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Disruption of Rhodopsin Dimerization in Mouse Rod Photoreceptors by Synthetic Peptides Targeting Dimer Interface

Sandeep Kumar, Alyssia Lambert, Jon Rainier, and Yingbin Fu

Abstract

Synthetic peptides derived from transmembrane segments of G protein-coupled receptors (GPCR) are used to disrupt GPCR dimer interface. This peptide competition technique is an effective approach to map the dimer interface of GPCR and its functional significance. Here we present a technique to deliver synthetic transmembrane peptides to living mouse rod photoreceptors to disrupt rhodopsin (a prototypical member of Class A GPCRs) dimer formation in the endoplasmic reticulum (ER). We have shown that rhodopsin helix H1- or H8-peptide caused mislocalization of rhodopsin to the perinuclear endoplasmic reticulum (ER).

Keywords

Rhodopsin; Cone opsin; Rhodopsin dimer; Dimerization; Protein trafficking; Peptide competition; Dimer interface; G protein-coupled receptor (GPCR); Rhodopsin helix peptides; Nanoparticle delivery

1 Introduction

Different strategies have been used to study the dimer interface of G protein-coupled receptors (GPCRs) and their functional significance. One effective approach is the peptide competition technique, in which synthetic peptides derived from transmembrane segments are used to disrupt the GPCR dimer interface [1–5]. If the peptide corresponds to a region involved in dimerization, it can compete for that domain and block dimerization. This is typically done in cell cultures where disruption of GPCR dimerization can be assayed by bioluminescence resonance energy transfer (BRET) or fluorescence resonance energy transfer (FRET).

Rhodopsin is a prototypical member of Class A GPCRs. Atomic-force microscopy has shown rhodopsin dimers are packed in rows [6], and biochemical experiments have demonstrated the existence of rhodopsin dimers in rod disc membranes [7–10]. Recent studies have adopted the peptide competition technique to study rhodopsin dimer interfaces in cell cultures and isolated bovine rod outer segment, suggesting the involvement of two interfaces (i.e., H1/H2 and H4/H5) between the two rhodopsin-protomers in the dimer [11].

In the current study, we developed a technique to deliver synthetic transmembrane peptides to living mouse rod photoreceptors to disrupt rhodopsin dimer formation in the endoplasmic reticulum (ER) [12]. To achieve long-term sustained release, we used biodegradable polylactic acid/polylactic acid–polyethylene oxide (PLA/PLA-PEO) nanoparticles (NPs) to deliver peptides to rods by subretinal injection [13]. An ER-targeting signal, KDEL, was

added at the C-terminus of the competing peptides in order to increase its effective concentration in the ER. We have used two animal models for this study, *S-opsin⁺Rho^{+/-}Lrat^{-/-}*, in which short-wavelength-sensitive cone opsin (S-opsin) was transgenically expressed in rods and also lacking chromophore, and wild-type C57Bl6 mice. Since cone opsins, but not rhodopsin, require chromophore for proper folding and trafficking to the photoreceptor's outer segment [14–18], the *S-opsin⁺Rho^{+/-}Lrat^{-/-}* mouse line provides a unique opportunity to explore rhodopsin dimer interface and the physiological relevance of dimerization in vivo. Introducing into *S-opsin⁺Rho^{+/-}Lrat^{-/-}* rods a competing R-opsin transmembrane-helix H1- or H8-peptide, but not H4- or H5-peptide, caused mislocalization of R-opsin and S-opsin to the perinuclear ER. A similar peptide-competition effect was observed even in wild-type rods. It should be mentioned that our work differs from other recent studies in two aspects: (1) it focuses on disruption of rhodopsin dimers in living rods; (2) it addresses rhodopsin-dimer formation in the ER, whereas other work addresses the state of rhodopsin after the protein has reached the ROS. It is possible that rhodopsin forms dimers first in the ER via the H1/H8 interface, followed by higher-order oligomerization involving multiple interfaces after reaching the ROS (perhaps promoted by the very high concentration of rhodopsin) [12]. In conclusion, the technique we described here is useful to study rhodopsin dimer interface and its physiological role in living photoreceptors.

2 Materials

All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) at Baylor College of Medicine and University of Utah, and performed in accordance with the ARVO Statement for the Use of Animal in Ophthalmic and Vision Research. Agents injected into or applied topically to the mice should be sterile and pharmaceutical grade (USP), if available according to federal regulations. Prepare all other analytical grade (ACS) reagent's solutions using ultrapure deionized water (18 MW cm at 25 °C). Store all reagents at room temperature (unless indicated otherwise).

