Energy Transport and its Function in Heptahelical Transmembrane Proteins

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Photoproteins such as bacteriorhodopsin (bR) and rhodopsin (Rho) need to effectively dissipate photoinduced excess energy to prevent themselves from damage. Another well-studied 7 transmembrane (TM) helices protein is the β2 adrenergic receptor (β2AR), a G protein-coupled receptor (GPCR) for which energy dissipation paths have been linked with allosteric communication. To study the vibrational energy transport in the active and inactive states of these proteins, a master equation approach [J. Chem. Phys. 152, 045103 (2020)] is employed, which uses scaling rules that allow to calculate energy transport rates solely based on the protein structure. Despite their overall structural similarities, the three TM proteins reveal quite different strategies to redistribute excess energy. While bR quickly removes the energy using the TM7 helix as a 'lightning rod', Rho exhibits a rather poor energy dissipation, which might eventually require the hydrolysis of the Schiff base between the protein and the retinal chromophore to prevent overheating. Heating the ligand adrenaline of β2AR, the resulting energy transport network of the protein is found to change significantly upon switching from the active to the inactive state. While the energy flow may highlight aspects of the interresidue couplings of β2AR, it seems not particularly suited to explain allosteric phenomena.

INTRODUCTION

Vibrational energy transport can be a critical aspect of protein functionality. In particular, proteins that employ or generate excess energy during their function need to quickly dissipate this energy to prevent damage to the protein. For example, enzymatic reactions may release enough heat to unfold a protein. Efficient energy dissipation is also crucial for photoproteins, which harness photonic energy via light-active cofactors, so-called chromophores, that can generate excess energies even higher than 2 eV. Following photoexcitation, this is achieved via ultrafast internal conversion into the electronic ground state and subsequent redistribution of the chromophore vibrational energy into low-frequency modes. Using time-resolved vibrational spectroscopy, the resulting flow of biomolecular energy along the protein backbone and via tertiary contacts such as hydrogen bonds, salt bridges and polar contacts can be monitored in space and time. Notably, these energy transport pathways are believed to be linked to channels of allosteric communication, which is of key importance for protein signaling and regulation.

Well-known examples of photoproteins are the two protein families of microbial and visual rhodopsins. For the first family, the prime example is bacteriorhodopsin (bR), which was discovered over 40 years ago as the only protein in the purple membrane of Halobacterium salinarum. bR acts as light driven ion pump, using the energy of green light photons to pump protons out of a bacterial cell, creating a proton gradient for ATP production. The visual rhodopsin family is best represented by rhodopsin (Rho) itself, which is the photoreceptive protein located in the membrane of vertebrate retina rod cells and forms a subgroup of the G protein-coupled receptor protein (GPCR) superfamily. As can be seen in Fig. 1, both protein families share seven transmembrane helices (TM1–TM7) connected by intracellular loops (ICL) and extracellular loops (ECL) as well as a retinal chromophore bound to the protein core and covalently linked to the protein through a Schiff base via a lysine residue. Despite these global similarities, the details of the protein’s architecture differ significantly, and both classes appear to have evolved independently.

In both types of proteins, photon absorption induces an isomerization of retinal, which drives protein conformational and electrostatic changes necessary for the protein’s function. The main difference between bR and rhodopsin is found in the details of the photoreaction: bR undergoes a photocycle where the retinal starts in an
all-trans configuration and isomerizes into a 13-cis arrangement, with the all-trans configuration and the initial protein conformation being restored at the end of the photo reaction. In Rho, the retinal starts in a 11-cis configuration and irreversibly converts into the all-trans state, switching the protein from an inactive dark state to the active Meta II state. The all-trans retinal then needs to be chemically cleaved from the protein and be chemically converted back into its 11-cis configuration by photoisomerases. Despite these differences, ~2/3 of absorbed photons result in the isomerization in both bR and rhodopsin,\textsuperscript{20,30} which in turn means that 1/3 of all absorbed photons inject their full energy into the protein via internal conversion, as fluorescence in both proteins is negligible.\textsuperscript{20,30} Ultimately, only a part of the absorbed photonic energy is used to perform work,\textsuperscript{31} while the rest is dissipated into the protein. Furthermore, the all-trans retinal in Meta II is chemically cleaved from the opsin apoprotein, unbinds from it and isomerizes back to 11-cis retinal using additional proteins.\textsuperscript{32,33}

Apart from the photoprotein bR and Rho, we are also concerned with the \(\beta_2\) adrenergic receptor (\(\beta_2\)AR).\textsuperscript{27,34} which represents a well-investigated GPCR that undergoes an allosteric transition upon ligand binding. As displayed in Fig. 1, \(\beta_2\)AR is structurally highly similar to Rho, but is activated by the diffusible ligand adrenaline instead of a chromophore. By heating the ligand and following the resulting energy flow though \(\beta_2\)AR, one may study the change of the energy transport network when we switch switch from the active to the inactive state. In this way we connect to recent work\textsuperscript{35} that linked vibrational energy transport and allostery to \(\beta_2\)AR.

A number of approaches have been suggested to model protein energy flow based on molecular dynamics (MD) simulations.\textsuperscript{36–44} Moreover, several authors have proposed network-type models,\textsuperscript{45–52} which are derived, for instance, by computing the inter-residue energy flow to create an energy exchange network. To describe the energy transport in proteins between the different residues, Buchenberg et al. have proposed a master equation approach.\textsuperscript{49,51} The discovery of scaling rules\textsuperscript{51,53} allows for calculating the energy transport rates solely based on the protein structure. Hence, it can be applied on a single protein frame such as a crystal structure without the need for extensive MD simulations.

In this work, we use the master equation approach of Ref. 53 to study energy dissipation from retinal into both bR and Rho. Additionally, we consider the ligand-induced energy transport in \(\beta_2\)AR, in order to investigate a possible connection between energy dissipation paths and allostery. For all three proteins, high-resolution crystal structures are available in ground (inactive) states and active states.\textsuperscript{54–59} For each system, we compute the energy transport rate matrix, which includes both transport along the backbone and through contacts. We evaluate a coarse-grained depiction of the rates on the level of the secondary structures, which yields an overview over the connectivity of the different proteins. By heating the ligand or retinal, respectively, we visualize and discuss the resulting energy flow in some detail.

