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PROTHON: A Local Order Parameter-Based Method for Efficient Comparison of Protein Ensembles

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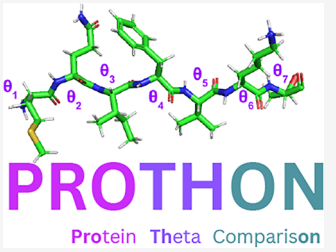
ABSTRACT: The comparison of protein conformational ensembles is of central importance in structural biology. However, there are few computational methods for ensemble comparison, and those that are readily available, such as ENCORE, utilize methods that are sufficiently computationally expensive to be prohibitive for large ensembles. Here, a new method is presented for efficient representation and comparison of protein conformational ensembles. The method is based on the representation of a protein ensemble as a vector of probability distribution functions (pdfs), with each pdf representing the distribution of a local structural property such as the number of contacts between C_β atoms. Dissimilarity between two conformational ensembles is quantified by the Jensen−Shannon distance between the corresponding set of probability distribution functions. The method is validated for conformational ensembles generated by molecular dynamics simulations of ubiquitin, as well as experimentally derived conformational ensembles of a 130 amino acid truncated form of human tau protein. In the ubiquitin ensemble data set, the method was up to 88 times faster than the existing ENCORE software, while simultaneously utilizing 48 times fewer computing cores. We make the method available as a Python package, called PROTHON, and provide a GitHub page with the Python source code at https://github.com/PlotkinLab/Prothon.



INTRODUCTION

One of the fundamental principles that has influenced protein science is the structure−function paradigm: The idea that, for proteins with a reliable and stable three-dimensional (3D) structure, the fold invariably determines the protein's functions.[1−3] Protein structures are central to molecular biology and are used for example in synthetic biology[4−7] and in drug design.[6,8,9]

Comparing protein structures is a common and useful practice in structural biology for the understanding of functional and evolutionary relationships.[1−3,10−12] Consequently, several quantitative measures for comparing protein structures have been developed; the most commonly used dissimilarity measure is the distance-based global root-mean-square deviation (RMSD) of atomic positions. This has, however, been shown to be one of the least representative of the degree of structural dissimilarity compared to contact-based methods, such as C_β−C_β pairwise distances, that at least in some cases are more robust and relevant.[13] Other limitations, such as the dependence of RMSD on the accuracy of the superposition of protein structures, and its sensitivity to protein length and particularly the presence of flexible regions, have motivated more accurate measures used in Critical Assessment of techniques for protein Structure Prediction (CASP) competitions. Here metrics such as the global distance test total score (GDT-TS) and template modeling score (TM-score) are commonly used to quantify the similarity between computationally predicted structures and experimentally determined structures.[13−16] In estimating the kinetic proximity of partially disordered protein structures to the native state, generalizations of geometrical Euclidean distance have been shown to be the most accurate metric of proximity between two structures.[17,18]

Proteins are dynamic systems and explore a vast conformational space. Thus, comparisons between individual structures often need to be generalized to comparisons between ensembles of structures to accurately characterize macrostates of proteins. Unfolded states, partially unfolded states, or intrinsically disordered proteins (IDPs) require an ensemble description to properly characterize them. IDPs do not have a well-defined native structure but exist as an equilibrium ensemble of diverse conformations, which interconvert rapidly.[19−24] Comparisons between ensembles of structures have thus been developed to treat these systems.

Unlike protein structure comparison, for which many metrics are available, efficient methods and software for comparing protein structural ensembles are much less common. The first attempt to quantitatively measure the dissimilarity between structural ensembles was an extension of the global RMSD.[25] A different approach was proposed by Lindorff-Larsen and Ferkinghoff-Borg,[26] which involves the estimation of the underlying probability distributions of

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protein conformational ensembles and the quantification of the overlap between these probability distributions using a symmetrized form of the Kullback−Leibler divergence.[26−28] Three methods were proposed for estimating the underlying probability densities, including the quasi-harmonic approximation, conformational clustering, and dimensionality reduction;[26] all three methods had serious limitations pointed out by the authors. While the quasi-harmonic approximation method is relatively fast, it is only effective for ensembles that can be described by a multivariate normal distribution. The other two methods require the calculation of all pairwise global RMSD values, a calculation scaling as $\frac{1}{2}(M_1 + M_2)(M_1 + M_2 + 1)$, where M_1 and M_2 are the two ensemble sizes to be compared, which can be prohibitively expensive for large ensembles, due to the optimization of the structural superposition of structures that is required. For example, using the current computing power on the Digital Research Alliance of Canada Cedar computing cluster (https://alliancecan.ca), to compare two ensembles of a peptide of 76 amino acids, each of 15000 structures, takes 58 h (2 days and 10 h) on a single core, and 7 h on 48 cores running in parallel.

