Supplementary Material

Domain Dissection and Characterization of the Aminoglycoside Resistance Enzyme ANT(3")-Ii/AAC(6')-IId from *Serratia marcescens*

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Table S1: Primers used for the PCR amplification of various constructs (full-length and truncated) of *ant(3")-Ii/aac(6')-IId*

gene	5' primer	3' primer
ant(3")-Ii/aac(6')-IId (NHis)	TTAGACCATATGAGTAACGCAG	TCGATGCTCGAGTTAGGCATCCTGCG
ant(3")-Ii/aac(6')-IId (CHis)	TTAGACCATATGAGTAACGCAG	ATGGAACTCGAGGGCATCACTGCG
aac(6')-IId(261-463) (NHis)	GCCACTCATATGCTTGGTGCCCATGCC	TCGATGCTCGAGTTAGGCATCCTGCG
aac(6')-IId(261-463) (CHis)	GCCACTCATATGCTTGGTGCCCATGCC	TCGATGCTCGAGTTAGGCATCCTGCG
aac(6')-IId(264-463) (NHis)	CTTGGTCATATGCCAGTGATGTCTAAAAC	TCGATGCTCGAGTTAGGCATCCTGCG
aac(6')-IId(264-463) (CHis)	CTTGGTCATATGCCAGTGATGTCTAAAAC	TCGATGCTCGAGTTAGGCATCCTGCG
aac(6')-IId(267-463) (NHis)	ATGCCACATATGTCTAAAACAAAGTTAG	TCGATGCTCGAGTTAGGCATCCTGCG
aac(6')-IId(267-463) (CHis)	ATGCCACATATGTCTAAAACAAAGTTAG	TCGATGCTCGAGTTAGGCATCCTGCG
ant(3")-Ii(1-260) (CHis)	TTAGACCATATGAGTAACGCAG	CATGGCCTCGAGCAATTTAGTGGCTTC
ant(3")-Ii(1-260) (NHis)	TTAGACCATATGAGTAACGCAG	TGGCATCTCGAGTCACAATTTAGTGGCTTC
ant(3")-Ii(1-263) (CHis)	TTAGACCATATGAGTAACGCAG	CATCACCTCGAGGGCACCAAGCAATTTAG
ant(3")-Ii(1-263) (NHis)	TTAGACCATATGAGTAACGCAG	AGACATCTCGAGTCAGGCACCAAGCAATTTAG
ant(3")-Ii(1-266) (CHis)	TTAGACCATATGAGTAACGCAG	TGTCACCTCGAGCACTGGCATGGCACCAAG
ant(3")-Ii(1-266) (NHis)	TTAGACCATATGAGTAACGCAG	CTTTGTCTCGAGTCACACTGGCATGGCACCAAG

The introduced restriction sites are underlined for each primer. All of the 5' primers introduced an *Nde*I restriction site. All of the 3' primers introduced an *Xho*I restriction site.

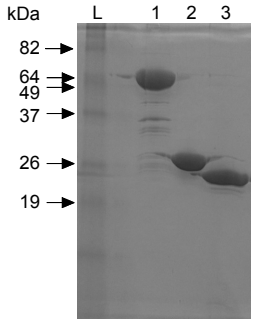

Fig. S1. Coomassie blue-stained 15% Tris-HCl SDS-PAGE gel showing the purified 55.0-kDa $NHis_{10}$-tagged ANT(3")-Ii/AAC(6')-IId (Lane 1), 30.3-kDa $CHis_6$-tagged ANT(3")-Ii(1-266) (Lane 2), and 24.0-kDa $NHis_6$-tagged AAC(6')-IId(267-463) (Lane 3). L = BenchMark™ Pre-Stained Ladder from Invitrogen. 6 μg of each protein was loaded on the gel.

