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Multifaceted evolution focused on maximal exploitation of domain knowledge for the consensus inference of Gene Regulatory Networks

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ABSTRACT

The inference of gene regulatory networks (GRNs) is a fundamental challenge in systems biology, aiming to decipher gene interactions from expression data. However, traditional inference techniques exhibit disparities in their results and a clear preference for specific datasets. To address this issue, we present BIO-INSIGHT (Biologically Informed Optimizer - INtegrating Software to Infer GRNs by Holistic Thinking), a parallel asynchronous many-objective evolutionary algorithm that optimizes the consensus among multiple inference methods guided by biologically relevant objectives. BIO-INSIGHT has been evaluated on an academic benchmark of 106 GRNs, comparing its performance against MO-GENECI and other consensus strategies. The results show a statistically significant improvement in AUROC and AUPR, demonstrating that biologically guided optimization outperforms primarily mathematical approaches. Additionally, BIO-INSIGHT was applied to gene expression data from patients with fibromyalgia, myalgic encephalomyelitis, and co-diagnosis of both diseases. The inferred networks revealed regulatory interactions specific to each condition, suggesting its clinical utility in biomarker identification and potential therapeutic targets. The robustness and ingenuity of BIO-INSIGHT consolidate its potential as an innovative tool for GRN inference, enabling the generation of more accurate and biologically feasible networks. The source code is hosted in a public GitHub repository under the MIT license: https://github.com/AdrianSeguraOrtiz/BIO-INSIGHT. Moreover, to facilitate its reproducibility and usage, the software associated with this implementation has been packaged into a Python library available on PyPI: https://pypi.org/project/GENECI/3.0.1/.

1. Introduction

Gene regulatory networks (GRNs) are complex systems through which cells control gene expression, determining how genes are activated or deactivated to produce specific proteins. These networks are essential for tasks such as: enabling organisms to respond to environmental changes, carrying out cellular functions, and developing complex structures during growth and differentiation [1]. Understanding them is not only fundamental for deciphering the underlying mechanisms of organism development and function, but also crucial for identifying new therapeutic targets [2,3] and advancing personalized medicine [4,5].

Mapping these intricate networks requires deciphering the complex relationships between genes and their regulators. A common approach to achieving this goal is through the analysis of expression data, leveraging a wide range of computational strategies, each with its own methodological foundations [6–8]. From information theory-based methods [9–11] to advanced machine learning models [12,13], researchers employ diverse approaches to unravel the regulatory dialogues encoded within cells.

However, inferring GRNs remains a challenging task due to the inherent complexity of gene regulation and several well-documented limitations in current methodologies. A key issue is the high variability in the networks inferred by different techniques, even when applied to the same dataset [14]. This variability not only reflects methodological differences, but also reveals a form of specialization, whereby certain techniques perform remarkably well on specific types of networks or experimental conditions, but yield suboptimal results in others [15]. This inconsistency complicates the selection of inference tools and reduces

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confidence in their general applicability. Additionally, gene expression data is inherently noisy, which may lead to the inclusion of spurious associations or the omission of true regulatory links [16]. Finally, many existing approaches are primarily driven by mathematical or statistical criteria, with little or no integration of biological context [17]. In the absence of biologically informed guidance, the inferred networks may lack functional relevance or be difficult to interpret experimentally.

While mathematical rigour is indispensable, the hypothesis of this study is that: *integrating multiple inference techniques into a biologically guided consensus framework can exploit their methodological complementarity and mitigate individual weaknesses, ultimately enhancing the quality of GRN reconstruction by generating networks that are more robust, coherent, and biologically interpretable.*

Recent advances, such as those introduced by MO-GENECI [18], have demonstrated that initial versions of this approach yield promising results. However, the biological complexity of GRNs requires greater attention and coverage beyond the degree distribution of their nodes and the enumeration of a predefined set of motifs. This expansion of knowledge must be carried out while maintaining the balance of opposing objectives, ensuring proper alignment with inference accuracy, and preserving computational feasibility for large-scale networks.

To overcome these challenges, this work introduces **BIO-INSIGHT (Biologically Informed Optimizer - INtegrating Software to Infer GRNs by Holistic Thinking)**, an innovative algorithmic approach that brings three main contributions to the current state of the art:

- **Objective space with high biological coverage**: Beyond the aspects already explored in the current literature, this proposal incorporates three main novelties: (1) the study of the structural influence of genes within the network, (2) network dynamism and stability under perturbations, and (3) analysis of the gene regulatory system to reduce non-essential interactions caused by weighting mechanisms that tend to excessively encourage regulatory redundancies.
- **Novel architecture adapted to high computational costs**: A new asynchronous and parallel evolutionary model is introduced, enabling simultaneous evaluations even across different generations. Additionally, objectives derived from previous proposals in the literature have been refactored to eliminate unnecessary intermediate conversions and implement internal caching systems to avoid redundant computations. This, along with other refactorizations and technical strategies discussed in later sections, helps to partially offset the computational complexity introduced by adding three high-cost dimensions. As a result, the approach maintains computational feasibility for network sizes comparable to those addressed in the closest related literature, even though BIO-INSIGHT operates in an objective space twice as large.
- **Biological validation and clinical relevance of the model**: In contrast to approaches focused solely on predictive performance or algorithmic design, BIO-INSIGHT has been evaluated on real-world gene expression data from fibromyalgia and myalgic encephalomyelitis patients, demonstrating its ability to identify biologically meaningful and reproducible gene interactions. The inferred networks enabled the distinction between clinically overlapping conditions, revealed regulatory alterations consistently absent in disease groups, and uncovered condition-specific interactions with potential biomarker value. Several of these findings are supported by existing literature or experimental validation, reinforcing the usefulness of BIO-INSIGHT as a tool for uncovering disease mechanisms in complex conditions with no validated biomarkers.

In addition to these main contributions, this article addresses a series of research questions (RQs) that support and justify the scientific contribution of this proposal:

RQ1: Is there sufficient disparity in the networks inferred by different high-precision techniques to justify the motivation for developing new consensus methods?
RQ2: Does the consideration of multiple biological aspects still maintain the opposition of objectives at the evolutionary core of the proposal?
RQ3: Is there a correlation between the individual accuracy of inference techniques and their contribution to the optimal consensus in BIO-INSIGHT?
RQ4: Do the networks inferred by BIO-INSIGHT exhibit a biologically coherent structure in relation to known GRNs?
RQ5: Is the optimization of BIO-INSIGHT's high-biological-coverage objective space properly aligned with improving the quality of GRN inference?
RQ6: Is BIO-INSIGHT's optimization process redundant concerning the knowledge already acquired by networks during their inference through individual techniques?

BIO-INSIGHT has been tested on an extensive academic benchmark of 106 gene networks. Results demonstrate that using BIO-INSIGHT leads to a statistically significant improvement in AUROC and AUPR metrics compared to other consensus strategies, including MO-GENECI [18]. Since this technique has consistently outperformed a broad set of 26 widely recognized machine learning techniques, its inclusion as a reference point simplifies comparisons with state-of-the-art, avoiding redundant evaluations.

Additionally, a final subsection presents the results of applying BIO-INSIGHT to a gene expression dataset from patients with various pathologies, including fibromyalgia, myalgic encephalomyelitis, and the co-diagnosis of both diseases. The analysis focuses on detecting additional or missing gene regulations compared to the interactions inferred in healthy control group patients. These findings provide new insights into the molecular mechanisms underlying these diseases, facilitating the identification of potential biomarkers and therapeutic targets.

The article is divided into five main sections. Section 2 reviews current efforts and literature proposals for inferring gene regulatory networks from expression data, emphasizing consensus mechanisms. Section 3 introduces the algorithmic approach of this study, explaining the asynchronous and parallel many-objective model used, computational strategies for high-cost evaluations, and how various fitness functions were encoded. Section 4 details the experimental setup, including the networks to be inferred, parameter configurations, and execution strategy. Section 5 analyses the experimental results, comparing the accuracy of this approach with other inference methods. The final Section 6 concludes the study, highlighting its contributions to biomedicine and potential future implications.

2. State of the art

In the field of gene regulatory network (GRN) inference, the consolidation of different computational approaches through consensus techniques represents a growing area of interest [15,19–21]. Several studies have addressed this challenge, yet each presents limitations that underscore the complexity of the problem and the need for continuous innovation in this domain.

Some proposals, such as those presented in [22,23], have adopted strategies based on the use of reference information or gold standards. However, this approach invalidates their ability to infer networks in contexts where such prior information is unavailable, namely, in real-world scientific environments, where the goal is to uncover novel interactions of biological relevance.

Conversely, EnGRaiN [24] and EnsInfer [15] represent recent efforts to achieve consensus from a predominantly mathematical perspective, overlooking considerations of the underlying biological context. Moreover, EnGRaiN [24] relies on a supervised procedure that similarly

requires prior knowledge of interactions, thus limiting its applicability. Meanwhile, EnsInfer [15] focuses its experimentation on networks from the DREAM challenges [25] which, as identified in GENECI [19], may introduce bias in the results due to the specialization of certain techniques for this specific set of problems.

Other approaches, such as the one proposed in [20], rely on the consensus of simple machine learning methodologies, while [21] proposes a refinement that focuses solely on network topology, neglecting other important biological considerations.

Finally, MO-GENECI [18] emerges as an advanced proposal that, despite its achievements, requires greater attention to the biological complexity of GRNs, extending beyond mere degree distribution and motif detection in networks.

Despite these limitations, the recent publication of these consensus-based approaches strongly justifies the ongoing research interest in this field. The need to develop methodologies that not only effectively integrate the various computational efforts available in the literature, but also incorporate biological considerations more explicitly and comprehensively is evident. The current proposal, BIO-INSIGHT, is a step forward in this direction, as it not only promises to overcome current limitations, but also to significantly advance our ability to infer gene regulatory networks in an accurate and biologically meaningful manner.

3. Algorithmic proposal

BIO-INSIGHT (Biologically Informed Optimizer – INtegrating Software to Infer GRNs by Holistic Thinking) introduces a novel and intelligent consensus system for GRN inference, guided by the biological context of the data. This biologically informed strategy enhances both the interpretability and reliability of the inferred gene regulatory networks, making it a major advancement over traditional approaches.

