Molecular Cell, Volume 82

Supplemental information

Crystal structure of human NADK2 reveals

a dimeric organization and active site

occlusion by lysine acetylation

Charline Mary, Mona Hoseini Soflaee, Rushendhiran Kesavan, Muriel Gelin, Harrison Brown, G. Zacharias, Thomas P. Mathews, Andrew Lemoff, Corinne Lionne, Gilles Labesse, and Gerta Hoxhaj

Site	Peptide sequence	Peptide modification	Abundance of modification (10 ⁶)	Total peptide abundance (10 ⁶)	% modified peptide	Phosphosite ^{®.}
T229	LYLEGtGINPVPVDLHEQQLSLNQHNR	T6(Phospho)	0.08	0.10	80.0	
S289	AsYYEISVDDGPWEK	S2(Phospho)	0.05	70.99	0.07	1 LTP
K76	VVVVA <mark>k</mark> TTR	K6(Acetyl)	4.85	9.84	49.28	
K98	YAELSEEDL <mark>k</mark> QLLALK	K10(Acetyl)	0.51	1127.97	0.04	1 HTP
K104	QLLALKGSSYSGLLER	K6(Acetyl)	0.87	5.48	15.87	
K104	QLLALKGSSYSGLLER	K6(Succinyl)	1.62	5.48	29.56	
K170	WADAVIAAGGDGTMLLAAS <mark>k</mark> VLDR	K20(Acetyl)	0.58	516.54	0.11	1 HTP
K176	L <mark>k</mark> PVIGVNTDPER	K2(Acetyl)	2.11	2585.11	0.08	
K304	QkSSGLNLCTGTGSK	K2(Acetyl)	0.27	0.7	38.57	2 HTP
K317	SSGLNLCTGTGS <mark>k</mark> AWSFNINR	K13(Acetyl)	ND	459.21	ND	
K339	VATQAVEDVLNIAkR	K14(Acetyl)	4.86	3473.97	0.14	4 HTP
K339	VATQAVEDVLNIAkR	K14(Succinyl)	0.22	3473.97	0.006	1 HTP
K355	ELVEkVTNEYNESLLYSPEEPK	K5(Acetyl)	1.15	5.00	23.00	1 HTP
K397	CFSSkVCVR	K5(Acetyl)	0.71	1.23	57.72	2 HTP

Table S1. Post-translational modifications of NADK2 identified from HEK293E cells expressing NADK2-FLAG. Related to Figure 5. Modified amino acids are shown in red. Percentage of modified peptide represents the ratio of the modified peptide abundance to the total peptide abundance. HTP and LTP refer to high and low throughput data, respectively curated from PhosphoSitePlus[®].



Figure S1: Crystal structure of NADK2. Related to Figure 1.

(A) Crystal structure of the NADK2 in the apo-form or bound to NADP⁺. The monomeric structure of NADK2 is shown in red and green ribbons for the NADP-bound and the apo-forms, respectively. The NADP⁺ molecule (sticks) and the calcium atom (violet sphere) are also shown.

(B) Crystal structure of the NADK2 monomer. The monomer crystal structure is colored in green (N-terminal domain) and red (C-terminus domain). The NADP⁺ molecule (CPK spheres) and the calcium atom (green sphere) are also shown. CPK: Space-filling calotte.

(C) His-NADK2 purity isolated from *E. coli* and subjected to size exclusion chromatography was visualized with Coomassie Blue staining. Lanes 1, 2, 3 indicate the different elution fractions represented on the SEC-MALS elution profile (D).

(D) SEC-MALS elution profile of purified His-NADK2 indicating elution as single peak corresponding to a dimer of 86.5 kDa.

(E) SAXS profile of purified NADK2. SAXS experimental data recorded at various concentrations (3, 6, and 12 mg/mL) were scaled to show a similar organization is observed over a large range of concentrations.

(F) Comparison of the crystal structure and the AlphaFold model of NADK2. The dimeric crystal structure is shown in a black ribbon. The AlphaFold2 model is colored in cyan for one monomer and in violet ribbon but for the three extensions (shown in green, yellow, and pink ribbons).

(**G**) Comparison of the crystal structures of dimeric NADK2 (top) and cytosolic tetrameric NADK (bottom) (PDB3PFN). The macromolecular surfaces are shown side-by-side for clarity and in the same color code as in Figure. 1B. A dotted pink circle shows extension 3 (aa 325-365), preventing the tetramerization of NADK2.

