Supplementary information

Biohybrid Photosynthetic Charge Accumulation as Detected by Flavin Semiquinone Formation in Ferredoxin-NADP$^+$ Reductase

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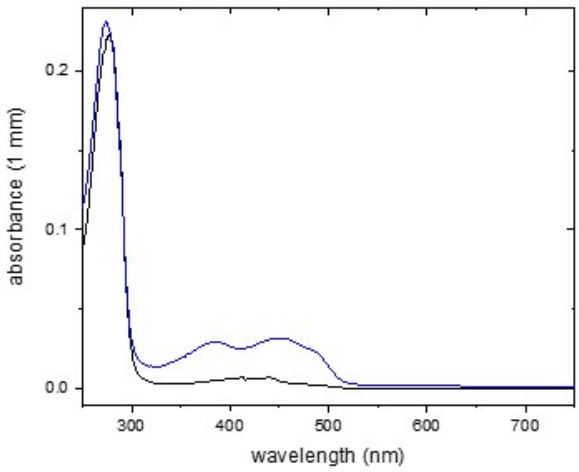

Figure S1: Absorption spectrum of (black) purified FNR, A_{280}/A_{456} = 54; and (blue) purified FNR reconstituted with ^{2}H-FMN, A_{280}/A_{456} = 6.9.

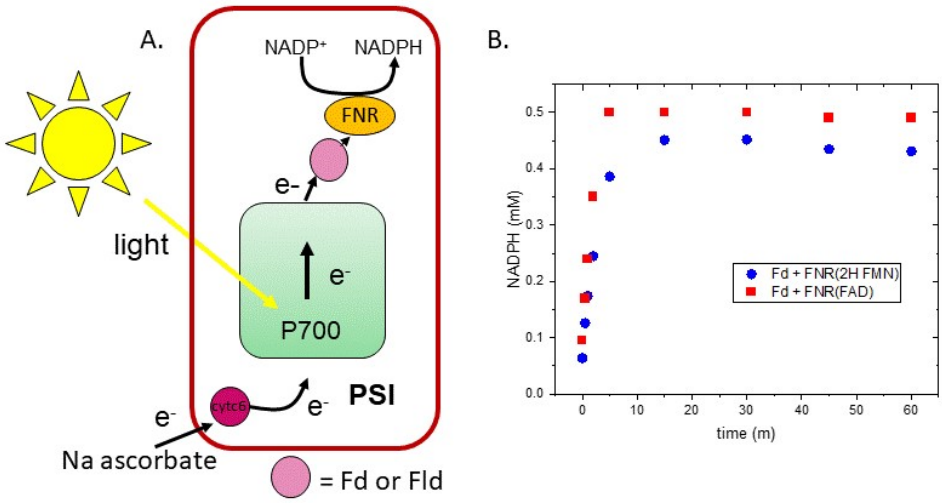

Figure S2: PSI-driven $NADP^+$ Reduction. A. Reaction scheme for the PSI-driven *in vitro* reduction of $NADP^+$. B. Time course of NADPH formation as monitored at 340 nm. The reaction conditions were: 20 mM Hepes pH 7.29, 3 mM $MgCl_2$, 2 mM sodium ascorbate, 0.03% n-dodecyl β-D-maltopyranoside, 10 µM cytochrome c_6, 0.5 mM $NADP^+$, 4 µM ferredoxin and 60 nM PSI. The reactions were differentiated with 0.5 µM FNR reconstituted with ^{2}H-FMN (blue circle), and 0.5 µM FNR reconstituted with FAD (red square). Rates (@ 5 min) 77,000 mol NADPH (mol PSI^{-1}) h^{-1} for FNR reconstituted with FMN and 100,400 mol NADPH (mol PSI^{-1}) h^{-1} for FNR reconstituted with FAD.

