

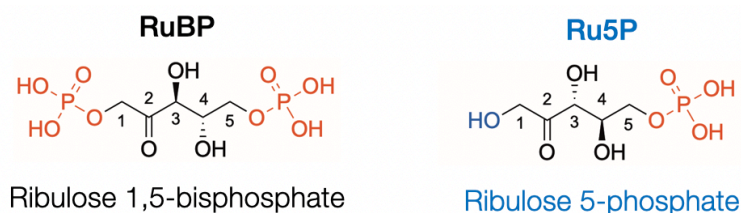
## Novel Carbon Fixation Reaction for Enhanced CO<sub>2</sub> Utilization

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Carbon fixation, the process by which inorganic carbon is converted to organic compounds, plays a critical role in sustaining the biosphere<sup>1</sup>. Advancing our ability to engineer carbon fixation reactions is essential to achieving energy and material sustainability. We aim to broaden the substrate specificity of the key enzyme in photosynthetic carbon fixation, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), enabling it to fix carbon dioxide (CO<sub>2</sub>) into a wider range of valuable compounds.

*Tk*-RuBisCO<sup>2</sup>, a RuBisCO from *Thermococcus kodakarensis* KOD1, exhibits remarkable thermostability, high carboxylase activity, and superior specificity at elevated temperatures. Its folded structure is more stable and can accommodate a greater number of mutations at standard temperatures compared to other RuBisCO types<sup>3</sup>. These unique properties make *Tk*-RuBisCO an excellent candidate for structure-function analysis and protein engineering to explore new CO<sub>2</sub> fixation reactions. We investigated the carboxylase activities of native and mutant *Tk*-RuBisCO using its natural substrate, ribulose 1,5-bisphosphate (RuBP), and a non-native substrate, ribulose 5-phosphate (Ru5P), to confirm their selectivity for a novel CO<sub>2</sub> fixation reaction. This approach has the potential to enhance industrial applications for efficient CO<sub>2</sub> utilization, a carbon-negative technology that supports the transition to a carbon neutral society.



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