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REVISED APPROACH TO STATISTICAL ANALYSIS OF IONIC CURRENT FLUCTUATIONS[*]

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We study properties of ion current through a high conductance locust potassium channel. Applying the p-variation test to the current signal we exclude the continuous time random walk (CTRW) as an underlying mechanism of the current fluctuations. Instead, we show that a fractional Brownian motion (FBM) should be considered as the most suitable stochastic model of the ionic channel action. Using the sample mean square displacement (sample MSD) test we bring to light the superdiffusive, persistent properties of the studied current. To illustrate the obtained results, we propose a simple hydrodynamic approximation of the effective charge transport.

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1. Introduction

Ionic currents are the most vitally important biophysical processes in living cells [1,2,3]. Determination of their nature is of importance for many reasons [4, 5, 6, 7]. For instance, ionic currents enable a living cell to control its volume, they generate and conduct electrical and chemical impulses, they also maintain ionic concentration desired for biological reactions. The problem of a detailed characteristics of the ionic channel action has been extensively studied in many papers [8, 9, 10, 11, 12] using statistical methods introduced decades ago. However, a recent great progress in statistical analysis of the time-series data and in understanding of the anomalous dynamics of complex systems [13,14,15,16] makes it reasonable to verify earlier conclusions. The new statistical tools, developed to distinguish between stochastic mechanisms leading to anomalous diffusion, are not only more user-friendly

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but also more precise. They apply to even short one-trajectory time-series recordings and go beyond the usual very basic claims of normal *vs* anomalous diffusion by seeking origins for the anomalous.

In this paper, we look for stochastic origins of the ionic current fluctuations in a data set that was recorded from cell-attached patches of adult locust extensor tibiae muscle fibers (see a sample of the data in Fig. 1). The complete data set consists of one record composed of $N = 250\,000$ values of the potassium current measured by the patch clamp technique at equal time intervals $\Delta t = 0.1$ [ms], total duration being 25 [s]. The uncertainty of measurements of the current is equal to $\delta I = 1$ [pA]. The time-series signal reflects the fact that the channel is not permanently open for conduction of ions but continuously switches between closed (low values of the current) and open (high values of the current) states. The states of low and high values of the current are of random nature resulting from *e.g.*, thermal fluctuations, variations of the voltage difference across the cell membrane, or from conformational changes of channel proteins. A different response of the channel to changes of the environmental variables in open and closed states leads to a non-Markovian property of the underlying stochastic process [9]. A detailed analysis of the above mentioned data set [10,11,12], based on statistical methods available about one decade ago, has indicated the FBM as the most probable stochastic process by means of which the non-Markovian long memory nature of the investigated potassium current can be explained. Now, we verify this conclusion by applying the p-variation [13,16] test. We show that a second fundamental model of anomalous diffusion dynamics, *i.e.* the CTRW, which can be viewed as a substantial alternative, has to

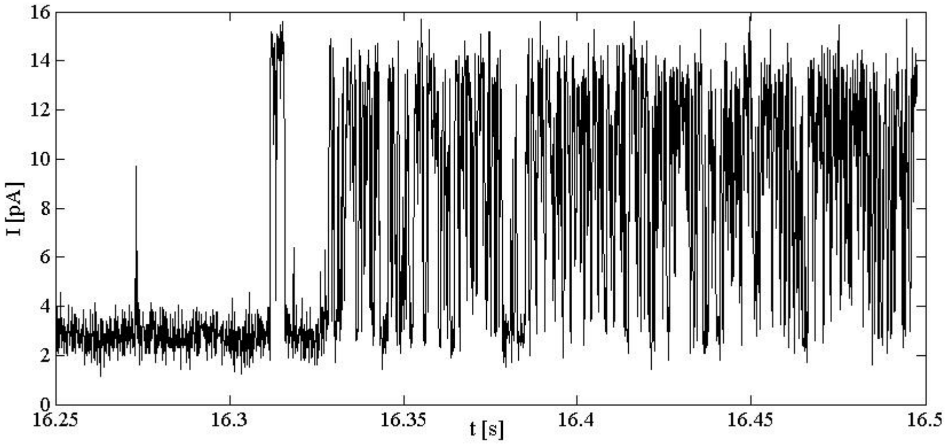

Fig. 1. A part of the potassium current signal. Two distinct states: of high (open channel) and low current (closed channel) can be seen.

be rejected. Using only this test, we are able to bring to light characteristic properties of the stochastic channel action, previously obtained by few different (like Hurst analysis, detrended fluctuation analysis, Orey index, autocorrelation analysis, *etc.*) statistical tools [9, 10, 11]. We also study the anomalous diffusion properties of the effective charge transport. For this purpose, we use the sample MSD introduced in papers [14, 15] for analysis of the one-trajectory time-series data.

