Supporting Information

Design of an in vitro multienzyme cascade system for the biosynthesis of nicotinamide mononucleotide

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#These authors contributed equally to this work.

Table S1 Strains and plasmids used in this work

Strains or plasmids	Description	Source
Strains		
E. coli BL21(DE3)	Used as host strain	Invitrogen
E.coli BL21-Pynp	*E. coli* BL21(DE3) harboring plasmid pET28a-*pynp*	This study
E.coli BL21-Pnp	*E. coli* BL21(DE3) harboring plasmid pET28a-*pnp*	This study
E.coli BL21-Nadr	*E. coli* BL21(DE3) harboring plasmid pET28a-*nadr*	This study
E.coli BL21-Apt	*E. coli* BL21(DE3) harboring plasmid pET28a-*apt*	This study
E.coli BL21-AMPase	*E. coli* BL21(DE3) harboring plasmid pET28a-*amn*	This study
E.coli BL21-Rppk	*E. coli* BL21(DE3) harboring plasmid pET28a-*prs*	This study
E.coli BL21-Nampt-HD	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-HD	This study
E.coli BL21-Nampt-HS	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-HS	This study
E.coli BL21-Nampt-MM	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-MM	This study
E.coli BL21-Nampt-LS	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-LS	This study
E.coli BL21-Nampt-CP	*E. coli BL21(DE3) harboring plasmid pET28a-nampt-CP*	This study
E.coli BL21-Nampt-SS	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-SS	This study
E.coli BL21-Nampt-MA	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-MA	This study
E.coli BL21-Nampt-EC	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-EC	This study
E.coli BL21-Nampt-XL	*E. coli* BL21(DE3) harboring plasmid pET28a-nampt-XL	This study
E.coli BL21-Ppk2	*E. coli* BL21(DE3) harboring plasmid pET28a-*ppk2*	This study
Plasmids		
pET28a	expression vector, KanR, P_{T7}, f1 ori	Invitrogen
pET28a-*pynp*	pET28a containing *pynp* from *Bacillus licheniformis*	This study
pET28a-*deoD*	pET28a containing *deoD* from *Klebsiella aerogenes*	This study
pET28a-*amn*	pET28a containing *amn* from *E. coli*	This study
pET28a-*prs*	pET28a containing *prs* from *E. coli*	This study
pET28a-*nadr*	pET28a containing *nadr* from *E. coli*	This study
pET28a-*apt*	pET28a containing *apt* from *E. coli*	This study
pET28a-nampt-HD	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Haemophilus ducreyi*	This study
pET28a-nampt-HS	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Homo sapiens*	This study
pET28a-nampt-MM	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Mus musculus*	This study
pET28a-nampt-LS	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Luteibacter* sp.	This study

pET28a-nampt-CP	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Chitinophaga pinensis*	This study
pET28a-nampt-SS	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Sphingopyxis sp. C-1*	This study
pET28a-nampt-MA	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Macaca mulatta*	This study
pET28a-nampt-EC	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Equus caballus*	This study
pET28a-nampt-XL	pET28a containing the synthetic codon-optimized coding sequence of NAMPT from *Xenopus laevis*	This study
pET28a-*ppk2*	pET28a containing synthetic *ppk2*	This study

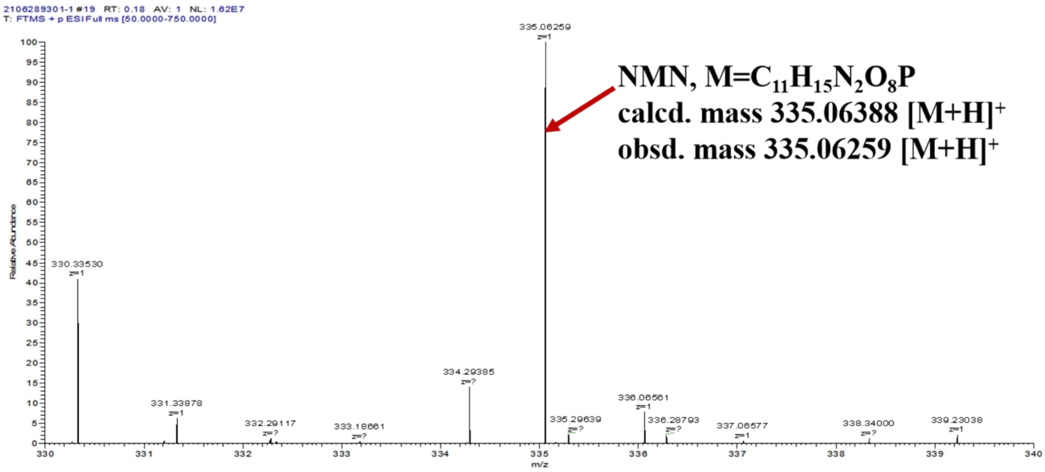
Table S2 Summary of enzymes used in this work

