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Malthusian fitness originating in protein stability in the present model

For typical proteins whose folding free energy ΔG satisfies $\exp(\beta\Delta G) \ll 1$, without loss of generality we can assume the Malthusian fitness of the single protein-coding genes to be equal to

$$m = -\kappa e^{\beta\Delta G} \quad \text{with } \kappa \geq 0 \tag{S.1}$$

where $\beta = 1/kT$, k is the Boltzmann constant, T is absolute temperature, and κ is a parameter whose meaning may depend on the situation; refer to Method for details. If the fitness costs of functional loss and toxicity due to misfolded proteins are both taken into account and assumed to be additive in the Malthusian fitness scale, κ will be defined as

$$\kappa = cA + \gamma \tag{S.2}$$

where c is fitness cost per misfolded protein (Geiler-Samerotte et al., 2011), A is the cellular abundance of a protein (Geiler-Samerotte et al., 2011), and γ is indispensability (Drummond and Wilke, 2008) and defined as $\gamma \equiv -\log(\text{deletion-strain growth rate / max growth rate})$. The parameter κ is assumed in the present analysis to take values in the range of $0 \leq \log 4N_e\kappa \leq 20$ with effective population size N_e, taking account of the values of the parameters, $c \sim 10^{-4}$ (Drummond and Wilke, 2008), $10 < A < 10^6$ (Ghaemmaghami et al., 2003), $\gamma = 10$ for essential genes (Drummond and Wilke, 2008), and $N_e \sim 10^4$ to 10^5 for vertebrates, $\sim 10^5$ to 10^6 for invertebrates, $\sim 10^7$ to 10^8 for unicellular eukaryotes, and $> 10^8$ for prokaryotes (Lynch and Conery, 2003).

Then, the selective advantage of a mutant protein is given as follows in Malthusian parameters as a function of the folding free energy (ΔG) of the wild-type protein, the stability change ($\Delta\Delta G$) of a mutant protein, and the parameter κ;

$$s \equiv m^{\text{mutant}} - m^{\text{wildtype}} = \kappa e^{\beta\Delta G}(1 - e^{\beta\Delta\Delta G}) \tag{S.3}$$

Equation (S.3) indicates that s is upper-bounded.

$$s \leq \kappa e^{\beta\Delta G} \tag{S.4}$$

Distribution of stability changes ($\Delta\Delta G$) due to single amino acid substitutions

Here, according to (Tokuriki et al., 2007), the distribution of folding free energy changes, $\Delta\Delta G$, of mutant proteins is assumed to be a bi-Gaussian function with mean depending on ΔG, in order to take into account the effects of structural constraint on evolutionary rate. The probability density function (PDF), $p(\Delta\Delta G)$, of $\Delta\Delta G$ for nonsynonymous substitutions is defined as

$$p(\Delta\Delta G) = \theta\mathcal{N}(\mu_s, \sigma_s) + (1 - \theta)\mathcal{N}(\mu_c, \sigma_c) \tag{S.5}$$

where $0 \leq \theta \leq 1$, and $\mathcal{N}(\mu, \sigma)$ is a normal distribution with mean μ and standard deviation σ. One of the two Gaussian distributions above, $\mathcal{N}(\mu_s, \sigma_s)$, results from substitutions on protein surfaces and is a narrow distribution with a mildly destabilizing mean $\Delta\Delta G$, whereas the other, $\mathcal{N}(\mu_c, \sigma_c)$, due to substitutions in protein cores is a wider distribution with a stronger destabilizing mean (Tokuriki et al., 2007). Since the majority of substitutions appear to be single nucleotide substitutions, the values of standard deviations (σ_s and σ_c) estimated in (Tokuriki et al., 2007) for single nucleotide substitutions are employed here; in kcal/mol units,

$$\mu_s = -0.139\,\Delta G - 0.168 \quad , \quad \sigma_s = 0.90 \tag{S.6}$$

$$\mu_c = -0.139\,\Delta G + 1.232 \quad , \quad \sigma_c = 1.93 \tag{S.7}$$

To analyze the dependences of the means, μ_s and μ_c, on ΔG, we plotted the observed values of $\Delta\Delta G$ of single amino acid mutants against ΔG of the wild type, which are collected in the ProTherm database (Kumar et al., 2006); the same analysis was done in (Serohijos et al., 2012). Fig. S.2 shows a significant dependence of $\Delta\Delta G$ on ΔG; the regression line is $\mu = -0.139\Delta G + 0.490$. The linear slopes of μ_s and μ_c are taken to be equal to the slope (-0.139) of the regression line. The intercepts have been estimated to satisfy the following two conditions.

1. Equations (S.6) and (S.7) satisfy $\mu_s(\Delta G_0) = 0.56$ and $\mu_c(\Delta G_0) = 1.96$, which were estimated for single nucleotide substitutions in (Tokuriki et al., 2007), at a certain value (ΔG_0) of ΔG.
2. The total mean of the two Gaussian functions agrees with the regression line, $\mu = -0.139\Delta G + 0.490$. The value of θ is taken to be 0.53, which is equal to the average of θ over proteins used in (Tokuriki et al., 2007).

A representative value, 7.550, of $\log 4N_e\kappa$ is determined in such way that the equilibrium value of ΔG is equal to $\Delta G_0 = -5.24$ introduced above. It is interesting that this value $\Delta G_e = -5.24$ kcal/mol agrees with the most probable value of ΔG in the observed distribution of protein stabilities shown in Fig. 1. The fraction θ of less-constrained residues such as most residues on protein surface is correlated with protein length for globular, monomeric proteins (Tokuriki et al., 2007); $\theta = 1.27 - 0.33 \cdot \log_{10}$(protein length) for $50 \leq$ length ≤ 330. (Tokuriki et al., 2007). However, residues taking part in protein–protein interactions may be regarded as core residues rather than surface residues.

Probability distributions of selective advantage, fixation rate and K_a/K_s

Now, we can consider the probability distributions of characteristic quantities that describe the evolution of genes. First of all, the PDF of selective advantage s, $p(s)$, of mutant genes can be represented by

$$\begin{aligned} p(s) &= -p(\Delta\Delta G)\frac{d\Delta\Delta G}{ds} \\ &= p(\Delta\Delta G)\frac{1}{\beta(\kappa e^{\beta\Delta G} - s)} \end{aligned} \tag{S.8}$$

where $\Delta\Delta G$ must be regarded as a function of s, that is, $\Delta\Delta G = \beta^{-1}\log(1 - s(\kappa\exp(\beta\Delta G))^{-1})$. The PDF of $4N_e s$, $p(4N_e s)$, may be more useful than $p(s)$.

$$p(4N_e s) = p(s)\frac{1}{4N_e} \tag{S.9}$$

The fixation probability u of a mutant gene with selective advantage s and gene frequency q in a duploid system is equal to (Crow and Kimura, 1970)

$$u(4N_e s) = \frac{1 - e^{-4N_e sq}}{1 - e^{-4N_e s}} \tag{S.10}$$

where $q = 1/(2N)$ for a single mutant gene in a population of size N. Population size is taken to be $N = 10^6$. Thus, the PDF of fixation probability u can be

represented by

$$p(u) = p(4N_e s)\frac{d4N_e s}{du} = p(4N_e s)\frac{(e^{4N_e s}-1)^2 e^{4N_e s(q-1)}}{q(e^{4N_e s}-1)-(e^{4N_e sq}-1)} \quad \text{(S.11)}$$

where s must be regarded as a function of u.

The ratio of the substitution rate per nonsynonymous site (K_a) for nonsynonymous substitutions with selective advantage s to the substitution rate per synonymous site (K_a) for nonsynonymous substitutions with s = 0 is

$$\frac{K_a}{K_s} = \frac{u(4N_e s)}{u(0)} = \frac{u(4N_e s)}{q} \quad \text{(S.12)}$$

$$\simeq \frac{4N_e s}{1-e^{-4N_e s}} \quad \text{for } \frac{|4N_e sq|}{2} \ll 1 \quad \text{(S.13)}$$

assuming that synonymous substitutions are completely neutral and mutation rates at both types of sites are the same. The PDF of K_a/K_s is

$$p(K_a/K_s) = p(u)\frac{du}{d(K_a/K_s)} = p(u)q \quad \text{(S.14)}$$

$$\simeq p(4N_e s)\frac{(e^{4N_e s}-1)^2}{e^{4N_e s}(e^{4N_e s}-1-4N_e s)} \text{ for } \frac{|4N_e sq|}{2} \ll 1 \quad \text{(S.15)}$$

In the range of $|4N_e sq|/2 \ll 1$, both K_a/K_s and $p(K_a/K_s)$ do not depend on $q = 1/(2N)$.