2. Statistical tools

2.1. The sample MSD test

The sample MSD, which is a time average of a square displacement, is a function of time difference τ between observations [14, 15]. If the considered process is ergodic it is equivalent to the classical MSD. For a discrete process $(X_i)_{i=0}^{N}$ the sample MSD reads

$$M_N(\tau) := \frac{1}{N-\tau+1} \sum_{k=0}^{N-\tau} (X_{k+\tau} - X_k)^2 \,. \tag{1}$$

It should be noted that in contrary to the classical MSD, $M_N(\tau)$ is a random variable. Its time dependence for $\tau \ll N$ allows one to distinguish stochastic mechanisms of the anomalous dynamics.

If the stochastic process, underlying the recorded signal, is a fractional Lévy α-stable motion (FLSM) with a self-similarity index H and a stability index α then

$$M_N(\tau) \overset{d}{\sim} \tau^{2d+1} \,. \tag{2}$$

The symbol $\overset{d}{\sim}$ denotes similarity in distribution and $d = H - 1/\alpha$ a memory parameter. The memory parameter taking values $d \neq 0$ points to anomalous diffusion dynamics, subdiffusive for $d < 0$ and superdiffusive for $d > 0$, respectively. For the FBM, which is a specific case of the FLSM with the stability index $\alpha = 2$, the memory parameter reads $d = H - 1/2$.

If the signal follows from a CTRW process, then

$$M_N(\tau) \overset{d}{\sim} \tau \,. \tag{3}$$

Due to different time dependences, the sample MSD can easily exclude either the FLSM or the CTRW model. This method is useful in cases when the classical MSD estimators cannot be applied (*e.g.*, when only one trajectory is available).

Results of the sample MSD test are enclosed in Fig. 2. The slope of the function $M_N(\tau)$ for small τ determines the value of the memory parameter d. Using linear regression for interval $\{1, 2, \ldots, 5000\}$ we estimate the value of this parameter $d = 0.3939 \pm 0.0002$ with the satisfying coefficient of determination $R^2 = 0.9998$. This result clearly shows that the CTRW model must be rejected. The positive value of the memory parameter points to superdiffusive properties of the charge transport through the channel.

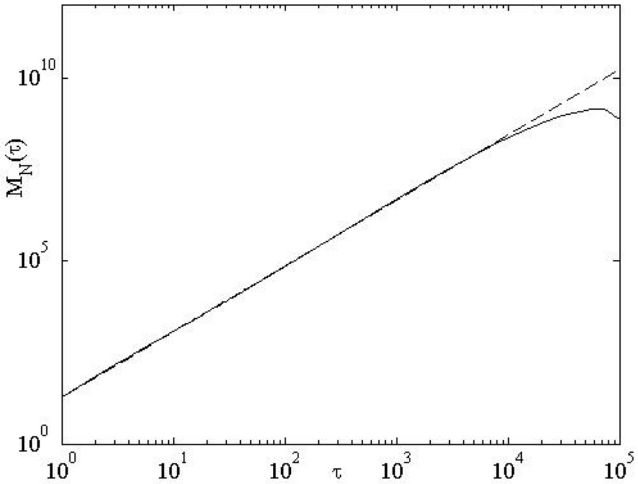

Fig. 2. The calculated sample MSD in a double logarithmic scale compared with linear fitting (dashed line). The slope of the function $M_N(\tau)$ for $\tau \ll N$ estimates the memory parameter $d = 0.3939 \pm 0.0002$. For too large τ the function $M_N(\tau)$ becomes irregular.

