Rates of Membrane-associated Reactions: Reduction of Dimensionality Revisited

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Abstract. The hypothesis that reactions associated with intracellular membranes enjoy a kinetic advantage from a reduced dimensionality for diffusion is inconsistent with available data on lateral diffusion rates, membrane-substrate affinities, and endogenous concentrations of enzymes and their aqueous substrates.

It follows from a probability theorem by Polya that a random walker confined to one or two dimensions (1d or 2d) is guaranteed to find a stationary target, wherever it is, but in three dimensions (3d) there is some chance he never will (Polya, 1921; Feller, 1957). Trurnit (1945) and Bucher (1953) suggested some time ago that the turnover numbers of certain membrane-bound enzymes might be enhanced if their aqueous substrates were to undergo guided 2d diffusion along membrane surfaces. In 1968 Adam and Delbrück put this hypothesis on quantitative grounds in proving that the diffusion-limited rate at which a surface-bound trap reacts with bulk-phase reactants can theoretically be enhanced, provided the reactants first adsorb to the surface and then diffuse in 2d before striking the trap. Echoing Dirac's (1931) reasoning that one would be surprised if nature had made no use of this possibility, they proposed that intracellular membranes enjoy a selective advantage because of an ability to adsorb and guide the diffusion of substrates to membrane-embedded enzymes. Indeed, they speculated that this advantage "contributed greatly to the evolutionary step from small, little-organized bacteria-like ancestors of cells to fully developed, internally compartmentalized unicellular organisms." A similar view is voiced by Eigen (1974), who calls reduction of dimensionality "nature's trick to overcome the barrier of diffusion control, and make multimolecular reaction processes at low concentrations more efficient." It is this device, according to Eigen, that explains the prevalence of membrane-bound enzymes in living systems.

In this paper we reappraise the dimensional reduction hypothesis of Adam and Delbrück as it pertains to reactions taking place on intracellular membranes. By dimensional reduction we refer specifically to the situation whereby a bulk phase reactant, e.g., an aqueous substrate, finds its membrane-bound target, e.g., an enzyme, not by purely 3d diffusive encounters, but by first adsorbing nonselectively to the membrane and then diffusing in 2d before reacting. We ask what magnitude of kinetic advantage this two-step searching process reasonably can be expected to afford in cellular reactions. Has natural selection actually entrained mechanisms that capitalize upon the theoretical rate advantages of the recurrent 2d random walk? In the first section we recount the Adam-Delbrück hypothesis (1968) describing Berg and Purcell's (1977) subsequent treatment of the effects of target density, and then estimate the theoretical advantages to be gained from two-step searching on intracellular membranes by using literature values of relevant parameters, e.g., diffusion coefficients, binding constants, and target densities. In the second section we offer a brief critique of experiments dealing with the effects of dimensionality on reaction kinetics in vitro. Finally, we consider other possible consequences of locating enzymes in membranes that in our opinion are more likely to influence biological reaction kinetics than are the special properties of 2d diffusion, per se.

Searching by Soluble Substrates for Membrane-bound Targets

Adam and Delbrück's Model

The gist of Polya's theorem is that on an isotropic random walk over an infinite 1d or 2d lattice there is unit probability that the walker will eventually visit every lattice point, whereas in 3d the probability is considerably less than 1 (Polya, 1921; Feller, 1957). This "critical" dependence of encounter probability on dimensionality provides a qualitative rationale for the proposal that internal membranes might function as "antennae," catching substrates and guiding their diffusion to membrane-embedded enzymes. But Polya's theorem concerns only the encounter probability, not the actual diffusion time required to encounter a target. As Adam and Delbrück...
requires a for biological membranes.

The average lifetime of soluble reactant molecules inside the sphere was then calculated. This led to a range of ratios of 2d to 3d translational diffusion coefficients \((D_2/D_3)\) and diffusion space size to target size \((b/a)\) over which combined surface and bulk-phase diffusion (two-step trapping) is favorable (Fig. 1). Notice that for a given \(b/a\), reduction of dimensionality can either enhance or retard the search, depending on how fast the adsorbed reactant diffuses.

No data on the surface diffusion rates of membrane-bound molecules were available in 1968, yet Adam and Delbrück argued that "from our present knowledge of diffusion coefficients in liquids, gels, and solids, it appears improbable that we will encounter in biological systems any ratios \(D_2/D_3\) much smaller than \(10^{-2}\." Given \(D_2/D_3 = 10^{-2}\) and a target radius \(a\) of 10 Å, Adam and Delbrück (1968) noted a "transition" in kinetics when the reaction compartment is about the size of a bacterium or a eukaryotic organelle: Two-step trapping becomes favorable when the size of the diffusion space is above \(\sim 1 \mu m\). The existence of this transition was qualitatively confirmed in recent computer simulations using lattice models of diffusion and reaction (Lee and Kozak, 1984).

