Comparative Network Reconstruction using mixed integer programming

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Abstract

Motivation: Signal-transduction networks are often aberrated in cancer cells, and new anti-cancer drugs that specifically target oncogenes involved in signaling show great clinical promise. However, the effectiveness of such targeted treatments is often hampered by innate or acquired resistance due to feedbacks, crosstalks or network adaptations in response to drug treatment. A quantitative understanding of these signaling networks and how they differ between cells with different oncogenic mutations or between sensitive and resistant cells can help in addressing this problem.

Results: Here, we present Comparative Network Reconstruction (CNR), a computational method to reconstruct signaling networks based on possibly incomplete perturbation data, and to identify which edges differ quantitatively between two or more signaling networks. Prior knowledge about network topology is not required but can straightforwardly be incorporated. We extensively tested our approach using simulated data and applied it to perturbation data from a BRAF mutant, PTPN11 KO cell line that developed resistance to BRAF inhibition. Comparing the reconstructed networks of sensitive and resistant cells suggests that the resistance mechanism involves re-establishing wild-type MAPK signaling, possibly through an alternative RAF-isoform.

Availability and implementation: CNR is available as a python module at https://github.com/NKI-CCB/cnr. Additionally, code to reproduce all figures is available at https://github.com/NKI-CCB/CNR-analyses.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Aberrations in cellular signal-transducing networks are one of the hallmarks of cancer (Hanahan and Weinberg, 2011), and many new anti-cancer drugs specifically target genes involved in signaling (Roberts and Der, 2007). Unfortunately, the effectiveness of targeted therapies is often limited by unexpected feedbacks or crosstalks (Klinger et al., 2013; Prahllad et al., 2012; Sun et al., 2014a), and resistance typically emerges due to network adaptations that reactivate inhibited pathways (Ahronian et al., 2015; Sun et al., 2014b). A better and more quantitative understanding of the interactions in such networks, and how these are remodeled under drug exposure, could potentially aid in the design of effective treatments.

Several types of computational approaches have been developed to characterize signaling networks. Ordinary differential equation-based models give detailed, dynamic and quantitative descriptions of a system (Fey et al., 2015). However, such models typically have many parameters that need to be estimated. Fitting these requires vast amounts of measurements and is computationally very expensive, limiting the scope to small systems of which the topology is already well known. Because of their relative simplicity, logic (or Boolean) models are suitable for larger systems (Saez-Rodriguez et al., 2009), and have been used to predict synergistic drug interactions (Flobak et al., 2015) and to identify differences in signaling network topologies between cell lines (Saez-Rodriguez et al., 2011).
However, these models are not quantitative and require discretization of the data.

Modular response analysis (MRA) is a framework that strikes a balance between the level of detail and scope of a model (Bruggeman et al., 2002; Khodolenko et al., 2002). MRA is a mathematical framework that quantifies the interaction strengths between network nodes based on perturbation data. It only considers interactions between ‘modules’, characterized by a single output affecting other modules. In the context of signaling networks, this means that only the active form of the kinase needs to be considered. MRA is considerably simpler than ODE-based models while retaining the relevant information about how network nodes influence each other. In its original formulation, MRA and similar methodologies (Gardner et al., 2003) require perturbations of all nodes, which is always feasible, for instance, because not all proteins are druggable. This problem can be solved by a maximum-likelihood approach using perturbation data from a BRAF mutant, PTPN11 KO cell line that acquired resistance to BRAF inhibition. The resistant cells are less sensitive to growth-factor stimulation, and have a negative response to BRAF inhibition. The resistant cells are therefore more sensitive to BRAF inhibition, more sensitive to growth-factor stimulation, and have a negative feedback from ERK to MEK, suggesting that the resistance mechanism involves re-establishing wild-type MAPK signaling.

2 Materials and methods

2.1 Formulation of the Comparative Network Reconstruction algorithm

Here, we briefly describe the CNR formalism. For more background information and details, please consult the Supplementary information.

