SUPPORTING INFORMATION

dGMP Binding to Thymidylate Kinase from *Plasmodium falciparum* shows half-site binding and induces protein dynamics at the dimer interface.

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Supplemental Figures

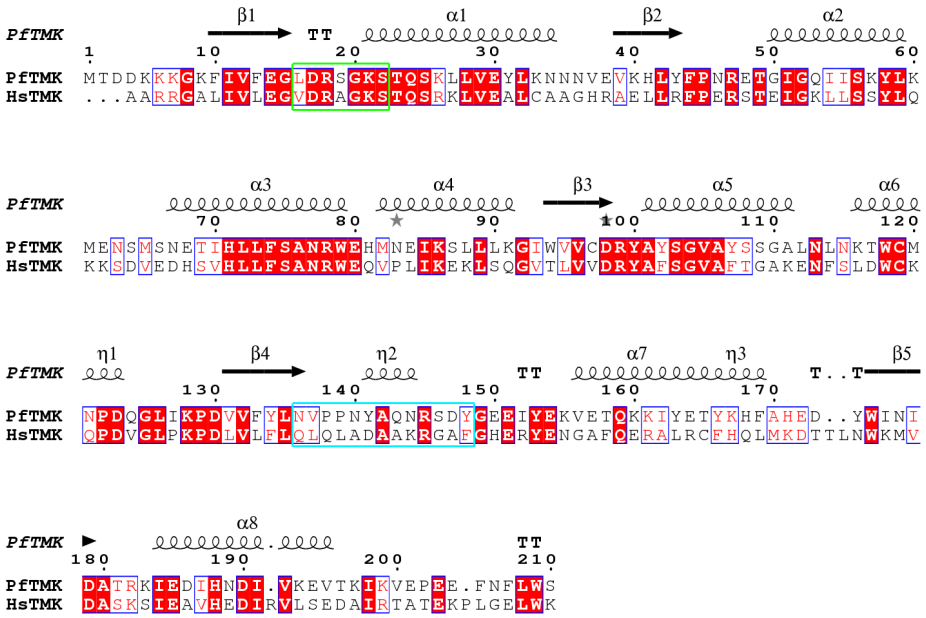

Figure S1. Sequence alignment of PfTMK and hsTMK. Sequence alignment showed 41% identity between the two amino acid sequences. The well-conserved P-loop is boxed in green, and poorly-conserved LID domain is boxed in cyan. This explains the low root-mean-squared deviation (RMSD) for superposition of the P-loops and high RMSD for the LID domains.

