Optimization of lag phase shapes the evolution of a bacterial enzyme

Bharat V. Adkar[1], Michael Manhart[1], Sanchari Bhattacharyya[1], Jian Tian[1,2], Michael Musharbash[1], Eugene I. Shakhnovich[1*]

[1]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford St., Cambridge, MA 02138, USA

[2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing, 100081, China

[*]Correspondence should be addressed to E.S. (shakhnovich@chemistry.harvard.edu)

Abstract

Mutations provide the variation that drives evolution, yet their effects on fitness remain poorly understood. Here we explore how mutations in the essential enzyme Adenylate Kinase (Adk) of *E. coli* affect multiple phases of population growth. We introduce a biophysical fitness landscape for these phases, showing how they depend on molecular and cellular properties of Adk. We find that Adk catalytic capacity in the cell (product of activity and abundance) is the major determinant of mutational fitness effects. We show that bacterial lag times are at a well-defined optimum with respect to Adk's catalytic capacity, while exponential growth rates are only weakly affected by variation in Adk. Direct pairwise competitions between strains show how environmental conditions modulate the outcome of a competition where growth rates and lag times have a tradeoff, altogether shedding light on the multidimensional nature of fitness and its importance in the evolutionary optimization of enzymes.

Introduction

Random mutagenesis is often used to assess the distribution of fitness effects in simple experimental models such as propagating viruses and microbes evolving under antibiotic stress[1,2]. However, the enormous size of sequence space severely constrains how much of the fitness landscape can be explored this way, and mechanistic and predictive insights from these experiments are further limited by a lack of knowledge of the molecular effects of mutations. Instead, a more targeted experimental approach relies on the concept of a biophysical fitness landscape, in which fitness effects of mutations are mapped through their effects on molecular traits of the mutated proteins. In this approach, biophysically-rational genetic variation is introduced on the chromosome, and the molecular and phenotypic effects of that variation are analyzed concurrently[3-6]. By mapping fitness effects to variation of molecular properties rather than to sequences of mutated proteins, we can dramatically reduce the dimensionality of the genotype-to-phenotype mapping. The underlying hypothesis is that variation in a small number of properly-selected molecular traits of mutated proteins can explain most of the resulting mutational variation in fitness, and that the relationship between these molecular traits and fitness is smooth and continuous. Several recent studies have supported this approach[5-7].

The relationship between sequence variation and fitness is further confounded by the fact that multiple life-history traits contribute to fitness[8], and the relative importance of these traits to the long-term evolutionary fate of a mutation may be highly dependent on environmental and ecological conditions. While multicellular organisms are generally described by a large number of traits (e.g., viability at various life phases, mating success, fecundity, etc.), unicellular microorganisms like bacteria and yeast are described by relatively fewer components of fitness, such as the time in lag phase, the exponential growth rate, and the overall yield at saturation. All these phases of growth contribute toward the outcome when in competition for limited resources, and hence determine fitness[3,9]. The relative importance of these different phases of bacterial growth in sculpting the fitness landscape depends on the conditions of growth and competition[10-12].

Overall, the challenge in quantitatively characterizing the fitness landscape is twofold: Understanding fitness in terms of contributions from different phases of growth, and linking each of these components to molecular and cellular traits. In this work, we address both challenges by

55 introducing biophysically-rational genetic variation in the *adk* locus that encodes the essential
56 *E. coli* enzyme Adenylate Kinase (Adk), and projecting the ensuing variations of fitness effects
57 (phenotypic components like growth rate and lag time) onto the biophysical traits of Adk. We
58 find that a unique combination of molecular and cellular traits of Adk — the product of
59 intracellular abundance and catalytic activity, which we term catalytic capacity — provides a
60 reliable predictor of fitness effects across the full range of phenotypic variation. Furthermore, we
61 find that the length of the lag phase is more sensitive to variation in Adk catalytic capacity than
62 is the exponential growth rate, so that the lag phase of the wild-type *E. coli* appears to be optimal
63 with respect to variation in Adk catalytic capacity.

64 **Results**

65 ***Biophysical properties of Adk mutants***

66 Destabilizing mutations have been shown to cause a drop in intracellular protein abundance,
67 mostly through a decrease in the folded fraction of the protein[3]. Hence in order to sample a broad
68 range of molecular and cellular traits of Adk protein below the wild-type levels, we chose a set
69 of 21 missense mutations at 6 different positions of *adk*. (Table S1 and Fig. 1). We selected
70 residues such that their accessible surface area was less than 10% and they were at least 6 Å
71 away from the catalytically-active sites of Adk, so that mutations at these residues were likely to
72 destabilize the protein[13]. For most mutants, we chose amino acid mutations that appeared only at
73 low frequency in an alignment of 895 homologous sequences of Adk. As intended, the purified
74 mutant proteins were destabilized over a wide range (~17 °C in terms of T_m, and ~7.5 kcal/mol
75 in terms of folding ΔG) (Table S1, Figs. 1B, S1, S2). In only one case (L209I) did we change
76 the *E. coli* sequence to the consensus amino acid at that position, and we found it in fact
77 stabilized the protein by ~1 kcal/mol (Table S1). Although most of the Adk mutants were less
78 stable than the wild-type (WT), they nevertheless existed predominantly as monomers in solution
79 (Fig. S3). However, several mutations in one position — V106H, V106N, and V106W — did
80 have significant fractions of proteins present in higher oligomeric forms, in addition to the
81 predominant monomeric species (Fig. S3). These proteins bound 4,4'-Dianilino-1,1'-binaphthyl-
82 5,5'-Disulfonate (Bis-ANS) dye to a higher degree compared to the rest of the mutants (Fig. S4),
83 indicating the presence of possible molten globule states in solution[14]. The proteostat dye that
84 reports on protein aggregation[4,15] also bound these mutants more strongly compared to others

85 (Fig. S4), clearly indicating a higher fraction of aggregated species. The catalytic efficiency
86 (k_{cat}/K_M) of the mutant Adk proteins was distributed broadly with most mutants showing a
87 lower activity than *E. coli* WT (Table S1, Figs. 1C, S5).

88 ***Intracellular abundance of Adk follows prediction from Boltzmann distribution***

89 We then incorporated each of the 21 *adk* mutations one-by-one into the *E. coli* chromosome
90 using a genome-editing approach based on homologous recombination[3,4]. We measured the *total*
91 intracellular abundance of WT and mutant Adk proteins using a quantitative western blot
92 (Table S2). The sigmoidal dependence of total intracellular Adk abundance on folding stability
93 (ΔG) (Fig. 1D) is well-described by the Boltzmann distribution for two-state unfolding proteins:

94 $$P_F = \frac{1}{1+\exp(\beta \Delta G)} \quad (1)$$

95 where P_F is the fraction of folded molecules in the ensemble of intracellular Adk and
96 $b = 1/k_B T$, with Boltzmann constant k_B and growth temperature T. The total measured
97 abundance of a protein is its amount in the cytoplasm at steady-state, achieved by a balance
98 between production and degradation. Since Adk is expressed from a constitutive promoter in the
99 cells, it is generally safe to assume that the rates of production of all mutants are similar. Under
100 this assumption, the sigmoidal dependence of abundance on stability clearly indicates that the
101 unfolded protein is degraded in the active medium of the cytoplasm.

102 ***Mutations in Adk affect lag times more than exponential growth rates***

103 Mutations in Adk affect both intracellular abundance (via folding stability) and catalytic activity
104 of the protein. Flux dynamics theory predicts, and experiments have confirmed, that the key
105 enzymatic parameter determining the flux through an enzymatic reaction chain is the quantity
106 which we call "catalytic capacity," defined as the product of intracellular abundance and
107 enzymatic efficiency k_{cat}/K_M [5,6,16]. To that end, we determined how two key components of
108 bacterial growth — the exponential growth rate and the lag time (Fig. 2A) — depend on the total
109 catalytic capacity of Adk in *E. coli* cells (Fig. 2B,C; also see *Methods* and Fig. S6-S8 for
110 estimation of growth parameters). We find that while only 3 out of 21 strains show a drop in

growth rate greater than 5% of WT, 17 strains show an increase in lag time for a similar change over the WT value (Table S2). This suggests that the mutations in Adk affect the lag phase more significantly than the exponential growth phase. One mechanism for producing longer apparent lag times is when a greater proportion of cells that come out of stationary phase are simply nonviable, as described in a recent study[17]. However, this appears not to be the major cause in our case, as lag times are fairly consistent across replicates (error bars in Fig. 2C) and do not negatively correlate with the number of viable cells (Fig. S9). We also find that the variation in total catalytic capacity of Adk correlates better with the variation in lag times (Spearman's rank correlation $\rho = -0.44$, $p = 0.057$) than with the variation in growth rates (Spearman's rank correlation $\rho = -0.08$, $p = 0.737$) (Fig. S10). The variation in lag times is also better explained by the variation in catalytic capacity than with any of the Adk properties separately (stability, abundance, or activity) (Fig. S10). Surprisingly, growth rate appears to tolerate a rather large drop in catalytic capacity of Adk, while lag time does not.

WT E. coli is positioned at the cusp of the biophysical fitness landscape for lag time

Since almost all the designed mutants were destabilizing and therefore have lower catalytic capacity than *E. coli* WT, they only provide sampling in the lower range of catalytic capacity. In studies so far, no evidence exists for changes in intracellular protein abundance for stabilizing mutations. Hence to determine the dependence of growth rate and lag time on catalytic capacity above WT levels, we over-expressed WT Adk from a pBAD plasmid (see Supplementary Methods). We observed no significant change in either growth rate or lag time at higher than endogenous catalytic capacity (Fig. 2B,C, S8, and Table S3). This means that while the growth rate appears to be insensitive to large changes in Adk catalytic capacity both below and above the wild-type level, WT catalytic capacity appears to be situated at the threshold of optimizing lag time. Next, we attempted to quantitatively compare the position of WT on these two fitness landscapes. To that end, we used a simple reciprocal Michaelis-Menten-like function to fit the relative growth times (growth time is reciprocal of growth rate μ) and lag times (Fig. S11, also see Eq. 3 and *Methods*). The fitting parameter b which characterizes the onset of curvature on the landscape (analogous to K_M in Michaelis-Menten equation for enzymatic rate) reports proximity of WT to the cusp on the landscape (see *Methods*). It was 0.006 for growth time and

140 0.019 for lag time as compared to normalized catalytic capacity of 1 for WT. This shows that
141 WT is situated closer to the cusp in terms of lag time as compared to growth time or growth rate.

142 ***Shorter lag imparts advantage at low carrying capacity: A computational model***

143 This data highlights the pleiotropic effects of mutations on different phases of bacterial
144 population growth, which raises the question of how pleiotropy shapes the evolutionary fate of a
145 mutation. We explore this issue by considering the outcome of binary competitions between
146 strains[18]. We first simulated binary competitions over a wide range of growth rates and lag times
147 in media conditions that allow for either 5-fold (low carrying capacity) or 500-fold (high
148 carrying capacity) increase over the initial population (Fig. 3A) (See *Methods*). We found that
149 there is a significant tradeoff between lag times and growth rates in determining the winners of
150 binary competitions, with lag playing a more important role at low carrying capacity (Fig. 3A),
151 implying that beneficial lag provides a greater fitness advantage under strongly nutrient-limiting
152 conditions.

153 ***Shorter lag imparts advantage at low carrying capacity: Experimental evidence***

154 To realize varying nutrient conditions in binary competition experiments, we explored the
155 growth of *E. coli* over a range of glucose concentrations, mimicking the variation of carrying
156 capacity in simulations, and found that only the carrying capacities are proportional to glucose
157 concentration with minimal effects on lag time and growth rate (Fig. 4). This suggests that
158 observing the outcome of the competition at different time snapshots in a nutrient-rich medium is
159 equivalent to running the competition at different glucose concentrations (carrying capacities).
160 To evaluate the predictions from simulations, we carried out two sets of binary competition
161 experiments based on the overall distribution of growth rates and lag times (Fig. 3B). First, we
162 selected strains exhibiting a tradeoff between growth rate (μ) and lag time (λ) ($\mu_1 > \mu_2$ and
163 $\lambda_{,1} > \lambda_{,2}$) (inset of Fig. 5B). Second, we tested competition between strains that differ in their lag
164 times but have nearly indistinguishable growth rates ($\mu_1 \approx \mu_2$ and $\lambda_1 > \lambda_2$) (inset of Fig. 5C). In
165 all cases a strain with shorter lag time is expected to dominate at lower carrying capacity
166 conditions (corresponding to the competition outcome at early time points), however this
167 advantage would be lost at later time points if its growth rate is lower than that of the competing
168 strain (Fig. 5A). In the second scenario, the advantage due to short lag is expected to persist even

169 at high carrying capacity conditions because the growth rates of the competing strains do not
170 differ. We estimated the relative proportions of the two strains by a qPCR-based mismatch
171 amplification mutation assay (MAMA) approach[19] (see *Methods* and Fig. S12). As expected in
172 the first scenario, L083F and V106H dominated at earlier time points when competed against
173 A093I and L209I, respectively, due to their shorter lag times ($\lambda_{L083F} < \lambda_{A093I}$ and $\lambda_{V106H} < \lambda_{L209I}$)
174 (Fig. 5B). Eventually their fraction dropped below 0.5 at later time points (equivalent to high
175 carrying capacity) where the growth rates determine the competition output ($\mu_{L083F} < \mu_{A093I}$ and
176 $\mu_{V106H} < \mu_{L209I}$) (Fig. 5B). Similarly, for the second scenario, despite having similar growth rates
177 ($\mu_{WT} \approx \mu_{Y182V} \approx \mu_{L209A}$), the fraction of WT was always maintained above 0.5 as it spends a
178 shorter time in the lag phase compared to Y182V and L209A (Fig. 5C). The early advantage to
179 WT due to its shorter lag phase determined the competition fitness throughout the whole growth
180 cycle.