### THEORY AND METHODS

#### Master Equation

Starting from a discretized diffusion equation, Buchenberg et al.\textsuperscript{51} derived the energy flow master equation

\[
\frac{dE_i(t)}{dt} = \sum_j [k_{ij}E_i(t) - k_{ji}E_j(t)],
\]

where \(E_i\) describes the kinetic energy of residue \(j\) and \(k_{ij}\) denotes the transport rate from residue \(i\) to \(j\). The corresponding energy transport times \(\tau_{ij}\) are obtained via \(\tau_{ij} = 1/k_{ij}\). The rate matrix \(k_{ij}\) contains the full information about the energy redistribution, and can be employed to predict the energy evolution of the system. Moreover, one can consider the solvent as an additional state with solvent rates \(k_{ps}\) and \(k_{sp}\) from the protein into the solvent and back.\textsuperscript{38} However, we do not include a solvent rate in this work, as the major protein surface is found at the membrane interface, and the protein-water interface above and below the membrane only leads to a non-specific removal of the excess heat.\textsuperscript{10,53} Protein-membrane lipid contacts are mostly hydrophobic, and as was found earlier,\textsuperscript{44,51} the energy transfer through such contacts can be safely neglected.

#### Scaling rules and Identification of Contacts

The scaling rules of biomolecular energy flow allow to describe the energy transport in terms of only a few parameters, yielding a simple and transferable model. The scaling rule for backbone transport yields the rates\textsuperscript{51}

\[
k_{ij}^B = \frac{D_B}{\langle x_{ij}\rangle^2} \sqrt{\frac{f_i}{f_j}},
\]

and for contact transport we obtain\textsuperscript{53}

\[
k_{ij}^C = \frac{D_C}{\langle q_{ij}\rangle^2} \sqrt{\frac{f_j}{f_i}}.
\]

Both rules follow from the diffusion equation with \(D_B\) and \(D_C\) being diffusion constants and \(f_i\) denoting the number of the degrees of freedom of residue \(i\). In case of the backbone transport, we consider the average square distance \(\langle x_{ij}\rangle^2\) along covalent bonds between all atoms of residues \(i\) and \(j\). In contrast to that, the contact transport only takes the atoms of selected contacts into account (see below) and is inversely proportional to the square mean distance \(\langle q_{ij}\rangle^2\) between those atoms. The diffusion constants were determined as \(D_B = 1.1 \text{nm}^2\text{ps}^{-1}\) and \(D_C = 2.1 \times 10^{-3} \text{nm}^2\text{ps}^{-1}\) in
Ref. 53 and serve as global constants, while the individual bond strength is reflected by \((x_{ij})^2\) and \((g_{ij})^2\), respectively. The application of these scaling rules allow us to predict the energy transport directly from the connectivity within a protein structure, providing the general means to model the energy flow in various proteins using solely a crystal structure.

Since nonpolar contacts were shown to be negligible for energy transport,\(^{34,54}\) they are omitted in this study. Moreover, as the residues that form strong polar contacts additionally also form hydrogen bonds (but with a smaller distance), it is sufficient to focus on hydrogen bonds for energy transport as a first approximation. Here we only include hydrogen bonds, where the distance between hydrogen atom and acceptor is smaller than 2.8 Å and the angle \(\theta\) between donor-acceptor-hydrogen is \(\theta \leq 30^\circ\). Concerning ionic interactions in the considered proteins, charged residues interact with each other via salt bridges, i.e., combined ionic-hydrogen bond interactions. Hence, this type of interaction is already approximately taken care of via the hydrogen bond analysis. We furthermore consider ionic interactions over distances larger than 4.5 Å to be damped out by other residues found between them, and thus so weak that their contribution to energy transfer is negligible.

\(\xi\) From these contacts, we compute the energy transport rates on the level of residues, as those are the states used in the master equation. To obtain a broader picture, we also consider the energy transport on the level of the protein’s secondary structures, where all rates between the residues of two secondary structures are combined into one rate. This method allows us to obtain a better overview of the connections between the different protein structures. The secondary structure rates can then be visualized in the protein structure to grasp the protein’s connectivity at one glance.

Quantum Corrections

The above scaling rules were parameterized from classical nonequilibrium MD simulations.\(^{53}\) However, classical mechanics approximate the quantum mechanics of anharmonic oscillators only well on short time scales.\(^{60-62}\) In order to reproduce the correct energy transport time scales, it is thus necessary to introduce a quantum correction to our classical approach. As a simple approximation, it may be sufficient to rescale the diffusion coefficients \(D_R\) and \(D_C\) obtained from the classical MD simulations by a common quantum correction factor \(Q\). By comparison of simulated and experimentally measured data for various test proteins, this factor was determined as \(Q = 3.1\) by Deniz et al.\(^{10}\) As in this work we no not include the dissipation into the solvent water (which typically needs no correction),\(^{58}\) the quantum correction is applied to all considered transport channels and therefore only serves as a general scaling factor to obtain the correct the time scales.

Structure Preparation

In order to compute the contact rates, we first have to identify the contacts that the protein forms. We apply our method to crystal structures, which were in part slightly modified. For br, we base our calculations on the recently resolved set of crystal structures by Weinert et al.\(^{59}\) We consider the inactive “dark” state (PDB 6RQP) with an all-trans retinal and the active state with 13-cis retinal (PDB 6RPH), which represents the subsequent N- and O-intermediates in the photocycle.\(^{23}\) In this case, the crystal structures already include hydrogen atoms, which we use for further contact analysis. For Rho, we consider the inactive dark state (PDB 1U19), where the retinal is bound in the cis state, and the Meta II state (PDB 3PQR), with the isomerized retinal in the all-trans state still bound to the protein. The results for \(\beta_2\)AR rely on crystal structures of the inactive\(^{55}\) (PDB 2RH1) and the active state\(^{57}\) (PDB 3P0G) with the docked native ligand adrenaline. To verify that docking a ligand is a viable approach for studying energy transmission in proteins, we additionally considered a crystal structure of an active \(\beta_2\)AR-adrenaline complex\(^{64}\) (PDB 4LDO). Missing hydrogen atoms are added to protein crystal structures with PROPKA3\(^{64,65}\) for a pH of 7 using the PDB2PQR web server.\(^{66,67}\) The missing intracellular loop 3 in the \(\beta_2\)AR was recovered via the SWISS-MODEL web server.\(^{68}\) And the native ligand adrenaline was added via docking using Autodock Vina.\(^{69}\) T4 lysozyme fusion proteins and active state stabilizing peptides or proteins were removed.

