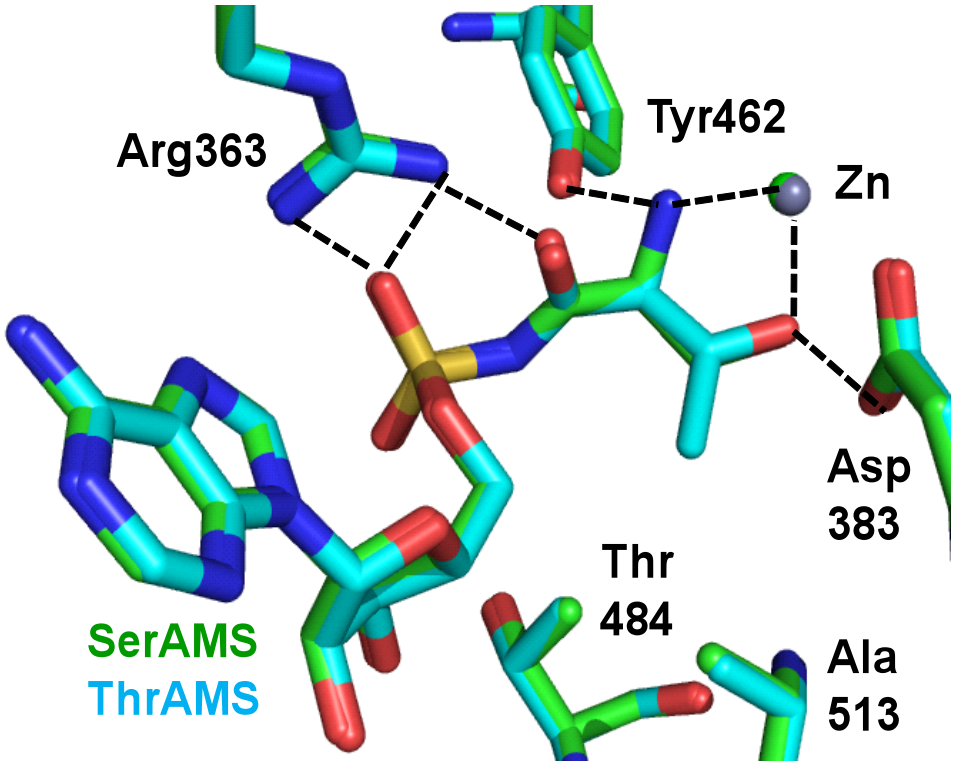
**Supplemental data for**

# AMINOACYL TRANSFER RATE DICTATES CHOICE OF EDITING PATHWAY IN THREONYL-tRNA SYNTHETASE

**Anand Minajigi[1] and Christopher S. Francklyn[1,2*]**

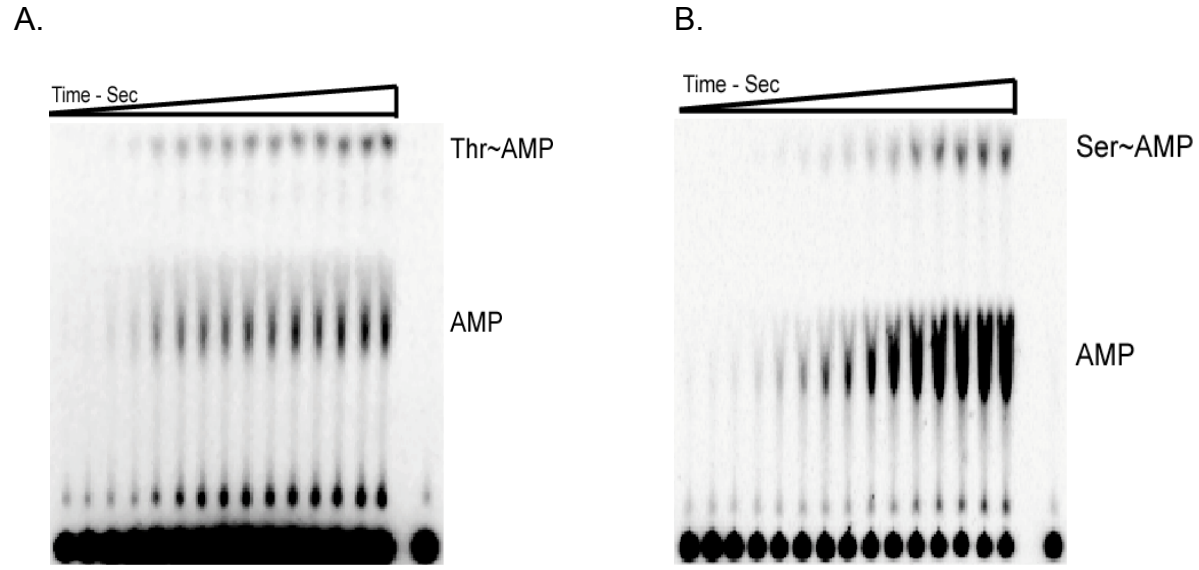
From the [1]Cell and Molecular Biology Program, [2]Department of Biochemistry, College of Medicine, Health Sciences Complex, 89 Beaumont Ave, University of Vermont, Burlington, Vermont 05405.

