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# Concentration effects on reactions in membranes: rhodopsin and transducin

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**The reaction rate of two laterally-diffusing species in a biological membrane shows a maximum at some concentration of reactants, because an increase in the concentration of reactants tends to increase the reaction rate by the law of mass action but decreases the diffusion rate of the reactants. The activation of transducin by rhodopsin in the disk membrane of the rod outer segment is described in terms of a steady-state diffusion model with concentration-dependent diffusion coefficients. The optimum concentrations of reactants are obtained from contour plots of the reaction rate as a function of rhodopsin and transducin concentrations, and the sensitivity of the results to the assumed values of the variables is examined. To determine whether the observed concentrations are in fact those yielding the maximum reaction rate, several variables must be known more accurately.**

## Introduction

Many processes in biological membranes involve the lateral diffusion, collision, and reaction of membrane-bound species. For example, the first steps in the action of insulin, epidermal growth factor, and nerve growth factor are the binding of hormone to its receptors, and the crosslinking of the mobile receptors. Electron transfer in chloroplasts, endoplasmic reticulum, and mitochondria is thought to require lateral diffusion of mobile redox carriers. In the retina, the first steps of the cascade leading to a nerve signal are the activation of rhodopsin by absorption of a photon, and the activation of transducin by rhodopsin [1–3].

At high concentrations, membrane proteins can obstruct diffusion significantly. An increase in the concentration of reactants therefore has two opposing effects. By the law of mass action, it increases the reaction rate, but it also decreases the reaction rate by lowering the diffusion coefficient of the reactants. The competition of these effects implies the existence of an optimum concentration of reactants giving the maximum reaction rate.

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We apply this argument to the activation of transducin by rhodopsin. The initial reactions of the cascade in the rod outer segment are as follows [4–21]:

$$R \xrightarrow{h\nu} R^* \quad (1a)$$

$$R^* + T \xrightarrow{k} R^* + T^* \quad (1b)$$

$$R^* + K \xrightarrow{\lambda} R + K \quad (1c)$$

Here R is rhodopsin; R* is light-activated rhodopsin (metarhodopsin II); T is transducin ($T_{\alpha\beta\gamma}$-GDP in the notation of Stryer; G-protein or $\Gamma$ in the notation of Liebman); T* is activated transducin ($T_\alpha$-GTP in the notation of Stryer); and K is the kinase deactivating rhodopsin. R and R* are integral proteins; T is a peripheral protein that releases a component on activation; and K is a soluble protein.

In the first reaction, absorption of a photon converts rhodopsin to the activated state. In the second reaction, each R* activates many transducin molecules, providing the first stage of amplification in the cascade. This reaction requires the binding of T to R*, the loss of GDP from T, the binding of GTP to T to form T*, and the dissociation of T* from R*. We assume that the concentration of GTP is high enough that the rate-determining step in Eqn. 1b is the encounter of R* with T. For our purposes, there is no need to consider the

rest of the cascade, in which a fragment of T* activates phosphodiesterase (PDE); PDE hydrolyzes cGMP to GMP, and the change in cGMP concentration turns off the sodium channels. The third reaction shown, the deactivation of R*, involves the phosphorylation of R* and the binding of arrestin to phosphorylated R* [22]. For simplicity, we treat these as a single reaction. The deactivation of T* by hydrolysis of GTP [23] is not treated explicitly.

In the case of the rhodopsin-transducin reaction, our argument is as follows. If the concentration of R is increased, more photons will be absorbed and more R* will be formed. But the increased concentration of R hinders the diffusion-controlled activation of T by R*. The competition of these effects leads to the existence of an optimum concentration of R and T, as proposed by Liebman et al. [16].

In this paper, we analyze the activation of transducin in terms of a steady-state diffusion model with concentration-dependent diffusion coefficients, evaluate the optimum concentrations of rhodopsin and transducin, examine the sensitivity of the results to the values of several variables, and identify the quantities which must be known more precisely to establish whether the observed concentrations are in fact optimal.

