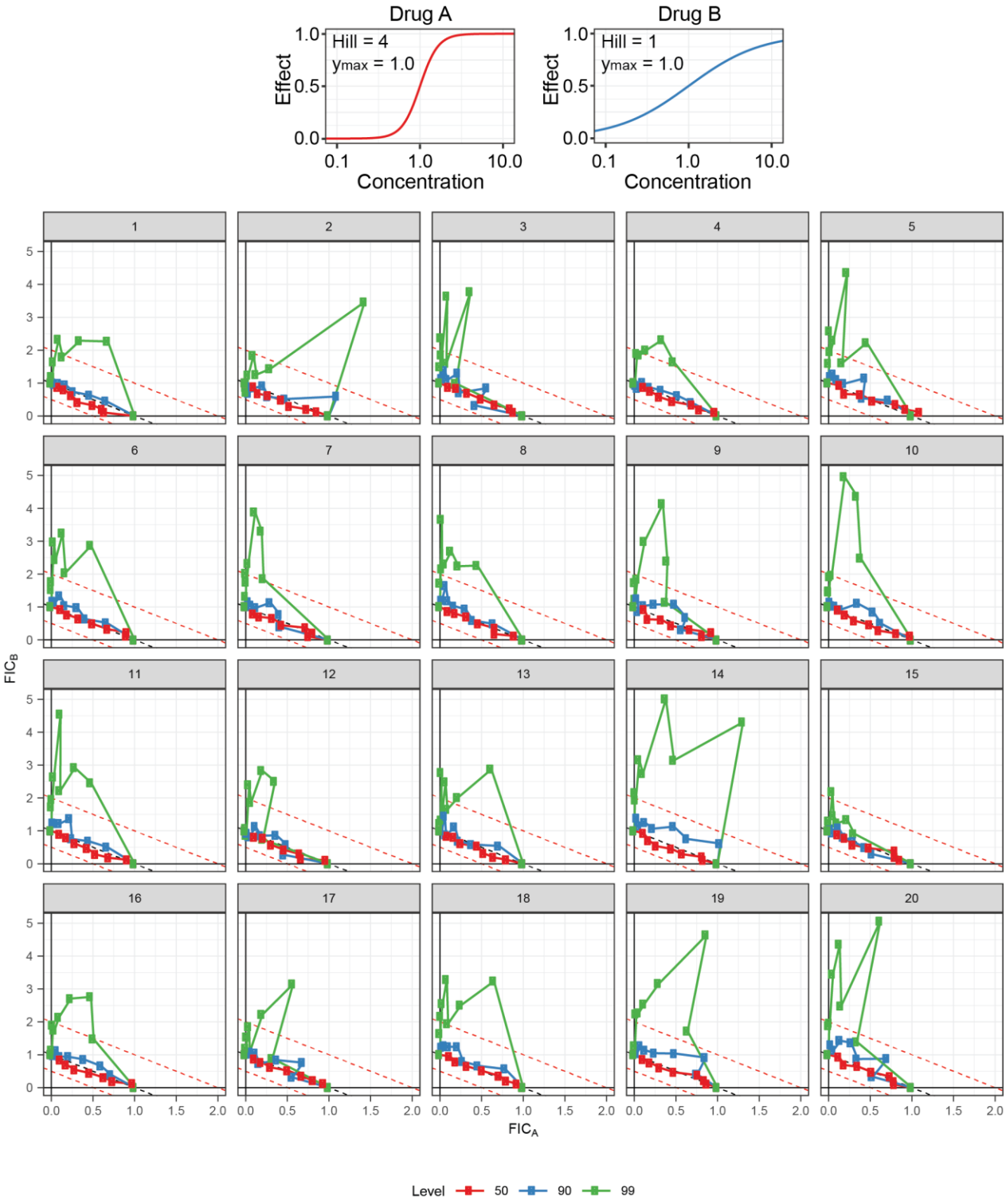
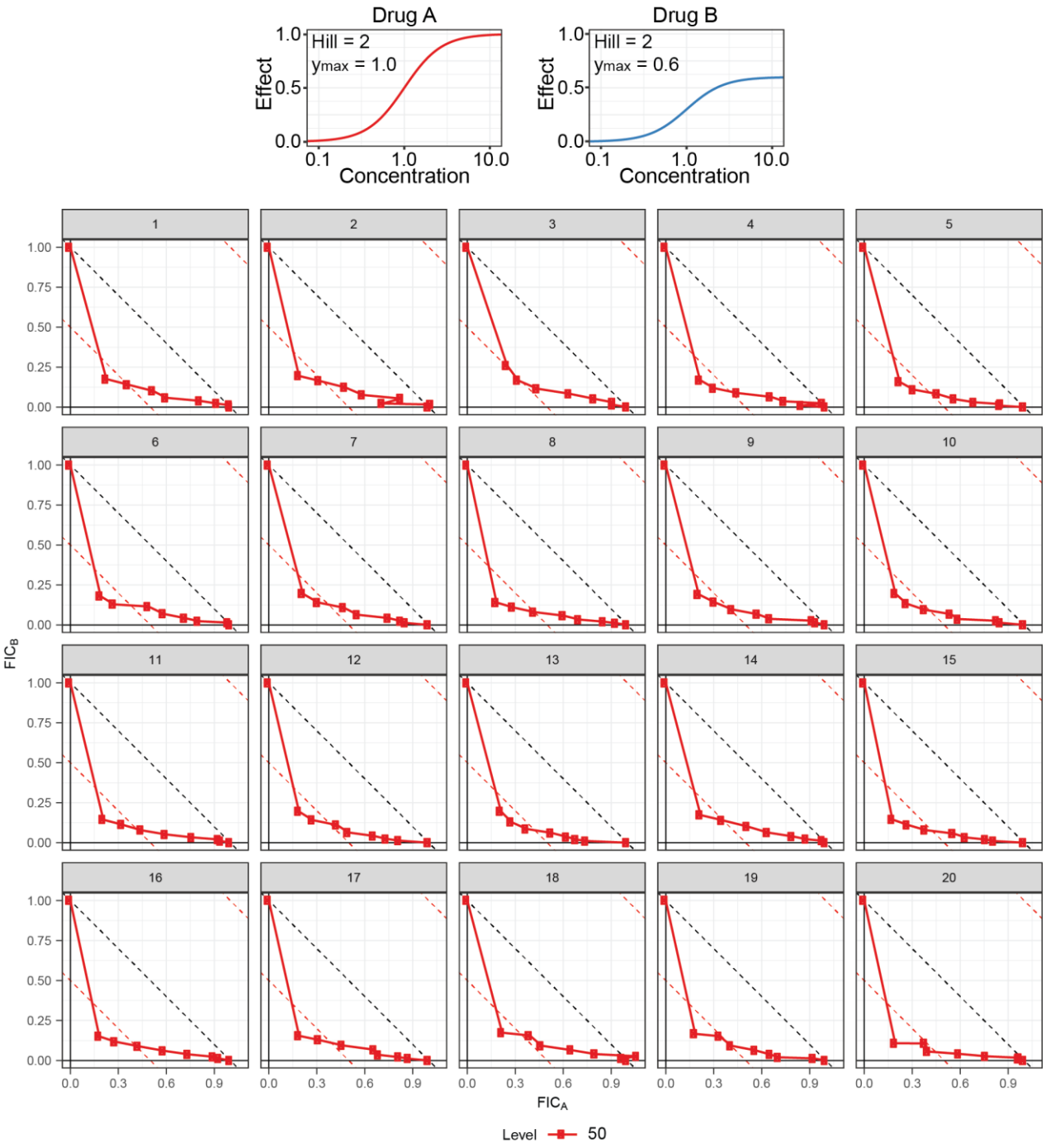
