**Paradigms, Innovations, and Biological Applications of RNA Velocity: A Comprehensive Review**

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**Abstract**

Single-cell RNA sequencing (scRNA-seq) enables unprecedented insights into cellular heterogeneity and lineage dynamics. RNA velocity, by modeling the temporal relationship between spliced and unspliced transcripts, extends this capability to predict future transcriptional states and uncover the directionality of cellular transitions. Since the introduction of foundational frameworks such as *Velocyto* and *scVelo*, an expanding array of computational tools has emerged, each based on distinct biophysical assumptions and modeling paradigms. To provide a structured overview of this rapidly evolving field, we categorize RNA velocity models into three classes according to their underlying approaches to transcriptional kinetics inference. These classes are: steady state methods, trajectory methods, and state extrapolation methods. For each category, we systematically analyze both the overarching principles and the individual methods, comparing their assumptions, kinetic models, and computational strategies, and assessing their respective strengths and limitations. To demonstrate the biological utility of these tools, we summarize representative applications of RNA velocity across developmental biology and diseased microenvironments. We further introduce emerging extensions of RNA velocity methods that go beyond classical splicing kinetics. Finally, we discuss existing limitations regarding model assumptions, preprocessing procedures, and velocity visualization, and offer practical recommendations for model selection and application. This review offers a comprehensive guide to the RNA velocity landscape, supporting its effective implementation in dynamic transcriptomic research.

**Keywords:** RNA velocity, single-cell RNA sequencing, dynamic transcriptomics, computational modeling, advanced inference strategies

**Issue Section:** Review

**Background**

Single-cell RNA sequencing (scRNA-seq) has revolutionized the study of biological systems by enabling the exploration of cellular heterogeneity, lineage tracing, and gene regulatory network dynamics at an unprecedented resolution [1-3]. This technology has provided critical insights into complex biological processes such as cellular development, differentiation, immune response, and tumor evolution, thereby paving the way for transformative advances in the field of developmental biology [3, 4]. The analysis of cellular development and differentiation presents a unique challenge, as cells traverse a continuous landscape of states rather than existing in discrete categories. Traditional trajectory inference methods reconstruct developmental paths by ordering cells according to their transcriptional similarity. This approach effectively creates a pseudotemporal sequence that reflects biological progression [3, 5, 6]. However, these methods are limited by the static nature of single-cell measurements, which only capture snapshots of cellular states. While trajectory inference has proven valuable for understanding cell fate decisions, the selection of appropriate methods depends heavily on the expected trajectory topology (linear, branching, or cyclic) and selected methods often require validation through multiple approaches [7].

RNA velocity analysis represents a significant advancement in this field, offering a more direct way to infer cellular dynamics. Unlike traditional trajectory inference methods that rely solely on transcriptional similarities, RNA velocity leverages the relative abundance of spliced and unspliced mRNA to predict future cell states. This approach, first introduced by La Manno et al. [8], captures directed dynamic information and predicts future cell states by distinguishing the relative abundances of unspliced pre-mRNA and spliced mature mRNA presented in scRNA-seq, based on a steady-state theory. By modeling transcriptional dynamics, RNA velocity infers the instantaneous rate of change in unspliced mRNA abundance (*ds*/*dt*) for individual genes from relative mRNA abundances, known as RNA velocity. A positive RNA velocity indicates an induction in transcriptional state and a negative velocity refers to a gene repression. This qualitative premise has profound implications for the analysis of scRNA-seq data. The experimentally observed transcriptome is a snapshot of a biological process [9]. By carefully integrating snapshot data with a causal model, it has become possible to reconstruct both the dynamics and direction of this process without prior knowledge or specialized experiments.

The first RNA velocity model, *Velocyto*, developed by La Manno et al. [8], captures directed dynamic information and predicts future cell states. It works by distinguishing the relative abundances of unspliced pre-mRNA and spliced mature mRNA detected in scRNA-seq, based on steady-state theory. This foundational model was subsequently enhanced by Bergen et al. [10] in *scVelo*, which implemented a more sophisticated dynamical model capable of inferring transcriptional dynamics together and assigning latent cell time. These two tools have established cornerstones for the RNA velocity inference and will be discussed in detail in the subsequent section on model descriptions [11, 12]. Followed by *Velocyto* and *scVelo*, numerous models [8, 10, 13-25] have been developed to elucidate transcriptional dynamics within single-cell transcriptomes, each leveraging distinct computational frameworks grounded in specific biophysical assumptions about transcriptional processes. For instance, certain methods utilize cell-shared kinetic rates, typically including transcription, splicing, and degradation rates, while others propose that these kinetic rates should vary across cells. Some methods incorporate latent variables, such as latent time and latent transcriptional states, to enhance model fitting. Several tools have expanded RNA velocity estimation by integrating multi-omics data, like chromatin accessibility and metabolic labeling.

Despite the rapid development of these tools, no comprehensive review has yet to provide a thorough comparison of their strengths, limitations and applicability across different biological contexts. In this review, we aim to fill this gap by categorizing existing RNA velocity computational tools based on their underlying assumptions and computational frameworks, and summarizing their applications in key biological scenarios. Furthermore, we discuss practical considerations for the use of RNA velocity tools, including current challenges and emerging opportunities for integrating multi-omics data and improving trajectory inference. By providing a clear overview of the field, this review aims to facilitate a more informed and effective application of RNA velocity tools in both fundamental and translational research contexts.

**Workflow and Implementation**

The typical RNA velocity analysis pipeline (**Figure 1A**) begins with an essential preprocessing step to distinguish between unspliced and spliced transcripts in the raw sequencing data. Several tools [8, 26-31] have been developed to quantify these abundances (**Figure 1B**) , enabling the construction of separate count matrices [32]. Given the inherent noise in single-cell RNA sequencing data, most analytical frameworks employ sophisticated data smoothing techniques (also known as data imputation) to extract reliable signals for velocity inference (**Figure 1C**). A prevalent approach involves computing the first-order moment (mean) across k-nearest neighbors (KNN) in the expression space. While the majority of protocols incorporate additional preprocessing steps, including library size normalization and log-transformation[11], the choice between using such processed data versus raw count data can influence RNA velocity results and is often dependent on the specific inference model employed. For instance, some alternative estimation frameworks (e.g. TopicVelo [15], Pyro-Velocity [21], and cell2fate [22]) are specifically designed to treat transcript dynamics as a discrete stochastic process, allowing the direct use of raw count matrices. This approach can better preserve biological signals, including informative noise and gene-gene interactions, and minimizing potential parameter estimation biases that might arise from extensive preprocessing [11] , a topic further explored in the Discussion section (see 'Current challenges and towards the better practice'). The subsequent phase involves estimating cell-specific RNA velocities in high-dimensional space by applying various biophysical models to fit unspliced and spliced transcript counts (**Figure 1D**). This process yields essential kinetic parameters, including transcription, splicing, and degradation rates. Depending on the chosen modeling framework, additional latent variables such as latent time, can be simultaneously inferred, further enhancing the resolution of transcriptional dynamics (**Supplementary Material. 1**).

