



## Original software publication

**scbursts: An R package for analysis and sorting of single-channel bursts**

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## ABSTRACT

Analysis of single-channel activity provides unparalleled insight into ion channel structure, function, and mechanism. An essential step in the analysis pipeline is the assignment and sorting of bursts of activity that can be attributed to a single ion channel. Here we present *scbursts*, an R package for the analysis and sorting of single-channel bursts. Using *scbursts*, idealized data from a variety of single-channel analysis software packages can be read directly into R, and parsed into bursts according to a user-defined critical closed duration. Bursts can be analyzed and sorted based on a variety of parameters, including burst open probability, and the number of openings per burst. Sorted bursts can then be exported in a standard format for downstream kinetic fitting. We provide examples illustrating the implementation and utility of *scbursts*, as well as how *scbursts* brings the statistical prowess and versatility of R to single-channel analysis.

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## Code metadata

Current Code version	1.6
Permanent link to code/repository used of this code version	<a href="https://CRAN.R-project.org/package=scbursts">https://CRAN.R-project.org/package=scbursts</a>
Legal Code License	LGPL-2.1
Code Versioning system used	git
Software Code Language used	R
Compilation requirements, Operating environments & dependencies	R, readxl, tibble. For compiling documentation: knitr, rmarkdown, pandoc, LaTeX,
If available Link to developer documentation/manual	<a href="https://cran.cnr.berkeley.edu/web/packages/scbursts/scbursts.pdf">https://cran.cnr.berkeley.edu/web/packages/scbursts/scbursts.pdf</a>
Support email for questions	<a href="mailto:blair.robert.drummond@gmail.com">blair.robert.drummond@gmail.com</a>

## Software metadata

Current software version	1.6
Permanent link to executables of this version	<a href="https://CRAN.R-project.org/package=scbursts">https://CRAN.R-project.org/package=scbursts</a>
Legal Software License	LGPL-2.1
Computing platform/Operating System	Linux, OS X, Microsoft Windows
Installation requirements & dependencies	R, readxl, tibble
If available Link to user manual – if formally published include a reference to the publication in the reference list	<a href="https://cran.cnr.berkeley.edu/web/packages/scbursts/vignettes/scbursts.pdf">https://cran.cnr.berkeley.edu/web/packages/scbursts/vignettes/scbursts.pdf</a>
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## 1. Introduction

The patch clamp technique continues to be the gold standard for single-molecule experiments in terms of both time resolution

and signal-to-noise ratio [1]. Developed in the 1970's and 80's, the technique allows the activity of individual ion channel proteins to be registered in real time [2–4]. The resulting analog signal is digitized, and then idealized, so that the sequence of channel openings and closings can be fit to kinetic schemes describing the underlying activity [5]. This has led to great insight into ion channel structure, function, and mechanism [1,6].

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One of the essential steps in the analysis pipeline is the determination of bursts of closely spaced openings and closings that can be attributed to the activity of a single channel [7]. Bursts are defined by a critical closed duration ( $\tau_{\text{crit}}$ ), which stipulates that openings separated by closings briefer than  $\tau_{\text{crit}}$  are part of the same burst, and originate from the same channel, whereas openings separated by closings longer than  $\tau_{\text{crit}}$  are from different bursts. In other words, a burst is a collection of closely spaced openings and closings that all originate from the same channel, and are flanked by extended closings longer than  $\tau_{\text{crit}}$  [7].  $\tau_{\text{crit}}$  is usually determined after idealization of single-channel data, and through examination of the distribution of closed dwell durations [8–11].

Once bursts have been identified, they are often sorted prior to kinetic fitting [12–14]. Bursts are sorted based on criteria such as the minimum number of openings per burst, or the probability that the channel is open during a given burst (i.e. burst  $P_{\text{open}}$ ) [15]. In some cases this sorting has been done manually, with the experimenter arbitrarily deciding which bursts to include for downstream analysis. In other cases, inaccessible homemade software solutions have been employed. Here we describe *scbursts*, an open-source and freely available R package designed for the analysis and sorting of single-channel bursts. We demonstrate the utility of analyzing bursts within the open-source R environment by showing how existing R packages can be called upon to objectively and transparently sort bursts for downstream kinetic analysis.

