Supporting information

Conferring the ability to utilize inorganic polyphosphate on ATP-specific NAD kinase

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Figures S1, S2, S3 and S4
Table S1

Figure S1

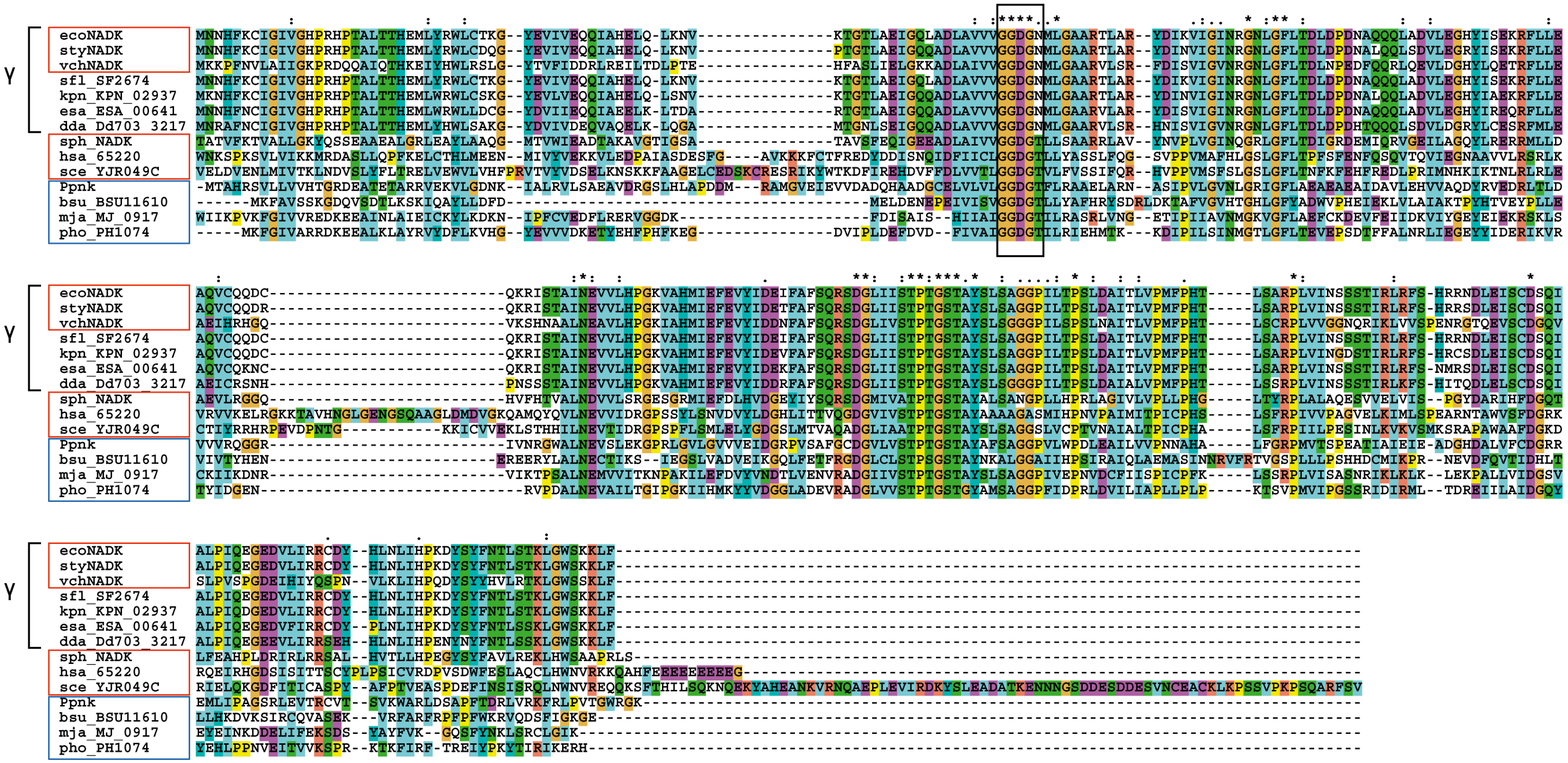
γ
ecoNADK
styNADK
vchNADK
sfl_SF2674
kpn_KPN_02937
esa_ESA_00641
dda_Dd703_3217
sph_NADK
hsa_65220
sce_YJR049C
Ppnk
bsu_BSU11610
mja_MJ_0917
pho_PH1074

