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Molecular dynamics simulations with replica-averaged structural restraints generate structural ensembles according to the maximum entropy principle

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In order to characterise the dynamics of proteins, a well-established method is to incorporate experimental parameters as replica-averaged structural restraints into molecular dynamics simulations. Here, we justify this approach in the case of interproton distance information provided by nuclear Overhauser effects by showing that it generates ensembles of conformations according to the maximum entropy principle. These results indicate that the use of replica-averaged structural restraints in molecular dynamics simulations, given a force field and a set of experimental data, can provide an accurate approximation of the unknown Boltzmann distribution of a system. © *2013 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4793625]

I. INTRODUCTION

Proteins are molecules involved in essentially all the complex biochemical reactions that take place in living organisms.[1] As in order to perform their functions they undergo conformational fluctuations on timescales ranging from nanoseconds to milliseconds and beyond,[2–7] it is important to develop methods capable of characterizing these motions. In this context, nuclear magnetic resonance (NMR) spectroscopy is a powerful technique that enables the determination of the structures and dynamics of proteins at atomic resolution.[8–12] Since NMR measurements produce values of observables resulting from time and ensemble averages, their interpretation is facilitated by considering ensembles of structures.[13–23] The accurate determination of such ensembles is, however, very challenging. If the measurements are carried out for proteins in fully folded states, an average structure can normally be defined, so that the ensembles can be represented in terms of conformational fluctuations around such average structure. These averages structures, however, do not describe very accurately the dynamical properties of more flexible states, since in these cases the conformational fluctuations are of larger amplitude.[19–22] In these cases, the ensembles of structures are very heterogeneous and include conformations that can range from very compact to very extended,[19–22,24] making it difficult to apply standard methods of structure determination.

In principle, one could generate an ensemble of conformations consistent with the Boltzmann distribution of a protein by running molecular dynamics or Monte Carlo simulations under appropriate conditions.[25] In this approach, the experimental measurements would be automatically consistent with the generated ensemble, provided that one has accurate methods of calculating the experimental observables from the available structures. A complication of this strategy, however, is that existing force fields results in only approximate values of the Boltzmann weights,[26,27] and thus it is often difficult to obtain a close agreement between calculated and measured parameters. To overcome this problem, it has been suggested to use experimental measurements as averaged structural restraints in molecular dynamics simulations.[16,18,23,28] This idea was first implemented for the case distances derived from nuclear Overhauser effects (NOE) by applying a penalty if the time-average of an NMR observable calculated from a molecular dynamics trajectory differs from experiment.[14,15,29–34] In an alternative approach, penalizing forces are applied if the calculated average distances at a given time across an ensemble of simulated molecules (or replicas) do not match the experimental ones. Since the early implementations of this approach in the case of the replica-averaged NOE distance restraints,[13,15,16] a variety of restraining algorithms, including simultaneous time and ensemble averaging,[35] have been developed for an array of experimental observables measured for native, transition, intermediate, and unfolded states.[17,36–42]

One of the challenges in using replica-averaged structural restraints in molecular dynamics simulations, however, is that while the experimentally-determined average values are known, the underlying distribution (i.e., ensembles of structures) from which they come are not.[19,37] There is thus an ambiguity arising from the fact that infinitely many distributions may exist with the given set of average values.[43] It would thus seem that determining ensemble of conformations from experimental information about just average values is an ill-defined problem. By using the maximum entropy principle,[44,45] however, it is possible to chose a special distribution (i.e., an ensemble of structures) among all those that are consistent with the experimentally-determined average values by imposing the average values themselves as thermodynamic constraints.[46,47] This particular maximum entropy distribution provides an accurate representation of the unknown Boltzmann distribution of the system. The problem of determining structural ensembles can thus be solved unambiguously without making any additional assumption apart from the requirement that the experimental data should be consistent with it in the sense of the maximum entropy principle. To implement the maximum entropy principle

in a computationally efficient manner, as we demonstrate in this paper in the case of NOE data, it is possible to use experimental measurements as replica-averaged structural restraints in molecular dynamics simulations.

