



# CPRISMA

COLORING PROTEINS BY INPUTS AND  
SETS OF MULTIPLE ALIGNMENTS

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# CPRISMA USER'S GUIDE

Version 1.0

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Contributions from

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## DESCRIPTION

CPRISMA is a bioinformatics program that gives color to multiple sequence alignment based on an input of numerical data.

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# 1 Introduction

CPRISMA (**C**oloring **P**Roteins by **I**nputs and **S**ets of **M**ultiple **A**lignments) is a command-line program designed to give color to protein multiple sequence alignments. CPRISMA was written in Python language and runs on Unix systems. Since we often want to compare sets of numerical protein variables at the primary structural level, CPRISMA is an ideal program to visualize and/or detect possible differences in specific amino acids or protein regions through a color palette. CPRISMA is a versatile tool that allows the user to incorporate the features that are more convenient to highlight.

The development of this program was inspired by previous studies where we sought to highlight regions for the non-structural (NS) protein 1 from several *Flavivirus* with particular physical-chemistry or structural behavior [1, 2, 3]. We thought it would be interesting to have a capable tool to make relationships between numerical information and sequence alignments, but for any external data set.

In this documentation, we have divided the Chapters following a logical order and using practical examples to understand each CPRISMA parameter. In Chapter 2, the installation/uninstallation and the type of input and output (I/O) files needed to run a simulation in CPRISMA will be treated. Chapter 3 will describe the basic functions, and Chapters 4, 5, 6, and 7 more complex variables. These six Chapters, we used as examples the NS1 proteins from Zika virus (ZIKV), Dengue variant 2 (DENV2), and West Nile viruses (WNV). Regarding ZIKV, we employ two strains from Uganda (UG) and Brazil (BR). Finally, in Chapter 8, we show three practical examples that correlate directly with three publications where we explored *i*) p*K<sub>a</sub>* shifts for several NS1<sub>ZIKV</sub> [1], *ii*) B-cell epitope prediction for NS1<sub>WNV(176–352)</sub> [2], and *iii*) the structural domains and biological interfaces for two ZIKV NS1 proteins [3].

Command lines, alert messages, or variables will be distinguished with the **Typewriter** font. Also, when a particular word/letter is preceded by a hyphen (-) in any title of this documentation it should be interpreted as a line command, otherwise, it should be interpreted



as a variable.

The current developer of CPRISMA is Sergio Alejandro Poveda Cuevas at the [Laboratory of Computational Biophysical Chemistry](#) (University of São Paulo). If you have any suggestions, bug reports, or general comments about CPRISMA, please contact us at [seapovedac@gmail.com](mailto:seapovedac@gmail.com).



## 2 Setup

### 2.1 Installation/unistallation

To install CPRISMA you must directly download the code from the GitHub repositories <https://github.com/seapovedac/cprisma/> or by executing the following command:

```
git clone https://github.com/seapovedac/cprisma.git
```

Then, you can invoke in that directory:

```
pip install .
```

Now your program should be in the OS environment variables. Typing in the terminal:

```
cprisma1
```

or

```
python -m cprisma
```

should return an error, requesting one of the input files for execution. If you want to be sure that there are no problems with the program, you can try to generate outputs from the “examples” directory.

To uninstall the program you can simply do it through the following command:

```
pip uninstall cprisma
```

### 2.2 Inputs

To run CPRISMA, a basic knowledge of Python is required, so a good understanding of the data types that this language uses (*e.g.* sets, tuples, lists, etc.) is essential to achieve the

---

<sup>1</sup>We will use this command throughout the documentation.



desired results<sup>2</sup>.

Hereafter, we will use as an example a data set of  $pK_a$ 's<sup>3</sup> for four NS1 from ZIKV (two different strains), DENV2, and WNV. Protein sequences were extracted from the NCBI (National Center for Biotechnology Information)<sup>4</sup>, and have the next accession codes: AY632535 (ZIKV-UG), KU365777 (ZIKV-BR), PR159-S1 (DENV2), and Q9Q6P4 (WNV). We will only take into account incomplete sequences (from residues 1 to 120) and the focus will be given to the titratable residues: Asp, Glu, Arg, His, Lys, and Tyr. Cys residues are not included, due to the fact that they are part of disulfide bridges.

<sup>5</sup>CPRISMA is a program that requires at least three file kinds in the run directory<sup>5</sup>:

1. The alignment to put the color called “alignment.dat”.
  2. A CSV file called “data\_input.csv”.
  3. A Python script called “array\_get.py”.

|         |                                                                       |
|---------|-----------------------------------------------------------------------|
| ZIKV-UG | DVGCSVDFSKKETRCGTVFIYNDVEAWRDRKYHPDSPRLLAAVKQAAWEEGICGISSV            |
| ZIKV-BR | DVGCSVDFSKKETRCGTVFVYNDVEAWRDRKYHPDSPRLLAAVKQAAWEDGICGISSV            |
| DENV2   | DSCGVVSWKNKELKCGSGIFITDNVHTWEQYKFQPESPSSLASAIQKAHEEGICGISSV           |
| WNV     | DTGCAIDISRQLRCGSGVFHINDVEAWMDRYKYYPTPQGLAKIIQKAHEGVCGLRSV             |
|         | * * * . . . * . * : * : * . * : * . * : * . * : * . * : * . * : *     |
| ZIKV-UG | SRMENIMWKSVEGELNAILEENGVQLTVVVGSVKNPWMWRGPQRLLPVVNELPHGWKAWGK         |
| ZIKV-BR | SRMENIMWRSVEGELNAILEENGVQLTVVVGSVKNPWMWRGPQRLLPVVNELPHGWKAWGK         |
| DENV2   | TRLENLMWKQTIPELNHLISENEVKLTIMTGDIGKIMQAGKRSLRPQPTELKSYWSKTWGK         |
| WNV     | SRLHQMWAVEAVKDELNTLKLKGENDVLSVVVEKQEGMYKSAKRLTATTEKLEIGWKAWGK         |
|         | * : * : * . * . * : * . * : * . * : * . * : * . * : * . * : * . * : * |

Figure 2.2.1: MUSCLE output in ClustalW format.

In addition to these files, others directories will appear (*e.g.*, “cprisma”, “examples”, so on), which contain all the scripts and other stuff necessary for the program. For now, let us focus on the three files mentioned above. To get the alignment data, you can directly go to the website program called MUSCLE (MULTiple Sequence Comparison by Log-Expectation)<sup>6</sup> and run it with the FASTA format protein sequences of your interest. You can modify the input parameters as wanted, nevertheless, it is essential that you keep ClustalW as the output format. You can also generate these multiple sequence alignments by invoking MUSCLE

---

<sup>2</sup>See also the official Python documentation: <https://docs.python.org/3.7/>.

<sup>3</sup>pK<sub>a</sub>'s were obtained through charges of ionizable residues calculated by Monte Carlo simulations [4, 5].

<sup>4</sup> See <https://www.ncbi.nlm.nih.gov/>.

<sup>5</sup>These files can be found in the directory “examples/Example\_0”.

<sup>6</sup>See <https://www.ebi.ac.uk/Tools/msa/muscle/>.



through Biopython<sup>7</sup>. A typical MUSCLE result appears in Figure 2.2.1. All the details about this type of output is previously described [6]. An improper alignment format can produce the following output:

The program stop! Improper alignment format. Check ClustalW format at  
<https://www.ebi.ac.uk/Tools/msa/muscle>.

|    | ZIKV-UG |          | ZIKV-BR |          | DENV2 |          | WNV |          |
|----|---------|----------|---------|----------|-------|----------|-----|----------|
|    | A       | B        | C       | D        | E     | F        | G   | H        |
| 1  |         |          |         |          |       |          |     |          |
| 2  | ASP     | 3.9      | ASP     | 3.9      | ASP   | 3.8899   | ASP | 3.7834   |
| 3  | ASP     | 3.09737  | ASP     | 3.38668  | LYS   | 10.48393 | ASP | 3.39189  |
| 4  | LYS     | 10.89431 | LYS     | 10.99729 | LYS   | 10.58842 | ARG | 12.78243 |
| 5  | LYS     | 10.68626 | LYS     | 10.69875 | GLU   | 3.79962  | GLU | 3.99138  |
| 6  | GLU     | 3.77967  | GLU     | 3.98917  | LYS   | 10.67679 | ARG | 12.39385 |
| 7  | ARG     | 12.58017 | ARG     | 12.2972  | ASP   | 3.39033  | HIS | 6.69314  |
| 8  | TYR     | 9.97794  | TYR     | 9.98935  | HIS   | 6.77441  | ASP | 3.58575  |
| 9  | ASP     | 3.49226  | ASP     | 3.79508  | GLU   | 4.08867  | GLU | 4.38775  |
| 10 | GLU     | 3.99375  | GLU     | 4.09717  | TYR   | 9.98403  | ASP | 3.48066  |
| 11 | ARG     | 12.5932  | ARG     | 13.38161 | LYS   | 10.9964  | ARG | 12.89923 |
| 12 | ASP     | 3.09964  | ASP     | 2.68828  | GLU   | 3.99943  | TYR | 9.79223  |
| 13 | ARG     | 13.28718 | ARG     | 12.97763 | LYS   | 11.08543 | LYS | 11.18316 |
| 14 | TYR     | 9.59769  | TYR     | 9.59592  | LYS   | 10.88321 | TYR | 10.19568 |
| 15 | LYS     | 11.08461 | LYS     | 10.99773 | HIS   | 6.3864   | TYR | 9.49347  |

Figure 2.2.2: Data input with  $pK_a$  values for four proteins. All the information has not been shown.

Our second type of file with CSV extension is the numerical data that will be used as a basis for defining the color. Figure 2.2.2 shows an example of how this file should be. We are working with residues up to position 120, however, Figure 2.2.2 does not show all the charged amino acids up to that position. Each system is divided into 2 columns where the information on the residue kind (with code of three letters in capital) and the numerical value (in our case  $pK_a$ ) will be contained.

*Note: If, for example, you eliminated the  $pK_a$  value of 2.68828 from row 12 and column D, CPRISMA will consider the absence of it as equal to 0.*

<sup>7</sup>For more details: <https://biopython.org/docs/1.75/api/Bio.Align.Applications.html>.



---

```
def array_get():
    # Tuple of residues
    target_residues = ()

    # Tuple of sequences name
    name_sequence = ()

    # Operations
    descriptor_ope = { 'n' }

    # Color
    descriptor_col = { 'nc' }

    # Visualization
    descriptor_vis = { 'ReY', 'DeN', 'LeY' }

    # Maximum restriction
    descriptor_mx = { 'nr' }

    # Array for comparisons
    dict_ref = {}

    # Array for operations
    dict_ope = {}

    # Array for color
    dict_col = {}

    # Array for visualization
    dict_vis = {}

    # Array for maximum restriction
    dict_mx = {}

    return target_residues, name_sequence, descriptor_ope, descriptor_col, descriptor_vis, descriptor_mx, dict_ref, dict_ope, dict_col, dict_vis, dict_mx
```

Figure 2.2.3: Example of how looks like the Python script “array\_get.py”.

Notice that there are 4 data sets for the 4 systems. In our example, the absence of any 8 columns in the CSV file can return the following error message:

The program stop! Number of columns are not complete in data\_input.csv.

On the other hand, the order as each protein appears in the alignment should match with the numerical data of the CSV file. For instance, the first dataset (highlighted by brackets) is related to the first protein (labeled as “ZIKV-UG”) in the alignment sequence and so on.

*Note: The program does not have the ability to distinguish which data set each sequence belongs to, so it is your responsibility to put this information in the correct order.*

Finally, it is crucial that the first row is empty, as indicated by the arrow in Figure 2.2.2.

*Note: Sometimes the numerical data length will not be the same between the systems, however, you can ignore this as long as there is a match regarding the order of appearance between the alignment and numerical data files.*

The third type of file is a Python script with the variables that will be used to give different instructions to the program and obtain the desired output (Figure 2.2.3)<sup>8</sup>.

---

<sup>8</sup>These variables and how they work will be better described in next Chapters.



---

## 2.3 Outputs

The two types of output files that CPRISMA generates are a log file called “cprisma.log” which shows relevant data from the comparisons that are made or data directly related to the input parameters, and a HTML file called “alignment.html” where the colored alignment will appear. As we will see later, due to the various features that CPRISMA has, it is better to observe with some examples how these two files work. Besides, it is worth mentioning that all the warning messages or error reports will appear in the log file. Finally, you can generate other types of files with specific commands (see Section [5.14](#)).



### 3 Basic commands

Our starting point will be the files located in "examples/Example\_0". To run CPRISMA, you can simply type `cprisma` in the terminal (at the working directory). Based on the files previously created (*i.e.*, "alignment.dat", "data\_input.csv", and "array\_get.py"), something you will notice is that a new log file called "cprisma.log" has been generated in your directory. In this file will appear a count of all the amino acids per system registered from "alignment.dat" (Figure 3.0.1). On the other hand, a count of total charged or hydrophobic residues will also show right away (Figure 3.0.2).

|         |  | --- Number of residues in alignment --- |   |   |   |   |   |    |    |   |    |    |    |   |   |   |    |   |   |   |    |   |
|---------|--|-----------------------------------------|---|---|---|---|---|----|----|---|----|----|----|---|---|---|----|---|---|---|----|---|
|         |  | A                                       | R | N | D | C | Q | E  | G  | H | I  | L  | K  | M | F | P | S  | T | W | Y | V  | Z |
| ZIKV-UG |  | 7                                       | 8 | 6 | 5 | 3 | 3 | 10 | 11 | 2 | 5  | 6  | 8  | 3 | 2 | 7 | 8  | 3 | 6 | 3 | 14 | 0 |
| ZIKV-BR |  | 7                                       | 9 | 6 | 6 | 3 | 3 | 9  | 11 | 2 | 4  | 6  | 7  | 3 | 2 | 7 | 8  | 3 | 6 | 3 | 15 | 0 |
| DENV2   |  | 4                                       | 4 | 5 | 3 | 3 | 6 | 10 | 9  | 3 | 10 | 9  | 13 | 3 | 2 | 5 | 10 | 9 | 5 | 2 | 5  | 0 |
| WNV     |  | 8                                       | 6 | 3 | 6 | 3 | 5 | 12 | 10 | 3 | 6  | 10 | 12 | 3 | 1 | 3 | 6  | 6 | 4 | 4 | 9  | 0 |

Figure 3.0.1: Amino acid count in the log file. Note that the last column is showing the residue called "Z", this is only a way to identify those residues that do not belong to the 20 essential amino acids.

```
--- Kind of residues in alignment ---  
ZIKV-UG charged=39 ['D', 'E', 'R', 'H', 'K', 'Y', 'C'], hydrophobic=50 ['A', 'I', 'L', 'M', 'F', 'P', 'W', 'V']  
ZIKV-BR charged=39 ['D', 'E', 'R', 'H', 'K', 'Y', 'C'], hydrophobic=50 ['A', 'I', 'L', 'M', 'F', 'P', 'W', 'V']  
DENV2 charged=38 ['D', 'E', 'R', 'H', 'K', 'Y', 'C'], hydrophobic=43 ['A', 'I', 'L', 'M', 'F', 'P', 'W', 'V']  
WNV charged=46 ['D', 'E', 'R', 'H', 'K', 'Y', 'C'], hydrophobic=44 ['A', 'I', 'L', 'M', 'F', 'P', 'W', 'V']  
Number of columns in data_input.csv is ok!
```

Figure 3.0.2: Kind of residues count in the log file.

*Note: For our example, in the output shown in Figure 3.0.2 it was considered the Cys residues as charged, and this should not be. Nonetheless, CPRISMA by default counts all charged amino acids without discriminating whether or not Cys' belong to disulfide bridges.*

You can see that the log file will report whether the number of columns in the CSV file is



---

equivalent to the number of systems in the alignment file (see the last text in Figure 3.0.2).

Other additional information that appears in the log file will be the numerical data of the CSV file (Figure 3.0.3). Although it may be redundant information because you know about that in advance, what is sought with this is to propose the user a double-check of the numerical data to avoid future problems.

```
--- Information in data_input.csv ---
```

| ZIKV-UG | ZIKV-UG_dat | ZIKV-BR | ZIKV-BR_dat | DENV2 | DENV2_dat | WNV | WNV_dat  |
|---------|-------------|---------|-------------|-------|-----------|-----|----------|
| ASP     | 3.90000     | ASP     | 3.90000     | ASP   | 3.88990   | ASP | 3.78340  |
| ASP     | 3.09737     | ASP     | 3.38668     | LYS   | 10.48393  | ASP | 3.39189  |
| LYS     | 10.89431    | LYS     | 10.99729    | LYS   | 10.58842  | ARG | 12.78243 |
| LYS     | 10.68626    | LYS     | 10.69875    | GLU   | 3.79962   | GLU | 3.99138  |
| GLU     | 3.77967     | GLU     | 3.98917     | LYS   | 10.67679  | ARG | 12.39385 |
| ARG     | 12.58017    | ARG     | 12.29720    | ASP   | 3.39033   | HIS | 6.69314  |
| TYR     | 9.97794     | TYR     | 9.98935     | HIS   | 6.77441   | ASP | 3.58575  |
| ASP     | 3.49226     | ASP     | 3.79508     | GLU   | 4.08867   | GLU | 4.38775  |
| GLU     | 3.99375     | GLU     | 4.09717     | TYR   | 9.98403   | ASP | 3.48066  |
| ARG     | 12.59320    | ARG     | 13.38161    | LYS   | 10.99640  | ARG | 12.89923 |
| ASP     | 3.09964     | ASP     | 2.68828     | GLU   | 3.99943   | TYR | 9.79223  |
| ARG     | 13.28718    | ARG     | 12.97763    | LYS   | 11.08543  | LYS | 11.18316 |
| TYR     | 9.59769     | TYR     | 9.59592     | LYS   | 10.88321  | TYR | 10.19568 |
| LYS     | 11.08461    | LYS     | 10.99773    | HIS   | 6.38640   | TYR | 9.49347  |
| TYR     | 9.99884     | TYR     | 9.68700     | GLU   | 3.78878   | GLU | 3.99996  |
| HIS     | 6.27657     | HIS     | 6.19907     | GLU   | 3.48118   | LYS | 10.69714 |

Figure 3.0.3: Numerical data in the log file. All the information has not been shown.

