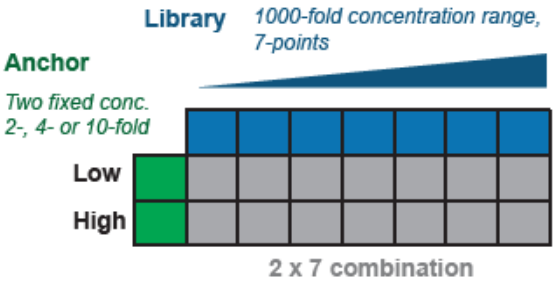
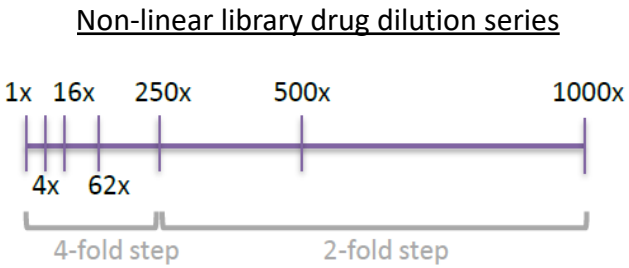
Documentation

Anchored design





To efficiently screen the combination space we used a 2x7 concentration matrix, referred to as an 'anchored' approach. Single-agent drug concentrations were optimised in a mini-screen (see "Screening").

Anchor drugs were screened at two fixed concentrations with a 2, 4, or 10-fold difference between them. **Library drugs** were screened at seven concentrations spanning a 1,000-fold range with a non-equidistant log2 design of two 4-fold steps followed by four 2-fold dilution steps starting at the lowest used concentration. The use of this design was based on the observation that the higher concentrations are most informative and would benefit from denser profiling.

Screening

Cell lines were acquired from commercial cell banks. To prevent cross-contamination or misidentification, all cell lines were profiled using a panel of 94 SNPs (Fluidigm, 96.96 Dynamic Array IFC). Short tandem repeat (STR) analysis was also performed, and cell line profiles were matched to those generated by the cell line repository. Further information on the cell lines used in this study, including their source and molecular profiling datasets can be found on cellmodelpassports.sanger.ac.uk.

Compounds were sourced from commercial vendors. Anchor and library concentrations were drug- and tissue-specific and determined from a small pilot screen, testing the drugs in 9-13 cell lines per tissue and selected to give moderate activity (60-90% viability) in the majority of models. Screening concentrations typically did not exceed 10 µM. On each plate, all single agents were screened at five and four technical replicates for anchors (each concentration) and libraries, respectively.

For **screening** in 1536-well plates, cell lines were transferred in 7.5 µL of their respective growth medium using XRD384 (FluidX) dispensers. The seeding density was optimised to ensure that each cell line was in the exponential growth phase at the end of the assay. The maximum density tested varied based on cell type, typically 5,000 cells/well for suspension cells and 1,250 cells/well for adherent cells. Assay plates were incubated for 24 hours then dosed with the test compounds using an Echo555 (Labcyte). After 72 hours of drug treatment cell viability was measured by adding 2.5 µL of CellTiter-Glo 2.0 (Promega) and quantification of luminescence.

Each 1536-well plate contained several wells with negative and positive controls to control quality of the screening data.

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Quality control

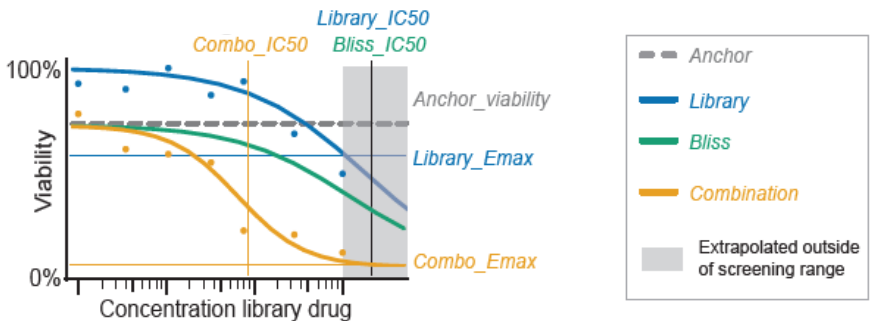
All screening plates contained negative control wells (untreated wells, n=6; DMSO-treated wells, n=126) and positive control wells (blanks, i.e. medium-only wells, n=28; Staurosporin treated wells, n=20; and MG-132 treated wells, n=20) distributed across the plate. Defined quality control criteria were applied to each plate.

