

Systems Biology

MIIC online: a web server to reconstruct causal or non-causal networks from non-perturbative data

Nadir Sella^{1,2}, Louis Verny^{1,2}, Guido Uguzzoni^{1,2}, Séverine Affeldt^{1,2,3} and Hervé Isambert^{1,2,*}

¹Institut Curie, PSL Research University, CNRS, UMR168, 26 rue d'Ulm, 75005 Paris, France,

²Sorbonne Universités, UPMC Univ Paris 06, 4, Place Jussieu, 75005 Paris, France and

³Current address: LIPADE, University of Paris Descartes, 45 rue des Saints Pères, 75006 Paris, France.

*To whom correspondence should be addressed.

Associate Editor: Jonathan Wren

Received on 16 August 2017; revised on 24 November 2017; accepted on 26 December 2017

Abstract

Summary: We present a web server running the MIIC algorithm, a network learning method combining constraint-based and information-theoretic frameworks to reconstruct causal, non-causal or mixed networks from non-perturbative data, without the need for an *a priori* choice on the class of reconstructed network. Starting from a fully connected network, the algorithm first removes dispensable edges by iteratively subtracting the most significant information contributions from indirect paths between each pair of variables. The remaining edges are then filtered based on their confidence assessment or oriented based on the signature of causality in observational data. MIIC online server can be used for a broad range of biological data, including possible unobserved (latent) variables, from single-cell gene expression data to protein sequence evolution, and outperforms or matches state-of-the-art methods for either causal or non-causal network reconstruction.

Availability: MIIC online can be freely accessed at <https://miic.curie.fr>

Contact: herve.isambert@curie.fr

Supplementary information: Supplementary Materials are available at *Bioinformatics* online.

1 Introduction

The reconstruction of graphical models has become ubiquitous to analyze the rapidly expanding, information-rich data of biological interest. However, to date, all available network reconstruction servers are restricted to specific types of data and make an *a priori* choice on the causal or non-causal nature of the underlying model, such as BNW (Ziebarth *et al.* (2013)) performing Bayesian Network reconstructions, LEGUMEGRN (Wang *et al.* (2013)) learning directed regulatory networks or EVFOLD (Marks *et al.* (2011)) and DCA (Morcos *et al.* (2011)) predicting undirected protein contact interactions from amino acid homologous sequences.

MIIC online server aims to fill this gap by learning the most appropriate causal, non-causal or mixed graphical model from the available data. MIIC can be used for a broad range of biological data, from single-cell transcriptomics or genomic alterations in tumor progression to long term evolution of proteins and genomes (Fig. 1 and Figs. S1-S4 and Verny *et al.* (2017)). MIIC online server is outlined below with more detailed information available in Supplementary Materials and online Tutorial and User Guide documentation available at <https://miic.curie.fr>.

2 Methods

2.1 MIIC algorithm

MIIC (Multivariate Information-based Inductive Causation) algorithm relies on a novel information-theoretic method that combines constraint-based learning approach and maximum likelihood framework (Verny *et al.* (2017), Affeldt *et al.* (2016), Affeldt *et al.* (2015)). Starting from a fully connected graph, MIIC iteratively removes dispensable edges, by uncovering significant information contributions from indirect paths, and orients the remaining edges, based on the signature of causality in observational data. MIIC also provides an edge specific confidence assessment of retained edges. The approach outperforms traditional search-and-score and constraint-based methods on a broad range of benchmark networks (Verny *et al.* (2017), Affeldt *et al.* (2016), Affeldt *et al.* (2015)). It achieves significantly better results with much fewer samples and is typically ten to hundred times faster than existing methods taking into account the causal effects of unobserved latent variables (Verny *et al.* (2017)).

2.2 MIIC online input and main options

MIIC online pipeline (Fig. S1) is a web interface for the MIIC algorithm. The **Workbench** is used to upload the user's dataset formatted as a table with variable names specified as column names (or row names). This is the only required input to reconstruct a network using basic (default) settings.

Algorithm advanced parameters. This section allows the user to specify the following parameters: (i) N_{eff} , the effective number of independent samples in the submitted dataset (also estimated by MIIC itself, see below), (ii) *Complexity criterion*, either MDL/BIC (Minimum Description Length/ Bayesian Information Criterion) or NML (Normalized Maximum Likelihood) (Affeldt et al. (2016)), (iii) *Orientation step* (optional), orienting 'v-structures', (iv) *Propagation step* (optional), if the orientation step is performed, and (v) *latent variables*, to take into account or ignore the causal effects of unobserved (latent) variables (Verny et al. (2017)).

Supplementary files. Supplementary files can be uploaded (optional), in particular, to exclude specific edges based on prior knowledge, to provide a user-defined network layout (x, y coordinates of nodes) or to provide an order of categorical (non-numerical) variables for assigning edge signs based on correlations or causal effects between variables.

Confidence cut. A threshold can be provided (default 10^{-2}) to filter retained edges based on their confidence estimated over a number of randomizations of the available data (default 100 randomizations).

3 Results

3.1 MIIC online output: visualization and analysis

Once the learning process is finished, the web server redirects the user to the results page, that contains several sections, displaying the network and reporting some analysis. The **Advanced visualization** tab uses a web-embedded Cytoscape display to visualize and interactively rearrange the resulting network. It is also possible to open a new browser tab ("Go!" button) to visualize and filter network edges based on their residual (partial) **correlation** or edge-specific **confidence** and save the graph visualization in various formats. The **Summary** tab contains all the processed information on each retained or deleted edges. The **Probabilities** tab contains information relative to the orientation and propagation steps of the network reconstruction and is relevant for a quantitative analysis of the causal relations in the data (see Supplementary Information). The **Centrality measures** tab provides a topological analysis of the network using graph theoretical measures. The **Cross correlation** tab displays a plot of the sample cross-correlation decay along with an exponential test. In the presence of correlation biases between successive samples, a warning message is displayed on the results page and an estimate of the effective number of independent samples, N_{eff} , is used to reconstruct a more reliable network model (Verny et al. (2017)). The **Download** tab allows to download all the results associated with a network reconstruction.

