Tutorial 5: Alchemical structure-based design simulation of type I and II kinase inhibitors

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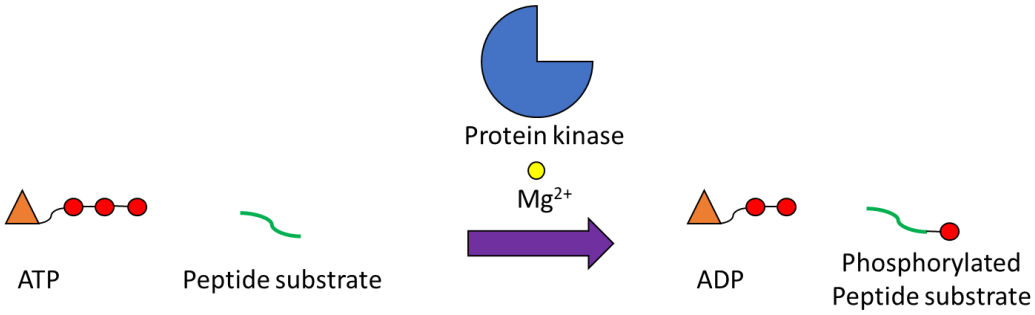
Background

Protein kinases are signaling enzymes that catalyze the transfer of phosphate from ATP to protein/peptide substrate. A typical reaction is of the scheme ATP + Peptide -> ADP + Peptide-P:



The phosphorylated substrate is frequently an "activated" form of the downstream protein that propagates the signal that activated the kinase. Kinases may be activated by extracellular binding of growth factors to promote cell growth and division. Thus, oncogenic kinases promote carcinogenesis through aberrant signaling activity.

A common strategy to treat cancers caused by overactive kinases is to prevent binding of ATP and subsequent substrate phosphorylation with ATP-competitive small molecule inhibitors. In this tutorial, we will use a new multifaceted Rosetta mover called `BCLFragmentMutateMover` to perform structure-based design of small molecule inhibitors of Abl kinase. `BCLFragmentMutateMover` is an implementation of the `FragmentMutateInterface` framework in the BCL and at the time of writing represents the primary functional connection at the BCL-Rosetta interface.

An important note about the intended functionality of the `BCLFragmentMutateMover` is that it was built to try and retain information about the binding pose of the ligand as the ligand is chemically perturbed. The idea here is that we want to avoid a global redocking of the ligand if we can because docking is expensive and not without error. Therefore, the BCL mutates attempt to preserve coordinate information as the design process is ongoing, and Rosetta refines the protein-ligand interaction space as needed with help from other algorithms (e.g., `FastRelax`). If you *do* want to redock after each round of design, this is perfectly fine and it can easily be done using standard RosettaLigand after chemical structure perturbation.

Part 1: Preparing a small molecule scaffold for design

In contrast to the reaction-based design approach in Tutorial #4, most* applications of the `BCLFragmentMutateMover` require a starting scaffold. One way in which a pipeline may proceed is to perform *de novo* reaction-based design as in Tutorial #4 and a second round of focused design on the most promising hits using the `BCLFragmentMutateMover`. For the purposes of this tutorial, we will start with a kinase inhibitor scaffold designed by Okram et al.[1]. The scaffold consists of an amino-substituted pyridopyrimidine heterocycle connected to a benzene ring.

This scaffold is interesting because it can support derivatization into either type I or type II inhibitors. Note that it contains a canonical hinge-binding motif, which is a set of 1-2 hydrogen bonds that mimic the hydrogen bond made by ATP in the binding pocket.

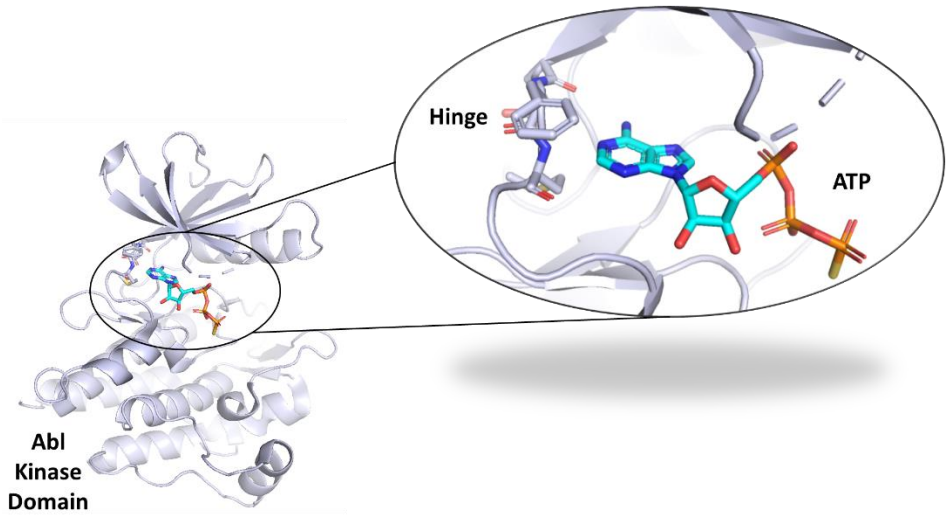


Figure 1. Illustration of the Abl kinase domain hinge region in complex with an ATP mimetic. Structure obtained from PDB ID 2G2F[2].

All right, before we get started, it will make your life easier if you set the path to your Rosetta directory as an environment variable. For example, the Rosetta directory is pre-installed in the Meiler Lab at `/sb/apps/bcl/Rosetta/main`. In bash, we can set this as an environment variable by doing the following:

```
export ROSETTA=/sb/apps/bcl/Rosetta/main
```

In tcsh, this is accomplished with a similar command:

```
setenv ROSETTA "/sb/apps/bcl/Rosetta/main"
```

Alternatively, feel free to modify your PATH and LD_LIBRARY_PATH variables. Throughout the remainder of the tutorial, we will assume that the environment variable ROSETTA has been set as described for simplicity. Assume the same for an equivalent BCL environment variable.

