quicR: An R Library for Streamlined Data Handling of Real-Time Quaking Induced Conversion Assays

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Abstract

Real-time quaking induced conversion (RT-QuIC) has quickly become a valuable diagnostic tool for protein misfolding disorders such as Creutzfeldt-Jakob disease and Parkinson's disease. Given that the technology is relatively new, academic and industry standards for quality filtering data and high throughput analysis of results have yet to be fully established. The open source R library, quicR, was developed to provide a standardized approach to RT-QuIC data analysis. quicR provides functions, which can be easily integrated into existing R workflows, for data curation, analysis, and vizualization.

Keywords: R package, RT-QuIC, prion, diagnostics, CJD, Parkinson's

Metadata

Nr.	Code metadata description	Metadata
C1	Current code version	V2.1.0
C2	Permanent link to code/repository used for this code version	`https://github.com/gage1145/quicR`
C3	Permanent link to Reproducible Capsule	`https://github.com/gage1145/quicR/releases/tag/v2.1.0`
C4	Legal Code License	GPL-3
C5	Code versioning system used	git
C6	Software code languages, tools, and services used	R
C7	Compilation requirements, operating environments & dependencies	R (>=4.1.0)
C8	If available Link to developer documentation/manual	`https://cran.r-project.org/web/packages/quicR/quicR.pdf`
C9	Support email for questions	rowde002@umn.edu

Table 1: Code metadata

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1. Motivation and significance

Real-time quaking induced conversion (RT-QuIC) is a cutting-edge diagnostic assay that has garnered significant attention for its ultra-sensitive detection of misfolded protein aggregates [1, 2]. The assay works by converting a recombinant protein substrate into an amyloid aggregate in the presence of a misfolded seed [1, 3, 4, 5, 6, 7, 8, 9, 10]. The assay's sensitivity and specificity make RT-QuIC a promising tool for diagnosing diseases such as prion disorders and other protein misfolding pathologies [11, 12, 13, 14]. However, the relatively recent development and novelty of the assay have left a gap in widely accepted academic and industry standards for data analysis and interpretation [15].

To address this gap, we introduce quicR, an open-source library, developed in R [16], dedicated to the cleaning, analysis, and visualization of RT-QuIC data. By consolidating key metrics and providing robust analytical tools, quicR aims to standardize the analysis pipeline and foster reproducibility within the field of quaking induced assays including related assays such as Nano-QuIC [17] and Micro-QuIC [18]. quicR is designed with both researchers and diagnosticians in mind, providing a user-friendly interface that integrates seamlessly with existing R workflows.

While universal diagnostic criteria for RT-QuIC have yet to be established, certain analytical metrics have emerged as valuable tools for interpreting assay results and kinetics. These include:

1. Time-to-threshold (TtT): The time required for the fluorescence signal to exceed a predefined threshold [5].
2. Rate of amyloid formation (RAF): A measure of the kinetics of aggregate growth, which provides insight into the relative quantity of misfolded seed [19].
3. Maxpoint ratio (MPR): A ratio-based metric measuring peak normalized fluorescence intensities [15].
4. Maximum slope (MS): The steepest rate of fluorescence increase, reflecting the most rapid phase of aggregation [20].

Together, these metrics enable researchers to characterize the kinetics of RT-QuIC reactions comprehensively, enhancing the rigor and reliability of diagnostic decisions.

In addition to analytical tools, quicR provides flexible and customizable visualization capabilities. Leveraging the powerful ggplot2 library [21], quicR enables users to generate high-quality, publication-ready figures. These visualizations can be further customized using the intuitive '+' syntax of ggplot2, allowing for tailored presentations of RT-QuIC data.