Assuming both the alignment and the numerical data match, the directory would be expected to have another new file called “alignment.html”, which contains the multiple sequence alignment with color. For our example, we will not get this file, instead, it displays the following message:

```
The program stop! Number of target-residues [ZIKV-UG] is not the same in the  
input files. Residue A: 0 (data_input.csv) != 7 (alignment.dat).
```

This error message is telling us that the number of Ala/Alanine residues (or A) for ZIKV-UG is not the same in both the alignment and the numerical data files. This error can be corrected by applying a specific command line, but, we must know the different possible parameters that can be used through CPRISMA at first. Executing:



---

```
cprisma -h
```

... the following optional arguments will be displayed:

|                     |                                                                                               |
|---------------------|-----------------------------------------------------------------------------------------------|
| <b>-h --help</b>    | show this help message and exit                                                               |
| <b>-v --version</b> | show program's version number and exit                                                        |
| <b>-ns [bool]</b>   | rename the sequences based on a tuple                                                         |
| <b>-va [int]</b>    | method to visualize the alignment                                                             |
| <b>-j [bool]</b>    | join sequences (only available for <b>va = 2</b> )                                            |
| <b>-hc [bool]</b>   | hide the conservation line                                                                    |
| <b>-n [int]</b>     | change the first and subsequent numbers in the alignment                                      |
| <b>-l [int]</b>     | number of residues per line                                                                   |
| <b>-tr [bool]</b>   | get a tuple of target-residues                                                                |
| <b>-a [int]</b>     | define the accuracy of the input data                                                         |
| <b>-t [bool]</b>    | get all information for the different comparisons in log file                                 |
| <b>-ck [bool]</b>   | check reference method                                                                        |
| <b>-rf [str]</b>    | method to compare sequences ('default', 'pair', 'multiple')                                   |
| <b>-lco [bool]</b>  | display a list of the available colors                                                        |
| <b>-ico [int]</b>   | multiply the intensity of the color                                                           |
| <b>-sco [int]</b>   | sequence color (when 'ssc' descriptor is applied)                                             |
| <b>-mco [int]</b>   | mutation color (when 'fmac' or 'pimc' descriptors are applied)                                |
| <b>-tco [bool]</b>  | display color at the level of 3D structure through pymol script                               |
| <b>-dop [bool]</b>  | get a operation descriptors dictionary (only available for <b>rf = 'multiple'</b> )           |
| <b>-dco [bool]</b>  | get a color descriptors dictionary (only available for <b>rf = 'multiple'</b> )               |
| <b>-dvi [bool]</b>  | get a visualization descriptors dictionary (only available for <b>rf = 'multiple'</b> )       |
| <b>-dmx [bool]</b>  | get a maximum restriction descriptors dictionary (only available for <b>rf = 'multiple'</b> ) |

Above you can see the possible flags implemented in CPRISMA. Note that the Python variable type is appearing inside of square brackets, and a short description is displayed as well.

Next, a description of them will be given in the next Sections and Chapters, bringing practical examples to better understand how they work. Some flags should be parsed in parallel with the "array\_get.py" script to avoid future misinterpretations and others may be interdependent.

The positional arguments **-h** (**--help**) and **-v** (**--version**) are the default commands that



---

will display the possible flags that can be written in the terminal and the version of the program, respectively. As these two commands are by default and self-explicit, in the following sections we will just focus on the CPRISMA parameters.

### 3.1 Target-residues (-tr)

The problem with the residue A of ZIKV-UG shown above is caused by the absence of numerical data for that amino acid. A possible solution could be to include in the CSV file, new rows named “ALA” followed by a column with  $pK_a$  values equal to 0 and respecting the order in which they appear for each protein.

*Note: Here we assume  $pK_a = 0$  as equivalent to no color, but this may vary depending on the type of data.*

But this process can become time-consuming because the  $pK_a$  values for residues of Val, Ser, Trp, and so on, would also have to be included in the CSV file. A more efficient way to exclude residues that are not needed is to run the `-tr` command. However, when running it you may find the following problem:

`The program stop! The variable to check the target-residues is True, but the tuple is equal to 0.`

The flag `-tr` is a Boolean command, which means that by default it is “False”, but when you invoke it becomes “True”. `-tr` calls the `target_residues` variable in the script “array\_get.py” (Figure 2.2.3). This one is a *tuple*<sup>1</sup> that must contain residues in one-letter format. When `-tr` is executed, the correlation is made between the CSV file and the residues reported in the tuple. So you should try to use the same types of amino acids that appear in the numerical data file to build the tuple. Common errors that can occur in the construction of the tuple are listed below:

1. An empty tuple will return an error like the one we see above.
2. Do not put all the kinds of residues that appear in the CSV file in the tuple.
3. To include a residue in the tuple that is not in the CSV file.

For our example, the tuple should be as follows:

---

<sup>1</sup>See <https://docs.python.org/3.7/tutorial/datastructures.html#tuples-and-sequences>.



---

```
target_residues = ('D', 'E', 'R', 'H', 'K', 'Y')
```

If we again run the program with the next command line:

```
cprisma -tr
```

... our log file will successfully return the following message:

```
Target-residues ('D', 'E', 'R', 'H', 'K', 'Y') match in alignment.dat and  
data_input.csv.
```

*Note: It is important to mention that if you have the numerical values of all residues that appear in a specific multiple sequence alignment, it is not necessary to explicitly define a tuple with the 20 essential amino acids in the script “array\_get.py” because the program already defines those 20 residues by default (see the practical example of Section 8.3).*

## 3.2 Accuracy (-a)

Sometimes the color may not have enough intensity depending on the numerical data employed. This happens due to the limits that the HTML color code itself may have or because the limits of the human vision. To improve the color intensity to highlight the regions that matter most to us in the sequence alignment is necessary to make additional adjustments.

In this section, let us focus on the accuracy of the data, which is changeable by means of **-a** command<sup>2</sup>. In Figure 3.0.3, it is observed that the precision of the data goes up to the fifth decimal place. As shown in Figure 3.2.1, after program processing one decimal per value will be displayed (which corresponds to the default condition). When two or more decimals are considered, it is necessary imperative to invoke the command **-a** followed by a positive integer number which refers to the decimal places, as follows:

```
cprisma -tr -a 3
```

In our example, we are ordering the program to only consider taking into account three decimal places. Remember that this command will only affect the possible values obtained from operations, and then, the color applied when a gradient is executed (see Chapters 4, 5,

---

<sup>2</sup>This is one of the possible methods to improve the color intensity. Other methods can also be applied (see Section 5.15 and Chapter 7).



---

and 6, respectively). Nevertheless, some information in the log file will not be affected by this parameter, like the statistical data reported there (Figure 3.2.2).

| --- Data processed --- |         |             |         |             |       |           |     |         |  |
|------------------------|---------|-------------|---------|-------------|-------|-----------|-----|---------|--|
|                        | ZIKV-UG | ZIKV-UG_dat | ZIKV-BR | ZIKV-BR_dat | DENV2 | DENV2_dat | WNV | WNV_dat |  |
| 0                      | ASP     | 3.9         | ASP     | 3.9         | ASP   | 3.9       | ASP | 3.8     |  |
| 1                      | ASP     | 3.1         | ASP     | 3.4         | XXX   | 0.0       | ASP | 3.4     |  |
| 2                      | XXX     | 0.0         | XXX     | 0.0         | LYS   | 10.5      | XXX | 0.0     |  |
| 3                      | LYS     | 10.9        | LYS     | 11.0        | XXX   | 0.0       | ARG | 12.8    |  |
| 4                      | LYS     | 10.7        | LYS     | 10.7        | LYS   | 10.6      | XXX | 0.0     |  |
| 5                      | GLU     | 3.8         | GLU     | 4.0         | GLU   | 3.8       | GLU | 4.0     |  |
| 6                      | ARG     | 12.6        | ARG     | 12.3        | LYS   | 10.7      | ARG | 12.4    |  |
| 7                      | TYR     | 10.0        | TYR     | 10.0        | XXX   | 0.0       | HIS | 6.7     |  |
| 8                      | XXX     | 0.0         | XXX     | 0.0         | ASP   | 3.4       | XXX | 0.0     |  |
| 9                      | ASP     | 3.5         | ASP     | 3.8         | XXX   | 0.0       | ASP | 3.6     |  |
| 10                     | GLU     | 4.0         | GLU     | 4.1         | HIS   | 6.8       | GLU | 4.4     |  |
| 11                     | ARG     | 12.6        | ARG     | 13.4        | XXX   | 0.0       | XXX | 0.0     |  |
| 12                     | ASP     | 3.1         | ASP     | 2.7         | GLU   | 4.1       | ASP | 3.5     |  |
| 13                     | ARG     | 13.3        | ARG     | 13.0        | XXX   | 0.0       | ARG | 12.9    |  |
| 14                     | TYR     | 9.6         | TYR     | 9.6         | TYR   | 10.0      | TYR | 9.8     |  |
| 15                     | LYS     | 11.1        | LYS     | 11.0        | LYS   | 11.0      | LYS | 11.2    |  |
| 16                     | TYR     | 10.0        | TYR     | 9.7         | XXX   | 0.0       | TYR | 10.2    |  |
| 17                     | HIS     | 6.3         | HIS     | 6.2         | XXX   | 0.0       | TYR | 9.5     |  |

Figure 3.2.1: Numerical data processed in the log file. Note that some rows are named “XXX”, which indicates that a gap has been inserted. These gaps are directly related to the alignment and are necessary for the reading and coloring of the sequences later. Notice that there is an index starting from 0 that number each row. All the information has not been shown.

| --- Statistics --- |           |           |           |           |
|--------------------|-----------|-----------|-----------|-----------|
|                    | ZIKV-UG   | ZIKV-BR   | DENV2     | WNV       |
| count              | 36.000000 | 36.000000 | 35.000000 | 43.000000 |
| mean               | 8.027069  | 8.065460  | 7.998239  | 7.820189  |
| std                | 3.957723  | 4.020073  | 3.578109  | 3.732822  |
| min                | 3.097370  | 2.688280  | 3.390330  | 3.384640  |
| 25%                | 3.964313  | 3.990678  | 3.892795  | 3.993175  |
| 50%                | 9.787815  | 9.641460  | 9.984030  | 9.493470  |
| 75%                | 11.078850 | 11.322598 | 10.883975 | 11.034720 |
| max                | 13.392970 | 13.485050 | 13.184100 | 13.491570 |

Figure 3.2.2: Statistical data for each protein in the log file.



### 3.3 Alignment visualization (-va)

At this point, you will have noticed that the file “alignment.html” appears in your directory (Figure 3.3.1). Note that the file does not have any color still<sup>3</sup>. By default, CPRISMA will separate each protein sequence keeping the conservation line for each of them [*i.e.*, the line that contains dots (“.”, “?”), asterisks (“\*”) or spaces (“ ”)]. Now it seems not to have much relevance to observe our alignment without comparisons among sequences. Nevertheless, as described later this kind of visualization can be very useful to detect independent color behaviors from sets of numerical data.

Figure 3.3.1: Alignment processed through visualization method 1 without sequence comparison.

Another way to visualize our alignment can be calling method 2 through the `-va` command,

---

<sup>3</sup>This aspect will be covered in Chapter 5.



as follows:

cprisma -tr -va 2

An output using this method appears in Figure 3.3.2. Notice that each sequence is being labeled at the end with the word “comparison” followed by a number (see brackets in Figure 3.3.2). This means that CPRISMA is not comparing the sequences but only analyzing each protein separately, like in the previous method.

Note: We can apply these same visualization methods but comparing our sequences. To see this aspect in more detail it is recommended to check Sections 3.9 and 3.10.

Figure 3.3.2: Alignment processed through visualization method 2 without sequence comparison.

### 3.4 Join (-j)

The `-j` command is a complement for the visualization method 2. With this command the space between the comparisons is reduced (compare Figures 3.3.2 and Figure 3.7.1). To get an alignment without spaces, you can invoke the following command:

cprisma -tr -va 2 -j



---

Practical examples using this flag appear in Chapter 8.

### 3.5 Numbering (-n)

To change the first and subsequent numbers that appear at the top of the alignment, the command `-n` can be executed followed by a positive integer number corresponding to the desired position. For instance, we can assume that our first number is 101, so:

```
cprisma -tr -va 2 -j -n 101
```

See the practical example of Section 8.2.

### 3.6 Number of residues per line (-l)

You can change the number of amino acids per line using the command `-l` followed by a positive integer number.

*Note: Remember that 80 residues per line will appear by default, so we recommend only use values greater than and multiples of 80 to have an optimal display of the numbering. You can use whatever amount of residues per line you prefer, but there may be times when the numbers will be cut off.*

Next, an example of a command-line for this feature appears:

```
cprisma -tr -va 2 -j -l 160
```

### 3.7 Name sequence (-ns)

Something that is useful when working with different sequences is renaming them with the command `-ns`. This command calls the variable `name_sequence` of the script “array\_get.py” (Figure 2.2.3) and makes a relationship between this variable (which is a tuple) and the number of proteins analyzed. Let us imagine that we build the following tuple:

```
name_sequence = ("Sequence 1", "Sequence 2", "Sequence 3")
```

Then, the next command-line is executed:

```
cprisma -tr -va 2 -j -ns
```



---

Since the number of “name sequences” is only 3 and the total of proteins is 4, CPRISMA will return the following error:

```
Variable check_name_seq = True but the sequences' number does not match with
tuple input ('Sequence 1', 'Sequence 2', 'Sequence 3')
```

Adding one more name to the tuple ('Sequence 1', 'Sequence 2', 'Sequence 3', 'Sequence 4'), the program will finally return the following message:

```
Variable check_name_seq = True, so the sequences' names have been changed.
```

Now you can see that both your alignment (Figure 3.7.1) and all the information in the log file have changed the sequences’ names.

```
1           20           40           60
Sequence 1 DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRYKYHPDSPRRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE comparison 1
Sequence 2 DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRYKYHPDSPRRLLAAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILE comparison 2
Sequence 3 DSGCVVSWKNKELKGCGSGIFITDNVHTWTEQYKQPESPSKLASAIQKAHEEGICGIRSVRTLENLMWKQITPELNHILS comparison 3
Sequence 4 DTGCAIDISRQELRGCGGVFIHNDVEAMDRYKYYPETPQGLAKIIQKAHKEGVCGLRSVSRLHQMWEAVKDELNTLLK comparison 4
      * *** : . . . : * . ** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * .
```

```
81           100          120
Sequence 1 ENGVQLTVVVGSKNPMWRGPQRLPVPVNELPHGWKAWGK comparison 1
Sequence 2 ENGVQLTVVVGSKNPMWRGPQRLPVPVNELPHGWKAWGK comparison 2
Sequence 3 ENEVKLTIMTGDIGKIMQAGKRSLRPQPTELKYSWKTWGK comparison 3
Sequence 4 ENGVDSLSSVEKQEGMYKSAPKRLLTATTEKLEIGWKAWGK comparison 4
      ** *.*::: . . . * : * .** :***
```

Figure 3.7.1: Alignment with renamed sequences.

### 3.8 Hide conservation line (-hc)

The `-hc` command hides the conservation line of the alignment (check the line with “\*”, “.”, “:” and spaces “ ” in Figure 3.7.1, for instance). `-hc` can be invoked following the command-line:

```
cprisma -tr -hc
```

Hiding the conservation line can be useful when we are using transcendental comparison methods (see Section 3.10).

See the practical example of Section 8.2 where `-hc` is employed.



---

### 3.9 Check reference method (-ck)

So far you have surely noticed the following message in your log file:

```
No comparison among the data.  
-- Set of comparisons: main_0 --  
    ZIKV-UG X no comparison  
-- Set of comparisons: main_1 --  
    ZIKV-BR X no comparison  
-- Set of comparisons: main_2 --  
    DENV2 X no comparison  
-- Set of comparisons: main_3 --  
    WNV X no comparison
```

For our example, the data of the protein systems are not being compared. But if you want to apply mathematical operations between your numerical data (see Chapter 4), it will be mandatory to compare the sequences. For instance, in our previous study, we wanted to compare the  $pK_a$ 's of ionizable residues among NS1's proteins from several ZIKV strains [1]. For that, it was calculated the difference of  $pK_a$ 's ( $\Delta pK_a$ ) among proteins and they were visualized with specific colors (see Section 5.10 and the practical example described in Section 8.1 as well).

Before doing any calculation, you must inform the program that it is desired to compare the data by means of the -ck flag. The command line to execute comparisons between the protein sequences appears below:

```
cprisma -tr -ck
```

Now, the following information will be found in the log file:

```
-- Set of comparisons: main_0 --  
    ZIKV-UG X ZIKV-BR DENV2 WNV
```



Note that now the sequences are being compared, so ZIKV-UG is the reference sequence, and ZIKV-BR, DENV2, and WNV are the target-sequences. Also, this comparison is being coupled to a group of comparisons called “`main_0`”. This is not the same as what was seen previously where different “`main's`” were generated. At the same time, you will have perceived that your HTML file (Figure 3.9.1) is different when compared to the previous outcomes (Figures 3.3.1 and 3.3.2). Technically, the alignment is the same as the input shown in Figure 2.2.1 but with a slightly different format.

Note: In Section 3.10, you will find more information on how to apply more transcendental comparisons.

|         |                                                                                             |     |    |
|---------|---------------------------------------------------------------------------------------------|-----|----|
| 1       | 20                                                                                          | 40  | 60 |
| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHDPSPRRLAAAVKQAWEEGICGISSVRMENIMWKSVEGELNAILE              |     |    |
| ZIKV-BR | DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRKYHDPSPRRLAAAVKQAWEDGICGISSVRMENIMWRSVEGELNAILE              |     |    |
| DENV2   | DSGCVVSWKNKELKGSGIFITDNVHTWTEQYKFQPESPSKLASAIQKAHEEGICGIRSVTRLENLMWKQTPELNHILS              |     |    |
| WNV     | DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYYPETPQGLAKIIQKAHKEGVCGRLSRVSRLEHQMWEAVKDELNTLLK           |     |    |
|         | * * * : . . . * . * : * : * : * . * : * : * : * : * : * : * : * : * : * : * : * : * : * :   |     |    |
| 81      | 100                                                                                         | 120 |    |
| ZIKV-UG | ENGVLQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWKAWGK                                                   |     |    |
| ZIKV-BR | ENGVLQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWKAWGK                                                   |     |    |
| DENV2   | ENEVKLTLIMTGDIGKIMQAGKRSLRPQPTELKYSWKTWGK                                                   |     |    |
| WNV     | ENGVDLSVVVEKQEGMYKSAPKRLTATTEKLEIGWKAWGK                                                    |     |    |
|         | * * . * : : . . : . . . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : |     |    |

Figure 3.9.1: Alignment processed through visualization method 1 with sequence comparison.

### 3.10 Reference method (-rf)

Whether -ck was executed, other additional information will appear in the log file:

Method used 'default' for the array: | main\_0 [{‘O’: [1, 2, 3]}] |

To understand what this means, a basic understanding of Python's data structure is necessary. Let us go a little back, just to correctly interpret these outcomes. Based on the previous message, note that you have one comparison that belongs to `main_0`. Figure 3.10.1 is showing an example of the CPRISMA default variable when `-rf` is invoked (pay attention to the Roman numerals). In Python, whenever one string type (II) and any type variables (VI) appear separated by a colon (V) inside brackets (I), it will be interpreted by the language as



---

a *dictionary*<sup>4</sup>. The numerals II and VI are also called *keys* and *values*, respectively. In our case, the key of our dictionary is the string ‘0’ and the value is a *list* of numbers from 1 to 3. Note that the lists have square brackets (III), which differentiates them from dictionaries<sup>5</sup>.

I    II    V       III           VI       III |  
↓    ↓    ↓       ↓            ↓        ↓ |  
{'0': [1, 2, 3]}

Figure 3.10.1: Dictionary created by CPRISMA. the meaning of Roman numerals is explained in the text.

Note: Based on the message cited at the beginning of this Section, see that your dictionary is also inside square brackets: `[{'0': [1, 2, 3]}]`<sup>6</sup>. In Subsection 3.10.3, we will explain in more detail the reason for that.

Please keep all these features in mind as they will be helpful in understanding the following sections. Furthermore, in some moments we will call the sets of comparisons as arrays.

To interpret the numbers in our dictionary (Figure 3.10.1), we simply have to relate the order of appearance for each sequence based on the multiple alignments. Thus, ‘0’ will be ZIKV-UG, ‘1’ will be ZIKV-BR, and so on.

There are three comparison methods used by CPRISMA: `default`, `pair`, and `multiple`, which are described below.

