CHL Lab
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Umbrella sampling / wham protocol

1. Run ir-md-umbrella-setup-slurm.sh and then ir-md-umbrella-md-slurm.sh.
2. Gather the output *dihedral*.xvg files in $protein-$site-$spoint-$length-$proc/raw.
3. Install the Grossfield Lab wham software (remember to set energy units to kJ/mol for gromacs outputs!!!): http://membrane.urmc.rochester.edu/content/wham, and read the documentation.
4. Run the following script (with modifications) to produce wham-2d inputs:

```
springx=0.0091385    #spring constant for CA-CB-SD-CE in units of kJ/mol/degree^2 (while dihrestraint.itp has it in kJ/mol/rad^2!)
springy=0.0091385    #spring constant for CA-CB-SD-CE in units of kJ/mol/degree^2 (while dihrestraint.itp has it in kJ/mol/rad^2!)
nheadings=16          #number of headings in *dihedralTime.xvg files
proc=md               #no need to change
for protein in holo.cam; do
for site in m72c; do
for spoint in n.1; do
for length in 250ps-umbrella; do
        cd $protein-$site-$spoint-$length-$proc
        mkdir processed
for center1 in $(seq 0 30 331); do
for center2 in $(seq 0 30 331); do
        awk -v nheadings=$nheadings  'NR>nheadings{print}' raw/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime1.xvg > processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime1-no_heading.xvg
        awk -v nheadings=$nheadings  'NR>nheadings{print}' raw/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime2.xvg > processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime2-no_heading.xvg
        awk 'FNR==NR{a[$1,1]=$1; a[$1,2]=$2;next}{print a[$1,1], a[$1,2], $2}' processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime1-no_heading.xvg processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime2-no_heading.xvg > processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-dihedralTime-combined.xvg
        rm processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-
```

```
dihedralTime1-no_heading.xvg
        rm processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-
dihedralTime2-no_heading.xvg
        (echo processed/$protein-$site-$spoint-$length-$center1-$center2-$proc-
dihedralTime-combined.xvg $center1 $center2 $springx $springy) >> holo.cam-
m72c-n.1-500ps-wham.inp
done
done
        cd ..
done
done
done
done
```

5. Run wham-2d:

```
wham-2d Px -180 180 36 Py -180 180 36 1e-12 300 0  holo.cam-m72c-n.
1-250ps-wham.inp holo.cam-m72c-n.1-250ps-wham.dat 1
```

Refer to manual for what each option means and weather you need to change them: http://membrane.urmc.rochester.edu/sites/default/files/wham/doc.html

6. Plot the output .dat file in wham-analysis.nb, and calculate double integrals under each dihedral combination with non-zero probabilities.