

1 Application of Modular Response Analysis to Medium- to Large-Size Biological

2 Systems

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4 Meriem Mekedem^{1,2,3}, Patrice Ravel^{1,2,3,4}, Jacques Colinge^{1,2,3,5,*}

5 ¹ Université de Montpellier, Montpellier, France

6 ² Institut de Recherche en Cancérologie de Montpellier, Inserm U1194, Montpellier, France

7 ³ Institut régional du Cancer Montpellier, Montpellier, France

8 ⁴ Faculté de Pharmacie, Université de Montpellier, Montpellier, France

9 ⁵ Faculté de Médecine, Université de Montpellier, Montpellier, France

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11 *Corresponding author

12 E-mail : jacques.colinge@inserm.fr

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14 Short title: Application of MRA to large biological systems

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17 **Abstract (300 words)**

18 The development of high-throughput genomic technologies associated with recent genetic perturbation
19 techniques such as short hairpin RNA (shRNA), gene trapping, or gene editing (CRISPR/Cas9) has made it
20 possible to obtain large perturbation data sets. These data sets are invaluable sources of information
21 regarding the function of genes, and they offer unique opportunities to reverse engineer gene
22 regulatory networks in specific cell types. Modular response analysis (MRA) is a well-accepted
23 mathematical modeling method that is precisely aimed at such network inference tasks, but its use has
24 been limited to rather small biological systems so far. In this study, we show that MRA can be employed
25 on large systems with almost 1,000 network components. In particular, we show that MRA performance
26 surpasses general-purpose mutual information-based algorithms. Part of these competitive results was
27 obtained by the application of a novel heuristic that pruned MRA-inferred interactions *a posteriori*. We
28 also exploited a block structure in MRA linear algebra to parallelize large system resolutions.

29

30 **Author Summary (150-200 words)**

31 The knowledge of gene and protein regulatory networks in specific cell types, including pathologic cells,
32 is an important endeavor in the post-genomic era. A particular type of data obtained through the
33 systematic perturbation of the actors of such networks enables the reconstruction of the latter and is
34 becoming available at a large scale (networks comprised of almost 1,000 genes). In this work, we
35 benchmark the performance of a classical methodology for such data called modular response analysis,
36 which has been so far applied to networks of modest sizes. We also propose improvements to increase
37 performance and to accelerate computations on large problems.

38

39 Introduction

40 The expression and activity of genes and proteins in cells are controlled by highly complex regulatory
41 networks involving genes and proteins themselves, but also non-coding RNAs, metabolites, etc. Despite
42 tremendous efforts in research, including all the developments of high-throughput genomic
43 technologies, a significant portion of this machinery remains uncharted. Moreover, dysregulations in
44 such networks are related to many diseases, and healthy cells of a same organism feature adjusted
45 regulatory networks depending on their cell types and states. Techniques, both experimental and
46 computational methodologies, that enable the inference of regulatory networks for different cells are
47 obviously of great interest.

48 Reference databases such as Reactome[1], KEGG[2], IntAct[3], or STRING[4] that compile our knowledge
49 of biological pathways or protein interactions have been established that provide valuable reference
50 maps. Due to their universal nature, these maps do not reflect natural and pathologic variations of
51 regulatory networks though some chosen disease pathways might be included. In principle, researchers
52 should generate data specific to the biological system of interest to assess the actual wiring of its
53 regulatory network. Specific data can be combined with reference databases in some algorithms, while
54 others only rely on *de novo* inferences. The field of systems biology has proposed many algorithms for
55 such a purpose involving different modeling approaches[5–7]. Obviously, algorithms must match the
56 type of data available to perform the inference such as a transcriptomes or proteomes obtained under
57 multiple conditions, time series, or perturbation data.

58 In this work, we are interested in the inference of regulatory networks based on systematic perturbation
59 data. That is, given a biological system of interest, which could be the whole cell, but also a small set of
60 related genes or proteins such as a pathway or part of a pathway, we have access to information
61 reporting the activity level of every component (gene/protein). Typical examples are transcript, protein,
62 or phosphorylated protein abundances. This information is available in basal condition as well as under
63 the systematic perturbation of each single component. When this type of data are obtained from a
64 biological system in a steady state, modular response analysis[8] (MRA) has been widely and successfully
65 applied[9]. The elegance of MRA is that it provides an efficient mathematical framework to estimate a
66 directed and weighted network representing the system regulatory network. Most applications of MRA
67 are limited to networks comprised of a modest number of modules (<10). In this study, we want to
68 explore the application of MRA to medium- (>50) and large-size (>500) systems. It entails a particular

69 implementation of the linear algebra at the heart of MRA to parallelize computations as well as the
70 introduction of a heuristic to prune the inferred networks *a posteriori* to improve accuracy.

71 As stated above, rewiring of regulatory networks is natural and necessary to yield a multitude of cell
72 types in higher organisms, and to adapt to distinct environmental conditions. Rewiring is also associated
73 with several diseases[10,11], an extreme case being cancer[12–14]. For instance, kinase signaling
74 cascades might be redirected in certain tumors to achieve drug resistance or to foster exaggerated cell
75 growth. MRA has been applied to a number of such cancer-related investigations[15,16] considering
76 rather small networks. Here, we take advantage of two published data sets that involve cancer cell lines
77 and provide systematic perturbation data compatible with MRA requirements. The first – medium-size –
78 data set[17] reports the transcriptional expression of 55 kinases and 6 non kinases under 11
79 experimental conditions (unstimulated plus 10 distinct stimulations). Under every condition, the
80 transcript levels of all the 61 genes were obtained by shallow RNA sequencing, including wild type cells
81 and cells with individual KOs of each gene. These data hence enable us to infer one network *per*
82 condition (11 networks) to discover how those 61 genes regulate themselves transcriptionally. The
83 second – large-size – data set was generated by the next generation of the Connectivity Map (CMap)
84 using its new L1000 platform[18]. Both shRNA- and CRISPR/Cas9-based systematic perturbations of
85 roughly 1,000, respectively 350, genes in 9, respectively 5, cell lines were released. These data enable us
86 to infer 9+5=14 networks.

87 We compare the performance of MRA, with and without the proposed pruning heuristic, to mutual
88 information (MI)-based methods that have found broad acceptance.

89

90 Results

91 *Network inference algorithms*

92 The availability of large functional genomics data collections (transcriptomes and/or proteomes) has led
93 to the development of a number of algorithms aimed at inferring interaction networks [7]. An essential
94 ingredient of most algorithms is the co-expression of genes (or proteins)[19], which can be captured by
95 simple correlation coefficients[20], mutual information (MI), or diverse statistical models[21]. There are
96 too many such algorithms to review them all here, but MI-based approaches seem to have provided off-
97 the-shelf, robust solutions that are widely used. We hence compare MRA to representatives of this
98 category such as CLR[22], MRNET[23], and ARACNE[24].

