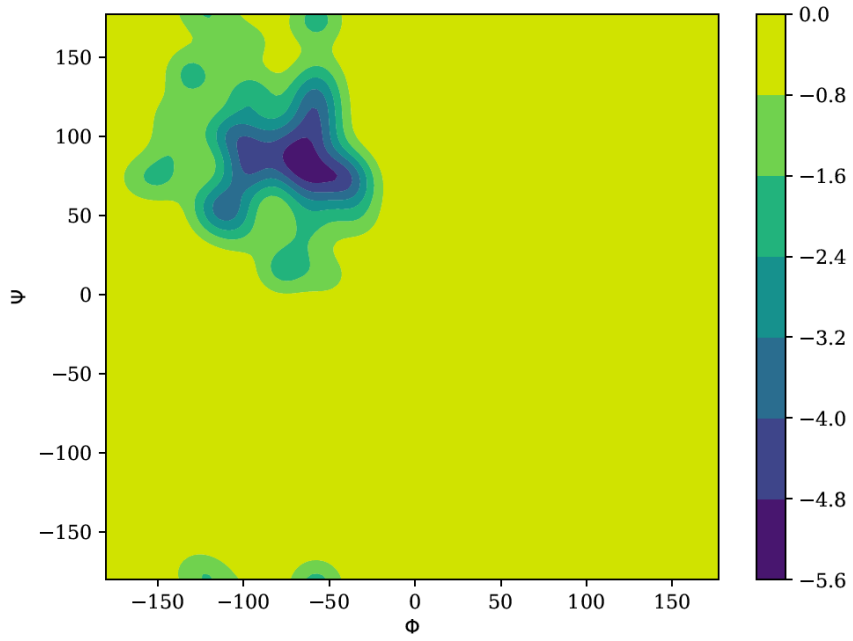
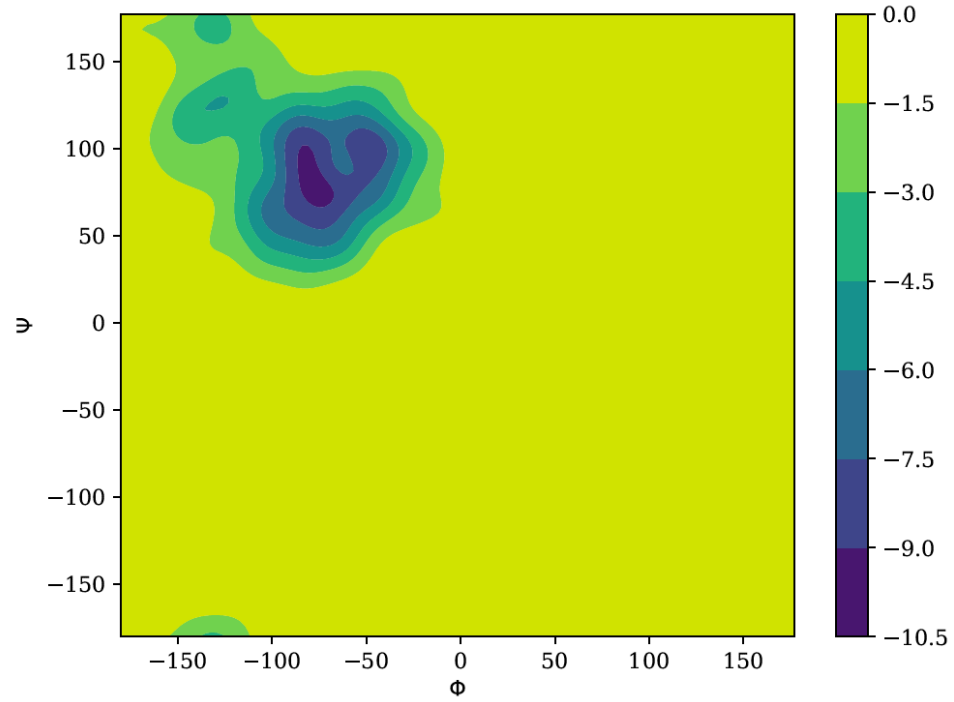
1. Alanine Dipeptide in Vacuum

1a. These codes were generated using `python alanine_meta_openmm77.py 1.0 .2 100 x` where 'x' equals 50, 100, 500, 1000.

