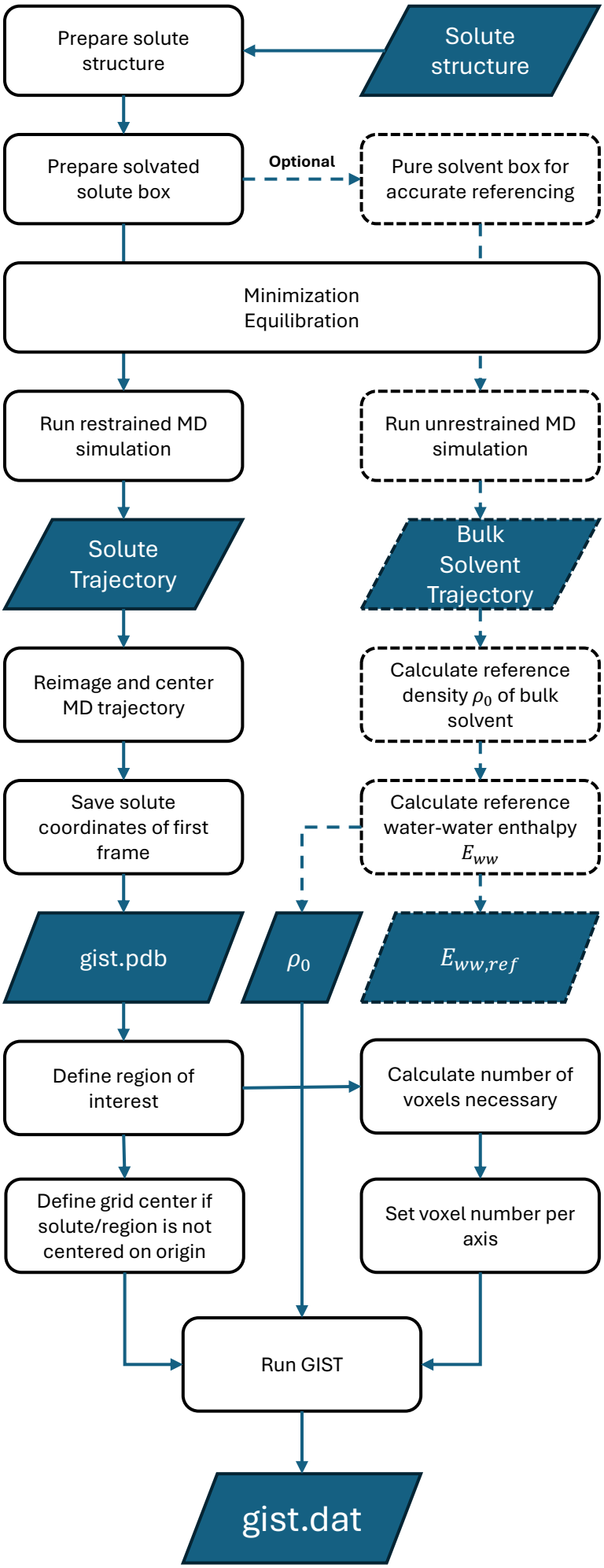


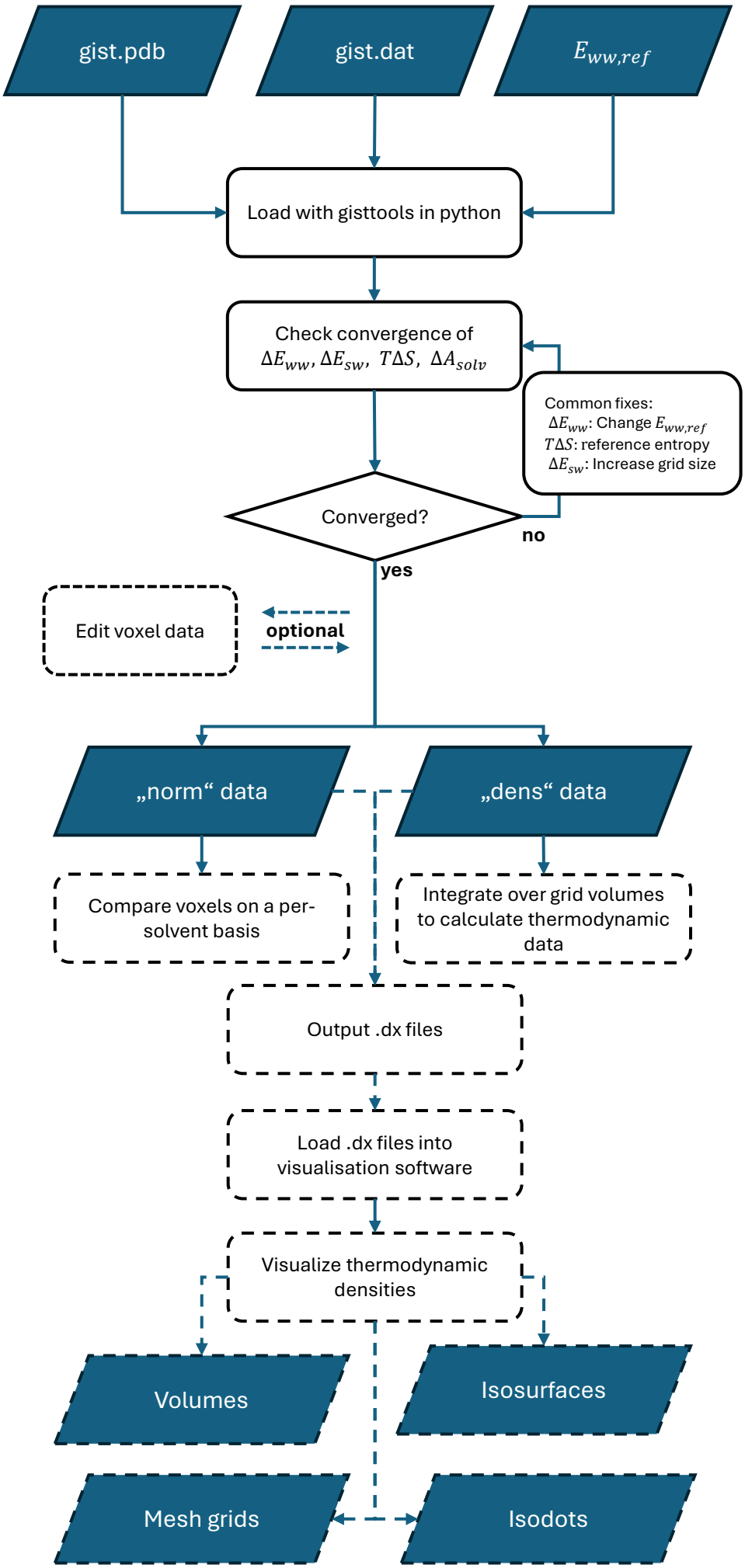
1. MD Simulation

- ❑ Add missing atoms
- ❑ Check protonation states
- ❑ Select force field
- ❑ Add solvent
  At least 10-15Å buffer or 3 solvation layers depending on solvent
- ❑ Optional: Remove solute to create bulk solvent box
- ❑ Restrain heavy atoms for solute simulation
  Harmonic restraint potential $V_r(x) = k_r \cdot \Delta x^2$ with k_r =10 - 100 kcal mol^{-1} Å^{-2}
- ❑ Run simulation
  ≥10 ns (100 ns ideal)
  ≥ 10 000 frames (100 000 ideal)
- ❑ Check RMSD of solute
  ≤ 0.5Å (0.1Å or lower ideal)

2. Prepare GIST settings

- ❑ Determine reference density ρ_0
  Calculate from reference bulk simulation or use tabulated value for the used solvent model.
- ❑ Determine grid size
  Use a small grid focused on a region of interest (e.g. the binding pocket) if interested in local solvent properties or a big grid encompassing at least 2 solvation layers or more around the solute for system properties.
- ❑ Determine voxel lenght
  Smaller voxels provide higher fidelity but require longer simulations for converged results. 0.5Å is recommended.
- ❑ Determine voxel number
  For each cartesion direction, the number of voxels necessary is the length of the region of interest divided by the voxel side length.

3. Run GIST in cpptraj



4a. Postprocess & Analysis

- ❑ Determine $E_{ww,ref}$
  Use tabulated value, calculate from reference simulation or autoreference from outer grid voxels, if the grid is large enough (> 3 solvent layers)
- ❑ Load gist.dat with gisttools
  Preset Eww,ref or use the autoreferencing. A pdb structure of the solute without water is necessary for this.
- ❑ Check convergence of thermodynamic properties
- ❑ Optional: edit voxel data
  For example, apply the fudge factor of 0.6 to the entropy column or set voxels far away from solute to 0.
- ❑ Use „norm“ data to compare voxel to voxel
- ❑ Use „dens“ data to integrate over voxels or for visualisation purposes

4b. Visualisation (optional)

- ❑ If properties were modified or newly created, output density files for visualisation
- ❑ Visualize .dx files
  e.g. with PyMOL or VMD