In addition to this core innovation, BIO-INSIGHT offers a comprehensive suite of complementary tools: interactive visualization modules, centralized access to datasets from well-known challenges, academic benchmark generation through multiple simulators, domain-specific validation metrics, and support for up to 26 well-established inference techniques.

To gain a more detailed understanding of the functionality and characteristics of this system, Fig. 1 presents a diagram illustrating the main workflow of BIO-INSIGHT. This diagram corresponds to the primary command of the tool (run) and takes as input the expression dataset from which the GRN is to be inferred. The set of available techniques for the initial inference phase includes: ARACNE [7], BC3NET [26], C3NET [27], CLR [10], GENIE3_RF [6], GRNBOOST2 [28], GENIE3_ET [6], MRNET [29], MRNETB [30], PCIT [31], TIGRESS [12], KBOOST [32], MEOMI [33], JUMP3 [8], NARROMI [34], CMI2NI [9], RSNET [35], PCACMI [11], LOCPCACMI [36], PLSNET [37], PIDC [38], PUC [38], GRNVBEM [39], LEAP [40], NONLINEARODES [13] and INFERELATOR [41].

Gene regulatory networks are, by nature, directed graphs, as they represent causal relationships between regulators and target genes. However, since some of the techniques integrated into BIO-INSIGHT produce undirected graphs while others infer directionality, this property has been deliberately omitted in the consensus process. This decision enables the unification of the output representations across different methods, avoiding structural incompatibilities and ensuring that no technique is excluded based on the type of graph it generates. At the same time, it facilitates the future incorporation of new tools, regardless of whether they infer directed or undirected networks.

Designing an architecture adapted to large-scale networks requires optimizing the utilization of available resources to minimize execution time. To achieve this, in the initial phase, inference techniques are executed in parallel within multiple Docker containers, each dynamically assigned a specific amount of resources based on prior computational costs and adapted to the available resources in the environment.

Algorithm 1 Evolutionary optimization of the consensus in BIO-INSIGHT

Input Confidence matrices from inference techniques M
Output Pareto front of consensus networks F

1: $P \leftarrow$ InitializePopulation()
2: **while** not TerminationCriterionMet() **do**
3: $O \leftarrow$ GenerateOffspring(P)
4: **for all** $I \in O$ **do**
5: $G \leftarrow$ BuildConsensusNetwork(I,M)
6: Parallel execution:
O1: Quality(I,G) (refactored from [19])
O2: Motif(I,G) (refactored from [18])
O3: EVDist(I,G) (Algorithm 2)
O4: ReduceNEInt(I,G) (Algorithm 3)
O5: DegreeDist(I,G) (retrieved from [18])
O6: Dynamicity(I,G) (Algorithm 4)
7: $P \leftarrow$ SelectNextGeneration($P \cup O$)
8: **return** $F \leftarrow$ ExtractParetoFront(P)

The results from these individual techniques are then provided as input to the algorithmic core of BIO-INSIGHT (see Algorithm 1). This asynchronous and parallel many-objective evolutionary algorithm optimizes a weighted voting system among different machine-learning techniques based on a broad set of biologically driven objectives. This algorithm, built upon MO-GENECI [18] and consequently, NSGA-II [42], has been implemented in Java using the JMetal framework [43].

Each individual is created as a weight vector (Line 1 in Algorithm 1) whose total sum equals one (simplex). Each vector assigns a decimal value between 0 and 1 to each inference technique to be integrated into the consensus. In every generation (Line 2 in Algorithm 1), individuals undergo crossover and mutation phases, using simplex-compatible operators described in [18], ensuring that offspring remain within the solution space (Line 3 in Algorithm 1). By optimizing the objective functions detailed in the following sections, the algorithm aims to determine the optimal vote distribution to construct the gene regulatory network (Line 5 in Algorithm 1) that best satisfies all objectives (Line 6 in Algorithm 1).

Among the main contributions of this algorithm compared to existing state-of-the-art implementations, the following stand out:

- Refactorization of objectives retrieved from the literature [18] (quality, degree distribution, and motifs), along with the implementation of three new fitness functions (eigenvector distribution, dynamicity, and reduce non-essential interactions).
- Design of an asynchronous and parallel implementation that, through the simultaneous evaluation of individuals — even from different generations — maintains computational feasibility for large-scale networks.
- Incorporation of an external evaluation file that allows reconsidering the inclusion of prematurely discarded individuals, a practice that has already proven successful in many-objective problems [44].
- Implementation of a concurrent caching system capable of handling simultaneous reads and writes from multiple threads to reduce the number of redundant evaluations. This approach has been particularly useful for objectives where the consensus network must be binarized before computing the fitness value, as different individuals may represent the same binary network.

The output of the algorithm consists of an approximated Pareto front, where each solution represents a potential gene regulatory network inferred through the consensus of all initial techniques (Line 8 in Algorithm 1). This front is supplemented with various graphical representations of the evolutionary process that took place during

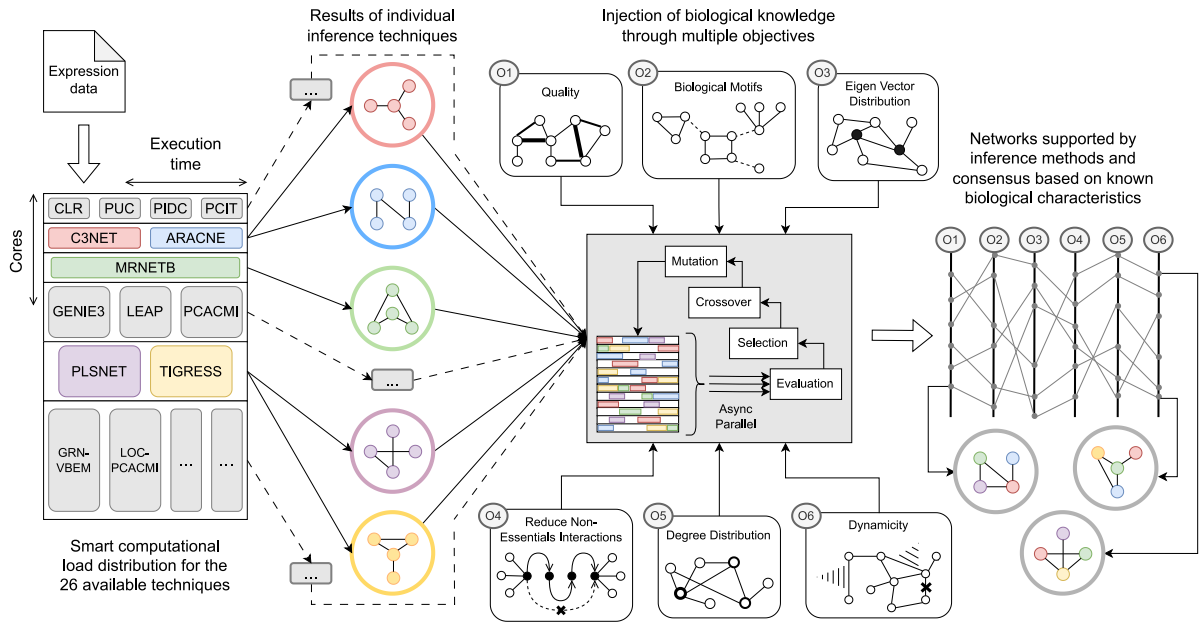


Fig. 1. Standard workflow of BIO-INSIGHT. Starting from gene expression data, the 26 available inference techniques are executed in parallel, with computational load distributed based on their expected cost (left). Each technique infers a different gene regulatory network, producing a set of individual solutions (centre-left). These networks are integrated through an asynchronous and parallel many-objective evolutionary algorithm, which optimizes a weighted voting system using six biologically driven objectives: interaction quality (O1), presence of biological motifs (O2), eigenvector centrality distribution (O3), reduction of non-essential interactions (O4), node degree distribution (O5), and dynamic stability (O6). Each individual in the population represents a set of weights for the inference techniques and is evaluated according to how well its resulting consensus network satisfies the biological objectives (centre). The evolutionary process generates a Pareto front of optimal trade-offs, where each solution corresponds to a consensus network that balances different biological properties. These final networks (right) are supported both by the inference techniques and by known structural characteristics of real-world gene regulatory systems.

execution, a parallel coordinates diagram depicting each solution in the front, as well as 2D and 3D plots resulting from grouping different objectives. These, along with the different network visualization tools implemented in the software package, allow domain experts to select the most appropriate solution based on their criteria.

The following subsections provide a detailed description of each objective function considered in BIO-INSIGHT. They include the sources from which their implementations or refactorings were obtained, and for newly created objectives, a comprehensive explanation of their design and implementation is provided.

3.1. Objective 1: Quality

This objective was retrieved from the first aggregative term of the proposal in [19], which was later reformulated in [18], simplifying its implementation. This latter implementation was retained in BIO-INSIGHT, as it demonstrated superior performance compared to the original version.

Although it is thoroughly described in [18], it is important to mention that the purpose of this function is to promote the emergence of networks whose interactions have high confidence levels, provided they are derived from individuals with consistent weight distributions, that is, individuals that assign greater importance to inference techniques whose values are more aligned with those of others.

To achieve this, the Quality function evaluates each interaction's quality based on consensus confidence and distance. The consensus is obtained through a weighted sum of the confidence values assigned by different techniques. The distance measures coherence among these techniques, penalizing significant discrepancies from the median while rewarding alignment with it. Only interactions with a quality score above the mean are used in the final calculation, which is defined as one minus the mean of these quality scores. Thus, this function minimizes this value, favouring networks with more coherent and high-quality interactions.

3.2. Objective 2: Motifs

The Motifs function, based on the one described in [18] under the same name, aims to favour consensus networks with a high density of recurrent structural patterns that have been previously identified as characteristic of biological networks: regulatory pathways, differentiation, bifurcation, and coupling. These motifs represent specific interaction configurations between genes and are fundamental for understanding the organization and dynamics of biological systems [45]. The number of detected motifs in the consensus network determines the final score of the function, encouraging networks with biologically relevant structures.

In [18], the individual's weight vector is first converted into a consensus network, which is then binarized to transform it into a directed graph using the JGrapht library [46]. The refactoring performed in BIO-INSIGHT optimizes this process by eliminating the intermediate step, allowing the direct conversion of confidence levels into a JGrapht graph without generating the adjacency matrix beforehand in each evaluation. This improvement has significantly reduced RAM consumption, particularly for large-scale networks. Moreover, it has been verified that this refactoring does not alter the results, ensuring that the function keeps producing the same values as in its original implementation.