## Theory

*Model of transducin activation*

Our model of the activation of transducin is as follows. A rhodopsin at the origin of the coordinate system is activated to R* by absorption of a photon, and is deactivated to R at a rate $\lambda$. Transducin molecules diffuse to the R* molecule, with a diffusion coefficient equal to the sum of the diffusion coefficients of R and T, and are converted to T*. The steady-state concentration of T is maintained by the T* deactivation reaction. We restrict the model to a fraction of activated rhodopsin low enough that a steady state can be maintained with T essentially undepleted.

In a pure two-dimensional diffusion-controlled reaction, the rate constant is time-dependent, and no steady state exists [24–26]. We avoid this problem by using a steady-state model in which the reactants are replenished.

The total concentration of rhodopsin is [R]; the concentration of activated rhodopsin is $[R^*]=\phi[R]$, where $\phi$ is the fraction activated; and the total concentration of transducin is [T]. All concentrations [X] of membrane-bound species X are in molecules per area; this is converted to the area fraction $C_X$ using the area per molecule $a_X$.

Wofsy and Goldstein presented a model of trapping in coated pits [27]. To describe the activation of transducin, we generalize this model to allow for an arbitrary probability of reaction on collision. Consider steady-state diffusion in an annulus with a sink at a radius $r=s$ and a reflecting boundary at $r=b$. Here $s$ is the radius of a rhodopsin molecule, and $b$ is the radius of a circle containing an average of one R* molecule. Then

$$[R^*]=1/\pi b^2 \tag{2}$$

We sketch the derivation here; the detailed derivation follows Appendix III of Ref. 27 closely. The steady-state diffusion equation for the transducin concentration $C$ is

$$D\nabla^2 C+S-\lambda C=0 \tag{3}$$

where the first term represents diffusion of T, the second term represents the production of T by the deactivation reaction, and the third term represents the redistribution of T around R* due to the random activation and deactivation of rhodopsin molecules. This equation is solved subject to the boundary conditions

$$\left.\frac{dC}{dr}\right|_{r=b}=0 \tag{4a}$$

$$D\left.\frac{dC}{dr}\right|_{r=s}=\kappa C(s) \tag{4b}$$

The parameter $\kappa$ is a measure of the effectiveness of rhodopsin at activating transducin. If $\kappa\to 0$, the inner boundary is perfectly reflecting, and no reaction occurs. If $\kappa\to\infty$, the inner boundary is perfectly absorbing, and reaction always occurs. Wofsy and Goldstein [27] treated the latter case.

If some collisions between R* and T are unproductive, R* is not a perfect absorber, and $\kappa$ is finite. Furthermore, during the time it takes for the R*-T reaction to occur, the R* is unavailable for reaction with another T. If this time is significant, $\kappa$ is finite. The relevant quantity is the ratio of the lifetime of the R*-T complex to the mean time between collisions of T with R*. (See appendix.) The interpretation of $\kappa$ is discussed in Refs. 28–30, among others.

The rate constant is

$$k=\frac{2\pi sD}{\langle C\rangle}\left.\frac{dC}{dr}\right|_{r=s} \tag{5}$$

where $\langle C\rangle$ is the average concentration of transducin in annulus. Then it can be shown that

$$k=\frac{\pi b^2\lambda f}{1-f} \tag{6}$$

where

$$f=\frac{2s\Sigma_1}{(b^2-s^2)\alpha[\Delta_{10}+Da\Sigma_1/\kappa]} \tag{7a}$$

$$\Sigma_1 = I_1(ab)K_1(as) - K_1(ab)I_1(as) \tag{7b}$$

$$\Delta_{10} = I_1(ab)K_0(as) + K_1(ab)I_0(as) \tag{7c}$$

$$\alpha = \sqrt{\lambda/D} \tag{7d}$$

and $I_n$ and $K_n$ are $n$th-order modified Bessel functions. In the limit as $\kappa \to \infty$, Eqn. 7a reduces to the expression of Wofsy and Goldstein [27].