2.1 Preparation of Nanoparticles

1. Rhodopsin helix peptides corresponding to H1 (WQFSMLA AYMFLLVLGFPINFLTYVTV), H4 (HAIMGVVFTWI MALACAAPPLV), H5 (ESFVIYMFVVHFTIPMIVIFFCY GQLV), H8 (NKQFRNCMLTTL), H4-KDEL (HAIMG VVFTWIMALACAAPPLVKDEL), and H8-KDEL (NKQFR NCMLTTLKDEL) were synthesized and purified by Selleck (Houston, TX, USA).
2. Dichloromethane (CH₂Cl₂, HPLC grade, >99.5%).
3. Acetone (HPLC grade).
4. *N,N*-dimethyl formamide (>99.5%).
5. Poly(vinyl alcohol) (89,000–98,000 MW).
6. (D,L)-polylactic acid ((D,L)-PLA, 10,000–18,000 MW).
7. (D,L)-polylactic acid–polyethyleneoxide ((D,L)-PLA–PEO (3750 MW PLA, 700 MW PEO).

8. Coumarin-6 (99%).
9. PLA–PEO stock solution: Prepared by dissolving 28.9 mg (D,L)-PLA–PEO in 0.289 mL acetone (*see Note 1*).
10. Coumarin-6 stock solution: Prepared by dissolving 12.2 mg of coumarin-6 in 25.05 mL CH₂Cl₂.
11. Phosphate buffered saline (PBS): Prepare a 10× PBS stock by dissolving the following in 800 mL deionized H₂O: 80 g NaCl, 2 g of KCl, 14.4 g Na₂HPO₄, and 2.4 g KH₂PO₄. Adjust pH to 7.4, bring the volume to 1 L with additional dH₂O. Prepare 1× PBS by dilution of one part with nine parts of dH₂O. Sterilize by autoclaving.
12. PBS buffer with different pHs: Make 1× PBS with pH 6.8, 7.0, and 7.4. Sterilized using 0.22 µm syringe filter.

2.2 Mouse Preparation and Subretinal Injection

1. Sterile water.
2. 70% Ethanol: To make a 70% ethanol solution, mix 70 mL of 100% ethanol with 30 mL of sterile water.
3. Alcohol wipes.
4. Tropicamide ophthalmic solution USP, 1.0% (Bausch & Lomb, USA)
5. Proparacaine, hydrochloride ophthalmic solution USP, 0.5% (Akorn, USA).
6. Gonok (Hypromellose ophthalmic demulcent solution), USP 2.5% (Akorn, USA).
7. 0.5% Erythromycin ophthalmic ointment (Bausch & Lomb, USA).
8. Ketamine (Vedco Inc.)/Xylazine (Lloyd Laboratories) (65–100/10–20 mg/kg body weight).
9. 30-gauge needle.
10. 1 mL syringe.
11. 32-gauge 5 µL Hamilton syringe (0.5 in. needle length, point style 3, Hamilton, USA).
12. Circular micro glass coverslips, 8 mm diameter.
13. Stereomicroscope.
14. Circulating water warming pad (*see Note 2*).

2.3 Eye Fixation and Sectioning

1. Dissection tools.
2. Transfer pipettes.

3. Red color ultrafine permanent markers.
4. Orbital shaker.
5. 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4: Weigh 4.5 g of NaOH pellets and dissolve in ~750 mL of dH₂O. Add 40 g of PFA powder and stir with magnetic bar at 50–55 °C until dissolved. Add 18.75 g of monobasic sodium phosphate monohydrate (NaH₂PO₄·H₂O). Stir until dissolved, then chill the fixative on ice. Adjust pH to 7.4. Bring volume to 1000 mL with dH₂O (*see Note 3*).
6. 0.1 M phosphate buffer, pH 7.4: Dissolve 4.5 g of NaOH pellets in to 750 mL of deionized H₂O. Then, add 18.75 g of NaH₂PO₄·H₂O and dissolve. Adjust pH to 7.4 and bring volume to 1 L.
7. 0.1 M phosphate buffer, 0.9% NaCl: For 100 mL, weigh 0.9 g of NaCl and dissolve in 100 mL of 0.1 M phosphate buffer. Stir until dissolved.
8. 15% sucrose in 0.1 M phosphate buffer, pH 7.4: Dissolve 15 g of sucrose in ~60 mL of 0.1 M phosphate buffer, bring up volume to 100 mL with additional phosphate buffer. Adjust pH to 7.2–7.4 if necessary.
9. 30% sucrose in 0.1 M phosphate buffer, pH 7.4: Dissolve 30 g sucrose in ~60 mL of 0.1 M phosphate buffer, bring up to 100 mL with additional phosphate buffer. Adjust pH to 7.4 if necessary.
10. Optimum cutting temperature (OCT) compound.
11. Tissue embedding mold.
12. Cryo-sectioning machine (Leica CM 3050, Leica, USA).