[Figure 1 insert here]

**Figure 1**. Workflow for RNA velocity analysis. **(A)** Raw scRNA-seq data acquisition. **(B)** Quantification of unspliced and spliced transcript abundances. **(C)** Count matrices pre-processing. Data normalization and neighborhood smoothing are included in classic workflow. **(D)** Estimation of RNA velocities by fitting spliced and unspliced counts to biophysical models, also yielding kinetic parameters and latent variables. **(E)** Visualization of high-dimensional velocity vectors in low-dimensional space via methods such as streamline plots and grid-averaged vector fields. **(F)** Downstream analyses.

Once high-dimensional velocity vectors are derived, they are embedded into a low-dimensional representation (**Figure 1E**), commonly generated through dimensionality reduction methods applied to the spliced count matrix. These methods include Uniform Manifold Approximation and Projection (UMAP) [33], t-distributed Stochastic Neighbor Embedding (t-SNE) [34], and Principal Component Analysis (PCA) [35] . For this projection, velocity vectors are typically mapped into the lower-dimensional space by calculating transition probabilities. The projected velocities are visualized through techniques such as streamline plots or grid-averaged vector fields. To improve clarity, additional post-processing—such as smoothing the velocity field using kernel methods and scaling vector magnitudes—is commonly applied. This enables clearer interpretation of cell state transitions and developmental trajectories.

RNA velocity frameworks have evolved beyond simple velocity estimation to incorporate sophisticated downstream analyses that facilitate interpretation of cellular dynamics and underlying molecular mechanisms (**Figure 1F**). Many RNA velocity models integrate inferred kinetic parameters and latent variables to support downstream analyses that provide deeper insights into dynamic processes and potential regulatory mechanisms. During the estimation phase, several models infer latent time alongside kinetic parameters [10, 13, 16, 18-22]. These temporal measurements serve as cellular internal clock, precisely tracking cell progression through biological processes [6, 7]. Various analytical tools also offer specialized functionalities. For instance, *scVelo* can identify key driver genes orchestrating the transcriptional dynamics captured in velocity estimates. Bayesian models provide additional insights by quantifying posterior uncertainty in velocity and kinetic parameter estimates, thereby enabling robust statistical assessment [18-22, 24].

The RNA velocity framework has led to the development of numerous specialized computational tools. Several tools [36-40] focus on trajectory analysis by integrating expression-based k-NN graphs with RNA velocity transition probabilities, enabling identification of initial, intermediate, and terminal cell populations during differentiation and reveal broad developmental patterns. Visualization has also been enhanced through tools like *scDEED* [41], *DynamicViz* [42], and *Ocelli* [43], which improve the representation of transition probability matrices in reduced dimensional space. For benchmarking purposes, simulators such as *scMultiSim* [44] and *SERGIO* [45]generate synthetic scRNA-seq data that mimics diverse cellular developmental trajectories.

This workflow was originally proposed by La Manno et al. [8] and subsequently refined by Bergen et al. in the *scVelo* framework [10]. Most later RNA velocity tools adopted this general paradigm, differing primarily in their approaches to kinetic prediction and velocity inference. Moreover, some methods are not only conceptually based on the *scVelo* framework, but also directly integrate *scVelo*'s preprocessing modules and visualization interfaces. While previous reviews have primarily focused on comparing *Velocyto* and *scVelo*, providing a comprehensive comparison of these two methods, delving into implementation details and discussing valuable aspects of data processing and visualization techniques [11, 12, 46]. However, recent advances in RNA velocity have introduced conceptual and technical recasting. These include the incorporation of more biophysically grounded dynamic processes and the optimization of computational frameworks for learning and interpreting transcriptional dynamics.

In the following sessions, we build upon *Velocyto* and *scVelo* to review existing RNA velocity methods, providing an extensive overview of current developments in RNA velocity computational models. We categorize these methods according to their paradigms for modeling transcriptional dynamics and systematically dissect their kinetic prediction and velocity inference strategies, along with the underlying biophysical assumptions. Furthermore, we map their applications to critical biological scenarios and discuss current challenges these methods are facing alongside emerging opportunities in basic research and clinical applications.

**Kinetics Prediction and Velocity Inference**

Based on distinct paradigms in learning transcriptional dynamics, we categorize RNA velocity methods into three classes: steady-state methods, trajectory methods, and state extrapolation methods (**Figure 2** and **Table 1**). Steady-state methods, such as *Velocyto*, solve transcriptional dynamics relying on steady-state assumption, presuming a constant splicing rate, and inferring kinetic rates using steady-state subpopulations. Trajectory methods, exemplified by *scVelo*, estimate kinetic parameters to construct phase portrait trajectories that align observed cells with their respective corresponding cell times. State extrapolation methods leverage expected future cell states to supervise the estimation and optimization of cell-level RNA velocity vectors. In the following sections, we introduce and compare representative RNA velocity models, with a focus on their design of computational frameworks as well as underlying modeling concepts (**Table 2**).

[Figure 2 insert here]

Figure 2. RNA velocity methods are categorized into three classes based on their paradigms in learning transcriptional dynamics. (A, B) steady-state methods, includs linear regression based on the steady-state ratio and inference based on minimizing Kullback–Leibler (KL) divergence between observed and predicted distributions. (C, D) trajectory-based methods, where either cell-shared or cell-specific latent trajectories are used to reconstruct cellular dynamics by minimizing the sum of displacements between observed and estimated states. (E, F) state extrapolation methods, which infer future states by minimizing cosine similarity or distance in phase portrait space or high-dimensional gene space.

Table 1. Summary of three categories RNA velocity computational methods.

[Table 1 insert here]

**Steady-state Methods**

La Manno et al. [8] proposed the first RNA velocity estimation framework, *Velocyto*, which is grounded in a steady-state assumption. This assumption posits that after transcriptional initiation, the abundances of unspliced and spliced mRNA for genes reach a steady-state equilibrium due to ongoing molecular degradation. When gene expression ceases, mRNA abundance gradually decays to zero. *Velocyto* introduces rigorous constraints on kinetic parameters in the rate equation (**Supplementary Material 1**), treating transcription and degradation rates as time-dependent variables to be estimated, while defining the splicing rate as a fixed constant (typically set to 1) across all genes to reduce model complexity and ensure tractability. For each gene, steady-state cells, where unspliced and spliced mRNA abundances reach minimal or maximal values, are identified (**Figure 2A**). A least-squares linear regression is then applied to these steady-state cells to analytically derive the degradation rate, also referred to as the steady-state ratio. Subsequently, RNA velocity is computed for each cell using the derived kinetic parameters and a closed-form equation (**Figure 1D**).

