

# Exploring the evolvability of an antibiotic resistance enzyme

Work with Merijn Salverda, Martijn Schenk, Ivan Szendro and Joachim Krug

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Laboratory of Genetics



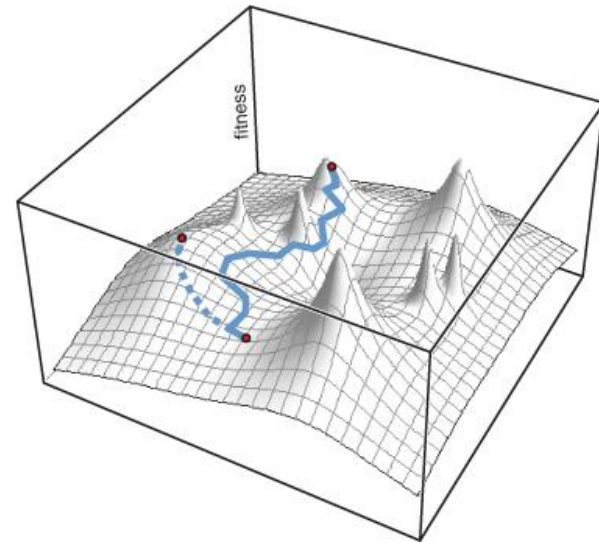
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# Determinants of evolvability

## 1. Fitness landscape

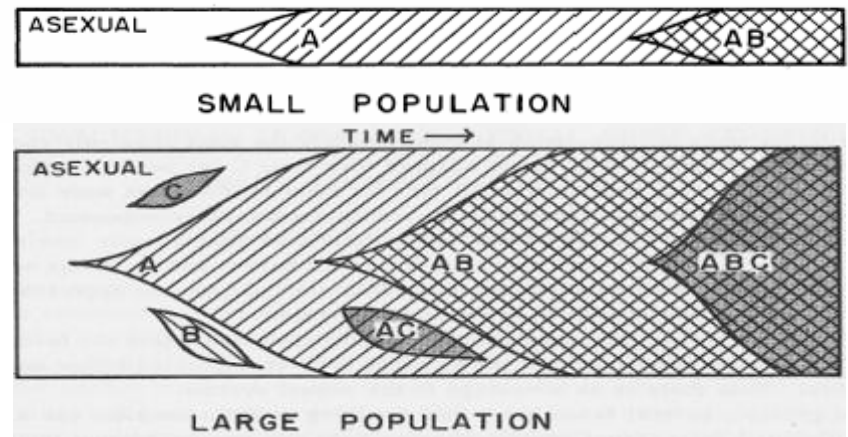
- fitness map of related genotypes
- topography depends on epistasis
- adaptation as hill-climbing process
- **what is possible?**



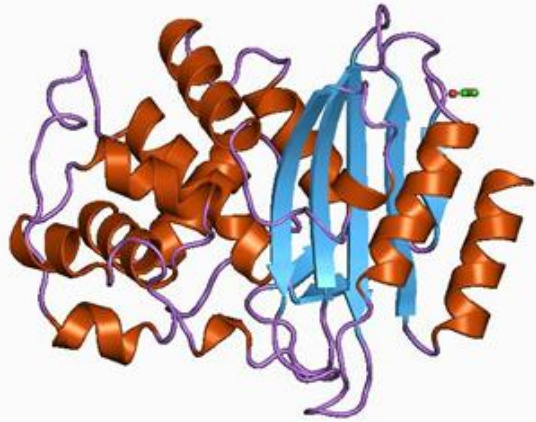
## 2. Population dynamics

$N$ ,  $U$  and  $r$

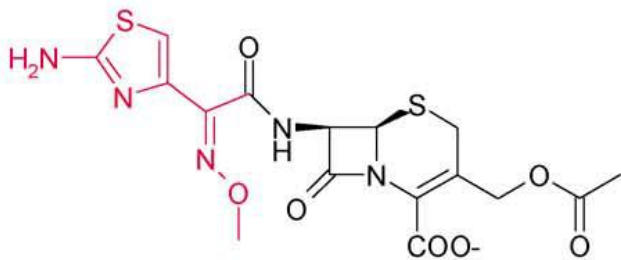
- genetic variation
- selection versus drift, clonal interference
- **what is realized?**



# The system: TEM-1 $\beta$ -lactamase



- Breaks down penicillins
- Ancestor of TEM-family of ESBLs
- Adaptation to 3<sup>rd</sup>-gen. cephalosporin cefotaxime via multiple mutations: study mutational pathways
- Control of evolutionary parameters
- Monitor and correlate genetic and phenotypic changes



cefotaxime (CTX)

# Outline

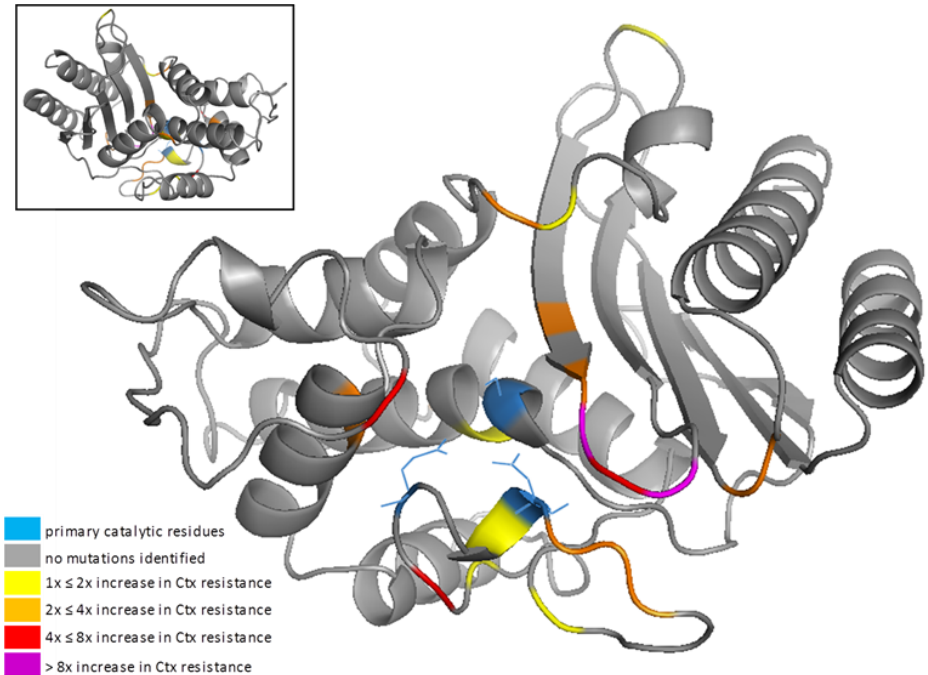
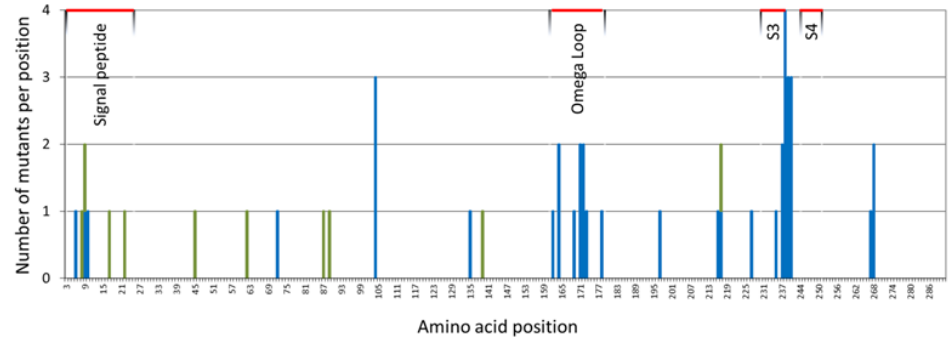
- I. Number and fitness effects of beneficial mutations
- II. Epistatic properties of beneficial mutations
- III. Divergence of mutational pathways in large populations
- IV. Evolvability as function of population size