The fraction of T activated during the lifetime $1/\lambda$ of an activated rhodopsin is $f$ [27]. The distance diffused by a transducin molecule during that time is $2/\alpha$. The mean time for activation of a T molecule at a random position in the annulus is

$$\tau = 1/k[\mathrm{R}^*] \tag{8}$$

The rate of activation of transducin is

$$\frac{\mathrm{d}[\mathrm{T}^*]}{\mathrm{d}t} = k[\mathrm{R}^*][\mathrm{T}] \tag{9}$$

To maintain a steady state, this must be balanced by the deactivation reaction.

*Concentration dependence of the diffusion coefficient*

Next, we consider the dependence of the lateral diffusion rate on the concentration of protein. The diffusion coefficient decreases monotonically with the area fraction of protein, as shown experimentally [31,32] and by Monte Carlo calculations of random walks of hexagons on a triangular lattice [33,34]. Both integral and peripheral proteins must be included in the area fraction because the integral proteins protrude from the membrane far enough to collide with the peripheral proteins.

Both rhodopsin and transducin are mobile. Transducin remains bound to the membrane, though; it does not move by 'hopping' into the aqueous phase, diffusing there, and then rebinding to the membrane [35]. For simplicity, we assume that rhodopsin is cylindrical. Then transducin encounters the same area fraction of obstacles as rhodopsin does, and its diffusion coefficient displays the same concentration dependence *.

The diffusion coefficient is therefore

$$D(C_{\mathrm{tot}}) = D_0 D^*(C_{\mathrm{tot}}) \tag{10}$$

where

$$D_0 = D_0(\mathrm{R}) + D_0(\mathrm{T}) \tag{11}$$

* This assumption could be removed, as suggested by Liebman et al. [16]. One would simply define different values of $C_{\mathrm{tot}}$ for R and T, and replace Eqns. 10 and 11 with

$$D(C_{\mathrm{tot}}) = D_0(\mathrm{R})D^*(C_{\mathrm{tot\,R}}) + D_0(\mathrm{T})D^*(C_{\mathrm{tot\,T}})$$

In view of the insensitivity of our results to the form of $D^*$, we use Eqns. 10 and 11.

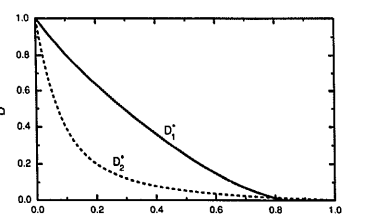


Fig. 1. Diffusion coefficients $D_1^*(C_{\mathrm{tot}})$ and $D_2^*(C_{\mathrm{tot}})$ as a function of the area fraction $C_{\mathrm{tot}}$ of protein. For native membrane, $C_{\mathrm{tot}} = 0.32$ under the assumptions stated in the text.

$D_0(\mathrm{R})$ and $D_0(\mathrm{T})$ are the diffusion coefficients of rhodopsin and transducin at infinite dilution, $D^*(C_{\mathrm{tot}})$ is the normalized concentration-dependent diffusion coefficient, and $C_{\mathrm{tot}}$ is the sum of the area fractions of rhodopsin, transducin, and PDE. We neglect the small area fractions of other integral and peripheral proteins.

The diffusion coefficients of rhodopsin and transducin have not been measured as a function of concentration, so we use two approximate expressions for $D^*(C_{\mathrm{tot}})$, as shown in Fig. 1. The first, $D_1^*$ (Eqn. 12 of Ref. 34), was calculated for hexagons of radius 1–4 lattice constants on a triangular lattice. For a lattice constant of 0.8 nm, as appropriate for a lipid bilayer, the radius of rhodopsin is 1.6 lattice constants (Eqn. 9 of Ref. 34). This expression for $D^*$ takes into account only the hard-core interaction of the diffusing particles. In the two systems for which experimental data are available, bacteriorhodopsin [31] and gramicidin [32], the observed concentration dependence is stronger than that given by $D_1^*$ [36], so we also use an expression $D_2^*$ roughly approximating these results (Eqns. 4–6 of Ref. 34, with $\gamma = 1$, $\alpha = 0.9$). Under physiological conditions, $C_{\mathrm{tot}} \approx 0.32$, as discussed later, so the diffusion coefficient is lowered significantly: $D_1^* = 0.46$, and $D_2^* = 0.11$.

