Supporting Information

Enzyme-substrate-cofactor dynamical networks revealed by high resolution field cycling relaxometry

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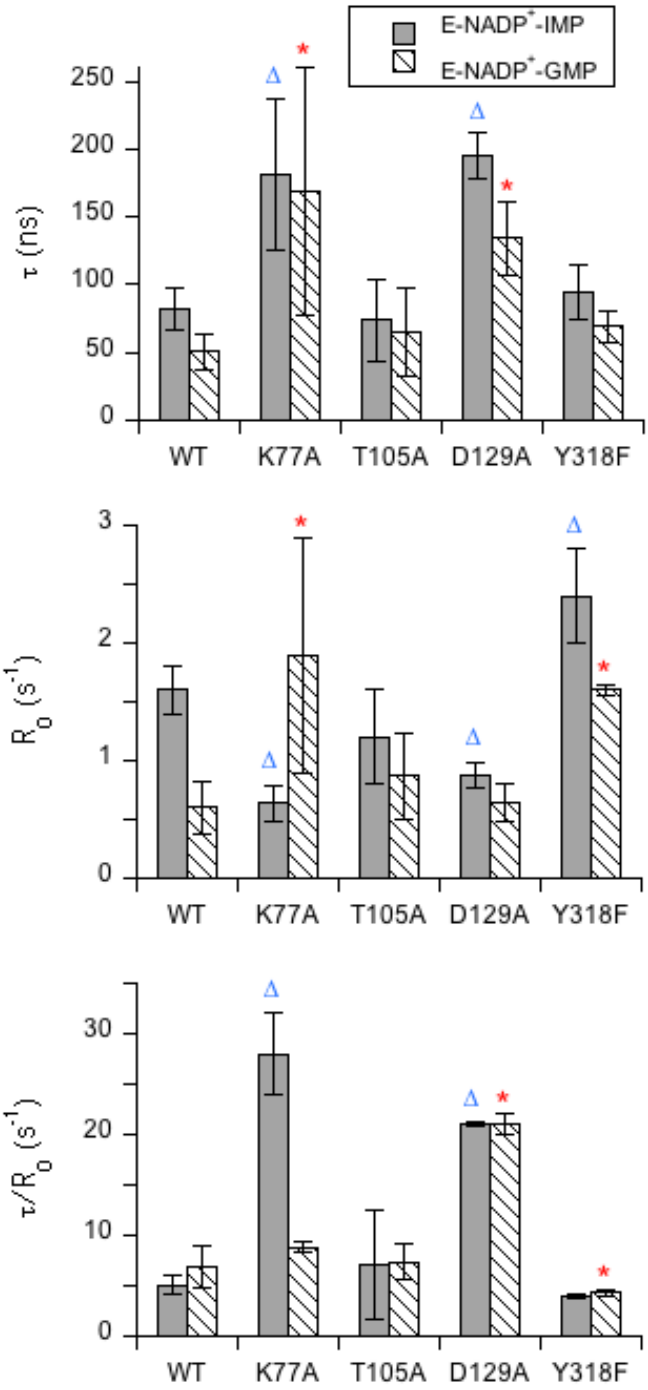

Mutant	P-value E-NADP$^+$-IMP	E-NADP$^+$-GMP
K77A	0.015	0.046
T105A	0.63	0.46
D129A	0.001	0.006
Y318F	0.43	0.12

K77A	0.004	0.048
T105A	0.14	0.28
D129A	0.016	0.84
Y318F	0.042	0.008

K77A	<0.0001	0.15
T105A	0.49	0.35
D129A	<0.0001	0.002
Y318F	0.24	0.013

Figure S1. ^{31}P field cycling parameters extracted for NADP$^+$ PP-2 compared to those for WT GMPR. P-value is from an unpaired t-test and comparisons with P<0.05 indicates the WT and mutant values are different. The parameters for this diphosphate nucleus are similar to those for PP-1 (in Figure 2).

