of these processes. Though this will be a significant investment, fast inhibitor degradation will lead to significant cost and material reductions compared to current detoxification methods (**Fig. 1**) and, as such, will be important in providing a green, cost-effective option for lignocellulose processing technology, as well as biofuel and biorefinery processes more generally.

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Competing financial interests

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DRUG DISCOVERY

Engineering drug combinations

The level of an individual protein in cells treated with combinations of drugs is best explained by simple linear superposition of the protein levels in response to single drugs. This finding may facilitate rational design of higher order drug combinations.

Scott J Dixon & Brent R Stockwell

As we learn more about disease mechanisms at the molecular level and move toward a more personalized approach to medicine, the need to adjust the composition of therapies for each individual patient continues to grow[1]. Drug combinations can target multiple sites within the same protein, countering the emergence of drug resistance, or multiple nodes within a molecular network, enabling the combinatorial control of biological systems[2,3] (**Fig. 1a**). These considerations make drug combinations leading candidates for personalized therapies. In fact, drug combinations are already in widespread clinical use. Two well-known examples are the three-drug combinations of reverse-transcriptase and protease inhibitors used to treat HIV infection[4] and the four-drug combination comprising DNA-damaging agents, a microtubule disruptor and a corticosteroid (cyclophosphamide, doxorubicin, vincristine and prednisone, together known as CHOP) used to treat non-Hodgkin's lymphoma[5]. Variations on these treatments exist that add even more drugs to the mix. Given this trend, one may ask: what is the most effective drug combination complexity, and how will we know when we get there? In nature, a bacterial endosymbiont growing on the antennae of certain wasp species releases a cocktail of nine different antibiotic compounds that together protect growing wasp larvae from a broad range of fungal and bacterial pathogens[6]. This suggests that we have far to go before achieving the same sophistication in designing drug combinations. Would ten-, fifty- or hundred-drug combinations be more effective than existing three- and four-drug combinations to combat diseases or selectively modulate cell function? How could such combinations be identified? Certainly at this level, both clinical trial-and-error and unbiased screening of all possible combinations of drugs become utterly impractical. We must therefore devise ways to better predict the effects of drug combinations on molecular and cellular networks. In a recent paper, Geva-Zatorsky *et al.*[7] focus on one aspect of this problem, investigating the effects of drug combinations on protein abundances in cells.

Geva-Zatorsky *et al.*[7] investigated what happens to protein levels in cells treated with various drugs. Building on previous work[8,9], they used automated image analysis to examine the expression levels of 15 functionally diverse yellow fluorescent protein (YFP)-tagged proteins in response to 13 different drugs and 19 drug combinations, over the course of 2 days in culture. They observed a surprisingly wide array of protein level changes over time; these changes were unique to each drug–protein pair. Thus, for example, the level of the ribosomal protein RPS3 increased in response to nocodazole but decreased in response to camptothecin; by contrast, the level of the nuclear lamin protein LMNA increased in response to both drugs.

What effect does the combination of two drugs have on specific protein levels? Remarkably, protein levels in cells treated with combinations of two drugs was best described by the weighted sum of the protein level in response to either drug alone (**Fig. 1b**). These weights (from 0 to 1) refer to how much each drug 'counts' toward the final level. The weights were protein specific and varied according to the concentration of drug tested, but they were constant over time and, for the most part, summed to 1. One important caveat is that not all drugs conformed to the linear superposition model. For unknown reasons, the effects of one compound, the phosphatidylinositol-3-OH kinase inhibitor wortmannin, could not be explained by linear superposition. It is not clear whether this is an isolated case or whether a significant fraction of all drugs will produce effects not explicable in terms of the superposition model.

