The NCIWEB Server: A Novel Implementation of the Noncovalent Interactions Index for Biomolecular Systems

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Trinidad Novoa,[†,‡] Rubén Laplaza,[†] Francesca Peccati,[¶] Franck Fuster,[†] and Julia Contreras-García[*,†]

†*Laboratoire de Chimie Théorique, LCT, Sorbonne Université , CNRS, F-75005 Paris, France.*

‡*Laboratoire Jacques-Louis Lions, LJLL, Sorbonne Université , CNRS, F-75005 Paris, France.*

¶*Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Building 801A, 48160 Derio, Spain.*

E-mail: contrera@lct.jussieu.fr

Abstract

It is well known that the activity and function of proteins is strictly correlated with their secondary, tertiary, and quaternary structures. Their biological role is regulated by their conformational flexibility and global fold, which in turn is largely governed by complex non-covalent interaction networks. Owing to the large size of proteins, the analysis of their non-covalent interaction networks is challenging, but can provide insights into the energetics of conformational changes or protein–protein and protein–ligand interactions. The NCI[1–3] (non-covalent interaction) index, based on the reduced density gradient, is a well-established tool for the detection of weak contacts in biological systems. In this work, we present a web-based application to expand the use of

this index to proteins, which only requires a molecular structure as input and provides a mapping of the number, type and strength of non-covalent interactions. Structure preparation is automated and allows direct importing from the PDB database, making this server (https://nciweb.dsi.upmc.fr) accessible to scientists with limited experience in bioinformatics. A quick overview of this tool and concise instructions are presented, together with an illustrative application.

Introduction

Among other systems of biological importance, it is well established that proteins can undergo important conformational transitions, including folding, unfolding and aggregation, which are governed by the rearrangement of intricate networks of Non-Covalent Interactions (NCIs).[4–9] By controlling NCIs, biomolecules are able to perform a variety of exceedingly complex tasks, including precise molecular recognition[10–12] and selective catalysis.[13,14]

Given the centrality of NCIs in regulating protein activity, their analysis has been the object of extensive computational efforts encompassing a wide range of approaches, from highly accurate ab initio calculations on small model systems[15] to the development of empirical energy functions that account for the entire biomolecule and environmental effects such as solvation.[16,17] Inherent difficulties in correctly representing NCIs in biomolecules originate from the small energy changes associated to individual interactions – often close to the accuracy limit of computational approaches – and from their large number.

The most common approach due to the enormous number of atoms has been to assign van der Waals interactions (vdW), steric clashes (SC) and hydrogen bonds (HBs) in terms of pair-wise distances and angles between atoms, and their van der Waals radii. This procedure, although lacking generality and giving rise to clear systematic errors (e.g. the distance at which a HB can occur is known to be overestimated) provides very fast estimations of non covalent interactions. More elaborate algorithms can be derived from quantum-mechanical calculations. These approaches have served to elucidate fundamental aspects of NCIs in small

systems. However, their high cost forbids their use on large biomolecular systems. Empirical energy functions typically tackle NCIs by discretizing them and summing the contribution of distinct interaction types. These approaches, while efficient, rely on empiricism to coarse grain the intrinsically delocalized nature of NCIs in proteins.[18]

Most generally, the NCI index relies on a general approach derived from density functional theory for the visualization and analysis of NCIs. While a rigorous description of the theoretical framework of this approach can be found elsewhere and is beyond the scope of this work,[3,19–21] the gist of the NCI index is to use the electron density of the system $\rho(r)$ to identify non-covalent interactions as the regions where i) the reduced density gradient s ($s(r) = 1/(2(3\pi 2)^{1/3}|\nabla\rho(r)|/\rho(r)^{4/3})$ vanishes, and ii) $\rho(r)$ is lower than that of a covalent interaction. Since the NCI index exclusively relies on the electron density, which can be reconstructed from atomic densities without complex sets of parameters to adjust empirically (Fig. 1a),[2,22–24] it has been used in systems ranging from small molecules,[25,26] to solid state chemistry,[27–29] to biomolecules,[30–32] for diverse applications including the understanding of reactivity,[33–35] cofactor and active site activity in biomolecules,[36,37] and performance of chemotherapy carriers.[38]

