI).

Microglial uptake of Aβ was investigated using EOC-20 microglial cells with or without SOBIN and 0.5μM α-sheet enriched Aβ oligomers tagged with either AlexaFluor 647 (for imaging) or tagged with AlexaFluor 555 (for florescence quantification) for 24h.

In vivo efficacy of SOBIN was evaluated in 3-month-old Tg2576 AD mice and WT controls treated for 12 months intranasally with vehicle or SOBIN dosed 3x/week. Mice were subjected to Morris water maze behavioral testing after 6 months of treatment. Brains were collected for Aβ plaque staining after 12 months of treatment.

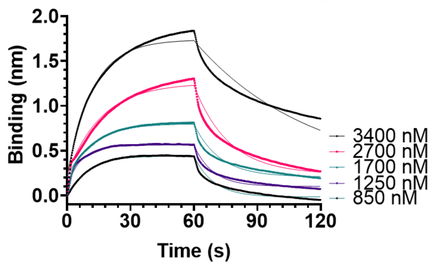
Results

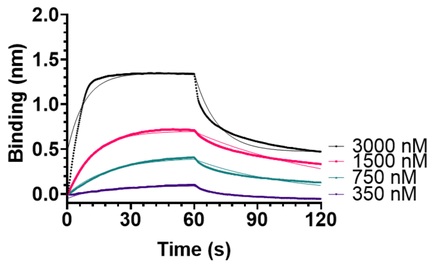
SOBIN binds Aβ oligomers 27,000-fold stronger than protofibrils



B. Binding affinity

Aβ Species	K_D (nM)
Aβ protofibril (βS)	92600 ± 9500
Aβ monomer (rc)	19200 ± 2500
Aβ oligomers (αS)	3.4 ± 2.4





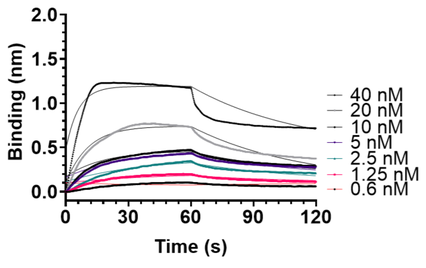


Figure 1. A) Schematic diagram of BLI experimental setup. **B**) Binding affinities for SOBIN dissociation from different Aβ species. **C-E**) Binding kinetics of SOBIN to different forms of Aβ. **C**) monomer random coil (rc), **D**) β-sheet protofibrils (βS), and **E**) α-sheet toxic oligomers (αS).

References

1. Hsia AY et al. (1999) *PNAS*. doi:10.1073/pnas.96.6.3228
2. Shea D et al. (2019) *PNAS*. doi:10.1073/pnas.1820585116
3. Maris NL et al (2018) *Biochem*. doi:10.1021/acs.biochem.7b00345

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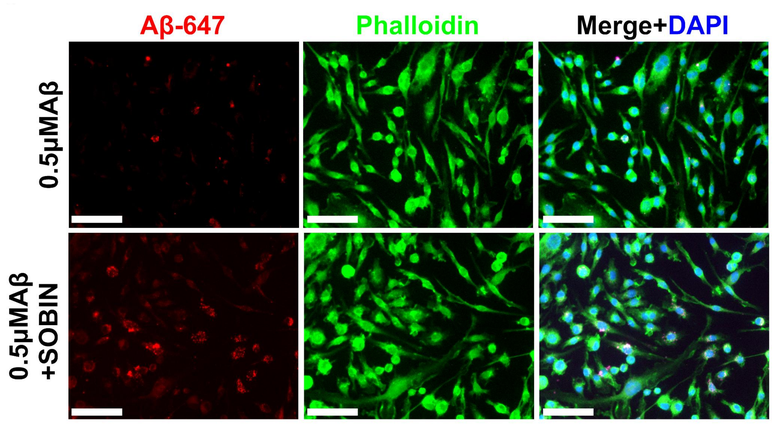
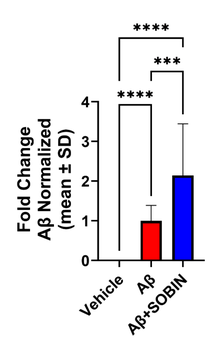
Results

SOBIN enhances microglial phagocytosis of toxic Aβ oligomers in a dose-dependent manner





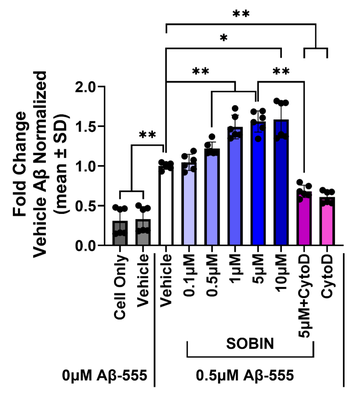
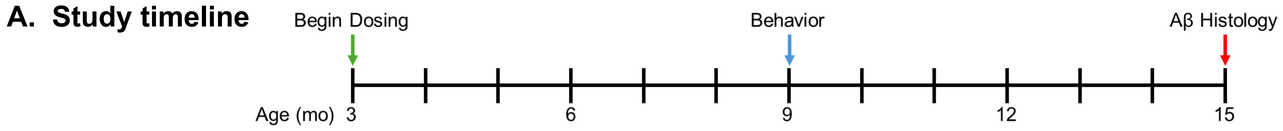
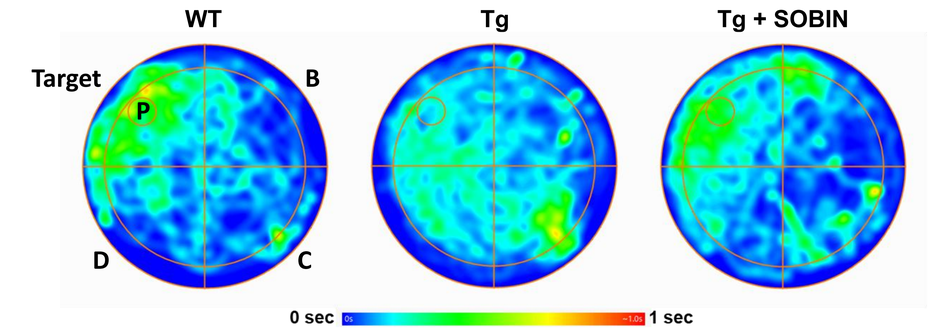


