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Supporting information for article:

Unravelling the shape and structural assembly of photosynthetic GAPDH–CP12–PRK complex from *Arabidopsis thaliana* by small-angle X-ray scattering analysis

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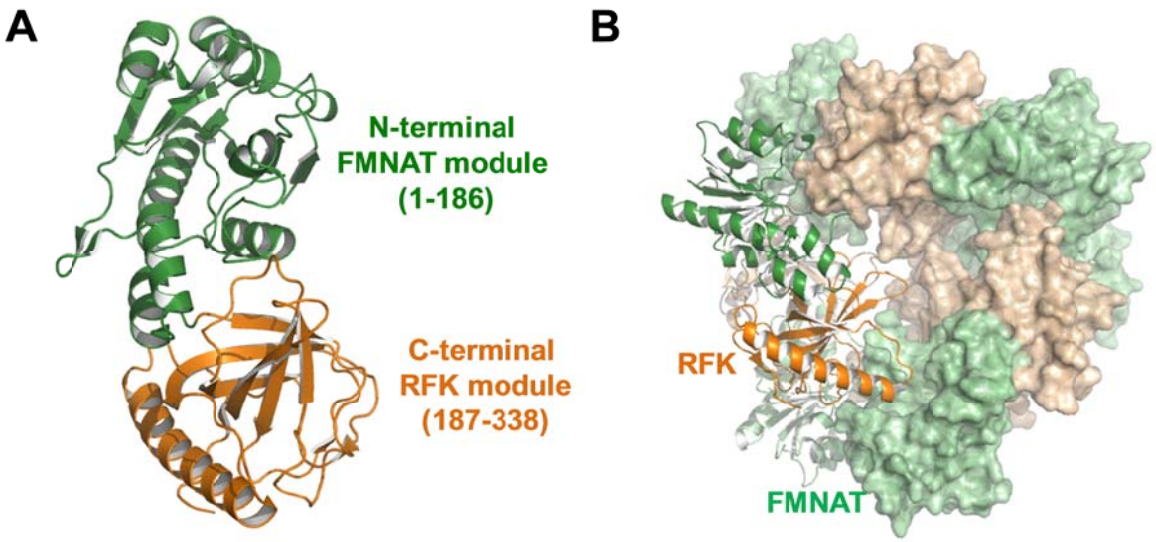

Figure S1. (A) Cartoon representation of the *Ca*FADS monomer (PDB 2X0K), with the FMNAT module coloured in green and the RFK module in orange. (B) Representation of the *Ca*FADS dimer of trimers showing the head-tail arrangement and the interactions between active sites of different protomers within the trimer. One of the protomers is represented as cartoon and the rest as surfaces.

