Metabolic fitness landscapes predict the evolution of antibiotic resistance

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Motivation

- Predictions of antibiotic resistance evolution is of major medical interest

- Metabolic models of drug action and resistance for ribosome-targeting antibiotics are well established (Greulich *et al.* 2019 *Mol Sys Biol.*)

- Unclear, how resistance affects cell growth at different drug levels

  → Study aim: Comparison of model predictions with empirical data

The metabolic fitness model
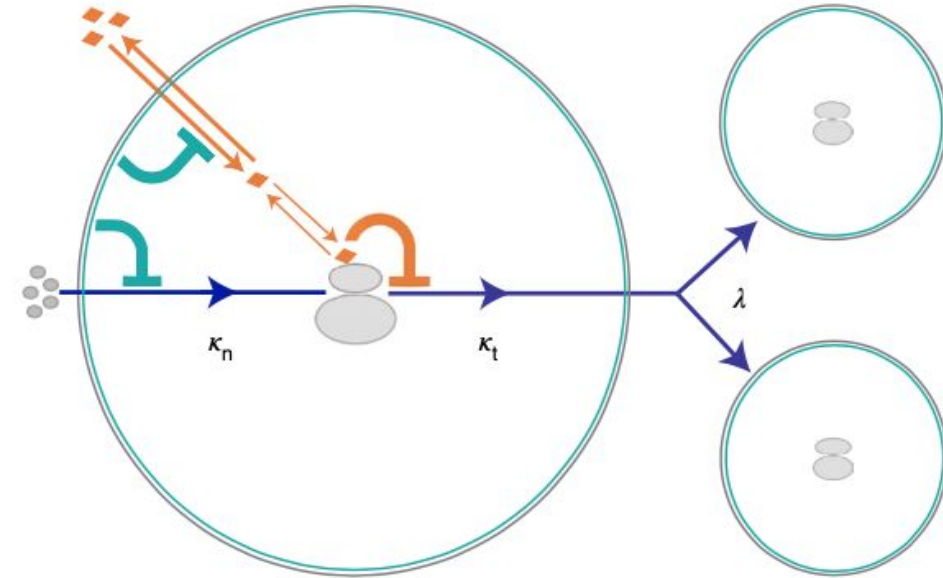
Key idea: drug action + resistance evolution → coupled perturbation
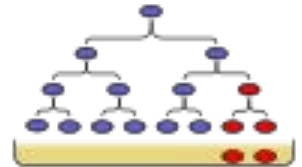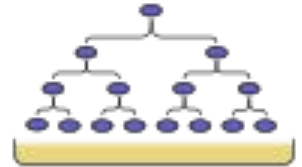
drug affects translational capacity (κ_t)

resistance affects nutritional capacity (κ_n)

Fitness=growth rate (λ)

Selection experiment: Luria-Delbrück assay

- wt *E. coli* strain
- LB (rich) and glycerol (minimal) medium
- streptomycin at different conc.
- 8 colonies/condition for WGS and growth rate measurement

$$G(d) = \lambda(d)/\lambda_0^{\text{wt}}$$
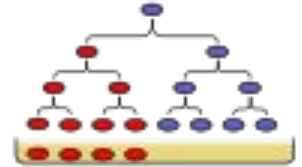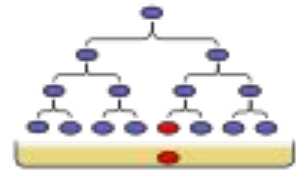
(B) Spontaneous mutation

82% of all membrane mutations are sequenced only in a single clone

(a)