Figure S1. Multiple alignment of the primary structures of ATP-specific NADKs (boxed in red), poly(P)/ATP-NADKs (boxed in blue), and γ-proteobacterial NADK homologs (indicated by γ). ATP-specific NADKs are ecoNADK (*Escherichia coli*), styNADK (*Salmonella enterica*), vchNADK (*Vibrio cholerae*), sph_NADK (*Sphingomonas* sp. A1), hsa_65220 (human), and sce_YJR049C (*Saccharomyces cerevisiae*). Poly(P)/ATP-NADKs are Ppnk (*Mycobacterium tuberculosis*), bsu_11610 (*Bacillus subtilis*), mja_MJ_0917 (*Methanococcus jannaschii*), and pho_PH1074 (*Pyrococcus horikoshii*). γ-Proteobacterial NADK homologs are ecoNADK, styNADK, vchNADKs, sfl_SF2674K (*Shigella flexneri*), kpn_KPN_02937 (*Klebsiella pneumoniae*), esa_ESA_00641 (*Cronobacter sakazakii*), and dda_Dd703_3217 (*Dickeya dadantii*).

The motif GGDGN and GGDGT are boxed in black. The motif GGDGN is conserved in the primary structures of some γ-proteobacterial NADK homologs, while the motif GGDGT is found in those of other ATP-specific NADKs and poly(P)/ATP-NADKs. We failed to identify the key conserved residues in all ATP-specific NADKs or all poly(P)/ATP-NADKs; see also Fig. 1.