### 3d and 2d Diffusion Rates In Vivo

Adam and Delbrück's guess for the lower limit to \(D_2/D_3\) was remarkably close to actual values of \(D_2/D_3\) observed for several diffusion probes in biological membranes, provided that a cytoplasmic viscosity significantly greater than that of pure water is assumed (Fig. 2, Table I). In Table I, experimental values of \(D_3\) are used along with values of \(D_3\) calculated assuming an average cytoplasmic viscosity 3.2 times that of pure water at the temperature of the diffusion measurement. The factor 3.2 is an average of 30 estimates on 14 different cell types from three kingdoms (Mastro et al., 1984; Lepok et al., 1983; Morse, 1977; Haak et al., 1976; Sachs and Latorre, 1974; Livingston et al., 1983; Burns, 1969; Lehman and Pollard, 1965; Horowitz, 1972; Hodgkin and Keynes, 1956; Horowitz and Moore, 1974; Caille and Hinke, 1974; Chang et al., 1972; Horowitz and Fenichel, 1970; Fenichel and Horowitz, 1969; Kushmerick and Podolsky, 1969). It includes eight different techniques using 19 probe molecules of \(\leq 16,900 \text{ mol wt}\). Large proteins are known to diffuse through cytoplasm at rates much less than 3.2 times that of their \(D_3\) in pure water (see, e.g., Mastro et al., 1984), but our concern here is with typical low molecular weight metabolites. Much greater cytoplasmic viscosities have also been reported even for small molecules (Keith and Snipes, 1974), it being suggested that "diffusion along the plane of the membrane or within the membrane structure may be faster and more efficient than through the aqueous protoplasm." It seems likely, however, that these very high viscosities were an artifact of the extremely hypertonic medium (~1.5 osM) in which the cells were bathed. If one includes only those estimates of viscosity based upon actual measurement of translational

![Figure 1](image1.png)

**Figure 1.** Criteria for an advantageous two-step search by soluble substrates for a single membrane-bound target, according to Adam and Delbrück (5; reprinted from Fig. 3 in reference 1). Contours show three different values (0.1, 1.0, and 10) for the ratio of mean lifetimes of soluble reactants \((t_2/t_3)\) for theoretical two-step (3d + 2d) vs. one-step (3d) searching process. \(D_2/D_3\) is the ratio of 2d to 3d diffusion coefficient, and \(b\) and \(a\) are the linear size of the diffusion space and the target radius, respectively. Stippled region is where two-step searching (reduction of dimensionality) impedes the trapping rate. Note that for \(b = 1 \mu m\) and \(a = 10 \mu m\), a modest 10-fold rate advantage requires a \(D_2/D_3 = 0.1\). As shown in Table I, this is an unlikely ratio for biological membranes.

![Figure 2](image2.png)

**Figure 2.** Histogram showing distribution of measured translational diffusion coefficients of membrane-associated molecules. Each square represents an independent measure of \(D_3\); i.e., a measurement from one laboratory, for one technique, for one cell type, and usually for one temperature. All artificial membranes were in a fluid state. In A "small hydrophobic molecules" includes n-valeramide, benzene, di-t-butyl nitrooxide, tetramethylpiperidine-N-oxyl, lindane, and others. In B and D "lipid" includes endogenous or synthetic phospho and neutral-lipids, fluorescent derivatives of lipids and synthetic amphiphaes, pyrene, spin-labeled derivatives of phospholipids, and fatty acids. In E, all diffusion coefficients below \(5 \times 10^{-10} \text{ cm}^2/\text{s}\) have been disregarded, but this is not to say that proteins in biomembranes do not move more slowly than this. A full list of references is available from the authors upon request.
Table I. Comparative Diffusion Rates on Membranes and in the Bulk