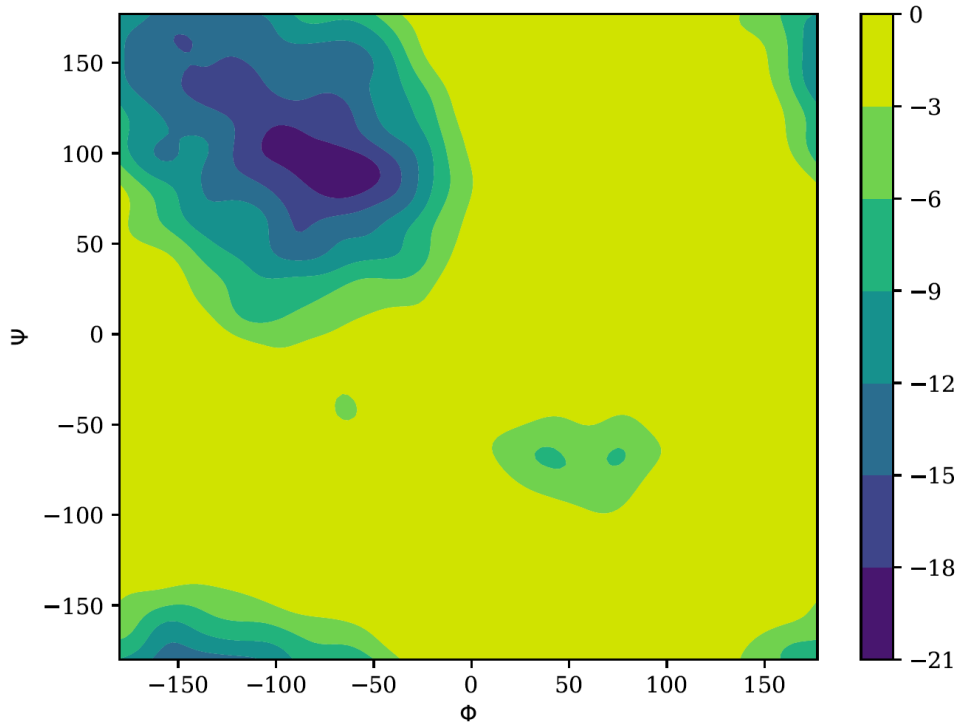
50 steps



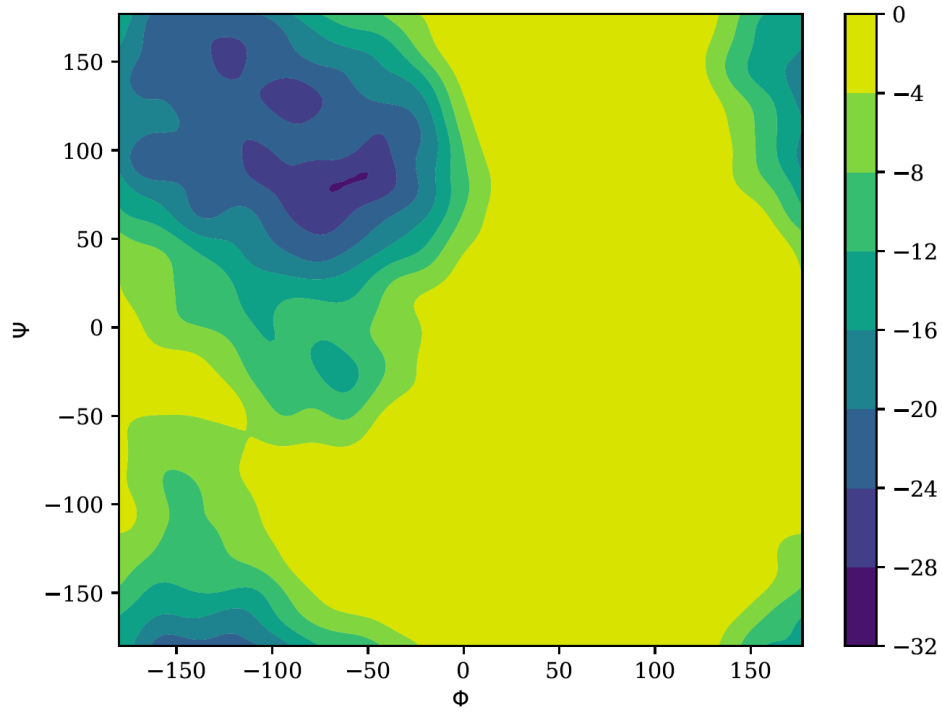
100 steps



500 steps



1000 steps



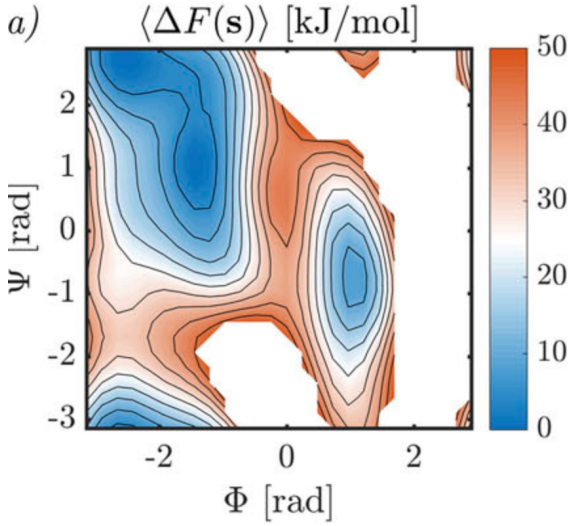
1b.

More minima, or dihedral angles corresponding to stable conformations, are discovered as the number of simulation steps increase. Noting that the colour bar is in KJ/mol, we also see that

the free energy minima become lower and lower, indicating that more phase space was explored. This is because Metadynamics works by adding Gaussians onto the potential energy surface/landscape (PES), and is only able to escape a minimum when a sufficient number of Gaussians have been added to surmount a potential energy barrier. The darker colour bar contours show that more Gaussians were added in those regions, indicating that more phase space was explored in that region. This is expected, since more steps are added in the simulation, allowing more phase space to be explored.

The minima seen in these simulations for the alanine dipeptide (henceforth abbreviated AD) are noted stable conformations of the molecule. These include the $C7_{eq}$ (Φ=-84.6 degrees, Ψ=73.0 degrees) and C5 (Φ=-165.7 degrees, Ψ=167.3 degrees) seen in all simulations, and $C7_{ax}$ (Φ=-74.6 degrees, Ψ=-62.0 degrees) seen only in the 500 steps simulation. While $C7_{eq}$ is seen in all 4 simulations, C5 and $C7_{ax}$ only emerge as the number of simulation steps increases. It's postulated that the $C7_{ax}$ is only seen in the third simulation since there's a non-deterministic nature in aspects of the openMM simulation such that the same simulation run twice will not yield identical contour plots. Since Ramachandran plots—the name given to these contour plots—provide angles, the plot wraps around left to right and top to bottom. This implies that the top right of the 1000 step plot, for example, is part of the extremity of the minima seen in the top left of the plot. All these conformation are from Mironiv et. al. (doi: **10.1002/jcc.25589**) , and were determined with Hartree-Fock (HF) level of theory for the 6-31G* basis set with MP2 reoptimization in the 6-311++G** basis.

