

Probing Biological Networks using Chemical Combinations

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1 Introduction.

Chemical combinations can provide a rich source of information about biological systems. Genetic deletion screens in yeast have already demonstrated that combination constraints reveal functional associations between genes^[4], and chemical combination screens have been able to distinguish between yeast mutant strains^[3]. Our screens for novel combination therapies^[1] produce a wide variety of response surfaces whose shapes differ markedly for different mechanisms (Fig. 1, left). This information could be especially useful for systems biology because: (1) phenotypic responses to increasing amounts of chemical agents yield more detailed combination effects than can be extracted from essentially digital genetic screens; (2) chemical probes target different cellular components than genetic deletions, providing different and more immediate constraints on the protein network; and (3) chemical combinations can be applied easily to disease-relevant systems, like human signaling networks, that are less amenable to genetic deletion screens.

2 Results and Conclusions.

To show the relationship between combination response surface shape and the target connection, we simulated a branched, unregulated pathway of linked Michaelis-Menten reactions, and calculated the final reaction velocity to determine the inhibition. Distinct patterns appeared for different target configurations (Fig. 1, right), and while the details (EC_{50} doses, transition doses, asymptotic effect levels, etc.) were sensitive to kinetic parameters, the surface shapes were not.

We also carried out a small combination screen using an Alamar Blue proliferation assay of *C. glabrata* yeast cultures (Fig. 2, left). The surfaces were compared to the models shown in Fig. 1 using chi-squared goodness-of-fit (Fig. 2, right). Within the sterol pathway, same-target combinations fit the additive model, as expected. Cross-target combinations produced potentiation-like surfaces, similar to that in Bactrim (Fig. 1). The consistency of effect matches the accepted view of the sterol pathway as a linear sequence of reactions, but the potentiation observed does not agree with the simulations in Fig. 1. Simulations do produce potentiation when negative feedback is introduced to regulate the pathway, as is indeed seen for sterol biosynthesis^[2]. Drug pairs across pathways produced very different combination profiles, and the compound in the most closely related pathway (C-10809, targeting cell-wall proteins) responded very similarly to the imidazoles.

In conclusion, the shapes of chemical combination responses relate to the connection between the compounds' targets in the biological network. Combination screens can thus provide sensitive new constraints on the nature of functional connections between targets which can be used for systems

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biology and chemical genetics applications. We will test the effectiveness of these constraints for *S. cerevisiae* models in collaboration with UC San Diego and the Institute for Systems Biology.

3 Figures.

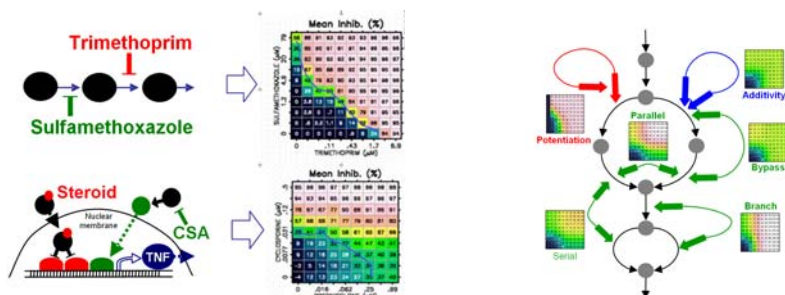


Figure 1: Different mechanisms yield distinct response surface shapes. In our therapeutic screens, the type of synergy for Bactrim (upper left) is very different from that of CSA-Steroid (lower left). Simulations of multiply inhibited pathways (right) also produce a variety of response surface shapes that depend on the target connection and are insensitive to kinetic parameters.

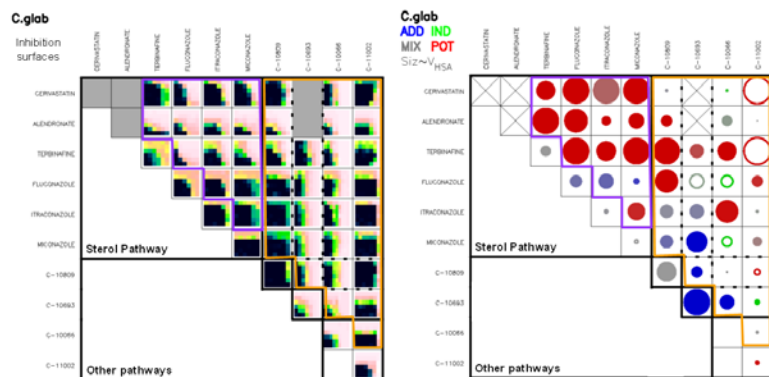


Figure 2: Results from a combination screen in *C. glabrata* proliferation of antifungal agents. The response surface for each pair of drugs (left) was compared to the models shown in Fig. 1, and the symbols (right) show the type of combination effect. Larger symbols indicate more combination effect, with empty symbols showing antagonism. Symbol colour shows which model produced the best fit, with the intensity indicating the certainty of model discrimination. Same-target pairs are additive, combinations between sterol pathway inhibitors consistently fit synergistic potentiation, and cross-pathway combinations produce a variety of different combination types.

4 References and bibliography.

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