99 MI is often preferred over correlation for its ability to detect nonlinear relationships. With a network
100 involving n genes whose expression levels are measured in m transcriptomes, we write X_i the discrete
101 distribution representing gene i expression. The MI between genes i and j is given by

$$102 \quad MI_{i,j} = H(X_i) + H(X_j) - H(X_i, X_j),$$

103 where $H(X) = -\sum_{k \in X} p(x_k) \ln(p(x_k))$ is the entropy of a discrete random variable X . There exist
104 different estimators for $H(X)$ that use the m available transcriptomes[25]. Networks of interactions
105 identified though MI, imposing a minimal threshold on MI values, are commonly called relevance
106 networks[26,27]. The CLR algorithm improves over relevance networks by introducing a row- and
107 column-wise z-score-like transformation of $MI_{i,j}$ to normalize the MI matrix into a $Z = (z_{i,j})$ matrix
108 before thresholding. Namely, for each gene i CLR computes

$$z_i = \max \left\{ 0; \frac{MI_{i,j} - \text{mean}(MI_{i,\cdot})}{\text{sd}(MI_{i,\cdot})} \right\}$$

109 and then

$$110 \quad z_{i,j} = \sqrt{z_i^2 + z_j^2}.$$

111

112 MRNET applies a greedy maximum relevance strategy to link each gene i to the gene j that has
113 maximum MI with it ($j = \arg \max MI_{i,j}$). Additional links are added recursively maximizing MI with both

114 the gene i and the already linked genes until a stop criterion based on redundancy is met. A further
115 approach by pruning was proposed by ARACNE authors, where as in relevance networks a common
116 threshold is applied to all the $M_{i,j}$ followed by the application of a pruning rule. This rule states that, if
117 gene i interacts with gene j through gene k , then $M_{i,j} \leq \min\{M_{i,k}; M_{k,j}\}$. Consequently, among each
118 triplet of nonzero MI after initial thresholding, the weakest interaction is removed.

119

120 ***The MRA and MRA+CLR algorithms***

121 Due to its ability to model biological systems at various resolutions, the MRA terminology for a system
122 component is a module. We follow this terminology and consider that the n modules composing the
123 system have their activity levels denoted by $x \in \mathbb{R}^n$. Here, modules are genes and x_i stands for gene i
124 transcript abundance. If we make the rather nonrestrictive assumption that relationships between
125 modules are modeled by a dynamical system

$$\dot{x} = f(x)$$

126 ($f(\cdot)$ must exist but it does need to be known), and the system is in a steady state at the time of
127 experimental measurements ($\dot{x} = 0$), MRA theory lets us compute an $n \times n$ matrix of local interaction
128 strengths $r = (r_{i,j})$ from a gene j to a gene i ($r_{i,j} = \frac{\partial x_i}{\partial x_j} x_j$). The matrix r is obtained from linear
129 algebraic computations based on the observed activity of each module in an unperturbed state, and
130 under the individual, successive perturbations of each module. Details are provided in MRA original
131 publication[8], reviews of MRA developments[9], or in our recent publication[15]. We use the notations
132 of this recent paper. In Materials and Methods, we provide a brief overview of MRA along with a
133 description of the particular way we implemented the linear algebra to take advantage of parallel
134 computing.

135 Returning to the regulatory network inference problem, the MRA local interaction matrix r provides us
136 with a direct estimate of this network. Interactions are signed with positive coefficients representing
137 activation and negative coefficients representing inhibition. Given the fact that we want to apply MRA to
138 large systems, where every module does not necessarily have a direct influence on all the others, we
139 also face the problem of thresholding or pruning. Within the context of this study, we call MRA the
140 direct use of MRA computations followed by a threshold on the absolute values of r coefficients (values
141 below a given threshold in absolute values are set to 0). We also adapted CLR heuristic (z-score-like

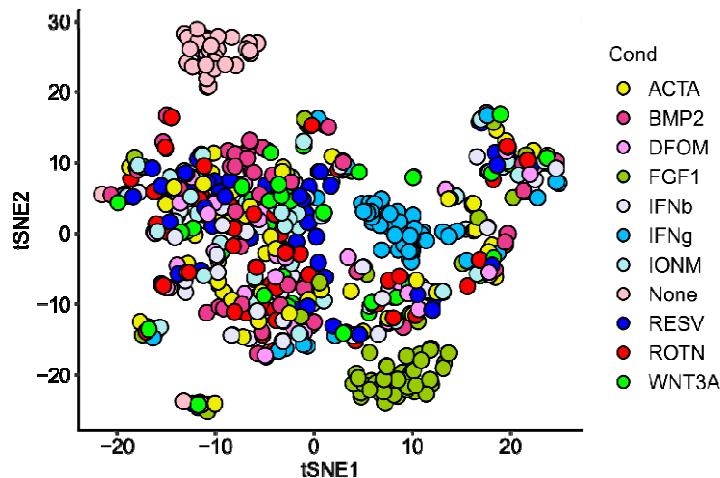
142 computation) to bring r coefficients to a more uniform scale before thresholding. We call this algorithm
143 MRA+CLR, see Materials and Methods for details.

144

145 ***Application to a medium-size data set***

146 Gapp *et al.*[17] published a data set, where they studied the transcriptional impact of the full knockouts
147 (KOs) of 55 tyrosine kinases and 6 non-kinases. We call this data set K61. The systematic perturbations
148 (KOs) of each gene as well as the unperturbed transcriptomes obviously constitute a *bona fide* MRA data
149 set. The transcriptomes were acquired under 11 conditions: no stimulation (None), FGF1, ACTA, BMP2,
150 IFNb, IFNg, WNT3A, ionomycin (IONM), resveratrol (RESV), rotenone (ROTN), and deferoxamine (DFOM)
151 stimulation. Stimulation were applied for 6 hours allowing the cells to adapt and reach a steady state or
152 near steady state. To facilitate the generation of full-KOs, human HAP1 haploid cells[28] were utilized.
153 The published transcriptomes were not limited to the expression of the 61 perturbed genes, but here,
154 due to the specifics of MRA, we limited the data to those 61 genes. Replicates were essentially averaged
155 (see Materials and Methods), resulting in a 61×61 matrix for each of the 11 conditions. Interestingly,
156 considering the complete transcriptomes, K61 authors showed in their publication that those clustered
157 primarily after the stimulatory condition. That is transcriptomes of different KOs obtained under the
158 same stimulation were closer to each other than transcriptomes of the same KO but under different
159 conditions. When reduced to the 61 genes of the network, this picture was less pronounced. In Fig. 1,
160 we see that None-, WNT3A-, and to a certain extent IFNg-stimulated transcriptomes clustered
161 separately thus potentially indicating rather different network wiring. The other conditions were not
162 really separated suggesting that more similar networks could take place.

