Origin of $1/f^{\alpha}$ Noise in Membrane Channel Currents

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The transport characteristics of nanofabricated synthetic pores of similar dimensions to those of biological channels is reported. By comparison of the ion current through single synthetic and biological channels we show that the $1/f^{\alpha}$ noise indeed originates from the channel's opening-closing process. Strong evidence has been provided that the latter is related to the underlying motions of channel wall constituents.

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The generating mechanism of $1/f^{\alpha}$ (flicker) noise (FN) is still unknown in spite of about 40 years of investigations [1,2]. Bezrukov and Winterhalter have recently suggested that FN in biological ion channels is caused by "the equilibrium conductance fluctuations related to the conformational flexibility of the channel pore structural constituents," i.e., that it is "not a fundamental property of nonequilibrium transport phenomena" [3]. Moreover, Bezrukov claims that "the $1/f$ noise measured in biological membranes is not an inherent property of ion transport. Rather, this type of noise is produced by fluctuator dynamics, that is, by random switching of the channels between their different conducting states" [2]. It implies that FN "reflects the complex hierarchy of equilibrium protein dynamics that modulate channel conductance" [3]. To check those conjectures we nanofabricated synthetic pores of similar dimensions to those of biological channels and studied their transport characteristics.

Synthetic channels (nanopores) [4,5] enable the measurements, which are impossible or very difficult to perform in biological materials. Moreover, with the advancement of nanotechnology, it is possible to produce polymeric membranes with one single nanopore [6], which makes the examination of individual pore transport characteristics much easier. We are also in a position to tailor the properties of the pore on a nanometer scale, giving it the desired properties. We decided to analyze the behavior of three voltage-gated channels: one biological (BIO) [7,8] and two synthetic double cone pores (one pore per sample) [4,5], nanofabricated in a polyethylene terephthalate (Hoechst, RN12) (PET) and polyimide foil [9] (Kapton 50HN, DuPont) (KPT). All three channels have a comparable diameter in a narrow part of about 2 nm; the height of the smaller cone in PET and KPT pores is similar to the length of the BIO channel and equal approximately to 20 nm. The magnitudes of measured potassium currents I are also comparable (cf. below).

The main structural differences between the three channels examined in this work seem to be as follows: The BIO channel (the large conductance locust potassium channel [7,8]) consists of four protein subunits, which create an inverted cone with charged entrances [10,11], structure common for the prokaryotic as well as eukaryotic potassium channels [12]. The ion current data were obtained from cell attached patches of adult locust (*Schistocerca gregaria*) extensor tibiae muscle fibers [8]. The PET and KPT channels were produced by irradiation of the polymeric foils with single accelerated Au ions of 2.2 GeV kinetic energy (linear accelerator UNILAC, GSI, Darmstadt), and subsequent chemical etching [4–6,9]. To produce double-conical pores, the procedure based on asymmetric etching was used. The foil was at first preetched on both sides, which brought about formation of two small cones. In the next step, a one-sided etching was performed, which completed formation of the second, bigger cone, which plays the role of support for the small one [9].

Preparation of the pore as well as the current measurements were performed in a conductivity cell, connected to the locally built electronic feedback circuit based on a current/voltage converter with pA sensitivity, controlled by software written in LABVIEW 5.0 (National Instruments). The output was digitized at 166 kHz using the National Instruments PCI-MIO-16XE-10 card, and sampled at 1 kHz. The experimental setup was placed on the Köttermann balance table. We used a closed copper Faraday cage. PET foil was etched in sodium hydroxide, while Kapton was etched in sodium hypochlorite [9,13]. In KPT this treatment results in a fairly smooth pore (channel) with relatively stable (unmoving) walls, which is a result of the chemical structure of the polymer, based on aromatic rings [13]. In PET on the other hand, there remain—sticking from the pore walls—charged with carboxylate groups, loose ends of cleaved polymer strands, so-called "dangling ends" [14], which move continuously in a more or less random fashion [15,16]. Ion current recordings through the synthetic nanopores were performed at symmetric electrolyte conditions, in 0.1 M KCl at pH 7 (phosphate buffer).