The first step in preparing our scaffold is to generate a Rosetta params file from a valid MDL SD file. Recall from first tutorial that we can check to make sure our scaffold meets some basic quality control criteria before continuing with it.

Navigate to the `BCL_Workshop_2022/Tutorial_5/inputs/ligands` directory.

```
cd BCL_Workshop_2022/Tutorial_5/inputs/ligands
```

Run `molecule:Filter` on the starting molecule that I manually pulled from the crystallographic structures published in Okram et al.[1]:

```
bcl.exe molecule:Filter \
-input_filenames starting_mol.raw.sdf \
-output_matched LIG.sdf \
-defined_atom_types \
-3d \
-add_h \
-neutralize
```

Next, generate the Rosetta params file with the following command:

```
${ROSETTA}/source/scripts/python/public/molfile_to_params.py \
-n LIG -p LIG LIG.sdf --root_atom=1 --centroid --extra_torsion_output
```

You should see the following message:

```
Centering ligands at (  18.095,   15.216,    4.160)
Atom names contain duplications -- renaming all atoms.
WARNING:  structure contains double bonds but no aromatic bonds
  Aromatic bonds must be identified explicitly --
  alternating single/double bonds (Kekule structure) won't cut it.
  This warning does not apply to you if your molecule really isn't
aromatic.
Total naive charge -1.690, desired charge 0.000, offsetting all atoms
by 0.055
WARNING: fragment 1 has 31 total atoms including H; protein residues
have 7 - 24 (DNA: 33)
Average 31.0 atoms (19.0 non-H atoms) per fragment
(Proteins average 15.5 atoms (7.8 non-H atoms) per residue)
Wrote PDB file LIG_0001.fa.pdb
Wrote params file LIG.fa.params
Wrote PDB file LIG_0001.cen.pdb
Wrote params file LIG.cen.params
```

A few notes:

1. The specification of the root atom during params generation is arbitrary. I just specified `root_atom` because it can influence atom indexing and this is important in a little bit.

2. This is the **only** time during the drug design process that we will need to make a params file. Throughout the remainder of this exercise, **all newly designed molecules will be parameterized**

automatically internally. We need this params file strictly to load the small molecule into Rosetta.

3. For small molecule docking, it may be beneficial to explicitly declare aromatic bonds instead of the Kekulized (alternating single/double bonds) structure. Recall that this can be achieved in most BCL applications by simply passing the `explicit_aromaticity` flag. This will cause aromatic substructures in the output SDF to have bond orders of 4 (indicating aromatic).

4. In our case here, it is **important that you use the Kekulized structure.** This is (currently) necessary for Rosetta to communicate bond orders reliably to the BCL data structures that will handle design. **Do not worry – the BCL will recompute aromaticity** every time the molecule is modified and assign the appropriate atom types and bond orders to the molecules when it is converted into a Rosetta object again.

The second step is not strictly mandatory; however, it is important if you want to be able to specify the parts of the scaffold that can be modified during design at the atom level (i.e., specify mutable atoms explicitly). The second step is to convert our params file into a molecule file (e.g., SDF or PDB).

```
${ROSETTA}/source/bin/restype_converter.bcl.linuxgccrelease \
-extra_res_fa LIG.fa.params \
-out:pdb
```

Why did we do this, you ask? **Because Rosetta does not preserve input atom indices. The atom indices of the initial input file, e.g., LIG.sdf, differ from the atom indices of the molecule after it is read from a params file**.

If you visualize the two molecules in PyMOL you can see the difference in atom indexing (from 0) by clicking `L → atom identifiers → rank` in the options panel on the top right.

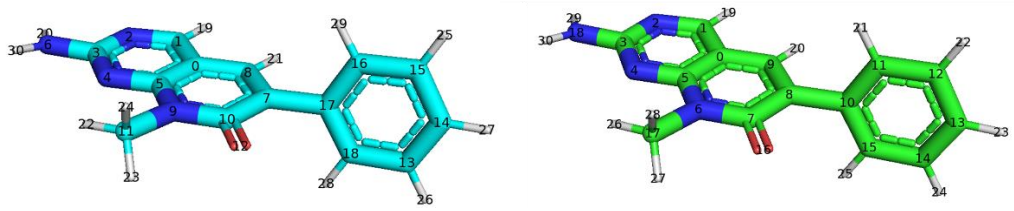


Figure 2. Rosetta atom indices on input scaffold molecule. (A) The PDB ligand "LIG_0001.fa.pdb" output from `molfile_to_params.py`. (B) The PDB ligand "LIG.pdb" output from restype_converter reading in the params file generated by `molfile_to_params.py`.

The indices that Rosetta will reference internally are the indices matching "LIG.pdb" (Figure 2, green molecule on the right) because they come from the interpretation of the params file. If you were to tell Rosetta "I want derivatize my scaffold at index 16" and you did not know that Rosetta renumbered the atoms of the original molecule, you would be very confused about why your oxygen atom was changing instead of your carbon atom.

Now we are ready to start using the BCL-Rosetta small molecule design mover!

Part 2: Intro to BCLFragmentMutateMover

During this section it may be helpful to open the slides from the talk so that you can reference the standalone BCL commands in `molecule:Mutate`.

Navigate to the BCL_Workshop_2022/Tutorial_5/scripts directory.

```
cd BCL_Workshop_2022/Tutorial_5/scripts
```

Open the "SimpleTest.intro.sh" script with vim or gedit or any other editor of your choice. This is a wrapper shell script to call the `rosetta_scripts.bcl.<os>release` application. The arguments are, in order, the RosettaScripts XML file, receptor PDB filename, ligand PDB filename ligand params filename, and an output prefix.