Inspired by *Velocyto*, Bergen et al. [10] developed *scVelo*, a comprehensive and extensible computational framework that integrates the original steady-state model proposed by *Velocyto* and extending it through the incorporation of a stochastic model. This stochastic model reformulates the first order transcriptional ODE by including second order moments (variance and covariance of unspliced and spliced counts) to account for stochasticity. Similar to the steady-state approach, a least-squares fit is employed to estimate degradation rates from steady-state populations. Subsequently, *MultiVelo* [13] further advanced these models by integrating chromatin accessibility information derived from ATAC-seq data [47]. In MultiVelo, transcriptional regulation is simplified through the abstraction of chromatin modifiers, pioneer factors, and transcription factors into a single rate parameter reflecting chromatin accessibility. Such transcriptional regulation is explicitly modeled by linking transcription rates to chromatin accessibility within the ODE framework, thereby enabling a more accurate and biologically informed estimation of kinetic parameters.

*VeloAE* [14] solves steady-state ratio and infers latent transcriptional dynamics within a learned low-dimensional representation using and autoencoder framework. Specifically, it uses a graph convolutional network (GCN) to smooth the pre-encoded latent cell states (latent unspliced and spliced matrices) based on neighboring cells, and then adopt an attentive combination module to reconstruct the input mRNA abundances in decoder. The autoencoder framework allows *veloAE* to capture biologically meaningful latent cell states by minimizing the reconstruction loss between input and output count matrices. In addition, a regression loss is introduced to supervise the fit of the steady-state ratio within low dimensional space. By learning low dimensional kinetics rates and RNA velocity, *VeloAE* is able to recover denoised transcriptional dynamics and mitigates the sparsity challenges often encountered in high-dimensional raw count data.

In contrast to other steady-state models, *TopicVelo* [15] employs a probabilistic topic modeling framework to disentangle potentially simultaneous processes. This model assumes that multiple biological processes (also called topics, e.g. proliferation, immune response, and system-specific processes) can be extracted from scRNA-seq by adopting Bayesian Nonnegative Matrix Factorization (BNMF), which identifies both process-specific gene signatures and cell-specific topic activity levels. Operating directly on raw count data, *TopicVelo* incorporates transcriptional bursting into a chemical master equation (CME) framework. The Gillespie algorithm [48] is then applied to each gene within each process to simulate the stochastic transcriptional dynamics and estimate the joint probabilistic distribution of unspliced and spliced counts at steady-state. Kinetic parameters are optimized by minimizing the Kullback-Leibler (KL) divergence between the inferred and experimentally observed joint distributions (**Figure 2B**). *TopicVelo* estimates process-specific kinetic rates and computes corresponding process-specific transition matrices which are then aggregated into a unified transition matrix estimates for each cell, weighted by the inferred topic proportions.

The steady-state RNA velocity inference framework has demonstrated utility in specific biological contexts. For example, in immune system disorders, steady-state methods have been used to reconstruct the epigenetic regulation of T cell differentiation, elucidating how clonal hematopoiesis gene knockouts influence T cell fate at distinct developmental stages [49]. In tumor microenvironments, these methods have provided insights into CD8+ T cell differentiation, tracing distinct trajectories leading to cytotoxic and exhausted T cell states, and identifying a stem-like T cell reservoir that sustains anti-tumor immunity [50]. Additionally, the probabilistic topic approach adopted by *TopicVelo* has uncovered distinct transcriptional programs within mixed cell populations, including the differentiation trajectory of bone marrow precursors into natural killer (NK) cells, clarifying lineage commitments between NK cells and innate lymphoid cells [51].

Despite their demonstrated applicability, steady-state assumptions impose substantial constraints that limit broader usability. The estimated steady-state ratio may diverge from the actual ratio in instances where not all steady states are observed, such as when transcription stops prematurely. By integrating topic modeling with the steady-state inference system, *TopicVelo* enables more precise analysis of steady-state gene expression patterns while also implicitly addressing some non-steady-state conditions. However, a key limitation remains: splicing is inherently gene-specific, with splicing rates exhibiting substantial variability across genes. The foundational assumptions of a constant splicing rate in all steady-state methods are frequently compromised, especially in cases where the population includes multiple heterogeneous subpopulations exhibiting distinct kinetic behaviors.

**Trajectory Methods**

*scVelo* [10] introduced a likelihood-based dynamical model for inferring RNA velocity that addresses complete gene-specific transcriptional dynamics, without relying on steady-state assumptions. This method solves full transcriptional dynamics by constructing phase trajectories, visual manifestations of the transcriptional dynamics governed by analytical solution of ODEs, illustrating how unspliced and spliced mRNA levels for a gene evolve over time as a function of the kinetic parameters (**Figure 2C and Supplementary Material 1**). To estimate these dynamics, the dynamical model utilizes an efficient expectation maximization (EM) framework, that jointly estimates gene-specific kinetic parameters along with gene-specific latent time and transcriptional states. In the expectation step, latent time is assigned to cells by minimizing the distance between observed cell states and their corresponding positions along the current trajectory estimate. Transcriptional states are assigned by associating a likelihood value to respective segments of the phase trajectory. In the maximization step, kinetic parameters are optimized to specify a phase trajectory that maximize the log-likelihood of all observed displacements between cells and their corresponding inferred transcriptional states (**Figure 2C**).

*MultiVelo* [13] extends the RNA velocity framework by integrating chromatin accessibility in EM framework. As previously illustrated in steady-state context, regulation of transcription process via chromatin accessibility is explicitly modeled into ODE system, allowing the model to account for upstream regulatory influences. Distinct phases or states of chromatin activity that a cell traverses as its time advances are modeled analogously to the stepwise transcription states used in *scVelo* dynamics model. Note that the fitted trajectory is extended to three-dimensional phase portrait, where chromatin accessibility constitutes an additional dimension alongside unspliced and spliced mRNA levels.

*UniTVelo* [16] adopts an EM framework similar to that of *scVelo*, while introduces a radial basis function (RBF) to model transcriptional dynamics and quantify RNA velocity in a top-down manner. This method addresses a core limitation in *scVelo*'s dynamical model: the complete transcriptional dynamic process across many genes is typically challenging to capture, leading to significant bias in phase trajectory fitting. Instead of modeling gene expression as discrete transcriptional states, *UniTVelo* employs a spliced-oriented design that defines spliced abundance as a smooth time-dependent function using RBFs. It then applies a linear dynamical system to compute the corresponding unspliced abundance, preserving a continuous relationship between unspliced and spliced RNA levels. Additionally, in the expectation step, *UniTVelo* introduces a gene-shared latent time, which unifies gene-specific latent times by aligning them based on a common cell order. This helps to resolve inconsistencies in inferred directionality across different genes and ensures a more coherent temporal structure.