In the calculations, we specify the area fractions of rhodopsin and transducin, and calculate the concentration-dependent diffusion coefficient from Eqn. 10. We then obtain the parameter $\alpha$ from Eqn. 7d, yielding $f$ from Eqns. 7a–7c, and the rate from Eqns. 6 and 9.

*Parameters*

Molecular areas and molar ratios are summarized by Liebman et al. [16]. Electron microscopy of two-dimensional crystals gives the area of a rhodopsin molecule as 7.5 nm$^2$ [37]. The area of transducin is less well-established. The Stokes radius of transducin is 3.75 nm [38], giving a molecular area $a_{\mathrm{T}} = 44.2$ nm$^2$. If the radius of transducin from electron micrographs of transducin-

enriched disk membranes [39,40] is corrected for the thickness of the Pt/C layer using the radius of rhodopsin in those micrographs as a standard, a radius of 3.60 nm is obtained, in reasonable agreement with the Stokes radius. A lower limit is obtained by assuming that transducin is a sphere of partial specific volume 0.735 $cm^3 \cdot g^{-1}$ and mass 85.5 kDa, giving $a_T = 26.8\ nm^2$. An upper limit is obtained using the radius from the electron micrographs with no correction for the thickness of the Pt/C layer, so that $a_T = 78.5\ nm^2$. This value is also obtained if the transducin molecule is assumed to be an oblate ellipsoid of axial ratio 5:1 [16]. If PDE is assumed to be spherical, its area is 45.6 $nm^2$.

Following Liebman et al. [16], we take the molar ratio of transducin to rhodopsin to be 0.1, and the ratio of PDE to transducin to be 0.17. The physiological concentration of rhodopsin is $[R] = 25\,000\ \mu m^{-2}$, so the area fraction $C_R$ is 0.19. The area fraction $C_T$ of transducin is 0.11 for $a_T = 44.2\ nm^2$, the area fraction $C_{PDE}$ of phosphodiesterase is 0.02, and $C_{tot} = 0.32$ in native membrane.

The radius of a rhodopsin molecule is 1.55 nm, and the average distance between rhodopsin molecules in native membrane is 6.8 nm. We assume a low fraction of rhodopsin bleached: $\phi = 10^{-5}$. With this value, the average distance $2b$ between excited rhodopsin molecules is 2300 nm.

As mentioned earlier, a limit on the value of $\phi$ is set by the assumption of steady-state kinetics. The time for the R*-T reaction is 1 ms [6] or less, and the time for the deactivation of T* is 30 s [6], so we require $\phi < 3 \cdot 10^{-5}$ to allow deactivation to keep up with activation. Values of $\phi$ of this magnitude are often used in kinetics measurements (e.g., Ref. 41).

The diffusion coefficient $D_0$ is the sum of the diffusion coefficients of rhodopsin and transducin at infinite dilution. Measured values for rhodopsin in frog rod outer segment are 0.35–0.55 $\mu m^2 \cdot s^{-1}$ [42,43]. Extrapolation to infinite dilution using the formula for $D_1^*$ gives $D_0(R) = 0.80 - 1.2\ \mu m^2 \cdot s^{-1}$; extrapolation using $D_2^*$ gives a value of $3 - 5\ \mu m^2 \cdot s^{-1}$.