## 2. Software framework

The *scbursts* package runs in R [16]. It comprises a set of functions for parsing idealized single-channel data into bursts so that they can be sorted and exported for kinetic analysis. The package also contains functions for importing idealized data from a number of popular single-channel analysis suites into the R environment (Table 1), and thus for unifying idealized single-channel data across platforms and analysis suites. To illustrate the utility of *scbursts*, and the flexibility afforded by working with single-channel data in R, we provide specific examples of *scbursts* implementation. All associated data files, as well as analogous workflows for events idealized using the different software packages are available online (GitHub, CRAN), and as Supplemental Files.

## 3. Implementation and illustrative examples

### 3.1. Importing idealized events and defining bursts in R

Burst analysis in *scbursts* begins with idealized single-channel data being imported into R. Depending on the method and software used for detection of single-channel events, idealized data can either be in the form of (a) a series of open or closed dwell durations, or (b) a series of transition times denoting when the channel transitioned between non-conducting (closed) and conducting (open) states (Table 1). Given that the latter case represents the more complicated of the two scenarios because it involves an additional step, here we provide an example of an analysis that

begins with idealized data in the form of conductance transition times.

Fig. 1 shows a continuous stretch of single-channel activity for a cell-attached patch containing the human adult muscle-type acetylcholine receptor (AChR) in the presence of agonist (30  $\mu\text{M}$  acetylcholine; Fig. 1A). For the depicted example, the single-channel data was idealized using the TAC single-channel analysis software package, which detects opening and closing transition times based on a 50% threshold-crossing criterion. For comparison, TAC idealized events are shown below the raw data (Fig. 1B). TAC saves idealized transition times as an “event” file with the extension “evt” (see “example1\_tac.evt” that is part of the *scbursts* package and Supplemental File 1.zip), which can be imported directly into the R environment by entering the following in the R workspace:

```
>evtfile <- "path/example1_tac.evt" (1)
```

where “path” denotes the directory for the appropriate R workspace folder containing the file “example1\_tac.evt”.

TAC event files contain header information pertaining to the single-channel recording that is not necessary for burst analysis within *scbursts*. To extract solely the transition times, enter the following:

```
>transitions <- evt.read(evtfile) (2)
```

The resulting “transitions” will be a list of times when the channel transitioned from closed to open (i.e. an opening = 1), or from open to closed (i.e. a closing = 0). Since the channel is either open or closed, a closing must precede each opening, and analogously each closing must follow an opening. To ensure this criterion is obeyed, *scbursts* has a built in feature where it will flag idealized data sets that contain two or more successive events (i.e. openings or closings) with the same conductance level. Upon defining bursts, the offending burst(s) can be removed from downstream analysis.

To convert the transition times into a series of open and closed dwell durations, *scbursts* has the built in *evt.to\_dwells* function. Taking into account whether the transition is an opening or a closing, this function subtracts the previous transition time from the current transition time to determine the duration of the associated open or closed dwell. To convert the transition times into dwell durations, simply enter:

```
>dwells <- evt.to_dwells(transitions) (3)
```