3.2 Examples of causal versus non-causal networks

3.2.1 Gene regulatory network in hematopoiesis

This first example concerns the reconstruction of a regulatory network from 2,167 single-cell gene expression profiles of blood stem cells from (Hamey et al. (2017)), see Supporting Information. Fig. 1 displays MIIC online results page with a zoomed view of the regulatory network including 34 transcription factors, see full network in Fig S2. MIIC predicted network exhibits a number of known central regulators such as *MECOM*|*EVII*, *GATA1* and *GATA2*, with regulatory interactions documented in the literature, such as *MECOM* → *PBX1*, *MECOM* → *GATA2* and *GATA2* → *TALI*|*SCL*, see Supporting Information for details. Note, in particular, that nearly all predicted edges are directed, as expected for a transcriptional regulatory network, with red edges indicating gene activation and blue edges indicating gene repression regulations.

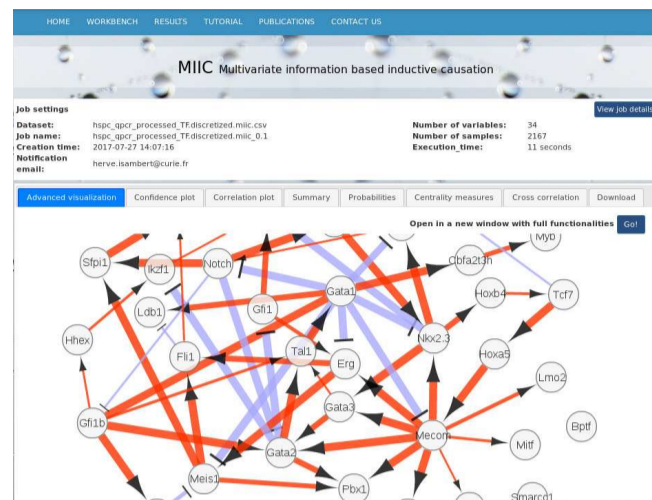


Fig. 1. View of MIIC online output page with a network visualization. It corresponds to a zoom of a regulatory network reconstructed from single-cell expression data from hematopoietic stem cell differentiation, Hamey et al. (2017), See full network in Fig S2.

3.2.2 Protein undirected contact map

By contrast, the second example concerns an inherently non-causal network corresponding to the physical contact map of amino acid residues within a protein structure reconstructed from 12,533 aligned homologous sequences of an abundant protein domain family: the *response regulator receiver domain* (Pfam code PF00072). MIIC contact prediction results are presented in Figs. S3 & S4 and provide similar accurate predictions of the protein contact map, without a *a priori* choice or bias on the causal or non-causal class of reconstructed networks, as compared with the state-of-the-art method for protein contact prediction, plmDCA Ekeberg et al. (2013), although MIIC performance on protein structures with fewer homologous sequences is found to be less accurate, Fig. S5.

Funding

This work has been supported by the Labex celtisphybio and Region IdF.

Conflict of Interest: none declared.

References

- Affeldt S, Verny L, Isambert H. (2016) 3off2: A network reconstruction algorithm based on 2-point and 3-point information statistics, *BMC Bioinformatics* **17** (Suppl 2), 12.
- Affeldt S, Isambert H (2015) Robust reconstruction of causal graphical models based on conditional 2-point and 3-point information, *Proceedings of the 31st Conference on Uncertainty in Artificial Intelligence (UAI)*, 42-51.
- Ekeberg, M., Lökvist, C., Lan, Y., Weigt, M., Aurell, E. (2013) Improved contact prediction in proteins: using pseudolikelihoods to infer Potts models. *Phys Rev E*, **87**(1), 012707.
- Hamey FK, et al. (2017) Reconstructing blood stem cell regulatory network models from single-cell molecular profiles, *Proc Natl Acad Sci USA* **114**(3), 5822-5829.
- Marks DS, et al. (2011) Protein 3D structure computed from evolutionary sequence variation, *PLoS one* **6**(12), e28766.
- Morcos F, et al. (2011) Direct-coupling analysis of residue coevolution captures native contacts across many protein families, *Proc Natl Acad Sci USA* **108**(49), E1293-E1301.
- Verny L, et al. (2017) Learning causal networks with latent variables from multivariate information in genomic data, *PLoS Comput Biol*, **13**(10):e1005662.
- Wang M, et al. (2013) LegumeGRN: a gene regulatory network prediction server for functional and comparative studies, *PLoS one* **8**(7), e67434.
- Ziebarth JD, Anindya B, Yan C. (2013) Bayesian Network Webserver: a comprehensive tool for biological network modeling, *Bioinformatics* **29**(21), 2801-2803.

Supplementary Materials

for manuscript

MIIC online: a web server to reconstruct causal or non-causal networks from non-perturbative data

Nadir Sella^{1,2}, Louis Verny^{1,2}, Guido Uguzzoni^{1,2}, Séverine Affeldt^{1,2,3} and Hervé Isambert^{1,2*}

¹Institut Curie, PSL Research University, CNRS, UMR168, 26 rue d'Ulm, 75005 Paris, France,

²Sorbonne Universités, UPMC Univ Paris 06, 4, Place Jussieu, 75005 Paris, France and

³ Current address: LIPADE, University of Paris Descartes, 45 rue des Saints Pères, 75006 Paris, France.

