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Importance of intermediary transitions and waveform in the enzyme–electric field interaction

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Abstract

The current theory of enzymes and electric field interaction does not account for all the observed data since we could not observe non-linear behavior of cell suspensions as anticipated by other authors. In our case, we used a pure sinusoidal source, however the experiments that do account for responses used a sum of a central sinusoidal and its harmonics frequencies. As a result, we suggest that the enzyme and electric interaction are favored when the field has a non-strictly sinusoidal waveform, and this behavior is related to the true intermediate transitions of the enzyme during its catalytic cycle. Therefore, we developed an iterative model of the interaction process based on previous models and actual trends. The model well verified all the previous simulations and showed that, for a non-symmetrical enzyme, the energy can harvest its maximal for non sinusoidal fields.

Keywords: Proteins; Harmonic; Non-linear; Simulation; Iterative model

1. Introduction

Macroscopic interaction between electric fields (EF) and biological media has always been a subject of concern, however, the molecular level has attracted the attention of researchers in the last decades. Fluctuating potentials close to the ATP synthase have been proposed as sources of complementary energy which can be converted by the enzyme during ATP synthesis [1–4]. Other enzymes, such as H^+- and Na^+/K^+-ATPase can harvest energy from an external EF and use it to pump ions across a membrane [4–8]. Theoretical analysis demonstrated that coupling can be made with sinusoidal, random or random telegraph function (RTF) fields [9–14], but chaotic waveforms have also been suggested [14]. The interaction process was explained with the electro-conformational coupling theory (ECC), which briefly proposes that both semi-cycles of a symmetric fluctuating EF affect alternatively two opposite transitions in a typical four states E1E2 enzyme, in a sort of resonant behavior [13].

In the 1990s, the frequency analysis worked out by Astumian and Robertson [15] led to the concept of non-linear monitoring of living cells, introduced by Woodward and co-workers [16–24]. The former monitored the metabolic state of some microorganisms (mainly *Saccharomyce cerevisiae*) applying an external "sinusoidal" EF. The phenomenon was attributed to the interaction between EF and membrane-bounded enzymes (generally H^+-ATPase) and justified with the cited literature. Even Woodward stated that the EF used was a pure sinusoidal, it can be observed that the real applied signal (see reference cell of Fig. 3 in [16]) included the original one and its harmonic spectrum. These experiments were repeated fairly unsuccessfully by other groups [25–29]. When we applied a true sinusoidal EF to the same suspension [16] no harmonic response was observed (unpublished results).

As the true input signal used by Woodward was a non-single-frequency EF, we hypothesize that the energy coupling between enzymes and electric fields is most favorable when the input

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signal is a multi-frequency electric field. We believe that true enzymes do not behave as symmetrically as assumed, and this leads to a misconception in the ECC. The optimal waveform that alternatively favors two opposite non-identical transitions must be much complex than a simple sinusoidal, random or stochastic signal. However there is not theoretical or practical evidence that confirms such hypothesis. This paper is twofold, first we will present a computational model for the field–enzyme interaction based in the ECC, and take into account previous simulations and experiments. It will be shown that the enzyme can use the alternate electric field and that it does work when the field is sinusoidal, square, RTF and random, as suggested previously. Second, we will show that a slightly asymmetrical enzyme (a much more realistic situation) will respond also to a complex EF in a distinguishable way from the single-frequency analysis. The frequency content (amplitude and phase) of such signal is closely related to the kinetics of the enzyme and the intermediary states.

2. The model

The model describes a single enzyme, and its average behavior is evaluated when many enzymes are simulated simultaneously, each one responding independently from the rest. The model can be fixed to any number of states and here we shall assume a typical four states E1E2 H^+-ATPase, as described in Fig. 1a. The enzyme, bounded in the plasmatic membrane, undergoes conformational changes pumping a single H^+ to the outside each time a new clockwise cycle is completed. For each cycle a molecule of ATP is hydrolyzed to ADP and P_i.

The model resembles the ratchet mechanisms, where each enzyme moves randomly forward and backward in equilibrium, but, at expense of energy consumption, the equilibrium can be shifted [14,30–33]. However, this model does not account for the ATP utilization, so it must be defined for the equilibrium or non-equilibrium state. In the equilibrium, each transition (forward or backward) has the same probability of occurrence and there is no net production caused by the enzyme. In non-equilibrium, ATP consumption is implicit, probabilities are shifted and work is done by the enzyme.

Each enzyme begins in a randomly selected state and it can iteratively change to other states, based on the probabilities of occurrence of each transition. The probability of any enzyme in the n-state of moving forward, backward or neutral are the $k_{n\to n+1}$, $k_{n\to n-1}$ and $k_{n\to n}$ parameters respectively. All three process are assumed to be transition, whether it is forward (or clockwise), backward (or counterclockwise) or neutral. The latter is a computational workaround to allow the enzyme to remain short at any state without suffering an immediate transition. In non-equilibrium (implicit ATP consumption) some transition are more probably to occur and their k-values are higher. In the absence of an electric field, a random number selects the direction of transition.

Any transition, once it has been randomly assigned, has a finite time of occurrence and the enzyme will be ignored until it is finished. Once the transition was completed, the enzyme is evaluated again to change its state. The EF is sampled at a frequency of 5000 samples per second and the sampling period (also the iteration period, 200 μs) defines the smallest time that an enzyme can remain in any state without suffering a new transition.

