

Computational procedure and data analysis protocol[†]

February 10, 2015

1 Characteristics of ion-current fluctuation

If the observed ion current, j_k , is sampled at rate $f_s = 1/\Delta t$ (Δt is the sampling period) the average current for a data record taped for a period $T = N_T \Delta t$ reads

$$j_{\text{av}}(T) = \frac{1}{N_T} \sum_{k=1}^{N_T} j_k \quad . \quad (1)$$

We also define the partial average for the record by using only the data that start from time $\tau = M_\tau \Delta t$, where $M_\tau = 0, 1, \dots, N_T - 1$,

$$j_{\text{av}}(\tau, T) = \frac{1}{N_T - M_\tau} \sum_{k=1}^{N_T - M_\tau} j_{k+M_\tau} \quad . \quad (2)$$

Clearly, $j_{\text{av}}(T) = j_{\text{av}}(0, T)$.

The auto-correlation function calculated for the data record is defined as follows,

$$R(\tau, T) = \frac{1}{N_T - M_\tau} \sum_{k=1}^{N_T - M_\tau} \left[j_k - j_{\text{av}}(T) \right] \left[j_{k+M_\tau} - j_{\text{av}}(\tau, T) \right] \quad . \quad (3)$$

⁰† This is the Electronic Supplementary Information (ESI) for *I. Bodrenko, H. Bajaj, P. Ruggerone, M. Winterhalter, and M. Ceccarelli, 'Analysis of fast channel gating: revealing substrate binding in the microsecond range'*

With the above definition, the squared standard deviation of the ion current, σ_j^2 , is equal to the auto-correlation function at zero time shift,

$$\sigma_j^2 = R(0, T) \quad . \quad (4)$$

The power spectral density (PSD) of a signal is formally defined as the Fourier transform of its auto-correlation function, $R(\tau) \equiv R(\tau, T \rightarrow \infty)$,

$$g(\omega) = \frac{1}{\pi} \int_0^{+\infty} R(\tau) \cos(\omega\tau) d\tau \quad . \quad (5)$$

Below, we will also use an alternative definition of the power spectral density by using the temporal frequency $f = \omega/2\pi$ instead of the angular frequency ω ,

$$G(f) \equiv 4\pi g(2\pi f) \quad . \quad (6)$$

Then, we define the finite-interval power spectral density as follows

$$g(\omega, \tau_{\max}, T) = \frac{1}{\pi} \int_0^{\tau_{\max}} R(\tau, T) \cos(\omega\tau) d\tau \quad , \quad (7)$$

so that

$$\lim_{\tau_{\max} \rightarrow \infty} \left[\lim_{T \rightarrow \infty} g(\omega, \tau_{\max}, T) \right] = g(\omega), \quad (8)$$

assuming that integrals and limits exist. If the correlation function decays sufficiently fast by increasing τ and if the correlation time of the process is smaller than $\tau_{\max} \ll T$, then one may expect that $g(\omega, \tau_{\max}, T) \approx g(\omega)$.

The auto-correlation function of the ion-current record, (3), is defined at discrete time shifts, $\tau_l = l\Delta t$, $l = 0, 1, \dots, M_\tau$, as

$$R_k^T \equiv R(\tau_k, T) \quad . \quad (9)$$

If a linear interpolation between the time points is assumed, then the auto-correlation function may be continuously represented over the interval $\tau \in [0, \tau_{\max}]$, where $\tau_{\max} = M_\tau \Delta t$, as

$$R(\tau, T) \approx \sum_{l=0}^{M_\tau-1} \left[\left(R_{l+1}^T - R_l^T \right) \frac{\tau - \tau_l}{\Delta t} + R_l^T \right] \text{rect} \left(\frac{\tau - \tau_l}{\Delta t} \right) \quad , \quad (10)$$

where $\text{rect}(x) = \theta(x) - \theta(x - 1)$ is the rectangular function on interval $[0, 1]$ and $\theta(x)$ the Heaviside step function. Then, the PSD at the characteristic frequencies may be approximately calculated as follows

