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Haiyan Jiang · Christian Blouin

Ab initio construction of all-atom loop conformations

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Abstract In this study, a new ab initio method named CLOOP has been developed to build all-atom loop conformations. In this method, a loop main-chain conformation is generated by sampling main-chain dihedral angles from a restrained φ/ψ set, and the side-chain conformations are built randomly. The CHARMM all-atom force field was used to evaluate the loop conformations. Soft core potentials were used to treat the non-bond interactions, and a designed energy-minimization technique was used to close and optimize the loop conformations. It is shown that the two strategies improve the computational efficiency and the loop-closure rate substantially compared to normal minimization methods. CLOOP was used to construct the conformations of 4-, 8-, and 12-residue loops in Fiser's test set. The average main-chain root-mean-square deviations obtained in 1,000 trials for the 10 different loops of each size are 0.33, 1.27, and 2.77 Å, respectively. CLOOP can build all-atom loop conformations with a sampling accuracy comparable with previous loop main-chain construction algorithms.

Keywords Loop modeling · Energy minimization · Loop closure · Soft core potential

H. Jiang (✉) · C. Blouin
Faculty of Computer Science, Dalhousie University,
Halifax, NS B3H 1W5, Canada
E-mail: hjiang@cs.dal.ca
Tel.: +1-902-4946702
Fax: +1-902-4941517

C. Blouin
Faculty of Computer Science, Dalhousie University,
Halifax, NS B3H 1W5, Canada

H. Jiang (✉) · C. Blouin
Department of Biochemistry and Molecular Biology,
Dalhousie University, Halifax, NS B3H 1W5, Canada

Introduction

Protein loops are polypeptides connecting more rigid structural elements of proteins. Because of this flexibility, they may not be structurally well defined [1]. Loops occur in a variety of lengths from only a few to as many as 30 residues, though the majority has less than 12 residues [2]. Protein loops play a key role in the function and specificity of proteins. They are often involved in active and binding sites, or take part in molecular recognition.

Protein loops have high structural flexibility and diversity. The prediction of protein loops is still one of the open problems in structural biology [3, 4]. In loop prediction, a loop-construction method is used to create a large number of conformational candidates. Subsequently, a loop-prediction algorithm selects the best conformation by optimizing and evaluating these candidates with an energy function [5–7]. A good loop-building method should solve the loop-closure problem, i.e. generate geometrically consistent loop structures with the rest of the protein chain [8], and sample near-native conformations.

Loop-conformation generation techniques can be classified into knowledge-based and ab initio approaches. Knowledge-based methods depend on a representative database of loops of the appropriate size with known 3D structures [9–12]. However, in general, there is no guarantee that the database is a homogeneous representation of all loops' conformational space. In addition, structural databases cannot provide representative ensembles for loops longer than 8 residues [13]. There are many efficient ab initio methods including random tweak [5, 14, 15], analytical methods [8, 16, 17], constraint optimization [18], scaling relaxation, [19] algorithm in robotics [4], and the contact-matrix method [20]. However, most of them only generate loop backbone conformations and need to cooperate with other methods to build all-atom conformations. Some other ab initio methods such as molecular dynamics [21], simulated annealing [2], and Monte Carlo [22, 23] sample low-energy loop conformations

with side-chain atoms by employing an energy function like a molecular mechanics force field or potential derived from statistical mechanics. However, these simulation-based methods are computationally expensive.

In this study, a new ab initio loop-construction method CLOOP has been developed to generate all-atom loop conformational ensembles. In CLOOP, loop candidates are built by sampling main-chain dihedrals from a restrained dihedral range and assigning side-chain dihedrals randomly. CLOOP uses an energy-minimization approach to solve the loop-closure problem. To improve the efficiency, a designed energy-minimization strategy is proposed to optimize the generated loop conformations with the CHARMM all-atom force field [24]. Furthermore, a soft core potential [25] is used to calculate the non-bonded energy. Soft-core potentials smooth the potential energy surface to facilitate crossing energy barriers. This technique has been proved successful for loop modeling using molecular dynamics [26–28]. In this article, we report that soft core potentials increased the loop-closure rate substantially. CLOOP successfully generated conformational ensembles with near-native loop conformations in good efficiency for the test loops up to 12 residues.

