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THE EFFECT OF DIFFUSION ON THE TRAPPING OF MEMBRANE-BOUND RECEPTORS BY LOCALIZED COATED PITS

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Localized coated pits are considered in the primary steps of receptor-mediated endocytosis. According to the pit reinsertion mechanism, we have modified our previous kinetic model and studied the effect of diffusion on the trapping rate constant (k^+). Using experimental data for low density lipoprotein (LDL) receptors on fibroblast cells, we found that the binding of receptors to coated pits is not totally diffusion controlled. For example, the process is less than 78% diffusion controlled if receptors are not allowed to escape the coated pits. However, due to the large uncertainties in the experimental parameters, a diffusion-controlled process cannot be ruled out. The greatest differences between localized and random reinsertion were found when the escaping rate constant (k^-) is much greater than the rate constant for invagination of the pits (λ_1). Under this condition, k^+ for localized reinsertion is no less than 39% diffusion controlled, while k^+ for random reinsertion shows no diffusion effect at all.

1. Introduction

Receptor-mediated endocytosis is one of the main pathways by which large molecules, such as hormones, growth factors, toxins, and some viruses enter the cell [1–4]. This process allows the cell to internalize efficiently specific ligands of physiological importance from its external environment. The receptors, which are mobile proteins embedded in the cell membrane [2,3], are able to bind a particular ligand. For example, in the case of low density lipoprotein (LDL), glycoprotein binds to the LDL's apoprotein B-100 [6]. After binding the ligand-receptor complex diffuses on the membrane and aggregates, prior to internalization, in specialized regions called coated pits. Aggregation could be independent of binding as in the case of LDL, where bound and unbound receptors have been observed in coated pits [7], or it could be exclusive for receptor-ligand complexes as found for epidermal growth factor (EGF) [8]. The coated pits are regions composed mainly of a protein called clathrin [9] and represent between 1 and 2% of the cell's surface [10]. These coated regions are the locations where the membrane invaginates, carrying with it the receptor-ligand complex.

A detailed knowledge of the molecular mechanisms involved in receptor-mediated endocytosis has not yet been obtained. Nevertheless, based on the slow receptor diffusion [11], aggregation to coated pits has been assumed to be a diffusion-controlled process [12]. As a consequence most of the reported calculations use Fick's diffusion equation [13–15] and ideas taken from the Smoluchowski theory of reactions in solutions [16]. With this approach necessary modifications are introduced to overcome the logarithmic divergence of Smoluchowski's theory in two dimensions. In contrast, Keizer et al. [17] (hereafter referred to as I) used the mechanistic statistical

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theory of nonequilibrium thermodynamics [18,19] to study the model suggested by Goldstein et al. [1]. Their approach is free of the divergence encountered in the two-dimension Smoluchowski theory and analyzes the effect of diffusion on the binding of receptors to coated pits by calculating the steady-state radial distribution function of receptors around coated pits. Based on experimental data for LDL receptors on human fibroblast cells, it was concluded in I that the effect of diffusion on the binding of LDL receptors to coated pits is no more than 84% diffusion controlled.

The model used in I allows three possible mechanisms for the pits' reinsertion. Two of them, random reinserted and static pits, were discussed in I. The third mechanism is a localized coated pit [20], in which the pit resurfaces at the same location where it was prior to investigation. This mechanism is supported by the observation of a thin neck [21–23] connecting the invaginated pit to the original location of the pit on the surface. In this communication, we extend the results obtained in I to the case of localized reinsertion of coated pits. In section 2, the receptor-pit radial distribution function is calculated. The effect of diffusion on the trapping rate constant is analyzed in section 3. Finally, in section 4, we compare our results with those obtained by Goldstein et al. [20].

2. Localized reinsertion

In contrast with I, where random reinsertion (RR) was assumed, we consider the case of localized reinsertion (LR) in the context of our kinetic model of receptor-mediated endocytosis [17]. Moreover, if invagination is assumed to be a first-order rate process, the rate of change of the number densities is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}\rho_{\mathrm{p}} = -\lambda_1\rho_{\mathrm{p}} + \lambda_2\rho_{\mathrm{ip}}, \tag{1}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\rho_{\mathrm{ip}} = \lambda_1\rho_{\mathrm{p}} - \lambda_2\rho_{\mathrm{ip}}, \tag{2}$$