**Supplemental Figure 1.**



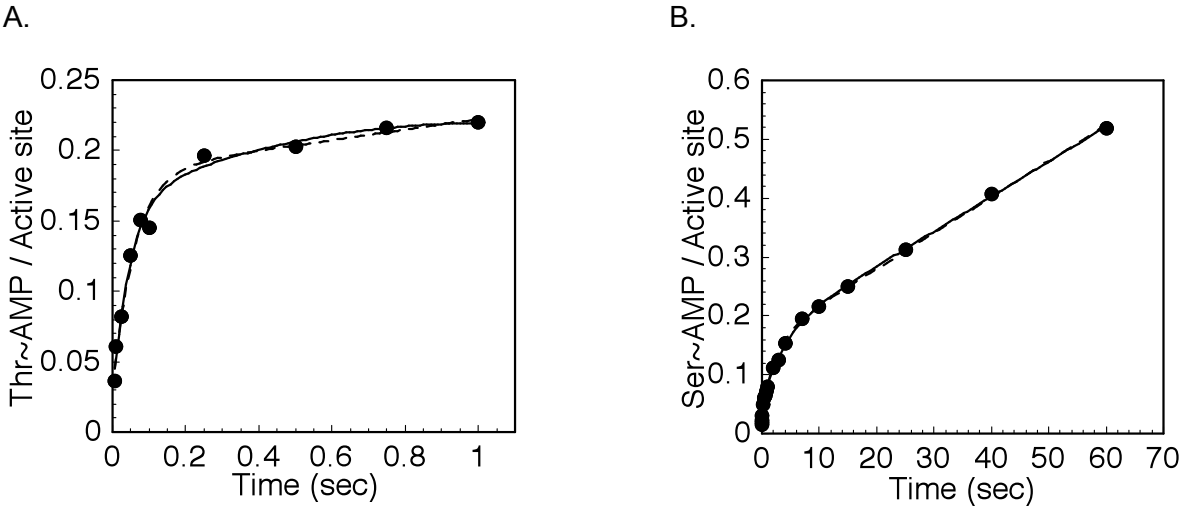

*Overlay of the ThrRS:ThrAMS and ThrRS:SerAMS active site complexes* (PDB id's 1EVL and 1FYF). The cognate adenylate is shown in cyan and the seryl~adenylate is in green. Active site residues that make contact to aminoacyl moieties of adenylates are shown.

**Supplemental Figure 2.**



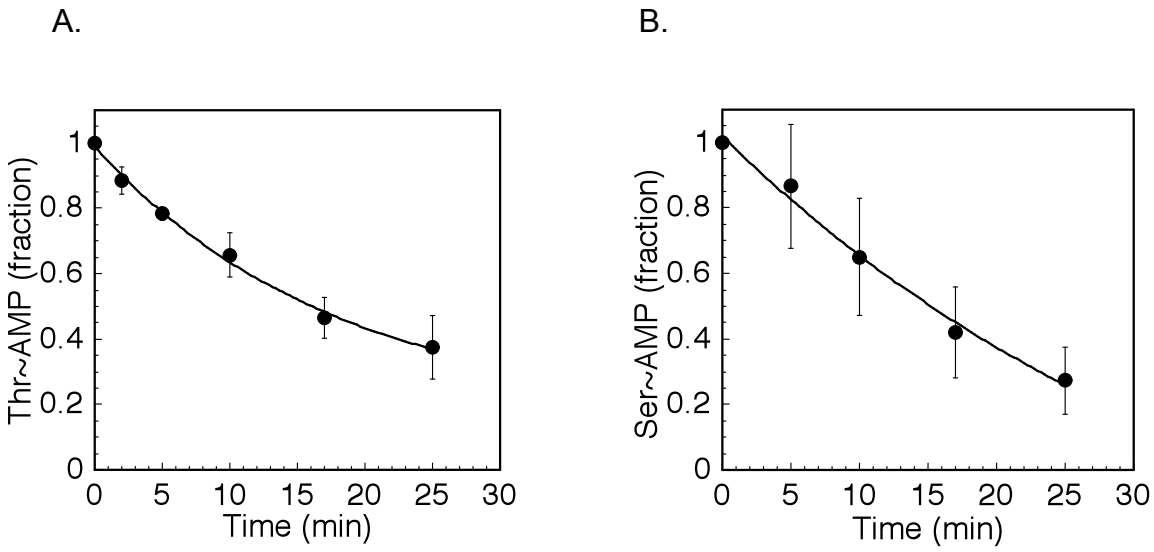

*Asymmetric amino acid activation by ThrRS.* The rapid quench experiments were performed at pH 8.0 and 37 °C in the absence of tRNA as described in Experimental Procedures, using 10 μM ThRS active sites, 100 μM [α-$^{32}$P] ATP, and 5 mM threonine or 150 mM serine. *(*A*),* TLC chromatogram showing the formation of [α-$^{32}$P] AMP and Thr~[α-$^{32}$P] AMP, and (B) TLC chromatogram showing the formation of [α-$^{32}$P] AMP and Ser~[α-$^{32}$P] AMP.

**Supplemental Figure 3.**




*Formation of adenylate under pre-steady state conditions:* The experimental conditions are same as described in supplemental Fig 2. The solid line represents a double exponential with a linear scale fit, while fit to a burst equation is presented as dashed line.

**Supplemental Figure 4.**




*Solvent-mediated adenylate hydrolysis is slower than the steady state rate of adenylate turnover.* (A), decay curve for threonyl-adenylate solvolysis in the absence of ThrRS. (B) decay curve for seryl-adenylate solvolysis. The adenylate formation reactions were carried out for five minutes in Buffer A at pH 8.0 and 37 °C as described in Experimental Procedures, employing 2 μM wild type ThrRS, 100 μM [α-$^{32}$P] ATP, and 5 mM threonine or 300 mM serine. The reaction was terminated by adding EDTA (pH 8.0) to 500 mM and SDS to 0.2% final. Hydrolysis of the adenylates was monitored by chromatography on PEI-TLC plates. The decrease in the adenylate fraction was plotted and analyzed by fitting to exponential decay equation as described in Experimental Procedures. Error bars represent mean ± S.D from three independent experiments.