181 Discussion

182 A complete mapping of mutational fitness effects would require sampling a practically infinite
183 number of mutations, an impossible proposition. Instead, we can project fitness onto a fairly
184 small number of molecular properties of proteins[5-7,20]. Within this paradigm, the identity of a
185 particular mutation does not matter as much as its effect on essential biochemical and
186 biophysical properties of the proteins in question. Our 21 engineered mutations in Adk, along
187 with the overexpression data, allow us to outline the biophysical fitness landscape, covering a
188 wide range of variation of the physical parameters of the Adk protein. This data shows that we
189 can collapse several molecular phenotypes into a single effective parameter – the product of
190 protein abundance and activity k_{cat}/K_M (catalytic capacity) – which quantitatively determines
191 the biophysical fitness landscape to a great extent (Fig. 2B,C). That is, Fig. 2 indicates that the
192 fitness effects of mutations can largely be predicted from their biophysical effects over a broad
193 range of catalytic capacity. Indeed, Adk catalytic capacity explains the variation in lag times to a
194 large extent (Fig. S10), validating the utility of a biophysical fitness landscape for mapping
195 fitness effects.

196 These results illustrate how the evolutionary endpoint of molecular traits may depend
197 fundamentally on the multidimensional nature of fitness, with the relative importance of different

198 components of fitness depending on the environment and lifestyle of the organism. It has been
199 argued that endogenous molecular traits are established as a result of mutation-selection
200 balance[21], with the final outcome depending on the relative strengths of selection and genetic
201 drift as determined by the population structure[22,23]. Here we encounter a more complex situation
202 where mutations in the essential enzyme Adk change multiple fitness components. In this case,
203 the mutation-selection balance apparently resulted in disparate outcomes for the two fitness
204 components with respect to the molecular trait, placing lag time at the cusp while keeping the
205 exponential growth rate farther within the plateau region of its respective biophysical fitness
206 landscape. Such an outcome may reflect different strengths of selection on growth and lag. The
207 relative strength of selection on these fitness components depends crucially on the environmental
208 conditions (e.g. nutrient availability, etc.)[24]. Our studies of binary competitions (Figs. 3 and 5)
209 highlight this scenario by showing how the environmental parameter of carrying capacity can
210 determine winners and losers in evolutionary dynamics. Although the lag time of a population
211 can depend not only on the environment but also on the population's specific history (e.g., how
212 long it was previously in stationary phase), the fundamental role of Adk in metabolism suggests
213 that its effects on lag time are likely to be common across conditions and histories. The deep
214 connection between ecological history of species and optimization of biophysical traits of their
215 proteins is a subject for valuable future studies.

216 Much of our current understanding of microbial cultures and fitness comes from experiments
217 done in the laboratory, where strains are typically grown under a large supply of nutrients. The
218 situation might be very different in the wild, however, where bacteria and other microbes have to
219 survive under harsh conditions of nutrient starvation, extreme temperature, and other
220 environmental stresses[25-27]. For example, *E. coli* is the predominant facultative anaerobe in the
221 gastrointestinal tract[28] which allows it to thrive in fluctuating environments of differing oxygen
222 concentrations along the GI tract (e.g., the small vs. the large intestine). In these circumstances,
223 organisms are likely to spend only a minute fraction of their life-cycle in the exponential growth
224 phase, while undergoing many cycles of lag-growth-saturation as new resources become
225 available and old ones are exhausted. It is therefore intuitive to expect that there has been strong
226 selection in favor of organisms that can not only divide rapidly during exponential growth, but
227 that can also wake up quickly from their lag phase and respond to newly available resources. Our
228 study demonstrates how this selection may shape individual molecular traits.

229 This work highlights the relationship between various components of fitness and the molecular
230 properties of modern enzymes — the endpoint of evolutionary selection. An interesting question
231 which is beyond the scope of current work is how modern variants emerged in evolutionary
232 dynamics. To that end mapping temporary reconstructed ancestral species onto biophysical
233 fitness landscape of Adk (and other enzymes) appears a promising direction of future research.

234

235 **Methods**

236 Selection of mutations: Mutations at relatively-buried positions generally result in decreased
237 stability and lower fitness[13,29]. Hence we selected the sites for mutagenesis with side-chain
238 accessibility of less than 10%. In addition, the selected sites were also away from the active-site
239 residues, or active-site contacting residues, and a minimum of 6 Å away from the inhibitor Ap5A
240 binding sites (pdb 1ake). The structure of Adk is divided into three domains: LID (residues 118-
241 160), NMP (residues 30-67), and Core (residues 1-29, 68-117, and 161-214). We define the
242 active-site residues as those whose accessible surface area changes by at least 5 $Å^2$ in the
243 presence of the inhibitor Ap5A. A similar criterion was used to define the residues contacting the
244 active site. Altogether 4 residues from the LID domain, 3 from the NMP domain, and 28 from
245 the Core domain satisfy these criteria. Of the 28 sites from the Core domain, we randomly chose
246 6 to mutate. We chose the identities of the mutations to span various sizes of the side chains and
247 a range of conservation. We derived the conservation from the multiple sequence alignment of
248 895 sequences for Adk collated from ExPASy database (as of Nov 2012).

249 Generation of mutant strains: We generated the strains with WT and mutant *adk* with
250 chloramphenicol- and kanamycin-resistance genes on either end of the *adk* gene using the
251 genome editing approach as described previously[3]. Since the *adk* gene is flanked by two repeat
252 regions (REPt44 and REPt45) on the wild-type chromosome, we extended the homology
253 required for recombination up to the middle of the adjacent genes.

254 Growth curve measurements and media conditions: WT and mutant strains were grown
255 overnight at 30 °C from single colonies in a supplemented M9 medium (0.2 % glucose, 1 mM
256 $MgSO_4$, 0.1 % casamino acids, and 0.5 μg/ml thiamine). OD600 was measured for all the strains
257 and then the cultures were normalized to whichever had the lowest OD. The normalized cultures

were diluted 1:100 in fresh supplemented M9 media and the growth curves were monitored in triplicates using Bioscreen C at 37 °C. We derived the growth parameters by fitting ln(OD) versus time with the four-parameter Gompertz function (see below). The error in replicates was found to be between 2-3% on an average, and it did not improve significantly upon increase in number of replicates.

Fitting growth data and estimation of growth parameters: In our study, we define lag time (λ) as the time required to achieve the maximum growth rate (μ) (Fig. 2A). Growth time (τ) was defined as reciprocal of growth rate μ. Since it has the same units as lag time, it is more convenient to use for the statistical analysis and data fitting (Fig. S11).

We used two different methods to infer these parameters: A) direct analysis of growth curve derivatives and B) fits to the Gompertz function (Fig. S6).

In method A, we took the growth rate as the maximum value of $\frac{\ln\left(OD(t)/OD(t-\Delta t)\right)}{\Delta t}$, where Δt is 15 minutes. The lag time was then the earliest time at which this maximum growth rate was achieved.

For method B we used the following four-parameter Gompertz function to fit ln(OD) vs. time plots:

$$\ln(OD) = \ln(OD_0) + K\exp\left[-\exp\left(-\frac{t-\lambda}{b}\right)\right] \tag{2}$$

where the carrying capacity is K, the maximum growth rate is $\mu = K/\left(b\cdot\exp(1)\right)$, and the lag time λ is the time taken to achieve the maximum growth rate.

For both the methods, we considered only data points with $OD600 \geq 0.02$. The instantaneous derivatives of all growth curves show presence of a distinct peak at OD600 values greater than 0.02 (Fig. S6), indicating monoauxic growth and also asserting that the derived growth parameters are unaffected due to ignoring the lower OD data.

The μ and λ estimated from the two aforementioned methods are strongly correlated (Pearson's $r = 0.80$, $p = 1.4\text{e}{-5}$ for μ, and $r = 0.71$, $p = 3.0\text{e-}4$ for λ) (Fig. S7). However, the uncertainty in the fitted parameters appears to be less than the uncertainty in the parameters obtained from the derivatives, which are limited by the low time-resolution of the experimental data (acquired at an interval of 15 min).

The growth rate (μ) and lag time (λ) appear to be statistically independent of each other across the Adk mutant strains (Spearman's $\rho = 0.31$, $p = 0.15$, Fig. 3B). Hence it is conceivable that selection can act separately on these two traits, which is further illustrated by the different fitness landscapes observed when projected onto the axis of catalytic capacity (Fig. 2B,C).

<u>Statistical tests for mutational variation in growth and lag phases</u>: We estimated the monotonic relationship between various growth traits and molecular/cellular properties of Adk mutant proteins using Spearman's rank correlation (Fig. S10). The agreement between growth parameters derived using instantaneous derivatives and Gompertz fit were estimated by Pearson's correlation coefficient (Fig. S7). We excluded V106N from all statistical analysis and data fitting as its lag time is ~13 s.d. away from the average lag time of all other strains.

<u>Quantification of the location of WT on the fitness landscapes:</u> A Michaelis-Menten-like elasticity curve function has been used previously[5,6,16,20] to fit the dependence of growth rate on catalytic capacity. Since we are considering growth and lag times rather than rates, we use a reciprocal form of the Michaelis-Menten-like function for fitting relative growth time (τ/τ_{WT}) and relative lag time (λ/λ_{WT}) vs. catalytic capacity (Fig. S11):

$$\text{Relative growth trait} = \frac{a \cdot (b + \text{Catalytic Capacity})}{\text{Catalytic Capacity}} \tag{3}$$

where a is the asymptotic value of the trait for infinitely large catalytic capacity, and b is the catalytic capacity when the trait equals twice the asymptotic value $(2a)$. Since catalytic capacity is normalized by WT, b serves as a measure of how close to the cusp the WT on the respective landscapes is. For fits in Fig. S11, we empirically set $a = 1$ which enables easy comparison of parameter b for lag time and growth time plots.

307 Simulation of binary competition: We simulated the competition of two strains by using the
308 Gompertz function (Eq. 2) to model the growth of individual strains. The initial population
309 (OD0) for both strains was equal, and growth ceases when $\Sigma\left(OD_t/OD_0\right)_i = K$, where K is the
310 carrying capacity. We considered two different values of carrying capacities (5 and 500). We set
311 μ_1 and λ_1 to values derived experimentally for WT Adk strain (Table S2), while the growth
312 rates and lag times for the second competing strain were varied randomly across the intervals
313 0.005 to 0.030 min^{-1} (for growth rate) and 50 to 300 min (for lag time).

314 Binary growth competition and quantification: The overnight cultures for individual strains were
315 grown for 16 hours at 30 °C. These cultures were mixed in 1:1 proportion, diluted to an OD of
316 0.01 in fresh supplemented M9 media, and then regrown at 37 °C. The samples were drawn at
317 different time points, and the OD was adjusted to 2.0, either by concentration or dilution. 5 µl of
318 OD 2.0 culture was eventually diluted in 45 µl of lysis solution (QuickExtract DNA extraction
319 solution (Epicentre)) to reach OD 0.2. Genomic DNA extracted from 50 µl of OD 0.2 culture
320 was diluted 5000 times and used as template. The individual strains in the competition were
321 differentially amplified using allele-specific primers and quantified by a qPCR-based mismatch
322 amplification mutation assay method [19] using QuantiTect SYBR Green PCR kit (Qiagen). A 150
323 bp long non-mutagenic amplicon of *adk* gene was amplified as a reference to quantify total
324 genomic DNA. The fraction of the competing strains was determined using the following
325 equation:

326 $$fraction = 2\wedge\left(\left(C_{t,ref} - C_{t,1}\right)_{competition} - \left(C_{t,ref} - C_{t,1}\right)_{pure}\right) \quad (4)$$

327 where C_t represents threshold cycle of qPCR, ref and 1 are the PCR reactions for amplifying
328 the reference and the first allele in competition, while $competition$ and $pure$ represent the
329 condition of culture.