II. MODELLING NOES WITH THE MAXIMUM ENTROPY PRINCIPLE

The acquisition of a nuclear Overhauser effect spectroscopy (NOESY) spectrum of a protein makes it possible to determine for each visible proton pair *ij* the value of $\langle \frac{1}{r_{ij}^6} \rangle$, where r_{ij} is the interproton distance in a given conformation and the average is taken as a time and ensemble average over the equilibrium distribution of the system.[19,34,37,48]

The question that we address here is how to use this NOE-derived interproton distance information to determine ensembles of structures representing the thermal fluctuations of proteins. The task is thus to find a probability distribution, $P(x)$, compatible with the measured NOEs, where x indicates the Cartesian coordinates of the atoms comprising the protein. For each proton pair *ij* for which a measurement is available, this probability distribution should satisfy the relationships

$$\int dx \frac{1}{r_{ij}^6} P(x) = \left\langle \frac{1}{r_{ij}^6} \right\rangle = I_{ij}, \tag{1}$$

where I_{ij} is proportional to the experimentally-determined integrated intensity of the peak corresponding to the proton pair *ij*. In this equation, the experimentally-determined integrated intensities (peak volumes) are converted into distance restraints by using the isolated spin pair approximation, which relates the volume to the inverse sixth power of the distance between the two interacting spins. As mentioned above, there is an infinite number of distributions that are solutions of Eq. (1). However, since the measurements are performed under equilibrium conditions, we also know that the distribution $P(x)$ should correspond to the Boltzmann distribution of the system

$$Q(x) = \frac{1}{Z} e^{-\beta E}. \tag{2}$$

The condition $P(x) = Q(x)$, which can be derived from the maximum entropy principle (MEP) applied to the average energy,[49] defines the distribution $P(x)$ that satisfies Eq. (1) and is consistent with the underlying energy function of the system.

If we could perform molecular dynamics simulations with the true force field E, we would generate the distribution $P(x) = Q(x)$, and thus satisfy Eq. (1). However, in practical applications we have only available an approximate expression E_{MM} (a molecular mechanics force field) of E, which corresponds to a distribution

$$P_{MM}(x) = \frac{1}{Z_{MM}} e^{-\beta E_{MM}}. \tag{3}$$

Therefore, as $Q(x)$ is not known, we would like to find the smallest possible perturbation of $P_{MM}(x)$ to find a distribution that satisfy at least approximately Eq. (1). This requirement can be met by searching the distribution $\tilde{P}(x)$ that minimizes the Kullback-Leibler divergence,[50]

$$D_{KL}(\tilde{P} \| P_{MM}) = \int dx \, \tilde{P}(x) \log \frac{\tilde{P}(x)}{P_{MM}(x)}, \tag{4}$$

with the boundary conditions

$$\begin{cases} \int dx \, \tilde{P}(x) = 1, \\ \int dx \frac{1}{r_{ij}^6} \tilde{P}(x) = I_{ij}, \end{cases} \tag{5}$$

for each proton pair for which a measurement is available. Equations (3) and (4) can be solved using the method of the Lagrange multipliers

$$\frac{\partial}{\partial P} \left\{ D_{KL}(\tilde{P} \| P_{MM}) + \kappa \left(\int dx \tilde{P}(x) - 1 \right) + \sum_{ij} \lambda_{ij} \left(\int dx \frac{1}{r_{ij}^6} \tilde{P}(x) - I_{ij} \right) \right\} = 0, \tag{6}$$

which provides

$$\tilde{P}(x) = \frac{1}{Z_\lambda} e^{-\frac{1}{kT}(E_{MM} - \sum_{ij} \lambda_{ij} \frac{1}{r_{ij}^6})}, \tag{7}$$

where Z_λ is the normalization factor (with $\lambda = \{\lambda_{ij}\}$), and the constants κ and λ_{ij} are the Lagrange multipliers. The λ_{ij} can be computed from

$$\frac{\partial}{\partial \lambda_{ij}} \log Z_\lambda = I_{ij}. \tag{8}$$

The accurate determination of the structure and dynamics of proteins can therefore be viewed as a re-parameterization of the force field E_{MM} in which we search for parameters λ_{ij} that minimally perturb the background force field by correcting for inaccuracies, so that at the end the NOE information is consistent with the resulting maximum-entropy-principle ensemble.