CNR is based on MRA (Kholodenko et al., 2002), a mathematical framework to identify and quantify the interactions in a network based on perturbation experiments. MRA links the measured response $R_k$ (defined as the log2-fold change in the steady-state activity of node $x_i$ after perturbation $j$) to the unobserved physical interaction strengths $r_{ij}$ (defined as the logarithmic partial derivative of $x_k$ with respect to $x_j$) and direct perturbation effects $s_{im}$ (the scaled direct effect of perturbation $n$ on $x_m$). The matrices of local and global response coefficients and the direct perturbation effects are related through the equation

$$r \cdot R = -s.$$  

(1)

CNR aims to find a network model that solves these equations for multiple cell lines simultaneously while minimizing model complexity and the number of differences between the reconstructed networks of the different cell lines.

Formally, given $N_n$ nodes in the network, $N_p$ perturbations and $N_c$ cell lines, and for $i,j \in \{1, 2, \ldots, N_n\}$, $n \in \{1, 2, \ldots, N_p\}$ and $x \in \{1, 2, \ldots, N_c\}$, the MIQP problem reads as follows:

\[
\text{minimize } \sum_{n=1}^{N_n} \left( \sum_{i,j=1}^{N_n} x_{ij} s_{in}^{n} + \eta \cdot s_{i}^{n} + \theta \cdot \left( d_{ij}^{n} + d_{diff}^{n} \right) \right) \\
\text{subject to: } \sum_{j=1}^{N_n} r_{ij}^n R_{n}^{x_{ij}} + s_{im}^{n} = e_{im}^{n} \text{ } \forall i,j,n,x \\
r_{ij}^{n} = 0 \Rightarrow r_{ij}^{n} = 0 \text{ } \forall i,j,x \\
d_{ij}^{n} = 0 \Rightarrow r_{ij}^{n} - r_{ij}^{mean} = 0 \text{ } \forall i,j,x \\
d_{diff}^{n} = 0 \Rightarrow s_{im}^{n} = 0 \text{ } \forall i,n,x \\
r_{ij}^{mean} = \sum_{x} r_{ij}^{n} / N_{c} \text{ } \forall i,j \\
s_{im}^{mean} = \sum_{x} s_{im}^{n} / N_{c} \text{ } \forall i,n \\
\quad p_{ij}^{diff}, p_{ij}^{diff}, p_{diff}^{mean} \in \{0, 1\} 
\]

where the $\eta$ represents the degree to which Equation (1) is satisfied, $\eta$ and $\theta$ are hyper parameters to tune the degree of penalization on the number of edges and difference between cell lines, respectively. $p_{ij}^{diff}$, $p_{ij}^{diff}$, and $p_{diff}^{mean}$ are indicator variables indicating whether an edge is present or not, and whether and edge or perturbation strength is allowed to differ between cell lines, respectively.

Prior information about network topology can be added as constraints of the form $p_{ij}^{diff} = 0$ or $p_{ij}^{diff} = 1$ for edges that are known to be absent or present, respectively. Similarly, information about the sign of an edge or perturbation can be added as a constraint of the form $r_{ij}^{n} > 0$ or $s_{im}^{n} \leq 0$.

The optimization problem is solved using IBM ILOG CPLEX solver (Version 12.7.1), which is freely available for academic use. Importantly, the MIQP solver guarantees optimal solutions (within small numerical tolerances). In some cases, we also want to obtained close-to-optimal solutions. For this, we use the CPLEX solution-pool functionality.

2.2 Simulating perturbation data

To generate simulated perturbation data, we used an ODE model of EGFR signaling developed by Orton et al. (2009). We calculated the steady state by integrating the model over a suitably long time-interval. All proteins were in their inactive state in the initial conditions. Activating mutations in BRAF, RAS and EGFR were modeled by setting all these proteins to the active state in the initial conditions, and setting the conversion rate to the inactive state to zero. We simulated perturbations as knockdowns of a protein by reducing the total amount of that protein by 50%, and recalculating the steady state. We calculated the global response coefficient for protein $i$ in response to perturbation $p$ as $R_p = \log_2(X_{ip}/X_{i0})$, where $X_{ip}$ and $X_{i0}$ are the perturbed and unperturbed steady-state concentrations of the active state of protein $i$, respectively. We added noise...
to the global response coefficients by multiplying them with a random number drawn from a normal distribution with mean ($\mu$) set to 1 and standard deviation ($\sigma$) set to the noise level. To calculate the true local response coefficients for each interacting pair of proteins, we numerically calculated the partial derivatives of the steady-state concentration of the downstream active protein with respect to a perturbation in the concentration of the upstream active protein (by keeping the concentration of all other active proteins fixed). To obtain the true direct perturbation effects of knockdown a protein, we reduced its total amount by 50%, fixed the concentrations of all other proteins, and calculated the new steady-state concentration of that protein in its active state.