330 Data availability: All raw data for growth curves of *adk* WT and mutant strains, as well as WT
331 overexpression in *E. coli* BW27783 strains, is included as Dataset 1.

332

333 **Figure Legends:**

334 Fig. 1: **Biophysical and intracellular properties.** (A) Crystal structure of Adenylate Kinase
335 from *E. coli* (PDB ID 4ake[30]). The core domain is colored in green, while the LID and NMP
336 domains are shown in white. The C_α atoms of active-site residues are shown in pink, and the blue
337 spheres represent the C_α atoms of the 6 buried positions which were mutated in this study. (B)
338 Histogram showing the distribution of folding free energies for all mutant proteins, as
339 determined by isothermal urea denaturation at 25 °C. The stability of WT is marked by a dashed
340 line. (C) Histogram of the catalytic activity parameter k_{cat}/K_M for all mutants. The dashed line
341 indicates the WT value. (D) Total intracellular abundance of mutant Adk proteins as a function
342 of ΔG at 37 °C. The abundances are normalized by the WT value. Each data point represents the
343 mean and error bars are standard deviation over two experiments. The dashed line represents the
344 fit to the Boltzmann distribution function described in Eq. 1, where k_B was 1.987 cal/mol/K. See
345 related Figs. S1-S5 and Table S1.

346 Fig. 2: **Traits of population growth.** (A) Schematic of estimation of lag time and growth rate.
347 The representative data points (solid gray circles) were plotted as ln(OD) vs time and was fitted
348 to a four parameter Gompertz function (Eq. 2) (cyan line). The red line is a tangent at the
349 inflection point of the function. The slope of the tangent is considered as the growth rate (μ) and
350 the time required to reach the maximum growth rate or the inflection point is taken as the lag
351 time (λ) (vertical dashed line). (B) Relative growth rate (μ/μ_{WT}) and (C) relative lag time
352 $(\lambda-\lambda_{WT})$ as functions of catalytic capacity which is defined as $\text{abundance}\times k_{cat}/K_M$. The
353 mutant data is shown in gray circles, whereas red circles represent the BW27783 strain with
354 varying degrees of overexpression of WT Adk from a pBAD plasmid. Data for WT is shown in
355 green. The data points represent mean and error bars represent standard deviation of parameters
356 derived from growth curves of 3 colonies (biological replicates) in triplicates (9 curves). See
357 related Figs. S6-S11 and Tables S2-S3. The solid gray arrow indicates the direction of increasing
358 fitness.

359 Fig. 3: **Binary growth competition**. The growth of individual strains was modeled as per
360 Gompertz equation (Eq. 2). The growth parameters for strain 1 were fixed to those obtained for

361 WT Adk (dashed gray lines) while those for strain 2 were generated randomly over a wide range
362 of growth rates (0.005 to 0.030 min^{-1}) and lag times (50 to 250 min). (A) Contour plot showing
363 fraction of strain 1 (WT) at saturation when the competition is carried out under two different
364 carrying capacities (red line indicates $K = 5$ while the black line indicates $K = 500$). The dashed
365 lines indicate neutrality region where both strains have equal proportions at saturation. The areas
366 below the neutrality line (filled with solid lines) represent the parameter space where strain 2
367 wins the competition (fraction of strain 2 > 0.5). (B) Scatter plot of growth rate (μ) versus lag
368 time (λ). The data points represent the mean and error bars the standard deviation of 6 to 9
369 measurements (see Table S2). The growth rate and lag time appear to be statistically independent
370 of each other across the Adk mutant strains (Spearman's $\rho = 0.31$, $p = 0.15$).

371 Fig. 4: **Growth curves at various nutrient concentration.** (A) Growth curves of strains with
372 WT Adk obtained under varying glucose concentrations in supplemented M9 medium. The fitted
373 growth curve parameters are shown as functions of glucose concentration: (B) carrying capacity
374 of ln(OD) as derived from Gompertz fitting, (C) relative growth rate $(\mu/\mu_{0.2})$, and (D) relative
375 lag time $(\lambda - \lambda_{0.2})$. The growth rates and lag times are estimated from analysis of growth curve
376 derivatives and are normalized relative to the respective values at 0.2% glucose concentration.

377 Fig. 5: **Tradeoffs between lag and exponential growth in binary competitions.** (A) Fraction
378 of the first strain as a function of time in simulated binary competitions. We modeled growth of
379 each strain using the Gompertz 4-parameter equation (Eq. 2) with experimentally measured
380 growth rate and lag time values. The initial OD for individual strains was assumed to be 0.006 at
381 the start of competition, and growth was assumed to saturate at OD of 0.6. Despite having
382 similar growth rates, the fraction of WT in WT + L209I and WT + Y182V competitions was
383 always above 0.5 owing to the advantage it gained due to shorter lag time (scenario 2 in the text).
384 L083F and V106H dominate at earlier time points (equivalent to low carrying capacities) due to
385 their short lag times compared to their respective competitors. However, at longer times (high
386 carrying capacities) the advantage due to lag is lost due to their lower growth rates. (B, C)
387 Experimental validations of the predictions in (A) using qPCR based mismatch amplification
388 mutation assay (MAMA). The fraction of competing strains was estimated using Eq. 4. The data

points are mean and error bars represent standard deviation of two measurements. See related Fig. S12. The growth rates and lag times for the competing pairs are shown in insets.

392 **References**

393 1 Sanjuan, R., Moya, A. & Elena, S. F. The distribution of fitness effects caused by single-nucleotide
394 substitutions in an RNA virus. *Proceedings of the National Academy of Sciences of the United*
395 *States of America* **101**, 8396-8401, doi:10.1073/pnas.0400146101 (2004).
396 2 Bershtein, S., Segal, M., Bekerman, R., Tokuriki, N. & Tawfik, D. S. Robustness-epistasis link
397 shapes the fitness landscape of a randomly drifting protein. *Nature* **444**, 929-932,
398 doi:10.1038/nature05385 (2006).
399 3 Bershtein, S., Mu, W. & Shakhnovich, E. I. Soluble oligomerization provides a beneficial fitness
400 effect on destabilizing mutations. *Proceedings of the National Academy of Sciences of the United*
401 *States of America* **109**, 4857-4862, doi:10.1073/pnas.1118157109 (2012).
402 4 Bershtein, S., Mu, W., Serohijos, A. W., Zhou, J. & Shakhnovich, E. I. Protein quality control acts
403 on folding intermediates to shape the effects of mutations on organismal fitness. *Mol Cell* **49**,
404 133-144, doi:10.1016/j.molcel.2012.11.004 (2013).
405 5 Bershtein, S. *et al.* Protein Homeostasis Imposes a Barrier on Functional Integration of
406 Horizontally Transferred Genes in Bacteria. *PLoS genetics* **11**, e1005612,
407 doi:10.1371/journal.pgen.1005612 (2015).
408 6 Rodrigues, J. V. *et al.* Biophysical principles predict fitness landscapes of drug resistance.
409 *Proceedings of the National Academy of Sciences of the United States of America* **113**, E1470-
410 1478, doi:10.1073/pnas.1601441113 (2016).
411 7 Gong, L. I., Suchard, M. A. & Bloom, J. D. Stability-mediated epistasis constrains the evolution of
412 an influenza protein. *Elife* **2**, e00631, doi:10.7554/eLife.00631 (2013).
413 8 Orr, H. A. Fitness and its role in evolutionary genetics. *Nature reviews. Genetics* **10**, 531-539,
414 doi:10.1038/nrg2603 (2009).
415 9 Elena, S. F. & Lenski, R. E. Evolution experiments with microorganisms: the dynamics and genetic
416 bases of adaptation. *Nature reviews. Genetics* **4**, 457-469, doi:10.1038/nrg1088 (2003).
417 10 Dykhuizen, D. E. & Hartl, D. L. Selection in chemostats. *Microbiological reviews* **47**, 150-168
418 (1983).
419 11 Biel, S. W. & Hartl, D. L. Evolution of transposons: natural selection for Tn5 in Escherichia coli
420 K12. *Genetics* **103**, 581-592 (1983).
421 12 Fridman, O., Goldberg, A., Ronin, I., Shoresh, N. & Balaban, N. Q. Optimization of lag time
422 underlies antibiotic tolerance in evolved bacterial populations. *Nature* **513**, 418-421,
423 doi:10.1038/nature13469 (2014).
424 13 Bajaj, K., Chakrabarti, P. & Varadarajan, R. Mutagenesis-based definitions and probes of residue
425 burial in proteins. *Proceedings of the National Academy of Sciences of the United States of*
426 *America* **102**, 16221-16226, doi:10.1073/pnas.0505089102 (2005).
427 14 Semisotnov, G. V. *et al.* Study of the "molten globule" intermediate state in protein folding by a
428 hydrophobic fluorescent probe. *Biopolymers* **31**, 119-128, doi:10.1002/bip.360310111 (1991).
429 15 Navarro, S. & Ventura, S. Fluorescent dye ProteoStat to detect and discriminate intracellular
430 amyloid-like aggregates in Escherichia coli. *Biotechnol J* **9**, 1259-1266,
431 doi:10.1002/biot.201400291 (2014).
432 16 Dykhuizen, D. E., Dean, A. M. & Hartl, D. L. Metabolic flux and fitness. *Genetics* **115**, 25-31
433 (1987).
434 17 Peters, J. M. *et al.* A Comprehensive, CRISPR-based Functional Analysis of Essential Genes in
435 Bacteria. *Cell* **165**, 1493-1506, doi:10.1016/j.cell.2016.05.003 (2016).

18 Hegreness, M., Shoresh, N., Hartl, D. & Kishony, R. An equivalence principle for the incorporation of favorable mutations in asexual populations. *Science* **311**, 1615-1617, doi:10.1126/science.1122469 (2006).
19 Cha, R. S., Zarbl, H., Keohavong, P. & Thilly, W. G. Mismatch amplification mutation assay (MAMA): application to the c-H-ras gene. *PCR methods and applications* **2**, 14-20 (1992).
20 Jiang, L., Mishra, P., Hietpas, R. T., Zeldovich, K. B. & Bolon, D. N. Latent effects of Hsp90 mutants revealed at reduced expression levels. *PLoS genetics* **9**, e1003600, doi:10.1371/journal.pgen.1003600 (2013).
21 Serohijos, A. W. & Shakhnovich, E. I. Merging molecular mechanism and evolution: theory and computation at the interface of biophysics and evolutionary population genetics. *Curr Opin Struct Biol* **26**, 84-91, doi:10.1016/j.sbi.2014.05.005 (2014).
22 Serohijos, A. W., Lee, S. Y. & Shakhnovich, E. I. Highly abundant proteins favor more stable 3D structures in yeast. *Biophys J* **104**, L1-3, doi:10.1016/j.bpj.2012.11.3838 (2013).
23 Hartl, D. L., Clark, A.G. *Principles of population genetics, 4th ed.*, (Sinauer, 2007).
24 Manhart, M., Adkar, B. V. & Shakhnovich, E. I. Tradeoffs between microbial growth phases lead to frequency-dependent and non-transitive selection. *bioRxiv*, doi:10.1101/096453 (2016).
25 Lambert, G., Liao, D., Vyawahare, S. & Austin, R. H. Anomalous spatial redistribution of competing bacteria under starvation conditions. *Journal of bacteriology* **193**, 1878-1883, doi:10.1128/JB.01430-10 (2011).
26 Palkova, Z. Multicellular microorganisms: laboratory versus nature. *EMBO reports* **5**, 470-476, doi:10.1038/sj.embor.7400145 (2004).
27 Hibbing, M. E., Fuqua, C., Parsek, M. R. & Peterson, S. B. Bacterial competition: surviving and thriving in the microbial jungle. *Nature reviews. Microbiology* **8**, 15-25, doi:10.1038/nrmicro2259 (2010).
28 Todar, K. Textbook of bacteriology. (2015).
29 Adkar, B. V. *et al.* Protein model discrimination using mutational sensitivity derived from deep sequencing. *Structure* **20**, 371-381, doi:10.1016/j.str.2011.11.021 (2012).
30 Muller, C. W., Schlauderer, G. J., Reinstein, J. & Schulz, G. E. Adenylate kinase motions during catalysis: an energetic counterweight balancing substrate binding. *Structure* **4**, 147-156 (1996).