Probability distributions of $\Delta\Delta G$, $4N_e s$, u, and K_a/K_s in fixed mutant genes

Now, let us think about fixed mutant genes. The PDF of the $\Delta\Delta G$ of fixed mutant genes is

$$p(\Delta\Delta G_{\text{fixed}}) = p(\Delta\Delta G)\frac{u(4N_e s(\Delta\Delta G))}{\langle u(4N_e s(\Delta\Delta G))\rangle} \quad \text{(S.16)}$$

$$\langle u\rangle \equiv \int_{-\infty}^{\infty} u(4N_e s)p(\Delta\Delta G)d\Delta\Delta G \quad \text{(S.17)}$$

$$= \int_{-\infty}^{4N_e\kappa\exp(\beta\Delta G)} u(4N_e s)p(4N_e s)d4N_e s \quad \text{(S.18)}$$

Likewise, the PDF of the selective advantage of fixed mutant genes is

$$p(4N_e s_{\text{fixed}}) = p(4N_e s)\frac{u(4N_e s)}{\langle u(4N_e s)\rangle} \quad \text{(S.19)}$$

and those of the u and K_a/K_s of fixed mutant genes are

$$p(u_{\text{fixed}}) = p(u)\frac{u}{\langle u\rangle} \tag{S.20}$$

$$p((\frac{K_a}{K_s})_{\text{fixed}}) = p(\frac{K_a}{K_s})\frac{u}{\langle u\rangle} = p(\frac{K_a}{K_s})\frac{\frac{K_a}{K_s}}{\langle\frac{K_a}{K_s}\rangle} \tag{S.21}$$

Then, the probabilities of $a < K_a/K_s < b$ and the averages of K_a/K_s over all mutants and also in fixed mutants can be calculated. The average of K_a/K_s in fixed mutants is equal to the ratio of the second moment to the first moment of K_a/K_s in all arising mutants.

$$\langle\frac{K_a}{K_s}\rangle_{\text{fixed}} = \langle(\frac{K_a}{K_s})^2\rangle/\langle\frac{K_a}{K_s}\rangle \tag{S.22}$$

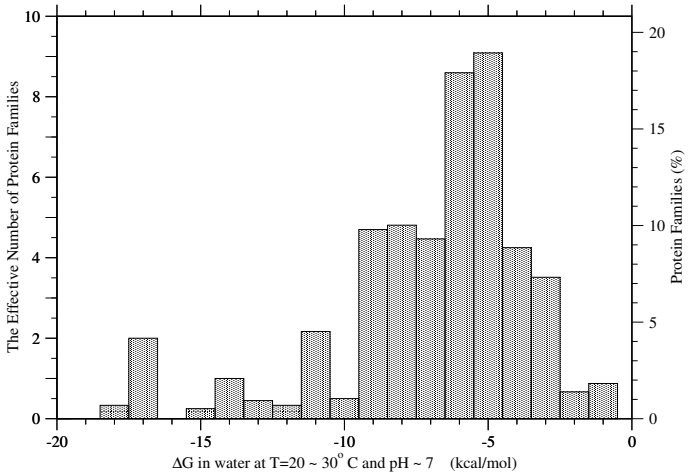

Figure S.1: **Distribution of folding free energies of monomeric protein families.** Stability data of monomeric proteins for which the item of dG_H2O or dG was obtained in the experimental condition of $6.7 \leq \mathrm{pH} \leq 7.3$ and $20°C \leq T \leq 30°C$ and their folding-unfolding transition is two state and reversible are extracted from the ProTherm (Kumar et al., 2006); in the case of dG only thermal transition data are used. Thermophilic proteins, and proteins observed with salts or additives are also removed. An equal sampling weight is assigned to each species of homologous protein, and the total sampling weight of each protein family is normalized to one. In the case in which multiple data exist for the same species of protein, its sampling weight is divided to each of the data. However, proteins whose stabilities are known may be samples biased from the protein universe. The value, $\Delta G_e = -5.24$ kcal/mol, of equilibrium stability at the representative parameter values, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$, agrees with the most probable value of ΔG in the distribution above. Also, the range of ΔG shown above is consistent with that range, -2 to -12.5 kcal/mol, expected from the present model. The kcal/mol unit is used for ΔG. A similar distribution was also compiled (Zeldovich et al., 2007).

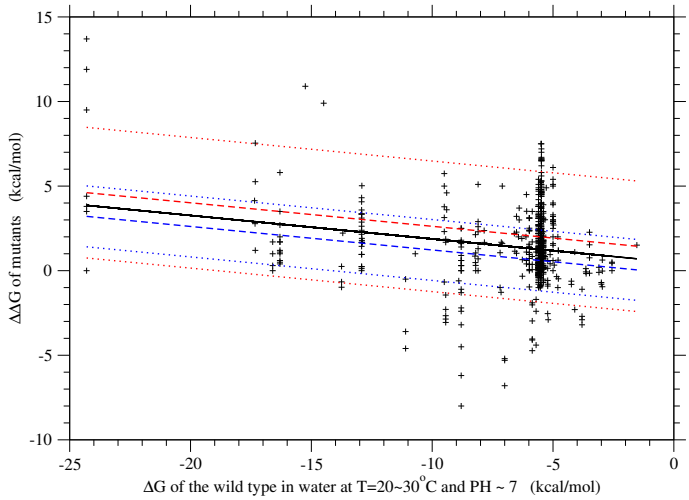

Figure S.2: **Dependence of stability changes, $\Delta\Delta G$, due to single amino acid substitutions on the protein stability, ΔG, of the wild type.** A solid line shows the regression line, $\Delta\Delta G = -0.139\Delta G + 0.490$; the correlation coefficient and p-value are equal to -0.20 and $< 10^{-7}$, respectively. Broken lines show two means of bi-Gaussian distributions, μ_s in blue and μ_c in red. Blue dotted lines show $\mu_s \pm 2\sigma_s$ and red dotted lines $\mu_c \pm 2\sigma_c$. See Eqs. (S.5), (S.6) and (S.7) for the bi-Gaussian distribution. Stability data of single amino acid mutants for which the items dG_H2O and ddG_H2O or dG and ddG were obtained in the experimental condition of $6.7 \leq$ pH ≤ 7.3 and $20°C \leq T \leq 30°C$ and their folding-unfolding transitions are two state and reversible are extracted from the ProTherm (Kumar et al., 2006). In the case of dG only thermal transition data are used. In the case in which multiple data exist for the same protein, only one of them is used. The kcal/mol unit is used for $\Delta\Delta G$ and ΔG. A similar distribution was also compiled (Serohijos et al., 2012).

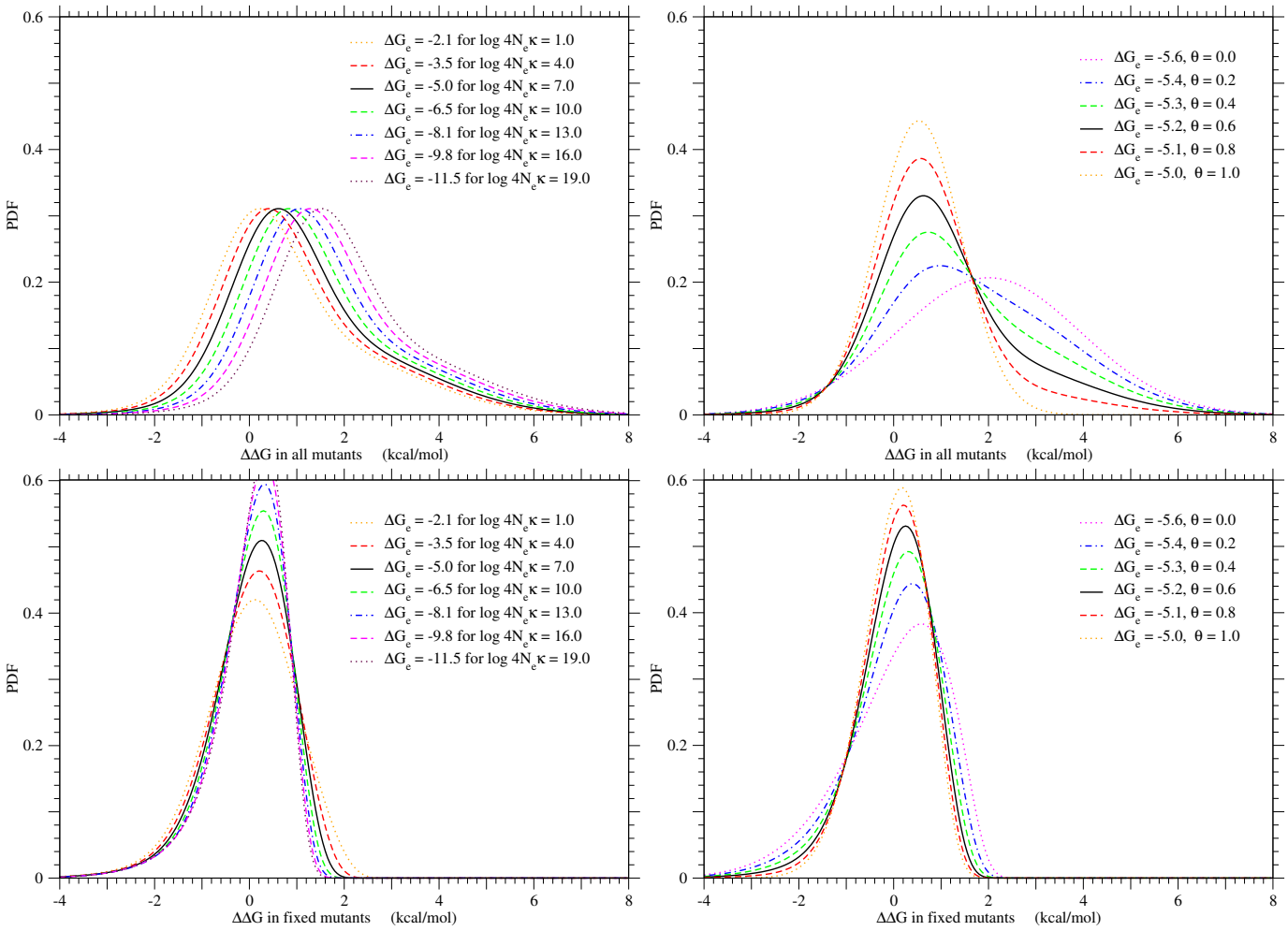

Figure S.3: **PDFs of stability changes, $\Delta\Delta G$, due to single amino acid substitutions in all mutants and in fixed mutants at equilibrium of protein stability, $\Delta G = \Delta G_e$.** The PDF of $\Delta\Delta G$ due to single amino acid substitutions in all arising mutants is assumed to be bi-Gaussian; see Eq. (S.5). Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed. The kcal/mol unit is used for $\Delta\Delta G$ and ΔG_e.

