QWENDY: Gene Regulatory Network Inference by Quadruple Covariance Matrices

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Abstract

Knowing gene regulatory networks (GRNs) is important for understanding various biological mechanisms. In this paper, we present a method, QWENDY, that uses single-cell gene expression data measured at four time points to infer GRNs. Based on a linear gene expression model, it solves the transformation of the covariance matrices. Unlike its predecessor WENDY, QWENDY avoids solving a non-convex optimization problem and produces a unique solution. We test the performance of QWENDY on two experimental data sets and two synthetic data sets. Compared to previously tested methods on the same data sets, QWENDY ranks the first on experimental data, although it does not perform well on synthetic data.

1 Introduction

One gene can activate or inhibit the expression of another gene. Genes and such regulation relations form a gene regulatory network (GRN). For n genes, the corresponding GRN is commonly expressed as an $n \times n$ matrix A, where $A_{ij} > 0/ = 0/ < 0$ means gene i has positive/no/negative regulation effect on gene j, and its absolute value represents the regulation strength. If the regulation relations of concerned genes are known, one can understand the corresponding biological process or even control it with gene perturbation. Therefore, knowledge of GRNs can be useful in developmental biology [1, 2, 3], and even in the study of macroscopic behavior [4, 5, 6]. Since it is difficult to determine the GRN directly, the common practice is to infer the GRN from gene expression data. Given new GRN inference methods, we can study how cells maintain homeostasis [7, 8] or be driven away from homeostasis and cause diseases [9, 10].

With new measurement techniques, such as scRNA-seq, one can measure the expression levels (mRNA counts) of different genes for a single cell. Since gene expression at the single cell level is random, this measurement can be repeated for different cells, and we can use those samples to obtain the probability distribution of different genes at this time point. Since the measurement kills cells, one cell can only be measured once. Therefore, we cannot obtain the joint probability distribution of gene expression levels at different time points. When we measure at multiple time points, we can only obtain a marginal probability distribution of different genes for each time point.

If the gene expression is at stationary, measurement at multiple time points will produce the same distribution. If the gene expression is away from stationary, such as after adding drugs or during development, we can measure the gene expression at multiple time points and obtain several different probability distributions. For single-cell expression data of n genes at one time point, we can calculate the mean value of each gene (n independent values) and the $n \times n$ covariance matrix ($n(n+1)/2$ independent values, since the covariance matrix is symmetric), while higher-order statistics are not numerically stable due to limited cell number. Therefore, we have $n + n(n+1)/2$ independent known values, much smaller than what is needed to fully determine the $n \times n$ GRN [11]. Therefore, we prefer the non-stationary distributions measured at multiple time points, since they contain enough information to infer the GRN.

For such single-cell gene expression data measured at multiple time points, although it is the most informative data type under current technology, there are only a few GRN methods developed specifically for this data type [12]. We have developed the WENDY method to infer the GRN with this data type [13]. It needs measurement at two time points, and solves the transformation between the covariance matrices of two probability distributions, where the transformation is determined by the GRN. However, it needs to solve a non-convex optimization problem, which has infinitely many solutions, and WENDY will output one solution, determined by the numerical solver chosen in the realization.

In this paper, we present an improved version of WENDY that needs data measured at four time points, and it is also based on solving the transformation between covariance matrices. This new method is named QWENDY, where Q stands for quadruple. With the help of data from more time points, QWENDY can uniquely determine the GRN. We will prove that under some assumptions, the output of QWENDY is the ground truth GRN. Besides, QWENDY does not need to conduct non-convex optimizations, but just matrix decompositions. Therefore, the output of QWENDY does not depend on the realization of the numerical computing procedure.

In a previous paper [14], we have tested 16 GRN inference methods on two experimental data sets and two synthetic data sets. In this paper, we test the performance of QWENDY on the same data sets and compare it with previous methods. In all 17 GRN inference methods, QWENDY ranks the first on experimental data, although it does not perform well on synthetic data. These results suggest that QWENDY is a promising GRN inference method

that warrants further testing and refinement.

Section 2 reviews other GRN inference methods. Section 3 introduces the QWENDY method, including a proof of its correctness. Section 4 tests the performance of QWENDY on four data sets. We finish with some discussions in Section 5.

2 Literature review

There have been numerous non-deep learning GRN inference methods, and they can be roughly classified as information-based and model-based.

Information-based methods [15, 16, 12] do not rely on models for gene expression, but treat GRN inference as a feature selection problem: for a target gene, select genes that can be used to predict the level of the target gene. A common obstacle for information-based methods is to distinguish between direct and indirect regulations.

Model-based methods construct concrete models for gene expression under regulation, and fit the expression data to the models to determine model parameters and reconstruct the GRN. Some methods [17] require measuring the same cell multiple times, which is not quite applicable for now. Some methods [18, 19] use average expression levels measured over many cells (bulk level), which do not utilize the rich information in the single-cell level measurements. Some methods [20] only work on single-cell data at one time point. For single-cell gene expression data measured at multiple time points, where each cell is measured only once, we only know one model-based method, WENDY. Therefore, we develop QWENDY as an alternative.

For more detailed summaries of traditional GRN inference methods, readers may refer to the literature review section of the WENDY paper [13].

Deep learning-based GRN inference methods [21, 22, 23, 24, 25] generally use neural networks as black boxes, without integrating them with gene expression models. Therefore, lacking of interpretability is a common problem.

We have developed the TRENDY method [14] to enhance the WENDY method with deep learning tools. Similar to TRENDY, there are some approaches to enhance GRNs inferred by other known methods [26, 27, 28, 29, 30], but they generally require extra knowledge of transcription factors or cannot fully determine the GRN.

For more detailed summaries of deep learning-based GRN inference methods and approaches to enhance existing methods, readers may refer to the literature review section of the WENDY paper [13].

Recently, there are some GRN inference methods related to large language models (LLMs), although it is questionable whether LLMs trained with natural language data are useful to the study of gene regulation.

Some researchers directly used LLMs as an oracle machine to generate answers, without further training the model. Azam et al. [31] asked different GRNs whether one gene regulates another gene. Afonja et al. [32] provided GPT-4 with potential transcription factors and asked it to generate a GRN.