The first XML file contains the following block to initialize the BCLFragmentMutateMover:

```
<BCLFragmentMutateMover name="help"
ligand_chain="X"
object_data_label="Help"
/>
```

Run this script with the following command-line

```
bash SimpleTest.intro.sh SimpleTest.intro_0.xml \
../inputs/receptor/Abl_Inactive_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb \
../inputs/ligands/LIG.fa.params \
HELP_0_
```

You will notice that the program crashes.

```
===> Failed to read
bcl::util::Implementation<bcl::chemistry::FragmentMutateInterface>,
error was: Given implementation "Help" is unknown, here are the
allowed values {AddBond, AddMedChem, Alchemy, Connect, Cyclize,
ExtendWithLinker, Fluorinate, Halogenate, React, RemoveAtom,
RemoveBond, RingSwap}
^^^ In serializer: Help
Failed at pre-read hook, exiting
```

This is expected because the `object_data_label` "Help" is invalid. **This is good,** though in the future maybe we'll clean it up so it looks more informative and less like the world is ending. For comparison, you obtain a similar error message if you try to pass "Help" as a mutate option in the standalone BCL app `molecule:Mutate`.

```
bcl.exe molecule:Mutate -implementation Help
```

These messages are telling us that the allowed mutate implementations are AddBond, AddMedChem, Alchemy, Connect, Cyclize, ExtendWithLinker, Fluorinate, Halogenate, React, RemoveAtom, RemoveBond, and RingSwap. This is helpful if you are not familiar with the available options.

But now what? What if you think that you want e.g., Alchemy, but you are not positive what it does or how to use it?

The next intro XML script expands on the `BCLFragmentMutateMover` definition:

```
<BCLFragmentMutateMover name="help"
ligand_chain="X"
object_data_label="Alchemy(help)"
/>
```

Run the following:

```
bash SimpleTest.intro.sh SimpleTest.intro_1.xml \
../inputs/receptor/Abl_Inactive_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb \
../inputs/ligands/LIG.fa.params \
HELP_1_
```

This will also stop early. It will display a description of the mutate:

```
===> Requested help: Transforms atom/element types inside of a
molecule
  Default label :
Alchemy(druglikeness_type=None,corina=0,mutable_atoms="",mutable_eleme
nts="",mutable_fragments="",complement_mutable_fragments=115,mutable_a
tom_comparison=Identity,mutable_bond_comparison=Identity,fixed_atoms="
",fixed_elements="",fixed_fragments="",complement_fixed_fragments=44,f
ixed_atom_comparison=Identity,fixed_bond_comparison=Identity,scaffold_
mol="",n_max_attempts=10,ov_shuffle_h=1,ov_reverse=0,allowed_elements=
H
    C O N
S,set_formal_charge=nan,set_chirality=X,restrict_to_bonded_h=0)
  Parameters:

...
...

Failed at reading arguments
=std=bcl::util=> RotamerLibraryFile reading files has run for 1.85372
seconds
```

Where I have written "…" there are descriptions of all the parameters that we can pass to the Alchemy mutate.

Once again, this is the information that is displayed if you were running the corresponding mutation from the BCL standalone version:

```
bcl.exe molecule:Mutate -implementation "Alchemy(help)"
```

Excellent! So now you have some familiarity with the basic syntax. Let's keep going and start designing some kinase inhibitors.

Part 3: Type I inhibitor design

Type I inhibitors sit directly in the ATP binding pocket. Our scaffold can still mimic additional ATP-protein interactions to increase binding affinity.

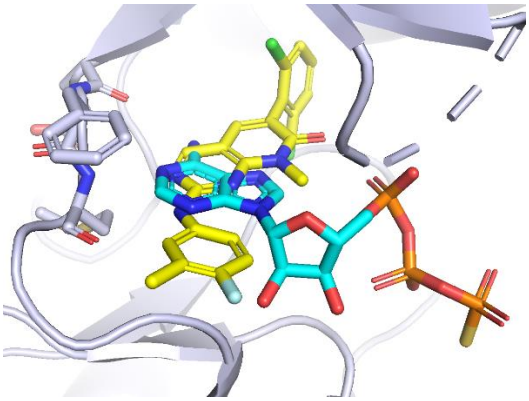


Figure 3. Overlap of ATP mimetic and Type I kinase inhibitor in Abl kinase domain. ATP and Abl structure obtained from PDB ID 2G2F[2]. Inhibitor constructed from analogous Type II inhibitor from PDB ID 2HIW[1].

In addition to the hinge binding hydrogen bonds, type I inhibitors often form hydrophobic-aromatic stacking interactions with the nucleotide-binding glycine-rich loop. Therefore, one rational design decision is to grow another aromatic ring system from our amino group. There are a lot of aromatic rings in the world and it's not obvious what the optimal choice will be to improve target engagement. So, let's sample a bunch and see which ones have the best interaction energies in Rosetta.

The first implementation of FragmentMutateInterface that we will initialize our `BCLFragmentMutateMover` mover with is `ExtendWithLinker`.

```
<BCLFragmentMutateMover name="extend_with_linker"
ligand_chain="X"
object_data_label="ExtendWithLinker(
ov_reverse=True,
ov_shuffle_h=False,
ring_library=%%rotamer_library%%/ring_libraries/
drug_ring_database.simple.aro.small.sdf.gz,
extend_within_prob=0.0,
direct_link_prob=100000,
druglikeness_type=None,
mutable_atoms=18)"
/>
```

We define our mover with the name `extend_with_linker` (I frequently use `ewl` for short). The ligand is identified as chain X. Now we need to initialize the BCL object within Rosetta via the `object_data_label` option. For reference, feel free to look at the Tutorial 5 intro talk PowerPoint slides.

