The Ducque Manual

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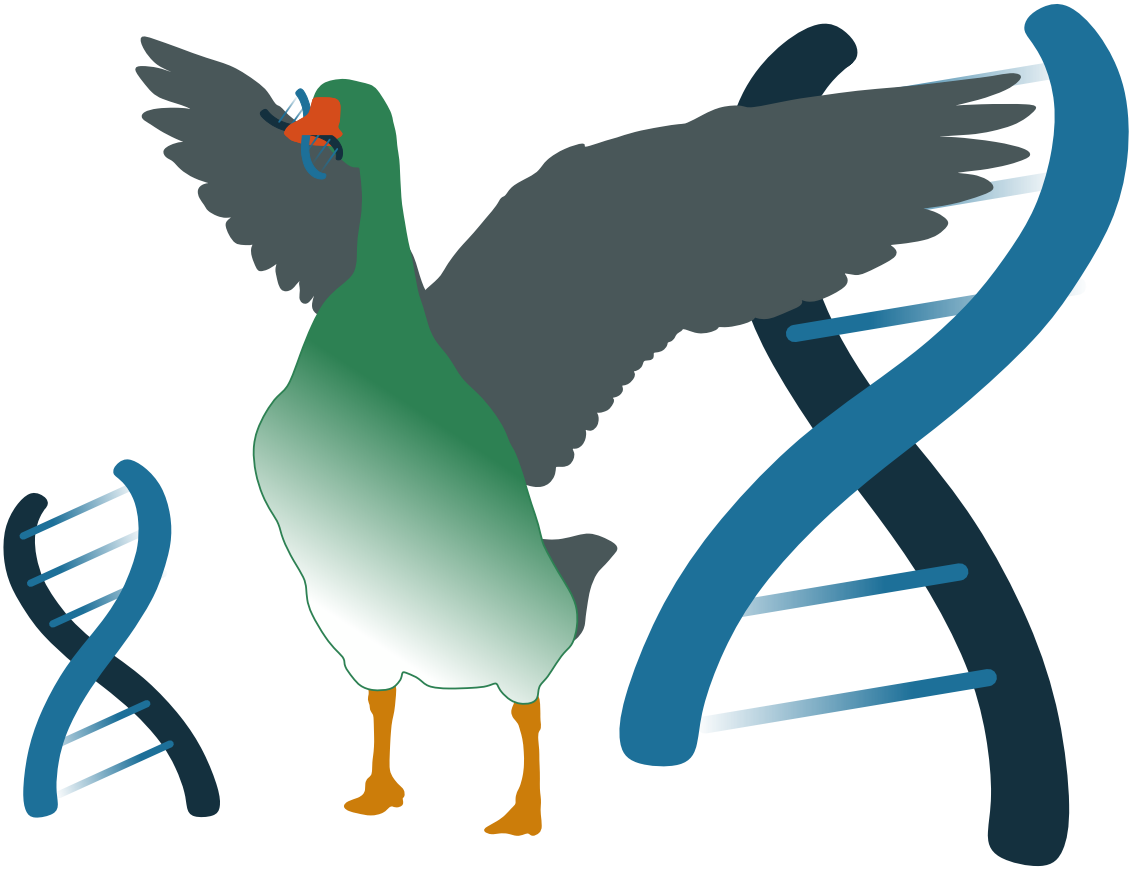

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Supervisor : prof. dr. Eveline Lescrinier
Co-promotor : prof. dr. Vitor Bernardes Pinheiro
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dr. Rinaldo Wander Montalvao for his guidance on the fundamentals of linear algebra.
dr. Charles-Alexandre Mattelaer for his guidance on Quantum Mechanics and without his experimental work, Ducque could have never been conceived.

1 Introduction

> There are no mistakes, only happy little accidents.
>
> Bob Ross

A software for the purpose of building native and synthetic nucleic acid duplexes.

Ducque (IPA : /dʌk/) stands for :

D Acronyms
U Are
C Rather
Q
U
E Tedious

The name was inspired much in the same way that the ORCA Quantum Mechanics package[1,2] was named. At the time of development, I had different name for Ducque. During one of my evaluation moments, one of the members of my advisory committee pointed out that with that name, I would probably not get enough traction, since Google searches gaves millions of results. Given that fact, I tried coming up with a new name for the model builder. Weeks later, as I was working, my hat that was resting on my desk in front of me caught my eye. You see, my hat has a tiny duck embroided on the front. With this idea, I "researched" ways to rewrite the word and get as little search engine results as possible, without degenerating the word itself and keeping it phonetically somewhat correct. Thusly, Ducque was born (a second time).

1.1 Modules

Ducque has five modules the user can access.

1. The build module will build the user the duplex structures we want to use for further experiments.
2. The transmute will be used to convert a specific nucleotide to a suitable input for Ducque to use as a building block.
3. The randomise module, will generate a randomised sequence that can be readily prompted to the build module to make a structure.
4. The xyz_pdb module exists because the many QM packages output xyz-formatted files after geometry optimisation. For most intents and purposes, the user might need to use pdb-formatted files. This module facilitates conversion from one format to the other through the CLI.
5. The GUI (Graphical User Interface) provides the user with clickable objects and predefined widgets to use Ducque, allowing the user to opt out of the CLI functionality. This module gives the user access to the build, transmute and the randomise module.

1.2 Package structure

path/to/Ducque

→ **bin/:** contains executable to run Ducque.
→ **docs/:** contains this pdf.
→ **ff/:** contains the pdf on *How_To_Build_A_Forcefield.pdf* .
→ **json/:** contains building blocks requires by Ducque to build duplex sequences; json format.
→ **src/:** contains Ducque's source code.
→ **transmute/:** contains the transmute input files; tinp format.
→ **xyz/:** contains the xyz_pdb input files.
→ LICENSE (MIT).
→ README.md (GitHub).
→ setup.sh : required to make Ducque executable.

2 Installing and running Ducque

2.1 Installation

The installation is done by cloning the repository, through the use of git. It is advised to clone the repository to the desired location at the $HOME-level ($HOME, $HOME/Desktop, ...).

Enter the Ducque directory and run the setup.sh script to give the correct permissions to the Ducque executable, located in Ducque/bin.
Finally, add the last line to your bash environment, by adding it to your $HOME/.bashrc. MacOS users may replace mentions of $HOME/.bashrc with $HOME/.bash_profile.

For future reference, the path/to/program is the location on your system where Ducque is installed. E.g., if Ducque is installed at $HOME/Desktop/Ducque, then $HOME/Desktop = path/to/program.

```
# Install on your system
$ git clone https://www.github.com/jrihon/Ducque.git # clone repo locally
$ cd Ducque

# Run the setup.sh script
$ chmod a+x setup.sh    # Give permission to run as an executable script
$ ./setup.sh

# Add to ~/.bashrc
export PATH="$PATH:path/to/program/Ducque/bin"
```

2.2 Environment

To install the correct packages for Ducque, it is advised to use the conda package manager for Python modules. Install conda.
To use Ducque, it is also advised to create a separate virtual environment so as to not override or break any dependencies in the base environment.

The user must activate the Ducque environment before installing the dependencies. The packages to install are NumPy, SciPy and Tkinter.

```
# Create a virtual environment using conda
$ conda create --name Ducque
# Enter the Ducque `conda environment`
$ conda activate Ducque

# Using the conda package manager install : NumPy, SciPy, Tkinter
$ conda install -c numpy scipy tk


# To leave the virtual environment
$ conda deactivate
```

To leave the virtual environment, the user can deactivate the conda environment to leave the Ducque environment.

