Supplementary Note 1: Simulated Synergy and Selectivity

Simulated combination screens with Gaussian noise

To describe how selectivity bias depends on single agent activities, synergistic interactions and analysis parameters, we simulated sets of combinations in two assays (Fig. S1). We examined synthetic screens with only noise (Fig. S2), as well as different levels of single agent and combination activity, and various choices of $\boldsymbol{S}_{cut}$ or replicate data handling (Fig. S3).

Two sets of 1,200 6x6 dose matrix data were generated for simulated combination screens between lists of agents with differing aspect ratios (#drugs in one list to the other). Active agents (with a defined probability) had a maximum inhibition level randomly assigned with a uniform distribution in a defined range and a sigmoidal response curve from no effect to the maximum level, transitioning between the third and fifth of six dosing points in a twofold dilution series. With a defined linkage probability, the activity translated unchanged to the other assay or was replaced by a freshly randomized activity. Each combination was generated in both assays as a 6x6 dose matrix, with synergy at a defined rate using no interaction, antagonistic Bliss canceling[1], moderate Bliss multiplicative[2], or strong Greco synergy[3] with interaction index $\alpha = 1$, and translation to the other assay at a synergy linkage rate. After constructing each response surface, random noise was overlaid with a Gaussian distribution at a defined level. For a simulated screen, all the resulting ***S*** and ***SI*** values were analyzed as above, using $\boldsymbol{Z}_{cut} = \max(\boldsymbol{Z}_{test})/2$ and $\boldsymbol{S}_{cut}$ chosen to extract a defined fraction of top synergies. The resulting SI distributions were compared to determine ***B*** as above.

We generated 108 simulated screens exploring how strongly ***B*** depends on the simulation parameters (Suppl. Data 1; Fig. S3). Each parameter was varied over 5 settings around middling values (usually 0.5) for each, for both the Bliss multiplicative and Greco synergy combination models. We also generated some screens having only noise without signal. We examined ***B***'s sensitivity curve to each parameter, and performed a multiple regression analysis to determine the relative contributions of each to the selectivity bias.

These simulations confirmed our expectation that ***B*** does not depend strongly on stochastic noise, but does depend on screen design parameters, agent activity, and the nature of synergistic interactions. For simulations with only Gaussian noise, the combinations do tend to have a higher average ***SI*** than the single agents (Suppl. Data 1), most likely owing to our choosing the most selective of a greater number of tries available in the combination space (4-6 fixed ratio slices compared to only two single agent curves). However, comparing the synergies to the unfiltered set of combinations removes this asymmetry, and there were no significant differences in average ***SI*** between the synergies and unfiltered combinations even at high noise levels. Comparing ***B*** measurements across interaction models[1,3], HSA shows no bias, Bliss models yield moderate levels, and the strongest ***B***s are for the Greco model, owing to the ***SI*** calculation's focus on potency shifts. The selectivity bias is sensitive to the parameters that describe agent activity, synergistic interactions, and assay linkage. Multiple regression (Fig. S4) shows that the synergy type and the agent activity linkage most strongly contribute to a prediction model for selectivity bias.

References

1. Lehár, J. et al. Chemical combination effects predict connectivity in biological systems. *Mol Syst Biol* **3**, 80 (2007).
2. Bliss, C.I. The toxicity of poisons applied jointly. *Ann. Appl. Biol.* **26**, 585-615 (1939).
3. Zimmermann, G.R., Lehár, J. & Keith, C.T. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today* **12**, 34-42 (2007).

Approach

- Two simulated assays (Test, Ctrl)
- ~50 agent drug lists (variable screen aspect ratios (#Ydrug/#Xdrug)
- Random agent activities per assay
- Random combination synergy per assay
- Variable synergy & activity linkage rates
- Simulate combinations as 6x6 matrices with 2x dilution factors.
- Gaussian noise overlaid to all matrices
- Explored different $\boldsymbol{S}_{cut}$ for $\boldsymbol{B}$ calculation

Parameters

Noise = std. dev. of Gaussian noise
Rdrug = aspect ratio for screened drug lists
Alev = mean inhibition level for active agents
Arang = range of inhibition levels for actives
Arate = rate of activity for single agents
Alink = translation rate from Test to Ctrl
Stype = synergy type between active agents
Srate = synergy rate between active agents
Slink = synergy translation rate
Sfrac = fraction of combinations with $\boldsymbol{S}>\boldsymbol{S}_{cut}$
Replic = Whether replicates are split or copy

Procedure for each simulated screen

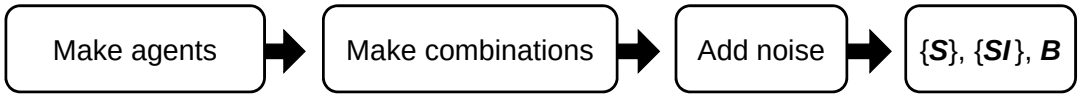

Figure S1. Simulation approach. Duplicate dose matrix screens with 50 agents were generated with differing levels of agent activity and synergy, and defined levels of linkage between two simulated assays, and with Gaussian noise overlaid at varying levels. Synergy ***S*** and selectivity ***SI*** were calculated as in Fig. 1, using $\boldsymbol{Z}_{cut} = \max(\boldsymbol{Z}_{test})/2$, and the selectivity bias ***B*** was measured for the top 5% of synergies.

