Quantitative Detection by Single Ion Channel Event Statistics: an Algorithmic Approach

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ABSTRACT

Ligand-gated ion channels electrical behaviour and, in particular, their conductance are influenced by the binding between receptive sites on channel surface and specific molecules. These channels, inserted in biomimetic membranes and in presence of a proper electronic system for acquiring and elaborating the electrical signal, could give us the possibility of detecting and quantifying concentrations of specific molecules in complex mixtures from ionic currents across the membrane. In this paper we propose an algorithm for extracting the information of target (gating) molecule concentration from an estimation of single-channel open-state probability (p) that, in turn, requires an estimation of the number of active channels.

Keywords: ligand-gated ion channels, algorithm.

1 ION CHANNELS

In biological world, life of cells is guaranteed by their ability to sense and to respond to a large variety of internal and external stimuli: this is allowed by the action of many different molecular sensors located in the cytoplasm or embedded across the cell membranes. In particular, excitable cells, like muscle or nerve cells, produce quick depolarizations in response to electrical, mechanical or chemical stimuli: this means that they can change their internal potential through a quick exchange of ions between cytoplasm and the external environment. Since the membrane structure of the cells is basically formed by a bimolecular layer of phospholipids (lipid bilayer), essentially impermeable to polar molecules, ions can flow across cell membrane thanks to the presence of ion channels, proteins that span the lipid bilayer and act like switches, allowing ionic current to flow opening and shutting in a stochastic way.

1.1 Ligand-gated ion channels

For a particular class of ion channels, ligand-gated ion channels, the stochastic properties of the gating processes are strongly influenced by binding between receptive sites located on the channel surface and specific target molecules. As a consequence, the open state probability of a single ion channel is function of the target molecule concentration in the fluid surrounding the receptive site. Every single channel behaves like a transducer that gives an appreciable current response (in the order of the pA) related a single molecule binding. In order to use these natural nanometric transducers for creating a new class of high performances sensors, some relevant problems must be overcome, related to technology and signal processing. Capability of having ligand-gated ion channels firmly embedded in planar biomimetic membranes, together with a proper electronic system for elaborating and recording the related electrical signal, is mandatory. Another crucial aspect is the signal processing of the electrical signal deriving from the channels. Since every ion channel behaves randomly, concentration measurement must be derived from a statistical analysis of it's open state probability distribution; moreover, it's not straightforward to control the exact number of channels that are inserted in a membrane. In essence, the system is composed by a random number of stochastic electrical sources that work in parallel. In this paper we propose an algorithm which, by analyzing the ionic current through a membrane embedding channels, extracts the information of the target molecule concentration (ligand) from an estimation of single channel open state probability (p) that, in turn, requires an estimation of the number of active channels.

2 ALGORITHMIC APPROACH

The estimation of the open state probability is achieved through a double step procedure, that involves a statistical analysis, based on probability density function of the current response, and an analysis based on the autocovariance of the current signal provided by the channels. These two approaches will be joined together to achieve the best performance of the overall method for large range of probability values.

2.1 PDF-based approach

A single ion channel's behaviour can be represented by a continuous time Markov chain, in which all states coincide with possible stable conformations of the protein: some of these are "open" states (allowing current to flow), the other ones are "shut" states. In many cases, all open states are characterized by the same conductance: the electrical signal deriving from a single ion channel is then

similar to a random telegraph signal and it's level can be represented, at a given time, as a Bernoulli random variable with open probability p and shut probability 1-p.
Let's suppose to have N ion channels of the same type inserted in a biomimetic membrane, which act independently from one another: the number of open channels can be represented, at a given time, as a binomial random variable N_{OPEN}, with N and p as parameters.
The instantaneous current I_1 from a single open channel, can be intended as a Gaussian random variable, characterized by a mean value $<I>_1$ and a variance σ^2 due to both instrumentation error (σ^2_{instr}) and intrinsic channel current variance (σ^2_{ch}).
The probability density function (PDF) for the current intensity provided by N channels of the same type embedded in a membrane is then:

$$f(I) = \sum_{j=0}^{N} w_j \frac{1}{\sqrt{2\pi}\,\sigma_j} \exp\left\{ -\frac{\left(I - < I >_j\right)}{2\sigma_j^2} \right\} \qquad (1)$$

where:

$$\sigma_j^2 = \sigma_{instr}^2 + j\sigma_{ch}^2 \qquad (2)$$

$$< I >_j = j < I >_1 \qquad (3)$$

$$w_j = \binom{N}{j} p^j (1-p)^{N-j} \qquad (4)$$

In particular, w_j is the probability of having N_{OPEN}=j.

Let's consider the histogram reporting the number of signal samples for given discrete-amplitude current bins. It can be described by a succession of couples: $C_m=(I_m, h_m)$, where m=1,2,…N_{BIN} (N_{BIN} is the number of bin used for discretize the current axis), I_m is the nominal current value for the m-th bin and h_m is the number of samples in the bin.