Methods

Loop generation method

CLOOP can build loop structures on the given anchor residues of a protein. In preparing the protein structure, the initial coordinates of loop atoms were generated randomly. After reading in the protein structure, the position of the central loop residue was first obtained by randomly generating a peptide trajectory for the first half of the loop. The position of the central residue was fixed as a new anchor residue so that the problem of building a loop was divided into constructing two smaller loops. Then, the backbone conformation was generated by sampling the main-chain dihedral φ and ψ either from a restricted dihedral range or randomly, with equal probability. The restricted dihedral range has 11 pairs of φ/ψ dihedral sub-ranges. It was obtained by adding 100 degrees variation to each state of the 11 φ/ψ set developed by Moult and James [29] for loop modeling. Besides, all ω were set to 180 degree. The side-chain dihedral χ of each loop residue was substituted with a random value. Having all the required dihedrals, the coordinates of loop segments were built by extending the ends of the anchor residues, and the unclosed loop candidates were obtained. Finally, a designed energy-minimization technique was used to optimize these structures.

Energy function

The CHARMM22 all-atom molecular mechanics force field [24] was used to calculate the energy of target loop conformations. In addition to the original energy terms, a soft core potential provided in the CHARMM software package was used to smooth non-bonded interactions (van der Waals and electrostatic energy terms).

$$E_{\text{nonbonded}} = E^{\text{CHARMM}}_{\text{nonbonded}}, \quad r \geqslant r_{\text{soft}} \tag{1}$$

$$E_{\text{nonbonded}} = k(r - r_{\text{soft}}) + E^{\text{CHARMM}}_{\text{nonbonded}}, \quad r < r_{\text{soft}} \tag{2}$$

where r is the distance of the two interacting atoms, r_{soft} is the switching distance for the soft core potential. In this study, the default value of r_{soft} 0.885 Å was used.

To improve the efficiency in energy calculation, the by-clusters-in-cubes (BYCC) method [30], provided by the CHARMM package, was selected to build the non-bonded interaction list.

Designed minimization strategy

The performance of CLOOP was investigated in two ways. One is to calculate the loop energy with a buffer region, and the other is loop only. The buffer region included a region extending up to 10 Å around the loop atoms. In energy minimization, only the loop atoms were allowed to move and all non-loop atoms, including those in the buffer region, were fixed.

The designed minimization (DM) strategy for the loop-closure problem has two stages. In the first stage, only the internal energy terms except the non-bonded interactions of the loop including bond, angle, dihedral, and improper, were considered. The conformational energies of the loop candidates were minimized with 200 iterations of steepest-descent and 200 iterations of conjugate-gradient minimization. In the second stage, the candidates were minimized further with the full CHARMM energy function including the van der Waals and electrostatic energy terms. The minimization procedure includes sequential steps of 100 iterations of steepest-descent, conjugate gradient, and adopted basis of Newton–Raphson minimization methods, respectively. The minimization threshold was set to 0.1 kcal mol^{-1}.

Loop closure criteria

In this study, if the distance of any two neighboring main-chain atoms in a loop candidate is lower than 1.8 Å, the candidate will be accepted as a closed loop conformation.

Implementation

CLOOP was written with the commands of the CHARMM molecular modeling package (Version 3.0b) [31]. All computations were performed on an Intel Pentium 4, 2.60 GHz processor, and the operation system was RedHat Linux 9.0.

Loop test set

The loop test set collected by Fiser et al. [2] was employed in this study. According to the alphabetical index, 10 test loops each were selected from the lists of 4-, 8-, and 12-residue loops. Several proteins with improper residue index and those reported problematic structures [18] were excluded. For larger loops, two proteins 351c and 1alc with one 18- and 31-residue loop, respectively, were used [20].

