Supplementary Information for

Dynamic characteristics of GMP reductase complexes revealed by high resolution ^{31}P field cycling NMR relaxometry

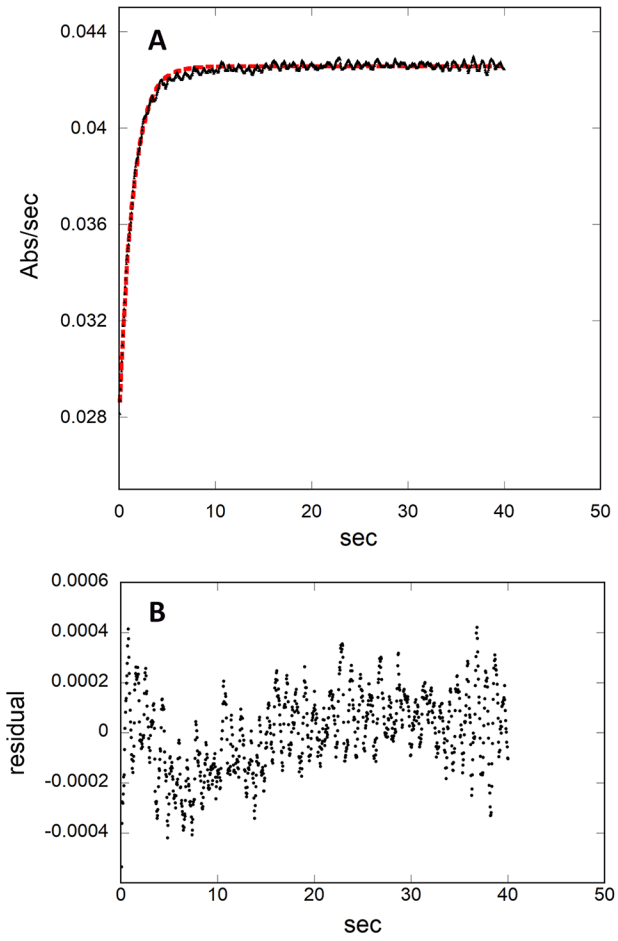
Masha M. Rosenberg[‡], Alfred G. Redfield[§], Mary F. Roberts[¶*] and Lizbeth Hedstrom[‡£*]

From the [‡] Department of Biology, Brandeis University, MS009, 415 South St., Waltham MA 02453-9110 USA
[§] Department of Biochemistry, Brandeis University, MS009, 415 South Street, Waltham, MA 02453-9110 USA
[¶] Department of Chemistry, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-9110 USA
[£] Department of Chemistry, Brandeis University, 415 South Street, Waltham, MA 02453-3808 USA.

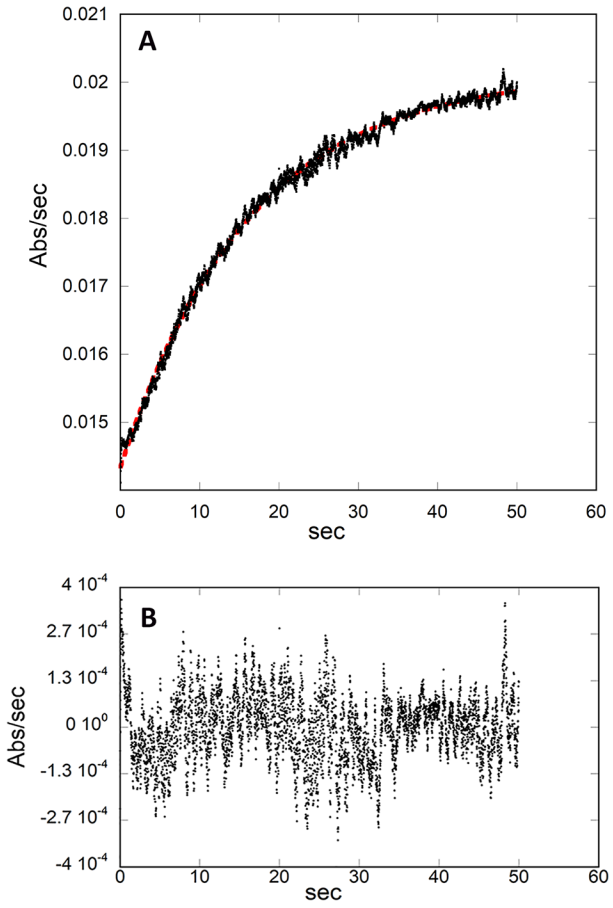
[*] To whom correspondence should be addressed: Lizbeth Hedstrom, Department of Biology, Brandeis University, MS009, 415 South Street, Waltham, MA 02453-9110 USA. Tel: 781-736-2333; Fax: 781-736-2349; E-mail: hedstrom@brandeis.edu; Mary Roberts, Department of Chemistry, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-9110. Tel: 617-552-3616; E-mail: mary.roberts@bc.edu.

