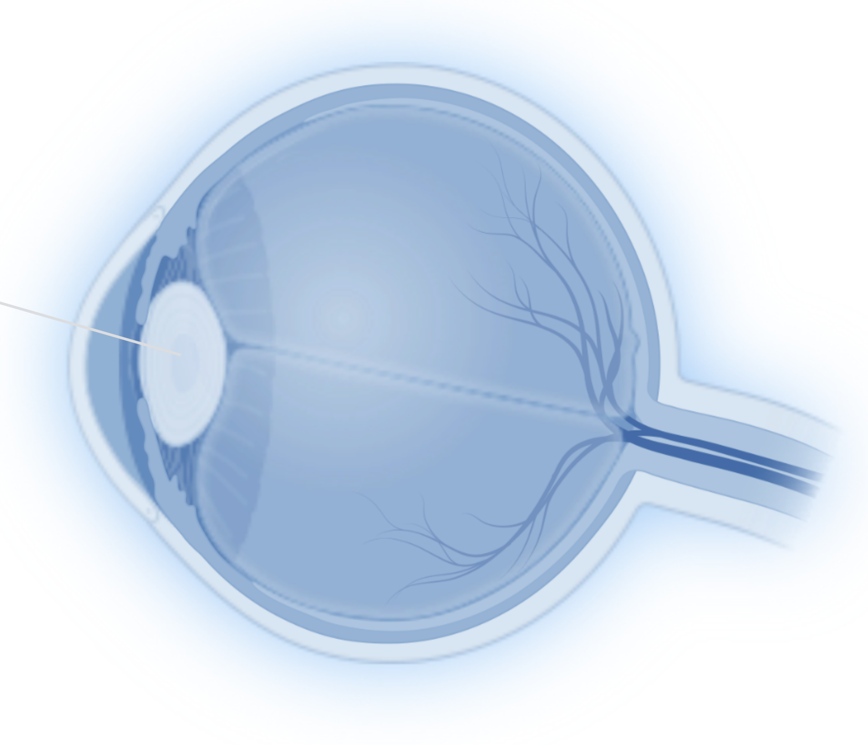
The Chemistry of Eyesight

Literature Discussion, Mar 28th 2023

Katherine Burton
MacMillan Group
Princeton University

The vertebrate optic lens

Crystallins and the chemistry of transparency

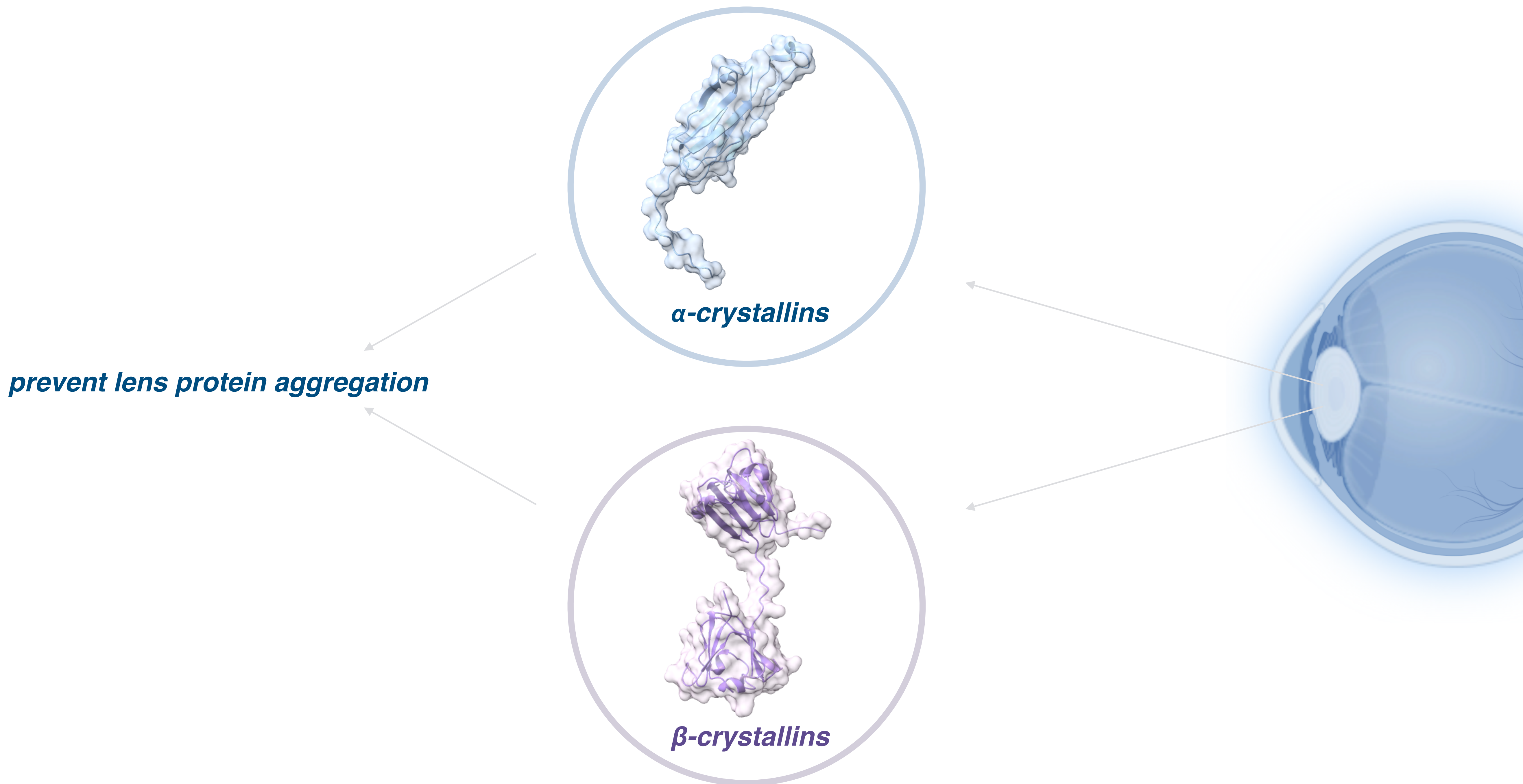


- ***Paracrystalline network***
- ***Low rate of protein turnover***
- ***Requires life-long stability***

Roskamp, K. W. et al. *Acc. Chem. Res.* **2020**. *53*, 863–874.

The vertebrate optic lens

Crystallins and the chemistry of transparency



Crystallin polydispersity enables fluid packing densities

Bari, K. J., Sharma, S. *J. Phys. Chem. B.* **2020**. *124*, 11041–11054.

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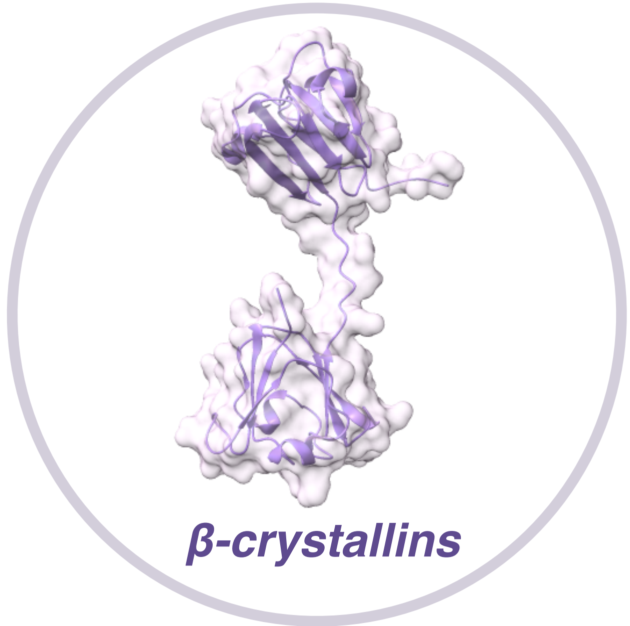

high degree of structural complexity

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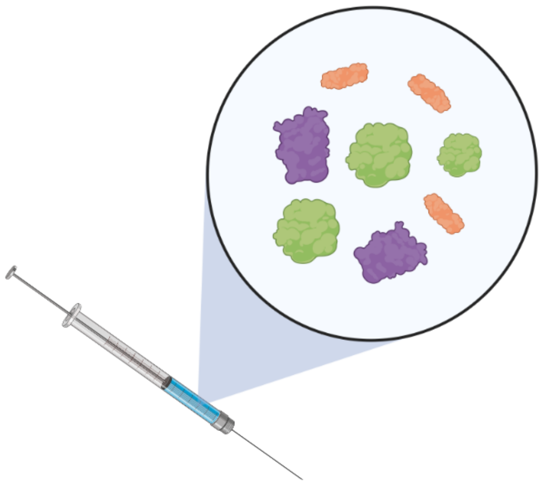
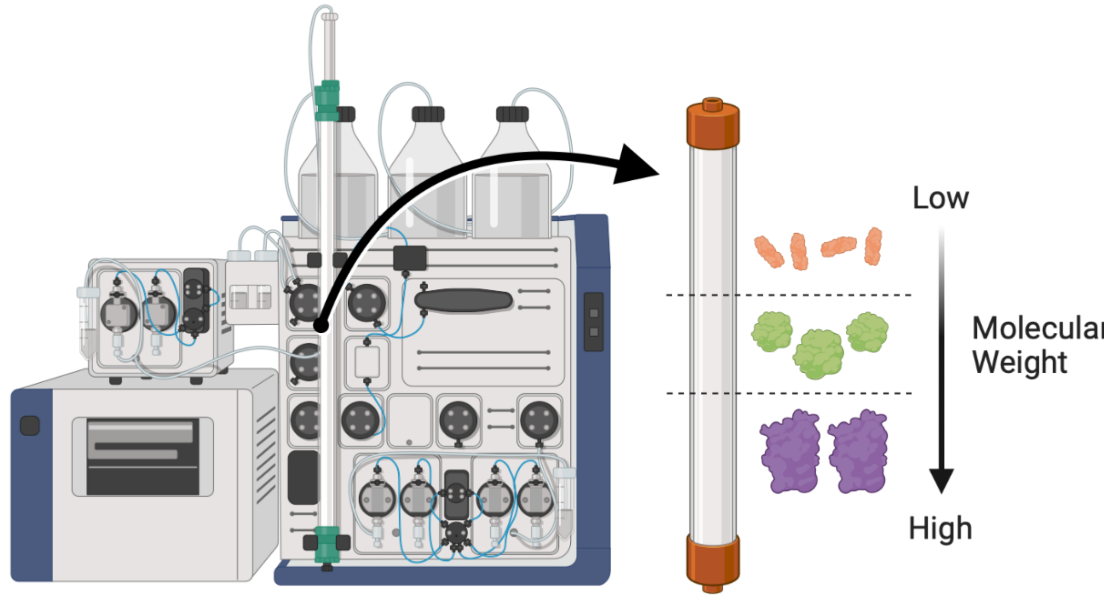
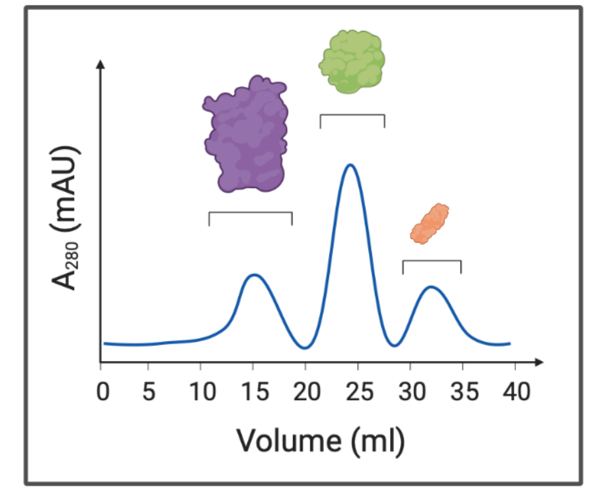
Roskamp, K. W. et al. *Acc. Chem. Res.* **2020**. *53*, 863–874.

Crystallin Protein Purification by Size-Exclusion Chromatography (SEC)







1. Injection

Heterogeneous protein loaded on FPLC

2. Elution

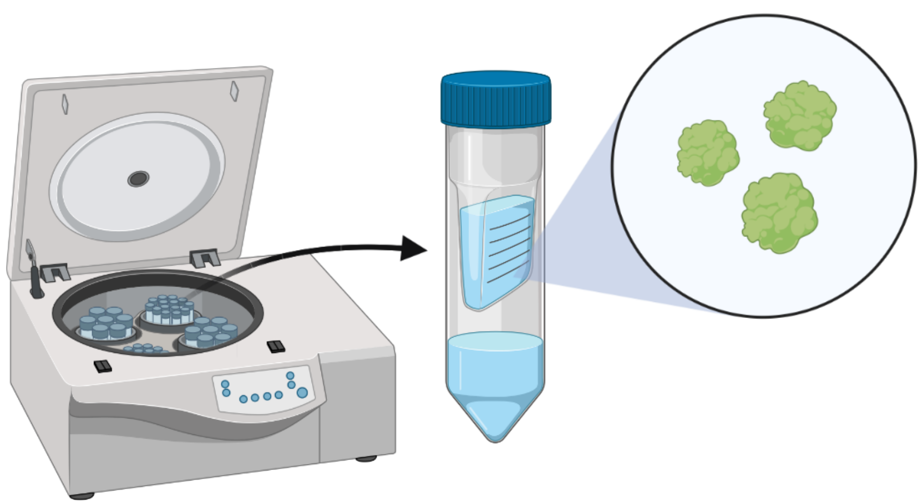
Proteins are separated by molecular weight

3. Abs Measurement

Abs measured for all eluted fractions

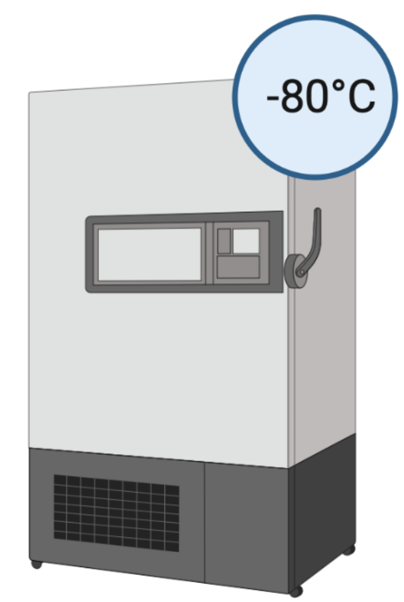
4. Concentration

Fractions of interest are mixed and concentrated



5. Storage

Purified proteins stored at -80 °C

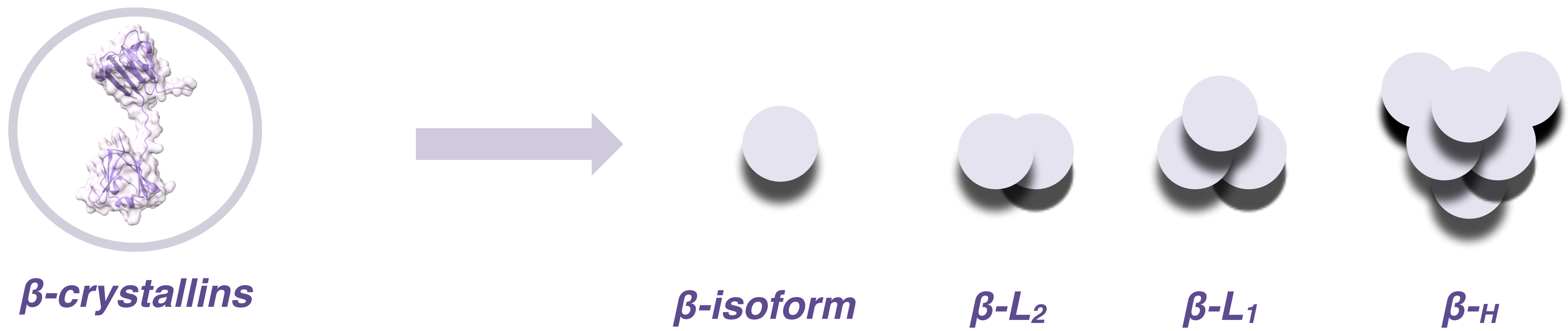


Acknowledgments to Biorender for generation of schematics

Bergman, M. R., Derive, L. F. *Proc. Natl. Acad. Sci.* **2022**. *119*, 1–10.