Sampling results evaluation

In the loop-building process, all the non-loop residues including the anchor residues were fixed and had the same coordinates as the crystal structure. Therefore, the root-mean-square deviation (RMSD) of the loop region between the predicted loop and the actual conformation reflects the overall difference of loop and protein. To evaluate the sampling results, each closed loop conformation was compared with the native loop structure using RMSD, which was calculated with the Cartesian coordinates of main-chain atoms N, C_α, C.

Results and discussion

Dividing the problem by generating the middle residue first

The loop-construction approach starts by generating the position of the loop central residue. This approach makes the closure of longer loops easier by subdividing the problem. Given two anchor residues (Depicted in CPK model in Fig. 1), the loop-construction procedure of CLOOP builds two loop segments from each anchor. As shown in Fig. 1a, the loop candidates built directly have large gaps. The large gaps make the task of minimizing these starting structures more difficult. Instead, CLOOP records and fixes the position of the central residue as a new anchor, and the trajectories of both halves are determined by sampling dihedrals as described previously (see Fig. 1b). Figure 1c shows the closed loop conformation obtained by minimizing the conformational energy of the structure shown in Fig. 1b.

The performance of CLOOP with and without generating the central residue first for the 32 test loops, including the 30 loops in Fiser's loop set, and the two larger loops of 351c and 1alc, is shown in Table 1. The numbers of closed loop conformations for the test loops with length longer than 4 were all increased when the loop conformations were constructed by generating the central residue first. However, the computational time remains similar for both methods. This result indicates that dividing the problem through a fixed central residue facilitates the convergence of the initial minimization step.

Optimizing the minimization strategy for loop closure

Three minimization strategies were tested specifically to address the loop-closure problem: (1) the effects of soft core potentials, (2) the DM strategy in which the loop structure is first minimized with only bonded terms and then with all terms of the energy function, and (3) the influence of the surrounding protein residues (buffer region) on the force-field energy of a loop conformation. For the effects of soft core potentials and the DM, the tests were performed by including or not including the buffer region in test loops with lengths ranging from 4 to 31 residues. The results are shown in Tables 2 and 3, respectively.

In the tests with buffer region (Table 2), the soft core potential substantially increased the number of closed conformations N_{closed}, while the computational time remained similar. For the test loop proteins with lengths 4, 8, 12, 18, and 31, N_{closed} with the soft core potential is 1.2, 2.7, 3.2, 2.3, and 5 times greater, respectively, than the value obtained by a minimization strategy treating the non-bonded components of the empirical force field normally.

Randomly generated conformations inevitably contain unrealistic geometries, including steric clashes. Because the energy penalty associated to long bonds is comparatively lower than that caused by steric clashes, it is hard for a minimization method to cross the high-potential barrier and ultimately close the loop. The use of soft core potentials is especially beneficial when the conformation of a loop is minimized in the presence of a buffer region, where the limited conformational space affects the rate of loop closure. The soft core potential makes loop closure easier by smoothing the energy landscape and reducing the height of barriers caused by steric clashes. Several earlier studies on the effect of soft core potentials on loop modeling focused on their molecular dynamics [26–28]. The results obtained in this study demonstrate that soft core potentials are also useful for energy minimization applied to the loop-closure problem.

The results of the DM procedure and the normal minimization (NM) are also compared in Table 2. In the NM, the target function always includes all energy terms of CHARMM force field. By comparing the test results of the DM and the NM without soft core potentials, it is clear that the major contribution of the DM approach is to save computational expense while it can also improve the closure rate in most cases. When the DM strategy was combined with soft core potentials in CLOOP, the DM approach decreased the computation time by about half. Furthermore, the numbers of closed conformations of all the test loops except those of size 8 and 351c_50–67 were increased further. Consequently, both soft core potentials and the DM strategy are effective when loop candidates are constructed with a buffer region.

