Evaluating the Photosensitizing Activity of OPE on Amyloid-β Fibrils

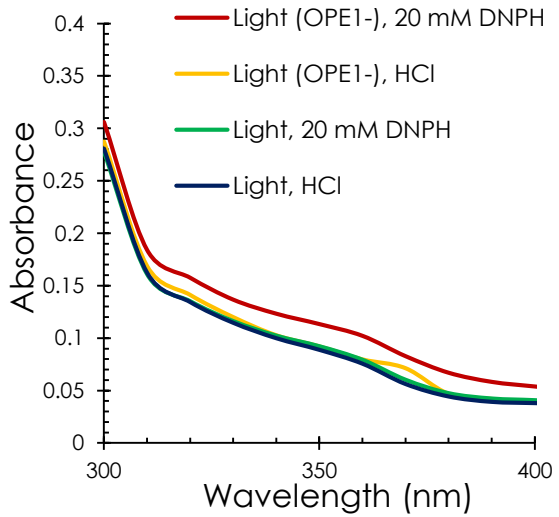
Julia Hammond[1,2], Adeline Fanni[2,3], Florencia Monge[2,3], Eva Chi[2]

[1]Biomedical Engineering, Rose-Hulman Institute of Technology, [2]Center for Biomedical Engineering, University of New Mexico, [3]Biomedical Sciences Graduate Program, University of New Mexico

Problem: Alzheimer's Disease (AD) is a neurodegenerative disease with no diagnostic method or cure. Amyloid beta fibrils, the hallmark pathology of AD, cause program cell death in the brain.

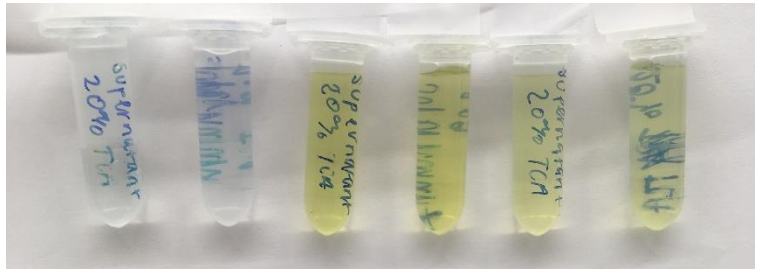
Goal: Evaluate the ability of OPE to sensitize the oxidation of Aβ-40 fibrils.

Results: The amyloid fibrils were used in the characterization of a chemical based assay using DNPH. Parameters including DNPH concentration, centrifugation time, and protein concentration were optimized. A known singlet oxygen generator methylene blue was able to cause significant oxidative damage to Aβ-40 fibrils (irradiated for 13 hours). OPE photobleaches after 30 minutes of irradiation, and is not able to oxidize Aβ-40 fibrils in that amount of time. Optimization of a irradiation procedure will need to be done for OPE to maximize oxidation without bleaching.



Absorbance spectra of Aβ-40 fibrils with light and light + oxidizer (25 min incubation)

Methods: A chemical assay using DNPH was used to qualitatively evaluate if oxidation of the Aβ-40 fibrils has occurred. The samples were irradiated at 420 nm for 25 minutes. Then, they were incubated in DNPH (HCl for control) for 1 hour, vortexing the samples every 15 minutes. The samples were then washed in a series of centrifugations with TCA, ethanol/ethyl acetate, and GdnHCl. 220 µL of each sample was loaded into the plate reader, and the absorption spectrum for each was read from 300-550nm.



Future work:

- Separate oxidized protein species (reverse phase HPLC)
- Obtain quantitative data of oxidation (mass spec)