Electronic Supplementary Material (ESI) for Lab on a Chip.

On-chip Phenotypic Investigation of Combinatory Antibiotic Effects by Generating Orthogonal Concentration Gradients

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Supplementary Note 1

One of the most crucial phenomena in our experiments is diffusion. We wish to obtain the diffusion coefficients of the antibiotics within the agarose gel medium in order to be able to accurately simulate their behavior. To obtain the diffusion coefficient of antibiotics within the 1.5% (w/v) agarose gel, we had to decide which model to use to best describe the behavior of the relevant molecules.

For our experiment, we first considered using the Ogston model, which assumes that the molecules are rigid: this model describes the diffusion of spherical molecules rather than long, snake-like particles.[1, 2] The model holds strongly in cases involving viscous flow over a long period of time. The limitation of the Ogston model and its equation $D/D_w = \exp(-\emptyset^{1/2} R_h/R_f)$ is that it ignores the hydrodynamic interactions between matrix fibers of the host fluid and the diffusing substance.

Therefore, we used a more rigorous version of the equation as suggested by Phillips et al.: $D/D_w = 1/\left\{1 + \left({R_h}^2/\kappa\right)^{1/2} + ({R_h}^2/\kappa)/3\right\}$, where D is the effective diffusion coefficient in the agarose gel, D_w is the diffusion coefficient in free solution, R_h the hydrodynamic radius of diffusing particles, and κ the Darcy permeability to water flow.[3] For the permeability, we used an equation relating it to the volume fraction $\emptyset$: $\kappa = (-3/20)({R_h}^2/\emptyset)(\ln\emptyset + 0.931)$.[4]

We calculated the diffusion coefficient of antibiotics using measurements of diffusion coefficient from a previous study and some assumptions. According to the study, the diffusion coefficient of humic substance SRFA (MW = 860 g/mol, R_h = 1.0 nm) was determined to be 2.5 × 10^{-10} m^2/s in 1.5% agarose gel, as measured by fluorescence correlation spectroscopy (at room temperature, pH 6.7).[5] The assumption for simplicity is that the antibiotic particle has spherical shape and the hydrodynamic radius of the particle is proportional to the cubic root of the molecular mass. As a result, we could estimate the diffusion coefficient of antibiotics in 1.5% agarose gel at room temperature (Supplementary Table 1).

Reference:

1. A. G. Ogston, B. Preston and J. Wells, *Proc. R. Soc. Lond. A*, 1973, **333**, 297-316.
2. A. Pluen, P. A. Netti, R. K. Jain and D. A. Berk, *Biophysical journal*, 1999, **77**, 542-552.
3. R. J. Phillips, W. M. Deen and J. F. Brady, *Journal of colloid and interface science*, 1990, **139**, 363-373.
4. E. M. Johnson and W. M. Deen, *AIChE Journal*, 1996, **42**, 1220-1224.
5. J. R. Lead, K. Starchev and K. J. Wilkinson, *Environmental Science & Technology*, 2003, **37**, 482-487.

Supplementary Table 1. The parameters for CFD simulation

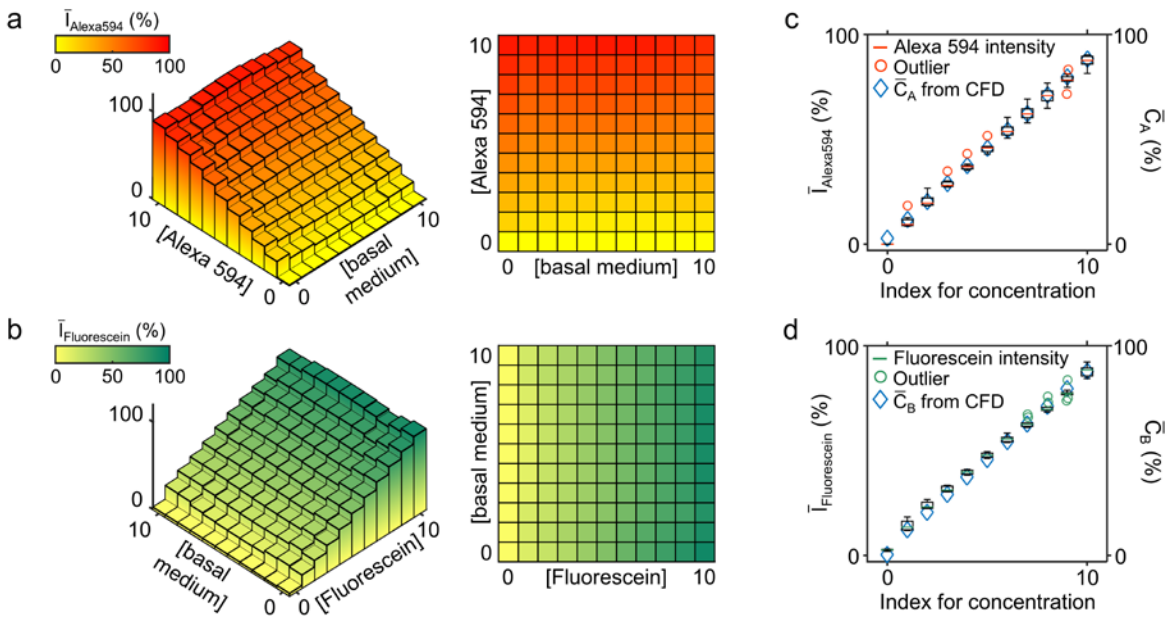
Parameter	Value
Width of the medium channel	0.6 mm
Width of the gel channel	0.8 mm
Flow rate per channel	2 μL/min
Diffusion coefficient of Fluorescein (MW = 332.3 g/mol)	0.008692 cm^2/h
Diffusion coefficient of Alexa 594 (MW = 819.9 g/mol)	0.008266 cm^2/h
Diffusion coefficient of MER (MW = 383.5 g/mol)	0.008646 cm^2/h
Diffusion coefficient of GEN (MW = 477.6 g/mol)	0.008562 cm^2/h
Diffusion coefficient of TET (MW = 444.4 g/mol)	0.008592 cm^2/h
Diffusion coefficient of LEV (MW = 361.4 g/mol)	0.008666 cm^2/h
Diffusion coefficient of COL (MW = 820.0 g/mol)	0.008266 cm^2/h
Water density	$1x10^3$ kg/m^3
Water viscosity	$1x10^{-3}$ kg/(m·s)

