Supplementary material

Figure 1. The *Hv*NTR2 SigmaA weighted 2Fo-Fc electron density contoured at a 1σ level and covering the (A) B9-B10 loop and the (B) B14-B15 loop, respectively.

Figure 2. Sequence alignment of NTRs from different plants. The NTRs and their accession numbers in parentheses are HvNTR1 (EU314717), HvNTR2 (EU250021) and HvNTRC from Hordeum vulgare (barley), TaNTR1 (Q8VX47) and TaNTR2 (TC297680) from Triticum aestivum (wheat), OsNTR1 (Q69PS6), OsNTR2 (Q6ZFU6) and OsNTRC (Q70G58) from Oryza sativa (rice), ZmNTR1 (EU966898), ZmNTR2 (BT054285) and ZmNTRC (BT037345) from Zea mays (maize), AtNTRA (Q39242), AtNTRB (Q39243) and AtNTRC (O22229) from Arabidopsis thaliana (mouse-ear cress), PtNTRA (AC149479), PtNTRB (XM_002317595) and PtNTRC (XM 002308899) from Populus trichocarpa (western balsam poplar) and MtNTRA and MtNTRC from Medicago truncatula (Barrel Medic, legume). The sequences where aligned using ClustalW and divided into 5 groups. Group 1 and 2 are both monocotyledon subgroups of the A/B type, group 3 is the dicotyledons type A/B, and group 4 and 5 are the monocotyledon and dicotyledon subgroups of the C type, respectively. Residues strictly conserved have a red background, residues well conserved within a group according to the Risler matrix are indicated by red letters, residues conserved between groups are boxed and residues conserved within a group, but showing significant differences between groups, have an orange background. The secondary structure of HvNTR2 was added using ESPript (Gouet et al., 1999), and coloured according to domain; blue is the FAD domain, green the NADPH domain and pink are the β -sheets functioning as a linker between the two. Residues making hydrogen bond to FAD are indicated by triangles, residues assumed to make hydrogen bonds to NADPH by stars and the active site cysteines by cyan circles. The alignment is followed by the phylogentic tree produced by the same ClustalW analysis and illustrated in TreeView.



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Figure 2

1	Hv2																																																							
	Ta2																																																							
