Supporting Information

Substrate Induced Formation of a Catalytically Competent Binuclear Center and Regulation of Reactivity in a Glycerophosphodiesterase from *Enterobacter aerogenes*

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Figure S1. Overall structure and oligomeric organization of GpdQ. *Upper Panel*. Monomer with active site metal ions shown as spheres and ligating residues as sticks. *Middle Panel*. Dimer with active site metal ions shown as spheres. *Lower Panel*. Trimer of dimers present in the asymmetric unit.

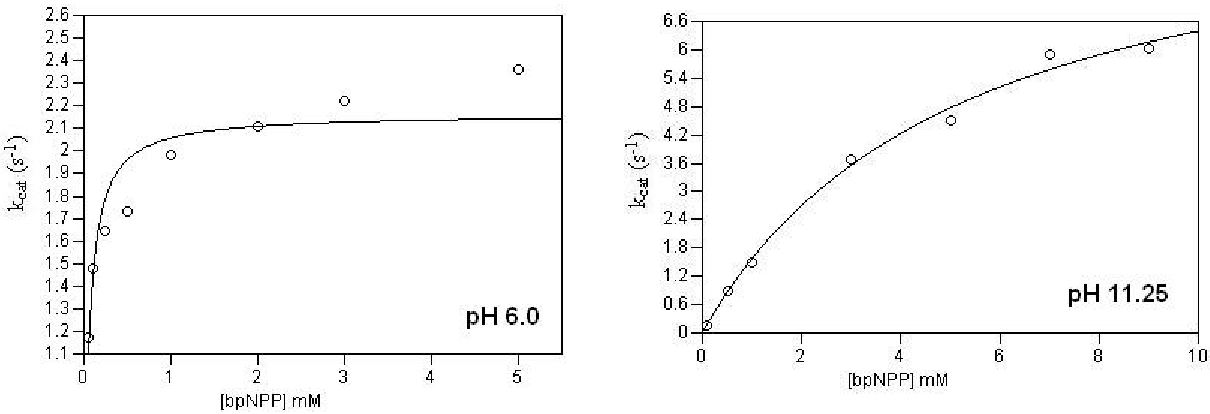

Figure S2. Examples of the effect of the substrate concentration (*bp*NPP) on the enzymatic activity of GpdQ. Rates were measured at pH 6.00 and pH 11.25.

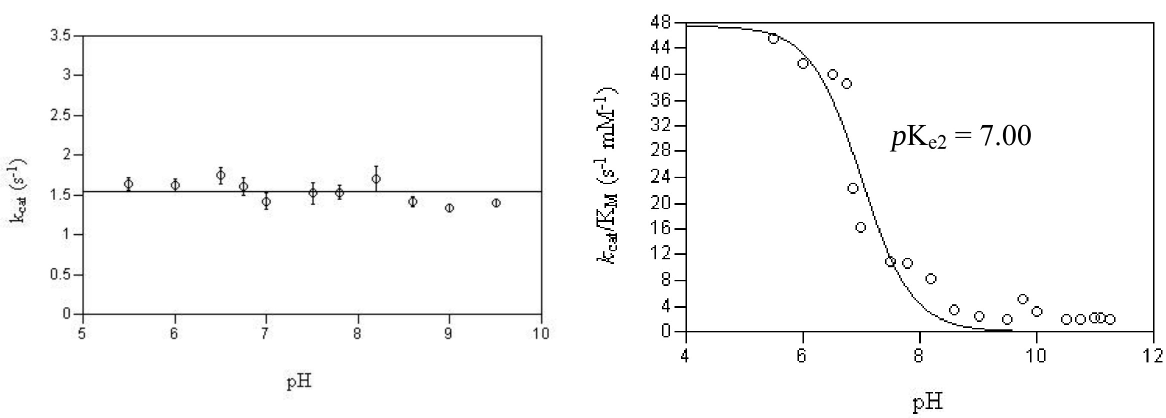

Figure S3. pH profile of k_{cat} and k_{cat}/K_M for the hydrolysis of *bp*NPP by GpdQ. The data in the lower panel were fit using a modified form of Equation 5, where $K_{e1} \gg [H^+]$:

$$\frac{k_{cat}}{K_M} = \frac{k_{cat}}{K_s\left(1 + \frac{K_{e2}}{[H^+]}\right)} \qquad \text{Equation S1}$$

