Supplementary Information

Modification of residue 42 of the active site loop with a lysine-mimetic sidechain rescues isochorismate-pyruvate lyase activity in *Pseudomonas aeruginosa* PchB

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Table S1: Catalytic constants of all controls and variants

	sample	K_m (μM)	k_{cat} (x10^{-3} s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
WT[a]	control	1.1 ± 0.1	130 ± 0	124,000 ± 4,000
	EtA	5.90 ± 0.03	232 ± 4	39,200 ± 500
	PrA	8.2 ± 0.2	168 ± 1	20,500 ± 700
K42A	control	30 ± 1	45 ± 1	1,510 ± 30
	EtA	48 ± 1	98 ± 1	2,060 ± 20
	PrA	60 ± 1	83 ± 1	1,370 ± 10
WT[b]	control	1.39 ± 0.03	178 ± 1	128,000 ± 3,000
	BrEA	3.13 ± 0.02	166 ± 1	52,900 ± 600
	BrEtOH	1.58 ± 0.02	167 ± 0	106,000 ± 1,000
K42C	control	134 ± 1	8.28 ± 0.04	61.6 ± 0.3
	BrEA	23 ± 1	94.6 ± 0.1	4,200 ± 100
	BrEtOH	125 ± 1	18.4 ± 0.1	147 ± 0
C7A/K42C	control	123 ± 2	8.96 ± 0.01	65 ± 1
	BrEA	15.6 ± 0.3	105 ± 1	6,700 ± 100
	BrEtOH	114 ± 2	12.8 ± 0	103 ± 1
C07A	control	2.10 ± 0.02	200 ± 10	93,000 ± 5,000
	BrEA	1.59 ± 0.03	190 ± 10	120,000 ± 9,000
	BrEtOH	1.73 ± 0.07	190 ± 10	109,000 ± 9,000

[a] exogenous amine conditions [b] covalent rescue conditions

Figure S1. Circular dichroism of PchB-variants and PchB-variants modified with bromoethylamine. All traces show that the protein is predominantly α-helical, despite mutation or modification with bromoethylamine. ● = wildtype PchB; ○ = wildtype PchB modified with bromoethylamine; ■ = C7A-PchB; □ = C7A-PchB modified with bromoethylamine; ◆ = K42C-PchB; ◇ = K42C-PchB modified with bromoethylamine; ▲ = C7A,K42C-PchB; △ = C7A,K42C-PchB modified with bromoethylamine

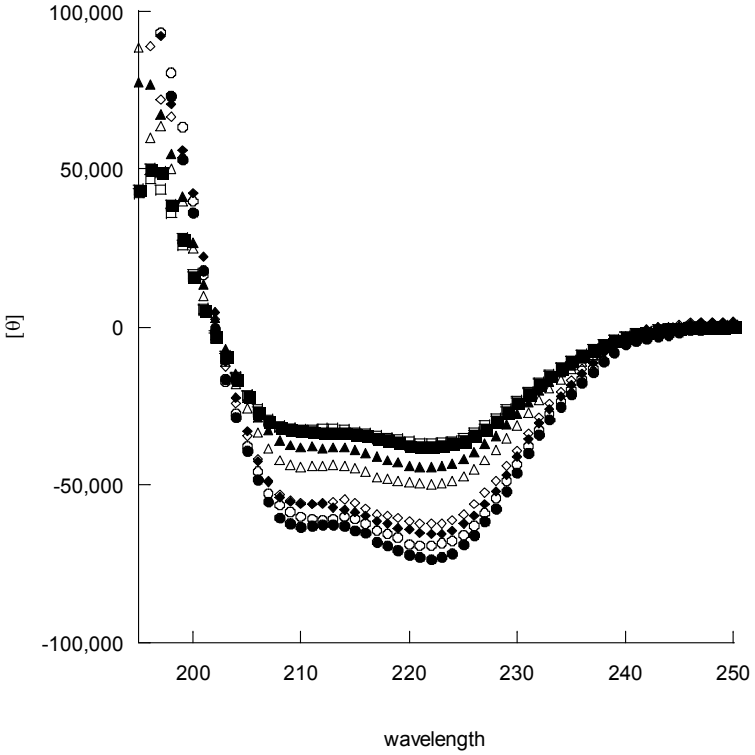
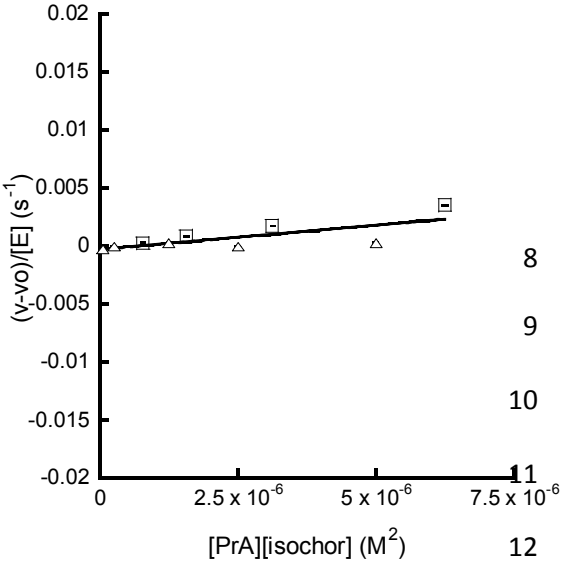
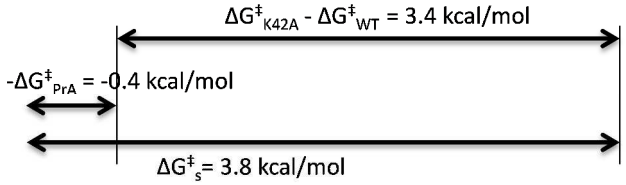