* Diffusion coefficients of molecules are considered based on 1.5% agarose gel.

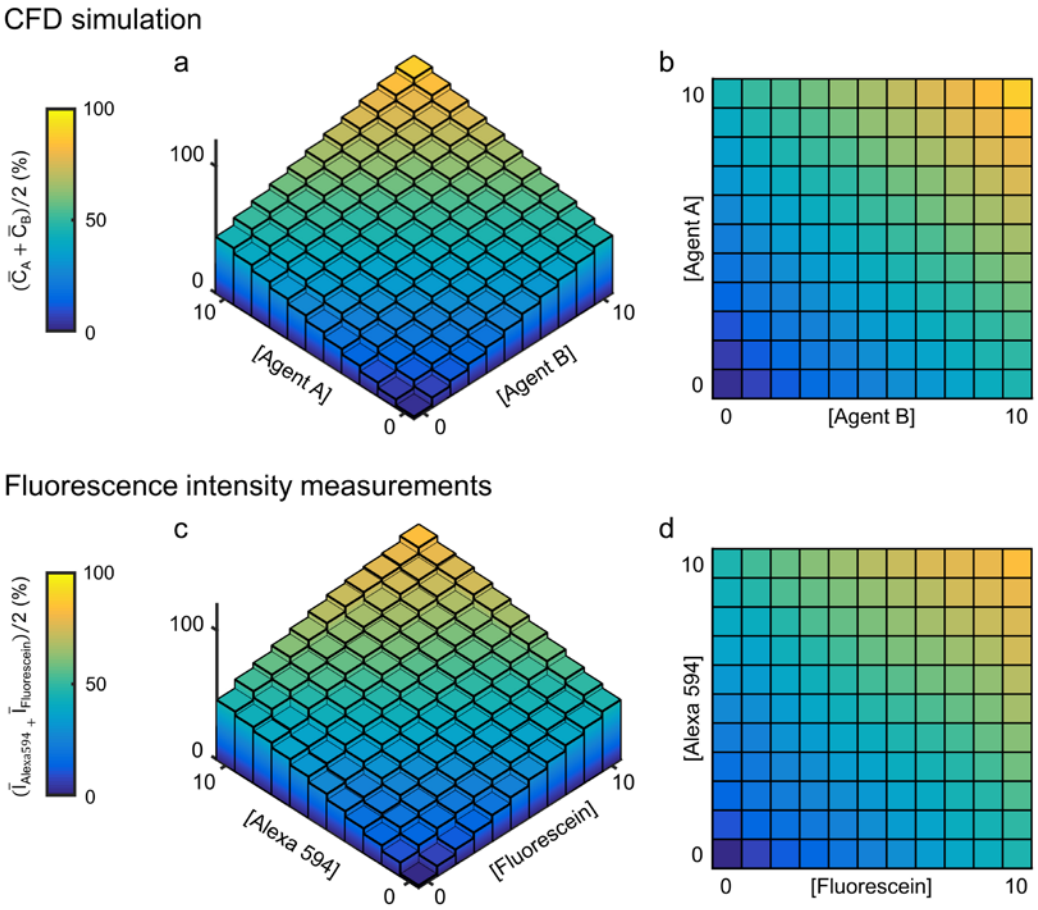
Supplementary Table 2. Index and corresponding agent concentrations (from CFD simulation)

