Production of Recombinant Soluble Aβ Oligomers with Split-luciferase Tags

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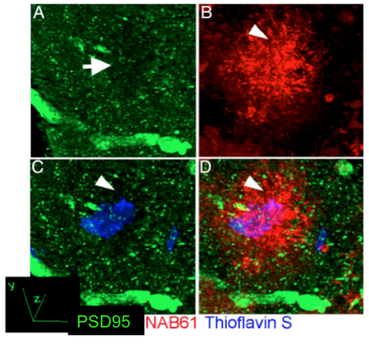
Soluble oligomers of Aβ peptide are cytotoxic and play a central role in Alzheimer's Disease, however, their mechanism of action remains unknown. We have made Aβ in bacteria and purified Aβ oligomers (AβOs) in order that we might describe their structure and oligomerization pathway. Purified, disease-relevant AβOs would also enable *in vitro* testing of proposed cytotoxic mechanisms. We have verifed that our soluble AβOs are similar in size and shape to patient-derived AβOs. Once we prove that our AβOs are cytotoxic, we will begin to study them using structural and biochemical techniques.

BACKGROUND:

Alzheimer's Disease and Aβ oligomers[1]

- **Alzheimers Disease** (AD) is a neurodegenerative disease with no effective treatment
  - 5.3 million patients in the US
  - 6th leading cause of death in the US
  - Over $ 200 Billion / year in healthcare costs in the US
- **Aβ peptides** are linked to AD progression
  - Aβ peptide is generated by cleavage of a larger APP protein
  - Insoluble Aβ is a major component of senile plaques
  - AD symptoms correlate with soluble oligomers Aβ (**see below**)
  - Soluble AB oligomers are known to be cytotoxic
- The **cytotoxic mechanism** for Aβ oligomers (AβOs) is currently unknown
  - Many hypotheses: Pore-forming ABOs? Inflammatory? Gene regulation? Oxidative stress? Alterations of tau protein?
  - Purified AβOs will enable us to test mechanism and drugs *in vitro*

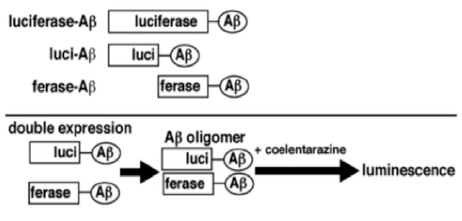
ABO's correlate with synapse loss[2]**:** This reconstructed 3D section (60x60x20μm) from array tomogram imaging (**right**) shows that loss of post-synaptic densities (PSD95, green) co-localize with soluble AβOs (NAB61, red) and are not limited to the area of the insoluble amyloid plaque (thioflavin S, blue).

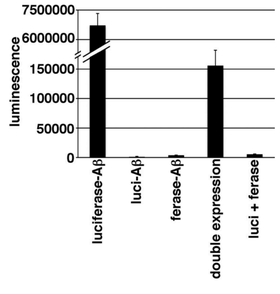


(Figure from Koffie, R., et al. 2009.)

A Luciferase Complementation System for Detecting AβOs[3]

- Two copies of Aβ42 peptide are each tagged with half of a luciferase molecule
- Both Aβ peptides are co-expressed in mammalian cells or bacteria (**below left**)
- Luciferase activity is detectable when AβOs are formed (**below right**)





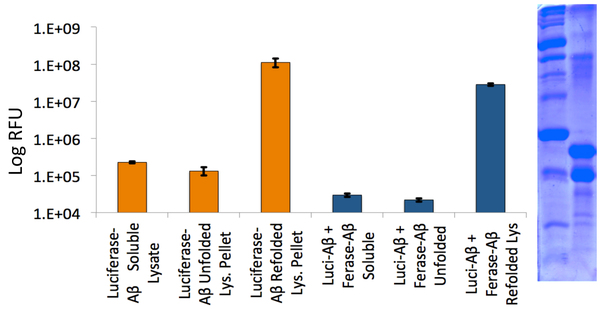
(Figure from Hashimoto, T., et al. 2011.)

RESULTS:

We CAN make soluble AβOs in bacteria!

