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Simon B. Hengeveld,* T. Malliavin,† J.H. Lin,‡ L. Liberti,§ A. Mucherino,*

*IRISA, University of Rennes 1, Rennes, France.
simon.hengeveld@irisa.fr, antonio.mucherino@irisa.fr

†Institut Pasteur, Paris, France.
therese.malliavin@pasteur.fr

‡Academia Sinica, Taipei, Taiwan.
jhlin@gate.sinica.edu.tw

§École Polytechnique, Palaiseau, France.
liberti@lix.polytechnique.fr

Abstract—**The Distance Geometry Problem (DGP) consists of finding the coordinates of a given set of points where the distances between some pairs of points are known. The DGP has several applications and one of the most relevant ones arises in the context of structural biology, where NMR experiments are performed to estimate distances between some atom pairs in a given molecule, and the possible conformations for the molecule are calculated through the formulation and the solution of a DGP. We focus our attention on DGP instances for which some special assumptions allow us to discretize the DGP search space and to potentially perform the complete enumeration of the solution set. We refer to the subclass of DGP instances satisfying such discretizability assumptions as the Discretizable DGP (DDGP). In this context, we propose a new procedure for the generation of DDGP instances where real data and simulated data (from known molecular models) can coexist. Our procedure can give rise to peculiar DDGP instances that we use for studying the impact of every distance type, involved in NMR protein structure determination, on the quality of the found solutions. Surprisingly, our experiments suggest that the distance types implying a larger effect on the solution quality are not the ones related to NMR data, but rather the more abundant, but much less informative, van der Waals distance type.**

I. Introduction

Given a simple weighted undirected graph $G = (V, E, d)$, the Distance Geometry Problem (DGP) in dimension 3 asks whether a realization $x : V \to \mathbb{R}^3$ exists such that all distance constraints

$$\forall \{u, v\} \in E, \quad ||x_u - x_v|| = d(u, v), \qquad (1)$$

are satisfied [14], where $|| \cdot ||$ is the Euclidean norm. Several real-life applications can be formulated as a DGP [21]. In this work, we will focus on the very important application in structural biology, where vertices of G represent atoms of a given molecule, and the distance information associated to the edges of the graph either reflects the geometry of its chemical structure, or it is experimentally obtained by Nuclear Magnetic Resonance (NMR) experiments [1]. In the context of this application, the employed distances come from different sources, and they can carry a different level of uncertainty on the actual distance value. Therefore, the equality constraints in equ. (1) can generally be replaced with inequality constraints where the lower and the upper bounds on the distances are taken into account. Throughout the article, we will confuse the two terms *vertex* and *atom*, as well as the two terms *edge* and *distance*.

The Discretizable DGP (DDGP[1]) [20] is a subclass of the more general DGP class. Let E' be the subset of edges in G that are related to *exact* distances, as opposite to *interval* distances, where a lower and an upper bound are actually given. As a consequence, $E \setminus E'$ is the subset of edges in G that contain all the interval distances.

Definition 1 *A simple weighted undirected graph G represents a DDGP instance in dimension 3 if and only if there exists a vertex ordering on V such that the following two assumptions are satisfied:*

(a) *$G[\{1, 2, 3\}]$ is a clique whose edges are in E';*
(b) *$\forall v \in \{4, \dots, |V|\}$, there exist u_1, u_2, and $u_3 \in V$ s.t.*
(b.1) *$u_1 < v$, $u_2 < v$, $u_3 < v$;*
(b.2) *$\{\{u_1, v\}, \{u_2, v\}\} \subset E'$ and $\{u_3, v\} \in E$;*
(b.3) *$d(u_1, u_3) < d(u_1, u_2) + d(u_2, u_3)$,*

where $G[\cdot]$ is the subgraph induced by the subset of vertices of V given in argument.

We can remark that assumption **(a)** is able to fix the coordinate space where the molecular conformations will thereafter constructed. Because of assumptions **(b.1)** and **(b.2)**, at least

[1] Accordingly to the definitions in [14] and [11], we should actually name the problem the "interval" DDGP and use the acronym iDDGP. Throughout this paper we are however going to use the acronym DDGP, for two reasons: (i) it makes notations lighter; (ii) we prefer to think of the DDGP as a general problem comprising exact and interval distances, where an instance with only exact distances is actually a special case.

3 other distinct atoms, preceding the current vertex v in the given vertex ordering, can be used as a reference for positioning the vertex v. Moreover, assumption **(b.2)** makes sure that at most one of the 3 available distances has a large enough degree of uncertainty to be considered as an interval distance, while at least two other distances can be considered as "exact". These two previous conditions ensure that, for every atom $v \in V$ to be placed, there is only a "small" subset (under some conditions, a discrete subset) of feasible positions for that atom. Finally, assumptions **(b.3)** ensures that the 3 reference atoms are not aligned in their assigned positions (the triangular inequality is strictly satisfied). Under these assumptions, the DDGP search space can be modeled as a tree [20], and, depending on the uncertainty associated to the third distance in assumption **(b.2)**, the nodes in this tree can either contain singletons (i.e., exact locations for the current atom), or rather (relatively) small subsets of feasible positions for the atom v. For more information about the theoretical background concerning this discretization process for the search space, the reader is referred to the citations above, and others therein.

Since DDGP search spaces are trees, the complete enumeration of the solution space is potentially possible (this is a rather hard task when the search space is continuous). This is a point of high interest in the context of structural biology, because the identification of a possible conformation for a molecule, which satisfies all distance constraints, does not deny the existence of another (even completely different) conformation where all distances are still satisfied. A fundamental advantage in the DDGP formulation stands in the fact that multiple solutions can be identified at once, and that no solutions can be discovered thereafter, not after a complete exploration of the search space has already been performed.