Wu et al. [33] provided GPT-4 with related papers and asked it to summarize regulations and form a GRN.

Some researchers worked on pre-trained LLMs and fine-tuned them with new data. Weng et al. [34] trained GPT-3.5 with related papers to obtain GenePT, a new LLM that can provide a high-dimensional embedding (a vector of real numbers) of each gene. Then this embedding was used to train a neural network to output the GRN. Yang et al. [35] trained BERT with scRNA-seq data to obtain scBERT, which can also provide gene embedding. Kommu et al. [36] used the embedding from scBERT to infer the GRN.

Some researchers trained new models from scratch. Cui et al. [37] trained a new model, scGPT, with expression data from many cells. The structure of scGPT is similar to other LLMs, although with a smaller size. This model can provide gene embedding, which was used to infer GRN.

For general applications of LLMs in bioinformatics, readers may refer to two reviews [38, 39].

3 Methods

3.1 Setup

At four time points $T = 0$, $T = t$, $T = 2t$, $T = 3t$, we measure the single-cell gene expression levels. For the expression levels of n genes from m cells at each time point, we treat them as m samples of an n-dimensional probability distribution. Then we first calculate the average level of each gene over m cells, and use graphical lasso to calculate the $n \times n$ covariance matrix for different genes. The $1 \times n$ expected levels at four time points are denoted as $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$. The covariance matrices at four time points are denoted as K_0, K_1, K_2, K_3. Notice that the process does not start from stationary, so that the probability distributions of n genes are different for different time points, and these $\mathbf{x}_i$ and K_i are not equal.

In the WENDY paper, the relationship for K_0, K_1 and the GRN A is derived after some approximations:

$$K_1 = (I + tA^{\mathrm{T}})K_0(I + tA),$$

where I is the $n \times n$ identity matrix. Define $B = I + tA$, we have

$$K_1 = B^{\mathrm{T}} K_0 B. \tag{1}$$

From this equation, WENDY directly solves B (and thus A) by minimizing $||K_1 - B^{\mathrm{T}} K_0 B||_{\mathrm{F}}^2$, where F is the Frobenius norm. This problem is non-convex and has infinitely many solutions. WENDY outputs one solution of them.

For K_2, K_3, similarly, we also have approximated equations

$$K_2 = B^{\mathrm{T}} K_1 B, \tag{2}$$

$$K_3 = B^{\mathrm{T}} K_2 B. \tag{3}$$

In this paper, we study whether we can better solve B with data from more time points, especially K_2, K_3. Since $B^{\mathrm{T}}KB = (-B)^{\mathrm{T}}K(-B)$, we cannot distinguish between B and $-B$ from K_0, K_1, K_2, K_3.

To solve this problem, we use $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$. In the WENDY paper, it is derived that

$$\mathbf{x}_1 = \mathbf{x}_0 B + t\mathbf{c}, \tag{4}$$

where $\mathbf{c}$ is an unknown vector. Similarly,

$$\mathbf{x}_2 = \mathbf{x}_1 B + t\mathbf{c}, \tag{5}$$

$$\mathbf{x}_3 = \mathbf{x}_2 B + t\mathbf{c}. \tag{6}$$

Since Eqs. 1–6 are approximated, there are two problems: **(1)** if these equations hold accurately, can we solve B; **(2)** if these equations do not quite hold, can we find B to minimize the error. We will present the QWENDY method that provides positive answers to both problems.

For the first problem, Theorem 1 proves that given K_0, K_1, K_2, K_3, $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$ that satisfy Eqs. 1–6 for some B_0, QWENDY can solve B_0 uniquely.

For the second problem, there are different interpretations.

One interpretation is to minimize

$$||K_1 - B^T K_0 B||_F^2 + ||K_2 - B^T K_1 B||_F^2 + ||K_3 - B^T K_2 B||_F^2$$

for any B. Unfortunately, this optimization problem is non-convex, making it difficult to solve.

Notice that for the gene expression data after interpretation, K_0 is measured earlier than K_3, making it farther from stationary, and more informative. Therefore, we want to emphasize more on K_0, K_1 than K_2, K_3. Our goal is to first find B that minimizes $K_1 - B^{\mathrm{T}}K_0B$; for such B (not unique), we further determine which minimizes $K_2 - B^{\mathrm{T}}K_1B$; for such B (still not unique), we finally determine which minimizes $K_3 - B^{\mathrm{T}}K_2B$. This time we can solve B uniquely up to a $\pm$ sign, which can be determined by $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$.

We will derive QWENDY by solving the second problem, and then prove that it also solves the first problem.

3.2 Algorithm details

In this section, given general covariance matrices K_0, K_1, K_2, K_3 that may not satisfy Eqs. 1–3 for any B_0, we introduce a procedure to calculate B that approximately solves Eqs. 1–3. Under some mild conditions (some matrices are invertible and have distinct eigenvalues), B can be uniquely determined up to a $\pm$ sign. Then we use $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$ to distinguish between B and $-B$. The whole procedure is named as the QWENDY method.

Step (1): For any B, we want to minimize

$$||K_1 - B^{\mathrm{T}}K_0B||_{\mathrm{F}}^2. \tag{7}$$

Assume K_0, K_1, K_2, K_3 are invertible. Consider Cholesky decompositions

$$K_1 = L_1 L_1^{\mathrm{T}},\ K_0 = L_0 L_0^{\mathrm{T}},$$

where L_1 and L_0 are lower-triangular and invertible. Define

$$O = L_1^{-1} B^{\mathrm{T}} L_0,$$

then the target Eq. 7 becomes

$$||L_1 L_1^{\mathrm{T}} - L_1 O O^{\mathrm{T}} L_1^{\mathrm{T}}||_{\mathrm{F}}^2.$$

Therefore, Eq. 7 is minimized to 0 if and only if O is orthonormal: $OO^{\mathrm{T}} = I$.

Since

$$B = L_0^{-\mathrm{T}} O^{\mathrm{T}} L_1^{\mathrm{T}},$$

we use K_0 and K_1 to restrict B to a space with the same dimension as the set of all orthonormal matrices.