On the other hand, large ensembles are now routinely generated from molecular dynamics (MD) simulations, and it is often useful to compare protein ensembles generated from MD simulations using different force fields, simulation parameters, solvent conditions, or amino acid sequences. For these practical situations involving large conformational ensembles, there is a need for an effective quantitative measure and software for comparing structural ensembles, which does not require the computationally expensive process of structural superposition.

To address the problem of the high computational cost required for optimal structural alignment, as well as the potential issues of accuracy mentioned above that are associated with the use of the global RMSD of atomic positions as a structural dissimilarity measure, contact-based measures and measures utilizing internal coordinates, for example, Ramachandran torsion angles, for structural comparison have previously been proposed.[13,29] More recently, a measure for comparing IDP ensembles based on C_α−C_α distance matrices was described in ref 30. Although the method described in ref 30 is superposition-independent, therefore requiring relatively lower computational cost, it only considers distance distribution averages rather than the full distributions themselves.

In the present work, we introduce and implement a new generalized method for the efficient representation and comparison of protein conformational ensembles. The method involves the utilization of the Jensen−Shannon Distance (JSD) metric for the quantification of the difference between probability distribution functions representing the distributions of a local structural property θ of polypeptide chains that constitute the protein ensemble under consideration. A related approach for comparing conformational ensembles was previously introduced by McClendon, Hua, Barreiro, and Jacobson, in which the Kullback−Leibler Divergence was used to quantify the differences between the distributions of the Ramachandran torsion angles.[31] In principle, any local structural property can be used in our method. Local structural properties including per residue solvent accessible surface area (SASA),[32,33] virtual C_α−C_α bond angle (CABA) and torsion angle (CATA),[34] C_α contact number (CACN), and C_β contact number (CBCN)[35,36] have been implemented in other contexts and could be applied to the method introduced here. In this manuscript, we use CBCN. The method of protein ensemble comparison described here was implemented using the Python programming language, and is made available as a Python package, called PROTHON (PROtein θ comparisON), for easy access to the computational structural biology scientific community. PROTHON provides an easy-to-use protein ensemble comparison program and a simple code framework for extension to include other local structural properties. We provide a GitHub page with the Python source code at https://github.com/PlotkinLab/Prothon.

DESCRIPTION

In this section, we describe the algorithm for the efficient representation and comparison of protein conformational ensembles.

Matrix Representation of a Protein Ensemble. Consider a protein ensemble with M conformations and N local structural property values (e.g., CBCN). Typically N is proportional to the number of amino acids in the chain. Let θ be a local structural property, such as the per-residue C_β contact number (CBCN)[35,36] (see section on C_β Contact Number Local Structural Property). In a given conformation m of the polypeptide chain, there are N θ values, and for a given local index such as a given residue, there are M θ values. Thus, θ is a function of (m, n), the conformation index and the residue index. As parametrized by the local property θ, the full ensemble of conformations can be represented by an $M \times N$ matrix, $\mathbb{X}$, with elements $X_{m,n}$ given by eq 1:

$$\mathbb{X} = \begin{pmatrix} \theta_{1,1} & \theta_{1,2} & \theta_{1,3} & \cdots & \theta_{1,n} & \cdots & \theta_{1,N} \\ \theta_{2,1} & \theta_{2,2} & \theta_{2,3} & \cdots & \theta_{2,n} & \cdots & \theta_{2,N} \\ \theta_{3,1} & \theta_{3,2} & \theta_{3,3} & \cdots & \theta_{3,n} & \cdots & \theta_{3,N} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ \theta_{m,1} & \theta_{m,2} & \theta_{m,3} & \cdots & \theta_{m,n} & \cdots & \theta_{m,N} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ \theta_{M,1} & \theta_{M,2} & \theta_{M,3} & \cdots & \theta_{M,n} & \cdots & \theta_{M,N} \end{pmatrix} \quad (1)$$

Each row of matrix $\mathbb{X}$ can be thought of as a vector representing the θ values in a conformation, e.g., $\boldsymbol{C}_m = |\theta_{m,1}, \theta_{m,2}, \theta_{m,3}, \cdots, \theta_{m,n}, \cdots, \theta_{m,N}\rangle$, and each column is an ensemble $\theta_n = (\theta_{1,n}, \theta_{2,n}, \theta_{3,n}, \cdots, \theta_{m,n}, \cdots, \theta_{M,n})$ of local values of θ across all conformations in the protein ensemble. A typical size of $\mathbb{X}$ might be a $(M, N) = 10{,}000 \times 100$ dimensional matrix.