3 Usage

3.1 Build

The Build module allows to build a structure with a simple query. The build inputfile are highlighted with the .binp file extensions (build-input).

```
$ Ducque --build INPUTFILE.binp
```

To allow for high customisability of model duplexes, the user can build the following way :

- Leading and complementary strand with the same chemistry (homoduplex) (Fig. 1A.)
- A difference in chemistry between the in both strands (heteroduplex) (Fig. 1B.)
- Complementary strand with various chemistries (custom antisense strands) (Fig. 1C.)

```
A.
--sequence DT, DC, DA, DA, DC, DG, DG, DT, DA
--complement HOMO
--pdbname dna_homo_duplex
```

```
B.
--sequence RA, RG, RU, RU, RG, RC, RC, RA, RU
--complement MNA
--pdbname rna_mna_heteroduplex
```

```
C.
--sequence   RC, RG,  RA,  RA, RG, RC, RC,  RA
--complement RG, RC, 2MU, 2MU, RC, RG, RG, 2MU
--pdbname rna_2ome-rna_heteroduplex
```

Figure 1: Example of possibilities of queries accepted by Ducque.

3.1.1 --sequence

A list of comma-separated values. The values are specific nucleic acids that are implemented into Ducque. A list of values readily available to the user is presented in Sec. 3.7.

3.1.2 --complement

This can be one of three different types of queries.

1. The HOMO input, to denote that the complementary strand should be built with the same chemistry as the leading strand. The complementary sequence is inferred from sequence query.
2. The specific chemistry for the complementary sequence, such as DNA, RNA, MNA etc. The complementary sequence is inferred from the sequence query.
3. A list of comma-separated values, as shown in Sec. 3.1.1. A list of values readily available to the user is presented in Sec. 3.7. This feature is only available in from the CLI, as this requires manual entry of the values.

3.1.3 --pdbname

The name of the output pdb-file. This can be appended by the .pdb extension, but is not required.

3.2 Transmute Nucleoside

The transmute module allows the user to incorporate nucleotides (building blocks) into Ducque's repository to be able to build nucleic acid duplexes with. The transmute inputfile are highlighted with the .tinp file extensions (transmute-input). The conversion to a suitable inputfile is stored as a json-formatted file in the repository itself. The json files are never to be written by hand and are mandated to be generated by the transmute module!

```
$ Ducque --transmute INPUTFILE.tinp
```

An example is given of the tinp file of DNA Adenosine :

```
--pdb dna_adenosine_2endo.pdb
--chemistry DNA
--conformation 2endo
--moiety nucleoside
--nucleobase A
--dihedrals -39.246, -151.431, 30.929, 156.517, 159.171, -98.922, -99.315
--bondangles 101.407, 118.980, 110.017, 115.788, 111.943, 119.045, 126.013
```

3.2.1 --pdb

Relative pathing to the pdb file of the molecule that the user wants to incorporate into the library. Nota bene: before using the pdb file, it has to be presented as an uncapped molecule. For example, when looking at Fig. 5, we see the HO5′ and HO3′ atoms capping the backbone. These atoms need to be removed before using the specific pdb file as an input for the transmute module! When finishing the building, terminal hydrogens are appended to either ends of the strands and their atomnames are inferred from the atom that bind the capping hydrogens.

3.2.2 --chemistry

The chemistry denotes the type of modification the molecule bears. For example: the standard deoxyribose nucleic acids are represented by adenosine, cytidine, guanosine and thymidine nucleosides, but are categorised under the DNA chemistry. The chemistry has direct relations to its key in Sec. 4.1.4 and Sec. 4.1.3.

3.2.3 --conformation

Ducque allows for multiple conformations to be built into the complementary strand of the generated duplex. For example: DNA chemistries are commonly determined in both the ${}^{2'}E$ and ${}^{3'}E$ configuration. Ducque allows that both are incorporated into the library (Sec. 4.1.2). The specific conformation is later inserted in the name of the json file to differentiate multiple conformers of the same chemistry and to avoid naming clashes in the repository.

3.2.4 --moiety

The Ducque software distinguishes two main part of molecule, being the nucleoside and the linker. Specifically, the building start with a linkerless nucleoside, to where to linker part is fitted on. Because

many different chemistries can share the same linker, and because it is useful to separate both parts when building and also on a coding-level, it was decided to allow nucleosides to be incorporated separately from the linker. Also, this dimishes boilerplate inputs. In Sec. 4.1.1, the user is mandated to couple the nucleoside chemistry and the type of linker together.

3.2.5 --nucleobase

The nucleobase flag is required to allow more flexibility in defining the residue name of the molecule. An earlier consideration was to infer the type of nucleobase from the residue name itself, but considering that we want to implement a method to accept any type of modified nucleobase in the future, the maintainer felt that this option might be more useful on a coding level and would disallow errors to propagate based on assumptions.
This flag represents the type of nucleobase that pertains to the nucleoside that the user wants to implement into Ducque. The nucleobase infers the complementary base with which it pairs; e.g. the G nucleobase infers basepairs with C.
For now, only purine and pyrimidine nucleobases are implementable. Technically, if the nucleobase modification entails only additional substituents and the naming of the atoms in the ring system remains identical to that of the natural bases, then it should also be implementable. This feature has not been tested.

3.2.6 --bondangles

The bondangles that define the backbone of the nucleotide.
The way the duplex is built is from bottom to top (e.g. from $3' \rightarrow 5'$ for DNA). The way this is reflected, among other things, is in the way the bondangles are required to be queried from the json files. In a set of a dihedral, meaning four (4) atoms, the latter three (3) are to be supplied as the bondangle of the respective backbone angle.
For example: the γ-dihedral contains the following set [C3′, C4′, C5′, O5′]. This means that the γ-bondangle contains the set [C4′, C5′, O5′] (Fig. 2). These values tend to be relatively stable across all different types of chemistries, given all atoms in a set are sp^3 hybridised. Therefore, one can use the same angles, pertaining to similar atoms involved in the bondangle, from other types of chemistries and it will build fine. For most, if not all, intents and purposes, the set of angles tend to remain relatively conserved. This means that, when implementing a new chemistry, the user should focus on the dihedrals.

IUPAC nomenclature states the backbone follows the Greek alphabet : $[\alpha, \beta, \gamma, \delta, \epsilon, \zeta]$.[3,4] This also involves the glycosidic dihedral of $[\chi]$ (Fig. 2A.). Since IUPAC follows the ordering of the alphabet, an additional backbone angle is added in case the type of chemistry requires an additional angle to be queried : $[\eta, (eta)]$.
Not all chemistries require all stated backbone angles and thus the software permits shorter backbone chains too, like PeptideNA and ThreoseNA, to be built all the same.

To emphasize : The angles given by the user to the building block are in fact the angles Ducque will use in order to build out the molecular structure! Giving erroneous bondangle values will result in *warped* molecular structures.

3.2.7 --dihedrals

The dihedral (torsion angles) that define the backbone of the nucleotide.
A dihedral constitutes an angle between four atoms. For example: the γ-dihedral contains the set

[C3′, C4′, C5′, O5′]. To procure these values, we redirect the reader to Sec. 4.2 of this manual for more information.

IUPAC nomenclature states this follows the Greek alphabet : $[\alpha, \beta, \gamma, \delta, \epsilon, \zeta]$.[3,4] This also involves the glycosidic angle of $[\chi]$ (Fig. 2B.). Since IUPAC follows the ordering of the alphabet, an additional backbone torsion is added in case the type of chemistry requires an additional angle to be queried : $[\eta, (eta)]$.
Not all chemistries require all stated backbone dihedrals and thus the software permits shorter backbone chains too, like PeptideNA and ThreoseNA, to be built all the same.

To emphasize : The dihedrals and angles given by the user to the building block are in fact the dihedral/angle Ducque will use in order to build out the molecular structure! Given erroneous dihedral values will result in *warped* molecular structures.