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Contributions:

BVA and EIS - designed research; BVA, SB, JT and MMu - performed experiments; BVA, MMa, SB and EIS - analyzed the data; BVA, MMa, SB and EIS - wrote the paper; All authors edited and approved the final version.

Fig. 1

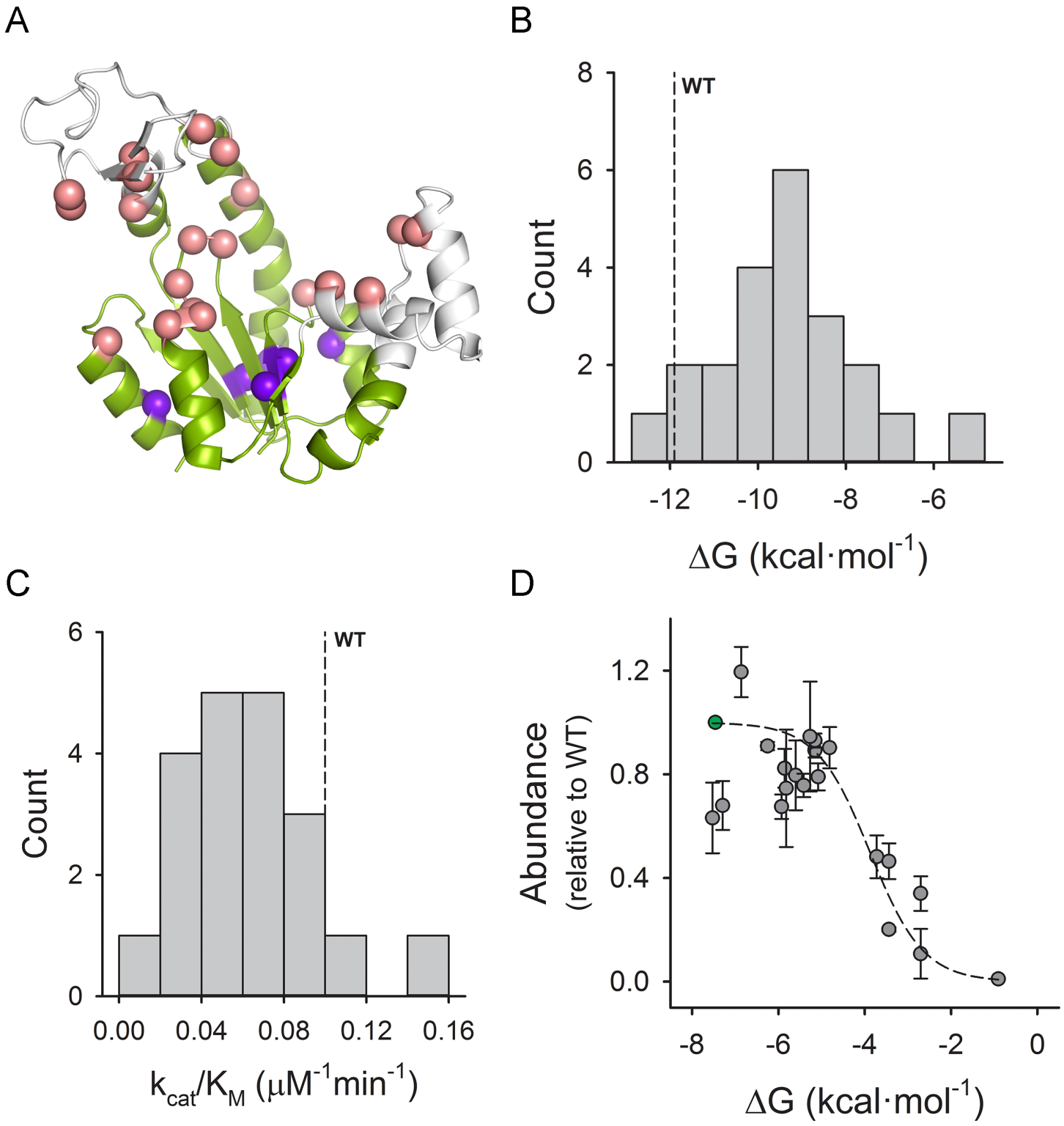

A
B
WT
Count
8
6
4
2
0
-12
-10
-8
-6
ΔG (kcal·mol-1)
C
WT
Count
6
4
2
0
0.00
0.04
0.08
0.12
0.16
kcat/KM (μM-1min-1)
D
Abundance
(relative to WT)
1.2
0.8
0.4
0.0
-8
-6
-4
-2
0
ΔG (kcal·mol-1)

Fig. 2

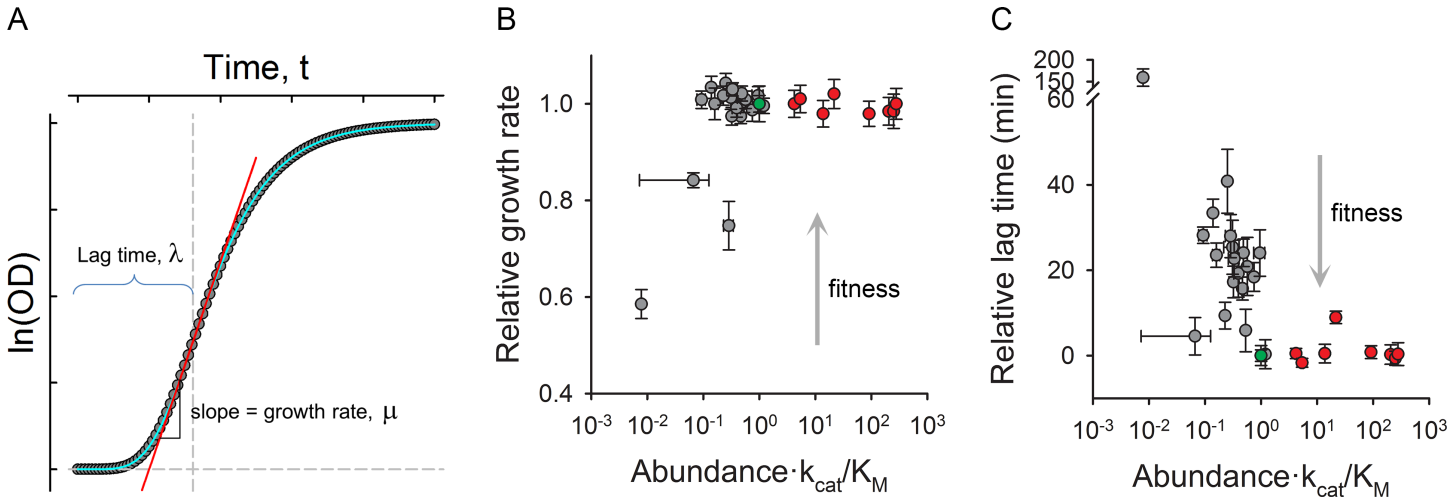

A
Time, t
ln(OD)
Lag time, λ
slope = growth rate, μ
B
Relative growth rate
1.0
0.8
0.6
0.4
fitness
Abundance·k_cat/K_M
C
Relative lag time (min)
200
150
60
40
20
0
fitness
Abundance·k_cat/K_M

Fig. 3

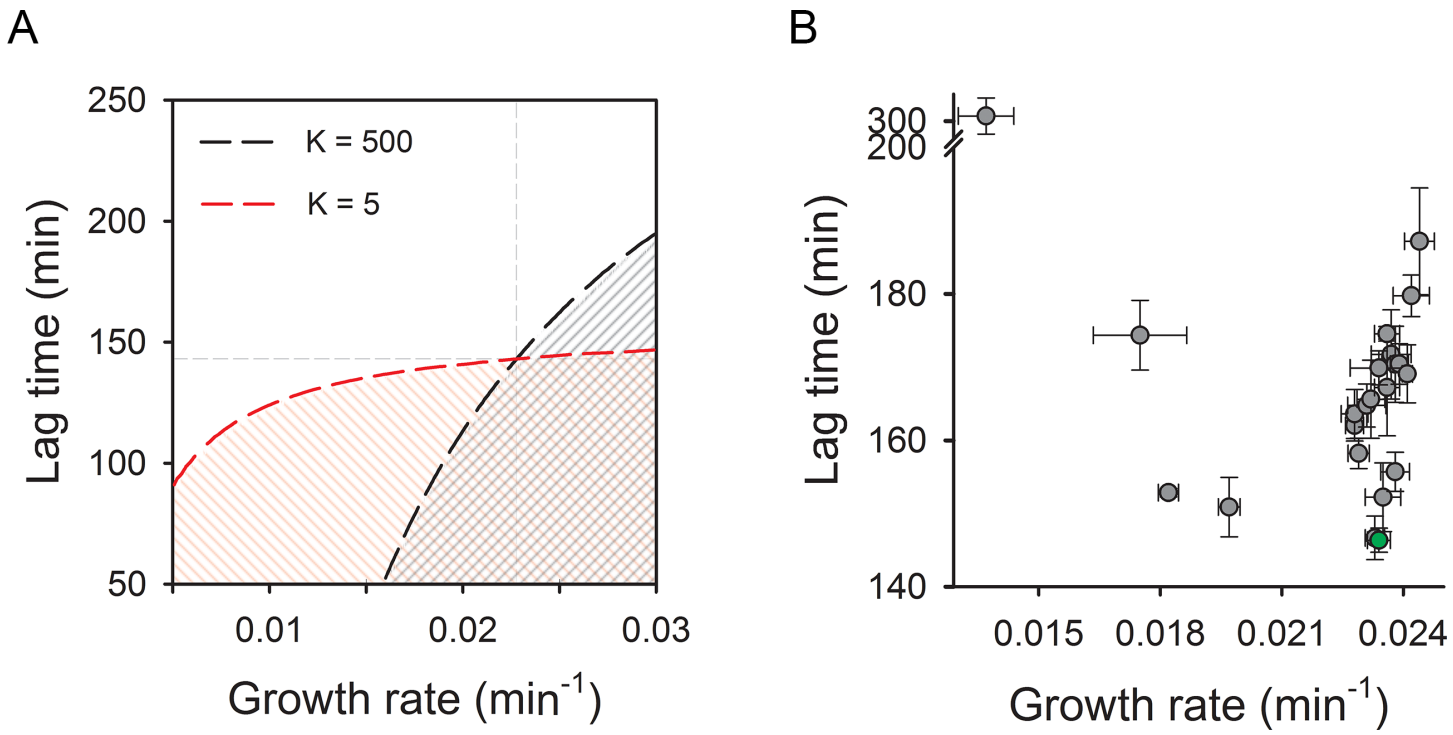

A
250
200
150
100
50
Lag time (min)
K = 500
K = 5
0.01
0.02
0.03
Growth rate (min-1)
B
300
200
180
160
140
Lag time (min)
0.015
0.018
0.021
0.024
Growth rate (min-1)

