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RESEARCH

An algorithm to enumerate all possible protein conformations verifying a set of distance constraints

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Abstract

Background: The determination of protein structures satisfying distance constraints is an important problem in structural biology. Whereas the most common method currently employed is simulated annealing, there have been other methods previously proposed in the literature. Most of them, however, are designed to find one solution only.

Results: In order to explore exhaustively the feasible conformational space, we propose here an interval Branch-and-Prune algorithm (*iBP*) to solve the Distance Geometry Problem (DGP) associated to protein structure determination. This algorithm is based on a discretization of the problem obtained by recursively constructing a search space having the structure of a tree, and by verifying whether the generated atomic positions are feasible or not by making use of pruning devices. The pruning devices used here are directly related to features of protein conformations.

Conclusions: We described the new algorithm *iBP* to generate protein conformations satisfying distance constraints, that would potentially allow a systematic exploration of the conformational space. The algorithm *iBP* has been applied on three α -helical peptides.

Keywords: Distance geometry, branch-and-prune algorithm, Molecular conformation, Protein structure, Nuclear Magnetic Resonance

1
2

3 Background

4 Protein structure determination is crucial for understanding protein function, as it
5 paves the way to the discovery of new chemical compounds and of new approaches
6 to control the biological processes.

7 The problem of protein structure determination is certainly a problem with mul-
8 tiple solutions, as proteins are flexible polymers. As most of the experimental tech-
9 niques of the structural biology obtain measurements averaged on an ensemble of
10 protein conformations, the usual approaches for structure determination intend to
11 find an average structure or a set of conformations describing fluctuations around
12 an average structure. A path intending to get a complete coverage of the confor-
13 mational space, given a series of constraints, is usually not taken, although such an
14 approach could provide precious information about the conformational equilibrium,
15 which is essential in the function of many proteins, as the HIV protease [1].

16 An important class of experimental methods for protein structure determination
17 is based on the measurement of inter-atomic distances and angles, such as Nu-
18 clear Magnetic Resonance (NMR) [2] and cross-linking coupled to mass spectrom-
19 etry [3]. In NMR, distance intervals between hydrogens are determined from the
20 measurement of nuclear Overhauser effects (NOE). The experimentally measured
21 distances are then used as constraints for protein structure calculations. Pure *in*
22 *silico* approaches have been also developed based on the use of inter-atomic dis-
23 tance constraints, such as homology modeling [4] or prediction of protein-protein
24 complexes [5] and ligand poses [6].

25 The Distance Geometry Problem (DGP) [7, 8] consists in identifying the sets of
26 points which satisfy a set of constraints based on relative distances between some
27 pairs of such points. The present work describes an algorithm developed to solve
28 DGP in the context of protein structure determination: the points represent the
29 protein atoms.

30 The DGP is a constraint satisfaction problem. Several approaches solve this prob-
31 lem by reformulating it [8] as a global optimization problem having a continuous
32 search domain, and whose objective function is generally a penalty function de-
33 signed to measure the violation of the distance constraints. Over the years, the
34 solution of DGPs arising in structural biology have been typically attempted by
35 Simulated Annealing (SA) approaches based on molecular dynamics [9]. Other pro-
36 posed approaches are based on various optimization methods as in [10]. As all
37 meta-heuristic approaches, SA may provide approximate solutions but does not de-
38 liver optimality certificates. In the case of protein structure determination, since
39 the optimization problem is a reformulation of a constraint satisfaction problem,
40 solutions given by SA can be successively verified by checking the violations of the
41 distance constraints. However, additional solutions may exist but go undetected
42 by SA. Thus, an algorithm for the systematic enumeration of the possible confor-
43 mations of a given protein could find a widespread field of application. Branch-
44 and-prune algorithms and similar were proposed in the general context of protein
45 structure determination [11–16], (see also [8] and references therein). However, these
46 studies primarily addressed the question of defining relative orientations of protein
47 monomers in symmetric oligomers, not the determination of all possible conforma-
48 tion of a polypeptide chain with a very large number of degrees of freedom from
49 distance constraints. Systematic exploration was proved to be useful in the case
50 of residual dipolar couplings (RDC) constraints [17], for exploring the sidechains
51 conformations [18, 19] and for assignment of NOEs, provided that the structure is
52 known [20]. For the structure determination from RDCs, it has been shown [21]
53 that when using RDCs but only sparse NOEs the problem can be solved in poly-
54 nomial time. Such approaches have also been used for structure determination in
55 X-ray crystallography for non-crystallographic symmetry by orienting and translat-
56 ing symmetric protein subunits [22]. To the best of our knowledge, in this paper
57 we present the first application of a Branch-and-Prune algorithm to the problem of
58 full protein structure determination based on unambiguous distance information.

59 Under certain conditions, DGPs can be discretized [23] (see below), which means
60 that the search domain for the corresponding optimization problem can be reduced
61 to a discrete set, which has the structure of a tree. The discretization makes the

62 enumeration of the entire solution set of DGP instances possible. This is important
 63 when the experimental constraints do not specify the protein conformation uniquely,
 64 i.e., more than one conformation satisfies all constraints. For solving discretized
 65 DGP, we employ an *interval* branch-and-prune (*iBP*) algorithm [24], which is based
 66 on the idea of recursively exploring the tree while generating new candidate atomic
 67 positions (branching phase) and to verify the feasibility of such positions (pruning
 68 phase) (Figure 1). By making use of pruning devices, branches rooted at infeasible
 69 positions can be discarded from the tree, so that the search can be reduced to the
 70 feasible parts of the tree (Figure 2). Pruning devices can be conceived and integrated
 71 in *iBP* to improve the performances of the pruning phase and thus of the algorithm.

72 In the present work, we first describe the branching phase and the pruning de-
 73 vices used to determine the solutions to the Distance Geometry problem. Then, an
 74 overall view of the method is given along with the use of the branching and pruning
 75 devices at different steps and the complexity of the algorithm is analyzed. We finally
 76 illustrate the algorithm application with three proteins for which α -helical regions
 77 are known along with few long-range NMR constraints (ie. constraints measured be-
 78 tween residues i and j such that $|i - j| > 3$ in the protein sequence). The obtained
 79 conformations display good stereochemical quality parameters, and the conforma-
 80 tional space explored is larger than the one sampled with traditional optimization
 81 methods such as simulated annealing.

82 Methods

83 In order to sample the conformational space of a protein, we use a Branch-and-
 84 Prune algorithm to build a tree in which each node represents a solution for one
 85 atomic position. We limit ourselves in the present work to the calculation of the
 86 backbone and $C\beta$ atomic coordinates.

87 The constraints used to generate atomic coordinates along the Branch-and-Prune
 88 algorithm are the following:

- 89 1 covalent distance constraints corresponding to bond lengths and bond angles,
 90 whose values are derived from high-resolution small molecule X-ray crystal
 91 structures [25];
- 92 2 NMR distance constraints;
- 93 3 van der Waals radii of atoms between non-bonded atom pairs (i, j) : a fraction
 94 of the sum of the van der Waals radii of each atom provides a lower bound to
 95 the corresponding inter-atomic distances:

$$96 \quad d_{ij} \geq \sigma(r_i^{vdw} + r_j^{vdw}), \quad (1)$$

97 where $\sigma \in [0, 1]$, and is typically around 0.85. The values for the radii are
 98 given in Table 1 [26, 27]. These lower bounds apply only in the cases where
 99 no larger lower bound has been determined from NMR distance constraints;

- 100 4 distances derived from the backbone torsion angles ϕ and ψ ;
- 101 5 hydrogen bonds in α -helix;
- 102 6 amino-acid chirality;
- 103 7 α -helix geometry.

104 The atom coordinates are calculated, one by one, following the atom order P_{ato}
 105 described in Figure 3 and previously proposed in [24]. In this order, some atoms are
 106 repeated to insure that any entered atom is defined by distance constraints with
 107 respect to three preceding atoms in P_{ato} [24]. The carbonyl oxygens and the atoms
 108 $C\beta$, which were not present in the order P_{ato} , are calculated separately.

109 Then, the tree is built using a recursive procedure to create each node of the tree.
 110 This procedure is called branching phase. The created nodes are then submitted to
 111 the pruning devices in order to decide whether the node should be kept or removed.
 112 If the node is removed, the possible branches starting from this node are also pruned.
 113 A pruning device is responsible for checking whether a partial solution is feasible,
 114 i.e. to check whether a set of embedded atoms fulfill the constraints (1)-(7) described
 115 above.

116 In the following, we describe the branching phase and the pruning devices. Then,
 117 the complexity of the algorithm is described from a theoretical point of view, before
 118 presenting some application cases.

119 Branching Devices

120 The tree parsed during *iBP* is formed by nodes, each corresponding to one set of
 121 atomic coordinates from the order P_{ato} (Figure 3) [24]. At each level of the tree,
 122 the atomic coordinates of the corresponding atom are calculated by making use of
 123 a recursive procedure, called branching phase. The current atom position is defined
 124 by distance constraints to three other atoms. These distances are obtained from
 125 the constraints (1-3) described above: (1) the covalent constraints, (2) the NMR
 126 distance constraints, (3) the van der Waals radii.

127 If the distance constraints specify a unique value rather than an interval, this
 128 signifies that the distances to three immediate predecessors from the current vertex
 129 are known: these are the centers of the three spheres, and the distances are the
 130 radii of these spheres. The position of the current vertex/atom is thus defined by
 131 the intersection of three spheres, so there are at most two solutions for the current
 132 atom position: this is called a 2-branching situation (Figure 4).