Step (2): For such B that minimizes Eq. 7, we want to find B that makes $B^{\mathrm{T}} K_1 B$ close to K_2. Here we do not minimize

$$||K_2 - B^{\mathrm{T}} K_1 B||_{\mathrm{F}}^2$$

as it is difficult. Instead, we want to minimize

$$||L_1^{-1}(K_2 - B^{\mathrm{T}} K_1 B) L_1^{-\mathrm{T}}||_{\mathrm{F}}^2 \tag{8}$$

among B that minimizes Eq. 7.

Assume that $L_0^{-1} K_1 L_0^{-\mathrm{T}}$ does not have repeated eigenvalues. Consider the eigenvalue decomposition decomposition

$$L_0^{-1} K_1 L_0^{-\mathrm{T}} = P_1 D_1 P_1^{\mathrm{T}},$$

where P_1 is orthonormal, and D_1 is diagonal with strictly increasing positive diagonal elements (eigenvalues), since $L_0^{-1} K_1 L_0^{-\mathrm{T}}$ is positive definite and symmetric. Similarly, assume that $L_1^{-1} K_2 L_1^{-\mathrm{T}}$ does not have repeated eigenvalues, and we have

$$L_1^{-1} K_2 L_1^{-\mathrm{T}} = P_2 D_2 P_2^{\mathrm{T}}$$

with orthonormal P_2 and diagonal D_2 with strictly increasing positive diagonal elements (eigenvalues). Now the target Eq. 8 equals

$$||P_2 D_2 P_2^{\mathrm{T}} - O P_1 D_1 P_1^{\mathrm{T}} O^{\mathrm{T}}||_{\mathrm{F}}^2 = ||D_2 - P_2^{\mathrm{T}} O P_1 D_1 P_1^{\mathrm{T}} O^{\mathrm{T}} P_2||_{\mathrm{F}}^2,$$

since P_2 is orthonormal and does not affect Frobenius norm. Define

$$W = P_2^{\mathrm{T}} O P_1,$$

which is orthonormal. Then

$$O = P_2 W P_1^{\mathrm{T}},$$

and Eq. 8 equals

$$||D_2 - W D_1 W^{\mathrm{T}}||_{\mathrm{F}}^2. \tag{9}$$

We use the following lemma to handle Eq. 9:

Lemma 1. *For diagonal D_1, D_2 with strictly increasing diagonal elements and any orthonormal W, Eq. 9 is minimized when W is diagonal, and the diagonal elements are ± 1. (There are 2^n possibilities for such W.)*

Proof. Denote the diagonal elements of D_1 as $\mathbf{d}_1 = [d_{1,1}, \ldots, d_{1,n}]$ with $d_{1,1} < d_{1,2} < \cdots, < d_{1,n-1} < d_{1,n}$, and similarly $\mathbf{d}_2 = [d_{2,1}, \ldots, d_{2,n}]$ with $d_{2,1} < d_{2,2} < \cdots, < d_{2,n-1} < d_{2,n}$ for D_2. We need to minimize the following norm with an orthonormal W.

$$||D_2 - WD_1W^{\mathrm{T}}||_{\mathrm{F}}^2 = ||D_2||_{\mathrm{F}}^2 + ||WD_1W^{\mathrm{T}}||_{\mathrm{F}}^2 - 2\sum_{i=1}^{n}[D_2 \otimes (WD_1W^{\mathrm{T}})]_{ii}$$

$$=||D_2||_{\mathrm{F}}^2 + ||D_1||_{\mathrm{F}}^2 - 2\sum_{i=1}^{n} d_{2,i} \sum_{j=1}^{n} W_{ij}^2 d_{1,j}$$

$$=||D_2||_{\mathrm{F}}^2 + ||D_1||_{\mathrm{F}}^2 - 2\mathbf{d}_1(W \otimes W)\mathbf{d}_2^{\mathrm{T}},$$

where $\otimes$ is the element-wise product. Thus we just need to maximize $\mathbf{d}_1(W \otimes W)\mathbf{d}_2^{\mathrm{T}}$. Notice that $W \otimes W$ is doubly-stochastic, meaning that it is non-negative, and each row or column has sum 1. By Birkhoff–von Neumann Theorem [40], $W \otimes W$ can be decomposed to

$$W \otimes W = \sum_{i=1}^{k} c_i Q_i,$$

where Q_i is a permutation matrix, $c_i > 0$, and $\sum_{i=1}^{k} c_i = 1$.

Due to the rearrangement inequality [41], for each permutation matrix Q_i,

$$\mathbf{d}_1 Q_i \mathbf{d}_2^{\mathrm{T}} \leq \mathbf{d}_1 I \mathbf{d}_2^{\mathrm{T}} = \mathbf{d}_1 \mathbf{d}_2^{\mathrm{T}},$$

where the equality holds if and only if $Q_i = I$.

Therefore,

$$\mathbf{d}_1(W \otimes W)\mathbf{d}_2^{\mathrm{T}} = \sum_{i=1}^{k} c_i \mathbf{d}_1 Q_i \mathbf{d}_2^{\mathrm{T}} \leq \sum_{i=1}^{k} c_i \mathbf{d}_1 \mathbf{d}_2^{\mathrm{T}} = \mathbf{d}_1 \mathbf{d}_2^{\mathrm{T}},$$

where the equality holds if and only if

$$W \otimes W = I,$$

meaning that W is diagonal, and diagonal elements are ± 1. □

We now have

$$B = L_0^{-\mathrm{T}} P_1 W P_2^{\mathrm{T}} L_1^{\mathrm{T}},$$

meaning that given K_0, K_1, K_2, we can restrict B to 2^n possibilities.