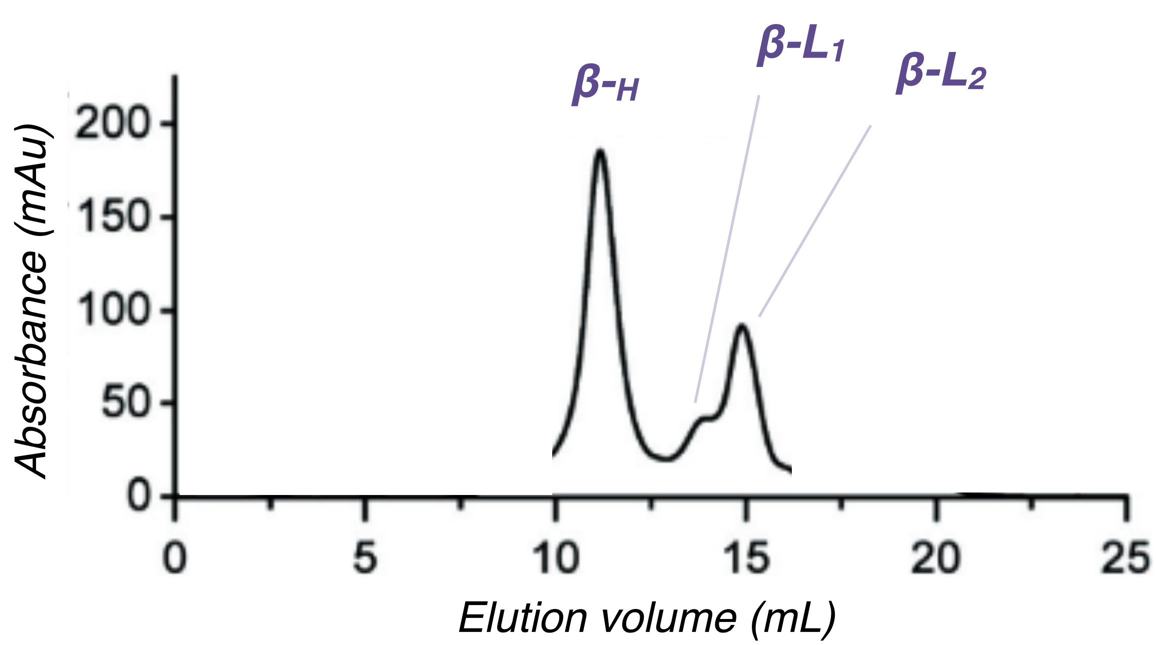
Crystallins and the chemistry of the optic lens

Polydispersity index of β-crystallins

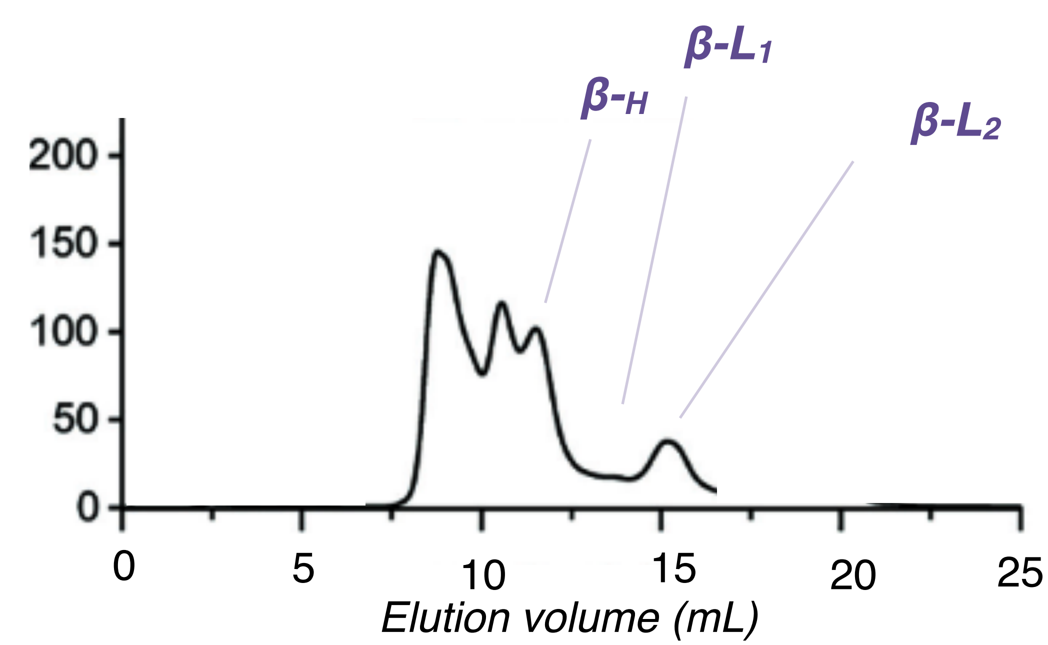


Polydispersity enables packing densities across the optic lens

β-crystallin distribution
Outer cortical lens



β-crystallin distribution
Nucleus



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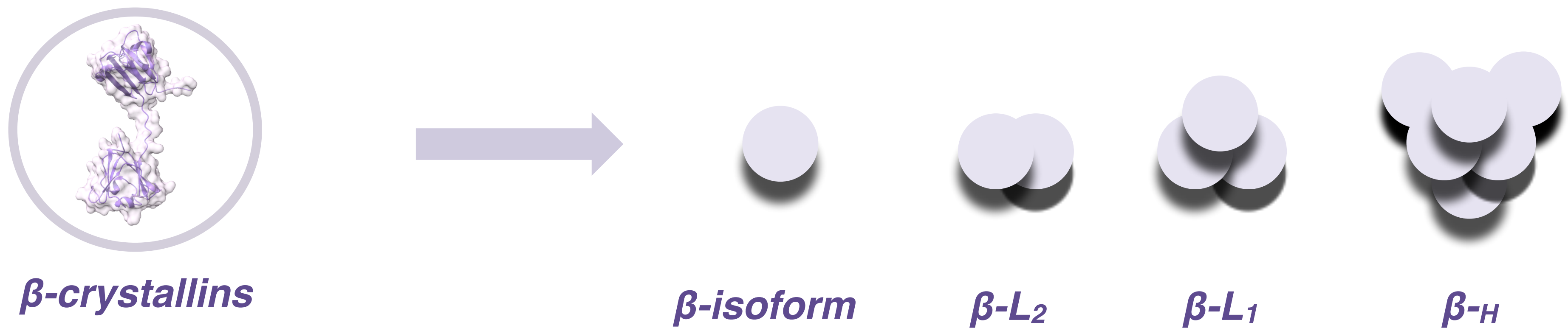

Cationic salts perturb oligomer size and surface charge

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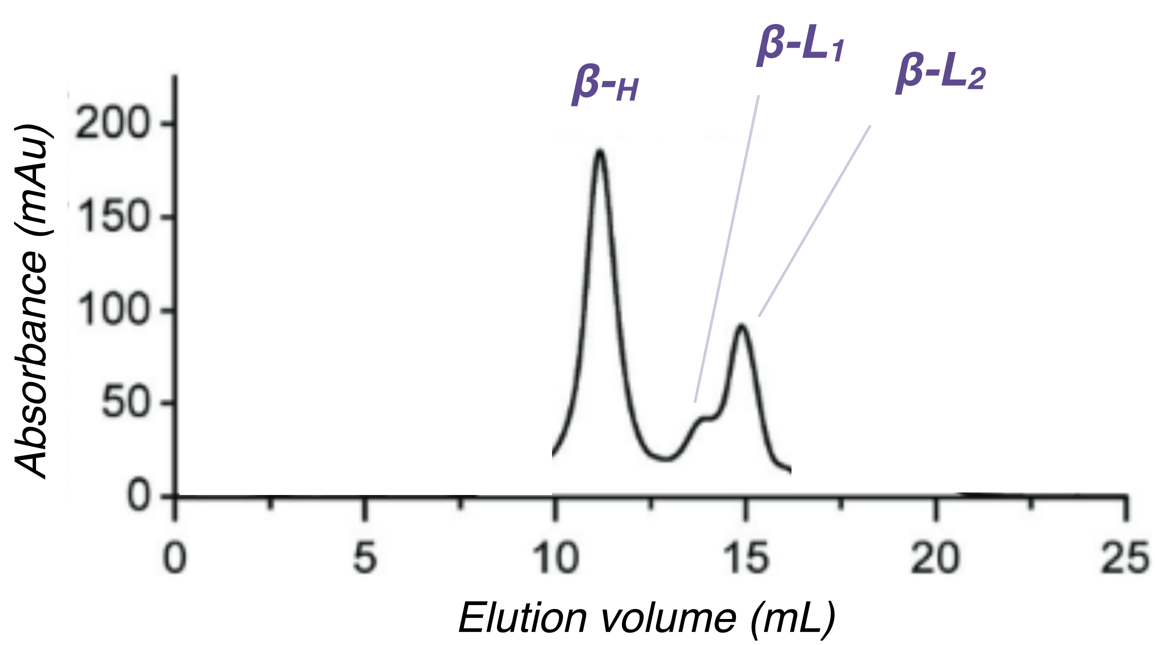

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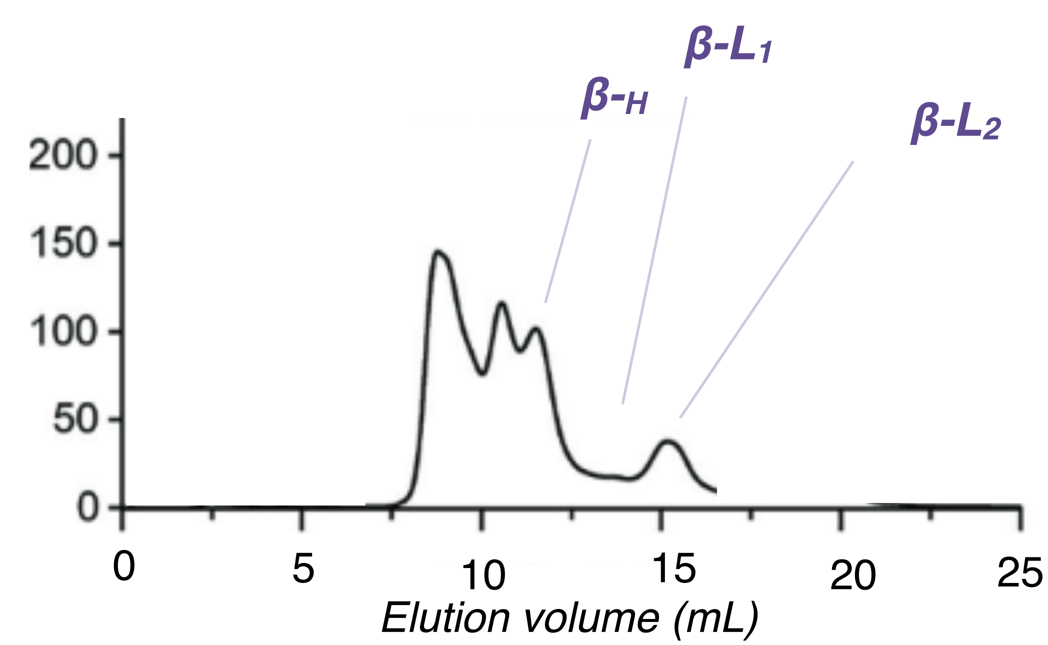

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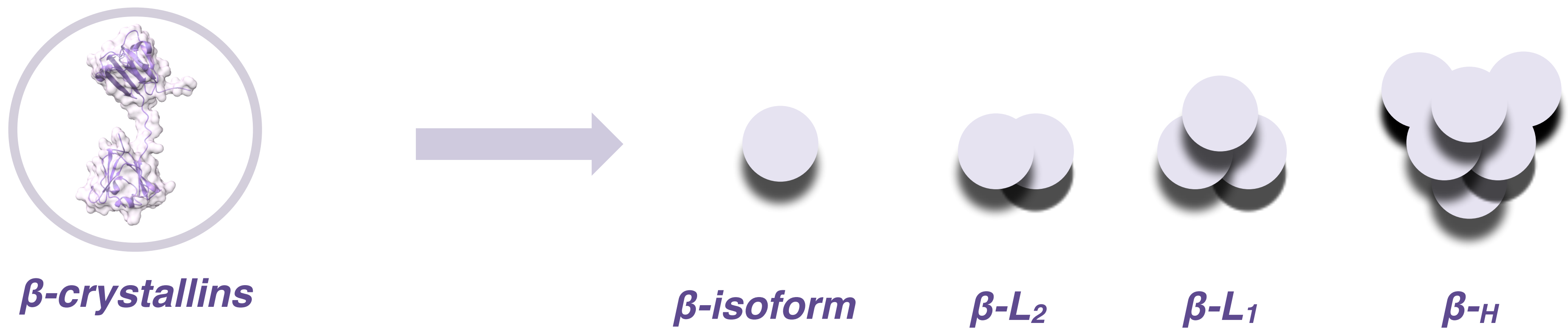

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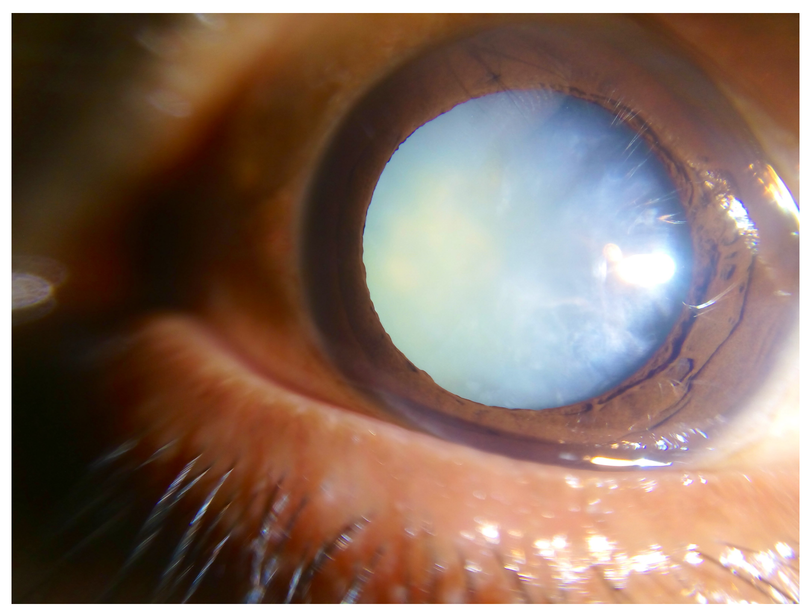

Age-related cataract formation

Bergman, M. R., Derive, L. F. *Proc. Natl. Acad. Sci.* **2022**. *119*, 1–10.

Crystallins and the chemistry of the optic lens

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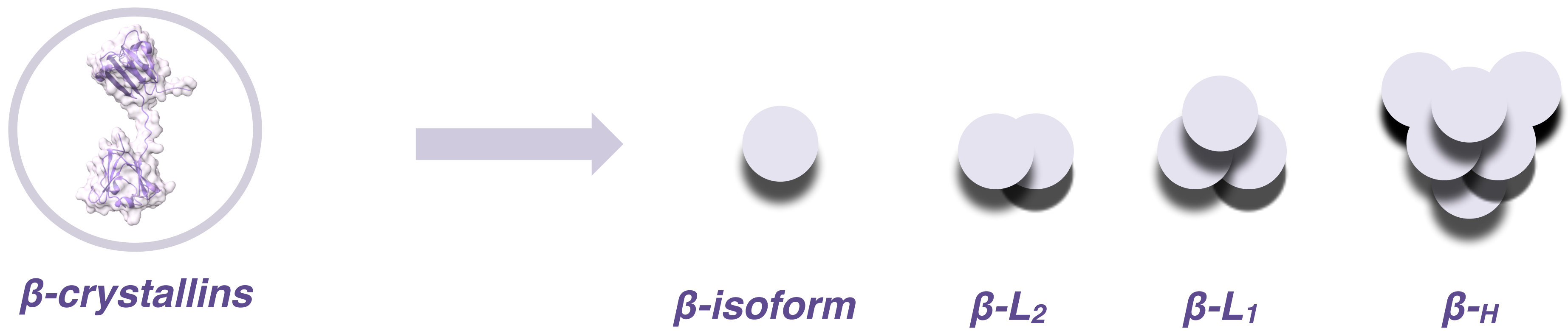

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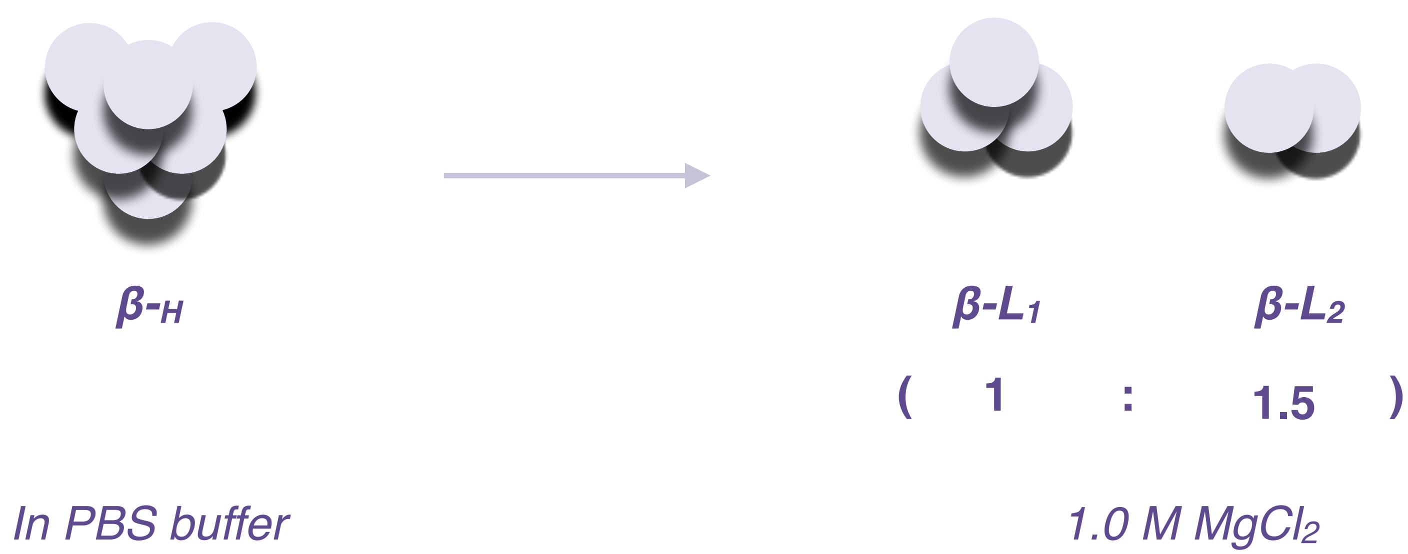

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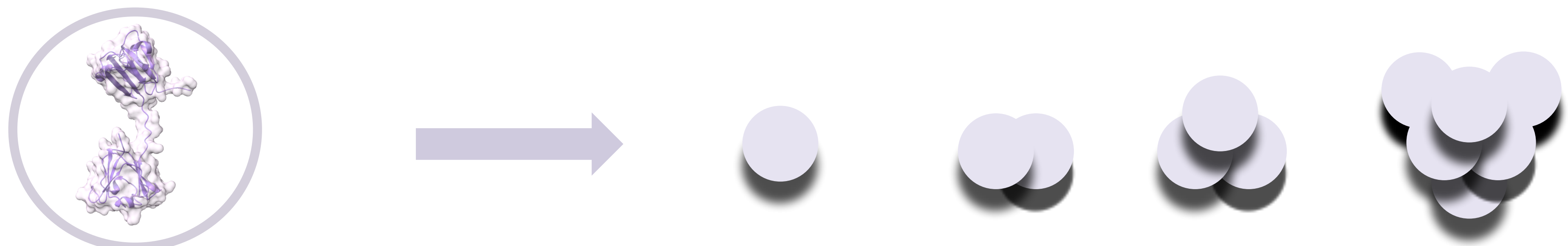


β-crystallins

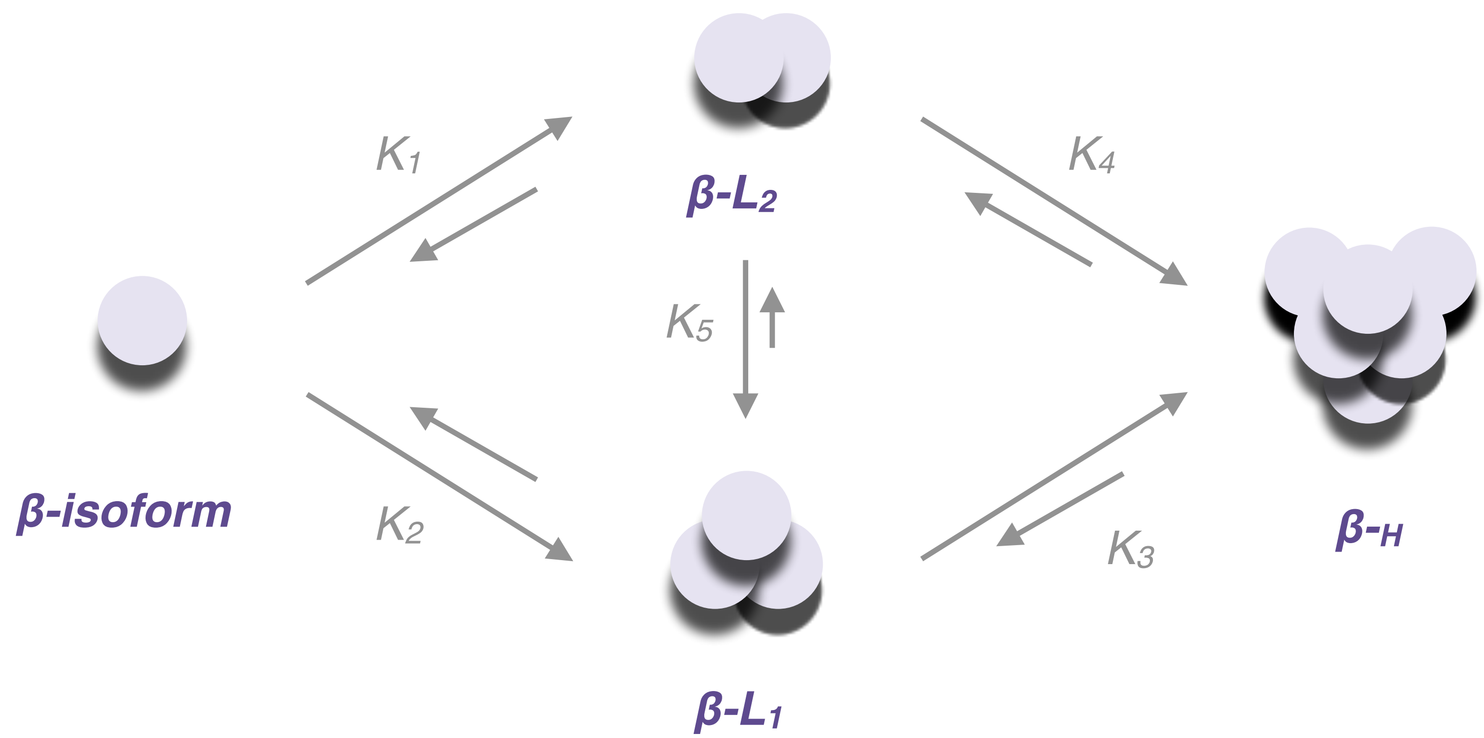
β-isoform ***β-L_2*** ***β-L_1*** ***β-$_H$***

Cationic salts perturb oligomer size and surface charge

Dissociation stops at dimers

Bergman, M. R., Derive, L. F. *Proc. Natl. Acad. Sci.* **2022**. *119*, 1–10.