The diffusion coefficient of membrane-bound transducin has not been measured, but values are available for other peripheral proteins. For the apolipoprotein ApoC-III in dipalmitoylphosphatidylcholine, ApoC-III in egg phosphatidylcholine, spectrin in dimyristoylphosphatidylcholine, and erythrocyte band 4.1 in dimyristoylphosphatidylcholine at 34–36 °C, values are 5.4–6.9 $\mu m^2 \cdot s^{-1}$ [44]. Band 4.1 is a peripheral protein of mass 80 kDa, binding to glycophorin [45], and the mass of transducin is 85.5 kDa, so the diffusion coefficient of band 4.1 is a plausible approximation for that of transducin.

We choose $D_0(R) = 1\ \mu m^2 \cdot s^{-1}$ and $D_0(T) = 6\ \mu m^2 \cdot s^{-1}$, so that $D_0 = 7\ \mu m^2 \cdot s^{-1}$. Note that the contribution from transducin is more important than that from rhodopsin. With the values we assume, $D_0(R)$ is only one-seventh of $D_0$. If we use the values for rhodopsin reconstituted in dimyristoylphosphatidylcholine, 3.3 $\mu m^2 \cdot s^{-1}$ [46] and band 4.1 in dimyristoylphosphatidylcholine, 6.2 $\mu m^2 \cdot s^{-1}$ [47], $D_0(R)$ is one-third of $D_0$. If we take the value for rhodopsin to be the highest extrapolated value and the value of transducin to be the lowest value given for a peripheral protein, $D_0(R)$ is one-half of $D_0$. So if rhodopsin were completely immobilized, as in invertebrate retinas [48,49], $D_0$ would decrease by one-sixth to one-half.

The measurements of Sitaramayya and Liebman [50] give a rate constant $\lambda$ for deactivation of R* of 1 $s^{-1}$ or faster. We choose a value of 20 $s^{-1}$ so that at the physiological concentrations of R and T, approximately 500 T are activated per R*, as observed experimentally [41,51]. (Higher rates have been observed [52]. The method of preparation of rod outer segments affects the concentrations of soluble species [53] and therefore the reaction rates.)

The values assumed for the variables are summarized in Table I; these values are used in all calculations unless stated otherwise.

TABLE I
*Assumed values of variables*

| | | |
|---|---|---|
| $a_R$ | 7.5 $nm^2$ | Molecular area of rhodopsin |
| [R] | 25000 $\mu m^{-2}$ | Concentration of rhodopsin |
| $C_R$ | 0.19 | Area fraction of rhodopsin |
| $a_T$ | 44.2 $nm^2$ | Molecular area of transducin |
| [T] | 2500 $\mu m^{-2}$ | Concentration of transducin |
| $C_T$ | 0.11 | Area fraction of transducin |
| $C_{PDE}$ | 0.02 | Area fraction of phosphodiesterase |
| $\phi$ | $10^{-5}$ | Fraction of rhodopsin activated |
| $D_0$ | 7 $\mu m^2 \cdot s^{-1}$ | Diffusion coefficient at infinite dilution (Eqn. 11) |
| $D^*$ | $D_1^*$ | Concentration-dependent diffusion coefficient (Eqn. 10) |
| $\kappa$ | $\infty\ \mu m \cdot s^{-1}$ | Microscopic reaction rate |
| $\lambda$ | 20 $s^{-1}$ | Deactivation rate of R* |

## Results and Discussion

### *Rate as a function of rhodopsin concentration at fixed transducin concentration*

The rate of transducin activation is shown in Fig. 2 for a fixed transducin concentration $C_T = 0.11$. The maximum rate is at an area fraction of rhodopsin of 0.30; the observed value of 0.19 gives a reaction rate of 87% of the maximum. The actual value of $C_R$ is expected to be lower than the calculated value to allow for minor integral and peripheral proteins [53] and the exclusion of rhodopsin from the rim of the disk [40].