Fig. 4

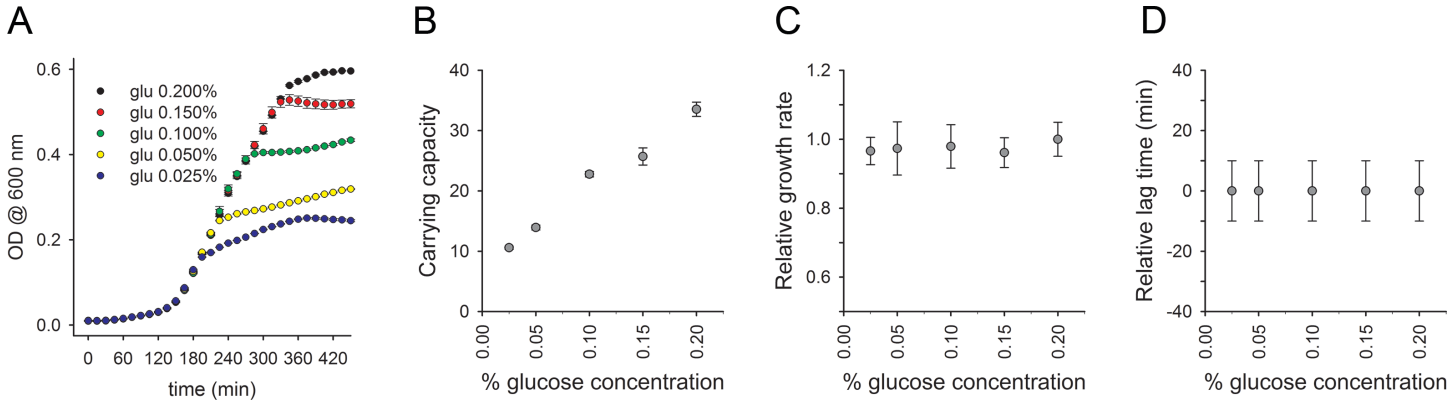
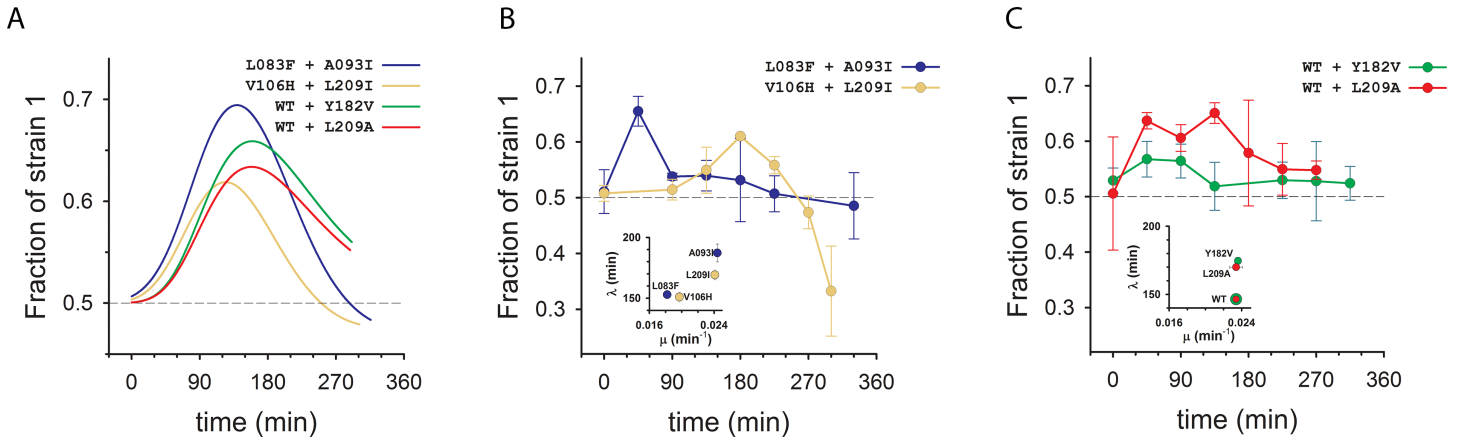

A
B
C
D
glu 0.200%
glu 0.150%
glu 0.100%
glu 0.050%
glu 0.025%
OD @ 600 nm
time (min)
Carrying capacity
Relative growth rate
Relative lag time (min)
% glucose concentration

Fig. 5


A
L083F + A093I
V106H + L209I
WT + Y182V
WT + L209A
Fraction of strain 1
0.7
0.6
0.5
0
90
180
270
360
time (min)
B
L083F + A093I
V106H + L209I
Fraction of strain 1
0.7
0.6
0.5
0.4
0.3
0
90
180
270
360
time (min)
λ (min)
200
150
A093I
L209I
L083F
V106H
0.016
0.024
μ (min⁻¹)
C
WT + Y182V
WT + L209A
Fraction of strain 1
0.7
0.6
0.5
0.4
0.3
0
90
180
270
360
time (min)
λ (min)
200
150
Y182V
L209A
WT
0.016
0.024
μ (min⁻¹)

Supplementary Methods

Mutagenesis and protein purification

Adenylate kinase (Adk) is encoded by the *adk* gene, which was cloned under the T7-*lac* promoter in pET28a(+) vector (Invitrogen) between *Nde*I and *Xho*I restriction sites. We carried out mutagenesis with a pair of 30-35 bp long, partially-complementary primers and the inverse PCR technique using KOD hot-start DNA polymerase. The mutations were centered in the complementary regions of the primers. The mutagenic plasmids were transformed in *E. coli* DH5α cells for faithful propagation and storage, and in *E. coli* BL21(DE3) for protein overexpression and purification. The His-tagged proteins were purified by Ni-NTA affinity chromatography (Qiagen) and subsequently passed through a HiLoad Superdex 75 pg column (GE). The monomeric peak was collected, concentrated and eventually stored in 10 mM potassium phosphate buffer (pH 7.2). The concentration of the proteins was measured by BCA assay (ThermoScientific) with BSA as standard.

Biophysical characterization

Thermal denaturation: We assessed the thermal stability of WT and mutant proteins by differential scanning calorimetry (nanoDSC, TA instruments) using 20 μM of protein. The scans were carried out from 10 to 90 °C at a scan rate of 90 °C/hr. The thermodynamic parameters were derived by fitting the data to a two-state unfolding model using NanoAnalyze (TA instruments). We also carried out thermal denaturation using the melt-curve module of BioRad CFX96, with Sypro Orange dye as a probe for unfolding as described earlier[1]. The dye was added to the final concentration of 5× in a 25 μl reaction volume containing 4 μM of protein in 10 mM potassium phosphate buffer (pH 7.2). The data were fit to a standard four-parameter sigmoidal equation to obtain apparent melting temperatures.

Urea denaturation: We carried out isothermal urea denaturation with WT and mutant proteins to assess the stability of the proteins to chemical denaturants. We incubated 5 μM of protein for ~4 hrs at 25 °C with varying concentrations of urea (0-8 M). The urea concentrations were estimated by refractive index measurements. The denaturation was monitored by measuring the ellipticity at 222 nm using a CD spectrometer (Jasco). The melt data was fitted assuming a model

of two-state unfolding with linear free energy as described earlier[2,3]. The m-value was fixed to 3300 cal/mol/M for fitting.

Gel filtration: We assessed the oligomeric status of purified proteins by gel filtration using 50 μg of protein on sephadex 75 analytical columns.

ANS and proteostat binding: We used 12 μM of bisANS for assessing binding to 2 μM of protein in 10 mM potassium phosphate buffer (pH 7.2). The excitation and emission wavelengths were set to 395 nm and 490 nm, respectively. 2 μM of protein was incubated with 3.5 mM of the proteostat dye in 1× assay buffer (Enzo LifeSciences). For this the excitation and emission wavelengths were set to 550 and 600 nm, respectively.

Enzyme activity: We measured the activity of Adk in terms of ADP formation by an end-point assay as described earlier[4]. Briefly, the concentration of AMP was fixed to 500 μM and ATP concentration was varied from 0 to 500 μM in an enzymatic reaction. 5 nM of Adk was used to initiate the reaction and 500 μM of Ap5A was used for quenching at 20, 40, and 60 second time points. The amount of ADP formed was measured by LDH-Pyruvate kinase-coupled reaction and the kinetic parameters were derived by fitting the data to the Michaelis-Menten equation.

Adk overexpression: The *adk* gene was cloned in a pBAD plasmid and transformed in the *E. coli* BW27783 strain (CGSC#12119). This strain constitutively expresses the arabinose transporter (araE) which enables uniform uptake of arabinose. The cells were induced with increasing concentrations of arabinose from 0 to 0.05%.

Intracellular protein abundance: Cells were grown in supplemented M9 medium for 4 hours at 37 °C, harvested and subsequently lysed with 1× BugBuster (Novagen) and 25 units/ml of Benzonase. Total amount of proteins in cell lysate was estimated by BCA assay. The specific fraction of Adk was determined by SDS-PAGE followed by western blot using rabbit anti-Adk polyclonal antibodies (custom- raised by Pacific Immunology).

Estimation of viable cells in saturating culture: The overnight culture was grown in supplemented M9 medium for 16 hours at 30 °C and the proportion of live:dead cells was measured using Live/Dead BacLight Bacterial Viability Kits (Molecular Probes) according to the manufacturer's instructions. Briefly, 1×10^8 cells (in a volume of 1ml) were mixed with 3 μl of a

1:1 proportion of Syto9 dye and Propidium Iodide (PI). The mixture was incubated in the dark for 15 minutes, following which the fluorescence was measured at 530 nm and 630 nm. Syto9 dye stains live cells and emits fluorescence at 530 nm (green), while PI stains dead cells and can be detected at 630 nm (red). The ratio of fluorescence values at 530 nm:630 nm corresponds to the proportion of live:dead cells in that sample which was eventually used to estimate the percentage of live cells in a sample, according to the manufacturer's instructions. An exponentially growing culture (considered as 100% live) and cells treated with 70% ethanol for 1 hour (considered 100% dead) were mixed in different known proportions, and their 530:630 nm ratio was used to generate a standard curve.

1. Niesen, F.H., Berglund, H. & Vedadi, M. The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. *Nat Protoc* **2**, 2212-21 (2007).
2. Chen, B.L. & Schellman, J.A. Low-temperature unfolding of a mutant of phage T4 lysozyme. 1. Equilibrium studies. *Biochemistry* **28**, 685-91 (1989).
3. Schellman, J.A. The thermodynamic stability of proteins. *Annu Rev Biophys Biophys Chem* **16**, 115-37 (1987).
4. Pena, M.I., Davlieva, M., Bennett, M.R., Olson, J.S. & Shamoo, Y. Evolutionary fates within a microbial population highlight an essential role for protein folding during natural selection. *Mol Syst Biol* **6**, 387 (2010).

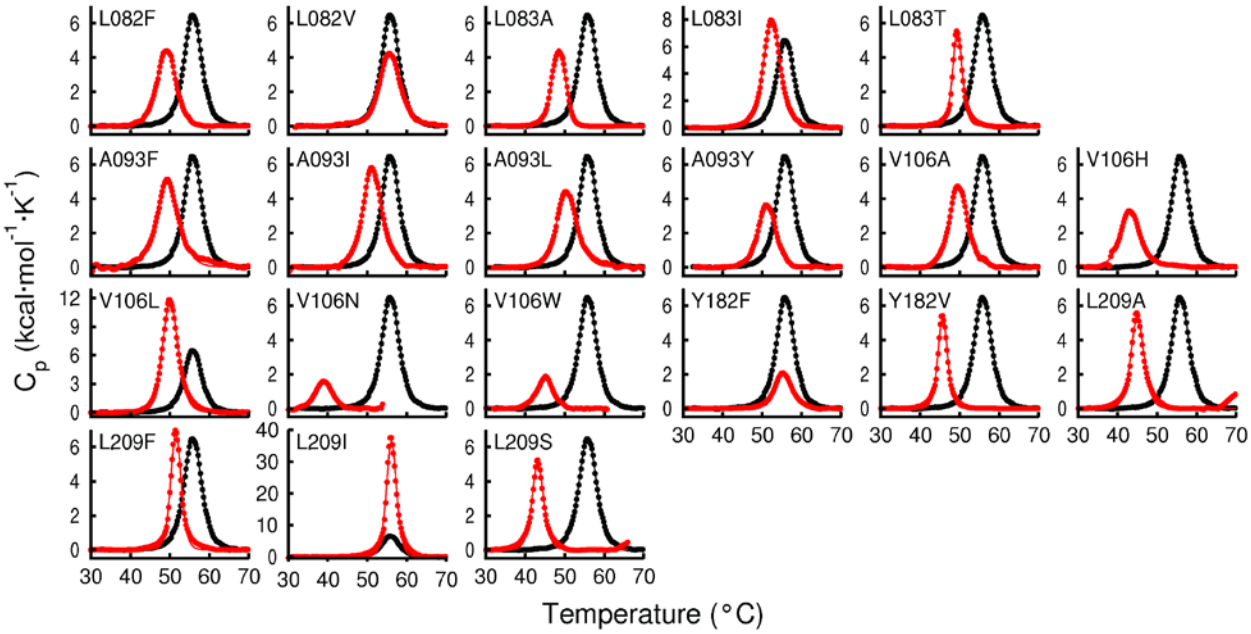


Fig S1: Thermal unfolding monitored by Differential Scanning Calorimetry (DSC) for WT (black trace) and 20 different Adk mutant proteins (red trace). The molar heat capacity (C_p) is shown as a function of temperature. The scan rate was 90 °C/hr. The data was fitted to a two-state thermal unfolding model to derive the thermodynamic parameters.

