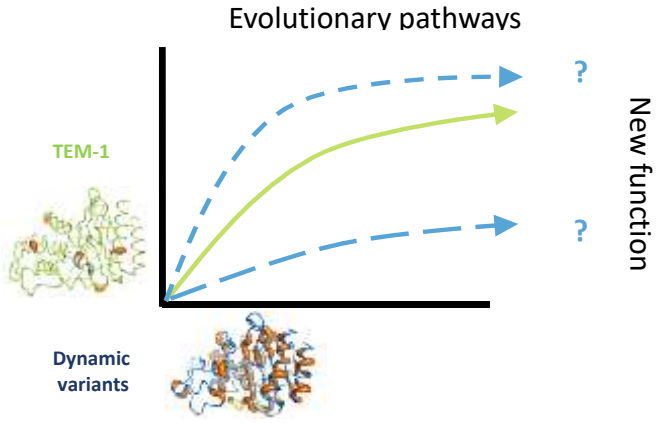
PROTEIN DYNAMICS AT SLOW TIMESCALES IN ENGINEERED ß-LACTAMASES DOES NOT LIMIT EVOLVABILITY

Lorea Alejaldre, Université de Montréal, Canada
l.alejaldre.ripalda@umontreal.ca
Ferran Sancho, Barcelona Supercomputing Center, Spain
Claudèle Lemay-St-Denis, Université de Montréal, Canada
Adem H-Parisien, Université de Montréal, Canada
Victor Guallar, Barcelona Supercomputing Center, Spain
Joelle N. Pelletier, Université de Montréal,Canada

Key Words: Epistasis, protein dynamics, ß-lactamase

Understanding the underlying mechanisms in the evolution of new protein functions is key to better directing enzyme engineering efforts. Intragenic epistasis (the non-additive interaction of mutations affecting function) is a key feature of protein evolution. For example, in TEM-1 ß-lactamase, the mutations E104K and G238S show positive epistasis in that their combination show a greater than expected increase in antibiotic resistance[1]. Here, we aim to understand the impact of protein dynamics at slow timescales on epistasis. Large conformational rearrangements associated with ligand-binding, turnover of substrate or allostery occur at this timescale[2]. The readily evolvable antibiotic-resistant TEM-1 ß-lactamase and two of its variants that are more dynamic at slow timescales serve as models for this study. Our models show similar catalytic activity and substrate recognition, thermal stability, as well as conserved motions in fast (ps-ns) and intermediate (ns-μs) timescales but different motions at slow timescales (μs-ms)[123].

In this study, we use two different approaches to examine the effect of protein dynamics on epistasis. First, we introduced the epistatic mutations E104K and G238S into our dynamic variants. These mutations confer high resistance to the antibiotic cefotaxime in TEM-1, increasing catalytic efficiency ~250-fold. The dynamic variants present similar kinetic values and increase in catalytic efficiency as does TEM-1 when the epistatic mutations are introduced. Molecular dynamic simulations in the presence of cefotaxime support these observations, as the presence of the epistatic mutations correlates with an increase in catalytically-competent conformers. Secondly, we performed directed molecular evolution in TEM-1 and its dynamic variants towards the hydrolysis of the antibiotic cefotaxime. We examined whether mutational pathways accessible to TEM-1 are also available in the context of increased dynamics at the timescale of turnover.
Overall, our work highlights that protein dynamics at slow timescales does not hinder the evolution of new activity in TEM-1 ß-lactamase engineered variants. Furthermore, epistasis can be maintained despite differences in dynamics at slow timescales.



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