The effects of soft core potentials and the DM technique when CLOOP was run without a buffer region (loop only) were also tested and the results are shown in

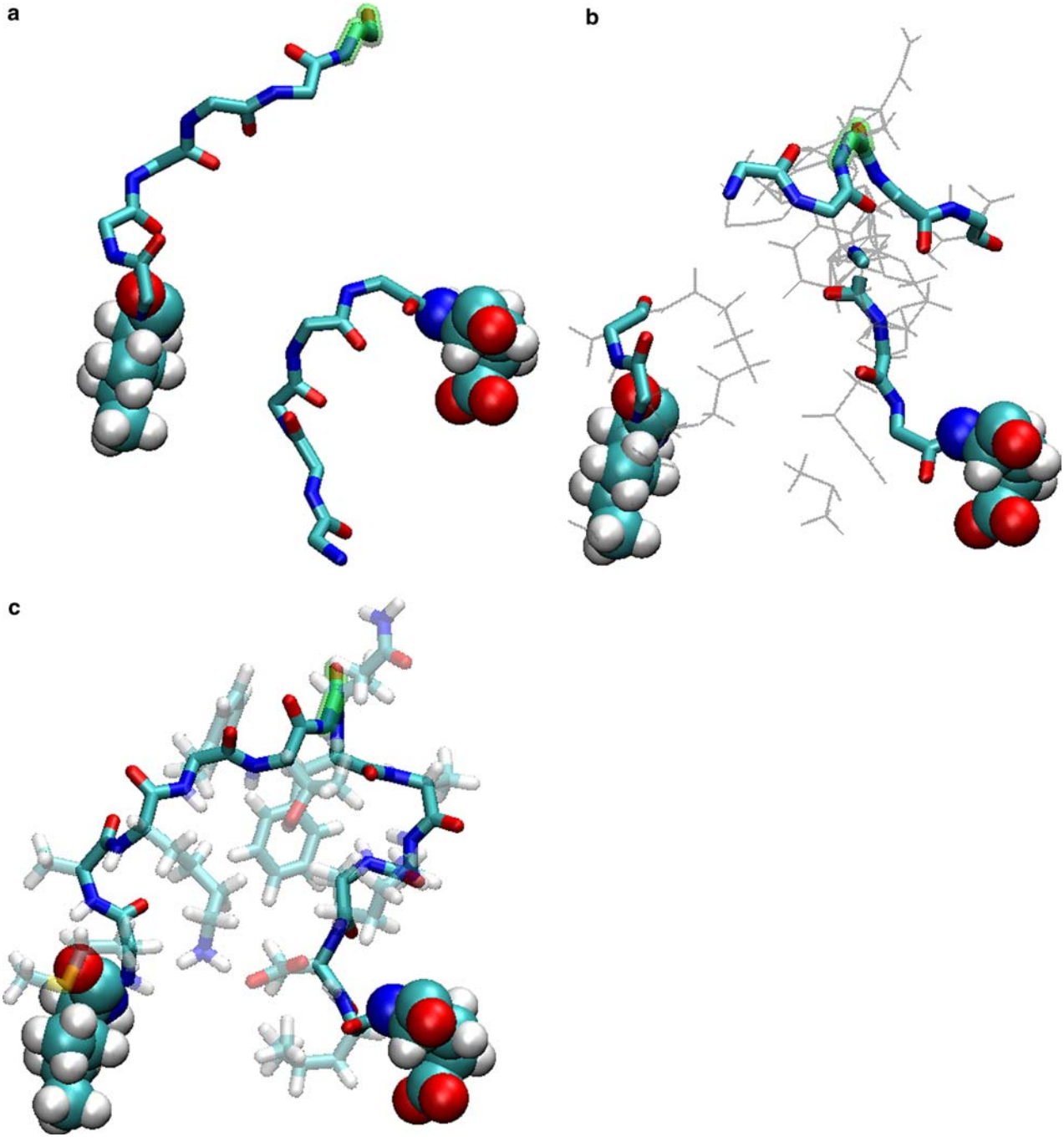

Fig. 1 Schematic diagram of the loop construction approach of CLOOP. **a** Generate main-chain trajectory on each anchor residue (shown in VDW mode) and obtain the position of middle residue (highlighted in *green*). **b** Keep the middle residue as the third anchor and build an initial all-atom loop structure. Side chain is depicted with *gray line*. **c** Close loop via energy minimization. This is the all-atom conformation, and side chain is shown in *transparent*

Table 3. Without the buffer region, the energy barrier caused by steric clashes is much lower, and loop closure is easier to obtain. Even using the NM approach, the closure rate is satisfactory, and the computational time is shorter than the method with buffer. As the test results given in Table 3 shows the soft core potentials, and the DM procedure do not give any improvements when the loop/protein contacts are not considered.

