scMultiSim: simulation of multi-modality single cell data guided by cell-cell interactions and gene regulatory networks

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Simulated single-cell data is widely used to aid in designing and benchmarking computational methods due to the scarcity of experimental ground truth. Recently, an increasing number of computational methods have been developed to address various computational problems with single-cell data, including cell clustering, trajectory inference, integration of data from multiple batches and modalities, inference of gene regulatory networks (GRNs) and cell-cell interactions (CCIs). Simulators that are designed to test a certain computational problem model only the particular factors that affect the output data; whereas modelling as many biological factors as possible into the simulation allows the generated data to have realistic complexity and can be used to benchmark a wide range of computational methods.

Here, we present scMultiSim, an *in silico* simulator that generates multi-modality data of single cells, including gene expression, chromatin accessibility, RNA velocity, and spatial cell locations while accounting for the relationships between modalities. We proposed a unified framework to jointly model biological factors including cell identity, CCIs, with-in-cell GRNs and chromatin accessibility, so all their effects simultaneously present in the output data. Users enjoy unparalleled flexibility by having full control of the cell population and the ability to fine-tune each factor's effect on the underlying model. We also provide options to simulate technical variations including batch effects to make the output resemble real data. We verified the simulated biological effects and demonstrated scMultiSim's applications by benchmarking computational tasks on single-cell single-omics or multi-omics data: cell clustering and trajectory inference, multi-modal and multi-batch data integration, RNA velocity estimation, GRN inference and analysis of cell-cell interaction using spatially resolved gene expression data. To our knowledge, scMultiSim is the only simulator of single cell data that can perform benchmarking for all these four challenging computational tasks.

Introduction

In recent years, technologies which profile the transcriptome and other modalities (multi-omics) of single cells have brought remarkable advances in our understanding of cellular mechanisms [63]. For example, technologies were developed to profile the chromatin accessibility jointly with the gene expression data [10; 8; 42]; abundance of surface proteins can also be profiled together with the transcriptome [58; 49]; in addition, spatial locations of cells can be measured together with transcriptome profiles using imaging based technologies [54; 19; 65] or sequencing-based [57; 52] technologies.

The multi-omics data of single cells allow researchers to study the state of cells in a more comprehensive manner, and more importantly, to explore the relationships between modalities and the causality across hierarchies [18]. Computational methods have been developed to exploit the single cell multi-omics and spatial omics data, including data integration methods [60; 66; 1; 72; 37], and methods to infer cell-cell interactions (CCIs) [5; 6; 55; 4; 43].

Before the availability of single cell multi-omics data, using only single cell RNA-sequencing (scRNA-seq) data, methods were developed to infer gene regulatory networks (GRNs) [50]. Most of these methods consider the transcription factors (TFs) as the only factor that affects the observed gene-expression data. However, the observed gene-expression data is affected by multiple factors, one of which is the chromatin accessibility of the corresponding regions for a target gene. Therefore, new methods have been developed to use both scRNA-seq and scATAC-seq data to infer GRNs [31; 64; 70]. Similarly, methods for CCI inference include those using only scRNA-seq data [4; 27; 30] and the ones that use both the gene expression and the spatial location modalities [16; 55; 5; 6]. Finally, using scRNA-seq data, RNA velocity can be inferred from the unspliced and spliced counts to indicate the state of each cell in the near future [36; 3]. Recently, methods were proposed to infer RNA velocity from jointly profiled chromatin accessibility and transcriptomics data [39].

Overall, a large number of computational methods have been developed using scRNA-seq data or single cell multi- and spatial-omics data [68]. However, the scarcity of *ground truth* in experimental data makes it difficult to evaluate the performance of proposed computational methods. Therefore, *de novo* simulators, which generate data by modeling biological mechanisms and provide ground truth for method benchmarking, have been widely used to evaluate the accuracy of computational methods. For example, SymSim [71] provides ground truth cell identity and gene identity and thus can benchmark clustering, trajectory inference and differential expression detection; SERGIO [14], BEELINE [50] and dyngen [7] can simulate scRNA-seq data with given ground truth GRNs; SERGIO, dyngen and VeloSim [73] can provide ground truth RNA velocity for testing RNA velocity inference methods. mistyR [62] generates single cell gene expression data from a given CCI network and can test CCI inference methods. With the *de novo* simulators, users can easily control input parameters and obtain the exact ground truth. Crowell *et al* [11] also discussed another category of single cell data simulators, named the reference-based methods, which learn a generative model from a given real dataset and generate synthetic

data [12; 61; 56; 2]. By design, these methods can output datasets that mimic the input reference data, but their flexibility can be limited by the specific reference dataset. These methods can provide ground truth cluster labels by using annotations in the reference dataset or pre-determining them when running the simulation, but none of the reference-based methods provides ground truth information that are rarely available via domain knowledge like GRNs, CCIs, or RNA velocity.

We consider that a desirable single cell simulator should: (1) generate as many modalities as possible so as to best represent a cell; (2) models as many biological factors that affect the output data as possible so that the output data has realistic complexity; (3) provides ground truth of the biological factors for the purpose of benchmarking various computational methods. Most of existing simulators generate only scRNA-seq data, and some generate only scATAC-seq data [46; 38]. Simulators that can generate more than one modality include dyngen and SERGIO (that output unspliced and spliced counts with ground truth RNA velocity), as well as a reference-based simulator scDesign3 [56], which can generate two modalities each with high dimensionality (*eg.* scRNA-seq and DNA methylation data), or one high-dimensional modality (*eg.* scRNA-seq) and spatial location data, depending on the input reference dataset (Table S1).

In terms of the biological factors modeled in the simulator, existing *de novo* simulators model only one or a small subset of the following biological factors: cell identity (cluster labels or positions on cell trajectories), chromatin accessibility, GRNs, spatial cell locations and cell-cell interactions (Table S1). Data generated by reference-based simulators can inherently have these effects but it is challenging to obtain the ground truth of the biological factors for these simulators.

In this paper, we present scMultiSim, a unified framework where *all* the above biological factors are modelled, and it also models technical noise including batch effects (Fig. 1a). For each single cell, it outputs the following modalities: unspliced and spliced mRNA counts, chromatin accessibility and spatial location, while considering the cross-modality relationships. It provides ground truth cell identity, RNA velocity, GRNs and CCIs, as well as relationships between chromatin accessibility and transcriptome data. Therefore, it can be used to evaluate methods for the following computational tasks with the same dataset: clustering or trajectory inference, multi-modal and multi-batch data integration, RNA velocity estimation, GRN inference and CCI inference (Table S1). When testing methods for a specific task, users can easily tune the effect of each biological factor on the output data to investigate how the performance of the methods are affected by each factor. scMultiSim is the most versatile simulator to date in terms of its benchmarking applications. Thanks to its flexible design, some extended functions can be easily added, *eg*, the GRNs can be made into cell-type-specific or cell-specific GRNs.

In the following, we first briefly introduce the core concepts and the simulation process of scMultiSim, then demonstrate its capability of simulating various biological factors simultaneously by validating each of their effects in the data. Finally, we showcase the applications of scMultiSim by benchmarking a wide variety of computational tools for the tasks mentioned above.

Results

scMultiSim overview

The kinetic model and control of intrinsic noise. In general, scMultiSim runs the simulation in two phases (Fig. 1b). The first is to generate the true gene expression levels in cells ("true counts"); then, we add technical noise such as library preparation noise and batch effects to get scRNA-seq and scATAC-seq data that is statistically comparable to real data ("observed counts"). In the first phase, we use the widely-accepted kinetic model [48] to simulate gene expression dynamics and generate the RNA velocity. scMultiSim models the cellular heterogeneity and stochasticity of gene regulation effects through a mechanism with two main concepts: Cell Identity Factors (CIFs) and Gene Identity Vectors (GIVs); it is used to generate both the scATAC-seq data and prepare the parameters for the kinetic model. We also expand this mechanism to incorporate GRNs or cell-specific GRNs and spatial cell-cell interactions.

scMultiSim extends the idea of SymSim [71] when simulating the gene expression. The kinetic model has four parameters for each gene in each cell, namely k_{on}, k_{off}, s and d. A gene can switch between *on* and *off* states, where k_{on} and k_{off} are the rates of becoming *on* and *off*. When a gene is in the *on* state (which can be interpreted as promoter activation), mRNAs are synthesized at rate s and degrade at a rate d. It is common to fix d at 1 and use the relative values for the other three parameters [45], therefore we need to model the remaining three parameters for each gene in each cell. The central part of our method will focus on encoding the heterogeneity and complex regulation effects in cells and genes into the kinetic parameters, producing the k_{on}, k_{off} and s parameters for each cell-gene combination, resulting in a cell$\times$gene matrix for each parameter.

Under the kinetic model, we provide two modes to generate true counts from the parameters (Fig. 1b): (1) the full kinetic model, where we explicitly let genes undergo several cell cycles with state changes, and the spliced/unspliced RNA counts are calculated (Methods). RNA velocity ground truth is also produced in this mode since the RNA synthesize rate is known; (2) the Beta-Poisson model, which is equivalent to the kinetic model's master equation [32] (Methods). When RNA velocity is unneeded, users can switch to the Beta-Poisson model for faster running time. We also introduce an intrinsic noise parameter σ_i that controls the amount of intrinsic noise caused by the transcriptional burst and the snapshot nature of scRNA-seq data (Methods). This parameter allows users to investigate the effect of intrinsic noise on the performance of the computational methods.

Modeling cellular heterogeneity and various biological effects. Compared to existing simulators, scMultiSim provides unprecedented flexibility to users that (i) encodes various types of biological factors simultaneously, including cell population, chromatin accessibility, GRN and cell-cell interaction, (ii) provides easy ways to adjust the effect of each factor, and (ii) produces arbitrary user-defined trajectories in the simulated data while maintaining all the biological effects. In order to model all these factors, we introduce the main concepts of our mechanism: *Cell Identity Factors (CIF)* and *Gene Identity Vectors (GIV)*, which are low-dimensional

representations of cells and genes (Fig. 2a).

The CIF of a cell is a 1D vector representing various biological factors that contributes to the cellular heterogeneity, such as concentrations of proteins and morphological properties. While the inherent noise in the transcription process is captured by the kinetic model, the CIF captures the extrinsic variation that controls the expression pattern, which is similar to the EVF concept in SymSim [71].

The values in the GIV of a gene act as the weights of the corresponding factors in the CIF, *i.e.*, how strong the corresponding CIF can affect the gene (Fig. 2a). For example, a higher value in the k_{on} GIV vector $\text{giv}_i^{k_{on}}$ can be interpreted as: higher concentration of the corresponding cell factor $\text{cif}_i^{k_{on}}$ causes a higher activation rate for the gene's promoter. In short, CIF encodes the *cell identity*, while GIV encodes the *strength of biological effects*. Therefore, by multiplying the CIF and GIV matrix, we are able to get a $n_{\text{cell}} \times n_{\text{gene}}$ matrix, which is the desired kinetic parameter matrix with the cell and gene effects encoded.

The biological factors including the underlying cell trajectories or clusters, GRNs and cell-cell interactions (CCIs) that affect the scRNA-seq data output are encoded in the CIF and the DE genes, target genes in the GRN and CCI networks are encoded in the GIVs. A CIF vector can be divided further into four segments (Fig. 2a), each represents one type of extrinsic variation:

(i) Non-differential CIFs (**non-diff-CIF**) model the inherent cellular heterogeneity. They represent various environmental factors or conditions that are shared across all cells and are sampled from a Gaussian distribution with standard deviation σ_{cif}.

(ii) Differential CIFs (**diff-CIF**) control the user-desired cell population. These are the biological conditions that are unique to certain cell types. These factors lead to different cell types in the data. For a heterogeneous cell population, cells have different development statuses and types. Values for diff-CIFs are used to represent these cell differential factors, which are generated based on the user-input cell differential tree. When generating data for cells from more than one cell types, the minimal user input of scMultiSim is the cell differential tree, which controls the cell types (for discrete population) or trajectories (for continuous population) in the output. The process of generating diff-CIFs is described in Methods.

