Dear Dr. Shuangge Ma (Editor in Chief) and Dr. Zhenxia Chen (Deputy Editor), and Reviewers,

**OVERALL COMMENTS:**

We are most thankful to all of you for taking the time to carefully review our manuscript. We sincerely appreciate your insightful comments and valuable suggestions, which have helped us significantly improve the quality of our review.

In this document, please kindly find our detailed responses to your helpful comments below and the corresponding revisions/corrections in the resubmitted files.

Thank you once again for your constructive feedback and kind support.

**GENERAL:**

Overall, we have made careful revisions to our manuscript on the basis of using and incorporating as needed, the helpful and constructive comments by the expert reviewers. We believe these changes have substantially improved the quality and strength of our scientific work and corresponding submission.

这里添加对于 reviewers 修改意见的整体回复以及总结

These revisions have enhanced the rigor and robustness of our conclusions, and we believe the manuscript has significantly improved as a result. Thank you once again for your insightful comments and helpful suggestions.

**Reviewer: 1**

Comments 1:  
Please thoroughly check the manuscript for consistency in the use of spaces and punctuation marks to ensure formatting uniformity throughout the text.

Response to comment 1:

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Comments 2:  
In the 'Trajectory Methods' section, the introduction of veloVI should be moved to the beginning of the corresponding paragraph to maintain structural consistency. Additionally, the sentence 'This model encodes the unspliced and spliced abundances into a latent cell representation' contains an ambiguous use of the term 'model'. Please clarify whether it refers to veloVI or VEA.

Response to comment 2:

We thank the reviewer for this valuable suggestion to improve structural consistency and clarity. As recommended, we have revised the paragraph discussing *veloVI* in the 'Trajectory Methods' section.

1. The introduction of *veloVI* has been moved to the beginning of the paragraph. We now first introduce *veloVI* and its use of a variational autoencoder (VAE) framework, and then briefly explain what a VAE is.
2. We have clarified the ambiguous use of the term 'model'. The sentence 'This model encodes the unspliced and spliced abundances into a latent cell representation' has been revised to explicitly state '*veloVI* encodes...' to ensure clarity.

We believe these changes have improved the flow and readability of this section. The revised text can be found on Page [X], Lines [Y-Z].

Comments 3:  
The suggestions for the future application of RNA velocity in the discussion should be more explicit and feasible.

Response to comment 3:

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Comments 4:  
Some sentences are overly long and densely populated with technical terms. They should be appropriately revised to enhance readability.

Response to comment 4:

We thank the reviewer for their valuable feedback on improving the manuscript's readability. We have carefully reviewed the entire manuscript and made efforts to simplify sentence structures and reduce overly technical jargon where possible. We believe these revisions have enhanced the overall readability and flow of the manuscript for a broader audience.

**Reviewer: 2**

Comments 1:  
The methods for RNA velocity are thoroughly explained in great detail.

Response to comment 1:

We are grateful to the reviewer for acknowledging the thoroughness and detail in our explanation of RNA velocity methods.

Comments 2:  
In the preprocessing stage of Figure 1, I mention that data can either be log2 normalized and library normalized or used as raw data. Could you clarify whether there is any difference in RNA velocity results when using processed data versus raw data?

Response to comment 2:

We thank the reviewer for this pertinent question regarding the differences in RNA velocity results when using processed versus raw data. We have addressed this by revising the 'Workflow and Implementation' section (Page [X], Lines [Y-Z]) and by ensuring our Discussion section ('Current challenges and towards the better practice') further elaborates on the implications of preprocessing choices.

To specifically clarify the differences in the RNA velocity results:

1. **Impact on Data Characteristics and Signal Preservation:** Traditional preprocessing (e.g., normalization, smoothing) can alter data by reducing noise but may also remove subtle biological signals or introduce biases. Using raw counts, often with specifically designed models (e.g., TopicVelo [15], Pyro-Velocity [21], cell2fate [22] as cited in our manuscript), aims to preserve the discrete and stochastic nature of gene expression [11].
2. **Influence on Velocity Estimation and Interpretation:** Consequently, processed data might yield smoother velocity fields, potentially masking finer dynamics, while raw data can reveal more complex patterns if the model appropriately handles its inherent variability. The choice significantly influences the resulting velocity estimations and biological interpretations.
3. **Model-Specific Suitability:** The suitability of raw versus processed data is often dictated by the underlying assumptions and design of the chosen RNA velocity model.

Our revised text now explicitly states that the choice of input data can influence results and is model-dependent. We also guide the reader to our Discussion section ('Current challenges and towards the better practice') for a more comprehensive examination of preprocessing implications.

We hope this detailed explanation, in conjunction with the manuscript revisions, fully addresses the reviewer's query.

Comments 3:  
I noticed that in Table 3, almost all the applications are based on the scVelo algorithm. However, the abstract of the paper aims to provide model selection and practical recommendations. This appears inconsistent with the content, as it predominantly focuses on a single method.

Response to comment 3:

We appreciate the reviewer's insightful comment regarding the diversity of applications presented and its alignment with our aim to provide model selection guidance. We agree that showcasing a broader range of tool applications is important. In response, we have conducted a comprehensive search for published applications of the RNA velocity tools discussed in our review. We have now updated the 'Application of RNA Velocity under Various Biological Scenarios' section (and correspondingly, **Table 3**) by incorporating an additional **9 published studies** that utilize RNA velocity tools other than *scVelo*. These include applications of *UniTVelo* (4 studies), *cellDancer* (2 studies), *LatentVelo* (1 study), *MultiVelo* (1 study), and *Dynamo* (1 study).

We also acknowledge that some of the newer tools covered in our review (such as *veloAE*, *cell2fate*, and *SymVelo*) currently have limited or no published applications in distinct biological studies beyond their initial introduction or benchmarking papers. This reflects the cutting-edge nature of these methods and the typical timeline for broader adoption and application in the field. Our review aims to be comprehensive in covering these newer methods' principles, and we anticipate their application scope will expand in the future.

Moreover, it is indeed true, as the reviewer implicitly noted, that *scVelo* has seen widespread adoption, having been applied in hundreds of studies, far exceeding other methods to date. A primary motivation for this review is precisely to look beyond the most established tools. We aim to systematically introduce and categorize the principles and innovations of many newer and diverse RNA velocity methods, thereby encouraging the research community to explore and consider these alternatives for their specific biological questions and datasets.

We believe that by both acknowledging scVelo's prevalence and actively expanding the documented applications of other tools, we provide a more balanced view of the application landscape. This better supports the manuscript's goal of offering practical recommendations and encouraging the thoughtful selection of appropriate models beyond the default. The revised section can be found on Page [X], Lines [Y-Z].

Comments 4:  
A significant portion of the manuscript is dedicated to discussing the principles and limitations of these algorithms. However, the section on model selection and practical recommendations is rather sparse. It would be helpful if this topic were given its own dedicated section, rather than simply recommending the use of scVelo without further elaboration. I suggest that more detailed guidance be provided for selecting the most suitable model for different scenarios.

Response to comment 4:

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