Protein Ensemble Represented as a Vector of Local Ensembles. The above matrix representing a protein ensemble, as described above in Matrix Representation of a Protein Ensemble, can alternatively be written as a vector of local ensembles θ_n (see eq 2).

$$\boldsymbol{X} = |\{\theta_1\}, \{\theta_2\}, \{\theta_3\}, \cdots\{\theta_n\}\cdots\{\theta_N\}\rangle \equiv |\theta_1, \theta_2, \theta_3, \cdots, \theta_n, \cdots, \theta_N\rangle \quad (2)$$

Each *ensemble* θ_n can further be represented by a probability distribution function (pdf); e.g., the ensemble of θ_1 can be represented as $p(\theta_1) \equiv p_1$. The conformational ensemble of the whole protein may then be written as

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J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

$$\boldsymbol{X}_p = |p_1, p_2, p_3, \cdots, p_n, \cdots, p_N\rangle \tag{3}$$

In the present work, we represent the pdfs in eq 3 by one-dimensional (1D) distribution functions. The probability distribution function p_n representing each θ_n is determined by Gaussian kernel-density estimation with the Silverman bandwidth estimator method,[37] which is implemented here using SciPy.[38] A conventional histogram with suitable choice of binning may be used as well; however, we choose Gaussian kernel-density estimation here because of its advantages of a smoother estimate for the density distribution, which is convenient for ensemble comparison. To implement the ensemble comparison analysis below, we compare two (or more) distributions p_n^X and p_n^Y for the same θ_n as follows. The minimum and maximum sampled values of θ_n are first determined; then the values of p_n^X and p_n^Y at $\nu = 100$ equally spaced points between $\theta_{\min}$ and $\theta_{\max}$ are obtained. Each distribution is represented by $\nu = 100$ values:

$$\begin{aligned} p_n^X &= \{p_{1,n}^X, p_{2,n}^X, p_{3,n}^X, \cdots, p_{\nu,n}^X\} \\ p_n^Y &= \{p_{1,n}^Y, p_{2,n}^Y, p_{3,n}^Y, \cdots, p_{\nu,n}^Y\} \end{aligned} \tag{4}$$

The value ν may be increased beyond 100 if necessary. The choice of $\theta_{\min}$ and $\theta_{\max}$ typically results in one or more of the distributions having values of zero near one or both of the limits of θ.

Quantifying Similarity between Protein Ensembles. Here, we propose an efficient and effective dissimilarity measure for protein conformational ensembles, by comparing pdfs representing a local property distribution using the Jensen−Shannon Distance (JSD) metric. The JSD is an information theory-based measure for comparing two probability distributions. A related nonmetric version of the JSD, the Jensen−Shannon Divergence, has been used to quantify the dissimilarity between protein ensembles in a previous study.[28] Employing this procedure in ref 28 can require high computational cost, however, and may have limited practical applicability to large ensembles.

The ensemble representation as described in Protein Ensemble Represented as a Vector of Local Ensembles above allows for the calculation of both local and global similarity between two protein ensembles. Given two protein ensembles $\boldsymbol{X} = |p_1^X, p_2^X, p_3^X, \cdots, p_n^X, \cdots, p_N^X\rangle$ and $\boldsymbol{Y} = |p_1^Y, p_2^Y, p_3^Y, \cdots, p_n^Y, \cdots, p_N^Y\rangle$, which are represented as vectors of pdfs (p_n^X and p_n^Y) of a local structural property, the dissimilarity between ensembles **X** and **Y** is estimated as the N-dimensional vector, termed here as the dissimilarity vector, given by

$$\boldsymbol{D} = |\mathcal{D}_1, \mathcal{D}_2, \mathcal{D}_3, \cdots, \mathcal{D}_n, \cdots, \mathcal{D}_N\rangle \tag{5}$$

where each component of **D**, $\mathcal{D}_n = \mathcal{D}(p_n^X, p_n^Y)$, is the JSD between the probability distributions representing the corresponding local structural property values θ_n in the polypeptide chain in the ensembles **X** and **Y**. The JSD is defined as

$$\mathcal{D}_n(p_n^X, p_n^Y) = \sqrt{\frac{1}{2}\left[D_{\mathrm{KL}}(p_n^X, p_n) + D_{\mathrm{KL}}(p_n^Y, p_n)\right]}^{1/2} \tag{6}$$

where

$$p_n = (p_n^X + p_n^Y)/2 \tag{7}$$

and, e.g.,

$$D_{\mathrm{KL}}(p_n^X, p_n) = \int \mathrm{d}\theta\, p_n^X \log_2\left(\frac{p_n^X}{p_n}\right) \equiv \sum_{i=1}^{\nu} p_{i,n}^X \log_2\left(\frac{p_{i,n}^X}{p_{i,n}}\right) \tag{8}$$

is the Kullback−Leibler divergence between probability distributions p_n^X and p_n.[27] $\mathcal{D}_n$ has a lower and upper bound of 0 and 1, respectively. A value of 0 indicates that two ensembles are identical while a value of 1 suggests that they are completely different.

