Engineered Melanopsin Mutants for Optogenetic Vision Restoration

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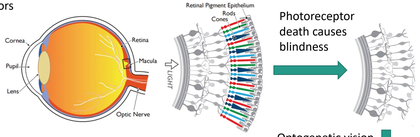
ADVERUM

BACKGROUND

Photoreceptors are the cells responsible for sensing light in the retina



Photoreceptor death causes blindness

Optogenetic vision restoration

Endowing the surviving retinal cells with light sensitivity could restore sight to the retina

Many causes of photoreceptor death

Genetic Diseases	Common Diseases	Accidental
Retinitis Pigmentosa Leber's Congenital Amaurosis	Late-Stage Macular Degeneration Geographic Atrophy	Poison Trauma

Best in class optogenetic product features

The optogenetic protein must be fast, sensitive, and readily supplied with chromophore

Opsin	Within legal light limit in Europe?	Can sense ambient light?	Able to adapt to light?	Chromophore regeneration?	Fast in non-photoreceptor?
ChR2	N	N	N	Y	Y
ChrimsonR	Y	N	N	Y	Y
MCO1	Y	N	N	Y	Y
Rhodopsin	Y	Y	Y	N	N
MW-Opsin	Y	Y	Y	N	N
Melanopsin	Y	Y	Y	Y	N
CaMeRaOpsin	Y	Y	Y	Y	Y

AAV for optogenetics delivery

The optogenetic protein must be expressed in as many target cells as possible

- Intravitreal injections are non-invasive and expose the entire retina to AAV

(1) Naturally occurring AAV serotypes poorly transduce the retina from the vitreous

(2) Designer AAV2.7m8 was developed for improved retinal transduction from the vitreous and has shown tolerability in humans

(3) AAV-LSV1 was developed by a directed evolution screen in NHP for improved retinal transduction from the vitreous

RESULTS

- Successful development of fluorescent calcium imaging assays enabled rapid screening of melanopsin mutants.
- Many point mutations, truncations and chimeras were found to increase the maximum amplitude in the ON assay (Figure 1A) or decrease TauOFF in the OFF assay (Figure 1B).
- Some point mutations were found to synergize with certain truncation or chimera mutants.

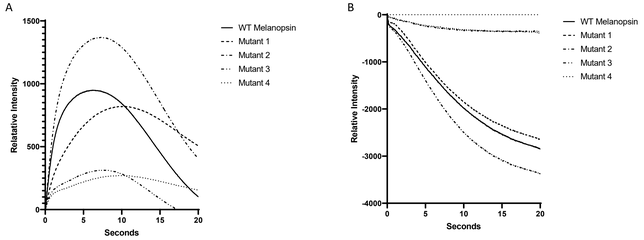


Figure 1. Melanopsin and Melanopsin Mutant Fluorescent Calcium Traces over time.
(A) Example ON assay fluorescent calcium trace. HEK293T Cells were transfected with melanopsin, melanopsin mutant, or GFP with GCaMP6 and imaged in the ImageXpress Micro (Molecular Devices) at 480 nm. (B) Example OFF assay fluorescent calcium trace. HEK293T Cells were transfected with melanopsin, melanopsin mutant, or mCherry with RGECO and imaged at 590 nm after a brief 1 s pulse of 480 nm light.

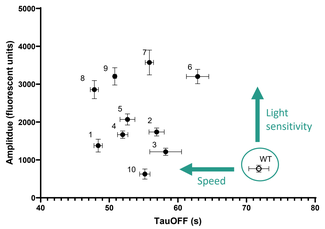


Figure 2. Comparison of Amplitude and TauOFF of Melanopsin and its Second Round Mutants.
Comparison of the amplitude of the ON response versus the TauOFF from the OFF assay for selected second round mutants. Second round mutants were truncations or chimeras with synergistic point mutations. Mutants with increased amplitudes and decreased TauOFF were selected for follow on studies. Error bars = SEM

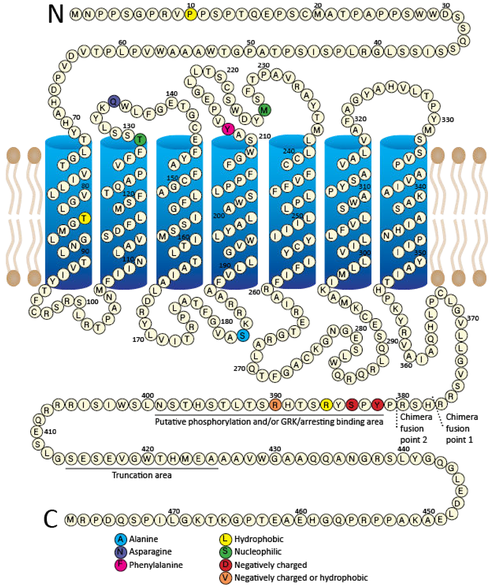


Figure 3. Schematic of Melanopsin Mutations.
Schematic of melanopsin with its human WT amino acids. Amino acids near the N-terminus and above the plasma membrane are in the extracellular space. Amino acids within blue columns are within the membrane. Amino acids underneath the plasma membrane near the C-terminus are intracellular. Highlighted amino acids and noted truncation areas and chimera fusion areas yielded mutants that had an increased ON amplitude or decreased TauOFF.