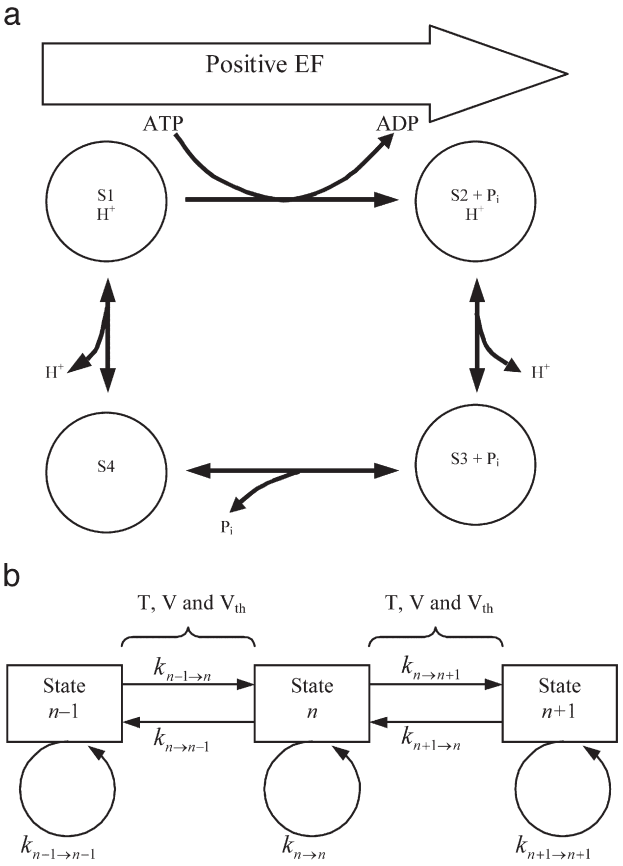

Fig. 1. a) Four state model of the H^+-ATPase and b) parameters involved in the computational model. $S_1 \ldots S_4$ are the states of the enzyme, T, V, V_{th} stand, respectively, for the time involved in the transition, the field sensitivity of the transition and a field threshold which may block the transition.

The time consumed in the forward, backward or neutral transition from the n-state is defined respectively by $T_{n\to n+1}$, $T_{n\to n-1}$ and $T_{n\to n}$. The time involved for each transition is not the same, since some transitions can be fast (ion binding) while others can be time consuming (complex reactions with intermediate products or domains movements). The forward and backward time are assigned to be longer than the iteration period. However, the neutral transition must be as brief as possible, and the smallest time possible distinguished by the model is the iteration period. Consequently, when an enzyme remains in its state (neutral transition), the next iteration is evaluated again, with no delaying in between.

According to the ECC, a transmembrane enzyme that possesses two conformational forms with different charge distributions, or electric moments, can convert from one form to another under the influence of an EF [4,10,12,15]. In our model it will be assumed that an electric field only changes the activation barrier and the probability of occurrence of the reaction increases or decreases, but both the sign of ΔG and the time of the reaction are not altered. The influence of the field over the transition $n \to n+1$ is modeled with the $S_{n\to n+1}$ parameter. It defines the sensitivity of the transition with a positive field. If $S_{n\to n+1}$ is greater than 0, the transition will

increase its probability of occurrence, while it will decrease if $S_{n\to n+1}$ is negative. A negative field favors transition with negative S-values, and vice versa. Mathematically, the probability of occurrence of the $n\to n+1$ transition could be described by:

$$k'_{n\to n+1} = k_{n\to n+1} \times 10^{S_{n\to n+1}\times \Delta V}$$

where $k'_{n\to n+1}$ is the modified probability and ΔV is the potential difference across the membrane induced by an applied electric field. As the sum of the new k-values for the n-state is not equal to one, they are normalized respect to the sum of all of them:

$$k''_{n\to n+1} = k'_{n\to n+1} \Big/ \sum_{i=-1}^{1} k'_{n\to n+i}$$

where $k''_{n\to n+1}$ is the modified and normalized probability, which is used subsequently. The exponential relationship adopted is based on the dependency between the pseudo-unimolecular rate coefficients and the shift in transmembrane electric potential described previously [10].

In our simulation, enzymes can be placed all parallel to the EF (maximum energy conversion) or can be placed randomly over a spherical cell. In such case, the EF and ΔV are related by $\Delta V = 1.5bE\cos\theta$ where θ is the angle between the direction of the field and the radius to the point considered on the membrane, b is the outer radii of the cell, and E is the externally applied EF [34]. As dimensions are not taken into account in our simulation, we assume that ΔV directly relates to $E\cos\theta$. A random θ between 0 and π is generated for each enzyme and maintained constant along the simulation. If enzymes are assumed parallel, θ-values are set to zero.

A high electric field is expected to rearrange the dipoles (and maybe the domains) of the molecule in such way that the enzyme is locked into a specific state and the wheel of Fig. 1a may fail to turn in full cycles [30,35]. This concept is introduced with the E_{th} value for each transition which defines a threshold field that turns the transition unviable. The mathematical treatment for the $n\to n+1$ transition with threshold is given by

$$k'_{n\to n+1} = k_{n\to n+1} \times 10^{S_{n\to n+1}\times E\cos\theta} \times \left[\frac{1}{1+10^{4\times\left[|E\cos\theta|-E_{th_{n\to n+1}}\right]}}\right]$$

where the term between brackets decreases dramatically when the local field exceeds the threshold. All the parameters that govern each transition are summarized in Fig. 1b.

Each simulation was run with a variable number of enzymes (typically 500), 5000 iterations at a frequency of 5000 iteration per second. First, we will analyze the overall behavior of a simple enzyme without and with a fixed EF. Then we will vary the frequency, amplitude and waveform of the EF to finally evaluate a second type of enzyme under the same conditions. When a fixed EF is used, the average occupancy (Ao) and average transitions (At) parameters will be analyzed. For each state, Ao, is the percentage of times that a single enzyme transitioned to it, relative to all the transitions registered. At is also calculated for each state, and it indicates the percentage of time that any enzyme transitioned from that state, relative to all transitions registered. Both are calculated after running the entire simulation and taking into account all simulated enzymes.

When a varying EF is used, the model will be evaluated measuring the increment or decrement in H^+ pumped per enzyme expressed as percentage to the amount pumped without EF (known as *normalized cycles per enzyme*, *NCE*).