Index for concentrations	Concentrations of an agent in horizontal gradient (%)	Concentrations of an agent in vertical gradient (%)
10	87.9 (83.7-92.1)	88.25 (84.0-92.5)
9	79.5 (75.3-83.7)	79.75 (75.5-84.0)
8	71.1 (66.0-75.3)	71.25 (67.0-75.5)
7	62.7 (58.5-66.9)	62.25 (58.5-67.0)
6	54.3 (50.1-58.5)	54.30 (50.1-58.5)
5	45.85 (41.6-50.1)	45.85 (41.6-50.1)
4	37.4 (33.2-41.6)	37.35 (33.1-41.6)
3	29.0 (24.8-33.2)	28.85 (24.6-33.1)
2	20.6 (16.4-24.8)	20.35 (16.1-24.6)
1	12.2 (8.0-16.4)	11.85 (7.6-16.1)
0	0.3*	2.83*

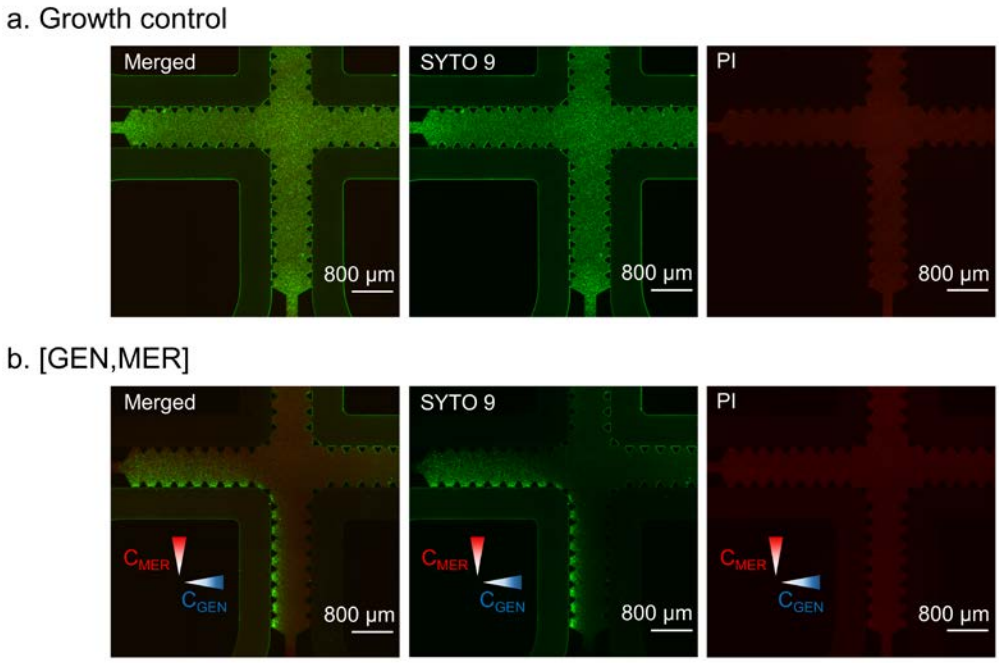
*The maximum values measured at the middle of gel region (Portion 2 and Portion 3, respectively).



Supplementary Figure 1. Comparison of fluorescence intensity measurements and CFD simulation. **a,b,** Normalized intensities of Fluorescence probes Alexa 594 (**a**) and Fluorescein (**b**) are expressed in matrices. The basal xy-plane indicates pairwise concentrations of the probe and basal medium. **c,d,** Box plots show good agreements between CFD simulations and fluorescence experiments in both vertical gradient (**c**) and horizontal gradient (**d**).



Supplementary Figure 2. Comparison between the results of CFD simulations and Fluorescence experiments. **a,b,** The matrices show the averaged concentrations of two agents (**a**) or the averaged intensity of two probes (**b**) over a grid of concentration combinations. The root mean squared error between the matrices was 2.08%.



Supplementary Figure 3. Live/Dead staining with the fluorophores SYTO 9 (Green) and propidium iodide (PI, red) for the cases of growth control (**a**) and [GEN,MER] (**b**). The fluorophores were supplemented to all media at 6 h of testing, and the images were obtained using a fluorescence microscope at 7 h of testing.

Supplementary Table 3. Synergy scores for on-chip antibiotic interaction with itself