To handle the structural protein information, we use MDAnalysis\(^{70,71}\)

For MD simulations of \(\beta_2\)AR, the same structure preparation protocol was employed. In the case of the active receptor, we employed PDB structure 4LDO with the nanobody present to stabilize the receptor. The complex was embedded into a POPC lipid bilayer surrounded by water using INFLATEGRO2.\(^{72}\)

A. Molecular Dynamics Simulations

Atomic interactions were described by the Amber99SB\(^{73}\) force field combined with the Berger lipid force field,\(^{75,76}\) and the TIP3P water model.\(^{77}\) Adrenaline parameters were generated using Antechamber\(^{78}\) and Acype\(^{79}\) with atomic parameters derived from GAFF parameters\(^{80}\) and AM1-BCC atomic charges.\(^{81,82}\)

Simulations were carried out using Gromacs v2018 (Ref. 83) in a CPU/GPU hybrid implementation. Van der Waals interactions were calculated with a cut-off of 1 nm, electrostatic interactions using the particle mesh Ewald method\(^{84}\) with a minimal real-space cut-off of 1 nm. All covalent bonds with hydrogen atoms were constrained using LINCS.\(^{85}\) After an initial steepest descent minimization with positional restraints of protein and ligand and heavy atoms, an initial 10 ns equilibration MD simulation in the NPT ensemble was performed with a 2 fs time step and positional restraints of protein and ligand.
Heavy atoms. A temperature of 300 K was kept constant using the Nosé-Hoover thermostat\textsuperscript{86,87} (coupling time constant of 0.2 ps), the pressure was kept constant at 1 bar using the Berendsen barostat\textsuperscript{88} (coupling time constant of 0.5 ps) with semi-isotropic pressure coupling, followed by a second steepest descent minimisation without restraints and a short 0.1 ns equilibration MD simulation in the NPT ensemble. Then, 120 ns of free MD simulations were carried out, switching the barostat to the Parrinello-Rahman barostat.\textsuperscript{89} Snapshots were collected each 20 ns, resulting in a total of 7 structures.

**Heating Process and Visualization**

Performing extensive nonequilibrium MD simulations of the energy flow in proteins, Gulzar et al.\textsuperscript{43} approximated the initial photoexcitation by an instantaneous temperature jump, where the resulting excess energy $\Delta T$ of the chromophore is chosen to match the $S_0 \rightarrow S_1$ excitation energy of $\approx 2$ eV, resulting in $\Delta T \approx 600$ K. In the master equation simulations of the energy flow, we use this temperature change as initial condition at time $t = 0$ to define the initial energy of the chosen heater system (i.e., retinal for bR and Rho and the ligand for $\beta_2$AR). We note that this choice does only affect the observed temperature scale but not the energy transfer dynamics of the master equation.

Solving the master equation (1) iteratively, we obtain the time evolution of the energy of every residue of the protein. To illustrate the energy transport in a more visual manner, we utilize these temperature curves to color the residues for each time step using VMD.\textsuperscript{90} This visualization allows us to watch the energy distribution in form of a movie and choose suitable time frames for representation. In the end, the energy distributes until equilibrium is reached and the equipartition theorem is fulfilled.

**RESULTS**

**Bacteriorhodopsin**

Starting with bR, we find 34 contacts for the inactive state and 32 for the active state, which we list in Tab. S1. To analyze how the system deals with the energy of the absorbed photons, we heat the retinal chromophore, which is covalently bound to Lys216 in TM7.

After computing all contact and backbone rates, we group them according to the involved secondary structures. The resulting secondary structure contact rates can be found in Tab. I and Fig. 2. The rates provide a rough picture of the connectivity between the different parts of the protein. The two states mostly agree in connectivity and absolute value of the rates. Though the largest structural difference between both states is the outward motion of helices E and F\textsuperscript{59} (here TM5 and TM6), the largest differences in the rates are found for helix G (here TM7). This is most likely due to the weakening of the Tyr185-Asp212 hydrogen bond, which elongates from 2.6 Å in the dark state to 3.1 Å in the active state. In general, there seem to be hardly any noticeable changes in the energy transport upon the activation of the protein. The main reason for this is that most contacts within the core of bR are hydrophobic and only a handful of contacts break during the photocycle. Furthermore, TM7 exhibits only two polar contacts via Asp204 and Asp212, hence energy transport via the backbone chain should dominate in both investigated structures.

Figure 3 shows both states of the system at 10 ps after heating the ligand impulsively (see Methods). As discussed above, the energy transport in the two states is very similar in behavior. We can observe that the energy flows through the covalent bond into TM7 and spreads in both directions from there. Additionally, TM3 and TM2 obtain small amounts of energy through contact...
transport. However, as presumed, backbone transport strongly dominates. We note that the C-terminus of TM7 quickly receives a large amount of kinetic energy. In bR crystal structures, the 16 amino acid C-terminus is not resolved, and forms a random coil\textsuperscript{91} that is embedded in the intracellular water bulk. Its removal does not affect the protein’s folding and function as a proton pump,\textsuperscript{92} but has been linked to protein stability.\textsuperscript{93} Naturally, these amino acids will exhibit contacts with water molecules, and form an efficient position for dissipation of excess energy into the solvent. Figuratively speaking, the TM7 helix seems to act like a ”lightning rod” providing a direct energy transfer path to the C-terminus, which forms a ”grounding rod” to effectively dissipate excess energy into the solvent.

**Rhodopsin**

We now turn to the analysis of energy transfer in Rho. We find 51 contacts between different residues for the dark state and 50 for the Meta II state, which are listed in Tab. S2. The secondary structure rates can be found in Tab. II with an illustration in Fig. 4. The inactive state contains slightly more internal connections and thus seems to be better at dissipating energy. Especially the two helices TM1 and TM7 are strongly connected with a large contact rate. In contrast to this, the active state appears to be more isolated, as many of the contacts break through changes in the structure upon activation.