### 3.10.1 Default

The `default` method of CPRISMA compares the first sequence with the rest. You can explicitly invoke this method via the `-rf` flag followed by the word “`default`” like this:

```
cprisma -tr -ck -rf default
```

Note: Remember, due to this comparison method is the optional argument by `default` (as its name implies), CPRISMA will always return a dictionary with this structure when you run `-ck` (i.e., you do not need to type `-rf default`, explicitly)

---

<sup>4</sup>See <https://docs.python.org/3.7/tutorial/datastructures.html#dictionaries>.

<sup>5</sup>See <https://docs.python.org/3.7/tutorial/datastructures.html#more-on-lists>.

<sup>6</sup>We have changed the color to purple for some square brackets to distinguish easily each list.



### Comparison: 1

```
      1          20          40          60
ZIKV-UG DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRYKYHPDSPRLAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE
ZIKV-BR DVGCSVDFSKKETRCGTGVFYNDVEAWRDRYKYHPDSPRLAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILE
* * * : . .::* .**;*: ::* :* .::*; *;* ** ::*.*;*: **;*: *: ** : *** ;*.

      81         100         120
ZIKV-UG ENGVQLTVVVGSKNPMWRGPQR LPVPVNELPHGKAWGK
ZIKV-BR ENGVQLTVVVGSKNPMWRGPQR LPVPVNELPHGKAWGK
*** *.*;:: . .: . . . * :* .**;***
```

### Comparison: 2

```
      1          20          40          60
DENV2 DSGCVVSWKNKELKGSGIFITDNVHTWTEQYKFQPESPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS
WNV DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYYPETPQGLAKIIQKAHKEGVGLRSVRLEHQMWAEAVKDELNTLLK
* * * : . .::* .**;*: ::* :* .::*; *;* ** ::*.*;*: **;*: *: ** : *** ;*.

      81         100         120
DENV2 ENEVKLTIMTGDIGIMQAGKRSLRQPTELKYSWKTWGK
WNV ENGVDSLSSVVEKQEGMYKSAPKRILTATTEKLEIGWKAWGK
*** *.*;:: . .: . . . * :* .**;***
```

Figure 3.10.2: Alignment processed with `pair` comparison method.

### 3.10.2 Pair

Another comparison method is called `pair`. To invoke it, we can type in terminal:

```
cprisma -tr -ck -rf pair
```

Now, our log file will contain the following information:

```
Method used 'pair' for the array: | main_0 [{‘0’: [1]}] | | main_2 [{‘2’: [3]}] |

-- Set of comparisons: main_0 --
ZIKV-UG X ZIKV-BR

-- Set of comparisons: main_2 --
DENV2 X WNV
```

Note that two arrays have now been created, which can be interpreted as two independent sets



---

of comparisons and they belong to the `main`'s: `main_0` and `main_2`. One where 0 (ZIKV-UG) and 1 (ZIKV-BR) sequences are the `referencecomparison1` and `targetcomparison1`, respectively; and another where 2 (DENV2) and 3 (WNV) sequences are the `referencecomparison2` and `targetcomparison2`, respectively. This will also be reflected in our HTML file (Figure 3.10.2)<sup>7</sup>.

It is important to mention that for CPRISMA to work correctly with the `pair` comparison method, the total number of sequences is needed to be even. Otherwise, it will return to the method for `default` with the following message:

Attention!!! Method selected by user is ‘pair’ but number of sequences (=3<sup>8</sup>) is not a even number. The method return to ‘default’.

### 3.10.3 Multiple

Among all the comparison methods, `multiple` can be the most complex to understand. With this method, many types of comparisons can be made between a set of sequences (*e.g.* see all the practical examples in Chapter 8). However, before invoking it you must have built a dictionary compatible with the CPRISMA format array. In the script “array\_get.py” a variable called `dict_ref` will appear (Figure 2.2.3). From this, you can build an array like this:

```
dict_ref = { 'main_0' : [ {'0':[1, 2, 3]}, {'0':[]} ] ,  
            'main_1' : [ {'1':[0, 2]}, {'1':[0, 2, 3]} ] ,  
            'main_2' : [ {'2':[0]} ] }
```

At first glance, this type of arrangement may seem tricky, but give us a chance to explain it to make it easier.

The CPRISMA convention is going to call the “external layer” of the array with the names: “first-key” (or “*main key*”) and “first-value” (or “*main value*”). Then, the first-keys will be all `main`'s typed in the array, *i.e.*, `main_0`, `main_1`, and `main_2`; and the first-values will be the lists: `[{'0': [1, 2, 3]}, {'0': []}]`, `[{'1': [0, 2]}, {'1': [0, 2, 3]}]`, and `[{'2': [0]}]`<sup>9</sup>. Each element of the list/first-value is equivalent to a comparison and they have a “second-key” (or “*subkey*”) and a “second-value” (or “*subvalue*”). The subkeys are the

---

<sup>7</sup>We suggest the user to also explore visualization method 2 (see Section 3.3).

<sup>8</sup>A data set of 3 proteins was used to compare them (only as an example).

<sup>9</sup>We have changed the color to purple for some square brackets to distinguish easily each list.



string numbers that represent the sequence references and the subvalues are the lists with the target proteins. Notice that each cluster of first-key/first-value pairs is separated by a comma and they are so-called like “set of comparisons”<sup>10</sup>. Besides, each comparison in the list/first-value is separated by a comma as well. Please, avoid confusing this! For the sake of convenience, in Figure 3.10.3, we have made a sketch for `main_0`, where you can follow each characteristic of the CPRISMA array.

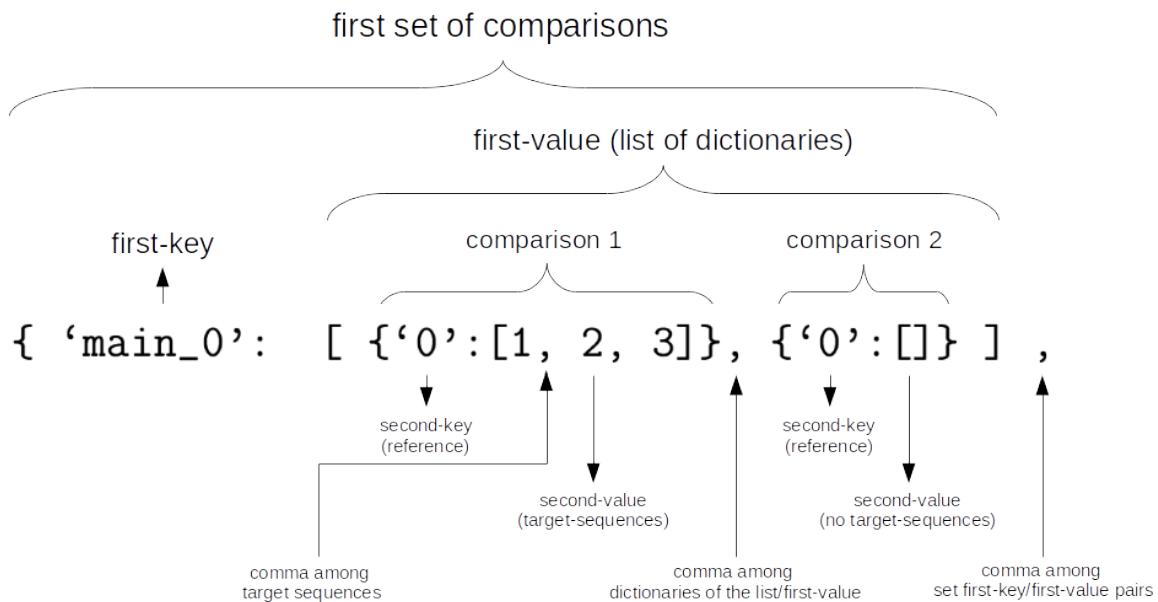


Figure 3.10.3: Example of the structure of the CPRISMA array when is used `multiple` method.

Maybe, you can also perceive that each `main` has the same reference number of a particular set of comparisons. For example, `main_1` has comparisons 3 and 4 which are `{'1': [0, 2]}` and `{'1': [0, 2, 3]}`, respectively, so the reference sequences ‘1’ match with the number of the `main` (*i.e.*, `main_1`). The same goes for `main_0` and `main_2`. However, it should be noted that you can use whatever name you prefer for each “`main`”, to better identify your comparisons. Just make sure they are different from each other. For practical purposes, we will use the standard name as already seen in the previous and following examples.

*Note: Check that comparison 2 has a list with no elements or empty: `{'0': []}`. CPRISMA*

<sup>10</sup>Identify this same structure when using the `default` and `pair` methods.



will interpret this dictionary as “without comparison” (or without target-sequences).

| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE   |  |  | comparison 1 |
|---------|-----------------------------------------------------------------------------------|--|--|--------------|
| ZIKV-BR | DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRKYHPDSPRLLAAAVKQAWEDGICGISSVSRMENIMWKSVEGELNAILE   |  |  |              |
| DENV2   | DSGCVVSWKNKELKGCGSIFICITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS |  |  |              |
| WNV     | DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYYPETPQGLAKIIQKAHEVGCLRSVSRLEHQMWAEVKDELNTLLK    |  |  |              |
| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE   |  |  | comparison 2 |
| ZIKV-BR | DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRKYHPDSPRLLAAAVKQAWEDGICGISSVSRMENIMWKSVEGELNAILE   |  |  | comparison 3 |
| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE   |  |  |              |
| DENV2   | DSGCVVSWKNKELKGCGSIFICITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS |  |  |              |
| ZIKV-BR | DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRKYHPDSPRLLAAAVKQAWEDGICGISSVSRMENIMWKSVEGELNAILE   |  |  | comparison 4 |
| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE   |  |  |              |
| DENV2   | DSGCVVSWKNKELKGCGSIFICITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS |  |  |              |
| WNV     | DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYYPETPQGLAKIIQKAHEVGCLRSVSRLEHQMWAEVKDELNTLLK    |  |  |              |
| DENV2   | DSGCVVSWKNKELKGCGSIFICITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS |  |  | comparison 5 |
| ZIKV-UG | DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE   |  |  |              |

Figure 3.10.4: Alignment processed with multiple comparison method. Not all alignment is being shown.

If the array is well constructed, we can invoke the following command:

cprisma -tr -ck -rf multiple

Then, in our log file the comparisons will appear as follows:

```
-- Set of comparisons: main_0 --
ZIKV-UG X ZIKV-BR DENV2 WNV

ZIKV-UG X no comparison

-- Set of comparisons: main_1 --
ZIKV-BR X ZIKV-UG DENV2

ZIKV-BR X ZIKV-UG DENV2 WNV

-- Set of comparisons: main_2 --
```



---

DENV2 X ZIKV-UG

Our HTML file will show an output like the one shown in Figure 3.10.4 (to facilitate the visualization, method 2 was used, it is recommended to see Section 3.3).

Finally, if the array is not well built, CPRISMA will always return to the `default` value. In addition, it will inform you in detail where the errors are. Based on this, you can correct them yourself (the program will guide you). Warning/error messages for `multiple` command will not appear in this documentation, as we prefer that the user be trained in understanding the construction of CPRISMA arrays.

See in Chapter 8 the possibilities that can be declared in `dict_ref` using `multiple` comparison method .

### 3.11 Total information (-t)

To have the verbose version of the log file, you can invoke the following command:

```
cprisma -tr -t
```

Now, the log file will show in detail the operations that you are applying to each set of comparisons (see Chapter 4). In turn, a relationship between each comparison and the color assigned to it will be shown (see Chapter 5). Also, if a maximum restriction is applied (see Chapter 7), you can visualize the lists of values used to normalize the color displayed on the HTML file (whether or not math operations are performed).



## 4 Operation (descriptor\_ope)

CPRISMA has a feature called “operation” that allows the user to compute basic mathematical operations among the data. To execute this feature, it is *mandatory* to have at least one comparison between a particular reference and target proteins. For the following examples, we will always assume the `default` comparison method (see Subsection 3.10.1), with the exception of Section 4.5.

Generally, the program will always read the variable called `descriptor_ope` from the Python script “array\_get.py” (Figure 2.2.3). Then, CPRISMA will perform a certain operation between the vector  $\mathbf{r} = [r_j]$  (reference) and the matrix  $\mathbf{T} = [t_{ij}]$  [target(s)]. Based on the data processed and displayed in the log file, for our example using  $pK_a$  values, the vector  $\mathbf{r}$ , and matrix  $\mathbf{T}$  have dimensions  $56 \times 1$  and  $56 \times 3$ , respectively. Something that should be noted is that CPRISMA takes advantage of the `numpy` features to apply broadcasting and thus improve the speed of matrix operations. For this version of CPRISMA, you can perform 3 types of calculations: `delta/d` ( $\Delta$ ), absolute `delta/da` ( $|\Delta|$ ), and multiplication/`m`. Although, it is possible to not calculate any operation (`none/n`) as well. For CPRISMA, these 4 possibilities are called *descriptors*.

*Note: We highlight that this nomenclature (i.e., descriptor) also applies to other features in addition to operations, such as color, visualization, and maximum restriction (see Chapters 5, 6, and 7, respectively).*

Finally, the numerical data generated through the operations will serve as the basis to put the color in the multiple sequence alignments.

### 4.1 None (n)

When it is desired to keep the same numerical values of the input (*i.e.*, the data of the CSV file), you can do this by declaring the descriptor `n` in the “array\_get.py” file, as follows:

```
descriptor_ope = { 'n' }
```



---

Perceive that the `n` descriptor is a string variable inside a pair of brackets. This data structure corresponds to a *set* variable type and should not be confused with a dictionary<sup>1</sup>.

After having run CPRISMA, your log file will have various information about the operation applied. For now, we should only focus on the next<sup>2</sup>:

```
main  comparison  descriptor
main_0          1           n
```

This statement is simply saying that `n` descriptor was considered for comparison 1, in the `main_0` comparison set.

*Note: Sometimes, it could be declared this descriptor or another wrongly. To avoid that the program stops, it will always return to `n` descriptor (default parameter), with some additional error messages in your log file, which will guide you to correct the problem.*

## 4.2 Delta (`d`)

When the `d` descriptor is invoked like this:

```
descriptor_ope = { 'd' }
```

... the program will do a conventional  $\Delta$  calculation between `r` and `T` (Equation 4.2.1).

$$\mathbf{W} = \mathbf{T} - \mathbf{r}, \quad (4.2.1)$$

where `W` is the new data to put color (see Chapter 5).

## 4.3 Absolute delta (`da`)

To declare the `da` descriptor, it is possible as follow:

```
descriptor_ope = { 'da' }
```

<sup>1</sup>See <https://docs.python.org/3.7/tutorial/datastructures.html#sets>.

<sup>2</sup>When using a different operation descriptor, respectively, its letter will appear in the `descriptor` column.



---

CPRISMA will apply the  $\Delta$  calculation as before (Section 4.2), but using the absolute value:

$$\mathbf{W} = |\mathbf{T} - \mathbf{r}| \quad (4.3.1)$$

## 4.4 Multiply (`m`)

To declare the `m` descriptor, you can invoke:

```
descriptor_ope = { 'm' }
```

The program will multiply the data as shown in Equation 4.4.1.

$$\mathbf{W} = \mathbf{T} \times \mathbf{r} \quad (4.4.1)$$

## 4.5 Operation descriptors dictionary (-dop)

One can run the following command using `multiple` comparison method:

```
cprisma -tr -ck -rf multiple
```

We have started from the next CPRISMA array (see Subsection 3.10.3)<sup>3</sup>:

```
dict_ref = { 'main_0': [ {'0': [1, 2, 3]}, {'0': []} ] ,  
            'main_1': [ {'1': [0, 2]}, {'1': [0, 2, 3]} ] ,  
            'main_2': [ {'2': [0]} ] }
```

Suppose we are doing a  $\Delta$  operation (*i.e.*, `descriptor_ope = { 'd' }`). Then, the following information will appear in the log file:

Comparing `dict_ref` with `dict_ope`:

```
{'0': [1, 2, 3]} ..... d  
{'0': []} ..... d  
{'1': [0, 2]} ..... d
```

---

<sup>3</sup>Note that we have differentiated each comparison with a different color to aid the reader.



---

```
{'1': [0, 2, 3]} ..... d  
{'2': [0]} ..... d
```

The array of the feature ‘operation’ `dict_ope` is compatible with the array of comparison sequences `dict_ref`!

Note: Comparison 2 does not have a target-sequence (*i.e.*, `{'0': []}`), so operations like `d`, `da`, or `m` are not applied.

This information is showing us a relationship between each comparison and the type of operation that it is applied for each one. For all comparisons, a  $\Delta$  is computed among its specific reference and target-sequences.

Nonetheless, we are not always going to calculate a single operation for all comparisons. To perform a different math calculation for each reference/target(s) relationship, an array of operation descriptors called `dict_ope` should be built in our “array\_get.py” script. We shall follow very similar rules to those we saw earlier for the `multiple` method (see Subsection 3.10.3). Our dictionary of operations must have an equivalent number of `main`’s as well as in `dict_ref` (*i.e.*, `main_0`, `main_1`, and `main_2`). In turn, for each `main` (first-key), a list of operation descriptors (first-values) should be invoked<sup>4</sup>. Based on this, suppose we construct the following operation-array<sup>5</sup>:

```
dict_ope = { 'main_0' : [ {'d'} , {'n'} ] ,  
            'main_1' : [ {'m'} , {'d'} ] ,  
            'main_2' : [ {'da'} ] }
```

Invoking the `-dop` command you will order to CPRISMA to take into account the `dict_ope` dictionary of the “array\_get.py” script:

```
cprisma -tr -ck -rf multiple -dop
```

Now, our log file will show the operation applied for each comparison:

Comparing `dict_ref` with `dict_ope`:

---

<sup>4</sup>For the other features (*i.e.*, color, visualization, and maximum restriction), these first-values will always be a list of descriptors with the same number of elements equivalent to the number of comparisons.

<sup>5</sup>Note that we have differentiated each operation descriptor for each comparison with a different color to aid the reader.



---

```
{'0': [1, 2, 3]} ..... d
{'0': []} ..... n
{'1': [0, 2]} ..... m
{'1': [0, 2, 3]} ..... d
{'2': [0]} ..... da
```

The array of the feature ‘operation’ `dict_ope` is compatible with the array of comparison sequences `dict_ref`!

... and the next table:

| main   | comparison | descriptor |
|--------|------------|------------|
| main_0 | 1          | d          |
| main_0 | 2          | n          |
| main_1 | 3          | m          |
| main_1 | 4          | d          |
| main_2 | 5          | da         |

It is important to mention that `-dop` will only work with the `multiple` comparison method.

*Note: To check how the numerical calculations are applied between each row of the processed input file, the `-t` flag can be employed (see Section 3.11). Figure 7.0.1 shows an example of how that part of the log file looks like.*

*Note: If the array of `dict_ope` is wrongly built, all descriptors will be transformed to `n` (default parameter) for each comparison and additional information about where the errors are will be displayed.*



## 5 Color (descriptor\_col)

So far, we have intentionally seen situations where our multiple sequence alignment is colorless. This is because we did not want to work with many features simultaneously. This Chapter will describe how to use the CPRISMA feature called “color”.

