Supporting Information

NAD$^+$ biosynthesis in Bacteria is subject to global Carbon/Nitrogen control via PII signaling

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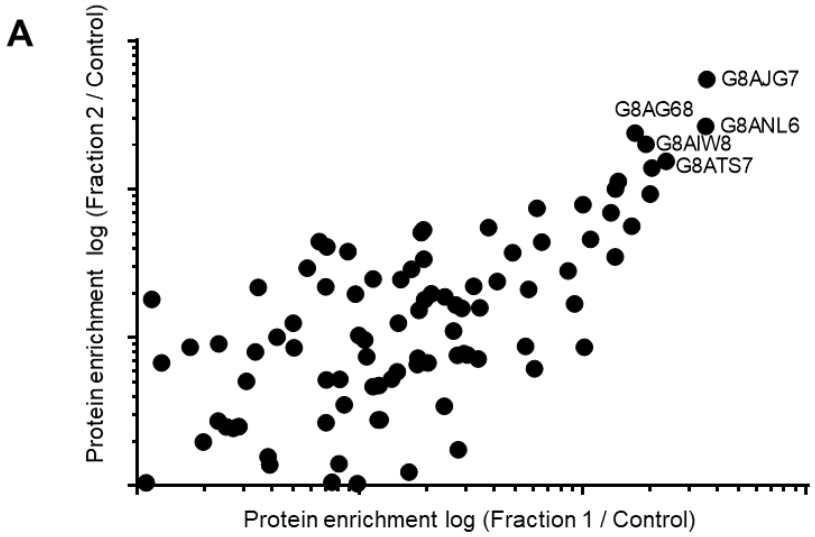
Table S2 - Bacterial strains and plasmids

Strain/plasmid	Genotype/phenotype	Source/reference
***Synechocystis* sp.**		
PCC 6803	Wild-type	(49)
A. brasilense		
FP2	Wild-type	(50)
LFH3	Nal^r, Δ*glnB* Nif$^-$	(51)
7611	Nif$^+$ *glnZ* ::Ω, Sp^r Sm^r	(25)
2812	*glnB*::kan/ *glnZ*:: Ω , Sm^r Km^r	(25)
E . coli		
DH10B	Sm^r ; F, [*pro*AB$^+$ *lacZ*M15]	Invitrogen
Rosetta (DE) pLysS	F- ompT hsdSB(rB- mB-) gal dcm (DE3) pLysSRARE	Novagen
Lemo21 (DE3)	Expresses T7 RNA polymerase	New England Labs Inc.
Plasmid	**Characteristics**	**Reference**
pET28a	Km^r Expression vector	Agilent
pET29a	Km^r Expression vector	Agilent
pTEV5	Amp^r Expression vector, His tag followed by TEV cleavage site	(52)
pASnade2	Km^r. Express *H. seropedicae* NadE2 with a N-terminal 6x His tag in pET28a	(7)

pLHnade2	Km^r. Express native *A. brasilense* NadE2 in pET29a	(7)
pASnadESc	Amp^r. Express *Synechocystis* sp. strain PCC 6803 NadE with a N-terminal 6x His tag in pTEV5	This work
pMSA4Δ42-54	Km^r. Express native *A. brasilense* GlnZΔ42-54 in pET29a	(45)
pGlnZΔTloop	Km^r. Express *A. brasilense* GlnZΔ42-54 with a N-terminal 6x His tag in pET28a	This work
pMSA3	Km^r. Express *A.brasilense* GlnZ with a N-terminal 6x His tag in pET28a	(53)
pMSA4	Km^r. Express native *A. brasilense* GlnZ in pET29a	(54)
pLH25	Km^r. Express *A.brasilense* GlnB with a N-terminal 6x His tag in pET28a	(55)
pASGlnDAb	Amp^r. Express *A. brasilense* GlnD with a N-terminal 6x His tag in pTEV5	This work