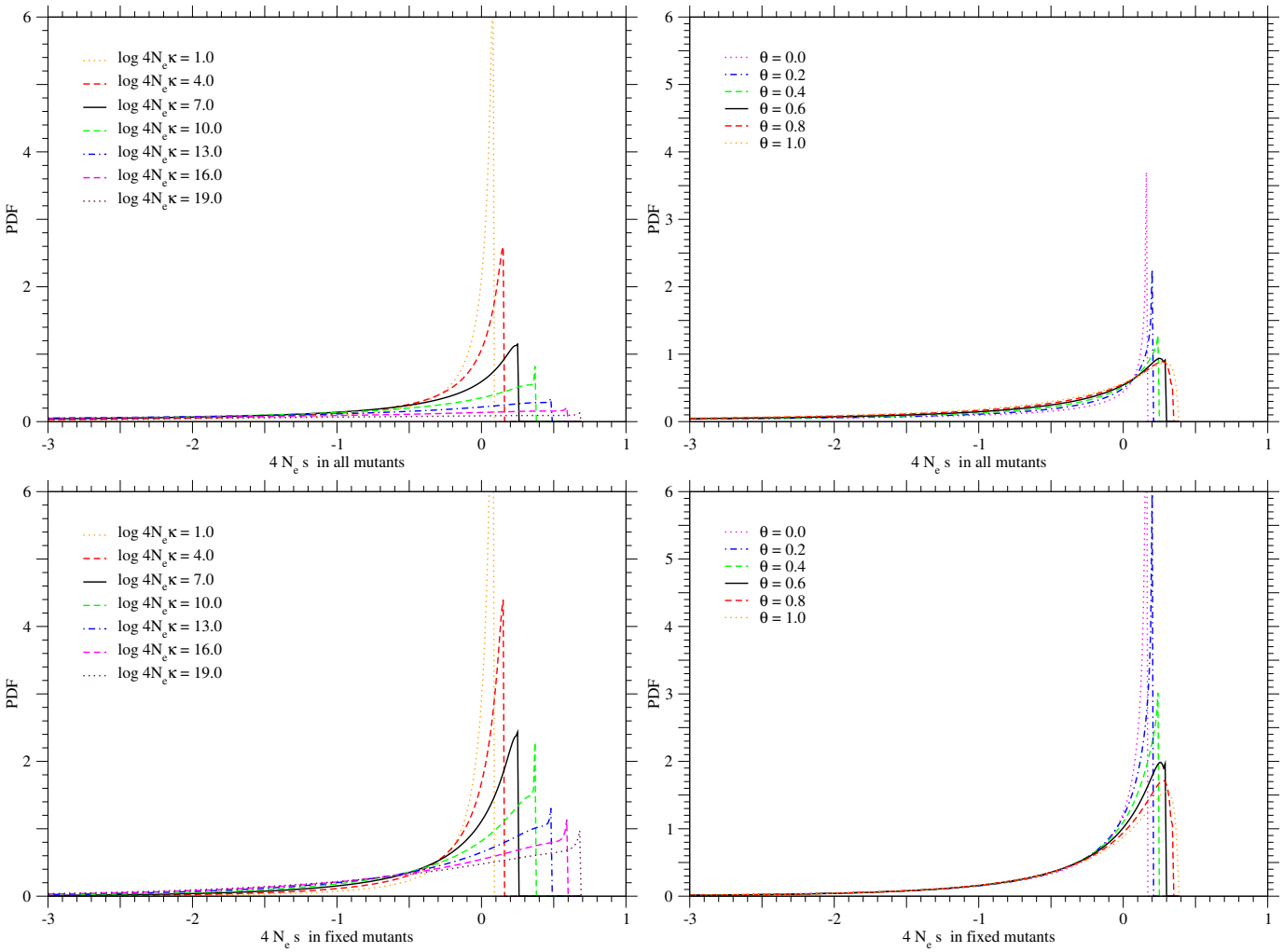

Figure S.4: **PDFs of $4N_e s$ in all mutants and in fixed mutants at equilibrium of protein stability, $\Delta G = \Delta G_e$.** Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed.

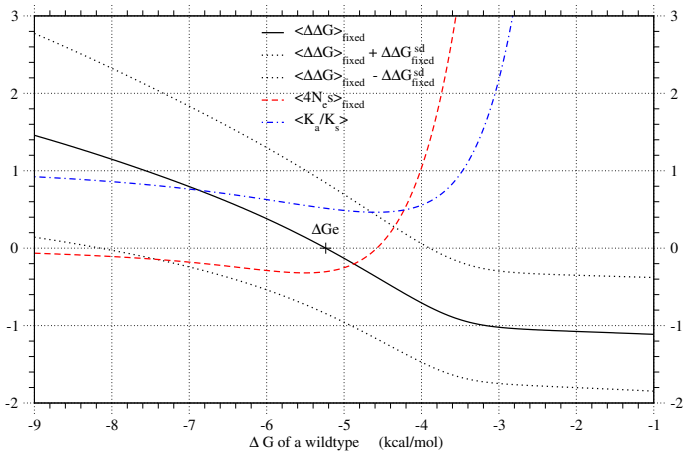

Figure S.5: **The average, $\langle\Delta\Delta G\rangle_{\mathbf{fixed}}$, of stability changes over fixed mutants versus protein stability, ΔG, of the wild type.** ΔG_e, where $\langle\Delta\Delta G\rangle = 0$, is the stable equilibrium value of folding free energy, ΔG, in protein evolution. The averages of $\Delta\Delta G$, $4N_e s$, and K_a/Ks over fixed mutants are plotted against protein stability, ΔG, of the wild type by solid, broken, and dash-dot lines, respectively. Thick dotted lines show the values of $\langle\Delta\Delta G\rangle_{\rm fixed} \pm \Delta\Delta G^{\rm sd}_{\rm fixed}$, where $\Delta\Delta G^{\rm sd}_{\rm fixed}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants. $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed. The kcal/mol unit is used for $\Delta\Delta G$.

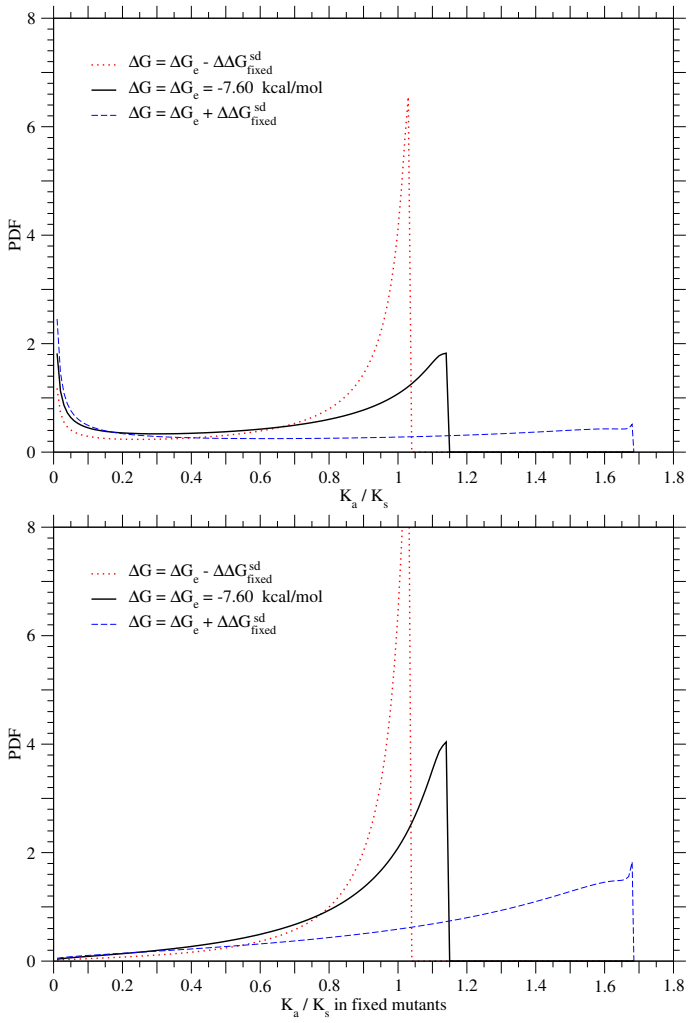

Figure S.6: **Dependence of the PDF of K_a/K_s on protein stability, ΔG, of the wild type in all mutants or in fixed mutants only.** $\Delta\Delta G^{\rm sd}_{\rm fixed}$ is the standard deviation (0.84 kcal/mol) of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed. The kcal/mol unit is used for ΔG_e.

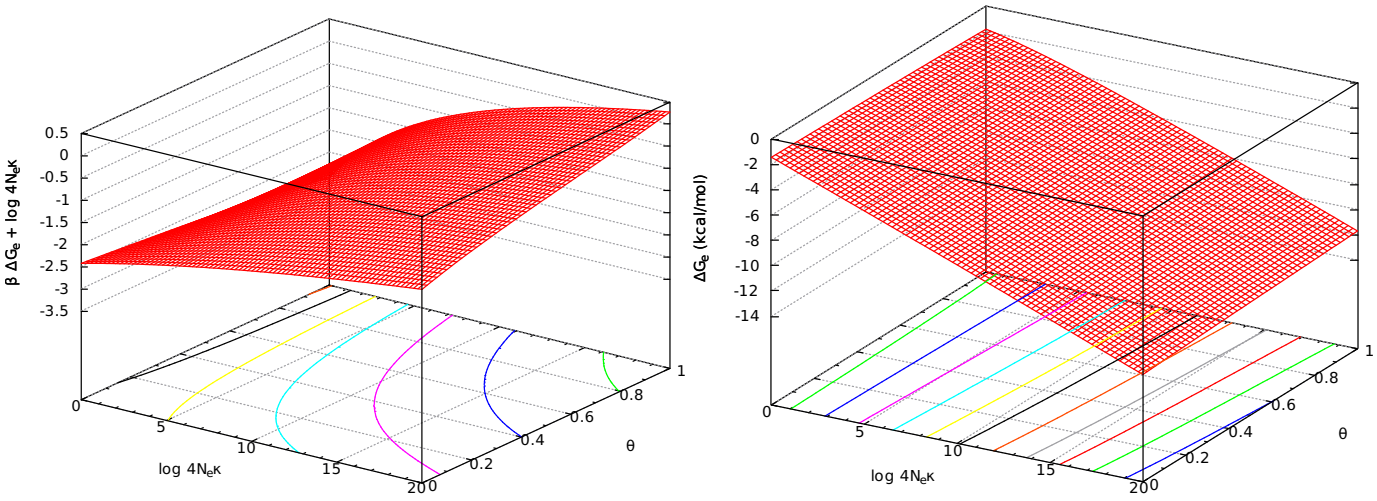

Figure S.7: **Dependence of equilibrium stability, ΔG_e, on parameters, $4N_e\kappa$ and θ.** ΔG_e is the equilibrium value of folding free energy, ΔG, in protein evolution. The value of $\beta\Delta G_e + \log 4N_e\kappa$ is the upper bound of $\log 4N_e s$, and would be constant if the mean of $\Delta\Delta G$ in all arising mutants did not depend on ΔG; see Eq. (S.3). The kcal/mol unit is used for ΔG_e.