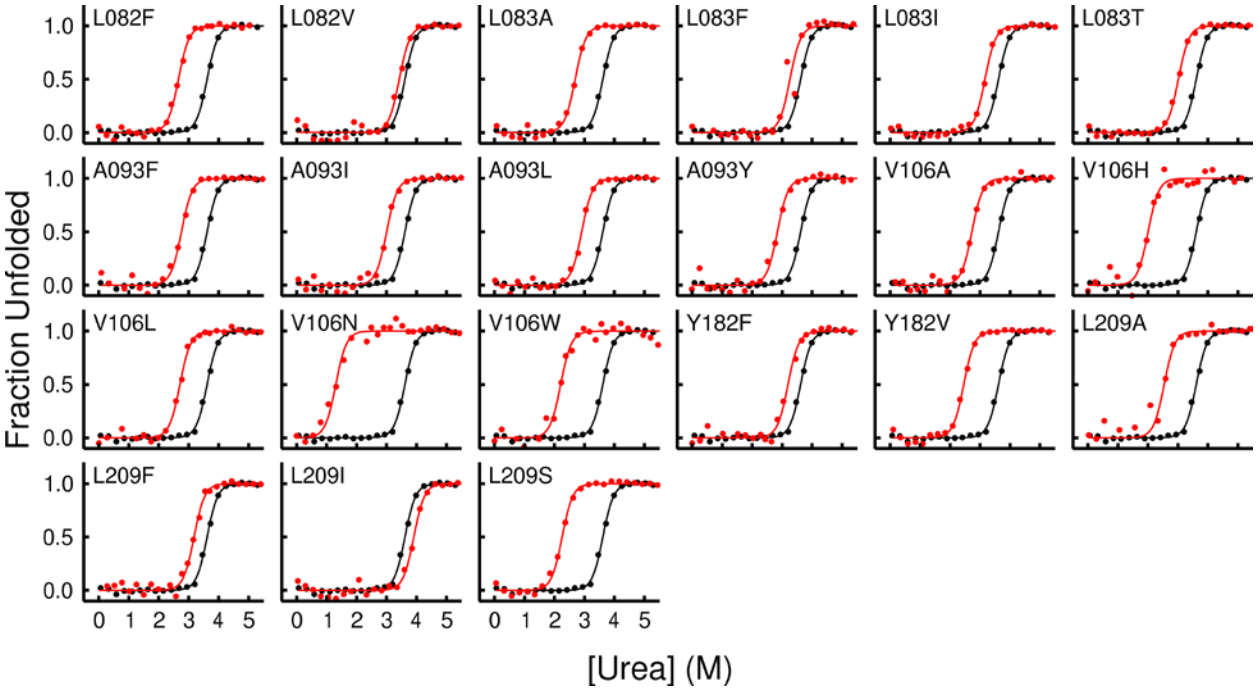


Fig S2: Isothermal urea denaturation curves at 25 °C for WT (black dots) and mutant Adk proteins (red dots). The fraction unfolded (Fu) is plotted as a function of denaturant concentration. Protein denaturation was monitored by recording the CD signal at 222 nm. The data was fit to a two-state unfolding model.

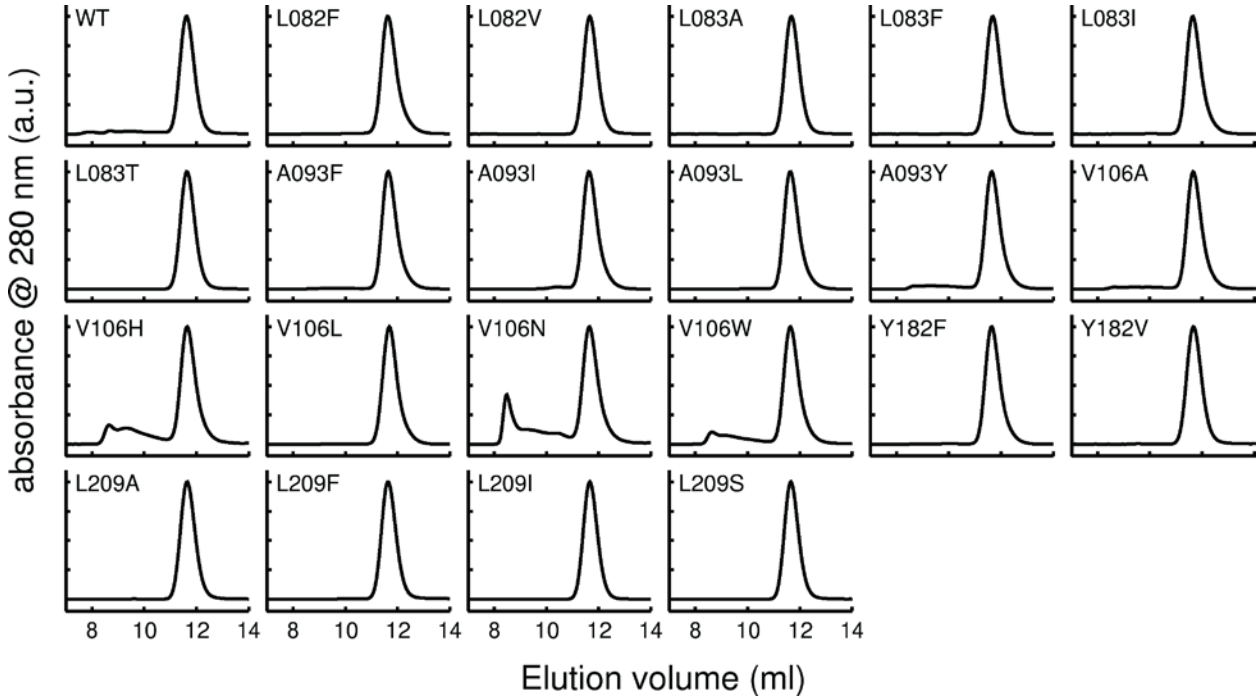


Fig S3: Analytical gel-filtration profile of WT and 20 mutant Adk proteins on a Superdex-75 column at room-temperature. The absorbance at 280 nm is shown as a function of elution volume. For comparison all the monomeric peaks were normalized to 1. WT Adk along with most other mutant proteins elutes at the expected position for a monomer. Exceptions were V106H, V106N and V106W, where additional peaks appear at much higher molecular weights.

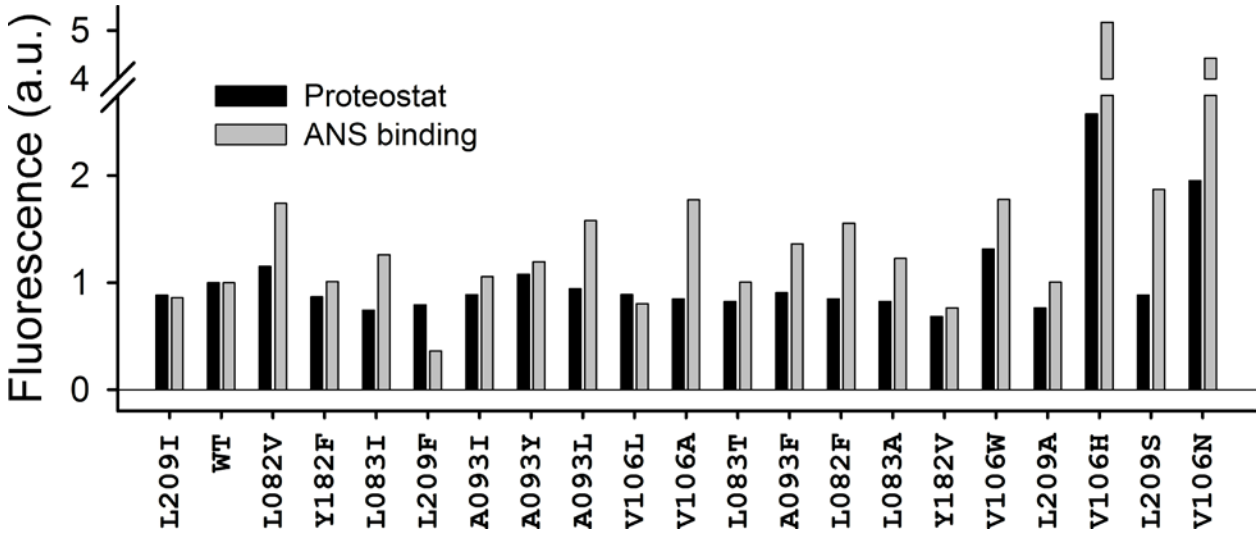


Fig S4: Aggregation propensity and molten-globule states of mutant proteins. Bar plots represent the extent of ProteoStat and ANS binding to WT and mutant Adk proteins. The proteins on the x-axis are arranged in decreasing order of stability from left to right.

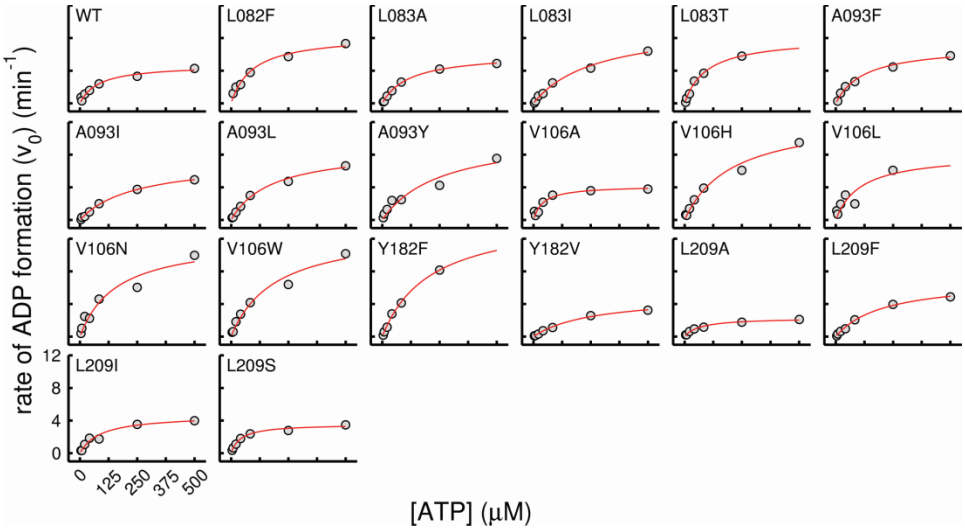


Fig S5: Enzyme activity of Adk mutants at 25 °C measured as described in Supplementary methods. The initial velocity, shown as a function of ATP concentration, was calculated as the amount of ADP produced per minute by 1 nmol of Adenylate Kinase. The concentration of AMP in all experiments was fixed to 500 μM. The data (gray circles) was fitted using the Michaelis-Menten equation of enzyme activity to extract relevant parameters (fitted line in red).

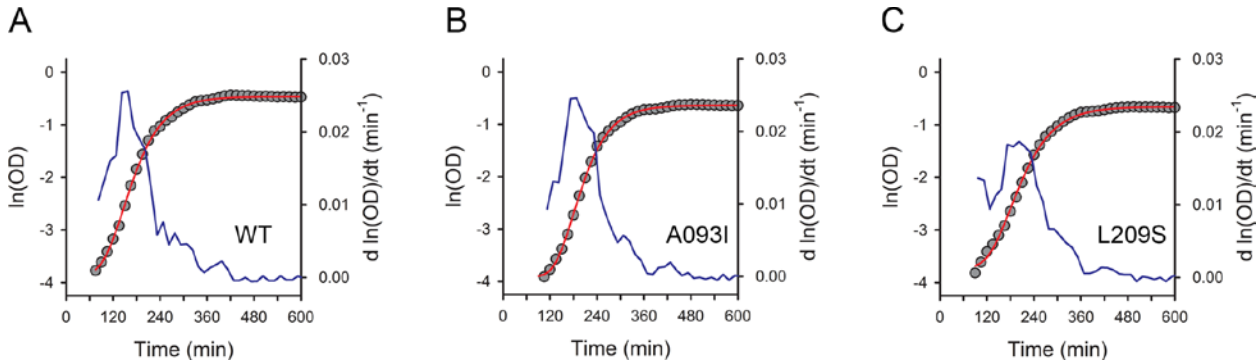


Fig S6: Representative growth curves of (A) WT, (B) A093I, and (C) L209S strains. Each growth curve is shown as ln(OD) vs time plot (left y-axis). The experimental data is shown in gray circles and the Gompertz fit is shown in solid red line. The instantaneous time derivative of the ln(OD) data is shown in blue line (right y-axis). The strains were chosen to illustrate the quality of the fit across different range of growth rates and lag times (see Table S2 for growth parameters).