2.4 Immunohistochemistry

1. Hydrophobic PAP Pen.
2. 1× PBST: 0.6% v/v Triton X-100 in 1× PBS, pH 7.4.
3. Blocking buffer: 1% v/v BSA in 1× PBST, pH 7.4.
4. Primary antibody: anti-S-opsin (MBO) [19], anti-M-opsin (AB5405; Millipore), anti-Rhodopsin (1D4) [20], anti-Calnexin (sc-11397; Santa Cruz Biotechnology), and anti-CNGA1 (1D1) [21].
5. Secondary antibody: Cy3 donkey anti-mouse IgG (#715-165-150), Alexa 488 donkey anti-rabbit IgG (#711-545-152) (both are from Jackson ImmunoResearch Laboratories, USA).
6. DAPI.
7. Glass slides.
8. Coverslips.
9. Aqueous mounting medium.

10. Confocal fluorescent microscope.

3 Methods

3.1 Preparation of Control Nanoparticles

1. Add 410 μL of the coumarin-6 stock solution and 50 μL of the PLA-PEO stock solution to a solution of 50 mg of (D,L)-PLA (10,000–18,000 MW) in 2 mL CH_2Cl_2 .
2. Sonicate the resulting mixture using a probe sonicator (smallest tip) for 30 s.
3. Add 10 mL of a 5% (w/v) solution of polyvinyl alcohol (PVA) in dH_2O (500 mg of PVA in 10 mL dH_2O) and again sonicate the resulting mixture using a probe sonicator (smallest tip) for 30 s.
4. Gently pour the resulting emulsion into 50 mL of a 0.5% (w/v) solution of PVA in dH_2O (250 mg of PVA in 50 mL of dH_2O). Stir the resulting mixture overnight to evaporate the organic solvents.
5. Collect the solid nanoparticles after centrifugation at $13131 \times g$ for 40 min. Wash three times with dH_2O water, and lyophilize overnight.

3.2 Preparation of Peptide-Conjugated Nanoparticles

1. Add sequentially 10 μL of the PLA-PEO stock solution, 82 μL coumarin-6 stock solution, and a solution of 3.42 μmol of the transmembrane peptide (*see Table 1*) in 0.12 mL of DMF (*see Note 4*) to a solution of 10 mg of (D,L)-PLA (10,000–18,000 MW) in 0.8 mL of CH_2Cl_2 .
2. Sonicate the resulting mixture using a probe sonicator (smallest tip) for 30 s.
3. Add 2 mL of a 5% (w/v) solution of polyvinyl alcohol (PVA) in dH_2O (100 mg of PVA in 2 mL dH_2O) and sonicate the resulting mixture using a probe sonicator for 30 s.
4. Gently pour the resulting emulsion into 10 mL of a 0.5% (w/v) solution of PVA in dH_2O (50 mg of PVA in 10 mL of dH_2O). Stir the resulting mixture overnight to evaporate the organic solvents.
5. Collect the solid nanoparticles after centrifugation at $13131 \times g$ for 40 min. Wash three times with dH_2O water, and lyophilize overnight (*see Note 5*).

3.3 Nanoparticle Solubility

Check the solubility of nanoparticles in phosphate buffer saline (PBS) with different pHs (*see Note 6*).

3.4 Nanoparticle Dosages

Determine the optimal nanoparticle dosage by immunohistochemistry of injected eyes, which should be selected based on maximizing dimer disruption and low retinal toxicity. For

our experiments, we used a total of 2.25 μg (1.5 μL of 1.5 mg/mL) of peptide-conjugated nanoparticles for one eye.