163



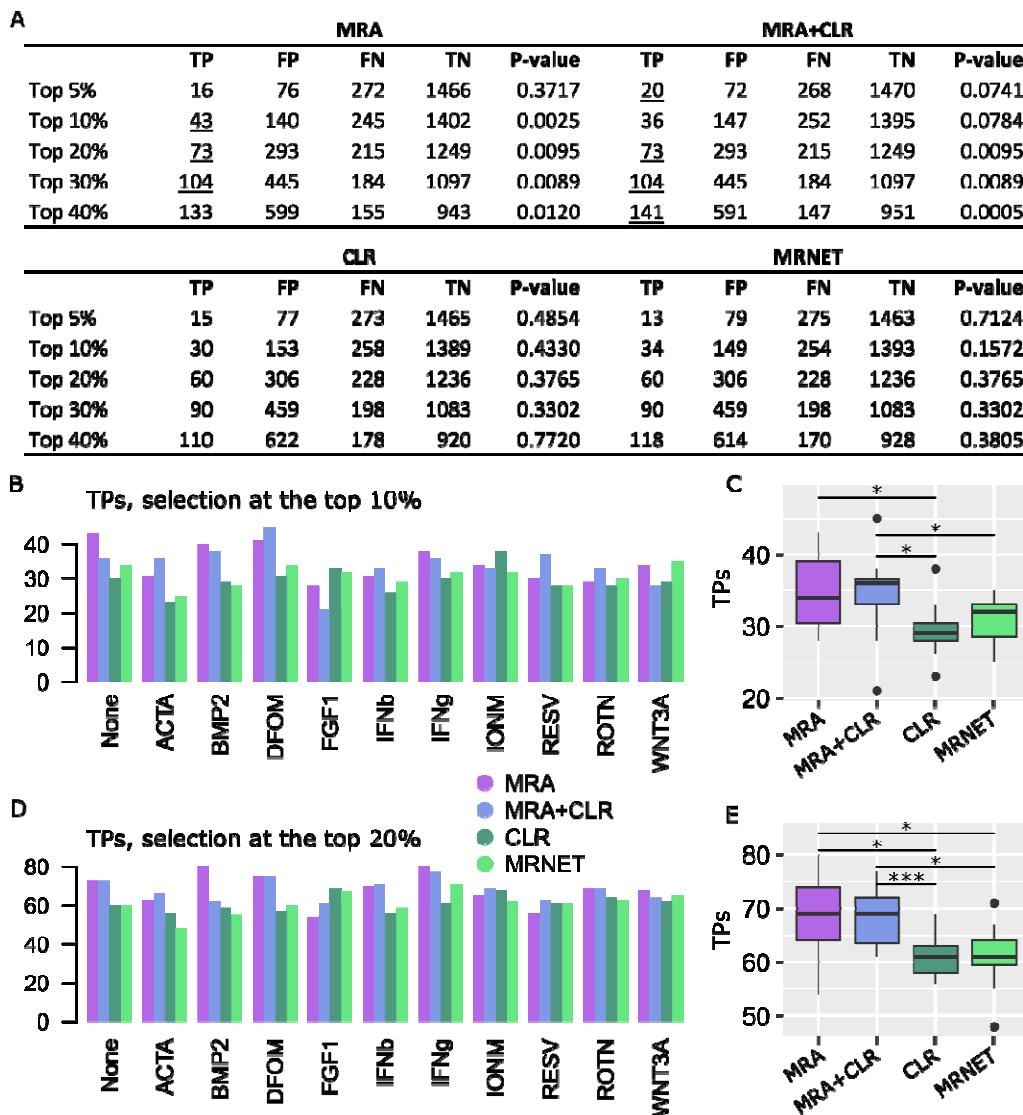
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165 **Figure 1.** t-distributed stochastic neighbor embedding (t-SNE) 2D projection of the 61 11 transcriptomes of the
166 K61 data set.

167

168 We applied MRA, MRA+CLR, CLR, MRNET, and ARACNE to the K61 data set, the later 3 algorithms
169 implementations were provided by the minet BioConductor package[25]. To estimate performance, we
170 compared our results with the STRING database[4] due to its broad content. In fact, working with
171 transcriptomic data, the inferred networks might overlap protein complexes as well as certain parts of
172 known pathways, but they might also unravel different types of relationships such as genetic
173 interactions, strong co-regulation, etc. Physical interaction of well-described pathway databases[1,3]
174 might thus be too restrictive. To apply a uniform selection mechanism to all of the algorithms, we simply
175 took the top 5%, 10%, 20%, 30% and 40% scores of the returned interaction matrices and determined
176 the intersection with STRING. This resulted in confusion matrices reporting true/false positives (TPs/FPs)
177 and true/false negatives (TNs/FNs) along with a P-value for the significance of the STRING intersection
178 (hypergeometric test). A representative example (None condition) is featured in Fig. 2A, while the
179 complete results are in Suppl. Table 1. Given the limited overlap between STRING and our data, and the
180 rather large numbers involved in the confusion matrices, we found the P-values rather unstable (small
181 differences in confusion matrices might cause important changes in terms of P-values). They should
182 hence be regarded as indicative only. Because we used a constant reference (STRING), and all the
183 algorithm scores were selected in identical numbers, reporting the number of TPs gives a clear
184 indication of the relative algorithm performances. In Fig. 2B-E we provide these numbers at the top 10%
185 and the top 20% selection levels. ARACNE implementation in minet did not perform well, typically
186 reaching half of CLR or MRNET TPs. Accordingly, ARACNE performance is not reported in Fig. 2, but in

187 Suppl. Table 1 only. The CLR heuristic applied on top of MRA did not provide much performance
 188 increase, but it resulted in more stable performances thus making it nonetheless an attractive option.

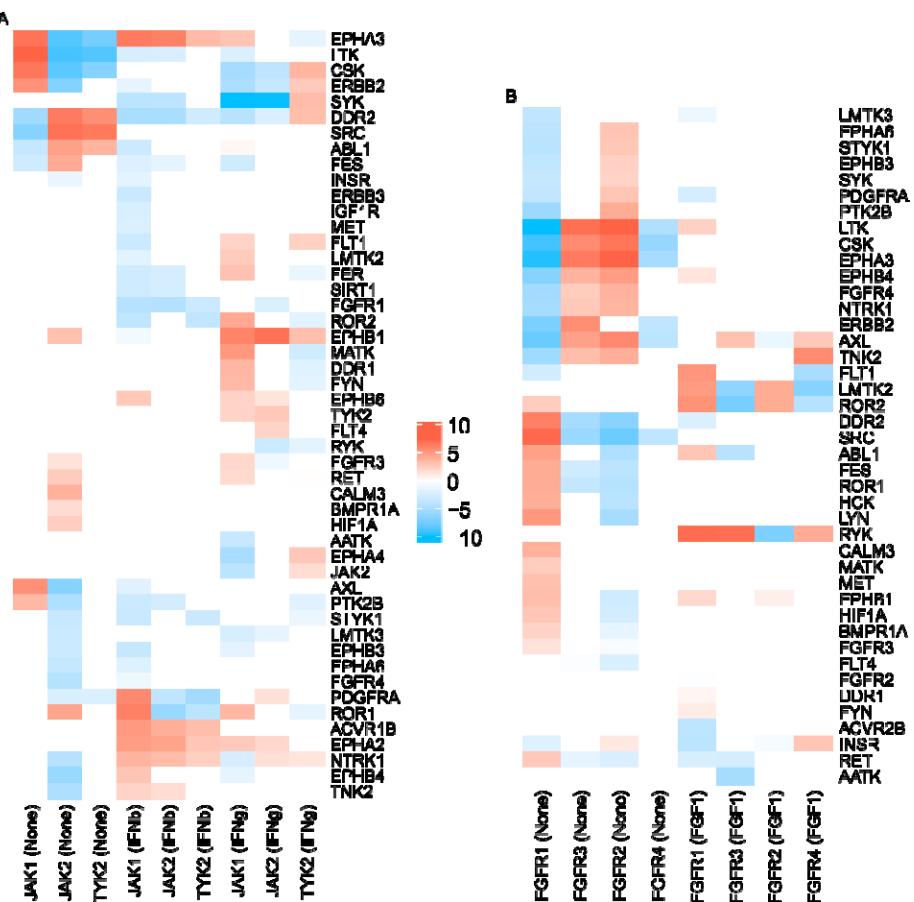


189
 190 **Figure 2.** Performance on K61 data. **A.** Representative confusion matrices for the None condition. **B.** TP numbers at
 191 the top 10% selection level. **C.** Comparison between the algorithm TP numbers (Wilcoxon test, 2-sided, *P < 0.05).
 192 **D.** TP numbers at the top 20% selection level. **E.** Comparison between the algorithm TP numbers (Wilcoxon test, 2-
 193 sided, *P < 0.05, ***P<0.005).

