

 COMMENTARY

# Pairwise interactions and the battle against combinatorics in multidrug therapies

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Drugs are often used in combination to treat bacterial infections, viruses, and cancer. Drug combinations may exhibit increased potency, decreased dosage-related side effects, and even the capacity to slow the emergence of resistance. The increased efficacy imparted by combining drugs—so-called drug “synergy”—has been a topic of fervent interest for decades (1), while recent studies have also highlighted the evolutionary impacts of counteracting (“antagonistic”) combinations (2–4). The spectrum of potential drug–drug interactions is rich and multifaceted, offering the promise of optimized combination therapies tailored to specific treatment objectives (5). Unfortunately, the inherent flexibility of combination therapy also presents a considerable practical hurdle: the number of possible drug combinations grows exponentially with the number of drugs, making exhaustive screening with even a modest number of drugs intractable. In PNAS, Zimmer et al. (6) develop a robust method for predicting the effects of multidrug combinations for microbial infections and cancer, potentially sidestepping the combinatorial explosion that limits systematic design of combination therapies.

Comprehensively testing the efficacy of  $N$  drugs at  $D$  doses requires  $D^N$  measurements, and this number grows unwieldy for even a modest number of drugs. For example, evaluating a 10-drug combination at 10 doses requires 10 billion measurements (Fig. 1). To put this in perspective, consider that a high-throughput screen capable of evaluating  $10^5$  drug combinations per day—a rate on par with some large-scale research facilities—would require more than 270 y to fully characterize all possible drug dosages. In addition to the overwhelming time cost, brute-force approaches are practically limited by the cost of drugs and potential scarcity of the biological samples.

A number of promising strategies have emerged to combat this combinatorial explosion. As our molecular and structural understanding of drug action and the targeted intracellular signaling pathways continues to mature, detailed computational models provide an avenue for rapidly evaluating drug efficacy in silico

(7, 8). Unfortunately, the required mechanistic insight is not always available, and these methods remain fundamentally limited by the problem’s exponentially growing complexity. Rather than relying on mechanistic models, Zimmer et al. attempted to evade the combinatorial explosion by leveraging a striking property commonly observed in many-body physical systems: the behavior of the composite system can often be explained by considering the aggregate behavior of smaller, tractable subsystems. For example, the statistical properties of neural populations (9, 10), the expression patterns of gene networks (11), the behavior of animal flocks (12), and even the voting tendencies of the US Supreme Court (13) can be largely explained by interactions between pairs of constituents—neurons, genes, birds, or justices. In physics parlance, higher-order interactions can often be decomposed—at least approximately—into a simple combination of lower-order interactions. The simplification to pairwise interactions is particularly significant, as the number of pairs grows quadratically—not exponentially—with  $N$ . In the context of drug combinations, screening all pairwise combinations of 10 drugs at 10 doses requires on the order of  $10^3$  measurements—less than a day with our hypothetical high-throughput screen.

Indeed, several recent studies have indicated that the effects of drug pairs may dominate features of the multidrug response, including the inhibitory strength of antiretroviral combinations (14), the dynamics of proteins in cancer cells (15), promoter activity of bacteria (16), and calcium signaling in human platelets (17). Perhaps most relevant, recent work in bacteria demonstrated that the inhibitory effects of antibiotic combinations could be predicted based on the effects of the drugs in pairs (18). Collectively, these studies highlight the promise of pairwise approximations for predicting multidrug effects.

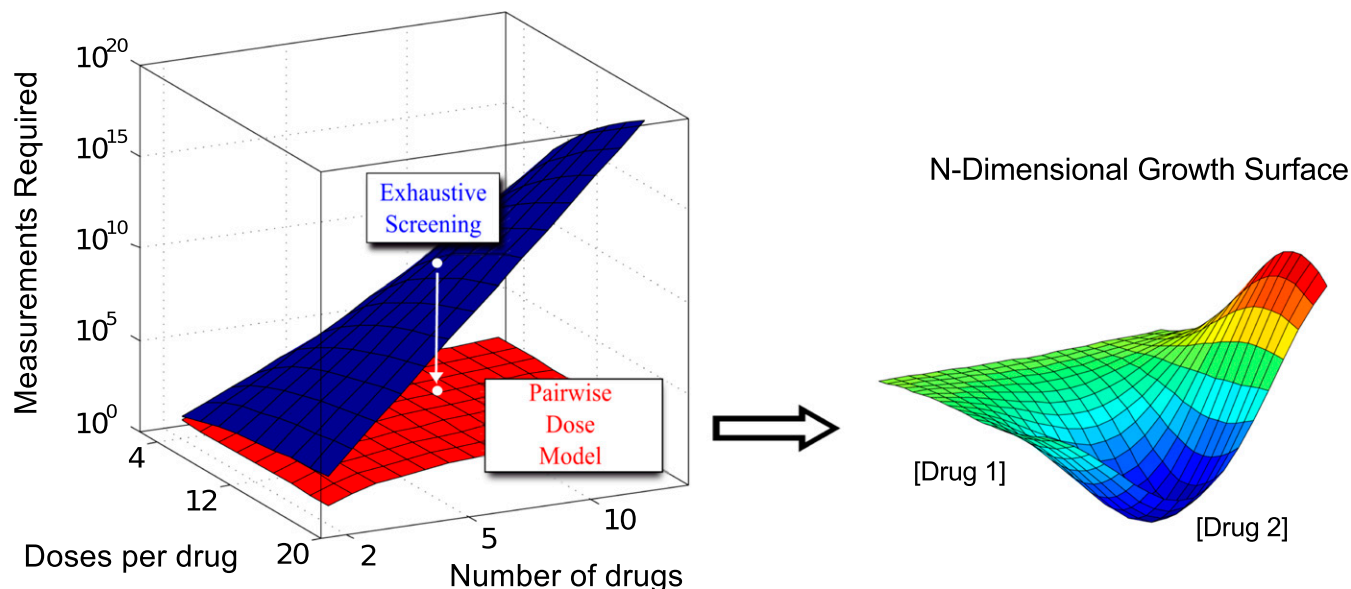
The study by Zimmer et al. (6) provides several innovative and fundamental advances over previous work, potentially opening the door to widespread practical application of pairwise approximations to multidrug treatments. First, they incorporate a pairwise

