

Impact of drug action in TB: A study using protein–protein influence networks and flux balance analysis

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Since most drugs currently in clinical use have not been designed either with the advantage of structural information or with that of rational target identification, we have no idea even where several mechanisms may be operating independently or together to inactivate the drug. Further, the emergence of resistance makes the drugs much less useful than anticipated. Though bioavailability studies are available in some cases, they often do not tell us how much of the drug actually binds to its intended target and more importantly what are all the other changes that the drug is effecting, which we have absolutely no clue about.

The reductionist approach of understanding proteins individually is obviously not sufficient for these purposes. Systems biology approaches therefore become essential to get a more holistic perspective. Besra and co-workers [1] have studied the effects of drugs on the whole *Mycobacterium tuberculosis* genome using microarrays, which capture those genes that are regulated in response to six anti-tubercular drugs. We have used organism-level metabolome models and analysed the effects on reaction fluxes, upon inhibition of the intended targets by six different anti-tubercular drugs.

What really matters to the functioning of the cell are the metabolites, whose biosynthesis, degradation and numerous inter-conversions are being driven predominantly by proteins and often also indirectly by gene regulations. Using this metabolome layer, we can easily derive protein-protein influences, which capture the influence a protein would have on the function of another protein through their respective metabolites (currency metabolites are excluded from this analysis). Including metabolite driven influences is analogous to identifying the full set of roads that exist in a city, that are either always operational or capable of becoming operational when the need arises due to reasons such as road-blocks or increased or decreased throughput elsewhere. We have augmented these influences with the high-confidence predicted 'interactions' (score ≥ 0.7) from the STRING database.

Many of the genes regulated in the microarray experiments were found to form networks

with connection density an order of magnitude more than the complete network. We also identified the influences between the genes that were commonly regulated. Of the 36 genes that were regulated by four or more drugs, 10 were linked between themselves. These turned out to be important proteins in pathways such as mycolic acid synthesis and respiration. The results of the microarray experiments can thus be rationalised in terms of the protein-protein influence network.

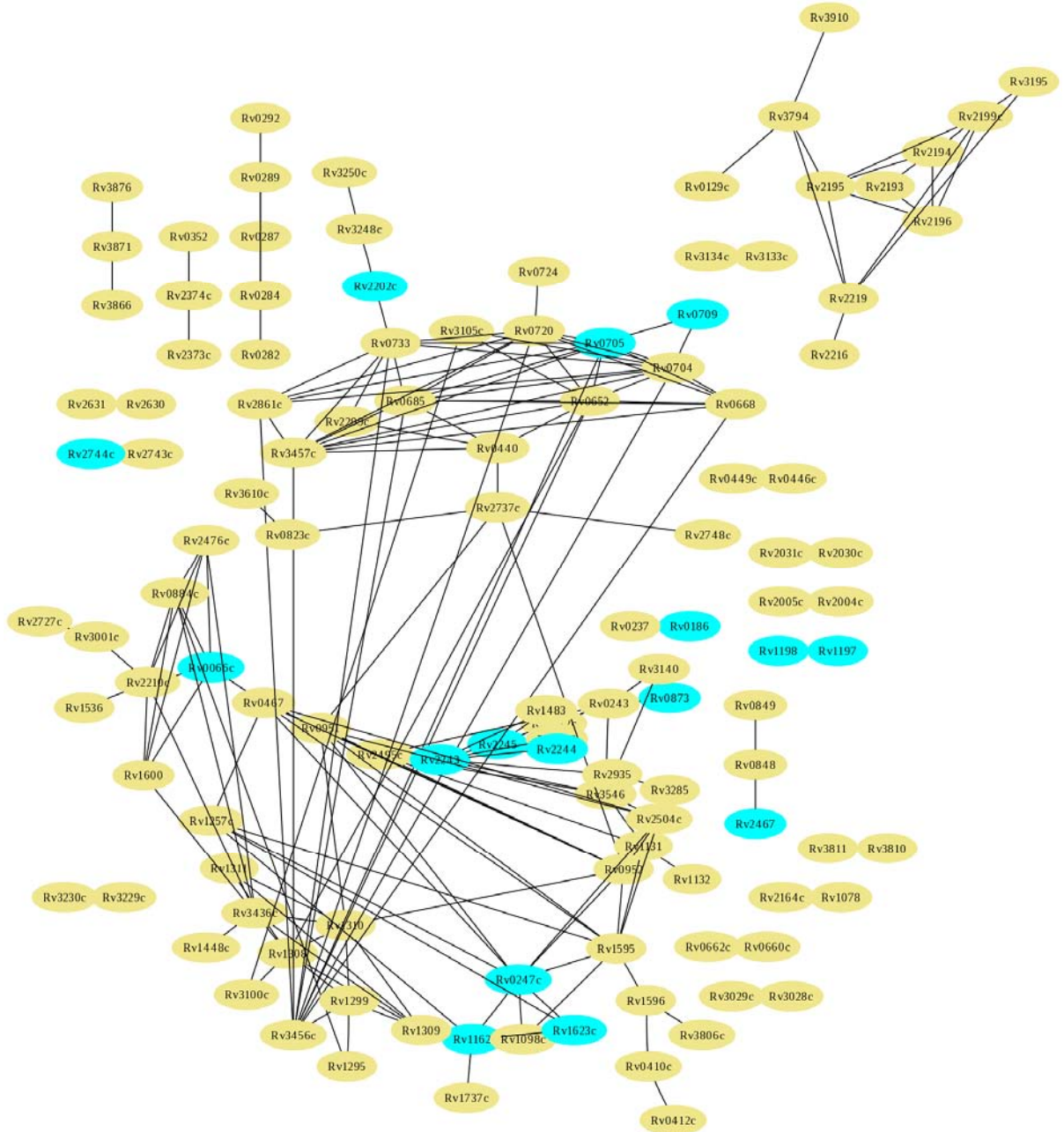


Figure 1: Influence network formed by genes regulated in response to isoxyl. Nodes coloured cyan represent those that are regulated in response to four or more drugs.

Drug	Proteins regulated in response to drug	Proteins linked to one another in influence network [N]	Total possible links [N(N-1)]	Number of links in network (% of total)
SRI#221	207	64	4,032	110 (2.728)
SRI#9190	146	41	1,640	60 (3.659)
SRI#967	146	33	1,056	44 (4.167)
Isoniazid	187	65	4,160	124 (2.981)
Isoxyl	252	120	14,280	398 (2.787)
Tetrahydrolipstatin	232	88	7,656	180 (2.351)
Complete Network	—	3317	10,999,172	33,910 (0.308)

Table 1: Comparison of linkages between proteins regulated in the presence of drugs

Flux Balance Analysis was also performed, to simulate the effect of drug action, for isoniazid and isoxyl, using different models [2-4]. In the genome-scale models, it turned out that some critical reactions corresponding to the production of ADP and NADP were positively modulated.

The studies reported here form a ready framework for understanding mechanisms for emergence of drug resistance and also provide strategies for multi-target inhibition of drugs for combination chemotherapy. New mechanistic insights for drugs with unknown targets can also be obtained by analysing the networks containing regulated genes.

References

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