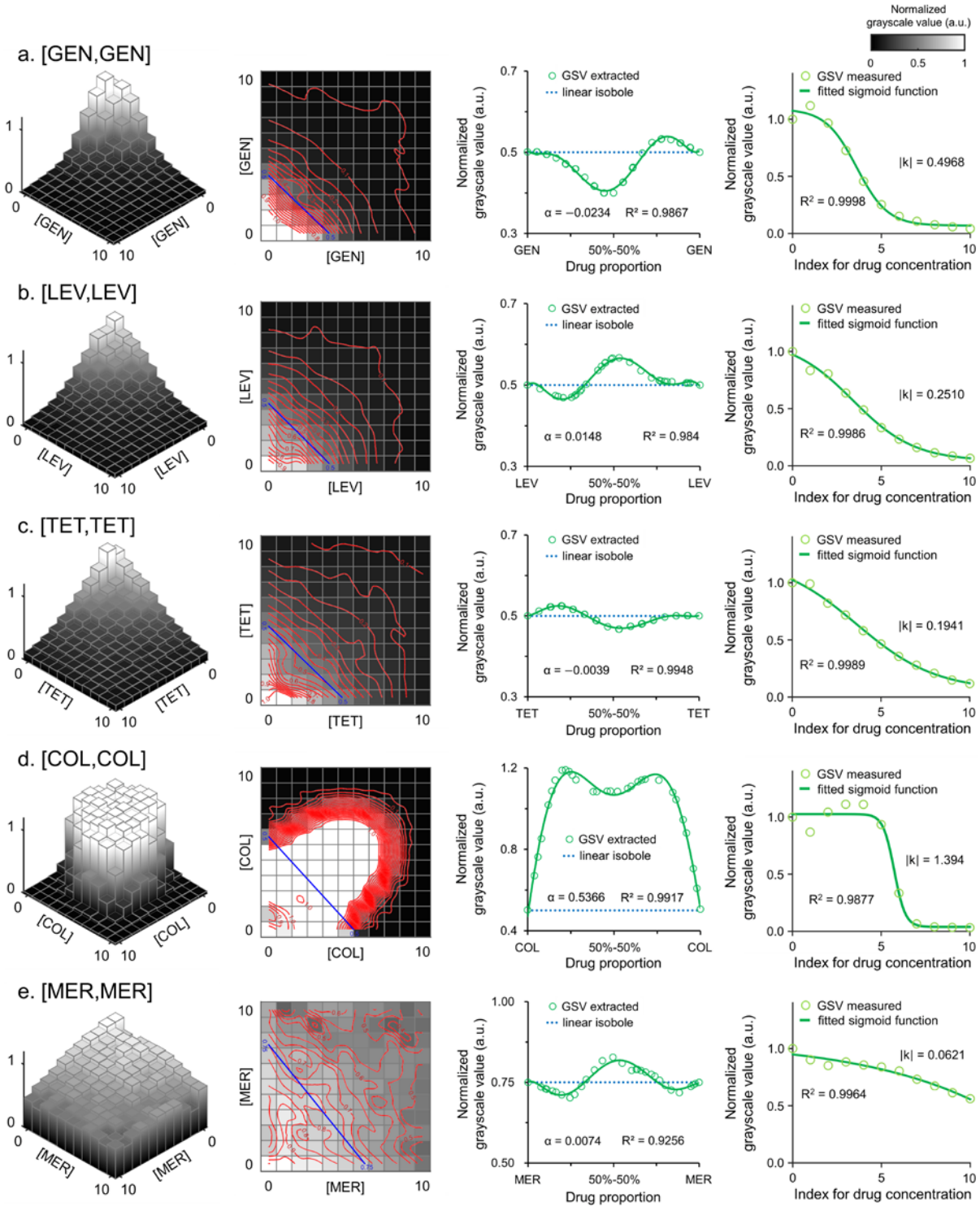
Antibiotic B in horizontal gradient	Antibiotic A in vertical gradient	Synergy score α (from Loewe Additivity)	Synergy score β (from Bliss Independence)
GEN	GEN	-0.0234	0.5831
LEV	LEV	0.0148	0.9344
TET	TET	-0.0039	0.8169
MER	MER	0.0074	1.0172
COL	COL	0.5366	1.1422
	Mean*	-0.0013	0.8988
	S.D.*	0.0144	0.1902

* The results of COL are excluded from the statistics of α.

Supplementary Table 4. Parameters from the dose-response profiles for on-chip antibiotic interaction with itself