Step (3): For such B that minimizes Eq. 8 among B that minimizes Eq. 7, we want to find B that makes $B^{\mathrm{T}} K_2 B$ close to K_3. Here we do not minimize

$$||K_3 - B^{\mathrm{T}} K_2 B||_{\mathrm{F}}^2$$

as it is difficult. Instead, we want to minimize

$$||L_1^{-1}(K_3 - B^{\mathrm{T}}K_2B)L_1^{-\mathrm{T}}||_{\mathrm{F}}^2. \tag{10}$$

Define

$$G = P_2^{\mathrm{T}}L_1^{-1}K_3L_1^{-\mathrm{T}}P_2,$$

and

$$H = P_1^{\mathrm{T}}L_0^{-1}K_2L_0^{-\mathrm{T}}P_1.$$

Eq. 10 equals

$$\begin{aligned}
&||P_2^{\mathrm{T}}L_1^{-1}K_3L_1^{-\mathrm{T}}P_2 - WP_1^{\mathrm{T}}L_0^{-1}K_2L_0^{-\mathrm{T}}P_1W||_{\mathrm{F}}^2 \\
=&||G - WHW||_{\mathrm{F}}^2 = ||G||_{\mathrm{F}}^2 + ||WHW||_{\mathrm{F}}^2 - 2||G \otimes (WHW)||_{\mathrm{F}}^2 \\
=&||G||_{\mathrm{F}}^2 + ||H||_{\mathrm{F}}^2 - 2||G \otimes (WHW)||_{\mathrm{F}}^2.
\end{aligned} \tag{11}$$

We want to find diagonal W with ± 1 that minimizes Eq. 10, which is equivalent to maximizing $||G \otimes (WHW)||_{\mathrm{F}}^2$. Define $C = G \otimes H$, which is still positive definite and symmetric by Schur product theorem [42]. Assume that C does not have repeated eigenvalues. Denote the diagonal elements of W by $\mathbf{w} = [w_1, \ldots, w_n]$. Then we need to maximize

$$||G \otimes (WHW)||_{\mathrm{F}}^2 = \mathbf{w}C\mathbf{w}^{\mathrm{T}}.$$

Now we relax this problem from $\mathbf{w}$ with $w_i = \pm 1$ to general $\mathbf{v}$ with $||\mathbf{v}||_2^2 = n$:

$$\max_{||\mathbf{v}||_2^2 = n} \mathbf{v}C\mathbf{v}^{\mathrm{T}}.$$

The unique solution (up to a $\pm$ sign) is the eigenvector that corresponds to the largest eigenvalue of C.

After obtaining $\mathbf{v}$, we just need to project it to $[w_1, \ldots, w_n]$ with $w_i = \pm 1$ by taking the sign of each term: $w_i = \mathrm{sign}(v_i)$. Then construct W by putting $[w_1, \ldots, w_n]$ on the diagonal.

This relaxation does not guarantee obtaining the optimal solution for Eq. 11, but we find that it produces the correct answer in almost all simulations. Alternatively, we can directly determine the optimal W for Eq. 11 by brute-force search, as there are finitely many (2^n) possibilities of W.

With

$$B = L_0^{-\mathrm{T}}P_1WP_2^{\mathrm{T}}L_1^{\mathrm{T}},$$

given K_0, K_1, K_2, K_3, we can uniquely determine B up to a $\pm$ sign. Since $B^{\mathrm{T}}KB = (-B)^{\mathrm{T}}K(-B)$, more K_i cannot provide more information.

Step (4): For B and $-B$ from Step (3), we determine which satisfies Eqs. 4–6 better. Notice that Eqs. 4–6 share the same unknown $\mathbf{c}$. Define

$$\mathbf{c}_0 = (\mathbf{x}_1 - \mathbf{x}_0B)/t,\ \mathbf{c}_1 = (\mathbf{x}_2 - \mathbf{x}_1B)/t,\ \mathbf{c}_2 = (\mathbf{x}_3 - \mathbf{x}_2B)/t,\ \bar{\mathbf{c}} = (\mathbf{c}_0 + \mathbf{c}_1 + \mathbf{c}_2)/3.$$

Then the error for fitting Eqs. 4–6 is

$$||\mathbf{c}_0 - \bar{\mathbf{c}}||_2^2 + ||\mathbf{c}_1 - \bar{\mathbf{c}}||_2^2 + ||\mathbf{c}_2 - \bar{\mathbf{c}}||_2^2 = ||\mathbf{c}_0||_2^2 + ||\mathbf{c}_1||_2^2 + ||\mathbf{c}_2||_2^2 - 3||\bar{\mathbf{c}}||_2^2.$$

We just need to compare the errors of B and $-B$ and choose the smaller one as the output.

With expression data from four time points, we uniquely determine B. See Algorithm 1 for the workflow of the QWENDY method.

1. **Input** expression levels of n genes over m cells at time points 0, t, $2t$, $3t$
2. **Calculate** covariance matrices K_0, K_1, K_2, K_3, and mean levels $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$
3. **Calculate** Cholesky decomposition

$$K_1 = L_1 L_1^{\mathrm{T}},\ K_0 = L_0 L_0^{\mathrm{T}}$$

   Calculate eigenvalue decomposition

$$L_0^{-1} K_1 L_0^{-\mathrm{T}} = P_1 D_1 P_1^{\mathrm{T}},\ L_1^{-1} K_2 L_1^{-\mathrm{T}} = P_2 D_2 P_2^{\mathrm{T}}$$

   Calculate

$$C = (P_2^{\mathrm{T}} L_1^{-1} K_3 L_1^{-\mathrm{T}} P_2) \otimes (P_1^{\mathrm{T}} L_0^{-1} K_2 L_0^{-\mathrm{T}} P_1)$$

4. **Calculate** $\mathbf{v}$, the eigenvector that corresponds to the largest eigenvalue of C, and the projection $\mathbf{w}$ with $w_i = \mathrm{sign}(v_i)$

   Construct W with $\mathbf{w}$ on diagonal
5. **Calculate** $B = L_0^{-\mathrm{T}} P_1 W P_2^{\mathrm{T}} L_1^{\mathrm{T}}$

   Compare total squared errors of B and $-B$ for Eqs. 4–6
6. **Output** B or $-B$, the one that corresponds to the smaller error, and the GRN $A = (B - I)/t$

Algorithm 1: Workflow of the QWENDY method.

