

Supplementary Materials

NSMB-A20845C

Title: Aminoacylation of tRNA with phosphoserine for synthesis of cysteinyl-tRNA<sup>Cys</sup>

Authors: Chun-Mei Zhang, Cuiping Liu, Simon Slater, and Ya-Ming Hou

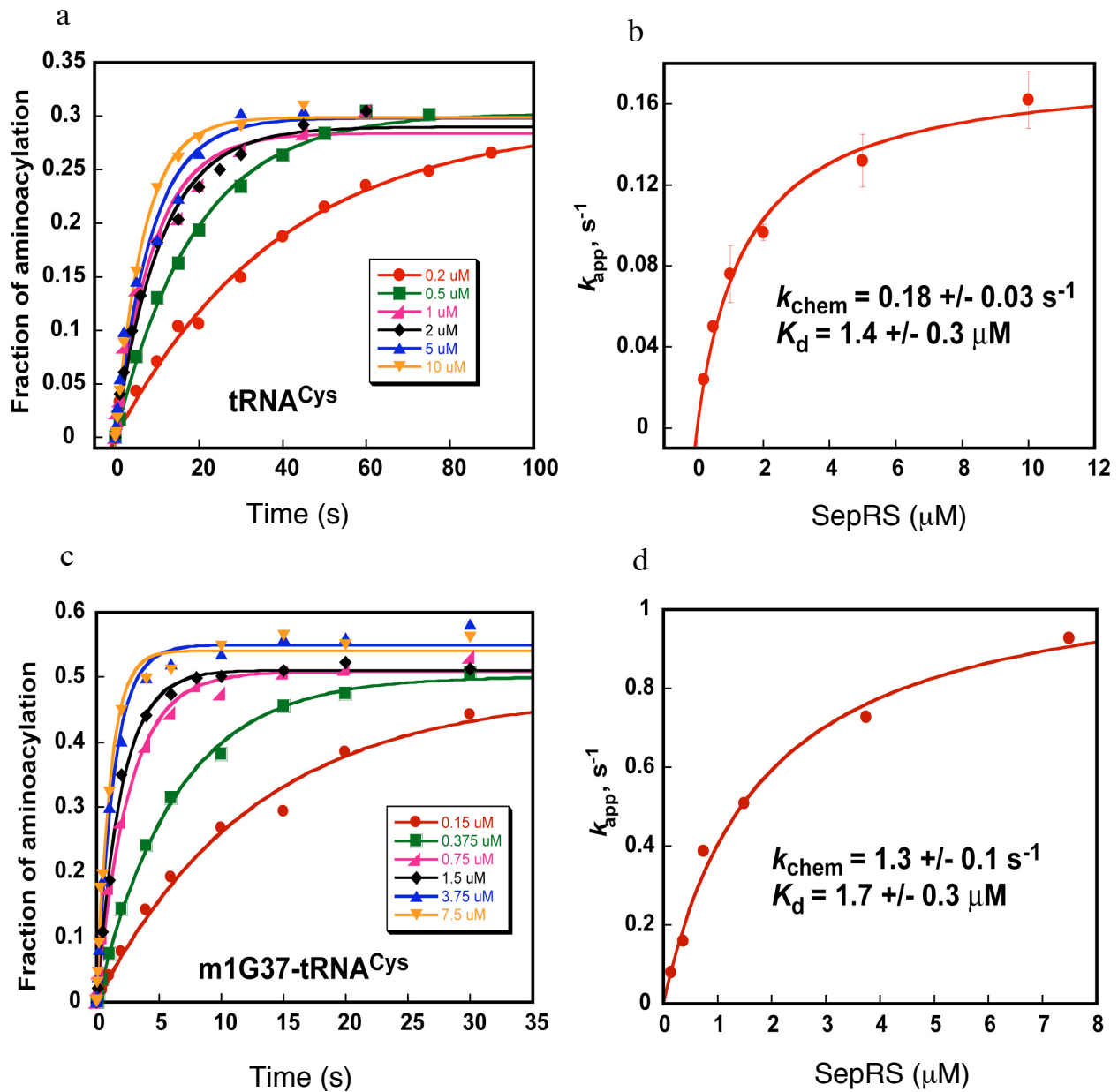


Figure 1 Single turnover kinetics of aminoacylation of *M. jannaschii*  $tRNA^{Cys}$  by *M. jannaschii* SepRS. (A) Time courses of aminoacylation of unmodified transcript by SepRS (0.2 to 10  $\mu M$ ). (B) Replot of  $k_{app}$  vs SepRS concentrations to a hyperbolic equation. (C) Time courses of aminoacylation of m1G37-transcript by SepRS (0.15 to 7.5  $\mu M$ ). (D) Replot of  $k_{app}$  vs SepRS concentrations to a hyperbolic equation. The derived  $k_{chem}$  and  $K_d$  were the average of 2 determinations. The  $\pm$  term refers to standard deviations.