133 When a distance is not uniquely defined, but rather defined by lower and upper
 134 bounds, i.e. $d_{i,j} \in [l_{i,j}, u_{i,j}]$, this distance is uniformly discretized by sampling $b \geq 1$
 135 values in $[l_{i,j}, u_{i,j}]$, as depicted in Figure 5.

$$136 \quad \tilde{d}_i = \left\{ l_{i,i-3} + (t-1) \frac{(u_{i,i-3} - l_{i,i-3})}{b} : t = 1, \dots, b \right\}. \quad (2)$$

137 In this case, we have a b -branching situation.

138 The algorithm used for calculating the atom coordinates is then applied to each
 139 set of \tilde{d}_i values sampled for the distance constraints. The choice of the *discretization*
 140 *factor* b is a crucial point: a small value might lead to an infeasible problem because
 141 we may not select any feasible distance; a larger value increases the computational
 142 burden. In general, the finer the discretization, the more accurate the computation
 143 is, but it is not trivial to figure out the optimal value for b . One way to choose b
 144 is to consider that the number of nodes in the search tree is bounded by $3 + (2^l b^k)$,
 145 where l is the number of tree levels where we have a 2-branching situation, and k is

146 the number of tree levels where we have a b-branching situation [28]. Appropriate
147 values of b should result in a manageable number of nodes.

148 Given the position of the three previous atoms $k-3$, $k-2$, $k-1$ in the order
149 P_{ato} and given the constraints to these atoms of the atom k to be embedded, the
150 position of k is calculated by a recursive matrix multiplication by making use of
151 the set of distances $d = \{d_{k,k-1}, d_{k,k-2}, d_{k,k-3}\}$ between the previous atoms and
152 k . Although there are several methods to compute sphere intersections [29], in our
153 experience, the best trade-off between efficiency and numerical stability is given by
154 the use of recursion matrices [23], and of the two following angles: (i) the torsion
155 angle ω_3 formed by atoms $\{k, k-1, k-2, k-3\}$ which depends on the distance
156 between k and $k-3$, (ii) the angle θ_2 formed by atoms $\{k, k-1, k-2\}$.

157 The recursion is applied through the equation:

$$158 \begin{bmatrix} x_k \\ y_k \\ z_k \\ 1 \end{bmatrix} = B_1 B_2 B_3 \dots B_k(d, \sigma) \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} = Q_{k-1} B_k(d, \sigma) \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} = Q_k \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \end{bmatrix}, \quad (3)$$

159 where:

$$160 B_k(d, \sigma) = \begin{bmatrix} -\cos \theta_2 & -\sigma \sin \theta_2 & 0 & -d_{k,k-1} \cos \theta_2 \\ \sigma \sin \theta_2 \cos \omega_3 & -\cos \theta_2 \cos \omega_3 & -\sin \omega_3 & \sigma d_{k,k-1} \sin \theta_2 \cos \omega_3 \\ \sigma \sin \theta_2 \sin \omega_3 & -\cos \theta_2 \sin \omega_3 & \cos \omega_3 & \sigma d_{k,k-1} \sin \theta_2 \sin \omega_3 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad (4)$$

161 and $\sigma \in \{+1, -1\}$. The series of recursion matrices is initialized as:

$$162 B_1 = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, B_2 = \begin{bmatrix} -1 & 0 & 0 & -d_{2,1} \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad (5)$$

$$B_3 = \begin{bmatrix} -\cos \theta_3 & -\sin \theta_3 & 0 & -d_{3,2} \cos \theta_3 \\ \sin \theta_3 & -\cos \theta_3 & 0 & d_{3,2} \cos \theta_3 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}.$$

163 $d_{2,1}$ being the distance between the first and the second atom, and $d_{3,2}$ the distance
164 between the third and the second atom in the order P_{ato} .

165 The total number of B_k matrices to be calculated along the parsing of the tree
166 is bounded by $2 | P_{\text{ato}} | b$, where $| P_{\text{ato}} |$ is the size of the ordered atom list P_{ato} .
167 The product $Q_{k-1} B_k$ is calculated in two steps: (1) the fourth column of Q_k , which
168 gives us the coordinates of k , is computed; (2) only if k is not pruned, the three
169 remaining columns are computed.

170 We must distinguish two cases when embedding an atom k . If it is the first appear-
171 ance of k in P_{ato} , we use equation (3) to compute all possible embeddings of k for

172 $\sigma \in \{+1, -1\}$ and the set of distances d . If it is not the first appearance of k in P_{ato} ,
 173 we need to take into account the fact that numerical instabilities generate matrices
 174 which will lead to slightly different coordinates for k than those computed the first
 175 time. In order to decrease the impact of these numerical errors, we compute the set
 176 of distances d , the angles θ_2, ω_3 and for $\sigma \in \{+1, -1\}$ the corresponding matrices
 177 $B_k(d, +1), B_k(d, -1)$, which lead to two possible embeddings of k (Equation 3), as
 178 $k^+ = Q_{k-1}B_k(d, +1)$ and $k^- = Q_{k-1}B_k(d, -1)$. We choose the value of k that
 179 yields the updated coordinates of k being the closest to the previous coordinates of
 180 this atom.

181 Each carbonyl oxygen O^{i-1} is uniquely determined for residue i , once C^{i-1}, N^i
 182 and H^i have been embedded, since these atoms are all part of the peptide plane [30].
 183 As is common practice (see, e.g., [31–33]), we fix here the torsion angle ω of the
 184 peptide plane to -180° or 0° . In a previous implementation [34], the positions of
 185 the carboxylic oxygens were not stored. Although this approach leads to memory
 186 savings, the availability of carboxylic oxygen positions can improve the definition
 187 of the α -helix secondary structure.

188 The positions of the carbonyl oxygens are thus now calculated in the following way.
 189 If $k = O^{i-1}$ is the carboxylic oxygen atom located at the vertex k , and $\{v_1, v_2, v_3\}$
 190 are the vertices corresponding to atoms $\{C^{i-1}, N^i, H^i\}$, belonging on the same
 191 peptide plane π , we denote n_π the normal vector to π . The coordinates of k can
 192 then be computed by solving the following non-linear system:

$$193 \quad \begin{cases} \|k - v_i\|^2 = d_{ki}^2, & i = 1, 2, 3 \\ n_\pi^T(v_1 - k) = 0 \end{cases} \quad (6)$$

194 where d_{ki} are the distances between atoms k and i . Using an approach similar to
 195 those employed in [35], we obtain the equivalent linear system:

$$196 \quad \begin{cases} 2(v_2 - v_1)^T k = d_{k1}^2 - d_{k2}^2 - \|v_1\|^2 + \|v_2\|^2 \\ 2(v_3 - v_1)^T k = d_{k1}^2 - d_{k3}^2 - \|v_1\|^2 + \|v_3\|^2 \\ n_\pi^T(v_1 - k) = 0 \end{cases} \quad (7)$$

197 The parameter d_{k1} is the length of the bond connecting O^{i-1} and C^{i-1} , the param-
 198 eters d_{k2} and d_{k3} are the distances between $k = O^{i-1}$ and N^i, H^i , calculated from
 199 bond angles and bond lengths between atoms of the peptide plane, and the angle ω
 200 of 180° in a *trans* peptide plane. The case of the *cis* peptide plane can be treated
 201 in the same way, modifying the value of ω to be 0° .

202 Following the idea proposed for carbonyl oxygens, the coordinates k of a C_β atom
 203 can be computed from previously calculated atoms, because the four distances of
 204 k to atoms $\{v_1 = C\alpha, v_2 = H\alpha, v_3 = N, v_4 = C\}$ are exactly known, and because
 205 these five atoms are not coplanar. The coordinates k are calculated by solving the
 206 linear system:

$$207 \quad \begin{cases} 2(v_2 - v_1)^T k = d_{k1}^2 - d_{k2}^2 - \|v_1\|^2 + \|v_2\|^2 \\ 2(v_3 - v_1)^T k = d_{k1}^2 - d_{k3}^2 - \|v_1\|^2 + \|v_3\|^2 \\ 2(v_4 - v_1)^T k = d_{k1}^2 - d_{k4}^2 - \|v_1\|^2 + \|v_4\|^2 \end{cases} \quad (8)$$

208 The parameter d_{k1} is the length of the bond connecting $k = C\beta$ and $C\alpha$, the
 209 parameters d_{k2} , d_{k3} and d_{k4} are the distances between $k = C\beta$ and $H\alpha$, N , C ,
 210 calculated from bond angles and bond lengths between these atoms.

211 Pruning Devices

212 Once the set of possible coordinates of the atom k has been determined in the
 213 branching phase described above, pruning devices are used to check whether the
 214 coordinates of k are feasible. In some cases described below, the coordinates of k
 215 along with the coordinates of previously embedded atoms are checked together. If
 216 the check is negative, the solution obtained for k is discarded, which prunes all
 217 tree branches originating from the node k . In this section, we present the pruning
 218 devices used to accept or discard the coordinates of the atom k generated by the
 219 branching devices. The pruning device applies all these tests as soon as the involved
 220 atoms have been embedded.

221 Direct Distance Feasibility (DDF)

222 As the coordinates for an atom k are determined, we first check that all distances
 223 between k and the other embedded atoms respect the given lower and upper bounds
 224 arising from the constraints (1-3) listed in section ‘‘Solving the DGP with iBP’’.

225 Torsion Angle Feasibility (TAF)

226 The values of the backbone torsion angles ϕ , ψ , are used as a pruning device, check-
 227 ing whether they are located in the permitted regions of the Ramachandran plot.
 228 The pruning device, first introduced in [34], is implemented in the following way.
 229 The torsion angle ξ_{ijkl} defined by a quadruple of atoms $\{i, j, k, l\}$ falls into a domain
 230 Ξ_{ijkl} , up to a certain tolerance $\epsilon_t > 0$. In general, Ξ_{ijkl} is the union of κ dis-joined
 231 intervals, i.e.

$$232 \quad \Xi_{ijkl} = \bigcup_{c=1}^{\kappa} \Xi_{ijkl}^c \quad (9)$$

233 From the bounds on a torsion angle ξ_{ijkl} it is possible to derive bounds on the
 234 distance d_{il} , noticing that

$$235 \quad d_{il}(\xi_{ijkl}) = \sqrt{d_{ij}^2 + d_{lj}^2 - 2(\cos(\xi_{ijkl})\sqrt{ef + bc})d_{ij}d_{lj}}, \quad (10)$$

236 where:

$$237 \quad b = \frac{1}{2} \frac{d_{lj}^2 + d_{jk}^2 - d_{lk}^2}{d_{lj}d_{kj}}$$

$$238 \quad c = \frac{1}{2} \frac{d_{ij}^2 + d_{jk}^2 - d_{ik}^2}{d_{ij}d_{jk}}$$

$$239 \quad e = 1 - b^2, f = 1 - c^2.$$

242 Taking the maximum and minimum values of $d(\xi_{ijkl})$ for $\xi_{ijkl} \in \Xi_{ijkl}$, we obtain
 243 an interval $[l_{il}, u_{il}]$ for the distance d_{il} . The sign of the angle ξ_{ijkl} is used as an
 244 additional pruning criterion along with the d_{il} interval.