(iii) CIFs corresponding to Transcription Factors (**tf-CIF**) control the GRN effects. This segment, together with the TF segment in the GIV, model how a TF can affect expression of genes in the cell (Methods). Its length equals to the number of TFs. In other words, the GRN is encoded in the TF part of CIFs and GIVs.

(iv) CIFs corresponding to ligands (**lig-CIF**) from neighboring cells control the effect of CCI. If CCI simulation is enabled, this segment models effects from ligands of a neighbor cell. The "ligand" segments in the CIF and GIV together encode the given CCIs, using a similar method as the GRN. However, now the cell's receptor is affected by ligands of multiple neighbors (Fig. 2a). We also generate the corresponding GIV matrices carefully considering the nature of the kinetic model (Methods).

The simulation process. Fig. 1b shows an overview of the simulation process. First, as we consider that the chromatin accessibility of a cell affects its gene expression, we generate scATAC-seq data before generating scRNA-seq data. The scATAC-seq data also follows a pre-defined clustering or trajectory structure, which can be pre-defined using the same tree as the one used for the scRNA-seq data, or a different tree if needed. Details on generating the scATAC-seq data is included in Methods.

Afterwards, scRNA-seq data can be generated in different modes: with or without CCIs and spatial locations, and with or without outputting RNA velocity data. In all modes, CIF and GIV matrices are used to generate the three kinetic parameters for each gene in each cell. The scATAC-seq data affects scRNA-seq data through the k_{on} parameter, because chromatin accessibility controls the activated status of genes (Methods).

If users specify to generate RNA velocity, the full kinetic model is used (as opposed to the Beta-Poisson model), cells undergo several cycles before the spliced and unspliced counts are outputted. In each cycle, the cell may switch between on/off state with probabilities determined by the parameters (Methods). Otherwise, if the Beta-Poisson model was used, the true counts are simulated according to the equations. No RNA velocity and unspliced count data is outputted in this mode.

Simulating cell-cell interaction. If it is specified to generate spatial-aware single cell gene expression data including cell spatial locations and CCI effects, scMultiSim uses a multiple-step approach that considers both time and space – as shown in Fig. 1b "Spatial CCI mode". Each layer corresponds to a time point. Cells are placed in a grid as described before, and one cell is added to the grid at each step, representing a newborn cell. Strategies are designed to ensure cells have a similar number of neighbors in the grid, and similar cell types are closely located (Methods). We output data of cells at the final time point, which contains the accumulated CCI effects during the cells' developmental process.

The minimal user input for simulating CCI is a list of ligand-receptor pairs. scMultiSim can provide ground truth CCIs both at the single cell level and at the cell type level. It is considered that CCIs between certain cell types generally happen more than CCIs between single cells of the same cell type, and this is modeled in scMultiSim. User can choose to input the ground truth specifying whether a ligand-receptor pair is enabled between two cell types to further control the behavior.

Extended functions. In addition to the standard functions of scMultiSim, it can incorporate extended functions that consider more realistic scenarios in biology. (1) The GRN can be set to a cell-specific and cell-type-specific mode. In this mode, random GRN edges are generated or deleted gradually along the pseudotime at a user-controlled speed. When simulating each cell, the tf-GIV will be filled with the current GRN effect matrix. The cell-specific GRN ground truth is outputted in this mode. (2) The scATAC-seq data and the scRNA-seq data can follow different trajectory or clustering structures. (3) The cell clusters can form less regular shapes than the current convex shapes.

Technical variations and batch effects. The steps described above generate "true" mRNA counts (and unspliced counts if RNA velocity mode is enabled) in the cells. As the second phase, scMultiSim simulates key experimental steps in wetlabs that lead to technical noises in the data and output observed scRNA-seq data. scMultiSim also adds batch effects and can output datasets from a specified number of batches. Users can control the amount of technical noise and batch effects between batches. These procedures are described in Methods. Next, we show the various output of scMultiSim and validate the effects present in the simulated data.

Design of simulation and datasets

We have generated a variety of datasets using different parameter configurations to show the effects in the data and to benchmark computational methods. These datasets contain *main datasets* and *auxiliary datasets*. The main datasets are 144 datasets with different configurations of $\sigma_{\text{cif}} \in \{0.1, 0.5\}$, $n_{\text{cell}} \in \{500, 800\}$, and $n_{\text{gene}} \in \{110, 200, 500\}$. They contain all effects scMultiSim can simulate, including GRN, chromatin accessibility, cell-cell interaction, technical noise and batch effect. The two σ_{cif} values, 0.1 and 0.5 respectively, generates relatively clear and noisy trajectory in the gene expression space with the above settings. Therefore, these 144 main datasets enclose a wide range of variety that covers different numbers of cells, genes and trajectory shapes to alleviate possible bias.

As shown in Table 1, we label the main datasets in the following format: `M{p}{c}{s}`; where the first letter M denotes the main dataset, then a letter $p \in \{L, T, D\}$ specifying the cell population: linear trajectory, tree trajectory or discrete. Next, a number $c \in [1, 12]$ indicates one configuration of σ_{cif}, n_{cell}, and n_{gene}. Finally, a lowercase latter $s \in \{a, b, c, d\}$ represents random seed 1-4. For example, dataset `MD5c` uses a discrete cell population, $\sigma_{\text{cif}} = 0.1$, 800 cells, 200 genes and random seed 3.

To further explore the effect of other parameters or to be compatible with some computational methods imposing constrains on the input, we also generated several auxiliary datasets (Table 2), which contains fewer types of effect. In the remaining article, we will primarily use the main datasets `M` for demonstration and benchmarking, and auxiliary datasets to show additional and supplementary results.

scMultiSim generates multi-batch and multi-modality data from pre-defined clustering or trajectories

A major advantage of scMultiSim is its ability to generate coupled scRNA-seq and scATAC-seq data from user designated clustering or trajectories. It provides parameters to control the structure of cell populations. First, the user can choose to generate "continuous" or "discrete" populations, and input a tree that represent the cell trajectories (in the case of "continuous" populations) or relationship between clusters (in the case of "discrete" populations). We name this tree the "differentiation tree", and an example is shown in Fig. 2b. Then, users can use these three parameters: intrinsic noise σ_i, CIF sigma σ_{cif} and Diff-to-nonDiff CIF ratio r_d, to control how clean or noisy the population structure is in the data (Fig. 2c-e). scMultiSim provides three example trees:

Phyla1, Phyla3 and Phyla5, each having 1, 3, and 5 leaves as shown in Fig. 2b. The main datasets were simulated using these trees (Table 1); From the differentiation tree, scMultiSim generates both discrete and continuous cell populations (Fig. 2c).

For the continuous population, we visualize a dataset `MT3a` generated using tree Phyla3 in Fig. 2c. We can observe that the trajectories corresponding to the input differentiation tree are clearly visible, for both the scRNA-seq and the scATAC-seq modality. For the discrete population, we visualize dataset `MD3a` and `MD9a` generated with tree Phyla5 in Fig. 2d. The parameter σ_{cif} controls the standard deviation of the CIF, therefore with a smaller σ_{cif}, the clusters are tighter and better separated from each other. We then used the auxiliary dataset `A` (Table 2) to explore the effect of the intrinsic noise parameter σ_i and Diff-to-nonDiff CIF ratio r_d (Fig. 2e). The results were generated using Phyla5 continuous mode using the same CIF sigma σ_{cif}. With a smaller Diff-to-nonDiff CIF ratio r_d, the trajectory is vague and more randomness is introduced, as the tree structure is encoded in the differential CIFs. With a smaller intrinsic noise σ_i, a fraction of the expression value is directly calculated from kinetic parameters without sampling from the Poisson model. As a result, the trajectory is more prominent. These patterns are much cleaner than real data because real data always has intrinsic noise.

Coupling between scATAC-seq and scRNA-seq data. In paired scATAC-seq and scRNA-seq data, these two data modalities are not independent of each other as it is commonly considered that a gene's expression level is affected by the chromatin accessibility of the corresponding regions, and if a gene's associated regions are accessible, this gene is more likely to be expressed. This mechanism can be naturally modeled in scMultiSim through the kinetic parameter k_{on} (Methods).

We provide a user-adjustable parameter, the ATAC-effect E_a, to control the extent of scATAC-seq data on k_{on}. In order to validate the connection between the scATAC-seq and scRNA-seq data, we calculate the mean Spearman correlation between these two modalities for each gene. Since one gene can be controlled by one or multiple chromatin regions in scMultiSim, the effect is complicated and the correlation is less prominent. In Fig. 2f, we show the correlations under different E_a values (ranges between 0 and 1). An averaged 0.2-0.3 correlation can be observed using the default value (0.5), and the correlation increases with E_a. This results show that the connection between scATAC-seq and scRNA-seq data is modeled in the single cell multi-omics data generated by scMultiSim.

scMultiSim simulates technical noise and batch effect. The single cell gene expression data shown in Figs. 2c,d,f are "true" mRNA counts which do not have technical noise. scMultiSim adds technical noise including batch effects to the true counts to obtain observed counts (Methods). The amount of technical noise and batch effects can be set through parameters. A parameter E_{batch} can adjust the amount of batch effect and users can specify the number of batches.

Fig. 2g shows the observed mRNA counts of dataset `MD9a` (true counts shown in Fig. 2d). The left plot corresponds to number of batches equal to one, and the right plot correspond to number of batches being two.

Technical noise and batch effects are also added to the scATAC-seq matrix. We further use auxiliary dataset `A` to verify that the amount of technical noise and batch effect can be adjusted on both scRNA-seq and scATAC-seq modalities, in terms of both continuous and discrete populations (Fig. S2). The main parameter for technical noise we vary here is α, which denotes the capture efficiency that affects the detection ability of the dataset, and lower α corresponds to worse data quality.

scMultiSim generates RNA velocity data

If RNA velocity simulation is enabled, the kinetic model outputs the velocity ground truth using the RNA splicing and degradation rates. The Phyla5 tree in Fig. 2b is used to generate the results in Fig. 2g. Fig. 2g shows both the true spliced and unspliced counts, and the RNA velocity averaged by kNN, which can be used to benchmark RNA velocity estimation methods. The velocity vectors follows the cell trajectory (backbone and directions shown in red), which is specified using the user-inputted differentiation tree.

scMultiSim generates single cell gene expression data driven by GRNs and cell-cell interactions

The strength of scMultiSim also resides in its ability to incorporate the effect of GRN and CCI while preserving the pre-defined trajectory structures. In this section, we show that the GRN and CCI effects both exist in the simulated expression data. The main datasets (Table 1) used the 100-gene GRN from [15] as the ground truth GRN, which is visualized in Fig. 3a. Cross-cell CCIs are also incorporated during the simulation. The CCIs are connected to the within-cell GRNs by adding ligand-receptor pairs from gene 99,101-104 to gene 91, 2, 6, 10 (TFs), and 8 (non-TF) in the GRN (Fig. 3a). The same GRN is used in all the cells for the main datasets, but each cell can also use cell-specific GRNs when the user specifies the option *dynamic.GRN*. Next, we used one dataset (`MT3a`, with a tree trajectory, 500 genes, 500 cells, $\sigma_{\text{cif}} = 0.1$) to inspect the simulated effects in details (Fig. 3b-e).

GRN guided expression data. We illustrate the gene regulation effects for dataset `MT3a` using a gene module correlation heatmap as shown in Fig.3b. The clustered heatmap is generated using Spearman correlations between each pair of regulated genes. Each color on the top or left side represents a TF in the GRN. The figure clearly shows that gene modules regulated by the same TF (genes with the same color) tend to be grouped together, while having higher correlations with each other. Thus, the heatmap suggests that the effect of GRN presents in the expression data. We also visualize the expression of a particular regulator-target pair (gene 19-20) along one lineage (4-5-3) in Fig. 3c. Correlation between the regulator and target RNA expression can be immediately observed. Furthermore, we plot the accessibility levels for the corresponding chromatin of gene 19 in Fig.3c, where significant drops in gene 19's expression occur when the related chromatin region is closed.