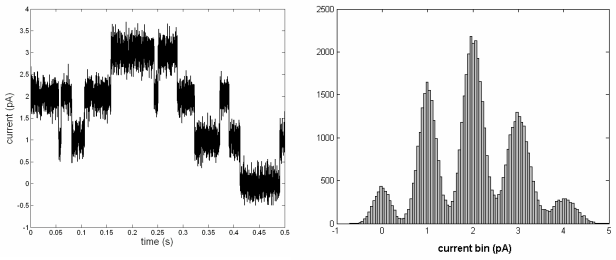

Figure 1: Left. Signals from Monte Carlo simulations of ion channels behaviour, with an added Gaussian noise having $\sigma_{instr} = 0.5 \times < I >_1$, $\sigma_{ch} = 0.03 \times < I >_1$. Right. histogram derived from it and used for PDF fitting.

The succession of values, normalized in order to have an unitary subtended area, becomes: $\tilde{c}_m = (I_m, \tilde{h}_m)$, where

$$\tilde{h}_m = \frac{h_m}{\Delta I \sum_{m=1}^{N_{BIN}} hm}, \quad \forall m = 1, 2, ..., N_{BIN} \qquad (5)$$

The normalized values of simulated ion current $\tilde{c}_m$, are well-fitted by the current probability density function f(I).

In the hypothesis that the number N of active channels correctly implanted in the membrane doesn't change during data recording, assuming that the standard deviation of the superimposed instrument-related Gaussian noise σ_{instr} is known from a previous initial calibration and that σ_{ch} is known from the knowledge of the channel's behaviour, it's possible to estimate p and N by fitting the distribution of normalized experimental data with the probability density function f(I). (eq.1)
The fitting is achieved by a numerical procedure through a minimization of the square error function $\Theta(p,N)$ according to:

$$\Theta(p, N) = \sum_{m=1}^{N_{BIN}} \left[f(I) - \tilde{h}_m \right]^2 \qquad (6)$$

The algorithm estimates the single channel open state probability for each possible value for the number of channels that are present in the membrane (this number is still unknown at this stage) and stores such probability values in a vector with dimension given by the maximum considered number of channels (N_{MAX}). The value of the vector corresponding to the minimum square error is actually the correct value of p (and its position corresponds to the correct number of channels) if p is not too low (0.1 or greater).

2.2 Autocorrelation-based approach

Let's consider an ion channel that can be described by a two-state reaction, characterized by two constant rates α (shut rate) and β (open rate). The associated current signal can be modelled as a random telegraph signal, that is a purely random signal which may assume two distinct values: "0", corresponding to the shut channel condition, and "1", the open state condition.

$$\text{Shut state} \underset{\alpha}{\overset{\beta}{\rightleftarrows}} \text{Open state} \qquad (7)$$

If the ligand molecule is an agonist, open rate β will be function of it's molar concentration; if, instead, the ligand

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molecule is a blocker, shut rate α will be dependent on the concentration.

For such a system, autocorrelation function of the total current through N identical, statistically independent channels, is given by:

$$A(s) = \frac{1}{\alpha}\left(\frac{\alpha+\beta}{\alpha\beta}\right)^2 \left\{\frac{1}{\beta}\exp\left[-(\alpha+\beta)\,|s|\right] + \frac{1}{\alpha}\right\} \quad (8)$$

The autocorrelation function A(s) is a simple exponential and does not depend on the number of channels N having its time constant $\tau = 1/(\alpha+\beta)$. Considering the case of an agonist ligand molecule, since the rate α is constant and known, autocorrelation function can be used for estimating, through a square error minimization fitting, the open rate β, that is a function of open probability:

$$p = \frac{\beta}{\beta+\alpha} \quad (9)$$

Autocorrelation-based estimation method allows us to estimate the variable β without caring about the number of channels inserted in the membrane; nevertheless it produces a rougher estimation than the PDF-based method previously described.

2.3 Two-steps approach

PDF-based approach provides a better open probability estimation compared to the autocovariance fitting, but run into difficulties when open probabilities are very low as it often fails in estimating the number of channels N. If N were known, it could be possible to obtain accurate estimations of p also when p is very low, as reported in table 1; however a wrong estimation of N, inevitably brings an error in estimating p. Since PDF-based algorithm can give as an output a vector containing the estimated probabilities for all the possible values of N (with $N \le N_{MAX}$), knowing a rough measure of p, obtained from the autocovariance fitting (not depending on N), is possible to recover the correct value in the vector, corresponding to the correct (and more accurate) measure of p (see fig. 3). The position of the picked value of p pinpoints the true number of channels N. Another possible way for extracting the correct value of p from the vector, using an array-based system, is reported in section 4.