As before we heat the retinal and observe how the energy distributes from there. Fig. 5 shows the two states of rhodopsin 10 ps after the heating process. The energy flows into TM7 through the covalent bond and quickly spreads in both directions along the backbone. However, the energy does not reach further than the ends of this helix. In the inactive state, we can additionally observe contact transport from TM7 into TM1 and TM3, even though the energy flow towards TM3 seems negligible. The main path for this energy transfer is the salt bridge between the protonated Schiff base of the retinal and Glu113, which is a major structural feature to stabilize the dark state.\textsuperscript{94} In the active state, on the other hand, no noticeable contact transport can be observed. This agrees with the lower connectivity we deduced from the rates.

In comparison with bR, we note that the excess energy is not quickly transferred to solvent-exposed unstructured domains, but remain in the core of the transmembrane helix bundle. In Rho, the C-terminus serves as important structural feature for signal transduction.\textsuperscript{95}

### Table II. Rates between the different secondary structures in Rho for both states. The rates are given in ps$^{-1}$.

| Sec. Struct. | Rates |
|--------------|-------|
| Sec. 1       | Sec. 2 | Inactive | Active |
| N-Term TM2   | 0.10   |          |        |
| N-Term ECL1  | 0.30   | 0.11     |        |
| N-Term ECL2  | 0.21   |          |        |
| N-Term ECL3  | 0.16   |          |        |
| TM1 TM2      | 0.16   | 0.25     |        |
| TM1 TM7      | 0.41   | 0.15     |        |
| TM2 TM4      | 0.22   | 0.13     |        |
| TM2 ECL2     | 0.14   | 0.15     |        |
| TM3 ECL2     | 0.14   | 0.15     |        |
| TM3 TM5      | 0.15   |          |        |
| TM3 TM6      | 0.18   |          |        |
| TM3 TM7      | 0.11   |          |        |
| TM4 TM5      | 0.35   | 0.49     |        |
| ECL2 TM6     | 0.17   | 0.14     |        |
| TM5 TM6      | 0.39   |          |        |
| TM6 H8       | 0.10   |          |        |
| TM7 H8       | 0.17   |          |        |

\textsuperscript{95} The C-terminus of the Rho protein is an important structural feature for signal transduction.
and thus cannot serve as "grounding rod" as in bR. Instead, it appears that excess energy is distributed into the whole protein, and then slowly released from there to its environment. We notice that the salt bridge between the Schiff base and Glu113 ruptures only after retinal isomerization,20 i.e., after the decay back into the retinal electronic ground state. Therefore, this salt bridge can indeed serve as energy dissipation channel. We finally note that the all-trans retinal in the active state still can absorb photons, which do not contribute to protein function anymore. Hydrolysis of the Schiff base on the formation of opsin may therefore not only serve for the reloading with 11-cis retinal, but also for the protection of rhodopsin from excess photon energy.

It has been shown that in both bR and Rho, protein-internal water molecules are essential for protein function and connect distant amino acid side chains to each other via hydrogen bonds. We therefore tested for the example of Rho how much the treatment of water molecules observed in crystal structures as contact residues would contribute to energy transfer from the retinal to the protein. As shown in Tab. S3, we found a significant number of contacts with internal water molecules (37 in the inactive and 17 in the active state), however, the respective contact rates are mostly small. Hence, these water molecules represent dead ends for the energy transfer and do not significantly change the time-dependent energy distribution (Fig. S1). Protein-internal water molecules therefore are negligible for the overall energy transport in Rho.

β2 adrenergic receptor

Finally, we explore if the lessons learned from energy transfer in bR and Rho can be transferred to the energy transport in β2AR. As stated before, β2AR binds a ligand by forming polar and nonpolar contacts. In contrast to the other two systems, where the retinal was covalently bound to the protein and the energy entered the protein through backbone transport along those bonds, here the energy flows into the protein through contact transport. Moreover, both states form at least 2 ligand contacts and the energy consequently already reaches different parts of the protein directly after the heating. The contacts formed with the ligand are shown in Fig. 6 and listed in Tab. III. In both states, the ligand binds to TM3 via a salt bridge / hydrogen bond combination with Asp113 and to TM7 via a bidentate hydrogen bond with Asn312. Additionally, the inactive state forms another contact through TM5 via Ser203, however, only with a small rate.

TABLE III. Rates of the contacts formed between the ligand adrenaline and β2AR, given in ps−1. "Adr" denotes adrenaline.

| Residues | Rates |
|----------|-------|
| Res 1    | Res 2 | Inactive | Active |
| Adr Asp113 | 0.34 | 0.27 |
| Adr Ser203 | 0.12 |
| Adr Asn312 | 0.14 | 0.14 |

FIG. 6. Illustration of the contacts rates of β2AR for the inactive state (left) and the active state (right).

From the contact analysis of β2AR we find 61 contact rates in the inactive state and 50 in the active. This is a larger difference than in the two proteins before and already indicates that the protein has a higher connectivity before the activation. The contact rates are listed in Tab. S4. Considering the secondary structure rates displayed Fig. 7, the energy transport seems to completely change between the two states. A list of the rates can be found in in Tab. IV. Many of those changes involve TM5 and TM6, which are known for their outward movements when the protein transitions into the active state.57

In Fig. 8 we can find a snapshot of the protein 10 ps after heating the ligand. In both states, energy reaches TM3 and TM7 and travels in both directions along the backbone, respectively. In the active state, more energy reaches TM7. We can also see that some energy flows through the third contact in the inactive state to TM5, but only a minor amount compared to the other two he-
TABLE IV. Secondary structures in β2AR shown for both states. The rates are given in ps$^{-1}$.

| Sec. Struct. | Rates |
|--------------|-------|
| Sec 1 | Sec 2 | Inactive | Active |
| N-Term ECL1 | 0.25 |
| TM1 | TM7 | 0.11 | 0.17 |
| TM2 | TM3 | 0.39 | 0.40 |
| TM2 | ECL2 | 0.15 |
| TM2 | TM7 | 0.12 |
| ECL1 | ECL2 | 0.11 |
| TM3 | TM4 | 0.19 |
| TM3 | ECL2 | 0.71 | 0.11 |
| TM3 | TM5 | 0.17 |
| TM3 | TM7 | 0.18 | 0.28 |
| ICL2 | ICL3 | 0.18 |
| TM4 | TM5 | 0.14 |
| ECL2 | TM7 | 0.24 | 0.26 |
| TM5 | TM6 | 0.29 | 0.19 |
| TM6 | TM7 | 0.19 | 0.54 |
| TM6 | ICL4 | 0.13 |
| TM7 | H8 | 0.23 |

FIG. 7. The secondary structure contact rates of β2AR visualized in a snapshot of the protein through black arrows with an arrow line width is proportional to the rates.