245 *Dijkstra Shortest-Path (DSP)*

246 As introduced in [23], we can exploit the fact that the distances are Euclidean
 247 to improve the *iBP* pruning capabilities. We extend and generalize the procedure
 248 presented in [36] in the following way. We introduce an auxiliary graph G^+ with the
 249 same topology as the graph connecting the atoms in the protein, but such that the
 250 weight of each edge (i, j) is the upper bound of the distance d_{ij} . For every pair of
 251 atoms i, j , the shortest-path between i, j in G^+ is a valid over-estimate of d_{ij} . Thus
 252 we used an all-to-all shortest-path algorithm, the Floyd-Warshall algorithm [37], to
 253 refine the upper bound for each pair of atoms.

254 The Dijkstra Shortest-Path pruning device uses the refined upper bounds of inter-
 255 atomic distances in the following way. According to Lemma 4 in [23], for an atom
 256 k and for each atom pair i, j such that $i < j < k$ in the order P_{ato} and for which
 257 d_{ik} is known, the embedding of k can be pruned if:

$$258 \quad \|i - j\| - d_{ik} > u_{jk} \quad (11)$$

259 where u_{jk} is the upper bound of the atom pair (j, k) obtained using the Floyd-
 260 Warshall algorithm [37].

261 *Chirality (CHI)*

262 The pruning of atom coordinates through the amino-acid chirality is implemented
 263 through the so-called CORN rule of thumb: in amino acids, the groups COOH, R
 264 (sidechain), NH₂ and H are bonded to the chiral center C α carbon. Starting with
 265 the hydrogen atom away from the viewer, if these groups are arranged clockwise
 266 around the C α carbon, then the amino-acid is in the D-form. If these groups are
 267 arranged counter-clockwise, the amino-acid is in the L-form. The CORN rule was
 268 restated by imposing that the torsion angle defined by the atoms $C, C\beta, N, H\alpha$ of
 269 residue i for the D-form or $C, N, C\beta, H\alpha$ of residue i for the L-form, is positive.

270 *α -helix secondary structure*

271 We proposed the use of α helix information as a pruning device in the context
 272 of the *iBP* algorithm first in [34]. The α helix location can be determined from
 273 an analysis of the NMR chemical shifts by TALOS [38]. Four criteria are used to
 274 enforce the formation of an α helix: (i) the formation of backbone hydrogen bonds
 275 between amide hydrogens and carbonyl oxygens, (ii) the alignment of the amide and
 276 carbonyl functions checked by a qualitative condition on the energy of the hydrogen
 277 bond, (iii) the definition of backbone ϕ and ψ torsion angles already described in
 278 the Torsion Angle Feasibility, (iv) the definition of three additional angles θ , θ' and
 279 θ'' similar to the ones introduced by Grishaev et al [39].

280 On a sequence of $m + 1$ contiguous residues $I_\alpha = \{i, i + 1, \dots, i + m\}$ forming an α
 281 helix, for any pair of residues $(i - 4, i)$ belonging to I_α , the lower and upper bounds
 282 on the distance between the carboxylic oxygen O^{i-4} and the amide hydrogen H^i
 283 should be compatible with the formation of an hydrogen bond. The upper and lower
 284 bounds are defined in an input parameter file of *iBP*, and were set to 1.9 and 3.0 Å
 285 in the present work.

286 The condition checking the alignment of atoms involved in the hydrogen bond is
 287 implemented by calculating a local energy information defined in the DSSP package
 288 [40]:

$$289 \quad q_1 q_2 \left[\frac{1}{d_{O_{i-4}N_i}} + \frac{1}{d_{C_{i-4}H_i}} - \frac{1}{d_{O_{i-4}H_i}} - \frac{1}{d_{C_{i-4}N_i}} \right] \cdot f < -0.5, \quad (12)$$

290 with $q_1 = 0.42$, $q_2 = 0.2$ and $f = 332$, and d_{AB} correspond to the distance between
 291 atoms A and B .

292 The last criterion enforces the angles θ , θ' , θ'' to be respectively into the interval
 293 values $0/70^\circ$, $0/90^\circ$ and $110/180^\circ$.

294 Implementation Details

295 In this section we provide an overview of the main implementation features. The *iBP*
 296 algorithm has been coded in C++ with extensive use of template meta-programming
 297 [41], STL [42, 43], and BOOST (www.boost.org). Linear systems, as for instance
 298 (7), are solved using the LAPACK library [44].

299 Discretizable DGP instances were represented by simple weighted undirected
 300 graphs $G = (V, E, d)$, which were handled by the Boost Graph Library (BGL) [45].
 301 The points in \mathbb{R}^3 were represented using the Boost Geometry Library (also known
 302 as Generic Geometry Library, GGL: www.boost.org).

303 Constraints on distances, angles or energy are typically expressed by enforcing a
 304 variable x to take values in a domain \mathcal{D} , which is generally the union of intervals
 305 and singletons:

$$306 \quad \mathcal{D} = \left\{ \bigcup_{j=1}^m \bar{x}_j \right\} \cup \left\{ \bigcup_{i=1}^k [x_i^l, x_i^u] \right\}. \quad (13)$$

307 The Boost Interval Library (BIL – see [46, 47]) was used to store such representa-
 308 tion, and to perform basic operations for intervals and singletons. On top of the
 309 BIL, we define the type `domain` which contains a set of intervals and operations as
 310 intersection, scaling, etc. The BIL allows also to select the underlining data format
 311 for the interval (single/double precision real, integer).

312 Theory

313 In this section we give some details about the worst-case asymptotic complexity
 314 behavior of the *iBP* algorithm. The description given above includes many details
 315 which are useful for finding the structure of proteins but which somewhat complicate

316 the precise mathematical treatment. We first give a very brief abstract description
 317 of the *iBP* and of the formal problem it solves, and then proceed to discuss its
 318 complexity.

319 Formally speaking, the DGP is the following decision problem: given an integer
 320 $K > 0$, a simple undirected graph $G = (V, E)$ and an edge weight function $d : E \rightarrow \mathbb{R}_+$,
 321 is there a realization $x : V \rightarrow \mathbb{R}^K$ such that for each $\{u, v\} \in E$ we have
 322 $\|x_u - x_v\|_2 = d_{uv}$? Note that we are writing x_u for $x(u)$ and d_{uv} for $d(u, v)$. We also
 323 remark that in the more “applied” interpretation given in the preceding section,
 324 the range of the edge function d is $\mathbb{I}\mathbb{R}_+$, i.e. the set of all non-negative closed real
 325 intervals, and $K = 3$. The DGP is **NP**-hard for any $K > 1$ and **NP**-complete for
 326 $K = 1$ [48]. Since we are interested in finding *all* solutions of the DGP rather than
 327 just one, we denote by X the set of all realizations of G .

328 Assumptions on the DGP input data

329 In fact, due to the fact that our data come from a protein structure setting, we can
 330 also make the following assumptions about G and d :

- 331 1 there is an order $1, 2, \dots, n$ on the vertices such that $1, 2, 3$ is a triangle in the
 332 graph G and, for each vertex $v > 3$, v is adjacent to $v - 1, v - 2, v - 3$;
- 333 2 the set of edges E can be partitioned in two subsets E_D and E_P , such that
 334 E_P consists of all edges $\{u, v\}$ with $v > 4$ and $|v - u| > 3$, and $E_D = E \setminus E_P$;
- 335 3 E_D can be further subdivided in E'_D and E''_D , so that E'_D consists of all edges
 336 $\{u, v\}$ with $|v - u| = 3$, and $E'_D = E_D \setminus E''_D$;
- 337 4 the distance function d is such that: (a) d_{uv} is a scalar for each $\{u, v\} \in E'_D$;
 338 (b) d_{uv} consists of a discrete set of b scalars for each $\{u, v\} \in E''_D$; (c) d_{uv} is
 339 a general interval for all $\{u, v\} \in E_P$.

340 We remark that the above definitions can be appropriately extended to Euclidean
 341 spaces of any dimension $K > 0$, not just $K = 3$. We call E_D the *discretization edges*
 342 and E_P the *pruning edges*. Discretization edges ensure that the graph G is rigid,
 343 which implies that there are finitely many realizations of G in \mathbb{R}^K . Pruning edges
 344 make some of those realizations infeasible, and thereby make the solution set X
 345 smaller. A few remarks are in order:

- 346 • we consider that distances which are known because of covalent bond relations
 347 are sufficiently precise to be represented by a scalar;
- 348 • we consider that distances which are known from NOESY (or other) experi-
 349 ments can be represented by intervals;
- 350 • we assume that a limited number of the intervals can be discretized into sets
 351 containing a finite number b of values within the intervals;
- 352 • the edges in E'_D represent atom pairs of the form $\{v, v - 1\}$ or $\{v, v - 2\}$ for
 353 any $v > 2$: these are involved in covalent bonds;
- 354 • the edges in E''_D represent atom pairs which are assigned a certain number b
 355 of possible values (optionally $b = 1$ for certain pairs);
- 356 • the edges in E_P represent atom pairs for which the distance might be a general
 357 interval.

