Proteins at the edge: How protein activity and stability correlate to fitness

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Abstract

Changes in protein structure at the atomic level are the basis for protein evolution and are critical for adaptation. Continuous evolution of bacterial populations can be used to determine mutational pathways and adaptive mechanisms of increased fitness during natural selection. A more precise understanding of the biophysical origins of fitness is central to predicting evolutionary outcomes.

In vitro biophysical analysis of the five double-mutant enzymes correlates to *in vivo* fitness. Folding studies show a general trend of decreased unfolding rate compared to Q199R, with the exception of A193V which also exhibits increased folding rate. Activity assays that monitor ADP production reveal that higher activity at specific temperatures correlates with the success of the strains at their respective temperatures. The activity profiles correspond so closely to the success of the mutants over the range of temperatures that activity may serve as a proxy for fitness. Crystal structures of the mutant enzymes reflect a diverse set of mechanisms by which stability is increased and activity is maintained. Together, these mutants illustrate both great and subtle adaptations produced through the smallest unit of evolutionary change.

Background

In the '**weak link**' approach, a functional and **essential** gene within the chromosome of the organism is replaced by a maladapted ortholog. A weak link was created by replacing the native adenylate kinase (*adk*) gene of *Geobacillus stearothermophilus*, a thermophile, with that of *Bacillus subtilis*, a mesophile, to produce the strain NUB3621-R:ThEV. Adenylate kinase is essential to adenylate homeostasis within the cell (Figure 1). Introducing the weak link into *G. stearothermophilus* produces a **temperature-sensitive** phenotype unable to survive at higher temperatures that would otherwise be habitable (Figure 2). Within a population undergoing selection for growth at higher non-permissive temperatures (Figure 3), mutations in *adk* facilitate growth by increasing enzyme activity and stability. The initial mutant, Q199R, rose to fixation and served as the background for all double-mutants that followed (Figure 4).

$$\text{ATP} + \text{AMP} \underset{}{\overset{\text{AK}}{\rightleftharpoons}} \text{ADP} + \text{ADP} \qquad \text{Energy Charge} = \frac{[\text{ATP}] + \tfrac{1}{2}[\text{ADP}]}{[\text{AMP}] + [\text{ATP}] + [\text{ADP}]}$$

Figure 1. **The Weak Link** Adenylate Kinase (AK) is essential to maintain adenylate homeostasis, the energy charge the cell uses to do work.

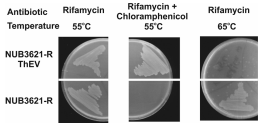


Figure 2. **Temperature-sensitive phenotype of NUB3621-R:ThEV cells** Chloramphenicol is used to select for recombination events that replaces the endogenous *adk*.

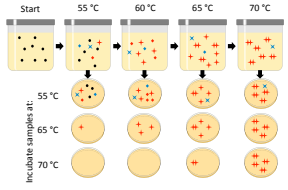


Figure 3. **Continuous evolution in a turbidostat.** The progenitor strain was grown for 48 hrs at 55 °C (permissive), after which the temperature was increased 0.5 °C/day to 70 °C.

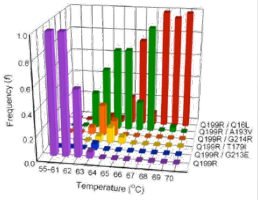
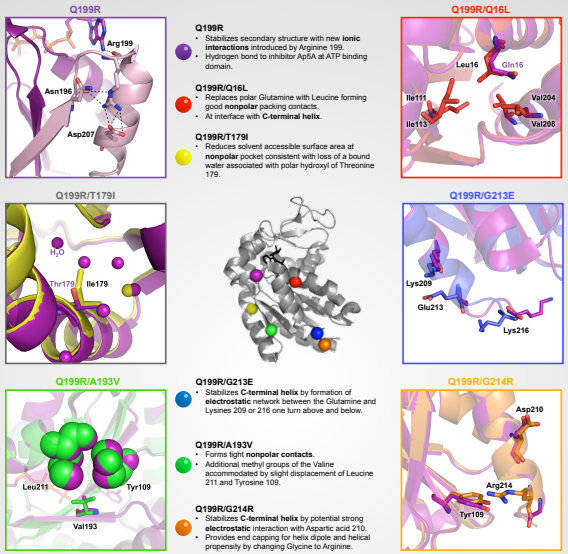


Figure 4. **Frequency of AK mutants in the turbidostat as a function of temperature.** For this histogram, 242 strains were isolated and sequenced.

