Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information

Nucleoside triphosphate cosubstrates control the substrate profile and efficiency of aminoglycoside 3'-*O*-phosphotransferase type IIa

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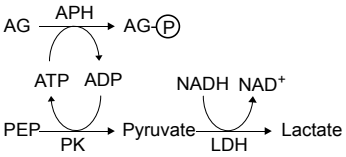

Fig. S1. Schematic of the coupled UV-Vis assay used in this study for determination of substrate and cosubstrate profiles and kinetics.

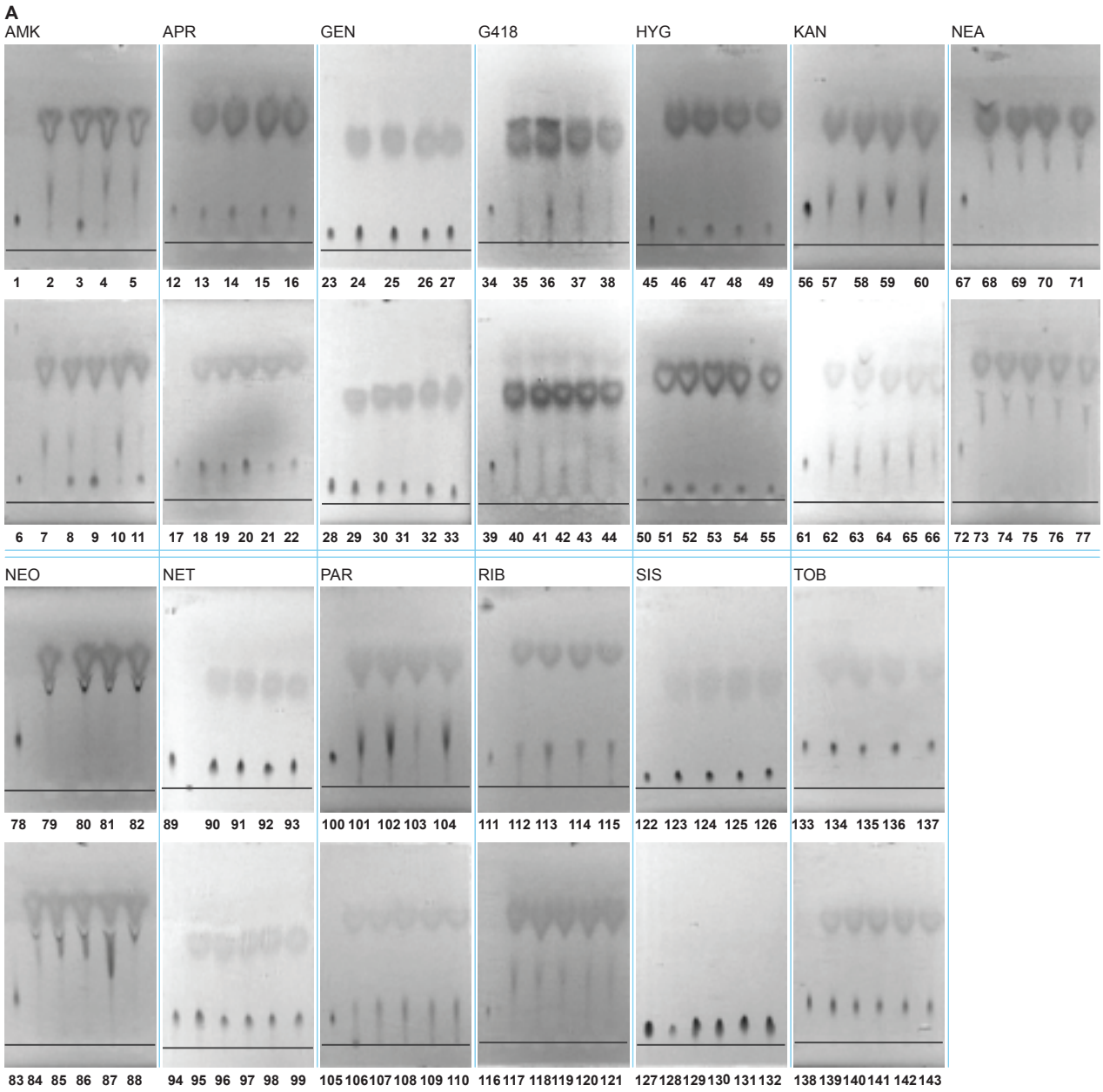

Fig. S2A. Pictures of TLC plates of APH(3')-IIa reacting with various (d)NTPs and AGs. The corresponding R_f values for each lane are listed in Fig. S4B presented on next page. *Note*: TLC images with ATP and the corresponding R_f values are summarized in Fig. 2.