Figure S2

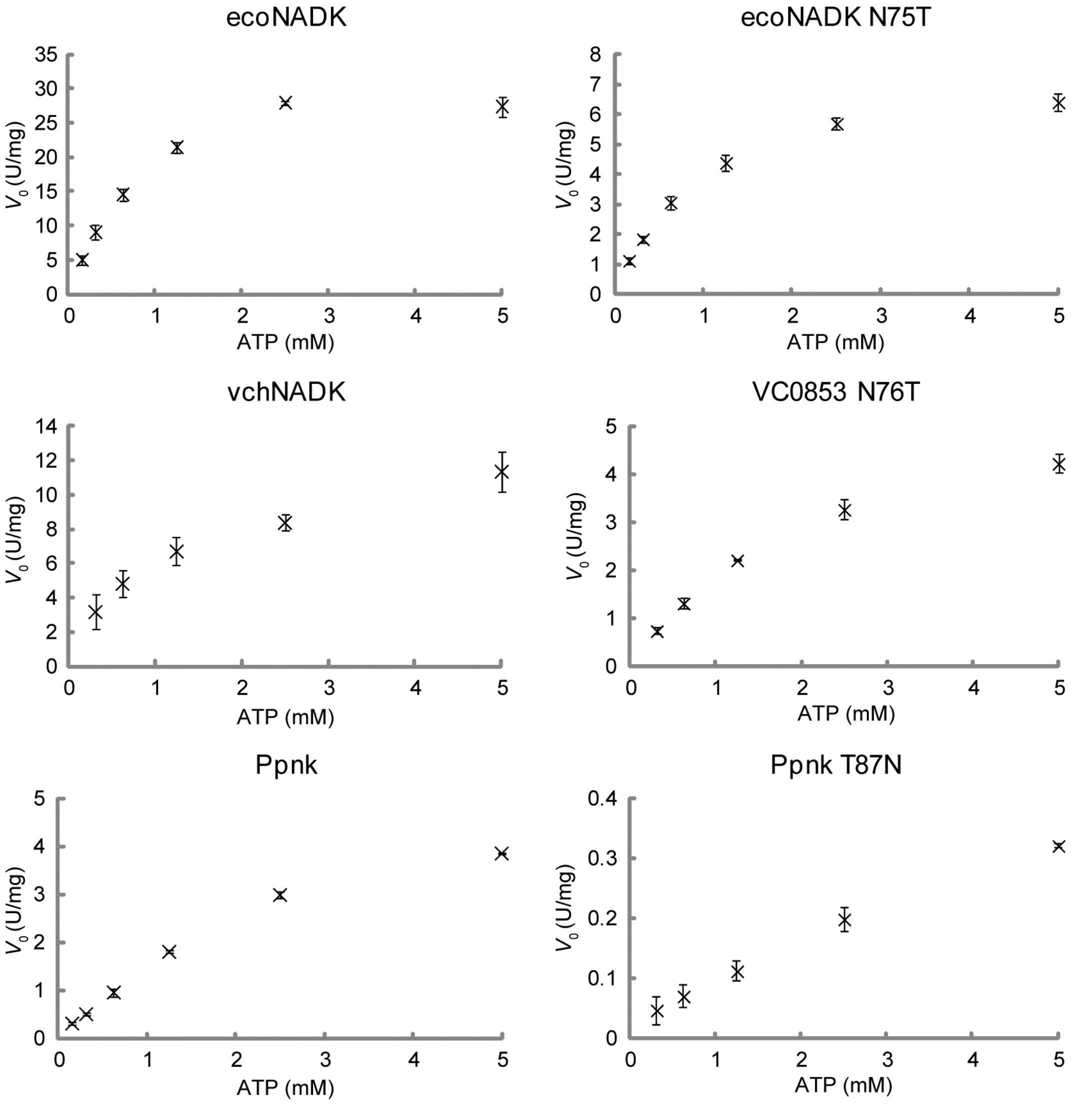

Figure S2. The data used to determine k_{cat} and K_{m} of each NADK toward ATP. Means and standard deviations of three independent experiments are shown.

