Kinetic Mechanism of Human Histidine Triad Nucleotide Binding Protein 1 (Hint1)

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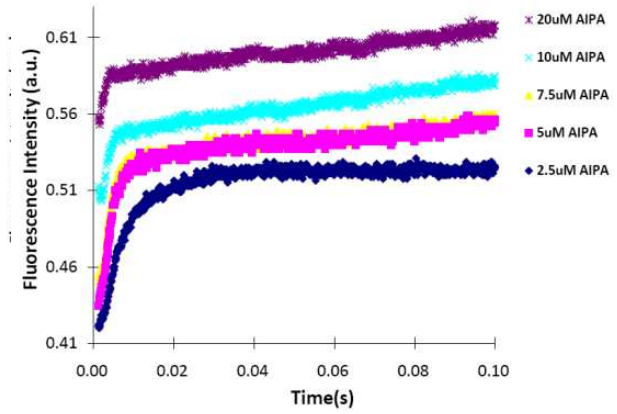
Supporting information

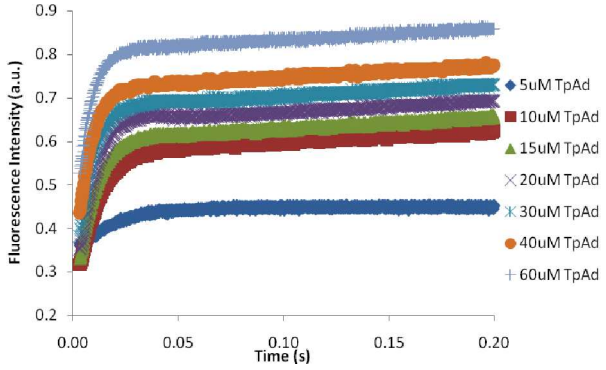
Supplemental Figure S1-S4

Figure S1 Pre-steady state kinetics of formation of the adenylated enzyme intermediate. (A) Stopped flow traces using varied concentration of AIPA exhibited a biphasic manner, a burst phase followed by a linear phase. (B) Stopped flow traces using varied concentration of TpAd. (C) Plot of the determined burst rate constants as a function of the AIPA concentration according to Eq 2. (D) Plot of the determined burst rate constants as a function of the TpAd concentration according to Eq 2.

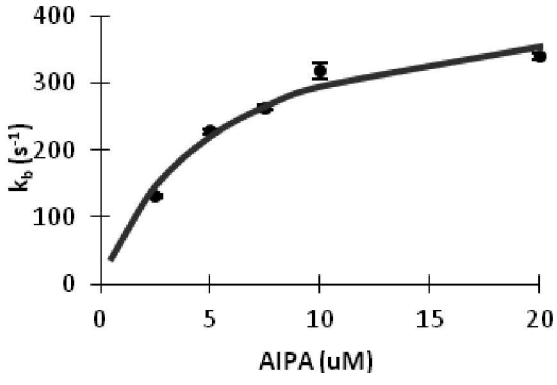
A



B



C.



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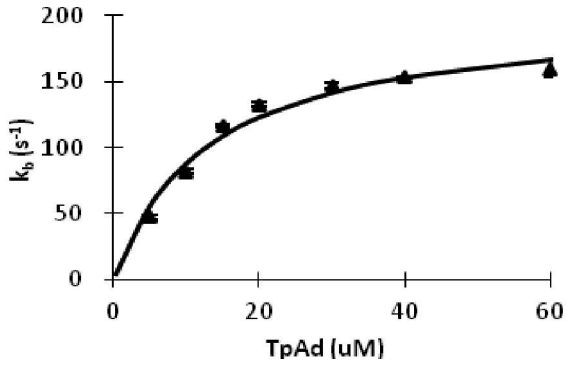


Figure S2 Dissociation constant (K_d) of AMP determined by fluorescence titration of hH1. The fluorescence of WT human Hint1 (6μM) was titrated by addition of AMP. Data were fitted using eq 15 and K_d =194 ± 9) μM.