Structure



Q199R
- Stabilizes secondary structure with new **ionic interactions** introduced by Arginine 199.
- Hydrogen bond to inhibitor Ap5A at ATP binding domain.

Q199R/Q16L
- Replaces polar Glutamine with Leucine forming good **nonpolar** packing contacts.
- At interface with **C-terminal helix**.

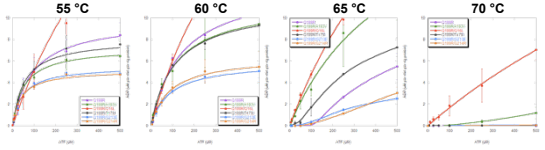
Q199R/T179I
- Reduces solvent accessible surface area at **nonpolar** pocket consistent with loss of a bound water associated with polar hydroxyl of Threonine 179.

Q199R/G213E
- Stabilizes **C-terminal helix** by formation of **electrostatic** network between the Glutamine and Lysines 209 or 216 one turn above and below.

Q199R/A193V
- Forms tight **nonpolar contacts**.
- Additional methyl groups of the Valine accommodated by slight displacement of Leucine 211 and Tyrosine 109.

Q199R/G214R
- Stabilizes **C-terminal helix** by potential strong **electrostatic** interaction with Aspartic acid 210.
- Provides end capping for helix dipole and helical propensity by changing Glycine to Arginine.

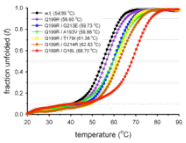
Activity



Effect of substrate concentration on the initial velocity of ADP production at various temperatures.

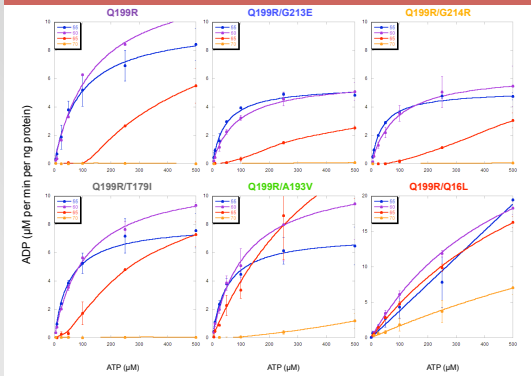
- **The success of a particular mutation is reflected in its ability maintain activity where others fail.**
- Q199R/Q16L is the only mutant isolated at 70 °C. It is also the only mutant with significant activity at 70 °C.

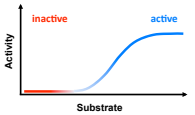
Stability



Unfolding of the mutant AK proteins were monitored by changes in circular dichroism at 220 nm. **All mutants showed increased stability compared to Wild-Type *B. subtilis* AK.**

Kinetics





Straying from Michaelis-Menten kinetics
Sigmoidal (yet not cooperative) kinetic curves are observed at 65 and 70 °C for all mutants except Q199R/Q16L.

Conclusions

- **Crystal structures of mutants display different stabilization mechanisms that include new electrostatic interactions (Q199R), stabilization of the C-terminal helix (G213E and G214R), and nonpolar packing (Q16L and T179I).**
- **Kinetic studies reveal activity profiles that correspond closely to success of the mutants in the turbidostat. Can activity be used as a proxy for fitness in simulation?**
- **Kinetics of enzymes on the precipice of stability stray from Michaelis-Menten kinetics. Can we model the sigmoidal kinetics?**

References

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Funding

This work was supported by the **National Science Foundation** (0212417/0641792) and the **Welch Foundation** (C1584)