*Dynamo* [17] uses metabolic labeling to facilitate the study of transcriptional dynamics. It observes transcriptional dynamics in real time, enabling the estimation of RNA velocity and kinetic parameters on an absolute time scale. *Dynamo* fits trajectories using nonlinear regression based on experimentally measured cell time, rather than employing an EM framework that treats cell time as a latent variable for joint inference. By incorporating real-time developmental information, this method allows for more accurate extrapolation of cell states across time and improves the biological interpretability of inferred dynamics. As a result, RNA velocity estimates and kinetic parameters are expressed in absolute temporal units, providing a more faithful reconstruction of dynamic transcriptional processes across cells.

As an alternative to EM-based approaches, *veloVI* [18] implements a variational autoencoder (VAE) framework for RNA velocity estimation. A VAE is a generative model that leverages the principles of Bayesian inference to learn the distribution of input data and generate new samples. In this context, *veloVI* encodes the unspliced and spliced abundances into a latent cell representation, which further encodes gene-specific latent transcriptional state assignment and latent time for each cell. A transcriptional ODE with a similar formulation of *scVelo* is utilized to reconstruct the input unspliced and spliced matrices by adopting learnt latent transcriptional state and time. The reconstruction process can be interpreted as mapping observed cell states onto a cell-shared trajectory, with latent time anchoring each cell’s position along that trajectory. This mechanism conceptually aligns with the notion of the maximization step in EM methods (**Figure 2C**). By leveraging a variational inference framework, *veloVI* enables RNA velocity to be modeled as a posterior predictive distribution, thus allowing for explicit quantification of uncertainty in both latent variables and velocity estimates. Furthermore, this model can be extended to capture cell-specific transcription rates by introducing an inhomogeneity term, allowing transcription rates to vary dynamically over time and better capture complex transcriptional processes.

*VeloVAE* [19] applies a similar VAE framework, whereas it models cell-specific transcription rates through lineage-dependent ODEs. This model first encodes a gene-shared latent time and latent cell state for each cell. A neural network is then used to infer adjustments to transcription rates based on each cell’s latent state, leveraging the biological intuition that nearby cells in the latent space tend to exhibit similar transcriptional kinetics [52]. Consequently, mRNA abundances are reconstructed based on the transcriptional dynamic ODEs in a similar manner as *veloVI*. However, since transcription rates are cell-specific, each cell effectively follows its own dynamic trajectory in phase space (**Figure 2D**). In addition, *VeloVAE* can be further extended to include a branching ODE system, allowing the model to infer lineage bifurcations and learn cell type–specific kinetic parameters across distinct developmental paths.

*LatentVelo* [20] computes a low-dimensional representation of gene dynamics by embedding cells into a latent space within a VAE framework and straightly estimates trajectory in low-dimension phase portrait. Latent dynamics is learnt via a structured neural ODEs system, which does not explicitly model transcription, splicing, and degradation rates. Instead, it implements structured neural networks to represent the interactions across each component of transcriptional ODE. An external latent regulatory state is also included into the ODEs system, enabling the estimation of lineage development and lineage-specific kinetic parameters. Hyperparameters within the neural network can capture higher-order dynamical signals and fit complex, non-linear trajectories. In this framework, latent dynamics are learnt by reconstructing both the embedded low-dimensional cell states and the original high-dimensional expression profiles, simultaneously optimizing the VAE framework. Additionally, batch correction and additional information such as annotated cell-type labels, temporal information, and chromatin accessibility can be incorporated into the latent dynamics learning process to further refine latent dynamics learning.

*Pyro-Velocity* [21] recasts RNA velocity estimation as a latent variable posterior inference task, leveraging automatic differentiation variational inference (ADVI) to perform fully Bayesian inference. The model is conditioned directly on raw count data, enabling the estimation of posterior distributions and providing explicit quantification of uncertainty in both RNA velocity and kinetic parameters. Like *scVelo*, *Pyro-Velocity* employs transcriptional ODEs parameterized by gene-specific kinetic rates and stepwise transcriptional states. However, instead of assigning a separate latent time per gene, it fits a shared latent time jointly across all genes based on the entire count matrix. Model optimization is in a similar manner of VAE methods, learning kinetic parameters and other latent variables to reconstruct the input data through phase trajectory.

Building on *Pyro-Velocity*, *cell2fate* [22] introduces a modular approach to RNA velocity by linearizing the transcriptional ODEs, enabling a biophysically informed, fully Bayesian model solvable via ADVI. *cell2fate* adopts a similar notion as *TopicVelo* that a single cell’s dynamics may arise from the superposition of multiple biological processes. To capture these complex dynamics, it models time-dependent transcription rates as a linear combination of multiple dynamic modules within the ODEs system. Dynamics of each *module* are defined by a cell-specific time scale with on/off switch times, corresponding rates, and a gene loading parameter. Like *Pyro-Velocity*, *cell2fate* operates directly on raw count matrices, jointly estimating latent variables and velocities while explicitly quantifying uncertainty, with the goal of reconstructing the input data. Additionally, the model can include refinements to account for technical factors such as ambient RNA, data overdispersion, and batch effects. Furthermore, *cell2fate* supports integration with spatial transcriptomics, allowing the mapping of inferred modules to spatial tissue architectures, thereby extending its applicability to spatially resolved developmental and disease systems.

As the most widely adopted RNA velocity methods, trajectory-based approaches have demonstrated substantial utility in resolving cellular differentiation trajectories, refining lineage inference, and integrating multi-omics data. For example, *scVelo* has been instrumental in elucidating neural development, identifying distinct oligodendrocyte precursor cell (OPC) subpopulations and reconstructing their differentiation trajectories in the human forebrain [53]. Additionally, these methods have provided insights into leukemia progression, capturing the unidirectional transition of chronic lymphocytic leukemia (CLL) cells within the lymph node microenvironment and challenging the traditional model of bidirectional movement between blood and lymphoid tissues [54]. The integration of RNA velocity with ATAC-seq data through *MultiVelo* has revealed the interplay between transcriptional regulation and chromatin accessibility, distinguishing between continuous and hierarchical differentiation patterns in human retinal development [55]. Collectively, the trajectory-based approaches extend the capabilities of RNA velocity beyond steady-state limitations, offer flexible modeling of complex, nonlinear transcriptional dynamics, and often incorporate latent time or regulatory states to enhance biological interpretability. However, these methods remain sensitive to incomplete or partial trajectories and rely on ordinary differential equation (ODE) formulations that may not fully capture multifaceted or branching kinetic processes.

**State Extrapolation Methods**

*cellDancer* [23] infers RNA velocity for each cell by leveraging expression states from its neighboring cells, and propagates a series of local velocities to provide single-cell resolution inference of transcriptional kinetics. For each gene, this model trains an independent deep neural network (DNN) to calculate cell-specific kinetic rates. Future expression states are extrapolated by modeling short-term changes in both unspliced and spliced mRNA abundances, governed by transcriptional dynamics ODEs. The optimization objective is to maximize the global cosine similarity between extrapolated cell states and their observed neighbors in the phase portrait (**Figure 2E**). The expected future state is selected as the neighbor with the highest cosine similarity, which serves as a guide for velocity vector refinement. By directly incorporating local velocity vector into the training process and learning cell-specific reaction rates, *cellDancer* effectively captures multi-rate kinetic regimes, ensuring a more accurate and nuanced representation of transcriptional kinetics.