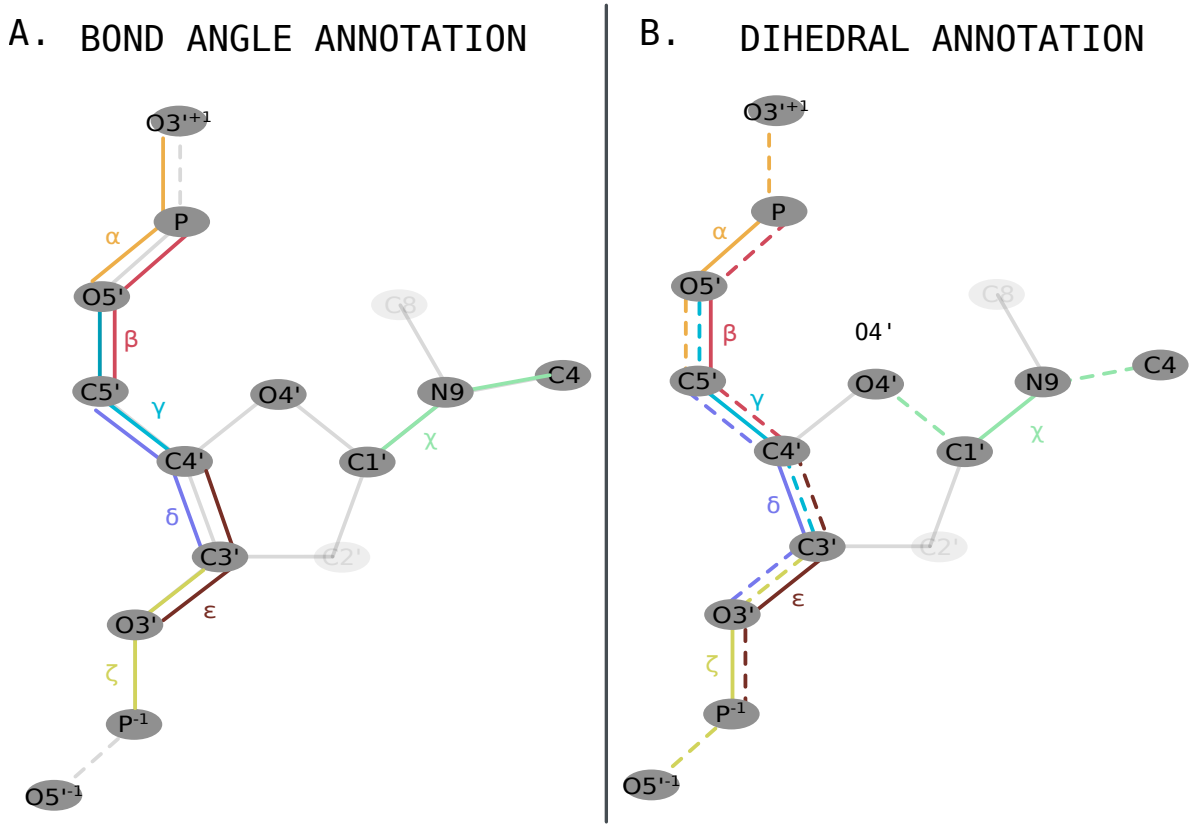


Figure 2: Representation of the **A.** bond angles and **B.** dihedrals in the backbone of nucleic acid nucleotides. This represents how one can consider inputting the values for their own type of chemistries. Notice the bottom to top direction in the annotated bond angles (**A.**).

3.3 Transmute Linker (tlinker)

Implementing the linker into the *src* of Ducque can be found at Sec.4.1.5.

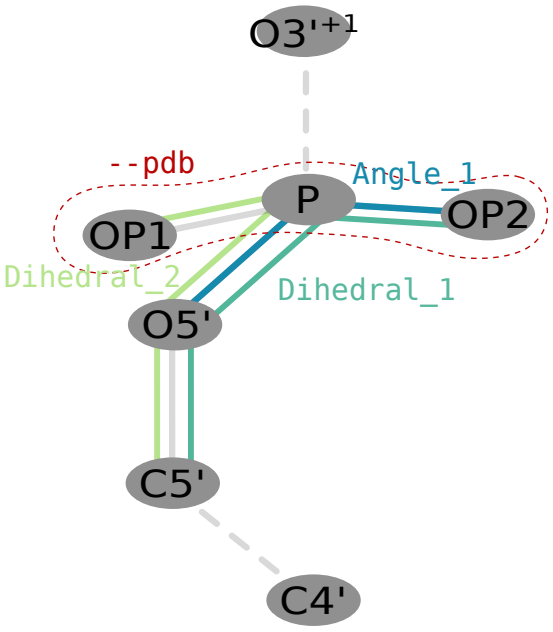


Figure 3: blabla

3.3.1 --pdb

Relative pathing to the pdb file of the molecule that the user wants to incorporate into the library. Looking over at Fig. 3, it is only asked of the user to supply the linker part. This makes some linkers extremely versatile in usage.
For example: even the the MNA nucleotides are synthetised with a phosphoramidate bond, the phosphate linker can still be used to build out the structure. The phosphate linker only consists of the set [P, OP1, OP2].

3.3.2 --chemistry

The chemistry denotes the type of modification the molecule bears. For example: the commonly used phosphate linker is just annotated as the PHOSPHATE moiety. The chemistry has direct relations to its key in Sec. 4.1.4 and Sec. 4.1.5.

3.3.3 --moiety

The Ducque software distinguishes two main part of molecule, being the nucleoside and the linker. Specifically, the building start with a linkerless nucleoside, to where to linker part is fitted on. Because many different chemistries can share the same linker, and because it is useful to separate both parts when building and also on a coding-level, it was decided to allow nucleosides to be incorporated separately from the linker. Also, this dimishes boilerplate inputs. In Sec. 4.1.1, the user is mandated to couple the nucleoside chemistry and the type of linker together.

3.3.4 --conformation

The conformation flag allows the user to distinguish the linker inputfiles when a stereocenter comes into play, or allows just simple insertion of the linker with the NONE keyword, like with DNA phosphate. However, most linkers, like thiophosphates, do contain a stereocenter. It is up to the user to know which R- or S-configuration the linker adopt and to annotate this correctly. For obvious reasons, these are the only valid set of options: [NONE, R, S].
The added benefit to this is that a racemised mixture of linkers can be queried through Ducque, containing both R and S linkers of the same chemistry. Another useful thing is that the user can swap the values for dihedral_1 and dihedral_2 with eachother in order to swap the stereoisomer, when transmuting the linker as an input for Ducque. For example, for S-Thiophosphate, the dihedral values are (1: -155.047, 2: 76.677), while for R-Thiophosphate the values read (1: 76.677, 2: -155.047).

3.3.5 --bondangles

The angle defines the orientation of the linker.
For example: the angle_1 (Fig. 3) contains the set [O5′, P, OP2]. Generally, the user may attribute an angle between $109° \leq X \leq 120°$, as the main purpose of the linker is to make minimal clashes with the backbone itself and be somewhat correctly oriented. The rest is handled by the simulation engine and a suitable forcefield. This is also the reason why there is a single angle value to prompt, as this bond angle can be relatively well generalised for building purposes.
To emphasize : The dihedrals and angles given by the user to the building block are in fact the dihedral/angle Ducque will use in order to build out the molecular structure! Given erroneous dihedral values will result in *warped* molecular structures.

3.3.6 --dihedrals

The dihedral (torsion angles) defines the orientation of the linker.
A dihedral constitutes an angle between four atoms. For example: the dihedral_1 (Fig. 3) contains the set [C5′, O5′, P, OP2], the dihedral_2 contains the set [C5′, O5′, P, OP1]. Generally, the user may attribute relatively the same dihedrals used by the phosphate linker for their own synthetic linkers, as the main purpose of the linker is to make minimal clashes with the backbone itself and be somewhat correctly oriented. The rest is handled by the simulation engine and a suitable forcefield.
To emphasize : The dihedrals and angles given by the user to the building block are in fact the dihedral/angle Ducque will use in order to build out the molecular structure! Given erroneous dihedral values will result in *warped* molecular structures.

3.4 Randomise

This module is implemented to facilitate writing and producing sequences.
The randomisation inputfile are highlighted with the .rinp file extensions (randomise input).

```
$ Ducque --randomise INPUTFILE.rinp
```

- A variable sequence with a specified chemistry (Fig. 4.A).
- A way to prepend a given sequence with a prompted chemistry. This method is not a randomisation, but allows the user to write the desired sequence faster. This functionality was more fit to be introduced in this module than anywhere else (Fig. 4.B).

