Structure of glaucoma-associated myocilin prefibrillar oligomers

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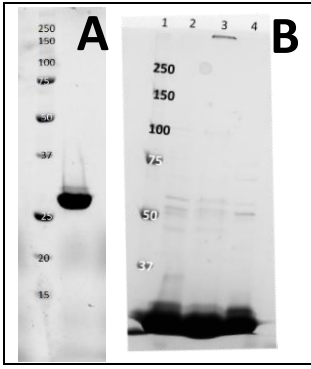

Figure 1. 8% polyacrylamide SDS PAGE of purified mOLF WT monomer and oligomers. (A) 200 uM mOLF WT oligomers were run in an 8% SDS PAGE gel in an attempt to determine the size of these particles. The lanes are a (1) molecular mass standards, (2) 200 uM aggregate sample, (3) 200 uM aggregate sample that was boiled at 95C for 3 minutes prior to being loaded and (4) a 30 kDa monomer mOLF WT control. Only the boiled aggregate sample was able to enter the stacking gel, and neither sample was able to enter the resolving gel. This suggests the large nature of these particles. (B) Purified 30 kDa mOLF WT, verifying that all contaminants can be removed from this protein during purification.

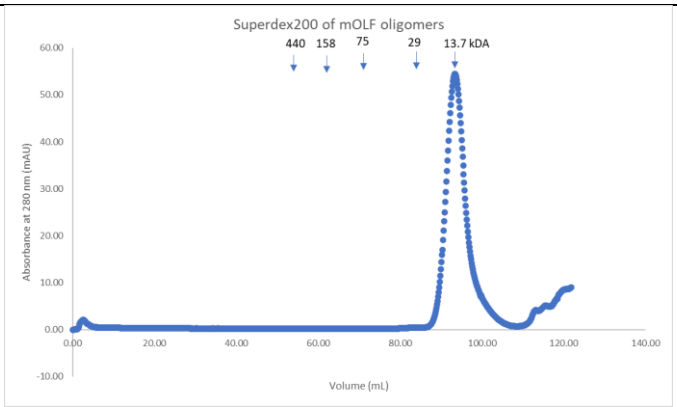

Figure 2. HiLoad 16/60 Superdex 200 chromatograph of mOLF oligomers. 200 uM mOLF WT was aggregated at 37C for ~66 hours and the sample was observed to be cloudy, verifying the presence of aggregates. The oligomers were then filtered through a 0.4 uM syringe filter and run over a sup200 SEC column. No monomer (~30 kDa) or higher order species were observed in the trace, suggesting the sample was lost to the filter.

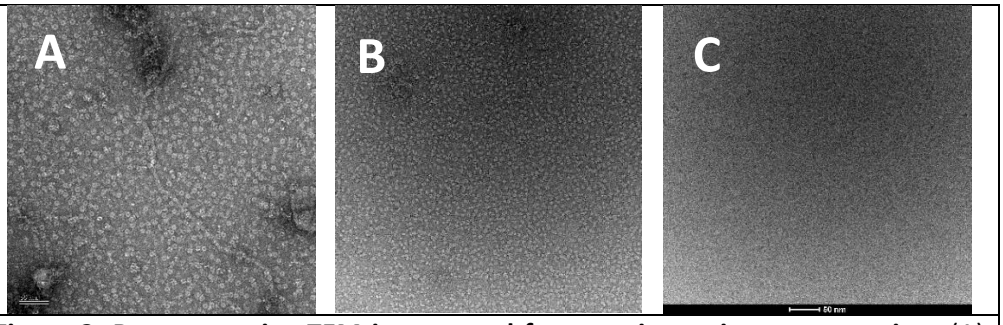

Figure 3. Representative TEM image used for negative stain reconstruction. (A) Original screen of 200 uM mOLF WT oligomers from Spring 2021. The carbon coated copper grid was prepared by incubation with an undiluted sample and stained with 3% Uranyl Acetate (UA). The grid is coated in ~5 nm oligomers with a central pore. (B) A fresh sample of 200 uM mOLF WT oligomers was diluted 5-fold with phosphate buffer and deposited on a carbon coated copper grid and stained with 0.7 Uranyl Formate (UF). This is a representation of ~100 images collected for a negative stain reconstruction. Small oligomeric particles with a central pore are present across the entire grid. (C) Cryo-screen of these 200 uM oligomers on quantifoil holey carbon grids, revealing the presence of particles across the entire grid.

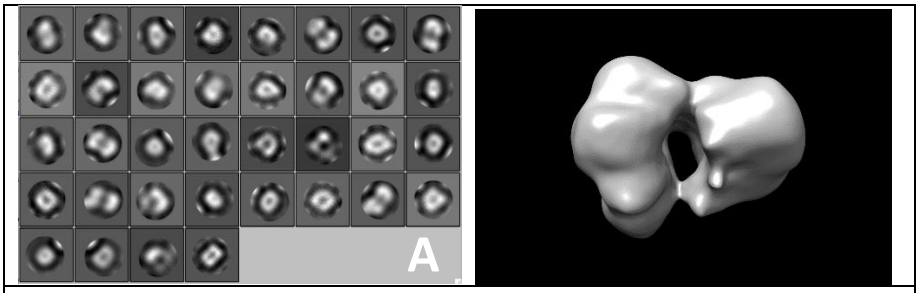

Figure 4. 2D reconstruction of mOLF WT oligomers. (A) 2D class averages of mOLF oligomers based off 125,000 particles from negative stain TEM, revealing a potential tetramer or two tetramer conformation. (B) 3D initial model of mOLF oligomers.