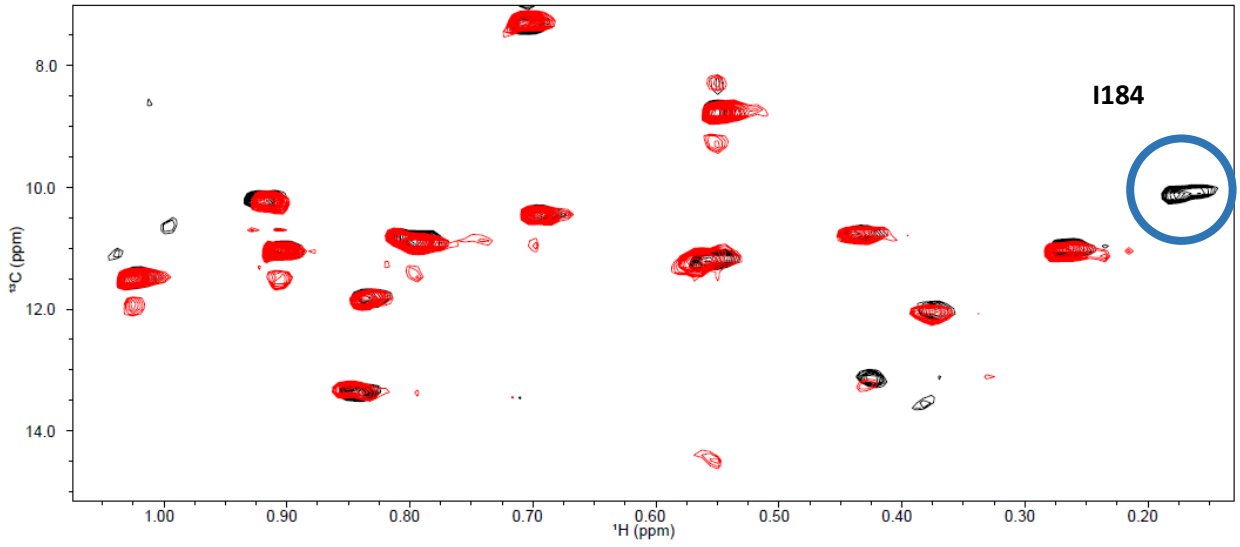

Figure S2. Isoleucine region of overlay of WT-PfTMK (black) and I184V spectra (red). From the overlay spectra, the WT spectrum exhibits one additional peak than the I184V spectrum (highlighted with circle), allowing the assignment of this peak to I184. Obtained with saturating dTMP and ADP and 10 mM Mg^{2+}.

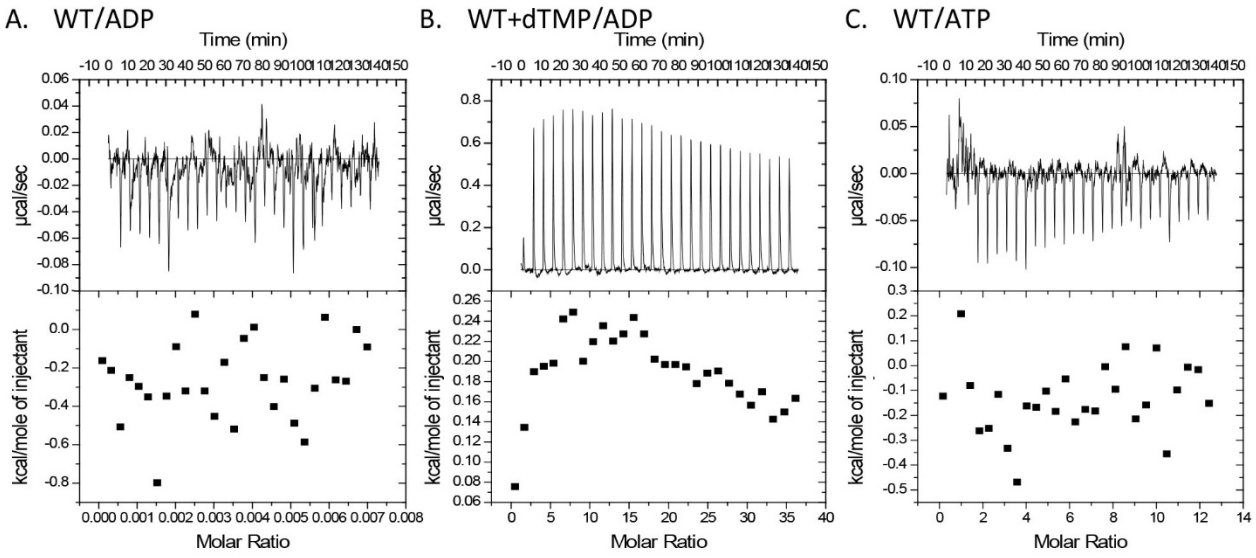

Figure S3. ITC profiles of ADP and ATP binding to WT PfTMK. Panel A: ITC profiles of ADP binding to PfTMK; Panel B: ITC profiles of ADP binding to dTMP-PfTMK complex; Panel C: ITC profiles of ATP binding to PfTMK. All top panels illustrate the raw titration data, bottom panel shows integrated binding isotherms and fitted results. The binding isotherms suggest that ADP and ATP bind to PfTMK in a mode that produce little heat change.

