Supporting information for:

The allosteric impact of the variable insert loop in Vaccinia H1-related (VHR) phosphatase

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Table S1: R_{ex} values and uncertainties (s^{-1}) for each residue of apo, WO_4-bound WT and WO_4-bound N74A. The numbers in red indicate those that are above 2σ of the mean. The dashes indicate residue for which the R_{ex} value could not be determined.

Residue	Apo WT	WO_4 WT	WO_4 N74A
S2	1.1 ± 0.6	-1.1 ± 0.5	-
G3	-0.9 ± 0.6	-1.7 ± 0.6	-2.9 ± 0.7
S4	-1.6 ± 0.6	-2.1 ± 0.6	-2.2 ± 0.6
F5	-0.1 ± 0.7	-1.3 ± 0.7	-1.3 ± 0.7
E6	-2. ± 0.7	-0.5 ± 0.7	-0.6 ± 0.7
L7	1.5 ± 0.6	2.2 ± 0.6	3.5 ± 0.6
S8	-0.9 ± 0.6	-3.5 ± 1.	-0.9 ± 0.7
V9	-2.2 ± 0.5	-3. ± 0.5	-
Q10	-	-1.5 ± 0.6	-0.4 ± 0.5
D11	-1.2 ± 0.5	-1.6 ± 0.6	-0.6 ± 0.5
L12	-1.8 ± 0.5	-2.6 ± 0.6	-2.1 ± 0.5
N13	-1.5 ± 0.5	**6.6 ± 0.6**	-
D14	-0.1 ± 0.5	-4.8 ± 0.9	0.6 ± 0.7
L15	0.2 ± 0.5	1.1 ± 0.7	-0.4 ± 0.5
L16	-1.3 ± 0.5	-	-0.5 ± 0.5
S17	-2.1 ± 0.5	-1.4 ± 0.6	-1.9 ± 0.5
D18	-1.3 ± 0.6	-1.7 ± 0.6	-0.3 ± 0.6
G19	3.3 ± 0.6	**9. ± 0.8**	**10.2 ± 0.8**
S20	-1.7 ± 0.7	-1.7 ± 0.7	-1.3 ± 0.7
G21	-0.5 ± 0.6	-1.6 ± 0.6	-0.3 ± 0.6
C22	0.2 ± 0.6	-0.4 ± 0.6	0. ± 0.6
Y23	1.1 ± 0.6	0.6 ± 0.6	2.7 ± 0.6
S24	-2.2 ± 0.6	-0.9 ± 0.9	-2.3 ± 0.9
L25	-	-	-
S27	-	0.8 ± 0.5	1.2 ± 0.5
Q28	1.1 ± 0.5	2.5 ± 0.8	3.5 ± 0.6
C30	1.5 ± 0.5	3. ± 0.6	4.1 ± 0.6
N31	-	0.8 ± 1.3	3.3 ± 1.3
E32	-1.4 ± 0.5	-1.2 ± 0.5	-0.7 ± 0.5
V33	-2.2 ± 0.6	-2.3 ± 1.	0.5 ± 0.5
T34	4.2 ± 0.5	3. ± 0.9	**5.3 ± 0.6**
R36	**6.7 ± 2.7**	-	-
I37	-3.2 ± 0.5	-3.6 ± 1.3	-4.1 ± 0.6
Y38	-2.4 ± 0.5	-2.2 ± 0.8	-2.9 ± 0.6
V39	-1.1 ± 0.4	-2.6 ± 0.5	-2.1 ± 0.4
G40	0.5 ± 0.5	3.9 ± 0.6	**4.8 ± 0.7**
N41	3. ± 0.6	3.5 ± 2.5	**10.9 ± 0.8**
A42	1.1 ± 0.6	1.4 ± 0.8	-
S43	-1.5 ± 0.5	1.3 ± 0.7	3.3 ± 2.5
V44	-	2.1 ± 1.4	2.5 ± 0.9
A45	1.2 ± 0.7	1.3 ± 2.5	**9.1 ± 2.6**
Q46	3.9 ± 0.6	-	-
D47	-3.9 ± 0.5	-2.7 ± 0.7	2.7 ± 0.6
I48	-0.6 ± 0.4	0.4 ± 0.4	2. ± 0.5
K50	-3.1 ± 0.5	-2.2 ± 0.5	-2.3 ± 0.5
L51	-2.3 ± 0.5	-2.2 ± 0.7	0. ± 0.5
Q52	-	-4.4 ± 0.8	-3.7 ± 0.5
K53	-2.2 ± 0.4	-3.9 ± 0.6	-2.1 ± 0.4