3.5 Mouse Preparation

1. Restrain and hold the mouse firmly. Dilate mouse eye with 1% tropicamide ophthalmic solution by squeezing the bottle right above the cornea (*see Note 7*). Repeat the procedure for the other eye. Return mouse to the original cage and wait for 5–10 min.
2. Weigh the mouse on a scale to determine the amount of anesthesia (Ketamine/Xylazine 65–100/10–20 mg/kg) needed (*see Note 8*).
3. Retrieve a sterile 1-mL syringe along with a 30-gauge needle and fill with the volume required for anesthesia. Restrain and hold the mouse firmly and inject the mouse intraperitoneally with the anesthetics. Wait until the mouse is fully anesthetized, which takes ~5 min (*see Notes 9 and 10*).
4. Hold the mouse in hand and apply 1 drop of local anesthetic agent Proparacaine Hydrochloride Ophthalmic Solution directly on the mouse cornea and wait for at least 30 s.
5. Retrieve Hamilton syringe along with a 32-gauge blunt needle and fill with 1.5 μL of control nanoparticles or nanoparticles containing rhodopsin helix peptides.

3.6 Subretinal Injection (See Notes 11 and 12)

1. Turn on the circulating water warming pad device.
2. Place a sterile pad under the operating stereomicroscope. Replace pad for each mouse.
3. Place the fully anesthetized mouse with good pupil dilation on the pad with the eye facing the ceiling and the body lying parallel to the stereomicroscope.
4. Gently retract the mouse eyelids with two fingers and apply a small drop of Gonak to the eye. Then, place an 8-mm circular glass coverslip at an angle so that retinal vessels become visible.
5. Using a 30-gauge needle to make a sclerotomy 1-mm posterior to the limbus without hitting the vessels.
6. Insert a 32-gauge blunt needle attached to a 5- μL Hamilton syringe, filled with NP containing rhodopsin helix peptide, through the sclerotomy with 45 °C angle, importantly without touching the lens into the subretinal space by passing through the vitreous cavity and retinal layers (*see Note 13*).
7. Immediately stop advancing the needle when there is a small resistance on the other end of the eye (*see Note 14*).
8. Slowly inject 1–1.5 μL of nanoparticles containing peptides into the subretinal space by applying constant pressure on the syringe plunger.

9. After nanoparticle delivery, leave the 32-gauge needle at the injection site for several seconds. This will help NP to get distributed into the subretinal space.
10. Slowly remove the 32-gauge blunt needle attached to a 5- μ L Hamilton syringe through the sclerotomy from the subretinal space without touching the lens.
11. Check the formation of bubble like appearance and any sign of hemorrhage (*see Note 15*).
12. Wipe off Gonak with sterile cotton tipped applicator from the injected eye and apply 0.5% erythromycin ophthalmic ointment.
13. Repeat steps 3–12 and inject the same volume of control coumarin nanoparticles into the other eye of the same mouse.
14. Leave the mouse on the circulating water warming pad for recovery from the anesthesia. After recovery, return mouse to the original cage and holding area.
15. Reapply 0.5% erythromycin ophthalmic ointment on to the eyes 72 h after injection.

3.7 Eye Enucleation, Fixation, Embedding, and Cutting

Euthanize the mice 6 days after subretinal injection by following the approved animal protocol.

1. Before enucleation of the mouse eye, remember to mark the injection site with a red color permanent marker.
2. Wash the eye cup in 1 $\times$ PBS pH 7.4 and place it under a stereomicroscope. Trim off extra muscles and optic nerve from the globe.
3. Release intraocular pressure by inserting a 30-gauge needle just posterior to the limbus or through the center of cornea. Both methods would work equally well.
4. Grab the tissue with a tweezer from the needle's inserted site and make a small slit with a micro surgical scissor at the limbus or cornea. Submerge the eye globe in 4% PFA in a vial and place on an orbital shaker for gentle agitation for 30 min at room temperature (*see Note 16*).
5. Carefully take out the eye cup from the vial using transfer pipette and remove the cornea and iris.
6. Resubmerge the eye cup in fresh 4% PFA in a vial. Place the vial for agitation on an orbital shaker at 4 °C overnight.
7. Cryoprotect tissue by submerging eye cup in graded sucrose solutions, 30 min in 10% sucrose, 30 min at 15% sucrose, and then overnight at 4 °C in 30% sucrose.
8. Following morning, remove the lens from the eye cup and replace 30% sucrose solution inside of eye cup with Tissue-Tek OCT medium.
9. Embed the eye cup in OCT medium by placing the red-color marked injection site facing down in the CryoMold. We usually embed 4–8 eyes per mold. Snap-

freeze the OCT in a dry ice–ethanol bath, cut the block immediately or store at -80°C .