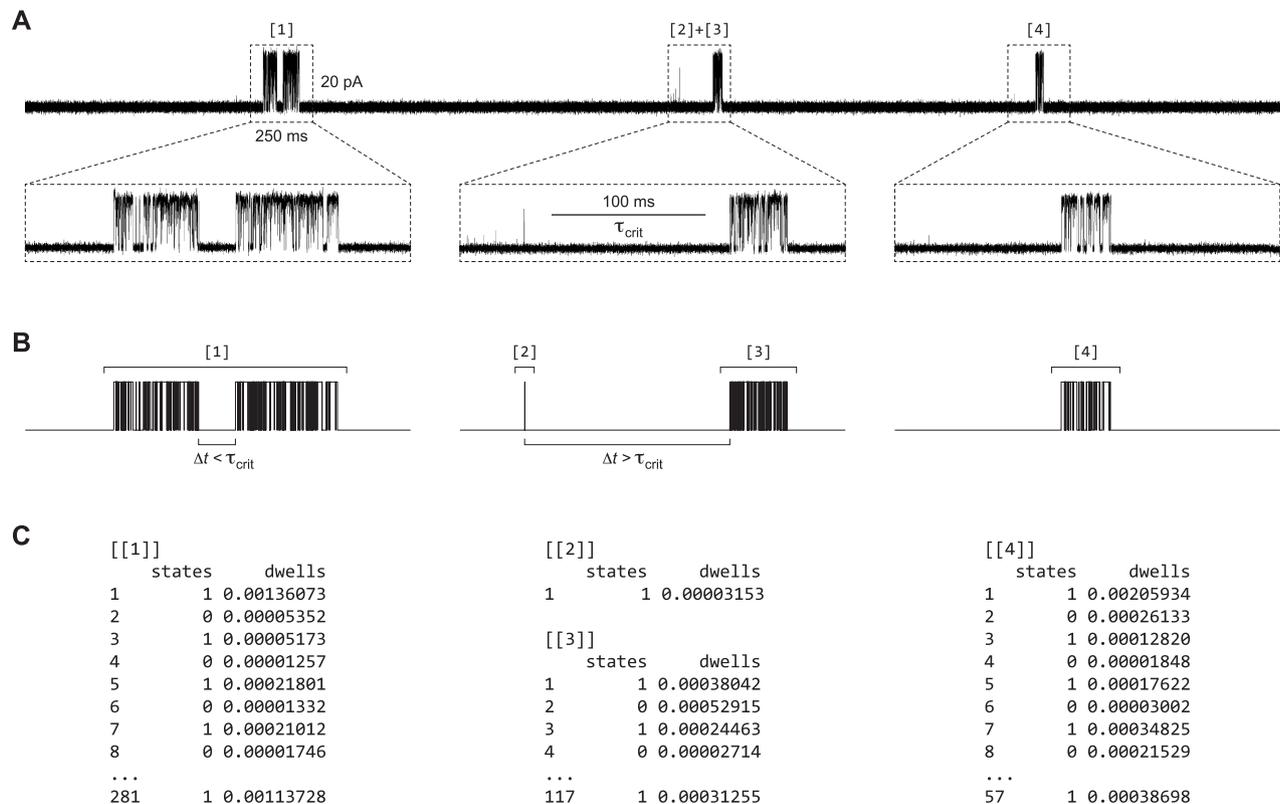
The resulting “dwells” consists of a series of alternating open and closed durations (see “example1\_tac\_Rworkflow.txt” within Supplemental File 1.zip). To divide the series of dwells into bursts of single-channel activity, it is necessary to specify a critical closed duration ( $\tau_{\text{crit}}$ ). The single-channel recording depicted in Fig. 1 contains relatively few bursts, and a  $\tau_{\text{crit}}$  of 100 ms was arbitrarily specified for illustrative purposes. To specify a  $\tau_{\text{crit}}$  of 100 ms and define bursts, enter the following:

```
>bursts <- bursts.defined_by_tcrit(dwells, 100, units = "ms") (4)
```

where a critical closed duration ( $\tau_{\text{crit}}$ ) of 100 ms is entered. The default units are seconds, but can be specified as above.

**Table 1**  
Single-channel analysis software packages compatible with *scbursts*.

Software	Detection criteria	Transitions or dwells	File extension	URL
Clampfit	50% Threshold	Dwells	*.xls	<a href="https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite">https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite</a>
QUB	Segmental k-means	Dwells	*.dwt	<a href="https://qub.mandelics.com/">https://qub.mandelics.com/</a>
SCAN	Timecourse	Dwells	*.txt	<a href="https://github.com/DCPROGS">https://github.com/DCPROGS</a>
TAC	50% Threshold	Transitions	*.evt	<a href="https://www.bruyton.com/TAC/index.html">https://www.bruyton.com/TAC/index.html</a>



**Fig. 1.** Bursts of single-channel activity. (A) Continuous stretch of a patch clamp recording of the human adult muscle-type acetylcholine receptor in the presence of 30  $\mu$ M acetylcholine. The recording contains several bursts of single-channel activity, which are numbered [1], [2], [3], and [4], from left to right. Openings are upward deflections, and the boxed regions, shown expanded below, highlight individual bursts. The boxes themselves represent scale bars with their “y” and “x” axes indicating 20 pA and 250 ms, respectively. A scale bar denoting the critical closed duration (i.e.  $\tau_{crit} = 100$  ms) used to define bursts in the present example, is shown in the middle expansion for reference. Traces were digitally filtered to 10 kHz. (B) Idealized opening and closing events for the expanded bursts shown in “A”. Select closings within (i.e.  $\Delta t < \tau_{crit}$ ; left) or between (i.e.  $\Delta t > \tau_{crit}$ ; middle) bursts are highlighted below the idealizations. (C) *scbursts* representations for the idealized bursts depicted in “B”. Each burst is numbered (e.g. [[1]]) and stored as a collection of alternating openings (state = 1) and closings (state = 0), with durations denoted in seconds. Note that bursts [1], [2], [3], and [4] contain 281, 1, 117, and 57 events (includes both openings and closings), but only select events are shown due to space limitations.