L54	-0.7 ± 0.5	-2.8 ± 0.9	0.7 ± 0.5
G55	-3.2 ± 0.4	-3.9 ± 0.6	-4.9 ± 0.5
I56	-0.4 ± 0.5	-3.7 ± 0.8	-2.6 ± 0.6
T57	-	3.8 ± 2.7	-2. ± 2.5
H58	3.8 ± 0.5	-	-
V59	-	-	1.7 ± 0.8
L60	-3.9 ± 0.4	-4.3 ± 0.6	-2.5 ± 0.5
N61	-0.6 ± 0.5	-1.5 ± 0.6	2.1 ± 0.5
A62	1.7 ± 0.6	**15.7 ± 2.3**	-
A63	**9.8 ± 1.**	-	-
E64	-2.5 ± 0.5	2.5 ± 2.5	-
G65	-	**37. ± 10.6**	-
R66	**12.9 ± 2.6**	**31.9 ± 5.8**	-
M69	-	-	-
N72	**24.6 ± 3.6**	**24. ± 3.**	-
T73	2.7 ± 0.5	**6.5 ± 0.6**	**47.7 ± 19.1**
N74	-	**30.1 ± 10.8**	-
A75	0.7 ± 0.4	3.8 ± 0.5	-
N76	-0.5 ± 0.5	1.4 ± 0.7	-
F77	-0.3 ± 0.5	1.4 ± 0.8	-2.4 ± 1.4
Y78	**4.7 ± 0.6**	4.3 ± 1.4	0.6 ± 0.8
D80	0.6 ± 0.6	-1.5 ± 1.3	-
S81	1.6 ± 0.5	-2.2 ± 1.3	-2.3 ± 0.8
I83	-3.5 ± 0.9	-2.9 ± 2.5	-3.8 ± 2.5
T84	-0.7 ± 0.4	0.8 ± 0.7	0. ± 0.5
Y85	-4. ± 0.4	-3.7 ± 0.5	-1.5 ± 0.5
L86	-4.3 ± 0.4	-5. ± 0.6	-4.2 ± 0.4
G87	-1.1 ± 0.4	0.8 ± 0.5	-0.1 ± 0.4
I88	-0.7 ± 0.6	3. ± 2.6	-
K89	**9.3 ± 0.6**	**15.4 ± 1.**	-
A90	-	**14.5 ± 3.6**	-
D92	**10.7 ± 1.6**	-	-
T93	-	**5.4 ± 0.8**	-
Q94	0.8 ± 0.6	**6.7 ± 0.6**	-
E95	**24.5 ± 2.2**	**11.7 ± 2.6**	-
F96	**10. ± 2.5**	**14.4 ± 2.6**	-
N97	-	**8.6 ± 2.5**	-
L98	**15.1 ± 1.5**	-	-
S99	3.5 ± 0.8	**11.6 ± 2.6**	-
A100	-2.3 ± 0.9	-	-
Y101	0.8 ± 0.5	**6.3 ± 1.4**	-
F102	-4.3 ± 0.5	-3.2 ± 0.9	0.2 ± 0.7
E103	-2.7 ± 0.4	-4. ± 0.5	-3.9 ± 0.5
R104	-2.4 ± 0.4	-	-3.9 ± 0.4
A105	-3.7 ± 0.5	-4.3 ± 0.6	-4.4 ± 0.6
A106	-1.9 ± 0.4	1.7 ± 0.9	-3.8 ± 0.5
D107	-	-3.7 ± 0.5	-4.7 ± 0.5
F108	-3.8 ± 0.5	-4.5 ± 0.6	-3.6 ± 0.5
I109	-	-2.3 ± 0.6	-3.1 ± 0.5
D110	-0.4 ± 0.5	-1.9 ± 0.6	-2.4 ± 0.5
Q111	-2.2 ± 0.4	-1.2 ± 0.5	-1.8 ± 0.5
A112	-3.1 ± 0.5	-3.7 ± 0.6	-4.5 ± 0.5
L113	2.5 ± 0.5	1.7 ± 0.5	1. ± 0.4
A114	-3.7 ± 0.5	-4.1 ± 0.5	-5. ± 0.5

