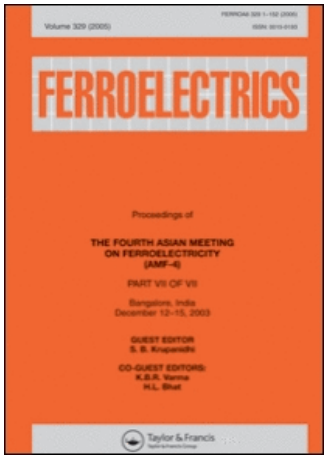

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Mechanisms for the interaction between nonstationary electric fields and biological systems I. Linear dielectric theory and its limitations

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MECHANISMS FOR THE INTERACTION BETWEEN NONSTATIONARY ELECTRIC FIELDS AND BIOLOGICAL SYSTEMS I. LINEAR DIELECTRIC THEORY AND ITS LIMITATIONS

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Our interest in the role of electric interactions in enzyme catalysis and biological free-energy transduction prompts us to examine to what extent dielectric phenomena, experiments, and theory may bear on these issues. In this paper we review linear dielectric theory and show that issues of interest for catalysis and free energy transduction lie outside the scope of linear dielectric theory (even in a somewhat extended form). This is due to the rather strict limitations imposed by the definition of linearity, which we discuss in detail. The review given here will provide a basis for the elaboration of nonlinear dielectric theory able to address the interaction between nonstationary electric fields and enzyme catalysis.

1. INTRODUCTION

Electric potentials play central roles in organisms, both in free-energy transduction [1,2] and in signal transmission [3,4]. Consequently, it is hardly surprising that exogenous electric fields have been shown to affect the physiology of living systems [5–10]. Yet, three problems surround our understanding of such physiological effects. The first [11,12] is that at frequencies below 1 MHz or so, the impedance (= effective resistance) of biomass is so much higher than that of extracellular fluids that, especially without connecting electrodes, electric fields in the air will hardly penetrate organisms.

The second problem occurs more generally in cell biology: even if one knew what the effects of electric fields were at the level of the elementary biochemical and biophysical reactions, it would still be a nontrivial problem to relate these effects to observations at the physiological level. It is only recently that

developments in theoretical biology, such as the emergence of mosaic non equilibrium thermodynamics and metabolic control theory (reviewed in [2]) have built the first pillars in the gap between molecular and physiological events. Both theories have served to identify the conceptual differences between fluxes through metabolic pathways and the turnover numbers of enzymes that participate in them [2,13–24]. Yet, even these theories are not sufficient to completely illuminate the complexities of organized cellular metabolism [17 and references therein].

These two twin papers are concerned with a third major problem, i.e., the problem that, especially in an area as interdisciplinary as bioelectrochemistry, it is often unclear as to what are reasonable expectations of how a dynamic electrical field can affect the turnover number of an enzyme, and therefore what constitutes a satisfactory explanation of a bioelectrical phenomenon at the enzyme level [25]. The search for mechanistic explanations has been furthered by experimental demonstrations in well-defined *in vitro* systems of effects of nonstationary electric fields on single biochemical reactions (reviewed in [20] and [26]).

In these two papers therefore, we combine dielectric theory, the theory of enzyme kinetics and non equilibrium thermodynamic principles to find out how dynamic electric fields and enzyme catalysis may interact. One subsequent aim is to investigate if dynamic electric fields may already play an endogenous role in processes of biological free-energy transduction. A second will be to design a variant of dielectric spectroscopy that may be exclusively sensitive to detecting enzyme properties that are involved in such processes. In the present paper, we will consider the limitations of linear dielectric theory *vis a vis* the mechanistic description of biological processes. It will become apparent that, despite the immense body of work that has been carried out on the linear, passive electrical properties of biological systems (reviews: [25,27–32]), linear dielectric theory is incongruent with enzyme kinetics and not apt to describe the interactions of enzymes with dynamic electric fields. We conclude that an extension to the non-linear domain, and the development of the formalisms relevant to such non-linear dielectric properties, represent urgent necessities. The accompanying article provides one such extension.

2. THE "LINEAR", PASSIVE ELECTRICAL PROPERTIES OF CONDENSED MATTER

2.1. Permittivity and conductivity for a single relaxation process: Debye dispersion

In this section we shall summarize linear dielectric theory. As discussed in several biologically orientated reviews and monographs (e.g. [27–32]), the "linear", passive electrical properties of condensed matter, including biological systems, are completely characterized by their frequency-dependent conductivity $\sigma(\Omega)$ and permittivity $\epsilon(\Omega)$ (where Ω is the frequency of the sinusoidal input electric field). $\sigma(\Omega)$ and $\epsilon(\Omega)$ are commonly assessed by measuring the conductance, G, and electrical capacitance, C, of an electrochemical cell containing the substance of interest. In a standard set-up for dielectric experiments, one places a sample between two electrodes, and measures the potential difference developed as a

result of the movement of charge (by the use of an external current source). Alternatively, one sets a certain voltage and determines how much charge must move between the electrodes to sustain this voltage. In practice, applied voltages or currents are not constant but vary periodically with time. In ideal cases the applied current or voltage is a sinusoid of a single frequency. It is simplest to view the electrodes as the two plates of a parallel-plate capacitor. The electric field between the plates of the capacitor is determined by the total displacement of charge, consisting of the charge (D) displaced from one plate to the other by the external current source and the charge displacement resulting from the electric polarization (P) of the material between the plates. Since the net displacement of charge amounts to $D - P$, electrostatic considerations require that for the electric field E:

$$\epsilon_0 E = D - P \tag{1}$$

where ϵ_0 is the permittivity of free space ($= 8.854 \times 10^{-12}\,\mathrm{F\,m^{-1}}$). The polarization of the material between the capacitor plates consists of the movement of charges and the reorientation of permanent dipoles, as well as the induction of dipole moments [28]. Since these processes take time, the polarization and the dielectric displacement will lag behind the electric potential. The time lag (or rather the relaxation time) will depend on the magnitude of the apparent resistance versus polarization as well as on the extent of polarization (the apparent capacitance).