The DDGP is NP-hard [10], as well as the more general DGP [25]. However, for the DDGP case, a specific algorithmic framework has been proposed in [12] to explore the search tree generated by the discretization process. This framework has been shown to work well in practice and is generally known under the name of Branch-and-Prune (BP) algorithm. The main idea is to exploit the distances that are available because of the assumptions in Def. 1 to construct the search tree recursively in a depth-first fashion, and to use additional distances (that may be available or not) for pruning purposes: whenever those additional distances are not satisfied by the computed candidate positions, the currently explored branch of the tree is pruned, and the search is backtracked. This mechanism of alternating branching and pruning phases allows the search to focus on the parts of the search tree where it is more likely to discover solutions. More general details about the BP framework can be found in Section II, together with some specific information about the BP implementation that we will use in the computational experiments below.

In previous works on the DDGP, NMR data were simulated from known molecular models extracted from PDB files [2]. As research went on (see for example [4, 11, 17, 23]), the considered DDGP instances approached more and more the *genuine* NMR data. Initially proposed for instances containing exact distances only [12], the BP framework was extended to interval distances in [11]; it was then associated to a coarse-grained representation (to better deal with uncertainty) in [23], and to a multi-threading-like approach (to deal with larger instances) in [17]. Together with these NMR-derived distances, other distances, rather obtained from the chemical structure of the considered molecules, are also included in the DDGP instances. It was noticed in fact that these additional distances give a fundamental help in determining the final molecular conformations, by guiding the solvers towards feasible solutions.

In this work, we will make a considerable step forward, by using for the very first time real NMR data in our experiments. Moreover, we will present a computational study where we will mix real and simulated data, with the main aim of analyzing the impact of every involved type of distances on the obtained solutions. When all distance types are simulated except one specific type, then we expect to observe how important this specific type is for the determination of the molecular structure. As expected, our computational experiments seem to suggest that some distances actually have a larger impact than others on the quality of the obtained results. Unexpectedly, however, these do not seem to be the NMR-related distances.

The rest of the paper is organized as follows. In Section II, we will briefly review the main ideas behind the BP framework. We pay particular attention to its implementation distributed under the name MDjeep, where a coarse-grained representation of the solutions is introduced to deal with the uncertainty on the interval distances. In Section III, we will detail a procedure for the generation, from the original set of NMR raw data, of DDGP instances where additional distance information, obtained by analyzing the chemical structure of the molecule at hand, is also included. Finally, Section IV will be devoted to our computational experiments, where simulated and original NMR distances will coexist and form peculiar instances that we will use to study the impact of every distance type on the conformations that MDjeep is able to find. Conclusions will be drawn in Section V, with some possible future works.

II. MDjeep

MDjeep[2] is a freeware implementation of the BP framework for DDGPs [22]. This main algorithmic framework can be implemented in the context of the DGP when the two discretization assumptions in Def. 1 are satisfied, because they ensure that the DGP search space has the structure of a tree (see Introduction). Algorithms based on the BP framework can therefore recursively construct this search tree (this is the "branching phase", which exploits the distances that are known by the assumptions), and to immediately verify the feasibility of newly generated tree branches (this second phase is named "pruning phase", because it actually allows for removing some branches from the search tree). While the pruning phase can

[2] https://github.com/mucherino/mdjeep

be applied alike to both exact and interval distances, because the verification of one equality (exact case) is simply replaced by two inequalities (interval case) to verify, the outcome of the branching phase is essentially different in the two situations. When all involved distances are exact, the set of feasible positions for a certain atom of the molecule (placed on a common layer of the search tree) is discrete and finite [13]. Instead, when interval distances are involved, disjoint and continuous subsets of feasible points can be identified for some atoms forming the molecule [11].

The use of a coarse-grained representation of the search space allows us to efficiently deal with the continuous feasible subsets of potential atomic positions while preserving the general tree structure [23, 24]. As its name suggests, this particular representation replaces every feasible subset of atomic positions, that is disjoint from all other possible positions related to the same atom, with a rough approximation of the subset. In this work, we approximate these subsets with three-dimensional boxes that are supposed to contain the *true* position of the atom (box-shaped space regions were also employed in the preliminary experiments presented in [23]). An initial guess of the actual position of the atom inside its own box is attempted at the time the box is created; this position is subsequently refined (via local optimization) for guaranteeing (when possible) the satisfaction of additional distances related to the current atom, including the ones that may come to play on further layers of the search tree.

In the experiments we will present in this work, we will consider the version `0.3.2` of MDJEEP. Since its version `0.3.0`, in fact, MDJEEP is integrated with the coarse-grained representation which allows for solving instances containing interval data [19]. For more information about this freeware, the reader is referred to the cited articles, as well as to the dedicated `GitHub` repository.

III. From NMR raw data to DDGP instances

Nuclear Magnetic Resonance (NMR) spectroscopy is able to provide data about the given molecule from which one can derive estimations of distances between pairs of atoms (mainly pairs of hydrogen atoms), together with some estimations on some of the typical torsion angles that can defined on the protein structure [7]. We can consider that these estimations are very rough: the distances between two given hydrogen atoms, for example, is given in ranges from 1.8 up to 5Å [28]. Moreover, these estimated distances generally only concern *short* distances, while the non-detection of a distance does not necessarily imply that it is longer than a "short" distance. Finally, it is sometimes hard to identify the correct pairs of atoms related to a given NMR distance, which can lead to the definition of distance constraints where a subset of potential atom pairs is given, and not only one unique pair [15, 16].