# I. Number of beneficial mutations



Martijn Schenk

- PCR mutagenesis
- Mutant isolation at low [Ctx]
- Sequencing > 300 isolates
- **48 unique beneficial** mutations (increased resistance), including **10 synonymous mutations**
- Maximum likelihood estimate (based on frequency unique mutations):  $\geq 87$  (**3.4%**) of 2,583 possible mutations is beneficial

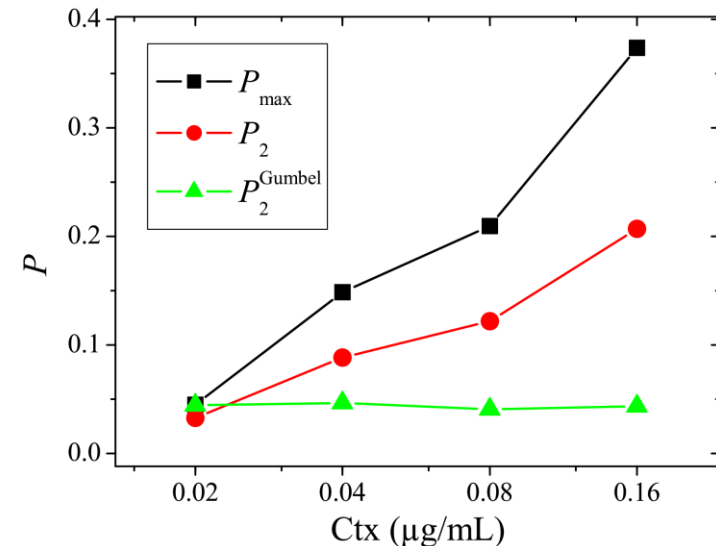
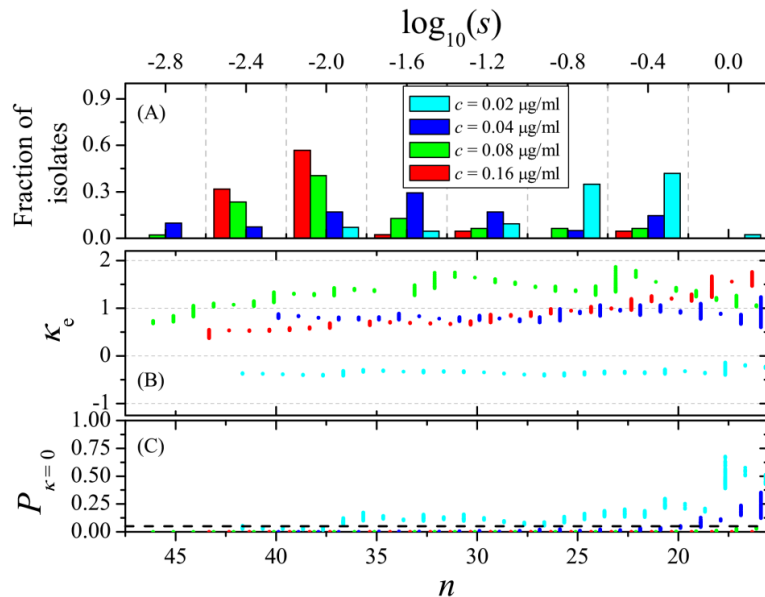


# I. Distribution of mutation effects

- Estimated shape parameter,  $\kappa$ , by fitting generalized Pareto distribution to effects of 48 mutants:

$\kappa = 0$ : **Gumbel**,  $\kappa < 0$ : **Weibull** (truncated),  $\kappa > 0$ : **Fréchet** (heavy tail)

- **Resistance** effects follow **Fréchet**-type distribution
- **Fitness** effects (inferred from survival data) follow also **Fréchet**
- Exceptionally large-effect mutations **increase repeatability of evolution**



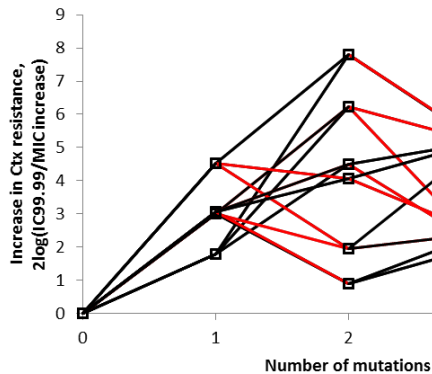
# II. Comparative analysis of epistasis



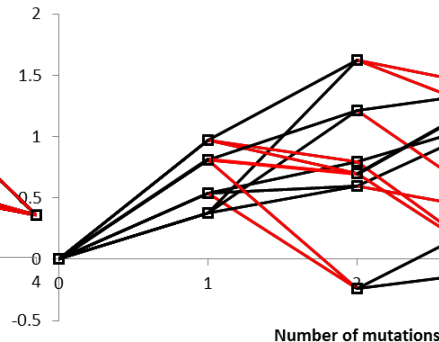
Martijn Schenk

- Fitness landscape involving 4 large and 4 small-effect beneficial mutations
- Constructed all  $2^4=16$  mutation combinations and measured Ctx resistance

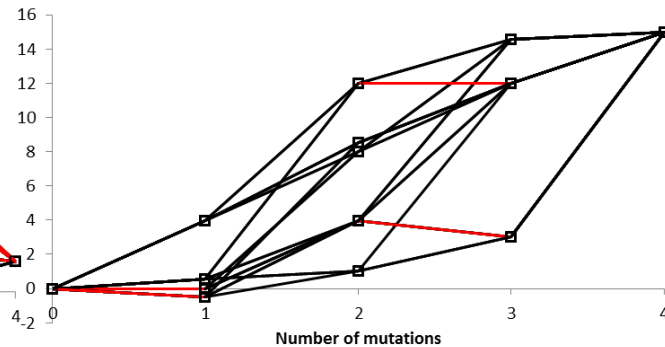
Large effect



Small effect



Joint effect



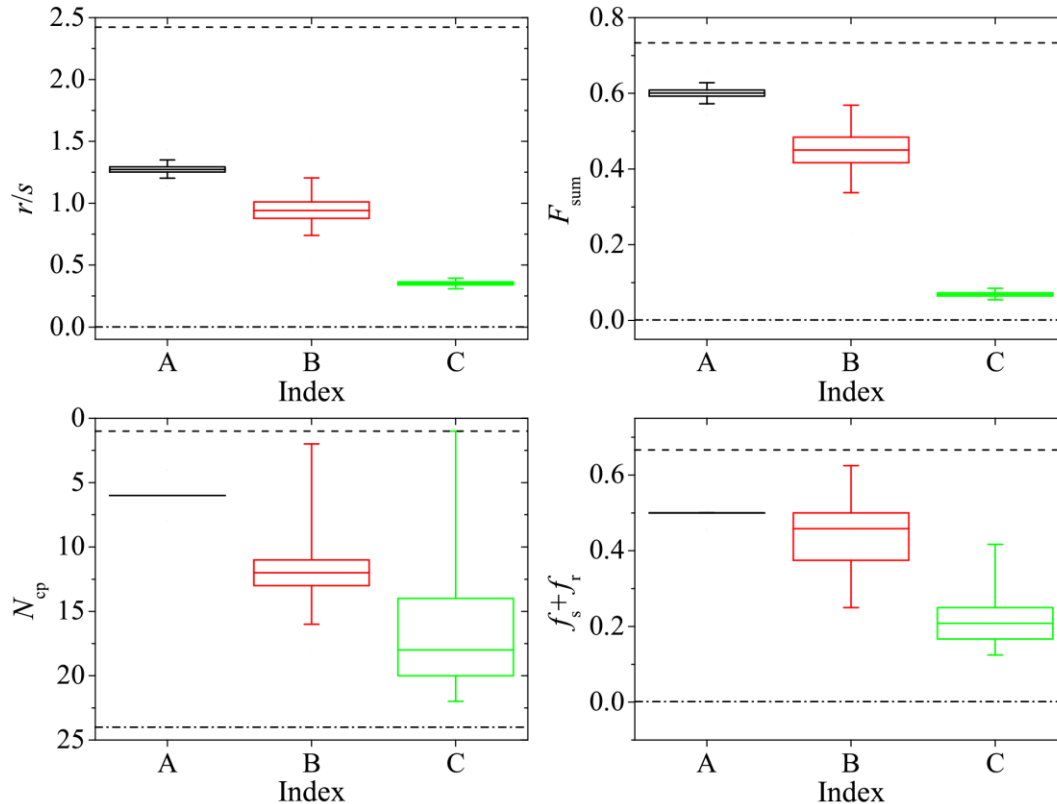
(D.M. Weinreich *et al.* 2006 *Science*)