Using the NCI index, interactions in small systems such as those in Fig. 1b-c can be dissected. In the example system containing an acetic acid and a uracil molecule, four NCI regions are identified. Qualitative information on the nature of the interaction can be obtained by considering the value of the electron density and the second eigenvalue of the Laplacian (λ_2), which is negative for attractive interactions and positive for repulsive clashes. At higher positive values of $\text{sign}(\lambda_2)\rho$, in red, we recognize the repulsive interaction regions inside the uracil. For very negative values, in blue, we find hydrogen bonds between the molecules. Finally, very small values of the density point out van der Waals interactions which are not driven by first-order electrostatics nor have a covalent component, pictured in green. A weak van der Waals can be seen as a thin green disk in Fig. 1b and the accompanying through towards $s(r) = 0$ near $\text{sign}(\lambda_2)\rho = 0.01$ a.u. in Fig. 1c, while the

existence of two distinct hydrogen bonds can be seen by the troughs at $\text{sign}(\lambda_2)\rho = -0.04$ and -0.05 a.u. respectively, with the latter being slightly stronger and corresponding to the O-H hydrogen bond. We direct the reader towards a recent review for further details concerning the interpretation of the NCI index.[3]

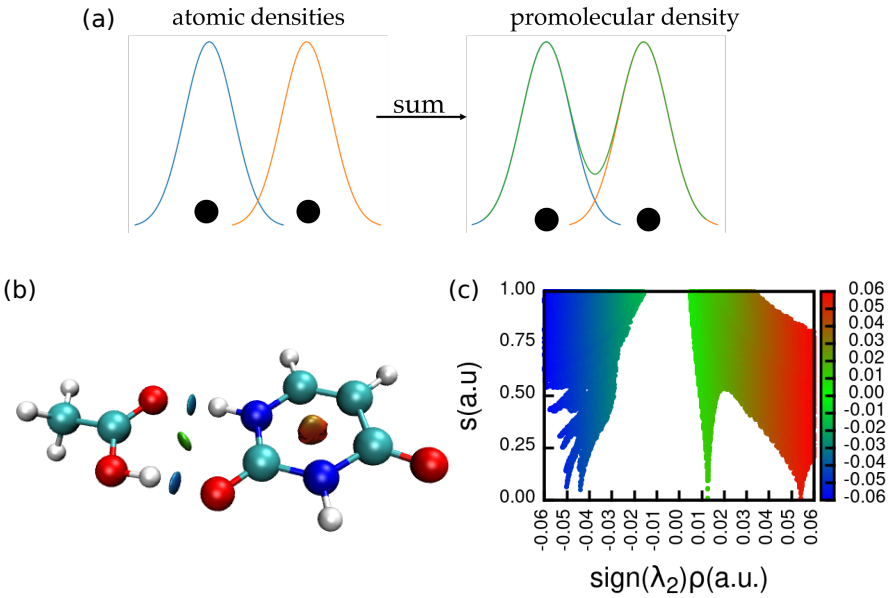

Figure 1: (a) Schematic representation of the promolecular density in one dimension. (b) 3D representation of non-covalent interactions in the AcOH-uracil system and (c) its respective 2D plot. Attractive (blue), van der Waals (green) and repulsive (red) non-covalent interactions are detected.

