Supporting Information

Structural Flexibility enhances the Reactivity of the Bioremediator Glycerophosphodiesterase by Fine-Tuning its Mechanism of Hydrolysis

Kieran S. Hadler,[a] Nataša Mitić,[a] Fernanda Ely,[a] Graeme R. Hanson,[b] Lawrence R Gahan,[a] James A. Larrabee,[c] David L. Ollis,[d] Gerhard Schenk [a*]

[a]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland, 4072, Australia; [b]Centre for Magnetic Resonance, The University of Queensland, St Lucia, Queensland, 4072, Australia; [c]Department of Chemistry and Biochemistry, Middlebury College, Middlebury, VT, 05753, USA; [d]Research School of Chemistry, Australian National University, Canberra, ACT, 0200, Australia;.

Address for correspondence:

School of Chemistry and Molecular Biosciences

University of Queensland

St Lucia, Queensland, 4072

Australia

Phone: +61 7 3365 4144

Fax: +61 7 3365 4299

Email: schenk@uq.edu.au

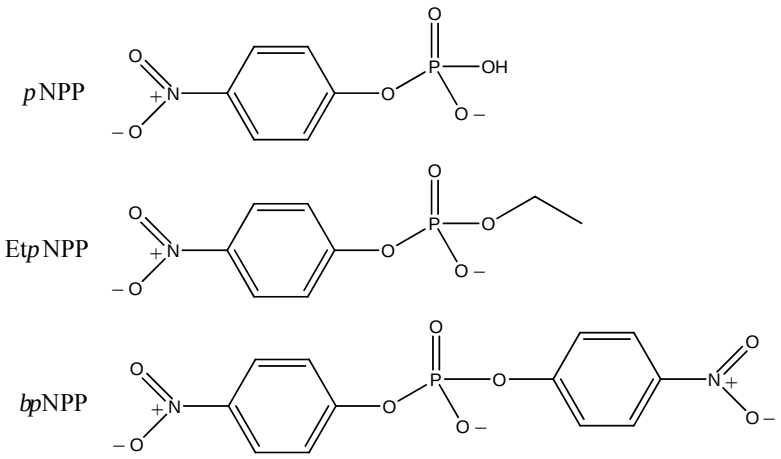

Figure S1. Chemical structures of the substrates *p*-nitrophenyl phosphate (*p*NPP), ethyl *p*-nitrophenyl phosphate (Et*p*NPP), and *bis(p*-nitrophenyl) phosphate (*bp*NPP).

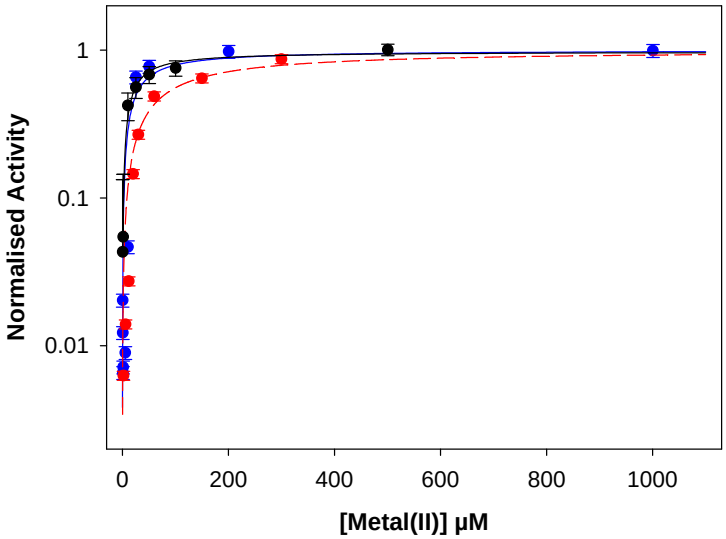

Figure S2. Determination of metal binding to GpdQ by measuring activity (using 5 mM *bp*NPP) as a function of added Co(II), Mn(II), or Cd(II) (blue, red, and black, respectively). The data were fit to the following equations as described elsewhere:[10]

$$r = \frac{n[M]_{free}}{K_d + [M]_{free}} \qquad \text{(S1)}$$

where r is the binding function, n is the number of sites associated with K_d, and $[M]_{free}$ is the free metal ion concentration. $[M]_{free}$ was calculated from eq. S2, where $[M]_{total}$ is the total concentration of metal ions added, and $[E]$ is the concentration of enzyme.