Crystallins and the chemistry of the optic lens



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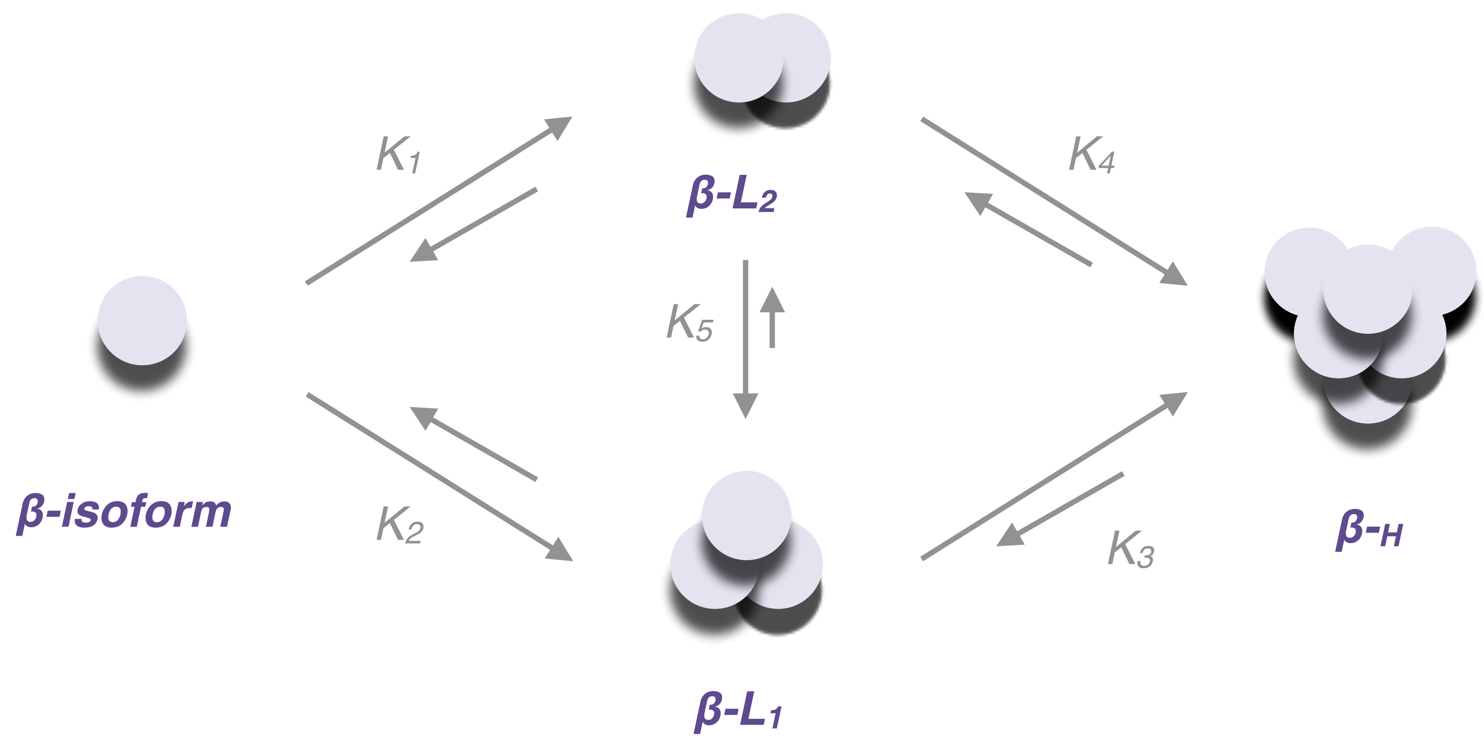


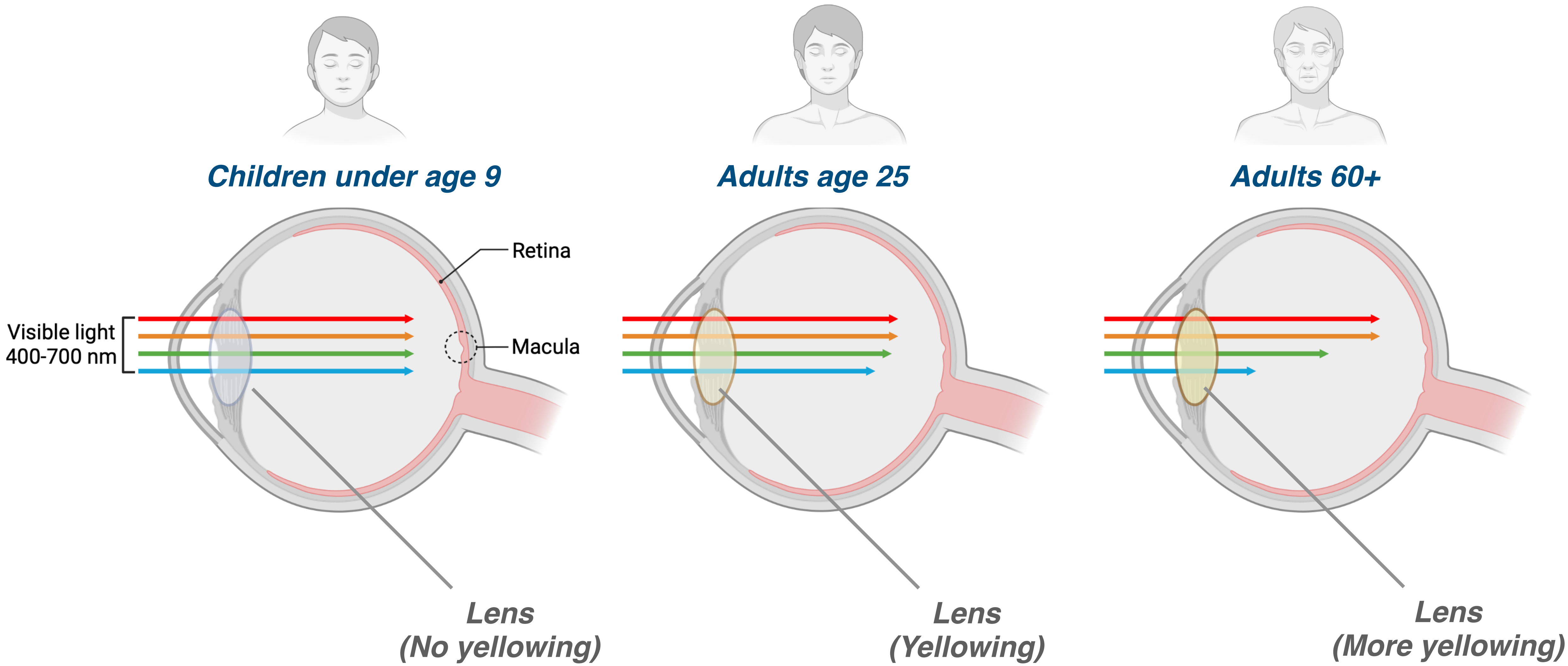
Cationic salts perturb oligomer size and surface charge

$K_1 \leq K_2 >> K_3 > K_4 > K_5 > 1$

Multistep chemical equilibrium favors higher order assembly

Bergman, M. R., Derive, L. F. *Proc. Natl. Acad. Sci.* **2022**. *119*, 1–10.

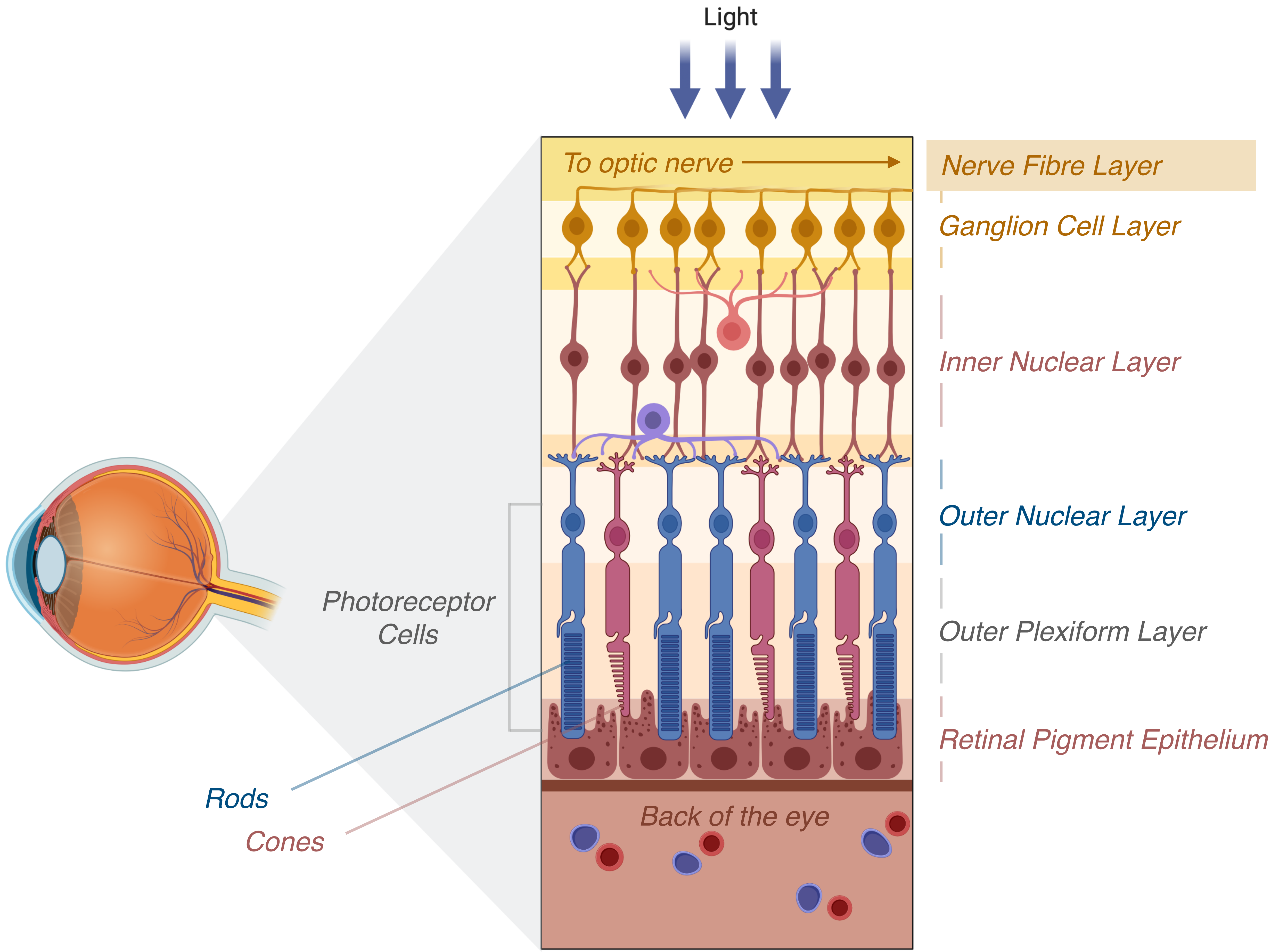
Age-related effects on vision



Age-related changes in divalent cation homeostasis impact vision

Bloemendal, H. et al. *Prog. Biophys. Mol. Bio.* **2004**. *86*, 407–485.

Retinal structure and function



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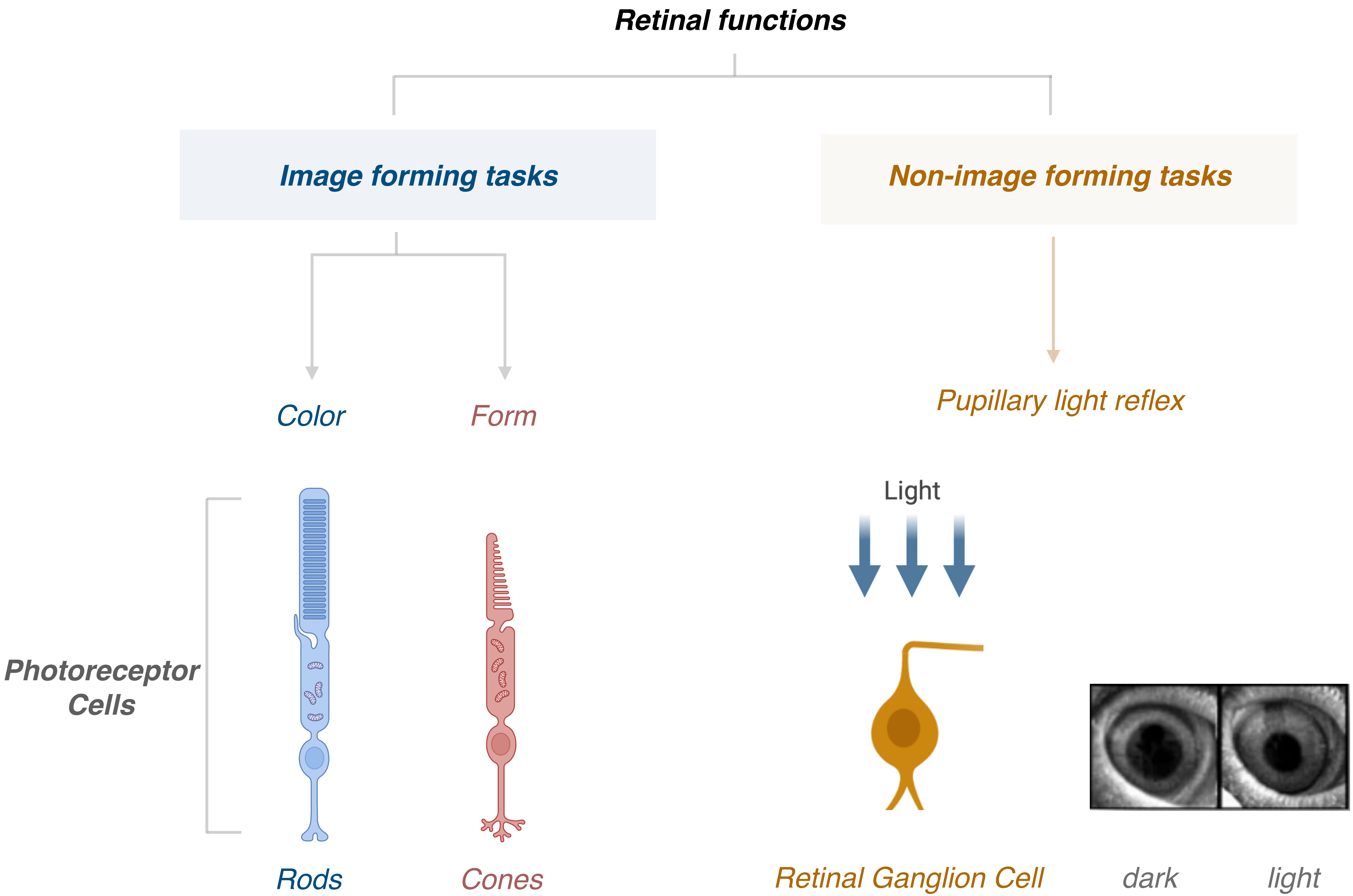

Guido, M. E. Et al. *Cell. Mol. Neurobio.* **2020**. *42*, 59–83.

