We thank reviewer 2 and the editor for the additional comments. In response to the specific points raised, we have prepared a revised version of the manuscript as outlined below.

From Reviewer 2:

1. p17, line 336: Amino acids with stereospecific 13 C enrichment of single methyl groups are related to SAIL amino acids. They are expensive but commercially available. They are produced by a former spin-off of the Kainosho laboratory and distributed by Cortec Net.

The statement has been revised to address the reviewer's comment regarding the availability of the amino acids.

2. Table S1. The price indicated for L-serine is for 200 mg and not for 1 gr. For several amino acids (Val, Asp, Glu, Phe, ..) the price corresponds to a racemic DL mixture. Are D-amino acids converted and incorporated into proteins? If not authors should specify whether they recommend doubling the amounts required compared to the use of L-amino acids. Alternatively, authors can provide the cost of L-amino acids in the table S1. Based on the changes, the authors should update the deuteration cost estimate in the manuscript.

The amino acid list has been amended in accordance with the reviewer's request to address concerns regarding pricing and the presence of racemic DL mixtures. Furthermore, the manuscript's deuteration cost estimate has been updated based on these revisions.

Additional suggestions from editor:

1. P. 6, L112. Provide force of centrifugation in units of g.
The force of centrifugation has been provided in units of g.

2. P. 5, L113 and 115. Provide weight of cells and volume for resuspension.
The weight of cells and the volume for resuspension have been included.

3. P. 7 L155. Provide weight of cells and volume for resuspension.
The weight of cells and the volume for resuspension have been specified.

4. P. 8. L208. Either remove the word high, or provide information of what it is high in relation to, with appropriate statistical test.
The word "high" has been removed

5. (P11 L231) To improve clarity use eCell CFPS instead of CFPS when referring to your method and only CFPS when referring to use of cell extracts. Revise throughout. For example the sentence on p. 11 L. 244-247, it is unclear if this was done here using eCell CFPS or previously using CFPS by the cited work.
This clarification has been applied throughout the manuscript.

6. P. 14 L275. Note the amount of glucose needed or remove the statement.
The amount of glucose needed has been noted

7. P. 15 L290. Change to "some of the advantages" as it does not inherit all advantages, as also noted by the reviewers.
The phrase has been modified to "some of the advantages" to acknowledge that not all advantages are inherited, as noted by the reviewers.

8. P. 15 L291. Either remove "compatibility with toxic proteins" or provide a reference for eCells. Alternatively change wording to clarify that this is a speculation, i.e. "likely to also be compatible with toxic proteins".

The wording regarding “compatibility with toxic proteins” has been revised to clarify that it is speculative

9. P. 15 L293. Please add “likely” or similar or provide reference or data to support the statement.
The word “likely” has been added

10. P16 L305. Clarify “extraordinarily low cost” by providing what this is relative to and justify relevance of comparison as appropriate. It is confusing that it is extraordinary, and also inefficient and in fact alternative methods are preferred by the authors.
The phrase “extraordinarily low cost” has been clarified.

11. P. 18 L. 375. Please provide data for and clarify “too long either”.
“too long either” has been clarified.

12. P. 19. L. 383. Remove second “ready”.
The second occurrence of the word “ready” has been removed.

Finally, I agree with reviewer-1’s request that:
“Figures 3 and 4: please add examples of 1D traces at appropriate locations to show whether or not there is residual 2abelling for valine, leucine or pro-R methyl groups.”

I appreciate that lower contours are provided, but 1D traces provide a more clear visual understanding of the S/N and are simpler for visual comparison of heights than contour plots. This can be provided in the SI section.

The requested examples of 1D traces demonstrating residual labeling for valine, leucine, or pro-R methyl groups have been included in the supplementary data.