b

Resistance evolution

Drug action

$$\lambda(\kappa_n, \kappa_t) = C\frac{\kappa_n \kappa_t}{\kappa_n + \kappa_t}$$

Growth rate, λ/λ_0^{wt}

1.0

0.5

0

Translational capacity, κ_t/κ_t^0

1.0

0.5

0

Nutritional capacity, κ_n/κ_t^0

2

4

6
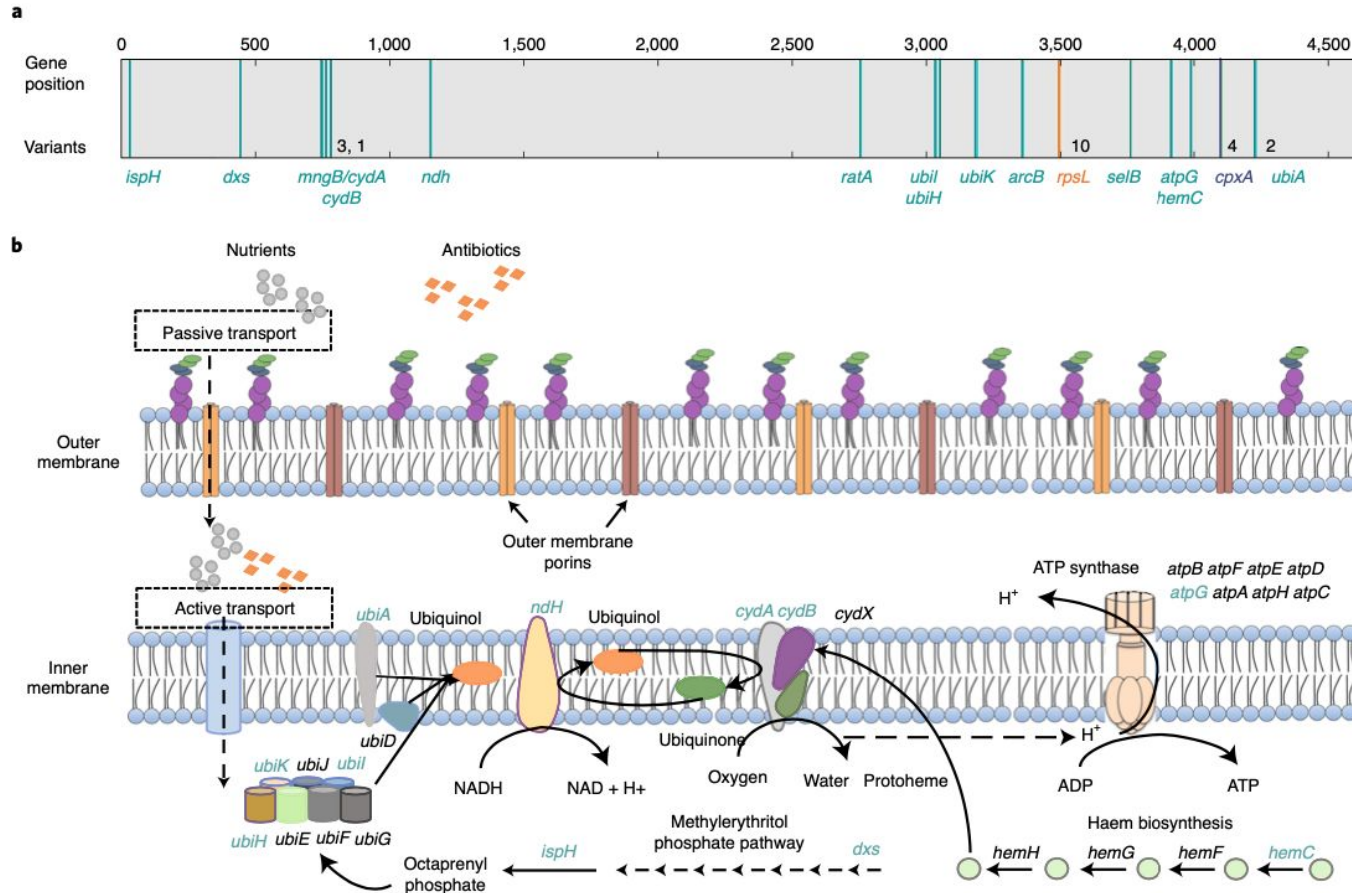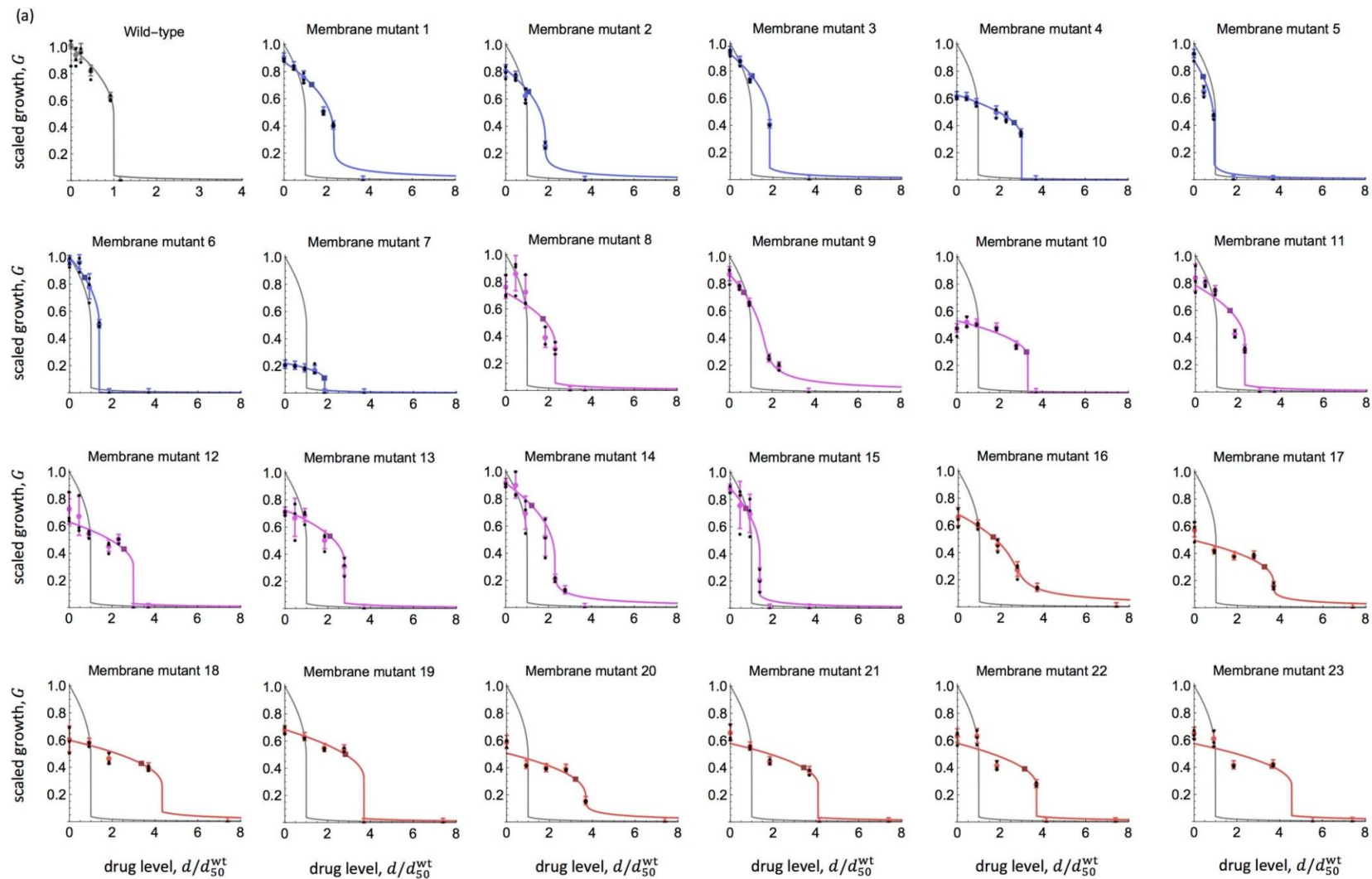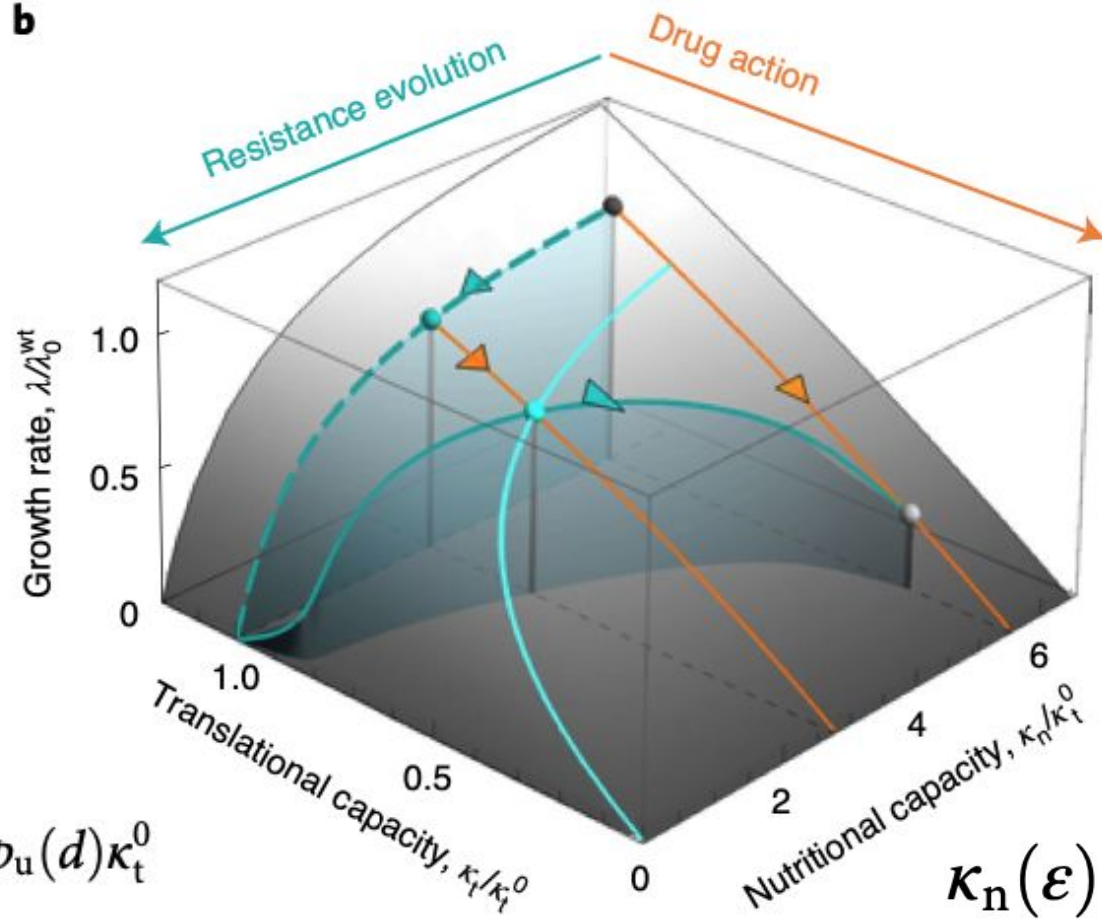
$$\kappa_t(d) = p_u(d)\kappa_t^0$$