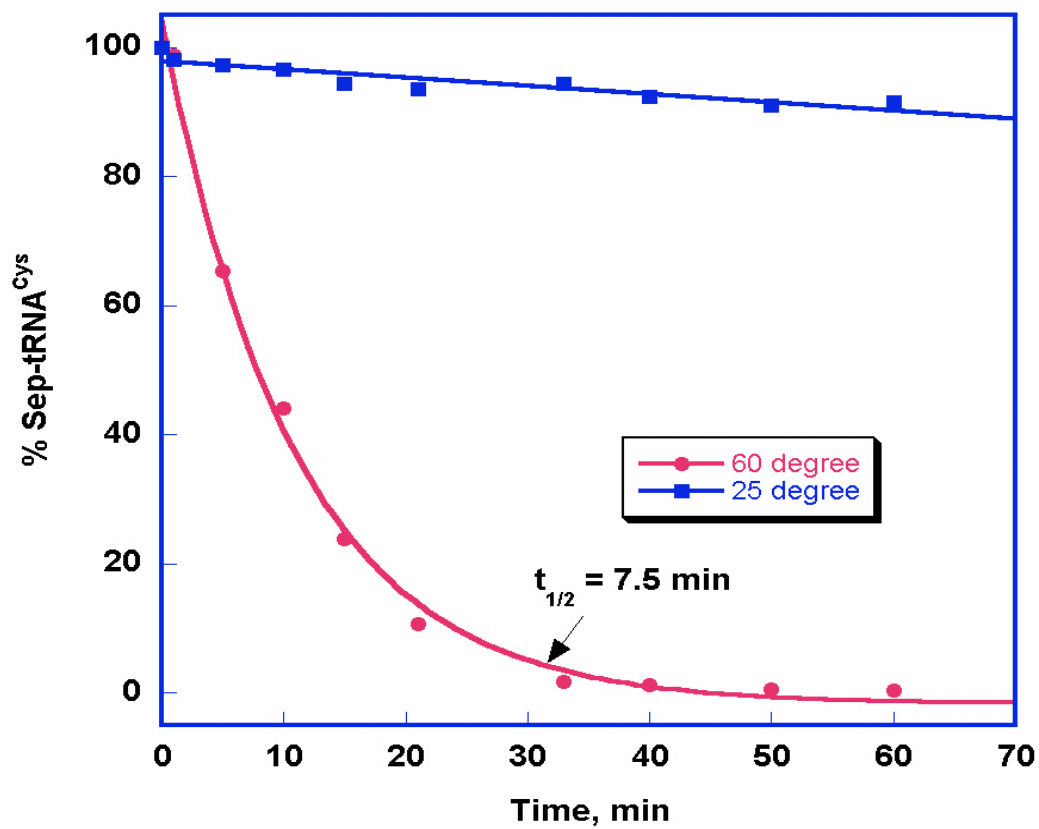


Figure 2 Stability of *M. jannaschii* Sep-tRNA<sup>Cys</sup> measured at 60 °C (red) and at 25 °C (blue). The tRNA transcript was labeled with  $\alpha$ -<sup>32</sup>P-A76 (5  $\mu$ M) and was aminoacylated with Sep by SepRS. After phenol extraction and ethanol precipitation, Sep-tRNA<sup>Cys</sup> was resuspended in 1 x aminoacylation buffer (30 mM NaAc, pH 6.0, 20 mM KCl, 10 mM MgCl<sub>2</sub>, and 5 mM DTT) and incubated at the specified temperatures. At specific time points, an aliquot was removed and digested with P1 nuclease. Deacylation of Sep-tRNA<sup>Cys</sup> was monitored by PEI cellulose plates.

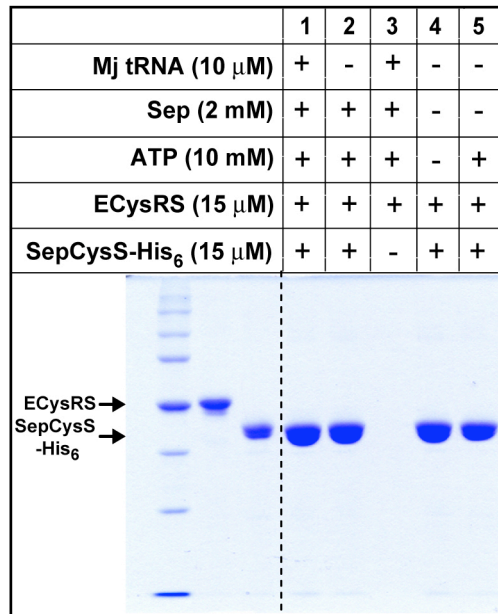


Figure 3 Lack of pull-down of *E. coli* CysRS by *M. jannaschii* SepCysS analyzed by SDS-PAGE.

Table 1. Steady-state kinetics

tRNA	<i>M.j</i> SepRS vs. <i>M.j</i> tRNA <sup>Cys(a)</sup>				<i>E.coli</i> CysRS vs. <i>E.coli</i> tRNA <sup>Cys</sup>	
