

Atomic resolution structure of spinach rubisco reveals protons and dynamics

Nicholas Croy¹, Guillaume Gaullier¹, Patricia Saura², Simonas Masiulis³, Asmit Bhowmick⁴, Jan Kern⁴, Oliver Raschdorf³, Inger Andersson^{5,6}, Ville R. I. Kaila², Cecilia Blikstad¹, Johannes Messinger^{1,7}

¹Department of Chemistry – Ångström, Uppsala University, Uppsala, Sweden.

²Department of Biochemistry and Biophysics, Stockholm University, Arrhenius Laboratories for Natural Sciences, Stockholm, Sweden.

³Materials and Structural Analysis Division, Thermo Fisher Scientific, Eindhoven, the Netherlands.

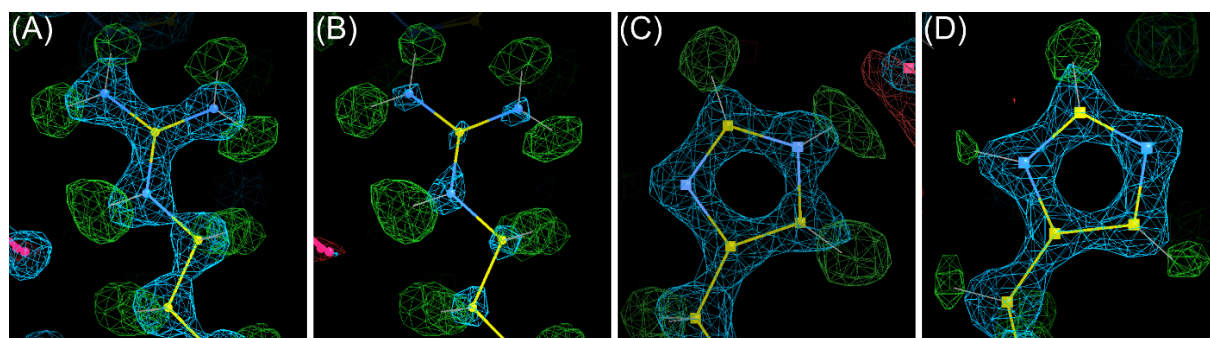
⁴Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

⁵Laboratory of Molecular Biophysics, Department of Cellular and Molecular Biology, Uppsala University, Uppsala, Sweden.

⁶The European Extreme Light Infrastructure, Dolni Brezany, Czech Republic.

⁷Department of Plant Physiology, Umeå University, Umeå, Sweden.

Photosynthetic organisms sustain life on Earth by storing solar energy in biomass. Central to this process is rubisco, the enzyme that catalyzes the fixation of CO₂ to ribulose-1,5-bisphosphate, providing the primary gateway for inorganic carbon into the biosphere. Rubisco's catalytic efficiency is a major determinant of crop productivity and global carbon flux, making it a longstanding target for protein engineering. Yet, attempts to enhance its performance through rational design have met limited success due to an incomplete understanding of rubisco's catalytic mechanism. Here, we report an atomic resolution (1.25 Å) cryo-EM structure of spinach rubisco in complex with the transition-state analogue 2-carboxyarabinitol-1,5-bisphosphate. Supported by large-scale quantum/classical (QM/MM) calculations, our structural analysis reveals protonation equilibria within the active site and unexpected structural flexibility across large protein regions despite the exceptionally tight ligand binding. Our findings provide new insight into the complex interplay of protonation equilibria and conformational sampling, suggesting a novel basis for rubisco's rational redesign utilizing strategies that rely on a combination of dynamic and electrostatic control.



Atomic resolution data reveal protons. F_o maps (blue) are displayed at a contour level of 9.4σ for R295 (A), H325 (C), and H327 (D) and at a contour level of 22σ for R295 (B). H-omit F_o-F_c maps (green/red) are displayed at a contour level of 10.5σ . The green densities indicate the positions of hydrogen atoms. All panels were generated with Coot using the consensus structure H-omit mtz file generated by the Servalcat “fofc” command.

Reference

N. Croy, bioRxiv 2025.12.22.696010, (2025)