Numerical results for the standard conditions of Table I are as follows. For $\phi = 10^{-5}$, $[R^*] = 0.25\ \mu m^{-2}$

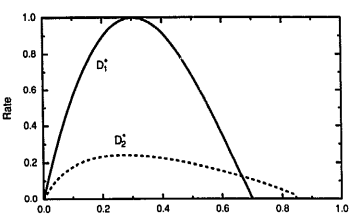


Fig. 2. Reaction rate as a function of the area fraction of rhodopsin for both expressions for $D^*$. Rates are normalized to the maximum value for $D_1^*$. The area fraction of transducin is fixed at 0.11.

and $[T] = 2500\ \mu m^{-2}$. We have $C_{tot} = 0.32$, so $D^* = 0.46$. The deactivation rate $\lambda = 20\ s^{-1}$, so the lifetime of R* is 50 ms. The rate is $d[T^*]/dt = 2360\ \mu m^{-2} \cdot s^{-1}$, and $k = 3.75\ \mu m^2 \cdot s^{-1}$. From Eqn. 8, the mean time for activation of a T molecule at a random position in the annulus is $\tau = 1.1$ s. There are 10 000 molecules of T per molecule of R*, and the fraction of T activated is $f = 0.045$, so each R* activates 450 T molecules, as required. The time for the activation of one T is then 50 ms/450 = 0.1 ms. Since $\kappa$ is assumed to be infinite, each collision of a T with R* leads to activation, so the mean time between collisions is also 0.1 ms. For this choice of parameters to be consistent, the lifetime of the R*-T complex must be no more than 0.1 ms.

Were we to take $\lambda = 1\ s^{-1}$, each R* would activate 4 700 transducins for infinite $\kappa$. We would then have to take $\kappa = 25\ \mu m \cdot s^{-1}$ to reduce the number of transducins activated per R* to 540. Such a value of $\kappa$ would imply that many collisions are needed for activation. Thus, with a consistent set of experimental rates (ideally, with all rates measured on the same preparation), it would be possible to determine the degree of diffusion control of the reaction.

The shape of the curve in Fig. 2 is insensitive to the choice of $D_0$; doubling or halving the value does not change the shape of the curve, but the maximum rate varies. approximately in proportion to $D_0$. (So an invertebrate receptor with immobile rhodopsin would have the same optimum as a vertebrate receptor with mobile rhodopsin, but the maximum rate would be lower.) Using $D_2^*$ instead of $D_1^*$ broadens the maximum and shifts its position slightly, as shown in Fig. 2.

If the molecular area of transducin is varied from the lower limit of 26.4 nm$^2$ to the standard value of 44.2 nm$^2$ to the upper limit of 78.5 nm$^2$, the position of the maximum shifts from $C_R = 0.32$ to 0.30 to 0.27.

If $\kappa$ is finite, the maximum shifts to much higher values: $C_R = 0.66$ for $\kappa = 1$, 0.59 for $\kappa = 10$, 0.45 for $\kappa = 100$, and 0.34 for $\kappa = 1\,000$.

If factors later in the cascade constrain the transducin concentration to the observed value, our results show that the observed rhodopsin concentration gives a rate near the maximum value. Other factors in the rod outer segment are arranged to give a high reaction rate [16,19,20]. For example, the photoreaction is an isomerization; there are several T* molecules per PDE, weakly associated with PDE; and the reaction rate of PDE is high enough to approach the diffusion limit.

The existence of an optimum rhodopsin concentration may explain the observation that the concentration of visual pigment in rod outer segments is approximately constant among species [54,55], but the length of the rods varies according to the environment of the species [54]. For example, in humans the length is 28 $\mu$m, and in frogs, 50 $\mu$m, while in some deep-sea fishes, the length may be as much as 600 $\mu$m [54,56].