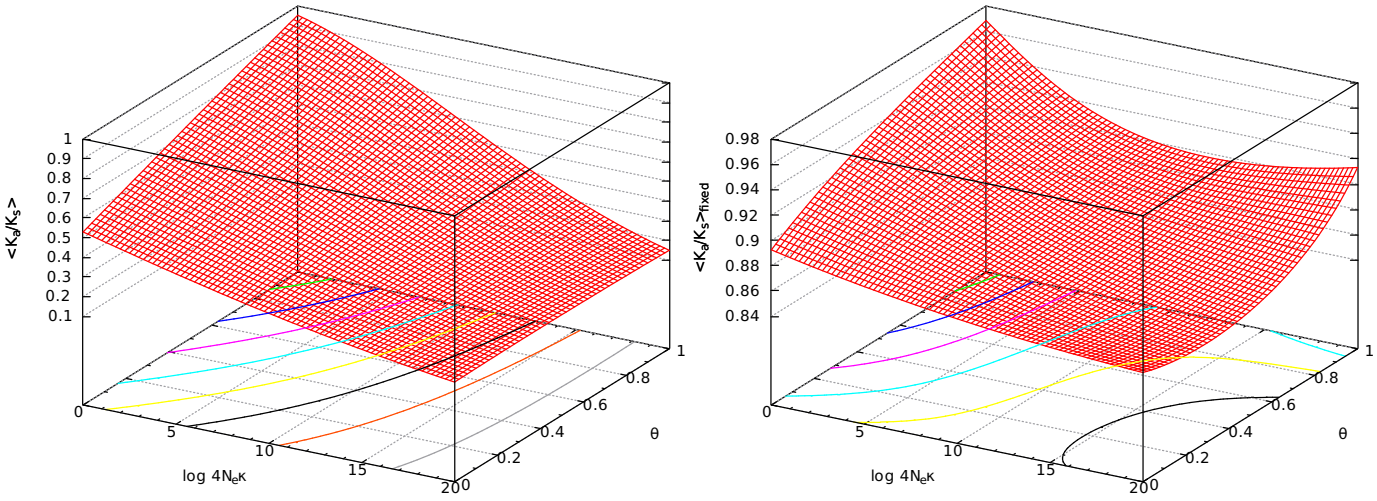

Figure S.8: **The average of K_a/K_s over all mutants or over fixed mutants only at equilibrium of protein stability, $\Delta G = \Delta G_e$.**

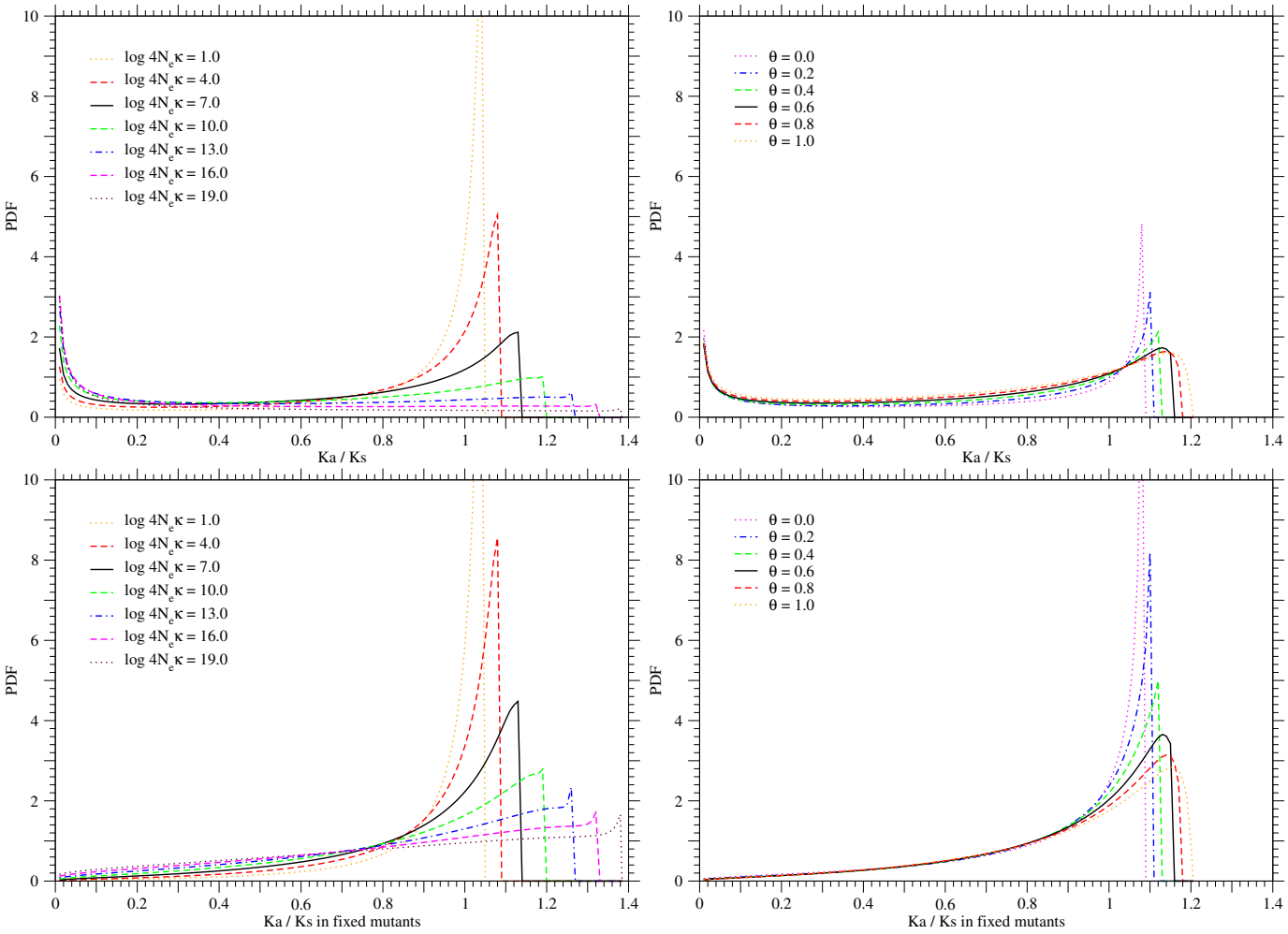

Figure S.9: **PDFs of K_a/K_s in all mutants and in fixed mutants only at equilibrium of protein stability, $\Delta G = \Delta G_e$.** Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed.

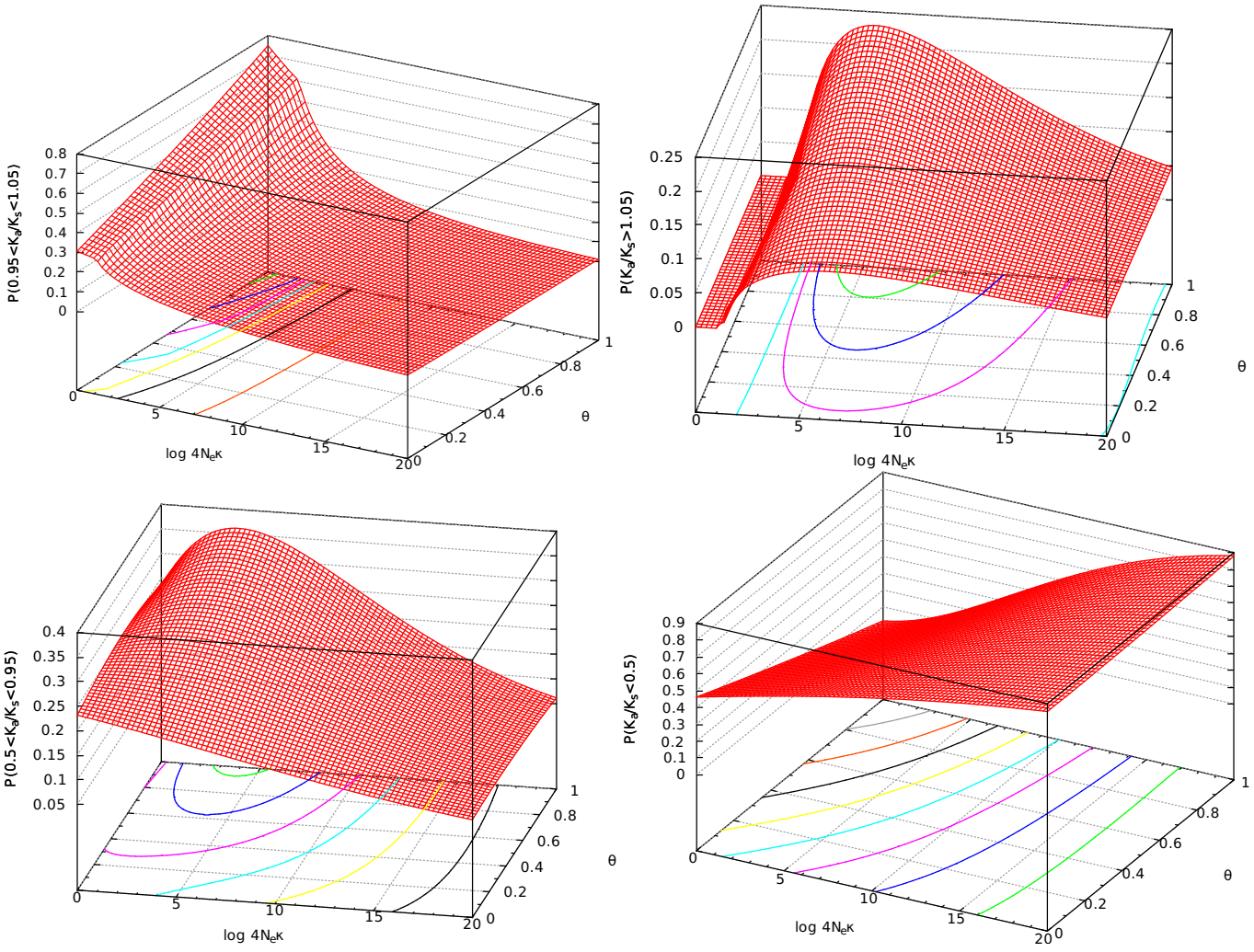

Figure S.10: **Probability of each selection category in all mutants at equilibrium of protein stability,** $\Delta G = \Delta G_e$**.** Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively.

