Supporting Information

High-throughput molecular dynamics-based alchemical free energy calculations for predicting the binding free energy change associated with the selected omicron mutations in the spike receptor-binding domain of SARS-CoV-2

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Results of conventional MD simulations:

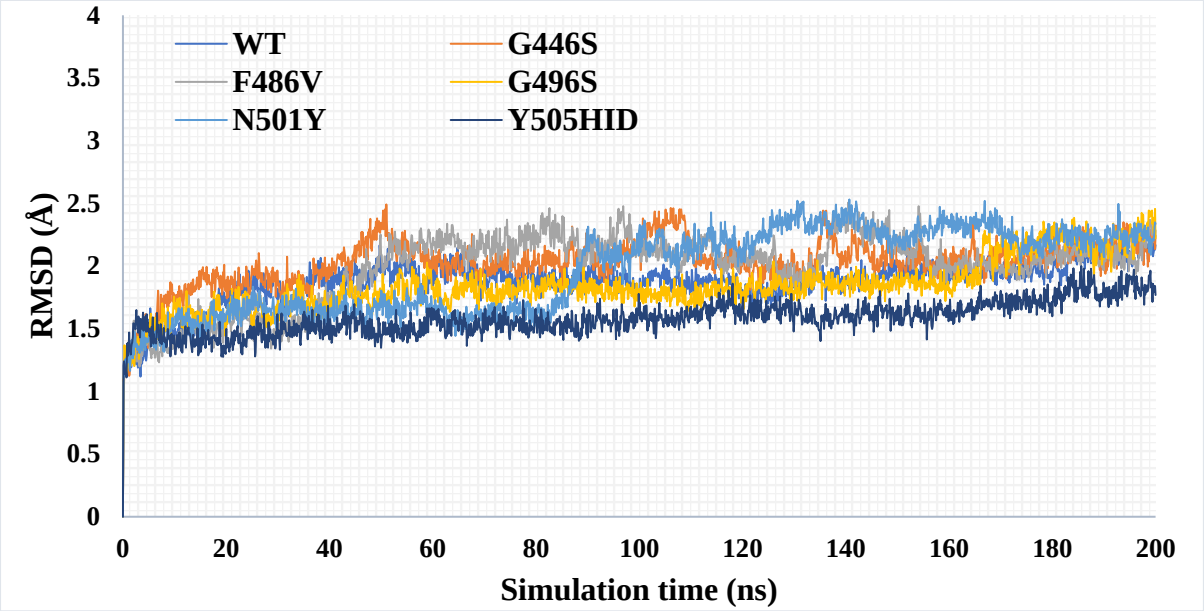
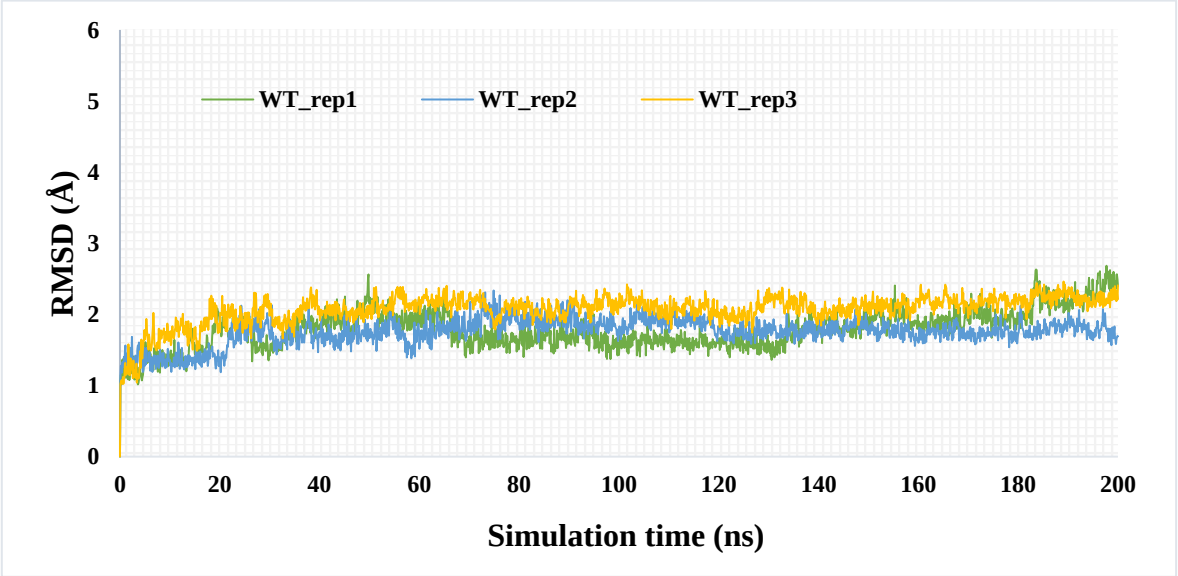
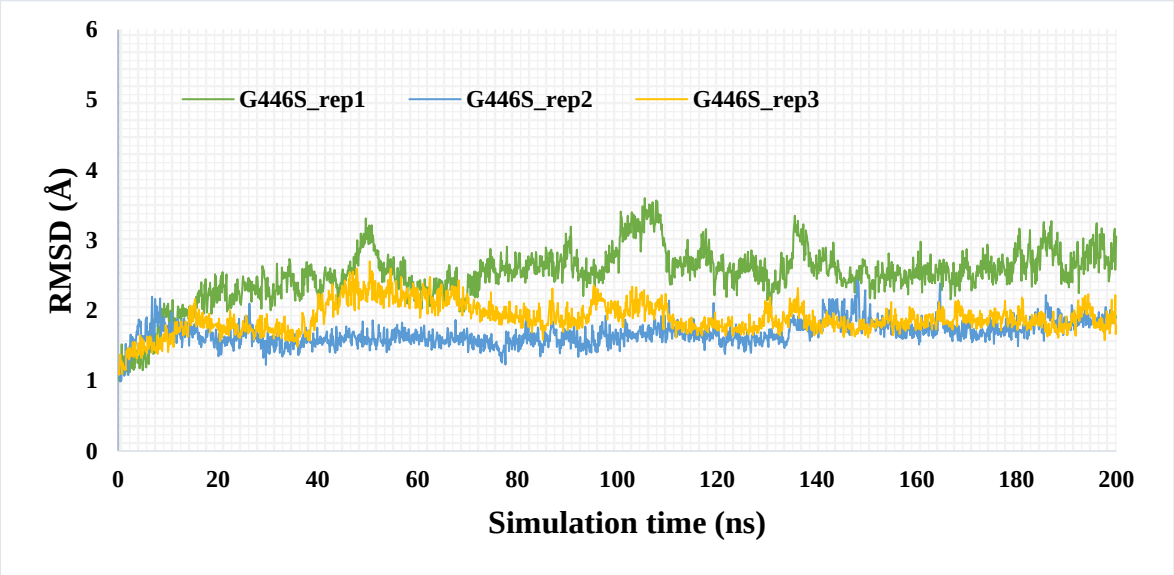
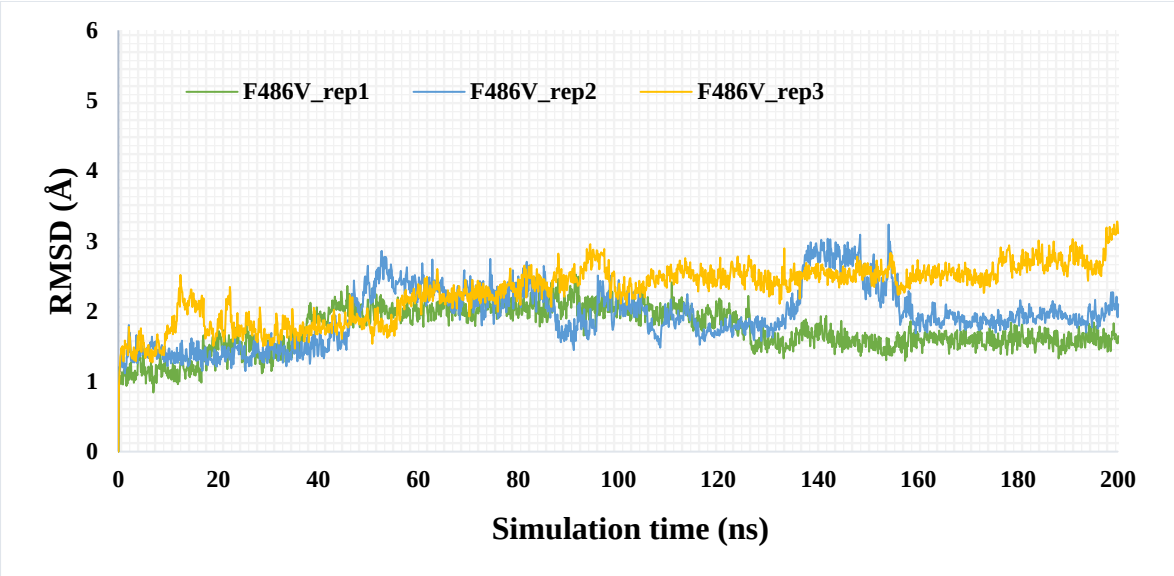


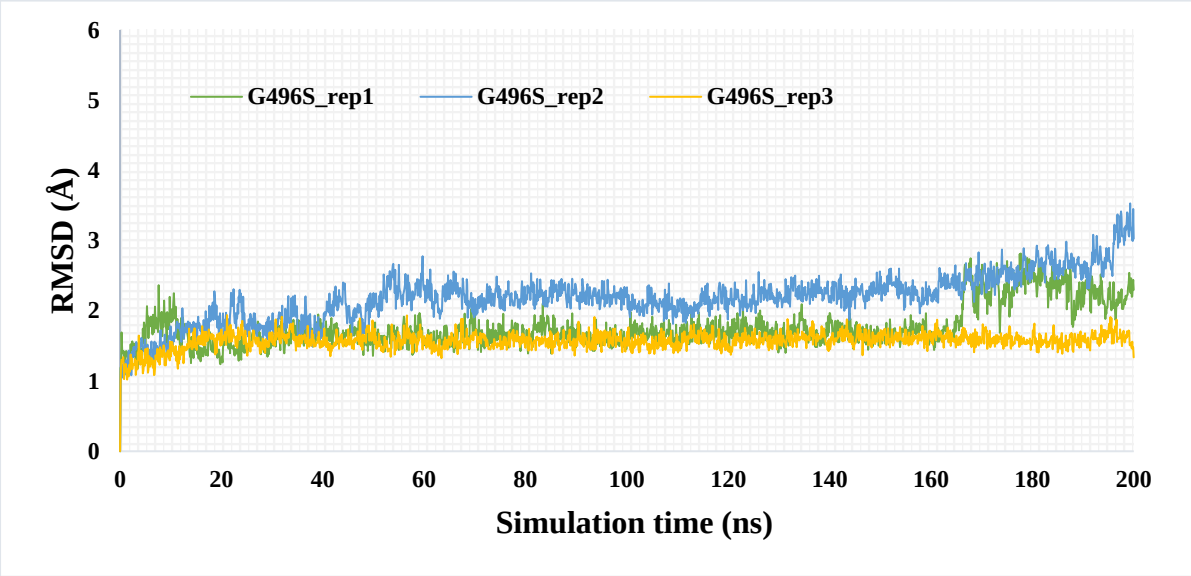
Figure S1. *The* ***averaged*** *root-mean-square deviation (RMSD) of the wildtype (WT) and mutated protein structures of the spike RBD in complex with hACE2 during* ***three replicates*** *of* ***200-ns*** *MD simulations. HID – histidine protonated at the delta nitrogen.*