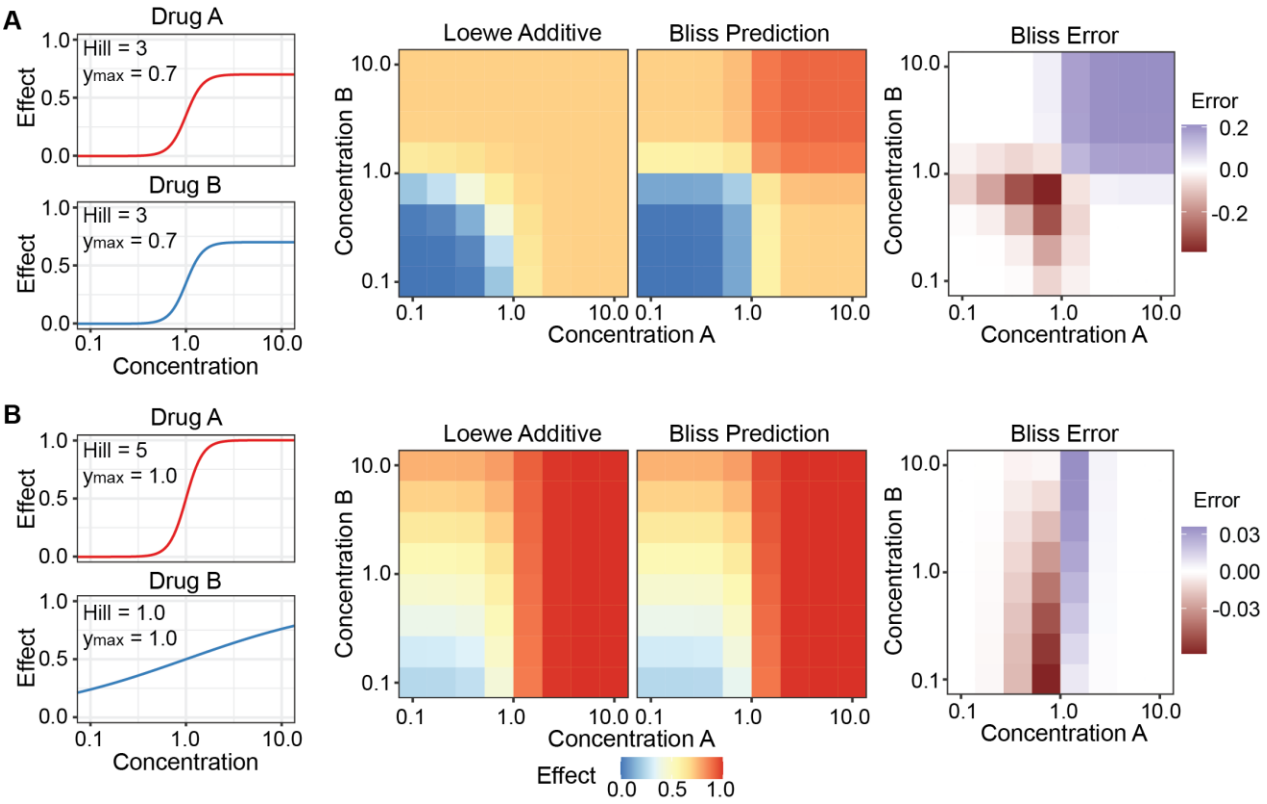
Supplementary materials