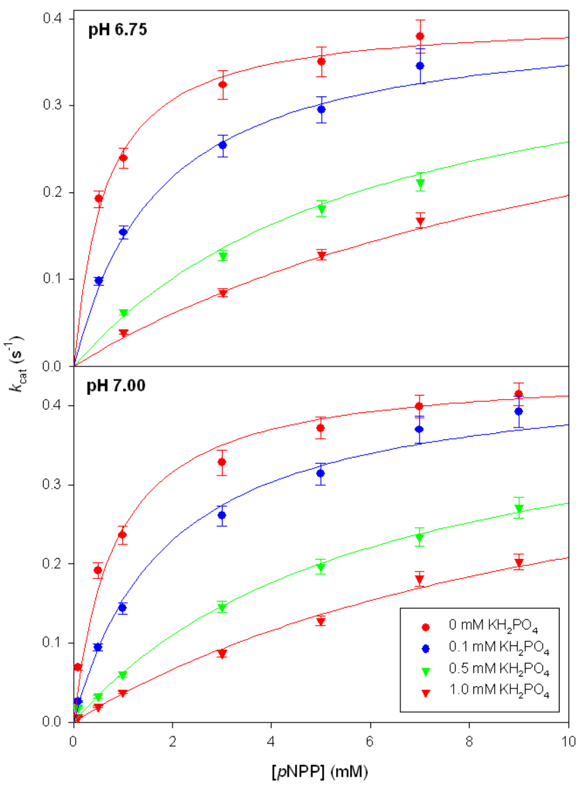

Figure S4. Effect of inhibition of 0 mM, 0.1 mM, 0.5 mM and 1.0 mM KH_2PO_4 towards the hydrolysis of *p*NPP. Data were measured at pH 7.0 and pH 6.75 under identical conditions and data were analyzed using Equation 6. The mode of inhibition is competitive with K_i = 78(6) and 57(4) µM, respectively.

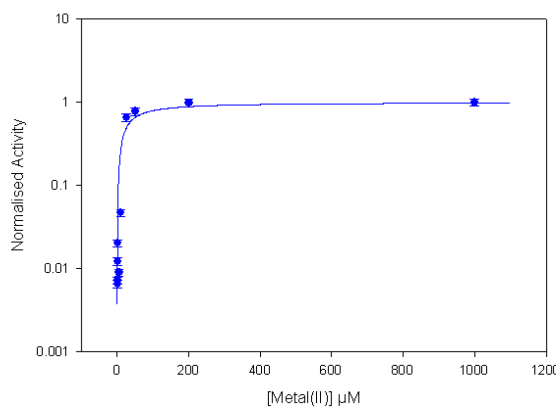

Figure S5. Determination of metal binding to GpdQ by measuring activity as a function of added Co^{II}.

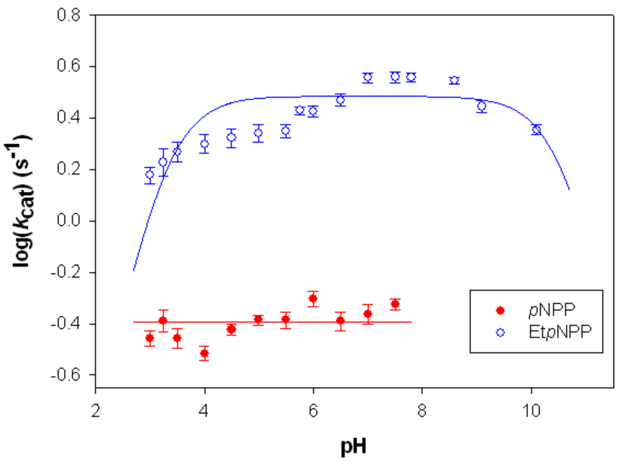

Figure S6. pH dependence of k_{cat} for the hydrolysis of Et*p*NPP and *p*NPP where the former has been fit to an equation derived for a model with two protonation equilibria:

$$k_{cat} = \frac{k_{cat,\max}}{1 + \frac{[H^+]}{K_{es1}} + \frac{K_{es2}}{[H^+]}} \qquad \text{Equation S2}$$

The effect of a viscogen (30% sucrose) on the value of k_{cat}/K_M for the hydrolysis of Et*p*NPP was also measured at several pHs. At pH 5.5: $k_{cat}/K_M = 10.7 \pm 1.6\ s^{-1}\ mM^{-1}$ (no sucrose), $9.7 \pm 1.3\ s^{-1}\ mM^{-1}$ (with 30% sucrose); at pH 7.0: $k_{cat}/K_M = 5.9 \pm 0.9\ s^{-1}\ mM^{-1}$ (no sucrose), $4.9 \pm 0.7\ s^{-1}\ mM^{-1}$ (with 30% sucrose); at pH 8.6: $k_{cat}/K_M = 0.42 \pm 0.06\ s^{-1}\ mM^{-1}$ (no sucrose), $0.33 \pm 0.05\ s^{-1}\ mM^{-1}$ (with 30% sucrose).

Full list of authors for references 61 and 89:

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