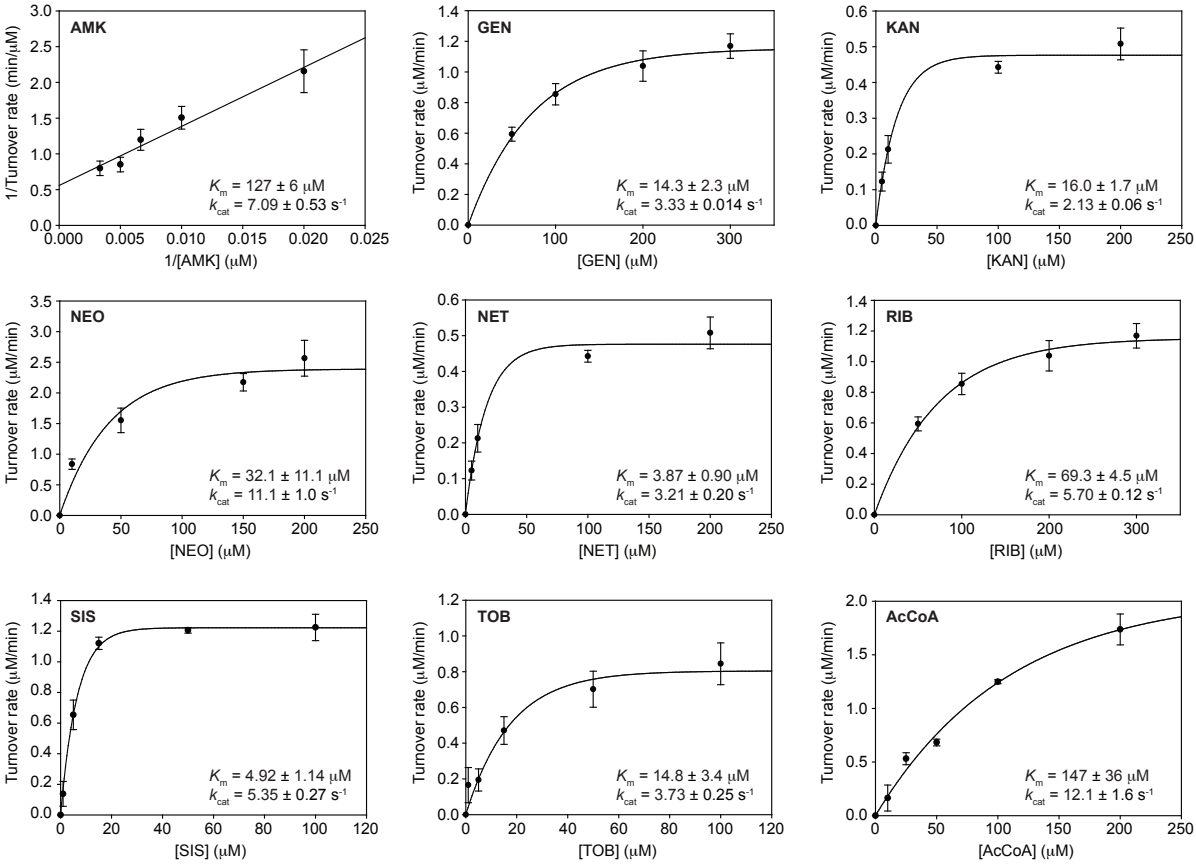

Fig. S2. Michaelis-Menten and Lineweaver-Burk kinetics for a variety of AGs and AcCoA during acetylation by AAC(6')-IId(267-463).