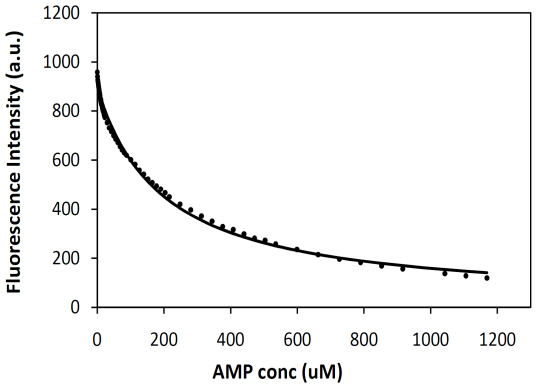


Figure S3 Determination of *Ki* for AMP in catalytic trapping experiments. k_b' and k_b represent the burst rates in the presence and absence of AMP, respectively. A K_i of 136μM was estimated by fitting the curve with eq. 18.

$$k_b' = k_b/(1+[\mathrm{AMP}]/K_i) \qquad \textbf{Eq. 18}$$

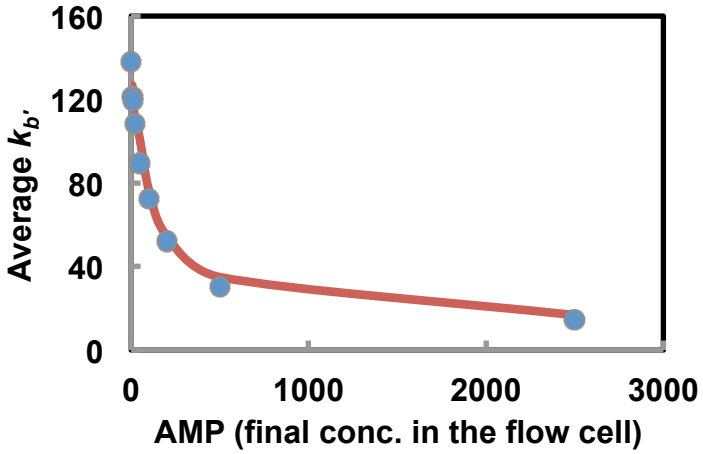
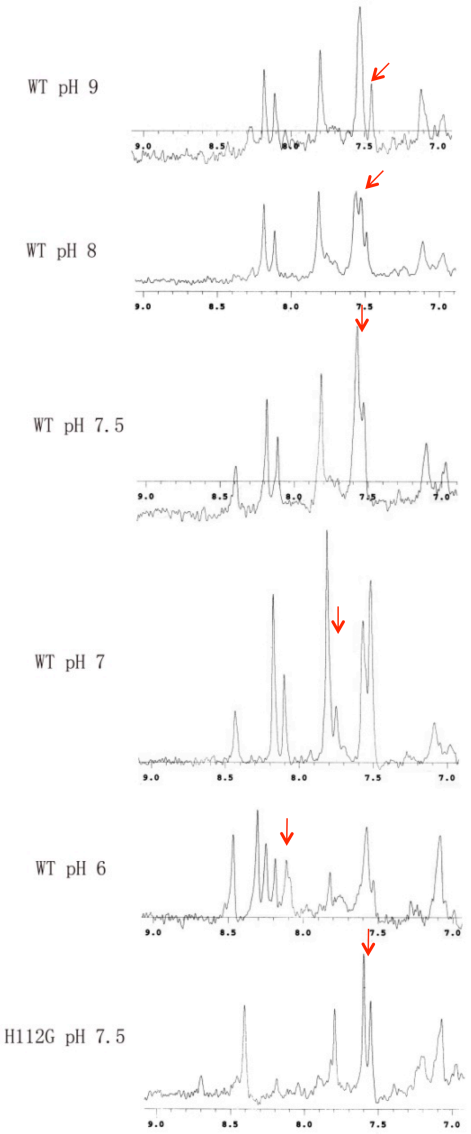


Figure S4 NMR analysis of histidine pKa in human Hint1. (A) Representative 1D proton TOCSY spectra for WT and H112G Hint1; (B) pKa titration curve for H112.

A.



B.