10. When ready to cut, set the cryostat's chamber and objective temperature at -20°C . Allow OCT block to get equilibrated to the cutting temperature set in the machine, which takes minimum 30–45 min, orientate the position of the OCT block, and collect 12–16 μm thick sections serially on “Superfrost Plus” slides (see Note 17).
11. Air-dry the slides for an hour at room temperature and then store at -80°C .

3.8 Immunohistochemistry (IHC)

Rhodopsin mistrafficking is not widespread in WT mice likely due to the availability of nanoparticles (see Note 18). It is important to pick up enough slides to cover the entire eye cup, i.e., close to and far away from the injection site. In our experience, four slides (six sections/slide, every 4th or 5th slides, e.g., 1, 4, 8, 12) provide good coverage in the first IHC experiment (see Note 19). We excluded the injection site in all analyses in order to avoid any injury effect.

1. Thaw retinal sections approximately for 30 min at room temperature.
2. Draw a line surrounding the retinal sections with hydrophobic PAP pen and let it dry at room temperature.
3. Wash the retinal sections with $1\times\text{PBS } 3\times 5\text{ min}$.
4. Permeabilized sections by incubating in PBST for 5 min at room temperature.
5. Rinse gently in $1\times\text{PBS } 3\times 5\text{ min}$.
6. Cover the sections with blocking buffer for 1 h at room temperature.
7. Replace blocking buffer with the blocking buffer containing primary antibodies and incubate overnight at 4°C .
8. Following morning, rinse the slide gently in $1\times\text{PBS } 3\times 5\text{ min}$.
9. Incubate sections with blocking buffer containing secondary antibodies for 1 h at room temperature.
10. Rinse gently in $1\times\text{PBS } 3\times 5\text{ min}$.
11. For nuclei staining, incubate section for 10 min at room temperature with $1\times\text{PBS}$ containing DAPI.
12. Rinse gently in $1\times\text{PBS } 3\times 5\text{ min}$.
13. Mount the sections in mounting medium and cover the sections with glass coverslip.
14. Scan slides with a Confocal Fluorescent Microscope. Representative results from our published work were shown (Disruption of S-opsin and R-opsin heterodimer in *S-opsin⁺Rho^{+/-}Lrat^{-/-}* rods in Fig. 1; disruption of rhodopsin homodimer in WT rods in Fig. 2) [12].

4 Notes

1. The PLA-PEO stock solution is stable for at least 1 month when stored at -20°C .
2. Circulating water devices offer excellent thermostatic control. Water is warmed by a control unit and then pumped through a pad, where the heat is transferred to the animal. Check the mouse every 10–15 min and turn the body from side to side until recovered.
3. While preparing 4% PFA, heat should never get above 60°C . Prepare 4% PFA fresh to ensure good fixation.
4. The relative amounts of CH_2Cl_2 for dissolving the PLA and DMF for dissolving the transmembrane peptides were modified for some peptides to ensure that the peptides and PLA were in solution.
5. It is necessary to subject nanoparticles to bath sonication for 5 s to redisperse them in water.
6. Nanoparticles aggregation can cause toxicity to the retina in the injected eye. We have found that pH plays a major role in nanoparticles solubility. It is important to test buffers with several pHs, ranging from acidic to alkali condition. Select an optimal pH so that nanoparticles are well dispersed and do not form visible aggregates. For our nanoparticles preparation, pH 6.8–7.4 worked equally well. Therefore, we choose neutral pH 7.0 for our experiments. Sometimes nanoparticles are dissolved better in acidic pH, e.g., 6.0 or 6.5. In this case, use MES or MOPS buffer.
7. It is important to wait for minimum 5 min to get enough pupil dilation. A good dilated pupil provides a better and wider view of retina vasculature under stereomicroscope, which makes it easy to select the injection site with no retinal vessels.
8. Anesthesia overdose causes cataract, which makes it difficult to find a good injection site (e.g., free of retinal vessels) and increase the rate of bad subretinal injection (e.g., hemorrhage).
9. Be careful not to introduce any air bubbles into the syringe.
10. Before performing subretinal injection, monitor the depth of anesthesia by assessing toe reflexes on a mouse. Mouse should not respond to toe pinch under deep anesthesia.
11. Good technique in subretinal injection is essential for the success of this experiment. Practice subretinal injection until you are confident about the technique. We also recommend to have a two-person team to perform subretinal injection. One person inserts and holds the syringe while the other person presses the plunger.