(H) Electron density around the NADP⁺ in the NADK2-NADP⁺-Mg²⁺ complex. The NADP molecule is shown in CPK sticks, and the metal atom is shown as a green sphere. The corresponding electron density was computed as an omit map and the Fourier difference density was contoured at 2.5 σ .

(I) Electron density around the NADP⁺ in the NADK2-NADP⁺-Ca²⁺ complex. Same as (H), but for the calcium ion shown as a green sphere.

(J) Electron density around the NADP⁺ in the NADK2-NADP⁺-Fe³⁺ complex. Same as (H), but for the iron ion shown as a green sphere.

Fig. S2

mtnadk_human		<u>ααααααααααα</u>	<u> 202000000</u>	00000000000000000000000000000000000000	0000000000000	β2 η1
mtnadk_human mtnadk_xenli mtnadk_danre mtnadk_drome mtnadk_arome nadk1_human pos5_yeast ppnk_myctu	ADGGFRPSRVVVVAFT SAEGFRPARVAVVAFT AECAFRPAKVAVVAFT EKPSFTPKLKRALVVTKI LTWNKSPKSVLVIKK LIWQNPLQNVVITKKF MTAHRSVLLVVHT	TRYEPEQORYRYA TRYEPEQORYRSS TRYEPEQORYRSS SRYEPEQORYRYA SRYEPEQORYRYA TRYEPEKRVNK R	ELSEEDLKQLLALKG GLSEAELRDLLALKG GLSEEDLKQLLALKG GCSDEQLQKLKDRG GCSDEQLATLLKKRG extention 1	SYSGLLERHHINT SSYNGLLQRYNIHS SSYNGLLQRYNIHS SSYGLLERHNIHT TDVEMVLHLHKVHKI DSDYGRLLSKHKIHHS 	NVEHILDSLRN. NVEHIVQSLQK. NVGHIVESLQK. FFERRVQSPQD. YLNTLQRELEN. PFFKELCTHLME. ANVEFITHLHES. TARRVEKVLGD.	EGTEVREVEREY EGTDVREVKEREY QGIEVRVVKRGY VGCEVKLSSRLEP AGTESREVRFGY BINIVVEKKVLE ENMIVVEKKVLE NKIALRVLSAEAV
mtnadk_human		0000	<u>ρο</u> <u>β3</u> 160 <u>ρο</u> ρο	17 0	β4	۵۵ موم
mtnadk_human mtnadk_kenli mtnadk_danre mtnadk_drome mtnadk_caceel nadk1_human pos5_yeast ppnk_myctu	DPAIASDESFGAVKKE EISQDFKSPLENDPNF DRGSLHLAPDDMRAMG	DETV DETV DETV RSSLSKDVM FCTP.REDVDIS PHILYTGPEQDIV VEIEVVDADQHAA	RWADAYIAAGGDGT RWADAIISAGGDGT RWADAIISAGGDGT SWADYIVPYGGDGT DWADAYSAGGGT NGIDFICLGGDGT NRTDLLYTLGGDGT DG <u>CELVLVIGGDGT</u>	TLAASKVL TLAASKVQ LVASKVY TLSAGRASPLFALS MASSRVR YASSLFQ THGVSMFG TRAAELAR	REKPVIGVNTDP RFKPVIGVNTDP KSKPVLGVNTDP QKTPIVGPNSDP KHKPVIGINTDP SVFVMAPHLG QVPPVLAPALG ASIPVLGVNLG	SRSECHLCLPVRYT ERSECHLCLPVRYT ERSECHLCLPVRYT HSEGRLMLPKHYS 20SECYMCLMRKLP SLCFLTPFSP RIGFLAEAEA
mtnadk_human 2	ο <u>οοοοοοοοο</u> - οο <u>21ο</u>	β5 β6	β7 23 0 24 0	0000000000 250	260	
mtnadk_human mtnadk_xenli mtnadk_danre mtnadk_drome mtnadk_caeel nadk1_human por5_yeast ppnk_myctu	H. SPPEALQK FYRGE W. SPPEALQKLYRGE H. APSEALQKLRKOP D. NPADAVSRIKSOD E. NPADAVSRIKSOD E. NFQSQVTQVIEONA K. EHKKVPQEVISSRA E. AIDAVLEHVVAQD	RWLWBORIRLYLB RWQWQQRIRLYLB RWQWQQRIRMHLB RWQWHSRVRTMHL RWMHSSRVRTTML AVVLSRLKVRVV KCLHRTRLBCHLK RVEDRLTLDVVVR	GTGINP.VPVDLHE GTGINL.TPVDLHE GTGINP.TPVDLHE GSNGNIPEPTDLFEL GDDGIS.DAIELHD KELRGK.KTAVHN KKD	QQLSLNQHNRALNIH QQLSLEQHNKAHNSG LQLSLQUHSKAHRI TTEVKMEQVSTAPEMI QQLNRDPATTRWTDH GLGENGSQ	RAHDER. LEQKSV. TGTQSSTP. DQDMAY. IPRSPAREIEECMS ention 2	SLS PPVK KRMISEA