where ρ_{p} and ρ_{ip} denote the number density of surface and invaginated pits, respectively, and λ_1^{-1} and λ_2^{-1} are the corresponding average lifetimes. These equations, which replace eq. 2 in I, are the only differences in the rate of change equations between the RR and LR models. Thus, on average, the LR model satisfies the following equations:

$$\frac{\mathrm{d}}{\mathrm{d}t}\bar{\rho}_{\mathrm{p}} = -\lambda_1\bar{\rho}_{\mathrm{p}} + \lambda_2\bar{\rho}_{\mathrm{ip}} \tag{3}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\bar{\rho}_{\mathrm{ip}} = \lambda_1\bar{\rho}_{\mathrm{p}} - \lambda_2\bar{\rho}_{\mathrm{ip}} \tag{4}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\bar{\rho}_{\mathrm{r}} = -k^+\bar{\rho}_{\mathrm{p}}\bar{\rho}_{\mathrm{r}} + k^-\bar{\rho}_{\mathrm{rp}} + K_{\mathrm{r}} \tag{5a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\bar{\rho}_{\mathrm{rp}} = k^+\bar{\rho}_{\mathrm{p}}\bar{\rho}_{\mathrm{r}} - k^-\bar{\rho}_{\mathrm{rp}} - \lambda_1\bar{\rho}_{\mathrm{rp}}, \tag{6}$$

where $\bar{\rho}_{\mathrm{r}}$ and $\bar{\rho}_{\mathrm{rp}}$ are the average number density for receptors and receptors in pits. k^+ and k^- are the binding and escaping rate constants, and K_{r} the rate of receptor reinsertion. In the calculation of eqs. 3–6, we have assumed a uniform system. This means a vanishing contribution to the average eq. 5 due to receptor diffusion:

$$\left(\frac{\partial\rho_{\mathrm{p}}}{\partial t}\right)_{\mathrm{Diff}} = D\nabla^2\rho_{\mathrm{p}} \tag{5b}$$

The solution to the steady-state equations is

$$\bar{\rho}_{\mathrm{ip}}^{\mathrm{ss}} = \frac{\lambda_1}{\lambda_2}\bar{\rho}_{\mathrm{p}}^{\mathrm{ss}} \tag{7}$$

$$\bar{\rho}_{\mathrm{r}}^{\mathrm{ss}} = \bar{\rho}_{\mathrm{rp}}^{\mathrm{ss}}\frac{(\lambda_1 + k^-)}{k^+\bar{\rho}_{\mathrm{p}}^{\mathrm{ss}}} \tag{8}$$

$$\bar{\rho}_{\mathrm{rp}}^{\mathrm{ss}} = K_{\mathrm{r}}/\lambda_1 \tag{9}$$

According to the mechanistic statistical theory of nonequilibrium thermodynamics (also known as the fluctuation-dissipation (F-D) theory) [18,19], fluctuations around the steady state satisfy the following equations (for details see I)

$$\frac{\partial}{\partial t}\delta\boldsymbol{\rho} = \boldsymbol{H}\delta\boldsymbol{\rho} + \tilde{\boldsymbol{f}}, \tag{10}$$

where $\delta\boldsymbol{\rho}^{\mathrm{T}} = (\delta\rho_1, \delta\rho_2, \delta\rho_3)$ and $\tilde{\boldsymbol{f}}^{\mathrm{T}} = (\tilde{f}_1, \tilde{f}_2, \tilde{f}_3)$ are vectors, $\boldsymbol{H}$ is a 3×3 matrix, and the indices are $p = 1$, $r = 2$ and $rp = 3$. Note that fluctuations in the density of invaginated pits, $\delta\rho_{\mathrm{ip}}$, do not appear in eq. 10, because the total number of pits either on the surface or invaginated is constant, which means that $\delta\rho_{\mathrm{ip}}$ are not independent fluctuations, i.e., $\delta\rho_{\mathrm{p}} = -\delta\rho_{\mathrm{ip}}$. The relaxation ma-

trix, $\boldsymbol{H}$, is given by

$$\boldsymbol{H}=\begin{bmatrix} -(\lambda_1+\lambda_2) & 0 & 0 \\ -k^+\bar{\rho}_r^{ss} & D\nabla_r^2-k^+\bar{\rho}_p^{ss} & k^- \\ k^+\bar{\rho}_r^{ss} & k^+\bar{\rho}_p^{ss} & -(\lambda_1+k^-) \end{bmatrix}\delta(r-r'). \tag{11}$$