Supplementary figure S1. Fractional inhibitory coefficient (FIC) analysis of 20 random simulations of an experiment combining two compounds with differing Hill slopes (1 and 4). Plot 1 is presented in **Figure 1C**.



Supplementary figure S2. Fractional inhibitory coefficient (FIC) analysis of 20 random simulations of an experiment combining two compounds with different maximum efficacies (y_{max}) but the same Hill slopes. Every plot shows strong synergy (FIC < 0.5). Because the maximum efficacy of Drug B is 0.6, the FIC is undefined for the 90% and 99% levels.



Supplementary figure S3. (a) A drug combination in which Drug A is the same as Drug B (left) produces different predictions under Loewe additivity and Bliss independence (center). This results in a false landscape of Bliss synergy and antagonism (right). **(b)** Similar to (A) but using a combination of fully efficacious drugs (maximum effect = 1.0) with differing Hill slopes.

Supplementary table S1. Name, abbreviation, target, mechanistic class, and inclusion status of the 38 compounds in the Merck OncoPolyPharmacology Screen (OPPS).

Drug	Abbreviation	Target	Mechanistic class	Status
5-FU	5FU	Thymidylate synthase	Anti-folate	Included
Methotrexate	MTX	Dihydrofolate reductase	Anti-folate	Included
Cyclophosphamide	CYC	DNA damage	DNA Damage	Excluded
Mitomycine	MIT	DNA damage	DNA Damage	Included
Temozolomide	TMZ	DNA alkylator	DNA damage	Excluded
AZD1775 (Adavosertib)	ADA	Wee1	Cell cycle checkpoint	Included
MK-8776	CHK1	CHEK1	Cell cycle checkpoint	Included
Erlotinib	ERL	Epidermal growth factor receptor (EGFR)	EGFR	Included
Lapatinib	LAP	EGFR/ERBB2	EGFR	Included
Zolinza (Vorinostat)	VOR	pan-HDAC	Singleton	Included
Paclitaxel	PAC	Microtubule	Microtubule	Included
Vinblastine	VNB	Microtubule	Microtubule	Included
Vinorelbine	VNR	Microtubule	Microtubule	Included
Dasatinib	DAS	BCR/Abl	Other kinase	Included
Dinaciclib	DIN	Cyclin-dependent kinases	Other kinase	Included
MK-5108	AKA	AURKA	Other kinase	Included
PD325901	MEK	MEK	Other kinase	Included
Sorafenib	SOR	Multiple kinase targets	Other kinase	Included
Sunitinib	SUN	Multiple kinase targets	Other kinase	Included
ABT-888 (Veliparib)	VEL	PARP	PARP	Excluded
MK-4827 (Niraparib)	NIR	PARP	PARP	Included
BEZ-235 (Dactolisib)	DAC	PI3K/mTOR	PI3K/AKT/mTOR	Included
MK-2206	AKT	AKT	PI3K/AKT/mTOR	Included
MK-8669 (Ridaforolimus)	RID	mTor	PI3K/AKT/mTOR	Included
Carboplatin	CAR	DNA damage	Platinum	Included
Oxaliplatin	OXA	DNA damage	Platinum	Included
Bortezomib	BOR	Proteosome	Singleton	Included

Dexamethasone	DEX	Glucocorticoid receptor	Singleton	Excluded
Geldanamycin	GEL	HSP90	Singleton	Included
Gemcitabine	GEM	Ribonucleotide reductase	Singleton	Included
L778123	FTI	Farnesyltransferase/ geranylgeranyltransferase	Singleton	Included
Metformin	MET	Energy homeostasis	Singleton	Excluded
MK-4541	ARM	Selective androgen receptor modulator	Singleton	Excluded
MRK-003	GSI	Gamma-secretase	Singleton	Included
Doxorubicin	DOX	Topoisomerase II	Topoisomerase	Included
Etoposide	ETO	Topoisomerase II	Topoisomerase	Included
SN-38	SN38	Topoisomerase I	Topoisomerase	Included
Topotecan	TOP	Topoisomerase I	Topoisomerase	Included

HDAC, histone deacetylase; HSP90, Heat shock protein 90; mTOR, mammalian target of rapamycin; PARP, poly ADP ribose polymerase; PI3K, phosphatidylinositol 3-kinase.