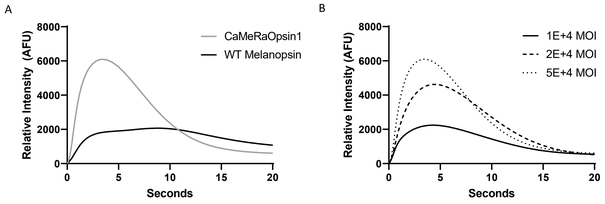


Figure 4. ON Assay Traces from Cells Transduced with AAV2.7m8 Carrying Melanopsin or CaMeRaOpsin
(A) HEK293T transduced with AAV2.7m8-CMV-CaMeRaOpsin or AAV2.7m8-CMV-Melanopsin at MOI of $5x10^4$ vg and imaged with Calcium 6 dye in the ImageXpress Micro (Molecular Devices) at 480 nm. AAV were produced by suspension AAVMAX cells and purified by AVB column followed by CsCl ultracentrifugation. (B) HEK293T transduced with different MOIs of AAV2.7m8-CMV-CaMeRaOpsin. Less AAV transduced resulted in a lower maximum amplification.

- AAV2.7m8 packaged with CMV-Melanopsin or CMV-CaMeRaOpsin successfully generate light response in HEK293T.
  - The light response of CaMeRaOpsin is greater and faster than Melanopsin.
- The amplitude of the light response varies with the dose of AAV2.7m8-CMV-CaMeRaOpsin applied.

CONCLUSIONS & NEXT STEPS

- ✓ Fluorescent calcium imaging can be used to screen melanopsin mutants.
- ✓ Point mutations as well as truncations and chimeras at the CTD of melanopsin can improve speed and light sensitivity.
- ✓ AAV2.7m8 can effectively deliver CaMeRaOpsin as well as Melanopsin. The improved kinetics in CaMeRaOpsins suggests they could be utilized as a therapeutic transgene for optogenetic vision restoration.
- ➢ Next, CaMeRaOpsin will be preclinically evaluated.
  - ➢ Expression target of ≥1% retinal ganglion cells*.

*See our cellular viral genome and mRNA visualization capabilities at poster #1157, *Intravitreal Delivery of Ixo-vec for Neovascular Age-related Macular Degeneration Results in Widespread Expression of Aflibercept in Cynomolgus Monkey Eyes as Confirmed Using In-situ Hybridization* by Julio Delano Nieves, Gustavo de Alencastro, Kelly Michiko Hanna, Jenny Vo, Stephanie Lussier, Ruslan N. Grishanin, & Brigit E. Riley .

METHODS

Make Fast Melanopsin → Calcium Mediating Rapid Opsin (CaMeRaOpsin)

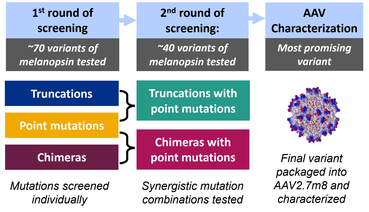
Melanopsin has all the best optogenetics in class features except it is slow

- Slow deactivation prevents rapid successive reactivation

Protein engineering can speed up melanopsin – especially at the C-Terminal Domain (CTD) where deactivation occurs

Melanopsin Mutational Strategies	
Mutate the C-Terminal Domain (CTD)	Incorporate specific mutations to enhance arrestin coupling, to putative PKC sites
Truncate the CTD	Previously shown in the literature to increase speed
CTD chimeras	Fuse the CTD from other GPCRs onto melanopsin
Other point mutations outside the CTD	Incorporate other known point mutation to enhance melanopsin function like enhance retinal release, enhance OFF kinetics, larger calcium response, faster desensitization kinetics

CaMeRaOpsin Development Strategy

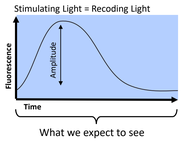


Mutant Melanopsin Screening – Genetically Encoded Fluorescent Calcium Indicator Imaging

- Melanopsin light activation causes an intracellular increase in calcium
- Co-expressing a genetically encoded fluorescent calcium indicator with melanopsin (or mutant) allows for the measurement of [calcium] over time as fluorescence over time
- Select for mutants that strongly respond to light (high amplitude) and quickly return to baseline (small TauOFF)

ON Assay

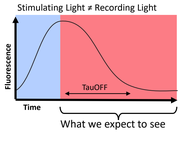
Co-transfect HEK293T with Melanopsin (or mutant) & GCaMP6. Melanopsin is activated by ~480 nm light, the same light used to record GCaMP6. Record cells at 480 nm light.



Measures light response (amplitude of wave)

OFF Assay

Co-transfect HEK293T with Melanopsin (or mutant) & RGECO. Melanopsin is activated by ~480 nm light, but RGECO is recorded with 590 nm light. Briefly stimulating with 480 nm light to activate melanopsin, then record RGECO at 590 nm.



Measures light response decay (TauOFF)

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(3) Baker, C. K., Bennett, A., Belbellaa, B., Nieves, J., Tai, A., McKenna, R., Cepeda, D., Agbandje-McKenna, M., & Riley, B. E. April 2022. Novel Capsid LSV1 Has a Unique 3D Structure at the Loop Substitution Area-Confers Superior Retinal Transduction from Intravitreal Injection. *Molecular Therapy* 30(4):575

Disclosures:

I am an employee of Adverum Biotechnologies and hold shares in the company.