2.3 Cells and cell culture

All cell lines were derived from the BRAFV600E mutant VACO432 colorectal cancer cell line. Generation of the VACO432 PTPN11 KO clones is described by Prahallad et al. (2015). Persistence cells were generated by prolonged culturing of VACO432 PTPN11 KO cells in the presence of 2 μM Vemurafenib and selecting surviving colonies. Cell lines were cultured in RPMI supplemented with 10% fetal calf serum (FCS) 1% Glutamine and 1% Penicillin/Streptomycin (Gibco). The persister cell line was not maintained.

2.4 Perturbation experiments

Prior to the perturbation experiments, cells were synchronized by serum starvation during 24 hours. During this period, the persistor cells were kept exposed to Vemurafenib. At $t = -60$ minutes, cells were treated with an inhibitor (BRAF: Vemurafenib at 2 μM, MEK: Selumetinib at 1 μM, ERK: SCH772984 at 1 μM, AKT: MK2206 at 1 μM or PI3Ki: GDC0941 at 1 μM; All SelleckChem). At $t = 0$, cells were stimulated with growth factor (EGF at 20 ng ml $^{-1}$, HGF at 25 ng ml $^{-1}$ or NRG1 at 25 ng ml $^{-1}$; all R&D systems) and at $t = 30$ minutes were harvested by washing with ice-cold PBS and lysing with Bio-Plex Pro Cell Signaling Reagent Kit (Bio-Rad) lysis buffer. Cell lysate was analyzed with the Bio-Plex Protein Array system (Bio-Rad, Hercules, CA) according to the suppliers protocol as described previously (Klinger et al., 2013). The following phospho-sites were measured: EGFR Y1068, MEK S217/S221, ERK1/2 T202/Y204, Y125, Y187, p90RSK S380, RPS6 S235/236, PI3K p85S15, AKT S473, mTOR S2448, and GSK3A/B S9.

3 Results

3.1 An efficient method for network reconstruction from perturbation data

We have developed CNR, a computational framework to (i) reconstruct and quantify interactions between signaling proteins based on potentially incomplete perturbation experiments; (ii) doing so in multiple cell lines simultaneously and (iii) with the possibility to use prior data on the network topology and edge signs (Fig. 1). CNR is a reformulation and extension of MRA (Kholodenko et al., 2002) as an MIQP problem.

Reconstruction of a network using MRA requires each node of the network to be perturbed, because otherwise the problem is under-determined. This is typically not feasible, for instance due to cost constraints or simply because not all nodes can be perturbed by e.g. applying a drug. Furthermore, in the classical formulation, it is not possible to simultaneously reconstruct the networks of several cell lines, which complicates their comparison. CNR solves these problems.

CNR takes the log2-fold changes of signaling proteins in response to perturbations as input, together with annotations of which nodes are perturbed (Fig. 1). Optionally, information about the presence, absence or signs of interactions can also be supplied (Fig. 1). CNR then tries to find a network model that (i) fits the perturbations data; (ii) maintains the same topology for all cell lines; (iii) penalizes model complexity (i.e. the number of edges in the network) to prevent over-fitting and (iv) penalizes the number of edges that differ between cell lines to facilitate identification of the most relevant differences. The latter has the additional advantage of reducing the number of parameters, since most edges are described by the same parameter in all cell lines.

The output of CNR is a network quantification for each cell line, in combination with a quantification of the strengths of the effects of perturbations on their direct targets (Fig. 1). The interaction strength between a pair of nodes consisting of a source and target node is referred to as the local response coefficient. This coefficient is interpreted as the percentage change in the target node activity in response to a 1% change in the source node activity.