We propose two types of enzymes. The first one is very similar to the previously proposed [8,14,30] with identical parameters (k, S, E_{th} and T) between transitions $1\to2$ and $3\to4$, and also between $2\to3$ and $4\to1$. Transitions $2\to3$ and $4\to1$ (ion binding and releasing) are supposed to be very favorable and reactive, while the others are not as favorable, acting bottleneck-like. The latter enzyme is slightly different than the former, and we assume that one of the transitions ($1\to2$) is not as influenced by the EF as the other ($3\to4$), and their S- and E_{th}-values are different. The parameters for each enzyme are presented in Table 1. These enzymes were tested without EF and also with sinusoidal, square, and random (RTF, Gaussian and uniform) fields. The second enzyme was also evaluated against the *pseudo-square* EF shown in Fig. 2.

3. Simulations

The main features of enzyme 1 in the absence and presence of a fixed sinusoidal EF are depicted in Fig. 3. The overall process is a clockwise reactive cycle. It always has more forward than backward transitions and a net H^+ pumping, however the state distribution of each enzyme along the entire simulation is not uniform. In absence of EF, there is an increased Ao of enzymes in states 1 and 3, it spends 85% of the time between these two states (subset a). When an EF (of proper amplitude and frequency) is applied, the equilibrium is altered, and Ao is shifted from states 1 and 3 to states 2 and 4 (subset a), attached to an increased forward At from states 1 and 3 (data not shown).

Subset b) shows the temporal evolution for all the enzymes with sinusoidal EF, when the enzymes are place parallel to the EF and also when they are supposed randomly distributed over the membrane. We will analyze first the former situation, and then we shall extend to the latter. The process starts with a

Table 1
Parameters of the enzyme model for both types of enzymes

	Parameter	State 1	State 2	State 3	State 4
Enzyme 1	$k_{n+1\to n}$	0.055	0.45	0.055	0.45
	$k_{n\to n}$	0.045	0.1	0.045	0.1
	$k_{n\to n+1}$	0.9	0.45	0.9	0.45
	$S_{n\to n+1}$	1	0	1	0
	$T_{n\to n+1}$ (s)	0.01	0.002	0.01	0.002
	$E_{th_{n\to n+1}}$ (V/cm)	10	10	10	10
Enzyme 2	$k_{n+1\to n}$	0.055	0.45	0.055	0.45
	$k_{n\to n}$	0.045	0.1	0.045	0.1
	$k_{n\to n+1}$	0.9	0.45	0.9	0.45
	$S_{n\to n+1}$	1	0	−0.2	0
	$T_{n\to n+1}$ (s)	0.01	0.002	0.01	0.002
	$E_{th_{n\to n+1}}$ (V/cm)	1	10	10	10

Enzyme 2 is almost identical to enzyme 1, it only differs in parameters $T_{n\to n+1}$ and $E_{th_{n\to n+1}}$.

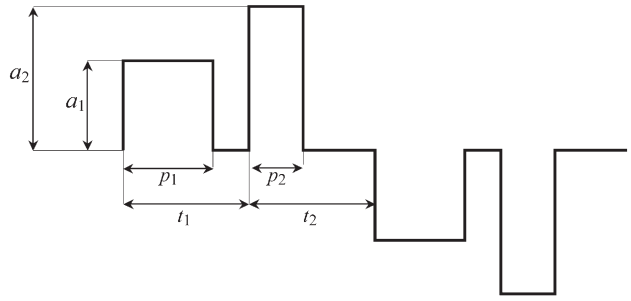

Fig. 2. Pseudo-square EF. The signal is composed by two symmetrical hemicycles, while each hemicycle is composed by two consecutive square-wave pulses. Each pulse is defined by its amplitude (a_x), duration (t_x), and the percentage of time (p_x) that maintains that amplitude a_x. The remaining time $t_x \times (1 - p_x/100)$ its amplitude is zero.

randomized occupancy of 25% at all states and it quickly reorganizes due to the presence of the EF. This transitory is not shown, and the two cycles can be considered stationary and they will be described briefly. When the field is increasing to the positive values, almost all enzymes are at state 1, and only a minor part are in state 4 ($t = 0.05$ s). During the beginning of the positive hemicycle, almost all enzymes in state 4 goes 1 (very reactive) and close to 90% of the enzymes are in state 1. Transition 1 to 2 is very favored by the presence of the positive field, and almost all enzymes have transitioned by the middle of the hemicycle. There is also a minor backward transition from state 4 to 3 due to the presence of the positive field. State 2 occupancy, as expected, increases but enzymes in this state are very reactive and quickly turn to state 3. When the positive field has ceased, most of the enzymes have turned from 1 to 3 through the forward path. As the negative hemicycle increases, the transition $3 \rightarrow 4$ becomes favorable and state 3 is depleted fast, it replenishes state 1 and it completes a full cycle. This behavior is repeated for each cycle of the EF.

For the randomly placed simulation, enzymes start also with a 25% occupancy for every state. However, not all enzymes are favored by the same EF. Those placed perpendicular to the EF ($\theta \approx \pi/2$) are almost unaffected, while some can be affected or disaffected by the same field. This produced an average occupancy close to 45% for states 1 and 3 with a cyclic behavior that it is repeated for each hemicycle.

Fig. 4 shows the overall effect of the field when both frequency and magnitude were changed (1 Hz $\leq f \leq$ 200 Hz; 0.1 V/cm $\leq E \leq$ 10 V/cm) and a different signal was applied. The surface shows a nearly flat behavior up to 0.3 V/cm for the sinusoidal EF (subset a), with no effect in the average pumping of H^+. As the field grows, H^+ pumping increases and decreases, depending on the frequency and field ranges evaluated. The H^+ pumped increases up to 100% for frequencies of 39.6 Hz, 120 Hz and 200 Hz 128% and 133% respectively, as the field increases up to 10 V/cm., The pumping decreases dramatically for the mid-ranges frequencies, lower than 60% below the steady state response when the AC field is higher than 1 V/cm. A square-wave EF leads to similar results as the sinusoidal EF (subset b).

