- open directory PaLoXY/Single_template/
- copy all files from Single_template_sample_files/ into this working directory

prepare alignment file
- copy FASTA sequence of target protein from protein_db_PilA_curated.fasta into target.ali

template selection
- `python build_profile.py > build_profile.log`
- open build_profile.prf & determine the best PDB structure based on:
  1. sequence identity (second to last column): >25-30% is good
  2. E-value: all <0.01 are shown, the lower the better
  3. if needed, check structure resolution (Å): the lower the better

download the template structure from PDB
- name if as template.pdb
- if present, remove the extra chains and name the resulting file using chain identifier, e.g. templateA.pdb (needed for profile building)
  otherwise just copy the same file once more and name it templateA.pdb

align sequences of target and template
- if needed, correct the chain name in align_2d.py
  NOTE: it assumes the template is **chain A**
- `python align_2d.py > align_2d.log`
- you can see the alignment in target-templateA.pap

do the modelling and evaluate resulting models
- if needed, correct the chain name in model_single.py
  NOTE: it assumes the template is **chain A**
- `python model_single.py > model_single.log` - produces 30 models
- open model_single.log and at the bottom of the file look into the models' scores
- **select the one with the lowest DOPE (most negative), given that its GA341 is >0.70**
  (DOPE is the most reliable measure for separating native-like vs decoys, but has model-dependent values; GA341 is model-independent score [0,1], closer to 1 is better and >0.60-0.70 is a good model)
- rename it to best_model_single.pdb

#model evaluation
- `python evaluate_model.py > evaluate_model.log`
- if needed, correct the chain name in evaluate_template.py
  NOTE: it assumes the template is **chain A**
- `python evaluate_template.py > evaluate_template.log`
- `python plot_profiles.py`
- look at the DOPE plot and decide whether refinement is needed:
  - NO - rename best_model_single.pdb into PaLoXY_final_model.pdb
  - YES
    - if multiple templates had seq. iden. > 30%, go to multiple templates modelling
    - otherwise try the loop refinement

building model with multiple templates

- open subfolder Multiple_templates/
- download all or several very good pdb files that could serve as templates according to data in build_profile.prf in Single_templates/ folder
- copy all files from Mult_template_sample_files/ into this working directory
- align multiple templates:
  add names of template PDB IDs in salign.py
  `python salign.py > salign.log`
- align that MSA with the target:
  copy target.ali from Single_templates/ to this folder
  `python align2d_mult.py > align2d_mult.log`
- in model_mult.py add PDB IDs of templates
  `python model_mult.py > model_mult.log` - makes 30 models
- select the best model based on DOPE and GA341 and name it as best_model_multiple.pdb
- copy *.profile from Single_templates/ to this folder
- `python plot_profiles.py`
- based on the DOPE profile, decide whether to go for loop refinement

loop refinement

- open a subfolder L1/
- copy all files from LR_sample_files/ into working directory
- copy best_model_single.pdb or best_model_multiple.pdb into the folder
- open loop_refinement.py to add loop positions and choose the pdb file name (single/multiple)
- `python loop_refinement.py > loop_refinement.log` - produces 30 models, slow method
- open loop_refinement.log & select the best model with refined loop by finding the lowest value of molpdf, name it as best_model_L1.pdb
- `python evaluate_model_loop.py > evaluate_model_loop.log`
- open plot_profiles_loop.py & add loop range (e.g. loop_pos = 25-35)
- `cp ../*ali .` (from Multiple_templates/ if it exists, otherwise from Single_template/)
- `cp ../*profile` (from both Multiple_templates/ and Single_template/ if both exist)
- `python plot_profiles_loop_single.py` or `python plot_profiles_loop_mult.py`
- look at the DOPE plot and decide whether another loop refinement is needed:
  - NO - rename the best model pdb file into PaLoXY_final_model.pdb and copy to master folder
  - YES - rename the best model pdb file into best_model_L1.pdb go for loop another refinement

`# loop refinement no2`

- take care to **add *.profile file of previous loop_refinement (i.e. L1), and also add newest best model to the graph** (adjusting evaluate_model.py and plot_profiles.py needed)

additional evaluation

- compare model and template at ProSA-web:
  https://prosa.services.came.sbg.ac.at/prosa.php
- check model with SaliLab Model Evaluation Server:
  https://modbase.compbio.ucsf.edu/evaluation//
  the GA341 value >0.7 means the model is reliable (>95% chance that the fold is correct, meaning that > 30% of Cα superpose within 3.5 Å of their correct positions)

run optimization of final model in Amber