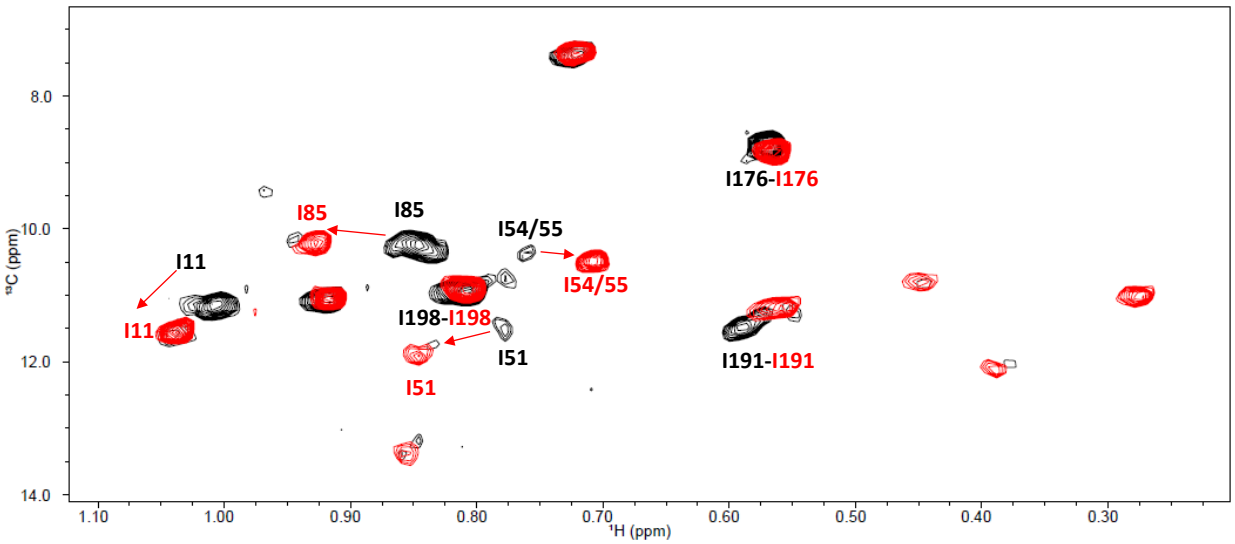

Figure S4. Overlay of 2D HMQC spectrum of WT-PfTMK with no dTMP (black) and spectrum of WT-PfTMK with 1 mM dTMP (red). Only the isoleucine region is depicted here, peak position with no dTMP bound is denoted in black, peak position with 1 mM dTMP is denoted in red. Chemical shift perturbation and peak movement is denoted in red.