Q115	-0.6 ± 0.4	-1. ± 0.4	-1.5 ± 0.4
K116	-0.8 ± 0.5	-0.1 ± 0.5	0.4 ± 0.7
G118	-1.7 ± 0.6	-2.9 ± 0.8	-4.2 ± 0.7
R119	**4.9 ± 1.4**	-	-
V120	-3. ± 0.5	-3.1 ± 0.7	-5.2 ± 0.5
L121	-3.6 ± 0.5	-5.4 ± 0.6	-4.8 ± 0.5
V122	-2.2 ± 0.5	-2.8 ± 0.6	-4.5 ± 0.5
H123	-3.2 ± 0.5	-0.4 ± 0.5	1.1 ± 0.5
C124	**4.6 ± 1.4**	-	**30.8 ± 14.5**
Y128	-	2.8 ± 1.	2.1 ± 0.8
S129	0.9 ± 0.5	2.2 ± 0.6	-
T133	1.6 ± 0.6	1. ± 2.6	-
L134	0.3 ± 0.6	0.7 ± 2.5	**9.4 ± 2.7**
V135	-3.6 ± 0.5	-2.9 ± 1.3	-3.2 ± 0.6
I136	-	-	-2.4 ± 0.7
A137	-2.1 ± 0.5	-3.6 ± 0.9	-2.6 ± 0.6
Y138	-2. ± 0.5	-3.6 ± 1.3	-
L139	-2.8 ± 0.5	-3. ± 0.8	-3.3 ± 0.6
M140	-3.5 ± 0.5	-2.2 ± 0.6	-
M141	-0.3 ± 0.5	-2. ± 0.8	-3. ± 0.6
R142	-0.2 ± 0.6	-2.7 ± 1.3	-2.1 ± 0.6
Q143	-1.7 ± 0.5	-5. ± 2.5	-1.4 ± 0.7
K144	-3.5 ± 0.5	-4.3 ± 0.6	-5.4 ± 0.5
M145	-2.3 ± 0.4	-4. ± 0.5	-5.3 ± 0.4
D146	-	-	-
V147	-0.8 ± 0.4	-2. ± 0.6	-1.5 ± 0.4
K148	-2.7 ± 0.5	-3.4 ± 0.6	-3.3 ± 0.5
S149	-0.1 ± 0.5	-	-2.1 ± 0.8
A150	-5.2 ± 0.5	-3.9 ± 0.9	-4.3 ± 0.5
L151	-	0.1 ± 0.5	-2.7 ± 0.5
S152	-0.5 ± 0.5	-1.5 ± 0.8	-1.9 ± 0.5
I153	-2.7 ± 0.6	-2. ± 0.6	-2.6 ± 0.6
V154	-3.4 ± 0.4	-4.3 ± 0.9	-4.6 ± 0.5
R155	-	-0.8 ± 0.7	-0.3 ± 0.6
Q156	-2.2 ± 0.6	-	-
N157	1.4 ± 0.5	-3.2 ± 2.5	-1.3 ± 0.6
R158	0.3 ± 0.5	**4.6 ± 1.4**	4.1 ± 0.8
E159	2.2 ± 0.5	3.9 ± 1.	0. ± 0.7
G161	**5.8 ± 1.1**	**6.7 ± 2.6**	**8.7 ± 1.7**
N163	0.5 ± 0.5	2.6 ± 2.5	**7.3 ± 1.5**
D164	**5.5 ± 0.6**	**5.4 ± 0.7**	**14.4 ± 1.1**
G165	0.5 ± 0.7	-	**16.9 ± 5.7**
F166	1.4 ± 0.8	-	**5.2 ± 1.1**
L167	-	1. ± 2.5	4.1 ± 2.5
A168	-2.3 ± 1.3	**5.4 ± 1.1**	-
Q169	0.1 ± 1.	**7.1 ± 2.6**	-
L170	-1.5 ± 0.6	1. ± 1.	0.1 ± 2.5
C171	-	-2.7 ± 0.9	-4. ± 2.4
Q172	-1.4 ± 0.5	-2.3 ± 1.3	-1.6 ± 0.7
L173	-2.6 ± 0.4	-2.3 ± 0.6	-3.2 ± 0.5
N174	-1.4 ± 0.5	-1.8 ± 0.7	-2.9 ± 0.5
D175	-	-	-
R176	-4.3 ± 0.4	-4.1 ± 0.7	-5.3 ± 0.4
L177	-	-2.8 ± 0.7	-4. ± 0.5

A178	-3.1 ± 0.5	-2.5 ± 0.6	-3.5 ± 0.5
K179	-1.3 ± 0.4	-1.2 ± 0.5	-2.6 ± 0.4
E180	-1.7 ± 0.5	-0.6 ± 0.5	-1.9 ± 0.5
G181	-2.7 ± 0.5	-3.4 ± 0.5	-4.2 ± 0.5
K182	-1. ± 0.5	-2.9 ± 0.5	-3.2 ± 0.5
L183	-0.9 ± 0.5	-1.2 ± 0.5	-1.3 ± 0.5
K184	-1.7 ± 0.7	-0.9 ± 0.7	-1.7 ± 0.7