Accuracy of CLOOP in loop conformation sampling

CLOOP was used to construct the conformations of Fiser's loop test set at lengths 4, 8, and 12. The performance of CLOOP with and without buffer region in 1,000 trials is summarized in Tables 4 and 5, respectively. In the tables, the values of $RMSD_{best}$ is the RMSD for the constructed loop most similar to the

Table 1 Comparison of the performance of CLOOP with and without generating the middle residue first, the conformational energy of loop was calculated with buffer region and the number of total generated loop candidates is 100

Loop	Length	Without		With	
		N^{a}_{closed}	Time (min)	N_{closed}	Time (min)
Fiser's Loop Set[b]	4	99	2.4	99	2.4
	8	64	5.6	92	5.2
	12	44	7.2	77	7.2
351c_50–67	18	74	4.4	81	4.6
1alc_61–91	31	56	7.9	63	9.3

[a]Number of closed loop conformations
[b]There are total 30 test loops in Fiser's loop set with 10 loops for each length, test results are the averaged values

Table 2 Effects of the soft core potential and the designed minimization (DM) strategy in CLOOP when loop conformational energy was calculated with a buffer region, and the number of total generated loop candidates is 100

Loop	Length	NM		DM		SCP + NM		SCP + DM	
		N^{a}_{closed}	Time (min)	N_{closed}	Time (min)	N_{closed}	Time (min)	N_{closed}	Time (min)
Fiser's loop set[b]	4	78	3.9	93	2.5	96	3.7	99	2.4
	8	35	8.3	53	5.6	93	8.4	92	5.2
	12	22	12.8	32	7.9	70	12.6	77	7.2
351c_50–67	18	40	9.1	34	5.8	90	7.3	81	4.6
1alc_61–91	31	9	17.5	4	9.9	45	17.4	63	9.3

NM normal minimization*SCP* soft core potential*DM* designed minimization
[a]Number of closed loop conformations
[b]There are total 30 test loops in Fiser's loop set with ten loops for each length, test results are the averaged values

Table 3 Effects of the SCP and the DM in CLOOP when loop conformational energy was calculated with loop atoms only, and the number of total generated loop candidates is 100

Loop	Length	NM		DM		SCP + NM		SCP + DM	
		N^{a}_{closed}	Time (min)	N_{closed}	Time (min)	N_{closed}	Time (min)	N_{closed}	Time (min)
Fiser's loop set[b]	4	99	0.8	99	0.8	99	0.8	99	0.8
	8	99	1.7	99	1.6	99	1.6	99	1.6
	12	99	1.8	99	1.7	98	1.8	98	1.7
351c_50–67	18	99	1.6	99	1.5	98	1.6	98	1.5
1alc_61–91	31	98	3.0	97	2.6	99	2.9	97	2.7

NM normal minimization*SCP* soft core potential*DM* designed minimization
[a]Number of closed loop conformations
[b]There are total 30 test loops in Fiser's loop set with 10 loops for each length, test results are the averaged values

experimentally solved loop. $RMSD_{best}$ is therefore a lower bound for the accuracy of a loop-prediction evaluator, given the set provided by CLOOP. The value $RMSD_E$ is the average RMSD for the ensemble of generated loop conformations. A powerful loop-construction method should be evaluated by the overall quality of near-optimal conformational sampling ($RMSD_E$).