In small systems, $\rho(r)$ can be obtained from an approximate wavefunction, generally resulting from quantum-mechanical computations. As solving the electronic structure of larger systems becomes exponentially more costly, usually either an experimental electron density, resulting from an X-ray diffraction experiment, or a promolecular density of choice are used. The promolecular density[2,22–24] (Fig. 1a) is obtained as a sum of atomic densities, which in turn are tabulated and fitted so that their first and second derivatives can be obtained analytically, thus making the calculation of the NCI quantities extremely efficient. Despite the simplicity of the latter approach, promolecular and DFT densities have been shown to yield

qualitatively equivalent results, meaning that non-covalent interactions are correctly identified in both cases.[2,20] We note that the promolecular density library currently available in NCIPLOT4 (and the NCIWEB server) was derived from neutral atoms. Nevertheless, charged residues lead to stronger interactions,[39] which are reflected on the distances and hence on the densities.[20] This functionality will be extended in the future through the use of localized molecular orbitals derived from charged fragments.[36,37]

Overall, NCI index provides an intermediate accuracy option: distances are taken into account just as in the case of radii, but environments reveal their influence (since all atomic densities contribute). Since these atomic densities are approximate, quantum mechanical calculations are not involved, thus making it an efficient approach for the analysis of non covalent interactions in biosystems.

As far as biomolecules are concerned, the NCI index presents unique advantages with respect to structural or data-driven approaches. It does not require any *a priori* parametrization or definition of particular NCIs (i.e., it does not require any notion of what types of interactions are present). Although the NCI index does not provide interaction energies, it has been shown that the integral of the density in the NCI regions are proportional to the strength of the interactions.[21,37,40] Thus, it can provide complementary information for the visualization, identification and monitoring of NCIs in such systems. Hence, they have also been included in NCIWEB (see Sec.).

In spite of its notable advantages, applications of the NCI index for biomolecules have been limited due to the technical difficulties of command line software. Thus, in this work we present the NCIWEB server, an accessible and convenient platform to perform the NCI index analysis on biomolecules. Through an interactive browser-based graphical user interface, it automates all the pre-processing steps and carries out the necessary computations server-side using the NCIPLOT4 program.[21] Moreover, the computational efficiency of the approach as implemented in the web server allows the analysis of large structures (over 500 residues) in less than 1 hour.

The NCIWEB server

Although the treatment of large systems is feasible with NCIPLOT4, it is yet not necessarily easy due to the necessary preprocessing and post-processing steps, which may be challenging for the structural biology and broader biochemistry community. These include dealing with the protonation of all species, selecting the relevant fragments or chains from the input structure, choosing conformations of relevant residues, and defining the type of NCI computation to perform depending on the interactions of interest.

For this reason, we have developed the NCIWEB server, a web-based graphical user interface to NCIPLOT4. The server aims to provide not only access to all the new features in NCIPLOT4 highlighted above, but also an attractive and intuitive GUI and a complete preparation and analysis pipeline. Additionally, we hope that the guidance provided by the interface will minimize wrongly performed and interpreted NCI computations.

The architecture and functionalities of the NCIWEB server will be explained in what follows.

Workflow

The workflow of NCIWEB can be summarized as follows (labels refer to Fig. 2).

1. The structure in PDB format is loaded by the user or imported form the Protein Data Bank;

2. Preprocessing begins by automatically identifying protein (A, B, C) and non-protein residues such as ligands (L1, L2), water (w) and ions; if not otherwise specified, ions and water are removed;