Additionally, it is worth noting that implementing the concurrent caching system developed in this proposal has been particularly beneficial for this objective function. Although individuals in the population encode different weighted networks, the previously discussed binarization step can cause several of them to converge on the same binary representation, resulting in redundant assessments. Thanks to the cache system, the corresponding evaluations are now efficiently optimized, hence saving processing time and improving the algorithm's overall performance.

3.3. Objective 3: Eigen vector distribution

This fitness function evaluates the distribution of gene influence within the consensus networks by computing eigenvector centrality

Algorithm 2 Third objective: Eigenvector Distribution.

Input Consensus list with confidence values c
Output Value of the fitness function $result$
1: key = getHashCode(c)
2: **if** $cache.containsKey(key)$ **then**
3: $result = cache.get(key)$
4: **else**
5: $graph$ = getWeightedGraph(c)
6: $eigenvectorScores$ = eigenvectorCentrality($graph$)
7: $result$ = goodnessFitParetoTest($eigenvectorScores$)
8: $cache.put(key, result)$
9: **return** $result$

[47]. This metric measures the importance of a node in a network, assigning higher values to nodes connected to other highly influential nodes.

It is well known that the topology of biological networks often follows a power-law distribution [48], where a few nodes (genes) act as highly connected hubs, while the majority exhibit only a few connections. This pattern has also been observed for more complex centrality metrics, such as eigenvector centrality [49], where influence tends to be concentrated in a few key nodes, reflecting the typical hierarchical and modular structure of biological networks.

Unlike node degree, which is simply based on the number of interconnections a node has, eigenvector centrality focuses on the significance of these interactions, prioritizing quality over quantity. As a result, Objective 5 and this objective, although both optimizing network topology, focus on different yet complementary aspects. While the former may favour overall structural connectivity, the latter emphasizes the functional importance of nodes within the network. This distinction has been shown to create some trade-offs during optimization: nodes with low degrees can exhibit high relevance due to their connection with key genes, while conversely, nodes with many connections may have limited global influence due to the low relevance of their interactions.

The implementation of this fitness function is outlined in Algorithm 2 and analyses the consensus network computed from the individual. First, a *hash* key is generated to check whether the evaluation has already been performed and stored in the cache (line 1 of Algorithm 2). If the network has been previously evaluated, the result is retrieved from the cache (line 3 of Algorithm 2).

Otherwise, a weighted graph is constructed from the consensus network (line 5 of Algorithm 2), and eigenvector centrality is computed for each node using the JGrapht implementation (line 6 of Algorithm 2). Subsequently, these values undergo a goodness-of-fit test for a Pareto distribution (line 7 of Algorithm 2, implemented according to [50]). Finally, the result is stored in the cache and returned as the final value of the fitness function (lines 8 and 9 of Algorithm 2).

3.4. Objective 4: Reduce non-essentials interactions

This fitness function evaluates the structure of the consensus networks by analysing the edge betweenness centrality [51], with the goal of identifying and favouring networks in which non-essential interactions have been discarded. Edge betweenness centrality measures the importance of each edge in the network based on the number of shortest paths that pass through it. Edges with high centrality act as critical bridges connecting different parts of the network, whereas edges with low centrality often represent redundant or less relevant interactions.

The purpose of this function is to penalize dense gene networks containing numerous superfluous or redundant interactions (with low centrality), favouring those in which only the most essential connections (with high centrality) are retained to maintain network cohesion and functionality. Networks with fewer redundant interactions are not only easier to analyse but also tend to better reflect the true underlying regulatory architecture [27].

Algorithm 3 Fourth objective: Reduce Non-Essential Interactions.

Input Consensus list with confidence values c, Number of decimal places in the rounding (dependent on the evolutionary stage in which the evaluation is taking place) $decimals$
Output Value of the fitness function $result$
1: key = getRoundedHashCode(c, decimals)
2: **if** $cache.containsKey(key)$ **then**
3: $result = cache.get(key)$
4: **else**
5: $graph$ = getWeightedGraph(c, decimals)
6: $edgeBetweenness$ = edgeBetweenness($graph$)
7: sort($edgeBetweenness$)
8: $mean$ = calculateWeightedMean($edgeBetweenness$)
9: $result = 1/mean$
10: $cache.put(key, result)$
11: **return** $result$

This fitness function is the most computationally expensive in BIO-INSIGHT. For this reason, two measures have been implemented:

1. **Adaptive approach in evaluation management through caching**. In the early exploratory stages of the evolutionary algorithm, the cache uses lists of confidence values from the consensus network rounded to fewer decimal places as keys. This increased rounding enhances the likelihood of reusing previous evaluations, thereby avoiding the computational cost of evaluating highly similar networks, an aspect that is not critical in this exploratory phase. As the algorithm progresses towards the exploitation and refinement stages, the precision of these values is gradually increased (using more decimal places), allowing for finer distinctions between similar solutions, where small differences may be crucial in identifying higher-quality configurations.
2. **More efficient implementation of the edge betweenness centrality metric calculation**. Taking JGrapht's original implementation of this score as a reference, a less computationally expensive alternative[1] has been implemented using Dijkstra's algorithm. The results of this implementation have been verified to be identical to those obtained using the library's original version.

The implementation of this fitness function is detailed in Algorithm 3. The process begins by generating a rounded *hash* key based on the consensus network (line 1 of Algorithm 3) to check whether the result is already stored in the cache. If it is, the result is retrieved directly (line 3 of Algorithm 3).

Otherwise, a weighted graph is constructed from the consensus network (line 5 of Algorithm 3), and edge betweenness centrality is computed using Dijkstra's algorithm (line 6 of Algorithm 3). The centrality values are then sorted in ascending order (line 7 of Algorithm 3), ensuring that the least relevant interactions appear first.

Next, a weighted average of the centralities is computed (line 8 of Algorithm 3), assigning greater weight to edges with low betweenness (those at the beginning of the list). The final value of this minimization-oriented function is obtained as the inverse of this weighted average

[1] Optimized implementation of Edge Betweenness Centrality metric https://github.com/AdrianSeguraOrtiz/BIO-INSIGHT/blob/main/EAGRN-JMetal/src/main/java/eagrn/fitnessfunction/impl/topology/EdgeBetweennessCalculatorDijkstra.java.

(line 9 of Algorithm 3), favouring networks in which even the least relevant interactions have high centrality values. Finally, the result is stored in the cache and returned (lines 10 and 11 of Algorithm 3).

3.5. Objective 5: Degree distribution

The Degree distribution function is retrieved from [18] and aims to promote the creation of consensus networks that follow a scale-free degree distribution, a typical pattern in biological networks. In these networks, most nodes have only a few connections, while a few hubs concentrate most interactions [48].

To achieve this, the function calculates the degree of each node by summing the decimal confidence levels of the interactions in which the gene acts as either a source or a target. Then, the goodness-of-fit test described in [50] is applied to assess how well the degree distribution of the consensus network aligns with a Pareto distribution, which is characteristic of scale-free networks. The result of this test, which reflects the probability that the network follows this distribution, is the value returned by the function.

3.6. Objective 6: Dynamicity

This fitness function evaluates the dynamic stability of the consensus networks. To achieve this, it models the temporal evolution of gene activity using a system of nonlinear ordinary differential equations (ODEs) based on the model presented in [52], applying certain simplifications to formulate it directly from the adjacency matrix of the inferred network.

Given a set of N genes in the network, each node i is represented by a variable $y_i(t)$, which denotes its expression level over time. The temporal evolution of each node is governed by the following differential equation:

$$\frac{dy_i}{dt} = f\left(\sum_j A_{ji} y_j\right) - y_i \tag{1}$$

where A_{ji} is the weight of the connection between nodes j and i in the inferred adjacency matrix, $f(x)$ is a nonlinear activation function that models gene regulation, and the term $-y_i$ represents the natural degradation of gene expression, preventing the system from growing without constraints.

The activation function used in this model is the Hill function [53], due to its widespread and traditional use in modelling transcriptional regulation interactions in GRNs [54,55]:

$$f(x) = \frac{x^n}{k^n + x^n} \tag{2}$$

where n controls the degree of nonlinearity in the system by modulating the cooperative response of the network, and k adjusts the threshold at which the input signal significantly affects activation. This formulation introduces saturation in the response of each node, reflecting that gene regulation does not respond linearly to stimuli, but instead exhibits threshold behaviours and cooperation among regulators.

In this model, after several experimental tests, the values $n = 2$ and $k = 0.5$ have been set to capture a biologically plausible dynamic from a general perspective. On the one hand, a value of $n = 2$ balances a gradual response and cooperative behaviour, ensuring that activation does not occur linearly or abruptly. On the other hand, the value $k = 0.5$ allows the system's response to be triggered by moderate signals without requiring extreme stimuli or overreacting to small fluctuations.

The dynamic stability of a biological network is a key indicator of its functional robustness. Stable networks tend to maintain their structure and functionality in response to internal or external perturbations. This fitness function is designed to favour individuals whose consensus networks exhibit well-defined temporal evolution and quickly converge to a state, avoiding chaotic systems or those highly sensitive to perturbations.

Algorithm 4 Sixth objective: Dynamicity

Input Consensus list with confidence values c
Output Value of the fitness function $result$
1: $adjacencyMatrix$ = getFloatMatrix(c)
2: $model$ = createODEModel($adjacencyMatrix$)
3: $integrator$ = DormandPrince54Integrator()
4: $initState$ = [1.0, 1.0, ..., 1.0]
5: $finalState$ = integrate($integrator$, $model$, $initState$)
6: $result$ = average($finalState$)
7: **return** $result$

In this case, it was decided not to use the caching system because the computational cost of this objective function is not as high as that of other functions that require complex calculations on networks using JGrapht. Performance analysis showed that storing all evaluations in a cache would result in higher memory and resource consumption than simply executing the function whenever needed.

The implementation of this fitness function is described in Algorithm 4. First, the consensus network is transformed into a weighted adjacency matrix (line 1 of Algorithm 4). This matrix is then used to construct a system of nonlinear ordinary differential equations (ODEs) that simulates the system's dynamics (line 2 of Algorithm 4).