The resulting bursts all begin and end with an opening, and are separated from each other by a closing longer than the specified  $\tau_{crit}$ . Note that had  $\tau_{crit}$  been  $\sim 20$  ms (or less), burst #1 would have been defined as two separate bursts. Alternatively, had  $\tau_{crit}$  been  $\sim 150$  ms (or greater), bursts #2 and #3 would have been combined into a single burst. Thus, even this small data set illustrates some important nuances to selecting an appropriate  $\tau_{crit}$ . Furthermore, the behavior of ion channels is often hierarchical, with bursts nested within “clusters” of bursts, and clusters nested within “super-clusters” of clusters [7]. Defining clusters and super-clusters within *scbursts* is as simple as specifying increasingly long critical closed durations.

The bursts defined above can be analyzed and sorted based on a variety of criteria. For example, one of the “bursts” defined in Fig. 1 is an isolated individual opening (burst #2). To discard “bursts” that contain fewer than a specified number of openings (in this case 2) use the following script:

```
> twoplus <- function(seg){return(segment.count_open(seg)>= 2)}
(5)
> bursts_twoplus <- bursts.select(bursts,twoplus)
```

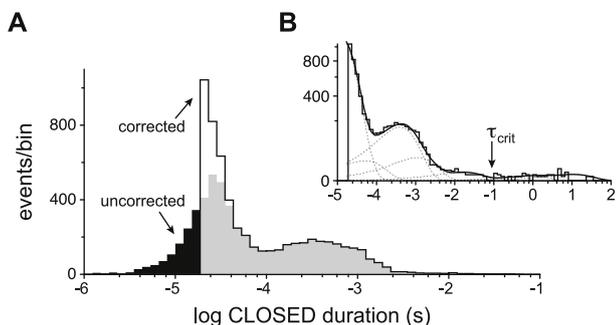
The resulting “bursts\_twoplus” only contains bursts with two or more openings, and so burst #2 has been discarded.

### 3.2. Sorting bursts based on burst $P_{open}$

The previous example contains relatively few bursts, and does not constitute enough data to be included in a standard kinetic

analysis. For larger data sets, comprising many bursts,  $\tau_{crit}$  is typically determined from the distribution of closed dwell durations, and inspection of a closed dwell duration histogram (Fig. 2) [10]. In the case of the AChR,  $\tau_{crit}$  is often specified by the point of intersection between the exponential fitted to the slowest activation component, and the exponential fitted to the fastest desensitization component [11]. Defining  $\tau_{crit}$  as the point of intersection between two components minimizes the number of incorrectly classified events [9]. To illustrate the assignment of  $\tau_{crit}$  based on a dwell duration histogram, as well as the ability of *scbursts* to sort bursts, we have included a larger data set (“example2.dwt” is part of the *scbursts* package and is also included in Supplemental File 2.zip) that contains a sufficient number of bursts required for downstream kinetic fitting.

For the present example, the single-channel data was filtered digitally with a 10 kHz Gaussian filter to improve the signal to noise ratio and ensure reliable detection of openings and closings using the 50% threshold-crossing criterion. Digital filtering, combined with the built in response time of the patch clamp, produce a step response that limits the time resolution of the single-channel data. Practically, this means that events briefer than a duration specified by the risetime of the filtered step response do not cross the 50% threshold, and thus are not detected by the threshold-crossing criterion. Furthermore, the measured durations of openings and closings whose duration approaches this “dead time” are systematically influenced by the filtered step response, and should be corrected prior to kinetic analysis [5]. *scbursts* can correct measured dwell durations based on a filtered



**Fig. 2.** Closed duration histogram of the human muscle-type acetylcholine receptor in the presence of 30  $\mu\text{M}$  acetylcholine. (A) Both uncorrected (black fill) and corrected (white fill) closed duration histograms are overlaid on the same plot, which has a linear y-axis to facilitate comparisons of corrected and uncorrected closed dwell durations. Dwell duration correction primarily affects dwells whose duration is close to the instrument dead time. (B) The same corrected closed duration histogram as in “A”, but where the y-axis is plotted with a square root scale [17]. The closed duration histogram has been fit (solid line) by a sum of exponentials (dashed lines). The square root scale of the y-axis makes minor closed components arising from long sojourns in closed desensitized states (i.e. closings between bursts) visible, thereby facilitating identification of  $\tau_{\text{crit}}$ , which is defined as the intersection between the longest closed component attributable to activation, and the shortest closed component attributable to desensitization [11].

