em1.mdp

```
; Parameters describing the details of the Energy Minimization protocol
define = -DPOSRES             ; Define any restrain ("-DPOSRES" OR "-DSTRONG_POSRES" OR leave blank if there is no restrain)
integrator      = cg          ; EM Algorithm (steep = steepest descent; cg = conjugate gradiant; l-bfgs = Low-memory Broyden-Fletcher-Goldfarb-Shanno quasi-Newtonian minimizer)
emtol           = 100.0       ; Minimization is stopped when the maximum force on an atom is less than the given value (kJ/mol/nm)
emstep          = 0.01        ; Initial step size (nm)
nsteps          = 50000               ; Maximum number of (energy minimization) steps to be performed

; Parameters describing neighbors searching and details about interaction calculations
nstlist         = 1           ; Neighbor list update frequency (after every given number of steps)
ns_type                 = grid          ; Neighbor list search method (simple, grid)
rlist           = 1.2         ; Neighbor list search cut-off (nm)
coulombtype     = PME         ; Long range electrostatic interactions treatment (cut-off, Ewald, PME)
rcoulomb        = 1.2         ; Short-range electrostatic cut-off (nm)
rvdw            = 1.2         ; Short-range van der Waals cut-off (nm)
pbc             = xyz         ; Direction in which to use Perodic Boundary Conditions (xyz, xy, no)
```

em2.mdp

```
; Parameters describing the details of the Energy Minimization protocol
define          =           ; Define any restrain ("-DPOSRES" OR "-DSTRONG_POSRES" OR leave blank if there is no restrain)
integrator      = cg        ; EM Algorithm (steep = steepest descent; cg = conjugate gradiant; l-bfgs = Low-memory Broyden-Fletcher-Goldfarb-Shanno quasi-Newtonian minimizer)
nstcgsteep      = 1000      ; Frequency of performing 1 steepest descent step while doing conjugate gradient energy minimization
emtol           = 100.0     ; Minimization is stopped when the maximum force on an atom is less than the given value (kJ/mol/nm)
emstep          = 0.01      ; Initial step size (nm)
nsteps          = 50000             ; Maximum number of (energy minimization) steps to be performed

; Parameters describing neighbors searching and details about interaction calculations
nstlist         = 1         ; Neighbor list update frequency (after every given number of steps)
ns_type                 = grid          ; Neighbor list search method (simple, grid)
rlist           = 1.2       ; Neighbor list search cut-off (nm)
coulombtype     = PME       ; Long range electrostatic interactions treatment (cut-off, Ewald, PME)
rcoulomb        = 1.2       ; Short-range electrostatic cut-off (nm)
rvdw            = 1.2       ; Short-range van der Waals cut-off (nm)
pbc             = xyz       ; Direction in which to use Perodic Boundary Conditions (xyz, xy, no)
```

heating.mdp

```
define          = -DPOSRES ; Protein is position restrained (uses the posres.itp file information)
; Parameters describing the details of the NVT simulation protocol
integrator      = md            ; Algorithm ("md" = molecular dynamics [leap-frog integrator]; "md-vv" = md using velocity verlet; sd = stochastic dynamics)
dt              = 0.002                 ; Time-step (ps)
nsteps          = 250000        ; 500 ps

; Parameters controlling output writing
nstxout         = 1000          ; Write coordinates to output .trr file every 2 ps
nstvout         = 1000          ; Write velocities to output .trr file every 2 ps
nstenergy       = 1000          ; Write energies to output .edr file every 2 ps
nstlog          = 1000          ; Write output to .log file every 2 ps

; Parameters describing neighbors searching and details about interaction calculations
cutoff_scheme   = Verlet
ns_type                 = grid          ; Neighbor list search method (simple, grid)
nstlist         = 10            ; Neighbor list update frequency (after every given number of steps)
rlist           = 1.2           ; Neighbor list search cut-off distance (nm)
rcoulomb        = 1.2           ; Short-range Coulombic interactions cut-off distance (nm)
rvdw            = 1.2           ; Short-range van der Waals cutoff distance (nm)
pbc             = xyz           ; Direction in which to use Perodic Boundary Conditions (xyz, xy, no)

; Parameters for treating bonded interactions
continuation    = no            ; Whether a fresh start or a continuation from a previous run (yes/no)
constraint_algorithm = LINCS        ; Constraint algorithm (LINCS / SHAKE)
constraints     = all-bonds     ; Which bonds/angles to constrain (all-bonds / hbonds / none / all-angles / h-angles)
lincs_iter      = 1             ; Number of iterations to correct for rotational lengthening in LINCS (related to accuracy)
lincs_order     = 4             ; Highest order in the expansion of the constraint coupling matrix (related to accuracy)

; Parameters for treating electrostatic interactions
coulombtype     = PME           ; Long range electrostatic interactions treatment (cut-off, Ewald, PME)
pme_order       = 4             ; Interpolation order for PME (cubic interpolation is represented by 4)
fourierspacing  = 0.12          ; Maximum grid spacing for FFT grid using PME (nm)

; Temperature coupling parameters
tcoupl          = berendsen         ; Modified Berendsen thermostat using velocity rescaling
tc-grps         = Protein_MOP POPC SOL_NA_CL        ; Define groups to be coupled separately to temperature bath, same groups should be defined in index.ndx
tau_t           = 0.5   0.5     0.5     ; Group-wise coupling time constant (ps)
ref_t           = 60    60      60      ; Group-wise reference temperature (K)

; annealing
annealing = single single single
annealing_npoints = 5 5 5
annealing_time = 0 100 200 300 400 0 100 200 300 400 0 100 200 300 400
annealing_temp = 60 120 180 240 300 60 120 180 240 300 60 120 180 240 300
```

```
; Pressure coupling parameters
pcoupl          = berendsen       ; Pressure coupler used under NPT conditions
pcoupltype      = semiisotropic                 ; Isotropic scaling in the x-y direction, independent
of the z direction
tau_p           = 5.0                   ; Coupling time constant (ps)
ref_p           = 1.0   1.0             ; Reference pressure for coupling, x-y, z directions (bar)
compressibility = 4.5e-5        4.5e-5    ; Isothermal compressibility (bar^-1)
refcoord_scaling= all                   ; Scale reference coordinates for the position restraints

; Miscellaneous control parameters
; Dispersion correction
DispCorr        = EnerPres              ; Dispersion corrections for Energy and Pressure for vdW cut-
off
; Initial Velocity Generation
gen_vel                 = yes                   ; Generate Velocities
; Centre of mass (COM) motion removal relative to the specified groups
nstcomm                 = 1                     ; COM removal frequency (steps)
comm_mode   = Linear                ; Remove COM translation (linear / angular / no)
comm_grps   = Protein_MOP POPC SOL_NA_CL        ; COM removal relative to the specified
groups
```