The use of the RTF (subset c) did show a minor positive interaction of 4% when the field amplitude was 0.5 V/cm and its frequency 2 kHz. Random (subset d) noise produced a similar result, with a maximum value of 3.7% at different field amplitudes.

Thereafter, we evaluated the second enzyme, under the same frequency–voltage ranges. The output of this system (Fig. 5a) was more irregular and lower than the former for the sinusoidal EF, and the maximal values were at field amplitudes slightly higher than 1 V/cm. Higher amplitudes produced a decrease in proton pumping. These maximums were observed at frequencies of 36 Hz, 114 Hz and 196 Hz at 3.4%, 12% and 21% respectively. The square EF (subset b) leads to similar results. Noise EF produced a smaller effect than the previous enzyme, with maximums between 2% and 3% (subset c and d).

As this system has different sensitivities and threshold to the EF in the bottleneck transitions, it is expected that each transition ($1 \rightarrow 2$ and $3 \rightarrow 4$) would react favorably at different voltage values. However, as each hemicycle favors simultaneously both transitions (of enzymes in opposite sites of the membrane) there is a restriction with the field amplitude. It can

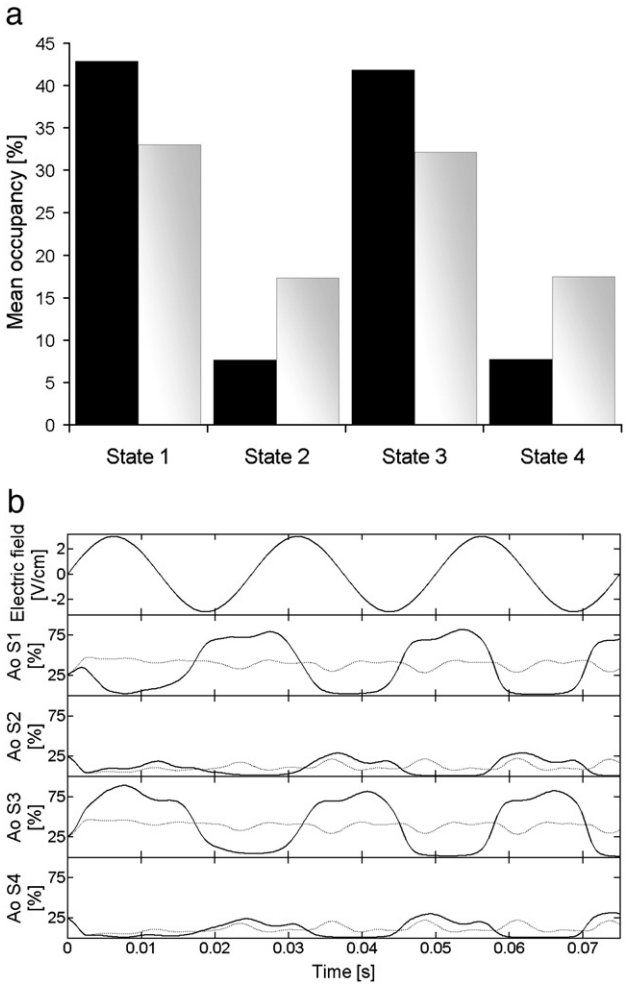

Fig. 3. a) Average occupancy for each state with (black) and without EF (2000 enzymes, 2 s of simulation, $\theta = 0$ for all enzymes, 40 Hz 2 V_{pp} sinusoidal), and b) The sinusoidal EF and time evolution of state occupancy with the enzymes placed both parallel (—) and randomly (– – –) respect to the EF (20,000 enzymes, 0.1 s of simulation, 40 Hz 2 V_{pp} for parallel enzymes and 6 V_{pp} for random enzymes).

be high because it blocks sensitive transitions (1 → 2), but it can be low because it does not favor the insensitive one (3 → 4). By solving this discrepancy, it was proposed that each hemicycle could be divided into two square cycles, each one favoring different transitions. We proposed the pseudo-square signal shown in Fig. 2, and tried several parameters. Fig. 5e shows the result of applying a pseudo-square signal with fixed parameters *a* and *p*, while *t* was variable with the frequency. The surface obtained was similar to the previous one, having maximums at the same frequency and voltage ranges, but the values observed were 7.76%, 19.6% and 30.2%. Other combinations of *t*, *a* and *p* parameters for the pseudo-square signal at these frequencies could obtain even higher responses, as shown in Fig. 5f.

4. Discussion

The model presented enables to simulate the particular and overall performance of a single-enzyme system, respectively, and its time distribution of states resembles the theoretical models previously published [10]. However, our description adds the state distribution of enzymes randomly placed over the membrane, and thus, it becomes a more realistic.

The *k*-values are chosen to ensure an average positive pumping in a steady state condition: the equilibrium has been shifted and ATP consumption is assumed. These values are fixed during simulation representing unaltered biochemical conditions (ATP availability and H^+ concentration). These parameters could be varied during simulation to represent real progression of substrate and product, and a new equation should be introduced. However, this work has been focused on the enzyme and EF interaction, not o the biochemistry associated to the enzyme.

Time constant assumed for transitions are clearly out of scale (a catalytic cycle is completed in more than 10 ms), which is far away from the real time. However, the iteration period must be, as much, the time involved in the fastest process or reaction. Time involved in real proteins motion range from 10^{12}–10^{13} s^{-1} (electron transport) to 10^{9}–10^{5} s^{-1} (local opening/closing of folding/unfolding) [36]. For an even more realistic simulation, each run would take about 10^{6} iterations, instead the currently 5000 used, becoming unpractical.