3.3 Correctness of the QWENDY method

Theorem 1. *If covariance matrices K_0, K_1, K_2, K_3 and mean levels $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$ satisfy Eqs. 1–6 for some B_0, then QWENDY will output B_0.*

Proof. If $K_1 = B_0^{\mathrm{T}} K_0 B_0$, then

$$(L_1^{-1} B_0^{\mathrm{T}} L_0)(L_0^{\mathrm{T}} B_0 L_1^{-\mathrm{T}}) = I,$$

and $L_1^{-1} B_0^{\mathrm{T}} L_0 = O_0$ for some orthonormal O_0. Thus

$$B_0 = L_0^{-\mathrm{T}} O_0^{\mathrm{T}} L_1^{\mathrm{T}},$$

which is among the calculated B in Step (1).

If $K_2 = B_0^{\mathrm{T}} K_1 B_0$, then

$$D_2 = P_2^{\mathrm{T}} O_0 P_1 D_1 P_1^{\mathrm{T}} O_0^{\mathrm{T}} P_2.$$

Since $P_2^{\mathrm{T}} O_0 P_1$ is orthonormal, D_1 and D_2 are similar and have the same eigenvalues. Therefore, D_1 and D_2 as diagonal matrices with increasing diagonal values are equal. Assume that the diagonal elements $d_1, \ldots, d_n$ of D_1 are distinct. Define $W_0 = P_2^{\mathrm{T}} O_0 P_1$, then

$$D_1 W_0 = W_0 D_1,$$

and $W_0[i,j]d_i = W_0[i,j]d_j$. For $i \neq j$, we have $d_i \neq d_j$, meaning that $W_0[i,j] = 0$. Now W_0 is diagonal and orthonormal, implying that its diagonal elements are 1 or -1, and

$$B_0 = L_0^{-\mathrm{T}} P_1 W_0 P_2^{\mathrm{T}} L_1^{\mathrm{T}}$$

is among the calculated B in Step (2).

If $K_3 = B_0^{\mathrm{T}} K_2 B_0$, then

$$P_2^{\mathrm{T}} L_1^{-1} K_3 L_1^{-\mathrm{T}} P_2 = W_0 P_1^{\mathrm{T}} L_0^{-1} K_2 L_0^{-\mathrm{T}} P_1 W_0.$$

Define $\mathbf{w}_0 = [w_1, \ldots, w_n]$ to be the diagonal elements of W_0. Then from Step (3), W_0 minimizes Eq. 10, and $\mathbf{w}_0$ is the solution to

$$\max_{||\mathbf{v}||_2^2 = n} \mathbf{v} C \mathbf{v}^{\mathrm{T}},$$

namely the eigenvector of the largest eigenvalue. Since $w_i = \pm 1$, the projection $w_i = \mathrm{sign}(v_i)$ has no effect, and

$$B_0 = L_0^{-\mathrm{T}} P_1 W_0 P_2^{\mathrm{T}} L_1^{\mathrm{T}}$$

is the unique B (up to a $\pm$ sign) calculated in Step (3).

From Eqs. 4–6,

$$\mathbf{x}_1 - \mathbf{x}_0 B_0 = \mathbf{x}_2 - \mathbf{x}_1 B_0.$$

Assume $\mathbf{x}_1 \neq \mathbf{x}_2$, then

$$\mathbf{x}_1 - \mathbf{x}_0(-B_0) \neq \mathbf{x}_2 - \mathbf{x}_1(-B_0).$$

Therefore, $-B_0$ does not satisfy Eqs. 4–6, and Step (4) chooses the correct B_0 from the two possibilities in Step (3). □

4 Performance on synthetic and experimental data

The same as in the WENDY paper and the TRENDY paper [13, 14], we test the QWENDY method on two synthetic data sets and two experimental data

sets. SINC data set [14] and DREAM4 data set [43] are generated by simulating stochastic differential equation systems. THP-1 data set is from monocytic THP-1 human myeloid leukemia cells [44]. hESC data set is from human embryonic stem cell-derived progenitor cells[45]. THP-1 and hESC data sets each has only one group of data (one ground truth GRN and the corresponding expression levels); DREAM4 data set has five groups of data; SINC data set has 1000 groups of data.

For SINC data set and DREAM4 data set, we use data from any four consecutive time points and take average. For THP-1 data set and hESC data set, we choose any four time points with equal difference. This is due to the limitation that QWENDY is derived for four evenly spaced time points.

We test the performance of the QWENDY method on these four data sets and compare it with the performance of 16 methods tested on the same data sets in the TRENDY paper [14]. We compare the inferred GRN and the ground truth GRN by calculating the AUROC and AUPRC scores [13]. These two scores, both between 0 and 1, evaluate the level of matching, where 1 means perfect match, and 0 means perfect mismatch.

See Table 1 for the performance of QWENDY, compared with other 16 previously tested methods. Although it does not perform very well on synthetic data sets, QWENDY has the best performance on experimental data sets.

5 Discussion

In this paper, we present QWENDY, a GRN inference method that requires single-cell gene expression data measured at four time points. QWENDY ranks the first in 17 GRN inference methods on experimental data sets. Notice that each experimental data set only has one group of data, meaning that the performance of each method has a large uncertainty level.

The QWENDY method requires measurements at four **evenly spaced** time points t_0, t_1, t_2, t_3, meaning that $t_1 - t_0 = t_2 - t_1 = t_3 - t_2$. Otherwise, the dynamics of covariance matrices is much more complicated, and we do not have an explicit solution. One possible strategy is to use the approximation

$$I + (t_2 - t_1)A \approx \frac{t_2 - t_1}{t_1 - t_0} \left[I + (t_1 - t_0)A \right],$$

so that different B in Eqs. 1-3 only differ by a constant factor. This means that we can relocate this factor to K_i, and B is the same for all equations.

Since QWENDY needs four time points, the duration of the whole experiment might be too long, so that the dynamics of gene expression under regulation might have changed during this time. QWENDY is based on a time-homogeneous model, which might fail in this situation.

Although QWENDY can fully determine the GRN, the diagonal elements that represent the regulation of one gene to itself (autoregulation) can be mixed with natural mRNA synthesis and degradation [46]. Therefore, it is not recommended to infer the autoregulation with QWENDY or its variants.