Remember that the color applied is working with the HTML language. The file employed by CPRISMA to apply the color is located at “cprisma/colors.csv”. Figure 5.0.1 shows some rows from this file. The cells are divided into four columns with the heads “General”, “Name”, “Hex”, and “RGB”. CPRISMA will only invoke the column labeled RGB to give color, the other information is just to help the user to identify each color easily. It is important to mention that all this stuff is based on the official HTML site about color code<sup>1</sup>. For quick access to this list of colors, you can invoke the `-lco` command described in Section 5.16.

|   | A        | B           | C       | D                  |
|---|----------|-------------|---------|--------------------|
| 1 | General  | Name        | Hex     | RGB                |
| 2 | Red HTML | IndianRed   | #CD5C5C | rgb(205, 92, 92)   |
| 3 | Red HTML | LightCoral  | #F08080 | rgb(240, 128, 128) |
| 4 | Red HTML | Salmon      | #FA8072 | rgb(250, 128, 114) |
| 5 | Red HTML | DarkSalmon  | #E9967A | rgb(233, 150, 122) |
| 6 | Red HTML | LightSalmon | #FFA07A | rgb(255, 160, 122) |
| 7 | Red HTML | Crimson     | #DC143C | rgb(220, 20, 60)   |
| 8 | Red HTML | Red         | #FF0000 | rgb(255, 0, 0)     |

Figure 5.0.1: CSV file with color information. All the rows have not been shown.

For the color feature, we also have a set variable called `descriptor_col` in the script “array\_get.py” (Figure 2.2.3). The color feature has 10 different descriptors (`nc`, `ssc`, `fsc`, `fac`, `fmac`, `pic`, `pimc`, `tc`, `tmc`, and `pkac`) that will be described below.

For most next examples the following command is being executed:

---

<sup>1</sup>See <https://htmlcolorcodes.com/color-names/>.



---

```
cprisma -tr -ck
```

Furthermore, the variable `descriptor_ope` in the Python script “array\_get.py” was typed as follows:

```
descriptor_ope = { 'd' }
```

## 5.1 No color (nc)

To use the descriptor `nc`, you can declare it like this:

```
descriptor_col = { 'nc' }2
```

As the name implies, you are not adding any color to the alignment. Now, your log file will have various information about the color feature applied. We should only focus on the next:

| main   | comparison | descriptor | sequence | residue | mutation | style | threshold |
|--------|------------|------------|----------|---------|----------|-------|-----------|
| main_0 | 1          | nc         | NaN      | NaN     | NaN      | NaN   | NaN       |

This table is simply saying that the `nc` descriptor was considered for comparison 1, in the `main_0` comparison set (for now ignore the rest of columns in that log message).

*Note: Sometimes, some descriptors can be declared wrongly. To avoid that the program stops, it will always return to the nc descriptor (default parameter), with some additional error messages in your log file, which will guide you to correct the problem.*

## 5.2 Same sequence color (ssc)

Suppose that you want to color a set of sequences with the same color. For this we declare:

```
descriptor_col = { 'ssc' }
```

The log file will report as `[same]` in the `sequence` column:

| main   | comparison | descriptor | sequence | residue | mutation | style | threshold |
|--------|------------|------------|----------|---------|----------|-------|-----------|
| main_0 | 1          | ssc        | [same]   | NaN     | NaN      | NaN   | NaN       |

Furthermore, the HTML file now has a colored alignment (Figure 5.2.1).

---

<sup>2</sup>For all alignment examples of Chapter 3, this was the variable executed.



Figure 5.2.1: Alignment colored through `ssc` descriptor.

Note that only the target-residues in all the proteins are being colored with Red (see Section 3.1) and the reference (*i.e.*, ZIKV-UG) is not being examined. The default color for the `ssc` descriptor is Red, which corresponds to the 6th row when executed `-lco` (see Section 5.16). You can change that color by invoking the `-sco` flag described in Section 5.12.

### 5.3 Free sequence color (fsc)

To give a different color to each *target-sequence*, the descriptor `fsc` can be declared as follows:

```
descriptor_col = { 'fsc' : [6, 54, 94] }
```

Notice that now is not being invoked a set variable type but a dictionary. Our key will be the descriptor `fsc` (string type) and our value is a list of number colors (see Section 5.16) that corresponds to the total number of target-sequences and their order of appearance (*i.e.*, colors 6, 54, and 94 for ZIKV-BR, DENV2, and WNV, respectively). After running the program, the log file will show the following information:

```
main comparison descriptor      sequence residue mutation style threshold
main_0          1           fsc  [6, 54, 94]       NaN      NaN      NaN      NaN
```

... and the HTML file will now display a color for each target-sequence (Figure 5.3.1). Assuming you will do quick tests and do not care about the color kind assigned to each target-sequence, it is possible to declare your variable like this:

```
descriptor_col = { 'fsc' : ['random'] }
```



When the word ‘random’ is typed into the list, CPRISMA will give each target-sequence a random color.

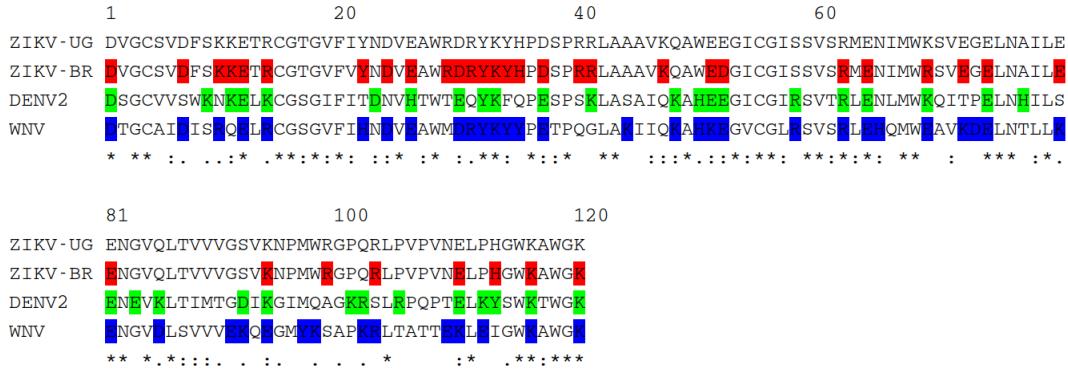


Figure 5.3.1: Alignment colored through `fsc` descriptor.

#### 5.4 Free amino acid color (fac)

To color each *target-residue* for the target-sequence(s), the **descriptor\_col** can be invoked like this:

```
descriptor_col = { 'fac' : [6, 20, 22, 54, 94, 46] }
```

Look at the list has 6 different color numbers. That number of elements is correlated to the number of target-residues declared in the tuple `target_residues` of the script “array\_get.py” (see Section 3.1).

Note: If you need to assess the 20 essential amino acids, you will need to declare 20 color numbers in that list or use random option.

Later, the log file will report this list of color numbers in the **residue** column like this:

| main   | comparison | descriptor | sequence | residue | mutation   | style | threshold |     |
|--------|------------|------------|----------|---------|------------|-------|-----------|-----|
| main_0 |            | 1          | fac      | NaN     | [6, 20...] | NaN   | NaN       | NaN |

... and the HTML file will now display a color for each target-residue (Figure 5.4.1). As with the `fsc` descriptor, random colors for your target-residues can be declared as follows:

```
descriptor_col = { 'fac' : ['random'] }
```



Figure 5.4.1: Alignment colored through `fac` descriptor.

## 5.5 Free amino acid and mutation color (fmac)

The `fmac` descriptor is a copy of the `fac` one, but with the capacity to distinguish regions where there are mutations between the reference and target-sequence(s). To execute it, you can declare your variable `descriptor_col` like this:

```
descriptor_col = { 'fmac' : [6, 20, 22, 54, 94, 46]3 }
```

Now the log file will also inform you that mutations are being differentiated by means of the **True** Boolean in the **mutation** column:

| main   | comparison | descriptor | sequence | residue | mutation   | style | threshold |     |
|--------|------------|------------|----------|---------|------------|-------|-----------|-----|
| main_0 |            | 1          | fmac     | NaN     | [6, 20...] | True  | NaN       | NaN |

A typical HTML outcome using the descriptor `fmac` appears in Figure 5.5.1. See that mutations are colored in gray. Whether you want to change the Gray color of the mutations (default), it is possible through the `-mco` command (see Section 5.13).

<sup>3</sup>Remember that the `random` method could be employed here.

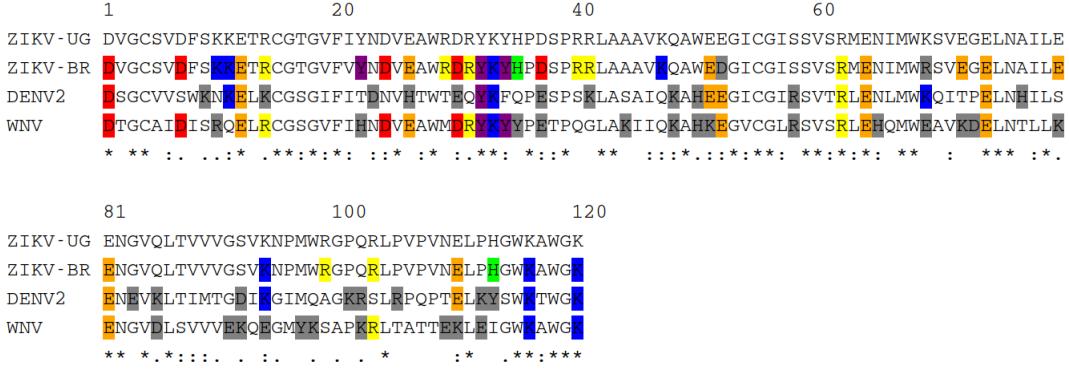


Figure 5.5.1: Alignment colored through `fmac` descriptor.

## 5.6 Color by position index (pic)

When it is desired to highlight only specific regions of the alignment, the `pic` descriptor can be invoked<sup>4</sup>:

```
descriptor_col = { 'pic' : [ { '1' : [6, 'n', '1-2', '3', '4-8'] } ,
                            { '1' : [94, 'b', '11-27'] } ,
                            { '2' : [75, 'd', '3', '30-55'] } ,
                            { '3' : [54, 'u', '20-35'] } ] }
```

This can be one of the most complex color descriptors to implement. The descriptor `pic` is a dictionary type where its key will be the string '`pic`', and the value is a list of dictionaries with several parameters (the orange square brackets delimit the list and purple brackets the dictionaries).

We have seen this arrangement structure before (see Subsection 3.10.3). However, when `pic` is called, we have some differences. Our second-key will be a number (string type), from one of the target-sequence(s) (for our example, '`1`', '`2`', and '`3`', or ZIKV-BR, DENV2, and WNV, respectively).

*Note: It is imperative that you respect the order in which the target-sequences appear in a specific comparison. However, note that you can call the same sequence more than once as*

---

<sup>4</sup>Some brackets, letters, and numbers are colored to aid the reader.



'1' in our example.

The second-value is a list of parameters where the first (in red letters) and second (in green letters) refer, respectively, to the positive integer color number (see Section 5.16) and the font style [‘n’ (“none”), ‘b’ (“**bold**”), ‘i’ (“*italic*”), ‘d’ (“~~deleted~~”), and ‘u’ (“underlined”)]. From the third (in blue), we find the intervals (string type) that we want to color with the format: ‘number<sub>1</sub>-number<sub>2</sub>’. The intervals must be from a lower to a higher number and sometimes only one can be considered (for our example see ‘3’ in blue). You should respect this consistency! The numbers of the intervals that you must implement are the same when the input data is processed (see the index of each row in Figure 3.2.1 or the “Data processed” section of the log file). Remember, your intervals should be related to that index and not the alignment position!

Note: Be careful about overlaps that can occur between the intervals.

When CPRISMA is run, the program will display the following information in the log file:

Figure 5.6.1: Alignment colored through `pic` descriptor.

Notice that for the `pic` descriptor, the `sequence` column is not showing the color as we saw



with the **fsc** descriptor, instead, it is displaying the number of the target protein that is being colored. To find the color, you must see the first element in the list that appears in the **residue** column [for our example the color values will be 6 (Red), 94 (Blue), 75 (Cyan), and 54 (Lime)]. The next elements on the same list will be the highlighted residues<sup>5</sup>. Moreover, the font letter that is being applied for each region will appear in the **style** column. The HTML file is going to display specific regions based on the position index of the processed data (Figure 5.6.1). Make correlations with both that graph and the table above. Note that if the interval matches with a gap, this region will not be colored. For instance, the interval ‘1-2’ (sequence<sub>1</sub>/ZIKV-BR) presents a gap at position 2 (Figure 3.2.1), as this position coincides with a Lys residue for DENV2 and one Ser residue for ZIKV-BR, no color should be assigned to that region of ZIKV-BR, due to the Ser is not a target-residue.

Figure 5.6.2: Alignment colored through pimc descriptor.

## 5.7 Color by position index and mutation (pimc)

The `pimc` descriptor can be declared when it is desired to put color based on an index and distinguish point mutations<sup>6</sup>. `pimc` is a copy of `pic`, so we can simply change `pic` by `pimc` from the previous array to get the following information:

<sup>5</sup>In the table they are being replaced by points.

<sup>6</sup>Imagine that this descriptor is equivalent to `fmac` or `tmc` (see Sections 5.5 or 5.9, respectively).




---

| main   | comparison | descriptor | sequence | residue | mutation | style | threshold |
|--------|------------|------------|----------|---------|----------|-------|-----------|
| main_0 | 1          | pimc       | [1]      | [6...]  | True     | n     | NaN       |
| main_0 | 1          | pimc       | [1]      | [94...] | True     | b     | NaN       |
| main_0 | 1          | pimc       | [2]      | [75...] | True     | d     | NaN       |
| main_0 | 1          | pimc       | [3]      | [54...] | True     | u     | NaN       |

Check that the `mutation` column appears with the Boolean value equal to `True`. If you prefer another color for the mutations, remember that it is also possible to implement the `-mco` flag (see Section 5.13).

The HTML file will be like shown in Figure 5.6.2.

A practical example using this descriptor appears in Section 8.3. Also, the implementation of the `pimc` descriptor appears in a previously published study [3].



Figure 5.7.1: Alignment colored through `tc` descriptor.

## 5.8 Color by threshold (`tc`)

To discriminate the numeric values of the input data by means of color and using a threshold, it is possible through the following declaration:

```
descriptor_col = { 'tc' : [94, 0.1, '>='] }
```

The descriptor `tc` is a dictionary where its value is a list with three elements. In the first position of that list, you must put a positive integer number that is related to the type of color desired (see Section 5.16). In the second position, there is an `int/float` number that defines



the threshold, and the third element, must be a string that refers to a symbol of order relation (*i.e.*, ‘ $\geq$ ’, ‘ $\leq$ ’, ‘ $>$ ’, ‘ $<$ ’, ‘ $\equiv$ ’, ‘ $\neq$ ’). For our example, we are ordering the program to color in Blue only those  $\Delta pK_a$  of the target-residues that are greater equal than 0.1.

Subsequently, the log file will report the color number in the **sequence** column, and the two last parameters of the **tc** descriptor list in the **threshold** column, like this:

| main   | comparison | descriptor | sequence | residue | mutation | style | threshold |         |
|--------|------------|------------|----------|---------|----------|-------|-----------|---------|
| main_0 |            | 1          | tc       | [94]    | NaN      | NaN   | NaN       | 0.1, >= |

The HTML file will now display a color for each target-residue following the threshold condition (Figure 5.7.1).

A practical example using this descriptor appears in Section 8.2. The implementation of the `tc` descriptor appears in a previously published study [2].

Figure 5.8.1: Alignment colored through `tmc` descriptor.

## 5.9 Color by threshold and mutation (tmc)

The descriptor `tmc` is the version of `tc` that will simultaneously discriminate mutations. This follows the same `tc` rules and is invoked as follows:

```
descriptor col = { 'tmc' : [94, 0.1, '>='] }
```

The log file will report the next information:



---

```
main  comparison  descriptor  sequence  residue  mutation  style  threshold
main_0          1           tc        [94]      NaN       True      NaN     0.1, >=
```

Look that a Boolean value equal to `True` appears in the `mutation` column. If you prefer another color for the mutations remember that it is also possible to implement the `-mco` flag (see Section 5.13).

The HTML file will be like shown in Figure 5.8.1. Note that CPRISMA only taking into account mutations that are matching the declared threshold condition.

## 5.10 $pK_a$ color (pkac)

To use the `pkac` descriptor you must always do a  $\Delta$  operation among *ionizable residues* (see Section 4.2). To execute this descriptor, your “`array_get.py`” script should contain the following instructions:

```
descriptor_col = { 'pkac' : True/False7 }
```

*Note: It is imperative you declare the `descriptor_ope` to be equal to { ‘d’ } to get the expected results!*

The descriptor `pkac` is able to distinguish between those negative and positive  $\Delta pK_a$  of the ionizable groups and will assign Red and Blue colors, respectively. Observe that the `descriptor_col` dictionary has a Boolean variable as a value. You can interpret `False` or `True` for cases where you do not require (as `fac/pic/tc` descriptors) or require (as `fmac/pimc/tmc` descriptors) to differentiate point mutations, respectively. If `True`, mutations will be highlighted in two ways:

1. When there is a shift from acid residue to a basic one (Lime color will be assigned).
2. Vice versa (Yellow color will be assigned).

A typical output in the log file when the `pkac` descriptor is invoked with `False` Boolean appears below:

```
main  comparison  descriptor  sequence      residue  mutation  style  threshold
main_0          1           pkac        NaN  [ionizable]      NaN      NaN     NaN
```

---

<sup>7</sup>Select only one Boolean option.

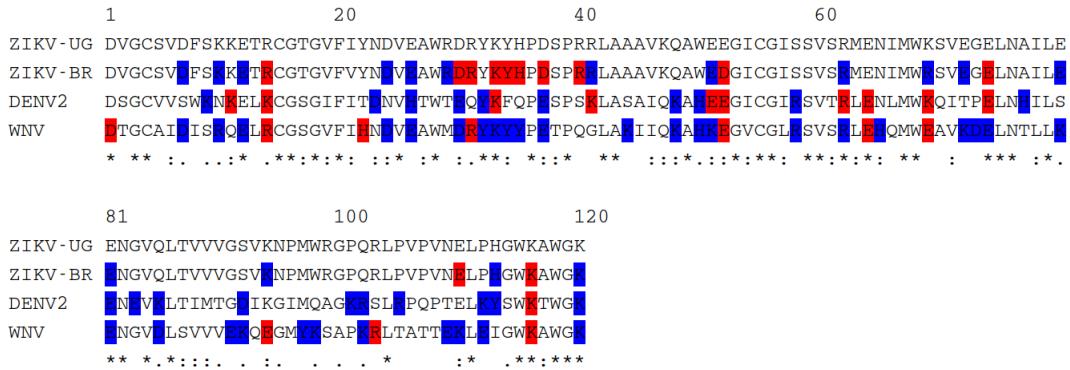


Figure 5.10.1: Alignment colored through `pkac` descriptor and invoking `False` as value.

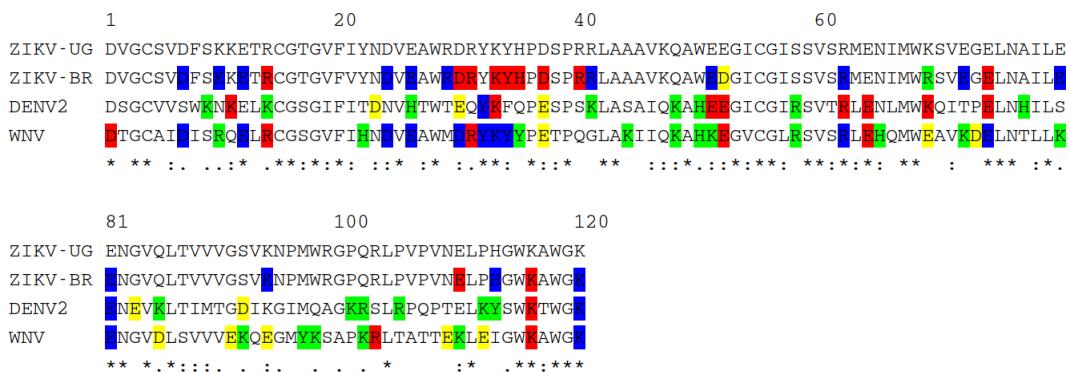


Figure 5.10.2: Alignment colored through `pkac` descriptor and invoking `True` as value.