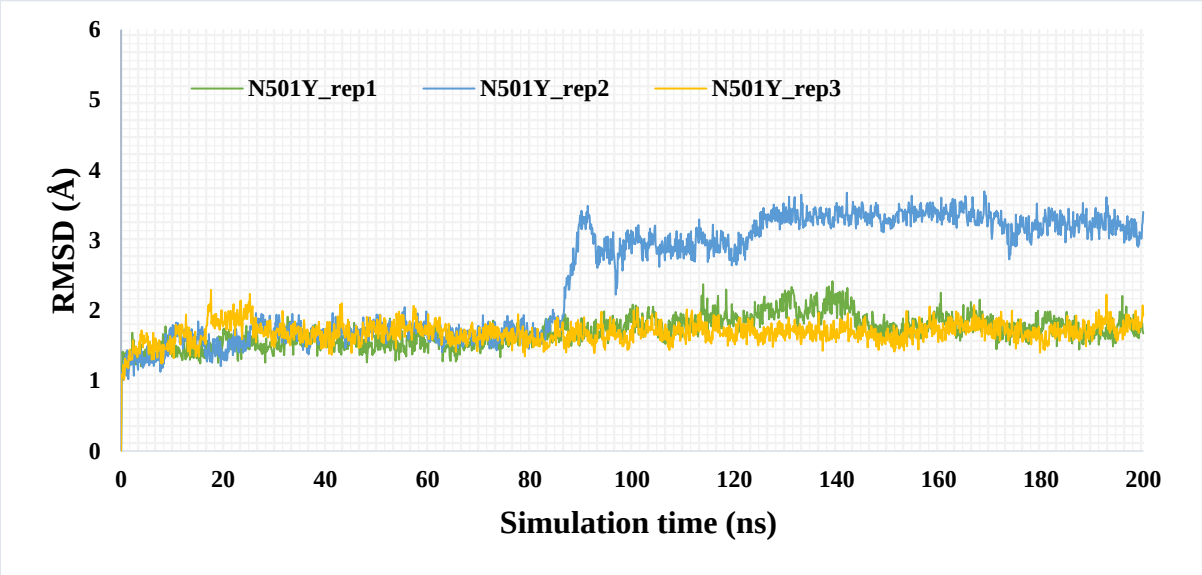
The RMSD plots for each individual MD run in Figure S1 are shown here below for WT and on pages S3-S5 for the mutants:

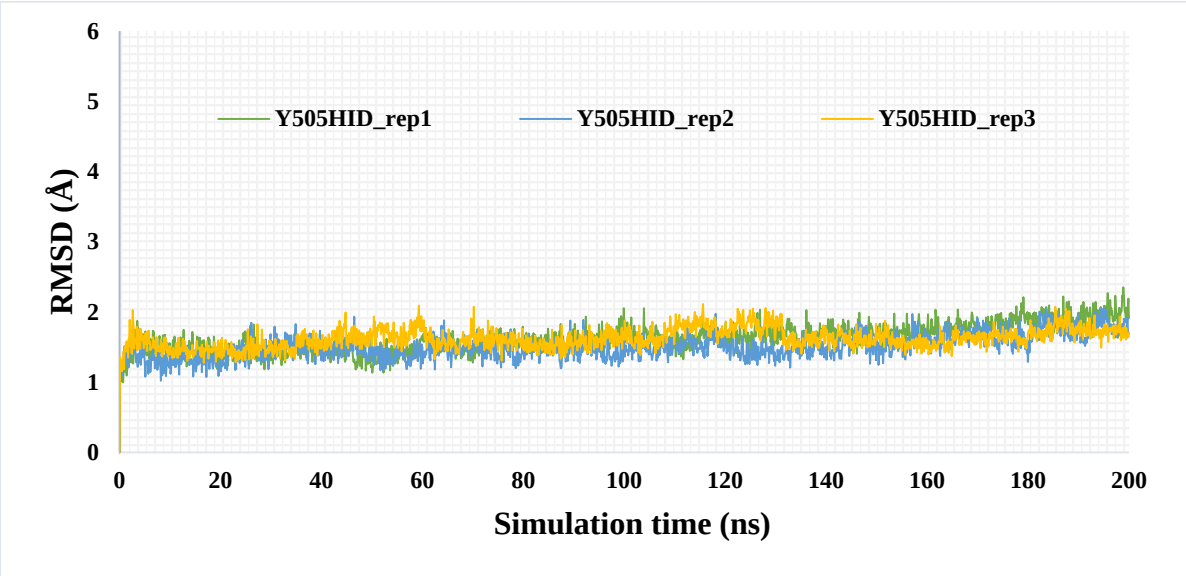



G446S_rep1
G446S_rep2
G446S_rep3
RMSD (Å)
Simulation time (ns)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
200


F486V_rep1
F486V_rep2
F486V_rep3
RMSD (Å)
Simulation time (ns)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
200


G496S_rep1
G496S_rep2
G496S_rep3
RMSD (Å)
Simulation time (ns)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
200


N501Y_rep1
N501Y_rep2
N501Y_rep3
RMSD (Å)
Simulation time (ns)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
200


Y505HID_rep1
Y505HID_rep2
Y505HID_rep3
RMSD (Å)
Simulation time (ns)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
200

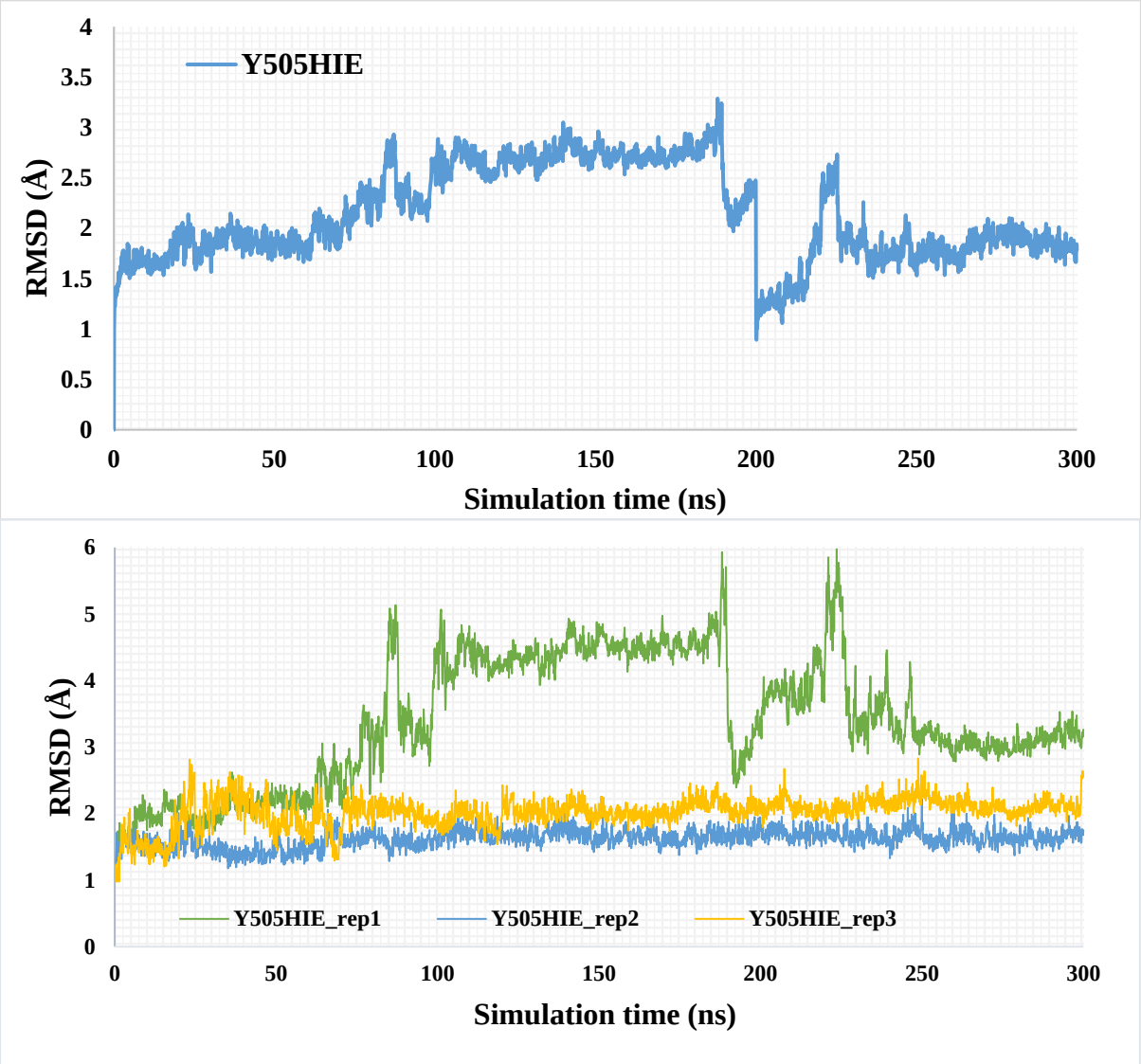


Figure S2. *The root-mean-square deviation (RMSD) of the* ***Y505HIE*** *mutant protein structure of the spike RBD in complex with hACE2 during three replicates of* ***300-ns*** *MD simulations. Top: average of three simulations. Bottom: RMSD for the individual simulations. HIE – histidine protonated at the epsilon nitrogen.*