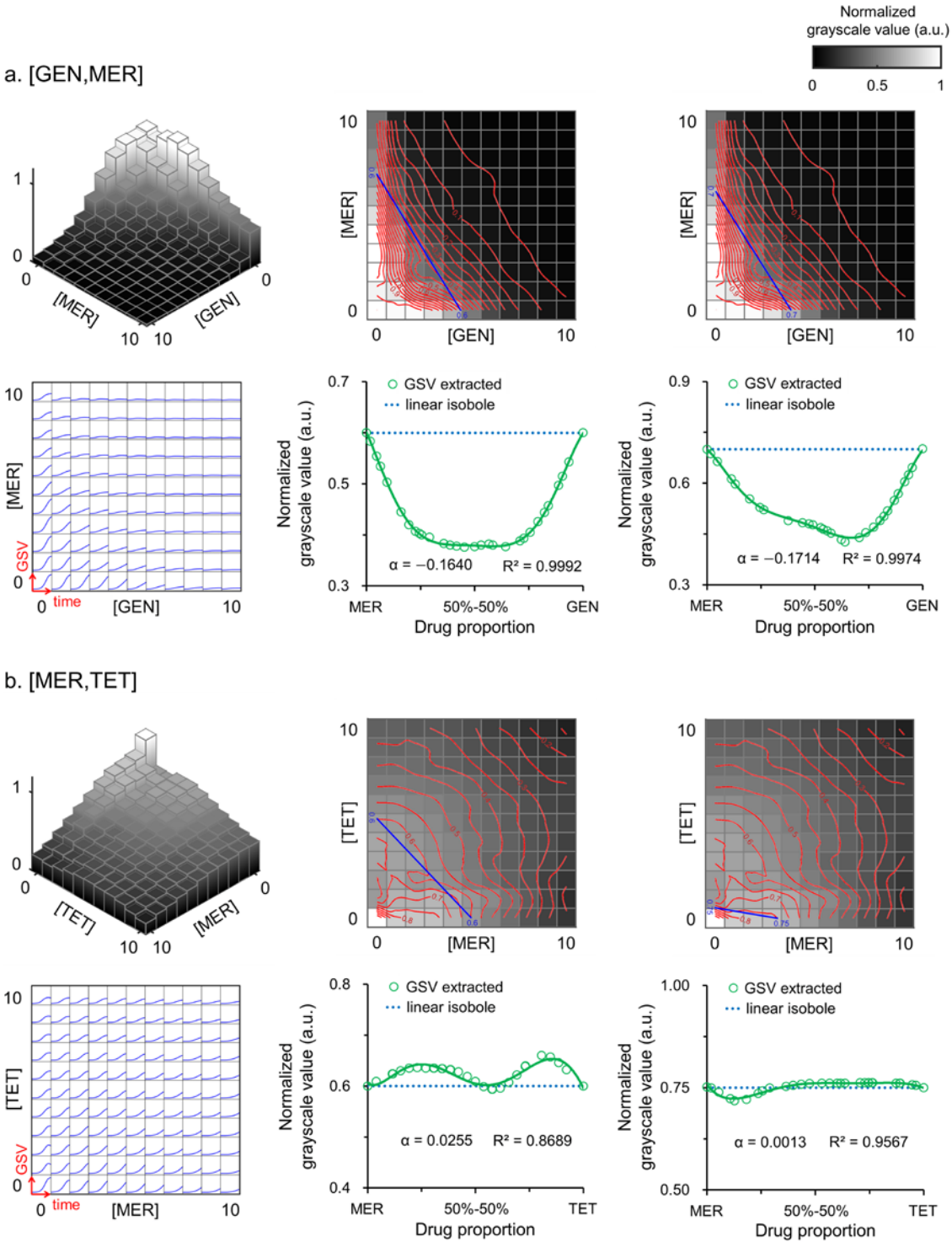
Antibiotic B in horizontal gradient	Antibiotic A in vertical gradient	IC50 (μg/mL)	Steepness |k|
GEN	GEN	0.4	0.4968
LEV	LEV	0.7	0.2510
TET	TET	5.2	0.1941
MER	MER	N.A.*	0.0621
COL	COL	1.6	1.3940

* The $\overline{GSV}$ recorded was 0.56 at the highest MER concentration of 0.88 μg/mL.



Supplementary Figure 4. On-chip testing of antibiotic interaction with itself. **a-e,** Checkerboards, combinatory response profiles, and dose-response profiles exhibit non-interacting behaviors for GEN (**a**), LEV (**b**), TET (**c**), and MER (**e**), but self-interacting behavior for COL (**d**).