When implemented with a buffer region, CLOOP sampled the near-native conformations for the test loop set. As shown in Table 4, the average main-chain RMSD values of the best conformations are 0.33, 1.27, and 2.77 Å for 4-, 8-, and 12-residue loops, respectively. The comparisons of the best-generated loop conformation with X-ray structure coordinates for different size loops 1gpr_123–126, 135l_84–91, and 1pmy_77–88 are shown in Fig. 2. The results indicate the loop conformations with lowest RMSD in 1,000 candidates sampled by CLOOP are very close to their native structures. The closure rate for loops of 4, 8, and 12 is 98.3, 92.2, and 76.7%, respectively, and the computational time grows linearly with the loop size. The results obtained by CLOOP without buffer are listed in Table 5. The average minimum RMSD of the sampled loop conformations at length 4, 8, and 12 is 0.64, 2.23, and 4.26 Å, respectively. It is obvious that the sampling method is more accurate when the contacts of loop and the buffer region are considered. This accuracy has, however, a computational cost in terms of throughput. Compared with the results indicated in Table 5, CLOOP with buffer is about three to four times slower.

In conclusion, CLOOP with buffer region is more accurate. It is more suitable for the construction of loop conformational ensembles. The results thus demonstrate

Table 4 Performance of CLOOP in 1,000 trials, the conformational energy of loop was calculated with a buffer region

Length 4		Length 8		Length 12	
Loop	$RMSD^{a}_{best}$	Loop	$RMSD_{best}$	Loop	$RMSD_{best}$
1aaj_82–85	0.28	135l_84–91	1.10	154l_153–164	3.13
1ads_99–102	0.47	1alc_34–41	1.62	1arp_201–212	2.64
1cbs_21–24	0.28	1btl_50–57	1.42	1ctm_9–20	3.00
1fkf_42–45	0.27	1cbs_55–62	1.24	1eco_35–46	2.60
1frd_59–62	0.42	1ddt_127–134	1.28	1ede_150–161	2.45
1gpr_123–126	0.22	1fnd_262–269	1.13	1ezm_122–133	2.42
1iab_100–103	0.31	1gky_72–79	1.17	1hfc_165–176	3.36
1mba_97–100	0.28	1iab_48–55	1.17	1msc_9–20	2.97
1nfp_37–40	0.38	1nar_192–199	1.32	1pbe_129–140	2.78
1pbe_117–120	0.35	1phf_85–92	1.23	1pmy_77–88	2.33
Average	0.33	Average	1.27	Average	2.77
Average $RMSD^{b}_{E}$	1.82	Average $RMSD_{E}$	4.04	Average $RMSD_{E}$	6.79
Average Time [c] (min)	23.6	Average Time (min)	49.0	Average Time (min)	70.1
Average N^{d}_{closed}	983	Average N_{closed}	922	Average N_{closed}	767

[a]Main-chain RMSD of the best conformation generated by CLOOP from X-ray structure
[b]Average main-chain RMSD of the conformational ensemble including all the good conformations
[c]Average computational time to generate 1,000 loop candidates for the test loop at certain length
[d]Average number of closed loop conformations

Table 5 Performance of CLOOP in 1,000 trials, the conformational energy of loop was calculated without buffer region

Length 4		Length 8		Length 12	
Loop	$RMSD^{a}_{best}$	Loop	$RMSD_{best}$	Loop	$RMSD_{best}$
1aaj_82–85	0.62	135l_84–91	1.85	154l_153–164	2.83
1ads_99–102	0.86	1alc_34–41	2.17	1arp_201–212	2.85
1cbs_21–24	0.86	1btl_50–57	2.63	1ctm_9–20	3.63
1fkf_42–45	0.62	1cbs_55–62	1.20	1eco_35–46	4.96
1frd_59–62	0.45	1ddt_127–134	2.11	1ede_150–161	3.43
1gpr_123–126	0.80	1fnd_262–269	2.12	1ezm_122–133	4.33
1iab_100–103	0.53	1gky_72–79	4.45	1hfc_165–176	6.32
1mba_97–100	0.70	1iab_48–55	2.10	1msc_9–20	4.71
1nfp_37–40	0.43	1nar_192–199	1.60	1pbe_129–140	5.41
1pbe_117–120	0.51	1phf_85–92	2.11	1pmy_77–88	4.08
Average	0.64	Average	2.23	Average	4.26
Average $RMSD^{b}_{E}$	2.50	Average $RMSD_{E}$	6.86	Average $RMSD_{E}$	10.25
Average Time [c] (min)	7.4	Average Time (min)	13.7	Average Time (min)	16.1
Average N^{d}_{closed}	997	Average N_{closed}	995	Average N_{closed}	991