B

Lane	AG	NTP	R_f	Lane	AG	NTP	R_f	Lane	AG	NTP	R_f	Lane	AG	NTP	R_f	Lane	AG	NTP	R_f
1	AMK	-	0.17	**34**	G418	-	0.17	**67**	NEA	-	0.27	**100**	PAR	-	0.15	**133**	TOB	-	0.29
2		GTP	0.32	**35**		GTP	0.17	**68**		GTP	0.57	**101**		GTP	0.20	**134**		GTP	0.29
3		CTP	0.15, 0.38	**36**		CTP	0.17	**69**		CTP	0.57	**102**		CTP	0.22	**135**		CTP	0.62
4		UTP	0.33	**37**		UTP	0.19	**70**		UTP	0.57	**103**		UTP	0.24	**136**		UTP	0.62
5		ITP	0.33	**38**		ITP	0.22	**71**		ITP	0.57	**104**		ITP	0.22	**137**		ITP	0.07
6		-	0.11	**39**		-	0.17	**72**		-	0.20	**105**		-	0.14	**138**		-	0.07
7		dATP	0.31	**40**		dATP	0.26	**73**		dATP	0.50	**106**		dATP	0.20	**139**		dATP	0.09
8		dGTP	0.11, 0.33	**41**		dGTP	0.28	**74**		dGTP	0.52	**107**		dGTP	0.20	**140**		dGTP	0.12
9		dCTP	0.11	**42**		dCTP	0.17	**75**		dCTP	0.52	**108**		dCTP	0.22	**141**		dCTP	0.12
10		TTP	0.31	**43**		TTP	0.26	**76**		TTP	0.51	**109**		TTP	0.20	**142**		TTP	0.21
11		dUTP	0.11	**44**		dUTP	0.26	**77**		dUTP	0.45	**110**		dUTP	0.20	**143**		dUTP	0.21
12	APR	-	0.18	**45**	HYG	-	0.13	**78**	NEO	-	0.30	**111**	RIB	-	0.15				
13		GTP	0.18	**46**		GTP	0.10	**79**		GTP	0.58	**112**		GTP	0.21				
14		CTP	0.18	**47**		CTP	0.10	**80**		CTP	0.58	**113**		CTP	0.26				
15		UTP	0.18	**48**		UTP	0.10	**81**		UTP	0.58	**114**		UTP	0.26				
16		ITP	0.15	**49**		ITP	0.10	**82**		ITP	0.58	**115**		ITP	0.02				
17		-	0.19	**50**		-	0.08	**83**		-	0.25	**116**		-	0.03				
18		dATP	0.19	**51**		dATP	0.06	**84**		dATP	0.55	**117**		dATP	0.05				
19		dGTP	0.19	**52**		dGTP	0.06	**85**		dGTP	0.55	**118**		dGTP	0.30				
20		dCTP	0.18	**53**		dCTP	0.06	**86**		dCTP	0.55	**119**		dCTP	0.29				
21		TTP	0.18	**54**		TTP	0.08	**87**		TTP	0.20, 0.56	**120**		TTP	0.28				
22		dUTP	0.18	**55**		dUTP	0.08	**88**		dUTP	0.58	**121**		dUTP	0.18				
23	GEN	-	0.08	**56**	KAN	-	0.21	**89**	NET	-	0.16	**122**	SIS	-	0.08				
24		GTP	0.08	**57**		GTP	0.25	**90**		GTP	0.15	**123**		GTP	0.08				
25		CTP	0.08	**58**		CTP	0.25	**91**		CTP	0.15	**124**		CTP	0.08				
26		UTP	0.09	**59**		UTP	0.27	**92**		UTP	0.15	**125**		UTP	0.09				
27		ITP	0.09	**60**		ITP	0.27	**93**		ITP	0.16	**126**		ITP	0.09				
28		-	0.09	**61**		-	0.19	**94**		-	0.18	**127**		-	0.09				
29		dATP	0.09	**62**		dATP	0.28	**95**		dATP	0.18	**128**		dATP	0.09				
30		dGTP	0.09	**63**		dGTP	0.28	**96**		dGTP	0.20	**129**		dGTP	0.09				
31		dCTP	0.09	**64**		dCTP	0.19, 0.26	**97**		dCTP	0.18	**130**		dCTP	0.09				
32		TTP	0.09	**65**		TTP	0.26	**98**		TTP	0.17	**131**		TTP	0.09				
33		dUTP	0.09	**66**		dUTP	0.26	**99**		dUTP	0.17	**132**		dUTP	0.09				

Fig. S2B. R_f values for each lane of TLC sample listed in Fig. S4A presented on the previous page. *Note*: TLC images with ATP and the corresponding R_f values are summarized in Fig. 2.