In linear dielectric theory it is assumed that the polarization current is linearly related to the field, or:

$$dP/dt = k_E E - k_{-1} P \tag{2}$$

k_E and k_{-1} are first-order rate constants. This equation also expresses the fact that, because the polarization involves the ordering of molecules as well as the increase of counteracting forces, the rate of polarization is inhibited by the extent of polarization. This inhibition is also taken to be linear. After a step change in electric field, the polarization will approach the value:

$$P_\infty = E\epsilon_0(\epsilon - 1) \tag{3}$$

P_∞ is the polarization at t infinity. The dielectric displacement will approach:

$$D = \epsilon_0 \epsilon E \tag{4}$$

with:

$$\epsilon = \epsilon_s \equiv (1 + k_E/k_{-1})/\epsilon_0 \tag{5}$$

ϵ_s is the "relative" dielectric "constant" of the material between the plates relative to the dielectric constant of vacuum (ϵ_0), for direct current ("DC") measurements.

In the case of a step change in E the polarization will approach the magnitude given by Equation 3 in an exponential manner, with a time constant of $1/k_{-1}$. When the applied electric field is not a step function but continuously varying with a frequency $\Omega/(2\pi)$ as given by $\cos(\Omega t)$, the dielectric displacement will behave as the same cosine, but with a phase shift. This can be seen by solving the differential equation Equation 2 for $E = E_0 \cos(\Omega t)$. The simplest way to do this for the stationary state is to assume that:

$$P \equiv P_0 \cos(\Omega t - \theta) \tag{6}$$

and insert this into Equation 2. The result is:

$$\tan(\theta) = \Omega/k_{-1} \tag{7}$$

and:

$$P_0/E_0 = \epsilon_0(\epsilon_s - 1)/\sqrt{[1 + (\Omega/k_{-1})^2]} \tag{8}$$

θ is the phase angle between the polarization and the electric field, indicating what fraction of the cycle lies between the moment the polarization is maximal and the moment at which the field is maximal. Equation 7 shows that the phase lag between the polarization and the electric field will become greater the faster the field oscillates up to a value of 90° at very high frequencies. The same ratio between frequency and relaxation rate constant of the dielectric (k_{-1}) enters the expression (Equation 8) for the amplitude of the polarization relative to that of the field: at low field frequencies the ratio of polarization to field amplitude is the same as in the case of a constant electric field (Equation 3), whilst at high frequencies the amplitude of the polarization tends to zero.

The variation with time of a sinusoidal electric field can be described as the projection on the x-axis of the position of a point that moves in a circle around the origin at a distance E_0 from it and with an angular velocity of Ω radians per second. In such a description the polarization can be described by a point moving on a circle with radius P_0, again around the origin, at the same angular velocity, lagging an angle θ behind the point describing the oscillating electric field. Such a description is in fact achieved (cf., Figure 1) by treating the oscillating electric field as the real part of a complex quantity (the real part is the projection on the x (or 'real-') axis of a point in the complex plane) and the oscillating polarization as the real part of another complex quantity:

$$\mathbf{E} = E_0 \exp(\mathbf{j}\Omega t) \tag{9}$$

and

$$\mathbf{P} = P_0 \exp[\mathbf{j}(\Omega t - \theta)] \tag{10}$$

(Complex quantities are in bold type.) $\mathbf{j}$ represents $\sqrt{(-1)}$. P_0/E_0 and θ are given by Equations 8 and 7 respectively. One of the advantages of this treatment is that

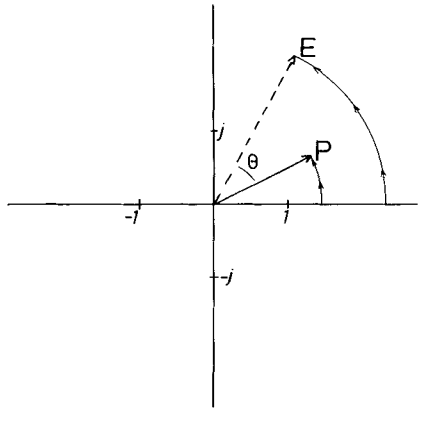

FIGURE 1 The relationship between polarization and the electrical field inducing it. For further details, see text.

Equation 3 is retained, be it that now:

$$\boldsymbol{\epsilon} - 1 = \mathbf{P}/(\epsilon_0 \mathbf{E}) = [P_0/(\epsilon_0 E_0)] \exp(-\mathbf{j}\theta) \equiv \epsilon' - 1 - \mathbf{j}\epsilon'' \tag{11}$$

Here, the real part of the permittivity is:

$$\epsilon' = 1 + (\epsilon_s - 1)/\{1 + (\Omega/k_{-1})^2\} \tag{12}$$

and the so-called dielectric loss ϵ'' equals:

$$\epsilon'' = \{(\Omega/k_{-1})(\epsilon_s - 1)/[1 + (\Omega/k_{-1})^2]\} + \sigma_s/(\Omega\epsilon_0) \tag{13}$$

These equations characterize the so-called Debye dispersion, which is the best known variation of the complex dielectric permittivity with the frequency of the electric field. They follow from the direct solution of the differential equation (Equation 2). They are, of course, consistent with Equations 7 and 8, except that the term $\sigma_s/(\Omega\epsilon_0)$ has been added to the equation for the dielectric loss. This term results from any frequency-independent conductivity, giving rise to an apparent polarization in phase with the electric field. With this treatment the relationship between the charge displacement and the electric field also remains simple:

$$\mathbf{D} = \epsilon_0 \boldsymbol{\epsilon} \mathbf{E} \tag{14}$$

In dielectric measurements one often measures the current between the electrodes rather than the dielectric displacement (using $d\mathbf{E}/dt = \mathbf{j}\Omega\mathbf{E}$):

$$\mathbf{I} = d\mathbf{D}/dt = \boldsymbol{\sigma}\mathbf{E} \equiv (\sigma' + \mathbf{j}\sigma'')\mathbf{E} \tag{15}$$

with the complex conductivity, $\boldsymbol{\sigma}$, consisting of the real component σ' and the imaginary component σ'', being:

$$\boldsymbol{\sigma}/\epsilon_0 = \mathbf{j}\Omega\boldsymbol{\epsilon} = \Omega\epsilon'' + \mathbf{j}\Omega\epsilon' \tag{16}$$

