October 14, 2005

ANALYSIS OF ION CURRENT SIGNAL IN TIME

I. Patch Clamp Technique

Patch clamp technique was developed to measure ion currents through single channels in biological membranes. The key element of the experimental set-up is a glass micropipette with opening ~50 – 200 nm. The pipette is filled with electrolyte e.g. 1 M KCl and it contains an Ag/AgCl electrode. This pipette is brought into contact with the cell surface and a little suction is applied to assure a seal between the pipette and the cell membrane. This suction prevents the leakage current to flow 'around' the pipette. The cross section area of the pipette is so small that on average it covers an area of a membrane with a single channel. By applying a voltage between the electrode in the pipette and another electrode inserted in the cell or in a Petri dish in which the bio-cell or membrane is placed, an ion current is flowing through that single channel. Four 'modes' of patch clamp technique are distinguished (Figure 1).

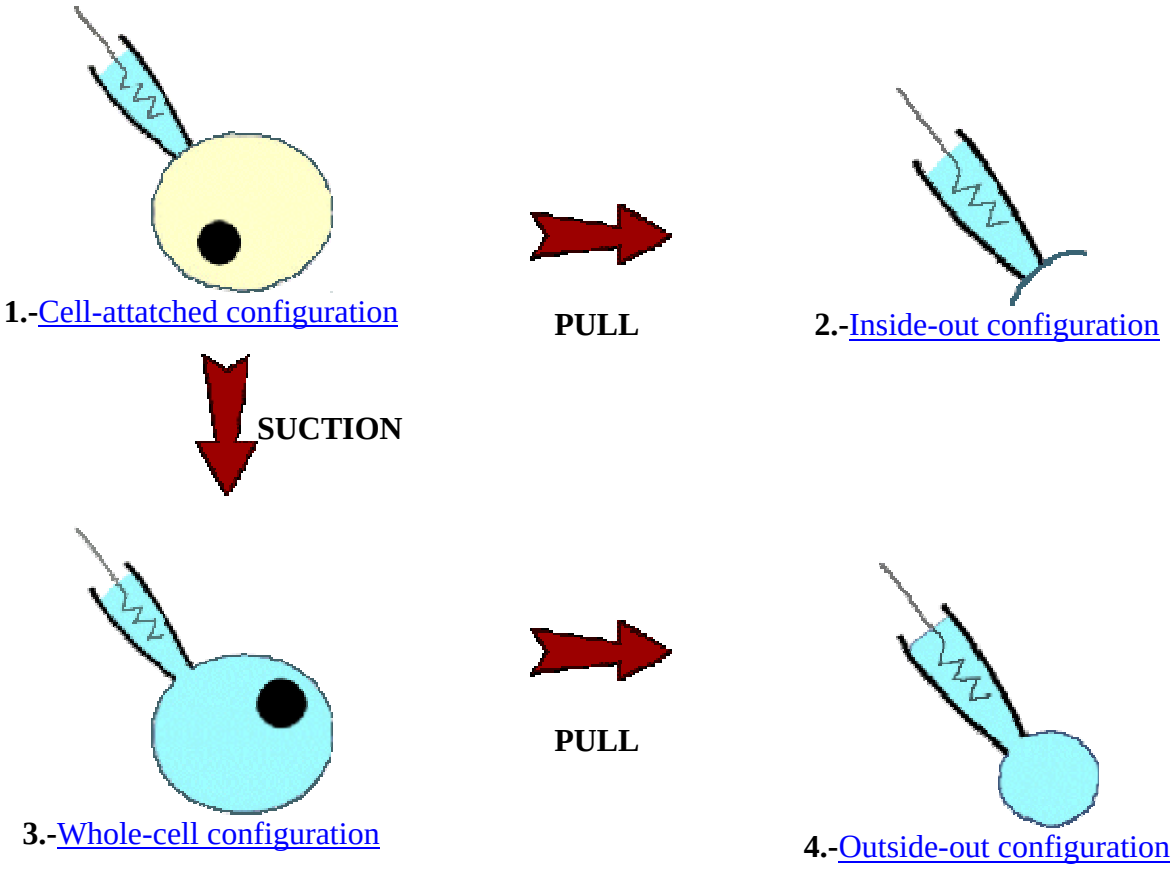

Figure 1 Four different configurations in patch-clamp measurements [http://medweb.bham.ac.uk/research/calcium/Homeostasis/Patchclamp.html].