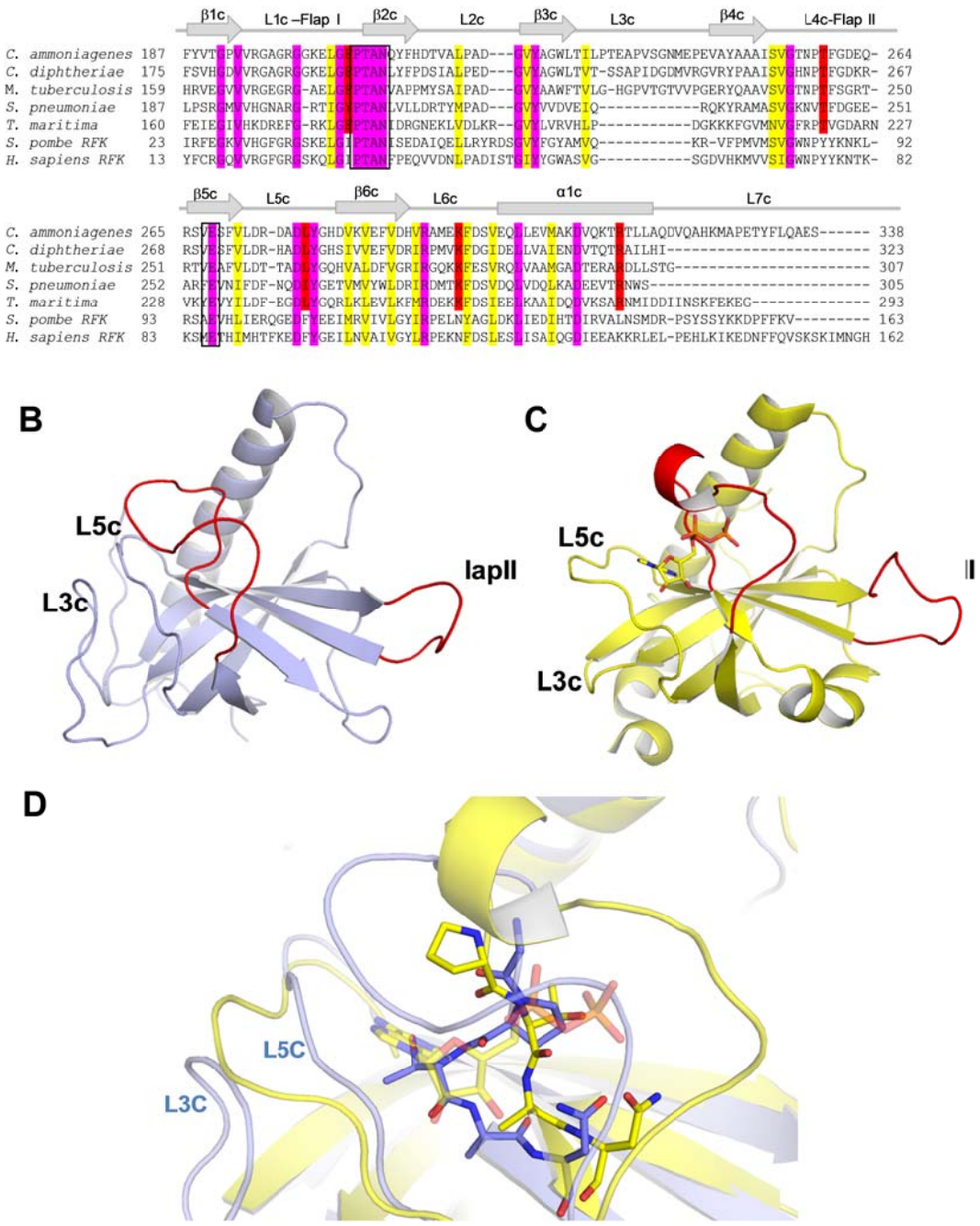

Figure S2. (A) Sequence alignment of the RFK modules of selected bifunctional FADSs and monfunctional RFKs. Conserved motifs are coloured in purple and yellow. Specific residues of prokaryotic RFKs are coloured in red. Secondary structure elements are shown at the top. (B) Cartoon representation of the free RFK module of *Ca*FADS. The loops L1c-FlapI and L4c-FlapII are highlighted in red. (C) Cartoon representation of monofunctional RFK from *S. pombe* in complex with ADP. The same two regions highlighted in B are also shown in red. (D) Detail of the conformation of the PTAN environment in *Ca*FADS (CPK coloured with carbons in blue) and the RFK from *S. pombe* (CPK coloured with carbons in yellow). The ADP molecule bound to *S. pombe* RFK is shown in transparent yellow CPK.