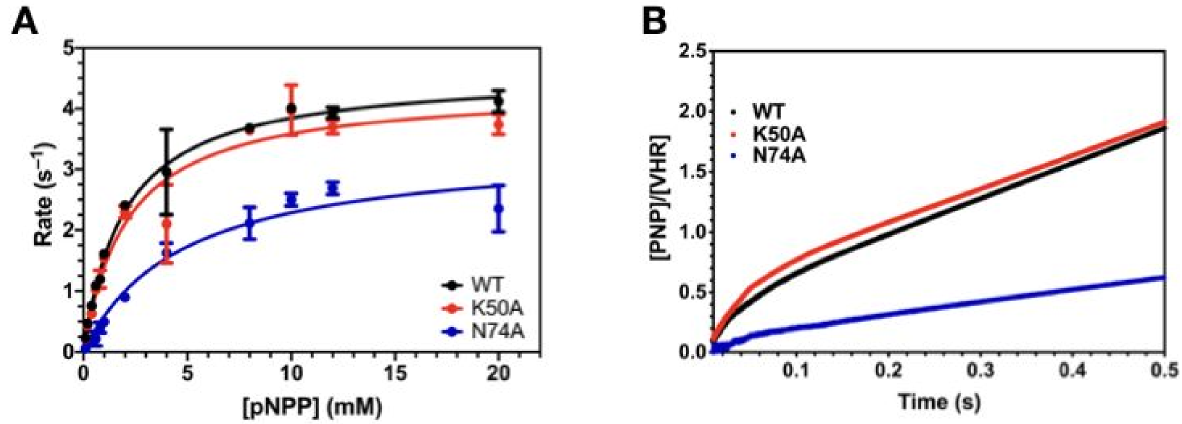

Figure S1. Enzyme kinetics for VHR. A) Steady-state kinetics of WT, K50A, and N74A with the pseudo-substrate pNPP. Curves are nonlinear fits with the Michaelis-Menten equation. B) Pre-steady-state kinetics of WT, K50A, and N74A with pNPP. The curves are non-linear fits to equation 1 in the main text. The kinetic parameters for each enzyme are given in the main text in Table 1.