Cell spatial locations. scMultiSim provides convenient helper methods to visualize the cell spatial locations as in Fig. 3d (dataset `MT3a`). For each ligand-receptor pair, arrows can be displayed between cells to show the

direction of cell-cell interactions. Multiple strategies are designed in scMultiSim to place the cells in the grid; the default strategy used here is that a newborn cell has a higher probability p_t of staying with a cell of the same type. Therefore, small cell clusters can be observed in the final result, but overall different cell types still mix well to enable more interactions between cell types. In real data, how likely cells from the same cell type locate together depends on the tissue type, and scMultiSim provides a probability parameter to tune this pattern. Strong spatial cluster patterns can be observed in the left figure in Fig. 3f, which was generated using $p_t = 1$. The right figure in Fig. 3f uses $p_t = 0.8$, where cells from the same cell type are more spread out in the grid.

Correlations between cells with CCI. scMultiSim generates CCIs between single cells as well as between cell types. We validate the simulated CCI effects by comparing the correlations between (i) neighboring cells with CCI, (ii) neighboring cells without CCI, and (iii) non-neighbor cells. Only cells at the last time point are considered when calculating the correlations. When calculating correlation with non-neighbors, four non-adjacent cells are randomly sampled for the current cell to keep the data scale comparable. Cell pairs of the same type are ignored while calculating the correlations because they tend to have similar expressions. Fig. 3e shows that gene expressions of neighboring cells with CCI have an average correlation of 0.1, while cells without CCI have approximately zero correlation, which is expected (using dataset `MT3a`). We noticed that neighboring cells without CCI still have slightly higher correlation compared to non-neighbor cells; and we speculate that this is because the cells are evolving to new cell types over time, so the CCI effect involved in an earlier cell type may remain in the final step.

scMultiSim generates realistic data

In this section, we show that scMultiSim's output can statistically resemble real data. We demonstrate scMultiSim's ability of generating realistic scRNA-seq data by matching to a referencing seqFISH+ dataset [16] (Fig. 3g), and compared it with dyngen [7]. dyngen is used as an existing simulator to compare with scMultiSim because it shares a few functionalities with scMultiSim. We compare the simulated data with real data in terms of the following properties: library size, zero count per cell, zero count per gene, mean count per gene, variation per gene, and the ratio between zero count and mean count per gene.

Fig. 3g shows that both scMultiSim and dyngen are able to simulate data with proper variation per gene. However, dyngen overestimates the library size, resulting in lower zero counts and larger mean count per gene; while scMultiSim successfully simulates the distribution in the experimental data. There is also usually a negative correlation between zero counts and mean counts in real data, and scMultiSim is able to simulate this relationship, matching well with the reference data.

Benchmarking clustering and trajectory inference methods

We first applied scMultiSim to test methods for two classic problems: cell clustering and trajectory inference, using the scRNA-seq modality in our main datasets (Table 1). We tested five clustering methods, PCA-KMeans, CIDR [40], SC3 [34], TSCAN [29], and Seurat [24] (Fig. 4a). Since Seurat does not provide direct control over the number of clusters, we varied the resolution parameter instead and plotted using the number of clusters in the results. From Fig. 4a, all methods have the best performance when the cluster number is the actual value, which is expected. In general, Seurat and SC3 have better performance than the others. We also show the comparison separately for $\sigma_{\text{cif}} = 0.1$ and $\sigma_{\text{cif}} = 0.1$ in Fig. S3a-b. Additionally, Seurat's recommended resolution range (0.4-1.2) provides a accurate estimation of the number of clusters (Fig. S3c).

We benchmarked the performance of five trajectory inference methods (PAGA [67], Monocle [51], Slingshot [59], MST [53], pCreode [26]) on tree-structured trajectories using the `MT` datasets. The result is shown in Fig. 4b, where we calculated the R^2 and kNN purity (for each cell, the Jaccard Index of the cell's kNN neighborhood in the inferred pseudotime vs. the ground truth pseudotime) for each separate lineage in each dataset. Overall, PAGA Tree and Slingshot has the best performance, which is in line with results shown in previous benchmarking efforts [53; 71]. We also show the separate results of $\sigma_{\text{cif}} = 0.1$ and $\sigma_{\text{cif}} = 0.5$ in Fig. S4a-b. Furthermore, we tested the curve-fitting performance a simpler single-lineage dataset `ML1a` in Fig. S4d, and the result was in line with a previous result shown in scDesign3 [56], which used a similar linear trajectory.

Benchmarking challenging computational tasks

We next show that scMultiSim can be used to benchmark challenging computational tasks in single cell genomics, which are recently actively studied: multi-modal data integration, RNA velocity estimation, GRN inference and CCI inference using spatially resolved single cell gene expression data. Using scMultiSim, we studied the performance of several recent methods on each task, and also investigated the effect of particular parameters for some of the benchmarks. As far as we know, scMultiSim is the only simulator that can benchmark all these tasks. It is noteworthy that our goal is not performing a comprehensive benchmarking but showing evidence of scMultiSim's "future-ready" capability. We expect these benchmarks can encourage forthcoming researchers to discover more use cases of scMultiSim.

Benchmarking multi-modal data integration methods

A number of computational methods have been proposed to integrate single cell genomics data from multiple modalities and multiple batches [1]. We benchmarked three recently proposed multi-modal integration methods: Seurat bridge integration [25], UINMF [35] and Cobolt [22] that can integrate data matrices from multiple batches and modalities. We use whole 144 main datasets to test their performance under various types of cell poopulation. Each main dataset is divided into three batches, then the scRNA data from batch 2 and scATAC data from

batch 3 are dropped intentionally to mimic a real scenario where some modalities are lacking (Fig.4d). We use the following metrics to evaluate the performance of the integration methods: Adjusted Rand Index (ARI) and Normalized mutual information (NMI) as the metrics for cluster identity preservation, and Graph Connectivity and average silhouette width (ASW) as metrics for batch mixing (Methods). These metrics were used in a recent paper on benchmarking single cell data integration methods [41].

The result is shown in Fig. 4c. Since Seurat does not output the latent embedding for the "bridge" dataset (batch 1), only the two matrices from batch 2 and 3 (colored in Fig.4c) were used for evaluation. We observe that Seurat bridge integration has the best performance on both continuous and discrete datasets in integrating cell types across modalities and batches. UINMF also has comparable ARI and NMI values with Seurat for discrete populations, but its performance is inferior on continuous datasets. Cobolt is the least satisfactory one among the three. When looking at the capability of merging different batches, Cobolt ranks at the top, while UINMF has the worst performance producing well-mixed batches. The results also suggest that continuous data is more challenging for the integration methods due to the unclear cluster boundaries.

We also visualized the integration results in Fig. S5, which helped us to understand the characteristic of each method's behavior. We noticed that while Seurat has lower graph connectivity and ASW scores, different batches are located closely (but do not overlap) in the visualized latent space. This means that Seurat may be able to identify cells from each batches, but chooses to separate the reference and query data in the latent space. Further inspection of the predicted cell types outputted by Seurat shows very accurate results, indicating that the latent embedding alone cannot represent Seurat's batch merging ability.

Benchmarking RNA velocity estimation methods

We demonstrate scMultiSim's ability of benchmarking RNA velocity estimation methods by running two representative RNA velocity inference methods, scVelo [3] and VeloCyto [36], on the simulated data. We compare all three models in scVelo package, including the deterministic, stochastic, and dynamical models. The auxiliary dataset `V` (Table 2) was used, which contains 72 datasets of different number of cells and genes, with or without GRN. We evaluate the accuracy of inferred RNA velocity using cosine similarity score. The score measures the degree of mismatch between the direction of inferred and ground truth velocity, where a higher score shows a better inference result (Methods).

From the result shown in Fig. 4f, scVelo's deterministic model has the highest cosine similarity score on all datasets. On the other hand, the dynamical model of scVelo, being considered a generalized version of VeloCyto, does not produce the best result. Interestingly, Gorin et al. also discussed a similar performance issue of the dynamical scVelo. They mentioned that the mismatch between the implicit assumption of dynamical scVelo and the true biological dynamics could be the cause of the performance issue [23]. In spite of the performance differences, the similarity scores are shown to be only around 0.2 for all methods. We suspect that it is the

intrinsic noise within the simulated dataset that affect the inference accuracy of all methods. We further conduct experiments comparing the accuracy of inferred RNA velocity with and without kNN smoothing (Methods). By conducting kNN smoothing, the inferred RNA velocity of each cell is further averaged with the velocity of all its neighboring cells. Since kNN smoothing helps to reduce the noise effect on the inferred velocity, we expect that the overall performance should improve after the smoothing. The experiment results validate our assumption (Fig. 4e), where the average performance of all methods increases to 0.63. The experiments show that the intrinsic noise within the sequencing dataset heavily affects the accuracy of RNA velocity inference methods, and it is still a challenging task to infer RNA velocity from noisy scRNA-seq datasets.

Benchmarking GRN inference methods

Using scMultiSim, we benchmarked 11 GRN inference methods which were compared in a previous benchmarking paper [50]. Using the predicted networks, we calculate the AUROC (area under receiver operating characteristic curve) as well as the AUPRC (area under precision-recall curve) ratio, which is the AUPRC divided by the baseline value (in our case, the AUPRC of a random predictor, which equals to the network density of the ground truth network), which was also used in previous benchmarking work [50].

We show results on the 144 main datasets in Fig. 5a. To further inspect the performance in a less-noisy scenario, we also generated auxiliary datasets `G` (Table 2) with a linear trajetory and without CCI effect. We benchmarked the methods using true counts and observed counts in `G`, respectively. The result of `G` is shown in Fig. 5b. All datasets use the same 100-gene GRN from [15]. We observed that PIDC [9] has the best overall performance, especially on true counts. Other methods like GENIE3 [28] and GRNBOOST2 [44] also have noteworthy precision. We then examined the effect of technical noise on the performance of GRN inference methods. On observed counts, both the AUPRC ratio and AUROC value suffer from a decline, indicating that it is significantly harder to infer the GRN from noisy data. However, PIDC continues to have the highest AUPRC and AUROC values, showing that its performance is more resistant to technical noises. SINCERITIES [47], PPCOR [33] and SINGE [13] perform well and beat GENIE3 and GRNBOOST2 on observed counts. Nevertheless, the absolute AUPRC values of all methods, even on true counts, are still far from satisfying, indicating that GRN inference is still a challenging problem.

Notably, the ordering of the methods tested using true counts is generally consistent with the ordering reported in [50] even though a different ground truth GRN was used. This fact not only validates the previous results but also suggests that scMultiSim can generate GRN-incorporated gene expression data comparable to other simulators. It indicates scMultiSim's practicality in benchmarking computational methods that involve GRNs.

Benchmarking CCI inference methods

We benchmarked three CCI inference methods based on spatially resolved single cell gene expression data, namely Giotto [16], SpaOTsc [5] and SpaTalk [55]. We run Giotto and SpaOTsc on the main datasets and show the result in Fig. 5c. Since SpaTalk needs a mininum of 3 genes from the receptor to a downstream activated TF, we also generated an auxiliary dataset `G` (Table 2) using an artificial GRN with long pathways to satisfy such requirement (Fig. S6a). There are totally eight `G` datasets with 500 cells, 200 genes and a linear trajectory, and the result is shown in fig. S6b. Again, we used AUPRC and AUROC as the metrics. When calculating the PRC and ROC curves, we applied different thresholds on the significance score for Giotto and the Bonferroni corrected p-values for SpaTalk. Considering both AUROC and AUPRC, Giotto has the best performance with an average AUROC of 0.68 and AUPRC of 0.54. SpaTalk outputs too many identical p-values for different datasets, causing the ROC and PRC curves to look unusual. We speculate that it is due to the artificial GRN still being too simple, indicating that SpaTalk is only designed for large-scale experimental data. Nevertheless, it has noteworthy performance in terms of AUROC and AUPRC values but is less accurate and stable than Giotto. The benchmarking results show that Giotto could be a versatile yet robust choice for CCI inference.