As already mentioned in the introductory part of this paper, we do not only include NMR data in our instances, but complete the overall distance information given by NMR with some additional distance information derived from analyzing different properties of the molecule. This additional distance information is fundamental to guide the solvers towards the feasible conformations. Moreover, in the specific case of the BP framework implemented by MDJEEP, it is important to point out that the assumptions in Def. 1 could not be satisfied without the explicit introduction of these additional distances [18].

In this section, we will present a procedure for creating DDGP instances by combining the NMR raw information with the additional distances that can be derived by analyzing the chemical structure of proteins. Listed below are five different *types* of distances that we will consider when building our DDGP instances:

Type1 lower bounds on the pairwise distances between all of the atoms, based on their van der Waals radii;

Type2 force field derived distances, which we can separate in two sub-types: (i) bond distances between covalently bonded atoms, and (ii) the distances calculated from the bond distances and the bond angles using the cosine formula;

Type3 NMR distances: these are interval distances, based on the measurements from NMR spectroscopy;

Type4 NMR torsion angles: the torsion angles are related to triplets of consecutive chemical bonds, involving four sorted atoms of the molecule, from which a distance value between the first and last atoms can be derived;

Type5 finally, distances derived from the minimal and maximal extension of all torsion angles which can be defined in the molecular conformation (except for those already defined in **type4**).

Notice that **type1** concerns interval distances where only a lower bound is given; the upper bound is infinite in theory but in practice it can be set to a sufficiently large value to cover the entire protein. These upper bounds can be tightened by using triangular inequality based on the other distances available in the instance. However, oftentimes these upper bounds remain quite large w.r.t. those related to other interval distances. We point out that the **type2** includes two sub-types for the following reason: in both cases, we need to use the same bond length distances extracted from a force field. Finally, the last two types are closely related: whenever a torsion angle is not defined through NMR (**type4**), we consider its minimal and maximal extension to estimate the distance between the first and last atom in the corresponding quadruplet (**type5**).

Our procedure is based on the idea to build-up the graph $G = (V, E, d)$ by adding more and more information (about atoms at first, about the various distance types then) in order to enrich it as much as possible. Figure 2 visualizes how the graph changes after each step in the process, showing the procedure for the first two amino acids of the protein `2jmy` in the Protein Data Bank (PDB) [2].

At the beginning, we are given an NMR file, and an empty graph G. This NMR file is in the form of a STAR file [27], which are files that are available in the PDB for protein models obtained through NMR experiments. Generally, NMR files contain a string, describing the amino acid chain of the corresponding protein. Each amino acid has a defined structure and behaviour when forming peptide bonds in the backbone of a protein. Based on the protein structure, we begin by constructing the vertex set V of G, one amino acid at a time. When adding every amino acid, our procedure reflects the natural behaviour of the amino acid during the protein synthesis. Therefore, when constructing the graph, totally three atoms are removed from the two amino acids forming the peptide bond, which thereafter form a water molecule, a byproduct of this event. The amino acids with missing atoms after the construction of the peptide bond are also referred to as *residues*. The first amino acid of the protein is referred to as the N-terminus, and the final amino acid of the protein sequence as the C-terminus. Compared to non-terminal residues, the N-terminal amino acid has two extra hydrogen atoms attached to the first nitrogen atom, while the amino acid that contains the C-terminus has an extra oxygen atom[3]. Figure 1 shows an example of these special cases for a simple chain only containing two amino acids.

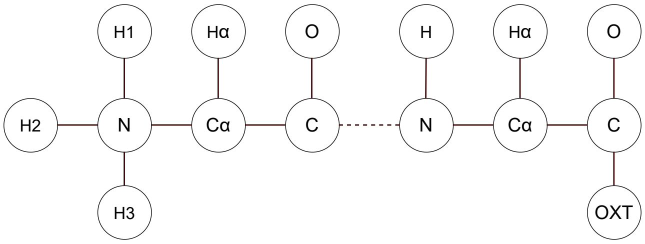
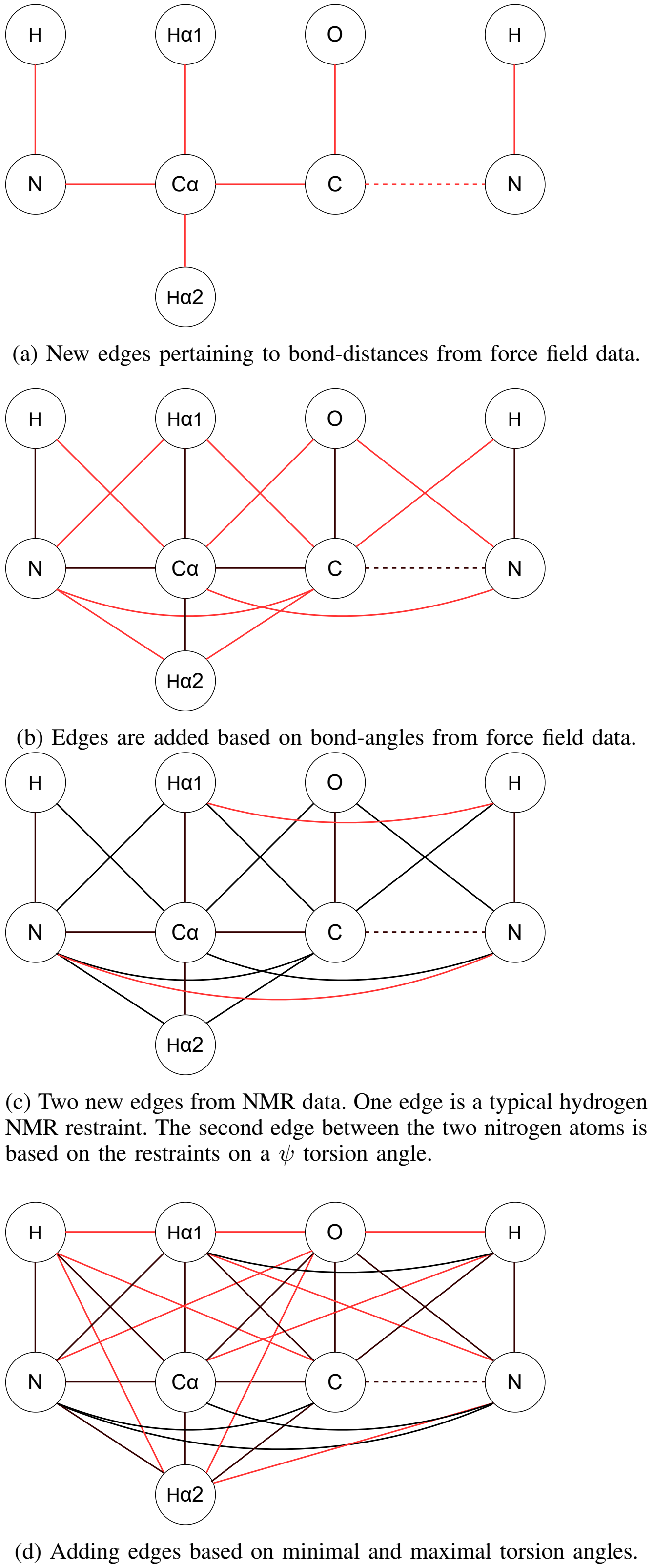


