

Nucleic Acids Flexibility

Tutorial, v1.0

Table of Contents

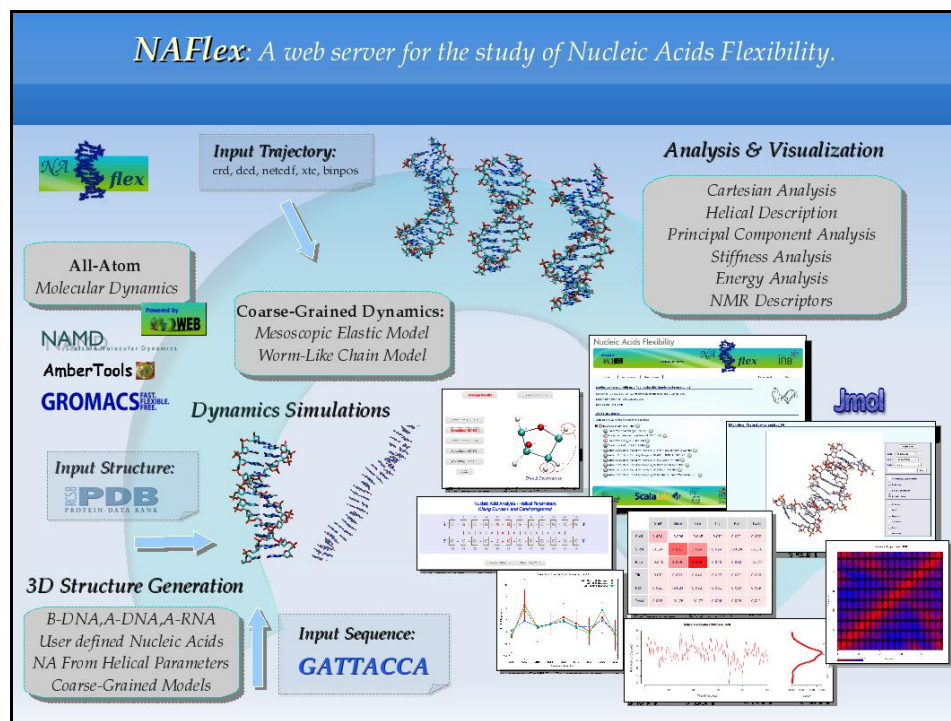
NAFlex Tutorials	3
Atomistic Molecular Dynamics Setup Tutorial	3
1.- Registration	4
2.- Starting Project	5
3.- Checking the structure	6
4.- Structure Setup	7
5.- Waiting Results	8
6.- Getting Results	10
Nucleic Acids Flexibility Analysis Tutorial	11
1.- Helical Parameters	13
2.- Stiffness Constants.....	15
3.- Principal Component Analysis	17
4.- Nuclear Magnetic Resonance Observables: J-Coupling	18
5.- Nuclear Magnetic Resonance Observables: NOE's.....	20
6.- Canonical Hydrogen Bonds Analysis	24
7.- Atom Pairs Distances.....	26
8.- HB/Stacking Energies.....	27
9.- Distance Contact Maps.....	29
Coarse-Grained Trajectory Analysis Tutorial.....	30
1.- Coarse-Grained Elastic Mesoscopic Model Flexibility Analysis	31
2.- Coarse-Grained Worm-Like Chain Model Flexibility Analysis.....	33

NAFlex Tutorials

NAFlex Tutorials

NAFlex is an on-line web server that offers a variety of simulation methods to obtain a dynamic view of a Nucleic Acid Structures and also a set of analysis packages for mining the resulting nucleic acids trajectories. In this help section user can find a set of tutorials explaining step by step what to do for using the most important functionalities of the server:

- **Atomistic Molecular Dynamics Setup Tutorial:**
How to properly prepare a nucleic acid structure in order to use it in a Molecular Dynamics Simulation.
- **Nucleic Acids Flexibility Analysis Tutorial:**
How to run the set of different Flexibility analysis offered by the server from a Molecular Dynamics trajectory: Helical Parameters, Principal Components (PCA), HB/Stacking energies, NMR-observables, etc.
- **Coarse-Grained Trajectory Analysis Tutorial:**
How to run the set of Flexibility analysis offered by the server from a Coarse-Grained generated trajectory: Distance Contact Maps and Stiffness Constants (the later only for Elastic Mesoscopic Model).



NAFlex Overview

NAFlex Atomistic Molecular Dynamics Setup Tutorial

NAFlex Atomistic Molecular Dynamics Setup Tutorial

NAFlex provides a friendly environment to setup new systems and run test simulations. With this short tutorial, you will be able to prepare a molecule to run a molecular dynamics simulation, following just a few steps.

1. Registration
2. Starting Project
3. Checking the Structure
4. Structure Setup
5. Waiting Results
6. Getting Results

Tutorial Steps

1. Registration

The first thing to do is choose between working as an *anonymous* user or alternatively as a *registered* user. We **strongly** recommend working as a registered user, as it has some important advantages.

Anonymous user's projects are completely removed once the user is disconnected and also when session expires (after some minutes of inactivity), and therefore working as anonymous user is only suited for a first impression of the web server.

Registration process will just take a minute --> Registration.

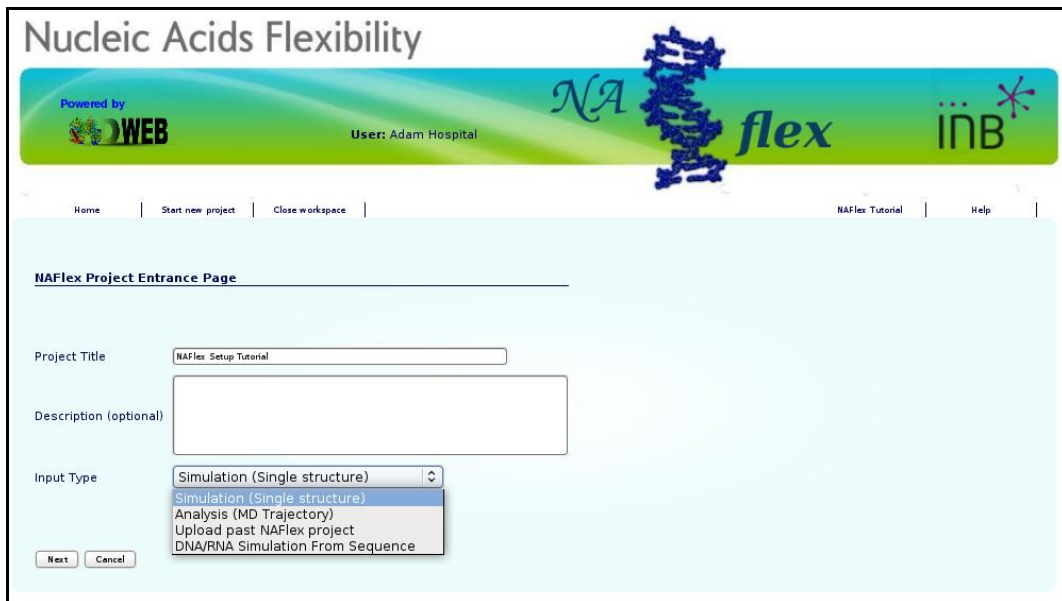
Once logged in, the user **workspace** appears. In this **workspace**, all projects of the user will be shown.

Now, we are ready to start our first NAFlex project.



2. Starting Project

NAFlex user can choose between four different kind of inputs, *Simulation*, *Analysis*, *Upload a past NAFlex project* and *DNA/RNA simulation from sequence*. In this tutorial, we will see an example of a Setup (Simulation) project.

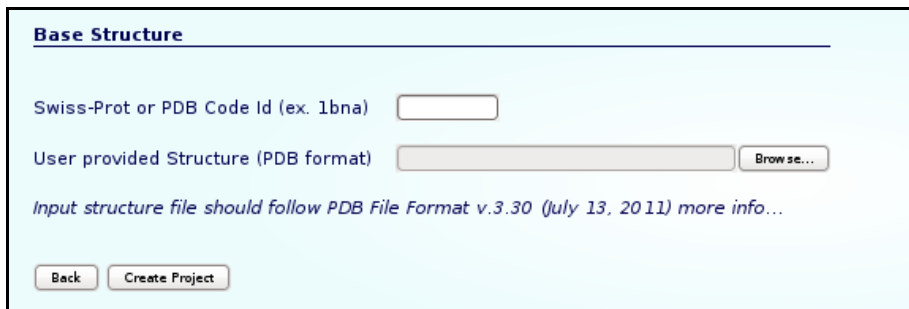


The screenshot shows the NAFlex Project Entrance Page. The header includes the text "Nucleic Acids Flexibility" and "Powered by OWEB". The user is identified as "User: Adam Hospital". The page features a navigation menu with "Home", "Start new project", "Close workspace", "NAFlex Tutorial", and "Help". The main content area is titled "NAFlex Project Entrance Page" and contains the following fields:

- Project Title:
- Description (optional):
- Input Type: A dropdown menu with the following options:
 - Simulation (Single structure) (selected)
 - Simulation (Single structure)
 - Analysis (MD Trajectory)
 - Upload past NAFlex project
 - DNA/RNA Simulation From Sequence

At the bottom of the form are "Next" and "Cancel" buttons.

You will be asked for just three easy inputs: a project title, an optional description of the project, and an input structure, either as a **PDB code / Swiss-Prot code** or directly as a **PDB structure file**.



The screenshot shows the "Base Structure" input form. It contains the following fields and buttons:

- Swiss-Prot or PDB Code Id (ex. 1bna):
- User provided Structure (PDB format):
- Input structure file should follow PDB File Format v.3.30 (July 13, 2011) more info...
- Buttons:

3. Checking the Structure


Just after creating the project, you will be redirected to the so-called *Checking page*. In this page, **NAFlex** will show a list of possible problems found in the input structure that can affect to a future Molecular Dynamics Simulation. User can interactively inspect these problems thanks to a Jmol applet coupled to each of the problems, helping to locate them on the real tridimensional structure.

In this particular case, we are trying to work with the well-known *B-DNA dodecamer d(CpGpCpGpApApTpTpCpGpCpG)* (pdb code 1bna). At the right part of the screen, you will find all the possible problems identified, whereas at the left part of the screen the Jmol applet with the structure loaded will be shown.