358 We remark that the order on V was initially intended to follow the protein backbone
 359 [49], but new orders which better exploit the hydrogen atoms in or close to the
 360 backbone have been defined in [50, 51]: these are the orders on which the above
 361 assumptions are based.

362 The DGP with the restrictions above, but where all intervals are replaced by
 363 scalars, is called DISCRETIZABLE MOLECULAR DGP (DMDGP). Both the DMDGP
 364 and its generalization to any K (denoted by K DMDGP) are **NP**-hard [52, 53]. The
 365 problem defined above, involving intervals, obviously contains the DMDGP as a
 366 sub-case and is hence also **NP**-hard by inclusion.

367 When all distances are precise

368 We first focus on the simplest case, where all intervals are replaced by scalar values.
 369 Then $d : E \rightarrow \mathbb{R}_+$, and $b = 1$. In this simplified setting, the *i*BP is simply called
 370 BP [52], and the order on V is called a *contiguous trilateration order* [54] or a
 371 *DMDGP order* [55].

372 The BP can be defined as a recursive procedure: assuming we already found a
 373 realization x_1, \dots, x_{v-1} for the vertices $1, \dots, v-1$, and that we mean to find a
 374 consistent realization x_v for v , the discretization edges E_D guarantee that there
 375 will be at most two positions for x_v compatible with the distances restricted to
 376 E_D [49]. This can be intuitively understood in \mathbb{R}^3 by considering the intersection of
 377 three spheres centered at $x_{v-1}, x_{v-2}, x_{v-3}$ with radii $d_{v,v-1}, d_{v,v-2}, d_{v,v-3}$: the first
 378 two spheres either do not meet or their intersection is in general a circle, and the
 379 intersection of the third sphere with this circle is either empty or consists in general
 380 of two points [56]. We can now consider the distances defined on pruning edges in
 381 E_P , linking v to its preceding vertices in order to accept or reject these two points.
 382 For each accepted point we recursively call BP with v replaced by $v+1$, for all $v < n$.
 383 When $v = n$ we have a valid realization of the graph: we save it in X , and proceed
 384 to complete the recursive search. This yields a search tree which is explored depth-
 385 first. The recursion starts after placing the initial triangle 1, 2, 3 (either arbitrarily
 386 or by using BP restricted to subspaces), so this tree starts branching at level 4. It
 387 can be proved that, at completion, X contains all incongruent (modulo translations
 388 and rotations) realizations of G .

389 In the case where $E_P = \emptyset$, the search tree is a complete binary tree with 2^{n-3}
 390 nodes at the n -th (and last) level: in other words, its depth is n and its width is
 391 2^{n-3} . This is the worst case, since the BP must explore all of the nodes in the
 392 tree, and proves that the BP (and hence the *i*BP, since it generalizes the BP) is an
 393 exponential-time algorithm in n .

394 When $E_P \neq \emptyset$, it was shown that X almost always contains a number of solutions
 395 which is either zero or a power of two [55]; this discovery led to a set of results
 396 where the BP search tree width can be kept polynomial in n during the search [53].
 397 Since the exponential behavior is only due to the tree width, this yields a set of
 398 cases where the BP is actually fixed-parameter tractable (FPT). Throughout all our
 399 experiments with protein data we were always able to fix the parameter controlling
 400 the exponential growth of the tree width to a universal constant, which makes BP
 401 “polynomial on proteins” (this is an informal statement — the precise statement is
 402 given in [53]).

403 Intervals and discrete distance sets

404 The theory supporting the case where d might map edges to discrete sets of dis-
405 tance values or intervals, which is the case treated in this paper, is not so clearly
406 understood yet. As it generalizes the simpler case sketched above, in a certain sense
407 it inherits its properties, but this is an oversimplification: for instance, if all inter-
408 vals are $[0, \infty]$, it is obvious that the problem is easy independently of the graph
409 topology, since every realization is valid.

410 Some bounds on the cardinality of X in the presence of discrete sets and intervals
411 are given in [55]. Our understanding is that if the intervals are small enough, the
412 theory which led to fixed-parameter tractability goes through with few changes,
413 but we have no way so far of establishing an aprioristic maximum width for the
414 intervals. If the intervals are very large the problem might become tractable, as
415 mentioned above, for the purposes of finding at least one solution. The *iBP* would
416 still behave exponentially, however.

417 Results-Discussion

418 We applied the presented algorithm to three examples of proteins displaying α -heli-
419 cal secondary structures. Before presenting the obtained results, we emphasize that
420 the method proposed here has a completely different philosophy than classical opti-
421 mization approaches commonly used in the field of NMR structure determination.
422 In the present approach, each constraint is treated in the strict sense, that is, no
423 violation, however small, is tolerated. This is why we consistently use the word
424 *constraint* in the paper. This is what potentially allows us to systematically explore
425 the entire search space. However, the use of the procedure demands that the data
426 have been pre-processed accordingly, and all geometric inconsistencies that exist in
427 three-dimensional space have been removed.

428 For the proteins studied here, if one includes the ensemble of NMR interval dis-
429 tance constraints stored in the .mr file at the Protein Data Bank (PDB) [57] as well
430 as all pruning devices described above, all solutions are pruned out, indicating that
431 no solution to the distance geometry problem exists with the deposited data. This
432 is not really surprising, since the optimization algorithms generally used in NMR
433 structure determination are based on optimization of a target function or hybrid
434 energy rather than on strict constraint satisfaction. That is, there is always a phase
435 where the algorithm tries to find a trade-off when inconsistencies exist between
436 constraints. The optimization thus produces solutions in which chemical and NMR
437 constraints are optimized, but in which small violations are always present. These
438 inconsistencies are present in any structure determination, in particular because
439 distance constraints are imprecise, due to experimental limitations.

440 Since the data in the PDB for the examples presented here were not pre-processed
441 the way our algorithm requires, we decided to use a subset of the stored data sets:
442 the definition of α -helix regions and a few long-range distance constraints arbitrary
443 selected from the set of NMR constraints for structures with more than one α -helix.
444 In order to further reduce the risk of all solutions being pruned, we used tolerance
445 values for atomic positions and angles between atoms (Table 2).

446 The three examples we chose to illustrate the algorithm display an increasing
447 structural complexity: (i) a single α helix, corresponding to the structure of pep-
448 tide CM15 determined in micelles (PDB id: 2JMY [58]), (ii) an α helical hairpin
449 (PDB id: 2KXA [59]), (iii) the insecticidal toxin TAITX-1a, formed as a bundle of
450 four α helices, restrained by three disulphide bridges (PDB id: 2KSL). The main
451 characteristics of the studied proteins are given in Table 2. All three examples were
452 originally determined by Nuclear Magnetic Resonance (NMR), and the correspond-
453 ing constraint lists are available from the PDB. The analysis by PROCHECK [60]
454 of the Ramachandran diagram of these three PDB structures shows that more than
455 85% of the residues are located in the core region. For 2KXA and 2KSL, more than
456 95% of the residues are located in the core and allowed region, whereas in 2JMY,
457 7% of the residues are located in the generously allowed region. For 2KXA, one
458 PRO residue was replaced by an ALA, as the PRO cycle has not yet been included
459 in the current version of the *iBP* algorithm.

460 We generated conformations using the branching phase and the pruning devices
461 described above. The long-range constraints added for the calculations of 2KXA and
462 2KSL, are: (i) for 2KXA, one constraint between $H\alpha$ hydrogen and carbonyl oxygen
463 of Ala-5 and Met-17, enforcing the pairing of the two α -helices, (ii) for 2KSL, three
464 constraints between Carbons β of Cys-7 and Cys-37, of Cys-23 and Cys-33 and of
465 Cys-26 and Cys-46, corresponding to the formation of the three disulphide bridges.

466 For all calculations, except the one of 2JMY with the α helix defined along the
467 whole sequence, the obtained conformations were filtered according to the coordi-
468 nate root mean-squared deviation (RMSD: 1.5 Å) with respect to the previously
469 obtained conformation in the *iBP* procedure. Enforcing an RMSD value larger than
470 1.5 Å between two successively stored conformations, avoids an oversampling of the
471 conformational space. Each calculation was stopped after storing 10000 filtered con-
472 formations.

473 For our three examples, five calculations were performed in total: three on 2JMY
474 with different definitions of the α helix (residues 1-15, 3-13 and 5-11), and one each
475 for 2KXA and 2KSL. For the first calculation on 2JMY, one conformation was
476 obtained and saved. The second and third calculations on 2JMY were quite short,
477 of the order of minutes (Table 2), which is due to the small size of the corresponding
478 tree. For the 2KXA and 2KSL calculations, 10000 conformations were obtained in
479 about 30 mins of calculation. Large total numbers of conformations were generated:
480 this number increases from $\sim 634,000$ (2JMY.1) up to $\sim 3,400,000$ (2KXA) with the
481 size of the considered problem, depending on the number of residues and on the
482 number of constraints. Despite 2KSL being the largest example, the second smallest
483 number of conformations was generated, which is the sign of a severe pruning arising
484 from a rather restricted conformational space.

485 The reliability of the obtained conformations was checked in three ways. First,
486 the whole set of NMR constraints deposited along with the PDB entries and involv-
487 ing backbone hydrogens, were probed on the conformations. Second, the quality
488 of the obtained conformations was checked using PROCHECK [60] analysis of the
489 Ramachandran plot. Third, the obtained conformations were clustered with an un-
490 supervised clustering method, namely the self-organizing map or SOM [61–63], in
491 order to investigate the properties of sampled conformations.