12. Between each eye injection, proper cleaning and washing of the Hamilton syringe and needle is essential to minimize the chances of infection. After performing each injection, carefully wipe off the 32-gauge needle with an alcohol wipe and wash the syringe thoroughly with 70% ethanol followed by sterile water. For washing, place 5–10 Eppendorf tubes (1.5 mL) on a stand in a row for each 70% ethanol and sterile water. Perform serial washing first with 70% ethanol and then with sterile water.
13. Move the mouse head and glass coverslip around until you get the best retinal vasculature view and then insert the 32-gauge needle at 45 °C angle without touching the lens. Lens damage interferes with in vivo imaging like fundus photography and angiography.
14. Resistance is a signal that the blunt needle has encountered the RPE and well placed into the subretinal space. If you do not stop, needle will damage the RPE layer and you might inject into the choroid vasculature or cause further damage in RPE/choroid.
15. In the injected eye, bubble formation without any sign of hemorrhage is the hallmark of a successful subretinal injection. Exclude the eyes from the analysis with no bubble formation and/or with hemorrhage.
16. We use 3–4 mL of 4% PFA and sucrose solution for one eye.
17. Remember to number the slides. It is important to collect serial sections from the whole eye. We usually collect 20 slides, and 6–8 sections per slide.
18. In transgenic *S-opsin⁺Rho^{+/-}Lrat^{-/-}* mice, H1- and H8-induced opsin mislocalization was wide spread (>1/3 of the retina). In WT, H8-KDEL-induced opsin mislocalization was more localized to near the injection site (100–200 µm). This was likely due to stronger homomeric R–R interaction in WT rods.
19. If you find partial mistrafficking in some sections on one slide, you can usually find the best area for mistrafficking by selecting the neighboring slides for another “follow-up” IHC.

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Fig. 1.

Peptide-competition experiments in *S-opsin*⁺*Rho*^{+/-}*Lrat*^{-/-} rods. (a) Subretinal injection of nanoparticle-encapsulated R-opsin H1 and 8 peptides but not R-opsin H4 and H5 peptides caused mislocalization of both R-opsin and S-opsin in *S-opsin*⁺*Rho*^{+/-}*Lrat*^{-/-} rods. Mouse retinas were stained with anti-R-opsin (red) and anti-S-opsin (green) antibodies. White arrows indicate mislocalized R-opsin and S-opsin in the perinuclear region. Yellow arrows indicate mislocalized R-opsin and S-opsin in the inner segment. Green arrows indicate retinal blood vessels in the OPL labeled by the Cy3-conjugated goat anti-mouse secondary antibody. (b) Mislocalized R-opsin caused by subretinal injection of R-opsin H8 peptide colocalizes with the ER marker calnexin in *S-opsin*⁺*Rho*^{+/-}*Lrat*^{-/-} rods. *S-opsin*⁺*Rho*^{+/-}*Lrat*^{-/-} mice were injected with nanoparticle-encapsulated R-opsin H8 peptide. Mouse retinas were stained with R-opsin (red) and calnexin (green) antibodies. Nuclei were stained with DAPI (blue). White arrows indicate the colocalization of mislocalized R-opsin with calnexin in the perinuclear region. NP nanoparticle control, ROS rod outer segments, RIS rod inner segments, ONL outer nuclear layer, OPL outer plexiform layer. Scale bar, 10 μ m.

Reproduced from [12]

Fig. 2.

Peptide-competition experiments in *WT* rods. Subretinal injection of nanoparticle-encapsulated R-opsin H8-KDEL peptide but not R-opsin H4-KDEL peptide caused mislocalization of rhodopsin in *WT* rods. Mouse retinas were stained with anti-R-opsin (red) and anti-calnexin (green) antibodies. White arrows indicate mislocalized R-opsin in the perinuclear ER region, which is colocalized with the ER marker calnexin. Yellow arrows indicate mislocalized R-opsin in the inner segment. Scale bar, 10 μm. Reproduced from [12]

Table 1
Amounts of transmembrane peptide used in the preparation of the nanoparticles

Transmembrane peptide	Amount (mg)
H1	11.67
H4	7.90
H5	11.08
H8	5.02
H4-KDEL	9.56
H8-KDEL	6.70