Discussion

We presented scMultiSim, a simulator of single cell multi-omics data which is able to simulate biological factors including cell population, chromatin accessibility, RNA velocity, GRN and spatial cell-cell interactions. We verified presence of the simulated factors in the output data and the relationship between modalities, as well as demonstrated its applications through benchmarks on various computational problems. Furthermore, by obtaining consistent benchmarking results with previous works like BEELINE [50] and dyngen [7], the simulated biological effects are validated to be practical and ready for real-world use.

Compared to existing simulators that focus on simulating one biological factor, data simulated by scMultiSim involves more biological factors, therefore has additional complexity similar to real data. Researchers using scMultiSim can therefore better estimate their methods' real-world performance on noisy experimental data. Furthermore, with the coupled data modalities in the output, researchers can benchmark their computational methods utilizing multiple modalities, which was previously impossible.

scMultisim's model is easy to extend to include more biological factors and modalities. Its flexibility and many functionalities are enabled by its mechanistic nature, which considers biological mechanisms as much as possible. Moreover, the framework we used to model chromatin regions (RIV) and genes (GIV) can also be expanded to other data modalities, like the protein abundance data. We have shown that our CIF/GIV model is versatile enough to not only mathematically represent the effects, but also incorporate various biological mechanisms.

scMultiSim has the following features that promote its usability: (1) requiring minimal and easy-to-construct input. For example, users do not need to prepare a backbone for the trajectory to control the cell population; instead, only a plain R phylogenetic tree or a text file with the newick format tree is needed. (2) transparent parameters. The parameters in scMultiSim are self-explanatory with a clear effect on the result. The user explicitly sets crucial metrics such as the number of cells and genes. (3) separated biological effects provide great flexibility. For example, GRNs can affect cell population shapes. However, obtaining the desired trajectory using GRN alone is difficult without explicit control of the cell population. Thanks to the separated diff and non-diff CIF mechanism, in scMultiSim, it is possible to set the trajectory to any shape without affecting the GRN effects. Users can also let the GRN control the trajectory by increasing the number of non-diff CIF.

We underline that scMultiSim's major advantage is encoding various factors using a single versatile model, thus creating a comprehensive multi-modal simulator that can benchmark an unprecedented range of computational methods. More importantly, the coupled data modalities in the output jointly provide more information than a single modality, which meets the needs of designing and benchmarking new methods on tasks involving multi-omics data. We believe that scMultiSim could be a powerful tool fostering new computational methods for single-cell multi-omics data. With more subsequent benchmarks, it can also help researchers choose their proper tool based on the kind of data they have.

Methods

A. The Beta-Poisson model and intrinsic noise

The master equation of the kinetic model represents the steady state distribution of a gene's expression level given its kinetic parameters, k_{on}, k_{off}, and s [45]. The Beta-Poisson model was shown to be equivalent to the master equation [32] with faster calculation. The gene expression level x (which is also the mRNA count) can be sampled from the following distribution:

$$y = \text{Beta}(k_{on}, k_{off})$$

$$x = \text{Poisson}(y \cdot s)$$

Using the above Beta-Poisson distribution to generate the gene expression level is one mode to obtain mRNA count for a gene in a cell. This works if we only need to generate the spliced mRNA counts. If users also need to generate unspliced mRNA counts and RNA velocity, the other mode, called the "full kinetic model" is used. The Beta-Poisson model is used by default when only generating spliced counts for lower running time.

The sampling process from the Beta-Poisson distribution to obtain x introduces intrinsic noise to the data, which corresponds to the intrinsic noise in real data caused by transcription burst. The theoretical mean of the kinetic model, which is $(\frac{k_{on}}{k_{on}+k_{off}} \cdot s)$, corresponds to the gene expression level of the gene with no intrinsic noise. We introduced parameter σ_i which controls the intrinsic noise through adjusting the weight between the random samples from the Poisson distribution and the theoretical mean:

$$x_{\sigma_i} = \sigma_i \cdot x + (1 - \sigma_i) \cdot (\frac{k_{on}}{k_{on} + k_{off}} \cdot s)$$

The intrinsic noise in scRNA-seq data is hard to reduce in experiments due to the snapshot nature of scRNA-seq data. The parameter σ_i allows users to investigate the effect of intrinsic noise on the performance of the computational methods.

B. Cell Identity Factors (CIFs) and Gene Identity Vectors (GIVs)

The length of the CIF and GIV, denoted by n_{cif}, can be adjusted by the user. Overall, we have a $n_{\text{cell}} \times n_{\text{cif}}$ CIF matrix for each kinetic parameter (Fig. S1a), where each row is the CIF vector of a cell. Correspondingly, we also have the $n_{\text{cif}} \times n_{\text{gene}}$ Gene Identity Vectors (GIV) matrix, (Fig. S1b) where each column is linked to a gene, acting as the weight of the corresponding row in the CIF matrix, i.e. how strong the corresponding CIF can affect the gene. For example, a higher value in the k_{on} GIV vector $\text{giv}_i^{k_{on}}$ can be interpreted as: higher concentration of the corresponding cell factor $\text{cif}_i^{k_{on}}$ causes a higher activation rate for the gene's promoter. In short, CIF encodes the *cell identity*, while GIV encodes the *strength of biological effects*. Therefore, by multiplying the CIF and GIV matrix, we are able to get a $n_{\text{cell}} \times n_{\text{gene}}$ matrix, which is the desired kinetic parameter matrix with the cell and gene effects encoded.

C. diff-CIF generates user-controlled trajectories or clusters.

When generating data for cells from more than one cell types, the minimal user input of scMultiSim is the cell differentiation tree, which controls the cell types (for discrete population) or trajectories (for continuous population) in the output. The generated scRNA-seq and scATAC-seq data reflect the tree structure through the diff-CIF vectors. The diff-CIF vectors are generated as following: starting from the root of the tree, a Gaussian random walk along the tree (Fig. 2a) is performed for each cell to generate the $n_{\text{diff-CIF}}$ dimension diff-CIF vector. Parameter σ_{cif} controls the standard deviation of the random walk, therefore a larger σ_{cif} will produce looser and noisier trajectory structures. Another parameter r_d is used to control the relative number of diff-CIF to non-diff-CIF. With a larger r_d, trajectories are clear and crisp in the output; with a smaller r_d, the trajectory is vague, and shape of the cell population is more controlled by other factors like GRN. For a discrete population, only the cell types at the tree tips are used; then cells of each type are shifted by a Gaussian distribution, controlled by the same σ_{cif} parameter. Therefore, a smaller σ_{cif} will produce clearer cluster boundaries.

For a heterogeneous cell population, cells have different development stages and types. Users should input a cell differentiation tree where each node represents a cell type. The tree provides a backbone for the trajectory in the cell population. Each dimension of the diff-CIF vector is sampled along the tree via browning motion. First, cells start at the root of the tree; then for each dimension, the diff-CIF value for all cells $\mathbf{v}$ is

$$v_i = \sum_{j=1}^{i} q_j \text{ where } q_j = \mathcal{N}(0, \sigma_j).$$

σ_j is the distance along the tree between cell j and $j-1$. Alternatively, users can use an impulse model (using implementation in SymSim). The lengths of the non-diff-CIF and diff-CIF vectors can be controlled by the user. More diff-CIFs will result in a more clear trajectory pattern in the cell population, which corresponds to the input tree. With very few diff-CIFs, the cell population is mainly controlled by the GRN.

D. tf-CIF and GIV encode the GRN effects

To encode GRN effect in the simulated single cell gene expression data, the GIVs and CIFs are designed to include a "TF part" (Fig. S1a). Cells are generated one by one along the given cell differentiation tree, where the expression of the TFs in the tth cell affect the gene expression of cell $t+1$. Formally, the ith position of the TF part (corresponding to the ith TF) of in the CIV of cell $t+1$ is calculated as:

$$\text{tf-CIF}_i^{(t+1)} = \frac{\mathbf{x}_i^{(t)}}{\mathbf{x}_i^{(t)} + \frac{1}{n}\sum_l \mathbf{x}_l^{(t)}} \qquad \forall i \in \text{TFs} \tag{1}$$

where $\mathbf{x}_i^{(t)}$ is the expression level of the ith TF in the tth cell. The corresponding tf-CIF for the root cell is sampled randomly from the Gaussian distribution $\mathcal{N}_{\text{cif}}$ supplied by the user.

The TF part of the GIV for a gene also has length of n_{TF}(Fig. S1b). Considering all genes, we have a $n_{\text{gene}} \times n_{\text{TF}}$ matrix, which we call the GRN effect matrix. This matrix encodes the ground truth GRN that is supplied by

the user. Naturally, the GRN effect matrix is included in the GIV when calculating the s parameter, where the value at (i, j) is the regulation strength of TF j on gene i. Therefore, a larger regulation strength will lead to higher s, and consequently, higher expression for the target genes. For k_{on} and k_{off}, the tf-CIF vector is sampled using $\mathcal{N}_{\text{cif}}$, assuming that the GRN does not affect the active state of a gene. However, in the scenario where it is desired to model GRN effect also in k_{on} and k_{off}, similar GRN effect matrix for s can be used for k_{on} and k_{off}.

E. lig-CIF and GIV encode cell-cell interactions

When simulating spatial transcriptomics data with CCI effects, we used a 2-D $k \times k$ grid to model the spatial locations of cells (Fig. S1d). The grid size k is large enough to accommodate the n cells (can be specified by the user; if not provided, use 250% of cell number by default). A cell can have at most n_{nbs} neighbors with CCI (within the blue circle's range in Fig. 2a, and this radius can be adjusted). Therefore, the ligand CIF and GIV are of length $n_{\text{lig}} \cdot n_{\text{nbs}}$, where n_{lig} is the number of ligands.

The lig-GIV vector contains the CCI strength values, for example, the "n2 lg3" entry in Fig. 2a indicates how strong the ligand 3 from the neighbor at position 2 can affect the receptor 2 of this cell. The lig-CIF of each cell will inherit from its previous cell during the simulation process, which is similar to the tf-CIF mentioned above. Each entry of the lig-CIF vector corresponds to a ligand from one neighbor. The same Gaussian distribution $\mathcal{N}_{\text{cif}}$ is used for k_{on} and k_{off}. For s, due to the similarity of the ligand-receptor pairs and the TF-target pairs, we use a similar strategy as tf-CIF (shown in Eq. 1): cell i's lig-CIF is the normalized vector of cell $i-1$'s gene expression counts of the ligand genes (See Fig. 2a, Fig. S1).

At each step t, a new cell is considered to be born and added to the grid. When adding a new cell, it has a probability of p_n to be a neighbor of an existing cell with the same cell type. A pre-defined cell differentiation tree is required as input to define the differentiation topology in the cells. A new cell will always be in the initial state at the root of the differential tree. At each step, an existing cell moves forwards along a random path in the cell differential tree, representing the cell development. The gene expressions in the final step are output as the observed data. Fig. S1 shows the structure for the CCI mode.