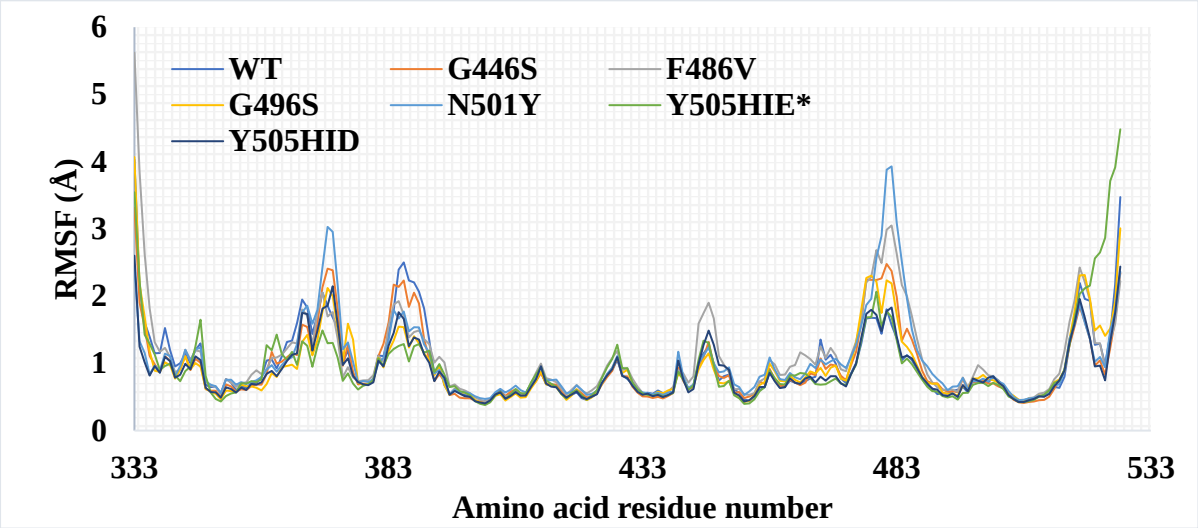
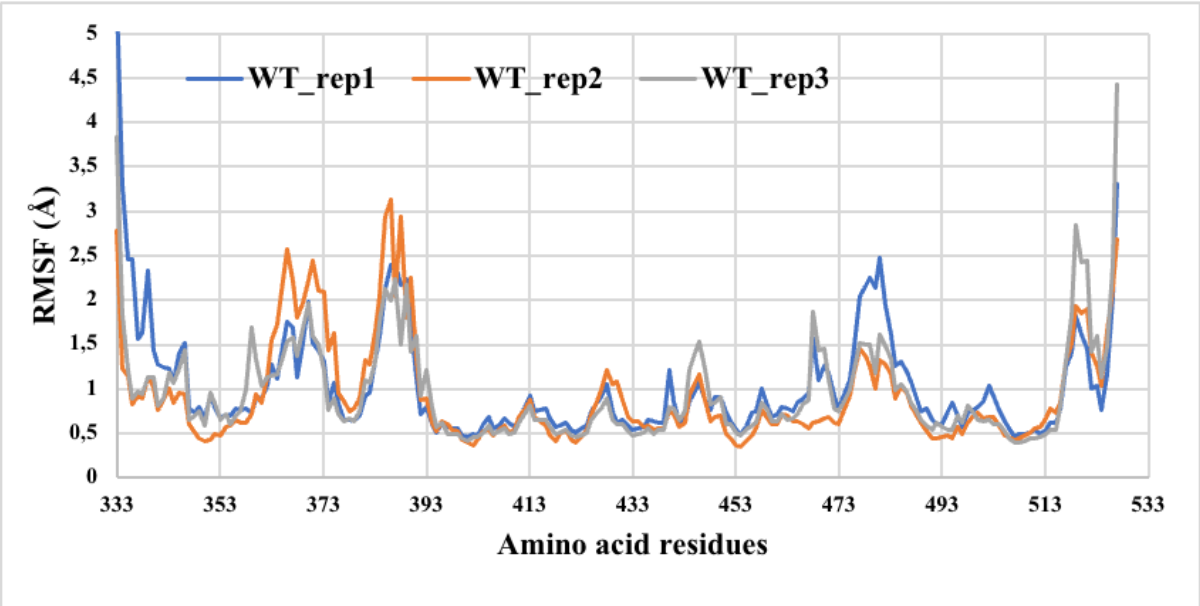
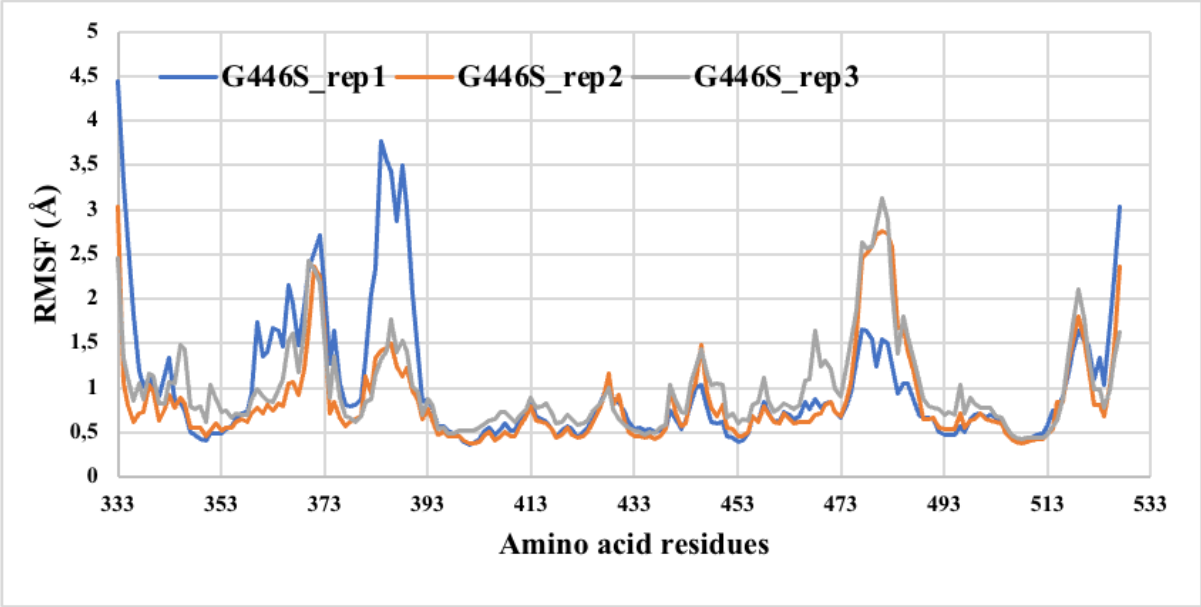
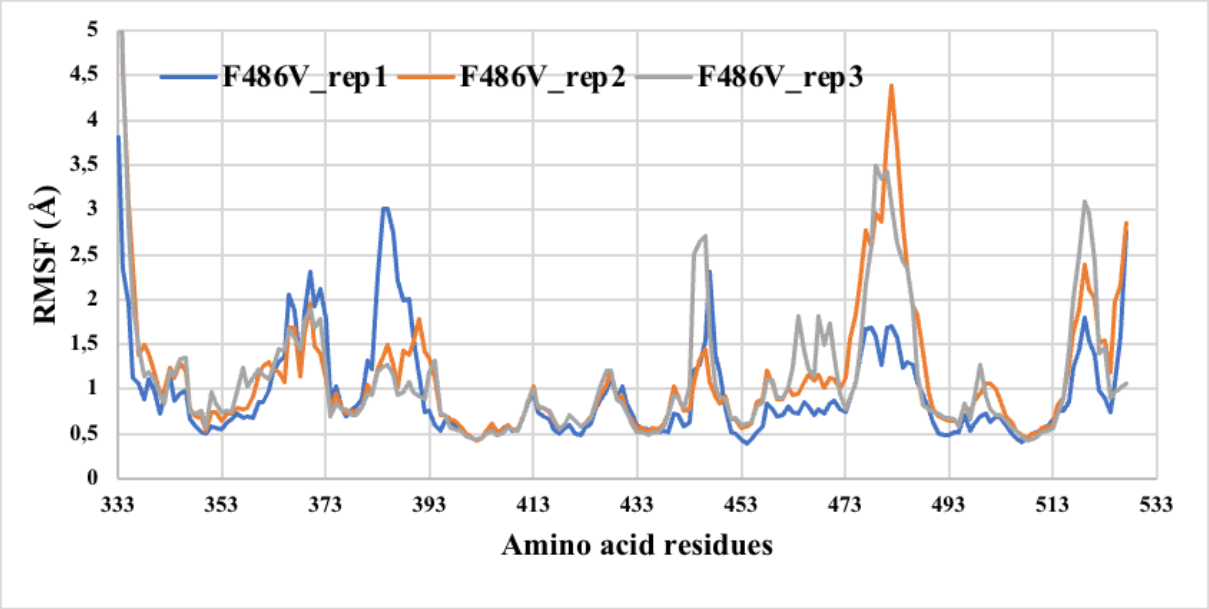


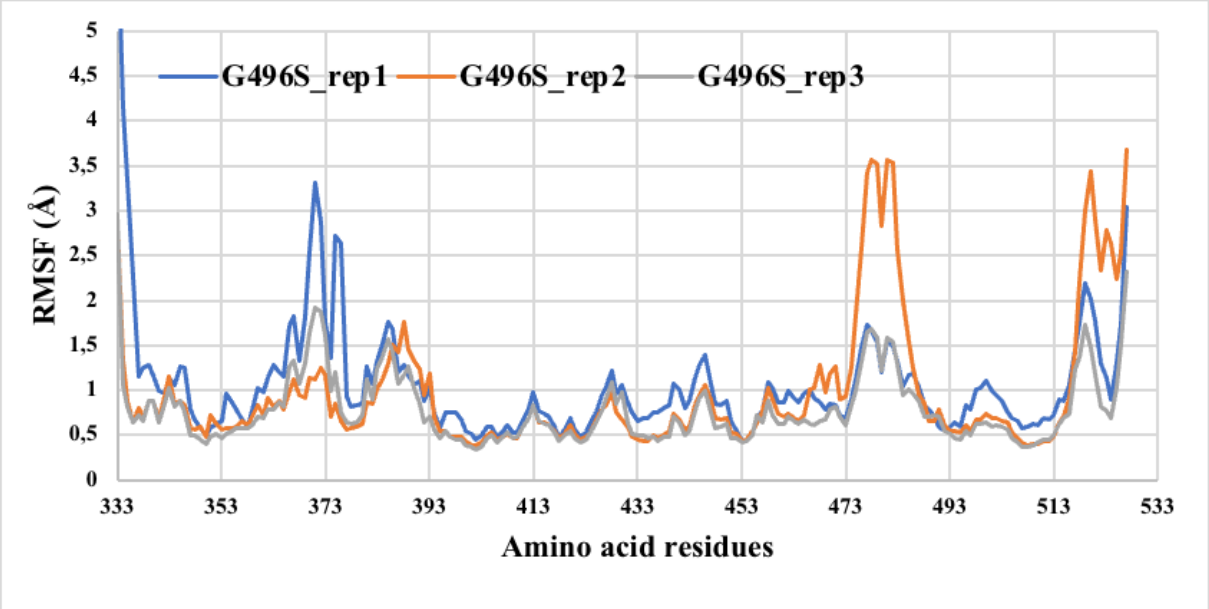
Figure S3. *The averaged root-mean-square fluctuation (RMSF) of the WT and mutated protein structures of spike RBD in complex with hACE2 during three replicates of 200-ns MD simulations (*Y505HIE mutant was simulated for 300 ns). HID – histidine protonated at the delta nitrogen; HIE – histidine protonated at the epsilon nitrogen.*

