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Activation of the G-Protein-Coupled Receptor Rhodopsin by Water

Udeep Chawla[†], Suchithranga M. D. C. Perera[†], Steven D. E. Fried, Anna R. Eitel, Blake Mertz, Nipuna Weerasinghe, Michael C. Pitman, Andrey V. Struts und Michael F. Brown*

Abstract: Visual rhodopsin is an important archetype for G-protein-coupled receptors, which are membrane proteins implicated in cellular signal transduction. Herein, we show experimentally that approximately 80 water molecules flood rhodopsin upon light absorption to form a solvent-swollen active state. An influx of mobile water is necessary for activating the photoreceptor, and this finding is supported by molecular dynamics (MD) simulations. Combined force-based measurements involving osmotic and hydrostatic pressure indicate the expansion occurs by changes in cavity volumes, together with greater hydration in the active metarhodopsin-II state. Moreover, we discovered that binding and release of the C-terminal helix of transducin is coupled to hydration changes as may occur in visual signal amplification. Hydration–dehydration explains signaling by a dynamic allosteric mechanism, in which the soft membrane matter (lipids and water) has a pivotal role in the catalytic G-protein cycle.

Introduction

G-protein-coupled receptors (GPCRs) are integral membrane proteins responsible for signal transduction across cellular membranes.^[1] Rhodopsin is the vertebrate GPCR^[2] responsible for scotopic (dim light) vision,^[3] and exhibits amazing fidelity, sensitivity, and signal amplification rate.^[4] Although membrane lipids are known to affect rhodopsin function,^[1a,5] there is considerable uncertainty about the role of water in the catalytic G-protein cycle of the *Rhodopsin* (Family A) GPCRs, which include the opioid and cannabinoid receptors,^[6] the A_{2A} adenosine receptor,^[7] and the β_2 -adrenergic receptor. Even with the latest GPCR structures,^[6,8] understanding the chemistry of transmembrane receptor signaling^[1b,9] requires mechanistic insights involving water and lipids (soft matter)^[5b,c,e,10] that crystallography alone

cannot provide.^[11] Here we investigated the light activation of rhodopsin in its natural omega-3 polyunsaturated lipid membranes under conditions of controlled hydration.^[12] Most arresting, we discovered that polymer osmolytes with a large molar mass favor the inactive MI state (closed conformation) by withdrawing water from the receptor core. According to Le Chatelier's Principle, an influx of water occurs upon light activation.^[12a] But surprisingly, we also found that small organic osmolytes increase the active (open) MII fraction.^[12] Further, we established that increased hydration drives binding of the C-terminal α -helix of the cognate G-protein to rhodopsin, while dehydration causes its unbinding. In addition to structural water^[13] and aqueous channels^[14] or hydrogen-bonded networks,^[13c,15] bulk water directly affects rhodopsin function, affording new insights into the G-protein cycle.

Results

We hypothesized that rhodopsin function within the lipid bilayer (Figure 1a) is coupled to large-scale changes in internal protein hydration. Light activation of rhodopsin is triggered by retinal isomerization from 11-*cis* to all-*trans*, altering its orientation within the binding pocket^[17] (Figure 1b). Experimental results over the past 15 years support

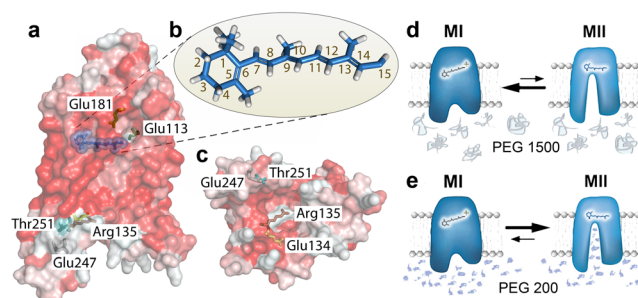


Figure 1. Rhodopsin activation is shifted either forward or backward depending on the osmolyte size. a) Metarhodopsin-II state (MII) viewed from the membrane plane (PDB code 3PQR; red indicates greater hydrophobicity). In the active MII state, the retinylidene Schiff base with Lys²⁹⁶ is deprotonated and its interaction with the Glu¹¹³/Glu¹⁸¹ complex counterion (ionic lock) is broken. b) All-*trans* retinylidene ligand in MII state with chemical numbering scheme. c) Active MII state viewed from the cytoplasmic side showing channel into the protein core. Amino acid residues Glu¹³⁴ and Arg¹³⁵ (second ionic lock) together with Tyr¹³⁶ comprise the E(D)RY motif and Glu²⁴⁷ and Thr²⁵¹ are sites of constitutively active mutations. d) Large polymer osmolytes are excluded from the protein to withdraw water and stabilize the inactive (closed) MI state. e) Small osmolytes penetrate the protein core thus stabilizing the active MII (open) state.

