Assumed steps that created the data and confusions to clarify:

1. Gromacs patched with plumed, the CHARMM22\* force field (54), and TIP3P water model.  
   **How was the CHARM22 force field integrated with Gromacs?  
   How was the topology file created? Gromacs doesn’t support CHARMM22?**
2. The initial conformation of Aβ42 was prepared as a linear peptide using PyMOL
3. A preliminary in vacuo molecular dynamics simulation was performed for 1 ns to collapse the extended conformation. The template pdb file is the one created afterwards. **I think?**
4. This structure was solvated in a rhombic dodecahedron box with an initial volume of 362 nm3 containing 11,746 water molecules.  
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   Description automatically generated  
   **Why does it say this?**
5. The solvated system was minimized using the steepest descent algorithm with a target maximum force of 1000 kJ mol−1 nm−1
6. A pool of 48 initial conformations was extracted from a preliminary 2-ns simulation at 600 K in the NVT ensemble.  
   The 48 .gro files in the system directory represent these 48 structures?
7. Equilibration was then performed in the canonical (NVT) ensemble for 500 ps at 278 K using the Bussi-Donadio-Parrinello thermostat (56) and for 500 ps at 278 K in the isothermal-isobaric (NPT) ensemble using Berendsen pressure coupling (57) with position restraints on heavy atoms.
8. Production runs were executed in the NPT ensemble at 278 K using the Parrinello-Rahman barostat (58). A time step of 2 fs was used together with LINCS constraints on all bonds (59). The van der Waals interactions were cut off at 1.2 nm, and the particle-mesh Ewald method was used for electrostatic interactions.  
   LINCS constraints?   
   In the production run, was NVT coupling on or not?

Confusions in plumed.date file

* BIASFACTOR=24 #10\*SQRT(NUM\_OF\_CVS)
* I also had a question about the number of MPI processes to use and how it links with Parallel bias metadynamics. I noticed that   
    
  My understanding of parallel bias metadynamics was that the CVs to bias are distributed among parallel simulations rather than all CVs being biased in the same simulation. Therefore, if I run 6 MPI processes, and give 6 confirmations of AB42 as input, Plumed would bias each simulation with one of the 6 CVs. However, using the WALKERS\_MPI flag would mean that each CV would be biased in multiple simulations. Therefore, using 48 confirmations and 48 MPI processes instead of 6 would mean that each CV would be biased

Todo

* Understand the collective variables defined
* Understand how NMR data is incorported
* Understand the entire plumed file
* See how to run nvt and npt equilibration in parallel
* See how to run simulations with checkpointing

Decisions to make before performing MD simulations:

* Which force field to use?
* Which method to use for NVT and NPT equilibration
* How to choose different mdp values