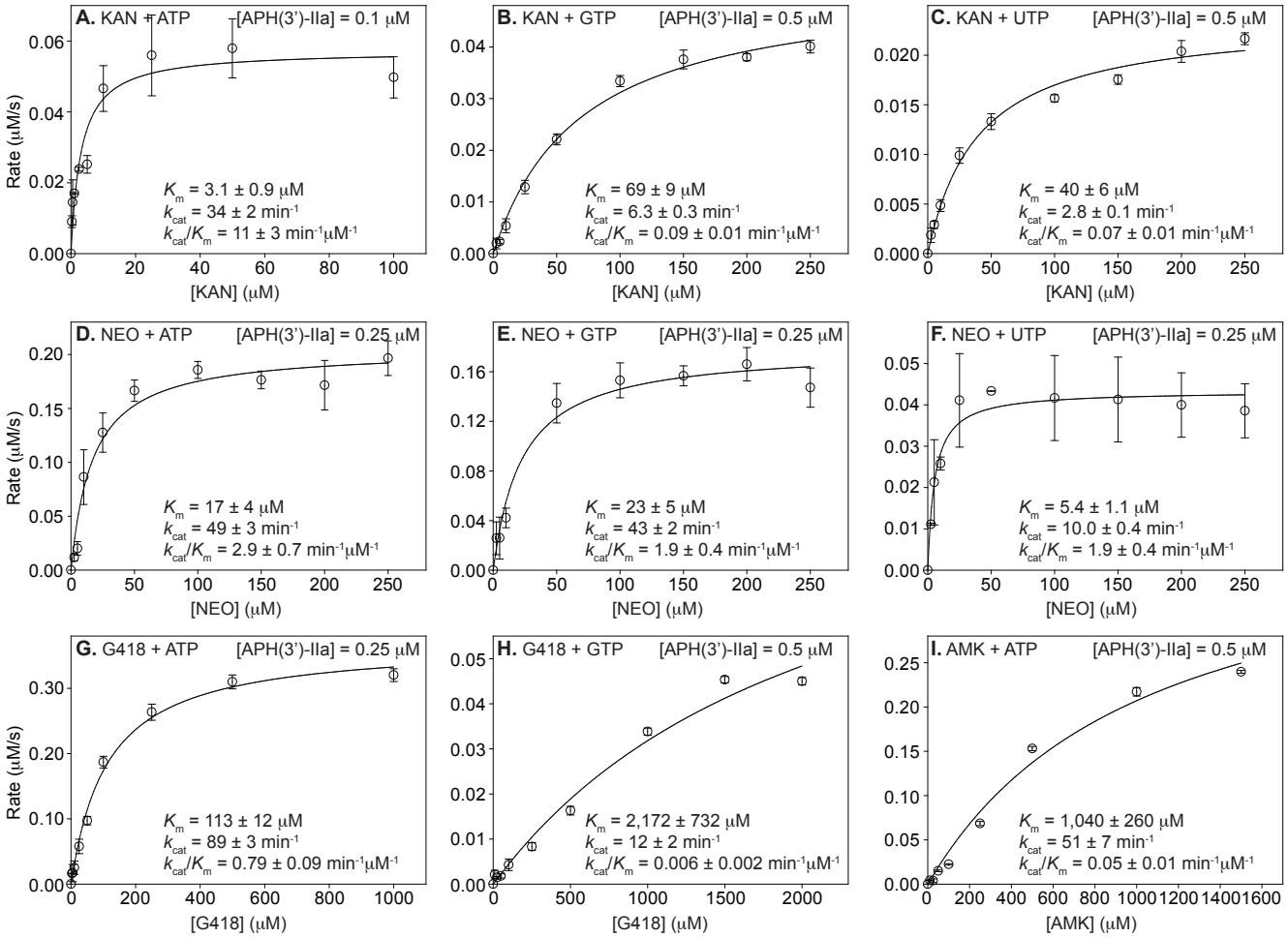

Fig. S3. Michaelis-Menten graphs with the determined kinetics parameters of APH(3')-IIa with respect to various AG substrates in the presence of various NTP cosubstrates (at 2 mM).