Figure S3

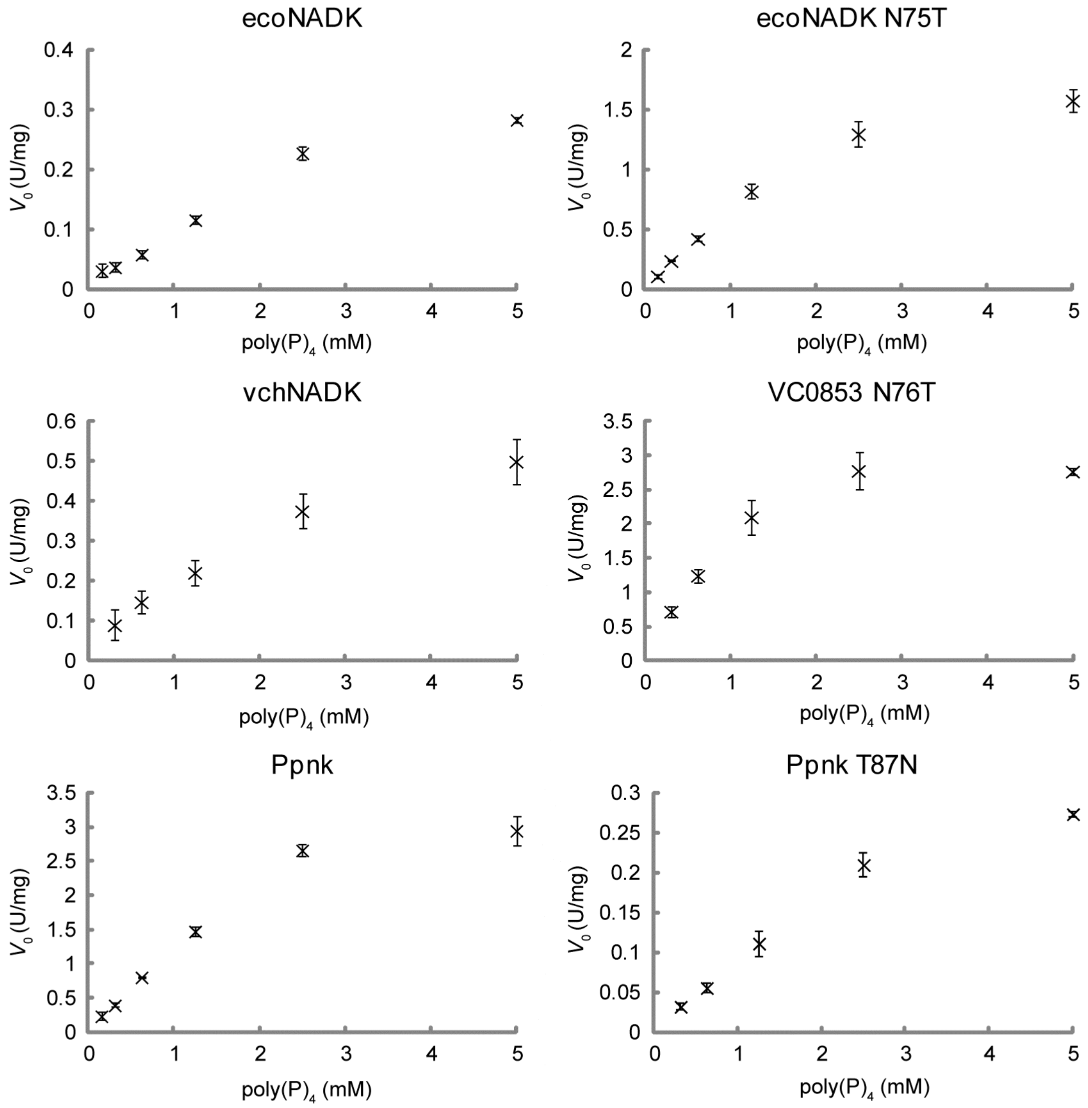

Figure S3. The data used to determine k_{cat} and K_{m} of each NADK toward poly(P)$_4$. Means and standard deviations of three independent experiments are shown.

Figure S4

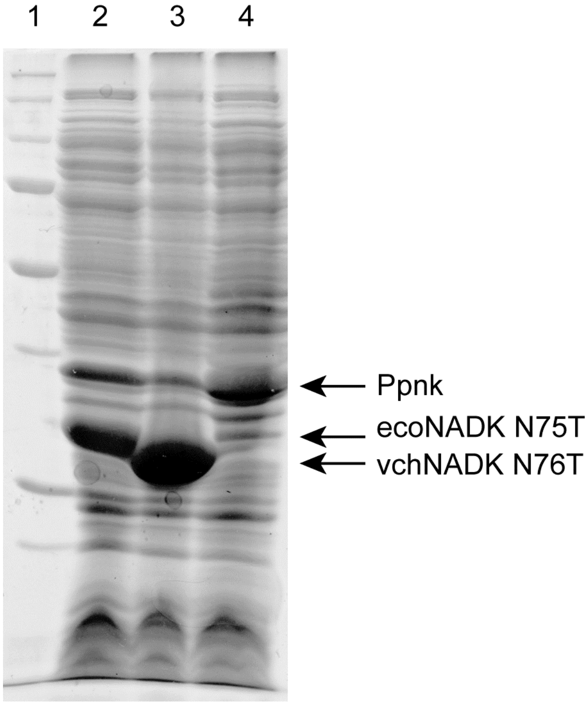

Figure S4. SDS-PAGE of extracts of cells expressing NADK. Cells were suspended in 100 mM Tris-HCl (pH 7.5) and ultrasonically disrupted at 9 kHz (Insonator model 201M; Kubota, Osaka, Japan) at 0°C for 10 min, and then centrifuged at 28,000 × *g* for 20 min at 4°C. The resultant clear supernatant was used as a cell extract, which was subjected to SDS-PAGE (12.5% gel). The remaining cells were immobilized and used for mass production of $NADP^+$. Lane 1: Protein markers (Bio-Rad, Hercules, CA). Lanes 2–4: cell extracts (50 µg protein) of cells expressing ecoNADK N75T (lane 2), vchNADK N76T (lane 3), and Ppnk (lane 4).

Table S1. Amino acid residue of γ-proteobacterial NADK corresponding to Asn-75 of ecoNADK