194

195 In their article, K61 authors discussed interesting differences in JAK1 *versus* JAK2 and TYK2 signaling,
 196 three members of the JAK family. In particular, they found that JAK1 KO cells were insensitive to IFNb
 197 and IFNg stimulation, while JAK2 and TYK2 KO cells responded normally although, in general, all these

198 proteins are known to contribute to transcriptional response upon type I and II interferon stimuli[29]. To
199 illustrate how network inference might provide some clue on such differences, we report in Fig. 3A the
200 MRA+CLR-inferred transcriptional interaction strengths between those three genes and their targets
201 under the unstimulated (None), IFNb, and IFNg conditions. In the absence of stimulation, we clearly
202 notice opposed influences of JAK1 on its targets compared to JAK2 and TYR2 (first three columns), which
203 already indicate different signal transduction capabilities. Upon IFNb stimulation, the interactions are
204 closer with opposed action on ROR1 and PDFGRA. JAK2 and TYR2 remained highly similar in this
205 condition. IFNg stimulation induced three different patterns with ROR1 transcriptional inhibition
206 remaining a specific mark of JAK1. Gapp *et al.* also found differences in FGF receptors. FGF-induced
207 response was attenuated in FGFR1 and FGFR3 KO cells, but preserved in FGFR2 and FGFR4 KO cells. In
208 Figure 3B, we notice an almost perfect inversion of the activation/inhibition pattern between FGFR1
209 versus FGFR2 and FGFR3. FGFR4 adopted a very different configuration with limited interactions. This
210 observation already indicates a distinct role for FGFR1. Upon FGF stimulation, the interactions are more
211 patchy, but certain oppositions can be found such as a strong inhibitory action of FGFR1 and FGFR3 on
212 RYK transcription.



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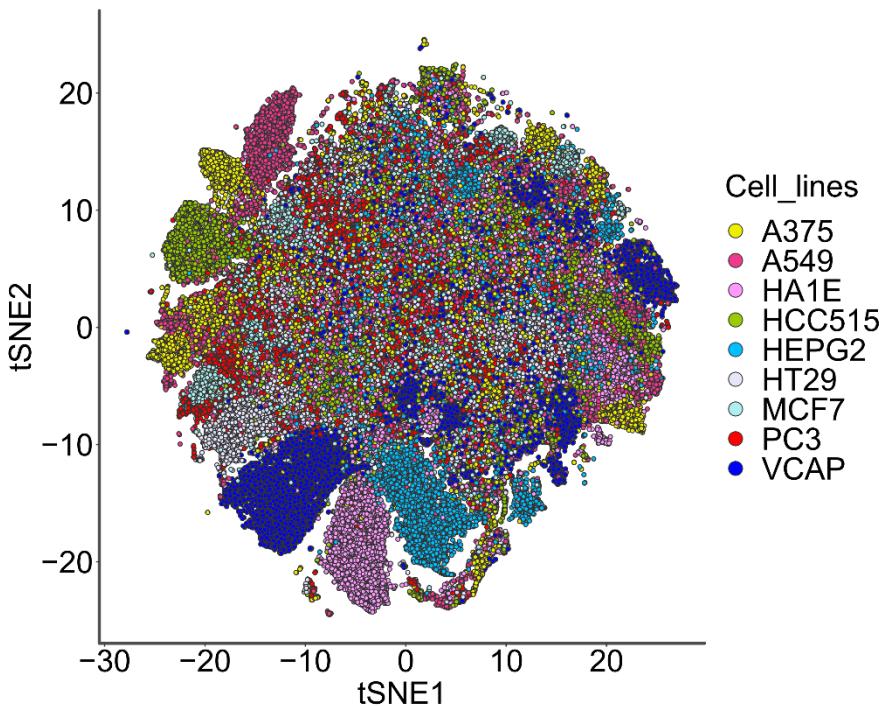
214 **Figure 3.** MRA+CLR-inferred interactions (top 20% selected). **A.** Interaction strengths (in \log_2 with sign preserved)
215 between JAK1, JAK2, and TYR2 and their targets. Stimulatory conditions are in brackets (None, IFNb, IFNg) **B.**
216 Interaction strengths between FGFR1, FGFR2, FGFR3, and FGFR4 and their targets.

217

218 ***Application to a large-size data set***

219 CMap next generation platform L1000[18] has recently released (December 2020) a new batch of data.
220 These data are in majority comprised of transcriptomes obtained in reference cancer cell lines under a
221 large number of perturbations with chemical agents, but most importantly shRNA-induced knockdowns
222 and CRISPR/Cas9 KOs. L1000 cost effective design entailed the identification of roughly 1,000 *hallmark*
223 genes from which a large proportion of the whole transcriptome can be inferred. The L1000 platform
224 only measures the expression of the hallmark genes experimentally. Two subsets of these data interest
225 us.

226 A first data set is composed of the almost systematic shRNA perturbation of all the hallmark genes, thus
227 providing an expression matrix close to $1,000 \times 1,000$ in size for 9 human cell lines: A375 (metastatic
228 melanoma), A549 (lung adenocarcinoma), HCC515 (non-small cell lung cancer, adenocarcinoma), HT29
229 (colorectal adenocarcinoma), HEPG2 (hepatocellular carcinoma), MCF7 (breast adenocarcinoma), PC3
230 (metastatic prostate adenocarcinoma), VCAP (metastatic prostate cancer), and HA1E (normal kidney
231 cells). To alleviate shRNA off-target effects, L1000 employed multiple hairpins, which were integrated
232 into a consensus gene signature (CSG) that the authors showed to be essentially devoid of off-target
233 consequences[18]. Cells were harvested 96 hours after shRNA perturbation leaving time to reach a
234 steady state that is compatible to shRNA common use. Due to variation in data production, the actual
235 matrix sizes ranged from 815×815 (MCF7) to 938×938 (A375). Interestingly, the t-SNE 2D projection of
236 all the L1000 shRNA transcriptomes used here clearly indicate cell line specific subnetworks as well as
237 shared, core parts (Fig. 4).