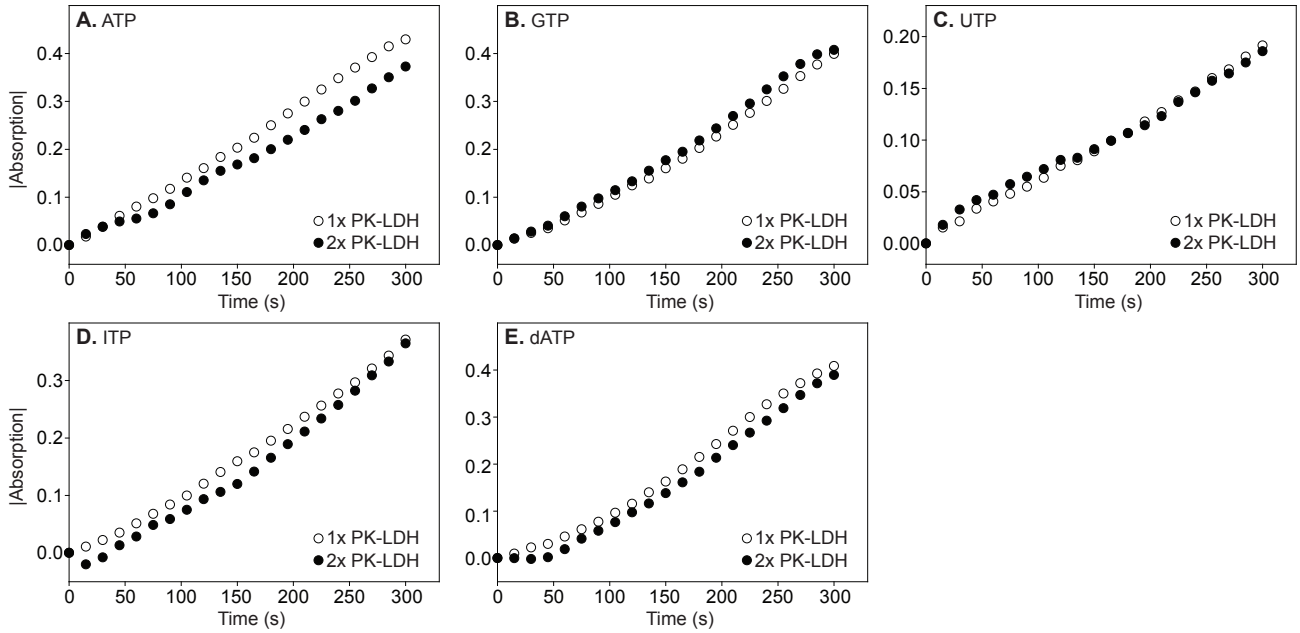

Fig. S4. Time course reaction for each NTP with APH(3')-IIa enzyme with either 1× or 2× PK-LDH concentration in the reaction in order to prove that APH(3')-IIa kinetics shown in Table 2 and Fig. S3 were not limited by the coupled reaction used in UV-Vis assays.

