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Rigidity and Flexibility of Protein–Nucleic Acid Complexes

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***Abstract*—The study of protein–nucleic acid complexes is relevant for the understanding of many biological processes, including transcription, translation, replication, and recombination. The individual molecules in such complexes must be rigid enough to allow geometric matching of complementary shapes, yet sufficiently flexible to perform their functions.**

In this paper, we present a newly developed extension to KINARI-Web, our freely available server for biomolecular rigidity analysis, to permit the analysis of PDB files containing nucleic acids and protein–nucleic acid complexes. Previously, only the protein portion of these complexes could be analyzed by KINARI. To the best of our knowledge, no other publicly available rigidity analysis software has this capability.

We demonstrate this new feature by performing *in silico* rigidity studies on two data sets of protein–nucleic acid complexes, both in the absence and presence of nucleic acids. We find that the inclusion of nucleic acids significantly alters the rigidity of 40% of the 506 structures we analyzed.

I. Introduction

Biological molecules such as proteins and nucleic acids are not static structures. They undergo both local and global changes in conformation. Our primary goal is to understand the global, large-scale flexibility behavior, during which some of the molecule's regions (called "rigid clusters") retain their structure; indeed, they provide important information for elucidating the functions performed by these bio-molecules. Since experimental methods for visualizing molecular motions are expensive, difficult to interpret and give limited information, they are complemented by computational methods. Classical simulations based on molecular dynamics are however slow and better suited for studying fast, local motions. Rigidity analysis is an alternative, efficient, graph-based computational method that decomposes a macromolecule into a series of interconnected rigid clusters, thus providing insights into possible large-scale motions of the macromolecule. The work presented here is built upon KINARI-Web [1], a web server for kinematic and rigidity analysis developed in the research group of the last author, and freely available at http://kinari.cs.umass.edu. KINARI works with protein structure information extracted directly from the Protein Data Bank (PDB).

Although the vast majority of PDB files (92.6%) contain only proteins, the remaining files have structural information about nucleic acids and nucleic acids complexed with proteins. In this paper, we focus on rigidity analysis of protein–nucleic acid complexes and we present our recent extension of KINARI-Web to analyze these structures. To the best of our knowledge, no other publicly available software tool can perform this kind of analysis.

We demonstrate the usefulness of this new tool by analyzing the rigidity of the protein complexes in the absence and presence of nucleic acids. We found that for many, but not all of the structures, the inclusion of the nucleic acids had a pronounced effect on the rigidity of the entire complex. An example of rigidity analysis performed on a zinc finger binding domain bound to DNA (PDB ID: 2QKB) is shown in Figure 1. The results of this kind of analysis shed light on the stability and functions of these structures, and allow for the exploration of interactions between nucleic acids and proteins.

(a) protein (b) nucleic acids (c) complex

Figure 1. Rigidity analysis of Human RNase H catalytic domain (PDB ID: 2QKB). Each different colored region represents a rigid cluster. Only clusters containing more than 15 atoms are shown, and atoms not contained in these rigid clusters are shown in ball and stick representation. When the rigidity of only the protein portion is analyzed, two distinct rigid clusters are detected (a). The nucleic acid portion of the structure forms one large rigid cluster (b). Analyzing the entire biomolecule reveals the effect that the nucleic acids have on the rigidity of the complex (c); the two separate protein rigid clusters merge with the nucleic acid into a single large cluster.

II. Background

A. Computational Techniques

Because proteins motions are difficult to observe directly, computational techniques are used to simulate them. Physics-based molecular dynamics (MD) simulates macromolecule motions, but unfortunately is computationally intensive. As a result, only motions on short time scales can be effectively calculated. Another technique, the Gaussian Network Model (GNM) [2], models a protein or nucleic acid macromolecule as a series of spring interactions between residues within a cutoff distance. GNM calculates a collection of normal modes, and the slowest of them

is used to calculate the molecule's motion. oGNM [3] is one web tool that relies on the Gaussian Network Model, but it does not provide features to analyze the normal modes of a protein in the presence and absence of nucleic acids. Also, when two distinct conformations are available, software such as RigidFinder [4] will identify rigid regions of the molecule by placing residues in a rigid cluster if they are located within a cutoff distance in the two different conformations. Although RigidFinder can be used to analyze both proteins and nucleic acids, it is limited to the analysis of the small number of structures for which multiple crystallized conformations exist in the Protein Data Bank (PDB) [5].

