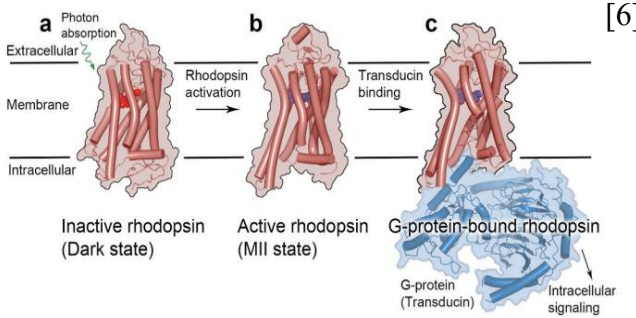
Invisible Forces Behind G-Protein Coupled Receptors

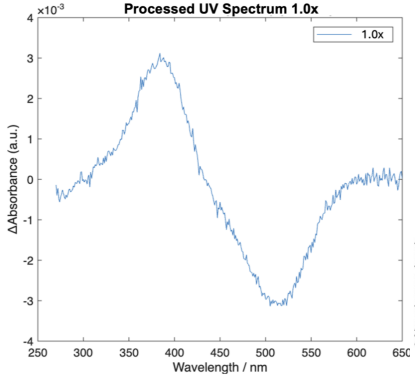
Research Question

How does varying salt concentrations in natural biological membranes regulate the structure, activation, and function of rhodopsin?



[6]

Data Analysis & Results



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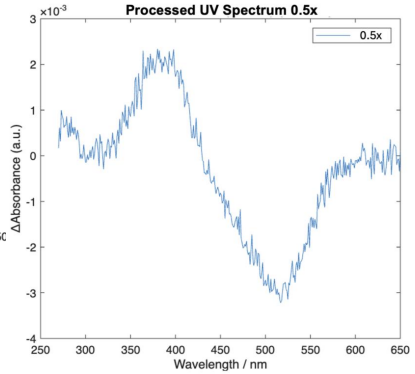

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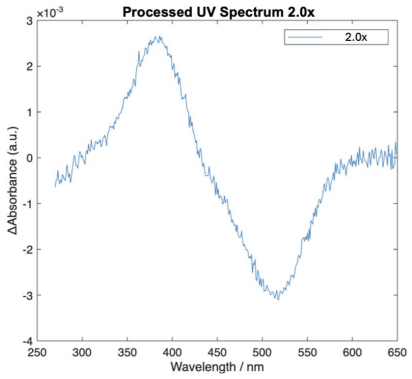

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Methodology

1. Prepared rhodopsin samples with identical constants
2. Varied ionic conditions using different concentrations of ions
3. Collected dark-state UV-Vis absorption spectra
4. Light-activated samples and recorded spectra
5. Generated difference spectra
6. Analyzed absorbance changes at key wavelengths (Meta I/II).
7. Chose representative samples and applied statistical analysis

Conclusion & Discussion

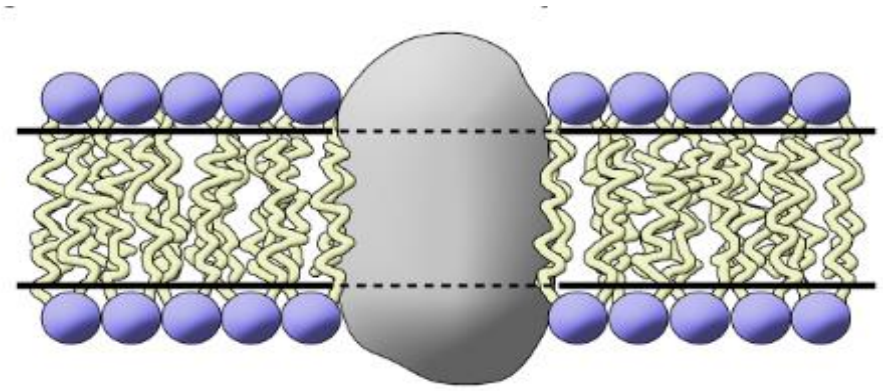
Changes in ionic strength altered the Meta I/Meta II equilibrium without shifting spectral features, suggesting that electrostatic screening of charged residues modulates rhodopsin activation.