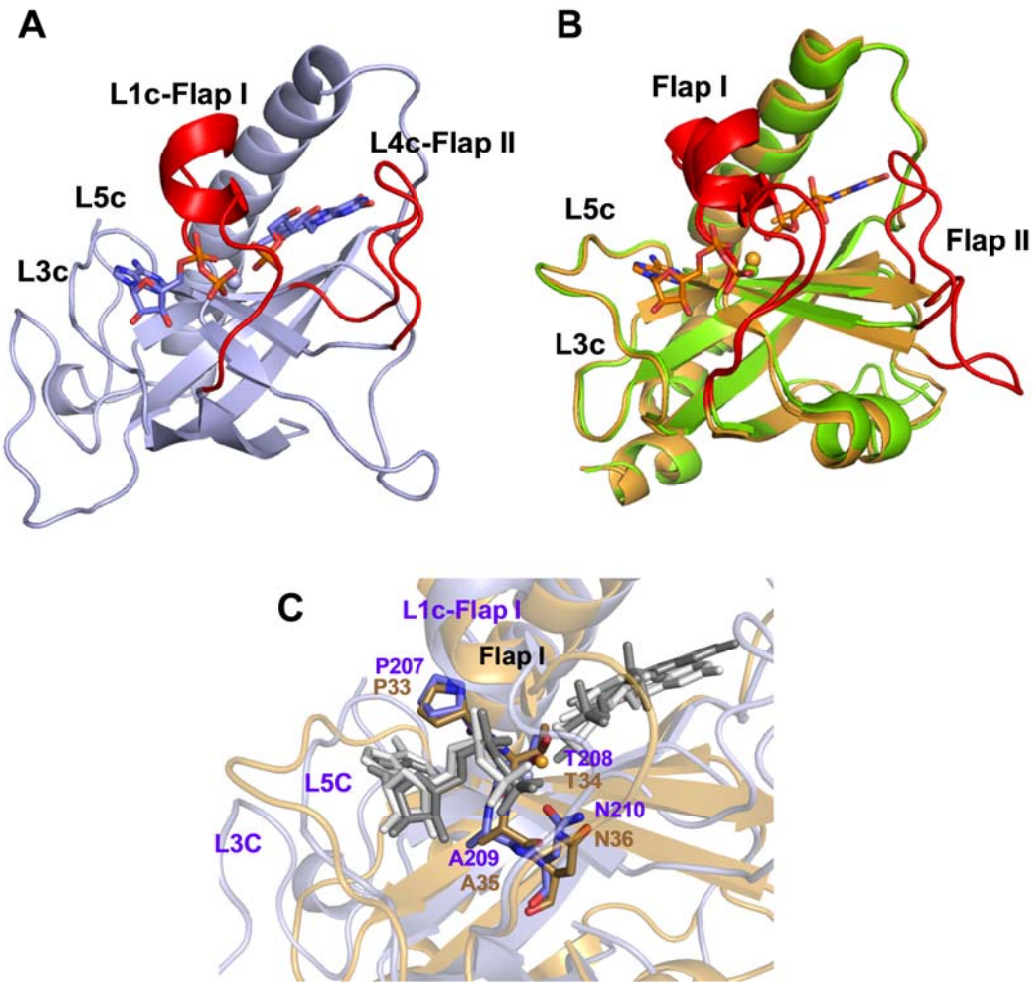

Figure S3. (A) Cartoon representation of the ternary complex of the RFK module of *Ca*FADS (blue; PDB 5A89). L1c-FlapI and L4c-FlapII are highlighted in red. FMN and ADP-Mg^{2+} are shown in CPK with carbons in blue. (B) Cartoon representation of monofunctional *Hs*RFK in complex with FMN and ADP-Mg^{2+} as obtained by soaking (light brown; PDB 1P4M) and co-crystallyzation (green; PDB 1Q9S). The same two regions highlighted in A are also shown in red. FMN and ADP-Mg^{2+} in CPK with carbons in orange (C) Detail of the conformation of the PTAN environment in the ternary complex of the *Ca*FADS RFK module (CPK coloured with carbons in blue) and in the ternary complex of *Hs*RFK (CPK coloured with carbons in light brown). The FMN and ADP-Mg^{2+} molecules bound to CaRFK module and *Hs*RFK are shown in light and dark grey CPK, respectively. Mg^{2+} atoms are represented as blue and light brown/ green spheres for *Ca*RFK module and *Hs*RFK, respectively.