Fig. 1: Illustration of two amino acids forming a peptide bond (dashed). Side-chains are omitted in the figures for clarity.

While adding these atoms to the graph, we make sure to partition the graph into different clusters. The clusters represent each individual amino acid, such that the combination of a cluster and an atom identifier is unique. This way, when we are adding distances from i.e. NMR files, we know which atoms in the NMR file pertain to which vertices in our graph. Once the vertex set V is filled, we start adding the various distance types, as detailed in the following subsections.

Type1*: lower bounds based on Van der Waals radii*

The van der Waals radius r_u of an atom $u \in V$ is the radius of an imaginary sphere which represents the closest distance any other atom v can approach u [3]. This means that these radii can be used to provide an expected lower bound to the distance between any pair of atoms. For any pair of two vertices $u, v \in V$, we add an edge e to G based on this van der Waals minimal distance. We sum the van der Waals radii r_u and r_v, and finally take 80% of the sum as the lower bound on the distance. This radius allows us to describe the electron cloud around the nucleus of an atom as a sphere. However, due to polarisability, these clouds are never *real*



(a) New edges pertaining to bond-distances from force field data.

(b) Edges are added based on bond-angles from force field data.

(c) Two new edges from NMR data. One edge is a typical hydrogen NMR restraint. The second edge between the two nitrogen atoms is based on the restraints on a ψ torsion angle.

(d) Adding edges based on minimal and maximal torsion angles.

Fig. 2: The evolution of the distance graph G. The figure shows a glycine and two atoms of a next amino acid in the sequence, connected by a peptide bond (dashed line). The edges that are added at each step are colored in red (light gray in gray scale); the pre-existing edges are instead marked in black. Note that we do not include the van der Waals lower bounds.

[3] This explanation may sound naïve to many people used to work in the context of structural biology. However, we decided to include this paragraph in the text because the bioinformatics community also includes pure computer scientists who may not be completely familiar with the biological mechanisms that our procedure attempts to reproduce by acting on G.

spheres, so that a pair of atoms can actually be closer than the sum of their van der Waals radii. In other words, the spheres in the var der Waals model can be considered as *soft* spheres, meaning that they can actually slightly penetrate one another, which is why we use 80% of this sum as the lower bound on our distances. On the one hand, van der Waals distances are not very informative, because they only carry a lower bound, while their upper bound is generally very large (initially set to infinity, it can be improved by using triangular inequalities involving related distances). On the other hand, however, these distances are very abundant, because they can be defined for every pair of atoms which are not bonded. Because of this high abundance, we do not explicitely report these distances in Figure 2.

Type2*: exact distances based on force fields*

Force fields are computational methods that allow us to estimate the forces between atoms within molecules. Using these forces, we can obtain rather precise estimates of distances between bonded atoms. These distances are added as edges to G, as shown in Figure 2a. Another useful piece of information that can be computed using force fields is the the angle between atoms bonded to a common atom. Using this bond angle, we can then also compute the distance between these two atoms. This can be done by using the law of cosines, as two sides and one angle are known. Examples of these distance edges are shown in Figure 2b, highlighted in red (light gray in gray scale).

Type3*: interval distances from raw NMR data*

The NMR file type that we focused on in the practical implementation is the STAR file type [27]. This is predominantly because the NMR STAR files include a description of the residue chain. Other NMR files could also be used, as long as they are somehow paired with information about the sequence of amino acids. These NMR files generally include two kinds of restraints. The first kind of restraints (which is related to our **type3** distance) are direct constraints on distances. They typically describe distances between hydrogen atoms, and can only be measured for atoms that are not further away from each other than 5 to 6Å. Furthermore, these constraints lead to interval distances whereby the intervals can be quite large. An illustration of such a distance between hydrogen atoms can be found in Figure 2c, between the atoms H_α^1 and H. In this work, whenever there is uncertainty on the distance assignment, we simply look at the corresponding PDB file and we fix the assignment to the right pair of atoms.