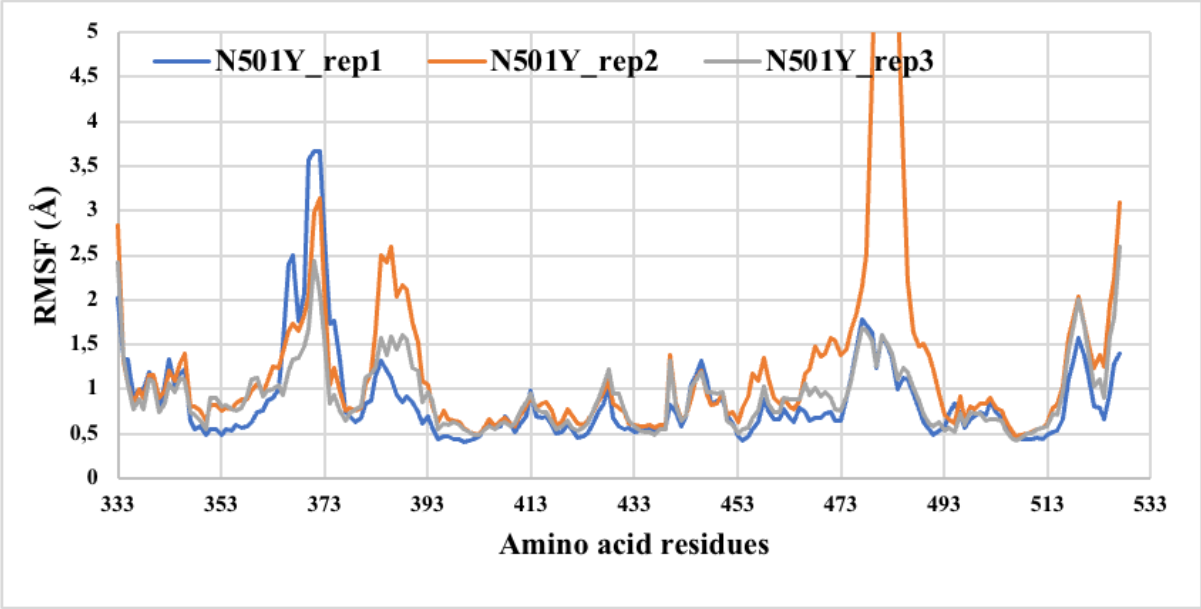
The RMSF plots for each individual MD run are shown here below for WT and on pages S7 and S8 for the mutants:

