Supplemental Data

Fig. S1: **Gel filtration chromatogram.**
Absorbance was detected at three characteristic wavelengths: 620 nm for phycocyanin, 280 nm for peptide and 450 nm for FAD. Three fractions named F1, F2 and F3 were recovered. The first and major fraction F1 containing FNR_L-PC was used for oxidase and reductase activities. The fractions F2 and F3 are of lower molecular weight and were analyzed together with F1 *via* SDS-PAGE. Inset: the expanded region of the chromatogram clearly illustrates that the minor fractions F2 and F3 contain primarily FAD and PC, respectively.

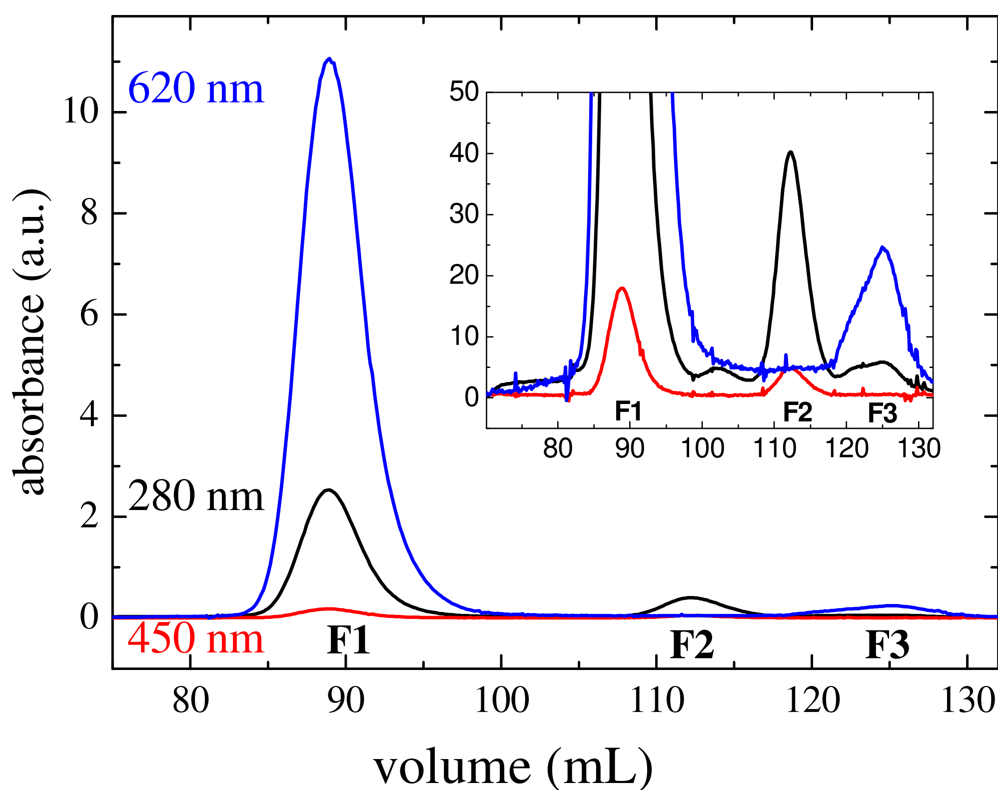
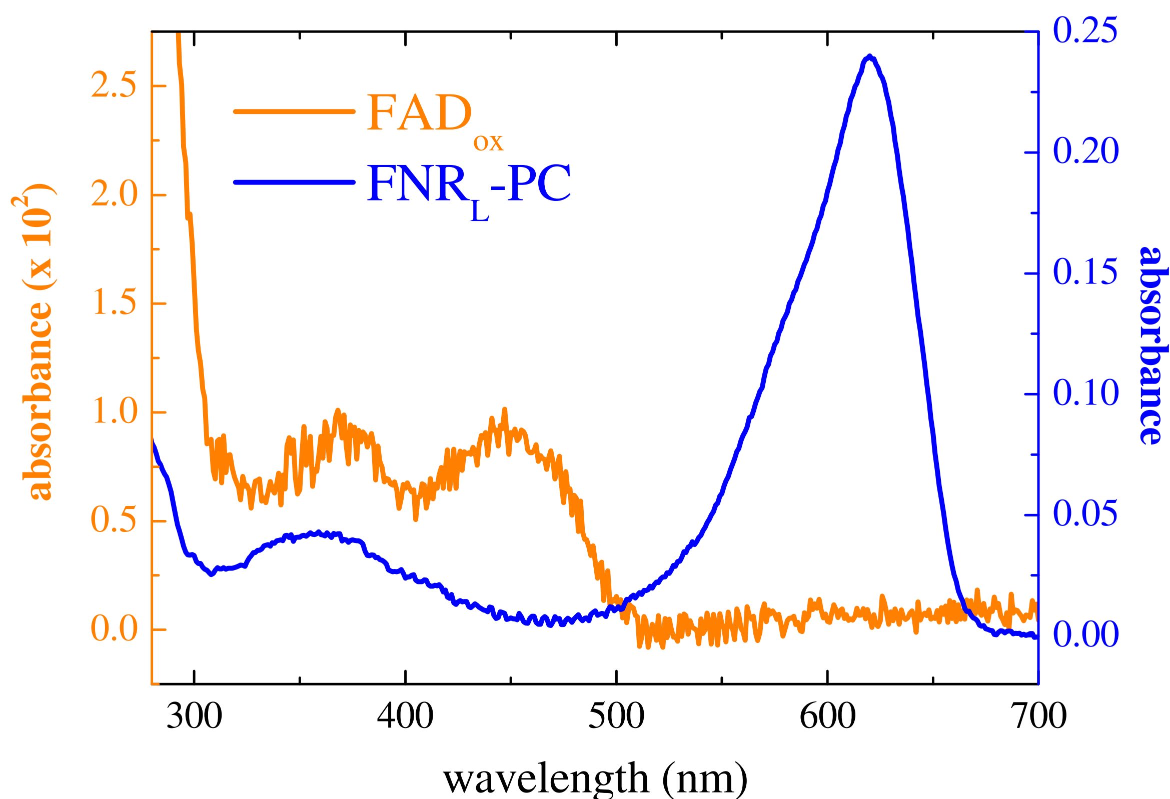