Figure 2. A) Representative images of cells exposed to toxic Aβ oligomers (red, Aβ with AlexaFluor 647 dye) alone (top row) or Aβ oligomers + 5μM SOBIN (bottom row). Cell bodies (phalloidin) shown in green (middle column). Merged Aβ-647 and phalloidin signals with nuclear DAPI staining (blue) shown in the right column. Scale bar, 50 μm. **B**) Quantification of percent area overlap between red and green signals. Data presented as mean + SD for *n*=28-30 images/group. **C**) Fluorescence intensity quantification of SOBIN-facilitated uptake of oligomers in microglia cells. Cytochalasin D (CytoD) was used to confirm SOBIN's effect on phagocytosis. Data are presented as mean ± SD. *n*=6. * p<0.05, ** *p*<0.01, *** *p*<0.001, **** *p*<0.0001.

SOBIN treatment improves short-term memory in 9-month-old Tg2576 AD mice





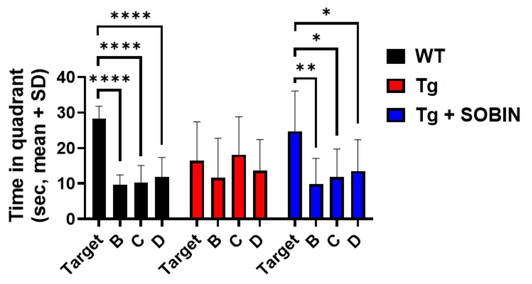
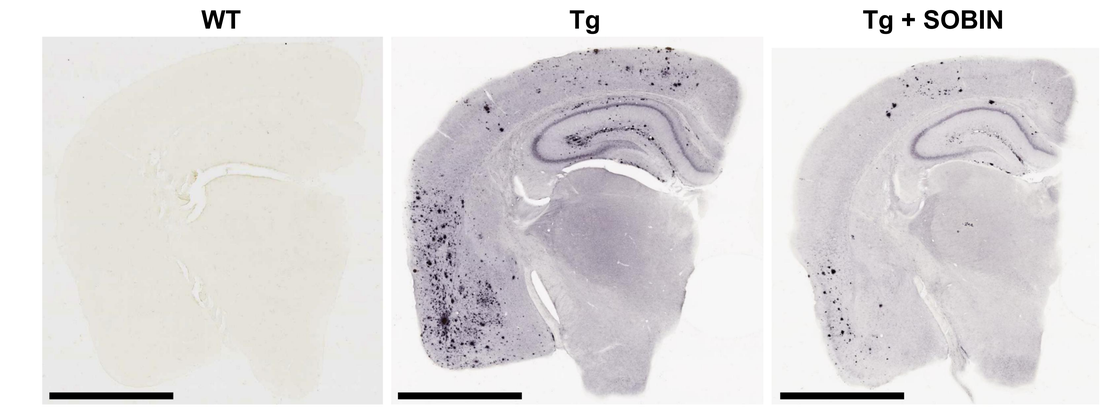


Figure 3. A) Experimental timeline of Tg2576 AD mouse (Tg) efficacy study. Mice were dosed 3x/week intranasally with either Vehicle or 100 μg SOBIN for 12 months. **B**) Group average heatmaps of short-term memory probe trial in the Morris water maze performed after 6 months of dosing. The submerged platform (P) was in the upper left target quadrant. Mice were initially placed in quadrant C. **C**) Quantification of time spent in each quandrant during the short-term memory probe. WT *n*=8; Tg *n*=16; Tg + SOBIN *n*=9. * *p*<0.05, ** *p*<0.01, **** *p*<0.0001.

SOBIN treatment reduces Aβ plaque burden in 15-month-old AD mice



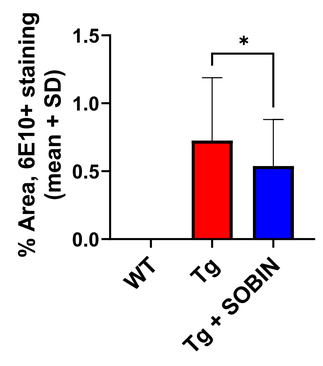


Figure 4. A) Representative brain slice images with Aβ staining (6E10 antibody). Scale bar, 5mm. B) Quantification of the percent area covered by 6E10 staining of 10 sections per animal for a total of *n*=70 sections. Bars represent mean + SD. * *p* < 0.05. WT, wild type. Tg, transgenic.

Conclusions

- SOBIN is highly selective for toxic α-sheet Aβ oligomers over other species of Aβ
- SOBIN enhances phagocytosis of toxic α-sheet Aβ oligomers by microglial cells
- 6 months of SOBIN treatment improves short-term memory in the Tg2576 mouse model
- 12 months of SOBIN treatment reduces plaque burden in the Tg2576 mouse model