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Supporting information for article:

Structural basis of a novel activity of bacterial 6-pyruvoyl tetrahydropterin synthase homologues distinguished from mammalian 6-pyruvoyl tetrahydropterin synthase activity

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Supporting Information

S1. Chemical cross-linking by glutaraldehyde

The eCTPS protein was concentrated and exchanged to 10 mg ml^{-1} in 20 m*M* Hepes pH 8.0. A reaction mixture was prepared in 20 µl containing 28 µg of the eCTPS protein and glutaraldehyde in final concentration varying from 0.002% to 0.0015%. After the mixture was incubated at 310 K for 5 min, the reaction was quenched at 310 K for 15 minutes by adding 1 *M* Tris pH 7.5 to a final concentration 50 m*M*. Then, each reaction mixture was examined by SDS-PAGE.

Table S1 Primers used for site-directed mutagenesis

Mutation	Forward primer	Reverse primer
C27A	ggagggcataaagctggtcgcctgcac	gtgcaggcgaccagctttatgcccttc
W51A	ccgcatacgggcgcgattatcgatttc	gaaatcgataatcgcgcccgtatgcgg
W51F	ccgcatacgggcttcattatcgat	gaaatcgataatgaagccccgtatgcgg
W51M	ccgcatacgggcatgattatcgat	gaaatcgataatcatgccccgtatgcgg
F55A	tggattatcgatgccgctgaactaaaa	ttttagttcagcggcatcgataatcca
F55L	tggattatcgatctcgctgaactaaaa	ttttagttcagcgagatcgataatcca
D70A	tacgagcgcctcgctcaccattatctc	gagataatggtgagcgaggcgctcgta
D70S	tacgagcgcctctctcaccattatctc	gagataatggtgagagaggcgctcgta
D70V	tacgagcgcctcgttcaccattatctc	gagataatggtgaacgaggcgctcgta
H71A	gagcgcctcgatgcccattatctcaat	attgagataatgggcatcgaggcgctc
E110A	gtgatggtaaaagcaacctgcaccgca	tgcggtgcaggatgcttttaccatcac

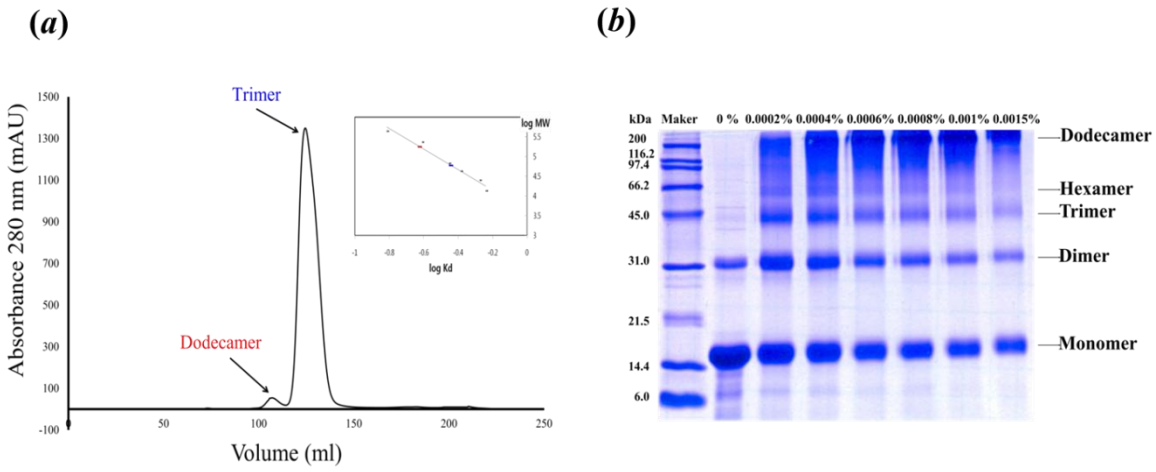

Figure S1 **Analytical oligomerization state of eCTPS.** (*a*) Two peaks corresponding to trimer and dodecamer size were observed. The scale at the bottom indicates the elution volume. Inset, semi log plot of the molecular mass of all the standard proteins used versus their logK_d values. (*b*) SDS-PAGE analysis of chemical cross-linked eCTPS by glutaraldehyde. eCTPS (28µg) treated with varying concentrations of glutaraldehyde was separated by SDS-PAGE. eCTPS molecular sizes corresponding to monomer, dimer, trimer, hexamer and dodecamer were marked as arrows.

