

Supplementary information

Determinants for tRNA-dependent pre-transfer editing in the synthetic site of isoleucyl-tRNA synthetase

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Table S1. Parallel formation of AMP and aa-tRNA^{Val} by wild-type and mutant *E. coli* ValRS.

	$k_{\text{AMP}}^a / \text{s}^{-1}$	$k_{\text{aa-tRNA}}^a / \text{s}^{-1}$	$k_{\text{AMP}} / k_{\text{aa-tRNA}}$	$k_{\text{ed}}^b / \text{s}^{-1}$	$k_{\text{ed-tRNA}}^a / \text{s}^{-1}$	$k_{\text{ed}} / k_{\text{ed-tRNA}}$
wt + Thr	6.7 ± 1.15	0.044 ± 0.005	149	6.655	0.40 ± 0.04	16.6
D286A + Thr	1.29 ± 0.19	0.9 ± 0.08	1.43	0.39	0.40 ± 0.06	0.975
wt + Val	1.78 ± 0.26	1.6 ± 0.04	1.1	0.18	0.007 ± 0.002	
D286A + Val	1.44 ± 0.34	1.19 ± 0.13	1.2	0.24	0.005 ± 0.002	

The values represent the mean \pm S.D. of at least three independent experiments.

The reaction conditions are as described in the main text.

^a k_{AMP} and $k_{\text{ed-tRNA}}$ represent measured rate constants for formation of AMP in the presence and absence of tRNA^{Val}, respectively. $k_{\text{aa-tRNA}}$ represents rate constant for formation of aa-tRNA^{Val} measured under the same conditions as k_{AMP} .

^b k_{ed} represents rate constant for tRNA-dependent editing and is calculated as a difference between the rate constant for AMP formation in the presence of tRNA^{Val} and the rate constant for aa-tRNA^{Val} formation.

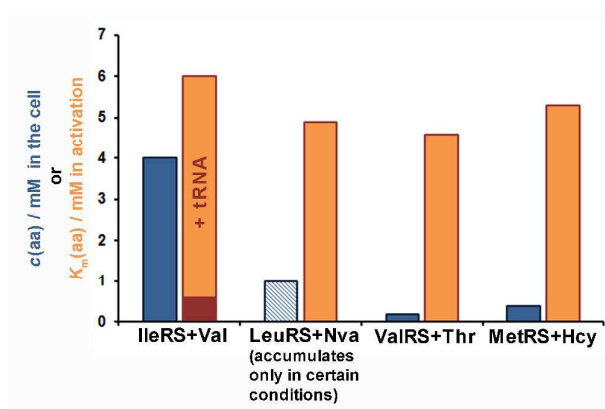


Figure S1. Comparison of the K_m values in the activation of noncognate amino acids with their concentration in the cell. Data are presented only for for class I editing aaRS. For IleRS, K_m in activation for valine is 0.46 - 1 mM (dark red rectangle), but rises approximately 10-fold in the presence of tRNA (Dulic et al, 2010, Cveticic and Gruic, unpublished results). K_m 's are as published in Cveticic et al, 2012, Dulic et al, 2010, Kim et al, 1993. Concentrations of amino acids are as published in Bennet et al, 2009 and Soini et al, 2008.

References:

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