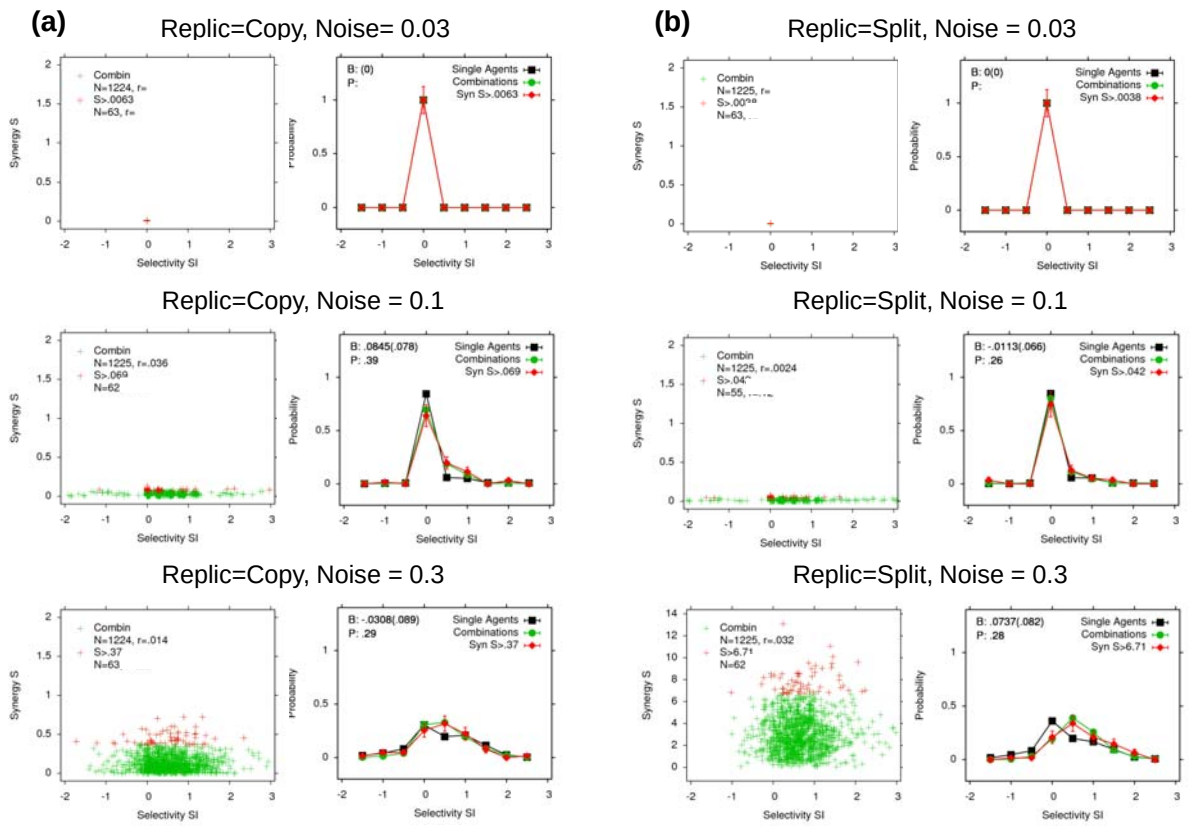

Figure S2. Selectivity bias in simulated screens with only noise. Each pair of charts show the ***S*** vs. ***SI*** correlations and the ***SI*** distributions compared to those for the more selective single agent in each combination, either for independent replicates (a) or split matrices (b). As the noise level increases, first the selectivity and then the synergy scores get spread out. Meeting expectations, no cases show significant ***B***. Split matrix results are indistinguishable from those for true replicates except that they have larger deviations in both ***SI*** and ***B***, as expected given the reduced data sampling resulting from the split. This shows that using separated replicates avoids spurious ***B*** measurements arising from stochastic noise in the data.