2.2. The p-variation test

The p-variation [13, 16] of a stochastic process $X(t)$, defined on time interval $[0, T]$, reads

$$V_p(t) := \lim_{n\to\infty} V_p^n(t)\,, \tag{4}$$

with $V_p^n(t)$ defined as

$$V_p^n(t) := \sum_{j=1}^{2^n-1} \left| X\left(\min\left\{\frac{(j+1)T}{2^n}, t\right\}\right) - X\left(\min\left\{\frac{jT}{2^n}, t\right\}\right)\right|^p . \tag{5}$$

This complicated formula should be interpreted as a sum of terms $|X(t_{i+1}^n) - X(t_i^n)|^p$ with a sequence $(t_i^n)_{i=1}^N$ being thickening binary divisions of the interval $[0, T]$. One must not confuse this notion with p-variation treated as supremum of sums taken over all divisions of $[0, T]$ which also appears in literature [17].

The p-variation method allows us to differ between stochastic origins of the analysed time-series signal. If the underlying process is the FBM then $V_p(t)$ takes the following values

$$V_p(t) = \begin{cases} \infty & p < 1/H\,, \\ t\,\mathrm{E}\left(|B_H(1)|^{1/H}\right) & p = 1/H\,, \\ 0 & p > 1/H\,, \end{cases} \tag{6}$$

where $B_H(t)$ denotes the FBM, H the self-similarity index and the expected value $\mathrm{E}(|B_H(1)|^{1/H})$ is a positive constant, not important in our studies. In the case of FLSM, the values of the p-variation depend on whether the memory parameter d is positive or negative. If $d < 0$ than

$$V_p(t) = 0\,, \qquad \text{for all } p\,. \tag{7}$$

If $d > 0$ the p-variation behaves in similar manner as in the case of FBM, namely

$$V_p(t) = \begin{cases} \infty & p < 1/H\,, \\ 0 & p > 1/H\,. \end{cases} \tag{8}$$

On the other hand, for a CTRW process the p-variation $V_p(t)$ satisfies

$$V_p(t) = \begin{cases} \infty & p < 2\,, \\ S_\alpha(t) & p = 2\,, \\ 0 & p > 2\,, \end{cases} \tag{9}$$

where $S_\alpha(t)$ is an inverse subordinator [18] which is an α-stable stochastic process.

The above formulas allow one to distinguish between the FBM, FLSM and CTRW processes only if estimation of the p-variation $V_p(t)$ is done with a good enough precision. It has been shown [13] that this test brings very good and sufficient results for a data set containing more than 2^9 experimental points distributed in equal time intervals.

To verify results for the potassium current signal, obtained previously by Mercik [10, 11, 12], we follow the p-variation method originally proposed in [13]. We analyse the time-series signal $X_0, X_1, \ldots, X_N$, where $X_n = \sum_{i=0}^{n} I(i)$ is a total flow of the ionic current in discrete time moments t up to $T = n\Delta t$. The values taken by the p-variation $V_p(t)$ can be estimated numerically from the signal cut to a subset of length M, defined by the largest power of 2 less than N. Next, on this subset we construct a sequence $V_p^n(t)$, calculated as sums over binary divisions containing $2, 4, \ldots, 2^{M-1}$ equal subintervals. In this case, the sequence $V_p^n(t)$ can be described in simpler than (5) form

$$V_p^n(t) = \sum_i |X_{i2^{M-n}} - X_{(i-1)2^{M-n}}|^p\,, \tag{10}$$

where the summation index i fulfils $0 \leq (i-1)2^{M-n}$ and $i2^{M-n} \leq t$. As a consequence, $V_p^n(t)$ for $n \in \{1, 2, \ldots, M\}$ is a sequence of simple functions constant on intervals of decreasing lengths 2^{M-n}. To find the time dependence of the p-variation, it is enough to perform the numerical calculations only for few largest n using the following relations

$$\begin{aligned} V_p^{M-1}(t) &= |X_2 - X_0|^p + |X_4 - X_2|^p + \ldots + |X_{\tau_2} - X_{\tau_2 - 2}|^p \,, \\ V_p^{M-2}(t) &= |X_4 - X_0|^p + |X_8 - X_4|^p + \ldots + |X_{\tau_4} - X_{\tau_4 - 4}|^p \,, \\ V_p^{M-3}(t) &= |X_8 - X_0|^p + |X_{16} - X_8|^p + \ldots + |X_{\tau_8} - X_{\tau_8 - 8}|^p \,, \qquad (11) \\ &\vdots \end{aligned}$$

where $\tau_2, \tau_4, \tau_8, \ldots$ are the largest numbers less than t and divisible by $2, 4, 8, \ldots$, respectively.