B. Rigidity Analysis and the Pebble Game Algorithm

Rigidity analysis is a graph-based method for calculating the rigid clusters of a macromolecule. A mechanical model of the molecule is first constructed, made up of bars, bodies, and hinges. Small groups of atoms that are chemically bonded with no degrees of freedom among them are modeled as rigid bodies; they are interconnected by either fixed-length bars or hinges, depending on the type and number of chemical interactions (both covalent and non-covalent) between the corresponding atoms. The result is a bar-body-hinge framework, which is then turned into a (multi)-graph, in which each body is a vertex, each bar an edge, and each hinge five edges. The rigidity analysis of these particular structures is well understood mathematically [6], and allows for the usage of an efficient, combinatorial algorithm, called the pebble game [7]. It decomposes the graph into clusters, from which the rigid and flexible regions of the macromolecule are subsequently inferred. Variations of this method have been used in several studies, including the analysis of glass networks and proteins [8], the study of rigidity properties of protein mutants [9], and the study of protein unfolding [10].

III. Material and Methods

A. KINARI-Web for Nucleic Acid Rigidity Analysis

To analyze the rigidity of a macromolecule, its PDB file is converted into a list of atoms, and the constraints between the atoms are calculated. KINARI-Web divides this process (called curation) into four steps: 1) select chains, waters, ligands, and non-standard residues, 2) add hydrogen atoms using the software package Reduce [11], 3) calculate interactions between atoms, and 4) prune the interactions [1]. We have made several modifications to the curation steps of KINARI-Web to recognize the presence of protein, DNA, and RNA molecules based on the names of the residues. KINARI's procedure for identifying covalent bonds was extended to recognize nucleic acid residues and to place interactions accordingly. The bulk of the extension to curation, described next, concerns the weaker interactions: hydrogen bonds and hydrophobics.

1) Methods for Hydrogen Bond Identification: KINARI-Web could use either HBPlus [12] or bndlst [13] to identify hydrogen bonds in macromolecules. Although these two methods find similar sets of hydrogen bonds for protein PDB files, they produce greatly different numbers for RNA PDB files. Therefore, we had to determine which of the two methods should be used as the default to detect hydrogen bonds in nucleotide residues.

We compared the output of HBPlus and bndlst to a third program, FR3D [14]. It finds 3D structural motifs, including hydrogen bonds, in RNA PDB files (KINARI cannot use FR3D directly because it identifies residue-level rather than atom-level interactions). Since HBPlus and FR3D found a similar number of hydrogen bonds, we selected HBPlus to be the default method for detecting hydrogen bonds in nucleic acids. HBPlus is also KINARI's default option for proteins, used for profiling and validating of the software [15].

HBPlus is set up to process the connectivities of some RNA residues (A, C, G, T). Because our extension to KINARI aims at processing not just RNA but also DNA molecules (whose residues are labeled DA, DC, DG, DT in PDB files), we had to add the structures of these molecules when invoking HBPlus. We also added uridine (DU and U) and inosine (DI and I) residues, because these are considered standard residues in the PDB. In addition, KINARI was modified to pre-processes PDB files to convert the PDB nomenclature to match that of HBPlus. Without pre-processing, HBPlus misses some hydrogen bonds and does not identify base-phosphate and base-ribose interactions. These bonds and interactions can have a marked effect on the molecule's rigidity, and it has been shown that they play a significant role in RNA folding [16]. Note that KINARI does not yet identify carbon base-phosphate interactions, but these are much weaker (-0.1 to 1.1 kcal/mol) than other base-phosphate interactions (-2.8 to -10.1 kcal/mol).