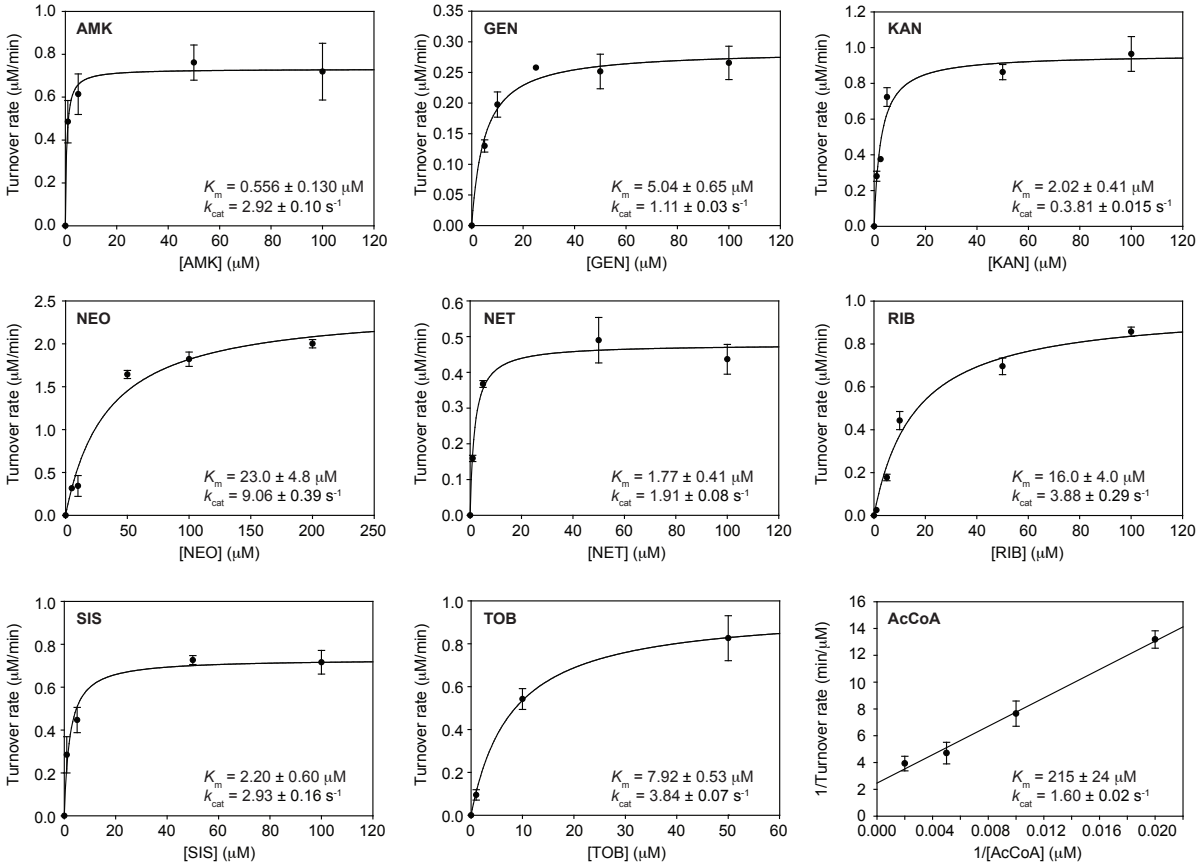

Fig. S3. Michaelis-Menten and Lineweaver-Burk kinetics for a variety of AGs and AcCoA during acetylation by ANT(3")-Ii/AAC(6')-IId.

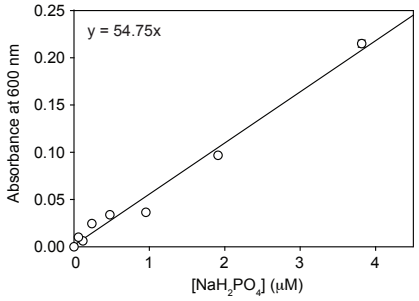

Fig. S4. Standard curve for P_i concentrations.