The global dissimilarity D between ensembles **X** and **Y** is calculated as the average over the N local dissimilarities.

$$D = \frac{1}{N}\sum_{n=1}^{N} \mathcal{D}_n \tag{9}$$

Motif dissimilarity between ensembles can also be determined in a similar fashion by averaging over local structural property values belonging to a particular motif. The dissimilarity vector in eq 5 allows ensemble comparisons for proteins with both structured and disordered domains.

C_β Contact Number Local Structural Property. The C_β contact number (CBCN) implemented in PROTHON is defined by the number of C_β atoms on other residues separated by 3 or more amino acids from a given residue that are located within a sphere of radius r_o centered on the C_β atom of the residue of interest. Here we implement this criterion with a smooth cutoff function:

$$\mathrm{CBCN}(i) = \sum_{\substack{j=1 \\ |j-i|>2}}^{N} \frac{1}{1 + \exp[\beta(r_{ij} - r_o)]} \tag{10}$$

where the sum runs over the N C_β atoms j that may be in contact with the C_β atom i such that $|j - i| > 2$, and r_{ij} is the distance between C_β atoms i and j, belonging to residues a and b with a sequence separation $|a - b| > 2$. The parameters β and r_o were taken to be 50 nm^{-1} and 1 nm, respectively. A similar method for calculating contact number was previously used by Kinjo, Horimoto, and Nishikawa[35] and Yuan.[36]

Measuring Significant Dissimilarity. To compare two protein ensembles $\mathbb{X}$ and $\mathbb{Y}$ with M_x and M_y number of conformations, respectively, we consider only local dissimilarities (i.e., $\mathcal{D}_n$) between the ensembles that are statistically significant relative to dissimilarities within each ensemble. $\mathcal{S}$ samples of each ensemble are generated by randomly sampling (with replacement) 1000 conformations from ensembles $\mathbb{X}$ and $\mathbb{Y}$, respectively. The local dissimilarities $\mathcal{D}_n$ are then calculated between the $\mathcal{S}$ samples within the same ensemble and across different ensembles, which results in $\mathcal{S}^2$ interensemble sample dissimilarities and $2 \times \binom{\mathcal{S}}{2}$ intra-ensemble sample dissimilarities. In this present work, we take $\mathcal{S} = 5$ samples, so that there are 25 interensemble sample dissimilarities and 20 intraensemble sample dissimilarities (10 for $\mathbb{X}$ and $\mathbb{Y}$, respectively). The statistical significance of local dissimilarities $\mathcal{D}_n$ are calculated using the Mann−Whitney U test,[38−40] which is a nonparametric test of the null hypothesis: the probability of x being greater than y is equal to the probability of y being greater than x for randomly selected values x and y from two populations. Only dissimilarities $\mathcal{D}_n$ with p-values <0.05 are taken to be statistically significant.

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VALIDATION

The validity and computational efficiency of the introduced method for the comparison of protein ensembles implemented in PROTHON is demonstrated in the sections below with computationally generated, experimentally derived, structured, and intrinsically disordered protein ensembles.

Comparing Computationally Generated Ensembles of Ubiquitin. Ubiquitin is a regulatory protein that exists in all eukaryotic cells. It performs various functions through conjugation to many different target proteins.[41] Ubiquitin consists of 76 amino acids and has a molecular mass of about 8.6 kDa.

To generate conformational ensembles for the natively structured protein ubiquitin, we used the 3D coordinates of its atoms downloaded from the protein data bank (PDB ID: 1UBQ[42]) as the initial state to perform molecular dynamics (MD) simulations with the CHARMM36m force field[43] using GROMACS (version 2019.2).[44] All simulations were performed in explicit solvent (TIP3P water model, and 150 mM NaCl) at 300 K and 1 bar with periodic boundary conditions. Five independent MD runs were performed for the folded state, and five independent MD runs were performed for five partially folded states of ubiquitin. Each MD run was 50 ns. The partially folded states were obtained by performing global unfolding MD simulations, using PLUMED,[45] along a collective coordinate Q defining the fraction of native contacts:

$$Q(C) = \frac{1}{K}\sum_{(i,j)} \frac{1}{1 + \exp[\beta(r_{ij}(C) - \lambda r_{ij}^{0})]} \tag{11}$$