3. The initial structure is split according to the chain ID into fragments (A, B, C, L1 and L2);

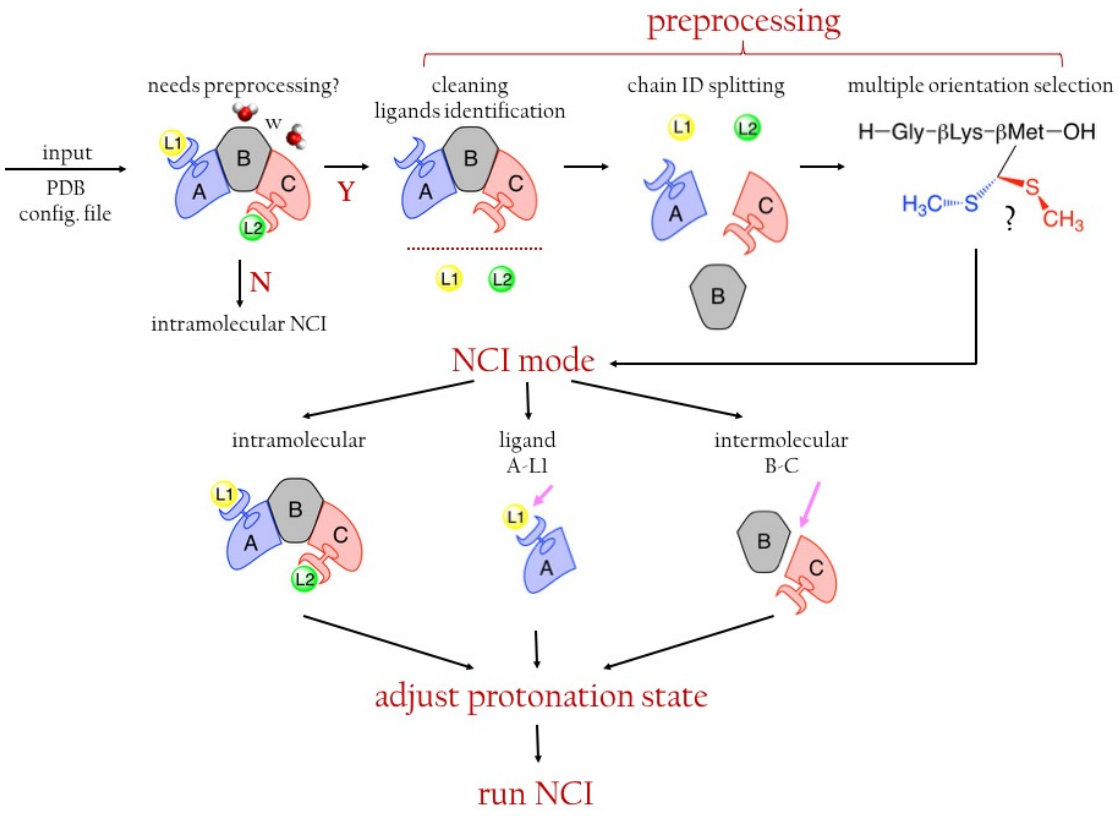

Figure 2: Schematic representation of the workflow of NCIWEB from the input of the PDB structure to the submission of the NCI calculation.

4. If alternate locations for the same residue are present, they are identified automatically; the user is prompted to choose a single location for each of these residues;

5. The user chooses one between the three types of NCI calculation: i) intramolecular, which computes the NCIs of the whole system; ii) ligand, which computes the noncovalent interactions of a fragment with its surroundings within a radius of 4 Å (L1-A); iii) intermolecular, which computes the NCIs between two sets of pre-defined fragments, in this case a set composed of fragment B and a set composed of fragment C (see Supporting Information for more details);

6. The protonation state is adjusted to standard conditions (pH = 7.4) using OpenBabel, by considering the pK_a values of the functional groups present in the molecule;[41]

7. The NCI calculation is run;

8. Results including the 2D and 3D NCI plots and the integrals of the density in the NCI regions are made available to the user through a link for online visualization, that remains active for 48 hours, where the results can also be downloaded.

Alternatively, the structure can be loaded in an XMOL format or a Gaussian wfn file. In these cases, an intramolecular NCI calculation is performed without preprocessing, as there is no chain or subunit information available to split the structure into fragments.

Tutorial example

As an example we have chosen PDB 5XH3, a complex of PET hydrolase with HEMT.[42] We aim at visualizing the interactions in between the enzyme and the HEMT ligand in order to understand its binding mode. The following steps are performed:

1. Screen 1 (Fig. 3a): The PDB is directly uploaded and the Ligand option is chosen, since the NCIs of interest are between the HEMT ligand and its binding pocket. An intermolecular computation would process the entirety of the protein searching for

NCIs in vain. Both entities are protonated. The email where results will be sent to is filled in.