Schenk, Szendro, Salverda, Krug & de Visser, 2013 *Mol. Biol. Evol.* 30: 1779-1787

- Sign epistasis limits the combined benefit of individually-beneficial mutations

# II. Pattern of epistasis

A. Large effect, B. Small effect, C. Joint effect



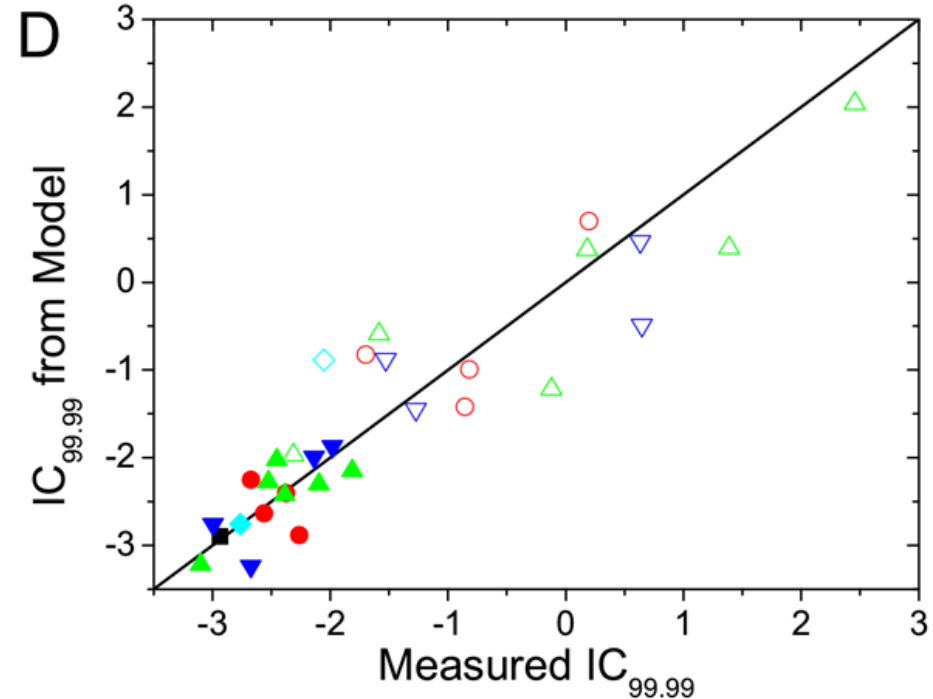
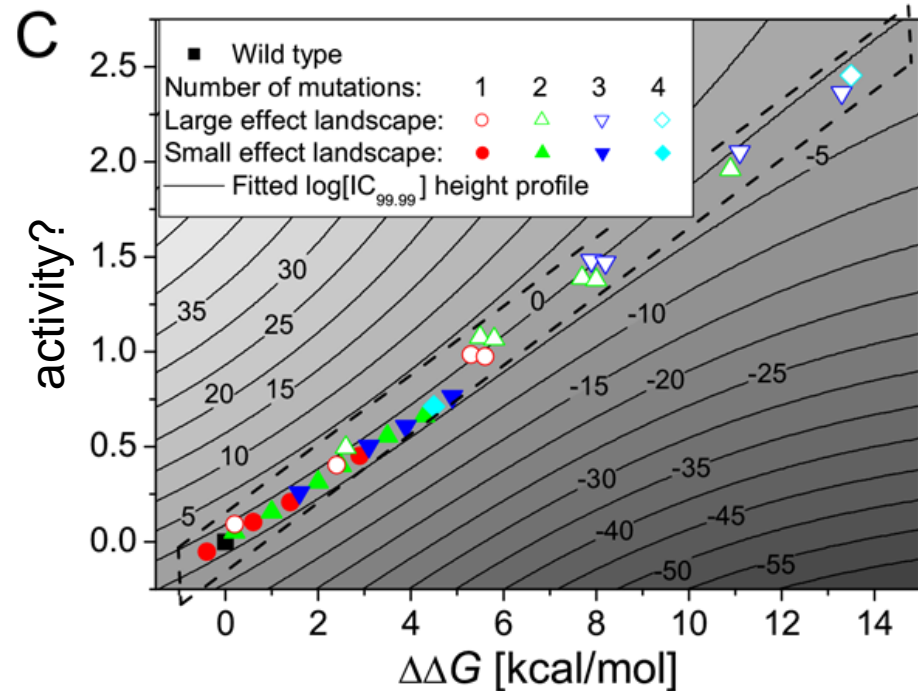
Consistent rank for different epistasis measures:

Large > Small > Joint

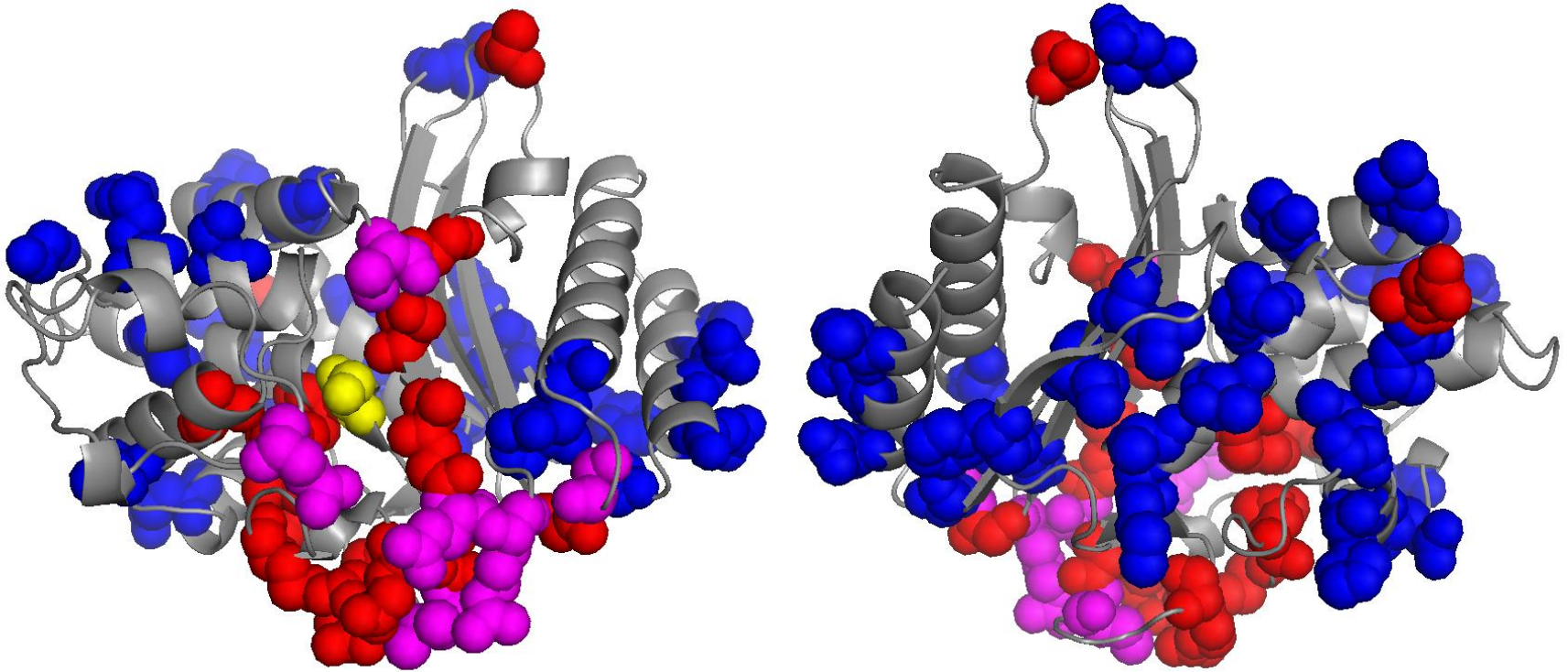
1. More epistasis among **individually** than **collectively** beneficial mutations
2. More epistasis among **large** than **small**-effect mutations