In eq 11, the sum runs over K pairs of native contacts (i, j), where K is determined from all atom pairs within 0.45 nm in the native structure. $r_{ij}(C)$ is the distance between atoms i and j in conformation C, belonging to residues a and b with a sequence separation $|a - b| > 3$, and r_{ij}^{0} is the native distance between atoms i and j. The parameters β and λ were taken to be 50 nm^{-1} and 1.5, respectively. A similar method for defining fraction of native contacts has been used in several previous studies; see, e.g., refs 46 and 47.

A time-dependent harmonic potential $V(Q, t)$

$$V(Q, t) = \frac{1}{2}k(Q - Q_0(t))^2 \tag{12}$$

was applied to move the center of the bias linearly from $Q_0 = 1$ to $Q_0 = 0.95, 0.90, 0.85, 0.80, 0.75$, respectively, for 5 partially folded states. The global unfolding bias was applied for 10 ns and then held fixed for 40 ns. The spring constant k in eq 12 was taken to be 10^7 $kJ{\cdot}mol^{-1}$.

Conformations were sampled at equal time intervals of 40 ps from the last 40 ns of five independent simulations for each of the above values of Q_0, giving a total of $\frac{40000}{40} \times 5 = 5000$ conformations in each of the folded and partially folded ensembles. Figure 1 shows randomly selected structures from the folded ensemble and for five partially folded ensembles that correspond to the five Q_0 values.

The degree of nativeness (or foldedness) of ubiquitin was taken as the fraction of native contacts Q. To visualize these ensembles, we represented them as CBCN $M \times N$ matrices (see Matrix Representation of a Protein Ensemble) and as pairwise RMSD $M \times M$ matrices (as used in ENCORE[28]), where $M = 30000$ is the total number of structures and $N = 70$ is the number of C_β atoms in each structure. The high

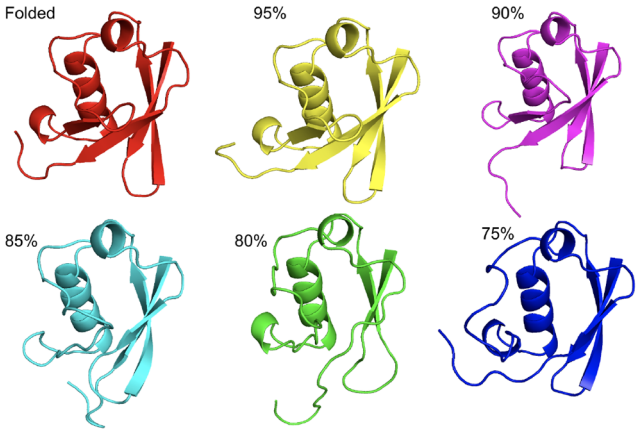

Figure 1. Representative structures from ubiquitin ensembles. The folded (red) and partially folded [95% folded (yellow), 90% folded (magenta), 85% folded (cyan), 80% folded (green), and 75% folded (blue)] ubiquitin ensembles. All images were rendered with PyMOL molecular visualization system (https://pymol.org).

dimensional CBCN and RMSD matrices were then reduced to two dimensions (2D) using principal component analysis (PCA[48]), multidimensional scaling (MDS[49]), and t-distributed stochastic neighbor embedding (t-SNE[50]) dimensionality reduction methods (see Figure 2). PCA, MDS, and t-SNE were implemented using Scikit-learn.[51]

In Figure 2, the ensembles are color coded as folded (red) and partially folded (yellow, 95% folded; magenta, 90% folded; cyan, 85% folded; green, 80% folded; blue, 75% folded). At this 2D level of resolution, where the PCA explained variance is ≈40%, the ensembles are more readily distinguishable for the CBCN matrix representation (Figure 2 panels A, B, and C) than for the pairwise RMSD matrix representation (Figure 2 panels D, E, and F). As expected for these ubiquitin ensembles, the proximity in the projected space of structures in the partially folded ensembles to structures in the natively folded ensemble decreases with decreasing degree of foldedness (e.g., yellow data points are closer to the red than blues are to the red), and the degree of spread increases with decreasing degree of foldedness (i.e., there is more spatial variation in the projected spaces for blue than there is for yellow) (see Figure 2 top panels A−C). These observed relationships between the ensembles using the CBCN matrix are visually absent when using the RMSD matrix representation (see Figure 2 bottom panels D−F).