[*] Dr. U. Chawla,^[†] Dr. S. M. D. C. Perera,^[†] S. D. E. Fried, A. R. Eitel, Dr. B. Mertz, N. Weerasinghe, Dr. M. C. Pitman, Dr. A. V. Struts, Prof. Dr. M. F. Brown
Department of Chemistry and Biochemistry, University of Arizona
Tucson, AZ 85721 (USA)
E-Mail: mfbrown@u.arizona.edu

Dr. A. V. Struts
Laboratory of Biomolecular NMR, St. Petersburg State University
St. Petersburg 199034 (Russia)
Prof. Dr. M. F. Brown
Department of Physics, University of Arizona
Tucson, AZ 85721 (USA)

[†] These authors contributed equally to this work.

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the general reaction scheme^[15,16,18] $\text{Rh} + h\nu \rightarrow \text{MI} \rightleftharpoons \text{MII}_a \rightleftharpoons \text{MII}_b + \text{H}_3\text{O}^+ \rightleftharpoons \text{MII}_b\text{H}^+$, where Rh is dark-state rhodopsin with the 11-*cis* retinal chromophore covalently bound to Lys²⁹⁶ by a Schiff base linkage.^[19] An equilibrium between metarhodopsin-I (MI) and metarhodopsin-II (MII_a and MII_b substates) yields the activated state (metarhodopsin equilibrium). Two protonation switches^[15,16] open a binding cleft into the protein core for the cognate G-protein transducin (G_t),^[20] and are affected by constitutive mutations of the receptor^[21] (Figures 1 a,c).

Polymer Osmolytes Reversibly Shift the Metarhodopsin Equilibrium

To investigate the role of water, we introduced water-soluble polymers (polyethylene glycol, PEG) (Figures 1 d,e), because relatively high osmotic pressures (Π) can be achieved (> 10 MPa).^[22] Electronic (UV/Vis) spectroscopy was applied to monitor the active MII fraction (θ)^[16b] formed by light absorption under controlled hydration conditions, as determined from the absorption differences of the MI and MII states (Figure 2 a; Supporting Information, Figures S1 and S2; for experimental details, see the Supporting Information). Our approach is based on the thermodynamic properties of solutions and is complementary to direct structural methods used in biophysics. Hydrophilic polymers like PEG are entropically excluded from the membrane; and by their colligative action they lower the chemical potential of water (Figure 1 d). The withdrawal of water from lipid membranes by PEG osmolytes is shown by solid-state ²H NMR spectroscopy,^[23] which corresponds to investigating bilayer properties using fluorescent probes like diphenylhexatriene (DPH).^[24]

Our methods quantify the amount of water involved in receptor activation and are consistent with cryo-electron microscopy (EM) studies.^[8b,c] The pH titration curves for rhodopsin establish how the polymer osmolytes reversibly shift the metarhodopsin equilibrium to either the inactive (closed) MI state or the active (open) MII state (Figure 2 b; Supporting Information, Table S1). For a protein like rhodopsin, by the Law of Mass Action, the back shifting of the activation equilibrium means that in the forward direction an influx (flood) of water occurs. Surprisingly, the nearly linear isotherms for different osmolytes ($\ln K$ versus Π) (Figure 2 c) reveal a negative slope for large molar mass (M_r) osmolytes (PEG 1500 and PEG 400), yet a positive slope for small osmolytes (PEG 300 and PEG 200). A negative slope (Figure 2 c) is due to a water influx in the forward reaction, while a positive slope implies an opposite efflux. Hence, an increase or decrease in osmotic stress (pressure) drives the activation equilibrium back and forth to states with smaller or greater hydrated volume, respectively^[22b] (Figures 2 b,c).

Hydration Underlies the Effects of Chemically Modified Retinoids and Constitutive Mutations on Receptor Activation

Next, we asked the question: Are there parallels to rhodopsin mutations in diseases of vision, like *retinitis*

