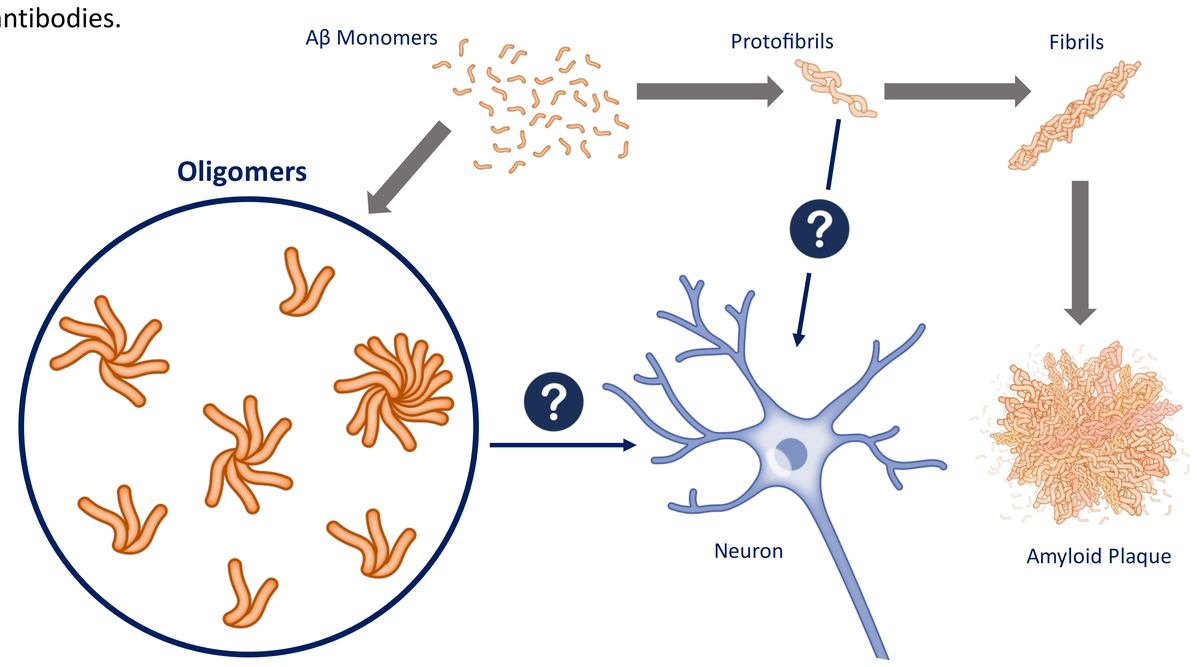
Binding of Soluble Amyloid Beta Oligomer Species to Human iPSC-Derived Excitatory Neurons Assessed Using a Panel of Amyloid Beta Antibodies

Erika N. Cline[1], Wei-Li Kuan[2], Sanofar Jainul Abdeen[3], James Cruse[3], Adam Kinne[3], Mahsan Rafizadeh[1], Martin Crossley[2], James Smith[2], Clare Jones[2], Jasna Jerecic[1]

(1) Acumen Pharmaceuticals Inc., Charlottesville, VA, USA; (2) Talisman Therapeutics, Cambridge, UK; (3) B2S Life Sciences, Indianapolis, IN, USA