2. Screen 2 (Fig. 3b): The fragments are chosen. Firstly, the HEMT is chosen as the ligand. It can be visualized by clicking on the eye symbol 👁 . The only chain is automatically chosen at the second line.

3. Screen 3 (Fig. 3c): The target ligand is chosen. In this case the HEMT from the previous screen is the only option. The calculation is sent. The user is provided with a reference number on the screen and by email, which is sent to the address provided in Screen 1 when the calculation is launched in the cluster.

4. Screen 4 (Fig. 3d): The user will receive another email when the calculation is done, with a link to visualize and download the results. More specifically, an image file containing the 2D plot of s against $\text{sign}(\lambda_2)\rho$ is displayed, along with an interactive visualization of the 3D isosurfaces representing the NCI regions. The resulting integrals of ρ and $\text{sign}(\lambda_2)\rho$ in the different intervals of the latter can be graphically visualized, as shown in Fig. S1. This and other files are available for download (see Supporting Information for further detail).

A closer inspection of Fig. 3d reveals the existence of a distinctly localized hydrogen bond between an oxigen atom in the 4-carboxylate group of the HEMT ligand and a hydrogen in residue MET132 of the hydrolase.

The steps are analogous for an intermolecular calculation, but instead of choosing a ligand, the user must specify the chains involved in the interaction. An example is the interaction between ribonuclease A and the ribonuclease inhibitor of the PDB 1DFJ[43] that turns out to be delocalized, therefore corresponding to a van der Waals region (see Fig. 4). A detailed description of how to perform this calculation is provided in the Supporting Information. For the intramolecular case, the steps are simpler as there are no target ligands

or chains to choose, as can be seen in Fig. S3 of the Supporting Information. For this type of calculation, the user can upload their own custom PDB, XMOL or Gaussian .wfn file.

Note that the NCIWEB visualization interface, although very useful to take a first glimpse at the results, does not provide many options. However, the files available for download allow for local, custom visualization (see Fig. S4).

