1. Load the **lastframe.pdb** of the equilibrated structure. In VMD TK Console write:

   set allatoms [atomselect top all]
   $allatoms set beta 0
   $allatoms set occupancy 0
   $allatoms writepdb new.ref (This will save new.ref file at your current directory)

2. To create the **smd.ref** file, open **new.ref** text file and modify the **B column of the SMD atom** from 0 to 1. Save the file as **smd.ref.**

   For potassium;

```
ATOM   2265  OE2 GLU P 149     -23.296 -16.076 -19.389  0.00  0.00       P
ATOM   2266  C   GLU P 149     -21.258 -12.399 -21.170  0.00  0.00       P
ATOM   2267  OT1 GLU P 149     -21.625 -12.078 -22.301  0.00  0.00       P
ATOM   2268  OT2 GLU P 149     -21.014 -11.520 -20.255  0.00  0.00       P
ATOM   2269  POT POT K 158       4.404 -18.349 -17.171  1.00  0.00       K
ATOM   2270  OH2 TIP3W   2      39.634  37.307  -6.943  0.00  0.00       WT1
ATOM   2271  H1  TIP3W   2      39.465  37.036  -7.845  0.00  0.00       WT1
ATOM   2272  H2  TIP3W   2      40.482  36.916  -6.730  0.00  0.00       WT1
```

   For ammonium ;

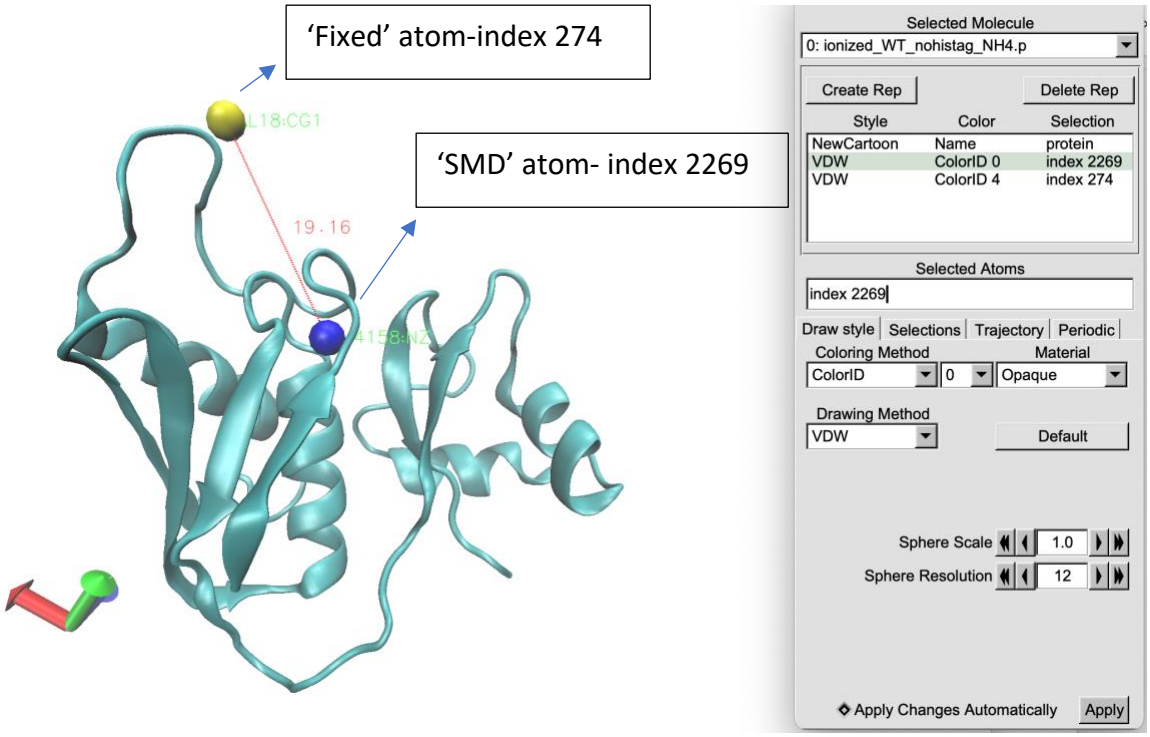
```
ATOM   2267  OT1 GLU P 149     -16.332  -1.990  -7.732  0.00  0.00       P
ATOM   2268  OT2 GLU P 149     -17.044   0.047  -8.332  0.00  0.00       P
ATOM   2269  HZ1 NH4 N 158       9.175   8.018  -5.961  0.00  0.00       N
ATOM   2270  NZ  NH4 N 158       8.939   7.754  -6.939  1.00  0.00       N
ATOM   2271  HZ2 NH4 N 158       9.374   7.866  -6.001  0.00  0.00       N
ATOM   2272  HZ3 NH4 N 158       8.986   7.930  -5.915  0.00  0.00       N
ATOM   2273  HZ4 NH4 N 158       9.444   8.059  -6.083  0.00  0.00       N
ATOM   2274  OH2 TIP3W   1     -20.992  17.539   6.446  0.00  0.00       WT1
ATOM   2275  H1  TIP3W   1     -20.357  16.833   6.570  0.00  0.00       WT1
```

