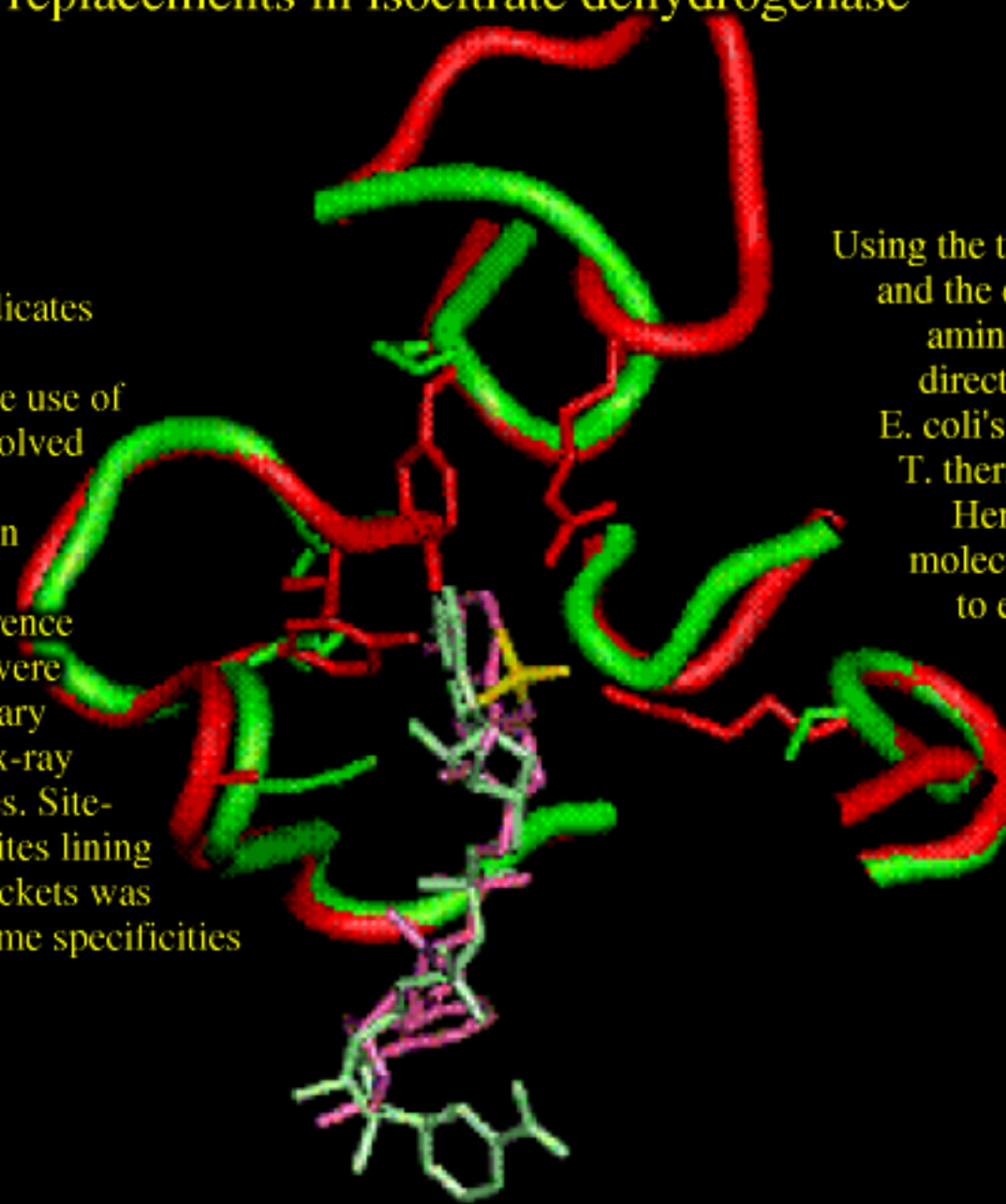


Protein engineering reveals ancient adaptive replacements in isocitrate dehydrogenase

Evolutionary analysis indicates that eubacterial isocitrate dehydrogenases that make use of a NADP cofactor first evolved from an NAD-dependent precursor about 3.5 billion years ago. Amino acids responsible for this difference in coenzyme specificity were identified from evolutionary reconstruction and from x-ray crystallographic structures. Site-directed mutagenesis at sites lining the coenzyme binding pockets was used to invert the coenzyme specificities of both enzymes.



Using the three dimensional structure and the evolutionary history of the amino acids we engineered site directed mutations that changed *E. coli*'s enzyme to use NAD and *T. thermophilus*'s to use NADP. Hence the adaptive history of molecular evolution is amenable to experimental investigation.