Preparation and qualification of soluble AβOs for use in bioanalytical assays supporting AD therapeutics

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Introduction

Objectives

Soluble amyloid beta oligomers (sAβOs) accumulate early in Alzheimer's disease (AD) and substantial experimental evidence indicates that sAβOs trigger AD-related neuropathologies as well as impairment in learning and memory. Despite this, the sAβO structures contributing to the neurotoxic effects in the AD brain remain ill-defined due to their low concentration, instability, and heterogeneity, impeding the effective design and use of sAβO reference standards in bioanalytical assays. sAβO assays, in combination with assays for Tau and Aβ proteoforms, could become a tool for earlier diagnosis of neurodegenerative disease subtypes as well as for measurement of sAβO-targeting drug pharmacokinetics, target engagement, or treatment efficacy in clinical trials. At present, no assays for sAβOs have proven robustness and clinical performance, due at least in part to the lack of readily available, well-characterized, critical raw materials, including antibodies and reference materials for preparation of sAβO calibrators and quality control specimens.

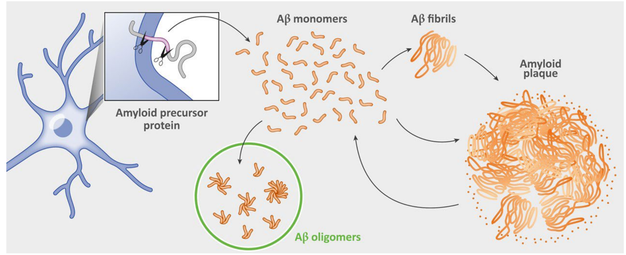
Methods

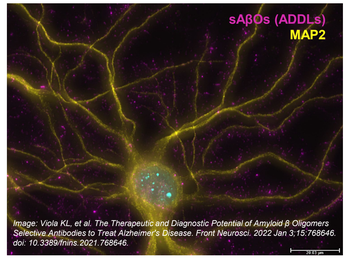
We have used amyloid-derived diffusible ligands (ADDLs) as an sAβO standard integrated into different assays designs. As a proof-of-concept, we have utilized these ADDL assays to study the specificity and selectivity of antibodies targeting sAβOs. All assays utilized the Mesoscale Discovery (MSD) technology and were conducted in the laboratories of B2S LifeSciences (Indianapolis, IN).

RESEARCH HIGHLIGHTS

- Soluble amyloid beta oligomers (sAβOs) accumulate early in AD and trigger neuropathologies and cognitive impairment.
- The non-abundance, instability, and heterogeneity of sAβOs has impeded their effective use as reference standards in bioanalytical assays.
- Here, we demonstrate the utility of ADDLs as a synthetic reference standard for sAβOs to study antibody specificity and selectivity.
- Other expected uses are: (i) as a calibrator in immunoassays aimed at quantitation of sAβO levels as a function of AD pathogenesis; or (ii) to screen for the presence of sAβO auto-antibodies in biofluids.

Soluble AβOs are AD neurotoxins

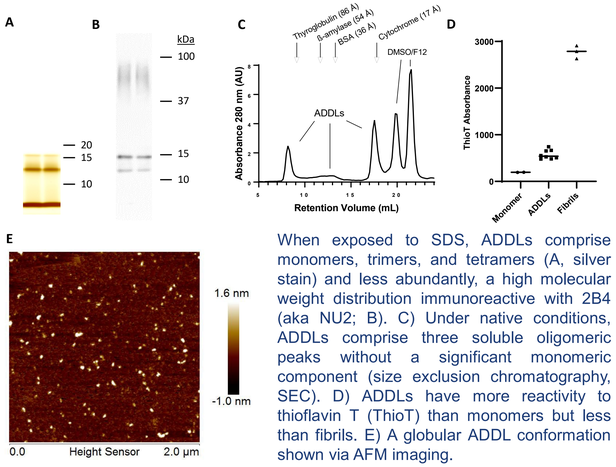




SOLUBLE AβOS (sAβOS) ARE DISTINCT FROM FIBRILS AND EXPERIMENTALLY INDUCE MANY FACETS OF AD PATHOGENESIS. Left: Aβ monomers can aggregate into either fibrils or sAβOs, the latter of which can bind cultured neurons in a punctate manner (middle) consistent with synapse binding. Right: Downstream of synaptic binding, sAβOs (including ADDLs) have been shown to induce many aspects of AD pathogenesis (reviewed in Cline et al 2018 *J Alzheimers Dis* **64**:S1).