[a]Main-chain RMSD of the best conformation generated by CLOOP from X-ray structure
[b]Average main-chain RMSD of the conformational ensemble including all the good conformations
[c]Average computational time to generate 1,000 loop candidates for the test loop at certain length
[d]Average number of closed loop conformations

the importance of considering the contacts of a loop with its environment, which can only be considered with an all-atom model.

The performance of CLOOP with buffer is compared with RAPPER, a successful loop-construction method that was also tested with Fiser's loop set [18]. In the ensemble with 1,000 conformations generated by RAPPER, the average best main-chain RMSD from the native structures is 0.43, 1.11, and 2.21 Å for 4-, 8-, and 12-residue loops, respectively. Therefore, the sampling accuracy of the CLOOP algorithm with buffer is comparable, if marginally inferior to that of RAPPER. $RMSD_{E}$ of CLOOP is similar to RAPPER, which is 1.82, 4.04, and 6.79 Å (Table 4) for 4-, 8-, and 12-residue loops, respectively, compared with 1.65, 4.16, and 6.96 Å of RAPPER. The average computational time of RAPPER, obtained on a 900-MHz AMD Athlon processor, is 57.9, 145.6, and 401.8 min for 4-, 8-, and 12-residue loops, respectively. Even though our computer is faster (CLOOP was run on an Intel Pentium 4, 2.60 GHz processor), a crude conversion of computational time for CLOOP with buffer is about 200 min for 12-residue loops, which is only half of the computational time for RAPPER. For smaller loops with four and eight amino acids, the efficiencies of the two methods are similar.

Two other algorithms have been developed recently, including the divide-and-conquer method [17] and the cyclic coordinate descent (CCD) algorithm, [4] that are quite efficient in constructing loop main-chain