Comparing the energies here to Metadynamics simulations in literature (ex. Gimondi et al., J. Chem. Phys. 2018), we see the relative Helmholtz Free Energy is calculated as 50 KJ/mol between the global minima and the potential energy barrier's height. It's expected that if this simulation was run for more way than 1000 steps, eventually the code would reproduce this Ramachandran plot seen in literature. Also, the extra minima seen only in the 500 step simulation is indeed a minimum seen in literature, and not an error in the theory.

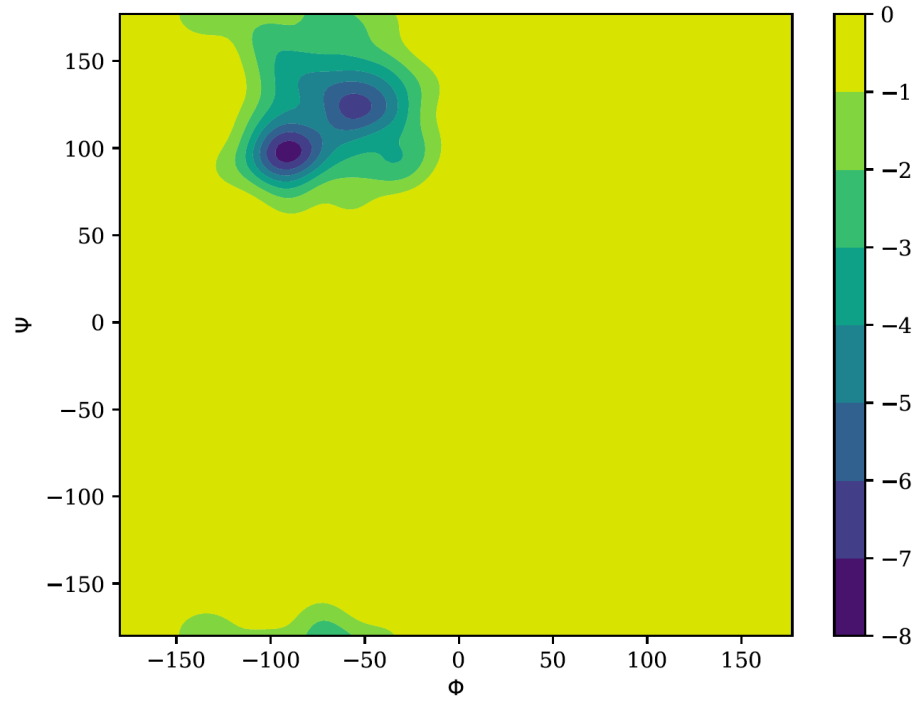


Ramachandran Plot for AD in Vacuum's Helmholtz Energy (Gimondi et al., J. Chem. Phys. 2018)