$$\begin{aligned}
g(\omega_l, \tau_{\max}, T) &\approx \frac{\Delta t}{\pi} \text{sinc}\left(\frac{\omega_l \Delta t}{2}\right) \sum_{k=0}^{M_\tau-1} \frac{R_{k+1}^T + R_k^T}{2} \cos\left[\omega_l \left(\tau_k + \frac{\Delta t}{2}\right)\right] + \\
&+ \frac{\Delta t}{\pi} \text{sinc}^{(1)}\left(\frac{\omega_l \Delta t}{2}\right) \sum_{k=0}^{M_\tau-1} \frac{R_{k+1}^T - R_k^T}{2} \sin\left[\omega_l \left(\tau_k + \frac{\Delta t}{2}\right)\right] \\
\omega_l &= \frac{2\pi}{\Delta t} l; \quad l = 0, 1, \dots, M_\tau
\end{aligned} \tag{11}$$

Here, $\text{sinc}(x) = \sin(x)/x$ and $\text{sinc}^{(1)}(x) = [x \cos(x) - \sin(x)]/x^2$.

2 Filter and sampling corrections

In the electrophysiology experiments, the ion-current signal is filtered by an analogous low-pass linear filter and then sampled at a specific rate, $f_s = 1/\Delta t$. The sampling procedure consists of averaging a signal for the period Δt and, thus, is also a linear filter applied after the first analogous one. The average value of the ion-current signal, j_{av} , is not affected by a linear filter whereas the dispersion, the auto-correlation function, and the PSD are modified by both filters. If the transfer function (its Fourier transform) of the analogous filter is $H_a(\omega)$ and that of the sampling filter $H_s(\omega)$, then the relation between the PSD of the original ion current, $g_o(\omega)$, and the one of the signal after successive application of the two filters reads,

$$g(\omega) = g_o(\omega) |H_a(\omega)|^2 |H_s(\omega)|^2 \quad . \tag{13}$$

A 4-order (4-pole) low-pass Bessel filter is used in the current measurements. The transfer function of the Bessel filter of order n is designed as follows,

$$H_n(\omega) = \frac{\theta_n(0)}{\theta_n(i\omega/\omega_0)}, \tag{14}$$

where $\theta_n(x)$ is the reverse Bessel polynomial of order n ; ω_0 is a frequency chosen to give the desired cut-off frequency. For the 4-order low-pass Bessel

filter, $\theta_n(x) = x^4 + 10x^3 + 45x^2 + 105x + 105$, and the absolute square of the transfer function reads,

$$|H_a(\omega)|^2 = \frac{11025}{\left(\frac{\omega}{\omega_0}\right)^8 + 10\left(\frac{\omega}{\omega_0}\right)^6 + 135\left(\frac{\omega}{\omega_0}\right)^4 + 1575\left(\frac{\omega}{\omega_0}\right)^2 + 11025} \quad (15)$$

Here, $\omega_0 = 2\pi f_c/x_c$, where f_c is the cutoff frequency, $x_c \approx 2.114$ the scaling factor determined by the following condition, $|H_a(2\pi f_c)|^2 = 1/2$.

The absolute square of the PSD of the sampling filter is

$$|H_s(\omega)|^2 = \left(\frac{\sin\left(\frac{\omega\Delta t}{2}\right)}{\frac{\omega\Delta t}{2}} \right)^2, \quad (16)$$

where Δt is the sampling interval.

Note that formally at small frequencies, $\omega \approx 0$, the transfer functions are close to 1 and do not modify the PSD,

$$g(\omega)|_{\omega \approx 0} \approx g_o(\omega) \quad . \quad (17)$$

At large frequencies, $\omega > \omega_0$; $\omega > 1/\Delta t$, the original PSD is strongly reduced by a factor ω^{10} ,

$$g(\omega)|_{\omega \rightarrow \infty} \sim g_o(\omega) \frac{1}{\omega^{10}} \quad . \quad (18)$$

Thus, if the correlation time τ of the ion-current signal, which determines the width of the PSD, is close to or smaller than the sampling interval Δt or the inverse filter cutoff frequency f_c , then corrections associated with the filters are very important for the PSD analysis.