`ExtendWithLinker` grows molecules using an assortment of linkers either internally or off a terminal heavy atom. Like all the `FragmentMutateInterface`-derived objects, if left unspecified `ExtendWithLinker` is given free rein to make random changes to your molecule (i.e., it acts on any

atom in the molecule, it randomly selects rings to either append or use as bridges, it does not filter for druglikeness, and the relative probabilities of extending with rings, single elements, or chains are approximately equal).

Here, we restrain the behavior of `ExtendWithLinker` to model our rational design decision. We set the `mutable_atoms` index to 18, corresponding to the amino nitrogen atom. The mutable and fixed atoms/elements/fragments options are common for all of these mutates. The ring library is taken from one of the default libraries provided with the BCL in the `${ROSETTA}/source/external/bcl/rotamer_library/ring_libraries/` directory. The path to the rotamer library is set with a variable defined in the wrapper shell script. We set the relative probability of a direct link arbitrarily high (if you read the help menu, it will indicate that the probabilities for the different linking strategies are relative to one another) to ensure that our chosen ring is directly bonded to the amino nitrogen.

There are several other options here that are common to other mutates. The `ov_reverse` and `ov_shuffle_h` flags are options related to "opening valences". We cannot form a new covalent bond between the amino nitrogen and an atom in the aromatic ring randomly selected from the user-specified pool of rings if we do not remove an existing bond. If we just force a bond willy-nilly, then we are frequently going to encounter invalid atom types. To avoid this issue, we remove hydrogen atoms to open a valence prior to making a bond.

But there are two hydrogen atoms. Which one do we remove? By default, we randomly select one to remove. Chemically, it does not matter which one we remove because they are symmetric. However, because `BCLFragmentMutateMover` attempts to preserve binding mode information, the hydrogen atom we remove has a substantial impact on the pose.

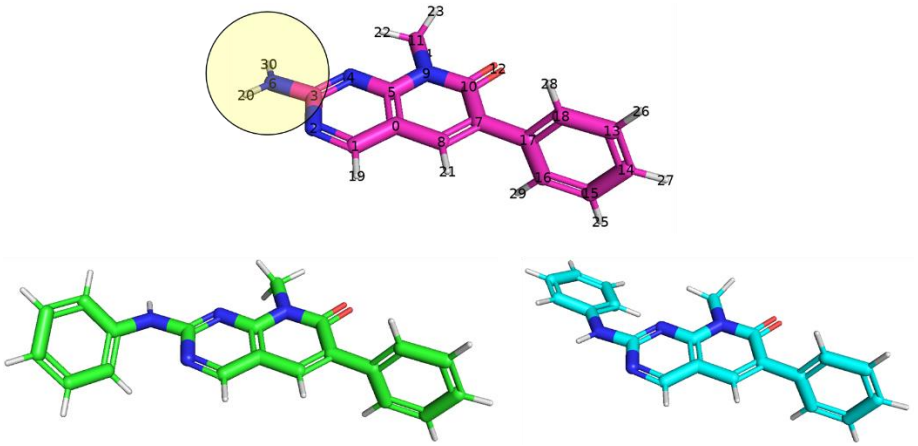


Figure 4. Effect of hydrogen atom selection on extension of a benzene ring from a primary amine. The magenta structure is our starting compound. Using the `ExtendWithLinker` mutate to grow from atom 6, we extend into a new benzene ring. If we disable random hydrogen atom selection (`ov_shuffle_h=0`) and start from the lower hydrogen atom index (`ov_reverse=0`) we get the addition as shown in the bottom left (green). If we disable random hydrogen atom selection and start from the higher hydrogen atom index (`ov_reverse=1`) we get the addition as shown in the bottom right (blue).

To target the correct hydrogen atom for removal, first we turn off the random hydrogen atom selection by passing `ov_shuffle_h=0`. Then we note that the index that we want to remove is the higher index. The default behavior is remove the lowest index hydrogen atom first. Therefore, we set `ov_reverse=1` to instead start removal from the highest index.

To recreate the structures in the image shown above, first navigate to the small molecule input directory.

```
cd BCL_Workshop_2022/Tutorial_5/inputs/ligands
```

Run ExtendWithLinker quickly using the BCL standalone app `molecule:Mutate`.

```
bcl.exe molecule:Mutate \
-input_filenames LIG.sdf \
-implementation "ExtendWithLinker(\
mutable_atoms=6,\
direct_link_prob=10000,\
ov_reverse=0,ov_shuffle_h=0,\
ring_library= \
$BCL/rotamer_library/ring_libraries/individual_rings/000.sdf.gz)" \
-output ../../outputs/T1L.benzene.0.sdf
```

Do the same thing for the other hydrogen atom.

```
bcl.exe molecule:Mutate \
-input_filenames LIG.sdf \
-implementation "ExtendWithLinker(\
mutable_atoms=6,\
direct_link_prob=10000,\
ov_reverse=1,ov_shuffle_h=0,\
ring_library= \
$BCL/rotamer_library/ring_libraries/individual_rings/000.sdf.gz)" \
-output ../../outputs/T1L.benzene.1.sdf
```

And that's that! By adding this mover to our protocol, we will sample aromatic rings linked to the amino nitrogen, and we will preserve the hinge hydrogen bond donor from our ligand (unless Rosetta perturbs the pose substantially during a subsequent `FastRelax`).