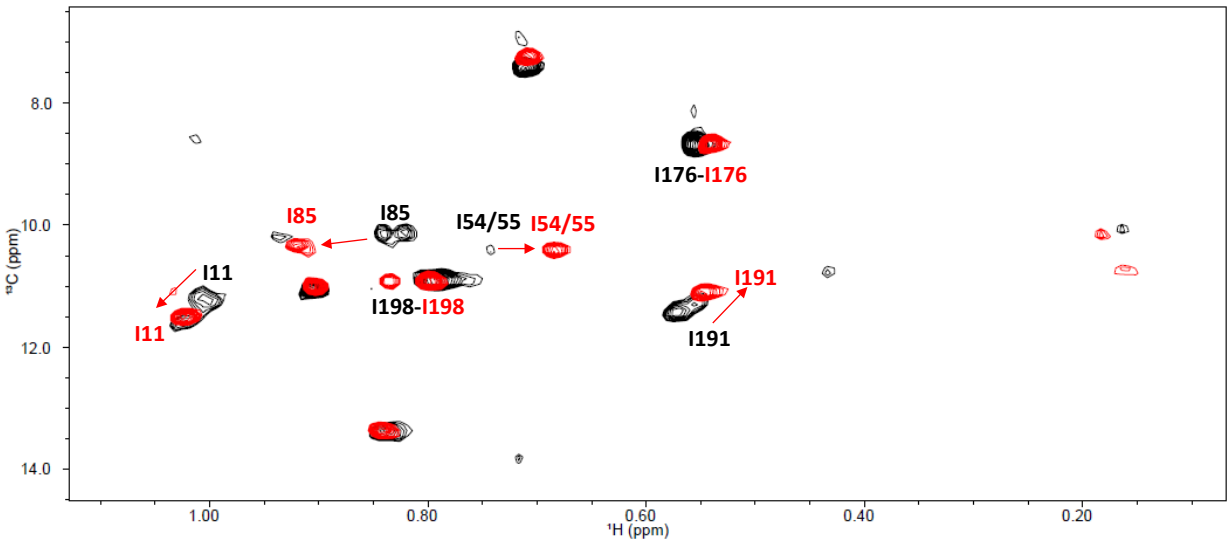

Figure S5. Overlay of 2D HMQC spectrum of WT-PfTMK with no dGMP (black) and spectrum of WT-PfTMK with 1 mM dGMP (red). Only the isoleucine region is depicted here, peak position with no dGMP bound is denoted in black, peak position with 1 mM dGMP is denoted in red. Chemical shift perturbation and peak movement is denoted in red.

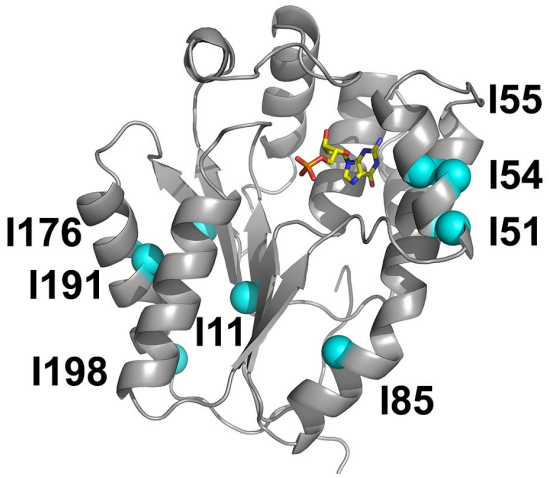

Figure S6. Ile residues that show chemical shift changes due to binding of TMP or dGMP.