md1.mdp

```
; Parameters describing the details of the NVT simulation protocol
integrator      = md            ; Algorithm ("md" = molecular dynamics [leap-frog integrator]; "md-vv" = md using velocity verlet; sd = stochastic dynamics)
dt              = 0.002         ; Time-step (ps)
nsteps          = 250000000  ; 500 ns

; Parameters controlling output writing
nstxout         = 10000         ; Write coordinates to output .trr file every 20 ps
nstvout         = 10000         ; Write velocities to output .trr file every 20 ps
nstenergy       = 10000         ; Write energies to output .edr file every 20 ps
nstlog          = 10000         ; Write output to .log file every 20 ps

; Parameters describing neighbors searching and details about interaction calculations
cutoff_scheme   = Verlet
ns_type                 = grid          ; Neighbor list search method (simple, grid)
nstlist         = 10            ; Neighbor list update frequency (after every given number of steps)
rlist           = 1.2           ; Neighbor list search cut-off distance (nm)
rcoulomb        = 1.2           ; Short-range Coulombic interactions cut-off distance (nm)
rvdw            = 1.2           ; Short-range van der Waals cutoff distance (nm)
pbc             = xyz           ; Direction in which to use Perodic Boundary Conditions (xyz, xy, no)

; Parameters for treating bonded interactions
continuation    = yes           ; Whether a fresh start or a continuation from a previous run (yes/no)
constraint_algorithm = LINCS        ; Constraint algorithm (LINCS / SHAKE)
constraints     = all-bonds     ; Which bonds/angles to constrain (all-bonds / hbonds / none / all-angles / h-angles)
lincs_iter      = 1             ; Number of iterations to correct for rotational lengthening in LINCS (related to accuracy)
lincs_order     = 4             ; Highest order in the expansion of the constraint coupling matrix (related to accuracy)

; Parameters for treating electrostatic interactions
coulombtype   = PME         ; Long range electrostatic interactions treatment (cut-off, Ewald, PME)
pme_order     = 4           ; Interpolation order for PME (cubic interpolation is represented by 4)
fourierspacing= 0.12        ; Maximum grid spacing for FFT grid using PME (nm)

; Temperature coupling parameters
tcoupl          = Nose-Hoover               ; Modified Berendsen thermostat using velocity rescaling
tc-grps         = Protein_MOP POPC SOL_NA_CL        ; Define groups to be coupled separately to temperature bath
tau_t           = 0.5   0.5     0.5     ; Group-wise coupling time constant (ps)
ref_t           = 300   300     300     ; Group-wise reference temperature (K)

; Pressure coupling parameters
pcoupl          = Parrinello-Rahman     ; Pressure coupler used under NPT conditions
pcoupltype      = semiisotropic                 ; Isotropic scaling
tau_p           = 2.0                   ; Coupling time constant (ps)
ref_p           = 1.0   1.0             ; Reference pressure for coupling, x-y, z directions (bar)
compressibility = 4.5e-5        4.5e-5      ; Isothermal compressibility (bar^-1)
```

```
; Miscellaneous control parameters
; Dispersion correction
DispCorr        = EnerPres              ; Dispersion corrections for Energy and Pressure for vdW cut-off
; Initial Velocity Generation
gen_vel         = no                    ; Velocity is read from the previous run
; Centre of mass (COM) motion removal relative to the specified groups
nstcomm         = 1                     ; COM removal frequency (steps)
comm_mode       = Linear                ; Remove COM translation (linear / angular / no)
comm_grps       = Protein_MOP POPC SOL_NA_CL        ; COM removal relative to the specified groups
```