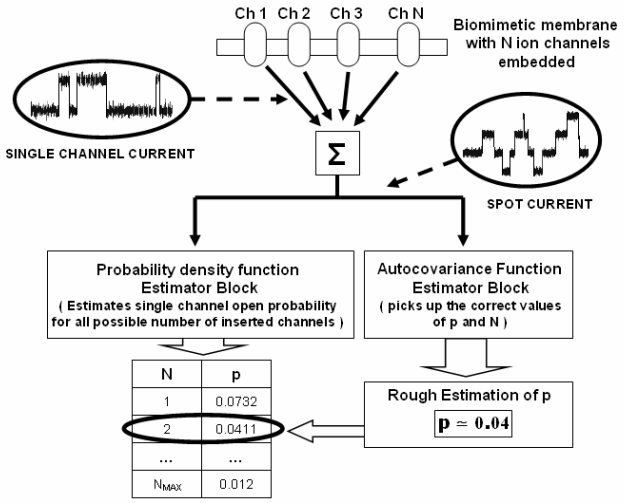

Figure 2: Block diagram of the two-steps algorithm for data processing. Estimation from autocovariance fitting is rougher than estimation based on PDF-fitting, and is used for picking up the corrects values of N and p.

3 APPLICATION TO THE ACH MUSCARINIC RECEPTOR

The Muscarinic Receptor activated by Acetylcholine (ACh) is a ligand-gated ion channel which can be modeled using the three-states Markov chain illustrated in equation (9) (Katz-Miledi model).

$$\boxed{\text{Shut} \underset{K_{DISS}}{\rightleftarrows} \text{Shut}^*} \overset{\beta'}{\underset{\alpha}{\rightleftarrows}} \text{Open}^* \quad (10)$$

The channel can't open when its receptive site is not bound (shut state): only bounding an Ach molecule (going to shut* state) it can pass in the open state

Therefore, in the two states inside the frame the receptor is shut, both in absence and in presence of Ach molecule bound. Since the binding steps are very fast compared with the subsequent conformation change that open the pore of the channel, the vacant and occupied states can be considered always close to equilibrium [1]; therefore they essentially behave as a single (shut) state.

where the superscript "*" indicates Ach bound, K_{DISS} is the dissociation constant. Muscarinic Receptor's behaviour can be represented by the two state Markov chain reported in equation (7), in which the open rate β' is function of the Acetylcholine molar concentration [Ach], in order to look up to the fact that the shut state spends part of it's time in absence of Ach bound, and in this situation opening is impossible. β' is given by [1].

$$\beta' = \beta \frac{[\mathrm{Ach}]}{[\mathrm{Ach}] + K_{DISS}} \quad (11)$$

where β is the open constant rate in case of ligand saturation. Open channel probability is then a not decreasing function of [Ach].

$$p = \frac{\beta[\mathrm{Ach}]}{\beta[\mathrm{Ach}] + \alpha(K_{DISS} + [\mathrm{Ach}])} \quad (12)$$

3.1 Simulation of [Ach] Receptors

In order to test the algorithm, a Monte Carlo simulator of the Ach Muscarinic Receptor was developed: it reproduces the stochastic current response of a population of ligand-gated ion channels in presence of additive Gaussian noise. The adopted rates $\alpha = 10.1 s^{-1}$, $\beta = 12\ s^{-1}$ and dissociation constant $K_{DISS} = 1.7\ \mu M^{-1}$ refer to [4], which describes voltage-clamp experiments conducted on S-A node of rabbit heart, in presence of neostigmine for avoiding cholinesterase effects. Standard deviation of Gaussian noise used for simulations is $0.5 \times \langle I \rangle_1$. Each simulated acquisition lasts for 2 hours.
Results are reported in table 1.

Open State Probability	Corresponding [Ach]	Mean Percentage Error
0.001	1.43 nM	21.20%
0.005	7.22 nM	2.93%
0.01	14.58 nM	3.02%
0.05	78.80 nM	0.86%
0.1	175.38 nM	0.58%
0.5	9.04 uM	0.15%

Table 1: Results of the estimation of p. 20 simulations per group were performed, each with a number of inserted channels between 1 and 12.

4 ARRAY SYSTEM

Considering an array approach, in which a large number of spots, each containing a planar artificial membrane with a limited number of ligand-gated embedded ion channels and an electronic interface for collecting the data, algorithm performance could significantly improve: simple averaging among values of p derived from single spots analysis produces an error reduction as high as. Moreover, interpolating data could allow to produce a right estimation also of very low values of p. In fact, for example, when the number of channels N of a single spot is overestimated, p is underestimated and vice versa; usually, when a spot contains only one channel, estimation of N is correct. Having a great number of spots and setting the channels inserting procedures in order to have a low mean number of channels per spot (also if it means to have some empty spots), could virtually assure to have some spots containing only one channel. These spots will be recognize because they will register the lower probability p between the spots with an estimated N equal to one, and they will report the correct value of p.

5 CONCLUSION

The proposed algorithm, especially if coupled with an array-organized system, can estimate also very low single channel open probabilities (0.005), with a mean percentage error less than 3%, starting from a few hours data acquisition. Obviously, longer acquisition could bring to better results, but at present, is difficult to achieve biomimetic membranes stable for more than few hours and without a significant reduction of the number of the channels (rundown effect). Another issue affecting sensors based on ligand-gated ion channels, is that usually their dose-response curve does not allow sensitivities below a few nM, thus limiting the capability to estimate very low concentrations. In principle, it would be significant to develop new chimeric channels, characterized by long average time in the bond state and gated by molecules with practical applications.

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