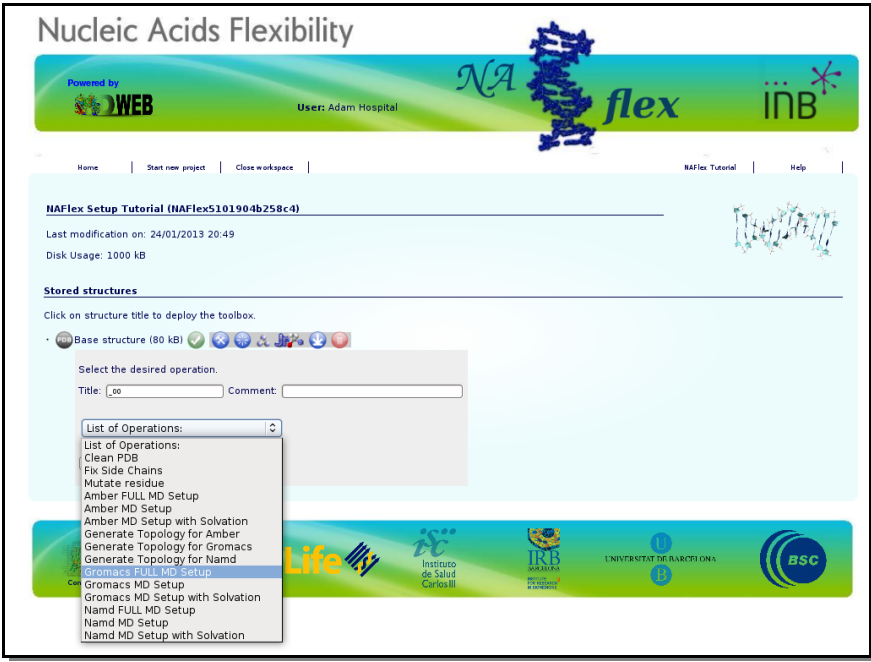
When clicking the next button, an **intermediate information page** will appear, listing the most important information chosen in the previous *Checking* page, i.e. **alternate locations** and **ligands discarded/taken**, and showing the part of the structure (chain/s) chosen for the project in a Jmol applet.

Parameter	Value
PDB code	1bna
Selected Chains	All from A,B

4. Structure Setup

At this point, we already have our structure loaded into **NAFlex** workspace. Now we are going to choose a **workflow** from the list of possible operations, after clicking at the *New Operation* icon . Our goal is obtain a system completely prepared to begin a Molecular Dynamic Simulation with **Gromacs** package. So we will click at the operation named: **Gromacs FULL MD Setup**. Note that choosing operation **Namd FULL MD Setup**, a system for **Namd** Package with **Charmm** forcefield will be prepared, and choosing **Amber FULL MD Setup**, a system for **Amber** Package with **Amber** forcefields will be prepared.

Gromacs FULL MD Setup will run a complete **Molecular Dynamics Setup** to the input structure. That means going from a protein/nucleic acid structure to a system formed by the molecule submerged into a solvent box, surrounded by ions up to a given concentration, and energetically equilibrated. For a more extended information about the steps done by the workflow, see the corresponding **MDWeb** help section (**Gromacs Workflow FULL Setup**).

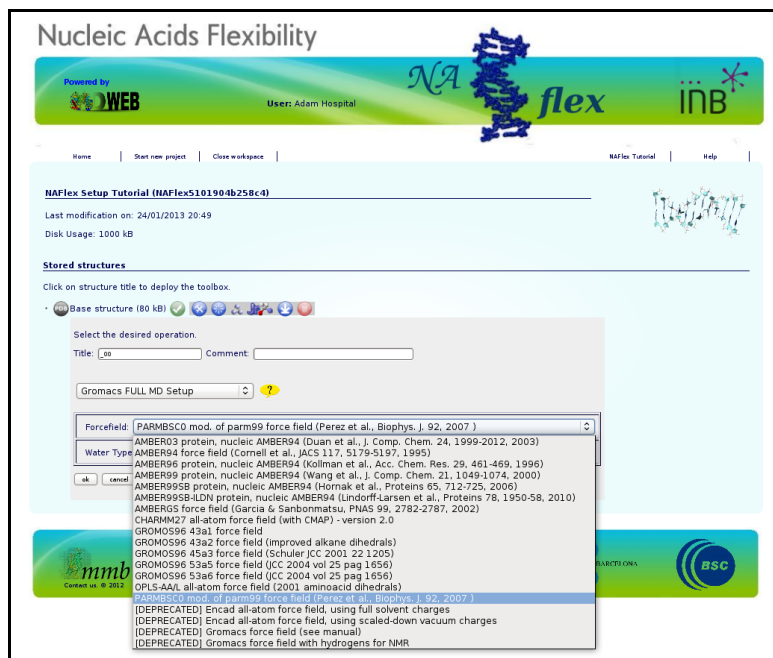


The screenshot displays the NAFlex web interface. At the top, it says "Nucleic Acids Flexibility" and "Powered by MDWEB". The user is identified as "Adam Hospital". Below the navigation bar, the current workspace is titled "NAFlex Setup Tutorial (NAFlex5101904b258c4)". It shows the last modification time as "24/01/2013 20:49" and disk usage as "1000 kB". Under the "Stored structures" section, there is a "Base structure (80 kb)" with a toolbox icon. A dropdown menu for "List of Operations" is open, showing a list of operations including "Clean PDB", "Fix Side Chains", "Mutate residue", "Amber FULL MD Setup", "Amber MD Setup", "Amber MD Setup with Solvation", "Generate Topology for Amber", "Generate Topology for Gromacs", "Generate Topology for Namd", "Gromacs FULL MD Setup" (which is highlighted), "Gromacs MD Setup", "Gromacs MD Setup with Solvation", "Namd FULL MD Setup", "Namd MD Setup", and "Namd MD Setup with Solvation".

After clicking at the workflow **Gromacs FULL MD Setup**, it will give us the possibility to choose between a set of different **forcefields** and **Water Type**. A force field has three components: equations defining the potential energy of a molecular system as a function of atomic coordinates, atom types, and parameter sets that fit the equations to experimental data. We will just keep the option that is active by default in **NAFlex**. In this case, **PARMBSC0 forcefield**, a modified version of the Amber parm99 forcefield to improve it specifically to run Nucleic Acids simulations. For the Water Type we will just keep the most used solvent model in combination with Amber forcefields that is the TIP3P model.

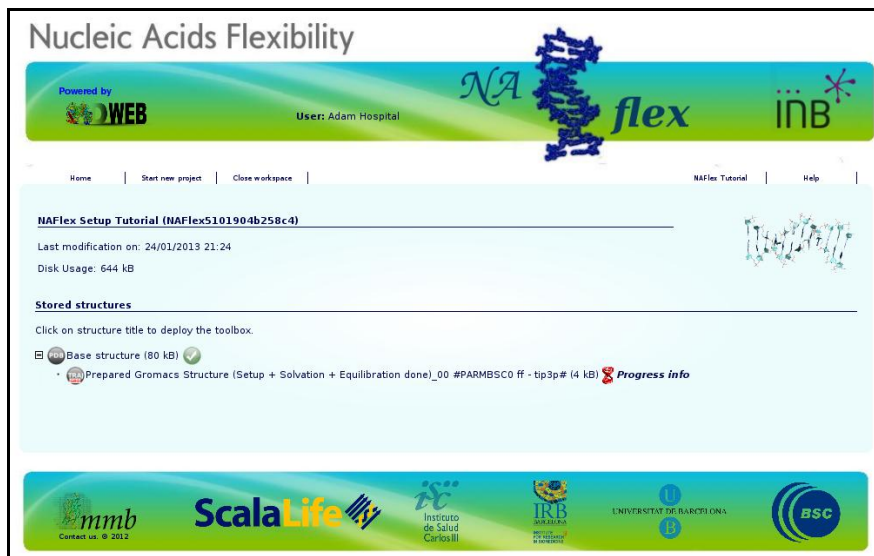
For more information about differences between force fields and water models, please refer to the corresponding MD program manuals. In this case, user can find these information in Gromacs manual.

Links to all the web homepages of the MD programs used in **NAFlex** (where Manuals are freely available) can be found in the corresponding help section (**Related Links**).

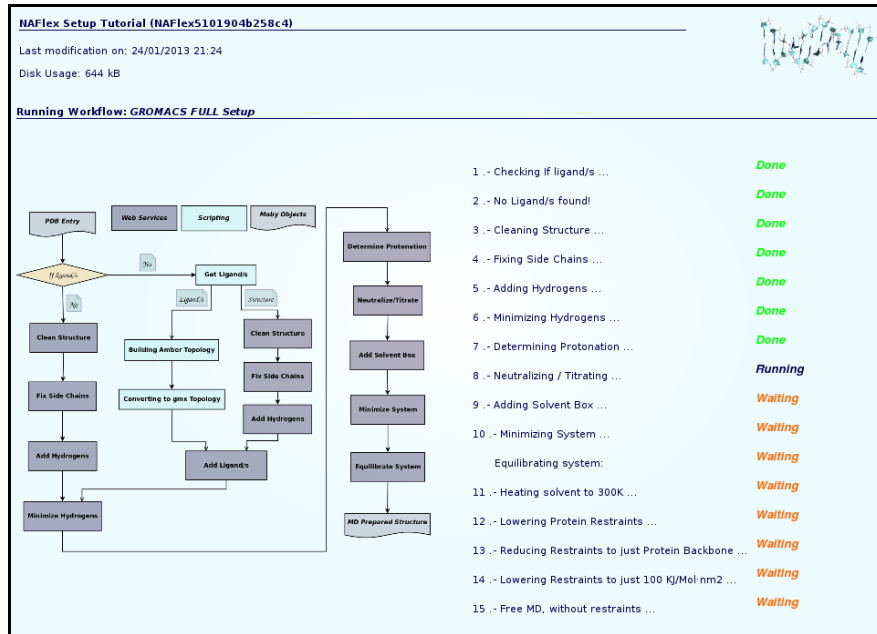


5. Waiting Results

Once the workflow is launched, we just have to wait for the results. **NAFlex** will be automatically reloaded from time to time after checking if the process has already finished. Meanwhile, you can follow the progress of it clicking at **Progress Info** whenever you want.




When clicking at **Progress Info**, a new page will be opened showing the progress of the workflow step by step, accompanied by a figure of the whole workflow. This way, you will be able to know at any moment which service is being executed, how many steps have been already computed, and how many steps left.