Additionally, we calculated the energy transfer within a crystal structure of an active β2AR bound to adrenaline. The results are presented in Tabs. S5 to S7 and Fig. S2. Although we observe differences in some rates, especially a decrease of the transfer rate between adrenaline and Asp113 by a factor of ~2, the overall time course of energy transfer is very similar to our results with the docked ligand. As the hydrogen bond energy transfer forms the bottleneck step in vibrational energy transport in β2AR, the overall time course of the transfer is not affected as long as $D_B \gg D_C$. This observation supporting our approach to evaluate energy transport within β2AR based on docking adrenaline into two receptor structures that were not crystallized with this ligand.

Lastly, to check if a single crystal structure is sufficient to predict the overall energy flow, we compared to energy transport results obtained for a set of snapshots from MD simulations (see Methods). Tables S8 to S10 show that the number of contacts increases by a factor of ~3, i.e., 141 (active) and 158 (inactive receptor) contacts in MD simulations vs. 51 (active) and 66 (inactive receptor) contacts in the crystal structure. As the majority shows only weak couplings, however, the additional contacts do not cause a significant change of the time evolution of the energy distribution (Fig. S3). At least in the case of 7TM proteins, using a single crystal structure seems sufficient to reflect the overall vibrational energy transfer.

**DISCUSSION AND CONCLUSION**

We have presented a computational approach that allows us to readily compute the energy transport in any protein. As input we solely need a protein structure, which e.g. can be deduced from crystal structural data. Hence our master equation model may account for potential structural heterogeneity only through an average over various structures. We note that heterogeneous averaging should be appropriate, because of the timescale separation between the fast (ps) energy transport and the slow (ns to ms) conformational dynamics. Moreover, the scaling rules rest on the assumption of a diffusive energy flow, which is expected to be valid for a protein at room temperature. Finally, we have focused on backbone and contacts transport between standard residues, and have not specifically parameterized specific cofactors, such as chromophores. This should be sufficient, when we are interested in the overall energy flow of the protein, which is the goal of this paper. The model needs to be extended, when we want to compare to a specific experiment as in Ref. 10.
We applied the method to three different systems, bR, Rho and β2AR, all from the group of heptahelical transmembrane proteins. The first two are photoreceptors, which are activated by the absorption of an incoming photon, and are especially interesting to consider as they need to dissipate the energy of the absorbed light quantum. Even though the three systems appear similar in structure, their energy transport mechanisms differ vastly.

bR manages to quickly direct excess energy out of the protein and thus seems well suited to deal with the photon that it absorbs through the retinal. As the two investigated states are very similar in their secondary structure rates, they seem equally well suited for dissipating excess energy out of the protein. The TM7 helix seems to act like a “lightning rod” as it directs the energy out of the protein and into the solvent.

Rho, on the other hand, exhibits a rather poor energy dissipation: excess energy mostly spreads along the backbone within TM7, but does not quickly reach a part of the protein where it could dissipate into a surrounding medium. Moreover, despite still covalently binding the retinal cofactor, the active state seems less suited for quickly distributing energy, which might potentially lead to overheating by additionally absorbed photons. One may speculate that this problem is potentially solved by the hydrolysis of the Schiff base between protein and retinal and its subsequent dissociation.

We finally considered β2AR, which does not belong to the group of photoreceptive proteins. When heating the ligand, the energy flows into the protein via contacts with the ligand and is thus already much more distributed than in the other systems, where only one entry way into the main bulk of the protein is given. However, from these entry points, the energy does not reach far and is consequently not dissipated much in the protein. Because there is no photoexcitation present in this protein, and adrenaline binding introduces a negligible amount of energy into the protein, however, there is also no need for an evolutionary adaption for the protein for optimal energy dissipation.

Another reason to consider the energy flow in β2AR is that its energy transport pathways are believed to be linked to channels of allosteric communication.35 Allostery may be mediated by structural and dynamical changes of a protein, and we indeed observed structural differences between the inactive and active state of β2AR. These conformational changes are typically associated with changes of interresidue contacts, which stabilize the two states. Since the energy transport in a protein is also mediated by interresidue contacts, various authors have studied potential interrelations of the two processes.51 While it seems appealing to rationalize elusive allosteric transitions via simple visualizations of vibrational energy transport (as, e.g., shown in Figs. 3, 5 and 8), there are several limitations to this analogy.

First off, it should be stressed that energy transport and allostery constitute different physical processes. Energy transport reflects the instantaneous mechanical connectivity of the protein, and occurs therefore on a picosecond timescale. Allostery, on the other hand, is believed to reflect structural changes (e.g., changes of interresidue contacts or side chain dihedral angles) which typically take place on a micro- or millisecond timescale. Secondly, our scaling rule for contact transport [Eq. (3)] provides a direct relation between protein energy flow and polar contacts. Within this approximation, the contact energy transport only gives information about the contacts that is already known from the protein structure. Finally, nonpolar contacts are negligible for energy transport,44 but are clearly important for allostery. In the case of β2AR, for example, Fleetwood et al.101 recently established the importance of hydrophobic contacts forming microswitches. While the energy flow may highlight aspects of the interresidue couplings of a protein, it therefore seems not particularly suited to explain allospheric phenomena.

Supplementary Information
Ten Supplementary Tables detailing on energy transfer rates and three Supplementary Figures displaying the energy distribution in protein over time.