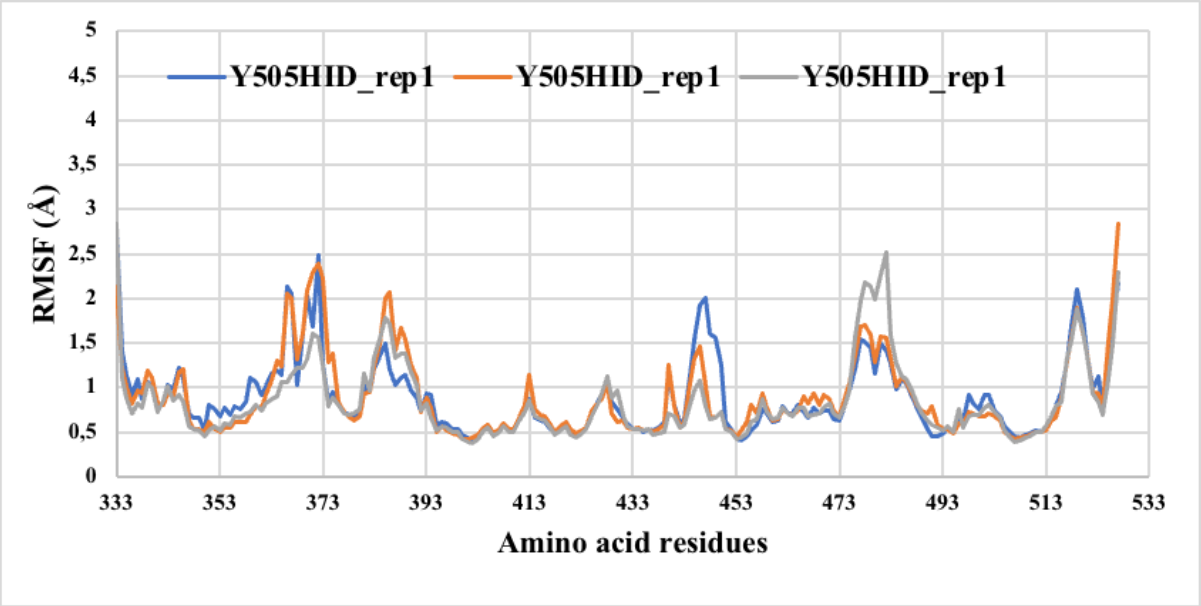


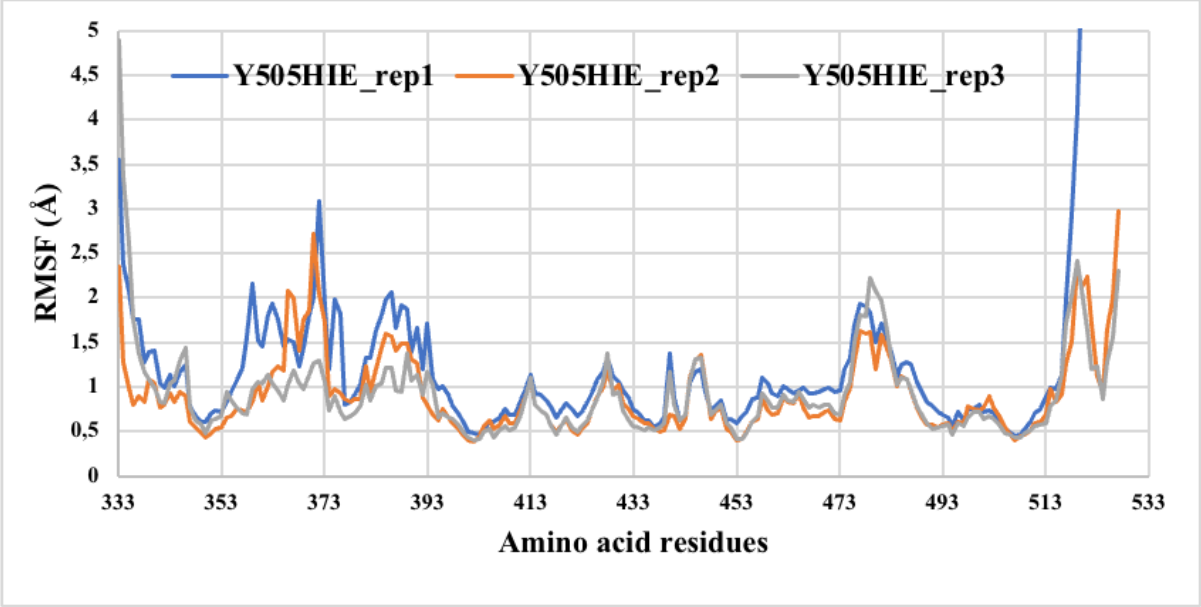

G446S_rep1
G446S_rep2
G446S_rep3
RMSF (Å)
Amino acid residues


F486V_rep1
F486V_rep2
F486V_rep3
RMSF (Å)
Amino acid residues


G496S_rep1
G496S_rep2
G496S_rep3
RMSF (Å)
Amino acid residues


N501Y_rep1
N501Y_rep2
N501Y_rep3
RMSF (Å)
Amino acid residues


Y505HID_rep1
Y505HID_rep1
Y505HID_rep1
RMSF (Å)
Amino acid residues


Y505HIE_rep1
Y505HIE_rep2
Y505HIE_rep3
RMSF (Å)
Amino acid residues

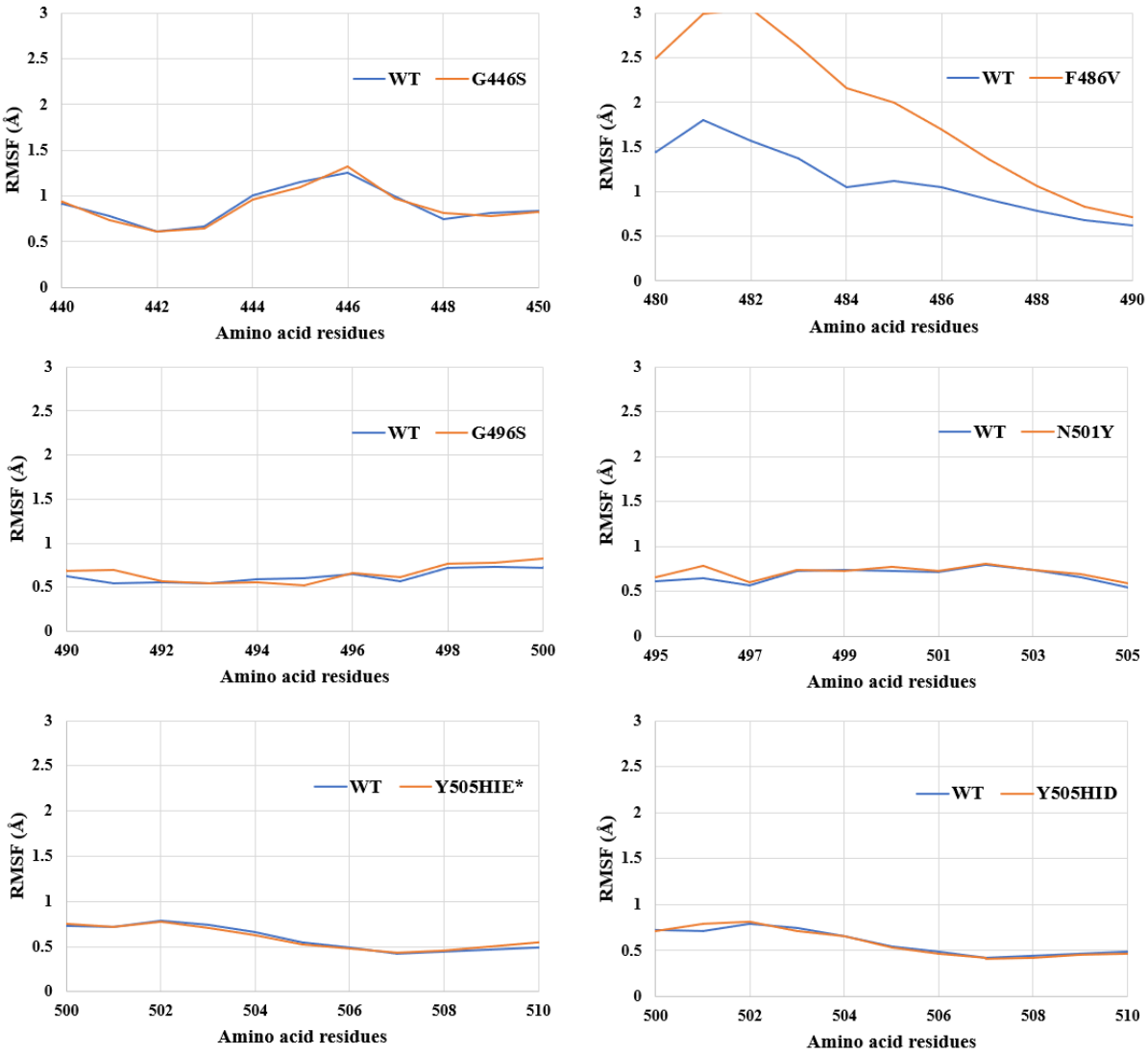


Figure S4. *Averaged root-mean-square fluctuation (RMSF) of the residues surrounding the individual mutations of spike RBD in complex with hACE2 during three replicates of 200-ns MD simulations (*Y505HIE mutant was simulated for 300 ns) compared with the RMSF of the corresponding residues in the WT complex. HID – histidine protonated at the delta nitrogen; HIE – histidine protonated at the epsilon nitrogen.*

Interaction analysis of spike RBD wildtype/mutant residues with hACE2 using conventional MD

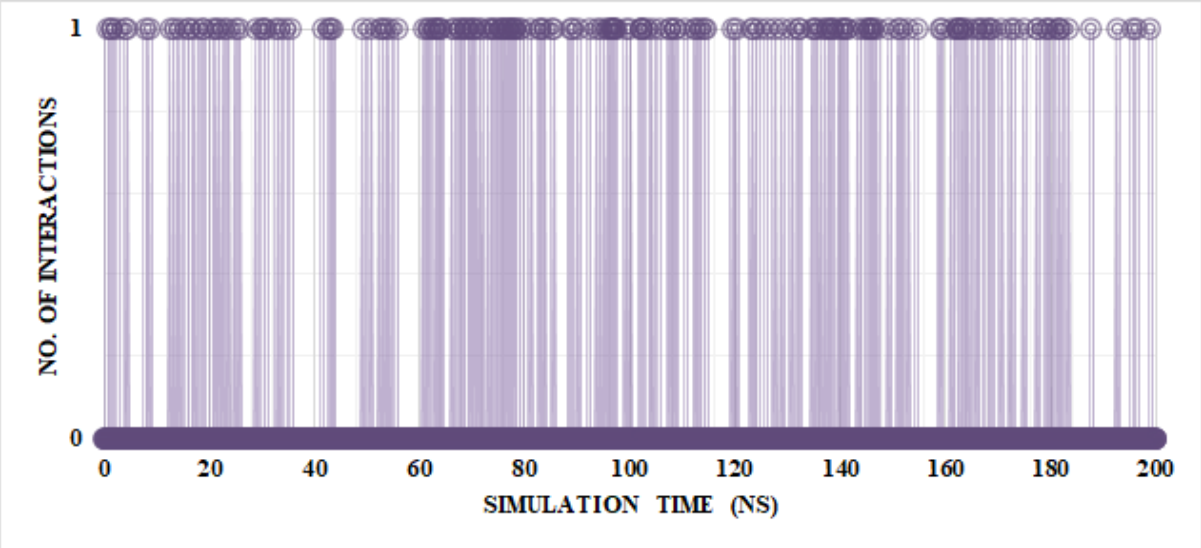


Figure S5. *Number of pi-pi stacking interactions between Phe486 of the wildtype spike RBD and Tyr83 of hACE2 during a 200-ns MD simulation (replica 3).*

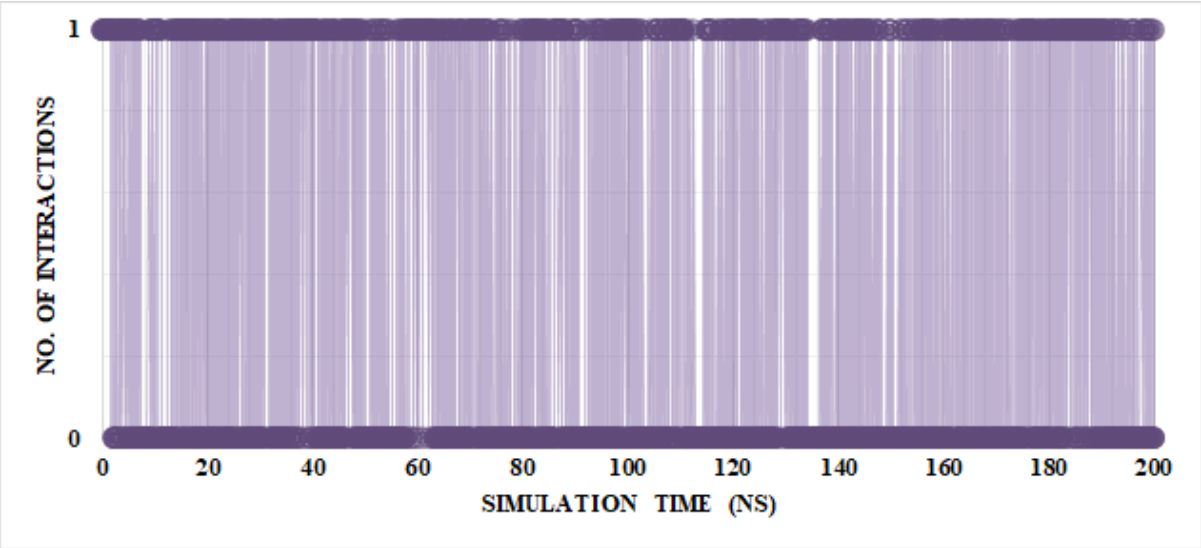


Figure S6. *Number of pi-pi stacking interactions between Tyr501 of the mutant spike RBD and Tyr41 of hACE2 during a 200-ns MD simulation (replica 3).*

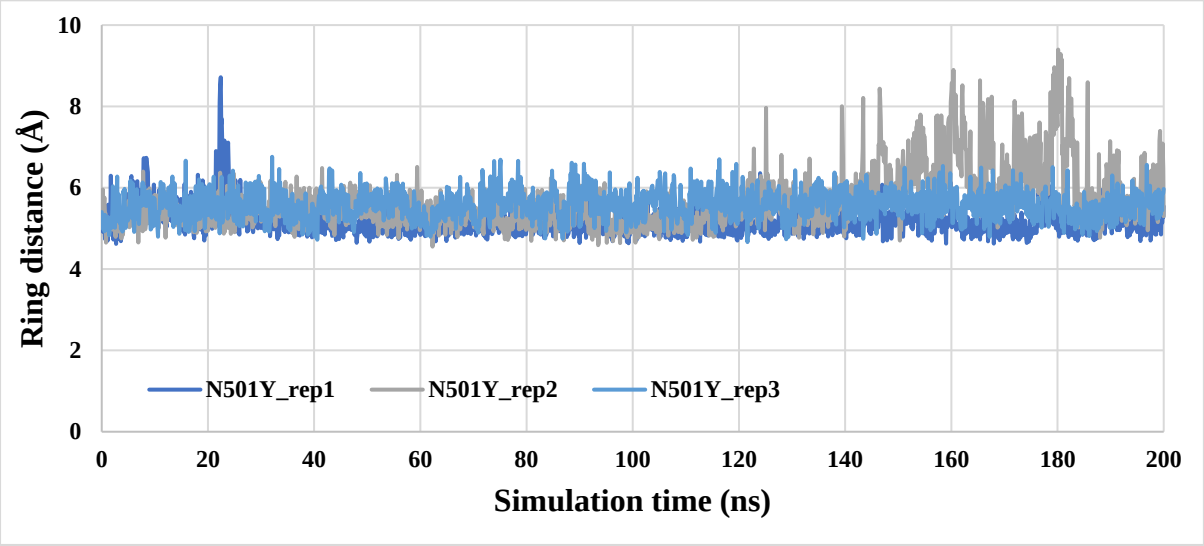


Figure S7. *The aromatic ring distance between Tyr501 of the mutant spike RBD and Tyr41 of hACE2 during three independent 200-ns MD simulations.*

Table S1. *Experimental binding affinities of SARS-CoV-2 spike RDB mutants converted to change in binding free energies (ΔΔG)*

SARS-CoV-2 spike RBD mutation	***Kd_{MUT} (nM)***	***Kd_{WT} (nM)***	***ΔΔG (kcal/mol)***	***Reference***
G446S	*46.9±3.18*	*29.4±2.62*	*0.28±0.12*	*(66)*
N501Y	*2.4±0.4*	*17±0.6*	*-1.17±0.24*	*(74)*
	3.0±2.1	*10±3.1*	*-0.72±0.23*	*(76)*
	5.5±2.4	*62.6±7.7*	*-1.45±0.69*	*(40)*
	10.7	*75.1*	*-1.16*	*(77)*
	0.40	*16.0*	*-2.20*	*(78)*

Note: The experimental Kd values were taken from the studies collected in Table 1 and converted to ΔG (kcal/mol) using equation ΔG=RTlnKd, where R is gas constant (1.9872 mol/cal) and T is temperature in Kelvin (300 K). The final ΔΔG was calculated using the equation $\Delta\Delta G=\Delta G_{MUT}-\Delta G_{WT}$.