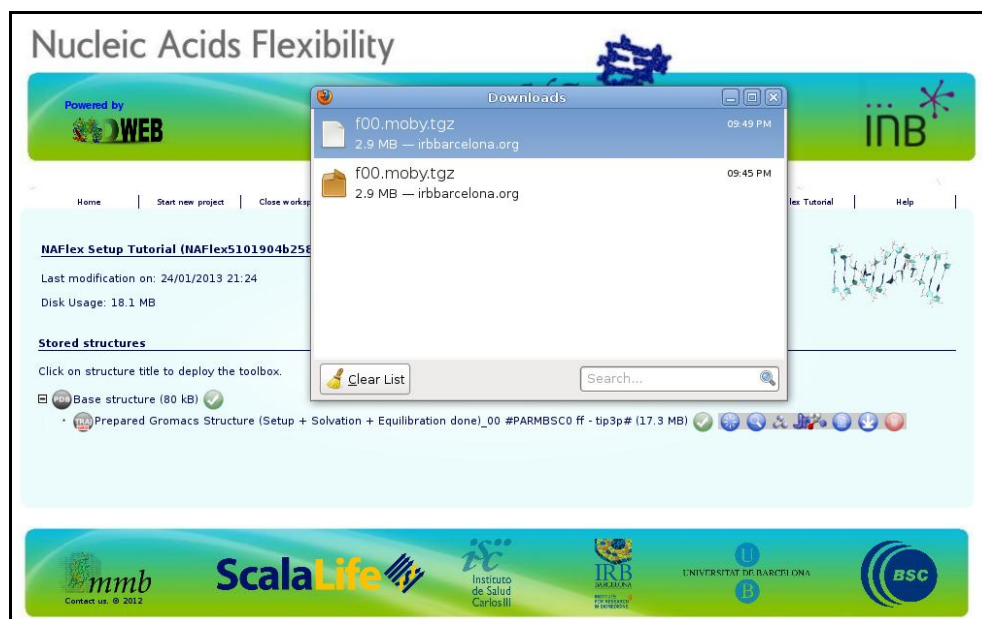


NAFlex offers you the possibility to leave your processes running (as sometimes they will be long processes), disconnect from the server, and reconnect again later on, to check the progress of your projects. The list of projects is easily accessible from the user workspace page (just clicking at **Home** link).

The screenshot shows the 'Nucleic Acids Flexibility' user workspace. At the top, it says 'Powered by WEB' and 'User: Adam Hospital'. Below the navigation bar (Home, Start new project, Close workspace, NAFlex Tutorial, Help), there is a section for 'Stored projects' with a total disk usage of 303.8 MB. The list of projects includes: DNA Simulation (98.5 MB), RNA Simulation (1.5 MB), Inql new mutants (202.8 MB), and NAFlex Setup Tutorial (1 MB). The footer contains logos for mmb, ScalaLife, Instituto de Salud Carlos III, IRB, UNIVERSITAT DE BARCELONA, and BSC.

6. Getting Results

Once the workflow has finished, you have the possibility to download the data generated, just clicking at the *download* icon .



Now you can work with your system, already prepared to run an MD Simulation with **Gromacs** package. The downloaded compressed file has all the necessary inputs to run the simulation, but still you will need to prepare the configuration file needed by gromacs to begin the run with some key parameters like the **MD ensemble**, **simulation time**, **time step**, etc. But don't worry, if you are not familiar at all with **Gromacs**, **MDWeb** will help you preparing these configuration input files for you. Check **MDWeb** help pages, and more specifically, the [Run Simulation Tutorial](#) section.

This example is done with Gromacs package, but **NAFlex** also offers a complete setup workflow for working with Namd package with **Charmm** forcefield or **Amber** forcefields. The complete list of workflows can be found at **MDWeb** help pages ([NAFlex List of Workflows](#)).

NAFlex Nucleic Acids Flexibility Analysis Tutorial

NAFlex Nucleic Acids Flexibility Analysis Tutorial

NAFlex provides a friendly environment to analyse your own generated molecular dynamics trajectories of nucleic acid structures.

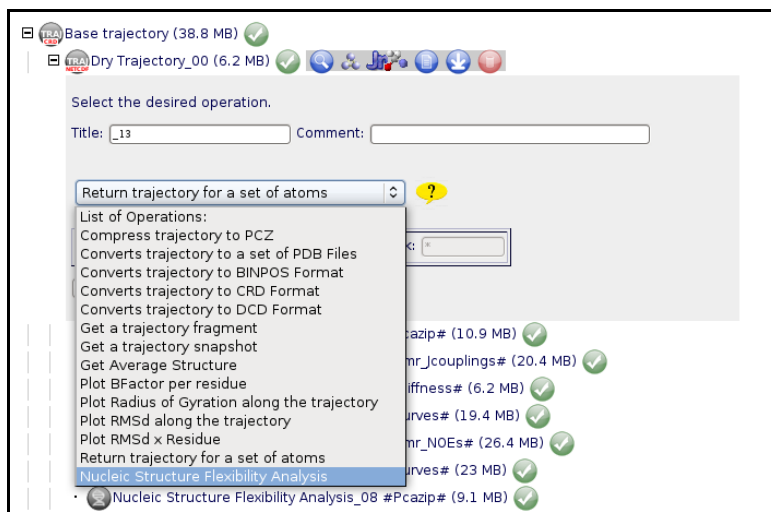
Please note that in this version of NAFlex, only **double-stranded, standard nucleic acids** can be analysed. **Mispaired** nucleotides, **single-stranded** nucleic acids, **triplexes** and **quadruplexes** are not currently supported in NAFlex flexibility analysis. **Modified nucleotides** are only allowed in some of the flexibility analysis operations.

User should follow three necessary steps before being able to analyse his/her own trajectory. These steps are *registration*, *starting project*, and *uploading a trajectory*. A short tutorial about these steps can be found in **Uploading a Trajectory** help section.

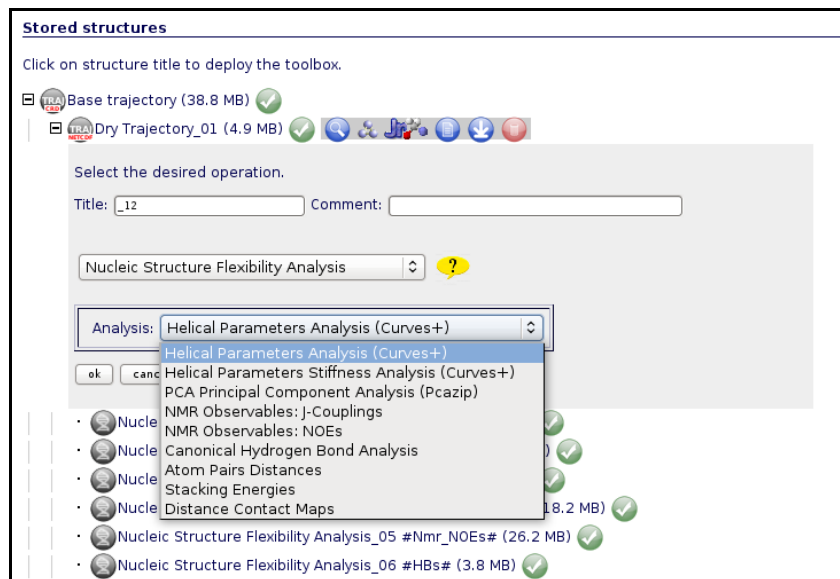
Once we have our trajectory uploaded, we can launch our Nucleic Acid Flexibility Analysis. NAFlex offers a set of Nucleic Acids flexibility analysis:


- **Helical Parameters**
- **Stiffness Constants**
- **Principal Components**
- **Nuclear Magnetic Resonance Observables: J-Couplings**
- **Nuclear Magnetic Resonance Observables: NOE's**
- **Canonical Hydrogen Bond Analysis**
- **Atom Pairs Distances**
- **HB/Stacking Energies**
- **Distance Contact Maps**

First of all, we have to identify the operation called *Nucleic Structure Flexibility Analysis* in the list of available operations:



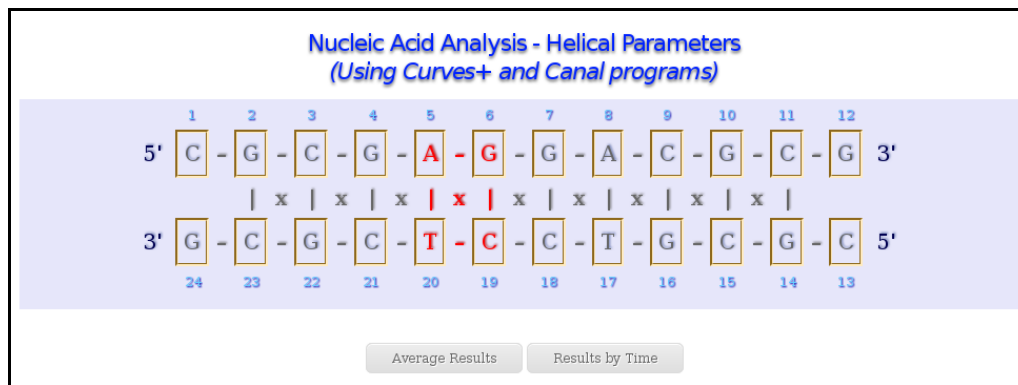
Then, we can choose between the list of possible analysis:



Each of the operations have its own web interface to graphically see and interpret the resulting data. These interfaces can be opened clicking at the *View*  icon. A short piece of information for every analysis can be found in the coming lines.

Helical Parameters

When analysing **Nucleic Acid Helical Parameters**, a graphical representation of the two sequence strands will appear on the screen. This representation allows a user-friendly selection of **nucleotides**, **base pairs** and **base pair steps** (tetramers), just clicking at the corresponding region of the sequence. For example, to select a base pair, user may click at the | symbol between nucleotides involved in the interesting tetramer, to select a base pair step, at the **x** symbol between nucleotides within the same strand, and just over a nucleotide one-letter code to select the corresponding nucleotide.