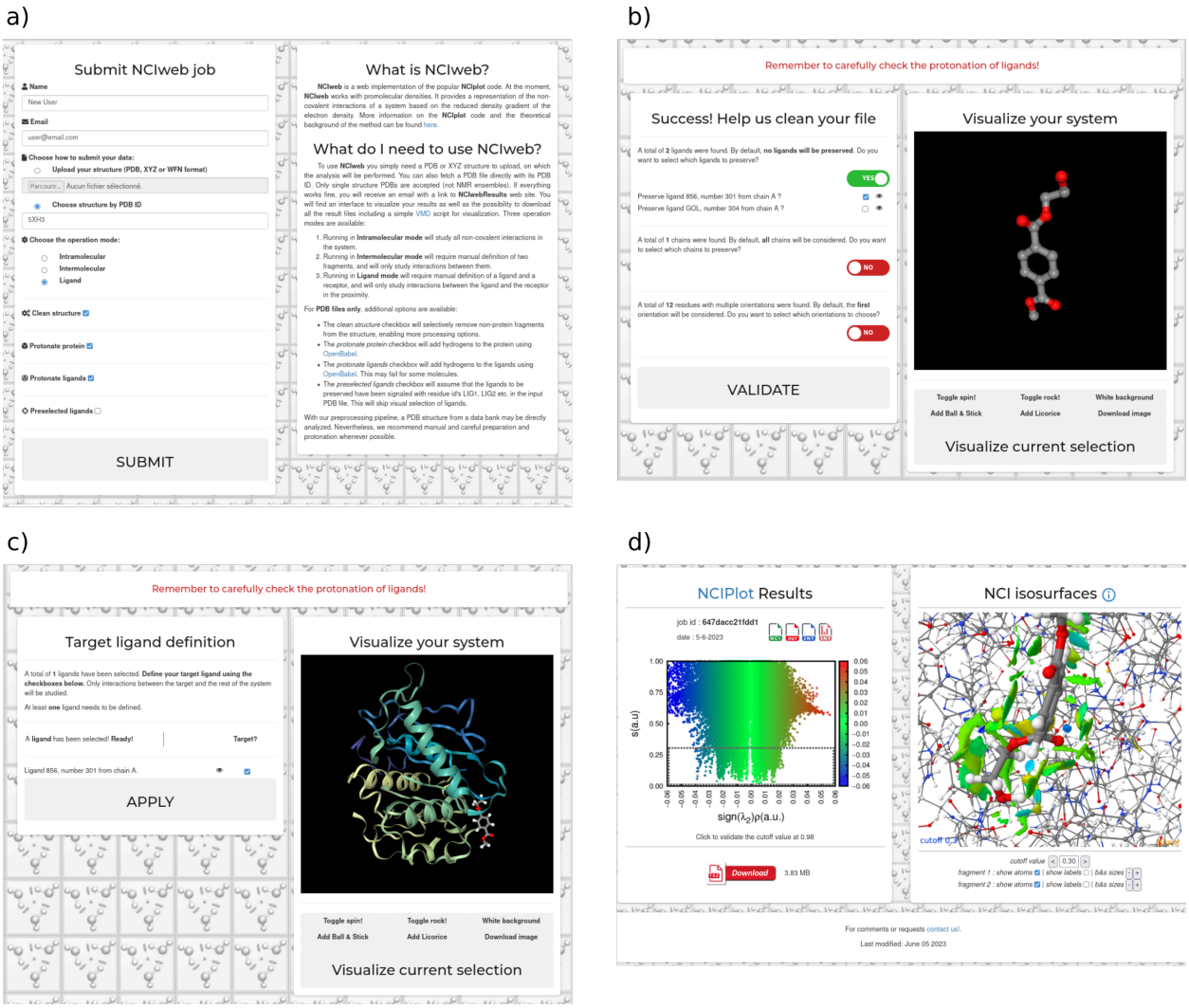

Figure 3: Steps to launch the NCIWEB calculations of the PET hydrolase with HEMT ligand (PDB ID: 5XH3). In a) screen 1 personal information is provided, the system is uploaded, the Ligand mode is chosen and structure protonation is requested; b) in screen 2 the ligand and chains are chosen, c) in screen 3 the target ligand is determined, and d) the resulting non-covalent interactions are visualized in their 2D representation (left) and as $s = 0.3$ a.u. isosurfaces (right) on the web server.