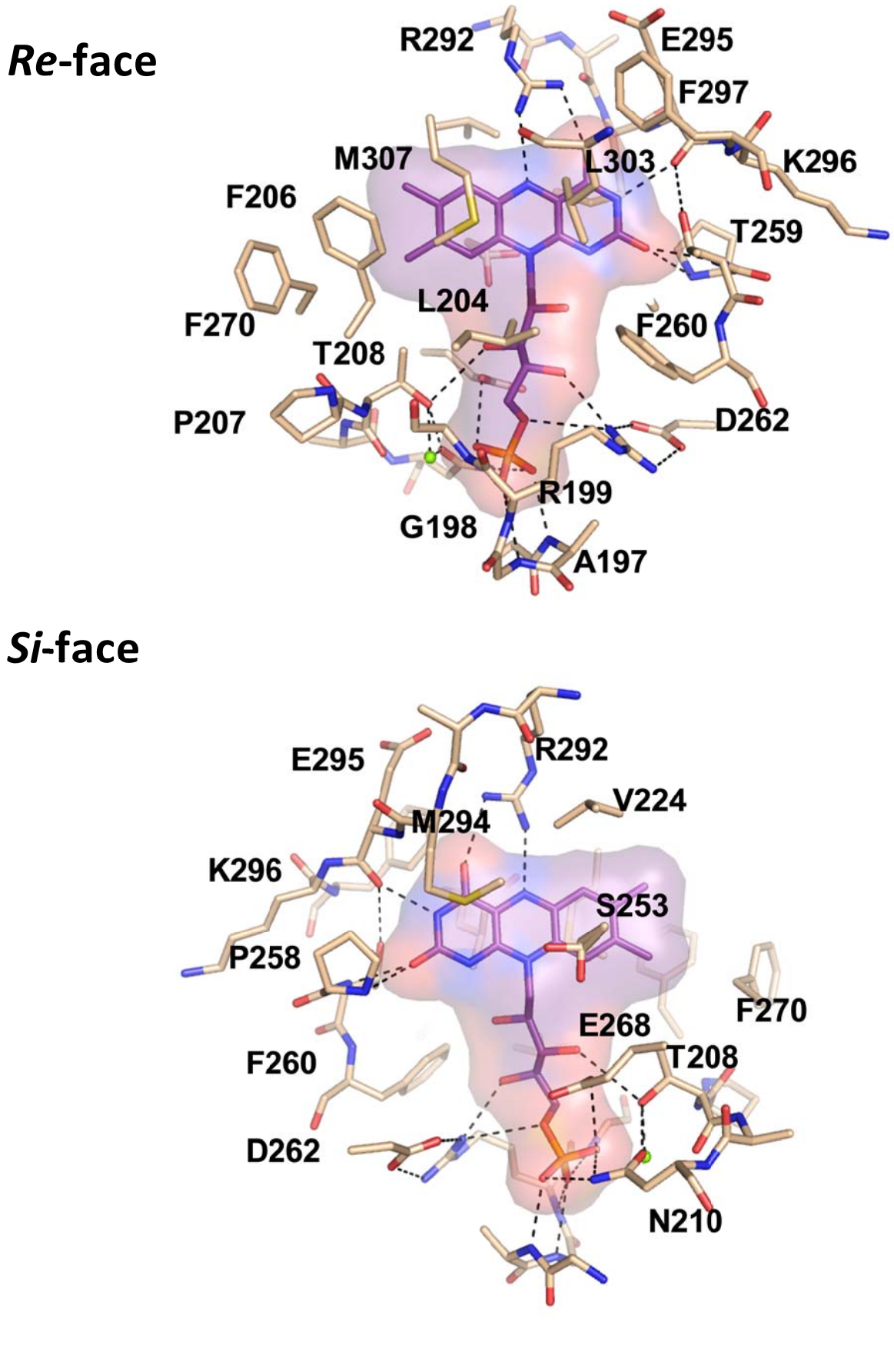

Figure S4. Detail of the residues at the *re*- and *si*-faces of the flavin.