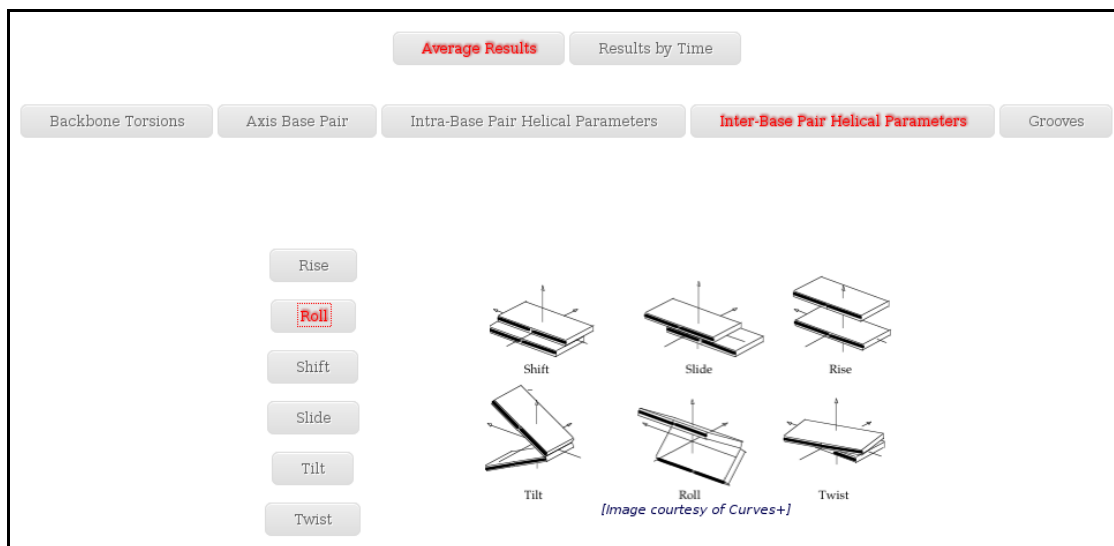
The **Helical Parameters** analysis is divided in two main results:

- **Average Results**
- **Results by Time**

Both sections share the same sub-sections:

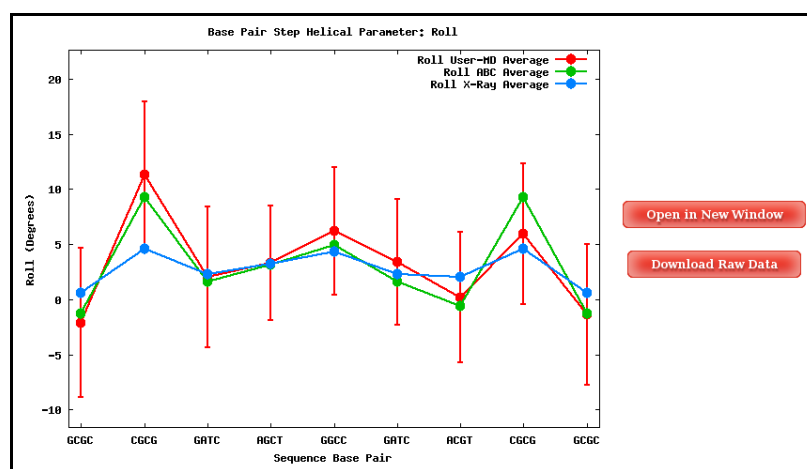
- **Backbone Torsions**
- **Axis Base Pair**
- **Intra-Base Pair Helical Parameters**
- **Inter-Base Pair Helical Parameters**
- **Grooves**

Each of the sub-sections is divided again into more sections that can be selected to obtain the corresponding plots.



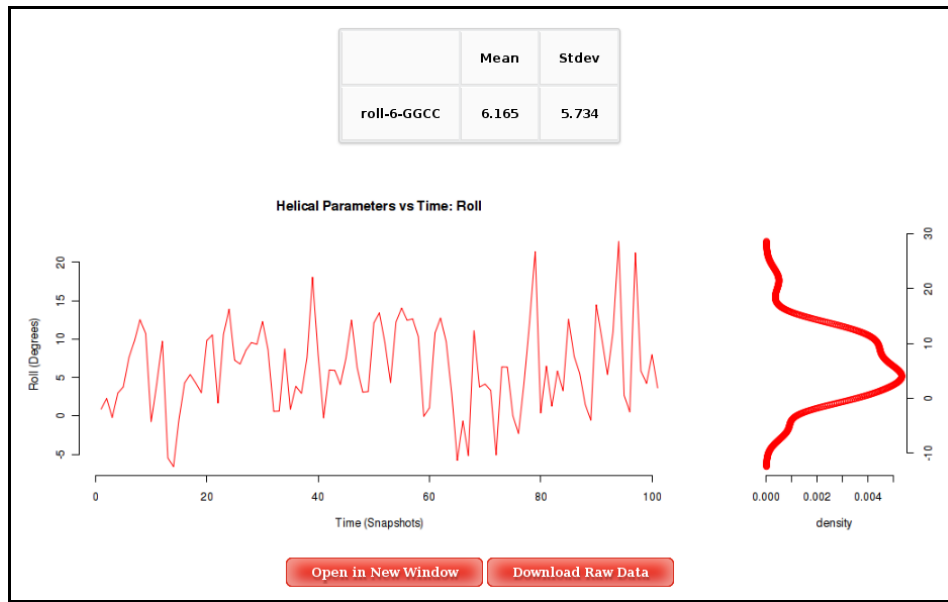
The **Average Results** section contains a set of plots generated after computing average values for all the trajectory snapshots. Plots and their associated raw data can be downloaded from the link provided.

When available, **published data** corresponding to the selected parameter set are plotted for comparison.



Into the **Results by Time** section, user can obtain plots of selected parameter vs time for the whole trajectory analysed, and for each of the corresponding primary entity, that depends on the parameter. For example, user will be asked to select a **nucleotide** to obtain **Backbone Torsions**, a **Base Pair** to obtain **Axis** and **Intra-Base Pair** parameters, and a **Base Pair Step** (tetramer) for **Inter-Base Pair** parameter and **Grooves**. The graphical representation of the sequence is very helpful when selecting the desired primary entities.

Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values.



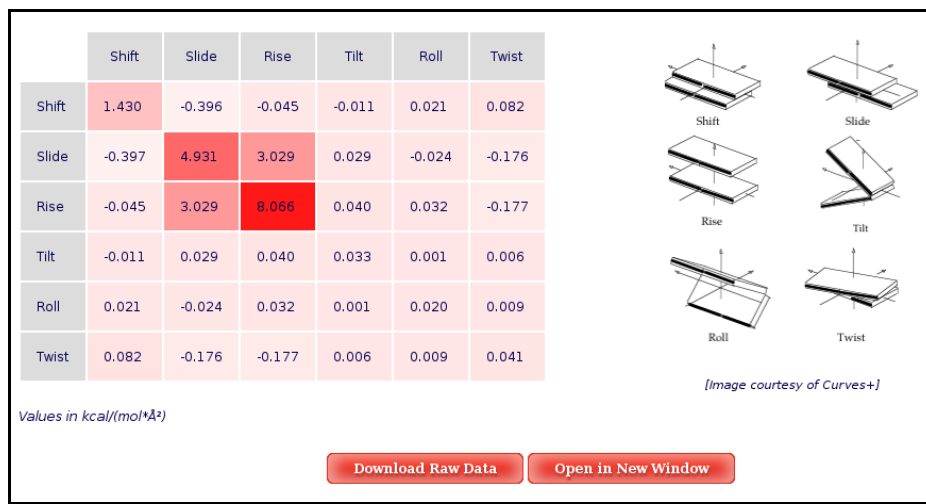
Stiffness Constants

When analysing **Nucleic Acid Stiffness Constants**, the resulting web page will show again the graphical representation of the sequence strands (*see previous section*).

The **Stiffness Constants** analysis is divided in two main sections:

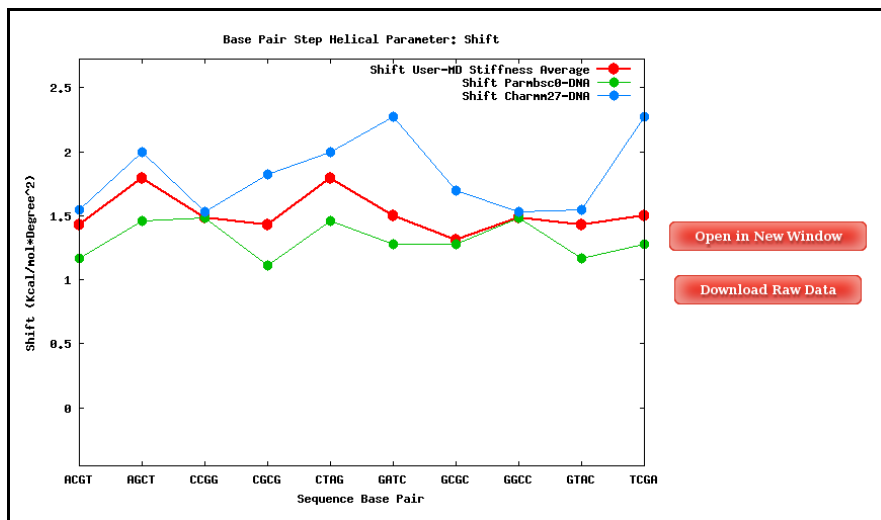
- **Average Results**
- **Results by Time**

The **Average Results** section contains a set of **Stiffness Matrices** (determined by the inversion of the covariance matrix in helical space) for each sequence **Base Pair Step**.

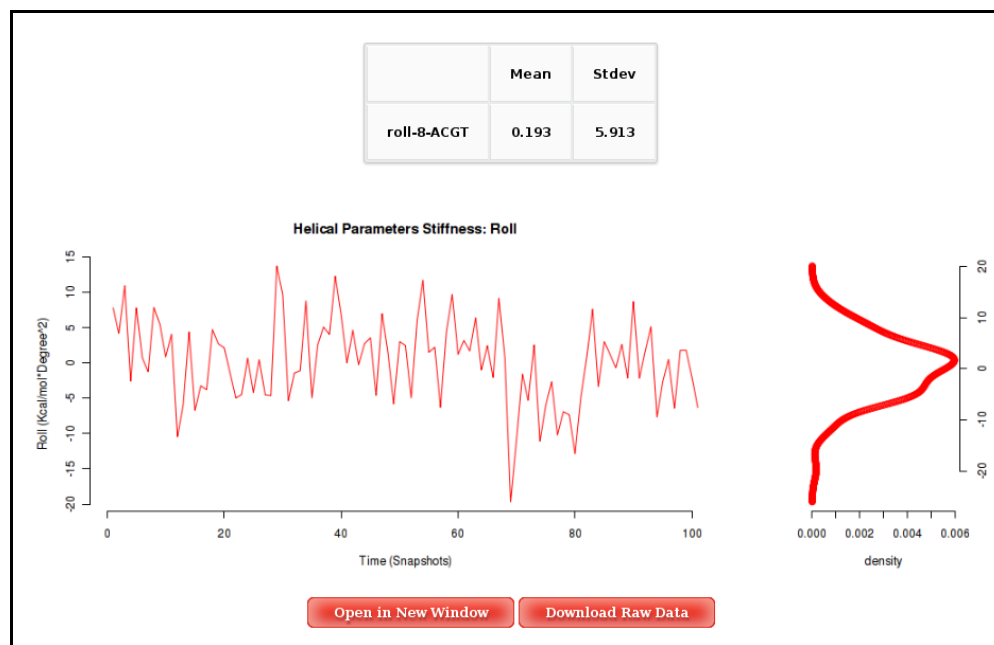


A set of average plots generated computing average values for all the **Stiffness Constants** associated to a **Base Pair Step** (average of the diagonal elements of the **Stiffness Matrices**) are also offered.

When available, published data corresponding to the selected parameter are plotted for comparison. Plots and its associated raw data can be downloaded from the link provided.