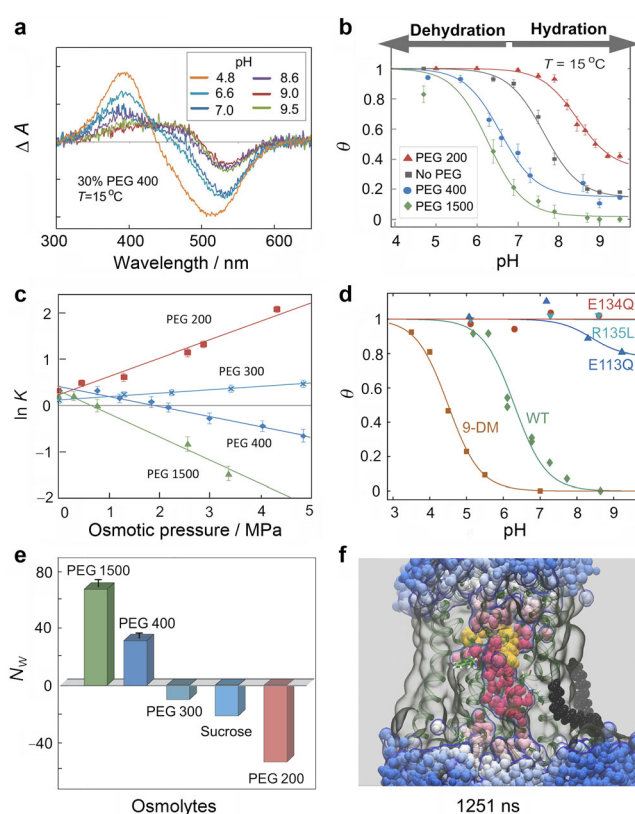


Figure 2. Water molecules flood into the transmembrane protein core of rhodopsin upon light activation. a) Representative difference UV/Vis absorption spectra of rhodopsin (photobleached minus dark state) for calculating the fraction (θ) of MII. Retinal disk membrane (RDM) samples at $T = 8^\circ\text{C}$ included 67 mM BTP buffer, 130 mM NaCl, and 2 mM MgCl_2 . b) Fraction of active MII state (θ) versus pH showing effect of controlled hydration ($T = 15^\circ\text{C}$) for osmolytes of different molar mass (M_r) (30–35% w/w polyethylene glycol, PEG). c) Metarhodopsin (MII/MI) ratio ($\ln K$) plotted versus osmotic pressure (Π) for different size PEG osmolytes (pH 7.4, $T = 15^\circ\text{C}$). d) Active MII fraction (θ) versus pH comparing wild-type (WT) rhodopsin in RDM ($T = 0^\circ\text{C}$) to constitutive mutants in egg phosphatidylcholine (PC) membranes ($T = 10^\circ\text{C}$) and to WT rhodopsin with retinoid antagonists (9-desmethyl-retinal, 9-DM) in RDM ($T = 20^\circ\text{C}$).^[21] e) Apparent number (N_w) of water molecules taken up or released from light-activated rhodopsin for polymer (PEG) osmolytes and sucrose. f) Snapshot of molecular dynamics (MD) simulation of rhodopsin 1.25 μs after retinal isomerization in silico.^[9a] Internal water molecules (red) flood the transducin binding cleft forming a channel to the retinal ligand.

pigmentosa, or to inhibitory retinoids acting as antagonists?^[21] For constitutively active mutations (e.g., E134Q, R135L, or E113Q) (see Figures 1 a, 2 d),^[21c] a forward shift to the active (open) MII state takes place, similar to small osmolytes (PEG 200) (Figure 2 d). Conversely, for inhibitory retinoids like 9-desmethyl-retinal (9-DM) (Figure 2 d), a back shift occurs to the inactive MI state, as seen for large molar mass (M_r) osmolytes (PEG 1500). We infer that the C9-methyl group maintains the open structure of MII needed for interaction with the G-protein (transducin). By removing the crucial C9-methyl group, an efflux of water collapses MII to the inactive MI state. Constitutive mutations of critical residues yield an opposite influx of mobile bulk water. Our results imply that



rhodopsin has greater conformational entropy upon light absorption,^[25] e.g., in analogy to intrinsically disordered proteins (IDPs). Small osmolytes increase the alkaline endpoint and decrease the pK_B for proton uptake (Figures 2b, 3; Supporting Information, Tables S2 and S3), while large osmolytes back shift the equilibrium, indicating a more collapsed state. Reversal of constitutive mutations by osmolytes may thus be possible.