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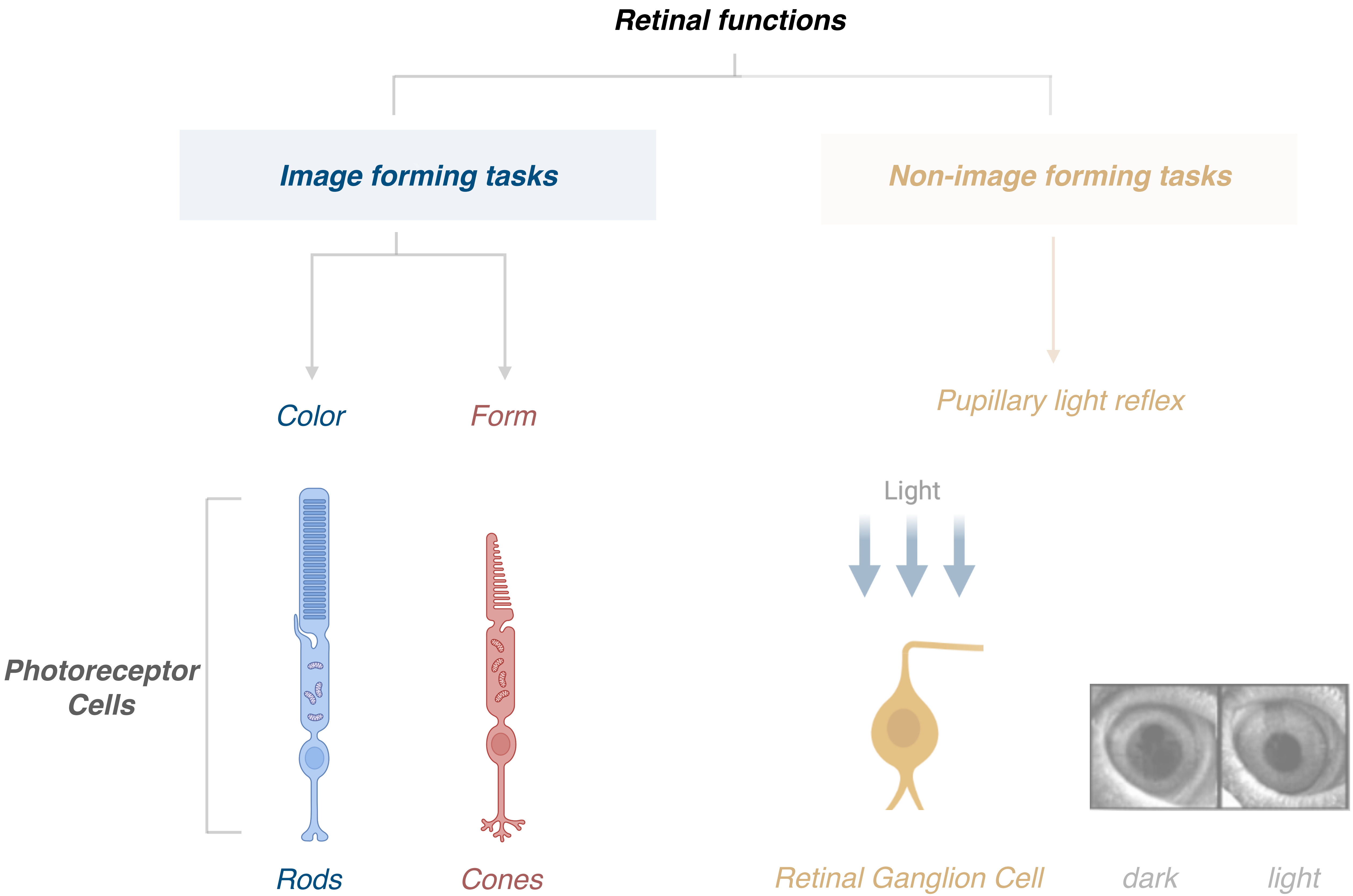

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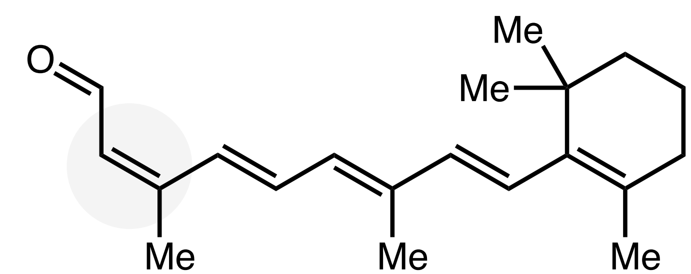
The molecular basis of vision

Protein + ***Chromophore***

***Opsins:* light-sensing receptor proteins**

- *G-protein coupled receptors (GPCRs)*
- *Retinal photoisomerases*

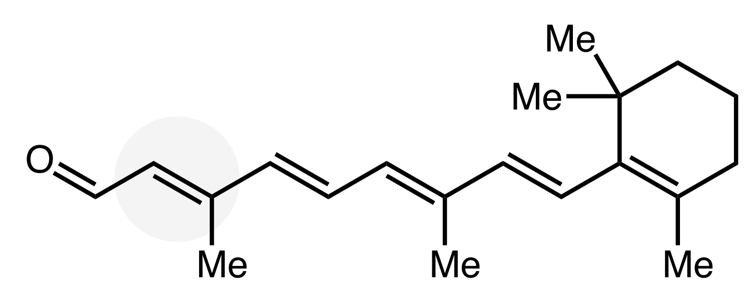
Retinal



11-cis-retinal

Resting

hv



11-trans-retinal

Active

Sato, K. Et al. *Nat. Comm.* **2018**. *1255*, 2491–2553.

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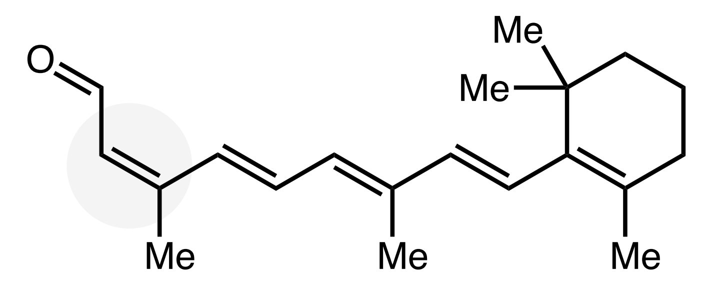

11-cis-retinal
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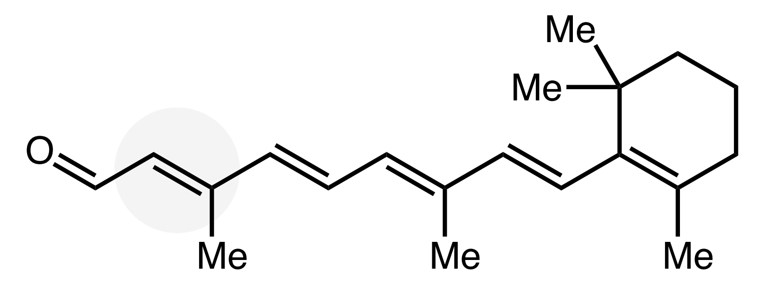

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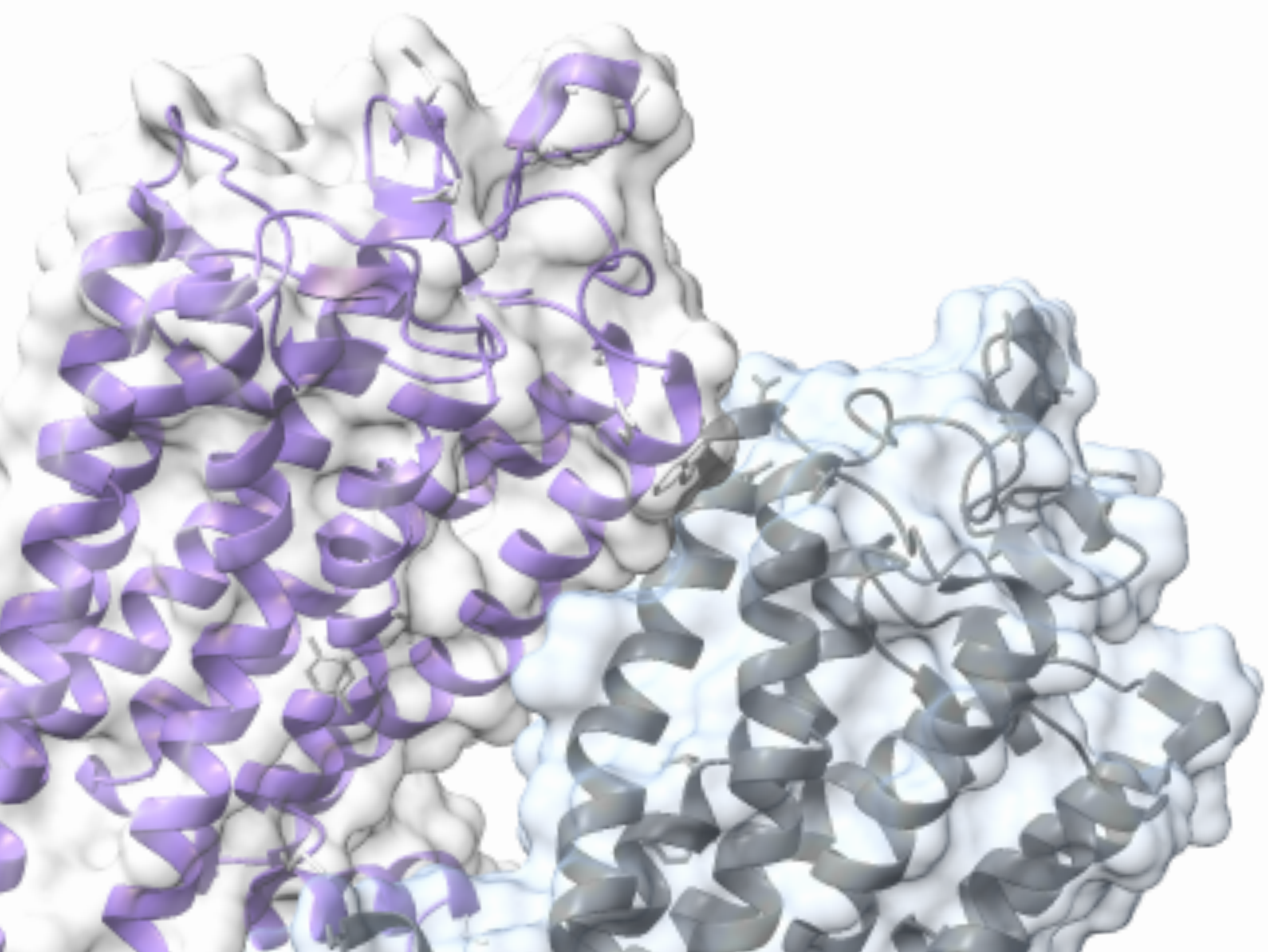
The molecular basis of vision

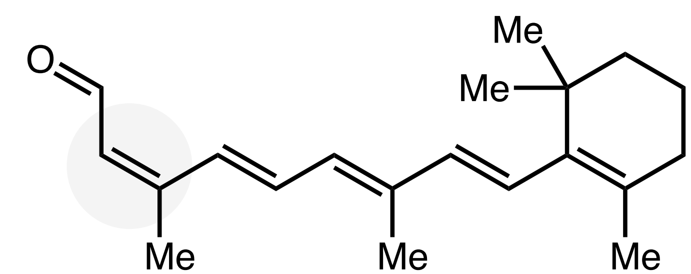
Protein + **Chromophore**

***Opsins:* light-sensing receptor proteins**

- ***Rhodopsin GPCRs***



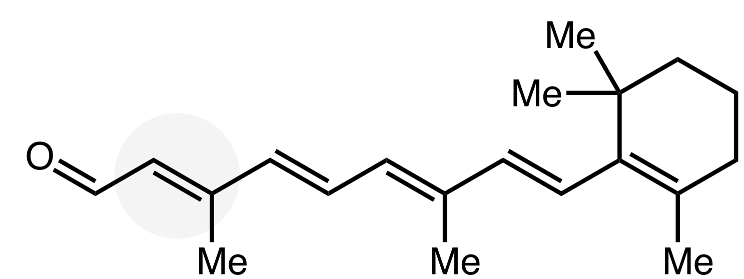
Retinal



11-cis-retinal

Resting

$\Phi_{cis\text{-}trans}$ **= 70%, 200 fs** *hν*



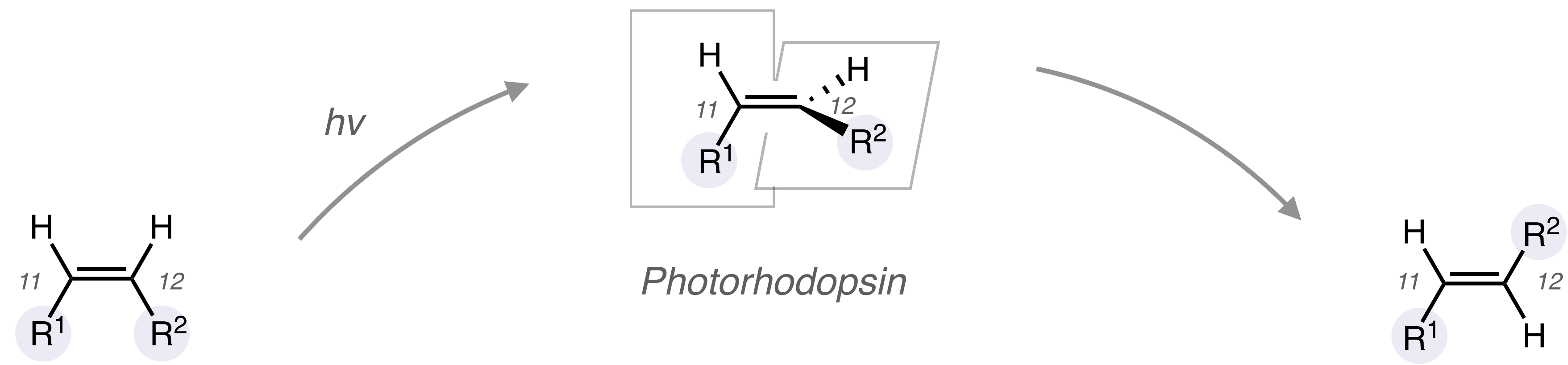
11-trans-retinal

Active

Yang, X. Et al. *Nat. Chem.* **2022**. *12*, 441–449.

The molecular basis of vision

Time-resolved serial femtosecond crystallography



Photorhodopsin

cis

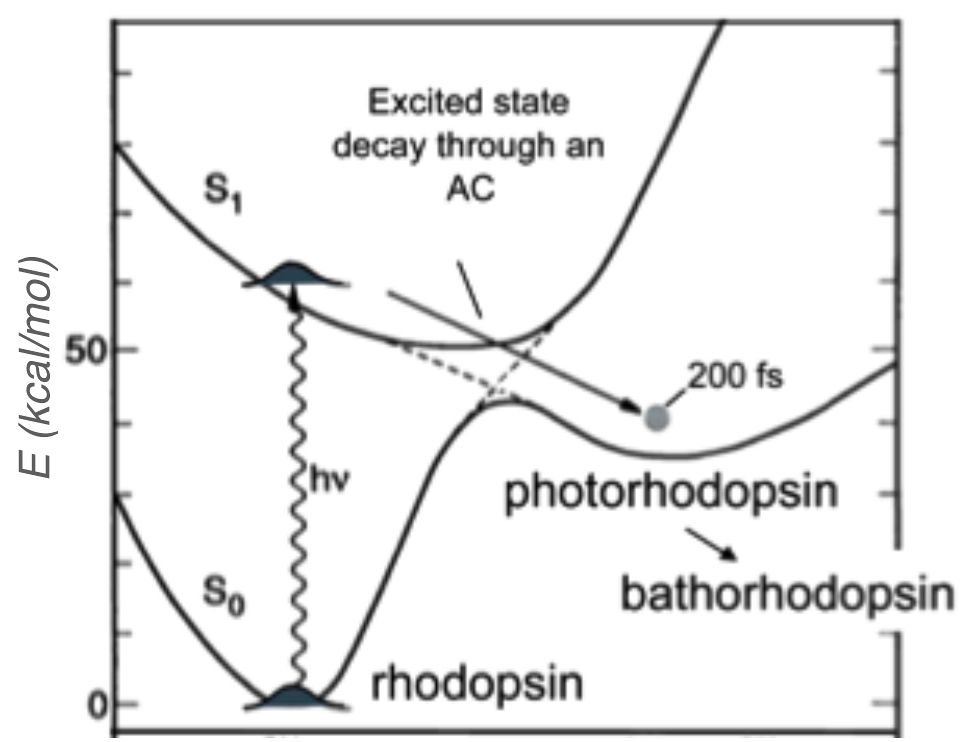
trans

- **Rhodopsin GPCRs**

Bathorhodopsin
200 fs

2D model:

Avoidant Crossing (AC) Mechanism for Ultrafast Rh Isomerization



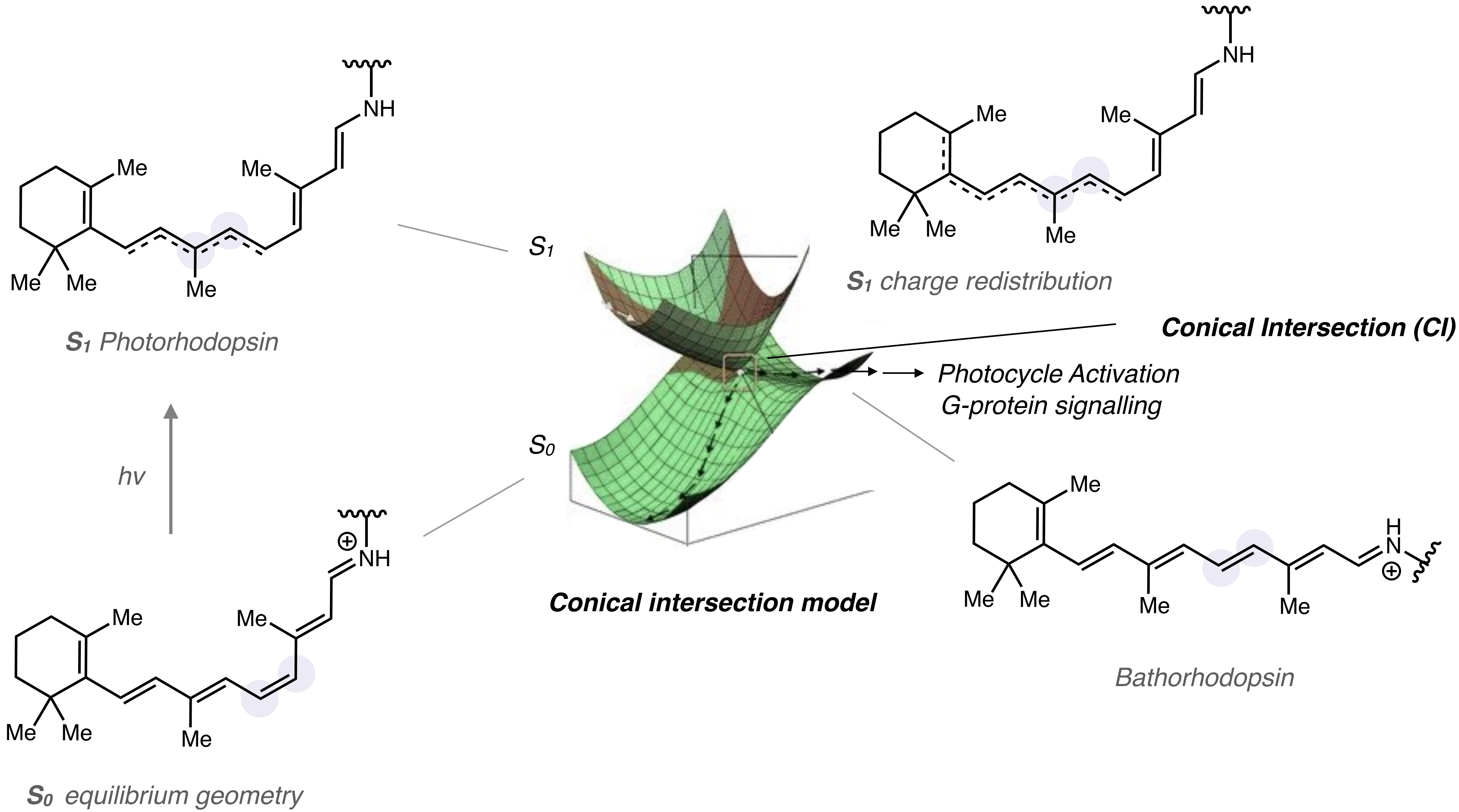
Reaction Coordinate

Gozem, S., Luk, H. L., Schapiro, I., Olivucci, M. *Chem. Rev.* **2017**. *117*, 13502–13565.