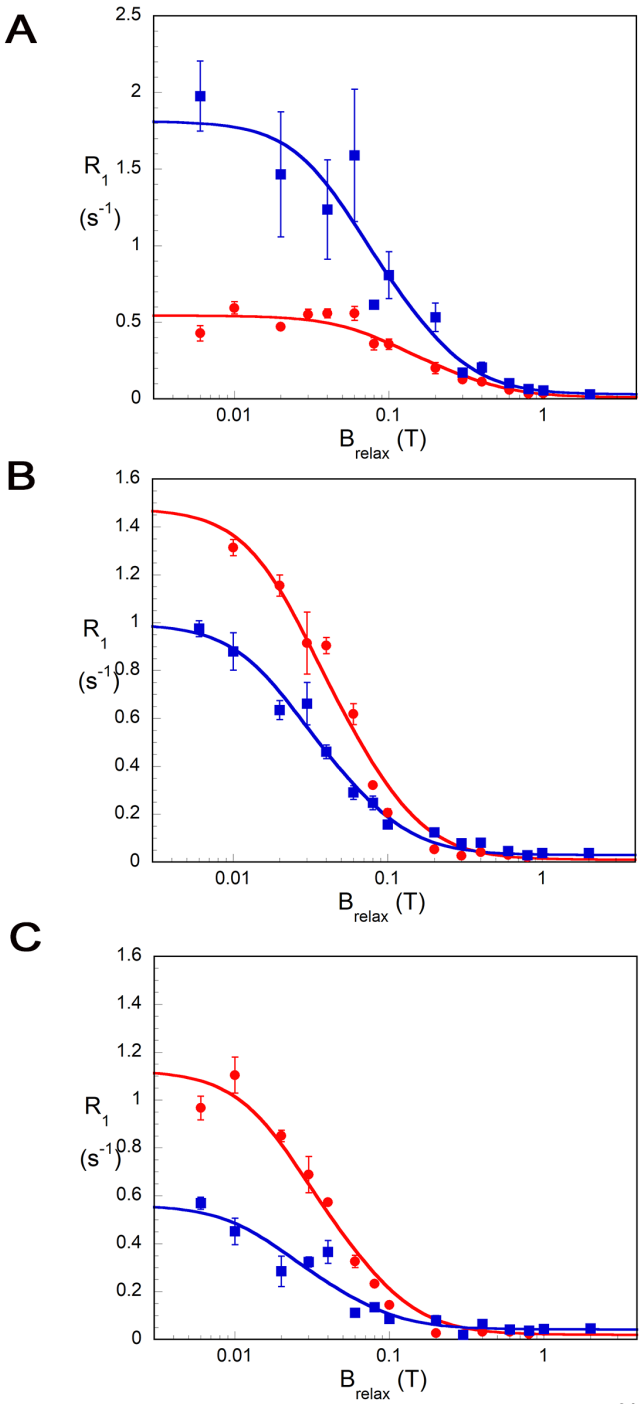

Figure S2. Magnetic field dependence of substrate and cofactor ^{31}P R_1 in K77A•GMP•NADP$^+$ and K77A•IMP•NADP$^+$ complexes. Samples (K77A•GMP•NADP$^+$ in *red* and K77A•IMP•NADP$^+$ in *blue*) contained 400 μM enzyme and 1.6 mM GMP/IMP and 1.6 mM NADP$^+$ in 75 mM Tris-HCl, pH 7.8, 100 mM KCl, 1 mM DTT and 0.5 mM EDTA: (*A*) substrate monophosphate, (*B*) cofactor monophosphate, and (*C*) cofactor diphosphates. The error bars are the standard error in R_1 from the exponential fit of signal magnitude versus time spent at the low field. The data shown are for one of the three independent experiments.