*DeepVelo* [24] employs a graph-based deep learning framework to estimate local velocity and extrapolate cell states within the high-dimensional splicing space. The method begins by identifying each cell’s KNN and encoding the resulting local neighborhood into a latent representation using a GCN. The GCN effectively captures local cell-cell relationships based on gene expression profiles. A downstream decoder network then predicts gene- and cell-specific kinetic parameters, which are used to extrapolate future cell states. In *DeepVelo*, transcriptional dynamics are learned by minimizing the cumulative displacement between predicted cell states and their expected neighbors (**Figure 2F**). Unlike *cellDancer*, which rely solely on the most similar neighboring cells, *DeepVelo* incorporates both downstream and upstream neighbors to supervise the optimization of forward and backward velocity vectors for each cell, thereby ensuring a more comprehensive inference of transcriptional dynamics.

*SymVelo* [25] is a dual-path framework that integrates high- and low-dimensional information to enhance RNA velocity estimation. This model employs a mutual learning framework, combining a state extrapolation branch and a steady-state branch to jointly capture transcriptional dynamics. The high-dimensional branch uses a neural ODE module called *SymNet*, a symbolic network designed to represent gene-specific kinetics via a generalized kinetic model, optimized in a manner similar to *DeepVelo*. In parallel, the low-dimensional branch adopts a *VeloAE*-inspired framework, learning RNA velocity in latent space. The inferred low-dimensional velocities are then used to supervise neighbor selection in the extrapolation branch. To reconcile potential mismatches between the two velocity spaces, Markov transition matrices are computed independently for each branch. A divergence loss between these matrices, combined with individual loss functions, is used to jointly train the model and capture complex cellular dynamics. This dual-path framework aligns the two branches via mutual learning, inheriting the robustness of low-dimensional representation learning while preserving biological interpretability through the high-dimensional branch. Furthermore, mutual learning ensures coverage of all cells across each latent dimension, enabling inter-gene information to guide the supervision of representation learning.

Compared to other RNA velocity approaches, state extrapolation methods offer superior capacities to capture lineage heterogeneity by dynamically predicting future cell states beyond static transcriptional snapshots. Their ability to estimate local velocity vectors at single-cell resolution and extrapolate expression states allows for a more refined reconstruction of transcriptional kinetics across diverse cellular trajectories. A key advantage of this approach is its robustness in handling multi-rate kinetic regimes, where transcription, splicing, and degradation rates vary across cell subpopulations. For example, *cellDancer* has demonstrated strong performance in resolving transcriptional boost genes, such as *Hba-x* and *Smim1*, during erythroid maturation in mouse gastrulation [23]. These genes undergo sudden transcriptional upregulation in the middle of erythroid differentiation, posing challenges for traditional RNA velocity models like *scVelo*, which failed to capture the induction phases of such MURK (multiple-rate kinetic) genes, whereas *cellDancer* accurately inferred their dynamics.

Given these advantages, state extrapolation methods may hold particular promise for future applications in highly heterogeneous biological systems, such as tumor evolution, immune cell differentiation, and dynamic transcriptional regulation in response to environmental stimuli. By enabling single-cell resolution estimation of RNA velocity in complex multi-lineage contexts, these models have the potential to improve the precision of cell fate predictions in developmental biology and disease modeling. Nevertheless, performance of state extrapolation methods is sensitive to the accuracy of neighbor selection, and the extrapolation process can be computationally demanding. Additionally, as relatively recent developments, their practical applications still remain limited. Beyond the benchmarking cases provided within original studies, these methods still require broader validation in diverse biological contexts to fully establish their reliability and generalizability.

Table 2. Summarization of RNA Velocity models.

[Table 2 insert here]

**Application of RNA Velocity under Various Biological Scenarios**

Through its unique capability of predicting future cell states by analyzing unspliced and spliced mRNA ratios, RNA velocity has provided unprecedented insights into cellular dynamics across diverse biological systems. These applications predominantly fall into three scenarios: differentiation and development, diseased and injured microenvironments, and tumor microenvironments. Specifically, in developmental biology, RNA velocity has significantly advanced our understanding of complex lineage relationships and temporal hierarchies, spanning from early embryonic development to tissue-specific differentiation. In disease research, this technique has uncovered abnormal cellular transitions and disrupted developmental trajectories, offering insights into disease progression, impaired regeneration, and key regulatory pathways that may serve as therapeutic targets. In tumor research, RNA velocity has helped reveal intratumoral heterogeneity, plasticity in cancer cell states, and dynamic interactions between tumor cells and immune populations within the microenvironment. A comprehensive summary of RNA velocity applications across these biological contexts is presented in **Table 3**, highlighting representative cases and their major findings. In the following sections, we further detail the specific roles of RNA velocity in each scenario, analyzing its contributions to developmental biology, disease research, and tumor microenvironments.

Table 3. Summarization of RNA velocity applications across biological scenarios.

[Table 3 insert here]

**Differentiation and Development**

Understanding cellular differentiation and lineage specification is a fundamental objective of single-cell omics research, as it provides crucial insights into how cells acquire distinct identities and functions across developmental stages. RNA velocity has emerged as a powerful tool in this domain, enabling researchers to reconstruct differentiation trajectories, infer cell fate decisions, and identify key transcriptional regulators driving developmental transitions. In the context of neural embryonic development, RNA velocity has enabled identification of distinct neural crest subpopulations and precise reconstruction of developmental trajectories, surpassing heuristic approaches by capturing subtle cell-state transitions [56, 57]. It has also elucidated directional lineage progression and regulatory gene dynamics during human forebrain oligodendrocyte precursor cell specification [53]. Mitic et al. utilized RNA velocity to uncover dynamic transitions of neural stem cells in the adult zebrafish telencephalon under both homeostatic and regenerative conditions [58]. Additionally, a study of human retinal development employed *MultiVelo*, integrating RNA velocity with chromatin accessibility data to refine analyses of retinal progenitor differentiation trajectories [55]. Stromal and immune cell studies leveraged RNA velocity to trace the differentiation trajectory of medullary thymic epithelial cells [59]. Similarly, Li et al. applied RNA velocity to dissect lineage relationships among human bone marrow stromal cells, identifying key regulatory genes along their differentiation paths [60]. *TopicVelo* was employed to reconstruct the differentiation trajectory from bone marrow precursors to classical NK cells, successfully capturing the lineage commitment that *scVelo* failed to resolve [51]. In studying B cells from multiple organs, *UniTVelo* confirmed the peripheral origin of thymic B cells [61]. In tissue-specific differentiation, RNA velocity has enabled identification of distinct adipocyte subpopulations and tracking of adipocyte differentiation trajectories [62]. It has also reconstructed absorptive enterocyte differentiation in the human intestine [63]. Additionally, *CellDancer* was used to trace the origin of differentiated intestinal cell types from intestinal stem cells [64]. RNA velocity has also been applied to human endometrial tissue studies. In pathological conditions, it revealed disrupted epithelial-mesenchymal transition in preeclampsia patients [65]. In normal regeneration, it showed that luminal cells exhibit high differentiation potential toward glandular cells [66]. *UniTVelo* has improved trajectory inference during pachynema progression in mouse testis spermatocytes and identified two waves of transcription in mouse visual cortex neurons [67, 68]. *LatentVelo* was used to uncover unexpected cell lineage transitions in human fetal lung development [69]. [67]Extending beyond animal systems, its application in plant development delineated shoot primordia differentiation in tomato callus [70], underscoring its versatility across biological domains. Collectively, RNA velocity has provided quantitative insights into cellular transitions, emphasizing its critical role in deciphering differentiation and developmental processes.