| Example          | $T$ (°C) | $D_2$ (cm$^2$/s) | $D_3$ (cm$^2$/s) | $D_2/D_3$ |
|------------------|---------|------------------|------------------|-----------|
| **A. Biological membranes** |         |                  |                  |           |
| NBD-PE$^*$       | 25      | $7.7 \times 10^{-9}$ | $7.5 \times 10^{-7}$ | 0.010     |
| DXC$^*$          | 29      | $9.2 \times 10^{-9}$ | $6.3 \times 10^{-7}$ | 0.015     |
| Alp-NBD$^4$      | 23      | $8.9 \times 10^{-9}$ | $9.9 \times 10^{-7}$ | 0.009     |
| Cytochrome c     | 20      | $1.9 \times 10^{-9}$ | $3.1 \times 10^{-7}$ | 0.006     |
| Integral proteins| 25      | $7.2 \times 10^{-9}$ | $7.2 \times 10^{-8}$ | 0.010     |
| All data on amphiphilic probes and pyrene | 26 | $1.2 \times 10^{-8}$ | $7.1 \times 10^{-7}$ | 0.015     |
| **B. Artificial lipid membranes (fluid)** |         |                  |                  |           |
| Stearic acid     | 25      | $1.6 \times 10^{-8}$ | $4.3 \times 10^{-6}$ | 0.004     |
| NBD-PE          | 27      | $5.6 \times 10^{-8}$ | $2.9 \times 10^{-6}$ | 0.019     |
| DXC$^*$          | 29      | $1.1 \times 10^{-7}$ | $2.5 \times 10^{-6}$ | 0.044     |
| All data on amphiphilic probes and pyrene | 30 | $8.2 \times 10^{-8}$ | $3.2 \times 10^{-6}$ | 0.029     |
| Integral proteins| 30      | $1.9 \times 10^{-8}$ | $7.9 \times 10^{-7}$ | 0.027     |
| n-Valeramide     | 20.6    | $6.5 \times 10^{-7}$ | $7.4 \times 10^{-6}$ | 0.088     |
| DTBN$^4$         | 37      | $1 \times 10^{-6}$ | $3 \times 10^{-6}$ | 0.33      |
| Benzene         | 22      | $3.7 \times 10^{-6}$ | $9.5 \times 10^{-6}$ | 0.39      |
| Lindane        | 37      | $1.2 \times 10^{-6}$ | $7.5 \times 10^{-6}$ | 0.16      |

For all but the integral proteins in biomembranes, the values of $D_2/D_3$ were calculated for each experimental measurement and then averaged, rather than formed by the ratio of the average $D$ values.

* Values of $D_2$ taken from data given in Fig. 2. $D_3$ for comparison with $D_2$ in biomembranes was calculated assuming the cytoplasmic viscosity ($\eta$) is 3.2 times that of pure water at the same temperature. In the calculation of $D_3$ values for comparison with $D_2$ values in artificial lipid membranes, the viscosity of pure water was assumed. The Stokes-Einstein relation $D_3 = kT/6\pi J R$, where $k$ is Boltzmann's constant, was used to calculate hypothetical $D_1$ values for integral proteins in reconstructed lipid membranes; the effective hydrodynamic radius $R$ was approximated as the radius of a sphere for each protein, assuming a specific volume of 0.73 g/cm$^3$. For lipids, lipid analogues, and small hydrophobic molecules $D_3$ was calculated from the molar volume, as outlined in Othmer and Thakar (1953).

$^*$ NBD-PE, nitrobenzoxadiazole-phosphatidylethanolamine.
$^4$ Alp-NBD, 7-(2-allylphenoxy)-2,2-dimethyl-6-hydroxy-1-(7-NBD)-1,4-diazahexane; it is a fluorescent $\beta$-adrenoceptor blocker.

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**Theoretical Effects of Target Density**

Reaction of soluble substrates with real membrane-bound "targets" differs in at least one important respect from the Adam-Delbrück model: The target density in biological systems is usually much higher than one per organelle. In 1977 Berg and Purcell considered the effect of target surface density on the reaction rate, assuming that the membrane either (a) perfectly reflects or (b) partially adsorbs substrate molecules that contact it in a nontarget area. They found that in case a the steady state diffusive flux of reactant should saturate when a small fraction of the total surface area is covered with perfect traps. As they pointed out, having once reached the surface, a molecule in random motion is likely to have multiple collisions with it before escaping. The probability ($P$) it will strike a trap once it has hit the surface is therefore much greater than the ratio of trap area to total surface area:

$$P = N_s/N_s + \pi a,$$

where $N_s$ is the number of traps (radius $s$) on the surface of a sphere with radius $a$ ($a > s$). ($P$ is related to the reactive flux $J$ by $P = J/J_{max}$, where $J_{max}$ is the flux to a completely absorbing sphere). Shoup and Szabo (1982) have confirmed this result using completely independent means. To reiterate, diffusional target searching at an inert (nonadsorbing) surface is far more effective than bullet-shooting analogies would predict.

For case b, Berg and Purcell (1977) first considered the case of an adsorbing surface with a single trap and obtained the same 2d mean lifetime ($t_{2d}$) as did Adam and Delbrück (1968). For the situation of multiple traps, they divided the total membrane area into cells, each containing a single trap, and found $t_{2d}$ by solving a 2d diffusion equation for each cell. The steady state flux ($J_2$) of adsorbed substrates into traps on the outer surface of a sphere was then expressed as the ratio $m/t_{2d}$, where $m$ is the average number of adsorbed substrate molecules. This equation is valid when the reactive flux is small as compared with the maximal flux, i.e., the flux onto a perfectly absorbing sphere. It implicitly assumes that equilibration of nonspecifically bound substrates with bulk phase substrates is rapid as compared with the rate of absorption by traps. Berg and Purcell put $m$ in terms of an energy of adsorption, but it is convenient for our purposes to have $m$ in terms of the empirical parameters $K_0$ or $K_0$, i.e., the equilibrium dissociation constant or the volume partition coefficient. With these substitutions, the Berg-Purcell criterion...
Table II. Theoretical Rate Advantage from Two-Step Searching by Aqueous Reactants for Hypothetical Membrane-bound Targets