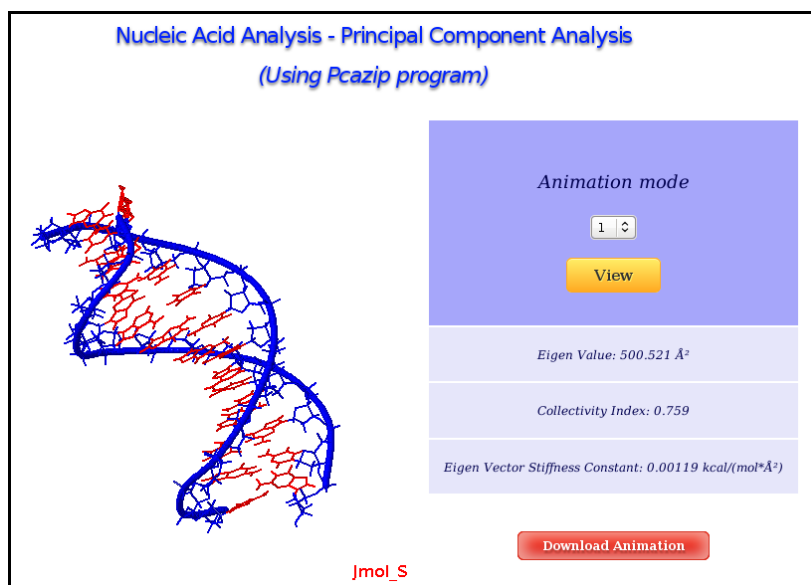


Into the **Results by Time** section, user can obtain plots of **Inter-Base Pair Helical Parameters** (for each **Base Pair Step**) vs. time for the whole trajectory analysed. User will be asked to select a **Base Pair Step** (tetramer) to obtain the corresponding plot. The graphical representation of the sequence is very helpful when selecting desired **Base Pair Steps**.

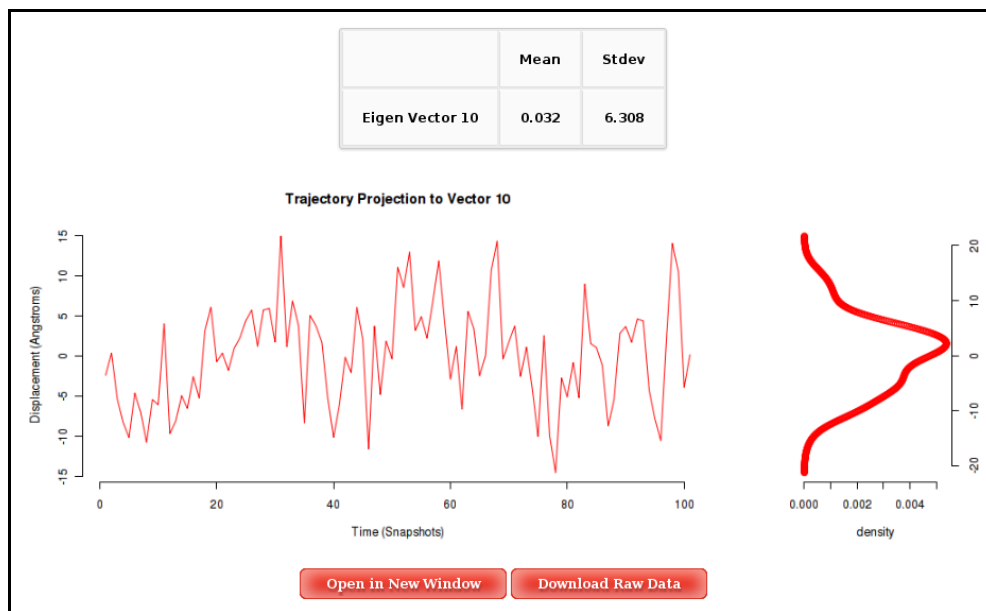


Principal Component Analysis

The **Principal Component Analysis** graphical interface offers the possibility of studying the real movements of the structure through the projections of the trajectory onto the different essential modes. An interactive **JMol applet** shows these movements, allowing user to translate, rotate and in general manipulate the visualization. The first 10 animation modes are offered for visualization and download. Associated values as **eigenvalues**, **collectivity indexes** and **eigenvector stiffness constants** are also shown.



For each of the first 10 **eigenvectors**, a plot containing the **displacement** (in Angstroms) of the projections of all the trajectory snapshots into the associated **eigenvector** from the first snapshot is also computed and shown. Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values. Raw data and plot are also easily downloadable.



Nuclear Magnetic Resonance Observables: J-Couplings

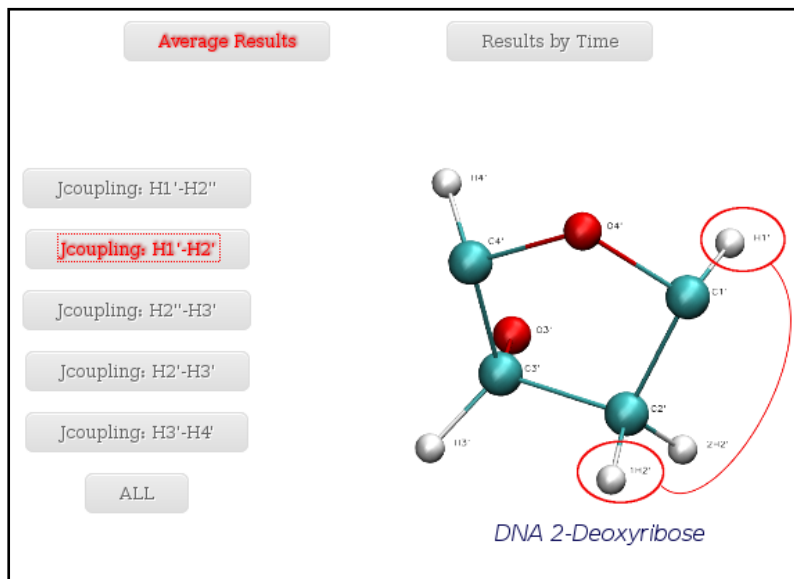
When analysing NMR **J-Couplings**, the resulting web page will show again the graphical representation of the sequence strands (*see Helical Parameters section*).

The **J-Couplings** analysis is divided in two main results:

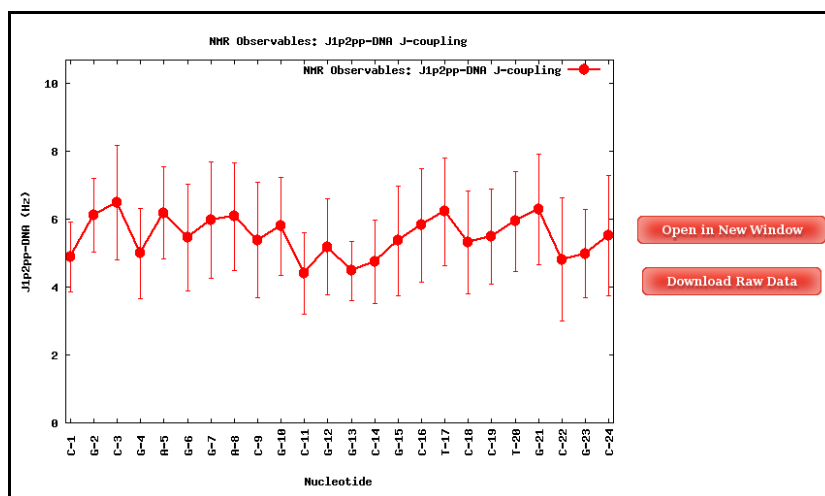
- **Average Results**
- **Results by Time**

The **Average Results** contain the **J-Couplings** observables for the **sugar** proton pairs:

- Jcoupling: **H1' - H2'**
- Jcoupling: **H2' - H3'**
- Jcoupling: **H3' - H4'**
- Jcoupling: **H1' - H2''**
(Only in DNA case)
- Jcoupling: **H2'' - H3'**
(Only in DNA case)



When selecting a specific proton pair, a plot with the average **J-Coupling** values for each of the nucleotides of the trajectory sequence will be shown.



Together with the proton pair options, it is also an **ALL** button. When selected, a table with all the average values (mean and standard deviation) for all the proton pairs and all the trajectory sequence nucleotides is shown. Both plots (with its associated raw data) and table can be easily downloaded.

Nucleotide Number	Jcoupling Type	Mean	Stdev
1	J1p2p-DNA	10.528	0.531
1	J1p2pp-DNA	4.886	1.039
1	J2p3p-DNA	6.824	0.984
1	J2pp3p-DNA	2.070	0.599
1	J3p4p-DNA	2.148	1.251
2	J1p2p-DNA	9.609	1.260

The **Results by Time** section contains the plots for the **J-Couplings** observables vs time for each of the sequence nucleotide. User will be asked to select a specific **Nucleotide** from the trajectory sequence (using the graphical representation of the sequence) to obtain the corresponding plot. Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values.



Nuclear Magnetic Resonance Observables: NOE's

Nuclear Magnetic Resonance **Nuclear Overhauser Effect (NOE)** can be used to determine intra/inter-molecular distances. Again, a graphical representation of the sequence strands (*see Helical Parameters section*) will be shown, helping in the selection of nucleotides/base pairs/base pair steps.

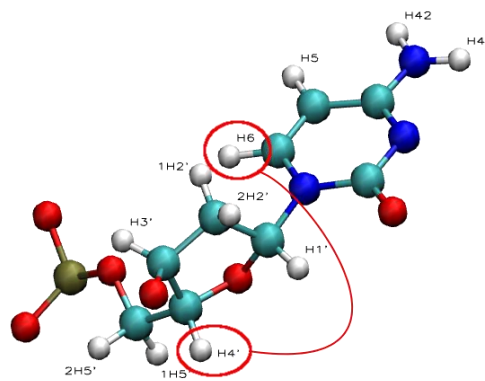
The **NOE** analysis is divided in two main results:

- **Average** Results
- Results **by Time**

The **Average Results** contain the **NOEs** distances for the next proton pairs:

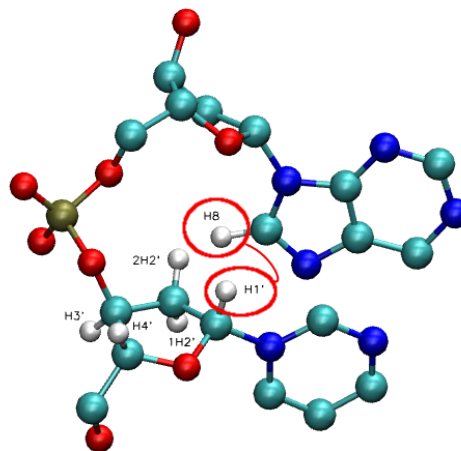
Intra-Nucleotide:

- NOE: **H1'** - **H2'** | **H1'** - **H3'** | **H1'** - **H4'**
- NOE: **H2'** - **H3'** | **H2'** - **H4'**
- NOE: **H3'** - **H4'**
- NOE: **H1'** - **H6/H8** | **H2'** - **H6/H8** | **H3'** - **H6/H8** | **H4'** - **H6/H8**
- NOE: **H5** - **H6**
- NOE: **H2''** - **H1'** | **H2''** - **H3'** | **H2''** - **H4'**
(Only in DNA case)
- NOE: **H2''** - **H6/H8**
(Only in DNA case)

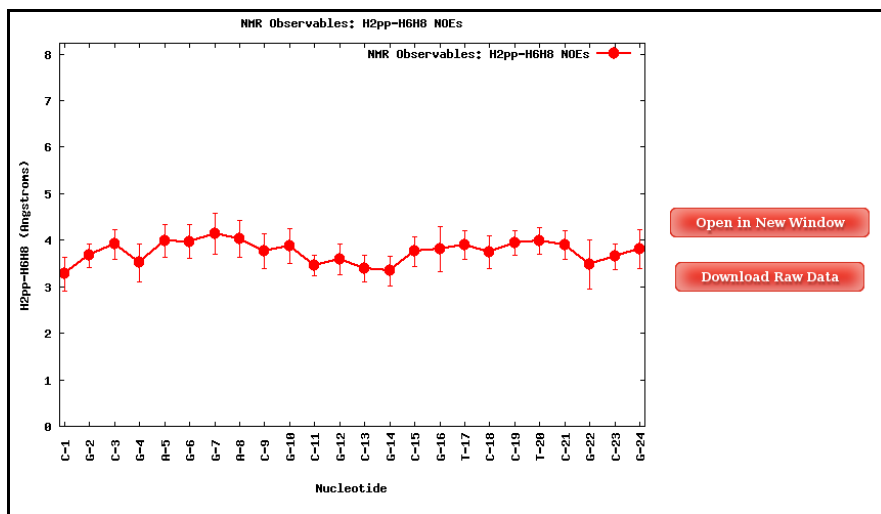


Inter-Nucleotide:

- NOE: **H1'** - **H6/H8 (+1)** | **H2'** - **H6/H8 (+1)** | **H3'** - **H6/H8 (+1)** | **H4'** - **H6/H8 (+1)**
- NOE: **H2''** - **H6/H8 (+1)**
(Only in DNA case)



When clicking one of the proton pairs, a plot containing the **average distances** (in Angstroms) for the specific proton pair for each of the nucleotides in the sequence will be shown. Plots and its associated raw data can be easily downloaded.



NAFlex offers the possibility to plot more than one of the **NOE distances** in the same plot. To do so, user should select the set of interesting proton pairs (with the checkboxes), and then click at **Show Selected Observables**.

H1'-H2'

H1'-H2''

H1'-H3'

H1'-H4'

H2'-H3'

H2'-H4'

H2''-H3'

H2''-H4'

H3'-H4'

H3''-H4'

H1'-H6/H8

H2'-H6/H8

H2''-H6/H8

H3'-H6/H8

H4'-H6/H8

H5-H6

H1'-H6/H8 (+1)

H2'-H6/H8 (+1)

H2''-H6/H8 (+1)

H3'-H6/H8 (+1)

H4'-H6/H8 (+1)

ALL

DNA 2-Deoxyribose

Sugar-Sugar Sugar-Base Sugar-Base-Step Check H1' Check H2' Check H2'' Check H3' Check H4' Uncheck All

Show Selected Observables

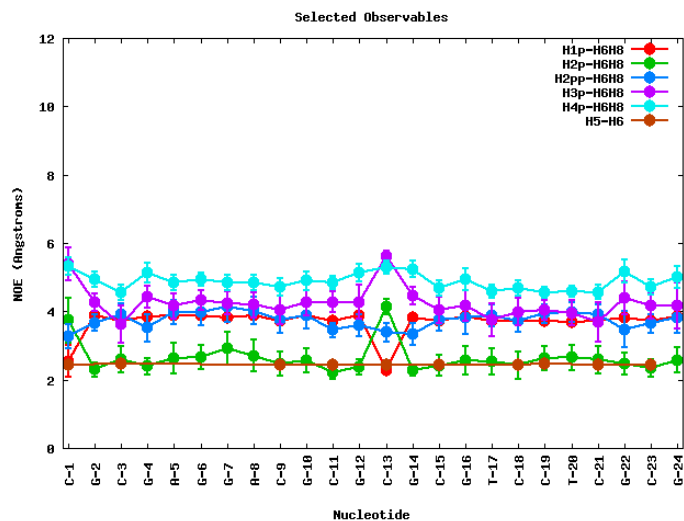
A set of buttons to automatically check interesting proton pairs are offered:

By proton pair position:

- **Sugar-Sugar:** Intra-Sugar proton pairs.
- **Sugar-Base:** Sugar-Base proton pairs.
- **Sugar-Base Step:** Sugar-Base Step proton pairs.

By specific proton:

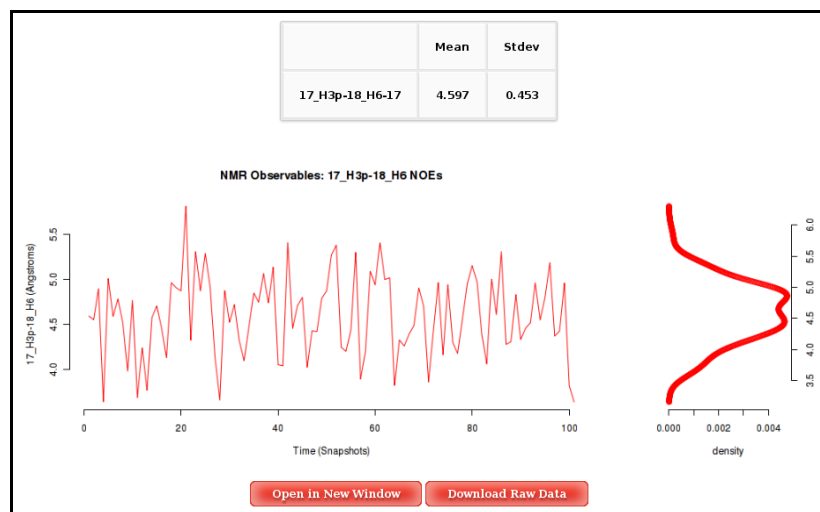
- **H1':** All proton pairs involving H1'
- **H2':** All proton pairs involving H2'
- **H3':** All proton pairs involving H3'
- **H4':** All proton pairs involving H4'
- **H2'':** All proton pairs involving H2'' (*Only in DNA case*)



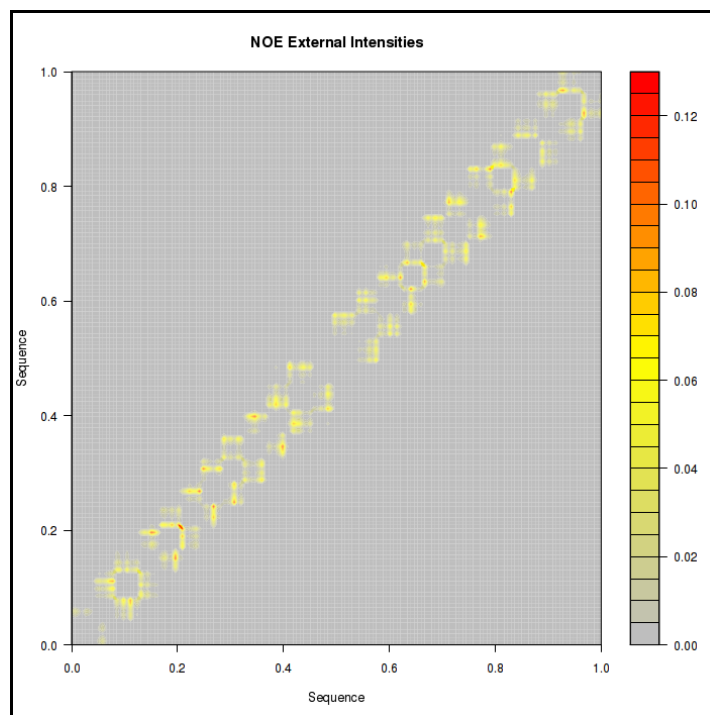
After clicking at the *Show Selected Observables* button, the corresponding plot will be computed, and a new web page will be opened. The plot is shown together with a table enclosing the raw data (mean and standard deviation) for the selected proton pairs. The table can be sorted by columns, helping then to obtain information for a specific **nucleotide number**, **nucleotide code**, or **NOE observable**.

Nucleotide Number <input type="text" value="1"/>	Nucleotide Code <input type="text" value="C"/>	NOE <input type="text" value="H1p-H6H8"/>	Mean <input type="text" value="2.5459"/>	Stdev <input type="text" value="0.4718"/>
1	C	H1p-H6H8	2.5459	0.4718
1	C	H2p-H6H8	3.7418	0.6597
1	C	H2pp-H6H8	3.2718	0.3678
1	C	H3p-H6H8	5.3815	0.4746
1	C	H4p-H6H8	5.3229	0.2576
1	C	H5-H6	2.4311	0.0728
2	G	H1p-H6H8	3.8676	0.0707

The **Results by Time** section contains the plots for the **NOE** observables vs time for each of the sequence nucleotide/base pair step. User will be asked to select a specific **Nucleotide** (if intra-nucleotide selected NOE) or **Base Pair Step** (if inter-nucleotide selected NOE) from the trajectory sequence (using the graphical representation of the sequence) to obtain the corresponding plot. Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values.



Proton pair distances are also transformed into **Intensities** and represented as a countour plot. Two pictures are available, one with the information of all the proton pairs studied in NOEs analysis, and another one with all the information not belonging to proton pairs from the same nucleotide (external).



Canonical Hydrogen Bond Analysis

To graphically analyse the canonical **Hydrogen Bond** interactions between bases, NAFlex offers an independent interface, again with a graphical representation of the sequence strands (*see Helical Parameters section*) to help in the selection of base pair steps.