To generate ground truth CCIs both at the cell types level and single cell level, scMultiSim pre-defines a ligand-receptor database, represented by a user input $m \times 3$ matrix S. There are m ligand-target pairs in total that correspond to each row of S. For each pair i, there are three parameters: ligand gene L_i, receptor gene T_i, and the effect E_i, representing how strongly the ligand can affect the expression of the receptor. For each cell type pair, the ground truth CCI beetween these two cell types are sampled from the ligand-receptor database (which correspond to the columns in S). For each neighboring cell pair, the ground truth CCIs between them follow the cell-type-level ground truth CCIs: if the two cells belong to two cell types C_1 and C_2 respectively (where C_1 can be the same as C_2), the CCIs between these two cells follow the CCIs defined in S corresponding to pair (C_1, C_2). Users can have further fine-grained control for each cell pair by letting it use a subset of ligand-receptor

pairs sampled from the cell-type level ground truth.

Although we collect cells at the last time point as our output (which is the case for real data), different cell types are guaranteed to present in the last step since the cells are added at different time steps, therefore having different development stages. In addition, we let the same cell (at the same location) having the same diff-CIF across different time steps, so the trajectory encoded in the diff-CIF is preserved in the final step. A cell's TF and ligand CIF for the current step are inherited from the previous one to make sure other factors stay the same.

F. Generating the Gene Identity Vectors

The GIV matrix is a $n_{\text{cif}} \times n_{\text{gene}}$ matrix, therefore it also can be divided into four submatrices as shown in Fig. S1b. For k_{on} and k_{off}, the nd-CIF and diff-CIF are sampled from distribution $\mathcal{G}$ as shown below:

$$\begin{cases} \mathcal{N}_{\text{giv}} & \text{w.p. } 1 - p_0^{\mathcal{G}} \\ 0 & \text{w.p. } p_0^{\mathcal{G}} \end{cases}$$

where $p_0^{\mathcal{G}}$ is a parameter specifying the probability of being zero, and $\mathcal{N}_{\text{giv}}$ is a user-adjustible gaussian distribution. tf-GIV and lig-GIV are all zeros since TF/ligands affect s only. For s, the tf-GIV submatrix is the GRN effect matrix, i.e. a $n_{\text{TF}} \times n_{\text{gene}}$ matrix where the entry at (i, j) is the regulation effect between TF i and gene j. Similarly, the lig-GIV submatrix is the cell-cell interaction effect matrix. The nd-GIV submatrix is sampled from $\mathcal{G}$. For diff-GIV, we do the following steps to the incorporate the connection between TFs and regulated genes: (1) Randomly select 2 GIV entries for each TF gene and give them a fixed small number. (2) For every target gene, it should use the same GIV vector as its regulators. If a gene has multiple regulators, its gene effects will be the combination of that of the regulators. This is achieved by multiplying the $n_{\text{diff}} \times n_{\text{TF}}$ GIV matrix in (1) and the $n_{\text{TF}} \times n_{\text{gene}}$ effect matrix. If a gene is both a TF and target, its GIV will be $0.5 \cdot ((1) + (2))$.

G. Preparing the kinetic parameters

The kinetic parameters, k_{on}, k_{off} and s are needed when generating single cell gene expression data (mRNA counts) using the kinetic model or Beta-Poisson distribution (Fig. 1b). While the basic idea is to get the parameter matrix using CIFs and GIVs (Fig. 1b), the three parameters go through different post-processing after the step of CIF $\times$ GIV. We first denote the result of CIF $\times$ GIV for k_{on}, k_{off} and s as M_1, M_2 and M_3, respectively.

(i) k_{on}. Since chromatin accessibility controls the activation of the genes, the scATAC-seq data is expected to affect the k_{on} parameter. We first prepare a $n_{\text{region}} \times n_{\text{gene}}$ 0-1 region-to-gene matrix Z using $\mathbf{r}$, where Z_{ij} indicates region i is associated with gene j (Z is outputted as the region-to-gene matrix). We multiply the scATAC-seq matrix with Z to get the $n_{\text{cell}} \times n_{\text{gene}}$ parameter matrix M_1'. Since the scATAC-seq data is sparse, there are many zeros in M_1'. Thus, we replace the zero entries in M_1' with the corresponding entries in M_1

(scaled to be smaller than the smallest non-zero entry in M_1') to help differentiate the zero entries. Finally, M_1' is sampled to match the distribution of k_{on} inferred from real data.

(ii) k_{off}. The parameters are obtained by scaling M_2 to match the real data distribution. For both k_{on} and k_{off}, it is possible to adjust the bimodality of gene expressions [71] through an optional bimodal factor B. A larger B will downscale both k_{on} and k_{off}, therefore increase the bimodality.

(iii) s. The parameters are obtained by scaling M_3 to match the distribution of s inferred from real data. Then, users can also used a "scale.s" parameter to linearly scale s. It allows us to adjust the size of cells – some datasets may tend to large cells and some tend to have small cells depending on the cell types being profiled.

When scaling a matrix (M_1', M_2, or M_3) to match a reference distribution (eg. the distributions of k_{on}, k_{off} and s estimated from real data), the procedure is as following: denoting the reference distribution by $\mathcal{D}$, the matrix to rescale by X, and the number of elements in X by n, we sample n ordered values from $\mathcal{D}$, then replace the data in X using the same order. scMultiSim uses the reference kinetic distribution parameters provided in SymSim [71], where the kinetic parameters are estimated from real data via an MCMC approach. The data used are the UMI-based dataset of 3005 cortex cells by Zeisel et al. [69], and a non-UMI-based dataset of 130 IL17-expressing T helper cells (Th17) by Gaublomme et al [21].

H. Simulating scATAC-seq data and relationship between scATAC-seq and scRNA-seq

Since scMultiSim incorporates the effect of chromatin accessibility in the gene expressions, the scATAC-seq data is simulated before the scRNA-seq data. The cell types in the scATAC-seq data can follow the same differentiation tree as in the scRNA-seq data (the scATAC-seq and scRNA-seq data share the same cells) or can follow a different tree (to reflect the difference between modalities).

Similar to GIV, we use a randomly sampled *Region Identity Vector (RIV)* matrix to represent the chromatin regions. Following the same mechanism, we multiply the CIF and RIV matrix, and obtained a "non-realistic scATAC-seq" data matrix. Next, the scATAC-seq data matrix is obtained by scaling the "non-realistic" scATAC-seq data to match a real distribution learned from real data. This is a step to capture the intrinsic variation of the chromatin accessibility pattern, which we will also apply for the kinetic parameters when generating gene expressions.

The RIV matrix is sampled from a distribution $\mathcal{R}$ similar to $\mathcal{G}$:

$$\begin{cases} \mathcal{N}_{\text{riv}} & \text{w.p. } 1 - p_0^{\mathcal{R}} \\ 0 & \text{w.p. } p_0^{\mathcal{R}} \end{cases}$$

where $p_0^{\mathcal{R}}$ is the probability of being zero and $\mathcal{N}_{\text{riv}}$ is a user-adjustable gaussian distribution. With the CIF and RIV matrices, the $n_{\text{cell}} \times n_{\text{region}}$ scATAC-seq can be generated by (1) multiplying the CIF matrix by the RIV matrix, (2) scale the matrix to match the real data distribution, and (3) adding intrinsic noise (sampled from a small

Gaussian) to the scATAC-seq data. In Step (2), we use the same rank-based scaling process as used for the kinetic parameters as described in Section "Preparing the kinetic parameters" above, and the real scATAC-seq data distribution is obtained from the dataset in [cite: Chen, Xi, et al. "A Rapid and Robust Method for Single Cell Chromatin Accessibility Profiling." Nature Communications, vol. 9, no. 1, Dec. 2018, p. 5345.].

To incorporate the relationship between scATAC-seq and scRNA-seq data, we use the scATAC-seq data to adjust the k_{on} parameter that is used to generate the scRNA-seq data, considering that chromatin accessibility affects the activated status of genes. First, a region-to-gene matrix (Fig.1b) is generated to represent the mapping between chromatin regions and genes, where a gene can be regulated by 1-3 consecutive regions. Users can input a region distribution vector $\mathbf{r}$, for example, $(0.1, 0.5, 0.4)$ which means a gene can be regulated by three regions, and the probability of it being regulated by one, two and three consecutive regions are 0.1, 0.5 and 0.4, respectively. Details on adjusting k_{on} by the scATAC-seq data is described in Methods (Section "Preparing the kinetic parameters".)

I. Generating RNA velocity with the full kinetic model

The starting spliced count x_s and unspliced count x_u for a cell are the previous cell's counts on the differential tree. For the first cell, the spliced/unspliced counts are

$$x_s = \frac{s \cdot k_{on} \cdot \beta}{k_{on} + k_{off}} \qquad x_u = \frac{s \cdot k_{on} \cdot d}{k_{on} + k_{off}}$$

where β and d respectively represents the splicing and degradation rate of genes. Both γ and d are sampled from a user-controlled normal distribution.

We set the cell cycle length to be $L = \frac{1}{k_{on}} + \frac{1}{k_{off}}$, and divide it into multiple steps. The number of steps follows $m = \left\lceil \frac{L}{\min(1/k_{on}, 1/k_{off})} \right\rceil$. We also provide an optional cell length factor η_L parameter to scale the cycle length if user desired. The probability of gene switching on or off are then calculated with $p_{\text{on}} = \frac{k_{on}}{m \cdot L}$ and $p_{\text{off}} = \frac{k_{off}}{m \cdot L}$. In each simulation step, we update the cell's current on/off state based on p_{on} and p_{off}, and generate the spliced/unspliced counts x_s and x_u. The spliced counts at step t is obtained by:

$$x_s^t = x_s^{t-1} + \frac{L}{m}(\beta \cdot x_u^{t-1} - d \cdot x_s^{t-1})$$

and the unspliced counts is obtained by:

$$x_u^t = \begin{cases} x_u^{t-1} + \frac{L}{m}(s - \beta \cdot x_u^{t-1}) & \text{if state is on} \\ x_u^{t-1} - \frac{L}{m}(\beta \cdot x_u^{t-1}) & \text{if state is off} \end{cases}$$

The outputted x_s and x_u are the values at the final step $t = m$. The ground truth RNA velocity is calculated as:

$$v = \beta \cdot x_u - d \cdot x_s$$

We obtain the KNN averaged RNA velocity by applying a Gaussian Kernel KNN on the raw velocity data, with $k = \lceil n_{\text{cell}}/50 \rceil$. Then we normalize the velocity by calculating each cell's normalization factor $s_i = |v_i|$, where v_i is the velocity vector for cell i.

J. Adding technical noise and batch effects to data

Technical noise is added to the true mRNA counts to generated observed counts (observed scRNA-seq data) (Fig. 1b). The workflow follows SymSim's approach [71]: we simulate multiple rounds of mRNA capture and PCR amplification, then sequencing and profiling with UMI or non-UMI protocols. The parameter α controls the capture efficiency, that is, the rate of subsampling of transcripts during capture step, which can vary in different cells, and user can specify it using a Normal distribution $\alpha \sim \mathcal{N}(\alpha_\mu, \alpha_\sigma)$. The sequencing depth $d \sim \mathcal{N}(d_\mu, d_\sigma)$ is another parameter that controls the quality of the observed data.

Batch effects are added by first dividing the cells into batches, then adding gene-specific and batch-specific Gaussian noise based on shift factors. For each gene j in batch i, the shift factor is sampled from Unif$(\mu_j - e_b, \mu_j + e_b)$, where $\mu_j \sim \mathcal{N}(0, 1)$, and e_b is the parameter controlling the strength of batch effects. We provide several settings for adding highly expressed genes to help researchers fit the housekeeping genes in real data. scMultiSim also supports cell- and gene-wise tuning of the mRNA capture efficiency during the PCR process; therefore per-cell and per-gene metrics (such as zero count proportion, count variance) in the observed data can be controlled separately.

For scATAC-seq data, as the data is sampled from real data we do not explicitly simulate the experimental steps. We do provide function to add batch effects to obtain multiple batches of scATAC-seq data.