| Reactant Membrane | Knee | cwa | D2/D3 | Advantage | Reference |
|-------------------|------|-----|-------|-----------|-----------|
| Apolipoprotein C-III Egg PC | 4.2 x 10^{-7} | 3.5 x 10^{-4} | 0.11 | No | Traubel et al., 1974; Vaz et al., 1979 |
| Cytochrome c Soy PC | 5.5 x 10^{-4} | 10^{-12} | 0.006 | No | Gute et al., 1983; Setlow and Pollard, 1962; Cannon and Ermann, 1980 |
| ACTH,-2a PA/PC (10:90) | 1.7 x 10^{-3} | 10^{-12} | 0.034 | No | Gysin and Schwyz, 1984 |
| Factor X (+2 mM Ca) PS/PC (16:84) | 3 x 10^{-7} | 10^{-12} | 0.034 | Yes (1-7x) | Nelsestuen, 1978 |
| Prothrombin (+2 mM Ca) PS/PC (16:84) | 1 x 10^{-6} | 10^{-12} | 0.034 | Yes (1-14x) | Nelsestuen, 1978 |
| Ovalbumin HFL cell PM | 1.2 x 10^{-4} | 10^{-12} | 0.017 | No | Krumins and Stotzky, 1980; Burghardt and Axelrod, 1981 |
| Lysozyme HFL cell PM | 2.5 x 10^{-5} | 10^{-12} | 0.017 | Yes (1-30x) | Krumins and Stotzky, 1980 |
| n-Valeramide Egg PC | 1.2 | 0.32 | 0.017 | No | Lange et al., 1974 |
| TEMPO DOPC | 20 | 0.31 | Yes (7x) | Dix et al., 1978 |
| Lindane DOPC | 1.3 x 10^{5} | 0.51 | Yes (31x) | Omann and Lakowicz, 1982 |

TEMPO, tetramethyl piperidine-N-oxyl; PC, phosphatidyicholine; DPPC, dipalmityl PC; DOPC, dioleoyl PC; PA, phosphatidic acid; PS, phosphatidylserine; PM, plasma membrane.

*K_m refers to equilibrium dissociation constant, *K_a for nonspecific membrane-reactant binding, or to the volume partition coefficient, *K_v units of molarity and the italicized *K_v values are dimensionless. *K_α = 0.0199 *K_v where *K_v is the mole fraction-based partition coefficient and *K_α is the weight-based partition coefficient.

1 *c_v is the concentration of the reactant in the aqueous phase. *J_2/*J_3 is assumed independent of *c_v for those reactants characterized by a partition coefficient.

2 *D_2/*D_3 is the ratio of 2d to 3d translational diffusion coefficients. Italic values indicate that *D_2 is known and *D_3 is either known or calculated as in Table I (assumes a cytoplasmic viscosity 3.2 times that of pure water at the same temperature). Values not in italics are hypothetical for illustration.

3 Theoretical rate advantage is determined by evaluation of *J_2/*J_3 in Eq. 2. We have assumed a dilute target density *p = 10^{9}/cm² (see text for details). The organelle radius *a = 0.5 µm and the effective reaction radius *s = 12Å + *r, where *r is the radius of reactant and 12Å is the radius of target. For proteins, *r is taken from the literature or estimated from the protein’s molecular weight, a specific volume of 0.7, and the assumption of a spherical shape; *r for ACTH is estimated from amino acid composition (Gysin and Schwyz, 1984), molar volumes of amino acid residues, and the assumption of a spherical shape. Values of *p_0 (in molecules per square centimeter) used in calculations are:

- ovalbumin (23.1, 1.06 x 10^{10}); lysozyme (15.3, 3.15 x 10^{10}); n-valeramide (3.8); TEMPO (3.2); lindane (4.7). An area per lipid molecule of 60 Å² was assumed.