The Dormand-Prince 5(4) integrator [56] is employed to solve the ODE system with high precision (line 3 of Algorithm 4). Homogeneous initial conditions are set, where all nodes start with a value of 1.0 (line 4 of Algorithm 4), and the system's evolution is simulated (line 5 of Algorithm 4).

Finally, the stability score is computed as the average of the final values of the nodes (line 6 of Algorithm 4). This score reflects the system's ability to maintain stable behaviour under the influence of the nonlinear interactions defined by the adjacency matrix. A value close to the initial state indicates stability, whereas significant deviations suggest instability or complex dynamic behaviours. The final value is returned as the fitness function's result (line 7 of Algorithm 4).

As seen in Algorithm 4, the implementation of this last objective is simple and designed to be as generic as possible, making it applicable to any network, regardless of its size and structure. This initial approach enables the exploration of the stability of consensus networks, with promising results that encourage future refinements in this aspect.

4. Experimentation

The first phase of the experimental design in this study aims to demonstrate that incorporating all the biological objectives described in the previous section not only improves the quality of the consensus networks compared to the state of the art, but also maintains computational feasibility, even for large-scale networks, thanks to the proposed architecture.

To achieve this, the first experiment is designed for a fair and rigorous comparison with the most recent and directly related algorithm, MO-GENECI [18], which has been shown to outperform a total of 26 widely used individual inference techniques. Since all the objective functions from MO-GENECI have been incorporated into this proposal, replicating its experimental conditions as closely as possible allows verification that the observed accuracy improvements are solely due to the integration of new biological knowledge introduced through the additional objectives.

For this reason, the same dataset used in its experimentation is employed in this phase, as it is also considered the most extensive and diverse academic benchmark constructed to date for the field of GRN inference. This benchmark consists of a total of 106 networks, with sizes of up to 2000 genes, sourced from up to ten different origins, including well-recognized challenges in the field (DREAM3 [57] and DREAM4 [25]), simulators (SysGenSIM [58], SynTReN [59], Rogers

Table 1
Summary of the academic benchmark extracted from MO-GENECi [18]. It is composed of a total of 106 inference problems that try to cover as much diversity as possible in order to obtain strong conclusions. Each network is subjected to each of the perturbations available for its case, obtaining a different set of data that make up an instance. Legend: KO (Knock-Out), KD (Knock-Down), and OE (Over-Expression).

Source	Networks	Sizes	Simulator	Disturbance	Instances
DREAM3 [57]	15	10, 50 and 100	DREAM team	–	15
DREAM4 [25]	10	10 and 100	DREAM team	–	10
IRMA [62]	1	5	Cell culture: RT-PCR	Switch on/off	2
TFLink [63]	4	12, 75, 163 and 371	SysGenSIM	Mixed	4
RegulonDB [64]	1	2234	SysGenSIM	Mixed	1
RegNetwork [65]	2	983 and 1033	SysGenSIM	Mixed	2
BioGRID [66]	32	6–1505	SysGenSIM	Mixed	32
GRNdb [67]	11	320–1598	SysGenSIM	Mixed	11
From scratch	4	20, 50, 100 and 200	SysGenSIM (EIPO Modular)	KO, KD and OE	12
From scratch	4	20, 50, 100 and 200	SysGenSIM (Scale Free)	KO, KD and OE	12
GRNdata [68]	2	300 and 1000	SynTReN	–	2
GRNdata [68]	1	1000	Rogers	–	1
GRNdata [68]	2	1565 and 2000	GeneNetWeaver	–	2

[60], and GeneNetWeaver [61]), in vivo networks such as IRMA [62], and a broad set of databases compiling verified GRNs through various procedures (TFLink [63], RegulonDB [64], RegNetwork [65], BioGRID [66], and GRNdb [67]).

The specifications of this diverse dataset are detailed in Table 1, which has been extracted from the original MO-GENECI paper [18]. This table summarizes the number of networks, their sizes, the simulation tools or experimental sources used to generate the data, the types of perturbations applied, and the resulting number of distinct inference instances. It highlights the breadth of the benchmark, encompassing GRNs derived from numerous sources and subjected to diverse experimental conditions, making it a comprehensive testbed for assessing inference performance.

The inference techniques to be integrated into the consensus are the same as those considered in MO-GENECI, specifically the 26 available techniques listed in the previous section. This ensures that the observed accuracy improvements are exclusively attributable to the algorithmic design and objective formulation of BIO-INSIGHT, rather than to the quality of the initial networks provided by the inference techniques. Similarly to what is stated in the article, some of these techniques are restricted to certain ranges of network size due to their high computational cost. This ensures that both proposals start from exactly the same baseline, ruling out the possibility that BIO-INSIGHT's improvements could be attributed to the initial quality of the inferred networks. Since both approaches share the same individual representation, the same crossover and mutation operators have been adopted, maintaining identical parameter settings and fixing the same values for population size (300) and number of evaluations (250,000). This guarantees that differences in accuracy between the two proposals are not due to the over-evolution of either one.

In addition to BIO-INSIGHT and MO-GENECI, other consensus strategies are incorporated into the comparison. These strategies should be capable of integrating any set of techniques and performing consensus in an unsupervised manner. That is, to ensure a fair comparison, the same inference techniques should be combined using a procedure that does not rely on labelled data. Otherwise, if accuracy is evaluated against the gold standard, such strategies would have a significant advantage by having prior, partial, or full access to that reference. Moreover, they are not conceptually comparable to this proposal, as supervised strategies are restricted to academic settings and cannot infer real-world, unexplored gene networks.

This excludes most of the proposals mentioned in the state of the art, either due to their supervised nature [22–24] or their lack of flexibility in accommodating any initial individual inference method [20,21]. A promising alternative would be EnsInfer [15]; however, despite its description not mentioning any supervised process, its algorithm requires confirmation of the existence of each interaction[2] as well as partitioning data into training, validation, and test sets.

Finally, due to the scarcity of feasible consensus proposals for algorithmic comparison, several simple strategies have been implemented as reference baselines: mean, median, weighted mean, and Bayesian fusion. The latter is inspired by the EnsInfer implementation, serving as an approximate substitute given the impossibility of its direct use.

Thus, the final comparison will be conducted between BIO-INSIGHT, MO-GENECI, and the remaining simple consensus strategies. This comparison also indirectly evaluates the set of 26 individual inference techniques, as MO-GENECI has already demonstrated superiority over all of them. The evaluation is performed by validating each resulting consensus network against the gold standards using AUPR and AUROC metrics. In the case of evolutionary algorithms that produce an approximated Pareto front, the median-quality solution and the best solution from the front will be considered representative samples.

To statistically validate the superiority of BIO-INSIGHT, a Friedman ranking test was applied over the benchmark of 106 gene networks, followed by Holm's non-parametric post-hoc procedure to adjust p-values and determine the significance of differences between methods. This methodology ensures a robust comparative analysis without assuming data normality, and it highlights which proposals achieve statistically better performance across the entire benchmark.

The second phase of the experimental design focuses on assessing the individual contribution of each objective function integrated into BIO-INSIGHT, in order to evaluate the innovation brought by the proposal and to test whether the simultaneous optimization of biologically driven yet potentially conflicting objectives leads to superior inference performance.

To this end, an ablation study was conducted by executing BIO-INSIGHT on all networks in the benchmark with fewer than 1000 genes (to ensure computational feasibility) under ten different configurations:

- The complete BIO-INSIGHT proposal, optimizing all objectives jointly in a many-objective framework.
- Nine mono-objective variants, each optimizing independently one of the biological aspects considered in the objective space.

It is worth noting that the *Motifs* objective actually integrates four distinct regulatory patterns —*regulatory pathways*, *differentiation*, *bifurcation*, and *coupling*— which are typically combined in a single score. However, for this ablation study, each motif type was optimized individually, in order to isolate their specific contributions. As a result, the number of mono-objective variants increases from six (one per original objective) to nine.

[2] Link to EnsInfer repository: https://github.com/IcyFermion/network_inference_ensemble/tree/main.

Each configuration was executed on the same subset of networks using identical inference inputs, parameters, and evolutionary operators, ensuring that the only variable factor was the objective function. The resulting consensus networks were evaluated using AUROC and AUPR against the gold standards. These metrics were then subjected to Friedman statistical ranking and Holm's post-hoc non-parametric tests to assess whether the full multi-objective optimization significantly outperforms any of the individual objective configurations.

Finally, the third experimental phase aims to demonstrate the real-world clinical applicability of this proposal once its accuracy in the academic domain has been validated in the previous phase. To achieve this, real-world gene expression data from patients with various pathologies, including fibromyalgia, myalgic encephalomyelitis, and the co-diagnosis of both diseases, have been used. The first step consists of dividing this dataset into four distinct groups: one for each pathology and a control group. Then, BIO-INSIGHT is executed on each subset, extracting an approximated Pareto front for each pathology. To reduce each front to a single representative consensus network, all the networks in the front were merged, storing for each interaction its frequency within the front and its average confidence score.

After obtaining the consensus network for each pathology, the designed analysis compares the presence or absence of interactions between different groups. In other words, it aims to observe, for example, whether there are interactions that are inferred in a specific clinical pathology, but not in the control group.

5. Results and discussion

The initial execution of individual inference techniques provides the input for the evolutionary algorithm in this proposal. Therefore, for each problem, the gene regulatory network inferred by each technique is obtained.

5.1. Performance analysis

Although the consensus of inference networks through BIO-INSIGHT does not require the prior computation of each technique's accuracy, this information is highly useful for making observations and future comparisons.

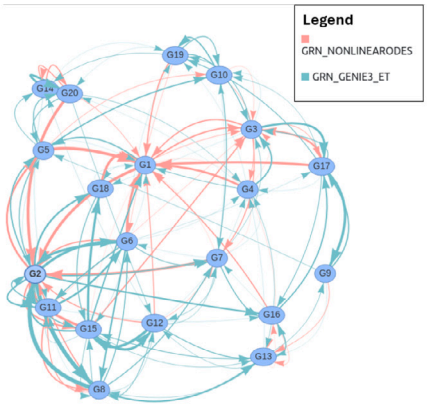
A noteworthy initial observation is the surprising disparity among the inferred networks, even when they exhibit similar accuracy. Across the results obtained from the 26 individual techniques, markedly different interactions can be identified, highlighting an apparent inconsistency among them. To illustrate this discrepancy, Fig. 2 presents the overlap of the two most accurate techniques in two specific problems.