1 User Documentation

A Tutorial and detailed User Guide documentations for MIIC online server are available at <https://miic.curie.fr>

2 MIIC online pipeline

The work-flow of MIIC online server is outlined in Fig. S1.

It consists of *i*) an input layer including the data, default or user-defined parameters and optional supplementary files uploaded by the user, *ii*) the algorithmic core of the network reconstruction and *iii*) an output layer with all interactive visualizations and analyses about the results.

MIIC algorithmic core (*ii*) includes three main steps detailed in the Methodological Sections of [Verny *et al.*, 2017].

These three algorithmic steps are summarized below:

Step1: Learning the network ‘skeleton’

Starting from a fully connected undirected graph, MIIC iteratively prunes the edges that are not required to account for the observed correlations in the available data, as the corresponding correlations can already been explained by indirect paths without the need for additional edges between some of the nodes. MIIC algorithm proceeds as follows based on information-theoretic results [Affeldt *et al.*, 2015, Affeldt *et al.*, 2016].

Given two nodes X and Y , MIIC looks for the most significant contributors susceptible to explain the mutual information between X and Y , $I(X; Y)$, and iteratively removes their contributions as,

$$I(X; Y|\{A_i\}) = I(X; Y) - I(X; Y; A_1) - I(X; Y; A_2|A_1) \dots - I(X; Y; A_i|\{A_{i-1}\}) \quad (1)$$

until the residual conditional mutual information between X and Y given $\{A_i\}$, $I(X; Y|\{A_i\})$, becomes lower than the associated complexity loss of the graphical model without the XY edge, $k_{X;Y|\{A_i\}}/N$, where N is the number of independent samples. Otherwise, if $I(X; Y|\{A_i\}) > k_{X;Y|\{A_i\}}/N$, the XY edge is retained, if no additional contributor can be found to account for the residual conditional mutual information between X and Y . This first step of MIIC algorithm returns an undirected graph, referred to as the network ‘skeleton’.

Step2: Edge filtering (optional) based on Confidence ratio

The edge filtering step (optional) allows to remove additional edges from the first network skeleton obtained in Step 1, according to an edge-specific confidence assessment [Verny *et al.*, 2017]. It is based on the probability to delete the edge XY between nodes X and Y , which can be estimated as,

$$P_{XY} = e^{-NI'(X;Y|\{A_i\})} \quad (2)$$

where N is the number of independent samples in the data and $I'(X;Y|\{A_i\}) = I(X;Y|\{A_i\}) - k_{X;Y|\{A_i\}}/N$.

The probability P_{XY} is then evaluated for each retained edge of the skeleton obtained using the actual dataset *versus* multiple randomized instances of the same dataset. This allows to compute the following edge-specific confidence ratio:

$$C_{XY} = \frac{P_{XY}}{\langle P_{XY}^{\text{rand}} \rangle} \quad (3)$$

where $\langle P_{XY}^{\text{rand}} \rangle$ is the mean probability to remove the edge XY averaged over multiple randomized datasets. Hence, a smaller confidence ratio, C_{XY} , implies a higher statistical confidence on the retained XY edge.

Step3: Edge orientation

Finally, given the skeleton obtained in Step 1, possibly filtered in Step 2, MIIC infers edge orientations based on the signature of causality in observational data as follows [Verny *et al.*, 2017].

First, MIIC sorts unshielded triples, *i.e.* $X - Z - Y$ without XY edge, by decreasing absolute value of their three-point conditional mutual information including finite size complexity correction, $|I'(X;Y;Z|\{A_i\})|$, where $\{A_i\}$ is the (possibly empty) set of contributors accounting for the removal of the XY edge, *i.e.* with $I'(X;Y|\{A_i\}) < 0$. Then, MIIC orients the XZ and/or ZY edges as,

- If $I'(X;Y;Z|\{A_i\}) < 0$, it forms a V-structure: $X* \rightarrow Z \leftarrow *Y$
- If $I'(X;Y;Z|\{A_i\}) > 0$ and $X* \rightarrow Z$, the second edge is oriented as to form a non-v-structure: $X* \rightarrow Z \rightarrow Y$

where the endpoint mark $*$ stands for either an arrow head $>$ or tail \leftarrow . This enables, in particular, to obtain bidirected edges, *e.g.* $Z \leftrightarrow Y$, shared by two v-structures, *e.g.* $X* \rightarrow Z \leftrightarrow Y \leftarrow *W$, which reflects the presence of unobserved (latent) causes such as, $Z \leftarrow - L \rightarrow - Y$.

3 Examples of causal versus non-causal networks

In this section we illustrate the use of MIIC `online` server with two examples of network reconstruction from real biological data.

The first example is an inherently causal network corresponding to directed regulatory interactions between specific transcription factors involved in hematopoietic stem cell differentiation, while the second example is a non-causal network corresponding to undirected symmetric physical interactions between amino acid residues in close contact in a protein structure.

As discussed in the main text, these different types of causal and non-causal networks cannot be reconstructed with the single existing online server, which are all designed to learn specific classes of directed *or* undirected networks without the possibility to compare between alternative classes of graphical models. This prevents all existing network reconstruction servers to uncover or rule out causality in observational data.

By contrast, **MIIC online** does not require the user to select *a priori* the type of causal or non-causal underlying model, as MIIC algorithm learns the most appropriate causal, non-causal or mixed model given the available data.

3.1 Reconstruction of regulatory networks from single cell expression data

This first example concerns the reconstruction of blood stem cell regulatory network models from single-cell molecular profiles. The mammalian blood system is maintained throughout the adult lifetime by hematopoietic stem cells (HSCs) that differentiate into all mature blood cell types. Differentiation of HSCs toward alternative lineages is controlled by transcription factors within organized regulatory programs that can be modeled as transcriptional regulatory networks.

