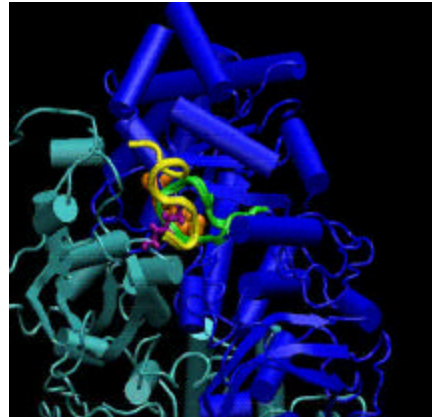


Targeted Molecular Dynamics Simulations of RuBisCO

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RuBisCO is the primary carbon fixation enzyme in *Synechococcus*, as well as in other bacteria and all plants. Its poor specificity and inefficiency represent a bottleneck for carbon sequestration and the photosynthetic process. In the Sandia National Laboratories-Oak Ridge National Laboratory Genomics:GTL project, “Carbon Sequestration in *Synechococcus* Sp.: From Molecular Machines to Hierarchical Modeling,” we are studying RuBisCO using the targeted molecular dynamics (TMD) method to show how structural changes in its binding niche gating mechanism alter the overall enzyme specificity and performance. *In silico* mutations of residues in the C-terminal region affect the computed free-energy barrier for the gating mechanism. This computed free-energy barrier can be used to predict the performance of a given RuBisCO mutant and its associated structure. Previous researchers have been frustrated to find that residues in the binding pocket are strictly conserved between species and cannot be altered without debilitating the enzyme. It is puzzling that the more distant residues, especially those found in the gating regions, cause variation in RuBisCO performance.

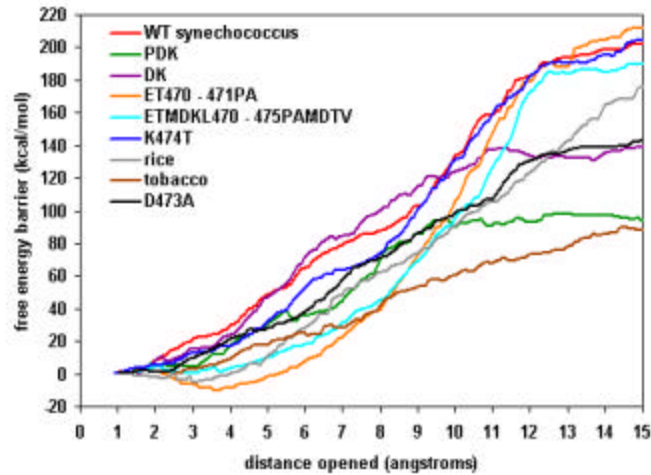


Two RuBisCO large subunits (blue and cyan) sandwiching a ribulose biphosphate molecule (orange) in the binding niche. The binding niche is gated by the C-terminus (yellow), K_{128} (purple), and loop 6 (green).

We added several new capabilities to our molecular dynamics code LAMMPS to enable our RuBisCO modeling. The new features speed up the modeling and increase its sampling efficiency for rare events such as gate openings and closings. Specifically, we added a general hierarchical timestepping capability, a pre-computation of Coulombic forces stored in tables, options to freeze portions of the model as rigid bodies and avoid unnecessary force computation, and the TMD algorithm mentioned above that enables portions of the gate to be rapidly opened and closed. LAMMPS was officially released on September 1, 2004 as an open-source parallel MD package available for download at (www.cs.sandia.gov/~sjplimp/lammps.html). Since its release, it has been downloaded over 2,000 times.

Using TMD and the other new features in LAMMPS, we have predicted the gating barriers for *Synechococcus*, rice, tobacco, and spinach RuBisCOs, as well as the following mutant forms of *Synechococcus* RuBisCO: D473A, K474T, ETMDKL470 – 475PAMDTV, ET470-471PA, the DK mutant, and the PDK mutant. As expected, higher specificity rice and tobacco forms of RuBisCO have smaller gating barriers than the WT *Synechococcus* RuBisCO. Also, destruction of a salt bridge between the C-terminus and the protein wall by D473A mutation reduces the gating barrier. The DK mutant, with almost the same specificity as WT, yields a work profile that is indeed very similar to

WT. The TMD approach enables discrimination between the RuBisCO forms and helps rationalize the effects of structural variations on enzyme performance.



Gating barriers for various forms of RuBisCO.

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