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

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].

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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

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

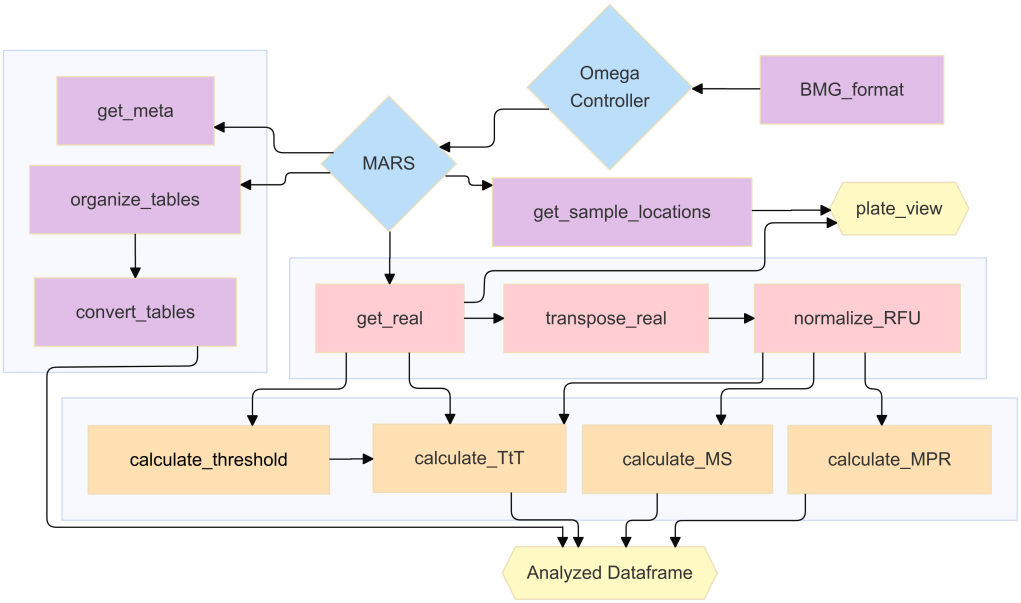
visualizations can be further customized using the intuitive '+' syntax of ggplot2, allowing for tailored presentations of RT-QuIC data.
By combining standardized metrics, advanced visualization tools, and a commitment to open source science, quicR serves as a foundational resource for the growing RT-QuIC 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 (Figure 1). With a focus on usability and reproducibility, the package is designed to standardize workflows and ensure compatibility across multiple laboratories.

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 RT-QuIC 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 data and apply transformations and normalization for downstream analysis.
3. Calculate critical analytical metrics, such as time-to-threshold (TtT), rate of amyloid formation (RAF), maxpoint ratio (MPR), and maximum slope (MS).
4. Vizualize raw and analyzed data.

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.

2.2.1. Input of Sample IDs into Omega Control Software

1. **BMG_format()**: 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. This function 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.

2.2.2. 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).

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

1. **organize_tables()**: returns a list of tables contained in the first sheet of the exported Excel sheet. These tables contain valuable metadata such as sample IDs, dilution factors, and microplate locations.
2. **convert_tables()**: accepts the tables outputted from **organize_tables** and converts them to columns in a dataframe.
3. **get_sample_locations()**: extracts the well locations for each sample. Output of this function is used as an argument for vizualizing a microplate-level view of real-time data.

2.3. 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.

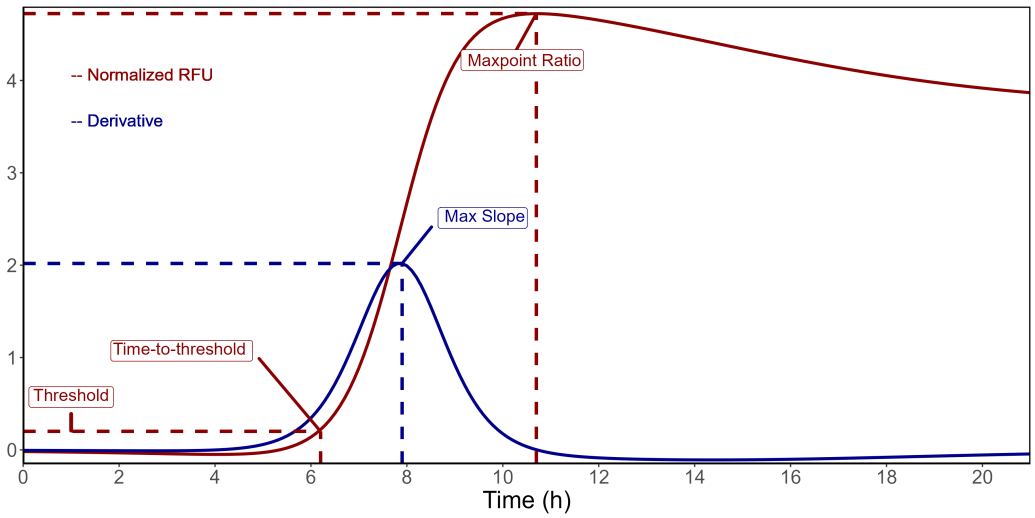
2.4. Calculations

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 Figure 2 for an example of the output of these functions.

1. **calculate_threhold()**: returns a value which is a given number of standard deviations above the average background fluorescence of the entire microplate.
2. **calculate_TtT()**: takes the real-time data and calculates the time in hours needed to reach a given threshold value.
3. **calculate_MPR()**: accepts raw or normalized data and returns the maximum value obtained during the run. If supplied with raw data, it will make a call to the normalize_RFU() function.
4. **calculate_MS()**: computes the approximate derivative of the real-time data and returns the maximum value obtained during the run.

Figure 2:



2.5. Vizualization

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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