Type4*: torsion angles from raw NMR data*

The second restraints that we can find in an NMR raw data file are bounds on different torsion angles within the protein backbone. A torsion angle, or *dihedral* angle, is defined by three consecutive bonds involving four atoms. The angle describes a rotation around the middle bond. Figure 3 gives an illustration of such a torsion angle γ. In the case of molecules, the points p_1 through p_4 describe four consecutive bonded atoms. The distances a, b and c then describe the bond distances. Finally, the angles α and β are bond angles that can be derived from force fields (see **type2**).

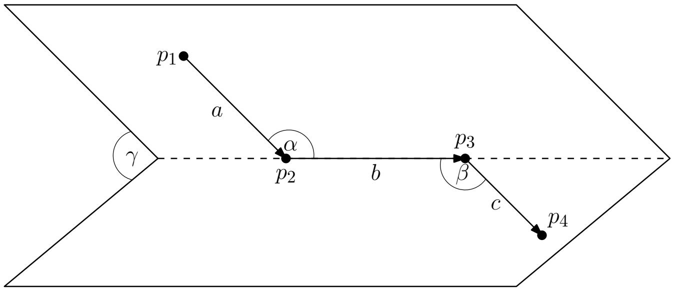


Fig. 3: An illustration of a dihedral angle given four points p_1, p_2, p_3 and p_4.

NMR data files give empirical constraints on two types of torsion angles, ϕ and ψ. The ϕ angles pertain to the rotation around the N-C$_\alpha$ bonds while the ψ angles relate to the C$_\alpha$-C bonds, both in the backbone. Using these torsion angles ($\gamma = \phi$ or ψ), and the distances (a, b) as well as the angles (α, β) obtained from the force field data, we can compute bounds on the distance between the first (p_1) and the last (p_4) atoms of the quadruplet. To do this, we compute the coordinates of the points p_1 and p_4 with the origin set at the midpoint of p_2p_3. We imagine the particular case where the torsion angle $\tau = 0$. Then, from this specific case we generalize for all other cases by rotating the vector $\overrightarrow{p_1p_2}$ around the x axis, with an angle of $\gamma/2$. We rotate $\overrightarrow{p_3p_4}$ in the opposite direction, with angle $-\gamma/2$. This gives us the below formulas for $p1$ and $p4$:

$$p_1 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\gamma/2 & -\sin\gamma/2 \\ 0 & \sin\gamma/2 & \cos\gamma/2 \end{pmatrix} \begin{pmatrix} -a\cos\alpha \\ +a\sin\alpha \\ 0 \end{pmatrix} - \begin{pmatrix} b/2 \\ 0 \\ 0 \end{pmatrix},$$

$$p_4 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\gamma/2 & \sin\gamma/2 \\ 0 & -\sin\gamma/2 & \cos\gamma/2 \end{pmatrix} \begin{pmatrix} -c\cos\beta \\ +c\sin\beta \\ 0 \end{pmatrix} + \begin{pmatrix} b/2 \\ 0 \\ 0 \end{pmatrix}.$$

All that remains is to compute the distance from $p1$ to $p4$ using the Euclidean distance formula:

$$\delta = \sqrt{(x_1 - x_4)^2 + (y_1 - y_4)^2 + (z_1 - z_4)^2}.$$

An example of such a distance is shown between the two nitrogen atoms in Figure 2c. This interval distance is based on the ψ torsion angle.

Type5*: "weak" interval distances based on minimal and maximal torsion angles*

Without considering the van der Waals distances (because they basically carry a lower bound), we can notice that, even after adding the distance types described in the previous sections (*types* 2, 3 and 4), the graph G does not correspond yet to an instance that satisfies the assumptions in Def. 1. This is visible in Figure 2c, which shows that we are still missing some distances to satisfy those assumptions.

To this aim, we add another (the last) type of interval distance: for every four atoms connected by three consecutive bonds, we derive the bounds on the distance value between the first and the fourth atom of the sequence. We compute these bounds based on the minimal (0°) and maximal (180°) torsion angles of the sequence (when information about this torsion angle was not given by NMR). These intervals are rather large, as they cover all possible values for the torsion angle, and they were already introduced in DDGP instances for guaranteeing the discretization [11]. Examples of edges added to G corresponding to these distance intervals are shown in Figure 2d.

After adding this last distance type, the graph G does satisfy the assumptions allowing for the discretization of the search space. The solver MDJEEP (see Section II) can therefore be invoked for the solution of the DDGP instance represented by the graph G.

IV. COMPUTATIONAL EXPERIMENTS

In previous works on the DDGP, the NMR instances were simulated by looking at some of the molecular models deposited on the PDB [2]. We say that this kind of artificially generated instances contain *simulated* data. MDJEEP was shown to perform quite well on those simulated data, and this fact strongly motivated the present work where real NMR data are instead going to be used. Moreover, since different types of distances can be identified in DDGP instances related to NMR experiments, we will present an experimental study where "genuine" NMR data will coexist with "simulated" distances. When we talk about genuine data, we mean the distances described in Section III, based on NMR data (STAR files), van der Waals radii and force field information. When we say simulated data, all distance types are generated by looking at the actual distances available from the atomic coordinates defined in the (first) model in the PDB files. We conducted these experiments with the aim of studying the impact of every distance type on the quality of the solutions that MDJEEP is able to find. All generated DDGP instances include both *backbone* atoms, as well as the *side chains* of the selected molecules. When force fields are involved in the computation of the distance types, we will make reference to the AMBER force field [5]. When we simulate the distance types, each type is simulated in a different way:

Type1 we take the actual distance values to play the role of lower bound in the van der Waals interval distance, while the upper bound is set to a symbolically high value;

Type2 we take the actual distances from the PDB model: this distance type is exact, but, to reflect the precision of force field parameters, only its first 3 decimal digits are taken into consideration;

Type3 and **Type4** we take the actual distances and we create an interval distance with a range of 1Å, where the true distance is randomly placed within this interval

Type5 we take the true torsion angle value and we compute the distance. Then, we create an interval with a range of 0.1Å, and the true distance is randomly placed within the interval.