		QWENDY	Best in other 16	Rank of QWENDY in all 17
SINC	AUROC	0.5107	0.8703	11th
	AUPRC	0.5537	0.7672	7th
DREAM4	AUROC	0.4987	0.5741	11th
	AUPRC	0.1844	0.2452	13th
Synthetic total		1.7475	2.3584	11th
THP-1	AUROC	0.5524	0.6261	8th
	AUPRC	0.4294	0.4205	1st
hESC	AUROC	0.6019	0.6233	3rd
	AUPRC	0.0435	0.0641	8th
Experimental total		1.6272	1.5783	1st
Overall total		3.3747	3.8497	9th

Table 1: AUROC and AUPRC scores of QWENDY on four data sets, compared with 16 previously tested methods

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Data and code availability

Data and code files used in this paper can be found in

`https://github.com/YueWangMathbio/QWENDY`

A Enhancing QWENDY with deep learning

A.1 Methods

WENDY method is derived from a simplified gene expression model, and the performance of WENDY is not satisfactory when the model does not fit with

experiments. We developed the TRENDY method [14], which trains a transformer model, a deep learning architecture that can be applied to various fields [47], to transform the input data to better fit the gene expression model. Then the transformed data will produce more accurate GRNs by WENDY. Such more accurate GRNs will be further enhanced by another transformer model.

We use the idea of TRENDY to enhance QWENDY. The enhanced version of QWENDY that trains two new transformer models is named TEQWENDY. Besides, we also present another approach that fine-tunes a large language model (LLM) to replace the transformer model, since it has a large pre-trained transformer section. This LLM-enhanced version is named LEQWENDY. The training of TEQWENDY and LEQWENDY uses synthetic data.

The QWENDY method is derived from Eqs. 1–6, especially Eqs. 1–3, which are a linear approximation of the actual nonlinear gene expression dynamics. Therefore, real K_0, K_1, K_2, K_3 might not fit with Eqs. 1–3, and it might not be feasible to apply QWENDY directly to real K_0, K_1, K_2, K_3. This problem already exists for the WENDY method, where the input matrices K_0, K_1 might not satisfy

$$K_1 = B^{\mathrm{T}} K_0 B.$$

For this problem, the TRENDY method [14] proposes that we can construct $K_1^* = B^{\mathrm{T}} K_0 B$, and train a model with input K_1 and target K_1^*, so that the output K_1' is close to K_1^*. Then we can apply WENDY to K_0, K_1' to obtain a more accurate GRN A_1. After that, we can train another model with input K_0, K_1, A_1, and the target is the true GRN A_{true}. Then the final output A_2 is more similar to the true GRN than A_1.

Inspired by TRENDY, we propose a similar solution to enhance QWENDY. See Algorithms S1,S2 for details. Since $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$ are only used to distinguish between B and $-B$, it is not necessary to train another model for them.

For those two matrix-learning models in Algorithms S1,S2, we can adopt the approach of TRENDY to construct a transformer structure with three sections: (1) Pre-process the inputs; (2) Use transformer encoder layers to learn high-dimensional representations of the inputs; (3) Construct outputs from the high-dimensional representations.

For training the transformer encoder layers in this structure, we present two approaches. One approach is to train new transformer encoder layers from scratch, the same as TRENDY. This structure is named "TE", meaning "transformer-enhanced".

The other approach is to integrate an LLM and fine-tune it. In general, an LLM has three sections: (1) Convert text to vector representations; (2) Use transformer (encoder layers, decoder layers, or both) to learn contextual relationships; (3) Convert model outputs back to natural language [48]. We can choose an LLM with pre-trained transformer encoder layers in the second section, and use them to replace the transformer encoder layers in the TE structure. In our practice, we use the encoder layers of the RoBERTa-large model [49], which have about 300 million parameters.

The pre-trained parameters will be kept frozen while we adopt a parameter-

1. **Repeat** generating random GRN A_{true} and corresponding gene expression data at time 0, t, $2t$, $3t$ from a gene expression simulator
2. **Calculate** covariance matrices K_0, K_1, K_2, K_3 and mean levels $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$, and then calculate

$$K_0^* = K_0,\ K_1^* = B^{\mathrm{T}} K_0^* B,\ K_2^* = B^{\mathrm{T}} K_1^* B,\ K_3^* = B^{\mathrm{T}} K_2^* B,$$

   where $B = I + tA_{\text{true}}$
3. **Train** matrix-learning model 1 with inputs K_0, K_1, K_2, K_3 and target K_0^*, K_1^*, K_2^*, K_3^*

   Call trained matrix-learning model 1 to calculate K_0', K_1', K_2', K_3' from K_0, K_1, K_2, K_3
4. **Call** QWENDY to calculate A_1 from K_0', K_1', K_2', K_3', $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$
5. **Train** matrix-learning model 2 with input A_1, K_0, K_1, K_2, K_3 and target A_{true}

Algorithm S1: Training workflow of LEQWENDY/TEQWENDY method. For those two matrix-learning models, LEQWENDY adopts LE structure, and TEQWENDY adopts TE structure.

1. **Input**: gene expression data at four equally spaced time points
2. **Calculate** covariance matrices K_0, K_1, K_2, K_3 and mean levels $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$
3. **Call** trained matrix-learning model 1 to calculate K_0', K_1', K_2', K_3' from K_0, K_1, K_2, K_3
4. **Call** QWENDY to calculate A_1 from K_0', K_1', K_2', K_3', $\mathbf{x}_0$, $\mathbf{x}_1$, $\mathbf{x}_2$, $\mathbf{x}_3$
5. **Call** trained matrix-learning model 2 to calculate A_2 from A_1, K_0, K_1, K_2, K_3
6. **Output**: inferred GRN A_2

Algorithm S2: Testing workflow of LEQWENDY/TEQWENDY method. For those two matrix-learning models, LEQWENDY adopts LE structure, and TEQWENDY adopts TE structure.

efficient fine-tuning method: LoRA [50]. Lower-rank matrices will be trained and added to the frozen encoding layers to obtain a new encoder in a cost-efficient way. The new structure with RoBERTa is named "LE", representing "LLM-enhanced".

We name Algorithms S1,S2 with TE structure as TEQWENDY, and Algorithms S1,S2 with LE structure as LEQWENDY. See Appendix for details of TE and LE structures. TEQWENDY has 4.7 million trainable parameters. LEQWENDY has 4.6 million trainable parameters, with 300 million non-trainable (frozen) parameters.