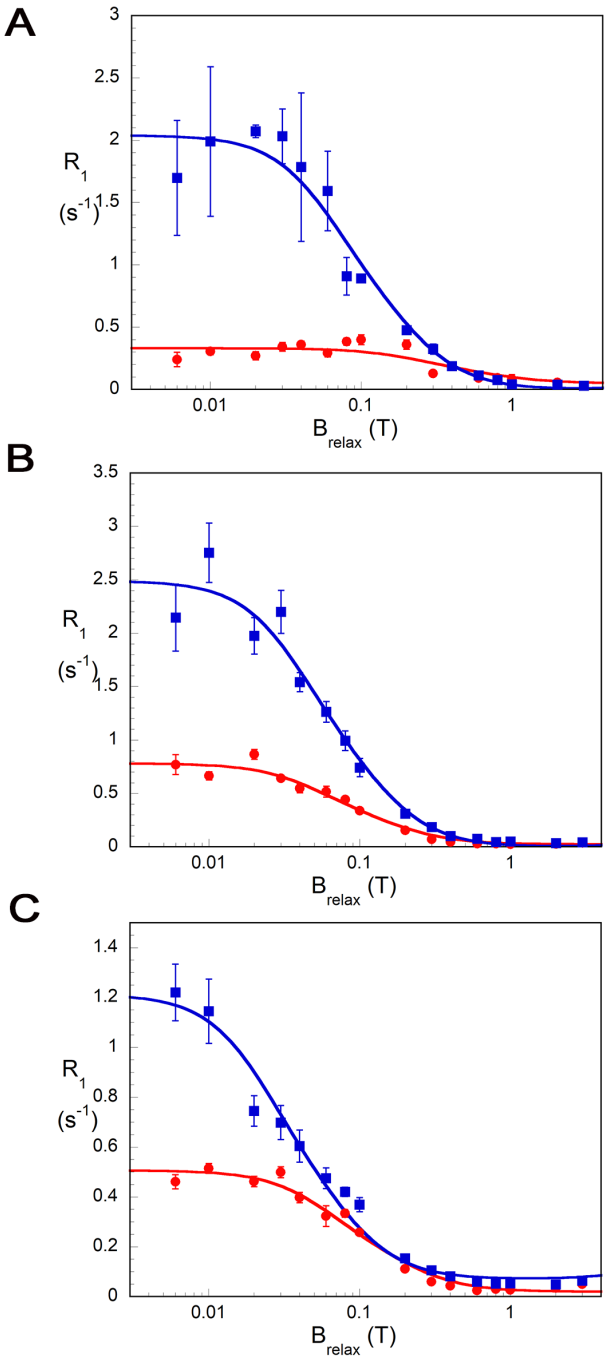

Figure S3. Magnetic field dependence of substrate and cofactor ^{31}P R_1 in T105A•GMP•NADP$^+$ and T105A•IMP•NADP$^+$ complexes. Samples (T105A•GMP•NADP$^+$ in *red* and T105A•IMP•NADP$^+$ in *blue*) contained 400 μM enzyme and 1.6 mM GMP/IMP and 1.6 mM NADP$^+$ in 75 mM Tris-HCl, pH 7.8, 100 mM KCl, 1 mM DTT and 0.5 mM EDTA: (*A*) substrate monophosphate, (*B*) cofactor monophosphate, and (*C*) cofactor diphosphates. The error bars are the standard error in R_1 from the exponential fit of signal magnitude versus time at low field. The data shown are for one of the three independent experiments.

