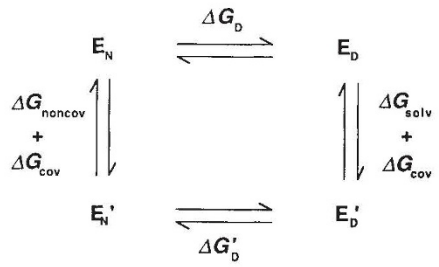

Fig. 3 Thermodynamic cycle relating the unfolding of wild-type (E) and mutant (E′) enzymes. On mutation, there is a change in the free energy of the protein (ΔG_{cov}) because of covalent changes such as the change in chemical bonds on going from Ile to Ala). ΔG_{cov} is the same for both the folded and unfolded states and so cancels out when comparing the right and left hand sides of the cycle. There are also changes in the noncovalent interactions, ΔG_{noncov} ($=G_{E_N'}-G_{E_N}$), on mutation. In the unfolded enzyme, the side chains of the amino acids are more, if not completely exposed to solvent and so $\Delta G_{noncov(D)}$ represents predominantly the changes in the solvation energy of the side chain, ΔG_{solv} ($=G_{E_D'}-G_{E_D}$), on mutation. ΔG_{noncov} for the folded state represents predominantly the loss of interaction energy between the enzyme and those parts of the side chain that are deleted on mutation. ΔG_{noncov} contains, in addition, the energy terms associated with any reorganization of enzyme and associated solvent on mutation. The experimentally determined quantity in our experiments is the difference in unfolding energy of the wild-type and mutant enzymes, $\Delta G_D - \Delta G_D'$. It is seen from the cycle that $\Delta G_D - \Delta G_D' = \Delta G_{noncov} - \Delta G_{solv}$.

ments of the destabilizing effects of cavities in enzymes. This points to a way of stabilizing protein structures: make substitutions to fill in holes that occur in the native structure.

Calculation of the free energy of folding (or unfolding) of proteins *ab initio* involves determining the free energies of the folded and unfolded states (Fig. 3, $\Delta G_D = G_{E_D} - G_{E_N}$, where E_N and E_D are the native and denatured forms of the enzyme). This requires calculating all the noncovalent interaction energies in the folded and unfolded proteins and the solvation energies. One fundamental unknown quantity is the noncovalent interaction energy in the unfolded state. This is probably not a single state but consists of a mixture of many different states of similar energies, in which the exposure of side chains to solvent is unknown and variable. This presents a stumbling block to calculation. It is important to relate the empirical data from the protein engineering experiments, $\Delta\Delta G_D$, ($=\Delta G_D - \Delta G_D'$) to the energies used in calculations. This is done by a simple thermodynamic cycle (Fig. 3) which considers the native and denatured states for wild-type (E_N and E_D) and mutant (E_N' and E_D') enzymes. In Fig. 3, the change in the noncovalent interaction energy in the native enzyme on removal of a side chain is defined by $\Delta G_{noncov} = G_{noncov(mut)} - G_{noncov(wt)}$ (where the subscripts refer to the noncovalent interactions in the folded mutant and wild-type enzymes). The equivalent change in noncovalent energy in the denatured state is termed ΔG_{solv} from Fig. 3:

$$\Delta G_D^{H_2O} - \Delta G_D'^{H_2O} = \Delta G_{noncov} - \Delta G_{solv} \quad (3)$$

First, it can be seen from equation (3) that the changes in free energy of unfolding are a function of the interactions in folded and denatured states. Second, equation (3) may be used to gather information about interactions in the denatured state as follows. ΔG_{noncov} may be calculated from the native structures of the wild-type and mutant enzymes using conventional computational methods. Recent developments in computational methods allow the direct calculation of the values of ΔG_{noncov} (and ΔG_{solv}) by the mathematical equivalent of mutating the side chains in slow increments[20–22]. If the side chain in the unfolded state is completely exposed to solvent, then ΔG_{noncov} is simply calculated from equation (3) using relative solvation energies of the amino acid side chains that are available from experiments on model compounds[23,24]. For example, ΔG_{solv} for Ile→Val is −0.12, Ile→Ala is −0.15 and Phe→Leu is 2.24 kcal mol^{-1} (ref. 23). Thus, if the side chain of Phe7 in denatured wild-type enzyme is completely exposed to solvent and so also is Leu7 in the mutant, then ΔG_{noncov} for Phe→Leu would be $4.6+2.2=6.8$ kcal mol^{-1}. Discrepancies between calculated and measured values of ΔG_{noncov} will give clues about side chain interactions in the unfolded states. Further mutational experiments will provide additional direct data on protein stability and more general information on interactions in native and denatured states.

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Errata

A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecules

J. H. Brown *et al.*
Nature **332,** 845–850 (1988).
In Fig. 3*a*, the I-A^d sequence numbers 10, 12, 14 should be replaced by the numbers 11, 13, 15 respectively; the word "first" in the seventh line, fourth paragraph of page 846 should be deleted.

Palaeoenvironmental trends in the history of trace fossils

D. J. Bottjer, M. L. Droser & D. Jablonski
Nature **333,** 252–255 (1988).
On page 253, left column, fourth paragraph down, a line of text is missing between the seventh and eighth lines. This line reads "features, and, most important to our study, avoid the circular".

A cellular analogue of visual cortical plasticity

Y. Frégnac, D. Shulz, S. Thorpe & E. Bienenstock
Nature **333,** 367–370 (1988).
The bottom line shown in Fig. 2 should be read in the reverse order, "LEFT RIGHT", instead of "RIGHT LEFT", such as that peri-stimulus histograms presented in the left column correspond to stimulation through the left eye (S^+), and those presented in the right column correspond to stimulation through the right eye (S^0).