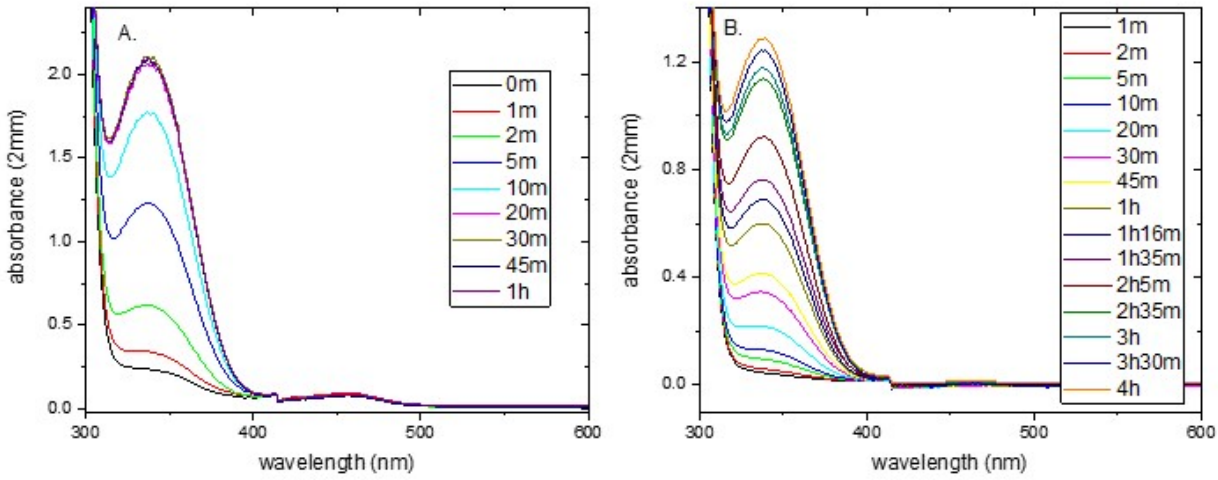

Figure S3: Time course absorption spectra of RuFld and RuFd driven $NADP^+$ reduction. The experimental conditions were: 20 mM Hepes pH 7.97, 100 mM sodium ascorbate, 2 mM $NADP^+$, 0.5 μM FNR and 4.8 μM (A) RuFld or (B) RuFd. The samples were placed in 2 mM cuvettes and illuminated with 455 nm LED (950 mA). The samples were removed from the light at specified times for UV-Vis spectral measurements. Rates of NADPH formation (@ 20 min): (A) 8500 mol NADPH (mol FNR)$^{-1}h^{-1}$ (B) 1050 mol NADPH (mol FNR)$^{-1}h^{-1}$.

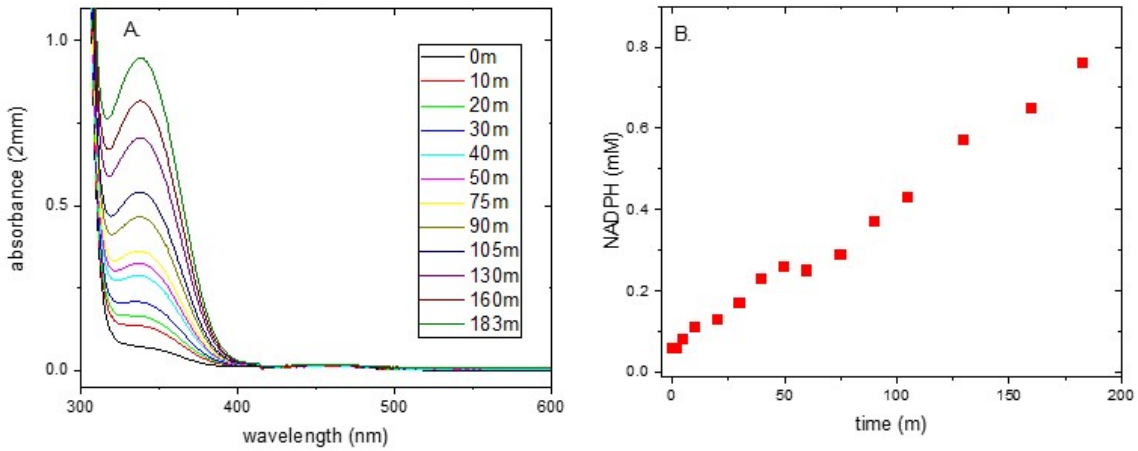

Figure S4: Time course absorption spectra of Ru-apoFld $NADP^+$ reduction. (A) Full UV-Vis spectrum taken at different times. (B) Plot of NADPH formed over time as determined by absorbance at 340 nm. The experimental conditions were: 20 mM Hepes pH 7.97, 100 mM sodium ascorbate, 2 mM $NADP^+$, 0.5 µM FNR and 4.8 µM Ru-apoFd. The sample was placed in 2 mM cuvette and illuminated with 455 nm LED (950 mA). The sample was removed from the light at specified times for UV-Vis spectral measurements. Rate of NADPH formation (@ 50 min): 630 mol NADPH $(mol\ FNR)^{-1}\ h^{-1}$.