## II. Explaining epistasis from nonlinear phenotype-resistance map



# 2<sup>nd</sup>-step beneficial mutations

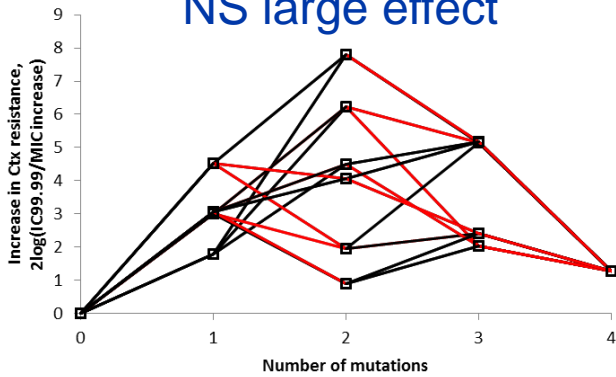


**35 2<sup>nd</sup>-step** beneficial mutations isolated in background of G238S:

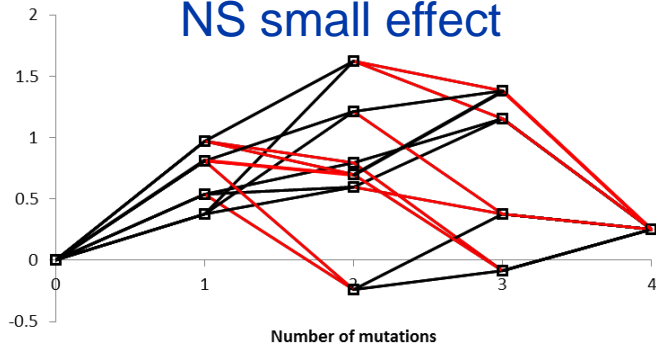
- **6 (15%) overlap** with **47 1<sup>st</sup>-step mutations** (19.1 expected if no epistasis and all 86 beneficial mutations still available,  $P < 0.001$ )
- no further synonymous mutations

# Synonymous mutation landscape

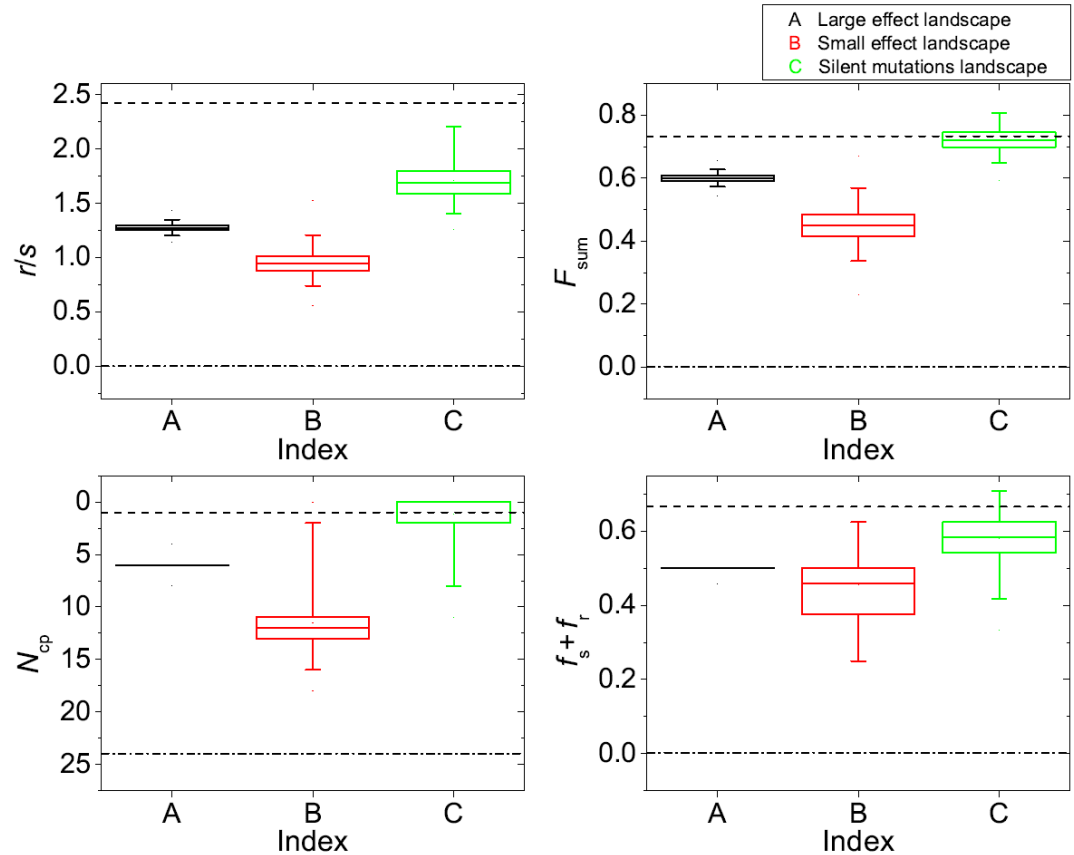
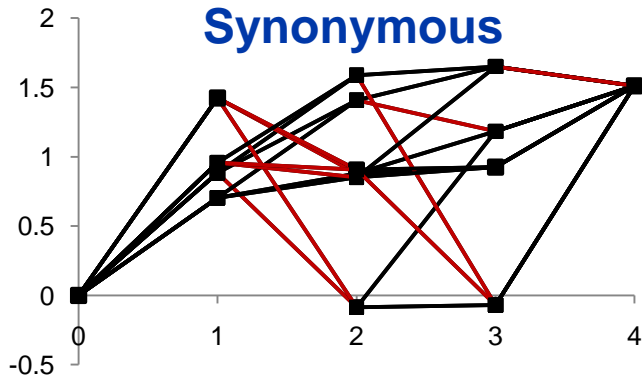
NS large effect



NS small effect



Synonymous



MF Schenk, IG Szendro, J Krug & JAGM de Visser, unpublished

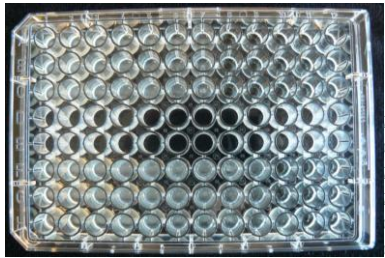
- Strong epistasis, including sign epistasis, also among synonymous mutations

# III. *In vitro* evolution



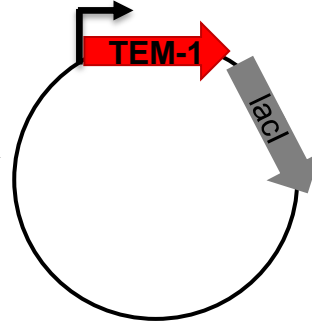
Merijn Salverda

Sequencing & MIC assay

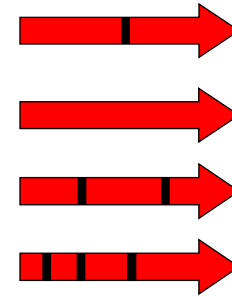


```
AGCGTAGGCTAGCTAGCTAGCTA
CGATCGATTTCGAAACGTAGGAG
AGCATGCAGTCAGGACTGGATAG
AGGGACAGATCCCTGTATCGCAG
GCTTAGCCTAGGCTTAGCTGGGG
CGATCCCTAGAATCGTTTCGATC
CCTAGCTTTATATAGATCTAGTC
```

pTac promoter

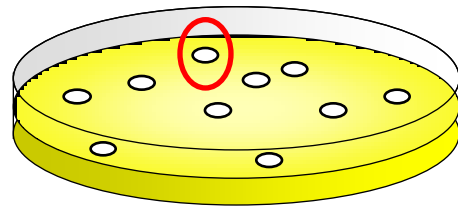
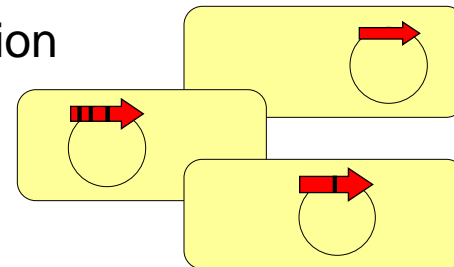