Ion current through the BIO channel was recorded by the patch-clamp technique [17] using a List EPC-7 Patch-Clamp amplifier. The patch pipettes contained 10 mM

NaCl, 180 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, and *p*H 6.8. Output was digitized at 22 kHz using a Sony PCM ES-701 and stored on standard videotape. Records were transferred to the hard disk of an IBM compatible PC via an analog-to-digital converter (Axon Instruments) sampling at 10 kHz [7].

BIO channel data are the same as in Ref. [7] and consist of stationary series of 250 000 values (total length 25 s) of potassium current ($0 < I < 20.5$ pA) excited at 60 mV. Corresponding PET pore data—stationary series of over 130 000 values, $-4.2 < I < 16.5$ pA, excited at 180 mV—were recorded at 1 ms intervals. In both cases the current fluctuates randomly between high (I_o) and low (I_c) values (open and closed channel), with most probable $I_o \approx 11$ pA and $I_c \approx 0$ pA. The only significant difference between BIO and PET data is their time characteristics: transitions between open and closed states in BIO channel are about 40 times faster than in PET ones (average dwell times $\tau_B \approx 1.7$ ms, $\tau_{\mathrm{PET}} \approx 73$ ms). Typical recordings of these currents are shown in Fig. 1(a).

The frequency (power) spectra $S(f)$ were calculated by dividing the experimental data into five to ten shorter segments, computing $S(f)$ for every segment separately, and averaging over segments. The obtained nonsmoothed power spectra are shown in Fig. 1(b) (for corresponding smoothed spectra, cf. below, Fig. 2). It is seen that the spectra for BIO (red) and for PET (black) series are almost identical in the frequency range $0.1 < f < 50$ Hz and show the $1/f^{\alpha}$ behavior with $\alpha = 1.05 \pm 0.05$. The conversion of the current values into a dichotomous (open-closed) series (cf. Ref. [7]) does not change the shape of $S(f)$.

In the above-described random series three presumably different stochastic processes can be singled out: closed-channel current fluctuations, open-channel ones, and random transitions between open and closed states. To find which of these is responsible for the appearance of flicker noise, we recorded the currents in a closed and in an open PET channel (stationary series of about 150 000 values each, excited at 60 mV, with average current $I_{\mathrm{av}} \approx 0$, and 250 mV, $I_{\mathrm{av}} \approx 59$ pA, respectively). At higher voltages the results are similar, with higher currents. The smoothed power spectra for these series are shown in Fig. 2 (black), together with smoothed $S(f)$ for the PET series recorded at 180 mV (black), and for the BIO series (red). The series of dwell times in open and closed states were calculated from both BIO and 180 mV PET series—the corresponding (smoothed) power spectra are also shown in Fig. 2 (blue). We found that the open-channel spectrum behaves as $f^{-\alpha}$ with $\alpha \approx 1.9$; to fragments of closed-channel and dwell-time spectra one can ascribe—with some amount of imagination—the $1/f$ behavior, though such a dependence is not obvious.

The results presented in Fig. 2 are still not decisive. To get a definitive answer, the new "mixed" data series were constructed by taking N_j successive values, N_j being the consecutive dwell times from the 180 mV PET series, alternately from (i) 60 mV (closed) and 250 mV (open) PET series, (ii) generated Gaussian-distributed values, peaked at 0 and at 59 pA, with widths adjusted to widths of experimental distributions in (i), and (iii) constant