step response with a given risetime, according to the following relationship described by Colquhoun and Sigworth [5]:

$$w_o = w_t + a_1 \exp\left(-\frac{w_t}{a_1} - a_2 w_t^2 - a_3 w_t^3\right), w_t > 0 \quad (\text{Eqn. 1})$$

where  $w_o$  is the actual dwell duration (i.e. dwell “original width”), and  $w_t$  is the dwell duration measured at threshold (i.e. dwell “width at threshold”). For simplicity, the risetime ( $T_r$ ) and a scaling factor are combined and represented by the coefficients  $a_1$ ,  $a_2$ , and  $a_3$  ( $a_1 = 0.5382 \bullet T_r$ ;  $a_2 = 0.837 \bullet T_r^{-2}$ ;  $a_3 = 1.120 \bullet T_r^{-3}$ ). To implement this correction within *scbursts*, all that is required is an accurate measure of the 10%–90% risetime of the filtered step response, which should be acquired under the same filter conditions used for idealization. To correct the measured dwell durations based on the above relationship, enter the following:

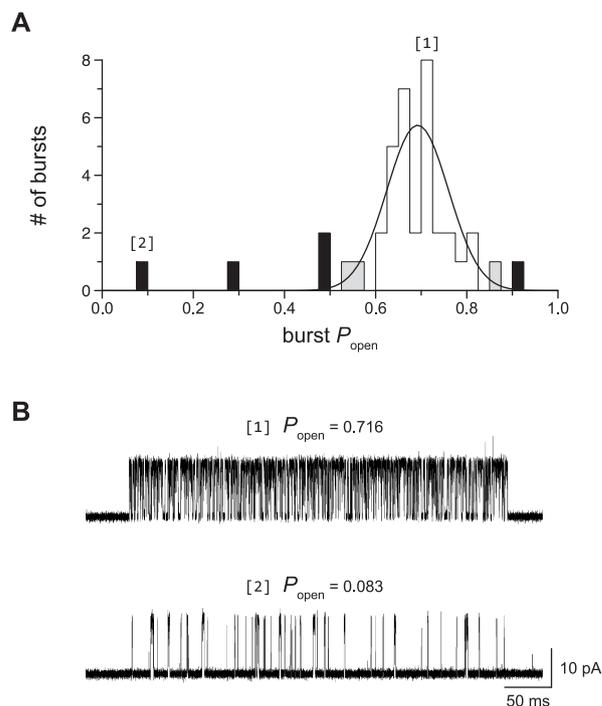
```
>dwells_c <- risetime.correct_gaussian(Tr = 35.0, dwells, unit = "us")
(6)
```

where a risetime ( $\text{Tr} = 35.0 \mu\text{s}$ ) is entered, and the default units are seconds, but can be specified as above.

As previously mentioned, brief dwells whose duration approach the dead time are most affected by the above correction. Thus the correction can be easily confirmed by comparing the durations of the briefest openings and closings in dwell time histograms of uncorrected and corrected dwells (Fig. 2A).

To define bursts with this larger data set, a  $\tau_{\text{crit}}$  of 92 ms was determined based on a corrected closed dwell duration histogram (Fig. 2B), and bursts were defined as outlined in step (4) above, but with a  $\tau_{\text{crit}}$  of 92 ms. “Bursts” with fewer than a specified number of openings (in this case three) were discarded as in step (6), and the resulting vectors were named “bursts\_threeplus”.

To determine the homogeneity of the present bursts, we determined the proportion of time that the channel spent open within each burst (i.e. burst  $P_{\text{open}}$ ), and binned these to plot a burst  $P_{\text{open}}$  histogram (Fig. 3A). From this histogram it is evident that the majority of bursts have a  $P_{\text{open}}$  distributed around a mean near 0.7. To remove aberrant bursts based on their  $P_{\text{open}}$ , we implemented an R protocol for identifying and objectively discarding outliers. The *extremevalues* R package determines which data in a data set fit a normal distribution, and thus which data points fall



**Fig. 3.** Analysis and sorting of bursts based on burst  $P_{\text{open}}$ . (A) The proportion of time spent open within each burst is binned into a burst  $P_{\text{open}}$  histogram. The *extremevalues* R package determines which bursts, based on their  $P_{\text{open}}$ , are part of a normal distribution (black line). Outliers not part of the normal distribution (black bars), as well as those bursts whose  $P_{\text{open}}$  is greater than two standard deviations from the mean (gray bars), are discarded from downstream kinetic analysis. (B) Selected bursts of single-channel activity with a  $P_{\text{open}}$  that places them in the indicated bins in “A”. Openings are upwards deflections, and traces were digitally filtered to 10 kHz.