There has been a microscopy technique developed for imagining cells and membranes based on the patch clamp technique, the so-called scanning ion conductance microscope [Hansma et al. Science 243 (1989) 641; Korchev et al. 1997 Biophys. J. 73 (1997) 653].
This technique also allows studying a distribution of ion channels in cell membranes [Nature Cell Biology 2 (2000) 616].

II. Analysis of Biological Signals.
Biological signals are often very complex. As an example, let's look at a signal of ion current through a single potassium channel in a locust muscle cell (Figure 2). Although the voltage applied across the membrane is constant, the ion current signal is not stable, but rather fluctuates between several levels. For this particular type of channel, ion current fluctuations are strongly voltage dependent (Figure 3).

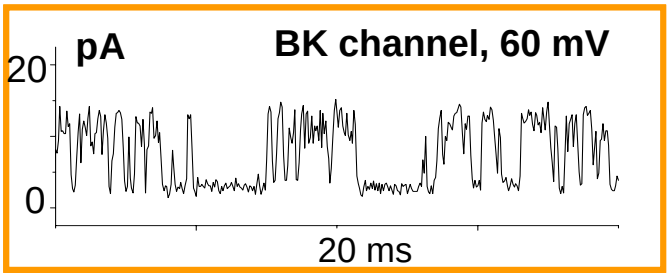

Figure 2 Ion current in time through a single voltage-gated channel in a locust muscle (courtesy of Prof. P.N.R Usherwood, University of Nottingham, UK).

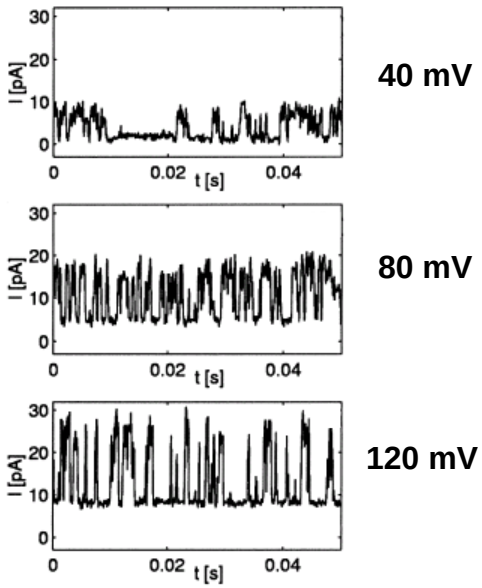

Figure 3 Ion current in time through a single voltage-gated channel in a locust muscle (courtesy of Prof. P.N.R Usherwood, University of Nottingham, UK), recorded for different voltages applied across the membrane.

There are several tools to characterize such a signal.

1. Basic statistics: calculating average values and standard deviation, maximum and minimum values.

2. Frequency histograms of ion current values: dividing the current signal into 'bins' and finding how frequently a value of ion current belonging to a given bin occurs in the signal. Frequency histograms give information on the number of current levels, called also 'states'. A histogram of the signal from Fig. 2 is shown in Fig. 4. It can be seen from the figure that all the current values fall into two groups of ion current values. The group close to 0 pA is called a closed state of the channel; the other groups of ion current signal, with average value significantly different than zero, is called an open state of the channel. Fitting the two histogram peaks e.g. by Gaussian distributions, allows finding a threshold value, which divides the ion current values into open and closed states. By integrating the two peaks of the histogram, it is also possible to calculate the probability of the channel opening and closing.