Hydrogen Bond analysis is divided in two main sections:

- **Average Results**
- **Results by Time**

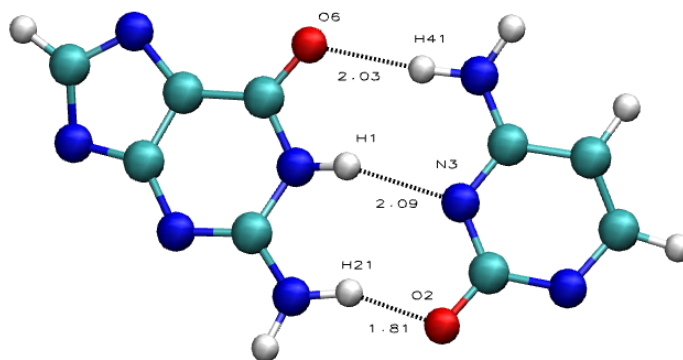
The **Average Results** contain the average **HBs** distances for the each of the base pairs in the sequence, divided in HB atom pairs:

Purine-Pyrimidine HBs:

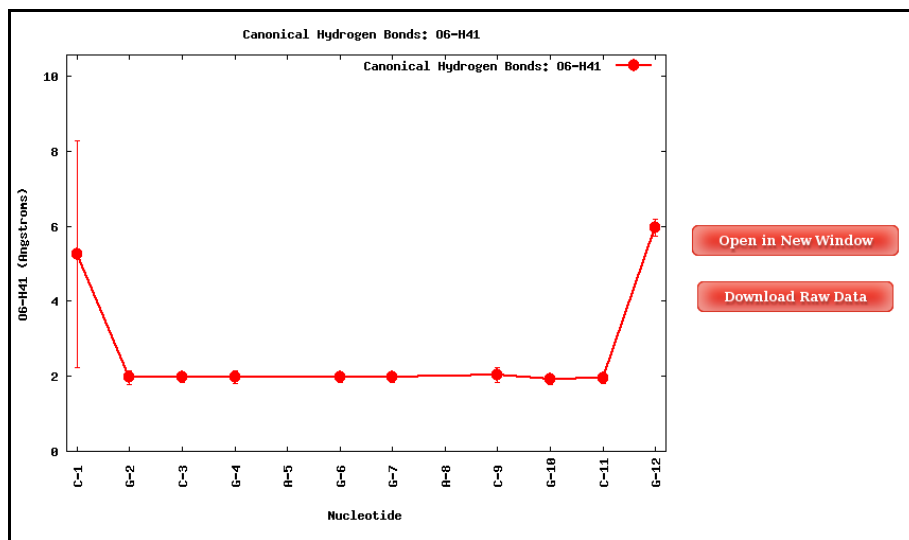
- Adenine N1 - Thymine/Uracil H3
- Adenine H61 - Thymine/Uracil O4

Pyrimidine-Purine HBs:

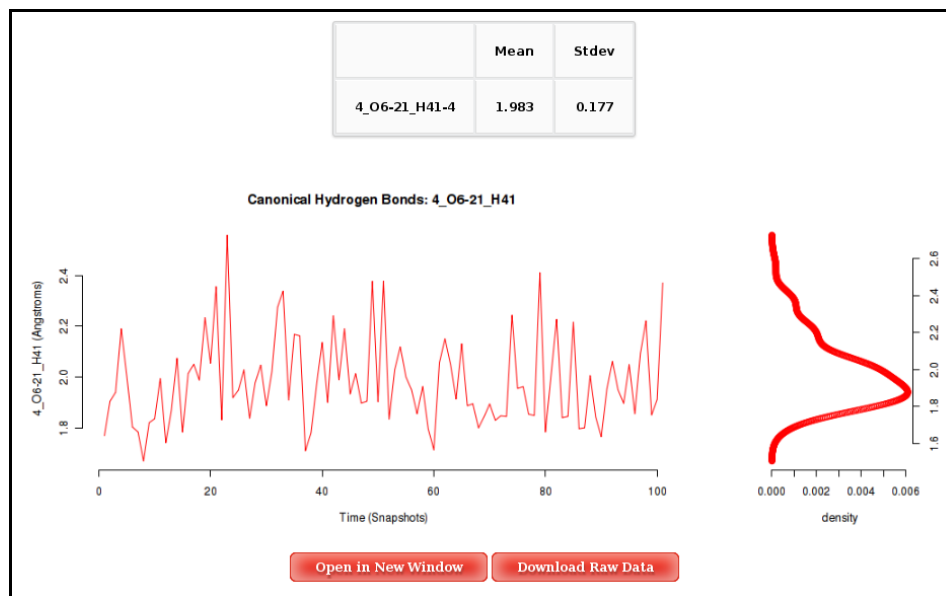
- Guanine O6 - Cytosine H41
- Guanine H1 - Cytosine N3
- Guanine H21 - Cytosine O2



Clicking one of the **Hydrogen Bond** pairs, a plot containing the **average distances** (in Angstroms) for the specific bond for each of the nucleotides in the sequence will be shown. Plots and its associated raw data can be easily downloaded.




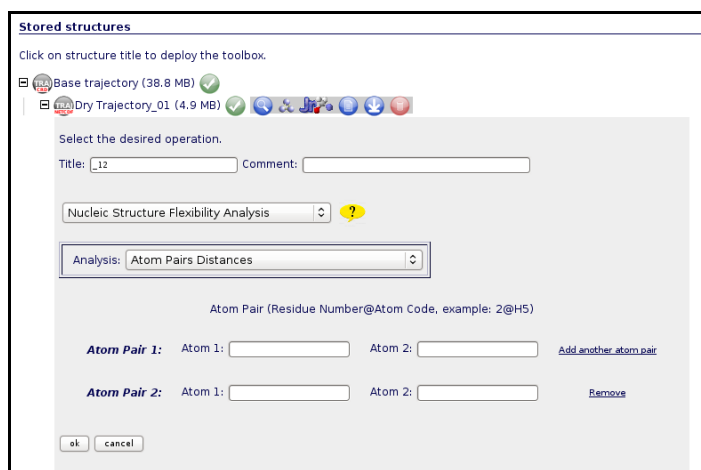
The **Results by Time** section contains the plots for the **Hydrogen Bond** distance (in Angstroms) vs time for each of the sequence base pairs. User will be asked to select a specific **base pair** from the trajectory sequence (using the graphical representation of the sequence) and a specific **hydrogen bond atom pair** to obtain the corresponding plot. Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values.



Atom Pairs Distances

Distances between atom pairs could be of interest when analysing flexibility from a nucleic acids simulation. For example, choosing atoms from the structure ends can give information about the global nucleic acid bending.

To run this analysis, user should introduce the desired atom pairs in the format: **Residue Number@Atom Code** (ex: 2@H5). Residue/atom codes can be confirmed using the Jmol visualizer (opening the trajectory, or even better opening just a single snapshot from the trajectory). Jmol is available in the **NAFlex** toolbox . The correct atom name and residue code can be obtained just clicking with the mouse on the desired atom (or keeping the mouse over the atom for a second).



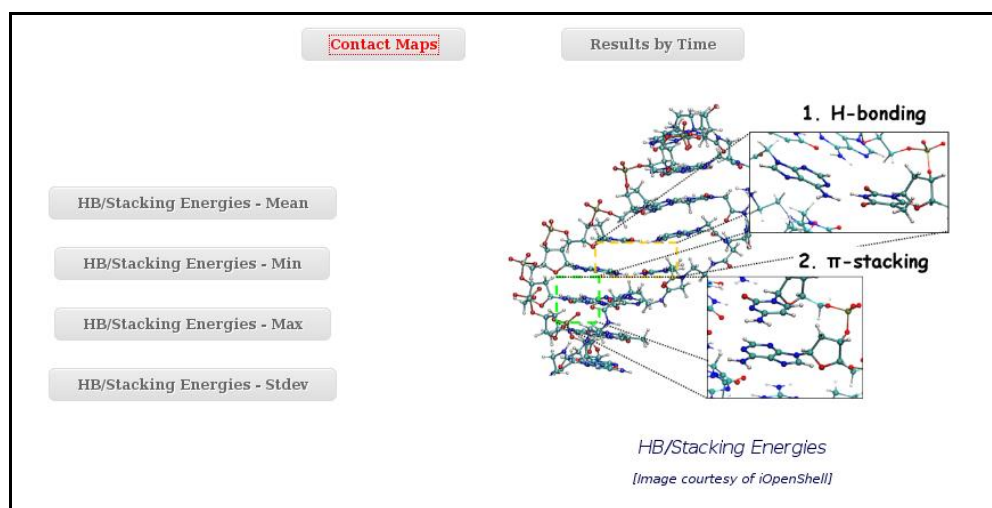
Once distances are computed, the graphical interface for this analysis will just show the different atom pairs **Time plot** with their corresponding associated histogram. Clicking at the desired atom pair button will launch the corresponding plot.



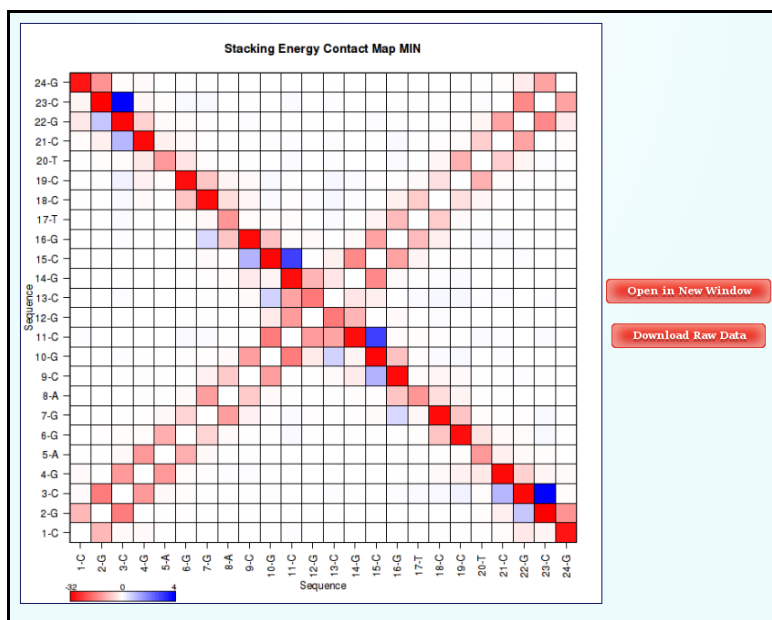
HB/Stacking Energies

To graphically analyse the **Base Pair Hydrogen Bond interaction energies** and the **Base Pair Stacking energies**, NAFlex offers an independent interface, including two different sections:

- **Contact Maps**
- **Results by Time**



Within the **Contact Maps** section, user can visualize **Contact Maps Plots** for the interaction energies between every base pair in the nucleic acid structure. In these plots, a square is painted for every nucleotide pair, assigning a color depending on the interaction energy value. Usually, high values in the top-left to bottom-right diagonal shows **HB interaction energies** for Base Pairs, while top-right to bottom-left diagonal shows **Stacking interaction energies**.



The **Results by Time** section contains the plots for the **Hydrogen Bond interaction energies** and the **Stacking energies** (in Kcal/mol) vs time for each of the sequence base pair/base pair step.

Again, a graphical representation of the sequence strands (*see Helical Parameters section*) to help in the selection of base pair/base pair steps is offered.

In order to see a **Hydrogen Bond** interaction plot, user will be asked to select a specific **base pair** from the trajectory sequence (using the graphical representation of the sequence, *see below*). Similarly, to see a **Stacking interaction** plots, user will be asked to select a specific **base pair** from the trajectory sequence. Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values.