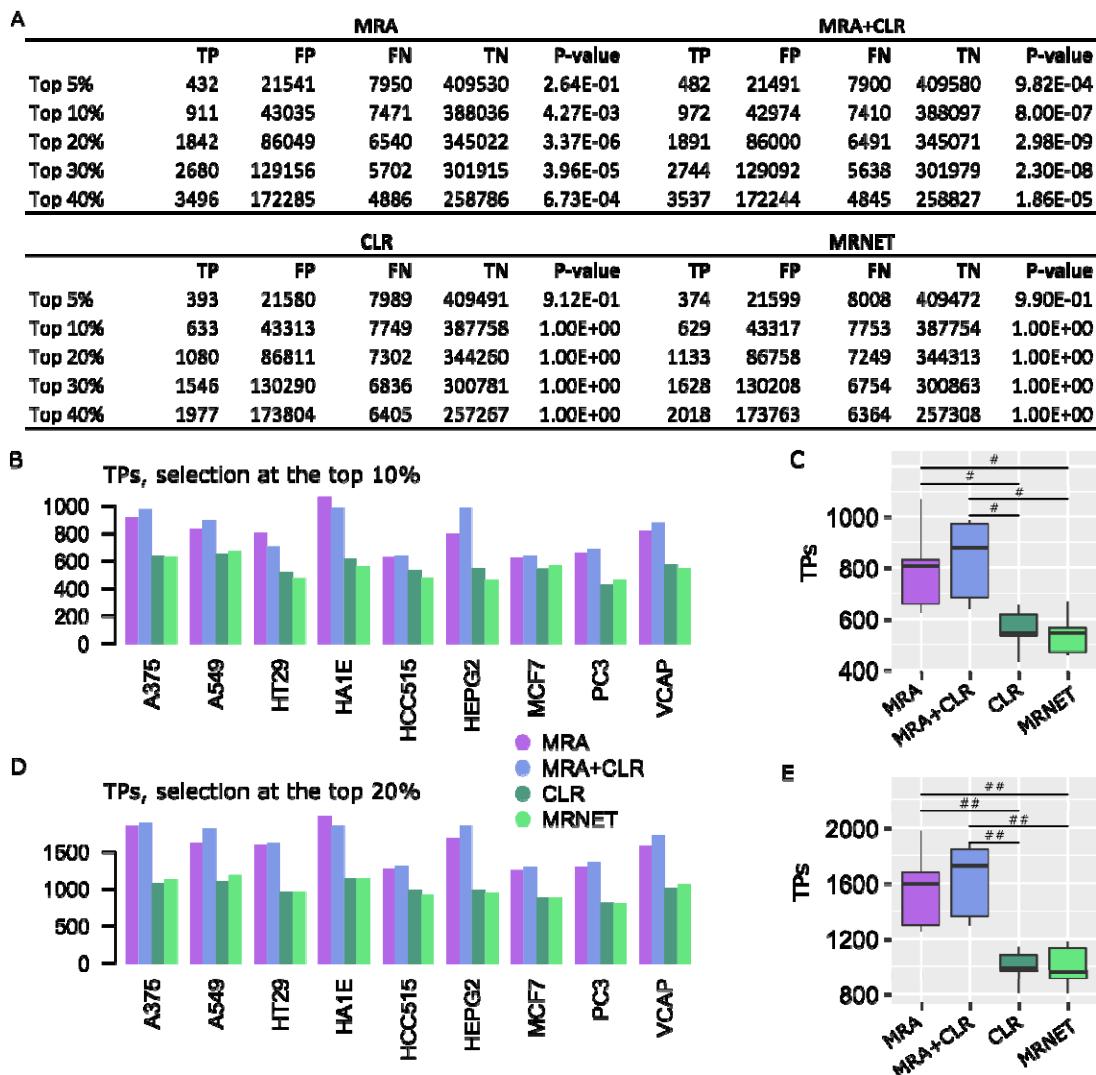


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239 **Figure 4.** t-SNE projection of L1000 shRNA data. We note well-separated clusters that are specific to certain cell
240 lines, *e.g.*, HA1E, VCAP, HCC515, HEPG2, A549, A375, as well as shared undistinguishable profile. This indicates
241 potential common and specific subnetworks across the cell lines.

242 We followed the same performance evaluation procedure as above for K61. A representative (A375
243 cells) confusion matrix is reported in Fig. 5A (full results in Suppl. Table 2), followed by TP numbers at
244 the top 10% and top 20% selection levels in Fig. 5B-E. With these larger matrices, but also knockdown
245 perturbations instead of KOs, MRA and MRA+CLR advantage was much augmented. Moreover, the CLR
246 heuristic not only attenuated performance variability, but it almost systematically outperformed MRA
247 alone.

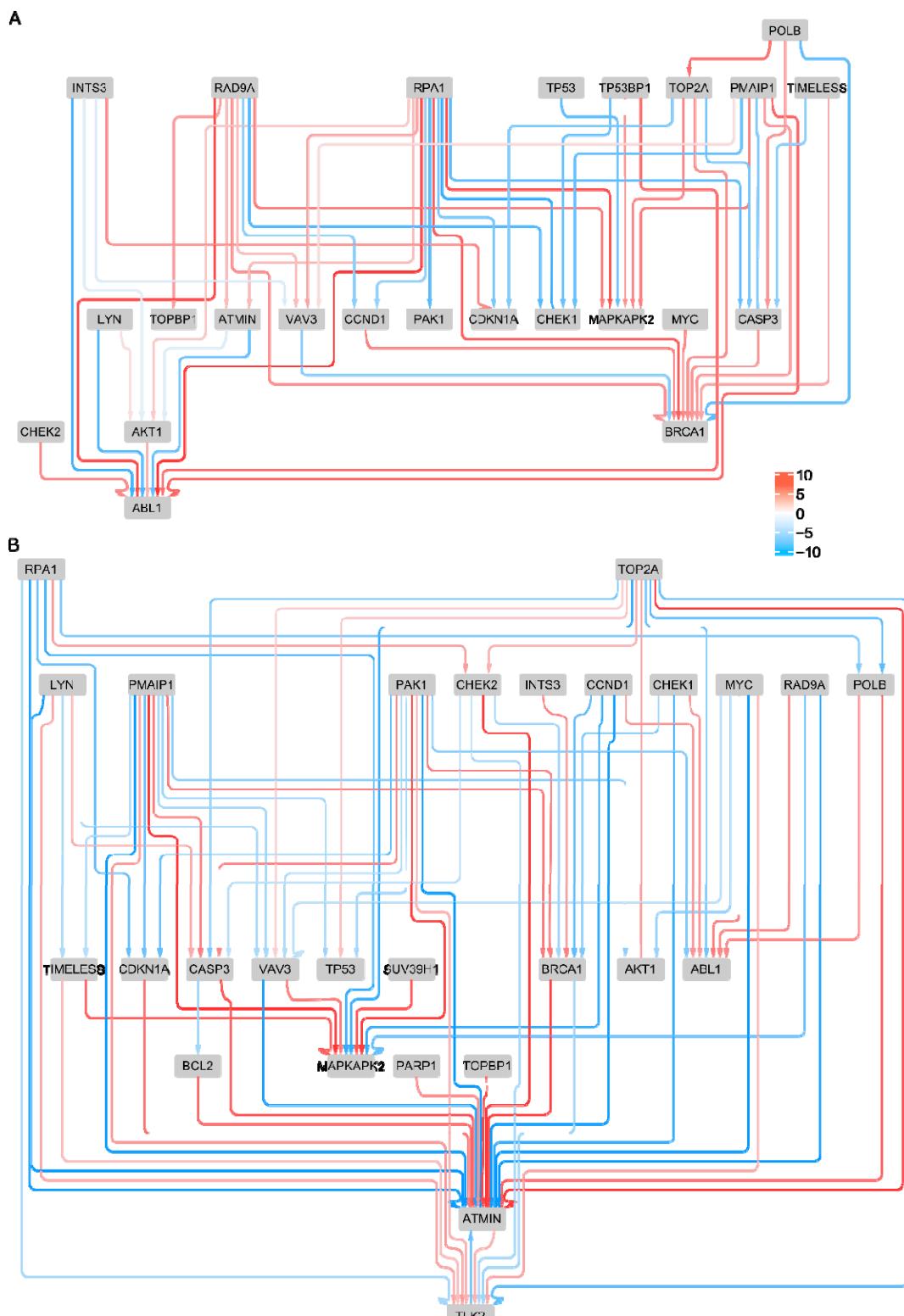
248 To illustrate the interest of network inference at this scale, we intersected MRA+CLR inferences in
249 normal kidney HA1E and melanoma A375 cells with a Gene Ontology term, *i.e.*, GO:0006974 cellular
250 response to DNA damage stimulus. In Fig. 6, we can notice the difference in connectivity between
251 normal cells and cells where this process is obviously exacerbated, in particular the regulation of ATMIN
252 a key molecule in DNA repair. This result is in agreement with the known rewiring of genetic networks in
253 response to DNA damage[30].



254

255 **Figure 5.** Performance on L1000 shRNA data. **A.** Representative confusion matrices for A375 cells. **B.** TP numbers at
 256 the top 10% selection level. **C.** Comparison between the algorithm TP numbers (Wilcoxon test, 2-sided, #P <
 257 0.001). **D.** TP numbers at the top 20% selection level. **E.** Comparison between the algorithm TP numbers (Wilcoxon
 258 test, 2-sided, #P < 0.001, ##P < 0.00005).