These results show that rhodopsin activation depends on an optimal ionic environment, highlighting the role of electrostatic interactions in regulating GPCR function.

G-Protein Coupled Receptors

- Largest family of membrane signaling proteins
- Heavily involved in cell signaling and cell interaction
- Target of ~ ⅓ of modern pharmaceuticals [3]

- Function depends on surrounding environment
- Native membranes are complex systems [1]
- Many studies use simplified conditions [1]



[2]

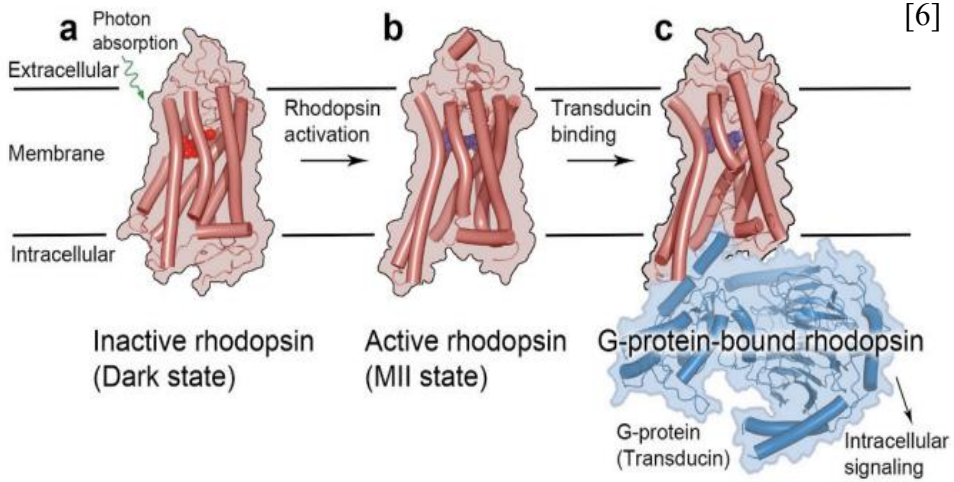
Fig. 1. Rhodopsin in the RDM (retinal disc membrane) membrane, surrounded by phospholipids. [2]

Rhodopsin as a Model GPCR

- Light-sensitive receptor in retinal photoreceptors [6]
- Initiates visual signaling cascade [6]
- Well-characterized activation pathway [6]

- **Dark state** - 500 nm
- **Meta I (inactive)** - 487 nm
- **Meta II (active)** - 380 nm

} UV-Visible Spectroscopy



[6]

Fig. 2. Rhodopsin, a GPCR, activates through shape changes triggered by light, leading to G-protein signaling. [6]

