Supporting Information for:

A Mechanistic Study of Protein Phosphatase-1 (PP1), A Catalytically Promiscuous Enzyme

Kinetic Isotope Effect Data Analysis.

For each isotope effect at least three reactions were run. The $^{15}N/^{14}N$ ratios were measured for the product (R_p) and of the remaining starting material (R_s) at partial reaction, as well as in the original mixture (R_o). The isotope effects were calculated using equations 1 and 2.[1]

$$\text{isotope effect} = \log (1 - f) / \log [(1 - f) (R_S / R_O)] \quad (1)$$

$$\text{isotope effect} = \log (1 - f) / \log (1 - f\ (R_P / R_O)) \quad (2)$$

For each isotope effect the value calculated from R_o and R_p (equation 1) and from R_o and R_s (equation 2) agreed within experimental error and these were averaged to give the results reported. The ^{15}N KIE is given directly from these equations. In the ^{18}O isotope effect experiments the observed KIEs given by the above equations were corrected for the ^{15}N isotope effect and for incomplete levels of isotopic incorporation.

Calculation of corrected ^{18}O kinetic isotope effects

In the ^{18}O isotope effect experiments the observed KIEs were corrected for the ^{15}N isotope effect and for incomplete levels of isotopic incorporation. The equations used for these corrections and their derivations have been described.[2]

For the labeled substrates and the mixtures used for the $^{18}k_{lg}$ experiments, the levels of isotopic incorporation and other quantities used in the correction equation were as follows. (Refer to figure 1S.)

pNPP

The fraction of ^{15}N, ^{18}O-labeled compound **A** in the remote-labeled mixture of **A** and **C** = b = 0.003653
The fraction of ^{15}N in the ^{15}N, ^{18}O-labeled compound **A** = x = 0.99,
The fraction of ^{18}O in the ^{15}N, ^{18}O-labeled compound **A** = y = 0.9012
The fraction of ^{15}N in the ^{14}N-labeled compound **C** = z = 0.0002

pNPMP

The fraction of ^{15}N, ^{18}O-labeled compound **D** in the remote-labeled mixture of **D** and **F** = b = 0.003701
The fraction of ^{15}N in the ^{15}N, ^{18}O-labeled compound **D** = x = 0.99,
The fraction of ^{18}O in the ^{15}N, ^{18}O-labeled compound **D** = y = 0.85
The fraction of ^{15}N in the ^{14}N-labeled compound **F** = z = 0.0003

For the labeled substrates and the mixtures used for the $^{18}k_{nonbridge}$ experiments, the levels of isotopic incorporation and other quantities used in the correction equation were as follows.

pNPP
The fraction of ^{15}N, ^{18}O-labeled compound **B** in the remote-labeled mixture of **B** and **C** = b = 0.003699
The fraction of ^{15}N in the ^{15}N, ^{18}O-labeled compound **B** = x = 0.99
The fraction of compound **B** with all three nonbridge oxygen atoms labeled = y = 0.895
The fraction of ^{15}N in the ^{14}N-labeled compound **C** = z = 0.0001

pNPMP
The fraction of ^{15}N, ^{18}O-labeled compound **E** in the remote-labeled mixture of **E** and **F** = b = 0.003237
The fraction of ^{15}N in the ^{15}N, ^{18}O-labeled compound **E** = x = 0.99
The fraction of compound **E** with both nonbridge oxygen atoms labeled = y = 0.732
The fraction of ^{15}N in the ^{14}N-labeled compound **F** = z = 0.0001

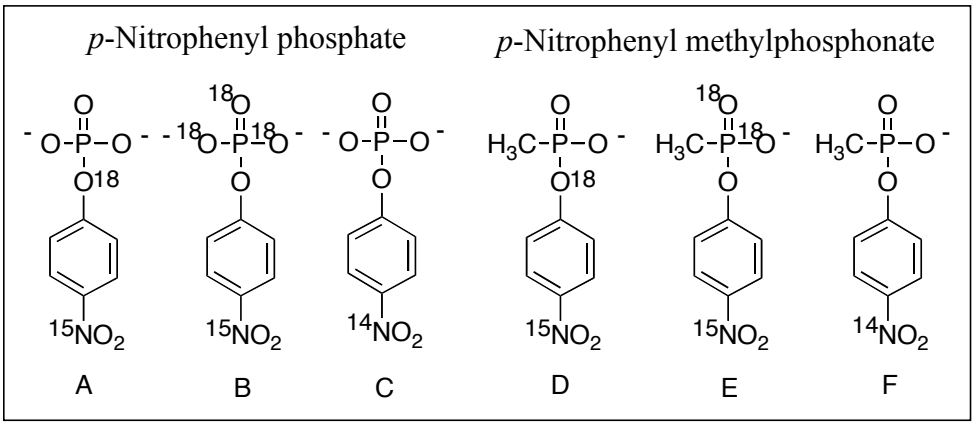

Figure 1S. Isotopic Isomers used in KIE Experiments

References

(1) Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* 1958, *1*, 15-76.
(2) Cleland, W. W. In *Isotope effects in chemistry and biology*; Kohen, A., Limbach, H.-H., Eds.; CRC Press: Boca Raton, FL, 2006, p 915-930; Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* 1984, *23*, 5479-88.