Notably, hydrophilic polymers (such as PEG) are expelled from the protein interior by steric exclusion, or because of the entropic penalty from the thin layer of water at the membrane surface.^[22b] Polymer confinement can be understood by a virtual Gibbs dividing surface separating the external solution from the proteolipid membrane (Figure 1d). An osmotic pressure (Π) develops that withdraws water from the protein. Large polymer osmolytes are completely excluded, while small organic molecules can wriggle into the protein core (Figures 1e). Using thermodynamic relations, we can count the number of water molecules that flood the protein interior (for details, see the Supporting Information). The value may be positive or negative, reflecting an influx or efflux that depends on the polymer size (Figure 2e). Our previous molecular dynamics (MD) simulations^[9a] (Figure 2f) give a physical view of the water influx upon activation, which may occur as early as in the MI state,^[26] and is consistent with water release from MII in the back direction.^[9b] Movement of transmembrane (TM) helix H6 away from the helical bundle^[16a,17a,b,27] (Figures 1a,c) yields a reversible influx of water into the G_i binding cleft and solvent channel to the retinal ligand (Figure 2f).^[9a,12a,13c,20b] Remarkably, the theoretical number of water molecules is nearly the same as from the experimental osmotic pressure dependence (≈ 70 – 80) (Figure 2c). The reactivity of the retinylidene Schiff base with hydroxylamine in the MII state^[5b,10a,28] also supports a solvent channel to the retinal-binding pocket (Figure 2f).^[20b] By contrast, other experimental studies indicate an opposite efflux,^[24] which is contradicted by radiolytic protein footprinting experiments,^[13c] and by site-directed spin labeling (SDSL) studies.^[29] Our findings immediately resolve these discrepancies (Figure 3).

Thermodynamic Relations Determine the Number of Water Molecules that Enter the Rhodopsin Protein Core

To quantitatively interpret the results, we consider osmotic pressure plus the associated water (i.e., the hydrated volume) as conjugate variables, analogous to hydrostatic pressure and total volume.^[12a,30] We do not introduce transmembrane osmotic or protonmotive gradients, because hydration is controlled through osmotic stress of the fully equilibrated membranes (see the Supporting Information).^[22b] Large osmolytes dehydrate the receptor, as they do not penetrate the protein core. Following Parsegian et al.,^[22b] the work of transporting water into or out of the protein interior is $w = -\Pi\Delta V_w$ where $\Delta V = \Delta V_w$ is the change in hydrated volume, and Π is the external osmotic pressure. Thermodynamics teaches us the equilibrium constant (K) depends on Π by

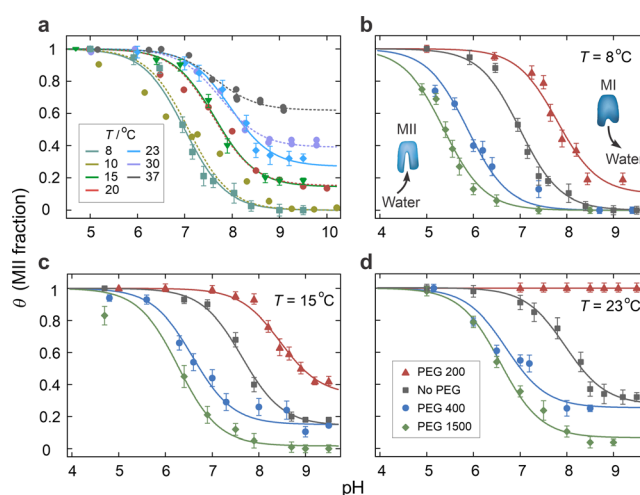


Figure 3. Effects of osmolytes at different temperatures provide evidence for an energy landscape mechanism (ELM) of rhodopsin activation. a) Plots of active MII fraction (θ) versus pH for rhodopsin in native RDM at various temperatures. The dashed lines are data from Ref. [16b]. b)–d) pH titration curves for rhodopsin in RDM containing various osmolytes (30–35 wt.% PEG 1500, PEG 400, no PEG, PEG 200) at $T = 8$, 15, and 23 °C, respectively. The metarhodopsin equilibrium is shifted towards either acidic or basic pH values depending on the osmolyte size. Note the nonzero alkaline endpoint fraction increases at the higher temperatures, and that the forward shift of the pK_A is positively correlated with a greater alkaline endpoint.

$$\left(\frac{\partial \ln K}{\partial \Pi}\right)_T = -\frac{\Delta V^\circ}{RT}, \quad (1)$$

where $\Delta V^\circ \approx N_w \bar{V}_w$ is the standard change in excess (partial) water volume of the initial and final states, the number of water molecules is N_w , and $\bar{V}_w$ is the water partial molar volume. For large osmolytes like PEG 1500, the increase in partial hydrated volume upon light activation is $\Delta V^\circ \approx 1.3 \text{ L mol}^{-1}$ rhodopsin ($2200 \text{ \AA}^3/\text{molecule}$)—a very large value indeed. This change in (hydrated) protein volume within the bilayer makes rhodopsin sensitive to the membrane soft matter, including both the lipids and surface water.^[5e] Sufficient (free) energy exists to perform this work ($\approx 10 \text{ kJ mol}^{-1}$) from photonic absorption and retinal isomerization ($\approx 239 \text{ kJ mol}^{-1}$), even in the absence of osmotic gradients or a protonmotive force.^[31] The large increase in partial water volume of rhodopsin upon photoactivation (≈ 70 – 80 waters, $\bar{V}_w^* = 18 \text{ mL mol}^{-1}$) (Figure 2e) far exceeds the changes due to hydrostatic pressure (equivalent to ≈ 3 – 6 waters), which involves cavities or structural waters,^[30,32] in addition to the thermal volume ($\Delta V^\circ \approx 60$ – 100 mL mol^{-1} protein).^[33] Osmotic stress experiments thus give striking validation of our previous theoretical MD simulations for rhodopsin^[9a,b] (Figure 2f).