Error-prone PCR



Transformation

Selection



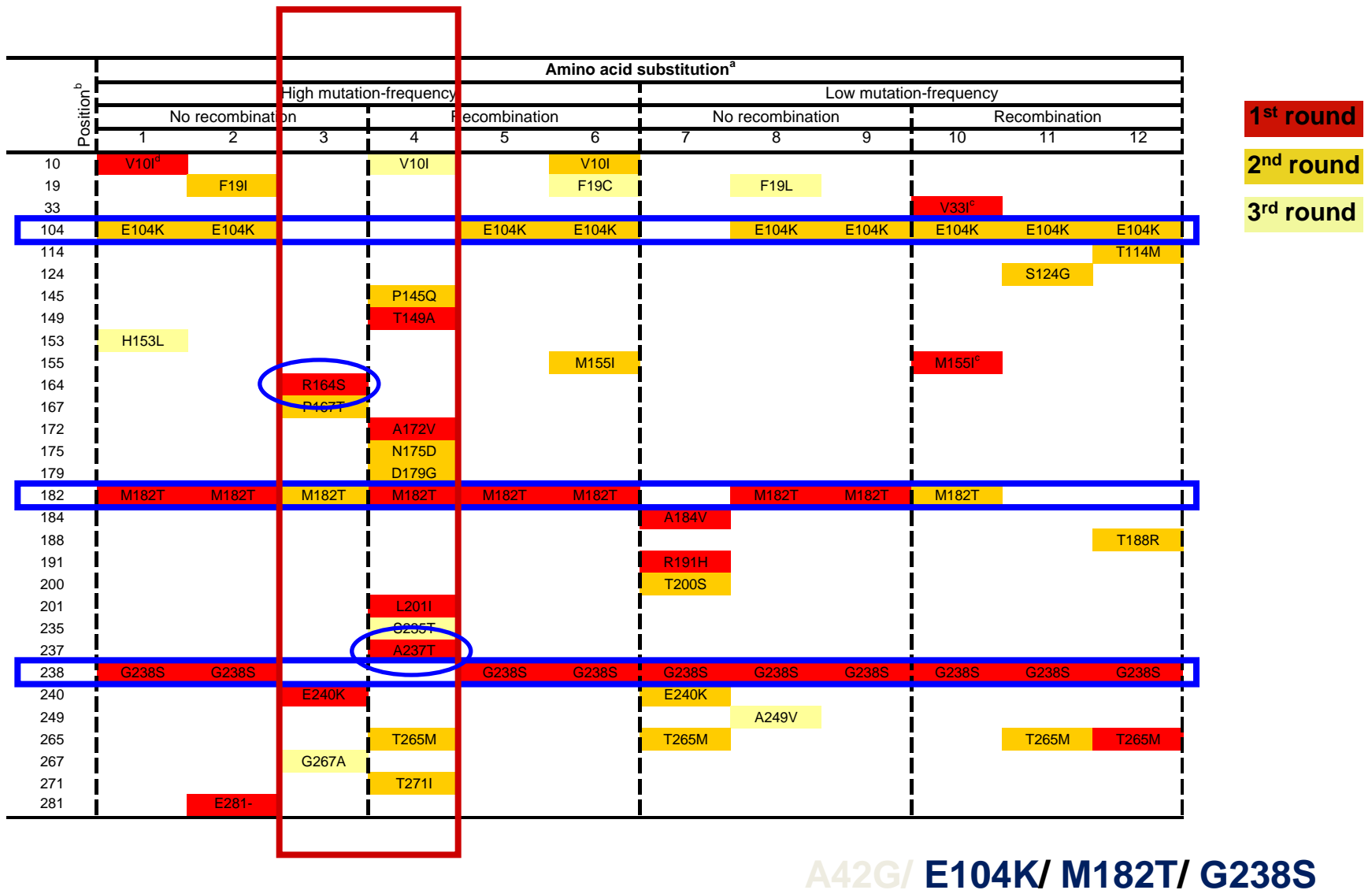
Clone isolation



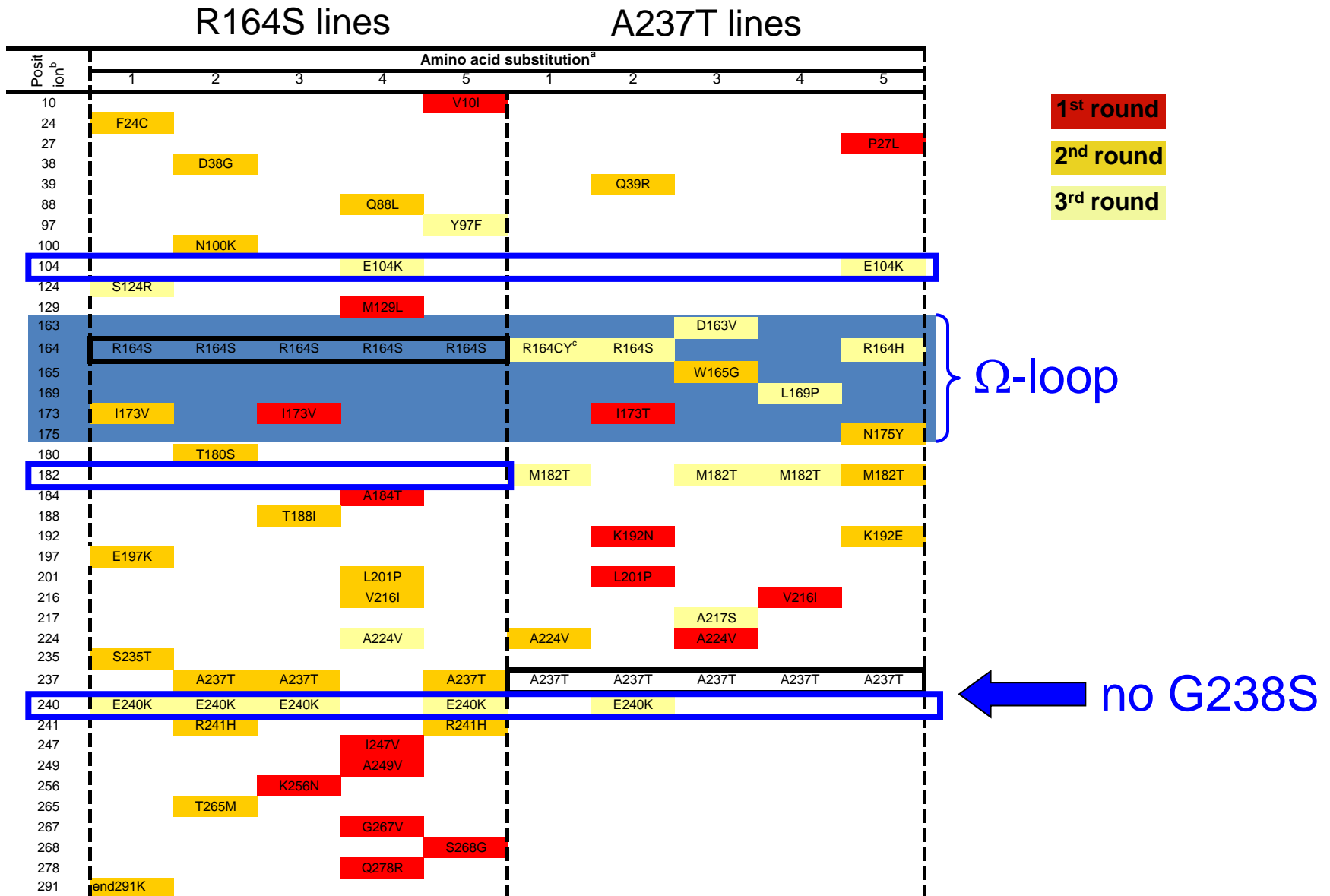
[Cefotaxime]