Simulation of biased FIC experiments

The FIC experiments were simulated using much the same methods as those used to simulate combination index experiments in our previous paper [11]. For each simulated experiment, each drug was tested in dose–response at 15 concentrations in a two-fold dilution centered on 1 µM; the two drugs were then tested in combination in a checkerboard of dose pairs, with 10 different combinations of both drugs, also in a two-fold dilution with a maximum of 32 µM. Within a simulated experiment, all measurements were simulated in triplicate. All experiments were simulated with Gaussian noise with a standard deviation of 7.5%. In addition, all concentrations were simulated as varying around the intended target concentration in a log normal distribution, such that the standard deviation of the natural logarithm of the simulated concentration was 0.953 (the natural logarithm of 1.1). All compounds had doses of median effect equal to 1 µM, initial effects of 0%, and maximal effects of 100%, unless otherwise indicated.

Additive surfaces were calculated using the iterative implicit method described by Greco *et al*. [36]. Briefly for each dose pair, the effect level was initially set at 50%; the right hand side of the equation for Loewe additivity was then calculated, and the effect level was increased or decreased depending on whether the expression evaluated to less than or greater than 1. By exponentially decreasing the size of the step, the effect could be numerically estimated with arbitrary precision.

Estimation of additive and Bliss independent surfaces

Loewe additive and Bliss surfaces were calculated directly from the dose response behaviors of the individual drugs. Bliss independent surfaces are particularly straightforward to calculate, as they are defined by the equation:

$$f_a = f_{a,A} + f_{a,B} - f_{a,A} f_{a,B}$$

where f_a is the fraction affected by the combination, $f_{a,A}$ is the fraction affected by the dose of A alone, and $f_{a,B}$ is the fraction affected by the dose of B alone. For example, in **Figure 1D**, when both concentrations are high, the one drug reaches an effect of 70% and the other reaches 35%, so the fractions affected by the two drugs are 0.7 and 0.35. The fraction affected by the combination (according to Bliss independence) is therefore

$$0.7 + 0.35 - 0.7 * 0.35 = 1.05 - 0.245 = 0.815$$

So, Bliss expects the combination to produce 81.5% effect.

Estimating additivity is less straightforward, but still entirely manageable. Loewe additive surfaces can be calculated as described in the previous section. When the maximal effect of one drug is smaller than that of the other, we use an extension of Loewe additivity called asymptotic additivity, defined in Twarog *et al.* [11]. Asymptotic additivity mirrors Loewe additivity at smaller doses of the partially active drug, but asymptotically approaches a partial effect at high doses,

behaving as though the dose of the fully active drug is being added to a partial dose (that which would produce the maximal effect of the partially active drug).

Estimation of extended interaction metrics

In our previous paper, three interaction metrics were fit or estimated for all combinations in the Merck Oncopolypharmacology screen: the BRAID interaction parameter kappa, the combination index, and a simple form of Bliss volume (not included in this analysis, as the updated version of Bliss volume is more robust and more informative). In addition, similarities were calculated using variation in potency (using the dose–response behavior of the analyzed compounds) and the BRAID therapeutic window measure, the index of achievable efficacy (IAE). For details of these fits, see Twarog *et al*. [11]. For the analysis in this paper, eleven additional interaction metrics were fit or estimated from the Merck dataset. To begin, we estimated two versions each of volumetric deviations from the highest-single-agent (HSA), Bliss independent, Loewe additive, and ZIP surfaces. The volumetric deviation calculations were based on the work of Vlot *et al*. [18], with adjustments to allow for more robust estimation across the full dataset, and to examine the impact of the volumetric weighting scheme adopted by the authors. In addition, all combinations were fit using the universal response surface approach (URSA) model of Greco *et al*. [36] with one interaction parameter (alpha), and with the MuSyC of Meyer *et al*. [38] with three interaction parameters (alpha1, alpha2, and beta).

For the eight volumetric deviations, the difference between the measured surface and the non-interacting surface was estimated for all 16 non-zero dose pairs tested. In the unweighted case, the average of these 16 differences was taken as the overall deviation from non-interaction; in the weighted case, dose pairs with the highest or lowest concentrations of one drug were given 70% weight, and those with the highest or lowest concentrations of both drugs were given 49% weight, and the weighted average of the differences was taken as the measured deviation from non-interaction. In the following sections we describe how each of the four difference surfaces was estimated.

Note: unless otherwise specified, equations were fit to data using the R non-linear optimization function ‘optim’.

HSA and Bliss volumes

HSA and Bliss are two of the simplest metrics, which probably explains their continued popularity. For the OPPS, for each drug pair and cell line, the individual dose response measurements were fit to a Hill model (the four parameter log-logistic equation). These dose response fits were used to estimate the effect of each drug alone at the four concentrations tested in combination (which were not reliably the same as those tested in dose response). The HSA response model predicts that the combined effect is the larger of the two effects; and because the measurements in the dataset were viabilities, this meant that the HSA prediction was simply the minimum of the two

individual predicted effects. Bliss independence, on the other hand, predicts that cells surviving the combination are a probabilistic conjunction of surviving each individual dose, so the Bliss independent prediction for a given dose pair was a product of the two individual predicted effects. Differences for each dose pair were calculated by subtracting the HSA or Bliss prediction from the average measured viability for that pair.