The distinct clusters of the ensemble with 75% natively folded ensemble (e.g., blue in Figure 2A) correspond to different independent simulations (see Figure S1). To enhance clarity and interpretation of the data, each ensemble in Figure 2 is plotted separately (nonoverlapping) in Figures S2−S7.

Figure 3A shows the dissimilarity of partially folded ensembles of ubiquitin from the natively folded ensemble at different degrees of partial nativeness, $75\% < Q < 95\%$, quantified using the C_β contact number (CBCN) local structural property as implemented in PROTHON, and the global RMSD structural property as implemented in ENCORE, both using the PCA dimensionality reduction method.[28] Both the CBCN local structural properties and the global RMSD structural property are able to distinguish the partially folded ensembles from the folded ensemble and

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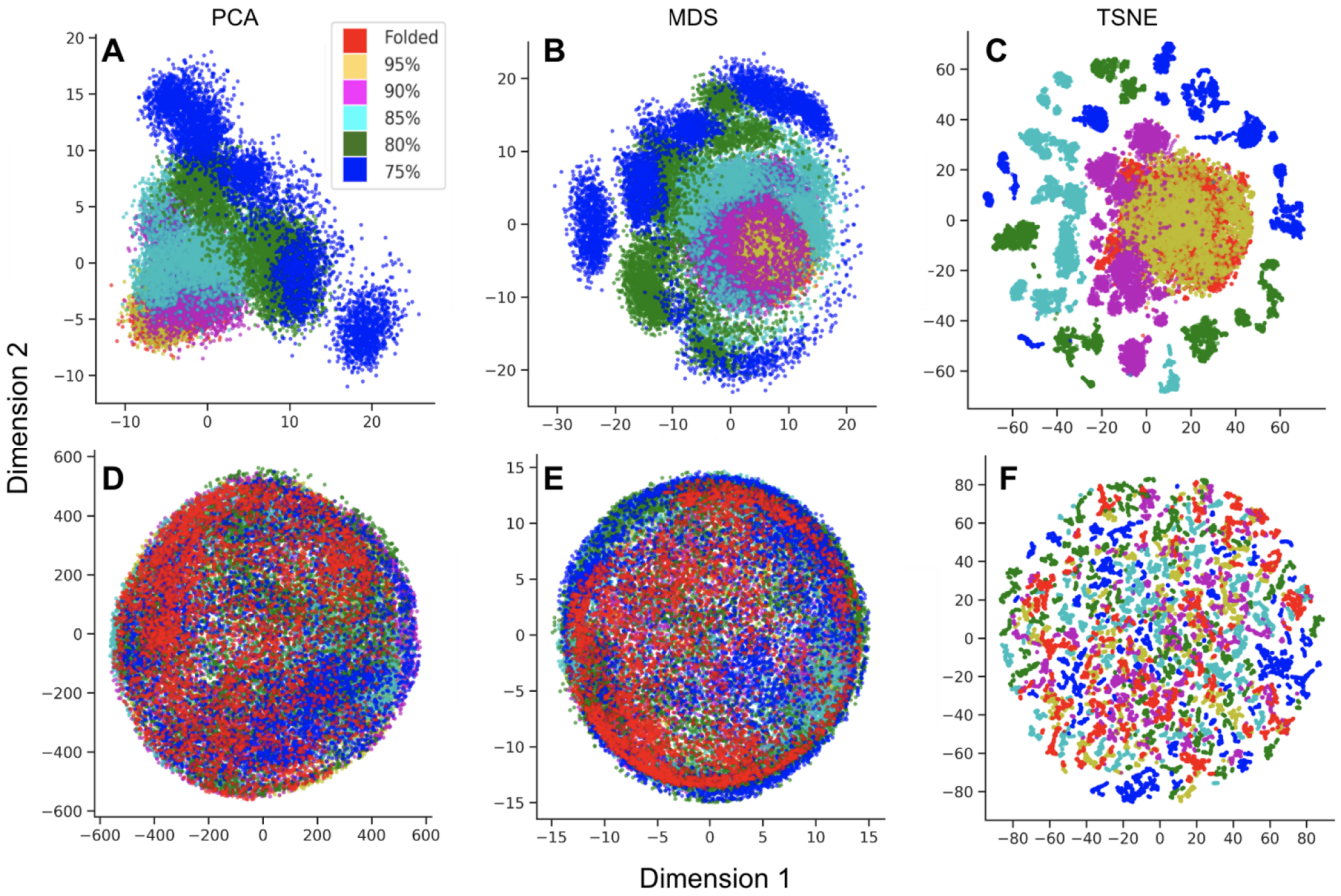