Expression and Purification

- His-tagged Luci-Aβ and Flag-tagged Ferase-Aβ were expressed in *E. coli*
- Our protein was mostly insoluble in lysate. The insoluble lysate pellet was solubilized by high pH and detergent
- After refolding by dialysis, luciferase activity comparable to intact Luciferase-Aβ (concentration estimated from pellet weight) was observed (**below left**)
- Further purification by His-tag affinity resin yielded a mostly pure sample (**below right**)



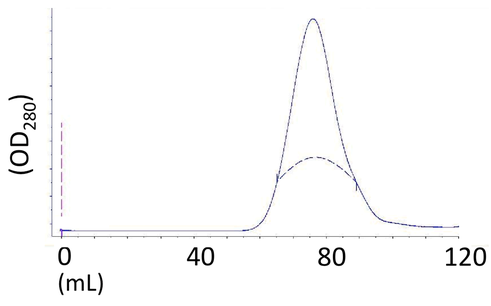
SDS-PAGE gel: This gel shows molecular weight markers (**left**) and an AβO sample (**right**). Note the two major bands for Luci-Aβ and Ferase-Aβ. The higher MW band is thought to be an SDS stable AβO fragment.

What SIZE are our AβOs?

Size Exclusion Chromatography

Technique: The diffusion rate of a molecule or oligomer through a matrix of porous beads is dependent on the size of that particle.

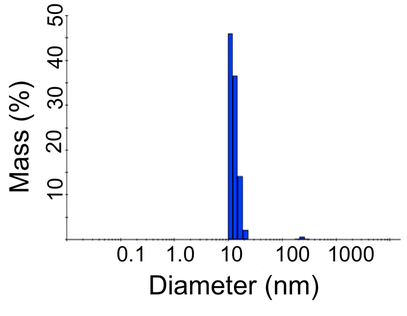
Results: Our purified sample of AβOs show monomodal population and weigh ~400-500 kilodaltons or approximately 24-30 monomers (**right**).



Dynamic Light Scattering

Technique: Rate of intensity fluctuations in reflected laser light are correlated with the size of the particles that reflected the light.

Results: AβOs are a monomodal population, about 10-18 nm diameter (**right**).



What SHAPE are our AβO's?

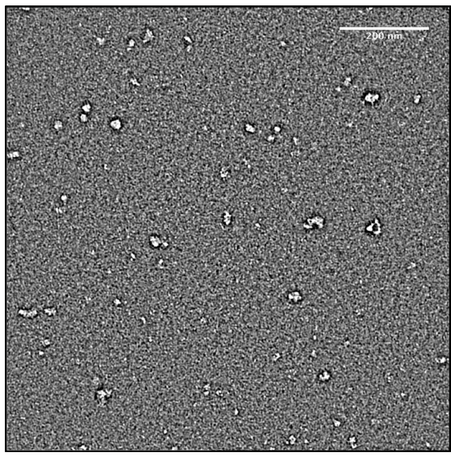
Transmission Electron Microscopy

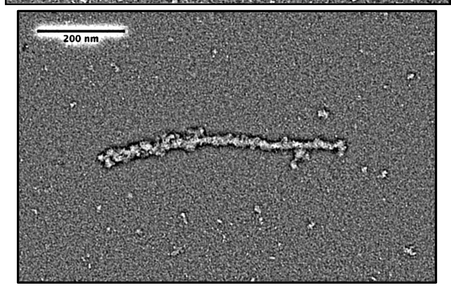
How: Samples were prepared at ~0.1mg/mL with negative stain techniques and viewed with an electron microscope

Results:

Aβ OLIGOMERS (**right top**)
- Globular but irregular shapes
- 2D area of 200-800nm for most particles

SHORT FIBRILS (**right bottom**)
- Fibril formation seems rare
- Could be evidence that our AβOs are on the known pathway to fibril formation and therefore relevant to AD





FUTURE DIRECTIONS:

Establish AD relevance
- Assay cytotoxicity in neuronal cell culture

Further structural characterization
- Test for Cross-beta structure with dye and antibody binding assays
- X-ray techniques that don't require crystals (SAXS, WAXS)
- More EM work

***In vitro* testing of existing mechanistic hypotheses**
- Pore-forming assays
- Immune activation assays

References:

1. Alzheimer's Association, www.alz.org

2. Koffie R., et al. (2009). Oligomeric amyloid β associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proceedings of the National Academy of Sciences*, *106*(10), 4012–4017.

3. Hashimoto, T., et al (2011). Characterization of Oligomer Formation of Amyloid-βPeptide Using a Split-luciferase Complementation Assay. *Journal of Biological Chemistry*, *286*(31), 27081–27091.

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