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# Supporting Information:
Energy Transport and its Function in heptahelical Proteins

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## I. SUPPLEMENTARY TABLES

Table S1. Contact between the different residues for the inactive and active state of bacteriorhodopsin. The rates are given in ps\(^{-1}\). According to convention, the residues start at ID 5.

| Residues | Rates |
|----------|-------|
|          | Inactive | Active |
| Res 1 | Res 2 |          |          |
| Arg7   | Met60   | 0.13     | 0.11     |
| Arg7   | Leu61   | 0.14     |          |
| Glu9   | Tyr79   | 0.26     | 0.16     |
| Leu13  | Thr17   | 0.17     | 0.17     |
| Met20  | Thr24   | 0.15     | 0.18     |
| Asp36  | Gly231  | 0.09     |          |
| Phe42  | Thr46   |          | 0.15     |
| Thr46  | Asp96   | 0.16     |          |
| Tyr57  | Asp212  | 0.16     | 0.15     |
| Ser59  | Tyr64   | 0.26     |          |
| Ser59  | Gly65   | 0.10     | 0.09     |
| Gly65  | Ala81   | 0.14     | 0.10     |
| Thr67  | Ile78   | 0.40     | 0.35     |
| Val69  | Asn76   | 0.28     | 0.11     |
| Phe71  | Asn76   | 0.08     |          |
| Asp85  | Thr89   | 0.20     |          |
| Trp86  | Thr90   | 0.14     | 0.18     |
| Trp86  | Asp212  |          | 0.11     |
| Ala98  | Ala103  | 0.16     | 0.12     |
| Asp102 | Lys159  | 0.21     |          |
| Ile117 | Thr121  | 0.15     | 0.16     |
| Met118 | Ser141  | 0.14     | 0.13     |
| Val124 | Thr128  | 0.19     | 0.21     |
| Ala126 | Arg134  | 0.20     | 0.21     |
| Thr128 | Arg134  | 0.21     | 0.18     |
| Arg134 | Glu194  | 0.24     | 0.17     |
| Trp138 | Thr142  | 0.11     | 0.12     |
| Trp138 | Pro186  | 0.12     | 0.08     |
| Arg164 | Phe230  | 0.25     |          |
| Pro165 | Ser169  | 0.17     | 0.14     |
| Glu166 | Thr170  | 0.16     |          |
| Leu174 | Thr178  | 0.15     |          |
| Val179 | Ser183  | 0.14     | 0.15     |
| Tyr185 | Asp212  | 0.15     | 0.10     |
| Leu190 | Ala196  | 0.14     | 0.14     |
| Ile191 | Val199  | 0.14     | 0.15     |
| Gly192 | Gly197  | 0.15     |          |
| Ser193 | Glu204  | 0.44     | 0.35     |
| Leu201 | Thr205  | 0.15     | 0.09     |
| Val210 | Ser214  | 0.16     |          |
Table S2. Contact rates between the different residues for the inactive and active state of rhodopsin. The rates are given in ps$^{-1}$. The residue IDs start at 1.

| Residues | Rates        |
|----------|--------------|
|          | Inactive | Active |
| Met1     | 0.08     |
| Asn2     | 0.16     |
| Thr4     | 0.42     | 0.16   |
| Thr4     | 0.25     | 0.26   |
| Pro7     | 0.21     |
| Asn15    | 0.20     |
| Arg21    | 0.16     | 0.12   |
| Ser22    | 0.13     | 0.12   |
| Pro23    | 0.20     |
| Gln28    | 0.11     |
| Tyr29    | 0.11     | 0.10   |
| Pro34    | 0.11     | 0.11   |
| Tyr43    | 0.22     |
| Gly51    | 0.27     | 0.30   |
| Ile54    | 0.19     | 0.16   |
| Asn55    | 0.16     | 0.25   |
| Asn55    | 0.18     | 0.15   |
| Thr58    | 0.21     | 0.16   |
| Tyr60    | 0.19     |
| Val61    | 0.19     |
| Thr62    | 0.21     |
| Val63    | 0.23     |
| Leu68    | 0.19     |
| Asn78    | 0.22     | 0.13   |
| Val87    | 0.08     |
| Phe88    | 0.20     |
| Gly89    | 0.28     |
| Gly90    | 0.21     |
| Thr93    | 0.19     |
| Thr94    | 0.16     | 0.19   |
| Tyr96    | 0.15     |
| Thr97    | 0.11     |
| Thr97    | 0.11     |
| Thr97    | 0.27     |
| Gln113   | 0.14     | 0.15   |
| Gln113   | 0.11     |
| Gly114   | 0.28     | 0.35   |
| Gln122   | 0.15     |
Table S3. Rates of Rhodopsin with surrounding water molecules. In the inactive state, we find 37 contacts with water (yielding 28 contact rates). In the active state we only identify 17 water contacts (16 contact rates). The rates are given in ps⁻¹.

| Residues | Rates     | Residues | Rates     |
|----------|-----------|----------|-----------|
|          | Inactive  | Active   | Inactive  | Active   |
| Gly3     | 0.16      | 0.16     | Cys185    | 0.06     |
| Glu5     |           |          | Cys187    | 0.07     |
| Asn8     | 0.08      |          | Tyr191    | 0.04     |
| Tyr10    | 0.11      |          | Tyr192    | 0.08     |
| Pro12    | 0.08      |          | Thr193    | 0.07     |
| Ser14    | 0.09      | 0.15     | Glu196    | 0.10     |
| Pro34    | 0.11      |          | Thr229    | 0.10     |
| Glu64    | 0.06      |          | Glu236    | 0.07     |
| Asn73    | 0.10      |          | Glu239    | 0.09     |
| Gly89    | 0.20      |          | Glu249    | 0.05     |
| Ser98    | 0.11      |          | Met257    | 0.09      |
| Gly113   | 0.08      |          | Ala260    | 0.10     |
| Gly120   | 0.07      |          | Cys264    | 0.12     |
| Ala153   | 0.12      |          | Gly284    | 0.27     |
| Pro171   | 0.11      |          | Pro291    | 0.08     |
| Ser176   | 0.11      |          | Ser298    | 0.23      |
| Tyr178   | 0.15      |          | Asn302    | 0.08     |
| Pro180   | 0.14      |          | Cys323    | 0.05     |
| Glu181   | 0.15      | 0.15     | Asp331    | 0.04     |
|          |           |          | Glu332    | 0.11     |
Table S4. Contact rates for the inactive and active state of β2AR. The rates are given in ps\(^{-1}\). According to convention, the residue IDs start at 29.