$$[M]_{free} = [M]_{total} - r[E] \qquad \text{(S2)}$$

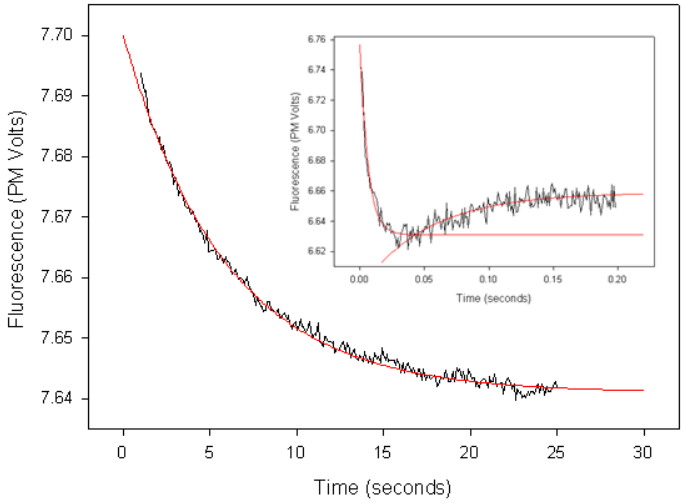

Figure S3. Sample progress curves showing the three fluorescence transient phases of the reaction between GpdQ and the substrate *p*NPP. *Main figure*: 5 µM GpdQ was reacted with 50.0 µM *p*NPP, and the progress was monitored over 25 s. *Inset*: 5 µM GpdQ was reacted with 500 µM *p*NPP, and fluorescence changes were monitored over 200 ms.

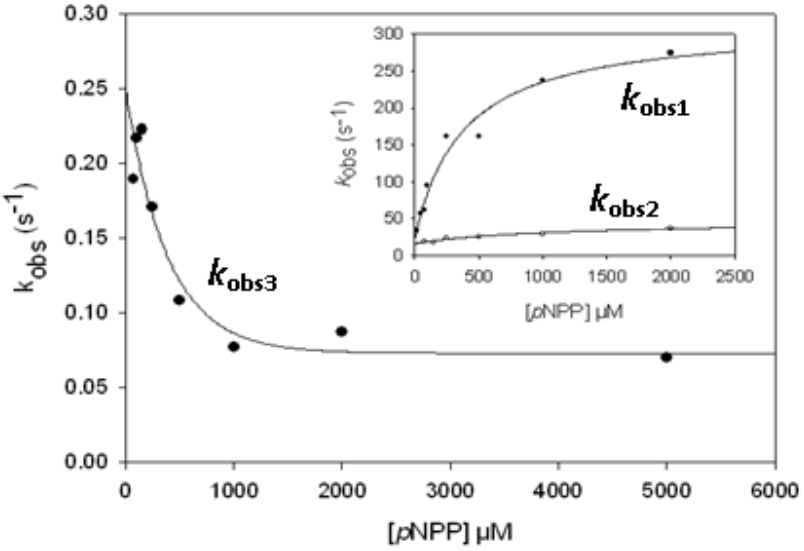

Figure S4. Effect of the concentration ([S]) of *p*NPP on the observed rates (k_{obs}) associated with the three fluorescence transients. The data were fit to equations described in the text.

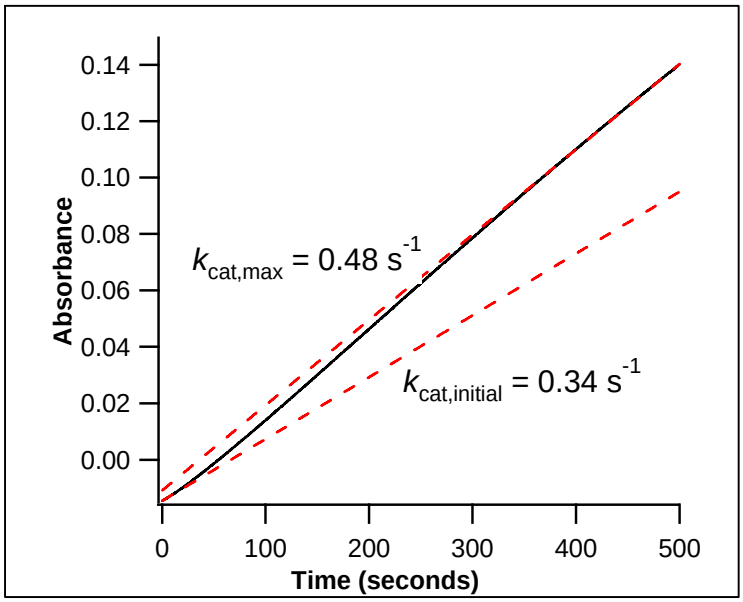

Figure S5. Example of the absorbance change measured over time for the reaction of GpdQ and *bp*NPP (5 mM). Note that the initial rate (k_{cat} = 0.34 s^{-1}) increases to the maximal rate ($k_{cat,max}$ = 0.48 s^{-1}) after 500 seconds.