Note: Remember, if you invoke the Boolean `True` as a value in the dictionary of `pkac` descriptor, then this parameter will be displayed on the `mutation` column as `True`.

Whenever the `pkac` descriptor is run, the `residue` column will show the information: `[ionizable]`. Examples of colored alignments by means of the `pkac` descriptor using `False` and `True` appear in Figures 5.10.1 and 5.10.2, respectively. See that this descriptor can be quite useful for data with negative or positive numbers even if they are not  $pK_a$  values!

A practical example appears in Section 8.1. Nevertheless, note that for these cases other features are being incorporated, such as color gradient (see Chapter 6). The implementation of the `pkac` descriptor appears in a previously published study [1].



## 5.11 Color descriptors dictionary (-dco)

When the `multiple` comparison method is executed, maybe you need to distinguish each reference/target(s) relationship with a specific color descriptor. For that, we must build an array of color descriptors through the `dict_col` variable of the script “array\_get.py”. To build it, you should follow the same rules as for `dict_ope` (see Section 4.5). For this example, we used the same dictionary for the `multiple` method as a base (see Subsection 3.10.3). Then, our CPRISMA color-array will be as follows<sup>8</sup>:

```
dict_col = { 'main_0' : [ { 'pimc' : [ { '1' : [63, 'b', '0-20'] } , { '3' : [96, 'u', '20-35'] } ] } , { 'ssc' } ] , 'main_1' : [ { 'fsc' : [2,80] } , { 'pkac' : True } ] , 'main_2' : [ { 'fac' : ['6, 20, 22, 54, 94, 46'] } ] }
```

Now we can execute the `-dco` flag to call the array `dict_col`.<sup>9</sup>

```
cprisma -tr -ck -rf multiple -va 2 -hc -sco 20 -mco 75 -dco
```

The log file will show the relationships between each comparison and color descriptor:

Comparing `dict_ref` with `dict_col`:

```
{'0': [1, 2, 3]} ..... { 'pimc' : ... }  
{'0': []} ..... { 'ssc' }  
{'1': [0, 2]} ..... { 'fsc' : [2,80] }  
{'1': [0, 2, 3]} ..... { 'pkac' : True }  
{'2': [0]} ..... { 'fac' : [6, 20, 22...] }
```

The array of the feature ‘color’ `dict_col` is compatible with the array of comparison sequences `dict_ref`!

... and a table with a summary for all color descriptor information per comparison:

<sup>8</sup>Note that we have differentiated each color descriptor for each comparison with a different color to aid the reader.

<sup>9</sup>Notice that additional parameters are being implemented.



| main   | comparison | descriptor | sequence | residue     | mutation | style | threshold |
|--------|------------|------------|----------|-------------|----------|-------|-----------|
| main_0 | 1          | pimc       | [1]      | [63...]     | True     | b     | NaN       |
| main_0 | 1          | pimc       | [3]      | [96...]     | True     | u     | NaN       |
| main_0 | 2          | ssc        | [same]   | NaN         | NaN      | NaN   | NaN       |
| main_1 | 3          | fsc        | [2, 80]  | NaN         | NaN      | NaN   | NaN       |
| main_1 | 4          | pkac       | NaN      | [ionizable] | True     | NaN   | NaN       |
| main_2 | 5          | fac        | NaN      | [6...]      | NaN      | NaN   | NaN       |

Figure 5.11.1 shows the alignment for the typed commands and the built `dict_col` array.

```

      1          20          40          60
ZIKV-UG DVGCSVDFSKETRCGTGVFIYNDVEAWRDRYKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE comparison 1
ZIKV-BR DVGCSVFSKETRCGTGVFIVNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWRSVEGELNAILE
DENV2   DSGCVVSWKNKELKGCGSIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS
WNV     DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYPETPQGLAKIIQKAHKIGVCGLRSVSLEHQMWAEAVKDNLNTLLK

      1          20          40          60
ZIKV-UG DVGCSVFSKETRCGTGVFIYNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE comparison 2
ZIKV-BR DVGCSVDFSKETRCGTGVFVYNDVEAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWRSVEGELNAILE
ZIKV-UG DVGCSVFSKETRCGTGVFIYNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE
DENV2   DSGCVVSWKNKELKGCGSIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS

      1          20          40          60
ZIKV-BR DVGCSVDFSKETRCGTGVFVYNDVEAWRDRYKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWRSVEGELNAILE comparison 3
ZIKV-UG DVGCSVFSKETRCGTGVFIYNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE
DENV2   DSGCVVSWKNKELKGCGSIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS

      1          20          40          60
ZIKV-BR DVGCSVDFSKETRCGTGVFVYNDVEAWRDRYKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWRSVEGELNAILE comparison 4
ZIKV-UG DVGCSVFSKETRCGTGVFIYNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE
DENV2   DSGCVVSWKNKELKGCGSIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS
WNV     DTGCAIDISRQELRCGSGVFIYNDVAWMDRYKYPETPQGLAKIIQKAHKIGVCGLRSVSLEHQMWAEAVKDNLNTLLK

DENV2   DSGCVVSWKNKELKGCGSIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITPELNHILS comparison 5
ZIKV-UG DVGCSVFSKETRCGTGVFIYNDVAWRDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE

```

Figure 5.11.1: Alignment for `multiple` comparison method and colored through an array of color descriptors `dict_col`. Not all alignment is being shown.

Note: If the array of `dict_col` is wrongly built, all descriptors will be transformed to `nc` (default parameter) for each comparison and additional information about where the errors are will be displayed.

Note: Remember that these complex color-arrays can be simultaneously combined (or not) with other transcendental CPRISMA arrangements of the other features as well (i.e., operation, visualization, and maximum restriction described in Sections 4.5, 6.1, and 7.14). See also the practical examples in Chapter 8.



---

## 5.12 Color sequence (-sco)

To change the default color of the `ssc` descriptor, you should leave your variable `descriptor_col` as declared above in Section 5.2 and execute the `-sco` flag followed by a positive integer number in the terminal, as follows:

```
cprisma -tr -ck -sco 51
```

Remember that this number should be equivalent to a positive integer number less equal than 142. For the command above we have changed the alignment color from Red to GreenYellow, which is at row-position 51 when `-lco` is executed (see Section 5.16).

## 5.13 Color mutation (-mco)

It is possible to change the color of the mutations by executing a command like the one below:

```
cprisma -tr -ck -mco 75
```

Remember to use the same rules as for the `-sco` flag (see Section 5.12). For the command above, the color of point mutations was changed from Gray to Cyan.

*Note: The flag `-mco` only works for `fmac`, `pimc`, and `tmc` color descriptors (see Sections 5.5, 5.7, and 5.9, respectively).*

## 5.14 Color on three-dimensional structure (-tco)

To observe the regions colored by CPRISMA at the three-dimensional structure level, the `-tco` flag can be useful. Using the case of Section 5.10 as an example<sup>10</sup>, we can execute:

```
cprisma -tr -ck -tco
```

This command will generate a directory called “3D\_representation” with various Pymol scripts for all proteins and comparisons. To visualize the protein, type the next command line:

```
pymol your-protein.pdb 3D_representation/main_0/main_0_comparison1_your-protein.pml
```

An example for ZIKV-BR appears in Figure 5.14.1. Notice that only the target-residues are taken into account. Furthermore, only the first 120 amino acids are being evaluated.

---

<sup>10</sup>Differences between mutations are being considered.

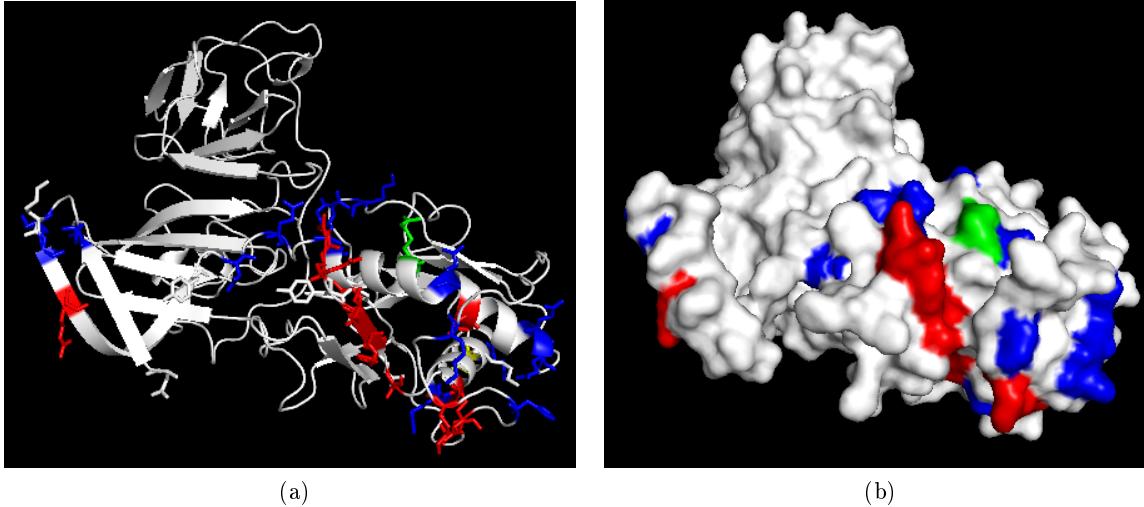


Figure 5.14.1: Three-dimensional structure for the monomeric NS1<sub>ZIKV-BR</sub> (PDB id 5GS6) using the pkac descriptor. (a) Ribbons representation (target-residues appear as sticks). (b) Surface representation. Visualizations were made from Pymol scripts generated through the -tco flag.

### 5.15 Color intensity (-ico)

Sometimes when we apply a gradient color (see Chapter 6) the intensity of this can not be the best due to several problems (as mentioned earlier in Section 3.2). An alternative to fix these dilemmas is to multiply the intensity of the color by a positive integer number invoking the `-ico` flag. As an example, you can execute it as appears below:

cprisma -tr -ck -ico 4

For the previous command line, we are multiplying by 4 the intensity of the color. We recommend the user to test with gradient colors and implement `-ico`.

## 5.16 List of colors (-lco)

To have quick access to the list of colors you can execute the command:

cprisma -lco



---

Figure 5.16.1 shows the first 10 rows displayed in the terminal as an example. See that an index appears numbering each color. This index will be useful to access the colors that CPRISMA applies. Remember, these colors are based on the website <https://htmlcolorcodes.com/color-names/>.

*Note: The possible number of colors is from 0 to 142.*

|    | General   | Name        | Hex     | RGB                |
|----|-----------|-------------|---------|--------------------|
| 0  | Red HTML  | IndianRed   | #CD5C5C | rgb(205, 92, 92)   |
| 1  | Red HTML  | LightCoral  | #F08080 | rgb(240, 128, 128) |
| 2  | Red HTML  | Salmon      | #FA8072 | rgb(250, 128, 114) |
| 3  | Red HTML  | DarkSalmon  | #E9967A | rgb(233, 150, 122) |
| 4  | Red HTML  | LightSalmon | #FFA07A | rgb(255, 160, 122) |
| 5  | Red HTML  | Crimson     | #DC143C | rgb(220, 20, 60)   |
| 6  | Red HTML  | Red         | #FF0000 | rgb(255, 0, 0)     |
| 7  | Red HTML  | FireBrick   | #B22222 | rgb(178, 34, 34)   |
| 8  | Red HTML  | DarkRed     | #8B0000 | rgb(139, 0, 0)     |
| 9  | Pink HTML | Pink        | #FFC0CB | rgb(255, 192, 203) |
| 10 | Pink HTML | LightPink   | #FFB6C1 | rgb(255, 182, 193) |

Figure 5.16.1: First 10 rows when invoked `-lco` command.



## 6 Visualization (descriptor\_vis)

Previously, in Chapters 3 and 5, we already saw some commands and variables that alter the display of alignments. In this Chapter, we will give an expansion focusing on three possible functionalities available on CPRISMA through the so-called “visualization” feature:

1. Reference display (`Re`).
2. Target-sequence(s) degrade (`De`).
3. Visualization of the letters (`Le`).

These three variables will be the descriptors of the “visualization” feature, and they are always declared simultaneously through the `descriptor_vis` of the “array\_get.py” script (Figure 2.2.3), as follows:

```
descriptor_vis = { 'ReY', 'DeN', 'LeY' }
```

The `descriptor_vis` is a set variable with three elements. Besides, each descriptor is accompanied by a letter Y (Yes) or N (No), which indicates whether or not this visualization feature is enabled, respectively.

Suppose we respectively declare the operation (see Chapter 4) and color (see Chapter 5) variables like this,

```
descriptor_ope = { 'd' }

descriptor_col = { 'pkac' : True }

... and we run the next command1:
```

```
cprisma -tr -ck
```

You may have noticed that your log file shows the following information:

---

<sup>1</sup>It is important to mention that for most of the examples in this Chapter we have used this command and these variables as declared.



---

|        | main | comparison | reference | degraded | letters |
|--------|------|------------|-----------|----------|---------|
| main_0 |      | 1          | True      | False    | True    |

Now, for each visualization descriptor, a Boolean variable is shown based on the relations: Y (True) and N (False).

*Note: Sometimes, some descriptors can be declared wrongly. To avoid that the program stops, it will always return to ‘ReY’, ‘DeN’, ‘LeY’ descriptors (default parameters), with some additional error messages in your log file, which will guide you to correct the problem.*

Early, the output alignment for these initial parameters was displayed (Figure 5.10.2). But now, let us change reference descriptor from ReY to ReN, as follows:

```
descriptor_vis = { 'ReN', 'DeN', 'LeY' }
```

As shown in Figure 6.0.1a, the “reference line” has disappeared. Since this sequence may not be relevant because it does not have any color, the Re descriptor allows us to remove it or not as appropriate.

Enabling the degrade, like this:

```
descriptor_vis = { 'ReY', 'DeY', 'LeY' }
```

... we can see the effect that our operations have when it is invoked the d descriptor on the descriptor\_ope (Figure 6.0.1b). Check that the color is on a scale from -13.3 to 13.3. This scale is normalized based on the maximum value derived from  $\Delta$  operation<sup>2</sup>. We observe that the point mutations have a greater impact on the rest of the  $\Delta pK_a$  values. Nevertheless, it is possible to apply maximum restriction methods to improve the color alignment display (see Chapter 7).

Finally, if we are not interested in visualizing the letters of the protein sequences, you could eliminate them by means of the following declaration:

---

<sup>2</sup>See explicitly the  $\Delta$  calculations in your log file with the -t flag (see Section 3.11).



1 20 40 60  
 ZIKV-BR DVGCSVDFSKKETRCGTGVFVYNIVLAWRDRYKYHPSPRLAAAVKQAWPDGICGISSVSRMENIMWRSVEGELNAILE  
 DENV2 DSGCVSVWNKELKGSGGIFITDNVHTWTEQKFQPEPSKLASAIQAHHEEGICGIGSVTRLENLMWKQITPELNHILS  
 WNV DTGCAIDISRQELRCGSVFIHNDVEAAMDYKYPETPQGLAKIIQAHKEGVCGLSVSRLEHQMWAEAVDDELNTLLK  
 \* \* . . . : \* . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* .

81 100 120  
 ZIKV-BR ENGVQLTVVVGSVNPMPWRGPQRPLPVVNELPHGWKAWGK  
 DENV2 ENEVKLTIMTGDIKGIMQACKRSLQPQPTELKYSWKTWKG  
 WNV ENGVDSLSSVVEKQEGMYKSAPKRLTATTEKLEIGWKAWGK  
 \* \* . \* : : . . . . \* : \* . \* : \* : \* : \* : \* : \* : \* : \* .

(a)

1 20 40 60  
 ZIKV-UG DVGCSVDFSKKETRCGTGVFIYNDVEAWRDRYKYHPDSPRLLAAAVKQAWEEGICGISSVSRMENIMWRSVEGELNAILE  
 ZIKV-BR DVGCSVDFSKKETRCGTGVFVYNDVEAWRDRYKYHPDSPRLLAAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILE  
 DENV2 DSGCVSVWNKELKGSGGIFITDNVHTWTEQKFQPEPSKLASAIQAHHEEGICGIGSVTRLENLMWKQITPELNHILS  
 WNV DTGCAIDISRQELRCGSVFIHNDVEAAMDYKYPETPQGLAKIIQAHKEGVCGLSVSRLEHQMWAEAVDDELNTLLK  
 \* \* . . . : \* . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* .

81 100 120  
 ZIKV-UG ENGVQLTVVVGSVKNPMPWRGPQRPLPVVNELPHGWKAWGK  
 ZIKV-BR ENGVQLTVVVGSVKNPMPWRGPQRPLPVVNELPHGWKAWGK  
 DENV2 ENEVKLTIMTGDIKGIMQACKRSLQPQPTELKYSWKTWKG  
 WNV ENGVDSLSSVVEKQEGMYKSAPKRLTATTEKLEIGWKAWGK  
 \* \* . \* : : . . . . \* : \* . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* .

-13.3 13.3  
 acid mutation basic mutation

(b)

1 20 40 60  
 ZIKV-UG  
 ZIKV-BR  
 DENV2  
 WNV  
 \* \* . . . : \* . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* : \* .

81 100 120  
 ZIKV-UG  
 ZIKV-BR  
 DENV2  
 WNV  
 \*\* . \* : : . . . . \* : \* . \* : \* : \* : \* : \* : \* : \* : \* : \* : \* .

(c)

Figure 6.0.1: Several alignments using different visualization descriptors. (a) Re descriptor with False Boolean (N). (b) De descriptor with True Boolean (Y). (c) Le descriptor with False Boolean (N).



```
descriptor_vis = { 'ReY', 'DeN', 'LeN' }
```

Figure 6.0.1c shows an alignment without the letters.

We recommend checking the practical examples in Sections 8.1 and 8.2 for a better understanding of the visualization descriptors.

## 6.1 Visualization descriptors dictionary (-dvi)

When it is applied complex comparisons with the `multiple` method (see Subsection 3.10.3), perhaps you will require to make a specific visualization based on `Re`, `De`, and `Le`, for each reference/target(s) relationship. For this, we can invoke the `-dvi` flag to get a visualization descriptors dictionary `dict_vis` from the “array\_get.py” script. It is important to mention that the same rules as already described for `dict_ope` were followed (see Section 4.5). For our example, the `dict_col` arrangement mentioned in Section 5.11 was employed. On the other hand, it will be assumed to be `d` for all comparisons (*i.e.*, `descriptor_ope = { 'd' }`) and the same array of comparisons described in Subsection 3.10.3. Our `dict_vis` variable will be declared as follows<sup>3</sup>:

```
dict_vis = { 'main_0' : [ { 'ReN', 'DeN', 'LeY' } , { 'ReY', 'DeY', 'LeY' } ] ,  
            'main_1' : [ { 'ReY', 'DeN', 'LeN' } , { 'ReY', 'DeY', 'LeY' } ] ,  
            'main_2' : [ { 'ReN', 'DeY', 'LeY' } ] }
```

Now we can execute the `-dvi` flag to call the array `dict_vis`<sup>4</sup>:

```
cprisma -tr -ck -rf multiple -va 2 -hc -sco 20 -mco 75 -dco -dvi
```

The log file will show the relationships between each comparison and visualization descriptors:

Comparing `dict_ref` with `dict_vis`:

```
{'0': [1, 2, 3]} ..... ['ReN', 'DeN', 'LeY']  
{'0': []} ..... ['ReY', 'DeY', 'LeY']  
{'1': [0, 2]} ..... ['ReY', 'DeN', 'LeN']
```

<sup>3</sup>Note that we have differentiated each set of visualization descriptors for each comparison with a different color to aid the reader.

