PBS Workflow details

The Mutation module specifies the ‘‘mutant library’’ to be screened and accommodates diverse construction strategies, including site-saturation mutagenesis, random mutagenesis, rational design, and other structure-based selections. Thermostability screening filters out thermally unstable variants based on ΔΔG_fold, with folding free energy evaluated by Rosetta cartesian_ΔΔG. In TS-analog binding screening, we exclude mutants predicted to weaken the rate-determining TS binding. Following a standard approximation, the pre-reaction complex serves as a TS analog to quantify mutational effects on binding enthalpy (MMPBSA) and a conformational-entropy proxy (active-site RMSD), both derived from MD trajectories (see Computational Implementation). From the remaining variants, Reactivity ranking prioritizes candidates by the electrostatic stabilization energy of the TS relative to the wild type (ΔE_elec), which reflects the expected change in catalytic activity upon mutation. The top ten mutants by ΔE_elec constitute the final PBS recommendations for experiment.PBS inherits its capacity to apply QM and MD modeling to hundreds of enzyme variants from the high-throughput EnzyHTP platform (5).

2.2 Performance metrics.

We evaluate PBS using two metrics: hit rate and function-enhancing speed (FES). *Hit rate* is the number of experimentally confirmed hits divided by the number of predicted candidates (here, 10), representing the probability that a recommended mutant improves function. *FES* is computed by identifying the variant with the greatest increase in turnover, then dividing its fold improvement by the total time consumption for discovery, quantifying how rapidly functional gains are achieved via unguided screening or PBS-guided experimentation.