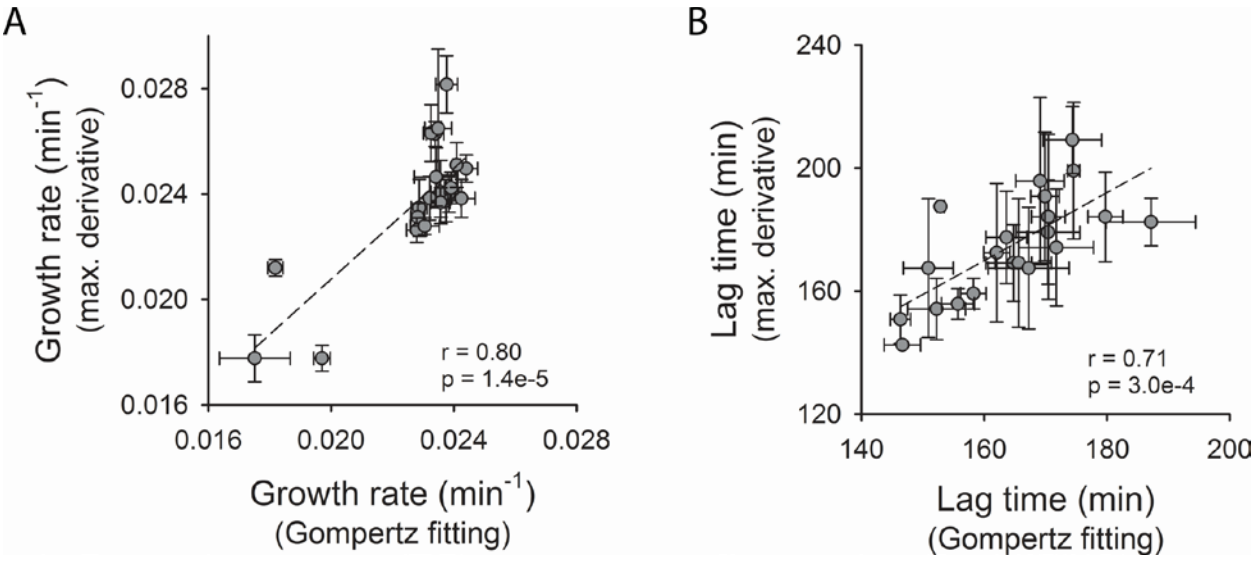


Fig S7: Correlation between growth parameters derived from Gompertz fitting and maximum-derivative method. The parameters derived from both the methods correlate very well as indicated by Pearson's correlation parameters (r and p-values). The data points represent mean and error bars are standard deviation of 6 or 9 measurements (see Table S2).

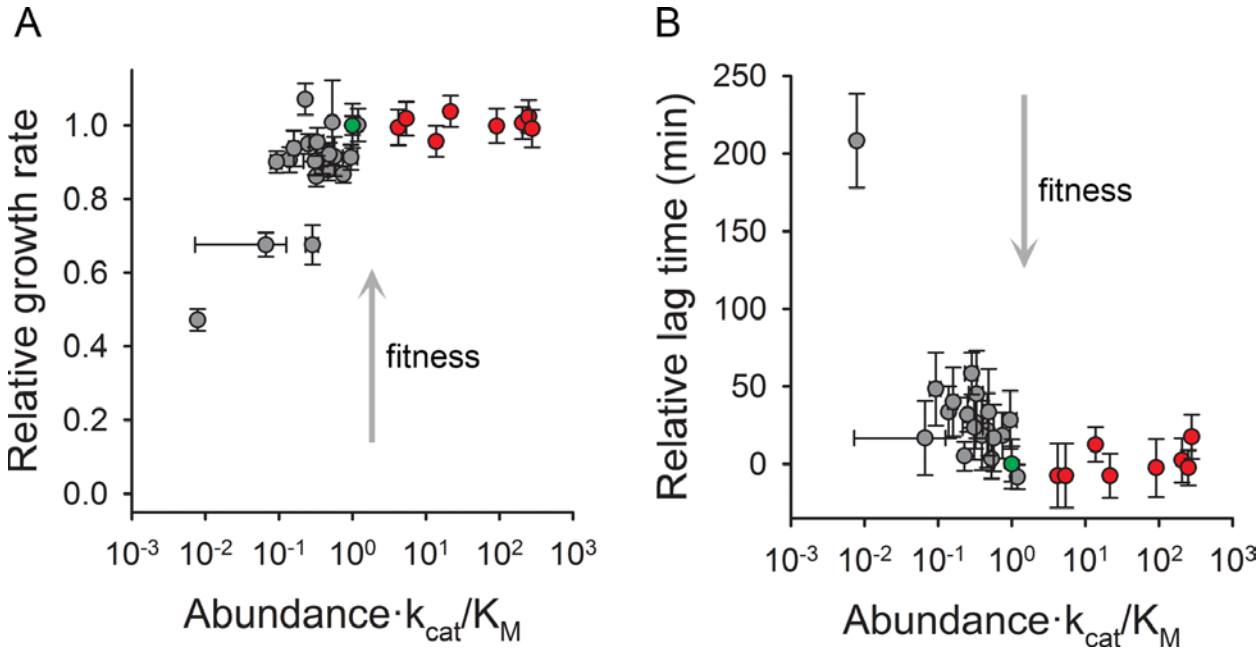


Fig S8: Traits of population growth. (A) Relative growth rates (μ/μ_{WT}) and (B) relative lag time $(\lambda-\lambda_{WT})$ obtained from analysis of growth curve derivatives shown as a function of catalytic capacity which is defined as abundance$\times k_{cat}/K_M$. The mutant data is shown in gray circles, whereas red circles represent the BW27783 strain with varying degrees of overexpression of WT Adk from a pBAD plasmid. Data for WT is shown in green. Fig 2 is an equivalent figure with growth rate and lag times obtained after fitting the raw data with Gompertz equation (Eq. 2).

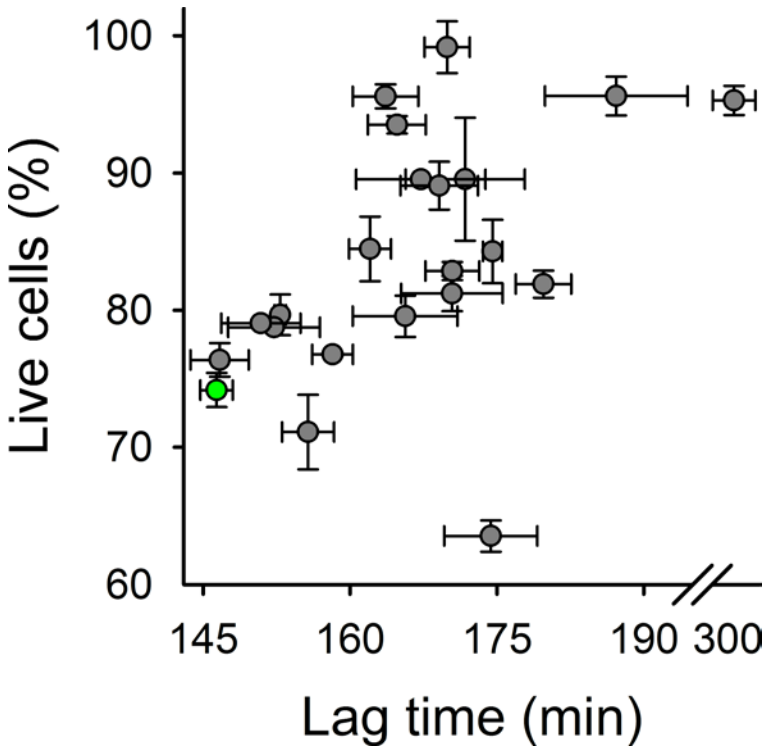
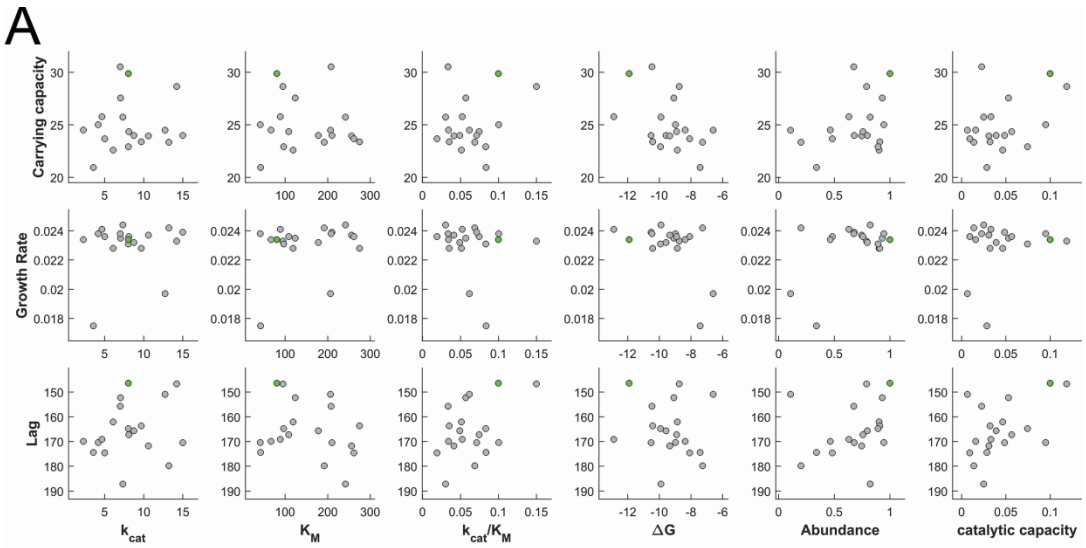


Fig S9: Percentage of live or viable cells of WT and mutant Adk strains at saturation (16 hours of growth) versus their population lag time. The cultures were grown overnight at 30 °C, and then stained using fluorescent dyes Syto9 (specific for live cells) and propidium iodide (specific for dead cells). The data points are mean and error bars represent standard deviation of 2 biological replicates. WT Adk strain is shown in green.

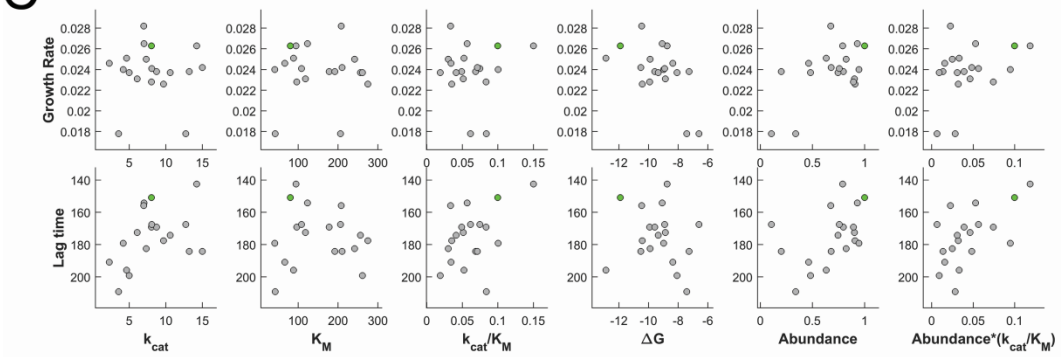
A



B

		k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($\mu M^{-1} \cdot min^{-1}$)	ΔG ($kcal \cdot mol^{-1}$)	Abundance	Abundance × (k_{cat}/K_M)
Carrying capacity	ρ	-0.02	-0.17	0.04	-0.38	0.22	0.24
	p-value	0.943	0.495	0.870	0.108	0.375	0.314
Growth rate (min^{-1})	ρ	0.04	0.20	-0.21	-0.32	-0.07	-0.08
	p-value	0.861	0.424	0.394	0.179	0.791	0.737
Lag time (min)	ρ	-0.19	0.20	-0.30	0.20	-0.39	-0.44
	p-value	0.442	0.412	0.212	0.420	0.103	0.057

C



D

		k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($\mu M^{-1} \cdot min^{-1}$)	ΔG ($kcal \cdot mol^{-1}$)	Abundance	Abundance × (k_{cat}/K_M)
Growth rate (min^{-1})	ρ	-0.06	-0.17	0.00	-0.44	0.24	0.32
	p-value	0.822	0.483	0.991	0.057	0.313	0.183
Lag time (min)	ρ	-0.42	0.03	-0.30	0.17	-0.48	-0.51
	p-value	0.077	0.903	0.209	0.486	0.038	0.027

Fig S10: Scatter plots of growth parameters (carrying capacity, growth rate and lag times) and molecular and cellular properties of Adk. Parameters were obtained using (A) Gompertz fit and (C) analysis of growth curve derivatives. Panels (B) and (D) show Spearman's correlation coefficients (ρ) and p-values for each of the sub-plots in panels (A) and (C) respectively. The

highest correlation values in each panel are highlighted in yellow. V106N was excluded from all correlation calculations.