Supplementary Figure 5. On-chip combinatory antibiotic testing. **a-j** Checkerboards, systematic time-killing graphs, and combinatory response profiles exhibit various patterns of pharmacological interactions between the antibiotics pairwise: [GEN,MER] (**a**), [MER,TET] (**b**), [MER,LEV] (**c**), [LEV,GEN] (**d**), [TET,LEV] (**e**), [TET,GEN] (**f**), [GEN,COL] (**g**), [TET,COL] (**h**), [LEV,COL] (**i**), and [MER,COL] (**j**).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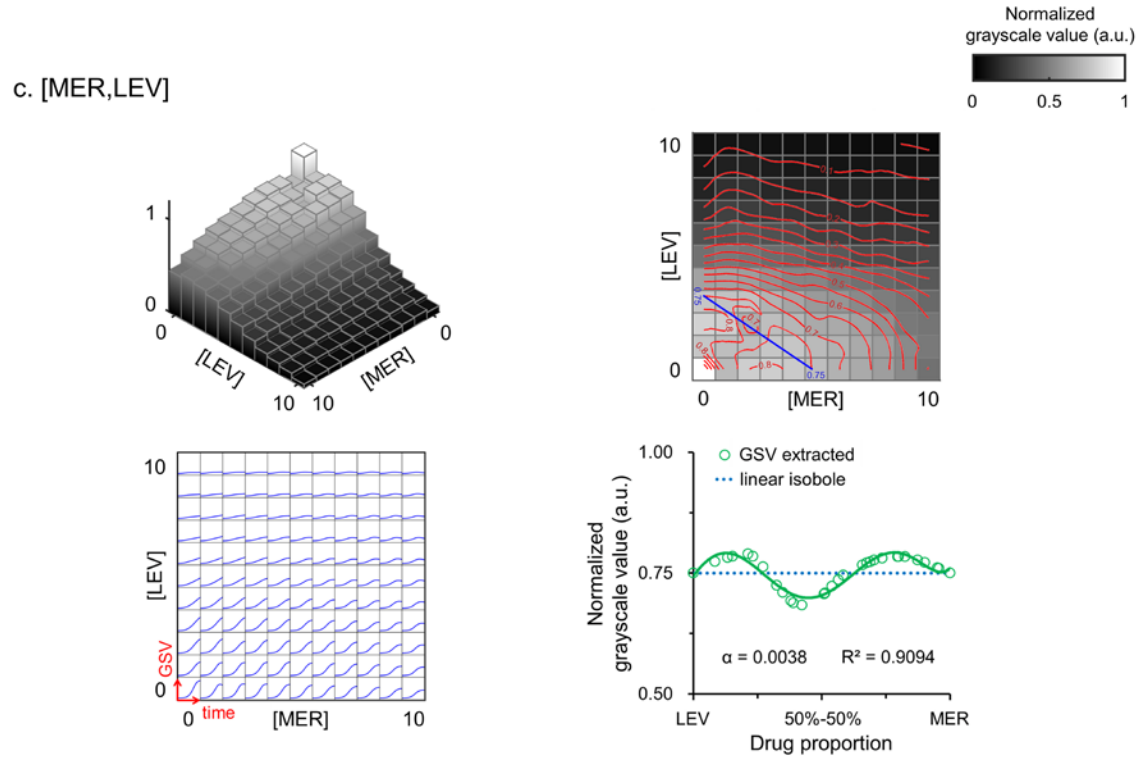
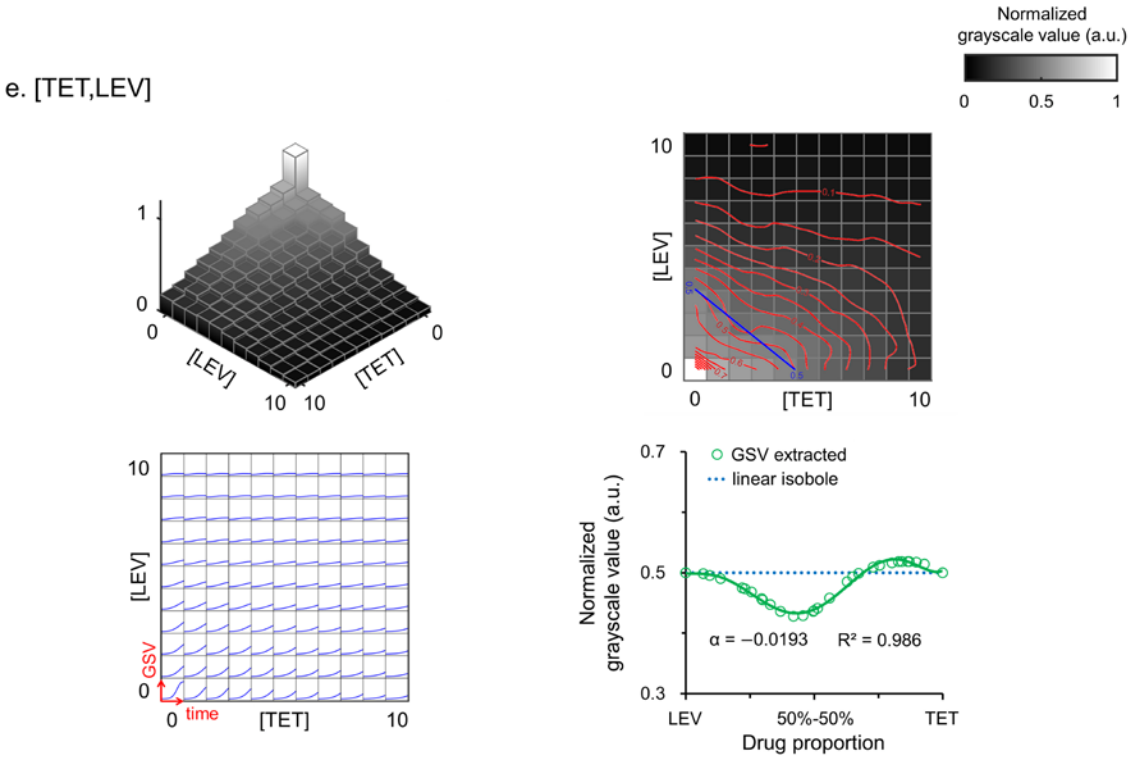
0
0.5
1
a. [GEN,MER]
[MER]
[GEN]
[TET]
GSV
time
Normalized grayscale value (a.u.)
GSV extracted
linear isobole
α = −0.1640
R² = 0.9992
α = −0.1714
R² = 0.9974
MER
50%-50%
GEN
Drug proportion
b. [MER,TET]
α = 0.0255
R² = 0.8689
α = 0.0013
R² = 0.9567
TET