492 The agreement of the obtained conformations with the backbone NMR constraints
493 deposited with the PDB structures was checked by calculating the distances between
494 the backbone hydrogens in each obtained conformation. The distances larger than
495 the upper bound of the constraint correspond to violations of this constraint. The
496 mean number of violated constraints along with the mean value of the difference to
497 the upper bound for these constraints were calculated on all conformations (Table
498 2). For the 2JMY calculation with the 1-15 α helix definition, no violation of the
499 NMR constraints could be observed. As expected, when the α helix definition is
500 reduced (2JMY_1 and 2JMY_2), the average number of violations increases as well
501 as the average maximum violation. Not surprisingly, the most violated constraints
502 involve residues located at the N and C terminal parts of the α -helix, TRP-2,
503 PHE-5, LYS-3, LYS-6 and VAL-11, VAL-14, LEU-15 for 2JMY_1 and 2JMY_2. The
504 largest violations and number of violations are of the same order or value for 2KXA
505 than for 2JMY_1 and 2JMY_2. In contrast, the largest violations and number of
506 violations are observed for 2KSL and involve residues CYS-33, GLU-34, PHE-38,
507 TYR-43. Such over-restraining of NMR structures have been put in evidence in
508 the past, through molecular dynamics simulations [64] and analysis of the structure
509 quality [65].

510 The average number of violations is similar for 2JMY_2, 2KXA and 2KSL, but
511 the average maximum violation for 2KSL is twice as large as that for 2JMY_2 and
512 2KXA. This might be due to the very restrained conformations of 2KSL, which
513 contain three disulphide bridges. Due to this restrained conformation, the NMR
514 constraint list is probably more prone to contain inconsistencies, and large mechan-
515 ical strain can be stored in the structure if one uses an optimization procedure such
516 as simulated annealing. In contrast, no mechanical strain whatsoever is generated by
517 the *i*BP algorithm, and the obtained conformations might have a stronger tendency
518 to deviate from the PDB conformations.

519 For each example, the obtained conformations were compared to the first conforma-
520 tion deposited in the PDB. Minimum RMSD values in the range 1.1-2.1 Å were
521 obtained for all targets, except 2KSL for which the minimum RMSD value was 3.0
522 Å. Thus the Branch-and-Prune algorithm was able to capture conformations close
523 to the PDB conformations, the larger value obtained for 2KSL arising from the
524 larger mechanical strain quoted above.

525 For each calculation, the conformation displaying the smallest number of NMR
526 constraint violations was compared to the first conformation deposited in the PDB.
527 The RMSD values are smaller than 1.5 Å for 2JMY and 2KXA. This shows that,
528 in the context of the *i*BP algorithm, the measured NMR constraints also push
529 the structure toward the PDB structure. For 2JMY_1 and 2JMY_2, the RMSD
530 value increases since the definition of the α helical region is shorter. For 2KSL, the
531 conformation displaying the smallest number of constraint violations, displays an
532 RMSD of 3.5 Å with the PDB first conformation, which agrees with the maximum
533 number of violations observed for this protein and with the minimum RMSD with
534 the PDB structure analyzed above.

535 From the PROCHECK [60] analysis, the percentage of residues located in core and
536 allowed Ramachandran regions, is larger than 95% for all targets except 2JMY_1,
537 2JMY_2, for which the percentages are about 80% due to the reduced definition of

538 the α helix. For all targets, the percentage of residues in disallowed regions is equal
539 to zero. The relatively important percentage of residues located in the allowed
540 region may arise from the systematic exploration performed by the Branch-and-
541 Prune algorithm, the strict nature of the constraints, and the nature of the pruning
542 devices.

543 In order to further probe the robustness of the proposed algorithm, *i*BP calcula-
544 tions on 2KXA and 2KSL have been performed, using input data degraded in the
545 following way: (i) the length of each α helix has been reduced by 1 residues at each
546 extremity, (ii) the lower and upper bounds of the long-range distance constraints
547 have been increased by 0.5 Å. The introduction of this noise into the α helical and
548 long-range constraints makes the *i*BP solution moving apart from the PDB struc-
549 ture, as the minimum RMSD to PDB structure changes from 1.1 to 2 Å for 2KXA,
550 and from 3.0 to 4.3 Å for 2KSL. Nevertheless, the quality of the Ramachandran
551 diagram remains satisfying, with 93.3% and 95.4% of the residues located in the
552 core and allowed regions of the Ramachandran plot for 2KXA and 2KSL.

553 The conformations were clustered using a self-organizing map (SOM) approach
554 [61, 62], on which the coordinate RMSD values between the conformers obtained
555 by Branch-and-Prune and the corresponding PDB structure, were projected on the
556 SOMs (Figure 6). These RMSD values lay in the 1.3-3.2 Å range for 2JMY_1, in the
557 2.4-4.9 Å range for 2JMY_2, in the 1.5-4.0 Å range for 2KXA, and in the 3.2-6.0 Å
558 for 2KSL.

559 In the SOMs for the four calculations (Figure 6), the RMSD values are colored
560 according to their RMSD from the PDB entry, violet color indicating values smaller
561 than the median value of the sampled RMSD value, green color indicating RMSD
562 values larger than this median value. For 2JMY_1, 2KXA and 2KSL, a larger num-
563 ber of neurons of the SOMs belongs to the second group, which is the sign of an
564 enhanced sampling of the conformational space with respect to the region sampled
565 by simulated annealing. For 2JMY_2, the inverse picture is observed, which may
566 arise from the more limited conformational space available to be sampled for a
567 unique α -helix.

568 In 2KSL and 2KXA SOMs, the protein conformations corresponding to the region
569 displaying the smallest coordinate RMSD values with respect to the PDB structure,
570 were extracted (Figure 7). These sets of conformers are similar to the superimposed
571 conformations obtained in a usual NMR calculation.

572 Conclusions

573 We proposed here a Branch-and-Prune algorithm (*i*BP) to solve the Distance Ge-
574 ometry Problem, in order to sample exhaustively the conformational space of the
575 backbone of α -helical proteins. The *i*BP algorithm bears a very slight reminiscence
576 to variable target function approaches for example implemented in DISMAN [66],
577 due to the sequential nature of introducing constraints and non-bonded interactions.
578 However, the precise way of introducing the constraints and non-bonded interac-
579 tions differs significantly, and DISMAN does not systematically search space but is
580 an optimization approach.

581 We introduced new pruning devices integrated in the *i*BP algorithm for DGP
582 with intervals and we tested our *i*BP implementation on the backbones of α -helical

583 proteins. Several pruning devices have been designed to enforce amino-acid chirality,
584 α -helix geometry and van der Waals steric hindrance. The algorithm allowed to
585 efficiently reconstruct backbone conformations of three α -helical peptides, of various
586 sizes, and for which the structure were previously solved by NMR. The obtained
587 solutions satisfy most of the NMR constraints involving backbone hydrogen bonds,
588 and display very acceptable Ramachandran statistics. The present work represents
589 a first successful step on the way to reconstruct protein structures using a branch-
590 and-prune algorithm applied to the Distance Geometry problem.

591 Applications where this approach could have significant advantages are cases
592 where there are few distances defining the tertiary structure of a protein, where
593 it is important to characterize the space of all solutions. It might also be useful as
594 part iterative automated assignment algorithms such as ARIA [67], CYANA [68]
595 or UNIO [69], where in a first iteration all solutions compatible with a few unam-
596 biguous long-range constraints could be generated to reduce the ambiguity of the
597 remaining constraints. Another application of the approach proposed here would be
598 to provide input molecular conformations to model the structure of multi-subunit
599 complexes into an electron microscopy density map [70].

600 Some limitations of the current version of *iBP* prevent for the moment its use
601 with real nuclear Overhauser effect (NOE) data. These limitations are the use of
602 unambiguous distance constraints, the non-inclusion of protein side-chains, the loss
603 of information intervals and the appropriate weighting of the various constraints
604 in order to overcome the inconsistencies contained among the whole constraint set.
605 Protein side-chains can be added to the protein backbone afterward. The discretiza-
606 tion of circle arcs could be tackled using algebraic geometry and geometric algebra
607 approaches [71]. The Bayesian approach [72] developed for the objective weighting
608 of various NMR constraints according to the data quality could be used to alle-
609 viate the inconsistency problems. The use of unambiguous distance constraints is
610 probably the most unavoidable aspect of the current set-up of the algorithm.

611 **Competing interests**

612 The authors declare that they have no competing interests.

613 **Author's contributions**

614 AC, TM, MN, LL and AM designed the work. AC implemented the algorithm. TM, GB and BB performed and
615 analyzed the application cases. AC, BB, AM, LL, CL, RA, MN and TM wrote the manuscript.

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625 **References**

- 626 1. Huang, X., Britto, M., Kear-Scott, J., Boone, C., Rocca, J., Simmerling, C., McKenna, R., Bieri, M., Gooley,
627 P., Dunn, B., Fanucci, G.: The role of select subtype polymorphisms on HIV-1 protease conformational
628 sampling and dynamics. *J Biol Chem* **289**, 17203–17214 (2014)
- 629 2. Kanelis, V., Forman-Kay, J., Kay, L.: Multidimensional NMR methods for protein structure determination.
630 *IUBMB Life* **52**, 291–302 (2001)
- 631 3. Sinz, A.: Chemical cross-linking and mass spectrometry for mapping three-dimensional structures of proteins
632 and protein complexes. *J Mass Spectrometry* **38**, 1225–1237 (2003)

