premin.in

initial minimisation to clean up bad contacts (restraints on protein so only water and couterions minimise)

```
&cntrl
     imin=1,                  perform an energy minimisation
     maxcyc=1000,             maximum number of cycles of minimization
     ncyc=100,                if ntmin=1 (default), switch from steepest descent to
                              conjugate gradient after ncyc cycles.
     cut=12.,                 nonbonded cutoff, in Angstroms.
     ntb=1,                   constant volume (default when igb and ntp are both 0)
     ntr=1,                   flag for restraining atoms using a harmonic potential
     ntpr=100                 every ntpr steps mdout and mdinfo are printed out
/
Hold protein fixed
10000.0
RES 1 461
END
END
```

sandermin1.in

minimisation with no restraints on the whole system

```
&cntrl
     imin=1,                  perform an energy minimisation
     maxcyc=2500,             maximum number of cycles of minimization
     ncyc=1000,               if ntmin=1 (default), switch from steepest descent to
                              conjugate gradient after ncyc cycles.
     cut=12.,                 nonbonded cutoff, in Angstroms.
     ntb=1,                   constant volume (default when igb and ntp are both 0)
     ntpr=100                 every ntpr steps mdout and mdinfo are printed out
/
```

02_Heat.in

NVE heating of the system from 0K to 100K with restraints on protein and solute (TNC, TNI, TNT, CAL) during 100 ps (50000 steps of MD simulation, timestep of 0.002 ps).

```
&cntrl
     imin=0,                  run MD with no minimisation
     ntx=1,                   only coordinates will be read from the inpcrd file
     irest=0,                 do not restart the simulation (run as new simulation)
     ntc=2,                   flag for SHAKE to perform bond length constraints
                (ntc=ntf=2 for TIP3P water --> bonds involving hydrogen are constrained)
     ntf=2,
     tol=0.0000001,     Relative geometrical tolerance for coordinate resetting in SHAKE
     ntr=1,                   flag for restraining atoms using a harmonic potential
     ig=-1,                   seed for the pseudo-random number generator is based on
                               current time and date, so each run is =!
     nstlim=50000,            number of MD steps to be performed
     ntt=3,       switch for temperature scaling. Use langevin dynamics with collision
                       frequency set by gamma_ln
     gamma_ln=1.0,     collision frequency in ps-1 when ntt=3
     ntpr=1000,         every ntpr steps mdout and mdinfo are printed out
     ntwr=10000,        every ntwr steps the restrt file is written
     ntwx=1000,         every ntwx the coordinates are written to the mdcrd file
     dt=0.002,          time step in ps
     nmropt=1,         NMR restraints and weight changes will be read
     ntb=1,            constant volume (default when igb and ntp are both 0)
     ntp=0,            no pressure scaling
     cut=8.0,          nonbonded cutoff, in Angstroms.
     ioutfm=1,         write the coordinate and velocity files (mdcrd, mdvel and inptraj) as
                        binary NetCDF trajectory
/
&wt type='TEMP0',
     istep1=0,
     istep2=50000,
     value1=0.0,
     value2=100.0 /
&wt type='END' /
Hold TNC TNT
10.0
RES 1 248
END
Hold bits of TNI
10.0
RES 290 408
END
Hold bits of CAL's
10.0
RES 459 461
END
END
```

03_Heat2.in

NPT heat up to 300K with weak restaints on protein and solute during 400 ps (200000 steps of MD simulation with 0.002 ps timestep)

```
&cntrl
      imin=0,                    run MD with no minimisation
      ntx=5,                     coordinates and velocities will be read
      irest=1,                   restart the simulation (reading coordinates and velocities
                                     from a previous saved restart file)
      ntc=2,                     flag for SHAKE to perform bond length constraints
            (ntc=ntf=2 for TIP3P water --> bonds involving hydrogen are constrained)
      ntf=2,
      tol=0.0000001,     Relative geometrical tolerance for coordinate resetting in SHAKE
      ntr=1,                     flag for restraining atoms using a harmonic potential
      ig=-1,                     seed for the pseudo-random number generator is based on
                              current time and date, so each run is =!
      nstlim=200000,         number of MD steps to be performed
      ntt=3,              switch for temperature scaling. Use langevin dynamics with collision
                              frequency set by gamma_ln
      gamma_ln=1.0,              collision  frequency in ps-1 when ntt=3
      ntpr=1000,                 every ntpr steps mdout and mdinfo are printed out
      ntwr=1000,                 every ntwr steps the restrt file is written
      ntwx=1000,                 every ntwx the coordinates are written to the mdcrd file
      dt=0.002,                  time step in ps
      nmropt=1,                NMR restraints and weight changes will be read
      ntb=2,                     constant pressure (default when ntp>0)
      ntp=1,                     flag for constant pressure dynamics. =1 is MD with
                              isotropic position scaling
      taup=2.0,                pressure relaxation time in ps (should be between 1.0 and 5.0)
      cut=8.0,                   nonbonded cutoff, in Angstroms.
      ioutfm=1,                write the coordinate and velocity files (mdcrd, mdvel and inptraj) as
                              binary NetCDF trajectory
      ntxo=2,                  format of the final coordinates, velocities and box size (if constant P or
                                  T run) written to file “restrt” is NetCDF
/
&wt type='TEMP0',
      istep1=0,
      istep2=100000,
      value1=0.0,
      value2=500.0 /
&wt type='END' /
Hold TNC TNT
10.0
RES 1 248
END
Hold bits of TNI
10.0
RES 290 408
END
Hold bits of CAL's
10.0
RES 459 461
END
END
```

05_Prod.in

Production run, so you need to set the write frequency of the print, restart and trajectory to appropriate values.

```
&cntrl
     imin=0,                          run MD with no minimisation
     ntx=5,                           coordinates and velocities will be read
     irest=1,                         restart the simulation (reading coordinates and
                                      velocities from a previous saved restart file)
     ntc=2,                           flag for SHAKE to perform bond length constraints
                (ntc=ntf=2 for TIP3P water --> bonds involving hydrogen are constrained)
     ntf=2,
     nstlim=20000000,                 number of MD steps to be performed
     ntt=3,                switch for temperature scaling. Use langevin dynamics with collision
                                frequency set by gamma_ln
     gamma_ln=1.0,                    collision  frequency in ps-1 when ntt=3
     temp0=300.0,            reference T at which the system is to be kept when ntt>0
     ntpr=5000,                       every ntpr steps mdout and mdinfo are printed out
     ntwr=500000,                     every ntwr steps the restrt file is written
     ntwx=5000,                       every ntwx the coordinates are written to the mdcrd file
     dt=0.0025,                       time step in ps
     ig=-1,                           seed for the pseudo-random number generator is based on
                                current time and date, so each run is =!
     ntb=2,                           constant pressure (default when ntp>0)
     ntp=1,                           flag for constant pressure dynamics. =1 is MD with
                                isotropic position scaling
     cut=8.0,                         nonbonded cutoff, in Angstroms.
     barostat=2,           Monte Carlo barostat is used to control the pressure
     ioutfm=1,                write the coordinate and velocity files (mdcrd, mdvel and inptraj) as
                                binary NetCDF trajectory
     ntxo=2,           format of the final coordinates, velocities and box size (if constant P or
                       T run) written to file “restrt” is NetCDF
/
```