<sup>4</sup>Notice that additional parameters are being implemented.



{'1': [0, 2, 3]} ..... ['ReY', 'DeY', 'LeY']

{'2': [0]} ..... ['ReN', 'DeY', 'LeY']

The array of the feature 'visualization' dict\_vis is compatible with the array of comparison sequences dict\_ref!

... and a table with a summary for all visualization descriptor information per comparison:

| main   | comparison | reference | degraded | letters |
|--------|------------|-----------|----------|---------|
| main_0 | 1          | False     | False    | True    |
| main_0 | 2          | True      | True     | True    |
| main_1 | 3          | True      | False    | False   |
| main_1 | 4          | True      | True     | True    |
| main_2 | 5          | False     | True     | True    |

1 20 40 60  
ZIKV-BR DVGCSVDFSKETRCGTGVFVYNDVEAWDRYKYHPDSPRLAAAVKQAWEDGICGISSVRMENIMWRSVEGELNAILE comparison 1  
DENV2 DSGCVVSWKNKELKGCGSGIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLWMWKQITPELNHILS  
WNV DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYPETPQGLAKIIQKAHKGVCGLRSVSILHQMWEEAVKDNLNTLLK

ZIKV-UG DVGCSVDFSKETRCGTGVFIYNDVEAWDRYKYHPDSPRLAAAVKQAWEEGICGISSVSRMENIMWKSVEGELNAILE comparison 2

ZIKV-BR comparison 3  
ZIKV-UG  
DENV2

ZIKV-BR DVGCSVDFSKETRCGTGVFVYNDVEAWDRYKYHPDSPRLAAAVKQAWEEGICGISSVRMENIMWKSVEGELNAILE comparison 4

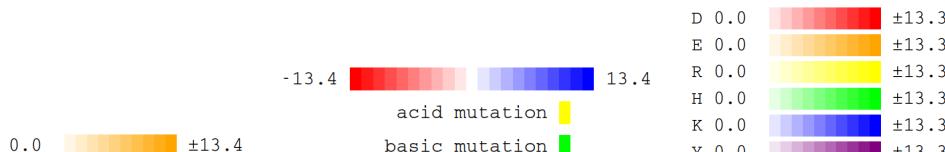
ZIKV-UG DVGCSVDFSKETRCGTGVFIYNDVEAWDRYKYHPDSPRLAAAVKQAWEEGICGISSVRMENIMWKSVEGELNAILE

DENV2 DSGCVVSWKNKELKGCGSGIFITDNVHTWTEQYKFQPEPSKLASAIQKAHEEGICGIRSVTRLENLWMWKQITPELNHILS

WNV DTGCAIDISRQELRCGSGVFIHNDVEAWMDRYKYPETPQGLAKIIQKAHKGVCGLRSVSRLEQQMWEAVKDELNTLLK

ZIKV-UG DVGCSVDFSKETRCGTGVFIYNDVEAWDRYKYHPDSPRLAAAVKQAWEEGICGISSVRMENIMWKSVEGELNAILE comparison 5

(a)



(b)

(c)

(d)

Figure 6.1.1: (a) Alignment for multiple comparison method and colored through the arrays of color (dict\_col) and visualization (dict\_vis) descriptors. Not all alignment is being shown. (b) Scale for comparison 2. (c) Scale for comparison 4. (d) Scale for comparison 5.



---

The HTML output for the previous conditions invoked is displayed in Figure 6.1.1a. Make the respective relationships between the previous table and that graph. Since we are invoking DeY for comparisons 2, 4, and 5, we are obtaining, respectively, their scales in the log file (Figures 6.1.1b, 6.1.1c, and 6.1.1d). Although comparison 2 does not have any target-sequence (*i.e.*, `{'0': []}`), the gradient color that you are seeing for this case is related to the reference protein. The case of comparison 4 is similar to what we saw earlier (Figure 6.0.1b), but now the reference sequence is ZIKV-BR. Finally, see that comparison 5 has separated a different color scale for each target-residue. This is because the color descriptor `fac` is being declared for that comparison (see Section 5.4). Nevertheless, the scale is normalized based on the greater value of the result of the operation `d` that is being applied for that comparison (*i.e.*,  $\pm 13.3$ ). You can see the independent behavior for each amino acid, but for that, you need to apply special maximum restriction methods (see Section 7.13).

*Note: Whether the array of `dict_vis` is wrongly built, all descriptors will be transformed to ReY, DeN, LeY (default parameters) for each comparison and additional information about where the errors are will be displayed.*



## 7 Maximum restriction (descriptor\_mxr)

To understand how the CPRISMA feature called “maximum restriction” works, let us suppose the next declared variables in the “array\_get.py” script<sup>1</sup>:

```
descriptor_ope = { 'd' }

descriptor_col = { 'pkac' : True }

descriptor_vis = { 'ReY', 'DeY', 'LeY' }
```

Then the following command is run in terminal<sup>2</sup>:

```
cprisma -tr -ck -t
```

The HTML output for this example was already cited earlier (Figure 6.0.1b). Although this output is correct, we are not clearly observing the  $pK_a$  shifts for many target-residues. Since we have invoked the `-t` flag (see Section 3.11), our log file will show in the operation feature part, how the  $\Delta$  data is being obtained (see Figure 7.0.1 as an example). The value of  $\Delta pK_a$  highlighted in row 13 (*i.e.*,  $-13.3$ ) is based on the subtraction  $pK_{a-\text{DENV2}} - pK_{a-\text{ZIKV-UG}}$ . This value matches with a mutation, due to a genetic indel (see row 13 in Figure 3.2.1). Thus, the scale of Figure 6.0.1b is being normalized as a function of this value, which corresponds to the maximum among the entire data set. Nonetheless, the regions that should be more Red or Blue cannot display that color because their  $\Delta pK_a$  are very low with respect to the greater value. For instance, when is calculated  $|0.1^{\Delta pK_a} / -13.3^{\Delta pK_a(\max)}|$ , it is observed that  $0.1$  is  $7.5 \times 10^{-3}$  smaller than the maximum value. From the point of view of the HTML code, this is negligible and the color should be equal to White.

To improve the color displayed when is invoked a gradient by DeY (see Chapter 6), we can use a maximum restriction method like: `rm`, `ra`, `ram`, `rpi`, `rt`, `rs`, `rsm`, `rsa`, `rsam`, `rspi`, and `rspim`, through the variable `descriptor_mxr` of the script “array\_get.py” (Figure 2.2.3).

---

<sup>1</sup>For a further explanation of these variables see Chapters 4, 5, and 6, respectively.

<sup>2</sup>Most of the examples below are using this command line.



ZIKV-UG X ZIKV-BR DENV2 WNV

|    | 0    | 1    | 2    | 3    | d  |      |       |       |  |
|----|------|------|------|------|----|------|-------|-------|--|
| 0  | 3.9  | 3.9  | 3.9  | 3.8  | -> | 0.0  | 0.0   | -0.1  |  |
| 1  | 3.1  | 3.4  | 0.0  | 3.4  | -> | 0.3  | -3.1  | 0.3   |  |
| 2  | 0.0  | 0.0  | 10.5 | 0.0  | -> | 0.0  | 10.5  | 0.0   |  |
| 3  | 10.9 | 11.0 | 0.0  | 12.8 | -> | 0.1  | -10.9 | 1.9   |  |
| 4  | 10.7 | 10.7 | 10.6 | 0.0  | -> | 0.0  | -0.1  | -10.7 |  |
| 5  | 3.8  | 4.0  | 3.8  | 4.0  | -> | 0.2  | 0.0   | 0.2   |  |
| 6  | 12.6 | 12.3 | 10.7 | 12.4 | -> | -0.3 | -1.9  | -0.2  |  |
| 7  | 10.0 | 10.0 | 0.0  | 6.7  | -> | 0.0  | -10.0 | -3.3  |  |
| 8  | 0.0  | 0.0  | 3.4  | 0.0  | -> | 0.0  | 3.4   | 0.0   |  |
| 9  | 3.5  | 3.8  | 0.0  | 3.6  | -> | 0.3  | -3.5  | 0.1   |  |
| 10 | 4.0  | 4.1  | 6.8  | 4.4  | -> | 0.1  | 2.8   | 0.4   |  |
| 11 | 12.6 | 13.4 | 0.0  | 0.0  | -> | 0.8  | -12.6 | -12.6 |  |
| 12 | 3.1  | 2.7  | 4.1  | 3.5  | -> | -0.4 | 1.0   | 0.4   |  |
| 13 | 13.3 | 13.0 | 0.0  | 12.9 | -> | -0.3 | -13.3 | -0.4  |  |
| 14 | 9.6  | 9.6  | 10.0 | 9.8  | -> | 0.0  | 0.4   | 0.2   |  |
| 15 | 11.1 | 11.0 | 11.0 | 11.2 | -> | -0.1 | -0.1  | 0.1   |  |
| 16 | 10.0 | 9.7  | 0.0  | 10.2 | -> | -0.3 | -10.0 | 0.2   |  |
| 17 | 6.3  | 6.2  | 0.0  | 9.5  | -> | -0.1 | -6.3  | 3.2   |  |

Figure 7.0.1: Example of the  $pK_a$  data for each target protein and its  $\Delta pK_a$  obtained after running `d` as an operation. The number highlighted in blue refers to the maximum value for this data set. All the information has not been shown.

## 7.1 No restriction (nr)

When it is not considered maximum restrictions we declare the `descriptor_mxr` as follows:

```
descriptor_mxr = { 'nr' }3
```

Note that `nr` descriptor is a string variable inside a pair of brackets.

When we run CPRISMA, our log file will show the following information:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | nr         | NaN      | NaN     | NaN      | NaN      | NaN       | NaN      |

This statement is simply saying that the `nr` descriptor was considered for comparison 1, in the `main_0` comparison set (for now ignore the rest of the columns in that log message). Besides this table, in the maximum restriction part of the log file, you will find a list with all values for the operations invoked, like this<sup>4</sup>:

```
list_max = [0.0, 0.3, 0.0, 0.1, 0.0, 0.2, -0.3, 0.0, 0.0, 0.3, 0.1, 0.8, -0.4, -0.3, 0.0,
```

<sup>3</sup>For all alignment examples of the preceding Chapters, this was the variable executed.

<sup>4</sup>These lists only appear when `-t` flag is executed (see Section 3.11).



-0.1, -0.3, -0.1, -0.5, -0.2, 0.1, 0.0, 0.0, 0.0, 0.0, 0.1, -0.6, 0.0, 0.1, 0.0, 0.0, 0.0, 1.7, 0.2, 0.0, -0.1, 0.0, 0.1, 0.3, 0.0, 0.0, 0.0, 0.0, 0.1, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, -0.2, 0.0, 0.1, -0.5, 0.3, 0.0, 0.0, -3.1, 10.5, -10.9, -0.1, 0.0, -1.9, -10.0, 3.4, -3.5, 2.8, -12.6, 1.0, -13.3, 0.4, -0.1, -10.0, -6.3, 0.7, -13.0, -1.8, 0.0, -11.0, 10.9, 6.4, -0.3, -0.8, 12.9, -0.2, -0.1, 0.0, -0.2, -3.8, 0.0, -0.2, 6.8, -4.2, 0.1, 4.2, 10.8, 0.0, 3.5, 0.0, 0.0, 0.0, -12.7, 10.2, 12.4, -12.9, 12.6, 0.0, 0.0, 11.2, 3.3, -0.2, 0.1, -0.1, 0.3, 0.0, 1.9, -10.7, 0.2, -0.2, -3.3, 0.0, 0.1, 0.4, -12.6, 0.4, -0.4, 0.2, 0.1, 0.2, 3.2, 0.7, -13.0, -12.9, 10.7, -11.0, 10.6, 6.1, 6.5, -0.9, 13.2, 0.1, -0.2, 6.8, -7.3, 7.9, 3.4, 0.1, 0.0, 7.2, 0.2, 0.0, 3.7, 4.0, 11.5, -6.7, 9.9, 10.9, -12.7, 0.0, 10.2, -0.5, 0.0, 3.6, 7.6, 4.2, -6.4, -0.1, 0.2]

A summary of this information should appear immediately, as follows:

|        | main | minimum (abs) | maximum (abs) | length |
|--------|------|---------------|---------------|--------|
| main_0 |      | 0.0           | 13.3          | 168    |

Notice that this table is showing the maximum and minimum numbers for the last list after applying the absolute value. This is because the HTML code will only work with positive numbers. Furthermore, the column `length` is showing us the total number of elements for that list ( $56^{\text{rows}} \times 3^{\text{target-proteins}} = 168$ ). As no restriction applies, all data is being considered.

*Note: Sometimes, some descriptors can be declared wrongly. To avoid that the program stops, it will always return to the `nr` descriptor (default parameter), with some additional error messages in your log file, which will guide you to correct the problem.*

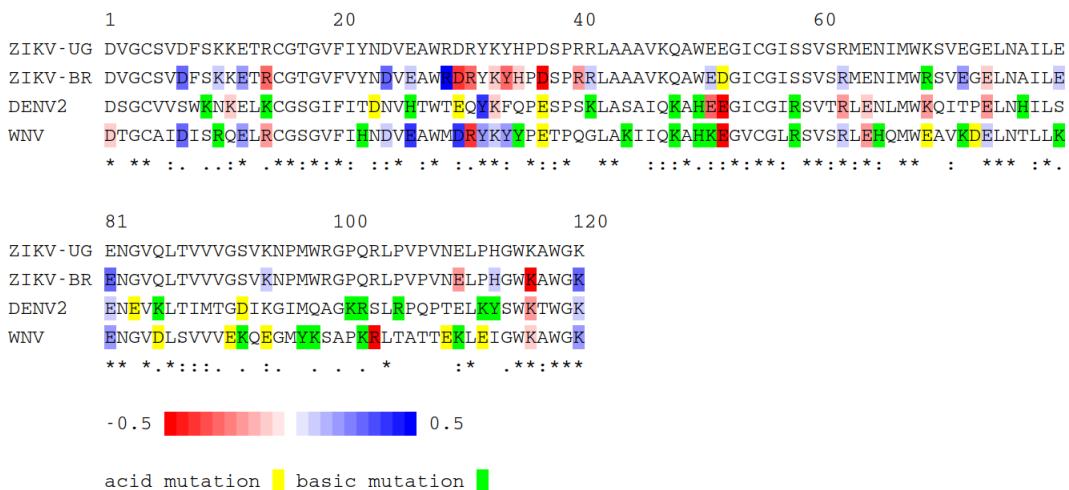


Figure 7.1.1: Alignment colored applying `rm` descriptor.



## 7.2 Restriction by mutation (rm)

For our example, notice that most of the high  $\Delta pK_a$  values correspond to regions where mutations occurred (compare Figures 3.2.1 and 7.0.1). We can declare the `rm` descriptor to avoid taking into account these “high  $\Delta pK_a$  values” through:

```
descriptor_mxr = { 'rm' }
```

After executing the program, our table for this CPRISMA feature will appear in the log file like this:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | rm         | NaN      | NaN     | NaN      | True     | NaN       | NaN      |

Notice that the `mutation` column has a Boolean equal to `True`. The list of  $\Delta pK_a$  values that are being considered to normalize the color now is reduced:

```
list_max = [0.0, 0.2, 0.0, -0.1, 0.1, 0.0, -0.1, 0.3, -0.5, 0.3, 0.0, 0.0, 0.0, 0.4, -0.1, -0.2, -0.1, -0.2, 0.1, -0.2, 0.1, -0.1, 0.2, 0.2, 0.1, 0.1, -0.2, 0.1, 0.2, -0.1, 0.2]
```

| main   | minimum (abs) | maximum (abs) | length |
|--------|---------------|---------------|--------|
| main_0 | 0.0           | 0.5           | 30     |

*Note: For the following sections, this information will not be displayed or discussed anymore. When a maximum restriction is invoked, it is understood that CPRISMA is removing or transforming the data to normalize and improve the color visual quality of the alignment.*

Changing the scale of these data allows us to observe with greater definition the color for each  $pK_a$  shifts (Figure 7.1.1). At this point, we can finally see the impact of the numerical data of the CSV input file after the  $\Delta$  operation is applied.

## 7.3 Restriction by amino acid (ra)

Suppose you do not want to consider the numerical values of some target-residues (see Section 3.1). To delete them you can invoke the `ra` descriptor, as mentioned bellow:

```
descriptor_mxr = { 'ra' : ['R', 'K'] }
```

The dictionary structure of the `ra` descriptor is composed by a key as a string (*i.e.*, ‘`ra`’) and a value as a list of target-residues (*i.e.*, `['R', 'K']`). For this example, CPRISMA is being



---

instructed to do not take into account the numerical values of the row where Arg and Lys residues match in the processed data (for instance, row 2, 3, and 4 in Figure 3.2.1).

*Note: When the `ra` descriptor is run, CPRISMA will looks for all the rows where the residue declared appears and the program will “remove” the entire row if it matches. Later, CPRISMA will calculate the maximum from these transformations. However, it is possible to use other specific descriptors like `rsa` (see Section 7.9) to have a more refined definition of maximum restriction.*

The log file will display the following information:

```
main  comparison  descriptor  sequence      residue   position    mutation   threshold  separate
main_0        1          ra        NaN     ['R', 'K']       NaN        NaN        NaN        NaN
```

See that the `residue` column is showing the list declared. The color scale is now normalized based on this condition.

## 7.4 Restriction by amino acid and mutation (ram)

The numerical values of target-residues and mutations can simultaneously constrained by means of:

```
descriptor_mxr = { 'ram' : ['R', 'K'] }
```

The `ram` descriptor is a combination of `rm` (see Section 7.2) and `ra` (see Section 7.3), therefore it will follow the same rules as for those descriptors.



Figure 7.4.1: Scale example invoking `ram` descriptor.

The log file will display the following information:

```
main  comparison  descriptor  sequence      residue   position    mutation   threshold  separate
main_0        1          ram        NaN     ['R', 'K']       NaN       True        NaN        NaN
```



---

Notice that the `mutation` column has a Boolean equal to `True`. Removing rows with Arg and Lys residues has changed the color scale. Now, it is displays a maximum of  $\pm 0.4$  instead of  $\pm 0.5$  (Figures 7.4.1 and 7.1.1, respectively).

## 7.5 Restriction by index position (rpi)

To have a maximum restriction for the exact position of specific operation values, the variable `descriptor_mxr` can be declared like this:

```
descriptor_mxr = { 'rpi' : [13, 45, 48] }
```

The `rpi` descriptor has a dictionary structure similar to `ra` (see Section 7.3). Although the list has numbers that match with the row index of the processed data (Figures 3.2.1 and 7.0.1), instead to target-residues. Remember, the numbers of the list should be related to that index and not the alignment position!