First, Fig. 2(a) compares the network inferred by NONLINEARODES with the one obtained by GENIE3_ET in a simulated 20-node network. The difference between them is evident: while GENIE3_ET favours a more dispersed topology, NONLINEARODES models gene regulation through a central hub, which is entirely ignored by the first technique.

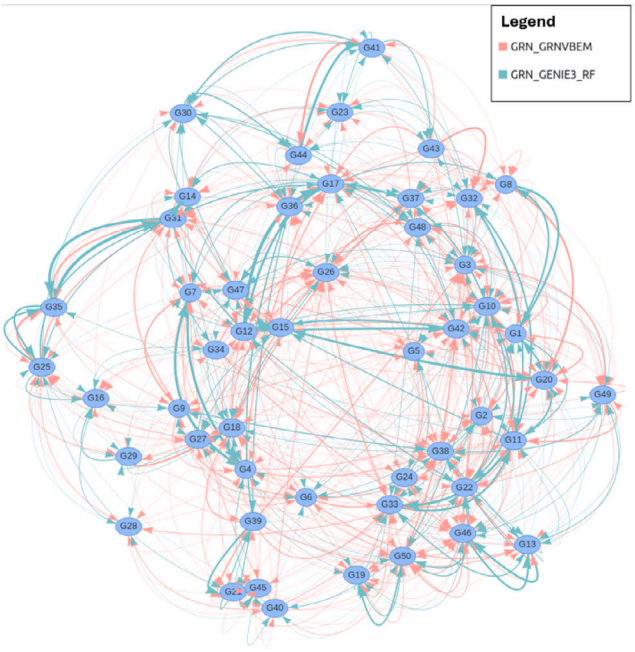
Second, Fig. 2(b) presents the comparison between GRNVBEM and GENIE3_RF in a 50-node network from the DREAM3 challenge. Although both networks appear to share a similar topological structure, the proposed interactions occur between different genes.

Answer to RQ1

Yes, the results show a significant disparity among the networks inferred by different high-accuracy techniques. The differences in topology and inferred interactions suggest a lack of coherence that could impact biological interpretation. This justifies the need to develop consensus methods integrating information from multiple approaches to obtain more robust and reliable representations.



(a) Simulated 20-node network with scale-free topology and overexpression perturbation. Confidence levels above 0.3.



(b) First yeast network from the DREAM3 challenge with a size of 50 nodes. Confidence levels above 0.2.

Fig. 2. Comparison of the networks inferred by the two most accurate techniques for a given problem. Each technique is represented by a different colour, arrows indicate the direction of regulation, and their thickness reflects the confidence level of the interaction.

After executing BIO-INSIGHT to consolidate the networks inferred by individual techniques, an approximated Pareto front is obtained for each problem. In scenarios with a high number of objectives, such as the one in this study, ensuring an adequate trade-off between them is crucial. The presence of overlapping objectives can hinder the effective exploration of the solution space, bias the dominance of specific objectives, and compromise the diversity of the Pareto front.

By visualizing various fronts obtained by BIO-INSIGHT through interactive parallel coordinate plots, a correct partial or even total conflict between the objectives of this proposal has been observed. However, statically representing this phenomenon in a single figure is challenging. Therefore, a specific sample has been selected, and a visualization has been designed to illustrate the opposition among all objectives in a single image.

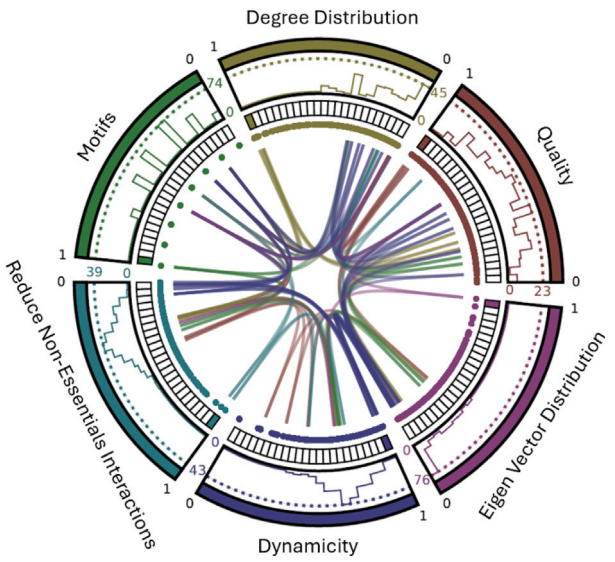


Fig. 3. Chord diagram for the solution front obtained by BIO-INSIGHT after optimizing the consensus of the techniques applied to the first network from the DREAM4 challenge, with a size of 10 nodes. In this diagram, each objective is represented as a circular trapezoid. Inside the trapezoid, a histogram illustrates the solutions' distribution across the corresponding objective's normalized values. Dashed lines indicate the maximum and minimum values within the histogram. Small boxes at the base of the circular trapezoid represent subsets of individuals grouped based on their proximity in the objective score. These boxes are initially interactive and allow the selection of individuals to be displayed in the core of the diagram. Since this document is static, a snapshot has been taken, selecting the worst-performing individuals for each fitness function to highlight the opposition between the objectives of the algorithm appropriately.

Fig. 3 presents a chord diagram for the approximated Pareto front obtained for one of the networks from the DREAM4 challenge. In this figure, the subset of individuals with the worst fitness values is selected for each objective. This allows for observing how sacrificing a specific objective enables individuals to achieve better fitness scores in the remaining objectives. For example, considering the worst-performing individuals in the Dynamicity objective, highlighted in dark purple, it is evident that they occupy highly optimized positions in other objectives, such as Reduce Non-Essential Interactions or Eigen Vector (metric) Distribution.

Answer to RQ2

Yes, the consideration of multiple biological aspects does not compromise the trade-off between objectives at the evolutionary core of the proposal. The exploration of the obtained fronts confirms the presence of a partial or total conflict among the objectives, ensuring a balanced and diverse optimization.

Although it has already been demonstrated that, due to specialization domains, no inference technique is generally more accurate than others [15], researchers might still be tempted to perform consensus using only techniques that have yielded good accuracy in other datasets. Therefore, it is interesting to analyse the weights assigned by the highest-accuracy individuals in the front to the different techniques and examine whether there is any correlation between these weights and the individual accuracy of the techniques.

It is natural to assume that BIO-INSIGHT would assign higher weights to the techniques with greater individual accuracy. However, this is not necessarily the case.

Fig. 4 presents a polar plot with the individuals from the front of one of the networks from the DREAM3 challenge, positioned based on the weights assigned to the different techniques and coloured according to their accuracy level. As observed, the highest-quality individuals (more yellow) are not necessarily concentrated near the techniques with the highest individual accuracy for the two accuracy metrics. This suggests that the algorithm adopts a holistic perspective, where consensus quality is not merely the sum of the individual accuracies of each technique. Thus, a balanced selection of less accurate techniques can generate high-quality networks, whereas consensus among highly accurate techniques does not necessarily guarantee higher accuracy.

Answer to RQ3

No, the results indicate that the optimization of consensus in BIO-INSIGHT does not align with an individualistic approach in which the accuracy of the techniques correlates with their level of participation in the consensus. Instead, the algorithm appears to adopt a more holistic perspective, where the combination of lower-accuracy techniques can lead to higher-quality consensus networks due to their complementarity.

After analysing the fitness values of the individuals and the weights they assign to the different techniques, it is essential to examine the characteristics of the consensus networks constructed from the individuals in the Pareto approximation front. Although the algorithmic comparison discussed later quantifies the accuracy of these networks against the gold standards, it is also important to verify that, in addition to being accurate, the consensus networks exhibit biologically coherent properties that ensure clear biological interpretability.

Fig. 5 presents the most accurate consensus gene regulatory network obtained by BIO-INSIGHT for one of the networks from the BioGRID repository. This network displays several characteristics that align with expectations in this domain: a scale-free topology with high connectivity in the core, a modular structure that enables the distinction of multiple functional communities, and a strong presence of regulatory motifs, such as a regulatory pathway (in orange), a clear feedforward loop (in light blue), and a biparallel motif (in grey).

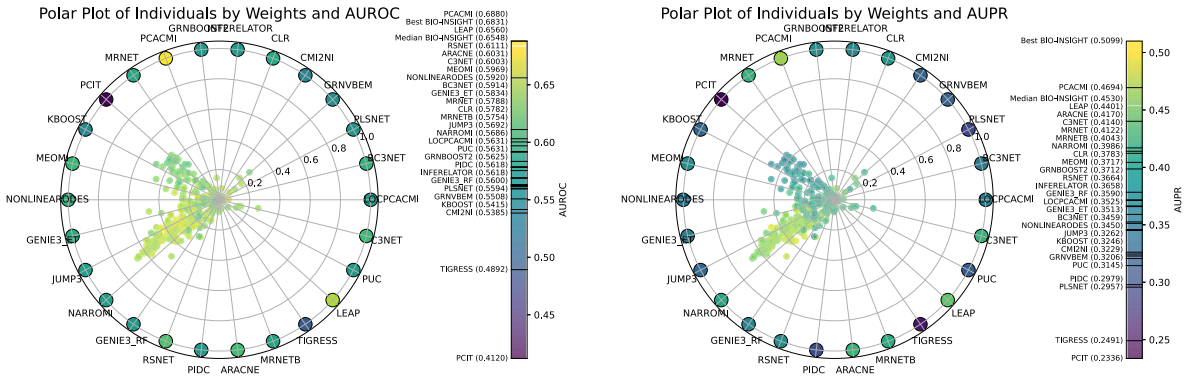
The presence of these characteristics in high-accuracy consensus networks inferred by BIO-INSIGHT validates the hypothesis of this study: an intelligent consensus of individual techniques, guided by a biologically comprehensive objective space, not only brings the results closer to biologically plausible scenarios, but also leads to a significant accuracy enhancement that cannot be achieved through the purely mathematical satisfaction of current literature proposals.

Answer to RQ4

Yes, the networks inferred by BIO-INSIGHT exhibit a biologically coherent structure consistent with current knowledge of GRNs. Their characteristics, such as topology, structure, and patterns, indicate that consensus guided by biologically comprehensive objectives not only enhances accuracy, but also generates networks with greater interpretability, aligning with the underlying biology.