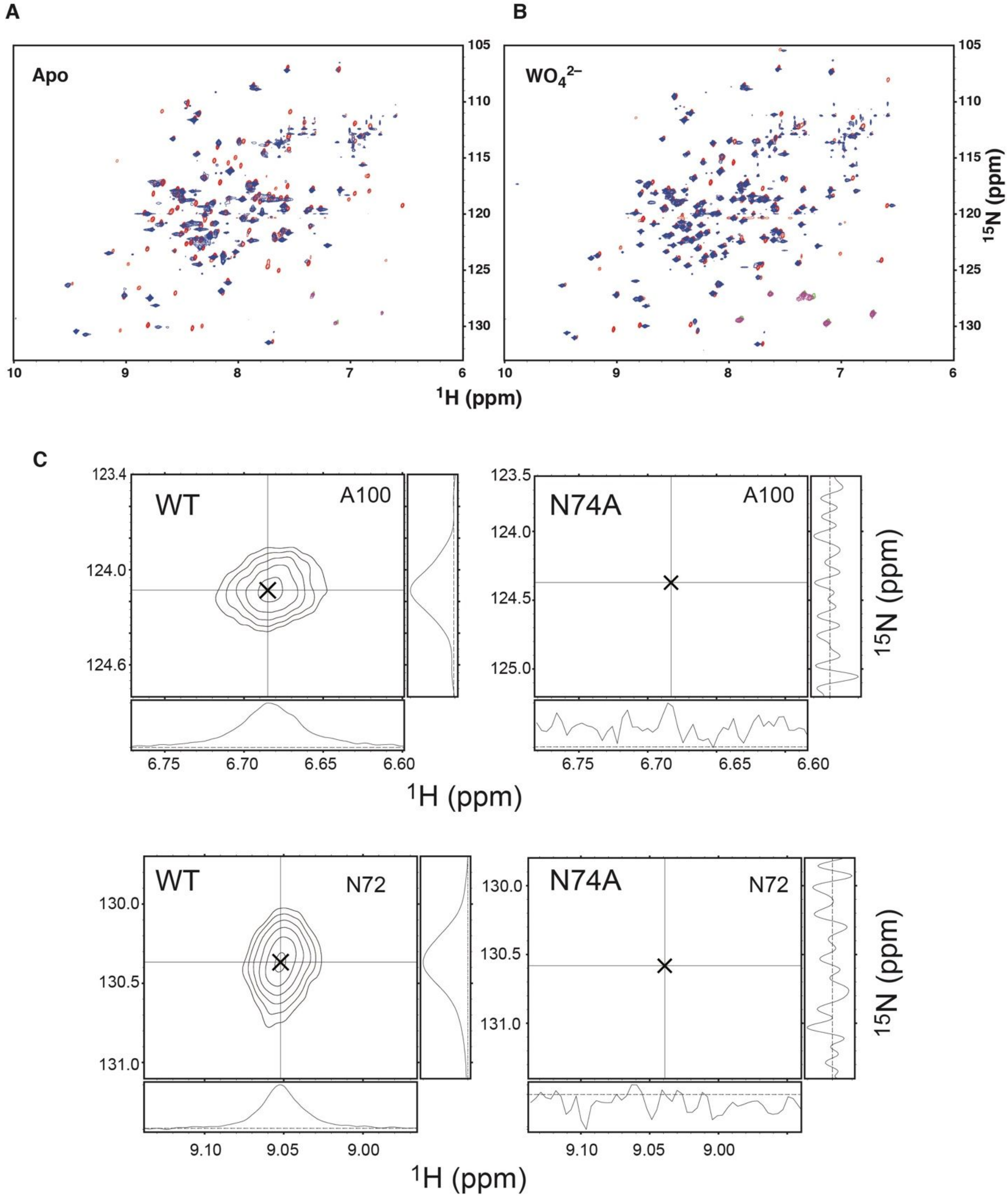

Figure S2. NMR spectra of VHR. A) ^1H-^{15}N-HSQC spectral overlays of WT (red) and N74A (blue). B) HSQC overlays of tungstate bound WT (red) and tungstate bound N74A (blue) VHR enzymes. Folded peaks for WT and N74A are shown as green and magenta colors, respectively. In C) 1D traces through the two-dimensional contour for A100 and N72 demonstrating the linebroadening that occurs in N74A bound to tungstate compared to WT bound to tungstate.

Configuration file used for molecular dynamics simulations.

```
#############################################################
## JOB DESCRIPTION                                         ##
#############################################################

## Production Run of  ##
## VHR in a Water Box ##


#############################################################
## ADJUSTABLE PARAMETERS                                   ##
#############################################################

structure          vhr_ionized.psf
coordinates        vhr_ionized.pdb

set temp           310
set inputname      vhr_ionized_production_Previous
set outputname     vhr_ionized_production_Next

binCoordinates     $inputname.coor;    # coordinates from last run
(binary)
binVelocities      $inputname.vel;     # velocities from last run (binary)
extendedSystem     $inputname.xsc;     # cell dimensions from last run
(binary)

#############################################################
## SIMULATION PARAMETERS                                   ##
#############################################################

# Input
paraTypeCharmm       on
parameters          toppar/par_all36m_prot.prm
parameters          toppar/par_all36_carb.prm
parameters          toppar/par_all36_lipid.prm
parameters          toppar/par_all36_na.prm
parameters          toppar/par_all35_ethers.prm
parameters          toppar/par_all36_cgenff.prm
parameters          toppar/toppar_water_ions_namd.str
parameters          toppar/WO4.prm
#temperature         $temp


# Force-Field Parameters
exclude             scaled1-4
1-4scaling          1.0
cutoff              12.0
switching           on
switchdist          10.0
pairlistdist        13.5


# 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     5     ;# damping coefficient (gamma) of 5/ps
langevinTemp        $temp
langevinHydrogen    off    ;# don't couple langevin bath to hydrogens


# Periodic Boundary Conditions
#cellBasisVector1    71.5    0.   0.
#cellBasisVector2     0.   65.0   0.
#cellBasisVector3     0.    0   82.0
#cellOrigin          -1.4  -0.1 -1.1

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    $temp


# Output
outputName          $outputname

restartfreq         500     ;# 500steps = every 1ps
dcdfreq             500
xstFreq             500
outputEnergies      100
outputPressure      100


#############################################################
## EXTRA PARAMETERS                                        ##
#############################################################
```

```
#fixedAtoms on
#fixedAtomsForces on
#fixedAtomsFile fix_protein.pdb
#fixedAtomsCol B

#############################################################
## EXECUTION SCRIPT                                        ##
#############################################################
# Minimization##
#minimize            100

##Heat##
#for {set i 1} {$i <= $temp } { incr i 1 } {
#langevinTemp $i
#reinitvels $i
#run 100
#}

##Run Simulation##
run 500000 ;# 1ns
```