$$\kappa_n(\varepsilon) = \varepsilon\,\kappa_n^{wt}$$

$$R(\varepsilon) = \frac{r^{wt}W(\varepsilon) + 1/(r^{wt}W(\varepsilon))}{\varepsilon(r^{wt} + 1/r^{wt})}$$

$$W(\varepsilon) = \frac{\varepsilon(q^{wt} + 1)}{(\varepsilon q^{wt} + 1)}$$

Trade-off
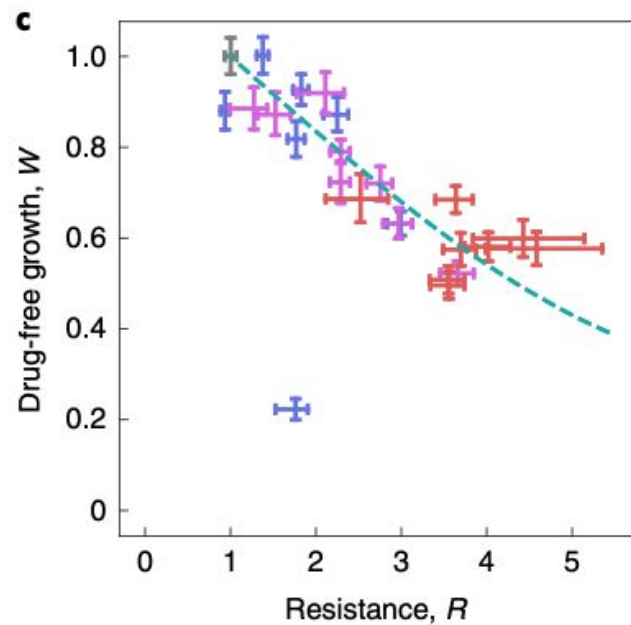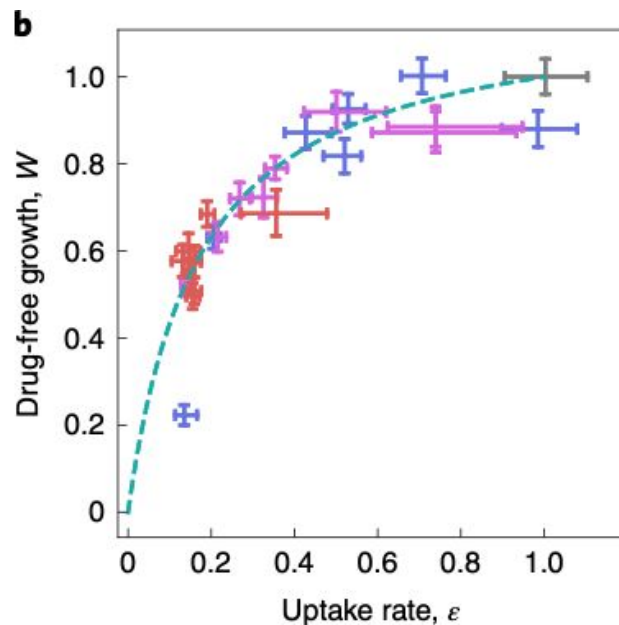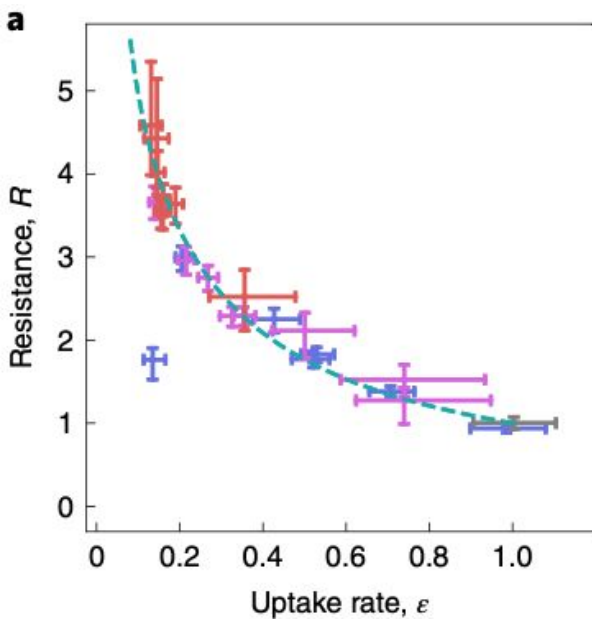
Max. fitness prediction match empirical data

$$G_c(\varepsilon) = \frac{\varepsilon(q^{wt} + 1)}{\varepsilon q^{wt} + 2}$$
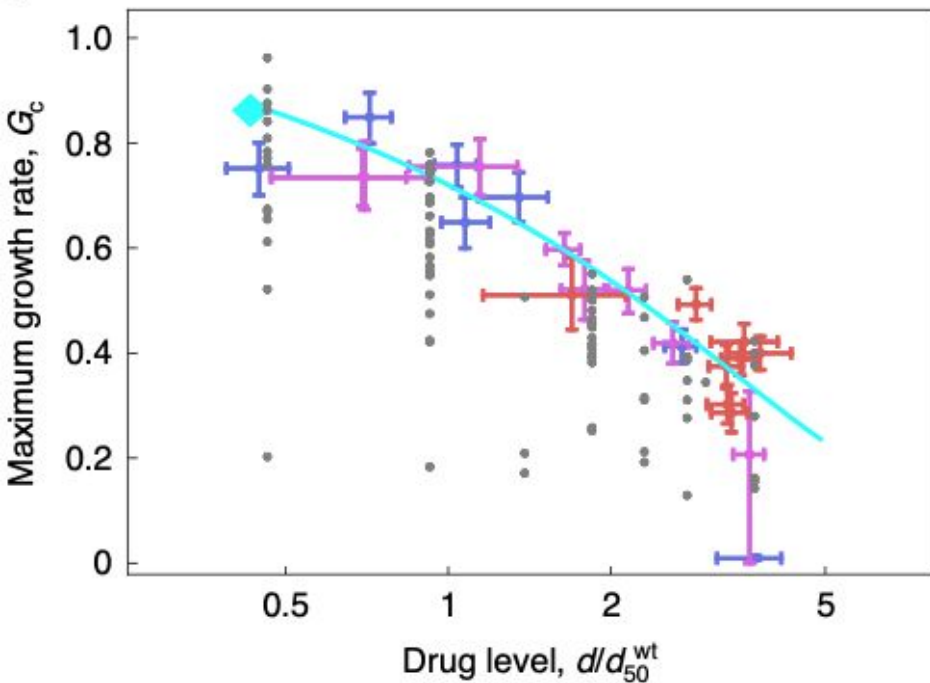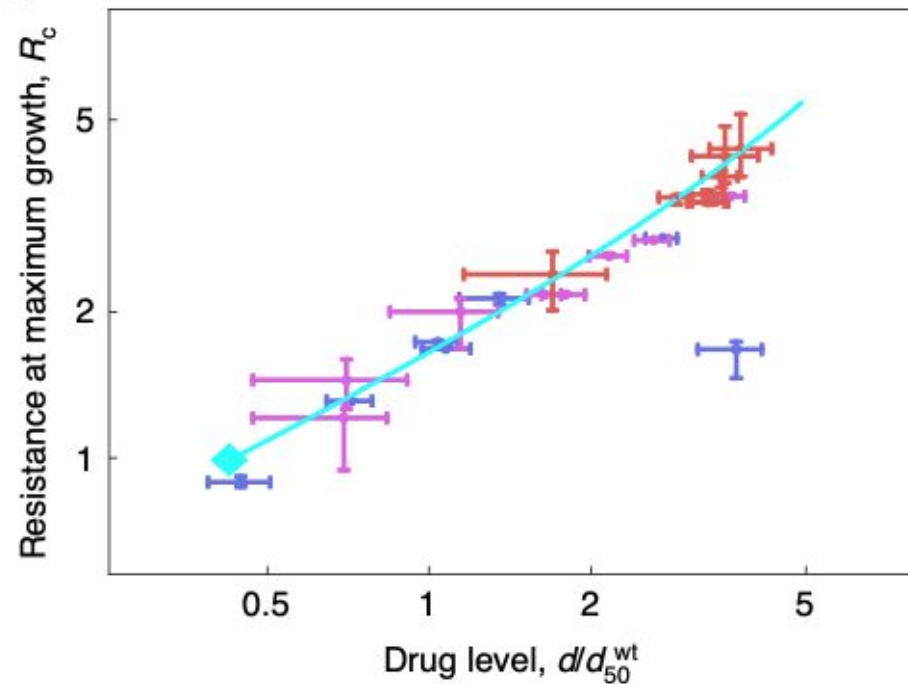
Predicting resistance mechanisms depending on drug level
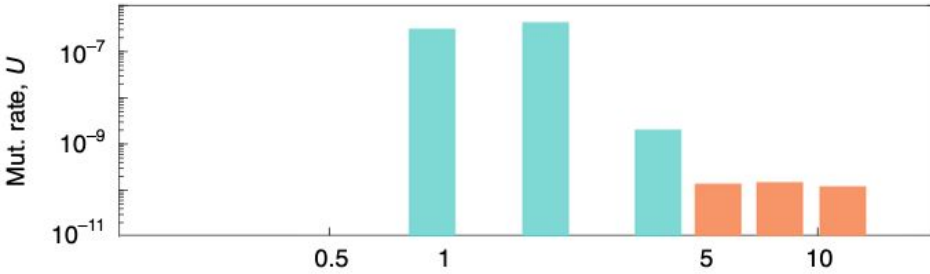
in rich medium:

low level: increasing ribosomal units #

intermediate: mutations decreasing import

high: mutations of drug-target
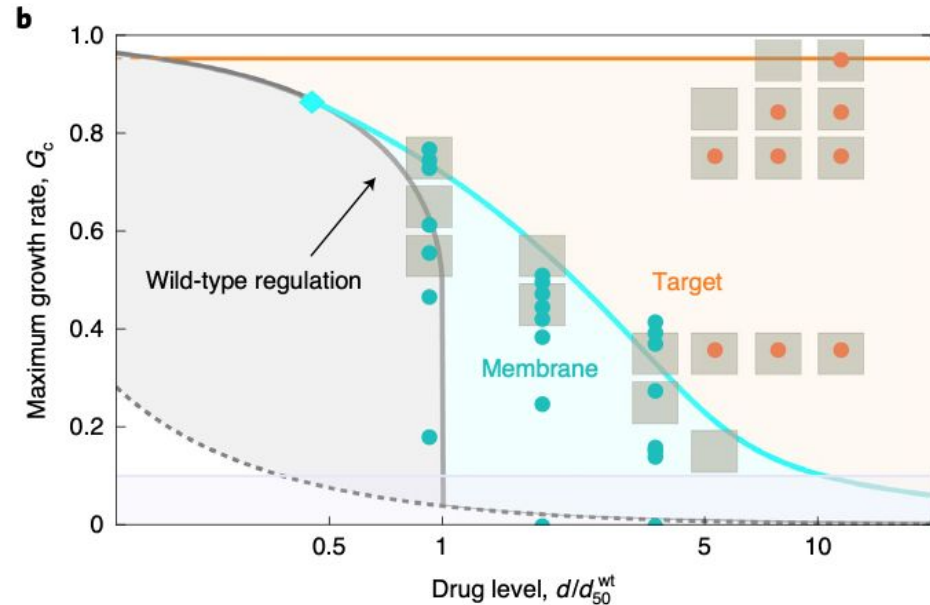
Fitting model to stress-response mutations in minimal environment
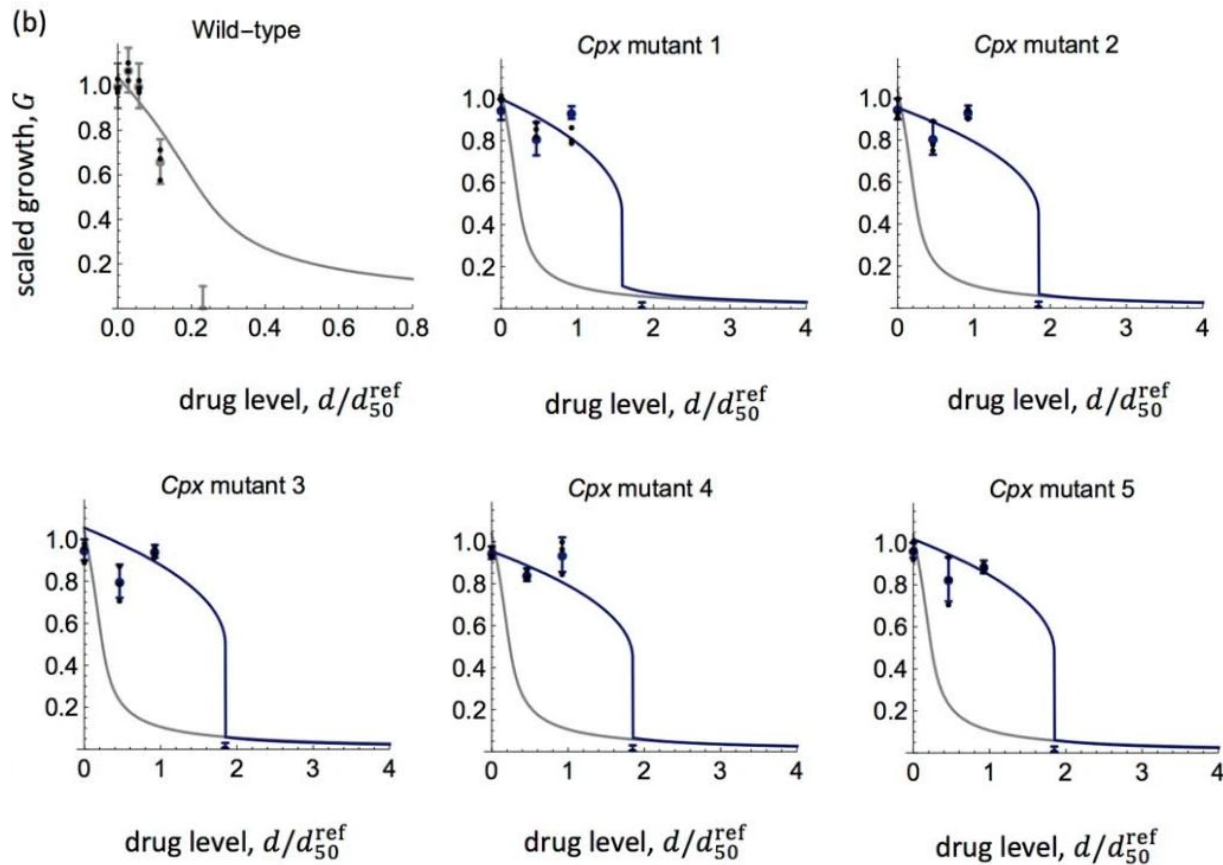
Predicting resistance mechanisms depending on drug level
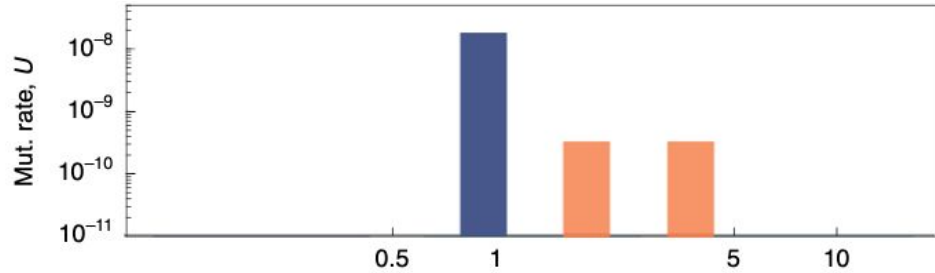
in minimal medium:

- membrane mutation are more costly in minimal medium

- mutation in stress response already at low to intermediate drug levels

- high: mutation in drug-target
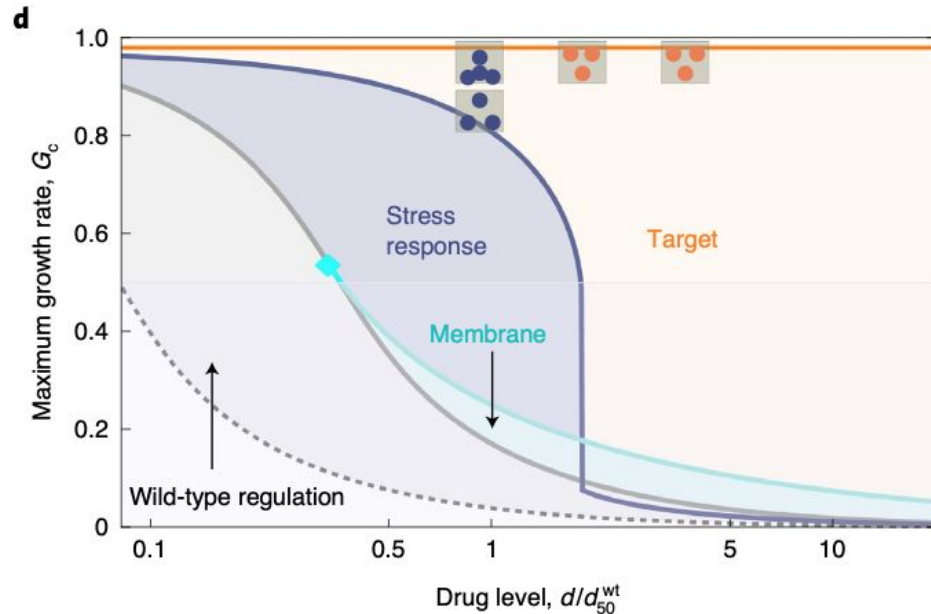
Discussion

- **Metabolic fitness** models predict the **optimal resistance** mechanism to depend not only on the **drug level** but also on **nutrient conditions**

- **Trade-off** functions can be regarded as Pareto fronts constraining the simultaneous optimization of drug resistance and growth in a drug-free medium