The random term, $\tilde{f}$, is a multivariant white noise that vanishes on the average and has the covariance matrix [18,19]

$$\langle \tilde{f}(r,t)\tilde{f}^{T}(r',t')\rangle=\gamma(r,r')\delta(t-t'). \tag{12}$$

γ is determined by the postulates of the F-D theory [18,19] and by the underlying molecular mechanisms involved in receptor-mediated endocytosis, namely

$$\gamma(r,r')=\begin{bmatrix} 2\lambda_1\bar{\rho}_p^{ss} & 0 & \lambda_1\bar{\rho}_{rp}^{ss} \\ 0 & k^+\bar{\rho}_r^{ss}\bar{\rho}_p^{ss} & -\left(k^+\bar{\rho}_r^{ss}\bar{\rho}_p^{ss}+k^-\bar{\rho}_{rp}^{ss}\right) \\ & +k^-\bar{\rho}_{rp}^{ss}-2D\nabla_r^2 & \\ \lambda_1\bar{\rho}_{rp}^{ss} & -\left(k^+\bar{\rho}_r^{ss}\bar{\rho}_p^{ss}+k^-\bar{\rho}_{rp}^{ss}\right) & k^+\bar{\rho}_r^{ss}\bar{\rho}_p^{ss} \\ & & +(k^-+\lambda_1\langle j\rangle)\bar{\rho}_{rp}^{ss} \end{bmatrix}\delta(r-r') \tag{13}$$

If we consider $\boldsymbol{H}$ and γ, the only differences between RR and LR are in the matrix elements $\boldsymbol{H}_{11}$ and γ_{11}.

Following Keizer's theory of rapid reactions [24], we need to calculate the radial distribution function of receptors around coated pits, g_{rp}. This function is related to the density-density correlation $\langle\delta\rho_r(r)\delta\rho_p(r')\rangle^{ss}$ by the following equation [25]

$$\langle\delta\rho_r(r)\delta\rho_p(r')\rangle^{ss}\equiv\sigma_{rp}^{ss}(r,r')=\bar{\rho}_r^{ss}\bar{\rho}_p^{ss}\left[g_{rp}(r,r')-1\right], \tag{14}$$

where the angular brackets represent the static average over the steady-state ensemble. Now, σ_{rp}^{ss} is known to satisfy the fluctuation-dissipation theorem [18,19] at steady state

$$\boldsymbol{H}\sigma+\sigma\boldsymbol{H}^{T}=-\gamma. \tag{15}$$

The solution to eq. 15 is easily obtained if we first Fourier transform eqs. 11, 13 and 14

$$\sigma_{ij}(k,k')=\frac{1}{(2\pi)^4}\int dr\int dr' e^{ik\cdot r-ik'\cdot r'}\sigma_{ij}(r,r') \tag{16}$$

After a straightforward calculation, one finds that the pit-receptor correlation function for $\lambda_1\neq 0$, given by $\sigma_{12}=\sigma_{21}$, is

$$\sigma_{12}(k,k')=\left[-\frac{k^+\bar{\rho}_r^{ss}\bar{\rho}_p^{ss}\bar{b}}{2D(\xi^2+k^2)}\right]\frac{\delta(k-k')}{(2\pi)^2} \tag{17}$$

where

$$\bar{b}=\lambda_1 b/(\lambda_1+k^-) \tag{18}$$

$$b=2\lambda_1/(\lambda_1+\lambda_2) \tag{19}$$

$$\xi=\left[\frac{\bar{\rho}_p^{ss}\tilde{b}k^+ +\lambda_1+\lambda_2}{D}\right]^{1/2} \tag{20a}$$

and

$$\tilde{b}=(2\lambda_1+\lambda_2)/(2\lambda_1+\lambda_2+k^-) \tag{20b}$$

Although eq. 17 is the same as the one obtained in I, $\bar{b}$ and the correlation length, i.e., ξ^{-1}, are different.