Supporting Table S1. Extrapolated ^{31}P-^{1}H distances.

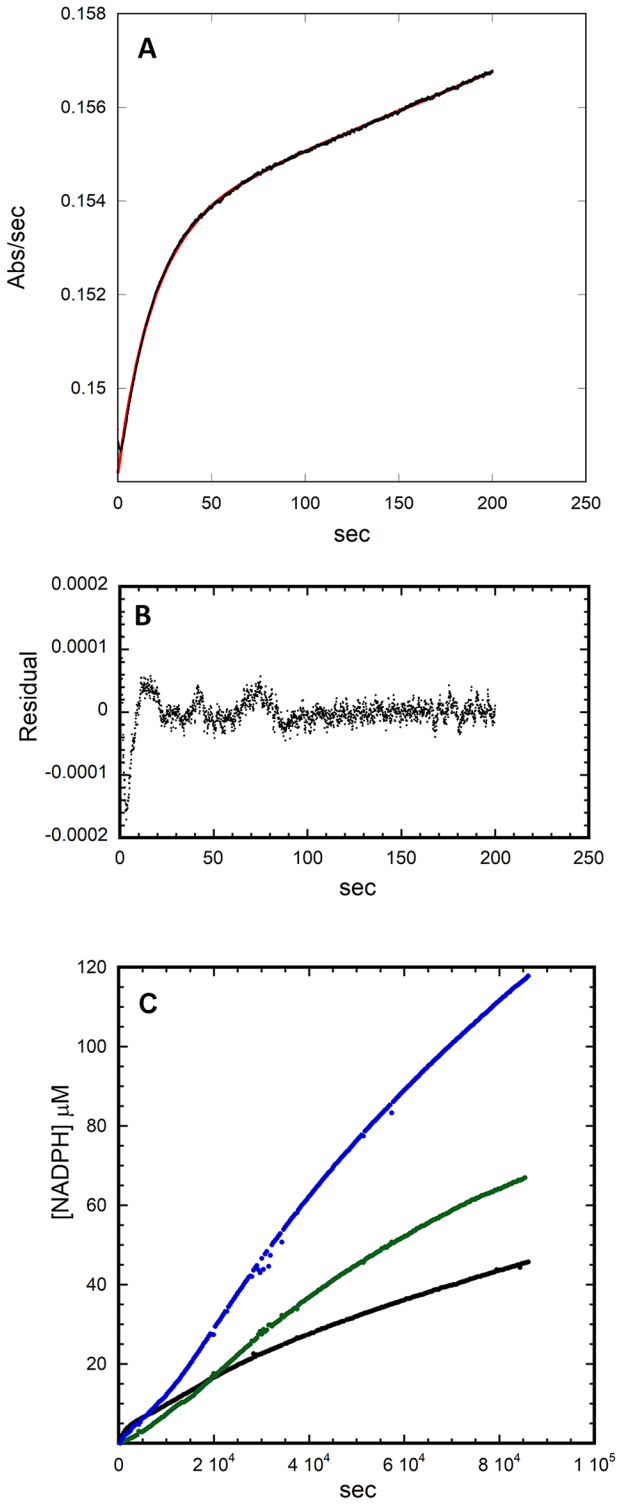
^{31}P distance	Exp.	IMP/GMP monoP	$NADP^+$ monoP	$NADP^+$ diP
WT•IMP •$NADP^+$	H_2O	2.9 ± 0.2	2.8 ± 0.2	3.0 ± 0.1
WT•GMP •$NADP^+$	H_2O	4.1 ± 0.2	3.1 ± 0.1	3.2 ± 0.1
WT•IMP •$NADP^+$	D_2O	3.4 ± 1.2	3.4 ± 1.6	3.6 ± 1.9
				3.5 ± 1.7
WT•GMP •$NADP^+$	D_2O	4.1 ± 2.0	3.7 ± 1.4	3.8 ± 0.8
				3.5 ± 0.9
WT•dIMP •$NADP^+$	H_2O	3.5 ± 1.8	3.6 ± 1.1	3.9 ± 1.0
				4.0 ± 1.0
WT•dGMP •$NADP^+$	H_2O	3.6 ± 1.8	3.1 ± 1.0	3.3 ± 1.0
				3.2 ± 1.0
D219A•IMP •$NADP^+$	H_2O	2.6 ± 0.9	2.8 ± 0.2	3.3 ± 0.2
				3.3 ± 0.3
D219A•GMP •$NADP^+$	H_2O	2.9 ± 1.2	3.2 ± 0.5	3.8 ± 0.6
				3.7 ± 0.7