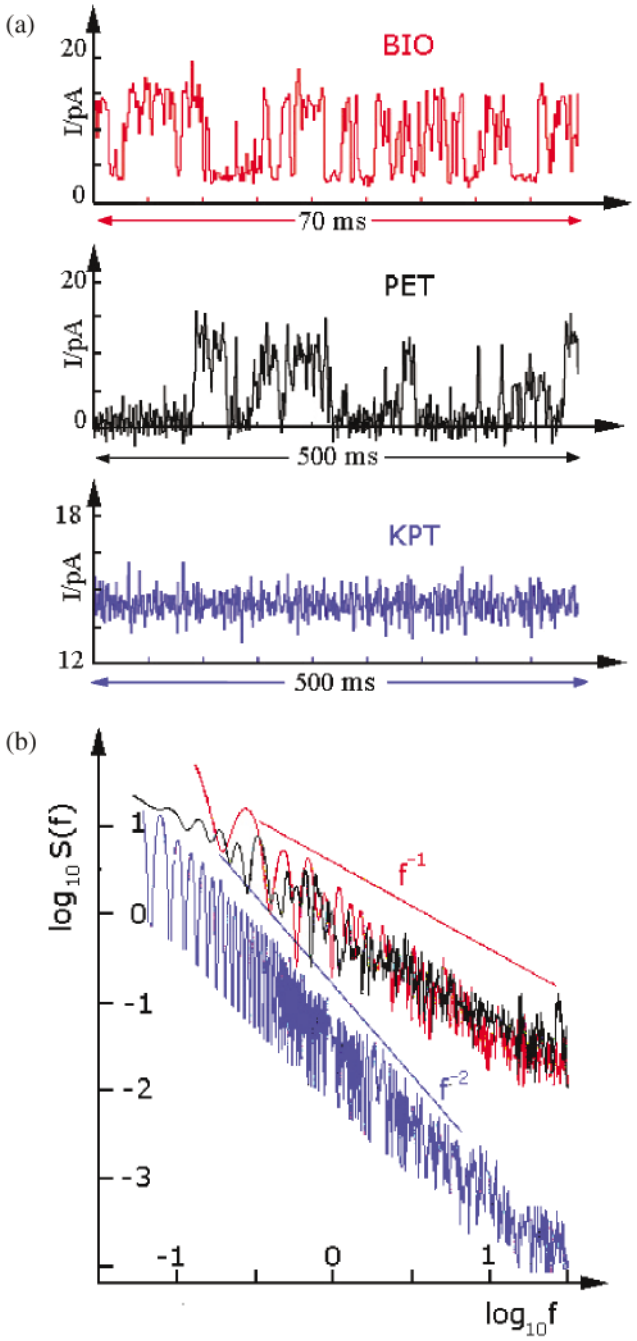

FIG. 1 (color). (a) K^+ current $I(t)$ through biological channel (BIO, red) excited at 60 mV, PET nanopore (PET, black) and Kapton nanopore (KPT, blue) at 180 mV. Note differences in scales. (b) Nonsmoothed power spectra of time series shown in (a) of biological BIO channel (red), and synthetic: PET (black) and KPT (blue) pores. BIO and PET channels work at bistable (open/closed) region, KPT channel—in monostable (open) region. $S(f)$ in $(\mathrm{pA})^2/\mathrm{Hz}$, f in Hz. KPT-pore data are shifted down by one decade unit; otherwise spectra would obscure each other.

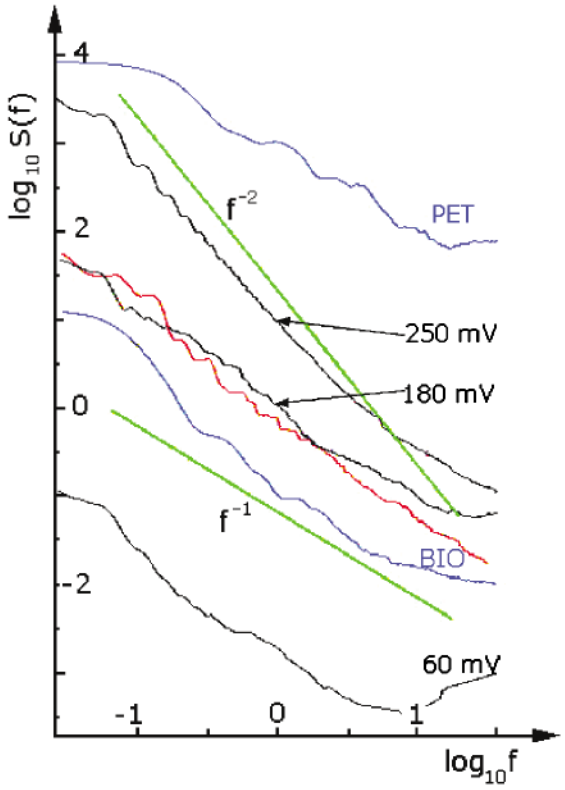

FIG. 2 (color). Smoothed power spectra. Potassium current series data: PET pore excited at different voltages (line labels, black), biological channel BIO (red)—these spectra are in $(\mathrm{pA})^2/\mathrm{Hz} = 10^{-24}\ \mathrm{A}^2 \cdot \mathrm{s}$; dwell-time series (blue) for bistable channels: biological (label BIO) and synthetic (label PET)—these spectra are in $(\mathrm{ms})^2/\mathrm{Hz} = 10^{-6}\ \mathrm{s}^3$.