ADDL Characteristics

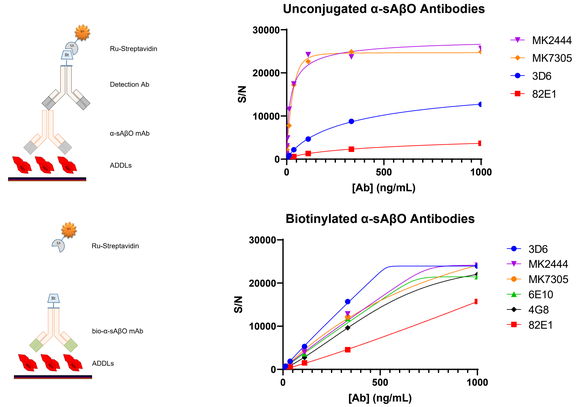
ADDLS ARE SOLUBLE, GLOBULAR OLIGOMERS OF AB WITH A WIDE SIZE DISTRIBUTION.



When exposed to SDS, ADDLs comprise monomers, trimers, and tetramers (A, silver stain) and less abundantly, a high molecular weight distribution immunoreactive with 2B4 (aka NU2; B). C) Under native conditions, ADDLs comprise three soluble oligomeric peaks without a significant monomeric component (size exclusion chromatography, SEC). D) ADDLs have more reactivity to thioflavin T (ThioT) than monomers but less than fibrils. E) A globular ADDL conformation shown via AFM imaging.

Results

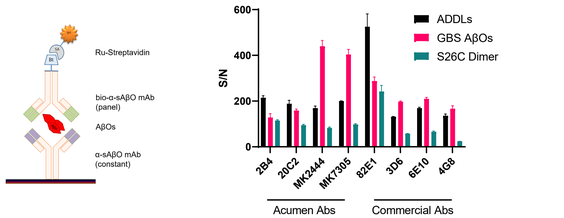
THE ADDL IMMUNOREACTIVITY OF A PANEL OF sAβO-TARGETING ANTIBODIES WAS EVALUATED ON ADDL-COATED MSD PLATES.



Analysis of biotinylated and non-biotinylated sAβO-targeting antibodies were shown to have variable immunoreactivity with ADDLs, informing their use in assays using ADDLs as reference standards. Assay formats are depicted schematically (left).
S/N = signal to noise ratio.

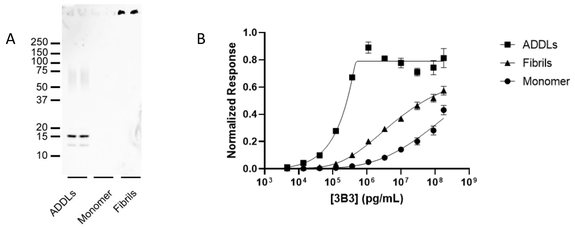
Results (cont.)

THE SPECIFICITY OF A PANEL OF sAβO-TARGETING ANTIBODIES TO ADDLS WAS COMPARED TO COMMERCIALLY-AVAILABLE sAβO REFERENCE STANDARDS.



Additional commercial sAβO reference standards, including stabilized sAβOs from Good Biomarker Sciences BV (GBS; Leiden, NL) (formerly produced by Crossbeta Biosciences) and S26C dimers (JPT Peptide Technologies GmbH), were used to further evaluate sAβO antibody selectivity/specificity. In this assay format (left), 82E1 (IBL; MN, USA) had the greatest ADDL immunoreactivity. Data are mean ± SEM, n=2. *S/N = signal to noise ratio.*

ADDLS CAN BE USED AS A TOOL TO DETERMINE SPECIFICITY AND SELECTIVITY OF ANTIBODIES FOR VARIOUS Aβ FORMS.



A) The antibody 20C2 (aka NU1) is shown via Western immunoblot that it is immunoreactive with SDS-stable ADDLs and fibrils, but not monomers. B) The antibody 3B3 is shown via ELISA to have > 7000-fold selectivity for ADDLs (EC50 = 192 ng/mL, 5PL regression) over monomers (EC50 ≥ 1.4 mg/mL) and > 40-fold selectivity for ADDLs over fibrils (EC50 ≥ 7.3 µg/mL). Data are mean ± SEM, n=4. In both (A-B), sAβOs are modeled by ADDLs and Aβ monomers by Aβ(1-40).