Coefficient of variation (CV) of the DMSO treated negative controls
CV = σN/μN, with σN the standard deviation of the negative control and μN the mean of the negative control
Threshold: CV ≤ 0.18

Z-factor
Z-factor = 1 - 3(σP + σN) / (|μP - μN|)*, with σN and σP the standard deviation of the negative and positive controls, and μN and μP the mean of the negative and positive controls, respectively
Threshold: Z-factor (per plate) ≥ 0.3 and Z-factor (mean within screen) ≥ 0.4
The Z-factors were calculated for all plates that indicate sensitivity of the cell lines to the positive control (ratio of NC1:PC ≥ 4). In case a cell line is insensitive to both positive control drugs, the Z-factors were calculated based on blank wells instead. Where a cell line was sensitive to both positive controls, it had to pass Z-factor thresholds for both positive controls.

Curve fitting

For each plate, the raw fluorescent intensity values were normalised to a relative viability scale (ranging from 0 to 1) using the blank (B) and negative control (NC) values (viability = (Treated cells - B)/(NC - B)). Anchor viability was determined from the mean across the five replicate wells screened on each plate. All library drug dose-responses were fitted as a 2-parameter sigmoid function [Vis et al., Pharmacogenomics, 2016]. The dose-response curves for the combinations were fitted similarly, but with two notable differences: 1) the cell line parameters were obtained from the library drug fits; 2) the maximum viability was capped at the anchor viability (rather than from 0 to 1). We use the 50% (inflection) point of the sigmoidal curve between zero and the anchor viability for both the expected Bliss and the observed combination. We extended the model to nest each replicate within the drug/cell line to obtain stable estimates from replicate experiments. To assess the quality of the fits, we computed the root mean square error (RMSE) and excluded curves with RMSE > 0.2. The Emax and the IC50 are based on the fitted curves. Emax is reported at the highest tested concentration for the drug.



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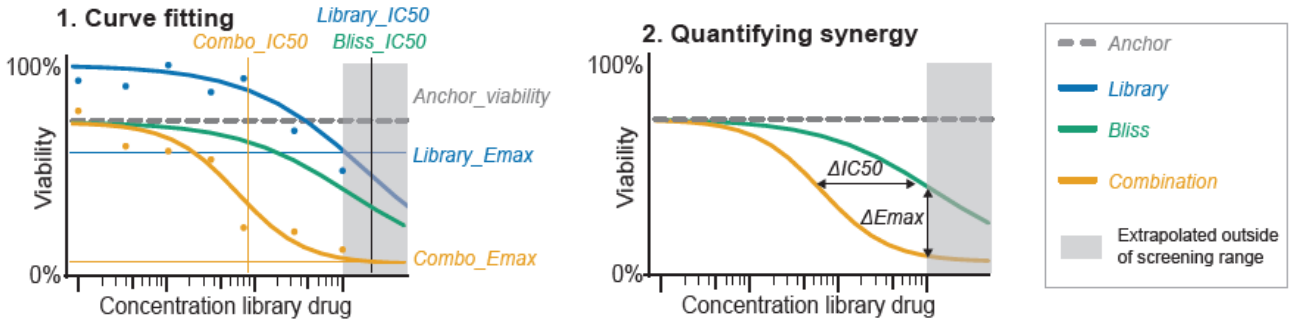
Classifying synergy

To detect synergy we compared observed combination responses to expected combination responses. For the latter, we used Bliss independence[1] of the response to the anchor and the library drug alone. Conceptually, every point on the Bliss dose response curve is defined as the product between the anchor viability and the corresponding point on the library dose response curve. Shifts in potency (ΔIC50) and in efficacy (ΔEmax) were calculated as the difference between the observed combination response and expected Bliss

ΔIC50 = Bliss_IC50 - combo_IC50 (reported on a log2 scale)

ΔEmax = Bliss_Emax - combo_Emax

A given measurement was synergistic if the combo_IC50 was less than twice the highest screened library concentration and either the ΔIC50 or the ΔEmax was above a specific threshold: ΔIC50 ≥ 3 (2^3= 8-fold shift in IC50) or the ΔEmax ≥ 0.2 (20% shift in viability). Replicate measurements of 'anchor-library-cell line' tuples were summarised as synergistic if half or more of the replicate measurements showed synergy. To summarise both anchor concentrations, we considered a 'combination-cell line' pair as synergistic if synergy was observed at either anchor concentration.



References

1. Bliss, C. I. The toxicity of poisons applied jointly 1. *Ann. Appl. Biol.* **26**, 585–615 (1939).