3. To create the constraints.ref file, again open new.ref and modify the **B column of Ca atoms of residues 7, 10, 75, 77 and 80 as 50.** This is the force constant that will be applied to Ca atoms of binding site residues to keep the protein at place during pulling. . Save the file as **constraints.ref**. An example of residue 7 is shown below:

```
ATOM    100  N   VAL P   7       2.540 -21.202 -20.427  0.00  0.00       P
ATOM    101  HN  VAL P   7       1.832 -20.654 -20.864  0.00  0.00       P
ATOM    102  CA  VAL P   7       3.897 -21.027 -21.032 50.00  0.00       P
ATOM    103  HA  VAL P   7       4.148 -22.005 -21.413  0.00  0.00       P
ATOM    104  CB  VAL P   7       3.870 -20.243 -22.383  0.00  0.00       P
ATOM    105  HB  VAL P   7       3.453 -19.215 -22.319  0.00  0.00       P
ATOM    106  CG1 VAL P   7       5.304 -20.063 -22.891  0.00  0.00       P
ATOM    107 HG11 VAL P   7       5.852 -19.264 -22.345  0.00  0.00       P
ATOM    108 HG12 VAL P   7       5.909 -20.994 -22.858  0.00  0.00       P
ATOM    109 HG13 VAL P   7       5.211 -19.647 -23.917  0.00  0.00       P
ATOM    110  CG2 VAL P   7       3.052 -21.092 -23.523  0.00  0.00       P
ATOM    111 HG21 VAL P   7       2.995 -20.489 -24.454  0.00  0.00       P
ATOM    112 HG22 VAL P   7       3.475 -22.102 -23.707  0.00  0.00       P
ATOM    113 HG23 VAL P   7       2.040 -21.231 -23.085  0.00  0.00       P
ATOM    114  C   VAL P   7       5.107 -20.648 -20.125  0.00  0.00       P
ATOM    115  O   VAL P   7       5.145 -19.608 -19.528  0.00  0.00       P
```

4. To determine SMD Direction, firstly find the **indexes** of the SMD atom (N of NH4 or K) and a fixed atom from the PDB file. Fixed atom will not be actually fixed during simulation. It is a reference atom to determine the direction of pulling. Note that the atom index in PDB file is 1 point higher than in VMD indexing.
   For example index of the N atom is **2270** in the PDB file of WT, but you need to type **index 2269** to visualize this atom in VMD.

```
2268 P    149   GLU   OT2   OC     -0.670000   15.9994   0
2269 N    158   NH4   HZ1   HGP2    0.330000    1.0080   0
2270 N    158   NH4   NZ    NG3P3  -0.320000   14.0070   0
2271 N    158   NH4   HZ2   HGP2    0.330000    1.0080   0
2272 N    158   NH4   HZ3   HGP2    0.330000    1.0080   0
2273 N    158   NH4   HZ4   HGP2    0.330000    1.0080   0
```



In VMD TkConsole, set the fixed atom and SMD atom by typing:
set fixedatom [atomselect top "index 274"]
set smdatom [atomselect top "index 2269"]

Get the direction vector between them:
set smdpos [lindex [$smdatom get {x y z}] 0]
set fixedpos [lindex [$fixedatom get {x y z}] 0]
vecnorm [vecsub $fixedpos $smdpos]

Calculated nx, ny and nz values (together with their +/- signs) will be written in .conf file as:

SMDDir nx ny nz