1. Prepare starting structure
    a. isolate binding helix (residues 23-46) from ACE2; save PDB of binding helix in complex with the spike protein RBD
    b. Relax native complex:
    ```
    $relax.static.linuxgccrelease
    -relax:constrain_relax_to_start_coords
    -relax:coord_constrain_sidechains -relax:ramp_constraints
    false -ex1 -ex2 -use_input_sc -flip_HNQ -no_optH false -s
    native_helix_rbd_complex.pdb
    ```
    c. Extract and renumber relaxed structure:
    ```
    $/farmshare/software/non-free/rosetta/3.12/tools/protein_t
    ools/scripts/clean_pdb.py
    native_helix_rbd_complex_relaxed.pdb ignorechain
    ```
2. Remodel
    a. Create blueprint file of the binding helix (chain A):
    ```
    $/farmshare/software/non-free/rosetta/3.12/tools/protein_t
    ools/scripts/clean_pdb.py
    native_helix_rbd_complex_relaxed.pdb A
    ```
    b. Created 22 blueprint files with python (see **helix_LLE_sub_blueprints** folder for blueprint files)
    c. Run remodel protocol on all blueprints to generate 5 designs from the starting helix-RBD complex:
    ```
    $for bp in helix_LLE_sub_blueprints/*; do
    remodel.static.linuxgccrelease -s
    native_helix_rbd_complex_relaxed_renumbered.pdb -blueprint
    $bp -jd2:no_output -remodel:quick_and_dirty -out:prefix
    complexed_LLE_sub_pdbs/${bp:36:21} -run:chain A; done
    ```
    d. Select the 12 designs with the lowest total energy scores for the next step (see **top12_remodel_designs** folder)
3. FastDesign and Docking
    a. Ran a FastDesign-Docking script (see **xml** file) on native structure and the 12 Remodel designs (see **sbatch** file)
4. NCAA Substitution with Pyrosetta
    a. Ran the ncaa_monte_carlo_substitution.py script to generate low-energy structures from 10,000 iterations of substitutions within the 16_5 remodeled helix (lowest-scoring design from step 3).
    b. After, ran the FastDesign-Docking mutant (see xml file) script on the mutant designs to generate docking scores for analysis with the sbatch file in the mutating folder.