Let's do a bit more rational design for our type I inhibitor simulations. The benzene ring of our scaffold is surrounded by several hydrocarbon sidechains. We can consider adding one or two substituents to form favorable Van der Waals interactions. To do this, we will use the `Alchemy` and `Halogenate` mutates.

```
<BCLFragmentMutateMover name="alchemy_core"
ligand_chain="X"
object_data_label="Alchemy(
druglikeness_type=None,
mutable_atoms=11 12 13 14 15,
allowed_elements=C,
restrict_to_bonded_h=1)"
/>
```

This defines an Alchemy mutate that will choose an atom from the benzene ring and change its bonded hydrogen atom into a carbon. If `restrict_to_bonded_h` was set to 0, Alchemy would modify the chosen atom directly.

```
<BCLFragmentMutateMover name="halogenate_core"
ligand_chain="X"
object_data_label="Halogenate(
druglikeness_type=None,
mutable_atoms=11 12 13 14 15,
reversible=1,
allowed_halogens=F Cl)"
/>
```

This defines a Halogenate mutate that will choose an atom from the benzene ring, open a valence by removing a hydrogen atom, and then add either a fluorine or chlorine. It is reversible, which means that this mutate has a 50% chance of removing a halogen from one of the mutable atoms.

You may ask "Can't we just use `Alchemy` to add halogens?" Sure, no problem. `Alchemy` has the ability to mutate element types in halogens or vice versa if desired. However, usually in `Alchemy` we have to worry about making sure that we have an atom type available with the desired formal charge if we are swapping heteroatom identities or some such thing. Halogens are actually a bit easier because the atom typing is far more restricted, so in some sense the logic in `Alchemy` is overkill for halogens. Moreover, halogens are special in medicinal chemistry and deserve an opportunity for special logic that is ill-placed in `Alchemy`. For example, we can restrict the addition of individual halogens to aromatic rings, and we are experimenting with optional biased probabilities based on stability of the halogenated substructure. Nevertheless, the choice is yours.

In any event, that is more of a code design issue. Here, we will create a `RandomMover` with equal probability of choosing `Alchemy` or `Halogenate`.

```
<RandomMover name="decorate_core" movers="halogenate_core,
alchemy_core" weights="0.5,0.5" repeats="2"/>
```

This will give us up to two substituents on our benzene ring. The substituents will be either a methyl, fluorine, or chlorine.

Now put it all together! The final protocol in the XML script will perform an initial minimization of the starting receptor with the scaffold, followed by `ExtendWithLinker` to add an aromatic ring to our amino nitrogen atom, followed by two repeats of `Alchemy` and/or `Halogenate` to add substituents to our benzene ring. Finally, we do a constrained `FastRelax` on the protein-ligand interface and score the complex.

Navigate to the output directory.

```
cd BCL_Workshop_2022/Tutorial_5/outputs/
```

```
bash ../scripts/SimpleTest.design_tk1.0.sh \
../scripts/SimpleTest.design_tk1.0.xml \
../inputs/receptor/Abl_Active_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb \
../inputs/ligands/LIG.fa.params \
DEMO.design.tk1.0_
```

How do the inhibitors you designed look compared to the Okram et al.[1] inhibitor? Are there similarities? Differences? Try additional mutates to see what else you can do.

One final note before we move on to Part 4: Have you noticed that you are able to perform sequential mutates on your molecule by referencing atom indices in your original scaffold? This is generally possible because new atoms are added to the end of the atom vector, so they do not change the numbering of our original indices. In our Alchemy mutate you may have wondered "Why is there this restrict_to_bonded_h" option? Can't we just specify an atom index for a hydrogen atom in the mutable_atoms option?" Well, you can, but it is probably a bad idea. **During the fragment cleaning, retyping, and 3D conformer generation phase after the mutation is made, hydrogen atoms are removed and re-added.** This changes the indexing of hydrogen atoms. So, stick with referencing heavy atom indices and you should be good.

But what do you do if the molecule undergoes a large substructure change, or greatly expand the initial molecule and want to make changes to the new region? Find out how we handle that in Part 4!

Part 4: Type II inhibitor design

Type II inhibitors also compete with ATP binding, but unlike Type I inhibitors they extend into a back hydrophobic pocket of the kinase domain.

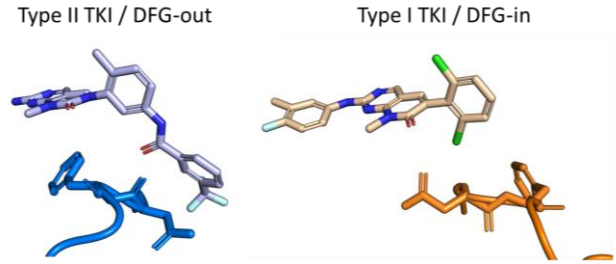


Figure 5. Comparison of Type I and Type II tyrosine kinase inhibitors.

Our rational design strategy will therefore start by extending the scaffold into the back hydrophobic pocket. Let's start with our trusty friend ExtendWithLinker again.

```
<BCLFragmentMutateMover name="extend_with_linker"
ligand_chain="X"
object_data_label="ExtendWithLinker(
ring_library=
%%rotamer_library%%/ring_libraries/individual_rings/000.sdf.gz,
extend_within_prob=0.0,
amide_link_prob=100000,
amide_n_attach_prob=1.0,
druglikeness_type=None,
mutable_atoms=14)"
/>
```

This time we will set an arbitrarily high probability for the amide linker. Amide linkers can be grown from two directions. The `amide_n_attach_prob` specifies the probability that the nitrogen atom of the amide will be covalently bonded to the chosen atom in our starting fragment. The remainder of the probability is the likelihood that the carbon atom in the amide will be covalently bonded to the chosen atom. We set a single mutable atom. We also set a single ring to use from our library – a benzene ring (for simplicity)! Feel free to peruse the ring library or use your own rings.

