

Protocol

- 1 Initial minimization:** Let solvent relax around restrained solute
 - 1 1000 steps (500 steepest descents/500 conjugate gradient)
 - 2 500 kcal/mol-Å restraints (solute)
- 2 Second minimization:** Let everything relax
 - 1 2500 steps (1000 steepest descents/1500 conjugate gradients)
- 3 Defrost (md1):** Begin constant volume to warm to proper temperature w/ restrained solute
 - 1 100 ~~20~~ ps *NVT*
 - 2 Langevin temperature control 0 → 300 K
 - 3 25 kcal/mol-Å restraints (solute)
- 4 Equilibration (md2):** Switch to constant pressure to get proper density while gradually releasing restraints on solute (5-stage release with strong restraints for first 40 ps while density is changing most rapidly)
 - 1 250 ~~100~~ ps *NPT*
 - 2 Langevin temperature control 300 K
 - 3 “Weak coupling” pressure control 1.0 bar (~ 1 atm)
 - 4 25 → 5 kcal/molÅ² restraints (solute)
 - 1 md2a. 25 kcal ~~20~~ 50 ps
 - 2 md2b. 20 kcal ~~20~~ 50 ps
 - 3 md2c. 15 kcal ~~20~~ 50 ps
 - 4 md2d. 10 kcal ~~20~~ 50 ps
 - 5 md2e. 5 kcal ~~20~~ 50 ps
- 5 Equilibration (md3):** Release solute constraints and collect data to isotropically scale box size to reflect average volume
 - 1 200 ~~80~~ ps *NPT*
 - 2 Langevin temperature control 300 K
 - 3 “Weak coupling” pressure control 1.0 bar (~ 1 atm)
 - 4 Calculate <volume> ** 1/3 and replace x,y,z in restart file
- 6 Equilibration (md4):** Switch to constant volume and equilibrate with scaled box size
 - 1 1 ns ~~150 ps~~ *NVT*
 - 2 Langevin temperature control 300 K
 - 3 ~~Scale velocities of final snapshot restart file to 300 K (scaleVelocities.scr)~~
- 7 ~Equilibration (md5):** Switch off temperature control and equilibrate~~
 - 1 100 ps *NVE*
- 8 Production**
 - 1 *NVE*
 - 2 Turn off removal of translational motion (nscm=0)
 - 3 Monitor temperature and Etot