Fig. S2: **FAD release from a FNR_L-PC complex.**

The blue trace (right y-scale) shows the characteristic absorption of an FNR_L-PC sample diluted to a concentration of 0.1 µM ($\varepsilon_{620\ nm}$ = 2.37 $\mu M^{-1}cm^{-1}$). The FAD cofactor was extracted by TCA from the initial sample (orange trace, left y-scale). FAD concentration was calculated to be 0.81 µM ($\varepsilon_{450\ nm}$ = 11,300 $M^{-1}cm^{-1}$). Pathlength: 1 cm. This procedure was applied on three different samples, leading to an occupancy of the FAD cofactor from 92 to 100 % in FNR_L-PC.



Measurement of the molar extinction coefficients of FNR_S and FNR_L from *Synechocystis* at 461 nm.

The concentration of an FNR_S sample was first estimated using an extinction coefficient of 10,500 $M^{-1}cm^{-1}$ at 461 nm (1, 2), leading to a value of 45.6 µM. The protein from a precise volume of this sample was precipitated by the addition of TCA (5% w/v final concentration) and the pellet was washed with 5% TCA. The pooled supernatants were extracted three times with diethyloxide to remove all traces of TCA and adjusted to 0.1 M Na phosphate pH 7.0 with a concentrated buffer (3). As expected, the absorption maximum of the extracted FAD was observed at 450 nm, and a concentration of 52.8 µM was calculated for the initial solution (instead of 45.6 µM) on the basis of a molar extinction of 11,300 cm^{-1} for free FAD (4). This allowed us to recalculate an extinction coefficient of 9,070 $M^{-1}cm^{-1}$ for FNR_S at 461 nm.

This coefficient was also measured using the complete release of FAD from FNR_S by SDS (5). A small volume of concentrated SDS was directly added (final concentration of 0.2%) in the spectrophotometer cuvette containing FNR_S, allowing the direct comparison of native (absorption maximum at 461 nm) and denatured FNR_S (absorption maximum of 450 nm with a coefficient of 11,300 $M^{-1}cm^{-1}$). This gave an FNR_S extinction coefficient of 9,000 ± 100 $M^{-1}cm^{-1}$ (six different measurements on six different FNR_S preparations). The same coefficient was found for recombinant FNR_L. Therefore the two different methods give identical results and the much more convenient SDS method validates the more complex TCA extraction method.