4 November 8, 1982.

5 Table II. Theoretical Rate Advantage from Two-Step Searching by Aqueous Reactants for Hypothetical Membrane-bound Targets

for a favorable two-step search becomes (see Table II):

\[
\frac{J_2}{J_3} \approx \left( \frac{\pi}{1.1s} \right) \frac{D_2}{D_3} \left( \frac{\rho_0}{K_\alpha + c_w} \right) \left( \ln \frac{1.2}{4\pi \rho s^2} \right)^{-1} \tag{2a}
\]

or

\[
\frac{J_2}{J_3} \approx \left( \frac{1.4 \times 10^{-6} \, \text{cm}}{s} \right) \frac{D_2}{D_3} \left( \frac{K_v}{\ln(1.2/4\pi \rho s^2)} \right) \tag{2b}
\]

\[D_2/D_3 \] and \[s\] are as defined above, \[\rho\] is the surface density of targets in molecules per square centimeter, \[c_w\] is the concentration of the reactant in the aqueous phase in molecules per cubic centimeter, \[K_\alpha\] is the equilibrium dissociation constant in molecules per cubic centimeter, and \[\rho_0\] is the surface density (number per square centimeter) of the nonspecifically adsorbed reactant when \[c_w\] is much greater than \[K_\alpha\]. \[K_v\] is the partition coefficient expressed as the ratio of molarity in the membrane to that in bulk solution. The ratio \[J_2/J_3\] must be greater than 1 for two-step searching to confer an advantage.

Nonspecific Membrane-Substrate Affinities

If one imagines that the bulk (cytosolic) concentration of a substrate is kept at a steady level by synthesis and consumption, then diffusion from the cytosol to the outer surface of an imaginary 1-µm-diam organelle may be modeled by Eq. 1. In Table II we have plugged values from the literature of the parameters into Eqs. 2a and 2b to get an idea of how many molecules might fit the criteria required for an advantageous two-step searching process on intracellular membranes. We do not imply that all of these molecules are found intracellularly, nor that they are actual substrates of membrane-bound enzymes; we use them just to fathom the range of affinities and \[D\] values expected for nonspecific binding.

There is an obvious lack in the literature of complete data on individual biochemical systems; until these data become available one is limited to such an approach. As in Table I, the \[D_3\] values pertain to a cytoplasmic viscosity 3.2 times that of pure water. Evidently, possession of a moderate affinity for membranes is no guarantee that a substrate will find its enzyme faster with the aid of surface diffusion than without, even when that diffusion is nearly as rapid as in the bulk. This is in sharp contrast to the assertion of Richter and Eigen that “Whenever there exists a nonspecific affinity of the membrane for the respective substrate molecules, a rate enhancement may be expected due to diffusion on that membrane” (Richter and Eigen, 1974).²

Successful application of dimensional reduction requires a...
Table III. Estimated Concentrations of Membrane-associated Enzymes and Their Aqueous Substrates