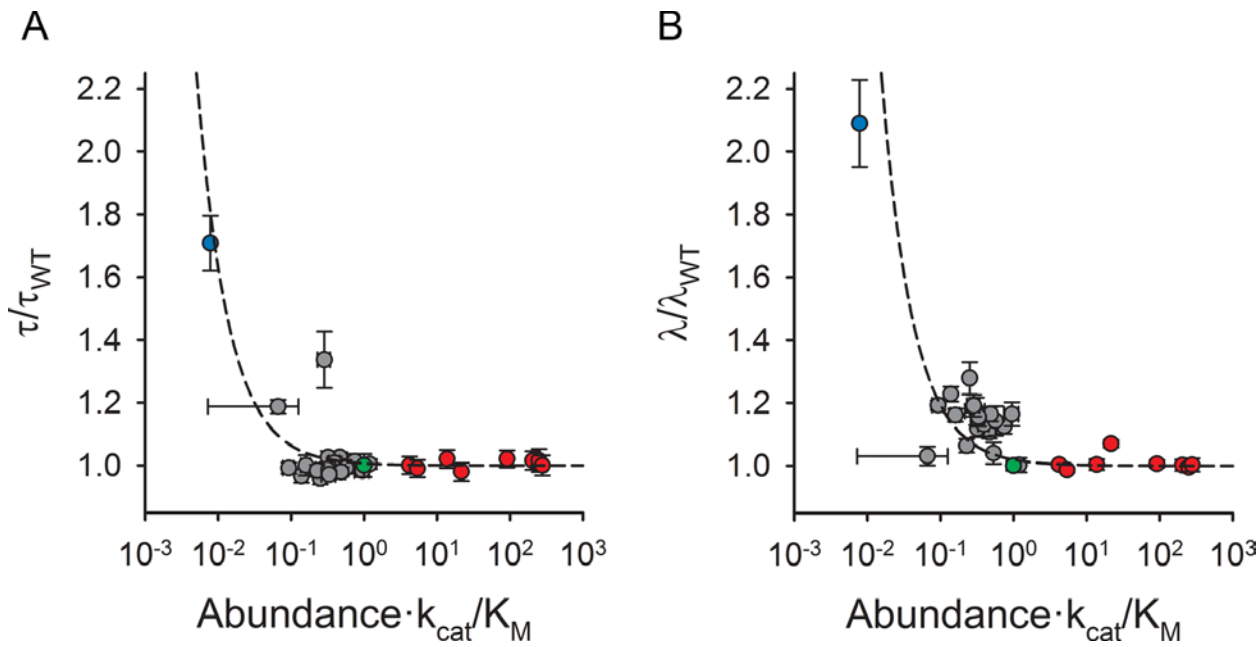


Fig S11: (A) Relative growth time (τ/τ_{WT}) and (B) relative lag time (λ/λ_{WT}) as a function of catalytic capacity $(\text{abundance}\times k_{cat}/K_M)$. The dashed line shows a fit to Eq. 3, where the asymptote (a) was assumed to be 1. The K_M-like parameter b for growth time was 0.006 and that for lag time was 0.019, which indicates that the WT catalytic capacity is closer to the cusp for lag time than for growth time. The mutant data is shown in gray circles, whereas the overexpression data is shown in red. In green is shown WT, while the blue circle indicates V106N which was omitted from the fitting. The error bars represent standard deviation of parameters derived from growth curves of 3 colonies (biological replicates) in triplicates (9 curves). See Table S2 and S3 for the parameters.

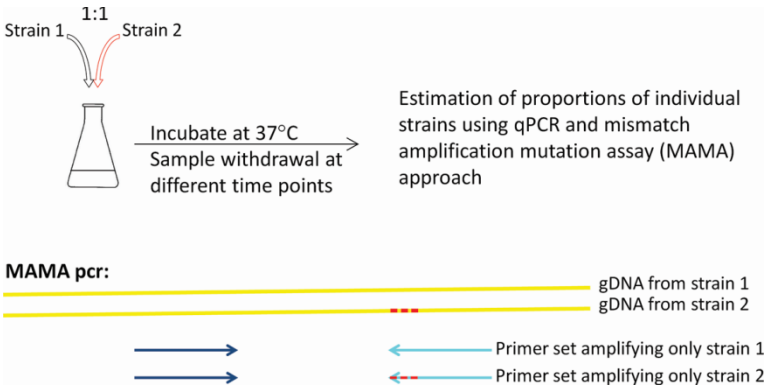


Fig S12: Schematic representation of binary growth competition experiments and estimation of relative proportion of competing strains. The strains (1) and (2) are mixed in 1:1 proportion and were grown at 37 °C. Samples were drawn at different time points, normalized for OD, and genomic DNA was extracted. The proportions of individual strains were estimated by a qPCR method employing mismatch amplification mutation assay method (see Methods). We designed a set of primers to differentially amplify the strains by matching the 3'-end of one of the primers to the site of mutation and using Taq DNA polymerase for amplification.

Table S1: Structural and biophysical parameters

adk strain	SC acc[a] (%)	Residue depth (Å)	Fraction of WT in MSA	Fraction of mutant in MSA[b]	T_m (DSC) (°C)	T_m (TFA)[c] (°C)	ΔG[d] (kcal/mol)	k_{cat}[e] (min^{-1})	K_M[f] (μM)	k_{cat}/K_M ($\mu M^{-1} min^{-1}$)
WT	--	--	--	--	55.9	53.8	-11.9	8.05	80.58	9.99E-02
L082F	0.0	8.8	0.57	0.00	49.2	47.4	-8.7	14.23	94.86	1.50E-01
L082V	0.0	8.8	0.57	0.14	55.7	53.2	-11.3	n.d.[g]	n.d.[g]	n.d.[g]
L083A	0.1	9.0	0.70	0.00	48.5	46.9	-8.9	6.10	118.73	5.13E-02
L083F	0.1	9.0	0.70	0.60	n.d.[g]	54.2	-10.8	n.d.[g]	n.d.[g]	n.d.[g]
L083I	0.1	9.0	0.70	0.23	52.4	50.7	-10.4	9.70	274.80	3.53E-02
L083T	0.1	9.0	0.70	0.00	49.4	49.3	-9.9	8.05	96.55	8.34E-02
A093F	0.0	5.9	0.85	0.00	49.4	47.2	-9.1	7.05	123.83	5.69E-02
A093I	0.0	5.9	0.85	0.00	51.3	49.7	-9.9	7.34	241.75	3.04E-02
A093L	0.0	5.9	0.85	0.07	50.6	47.9	-9.6	8.75	178.05	4.91E-02
A093Y	0.0	5.9	0.85	0.00	51.2	49.4	-9.3	10.61	256.23	4.14E-02
V106A	0.0	7.4	0.74	0.38	49.7	47.3	-9.0	4.19	41.78	1.00E-01
V106H	0.0	7.4	0.74	0.00	43.2	41.2	-6.6	12.74	206.54	6.17E-02
V106L	0.0	7.4	0.74	0.04	50.1	48.5	-8.9	8.10	108.60	7.46E-02
V106N	0.0	7.4	0.74	0.00	39.0	37.9	-4.8	12.07	158.06	7.64E-02
V106W	0.0	7.4	0.74	0.04	45.0	43.0	-7.3	13.22	192.05	6.88E-02
Y182F	7.2	6.3	0.86	0.14	55.4	53.8	-10.5	15.00	210.25	7.13E-02
Y182V	7.2	6.3	0.86	0.00	45.7	46.2	-8.1	5.01	261.53	1.92E-02
L209A	0.0	7.0	0.23	0.08	45.0	44.3	-8.4	2.30	66.94	3.43E-02
L209F	0.0	7.0	0.23	0.01	51.5	51.0	-10.5	7.00	208.17	3.36E-02
L209I[h]	0.0	7.0	0.23	0.58	56.1	55.5	-12.9	4.66	88.82	5.25E-02
L209S	0.0	7.0	0.23	0.00	43.2	42.4	-7.4	3.57	42.55	8.38E-02

[a] % sidechain accessibility calculated using coordinates of pdb 4ake

[b] fraction in multiple sequence alignment when WT amino acid is excluded

[c] melting temperture from thermofluor assay

[d] derived from isothermal urea denaturation experiments at 25C

[e] kcat for ADP formation

[f] KM for ATP

[g] not determined

[h] the only case in this dataset where fraction of mutant amino acid was greater that WT amino acid in MSA

Table S2: Intracellular abundance and growth parameters of adk mutants

adk strain	Carrying capacity, K[a]	s.d. in K[b]	Growth rate, μ[a] (min^{-1})	s.d. in μ[b]	Lag time, λ[a,c] (min)	s.d. in λ[b]	Abundance[d]	s.d. in abundance[e]
WT	29.9	3.0	0.0234	2.85E-04	146.4	1.7	1.00	0.00
L082F	28.7	2.9	0.0233	2.40E-04	146.7	3.0	0.79	0.05
L082V	24.4	0.8	0.0229	2.60E-04	158.2	2.1	n.d.[f]	n.d.[f]
L083A	22.6	0.8	0.0228	2.22E-04	162.0	2.1	0.90	0.08
L083F[g]	27.3	1.3	0.0182	2.48E-04	152.9	0.6	1.19	0.10
L083I	23.4	0.9	0.0228	3.30E-04	163.6	3.3	0.91	0.02
L083T	22.9	1.0	0.0231	4.72E-04	164.8	2.9	0.89	0.03
A093F	27.6	4.3	0.0235	4.37E-04	152.2	4.7	0.93	0.03
A093I[g]	25.7	0.5	0.0244	3.69E-04	187.2	7.3	0.82	0.07
A093L	24.0	1.0	0.0232	3.44E-04	165.6	5.3	0.80	0.13
A093Y	24.0	1.1	0.0237	4.90E-04	171.7	6.1	0.75	0.23
V106A	25.0	0.5	0.0238	3.28E-04	170.4	5.2	0.95	0.21
V106H[g]	24.5	2.2	0.0197	2.68E-04	150.9	4.1	0.11	0.10
V106L	24.4	2.2	0.0236	3.08E-04	167.2	6.6	0.76	0.05
V106N	5.9	0.3	0.0137	6.85E-04	305.8	20.0	0.01	0.00
V106W	23.4	1.0	0.0242	4.48E-04	179.7	2.8	0.20	0.01
Y182F	24.0	1.3	0.0239	2.15E-04	170.4	2.8	0.68	0.09
Y182V	23.7	1.1	0.0236	3.08E-04	174.6	1.0	0.48	0.08
L209A	24.5	1.3	0.0234	7.12E-04	169.9	2.3	0.46	0.07
L209F	30.5	1.8	0.0238	3.54E-04	155.7	2.7	0.68	0.05
L209I	25.8	1.2	0.0241	1.27E-04	169.1	4.0	0.63	0.14
L209S	20.9	1.5	0.0175	1.15E-03	174.4	4.8	0.34	0.07

[a] parameters derived by fitting Gompertz equation to ln(OD) vs time at 37 C

[b] standard deviation derived from 9 replicates (triplicates of 3 biological replicates)

[c] time required to achieve maximum growth rate

[d] abundance measured after 4h of growth at 37 C

[e] standard deviation derived 2 biological replicates

[f] not determined

[g] growth parameters derived for 6 replicates (triplicates of 2 biological replicates)

Table S3: Intracellular abundance and growth parameters of WT adk overexpression from pBAD plasmid in *E. coli* BW27783 strain

arabinose concentration (%)	Carrying capacity, K[a]	s.d. in K[b]	Growth rate, μ[a] (min^{-1})	s.d. in μ[b]	Lag time, λ[a,c] (min)	s.d. in λ[b]	Abundance[d]	s.d. in abundance[e]
no plasmid	29.5	2.5	0.0195	5.10E-04	126.7	1.0	1.00	0.10
0.00E+00	32.0	0.5	0.0195	2.00E-04	127.2	0.7	4.20	0.42
3.05E-06	30.9	1.8	0.0191	2.00E-04	127.2	2.0	13.73	1.37
1.22E-05	31.3	1.0	0.0197	2.00E-04	125.0	0.4	5.39	0.54
4.88E-05	27.1	1.0	0.0199	2.65E-04	135.7	1.1	21.55	2.15
1.95E-04	32.7	0.9	0.0191	1.00E-04	127.5	1.2	91.27	9.12
7.81E-04	31.6	2.5	0.0192	2.52E-04	127.0	2.1	205.31	20.51
3.13E-03	32.6	1.6	0.0192	4.73E-04	126.1	1.4	250.39	25.02
5.00E-02	28.4	3.1	0.0195	3.61E-04	127.1	2.5	278.69	27.84

[a] parameters derived by fitting Gompertz equation to ln(OD) vs time at 37 C

[b] standard deviation of 3 replicates

[c] time required to achieve maximum growth rate

[d] abundance measured after 4h of growth at 37 C

[e] standard deviation of 2 biological replicates