In the case of the FNR_L-PC complex, the huge absorption of phycocyanin indeed excluded both a direct measurement of the native FAD at 461 nm or of the SDS released form at 450 nm. The TCA precipitation procedure, which eliminates the phycocyanin contribution, was consequently used to estimate the FAD content of the complex (see Fig. S2)

1. Forti, G. (1966) *Brookhaven Symp. Biol.* **19,** 195-201
2. Foust, G. P., Mayhew, S. G., and Massey, V. (1969) *J. Biol. Chem.* **244,** 964-970
3. Engel, P. C. and Massey, V. (1971) *Biochem. J.* **125,** 879-887
4. Whitby, L. G. (1953) *Biochem. J.* **54,** 437-442
5. Aliverti, A., Curti, B., and Vanoni, M. A. (1999) *Methods Mol. Biol.* **131,** 9-23

Quantification of FNR apoproteins.

Two samples of recombinant FNR_S and FNR_L were calibrated on the basis of the FAD concentration ($\varepsilon_{461\ nm}$ = 9,000 $M^{-1}cm^{-1}$) and analysed for their protein content using the micro-BCA protein assay (Pierce Biotechnology). The protein amounts were found to be smaller than expected (92% and 91% of the calculated values for FNR_S and FNR_L, respectively), which must be ascribed to some underestimation by the micro-BCA assay (assuming an exact measurement would lead to a molar ratio of FAD to protein larger than 1). From these measurements, we conclude that there is no FAD free apoprotein in our FNR samples. 461/275 nm absorbance ratios of 0.128 and 0.122 were measured for recombinant FNR_S and FNR_L, respectively.

Table: **Comparison of the NADPH oxidase catalytic properties of the plant leaf and root FNR isoforms and of the cyanobacterial FNR_L-PC and FNR_S isoforms**.

Catalytic activity	leaf FNR	*vs*	root FNR	(Ref.)	FNR_L-PC	*vs*	FNR_S
Ferricyanide reduction							
K_m(NADPH) (µM)		**3-10 fold larger**				**30% smaller**	
	4.4	*vs*	0.39	(1)	40	*vs*	55
	35	*vs*	12	(2)			
k_{cat} (s^{-1})		**larger or similar**				**30% smaller**	
	44	*vs*	28	(1)	124	*vs*	174 s^{-1}
		500-520		(2)			
catalytic efficiency (k_{cat}/K_m) ($\mu M^{-1}s^{-1}$)		**3-7 fold smaller**				**similar**	
	10	*vs*	70	(1)		3.1-3.2	
	14	*vs*	43	(2)			
Fd mediated cyt *c* reduction							
K_m(leaf Fd) (µM)		**5-10 fold smaller**				**70% larger**[b]	
	3.3	*vs*	29	(1)	47	*vs*	28
	4.1	*vs*	43	(3)			
	2.8-4.0	*vs*	18-19	(4)			
	5.8	*vs*	26.7	(5)			
K_m(root Fd) (µM)		**similar**					
		3.0-3.4		(1)			
		4.1-4.7		(3)			
		4.7-4.9		(4)			
k_{cat} (s^{-1})		**3-4 fold smaller**[a]				**similar**[b]	
	120-130	*vs*	380	(1)		144-154	
	58-63	*vs*	207-212	(3)			
	60-80	*vs*	200-230	(4)			
	76-97	*vs*	210-230	(5)			

[a] Similar for leaf and root Fd.
[b] Measured with the main cyanobacterial Fd isoform.

1. Onda, Y., Matsumura, T., Kimata-Ariga, Y., Sakakibara, H., Sugiyama, T., and Hase, T. (2000) *Plant Physiol.* **123,** 1037-1045
2. Aliverti, A., Pandini, V., and Zanetti, G. (2004) *Biochim. Biophys. Acta* **1696,** 93-101
3. Hanke, G. T., Kurisu, G., Kusunoki, M., and Hase, T. (2004) *Photosynth. Res.* **81,** 317-327
4. Hanke, G. T., Kimata-Ariga, Y., Taniguchi, I., and Hase, T. (2004) *Plant Physiol.* **134,** 255-264
5. Gou, P., Hanke, G. T., Kimata-Ariga, Y., Standley, D. M., Kubo, A., Taniguchi, I., Nakamura, H., and Hase, T. (2006) *Biochemistry* **45,** 14389-14396