Name	Enzyme	EC number	NCBI accession number	Gene	Source
Pynp	pyrimidine-nucleoside phosphorylase	2.4.2.2	QBR18245.1	*pynp*	*Bacillus licheniformis*
Pnp	purine nucleoside phosphorylase	2.4.2.1	VDZ69745.1	*deoD*	*Klebsiella aerogenes*
NADR	ribosylnicotinamide kinase	2.7.1.22	NP_418807.4	*nadR*	*Escherichia coli*
AMPase	AMP nucleosidase	3.2.2.4	NP_416489.1	*amn*	*Escherichia coli*
RPPK	ribose-phosphate diphosphokinase	2.7.6.1	NP_415725.1	*prs*	*Escherichia coli*
Apt	adenine phosphoribosyltransferase	2.4.2.7	NP_415002.1	*apt*	*Escherichia coli*
Nampt-HD	nicotinamide phosphoribosyl transferase	2.4.2.12	WP_010945301.1	putative *nadV*	*Haemophilus ducreyi*
Nampt-HS	nicotinamide phosphoribosyl transferase	2.4.2.12	NP_005737.1	*nampt*	*Homo sapiens*
Nampt-MM	nicotinamide phosphoribosyl transferase	2.4.2.12	NP_067499.2	*nampt*	*Mus musculus*
Nampt-LS	nicotinamide phosphoribosyl transferase	2.4.2.12	SFW68293.1	*nampt*	*Luteibacter* sp.
Nampt-CP	nicotinamide phosphoribosyl transferase	2.4.2.12	WP_012788281.1	*nampt*	*Chitinophaga pinensis*
Nampt-SS	nicotinamide phosphoribosyl transferase	2.4.2.12	WP_062182430.1	*nampt*	*Sphingopyxis sp. C-1*
Nampt-MA	nicotinamide phosphoribosyl transferase	2.4.2.12	XP_014990151.1	*nampt*	*Macaca mulatta*
Nampt-EC	nicotinamide phosphoribosyl transferase	2.4.2.12	XP_023494644.1	*nampt*	*Equus caballus*
Nampt-XL	nicotinamide phosphoribosyl transferase	2.4.2.12	NP_001089988.1	*nampt*	*Xenopus laevis*
PPK2	polyphosphate kinase 2	2.7.4.1	RVP31066.1	*ppk2*	*Sinorhizobium meliloti*

Table S3 Primers used in this work

Name	Primer sequence (5'-3')
Pynp F	CATGCCATGGaacaccaccaccaccaccacATGAGAATGGTTGATATCATCACA
Pynp R	CGCGGATCCCTATTCCGTAATCACCGTATGCACAA
Pnp F	CATGCCATGGaacaccaccaccaccaccacATGGCCACCCCACACATTAA
Pnp R	CCCAAGCTTTTACTCTTTATCACCCAGCAGTACGG
NADR F	CATGCCATGGaacaccaccaccaccaccacATGTCGTCATTTGATTACCTGAAAACTGC
NADR R	CCGGAATTCTTATCTCTGCTCCCCCATCATCT
AMPase F	CATGCCATGGaacaccaccaccaccaccacATGAATAATAAGGGCTCCGGTCTG
AMPase R	CCCAAGCTTTTATCGGAACGGCGGCTC
RPPK F	CATGCCATGGaacaccaccaccaccaccacGTGCCTGATATGAAGCTTTTTGCT
RPPK R	CCGGAATTCTTAGTGTTCGAACATGGCAGAGAT
Apt F	CCCATGGaacaccaccaccaccaccacATGACCGCGACTGCACAG
APT R	GGAATTCTTAATGGCCCGGGAACGG

Underline indicates the cleavage sites of restriction enzyme and lowercase letters indicate the His Tag sequence.



NMN, M=$C_{11}H_{15}N_2O_8P$
calcd. mass 335.06388 $[M+H]^+$
obsd. mass 335.06259 $[M+H]^+$

Figure S1 MS result of the reaction product.

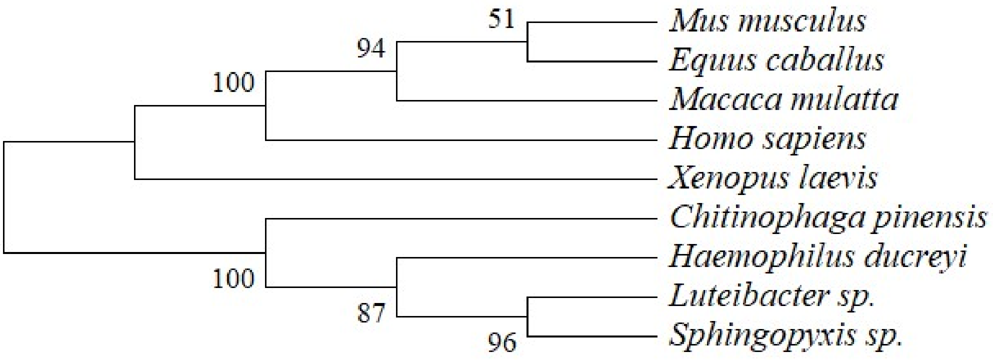


Figure S2 Amino-acid sequence homology analysis for nine different NAMPT.