Thank you for your attention

Gene	Protein location	Pathway	Pathway function	Likely functional effect of mutation
ispH	Cytoplasm	Methylerythritol-phosphate pathway	Precursor for ubiquinol/ubiquinone biosynthesis pathway	Reduced ubiquinol / ubiquinone levels
dxs	Cytoplasm	Methylerythritol-phosphate pathway	Precursor for ubiquinol/ubiquinone biosynthesis pathway	Reduced ubiquinol / ubiquinone levels
cydA-mngB	Inner membrane	NADH – cytochrome oxidase electron transfer	Cytochrome bd-I ubiquinol oxidase subunit 1	Reduced *cydA* expression
cydB	Inner membrane	NADH – cytochrome oxidase electron transfer	Cytochrome bd-I ubiquinol oxidase subunit 2	Reduced ubiquinone levels
ndh	Inner membrane	NADH – cytochrome oxidase electron transfer	Quinone oxidoreductase enzyme	Impaired cytochrome oxidase
ubiA	Inner membrane	Ubiquinone biosynthesis pathway	Ubiquinone biosynthesis	Reduced ubiquinol / ubiquinone levels
ubiI	Cytoplasm	Ubiquinone biosynthesis pathway	Ubiquinone biosynthesis	Reduced ubiquinol / ubiquinone levels
ubiH	Cytoplasm	Ubiquinone biosynthesis pathway	Ubiquinone biosynthesis	Reduced ubiquinol / ubiquinone levels
ubiK	Cytoplasm / Inner membrane	Ubiquinone biosynthesis pathway	Ubiquinone biosynthesis	Reduced ubiquinol / ubiquinone levels
atpG	Inner membrane	ATP biosynthesis pathway	ATP synthase subunit involved in ATP synthesis	Impaired ATP synthesis
hemC	Cytoplasm	Heme biosynthesis pathway	Synthesis of cofactors for ubiquinol oxidases (*cydA, cydB*)	Reduced ubiquinol / ubiquinone levels
arcB	Cytoplasm / inner membrane	NADH – cytochrome oxidase electron transfer, other pathways	Positive regulator of *cydA* expression [52,53]	Reduced *cydA* expression
selB	Cytoplasm	Electron transfer	Translation factor, insertion of seleno-cysteine into membrane peptides	Modification of membrane-bound dehydrogenases
ratA	Cytoplasm		Ribosome associated toxin / ubiquinone-binding protein	Reduced ubiquinone levels

Mutant number	$\dfrac{d_{LD}}{d_{50}^{wt}}$	$W = \dfrac{\lambda_0}{\lambda_0^{wt}}$	$\dfrac{d_*}{d_*^{wt}}$	$\dfrac{\lambda_*}{\lambda_*^{wt}}$
1	0.9	0.87 (0.83, 0.91)	3.90, (3.22, 4.60)	1.68, (1.26, 2.19)
2	0.9	0.82 (0.78, 0.86)	3.34, (3.21, 3.45)	1.73, (1.60, 1.82)
3	0.9	0.93 (0.89, 0.96)	2.65, (2.51, 2.76)	1.40, (1.31, 1.49)
4	0.9	0.63 (0.61, 0.66)	0.74, (0.70, 0.78)	0.15, (0.14, 0.16)
5	0.9	0.88 (0.84, 0.92)	1.39, (1.28, 1.51)	1.36, (1.23, 1.54)
6	0.9	1.00 (0.96, 1.04)	0.42, (0.40, 0.44)	0.30, (0.28, 0.32)
7	0.9	0.22 (0.20, 0.25)	2.68, (2.49, 2.92)	0.36, (0.30, 0.45)
8	1.8	0.72 (0.68, 0.77)	3.04, (2.84, 3.19)	0.99, (0.89, 1.06)
9	1.8	0.87 (0.83, 0.92)	3.59, (3.32, 3.83)	2.64, (2.14, 3.19)
10	1.8	0.52 (0.50, 0.55)	0.06, (0.06, 0.07)	0.01, (0.01, 0.01)
11	1.8	0.78 (0.76, 0.82)	2.93, (2.82, 3.03)	1.04, (0.99, 1.09)
12	1.8	0.63 (0.60, 0.67)	3.27, (3.10, 3.42)	0.70, (0.64, 0.75)
13	1.8	0.72 (0.68, 0.76)	3.40, (3.22, 3.58)	0.91, (0.85, 0.96)
14	1.8	0.92 (0.87, 0.97)	4.05, (3.79, 4.27)	2.01, (1.75, 2.41)
15	1.8	0.88 (0.84, 0.93)	1.80, (1.44, 2.12)	1.31, (1.04, 1.53)
16	3.7	0.68 (0.63, 0.74)	5.91, (5.23, 6.58)	2.08, (1.59, 2.67)
17	3.7	0.49 (0.47, 0.53)	6.65, (6.32, 6.94)	1.03, (0.93, 1.13)
18	3.7	0.60 (0.56, 0.64)	6.73, (6.17, 7.41)	0.97, (0.85, 1.07)
19	3.7	0.68 (0.66, 0.71)	3.91, (3.73, 4.07)	0.74, (0.69, 0.79)
20	3.7	0.51 (0.48, 0.54)	6.65, (6.35, 6.92)	1.06, (0.96, 1.16)
21	3.7	0.58 (0.55, 0.61)	4.71, (4.47, 4.94)	0.69, (0.63, 0.75)
22	3.7	0.58 (0.54, 0.61)	4.75, (4.50, 5.02)	0.76, (0.70, 0.82)
23	3.7	0.57 (0.54, 0.61)	5.94, (5.51, 6.39)	0.77, (0.67, 0.87)