3 Data analysis protocol

Selection of ion-current data records. For each concentration of a substrate one selects 3-5 independent ion-current records, lasting each T seconds. T must be much larger than the typical correlation time τ_c of the blockages due to substrate; if the latter is less than few milliseconds the former may be 5-10 sec. One has to make sure that the selected records are free from an apparent external noise, like symmetric spikes, etc.

Average current. At every substrate concentration, c , one calculates the average current j_{av} for each data record by using Eq. (1). Then, the mean value and the error estimate of the average current are computed for every concentration, $j_{\text{av}}(c)$. Thus, the shift of the average ion current at concentration c is given by

$$\langle \Delta j \rangle (c) = j_{\text{av}}(c) - j_{\text{av}}(0) \quad . \quad (19)$$

Ion-current frequency histogram. At every substrate concentration, one calculates the ion-current frequency histogram. At zero substrate concentration, the histogram usually contains a single peak corresponding to the ion current through open channel. If the channel closes spontaneously, a secondary peak (or peaks) may appear. By comparing the histogram with and without a substrate the secondary peak due to the channel blockages by the substrate molecule (different from the one due to channel gating) might be located. Once the secondary peak is identified, the shift of its position with respect to the position of the main peak provides an estimate to the Δj value, i.e., the absolute difference of the average current in the open monomer channel and that in the blocked one at the given applied potential (we assume that the concentration is small enough, so that the probability of simultaneous blockages of two monomer channels in the trimer is negligible). If Δj is determined, *Methods 1* or *4* are applicable to extract the kinetic parameters of the channel blocking. However, if the blockage events are fast enough the secondary peak may disappear, and only the asymmetry of the histogram is evident. In the latter case, Δj is not measurable directly, and *Method 2* or *3* should be used to determine it together with the kinetic parameters of the channel blocking.

Auto-correlation function and PSD. At every substrate concentration, c , one calculates the autocorrelation function, $R(\tau, T)$, and the PSD $G(f, \tau_{\text{max}}, T)$, for each data record by using Eqs. (3), (11), (6). τ_{max} must be much larger than the typical correlation time τ_c of the blockages due to substrate but smaller than T ; if τ_c is smaller than few milliseconds, τ_{max} may be equal to 20-100 ms.

Background and PSD of the blocking signal. The ion-current fluctuations for the open channel without a substrate represent the background. One computes the PSD of the background, $G_b(f)$, by averaging the PSDs

for the data records at $c = 0$. Successively, the PSD of the blocking signal is calculated for every substrate concentration and for each data record by subtracting the PSD of the background,

$$G_s(f, c) = G(f, c) - G_b(f) \quad . \quad (20)$$

The latter relation requires the non-trivial assumption that the ion-current fluctuations in an open channel do not correlate with the current fluctuations due to channel blocking by substrate molecules.

Frequency range. By comparing the signal PSDs, $G_s(f)$, for the same substrate concentration but different data records one notes that at small frequencies (below a certain f_{\min}) they differ significantly while at $f \geq f_{\min}$ they well coincide within the statistical errors. This low-frequency difference may come from slow incontrollable changes of the external conditions (e.g., temperature) but also from infrequent spontaneous channel gating. The upper frequency, f_{\max} , is selected so that for $f > f_{\max}$ the absolute value of the PSD is close to or smaller than the statistical errors. The choice of the frequency range, $f \in [f_{\min}, f_{\max}]$, therefore, identifies statistically significant values of the PSD and gets rid of the slow incontrollable processes.