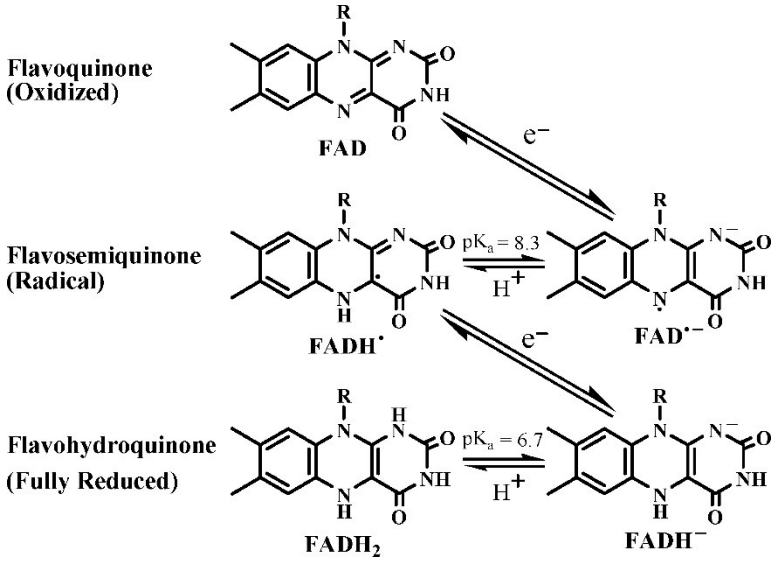

Figure S5: The three oxidation states of the flavin adenine dinucleotide (FAD). Figure taken from NIH Public Access.

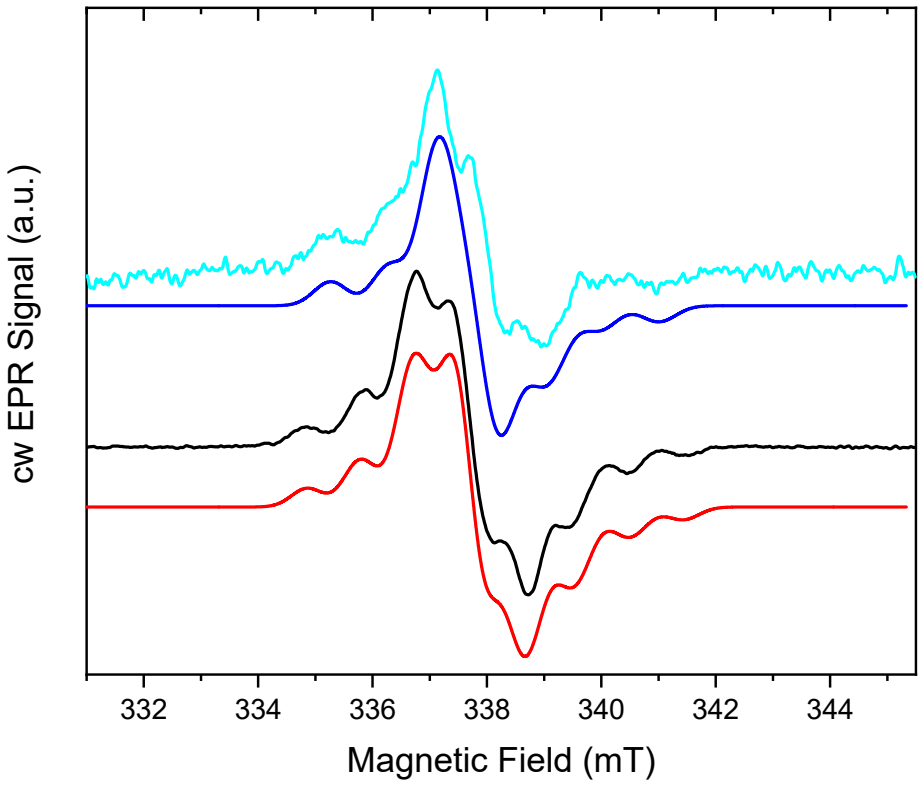

Figure S6: cw X-band EPR spectra of RuFd + FNR(^{2}H-FMN) in H_2O buffer (black) and in D_2O buffer (cyan) and the respective simulations (red, blue). The sample contained 700 µM RuFd, 325 µM FNR(^{2}H-FMN), 400 µM $NADP^+$, 0.1 mM sodium ascorbate, 140 mM NaCl, 20 mM Hepes, pH 7.97 and was exchanged into the D_2O buffer using 3000 MWCO centrifuge device (Amicon Ultra). Simulation parameters are summarized in Table S1. Note, that the spectral region around 338 mT in the D_2O buffer sample (cyan) is somewhat distorted due to the subtraction of the RuFd signal.