Going forward, the small polymer molecules enter the protein interior, e.g., the rhodopsin binding cleft for transducin (G_i) (Figure 2f). For partially excluded polymers ($< \text{PEG } 400$, Figure 1e), the apparent volume change is given by $\Delta V_{\text{app}} = \Delta V^\circ (1 - P)$ in terms of the partition coefficient P between the solution and the protein. Even then, the partial penetration of smaller osmolytes into the protein core can

only decrease the shift to MI, and not lead to reversal. By contrast, we observe a shift to the active MII state for small osmolytes (PEG 200), which mirrors that observed in previous studies of rhodopsin.^[24,34] One possible explanation is that this trend is due to the lipids, where dehydration increases the bilayer thickness as demonstrated by solid-state ²H NMR spectroscopy (Supporting Information, Figure S3).^[23,35] Dehydration can also lead to greater magnitude of the negative monolayer spontaneous curvature, as described by the flexible surface model (FSM).^[5c] Both effects will promote active MII formation in lipid bilayers.^[5e,10a,36] By conducting experiments for rhodopsin solubilized in micelles of detergents such as [(cholamidopropyl)dimethylammonio]-propanesulfonate (CHAPS) or *n*-dodecyl- β -D-maltoside (DDM), one can isolate the protein hydration effect from the lipid contribution. Direct comparability with recent small-angle neutron scattering (SANS)^[16c] and quasi-elastic neutron scattering (QENS) studies of rhodopsin hydration is then possible, which support water uptake in the active MII state. In this regard, experimental SDSL studies^[29a] of rhodopsin in DDM micelles have shown that the small osmolyte sucrose back shifts the population toward the MI component. Because the forward shifting to MII is absent in the detergent-solubilized system, a role of the lipid bilayer is supported in favoring the active state in the presence of small osmolytes.

Partial penetration of small osmolytes into the protein might also withdraw water from smaller internal cavities associated with the MI–MII transition.^[13a–c,e,17a] One example is offered by recent direct hydration experiments monitoring bound water by infrared spectroscopy in opsin and the E134Q mutant, suggesting that Glu¹³⁴ of the conserved E(D)RY motif is a hydration site at the protein-lipid interface, which dehydrates going from MII_b to the MII_b·H⁺ state.^[37] Local dehydration of such small protein regions is in line with MII stabilization by small osmolytes^[24] (see Figures 2b,d). The shift to the MII state could also be attributed to specific interaction of small osmolytes with the transducin binding cavity. Specific PEG-protein interactions are known to be inversely related to PEG size;^[38] however, we did not observe any substantial binding of small osmolytes to rhodopsin (see below). Distinguishing interpretations such as crowding or excluded volume^[39] from osmotic effects requires further measurements. The polymer size influences immediately explain previous osmotic stress results that have been erroneously interpreted by an opposite release of water from rhodopsin upon light activation.^[24,34]

Gating by Water Explains the Binding Affinity of Transducin to Rhodopsin in Physiological Signaling

Lastly, we asked the question: What is the connection to the G-protein activation cycle? To address how binding of the cognate G-protein is affected by hydration, we studied the influences of a high-affinity C-terminal peptide analogue of transducin^[17a,40] (Figure 4). Binding of the G_αCT2 peptide analogue (ILENLKDVGLF) stabilizes the active (open) MII state by conformational selection (so-called „extra MII“).^[4,41]

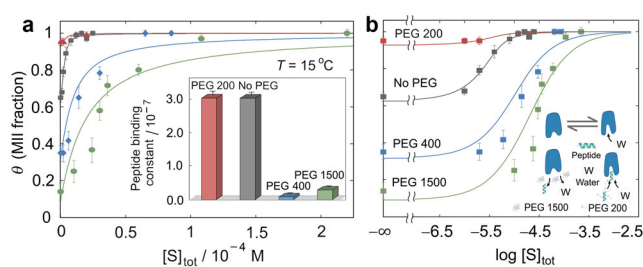


Figure 4. Binding of transducin C-terminal helix to rhodopsin is coupled to hydration while dehydration leads to unbinding. a) Active MII fraction (θ) in native RDM versus total concentration ($[S]_{\text{tot}}$) of transducin C-terminal peptide analogue (amino acid sequence ILENLKDVGLF) (pH 7.4, $T = 15^\circ\text{C}$). Data are fit to a single-site binding isotherm (see the Supporting Information). *Inset*: effect on peptide binding constant for different size polymer osmolytes. b) Active MII fraction (θ) versus $\log [S]_{\text{tot}}$ value. *Inset*: Cartoon depiction of how C-terminal peptide competes with large osmolytes (PEG 1500) yet is noncompetitive for small osmolytes (PEG 200).