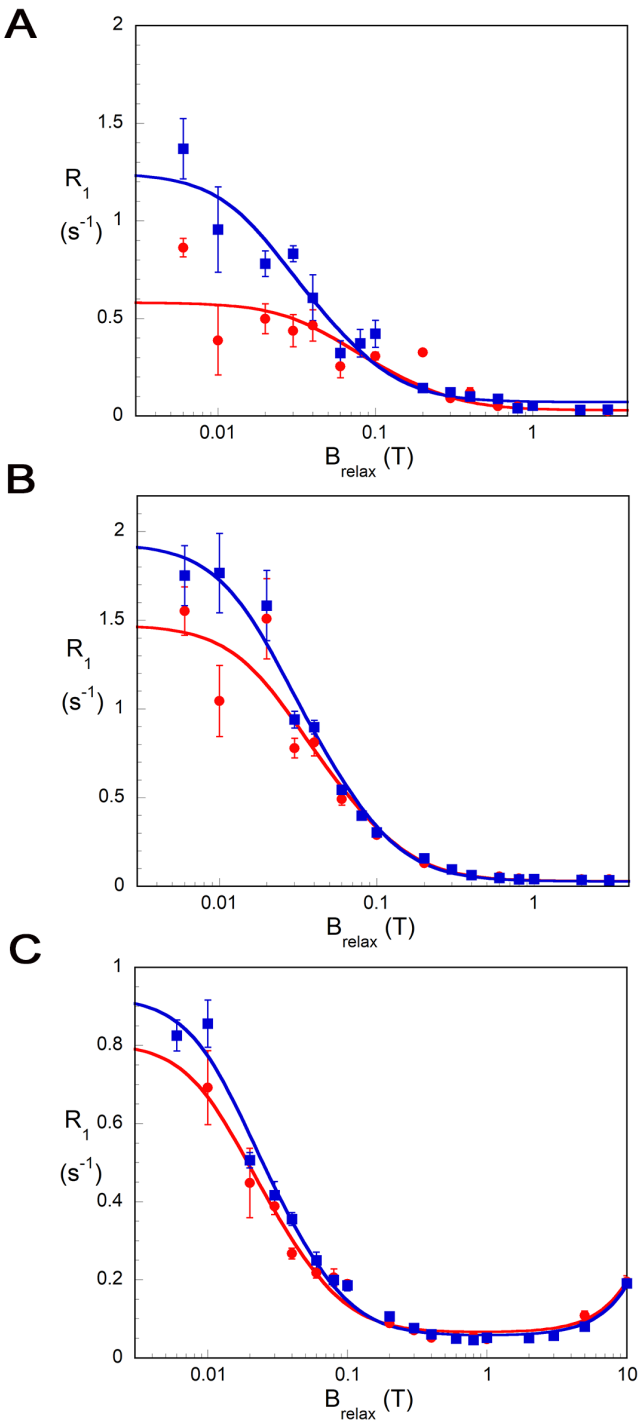

Figure S4. Magnetic field dependence of substrate and cofactor ^{31}P R_1 in D129A•GMP•NADP$^+$ and D129A•IMP•NADP$^+$ complexes. Samples (D129A•GMP•NADP$^+$ in *red* and D129A•IMP•NADP$^+$ in *blue*) contained 400 μM enzyme and 1.6 mM GMP/IMP and 1.6 mM NADP$^+$ in 75 mM Tris-HCl, pH 7.8, 100 mM KCl, 1 mM DTT and 0.5 mM EDTA: (*A*) substrate monophosphate, (*B*) cofactor monophosphate, and (*C*) cofactor diphosphates. The error bars are the standard error in R_1 from the exponential fit of signal magnitude versus time spent at low field. The data shown are for one of two independent experiments for the hydride transfer complex and for the single experiment for the deamination complex.

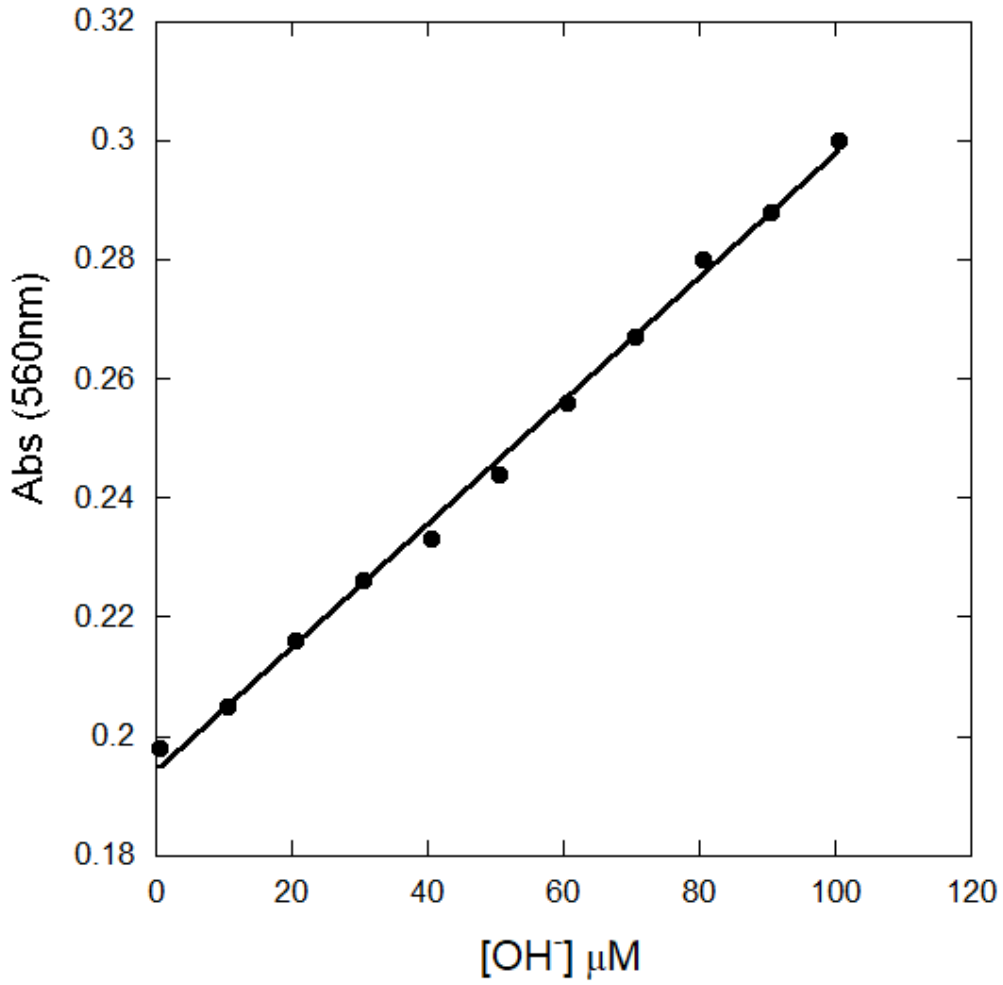

Figure S5. Standard curve for H^+ uptake experiments. The change in absorbance is 0.001/μM H^+.