mtnadk_human	β8	β ⁹ → →		β10	β11 β12	η2 α9 222 000000
mtnadk_human mtnadk_kani mtnadk_danre mtnadk_darre mtnadk_drem mtnadk_caeel nadk1_human pos5_yeast ppnk_myctu	β8 27 φ SEASGPQLLPVF AVSGPQLLPVF HDSPEAPHLLPVF KYKAKMKRVLPYI VEIPEVEKETVBLPVI AAGLDMDVGKQAMQYQ SNSSIVTF GRIVMRGW	β9 200 ALNEVPIGESLSS GLNEIPIGESLSS GLNEIPIGESLSS ALNEVPIGESLSS ALNEVPIGESLSS VLNEVVIDRGPSS ALNEVSLEKGPRL ★★	R. RVNYKSCKPRFTFSI R. R. R. H. G.	β10 290 30 ASY YEISVDG.I ASY YEISVDG.I ASY YEISIDG.I YSH LQLVLDHQD VSH LQLVLDHQD SN VDYLDGH.I LINLDIFIDGE.I VLGVVVEIDGR.I	β11 β12 90 310 WEKQKSSCLNVC WEKQKSSCLSIC WEKQKSSCLSIC WEKQKSSCLSIC VINKTKCSGLCVS WILKQKSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKCSGLCVS WILVSSCLSIC VINKTKSSCLS WILVSSCLSIC	η2 α9 9000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 1000000 32 10000000 32 10000000000 32 1000000000000 32 1000000000000000000000000000000000000
<pre>mtnadk_human mtnadk_kenli mtnadk_danre mtnadk_danre mtnadk_casel nadk1_human pos5_yeast ppnk_myctu mtnadk_human</pre>	β8 27 0	β9 280 ALNEVPTGESLSS ALNEVPTGESLSS GLNEIPTGESLSS ALNEVPTGESLSS VLNEVPTGESLSS MNDIPLHRGNSP ALNEVSLEKGPRL **	R. RVNYKSCKPRPTPSI R. R. Y. H. G. G. G. J G J G J G J G G G G G G G G	β10 290 30 ASYYEISVDDG.I RASYYEISVDDG.I SYYEISIDDG.I VSHLQLVLDHQD. VSHLQLVLDHQD.I LSNVDVYLDGH.I LSNVDVYLDGH.I LSNVDVYLDGH.I VLGVVVBIDGR.I 	β11 β12 β12 β12 β12 β12 β12 β12	12 09 200 000000 320 07 05 KAMS PN INR 07 05 KAMS YN INK 07 05 TS MH TS INR 07 05 TS MH TS INR 07 05 TS MH PN INK 07 05 TS MH PN INK 07 05 TA YAAAAGA 07 05 TA YAAAAGA 07 05 TA YAAAAGA 10 0 10 0 1
<pre>mtnadk_human mtnadk_xanli mtnadk_danre mtnadk_drome mtnadk_caeel nadk1_human pos5_yeast ppnk_myctu mtnadk_human mtnadk_kanre mtnadk_danre mtnadk_danre mtnadk_drome mtnadk_caeel nadk1_human pos5_yeast ppnk_myctu</pre>	β8 27 0 SEASGPQ LLPVF MUSPEAPHLLPVF KIYAKMKRYLPVI AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC GRIVMRGW AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC GRIVMRGW GRIVMRGW AGLONDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLOMDVGKQ AMQYC AGLONDVGKQ AMQYC AAGLOMDVGKQ AMQYC AAGLO	β9 280 ALNEVPICESLSS ALNEVPICESLSS CLNEIPICESLSS CLNEIPICESLSS VINEVVICESLSS VINEVVICESLSS VINEVVICESLSS VINEVVICESLSS ALNEVSLEKGPRL ALNEVSLEKGPRL ALNEVSLEKGPRL ALNEVSLEKGPRL 4 20 