Results of the alchemical free energy calculations – PMX standard output: Final free energy plots of the mutation free energy pipeline

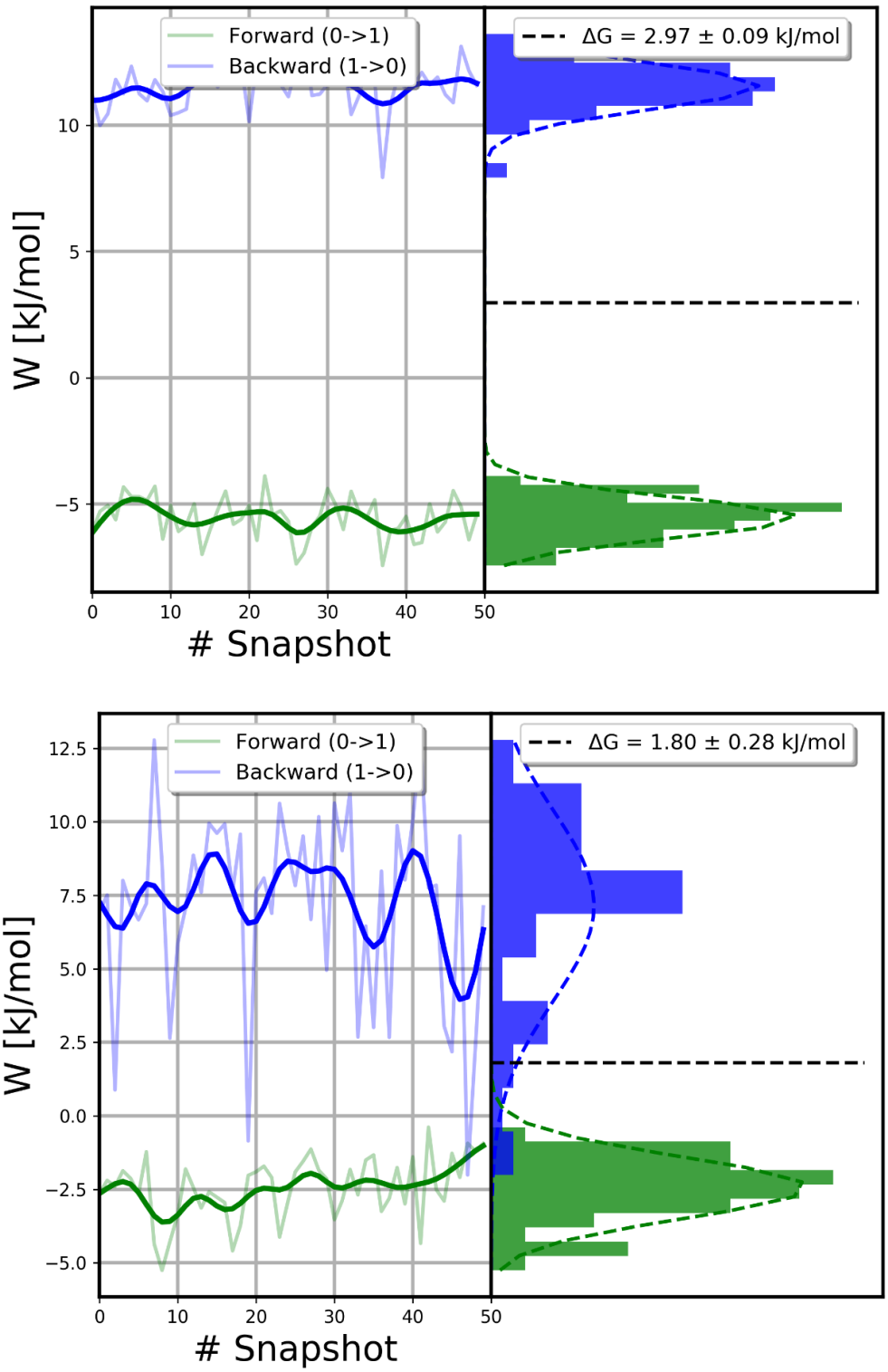


Figure S8. *Free energy plots for unbound (top) and bound (bottom) G446S (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

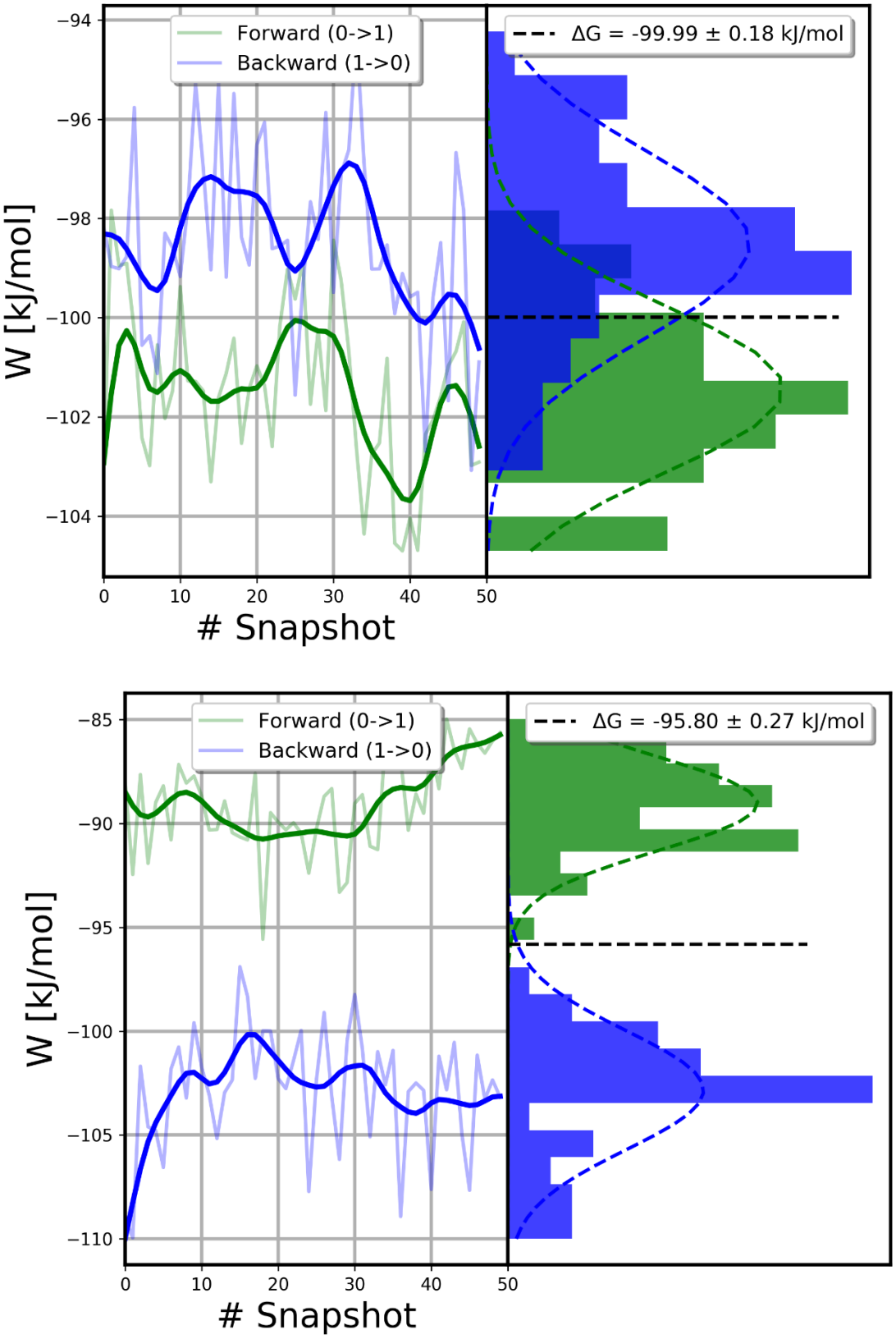


Figure S9. *Free energy plots for unbound (top) and bound (bottom) F486V (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

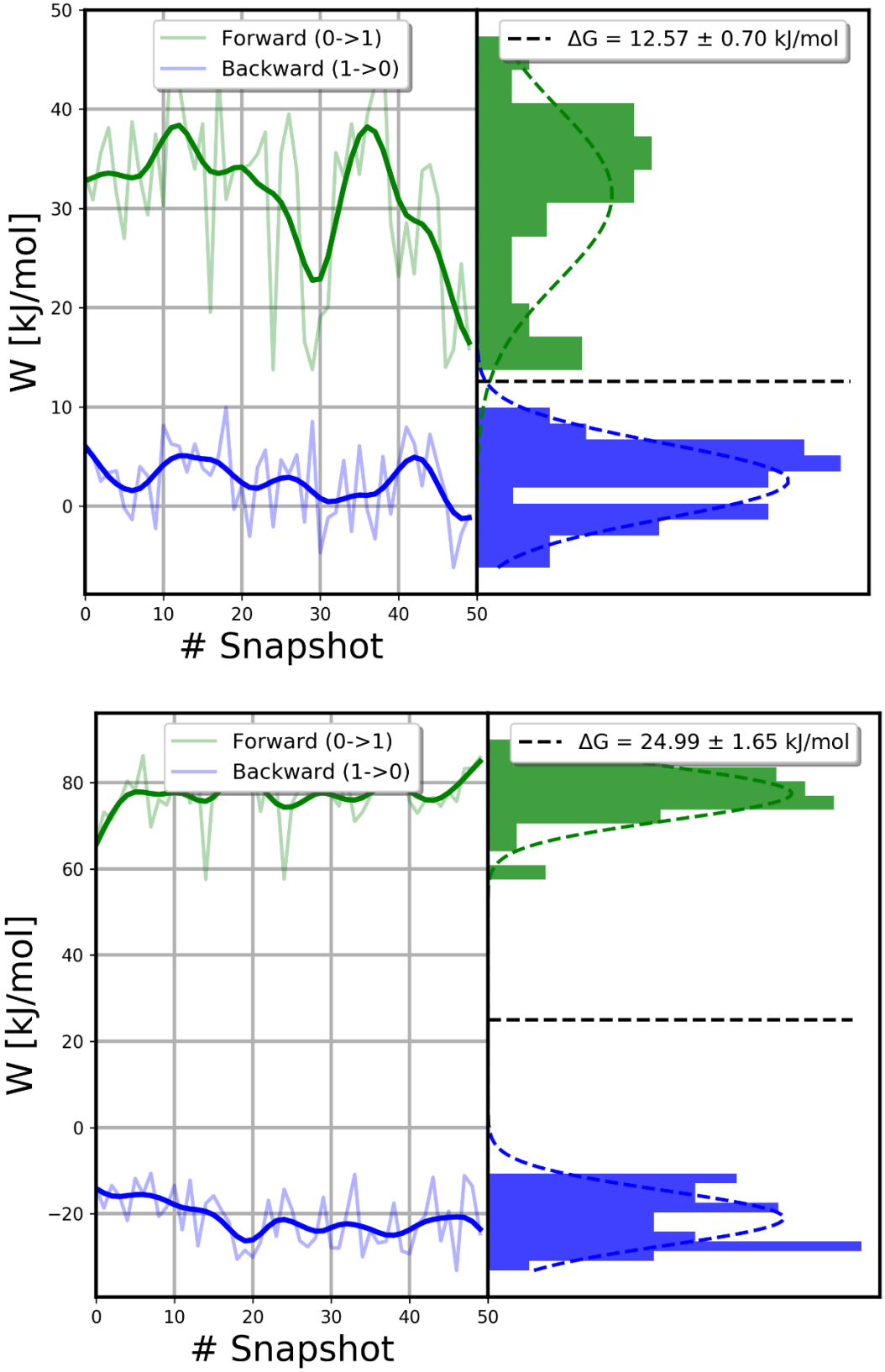


Figure S10. *Free energy plots for unbound (top) and bound (bottom) G496S (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