The inverse Fourier transform of eq. 17 yields

$$\langle\delta\rho_r(r)\delta\rho_p(r')\rangle^{ss}=-\frac{k^+\bar{b}}{4\pi D}\bar{\rho}_r^{ss}\bar{\rho}_p^{ss}K_0(\xi|r-r'|) \tag{21}$$

where K_0 is the McDonald function of order zero. Using eq. 14, the radial distribution function is

$$g_{rp}(r)=1-\frac{k^+\bar{b}}{4\pi D}K_0(\xi r). \tag{22}$$

Consequently the binding rate constant of receptors to coated pits is given by [17]

$$k^+=k^0 g_{rp}(R), \tag{23}$$

where k^0 is the intrinsic reactivity, and R the

radius of the coated pit at which binding occurs. Eq. 23 is a transcendental equation for k^+ where all the parameters, except k^- and k^0, have been measured experimentally. Even though the values of k^- and k^0 are not known, it is possible to study the effect of diffusion with the help of a second relation between k^+ and the experimental parameters. This relation is given by eq. 8

$$k^+ = \left[\frac{\bar{\rho}_{rp}^{ss}}{\bar{\rho}_r^{ss}}\right]\frac{1}{\bar{\rho}_p^{ss}}(\lambda_1 + k^-) \tag{24}$$

where the ratio $\rho \equiv \bar{\rho}_{rp}^{ss}/\bar{\rho}_r^{ss}$ has been measured [15]. Combining eqs. 22 and 23, one finds the following expression for the binding rate constant

$$k^+ = \frac{k^0[4\pi D/\bar{b}K_0(\xi R)]}{k^0 + [4\pi D/\bar{b}K_0(\xi R)]} \tag{25}$$

In the context of the present theory, we find that the condition for a diffusion-controlled process is given by

$$k^0 \gg 4\pi D/\bar{b}K_0(\xi R). \tag{26}$$

In this case the binding rate constant for a diffusion-controlled process is given by

$$k^+_{\text{Diff.Cont.}} \equiv k_D = 4\pi D/\bar{b}K_0(\xi R), \tag{27}$$

but eq. 27 has to be consistent with eq. 24. If we combine them, we obtain the following transcendental equation for k^-

$$K_0\left(R\sqrt{\frac{\lambda_1}{D}}\,x\right) = \frac{2\pi D(1+f_2)}{\lambda_1}\,\frac{\bar{\rho}_p^{ss}}{\rho} \tag{28}$$

where

$$x = \sqrt{1 + f_2 + \frac{\rho(2+f_2)(1+f_1)}{2+f_1+f_2}}\,. \tag{29}$$

The f terms are defined as $f_1 = k^-/\lambda_1$ and $f_2 = \lambda_2/\lambda_1$. Thus, if we find the solution to eq. 28, k^- is given by eq. 29 and k^+ by eq. 24.

3. The effect of diffusion

In order to analyze the effect of diffusion on the binding rate constant, we define the ratio

$$y \equiv k^+/k^0. \tag{30}$$

This ratio combined with eqs. 25 and 27 yields

$$y = 1/[1 + k^+/(k_D y)]. \tag{31}$$

From this expression one obtains the following relation

$$y = 1 - \frac{k^+}{k_D}. \tag{32}$$

If we substitute in this equation the values of k^+ and k_D given by eqs. 24 and 27, one gets

$$y = 1 - \left[\frac{\rho}{\bar{\rho}_p^{ss}}\right]\frac{\lambda_1}{2\pi D(1+f_2)}K_0\left(R\sqrt{\frac{\lambda_1}{D}}\,x\right) \tag{33}$$

with x given by eq. 29. Eq. 33 will allow us to examine the ratio k^+/k^0 over the plausible values of $f_1 = k^-/\lambda_1$. Note that the physical region is given by $y \geq 0$ where $y = 0$ is the diffusion-controlled case.

Table 1

Characteristic parameters for LDL receptors on human fibroblasts

Parameter	Symbol	Value	Source
Radius of coated pits	R	0.10 μm	ref. 15
Receptor diffusion constant	D	4.5×10^{-3} $(\mu m)^2/s$	ref. 8
Steady-state density of coated pits (37°C)	$\bar{\rho}_p^{ss}$	0.31 $(\mu m)^{-2}$	ref. 15
Invagination rate constant	λ_1	3.3×10^{-3}/s	ref. 15
Number ratio of receptors in pits to receptors out of pits	$\rho = \bar{\rho}_{rp}^{ss}/\bar{\rho}_r^{ss}$	2.2	ref. 15

Table 2

Parameters for the radial distribution function

Case	f_2	λ_1 (s^{-1})	D ($\times 10^{-3}$)($\mu m^2/s$)	A_1	A_2		
					$f_1 = 0$	$f_1 = 1$	$f_1 \gg 1$
LR1	1	1/300	4.5	0.418	0.176	0.200	0.252
LR2	2	1/14 [a]	60.0 [a]	0.449	0.249	0.278	0.374
LR3	2	1/300	4.5	0.279	0.196	0.220	0.296

[a] Value taken from ref. 19.