This query outputs a binp file that can be readily passed to the build module (Sec. 3.1).

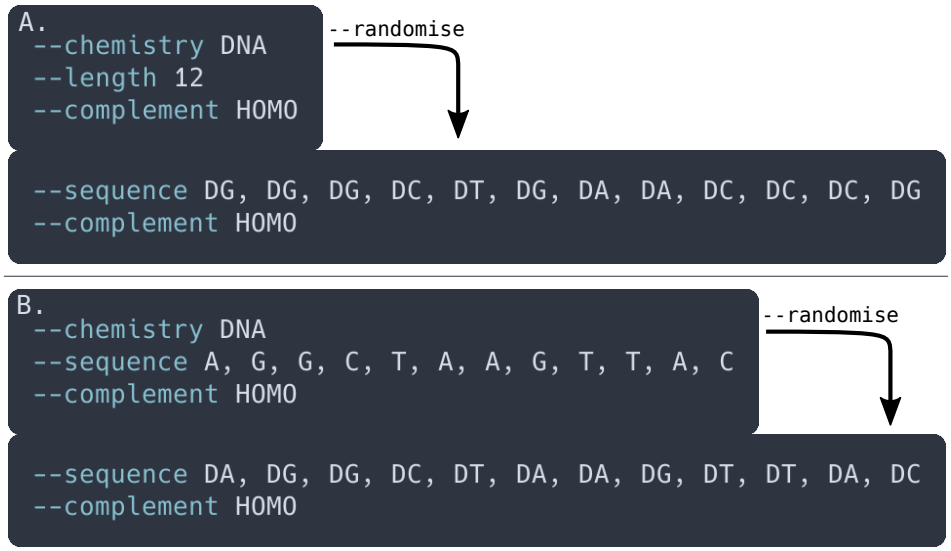


Figure 4: Examples of queries for the randomisation module.

3.4.1 --sequence

A mutually exclusive flag with --length. If a given sequence is given, then the length of it is already determined by the user. The sequence is a list of comma-separated values, consisting of one of five currently implemented nucleobases : A, C, G, T, U.

3.4.2 --length

A mutually exclusive flag with --sequence. This requires an integer argument to be passed into. It returns a randomised sequence, for a given chemistry, of the leading strand with a set length.

3.4.3 --chemistry

Prompt a chemistry type, from a list, to query Ducque for a correctly typed sequence. The type of chemistry is related to the keys of the entries given in Sec. 4.1.3.

3.4.4 --complement

The complement argument can be filled in at the randomisation stage, but its sole function is to pass the argument to the build module later. This is an optional argument.

3.5 XYZ → PDB

The xyz_pdb module allows the user to convert from an xyz to an pdb formatted file. Both are generally readable by all Molecular Viewing Software, but the pdb format is the only format accepted by Ducque, in order to transmute the molecule into a suitable building block for model building. An xyz file is a file consisting only of the atomic coordinates and the atomic elements.

```
$ Ducque --xyz_pdb INPUTFILE
```

```
--xyz dna_adenosine_2endo.xyz
--residue DA
--atomname_list HO5', O5', C5', H5'1, H5'2, C4', H4', O4', C1', ..., O3', HO3'
```

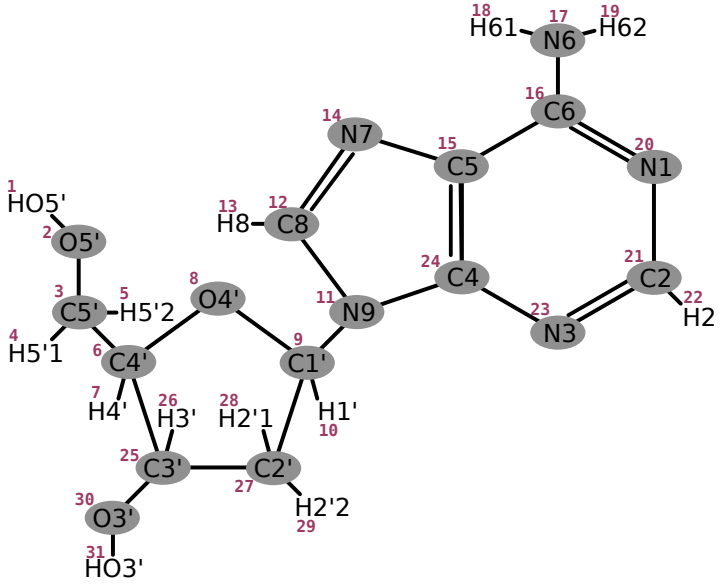


Figure 5: Depiction of the way in which atomnames are assigned according to the (AMBER Tree Structure.

3.5.1 --xyz

- Relative path to the xyz file the user wants to convert.
- A directory that contains xyz files, meaning multiple different files of the same molecule. When performing Conformational Sampling experiments, the user is left with all possible configurations a molecule can adopt. To facilitate conversion to the more useful pdb format, it suffices to query to name to the directory where those xyz files live. Again, this has to be a directory of a single type of molecule.

3.5.2 --residue

The name of residue attributed to the specific chemistry, as per the PDB format. String names can not exceed three (3) characters. The residue name has direct relations to its key in Sec. 4.1.1 and Sec. 4.1.2.

3.5.3 --atomname_list

The atom name attributed to the specific atom in the molecule, as per the PDB format. String names can not exceed four (4) characters per atom name. Fig. 5 follows the AMBER Tree Structure.

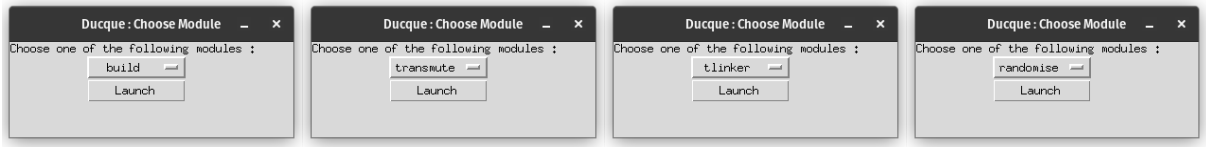
3.6 Graphical User Interface (GUI)

The GUI-module allows the user to access the other modules from a neat GUI instead of the CLI. The GUI is called by the following command, and specific modules are more quickly accessed by adding to the query :

```
# Ducque calls to GUI modules
$ Ducque --gui

# Query to open specific modules
$ Ducque --gui build
$ Ducque --gui transmute
$ Ducque --gui tlinker
$ Ducque --gui randomise
```

The first command gives the user the option to acces the modules, this defaults on build.



The xyz_pdb module was not made available as a GUI because it was difficult to implement a neat interface. As such, it is only available as a CLI command, where it performs better.

3.6.1 Build

We refer to Sec. 3.1 for the explanation on the various --build arguments.

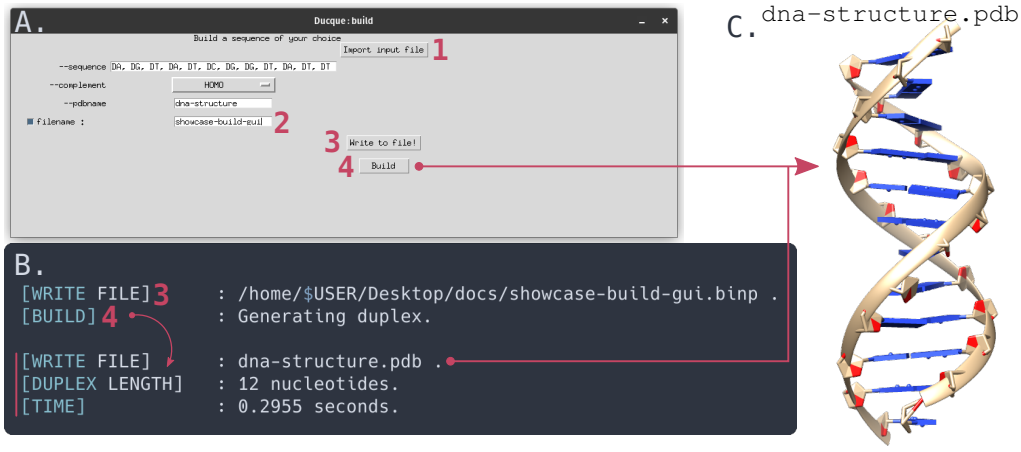


Figure 6: **A.** The build GUI. **B.** What gets outputted to stdout when using Ducque. **C.** The produced molecular structure.
1. Import a binp if desired or if possible. The user can also just write the query by hand. **2.** Optionally, rename the binp file to something else. **3.** Save the new binp, after having filled in the pdbname query. **4.** Build a model structure based on the produced binp file.