In the case of **Stacking interaction energies**, four different plots vs Time will be shown.

These plots corresponds to the Stacking energy between a nucleotide of the tetramer and its covalently bonded neighbour placed just above in the first strand (**nucleotides 2 and 3 in the picture**), between a nucleotide of the tetramer and its covalently bonded neighbour placed just above in the second strand (**nucleotides 1 and 4 in the picture**), between a nucleotide of the tetramer and the one just above its pair in the complementary strand (**nucleotides 2 and 1 in the picture**), and between a nucleotide of the tetramer and the one just under its pair in the complementary strand (**nucleotides 3 and 4 in the picture**).

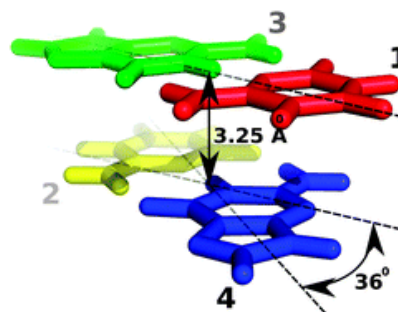
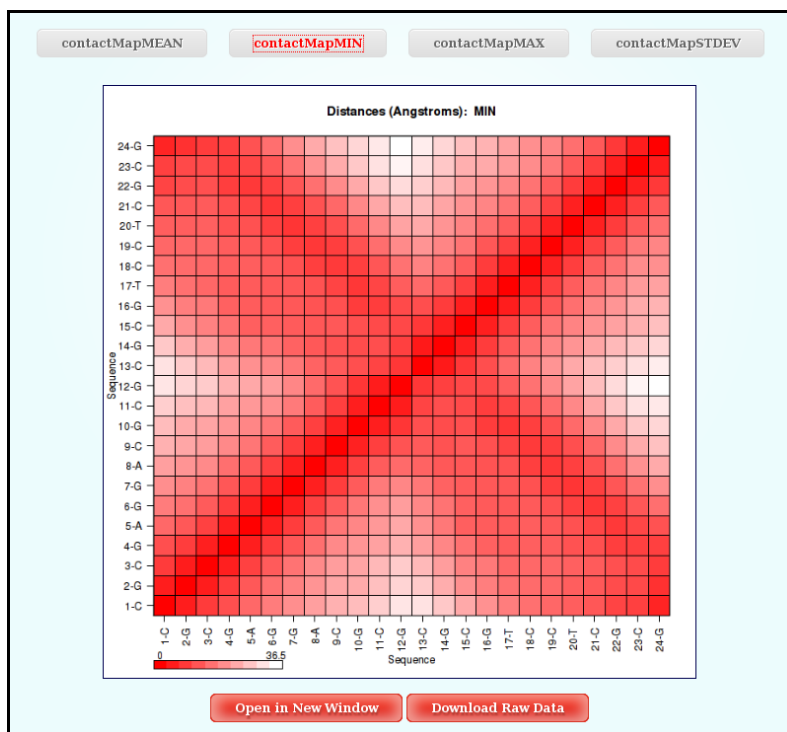


Image from Phys. Chem. Chem. Phys., 2010,12, 1369-1378.

Distance Contact Maps

In this analysis, user just have to choose between the different **Distance Contact Maps** offered by the server clicking at the corresponding button. The plot will be shown with a color legend, and like all the different plots generated by **NAFlex**, with the associated links giving the possibility to open it in a new window and download the raw data.



NAFlex Coarse-Grained Simulations Analysis Tutorial

NAFlex Coarse-Grained Simulations Analysis

NAFlex offers a couple of **Coarse-Grained** algorithms (low resolution, not atomistic) to quickly obtain a dynamic view of nucleic acid structures.

The resulting **Coarse-Grained trajectories** can not be analysed using the same set of tools offered by NAFlex for nucleic acids MD simulations, due to its low resolution. In fact, as a consequence of this coarse-grained reduction in complexity and resolution, many of the typical flexibility values such as **HB and Stacking energies, proton couplings and helical parameters** cannot be computed.

So, to obtain a graphical view of the flexibility of these **Coarse-Grained** trajectories, NAFlex implements a couple of specific analysis, taking into account the information that can be extracted from the low resolution trajectory generated.

The **Coarse-Grained** flexibility analysis are:

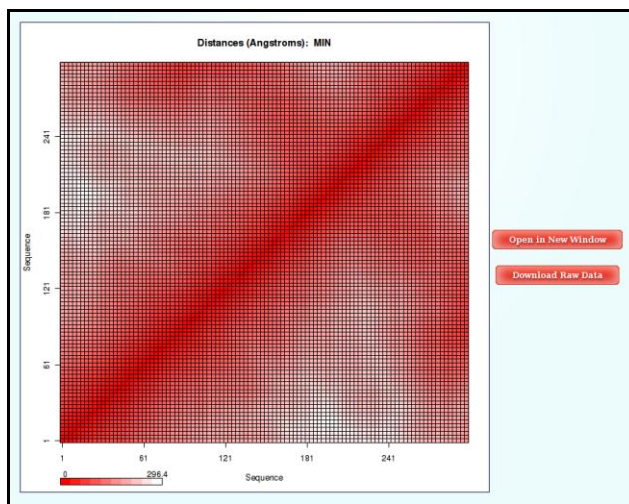
- **Coarse-Grained Elastic Mesoscopic Model Flexibility Analysis**
- **Coarse-Grained Worm-like Chain Model Flexibility Analysis**

Coarse-Grained Elastic Mesoscopic Model Flexibility Analysis

The **Montecarlo Coarse-Grained Flexibility Analysis** is divided in two main results:

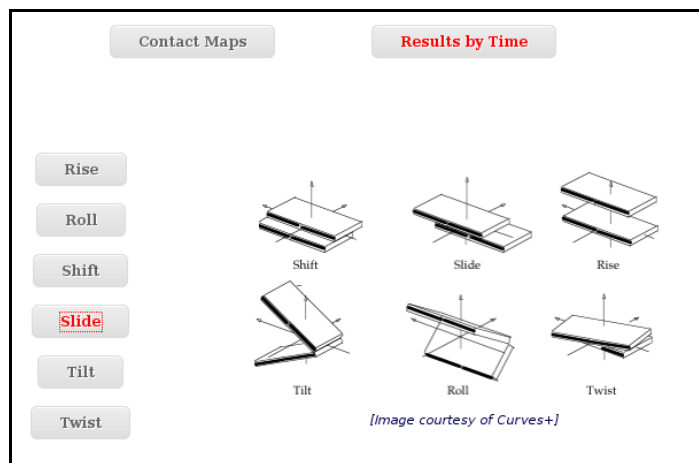
- **Distance Contact Maps**
- **Results by Time**

The **Distance Contact Maps** section contain a set of Distance Contact Maps Plots that offering a dynamic view of the whole molecule (if the analysis made is global) or of a specific molecule fragment (if the analysis made is local). Flexibility of the structure and movements that it undergoes during the dynamic simulation can be extracted from these informative plots.

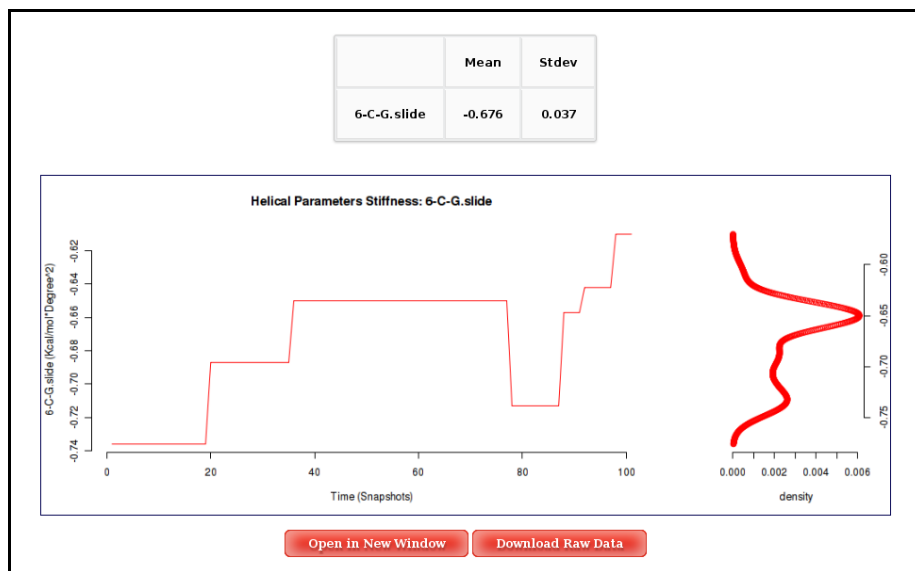


The **Results by Time** section contains information about helical parameters associated to the **Montecarlo** algorithm along the simulation.

Montecarlo Coarse-Grained simulations work at a Base level, not taking into account nucleic acid phosphates (backbone) neither sugar rings. As the algorithm changes DNA **Helical Parameters** (Stiffness constants associated to 6 Base Pair Step Helical Parameters: 3 local rotations -**roll**, **tilt** and **twist**- and 3 local translations -**slide**, **shift** and **rise**-), these values and their movements can be analysed vs time.



User can obtain plots of **Inter-Base Pair Helical Parameters** (for each **Base Pair Step**) vs time for the whole trajectory analysed. User will be asked to select a **Base Pair Step** (tetramer) to obtain the corresponding plot. The graphical representation of the sequence is very helpful when selecting desired **Base Pair Steps** (*see [Helical Params Help section](#)*). Plots generated contain the corresponding **histogram** attached, and an associated table with calculated **mean** and **standard deviation** values. Raw data and plot are also easily downloadable.



Coarse-Grained Worm-like Chain Model Flexibility Analysis

Worm-Like Chain Model Coarse-Grained simulations work at a very low resolution (**1 bead every 4 Base Pair Steps**), so reducing a lot the complexity of the system. For this reason, the only interesting and informative study that can be done to this extremely coarse-grained resulting trajectory is a **distance analysis**. And the chosen way to show the distance results is through **Contact Map Plots**.

Distances (for example distance **mean** or **minimum** distance) between all the pair of bases in a nucleic structure during a dynamic simulation can be very useful to obtain a picture of the **flexibility** of the structure and the movements that it undergoes during the dynamic simulation. In this case, resulting distances are those between coarse-grained beads (representing 4 base pair steps).

The most common way to look at this information is plotting it with **Contact Maps**. With these plots, a square is painted for every nucleotide (bead) pair, assigning a color depending on the value of the analysis (distance in this case). Usually, high values out of the diagonal (closest nucleotide pairs) are the most interesting and informative ones.