While hematopoiesis in adult has been extensively studied and well-characterized at cell population level, cell fate decisions are in fact made at the level of individual cells and lead to heterogeneous cell populations. Recent developments of high-throughput single-cell technologies, such as quantitative real-time PCR (qRT-PCR) and RNA sequencing (RNA-Seq), now provide unique tools to study such differentiation processes and corresponding regulatory networks at single-cell level.

In this section, we analyze the recent dataset obtained by [Hamey *et al.*, 2017], which contains qRT-PCR gene expression profiles of 48 genes including 34 transcription factors for 2,167 single HSCs and progenitor cells.

The input dataset used for **MIIC online** reconstruction includes all 34 transcription factors and has been discretized into binary levels corresponding to expressed *versus* non-expressed genes, as suggested by the clearly bimodal distributions of qRT-PCR expression profiles. As expected, no significant correlation bias between successive single cell samples is identified with **MIIC online** correlation analysis. Hence, all 2,167 single cell expression profiles can be considered as independent samples for the network reconstruction.

The network inferred by **MIIC online** is displayed in Fig. 1 of the main text (zoomed view) and Fig. S2 (full network). The edges in the reconstructed network have been filtered using a confidence ratio threshold of 10^{-1} (Step 2 of MIIC algorithm) and their width reflects their estimated confidence. They represent direct regulatory interactions between regulator and target transcription factors. In particular, we observe that nearly all predicted edges are directed, as expected for transcriptional regulatory networks, with red edges indicating gene activation and blue edges indicating gene repression regulations.

MIIC predicted network corresponds to a global transcriptional regulatory network, as it combines expression profiles of HSCs with different progenitor cell types [Hamey *et al.*, 2017]. This network exhibits a number of known central regulators such as *MECOM|EVII*, *GATA1* and *GATA2*, with regulatory interactions documented in the literature, such as *MECOM* \rightarrow *PBX1* [Yuan *et al.*, 2015], *MECOM* \rightarrow *GATA2* [Yuasa *et al.*, 2005] and *GATA2* \rightarrow *TAL1|SCL* [Chan *et al.*, 2006].

3.2 Reconstruction of residue-residue interaction network in protein structure from homolog genomic sequences

The three-dimensional structure similarity between homologous proteins imposes strong constraints on their sequence variability. This gives rise to correlated substitution patterns among amino acid residues at different sequence positions of a protein family. It has long been suggested that these correlations can be exploited to infer spatial contacts within the tertiary protein structure [Altschuh *et al.*, 1987][Neher, 1994]. In the last years several methods have been proposed to disentangle direct and indirect correlations, that represents one of the major difficulties for the success of the approach [Burger *et al.*, 2008] [Weigt *et al.*, 2009] [Morcos *et al.*, 2011] [Marks *et al.*, 2011].

In this section, we show the efficacy of MIIC algorithm to retrieve the internal protein contact network for a widely studied protein family: the *response regulator receiver domain* (Pfam code PF00072). This extremely abundant protein family is involved in bacterial signal transduction and acts as a transcription factor interacting with specific DNA binding domains. This family is especially suited to assess the performance of inference methods for protein contact network as (1) it contains a great number of sequenced proteins (63,624), (2) several protein structures belonging to this family have been experimentally resolved, and (3) it is a classical example that has already been studied in depth in the literature [Weigt *et al.*, 2009], [Uguzzoni *et al.*, 2017].

The input dataset consists of a multiple sequence alignment (MSA) including 112 positions of the homologous sequences, which can be downloaded from the Pfam database [Bateman *et al.*, 2004]. When the whole dataset including the 63,624 homologous sequences is used as input file on MIIC online server, a warning message appears in the Result page to indicate significant correlations between samples, which do not simply decay exponentially between successive sequences in the MSA. These correlations have been discussed in the literature and are due to the phylogeny, multiple-strain sequencing, and a biased selection of sequenced species. To overcome this issue, we have used a standard procedure to reduce the redundancy due to sequence bias [Morcos *et al.*, 2011]. Namely, we filtered the MSA by randomly selecting sequences that differ from each other for at least 30% of their positions and removing the other sequences from the MSA. After this preprocessing of the data, the resulting filtered MSA contains 12,533 sequences.

The results of MIIC network prediction are presented in Figs. S3 & S4. The edges in the reconstructed network represent the residue-residue physical proximity in the 3D structure. Using Pymol [DeLano *et al.*, 2017], we can visualize the contact predictions and overlay them to available crystallographic structures.

In Fig. S3, we report the contact predictions mapped on an experimentally resolved structure (*Inxs*) downloaded from the PDB database [Berman *et al.*, 1999]. Note that MIIC predictions provide an accurate description of the contact map of the protein (green edges). Quite remarkably, we also observe that MIIC does not predict any directed edges despite its lack of *a priori* restriction on the class of (undirected, directed or mixed) reconstructed network; this prevalence of undirected edges is in fact expected from the symmetry of the physical contacts between amino acid residues, by contrast to the asymmetric regulator-target gene relationships in the transcriptional regulation network described above. In addition, we found that most false positive contacts (red edges in left panel and red dots in right panel) are actually very close to true contacts in the *Inxs* protein structure (black dots in right panel) and are related to the intrinsic heterogeneity of the different protein structures within this large family. This is clearly apparent in Fig. S4, where MIIC predictions are compared to the union of 11 contact maps of homologous protein structures, see Fig. S4 caption. As a result, most of these apparently false positive contacts in the *Inxs* protein structure turn out to be true positive contacts once the structure heterogeneity of this large protein family is taken into account.