III. MODELLING NOES USING MOLECULAR SIMULATIONS WITH REPLICA-AVERAGED RESTRAINTS

While feasible in principle, the previous approach has the disadvantage that in order to model the dynamics accurately the force field has to be reparameterized, a procedure that can be very time consuming. It would be therefore advantageous to have a method that does not require the reparametrisation of the force field. A simple possibility[18] is to take N-copies of the system for which we want to study the dynamics and to enforce the averages with δ-function restraints

$$\tilde{P}_N(x_1, \ldots, x_N) = \frac{1}{Z^N} e^{-\beta \sum_\alpha E_{MM}(x_\alpha)} \prod_{ij} \delta \left[\frac{1}{N} \sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij} \right) \right], \tag{9}$$

where the index α runs over the N replicas, x_α indicates the Cartesian coordinates of the atoms comprising replica α, and

$r_{\alpha,ij}$ is the r_{ij} distance for replica α. Molecular dynamics simulations carried out in this way generate a set of conformations that we call a ''replica-averaged'' ensemble. The question is whether the modification introduced in this way is minimal in the MEP sense, i.e., if the replica-averaged ensemble satisfies the MEP. In Sec. IV, we will show that in the limit of N going to infinity the two methods are equivalent.

IV. EQUIVALENCE OF THE MAXIMUM-ENTROPY-PRINCIPLE AND REPLICA-AVERAGED ENSEMBLES

In order to establish the equivalence between the MEP and replica-averaged ensembles, we start from the expression for the partition function Z_N of a replica-averaged ensemble

$$Z_N = \int e^{-\beta \sum_\alpha E_{MM}(x_\alpha)} \times \prod_{ij} \delta\left[\frac{1}{N}\sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij}\right)\right] dx_1 \dots dx_N. \quad (10)$$

This expression is equivalent to

$$Z_N = \int e^{-\beta \sum_\alpha [E_{MM}(x_\alpha) + \frac{1}{N}\sum_{ij}\gamma_{ij}(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij})]} \times \prod_{ij} \delta\left[\frac{1}{N}\sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij}\right)\right] dx_1 \dots dx_N, \quad (11)$$

which holds for any set $\gamma = \{\gamma_{ij}\}$, in particular for the value of $\gamma = N\lambda$ that would enforce the right average in the MEP case. Therefore,

$$Z_N = \frac{1}{Z_\lambda^N} \int e^{-\beta \sum_\alpha [E_{MM}(x_\alpha) + \sum_{ij}\lambda_{ij}(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij})]} \times \prod_{ij} \delta\left[\frac{1}{N}\sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij}\right)\right] dx_1 \dots dx_N Z_\lambda^N. \quad (12)$$

In the limit $N \to \infty$, we get

$$Z_N \to Z_\lambda^N, \quad (13)$$

because the term

$$\frac{1}{Z_\lambda^N} \int e^{-\beta \sum_\alpha [E_{MM}(x_\alpha) + \sum_{ij}\lambda_{ij}(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij})]} \times \prod_{ij} \delta\left[\frac{1}{N}\sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij}\right)\right] dx_1 \dots dx_N \quad (14)$$

is, by construction, equal to 1 in the limit $N \to \infty$. Since Z_λ is obtained from the MEP, Eq. (13) demonstrates that the two ensembles are equivalent since the replicas become decoupled in the $N \to \infty$ limit.