K. Comparing statistical properties of simulated data with experimental data

To measure scMultiSim's ability to generate realistic data while incorporating all the effects, we compare the statistical properties of a real mouse somatosensory cortex seqFISH+ dataset [20] with simulated data generated using selected parameters. The dataset, with 10000 genes and spatial locations of 523 cells, is featured in Giotto [16]'s tutorial.

The scMultiSim simulated data has both GRN and CCI effect. The GRN used as input to scMultiSim is obtained as following: GENIE3 [28] was used to obtain an inferred GRN from the dataset, then after looking at the output edge importance values, the top 200 edges were utilized to form a reference GRN. We used this GRN (96 genes) and another randomly sampled 104 genes to generate a subsample of the data. We then simulated a dataset with 200 genes and 523 cells using scMultiSim. After observing the dimension reduction of the real dataset, a discrete cell population is assumed. We specify the cluster ground truth using the exact cell type labels in the dataset. There are 10 cell types in total. We also used Giotto [16] to infer the cell-cell interactions between cells.

We chose the top-seven most significant ligand-receptor pairs from Giotto's output, with p-value ≤ 0.01, more than 10 ligand and 10 receptor cells, and the largest `log2fc` values.

We used dyngen [7] as a baseline simulator to compare with scMultiSim. We generated a simulated dataset with dyngen, using the same GRN and number of cells. The cell types and cell-cell interaction ground truth was not provided since dyngen doesn't support them. Yet, we supplied the raw mouse SS cortex count matrix to dyngen's `experiment_params` as a reference dataset.

We used the following metrics to compare the distribution of simulated and experimental datasets, which is also used in [15]: library size (per cell), zero counts proportion (per cell), zero counts proportion (per gene), mean counts (per gene), counts variance (per gene), and relationship of zero counts and mean counts per gene.

L. Evaluation metrics for in benchmarking computational methods

The evaluation metrics used for multi-model data integration methods, Graph Connectivity and ASW, are described as following.

Graph Connectivity is defined as:

$$GC = \frac{1}{|C|} \sum_{c \in C} \frac{|LCC(c)|}{|c|}$$

where C is all cell types, $LCC(c)$ is in the largest connected component for cells of type c.

For the ASW:

$$batch\, ASW = \frac{1}{|M|} \sum_{k \in M} \frac{1}{|C_j|} \sum_{i \in C_j} 1 - |silhouette(i)|$$

where M is the set of all cell types, and C_j is all the cells of type j. We used the implementation in [41].

When evaluating RNA velocity inference methods, we used the *cosine similarity* between the averaged estimated velocity and the ground truth. Calculating the average of estimated velocity vectors is commonly used to reduce local noise [7]. In dyngen [7], averaged RNA velocities was calculated across cells at trajectory waypoints weighted through a Gaussian kernel using ground truth trajectory; while in scMultiSim, we averaged the raw velocity values by kNN with a Gaussian kernel and $k = n_{\text{cells}}/50$ to achieve a similar averaging effect. Finally, cosine similarity is calculated as:

$$\frac{1}{n_{\text{cells}}} \sum_i \frac{v_i \cdot u_i}{\|v_i\| \|u_i\|}$$

where v_i is the ground truth velocity vector for cell i, and u_i is the predicted velocity vector.

M. Details on running clustering methods

We used CODR 0.1.5, SC3 1.24.0, Seurat 4.1, and TSCAN 2.0. Parameters we specified are (1) SC3: `pct_dropout` $= [0, 100]$, (2) Seurat: `dims.use` $= 30$. For PCA-Kmeans, we simply ran Kmeans clustering on

the first 20 principle components using the default R implementation `prcomp` and `kmeans`. ARI is calculated by `adjustedRandIndex` from the R package `mclust`. Some code was adapted from [17].

N. Details on running trajectory inference methods

We used the latest dynverse [53] package to run the trajectory inference methods. When running them, we provide the correct root cell ID, number of starting clusters and number of ending clusters. The R^2 values are calculated between the inferred pseudotime and the ground truth for each separate lineage. The KNN purity value is calculated for each lineage as: for cell i, we obtain its KNN neighborhood N_i on the pseudotime with $k = 50$. Then the KNN purity for i is the Jaccard Index of N_i on the inferred pseudotime and N_i on the true pseudotime.

O. Details on running data integration methods

We use all the 144 main datasets. Technical noise and batch effects were added using default parameters (non-UMI, $\alpha \sim \mathcal{N}(0.1, 0.02)$, depth $\sim \mathcal{N}(10^5, 3000)$, ATAC observation probability 0.3). All integration methods were ran on the scRNA and scATAC data with technical noise and betch effect. For Seurat, we followed the vignette "Dictionary Learning for cross-modality integration" in Seurat 4.1.0 using the default parameters. For UINMF, we used the latest GitHub release. We followed the "UINMF integration of Dual-omics data" tutorial and ran the `optimizeALS` method using $k = 12$. For Cobolt, we used the GitHub version cd8015b, with 10 latent dimentions, learning rate 0.005. If the loss diverged, we automatically retry with learning rate 0.001. The metrics, including ARI, NMI, Graph Connectivity and ASW were computed using the scib [41] package.

P. Details on running RNA velocity estimation methods

We use the datasets `V` to benchmark RNA velocity inference methods as shown in Table 2. We used scVelo 0.2.4 and VeloCyto 0.17.17. We benchmarked scVelo with three modes: `deterministic`, `stochastic`, and `dynamical`. For VeloCyto, we used the default options.

Q. Details on running GRN inference methods

We use the BEELINE [50] framework to benchmark GRN inference methods. Apart from the main datasets, The dataset `G` (Table 2) was generated using the following configurations: The 100-gene GRN in Fig. 3, 1000 cells, 50 CIFs, $r_d = 0.2$, $\sigma_i = 1$, with other default parameters. Eight datasets were generated for random seed 1 to 8, and technical noise and batch effect was added using default parameters. We ran the BEELINE GitHub version 79775f0. In order to resolve runtime errors, all docker images were built locally, except that we used the provided images on Docker Hub for PIDC and Scribe. We use BEELINE's example workflow to infer GRN and

calculate the AUPRC ratio and AUROC for (a) true counts in the eight datasets, and (b) observed counts with batch effects in the eight datasets. Eleven methods were benchmarked in total: PIDC, GRNBoost2, GENIE3, Sincerities, PPCOR, LEAP, GRISLI, SINGE, GRNVBEM, Scribe and SCODE.

R. Details on running CCI inference methods

We generated 12 datasets using the following procedure. Apart from the main datasets, for each `C` dataset (Table 2), we first construct the GRN (Fig. S6a): (1) let genes 1-6 be the transcription factors. Sample 70 edges from gene 1-6 to gene 7-53. (2) Connect gene 7-53 (regulator) to gene 54-100 (target) consecutively. (3) Connect gene 54-100 to gene 110-156. In this way, we can generate a GRN with reasonable edge density and make sure that there are three downstream genes for each TF, which is required by SpaTalk. Then we construct the ligand-receptor pairs: let the ligands be gene 101-106 and receptors be gene 2, 6, 10, 8, 20, and 30. We divide a linear trajectory into 5 sections, corresponding to 5 cell types. Between each cell type pair (excluding same-type pairs), we sample 3-6 ligand-receptor pairs and enable cell-cell interactions with them for the two cell types. The dataset is then simulated using 160 genes in total, 500 cells, and 50 CIFs. We use the true counts to benchmark the methods.

To run SpaTalk, we modify the original `plot_lrpair_vln` method to return the p-value rom the Wilcoxon rank sum test directly, rather than drawing a figure. Before using the p-values to calculate the precision and recall, we adjusted them using Bonferroni correction:

$$\hat{p}_i = \max(p_i \cdot |p|, 1)$$

where p is the p-value vector for all cell type and ligand-receptor pairs. For Giotto, we used the R package 1.1.2 and followed the `mini_seqfish` vignette. For SpaOTsc, we used default parameters.

Data and Code Availability

The scMultiSim R package is availavle at https://github.com/ZhangLabGT/scMultiSim.

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Competing Interests Statement

The authors declared no competing interest.

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Tables

Name (#datasets)	Label	Trajectory	σ_{cif}	Cells	Genes	Velo	GRN	CCI	Seed
Main (144)	**M**	**L: Continuous Linear (Phyla1)** **T: Continuous Tree (Phyla3)** **D: Discrete (Phyla5)**	0.1	500	**1: 110 genes** **2: 200 genes** **3: 500 genes**	F	GRN_100	T	**a: 1** **b: 2** **c: 3** **d: 4**
				800	**4: 110 genes** **5: 200 genes** **6: 500 genes**				
			0.5	500	**7: 110 genes** **8: 200 genes** **9: 500 genes**				
				800	**10: 110 genes** **11: 200 genes** **12: 500 genes**				

Table 1. The main dataset contains 144 datasets with varying trajectory, σ_{cif},number of cells and genes. For each parameter configuration, four datasets are generated using different random seeds. We number the datasets for easy referencing in the text: starting with the letter M,then a letter {L,T,D} specifying the trajectory; followed by a number 1-12 identifying the configuration of σ_{cif}, number of cells and genes; and last, a lowercase letter a-d indicating the random seed. For example, MD5c uses a discrete cell population, $\sigma_{\text{cif}} = 0.1$, 800 cells, 200 genes and random seed 3. Phyla1, Phyla3 and Phyla5 are the input tree structure used to generate the cell populations, and they are shown in Fig. 2b.

Name (#datasets)	Label	Trajectory	σ_{cif}	Cells	Genes	Velo	GRN	CCI	Seed	Other Params
Auxiliary (144)	**A**	Tree (Phyla5) Discrete (5 clusters)	0.1, 0.5, 1	200	1000	F	GRN_100	F	1,2	$E_{\text{atac}} = 0.2, 0.5, 0.8$ $\sigma_i = 0.3, 1$ $r_d = 0.2, 0.8$
Velocity (72)	**V**	Tree (Phyla5)	0.1	500,750,1000	100,200,500	T	GRN_100 N/A	T	5-8	
Realistic (1)	**R**	Discrete (10 clusters)	0.1	523	200	F	Inferred	F	1	
Add. GRN (8)	**G**	Linear	0.1	110	1000	F	GRN_100	F	1-8	
Add. CCI (8)	**C**	Linear	0.1	200	500	F	Fig. S6	T	1-8	

Table 2. The auxiliary dataset and other datasets used in supplimental information.

