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Letter to the Editor

Method-Independent Effect in Testing for Detailed Balance in Ion Channel Gating

An ion channel in thermodynamic equilibrium is subject to the principle of detailed balance (microscopic reversibility). A violation of this principle would indicate the presence of an external energy source. Song and Magleby (1994) were the first to investigate the statistical methods to test for detailed balance and successfully applied this test to a four-state model in which the states are arranged in a circle.

Recently, Rothberg and Magleby (2001) published a "Letter to the Editor" in the *Biophysical Journal* concerning a paper from Wagner and Timmer (2000) that deals with testing for detailed balance in ion channel gating.

Wagner et al. (1999) showed that, for the loop-gating scheme sketched in Fig. 1, it is not possible to detect whether the ion channel is obeying the law of detailed balance if the dwell times for the two open states are equal. This is due to nonidentifiability of the parameters. In the case of almost equal open times, Wagner and Timmer (2000) found that a likelihood ratio test suffers from a loss of power for detecting a violation of the law of detailed balance. Rothberg and Magleby (2001) remarked that the likelihood ratio test is highly model-dependent and reviewed three additional methods to detect detailed balance including some that are model-independent.

We would like to emphasize that the nondecidability of detailed balance in the case of equal dwell times, and the loss of power in the case of similar dwell times, is not due to the model-based likelihood ratio test but a property of this gating scheme. It also holds to the three methods discussed by Rothberg and Magleby (2001).

To illustrate this fact, we apply the first of their model-free methods to the loop model. If the gating follows the principle of detailed balance, the two-dimensional dwell-time distributions of adjacent open and closed intervals in the forward and backward direction are equally distributed (Song and Magleby 1994). For different degrees of violation of detailed balance and different ratios of the open times, we calculate the probability of rejecting the null hypothesis of detailed balance. We choose the number of simulated open and closed dwell-times so that it corresponds approximately to a record of length of 105 s at a sampling rate of 5 kHz (2^{19} data points). This is the record length used by Wagner and Timmer (2000). The parameters of the model are given in Wagner and Timmer (2000). The

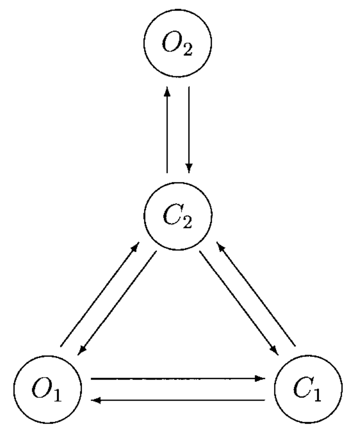

FIGURE 1 Loop gating scheme. We denote an open and a closed state by *O* and *C*, respectively.

degree of violation of detailed balance is determined by the value of *K*, which is defined as

$$K = \frac{\text{product of transition rates (clockwise)}}{\text{product of transition rates (counterclockwise)}}.$$

The results of our simulation study are summarized in Fig. 2. For equal open times, the rejection probability does not exceed the 5% level of the test, and, for nearly equal open times, the test suffers from a loss of power. Compared to the results from Wagner and Timmer (2000), the proposed method has even less power to detect a departure from the null hypothesis than does the likelihood ratio test.

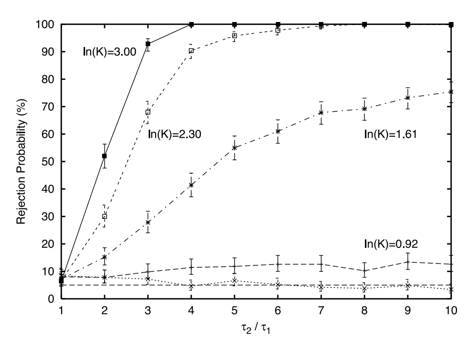

FIGURE 2 Rejection probability. The probability of rejecting the null hypothesis of detailed balance against the ratio of open times τ_2/τ_1 is shown for different values of ln(*K*) where *K* is the ratio of transition rates in clockwise and counterclockwise direction. The line at the bottom corresponds to a value of $\ln(K) = 0.22$. To calculate the rejection probability, we simulated 500 recordings for each τ_2/τ_1 and ln(*K*). The number of simulated dwell times per record ranges from 31,149 to 24,441, depending on the value of ln(*K*).

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This is expected theoretically because the likelihood ratio test is asymptotically locally most powerful among all invariant tests, see, e.g., Cox and Hinkley (1974).

In summary, the loss of power to detect a violation of detailed balance is a property of the loop model with equal dwell times due to nonidentifiability of the parameters and not due to the model dependence of the likelihood ratio test. It holds also for more complicated gating schemes.

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