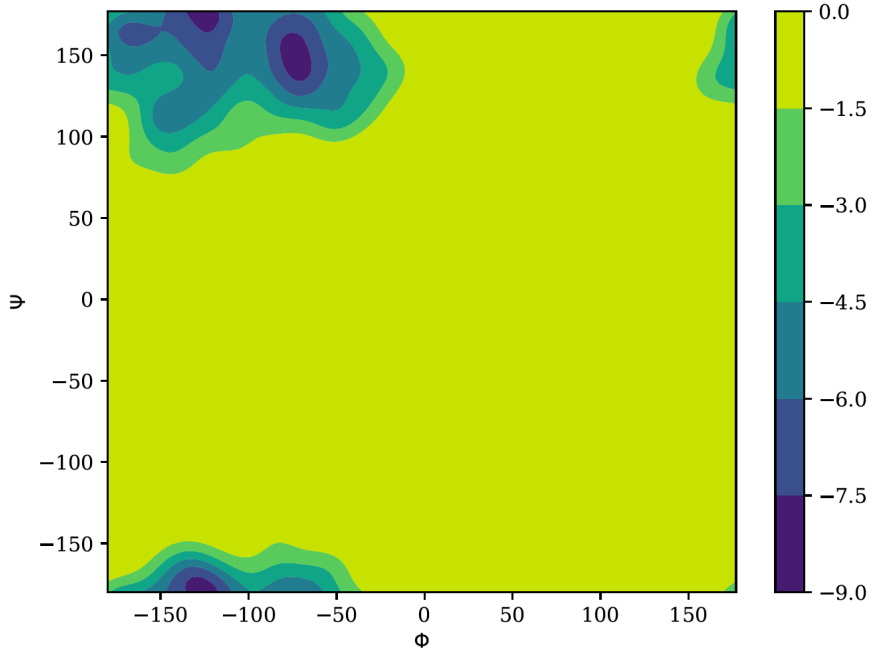
2. Alanine Dipeptide in Water

2a. These codes were generated using `python alanine_water_meta_openmm77.py 1.0 .2 100 x` where 'x' equals 50, 100, 500, 1000.