In Fig. 6A., we see the GUI of this module. The complement flag show a option-list from which we can query the type of chemistry we want. This list is dependent on the keys of the table defined in Sec. 4.1.3.

3.6.2 Transmute Nucleoside

We refer to Sec. 3.2 for the explanation on the different arguments.

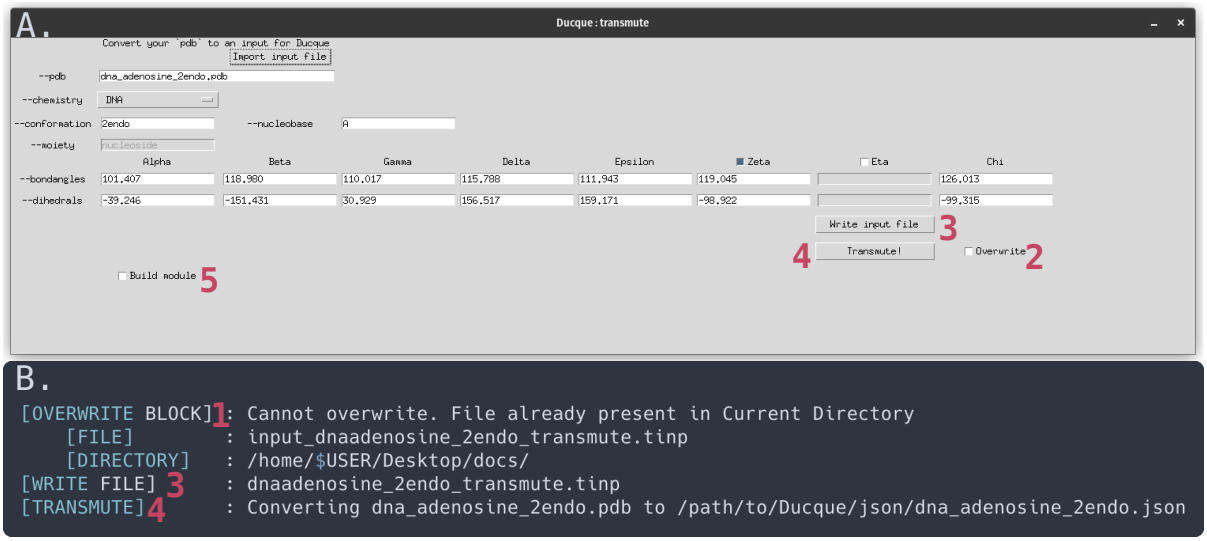


Figure 7: **A.** The transmute GUI. **B.** What gets outputted to stdout when using Ducque.
1. After having imported a specific tinp file and modified some of the data, we can write to the same file. However, to avoid accidental overwriting of the same file, the user can only write to the same file in the current working directory if the Overwrite button has been checked. This also prohibits from using the Transmute! button, if the json already exists in the path/to/Ducque/json directory. **2.** Check the button to allow writing to existing files on the system. **3.** With the given query, a tinp file will be written to the current working directory. **4.** Convert the tinp to a json file. **5.** This functionality is a built-in part of the build module, within the transmute module. This will come in later into play, but essentially facilitates the iterative approach to tweaking and modifying torsion angles for the given input by allowing transmuting and building within a few clicks (see Sec. 4.2).

In Fig. 7A., we see the GUI of this module. The chemistry flag show a option-list from which we can query the type of chemistry we want. This list is dependent on the keys of the table defined in Sec. 4.1.3.

3.6.3 Transmute Linker

We refer to Sec. 3.3 for the explanation on the different arguments. This procedure is nearly identical to Sec. 3.6.2

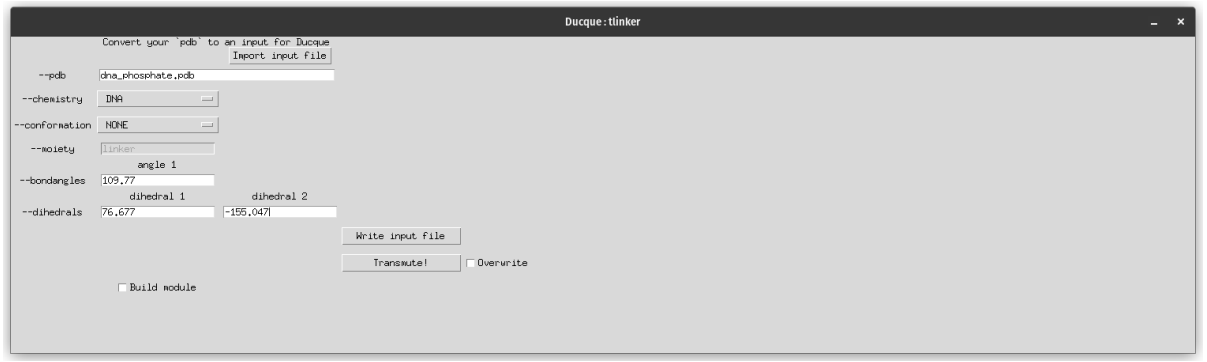


Figure 8: GUI module to implement new linker moieties.

3.6.4 Randomise

We refer to Sec. 3.4 for the explanation on the various --randomise arguments.

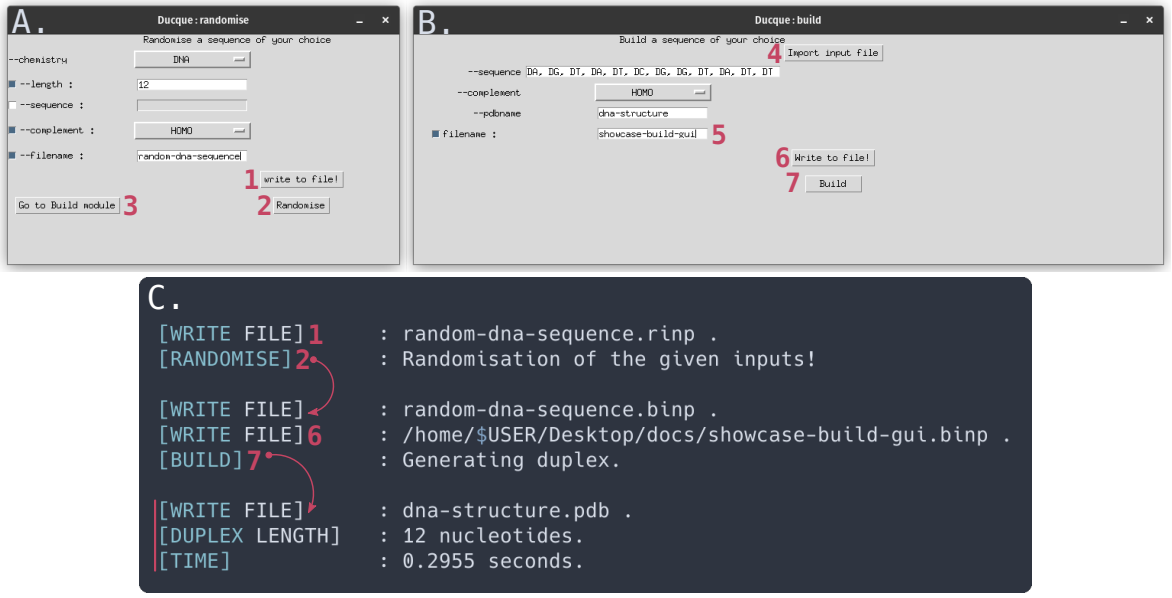


Figure 9: **A.** The randomise GUI. **B.** The build GUI. **C.** What gets outputted to stdout when using Ducque.
1. After filling in the query, write to a rinp file. **2.** Randomise an output based on the produced rinp file. This produces a binp file, with which one can readily build. **3.** Click the button to close the randomisation module and go straight to the build module. **4.** Import the created binp into the module to fill in the queries. **5.** Optionally, rename the .binp file to something else. **6.** Save the new binp, after having filled in the pdbname query. **7.** Build a model structure based on the produced binp file (Fig. 6C.).