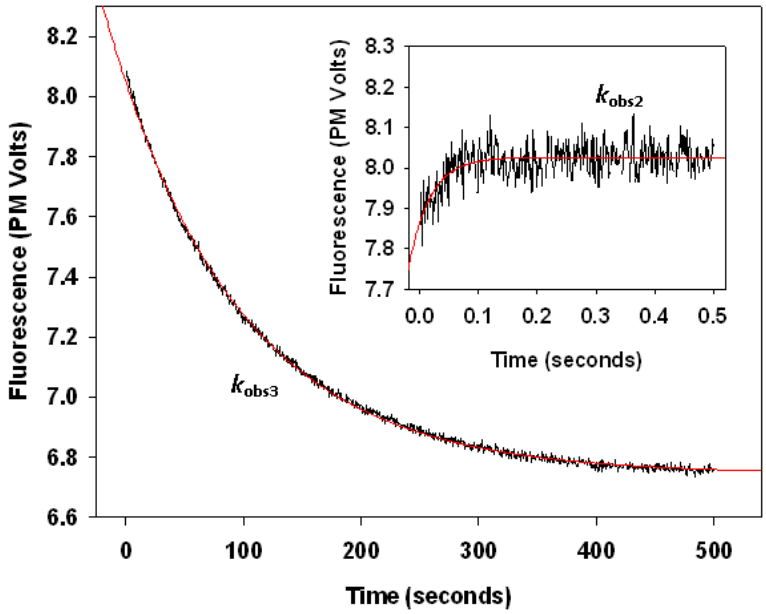

Figure S6. Reaction progress curves showing the changes in fluorescence during the reaction between N80A-GpdQ and the substrate *bp*NPP. *Main figure*: 1 µM N80A-GpdQ was reacted with 1.1 mM *bp*NPP and the progress was monitored over 500 s. The fitted k_{obs3} value was 0.540 ± 0.001 min^{-1} (*c.f.* k_{obs3} = 3.9 ± 0.1 min^{-1} for the wild type enzyme under identical conditions). *Inset*: 1 µM N80A-GpdQ was reacted with 120 µM *bp*NPP and fluorescence changes were monitored over 500 ms. The fitted k_{obs2} value was 28 ± 3 s^{-1}.

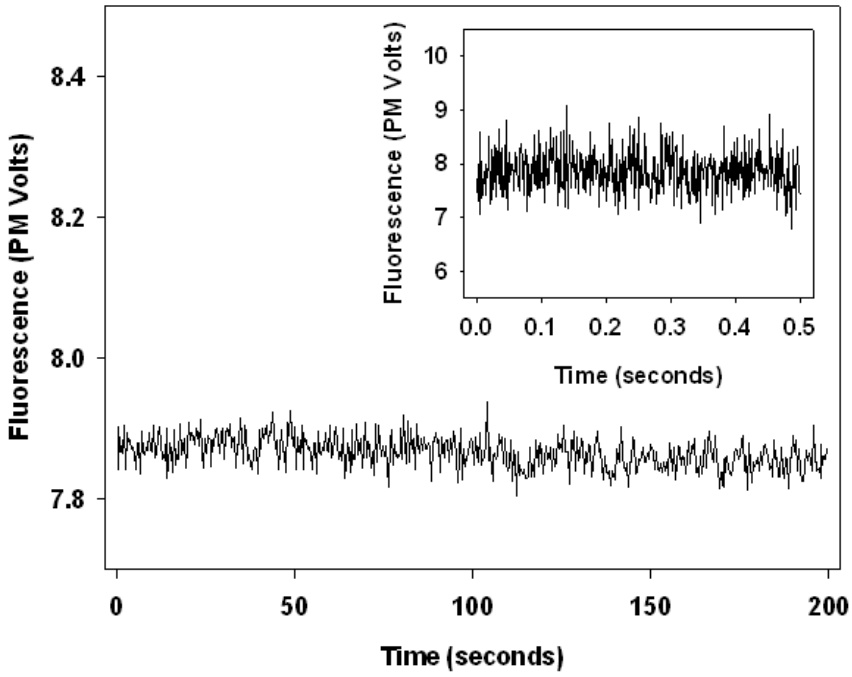

Figure S7. Reaction progress curves showing the changes in fluorescence during the reaction between N80D-GpdQ and the substrate *bp*NPP. *Main figure*: 5 μM N80D-GpdQ was reacted with 1.1 mM *bp*NPP and the progress was monitored over 200 s. *Inset*: 5 μM N80D-GpdQ was reacted with 120 μM *bp*NPP and fluorescence changes were monitored over 500 ms.