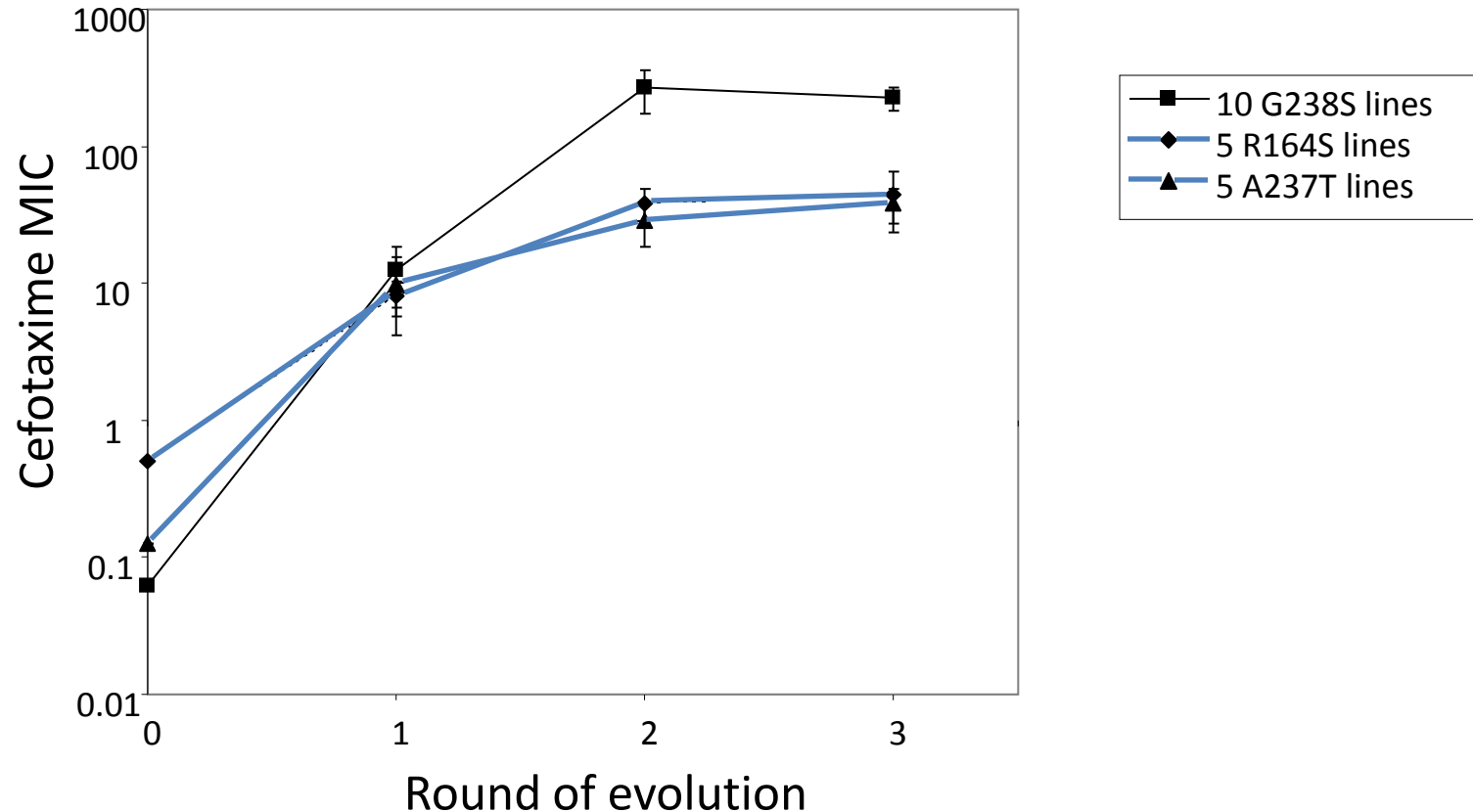
# III. Initial mutations direct adaptation



# III. Substitutions replay experiment



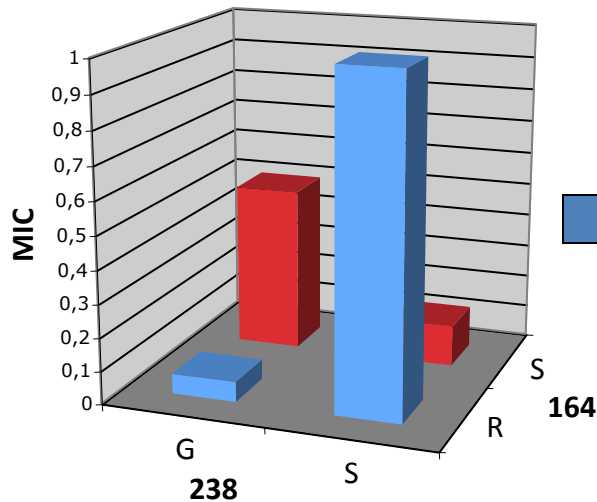
### III. Alternative pathways approach lower adaptive peaks



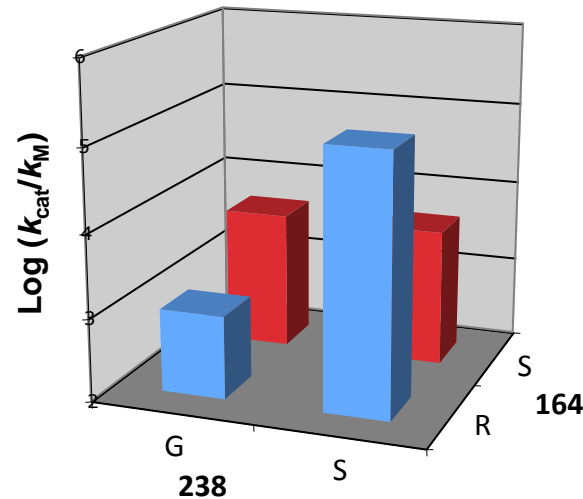
- Fitness landscape TEM-1 for Ctx<sup>R</sup> is rugged with multiple peaks
- Initial mutations largely determine which path is taken