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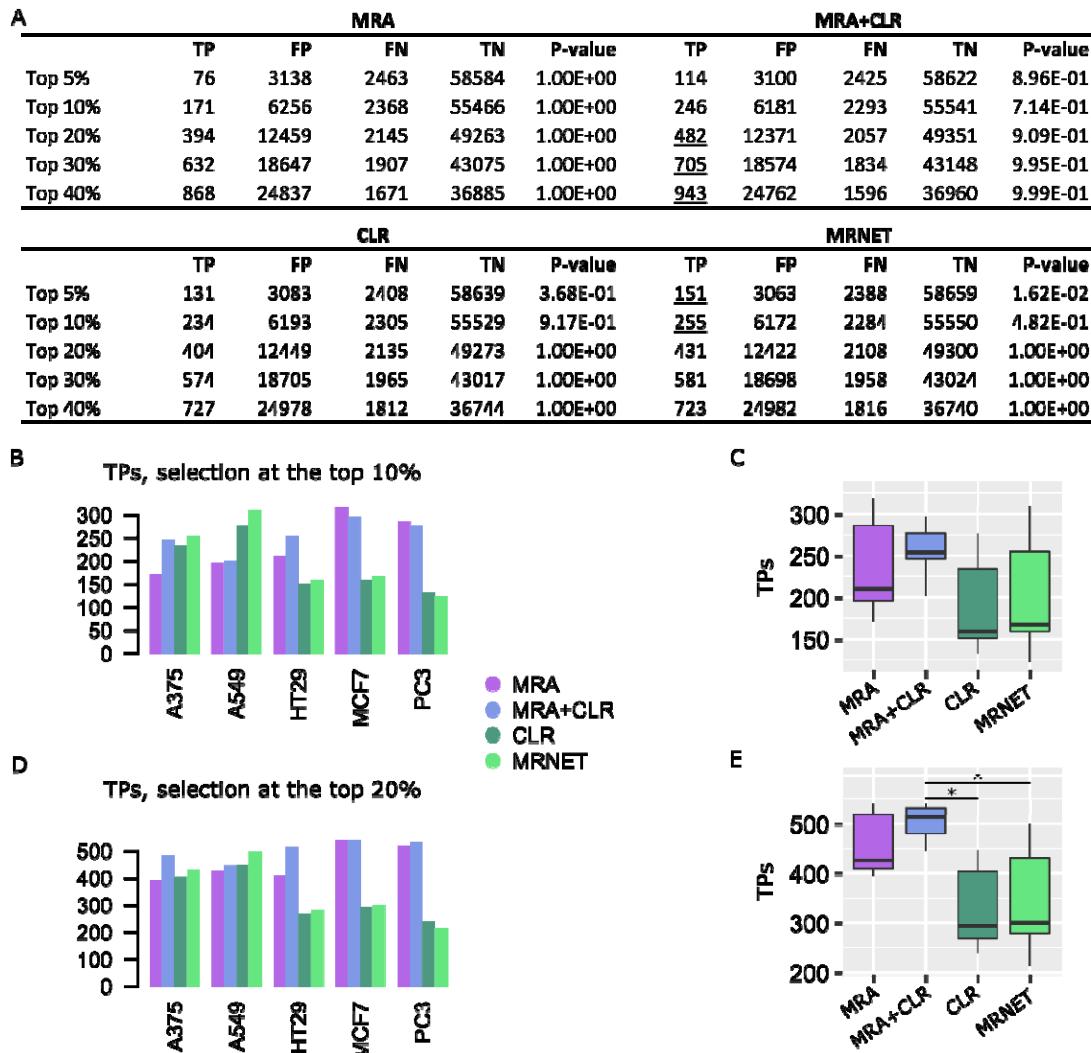
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261 **Figure 6.** Networks inferred with MRA+CLR (top 10% selection) in normal kidney cells (**A**) and melanoma cells (**B**)
262 for genes involved in cellular response to DNA damage stimulus (GO:0006974).

263

264 The second L1000 data set of interest is the CRISPR/Cas9 collection of KOs. These data were only
 265 available for five cell lines: A375, A549, HT29, MCF7, and PC3. The matrix sizes ranged from 343 343
 266 (MCF7) to 359 359 (A375). Performance results are featured in Fig. 7 and Suppl. Table 3. Although MRA
 267 and MRA+CLR again dominated the other algorithms, their advantage was less pronounced on these
 268 large, full KO data.

269



270

271 Figure 7. Performance on L1000 CRISPR/Cas9 data. **A**. Representative confusion matrices for A375 cells. **B**. TP
 272 numbers at the top 10% selection level. **C**. Comparison between the algorithm TP numbers. **D**. TP numbers at the
 273 top 20% selection level. **E**. Comparison between the algorithm TP numbers (Wilcoxon test, 2-sided, *P < 0.05).

274

275

276 **Discussion**

277 We presented a particular application of MRA to large biological systems and showed its competitive
278 performance compared to first-in-class MI-based inference methods. Obviously, MI-based methods
279 have a much broader spectrum of application, as they do not need specific and systematic perturbations
280 on the components of the biological system whose network is inferred. Nevertheless, when
281 perturbation data are available, our results suggest that a dedicated method, relying on a modeling
282 approach might deliver good performance in a robust fashion. The simple heuristic we proposed to
283 prune MRA inferences, which was adapted from the CLR algorithm, provided more stability in MRA
284 performance. In many cases, especially with very large systems ($n \approx 1,000$), this heuristic boosted
285 performance.

286 Although the number of data sets was limited, we could notice much superior improvement over MI-
287 based methods with L1000 shRNA knockdown perturbation data compared to the two full KO data sets.
288 This might relate to the linearization at the heart of MRA modeling, where the error depends on the
289 magnitude of perturbations (see our derivation of MRA through Taylor series expansion[15]). Very
290 strong perturbation such as full KOs might bring the data away from MRA area of safe application.

291

292 **Materials and Methods**

293

294 ***Modular response analysis***

295 We briefly recall the main MRA equations to facilitate the reading of this text, and to explain the
296 particular way we implemented the linear algebra. We assume that the biological system is comprised of
297 n modules whose activity levels are denoted by $x \in \mathbb{R}^n$. We further admit the existence of n intrinsic
298 parameters, $p \in \mathbb{R}^n$, one per module, and each of them can be perturbed by an elementary
299 perturbation. One can imagine x reporting mRNA abundances and perturbations induced by shRNAs for
300 instance. Lastly, we assume that there exist $S \subset \mathbb{R}^n \times \mathbb{R}^n$, an open subset, and $f: S \rightarrow \mathbb{R}^n$ of class \mathcal{C}^1 ,
301 *i.e.*, continuously differentiable, such that

302
$$\dot{x} = f(x, p). \quad (1)$$

303 We do not need to know $f(x, p) = (f_1(x, p), \dots, f_n(x, p))^t$ explicitly, but we need the existence of a
304 time $T > 0$ such that all the solutions, for any p and initial conditions of x , have reached a steady state,
305 *i.e.*,

$$\dot{x} = 0, \forall t > T.$$

306 The unperturbed, basal state of the modules is denoted $x(p^0) \in \mathbb{R}^n$ and it has corresponding
307 parameters $p^0 \in \mathbb{R}^n$. By the application of the implicit function theorem and Taylor expansion at the
308 first order [8,15], MRA relates the experimental observations of the global effect of perturbations to
309 local interaction strengths, *i.e.*, the matrix $r = (r_{i,j}) = \left(\frac{\partial x_i}{\partial x_j} \frac{x_j}{x_i} \right)$ that we mentioned in Results. Such local
310 interactions are obviously signed and non-symmetric. To compute r , we need to compute the relative
311 global change induced by each elementary perturbation in each module. These values are compiled in a
312 $n \times n$ matrix denoted $R = (R_{i,k})$ with

313
$$R_{i,k} = \left(\frac{\Delta x_i}{x_i} \right)_{q_k},$$

314 the relative difference in activity of module i upon Δp_k change induced by an elementary perturbation
315 q_k that touches module k only. The relationship between observational data in R and the local
316 interactions we want to estimate in r are provided by the following equations