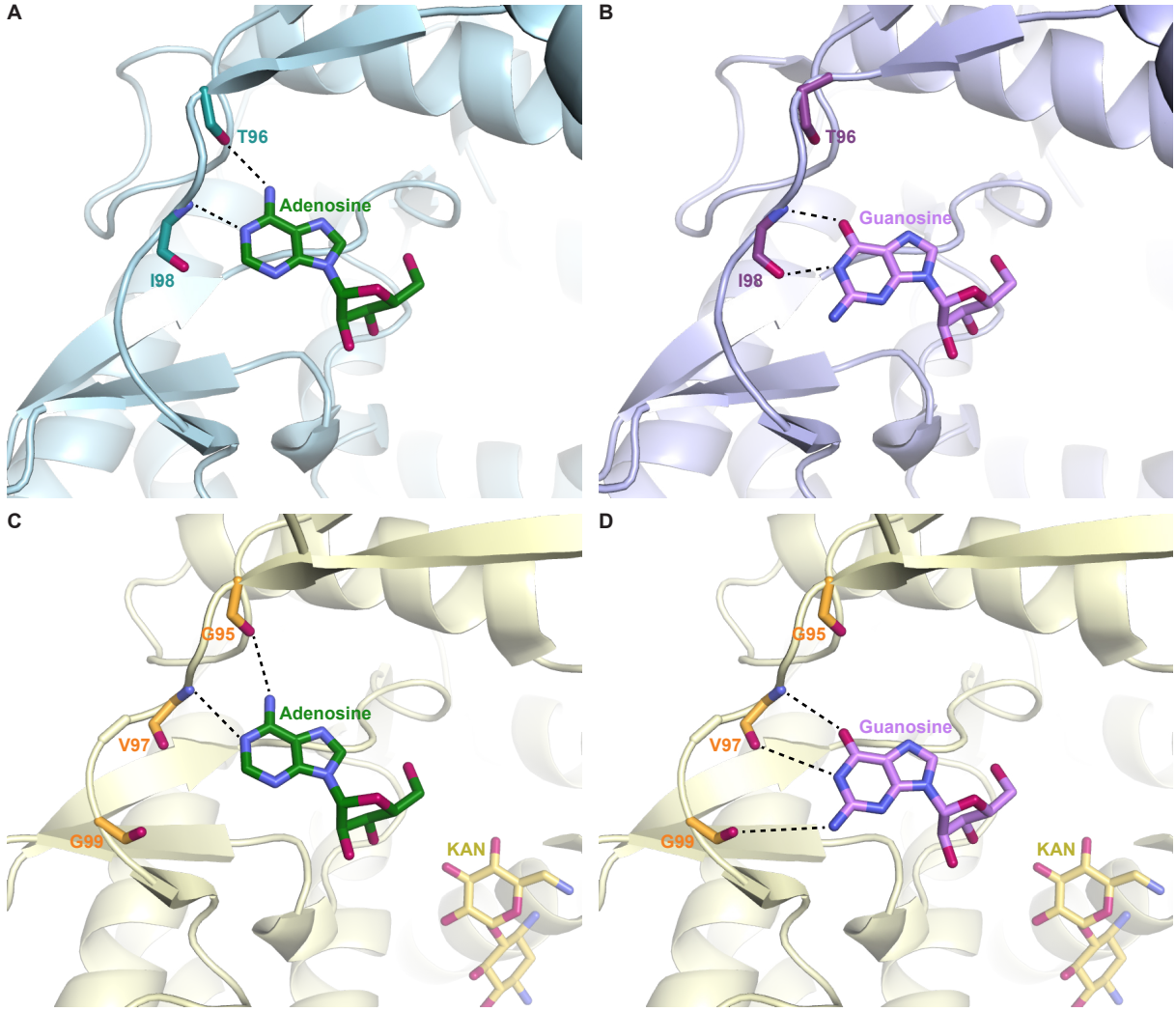

Fig. S5. Superimposition of crystal structures of APH(3')-IIa with KAN (PDB ID: 1ND4)[1] and APH(2")-IVa with adenosine and guanosine (PDB IDs: 4DT8 and 4DT9, respectively)[2]. **A.** APH(2")-IVa (in light teal) and adenosine in dark green with the backbones of Thr96 and Ile98 colored in dark teal, **B.** APH(2")-IVa (in light purple) and guanosine in purple with the backbone of Thr96 and Ile98 colored in dark purple, **C.** APH(3')-IIa (in pale yellow) with KAN (in bright yellow) and the adenosine from APH(2")-IVa structure in dark green and the backbone of Gly95, Val97, and Gly99 in orange, **D.** APH(3')-IIa (in pale yellow) with KAN (in bright yellow) and the guanosine from APH(2")-IVa structure in purple and the backbone of Gly95, Val97, and Gly99 in orange.

References

[1] Nurizzo, D., Shewry, S. C., Perlin, M. H., Brown, S. A., Dholakia, J. N., Fuchs, R. L., Deva, T., Baker, E. N., and Smith, C. A. (2003) The crystal structure of aminoglycoside-3'-phosphotransferase-IIa, an enzyme responsible for antibiotic resistance, *J. Mol. Biol. 327*, 491-506.

[2] Shi, K., and Berghuis, A. M. (2012) Structural basis for dual nucleotide selectivity of aminoglycoside 2"-phosphotransferase IVa provides insight on determinants of nucleotide specificity of aminoglycoside kinases, *J. Biol. Chem. 287*, 13094-13102.