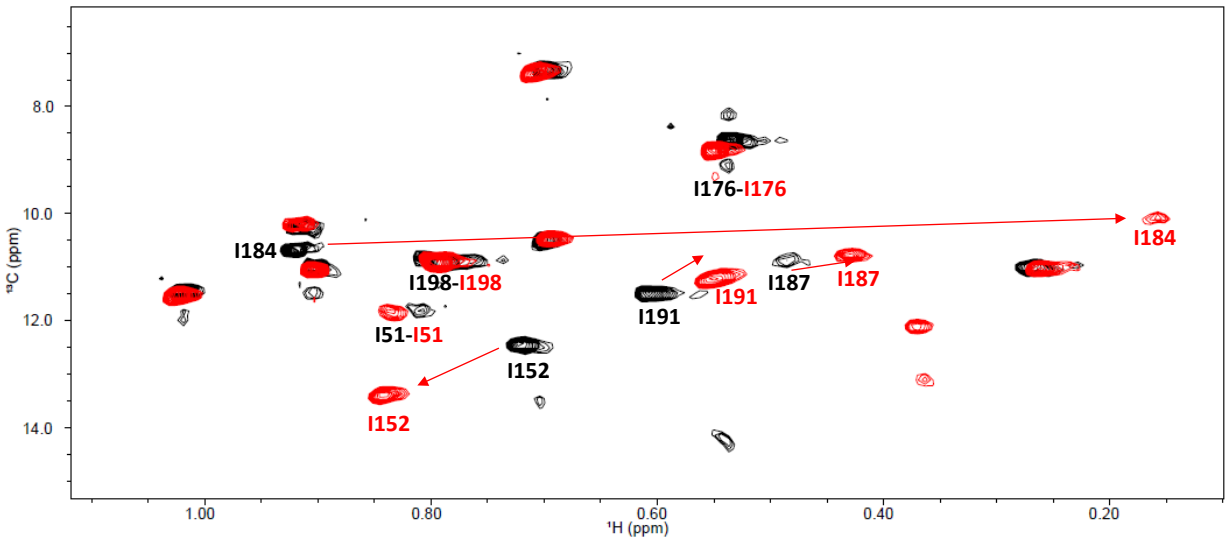

Figure S7. Overlay of 2D HMQC spectrum of WT-PfTMK with no ADP (black) and spectrum of WT-PfTMK with 10 mM ADP (red). Only the isoleucine region is depicted here, peak position under non-ADP condition is denoted in black, peak position under 10 mM ADP is denoted in red. Chemical shift perturbation and peak movement is denoted in red. I184 exhibited a significant chemical shift perturbation under ADP addition, as expected since I184 is close to the adenine ring.

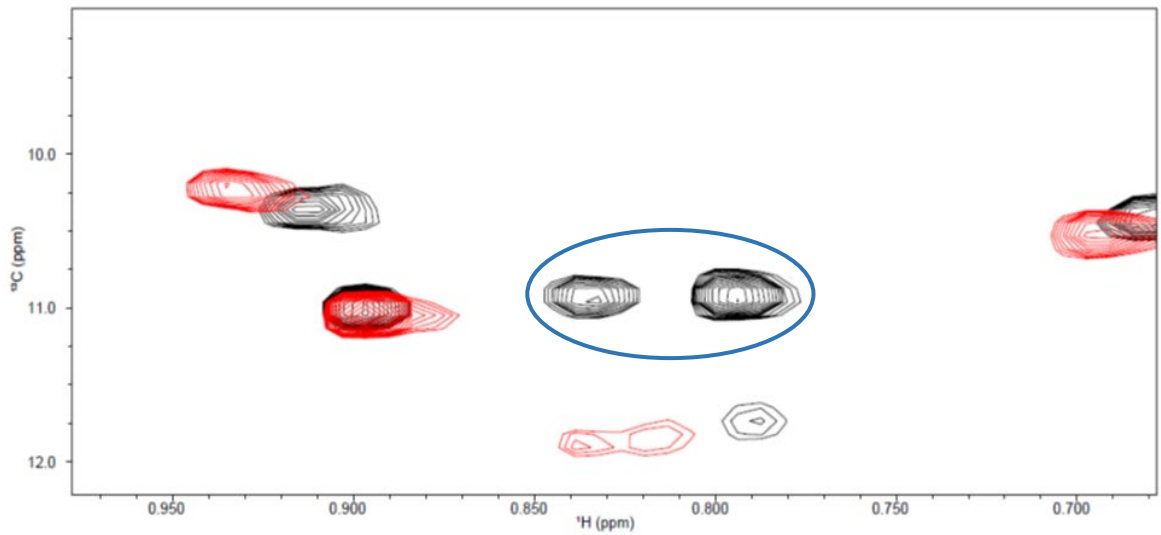

Figure S8. Overlay of 2D HMQC spectrum Isoleucine region of WT-PfTMK (black) and spectrum of I198V mutant (red) in saturated concentration of dGMP. I198V mutant lacks two peaks in the isoleucine region (highlighted with circle), suggesting peak splitting occurs in the dGMP saturated WT PfTMK sample.