	Zm2																							• •										•															•							•
	Os2		•	• •				•									•					•	•	• •			•	•				•		•														•	•							•
2	Hv1		•					•									•					•					•	•						•															•							•
	Tal							•									•							• •			•	•				•		•		•													•							•
	Os1		•	• •				•				•					•					•	•	• •			•	•				•		•										•				•	•		•					•
	Zm1																•					•		• •			•	•																					•							•
3	AtA							•									•						M	CV	ΙI	S	M	S	2 S	R	F	I	Γŀ	(S	L	F :	SΊ	A	G	G I	FΙ	L	G	S	A I	LS	Ν	Ρ.	Ρŝ	SΙ	A	T.	ΑF	'S	SS	S
	AtB		•					•		•		•	•				•					•	•	• •			•	•				•		•		•				•				•					•		•	•				•
	PtA		•	• •			•	•		•		•	•			•	•	-		•	•	•	•	• •		•	•	•		•	•	•		•	•	•			•	•				•	•			•	•		•	•				•
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4	HvC		•	• •			•	•		•	•	•	•	. F		•	•	•			•	•	•	• •			•	•			•	•		•	•	•			•	•			•	•			•	A	A A	A P	ΑP	A	ΑC) A	VI	D
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	MtC		M	Αſ	C A	ιK	Ι	G]	Ŀ.	F	G	V.	A î	ΓI	Ρ	Ρ	ΤI	Ηl	ΝF	ΙR	Ι.	Τ	Τ.	As	SS	H	Ηl	RI	FΙ	F	I	N S	SF	R	S	L			•	•		•	•	•	•	. R	T	R	SS	S S	SS	L'	ΤI	۶R	AS	S
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		B1/B2	A1		B3 3-	10 3-10	
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1 Hv2	MEGSAAA	P LRTRVC I I	GSGPAAHTAA	IYAARAELKI	V L <mark>F E G</mark> W M A	NDIA <mark>AGGQL</mark> I	TTTDVEN
Ta2	MEGS.AAAA	PLRTRVCII	GSGPAAH TAA	I YA <mark>ARAE</mark> LKI	V L <mark>F E G</mark> W M A	NDIA <mark>AGGQL</mark> I	TTTDVEN
Zm2	MEGSAAA	PLRTRIC <mark>I</mark> I	GSGPAAH TAA	IYA <mark>ARAE</mark> LKI	VL FEG WMA	NDIA <mark>AGGQL</mark> I	TTTDVEN
Os2	MEGSAGA	PLRTRVCII	GSGP SAH TAA	IYA <mark>ARAE</mark> LKI	VL <mark>FEG</mark> WLA	NDIA <mark>AGGQL</mark> I	TTTDVEN
2 Hv1	MEEAAAG	PLRTRVCII	GSGPAAH <mark>TAA</mark>	V YA<mark>ARAE</mark>LK I	VL <mark>FEG</mark> WLA	NDIA <mark>AGGQL</mark> I	TTTDVEN
Ta1	MEEAAAG	PLHTRVCII	GSGPAAH <mark>TAA</mark>	V YA<mark>ARAE</mark>LK I	VL FEG WLA	NDIA <mark>A<mark>GGQL</mark>I</mark>	TTTDVEN
Os1	MEEAAAG	PLRARVCII	GSGPAAH <mark>TAA</mark>	V YA<mark>ARAE</mark>LK I	VL <mark>FEG</mark> FLA	NDIA <mark>AGGQL</mark> I	TTTDVEN