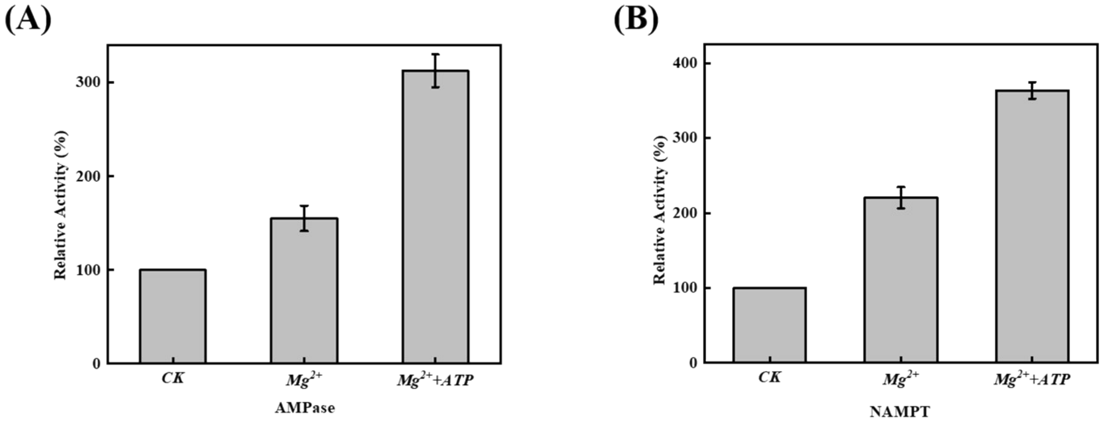
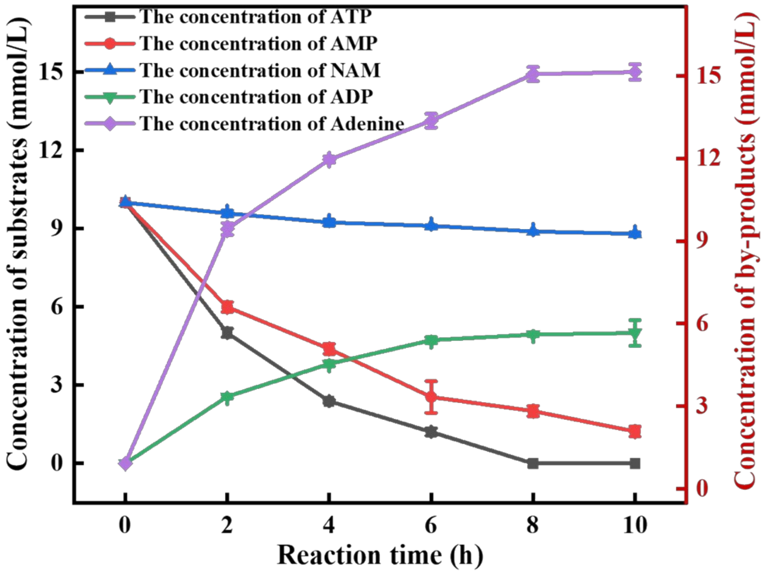
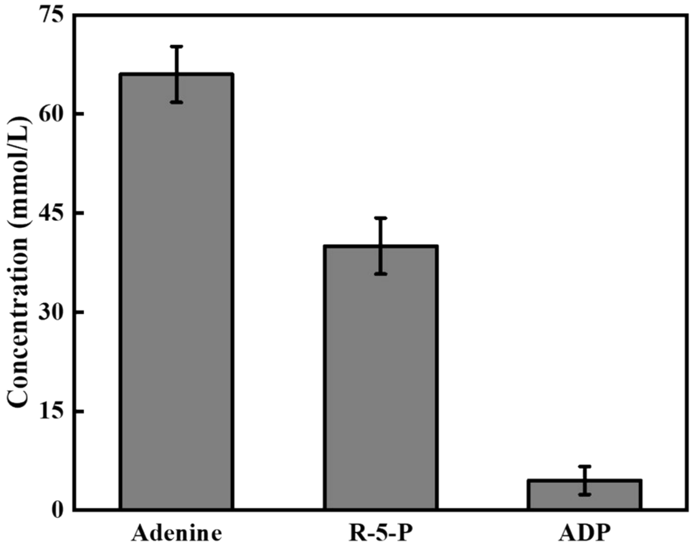


Figure S3 Effect of Mg^{2+} and ATP on enzyme activity of AMPase(A) and NAMPT(B). CK represents the control group.



Supplementary Figure S4 The consumption of ATP, AMP, and NAM, and the generation of by-products ADP and adenine under the condition of AMPase/RPPK/ NAMPT ratio of 1:6:10.



Supplementary Figure S5 The generation of by-products of adenine, R-5-P and ADP with the four-enzyme cascade catalysis system in vitro.