Combining theory and experiment to explain the evolution of antibiotic resistance

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Project description:
Beta-lactamases are enzymes that hydrolyse beta-lactams (e.g. penicillin); and are the most important resistance mechanism to these most important antibiotics. Beta-lactam resistance has been described as an "arms race" whereby introduction of new beta-lactams insusceptible to known enzymes leads to appearance of new enzymes or variants active against them. Sometimes, mutation at specific positions around the active site generates these new activities by modifying the catalytic mechanism or substrate binding pocket. However, in others changes at positions remote from the catalytic centre exert profound effects upon activity previously explained in terms of global stability and/or dynamics. Rationalising the effects of such remote mutations would both explain the activity of existing beta-lactamase variants and, potentially, predict how beta-lactamases might evolve to overcome new agents. Here we seek to apply recent advances in the fundamental understanding of enzymes (Macromolecular Rate Theory, MMRT) to study known beta-lactamase variants, responsible for antibiotic failure, with mutations remote from the active site, with the aim of explaining the evolution of this key resistance mechanism. MMRT (Arcus et al, Biochemistry, 2016) explains the temperature dependence of enzyme activity. Enzyme-catalysed reactions show an optimal temperature at which reaction rate is fastest and lose activity at temperatures below, or above, this value. At high temperatures this was ascribed to enzyme denaturation; i.e. thermal unfolding. However, experiments show this to be incorrect- many enzymes lose activity at increased temperatures but show no evidence of unfolding. MMRT holds that the activation heat capacity (change in heat capacity between ground and transition states) accounts for this behaviour and determines the optimum temperature for an enzyme-catalysed reaction. Excitingly, we have shown (Nature Communications 2018) that high-level molecular dynamics (MD) simulations can calculate activation heat capacities in agreement with experimental data, and further identify specific dynamic changes that account for this behaviour. Here we will investigate the temperature dependence of beta-lactam hydrolysis by known beta-lactamase variants to experimentally determine the activation heat capacity of reaction. This will be compared with values predicted by computational simulations, that will also explain how mutations in these variants exert their dynamic effects. The results will explain how remote mutations may modify activity towards specific antibiotic substrates, and thus understand how beta-lactamases may evolve in response to antibiotic pressure. The project will provide training in experimental (enzyme kinetics and X-ray crystallography) and computational (MD and quantum mechanics simulations) enzymology in the context of research into antimicrobial resistance.