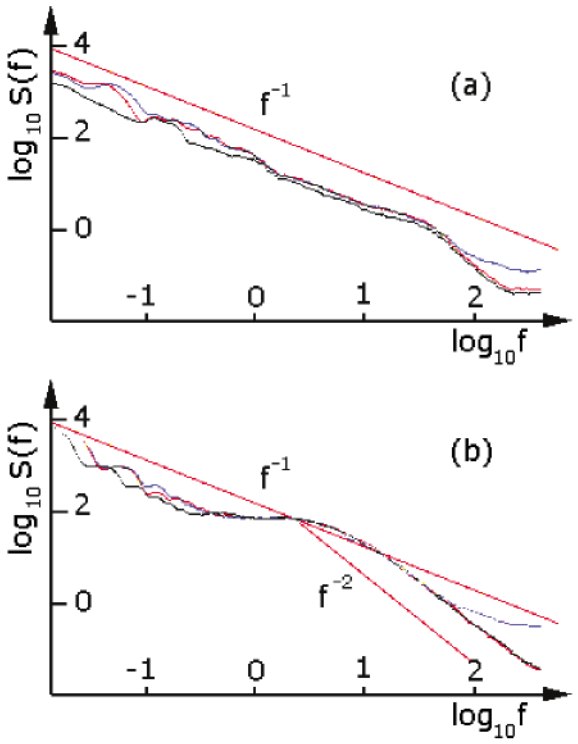

FIG. 3 (color). Power spectra for artificially mixed bistable K^+-current data. (a) Dwell times from PET potassium current series excited at 180 mV; (b) dwell times generated randomly with exponential distribution $P(t_d) = \exp(-t_d/\tau_{rnd})$; $\tau_{rnd} = \tau_{\mathrm{PET}} = 73$ ms. Current values taken as follows: black—PET 60 mV series (closed channel) and PET 250 mV series (open channel); blue—chosen randomly from two Gaussian distributions, peaked at 0 (closed channel) and 59 pA (open channel); red—constant values 0 (closed channel) and 59 pA (open channel).

values $I_c = 0$ and $I_o = 59$ pA. The resulting power spectra (smoothed) are shown in Fig. 3(a)—all three cases are almost identical and show distinct $1/f$ characteristics. Use of dwell times from BIO data gives a similar result, with $1/f^{\alpha}$, $\alpha = 1.1 \pm 0.1$ characteristics.

In order to provide an additional support for those results, the same procedure was repeated for a series of successive dwell times generated as a Markovian process with exponential dwell-time distribution with $\tau_{rnd} = \tau_{\mathrm{PET}}$. The result is shown in Fig. 3(b)—again all three cases [(i)–(iii) above] are almost identical, but their $S(f)$'s do not fall down as $1/f$.

These results provide a strong support to the hypothesis that the only source of flicker noise in voltage-gated potassium channels is the alternation of current between low (closed) and high (open) states, and that current fluctuations in these states do not influence in any significant way the shape of the power spectrum. This further supports the second Bezrukov's conjecture, mentioned in the opening section of this Letter.

On the other hand, there is also a distinct difference between experimental dwell-time series and the random Markovian one. We attribute this fact to the non-Markovian character of the experimental data [7]. One of properties of a Markov process is that it satisfies the Smoluchowski-Chapman-Kolmogorov (SCK) equation (for more details, cf. Ref. [7]). Therefore the deviation from that equation measures the degree of non-Markovianity:

$$D_{m,n}(t,\tau) = P(m,t|n,0) - \sum_{k=1}^{M} P(m,t|k,t-\tau)P(k,t-\tau|n,0), \quad (1)$$