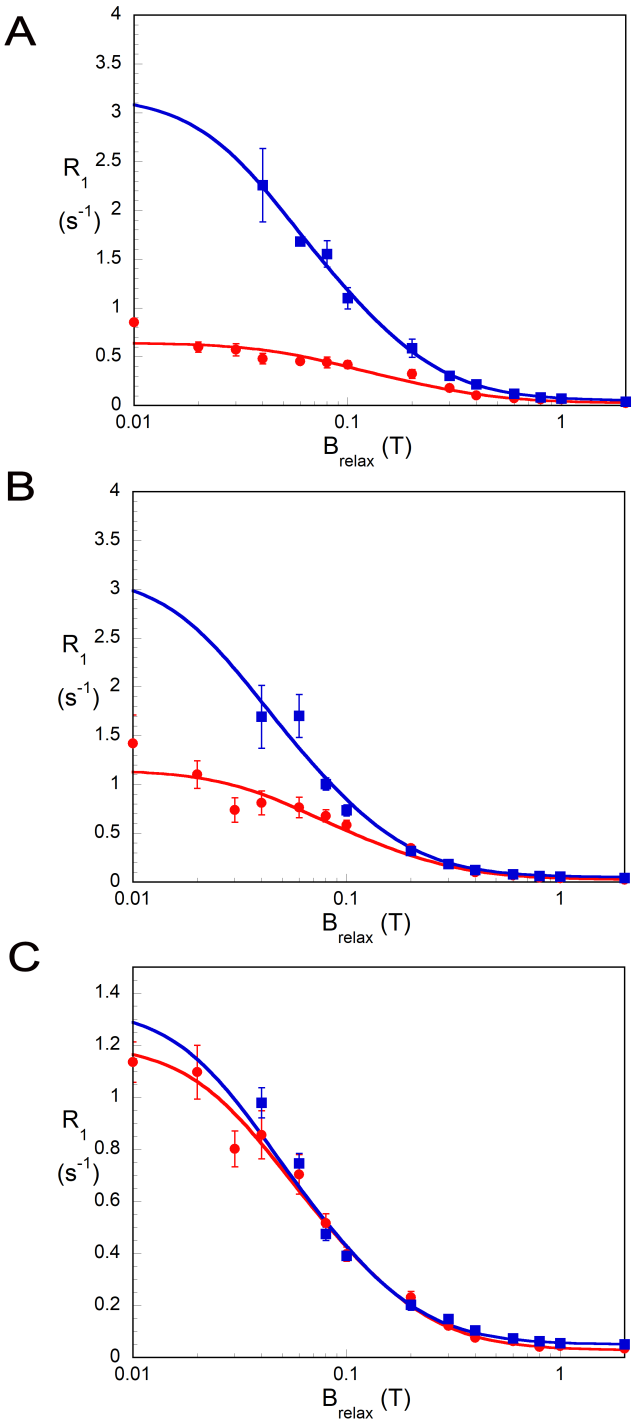

Figure S6. Magnetic field dependence of substrate and cofactor ^{31}P R_1 in Y318F•GMP•NADP$^+$ and Y318F•IMP•NADP$^+$ complexes. Samples (Y318F•GMP•NADP$^+$ in *red* and Y318F•IMP•NADP$^+$ in *blue*) contained 400 μM enzyme and 1.6 mM GMP/IMP and 1.6 mM NADP$^+$ in 75 mM Tris-HCl, pH 7.8, 100 mM KCl, 1 mM DTT and 0.5 mM EDTA: (*A*) substrate monophosphate, (*B*) cofactor monophosphate, and (*C*) cofactor diphosphates. The error bars are the standard error in R_1 from the exponential fit of signal magnitude versus time spent at low field. The data shown are for one of the two independent experiments.