It was previously acknowledged that two conditions are essential to observe interaction between enzymes and EF [10,12]: (i) asymmetry of state occupancy in the steady state and

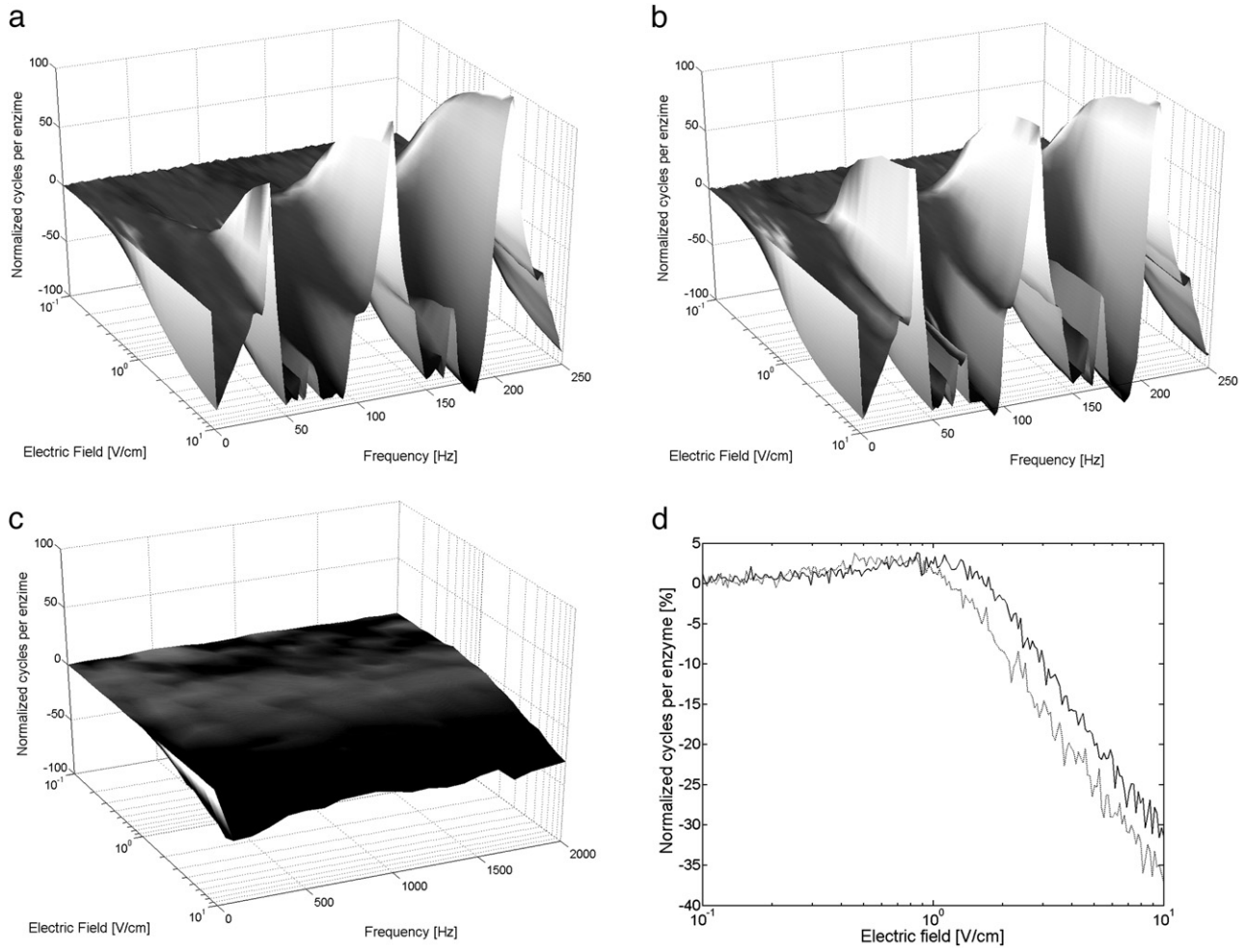

Fig. 4. *NCE* due to the presence of a fluctuating field: a) sinusoidal EF, b) square EF, c) RTF EF, and d) — white and – – – Gaussian EF. Calculation has been made with 500 enzymes, 1 s of simulation and random angles. Field amplitude indicates peak values in sinusoidal and square EF, standard deviation for RTF and random noise, and absolute maximal value for uniform noise.

