Cheat Sheet of the "Manual Fitting and Evaluation" Workflow

Note1: save your PyMOL session frequently!
Note2: a 3-button mouse would be extremely useful as you work with PyMOL.

1) Create a folder as your working directory
2) Select the receptor (and reference ligand, can be from the same PDB entry)
3) Start PyMOL
4) Download the specific chain(s) from the RCSB PDB (e.g. via PyMOL)
5) Check for any missing/mutated residues within the interested region
   a) PDB headers
   b) Visualize in 3D
   c) Corresponding journal article
6) Prepare receptor with the pdb2pqr server. Download the resulting .pqr file.
7) Check if the hydrogens are correct.
8) Restore essential cofactors to the protonated receptor (e.g. metal ions)
9) Reference complex preparation
   a) Extract the reference ligand from co-crystal
   b) Protonate the reference ligand from co-crystal
   c) (optional) re-combine the receptor-ligand complex to remove steric clashes of the protonated receptor/ligand
      i) Combine the protonated ligand with the protonated receptor
      ii) In "builder" fix receptor coordinates (all c-alphas, more (by res), done)
      iii) Sculpt to remove steric clashes due to the new hydrogens
      (Note the inaccuracies in ligand geometry!)
      iv) Extract the ligand again for geometry optimization
   d) Optimize the geometry of protonated reference ligand
      i) Download the optimize script and move it to your working directory
      ii) Create a folder named "ligand"
      iii) Save the extracted ligand in MDL SD format (.sdf) in the ligand folder
      iv) Open a terminal
         (1) Type `cd` followed by a space
         (2) Drag-and-drop your working directory to the terminal
         (3) Press enter
         (4) Type `bash optimize`
      v) Open the optimized ligand in pymol
   e) Re-combine the processed receptor and optimized ligand to make the reference complex
   f) Save the reference pdb to "complex" folder
10) Preparing a model of the "new" complex
   a) Copy the reference complex to a new object (copy 1)
   b) Fix receptor coordinates
   c) Modify the ligand as you like
   d) Use the sculpting tool to fit the new ligand by hand (optional). You may need a mouse to drag the molecule.
   e) Extract the new ligand
   f) Optimize the geometry of the new ligand (see 9(d)(ii-v)) and load the optimized ligand in PyMOL
   g) Copy the optimized new ligand to the object you extracted it from
   h) Save the complex as pdb in the "complex" folder
11) From the provided "scripts/MacOS/box-local" folder copy the script you want to your working directory
   a) Docking with the gridbox defined by ligand: "dock-autolist"
   b) Quick minimization only: "min-autolist"
   c) Quick minimization with flexible receptor: "min-flex-autolist"
12) Make sure there is no space in all the filename(s)!!!
13) Refine the ligand's pose and score its binding in a receptor:
   a) open a terminal
   b) activate the conda environment with smina installed
   c) cd to the directory with the scripts, then type:

```
bash xxx-autolist #where xxx is min, dock, min-flex etc depends on the method you are using
```

14) Open the results for visualization and analysis (pose, score, interactions(atomic distance, polar contacts, others))

FAQ(?):

1. Smina failed to reproduce the reference experimental structure during our method validation process.
    a. If the ligand has a **flexible** part, the default settings I chose may not be sufficient for the software to sample the "desired" conformation. There are a few possible solutions/workarounds:
        i. Due to the randomness in the conformation generation process, repeating the docking procedures several times may help you to finally achieve the targeted conformation.
            1. You may just remove the output file of the corresponding complex within the "docking/docking-flex/min/min-flex" folder and rerun the docking script.
            2. Alternatively, instead of removing you may duplicate the complex, rename it and rerun the docking script, so you can keep the output of both of the trials and compare the difference.
        ii. Assuming the starting structure to be ideal, you may also set the flexible part as rigid.
    b. Check your input structure:
        i. Is your receptor correctly protonated? Especially for active site histidine, the hydrogens may need to be adjusted.
        ii. Is your ligand correctly protonated as it should be under the targeted pH?
        iii. Are there any water bridges, cofactors or metal ions essential for ligand binding?
    c. If you need a specific metal coordination geometry, the current method may not be good enough for this purpose.
2. Bonds of the ligand messed up after geometry optimization!
    a. Make sure the bonds are correctly built in pymol before your exported it in SDF format.
3. The ligand gets away from the targeted site.
    a. The scripts I wrote for this workflow assume the ligand located in a near-target position and define the binding site around the input ligand structure. Inspect your complexes to see if the ligand located elsewhere.
4. Manually fitting all the ligands is too slow! I have 100 designs I would like to test!
    a. This workflow is mainly designed for educational purpose, aiming to let the user experience the process of docking.
    b. I am working on a set of scripts of the "standard" docking procedures, let me know if you would like to try them!