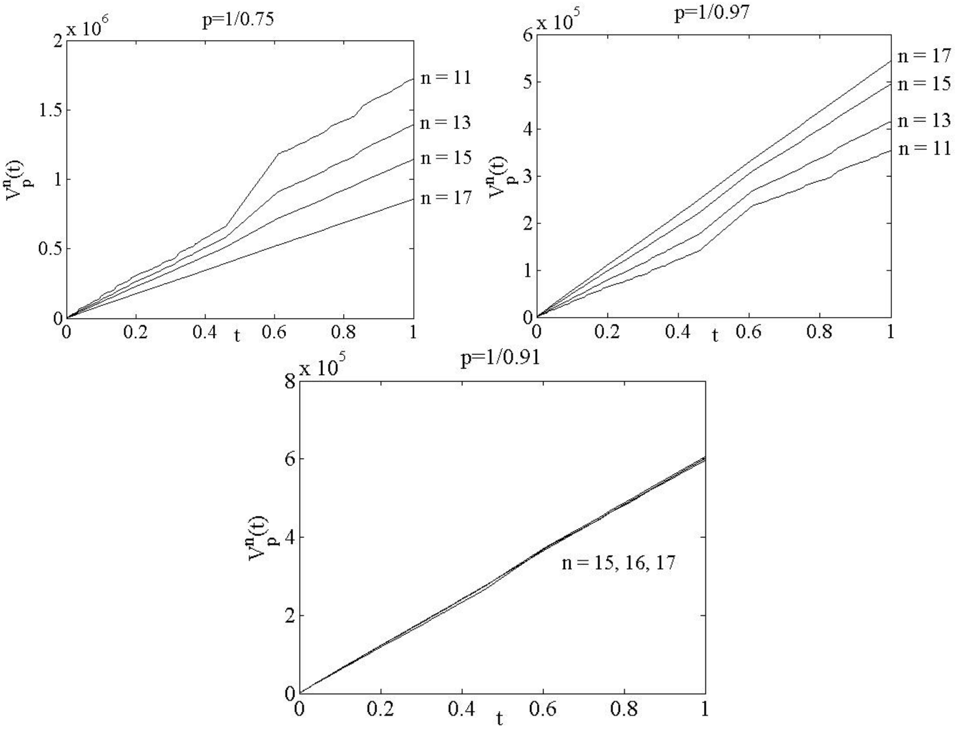

Fig. 3. Behaviour of the p-variation with growing number n: (a) for $p = 1/0.75$ a fast convergence of $V_p^n(t)$ toward 0 is observed, (b) for $p = 1/0.97$ divergence of the sequence $V_p^n(t)$ is noticed, (c) for $p = 1/0.91$ the sequence $V_p^n(t)$ oscillates around a common line $\mathrm{E}(|B_H(1)|^{1/H})$. Time scale normalized.

The time behaviour of $V_p^n(t)$ for different large n allows one to recognize divergence (for $p < 1/H$) or convergence (for $p > 1/H$) of the analysed sequence of the p-variations. Additionally, since a limit of $V_p^n(t)$ with growing n should be a linear function of time when $p = 1/H$, the minimum of square displacement between the functions $V_p^n(t)$ gives a good approximation of the value of p. Plot (a) of Fig. 3 presents a convergence of $V_p^n(t)$ toward 0 with the growing number n for $p > 1/H$. Plot (b) shows a divergence of the sequence $V_p^n(t)$ with growing n for $p < 1/H$. Finally, plot (c) shows a very fast convergence of the sequence $V_p^n(t)$ for values $p \approx 1/0.91$. In this case, the p-variation oscillates around the line $\mathrm{E}(|B_H(1)|^{1/H})$, where the self-similarity index $H \approx 0.91$ is in a very good agreement with the values of H estimated by different statistical methods [10, 11, 12]. Concluding, the p-variation test excluded the CTRW process as an acceptable model for the ionic current fluctuations. The obtained result suggests that the ionic current action may be modelled by means of a FBM with the self-similarity index suggesting a persistent character of the ion transport through the channel. Since the above estimation of the memory and self-similarity parameters suggest the value of the stability index $\alpha \approx 1.94$, as an alternative stochastic origin of the ionic current signal, a FLSM with the stability index close to 2 could be also considered. However, such a possibility has been already rejected by the statistical tests proposed in [19].