# III. Biochemical basis epistatic interaction

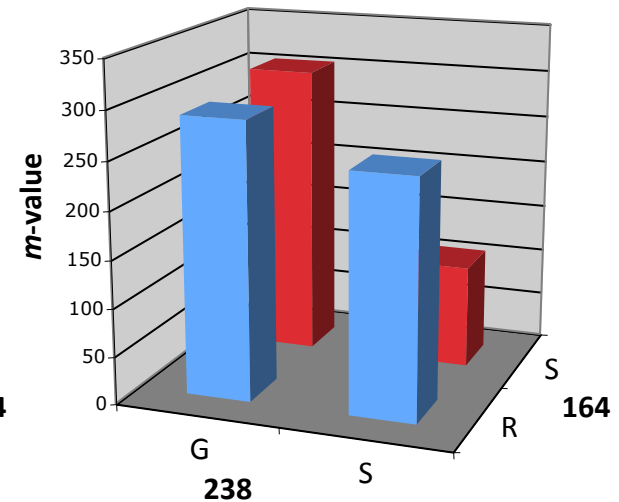
Cefotaxime resistance



Catalytic efficiency



Enzyme dosage



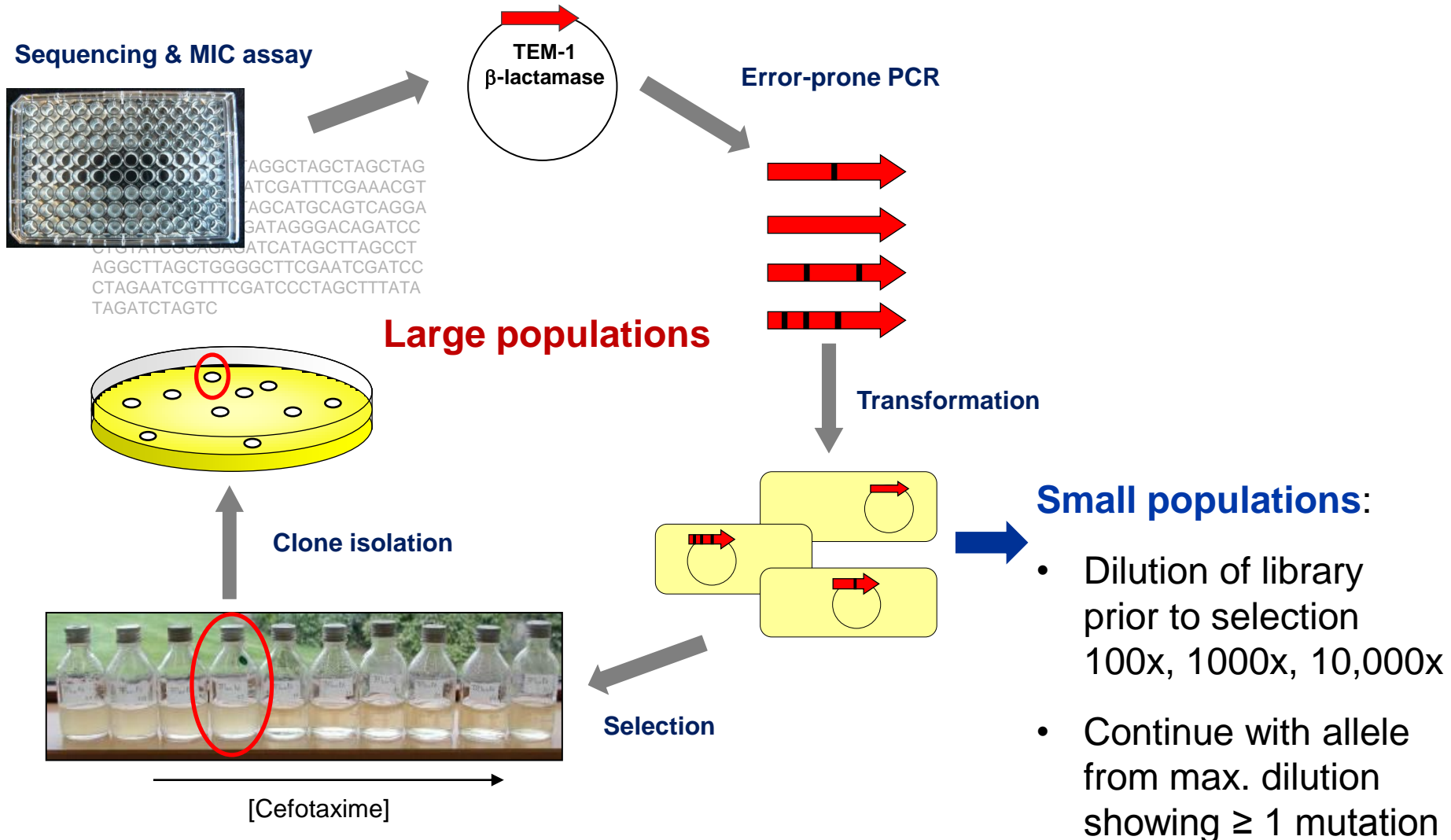
- Sign epistasis between beneficial mutations G238S and R164S results from combined epistatic effects at level of **activity** and **dosage** of properly folded enzyme



# IV. Evolvability and population size



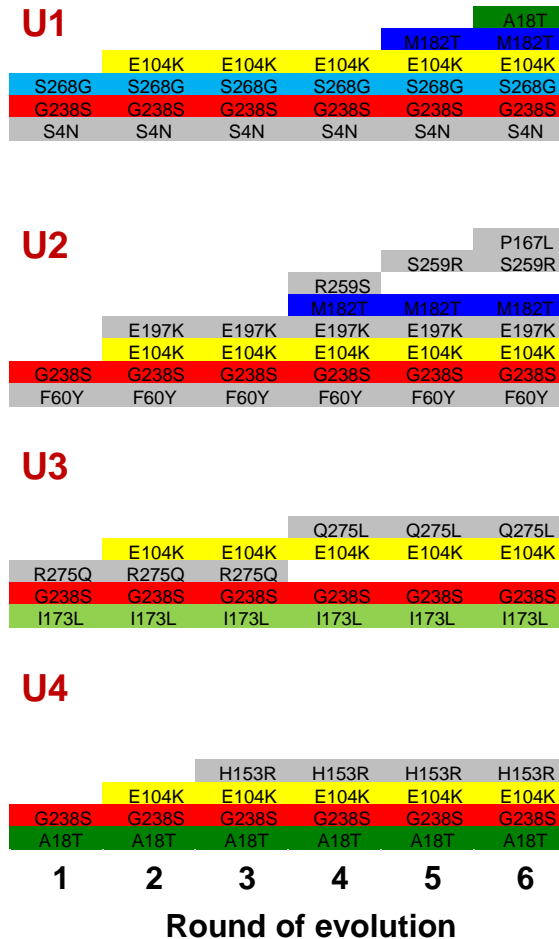
Merijn Salverda



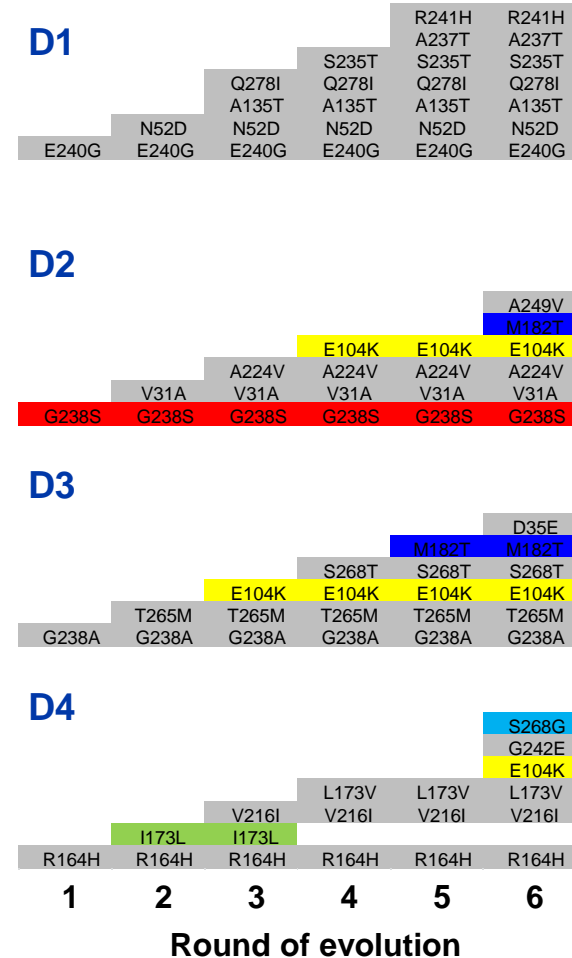
# IV. Small populations fix diverse mutations