| Enzyme/protein* | Membrane* | \( \rho \) | Substrate(s)* | \( K_m (\text{a}) \) | Notes |
|-----------------|------------|-------------|----------------|----------------------|-------|
| Phosphate translocat or <br> P-450c (dioxin-induced) <br> NADH dehydrogenase <br> ATP-ADP translocat or <br> P-450ins (uninduced) <br> Ca-Mg ATPase <br> Glyceral-3-P dehydrogenase <br> Nic-K ATPase <br> Ferrooxin-NADP+ reductase <br> H+ ATPase <br> p-Amino acid dehydrogenase <br> Ca-Mg ATPase <br> Dopamine-β-hydroxylase <br> SVF spike glycoproteins <br> HMG CoA reductase <br> α-6 Sialyltransferase <br> Epoxide hydrolase <br> H+ ATPase <br> Succinate dehydrogenase <br> Cyclic GMP phosphodiesterase <br> Pyridine nucleotide transhydrogenase <br> Glycerol-3-P acyl transferase <br> D-,L-Lactate dehydrogenase <br> NADH dehydrogenase <br> NADPH P-450 reductase <br> N-acetylglucosaminyl transferase I <br> Galactosyltransferase <br> UDP-galacturonyl-transferase (uninduced) <br> SVF spike glycoproteins <br> UDP-galactose:LPS galactosyltransferase <br> Carnitine palmityl-transferase <br> Phosphatidylinositoll synthase <br> Phosphatidylserine synthase <br> α-3 Sialyltransferase | CE (spinach) <br> ER (rab. liver) <br> PM (E. coli) <br> IMM (rat liver) <br> ER (rab. liver) <br> SR (avn. mus.) <br> PM (E. coli) <br> IMM (rat hrt.) <br> PM (E. coli) <br> CT (spinach) <br> IMM (rat) <br> ROS (bovine) <br> IMM (rat) <br> IMM (cow hrt.) <br> PM (E. coli) <br> PM (E. coli) <br> HMG (BHK cells) <br> GC (UT-1 cells) <br> GC (rat liver) <br> GC (E. coli) <br> GC (cow hrt.) <br> GC (E. coli) <br> GC (BHK cells) <br> ER (E. coli) <br> GC (HeLa cells) <br> GC (E. coli) <br> ER (E. coli) <br> GC (E. coli) <br> ER (BHK cells) <br> PM (S. typhimurium) <br> IMM (cow hrt.) <br> ER (yeast) <br> ER (yeast) <br> GC (E. coli) <br> GC (E. coli) <br> | 9 × 10^{-11} <br> 6.5 × 10^{-11} <br> 6 × 10^{-11} <br> 3 × 10^{-11} <br> 2.7 × 10^{-11} <br> 1.6-3.0 × 10^{-11} <br> 1.9 × 10^{-11} <br> 1.6-1.9 × 10^{-11} <br> >1.6 × 10^{-11} <br> 1.4 × 10^{-11} <br> 1.2 × 10^{-11} <br> 0.6-1.5 × 10^{-11} <br> 8.9 × 10^{-10} <br> 7.5 × 10^{-10} <br> 7 × 10^{-10} <br> 6.5 × 10^{-10} <br> 1.1-5.9 × 10^{-10} <br> 2.5-5.5 × 10^{-10} <br> 5.3 × 10^{-10} <br> 1.4-7.2 × 10^{-10} <br> 1.3-6.7 × 10^{-10} <br> 2.9 × 10^{-10} <br> 5.3 × 10^{-10} <br> 1.4-2.0 × 10^{-10} <br> 1.6 × 10^{-10} <br> 1.0 × 10^{-10} <br> 2.9 × 10^{-10} <br> 1.3 × 10^{-10} <br> >10^{-10} <br> 9 × 10^{-9} <br> | P3, 3PGA <br> NADH <br> ATP, ADP <br> ATP, ADP, P <br> ATP <br> ATP <br> P3, ADP <br> ATP <br> Octene-1,2-oxide <br> 3PGA <br> P <br> NADPH <br> UDP-NGlnAc <br> Glucose-6-P <br> NADH <br> NADPH <br> UDP-Gal <br> UDP-Gal <br> | 2.5 (7-10), 0.72 (2.2) <br> 0.05 <br> 0.15, 0.01 <br> ~1 <br> 5 <br> f, g <br> (0.2), (0.2) <br> 1.32 <br> 3-40 <br> >2.5-5.5 × 10^{-10} <br> Succinate <br> cGMP <br> NAD*, NADPH <br> (7) <br> 1 <br> 0.15 <br> 0.300 (0.14) <br> 0.006 (0.14) <br> 0.006 (0.14) <br> 0.006 (0.12–0.36) <br> 0.5-4 <br> 0.16 <br> 5.4 <br> 0.074 <br> 0.2-0.29 (2.5-4.8) <br> (0.1) <br> 0.58 <br> | a |
molecules, e.g., the endogenous and xenobiotic substrates for microsomal cytochrome P450's. These molecules spend a vast majority of their time in the membrane, and it is not too surprising that enzymes that act on them are also membrane bound. To argue that things are this way because of kinetic advantages due to 2d diffusion would be superfluous. Furthermore, the actual translation of small, rapidly diffusing molecules within the hydrophobic core may deviate enough from 2d to negate the theoretical rate advantages of the 2d random walk.

Perhaps the most serious consequence of promiscuous bonding is that, by definition, membranes throughout the cell will sequester substrates, diverting them from membranes containing the specific target enzymes. No such problems exist for nonselective trapping of air-borne pheromones by antennae of Bombyx mori (Adam and Delbrück, 1968), but for intracellular reactions in eukaryotes this concern is real: With a modest partition coefficient of 300 and a membrane thickness of 50 Å, stereologic analyses show that in a typical eukaryotic cell 95% of the substrate would be membrane bound, most of it to the wrong membrane (Buschmann and Manke, 1981; Blouin et al., 1977; Griffiths et al., 1984). Unless the enzymes that made the substrates were themselves localized near a particular set of target membranes, reduction of dimensionality could be quite deleterious.

**Substrate Concentrations In Vivo**

Adam and Delbrück (1968) specified that reduction of dimensionality would be most useful where "small numbers of molecules and their diffusion are involved," and this fact is evident in Eq. 2a and also in Table II, where most entries show a cross-over from impediment to advantage as the bulk concentration of reactants is lowered. Note, however, that the intracellular concentrations of aqueous metabolites acted on by membranous enzymes are often closer to milli- than to nanomolar (Table III) and cannot automatically be considered "small numbers." (We have used $K_m$ values as crude indicators of substrate levels when the latter were hard to find or equivocal, and have not corrected for depletion due to specific binding).

**Actual Target Densities In Vivo**

For those molecules in Table II that could experience a rate enhancement by guided diffusion, we must ask how realistic our dilute target approximation is. Eq. 2 is valid only for reactive fluxes that are small as compared with the maximal possible flux ($J_{1,\text{max}}$) reaching the surface, so for Table II we have assumed a target density ($\rho = 10^6$ cm$^{-2}$), giving $J_1/J_{1,\text{max}} = 0.03$ for a reaction radius $s = 17$ Å. Because of the saturation behavior noted above it is only when the lateral density of target enzymes is very low ($P \ll 1$) that two-step searching can yield a rate advantage.