317
$$\left(\frac{\Delta x_i}{x_i} \right)_{q_k} = \sum_{j \neq i} r_{i,j} \left(\frac{\Delta x_j}{x_j} \right)_{q_k}, \quad k \neq i, \quad (2)$$

318
$$\left(\frac{\Delta x_i}{x_i} \right)_{q_i} = \sum_{j \neq i} r_{i,j} \left(\frac{\Delta x_j}{x_j} \right)_{q_i} + \frac{\partial x_i}{\partial p_i} (p^0) \left(\frac{\Delta p_i}{x_i} \right). \quad (3)$$

319 By setting $r_{i,i} = -1$, Eqs (2) and (3) can be put together in matrix form and we obtain

320
$$rR = -P, \quad (4)$$

321 where P is a diagonal $n \times n$ matrix with

322
$$P_{i,i} = \frac{\partial x_i}{\partial p_i}(p^0) \left(\frac{\Delta p_i}{x_i} \right), \quad i \in \{1, \dots, n\}. \quad (5)$$

323 Eq. (3) can be solved in two steps: $r = -PR^{-1}$ and $r_{i,i} = -1$ imply $P_{i,i}(R^{-1})_{i,i} = 1$, thus

324
$$P_{i,i} = \frac{1}{(R^{-1})_{i,i}}.$$

325 Therefore,

326
$$r = -[\text{diag}(R^{-1})]^{-1}R^{-1}. \quad (6)$$

327 In practice, relative differences in R are often estimated with the more stable formula

328
$$R_{i,k} = 2 \left(\frac{x_i(p^0 + \Delta p_k) - x_i(p^0)}{x_i(p^0 + \Delta p_k) + x_i(p^0)} \right), \quad (7)$$

329 where we denote $x(p^0 + \Delta p)$ the steady-state corresponding to the changed parameters $p^0 + \Delta p$, i.e.,
330 the solution of $\dot{x}(p^0 + \Delta p) = f(x(p^0 + \Delta p), p^0 + \Delta p)$.

331

332 **Parallelized and stable linear algebra**

333 Eq. (6) requires the computation of the inverse of the matrix R , which is less efficient and less stable
334 than LU decomposition with pivot search[31]. These technical issues are usually irrelevant with small
335 systems, but in applications of MRA to larger biological systems they should be addressed.

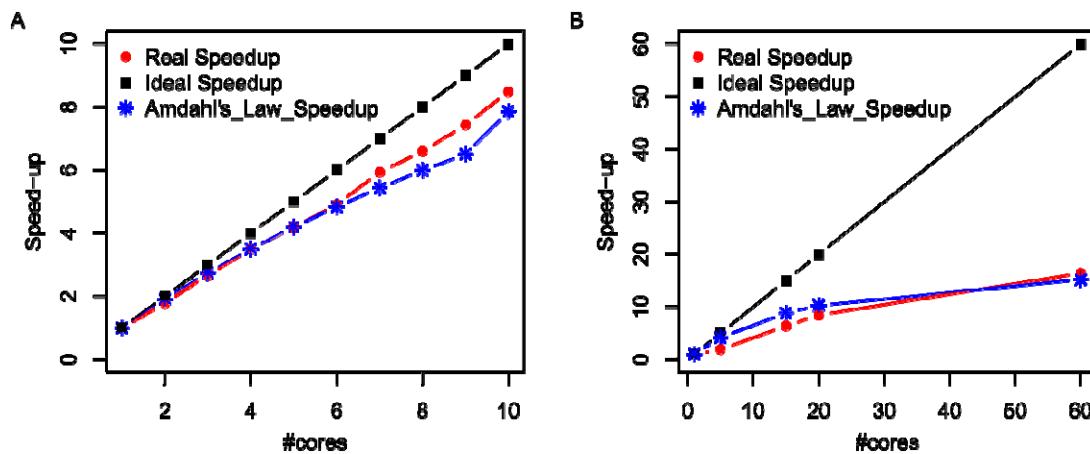
336 As several authors noticed, including in MRA original publication[8], the homogeneous Eq. (2) is
337 sufficient to compute r . Moreover, letting i take the values $1, \dots, n$, we remark that Eq. (2) defines n
338 systems of linear equations of dimension $n - 1$, which can be solved independently. In particular, those
339 systems can be solved on independent processors by performing the LU decomposition with pivot
340 search. Illustrative speedup curves are featured in Fig. 8. Depending on the size of n , each such
341 subsystem could itself benefit from a parallel solver if enough processors were available.

342 When Eq. (2) is solved for each value of i , it is straightforward to solve Eq. (3) to find $P_{i,i}$ values in case
343 those are required:

344
$$\left(\frac{\Delta x_i}{x_i} \right)_{q_i} = \sum_{j \neq i} r_{i,j} \left(\frac{\Delta x_j}{x_j} \right)_{q_i} + P_{i,i} \Leftrightarrow P_{i,i} = \sum_{j \neq i} r_{i,j} \left(\frac{\Delta x_j}{x_j} \right)_{q_i} - \left(\frac{\Delta x_i}{x_i} \right)_{q_i},$$

345 where Eq. (4) was used for the definition of .

346



347

348 **Figure 8.** Speedup curves. **A.** K61 data(None condition, 61 61 matrix). **B.** L1000 shRNA data (A375 cells, 938 938 matrix).

350

351 **CLR, MRNET, and ARACNE computations**

352 We used the implementation of these algorithms provided by the BioConductor R package minet[25].

353 The performance reported here reflects the performance of this specific implementation.

354

355 **CLR heuristic adapted to MRA**

356 We adapted the CLR normalization scheme by means of z-score computation to MRA matrix content.

357 From we thus derive a defined as follow:

358
$$\frac{r_i - \bar{r}}{\sigma_r}$$
, with σ_r the standard deviation of 's -th row,

359
$$\frac{c_j - \bar{c}}{\sigma_c}$$
, with σ_c the standard deviation of 's -th column,

360
$$\frac{r_i - \bar{r}}{\sigma_r} \frac{c_j - \bar{c}}{\sigma_c}$$
, and

361

362

363 ***Data sets preparation***

364 TK61 data were obtained on multiple 96-well plates. Accordingly, we tried to stick to this format
365 preparing data for MRA computations. We computed an R matrix for each plate and then simply
366 averaged the relevant R 's for each experimental condition to obtain the averaged R used in MRA. For
367 MI-based inferences, we averaged all the relevant values.

368 L1000 shRNA data were extracted at level 5 (L1000 terminology) where CGSs (integration of multiple
369 shRNA hairpins to alleviate off-target effects) were transformed into z-scores for normalization purposes
370 by the authors of the data. Consequently, values representing the abundance of a gene were no longer
371 positive numbers but just real numbers. Eq. (7) above was adapted to compute the relative changes in
372 MRA R matrices according to

$$R_{i,k} = 2 \left(\frac{CGS_i(p^0 + \Delta p_k) - CGS_i(p^0)}{|CGS_i(p^0 + \Delta p_k)| + |CGS_i(p^0)|} \right)$$

373 avoiding potential divisions by 0 in case of small values with opposed signs.

374 L1000 CRISPR/Cas9 data were averaged over replicates (also level 5).

375

376 ***Performance evaluation***

377 STRING as well as MI-based inference are devoid of direction of interaction and a sign. Therefore, the
378 intersection of inferences with STRING content only used the upper triangular part of matrices
379 representing the inferences (such matrices are symmetric anyway). To provide a fair comparison with
380 MRA and MRA+CLR, we filled the upper triangular part of r according to $r_{i,j} = \max\{|r_{i,j}|; |r_{j,i}|\}$, $i < j$.