V. TAKING INTO ACCOUNT EXPERIMENTAL ERRORS

In the previous discussion, we implicitly assumed that the experimental averages are known without errors. However, using the equivalence of replica-averaged and MEP ensembles, we can provide a generalization to the case in which also the experimental errors are known,

$$\tilde{P}_{N\sigma}(x_1, \dots, x_N, \{I_{ij}\}) = \frac{1}{Z} e^{-\beta \sum_\alpha E_{MM}(x_\alpha)} \times \prod_{ij} \delta\left[\frac{1}{N}\sum_\alpha \left(\frac{1}{r_{\alpha,ij}{}^6} - J_{ij}\right)\right] e^{-\sigma_{ij}^{-2}(J_{ij} - I_{ij})^2}, \quad (15)$$

where I_{ij} and J_{ij} are proportional, respectively, to the measured and actual NOE intensities (see Eq. (1)), and the σ_{ij} are the experimental errors on the I_{ij}. Integrating over the (not observed) J_{ij}, we get

$$\tilde{P}_{N\sigma}(x_1, \dots, x_N, \{I_{ij}\}) = \frac{1}{Z} e^{-\beta \sum_\alpha [E_{MM}(x_\alpha) + \frac{1}{N}\sum_{ij}\lambda_{ij}(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij})^2]}. \quad (16)$$

From this equation, we obtain that taking into account the experimental errors of the experimental observables is

TABLE I. List of the 34 distances used as restraints between non-consecutive Cα atoms in the simulations (see Figure 1).

THR_1	ILE_3	5.96854
THR_1	TYR_11	5.49480
THR_1	GLN_12	4.97910
TRP_2	GLN_4	5.92948
TRP_2	TRP_10	5.82122
TRP_2	TYR_11	4.64691
ILE_3	LYS_9	5.95770
ILE_3	TRP_10	5.13310
GLN_4	GLY_6	5.50290
GLN_4	THR_8	5.90928
GLN_4	LYS_9	4.01273
GLN_4	TRP_10	5.86225
ASN_5	SER_7	5.96451
ASN_5	THR_8	4.98059
ASN_5	LYS_9	5.79457
GLY_6	THR_8	5.58211
SER_7	LYS_9	5.91313
LYS_9	THR_20	5.94459
TRP_10	GLN_12	5.86253
TRP_10	LYS_17	5.97016
TRP_10	ILE_18	5.34757
TRP_10	TYR_19	4.51873
TRP_10	THR_20	5.88665
TYR_11	ASN_13	5.83155
TYR_11	ILE_18	5.04964
GLN_12	GLY_14	5.61545
GLN_12	SER_15	5.96628
GLN_12	THR_16	5.28133
GLN_12	LYS_17	4.45604
GLN_12	ILE_18	5.89949
ASN_13	SER_15	5.64519
ASN_13	THR_16	4.96147
GLY_14	THR_16	5.83866
THR_16	ILE_18	5.84907

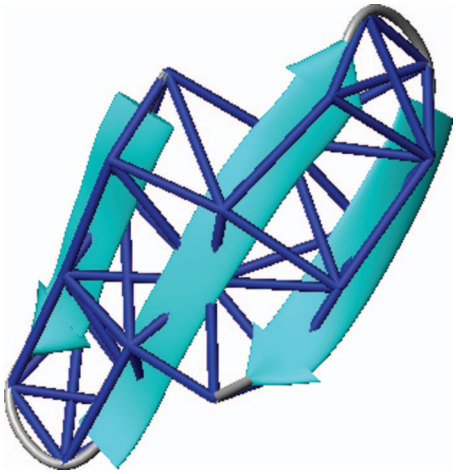

FIG. 1. Ribbon representation of native state structure of the GS peptide.[52,53] The NOE-like distances used in the calculations (Table I) are shown as blue bonds.

equivalent to performing simulations with the modified energy function

$$\tilde{E}_{N\sigma}(x_1, \ldots, x_N, \{I_{ij}\}) = \sum_{\alpha} \left[E_{MM}(x_\alpha) + \frac{1}{N} \sum_{ij} \frac{k_B T}{\sigma_{ij}^2} \left(\frac{1}{r_{\alpha,ij}{}^6} - I_{ij} \right)^2 \right]. \quad (17)$$

This expression provides a way to estimate the weight of the energy term with respect to the underlying force field.