Nevertheless, Geva-Zatorsky *et al.*[7] went on to ask whether it is possible to predict the effects on protein levels of higher order, three- and four-drug combinations using only the observed protein levels in two-drug combinations. In most cases, there was good agreement between the levels predicted from the weighted sums observed for the individual two-drug combinations and the observed levels in the three-drug and four-drug combination experiments (**Fig. 1b**). By implication, all that may be required to predict protein levels in response to any number of drugs is knowledge of each

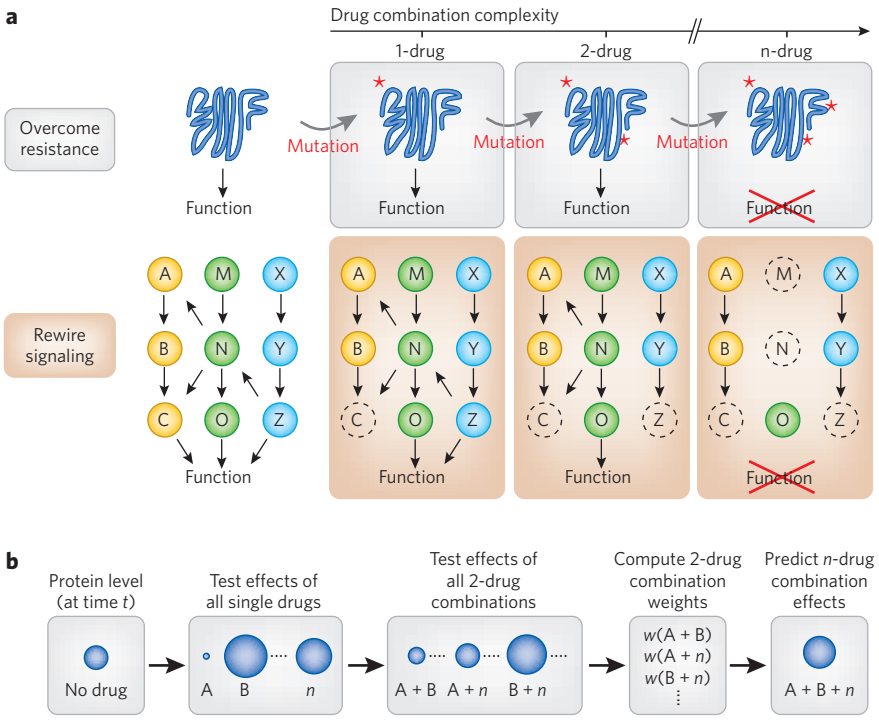

Figure 1 | The uses of drug combination therapies and how future therapies may be predicted. (**a**) Two well known uses for combination therapies: to prevent the emergence of drug-resistant pathogens or tumor cells by simultaneously targeting multiple sites on a key protein (blue squiggle), and to perturb the function of individual nodes within a signaling network to influence cell fate in a desired manner (for example, to induce death in a tumor cell). Higher order combinations of *n* drugs should be more effective in preventing both the emergence of drug resistance and in modulating cell function as they offer the potential to target more sites. (**b**) It may be possible to predict the abundance (size of the blue circle) of any given protein at a particular time point *t* in cells treated with *n* combinations of drugs by comparing the effects of all possible two-drug combinations, deriving the two-drug weights and computing the predicted effect on final protein level using the linear superposition model.

individual two-drug effect. Notably, given the linear superposition phenomenon, the tendency is for the protein levels in the higher order combinations to converge toward baseline as the effects of different drugs act to cancel each other out. This observation is consistent with previous findings that masking effects predominate in systematic two-drug combination experiments when the different drugs target different pathways[10].

This study helps us to understand the effects of drug combinations at the protein level but leaves several key questions unanswered. Most importantly, the relationship between protein levels and cellular phenotypes remains obscure. Indeed, previous work from the same group has demonstrated that the levels of many proteins are altered by drug treatments, but few of these changes are correlated with cell fate[9]. Thus, relating variations in the levels of specific proteins to desired phenotypic outcomes remains a crucial challenge. Additionally, it is unclear why so many randomly selected proteins vary in expression level in response to drugs at all. An important technical issue will be to rule out nonspecific effects on normal protein expression, activity and regulation associated with the essentially random insertion of the YFP cassette into the genome. Future work, using a broader range of compounds and test proteins in more model systems, will be essential to see whether the linear superposition model is broadly applicable, and whether it can be used to predict high-order drug combinations that produce clinically useful modulations of protein levels. If so, the first rationally designed 100-drug combination may not be so far off. ■

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