In comparisons among species, the amount of visual pigment is usually given as the absorptivity (absorbance/unit length). Our model suggests that the amount of visual pigment per unit area is constant (see Ref. 57), but the absorptivities may still vary if the visual pigments of different species have different molar

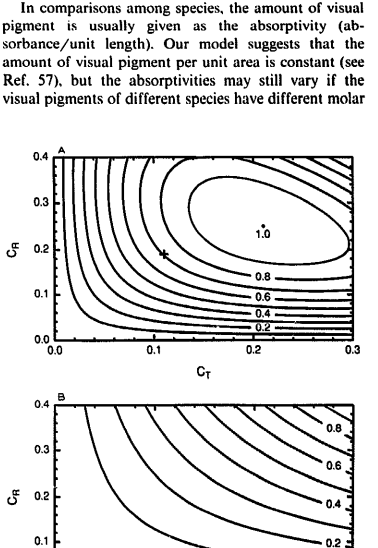


Fig. 3. Contour plot of the normalized reaction rate as a function of the area fractions of rhodopsin $C_R$ and transducin $C_T$. The observed values of $C_R$ and $C_T$ are indicated by the cross. (A) $D^* = D_1^*$. The value at the maximum is 3350 $\mu m^{-2} \cdot s^{-1}$. (B) $D^* = 1$. The rate simply increases with reactant concentrations.

absorption coefficients. The maximum in Fig. 2 is broad, so that values of $C_R$ between 0.20 and 0.41 give activation rates at least 90% of the maximum.

Our results suggest the possibility of visual disorders involving the regulation of protein synthesis.

*Rate as a function of the concentrations of rhodopsin and transducin*

Is it possible to obtain a faster response by allowing the concentrations of both rhodopsin and transducin to vary? Fig. 3A is a contour plot of the activation rate of transducin as a function of the area fractions of rhodopsin and transducin for $\kappa \to \infty$. The rates are normalized to one at the maximum. The rate is a maximum at $C_R = 0.25$, $C_T = 0.21$. At the observed area fractions of $C_R = 0.19$, $C_T = 0.11$, the rate is 71% of the maximum.

For a nontrivial optimum to exist, the diffusion coefficient must decrease with concentration. If the diffusion coefficient is constant, the rate simply increases with the concentrations of both reactants, as shown in Fig. 3B.

The form of the contour plot is insensitive to the value of the diffusion coefficient. Doubling or halving $D_0$ does not change the shape of the curves significantly, but the maximum rate changes, approximately in proportion to $D_0$. Using $D_2^*$ instead of $D_1^*$ shifts the position of the maximum slightly, to $C_R = 0.28$ and $C_T = 0.235$.

The contours are likewise insensitive to the deactivation rate of R* and to the value of $\phi$. Changing the value of $\lambda$ from 2 $s^{-1}$ to 20 $s^{-1}$ to 200 $s^{-1}$ changes the shape of the contour plot slightly and increases the maximum rate by one-third. Changing $\phi$ from $10^{-6}$ to $10^{-5}$ to $10^{-4}$ changes the shape of the contour slightly, and increases the maximum rate, approximately in proportion to $\phi$.

The contours are, however, sensitive to the probability of reaction when R* and T collide. So far, we have assumed that $\kappa \to \infty$, giving unit probability of reaction. If smaller values of $\kappa$ are used, the position of the maximum shifts to higher rhodopsin concentrations, as shown in Fig. 4.

Varying the molecular area of transducin has a minor effect on the contour plots, but a major effect on the interpretation of the results. In Fig. 3A, if $a_T = 44.2$ nm$^2$, the observed concentrations of rhodopsin and transducin yield a rate 71% of the maximum. But if the

