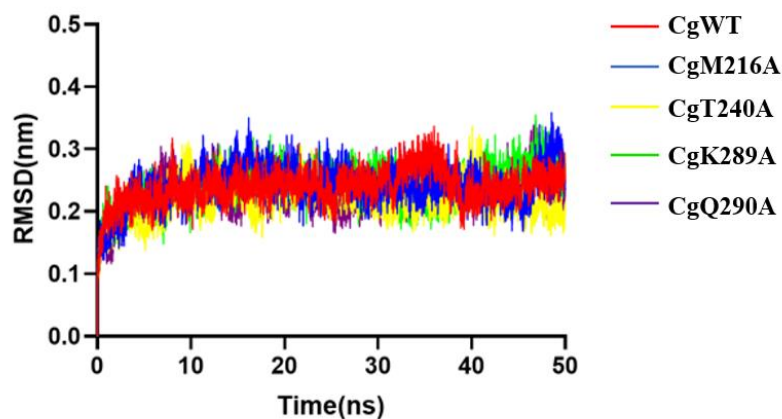


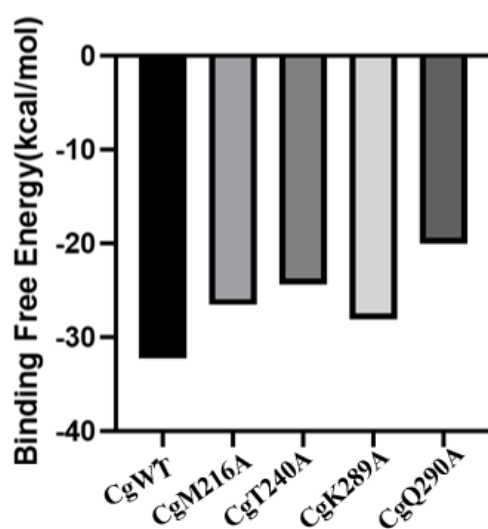
# Supplementary Material

**Table S1.** Primers used in this study.

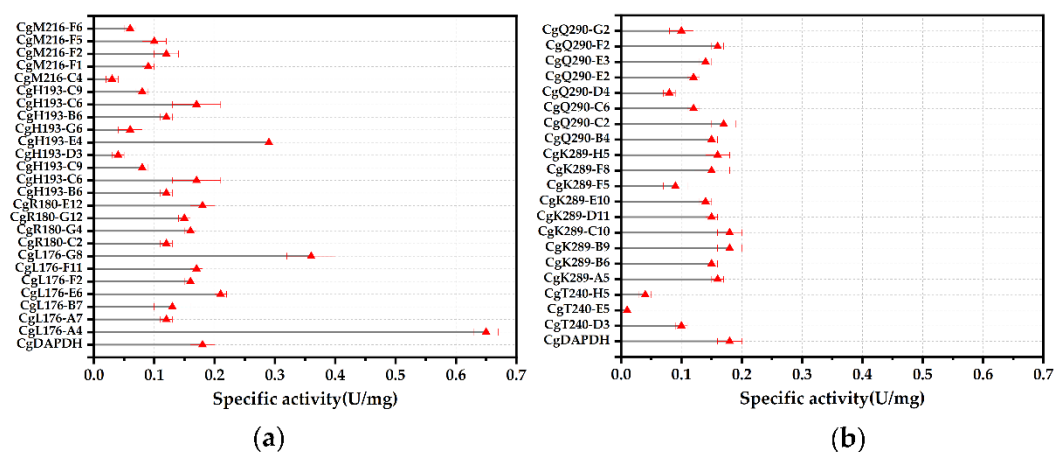
Primers	Sequence (5'-3' Direction)
CgT44A-F	CCTCGACACAAAGGCGCCAGTCTTTGATGT
CgT44A-R	GCGACATCAAAGACTGGCGCCTTTGTGTCG
CgP45A-F	CGACACAAAGACGGCGGTCTTTGATGTCGC
CgP45A-R	GACATCAAAGACCGCCGTCTTTGTGTCGAG
CgM216A-F	CGACATCCGCACCGCGCCTGATTACTTCGT
CgM216A-R	GAAGTAATCAGGCGCGGTGCGGATGTCGTT
CgH239A-F	TTCGACTCCGAGGCGACCGGCATGCCACAC
CgH239A-R	GTGGCATGCCGGTTCGCCTCGGAGTCGAAGG
CgT240A-F	TTCGACTCCGAGCACGCGGGCATGCCACAC
CgT240A-R	GTGGCATGCCCGCGTGCTCGGAGTCGAAGG
CgG241A-F	GACTCCGAGCACACCGCGATGCCACACGGT
CgG241A-R	CACCGTGTGGCATCGCGGTGTGCTCGGAGT
CgK289A-F	AGCTCACCGCATGGCGCAGCAGGGCCAAAG
CgK289A-R	TGGCCCTGCTGCGCCATGCGGTGAGCTCGG
CgQ290A-F	TCACCGCATGAAGGCGCAGGGCCAAAGCGG
CgQ290A-R	GCTTTGGCCCTGCGCCTTCATGCGGTGAGC
CgQ291A-F	CCGCATGAAGCAGGCGGGCCAAAGCGGAGC
CgQ291A-R	TCCGCTTTGGCCCGCCTGCTTCATGCGGTG
CgG292A-F	CGCATGAAGCAGCAGGCGCAAAGCGGAGCT
CgG292A-R	GAAAGCTCCGCTTTGCGCCTGCTGCTTCAT
CgL176X-F	ATCCGAAGACGCCNNKGAAAAGGCC
CgL176X-R	CCTTTTCMNNGGCGTCTTCGGATGG
CgR180X-F	CTGGAAAAGGCNNKCCGCGGCGAAG
CgR180X-R	TCGCCGCGMNNGGCCTTTTCCAGGG
CgH193X-F	GGAAAGCAAACCNNKAAGCGCCAATCCTTC
CgH193X-R	GCATTGGCGCTTMNNGGTTTGCTTTCGGT
CgM216X-F	ACGACATCCGCACCNKCCCTGATTACTTCG
CgM216X-R	AAGTAATCAGGMNNGGTGCGGATGTCGTTT
CgT240X-F	TTCGACTCCGAGCACNNKGGCATGCCACAC
CgT240X-R	GTGGCATGCCMNNGTGCTCGGAGTCGAAGG
CgK289X-F	CTCACCGCATGNNKCAGCAGGGCCAAAGCG
CgK289X-R	CTTTGGCCCTGCTGMNNCATGCGGTGAGCT
CgQ290X-F	GCTCACCGCATGAAGNNKCAGGGCCAAAGC
CgQ290X-R	TTTGGCCCTGMNNCTTCATGCGGTGAGCTG



**Figure S1.** RMSD analysis of CgDAPDH and alanine mutants MD simulation trajectory.



**Figure S2.** Comparison of substrate binding energy between CgDAPDH and alanine mutants.



**Figure S3.** Specific activities of CgDAPDH and its positive mutants towards pyruvic acid: (a) Specific activity of positive mutants at site L176, R180, H193 and M216; (b) Specific activity of positive mutants at site T240, K289 and Q290. The number after the mutant's name referred to the number on the 96 deep-well plates during the screening process.