Finally, when these results are compared with the state-of-the-art method for protein contact prediction, plmDCA [Ekeberg *et al.*, 2013], we find that MIIC predicts a similar list of contacts and achieves similar performance as plmDCA, as shown in Fig. S4 and Fig. S5 (upper panel). However, it is important to stress that MIIC predicts a finite list of 179 contacts, while plmDCA sorts all potential pairwise contacts using a rank but without predicting an explicit cutoff to distinguish between actual contacts and non-contacts. Note, also, that contacts involving residues closer than 5 AA along the sequence are not displayed in Figs. S3-S4 & S5, as they correspond to ‘trivial’ contacts and are possibly affected by small gap statistics in the MSA [Feinauer *et al.*, 2014]. Hence, Figs. S3 & S4 display in fact 75 long-distance contacts out of the 179 contacts predicted by MIIC and the first 75 potential long-distance contacts inferred by plmDCA. Interestingly, most of remaining long-distance false positive contacts, predicted by the two methods in Fig. S4, have been shown to correspond to intermolecular contacts across homodimers rather than intramolecular contacts within a single protein domain as reported in [Uguzzoni *et al.*, 2017]. Hence, while these predicted contacts are not in the tertiary structure, they nonetheless correspond to real coevolutionary signals in the MSA due to direct

physical interactions between individual monomers in the quaternary assembly of the protein homodimers.

To further assess the performance of MIIC on protein contact map predictions, we have analyzed two additional protein families containing fewer homologous sequences. These are the *1a3a:a* PDB protein structure with a total of 31,922 homologous sequences and the *1mb6:a* PDB protein structure with a total of 246 sequences.

We apply the same filtering procedure as for the *response regulator receiver domain (Inxs)* above. This amounts to filtering sequences with more than 70% identity to reduce phylogenetic or other sampling biases, which leads to significantly reduced datasets of only 2,897 out of 31,922 sequences for the *1a3a:a* structure and only 53 out of 246 sequences for the *1mb6:a* structure.

Comparisons of MIIC and plmDCA ranked predictions of protein map contacts are presented in Fig. S5 and show a lower accuracy of MIIC with respect to plmDCA for these two datasets containing fewer homologous sequences. Yet, we note that, unlike MIIC, plmDCA uses the complete homologous sequence datasets through a weighting scheme of similar sequences to compensate for phylogenetic or other sampling biases. By contrast, as noted earlier, MIIC has the useful feature of providing a finite number of (mostly correct) predictions, while plmDCA provides a ranked list of predictions including essentially all possible pairs without clear cut-off, Fig. S5.

References

- [Affeldt *et al.*, 2015] Affeldt S, Isambert H (2015) Robust reconstruction of causal graphical models based on conditional 2-point and 3-point information, *Proceedings of the 31st Conference on Uncertainty in Artificial Intelligence (UAI)*, 42-51.
- [Affeldt *et al.*, 2016] Affeldt S, Verny L, Isambert H. (2016) 3off2: A network reconstruction algorithm based on 2-point and 3-point information statistics, *BMC Bioinformatics* **17** (Suppl 2), 12.
- [Altschuhet *et al.*, 1987] D Altschuh, AM Lesk, AC Bloomer, A Klug. (1987) Correlation of co-ordinated amino acid substitutions with function in viruses related to tobacco mosaic virus. *J Mol Biol* **193**(4), 693-707.
- [Bateman *et al.*, 2004] Bateman, Alex, et al. (2004) The Pfam protein families database. *Nucl Acids Res* **32**.suppl: D138-D141.
- [Berman *et al.*, 1999] Berman, Helen M., et al. The Protein Data Bank, 1999-. International Tables for Crystallography Volume F: Crystallography of biological macromolecules. Springer Netherlands, 2006. 675-684.
- [Burger *et al.*, 2008] Burger L, Van Nimwegen E (2008) Accurate prediction of protein-protein interactions from sequence alignments using a Bayesian method. *Mol Syst Biol* **4**(1), 165.
- [Chan *et al.*, 2006] Chan WYI, Follows GA, Lacaud G, Pimanda JE, Landry JR, Kinston S, et al. (2006) The paralogous hematopoietic regulators *lyl1* and *scl* are coregulated by *ets* and *gata* factors, but *lyl1* cannot rescue the early *scl*^{-/-} phenotype. *Blood* **109**(5):1908-1916.
- [DeLano *et al.*, 2017] DeLano, Warren L. (2002) The PyMOL molecular graphics system. <http://pymol.org>.
- [Ekeberg *et al.*, 2013] Ekeberg, M., Lövkvist, C., Lan, Y., Weigt, M., Aurell, E. (2013). Improved contact prediction in proteins: using pseudolikelihoods to infer Potts models. *Phys Rev E*, **87**(1), 012707.
- [Feinauer *et al.*, 2014] Feinauer, C *et al.* (2014). Improving contact prediction along three dimensions. *PLoS Comput. Biol.* , **10**(10), e1003847.