**Diseased and Injured Microenvironments**

Building upon insights from normal development, RNA velocity has been extensively applied to pathological conditions, providing key insights into immune system disorders, developmental disorders, tissue repair, and regeneration. In immune disorders, this technique characterized monocyte fate decisions during inflammation [71] and revealed altered developmental trajectories of monocyte and T cell subsets in systemic lupus erythematosus [72]. In developmental disorders, RNA velocity demonstrated developmental stalling and abnormal endothelial cell differentiation in preeclampsia [73], and traced dynamic cell fate trajectories during lung epithelium regeneration [74]. Integration with tools such as *veloVI* refined predictions of neuronal state transitions and identified synaptic dysfunction associated with Alzheimer's disease [75]. Extending these insights into regenerative contexts, RNA velocity dissected tissue repair mechanisms and pathological remodeling events. It revealed bidirectional plasticity between fibroblast and macrophage populations in cardiac fibrotic microenvironments [76]. RNA velocity also inferred distinct differentiation patterns between healing and non-healing diabetic foot ulcers [77]. Furthermore, it mapped macrophage differentiation trajectories following myocardial infarction, predicting terminal states and monocyte origins [78]. It also supported continuous models of fibroblast activation during wound healing [79]. In summary, these studies highlight RNA velocity’s ability to capture aberrant cellular transitions and disrupted developmental trajectories in disease contexts, offering mechanistic insights that enhance our understanding of pathophysiology and inform therapeutic strategies.

**Tumor microenvironments**

As one of the most complex and recalcitrant frontiers in disease research, tumors present unique complexities due to their dynamically evolving microenvironments—heterogeneous ecosystems in which cancer cells, immune populations, and stromal components interact intricately. RNA velocity has emerged as a crucial tool for dissecting these interactions, offering insights into immune cell dynamics, cancer cell plasticity, and therapeutic responses within tumor microenvironments. Regarding immune cell dynamics, RNA velocity has provided multiple insights. It elucidated epigenetic regulation during T cell differentiation [49] and characterized distinct differentiation trajectories of CD8+ T cells [50]. Additionally, it identified a stem-like T cell reservoir within lymph nodes that sustains anti-tumor immunity [80] and mapped neutrophil differentiation in non-small cell lung cancer [81]. In studies of cancer cell plasticity, RNA velocity has clarified cellular origins of neuroendocrine prostate cancer [82]. It also demonstrated distinct developmental pathways in colorectal polyps [83], and revealed directional progression in chronic lymphocytic leukemia within lymphoid tissues [54]. Furthermore, RNA velocity provided mechanistic insights into therapeutic responses, demonstrating how PI3Kδ inhibition disrupts regulatory T cell development while promoting inflammatory T cell subsets, informing potential dosing strategies [84]. Additional applications in tumor contexts included analysis of clonal heterogeneity and developmental diversity in primary central nervous system lymphoma [85] and identification of hypoxia-induced cell state transitions in glioma stem cells [86]. Furthermore, *Dynamo* was used to reveal distinct lineage commitment pathways across immune archetypes in a study of bone metastasis ecosystems [87]. Despite its utility, challenges remain, including the frequent absence of ancestral cells in tumor samples and mutation-induced aberrant splicing, underscoring the necessity of complementary approaches such as chromosomal aberration analysis for more robust trajectory inference [85]. Despite these limitations, RNA velocity remains instrumental in dissecting tumor evolution and immune interactions, opening avenues for novel therapeutic strategies.

In conclusion, RNA velocity has demonstrated remarkable versatility and power across developmental biology, disease research, and cancer studies. Across all these fields, it has been particularly valuable for resolving cellular hierarchies, validating developmental trajectories, and identifying key molecular drivers of cell fate decisions. As technological advances continue to address current limitations, RNA velocity analysis is poised to remain a fundamental tool in understanding cellular dynamics in both normal and pathological conditions, ultimately contributing to advances in regenerative medicine, disease treatment, and cancer therapy. Despite the emergence of various novel RNA velocity models designed to address more complex cellular dynamics, their adoption in biological research remains limited. Biologists predominantly favor established, conservative approaches. In particular, *scVelo* remains the most widely used method due to its proven versatility in trajectory inference and differentiation prediction.

Nevertheless, several studies have highlighted critical limitations in *scVelo* models [11, 12, 46, 51, 88]. These include shortcomings in both steady-state (deterministic and stochastic) and trajectory (dynamical) models when resolving developmental dynamics and predicting future cell states in complex biological systems. [11, 12, 46, 51, 88]These limitations include inadequate representation of multi-rate kinetic regimes within heterogeneous subpopulations, biases introduced by conventional data preprocessing techniques, and insufficient complexity for accurately modeling tumor cell lineages and immune transitions. Addressing these challenges through robust modeling approaches that integrate additional data modalities and rely on fewer restrictive assumptions is vital for advancing RNA velocity methodologies. These issues will be comprehensively discussed in the subsequent section.

**Discussion**

The advent of RNA velocity has revolutionized single-cell transcriptomics by enabling dynamic predictions of cellular states through the temporal interplay of spliced and unspliced mRNAs. From its foundational models (*Velocyto* and *scVelo*) to advanced frameworks integrating multi-omics data or deep learning, these methods have illuminated cellular trajectories in developmental biology, disease progression, and tumor ecosystems. By capturing transcriptional kinetics, RNA velocity has resolved lineage hierarchies, identified fate-determining genes, and even challenged traditional paradigms—such as the unidirectional evolution of leukemia cells in lymphoid tissues. Its applications span diverse biological scales, from embryonic patterning to immune cell exhaustion, solidifying its role as a cornerstone of dynamic cellular analysis.

Despite these transformative insights, critical challenges persist. The reliance on steady-state assumptions, technical noise in single-cell data, and limitations in visualizing high-dimensional dynamics often constrain the accuracy and generalizability of RNA velocity predictions， particularly in systems with transient transcriptional states or heterogeneous kinetics. Conventional workflows may inadvertently obscure biological signals through preprocessing steps, while projections onto low-dimensional embeddings risk oversimplifying complex trajectories. These limitations highlight the need for methodological refinement and integrative validation. Here we synthesize recent advancements addressing these challenges, discuss extensions of RNA velocity beyond splicing kinetics, and propose practical guidelines to enhance the utility of RNA velocity in both basic research and clinical translation.