Rh = Rhodopsin

The molecular basis of vision

Ultrafast photochemical isomerization



Ultrafast chemical reaction: ~10^{15}

✓ ***geometric and electronic*** *structure of PES*

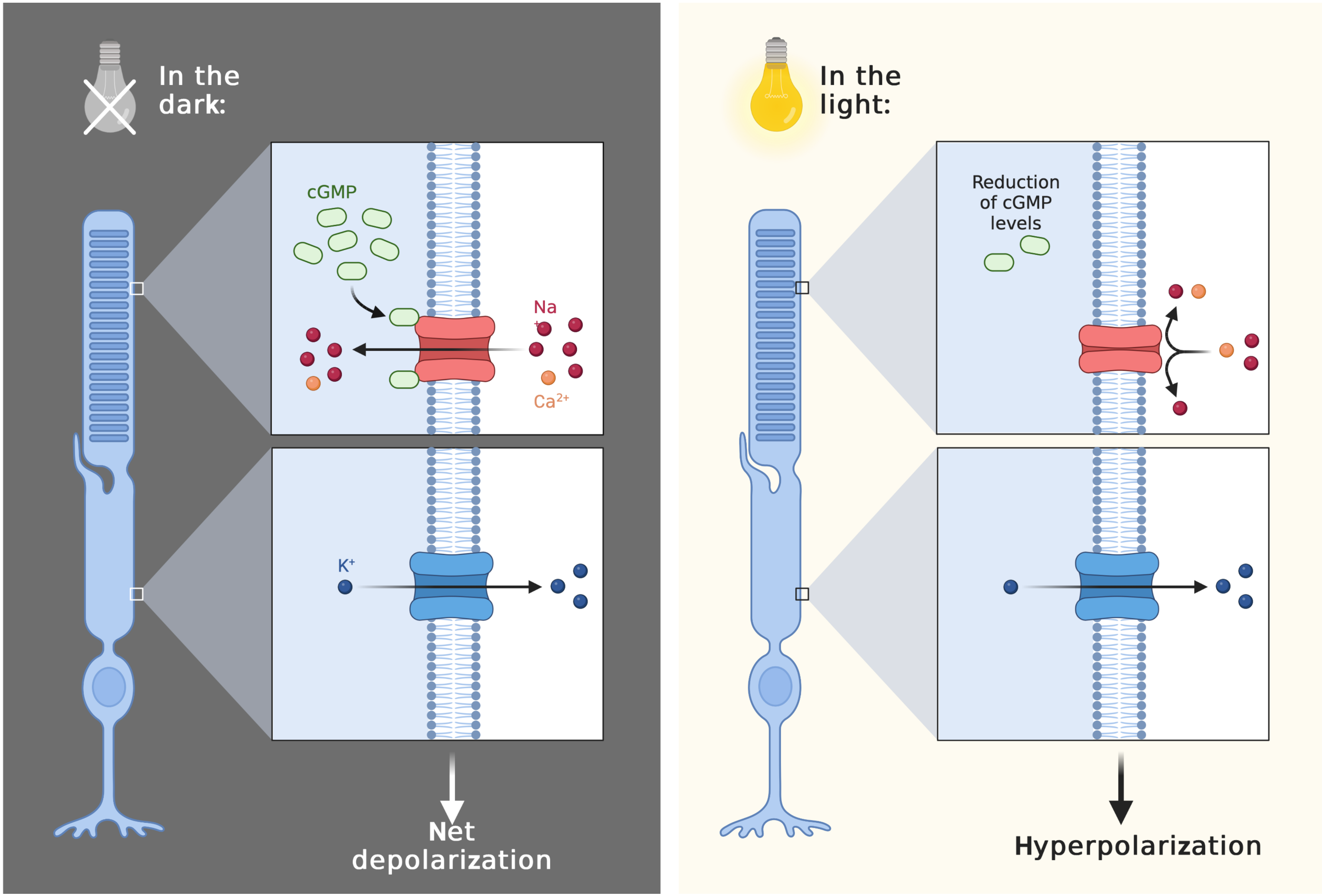
✓ ***barrierless*** *S_1 reaction path connecting Frank-Condon point to the CI*

Valsson, O. et al. *J. Chem. Theory. Comput.* **2013**. *9*, 2441–2454.

Gozem, S., Luk, H. L., Schapiro, I., Olivucci, M. *Chem. Rev.* **2017**. *117*, 13502–13565.

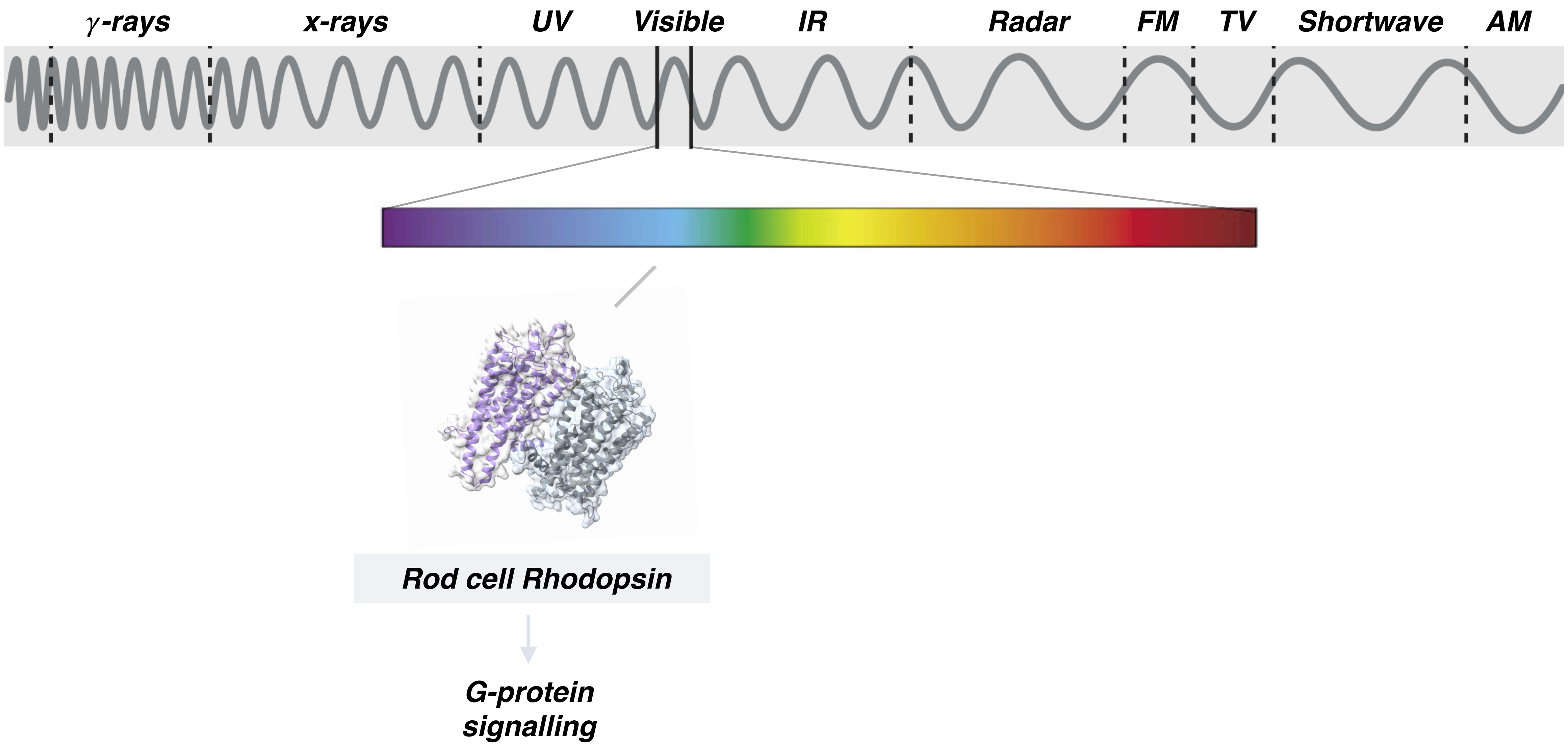
Rh = Rhodopsin

Light Induces Changes in Rhodopsin Electrical Activity



Sato, K. Et al. *Nat. Comm.* **2018**. *1255*, 2491–2553.

The conversion of photons into vision



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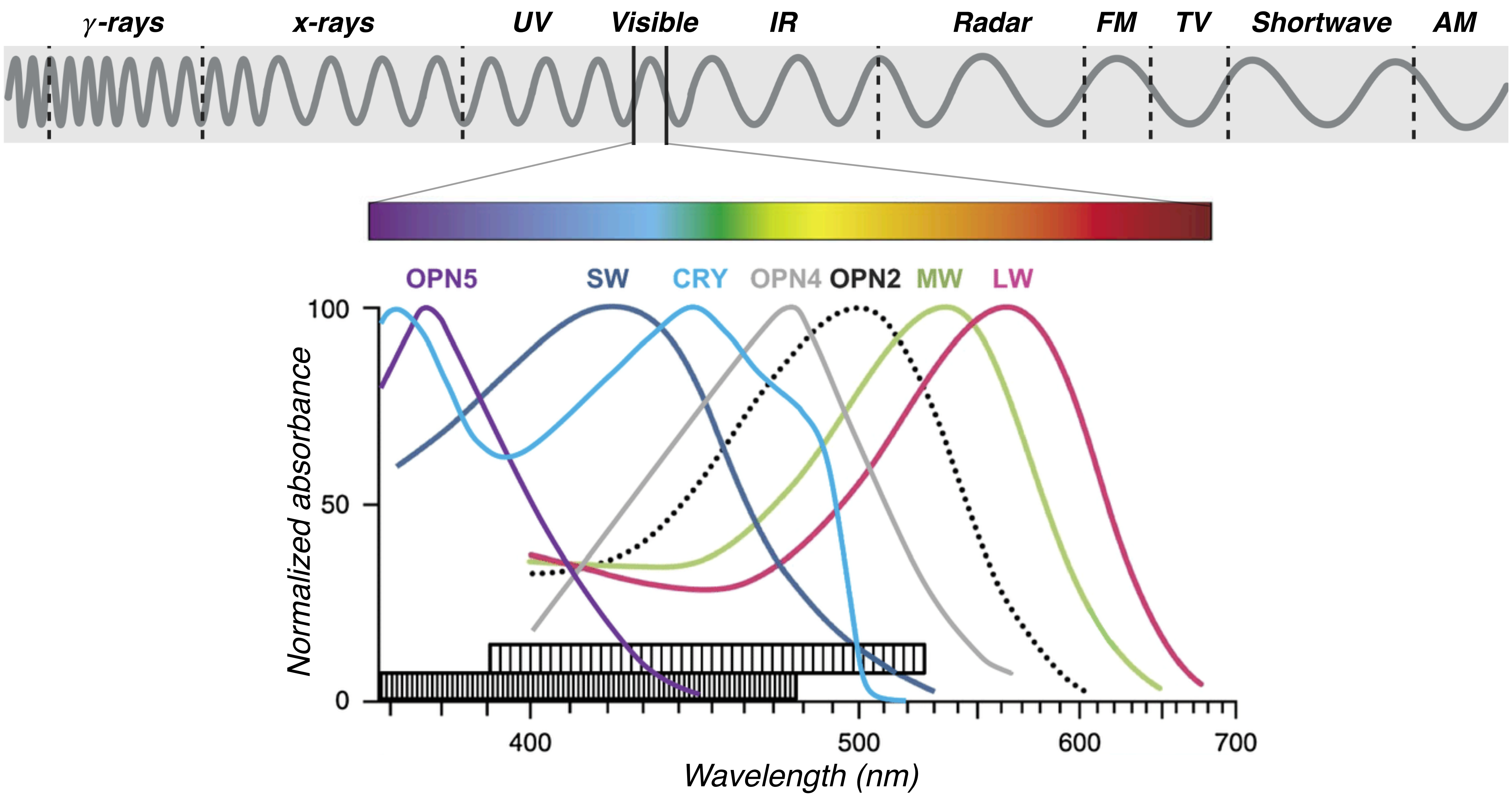
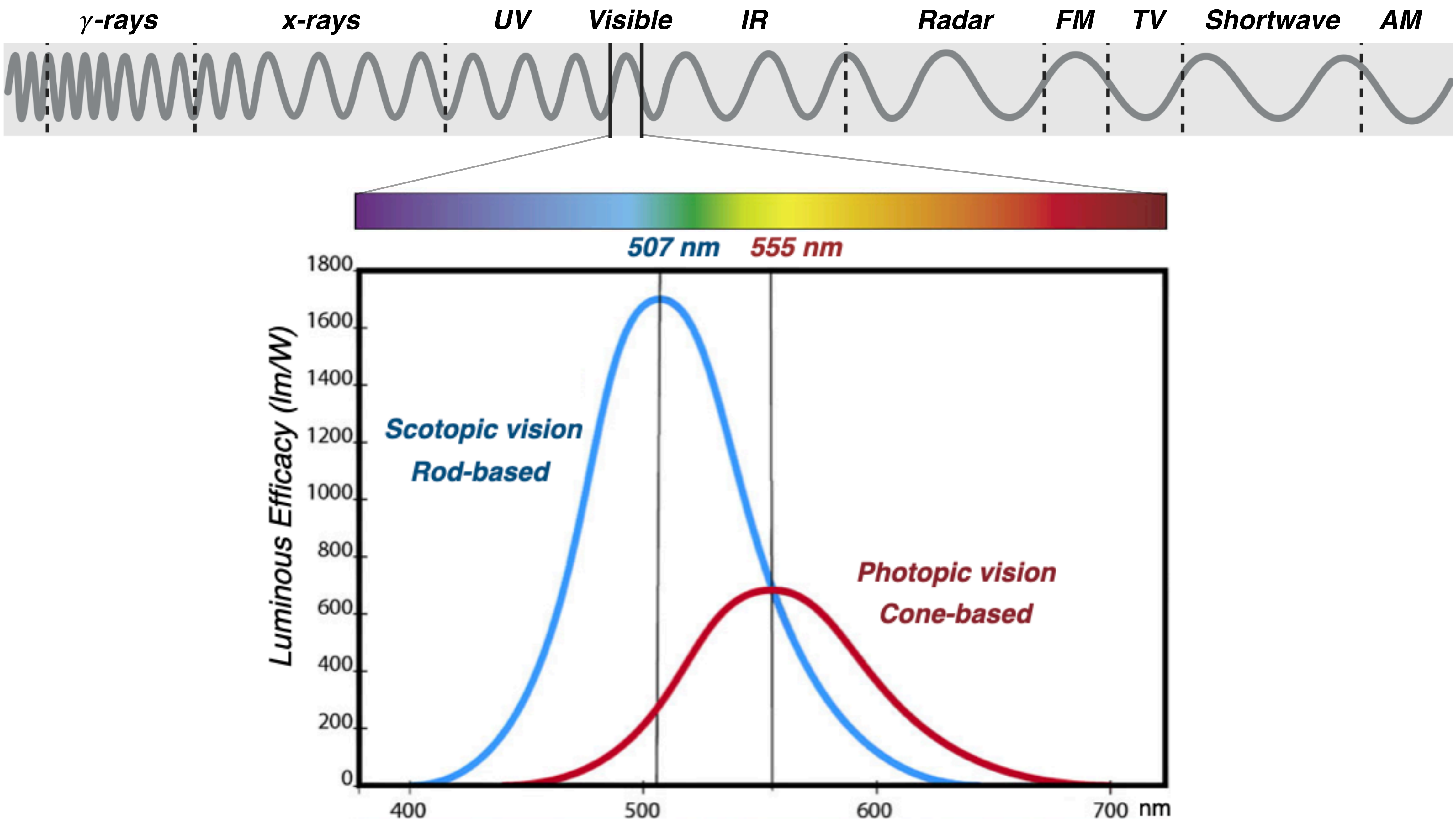


Absorption spectrum of human retinal opsins enable a wide illuminance range

Vanderstraeten, J., Gailly, P., Malkemper, P. *Cell. Mol. Life. Sci.* **2020**. *77*, 875–884.

The conversion of photons into vision



Purkinje Effect: luminance shift to blue under low illumination levels

Vanderstraeten, J., Gailly, P., Malkemper, P. *Cell. Mol. Life. Sci.* **2020**. *77*, 875–884.