- [Hamey *et al.*, 2017] Hamey FK, *et al.* (2017) Reconstructing blood stem cell regulatory network models from single-cell molecular profiles, *Proc Natl Acad Sci USA* **114**(3), 5822-5829.
- [Marks *et al.*, 2011] Marks, D. S., Colwell, L. J., Sheridan, R., Hopf, T. A., Pagnani, A., Zecchina, R., Sander, C. (2011). Protein 3D structure computed from evolutionary sequence variation. *PLoS one*, **6**(12), e28766.
- [Morcos *et al.*, 2011] Morcos F, *et al.* (2011) Direct-coupling analysis of residue coevolution captures native contacts across many protein families. *Proc Natl Acad Sci USA* **108**(49), E1293-E1301.
- [Neher, 1994] Neher E (1994) How frequent are correlated changes in families of protein sequences? *Proc Natl Acad Sci USA*, **91**(1), 98-102.
- [Uguzzoni *et al.*, 2017] Uguzzoni G, *et al.* (2017) Large-scale identification of coevolution signals across homo-oligomeric protein interfaces by direct coupling analysis. *Proc Natl Acad Sci USA* **114**(13), E2662-E2671.
- [Verny *et al.*, 2017] Verny L, Sella N, Affeldt S, Singh PP, Isambert H. (2017) Learning causal networks with latent variables from multivariate information in genomic data, *PLoS Comput Biol*, 13(10):e1005662.
- [Weigt *et al.*, 2009] Weigt, M., White, R. A., Szurmant, H., Hoch, J. A., Hwa, T. (2009) Identification of direct residue contacts in protein-protein interaction by message passing. *Proc Natl Acad Sci USA*, **106**(1), 67-72.
- [Yuan *et al.*, 2015] Yuan, X., Wang, X., Bi, K., Jiang, G. (2015). The role of EVI-1 in normal hematopoiesis and myeloid malignancies (Review). *International Journal of Oncology*, **47**, 2028-2036.
- [Yuasa *et al.*, 2005] Yuasa, H *et al.* (2005). Oncogenic transcription factor Evi1 regulates hematopoietic stem cell proliferation through GATA-2 expression. *The EMBO Journal*, **24**(11), 1976-1987.

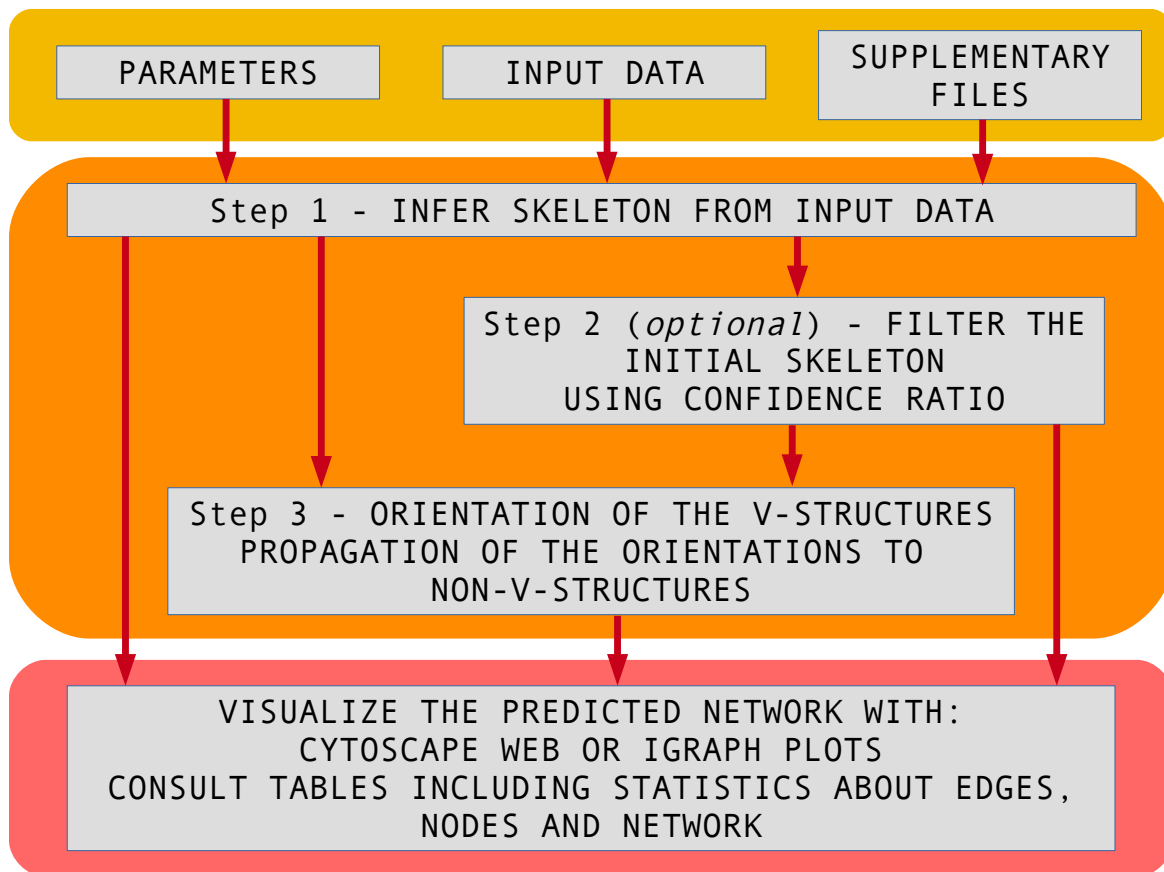


Figure S1: MIIC online server workflow. It consists of *i*) an input layer including the data, default or user-defined parameters and optional supplementary files uploaded by the user, *ii*) the algorithmic core of the network reconstruction including three main steps and *iii*) an output layer with all interactive visualizations and analyses about the results.