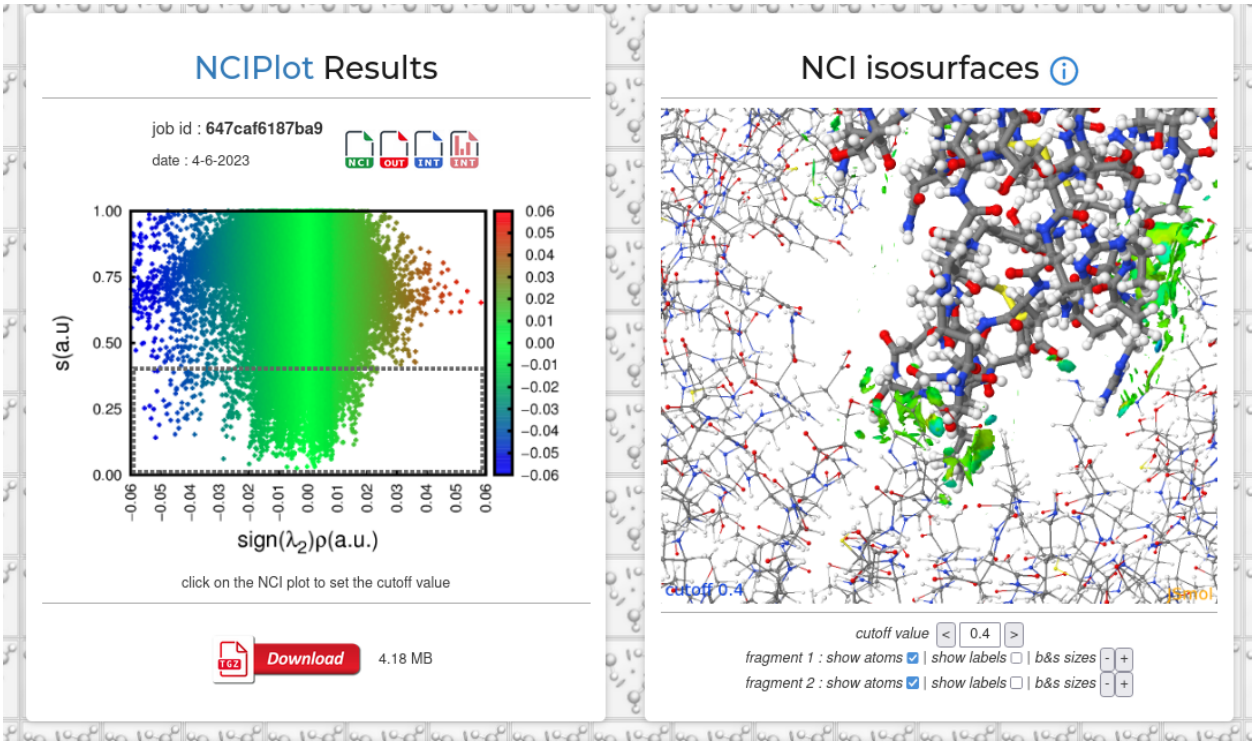

Figure 4: NCIWEB results for intermolecular interaction of ribonuclease inhibitor and ribonuclease A complex (PDB ID: 1DFJ), including the visualization of the resulting 2D NCI plot and of the 3D intermolecular NCI regions at $s = 0.4$.

Conclusions

We have presented the newly implemented NCIweb server, a user-friendly platform that makes it easy and accessible to find and classify regions of non-covalent interactions in large systems, enabling the study of NCIs in macromolecules of biological interest.

The promolecular approximation is used throughout, saving a considerable amount of computational time while maintaining a good description of attractive, repulsive and van der Waals interactions from first principles. To further optimize the performance of NCIWEB when the data is downloaded from the PDB, we have presented the intermolecular and ligand modes, that only search for NCIs in the regions of interest to the user. NCIWEB presents a simple and easy way of preprocessing the input structures in order to properly customize the computation and to fit to the user's needs. After the computation is performed internally, the server processes the output, offering convenient visualization and yielding the values

of the integrals of the density in the NCI regions, thus providing a quantitative estimate of the strength of the interactions. All of this makes it a powerful tool for a wide range of biomolecular applications, like studying protein–protein and protein–ligand interactions, and potential structural modification of complexes that could determine new biological functions and serve as a lead in the reverse engineering of desired compounds.

A thorough explanation on how to use the server has been laid out step-by-step, including examples for all the computation modes available. In this way, even an inexperienced user could successfully carry out a NCIPLOT calculation. This is exactly our aim: to expand the use of the NCI index formalism to new and diverse scientific communities.

Data and Software Availability

The NCIWEB server can be accesed through the following link: `https://nciweb.dsi.upmc.fr`. The output files for all of the examples on this manuscript are available in the *NCIWEB examples* Zenodo repository (`https://doi.org/10.5281/zenodo.7844872`).

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Supporting Information Available

Supporting information includes additional information about the formalism behind the NCI-index, a detailed description of how to visualize the results and what is available for download, and example calculations of intermolecular and intramolecular NCIs.

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