- 633 4. Marti-Renom, M., Stuart, A., Fiser, A., Sánchez, R., Melo, F., Sali, A.: Comparative protein structure modeling
634 of genes and genomes. *Annual Review Biophysical Biomolecular Structure* **29**, 291–325 (2000)
- 635 5. Vajda, S., Kozakov, D.: Convergence and combination of methods in protein-protein docking. *Current Opinion*
636 *Structural Biology* **19**, 164–170 (2009)
- 637 6. Bello, M., Martínez-Archundia, M., Correa-Basurto, J.: Automated docking for novel drug discovery. *Expert*
638 *Opinion Drug Discovery* **8**, 821–834 (2013)
- 639 7. Crippen, G., Havel, T.: *Distance Geometry and Molecular Conformation*. Wiley, New York (1988)
- 640 8. Liberti, L., Lavor, C., Maculan, N., Mucherino, A.: Euclidean distance geometry and applications. *SIAM Review*
641 **56**, 3–69 (2014)
- 642 9. Nilges, M., Gronenborn, A., Brünger, A., Clore, G.: Determination of three-dimensional structures of proteins
643 by simulated annealing with interproton distance restraints. Application to crambin, potato carboxypeptidase
644 inhibitor and barley serine proteinase inhibitor 2. *Protein Engineering* **2**, 27–38 (1988)
- 645 10. Alipanahi, B., Krislock, N., Ghodsi, A., Wolkowicz, H., Donaldson, L., Li, M.: Determining protein structures
646 from NOESY distance constraints by semidefinite programming. *Journal Computational Biology* **20**, 296–310
647 (2013)
- 648 11. Wang, C., Lozano-Pérez, T., Tidor, B.: AmbiPack: A Systematic Algorithm for Packing of Macromolecular
649 Structures With Ambiguous Distance Constraints. *Proteins* **32**, 26–42 (1998)
- 650 12. Potluri, S., Yan, A., Chou, J., Donald, B., Bailey-Kellogg, C.: Structure Determination of Symmetric
651 Homo-Oligomers by a Complete Search of Symmetry Configuration Space Using NMR Restraints and van der
652 Waals Packing. *Proteins* **65**, 203–219 (2006)
- 653 13. Potluri, S., Yan, A., Donald, B., Bailey-Kellogg, C.: A complete algorithm to resolve ambiguity for intersubunit
654 NOE assignment in structure determination of symmetric homo-oligomers. *Protein Science* **16**, 69–81 (2007)
- 655 14. Martin, J., Yan, A., Bailey-Kellogg, C., Zhou, P., Donald, B.: A geometric arrangement algorithm for structure
656 determination of symmetric protein homo-oligomers from NOEs and RDCs. *Journal Computational Biology* **18**,
657 1507–1523 (2011)
- 658 15. Martin, J., Yan, A., Bailey-Kellogg, C., Zhou, P., Donald, B.: A graphical method for analyzing distance
659 restraints using residual dipolar couplings for structure determination of symmetric protein homo-oligomers.
660 *Protein Science* **20**, 970–985 (2011)
- 661 16. Reardon, P., Sage, H., Dennison, S., Martin, J., Donald, B., Alam, S., Abd LD Spicer, B.H.: Structure of an
662 HIV-1-neutralizing antibody target, the lipid-bound gp41 envelope membrane proximal region trimer.
663 *Proceedings National Academy Sciences USA* **111**, 1391–1396 (2014)
- 664 17. Zeng, J., Boyles, J., Tripathy, C., Wang, L., Yan, A., Zhou, P., Donald, B.: High-resolution protein structure
665 determination starting with a global fold calculated from exact solutions to the rdc equations. *J Biomol NMR*
666 **45**, 265–281 (2009)
- 667 18. Gordon, D., Hom, G., Mayo, S., Pierce, N.: Exact rotamer optimization for protein design. *J Comput Chem* **24**,
668 232–243 (2003)
- 669 19. Kingsford, C., Chazelle, B., Singh, M.: Solving and analyzing side-chain positioning problems using linear and
670 integer programming. *Bioinformatics* **21**, 1028–1036 (2005)
- 671 20. Wang, L., Donald, B.: An efficient and accurate algorithm for assigning nuclear Overhauser effect restraints
672 using a rotamer library ensemble and residual dipolar couplings. The IEEE computational systems
673 bioinformatics conference (CSB), Stanford, CA, 189–202 (2005)
- 674 21. Wang, L., Mettu, R., Donald, B.: A polynomial-time algorithm for de novo protein backbone structure
675 determination from NMR data. *J Comput Biol* **13**, 1276–1288 (2006)
- 676 22. O’Neil, R., Lilien, R., Donald, B., Stroud, R., Anderson, A.: Phylogenetic classification of protozoa based on
677 the structure of the linker domain in the bifunctional enzyme, dihydrofolate reductase-thymidylate synthase. *J*
678 *Biol Chem* **278**, 52980–7 (2003)
- 679 23. Lavor, C., Liberti, L., Maculan, N., Mucherino, A.: The discretizable molecular distance geometry problem.
680 *Computational Optimization and Applications* **52**, 115–146 (2012)
- 681 24. Lavor, C., Liberti, L., Mucherino, A.: The interval Branch-and-Prune Algorithm for the Discretizable Molecular
682 Distance Geometry Problem with Inexact Distances. *Journal of Global Optimization* **56**, 855–871 (2013)
- 683 25. Engh, R.A., Huber, R.: Accurate bond and angle parameters for x-ray protein structure refinement. *Acta*
684 *Crystallographica Section A: Foundations of Crystallography* **47**(4), 392–400 (1991)
- 685 26. Rocchia, W., Alexov, E., Honig, B.: Extending the applicability of the nonlinear poisson-boltzmann equation:
686 Multiple dielectric constants and multivalent ions. *The Journal of Physical Chemistry B* **105**(28), 6507–6514
687 (2001)
- 688 27. Honig, B., Nicholls, A., et al.: Classical electrostatics in biology and chemistry. *Science* **268**(5214), 1144–1149
689 (1995)
- 690 28. Liberti, L., Masson, B., Lee, J., Lavor, C., Mucherino, A.: On the number of realizations of certain Henneberg
691 graphs arising in protein conformation. *Discrete Applied Mathematics* **165**, 213–232 (2014)
- 692 29. Coope, I.: Reliable computation of the points of intersection of n spheres in \mathbb{R}^n . *ANZIAM Journal* **42**, 461–477
693 (2000)
- 694 30. Berg, J., Tymoczko, J., Stryer, L.: *Biochemistry: International Edition*. WH Freeman & Co, New York (2006)
- 695 31. Güntert, P., Mumenthaler, C., Wüthrich, K.: Torsion angle dynamics for NMR structure calculation with the
696 new program DYANA. *J Mol Biol* **273**, 283–298 (1997)
- 697 32. Güntert, P., Wüthrich, K.: Sampling of conformation space in torsion angle dynamics calculations. *Comp Phys*
698 *Commun* **138**, 155–169 (2001)
- 699 33. López-Méndez, B., Güntert, P.: Automated protein structure determination from NMR spectra. *J Am Chem*
700 *Soc* **128**, 13112–13122 (2006)
- 701 34. Mucherino, A., Lavor, C., Malliavin, T., Liberti, L., Nilges, M., Maculan, N.: Influence of pruning devices on the
702 solution of molecular distance geometry problems. In: Pardalos, P., Rebennack, S. (eds.) *Lecture Notes in*
703 *Computer Science* 6630, pp. 206–217. Springer, Germany (2011)
- 704 35. Dong, Q., Wu, Z.: A geometric build-up algorithm for solving the molecular distance geometry problem with

