Divergent genetic evolution employs the same molecular mechanisms

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C. Frøhlich[I], A. Bunzel[II], K. Buda[III], N. Tokuriki[III]

[I]UiT The Arctic University of Norway, Tromsø, Norway, [II]ETH, Zurich, Switzerland, [III]UBC, Vancouver, Canada

Epistasis, defined as the non-additive impact of mutational effects, profoundly influences the adaptive landscapes of antimicrobial resistance (AMR) enzymes. Consequently, comprehending the molecular mechanisms governing epistasis and the evolutionary conservation of these effects across various adaptive pathways is imperative for predicting evolutionary outcomes and the resulting resistance phenotypes.
Using directed evolution, we investigated the extent to which the evolution of the β-lactamase OXA-48 toward the β-lactam ceftazidime is reproducible. We unveil that by repeating evolution three times, adaptation proceeded through completely distinct genotypic trajectories while reaching similar phenotypic maxima. Characterization of constructed fitness landscapes, comprising more than 150 mutational combinations, demonstrated that resistance development predominantly occurred through positive pairwise epistasis. We showed that epistasis was driven by changes in catalytic efficiency with the selection of an enzymatic burst phase. While ceftazidime binding is rate-limiting in the wild-type enzyme, the molecular mechanisms driving resistance through pairwise epistasis were highly conserved within each trajectory and driven by the interplay of improvements in substrate binding and catalysis. To assess cross-trajectorial compatibility, we combined mutations that improved either binding or catalysis from all trajectories. Combining only catalysis or binding enhancers did not lead to a boost in resistance. In contrast, 75% of the combinations that positively stimulated both binding and catalysis acted highly synergistically, significantly driving AMR.
While AMR can evolve through entirely distinct trajectories, we demonstrate that the molecular mechanisms by which epistasis leads to changes in susceptibility can be highly conserved. Our work emphasizes the dire need to understand the driving forces of epistasis, as this knowledge is crucial for predicting AMR development.