Supporting Figures



B

		Enrichment (log10)	
Uniprot	**Description**	**Fraction1/Control**	**Fraction2/Control**
G8AJG7	Orotate phosphoribosyltransferase	3,25	3,54
G8ANL6	Uncharacterized protein	3,24	3,06
G8AG68	Putative Phosphate dikinase regulatory protein	2,80	2,99
G8AIW8	Glutamine-dependent NAD(+) synthetase (NadE)	2,86	2,89
G8ATS7	Uncharacterized protein	2,99	2,74

Figure S1. Putative binding partners of the *A. brasilense* PII protein GlnZ. A) A 1 ml Ni-NTA column charged with His-tagged GlnZ protein was loaded with *A. brasilense* protein extracts in the presence of MgATP. After extensive washes, proteins were eluted with buffer containing MgATP and 1.5 mM of 2-OG and separated in 1.5 ml fractions (fractions 1 and 2). The amount of the proteins in each fraction was compared to the amount of proteins eluted from a control column, under the same conditions, by quantitative label free LC/MS/MS in technical triplicates. The graphic shows the log protein enrichment of the signal of each protein eluted the His-GlnZ column / control column in their respective 1.5 mL fractions. **B)** Details of the top 5 enriched proteins that were eluted from the His-GlnZ affinity column.