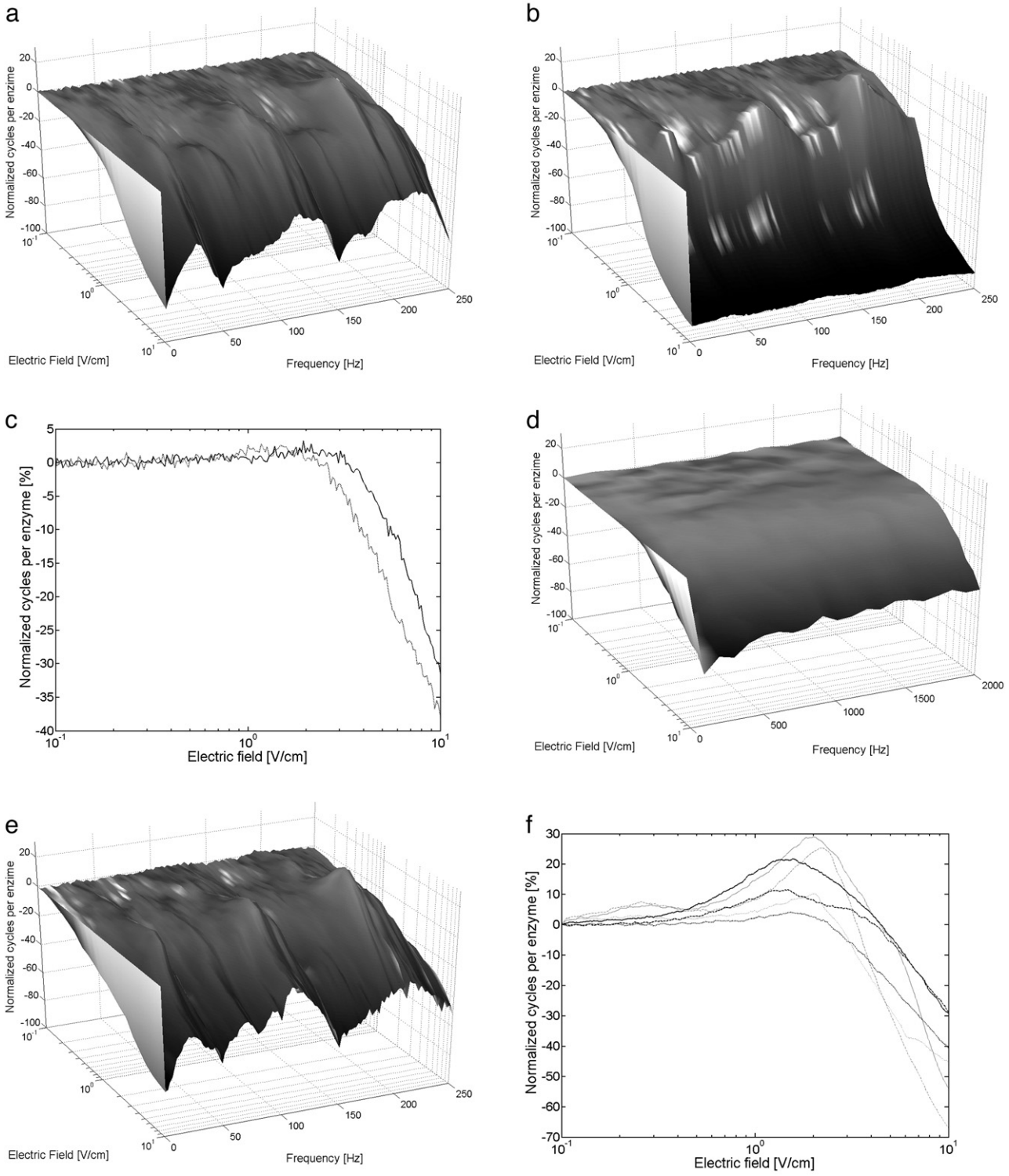

Fig. 5. *NCE* of enzyme 2 when different EFs were applied: a) sinusoidal, b) square, c) RTF, d) — white and - - - Gaussian, e) pseudo-square signal (as described in Fig. 2, with $t_1=t_2=1/(f\times 4)$; $a_1=0.55$; $a_2=4.15$; $p_1=100$; $p_2=60$) as a function of frequency and amplitude. Once the signal was generated, it was subsequently multiplied by the amplitude indicated as EF in the figure, and f) pseudo-square and equivalent sinusoidal as a function of amplitude: ⋯ pseudo-square with main frequency of 32.9 Hz ($t_1=7.05$; $t_2=8.14$; $a_1=0.475$; $a_2=5.03$; $p_1=100$; $p_2=65.24$), - - - pseudo-square with main frequency of 116.5 Hz ($t_1=2.12$; $t_2=2.17$; $a_1=0.473$; $a_2=4.28$; $p_1=97.5$; $p_2=88.7$), — pseudo-square with main frequency of 193 Hz ($t_1=1.31$; $t_2=1.28$; $a_1=0.551$; $a_2=4.15$; $p_1=100$; $p_2=49.6$), ⋯ sinusoidal of 36 Hz, - - - sinusoidal of 114 Hz, and — sinusoidal of 196 Hz; times are expressed in ms.

(ii) at least one transition must involve changes in the electric charge distribution or net movement of a (charged) ion across the membrane. Both conditions are applicable to this model, and they also should be announced coupled: at least one state must have a high average occupancy, and its forward transition must be sensible to the extern EF Transition $1\rightarrow 2$ and $3\rightarrow 4$ are

responsive to the EF in any of the models used herein, but states 1 and 3 also have the highest average occupancy. This led, within strict frequencies, to an increase in H^+ pumping due to the alternating EF. Otherwise, without accumulation in sates 1 and 3, the influence of the EF is too small to produce a major effect in the overall H^+ pumping (data not shown). If the enzyme–EF interaction were produced in other transitions ($2 \rightarrow 3$ or $4 \rightarrow 1$), but still accumulating in states 1 and 3, there would not be a noticeable effect either (data not shown).

Enzyme 1 interacted mostly to periodical alternating EF (sinusoidal and square) but showed poor interaction with randomly fluctuating fields (RTF, Gaussian or uniform noise). Random signals increased both forward and backward transitions, and they almost abolished neutral. However, if the transition time (forward and backward) is set too longer than used here, the result is a delaying of the enzyme respect to its normal catalytic cycle, and a decrease in the *NCE* is observed. If forward and backward transition times are close to the neutral, the random EF clearly favors H^+ pumping and the observed *NCE* is positive, close the values observed for periodic signals (data not shown).

When the field periodically fluctuates, a resonant behavior is observed when the field's period is close to the entire period of the enzyme ($1/f = T_{1\rightarrow 2} + T_{2\rightarrow 3} + \cdots + T_{n\rightarrow 1}$), with maximal increase in H^+ pumping. The resonant frequencies were the inverse of a full cycle for enzyme one (if all transition were forward) and its odd harmonics (120 Hz and 200 Hz). This behavior matches to the ECC theory described previously.