2) Identifying Hydrophobics: Hydrophobic interactions in proteins are identified in KINARI using a heuristic method [1], and their energies are calculated by the Leonard-Jones potential, using amber99 atom types [17]. In nucleic acids, stacking interactions (hydrophobic interactions between consecutive base pairs) are key to stabilizing the molecules. Towards their identification, we added nucleic acid amber atom types to our heuristic method for finding the Leonard-Jones potential of hydrophobic interactions in RNA and DNA molecules. Since KINARI-Web was designed and tested primarily for protein rigidity [1], the parameters used for finding and modeling hydrophobic interactions are protein-based, and have yet to be fine-tuned for nucleic acid rigidity analysis. KINARI's analysis of nucleic acids could be further improved, as a previous study [18] has been shown that using a similar protein-based method for identifying hydrophobics results in overly rigid RNA structures.

B. Analysis of Nucleic Acids with Modified Residues

Because many of the nucleic acid structures in the PDB contain modified residues, we also extended KINARI to allow for their processing. The PDB provides Crystallographic Information Files (cif) with information about the structure and connectivity of these non-standard residues. KINARI downloads cif files from the PDB and uses this information to correctly place single and double covalent bonds within the modified residue and between the modified residue and other residues in the structure. A mechanical model of the macromolecule is built, and rigidity analysis is performed, analyzing modified residues in the same manner as other residues. Although the functionality for analyzing these modified residues is implemented and available to users, it has yet to be thoroughly tested and validated.

C. Rigidity Analysis of Protein–Nucleic Acid Complexes With and Without Nucleic Acids

A data set of 40 high-resolution protein–nucleic acid complexes was compiled by searching the PDB for X-ray structures containing protein and DNA, protein and RNA, or protein with DNA and RNA. Structures with modified residues were omitted from this data set because KINARI's performance on these residues needs further exploration. This small dataset was specifically selected to allow for a visual inspection of the rigidity analysis results. A larger data set of 509 nucleic acid protein complexes, with molecular weights less than 50,000 kDa and resolution lower than 2.5Å, was then created for the automated data collection of our experiments.

To determine how or if the addition of nucleic acids affects the rigidity of protein–nucleic acid complexes, KINARI rigidity analysis was performed three times on each structure in both of the data sets: examining 1) the nucleic acid portion only, 2) the protein portion only, and 3) the entire complex. Rigidity analysis was performed using KINARI's default parameters, modeling single bonds as a hinge, double and resonance bonds as six bars, hydrogen bonds as hinges, and hydrophobic interactions as two bars [1]. Rigidity metrics, including the size of largest rigid cluster, number of clusters, average cluster size, rigidity order parameter [19], number of hydrogen bonds, number of hydrophobic interactions and atom clustering, were recorded.

To quantify how the addition of the nucleic acid affected the rigidity of the complex, the changes in the size of the largest rigid cluster (ΔLRC), number of clusters (ΔRC), number of hydrogen bonds ($\Delta HBonds$), and number of hydrophobic interactions ($\Delta HPhobes$) were calculated by subtracting the metric for only protein and only nucleic acid from the metric obtained for the entire complex.

Based on the visualization of the rigidity analysis results for the small, 40-structure dataset, the complexes were classified into four categories shown in Table I.

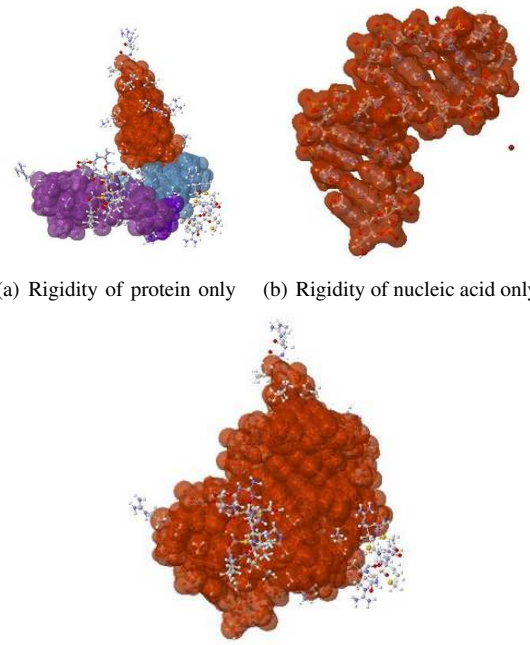
To help automate the classification of the structures in the larger, second dataset, we used the following ΔLRC calculation to further quantitatively classify the complexes:

$$\Delta LRC = LRC_{complex} - LRC_{protein} - LRC_{nucleicAcid}$$

Example rigidity results and a ΔLRC calculation are shown in Figure 2. Using the ΔLRC calculation, we arrived at a second classification scheme, shown in Table II.