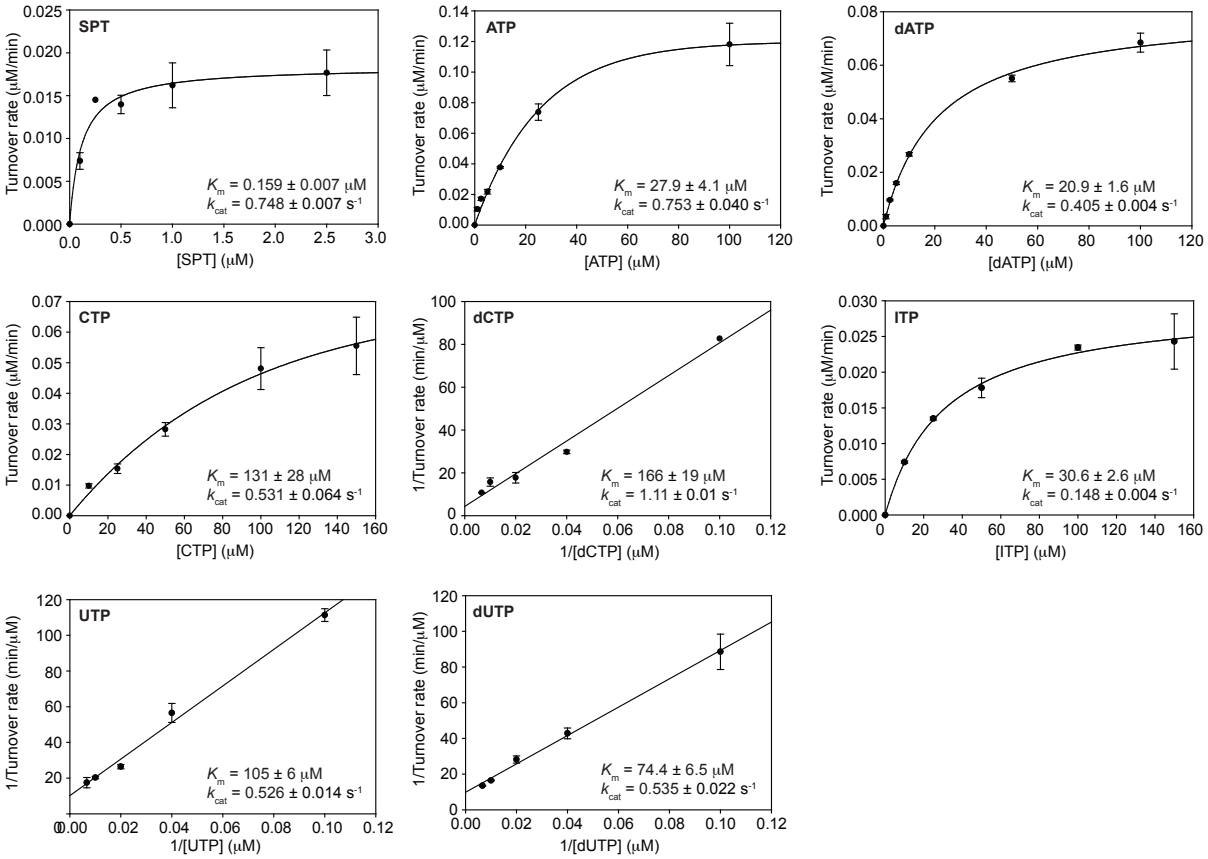

Fig. S5. Michaelis-Menten and Lineweaver-Burk kinetics for SPT as well as for a variety of NTPs and dNTPs during nucleotidylation by ANT(3")-Ii/AAC(6')-IId.

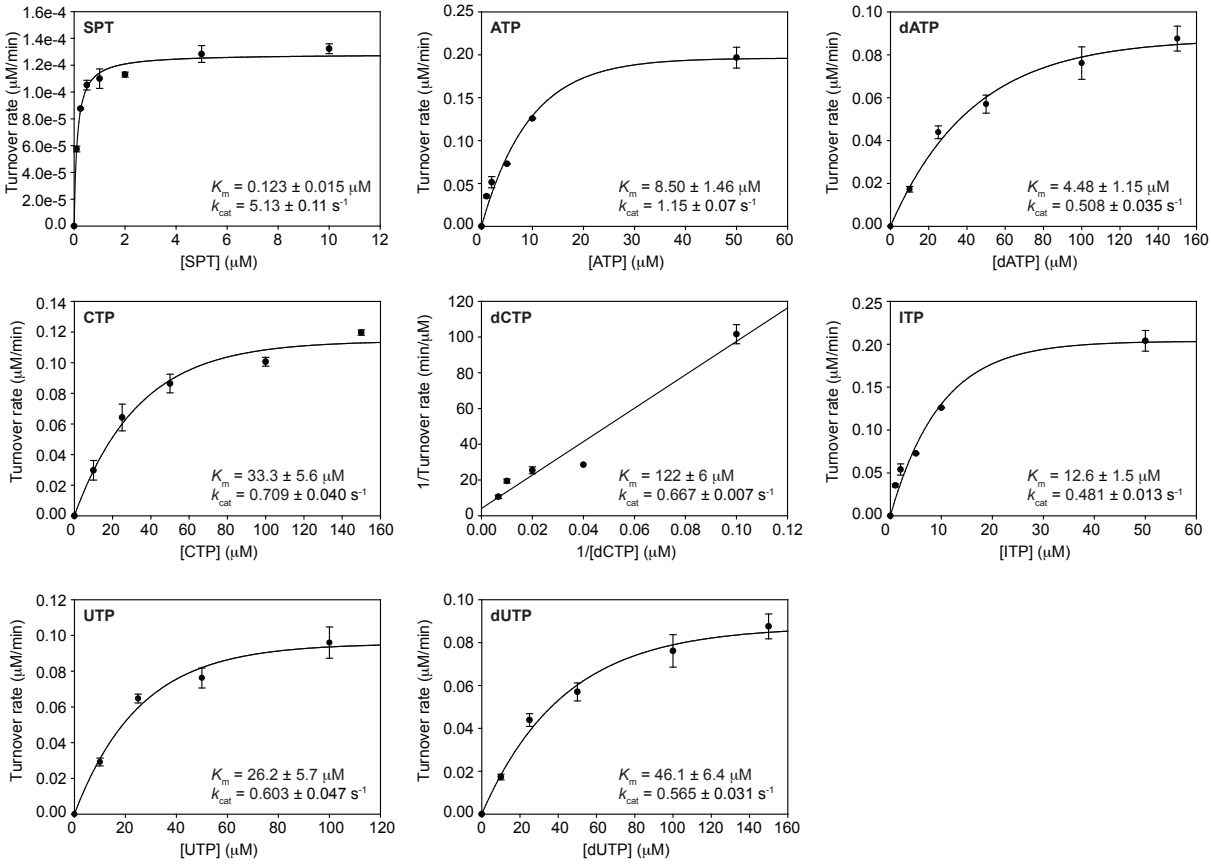

Fig. S6. Michaelis-Menten and Lineweaver-Burk kinetics for SPT as well as for a variety of NTPs and dNTPs during nucleotidylation by ANT(3")-Ii(1-266).