In Fig. 9A., we see the GUI of this module. The chemistry and the complement flag show a option-list from which we can query the type of chemistry we want. This list is dependent on the keys of the table defined in Sec. 4.1.3.

3.7 Available input by default

Deoxyribose NA (DNA)		Ribose NA (RNA)		β-homo NA (B-HOMODNA)	
DA	DC	RA	RC	DDA	DDC
DG	DT	RG	RU	DDG	DDT

Hexitol NA (HNA)		Xylose NA (XYNA)		Deoxyxlose NA (DXYNA)	
HA	HC	XA	XC	DXA	DXC
HG	HT	XG	XU	DXG	DXT

2-O-Methyl RNA (2-OME-RNA)		Cyclohexenyl NA (CENA)		Morpholino NA (MNA)	
2MA	2MC	CA	CC	MA	MC
2MG	2MU	CG	CT	MG	MT

2-Fluoro RNA (2-F-RNA)		S-Thiophoshate DNA (S-1DNA)		R-Thiophoshate DNA (S-2DNA)	
2FA	2FC	1DA	1DA	2DA	2DA
2FG	2FU	1DG	1DG	2DG	2DG

Figure 10: The headers contain the type of chemistry, with their abbreviation included in brackets. There are NINE (9) types of chemistries in total already implemented in Ducque.
A : Adenine, C : Cytosine, G : Guanine, T: Thymine, U: Uracil, NA: Nucleic Acid

3.8 Step-by-step instructions on how to start implementing nucleosides

This sections contains a checklist of the preparation and the execution to implementing new nucleosides.

1. Perform Conformational Sampling and curate the conformation of choice to implement and build with.[5]
2. Use the xyz_pdb (Sec. 3.5) function to convert the generated xyz to pdb files. Be mindful of the residue name that is given to the nucleoside, as this is important for later
3. Appending data to the TABLES
   (a) Sec. 4.1.1 requires the user to make residue name-inputfile.json pairs of the nucleoside the user wants to incorporate.
   (b) Sec. 4.1.2 requires the user to implement the same information as in the previous item, but allows the user to implement more than one different conformation of the same nucleoside
   (c) Sec. 4.1.3 is arguably the most important aspect. This calls for the user to make chemistry-backbone atoms pairs. This query is what allows Ducque to build with any type of backbone.
   (d) Sec. 4.1.4 is only necessary to specifiy the full name of the chemistry for the json file that will be transmuted for.
   (e) Sec. 4.1.5 requires the user to couple the chemistry nucleoside - chemistry linker together.
4. Start working towards transmuting (Sec. 3.2). Optimise bondangle and dihedral angle values (Sec. 4.2).
5. Randomise (Sec. 3.4) or query a specific sequence.
6. Build the desired model duplex (Sec. 3.1)

3.9 Step-by-step instructions on how to start implementing linkers

This sections contains a checklist of the preparation and the execution to implementing new linkers. Conformational Sampling is an option to derive the behaviour of the linker, but is by no means necessary for the model building iteself.

1. Design a linker through a preferred Molecular Viewing Software (UCSF Chimera, PyMol . . .).
2. Designing a new linker always entails making a new nucleoside chemistry as well.
   All molecules are annotated with a specific residue name (e.g. DNA Adenosine is DA, Sec. 3.5.2). A linker, by itself, needs to be associated with the nucleoside it is appended to. This means that the linker takes on the residue name of the nucleoside, to form a full nucleotide. Only the nucleoside json inputfiles have a residue name, which makes the linker much more modular to implement for other chemistries. The other side of the coin is that when a new linker is desired for implementation, a nucleoside chemistry has to be designed as well.
   For example: when implementing the thiophosphate linker (Sec. 3.7), a copy of the standard DNA nucleosides were made, where the residue name was altered to be unique for the thiophosphates. This is because when the user queries a specific sequence, the user does this by querying specific nucleotides (nucleoside-linker pairs, Sec.4.1.1). This means that an additional nucleoside json needs to be created to make a nucleotide, since we need to be able to distinguish this type of information explicitly to the library.
   Additionally, because the thiophosphate linker has a stereocenter, two copies were made of the DNA chemistry. The residue name for the S-isomer became [1DA, 1DG, 1DC, 1DT], for the R-isomer it [2DA, 2DG, 2DC, 2DT]. This naming is arbitrary and is there to allow the distinction between regular phosphate linkers and the two thiophosphate stereomers. While the nucleosides [DA, 1DA, 2DA] are identical in properties, they differ by residue name only and the specific linker to which they are coupled in the library (Sec. 4.1.1, 4.1.6).
3. Appending data to the TABLES
   (a) Sec. 4.1.1 requires the user to make nucleoside-linker (json format) pairs of the nucleoside the user wants to incorporate.
   (b) Sec. 4.1.2 requires the user to implement the same information as in the previous item, but allows the user to implement more than one different conformation of only the same nucleoside
   (c) Sec. 4.1.3 is arguably the most important aspect. This calls for the user to make chemistry-backbone atoms pairs. This query is what allows Ducque to build with any type of backbone. This is for both the nucleoside and linker chemistries.
   (d) Sec. 4.1.4 is only necessary to specifiy the full name of the chemistry for the json file that will be transmuted for.
   (e) Sec. 4.1.5 requires the user to couple the chemistry nucleoside - chemistry linker together.
4. Start working towards transmuting (Sec. 3.3). Optimise bondangle and dihedral angle values (Sec. 4.2). Generally speaking, the linker just needs to not clash with the surrounding atoms. The angle values here may well be an arbitrary value that evades clipping of the moieties. Subsequent minimisation and MD simulations will take care of the rest. To highlight, the phosphate and thiophosphate linkers have the exact same angle values.
5. Randomise (Sec. 3.4) or query a specific sequence.
6. Build the desired model duplex (Sec. 3.1)

4 User implementation of customised nucleic acids

4.1 How to add to Ducque's *src* files.

This section concerns with the incorporation of custom types of chemistries into Ducque's repository. This is done by modified one of the python files in the source code itself.

```
# Go to the src files
$ cd path/to/program/Ducque/src/ducquelib/

# Open file in your preferred text editor
$ vim library.py
```

The reason why a python script is modified, and not some sort of configuration file, is two-fold :

1. In order to create a configuration file, one needs to depend on various typed formats, like yml, json, toml etc. Though many libraries exist to parse these formats, they (especially the first two) tend to be somewhat finnicky if implemented by hand. To combat this issue, one would have to write a specified format and have it be parsed by such a library. Writing a specific format brings many I/O issues that bring more trouble than they are useful. When in reality, the only thing we'd need is a dictionary (a.k.a. table, hashmap ...) with the given inputs. This brings me to my second point.

2. What better parser can be asked to parse a native data structure, the dictionary, than the python interpreter itself. What the maintainer proposes is that errors in python source code are caught immediately. This means that, upon running Ducque, all code is checked and looked for syntax errors. If, and when, these errors are encountered, Ducque will not be called, which is the desired behaviour when such an error has been found. Even better, the Python Error Handling will return a message stating the exact problem of why the error has been encountered and thus one can fix their syntactic mistakes. This often involves missing a comma, square brackets or curly braces.