Table I
CLASSIFICATION BASED ON VISUAL INSPECTION

A	The largest protein and nucleic acid clusters combined to form one large cluster in the complex	The number of total clusters in the complex was reduced
B	The largest protein and nucleic acid clusters combined to form one large cluster in the complex	The number of total clusters in the complex was reduced, but to a lesser degree than category A
C	The largest protein and nucleic acid clusters combined	There was little or no additional change in rigidity
D	The largest protein and nucleic acid clusters remained separate	There was no change in rigidity



(a) Rigidity of protein only (b) Rigidity of nucleic acid only

(c) Rigidity of protein–nucleic acid complex

Figure 2. For PDB ID 1A1H, in (a), the largest protein rigid cluster contains 292 atoms, while the largest nucleic acid (b) cluster is made up of 649 atoms. The combined protein–nucleic acid complex (c) has a largest rigid cluster made up of 1490 atoms. The resulting $\Delta LRC = 1490 - 292 - 649 = 549$ atoms.

Since this analysis approach only looks at the change in the largest rigid cluster it may miss structures where the rigidity of the smaller clusters change. However, during our visual analysis, no structures were found where this was the case. Cutoffs for the ΔLRC for the different categories were selected to maximize the correspondence between the quantitative and visual classifications.

Table II
QUANTITATIVE CLASSIFICATION SCHEME FOR COMPLEXES

	I	II	III	IV
ΔLRC range	> 300	150 to 300	0 to 150	< 0

A negative ΔLRC (**category IV**) indicates the largest rigid cluster in the complex did not change, or only changed by a small amount, while a positive ΔLRC indicates the largest rigid cluster in the complex is larger than (or the size of) the largest rigid clusters in the protein only and nucleic acid only portions combined. We divided the structures with positive ΔLRC values into three separate categories based on the range of ΔLRC. A ΔLRC close to or equal to zero indicates the largest rigid clusters joined but there was little or no additional change in rigidity, so we place structures with ΔLRC between 0 and 150 in **category III**. Structures with $\Delta LRC \geq 300$ are placed in **category I**, because this indicates that the size of the largest rigid cluster is much greater than the combined largest rigid clusters of the protein and nucleic acid only portions. The structures with positive ΔLRC between 150 and 300 are placed in **category II**, indicating that the combined rigidity increased, but to a lesser extent than found in category I.

IV. RESULTS AND DISCUSSION

A. Visual Analysis of the Smaller Data Set

Of the 40 protein–nucleic acid complexes in the first data set, analysis with nucleic acids changed the rigidity of 18 structures, producing a large change in 10 structures (Category A) and a lesser change in 8 structures (Category B). In 15 structures, the protein and nucleic acid clusters joined in the complex, but there was little or no additional change in the protein rigidity (Category C). Finally, 7 structures exhibited no changes between analysis with and without nucleic acids present (Category D).

B. Quantitative Analysis of the Larger Data Set

Table III lists the quantitative (ΔLRC based) classifications 506 out of the 509 complexes in the larger dataset. Three structures were not classified because KINARI analysis produced error messages. In addition, the changes in the number of rigid clusters (ΔRC) hydrogen bonds ($\Delta HBonds$) and hydrophobic interactions ($\Delta HPhobes$) were calculated for each of the complexes. The average changes in the number of hydrogen bonds and hydrophobic interactions for each category are also listed in Table III.