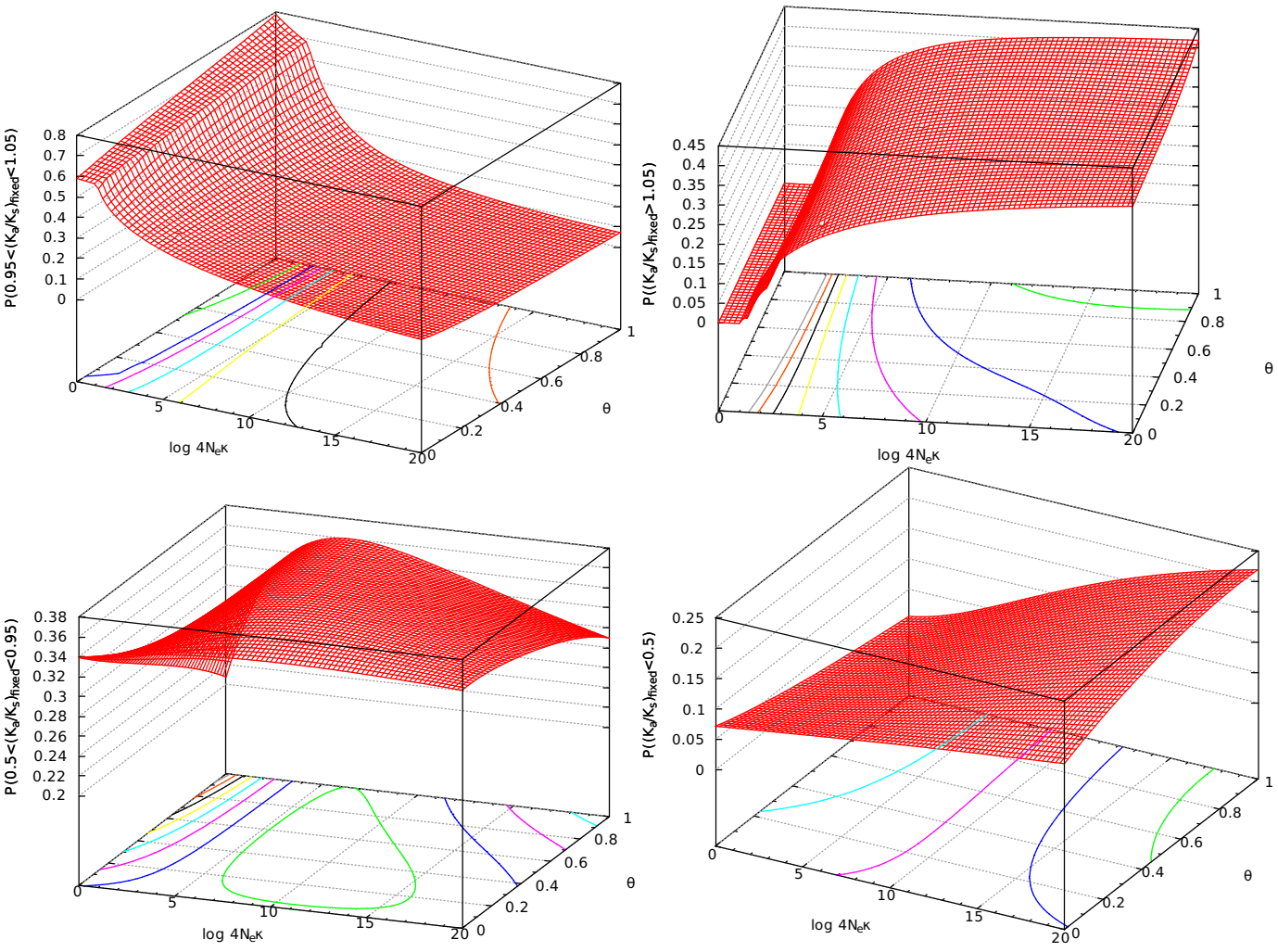

Figure S.11: **Probability of each selection category in fixed mutants at equilibrium of protein stability,** $\Delta G = \Delta G_e$**.** Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively.

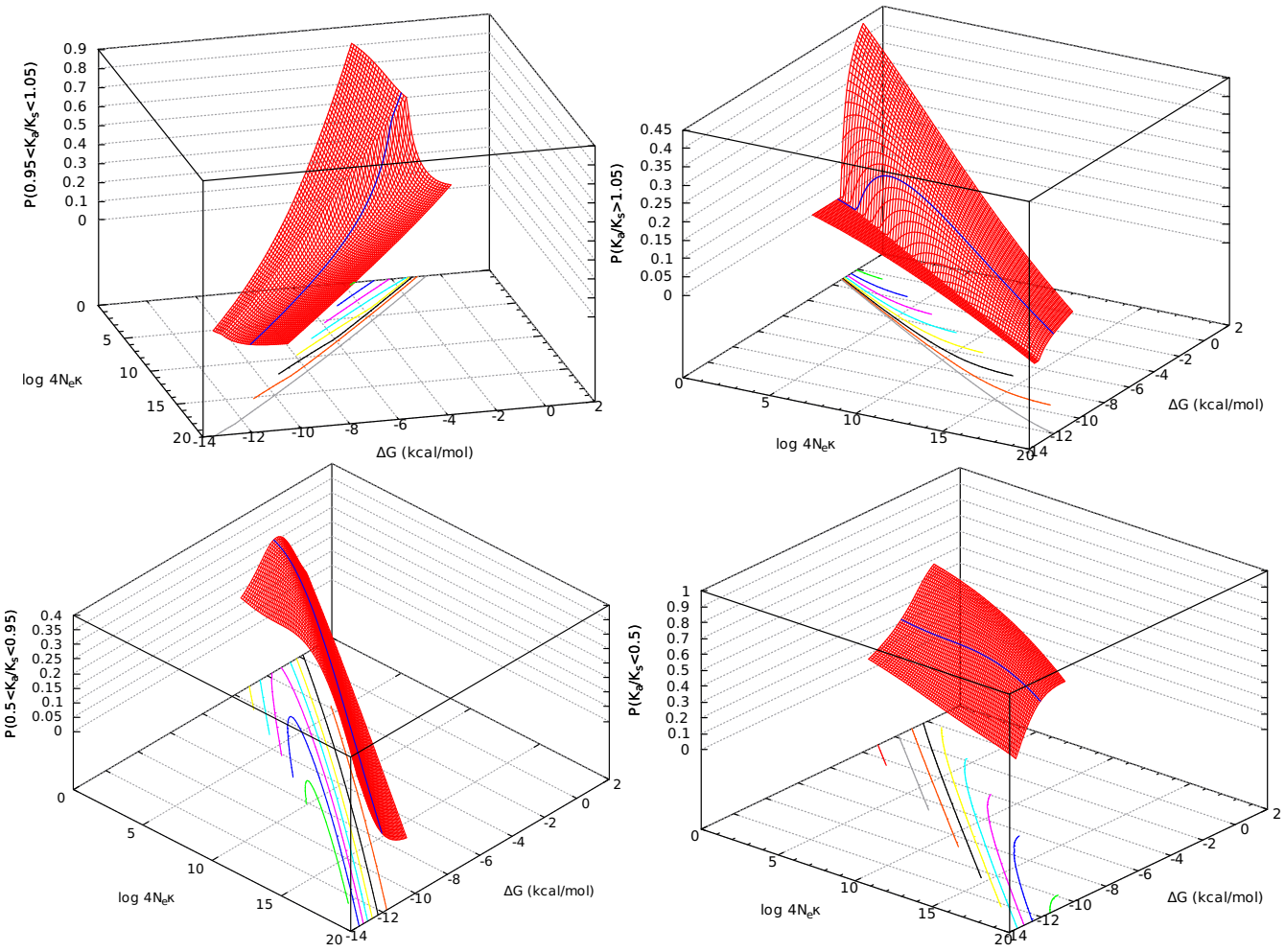

Figure S.12: **Dependence of the probability of each selection category in all mutants on $4N_e\kappa$ and ΔG.** A blue line on the surface grid shows $\Delta G = \Delta G_e$, which is the equilibrium value of ΔG in protein evolution. The range of ΔG shown in the figures is $|\Delta G - \Delta G_e| < 2 \cdot \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$, where $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively. $\theta = 0.53$ is employed. The kcal/mol unit is used for ΔG.