TEQWENDY and LEQWENDY are trained on synthetic data generated by [51, 12, 13, 14]

$$\mathrm{d}X_j(t) = V\left\{\beta\prod_{i=1}^{n}\left[1+(A_{\mathrm{true}})_{i,j}\frac{X_i(t)}{X_i(t)+1}\right]-\theta X_j(t)\right\}\mathrm{d}t+\sigma X_j(t)\mathrm{d}W_j(t), \tag{S1}$$

where $X_i(t)$ is the level of gene i at time t, $W_j(t)$ is a standard Brownian motion, and $V = 30$, $\beta = 1$, $\theta = 0.2$, $\sigma = 0.1$. There are 10^5 training samples, each with the expression levels of 10 genes for 100 cells, measured at four time points: 0.0, 0.1, 0.2, 0.3.

QWENDY requires that K_0, K_1, K_2, K_3 are symmetric and positive definite. Although the input K_i and the target K_i^* are naturally symmetric and positive definite, the learned output K_i' in Step (3) of Algorithms S1,S2 might not be positive definite, or even symmetric. Therefore, in the implementation of QWENDY, we add two extra steps to adjust the input covariance matrices:
1. If K_i is asymmetric, replace K_i by $(K_i + K_i^{\mathrm{T}})/2$.
2. If K_i is not positive definite, in the eigenvalue decomposition $K_i = O\Lambda O^{\mathrm{T}}$, where O is orthonormal, and Λ is diagonal, replace negative values of Λ by small positive values to obtain Λ', and replace K_i by $O\Lambda' O^{\mathrm{T}}$.

A.2 Performance

We also test the performance of TEQWENDY and LEQWENDY on the same data sets. See Table S1 for the scores. Compared with QWENDY, TEQWENDY performs better on synthetic data, but worse on experimental data. The overall performance of TEQWENDY is slightly better than QWENDY. Therefore, the improvement of TEQWENDY might be due to the overfitting on synthetic data, which does not work on experimental data. The performance of LEQWENDY is worse than QWENDY on both synthetic and experimental data, meaning that LLMs trained with natural language inputs might not directly help with the numerical task in GRN inference, even after task-specific fine-tuning.

Training TEQWENDY and LEQWENDY on data generated by Eq. S1 does not necessarily increase their performance on experimental data sets. This means that Eq. S1 might not faithfully reflect the gene expression dynamics. To better integrate deep learning techniques, we need better gene expression data generators. Another possibility is that the training data are only from

time 0.0 - 0.3. We could use data from later time points to increase the robustness of training.

		QWENDY	LEQ-WENDY	TEQ-WENDY
SINC	AUROC	0.5107	0.4990	0.5932
	AUPRC	0.5537	0.5183	0.6014
DREAM4	AUROC	0.4987	0.5164	0.5372
	AUPRC	0.1844	0.1823	0.2203
Synthetic total		1.7475	1.7160	1.9521
THP-1	AUROC	0.5524	0.5543	0.5415
	AUPRC	0.4294	0.3632	0.3801
hESC	AUROC	0.6019	0.5905	0.4815
	AUPRC	0.0435	0.0367	0.0317
Experimental total		1.6272	1.5447	1.4348
Overall total		3.3747	3.2607	3.3869
Overall rank in all 19		10th	11th	9th

Table S1: AUROC and AUPRC scores of QWENDY, LEQWENDY, and TEQWENDY on four data sets. The overall rank is for 19 methods: 16 previously tested methods, QWENDY, LEQWENDY, and TEQWENDY

A.3 Technical details

For all models in TEQWENDY and LEQWENDY, the loss function is mean squared error; the optimizer is Adam with learning rate 0.001; the number of training epochs is 100. After each epoch, we evaluate the model performance on a validation set with 1000 samples. The training stops early and rolls back to the best status if there is no improvement for consecutive 10 epochs.

See Algorithm S3 for the structure of the first half of TEQWENDY. The inputs are four covariance matrices K_0, K_1, K_2, K_3. The targets are four revised covariance matrices K_0^*, K_1^*, K_2^*, K_3^*. Notice that the first input matrix (K_0)

is not processed through these layers, since the target K_0^* equals K_0. Besides, K_1, K_2, K_3 are processed separately.

1. **Input**: four covariance matrices (4 groups of $n \times n$)
2. Linear embedding layer with dimension 1 to $d = 64$ (4 groups of $n \times n \times d$)
3. ReLU activation function (4 groups of $n \times n \times d$)
4. Linear embedding layer with dimension d to d (4 groups of $n \times n \times d$)
5. 2-D positional encoding layer (4 groups of $n \times n \times d$)
6. Flattening and concatenation (4 groups of $(n^2) \times d$)
7. 7 layers of transformer encoder with 4 heads, dimension d, feedforward dimension $4d$, dropout rate 0.1 (4 groups of $(n^2) \times d$)
8. Linear embedding layer with dimension d to d (4 groups of $(n^2) \times d$)
9. LeakyReLU activation function with $\alpha = 0.1$ (4 groups of $n \times n \times d$)
10. Linear embedding layer with dimension d to 1 (4 groups of $(n^2) \times 1$)
11. **Output**: reshaping into four matrices (4 groups of $n \times n$)

Algorithm S3: Structure of TEQWENDY method, first half. The shape of data after each layer is in the brackets.

The 2-D positional encoding layer incorporates spatial information of matrix to the input. It generates an $n \times n \times d$ array PE and adds it to the embedded input: For x and y in $1, 2, \ldots, n$ and j in $1, \ldots, d/4$,

$$\begin{aligned}
&\mathrm{PE}[x, y, 2j-1] = \cos[(x-1) \times 10^{-16(j-1)/d}],\\
&\mathrm{PE}[x, y, 2j] = \sin[(x-1) \times 10^{-16(j-1)/d}],\\
&\mathrm{PE}[x, y, 2j-1+d/2] = \cos[(y-1) \times 10^{-16(j-1)/d}],\\
&\mathrm{PE}[x, y, 2j+d/2] = \sin[(y-1) \times 10^{-16(j-1)/d}].
\end{aligned}$$

See Algorithm S4 for the structure of the second half of TEQWENDY. After obtaining the outputs K_0', K_1', K_2', K_3' from K_0, K_1, K_2, K_3 by the first half of TEQWENDY, call the QWENDY method to calculate the inferred GRN A_1 from K_0', K_1', K_2', K_3'. The inputs of the second half of TEQWENDY are four covariance matrices K_0, K_1, K_2, K_3, and the inferred GRN A_1. The target is the ground truth GRN A_{true}.