So, let's assume you were to run just that mutate. You would now have your original scaffold plus an amide linker connecting it from atom index 14 to a new benzene ring. How do we go about modifying the new substructure in subsequent design steps? We certainly do not necessarily know what the atom indices will be.

Well, you may have noticed from the help menu options that we can specify mutable atoms by more than just their indices. There are four options:
1. Do not specify anything and all atoms default to mutable
2. Mutable atoms – Specify mutable atoms by their index in the molecule (0-indexed)
3. Mutable elements – All atoms of the specified element types are set as mutable.
4. Mutable fragments – This option is very useful. You can pass SD files of fragments for substructure comparison to your current molecule. You can pass as many or few fragments as you want. Atoms in

matching substructures will be set to mutable. **These are cumulative.** There are two other options, `mutable_atom_comparison` and `mutable_bond_comparison` that allows users to specify the resolution of the substructure comparison. **Part of why this option is so useful is because there is also an option to invert the matched atoms.** If users set `complement_mutable_fragments` to true, then the mutable atoms are the ones that *did not* match in any of the substructure comparisons.

And if that is not good enough, **there are four identical options for setting fixed (non-mutable) atoms.** Importantly, fixed atoms are always set *after* the mutable atoms. This means that the fixed atom selections modify the mutable atoms and not vice versa. This is consistent with the default of setting all atoms to be mutable unless otherwise specified.

I am planning on adding a few more options so that users can selectively turn on or off more generic topological units, such as rings, chains, rigid fragments, etc. Stay tuned!

With this new knowledge, let's add a trifluoromethyl group to our newly created benzene ring.

```
<BCLFragmentMutateMover name="add_medchem"
ligand_chain="X"
object_data_label="AddMedChem(
medchem_library=%%medchem_fragments%%/trifluoromethyl.sdf.gz,
druglikeness_type=None,
mutable_fragments=%%mutfrag%%,
complement_mutable_fragments=1,
restrict_additions_to_aromatic_rings=1,
fixed_elements=N O)"
/>
```

The `mutfrag` variable will be defined in our wrapper shell script. In this case, it will just be the SDF corresponding to our scaffold. We want to invert the substructure match, so we set `complement_mutable_fragments` to true. We then say that `AddMedChem` cannot try to attach the trifluoromethyl group to nitrogen or oxygen atoms, which will prevent it from randomly selecting one of the amide heteroatoms. It could in principle select the carbon atom from the amide to try and bond to; however, there are no valences that can be opened, which would cause the move to fail and try again up to `n_max_mutates`. Alternatively, we can save it the trouble and specify `restrict_additions_to_aromatic_rings` as true. The only aromatic ring atoms not in our starting scaffold are the benzene ring carbon atoms.

Thus, this mover will add a trifluoromethyl group to one of only five available atom indices on the amide-linked new benzene ring (two of which are symmetric, leaving us with three unique positions). At this point, if you want to restrict the space available even further for the `AddMedChem` mutate, the best advice I have is to start anew with the structure obtained after `ExtendWithLinker` so that you can see the atom indices. Beyond the obvious observation that we can only be so specific about how to add atoms to other atoms that do not exist in the starting fragment, it is also important to note that this software was written to help with drug design, not drug drawing. For the latter, stay tuned as we see how to integrate these tools into FoldIt Drug Design.

Finally, let's add a small decoration to the core benzene ring using the Alchemy mutate.

```
<BCLFragmentMutateMover name="alchemy_core"
ligand_chain="X"
object_data_label="Alchemy(
druglikeness_type=None,
mutable_atoms=11 12,
allowed_elements=C,
restrict_to_bonded_h=1)"
/>
```

Put it all together and run the protocol.

```
bash ../scripts/SimpleTest.design_tk2.0.sh \
../scripts/SimpleTest.design_tk2.0.xml \
../inputs/receptor/Abl_Inactive_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb \
../inputs/ligands/LIG.fa.params \
DEMO.design.tk2.0_
```

This script provides everything you need to recreate the type II inhibitor in Okram et al.[1]; however, there is some stochasticity in the current design, and as we just discussed we have finite ability to create the exact molecule we want from scratch. But more importantly, how do the output molecules look? Do they appear to be type II inhibitors? Do they have similar features to type II inhibitor in Okram et al.[1] (or other TKIs you may have seen)?

Let's expand our design further. Check out the "SimpleTest.design_tk2.1.xml" script. How does it differ from the script we just ran? Do you expect more diversity or less in our designs? Run this protocol.

```
bash ../scripts/SimpleTest.design_tk2.1.sh \
../scripts/SimpleTest.design_tk2.1.xml \
../inputs/receptor/Abl_Inactive_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb \
../inputs/ligands/LIG.fa.params \
DEMO.design.tk2.1_
```

By this point, you have designed both type I and type II inhibitors. In Part 5, we will learn more about type I and II inhibitors and how we can begin to use our design protocols with receptor conformational sampling techniques.

<u>Part 4 Extension (Optional): Adding the Fluorinate mover to our toolbox</u>

In this tutorial we just used benzene as the base for our interactions with the back hydrophobic pocket. In principle, however, it does not need to be benzene. Here are a couple of extra things to try if you are interested:

1. Sample alternative rings. You can set your ring library to be an SDF with multiple rings, in which case one will be randomly selected each round, or you can sequentially iterate over SD files containing individual rings to enumerate.