Thus, the imaginary component of the dielectric permittivity, the dielectric loss, corresponds to a real conductivity divided by the frequency of the field:

$$\epsilon''(\Omega) = \sigma'(\Omega)/(\Omega\epsilon_0) = [\sigma_s + \sigma_p'(\Omega)]/(\Omega\epsilon_0) \tag{17}$$

where σ_s is the DC- or low-frequency conductivity and $\sigma_p'(\Omega)$ is the real, frequency dependent conductivity arising from electric polarization. Similarly, the imaginary conductivity (σ'') corresponds to a capacity (real dielectric permittivity, ϵ') multiplied by the frequency of the field:

$$\sigma''(\Omega) = \Omega\epsilon_0\epsilon' \tag{18}$$

It is common to make measurements in the frequency domain, that is to say to measure the voltage and current (and the phase angle θ between them) at a certain frequency, and repeat this at various frequencies so as to build up what is commonly referred to as a dielectric relaxation spectrum. This spectrum may be represented as (i) the amplitude (relative to the exciting field) of the dielectric displacement and the phase angle, (ii) the dielectric permittivity and the conductivity, (iii) the real and imaginary components of the complex permittivity, or (iv) the real and imaginary components of the complex conductivity, all as functions of frequency. For a simple system exhibiting but a single time constant (relaxation time), the dependencies are related through the relationship between

the conductivity and the imaginary part of the permittivity (Equation 17) and (Equations 12 and 13) the variation of ϵ' with ϵ'' as the frequency of the electric field is modulated. Thus:

$$\tau = [\epsilon'(\Omega) - 1]\epsilon_0/[\sigma'(\Omega) - \sigma_s] \tag{19}$$

The relaxation time $\tau = 1/k_{-1}$ (cf., Equation 2) is also given by $1/\tau = \Omega_c = 2\pi f_c$, where f_c is the characteristic frequency, i.e., that frequency at which the dispersion is half-completed as judged by measurement of either ϵ' or σ'. Equation 19 implies that as the frequency is scanned, the change in conductivity is directly proportional to the change in permittivity [28]. The dielectric loss peak of such a (Debye) dispersion is such that it has a breadth of 1.1 decades of frequency at half-peak height.

For each of the four ways of analyzing the dielectric dispersion one may also plot how one component varies with the other component, the frequency being the independent variable. A plot of ϵ'' versus ϵ' using frequency as the parameter gives a semicircle whose center lies on the abscissa and which has the maximum value of $\epsilon''(\Omega_c)$.

Since any periodic waveform may be described (via Fourier's theorem) as the sum of a set of sinusoids of defined amplitude and phase, it is also possible to determine the frequency response of a linear system by measuring the time course of current/voltage flowing in response to a voltage/current step (e.g. [27,33–37] and references therein), or to an extrinsically noisy input [38,39]. Additionally, it has been possible to investigate the kinetics of a number of chemical reactions by periodic field perturbation [40–44], following Schwarz's [45] presentation of a theory for the chemical contribution to the dielectric increment relevant for homogeneous solutions.

2.2. *A Physical Mechanism for "linear" dielectric relaxation*

For heuristic purposes we consider the simplest dielectric relaxation mechanism, namely the orientation of a hard dipolar sphere ("billiard ball", see Fig. 2) in a

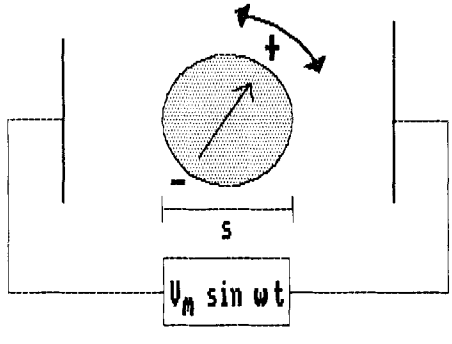

FIGURE 2 The dipolar billiard ball. This type of model constitutes the simplest and classical mechanism of dielectric relaxation. The system studied is taken to consist of a hard dipolar sphere which, in the presence of a sinusoidally-modulated field, will attempt to rotate in train with the field, its rotational mobility being independent of that of any other spheres in the ensemble, and constrained by hydrodynamic forces alone.

non-polarizable Newtonian fluid of macroviscosity η. Here we depict a body of dipole moment $\mu = qs$ Coulomb metres, consisting of a hard dipolar sphere containing point charges $+q$ and $-q$ separated by the diameter s. It is common to express the dipole moment in the non-SI unit Debye where $1\ D = 3.336 \times 10^{-30}$ Cm. The body in Figure 2 is representative of an ensemble of the same. We assume that the dipoles are sufficiently dilute that there are no dipole-dipole interactions. From the Stokes-Einstein relation, such a dipole will have a dielectric relaxation time $\tau = \eta a^3/(k_B T)$ where a is the radius of the sphere, k_B is Boltzmann's constant, η the viscosity of the medium and T is the absolute temperature. In the absence of an exogenous electrical field there will be a random orientation of the dipoles within the ensemble. In the presence of an electric field, the molecular dipoles will orient, which in our model is described by the orientation of the single dipole in the field till it makes an angle α with the field. The resulting component of the dipole moment μ_p in the direction of the field is:

$$\mu_p = \mu \cos(\alpha) \tag{20}$$

In a DC-electric field, the steady orientation of the model dipole (or the average orientation of an ensemble of such molecular dipoles) is dependent on the Boltzmann factor $\exp[-PE/(k_B T)]$. The average magnitude of $\cos(\alpha)$ is given by the Langevin function [28]:

$$\langle \cos \alpha \rangle = \coth(x) - 1/x \approx x/3 - x^3/45 + 2x^5/945 \tag{21}$$

where $x = \mu E/k_B T$. This relationship between $\langle \cos \alpha \rangle$ and x is strongly linear (note the absence of a second order term in x) for values of x up to about 1 [28,46], which is equivalent [46] to an electric field strength per unit dipole moment of 1.2×10^9 V (m Debye)$^{-1}$. In such cases $\langle \cos \alpha \rangle \approx \mu E/3k_B T$, and according to Equations 20 and 21 the average dipole moment becomes:

$$\langle \bar{\mu} \rangle \approx \mu^2 E/(3k_B T) - \mu^4 E^3/(45k_B T)^3 \tag{22}$$

Consequently, whenever $|\mu E| \leq k_B T$, the polarization depends linearly on the electric field.

The conditions of "linearity" employed in the derivation of the Debye equations (12,13) and required for the emergence of a voltage-independent dielectric permittivity, imply (see also above) that the polarization should be a linear function of the DC electric field (Set dP/dt to zero in Equation 2). Equation 22 demonstrates that for orientational polarization of dipoles this is only true within the linear regime of Equation 21. Since the orientation achieved in a sinusoidal field will always tend to be smaller than the orientation achieved in a DC-field of the same amplitude, $|\mu E| \leq k_B T$ also guarantees the applicability of linear dielectric theory for sinusoidal fields to orientational polarization. Put another way, the fact that in the positive going-part of an AC cycle only a minuscule fraction of the dipoles actually moves to align with the field means that their motions do not affect the dielectrically observable behavior during the negative-going phase of the same cycle. It is, most often, correctly considered that in the absence of the field *a priori* energies (i.e., probabilities) of all possible orientations of the dipole are identical, and that during the exposure to the field

the system remains close to internal equilibrium. It is also important to note that the value of E to be used in the general case is actually the local electric field strength (which in our simple example is the same as the macroscopic field strength), but this will in general be unknown, especially for the complex biological systems in which our interest lies.

