Supporting Information for

Rescue of glaucoma-causing mutant myocilin thermal stability by chemical chaperones

SUPPLEMENTAL METHODS

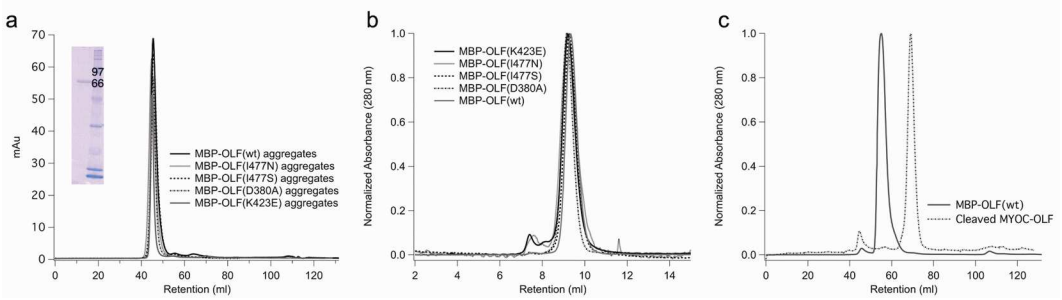
Disulfide bond characterization. The oxidation state of the two cysteines in OLFs was determined by utilizing a fluorogenic thiol-modifying reagent, ThioGlo® 1 (Calbiochem). A stock solution of ThioGlo® 1 was prepared by dilution in dimethylsulfoxide to a final concentration of 10 mM. ThioGlo® 1 was further diluted to 20 μM in Buffer B, along with 1-2 μM OLF with and without 5 mM tris(2-carboxyethyl)phosphine HCl (TCEP; Thermo Scientific). Fluorescence emission spectra of cysteine–ThioGlo® 1 protein adducts were recorded using excitation and emission wavelengths of 379 and 513 nm respectively, using a Shimadzu RF-5301 PC spectrofluorophotometer. The ThioGlo® 1 only or ThioGlo® 1 plus TCEP scans were subtracted from those of the OLF-containing protein samples. The assay was conducted in triplicate.

Circular Dichroism (CD). Prior to CD scans, monomeric MBP-OLF samples were purified once more on an analytical Superdex 75 gl column (GE Healthcare) to remove all traces of aggregate or MBP and then concentrated to 0.25-0.5 μM. CD spectra were acquired at 25 °C on a Jasco J-810 CD spectropolarimeter. Twenty consecutive scans ranging from 200 nm to 300 nm, using a bandwidth of 1 nm at a continuous scanning rate of 500 nm/min, were averaged for each sample.

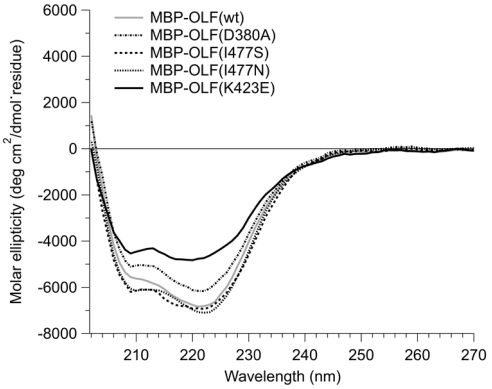
SUPPLEMENTAL FIGURES AND TABLES:

Sample	Fluorescence intensity (513 nm)
Cleaved MYOC-OLF	0.5 ± 0.3
Cleaved MYOC-OLF + TCEP	55.7 ± 2.6
MBP-OLF	0.9 ± 0.1
MBP-OLF + TCEP	46.6 ± 3.1
MBP-OLF(D380A)	0.9 ± 0.2
MBP-OLF(D380A) + TCEP	45.0 ± 2.0
MBP-OLF(I477S)	0.7 ± 0.4
MBP-OLF(I477S) + TCEP	60.9 ± 1.9
MBP-OLF(I477N)	1.5 ± 0.3
MBP-OLF(I477N) + TCEP	71.9 ± 1.5
MBP-OLF(K423E)	0.6 ± 0.2
MBP-OLF(K423E) + TCEP	60.5 ± 2.8

Supplemental Table S1 Characterization of disulfide bonds in OLF-containing proteins in this study. The thiol-modifying reagent ThioGlo® 1 only reacts with OLF-containing proteins when the reducing agent TCEP is added. Thus, OLF retains the single disulfide bond in all proteins characterized.



Supplemental Figure S1. Characterization of OLF and mutants by gel filtration. (a) Aggregates purified as in *Methods* that are reloaded onto preparative Superdex 75 column do not disaggregate into monomers. Inset: Fractionation of aggregates on SDS-PAGE under reducing conditions yields monomer MBP-OLF (~ 72 kDa). Right: molecular mass markers (kDa). (b) MBP-OLF monomers purified as in *Methods* that are reloaded onto analytical Superdex 75 column do not equilibrate to aggregates to any appreciable extent. (c) MBP-OLF(wt) and cleaved MYOC-OLF incubated at 4 °C for over a month do not aggregate to any appreciable extent.



Supplementary Figure S2. Characterization of MBP-OLF and mutants by CD. Overall, the shapes of the mutants are similar to MBP-OLF(wt), exhibiting a mixture of α-helical and β-sheet signatures expected of the fusion protein. Changes in intensity indicate some local perturbations, however.