Zm1	MEEAVAAAG	PLRTRVCII	GSGPAAH TAA	VYA <mark>ARAE</mark> LKI	VL <mark>FEG</mark> WLA	NDIA <mark>AGGQL</mark> I	TTTDVEN
3 AtA	SSSSSAAAAVD <mark>M</mark> E	ТНКТКУС <mark>І</mark> У	GSGP AAH TAA	IYASRAELKI	PLL FEG WMA	NDIA <mark>PGGQL</mark> I	TTTDVEN
AtB	MNG L E	THNTRLC <mark>I</mark> V	GSGPAAH <mark>TAA</mark>	I YA <mark>ARAE</mark> LKI	PLL FEG WMA	NDIA <mark>PGGQL</mark> I	TTTDVEN
PtA	ME	ELKTRVCII	GSGPAAH <mark>TAA</mark>	IYASRAELKI	PIL <mark>FEG</mark> WMA	NDIA <mark>P<mark>GGQL</mark>I</mark>	TTTDVEN
PtB	ME	ELKTRVCII	GSGPAGH <mark>TAA</mark>	IYASRAELKI	PIL FEG WMA	NDIA <mark>P<mark>GGQL</mark>I</mark>	TTTDVEN
MtA	ASATAMTDTTTLP	TVKTKLC <mark>I</mark> I	GSGPAAH <mark>TAA</mark>	VYA <mark>ARAE</mark> LKI	PIL FEG WMA	NDIA <mark>P<mark>GGQL</mark>I</mark>	TTTDVEN
4 HvC	EDAPASPPPSDPG	RGVENLV <mark>I</mark> I	GSGPAGY <mark>TAA</mark>	I YA<mark>ARA</mark>NLKI	V V F E G Y Q V	GGVP. <mark>GGQL</mark> N	4 TTTEVEN
OsC	EEAPASPPPSDLG	KGVENLV <mark>I</mark> I	GSGPAGY <mark>TAA</mark>	IYA <mark>ARA</mark> NLKI	V V F E G Y Q V	GGVP. <mark>GGQL</mark> N	4 T T T E VEN
ZmC	EETPASSPPSDAS	KGVENLV <mark>I</mark> I	GSGPAGY TAA	IYA <mark>ARA</mark> NLKI	VVFEG CQM	GGVP <mark>.</mark> GGQLN	4 TTTEVEN
MtC	SDTSSSSVASPG.	NAVENVVII	GSGPAGY <mark>TAA</mark>	IYA <mark>ARA</mark> NLKI	VV FEG YQM	GGVP. <mark>GGQL</mark> N	4 TTTEVEN
PtC	LFPAG.	KGIENVV <mark>I</mark> I	GSGPAGYTAA	IYA <mark>ARA</mark> NLKI	V V F E G Y Q V	GGVP. <mark>GGQL</mark> N	4TTTEVEN
AtC	ATANSPSSSSSGG	EIIENVVII	GSGPAGYTAA	IYAARANLKI	VVFEGYQM	GGVP. <mark>GGQL</mark> N	4TTTEVEN
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1	Hv2	FPGFI	PΤC	ΙM	ΙGΙ	DΙ	MI	DNC	RA	Q	SV	RF	G	ΤN	II:	LS	E	ΤV	ΤI	ΕV	DI	r s	ΑF	PI	F R	V	ſS	DS	T 1	ΓV	LAI	Σ	VV	VA	ΤG	AV	A	RR
	Ta2	FPGFI	ΡTΟ	ΙM	ΙGΙ	DΙ	MI	DNC	RA	Q	SΑ	RΕ	G	ΤN	II	LΣ	δE	ТΫ	7 T I	ΕV	DI	s S	ΑF	PI	R	V	[S	DS	ΤC	ΓV	LAI	Ъ	VV	VA	ΤG	AV	AI	RR
	Zm2	FPGFI	NG	ΙM	I <mark>G</mark> A	DI	S M I	D N C	RA	Q	SL	RΕ	G	ΤN	II	LΣ	δĒ	ΤV	ΤJ	ΑV	DI	s S	AC	PI	R	V	5 A	DS	T I	ΓV	LAI	D A	VI	VA	ΤG	AV	A]	RR
	Os2	FPGFI	ΡEΟ	ΙL	GG	ΕI	L M I	DRC	RA	Q	SΓ	RΕ	G	ΤS	I	ΙS	δĒ	ΤV	Τi	ΑV	DI	s S	ΑF	PI	R	Vi	ΑS	DS	T I	ΓV	LAI	D A	VV	VA	ΤG	AV	A I	RR
2	Hv1	FPGFI	P D C	ΙL	GI	DΙ	MI	DRC	RA	Q	SV	RΕ	G	ΤK	II	F S	Ξ	ТΝ	T :	s v	DI	s S	SF	PI	R	V	ΑS	DD	ΤV	7 V	ΗAΙ) S	VV	VA	ΤG	AV	A	RR