We will consider experimental parameters of LDL receptors on human fibroblasts, $f_2 = 1$ suggested by Goldstein et al. [20] and the values $f_2 = 2$ suggested by Willingham and Pastan [21,22]. For $f_2 = 1$ and the experimental parameters given in table 1, one finds the following equation:

$$y = 1 - \frac{1}{2.390} K_0(0.086x), \tag{34}$$

where x varies between 2.049 for $f_1 = 0$ and 2.933 for $f_1 \gg 1$. For these values of x, K_0 varies between 1.873 and 1.533. This means that k^+ varies between $0.78k_D$ and $0.645k_D$, where we have used eq. 32 to calculate the value of k^+.

For $f_2 = 2$, a value of $\lambda_1 = 7.15 \times 10\ s^{-1}$ has been suggested [20,23]. Now, if we take the other parameters from table 1, we find

$$y = 1 - \frac{1}{0.167} K_0(0.398x) \tag{35}$$

In this case for $f_1 = 0$ we have $x = 2.280$ and $K_0 = 0.481$, while for $f_1 \gg 1$ we have $x = 3.435$ and $K_0 = 0.254$. So, the second term on the right-hand side of eq. 35 is always greater than unity. This yields a negative value for y which is not a physical possibility. However, we cannot draw any definite conclusion since the value of λ_1, used in eq. 35, was observed on rodent Swiss 3T3 fibroblast cells [22] and not on human fibroblasts. Moreover, the value of the diffusion coefficient in table 1 [8] is different from those reported by Pastan et al. For the diffusion coefficient at 23°C, they found [23] values between 3×10^{-10} and 9×10^{-10} cm^2/s. Thus, if we take a diffusion constant equal to 6×10^{-10} cm^2/s and repeat the calculation that led to eq. 34, one finds the following equation

$$y = 1 - \frac{1}{2.227} K_0(0.109x) \tag{36}$$

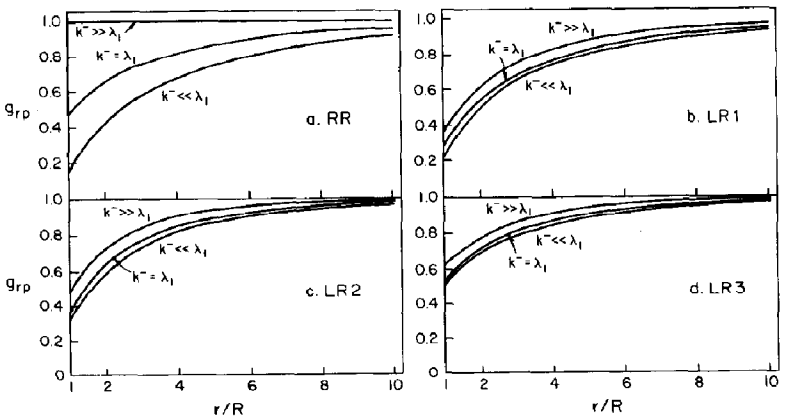

Fig. 1. The radial distribution of free LDL receptors around coated pits for random reinsertion and localized reinsertion. Parameter values taken from tables 1 and 2. The radial distance scale is the radius of a coated pit.

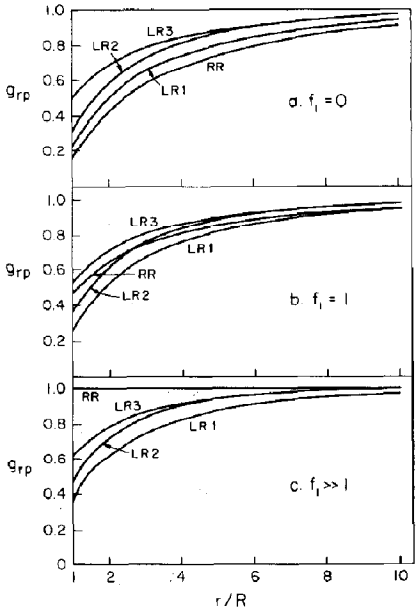

Fig. 2. Direct comparison of the radial distribution of free LDL receptors around coated pits under three possible conditions. (a) $k^- \ll \lambda_1$, (b) $k^- = \lambda_1$, (c) $k^- \gg \lambda_1$.