PSD fitting. At every substrate concentration, c , and for each data record one fits the model (filters-corrected) PSD,

$$G_m(f) = \frac{a}{1 + (2\pi f \tau_c)^2} |H_a(2\pi f)|^2 |H_s(2\pi f)|^2, \quad (21)$$

to the signal PSD, $G_s(f)$, in the frequency range $f \in [f_{\min}, f_{\max}]$, and obtains the Lorentzian factor a and the correlation time τ_c as well as the estimates of their uncertainties. Successively, the parameters are averaged over the data records for the same concentration of the substrate.

The weighted least squares method is used for the fitting. One minimizes the following scoring function,

$$S = \sum_{f_l \in [f_{\min}, f_{\max}]} \frac{[G_m(f_l) - G_s(f_l)]^2}{\sigma^2(f_l)}, \quad (22)$$

$$f_l = \frac{1}{\Delta t} l; \quad l = 0, 1, \dots \quad ; \quad (23)$$

where the frequency dependence of the weight function, $\sigma^2(f) = G_m(f)$, is selected to make more or less uniform the contribution of each f_i point to the sum of Eq. (22). We found that the choice $\sigma^2(f) = G_m(f)$ is better than $\sigma^2(f) = 1$ and than $\sigma^2(f) = G_m^2(f)$, as the former overweights the smaller-frequency region while the latter overestimates the higher frequency domain.

To estimate the fitting error (uncertainty) of the parameters, δa and $\delta \tau_c$ are determined so that

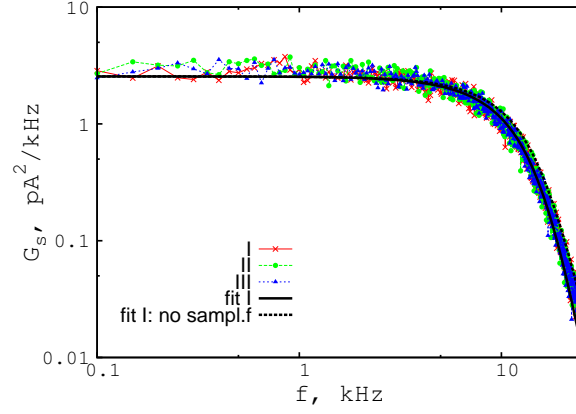
$$\min_{\tau'} S(a \pm \delta a, \tau') = 2S_{\min} \quad , \quad (24)$$

$$\min_{a'} S(a', \tau_c \pm \delta \tau_c) = 2S_{\min} \quad . \quad (25)$$

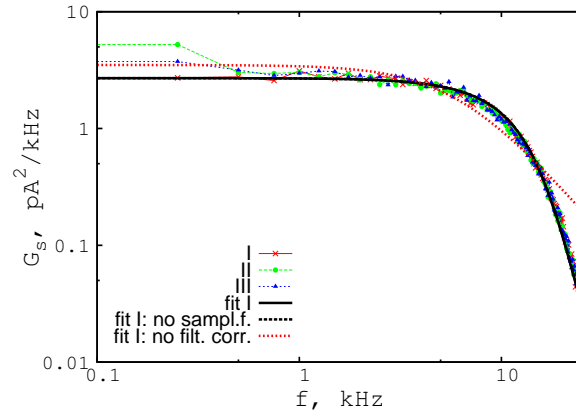
Here, $S_{\min} = \min_{a', \tau'} S(a', \tau') = S(a, \tau_c)$ is the minimum value of the scoring function calculated at the optimal values of a and τ_c .

Kinetic parameters. Finally, one uses one or, independently, several of the methods discussed in Section *Theory* to determine the kinetic parameters of the ion current blocking due to substrate molecules.

4 Double-logarithmic scale PSD plots



A



B

Figure 1: The signal PSD of the ion current fluctuations sampled at 50 kHz rate (A) and at 250 kHz rate (B). The thick solid line is the fit by the filters-corrected Lorentzian model; the dashed line is the same Lorentzian model but corrected for the Bessel filter only, i.e., without the sampling correction. The dotted line in (B) is the fit by the pure Lorentzian filters-incorrected model. These data are plotted in linear scale in Figure 3.