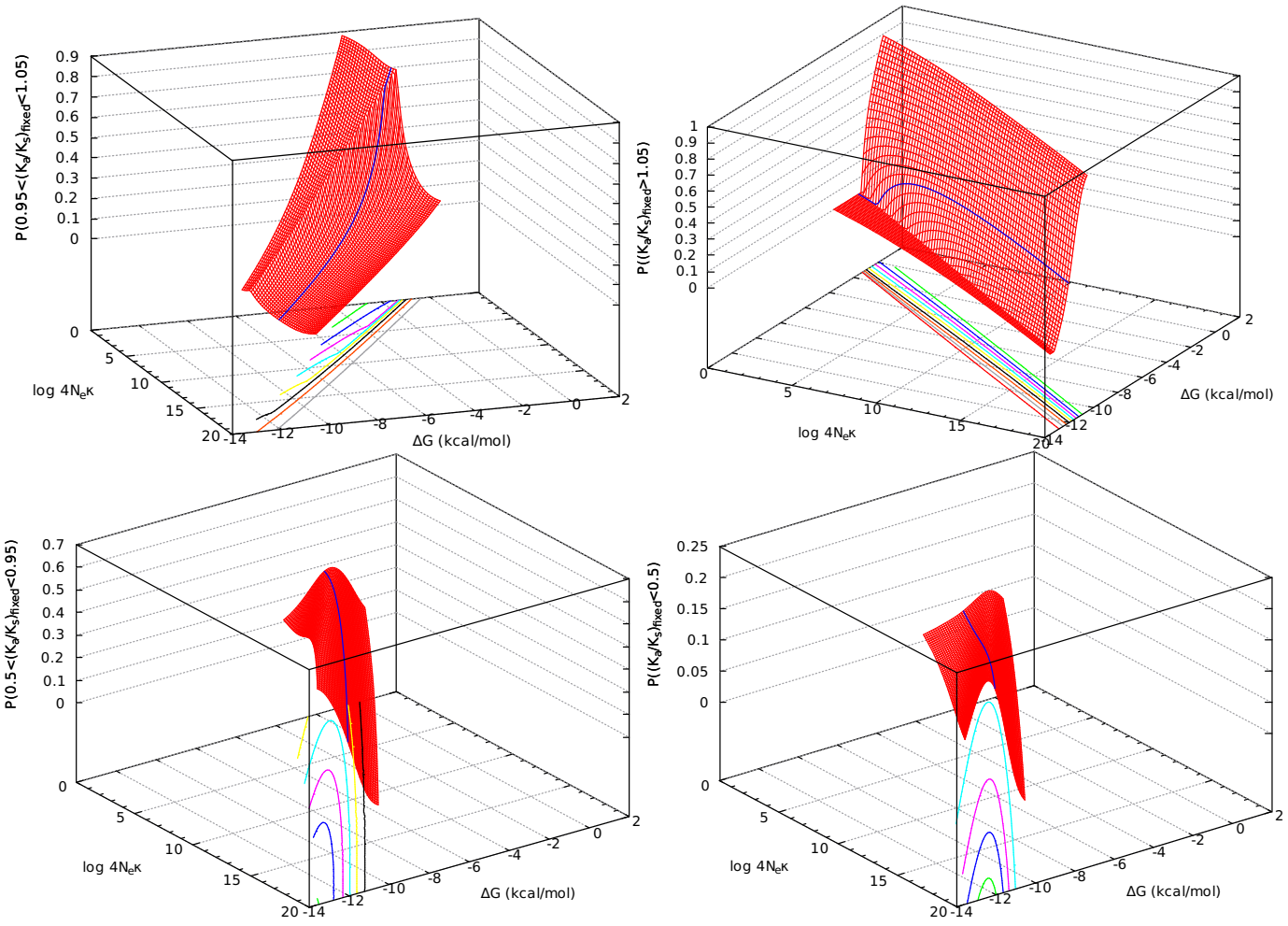

Figure S.13: **Dependence of the probability of each selection category in fixed mutants on $4N_e\kappa$ and ΔG.** A blue line on the surface grid shows $\Delta G = \Delta G_e$, which is the equilibrium value of ΔG in protein evolution. The range of ΔG shown in the figures is $|\Delta G - \Delta G_e| < 2 \cdot \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$, where $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively. $\theta = 0.53$ is employed. The kcal/mol unit is used for ΔG.

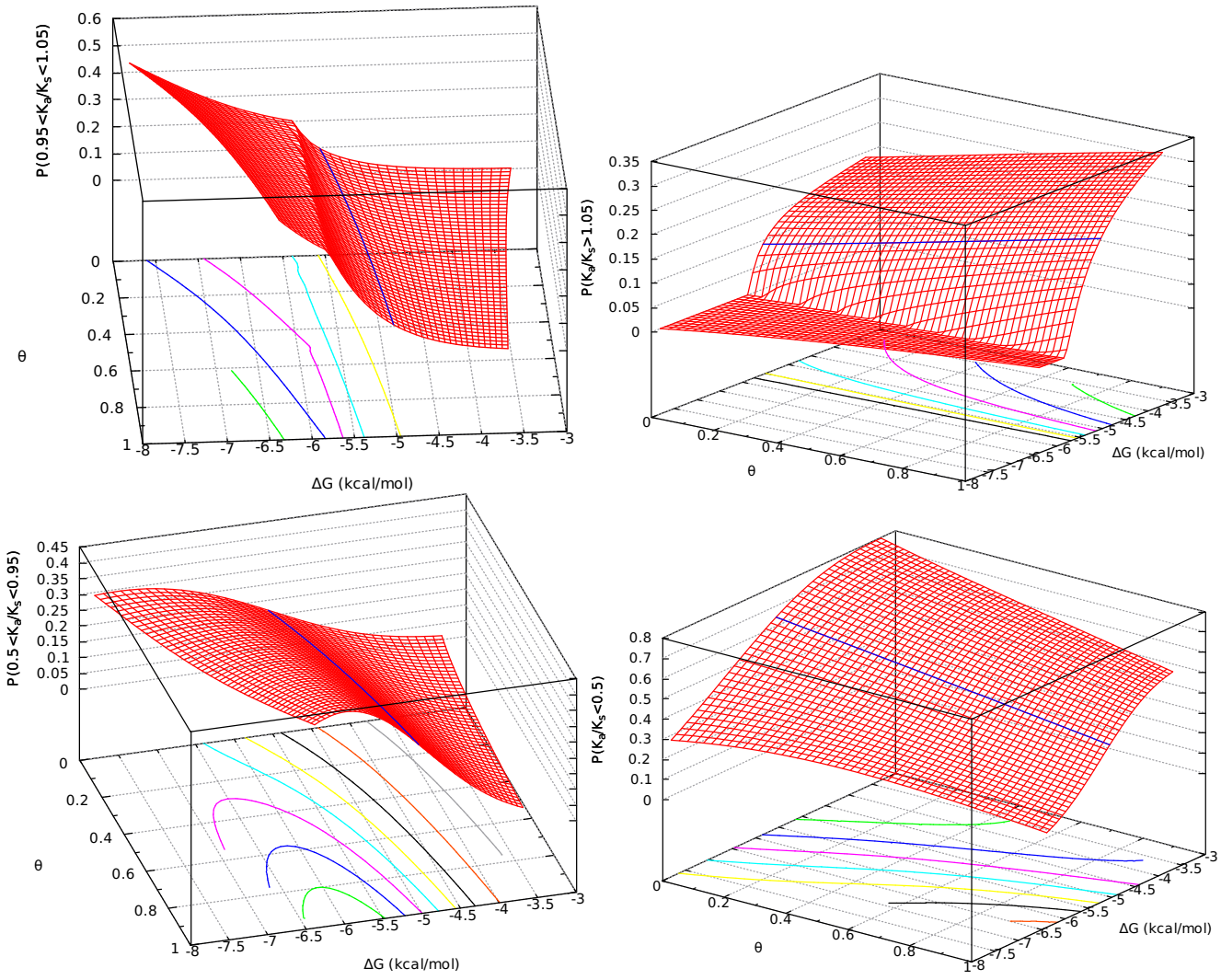

Figure S.14: **Dependence of the probability of each selection category in all mutants on θ and ΔG.** A blue line on the surface grid shows $\Delta G = \Delta G_e$, which is the equilibrium value of ΔG in protein evolution. The range of ΔG shown in the figures is $|\Delta G - \Delta G_e| < 2 \cdot \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$, where $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively. $\log 4N_e\kappa = 7.550$ is employed. The kcal/mol unit is used for ΔG.

