**Pipeline**

1. Protein-docking modeling software to get substrate-protein conformations at each key step
2. QM/MM MD simulation using amber at each of the 6 steps
3. Find free energy at each step

**List of parameters to consider for Amber**

1. Protein force field
   1. *GAFF* (general Amber force field): simpler, possibly [1]
      1. *ff14SB* for TIP3P water [2]
   2. May need to find more protein-specific force field [1]
      1. CHARMM 22 [3]
2. Choice of simulation program
   1. *Sander*: parallel processing (may not be possible with M1) [1]
      1. Might be the only way to access the QM/MM capabilities
   2. *Pmemd*: optimizes single-processor performance [1]
   3. *Nmode*: old, for <3000 atoms [1]
3. Water model
   1. Explicit water model [1]
      1. TIP3P [3]
      2. TIP4P
      3. TIP4P-Ew
      4. TIP5P
      5. SPC/E
      6. POL3
      7. OPC
         1. Shown to be more accurate for proteins [2]
   2. Implicit water model
      1. Replace discrete water molecule by “virtual water”
      2. Infinite continuum with some of the dielectric and hydrophobic properties of water [1]
      3. Many advantages of explicit model [1]
4. Boundary conditions
   1. Stochastic [3]
5. Ways to enhance conformational sampling
   1. Locally enhanced sampling (*LES*) [1]
      1. Reduces internal barrier heights
      2. Replaces portion of interest with multiple copies (mean-field approach)
      3. Prepared using *addles* module
      4. Useful for global optimization problems
         1. E.g., structure refinement, protein loop conformation optimization
      5. 3-5 copies suggested
         1. Single residues to improve protein sidechain rotamer sampling
         2. 3-4 residues to sample alternate backbone conformations
   2. Replica exchange molecular dynamics (*REMD*) [1]
      1. Simulated annealing by increasing the temperature of the system – provides more kinetic energy to overcome barriers to transitions
      2. Multiple copies are simulated at different temperatures
   3. Umbrella sampling [3]
6. QM/MM parameters
   1. Define QM and MM regions
      1. QM: substrate hydrocarbon framework, metal-pyrophosphate cluster PP-(Mg2+)3 (+ mutated side chain atoms)
      2. MM: remaining enzyme-solvent system
   2. “Treatment”
      1. DFT [3]
   3. Functional
      1. M06-2X [3]

**Workflow**

1. Choice of target PDB file [4]
   * 1. 3P5R, chain A (more resolved)
     2. 60 N-terminal residues are truncated to give active protein
     3. Components
        1. C- terminal domain: S553-V862
        2. N-terminal domain: M107-I135 and S349-Q552
           1. DDIPRLSANYHGDL important in catalysis
        3. Insertion domain: S136-Y348
2. Open conformation structure prediction using homology modelling [5]
   1. Identify template PDBs using BLAST
      1. Target:
         1. Active taxadiene synthase sequence (Arg84-Val862)
      2. Templates: [5]
         1. 3P5R, chain A
            1. Remove residues preceding Thr111, Lys836-Asp850, Ser569-Val581, hexa-histidine tag
         2. 1N23, chain A
         3. 3M02, chain A (not used)
         4. 2ONG, chain A (not used)
   2. Structurally align the two templates using Chimera
      1. Structure/structure alignment
   3. Align the result with the **active** taxadiene synthase and model 3D structure with Modeller
      1. Sequence/structure alignment
      2. Include Mg2+ of both TXS and BBPS
      3. Include FGP for TXS
         1. Include BBPS substrate?
      4. Best model: querysequence.B99990002.pdb
   4. Add MDDIP to N-terminus to generate active structure
   5. Relax the J-K loop (Lys836-Asp858)
      1. Had to constrain to Lys836-Ala761
3. Use AutoDock molecular docking software to generate protein-ligand systems for step C in taxadiene synthesis
   1. Make verticillen-12-yl ligand in PyMol
4. Prepare PDB file [6]
   1. Non-standard residues
      1. This is a more-complicated fix since our protein has metal atoms and a substrate, but I’m not sure what to do about it
   2. Metals [7]
      1. Mg2+ is a divalent ion but not very charged 🡪 use Li/Merz 12-6 ions for TIP3P water
         1. *Lib file*: atomic\_ions.lib
            1. Topologies for monoatomic ions
         2. *Frcmod file*: frcmod.ions234lm\_126\_tip3p
            1. Li/Merz ion parameters for +2 to +4 ions in TIP3 water (12-6 normal usage)
   3. Experimental methods [4]
      1. No disulfide bonds
      2. 2-fluorogeranylgeranyl diphosphate (FGP) substrate bound in active site
   4. Solvent molecules or crystallization buffer
      1. Water molecules were removed using PyMOL
      2. Substrate (FGP) was removed
      3. No other solvents or phosphates seen in the PDB file
   5. Missing electron density
      1. Use PyMOL to fill gaps
      2. Use Modeller ModLoop to relax loops
         1. Modeller deletes the metal ions 🡪 these were added back manually using the original positions
         2. Since only the added residues were relaxed and these are far from the active site, I assumed it was ok to keep the original Mg2+ ions
   6. Check for disulfide bonds
      1. None identified
   7. Check protonation states
      1. Does taxadiene synthase have any non-standard protonation states? None mentioned in the paper
      2. Histidine protonation predicted using the H++ program
         1. Standard parameters, pH 7.0
         2. Total charge: -19
5. *tleap.in, pdb.in:* Use LEaP to build protein system in explicit solvent [8]
   1. Load force fields
   2. Save molecule in gas
      1. *prmtop*: make topology
      2. *inpcrd*: make coordinate files
   3. Solvate protein in water
      1. Octahedral
      2. TIP3P explicit water system
      3. Stochastic boundary conditions
      4. 10.0 Å from structure
   4. Neutralize charge by adding NaCl
   5. Bring NaCl concentration to 150mM
6. Relax the system [8]
   1. Relax the surrounding water and ions
      1. *1min.in*
         1. Minimize the added water and ions
      2. *2mdheat.in*
         1. Heat up the solvent system from 100K to 298K over 1ns under constant volume
      3. *3md*.*in*
         1. Relax the solvent system at constant pressure
      4. *4md.in*
         1. Lower the restraints on the system by running the MD over 1ns at constant pressure and 298K with a smaller restraint of 10 kcal/mol•Å2
   2. Relax the backbone
      1. *5min.in*
         1. Minimize the system with restraints just on the backbone of the molecule (Ca, N, C atoms)
      2. *6md.in*
         1. Relax the system over 1ns at a constant pressure with backbone restrains set to 10 kcal/mol•Å2
      3. *7md.in*
         1. Reduce the backbone restrain force by relaxing for 1ns at a constant pressure with restraints on the backbone lowered to 1 kcal/mol•Å2
      4. *8md.in*
         1. Continue to reduce the backbone restrain force by relaxing for 1ns at a constant pressure with restraints on the backbone lowered to 0.1 kcal/mol•Å2
   3. Relax the system with no restraints
      1. *9md.in*
         1. 1ns of simulation time at constant pressure
7. Set up QM/MM system
   1. QM region: substrate, any mutated residues, and Mg2+
      1. Treated with DFT using the M06-2X functional
      2. May need to add link atom between Ca and Cb of mutant to cap the QM region
   2. Remaining enzyme and water represented by CHARMM22 force field

**Compute Canada**

ssh -Y [livtoft@cedar.computecanada.ca](mailto:livtoft@cedar.computecanada.ca)

**password:** Eib6R@!x6CYaqa@

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