3.2 In-silico evaluation

To test our method, we simulated perturbation experiments using an ODE-based dynamic model of the EGFR signaling developed by Orton et al. (2009) (Supplementary Fig. S1). Following Orton et al.,
we also generated BRAF, KRAS or EGFR mutant versions of the model by forcing these proteins to all be in their active state. We simulated network perturbations by ‘knocking down’ all the relevant nodes in the network, one by one, and using the resulting measurements as input to CNR.

We tested the performance of CNR by comparing network reconstructions to the known model from which the data were simulated. Note that since CNR aims to quantify the effect of a perturbation on the steady-state concentration of the active form of a protein only, the CNR network reconstruction will be much simpler than the original model. Nevertheless, the true interactions between active proteins are unambiguously defined (Fig. 2A).

Specifically, we wanted to test how noise in the data, the penalty on differences between cell line, or incomplete data affect the ability to reconstruct or quantify the network interactions. In the following sections, unless stated otherwise, we did not employ any information about the model topology for the reconstructions, and we added 10% noise to the input data.

Fig. 2. Orton model simulation and CNR results. For all results presented here, 10% noise was added to the simulated data. (A) Network topology of the modules in the Orton model. (B) Receiver-operator-curves of network reconstructions using noisy data of the wild-type model, the BRAF model and both combined, with \( \theta = 0 \). (C) Fitting error versus number of differing edges between cell lines indicate that most edges can be set to the same value without affecting model fit. (D) Network reconstructions of the wild-type and BRAF mutant model. Left: Network topology. Edges that differ between the model-reconstructions are highlighted. Green edges are positive, red ones negative. Right: Correlation between reconstructed and actual model parameters. (E) True (top) and False (bottom) positive rates of network reconstruction as function of the number of perturbations in the input data. (F) Spearman correlation between predicted and simulated model parameters (top) and response for perturbations that were not used in reconstructing the network (bottom).
3.2.1 The effect of measurement noise on topology reconstructions

We first tested the ability of our method to reconstruct the network topology when the data is noisy. To this end, we performed reconstructions with different levels of noise added to the input data, for different values of the $\eta$-hyperparameter (using $\theta=0$). By starting with a large $\eta$ and gradually lowering it, we get solutions with an increasing number of edges. From these solutions we calculated the true-positive rate (number of true-positive edges/total number of real edges) and the false-positive rate (number of false-positive edges/total number of real-negative edges). Figure 2B shows the receiver-operating characteristic (ROC) curve for reconstructions of the BRAF mutant (light blue) and the wild-type (gray) cell lines, and simultaneous reconstruction of both together (dark blue), when 10% noise is added to the data. A true-positive rate of $>0.8$ is attained while keeping the false-positive rate well below 0.05, so despite noisy data, a large majority of the edges can be correctly identified. (This is attained when using $\eta = 0.005$.) The performance of the wild-type + BRAF-mutant and wild-type alone reconstructions are comparable, and better than that of the BRAF-mutant alone. This is presumably because due to constitutive BRAF-activation nodes downstream of BRAF are nearly unresponsive to perturbations upstream of BRAF, making these less informative for identifying downstream edges. Similar results were obtained for the other mutants (Supplementary Fig. S2). As expected, increasing the noise levels reduces the performance, but even at 100% noise, the reconstruction performs much better than randomly reconstructing the network, represented by the dotted gray line where the true-positive rate equals the false-positive rate (Supplementary Fig. S2). Interestingly, the performance of the BRAF and the RAS mutant cell line reconstructions suffer less from increasing noise than that of the wild-type.

3.2.2 The effect of the penalty on cell line differences on model fit.

Next, we first tested how the penalty on the differences between cell lines affects the model fit to the data by performing reconstructions using different values for the $\theta$-hyperparameter (using a fixed $\eta = 0.005$). We performed this analysis by pairing the wild-type with the BRAF, RAS and EGFR mutants, respectively. In addition, we performed the analysis for all cell lines combined (Fig. 2C). For each of these analyses, a model with only a small number of differences ($\approx 10$, attained when $\theta = 0.01$) between the cell lines fits the data nearly as well as a model where all edges differ between the cell lines. The number of edges that differ between cell lines can thus be strongly decreased without affecting the fitting error, which is what one would expect when comparing isogenic cell lines that only differ by a single mutation. Adding a non-zero $\theta$-value does not influence the ROC-curves (Supplementary Fig. S2).