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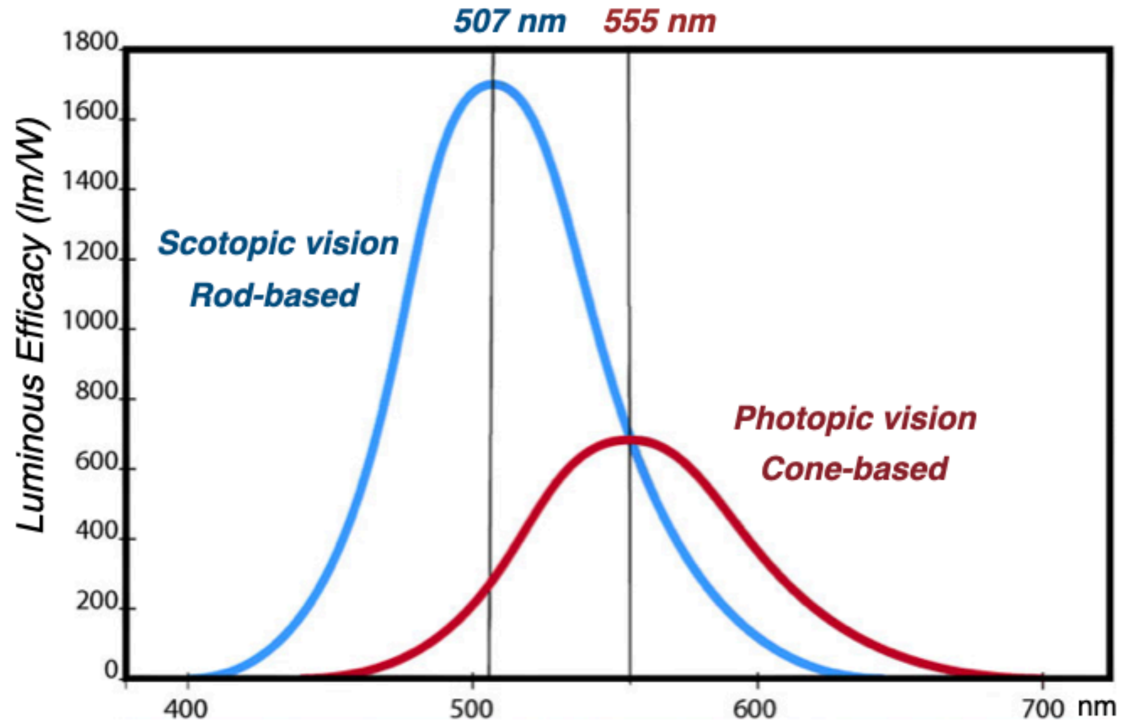

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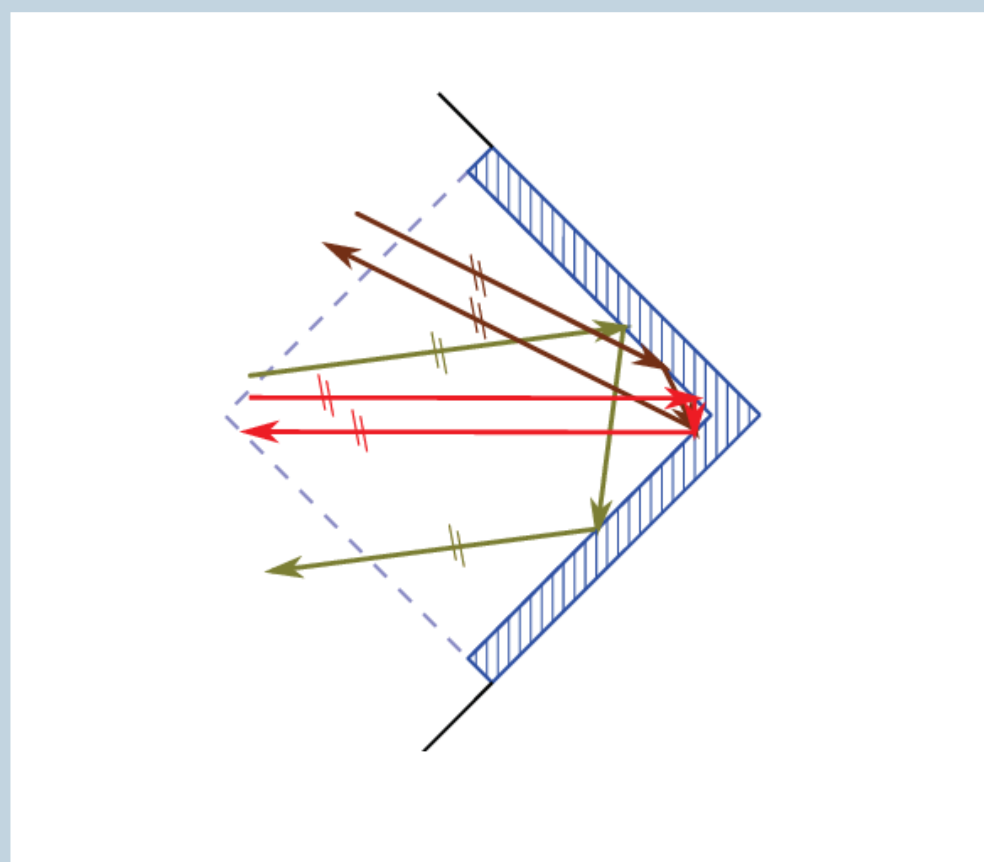
Retroreflectors in vision

Two types of retroreflectors:



1. Corner

- ***Reflect radiation back to its source with minimum scattering***

Acknowledgements to Rombo tools for schematic

Fosbury, R. A., Jeffrey, G. *Biol. Sci.* **2022**. *289*, 24754–24769.

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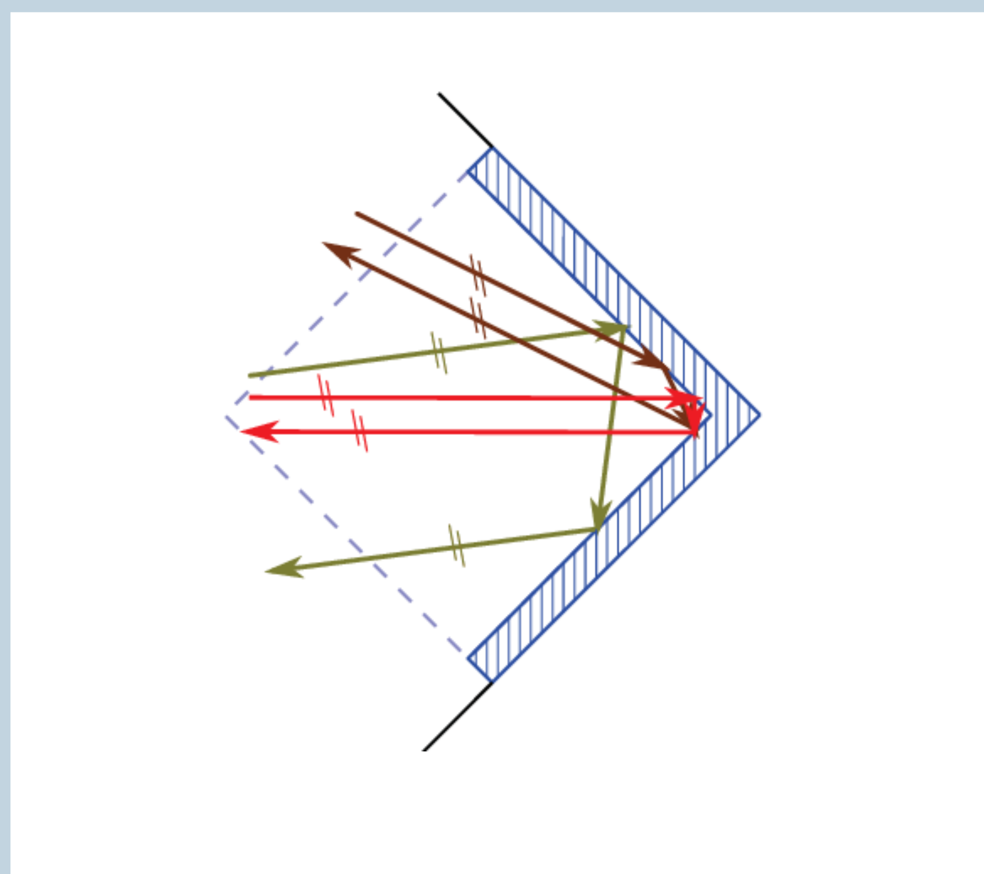

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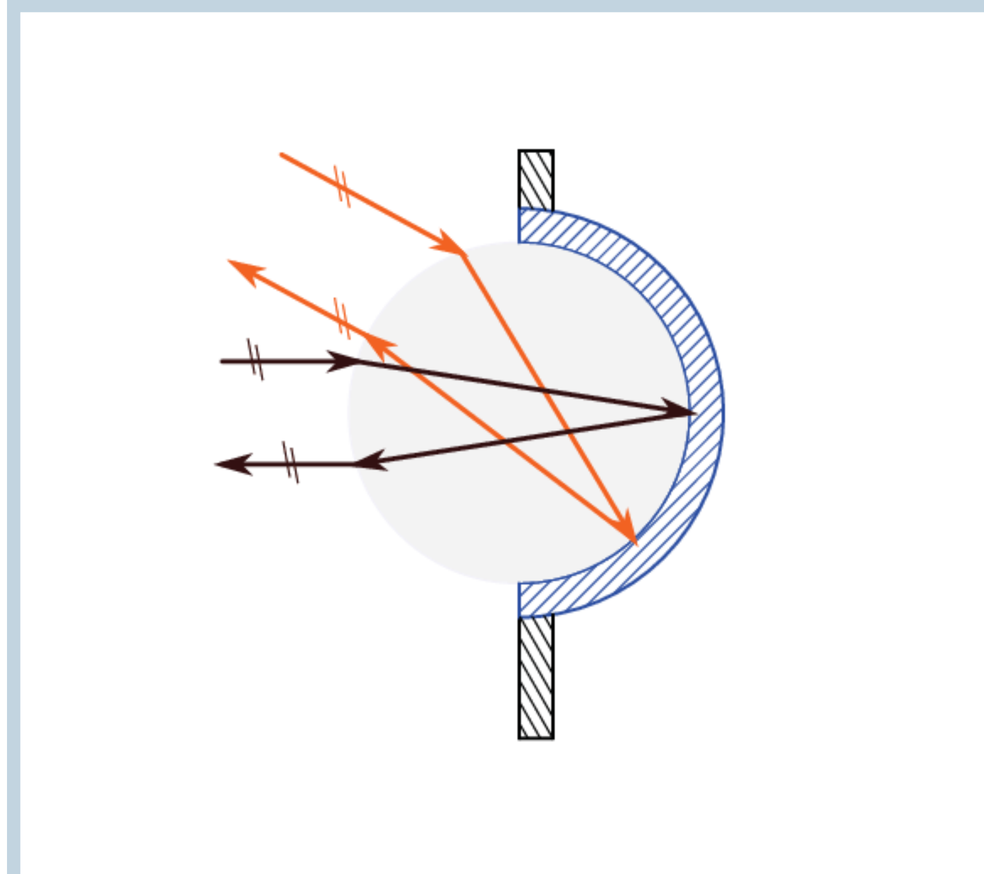

2. Spherical

- ***Reflect radiation back to its source with minimum scattering***

Acknowledgements to Rombo tools for schematics

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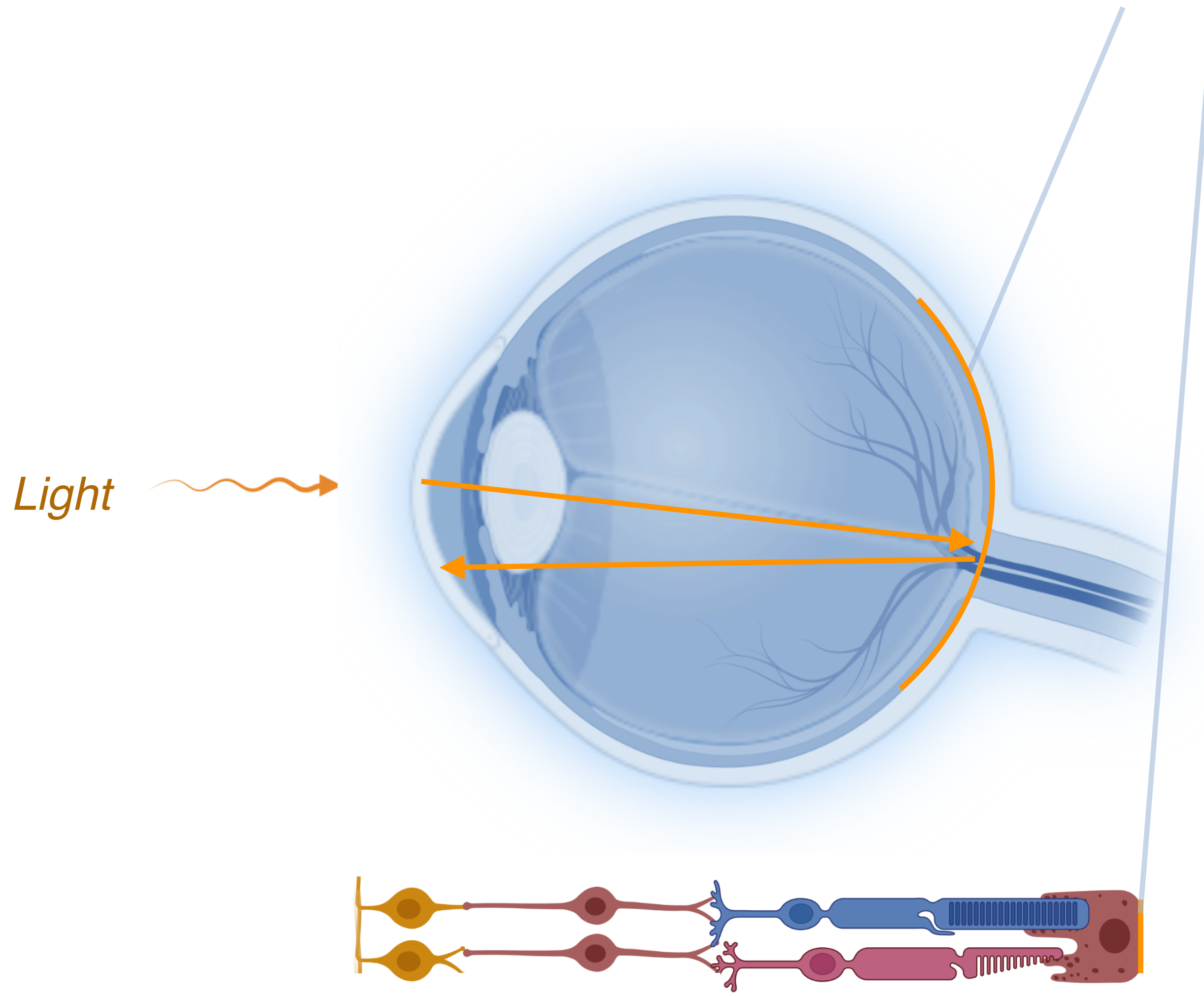
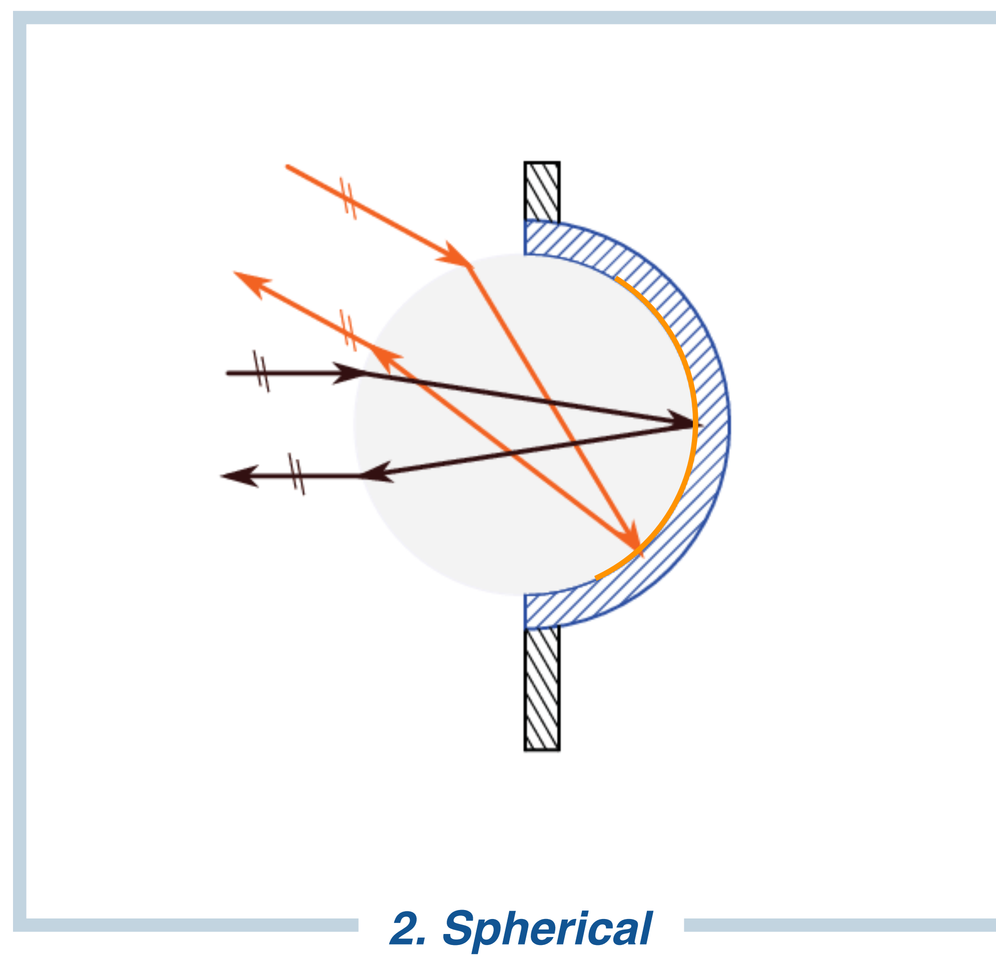
Retroreflectors in vision

Tapetum lucidum as a natural retroreflector





*Constructive interference of **incident** and **reflective** wave*

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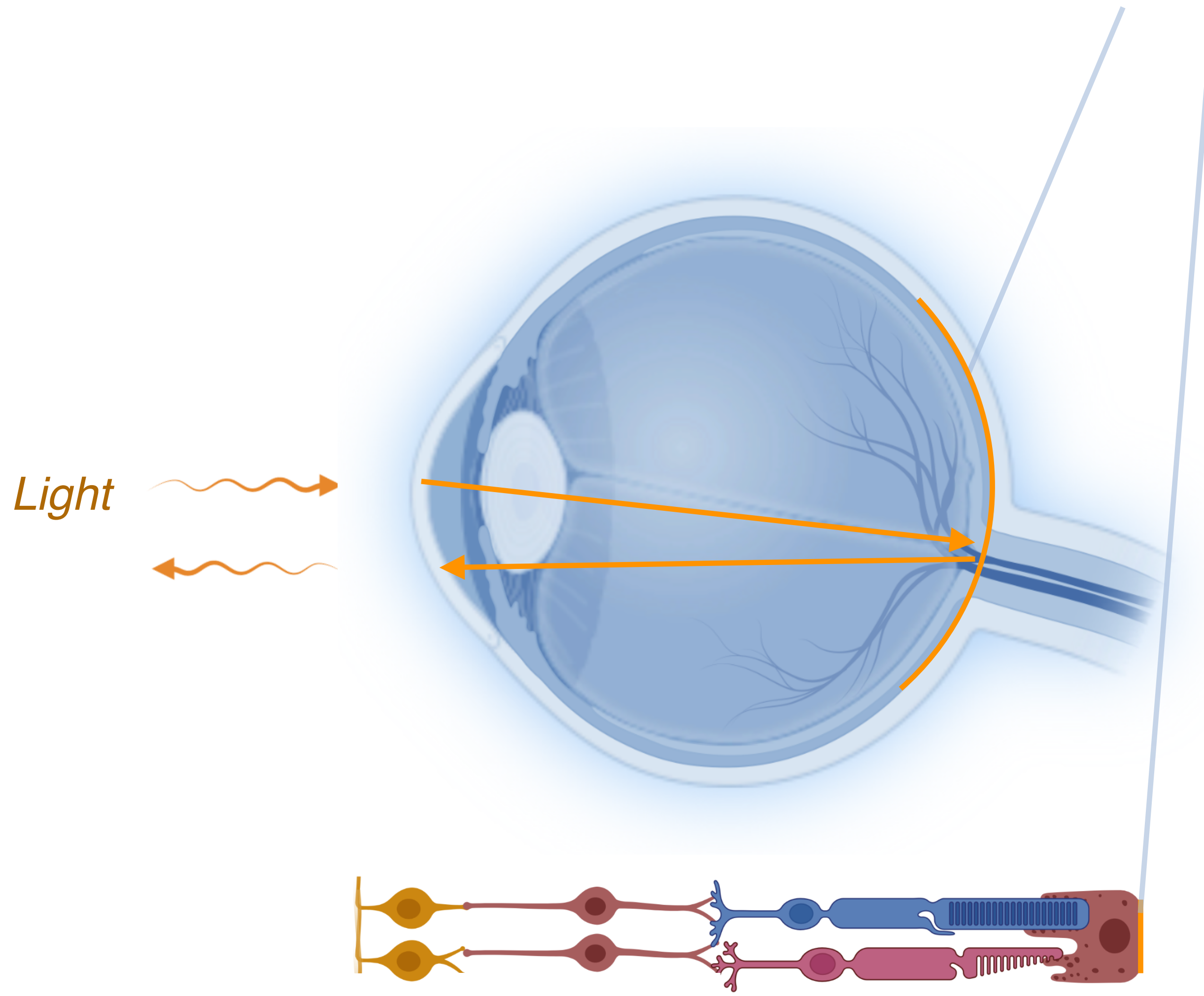