Loewe additive volumes

Estimation of a Loewe additive surface is a non-trivial problem, as Loewe additivity does not stipulate a closed-form equation for the resulting non-interacting surface. Instead, the Loewe additive surface is defined by the implicit equation

$$\frac{D_A}{ID_{X,A}} + \frac{D_B}{ID_{X,B}} = 1$$

where $ID_{X,A}$ is the dose of drug A alone which produces the combined effect of dose D_A of drug A and dose D_B of drug B, and $ID_{X,B}$ is the corresponding dose for drug B.

When both individual drugs are assumed to follow a Hill (log-logistic) model, this expands to:

$$\frac{D_A}{ID_{M,A}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{n_a}}} + \frac{D_B}{ID_{M,B}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{n_b}}} = 1$$

where $ID_{M,A}$ and n_a are the dose of median effect (often called the EC_{50}) and the Hill slope, respectively, of drug A, $ID_{M,B}$ and n_b are the corresponding parameters for drug B, E_0 and E_f are the minimal and maximal effects produced by the drugs, and E is the combined effect of the two doses in question. Evaluating a Loewe additive surface therefore reduces to solving this equation for E, given any dose pair D_A and D_B. Fortunately, by subtracting 1 from each side, solving the equation is converted to a problem of finding the root of the expression on the right (minus 1). This can be done quite efficiently in R using the 'uniroot' function.

However, one additional wrinkle remains. The Loewe additive surface is only well defined if the minimal and maximal effects of both individual dose–response curves are the same. When both Hill models are fit independently, there is no guarantee that this is the case; indeed, the parameters often differ greatly. To address this, for a given drug pair in a particular cell lines, we fit both dose–response behaviors with a single six-parameter model, with two parameters for the potency and Hill slope of drug A, two parameters for the potency and Hill slope of drug B, and a shared minimal and maximal effect. Once this fit was performed, the parameters were added to the equation above, and for each dose pair, the equation was solved for E to estimate the Loewe additive prediction. The deviation for each dose pair was then estimated by subtracting the Loewe additive prediction from the average viability for that dose pair.

ZIP volumes

The ZIP method does not in fact posit a new reference surface: the ZIP reference surface is simply a Bliss independent surface with two dose–response behaviors determined by a Hill (log-logistic) model. The difference between ZIP and the traditional Bliss volume method is in what ZIP compares to the reference surface: rather than calculating the difference between the raw measurements and the reference surface, ZIP first performs a particular 'smoothing' approach, and then compares the smoothed measurement to the reference surface.

The constraints determined by the ZIP method are difficult to determine; the original definition assumed that both dose–response curves share a common minimal and maximal effect, but the description of the method explicitly cited the need for a shared maximal effect as a limitation of Loewe additivity, so the authors probably intended the ZIP method to be used with differing maximal effects. However, the smoothing approach yields discontinuous results if the minimal effects of the two dose–response curves differ. Fitting dose–response curves with a shared minimal effect but diverging maximal effects was slightly beyond the scope of this analysis, so we instead constrained the minimal effects of both individual response curves to be 1, and fit the remaining three parameters of a four-parameter Hill model for both drugs. Then, for each combined level of drug A, we fit the dose–response behavior of drug B (combined with that level of drug A) to a four-parameter Hill model with the minimal effect fixed at the predicted viability with that level of drug A alone. Correspondingly, for each level of drug B, the dose response behavior of drug A (combined with that level of drug B) was fit, similarly constrained. The average of these two grids of partial dose–response curves constituted the smoothed surface of the ZIP method. The ZIP deviation for each dose pair was then calculated by subtracting the Bliss independent prediction at that dose pair (using the constrained Hill models fit above) from the ZIP-smoothed surface at that dose pair.

Estimation of URSA alpha

The URSA model is based directly on the definition of Loewe additivity. Like the Loewe additive surface, it is defined by an implicit equation, with an additional interaction term:

$$\frac{D_A}{ID_{M,A}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{n_a}}}+\frac{D_B}{ID_{M,B}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{n_b}}}+\alpha\frac{D_A}{ID_{M,A}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{2n_a}}}\frac{D_B}{ID_{M,B}\left(\frac{E-E_0}{E_f-E}\right)^{\frac{1}{2n_b}}}=1$$

When alpha is greater than zero, the potency of combined doses is increased, and the combination exhibits synergy; when alpha is less than zero, the combination exhibits antagonism. In their paper, Greco *et al*. [36] describe pseudo-code for evaluating an URSA surface with a given set of parameters; our implementation of the URSA model was based directly on this code. The resulting model function was used to fit the response surface parameters to each drug pair and cell line, using the R 'optim' nonlinear optimization function.