Let us now pretend that our sphere is actually a spheroidal protein molecule, which for present purposes is assumed to be in internal equilibrium with all its possible conformational and configurational states. (Arguments against the realism of this assumption are summarised for instance in [47–51].) The dielectric increment of the dispersion, $\delta\epsilon'$, which is a macroscopic observable, is related [52,53] to the dipole moment μ and the concentration of solute c mol m^{-3} in a solvent by:

$$\delta\epsilon' = cg\mu^2/(2\epsilon_0 RT) \tag{23}$$

where R is the gas constant and g is a parameter introduced by Kirkwood to account for local interactions between the dipole and the solvent (so that if $g = 1$, there are none). Of course, real proteins are both polarizable and exhibit a substantial and collective conformational flexibility, so that there will, in the case of such proteins, be other contributions to the observable dipole moment and dielectric increment than those caused by Debye-like rotation. However, it is usually taken that these effects are both small and fast [27,32,54,55], so that over the usual frequency range considered in this case (up to say 10 MHz) they are ignored at this stage of the development. Another reason why this cannot be accurate, however, is that the catalytically-relevant transitions in enzymes occur with time constants around a millisecond, and many of these transitions should be expected to be electrically active (e.g. [48,56]).

2.3. What is meant by "linear"

Referral to the simple example of relaxation of molecular polarizations given in the previous section as relevant for the dielectric behavior of actual systems carries with it many implicit assumptions, which are necessary to lead to linear dielectric behavior. Thus, if more than one (type of) dipole is present, the superposition principle is assumed to apply. That is, it is assumed that the dielectric relaxation spectrum is described by equations (12) and (13) summed over all of the dipoles present. This requires the motions of the (dipolar) particles to be independent of each other, and to be dependent only upon the strength of the exciting field. The field is assumed not to change the permanent dipole moment of the particles in the system. Collective motions are neglected, and the force exerted by the field on the dipoles is entirely independent of the instantaneous state of the system; thus, the particles rotate to and fro in train with the positive- and negative-going phases of the alternating voltage. After application of the electric field the dipoles in the system are assumed to approach equilibrium with first-order kinetics, leading to the production of heat by the expression of frictional forces between the protein dipole and the solvent bath. Further, any non-linearity occurring due to the properties of the Langevin function is assumed to result simply in a lower apparent admittance as the voltage

is increased, with the electric currents flowing only at the same sinusoidal frequency as the exciting voltage.

This enumeration of assumptions in the usual discussions of relaxation of an ensemble of oriented dipoles leaves the impression that linear dielectric theory is rather limited and only relevant for cases in which the various polarizations are completely independent of one another. We shall now attempt to examine this impression in more detail. To this end we shall first give a precise definition of the properties required for a system to exhibit "linear" dielectric behavior. This is important for our purposes, because there are many possibilities by which nonlinearities may be introduced into the system and it is our purpose to investigate what types of nonlinearities can induce the system to behave in a way such that it betrays its molecular mechanisms. In the back of our minds, we shall have enzymes as the system of principal interest.

Basically, a "linear" dielectric system is a system the electric behavior of which can be simulated by a linear electric network. A linear electric network consists of a number of ideal capacitances, resistances and inductances interconnected in any possible way. An ideal resistance is one in which the current is strictly proportional to the voltage. In an ideal capacitance the current is proportional to the change of the voltage with time, whereas the voltage across an inductance is proportional to the change of the current with time. Finally, there is an external voltage source.

Writing the external voltage as E, realizing that electric current is the analogue of the rate of change of the polarization and that polarization itself is the analogue of an internal voltage, the condition of linearity can also be formulated as:

$$dP/dt = \sum_k dP_k/dt = \sum_k \left(k_k E - \sum_m k_{-km} P_m \right) \tag{24}$$

where P_k is the component of the polarisation due to component k (cf. Equation 2).

This equation implies three conditions: The polarization may consist of a number of polarizations, each with its own relaxation depending (i) linearly on its own polarization and (ii) linearly on the electric field, (iii) in such a way that these dependencies are additive. Condition (i) means that k_{-m} be independent of P_m, condition (ii) means that k_k be independent of E, and condition (iii) means that k_{-m} be independent of E and k_m be independent of P_m.

In a simple case any polarization P_m is independent of any other polarization P_n. However, Equation 24 and the requirement of "linearity" does allow the different polarizations to depend on each other in the sense that the relaxation rate of any polarization depends linearly on any of the other polarizations. This generalization is important for the discussion of the dielectric behavior of enzymes. As most macromolecules, each enzyme can occur in many states which differ in various thermodynamic properties [2,26,46,56], including dipole moment. For catalysis it is essential that the enzyme can progress through a cycle of such states and hence important that transitions between some (but not all) states are possible. Denoting each enzyme state by its polarization (i.e., dipole moment divided by the total volume) the time dependence of each polarization depends linearly on all other polarizabilities [2]. The relaxation behavior of the entire system may

then be described by:

$$d\mathbf{p}/dt = -M\mathbf{p} + k_E\mathbf{E} \tag{25}$$

Here $\mathbf{p}$ represents a column vector of length n, where n is the number of independent polarizations. For enzymes this is the number of enzyme states minus 1. k_E represents a column vector of length n with rate constants. M is an $n \times n$ matrix with rate constants. We write $\mathbf{E}$ as $E_0 \exp(\mathbf{j}\Omega t)$ and $\mathbf{p}$ as $\mathbf{P}_0 \exp(\mathbf{j}\Omega t)$, where $\mathbf{P}_0$ is a vector of n components. The mth component (corresponding to the mth polarization or enzyme state) of $\mathbf{P}_0$ is written as $P_{m0} \exp(\mathbf{j}(\Omega t - \theta_m))$. P_{m0} is a real number indicating the amplitude of the mth component. $\mathbf{P}_0$ is a complex number comprising both the amplitude and the phase of the mth component in the usual fashion. Noting that $d\mathbf{p}/dt = \mathbf{j}\Omega\mathbf{p}$, multiplying the resulting form of Equation 25 by $\exp(-\mathbf{j}\Omega\mathbf{p})$ inv$(M + I\mathbf{j}\Omega)$ one obtains the stationary solution to Equation 25 as:

$$\mathbf{P}_0 = (M + I\mathbf{j}\Omega)^{-1} k_E E_0 \tag{26}$$

I represents the identity matrix ($n \times n$ with all zeros except for 1's on the main diagonal). Here it is assumed that the matrix $(M + I\mathbf{j}\Omega)$ is nonsingular. $(M + I\mathbf{j}\Omega)^{-1}$ is its inverse.