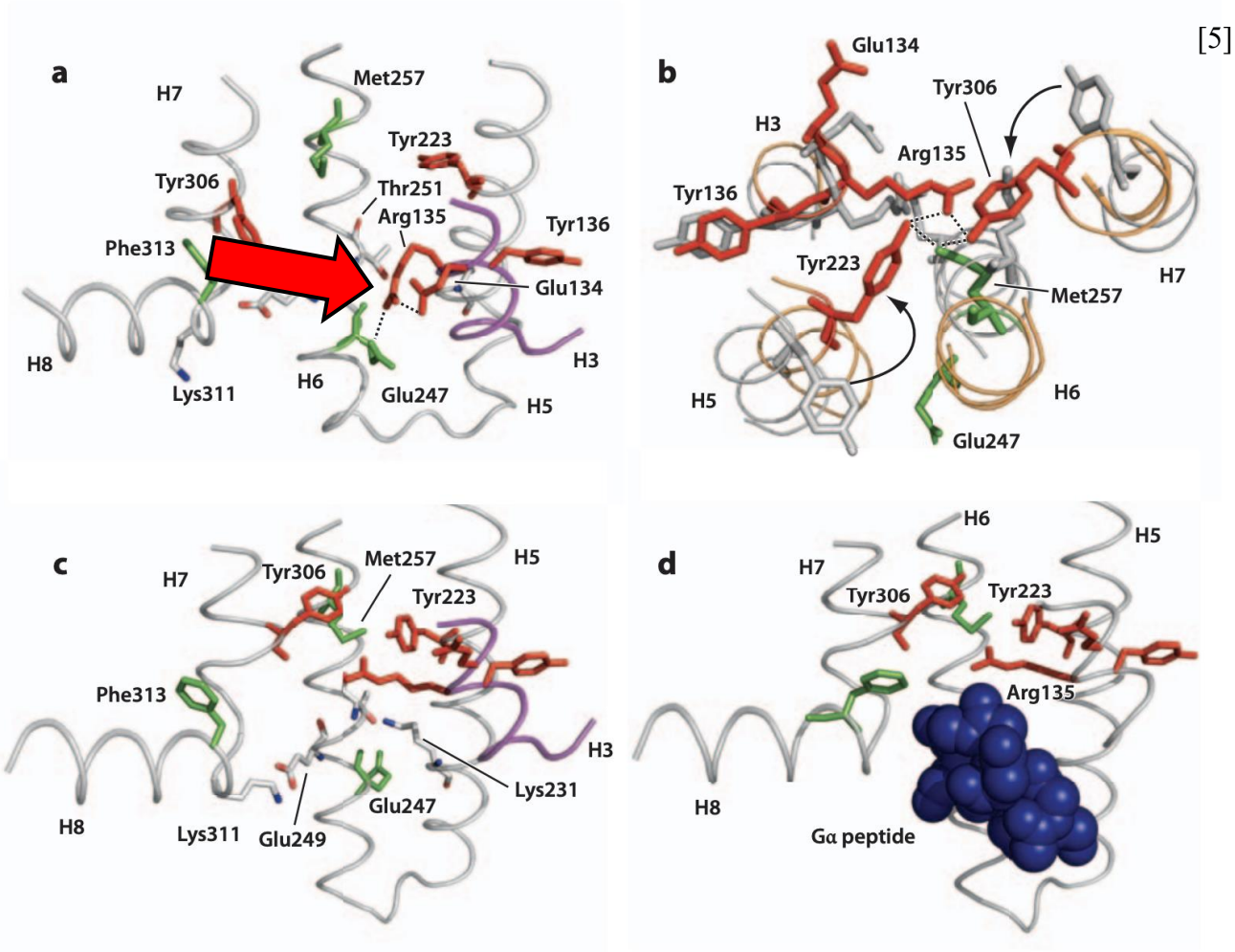
Inside Rhodopsin

- Two amino acids carry opposite charges:
  - **Glu134 (-)**
  - **Arg135 (+)**

- Opposite charges attract and form a stabilizing interaction
- Helps hold rhodopsin in the inactive **Meta I** state [5]

- Protonation removes Glu134's negative charge [5]
- Attraction weakens → protein shifts shape [5]
- Leads to the active **Meta II** state [5]

- Ions in solution interact with charged residues
- Charges can interact with surrounding ions instead of each other



[5]

Fig. 3. Ionic lock in rhodopsin showing Arg135–Glu247 interaction and the role of Glu134 in stabilizing the inactive state. [5]

Research Question

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Hypothesis

Increasing salt concentration will shift equilibrium toward the **Meta II (active) state** by screening electrostatic interactions.

Methodology

Rhodopsin samples prepared in controlled bis-tris propane (BTP) buffer

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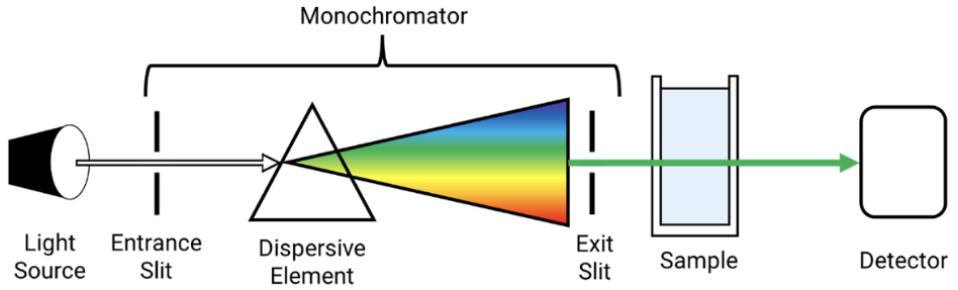
Ionic strength (NaCl & $MgCl_2$) varied: 0.5x, 1x (control), 2x