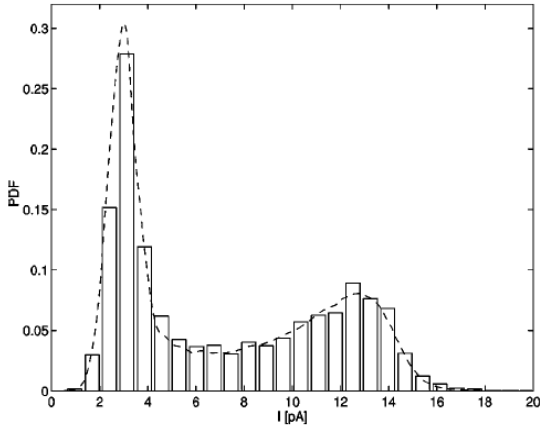

Figure 4 Histogram of ion current time series whose fragment is shown in Fig.2 (Mercik, Weron & Siwy PRE 60 (1999) 7343.

3. Changing the time series into a dichotomous signal and determination of dwell-times. Having the threshold value of ion current, it is possible to change the signal into series [0,1]. "0" state corresponds to the closed state of the channel, and state "1" corresponds to the open state of the channel. We measure then the duration of subsequent closed and open states $\{t_{o,i}\}$ and $\{t_{c,i}\}$.

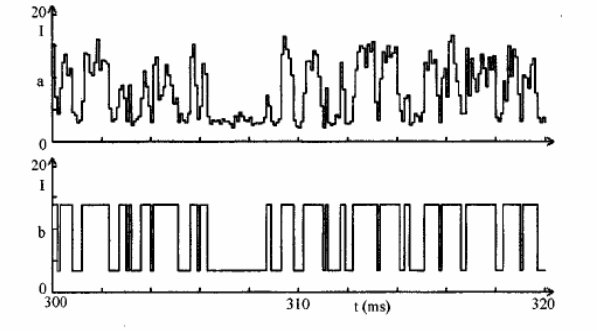

Figure 5. Example of changing the signal of ion current into a dichotomous signal Fulinski et al. PRE 58 (1998) 919.

4. Determination of dwell-times distribution; determination of frequency histograms of $\{t_{o,i}\}$ and $\{t_{c,i}\}$.

5. Modeling the channel kinetics as switching between two states: open and closed, with kinetics rates (probability of switching per unit time) k_o, and k_c.

$$C \underset{k_c}{\overset{k_o}{\Leftrightarrow}} O$$

The ion current values are treated therefore as a result of random switching between these two states.

Let's call $P(t)$ a probability that the channel is closed in the interval $[0,t]$. The probability that the channel remains closed over the interval $[0, t+\Delta t]$ is equal to the probability that the channel is closed up to the time t and will not open during the next interval. The latter probability is equal to $(1-k_o\Delta t)$.

$$P(t+\Delta t) = P(t)\ (1-k_o \Delta t)$$
$$P(t+\Delta t)/P(t) = 1-k_o \Delta t$$
$$\frac{P(t+\Delta t)-P(t)}{P(t)} = -k_o \Delta t$$

taking limit of $\Delta t \to 0$ and integrating between $[0, t]$ leads to the solution

$$\frac{dP(t)}{P(t)} = k_o dt$$
$$\ln P(t) = -k_o t$$
$$P(t) = A \exp(-k_o\, t)$$

$P(t)$ is the *cumulative probability functio*n. Probability per unit time that the channel is closed for a duration t is given therefore by the derivative of $P(t)$ in time i.e. by the *probability density function* $f(t)$:

$$f(t) = -\frac{dP(t)}{dt}$$

Exponential form of dwell-time histograms provides evidence that the channel kinetics can indeed be modeled by a simple two-state model with constant in time probabilities of opening and closing.

More complex channel kinetics involves k_o and k_c dependent on time e.g. $k_o(t)=Bt^{1-D}$. The kinetic rate constant can be found to be equal:

$$P(t) = e^{-[B/(2-D)]t^{2-D}}$$

The time dependent rate constants take into account the possibility of existence a whole spectrum of states of ion channel.