Introduction

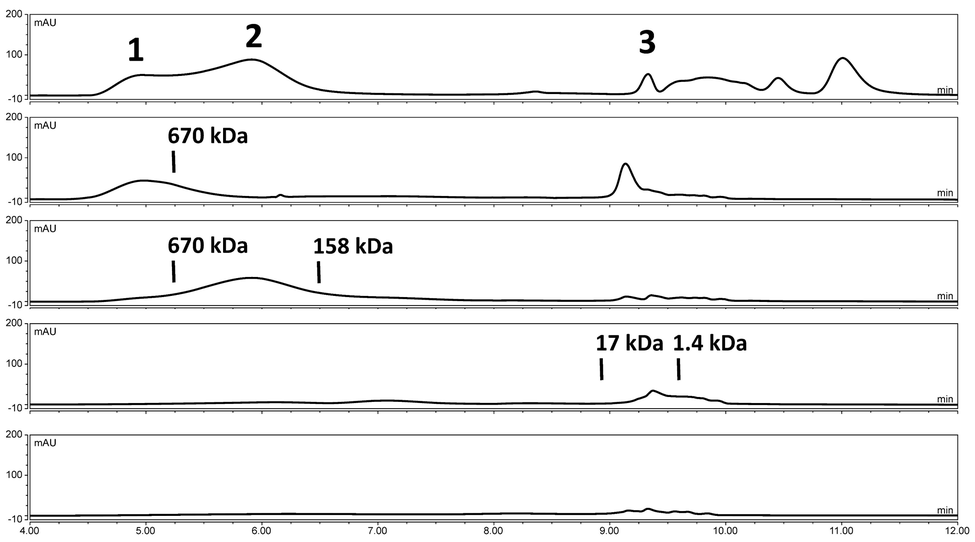
Soluble amyloid beta oligomers (sAβOs) accumulate in Alzheimer's disease (AD) and contribute to neuronal impairment through synaptic targeting. Myriad sAβO species have been identified in cultured cells, cerebrospinal fluid, and brain tissues from AD patients and animal models and their functionality assessed, with results varying by analytical method and antibody. However, it remains unclear which sAβO species are most relevant to AD pathogenesis. The objective of this study was to determine how sAβO size affects synaptic binding and immunoreactivity to a panel of anti-Aβ antibodies.



WHICH sAβ STRUCTURES ARE MOST RELEVANT TO AD SYNAPTIC IMPAIRMENT? In AD, Aβ monomers self-associate to form varying sizes and structures of sAβOs (circled), which cause neuronal dysfunction. Aβ monomers also form protofibrils and fibrils; the latter form deposits known as amyloid plaques. Question marks indicate unknowns regarding the exact structures of sAβ species most relevant to AD synaptic impairment.

Soluble Aβ Oligomer (sAβO) Preparations

Stable size fractions of sAβOs



CHEMICAL CROSS-LINKING STABILIZES SYNTHETIC sAβOS FOR REPRODUCIBLE SEPARATION OF MOLECULAR WEIGHT FRACTIONS. Acumen's synthetic sAβO preparation, amyloid beta derived diffusible ligands (ADDLs) (Chromy, et al., Biochemistry, 2003; 42(44): 12749), was stabilized via chemical crosslinking and fractionated into three size populations via size exclusion chromatography (above). According to analysis on multiple SEC columns with comparison globular molecular weight standards (indicated above the traces), these populations are > 670 kDa (HMW, Peak 1), 150-670 kDa (MMW, Peak 2), and monomer-trimer (LMW, Peak 3).

A

B

C

sAβO Preparation	EC50 (µM)
ADDLs (F12)	1.2
ADDLs (NB)	1.8
Stabilized ADDLs, Unfractionated	14
MMW (P2)	24
HMW (P1)	Not determined
LMW (P3)	Not determined

D

ELISA IMMUNOREACTIVITY OF sAβOS VARIES BY SIZE. The immunoreactivity of these stabilized sAβO size fractions was compared to non-stabilized ADDLs prepared in the standard Ham's F12 media (F12) or Neurobasal media (NB) via an ELISA assay. **(A)** The ELISA assay utilizes a sandwich format with two different anti-Aβ antibodies. Data are plotted for the non-stabilized **(B)** and stabilized **(D)** preparation; EC50s are shown in **(C)**. Data show that chemical crosslinking reduces the immunoreactivity of the preparation by 10-fold. In addition, the majority of the preparation's immunoreactivity is contributed by the mid-molecular weight (MMW) fraction, with a significantly smaller, and equal, contribution by the high- and low-molecular weight fractions (HMW, LMW).

