

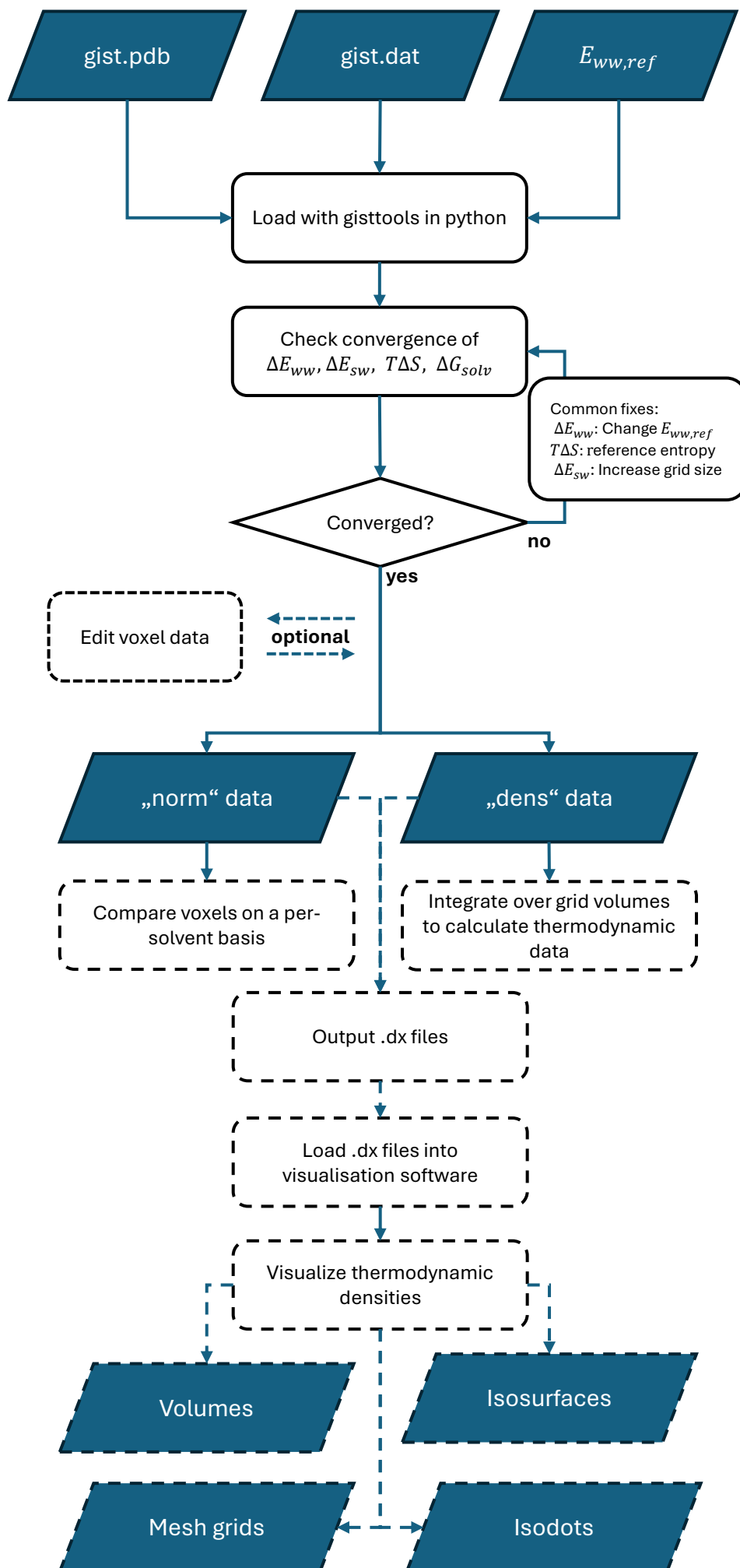
1. MD Simulation

- ☐ 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 $k_r = 10 - 100 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$
- ☐ Run simulation
 - $\geq 10 \text{ ns}$ (100 ns ideal)
 - $\geq 10\,000$ frames (100 000 ideal)
- ☐ Check RMSD of solute
 - $\leq 0.5 \text{ \AA}$ (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 length
 - Smaller voxels provide higher fidelity but require longer simulations for converged results. 0.5Å is recommended.
- ☐ Determine voxel number
 - For each cartesian 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 $E_{ww,ref}$ 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