Supporting Information

"Kinetic Isotope Effects for Alkaline Phosphatase Reactions: Implications for the Role of Active-Site Metal Ions in Catalysis."

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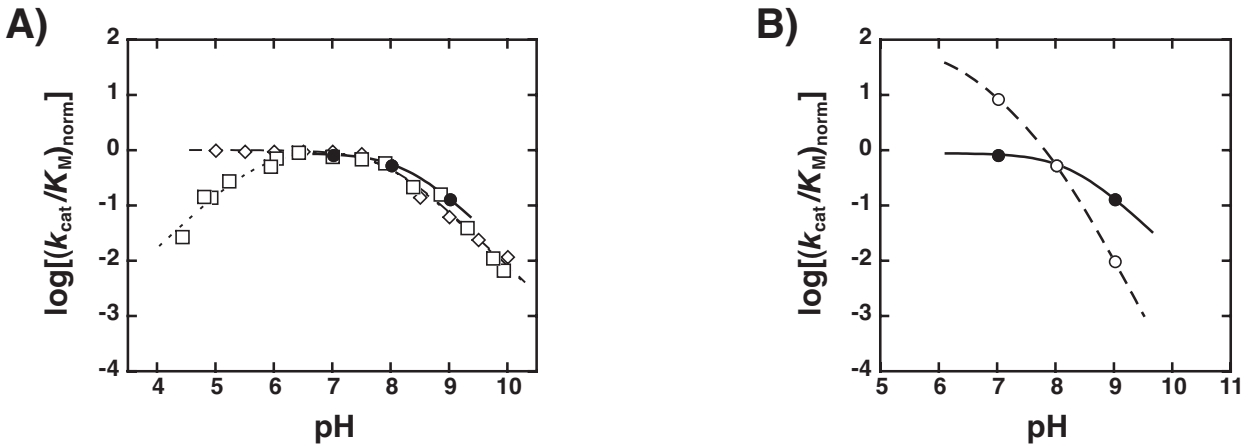
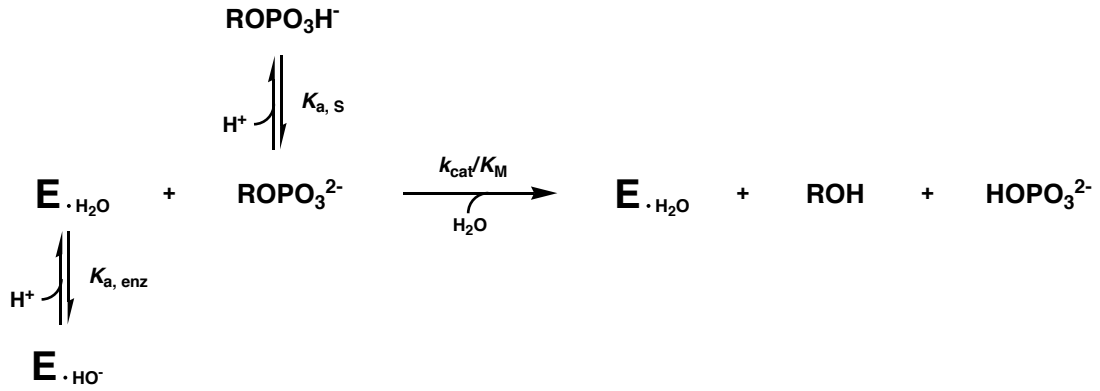