250 CONLSLPLN.REL KSLNVSLD.SDV TGLEVPLM.REP DSKNVMML.RQN CONLFOLTHGDKNA EXTENTION 3	R	β10 290 30 ASYYEISVDDGI ASYYEISVDDGI VSYEISVDDGI VSYEISIDDGI VSYEISIDDGI VSYYEIGINDA. VSYUGVULDHQD. VSYYEIGINDA. SYDVYLDGHI VSYUGVULDHQD. SYDVYLDGHI SYDVYLDGHI B13 370 380 SEPKILPSIREFII S	β11 β12 β13 WEK QKS SOLNIC WEK QKS SOLNIC WEK QKS SOLNIC WEK QKS SOLNIC WEK QKS SOLNIC WEK QKS SOLNIC WEK QKS SOLNIC UTTYQGDGVIVS JTRTTADGVALA SOL NRVPSSSRQRCPS NRVPSSSRQRCPS NRVPSSSQQRGPS VGVWPSPKTPKES VGVWPSPKTPKEPK SOL SOL SOL SOL SOL SOL SOL SOL	η2 α9 920 920 320 320 GTGSKAMSPNINK 510 GTGSKAMSYNINK 510 GTGSKAMSYNINK 510 GTGSKAMSYNINK 510 GTGSKAMSYNINK 510 GTGSKAMSYNINK 510 GTGSKAMSYNINK 500 GTGSKAMSYNINK 500 GTGSKAMSYNINK 500 GTGSKAMSYNINK 500 GTGSTAYSAS 600 SSKVCVRSR 700 A00 500 SSKVCVRSR 700 A00 500 SSKVCVRSR 700 DFVQTVPVSR 700 DDKICKSR 700 FDKICKISK 700 PRASHIKIKISPEATIAIENTEN 700
<pre>mtnadk_human mtnadk_kanni mtnadk_danre mtnadk_danre mtnadk_caeel nadk1_human pos5_yeast ppnk_myctu mtnadk_human mtnadk_kanre mtnadk_danre mtnadk_caeel nadk1_human pos5_yeast ppnk_myctu</pre>	β8 27 φ SEASGPQLIPVF AVSGPQLIPVF KYKAKMKRVLPVI VEIPEVERETVEIPVI AAGLDMDVGKQAMQYQ SNSSIVTF GRIVMRGW QQQQQQQQQQQQQQ 33 φ VATQAVEDVLNIAKFQ MSSQSVEELLNINYQE LVEQAVEDVLNIAKFA ITSRDVDDLLRSIPDQ LTEQCVQDLKKIVAEE 	β9 2800 ALNEVPIGESLSS ALNEVPIGESLSS GLNEIPIGESLSS GLNEVFIGESLSS JUNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIGESLSS VLNEVVIDRGPSS ALNEVSLEKGPRL ** 20 350 ONLSLPIN.REL KSLNVSLD.SDV TGLEVPLN.REP DSKNVMML.RQN CONLSLPINON GANON ALNEVPLON BJ7 BI8 420	R. RVNYKSCKPRPTPSI R. R. Y. H. G. WEKVTNEYNESLIYS VEKVTNEYNESLIYS VESVTDTYNESLIYS VESVTDTYNESLIYS SIT STIF STIF STIF STIF STIF	β10 290 30 ASYYEISVDDGI HRASYYEISVDDGI ASYYEISVDDGI VSYEISIDDGI VSYEISIDDGI VSYUGIVDHOA SYEIGINDA SYEISID SYEIGINDA SYEISID S	β11 β12 310 310 WEK QKS SCINCO WEK QKS SCINCO WITTY QGDO VIVS ITTY QGDO VIVS UTTTA DOVALA # MIL QKS SCITIC # B14 390 NR VPSSSRQ QC DOVIVS # NR VPSSSRQ QK GPP * NR VPSSSRQ QK GPP * NA TPP DPTD PRGPI * NA TPP DPTD PRGPI * US VM PSIK TFK BI * NA TP PTD PRGPI * LISPRPIV * MA SPOND # MA SPOND * MA SPOND *	η2 α9 320 320 GTGSKAWSPNINK STNINK GTGSKAWSYNINK STSWINSINK GTGSTSWHYSINK STSWINSINK GTGSTAYAAAQA STSWINSINK GTGSTAYAAAQA STSWINSINK GTGSTAYAAAQA STSWINSINK GTGSTAYAAAQA STSTAYAAAQA STSSTAYAAAQA STSTAYAAAQA STSTAYAAAQA STSTAYAAAQA STGSTAYAASAS STSTAYAAAQA STGSTAYAASAS STSTAYAAAQA STGSTAYAAASAS STSTAYAAAQA STGSTAYAAASAS STSTAYAAAQA STGSTAYAASAS STSTAYAAAQA ATTGSTAYAASAS STSTAYAAAQA SSKVCVRSR STSTAYASAS SSKVCVRSR STSTAR