VI. ILLUSTRATION OF THE REPLICA-AVERAGED APPROACH

The replica-averaged approach does not have free parameters except the number of replicas N. To be used in practice, it is therefore essential to define how many replicas are necessary to accurately describe the dynamics in the native enable. When N is too large, calculations are not practical. As we show here, however, N can be relatively small. In order to obtain this result, we study how the Shannon entropy[51]

$$S = \sum \pi_k log \pi_k, \quad (18)$$

where π_k indicates the probability of observing a given value for an observable, grows as a function of N. To determine this behaviour, we performed a set of molecular dynamics simulations using a model system, the 20-residue GS peptide,[52,53] and $N = 4$, 8, 16, 32, and 64. The GS peptide has been shown

TABLE II. Comparison of the entropy as a function of the number of replicas (see Figure 2) using only the full-length of the simulations and only the second half.

Nrep	Full-length	Half-length	Error %
4	0.121714	0.121963	0.0020
8	0.373462	0.373073	0.0010
16	0.540298	0.540746	0.0008
32	0.571818	0.571661	0.0003
64	0.576687	0.576863	0.0003

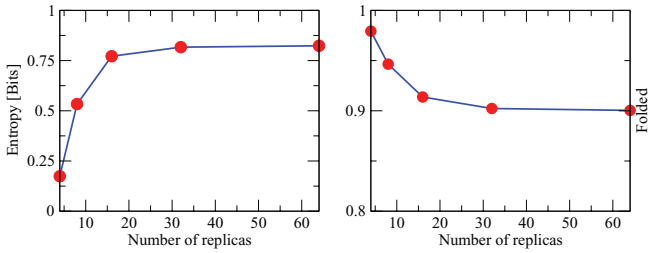

FIG. 2. Shannon entropy of the Cα-RMSD distribution and percentage of folded structures (RMSD < 2 Å) as a function of the number of replicas.

to reversibly fold into a triple-stranded antiparallel β-sheet conformation[52,53] and being relatively small, it represents an ideal system to perform simulations long enough to reach convergence.

For the GS peptide, we generated a set of 34 NOE-like distances (Table I) by considering the distances between all non-consecutive Cα atoms closer than 6 Å in the native state (Figure 1). Then, five molecular dynamics simulations were performed using $N = 4$, 8, 16, 32, and 64 replicas. All simulations were carried out using the replica-averaged distance restraints implemented in the molecular simulation package ALMOST.[52,53] The ALMOST software and scripts are available for download from the website (http://www.open-almost.org), or upon request from the authors. Simulations were run using the CHARMM 19 force field with neutralized ionic side-chains [1] in conjunction with an implicit solvation model based on the solvent accessible surface (SASA) [2]. The SHAKE algorithm [3] was used to fix the length of bonds of the covalent bonds having hydrogen atoms at one end. The leapfrog algorithm and an integration time step of 2 fs were used to integrate the Newton's equations of motion. All simulations were started from the native state and the temperature was kept close to 330 K by weak coupling to an external bath with a coupling constant of 5 ps [4]. The simulations were started from the native conformation and each of the five systems was simulated for 4 μs. At convergence (see Table II), we computed the Shannon entropy of the distribution of the root mean square distances (RMSDs) between Cα atoms from the native state. We found that the entropy converges for about 16 replicas (Figure 2), thus suggesting that the replica-averaged approach accurately describes the native state dynamics with a relatively small number of replicas, which can be simulated using standard computational resources. In our calculations, we found that the percentage of folded structures (Figure 2) is consistent with that found in previous studies[52,53] thus indicating that the replica-averaged restraints approach reproduces the correct behaviour of the system.

VII. CONCLUSIONS

In this work, we have illustrated in the case of NOE measurements that the incorporation of NMR information as replica-averaged structural restraints in molecular dynamics simulations generates a sampling of the conformational space of proteins consistent with the maximum entropy principle. The equivalence between the maximum entropy principle and the replica-averaging methods enables also the

estimation of the weight of the restraint term with respect to the underlying force field used in the molecular dynamics simulations. Therefore, the incorporation of experimental data as replica-averaged structural restrains in molecular dynamics simulations provides an accurate representation of the unknown Boltzmann distribution given an approximate force field and a set of experimental data.

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