The segment embedding layer generates different trainable d-dimensional vectors for all five inputs. Then each vector is copied into dimension $n \times n \times d$, and added to the embedded inputs. This layer incorporates the source of inputs. The

1. **Input**: four covariance matrices and one inferred GRN (5 groups of $n \times n$)
2. Linear embedding layer with dimension 1 to $d = 64$ (5 groups of $n \times n \times d$)
3. Segment embedding layer (5 groups of $n \times n \times d$)
4. 2-D positional encoding layer (5 groups of $n \times n \times d$)
5. Flattening and concatenation $((n^2) \times (5d))$
6. 3 layers of transformer encoder with 4 heads, dimension $5d$, feedforward dimension $20d$, dropout rate 0.1 $((n^2) \times (5d))$
7. Linear embedding layer with dimension $5d$ to 1 $((n^2) \times 1)$
8. **Output**: reshaping into one matrix $(n \times n)$

Algorithm S4: Structure of TEQWENDY method, second half. The shape of data after each layer is in the brackets.

final input of the transformer encoder layers is a matrix, with shape $k \times D$, where k is the total number of input values, and D is the representation dimension. For each location in $1, 2, \ldots, k$, the segment embedding marks which input matrix it is from, and the position encoding marks which position in the matrix it is from. These two layers solve the problem that the inputs are multiple matrices, but the inputs of transformer are representations of a 1-D sequence.

See Algorithm S5 for the structure of the first half of LEQWENDY. The inputs are four covariance matrices K_0, K_1, K_2, K_3. The targets are four revised covariance matrices K_0^*, K_1^*, K_2^*, K_3^*. Notice that the first input matrix (K_0) is not processed through these layers, since the target K_0^* equals K_0. Besides, K_1, K_2, K_3 are processed separately.

For each large pre-trained weight matrix W with size $p \times q$, LoRA freezes W and replace it by $W + \Delta W$. Here $\Delta W = (\text{LoRA-}\alpha/r)AB$, where the trainable A has size $p \times r$, and the trainable B has size $r \times q$. The total number of trainable parameters decreases from pq to $(p + q)r$, since $r \ll p, q$. The scaling factor LoRA-α controls the update rate.

See Algorithm S6 for the structure of the second half of LEQWENDY. After obtaining the outputs K_0', K_1', K_2', K_3' from K_0, K_1, K_2, K_3 by the first half of LEQWENDY, call the QWENDY method to calculate the inferred GRN A_1 from K_0', K_1', K_2', K_3'. The inputs of the second half of LEQWENDY are four covariance matrices K_0, K_1, K_2, K_3, and the inferred GRN A_1. The target is the ground truth GRN A_{true}. Since the transformer encoder part of the RoBERTa-large model has a fixed dimension 1024, we need to apply $d_1 = 192$ for each covariance matrix input, and $d_2 = 256$ for the GRN input, so that the

1. **Input**: four covariance matrices (4 groups of $n \times n$)
2. Linear embedding layer with dimension 1 to $d = 256$ (4 groups of $n \times n \times d$)
3. ReLU activation function (4 groups of $n \times n \times d$)
4. Linear embedding layer with dimension d to d (4 groups of $n \times n \times d$)
5. Segment embedding layer (4 groups of $n \times n \times d$)
6. 2-D positional encoding layer (4 groups of $n \times n \times d$)
7. Flattening and concatenation $((n^2) \times (4d))$
8. Transformer encoder part of the RoBERTa-large model, frozen: 24 layers of transformer encoder with 16 heads, dimension $4d$, feedforward dimension $16d$, dropout rate 0.1;

   Trainable LoRA layers with rank $r = 8$ and LoRA-$\alpha = 16$, added to each transformer encoder layer $((n^2) \times (4d))$
9. Four different linear embedding layers with dimension $4d$ to $2d$ (4 groups of $(n^2) \times (2d)$)
10. LeakyReLU activation function with $\alpha = 0.1$ (4 groups of $(n^2) \times (2d)$)
11. Four different linear embedding layers with dimension $2d$ to 1 (4 groups of $(n^2) \times 1$)
12. **Output**: reshaping into four matrices (4 groups of $n \times n$)

Algorithm S5: Structure of LEQWENDY method, first half. The shape of data after each layer is in the brackets.

total dimension is $d = 4d_1 + d_2 = 1024$.

1. **Input**: four covariance matrices and one inferred GRN (5 groups of $n \times n$)
2. Linear embedding layer with dimension 1 to $d_1 = 192$ or $d_2 = 256$ (4 groups of $n \times n \times d_1$ and 1 group of $n \times n \times d_2$)
3. Segment embedding layer (4 groups of $n \times n \times d_1$ and 1 group of $n \times n \times d_2$)
4. 2-D positional encoding layer (4 groups of $n \times n \times d_1$ and 1 group of $n \times n \times d_2$)
5. Flattening and concatenation $((n^2) \times (d = 4d_1 + d_2 = 1024))$
6. Transformer encoder part of the RoBERTa-large model, frozen: 24 layers of transformer encoder with 16 heads, dimension $4d$, feedforward dimension $16d$, dropout rate 0.1;

   Trainable LoRA layers with rank $r = 16$ and LoRA-$\alpha = 32$, added to each transformer encoder layer $((n^2) \times d)$
7. Linear embedding layer with dimension d to d $((n^2) \times d)$
8. LeakyReLU activation function with $\alpha = 0.1$ $((n^2) \times d)$
9. Linear embedding layer with dimension d to 1 $((n^2) \times 1)$
10. **Output**: reshaping into one matrix $(n \times n)$

Algorithm S6: Structure of LEQWENDY method, second half. The shape of data after each layer is in the brackets.

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