Resurrecting ancient ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) *in silico, in vitro,* and *in vivo*

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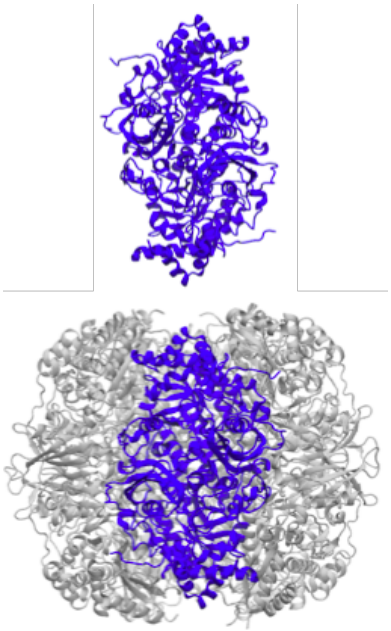
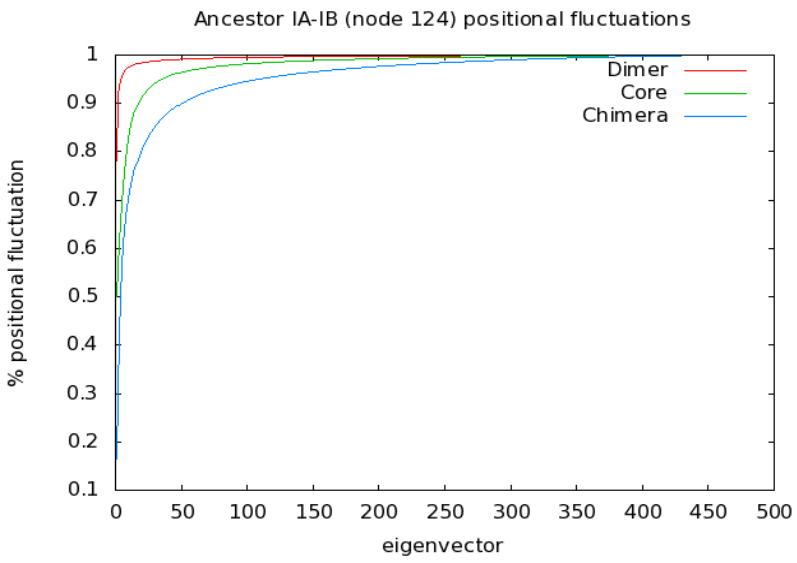
Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO) is the primary catalyst of biological carbon fixation. Although the active site is structurally similar across autotrophs, the tertiary structure varies from a dimer of catalytic large subunits to a hexadecamer that includes four catalytic dimers and eight structural small subunits. Studying the evolutionary history of RubisCO informs us of atmospheric and environmental conditions throughout the past, as well as into the future. By determining the structure—and therefore function—of ancestral Rubisco species, we can explore its kinetic properties and their environmental implications. The sequence and structure of catalytic dimers for several ancestral species are known, but it is believed these species existed with small subunits. Here we present a method of simulating these ancestral proteins as chimera within extant species in order to accurately recreate and analyze their solvation environments. These simulations inform experimental strategies, such as the inclusion of chaperonin proteins and small subunits, for expressing and characterizing ancestral RubisCO enzymes *in vitro* and *in vivo.*





The figure on top (left) is the known catalytic dimer structure of an ancestral RubisCO. The figure on bottom (left) is the ancestral dimer inserted into an existing hexadecameric structure. This chimeric structure more accurately recreates the solvation environment of the ancestral enzyme. The global motion of the dimer in each solvation environment was analyzed using Principal Component Analysis (PCA) in order to determine the most accurate strategy for protein expression *in vitro* and *in vivo* (right).