where $K_0(f_1 = 0) = 1.547$ and $K_0(f_1 \gg \lambda_1) = 1.173$. For this value, k^+ lies in a physical region between $0.69k_D$ and $0.52k_D$. Since $f_2 = 2$ is a plausible physical value [23], let us consider it along with λ_1 and D from table 1. The resulting equation is given by

$$y = 1 - \frac{1}{3.586} K_0(0.086x). \tag{37}$$

Here one finds a $k^+ = 0.49k_D$ for $f_1 = 0$ and $k^+ = 0.39k_D$ for $f_1 \gg 1$. Thus, care has to be taken when selecting and varying the parameters. Otherwise, erroneous conclusions could be drawn if the interdependence between the parameters is neglected.

Table 3
Correlation length

Case	$f_1 = 0$	$f = 1$	$f_1 \gg 1$
LR1	$5.7R$	$5.0R$	$4.0R$
LR2	$4.0R$	$3.6R$	$2.7R$
LR3	$5.1R$	$4.5R$	$3.4R$
RR	$6.5R$	$5.9R$	$5.0R$

For the cases discussed above, the radial distribution function for LDL receptors g_{rp} is given by

$$g_{rp}(r) = 1 - A_1 K_0(A_2 r/R), \tag{38}$$

where we have used eq. 28. The values of A_1 and A_2 have been listed in table 2, and g_{rp} is depicted in figs. 1 and 2. Fig. 1 shows the radial distribution functions for the different cases of the LR model, and it also includes the RR case obtained in I. Fig. 2 compares the LR and RR models for the same value of f_1. Finally, we have included the corresponding correlation lengths, ξ^{-1}, in table 3.

4. Discussion

The exact recycling mechanisms of coated pits in receptor-mediated endocytosis is not known. But, it is most probable that pit reinsertion is either random [20] or localized [21]. In the context of our kinetic model of receptor trapping in receptor-mediated endocytosis [17] we have analyzed both cases and found minor differences between the LR and RR models in the average equations, the relaxation matrix $\boldsymbol{H}$, and the correlation matrix γ. These minor differences are enough to produce considerably different diffusion effects on the trapping rate constant k^+ for each model. In the case of RR, we have found in I that k^+ could be no more than 84% diffusion controlled when k^- is much less than λ_1, and no diffusion effect is present when k^+ is much greater than λ_1. Contrasting with this behavior, the LR mechanism shows some diffusion effect for all possible values of k^-. For a quantitative comparison, LR1 will be used. In this case k^+ varies between 78 and 69% diffusion controlled. In all the cases discussed here, we found no physical solution to the diffusion-controlled case. Thus, despite experimental uncertainties in the data, one can state that in the LR model some effect of diffusion is always present. This result is depicted in fig. 1 in terms of the radial distribution function of receptors around a coated pit. In fig. 1a, $g_{rp}(r) = 1$ implies no diffusion effect, and the effect of diffusion is depicted by the dip of the curve near $r/R = 1$ for

possible values of the dissociation escaping rate constant. In fig. 2, one notices the greatest differences between the RR and LR models when $k^- \gg \lambda_1$.

Once the receptor-ligand complex reaches the pit, binding occurs with an intrinsic rate which is characterized by k^0, the intrinsic reactivity [22]. Combining eqs. 8 and 25, one is able to obtain an expression for k^0, namely

$$k^0 = \frac{4\pi D(1+f_1)}{\left[\frac{4\pi D\bar{\rho}_p^{ss}}{\rho\lambda_1}\right] - (1+f_1)\bar{b}K_0(R\xi)}, \tag{39}$$

where $\bar{b}$ and ξ are given for the RR model by eqs. 18–20 and for the RR model by eq. 32 in I. For the particular case $k^- = 0$, the corresponding values are $k^0_{LR1} = 4.67k^+_{LR1}$ and $k^0_{RR} = 6.29k^+_{RR}$. These values of the intrinsic reactivity do not satisfy the diffusion-controlled condition given by eq. 26. Consequently, even though receptor diffusion is slow, the actual binding at the boundary of the pit is not a rapid process. Thus, assuming a diffusion-controlled process based only on the slow diffusion of receptors is questionable. A better criterion is given by eq. 26, where the intrinsic reactivity and the diffusion-controlled rate constant are compared.