2. Forget the rings. Let's be creative. I will introduce you to the special purpose `Fluorinate` mutate. You may ask, "Why do we have a mutate specifically for fluorine when we can already manipulate fluorine atoms with `Halogenate`? If you really needed to use fluorine more dynamically, can't you just enable it in `Alchemy`?" Good questions. These are things we considered. The answer boils down to "fluorine is special." It has unique chemistry, and while it is often used to decorate scaffolds like chlorine or bromine or methyl or methoxy, it is also often used to saturate individual carbon atoms on aliphatic chains or non-aromatic rings. If you are new to small molecule drug design, you may have noticed that this effect can also be obtained with trifluoromethyl and trifluoromethoxy fragment additions in the `AddMedChem` mutate!

So, you will notice that the `Fluorinate` mutate contains several specific options to manage the minimum and maximum number of fluorine atoms that are added to a single carbon atom in a single move. Let's combine the `AddMedChem` and `Fluorinate` mutates to add an ethylamide and saturate the terminal carbon with fluorine atoms.

```
bcl.exe molecule:Mutate \
-input_filenames LIG.sdf \
-output LIG.amide_cf3.sdf \
-implementation "AddMedChem(\
mutable_atoms=13,\
medchem_library=$BCL/rotamer_library/medchem_fragments/ethylamide.sdf.
gz)" \
"Fluorinate(\
mutable_fragments=LIG.sdf,\
complement_mutable_fragments=1,\
fixed_elements=N O, \
n_min_f=3,\
n_min_h_sub=3)"
```

And let's take it a step further. Let's add a single carbon atom to our benzene ring first and *then* add the fluorinated ethylamide. This will give us a slightly longer chain.

```
bcl.exe molecule:Mutate \
-input_filenames LIG.sdf \
-output LIG.amide_cf3.sdf \
-implementation "Alchemy(\
mutable_atoms=13,\
allowed_elements=C,\
restrict_to_bonded_h=1\
)" \
"AddMedChem(\
mutable_fragments=LIG.sdf,\
complement_mutable_fragments=1,\
medchem_library=$BCL/rotamer_library/medchem_fragments/ethylamide.sdf.
gz)"\
"Fluorinate(\
mutable_fragments=LIG.sdf,\
complement_mutable_fragments=1,\
fixed_elements=N O,\
n_min_f=3,\
n_min_h_sub=3)”
```

Visualize your molecules in PyMOL. Try making a protocol in RosettaScripts to do the same design but in the context of a receptor binding pocket.

Part 5: Induced-fit inhibitor design

You may have noticed that in the two previous design examples that we used two different PDB files for the Abl kinase receptor structure. For the Type I inhibitor we used the active state (activation loop extended outward; DFG-in) and for the Type II inhibitor we used the inactive state (activation loop folded inward; DFG-out). Kinases can adopt many conformations that span the active and inactive states. The spectrum of available states differs between kinases, as well as the propensity for a kinase to be in one state or another. They are fascinatingly dynamic proteins!

Type II inhibitors require the kinase domain to be in either an inactive or intermediate state to bind. This is because the active state sterically occludes the back hydrophobic pocket that type II inhibitors target. In contrast, type I inhibitors have been observed in both active and inactive states[3]. Consistent with the observation that different kinases have different conformational preferences, it has become well-accepted that not all inhibitors bind to the same conformation across kinases[4].

Therefore, it may be of interest to sample multiple conformational states of a protein during drug design. This can allow the user to filter out molecules that are predicted to preferentially bind an undesired conformation, or to identify features that stabilize a desired conformation.

Rosetta has many powerful tools for sampling protein conformational changes: fragment insertion, generalized kinematic closure, thermodynamic movers for canonical sampling, etc. There are also tools for sampling the interfaces of protein-ligand complexes explicitly, such as the low-resolution `Transform` mover and `HighResDocker`.

The `Transform` mover can optionally incorporate protein flexibility through pre-generated conformational ensembles. Users can pass an ensemble of proteins that are rapidly scored with the `Transform` mover scoring grids. The protein with the best score is carried forward to subsequent stages of the docking protocol.

For this example, we will use a variant of this protocol to score our inhibitor designs on multiple pre-generated conformations of the same protein prior to high-resolution refinement. The benefit to this approach is that it can be done faster than on-the-fly sampling of large conformational changes, and it ensures consistency in the sampled conformational space between molecule designs.

We will use 16 unique conformations of Abl kinase rigorously generated by Meng et al. 2018 using extensive molecular dynamics (MD) simulations coupled with Markov state models (MSM)[5]. The approach taken by Meng et al. blindly recovered known publicly available Abl conformations as well as previously unreleased inhibitor-stabilized conformers from the private Lilly database[5].

Let's begin. The Abl conformers have already been obtained from the literature and stashed in the inputs/receptors/ directory. Make a list containing the full paths of each of the conformers.

```
cd BCL_Workshop_2022/Tutorial_5/inputs/receptor/conformers

readlink -e *.pdb > confs.list
```

Get the full path to the list

```
readlink -e confs.list
```

The script that we will use to run the RosettaScripts protocol is called “InducedFitDrugDesign_v1.sh”. In that script we define a variable CONFS_LIST. Define CONFS_LIST with the full path to “confs.list” on your workstation. As we did previously, also make sure the rotamer library and fragments variables are set to the correct directory.

Let’s look at the Transform mover in this example.

```
# Low resolution docking
<Transform name="transform" chain="X" box_size="6.0"
move_distance="0.2" angle="2.0" cycles="500" repeats="1"
temperature="%%temp%%" initial_perturb="0.0"
initial_angle_perturb="0.0" ensemble_proteins="%%confs_list%%"
use_main_model="false" />
```

It differs from how we usually set it up for ligand docking because here we are not using it to sample large ligand pose changes. We just want to do a small bit of optimization of the designed ligand in each receptor pocket. We have small distance and angle perturbations, 0.2 Angstroms and 2.0 degrees, respectively, and we disable initial perturbations. The `ensemble_proteins` option will take a variable corresponding to the PDB list we just made. The option `use_main_model` is set to false, indicating that the best scoring model is the one we will carry forward.