- 705 sparse distance data. *Journal of Global Optimization* **26**(3), 321–333 (2003)
- 706 36. Lavor, C., Liberti, L., Mucherino, A., Maculan, N.: On a discretizable subclass of instances of the molecular
707 distance geometry problem. In: *Proceedings of the 2009 ACM Symposium on Applied Computing*, pp. 804–805
708 (2009). ACM
- 709 37. Floyd, R.W.: Algorithm 97: shortest path. *Communications of the ACM* **5**(6), 345 (1962)
- 710 38. Shen, Y., Delaglio, F., Cornilescu, G., Bax, A.: TALOS+: a hybrid method for predicting protein backbone
711 torsion angles from NMR chemical shifts. *Journal Biomolecular NMR* **44**, 213–223 (2009)
- 712 39. Grishaev, A., Bax, A., et al.: An empirical backbone-backbone hydrogen-bonding potential in proteins and its
713 applications to nmr structure refinement and validation. *Journal of the American Chemical Society* **126**(23),
714 7281–7292 (2004)
- 715 40. Kabsch, W., Sander, C.: Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and
716 geometrical features. *Biopolymers* **22**(12), 2577–2637 (1983)
- 717 41. Abrahams, D., Gurtovoy, A.: *C++ Template Metaprogramming: Concepts, Tools, and Techniques from Boost
718 and Beyond*. Addison-Wesley Professional, Boston, Massachusetts (2004)
- 719 42. Austern, M.H.: *Generic Programming and the STL: Using and Extending the C++ Standard Template Library*.
720 Addison-Wesley Longman Publishing Co., Inc., Boston, Massachusetts (1998)
- 721 43. Josuttis, N.: *The C++ Standard Library: a Tutorial and Reference*. Addison-Wesley Professional, Boston,
722 Massachusetts (1999)
- 723 44. Anderson, E., Bai, Z., Bischof, C., Blackford, S., Demmel, J., Dongarra, J., Du Croz, J., Greenbaum, A.,
724 Hammarling, S., McKenney, A., Sorensen, D.: *LAPACK Users' Guide*, 3rd edn. Society for Industrial and
725 Applied Mathematics, Philadelphia, PA (1999)
- 726 45. Lee, L.-Q., Lumsdaine, A.: *The Boost Graph Library: User Guide and Reference Manual*. Addison-Wesley
727 Professional, Boston, Massachusetts (2002)
- 728 46. Brönnimann, H., Melquiond, G., Pion, S.: The design of the boost interval arithmetic library. *Theoretical
729 Computer Science* **351**(1), 111–118 (2006)
- 730 47. Brönnimann, H., Melquiond, G., Pion, S., et al.: The boost interval arithmetic library. In: *Real Numbers and
731 Computers*, pp. 65–80 (2003)
- 732 48. Saxe, J.: Embeddability of weighted graphs in k -space is strongly NP-hard. *Proceedings of 17th Allerton
733 Conference in Communications, Control and Computing*, 480–489 (1979)
- 734 49. Liberti, L., Lavor, C., Maculan, N.: A branch-and-prune algorithm for the molecular distance geometry problem.
735 *International Transactions in Operational Research* **15**, 1–17 (2008)
- 736 50. Lavor, C., Mucherino, A., Liberti, L., Maculan, N.: On the computation of protein backbones by using artificial
737 backbones of hydrogens. *Journal of Global Optimization* **50**, 329–344 (2011)
- 738 51. Costa, V., Mucherino, A., Lavor, C., Cassioli, A., Carvalho, L., Maculan, N.: Discretization orders for protein
739 side chains. *Journal of Global Optimization* **60**, 333–349 (2014)
- 740 52. Lavor, C., Liberti, L., Maculan, N., Mucherino, A.: The discretizable molecular distance geometry problem.
741 *Computational Optimization and Applications* **52**, 115–146 (2012)
- 742 53. Liberti, L., Lavor, C., Mucherino, A.: The discretizable molecular distance geometry problem seems easier on
743 proteins. In: Mucherino, A., Lavor, C., Liberti, L., Maculan, N. (eds.) *Distance Geometry: Theory, Methods,
744 and Applications*. Springer, New York (2013)
- 745 54. Cassioli, A., Günlük, O., Lavor, C., Liberti, L.: Discretization vertex orders for distance geometry. *Discrete
746 Applied Mathematics* (accepted)
- 747 55. Liberti, L., Masson, B., Lavor, C., Lee, J., Mucherino, A.: On the number of realizations of certain Henneberg
748 graphs arising in protein conformation. *Discrete Applied Mathematics* **165**, 213–232 (2014)
- 749 56. Lavor, C., Lee, J., Lee-St. John, A., Liberti, L., Mucherino, A., Sviridenko, M.: Discretization orders for
750 distance geometry problems. *Optimization Letters* **6**, 783–796 (2012)
- 751 57. Berman, H., Kleywegt, G., Nakamura, H., Markley, J.: The future of the Protein Data Bank. *Biopolymers* **99**,
752 218–222 (2013)
- 753 58. Respondek, M., Madl, T., Göbl, C., Golser, R., Zangger, K.: Mapping the orientation of helices in micelle-bound
754 peptides by paramagnetic relaxation waves. *Journal of the American Chemical Society* **129**, 5228–5234 (2007)
- 755 59. Lorieau, J., Louis, J., Bax, A.: The complete influenza hemagglutinin fusion domain adopts a tight helical
756 hairpin arrangement at the lipid:water interface. *Proceedings National Academy Sciences USA* **107**,
757 11341–11346 (2010)
- 758 60. Laskowski, R., MacArthur, M., Moss, D., Thornton, J.: PROCHECK: a program to check the stereochemical
759 quality of protein structure. *Journal Applied Crystallography* **26**, 283–291 (1993)
- 760 61. Miri, L., Bouvier, G., Kettani, A., Mikou, A., Wakrim, L., Nilges, M., Malliavin, T.: Stabilization of the
761 integrase-DNA complex by Mg²⁺ ions and prediction of key residues for binding HIV-1 integrase inhibitors.
762 *Proteins* **82**, 466–478 (2014)
- 763 62. Bouvier, G., Duclert-Savatier, N., Desdouits, N., Meziane-Cherif, D., Blondel, A., Courvalin, P., Nilges, M.,
764 Malliavin, T.: Functional motions modulating VanA ligand binding unraveled by self-organizing maps. *Journal
765 Chemical Information Modeling* **54**, 289–301 (2014)
- 766 63. Kohonen, T.: *Self-organizing Maps*. Springer, Heidelberg, Germany (2001)
- 767 64. Fan, H., Mark, A.: Relative stability of protein structures determined by X-ray crystallography or NMR
768 spectroscopy: a molecular dynamics simulation study. *Proteins* **53**, 111–120 (2003)
- 769 65. Nabuurs, S., Spronk, C., Vuister, G., Vriend, G.: Traditional biomolecular structure determination by NMR
770 spectroscopy allows for major errors. *PLoS Computational Biology* **2**, 9 (2006)
- 771 66. Braun, W., Gö, N.: Calculation of Protein Conformations by Proton-Proton Distance Constraints: A New
772 Efficient Algorithm. *Journal Molecular Biology* **186**, 611–626 (1985)
- 773 67. Rieping, W., Habeck, M., Bardiaux, B., Bernard, A., Malliavin, T., Nilges, M.: ARIA2: automated NOE
774 assignment and data integration in NMR structure calculation. *Bioinformatics* **23**, 381–382 (2007)
- 775 68. Guntert, P.: Automated NMR structure calculation with CYANA. *Methods Molecular Biology* **278**, 353–378

- 776 (2004)
- 777 69. Guerry, P., Herrmann, T.: Comprehensive automation for NMR structure determination of proteins. *Methods*
778 *Molecular Biology* **831**, 429–451 (2012)
- 779 70. Lasker, K., Sali, A., Wolfson, H.: Determining macromolecular assembly structures by molecular docking and
780 fitting into a electron density map. *Proteins* **78**, 3205–3211 (2010)
- 781 71. Lavor, C., Alves, R., Figueiredo, W., Petraglia, A., Maculan, N.: Clifford Algebra and the discretizable
782 molecular distance geometry problem. *Adv Appl Clifford Algebras*, (2015)
- 783 72. Bernard, A., Vranken, W., Bardiaux, B., Nilges, M., Malliavin, T.: Bayesian estimation of NMR restraint
784 potential and weight: a validation on a representative set of protein structures. *Proteins* **79**, 1525–1537 (2008)

785 Tables

Table 1 Van der Waals radii (see [26] and [27]).

atom	O	H	C	N
r^{vdw} (Å)	1.4	1.0	1.7	1.5

Table 2 Analysis of conformations obtained by the branch-and-pruning algorithm on the three proteins targets: 2JMY, 2KXA and 2KSL. 2JMY_1 and 2JMY_2 correspond to the target 2JMY with shorter definitions of α helices. The total number of generated conformations is given, along with the number conformations filtered according to RMSD values.

Proteins	2JMY	2JMY_1	2JMY_2	2KXA	2KSL
Number of residues	15	15	15	24	51
Number of vertices	107	107	107	170	359
Definition of α helices	1-15	3-13	5-11	1-11, 13-23	4-11, 13-27, 29-36, 41-50
Position tolerance (Å)	0.2	0.2	0.2	0.2	0.2
Angle tolerance (°)	2	2	2	4	4
<i>b</i> value	4	4	4	8	4
Number of long-range constraints	0	0	0	1	3
Number of saved conformations	1	10000	10000	10000	10000
Number of generated conformations	1	633,937	928,399	3,380,964	491,498
CPU time	-	1 min	1 min	25 min	31 min
Number of violated constraints ($> 1\text{Å}$)	0	4.0 ± 2.1	11.6 ± 3.6	9.6 ± 2.9	12.8 ± 1.1
Maximum violation (Å)	0	3.3 ± 1.4	4.8 ± 0.7	3.7 ± 1.0	8.1 ± 0.6
Minimum RMSD from PDB structure (Å)	1.4	1.3	2.1	1.1	3.0
RMSD from PDB structure for minimum violated conformations (Å)	1.4	2.9	2.8	1.3	3.5
PROCHECK					
core residues	100	65.7 ± 25.9	49.2 ± 7.6	60.4 ± 8.1	76.9 ± 2.4
allowed residues	0	17.9 ± 9.7	40.9 ± 8.3	39.6 ± 8.0	21.3 ± 2.8
gen.allow. residues	0	3.6 ± 4.8	9.9 ± 7.2	0.0 ± 0.0	1.9 ± 1.7
disall. residues	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

786 **Figures**

Figure 1 The *i*BP recursive algorithm. Description of the *i*BP algorithm.

Figure 2 The branch-and-prune search tree. Example of branch-and-prune search tree exploration. With solid line, we depict the path currently in use, with dotted arcs pruned paths, and with dashed arcs paths not yet explored. The squared node corresponds to a feasible solution.

Figure 3 Order P_{ato} of the atoms parsed during the branch-and-prune algorithm.

Figure 4 Intersection of three spheres. Intersection of three spheres, colored in yellow, green and cyan. The two points produced by the intersection are indicated with red spots.

Figure 5 Discretization of the distance constraints. An example of discretization of the distance $d_{i,i-3}$. The solid circle represents the result of the intersection of the spheres centered in $i-1, i-2$ with radii $d_{i,i-1}, d_{i,i-2}$, respectively. The distance $d_{i,i-3}$ is discretized accordingly to Equation (2) with $b = 5$: dotted circles represent the intersections of spheres centered in $i-3$ with radii in \tilde{d}_i with the plane containing the $i-3, i-2$ and $i-1$. Thick gray arcs represent the feasible regions for the atom i .

Figure 6 Clustering of the conformations obtained by the *i*BP algorithm. Self-organizing maps describing the clustering of the conformations obtained by the *i*BP algorithm on 2JMY, 2KXA and 2KSL. The contour plots (lines) represent the local similarity between the clustered conformations. The color scales (on plot left) extend from blue to red (from very similar to very dissimilar conformations). The small red points are drawn on the SOM neuron for which the largest local similarity is observed between conformations. Each SOM neuron is colored according to the average value of the coordinates RMSD of the neuron conformations with respect to the PDB structure. The color scales extend (on plot right) from purple to green (from very similar to very dissimilar to the PDB structure). The similarity between SOM neurons as well as the RMSD to the PDB structure are expressed in Å for comparison purposes.

Figure 7 Superimposed 2KXA and 2KSL conformations. Superimposition of 2KXA and 2KSL conformations extracted from the SOM, as the ones displaying the minimum coordinates RMSD with respect to the first conformer of the corresponding PDB structures. The N and C terminal extremities are labeled, and the conformations, drawn in cartoon, are colored from blue to red, according to the conformational index.

Figures

```
Algorithm 1: The iBP recursive algorithm.  
Input: atom index  $l$ , total number of atoms  $n$ , solution  $x$   
1 if  $l = n$  then  
   | /* Solution found! */  
2   return  
   /* Branching */  
3 compute set  $P_l$  of possible position of atom  $l$ ;  
4 foreach  $p \in P_l$  do  
   | /* Check for infeasibility (Pruning) */  
5   if  $p$  is feasible then  
   | | /* Value accepted */  
6   | |  $x^l \leftarrow p$ ;  
   | | /* Go to the next level */  
7   | |  $iBP(l+1, n, x)$ ;  
8 end
```

Figure 1: The *i*BP recursive algorithm. Description of the *i*BP algorithm.