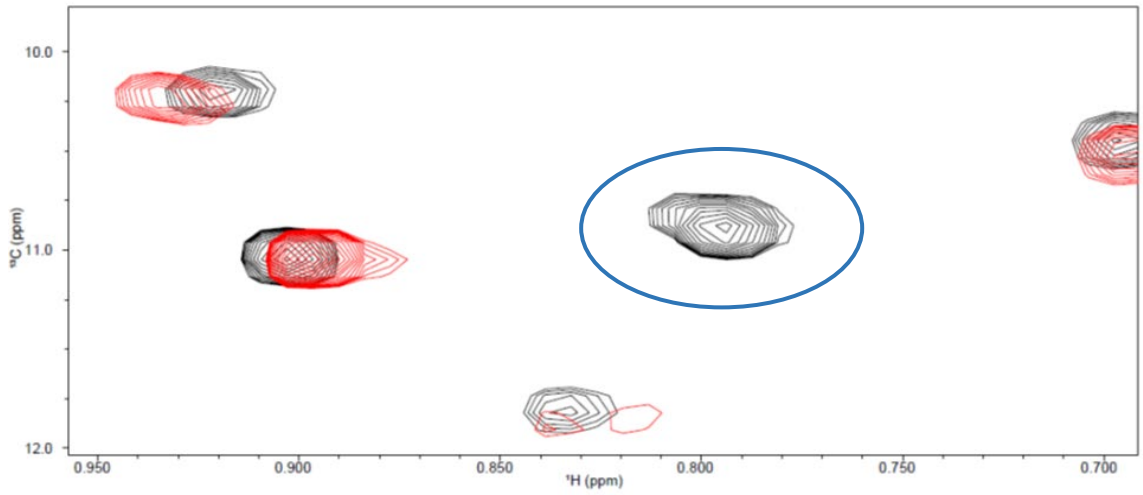

Figure S9. Overlay of 2D HMQC spectrum Isoleucine region of WT-PfTMK (black) and spectrum of I198V mutant (red) in saturated concentration of dTMP. Note that most peaks are consistent between WT and mutant spectrum, and the I198V mutant is missing only one peak in the isoleucine region (highlighted with circle).