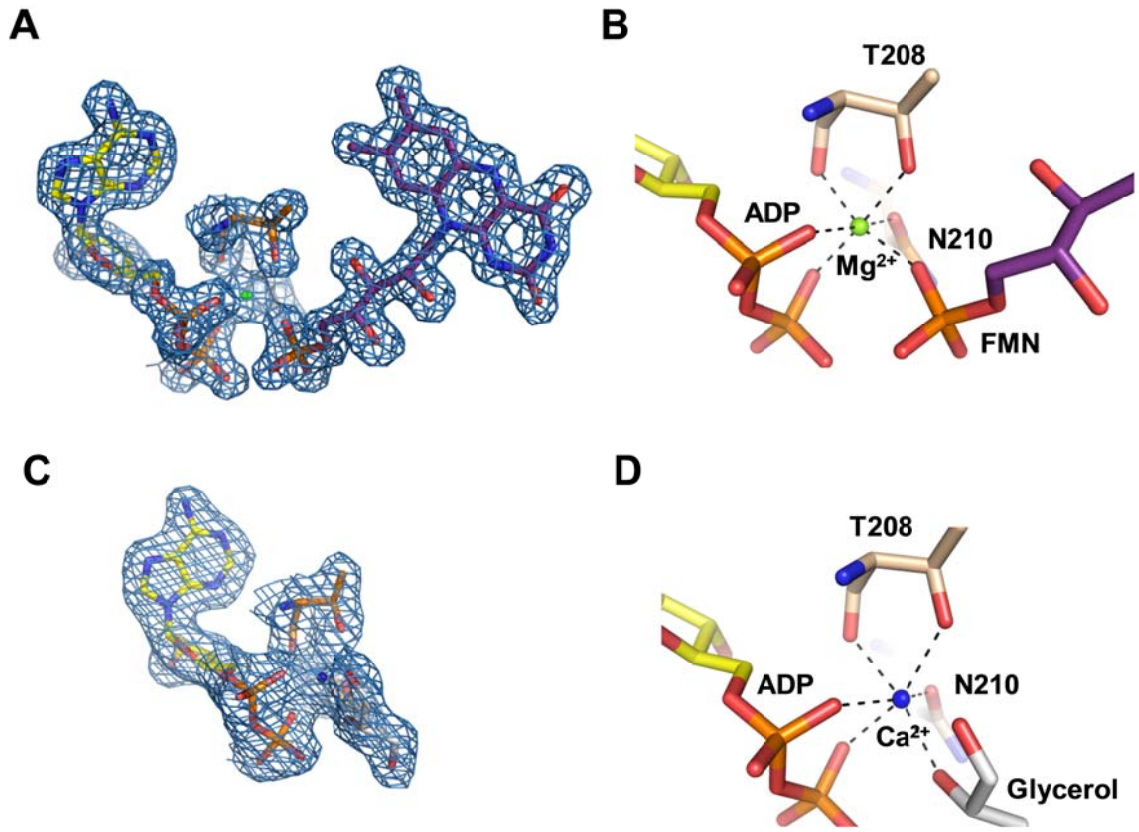

Figure S5. (A) $2F_oF_c$ elec tron density map around FMN, ADP and Mg^{2+} in the ternary complex. (B) Detailed view of the Mg^{2+} coordination in the ternary complex. (C) $2F_oF_c$ electron density map around ADP and Ca^{2+} in the binary complex. (D) Detailed view of the Ca^{2+} coordination in the binary complex.