Notice that a similar setup was considered in previous publications, as for example in [23].

Some of the DDGP instances that we will use in the experiments below only contain simulated distances; others are generated instead with the idea to mix those simulated data with real distances from NMR. In the latter situation, only one distance type per time is defined with real distances, while all the others are simulated. We repeat our experiments for 7 small proteins; one of the criteria for the selection is their small size. The considered PDB models for these proteins are shown in Figure 4, and some of the properties of the proteins are shown in Table I.

Our construction method for DDGP instances (see Section III) was implemented in Java programming language. It is able to read STAR-NMR files in input, and to give in output the DDGP instance text file formatted so that MDJEEP can directly read it. We remark that, after the graph G has been constructed, a vertex order, which allows the assumptions in Def. 1 to be satisfied, is associated to the graph by simply running the greedy algorithm proposed in [9].

The experiments were ran on a computer with a 64-bit Linux Mint operating system using a 8-core Intel(R) Core i7-7700HQ CPU at 2800 Mhz and 16 GB of main memory. The results of the experiments, where MDJEEP `0.3.2` is invoked to solve the constructed DDGP instances, are shown in Table II. The first row in the table indicates the experiments where all distance types were simulated; the last row is instead related to the experiments where only genuine non-simulated distances were included in the instance. Between the two extreme cases, the inner rows of the table show the results obtained with instances where only one distance type carries real distances, while all other distances are actually simulated.

After the solver MDJEEP has found one solution to the DDGP instances, in order to evaluate the quality of such solutions, we computed the lowest Root Mean Square Deviation (RMSD) between each solution and all available PDB models. The RMSD was computed after running our own implementation of the Kabsch alignment algorithm [8]. Our implementation also attempts, together with translations and rotations, to perform a total reflection of the protein models to improve the alignments.

As expected, very satisfactory results can be obtained in general when all distances are simulated. And as expected, when at least one of the distance types is not simulated, but rather the real data are taken into account, then the quality of the results lowers. However, we can notice a different impact on the quality for each distance type. The distance type that seems to give the largest impact is, unexpectedly, the one related to van der Waals distances (**type1**). Our instances are very rich in this distance type, because we can define a distance for every pair of atoms which are not bonded. This is the only type where the distances only indicate lower bounds and, when simulated, the actual distance value is taken for the lower bound. In genuine instances, instead, it is rather set to

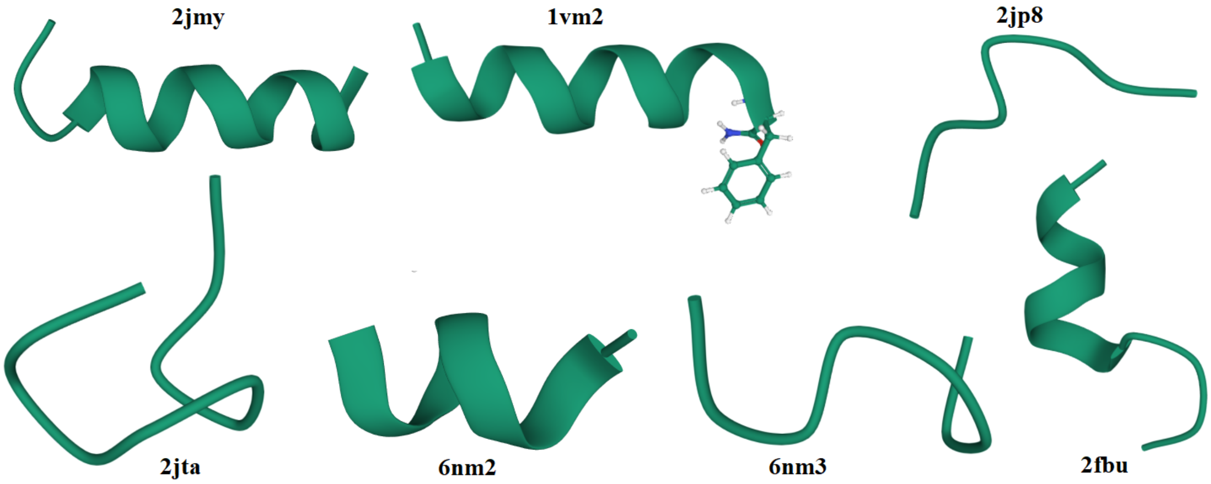


Fig. 4: The considered PDB model of the 7 considered proteins: `2jmy`, `1vm2`, `2jp8`, `2jta`, `6nm2`, `6nm3` and `2fbu` . The images were generated using Mol* [26], the PDB model viewer associated with the RCSB database.

Properties of protein	**2jmy**	**1vm2**	**2jp8**	**2jta**	**6nm2**	**6nm3**	**2fbu**
Number of atoms (including hydrogens)	282	212	120	157	182	182	216
Type1 distances (van der Waals lower bound)	37918	21120	6498	11386	15288	15381	21995
Type2 distances (Force field)	812	600	340	436	526	526	611
Type3 distances (NMR restraints)	171	91	25	35	185	90	48
Type4 distances (NMR torsion)	13	4	5	7	2	6	6
Type5 distances (Torsion min-max)	707	551	277	389	470	468	560
Classification	Antimicrobial	Antibiotic	Signaling	Signaling	Antimicrobial	Antimicrobial	Antimicrobial

TABLE I: Different properties of the proteins considered in this work. Includes the number of distances, per type, that can form our DDGP instances.