6. Autocorrelation function studies correlation between a given value of ion current at time t with values recorded at an earlier time: $t - \tau$.

$$R_x(\tau) = \langle x(t)x(t-\tau)\rangle = \lim_{T\to 0}\left\{\frac{1}{T}\int_{-T/2}^{T/2} x(t)x(t-\tau)dt\right\}$$

7. Power spectrum – Fourier analysis.

Fourier analysis is a technique by which a signal that varies in time or in space is decomposed into its constituent temporal or spatial frequency components.
A signal $x(t)$ of total duration T can be expressed as a Fourier series:

$$x(t) = \sum_{n=1}^{\infty} a_n \cos\frac{2\pi nt}{T} + b_n \sin\frac{2\pi nt}{T}$$

where a_n and b_n are calculated as:

$$a_n = 2\left\langle x(t)\cos\frac{2\pi nt}{T}\right\rangle = \frac{2}{T}\int_{-T/2}^{T/2} x(t)\cos\frac{2\pi nt}{T}dt \qquad n \geq 1$$

$$a_n = 2\left\langle x(t)\sin\frac{2\pi nt}{T}\right\rangle = \frac{2}{T}\int_{-T/2}^{T/2} x(t)\sin\frac{2\pi nt}{T}dt \qquad n \geq 1$$

The power spectrum $G_x(f)$ of a signal $x(t)$ is defined so that $G_x(f)\cdot\Delta f$ is the mean-square displacement of the signal in the frequency range (f, $f + \Delta f$). If the signal therefore is passed through a filter with a center frequency f Hz and bandwidth Δf Hz, then the value $G_x(f)\ \Delta f$ gives the mean-squared value of this filtered signal.

$$G_x(f)\cdot\Delta f \equiv \left\langle x^2_{f,\Delta f}(t)\right\rangle = 0.5\left(a_n^2 + b_n^2\right)$$

Relation between power spectrum and autocorrelation function:
Power spectrum is the Fourier transform of autocorrelation function.

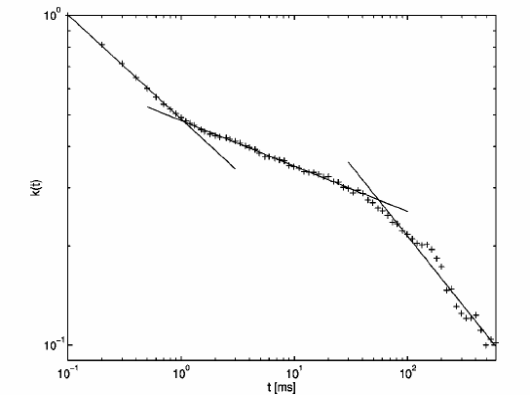

Figure 6. Autocorrelation function of the time series shown in Fig. 2.

8. Examination of stationary character of time signal.

III. Langevin Equation and Autocorrelation Function

Let's consider randomly moving particle. $F(t)$ is the thermal force acting on a molecule due to collisions with surrounding solvent molecules. It comprises very brief impulses with random direction, occurring at random times. The equation of motion of that molecule in response to this force is given by the so-called Langevin equation:

$$m\frac{d^2x}{dt^2}(t) + \gamma\frac{dx}{dt}(t) + \kappa x(t) = F(t)$$

The important feature of autocorrelation function is that it fulfills the equation of motion. This indicates that the autocorrelation function has the same form as the response of the molecule to the external force. This further means that by measuring thermal motion, calculating autocorrelation function and comparing it to the theoretical autocorrelation function predicted by a given model, we can obtain molecular parameters of stiffness and damping.

1. *Single-Channel Recording*, 2nd Ed. B. Sakmann, E. Neher (editors), Plenum Press, New York and London (1983).
2. J. Howard, *Mechanics of Motor Proteins and the Cytoskeleton*, Sinauer Associates Inc. Sunderland, Massachusetts (2001).
3. L.S. Liebovitch, J.M. Sullivan, *Biophys. J.* **52** (1987) 979.