Supplementary Figure S1. The partial reaction of IMP and $NADP^+$ catalyzed by GMPR WT. Enzyme (10 μM) was mixed with IMP (500 μM) and $NADP^+$ (500 μM) and the formation of NADPH is monitored by the change in absorbance at λ = 340 nm. The amount of NADPH formed is ~16% of the active sites. **A.** A representative progress curve comprised of the average absorbance readings from at least 5 "shots". The red line shows the fit to a single exponential equation. **B.** The residual errors on the fit in A.



Supplementary Figure S2. The partial reaction of dIMP and $NADP^+$ catalyzed by GMPR WT. Enzyme (10 μM) was mixed with dIMP (500 μM) and $NADP^+$ (500 μM) and the formation of NADPH is monitored by the change in absorbance at λ = 340 nm. The amount of NADPH formed is ~10% of the active sites. **A.** A representative progress curve comprised of the average absorbance readings from at least 5 "shots". The red line shows the fit to a single exponential equation. **B.** The residual errors on the fit in A.



Supplementary Figure S3. The partial reaction of IMP and $NADP^+$ catalyzed by GMPR mutant D219A. A. A representative progress curve of the presteady state reaction comprised of the average absorbance readings from at least 5 "shots". The red line shows the fit to a single exponential and a steady state. Enzyme (30 µM) was mixed with IMP (10 mM) and $NADP^+$ (3 mM) and the formation of NADPH is monitored by the change in absorbance at λ =

340 nm. The amount of NADPH formed is ~3% of the active sites after the burst phase. The apparent steady state rate is 0.001 s^{-1}. **B.** The residual errors on the fit in A. **C.** The partial reaction of IMP and $NADP^+$ catalyzed by D219A monitored for 24 hours. Formation of NADPH is monitored by the change in absorbance at λ = 340 nm. Initial rates were determined over the first 10 min. *Black line*: conditions of the pre-steady state experiment: enzyme (30 μM) was mixed with saturating IMP (10 mM) and $NADP^+$ (3 mM), initial rate = 6.3 x 10^{-5} s^{-1}; *Green line*: conditions of the field cycling experiment: enzyme (400 μM) was mixed with IMP (1.6 mM) and $NADP^+$ (1.6 mM), initial rate = 1.4 x 10^{-5} s^{-1}; *Blue line*: enzyme (400 μM) was mixed with saturating IMP (10 mM) and $NADP^+$ (3 mM), initial rate = 3.5 x 10^{-6} s^{-1}. These observations suggest that the enzyme is 41% saturated with IMP and $NADP^+$ under the conditions of the field cycling experiment.