The simultaneous occurrence of high accuracy and biologically coherent properties in the consensus networks generated by BIO-INSIGHT, along with their role in validating the hypothesis of this study, can only be explained in one way: the biological objectives designed in this work are somehow aligned with inference accuracy.

To demonstrate this, Fig. 6 presents the moving medians of the objectives sorted for each accuracy metric in the fronts of two specific BioGRID networks. In both cases, for the *Glycine max* network (Fig. 6(a)) and network *Strongylocentrotus purpuratus* (Fig. 6(b)), a clear relationship can be observed between the level of optimization of the different objectives and the AUPR and AUROC values of the individuals.



(a) Polar chart for AUROC (b) Polar chart for AUPR

Fig. 4. Polar plot locating each solution from the front obtained by BIO-INSIGHT for the second yeast network from the DREAM3 challenge, with a size of 10 nodes. In these plots, individuals are represented by points positioned according to the weights assigned to different techniques and are coloured based on their accuracy level. Additionally, larger markers represent simulated solutions (not part of the front), corresponding to assigning all the weight to a single technique. To the right of the radar, a sidebar displays the colour gradient associated with the accuracy metric in question. In this bar, black markers indicate the accuracy values of each technique, while white markers highlight the accuracy of BIO-INSIGHT's best solution as well as the median of the front.

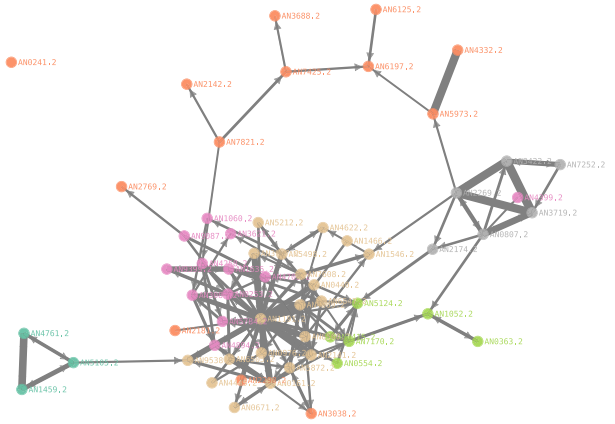


Fig. 5. Representation of the best solution obtained by BIO-INSIGHT for the *Emericella nidulans FGSC A4* network extracted from the BioGRID repository. Genes are coloured based on their neighbourhood, arrows indicate the direction of regulation, and their thickness represents the confidence level of the interaction.

Answer to RQ5

The conflicting objectives of BIO-INSIGHT have demonstrated that, despite not using labelled data at any point, their optimization is clearly related to inference accuracy. This helps validate the design of the objective functions, their appropriate directionality within this field, and their feasible use in real-world settings where the networks to be inferred are yet to be discovered.

It is essential to show that the accuracy enhancement, and thus the optimization performed by BIO-INSIGHT, does not duplicate or interfere with the initial learning occurring during the execution of individual inference techniques. Although these techniques are primarily based on mathematical principles that differ significantly from BIO-INSIGHT's objectives, some indirect alignment could still exist.

To rule out this possibility, Fig. 7 presents the moving medians of the weights assigned to each technique, sorted for each objective of the algorithm, in the front of one of the BioGRID networks. This representation reveals the wide interquartile ranges and the lack of directionality in the curves. This noise confirms that the objectives do not favour any particular technique individually. In other words, no techniques are specialized in a specific objective, nor do they individually contribute the biological knowledge that the objective represents.

Table 2
Friedman mean rank with Holm's adjusted p values (0.05) for AUPR.

AUPR		
Technique	$Friedman's Rank$	$Holm's Adj - p$
BEST_BIO-INSIGHT	**1.51887**	–
BEST_MO-GENECI	2.8396	8.6584e−05
MEAN_WEIGHTS	4.8349	1.2970e−22
RANK_AVERAGE	4.8962	3.1183e−23
MEDIAN_BIO-INSIGHT	5.0896	1.0415e−25
MEDIAN_WEIGHTS	5.4151	2.6040e−30
BAYESIAN_FUSION	5.5802	9.0595e−33
MEDIAN_MO-GENECI	5.8255	1.1532e−36

Answer to RQ6

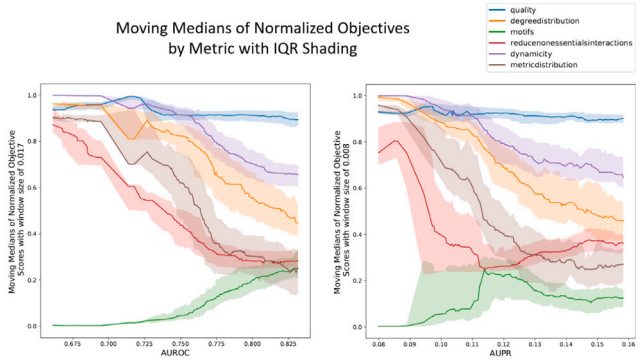
The carefully designed objective functions in this study have successfully demonstrated their complete independence from the methodologies integrated into the individual techniques. This means that applying BIO-INSIGHT after executing these techniques to optimize their consensus provides entirely novel information.

Answering each research question has justified the motivation and validated the design of the algorithmic proposal. However, to demonstrate the scientific contribution of this work, it is essential to conduct a rigorous accuracy comparison with existing state-of-the-art methodologies.

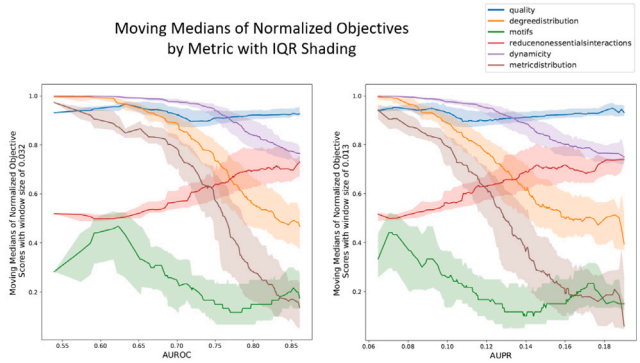
In Fig. 8, the AUPR and AUROC values are represented for a diverse set of networks inferred by BIO-INSIGHT, MO-GENECI, several consensus strategies, and the 26 individual inference techniques. The results show that BIO-INSIGHT clearly dominates in both accuracy metrics, consistently outperforming all other evaluated methodologies.

A Friedman statistical ranking with Holm's non-parametric tests has been conducted on the academic benchmark of 106 gene networks to corroborate this superiority in a statistically rigorous manner. Since MO-GENECI had already demonstrated in its original article [18] that it outperforms the 26 individual techniques using this same statistical test, these techniques have been excluded from this analysis, focusing instead on comparing consensus approaches.

In Tables 2 and 3, the statistical test results for the AUPR and AUROC metrics, respectively, are presented. BEST_BIO-INSIGHT achieves



(a) Network *Glycine max* extracted from the BioGRID repository.



(b) Network *Strongylocentrotus purpuratus* extracted from the BioGRID repository.

Fig. 6. In these plots, the moving medians of the normalized objective values for the individuals forming the front obtained by BIO-INSIGHT are represented and sorted for each accuracy metric. Additionally, each moving median is shaded by the interquartile range, allowing an outline of the diversity of objective values at each accuracy level.

Table 3
Friedman mean rank with Holm's adjusted p values (0.05) for AUROC.

AUROC		
Technique	$Friedman's Rank$	$Holm's Adj - p$
BEST_BIO-INSIGHT	**1.69811**	–
BEST_MO-GENECI	2.9387	2.2685e−04
MEDIAN_BIO-INSIGHT	4.2359	9.2290e−14
RANK_AVERAGE	4.8443	2.6078e−20
MEAN_WEIGHTS	5.1179	1.1488e−23
MEDIAN_MO-GENECI	5.3774	3.9178e−27
BAYESIAN_FUSION	5.7689	6.4410e−33
MEDIAN_WEIGHTS	6.0189	6.7039e−37

the highest average ranking in both metrics, significantly outperforming all other methodologies. The statistical comparison further confirms that this difference is significant, with p-values for the other techniques being extremely low and far from the commonly recognized 0.05 threshold.

It is important to highlight that BEST_MO-GENECI, the second-best method in both cases, is at a considerable distance from BIO-INSIGHT. Given that previous studies had already demonstrated that MO-GENECI outperforms the 26 individual inference techniques, these results indicate that BIO-INSIGHT surpasses it even more significantly. Therefore, any additional comparison with the individual inference techniques would be redundant, as their inferiority has already been indirectly established through the significant and consistent superiority of BIO-INSIGHT over MO-GENECI. Additionally, traditional consensus strategies, such as mean, median, and rank average, fall considerably

Table 4
Friedman mean rank with Holm's adjusted p values (0.05) for AUPR.

AUPR		
Configuration	$Friedman's Rank$	$Holm's Adj - p$
BEST_BIO-INSIGHT	**1.3779**	–
O6: Dynamicity	5.1337	4.1353e−16
O2-1: MotifsDifferentiation	5.5581	2.7626e−19
O3: EigenVectorDistribution	5.6454	7.2146e−20
O2-2: MotifsRegulatoryRoute	5.7616	8.8521e−21
O4: ReduceNEInteractions	6.0233	4.1025e−23
O1: Quality	6.0407	3.3516e−23
O2-3: MotifBifurcation	6.1570	2.9065e−24
O2-4: MotifCoupling	6.6512	2.9530e−29
O5: DegreeDistribution	6.6512	2.9530e−29

Table 5
Friedman mean rank with Holm's adjusted p values (0.05) for AUROC.

AUROC		
Configuration	$Friedman's Rank$	$Holm's Adj - p$
BEST_BIO-INSIGHT	**1.6570**	–
O6: Dynamicity	5.1744	2.5713e−14
O4: ReduceNEInteractions	5.3488	2.5701e−15
O3: EigenVectorDistribution	5.4942	2.8512e−16
O5: DegreeDistribution	5.8605	3.4785e−19
O1: Quality	5.9361	9.4989e−20
O2-3: MotifBifurcation	6.0581	9.2336e−21
O2-2: MotifsRegulatoryRoute	6.0930	5.1874e−21
O2-1: MotifsDifferentiation	6.1861	8.2152e−22
O2-4: MotifCoupling	7.1919	3.7083e−32

behind, sometimes even being outperformed by a random selection from the BIO-INSIGHT front rather than a proper selection made by a domain expert.

