Switching a Nitrilase from *Syechocystis sp.* PCC6803 to a Nitrile Hydratase by Rationally Regulating Reaction Pathways

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Computational Details

The system was minimized with position restraints of 50.0 kcal mol\(^{-1}\) Å\(^{-2}\) and 20.0 kcal mol\(^{-1}\) Å\(^{-2}\) in the first and second stages, respectively, and with no restraint in the third stage. The minimization for each stage took 20,000 steps to reach convergence. Then, the system was heated from 0 K to 300 K in 0.05 ns with a restraint of 10.0 kcal mol\(^{-1}\) Å\(^{-2}\) using the Andersen temperature-coupling scheme \(^1\). The next stage was the equilibration with a restraint of 2.0 kcal mol\(^{-1}\) Å\(^{-2}\) for 0.05 ns, followed by full system equilibration with no restraints for 0.5 ns. Finally, the MD simulation was performed at 300 K for 10 ns. Non-bonding interactions were calculated using a cutoff of 14 Å. The SHAKE algorithm \(^2\) was employed to restrain all bonds involving hydrogen atoms. Langevin dynamics \(^3\) was applied to regulate the temperature with a collision frequency of 2.0 ps\(^{-1}\). The pressure was controlled using the isotropic position scaling protocol.

Fig.S1  The amide formation (orange) and the relative activity (dark cyan) of all mutants, the catalytic activity of WT was set as 100%.