Binding of sAβ Species to Human iPSC-derived Excitatory Neurons

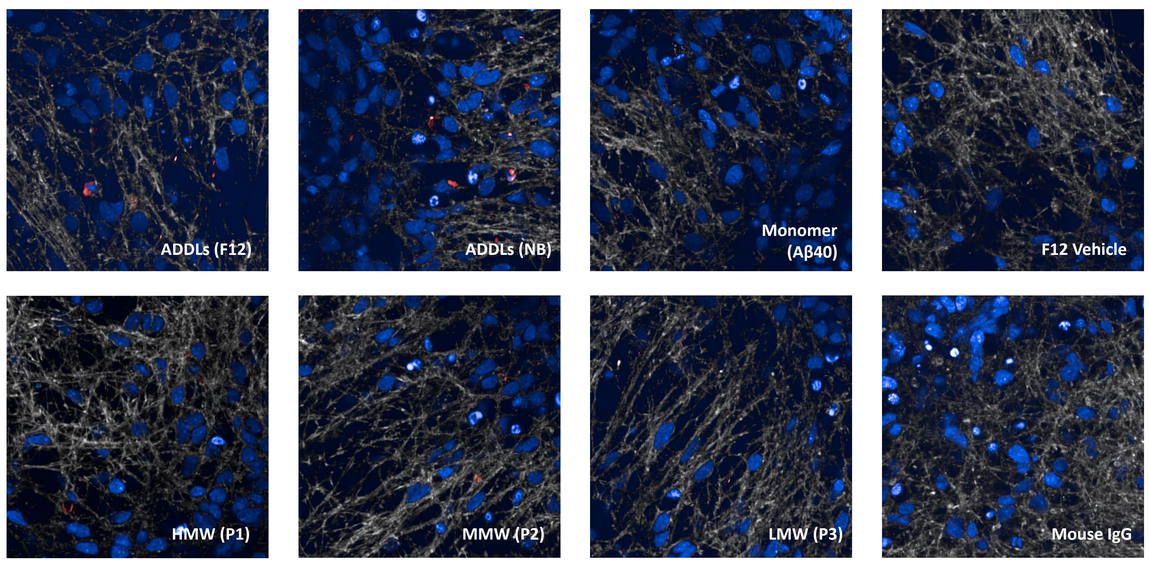
sAβ Addition to iPSC Neurons (30min) 50, 250, 500 nM → Fix & Stain (• Aβ (Antibody Panel) • Drebrin (Synapses)) → High Content Imaging → Image Analysis

sAβ Preparations	
Preparation	**Size Distribution (kDa)**
ADDLs (F12)	4.5-670
ADDLs (NB)	4.5-670
Stabilized, HMW (P1)	> 670
Stabilized, MMW (P2)	150-670
Stabilized, LMW (P3)	4.5-14
Aβ1-40	4.3

Aβ Antibody Panel		
Aβ Antibody	**Source**	**Antigen/Epitope**
82E1	IBL	Aβ N-terminus
2B4.6	Acumen	ADDLs
MK2444	Acumen	ADDLs
MK7305	Acumen	ADDLs

SEVERAL sAβ PREPARATIONS AND ANTIBODIES WERE USED TO QUANTIFY DIFFERENTIAL INTERACTION WITH HUMAN iPSC-DERIVED NEURONS. Heterogeneous synthetic sAβO preparations (ADDLs), stabilized size fractions (HMW, MMW, LMW), and Aβ monomer (Aβ1-40) were applied to human iPSC-derived cortical excitatory neurons for 30 min. Binding was assessed via immunofluorescence using the commercial anti-Aβ antibody 82E1 or a panel of anti-ADDL antibodies. Synaptic localization of the bound sAβ species was assessed via co-localization of the antibody fluorescence with the postsynaptic protein drebrin.