[4]

Fig. 4. Elements in a single beam UV-Visible spectrometer. [4]

Dark spectra taken (UV-Vis)

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Light Activation

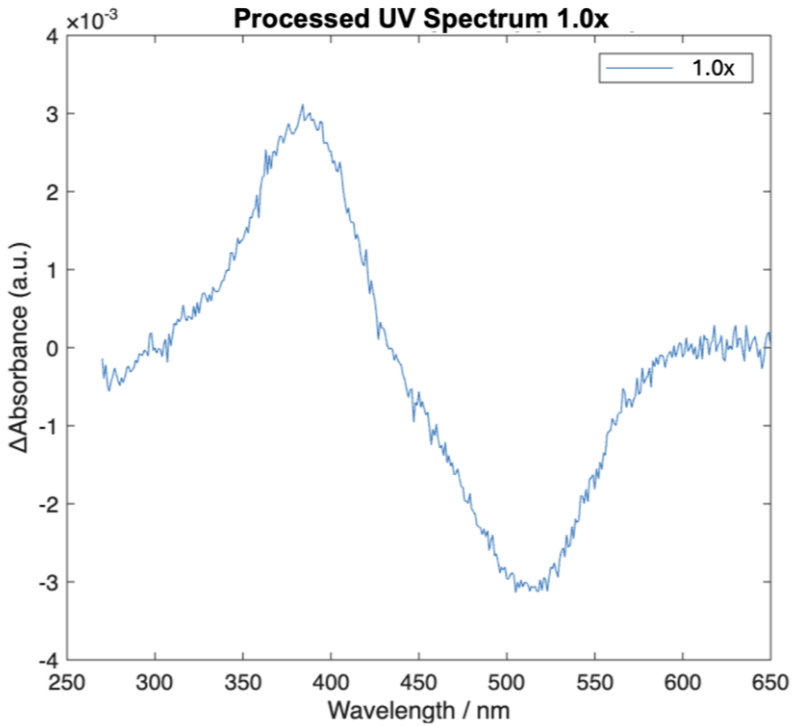
↓

Light Spectra taken (UV-Vis)

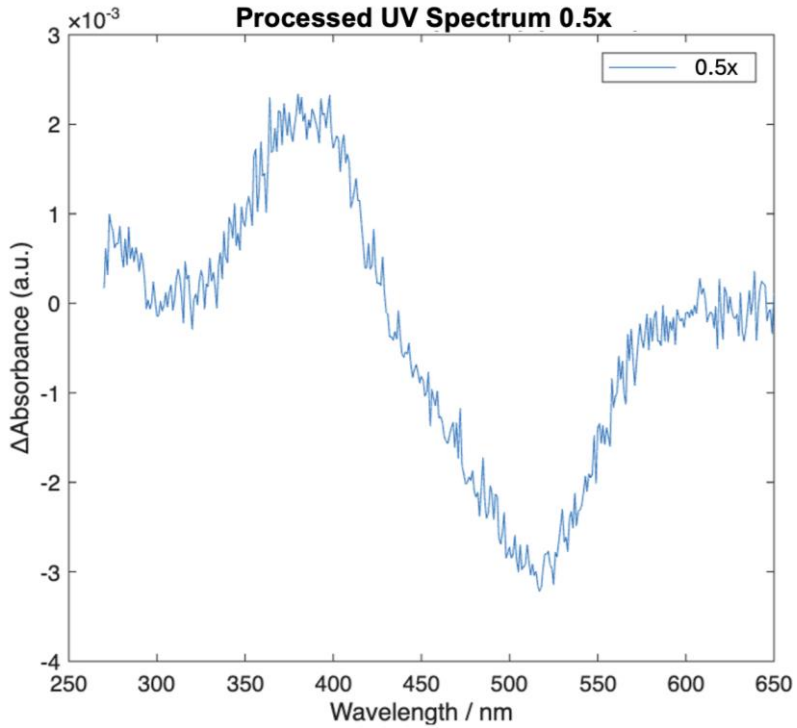
↓

Difference spectra generated & comparison of conformational states

Results



1.0x (Control)