Mutant number	$\varepsilon = \dfrac{\gamma_{in}}{\gamma_{in}^{wt}}$	$\dfrac{\gamma_{out}}{\gamma_{out}^{wt}}$	$R = \dfrac{d_{50}}{d_{50}^{wt}}$	$\dfrac{d_c}{d_{50}^{wt}}$
1	0.43 (0.38, 0.49)	3.01 (1.58, 4.80)	2.25 (2.09, 2.38)	1.35 (1.14, 1.53)
2	0.51 (0.47, 0.56)	3.06 (2.55, 3.30)	1.77 (1.67, 1.87)	1.07 (0.97, 1.19)
3	0.52 (0.49, 0.57)	1.96 (1.71, 2.21)	1.83 (1.73, 1.91)	1.04 (0.94, 1.13)
4	0.20 (0.19, 0.22)	0.02 (0.02, 0.03)	3.00 (2.83, 3.13)	2.71 (2.52, 2.88)
5	0.99 (0.90, 1.08)	1.88 (1.52, 2.39)	0.94 (0.88, 0.99)	0.45 (0.39, 0.51)
6	0.70 (0.66, 0.76)	0.09 (0.08, 0.10)	1.38 (1.31, 1.44)	0.71 (0.64, 0.78)
7	0.13 (0.11, 0.17)	0.13 (0.09, 0.21)	1.76 (1.53, 1.90)	3.71 (3.15, 4.14)
8	0.32 (0.30, 0.36)	0.99 (0.79, 1.13)	2.29 (2.16, 2.40)	1.79 (1.62, 1.95)
9	0.72 (0.59, 0.93)	7.12 (4.56, 10.20)	1.52 (1.32, 1.70)	0.70 (0.47, 0.91)
10	0.15 (0.13, 0.15)	0.00 (0.00, 0.00)	3.66 (3.46, 3.85)	3.62 (3.37, 3.85)
11	0.35 (0.33, 0.38)	1.07 (0.98, 1.18)	2.29 (2.17, 2.40)	1.64 (1.52, 1.76)
12	0.21 (0.20, 0.24)	0.49 (0.41, 0.57)	2.97 (2.79, 3.12)	2.61 (2.40, 2.79)
13	0.27 (0.24, 0.29)	0.83 (0.72, 0.92)	2.75 (2.59, 2.90)	2.16 (1.98, 2.33)
14	0.45 (0.42, 0.62)	4.14 (3.07, 5.81)	2.11 (1.78, 2.33)	1.14 (0.85, 1.34)
15	0.67 (0.62, 0.95)	1.76 (1.09, 2.35)	1.27 (0.99, 1.43)	0.69 (0.47, 0.84)
16	0.34 (0.27, 0.48)	4.51 (2.54, 7.12)	2.52 (2.11, 2.85)	1.70 (1.16, 2.13)
17	0.15 (0.14, 0.17)	1.08 (0.87, 1.27)	3.56 (3.34, 3.74)	3.35 (3.07, 3.59)
18	0.15 (0.11, 0.17)	0.95 (0.72, 1.15)	4.43 (3.84, 5.14)	3.54 (3.07, 4.08)
19	0.19 (0.17, 0.21)	0.55 (0.48, 0.62)	3.64 (3.40, 3.83)	2.88 (2.67, 3.07)
20	0.16 (0.14, 0.18)	1.13 (0.93, 1.33)	3.55 (3.33, 3.74)	3.29 (3.02, 3.53)
21	0.14 (0.13, 0.16)	0.48 (0.40, 0.56)	4.02 (3.74, 4.28)	3.48 (3.21, 3.74)
22	0.16 (0.14, 0.18)	0.58 (0.49, 0.68)	3.70 (3.49, 3.88)	3.28 (3.04, 3.52)
23	0.13 (0.11, 0.16)	0.60 (0.46, 0.75)	4.58 (3.99, 5.35)	3.78 (3.31, 4.32)

(a) Relative streptomycin uptake vs. Wild-type (1) and Membrane mutants #1 (2), #3 (3), #20 (4)

(b) Uptake rate, ε (constrained) vs. Uptake rate, ε

(c) Drug-free growth, W (constrained) vs. Drug-free growth, W

(d) Resistance, R (constrained) vs. Resistance, R

(a)

Resistance-cost tradeoff

Drug-free growth, W vs. Resistance, $R = d_{50}/d_{50}^{wt}$

Maximum-growth trajectory

Maximum growth rate, G_c vs. Drug level, d/d_{50}^{wt}

irreversible regime ($r^{wt} \gg 1$)

— regulation

— membrane evolution minimal model

— membrane evolution extended model

(b)

Resistance-cost tradeoff

Drug-free growth, W vs. Resistance, $R = d_{50}/d_{50}^{wt}$

Maximum-growth trajectory

Maximum growth rate, G_c vs. Drug level, d/d_{50}^{wt}

irreversible regime ($r^{wt} \gg 1$)

— regulation

— membrane evolution minimal model

— efflux pump evolution

(c)

Resistance-cost tradeoff

Drug-free growth, W vs. Resistance, $R = d_{50}/d_{50}^{wt}$

Maximum-growth trajectory

Maximum growth rate, G_c vs. Drug level, d/d_{50}^{wt}

reversible regime ($r^{wt} \lesssim 1$)

— regulation

— membrane evolution minimal model

— efflux pump evolution

(a)

(b) Selection at $d/d_{50}^{\mathrm{wt}} = 1.8$ Selection at $d/d_{50}^{\mathrm{wt}} = 3.7$ Selection at $d/d_{50}^{\mathrm{wt}} = 5.5$

(c) Selection at $d/d_{50}^{\mathrm{wt}} = 1.8$ Selection at $d/d_{50}^{\mathrm{wt}} = 3.7$ Selection at $d/d_{50}^{\mathrm{wt}} = 5.5$

(d) Selection at $d/d_{50}^{\mathrm{wt}} = 1.8$ Selection at $d/d_{50}^{\mathrm{wt}} = 3.7$ Selection at $d/d_{50}^{\mathrm{wt}} = 5.5$