Solution to eq. 28 implies a total diffusion-controlled process. For the parameters in table 1, we found no solution for both reinsertion mechanisms. However, the experimental uncertainties, as large as 33% in the case of the diffusion coefficient [8], could sustain solutions to eq. 28. The diffusion-controlled case with $k^- = 0$ has been studied in length by Goldstein, Wofsy and co-workers [11,14,15,20]. Their calculations use Fick's diffusion equation to estimate the rate constants by calculating the mean first-capture time for receptors. We could compare our results with theirs by using $k^- = 0$, and by assuming that the parameters are such that eq. 28 has a solution. Under these conditions, we recall the expressions for the trapping rate constants in the RR model and the static sink model (SS) from I

$$k^+_{RR} = \frac{4\pi D}{K_0\left[R\sqrt{\frac{\bar{\rho}_p^{ss}k^+_{RR}}{D} + \frac{\lambda_1}{D}}\right]} \tag{40a}$$

$$k^+_{SS} = \frac{1}{2}\frac{4\pi D}{K_0\left[R\sqrt{\frac{\bar{\rho}_p^{ss}k^+_{ss}}{D}}\right]} \tag{40b}$$

For the LR model, we have

$$k^+_{LR} = \frac{1}{\left[\frac{2}{1+f_2}\right]}\frac{4\pi D}{K_0\left[R\sqrt{\frac{\bar{\rho}_p^{ss}k^+_{LR}}{D} + \frac{\lambda_1}{D}(1+f_2)}\right]} \tag{41}$$

One might be tempted to take the infinite lifetime limit, i.e., $\lambda_1 \to 0$, in eqs. 40a and 41 thinking that it should lead to eq. 40b. Care has to be taken in this case. Since eqs. 40a and 41 were obtained by first solving eq. 15 for $\lambda_1 \neq 0$, one should take the limit ($\lambda_1 \to 0$) not of eqs. 40a and 41, but rather of eq. 15 and then solve for σ_{12}. In this limit and using eqs. 14 and 23, one obtains $\sigma_{12} = 0$, $g_{rp} = 1$ and $k^+ = k^0$ for both localized and random reinsertion. Thus, the models discussed here and in I describe an equilibrium state, i.e., $g_{rp} = 1$, when $\lambda_1 \to 0$. Moreover, the relaxation matrices, $\boldsymbol{H}$, for the localized and random reinsertion models do not reduce to the static sink's relaxation matrix in the limit of infinite lifetime [26]. In other words, the models discussed here and in I do not reduce to the model of static sinks [26] when $\lambda_1 \to 0$. As a result, one should take care and not expect to obtain the same rate constant [40b] in this limit.

If $f_2 \geq 1$, the following inequality is rigorously satisfied

$$k^+_{SS} < k^+_{RR} < k^+_{LR}. \tag{42}$$

This result is easily obtained, since the McDonald function of order zero is a monotonic decreasing function, and because the argument of K_0 for SS is less than the argument for RR, and this is less than the argument for LR.

Whether or not the inequality is satisfied will depend on the experimental data. However, for a low pit density, one intuitively expects that a random reinserted trap will eventually reappear in regions where no trapping has taken place. In contrast, a localized trap returns to the same location where trapping has already occurred. This means that a random reinserted pit should trap

more receptors than a localized pit. In other words, in the low pit density limit,

$$k^{+}_{RR} > k^{+}_{LR} > {}^{+}_{SS}$$

and, as a consequence, f_2 has to be less than unity ($f_2 < 1$) in this low density limit.

On the same intuitive physical grounds, one expects that for high pit densities a random inserted trap will not be able to reappear in regions where no trapping has taken place. On the other hand, a localized trap returns to a region that has had time to relax towards the receptor's bulk density. Consequently in the high pit density limit, one could expect that the relaxed regions encountered by localized pits would have more receptor than the regions encountered by random reinserted pits. Thus, for the high pit density limit, eq. 42 holds and f_2 has to be equal or greater than unity ($f_2 \geq 1$).

The solutions of the transcendental equations, eqs. 40a and 41, are listed in table 4. For ρ values within experimental error, the solutions are consistent with the steady-state condition, eq. 8. A quantitative comparison with Goldstein et al. is difficult since the experimental value of $\rho = \bar{\rho}^{ss}_{rp}/\bar{\rho}^{ss}_{r}$ is not known as a function of D. Indeed, if we were to make this comparison, we would have to know the value ρ for a six order of magnitude change in D. There is another basic difference between our calculations and Goldstein's model that makes a quantitative comparison even more difficult. We assume that the pits reappear on the surface empty, even if they reappear in regions with bulk receptor density. In our model the receptors are pushed away from the area when a pit reappears. Only when the pit is reinserted does it start trapping receptors again. On the other hand, Goldstein et al. assume instantaneous trapping of receptors when pit reinsertion occurs.