This matrix becomes singular when $\mathbf{j}\Omega$ is an eigenvalue of M. This implies that the system would respond to a step increase in an electric field by undamped oscillatory behavior. For the case of an oscillatory electric field one then would find strongly resonant behavior of the polarization at that value of the frequency of the input field. In biological dielectric spectroscopy such strongly resonant behavior is rarely observed (except of course at very high, >THz frequencies). This is not unexpected, since, for a relaxation matrix to have complex eigenvalues, the relaxation must involve relatively strong interaction of two or more processes. Such coupling most often requires the action of an enzyme. And, for a single enzyme, the relaxation matrix M cannot have purely imaginary eigenvalues, though its eigenvalues may be complex [57]. If the eigenvalues of a relaxation matrix have an imaginary component, then the response to a step in the electric field will be a damped oscillation and the response to an oscillatory electric field may exhibit a maximum with respect to frequency.

In the case that all the polarizabilities contribute equally to the overall polarization $\mathbf{P}$, Equation 26 leads to the following expression for the complex dielectric permittivity:

$$\epsilon(\Omega) = (1\ 1\ 1\ 1\ 1 \cdots 1)(M + I\mathbf{j}\Omega)^{-1} k_E \tag{27}$$

Thus, even though in this most general linear case the frequency-dependence of the real and imaginary parts of the complex permittivity do not obey the Debye equations 12 and 13, the complex permittivity is still independent of the amplitude of the electric field. Its real and imaginary components are determined by the relaxation rate constants of all the individual polarizabilities (contained in M) as well as by the rate constants by which the polarizations respond to changes in the electric field (the vector k_E).

Noting that the complex conjugate of the real k_E must equal k_E, and that M is the matrix of real rate constants, we take the complex conjugate of both sides of Equation 27 such that:

$$\epsilon(-\Omega) = \bar{\epsilon}(\Omega) \tag{28}$$

Here $\bar{\epsilon}(\Omega)$ is the complex conjugate of ϵ. Equation 28 can also be expressed as $\epsilon'(-\Omega) = \epsilon'(\Omega)$ and $\epsilon''(-\Omega) = -\epsilon''(\Omega)$. Equation 28 is an important symmetry relationship (see section 2.4) and it can be proven in a more general way: If one describes an electric field by $E_0 \exp(\mathbf{j}\Omega t)$, then the mathematically different description taking the negative frequency *and* the complex conjugate of that field, is physically identical (in both cases the field circles in the counterclockwise direction around the origin of the complex plane). In fact this is true for any periodic signal. Since both **D** and **E** in Equation 14 must have this symmetry property and because the complex conjugate of a ratio of numbers is the ratio of their complex conjugates, ϵ must also have this symmetry property. What tends to remain implicit in the above description, but is of utmost importance for what follows in Section 3 and in the accompanying article, is that the appearance of any frequency components (even harmonics) other than those of the input can never be explained in the context of linear dielectric theory: $\epsilon(\Omega)$ does not depend on what may happen at other frequencies. Note that while the theory presented here can describe the linear dielectric response of a system subjected to an input field with an arbitrary Fourier spectrum, the output spectrum in that case will contain, in principle, components at all input frequencies. This should not be mistaken for "nonlinear" behavior, which may be diagnosed (see below and in the accompanying paper) by the appearance of frequency components in the output which are not contained in the input.

2.4. *The Kronig-Kramers relations*

If one wishes to know both the real conductivity and the real permittivity at a certain frequency, then one can measure the in-phase and the 90° out-of-phase currents in response to a sinusoidal electric field of that frequency (cf., Equation 15 and 18). In the event that out-of-phase measurements are impossible, one may however make use of the so-called Kronig-Kramers relations, which relate ϵ' to the complete dielectric spectrum of ϵ'' and *vice versa*:

$$\epsilon'(\Omega) - \epsilon_\infty = -(2/\pi) \int_0^\infty \epsilon''(f) f / (f^2 - \Omega^2)\, df \tag{29}$$

$$\epsilon''(\Omega) = -(2/\pi)\Omega \int_0^\infty (\epsilon'(f) - \epsilon_\infty)/(f^2 - \Omega^2)\, df \tag{30}$$

The original proof of these equations exists in publications that are not easily accessible ([58,59]; for accessible accounts see [35,36,60,61]). Since we shall want to examine the requirements for the Kronig–Kramers relations to be valid in dielectrics, we shall now give a short proof of these relations, which is somewhat different from that given by Macdonald and Brachman [60].

For this purpose we define $\hat{\mathbf{e}}(\Omega)$ as the right hand side of Equation 29 minus j times the right hand side of Equation 30. Our task is to prove that $\hat{\mathbf{e}}(\Omega)$ is identical to $\boldsymbol{\epsilon}(\Omega) - \epsilon_\infty$. We may define $g(f)$ as $[\Omega(\epsilon' - \epsilon_\infty) - \mathbf{j}f\epsilon'']/(f^2 - \Omega^2)$ and then summarize Equations 29 and 30 by:

$$\hat{\mathbf{e}}(\Omega) \equiv 2\mathbf{j}/\pi \int_0^\infty \mathbf{g}(f)\, df = \mathbf{j}/\pi \int_{-\infty}^\infty \mathbf{g}(f)\, df \tag{31}$$

Here the integration is over the real axis and the improper integral should be interpreted in the Cauchy sense [62]. The second equality in Equation 31 follows from the symmetry property of ϵ expressed in Equation 28. Defining $\epsilon(\mathbf{f})$ as equal to $\epsilon(\mathrm{Re}(\mathbf{f}))$ (i.e., the dielectric permittivity at a complex frequency as the dielectric permittivity of the absolute magnitude of that frequency; note that this is possible in view of the symmetry properties of $\epsilon: \epsilon'(-f) = \epsilon'(f)$), we shall calculate the integral in the right-hand side of Equation 31 over a slightly different path. This path is described by the complex number $r\exp(j\delta)$. The first part of the path will be a straight line varying r from $-R$ to $+R$ ($R > \Omega$), keeping δ constant at δ_0. The second part of the path will lead along a circle at $r = R$ with δ increasing from δ_0 counterclockwise till $\pi + \delta_0$. The combination of the two paths yields a counterclockwise integral over a closed line in the complex plane.