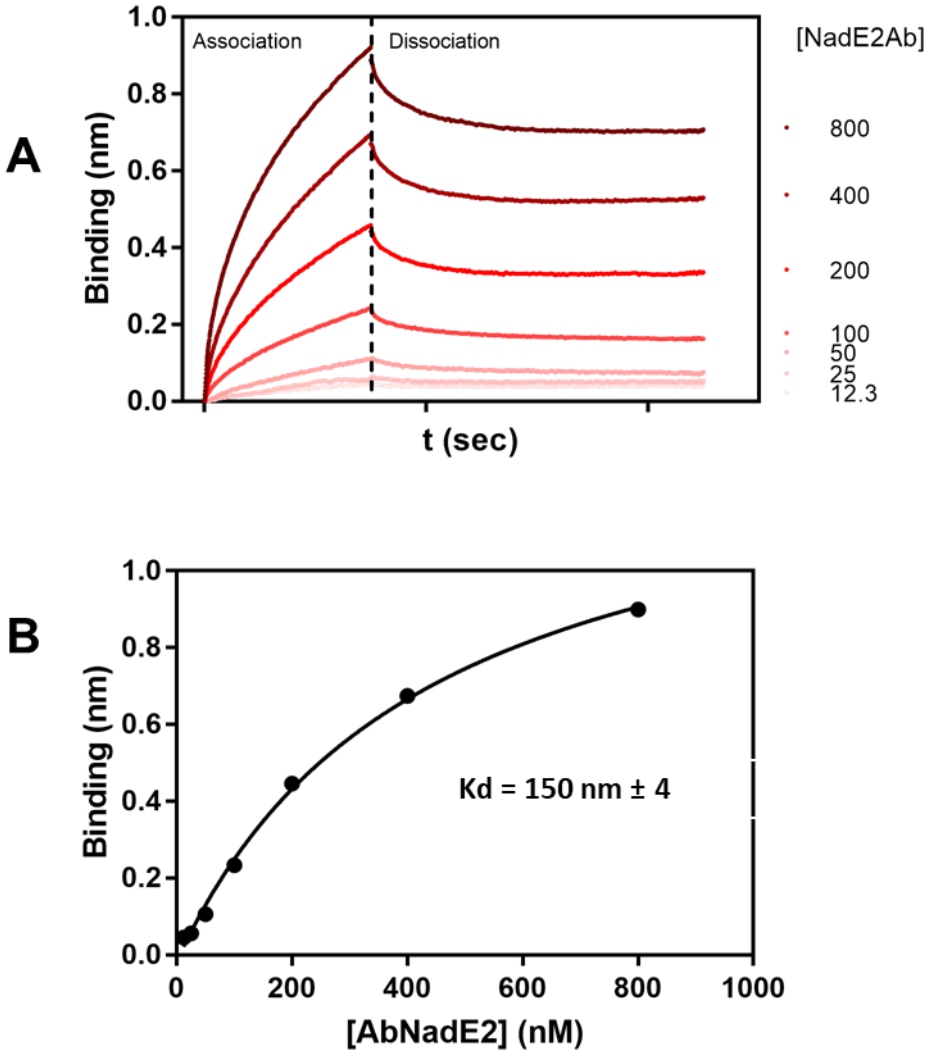


Figure S2. Determination of the dissociation constant of the His-GlnZ and AbNadE2 complex in the presence of ADP by Bio-layer Interferometry. A) The His-GlnZ was immobilized in the Ni-NTA sensor tip in a concentration of 2 µg/mL until saturation. The tip with His-GlnZ was then challenged in solution containing the indicated AbNadE2 concentrations in the presence of 1mM ADP. **B)** Plot reporting the Δλ spectral shift in nm vs AbNadE2 concentration. The binding was measured in an Octet K2 (Fortébio) and the Kd determined using the manufacturer´s software.

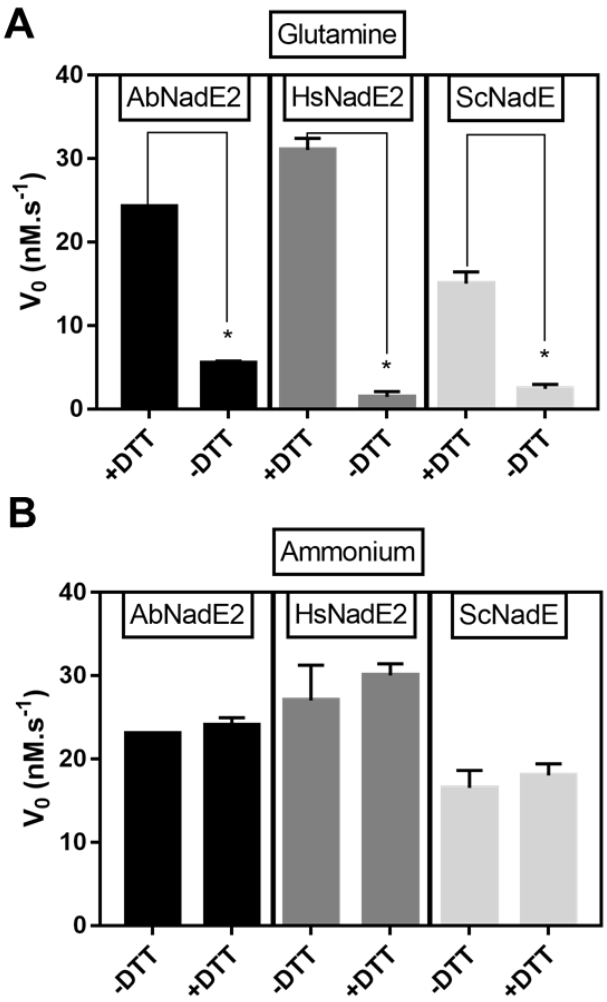


Figure S3. The NadE^{Gln} type 2 are activated in reductive environment when using L-glutamine as N-donor. NadE^{Gln} type 2 activity was continuously measured by determining NADH formation in assays coupled with alcohol dehydrogenase in the presence or absence of 10 mM Dithiothreitol (DTT) using 2 mM of L-glutamine as a N-donor (**A**) or 10 mM of ammonium as a N-donor **(B).** Proteins from *A. brasilense* (AbNadE2), *Herbaspirillum seropedicae* (HsNadE2) and *Synechocystis* sp. (ScNadE2) were assayed.