4.1.1 Adding a nucleoside-linker combination to form a defined nucleotide

This section allows the user to implement the correct combination of nucleoside and linker to Ducque's repository. The way this is formed is through {KEY : VALUE} pairs, more commonly referred to as a dictionary.
Important to note here is that the given conformation will be the conformation with which Ducque will build to leading strand, for the nucleoside-linker pair in TABLE_NUCLEOTIDES!

```
# GO TO +- line 18
# ------------------------------------------------------------------------------
#                   NUCLEOSIDE              ,             LINKER
# ------------------------------------------------------------------------------
DH = path/to/Ducque # DUCQUE_HOME
TABLE_NUCLEOTIDES = {
"DA" : [ DH + "dna_adenosine_2endo.json", DH + "dna_phosphate.json"],
"DG" : [ DH + "dna_guanosine_2endo.json", DH + "dna_phosphate.json"],
#                         ...                          ...
"FOO" : [ DH + "foo_nucleoside.json", DH + "bar_linker.json"],
}
```

- The KEY is the residue name (Sec. 3.5.2) that was used in the pdb file (Sec. 3.2.1), upon transmuting the parameters of the desired nucleoside. This KEY is a string and has to be ALL CAPS.
- The VALUE is a python list that contains two values : the name of the nucleoside and the name of the associated linker, as string-typed values.
- The DH variable (DUCQUE_HOME) is the place where Ducque lives on the user's machine; this is evaluated at runtime. The user needs only to prepend 'DH + ' to the name of the files pertaining to the nucleotide incorporated. To clarify, the inputfiles for Ducque are stored as json files in the path/to/Ducque/json/ directory, upon transmuting the inputs, so this removes a lot of boilerpate text in the repository.

4.1.2 Adding one ore more conformations of a nucleoside chemistry

This section is similar to the Sec. 4.1.1, but builds the complementary strand. This allows the user the implement multiple conformations of the same chemistry to be used while building that strand. Building out the complement involves several fitting procedures. If more than one conformation exist of a type of chemistry, Ducque will try its best to fit the best conformation to the leading strand and its adjacent stacking nucleotides.
Again, the {KEY : VALUE} pairs are crucial to our repository.

```
# GO TO +- line 75
# ----------------------------------------
#       COMPLEMENTARY REPOSITORY
# ----------------------------------------
TABLE_CONFORMATIONS = {
"DA": [ DH + "dna_adenosine_2endo.json", DH + "dna_adenosine_3endo.json"],
"RG": [ DH + "rna_guanosine_3endo.json"],
#                              ...                              ...
"FOO": [ DH + "foo_nucleoside_Xendo.json", DH + "foo_nucleoside_Yexo.json"],
}
```

Figure 11: To dictionary that is used to insert multiple conformations into Ducque for a specific residue, to build out the complementary strand.

- The KEY is the residue name (Sec. 3.5.2) that was used in the pdb file (Sec. 3.2.1), upon transmuting the parameters of the desired nucleoside. This KEY is a string and has to be ALL CAPS.
- The VALUE is a python list that contains two values : the name of the respective nucleoside conformations, as string-typed values.
- The DH variable (DUCQUE_HOME) is the place where Ducque lives on the user's machine; this is evaluated at runtime. The user needs only to prepend 'DH + ' to the name of the files pertaining to the nucleotide incorporated. To clarify, the inputfiles for Ducque are stored as json files in the path/to/Ducque/json/ directory, upon transmuting the inputs, so this removes a lot of boilerpate text in the repository.

4.1.3 Adding the backbone atoms by which Ducque needs to build

As stated before (Sec. 3.2.6), the software builds from bottom to top (e.g. for DNA: $3' \rightarrow 5'$).
This section defines the backbone by which the Ducque model builder should produce the structure with. In the example (Fig. 12), the DNA and MNA nucleic acid chemistries are given.
Again, the {KEY : VALUE} pairs are crucial to our repository.

```
# GO TO +- line 130
# ----------------------------------------
#        BACKBONE REPOSITORY
# ----------------------------------------
# Add atoms of the backbone in ASCENDING ORDER OF APPEARANCE!
TABLE_BACKBONE = {
"DNA" : ["O3'", "C3'", "C4'", "C5'", "O5'"],
"MNA" : ["N3'", "C4'", "C5'", "C6'", "O6'"],
"PHOSPHATE" : ["P"],
#                          ...
"FOO" : ["Z", "Y", "X", "W", "V"],
}
```

Figure 12: The dictionary that is used by Ducque to build along a given backbone. One of the most crucial aspects to Ducque and why it allows any backbone modification to be built with this software.

- The KEY is the chemistry that was used in the tinp (Sec. 3.2.2), upon transmuting the parameters of the desired nucleoside. This KEY is a string and has to be ALL CAPS.
- The VALUE is a python list that contains all atoms of the backbone, in ascending order (callback to Sec. 3.2.6). The list is a set of comma-separated string-typed values, that pertain to the atomnames (Sec. 3.5.3) in the prompted pdb file (Sec. 3.2.1).

Fig. 12 refers to the storage of the information on the DNA chemistry ($3' \rightarrow 5'$) and the MNA (Morpholino) chemistry ($3' \rightarrow 6'$). This is why it is important to be consistent with the nomenclature of the atoms in the molecule and the order in which they appear in the list.
This functionality is what gives Ducque its power, by being able to build with any type of backbone! Ducque will query its repository, based on the user's input values. If these do not align with each other, then Ducque will not be able to build the desired structure, so be precise with your inputs.

4.1.4 Adding transmutation specifications

This section only exists to add specifications to the json file that gets produced. The idea is that some additional information can go a long way, especially when considering future expansions of Ducque. This data is not necessary to build structure with, but is required to correctly transmute to a json file. This concerns the full nucleotide chemistry. Linker partners are annotated in Sec. 4.1.6.

```
# GO TO +- line 150
# ----------------------------------------
#       CHEMISTRY REPOSITORY
# ----------------------------------------
TABLE_CHEMISTRY = {
"DNA" : "Deoxyribonucleic acid",
"RNA" : "Ribonucleic acid",
"MNA" : "Morpholino Nucleic Acid",
#                           ...
"FOO" : "Foobar Nucleic Acid",
}
```

Figure 13: Couple a key-value pair of the chemistry and its full name.

4.1.5 Adding the atoms of the linker to orient it correctly

In Fig.14, we assign the atoms by which the Ducque needs to position to linker moiety with consideration to its position for the adjacent nucleic acids.

A.

```
# GO TO +- line 140
# ----------------------------------------
#       LINKER BACKBONE  REPOSITORY
# ----------------------------------------
TABLE_LINKER_BACKBONE = {
"PHOSPHATE" : ["P", "OP1", "OP2"],
"THIOPHOSPHATE" : ["P", "SP1", "OP2"],
#                               ...
"BAR" : ["X", "Y", "Z"],
}
```

B.

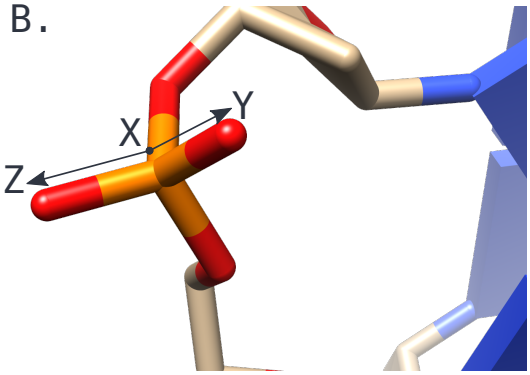


Figure 14: The dictionary that is used by Ducque to position the linker correctly to the nucleoside it is being appended to. The phosphate linker is used as an example.

The reason the user needs only to assign three atoms, is because the linker moiety can be abstracted to a plane (formed by three points). This means the linker only requires three assigned atoms to be positioned. The atoms prompted are of course correlated with the angle (Sec. 3.3.5) and dihedrals (Sec. 3.3.6) used to transmute to linker into a suitable json inputfile (Sec. 3.3).