Figures

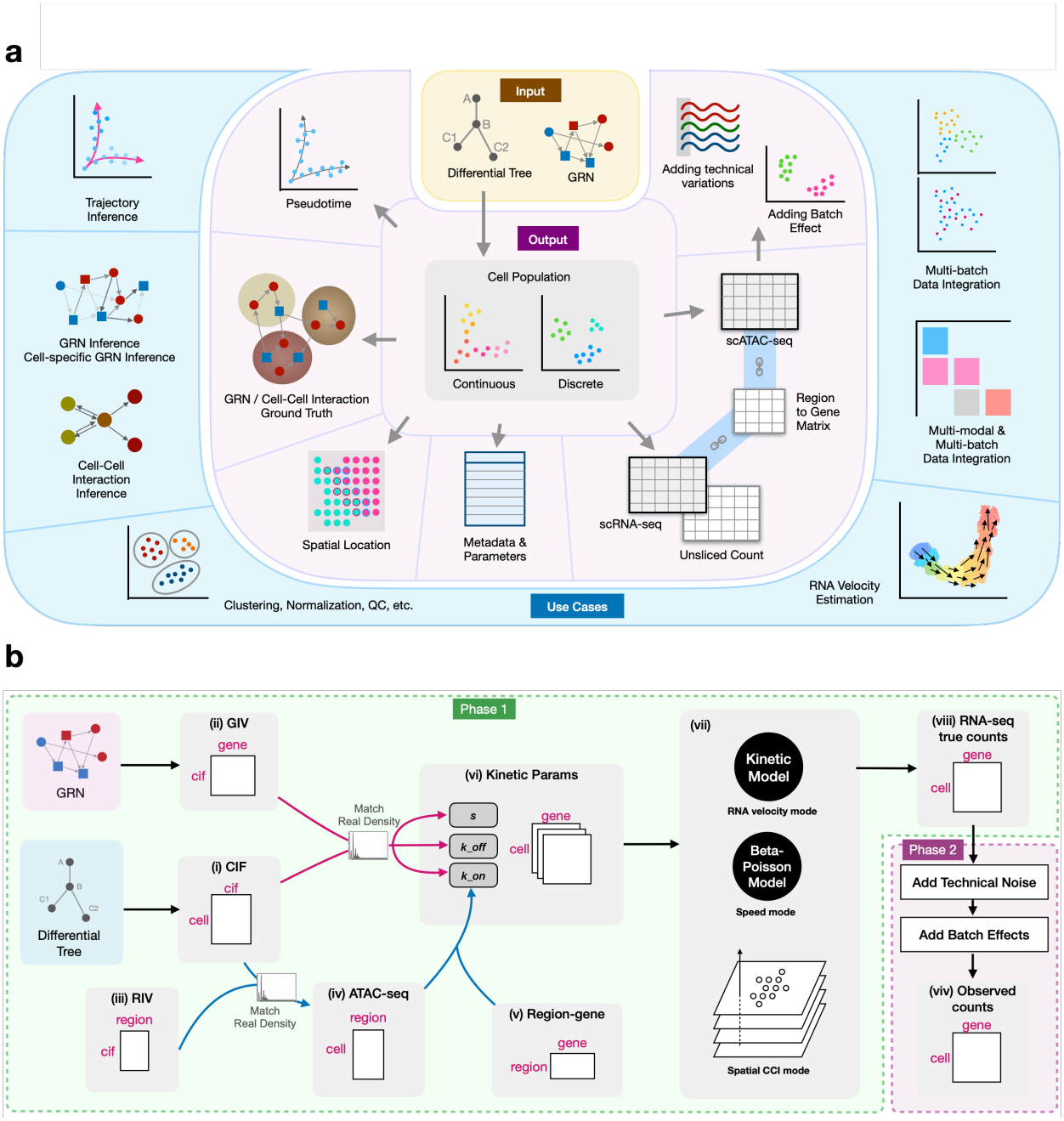

Figure 1. Overview of scMultiSim. See the next page for descriptions.

. Overview of scMultiSim. (a): The input, output, and use cases. The minimal required input is a cell differential tree describing the differentiation relationship of cell types. It controls the cell trajectory or clusters in the output. A user-input ground truth GRN is recommended to guide the simulation. Users can also provide ground truth for cell-cell interaction and control each simulated biological effect using various parameters. **(b)**: The overall structure of scMultiSim. The scATAC-seq data (iv) is firstly generated using CIF (i) and RIV (iii). The kinetic parameters used to generate scRNA-seq data (vi) is prepared using GIV (ii), CIF (i) and the scATAC-seq data with (**v**) a region-to-gene matrix. Using the parameters, either the full kinetic model (when RNA velocity is required), the Beta-Poisson model (when running speed matters), or the Spatial CCI mode (when CCI is enabled) will be used to generate the scRNA-seq data (vii). With the simulated true counts (viii), technical noise and batch effects can be added to obtain the observed counts.

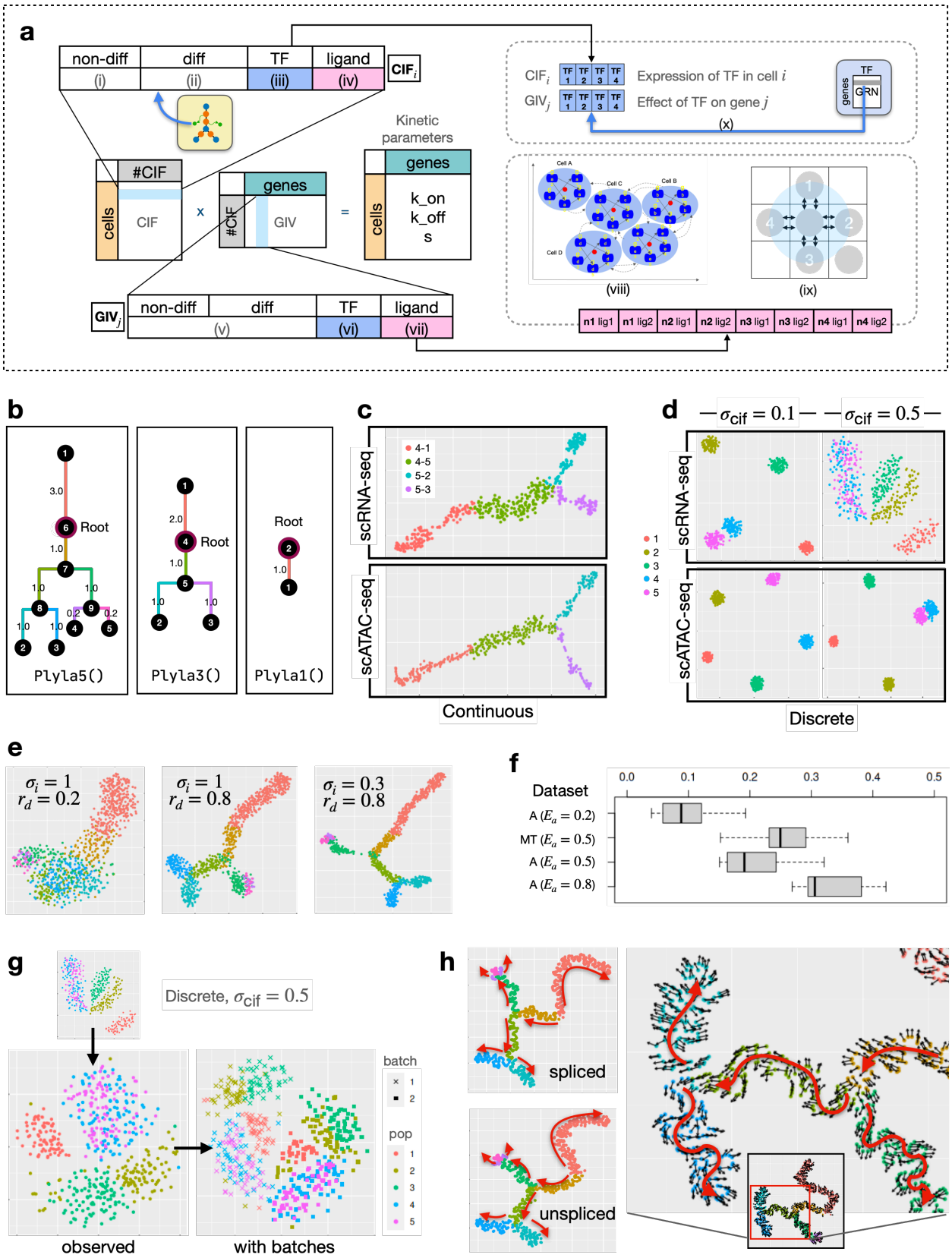

Figure 2. scMultiSim generates multi-modal single cell data from pre-defined cell clustering structure or trajectories. See the next page for descriptions.

. scMultiSim generates multi-modal single cell data from pre-defined cell clustering structure or trajectories. (a) The CIF and GIV matrix. We multiply the CIF and GIV matrix to get the cell$\times$gene matrix for each kinetic parameter. CIFs and GIVs are divided into segments to encode different biological effects, where each segment encodes a certain type of biological factor. A cellular heterogeneity is modeled in the CIF, and regulation effects are encoded in the corresponding GIV vector. (viii) is the illustration of the cell-cell interactions and in-cell GRN in our model. (ix) is the grid system representing spatial locations of cells. A cell can have at most four neighbors (labeled 1-4) within a certain range (blue circle). The cell at the bottom right corner is not a neighbor of the center cell. (**b**) Three trees are provided by scMultiSim and used to produce the datasets. Phyla1 is a linear trejectory, while Phyla3 and Phyla5 has 3 and 5 leaves, respectively. (**c**) t-SNE visualization of the paired scRNA-seq and scATAC-seq data (without adding technical noise) from the main dataset `MT3a` (continuous populations following tree Phyla3), both having $n_{\text{cell}} = n_{\text{gene}} = 500$. (**d**) t-SNE visualization of the paired scRNA-seq and scATAC-seq data (without adding technical noise) from the main datasets `MD3a` and `MD9a` (discrete populations with five clusters, following tree Phyla5). (**e**) Additional results showing the effect of σ_i and r_d using datasets A. (**f**) Additional results exploring the ATAC effect parameter using datasets A and MT. Averaged Spearman correlation between scATAC-seq and scRNA-seq data for genes affected by one chromatin region, from 20 datasets using various parameters (σ_i, σ_{cif}, r_d, continuous/discrete). (**g**) The observed RNA counts in dataset MD9a with added technical noise and batch effects. (**h**) The spliced true counts, unspliced true counts, and the RNA velocity ground truth from dataset V. The velocity vectors point to the directions of differentiation indicated by red arrows, from the tree root to leaves.

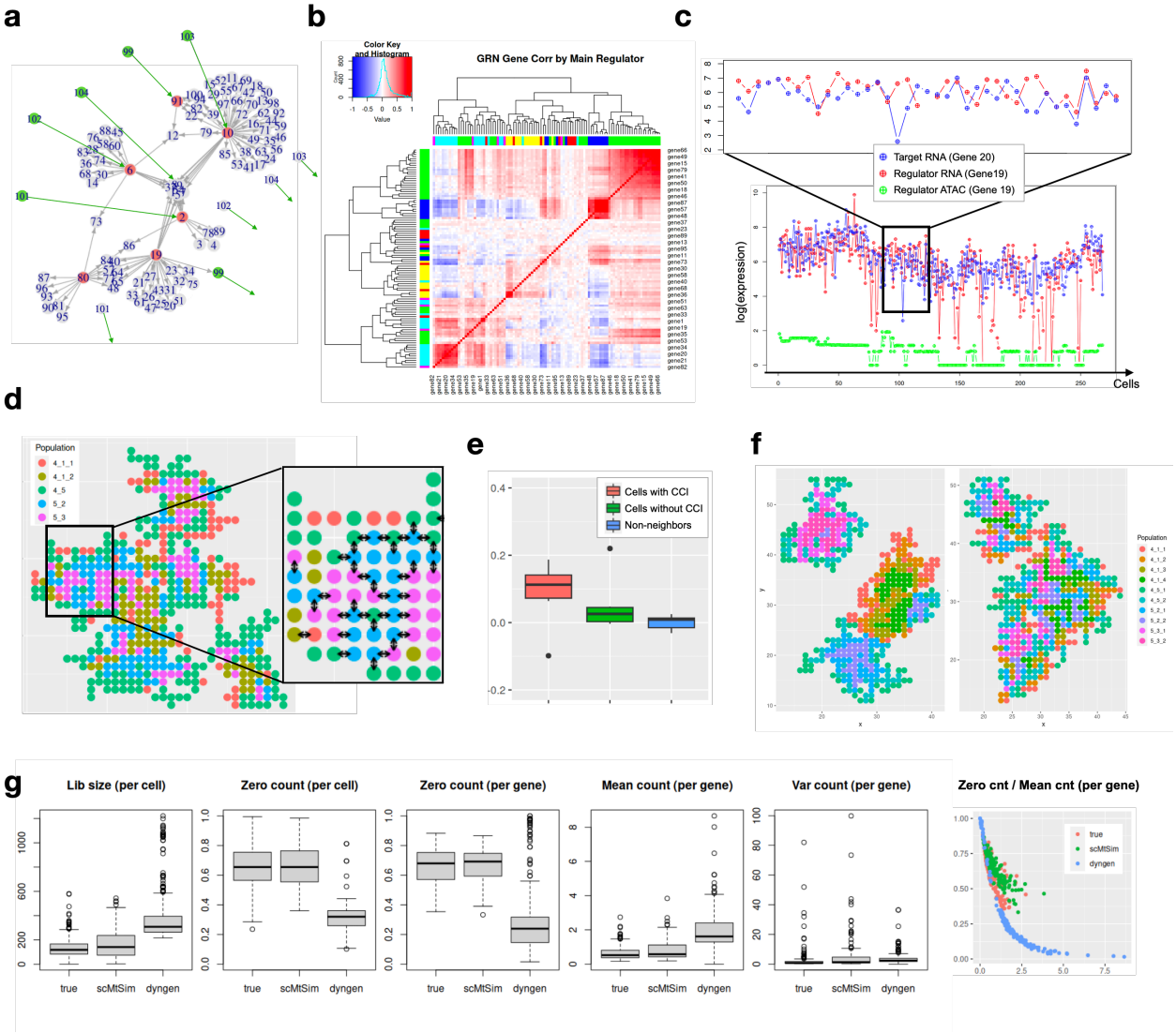

Figure 3. scMultiSim generates realistic single cell gene expression data driven by GRN and cell-cell interaction. See the next page for descriptions.