	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1}\text{s}^{-1}$ )	relative	$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1}\text{s}^{-1}$ )	relative
WT	$0.97 \pm 0.01$	$0.24 \pm 0.04$	$0.25 \pm 0.04$	1	$0.99 \pm 0.06^{(b)}$	1
U73G	$1.3 \pm 0.3$	$0.01 \pm 0.001$	$0.008 \pm 0.002$	0.03	$(7 \pm 0.1) \times 10^{-6}^{(b)}$	$7 \times 10^{-6}$
G34C	$19 \pm 5$	$0.08 \pm 0.01$	$0.004 \pm 0.001$	0.02	$(6 \pm 0.3) \times 10^{-4}^{(b)}$	$6 \times 10^{-4}$
C35U	$7.0 \pm 0.4$	$0.10 \pm 0.02$	$0.014 \pm 0.002$	0.06	$(3 \pm 0.1) \times 10^{-3}$	$3 \times 10^{-3}$
A36G	$22 \pm 2$	$0.17 \pm 0.01$	$0.008 \pm 0.001$	0.03	$(2 \pm 0.2) \times 10^{-3}$	$2 \times 10^{-3}$
G37A	$11.4 \pm 0.7$	$0.11 \pm 0.01$	$0.01 \pm 0.001$	0.04	Not determined	
A38G	$2.9 \pm 0.2$	$0.04 \pm 0.01$	$0.014 \pm 0.004$	0.06	Not determined	
G15C-C48G	$3.7 \pm 0.8$	$0.17 \pm 0.03$	$0.05 \pm 0.01$	0.2	$0.02 \pm 0.02$	0.02

(a) Transcripts of m1G37-*M.j* tRNA<sup>Cys</sup> were used except G37A.

(b) Data from Liu *et al.* (2007).