**Advancements in splicing-kinetic-based RNA velocity models**

By capturing the dynamics of splicing process, RNA velocity methods predict future transcriptional states of individual cells, facilitating the reconstruction of differentiation trajectories, elucidation of cellular heterogeneity, and enhanced understanding of disease progression mechanisms. Recent advances have expanded splicing-kinetic RNA velocity frameworks to broader biological contexts, including generalized cell dynamics and gene regulatory investigations. *Protaccel* [89] extends the RNA velocity paradigm by incorporating protein-level dynamic into steady-state methods, enabling the prediction of protein production rates directly from transcriptomic data. This approach enriches the interpretation of cellular state transitions by combining estimates of unprocessed transcripts and protein abundances to project future cell states. *SIRV* [90] integrates spatial transcriptomics data with traditional RNA velocity by aligning spatial information and spliced/unspliced expression matrices through domain adaptation. By employing spatial data as a reference, this method calculates RNA velocities within a spatial context, enabling the reconstruction of tissue-specific differentiation dynamics. *DeepKINET* [91], contrasting with typical trajectory methods, treats RNA velocity as a latent variable within a deep learning model to estimate splicing and degradation rates at single-cell resolution. It aims to reveal the heterogeneity in splicing and degradation rates across cells, thereby elucidating post-transcriptional regulatory mechanisms mediated by factors such as RNA-binding proteins.

RNA velocity methodologies have also been adapted specifically for studying cell-cycle dynamics, offering unique insights into temporal regulatory mechanisms. *DeepCycle* [92] infers RNA velocity by using an autoencoder (AE) framework to model the complex spliced-to-unspliced RNA relationship throughout the cell cycle, introducing a continuous latent variable termed “transcriptional phase” to characterize the periodic progression of cells. *VeloCycle* [93], applies manifold-constrained modeling to infer cell-cycle velocities, constraining velocity estimates onto a cell-cycle-specific manifold, thus revealing differences in cell-cycle progression rates among diverse cell types and physiological conditions. These specific extensions of RNA velocity allow for a deeper understanding of cell cycle regulation, modulations in cell cycle speed, and the dynamic control of cell cycle-related genes at the single-cell level.

Disregarding strictly splicing kinetics-based methods, several tools have been designed to infer cellular dynamics from expression profiles at the single-cell level by incorporating auxiliary information. For example, a generalized model of *Dynamo* [17] exclusively reliant on metabolic labeling to infer total RNA velocity, bypassing explicit consideration of splicing dynamics. This method extends the steady-state paradigm of *Velocyto* by positing stable relationships between newly labeled and total RNA pools under steady-state expression assumptions. *TFvelo* [94] recovers gene-specific cellular dynamics by examining transcription factor (TF) expression profiles rather than relying solely on splicing information. It postulates a characteristic clockwise trajectory in the joint expression space between TFs and their targets, mirroring the trajectory inferred from spliced and unspliced RNAs, thereby enabling analogous computational solutions. Similarly, *scKINETICS* [95] cellular phenotype transitions via a system of dynamic equations driven by gene expression regulators within genome-wide gene regulatory networks (GRNs), further broadening the applicability of velocity analyses beyond conventional splicing kinetics.

**Current challenges and towards the better practice**

Several recent reviews [11, 12, 32, 46, 96] have comprehensive discussed critical challenges that lead to failures in capturing transcriptional dynamics through RNA velocity methods, particularly regarding two conventional tools: *Velocyto* and *scVelo*. These studies highlight that many models rely on simplifying assumptions of transcriptional dynamics, such as steady-state kinetics or constant kinetic rates. However, these assumptions often do not hold in biological systems with complex transcriptional kinetics, result in incorrect inference of full transcriptional dynamics [46]. Some genes exhibit multiple kinetic regimes, such as transcriptional regulation within specific cell subpopulations (e.g. transcriptional boost) [88, 97] or lineage-dependent kinetics patterns [8, 10]. This manifests as genes that display multiple trajectories and secondary boost of induction in phase space. Steady-state methods, which infer kinetics that only presented in steady-state populations by using linear regression, struggle to resolve dynamic in heterogeneous subpopulations that deviate from steady-state. Methods such as *TopicVelo* relaxes this restriction by identifying distinct biological processes and separately modeling dynamics for each process.

Trajectory methods assign cell time and concurrently fit phase trajectories to solve the full dynamics of transcription. The dynamical model of *scVelo*, *veloVI*, and *Pyro-Velocity*, formulate transcriptional ODEs in a step-wise manner, where cells are assigned to transient states of induction and repression and two steady states (active and inactive). The induction and repression stages of the phase trajectory are fitted using non-steady-state cells, thereby partially reducing the interference from heterogeneous steady-state cells, which are often composed of highly differentiated mature subpopulations. However, stepwise trajectory methods suffer from limitations in capturing the complex kinetics within induction and repression stages. To address this, the dynamical model of *MultiVelo* extends transcriptional ODEs by incorporating chromatin accessibility, enabling it to model time-varying transcriptional rates. *veloAVE* and an extension of *veloVI* estimate lineage-dependent or time-dependent transcription rates directly from expression profile. *veloAVE* further utilize the inferred kinetic parameters to construct cell type transition graphs and fit branching ODEs tailored to bifurcating cell populations. *LatentVelo* reformulates transcriptional dynamics into structured neural ODEs and captures latent dynamics in a latent embedding of cell states. By modeling state-dependent transcriptional regulation, this approach effectively estimates complex lineage- and time-dependent kinetic rates. *cell2fate* employs a modular approach, disentangling cellular dynamics into multiple modules and modeling time-dependent transcription rates as a linear combination of these modules.

State extrapolation methods integrate inferred RNA velocity into dynamic learning by inferring cell-specific kinetics from neighboring cells rather than fitting global dynamics. Specifically, *cellDancer* infers cell-specific kinetics by estimating the unspliced and spliced velocities in phase space, whereas *DeepVelo* and *SymVelo* estimate velocities within the high-dimensional gene expression space, such as the high-dimensional spliced space. By accurately identifying the expected future state of each cell as the ground truth, these methods are able to capturing subtle kinetic variations within heterogeneous populations and across multiple lineages.