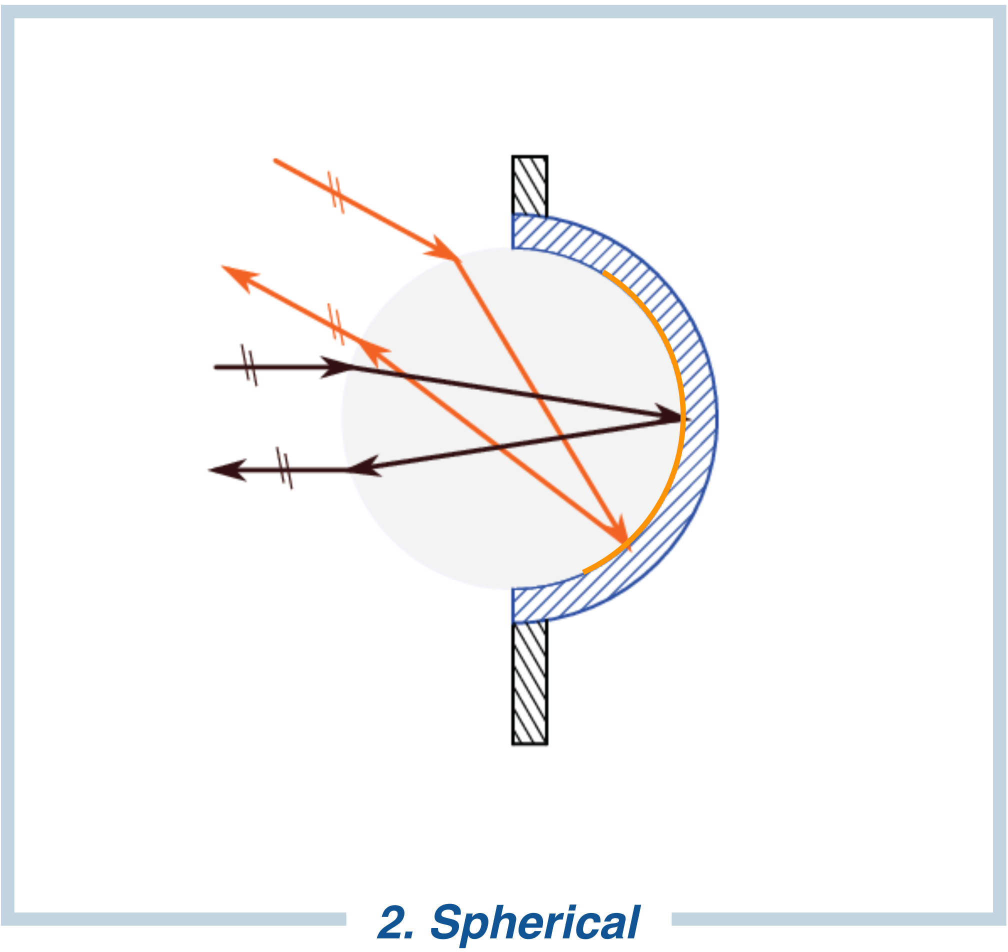

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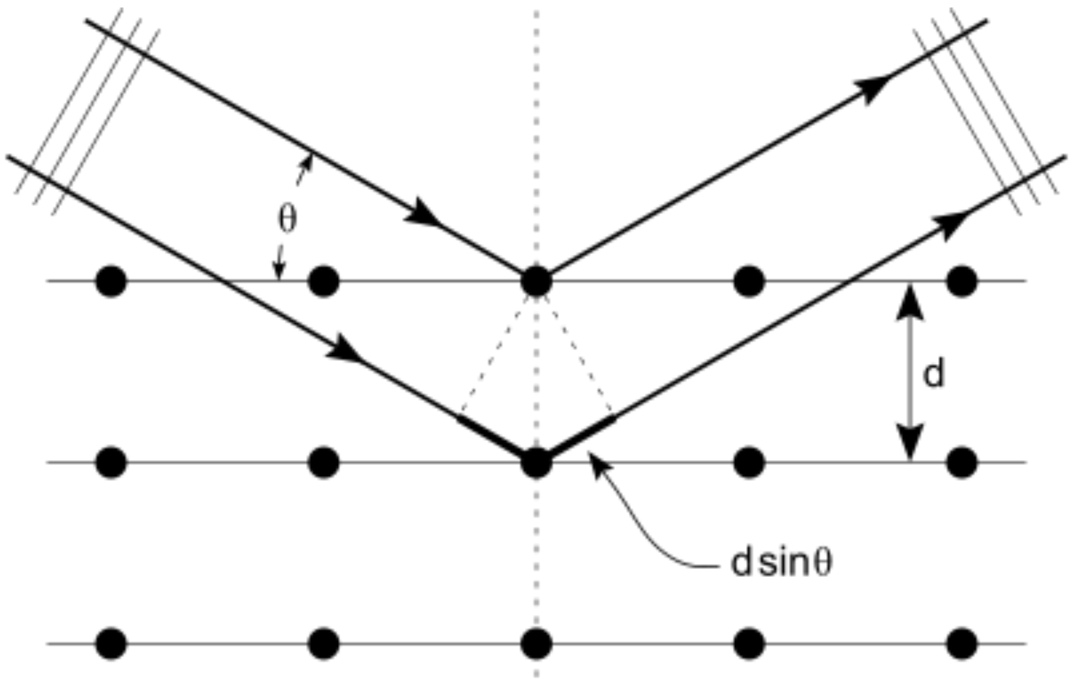
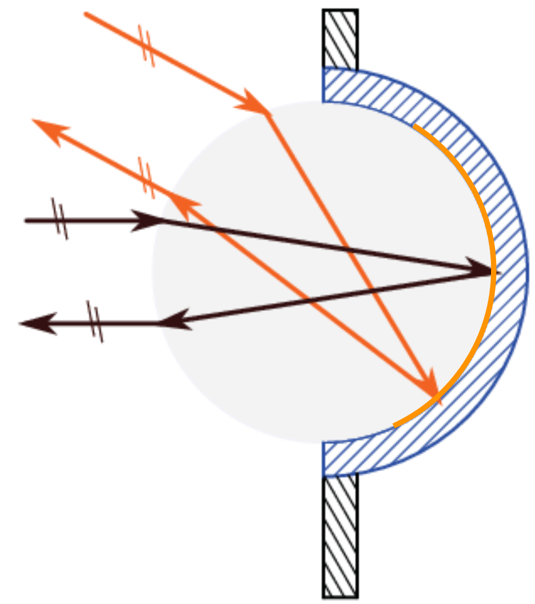
Retroreflectors in vision

Crystal lattice of the Tapetum lucidum alters vision



Bragg diffraction

$$n\lambda = 2d \sin\theta$$



2. Spherical

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Retroreflectors in vision

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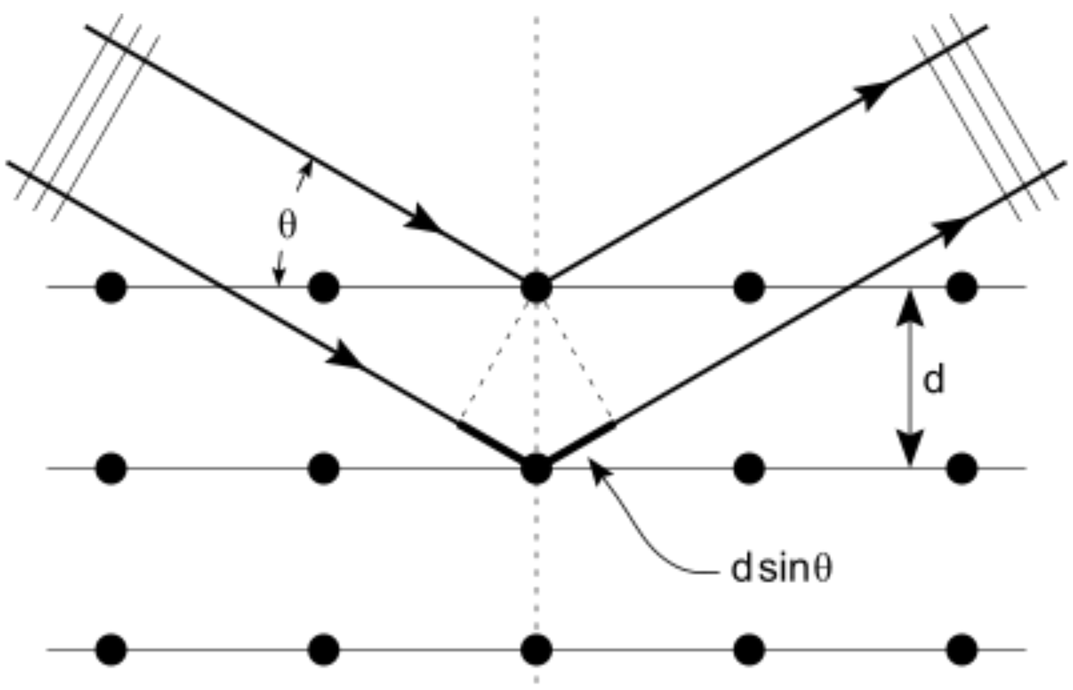

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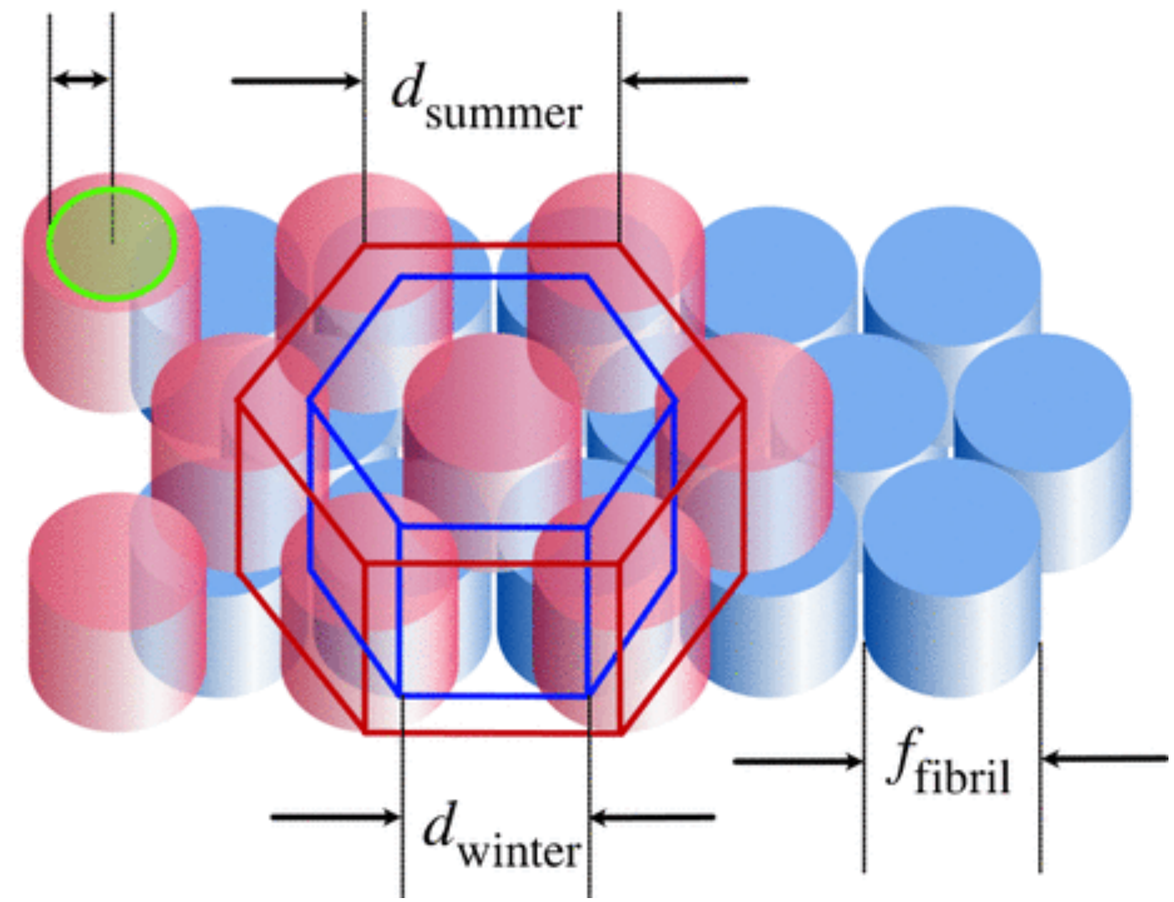

Tapetum lucidum crystal lattice

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Retroreflectors in vision

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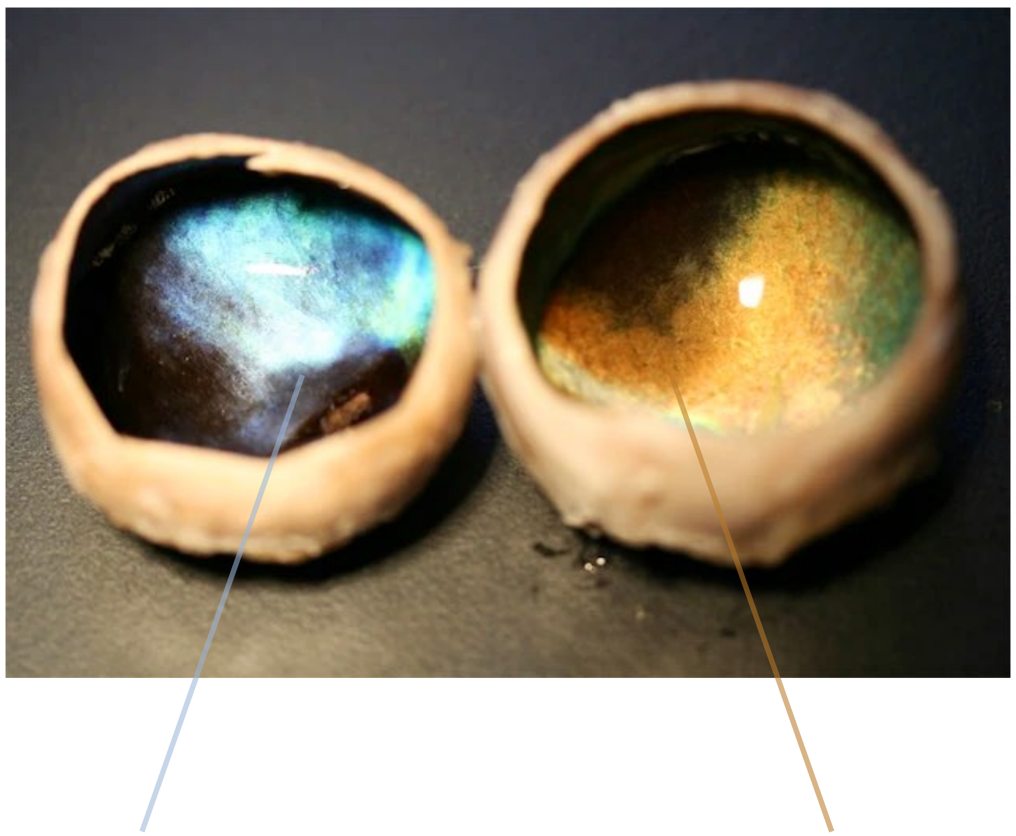
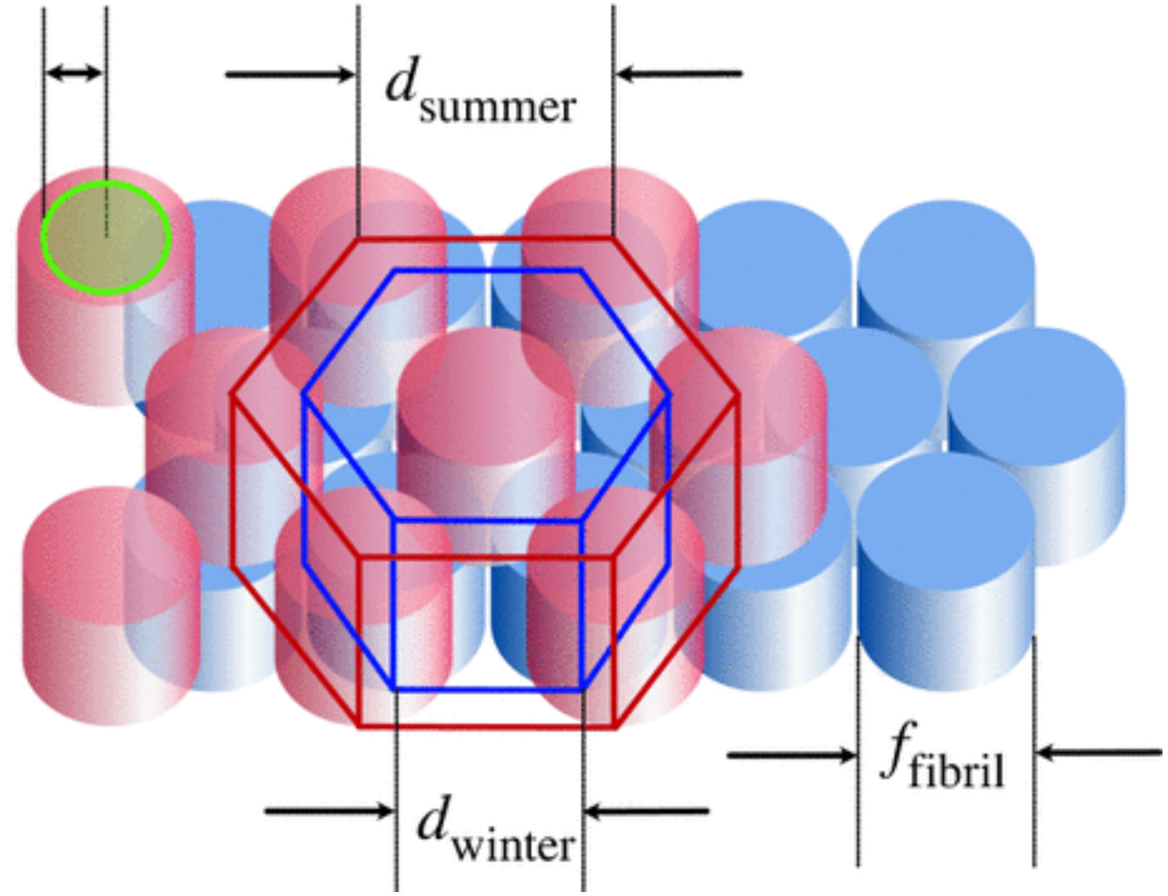