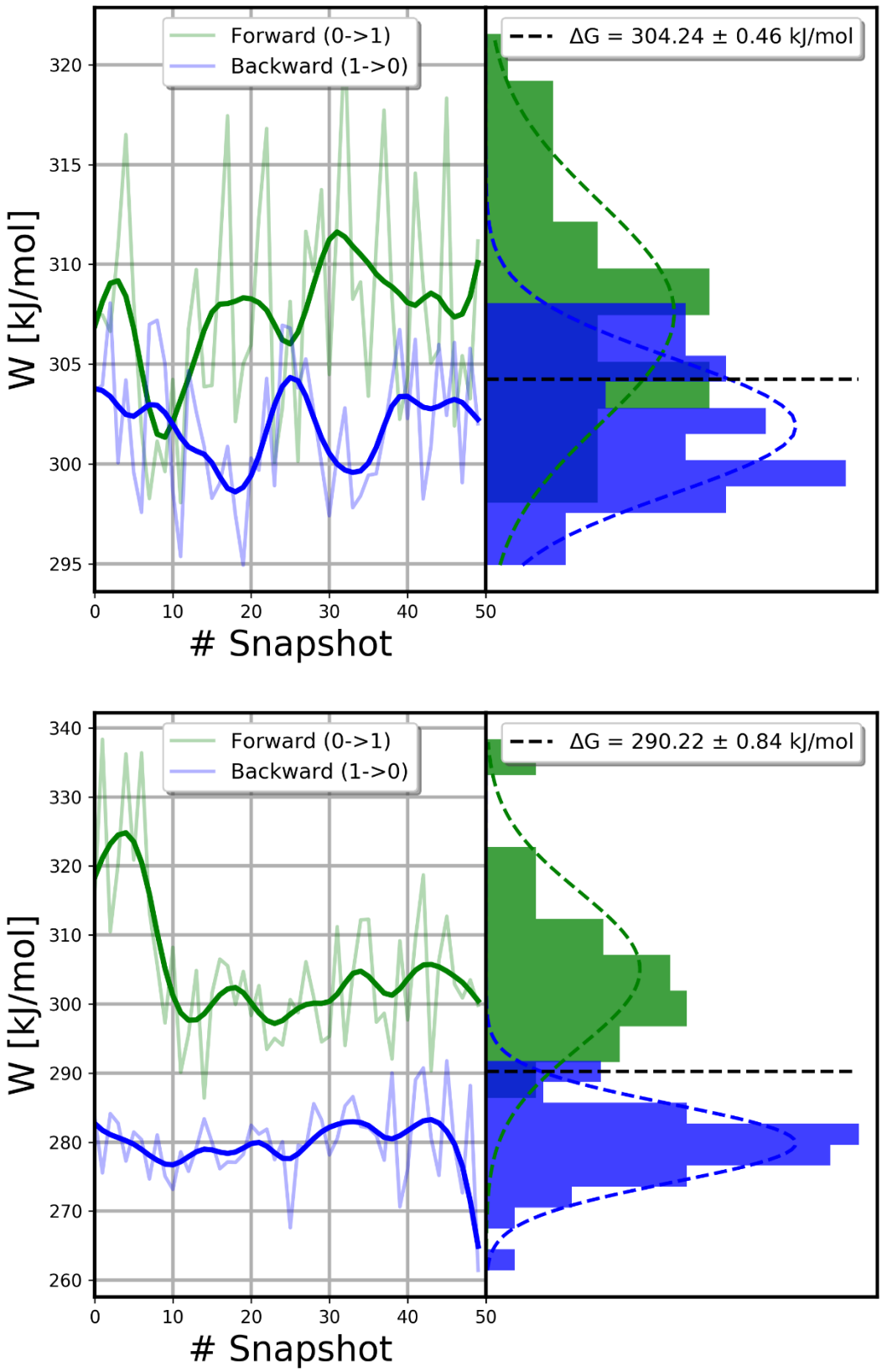


Figure S11. *Free energy plots for unbound (top) and bound (bottom) N501Y (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

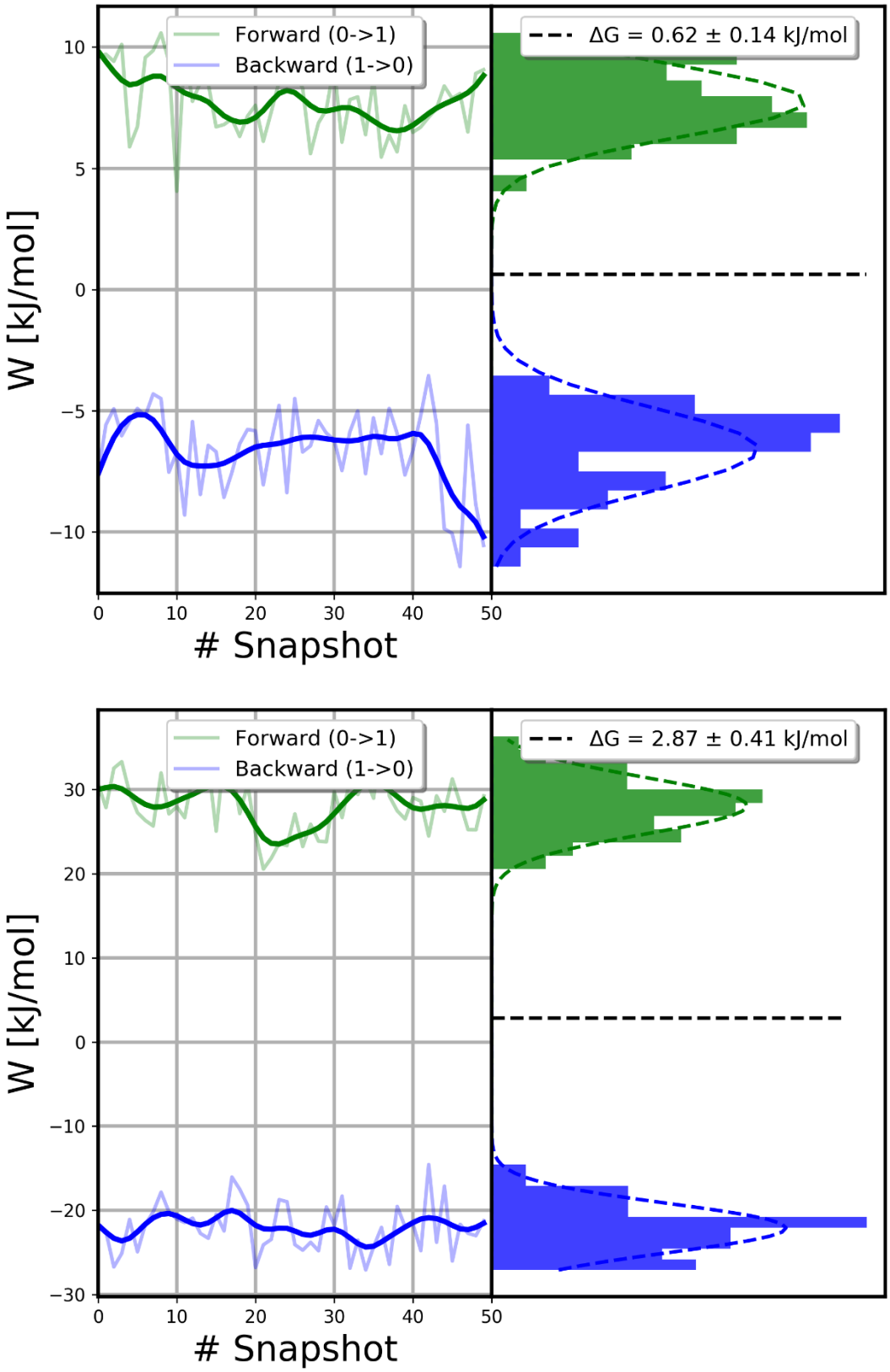


Figure S12. *Free energy plots for unbound (top) and bound (bottom) Y505HID (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