Normalized
grayscale value (a.u.)
0
0.5
1
c. [MER,LEV]
1
0
0
[LEV]
10
10
[MER]
0
10
[LEV]
0
[MER]
10
10
[LEV]
GSV
0
time
0
[MER]
10
1.00
0.75
0.50
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = 0.0038
R² = 0.9094
LEV
50%-50%
MER
Drug proportion
d. [LEV,GEN]
1
0
0
[GEN]
10
10
[LEV]
0
10
[GEN]
0
[LEV]
10
10
[GEN]
GSV
0
time
0
[LEV]
10
0.7
0.5
0.3
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = −0.0132
R² = 0.9993
GEN
50%-50%
LEV
Drug proportion


Normalized
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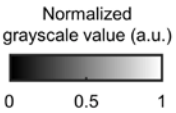
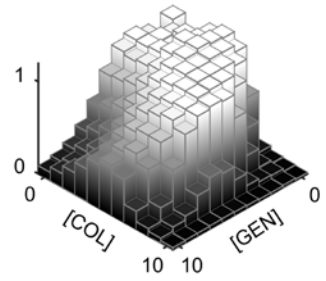
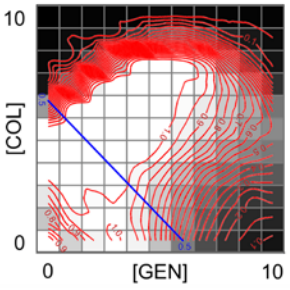
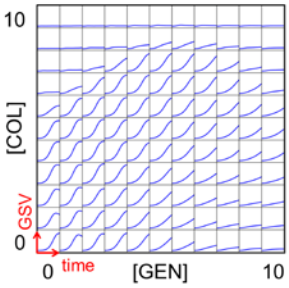
e. [TET,LEV]
1
0
0
[LEV]
10
10
[TET]
0
10
[LEV]
0
[TET]
10
10
[LEV]
GSV
0
time
0
[TET]
10
0.7
0.5
0.3
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = −0.0193
R² = 0.986
LEV
50%-50%
TET
Drug proportion
f. [TET,GEN]
1
0
0
[GEN]
10
10
[TET]
0
10
[GEN]
0
[TET]
10
10
[GEN]
GSV
0
time
0
[TET]
10
0.9
0.6
0.3
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = 0.1360
R² = 0.9926
GEN
50%-50%
TET
Drug proportion

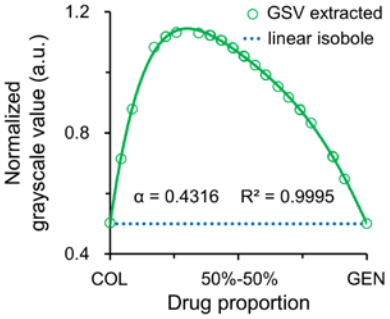
g. [GEN,COL]