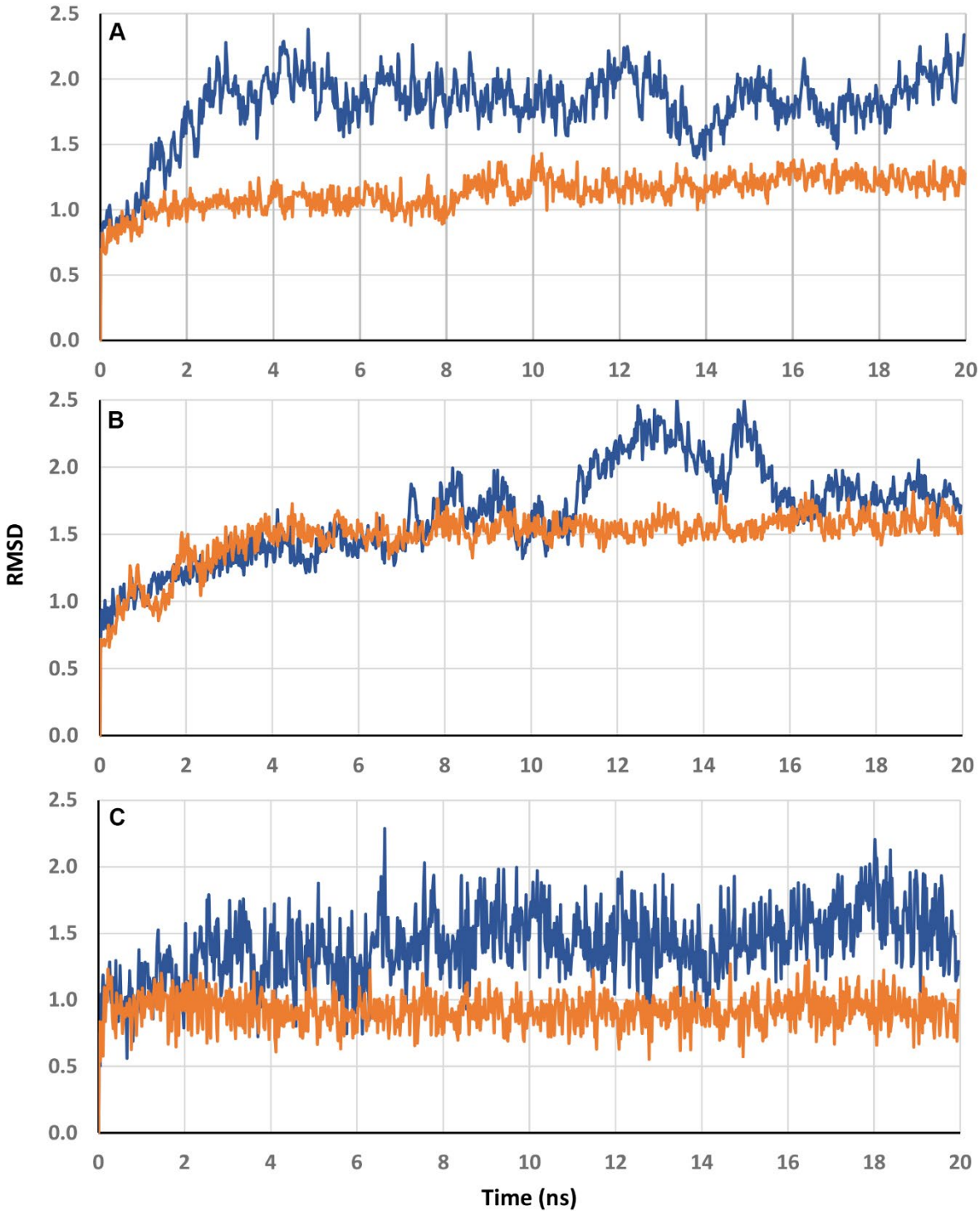

Figure S10: Root-mean-square deviation (RMSD) for hemi-liganded structures during 20 ns molecular dynamics calculation. Blue is for dGMP and orange is for TMP. A) subunit with bound nucleotide monophosphate (chain A), backbone atoms, B) subunit without bound nucleotide monophosphate (chain B), backbone atoms, C) bound nucleotide (dTMP or dGMP), all atoms.

Chart 1: NAMD Script for 20ns Molecular Dynamics Calculations.

```
structure          solvate.psf
coordinates        solv_center.pdb

bincoordinates     PfTMK_eq.restart.coor
binvelocities      PfTMK_eq.restart.vel
extendedSystem     PfTMK_eq.restart.xsc

set temperature    310
set outputname     PfTMK_20a_md

firsttimestep      0


# Input
paraTypeCharmm             on
parameters          par_all27_prot_na_lipids_XMP.inp
#temperature         $temperature

# Force-Field Parameters
exclude             scaled1-4
1-4scaling          1.0
cutoff              12.0
switching           on
switchdist          10.0
pairlistdist        14.0

# Integrator Parameters
timestep            2.0  ;# 2fs/step
rigidBonds          all  ;# needed for 2fs steps
nonbondedFreq       1
fullElectFrequency  2
stepspercycle       10

# Constant Temperature Control
langevin            on    ;# do langevin dynamics
langevinDamping     1     ;# damping coefficient (gamma) of 1/ps
langevinTemp        $temperature
langevinHydrogen    off    ;# don't couple langevin bath to hydrogens

# Periodic Boundary Conditions |min-max|
cellBasisVector1   56.23    0.   0.0
cellBasisVector2     0.0  100.70 0.0
cellBasisVector3     0.0    0   80.75
cellOrigin           0.0   0.0   0.0

wrapAll             on


# PME (for full-system periodic electrostatics)
PME                 yes
PMEGridSpacing      1.0
```

```
# Constant Pressure Control (variable volume)
useGroupPressure      yes ;# needed for rigidBonds
useFlexibleCell       no
useConstantArea       no

langevinPiston        on
langevinPistonTarget  1.01325 ;#  in bar -> 1 atm
langevinPistonPeriod  100.0
langevinPistonDecay   50.0
langevinPistonTemp    $temperature


# Output
outputName          $outputname

restartfreq         1000
dcdfreq             1000
xstFreq             1000
outputEnergies      200
outputPressure      200

run 10000000
```