where $k, m, n = 1, 2, \ldots, M$ number the current states. The value of the current belongs to the mth channel state, when it lies in the interval $mdI < I < (m+1)dI$, $m = 0, \ldots, M-1$, $dI = (I_{\max} - I_{\min})/M$, M being the assumed number of different channel states. The $P(m,t|n,s)$ is the current-current conditional probability that the current $I(t)$ is in the state number m, under the condition that at the earlier time $s < t$, $I(s)$ was in the state number n. The integral measure (mean square characteristics) of the non-Markovianity, defined in Ref. [7], reads

$$G = G(\tau, T) = \left[\frac{1}{T}\frac{1}{M^2}\sum_{m,n}^{M}\int_{\tau}^{\tau+T} dt D_{m,n}^2(t,\tau)\right]^{1/2}, \quad (2)$$

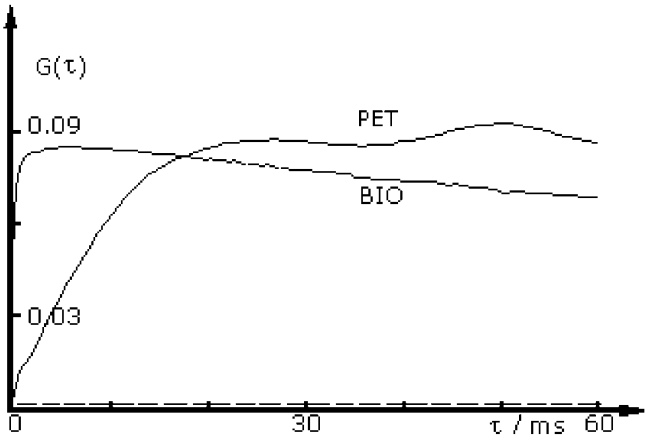

FIG. 4. Non-markovian measure $G(\tau)$ [Eq. (2)] for bistable potassium current data—biological (BIO) and synthetic PET excited at 180 mV; time range $T = 60$ ms. $G(\tau)$ for markovian dichotomous series is shown as a dashed line at the bottom.

where T is the range of the time t and τ is the shift in the SCK equation.

In our case, a natural number of states is $M = 2$, as in Ref. [7]. To obtain comparable results (because of different characteristic time scales mentioned above) $G(\tau, T)$ was calculated for $T = 60$ ms, and for different values of τ (Fig. 4). Maximal values $G_{\max} = \sup_\tau G(\tau, T)$ are $G_{\mathrm{BIO}}(5, 60) = 0.085$, $G_{\mathrm{PET}}(50, 60) = 0.090$, τ and T are in ms. For computer-generated Markovian dichotomous series of a comparable length $G = 0.0014$ and does not depend on time-lag τ. This confirms again the similarity of the processes underlying the dichotomous character of BIO and 180 mV PET currents.

Finally, the question arises of what induces the non-Markovian character of the bistable open-closed transitions, which seem to be responsible for the appearance of the flicker noise. According to the Bezrukov's conjecture mentioned above, the "random motion of the channel pore structural constituents" is the responsible agent. To check this, we performed the measurements of the potassium current in the Kapton pore.

KPT channel stationary series measured at 1 ms intervals and 180 mV contains over 130 000 values. The current fluctuates randomly around 15.4 pA [$14 < I <$ 17.3 pA, no closed states—cf. Fig. 1(a)]. The lack of transitions between open and closed states in KPT is the most significant difference between KPT and (BIO and PET) channels. This results in the power spectrum [Fig. 1(b), blue] with totally different characteristics, decaying as $1/f^2$. Identical results were obtained for KPT channel currents measured at 10 and 60 mV—in each case the KPT pore behaves as an open channel (identical to the PET open channel, e.g., at 250 mV), the only difference being lower current values.

The presented close similarity of the stochastic behavior of BIO and PET channels seems to be the result of the motions of subunits constituting the channel walls, whereas the KPT channel, which is lacking of such fluctuating obstacles, is characterized by fundamentally different stochastic behavior. It supports Bezrukov's conjecture on the fundamental role of the "equilibrium protein dynamics that modulate channel conductance" in creating flicker noise in a channel conductance. The presented results also provide additional strong evidence of the universality of $1/f$ noise.

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