*Note: When the `rpi` descriptor is typed, CPRISMA will looks for all the rows based on the index positions declared and the program will “remove” the entire row if matches. Later, CPRISMA will calculate the maximum from these transformations. Nevertheless, it is possible to use other specific descriptors like `rspi` (see Section 7.11) to have a more refined definition of maximum restriction.*

The log file will display the following information:

| main   | comparison | descriptor | sequence | residue | position     | mutation | threshold | separate |
|--------|------------|------------|----------|---------|--------------|----------|-----------|----------|
| main_0 | 1          | rpi        | NaN      | NaN     | [13, 45, 48] | NaN      | NaN       | NaN      |

See that in the `position` column, the list declared for the `rpi` descriptor appears. Based on this, the color scale will be normalized.

## 7.6 Restriction by threshold (rt)

A maximum restriction based on a threshold can be invoked through:

```
descriptor_mxr = { 'rt' : [0.0, '>='] }
```

Look that `rt` descriptor has a dictionary structure. The value of the dictionary is a list with two elements where the first shows a numerical value (float type), meanwhile the second is a



---

string that refers to a symbol of order relation (*i.e.*, ‘ $\geq$ ’, ‘ $\leq$ ’, ‘ $>$ ’, ‘ $<$ ’, ‘ $\equiv$ ’, ‘ $\neq$ ’). Based on our example, we are telling the program to only take into account those rows with values greater equal than 0.0. Whether the row presents any number in any column that does not meet that condition, then the value will be converted to 0.0 immediately.

*Note:* Occasionally, too strict threshold can be invoked and this could cause all values to be ignored. To avoid the program stops, by default, CPRISMA will normalize everything based on 1.0.

The table when invoked `rt` descriptor looks like this:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold      | separate |
|--------|------------|------------|----------|---------|----------|----------|----------------|----------|
| main_0 | 1          | rt         | NaN      | NaN     | NaN      | NaN      | [0.0, $\geq$ ] | NaN      |

When `-t` flag is invoked (see Section 3.11), the log file will display the following information:

| main   | minimum (abs) | maximum (abs) | length |
|--------|---------------|---------------|--------|
| main_0 | 0.0           | 13.2          | 168    |

See that the `length` column reports the same amount of data as when we have no restriction (*i.e.*, when `nr` is applied [see Section 7.1]). This is because all values that do not satisfy the threshold condition are being converted to 0.0 and are not being “eliminated”.

Finally, the new maximum for the color scale will be 13.2. Our maximum constraint has not changed much despite the conversion of the highest negative value (*i.e.*, from  $-13.3$  to 0.0). As the threshold condition cited above does not take into account values less than 0.0, the problem persists because, even with this restriction, the data still have “high” positive values like 13.2.

*Note:* Based on the last statement, it is important to mention that `rt` descriptor is more suitable for data with only one type of mathematical sign. For that, you could use `da` as an operation descriptor (see Section 4.3).

## 7.7 Restriction by sequence (rs)

In CPRISMA you can not consider the data of a whole sequence by invoking `descriptor_mxr` like this:



---

```
descriptor_mxr = { 'rs' : [2] }
```

The dictionary structure of the **rs** descriptor is similar to **ra** (see Section 7.3) or **rpi** (see Section 7.5). But now, the number in the list refers to a target-sequence.

The log file will display the following information:

```
main   comparison   descriptor   sequence   residue   position   mutation   threshold   separate
main_0       1           rs         [2]        NaN        NaN        NaN        NaN        NaN
```

For our example, we are not taking into account the data of the DENV2 sequence. Based on this, the color scale will be normalized.

*Note: Regarding the descriptor **rs**, the pair comparison method, array of comparisons like `{# : []}`, or cases where `-ck` is not invoked, CPRISMA will always return to the default value of the maximum restriction feature (i.e., `nr`).*

If you want to restrict the maximum values of target-residues or row position index from a specific sequence, we recommend Sections 7.9 and 7.11, respectively.

## 7.8 Restriction by sequence and mutation (**rsm**)

The data from a sequence and the places where mutations appear can simultaneously “removed” by means of:

```
descriptor_mxr = { 'rsm' : [2] }
```

The descriptor **rsm** is a combination of **rm** (see Section 7.2) and **rs** (see Section 7.7), so the same rules will apply for it.

The log file will display the following information:

```
main   comparison   descriptor   sequence   residue   position   mutation   threshold   separate
main_0       1           rsm         [2]        NaN        NaN       True        NaN        NaN
```

Notice that the **mutation** column has a Boolean equal to `True`. The scale of your HTML output will be normalized based on these parameters.



## 7.9 Restriction by sequence and amino acid (`rsa`)

Sometimes, it could be very radical to apply `ra` (see Section 7.3) due to many rows with useful values may be being removed. Nevertheless, to focus only on target-residues of a specific sequence, the `rsa` descriptor can be invoked. To declare this maximum restriction descriptor you can do it as follows:

```
descriptor_mxr = { 'rsa' : [ { '1' : ['R', 'K'] }, { '2' : ['D', 'Y'] } ] }
```

Notice that we saw this array structure before (see Subsection 3.10.3). Now, our value is a list of dictionaries. Each second-key represents the target-sequences of interest (*i.e.*, ‘1’ and ‘2’) and the second-values are the lists of target-residues to be discard (*i.e.*, ['R', 'K'] and ['D', 'Y'], respectively). For our example, CPRISMA is being instructed to just consider the sequences of ZIKV-BR (‘1’) and DENV2 (‘2’) and “to delete” the Arg and Lys residues; and the Asp and Tyr residues of that proteins, respectively. The descriptor `rsa` follows similar rules like `ra` but just examining the target-residues of the target-sequence of interest.

The log file will display the following information:

| main   | comparison | descriptor | sequence | residue    | position | mutation | threshold | separate |
|--------|------------|------------|----------|------------|----------|----------|-----------|----------|
| main_0 | 1          | rsa        | [1]      | ['R', 'K'] |          | NaN      | NaN       | NaN      |
| main_0 | 1          | rsa        | [2]      | ['D', 'Y'] |          | NaN      | NaN       | NaN      |

The color scale will be normalized based on these conditions.

*Note: Regarding the descriptor `rsa`, the pair comparison method, array of comparisons like `{# : []}`, or cases where `-ck` is not invoked, CPRISMA will always return to the default value of the maximum restriction feature (*i.e.*, `nr`).*

## 7.10 Restriction by sequence, amino acid, and mutation (`rsam`)

We can use a version of `rsa` that also restricts mutations simultaneously. For that, we invoke the maximum restriction variable by means of:

```
descriptor_mxr = { 'rsam' : [ { '1' : ['R', 'K'] }, { '2' : ['D', 'Y'] } ] }
```

The `rsam` is a combination of `rm` and `rsa` descriptors (see Sections 7.2 and 7.9, respectively), so it will follow similar rules as them. For this case, `rsam` will not take into account the mutations



---

that occur between the target-sequence of the second-keys [for our example, ‘1’ (ZIKV-BR) and ‘2’ (DENV2)] and the reference sequence [for our example, ‘0’ (ZIKV-UG)].

The log file will display the following information:

| main   | comparison | descriptor | sequence | residue    | position | mutation | threshold | separate |
|--------|------------|------------|----------|------------|----------|----------|-----------|----------|
| main_0 | 1          | rsa        | [1]      | ['R', 'K'] | NaN      | True     | NaN       | NaN      |
| main_0 | 1          | rsa        | [2]      | ['D', 'Y'] | NaN      | True     | NaN       | NaN      |

Notice that the `mutation` column has a Boolean equal to `True`. The color scale will be normalized based on these conditions.

## 7.11 Restriction by sequence and index position (`rspi`)

The `rspi` descriptor is similar to `rsa`, however, the second-value must have the index positions of the processed data (see the index in Figure 7.0.1). To declare `rspi`, we can do it by means of:

```
descriptor_mxr = { 'rspi' : [ { '1' : [0, 29] } , { '2' : [48] } ] }
```

The `rspi` descriptor shares the same rules as `rpi` (see Section 7.5) and `rsa` (see Section 7.9). For our example, CPRISMA is being instructed to just “delete” the positions 0 and 29 of ZIKV-BR (‘1’) and 48 of DENV2 (‘2’).

The log file will display the following information:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | rspi       | [1]      | NaN     | [0, 29]  | NaN      | NaN       | NaN      |
| main_0 | 1          | rspi       | [2]      | NaN     | [48]     | NaN      | NaN       | NaN      |

The color scale will be normalized based on these conditions.

*Note: Regarding the descriptor `rspi`, the pair comparison method, array of comparisons like `{# : []}`, or cases where `-ck` is not invoked, CPRISMA will always return to the default value of the maximum restriction feature (i.e., `nr`).*



---

## 7.12 Restriction by sequence, index position, and mutation (rspim)

The version of `rspi` that restricts places where point mutations occur is called `rspim` and can be declared as follows:

```
descriptor_mxr = { 'rspim' : [ { '1' : [0, 29] } , { '2' : [48] } ] }
```

The `rspim` is a combination of `rm` and `rspi` descriptors (see Sections 7.4 and 7.11, respectively), so it will follow similar rules as them<sup>5</sup>.

The log file will display the following information:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | rspim      | [1]      | NaN     | [0, 29]  | True     | NaN       | NaN      |
| main_0 | 1          | rspim      | [2]      | NaN     | [48]     | True     | NaN       | NaN      |

Notice that the `mutation` column has a Boolean equal to `True`. The scale of your HTML output will be normalized taking into account these parameters.

## 7.13 Restrictions by target-residues (Y)

Sometimes some target-residues have more influence than others when we apply a maximum restriction method. We can evaluate the independent maximum behavior for each target-residue by adding the letter '`Y`' to our descriptor in the following way:

```
descriptor_mxr = { 'nrY' }
```

When we run CPRISMA, our log file will show the following information:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | nr         | NaN      | NaN     | NaN      | NaN      | NaN       | True     |

Notice that the `separate` column is showing a Boolean variable as `True`. Whether `-t` flag is invoked (see Section 3.11), the log file will display the following information:

---

<sup>5</sup>See other considerations on how numerical data is removed at the places where mutations happen in Section 7.10 (although `rsam` and `rspim` are different maximum restriction methods they follow the same pattern of execution)



---

|        | main | minimum (abs) |                                  | maximum (abs) | length |
|--------|------|---------------|----------------------------------|---------------|--------|
| main_0 |      | 0.0           | [3.7, 7.3, 13.2, 6.8, 11.5, 9.9] |               | NaN    |

Now, the **maximum (abs)** column is displaying the maximum for each target-residue. In other words, the values for each amino acid will be normalized according to their respective maxima. This list and its order will match according to the target-residues that were declared in the tuple `target_residues` (see Section 3.1). The alignment of the HTML file for this new condition appears in Figure 7.13.1. Note that the scale for each target-residue can be observed. A first impression may seem like there is no difference between what we got earlier (Figure 6.0.1b). Although some regions are fainter in color (see some amino acids between positions 20 and 40 on the alignment). The intensity of the color is still not enough because of the maximum values are still very high due to the presence of mutations.

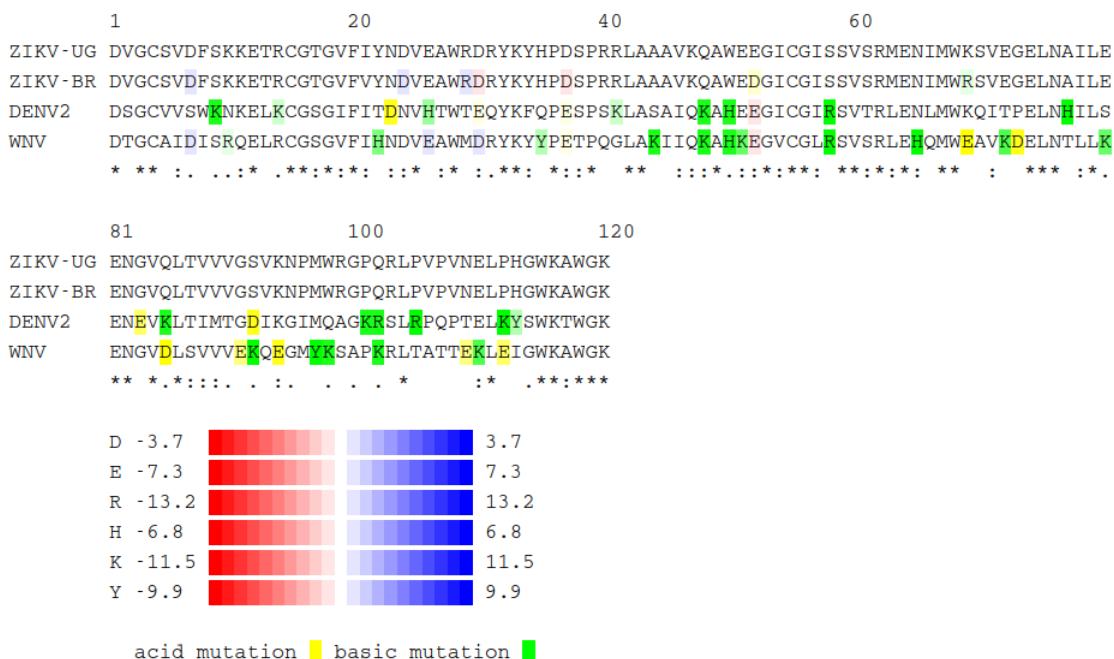


Figure 7.13.1: Alignment colored applying `nr` descriptor and the condition `Y` to separate the maximum per target-residue.

We can confirm this by invoking the color descriptor like this:

```
descriptor_col = { 'fac' : [6, 20, 22, 54, 94, 46] }
```



Based on that, we can clearly see the influence of the maximum for each target-residue (Figure 7.13.2). Furthermore, check how the most intense colors match with mutations.

Finally, you can test different descriptors and observe the behavior separately based on maximums for each target-residue. The base structure for invoking these conditions is shown as follows:

```
descriptor_mxr = { 'maximum_descriptorY' }  
  
descriptor_mxr = { 'maximum_descriptorY' : additional_parameters }
```

Note that we are considering situations where descriptors can be sets (e.g. `nr`, `rm`, and so on) or dictionaries (e.g., `rt`, `rsa`, `rspi`, and so on), respectively.

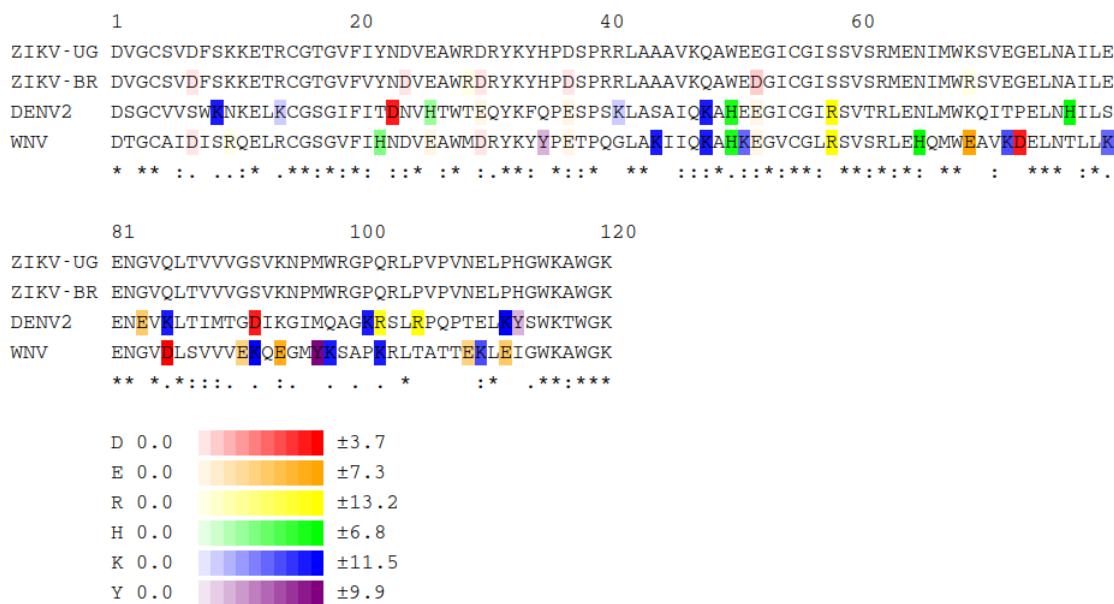


Figure 7.13.2: Alignment colored applying `nr` descriptor and the condition `Y` to separate the maximum per target-residue but using `fac` descriptor.

*Note: We recommend the user to test different combinations of maximum restriction and color (see Chapter 5) descriptors, to examine the impact of each case. Keep in mind the same rules for each maximum restriction descriptor that we saw previously.*



## 7.14 Maximum restriction descriptors dictionary (-dmx)

When the `multiple` comparison method is declared, it is possible that you need to distinguish each reference/target(s) relationship with a specific maximum restriction descriptor. For that, we must build an array of maximum descriptors through the `dict_mxr` variable of the script “array\_get.py”. To build it, you should follow the same rules as for `dict_ope` (see Section 4.5). For this example, we used the same dictionary for the `multiple` method as a base (see Subsection 3.10.3). The CPRISMA maximum-restriction-array will be declared as follows<sup>6</sup>:

```
dict_mxr = { 'main_0' : [ { 'rmY' } , { 'nr' } ] ,  
            'main_1' : [ { 'rspim' : [ { '2' : [ 47, 48, 50 ] } ] } ,  
                         { 'rsaY' : [ { '0' : [ 'R' ] } , { '3' : [ 'D' ] } ] }  
            ,  
            'main_2' : [ { 'rt' : [ 0, '>=' ] } ] }
```

Now we can execute the `-dmx` flag to call the array `dict_mxr`<sup>7</sup>:

```
cprisma -tr -ck -rf multiple -dmx
```

The log file will show the relationships between each comparison and maximum restriction descriptors:

Comparing `dict_ref` with `dict_mxr`:

```
{'0': [1, 2, 3]} ..... { 'rmY' }  
{'0': []} ..... { 'nr' }  
{'1': [0, 2]} ..... { 'rspim' : ... }  
{'1': [0, 2, 3]} ..... { 'rsaY' : ... }  
{'2': [0]} ..... { 'rt' : [0, '>=' ] }
```

The array of the feature ‘maximum’ `dict_mxr` is compatible with the array of comparison sequences `dict_ref`!

<sup>6</sup>Note that we have differentiated each maximum restriction descriptor for each comparison with a different color to aid the reader.

<sup>7</sup>Notice that additional parameters are being implemented.



---

... and a table with a summary for all maximum descriptor information per comparison:

| main   | comparison | descriptor | sequence | residue | position | mutation | threshold | separate |
|--------|------------|------------|----------|---------|----------|----------|-----------|----------|
| main_0 | 1          | rmY        | NaN      | NaN     | NaN      | True     | NaN       | True     |
| main_0 | 2          | nr         | NaN      | NaN     | NaN      | NaN      | NaN       | NaN      |
| main_1 | 3          | rspim      | 2        | NaN     | [47...]  | True     | NaN       | NaN      |
| main_1 | 4          | rsaY       | 0        | [R]     | NaN      | NaN      | NaN       | True     |
| main_1 | 4          | rsaY       | 3        | [D]     | NaN      | NaN      | NaN       | True     |
| main_2 | 5          | rt         | NaN      | NaN     | NaN      | NaN      | [0, >=]   | NaN      |

*Note: If the array of dict\_mxr is wrongly built, all descriptors will be transformed to nr (default parameter) for each comparison and additional information about where the errors are will be displayed.*



## 8 Practical Examples

The following examples bring together some of the features that we described earlier. Each is related to a previously published study [1, 2]. Furthermore, in the “examples” directory, you will find the scripts, alignments and other files that help as the basis for achieving the desired visualizations. Many details will not be given as we wish to motivate the reader to test and explore the characteristics of each example on their own. Each case follows the next order:

1. Command-line invoked.
2. Declared variables (as shown in Figure 2.2.3).
3. An image of the alignment.

Each example tries to approximate a certain image from a specific publication. In example 1 (see Section 8.1) we try to reproduce Figure 8 [1], in example 2 (see Section 8.2) Figure 5 (only chain A) [2], and in example 3 (see Section 8.3) Figure 1a [3].

