

# Supporting Information

Saen-oon et al. 10.1073/pnas.0808413105

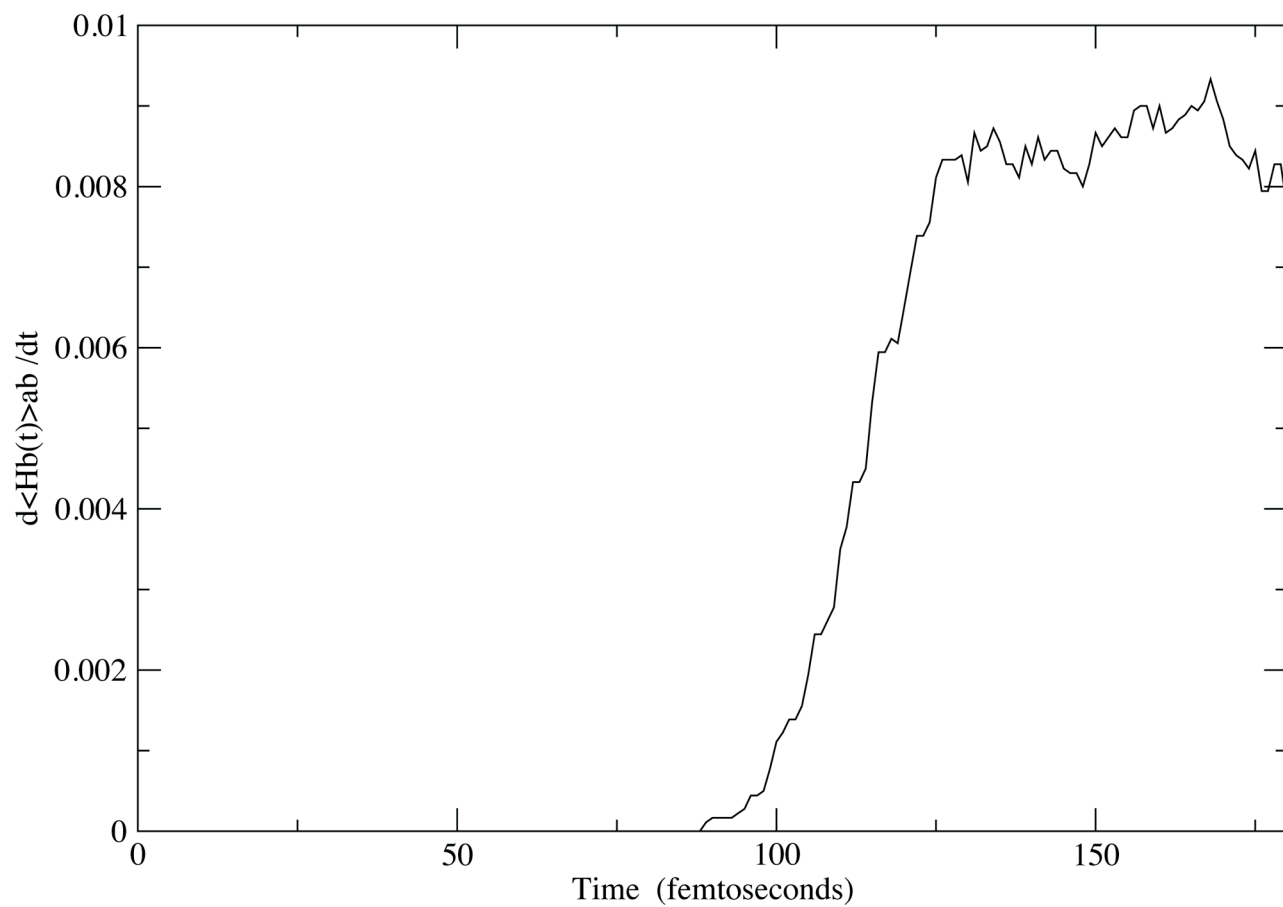
## SI Text

**Classical Molecular Dynamics Simulations.** The initial model was solvated in an orthorhombic box of TIP3P waters [Jorgensen WL, Chandrasekhar J, Madura J, Impey R, Klein M (1983) Comparison of simple potential functions for simulating liquid water. *J Chem Phys* 79:926] and the dimension of the box was chosen such that the minimum distance between solute and the edge of the box was 8 Å, giving a final dimension of  $96 \times 96 \times 65$  Å.  $\text{Na}^+$  and  $\text{Cl}^-$  ions were added to neutralize the system. The resulting system has total of 57,873 atoms (including 13,507 atoms of solute, 14,779 water molecules and 20 of  $\text{Na}^+$  and 6 of  $\text{Cl}^-$  ions). The SHAKE algorithm was applied to all MM bonds involving hydrogen atoms, and a 1-fs time step was used. Nonbonded interactions were calculated within 12 Å cutoff distance, and a smoothing function to gradually reduce to zero this contribution between 12 Å and 14 Å was applied. We performed stepwise minimizations to remove steric clashes between atoms of protein and water molecules. Initially, water molecules were minimized by 2,000 steps of steepest descent (SD) while the position of protein was retained with a harmonic force of 50 kcal/mol Å<sup>2</sup>. The harmonic force was lowered to 5 kcal/mol Å<sup>2</sup> for guanosine and phosphate molecules to retain their position as in crystal structure, while the protein and waters