3.2.3 Example reconstructions

Having established that CNR is capable of accurately reconstructing networks while strongly reducing the differences between cell lines, we next wanted to assess how interpretable the obtained differences between networks are. To this end, we reconstructed the wild-type and BRAF mutant models together, using $\eta = 0.005$ and $\theta = 0.01$, and visualized the resulting networks (Fig. 2D, left). The thickness of the arrows represents the magnitude of the reconstructed local response coefficients. The edges that differ between the cell lines are highlighted in color. As expected, in the BRAF-mutant, BRAF activation is unresponsive to RAP1 and more responsive to knockdown of BRAF itself. Furthermore, all other differences between the cell lines are downstream of BRAF. (In the mutant, the nodes downstream of BRAF are less responsive to changes in their direct upstream kinases due to saturation effects.) Similar results are obtained for the other mutants (Supplementary Fig. S3). This illustrates that a comparative reconstruction can identify where in the network two cell lines differ. Importantly, the reconstructed local response coefficients and direct perturbation effects correlate very well with their true value directly obtained from the Orton model (Fig. 2D, right). This indicates that CNR can be used to obtain quantitatively accurate model reconstructions from noisy data.

3.2.4 The effect of incomplete perturbation data

It is always not feasible to perturb each node in the network. To test how incomplete perturbation data affects the accuracy of network reconstruction, we performed reconstructions based on a reduced number of input perturbations. To this end, we randomly selected between 2 and 10 nodes to perturb, added 10% noise to the data, and performed the network reconstruction. (Because we expect the total fitting error to scale with the number of perturbations $N_p$, we scaled $\eta$ and $\theta$ using $\eta = 0.01 \cdot N_p/N_h$ and $\theta = 0.01 \cdot N_p/N_h$, where $N_h = 12$.) We repeated this 50 times for each number of perturbations. Figure 2E and Supplementary Figure S4 show the true-and false-positive rates of these reconstructions. As expected, the performance decreases with decreasing numbers of perturbations. However, with as few as two or four perturbations, we still attain performance levels well above random guessing. With low numbers of perturbations used as input, there is a clear benefit of combining multiple cell lines, as the panel combining all cell lines consistently has among the highest true-positive and lowest false-positive rates.

3.2.5 Predicting the effect of new perturbation

Sometimes one is more interested in network quantification and prediction of the effect of new perturbations from a systems were the topology is known, rather than identifying which edges are present. We therefore next tested how well CNR is capable of quantifying the model parameters—local response coefficients and direct perturbation effects—based on incomplete perturbation data. We again randomly selected between 2 and 10 perturbations, added 10% noise to the data, performed reconstructions this time with the correct network topology supplied, and compared the fitted parameters with the true parameters directly obtained from the Orton model (Fig. 2F and Supplementary Fig. S5, top). With as few as 6 perturbations, the median Spearman correlation is roughly 0.5, and for 8 perturbations it approaches 1.

We then used the obtained network-quantifications to predict the response to perturbations that were not used in quantifying the network. To quantify the predictive accuracy of these networks, we calculated the Spearman correlation between the predicted and true response for each perturbation. In calculating the correlation, we excluded the perturbed node itself since the direct-perturbation effect is not part of the prediction. Perturbations where none of the nodes respond are also excluded. Figure 2F and Supplementary Figure S5 (bottom) show that for as few as four or six perturbations, the median Spearman correlation between the predictions and the simulated responses is very close to 1. Interestingly, while the performance in edge reconstruction of the BRAF and RAS mutants were slightly better than those of the wild-type and EGFR mutants, their quantitative predictions are slightly worse (Supplementary Fig. S5).

3.3 Network quantification of PTPN11 KO cells with and without acquired resistance to BRAF inhibition

To test the ability of CNR in a real-world setting, we set out to identify the cause of resistance to targeted anti-cancer drugs by elucidating how the signaling network changed in resistant cells.