*Note: For more details on the methodological aspects, data collection, purpose, and others, you can find them in the respective publication.*

### 8.1 Example 1: $\Delta pK_a$ for several NS1<sub>ZIKV</sub>

Command-line:

```
cprisma -tr -ns -ck -rf multiple -va 2 -j -sco 142 -dop -dco -dvi -dmx
```

Variables:

```
target_residues = ('D', 'E', 'R', 'H', 'K', 'Y')

name_sequence = ('UG_chA_x-ray', 'UG_chB_x-ray', 'UG_chA_mod1', 'UG_chA_mod2',
'SE_chA_mod1', 'CAR_chA_mod1', 'MA_chA_mod1', 'TH_chA_mod1', 'YAP_chA_mod1',
'BR_chA_x-ray', 'BR_chB_x-ray', 'BR_chA_mod1', 'BR_chA_mod2', '')

descriptor_ope = {'n'}
```



```
descriptor_col = { 'nc' }  
descriptor_vis = { 'ReY', 'DeN', 'LeY' }  
descriptor_mxr = { 'nr' }
```

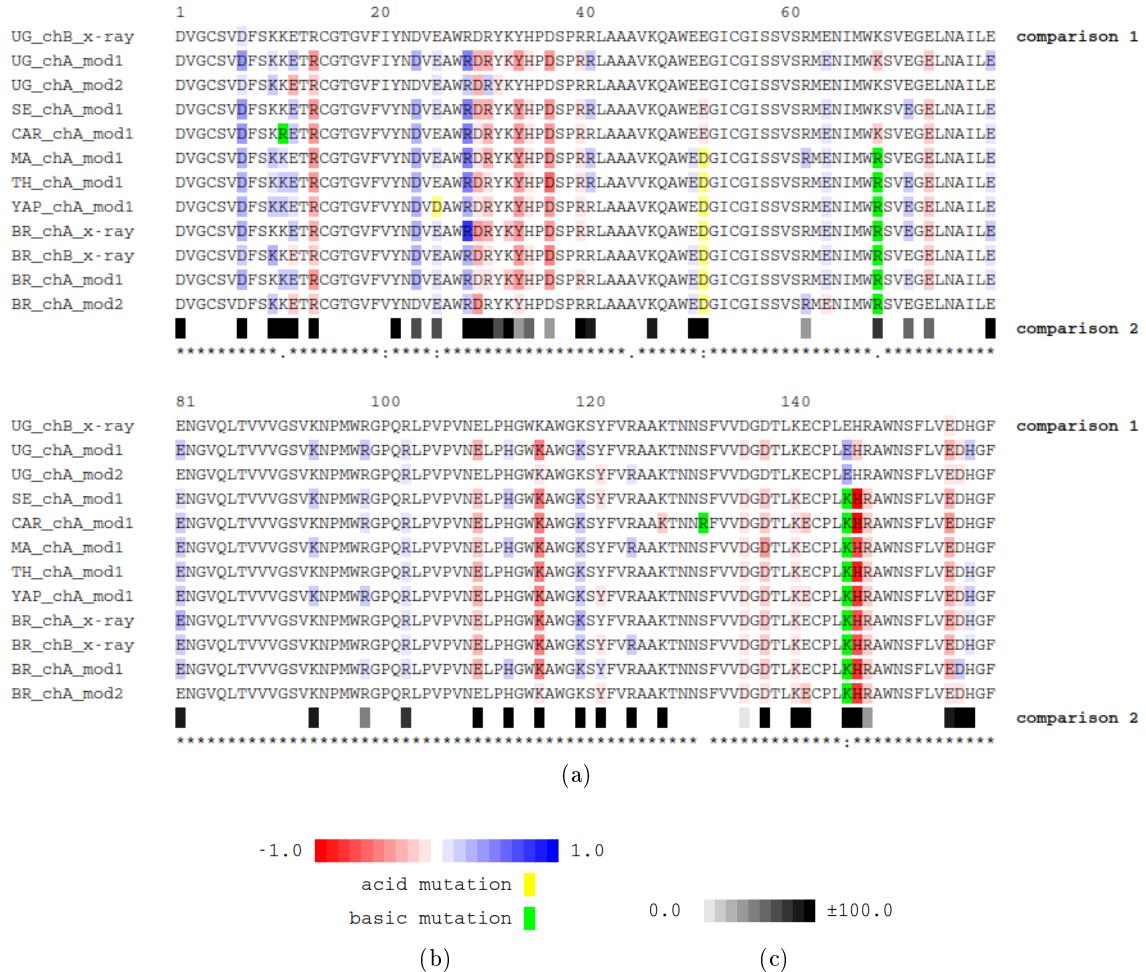


Figure 8.1.1: (a) Alignment for Example 1. Not all alignment is being shown. (b) Scale for comparison 1. (c) Scale for comparison 2.

```
dict_ref = { 'main_0' : [ { '0' : [ 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 ] } ] ,  
            'main_13' : [ { '13' : [ ] } ] }
```



---

```
dict_ope = { 'main_0' : [ { 'd' } ] ,  
            'main_13' : [ { 'n' } ] }  
  
dict_col = { 'main_0' : [ { 'pkac' : True } ] ,  
            'main_13' : [ { 'ssc' } ] }  
  
dict_vis = { 'main_0' : [ { 'ReN', 'DeY', 'LeY' } ] ,  
            'main_13' : [ { 'ReY', 'DeN', 'LeN' } ] }  
  
dict_mxr = { 'main_0' : [ { 'rm' } ] ,  
            'main_13' : [ { 'nr' } ] }
```

From these input parameters, we obtain the alignment that appears in Figure 8.1.1. Note that the last element of the tuple `name_sequence` does not have a name. Due to that, the alignment will not display any name for that sequence.

## 8.2 Example 2: B-cell epitope predictions on NS1<sub>WNV(176–352)</sub>

Command-line:

```
cprisma -tr -ck -rf multiple -ns -va 2 -j -hc -n 176 -sco 142 -a 3 -dop -dco  
-dvi
```

Variables

```
target_residues = ('D', 'E', 'R', 'H', 'K', 'Y')  
  
name_sequence = ('SASA', 'pKa_NS1 (alone)', 'PROCEEDpKa', 'PISA', 'DiscoTope',  
                 'ElliPro', 'SEPPA2', 'SEPPA3', 'Consensus', '')  
  
descriptor_ope = { 'n' }  
  
descriptor_col = { 'nc' }  
  
descriptor_vis = { 'ReY', 'DeN', 'LeY' }  
  
descriptor_mxr = { 'nr' }  
  
dict_ref = { 'main_0' : [ {'0' : [] } ] ,  
            'main_1' : [ {'1' : [2] } ] ,
```



---

```
'main_3' : [ {'3' : []} ] ,
'main_4' : [ {'4' : []} ] ,
'main_5' : [ {'5' : []} ] ,
'main_6' : [ {'6' : []} ] ,
'main_7' : [ {'7' : []} ] ,
'main_8' : [ {'8' : []} ] ,
'main_9' : [ {'9' : []} ] }

dict_ope = { 'main_0' : [ { 'n' } ] ,
'main_1' : [ { 'da' } ] ,
'main_3' : [ { 'n' } ] ,
'main_4' : [ { 'n' } ] ,
'main_5' : [ { 'n' } ] ,
'main_6' : [ { 'n' } ] ,
'main_7' : [ { 'n' } ] ,
'main_8' : [ { 'n' } ] ,
'main_9' : [ { 'n' } ] }

dict_col = { 'main_0' : [ { 'tc' : [54, 0, '>'] } ] ,
'main_1' : [ { 'tc' : [6, 0.007, '>'] } ] ,
'main_3' : [ { 'tc' : [75, 0, '>'] } ] ,
'main_4' : [ { 'tc' : [20, -6.85, '>='] } ] ,
'main_5' : [ { 'tc' : [64, 0, '>'] } ] ,
'main_6' : [ { 'tc' : [22, 0.049, '>='] } ] ,
'main_7' : [ { 'tc' : [96, 0.093, '>='] } ] ,
'main_8' : [ { 'tc' : [92, 0, '>'] } ] ,
'main_9' : [ { 'ssc' } ] }
```

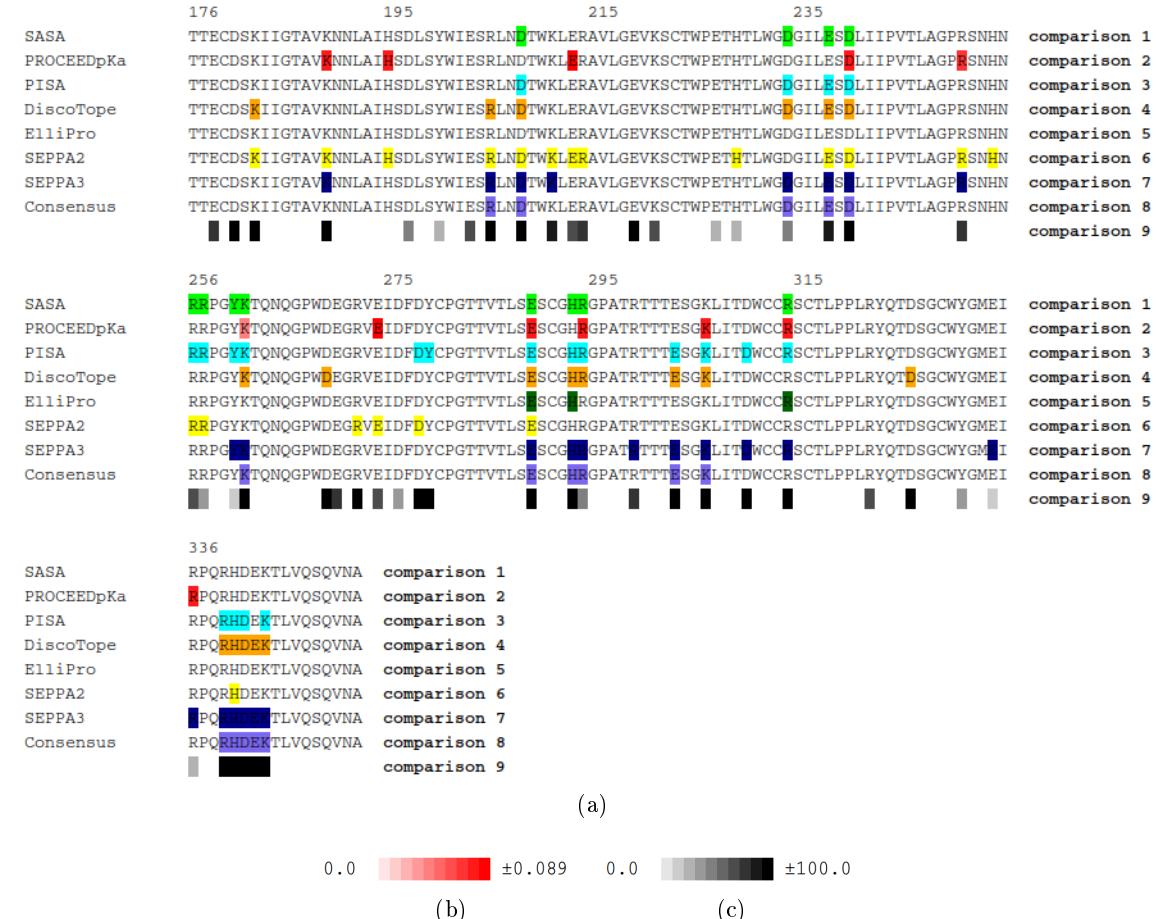


Figure 8.2.1: (a) Alignment for Example 2. (b) Scale for comparison 2. (c) Scale for comparison 9.

```
dict_vis = { 'main_0' : [ { 'ReY', 'DeN', 'LeY' } ] ,
             'main_1' : [ { 'ReN', 'DeY', 'LeY' } ] ,
             'main_3' : [ { 'ReY', 'DeN', 'LeY' } ] ,
             'main_4' : [ { 'ReY', 'DeN', 'LeY' } ] ,
             'main_5' : [ { 'ReY', 'DeN', 'LeY' } ] ,
             'main_6' : [ { 'ReY', 'DeN', 'LeY' } ] ,
             'main_7' : [ { 'ReY', 'DeN', 'LeY' } ] ,
```




---

```

'main_8' : [ { 'ReY', 'DeN', 'LeY' } ] ,
'main_9' : [ { 'ReY', 'DeY', 'LeN' } ] }

dict_mxr={}

```

As we are implementing the `tc` color descriptor (see Section 5.8) and the numerical data is dependent on the threshold, we recommend taking care with the accuracy. The output for example 2 appears in Figure 8.2.1. To obtain this outcome, we have considered an accuracy of 3 decimal places with the `-a` flag (see Section 3.2).

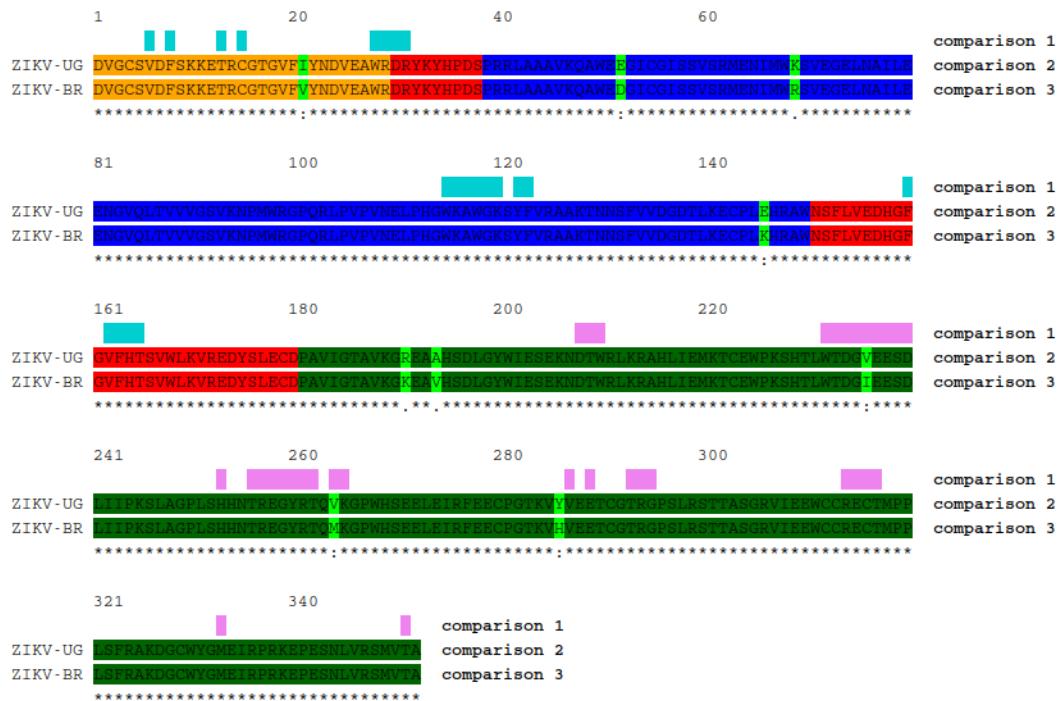


Figure 8.2.2: Alignment for Example 3.

### 8.3 Example 3: Protein structural domains for two $\text{NS1}_{\text{ZIKV}}$ and its biological interfaces

Command-line:

```
cprisma -va 2 -j -ns -ck -rf multiple -mco 54 -dco -dvi
```



---

Variables:

```
target_residues = ()  
  
name_sequence = ('', 'ZIKV-UG', 'ZIKV-BR')  
  
descriptor_ope = { 'n' }  
  
descriptor_col = { 'nc' }  
  
descriptor_vis = { 'ReY', 'DeN', 'LeY' }  
  
descriptor_mxr = { 'nr' }  
  
dict_ref = { 'main_0' : [ {'0' : []} ] ,  
            'main_2' : [ {'2' : [1]} ] ,  
            'main_1' : [ {'1' : [2]} ] }  
  
dict_ope = {}  
  
dict_col = { 'main_0' : [ { 'pic' : [ { '0' : [81, 'n', '5', '7', '12', '14',  
                                         '27-30', '114-119', '121-122',  
                                         '159', '161-164'] } ,  
                           { '0' : [35, 'n', '207-209', '231-239',  
                                     '252', '255-261', '263-264',  
                                     '286', '288', '292-294',  
                                     '313-316', '332', '350'] } ] } ,  
  
            'main_2' : [ { 'pimc' : [ { '1' : [20, 'n', '0-28'] } ,  
                                      { '1' : [6, 'n', '29-37'] } ,  
                                      { '1' : [94, 'n', '38-149'] } ,  
                                      { '1' : [6, 'n', '150-179'] } ,  
                                      { '1' : [64, 'n', '180-351'] } ] } ] ,  
  
            'main_1' : [ { 'pimc' : [ { '2' : [20, 'n', '0-28'] } ,  
                                      { '2' : [6, 'n', '29-37'] } ] } ] ,
```



---

```
{ '2' : [94, 'n', '38-149'] } ,  
{ '2' : [6, 'n', '150-179'] } ,  
{ '2' : [64, 'n', '180-351'] } ] } }  
  
dict_vis = { 'main_0' : [ { 'ReY', 'DeN', 'LeN' } ] ,  
             'main_2' : [ { 'ReN', 'DeN', 'LeY' } ] ,  
             'main_1' : [ { 'ReN', 'DeN', 'LeY' } ] }  
  
dict_mxr = {}
```

The output for example 3 appears in Figure 8.2.2. See that for `pimc` descriptor to distinguish the mutations (see Section 5.7), the `main`'s 1 and 2 in `dict_ref` have the sequences exchanged and we have hidden the references with the `ReN` visualization method (see Chapter 6).



## Bibliography

- [1] Sergio Alejandro Poveda-Cuevas, Catherine Etchebest, and Fernando Luís Barroso da Silva. Insights into the ZIKV NS1 virology from different strains through a fine analysis of physicochemical properties. *ACS omega*, 3(11):16212–16229, 2018.
- [2] Sergio Alejandro Poveda-Cuevas, Catherine Etchebest, and Fernando Luís Barroso da Silva. Identification of electrostatic epitopes in flavivirus by computer simulations: The PROCEEDpKa method. *Journal of Chemical Information and Modeling*, 60(2):944–963, 2020.
- [3] Sergio Alejandro Poveda-Cuevas, Fernando Luís Barroso da Silva, and Catherine Etchebest. How the strain origin of ZIKV NS1 protein impacts its dynamics and implications to their differential virulence. *Journal of Chemical Information and Modeling*, 61(3):1516–1530, 2021.
- [4] Fernando Luís Barroso da Silva and Donal MacKernan. Benchmarking a fast proton titration scheme in implicit solvent for biomolecular simulations. *Journal of Chemical Theory and Computation*, 13:2915–2929, 2017.
- [5] Fernando Luís Barroso da Silva and Luis Gustavo Dias. Development of constant-pH simulation methods in implicit solvent and applications in biomolecular systems. *Biophysical Reviews*, 9(5):699–728, 2017.
- [6] Robert Edgar. MUSCLE a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5(1):113, 2004.

*“One should never try to prove anything that is not almost obvious”*

Alexander Grothendieck