(For a description of the binding model, see the Supporting Information.) Our experiments imply that hydration favors binding of G_t to active MII in the G-protein cycle,^[20a,42] while dehydration favors its release (Figure 4). The results are consistent with recent cryo-EM structures of rhodopsin complexed with transducin or G_t^[8b,c] and indicate at least part of these water molecules (≈ 50) can be replaced by the transducin C-terminal helix. Fitting the binding isotherms (Figures 4a,b) (see the Supporting Information), we find that larger polymers (PEG 1500 and PEG 400) decrease the binding affinity by an order of magnitude (competitive) (Supporting Information, Table S4). For smaller osmolytes, the effect is absent ($M_r < 400$ Da) (noncompetitive)—an arresting discovery (Figure 4a). Detailed analysis shows that for large osmolytes, the peptide binding constant correlates with proton uptake by Glu¹³⁴ of the conserved E(D)RY sequence motif (Supporting Information, Table S4). Hence, water not only governs the equilibrium between active and inactive states^[12a,16b] of the receptor, but also affects the intrinsic binding of its cognate G-protein.^[2b–c,20a,42a]

Discussion

Pressure-Based Force Measurements Show a Volumetric Increase upon Light Exposure of Rhodopsin

Our findings reveal how the soft cellular matter comprising the water and lipids is likely to affect the transmembrane signaling in the case of rhodopsin. Integrating the current results with small-angle neutron scattering (SANS) studies of rhodopsin in detergent solutions,^[16c] and quasi-elastic neutron scattering (QENS) data for partially hydrated powders,^[43] affords new insights into an energy landscape mechanism versus a bimodal-switch model. According to our picture, rhodopsin is swollen by penetration of water into the protein core following the light exposure.^[12a,16c,43] Volumetric changes in protein shape are coupled (slaved) to the aqueous solvent, including the hydration shell and bulk solvent.^[16c,44] Upon



photon absorption, the elimination of voids or cavities due to the water penetration yields a solvent-swollen protein interior, with a further increase of hydration in the active MII state.^[12] Neutron scattering studies,^[16c,43b] as well as hydrostatic pressure^[45] and osmotic pressure measurements, thus point to greater hydrated volume upon forming the active MII state in the reaction mechanism.^[5b]

Metarhodopsin Equilibrium is Shifted by Chemically Nonspecific Osmotic Action

Effects of osmotic stress add an important new layer of information to studies of the influences of hydrostatic pressure on rhodopsin^[45] and other proteins.^[32b,46] Back shifting of the metarhodopsin equilibrium by either osmotic pressure or hydrostatic pressure reveals a lower-density, hydrated active state. Both favor the preactive MI state, yet for different reasons. While hydrostatic pressure acts upon a closed thermodynamic system—and thereby detects changes in the densities of the components (i.e., partial molar volumes)^[32a,33a,47]—osmotic pressure acts on an open system, and investigates changes in composition, water in this case.^[12a,23a] Indeed, the force-based measurements give evidence of a functional volumetric change that occurs in conjunction with light activation of rhodopsin. We propose that formation of active MII entails an interplay of hydration and packing forces,^[16c,43b] whereby the penetration of water into the protein core yields a solvent-swollen active state. Changes of helix packing density affect small voids or cavities within the protein, as described by Richards^[48] and by Hummer et al.^[49] Alterations in both internal packing and protein hydration underlie the dynamics of rhodopsin conformational transitions, giving a directed flow of energy throughout the photoreceptor molecule. Such changes of hydration can be structurally investigated by methods such as time-resolved fluorescence spectroscopy of polarity-sensitive fluorophores,^[50] and by ¹H Overhauser dynamic nuclear polarization (ODNP) relaxometry.^[51]

Influences of Pressure on Rhodopsin Conformational Equilibria Give Further Insights into the Activated State