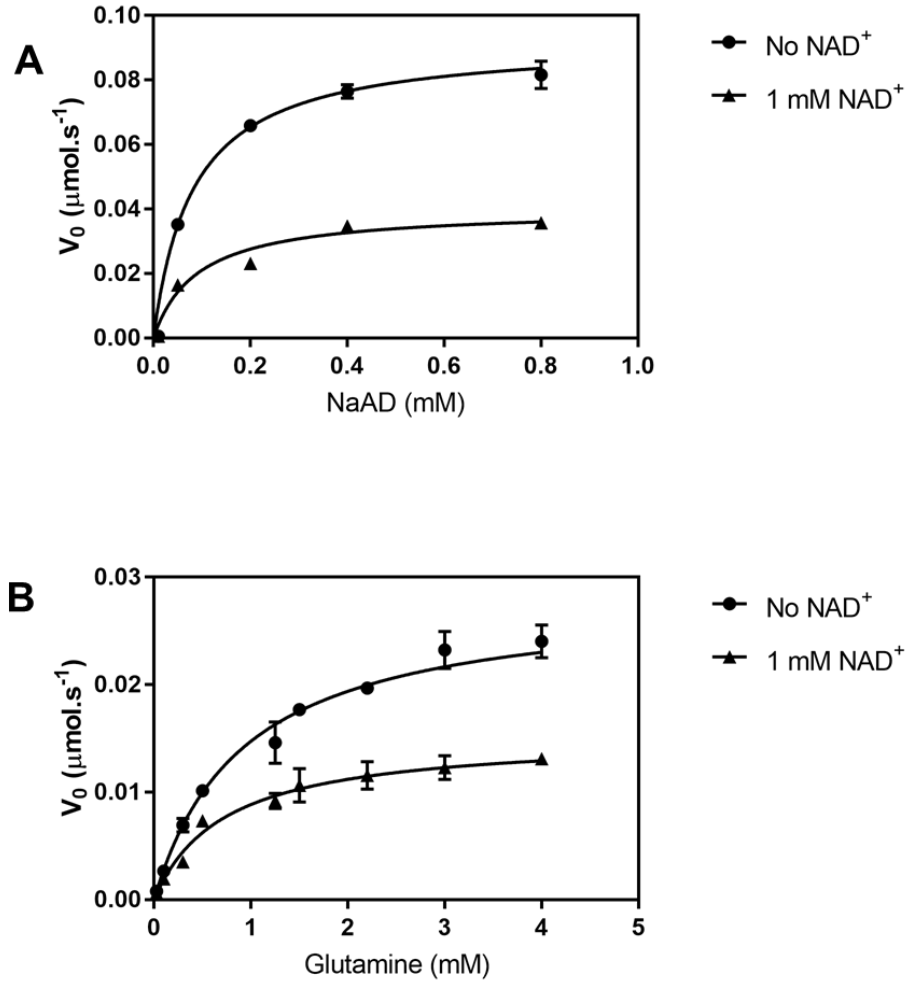


Figure S4. Effect of NAD^+ in the kinetic parameters of AbNadE2 reaction. A) Determination of initial velocities accordingly to varying NaAD concentrations in the presence and absence of 1mM NAD^+. A NaAD K_M of 0.08 mM and Vmax of 0.09 µmol.s^{-1} were obtained in the absence of NAD^+. In the presence of NAD^+ the values were K_M 0.09 mM and Vmax 0.04 µmol.s^{-1} . **B)** Determination of initial velocities accordingly to varying L-glutamine concentrations in the presence and absence of 1 mM NAD^+. A L-glutamine K_M of 0.72 mM and Vmax of 0.15 µmol.s^{-1} were obtained in the absence of NAD^+. In the presence of NAD^+ the values were K_M 0.92 mM and Vmax 0.028 µmol.s^{-1} .