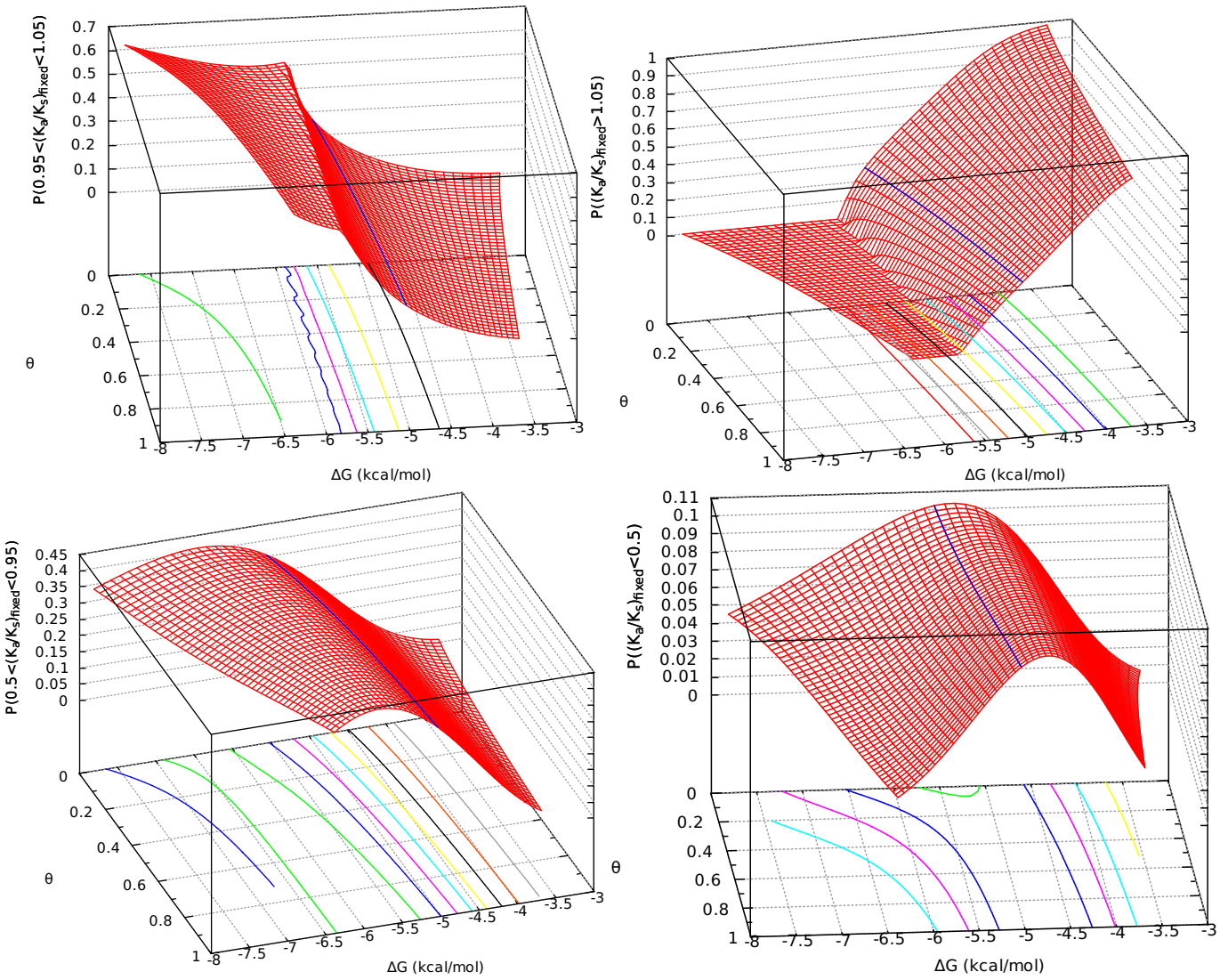

Figure S.15: **Dependence of the probability of each selection category in fixed mutants on θ and ΔG.** The blue line on the surface grid shows $\Delta G = \Delta G_e$, which is the equilibrium value of ΔG in protein evolution. The range of ΔG shown in the figures is $|\Delta G - \Delta G_e| < 2 \cdot \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$, where $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. Arbitrarily, the value of K_a/K_s is categorized into four classes; negative, slightly negative, nearly neutral, and positive selection categories in which K_a/K_s is within the ranges of $K_a/K_s \leq 0.5$, $0.5 < K_a/K_s \leq 0.95$, $0.95 < K_a/K_s \leq 1.05$, and $1.05 < K_a/K_s$, respectively. $\log 4N_e\kappa = 7.550$ is employed. The kcal/mol unit is used for ΔG.

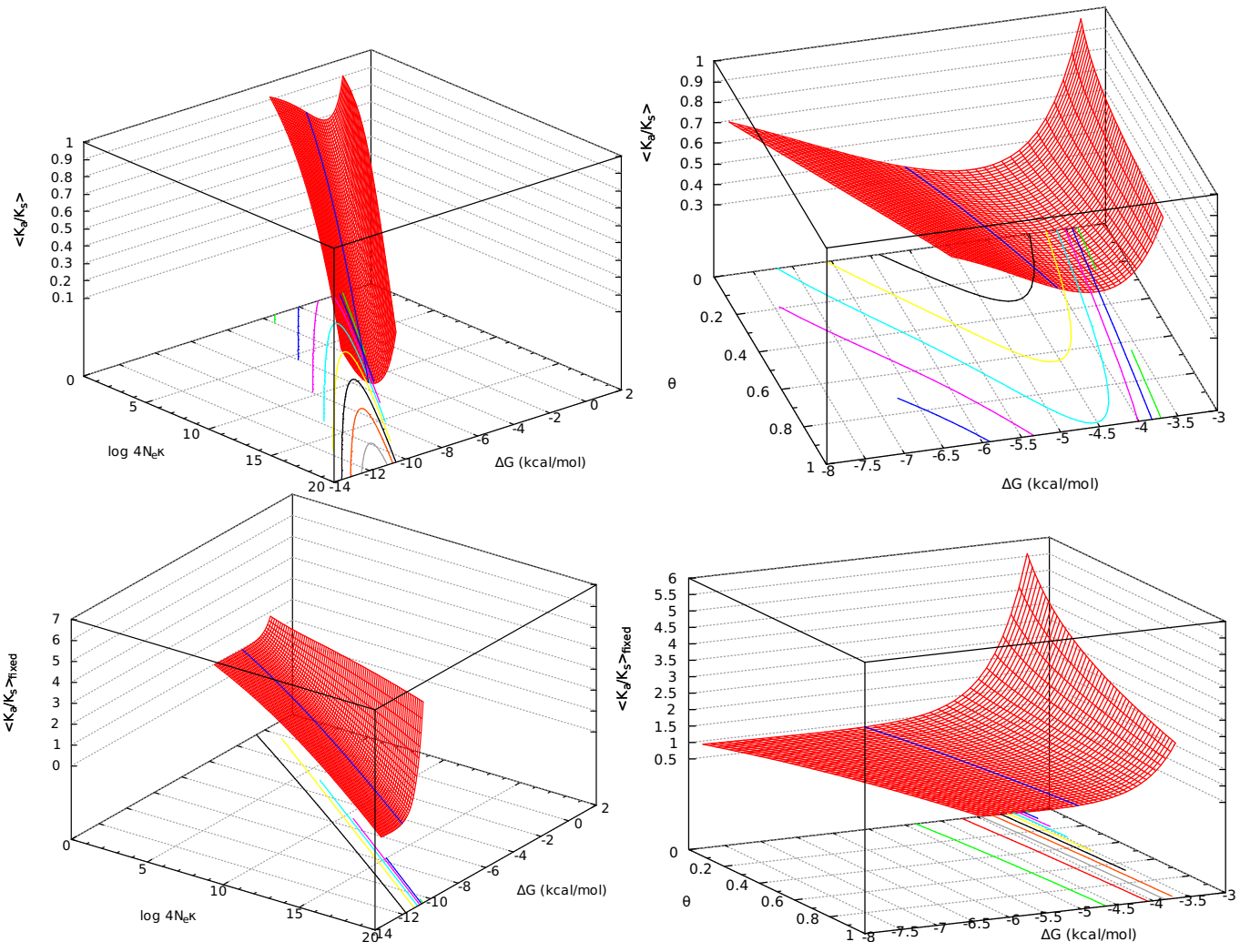

Figure S.16: **Dependence of the average of K_a/K_s over all mutants or over fixed mutants only on protein stability, ΔG, of the wild type.** A blue line on the surface grid shows $\Delta G = \Delta G_e$, which is the equilibrium value of ΔG in protein evolution. The range of ΔG shown in the figures is $|\Delta G - \Delta G_e| < 2 \cdot \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$, where $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$. Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed. The kcal/mol unit is used for ΔG.

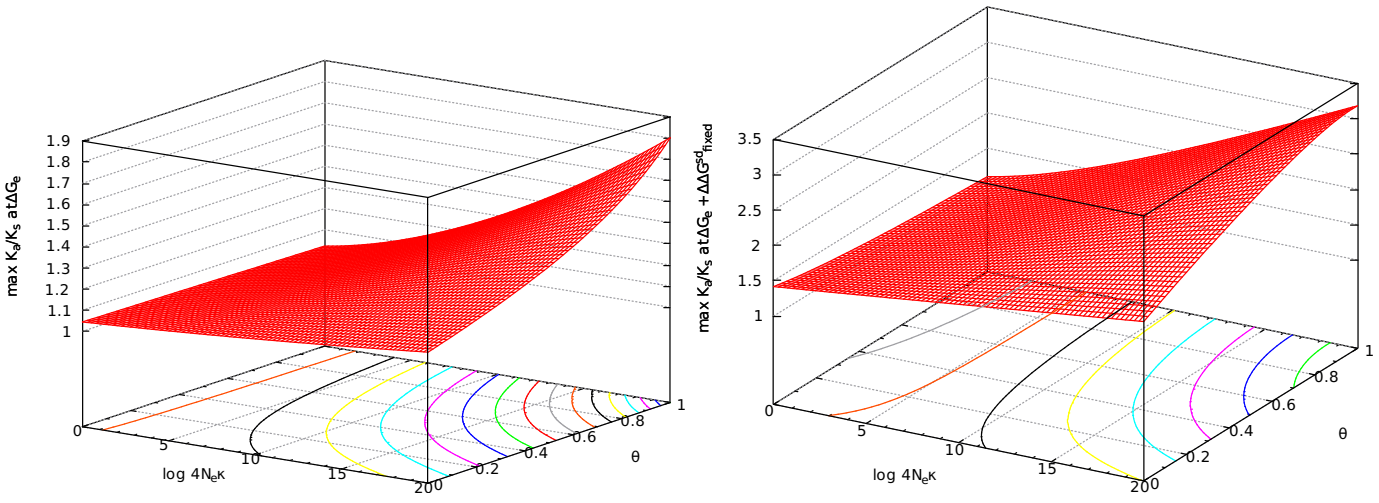

Figure S.17: **Dependence of** max K_a/K_s **on** $4N_e\kappa$ **and** θ**.** The maximum values of K_a/K_s, which correspond to the upper bound of selective advantage s (Eq. (S.4)), at $\Delta G = \Delta G_e$ and at $\Delta G = \Delta G_e + \Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ are plotted as a function of $\log 4N_e\kappa$ and θ; $\Delta\Delta G^{\mathrm{sd}}_{\mathrm{fixed}}$ is the standard deviation of $\Delta\Delta G$ over fixed mutants at $\Delta G = \Delta G_e$.