were further minimized with 1,000 steps of SD followed by adopted-basis Newton–Raphson (ABNR) until a convergence in RMSD gradient of 0.001 kcal/mol Å. The temperature of the system was slowly increased over the first 50 ps of the heating phase from 0 to 300 K of constant pressure MD. The temperature was checked every 2,000 steps to ensure it is within  $300 \pm 5$  K. The Langevin piston method was used to maintain constant pressure using the following parameters for the algorithm: collision frequency = 20 ps<sup>-1</sup>, piston mass = 400 amu, and the piston was coupled until 400 ps. The final equilibrated structures retained the interactions between guanosine/phosphate and catalytic site residues as observed in the crystal structure.

**Frequency Factor.** To confirm that the trajectories were long enough, the frequency factor was calculated from the time derivative of the path average ( $d \langle H_b(t) \rangle_{ab}/dt$ ) and the existence of a plateau was verified.

**Configurations for Transition State Ensembles.** The configurations for which the value of  $P_p$  was in the range 0.4–0.6 were identified as transition state structures, see Fig. 2. The structural parameters of all transition state configurations from 4 transition paths, each from a unique area of phase space, are given in [Tables S1–S4](#).



**Fig. S1.** The frequency factor was calculated from the time derivative of the path average ( $d\langle H_b(t) \rangle_{ab}/dt$ ). The product region (b) was defined using the the bond-breaking ( $C1'-N9$ ) and bond-forming ( $C1'-O_p$ ) as order parameters. The product region (b) included all configurations that had a bond length of  $C1'-N9$  longer than  $1.7\text{\AA}$  and a bond length of  $C1'-O_p$  shorter than  $1.8\text{\AA}$ . The time derivative plateaus at  $\approx 150$  fs.

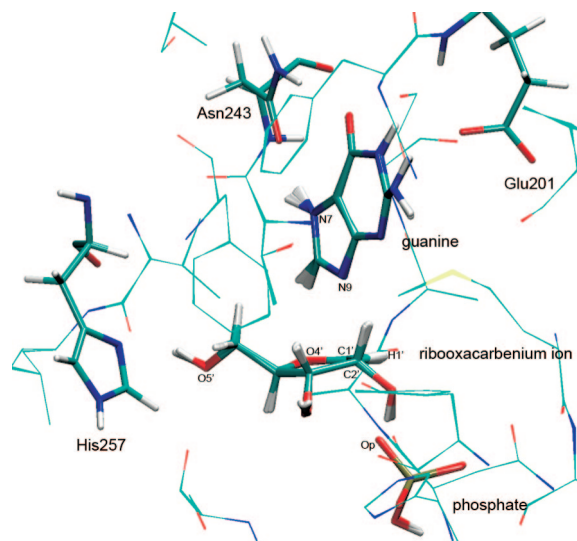
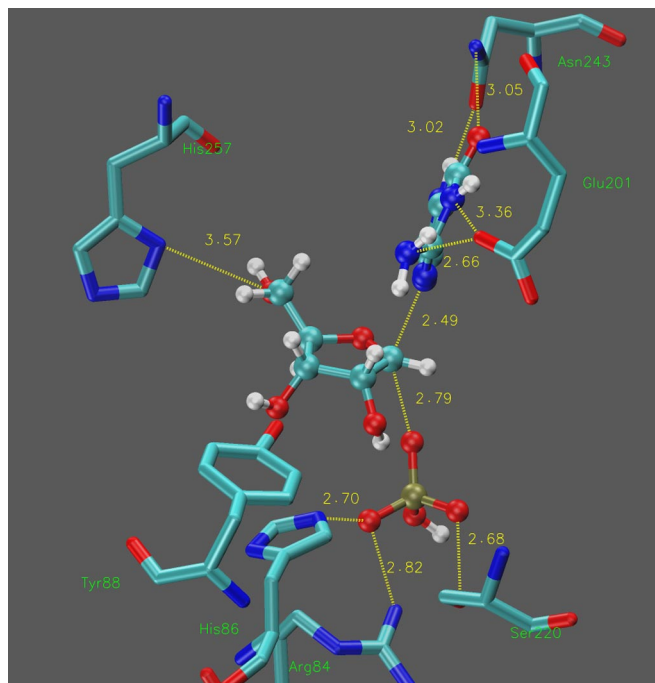


Fig. S2. Snapshot of 10 transition state structures taken from a single reactive trajectory (TS1).