. scMultiSim generates realistic single cell gene expression data driven by GRN and cell-cell interaction. (**a**) The GRN and CCIs used to generate the main datasets. Red nodes are TF genes and green nodes are ligand genes. Green edges are the added ligand-receptor pairs when simulating cell-cell interactions. (**b-e**) Results from dataset `MT3a`, which uses Phyla3, 500 genes, 500 cells and $\sigma_{\text{cif}} = 0.1$. (**b**) The gene module correlation heatmap. The color at left or top represents the regulating TF of the gene. Genes regulated by the same TF have higher correlations and tend to be grouped together. (**c**) The log-transformed expression of a specific TF-target gene pair (gene19-gene20) for all cells on one lineage (node 4-5-3 in Phyla3). Correlation between the TF and target expressions can be observed. We also show the chromatin accessibility level for the TF gene 19, averaged from the two corresponding chromatin regions of the gene. Significant lower expression can be observed when the level is zero, indicating the chromatin is closed. (**d**) The spatial location of cells, where each color represents a cell type. Arrows between two cells indicates that CCI exists between them for a specific ligand-receptor pair (gene101-gene2). By default, most cell-cell interactions occur between different cell types. (**e**) Gene expression correlation between (1) neighboring cells with CCI, (2) neighboring cells with CCI, and (3) non-neighbor cells. Cells with CCI has higher correlations. (**f**) scMultiSim provides options on the cell layout. We show the results of 1200 cells using same-type probability $p_n = 1.0$ and 0.8, respectively. When $p_n = 1.0$, same-type cells tend to cluster together, while $p_n = 0.8$ introduces more randomness. (**g**) Comparison between a real dataset and simulated data using multiple statistical measurements. Parameters were adjusted to match the real distribution as close as possible.

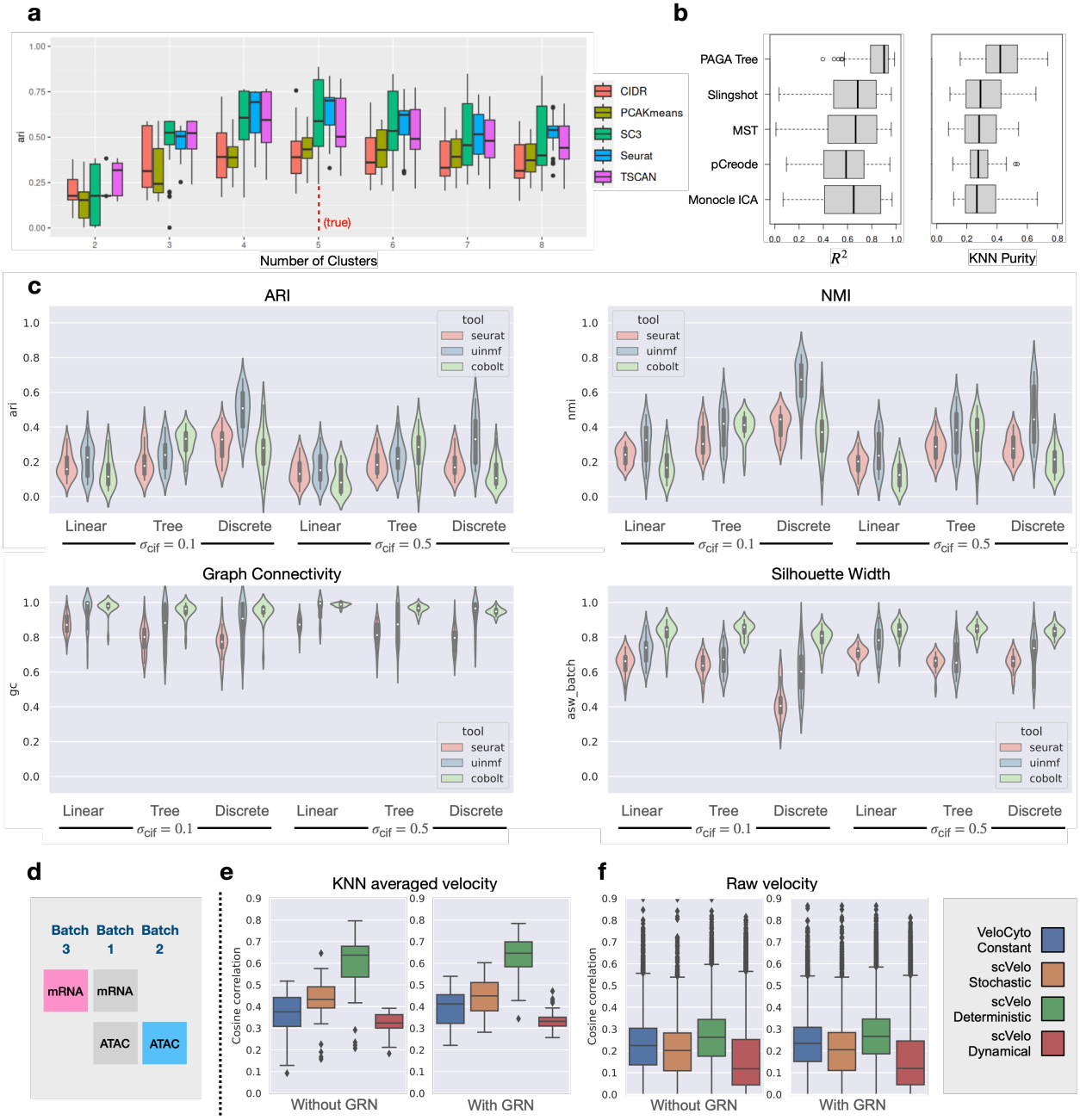

Figure 4. Benchmarking clustering, trajectory inference, multi-modal data integration and RNA velocity estimation methods. (**a**) Benchmarking clustering methods on dataset `MD` (discrete). Methods are grouped by number of clusters in the result. A higher ARI indicates better clustering. (**b**) Benchmarking trajectory inference methods on dataset `MT` (tree). Methods are evaluated based on their mean R^2 and KNN purity on each lineage (higher is better). (**c**) Benchmarking multi-modal data integration methods. Metrics for the methods: ARI, NMI (higher = better at preserving cell identities), graph connectivity and average silhouette width of batch (higher = better merging batches). (**d**) The task illustration of multi-modal data integration. Only cells in batch 1 and 3 (pink and blue matrices) are used for evaluation. (**e,f**) Benchmarking RNA velocity estimation methods on dataset `V`.

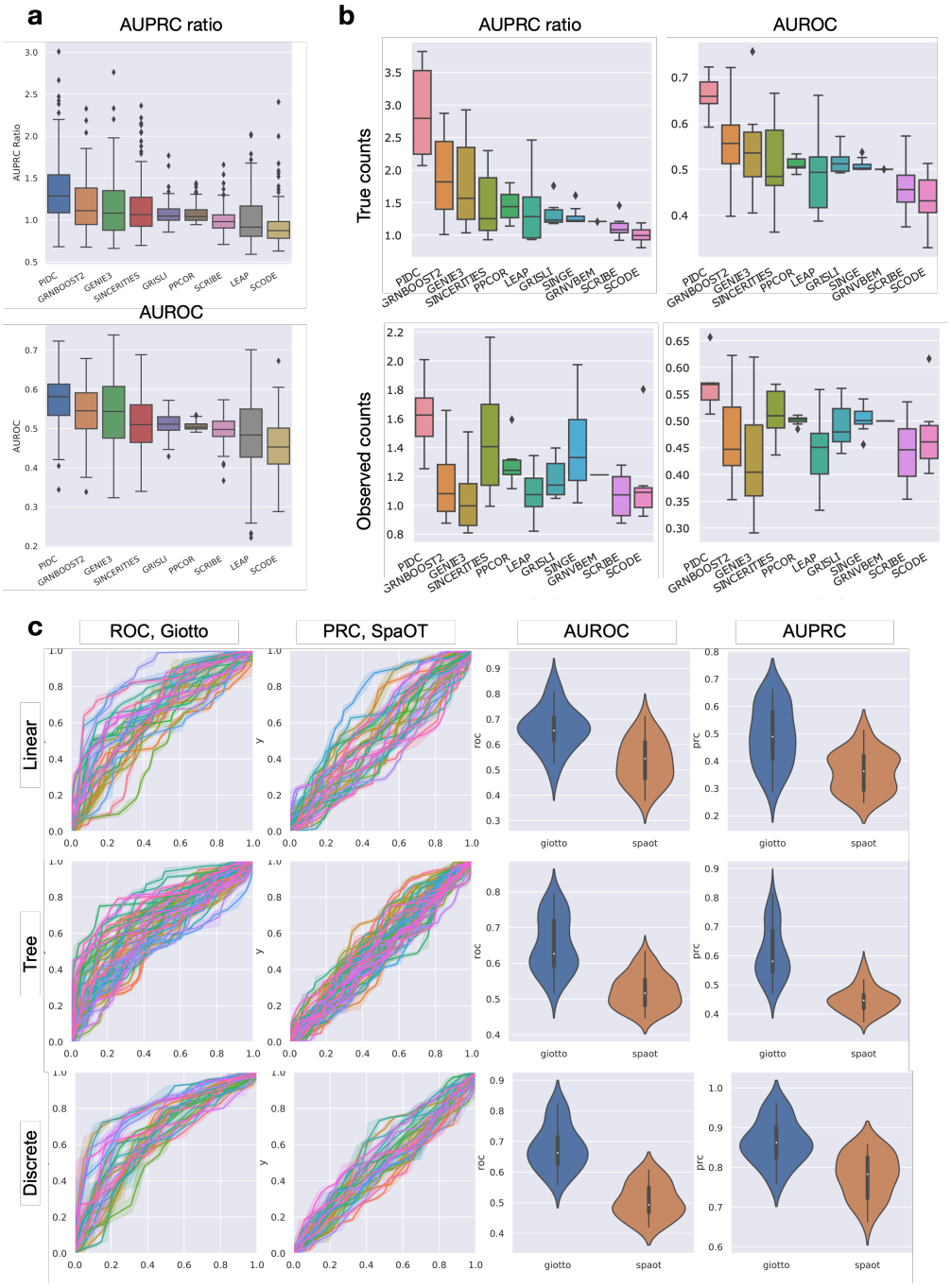

Figure 5. Benchmarking GRN inference and CCI inference methods. (**a**) Benchmarking GRN inference methods. the upper figure shows AUPRC ratios (versus a random classifier), and the lower figure shows AUROC values. (**b**) Benchmarking cell-cell interaction inference methods. Each curve in the ROC/PRC plots correspondings to one dataset. (**a**) Additional results on benchmarking GRN inference methods using datasets G that does not contain CCI effects. We also tested the performance on observed counts with technical noise.