Genuine distance types	**2jmy**	**1vm2**	**2jp8**	**2jta**	**6nm2**	**6nm3**	**2fbu**
none (simulated instance)	0.050	0.011	0.004	0.063	0.289	0.196	1.595
only **type1** (van der Waals)	5.076	0.289	1.442	0.535	0.730	3.036	3.339
only **type2** (force field bonds and angles)	0.012	0.343	0.021	0.291	0.319	0.210	0.839
only **type3** (NMR restraints)	0.005	0.568	0.009	0.771	0.462	0.355	0.612
only **type4** (NMR torsion angles)	0.007	0.660	0.010	0.554	0.225	0.244	1.596
only **type5** (min-max torsion angles)	1.587	1.485	3.332	–	0.504	0.473	–
all types (genuine NMR instance)	4.181	3.850	3.529	4.522	3.419	4.326	4.94

TABLE II: The effect of each of the different distance types on the accuracy of the solutions found by MDJEEP `0.3.2`. The DDGP instances partially contain genuine NMR-derived distance information, as well as simulated data. For the five experiments concerning the five different distance types, all distances except for the stated type were simulated. For every experiment, the RMSD value (in Å) obtained when comparing the found solution and all original models in the PDB entry are reported. The symbol "–" indicates that MDJEEP was able to find no solutions within 1-hour CPU time.

the 80% of the sum of the two van der Waals radii. Even if these two possible lower bounds (the real and the simulated one) do not differ much, the effect on the experiments is quite consistent. The main reasons for this result is probably due to the very generous presence of this distance type in our instances.

Table II shows that distances based on force fields have a rather light effect on the quality of the found solutions. In the case of the `2fbu` protein, the table even shows a reduction of about 50% on the RMSD value when genuine force field parameters are used at the place of simulated data (which correspond to the set of distances extracted from a known PDB model in this case), instead of reporting a decrease on the solution quality.

Next, distances and torsion angles derived from NMR (**type3** and **type4**) belong to the distance types that do not make the quality of the solution drastically change when simulated data are replaced by the genuine distances. For the `2jmy` protein, MDJEEP is actually able to obtain better results when using the real NMR data, instead of the simulated distances. One reason for this result is likely to be related to the quite scarce presence of NMR information. As shown in Table I, NMR-derived distances are generally much less abundant than the other distance types. It is important to point out, however, that the number of NMR-derived distances can actually change per each specific NMR experiment, and therefore their impact can be more or less pronounced depending on the quantity of distances that are actually available through NMR.

Finally, a distance type which shows a larger impact on the experiments is the **type5**. The reason for this is likely to be related to their total artificial nature. Introduced in previous publications on the DDGP for ensuring discretizability, they simply translate in distances the minimal and maximal extensions of the corresponding torsion angles. The artificial nature of this distance type is reflected in our Table II, which shows that the set of simulated data deprived of this distance type give rise to DDGP instances that MDJEEP cannot solve in a computational time comparable to the other presented experiments (which is, in less than 1 hour on our computational setup).

V. Conclusions and future works

We studied the impact of different distance types on experiments of protein structure determination where the involved distances are either simulated or real. Our approach is based on the discretization of the search space of the corresponding distance geometry problem, which defines a special class of instances we have referred throughout our paper to as the DDGP. To perform this study, we have introduced a new procedure for the generation of DDGP instances that can take into consideration genuine data and, at the same time, simulated data obtained through known models of the molecules at hand. Unexpectedly, our experiments indicate that the distance types that can have a larger impact on the quality of the obtained solutions are not the ones related to NMR or force fields, but mostly the van de Waals distances. We conclude that this result is the direct consequence of the high abundance in our generated instances of this type of distances, which goes in contrast with the quite scarce presence of NMR-derived distances.

Future works will need to confirm these new empirical results with further experiments on larger protein molecules, associated with a more accurate analysis. A side work to investigate in parallel consists in improving the performances of the solver that we used in our computational experiments, MDJEEP. In fact, the results of our present study can potentially help us in determining new ways to make the solver work more efficiently, as for example by making it become distance type aware. On the other side, a more efficient solver can potentially allow us to perform the presented study on larger molecules, which might confirm, or rather complete, our conclusions.

Finally, among the other directions for improving MDJEEP, in the same spirit of the present paper, we can mention to the idea of extend the branching mechanism to NMR distances with multiple (and therefore uncertain) assignments to some atom pairs, and to exploit force field information also for pruning purposes (see for example the preliminary studies in [6]).

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References

[1] F.C.L. Almeida, A.H. Moraes, F. Gomes-Neto, *An Overview on Protein Structure Determination by NMR, Historical and Future Perspectives of the Use of Distance Geometry Methods*. In: [21], 377–412, 2013.

[2] H. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, H. Weissig, I. Shindyalov, P. Bourne, *The Protein Data Bank*, Nucleic Acids Research **28**, 235–242, 2000.

[3] A. Bondi, *van der Waals Volumes and Radii*, Journal of Physical Chemistry **68**(3), 441–451, 1964.

[4] A. Cassioli, B. Bardiaux, G. Bouvier, A. Mucherino, R. Alves, L. Liberti, M. Nilges, C. Lavor, T.E. Malliavin, *An Algorithm to Enumerate all Possible Protein Conformations verifying a Set of Distance Restraints*, BMC Bioinformatics **16**:23, 15 pages, 2015.

[5] R. Engh, R. Huber, *Accurate Bond and Angle Parameters for X-ray Protein Structure Refinement*, Acta Crystallographica A **47**, 392–400, 1991.