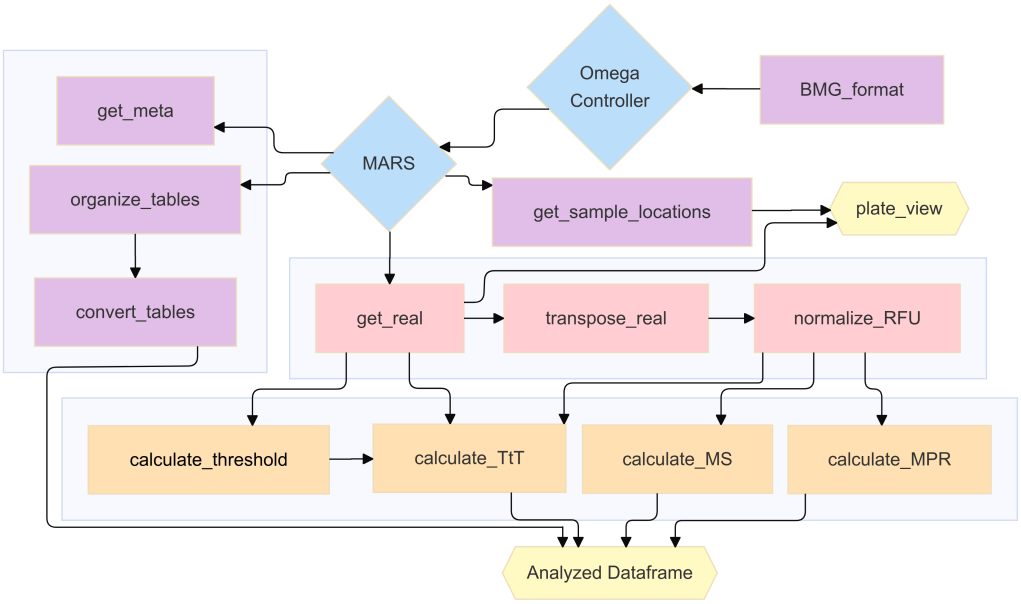
By combining standardized metrics, advanced visualization tools, and a commitment to open source science, quicR serves as a foundational resource for the growing RT-QuIC community. Its goal is to empower researchers to analyze and present their data with clarity, consistency, and cohesion.

2. Software description

2.1. Software architecture

quicR was developed to address the growing need for efficient data conversion, analysis, and visualization of RT-QuIC data. With a focus on usability and reproducibility, the package is designed to standardize workflows and ensure compatibility across multiple laboratories. Its primary input format is data exported as Excel workbooks from the proprietary MARS software (BMG Labtech, Ortenberg, Germany), providing seamless integration with existing experimental workflows. See Figure 1 for a detailed workflow.

Figure 1: Workflow hierarchy of the quicR package. Blue nodes indicate steps where BMG software is needed. Purple nodes indicate functions dedicated to handling metadata. Red nodes are functions that acquire and manipulate raw data. Orange nodes are functions which calculate some metric. Finally, yellow nodes represent data analysis endpoints.



2.2. Software functionalities

The implementation of the quicR package encompasses several streamlined processes designed to facilitate data input, cleaning, transformation, and

analysis of real-time fluorescence data. This section provides a comprehensive guide to utilizing the package's key functionalities, detailing how to:

1. Format and input sample data into Omega control software (BMG Labtech, Ortenberg, Germany).
2. Extract, clean, and organize metadata and raw fluorescence data.
3. Apply transformations and normalization to raw data for downstream analysis.
4. Calculate critical analytical metrics, such as time-to-threshold (TtT), rate of amyloid formation (RAF), maxpoint ratio (MPR), and maximum slope (MS).

These steps are designed to enhance reproducibility, minimize manual data handling, and enable seamless integration with the MARS software workflow. Through practical examples, this section illustrates how each function operates, along with expected input and output formats, ensuring clarity and ease of use for researchers.

2.3. Input of Sample IDs into Omega Control Software

The Omega control software allows input of a TXT file containing sample IDs, dilution factors, and their well locations. This file is uniquely formatted, and not easily reproduced manually. The function, "BMG_format", allows for input of a CSV file containing the plate layout (see Table 2 for proper formatting), and exports the formatted TXT file. The file can then be imported into the control software before running.

Table 2: Example CSV file plate layout for input into the "BMG_format" function. The top left corner should be cell "A1" in the CSV file. The top numbered row and the left-most lettered column should never be altered.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	P	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
B	P	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
C	P	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
D	P	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
E	N	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
F	N	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
G	N	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
H	N	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11

2.4. Data Cleaning and Transformation

The MARS software (BMG Labtech, Ortenberg, Germany) exports real-time data as an Excel workbook. Typically, the first sheet in the workbook will include microplate views of both raw data and metadata; however, the metadata on this page is what is most useful for downstream processes. Those tables include the "Sample IDa" and "Dilutions" tables (if dilutions were included in the MARS export). For much of the downstream analysis, it is crucial that the "Sample IDs" table was exported from MARS. If there is no table, the user can simply add it manually (see [@fig-sheet1] for proper formatting).

2.4.1. Retrieving Metadata

The metadata is defined as either sample-dependent or -independent. Sample-dependent metadata includes information such as sample IDs and dilution factors, whereas sample-independent metadata includes the date, time, reaction ID, etc.

The dependent metadata can be retrieved using the "organize_tables" and "convert_tables" functions. The former returns a list of tables, and the latter converts each table into a column in a single data frame.