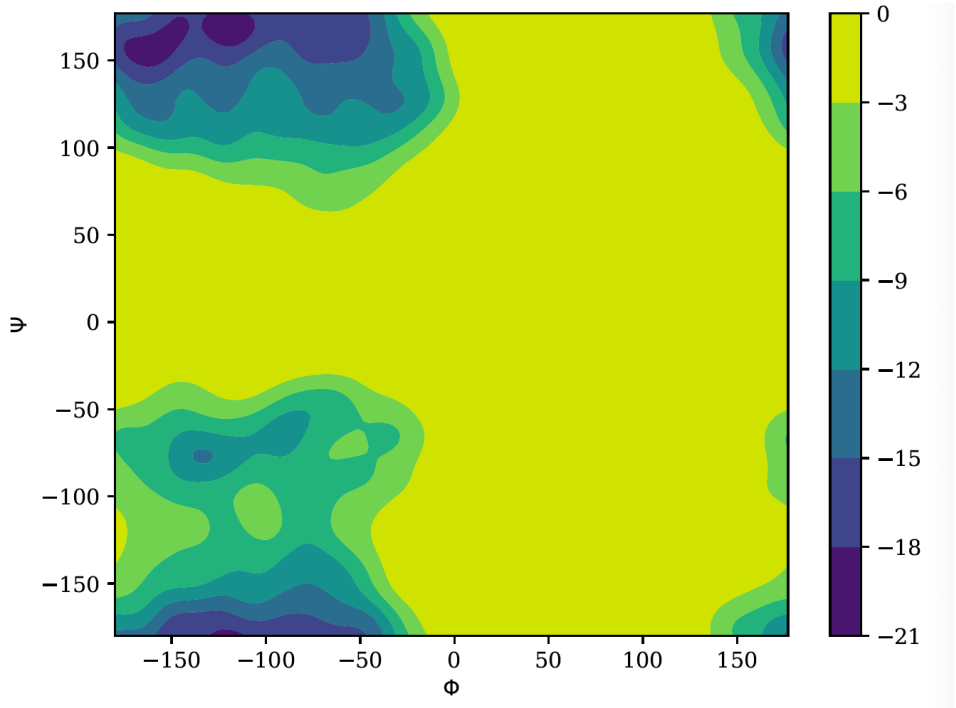
50 steps



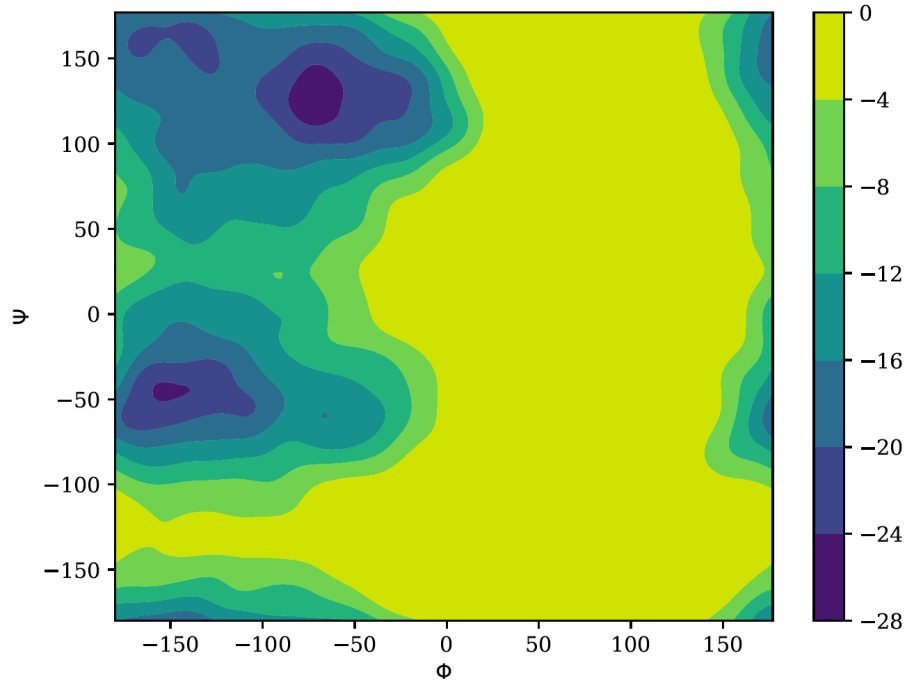
100 steps



500 steps



1000 steps

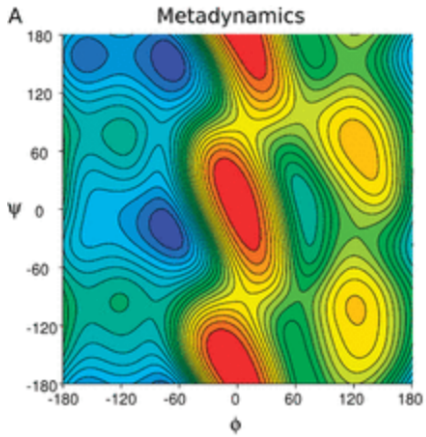


2b.

As the number of steps are increased, more phase space is explored and more minima begin to emerge. The $C7_{eq}$ and $C7_{ax}$ minima are not seen as is the case in the vacuum simulations, but the C5 is consistently noticeable (Φ=-165.7 degrees, Ψ=167.3 degrees). Also, in all simulations

except the 500 step one a new stable conformation, the Beta conformation (Φ=-57.6 degrees, Ψ=134.4 degrees), exists. This conformation is found through HF with the 6-31G**31 basis set. Notably, a new minimum with comparable depth to the C minima is discovered, the a' conformation (Φ=-161.7 degrees, Ψ=-55.4 degrees): this is found using a 4-21G basis set in HF. The Beta and a' conformations, unlike the C conformations discussed, are not discoverable at all levels of theory. (Comparison done with Mironiv et. al. (doi: **10.1002/jcc.25589**)).

Comparing the energies here to AD-in-water Metadynamics simulations in literature (ex. Vymetal et al., J.Phys. Chem. B 2010), a new minimum in the middle lower left quadrant is seen compared to the AD in vacuum simulation. Also, the PES results from these simulations are in good agreement with the paper. This paper shows a difference of 40KJ/mol between the minima trough and the barriers' crest in the middle of the Ramachandran plot, while the simulations show -28 KJ/mol. It's anticipated that if the simulation was run for more steps, such a large difference is obtained. It's also expected that the potential energy barriers are better realized in longer simulations.



Metadynamics plot with numerical integration (Vymetal et al., J.Phys. Chem. B 2010)

Just as in the vacuum simulations, we also see the phase space explored is more or less radial exploration from the starting point (or the minima, since that is where the simulation has spent most of its time), which helps ensure all directions are equally explore.

2c.

The water molecules form a solvation shell, or cage around the alanine dipeptide (AD) that restricts backbone rotation. Coupled with the hydrogen bonding that can take place between the peptide nitrogen and oxygen atoms with the water, and dipole-dipole interactions, a smaller absolute free energy (i.e. less stable conformation) is seen in the water simulations compared to the same simulations in vacuum.