Regardless of these difficulties, we at least understand the difference in the values of k^{+}_{ss}. Goldstein et al. [20] reported a mean capture time, $t_c = (k^{+}\bar{\rho}^{ss}_{p})^{-1}$, of 178 s. This value is obtained if one uses the Burg and Purcell [26] result in the limit of dilute ordered sinks

$$t_c D = \frac{b^2}{2}\left[\ln(b/R) - 3/4\right], \tag{43}$$

where $b^2 = (\pi\bar{\rho}^{ss}_{p})^{-1}$ and the term 3/4 depends on the way in which the boundaries are chosen [17]. In contrast, for completely random sinks, we showed in I that the dilute limit, independent of boundary conditions, is given by

$$t_c D = \frac{b^2}{2}\left[\ln(b/R) - 0.231\right]. \tag{44}$$

Recently, Goldstein (unpublished work) obtained eq. 44 using the Berg and Purcell method in the case of random coated pits. Hence, it seems that the differences in the low density limit between eqs. 43 and 44 depends not on the technique used to obtain them, but rather on the pit's distribution. This equation is the lowest approximation of the exact solution to the transcendental equation

$$t_c D = \frac{b^2}{2} K_0\left[\sqrt{R^2/t_c D}\right], \tag{45}$$

where eq. 45 was obtained by substituting t_c in eq. 40b. Moreover, for fixed values of $\bar{\rho}^{ss}_{p}$ and R, the

Table 4

Comparison between random reinsertion (RR) and localized reinsertion (LR) under diffusion-controlled conditions

D ($\times 10^{-3}$)($\mu m^2/s$)$^{-1}$	f_2	λ_i^{-1} (s^{-1})		LR	RR
4.5	1	300	$k^{+}/(10^{-2}\,\mu m^2/s)$	3.148	2.928
			t_c (s^{-1})	102.5	110.2
			ξ^{-1}/R	5.26	6.05
	0.895	316	$k^{+}/(10^{-2}\,\mu m^2/s)$	2.913	2.916
			t_c (s^{-1})	110.7	110.6
			ξ^{-1}/R	5.48	6.08
60	2	14	$k^{+}/(10^{-2}\,\mu m^2/s)$	77.978	40.877
			t_c (s^{-1})	4.1	7.9
			ξ^{-1}/R	3.63	5.5

product $t_c D$ is a constant. Using the values in table 1, we found a $t_c = 292$ s, which is the exact mean capture time in the case of long-lived static sinks. On the other hand, eq. 44 yields a $t_c = 238$ s. Thus, for the values of interest, eqs. 43 and 44 are poor approximations of the exact solution, and further iterations had to be considered (see I for details).

We als note that the parameters, $f_1 = 1$ and $\lambda_1^{-1} = 300$ s, used by Goldstein et al. do not coincide with the experimental values $f_1 = 0.895$ and $\lambda_1^{-1} = 316$ s. These minor quantitative differences produce significant qualitative changes in our calculations, as can be seen in table 4. For example, if we use the approximate values $f_2 = 1$ and $\lambda_1^{-1} = 300$ s, we find that k^+_{LR1} is greater than k^+_{RR}. Since receptor-mediated endocytosis can be considered as a process in the low pit density regime, this result contradicts our previous heuristic discussion and Goldstein's findings. This contradiction results from using the approximate values of the parameters in our equations. The inequality, $k^+_{RR} > k^+_{LR1}$, is obtained when the experimental values of f_2 and λ_1 are used. In this case, our results agree qualitatively with the dilute limit calculation of Goldstein et al. [20].

Our method, although exact, is extremely sensitive to the experimental parameters. Due to large uncertainties in the measurements, we were not able to determine whether or not pit reinsertion is random. Nevertheless, we were able to predict the differences between localized and random reinsertion under a partial or a total diffusion-controlled binding process. We expect that refined measurements, knowledge of the binding process at the pits' boundary, and the experimental determination of the radial distribution of receptor around coated pits will finally determine whether or not pits are localized.

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