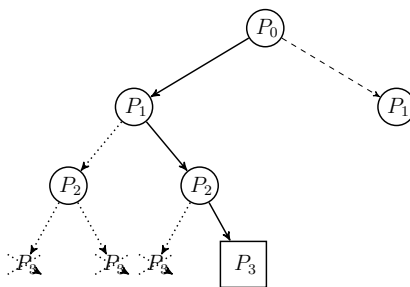


Figure 2: The branch-and-prune search tree. Example of branch-and-prune search tree exploration. With solid line, we depict the path currently in use, with dotted arcs pruned paths, and with dashed arcs paths not yet explored. The squared node corresponds to a feasible solution.

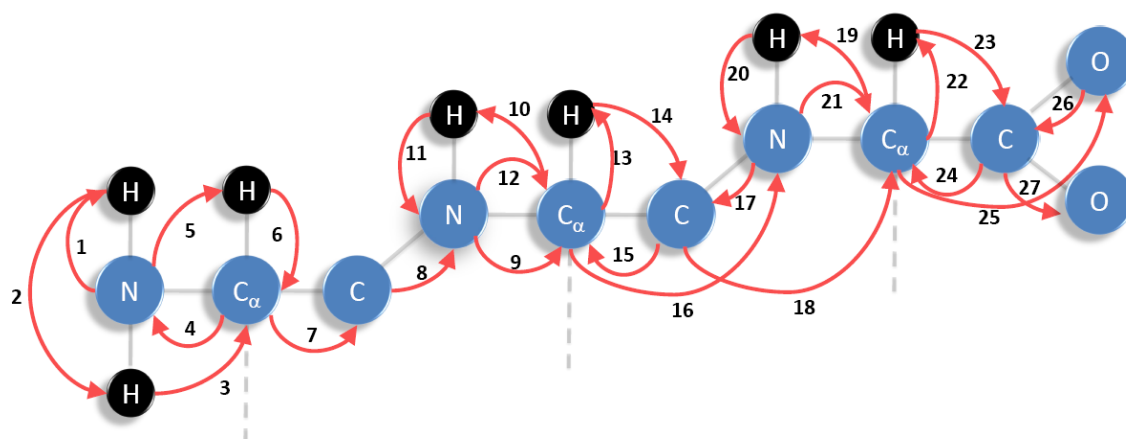


Figure 3: Order of the atoms P_{ato} parsed during the branch-and-prune algorithm.

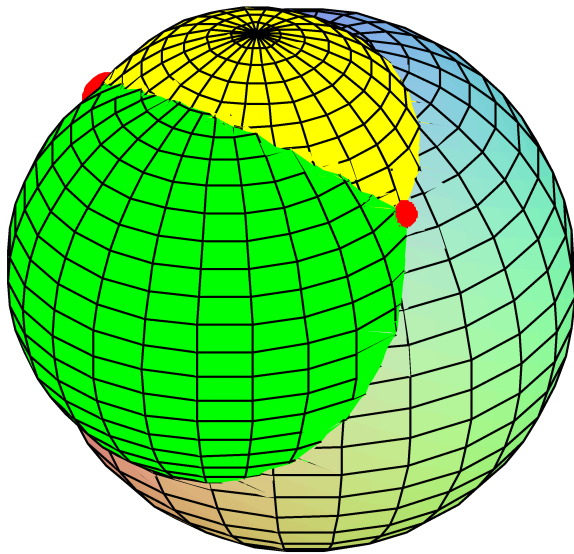


Figure 4: Intersection of three spheres. Intersection of three spheres, colored in yellow, green and cyan. The two points produced by the intersection are indicated with red spots.

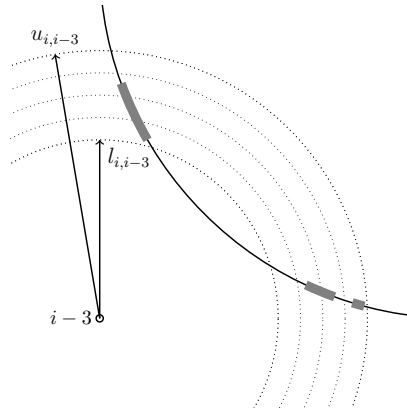


Figure 5: Discretization of the distance restraints. An example of discretization of the distance $d_{i,i-3}$. The solid circle represents the result of the intersection of the spheres centered in $i-1, i-2$ with radii $d_{i,i-1}, d_{i,i-2}$, respectively. The distance $d_{i,i-3}$ is discretized accordingly to Equation (??) with $b = 5$: dotted circles represent the intersections of spheres centered in $i-3$ with radii in \tilde{d}_i with the plane containing the $i-3, i-2$ and $i-1$. Thick gray arcs represent the feasible regions for the atom i .

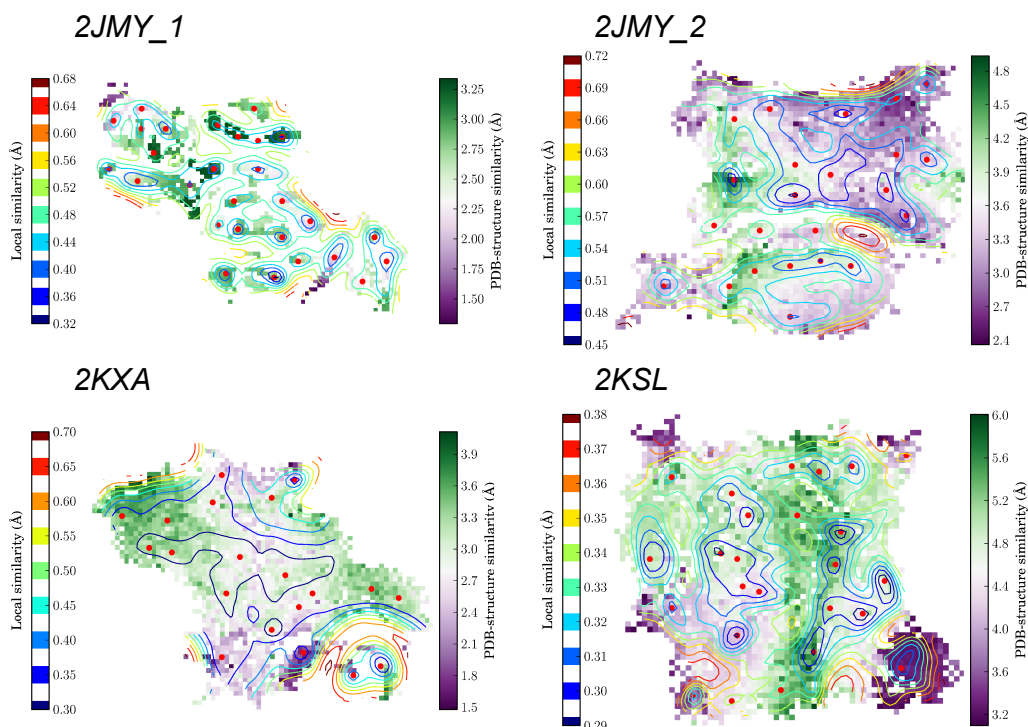


Figure 6: Clustering of the conformations obtained by the *iBP* algorithm. Self-organizing maps describing the clustering of the conformations obtained by the *iBP* algorithm on 2JMY, 2KXA and 2KSL. The contour plots (lines) represent the local similarity between the clustered conformations. The color scales (on plot left) extend from blue to red (from very similar to very dissimilar conformations). The small red points are drawn on the SOM neuron for which the largest local similarity is observed between conformations. Each SOM neuron is colored according to the average value of the coordinates RMSD of the neuron conformations with respect to the PDB structure. The color scales extend (on plot right) from purple to green (from very similar to very dissimilar to the PDB structure). The similarity between SOM neurons as well as the RMSD to the PDB structure are expressed in Å for comparison purposes.

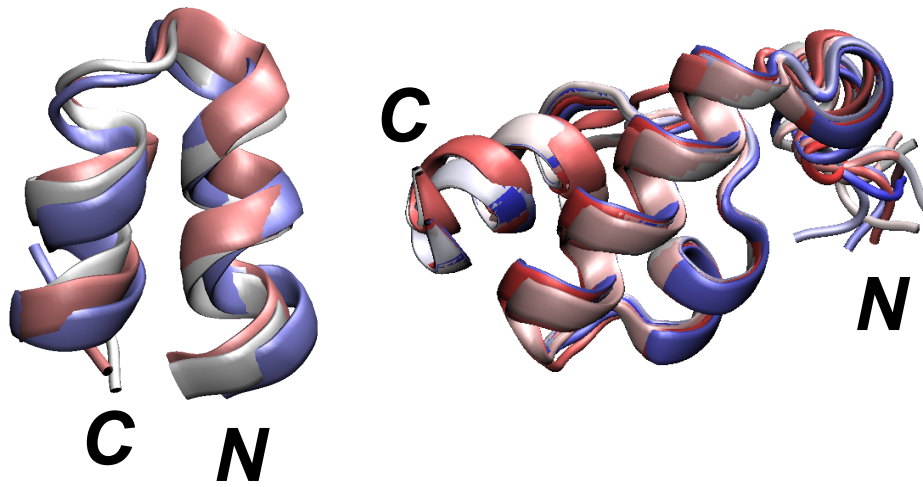


Figure 7: Superimposed 2KXA and 2KSL conformations. Superimposition of 2KXA and 2KSL conformations extracted from the SOM, as the ones displaying the minimum coordinates RMSD with respect to the first conformer of the corresponding PDB structures. The N and C terminal extremities are labeled, and the conformations, drawn in cartoon, are colored from blue to red, according to the conformational index.