**Movie S1.** A 250 fsec reactive trajectory generated by the TPS algorithm reveals the reaction mechanism as the reaction progresses from reactant (guanosine + phosphate) to product (guanine + ribose-1 phosphate). The reaction mechanism involves the cleavage of the N-ribosidic bond to form transition states with substantial ribooxacarbenium ion character, followed by conformational changes in the enzyme and the ribosyl group leading to migration of the anomeric carbon of the ribosyl group toward phosphate, to form the product ribose 1-phosphate. A movie of dynamic atomic motion for transition state passage (a 250-fs trajectory) is available at <http://risc1.aecom.yu.edu/suwipa/pnptps-movie.mpg>.

[Movie S1 \(MPG\)](#)

**Table S1. Structural parameters for ten PNP transition states, phase space 1**

Parameters	Reactant	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	TS10	Product
$P_p$	0	0.56	0.50	0.51	0.58	0.53	0.60	0.52	0.54	0.56	0.49	1
$d(\text{C1}' - \text{N9}), \text{\AA}$	1.47	2.49	2.51	2.52	2.54	2.56	2.57	2.58	2.59	2.60	2.61	3.45
$d(\text{C1}' - \text{O}^p), \text{\AA}$	3.15	2.79	2.78	2.77	2.76	2.75	2.75	2.75	2.75	2.75	2.75	1.50
$d(\text{O4}' - \text{O5}'), \text{\AA}$	3.10	2.99	2.96	2.93	2.90	2.88	2.86	2.86	2.85	2.85	2.85	3.32
$\phi_{\text{oxa}}, \text{deg.}^*$	100	179	178	177	176	175	175	176	177	178	180	159

\* $\phi_{\text{oxa}}$  is the dihedral angle of O4'-C1'-C2'-H1' monitoring the orbital hybridization of the C1' reactive center from  $sp^3$  to  $sp^2$ .

**Table S2. Structural parameters for fifteen PNP transition states, phase space 2**

Parameters	Reactant	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	TS10	TS11	TS12	TS13	TS14	TS15	Product
$P_p$	0	0.46	0.53	0.6	0.53	0.48	0.53	0.55	0.46	0.54	0.44	0.48	0.45	0.5	0.6	0.57	1
$d(\text{C1}' - \text{N9}), \text{\AA}$	1.45	2.45	2.47	2.48	2.50	2.51	2.53	2.54	2.56	2.57	2.58	2.59	2.60	2.61	2.62	2.63	3.46
$d(\text{C1}' - \text{O}^p), \text{\AA}$	3.18	2.84	2.82	2.81	2.79	2.78	2.77	2.77	2.76	2.76	2.76	2.76	2.76	2.76	2.76	2.77	1.37
$d(\text{O4}' - \text{O5}'), \text{\AA}$	2.98	3.04	3.02	2.99	2.96	2.93	2.90	2.88	2.87	2.85	2.85	2.85	2.85	2.86	2.86	2.87	3.19
$\phi_{\text{oxa}}, \text{deg.}^*$	113	177	179	179	178	177	176	175	175	175	176	177	179	178	175	172	136

\*See Table S1.

**Table S3. Structural parameters for six PNP transition states, phase space 3**

Parameters	Reactant	TS1	TS2	TS3	TS4	TS5	TS6	Product
$P_p$	0	0.41	0.48	0.58	0.57	0.53	0.51	1
$d(C1' - N9), \text{\AA}$	1.54	2.47	2.48	2.49	2.51	2.52	2.54	3.71
$d(C1' - O^p), \text{\AA}$	2.94	2.82	2.80	2.79	2.78	2.76	2.76	1.37
$d(O4' - O5'), \text{\AA}$	3.10	3.05	3.02	3.00	2.96	2.94	2.91	3.19
$\phi_{\text{oxa}}, \text{deg.}^*$	125	177	179	180	179	178	177	113

\*See Table S1.

**Table S4. Structural parameters for eleven PNP transition states, phase space 4**

Parameters	Reactant	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	TS10	TS11	Product
$P_p$	0	0.42	0.45	0.44	0.47	0.6	0.51	0.6	0.40	0.54	0.42	0.51	1
$d(\text{C1}' - \text{N9}), \text{\AA}$	1.62	2.48	2.49	2.50	2.51	2.53	2.54	2.55	2.57	2.59	2.61	2.61	3.39
$d(\text{C1}' - \text{O}^p), \text{\AA}$	2.78	2.80	2.79	2.78	2.77	2.76	2.75	2.74	2.74	2.74	2.74	2.74	1.51
$d(\text{O4}' - \text{O5}'), \text{\AA}$	3.10	3.05	3.03	3.00	2.97	2.94	2.91	2.89	2.87	2.86	2.86	2.86	3.37
$\phi_{\text{oxa}}, \text{deg.}^*$	132	177	179	180	180	178	178	177	177	179	178	176	123

\*See Table S1.