	Ta1	FPGFI	P D C	ΙL	GI	DΙ	ΜI	DRC	RA	Q	SV	RF	G	ΤK	II	F S	ΞĒ	ТΫ	T :	s v	DI	7 S	SF	PI	R	V	5 S	DD	ΤV	7 V	ΗAΙ) S	VV	VA	ΤG	AV	AI	RR
	Os1	FPGFI	P D C	ΙL	GΑ	DI	MI	DRC	RA	Q	SV	RΕ	G	ΤF	ιI.	LΊ	Ē	ΤV	ΤI	ΑV	DI	ΓS	SF	PI	R	V	ΑS	GD	ΤV	7 V	ΗAΙ	D A	VV	VA	ΤG	AV	A]	RR
	Zm1	FPGFI	P D C	ΙL	GA	D I	L M I	DRC	RA	Q	SΓ	RΕ	G	ΤK	II	LΊ	Ē	ΤV	7 T (ΓV	DI	s S	ΑF	PI	R	V	ΑS	DD	ΤV	7 V	ΗAΙ) S	VV	VA	ΤG	A V	A]	RR
з	AtA	FPGFI	? E 6	ΙL	GI	DI	V	ΕKΕ	RK	Q	SΕ	RΕ	G	ТΊ	I	FΊ	Ē	ΤV	7N I	ΚV	DI	r s	SK	(PI	ΓK	L	Т	DS	R'	ΓV	LAI) S	VI	ΙS	ΤG	AV	A	ΚR
	AtB	FPGFI	E C	ΙL	G٧	ΈI	ΤI	ΟKΕ	RK	Q	SΕ	RΕ	G	ТΊ	I	FΊ	Έ	ΤV	7 T I	ΚV	DI	s S	SK	(PI	ΓK	LI	Т	DS	ΚI	ΑI	LAI) A	VI	LΑ	ΤG	AV	A١	ΚR
	PtA	FPGFI	? E C	ΙM	(<mark>G</mark> G	DI	TI	ΕKΕ	RA	Q	SA	RΕ	G	ΤÇ	2 I I	FΊ	È	ТΝ	7 T 1	ΚV	ΝI	? S	ΚΊ	PI	E	VI	Т	DS	ΚI	RV	VAI) S	VI	VS	ΤG	AV	ΑI	ΚK
	PtB	FPGFI	? E G	ΙM	I G V	ΈI	TI	ΕKΕ	RA	Q	SΑ	RΕ	G	ΤÇ	ΣI	LΊ	È	ΤV	7 T 1	ΚV	DI	s S	RΊ	PI	ΞE	VI	Т	DS	ΚI	RV	VAI) S	VI	VA	ΤG	ΑA	ΑI	ΚK
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4	HvC	FPGFI	P D C	IΤ	GΡ	DΙ	MI	ΟKΝ	1 R K	Q	ΑE	RW	G	ΑE	L	ΗÇ	$2\mathbf{E}$	DV	7E]	ΓV	D	7 K	SF	PI	F V	II	RΒ	SD	RI	ΞV	KCI	ΙS	VI	ΙA	ΤG	ΑT	A I	ΚR
	OsC	FPGFI	P D C	VΤ	GΡ	DI	MI	DKΝ	1 R K	Qi	ΑE	RW	G	ΑE	L	ΗÇ	$2\mathbf{E}$	DV	7E 1	ΓV	N	7 K	SF	PI	ΓV	ΊI	RΒ	SD	RI	ΞV	KCI	ΗS	VI	ΙA	ΤG	AA	Αł	ΚR
	ZmC	FPGFI	P D C	ΙT	GΡ	DΙ	ΜI	DRN	1 R K	Q	ΑE	RW	G	ΑE	Ľ	ΥÇ	\mathbf{E}	DV	7E]	ΓV	N	7 K	SS	PI	τV	ΙI	RΒ	SD	RI	ΞV	KCI	ΗS	LΙ	ΙA	ΤG	ΑT	ΑI	ΚR
	MtC	FPGFI	P D C	ΙT	GΡ	DI	S M I	DR	1 R R	Q	ΑE	RW	G	ΑE	L	ΗE	E	DV	ΤE I	ΑI	D	7 K	ΤS	PI	Τ	V) S	SE	RI	ΚV	ΚSΙ	ΗT	VΙ	ΙA	ΤG	ΑT	ΑI	ΚR
	PtC	FPGFI	? D C	IΤ	GΡ	DΙ	MI	DRN	1 <mark>R</mark> R	Qi	ΑE	RW	G	ΑE	L	FÇ	$2\mathbf{E}$	DV	ΖĒ.	SΙ	D	7 K	SS	PI	ΓT	VI	٢S	<mark>S</mark> E	RI	ΚV	KCI	ΗS	ΙΙ	ΥA	ΤG	ΑT	A I	RR
	AtC	FPGFI	P D C	ΙT	GΡ	DΙ	M I	ΕKΝ	1 R K	Q	ΑE	RW	G	ΑE	L	ΥĒ	Е	DV	ΤE	SΙ	S	ΖT	ΤA	PI	Τ	V	2 T	SE	RI	ΚV	KCI	ΗS	ΙI	ΥA	ΤG	ΑT	A	RR

		B10	A3 3-10	B11	A4	B12
	130	140 🗕 🖉	150 200	160	170	180 190
1 Hv2	LYFSGSDT	YWNRGISACA	VCDGAAPIFR	NKPIAVIGG	GDSAMEEGNFLI	KYGSQVYIIHRRNT
Zm2	LHFPGSDA	.YWNRGISACA	VCDGAAPIFR VCDGAAPIFR	NKPIAVIGG	GD SAMEEGNEL GD S <mark>AMEE SNEL</mark>	KIGSQVIIIHK RNI KY GSH <mark>V</mark> YIIHRRNT
0s2 2 Hv1	LHFAGSDA	.YWNRGISACA	V <mark>CDGA</mark> APIFR V CDGA APIFR	NKPIAVIGG NKPIAVVGG	GDS <mark>A</mark> MEESNFL] GDSAMEEANFL]	KYGSHVYIIHRRNT KYGSRVYIIHRRDA