2.3. Statistical verification of the FBM model

In this section, we analyse the ionic current signal by means of another statistical test, introduced in [15]. This test allows for a more formal validation of the FBM model, indicated in Sec. 2.2 (also in [12]) as the most appropriate one to explain characteristics of the studied signal. We start from a null hypothesis which states that the data's p-variation yields the FBM with a given self-similarity index H. The opposite hypothesis simply contradicts this conclusion, *i.e.* it may point to any stochastic process with a different value of the p-variation. To verify such an alternative we examine the following statistics, defined for $m \leq n$

$$D_{m,n} = \frac{|V_p^m(T) - V_p^n(T)|}{V_p^n(T)}\,, \tag{12}$$

where $V_p^k(T)$ are approximations of the p-variation at time T. In order to accept or to reject the null hypothesis, a value $d_{m,n}$ of this statistics, derived from the experimental data, has to be compared with a constant D_γ, which is a quantile of order $(1-\gamma)$ defined by the following probabilistic relation

$$\Pr\left(\frac{|\tilde{V}_p^m(T)-\tilde{V}_p^n(T)|}{\tilde{V}_p^n(T)} \le D_\gamma\right) = 1-\gamma\,, \tag{13}$$

where $\tilde{V}_p^k(T)$ denotes the p-variation calculated from the numerically generated FBM with the self-similarity index H estimated from the data and γ is a given level of confidence. We reject the null hypothesis when

$$d_{m,n} > D_\gamma\,. \tag{14}$$

For the confidence level $\gamma = 0.05$, index $H = 0.91$, $m = 16$ and $n = 17$, we obtain $D_\gamma = 33 \times 10^{-4}$. At the same time, $D_{m,n}$ statistics, obtained from the ionic current signal, gives $d_{m,n} = 66 \times 10^{-4}$. This result yields rejection of the hypothesis that the ionic current signal is equivalent to a FBM trajectory. Calculations carried out for different m, n bring a similar result. Nevertheless, the FBM as a first approximation of the current fluctuations is sufficient, but must be treated with a care. It is only a stochastic model reflecting the main statistical characteristics of the complex relations between open and closed states of the ionic channel.

3. Anomalous effective charge transport

In order to illustrate the statistical characteristics obtained for the potassium current signal, we propose the following hydrodynamic approximation. The current recorded in the experiment is of the order of 1–10 [pA] with sampling frequency 10 [kHz]. The single current recording corresponds hence to flow of about 10^2–10^4 potassium ions. This suggests that we can track only a path of a mean charge representing the effective flow of ions and not a single ion itself. We assume that a flow of the potassium ions through the channel is equivalent to the flow of an ionic fluid with a charge density ρ. Because of unpredictable conformational changes of the channel proteins or electrical field and thermal fluctuations the flow of the ions is very unsteady. Regardless, the stochastic process underlying the detected signal [9, 10, 11] is stationary due to a temporal equilibrium of the cell membrane with its environment. The ionic fluid can be viewed as similar in properties to water, hence incompressible in studied circumstances. Because of that, although the channel is constantly changing its states (from open to a closed one and back), at its limits these changes affect only the velocity of the ions transport. Therefore, the flow of the ions can be represented by a movement of a cylinder of the ionic fluid with a constant cross section area S. In this approximation, at any instant of time the current $I(t)$ is proportional to the velocity $v(t)$ of the considered cylinder

$$I(t) = \rho S\, v(t)\,. \tag{15}$$

Let us remark that in living cells the charge density ρ cannot be time independent. The densities of ions inside and outside cell are different, so during channel's functioning the density must be changing in channel's neighbourhood. But, if the flow is one-way in some time interval, the density of charges at one of both sides of the membrane is not changing — the new portion of homogeneous liquid is replacing one that flown. In that case, we can still assume a constant charge density.

The non-Markovian nature of this process may be described in terms of a generalized Langevin equation [20]

$$m\frac{dv(t)}{dt} = -\int\limits_{-\infty}^{t} v(u)K(t-u)du + F_0 + N(t)\,, \tag{16}$$

where the memory kernel $K(t-u)$ is related to the friction, the deterministic force F_0 is related to the external electric field and is constant, a stochastic noise $N(t)$ is dependent on the channel thermal and conformational changes. This assumption holds when the deterministic force is small enough not to change important properties of the channel. In that case, linearity of (16) allows us to study separately the deterministic and stochastic contributions to motion of the ionic-fluid cylinder. Formally, we restrict to solution of (16) in the following form