Figure 2. Representation and dimensionality reduction of ubiquitin ensembles. The folded (red) and partially folded (yellow, 95% folded; magenta, 90% folded; cyan, 85% folded; green, 80% folded; blue, 75% folded) ubiquitin ensembles are represented using the PROTHON C_β contact number (CBCN) matrix (top panels A, B, C) and ENCORE RMSD matrix (bottom panels D, E, F). Dimensionality reduction to 2D using (left) principal component analysis (PCA) for (A) CBCN and (D) RMSD matrix representations, (middle) multidimensional scaling (MDS) for (B) CBCN and (E) RMSD matrix representations, and (right) t-distributed stochastic neighbor embedding (t-SNE) for (C) CBCN and (F) RMSD matrix representations.

correctly determine the order of nativeness without prior knowledge of how the ensembles were obtained (Figure 3A).

We also examine four additional local structural properties to demonstrate the generality of the PROTHON approach for ensemble comparison: the C_α contact number (CACN), virtual C_α−C_α bond angle (CABA) and torsion angle (CATA),[34] and per-residue solvent accessible surface area (SASA).[32,33] Figure S8 plots the dissimilarity vs the percentage of native contacts, indicating that all local structural properties accurately distinguish between the partially folded ensemble and the folded ensemble of ubiquitin. The order parameter we focus on here, CBCN, has among the largest relative differences between the ensembles and does not exhibit the same degree of nonlinear "plateauing" as some of the other order parameters do.

Figure 3 panels B and C show a heatmap grayscale-coded matrix of pairwise dissimilarity between the 5 partially folded ensembles of ubiquitin, as quantified using CBCN and RMSD, respectively. As expected, the dissimilarity increases (becomes lighter in the matrices) as one moves away from the diagonal in all 4 directions (up, down, left, and right). The dissimilarity is zero when comparing an ensemble with itself (black diagonal). The dissimilarity increases as one moves away from the diagonal when using RMSD as a dissimilarity measure, but an exception is noticeable: The ensemble with 75% native contacts is more dissimilar to the ensemble with 90% native contacts than it is to the ensemble with 95% native contacts; this nonmonotonicity is unexpected. These results show that the methods of protein ensemble representation and comparison as implemented in PROTHON may be more effective than those using RMSD.

Comparing Experimentally Derived Ensembles of the Tau K18 Domain. Tau is an intrinsically disordered protein (IDP), whose misfolding and aggregation are implicated in many neurodegenerative diseases including Alzheimer's disease (AD), Pick's disease (PiD), chronic traumatic encephalopathy (CTE), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP).[52−54] Tau K18 domain (tauK18) is a 130-residue truncated human tau protein consisting of the four microtuble binding repeats of tau. Five ensembles of tauK18 were download from the Protein Ensemble Database (PED ID: PED00017).[55] These ensembles represent different models of tauK18 that were derived by first sampling random coil conformations and then selecting those conformations that fit experimentally determined chemical shifts and residual dipolar couplings in NMR data.[56]

Table 1 shows the pairwise conformational dissimilarity of the 5 tauK18 ensembles. Conformational dissimilarity here was quantified using the C_β contact number (CBCN) local structural property implemented in PROTHON. The dissim-

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J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