The various partial contributions to the total volume change of a protein^[33a,52] include cavity or void reduction, interstitial solvation, hydrophobic hydration, and the hydrophobic effect. As originally set forth by Kauzmann,^[52a] based on small molecule data, contributions to the partial molar volume of a protein arise from the van der Waals volume of the constitutive protein atoms, the volume of structural voids or cavities, and changes in the solvent due to hydration. Typically, these contributions give negative values to the volumetric change of protein unfolding.^[32a,33a,47] The overall volume change depends on the magnitudes and signs of the various partial contributions, yielding the so-called protein volume paradox, as discussed by Brandts^[52b] and by Chalikian and Breslauer.^[33a] When a protein conformational transition occurs, the changes in hydration or intramolecular voids or

cavities are compensated by motions of the solute and solvent molecules. The additional large positive contribution from the thermal volume explains why the partial molar volumes for small molecule solutes are usually greater than the van der Waals volume.^[33] Based on scaled particle theory, the coupled vibrations of the solute and surrounding solvent give an added expansion of the macromolecule (including bound water and lipids for a membrane protein).

It follows that for a rhodopsin-like GPCR, the interplay of both packing and hydration forces together with coupling to the solvent yields activation of the receptor. As discussed by Perera et al.,^[16c] in the metarhodopsin equilibrium the active MII state is more hydrated and characterized by a greater thermal volume (shown by hydrostatic pressure studies^[45] and by our neutron scattering investigations^[16c]). The larger volumetric fluctuations in the active MII state^[16c] lead to back shifting of the metarhodopsin equilibrium to the MI state by hydrostatic pressure.^[45] Greater hydration of the active MII conformation is clearly shown by the effects of osmotic pressure (stress).^[12] Consequently, the two force-based measures taken together imply the active MII conformation has greater thermal fluctuations. A solvent-swollen state is present upon light activation, e.g., with internal waters that couple the ligand and G-protein binding sites of the photoreceptor.

Binding Affinity of Transducin to Rhodopsin is Modulated by the Soft Membrane Matter

The reciprocal effects of lipid structure, osmotic stress, and protein function demand entirely new ways of thinking about GPCR signaling in biomembranes (Figure 5). Control of the binding affinity of rhodopsin for transducin in the G-protein cycle is needed for rapid, high fidelity downstream signaling cascades. Experimentally, we find that binding and unbinding of a high-affinity peptide analogue of transducin is coupled to hydration changes in visual signal amplification. The influences of hydration support a water-mediated, sponge-like mechanism (as we now term it) for rhodopsin-based signaling. Rhodopsin must first bind transducin tightly, yet it cannot remain strongly bound. The G-protein must be expelled following nucleotide exchange to ensure rapid signal amplification. Cycling the on/off rates in conjunction with pre- and post-GTP–GDP exchange fulfills the requirements of high signal fidelity, due to a high on-rate, together with rapid signaling, which requires a high off-rate. For a multiscale reaction scheme^[15,16] of transducin binding and unbinding, light activation proceeds via the less hydrated („dry“) states (e.g., MI, MII_a) to an ensemble of hydrated („wet“) MII states. We further propose that the wet (hydrated) states are optimized for binding of transducin (G_t), while the dry (dehydrated) states favor its release. According to an osmotic piston analogy (Supporting Information, Figure S4), the retinal isomerization „pumps water into the well“ leading to binding of transducin. Similarly to large osmolytes, nucleotide exchange and dissociation of the heterotrimeric G_α and G_{βγ} subunits „pumps water back out of the well“.