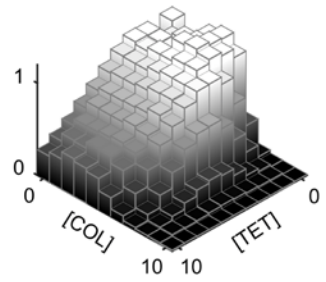


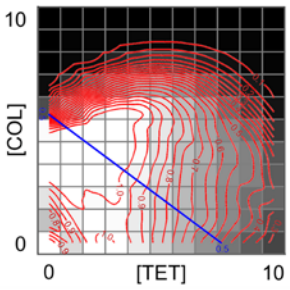


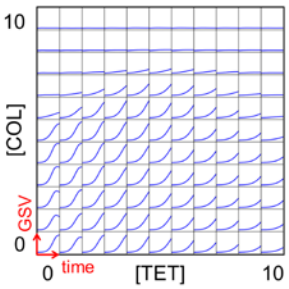


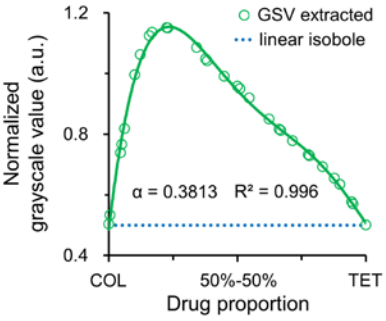


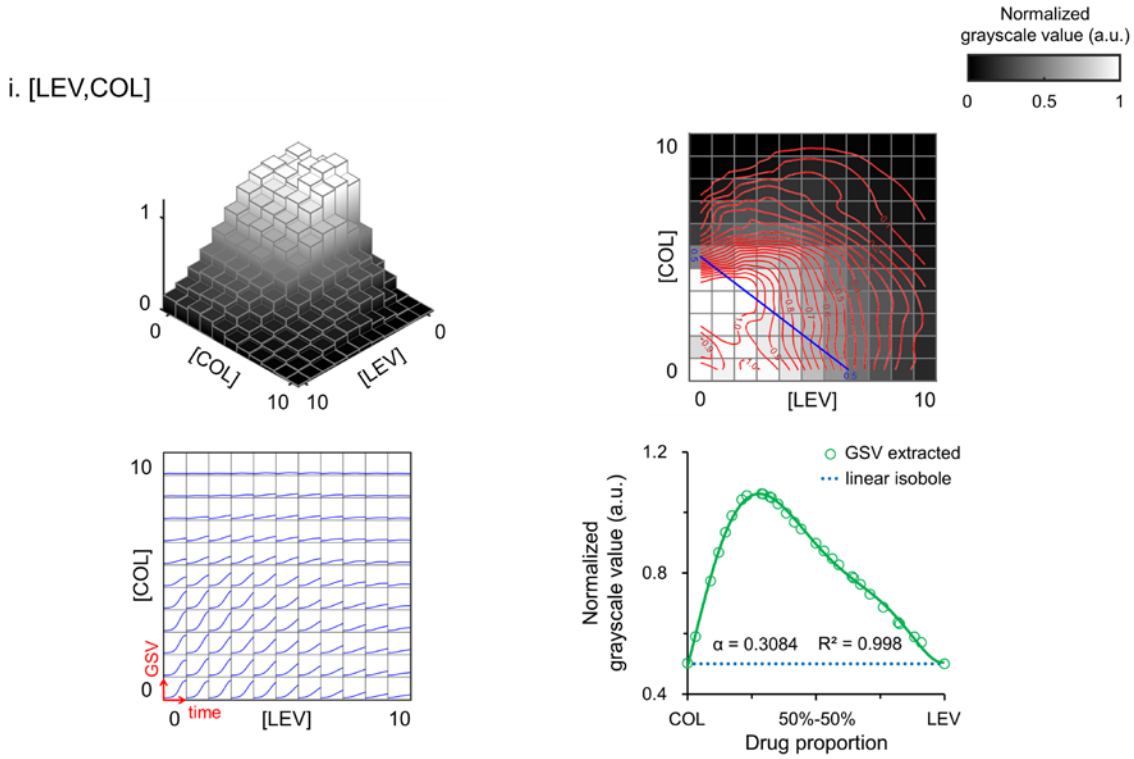
h. [TET,COL]

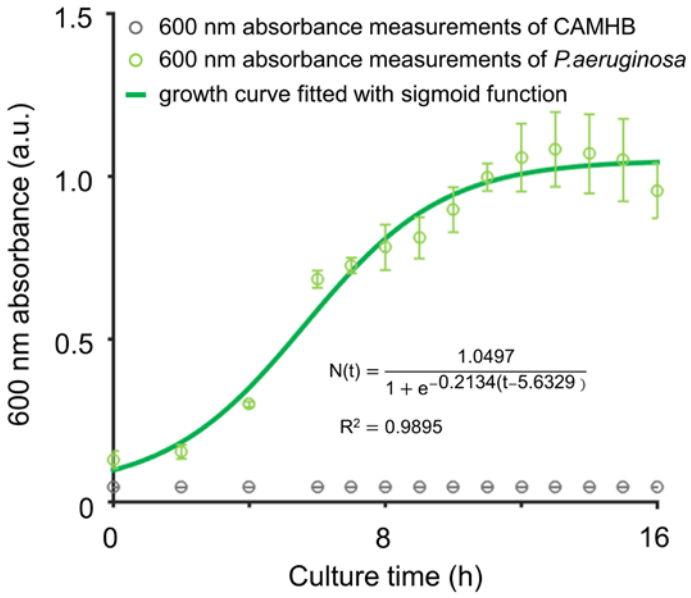









Normalized
grayscale value (a.u.)
0
0.5
1
i. [LEV,COL]
1
0
0
[COL]
10
10
[LEV]
0
10
[COL]
0
[LEV]
10
10
[COL]
GSV
0
time
0
[LEV]
10
1.2
0.8
0.4
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = 0.3084
R² = 0.998
COL
50%-50%
LEV
Drug proportion
j. [MER,COL]
1
0
0
[COL]
10
10
[MER]
0
10
[COL]
0
[MER]
10
10
[COL]
GSV
0
time
0
[MER]
10
1.2
0.9
0.6
Normalized
grayscale value (a.u.)
GSV extracted
linear isobole
α = 0.2254
R² = 0.9941
COL
50%-50%
MER
Drug proportion



Supplementary Figure 6. Growth curve of *P. aeruginosa* (ATCC 27853) on a 96-well plate at 37 °C. The inset equation represents fitted sigmoid function to measurements of 600 nm absorbance. Error bars indicate s.d. (n=3).