CHL Lab
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Cyanylation on protein + water + ions (the entire system)—manually

Good for: Inserting the MSCN probe and continuing a simulation from a frame in MD trajectory, with the waters / ions around the protein.

Strategy: Take the whole protein / water / ions complex with box dimensions (at desired representative structures; in the case of 2BBM, simply t=100.0 ns); modify the desired residue to MSCN; use pdb2gmx to convert the whole system to .gro file, together with the box dimensions, and generate the corresponding .top files; submit for MD run directly, and take the converged trajectory for analysis.

In Chimera (Pymol screws up residue listing):

1. Select residue to mutate (Tools->Sequence->Sequence; control+drag to select)
2. Mutagenesis: Tools->Structural editing->Rotamers; show all atoms / bonds to aid selection of a cysteine rotamer
3. Cyanylation: Tools->Structural editing->Build structure->SMILES string; Enter C#N; or -> PunChemCID; Enter 768
4. Tools->Command line; type “select #0#1” then "combine selected close true"
5. Delete H from HCN group: zoom out to find HCN group first. Then control-click to select H from HCN. Then Build structure->Modify structure->Delete (selected atoms/bonds)
6. Bond S-C: control-click to select S and C; use Shift to add second atom. Build structure->Adjust bonds->Add (all possible) bonds
7. Adjust SC bond length: control-click to select S-C bond. Build structure->Adjust bonds->Set length to 1.688
8. Adjust angles: control (shift) click to select CB-S and S-C bonds; Build structure->Adjust angles->100.6; then select S-C and C-N bonds; enter 177.8.
9. Adjust torsions: Action -> Ribbon -> hide. In command line, type “select #2:[MSCN residue number] z<7”. Actions -> Atoms/Bonds -> Show. Then select CB-S bond. Build structure->Adjust torsions->Activate. Adjust the value to make the torsion angle close to 180, but avoid steric clashes with nearby residues / waters.
10. Save as .pdb file.

Edit .pdb file:

1. Move C and N from res UNK to end of CYS. Modify residue and chain number.

2. Change names of S C N to SD CE NF.
3. Change residue name of cyanylated cysteine to MSCN. (Caution .pdb file formatting: “M” from “MSCN” should align with the first letter of other residues, even if this means “N” will be directly adjacent to the chain number without a blank in between.
4. Open in either Chimera or Pymol. Check to make sure that all arginines have NH1 cis to CD, and NH2 trans to CD. If not, switch the names of NH1, NH2 and attached hydrogens.
5. Copy headings from the wild type representative structure .pdb file to the mutated .pdb file for box dimensions.

In Gromacs:

1. pdb2gmx: gmx pdb2gmx -f $protein-$site.pdb -o $protein-$site.gro -p $protein-$site.top -water tip3p -ff amber99sbMSCN (or charmm36MSCN) -ter
2. Convert .gro file back to .pdb file. Check to make sure it is correct.
3. Compare box dimensions in the generated .pdb file to the wild type .pdb file. Make sure they are identical.

Use the .gro and .top files for simulation purposes. The following is just an example.
Note: make sure that your system does have initial velocities! Otherwise the initial temperature will be 0, and it can take a while for the system to heat up.

1. Modify 6-md.mdp to the correct parameters (and set gen-vel = yes and continuation = no)
2. Then: gmx grompp -f 6-md.mdp -c $protein-$site.gro -p $protein-$site.top -o $protein-$site-md.tpr
3. and submit for: gmx_mpi mdrun -v -deffnm $protein-$site-md