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Specifically, we wanted to understand how a PTPN11 KO colorectal cancer (CRC) cell line harboring an activating BRAFV600E mutation become resistant to BRAF inhibition with Vemurafenib.

3.3.1 PTPN11 KO and sensitivity to BRAF inhibition
Normally, BRAFV600E mutated CRC cells are unresponsive to BRAF inhibition due to a feedback loop that activates the receptor tyrosine kinase (RTK), EGFR, upon BRAF inhibition (Klinger et al., 2013; Prahallad et al., 2012). Recently, Prahallad et al. showed that knock-out of PTPN11—which is required to transduce signals from RTKs to the MAPK pathway—prevents this feedback loop from reactivating the MAPK signaling pathway (Prahallad et al., 2015) (Fig. 3A). However, when PTPN11 KO cells are cultured in the presence of a BRAF inhibitor, they eventually become resistant to this inhibitor.

3.3.2 Generation of persisters
To test how resistance to the BRAF inhibition in PTPN11 KO CRC cells emerges, we generated ‘persister’ cells by culturing a PTPN11 KO clone of the BRAFV600E mutated CRC cell line VACO432 in the presence of the BRAF inhibitor Vemurafenib. After roughly 2 weeks, persister cells emerged that were able to grow in the presence of Vemurafenib at a growth rate comparable to that of the parental line in the absence of drug (Fig. 3B).

3.3.3 Perturbation experiments
We systematically perturbed VACO432 parental, PTPN11 KO and the derived persister cells with combinations of growth factors and inhibitors targeting proteins in the PI3K-AKT and MAPK pathway, and measured the steady-state responses of the phosphorylation status of main proteins in the pathways (Fig. 3A and C). The log2-fold changes, relative to their unperturbed (i.e. uninhibited unstimulated) state, were used as input to the CNR optimization problem. As the MAPK and AKT pathways are relatively well characterized, we did not focus on the reconstruction of the pathway topology. Instead, we restricted the model to consist of known interactions and feedbacks obtained from the literature (Fig. 3A).

3.3.4 Modeling of perturbations
Some kinase inhibitors prevent activation of their target by blocking the phosphorylation of the kinase itself, whereas other work by preventing the kinase activity of the phosphorylated protein. We model the latter type of inhibitor as perturbing the substrates of its direct target. Specifically, the MEK and PI3K inhibitors are preventing the kinase activity of their targets and are thus modeled to perturb ERK and AKT, respectively. Since we did not measure BRAF, we cannot model the direct effect of BRAF inhibition on BRAF, but instead model it as a perturbation of MEK. Similarly, we did not measure activity of HER2 and MET, so we model HGF and NRG1 stimulation as affecting MEK, PI3K and AKT directly.

3.3.5 Network reconstruction
Figure 4A shows the network reconstruction of the three cell lines, and how they differ. We allowed for six differences between networks as it gives a good balance between model-fit and model-complexity (Supplementary Fig. S6). As expected, the response of MEK activation to BRAF inhibition is much smaller in the persisters compared to the parental and the PTPN11 KO cells. Furthermore, in the persisters the feedback from ERK to MEK is stronger and MEK is more responsive to receptor stimulation. Finally, the effect of EGFR on AKT is weaker in both the PTPN11 KO and the persister cells, indicating that PTPN11 might be involved in signaling to the AKT pathway as well as the MAPK pathway.

3.3.6 Robustness of network reconstruction
To test how robust these results are, we generated a large pool of solutions by varying the allowed number of differences between 0 and 16, and also considering close-to-optimal solution (with an objective value at most 1.5 times the best possible given the number of differences between cell lines). We counted how often each edge
differed between cell lines in the 168 solutions in the solution pool. There are a limited number of edges that occur in the majority of these solutions (Fig. 4B). These are (i) the response of MEK to BRAF inhibition; (ii) the effects of EGFR on MEK and AKT; (iii) the feedback from ERK to MEK and (iv) the responsiveness of MEK to growth-factor stimulation. Furthermore, the often-occurring differences were also quantitatively consistent in most solutions (Fig. 4C), and in agreement with the observations we made earlier: (i) MEK activity in persisters is less responsive to BRAF inhibition; (ii) more responsiveness to growth factor stimulations; (iii) persisters have a negative feedback from ERK to MEK and (iv) the connection between EGFR and AKT is stronger in the (PTPN11 WT) parental cells. This indicates that these results are robust and do not depend on the detailed settings in the network reconstruction.