Enzyme 2 shows interaction with sinusoidal, square, and random signals, but the maximal response observed for any of them was far away for the maximum observed for enzyme 1. With pseudo-square signals, the maximums observed were between 1.5 and 2 times bigger than sinusoidal or square signal. This indicates that, for non symmetric parameters, the most effective energy coupling can be accomplished with non typical signals.

Woodward stated that the energy transfer occurred with the 15 Hz sinusoidal signal [16], and the harmonic content observed was a consequence of the coupling. In spite of that true sinusoidal, EF did not account for the anticipated non-linear phenomenon, we would rather believe that the harmonic content of the input signal applied by Woodward could probably work in the same way that the signals shown here. Actually, square and pseudo-square wave (as described in Fig. 5) have very similar harmonic content, however, they rather differ in their phase response. An adequate phase for each harmonic is as important as its amplitude, because it represents the proper timing to favor (or not) each transition.

The E1E2 model explains intuitively that the enzymes alternatively exposes its binding site to the intra- an extracellular space, being these transitions equivalent (dielectrically) but in opposite directions, but this is, by far, false. It has been demonstrated that P-type ATPases does not expose alternatively a binding site to the intra- and extra-cellular place. The real process includes inter-domain motions and rearrangement of the transmembrane α-helix, but these intermediate steps do not have a strictly counterpart reaction [37,38]. Even more, the intermediate reaction have different equilibrium constant (see for example the energetics of a Ca^{++}-ATPase [39]) and, maybe, a different amount of states should be taken intro account, as suggested by [40]. In such scenario, an enzyme would require an extremely delicate EF to get the most favorable response. The waveform of the field could probably be segmented, with each segment matching (both in time and amplitude) each single transition. Some transitions may be sensitive to EF, while other may not, leading to intervals with and without extern EF. The analysis must also take into account the angle distribution of the enzymes respect the EF.

5. Conclusions

This simulation model fits the theoretical requirements imposed previously and shows the same results. Its advantage is the iterative process, which does not require mathematical formalism for the EF waveform. It adds new information about the state distribution when symmetric enzymes are randomly distributed.

Our original hypothesis, that a specific system may respond only to broad spectrum signals, has not been fully demonstrated. It was partially demonstrated by showing that a system can react to a broad spectrum signal in a way unpredicted by a single-frequency analysis. It also adds new information about the waveform of the field, indicating that periodic non sinusoidal signals can achieve better results than sinusoidal, tracing the relationship between the kinetics of the enzyme and the waveform of the signal. This analysis was carried on enzymes randomly distributed (as the experiments detailed in the references), however, different results can be obtained for non-randomly distributed enzymes (such as patch clamp procedures). To fully understand the interaction mechanism, much more analysis must be given to the kinetics of the enzyme, to the bottleneck transitions and to whether they are or not sensitive to the extern EF.

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References

[1] H.T. Witt, E. Schlodder, P. Graber, Membrane-bound ATP synthesis generated by an external electrical field, FEBS Letters 69 (1–2) (Oct. 1976) 272–276.

[2] J. Teissie, B.E. Knox, T.Y. Tsong, J. Wehrle, Synthesis of adenosine triphosphate in respiration-inhibited submitochondrial particles induced by microsecond electric pulses, PNAS 78 (12) (Dec. 1981) 7473–7477.

[3] B.E. Knox, T.Y. Tsong, Voltage-driven ATP synthesis by beef heart mitochondrial F0F1-ATPase, J. Biol. Chem. 259 (8) (Apr. 1984) 4757–4763.