Order	Genera	N	T	S	F	Y	H
Enterobacteriales	*Cronobacter*	3	-	-	-	-	-
	Dickeya	4	-	-	-	-	-
	Edwardsiella	2	-	-	-	-	-
	Enterobacter	5	-	-	-	-	-
	Erwinia	6	-	-	-	-	-
	Escherichia	55	-	-	-	-	-
	Klebsiella	7	-	-	-	-	-
	Pectobacterium	3	-	-	-	-	-
	Photorhabdus	2	-	-	-	-	-
	Pnatoea	6	-	-	-	-	-
	Proteus	1	-	-	-	-	-
	Providencia	1	-	-	-	-	-
	Rahnella	3	-	-	-	-	-
	Salmonella	26	-	-	-	-	-
	Serratia	5	-	-	-	-	-
	Shigella	9	-	-	-	-	-
	Xenorhabdus	2	-	-	-	-	-
	Yersinia	19	-	-	-	-	-
Pasteurellales	*Actinobacillus*	4	-	-	-	-	-
	Aggregatibacter	4	-	-	-	-	-
	Buchnera	10	-	-	-	-	-
	Haemophilus	12	-	-	-	-	-
	Mannheimia	1	-	-	-	-	-
	Pasteurella	4	-	-	-	-	-
	Sodalis	1	-	-	-	-	-
	Wigglesworthia	-	-	2	-	-	-
Vibrionales	*Aliivibrio*	3	-	-	-	-	-
	Listonella	1	-	-	-	-	-
	Photobacterium	16	-	-	-	-	-
	Vibrio	20	-	-	-	-	-
Aeromonadales	*Aeromonas*	3	-	-	-	-	-

Table S1. Continued

Order	Genera	N	T	S	F	Y	H
Alteromonadales	*Alteromonas*	-	-	2	-	-	-
	Colwellia	-	-	-	-	1	-
	Glaciecola	1	-	-	-	1	-
	Idiomarina	1	-	-	-	-	-
	Mariniobacter	-	-	3	-	-	-
	Oceanimonas	1	-	-	-	-	-
	Pseudoalteromonas	3	-	-	-	-	-
	Psychromonas	-	-	-	-	1	-
	Saccharophagus	-	-	1	-	-	-
	Shewanella	24	-	-	-	-	-
Oceanospirillales	*Alcanivorax*	-	-	1	-	-	-
	Chromohalobacter	-	-	1	-	-	-
	Hahella	-	-	1	-	-	-
	Halomonas	-	-	1	-	-	-
	Marinomonas	-	-	3	-	-	-
Pseudomonadales	*Acinetobacter*	-	-	13	-	-	-
	Azotobacter	-	-	1	-	-	-
	Cellvibrio	-	-	1	-	-	-
	Moraxella	-	-	1	-	-	-
	Pseudomonas	-	-	27	-	-	-
	Psychrobacter	-	-	3	-	-	-
Legionellales	*Coxiella*	-	-	5	-	-	-
	Legionella	-	-	7	-	-	-
Methylococcales	*Alkalilimnicola*	-	-	-	-	1	-
Chromatiales	*Allochromatium*	-	-	-	-	1	-
	Halorhodospira	-	-	-	-	1	-
	Halothiobacillus	-	-	-	-	1	-
	Methylococcus	-	-	-	-	1	-
	Methylomicrobium	-	-	-	-	1	-
	Methylomonas	-	-	-	-	1	-
	Nitrosococcus	-	-	-	-	3	-
	Thioalkalivibrio	-	-	-	-	2	-
Xanthomonadales	*Frateuria*	-	1	-	-	-	-
	Pseudoxanthomonas	-	2	-	-	-	-
	Stenotrophomonas	-	4	-	-	-	-
	Xanthomonas	-	11	-	-	-	-
	Xylella	-	5	-	-	-	-

Table S1. Continued

Order	Genera	N	T	S	F	Y	H
Thiotrichales	*Francisella*	15	-	-	-	-	-
	Methylophaga	-	-	-	-	2	-
	Thioalkalimicrobium	-	-	-	-	1	-
	Thiomicrospira	-	-	-	-	1	-
Acidithiobacillales	*Acidithiobacillus*	-	-	-	-	4	-
Cardiobacteriales	*Dichelobacter*	-	-	-	-	1	-
others	*Baumannia*	1	-	-	-	-	-
	Blochmannia	2	-	-	-	-	-
	Ferrimonas	1	-	-	-	-	-
	Gallibacterium	1	-	-	-	-	-
	Hamiltonella	1	-	-	-	-	-
	Histophilus	2	-	-	-	-	-
	Kangiella	-	-	1	-	-	-
	Riesia	-	-	1	-	-	-
	Ruthia	-	-	1	-	-	-
	Teredinibacter	-	-	1	-	-	-
	Tolumonas	-	-	-	-	-	1
	Unclassified	-	-	1	-	-	-
	Vesicomyosocius	-	-	1	-	-	-