

**SUPPORTING INFORMATION**

**Conformational States of Human Purine Nucleoside  
Phosphorylase at Rest, at Work and with Transition State  
Analogues<sup>†</sup>**

Achelle A. Edwards<sup>‡</sup>, Jeremiah D. Tipton<sup>§</sup>, Michael D. Brenowitz<sup>‡</sup>, Mark R. Emmett<sup>§, #</sup>,

Alan G. Marshall<sup>§, #</sup>, Gary B. Evans<sup>¶</sup>, Peter C. Tyler<sup>¶</sup> and Vern L. Schramm<sup>\*, ‡</sup>

<sup>‡</sup>*Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461*

<sup>§</sup>*Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory,*

*Tallahassee, FL 32310*

<sup>#</sup>*Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL*

*32306*

<sup>¶</sup>*Carbohydrate Chemistry Team, Industrial Research Ltd., Lower Hutt, New Zealand.*

Supporting Table 4: H/D exchange rate kinetics for peptide H/D exchange experiments in phosphate. <sup>a</sup>					
Peptide 79-86		VMMQGRFH		7 exchangeable hydrogens	
Enzyme Complex	-1.5 < k	-1.5 > k > 1.5	k > 1.5	# Ex 8 hrs	% Total 8 hrs
PNP + Phos	3.00	2.00	2.00	3.50	50.0
PNP + Phos + ImmH	5.00	1.50	0.50	1.00	14.3
PNP + Phos + DATMe-ImmH	4.00	1.50	1.50	2.00	28.6
PNP + Phos + Inosine	3.00	2.00	2.00	3.50	50.0
Peptide 195-205		VAGPSFETVAE		9 exchangeable hydrogens	
Enzyme Complex	-1.5 < k	-1.5 > k > 1.5	k > 1.5	# Ex 8 hrs	% Total 8 hrs
PNP + Phos	5.50	3.00	0.50	3.00	33.3
PNP + Phos + ImmH	8.00	0.50	0.50	0.50	5.6
PNP + Phos + DATMe-ImmH	7.50	1.00	0.50	1.00	11.1
PNP + Phos + Inosine	6.50	2.00	0.50	2.00	22.2
Peptide 208-232		VLQKLGADAVGMSTVPEVIVA RHCG		23 exchangeable hydrogens	
Enzyme Complex	-1.5 < k	-1.5 > k > 1.5	k > 1.5	# Ex 8 hrs	% Total 8 hrs
PNP + Phos	16.00	4.00	3.00	6.00	26.1
PNP + Phos + ImmH	18.50	2.00	2.50	3.50	15.2
PNP + Phos + DATMe-ImmH	18.00	1.50	3.50	4.00	17.4
PNP + Phos + Inosine	15.00	4.00	4.00	6.00	26.1
Peptide 241-248		ITNKVIMD		7 exchangeable hydrogens	
Enzyme Complex	-1.5 < k	-1.5 > k > 1.5	k > 1.5	# Ex 8 hrs	% Total 8 hrs
PNP + Phos	2.50	2.50	2.00	4.50	64.3
PNP + Phos + ImmH	5.00	0.00	2.00	2.00	28.6
PNP + Phos + DATMe-ImmH	4.50	0.50	2.00	2.00	28.6
PNP + Phos + Inosine	3.50	2.00	1.50	4.00	57.1
<sup>a</sup> The number of hydrogens exchanged in 8 hours and the percent of hydrogens exchanged in 8 hours is represented as # Ex 8 hrs and % Total 8 hrs, respectively. k is the exchange rate constant covering three ranges: slow ( $-1.5 \text{ h}^{-1} < k$ ); medium ( $-1.5 \text{ h}^{-1} < k < 1.5 \text{ h}^{-1}$ ); and fast ( $k > 1.5 \text{ h}^{-1}$ ) calculated-by use of MEM software.					

Supporting Table 5: Summary of corrected sedimentation coefficients obtained for inhibitor-PNP or substrate-PNP complexes and their paired apo-enzyme controls.			
PNP•PO <sub>4</sub>	ImmH	% change in <i>S</i>	Mean % change in <i>S</i>
5.920 ± 0.002	6.049 ± 0.003	2.18	2.21 ± 0.23
5.833 ± 0.011	5.976 ± 0.002	2.45	
6.126 ± 0.004	6.248 ± 0.001	1.99	
DATMe			
6.131 ± 0.003	6.213 ± 0.002	1.34	0.95 ± 0.38
5.860 ± 0.003	5.894 ± 0.001	0.58	
5.860 ± 0.003	5.915 ± 0.002	0.94	
Inosine			
5.920 ± 0.002	5.957 ± 0.008	0.63	0.68 ± 0.05
6.137 ± 0.002	6.181 ± 0.002	0.72	
6.140 ± 0.004	6.183 ± 0.002	0.70	

Every run includes PNP•PO<sub>4</sub> as in internal control for temperature and speed variations. The complexes with ImmH, DATMe and Inosine all sediment more rapidly than PNP•PO<sub>4</sub> alone (the internal control). The PNP•PO<sub>4</sub>•Inosine and PNP•PO<sub>4</sub>•DATMe-ImmH sediment within experimental errors of each other but both sediment significantly slower than PNP•PO<sub>4</sub>•ImmH.

SUPPORTIING FIGURE 1: Maximum entropy method (MEM) plots showing the H/D exchange rate constant distribution derived from the H/D exchange time courses in Figure 6 and exchange rate kinetics in Table 4 (Supporting Information), for peptides V79 – H86, V195 – E205, V208 – G232 and I241 – D248. PNP + phosphate (blue); michaelis complex (red); DATMe-ImmH complex (green); and ImmH complex (gold). Highlighted in red in the peptide sequence are the residues implicated in nucleoside and/or phosphate binding (see text). The number of amide hydrogens for each resolved peak in the rate constant distribution for PNP + phosphate and the ImmH complex is shown above that peak (see Table 4 in Supporting Information). Quantitation of fast to slowly exchanged amide protons is represented in the x-axis from right to left.

Supporting Figure 1.