4.1.6 Adding a nucleoside-linker pair explicitly

This final TABLE is the last step needed when implementing a new chemistry. The names correlate with the chemistry type annotated by the user (Sec. 3.2.2 for nucleosides, Sec. 3.3.2 for linkers).

```
# GO TO +- line 168
# ----------------------------------------
#           LINKER REPOSITORY
# ----------------------------------------
TABLE_LINKER = {
"DNA" : "PHOSPHATE",
"MNA" : "PHOSPHATE",
"S-1DNA" : "THIOPHOSPHATE",
"S-2DNA" : "THIOPHOSPHATE",
#                ...
"FOO" : "BAR",
}
```

Figure 15: Couple a key-value pair of the chemistry of the nucleoside-linker pair.

4.2 Defining the correct torsion angle for the given nucleoside

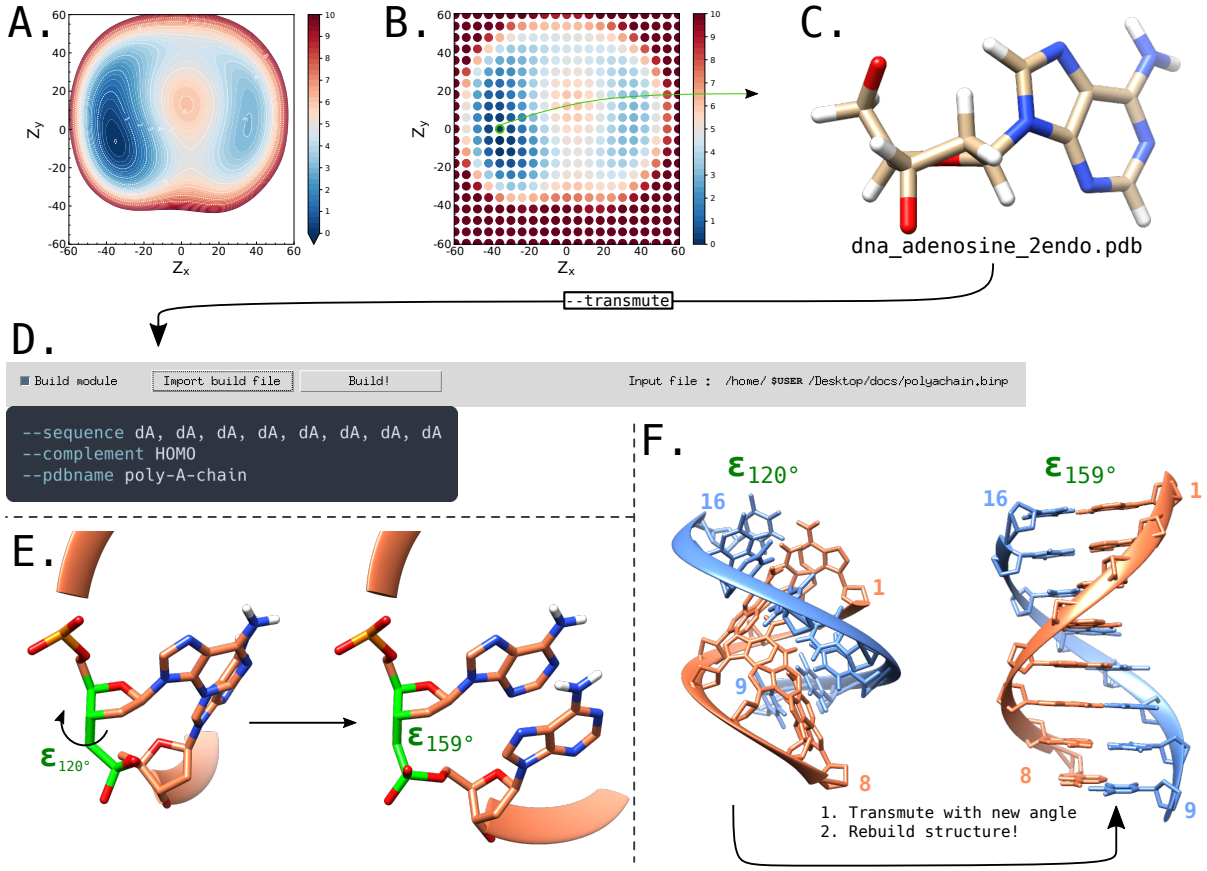


Figure 16: **A.** Potential Energy Surface (PES) of DNA Adenosine. **B.** A scatterplot that used to create the PES, by interpolating gridpoints. **C.** Uncapped DNA Adenosine, ready for conversion to json file. **D.** The build module, embedded into the transmute module. This makes for easy access to building new structures after modifying angle values. **E.** Closer look to how one can iteratively optimise backbone angle values. The complementary strand is not shown here to reduce visual noise. **F.** Leading strand in coral, complementary strand in cornflower. This exemplifies how the differences in backbone angle values can have a dramatic change on the resulting structure.

Fig. 16C. The user can curate the Conformational Sampling[5] landscape for a specific conformation to be built into Ducque's repository. Here is a depiction of the DNA Adenosine nucleoside ($^{2'}$E), prepaped as an uncapped nucleoside, ready to be transmuted by Ducque.
In Fig. 16D., the embedded build module is shown, which is a callback Sec.3.6.2-5. Here, the user loads in a finished binp to the Import build file button. Whenever the user makes changes to the produced json library file, upon transmuting, the user just has to click the Build! button to produce a new structure, which will build a structure with the recently modified backbone angle values. This makes it possible for the user to iteratively adjust and build structures without having to leave the transmute GUI.

The author suggest the following protocol, as devising the correct backbone dihedrals can be quite tricky when trying out for the first time.

- It is advisable that, whenever curating conformers from the landscape (Fig.16B.), that the same conformer is picked for all nucleobase types. It has been noticed that purines and

pyrimidines tend to have similar backbone angle values respectively. This makes the model building simpler; the goal to a good structure is building a model that is fit for upcoming simulations.
Additionally, it is mandated to flatten the nucleobase and glycosidic improper torsion. by means of geometry optimisation at a very cheap level (i.e. HF-3c). Optimising at this level will cause the substituents to lie in the plane of the nucleobase. The torsion, however, requires to be restraint at an angle of (-179.5°).
For example: For Adenosine, this would be the set [C1′, C8, N9, C4].

- As shown in Fig.16E., the structure can start from a starting value, here arbitrarily chosen to be $\epsilon_{120°}$. By opening your preferred Molecular Viewing Software (UCSF Chimera, PyMol ...), one can select a dihedral and rotate around it, up to the point that the basepairs stack well with eachother. Emulating, or at least trying to emulate, stacking conditions is a good indicator of going into the right direction.

- The leading strand is entirely dependent on the two following items :

  1. The conformation of the nucleoside prompted by the user. This is the most obvious one, but is mentioned nevertheless. The specific conformation the user employs in the model building will be of key importance in how the duplex turns out. This also means the geometry of the backbone of that conformer is important.
  2. The leading strand is built according to the β angle and the last two angles of the backbone, not accounting for the glycosidic bond angle.
     For example: DNA has will be built along the highlighted angles : [α, β, γ, δ, ϵ, ζ, χ].

  → This means that all other values angles, except for α, are already defined with the prompted conformation. They are required to be filled in by the user, when transmuting, for the sake of completeness!

- The α and ζ are more important for the complementary strand's associated fitting, but nucleobase positioning is the prime contributor for the complementary strand build process. This is why it is very important to keep nomenclature consistent in the nucleobases,[3] as basepairing rests on the shoulders of correctly named atoms to infer basepairs!

- Another suggestion is to use poly-N sequences to build the structure. This will make it far easier to modify angles of one type of nucleoside at one time.
  NB: in order to build a poly-N structure, the complement of N needs to be implemented too! Though the complement does not need to have its angles optimised just yet, as basepairing conditions are the driving factor in fitting the complementary strand when building the molecular structure.

References

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