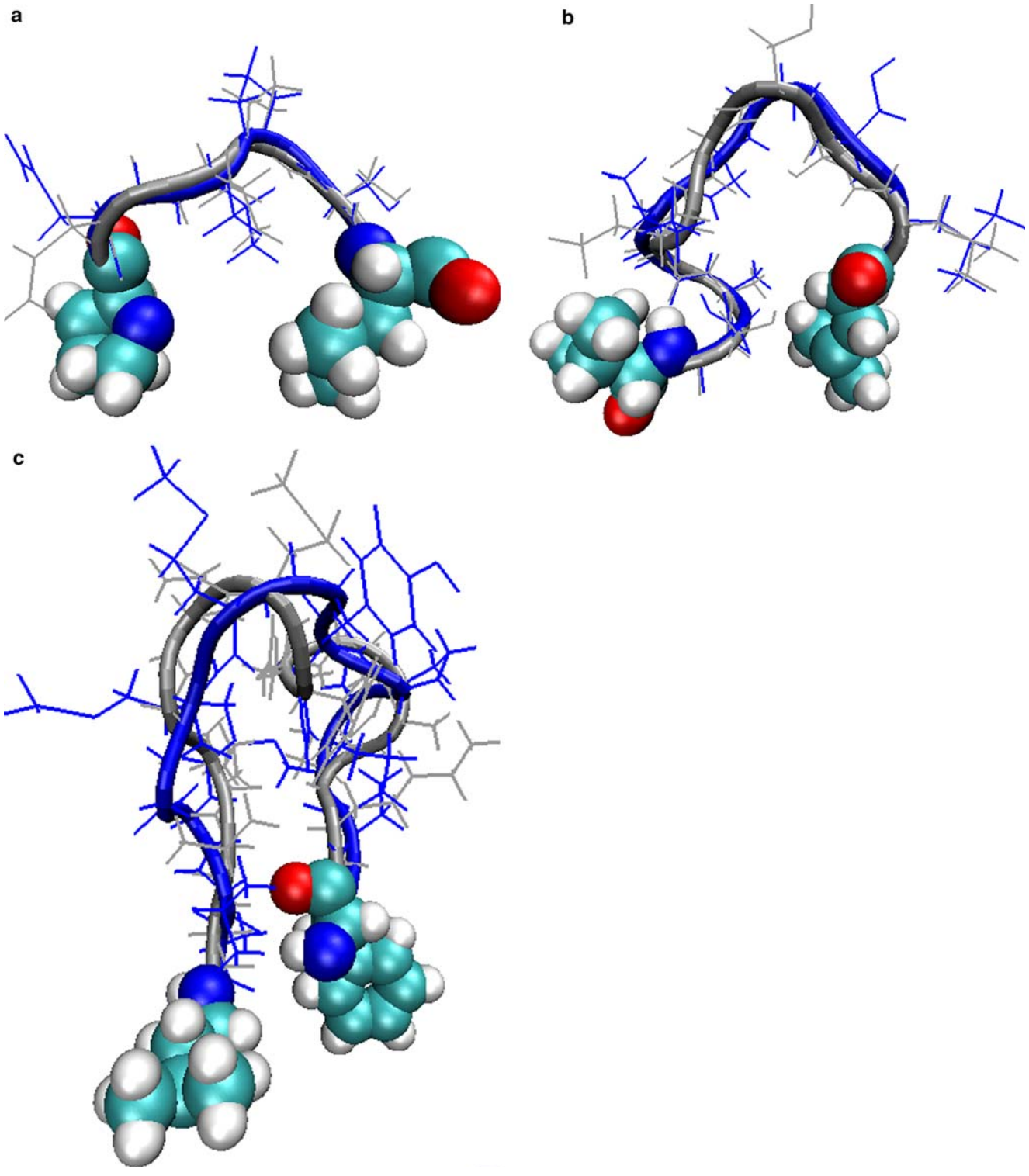

Fig. 2 Comparison of the best loop conformations (*blue*) in 1,000 trials generated by CLOOP with the X-ray structures (*gray*). **a** 4-residue loop 1gpr_123–126; **b** 8-residue loop 135l_84–91; **c** 12-residue loop 1pmy_77–88

conformations. The two methods are faster than CLOOP, but the sampling accuracy of CLOOP is better. It is noticeable that CLOOP builds the all-atom loop conformations, whereas all the methods mentioned above only generate loop main-chain structures, and an additional side-chain construction method in real loop modeling.

The sampling ability of CLOOP was also tested with an 18-residue loop 351c_50–67 and a 31-residue loop 1alc_61–91, but the method failed to give near-native

conformations. It is still a great challenge to model these long loops.

Conclusion

In this study, a new ab initio loop-construction method CLOOP based on energy minimization is proposed. We have demonstrated that the contribution of the protein to which a loop is attached facilitates the discrimination of near-optimal loop structures. The use of soft core potentials and a DM strategy focus the set of constructed loops to near-native loop conformations further. The construction process is an important step in loop prediction because it can offset the cost of evaluating obviously inadequate loop structures. CLOOP was used to construct the conformations of loops in Fiser's loop test set, and the average best main-chain RMSD is 0.33, 1.27, and 2.77 Å for 4-, 8-, and 12-residue loops, respectively. It can perform an all-atom loop construction in times comparable to current methods of main-chain atom only.

CLOOP was designed for real loop-modeling purposes. It can build any loop structure according to the given sequence on a scaffold protein. This method can be used to construct an all-atom loop conformations, and generate a good conformation ensemble of loops with size up to 12. The CLOOP program is available at http://morticia.cs.dal.ca/projects/CLOOP/.

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