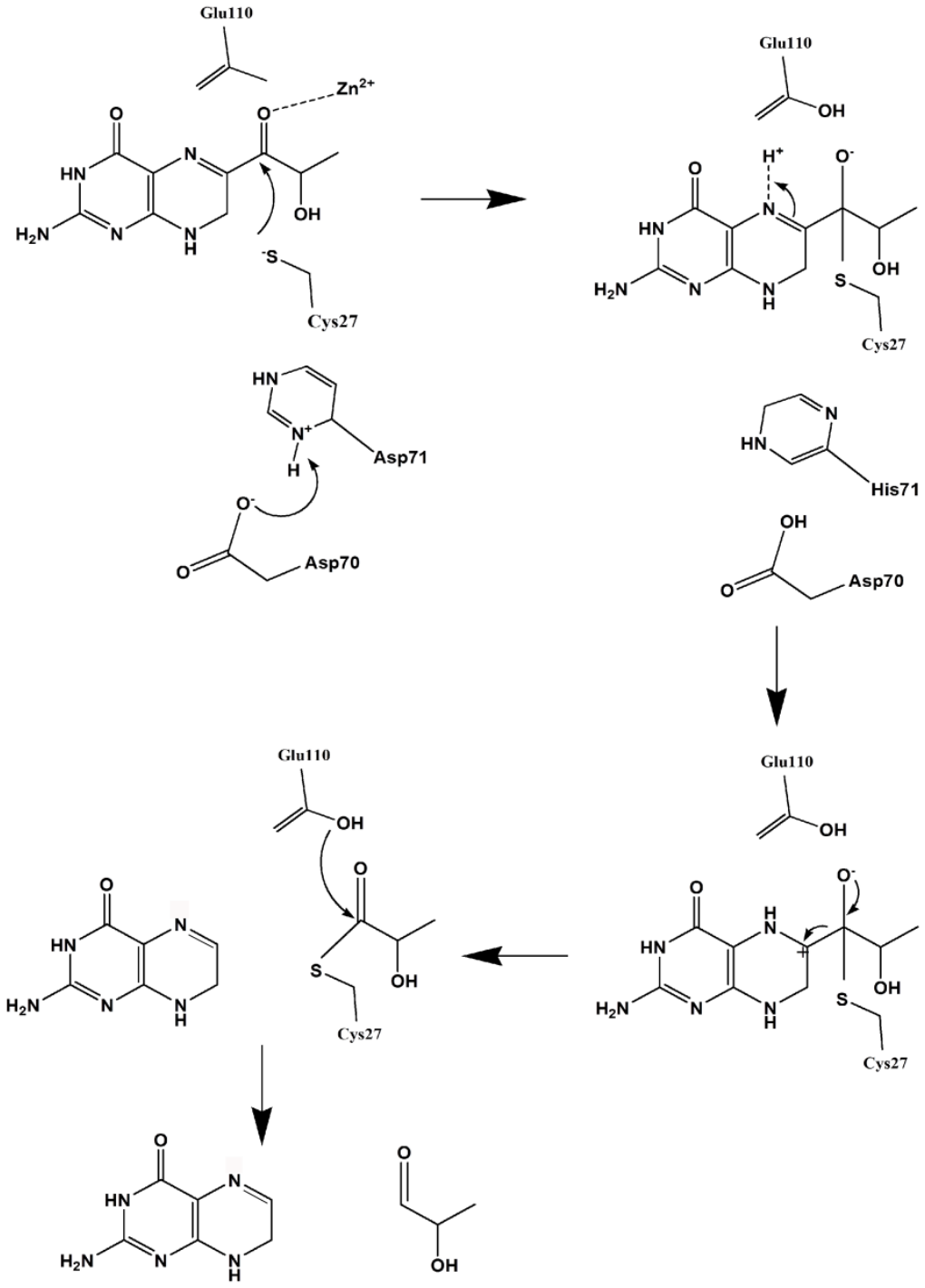

Figure S2 Proposal of eCTPS catalysis mechanism. The crystal structure of the $eCTPS_{C27A}$-sepiapterin complex provides insight on the chemical reaction mechanism of the SSCR activity in the bacterial PTPS. There is a catalytic triad consisting of Cys27, Asp70 and His71 in the active site. The role of the active residue Cys27 can be deduced from the biochemical and structural studies on mammalian PTPS enzymes. The nucleophilicity of Cys27 is strengthened by Asp70 and His71. The Cys27 attacks the C1' of sepiapterin side chain, which leads to an intermediate. At the second step the C1'-OH is reattached to the sulfur atom on Cys27 to form a keto-enol by tautomerization and then the

C6 side chain of sepiapterin is simultaneously cleaved out to produce dihydropterin. Then the side chain of Glu110 attacks the C1’ of cleaved side chain of sepiapterin, releasing the cleaved sepiapterin side chain and getting the Cys27 back to the starting point. The Glu110 was important for catalysis reaction in eCTPS enzyme. The catalytic triad is involved in the activation of nucleophile and Glu110 is essential to proposed proton transfer pathway.