To improve the transparency of the study, the AUROC and AUPR values obtained for each inferred network, using both the individual techniques and the consensus strategies including BIO-INSIGHT, have been provided as supplementary material.

The accuracy improvements introduced by this proposal involve a considerably high algorithmic complexity. Although the necessity of its implementation has been thoroughly justified through the resolution of various research questions, the computational cost of its execution remains a relevant aspect.

For this reason, from the beginning of BIO-INSIGHT's design, numerous measures were taken to minimize the impact of its complexity on execution time: the implementation of an asynchronous parallel model, the caching system, the elimination of unnecessary intermediate steps, the adaptive approach to managing evaluation storage, the efficient implementation of graph-based scoring methods from the literature, and more.

Although these optimizations cannot fully compensate for adding three extra dimensions to the search space, they have allowed BIO-INSIGHT's operational range to remain comparable to the closest previous strategy.

Fig. 9 compares the MO-GENECI algorithm and BIO-INSIGHT, clearly showing the exponential increase in BIO-INSIGHT's execution time as network size increases. Both algorithms were analysed using the same number of evaluations (250,000) and executed under the same computational resources (500 GB of RAM and 32 cores).

5.2. Objective function ablation study

Tables 4 and 5 present the results of the ablation study based on the Friedman ranking and Holm's post-hoc procedure, considering AUROC and AUPR as evaluation metrics. In both cases, the configuration that jointly optimizes all objectives (**BEST_BIO-INSIGHT**) obtains the best average rank with statistically significant differences (adjusted $p < 0.05$) with respect to all mono-objective variants.

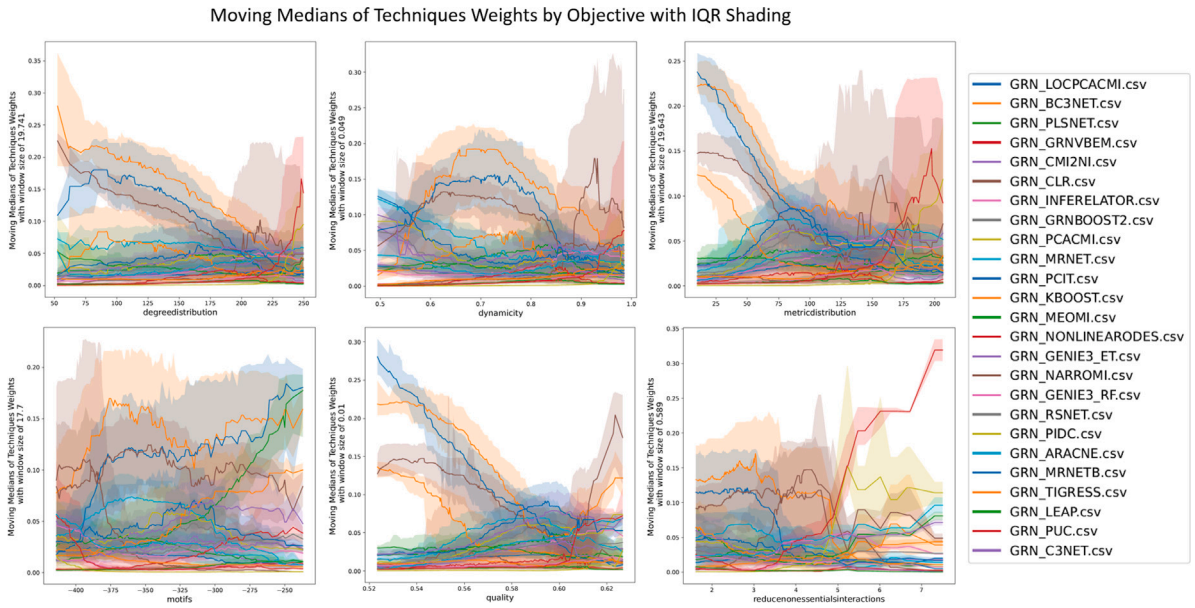


Fig. 7. In these plots, the moving medians of the weights assigned to each technique in the front obtained by BIO-INSIGHT for the *Glycine max* network from BioGRID are represented and sorted for each objective of the algorithm. Additionally, as in Fig. 6, each moving median is shaded by the interquartile range, which in this case outlines the diversity of the weights assigned to each technique at each objective function value.

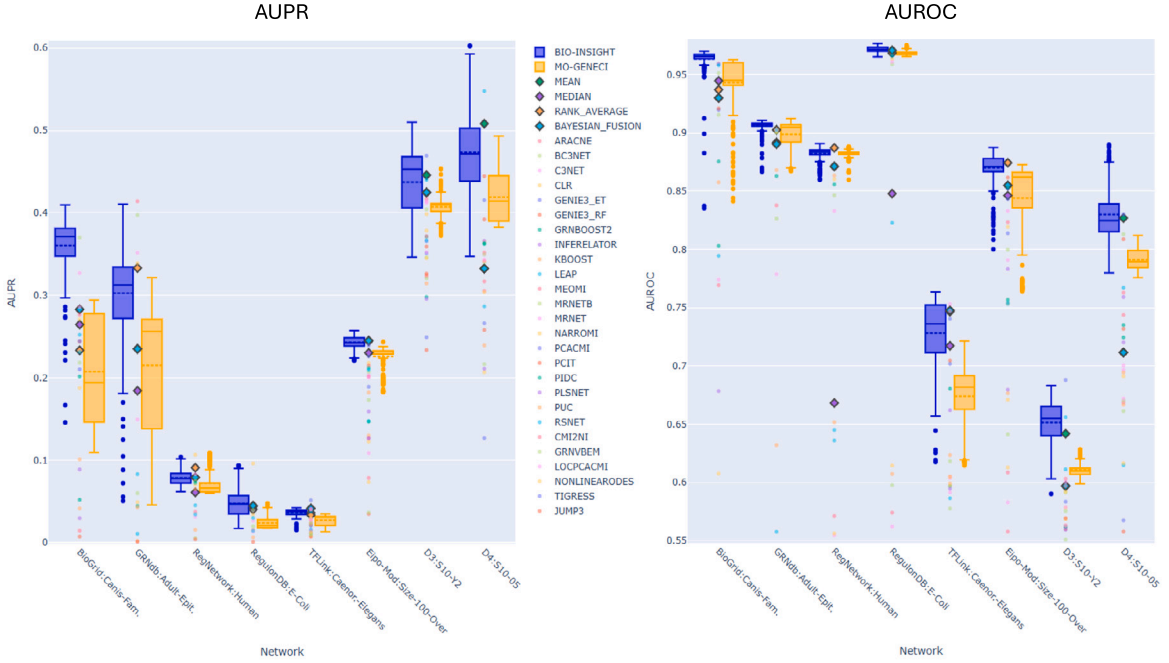


Fig. 8. Comparison of the AUPR and AUROC metrics obtained by BIO-INSIGHT (blue box) for a diverse set of networks from different sources, in relation to MO-GENECI (orange box), other consensus strategies (diamonds), and individual techniques (circles).

It is important to highlight that only the best-performing individual from the Pareto front is considered in this comparison. The median-quality solution was deliberately excluded, as it would correspond to an uninformed random selection within the front, and would therefore be at a disadvantage compared to mono-objective variants that always return the single optimal solution found.

These results confirm that the simultaneous optimization of multiple biologically grounded objectives leads to more accurate consensus networks than optimizing each one in isolation. This supports the main hypothesis of this study, which advocates for a holistic and multi-faceted approach to GRN inference.

In addition, the rankings provide valuable insights into the relevance of each objective. Notably, several of the best-ranked mono-objective variants correspond to newly proposed objectives not inherited from previous literature, such as *Dynamicity* (O6), *Reduce Non-Essential Interactions* (O4), and *Eigenvector Distribution* (O3). These consistently outperform classical objectives like *Quality* (O1) or *Degree Distribution* (O5), reinforcing the contribution and novelty of this proposal in terms of biological interpretability and inference accuracy.

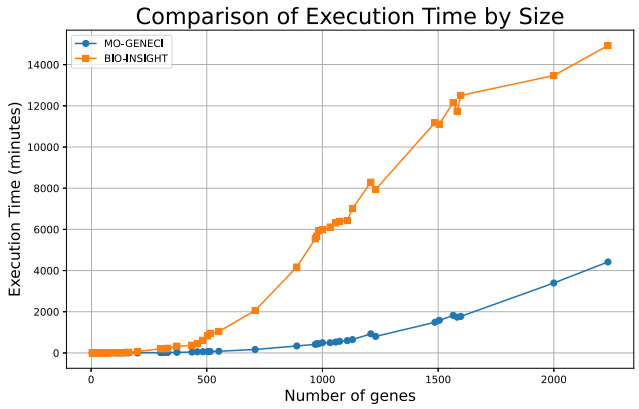


Fig. 9. Comparison of execution time depending on the network size for MO-GENECI (blue) and BIO-INSIGHT (orange).

5.3. Real-world clinical application

The performance of BIO-INSIGHT was also evaluated using non-simulated gene expression data from 43 female subjects: 8 diagnosed with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), 10 with Fibromyalgia (FM), 16 with both ME/CFS and FM (co-diagnosed from now on), and 9 healthy controls (GSE269048 dataset) [69]. ME/CFS and FM are chronic conditions characterized by fatigue, pain, and other disabling symptoms for which no validated biomarkers exist. Gene expression levels were measured using custom Affymetrix HERV-V3 microarrays [70], targeting 1559 genes related to immunity, inflammation, cancer, central nervous system functions, differentiation, telomere maintenance, chromatin structure, and gag-like genes. Data preprocessing and normalization steps prior to analysis are detailed in [69].