[6] D.S. Gonçalves, A. Mucherino, C. Lavor, *Energy-based Pruning Devices for the BP Algorithm for Distance Geometry*, IEEE Conference Proceedings, Federated Conference on Computer Science and Information Systems (FedCSIS13), Workshop on Computational Optimization (WCO13), Krakov, Poland, 335–340, 2013.

[7] R.K. Harris, *Nuclear Magnetic Resonance Spectroscopy*, 1986.

[8] W. Kabsch, *A Solution for the Best Rotation to Relate Two Sets of Vectors*, Acta Crystallographica, 1976.

[9] C. Lavor, J. Lee, A. Lee-St.John, L. Liberti, A. Mucherino, M. Sviridenko, *Discretization Orders for Distance Geometry Problems*, Optimization Letters **6**(4), 783–796, 2012.

[10] C. Lavor, L. Liberti, N. Maculan, A. Mucherino, *The Discretizable Molecular Distance Geometry Problem*, Computational Optimization and Applications **52**, 115–146, 2012.

[11] C. Lavor, L. Liberti, A. Mucherino, *The interval Branch-and-Prune Algorithm for the Discretizable Molecular Distance Geometry Problem with Inexact Distances*, Journal of Global Optimization **56**(3), 855–871, 2013.

[12] L. Liberti, C. Lavor, N. Maculan, *A Branch-and-Prune Algorithm for the Molecular Distance Geometry Problem*, International Transactions in Operational Research **15**, 1–17, 2008.

[13] L. Liberti, C. Lavor, A. Mucherino, N. Maculan, *Molecular Distance Geometry Methods: from Continuous to Discrete*, International Transactions in Operational Research **18**(1), 33–51, 2011.

[14] L. Liberti, C. Lavor, N. Maculan, A. Mucherino, *Euclidean Distance Geometry and Applications*, SIAM Review **56**(1), 3–69, 2014.

[15] J.P. Linge, M. Habeck, W. Rieping, M. Nilges, *ARIA: Automated NOE Assignment and NMR Structure Calculation*, Bioinformatics **19**, 315–316, 2003.

[16] M. Nilges, *Ambiguous Distance Data in the Calculation of NMR Structures*, Folding and Design **2**(1), S53–S57, 1997.

[17] T.E. Malliavin, A. Mucherino, C. Lavor, L. Liberti, *Systematic Exploration of Protein Conformational Space using a Distance Geometry Approach*, Journal of Chemical Information and Modeling **59**(10), 4486–4503, 2019.

[4] `https://math.stackexchange.com/users/305862/jean-marie`

[18] A. Mucherino, *A Pseudo de Bruijn Graph Representation for Discretization Orders for Distance Geometry*, Lecture Notes in Computer Science **9043**, Lecture Notes in Bioinformatics series, F. Ortuño and I. Rojas (Eds.), Proceedings of the 3^{rd} International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO15), Granada, Spain, 514–523, 2015.

[19] A. Mucherino, D.S. Gonçalves, L. Liberti, J-H. Lin, C. Lavor, N. Maculan, *MD-jeep: a New Release for Discretizable Distance Geometry Problems with Interval Data*, IEEE Conference Proceedings, Federated Conference on Computer Science and Information Systems (FedCSIS20), Workshop on Computational Optimization (WCO20), online event, 289–294, 2020.

[20] A. Mucherino, C. Lavor, L. Liberti, *The Discretizable Distance Geometry Problem*, Optimization Letters **6**(8), 1671–1686, 2012.

[21] A. Mucherino, C. Lavor, L. Liberti, N. Maculan (Eds.), *Distance Geometry: Theory, Methods and Applications*, 410 pages, Springer, 2013.

[22] A. Mucherino, L. Liberti, C. Lavor, *MD-jeep: an Implementation of a Branch & Prune Algorithm for Distance Geometry Problems*, Lectures Notes in Computer Science **6327**, K. Fukuda et al. (Eds.), Proceedings of the 3^{rd} International Congress on Mathematical Software (ICMS10), Kobe, Japan, 186–197, 2010.

[23] A. Mucherino, J-H. Lin, *An Efficient Exhaustive Search for the Discretizable Distance Geometry Problem with Interval Data*, IEEE Conference Proceedings, Federated Conference on Computer Science and Information Systems (FedCSIS19), Workshop on Computational Optimization (WCO19), Leipzig, Germany, 135–141, 2019.

[24] A. Mucherino, J-H. Lin, D.S. Gonçalves, *A Coarse-Grained Representation for Discretizable Distance Geometry with Interval Data*, Lecture Notes in Computer Science **11465**, Lecture Notes in Bioinformatics series, I. Rojas et al (Eds.), Proceedings of the 7^{th} International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO19), Part I, Granada, Spain, 3–13, 2019.

[25] J. Saxe, *Embeddability of Weighted Graphs in k-Space is Strongly NP-hard*, Proceedings of 17^{th} Allerton Conference in Communications, Control and Computing, 480–489, 1979.

[26] D. Sehnal, S. Bittrich, M. Deshpande, R. Svobodová, K. Berka, V. Bazgier, S. Velankar, S.K. Burley, J. Koca, A.S. Rose, *Mol* Viewer: Modern Web App for 3D Visualization and Analysis of Large Biomolecular Structures*, Nucleic Acids Research, 2021.

[27] E.L. Ulrich, K. Baskaran, H. Dashti, et al. *NMR-STAR: Comprehensive Ontology for Representing, Archiving and Exchanging Data from Nuclear Magnetic Resonance Spectroscopic Experiments*, J Biomol NMR 73, 5–9, 2019.

[28] K. Wuthrich, *NMR in Structural Biology: A Collection of Papers by Kurt Wuthrich*, Vol. 5. World Scientific, 1995.