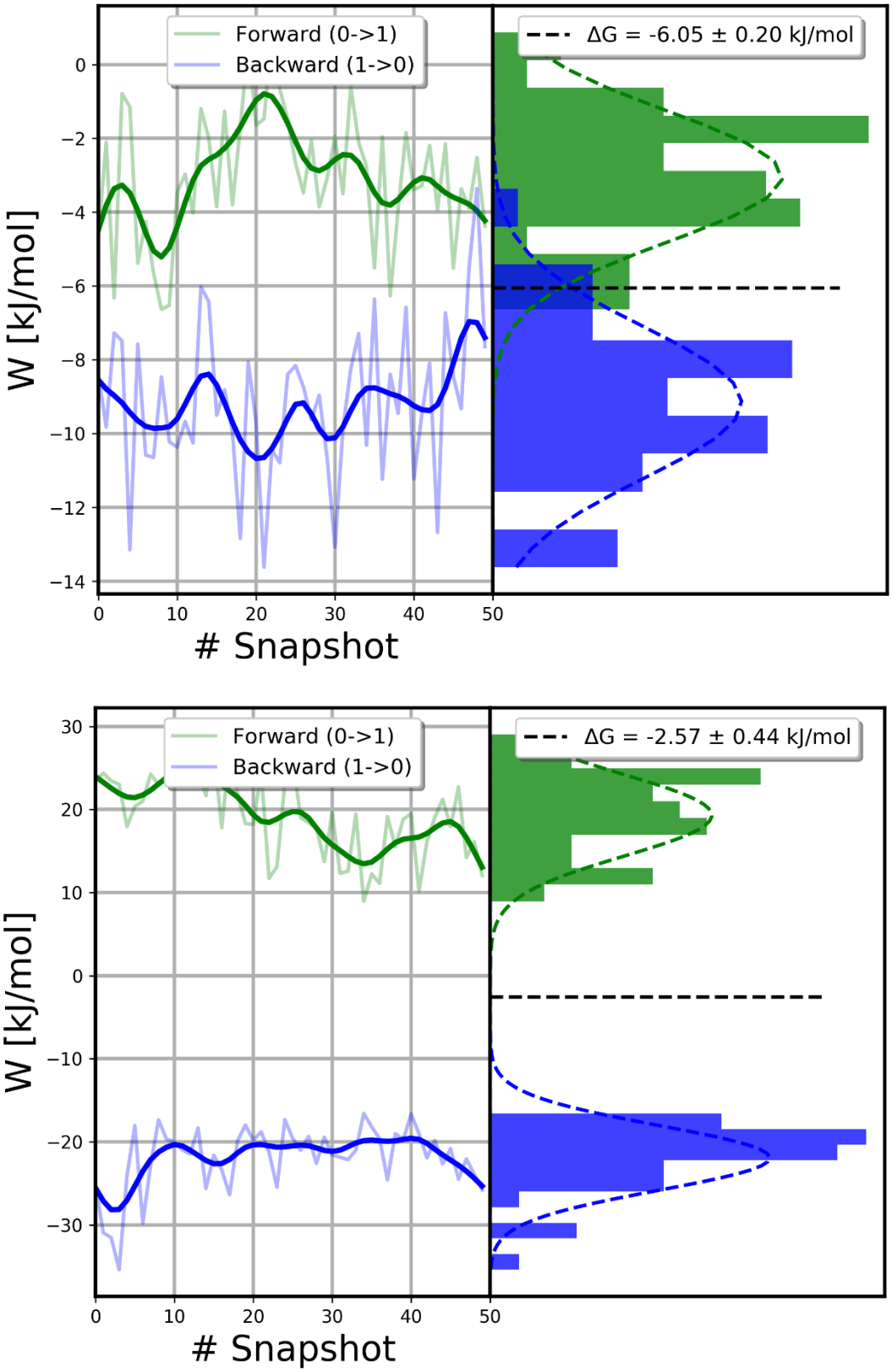


Figure S13. *Free energy plots for unbound (top) and bound (bottom) Y505HIE (each plot is the sum of 50 forward and 50 backward 5-ns non-equilibrium MD simulations).* ***Left:*** *Work values for the forward and backward transitions are plotted for every snapshot structure.* ***Right:*** *The work value distributions are depicted as histograms; their intersection gives the free energy difference.*

Input scripts for alchemical free energy calculations:

A. Forward

```
;==========================================================
; Non-equilibrium transition
;==========================================================

; RUN CONTROL
;----------------------------------------------------
integrator   = sd          ; stochastic leap-frog integrator
nsteps       = 2500000     ; 2 * 2500000 fs = 5 ns
dt           = 0.002       ; 2 fs
comm-mode    = Linear      ; remove center of mass translation
nstcomm      = 1           ; frequency for center of mass motion removal

; OUTPUT CONTROL
;----------------------------------------------------
nstxout                = 10000    ; save coordinates to .trr every 20 ps
nstvout                = 10000    ; save velocities to .trr every 20 ps
nstfout                = 10000    ; save forces to .trr every 20 ps
nstxout-compressed     = 10000    ; xtc compressed trajectory output every 20 ps
compressed-x-precision = 1000     ; precision with which to write to the compressed
   trajectory file
nstlog                 = 5000     ; update log file every 10 ps
nstenergy              = 1000     ; save energies every 2 ps
nstcalcenergy          = 1        ; calculate energies at every step

; BONDS
;----------------------------------------------------
constraint_algorithm   = lincs    ; holonomic constraints
constraints            = all-bonds  ; constrain all-bonds
lincs-iter             = 2        ; accuracy of LINCS (1 is default)
lincs-order            = 6        ; also related to accuracy (4 is default)
lincs-warnangle        = 30       ; maximum angle that a bond can rotate before LINCS will
   complain (30 is default)
continuation           = yes      ; formerly known as 'unconstrained-start' - useful for exact
   continuations and reruns

; NEIGHBOR SEARCHING
;----------------------------------------------------
cutoff-scheme          = Verlet ; group or Verlet
ns-type                = grid   ; search neighboring grid cells
nstlist                = 10     ; 20 fs (default is 10)
rlist                  = 1.0    ; short-range neighborlist cutoff (in nm)
pbc                    = xyz    ; 3D PBC

; ELECTROSTATICS & EWALD
;----------------------------------------------------
coulombtype      = PME                    ; Particle Mesh Ewald for long-range electrostatics
```

```
coulomb-modifier = Potential-shift-Verlet
rcoulomb        = 1.0                   ; short-range electrostatic cutoff (in nm)
ewald-geometry   = 3d                    ; Ewald sum is performed in all three dimensions
pme-order       = 4                     ; interpolation order for PME (default is 4)
fourierspacing   = 0.12                  ; grid spacing for FFT
ewald-rtol      = 1e-5                  ; relative strength of the Ewald-shifted direct potential at
   rcoulomb

; VAN DER WAALS
;----------------------------------------------------
vdw-type         = Cut-off      ; potential switched off at rvdw-switch to reach zero at rvdw
vdw-modifier      = Potential-shift-Verlet
rvdw             = 1.0          ; van der Waals cutoff (in nm)
DispCorr         = EnerPres     ; apply analytical long range dispersion corrections for
   Energy and Pressure

; TEMPERATURE COUPLING (Langevin)
;----------------------------------------------------
tc-grps          = System
tau-t            = 1.0
ref-t            = 298.15
gen-vel          = no           ; Velocity generation (if gen-vel is 'yes', continuation should be
   'no')
gen-seed         = -1

; PRESSURE COUPLING
;----------------------------------------------------
pcoupl           = Parrinello-Rahman
pcoupltype       = isotropic            ; uniform scaling of box vectors
tau_p            = 2.0                  ; time constant (ps)
ref_p            = 1.0                  ; reference pressure (bar)
compressibility  = 4.5e-05              ; isothermal compressibility of water (bar^-1)
refcoord-scaling = no

; FREE ENERGY
;----------------------------------------------------
free-energy       = yes
init-lambda       = 0     ; start from state A
delta-lambda      = 2.5e-07  ; complete transition in this number of steps
sc-coul           = yes   ; use soft-core also for coulombic interactions
sc-alpha          = 0.3   ; soft-core
sc-sigma          = 0.25  ;
sc-power          = 1     ;
nstdhdl           = 1     ; write to dhdl at each step
```

B. Reverse

```
;=====================================================
; Non-equilibrium transition
;=====================================================
```

```
; RUN CONTROL
;-----------------------------------------------------
integrator   = sd          ; stochastic leap-frog integrator
nsteps       = 2500000     ; 2 * 2500000 fs = 5 ns
dt           = 0.002       ; 2 fs
comm-mode    = Linear      ; remove center of mass translation
nstcomm      = 1           ; frequency for center of mass motion removal

; OUTPUT CONTROL
;-----------------------------------------------------
nstxout                = 10000     ; save coordinates to .trr every 20 ps
nstvout                = 10000     ; save velocities to .trr every 20 ps
nstfout                = 10000     ; save forces to .trr every 20 ps
nstxout-compressed     = 10000     ; xtc compressed trajectory output every 20 ps
compressed-x-precision = 1000      ; precision with which to write to the compressed
   trajectory file
nstlog                 = 5000      ; update log file every 10 ps
nstenergy              = 1000      ; save energies every 2 ps
nstcalcenergy          = 1         ; calculate energies at every step

; BONDS
;-----------------------------------------------------
constraint_algorithm   = lincs     ; holonomic constraints
constraints            = all-bonds ; constrain all bonds
lincs-iter             = 2         ; accuracy of LINCS (1 is default)
lincs-order            = 6         ; also related to accuracy (4 is default)
lincs-warnangle        = 30        ; maximum angle that a bond can rotate before LINCS will
   complain (30 is default)
continuation           = yes       ; formerly known as 'unconstrained-start' - useful for exact
   continuations and reruns

; NEIGHBOR SEARCHING
;-----------------------------------------------------
cutoff-scheme          = Verlet ; group or Verlet
ns-type                = grid   ; search neighboring grid cells
nstlist                = 10     ; 20 fs (default is 10)
rlist                  = 1.0    ; short-range neighborlist cutoff (in nm)
pbc                    = xyz    ; 3D PBC

; ELECTROSTATICS & EWALD
;-----------------------------------------------------
coulombtype      = PME                    ; Particle Mesh Ewald for long-range electrostatics
coulomb-modifier = Potential-shift-Verlet
rcoulomb         = 1.0                    ; short-range electrostatic cutoff (in nm)
ewald-geometry   = 3d                     ; Ewald sum is performed in all three dimensions
pme-order        = 4                      ; interpolation order for PME (default is 4)
fourierspacing   = 0.12                   ; grid spacing for FFT
ewald-rtol       = 1e-5                   ; relative strength of the Ewald-shifted direct potential at
   rcoulomb

; VAN DER WAALS
```

```
;----------------------------------------------------
vdw-type          = Cut-off      ; potential switched off at rvdw-switch to reach zero at rvdw
vdw-modifier      = Potential-shift-Verlet
rvdw              = 1.0          ; van der Waals cutoff (in nm)
DispCorr          = EnerPres     ; apply analytical long range dispersion corrections for
   Energy and Pressure

; TEMPERATURE COUPLING (Langevin)
;----------------------------------------------------
tc-grps           = System
tau-t             = 1.0
ref-t             = 298.15
gen-vel           = no           ; Velocity generation (if gen-vel is 'yes', continuation should be
   'no')
gen-seed          = -1

; PRESSURE COUPLING
;----------------------------------------------------
pcoupl            = Parrinello-Rahman
pcoupltype        = isotropic            ; uniform scaling of box vectors
tau_p             = 2.0                  ; time constant (ps)
ref_p             = 1.0                  ; reference pressure (bar)
compressibility   = 4.5e-05              ; isothermal compressibility of water (bar^-1)
refcoord-scaling = no

; FREE ENERGY
;----------------------------------------------------
free-energy       = yes
init-lambda       = 1          ; start from state B
delta-lambda      = -2.5e-07  ; complete transition in this number of steps
sc-coul           = yes   ; use soft-core also for coulombic interactions
sc-alpha          = 0.3   ; soft-core
sc-sigma          = 0.25  ;
sc-power          = 1     ;
nstdhdl           = 1     ; write to dhdl at each step
```