Using these data, BIO-INSIGHT predicted pairs of interacting genes for each condition. After applying the approximate Pareto front reduction discussed in Section 4, it could be observed that all inferred interactions for the same pathology appear in 100% of the solutions of the corresponding front (frequency = 1), which is evidence of the stability and consistency of the predictions. Results were compared between disease groups and controls by calculating differences in the average weight of interactions. A threshold of $|0.4|$ was applied to identify significant differences (Table S1 in Suplementary Material). In the case of duplicate interactions, where genes could act as both regulators and regulated, only the pair with the highest average weight difference was retained (Table S2 in Suplementary Material). When each disease study group was compared to the healthy control group, 32 additional and 35 absent gene interactions were identified in ME/CFS, while fewer interactions appeared related to FM and the co-diagnosed groups, most seemingly absent with respect to controls. Specifically, 40 gene regulations were found absent in FM, with only 2 additional for this condition, and 41 were absent in the co-diagnosed group, which displayed 6 disease-associated additional interactions. Some of these genes, i.e. CD74 and TAB2 in the ME/CFS group, were also found to be differentially expressed [69], in support of potential biological relevance of the predicted interactions (Table S2 in Suplementary Material). Overrepresentation analysis (ORA) of the interacting gene sets for each condition, using Gene Ontology terms for Biological Processes, revealed that cytoplasmic translation, immune response and programmed cell death pathways could be affected across all disease groups (Fig. 10A). These findings align with the existing literature, supporting a role for immune abnormalities in ME/CFS and FM [71]. Furthermore, alterations in apoptotic signalling [72,73] and protein synthesis [74–76] can lead to immune dysregulation, potentially contributing to the clinical manifestations observed in these patients.

Given the overlapping clinical features of ME/CFS and FM, and the lack of specific biomarkers, we expanded the analysis to compare potential gene interactions across ME/CFS, FM, and co-diagnosed regulatory networks. This approach identified significant gene-gene interactions, particularly in the ME/CFS study group, most absent in the FM (36/38) or co-diagnosed (38/41) groups (Table S2 in Suplementary Material). To further elucidate disease-specific or commonly absent interactions across all groups, the intersection between group comparisons is obtained. Venn diagrams are used to illustrate the specific or common regulatory interactions across these conditions (Fig. 10B). A set of 25 gene-gene interactions are predicted to be missing across all disease groups (Table S3 in Suplementary Material, control unique intersections), including gene pairs such as IL6-CCL8, TNF-TAB2, or RPS10-RPL19, involved in critical pathways like cytokine signalling, immune activation, and protein synthesis, respectively (Fig. 10B-C). Conversely, 27 gene-gene interactions related to programmed cell death are uniquely present in ME/CFS (Table S3 in Suplementary Material and Fig. 10B-C), suggesting distinct regulatory alterations specific for this condition. Among them the CD74-EIF4G2 interaction, involving a cell surface protein that participates in several immune processes, including inflammatory or autoimmune diseases [77] and a protein involved in the regulation of protein synthesis, leading to immune dysregulation when its function is impaired [74–76] should be highlighted for the available validating information. In addition to CD74, a human endogenous retrovirus (HERV) (the MLT1_5q32 element) encoded in one of its introns, were found differentially expressed in ME/CFS, supporting its potential implication in this disease [69]. Furthermore, CD74 gene's potential role linking monocyte functioning and neurological symptoms in ME/CFS, with biomarker value, had been previously described by [78]. On another hand, the physical interaction between CD74 and EIF4G2 is experimentally confirmed by [79] using Affinity Capture-MS, further validating BIO-INSIGHT's predictive capacity and underscoring its utility in identifying clinically relevant gene predictions. These results demonstrate that BIO-INSIGHT provides a valuable tool for understanding the molecular underpinnings of ME/CFS and FM, offering insights into gene regulatory networks that could inform future therapeutic strategies.

6. Conclusions

This article has introduced BIO-INSIGHT, a software package designed for gene regulatory network consensus inference, whose core consists of an asynchronous and parallel many-objective evolutionary algorithm that optimizes a weighted voting system among various machine learning techniques, based on a comprehensive set of biologically driven objectives.

The experimentation conducted on an extensive and diverse academic benchmark of 106 gene networks has demonstrated that BIO-INSIGHT significantly outperforms state-of-the-art methodologies in accuracy (AUPR and AUROC), including individual techniques and consensus strategies and algorithms.

Additionally, the discussion of the results has addressed a total of six research questions, whose answers have allowed for justifying the motivation behind the proposal, ensuring an appropriate trade-off between objectives that validates the algorithmic design, establishing a necessary holistic perspective in the field, demonstrating the enrichment of the biological interpretability of consensus networks, verifying a correct alignment between the objective space and inference accuracy, and finally, confirming BIO-INSIGHT's complete independence from the methodologies integrated into individual techniques.

The high accuracy and robustness demonstrated by BIO-INSIGHT in the academic benchmark have motivated its application to real-world gene expression datasets. In an additional analysis, BIO-INSIGHT has showcased its clinical potential by identifying characteristic interactions for different treated diseases, suggesting its usefulness in exploring novel underlying biological mechanisms and discovering potential biomarkers.

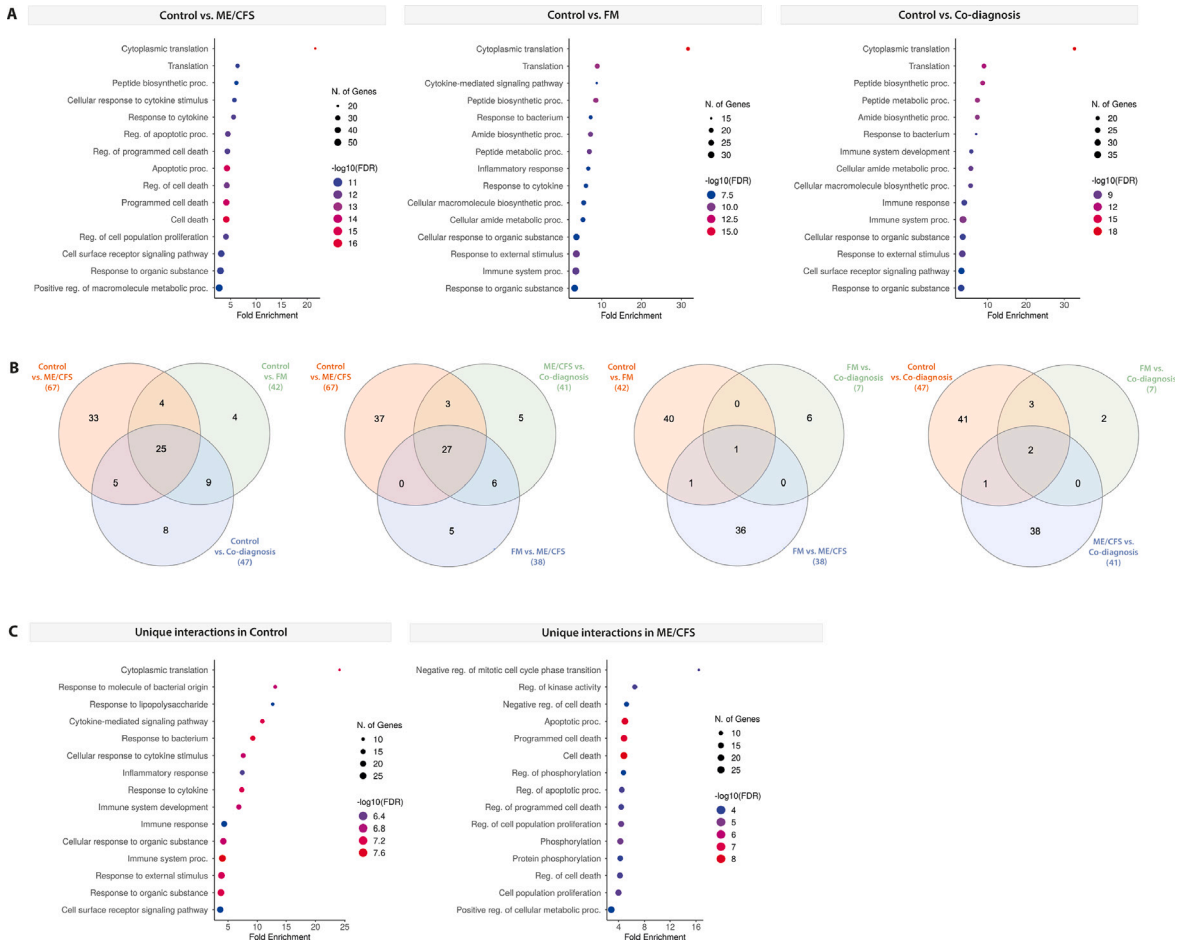


Fig. 10. Differential gene enrichment and unique interactions across conditions. (A) Pathway enrichment analysis comparing gene expression between control and ME/CFS, FM, and co-diagnosis study groups. Dot size represents the number of genes involved while colour indicates the -log10(FDR) significance. (B) Venn diagrams displaying the overlap of predicted gene interactions across study groups. (C) Unique pathway interactions for the control and ME/CFS groups, highlighting significantly enriched biological processes.

Although the present study has employed the same inference techniques as MO-GENECI to ensure a fair methodological comparison, the current implementation of BIO-INSIGHT allows for the inclusion of results from any external inference method. As a future line of work, we foresee the integration of new tools into the software package, including recent developments based on deep learning, thereby enhancing the flexibility and adaptability of the framework to upcoming advances in the field.

Despite the promising results, certain limitations are anticipated, which pave the way for future research directions. Specifically, the current encoding of individuals as weight vectors, while effective, may prove too simplistic given the increasing complexity of the biological objective space introduced. This potential mismatch between the search space's expressiveness and the encoding's flexibility could hinder the exploration of more sophisticated configurations. To address this, it is worth considering the development of new encodings that allow for more flexible and adaptive selection within the contents of each inference technique. Additionally, to improve computational efficiency, the integration of preliminary clustering steps over the inferred networks is being explored to alternate between global and local optimization phases. This strategy would allow computational resources to focus on highly conflicting regions among techniques while avoiding redundancy in clearer, more consistent areas of the solution space.

CRediT authorship contribution statement

Adrián Segura-Ortiz: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Karen Giménez-Orenga:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **José García-Nieto:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Elisa Oltra:** Supervision, Conceptualization. **José F. Aldana-Montes:** Supervision, Funding acquisition.

Ethics statement

The research presented in this manuscript does not require an Ethics Statement, as it does not involve any new experimentation with human or animal subjects. All analyses were conducted using either simulated data derived from publicly available databases or real-world expression data obtained from previously published studies.

The real-world datasets used in this work were generated in past research efforts that followed appropriate ethical guidelines and obtained the necessary approvals at the time of their collection. No identifiable personal or sensitive information was used in this study.

As such, no new ethical approval was required for the purposes of the current computational and inferential analyses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.compbiomed.2025.110632.

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