outside the normal distribution [18]. To ensure homogeneity of bursts for downstream kinetic analysis, we used *extremevalues* to determine which bursts were normally distributed based on their  $P_{\text{open}}$ , and removed bursts whose  $P_{\text{open}}$  was more than two standard deviations from the mean (Fig. 3; see Supplemental File 2.zip for implementation of *extremevalues*, as well as a complete workflow for the present example). The resulting homogeneous bursts can be exported as a “dwt” file for kinetic analysis in QUB/MIL.

Some patches exhibit heterogeneous burst activity [19]. In such instances, it may be beneficial to isolate divergent subpopulations of bursts so that they can be analyzed separately. Supplementary Figure 1 depicts a scenario with at least two subpopulations of bursts with a different mean  $P_{\text{open}}$ . To isolate bursts in each of these subpopulations, we again turned to an existing R package. The *mixtools* package contains functions for approximating statistical parameters of each component in a complex distribution [20]. As an initial approximation, *mixtools* was used to estimate the mean and standard deviation of the two components of bursts with divergent  $P_{\text{open}}$ . Based on these initial estimates, bursts within two standard deviations of either the high or low  $P_{\text{open}}$  bursts were removed, and in each case the remaining bursts were then subjected to the above *extremevalues* protocol for identifying outliers.

Although the above examples include only two conductance levels (i.e. 0 or 1), some channels display multiple conductance levels within individual bursts [21]. Idealized single-channel data that contains any number of conductance levels can be imported into R using *scbursts* (for a detailed example see Supplementary Figure 2, as well Supplemental File 4.zip that contains “example4.dwt”, which is also part of the *scbursts* package).

## 4. Impact

R and the *scbursts* package are open source, cross-platform, extensible, and freely available, thereby facilitating transparency and objectivity in the sorting of single-channel bursts. By allowing single-channel data to be imported into the R environment, *scbursts* brings the statistical prowess and versatility of R to single-channel analysis. The R environment is constantly growing, and packages with new applications and potential uses for single-channel analysis continue to be added to the R family. Indeed, to select bursts based on their  $P_{open}$ , we turned to existing packages within R (*extremevalues* and *mixtools*), which allowed us to objectively discard bursts based on their  $P_{open}$  prior to kinetic analysis. These examples directly demonstrate the ease with which single-channel burst analysis can be implemented within R, as well as the potential for R to improve the transparency and objectivity of the burst sorting process, and thus overall single-channel analysis.

By virtue of being able to read idealized data from a variety of single-channel analysis software packages, *scbursts* unifies single-channel data in a common format within the R environment. We anticipate that this will facilitate meaningful comparisons between different idealization algorithms and software packages, ensuring that maximum information is extracted from precious single-channel data. This is particularly pertinent given the increasing complexity of mechanistic schemes used to explain single-channel activity [22–24], and the push to increase the time resolution of single-channel data in order to measure intermediate states [25].

## 5. Conclusions

We have introduced *scbursts*, an R package for parsing idealized single-channel data into bursts, and then analyzing and sorting bursts for downstream kinetic analysis. Idealized data, from a variety of single-channel analysis software packages, can be read directly into the R environment using standard R scripting. Data represented as closed-to-open and open-to-closed transition times can be converted into the corresponding series of open and closed dwell durations. Measured durations that are systematically altered as a result of filtering can be corrected based on a known risetime, and then parsed into bursts of single-channel activity defined by a user-specified critical closed duration ( $\tau_{crit}$ ). The resulting bursts can then be analyzed and sorted based on a variety of parameters, such as the number of openings per burst, or burst open probability ( $P_{open}$ ). Finally, although *scbursts* was designed specifically for single-channel data, it can be used to analyze other single-molecule data, and any multi-state stochastic process exhibiting burst-like activity.

### Declaration of competing interest

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

### CRedit authorship contribution statement

**Blair R. Drummond:** Conceptualization, Methodology, Software, Writing - review & editing. **Christian J.G. Tessier:** Formal analysis, Methodology, Validation, Writing - review & editing. **Mathieu F. Dextraze:** Methodology, Software, Validation, Writing - review & editing. **Corrie J.B. daCosta:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - original draft, Writing - review & editing.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.softx.2019.100285>.

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