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See companion article on page 10442.

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**Fig. 1.** The experimental demands of exhaustive combination screening increase dramatically with the number of drugs and doses (blue surface). The pairwise dose model introduced in ref. 6 reduces the experimental burden by multiple orders of magnitude (red surface). For a screen of 10 drugs at 10 dosages, the number of required measurements is reduced from  $10^{10}$  (top white circle) to roughly  $10^2$  (bottom white circle), allowing for a quantitative prediction of the multidrug response surface (Right, schematic) that is robust to measurement noise and missing data.

approximation into a phenomenological dose–response model. The model accounts for observed interactions between drug pairs by assuming that each drug has the potential to rescale the effective concentration of the other. Similar approaches have been recently used to describe the effects of resistance-conferring mutations on two-drug mixtures (2, 19). By extending their two-drug model to  $N$ -drug combinations, Zimmer et al. leverage both the power of the pairwise approximation as well as the inherent simplicity of concentration rescaling. To illustrate the advantage of this approach, consider the task of predicting the effects of a three-drug combination, where the concentrations of the three drugs are  $D_1$ ,  $D_2$ , and  $D_3$ , respectively. To make this prediction, the model from Zimmer et al. incorporates not only the single drug ( $D_1$ ;  $D_2$ ;  $D_3$ ) and pairwise ( $D_1 + D_2$ ;  $D_1 + D_3$ ;  $D_2 + D_3$ ) measurements at these concentrations but also potentially measurements at other doses. Furthermore, their method allows one to predict the effects of dosage combinations even when the complete collection of single-drug and pairwise measurements is not available. In essence, their model exploits the inherent smoothness of dose–response surfaces—which is naturally embedded in many pharmacology models (1)—to minimize the effects of experimental noise and missing data. As a result, they are able to apply their approach to new combinations of anticancer drugs and significantly improve upon previous approaches. Even more strikingly, they are able to estimate the full  $N$ -drug response surface using only a small fraction of the pairwise measurements, making this method ideal for optimizing therapies (Fig. 1). Using their estimate of  $\sim 10$  measurements per drug pair, our hypothetical 10-drug screen would now be reduced to several hundred measurements—a task easily achievable even for modest-sized academic laboratories. As an elegant proof of principle, they optimize a combination of three antibiotics to achieve

growth inhibition comparable to single-drug therapy but with a four-fold reduction in drug concentration (6).

The practical implications of robust, multidimensional strategies for predicting drug combination effects are far-reaching. These approaches represent an additional step toward individualized, precision medicine—where, for example, infections are treated with optimized combination therapies based on real-time information about genetic and phenotypic composition of particular microbial populations. Interestingly, the results also raise theoretical questions at the interface of cell biology and statistical physics. In many physical systems, such as a dilute gas, the dominance of pairwise interactions intuitively follows from the fact that interactions are spatially localized, making higher-order interactions—for example, three-body molecular collisions—statistically unlikely. By contrast, in the context of drug combinations, interactions often do not arise from direct molecular or chemical interactions between drugs. Instead, drugs represent generalized perturbations to the intracellular networks governing cell growth and proliferation (see, for example, ref. 20). In this sense, drug interactions stem from indirect coupling between multiple perturbations to a complex network. As a result, the relative strengths of higher- and lower-order interactions are not immediately clear, and elucidating the mechanisms underlying the functional dominance of drug pairs—whether biochemical, biological, or statistical—remains an open theoretical question. Nevertheless, the results from ref. 6—and the remarkable success of pairwise approximations for predicting the multidrug response across organisms—may hint at evolved topological or statistical constraints on these networks. These findings therefore have the potential to spawn new research directions linking network theory, complex systems, and biomedicine.

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