| Residues | Rates  |
|----------|--------|
|          | Inactive | Active |
| Glu30    | Lys97    | 0.25   |
| Gly37    | Ser41    | 0.24   |
| Ile47    | Asn51    | 0.13   |
| Asn51    | Ser319   | 0.11   |
| Val52    | Thr56    | 0.21   |
| Ala57    | Phe61    | 0.25   |
| Ala59    | Gln65    | 0.21   |
| Leu64    | Asn69    | 0.17   |
| Thr68    | Asp130   | 0.21   |
| Asn69    | Thr73    | 0.11   |
| Asp79    | Ser120   | 0.16   |
| Asp79    | Ser319   | 0.12   |
| Val86    | Trp109   | 0.19   |
| His93    | Cys191   | 0.15   |
| Trp99    | Thr189   | 0.11   |
| Asn103   | Ala186   | 0.11   |
| Cys106   | Thr110   | 0.23   |
| Glu107   | Ser111   | 0.18   |
| Glu107   | Gln170   | 0.19   |
| Gly107   | Tyr174   | 0.30   |
| Asp113   | Tyr316   | 0.18   |
| Asp113   | Adr343   | 0.71   |
| Val114   | Thr118   | 0.19   |
| Cys116   | Ser120   | 0.23   |
| Ala119   | Thr123   | 0.26   |
| Glu122   | Val206   | 0.17   |
| Asp130   | Ser143   | 0.21   |
| Arg131   | Tyr141   | 0.15   |
| Arg131   | Gln142   | 0.16   |
| Tyr132   | Thr136   | 0.12   |
| Ala134   | Tyr141   | 0.17   |
| Lys140   | Gln243   | 0.18   |
| Lys147   | Arg151   | 0.17   |
| Val157   | Ser161   | 0.16   |
| Val160   | Thr164   | 0.20   |
| Ser161   | Ser165   | 0.20   |
| Thr164   | Tyr199   | 0.14   |
| Pro168   | Tyr174   | 0.20   |
| Trp173   | Asn196   | 0.16   |
| Tyr174   | Asn196   | 0.10   |
| Arg175   | Asn196   | 0.14   |
| Ala176   | Tyr185   | 0.34   |

Table S5. Rates of the contacts formed between the ligand adrenaline and β2AR for the 4LDO state and the 3P0G state. The rates are given in ps\(^{-1}\).

| Residues | Rates  |
|----------|--------|
|          | Inactive | Active |
| Ala176  | Phe194  | 0.18   |
| Gln179  | Asn183  | 0.14   |
| Tyr185  | Phe194  | 0.13   |
| Asp192  | Lys305  | 0.24   |
| Tyr199  | Ser203  | 0.15   |
| Ala200  | Ser204  | 0.17   |
| Ser203  | Asn343  | 0.29   |
| Tyr209  | Phe290  | 0.14   |
| Val216  | Ser220  | 0.21   |
| Tyr219  | Leu272  | 0.15   |
| Arg221  | Gln225  | 0.18   |
| Gln225  | Gln229  | 0.18   |
| Arg228  | Gln249  | 0.34   |
| Gln231  | Gln268  | 0.09   |
| Asp234  | Arg239  | 0.52   |
| Asp234  | Asp260  | 0.55   |
| Arg239  | His256  | 0.13   |
| Phe240  | Thr254  | 0.18   |
| His241  | Gly252  | 0.10   |
| Val242  | Gln250  | 0.17   |
| Gln243  | Gln249  | 0.13   |
| Asn244  | Val248  | 0.18   |
| Glu249  | Arg253  | 0.43   |
| Lys270  | Thr274  | 0.15   |
| Lys270  | Arg328  | 0.13   |
| Ile277  | Thr281  | 0.20   |
| Ile278  | Tyr286  | 0.24   |
| Met279  | Tyr283  | 0.22   |
| Trp286  | Asn318  | 0.09   |
| Phe289  | Asn293  | 0.16   |
| Asn293  | Tyr308  | 0.19   |
| Val295  | Gln299  | 0.14   |
| His296  | Asn301  | 0.12   |
| His296  | Ile303  | 0.09   |
| Asn312  | Asn343  | 0.27   |
| Gly315  | Ser319  | 0.11   |
| Tyr316  | Gly320  | 0.08   |
| Asn318  | Asn322  | 0.15   |
| Cys327  | Arg333  | 0.23   |
| Arg328  | Arg333  | 0.20   |
| Gln337  | Leu342  | 0.17   |
**Table S6.** Secondary structure rates for both active states, 4LDO (left) and 3P0G (right). The rates are given in ps$^{-1}$.

| Sec. Struct. | Rates |
|--------------|-------|
| N-Term ECL1  | 0.25  |
| TM1 TM7      | 0.11  |
| ICL1 H8      | 0.09  |
| TM2 TM3      | 0.49  |
| TM3 TM4      | 0.19  |
| TM3 ECL2     | 1.03  |
| TM3 TM5      | 0.19  |
| TM3 TM7      | 0.24  |
| TM4 TM5      | 0.21  |
| TM5 TM6      | 0.12  |
| TM6 TM7      | 0.29  |

**Table S7.** Contact rates for 4LDO active state of $\beta_2$AR. The rates are given in ps$^{-1}$. According to convention, the residue IDs start at 29.

| Residues | Rates |
|----------|-------|
| Glu30 Lys97 | 0.25 |
| Gly37 Ser41 | 0.16  |
| Ile47 Asn51 | 0.15  |
| Asn51 Ser319 | 0.11  |
| Val52 Thr56 | 0.15  |
| Ala57 Phe61 | 0.25  |
| Arg63 Asp331 | 0.09  |
| Leu64 Asn69 | 0.15  |
| Thr68 Asp130 | 0.12  |
| Asn69 Thr73 | 0.15  |
| Asp79 Ser120 | 0.14  |
| Val86 Trp109 | 0.23  |
| Ala92 Met96 | 0.23  |
| Asn103 Ala186 | 0.11  |
| Asn103 Gln187 | 0.16  |
| Asn103 Glu188 | 0.22  |
| Cys106 Thr110 | 0.17  |
| Glu107 Ser111 | 0.12  |
| Glu107 Gln170 | 0.19  |
| Glu107 Tyr174 | 0.23  |
| Glu107 Arg175 | 0.42  |
| Asp113 Tyr316 | 0.24  |
| Asp113 Asn343 | 0.22  |
| Val114 Thr118 | 0.18  |
| Ala119 Thr123 | 0.19  |
| Glu122 Val206 | 0.09  |
| Asp130 Tyr141 | 0.22  |
| Tyr132 Thr136 | 0.13  |
| Tyr132 Glu225 | 0.09  |
| Leu144 Lys149 | 0.22  |
| Val157 Ser161 | 0.13  |
| Val160 Thr164 | 0.20  |
| Ser161 Ser165 | 0.18  |
| Thr164 Tyr199 | 0.21  |
| Pro168 Trp173 | 0.24  |
| Pro168 Tyr174 | 0.14  |
| Trp173 Asn196 | 0.09  |