Figure S2. Sequence alignment for NADK2 with several tetrameric NADKs. Related to Figures 1 and 2.

The sequence of the NADK2 from human (Q4G0N4), *D. rerio (Q6DBS0), X. tropicalis* (Q08CZ6), *D. melanogaster* (Q7JW73), *C. elegans* (Q9XXI6) were aligned with the sequence of three tetrameric NADKs (human NADK2 (O95544); yeast mitochondrial NADK (POS5, Q06892) and *M. tuberculosis* NADK (ppnK, P9WHV7) using ViTO (Catherinot and Labesse, 2004).



Figure S3: Mitochondrial localization and function of NADK2 phospho-deficient- and acetylation-deficient mutants. Related to Figures 3 and 5.

(A) Fractional abundance of glutamine (M+5) from Δ NADK2 HEK-293E cells stably expressing either empty vector, NADK2 WT, or NADK2 variants with the indicated deleted extensions (Δ 78-114, Δ 231-266, Δ 325-365) labeled for 3 h with ¹³C₅ glutamine. Related to Fig. 3G.

(B) Representative images of localization of the NADK2-Flag WT or S/A variants to mitochondria (red) detected by immunofluorescence with a FLAG epitope antibody (green). Δ NADK2 HEK-293E cells were transiently transfected with NADK2 WT or the indicated NADK2 phospho-mutants (S/A). Mitochondria were stained with MitoTrackerTM (red) and nuclei with Hoechst (gray). Scale bars, 2 µm.

(C) As in (B), but from NADK2 HEK-293E cells were transiently transfected with NADK2 WT or the indicated NADK2 acetylation-mutants (K/Q). Scale bars, 2 μ m.

(**D**) Fractional abundance of glutamine (M+5), but from Δ NADK2 HEK-293E cells stably expressing either empty vector, NADK2 wild-type, or the indicated NADK2 phospho-mutants (S/A) labeled for 3 h with ¹³C₅ glutamine. Related to Fig. 5D.

(E) Fractional abundance of glutamine (M+5), but from Δ NADK2 HEK-293E cells stably expressing either empty vector, NADK2 wild-type, or the indicated NADK2 acetylation-mutants (K/Q) labeled for 3 h with ¹³C₅ glutamine. Related to Fig. 5F.

(F) Representative images of spheroids from \triangle NADK2 HEK-293E cells stably expressing either NADK2 WT or NADK2 S188A cultured without proline. Related to Fig. 5I.

(G) Immunoblots from Δ NADK2 HEK-293E cells transiently transfected with either empty vector (EV), WT NADK2 or NADK2 S188A, K76Q or K304Q that were treated with 1 mM DSS for 1 hour. Monomeric and dimeric NADK2 and the β -actin control are shown.

(H) Normalized peak area of mitochondrial NAD⁺ (M+4) from labeling with ${}^{13}C_{3}$ - ${}^{15}N$ -Nicotinamide (M+4). Δ NADK2 HEK-293E cells stably expressing HA-Mito were transiently transfected with empty vector (EV), WT NADK2 or NADK2 K76Q, K304Q or S188A mutants. Related to Fig. 5L.

Data are presented as the mean \pm s.d from n=3 (D, E, H) of biologically independent samples. Data are representative of at least two independent experiments. Multiple comparisons were calculated using one-way ANOVA and Tukey's post hoc test.



Figure S4: Structural basis for the regulation of human NADK2 by post-translational modification events. Related to Fig. 5.

(A) Enzymatic cycle of NADK2 as deduced from the crystal structures of the NADP⁺ bound form and the apo form as in Figure 2D, but with the three side-chains neighboring the NADP⁺ molecule now showing post-translation modification (K76 and S188 from one monomer and K304 from a second monomer). Proteins are in grey ribbon, while ligands are in colored sticks (ATP/ATP are in blue; NAD⁺/NADP⁺ in orange).

(**B**) Schematic representation of the impact of K76 acetylation, K304 acetylation, and S188 phosphorylation (Top panels) or the directed mutagenesis (bottom panels) predicted from manual modeling of the residue modification in the complex with NAD⁺ or NADP⁺, as well as docking of ADP or ATP molecules. Figures were drawn using Pymol. Proteins are in grey ribbon, while ligands are in colored sticks (ATP/ATP are in blue; NAD⁺/NADP⁺ in orange). Mutated or modified side-chains are shown in green sticks.