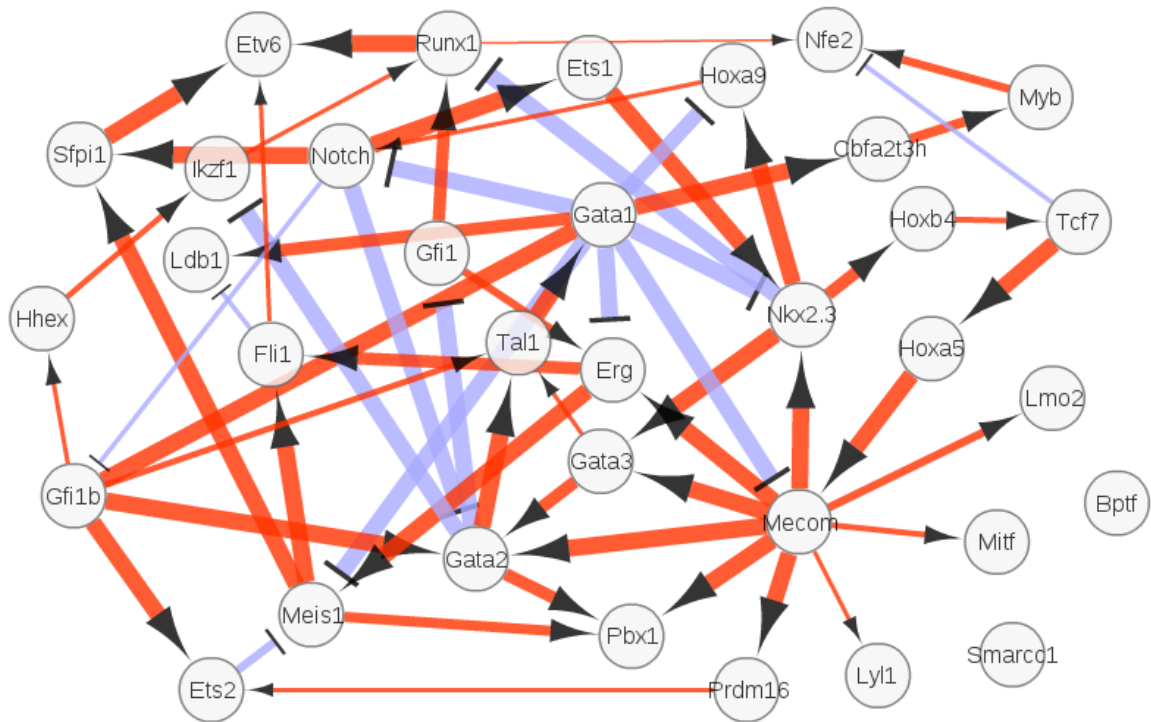


Figure S2: MIIC online reconstruction of the regulatory network of hematopoietic stem cell differentiation from single-cell expression data taken from [Hamey *et al.*, 2017]. See main text and Supplementary Materials for detailed information.

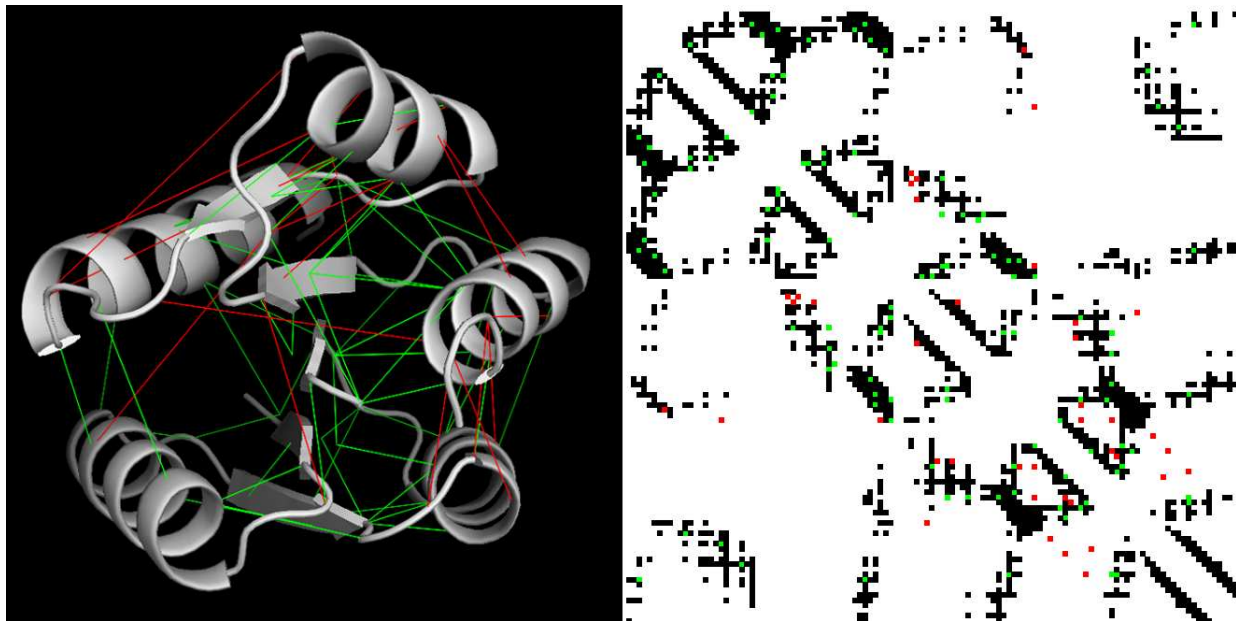


Figure S3: MIIC residue-residue contact predictions of the response regulator receiver domain (PF00072) mapped on an experimentally resolved structure (*Inxs* PDB). Contacts are defined as residues with a proximity of less than 8Å. Left panel: protein 3D structure with correct predictions in green and apparent errors in red, see however Fig. S4. Right panel: 2D contact map with experimental contacts in black and predictions with same color code as in the left panel.

