Thirty mutants of E. coli dihydrofolate reductase (DHFR) have been made by site-directed mutagenesis. The variants were designed to probe the structure-function relationships in DHFR with respect to the catalytic mechanism, coenzyme specificity, structural stability and inhibitor binding. The high resolution (1.9Å-2.0Å) crystal structure of a dozen of these variants is now known. This makes possible a detailed structural analysis of the effects of these specific amino acid substitutions. In general, few changes are observed in the enzyme structure. Some significant changes are found, however, in the case of Gly 95→ala where a cis-peptide bond is converted to the trans conformation and in the case of Asp 97→ala where the backbone shifts to accommodate the additional methyene group of the glutamate side chain. Where large van der Waals gaps are formed by the amino acid substitution, highly ordered water molecules fill the space. The effects of these amino acid substitutions on the enzymes’ function will be presented.