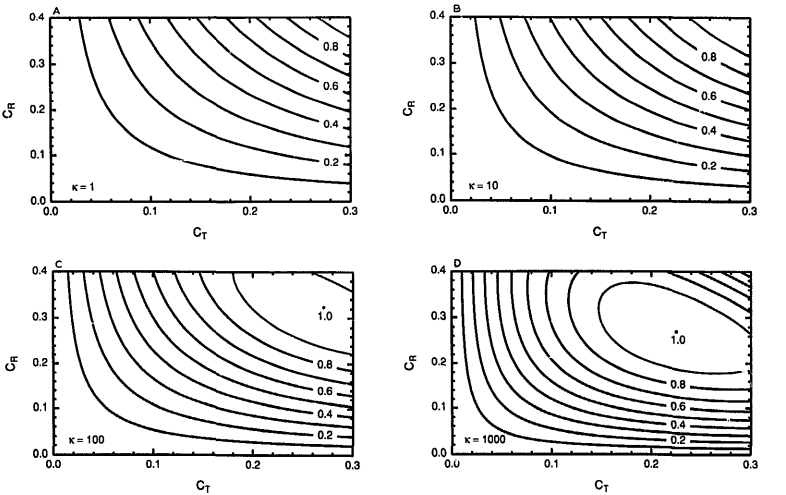


Fig. 4. Contour plots of the normalized reaction rate as a function of the area fractions of rhodopsin and transducin for the indicated values of $\kappa$. The contours for $\kappa = 1$ are close to those in Fig. 3B. As $\kappa$ increases, the maximum shifts to lower rhodopsin concentrations, and the contours approach those in Fig. 3A. If $\kappa \geq 10^5$, the contours are indistinguishable from those in Fig. 3A for $\kappa$ infinite.

area is equal to the upper limit $a_T = 78.5$ nm$^2$, then the observed value of [T] gives $C_T = 0.20$, and the area fractions fall close to the optimum, yielding a rate 93% of the maximum.

If the size of the transducin molecule is near the upper limit, and $\kappa \to \infty$, our results strongly support the conjecture of Liebman et al. [16] that the rhodopsin and transducin concentrations in the rod outer segment are the values yielding the maximum reaction rate.

If the size of transducin is smaller, the reaction rate falls below the maximum. In this case, our results suggest that if the visual cascade is adapted for speed, some other step in the cascade is rate-determining. Measurements of cytoplasmic diffusion [58,59] indicate that the rate-determining factor in the rod outer segment is diffusion in the cytoplasm, not diffusion in the membrane.

To establish which case is true, more information is needed: the shape of transducin, the probability of reaction on collision, and the diffusion coefficients of rhodopsin and transducin as a function of concentration in rod disk membranes.

The treatment presented here could be extended. We have assumed the simplest mechanism, but Wessling-Resnick and Johnson [60,61] have proposed mechanisms involving cooperative behavior, and Liebman et al. [16] have argued that T* remains bound to the membrane and forms a weak complex with PDE [62]. The reactions not treated here could be included, and the restriction to steady-state reactions could be removed. Ultimately, the entire cascade could be modeled [63]. We expect that these extensions will also show an optimum concentration of reactants.

We have assumed that the concentrations are optimized for speed of response at low light levels. It is entirely possible that nature may have carried out a more subtle optimization.

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**Appendix**

The order of magnitude of $\kappa$ can be estimated by a simple argument. Razi Naqvi et al. [28] present a lattice model of one-dimensional diffusion, and show that $\kappa/D = (1 - P)/lP$, where $l$ is the lattice spacing and $P$ is the probability of reflection at the reactive site. Then

$$P = \frac{1}{1 + l\kappa/D}$$

If $D = 0.46 \times 7.0\ \mu m^2 \cdot s^{-1}$ and $l = 0.8$ nm, then $P = 1/2$ when $\kappa = 4025\ \mu m \cdot s^{-1}$, and $P = 0.04$ at $\kappa = 10^5$. This indicates why, in the contour plots (Figs. 4A–4D), values of $\kappa \geq 10^5$ are needed to give contours close to those with $\kappa \to \infty$.

Consider the equilibrium between a blocked and an unblocked state:

$$\text{blocked} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{unblocked}$$

The blocked state corresponds to the R*-T complex, and the unblocked state corresponds to free R*. So $\alpha$ is the reciprocal of the lifetime $\tau$(R*-T) of the complex and $\beta$ is the reciprocal of the mean time $\tau$(coll) between collisions. Then $P = \beta/(\alpha + \beta)$, and

$$\kappa/D = \alpha/l\beta = \tau(\text{coll})/l\tau(\text{R*-T})$$

So if $\tau$(R*-T) $\ll \tau$(coll), then $\kappa \to \infty$, and R* is a perfect absorber.

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