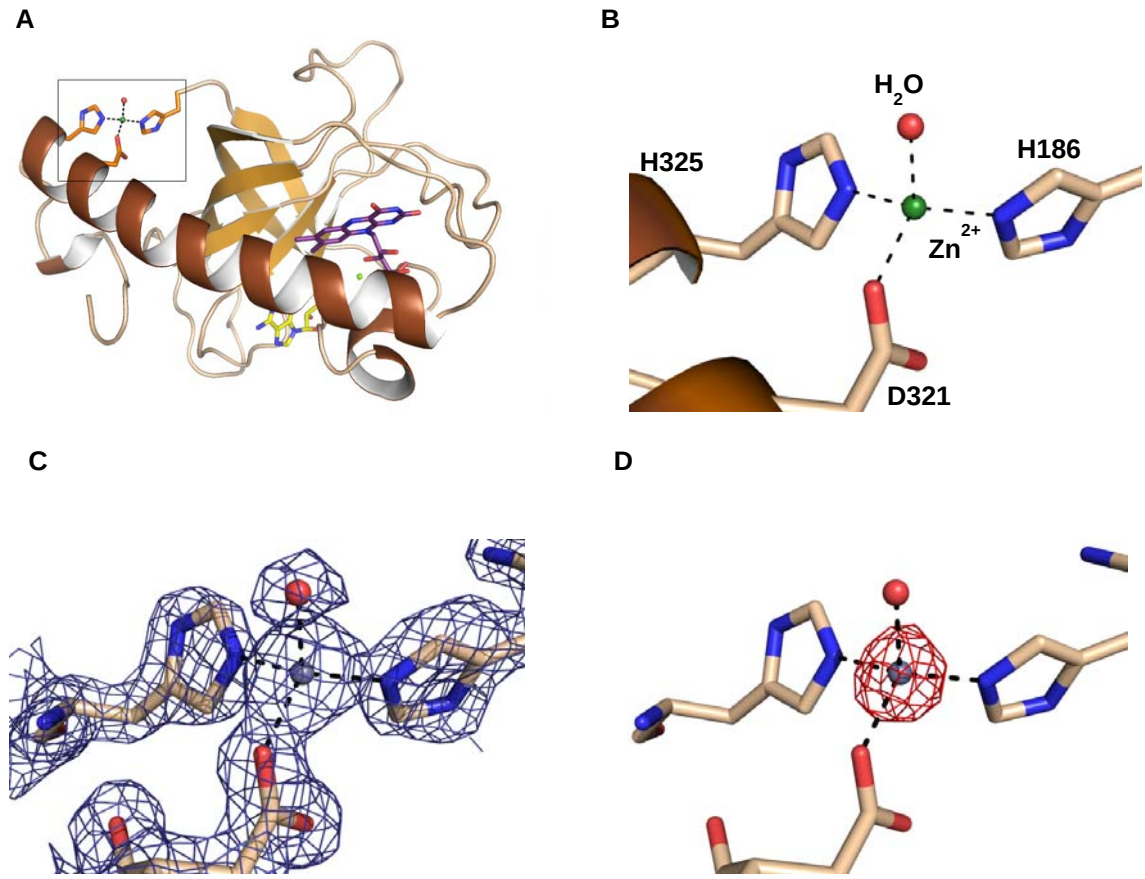

Figure S6. (A) Zn^{2+} binding in the ternary complex. (B) Coordination of the Zn^{2+} ion. (C) $2F_oF_c$ electron density map around Zn^{2+} ion. (D) Anomalous map around the Zn^{2+}ion.