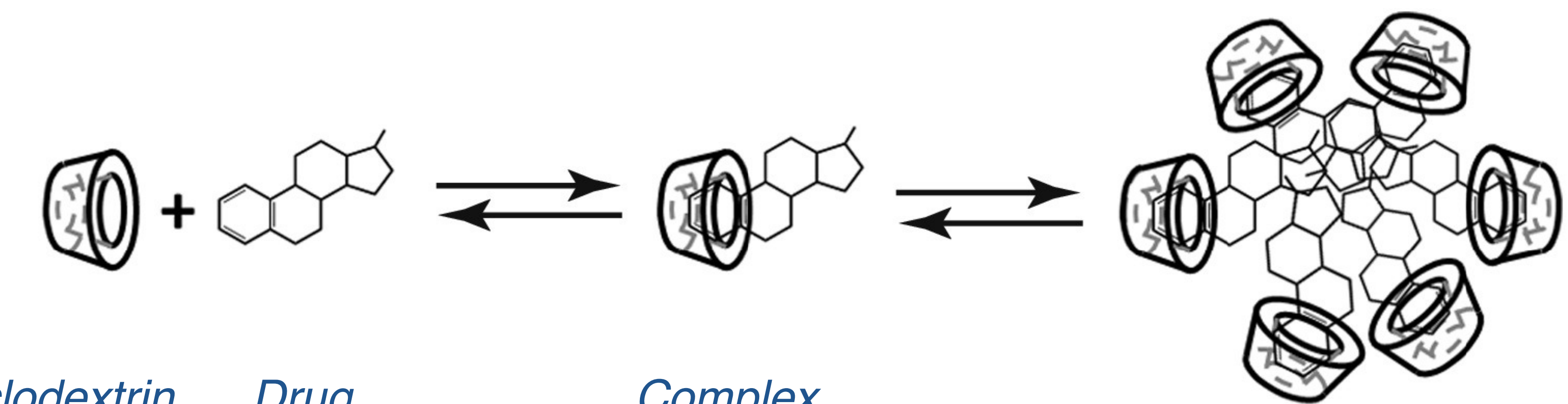
Tapetum lucidum crystal lattice

Fosbury, R. A., Jeffrey, G. *Biol. Sci.* **2022**. *289*, 24754–24769.

Drugging the retina

3 key delivery obstacles:

Solubility



Cyclodextrin *Drug* *Complex*

Nanoparticle

Hydrophilicity

***Aqueous** tear fluid and exterior mucus layer*

$V_{tear} \sim 7\ \mu L$

$K_{turnover} \sim 1.2\ \mu L/min$

$\tau_{corneal} \sim 3\ min$

Lipophilicity

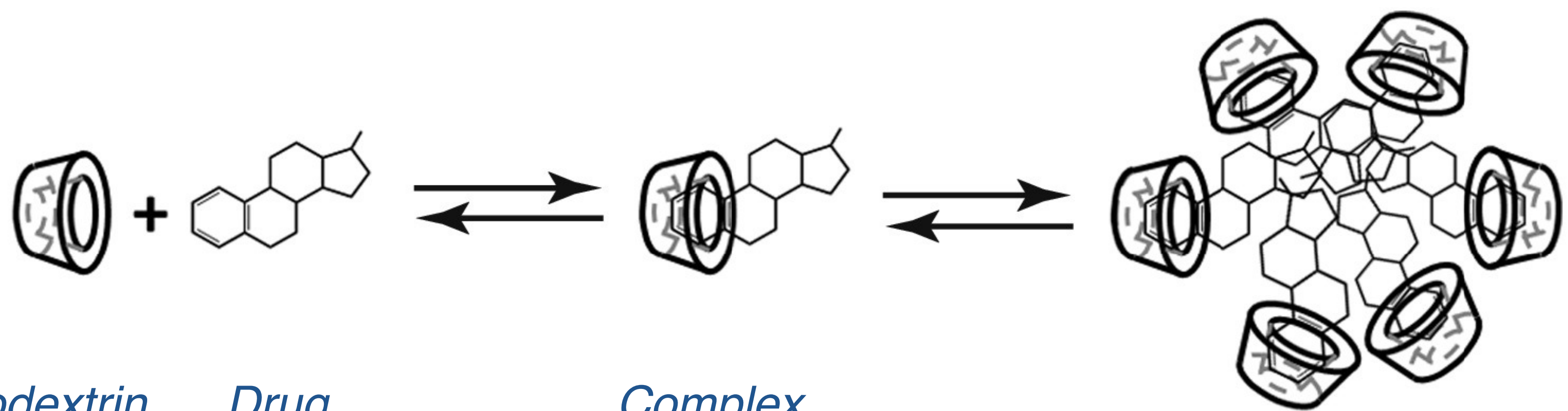
***Corneal** and **conjunctival** membrane barriers*

Contact lens supports

Nanoparticle delivery

Ramsay, E. et al. *Eur. J. Pharm. Biopharm.* **2019**. *143*, 18–23

Nanocarriers for anterior eye segment diseases:



Cyclodextrin *Drug* *Complex*

Nanoparticle

FDA-approved nanocarriers:

Durezol®*, Novartis*
Difluprednate → *Postoperative ocular inflammation*

Cequa®*, Upjohn*
Cyclosporine → *Dry eyes*

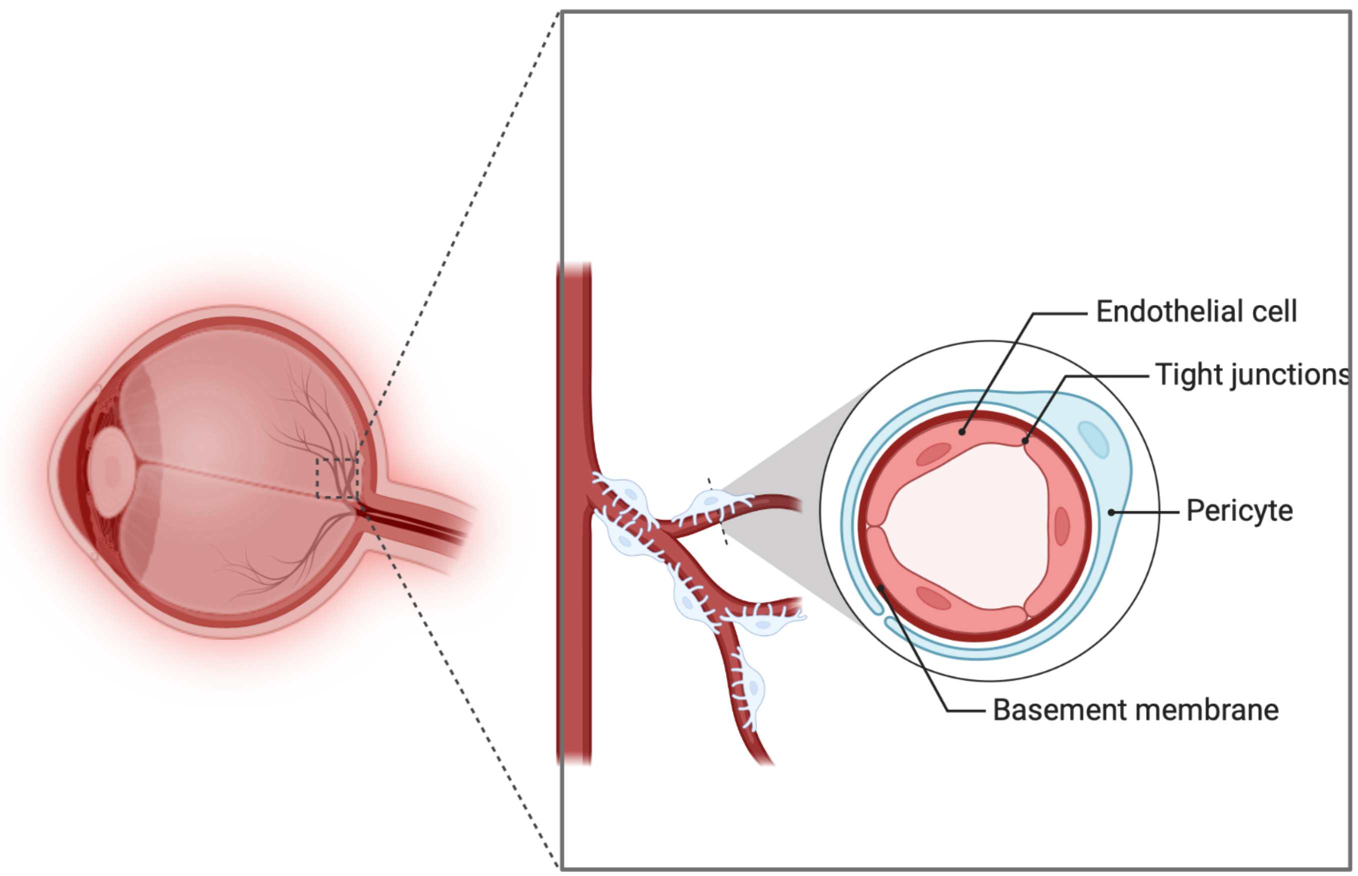
Eysuvis®*, Santen*
Cyclosporine → *Conjunctivitis*

Onugwu, A. L. et al. *J. Control. Release.* **2023**. *354*, 465–488

Drugging the retina

3 key delivery obstacles:

Solubility | *Blood-retina barrier* | *Potency*



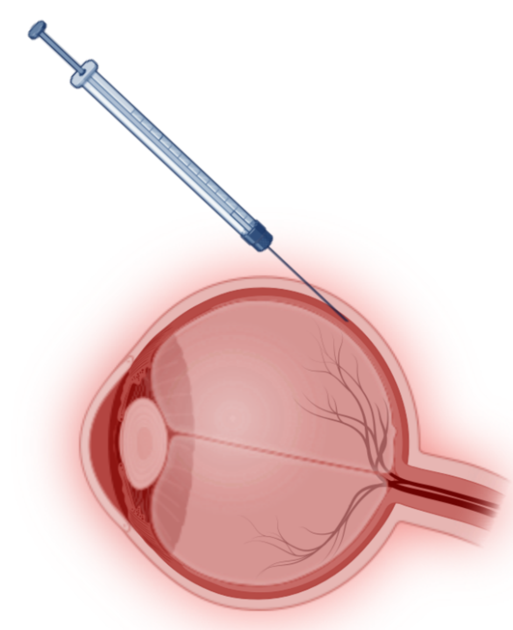
del Amo, E. M. et al. *Prog. Retin.* **2017**. *57*, 134–185

Drugging the retina

3 key delivery obstacles:

Solubility | *Blood-retina barrier* | *Potency*

Subretinal injection



Monoclonal antibody therapeutics → ***anti-VEGF treatments*** → ***Lucentis®****, Genentech*; ***Aflibercept®****, Bayer*

Adeno-associated virus therapeutics → ***Gene therapy*** → ***Luxturna®****, Novartis*

Maguire, A. B. et al. *Mol. Ther.* **2021**. *29*, 442–463

del Amo, E. M. et al. *Prog. Retin.* **2017**. *57*, 134–185

Topical drug delivery: 2 possible routes of entry

Corneal route:

Cornea

↓

Aqueous humor

↓

Aqueous intraocular tissues

↓

Posterior segment

Scleral route:

Conjunctiva

↓

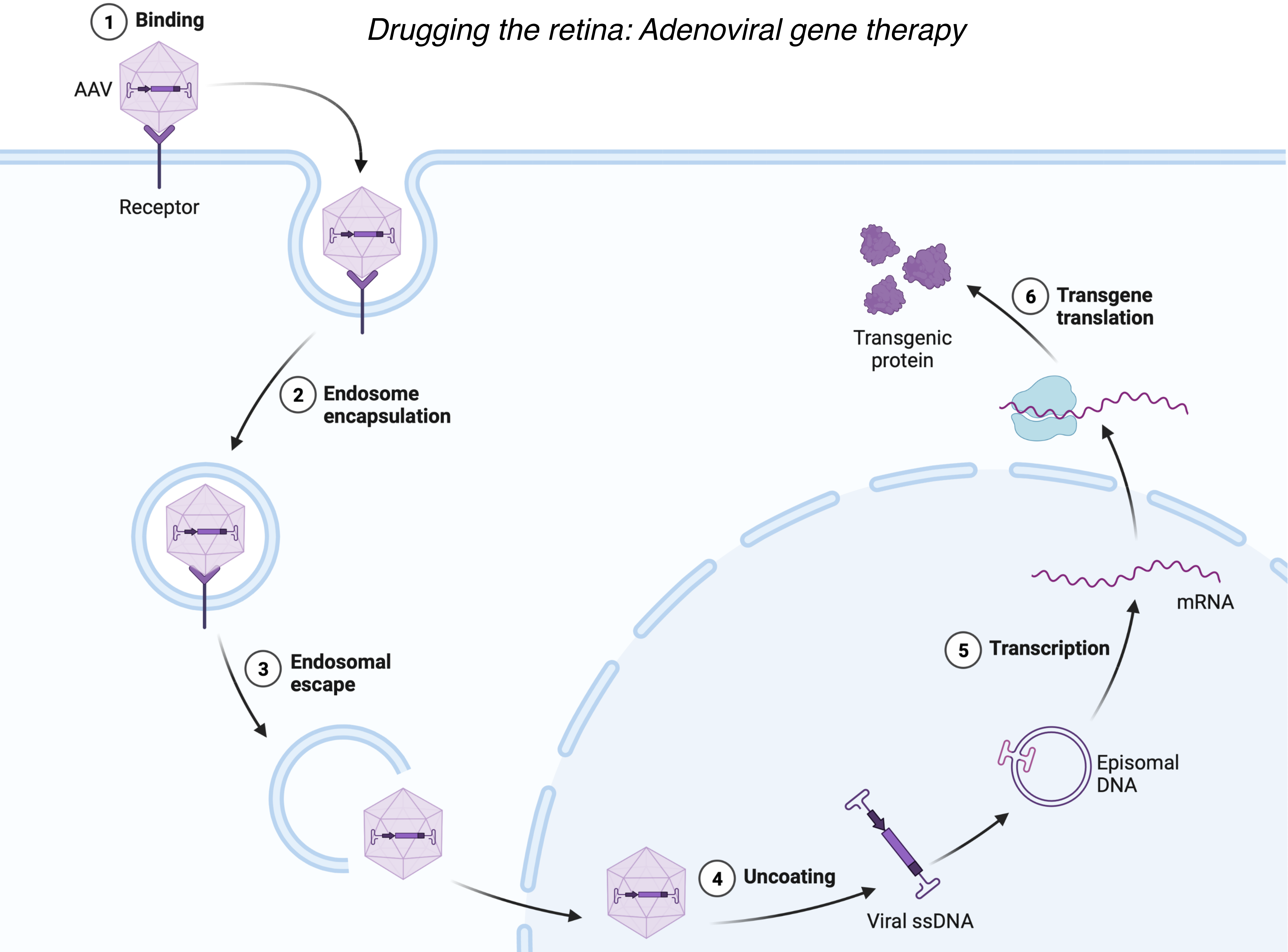
Sclera

↓

Retinal epithelium

Ramsay, E. et al. *Eur. J. Pharm. Biopharm.* **2019**. *143*, 18–23

Drugging the retina: Adenoviral gene therapy



Summary overview: The chemistry of eyesight

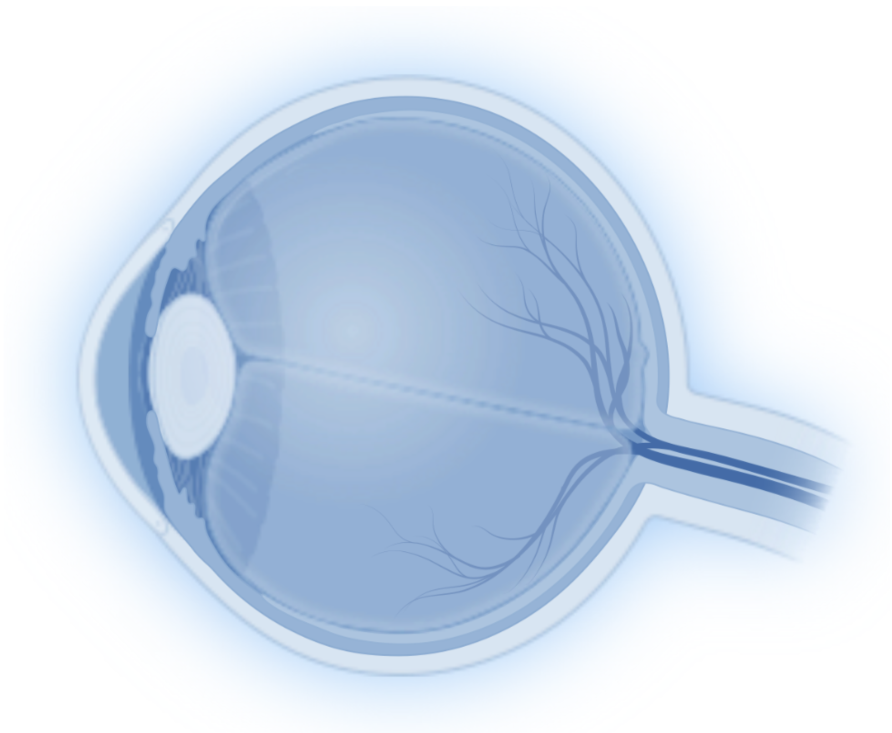


Fig 1. The mammalian eye

Cation homeostasis regulates protein aggregation

- *Hydrodynamic kinetics and cataracts*
- *Yellowing causes blue filtration through the lens*

Ultrafast isomerization of rhodopsin

- *Quantum efficiency of biological process*

Absorption spectra of scotopic versus photopic vision

- *Using abs spectra to understand night vision*

Thin film retroreflectors in nature

- *Crystal packing lattice changes with seasons*

Medicinal chemistry: challenges with drugging the eye

- *FDA-approved treatments for viral vector therapy*
- *FDA-approved VEG-F therapy*

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