A



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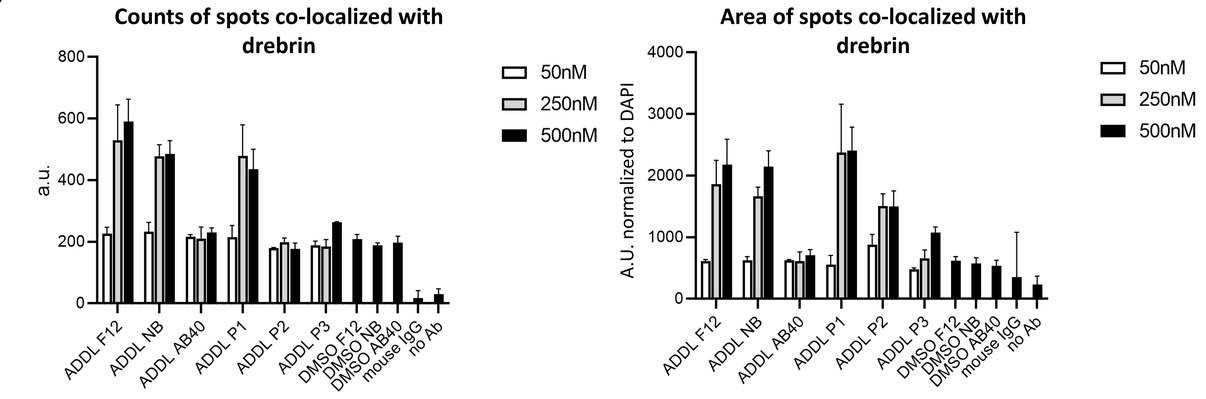

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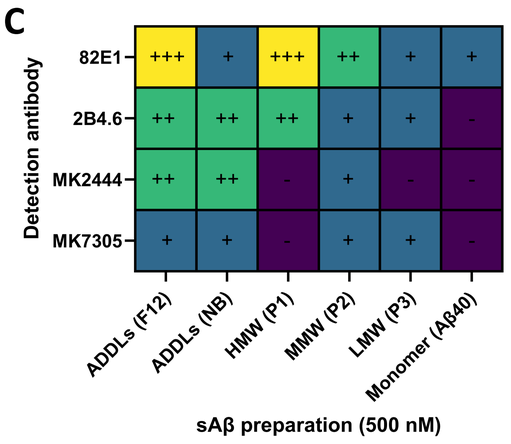

ANTIBODY DETECTION OF EACH sAβ SPECIES BOUND TO HUMAN iPSC-DERIVED NEURONS DEPENDS ON SIZE. RESULTS FURTHER VARY BY ANTIBODY.

(A) Representative immunofluorescent images of all sAβ preparations and negative controls using the anti-ADDL antibody 2B4.6 (red). Nuclei are visualized via DAPI (blue), and synapses via drebrin (white). **(B)** Quantification of 2B4.6 puncta co-localized with drebrin. Data are plotted as mean ± SD (n=3 wells/condition, 49 fields of view per well). **(C)** Binding of all sAβ preparations detected with each antibody summarized: - (no binding above background); + (low level of binding); ++ (moderate binding); +++ (high level of binding).

RESEARCH HIGHLIGHTS

- Chemical cross-linking of synthetic sAβOs enables reproducible preparation of high, mid, & low molecular weight sAβO fractions
- Biochemical & cell assays show that sAβO conformation & epitope accessibility varies by size, impacting antibody detection of synaptic binding
- Comparing results across the two assays:
  - Unfractionated sAβ preparations tend to perform the best. In the ELISA, most of the antibody binding is contributed by MMW sAβOs; in the iPSC assay, by HMW & MMW sAβOs.
  - LMW sAβ species (including monomer) show lowest immunoreactivity.
- Overall, results further underscore the importance of:
  - Careful characterization and consideration of sAβO preparations and detection antibodies when bridging results across different assays & studies
  - Contribution of sAβO structure to function in AD, such as synaptic binding
- Ultimately, this study indicates that design of novel AD diagnostics & therapeutics may benefit from consideration of sAβO size.

SCAN ME