Another significant challenge arises from insufficient observations of transcriptional dynamics within specific subpopulations, posing challenges for the accurate estimation of RNA velocity [46]. This arises when a gene is active only during a brief window of the observed process, such as monotonous upregulation at the end or downregulation at the beginning of a developmental process. For steady-state methods, the lack of observations in steady-state populations violates the basic steady-state assumptions, leading to inaccuracies in linear regression. Moreover, incomplete dynamics often result in cells in phase space being distributed along a straight line rather than a curve. Trajectory methods, such as *scVelo*, therefore struggle to determine whether a trajectory is in the upregulation or downregulation phase. To overcome this challenge, *MultiVelo* incorporates chromatin accessibility by incorporating open or closed states of chromatin to infer transcriptional states. Methods like *UniTVelo* assign a unified cell time and aggregate dynamic information across all genes to enhance the identification of transcriptional states for genes with incomplete dynamics. The use of unified cell time also helps mitigate overfitting caused by high technical noise and the complexity of gene activities. *Dynamo* further refines this approach by directly extracting cellular real-time information from metabolic labeling data. State extrapolation methods infer local kinetics without relying on complete dynamics, thus enabling them to robustly handle cases of partial observation of dynamics.

Conventional data pre-processing procedures (**Figure 1 A-C**) may significantly disorder model inference and downstream dynamics visualization. The standard RNA velocity workflow begins by distinguishing unspliced and spliced matrices from raw data. This binary classification of transcripts overlooks potential transient and terminal isoforms of RNAs arising from alternative splicing mechanisms [11]. Such informative ambient RNAs can be detected by quantification tools, yet they are excluded in most methods, except for *cell2fate*. Moreover, quantified count matrices comprise substantial noise arising from the low-copy number regime in single-cell RNA synthesis, as well as technical noise inherent in scRNA-seq measurements [11, 46, 98]. Conventional RNA velocity methods address these issues by employing cell size normalization and KNN smoothing to remove noise, while also filtering for high-quality cells and highly variable genes to facilitate kinetics prediction and velocity inference. Methods such as *VeloAE*, *LatentVelo*, and *SymVelo* further denoise data by encoding unspliced and spliced counts into a low-dimensional space to infer latent dynamics. However, the noise in single-cell data is presumed to be informative and aligns with the discrete stochastic nature of gene expression [99, 100]. Count normalization may diminish the interpretability of the discrete data [11, 101, 102]. Nearest neighbor imputation has been mathematically demonstrated to introduce model-agnostic corrections, which can lead to distortions in high-dimensional RNA velocity and stream plot visualizations [11, 46]. Recognizing these limitations and the informative potential of unprocessed data, methods such as *Pyro-Velocity*, *cell2fate*, and *TopicVelo* directly leverage unprocessed discrete raw counts for dynamics inference. By doing so, these approaches aim to avoid the aforementioned pitfalls, diminish the reliance on *ad hoc* parameter tuning in data pre-processing, and model the inherent stochasticity of gene expression more faithfully.

The common practice of projecting RNA velocity vectors onto low-dimensional embeddings for visualization may obscure meaningful dynamics or introduce artifacts that do not accurately reflect the underlying biology [11, 46]. Both local neighborhoods and the global topology heavily depend on the KNN neighbor graph, which is susceptible to noise and lacks interpretability. Therefore, when exploring cellular developmental trajectories or potential dynamics, it is advisable to rely on the analytical tools provided by RNA velocity methods or the latent processes governing transcriptional variation. For example, kinetics of identified driver genes can be used to resolve underlying dominant dynamics. The uncertainty of velocity and latent variables can be quantified to assess the robustness of dynamics during cell development. Moreover, methods such as *LatentVelo* not only infer latent dynamics but also jointly learn the latent unspliced and spliced states. This dynamics-informed embedding enables stream plots to more accurately represent cellular developmental trajectories.

In practical applications of RNA velocity analysis, several key considerations and recommendations can ensure robust and insightful results. Firstly, it is essential to critically assess whether the single-cell data and the biological context under investigation are suitable for RNA velocity analysis. For instance, caution should be exercised when analyzing samples characterized by high heterogeneity, such as tumors, or samples displaying subtle or indistinct transcriptional dynamics. In such scenarios, employing RNA velocity tools capable of quantifying uncertainty or robustness, such as Bayesian or variational inference-based models (e.g., *veloVI*, *cell2fate*, *Pyro-Velocity*), can offer valuable reliability assessments and inform decision-making regarding the appropriateness of further analysis. Secondly, tool selection plays a pivotal role in RNA velocity studies. Traditional methods, notably *scVelo*, have demonstrated limitations and biases under certain conditions, emphasizing the need to consider advanced computational approaches. Depending on the research goals and prior biological knowledge (e.g., known cell-cycle dynamics, branching trajectories, or lineage-specific transcriptional kinetics), researchers are encouraged to evaluate multiple state-of-the-art RNA velocity models, including trajectory-based methods (e.g., *MultiVelo*, *LatentVelo*, *VeloVAE*) and state extrapolation methods (e.g., *cellDancer*, *DeepVelo*, *SymVelo*), to ensure the accuracy and relevance of inferred cellular trajectories. Thirdly, caution is advised when interpreting developmental trajectories derived from RNA velocity. Rather than solely relying on two-dimensional visualization techniques such as streamline plots or embedding-based velocity representations, it is recommended to integrate additional analytical strategies provided by RNA velocity tools. For example, examining gene-specific phase portraits, identifying driver genes through kinetic parameters, and utilizing downstream analyses like latent-time inference, cell-fate prediction, or uncertainty quantification can greatly enhance biological interpretation. Finally, continuous validation through independent experimental evidence or orthogonal bioinformatics analyses, such as lineage tracing experiments, multi-omic data integration, or validation of identified regulatory mechanisms, is highly beneficial to substantiate RNA velocity results, thereby solidifying its biological insights and facilitating its broader acceptance in biological research.

**Key Points**

1. This review systematically categorizes RNA velocity computational tools into three paradigms: steady-state, trajectory-based, and state extrapolation methods, outlining their assumptions, modeling strategies, and kinetic inference approaches.
2. The article provides a comprehensive comparison of representative RNA velocity models, highlighting their innovations, technical frameworks, and how they handle cell-specific transcriptional dynamics.
3. Practical guidance is offered for the selection and application of RNA velocity methods, including discussions of preprocessing pitfalls, model limitations, and trajectory visualization artifacts.
4. A detailed summary of biological applications is presented, demonstrating how RNA velocity has been used to study cellular differentiation, immune regulation, disease progression, and tumor microenvironmental dynamics.
5. The review outlines future directions and current challenges, emphasizing the need for robust inference under heterogeneous kinetics, integrative multi-omics modeling, and broader validation in complex biological systems.

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**Data availability**

Not applicable.

**Code availability**

Not applicable.

**Authors’ contributions**

Y.W. conducted the literature review and contributed to all aspects of manuscript preparation, including conceptualization, analysis, writing, and editing. J.L. contributed to the writing of the *Background* and *Applications* sections and participated in manuscript review. H.Z. contributed to the writing of the *Methods* section. S.L. was responsible for figure generation and graphical illustrations. D.H. contributed to manuscript review and provided supervision. L.F. provided funding support. X.L. supervised the project, contributed to manuscript review, and provided overall guidance throughout the study. All authors read and approved the final manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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