Title: A Shiny App for Determining Labeling Duration for Two-Sample Experiments

In this study, we present a web application developed using the R programming language and the Shiny framework. This application is designed to assist researchers in planning heavy water labeling experiments using a two-sample approach. The web application effectively determines the optimal labeling duration for a given peptide based on essential experimental parameters. The tool provides two input modes, illustrated in Figure A and Figure B. The summary of the input and outputs is presented in Table 1

In Figure A, the application uses a single expected turnover rate, along with the peptide sequence, body water enrichment (BWE), and ΔI₀ (the smallest detectable change in mono isotope relative abundance). Users can also specify the maximum labeling duration with a slider. Using these inputs, the application simulates the labeling curve over time and identifies the time window where the relative Mon isotope RA, I₀(t), falls between the theoretical upper and lower bounds (represented by red and black dashed lines, respectively). This interval, visually indicated by the green shaded region on the plot, corresponds to the recommended minimum and maximum labeling durations for the specified peptide and conditions.

In Figure B, the application takes a range of turnover rates (the slowest and fastest expected rates), which is useful when turnover is uncertain or varies across conditions. The labeling curves are simulated for both the lower and upper turnover rates (shown as black and blue points, respectively). The intersection of these simulations with the detection thresholds defines the valid labeling window, again indicated by the shaded region in the plot.

For both input modes, the application provides a graphical plot and a text summary indicating the recommended minimum and maximum labeling durations for reliable estimation of turnover rate using a two-sample approach. This tool helps guide the design of a single-deuterium labeled experiment by identifying the earliest and latest plausible duration that can be used to determine the turnover rates accurately.

A screenshot of a computer

AI-generated content may be incorrect.

Figure A)

A screenshot of a computer

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Figure B)

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| **Input** | **Description** |
| *Peptide* | Amino acid sequence used to calculate the number of exchangeable hydrogens (NEH). |
| *Expected Turnover Rate (k)* | Turnover rate estimate (used in single-rate mode, Figure A). |
| *Expected Slowest Turnover Rate* | Lower bound of expected turnover rate range (used in range mode, Figure B). |
| *Expected Fastest Turnover Rate* | Upper bound of expected turnover rate range (used in range mode, Figure B). |
| *Body Water Enrichment (BWE)* | Fraction of enriched water in the system |
| *ΔI₀* | Minimum detectable change in isotopic enrichment, defining sensitivity threshold. |
| *Max. Labeling Duration* | User-defined upper limit on the number of days for labeling simulation. |

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| **Output** | **Description** |
| *I₀ Depletion Curves* | Simulated I₀(t) values for one or more turnover rates , shown as black/blue dots. |
| *Detection Bounds* | Theoretical upper (red) and lower (black) detection thresholds. |
| *Valid Labeling Window* | Time range (green shaded area) where I₀(t) falls between detection bounds. |
| *Minimum Labeling Duration* | The plausible earliest labeling duration for the two-sample approach |
| *Maximum Labeling Duration* | The plausible latest labeling duration for the two-sample approach |