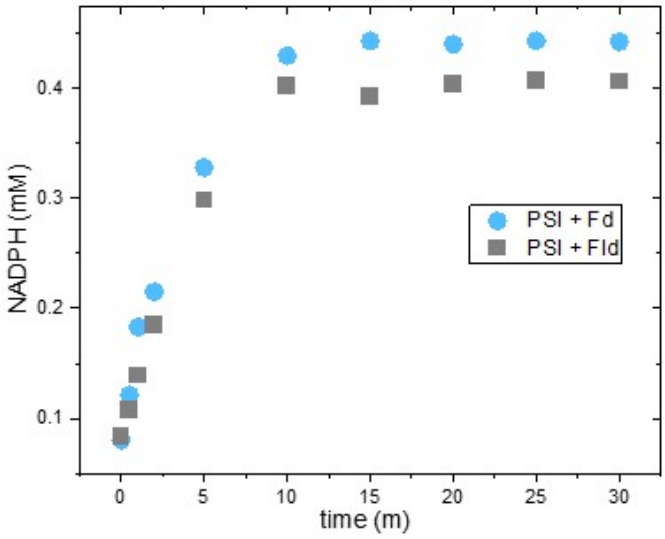

Figure S7: PSI-driven $NADP^+$ reduction comparing Fd and Fld as the shuttle proteins between PSI and FNR. Time course of NADPH formation as monitored at 340 nm. The reaction conditions were: 20 mM Hepes pH 7.29, 3 mM $MgCl_2$, 2 mM sodium ascorbate, 0.03% n-dodecyl β-D-maltopyranoside, 10 µM cytochrome c_6, 0.5 mM $NADP^+$, and 60 nM PSI. The reactions were differentiated with 4 µM Fd (blue circle) or 4 µM Fld (grey square). Rates (@ 5 min) 92,500 mol NADPH (mol PSI^{-1}) h^{-1} for assay using Fld and 100,400 mol NADPH (mol PSI^{-1}) h^{-1} for assay using Fd.

Table S1: Simulation parameters (g-tensor, ^{1}H and ^{14}N hyperfine tensors) for the EPR spectra of the flavin radicals in deuterated RuFld biohybrid ^{2}H-Fld in H_2O buffer (black, Figure 8A), and FNR(^{2}H-FMN) in H_2O buffer (black, Figure S6) and in D_2O buffer (cyan, Figure S6). Non-exchangeable protons of the isoalloxazine moiety are not listed.

Neutral Flavin Semiquinone			
	^{2}H-Fld (this work)	FNR(^{2}H-FMN) in H_2O (this work)	FNR(^{2}H-FMN) in D_2O (this work)
g-tensor	2.0044, 2.0036, 2.0021	2.0044, 2.0036, 2.0021	2.0044, 2.0036, 2.0021
A(^{14}N(5))/MHz [a]	0, 0, 50.5	0, 0, 51	0, 0, 51
A(^{14}N(10))/MHz [a]	0, 0, 25	0, 0, 29	0, 0, 29
A(^{1}H(5))/MHz [a]	5, 44, 25	3, 31, 23	(3, 31, 23)/6.5 for ^{2}H

[a] The sign of the hyperfine coupling constants cannot be determined from the EPR spectra.

Table S2: Simulation parameters (g-tensor, ^{1}H and ^{14}N hyperfine tensors) for the EPR/ENDOR spectra of neutral protein bound flavin radicals and anionic protein bound flavin radicals. Non-exchangeable protons of the isoalloxazine moiety are not listed. All examples are taken from Okafuji et al., *J. Phys. Chem. B*, 112, 3568- 3574 (**2008**) and references cited therein (for Table 1).

	Neutral Flavin Semiquinone			Anionic Flavin Semiquinone	
	A. niger glucose oxidase pH 5	*E. coli* DNA photolyase	*Na^+-NQR*	*A. niger* glucose oxidase pH 10	*Na^+-NQR*
g-tensor	2.0043, 2.0036, 2.0021	2.00431, 2.00360, 2.00217	2.00425, 2.00360, 2.00227	2.00429, 2.00389, 2.00216	2.00436, 2.00402, 2.00228
A(^{14}N(5))/MHz [a]	0, 0, 53	0, 0, 50.1	0.2, 0.2, 52.2	0, 0, 53	2.3, 2.3, 57.6
A(^{14}N(10))/MHz [a]	0, 0, 30	0, 0, 31.7	2.0, 2.0, 28.9	0, 0, 25	1.6, 1.6, 28.9
A(^{1}H(5))/MHz [a]	10, 33.9, 24.1	8.5, 37.0, 24.9	0.2, 38.6, 25.8	-	-

[a] The sign of the hyperfine coupling constants cannot be determined from the EPR spectra.