Estimation of MuSyC interaction parameters

The MuSyC model of combined actions is defined in terms of transitions between four compartments; the predicted effect is a weighted average of the effect levels observed in these four compartments, with the weights determined by the equilibrium occupancies of those compartments as determined by the transition rates between them. Those transition rates are governed by the doses of drugs and six (or eight) of the ten (or twelve) overall response surface parameters. (The full MuSyC model allows for two interaction parameters, gamma1 and gamma2, which affect the Hill slope of the individual drugs, but the effects are small, and they are hence generally assumed to be 1 in MuSyC analyses. We do the same here.) |The original paper defined the model only in differential equation terms, but it is in fact possible to solve the relevant system of equations for an explicit expression of the MuSyC response surface:

$$E = \frac{E_0 f_0 + E_1 f_1 + E_2 f_2 + E_3 f_3}{f_0 + f_1 + f_2 + f_3}$$

where the E_i are the observed effect levels of the four compartments, and the f_i are expressions for the relative occupancy of the four compartments. They are defined as:

$$f_0 = 2 + \left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a} + \left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b}$$

$$f_1 = 2\left(\frac{D_A}{ID_{M,A}}\right)^{n_a} + \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a} + \left(\frac{D_B}{ID_{M,B}}\right)^{n_b}\left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a}$$

$$f_2 = 2\left(\frac{D_B}{ID_{M,B}}\right)^{n_b} + \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b} + \left(\frac{D_B}{ID_{M,B}}\right)^{n_b}\left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b}$$

$$f_3 = \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b} + \left(\frac{D_B}{ID_{M,B}}\right)^{n_b}\left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a} + \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b} + \left(\frac{D_B}{ID_{M,B}}\right)^{n_b}\left(\frac{\alpha_2 D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{\alpha_1 D_B}{ID_{M,B}}\right)^{n_b}$$

As a note of interest, when the interaction parameters are set to 1, this expression simplifies considerably to:

$$E = \frac{E_0 + E_1\left(\frac{D_A}{ID_{M,A}}\right)^{n_a} + E_2\left(\frac{D_B}{ID_{M,B}}\right)^{n_b} + E_3\left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{D_B}{ID_{M,B}}\right)^{n_b}}{1 + \left(\frac{D_A}{ID_{M,A}}\right)^{n_a} + \left(\frac{D_B}{ID_{M,B}}\right)^{n_b} + \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}\left(\frac{D_B}{ID_{M,B}}\right)^{n_b}}$$

and when the E_i are correctly chosen, this expression is equivalent to the Bliss independent combination of Hill model dose–response curves, so MuSyC is indeed an extension of Bliss independence.

Armed with these expressions, fitting MuSyC was simply a matter of fitting the (10-parameter) model to a given set of combined action data. This was done using the R function ‘optim’.

Clustering analysis of interaction metrics

To evaluate the informativeness of the estimated metrics, we chose to compare the organization of the tested compounds according to each metric (represented by a hierarchical clustering) with a known classification of that compound's mechanism of action. To achieve these clusterings, we first needed to determine the similarity between two compounds according to a given metric. The most sensible approach to this seemed to be to take that metric (e.g. URSA alpha), estimate it for a given compound in all available contexts (i.e. partner drugs and cell lines), and then do the same for a second compound. The similarity between those two compounds is then the similarity between the values of the interaction metrics across all contexts in which both compounds were evaluated.

Because the interaction metrics in question measure deviations in either direction from non-interaction, we chose to evaluate the similarity between two 'interaction profiles' using the cosine similarity metric, which captures the degree to which the signs and magnitudes of two vectors elements vary in the same way. However, in order to apply the cosine similarity, the metrics needed to be mapped into a space in which deviations lie in an approximately symmetric fashion on either side of 0. For the volumetric deviations and the MuSyC parameter beta, no transformation was necessary; however, for the combination index and the response surface parameters, transformations were needed to coerce values into the correct space. The six metrics were transformed as follows:

$$\lambda_{CI} = \ln CI$$

$$\lambda_{UA} = \ln \frac{\sqrt{\alpha_{URSA} + 1} + 1}{2}$$

$$\lambda_{BK} = \ln \frac{\kappa + 2}{2}$$

$$\lambda_{MA1} = \ln \alpha_1$$

$$\lambda_{MA2} = \ln \alpha_2$$

These transformations might appear rather arbitrary, but they all occupy a roughly equivalent space:

- λ_{CI} is the natural logarithm of the ratio between the EC_{50} of an equal ratio combination of two compounds with identical dose–response parameters and a given CI value and the expected EC_{50} of an additive combination of the same compounds.
- λ_{UA} is the natural logarithm of the ratio between the expected EC_{50} of an equal ratio combination of an additive combination of two compounds with identical dose–response parameters and the EC_{50} of the same combination with the URSA interaction parameter α_{URSA}.
- λ_{BK} is the natural logarithm of the ratio between the expected EC_{50} of an equal ratio combination of an additive combination of two compounds with identical dose–response parameters and the EC_{50} of the same combination with the BRAID interaction parameter kappa.

- λ_{MA1} is the natural logarithm of the ratio between the EC_{50} of drug B alone and the EC_{50} of drug B in the presence of high levels of drug A in a MuSyC combination with parameter α_1.
- λ_{MA2} is the natural logarithm of the ratio between the EC_{50} of drug A alone and the EC_{50} of drug A in the presence of high levels of drug B in a MuSyC combination with parameter α_2.

In these transformed spaces, values are roughly symmetrically distributed around 0, and positive and negative deviations of equal magnitude have similar (if opposing) effects on the given models.

Two additional values were evaluated using clustering: potency (the ratio of the highest concentration tested for a given compound and the EC_{50} of that compound in each cell-line) and the BRAID IAE (a measure of combined potency). As neither of these metrics represent a deviation from a given reference model, similarity between sets of potencies or sets of IAEs was measured using simple correlation.