| Residues | Rates |
|----------|-------|
| Tyr174 Asn196 | 0.09  |
| Arg175 Asn196 | 0.14  |
| Ala176 Phe194 | 0.18  |
| Gln179 Asn183 | 0.17  |
| Tyr185 Phe194 | 0.13  |
| Asp192 Lys305 | 0.26  |
| Tyr199 Ser203 | 0.15  |
| Ala200 Ser204 | 0.18  |
| Ser203 Ser207 | 0.19  |
| Tyr209 Phe290 | 0.12  |
| Val216 Ser220 | 0.21  |
| Arg221 Glu225 | 0.07  |
| Gln225 Gln229 | 0.22  |
| Gln231 Lys235 | 0.19  |
| Gln231 Glu268 | 0.09  |
| Asp234 Arg239 | 0.31  |
| Lys235 Ser262 | 0.08  |
| Ser236 Asp251 | 0.48  |
| Gln237 Arg253 | 0.20  |
| Gly238 Asn244 | 0.21  |
| Gln249 Arg253 | 0.43  |
| Gly255 Ser262 | 0.17  |
| Leu258 Ser262 | 0.07  |
| Lys270 Thr274 | 0.12  |
| Ile277 Thr281 | 0.11  |
| Ile278 Tyr326 | 0.24  |
| Met279 Thr283 | 0.20  |
| Trp286 Asn318 | 0.09  |
| Asn293 Tyr308 | 0.29  |
| Val295 Gln299 | 0.14  |
| His296 Asn301 | 0.08  |
| Asn312 Asn343 | 0.24  |
| Asn318 Asn322 | 0.22  |
| Asn322 Tyr326 | 0.19  |
| Arg328 Arg333 | 0.20  |
| Gln337 Leu342 | 0.14  |
Table S8. Contact rates between the different residues for the inactive and active state of β2AR based on 7 snapshots from the trajectory. The rates are given in ps⁻¹. The residue IDs start at 1.

| Res 1   | Res 2   | Residues | Rates   | Res 1   | Res 2   | Residues | Rates   | Res 1   | Res 2   | Residues | Rates   |
|---------|---------|----------|---------|---------|---------|----------|---------|---------|---------|----------|---------|
| Val33   | Gln37   | Asp133   | 0.02    | Val135  | Gln525  | Ser235   | 0.20    | Ser236  | Gln37   | Arg239   | 0.07    |
| Lys97   | Ser41   | Gly338   | 0.22    | Ser135  | Gln229  | Thr136   | 0.04    | Thr136  | Gln229  | Ser236   | 0.07    |
| Ser41   | Asn51   | Val52    | 0.15    | Asp331  | Asn333  | Tyr316   | 0.16    | Arg175  | Asn196  | Arg259   | 0.12    |
| Gly338  | Ser319  | Thr56    | 0.13    | Arg63   | Val157  | Lys140   | 0.10    | Ser143  | Lys149  | Arg239   | 0.07    |
| Asn333  | Val52   | Phe61    | 0.21    | Ala57   | Ser161  | Thr164   | 0.19    | Arg259  | Gln250  | Gly238   | 0.03    |
| Val52   | Cys327  | Ala58    | 0.30    | Ala58   | Ser165  | Arg151   | 0.05    | Arg259  | Gln250  | Arg239   | 0.07    |
| Ala58   | Arg333  | Glu56    | 0.03    | Arg63   | Thr199  | Val160   | 0.18    | Arg250  | Ser235  | Gly238   | 0.02    |
| Arg333  | Ser333  | Lys140   | 0.05    | Gly65   | Thr174  | Gln207   | 0.09    | Arg250  | Gln250  | Arg239   | 0.12    |

The rates are given in ps⁻¹.
Table S9. Rates of the contacts formed between the ligand adrenaline and β2AR for the active and the inactive state using 7 frames from the trajectory. The rates are given in ps$^{-1}$.

| Residues | Rates |
|----------|-------|
|          | Res 1 | Res 2 | Inactive | Active |
| Adr      | Asp113| 0.29  | 0.24     |
| Adr      | Ser203| 0.02  |          |
| Adr      | Asn312| 0.14  | 0.15     |

Table S10. Secondary structure rates for the active and the inactive state of β2AR based on 7 snapshot from the simulation trajectory. The rates are given in ps$^{-1}$.

| Sec. Struct. | Rates |
|--------------|-------|
|              | Sec 1 | Sec 2 | Inactive | Active |
| N-Term       | ECL1  | 0.04  |          |
| TM1          | TM7   | 0.13  | 0.12     |
| ICL1         | H8    | 0.14  | 0.15     |
| TM2          | TM3   | 0.40  | 0.31     |
| TM2          | TM4   | 0.07  |          |
| TM2          | ECL2  | 0.12  | 0.06     |
| TM2          | TM7   | 0.23  | 0.05     |
| ECL1         | ECL2  | 0.05  | 0.07     |
| ECL1         | TM7   | 0.05  |          |
| TM3          | TM4   | 0.02  | 0.03     |
| TM3          | ECL2  | 0.92  | 0.24     |
| TM3          | TM5   | 0.12  | 0.20     |
| TM3          | ICL3  | 0.01  | 0.02     |
| TM3          | TM7   | 0.28  | 0.26     |
| ICL2         | ICL3  | 0.05  |          |
| ICL2         | TM6   | 0.10  |          |
| TM4          | TM5   | 0.18  |          |
| ECL2         | TM6   | 0.02  |          |
| ECL2         | TM7   | 0.20  | 0.22     |
| TM5          | TM6   | 0.32  | 0.34     |
| TM6          | TM7   | 0.06  | 0.44     |
| TM6          | ICL4  | 0.02  |          |
| TM6          | H8    | 0.11  |          |
| TM7          | H8    | 0.18  |          |
II. SUPPLEMENTARY FIGURES

Figure S1. Rhodopsin shown in the inactive and active state including water molecules 10 ps after heating the retinal. The water molecules are depicted as spheres.

Figure S2. The two active states of $\beta_2AR$ (4LDO on the left and 3P0G on the right side) shown 10 ps after heating the ligand.
Figure S3. β2AR shown in the inactive and active state 10 ps after heating the ligand. The results are based on the contacts computed from 7 snapshots from the simulation trajectory.