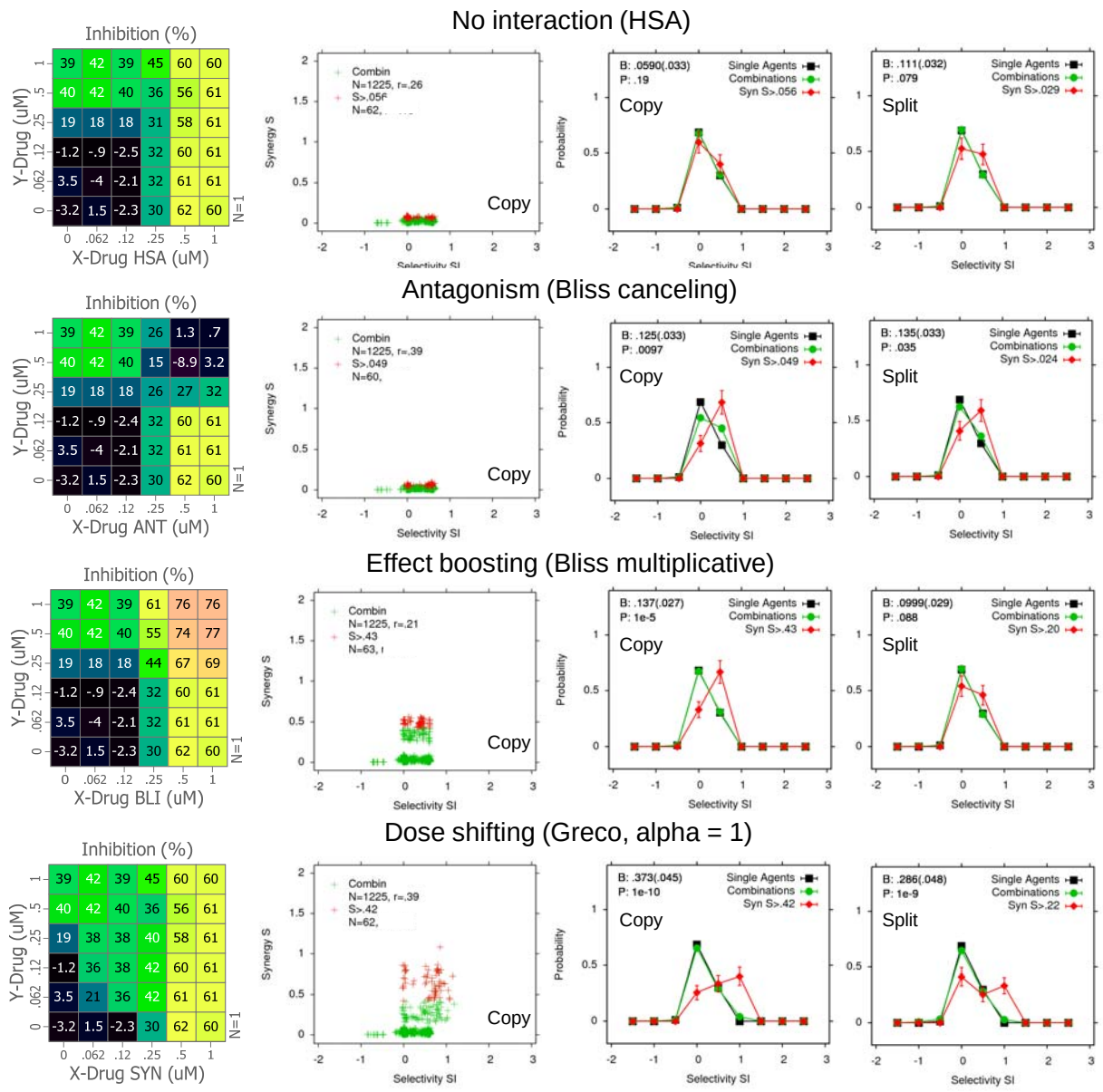

Figure S3. Selectivity bias for different interaction models[1,3] with a low noise level (0.03), unit aspect ratio, 5% hit rate for Syn, and middling values (0.5) for all other parameters. In each case we show the modeled synergy (left), the ***S*** vs. ***SI*** correlation (center) and the ***SI*** distribution (right) for the top 5% synergies compared to all combinations, reporting ***B*** ($2\sigma_B$). The rightmost panels show the distributions for split replicates. The HSA model shows no selectivity bias, while the Bliss models yield moderate levels of ***B***. Screens with antagonisms generate weak selectivity with no synergy, and multiplicative synergies also yield only weak bias levels since they do not produce strong dose shifting. The strongest selectivity occurs for the Greco model, owing to our ***SI*** calculation's focus on potency shifts. These simulations show that even with introduced signals, selectivity shifts only occur when there are real interactions (synergies or antagonisms). Results for split matrices are similar to those for copies.