$$v(t) = v_{\mathrm{D}} + v_{\mathrm{S}}(t) \tag{17}$$

with v_{D} corresponding to overdamped condition with the force F_0 equilibrating friction. The residual part $v_{\mathrm{S}}(t)$ gives insight into the stochastic properties of the channel itself. Without an external field the channel maintains charge density equilibrium, therefore $\langle v_{\mathrm{S}}(t)\rangle = 0$ and accordingly $\langle v(t)\rangle = v_{\mathrm{D}}$. Respectively, in terms of the current these relations read $I(t) = I_{\mathrm{D}} + I_{\mathrm{S}}(t)$ and $\langle I(t)\rangle = I_{\mathrm{D}}$. The stochastic term $I_{\mathrm{S}}(t)$ contributes to the ionic flow in both states of the channel (see Fig. 1). Closing of the channel simply decreases the total flow rate and opening increases it.

The closed and open states of the channel can be represented by movements of the cylinder with different, but constant speeds in those states. Consequently, the effective charge $Q(t)$ is proportional to the displacement $r_{\mathrm{S}}(t) = \int_0^t v_{\mathrm{S}}(u)du$ of the cylinder

$$Q(t) = \rho S\, r_{\mathrm{S}}(t) = \int\limits_{0}^{t} \left(I(u) - I_{\mathrm{D}}\right) du\,. \tag{18}$$

The power-law decay of the current autocorrelation, shown in [11, 12], together with stationarity of the data, are sufficient to prove ergodicity of the studied process using well-known Kchinchin theorem [21]. This fact allows to take the sample mean current $\bar{I} = 6.96$ [pA] as the most natural estimator of I_{D}. Furthermore, it rejects the CTRW as an acceptable model of the process because of its non-ergodicity.

The power-law decay of the autocorrelation function also suggests that the covariation of two separated shifts of the effective charge likewise has a power-law property, what is a signature of anomalous diffusion. We verify this conclusion by means of the sample MSD method. Using this method, we bring to light the persistent character ($1/2 < H < 0$) of the effective charge superdiffusive transport (see Fig. 4). It is clear from this plot that increases in values of the charge $Q(t)$ flowing through the membrane channel are more likely to be followed by increases and, conversely, every decreases are more likely followed by decreases.

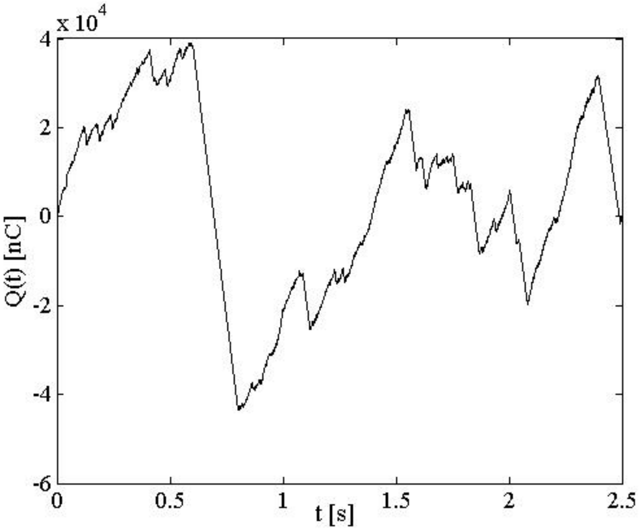

Fig. 4. The effective charge $Q(t)$ flowing through the channel as a function of time. The persistent character of the process is shown. The long decreasing jumps are caused by long closing times of the channel.

4. Conclusions

The main objective of the paper was to present what information on stochastic origins of the observed ionic current fluctuations can be gathered by using the new, recently developed [13, 14, 15] statistical tools. We have shown that the p-variation test is a very simple and efficient method to distinguish between the FBM (or the FLSM) and CTRW properties of the studied time series, in addition estimating the self-similarity index H. By using only this single test, we have excluded the CTRW process as an origin of the current fluctuations. The earlier suggestion by Mercik [10,11,12], that the most probable stochastic model of the ionic channel action is the FBM, was hence verified. Our results have shown, however, important limitations of this model. We also studied the ionic current by means of another new statistical tool, namely the sample MSD test. This test has brought to light the anomalous, in particular superdiffusive type of the effective charge transport through the membrane channel. In contrary to most of the statistical methods, used in the last decades in studies of the ionic currents, the new statistical tests work even if we deal with one time-series trajectory.

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