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

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
* Meaning of different mdp values

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