Table III
CATEGORIZATION AND STATISTICS FOR THE LARGER DATA SET

	I	II	III	IV
Number of Files	159	44	176	127
Average ΔLRC	801.2	214	49.01	-438.7
Average ΔRC	-73.38	-43.32	-15.18	-3.213
Average $\Delta HBonds$	10.66	9.091	5.864	2.228
Average $\Delta HPhobes$	16.86	20.57	7.551	5.315

Although larger ΔLRC values (and hence different categories) are associated with larger average $\Delta HBonds$ and $\Delta HPhobes$, there does not appear to be a direct relationship between the values of $\Delta HBonds$ and $\Delta HPhobes$ and the categorization. This was surprising. For some structures, several hydrogen bonds or hydrophobic interactions are added and there is little or no impact on rigidity of the complex, while for others, only a small number of interactions are added but these have a marked affect. This indicates that many of these non-covalent bonds are redundant. In the next sections we give four case studies that highlight interesting rigidity results.

Case study 1 - 1ZBL: Addition of nucleic acid joins two largest protein clusters

PDB file 1ZBL contains the structure of an RNase H bound to a DNA/RNA hybrid [20]. It is an endonuclease that non-specifically cleaves RNAs contained in DNA/RNA hybrids. Figure 3 shows the rigid decomposition of both the nucleic acid and protein portions and the entire complex. In the absence of nucleic acids, the protein is divided into two large rigid clusters, and in the absence of protein, the DNA/RNA hybrid strand is decomposed into one large rigid cluster. Analysis of both together causes the two protein and one nucleic acid clusters to combine, forming one large rigid cluster. The ΔLRC, $\Delta HBonds$, and $\Delta HPhobes$ values for 1ZBL are listed in Table IV. For 1ZBL, ΔLRC is 1445, and $\Delta HBonds$ and $\Delta HPhobes$ are 5. This indicates that five hydrogen and hydrophobic interactions form between the protein and nucleic acid subunits, which have an impact on the rigidity, increasing the size of the largest rigid cluster by 1,445 atoms.

(a) Protein (b) Nucleic acid (c) Protein–nucleic acid complex

Figure 3. **Case Study 1:** KINARI-Web visualization of 1ZBL rigidity. Including a rigid nucleic acid segment (b) with two far-separated protein segments (a), produces one large rigid complex.

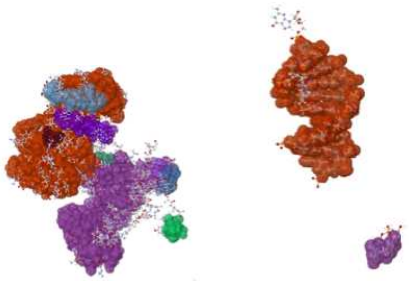
Table IV
KINARI-WEB GENERATED RIGIDITY STATISTICS FOR CASE STUDIES

PDB ID	Category	ΔLRC	$\Delta HBonds$	$\Delta HPhobes$
1ZBL	I	1445	5	5
3HXM	II	230	3	11
2G8I	III	11	4	0
2NQ9	IV	-319	0	3

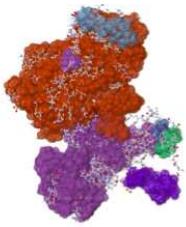
Case study 2 - 3HXM: The size of the largest rigid cluster increased, but to a lesser extent

PDB file 3HXM contains the structure of the Argonaute protein bound to a target RNA molecule [21]. Argonaute is part of the RNA-induced silencing complex (RISC),

and functions by recognizing and cleaving specific RNA sequences. Rigidity analysis was performed on the protein and nucleic acid portions separately, and on the entire 3HXM complex. Figure 4 shows these three different rigid cluster decompositions. Analysis of only the protein portion divides the structure into two large rigid clusters and several smaller clusters. In the absence of protein, the nucleic acid portion is decomposed into one large rigid cluster consisting of 517 atoms, and another smaller rigid cluster. When the entire complex is analyzed together, the larger of the two protein clusters (containing 3072 atoms) combines with the large nucleic acid cluster to form a rigid cluster with 3819 atoms. The second of the two large protein clusters remains separate from the combined protein–nucleic acid cluster. In this case, the addition of nucleic acid increased the rigidity of the complex, but to a lesser extent than in complexes like 1ZBL. The ΔLRC value of 230 (see Table IV) indicates that additional atoms become a part of the new largest rigid cluster. Three hydrogen bonds and eleven hydrophobic interactions form between the nucleic acid and protein portions.