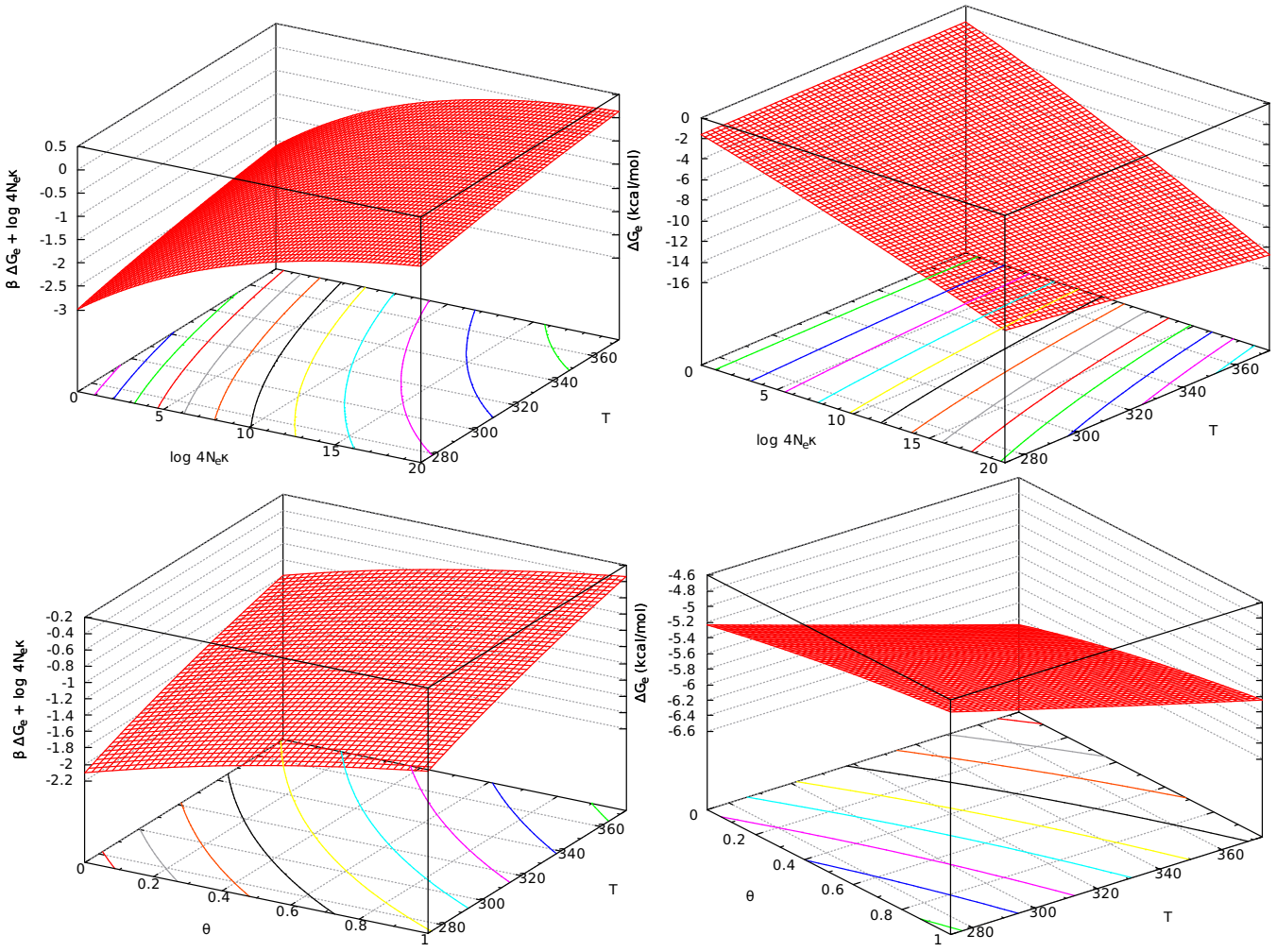

Figure S.18: **Dependence of equilibrium stability, ΔG_e, on parameters, $4N_e\kappa$, θ and T.** ΔG_e is the equilibrium value of folding free energy, ΔG, in protein evolution. T is absolute temperature; $\beta = 1/kT$, where k is the Boltzmann constant. Equations (22), (23) and (24) are assumed for the distribution of $\Delta\Delta G$ and its dependency on ΔG; they are assumed to be independent of T. Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed. The value of $\beta\Delta G_e + \log 4N_e\kappa$ is the upper bound of $\log 4N_e s$, and would not depend on $\log 4N_e\kappa$ if the mean of $\Delta\Delta G$ in all arising mutants did not depend on ΔG; see Eq. (S.3). The kcal/mol unit is used for ΔG_e.

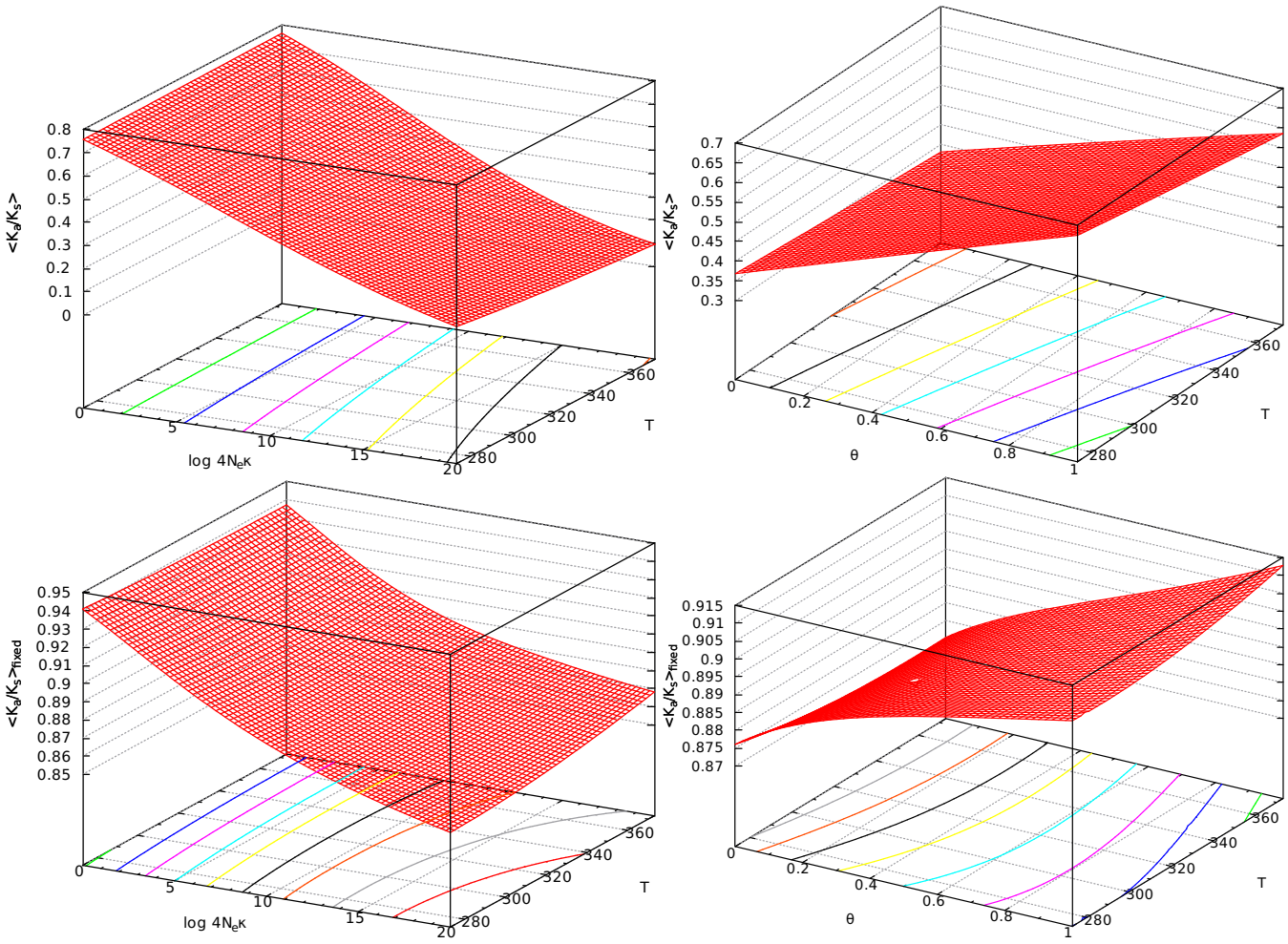

Figure S.19: **The average of K_a/K_s over all mutants or over fixed mutants only at equilibrium of protein stability, $\Delta G = \Delta G_e$: Dependence on temperature.** T is absolute temperature. Equations (22), (23) and (24) are assumed for the distribution of $\Delta\Delta G$ and its dependency on ΔG; they are assumed to be independent of T. Unless specified, $\log 4N_e\kappa = 7.550$ and $\theta = 0.53$ are employed.

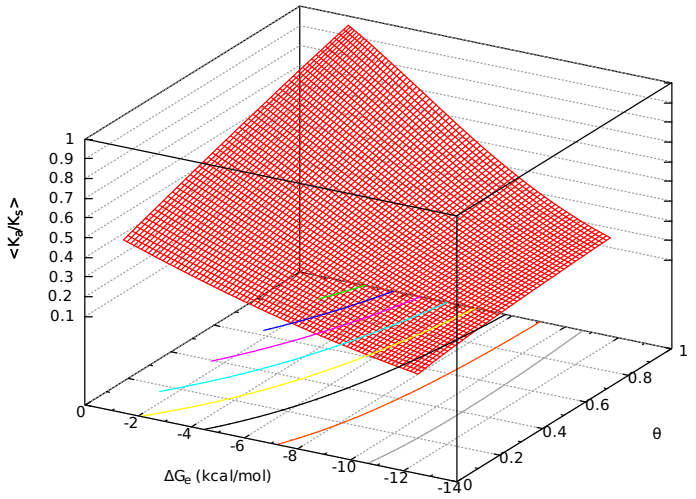

Figure S.20: **The average of K_a/K_s over all mutants as a function of ΔG_e and θ.**