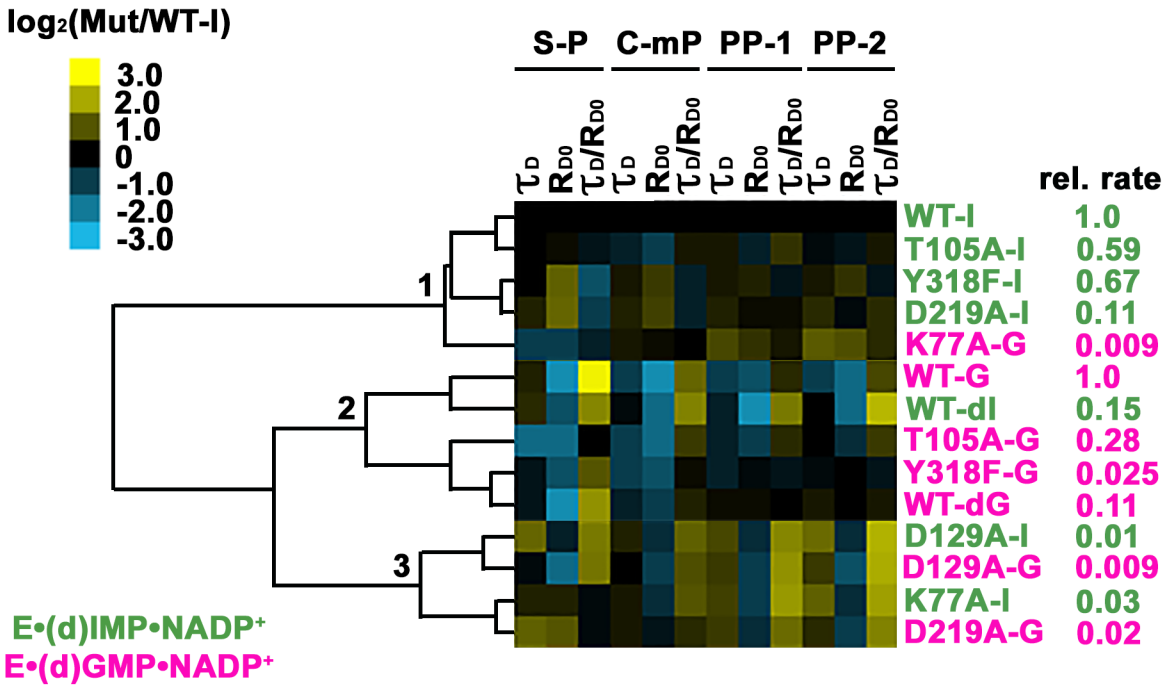

Figure S7. Heat map of cluster analysis. Field cycling parameters were expressed as the ratio of mutant to wild-type hydride transfer complex and converted to $\log_2$. Hydride transfer complexes are green, deamination complexes are magenta. Relative rates compared to the relevant wild-type complex are shown, for the partial hydride transfer in green and the back reaction in magenta. Enzyme complexes were clustered using Euclidean distance algorithm with centroid linkages in Cluster 3.0 [1]. Results were visualized using Tree View [2]. S-P, substrate monophosphate; C-mP, cofactor monophosphate; PP-1, cofactor diphosphate 1; PP-2, cofactor diphosphate 2.

Table S1. Field cycling parameters for substrate in mutant NADP$^+$ complexes. Values are the average of three independent experiments unless otherwise noted.

^{31}P	Enzyme	Hydride transfer complex (IMP+NADP$^+$)			Deamination complex (GMP+NADP$^+$)		
		τ_D (ns)	R_{D0} (s^{-1})	τ_D/R_{D0} x 10^8 (s^2)	τ_D (ns)	R_{D0} (s^{-1})	τ_D/R_{D0} x 10^8 (s^2)
Substrate mono-P	WT	55±12	1.8±0.9	3.3±1.2	72±13	0.3±0.02	24±5
	K77A [a]	74±23	2.4±0.7	3.1±0.3	26±1	0.64±0.16	4.1±1.1
	T105A	55±14	2.1±0.5	2.7±1.2	16±5 [a]	0.4±0.3 [a]	3.4±0.8 [a]
	D129A [a]	126±20	1.4±0.7	10±6	48±18 [b]	0.6±0.1 [b]	8.7±0.4 [b]
	Y318F	55±3 [a]	4.0±1.6 [a]	1.4±0.6 [a]	46±13	0.7±0.1	6.5±1.3
NADP mono-P	WT	103±23	2.8±0.2	3.8±1.0	47±11	0.6±0.1	7.5±2.8
	K77A	124±14	1.1±0.2 (1.6±0.3) [c]	11±1 (7.7±0.7) [c]	121±35	2.0±0.8 (3.2±1.3) [d]	6.1±0.5 (3.8±0.8) [d]
	T105A	67±18	1.5±0.7	5.0±2.4	49±1 [a]	0.79±0.03 [a]	6.2±0.4 [a]
	D129A [a]	131±28	1.6±0.5	8.6±1.0	100±2	1.42±0.03	7.1±0.3
	Y318F	118±29 [a]	4.6±1.5 [a]	2.6±0.2 [a]	49±5	1.2±0.1	4.1±0.1
NADP PP1	WT	88±5	1.4±0.3	6.6±1.2	44±6	0.49±0.11	9.5±2.1
	K77A	147±31	0.44±0.06 (0.65±0.09) [c]	28±18 (19±12) [c]	161±58	1.3±0.3 (2.1±0.5) [d]	12.5±2.1 (7.8±1.3) [d]
	T105A	102±14	0.99±0.47	12±6	56±15 [a]	0.64±0.16 [a]	8.8±0.7 [a]
	D129A [a]	160±1	0.79±0.11	20±3	142±40	0.66±0.11	21±2
	Y318F	102±11	1.8±0.2	5.58±0.02	61±9	1.2±0.2	5.0±0.6
NADP PP2	WT	82±15	1.6±0.2	5.1±1.0	51±13	0.60±0.22	8.9±2.0
	K77A [a]	181±56	0.64±0.15 (0.95 ± 22) [c]	28±4 (19±2.7) [c]	169±91	1.9±1.0 (3.1±1.6) [d]	8.8±0.5 (5.4±0.3) [d]
	T105A	74±30	1.2±0.4	7.1±5.5	65±33 [a]	0.87±0.37 [a]	7.4±1.8 [a]
	D129A [a]	195±17	0.88±0.11	21±2	134±27	0.64±0.16	21±1
	Y318F	95±20 [a]	2.4±0.4 [a]	4.0±0.2 [a]	69±12	1.6±0.04	4.3±0.4

[a] The number of experiments, N, was 2.