381

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384

385

386 **References**

- 387 1. Matthews L, Gopinath G, Gillespie M, Caudy M, Croft D, de Bono B, et al. Reactome knowledgebase
388 of human biological pathways and processes. *Nucleic Acids Res.* 2009;37: D619-22.
389 doi:10.1093/nar/gkn863
- 390 2. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, et al. KEGG for linking genomes to life
391 and the environment. *Nucleic Acids Res.* 2008;36: D480-4.
- 392 3. Kerrien S, Alam-Faruque Y, Aranda B, Bancarz I, Bridge A, Derow C, et al. IntAct--open source
393 resource for molecular interaction data. *Nucleic Acids Res.* 2007;35: D561-5.
394 doi:10.1093/nar/gkl958
- 395 4. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The STRING database
396 in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids
397 Res.* 2011;39: D561-8. doi:10.1093/nar/gkq973
- 398 5. Bansal M, Belcastro V, Ambesi-Impiombato A, di Bernardo D. How to infer gene networks from
399 expression profiles. *Molecular Systems Biology.* 2007;3: 78. doi:10.1038/msb4100120
- 400 6. Babtie AC, Stumpf MPH, Thorne T. Gene Regulatory Network Inference. In: Wolkenhauer O, editor.
401 Systems Medicine. Oxford: Academic Press; 2021. pp. 86–95. doi:10.1016/B978-0-12-801238-
402 3.11346-7
- 403 7. Emmert-Streib F, Dehmer M, Haibe-Kains B. Gene regulatory networks and their applications:
404 understanding biological and medical problems in terms of networks. *Front Cell Dev Biol.* 2014;2.
405 doi:10.3389/fcell.2014.00038
- 406 8. Kholodenko BN, Kiyatkin A, Bruggeman FJ, Sontag E, Westerhoff HV, Hoek JB. Untangling the wires:
407 a strategy to trace functional interactions in signaling and gene networks. *Proc Natl Acad Sci U S A.*
408 2002;99: 12841–6. doi:10.1073/pnas.192442699
- 409 9. Santra T, Rukhlenko O, Zhernovkov V, Kholodenko BN. Reconstructing static and dynamic models of
410 signaling pathways using Modular Response Analysis. *Current Opinion in Systems Biology.* 2018;9:
411 11–21. doi:10.1016/j.coisb.2018.02.003
- 412 10. Hu JX, Thomas CE, Brunak S. Network biology concepts in complex disease comorbidities. *Nat Rev
413 Genet.* 2016;17: 615–629. doi:10.1038/nrg.2016.87
- 414 11. Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, et al. Architecture of the human
415 interactome defines protein communities and disease networks. *Nature.* 2017;545: 505–509.
416 doi:10.1038/nature22366
- 417 12. Assi SA, Imperato MR, Coleman DJL, Pickin A, Potluri S, Ptasińska A, et al. Subtype-specific regulatory
418 network rewiring in acute myeloid leukemia. *Nat Genet.* 2019;51: 151–162. doi:10.1038/s41588-
419 018-0270-1
- 420 13. Pawson T, Warner N. Oncogenic re-wiring of cellular signaling pathways. *Oncogene.* 2007;26: 1268–
421 1275. doi:10.1038/sj.onc.1210255

422 14. Weinstein IB, Joe A. Oncogene addiction. *Cancer Res.* 2008;68: 3077–3080; discussion 3080.
423 doi:10.1158/0008-5472.CAN-07-3293

424 15. Jimenez-Dominguez G, Ravel P, Jalaguier S, Cavaillès V, Colinge J. An R package for generic modular
425 response analysis and its application to estrogen and retinoic acid receptor crosstalk. *Sci Rep.*
426 2021;11: 7272. doi:10.1038/s41598-021-86544-0

427 16. Klinger B, Sieber A, Fritsche-Guenther R, Witzel F, Berry L, Schumacher D, et al. Network
428 quantification of EGFR signaling unveils potential for targeted combination therapy. *Mol Syst Biol.*
429 2013;9: 673. doi:10.1038/msb.2013.29

430 17. Gapp BV, Konopka T, Penz T, Dalal V, Bürckstümmer T, Bock C, et al. Parallel reverse genetic
431 screening in mutant human cells using transcriptomics. *Molecular Systems Biology.* 2016;12: 879.
432 doi:10.15252/msb.20166890

433 18. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, et al. A Next Generation
434 Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. *Cell.* 2017;171: 1437-1452.e17.
435 doi:10.1016/j.cell.2017.10.049

436 19. Horvath S, Dong J. Geometric interpretation of gene coexpression network analysis. *PLoS Comput
437 Biol.* 2008;4: e1000117. doi:10.1371/journal.pcbi.1000117

438 20. Obayashi T, Hayashi S, Shibaoka M, Saeki M, Ohta H, Kinoshita K. COXPRESdb: a database of
439 coexpressed gene networks in mammals. *Nucleic Acids Res.* 2008;36: D77-82.
440 doi:10.1093/nar/gkm840

441 21. Wang YXR, Huang H. Review on statistical methods for gene network reconstruction using
442 expression data. *J Theor Biol.* 2014;362: 53–61. doi:10.1016/j.jtbi.2014.03.040

443 22. Faith JJ, Hayete B, Thaden JT, Mogno I, Wierzbowski J, Cottarel G, et al. Large-scale mapping and
444 validation of *Escherichia coli* transcriptional regulation from a compendium of expression profiles.
445 *PLoS Biol.* 2007;5: e8. doi:10.1371/journal.pbio.0050008

446 23. Meyer PE, Kontos K, Lafitte F, Bontempi G. Information-theoretic inference of large transcriptional
447 regulatory networks. *EURASIP J Bioinform Syst Biol.* 2007; 79879. doi:10.1155/2007/79879

448 24. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Favera RD, et al. ARACNE: An
449 Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context.
450 *BMC Bioinformatics.* 2006;7: S7. doi:10.1186/1471-2105-7-S1-S7

451 25. Meyer PE, Lafitte F, Bontempi G. minet: A R/Bioconductor Package for Inferring Large
452 Transcriptional Networks Using Mutual Information. *BMC Bioinformatics.* 2008;9: 461.
453 doi:10.1186/1471-2105-9-461

454 26. Butte AJ, Kohane IS. Mutual information relevance networks: functional genomic clustering using
455 pairwise entropy measurements. *Pac Symp Biocomput.* 2000; 418–429.
456 doi:10.1142/9789814447331_0040

457 27. Butte AJ, Tamayo P, Slonim D, Golub TR, Kohane IS. Discovering functional relationships between
458 RNA expression and chemotherapeutic susceptibility using relevance networks. PNAS. 2000;97:
459 12182–12186.

460 28. Carette JE, Guimaraes CP, Varadarajan M, Park AS, Wuethrich I, Godarova A, et al. Haploid genetic
461 screens in human cells identify host factors used by pathogens. Science. 2009;326: 1231–1235.
462 doi:10.1126/science.1178955

463 29. Rane SG, Reddy EP. Janus kinases: components of multiple signaling pathways. Oncogene. 2000;19:
464 5662–5679. doi:10.1038/sj.onc.1203925

465 30. Bandyopadhyay S, Mehta M, Kuo D, Sung M-K, Chuang R, Jaehnig EJ, et al. Rewiring of genetic
466 networks in response to DNA damage. Science. 2010;330: 1385–1389. doi:10.1126/science.1195618

467 31. Golub GH, Loan CFV. Matrix Computations. JHU Press; 2013.

468

469

470 **Data availability**

471 Data used in this work were made publicly available by their respective authors.

472

473 **Supporting information caption**

474 **Supplementary Table 1.** Confusion matrices on the K61 data set.

475 **Supplementary Table 2.** Confusion matrices on the L1000 shRNA data set.

476 **Supplementary Table 3.** Confusion matrices on the L1000 CRISPR/Cas9 data set.

477