(a) Protein (b) Nucleic acids



(c) Complex

Figure 4. **Case Study 2:** KINARI-Web visualization of 3HXM rigidity. Incorporating nucleic acid (b) with a largely flexible protein (a) alters the rigidity of the complex (c), but does not result in as large a change in rigidity as in PDB file 1ZBL.

Case study 3 - 2G8I: The nucleic acid and protein largest rigid clusters joined, but little other change in rigidity

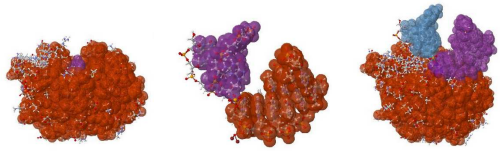
2G8I contains the structure of a reaction intermediate of RNase H bound to a DNA/RNA hybrid [22]. Figure 5 shows the rigidity results. When protein and nucleic acid are analyzed separately, their rigid decompositions primarily consist of one large cluster each, of 1313 and 329 atoms respectively. Analysis of the entire complex produces one large rigid cluster containing 1653 atoms. The calculated ΔLRC value (Table IV) indicates that the nucleic acid and protein portions combined to form one large rigid cluster that is only slighlty (11 atoms) larger than the clusters of the two separate components. Four hydrogen bonds exist between the protein and nucleic acid portions, and no additional hydrophobic interactions between the two complexes are formed.

(a) Protein (b) Nucleic acid (c) Complex

Figure 5. **Case Study 3:** KINARI-Web visualization of 2G8I rigidity. Analyzing the protein (a) along with a rigid DNA/RNA strand (b), results in a large rigid cluster (c) that is only slightly larger than the sum of the protein and DNA/RNA rigid clusters.

Case study 4 - 2NQ9: No change in rigidity after adding nucleic acid

PDB file 2NQ9 contains an *Escherichia coli* endonuclease complexed with DNA. This particular endonuclease is involved in the excision and repair of DNA bases [23]. Analysis of the DNA portion of this complex produced two large rigid clusters (see Figure 6) while analysis of only the protein portion produced one large rigid cluster. When analyzed together, the large rigid clusters did not change, leading to a ΔLRC of -319 (see Table IV), which is the size of the DNA largest rigid cluster. There were no hydrogen bonds and three hydrophobic interactions between the nucleic acid and protein portions, but these hydrophobics were not sufficient to alter rigidity the of the complex.



(a) Protein (b) Nucleic acid (c) Complex

Figure 6. **Case Study 4:** KINARI-Web visualization of 2NQ9 rigidity. Although three hydrophobic bonds exist between the protein (a) and nucleic acid (b) portions, nucleic acid does not have any effect on the rigidity of the complex (c).

V. Conclusion

We have extended KINARI-Web, our server for protein rigidity analysis, to analyze the rigidity of nucleic acids and protein–nucleic acid complexes. Until now, rigidity analysis of DNA and RNA structures was not possible in KINARI. Using our software, we performed preliminary studies of the rigidity of hundreds of complexes in the absence and presence of nucleic acids.

Adding nucleic acids markedly impacted the rigidity of many (203 out of 506 files) but not all of the complexes examined. Consequently, based on our proof-of-concept

analysis, one might hypothesize that to gain accurate information into the rigidity of proteins contained in protein–nucleic acid complexes, it is important to analyze these proteins with nucleic acids present.

The implementation of this extension and our preliminary studies into the rigidity of protein–nucleic acid complexes is a first step toward accurate rigidity analysis of nucleic acids. In the future, we will improve our methods for identifying and modeling hydrophobic interactions in nucleic acids. The performance of our software on nucleic acids with modified residues will also be examined.

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Contributions

IS designed the research and lead the project. EF developed the new curation and nucleic acid modeling features and FJ integrated them into KINARI. SS performed the experiments; FJ and EF developed the case studies. IS, FJ, and EF wrote the paper.

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