[b] Of two field cycling runs for this complex, only one had sufficient data to extract GMP t and R_{D0}. The errors for the D129A GMP parameters are from the goodness of fit of the field cycling data for that one sample. The NADP$^+$ resonances were measurable in both samples so that for all the D129A cofactor parameters the error is the variance from the mean of the two samples.

[c] Adjusted for F_b(NADP) = 0.67

[d] Adjusted for F_b(NADP) = 0.62

Table S2. **Comparisons of mutant protein τ_D, R_{D0}, and τ_D/R_{D0} ^{31}P parameters in the hydride transfer (HT), E-NADP$^+$-IMP, and deamination (DA) E-NADP$^+$-GMP complexes.** [a]

GMPR[b]	τ_D	R_{D0}	τ_D/R_{D0}
Substrate:			
K77A	τ_D (IMP) > τ_D (GMP) P = 0.047	R_{D0} (IMP) > R_{D0} (GMP) P = 0.035	τ_D/R_{D0} (IMP) = τ_D/R_{D0} (GMP)
T105A	τ_D (IMP) > τ_D (GMP) P = 0.037	R_{D0} (IMP) > R_{D0} (GMP) P = 0.027	τ_D/R_{D0} (IMP) = τ_D/R_{D0} (GMP)
D129A	–	–	–
Y318F	τ_D (IMP) = τ_D (GMP)	R_{D0} (IMP) > R_{D0} (GMP) P = 0.030	τ_D/R_{D0} (HT) < τ_D/R_{D0} (DA) P = 0.016
NADP$^+$ mono-P			
K77A	τ_D (NADP$^+$)$_{HT}$ = τ_D (NADP$^+$)$_{DA}$	R_{D0} (NADP$^+$)$_{HA}$ = R_{D0} (NADP$^+$)$_{DA}$	τ_D/R_{D0} (NADP$^+$)$_{HA}$ > τ_D/R_{D0}(NADP$^+$)$_{DA}$ P = 0.0001
T105A	τ_D (NADP$^+$)$_{HT}$ = τ_D (NADP$^+$)$_{DA}$	R_{D0} (NADP$^+$)$_{HA}$ = R_{D0} (NADP$^+$)$_{DA}$	τ_D/R_{D0} (NADP$^+$)$_{HA}$= τ_D/R_{D0} (NADP$^+$)$_{DA}$
D129A	τ_D (NADP$^+$)$_{HT}$ = τ_D (NADP$^+$)$_{DA}$	R_{D0} (NADP$^+$)$_{HA}$ = R_{D0} (NADP$^+$)$_{DA}$	τ_D/R_{D0} (NADP$^+$)$_{HA}$= τ_D/R_{D0} (NADP$^+$)$_{DA}$
Y318F	τ_D (NADP$^+$)$_{HT}$ > τ_D (NADP$^+$)$_{DA}$ P = 0.022	R_{D0} (NADP$^+$)$_{HA}$ > R_{D0} (NADP$^+$)$_{DA}$ P = 0.024	τ_D/R_{D0} (NADP$^+$)$_{HA}$< τ_D/R_{D0} (NADP$^+$)$_{DA}$ P = 0.0003
NADP$^+$PP-1			
K77A	τ_D (PP-1)$_{HT}$ = τ_D (PP-1)$_{DA}$	R_{D0} (PP-1)$_{HA}$ < R_0 (PP-1)$_{DA}$ P = 0.0014	τ/R_0 (PP-1)$_{HT}$ > τ/R_0 (PP-1)$_{DA}$ P = 0.0078
T105A	τ_D (PP-1)$_{HT}$ = τ_D (PP-1)$_{DA}$	R_{D0} (PP-1)$_{HA}$ = R_0 (PP-1)$_{DA}$	τ/R_0 (PP-1)$_{HT}$ = τ/R_0 (PP-1)$_{DA}$
D129A	τ_D (PP-1)$_{HT}$ = τ_D (PP-1)$_{DA}$	R_{D0} (PP-1)$_{HA}$ = R_0 (PP-1)$_{DA}$	τ/R_0 (PP-1)$_{HT}$ = τ/R_0 (PP-1)$_{DA}$
Y318F	τ_D (PP-1)$_{HT}$ > τ_D (PP-1)$_{DA}$ P = 0.017	R_{D0} (PP-1)$_{HA}$ > R_0 (PP-1)$_{DA}$ P = 0.029	τ/R_0 (PP-1)$_{HT}$ = τ/R_0 (PP-1)$_{DA}$

[a] P values < 0.05 are given and the direction of the comparison of τ_D, R_{D0}, or τ_D/R_{D0} is indicated for a given GMPR. These data should be To be compared to WT parameters in Table 1 of the main text.