According to the Cauchy theorem [62] the numerical value of the latter integral equals the 'residue' $2\pi\mathbf{j}\mathbf{h}(\mathbf{f}_0)$, where $\mathbf{h}(\mathbf{f}) \equiv g(\mathbf{f})(\mathbf{f} - \mathbf{f}_0)$ and $\mathbf{f}_0$ is a point where the function $\mathbf{g}$ is singular (becomes infinite). If δ_0 lies in between 0 and π, the singular point is at $\mathbf{f} = -\Omega$. Because of the symmetry properties of ϵ' and ϵ'', $\mathbf{h}(-\Omega) = -[\epsilon(\Omega) - \epsilon_\infty]/2$, so that the complete circular integral of $\hat{\mathbf{e}}$ equals $\epsilon - \epsilon_\infty$. The integral over the arc at $r = R$, with δ varying between δ_0 and $-\delta_0$ goes to zero as R goes to infinity, provided that $\epsilon''(\Omega)$ goes to zero as Ω goes to infinity. Our final act is to let δ_0 go to zero, such that the circular integral converges to the integral over the real axis from $-\infty$ to $+\infty$ of $\hat{\mathbf{e}}(\Omega)$ (it may be noted that if δ_0 were taken negative, the singular point would lie at $+\Omega$, which with the existing symmetry properties of $\mathbf{g}$ leads to the same result). Thus $\hat{\mathbf{e}}(\Omega)$ is identical to $\epsilon(\Omega) - \epsilon_\infty$, and q.e.d.

To summarise, the only requirements for the validity of the Kronig–Kramers relations were the symmetry relationship expressed in Equation 28, that the function $\Omega[\epsilon'(f) - \epsilon_\infty] - \mathbf{j}\epsilon''(f)f = g(f)(f^2 - \Omega^2)$ be analytic within the region surrounded by the circular integral (e.g., that the function does not contain any poles), and that $\epsilon''(\Omega)$ go to zero as the frequency goes to infinity. As we reviewed above, the symmetry requirements follow from the usual requirement of "linearity".

2.5. *Empirical descriptions of actual dielectric spectra*

As intimated, real systems, and especially biological ones, are complicated and contain many polarizations. As witnessed by Equation 26, this leads to the broadening of peaks and of transitions in the dielectric spectra. Biological work tends to describe the inevitable broadening of a given dielectric dispersion in terms of a distribution of relaxation times according to the superposition principle. Whilst [63] a host of relaxation time distribution functions can account for almost any data (on a given dispersion) within experimental error, it has become common in biological work to use the empirical Cole-Cole [64] relationship, which for the frequency-dependent complex permittivity ϵ, of which the Debye equations (12 and 13) are a special case, is:

$$\epsilon(\Omega) = \epsilon_\infty + (\epsilon_s - \epsilon_\infty)/[1 + (\mathbf{j}\Omega\tau)^{1-\alpha}] \tag{32}$$

The Cole-Cole α accounts for (or at least is used to describe the magnitude of)

the distribution of relaxation times, and has the property that a line drawn between the points $(\epsilon_s, 0)$ or $(\epsilon_\infty, 0)$ and the center of the semi-circular locus in a plot of the imaginary part ϵ'' versus the real part ϵ' of the permittivity (after any DC conductivity has been subtracted) makes an angle $\alpha\pi/2$ radians with the abscissa. For the Debye equations, $\alpha = 0$ and the characteristic frequency is that at which ϵ'' takes its maximum value. A similar 'complex conductivity' or admittance plot of the imaginary versus the real part of the conductivity may also be drawn [30,37,65–68].

It is of interest that except for some values of α the expression given by Cole and Cole [64] for the permittivity (i.e., Equation 32) is not consistent with the symmetry properties (Equation 28) of $\boldsymbol{\epsilon}(\Omega)$ required for the proof of the Kronig–Kramers relationship: $\boldsymbol{\epsilon}(-\Omega)$ in Equation 32 is not the complex conjugate of $\boldsymbol{\epsilon}(\Omega)$ [60]. The dielectric dispersion proposed by Davidson and Cole [69]:

$$\boldsymbol{\epsilon}(\Omega) = (1 + \mathbf{j}\Omega\tau)^{-\beta} \quad \text{with} \quad 0 > \beta > 1 \tag{33}$$

is consistent with the Kronig–Kramers relationships [60]. Boyd [70] discusses a variety of other empirical modifications of the Debye equation that have found use in polymer work, whilst Grant and colleagues [27] describe circumstances in which various dielectric relaxations are deconvoluted in terms of overlapping Debye-like and Cole-Cole dispersions together with the statistical arguments which may be used to defend such a procedure.

In general, any asymmetry in a complex permittivity plot may be ascribed to a variable (frequency-dependent) interaction or coupling of the motions of the dipolar species with the surrounding (and possibly itself) dipolar medium or matrix. Jonscher [35] and Dissado and Hill [71] give arguments in favor of the view that such motions are a general property of solids, whilst Dissado [72] summarises this view for proteins.

As discussed for instance by Schanne and Ceretti [73] and by Foster and Schwan [30], the relaxation time determined from permittivity measurements is not normally equal to that determined from conductivity measurements when $\alpha \neq 0$, and although it is stated [30] that "in general" the characteristic frequency determined from the complex admittance plane is higher than that obtained from the complex permittivity plane, a general truth does not yet seem to have been discerned. We will argue shortly in an accompanying article [74] that additional complexities, which have thus far apparently escaped discussion, lead to the view that many real biological systems might possess dipolar structures whose constitution is such that they cannot even in principle be expected to possess only the simple dielectric behavior alluded to above.