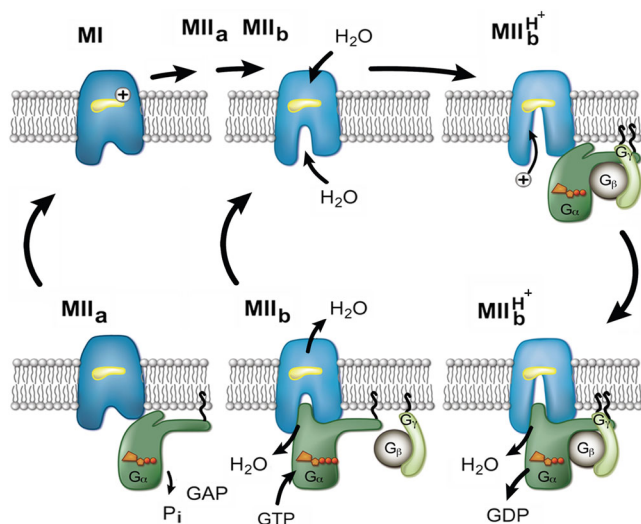


Figure 5. Hydration–dehydration cycling explains catalytic activation of transducin by a sponge-like allosteric mechanism. Coupling of water uptake and release by rhodopsin (blue) to binding and unbinding of the G-protein transducin (green) accounts for high-fidelity rapid visual signaling. (*Upper left*) Rhodopsin enters cycle in the low-hydration, low-affinity MI state by visible light isomerization of the protonated retinylidene Schiff base (SB) (yellow). (*Upper middle*) In the MII_b substate,^[16a] the SB becomes deprotonated, breaking the ionic lock to the Glu¹¹³/Glu¹⁸¹ complex counterion (first protonation switch). TM helix H6 tilts away from the helical bundle,^[16a] initiating a flux of water into the transducin-binding cleft^[9a] (MII_b). (*Upper right*) Water influx into the protein interior enables proton uptake via Glu¹³⁴ of the conserved ERY motif giving the high-hydration, high-affinity MII_bH⁺ substate (second protonation switch). (*Lower right*) binding of G_t-GDP yields the receptor–G-protein complex. Exposure of the transducin binding cleft allows binding of G_t-GDP by the α5 helix of the transducin C-terminus analogously to small osmolytes. (*Lower middle*) Exchange of GTP for GDP yields dissociation of the transducin G_{βγ} subunits, withdrawing water and dehydrating rhodopsin in analogy to large polymer osmolytes, giving the partially hydrated MII_b substate. (*Lower left*) Transducin catalyzes its own release by pinching off the G_α-GTP subunit as the carrier of the light signal. The GTPase-activating protein (GAP) terminates signaling by GTP hydrolysis on transducin.

Up and down regulation of the G-protein-binding affinity by hydration is clearly one way of controlling downstream signaling (Figure 4). Binding of transducin (G_t-GDP) locks rhodopsin in the partially hydrated state, until GTP–GDP exchange occurs and the G_{βγ} subunits dissociate from the G_α-GTP subunit. The GTP–GDP exchange marks the point in the cycle where accelerating overall G-protein turnover requires switching from a high on-rate to a high off-rate. Once GTP is bound to the G_α subunit, the binding interface to the G_{βγ} subunits is destabilized. The greater electrostatic charge on GTP (relative to GDP) increases the local hydration at the expense of the G_α-G_{βγ} binding interface, thus favoring dissociation of the heterotrimer subunits. As hydration of the G_α-G_{βγ} binding interface proceeds, water is withdrawn from the interior of rhodopsin. Dehydration closes the transducin-binding cleft of rhodopsin, pinching off or expelling the G_α-GTP subunit, and hence the off-rate is increased. The „extra MII“ is thereby converted back to the inactive MI or MII_a substates for another round of the

catalytic G-protein cycle. Transducin subunit dissociation acts analogously to a large osmolyte, withdrawing water from the rhodopsin interior with its binding cleft. In this way, transducin catalyzes its own release from the active receptor in the catalytic G-protein cycle (Figure 5).

Conclusion

Our findings recast a new view of the role of biological soft matter (water plus lipids) in GPCR activation mechanisms. Experiments for the first time reveal the striking functional influences of water on the equilibrium between inactive and active states of a 7TM receptor. Water and lipids act as dynamic allosteric modulators that synergistically control rhodopsin interaction with effector proteins in the visual process. We propose that hydration–dehydration cycling of rhodopsin has evolved together with GTP–GDP exchange on transducin for enhanced turnover, while preserving high fidelity activation of effector proteins. Influences of both lipids and water point toward an energy landscape (ensemble) mechanism for the activation process. Water is coupled to up and down regulation of transducin binding to rhodopsin in the catalytic reaction cycle. Furthermore, its role suggests potential new routes to druggable interactions involving GPCRs in pharmacological applications. Whether changes in hydration occur as a part of the G-protein cycle for other GPCRs remains as a question of great immediacy for the chemistry of receptor signaling in lipid membranes.

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Conflict of interest

The authors declare no conflict of interest.

Stichwörter: GPCRs · membrane lipids · membrane proteins · osmotic stress · rhodopsin



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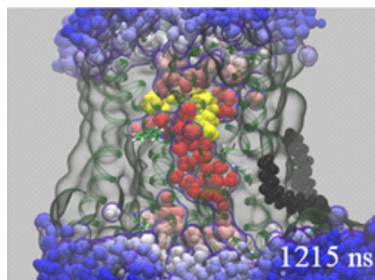
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Biophysics

U. Chawla, S. M. D. C. Perera,
S. D. E. Fried, A. R. Eitel, B. Mertz,
N. Weerasinghe, M. C. Pitman,
A. V. Struts,
M. F. Brown* ————— ■■■-■■■

Activation of the G-Protein-Coupled
Receptor Rhodopsin by Water



Water molecules flood the protein core of visual rhodopsin to form a solvent-swollen active state upon light absorption. Binding of a peptide analogue of transducin is coupled to hydration changes in catalytic activation of the G-protein. The soft membrane matter comprises lipids and water, which interact with rhodopsin in the signaling mechanism to control visual function.