For the estimation of similarity and clustering, six of the 38 compounds tested in the OPPS were excluded: veliparib (ABT-888), cyclophosphamide, dexamethasone, metformin, MK-4541, and temozolomide. These compounds showed broadly low efficacy across the dataset, and because interaction values are highly unstable when one or more of the compounds exhibit little to no activity, including these six compounds significantly degraded the informativeness of the interaction metrics.

With a suitably transformed value in hand for each of the 13 interaction metrics and two potency metrics, the cosine similarity between each pair of compounds interaction profile was estimated for all compound pairs. The resulting similarity matrices were then used to construct a hierarchical clustering of all 32 compounds. This clustering was then compared at each level of granularity with the known partition of compounds into mechanistic classes using the adjusted Rand index (ARI) (a zero-centered measure of agreement between two partitions). The mean ARI across all granularities was used as the measure of clustering accuracy for all metrics.

Extension of BRAID and URSA to three compounds

Both BRAID and URSA can be described by similarly structured implicit equations. In the case of URSA, this implicit equation is the definition of the model, as it cannot be algebraically solved for a closed from expression of the predicted effect. This equation is:

$$1 = \frac{D_A}{ID_{M,A}\left(\frac{E-E_0}{E_f-E}\right)^{1/n_a}} + \frac{D_B}{ID_{M,B}\left(\frac{E-E_0}{E_f-E}\right)^{1/n_b}} + \alpha \frac{D_A}{ID_{M,A}\left(\frac{E-E_0}{E_f-E}\right)^{1/2n_a}} \frac{D_B}{ID_{M,B}\left(\frac{E-E_0}{E_f-E}\right)^{1/2n_b}}$$

where D_A and D_B are the doses of drugs A and B, respectively, $ID_{M,A}$ and $ID_{M,B}$ are the doses of median effect (also known as the EC_{50}), n_a and n_b are the Hill slopes, E_0 and E_f are the effects observed in the presence of no drug and maximal levels of drug, respectively, and E is the

predicted effect of both doses together. Note that URSA does not allow for differing maximal effects.

In addition, although it is not usually expressed in this way, the definition of the BRAID response surface model is equivalent to the following implicit equation:

$$\left(\frac{E-E_0}{E_f-E}\right)^{1/n} = \left(\frac{E_A-E_0}{E_f-E_A}\right)^{1/n} + \left(\frac{E_B-E_0}{E_f-E_B}\right)^{1/n} + \kappa\left(\frac{E_A-E_0}{E_f-E_A}\right)^{1/2n}\left(\frac{E_B-E_0}{E_f-E_B}\right)^{1/2n}$$

where E_0 is the effect when no drug is present, E_f is the larger of the two maximal effects, E_A is the predicted effect of the dose of drug A alone, E_B is the predicted effect of the dose of drug B alone, E is the combined effect of both drugs, and n is the geometric mean of the two hill slopes. Both of these models introduce interaction to an otherwise 'additive' surface through an interaction term that is a kind of product of the first two terms, with an interaction parameter as a coefficient. Viewed this way, it is easy to see how one might extend these models to a third drug. In the case of URSA, a natural extension would be:

$$\begin{aligned}1 = \frac{D_A}{ID_{M,A}R^{1/n_a}} + \frac{D_B}{ID_{M,B}R^{1/n_b}} + \frac{D_C}{ID_{M,A}R^{1/n_c}} + \alpha_{AB}\frac{D_A}{ID_{M,A}R^{1/2n_a}}\frac{D_B}{ID_{M,B}R^{1/2n_b}} \\ + \alpha_{AC}\frac{D_A}{ID_{M,A}R^{1/2n_a}}\frac{D_C}{ID_{M,C}R^{1/2n_c}} + \alpha_{BC}\frac{D_B}{ID_{M,B}R^{1/2n_b}}\frac{D_C}{ID_{M,C}R^{1/2n_c}} \\ + \alpha_{ABC}\frac{D_A}{ID_{M,A}R^{1/3n_a}}\frac{D_B}{ID_{M,B}R^{1/3n_b}}\frac{D_C}{ID_{M,C}R^{1/3n_c}}\end{aligned}$$

where we have use R to represent the expression

$$\frac{E-E_0}{E_F-E}$$

to simplify the total equation.

Similarly, the BRAID implicit equation can be extended thus:

$$\begin{aligned}R^{1/n} = R_A^{1/n} + R_B^{1/n} + R_C^{1/n} + \kappa_{AB}R_A^{1/2n}R_B^{1/2n} + \kappa_{AC}R_A^{1/2n}R_C^{1/2n} + \kappa_{BC}R_B^{1/2n}R_C^{1/2n} \\ + \kappa_{ABC}R_A^{1/3n}R_B^{1/3n}R_C^{1/3n}\end{aligned}$$

where we have defined R_A, R_B, and R_C to represent the expressions:
$\frac{E_A-E_0}{E_f-E_A}$, $\frac{E_B-E_0}{E_f-E_B}$, and $\frac{E_C-E_0}{E_f-E_C}$, respectively.