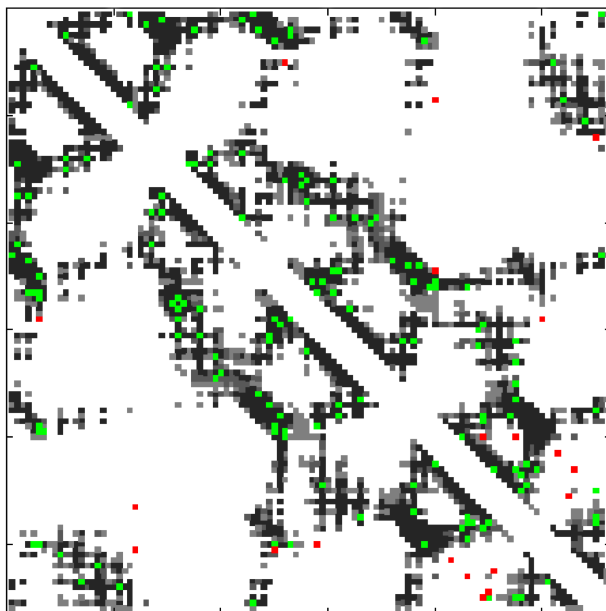


Figure S4: Contact map predictions of MIIC (upper triangular region) and plmDCA (lower triangular region) compared with the union of 11 experimental contact maps (from the following PDB structures: *1nxs*, *1zes*, *2pln*, *2zwm*, *3nnn*, *3r0j*, *2rdm*, *6chy*, *1l5y*, *2vuh*, *4l4u*). Structural contacts are displayed in black (if shared in all 11 models) or gray (if present in at least one of the 11 structures), while correct and erroneous predictions are shown in green and red, respectively. Note that the two methods present only small differences in the number of correct and erroneous predictions. Besides, many of the apparently erroneous contact predictions are in fact due to intermolecular interactions across the protein homodimers [Uguzzoni *et al.*, 2017].

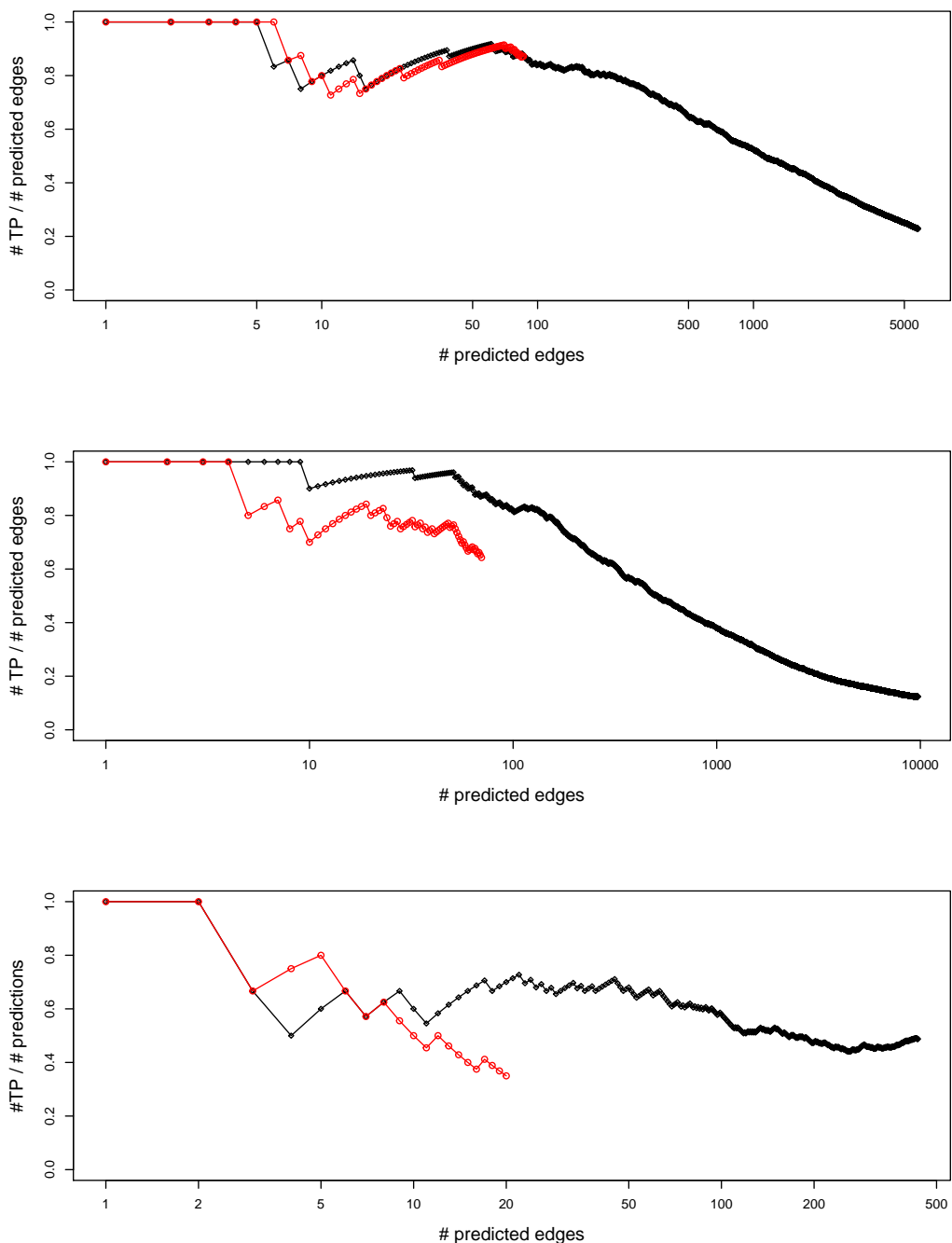


Figure S5: Fraction of true positive (TP) contacts amongst the first predicted pairs ranked by MIIC (red curves) and plmDCA (black curves) for three protein structures: *1nxs* (Figs S3 & S4), *1a3a:a* and *1mb6:a*. PlmDCA predictions make use of the full datasets which requires a reweighting scheme to compensate for sampling biases of similar sequences. By contrast, MIIC results are based on reduced datasets filtering out sequences with more than 70% identity. This corresponds to reduced datasets including 12,533 out of 63,624 sequences for *1nxs* (upper panel), 2,897 out of 31,922 sequences for *1a3a:a* (middle panel) and 53 out of 246 sequences for *1mb6:a* (lower panel). Note, however, that MIIC predicts a finite number of contacts, while plmDCA ranks predictions without a clear cut-off.