We have defined a number of BCLFragmentMutateMover movers that are very similar to the ones we used in Parts 3 and 4. Take some time to go through them and make sure you fully understand what each is doing. One thing you will notice is that we are now using `druglikeness_type=IsConstitutionDruglike` instead of `druglikeness_type=None`. This acts as an internal filter to help prevent the design of grotesque molecules. To see what this filter does, run the following:

```
bcl.exe molecule:Properties -add "IsConstitutionDruglike(help)"
```

Finally, a note on the configurational sampling in this protocol. The initial minimization may bias the simulation toward the input conformer. Therefore, for production simulations it is advised that multiple independent trials are run starting from each conformer, or alternatively that the minimization step is removed, and each conformer is minimized with the scaffold outside of this protocol prior to starting.

Let’s run this protocol!

```
cd BCL_Workshop_2022/Tutorial_5/outputs/
```

First, for type I inhibitors:

```
i=1; bash ../scripts/InducedFitDrugDesign_v1.sh \
../scripts/InducedFitDrugDesign_v1.xml \
../inputs/receptor/conformers/ct7b01170_si_004_0001_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb ../inputs/ligands/LIG \
1.2 $i IF_t${i}_ &
```

Second, for type II inhibitors:

```
i=2; bash ../scripts/InducedFitDrugDesign_v1.sh \
../scripts/InducedFitDrugDesign_v1.xml \
../inputs/receptor/conformers/ct7b01170_si_004_0001_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb ../inputs/ligands/LIG \
1.2 $i TEST_t${i}_ &
```

Finally, there is a RandomMover defined that will randomly select either a type I or type II design pattern if the "TYPE" variable in the wrapper shell script is set to "1-2".

```
i=1-2; bash ../scripts/InducedFitDrugDesign_v1.sh \
../scripts/InducedFitDrugDesign_v1.xml \
../inputs/receptor/conformers/ct7b01170_si_004_0001_A.pdb \
../inputs/ligands/LIG_0001.fa.pdb ../inputs/ligands/LIG \
1.2 $i TEST_t${i}_ &
```

Now visualize your output structures. How did they turn out? Are the type II inhibitors more frequently in an inactive state than an active state? Do the type I inhibitors have a conformational state preference? Note that we initialized these simulations with a receptor in the active conformation. If you start with a different protein conformer, does the output follow a similar trend?

In a preliminary benchmark in an earlier version of the BCL-Rosetta integration, we used a related set of mutate options to create type I and II inhibitors and found the following distribution of states:

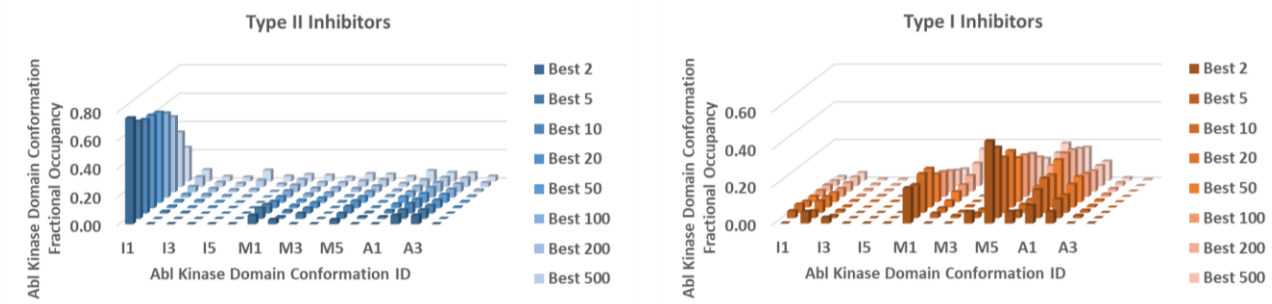


Figure 6. Induced-fit Abl kinase domain conformations during drug design.

Congratulations! You have completed Tutorial 5. Please feel free to continue tinkering with the options. If you have any questions, do not hesitate to ask.

References

(1) Okram, B.; Nagle, A.; Adrián, F. J.; Lee, C.; Ren, P.; Wang, X.; Sim, T.; Xie, Y.; Wang, X.; Xia, G.; Spraggon, G.; Warmuth, M.; Liu, Y.; Gray, N. S. A General Strategy for Creating "Inactive-Conformation" Abl Inhibitors. *Chemistry & Biology* **2006**, *13* (7), 779–786. https://doi.org/10.1016/j.chembiol.2006.05.015.

(2) Levinson, N. M.; Kuchment, O.; Shen, K.; Young, M. A.; Koldobskiy, M.; Karplus, M.; Cole, P. A.; Kuriyan, J. A Src-Like Inactive Conformation in the Abl Tyrosine Kinase Domain. *PLOS Biology* **2006**, *4* (5), e144. https://doi.org/10.1371/journal.pbio.0040144.

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(4) Hanson, S. M.; Georghiou, G.; Thakur, M. K.; Miller, W. T.; Rest, J. S.; Chodera, J. D.; Seeliger, M. A. What Makes a Kinase Promiscuous for Inhibitors? *Cell Chem Biol* **2019**, *26* (3), 390-399.e5. https://doi.org/10.1016/j.chembiol.2018.11.005.

(5) Meng, Y.; Gao, C.; Clawson, D. K.; Atwell, S.; Russell, M.; Vieth, M.; Roux, B. Predicting the Conformational Variability of Abl Tyrosine Kinase Using Molecular Dynamics Simulations and Markov State Models. *J. Chem. Theory Comput.* **2018**, *14* (5), 2721–2732. https://doi.org/10.1021/acs.jctc.7b01170.