2.6. Free-energy exchange

In dielectrics, electric work is performed on the sample. Taken per unit of time, this work becomes power and is denoted by W_{in}:

$$W_{in} = \mathrm{Re}(\mathbf{E})\,\mathrm{Re}(d\mathbf{D}/dt) \tag{34}$$

$\mathrm{Re}(\mathbf{z})$ refers to the real component of a complex quantity $\mathbf{z}$. Here it has been used (Equation 15) that the current is the time derivative of the dielectric displace-

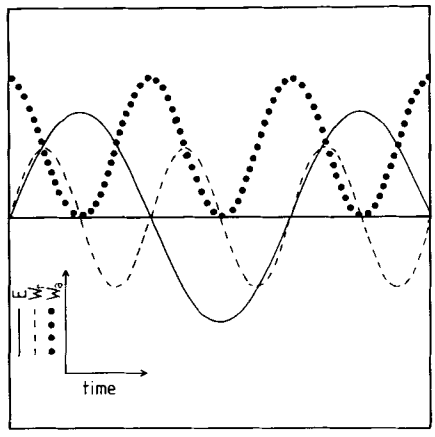

FIGURE 3 Free-energy exchange and absorption in dielectrics. E represents the electrical field (——), W_r the power reversibly exchanged between the field and the system (– – – – –) and W_a the power irreversibly absorbed by the system (· · · · ·).

ment, i.e., DC leakage currents are not considered. The input free energy will be the integral of W_{in} over time. Within each field cycle, the input power will vary with time. If the system is in a stationary state, then the input free energy per field cycle will be the same for subsequent cycles.

Using Equation 15, the power can be distinguished into two terms, W_r and W_a:

$$W_r = \tfrac{1}{2}E_0^2\Omega\epsilon_0\epsilon' \sin(2\Omega t) \tag{35}$$

$$W_a = \tfrac{1}{2}E_0^2\Omega\epsilon_0\epsilon''[1 + \cos(2\Omega t)] \tag{36}$$

In Figure 3 W_r (– – –) and W_a (· · ·) are shown together with the input potential (——) for an example where the phase angle happens to be 45°. In the first quarter of the field cycle W_r is positive (increasing towards $\frac{1}{2}\epsilon_0\Omega/\epsilon' E_0^2$), but in the second quarter W_r turns negative and even such that the time integral of W_r over half a field cycle equals zero: W_r is free energy that is reversibly exchanged between the input electric field and the dielectric sample.

In contrast, W_a never becomes negative: it represents free energy irreversibly absorbed from the electric field. The time integral of W_a over $2m$ half field cycles amounts to:

$$G_a(m) \equiv \int_0^{2\pi m} W_a \, dt = m\pi\epsilon''\epsilon_0 E_0^2 \tag{37}$$

Thus, the free-energy absorbed from the field over complete field cycles is directly proportional to the "dielectric loss" factor ϵ'', which reflects a "resistive" element. Conversely, the real component of the dielectric permittivity, ϵ', should be thought of as a capacitative (storage) factor rather than a loss. Indeed, when a dielectric system with a single relaxation time is modelled by a resistor and a capacitor in series, ϵ' and ϵ'' are proportional to $1/(\Omega C)$ and R respectively (at any given frequency). It should be noted however, that in general ϵ' is not independent of the magnitudes of resistances in the actual system, nor is ϵ'' in general independent of capacitances [37].

It has been shown, both experimentally [75] and theoretically [26,76,77] that

biological systems can transduce free energy from an oscillating electric field to net chemical or transport work. We shall now ask whether such free-energy transduction is described by equations of the type used in "linear" dielectric theory. For free-energy transduction to occur, an output process, such as the conversion of S to P, must be coupled to the absorption of free energy from the field. Such coupling may be mediated by a catalyst, such as an enzyme. The simplest case is that of a 2-state enzyme (cf., Figure 3 of the accompanying paper). Thus, one mechanism for transition of such a protein from its state 1 to its state 2 may involve the association of S to the protein, the conversion of S to P and the dissociation of P from the protein. If the protein can go back to state 1 through some other route (i.e., without reconverting P to S), then it becomes an enzyme capable of interconverting S and P catalytically. Denoting the enzyme's state probabilities by their polarizations $\mathbf{P}_1$ and $\mathbf{P}_2$ respectively (cf. Equation 25), the rate at which the conversion of S to P occurs will be given by:

$$v = k_c \mathbf{P}_1[S] - k_{-c}\mathbf{P}_2[P] + k_E\mathbf{E} \tag{38}$$

Although in principle, k_c and k_{-c} should be parameters that are dependent on the electric field (see section 2.1 of the accompanying paper [74]), the "linearity" requirement of linear dielectric descriptions, demands that we naively approximate the field dependence by the additive linear term $k_E\mathbf{E}$. As indicated by Equation 26, $\mathbf{P}_1$ and $\mathbf{P}_2$ are both equal to the complex electric field multiplied by a time independent complex number. Taking the average of Equation 38 over a complete field cycle and using that the rate constants and the concentrations of S and P are time independent, it is found that the average reaction rate $\langle v \rangle$ will amount to zero.

Consequently, to the extent that an enzyme catalytic flux can be modelled by equations deriving from linear dielectrics, that enzyme cannot transduce free-energy from the exciting electric field to any output process, nor can the oscillating electric field be shown to have any catalytic effects [cf., 56].

We wish to stress that this is a limitation of the theory rather than a limitation of the enzyme. Because in any enzyme catalytic cycle, there are at least two routes connecting two different enzyme states, the flux through a single enzyme transition cannot be equated to the change in time of a polarization, hence is not a dielectric property of the system. Indeed, it is possible that a system for which the dielectric properties are perfectly well described by the linear theory, may nevertheless be able to transduce work from the exciting field. In the accompanying paper, we shall indicate which deviations from the "linear" equations may allow one to describe a system to transduce free energy from the electric field to a chemical or transport process.

In the absence of transduction to an output process, the free energy absorbed from the field must either be exported as a travelling wave (which would amount to scattering), or (which is more likely in the usual case) be dissipated.

3. NONLINEAR DIELECTRIC PROPERTIES

Notwithstanding the advantage that its description is relatively simple, the "linear" dielectric domain has the disadvantage that it is relatively uninformative

about molecular mechanisms. Many (linear) dielectric relaxation mechanisms are presently even in principle indistinguishable from one another by electrical methods alone (since they are observed macroscopically simply by measuring ϵ' and σ' as a function of frequency). At frequencies of present interest (say <10–20 GHz), it is argued implicitly, the system succeeds in remaining in thermal equilibrium because typical molecules can exchange heat quanta with the solvent bath at infra-red frequencies ($\approx 10^{13}$ Hz) through the bending, stretching, and rotational modes of chemical bonds. In particular, frequency-domain measurements are made with V_m, the dielectric current i_m, and θ being the macroscopic observables and with the assumption that the system actually is in a (strongly) stationary state [28]. Any field-induced temperature rise, caused by dissipative processes, is considered to be negligibly small or at least expressed only through the Boltzmann factor, in which case the rotational relaxation time of the dipoles would be reduced. In the hope that the nonlinear domain will prove much more informative with regards to molecular mechanisms, we shall now discuss nonlinear dielectric phenomena in somewhat more detail.