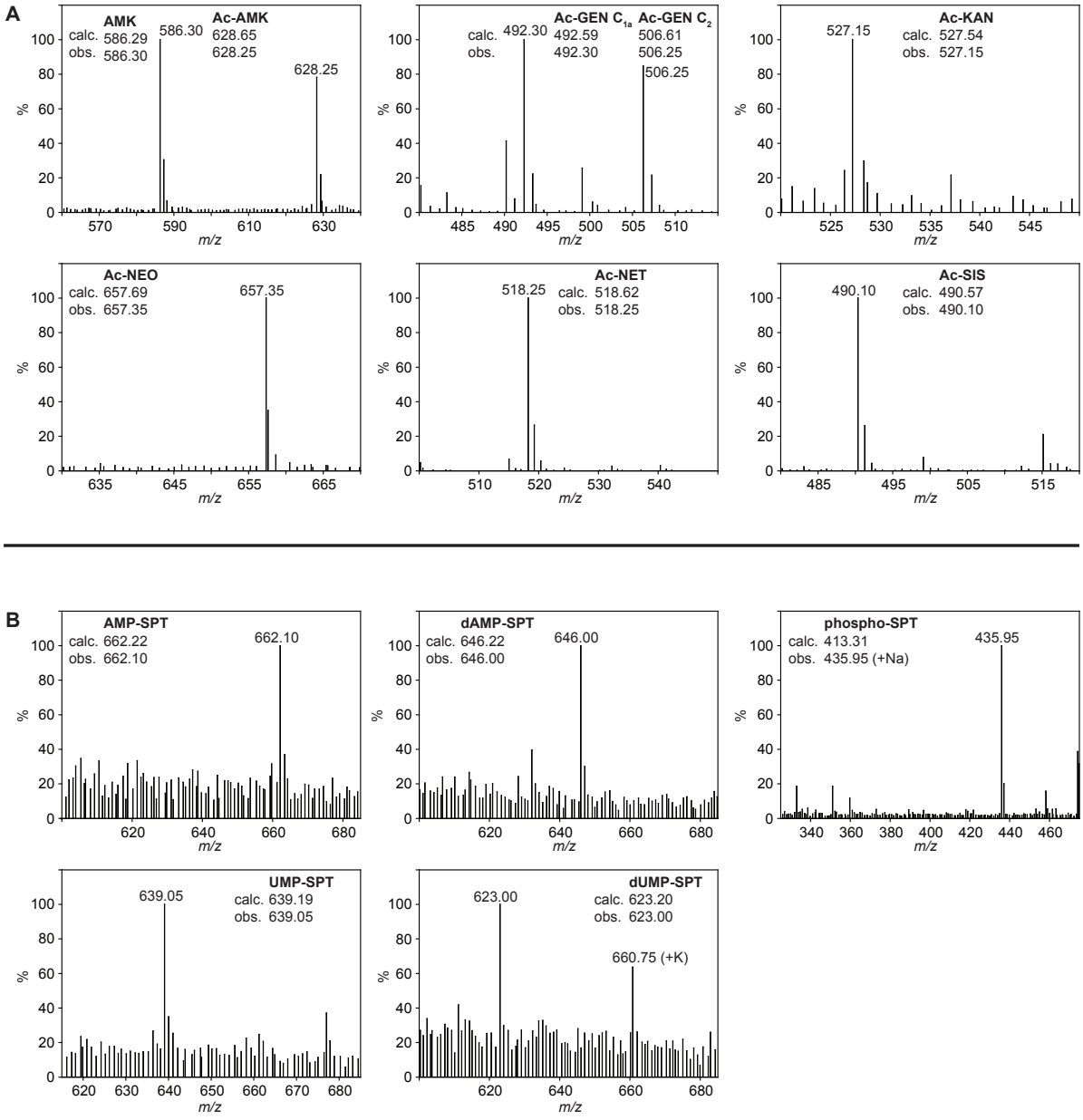

Fig. S7. Representative mass spectra for **A.** the acetylation of various AGs, and **B.** nucleotidylation of SPT with various NTPs, dNTPs, and TP by ANT(3")-Ii/AAC(6')-IId.