Figure S2: Lack of activation of K42A-PchB by added propylamine. Concentrations of propylamine (△) are: 1, 5, 25, 50, and 100 mM (isochorismate constant at 50 μM). Concentrations of isochorismate (□) are: 1.6, 3.2, 6.3 and 12.5 μM mM (propylamine constant at 500 mM), plotted as found in reference (*1*). The calculations are generated based on the work of Richard and colleagues (*1, 2*). On the right is a comparison plot generated like those found in Go *et al.* (*2*), which highlights that in the case of PchB the connection energy is larger than the overall stabilization in the transition state between the K42A variant and wildtype.





1. k_{cat}/K_mK_d = slope of line = 420 $M^{-2}s^{-1}$

2. $\frac{k_{cat}/K_mK_d}{(k_{cat}/K_m)_{K42A}} = \frac{420M^{-2}s^{-1}}{850M^{-1}s^{-1}} = \mathbf{0.5M^{-1}} = \frac{1}{K_d^{\ddagger}}$

3. $\Delta G_{PrA}^{\ddagger} = -RTln\left(\frac{1}{K_d^{\ddagger}}\right) = -(1.98\ cal\ K^{-1}\ mol^{-1})(298\ K)\ ln(0.5M^{-1}) = \mathbf{0.4\ kcal\ mol^{-1}}$

4. $\frac{(k_{cat}/K_m)_{WT}}{k_{cat}/K_mK_d} = \frac{258{,}000\ M^{-1}s^{-1}}{420M^{-2}s^{-1}} = \mathbf{610\ M}$ = Effective molarity (EM) of the Lys42 sidechain

5. $\Delta G_S^{\ddagger} = RTln(EM) = (1.98\ cal\ K^{-1}\ mol^{-1})(298\ K)\ ln(610\ M) = \mathbf{3.8\ kcal\ mol^{-1}}$

6. $\frac{(k_{cat}/K_m)_{WT}}{(k_{cat}/K_m)_{K42A}} = \frac{258{,}000\ M^{-1}s^{-1}}{850M^{-1}s^{-1}} = \mathbf{300}$ or $\frac{EM}{K_d^{\ddagger}} = \mathbf{300}$

7. $\Delta G_{K42A}^{\ddagger} - \Delta G_{WT}^{\ddagger} = RTln\left(\frac{EM}{K_d^{\ddagger}}\right) = RTln(300) = \mathbf{3.4\ kcal\ mol^{-1}}$

8. $\Delta G_{K42A}^{\ddagger} - \Delta G_{WT}^{\ddagger} = \Delta G_S^{\ddagger} - \Delta G_{PrA}^{\ddagger}$

$$or\ \mathbf{3.4\ kcal\ mol^{-1}} = \mathbf{3.8\ kcal\ mol^{-1}} - \mathbf{0.4\ kcal\ mol^{-1}}$$

References

1. Barnett, S. A., Amyes, T. L., Wood, B. M., Gerlt, J. A., and Richard, J. P. (2010) Activation of R235A mutant orotidine 5'-monophosphate decarboxylase by the guanidinium cation: effective molarity of the cationic side chain of Arg-235, *Biochemistry 49*, 824-826.
2. Go, M. K., Amyes, T. L., and Richard, J. P. (2010) Rescue of K12G triosephosphate isomerase by ammonium cations: the reaction of an enzyme in pieces, *J. Am. Chem. Soc. 132*, 13525-13532.