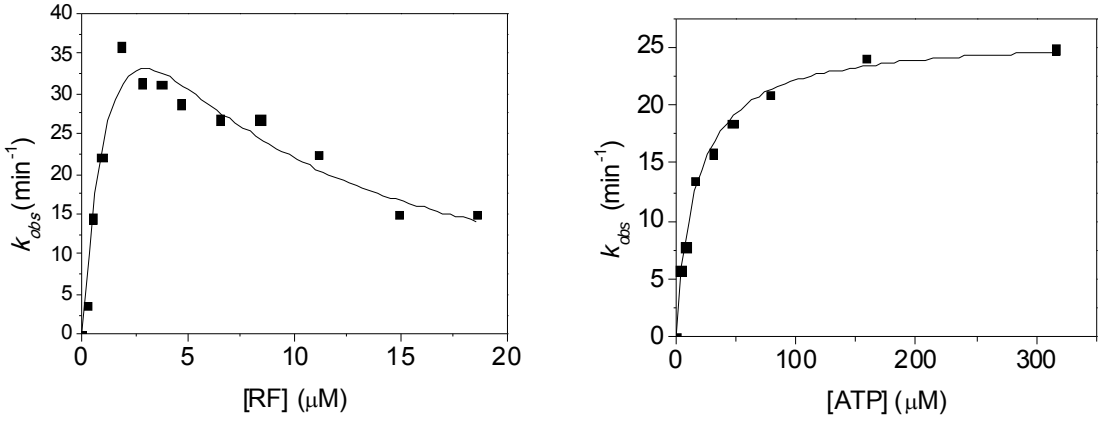

Figure S7. Steady-state rates for the RFK activity of Δ(1-182)*Ca*FADS as a function of (A) the RF concentration at saturating ATP concentrations and (B) as a function of the ATP concentration at RF concentration giving maximal activity. All the experiments were assayed in 20 mM PIPES, 0.8 mM $MgCl_2$, pH 7.0 at 25 ºC.

Supporting Table

Table S1 Crystal structures of proteins involved in FMN and FAD biosynthesis.

Organism	Protein	PDB code
Eukaryotes		
Homo Sapiens	RFK:ADP-Mg^{2+}	1NB0
	RFK:RF:ADP-Mg^{2+}	1NB9
	RFK:FMN:ADP-Mg^{2+} (1)	1P4M
	RFK:FMN:ADP-Mg^{2+} (1)	1Q9S
Schizosaccharomyces pombe	RFK	1N05
	RFK:ADP	1N06
	RFK:ADP:FMN	1N07
	RFK:ADP-Zn^{2+}	1N08
Tripanosoma brucei	RFK	3BNW
Candida glabrata	$FMNAT_{eukaryote}$	3FWK
	$FMNAT_{eukaryote}$:ATP	3G59
	$FMNAT_{eukaryote}$ FMN:AMP-CCP	3G5A
	$FMNAT_{eukaryote}$ FAD:PPi	3G6K
Saccharomyces cerevisiae	$FMNAT_{eukaryote}$:FAD	2WSI
Archaeas		
Methanocaldococcus jannaschii	$RFK_{archaea}$	2P3M (RMN)
	$RFK_{archaea}$ P	2VBS
	$RFK_{archaea}$: P:CDP	2VBT
	$RFK_{archaea}$: CDP	2VBU
	$RFK_{archaea}$:CDP:FMN	2VBV
Thermoplasma acidophilum	$RFK_{archaea}$	3CTA
Prokaryotes		
Corynnebacterium ammoniagenes	FADS:PPi	2X0K
Thermotoga maritima	FADS	1MRZ
	FADS, crystal form II	2I1L
	FADS:lumichrome	1S4M
	FADS:ADP	1T6X
	FADS:ADP:AMP:FMN	1T6Y
	FADS:RF	1T6Z
Streptococcus pneumoniae	FADS(2)	3OP1

(1) Both RFKs and FADS co-purify with flavin cofactors, which are removed during purification process. The structure of the ternary complex was solved with the protein co-purified with both products (1Q9S) and with crystals of apo-protein soaked with ligands (1P4M), resulting in two different structures.

(2) Crystal structure of the FADS from *S. pneumoniae* is annotated in the PDB as Macrolide-efflux protein, although it is a bifunctional FAD synthetase. It contains many small molecules located in the active sites, suggesting a residual occupation with flavin and/or adenine nucleotides that might co-purify with the protein.