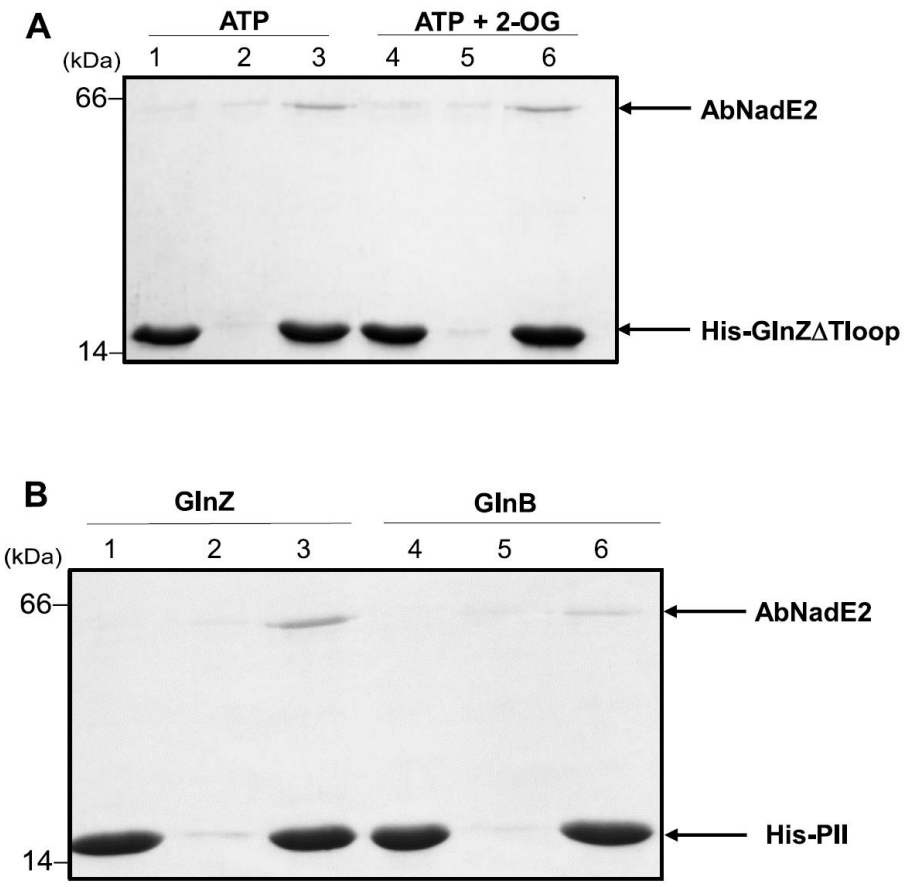


Figure S5. ***In vitro*** **formation of the NadE2-GlnZ complex does not require the GlnZ T loop and AbNadE2 preferentially interacts with the PII protein GlnZ**. **A)** Complex formation was assessed by pull-down using Ni^{2+} beads. Reactions were performed in the presence of the ATP and 2-OG at 1mM, as indicated. Binding reactions were conducted in 400 µl of buffer (50 mM Tris HCl; 100 mM NaCl; glycerol 10%; 20 mM Imidazole; 0,05% Tween 20; 5 mM $MgCl_2$). The fractions eluted from the Ni^{2+} beads were analyzed by SDS-PAGE and the gel was stained with Coomassie Blue. Lanes 1 and 4, HisGlnZ only. Lanes 2 and 5, AbNadE2 only. Lanes 3 and 6, mixture of HisGlnZ and AbNadE2. **B)** Complex formation was assessed by pull-down using Ni^{2+} beads. Reactions were performed under fixed concentration ATP 1 mM. Binding reactions were conducted in 400 µl of buffer (50 mM Tris HCl; 100 mM NaCl; glycerol 10%; 20 mM Imidazole; 0,05% Tween 20; 5 mM $MgCl_2$). The fractions eluted from the Ni^{2+} beads were analyzed by SDS-PAGE 15% and the gel was stained with Coomassie Blue. Lanes 1 and 4, His-PII only (GlnB or GlnZ as indicated). Lanes 2 and 5, AnNadE2 only. Lanes 3 and 6, mixture of His-PII and AbNadE2.

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