Figure S1. The mNBP dianion is the substrate for AP-catalyzed hydrolysis. (A) Overlay of the pH-rate profiles for the R166S AP-catalyzed reactions of mNBP (●, solid line) and pNPP (◇, dashed line) and the wt AP-catalyzed reaction of ethyl phosphate (□, dotted line). Data for pNPP and ethyl phosphate are from previous work (S1, S2). The values of k_{cat}/K_M were normalized by dividing by the maximum value of k_{cat}/K_M. The fit for ethyl phosphate (dotted line) is to a model with two rate-controlling ionizations (Scheme S1 and eq S1) and gives pK_a values of 5.6 ± 0.1 and 7.9 ± 0.1, respectively. The acidic limb corresponds to protonation of the phosphate monoester dianion substrate to give a phosphate monoester monoanion, which is not a substrate for AP (S2). The basic limb has been suggested to reflect deprotonation of a Zn^{2+}-coordinated water molecule to form an inactive metal-hydroxide complex (S2). The fits for mNBP (solid line) and pNPP (dashed line) were to a model with a single rate-controlling ionization (eq S2) because the data do not extend below the pK_a values for these substrates (6.2 for mNBP (S3) and 4.8 for pNPP (S4)). The fits gave pK_a values of 8.2 ± 0.1 and 7.9 ± 0.1, respectively, and the decrease in $(k_{cat}/K_M)_{norm}$ at high pH presumably arises from ionization of a Zn^{2+}-coordinated water molecule (S1, S2). (B) Comparison of the observed pH-rate profile for AP-catalyzed mNBP hydrolysis (●, solid line) versus that which would be expected if mNBP reacted via an alternative mechanism in which the monoanion is the substrate for catalysis (○, dashed line). To facilitate comparison, the values of $(k_{cat}/K_M)_{norm}$ were assumed to be equal at pH 8.0. If the monoanion of mNBP were the true substrate of AP (Scheme S2), the pH-rate profile would follow the dependence shown in eq S3 (dashed line), which diverges from the observed pH-rate profile.

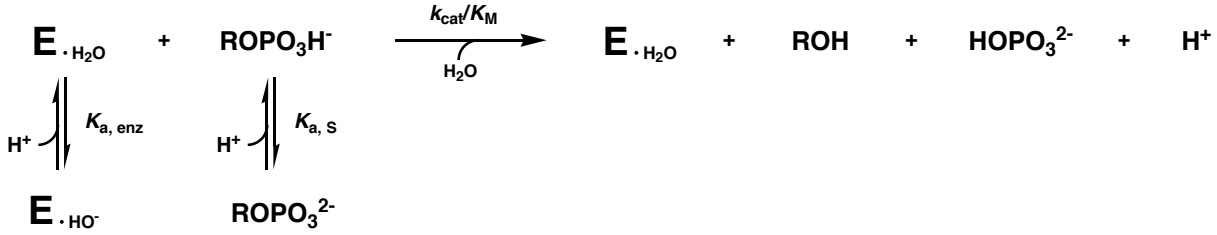
Scheme S1: mNBP reacts as a dianion



$$(k_{cat}/K_M)_{obs} = \frac{(k_{cat}/K_M)}{(1+10^{pH-pK_{a,enz}})(1+10^{pK_{a,S}-pH})} \quad \text{(S1)}$$

$$(k_{cat}/K_M)_{obs} \cong \frac{(k_{cat}/K_M)}{(1+10^{pH-pK_{a,enz}})} \quad , \quad (pH >> pK_{a,S}) \quad \text{(S2)}$$

Scheme S2: mNBP reacts as a monoanion



$$(k_{cat}/K_M)_{obs} = \frac{(k_{cat}/K_M)}{(1+10^{pH-pK_{a,enz}})(1+10^{pH-pK_{a,S}})} \quad \text{(S3)}$$

Supporting References

S1. O'Brien, P. J.; Herschlag, D., *Biochemistry* **2001**, *40*, 5691-5699.
S2. O'Brien, P. J.; Herschlag, D., *Biochemistry* **2002**, *41*, 3207-3225.
S3. Grzyska, P. K.; Czyryca, P. G.; Purcell, J.; Hengge, A. C., *J. Am. Chem. Soc.* **2003**, *125*, 13106-13111.
S4. Zalatan, J. G.; Fenn, T. D.; Brunger, A. T.; Herschlag, D., *Biochemistry* **2006**, *45*, 9788-9803.