3.1. *Prelude: Motions of proteins in spherical shell bilayers*

We considered above the simplest example of dielectric relaxation, namely the orientation of a spherical dipole (representing an aqueous globular protein) by an alternating field. As discussed *in extenso* in reviews of the dielectric behavior of proteins [27,54,78,79], changing the shape of the protein dipole to a spheroid or ellipsoid merely serves to broaden such dielectric relaxations until (for high axial ratios) two separable dispersions may be observed. All of the other implicit assumptions remain, including that pertaining to the lack of effect of the electric field on the permanent dipole moment of the protein, and that the system attains a genuine steady state which is unchanged from the initial condition by the application of the periodic field ($\langle E \rangle = 0$). If we put our protein in a biological membrane constituting a vesicle, some important differences arise (Figure 4A & B). As discussed elsewhere [32,46], the forces acting on membrane proteins are such that any lateral motions they may have will be confined to the spherical shell bilayer. If such proteins themselves possess a net charge or dipole moment oriented as in Figure 4, the imposition of the exogenous electrical field will, as pointed out by Zimmermann and Vienken [80], tend to drive them towards the poles of the vesicle even though the field oscillations may be symmetric about zero. This is because the field-induced force depends upon the cosine of the angle between the dipole moment and the field and this is constantly changing as the proteins move. Thus, the "passive" electrical properties of such an arrangement are changed by the electrical field and the dipolar structure of the system is changed by the applied electrical field. (Obviously, this alone could lead in a simple manner to significant changes in enzymatic activities [32,81–84]). Given that one generally measures the dielectric properties at a certain frequency after a few cycles (in frequency-domain methods) or at a constantly changing time (in time-domain methods), it is evident that the assumption of genuine linearity for such a system is untenable (except if $\mu E \ll k_B T$): the dielectric behavior of the system is not a constant but changes due to the imposition of the electric field. A

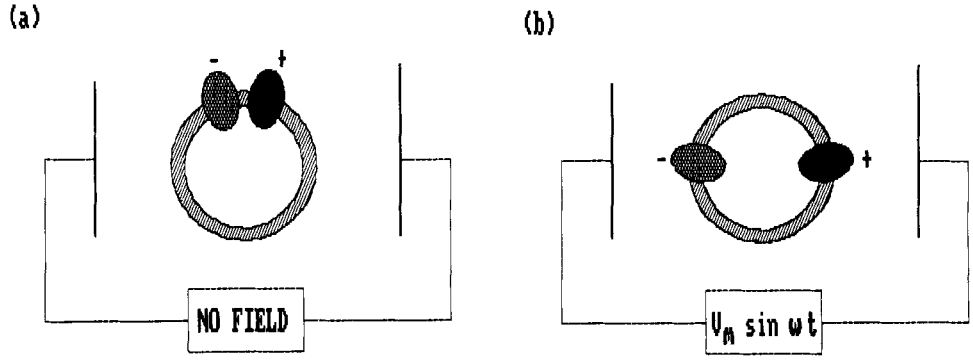

FIGURE 4 Nonlinear dielectric behavior of proteins constrained in a spherical shell bilayer. (a) In the absence of an exogenous AC field, the "equilibrium" position of two proteins might be as indicated, if for no other reason than because of the electrostatic attraction between the charges indicated. (b) In the presence of an exogenous electrical field, the proteins will tend to move so as to minimise their dipolar interaction with the field vector. Because that interaction is greater the greater is the angle between the protein's individual dipole and the field vector, any field-induced lateral motion of the proteins from their position(s) in (a) towards the poles of the cell adjacent to the electrodes will decrease the net dipole moment such that during the opposite cycle of the field the force tending to move the protein back to its 'starting' position will be less than that which moved it away from it in the first place. In other words, (i) there will be an "induced" dipole moment under such circumstances, (ii) this will tend to be "irreversible" and hence nonlinear with the field, and (iii) there may be changes in enzymatic activity consequent upon the disentanglement of the proteins in (a).

steady state will, given sufficient time, be reached such that after enough cycles of the field the state of the system is identical to that pertaining at the same point in the preceding cycle. This steady state will not, however, be described by an effectively random orientation of dipoles at points where the field strength is zero. This contrasts with the situation of Figure 2 where we considered a permanent dipole which, after the application of say a positive-going AC cycle, would seek to return to a "random" orientation by thermal means, any such motions being accompanied by a dissipative current equal in magnitude but opposite in sign to that which accompanied its orientation in the first place. In our membrane-located system of Figure 4, however, the size of the vector dipole which is seeking to return to its "starting" position is now (Figure 4B) different from that at the start (Figure 4A). Indeed, the fact that we have moved our proteins from their original (field-free) and presumably "stable" positions means that they may now relax to a new disposition within the membrane, a position which would form the starting point for any new assessment of the dielectric behavior of the system. In particular, the frequencies (rate constants) for such motions are likely to be different from those existing prior to the imposition of the electric field, so that the displacement current arising from the return of the proteins to their starting positions will be at a different frequency from that which caused them to move in the first place during transitions from one steady state to another. As pointed out before [32], it is clear that a more detailed assessment than that currently practiced is required for the proper description of the dielectric behavior of membrane vesicle systems.

This is of course still a very simple example; additional complexities will arise (a) because real membrane vesicles are rarely strictly spherical, (b) because there are likely to be electroosmotic forces [32,46,85,86] which, though as yet uncertain in magnitude and even in nature, will act to oppose the "lateral electrophoresis" alluded to above, and (c) because the vesicle itself will tend to rotate (more slowly) due to the imposition of the electrical field.

In the accompanying article [74], we will show, using elementary thermodynamic and kinetic principles, that energy input into a system of chemically reacting protein as a sinusoidal electric field may be dissipated through a displacement current with frequency components different from the input sine wave. This constitutes a clear transgression of the limits of "linear" dielectrics, in which output and input have the same frequency [37]. Additionally [76,87,88], energy from an oscillating or noisy electric field can be conserved as chemical or transport work through the action of a membrane enzyme. This is because the dynamic field may drive the enzyme backward through its catalytic cycle. Rather than a peculiarity, the interaction of nonstationary (local) electric fields and enzyme transitions may well be an essential element of biological free-energy transduction [26,56,77,89,90]

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