What is known about the lateral densities of membranous enzymes? Are they low enough for two-step searching to confer an advantage? The densities listed in Table III span three orders of magnitude, yet the density assumed in our calculations for Table II is beneath the low end of the spectrum. In fact, 84% of the proteins are present at a density of at least $10^9$ cm$^{-2}$, which means that for all these the maximum possible enhancement according to the steady state model is $\leq 3 \times$, under ideal conditions of $K_m$, $D_2/D_1$, and $c_{\text{eff}}$. Although the numbers in Table III are inexact, they were obtained by a variety of methods for organisms in four separate kingdoms; most are better than order-of-magnitude estimates. Thus it appears that the frequency of enzymes that may benefit appreciably from two-step searching on intracellular membranes is low.\footnote{A few entries are for plasma membrane enzymes of procaroytes and one is for a mammalian plasma membrane enzyme. It is interesting that the $p$ values for these systems fall in the same range as those for enzymes of the intracellular organelles of eukaryotes. Because the dimensions of most bacteria are also comparable to those of eukaryotic organelles, one is tempted to suggest that two-step searching is unlikely to be important in either system. But the main reason for including these values is to broaden the scope of the Table, which is interesting outside the immediate context of reduction of dimensionality.} It would be surprising if a non-steady state calculation analogous to that of Adam and Delbrück (1968) would lead to a conclusion drastically different from that reached here, although such a calculation has yet to be performed (but see Torney et al., 1985).

**What Happens in the Absence of Surface Diffusion?**

Without surface diffusion, when one reactant in an elementary bimolecular reaction is removed from the bulk and placed on a surface, the rate constant (in 3d units) can only go down (Astumian and Schelly, 1984). Schurr (1970) also concluded that in the absence of surface diffusion (and neglecting any ability of the membrane to concentrate substrate), when a single type of enzyme is immobilized $V_\text{max}$ can only go down or remain the same, and the reverse is true for $K_m$. This potential for slowing reactions by embedding enzymes in membranes makes the possibility seem even more remote that membrane-enzyme association was entrained primarily because of a kinetic advantage due to dimensional reduction (Eigen, 1974).

**Experimental Systems**

**Redox Reactions at Surfaces**

Most discussions on the role of dimensional reduction in biology and chemistry have been speculative or theoretical in nature, but some quantitative experiments have been done. It is known that disproportionation of certain radical ions occurs at a rate $10^2-10^3$ times greater on micelle surfaces than in the bulk (Frank et al., 1976; Henglein and Prosko, 1978). Photooxidation of cytochromes c and c$_2$ by bacterial photosynthetic reaction centers incorporated into phospholipid vesicles can be eight times faster when the cytochromes are adsorbed to the membrane than when they are diffusing in the bulk (Overfield and Wraith, 1980). These rate enhancements have been attributed to a reduced dimensionality for diffusion, although a simple calculation shows that the effective 3d concentrations of the adsorbed reactants could have contributed substantially to the observed rate enhancements. Note also that the maximal eightfold enhancement observed for bacterial reaction centers may bear little relevance to photosynthetic function in Rhodopseudomonas spheroides, since it occurs only at an ionic strength well above that of the periplasmic space, which contains cytochrome c$_2$ (Overfield and Wraith, 1980). At "physiological" ionic strength the reaction of adsorbed cytochromes is retarded ($\approx 10$ times) as compared with that of bulk-dissolved cytochromes.
Bacteriophage Infection

It has been argued that in certain bacterial species lipopolysaccharide mediates an initial “nonspecific” adsorption of bacteriophage, which then facilitates binding of the phage to other specific surface receptors via an Adam-Delbrück two-step process (Heller and Braun, 1979; Wong et al., 1978). This argument is questionable on several counts: (a) The data on infection of Salmonella anatum by λ (Heller and Braun, 1979) fit a two-step better than a one-step search, but this two-step search is slower than the one-step. (b) Interaction of phage with lipopolysaccharide is a highly specific event (Lindberg, 1973), hardly the nonspecific adsorption envisioned by Adam and Delbrück (1968): Slight mutations in the lipopolysaccharide structure of Escherichia coli F completely reverse the tail fiber-mediated enhancement in the rate of T5 phage adsorption reported by Heller and Braun (1979). (c) Since the enhancement observed with the latter system is mediated by phage tail fibers, one has to be sure that they alone are not increasing the capture cross-section, but no relevant calculations were performed. Phage-bacterium binding can be nearly as rapid as the theoretical diffusion-controlled rate even at very low receptor densities, and a hyperbolic dependence of rate constant on receptor density is observed upon systematic manipulation of the latter (Schwartz, 1976). It is interesting that these findings are exactly those predicted by Berg and Purcell (Eq. 1) for simple one-step (3d) searching.