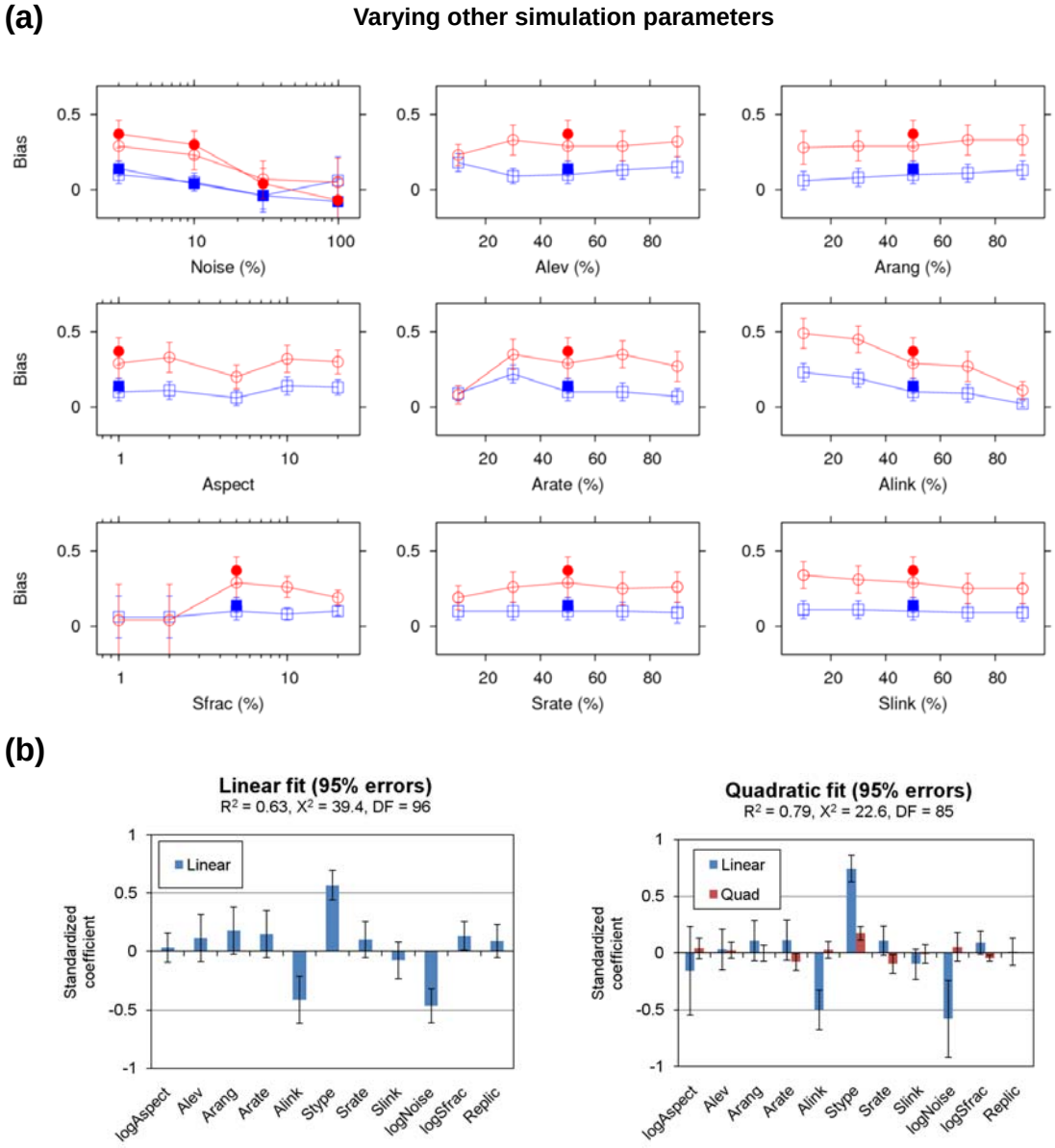

Figure S4. Effect of simulation parameters on selectivity bias. (a) Sensitivity analyses show how ***B*** responds to varying each of the parameters, for the effect boosting Bliss multiplicative and dose shifting Greco synergy model. The Greco model produces stronger responses to each parameter. (b) Multiple regression analysis showing the standardized coefficients for linear and quadratic dependencies with 95% confidence ranges illustrates which simulation parameters have the greatest influence. Both a linear and a quadratic regression model are shown, higher orders yielding insignificant changes to chi-squared $\boldsymbol{X}^2$. As expected, the synergy type, activity linkage and noise level predominate. These simulations show that when there are real interactions, the level of selectivity depends on how closely linked the drug activities are between the assays, or how context-dependent they are. Also, increasing noise reduces ***B***, making stochastic artifacts an unlikely explanation for experimental selectivity biases.