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.

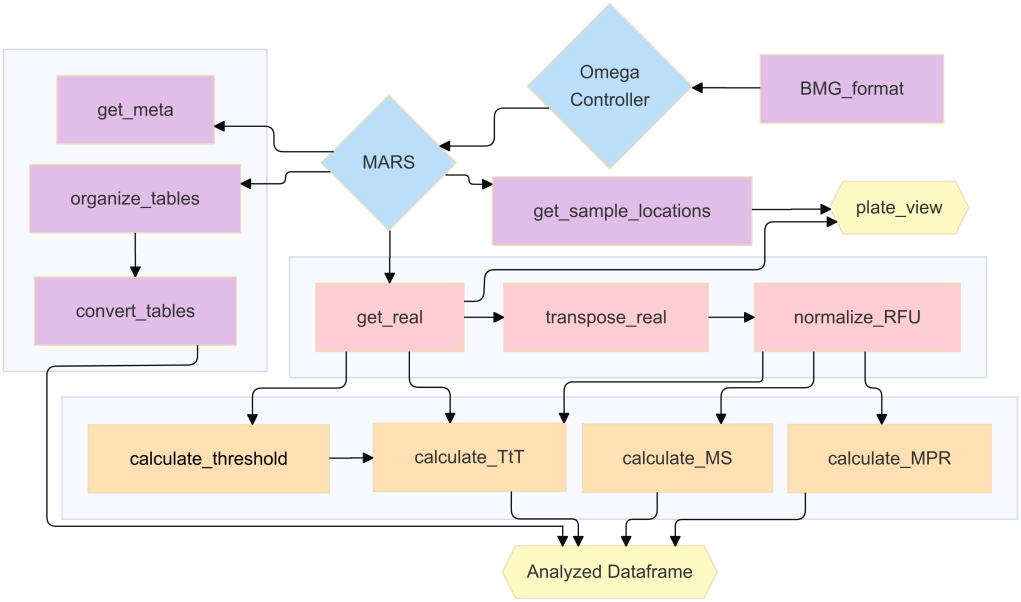
By combining standardized metrics, advanced visualization tools, and a commitment to open source science, quicR serves as a foundational resource for the growing RT-QuIC community. Its goal is to empower researchers to analyze and present their data with clarity, consistency, and cohesion.

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

Present the major functionalities of the software.

2.3. Sample code snippets analysis (optional)

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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