Fatty Acid Biosynthesis

Sumper and Trauble (1973) proposed that in yeast, fatty acyl-coenzyme A is released from the fatty acid synthetase complex only upon collision of fatty acid synthetase complex with an acceptor membrane, whereupon it diffuses laterally until it finds an enzyme that processes it. This is certainly an appealing mechanism, since free fatty acids and fatty acyl-coenzyme A bind to and inhibit some soluble enzymes. But in attributing a kinetic advantage to this hypothetical scheme, the authors did not consider the saturation effects of target density discussed in the previous section, i.e., they used the bullet-shooting analogy for diffusion. If one modifies their calculated 3d mean lifetime with the probability factor of Berg and Purcell (1977) the purported rate advantage drops to nearly zero.

Polypeptide Hormone Binding

Several polypeptide hormones such as glucagon, ACTH, calcitonin, and β-endorphin have amphiphilic structures that are essential for biological activity (Kaiser and Kézdy, 1984). Gysin and Schwyzer (1984) suggest that this amphiphilicity accelerates binding of the hormones to membrane-associated receptors by promoting two-step searching. The Kd for nonspecific binding of ACTH to negatively charged lipid vesicles is fairly low (10^{-4} M) (Gysin and Schwyzer, 1984), but the important 2d diffusion coefficient has not been measured. In any case, two-step searching via nonspecific binding of hormones to membranes seems counterproductive, since these hormones exist at extremely low concentration (10^{-6} to 10^{-12} M) (Tepperman, 1980), and the relatively high nonspecific affinity required for an advantageous 2d search would tie up precious hormone in nontarget tissues throughout the body. References to further discussion on possible biological applications of dimensional reduction can be found in the review by Axelrod (1983).

Other Considerations of Membrane-associated Reactions

In this paper we have focused on just one aspect of the kinetics of membrane-associated reactions, namely, potential rate enhancements arising solely from the recurrent nature of the 2d random walk. However, localization of enzymes in membranes could conceivably yield several benefits that are independent of surface diffusion, kinetic and otherwise. Reduced transient times have been observed in artificial immobilized multienzyme systems (Mosbach, 1976; Welch and Gaertner, 1975), and may facilitate rapid adjustment to differing metabolic rates in vivo (Gaertner, 1978). In some sequential reactions even the steady state rates might be increased by proximity of the enzymes or diffusional resistances (unstirred layers) near the membrane surface (Gaertner, 1978). The local compartmentation of intermediates responsible for these effects also lowers the bulk substrate concentration necessary to sustain a given steady state rate. Atkinson (1969) has emphasized the finite solvent capacity of intracellular water and suggests that devices that maintain a low bulk concentration of substrates are favored by natural selection. “Channeling” of common intermediates through anabolic or catabolic pathways is often considered a function of multienzyme assemblies, be they on the membrane or elsewhere (Welch, 1977; Freedman, 1981). Even for a single enzyme, partitioning of substrates into or on the membrane could yield higher effective concentrations, and maintain orientations favorable for reaction (Fendler and Fendler, 1975; Nesheim et al., 1981). Increased sensitivity to small changes in the concentrations of substrate, product, and effector molecules opens new possibilities for regulation mechanisms with immobilized enzymes (Engasser and Horvath, 1976; Lecoq et al., 1975). Most of these potential advantages have yet to be demonstrated for multienzyme systems held together only by a phospholipid bilayer; but in comparison with these and other very obvious nonkinetic factors, the contribution that dimensional reduction, i.e., two-step searching, makes to any “selective advantages” of intracellular membranes would seem to pale.

Conclusions

The theoretical predictions of Adam and Delbrück (1968) have been tested in model 2d systems. As predicted, dimensional reduction or two-step searching can enhance as well as retard reaction rates. It remains to be seen whether the rate enhancements actually derive from the special properties of 2d diffusion and reaction or from other sources such as increased local reactant concentrations. The hypothesis that dimensional reduction was entailed by natural selection demands a delicate balance between two seemingly opposing factors: rapid 2d diffusion and strong nonspecific attraction. Examination of available data on diffusion coefficients and binding constants, scarce and imprecise as they may be, suggests that this balance is not generally met. Even when it is met, theory predicts diminishing returns from dimensional reduction as the target density and bulk phase substrate concentration are increased. From estimates of the lateral densities of several membranous enzymes and from the endogenous levels of their substrates, it appears that the maximum rate advantage to be expected from two-step searching on intracellular membranes is in most cases small to nil. Thus, the teleological notion that internal membranes may
somehow have “capitalized” on Polya’s theorem remains a cure in search of a disease.

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