Tal	LHFAGSDA	FWNRGITACA	V CDGA A P IFR	NKPIA <mark>V</mark> VGG	GDS <mark>A</mark> MEEANFL7	KY GSR V YIIH R RDA
Osl Zml	LHFAGSDA	.FWNRGISACA	VCDGAAPIFR VCDGA <mark>AP</mark> IFR	NKPIAVVGG NKPIA <mark>V</mark> VGG	GDSAMEEANFLI GDS <mark>A</mark> MEEANFLI	KYGSRVYIIHRRNA KYGSQVYIIHRRSD
3 AtA	LSFTGSGEGNG	GFWNRGISACA	VCDGAAPIFR	NKPLVVIGG	GDS <mark>A</mark> MEEANFL] CDSAMEEANFL]	KYGSKVYIIHRRDT
PtA	LSFAGSET	.FWNRGISACA	V CDGA APIFR	EKPLAVIGG	GDS <mark>A</mark> MEEANFLI	KY GSK V YIIH R RDT
PtB MtA	LNFAGSEK LPFTGSGDGPN	.FWNKGISACA GYWNRGISACA	VCDGAAPIFR VCDGAAPIFR	EKALAVIGG. NKPLAVIGG	G D S A M E E A N F L 1 G D S A M E E A T F L 1	KYGTKVYIIHRRDT KYGSEVYIIHRRDT
4 HvC		.FWSRGISACA	ICDGASPLYK	GQVLAVVGG	GDT <mark>ATEE</mark> AIYLI CDTATEEAIYLI	KYACHVHLLVRRDQ
ZmC	LRLPREDE	.FWSRGISACA	ICDGASPLFK ICDGASPLFK	GQVLA <mark>V</mark> VGG	GDVATEEAVYLI	KIARHVHLLVRKDQ
MtC PtC		.FWSRGISACA	ICDGASPLFK ICDGASPLFK	GQILAVVGG ROVLAVVGG	GDTATEEALY <mark>L</mark> GDTATEEALYL	KYARHVHLLVRRDQ
Ato	LRLPREEE	FWSRGISACA	I CDGA<mark>SP</mark>LFK	GOVLAVVGG	GDTATEEALYLI	KYAR H V HLLV R RDQ
		•	•		*	*





<i>Ec</i> NTR	HvNTR2	AtNTR-B	Interaction type
Thr156	Ser168	Ser170	Two hydrogen bonds
Arg176	Arg188	Arg190	Two hydrogen bonds
Arg181	Arg193	Arg195	Hydrogen bond
Ile243	Ile253	Ile255	Hydrogen bond
Arg293	Arg300	Arg302	Hydrogen bond
Leu119	Leu129	Leu127	van der Waals
Ile151	Ile163	Ile165	van der Waals
Gly154	Gly166	Gly168	van der Waals
Glu159	Glu171	Glu173	van der Waals
His175	His187	His189	van der Waals
Arg177	Arg189	Arg191	van der Waals
Glu183	Ser195	Ser197	van der Waals
Gly244	Gly254	Gly256	van der Waals
His245	His255	His257	van der Waals
Asn260	Asp271	Asp273	van der Waals
Tyr262	<i>Tyr273</i>	<i>Tyr</i> 275	van der Waals
His290	Lys297	Lys299	van der Waals

Table 1Amino acid residues involved in NADP+ binding to *Ec*NTR in the FO conformation (pdbaccession: 1tdf) and the corresponding residues of *Hv*NTR2 and *At*NTR-B identified by superpositionof the three structures (Wallace *et al.*, 1995).

Hydrogen bond cut-off < 3.35Å and hydrophobic interaction cut-off < 3.90 Å. Residues in italics belong to the FAD domain.