## Amino acid substitutions

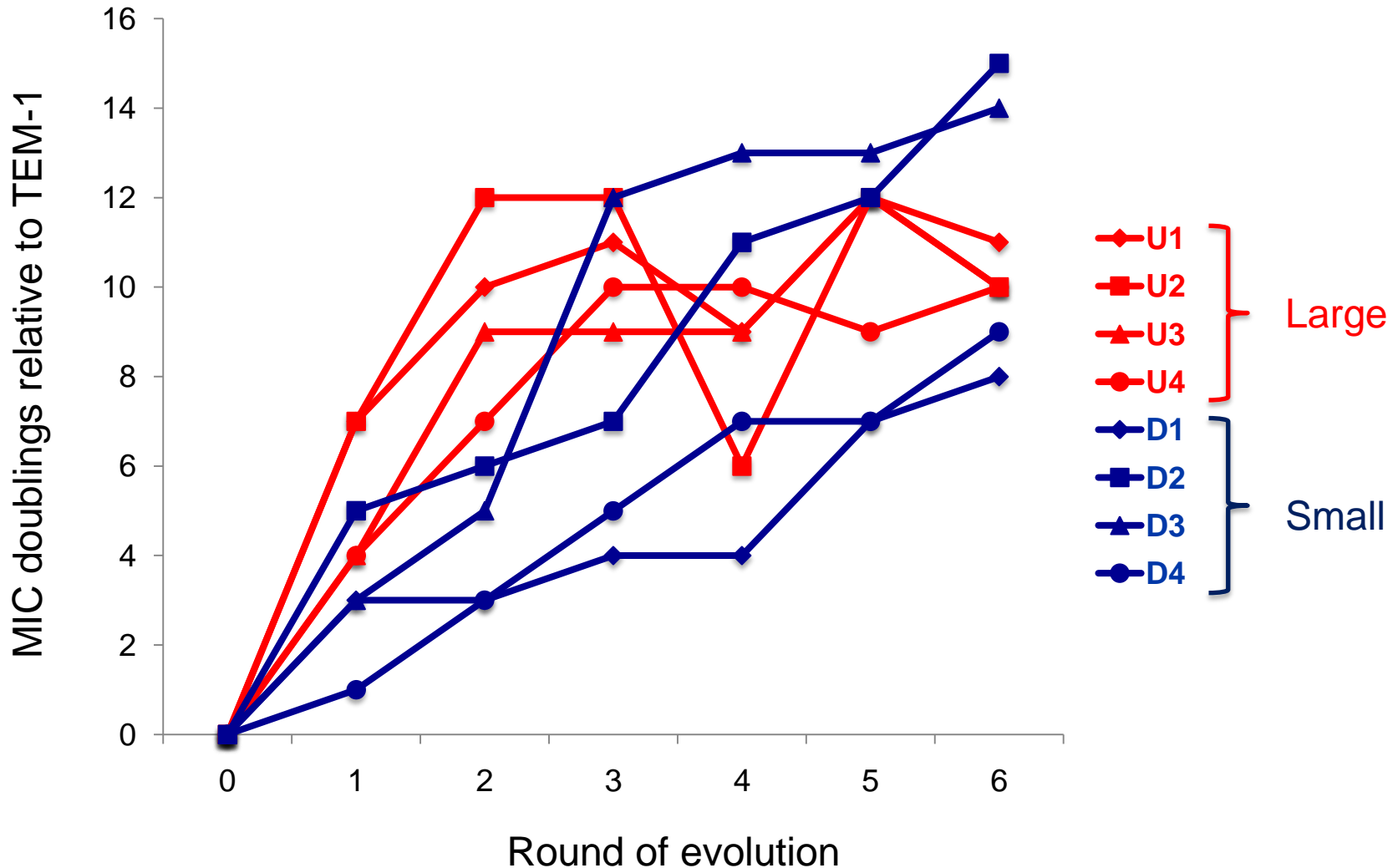
### Large populations



### Small populations

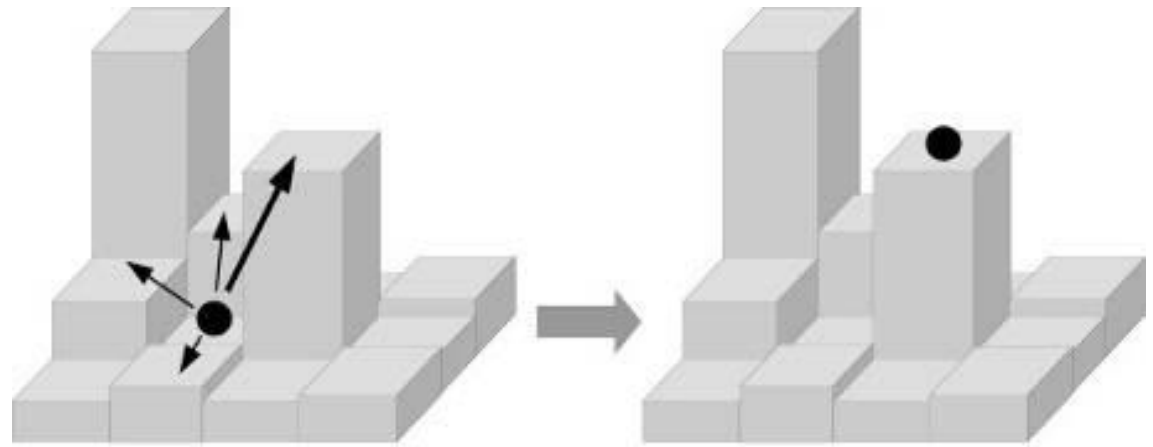
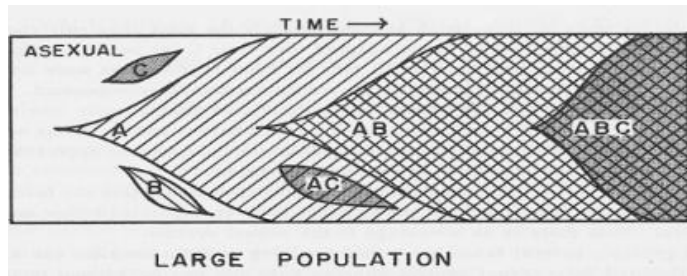


# IV. Small populations adapt slower initially, but sometimes end higher

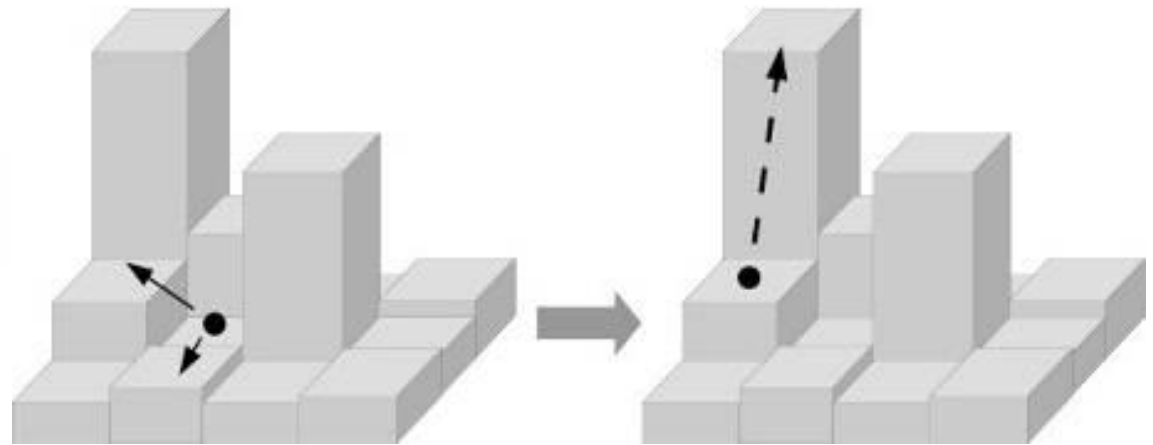


# IV. Chance events facilitate adaptation

large pop



small pop



(From Handel & Rozen 2009 *BMC Evol. Biol.*)

# Summary & outlook

- ~3% of all point mutations in TEM-1  $\beta$ -lactamase is **beneficial** in presence of Ctx, including **synonymous** and **exceptional-benefit** ones
- These mutations show **pervasive sign epistasis**, particularly if (i) their effects are large, and (ii) only their individual benefit is known
- A simple **nonlinear dependence of resistance on phenotypes displaying tradeoffs** can explain observed epistasis
- **Factors enhancing parallel evolution**: (i) mutations of exceptional benefit, (ii) epistatic constraints, and (iii) large population size
- Epistasis constrains number of accessible pathways, **but also the rate of adaptation?**
- **Chance events** in small populations may increase adaptive variability on rugged fitness landscapes and probability to find new maxima

# Acknowledgement

- Merijn Salverda
- Martijn Schenk
- Ivan Szendro
- Joachim Krug
- Fons Debets
- Eynat Dellus
- Florian Gorter
- Rolf Hoekstra
- Jeroen Koomen
- Bertha Koopmanschap
- Dan Tawfik
- John van der Oost
- Mark Zwart