3.3.7 Interpretation
The CNR results suggest a model in which the persister cells became resistant by re-establishing normal (RAF-wild-type) MAPK signaling, by, for instance, using an alternative RAF-isof orm (Fig. 4D). Such a model explains the reduced response of MEK to Vemurafenib, which specifically targets BRAF\textsuperscript{V600E} and not wild-type RAF. It explains the stronger negative feedback from ERK to MEK, which is mediated through RAF and thus weak if BRAF is constitutively active. It also explains the increased responsiveness of MEK to EGFR and other growth factors, which are also mediated through RAF and thus weak if BRAF is constitutively active. Consistent with this hypothesis, pERK is activated in the persisters and RAS is constitutively loaded with GTP (Fig. 4E). This hypothesis suggests that the persister cells might be sensitive to pan-RAF inhibition.

4 Discussion
Understanding which targeted therapies are effective in what context, and how cells adapt to become resistant, requires a quantitative understanding of signaling networks and how they differ between cells. In this study we developed CNR, a computational method to reconstruct signaling networks based on perturbation data. CNR focuses on identifying quantitative differences between a set of cell lines (or other experimental models), as this is often the relevant biological question. Using simulated data we showed that CNR works well with noisy and incomplete data.

We applied CNR to perturbation data we generated from BRAF\textsuperscript{V600E} mutated, PTPN11 KO colorectal cancer cells that were...
made resistant to the BRAF inhibitor Vemurafenib through continuous exposure to sub-lethal doses of the drug. The network reconstructions suggested a reversion to RAF wild-type-like MAPK signaling in the resistant cells that would likely make them sensitive to pan-RAF inhibition. This hypothesis is consistent with the observation that both in vitro and in patients resistance to combined BRAF/EGFR inhibition is mediated by alterations that reactivate MAPK pathway activity (Ahronian et al., 2015; Hazar-Rethinam et al., 2018). It also raises a number of other interesting questions, such as how RAF can be activated and respond to growth factor stimulation in the absence of PTPN11.

CNR is formulated as an MIQP problem. Theoretically, such problems are NP-hard, and the search space increases exponentially with the number of nodes in the network. In practice the MIQP problem associated with CNR can typically be efficiently solved. For example, a typical reconstruction for the 12-node Orton model is optimized in under a second on a standard laptop computer. Parameter-setting that lead to highly connected networks are somewhat slower (Supplementary Fig. S7), but since most proteins interact with a limited set of other proteins, biological networks are sparse and the highly connected solutions are not relevant. In addition, the run time can be strongly reduced by incorporating prior information, for instance by providing a starting network with well-known interactions and allowing for a limited number of additional edges.

Some other quantitative network reconstruction approaches, notably those based on maximum-likelihood optimization (Dorel et al., 2018; Klinger et al., 2013) and Bayesian statistics (Halasz et al., 2016; Jastrzebski et al., 2018; Thijssen et al., 2018), are statistically more rigorous defined than CNR. However, the main advantages of CNR compared to these approaches is its flexibility regarding prior knowledge of the network topology or edge signs. CNR does not require such knowledge, but it can straightforwardly be incorporated when it is available. An additional benefit of CNR is the efficiency, making it scalable to much larger networks than other quantitative approaches.

In this work we focused on signaling networks, but CNR can be applied to any perturbation dataset were the targets of the perturbations are known. We used a L0-penalty for the differences between networks (an edge is either different or not) because such results are convenient to interpret as only a subset of edges need to be inspected. However, the penalty can be replaced with an L1 or L2-penalty (The absolute or square of the difference between two edges, respectively), which in some cases better resembles the biological problem, and is expected be faster to solve.

To conclude, CNR provides a flexible and efficient way to reconstruct networks from perturbation data. Using simulations, we demonstrated its ability to accurately reconstruct networks when data is noisy and incomplete. Application to a real-world problem showed its robustness and potential in formulating hypotheses on biological problem.

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