2.4.2. Sample Locations

Samples locations can be extracted based on their well ID. The "get_sample_locations" function accepts additional arguments if dilution factors were exported from MARS. The "dilution_bool" argument can be set to TRUE if dilutions are to be included in the output. The "dilution_fun" argument will supply a function for transforming the dilution factors (e.g. if the user would want to perform a log transformation). The user can supply a delimiter using the "sep" argument. The output of this function is critical as an argument in the "plate_view" function which is further explained in the visualization section.

2.5. Retrieving and Manipulating Raw Data

The raw, real-time data is typically found on the second sheet of the Excel workbook exported from MARS. There are three functions dedicated to the retrieval and cleaning of raw data.

1. **get_real**: Retrieves the raw data from the Excel file, and outputs it as a dataframe.
2. **transpose_real**: Swaps the rows and columns which makes some downstream analyses easier.
3. **normalize_RFU**: normalizes the raw data by dividing each read by background fluorescence at a given cycle.

2.5.1. Retrieve Raw Data

Raw data can be retrieved using the "get_real" function. The logical argument, "ordered", indicates whether the user would prefer the columns to be ordered by well or by sample ID. By default, it is FALSE which will order the data by well. This should almost always be the case for easier integration with other downstream functions. Additionally, since there can be more than one instance of real-time data (depending on if the user added some calculations in MARS), "get_real" returns a list of dataframes. Therefore, the output must be indexed to access the data frame of interest.

2.5.2. Transpose Raw Data

This data is structured such that each sample is its own column (variable) and each row (observation) is a time point. While this format is technically correct, a transposed format is more ideal for some downstream manipulation. This operation is performed using the function, "transpose_real". After transposition, each time point is an individual column (variable), and each sample is an individual row (observation).

2.5.3. Normalize Raw Data

The function "normalize_RFU" will convert the raw data into a background normalized data set. The function includes two additional arguments, "bg_cycle" (the cycle which will be used as the background fluorescence value) and "transposed" (if FALSE, will make a call to the "transpose_real" function). Note that the fourth time point is all "1's" since this was designated the background cycle.

2.6. Calculations

There are three analytical metrics with dedicated functions: time-to-threshold (TtT), maxpoint ratio (MPR), and maximum slope (MS). The rate of amyloid formation does not have a designated function since it is simply the reciprocal of the time-to-threshold (1/TtT). Each function below accepts input from the "transpose_real" or the "normalize_RFU" functions. See @tbl-metrics for an example of the output of these functions.

2.6.1. Thresholds

Many publications have different methods of determining thresholds. By convention, the most popular method is to take the average background fluorescence of the every well and add some multiple of standard deviations [15]. The quicR package provides the "calculate_threshold" function for this purpose. A value can be provided as the optional argument, "multiplier", which will be applied to the standard deviation.

2.6.2. Time-to-threshold and Rate of Amyloid Formation

TtT is calculated using the "calculate_TtT" function. The function must be supplied a threshold; default value is 2 (i.e. twice the background fluorescence if the data is normalized). A starting column should also be given as an integer; default value is 3. This is essentially asking how many columns of metadata are included before the fluorescence reads begin.

TtT is calculated by iterating through each row and checking if a value is greater than the threshold. If the value is greater, the slope of the previous time-point to the current time-point is calculated, and the time intersection of the current read is returned.

2.6.3. Maxpoint Ratio

MPR is calculated by the "calculate_MPR" function. Data must be normalized in order to derive this metric. In a normalized data set, the MPR is simply the maximum value achieved during the run. Raw data can be passed to this function, but the argument "data_is_norm", must be set to FALSE. This will pass the raw data to "normalize_RFU" before calculating the MPR values.

2.6.4. Maximum Slope

MS is calculated by the "calculate_MS" function. The function iterates through each row using a rolling window which can be adjusted (default value is 3). Given the window size, the slope is calculated based on change in fluorescence divided by the range of the window. The MS is simply the largest slope value recorded. The units are typically reported as ΔRFU/h.

3. Illustrative examples

4. Impact

5. Conclusions

quicR offers a powerful solution for the cleaning, analysis, and visualization of RT-QuIC data, addressing critical needs in a rapidly evolving field. By enabling consistent data handling and interpretation, quicR lays the groundwork for improved diagnostic consistency and reproducibility. The package's open-source nature ensures that it will continue to evolve, integrating new insights and technologies as they emerge.

As RT-QuIC technology advances, tools like quicR will play a pivotal role in bridging the gap between assay development and practical application. By equipping researchers with reliable, standardized tools, quicR not only supports the study of prion and protein misfolding disorders but also serves as a model for the development of software solutions in other diagnostic fields.

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