It should be noted that in all of these cases, we are implicitly or explicitly solving for R (from which E, the predicted effect, can easily be calculated), so it is necessary to select our interaction parameter values such that R can be solved for all possible dose values. This is why, in the two-drug BRAID model, kappa (κ) cannot go below –2, and why for any negative value of α, there are dose pairs for which the URSA surface is ill-defined. When extending to the four parameter three-drug interaction, we have found it is best to constrain κ values to be greater than –1, as determining the precise combinations of κ values that are valid can be very difficult.

Generating non-traditional BRAID surfaces

The oppositional surface is generated by flipping the surface along the x-axis (in log-log space), which is achieved mathematically by taking the multiplicative inverse of the dose of drug B (relative to the dose of median effect). The dose of drug B always appears in the BRAID equation as a ratio of the dose of median effect raised to the power of the Hill slope, so this is equivalent to inverting the Hill slope wherever it appears. What was the maximal effect of drug B is now the effect when no drug is present; what was the effect when no drug is present is now the maximal effect of drug B. The role of the maximal effect of drug A (and maximal effect overall, as the creation of the oppositional surface assumes that it is drug B that produces a partial effect) is unchanged. The oppositional BRAID model can therefore be written:

$$E_{Opp}(D_A, D_B) = E_{f,B} + \frac{E_{f,A} - E_{f,B}}{1 + \left(\widetilde{D}_A^{1/\sqrt{n_a n_b}} + \widetilde{D}_B^{1/\sqrt{n_a n_b}} + \kappa \widetilde{D}_A^{1/2\sqrt{n_a n_b}} \widetilde{D}_B^{1/2\sqrt{n_a n_b}}\right)^{-\sqrt{n_a n_b}}}$$

$$\widetilde{D}_A = \left(\frac{D_A}{ID_{M,A}}\right)^{n_a}$$

$$\widetilde{D}_B = \frac{\left(\frac{E_0 - E_{f,B}}{E_{f,A} - E_{f,B}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{-n_b}}{1 + \left(1 - \frac{E_0 - E_{f,B}}{E_{f,A} - E_{f,B}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{-n_b}}$$

The protective BRAID surface involves very similar manipulations: inverting the Hill slope of drug A and rearranging the initial and maximal effects. This gives us:

$$E_{Pro}(D_A, D_B) = E_{f,A} + \frac{E_0 - E_{f,A}}{1 + \left(\widetilde{D}_A^{1/\sqrt{n_a n_b}} + \widetilde{D}_B^{1/\sqrt{n_a n_b}} + \kappa \widetilde{D}_A^{1/2\sqrt{n_a n_b}} \widetilde{D}_B^{1/2\sqrt{n_a n_b}}\right)^{-\sqrt{n_a n_b}}}$$

$$\widetilde{D}_A = \left(\frac{D_A}{ID_{M,A}}\right)^{-n_a}$$

$$\widetilde{D}_B = \frac{\left(\frac{E_{f,AB} - E_{f,A}}{E_0 - E_{f,A}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{n_b}}{1 + \left(1 - \frac{E_{f,AB} - E_{f,A}}{E_0 - E_{f,A}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{n_b}}$$

where $E_{f,AB}$ is the attenuated maximal effect that high doses of drug B induce for high levels of drug A.

Finally, inverting both Hill slopes and a third rearrangement of minimal and maximal effects produces the adjuvant BRAID surface:

$$E_{Adj}(D_A, D_B) = E_{f,AB} + \frac{E_0 - E_{f,AB}}{1 + \left(\widetilde{D}_A^{1/\sqrt{n_a n_b}} + \widetilde{D}_B^{1/\sqrt{n_a n_b}} + \kappa \widetilde{D}_A^{1/2\sqrt{n_a n_b}} \widetilde{D}_B^{1/2\sqrt{n_a n_b}}\right)^{-\sqrt{n_a n_b}}}$$

$$\widetilde{D}_A = \left(\frac{D_A}{ID_{M,A}}\right)^{-n_a}$$

$$\widetilde{D}_B = \frac{\left(\frac{E_{f,A} - E_{f,AB}}{E_0 - E_{f,AB}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{-n_b}}{1 + \left(1 - \frac{E_{f,A} - E_{f,AB}}{E_0 - E_{f,AB}}\right)\left(\frac{D_B}{ID_{M,B}}\right)^{-n_b}}$$

where $E_{f,AB}$ is the enhanced maximal effect produced by high doses of drug A combined with high doses of drug B.

Each of these transformations can be fit quite easily using existing BRAID code in the 'braidrm' package. For the oppositional surface, the concentrations of the second drug should be inverted (multiplicatively); for the protective surfaces, the concentrations of the first drug should be inverted; and for the adjuvant surface, both concentrations should be inverted. In addition, any defaults or constraints on the minimal and maximal effects must be appropriately rearranged to match the structure of the original BRAID equation. So, for the oppositional surface, the final four parameters will be ordered:

$$(\ldots, E_{f,B}, E_{f,AB}, E_0, E_{f,A})$$

For the protective surface, they are ordered:

$$(\ldots, E_{f,A}, E_0, E_{f,AB}, E_{f,B})$$

And for the adjuvant surface, they are ordered:

$$(\ldots, E_{f,AB}, E_{f,B}, E_{f,A}, E_0).$$