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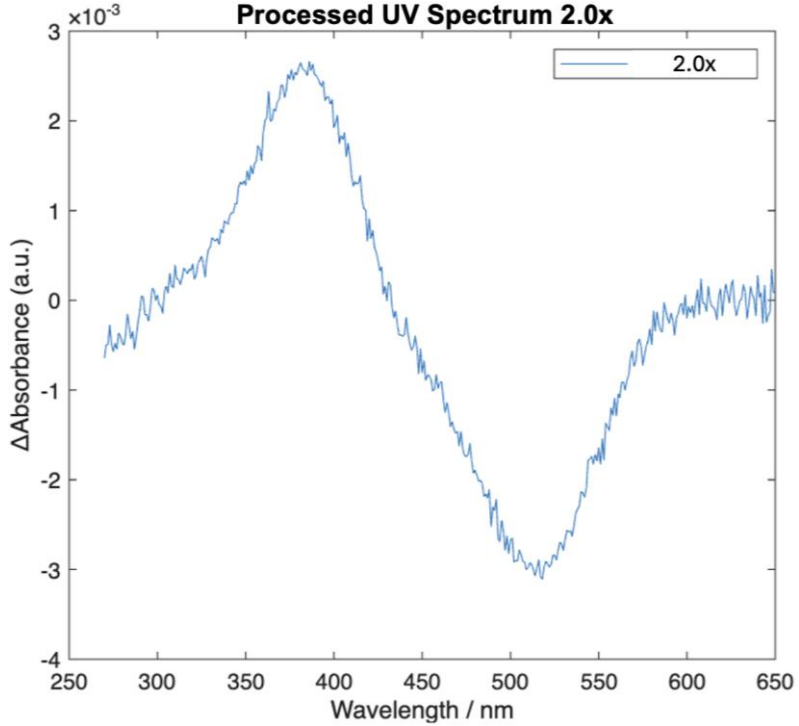

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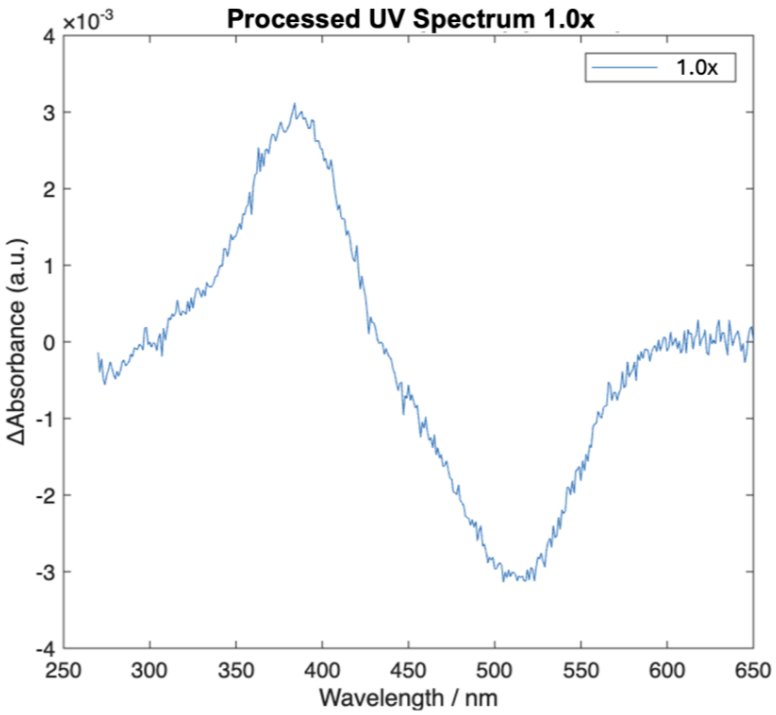
Discussion & Conclusion