[b] Since only one run of the DA had enough data to extract GMP parameters for this mutant, a statistical analysis of how the two substrates compare cannot be done.

Table S3. Field cycling parameters organized in clusters. Green are hydride transfer complexes, pink are deamination complexes. Data for D219A, dIMP and dGMP complexes are from [3]. For K77A and D219A, cluster analysis utilized values of R_{D0} and τ_D/R_{D0} corrected for fraction bound (corrected values shown in parentheses).

Enzyme	(d)NMP			NADP monoP			NADP PP1		
	τ_D (ns)	R_{D0} (s^{-1})	τ_D/R_{D0} x 10^8 (s^2)	τ_D (ns)	R_{D0}(s^{-1})	τ_D/R_{D0} x 10^8 (s^2)	τ_D (ns)	R_{D0} (s^{-1})	τ_D/R_{D0} x 10^8 (s^2)
Cluster 1									
WT	55±12	1.8±0.9	3.3±1.2	103±23	2.8±0.2	3.8±1.0	88±5	1.4±0.3	6.6±1.2
T105A	55±14	2.1±0.5	2.7±1.2	67±18	1.5±0.7	5.0±2.4	102±14	0.99±0.47	12±6
Y318F	55±3 [a]	4.0±1.6 [a]	1.4±0.6 [a]	118±29 [a]	4.6±1.5 [a]	2.6±0.2 [a]	102±11	1.8±0.2	5.58±0.02
D219A	70±26	1.7±0.3 (4.3±0.7)[c]	4.1±1.7 (1.6±0.7)[c]	139±9	1.95±0.02 (4.88±0.05)[c]	7.1±0.5 (2.8±0.2)[c]	113±6	0.60±0.02 (1.50±0.05)[c]	18±1 (7.2±0.4)[c]
K77A[a]	26±1	0.64±0.16	4.1±1.1	121±35	2.02±0.77 (3.3±1.2)[d]	6.1±0.5 (3.2±1.3)[d]	161±58	1.3±0.3 (2.1±0.5)[d]	12.5±2.1 (7.8±1.3)[d]
Cluster 2									
WT	72±13	0.3±0.02	24±5	47±11	0.6±0.1	7.5±2.8	44±6	0.49±0.11	9.5±2.1
WT(dIMP)	75±30	0.8±0.3	10±5	92±18	0.8±0.2	11±3	61±23	0.3±0.1	18±8
T105A	16±5 [a]	0.4±0.3 [a]	3.4±0.8 [a]	49±1 [a]	0.79±0.03 [a]	6.2±0.4 [a]	156±15 [a]	0.64±0.16 [a]	8.8±0.7 [a]
Y318F	46±13	0.7±0.1	6.5±1.3	49±5	1.2±0.1	4.1±0.1	61±9	1.2±0.2	5.0±0.6
WT(dGMP)	43±12	0.4±0.2	11±6	75±8	1.5±0.5	5±2	101±27	1.6±0.5	6.3±3
Cluster 3									
D129A[a]	126±20	1.4±0.7	10±6	131±28	1.6±0.5	8.6±1.0	160±1	0.79±0.11	20±3
D129A[a]	48±18 [b]	0.6±0.1 [b]	8.7±0.4 [b]	100±2	1.42±0.03	7.1±0.3	142±40	0.66±0.11	21±2
K77A[a]	74±23	2.4±0.7	3.1±0.3	124±14	1.1±0.2 (1.6±0.3)[c]	11±1 (7.7±0.7) [c]	147±31	0.44±0.06 (0.66±0.09)[c]	28±18 (19±12)[c]
D219A	120±34	3.8±1.2	3.1±1.3	125±8	2.3±0.3	5.3±0.8	137±32	0.86±0.15	16±5

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[3] Rosenberg, M. M., Redfield, A. G., Roberts, M. F., and Hedstrom, L. (2018) Dynamic Characteristics of Guanosine-5'-monophosphate Reductase Complexes Revealed by High-Resolution (31)P Field-Cycling NMR Relaxometry, *Biochemistry 57*, 3146-3154.