[4] T.Y. Tsong, R.D. Astumian, 863 — absorption and conversion of electric field energy by membrane bound ATPases, Bioelectrochemistry and Bioenergetics 15 (3) (June 1986) 457–476.
[5] J. Teissie, T.Y. Tsong, Evidence of voltage-induced channel opening in Na/K ATPase of human erythrocyte membrane, Journal of Membrane Biology 55 (2) (June 1980) 133–140.
[6] H.S. Engin, Y.T. Tian, Stimulation of a ouabain-sensitive Rb+ uptake in human erthrocytes with an external electric field, Journal of Membrane Biology V74 (3) (Oct.1983) 191–201.
[7] E.H. Serpersu, T.Y. Tsong, Activation of electrogenic Rb+ transport of (Na,K)-ATPase by an electric field, J. Biol. Chem. 259 (11) (June 1984) 7155–7162.
[8] D.S. Liu, R.D. Astumian, T.Y. Tsong, Activation of Na+ and K+ pumping modes of (Na,K)-ATPase by an oscillating electric field, J. Biol. Chem. 265 (13) (May 1990) 7260–7267.
[9] H.V. Westerhoff, Y.D. Chen, Stochastic free energy transduction, PNAS 82 (10) (May 1985) 3222–3226.
[10] H.V. Westerhoff, T.Y. Tsong, P.B. Chock, Y.D. Chen, R.D. Astumian, How enzymes can capture and transmit free energy from an oscillating electric field, PNAS 83 (13) (July 1986) 4734–4738.
[11] R.D. Astumian, P.B. Chock, T.Y. Tsong, Y.D. Chen, H.V. Westerhoff, Can free energy be transduced from electric noise? PNAS 84 (2) (Jan. 1987) 434–438.
[12] Y.D. Chen, Asymmetry and external noise-induced free energy transduction, PNAS 84 (3) (Feb. 1987) 729–733.
[13] T.Y. Tsong, D.S. Liu, F. Chauvin, A. Gaigalas, R.D. Astumian, Electroconformational coupling (ECC): an electric field induced enzyme oscillation for cellular energy and signal transductions, Bioelectrochemistry and Bioenergetics 21 (3) (June 1989) 319–331.
[14] T.Y. Tsong, C.H. Chang, Ion pump as Brownian motor: theory of electroconformational coupling and proof of ratchet mechanism for Na,K-ATPase action, Physica A: Statistical Mechanics and its Applications 321 (1–2) (Apr. 2003) 124–138.
[15] R.D. Astumian, B. Robertson, Nonlinear effect of an oscillating electric field on membrane proteins, The Journal of Chemical Physics 91 (8) (Oct. 1989) 4891–4901.
[16] A.M. Woodward, D.B. Kell, On the nonlinear dielectric properties of biological systems: *Saccharomyces cerevisiae*, Bioelectrochemistry and Bioenergetics 24 (2) (Oct. 1990) 83–100.
[17] A.M. Woodward, D.B. Kell, On the relationship between the nonlinear dielectric properties and respiratory activity of the obligately aerobic bacterium *Micrococcus luteus*, Journal of Electroanalytical Chemistry 321 (3) (Dec. 1991) 423–439.
[18] A.M. Woodward, D.B. Kell, On the relationship between the nonlinear dielectric properties and respiratory activity of the obligately aerobic bacterium *Micrococcus luteus*, Bioelectrochemistry and Bioenergetics 26 (3) (Dec. 1991) 423–439.
[19] A.M. Woodward, D.B. Kell, Confirmation by using mutant strains that the membrane-bound H+-ATPase is the major source of non-linear dielectricity in *Saccharomyces cerevisiae*, FEMS Microbiology Letters 84 (1) (Nov. 1991) 91–95.
[20] A.M. Woodward, D.B. Kell, Dual-frequency excitation: a novel method for probing the nonlinear dielectric properties of biological systems, and its application to suspensions of S. cerevisiae, Journal of Electroanalytical Chemistry 320 (3) (June 1991) 395–413.
[21] A. McShea, A.M. Woodward, D.B. Kell, Non-linear dielectric properties of *Rhodobacter capsulatus*, Bioelectrochemistry and Bioenergetics 29 (2) (Dec. 1992) 205–214.
[22] A.M. Woodward, A. Jones, X.Z. Zhang, J. Rowland, D.B. Kell, Rapid and non-invasive quantification of metabolic substrates in biological cell suspensions using non-linear dielectric spectroscopy with multivariate calibration and artificial neural networks. Principles and applications, Bioelectrochemistry and Bioenergetics 40 (2) (Aug. 1996) 99–132.
[23] A.M. Woodward, R.J. Gilbert, D.B. Kell, Genetic programming as an analytical tool for non-linear dielectric spectroscopy, Bioelectrochemistry and Bioenergetics 48 (2) (May 1999) 389–396.
[24] A.M. Woodward, E.A. Davies, S. Denyer, C. Olliff, D.B. Kell, Non-linear dielectric spectroscopy: antifouling and stabilisation of electrodes by a polymer coating, Bioelectrochemistry 51 (1) (Feb. 2000) 13–20.
[25] B.C. Blake-Coleman, M.J. Hutchings, P. Silley, Harmonic 'signatures' of microorganisms, Biosensors and Bioelectronics 9 (3) (1994) 231–242.
[26] M.J. Hutchings, B.C. Blake-Coleman, P. Silley, Harmonic generation in 'non-linear' biological systems, Biosensors and Bioelectronics 9 (2) (1994) 91–103.
[27] D. Nawarathna, J. Miller, J.R. Claycomb, G. Cardenas, D. Warmflash, Harmonic response of cellular membrane pumps to low frequency electric fields, Physical Review Letters 95 (15) (Oct. 2005) 158103–158104.
[28] D. Nawarathna, J.R. Claycomb, J. Miller, M.J. Benedik, Nonlinear dielectric spectroscopy of live cells using superconducting quantum interference devices, Applied Physics Letters 86 (2) (Jan. 2005) 023902–023903.
[29] E.F. Treo, C.J. Felice, R.E. Madrid, Non linear dielectric properties of microbiological suspensions at electrode–electrolyte interfaces, 27th IEEE EMBS Annual International Conference, September, 2005, Shanghai, China. 2005.
[30] T.Y. Tsong, T.D. Xie, Ion pump as molecular ratchet and effects of noise: electric activation of cation pumping by Na,K-ATPase, Applied Physics A: Materials Science & Processing V75 (2) (Aug. 2002) 345–352.
[31] R.D. Astumian, Protein conformational fluctuations and free-energy transduction, Applied Physics A: Materials Science & Processing V75 (2) (Aug. 2002) 193–206.
[32] R.D. Astumian, Adiabatic pumping mechanism for ion motive ATPases, Physical Review Letters 91 (11) (Sept. 2003) 118102–118104.
[33] R.D. Astumian, Biasing the random walk of a molecular motor, Journal of Physics: Condensed Matter 17 (47) (2005) S3753–S3766.
[34] J. Teissie, T.Y. Tsong, Electric field induced transient pores in phospholipid bilayer vesicles, Biochemistry 20 (6) (Mar. 1981) 1548–1554.
[35] T.Y. Tsong, R.D. Astumian, Electroconformational coupling and membrane protein function, Progress in Biophysics and Molecular Biology 50 (1) (1987) 1–45.
[36] K.E. van Holde, W.C. Johnson, P.S. Ho, Principles of Physical Biochemistry, Prentice-Hall, Englewood Cliffs, NJ, 1998.
[37] G.A. Scarborough, Molecular mechanism of the P-Type ATPases, Journal of Bioenergetics and Biomembranes 34 (4) (Aug. 2002) 235–250.
[38] G.A. Scarborough, Crystallization, structure and dynamics of the proton-translocating P-type ATPase, J Exp Biol 203 (1) (Jan. 2000) 147–154.
[39] C.M. Pickart, W.P. Jencks, Energetics of the calcium-transporting ATPase, Journal of Biological Chemistry 259 (3) (Feb. 1984) 1629–1643.
[40] G.A. Scarborough, Why we must move on from the E1E2 model for the reaction cycle of the P-type ATPases, Journal of Bioenergetics and Biomembranes 35 (3) (June 2003) 193–201.