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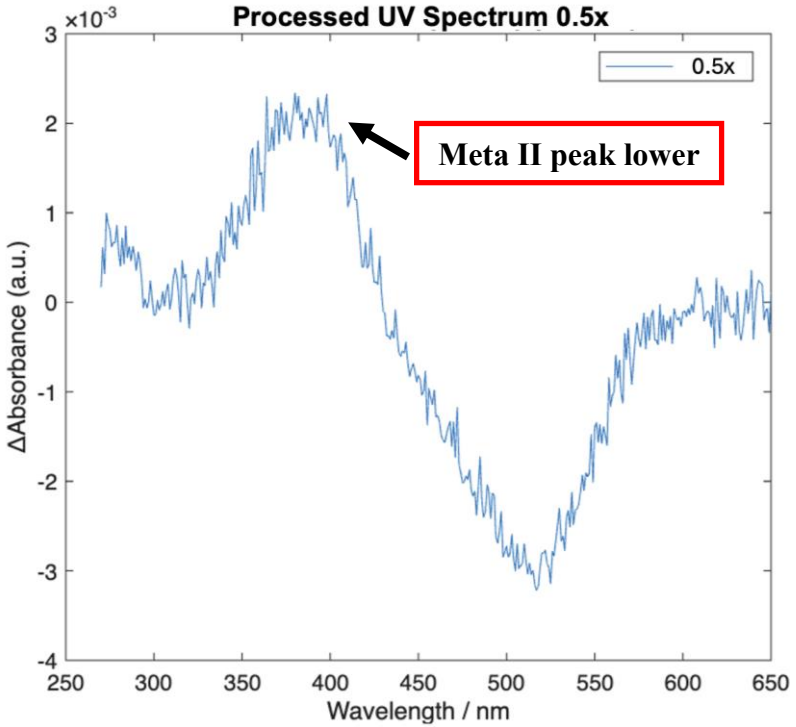

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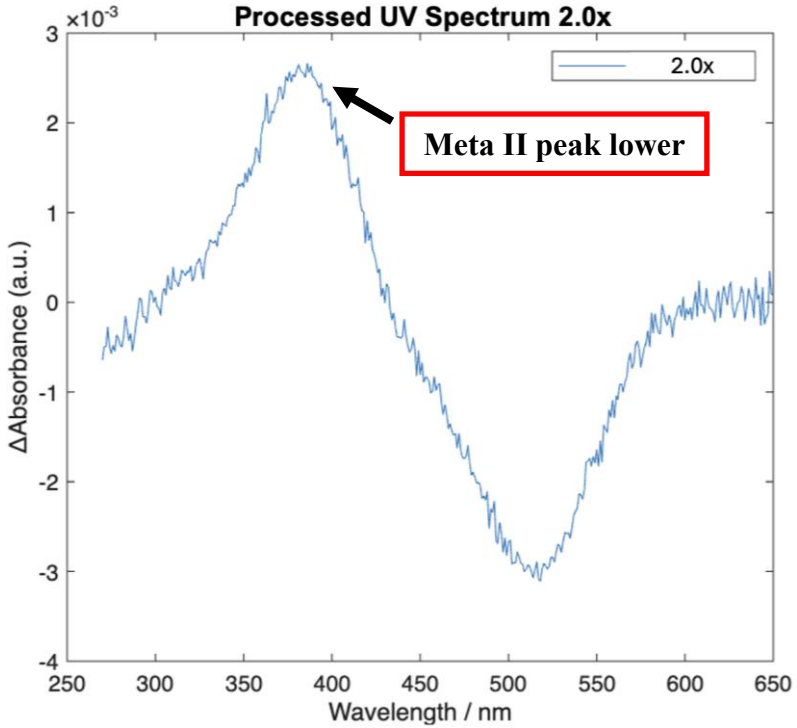

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References

- Bachler, Z. T., & Brown, M. F. (2024). Hidden water's influence on rhodopsin activation. *Biophys. J.*, 123(24), 4167–4179.
- Brown, M. F. (2012). Curvature forces in membrane lipid–protein interactions. *Biochem.*, 51(49), 9782–9795.
- Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov.* 2017;16(12):829-842.
- JASCO. (n.d.). *UV-visible spectroscopy instrumentation* [Figure]. JASCO Learning Center.
- Smith, S. O. (2010). Structure and activation of the visual pigment rhodopsin. *Annual Review of Biophysics, 39*, 309–328.
- Struts, A. V., Barmasov, A. V., Fried, S. D. E., Hewage, K. S. K., Perera, S. M. D. C., & Brown, M. F. (2024). Osmotic stress studies of G-protein-coupled receptor rhodopsin activation. *Biophys. J., Chem.*, 304, 107112.