Creating non-standard amino acids library files.

# Creating a PDB (middle chain residues)

1. Open PyMOL.

A screenshot of a computer

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1. Open the PyMOL building tab.

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1. Move to the ‘Protein’ tab.

Graphical user interface, application

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1. Build the backbone structure of the peptide for ATB upload in the form Ace-Ala-Ala-Ala-NMe. To begin the peptide, after selecting the Ace click “Create as New Object”. After adding the NMe as the final residue, click “Done”. For N- or C-termini please see page 4.

Graphical user interface, application

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1. Return to the chemical option menu. From this menu the base central alanine can be modified to become the non-canonical amino acid of interest. Atom type can be changed by selecting an element, chemical group or cyclic structure, and clicking on the atom you wish to change. Further options are present at the bottom of the chemical menu, where hydrogens can be added, atoms can be removed, charges can be changed, bonds created or deleted, and covalent bond type (single, double or triple) can be altered.
   1. If an element type is required that is not present on the table, it can be introduced using the command ‘alter pk1,elem= X’ with the element symbol in the X position.

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1. To create D-amino acids, select the Invert atoms button. This will prompt the user to make three atom selections. The first picked atom (pk1) is the Cα atom. The second atom to be picked is the backbone N and the last atom is the CO backbone atom. The molecule will visibly flip from an L- to a D-amino acid.

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1. The final step of the building process is to run energy minimisation the model using the Clean button. This is particularly important when introducing bonds or with larger, complex sidechains. There is a limit of 999 atoms for this function.

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# Creating a PDB (N- or C-terminal residue)

For this protocol, the above steps 1-3 are identical

## N-terminal

1. Build the backbone structure of the peptide for ATB upload in the form Ala-Ala-NMe. To begin the peptide, after selecting the Ala click “Create as New Object”. After adding the NMe as the final residue, click “Done”. Return to the N-terminal Ala, and select ‘Fix H’, before clicking the nitrogen. This will give you a NH2 terminal.

Graphical user interface, application

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1. Return to the chemical option menu. From this menu the N-terminal alanine can be modified to become the non-canonical amino acid of interest. Atom type can be changed by selecting an element, chemical group or cyclic structure, and clicking on the atom you wish to change. Further options are present at the bottom of the chemical menu, where hydrogens can be added, atoms can be removed, charges can be changed, bonds created or deleted, and covalent bond type (single, double or triple) can be altered.
   1. If an element type is required that is not present on the table, it can be introduced using the command ‘alter pk1,elem= X’ with the element symbol in the X position.
2. The final step of the building process is to run energy minimisation the model using the Clean button. This is particularly important when introducing bonds or with larger, complex sidechains. There is a limit of 999 atoms for this function.

## C-terminal

1. Build the backbone structure of the peptide for ATB upload in the form Ace-Ala-Ala. To begin the peptide, after selecting the Ace click “Create as New Object”. After adding the Ala as the final residue, click “Done”. Return to the C-terminal Ala, and select ‘Fix H’, before clicking the carbon. After this, select the hydrogen atom you have just created and change it to an oxygen, this should change it to an OH group. Your C-terminal will now be a COOH.

Graphical user interface, application

Description automatically generated

A screenshot of a computer

Description automatically generated with medium confidence

Shape, arrow

Description automatically generated

1. Return to the chemical option menu. From this menu the C-terminal alanine can be modified to become the non-canonical amino acid of interest. Atom type can be changed by selecting an element, chemical group or cyclic structure, and clicking on the atom you wish to change. Further options are present at the bottom of the chemical menu, where hydrogens can be added, atoms can be removed, charges can be changed, bonds created or deleted, and covalent bond type (single, double or triple) can be altered.
   1. If an element type is required that is not present on the table, it can be introduced using the command ‘alter pk1,elem= X’ with the element symbol in the X position.
2. The final step of the building process is to run energy minimisation the model using the Clean button. This is particularly important when introducing bonds or with larger, complex sidechains. There is a limit of 999 atoms for this function.

# Saving PyMOL as a PDB

1. After building your molecule, the next step is to save it ready for export to the ATB. To do this, open the file menu
2. Click on Export Molecule
3. The first GUI the opens, click save
4. This will then bring you to a files page
5. From here you can navigate to the folder you wish to save it to
6. Ensure it is named correctly, and the Save as type is a PDB extension (\*.pdb)

# Uploading your ncAA to the ABT

1. Visit the ATB: <https://atb.uq.edu.au/index.py> and log in
2. Navigate to the Submit page

Graphical user interface, text, website

Description automatically generated

1. Once in the submission portal
   1. Select Molecule type as amino acid from the drop down menu
   2. Set the molecule Net charge
   3. Upload the PDB from your computer
   4. Click Next

Graphical user interface, text, application, email

Description automatically generated

1. Confirm the submission and Submit This Molecule

Graphical user interface, text, application

Description automatically generated

1. If your molecule has already been submitted to the ATB you will be informed at this stage
   1. If your molecule is similar to one previously submitted, you will be given the option to submit anyway
   2. If your molecule is identical, you will be redirected to the previously submitted molecule’s page
2. After a successful submission you will be directed to a page which gives you two options
   1. You can follow on to the molecule’s page and follow its progress there
   2. You can Submit another molecule

Graphical user interface, text, application, email

Description automatically generated

1. An email will be sent to you which will contain the atom’s MOLID number for easy location in the ATB. This can also be found under your account in the Submitted Molecules page.