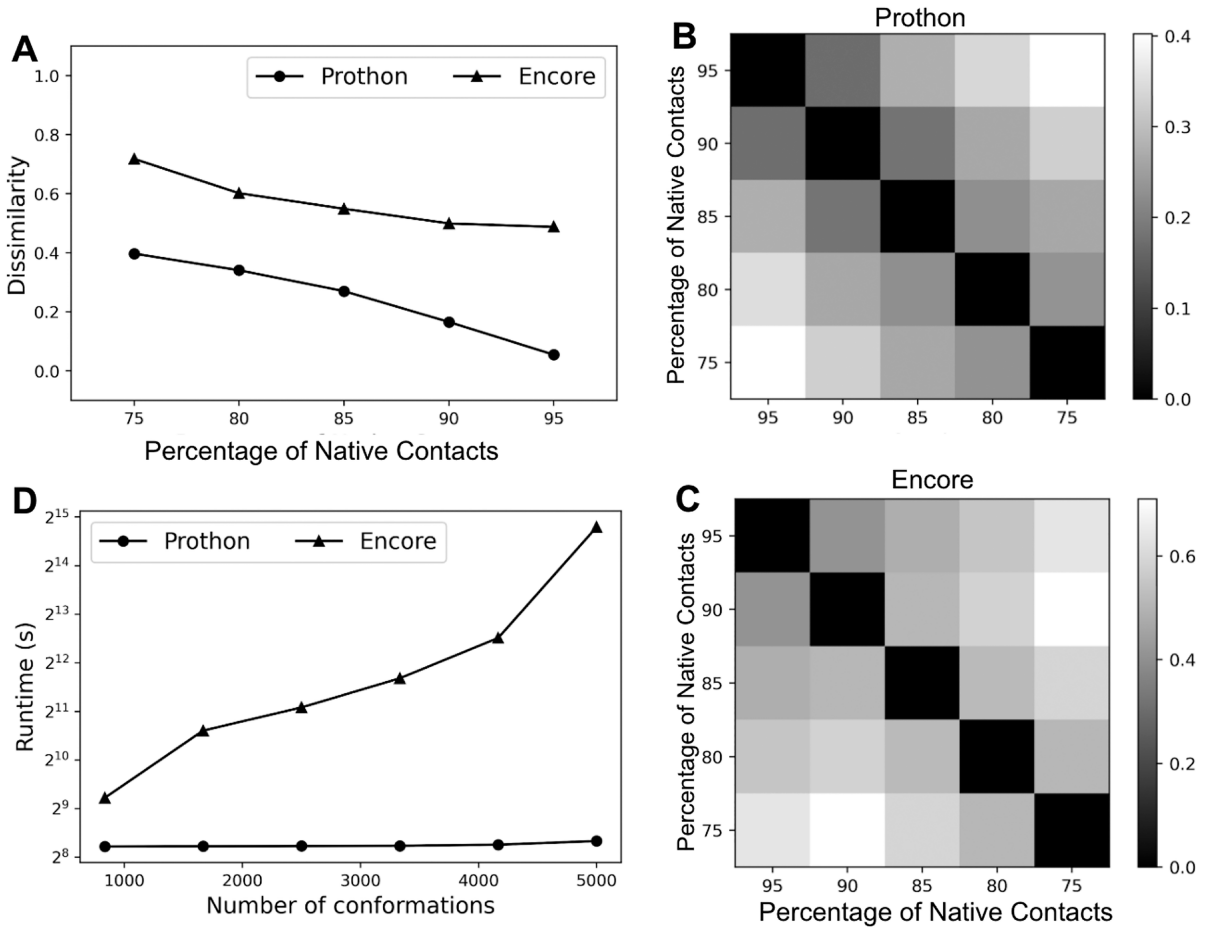

Figure 3. Comparing partially folded ensembles of ubiquitin. (A) Dissimilarity between partially folded ensembles (75% < Q < 95%) and the folded equilibrium ensemble of ubiquitin. Dissimilarity is quantified using the local structural property of the C_β contact number (circles) implemented in PROTHON, and RMSD similarity is implemented in ENCORE (triangles). (B) Pairwise dissimilarity between the 5 partially folded ensembles of ubiquitin in panel (A) using PROTHON. (C) Pairwise dissimilarity between the 5 partially folded ensembles of ubiquitin in panel (A) using ENCORE.[28] (D) Wall-clock time (computational efficiency) of PROTHON compared with ENCORE. ENCORE calculations were run on 48 cores; PROTHON calculations were run on a single core.

Table 1. Comparing Intrinsically Disordered Ensembles of Tau K18[a]

Dissimilarity	Ensemble 2	Ensemble 3	Ensemble 4	Ensemble 5
Ensemble 1	0.029	0.028	0.031	0.028
Ensemble 2	-	0.026	0.029	0.028
Ensemble 3	-	-	0.026	0.027
Ensemble 4	-	-	-	0.029

[a]The pairwise dissimilarity between 5 ensembles of tau K18 domain quantified using the C_β contact number local structural property implemented in PROTHON.

ilarity values are all small (average dissimilarity ≈0.03), suggesting that all 5 generated ensembles represent similar ensembles, in agreement with the result obtained by Lazar et al.[30]

Computational Efficiency. To evaluate the computational efficiency of PROTHON, we recalculated the pairwise dissimilarity between the 5 ubiquitin partially folded ensembles described in Comparing Computationally Generated Ensembles of Ubiquitin. The calculation of the pairwise ensemble dissimilarity was repeated 6 times using PROTHON, and also using the publicly available program ENCORE.[28] For the ith run, where i, $1 \le i \le 6$, a subset of conformations were selected from the 5000 total conformations that make up each ensemble by choosing conformations at i regular frame intervals, for a total of $\approx\frac{1}{i} \times 5000$ conformations in each ensemble; i.e., every ith frame is sampled, for $1 \le i \le 6$. Since PROTHON is currently a serial code, each run used a single core on a 2020 MacBook Pro, while 48 cores were available for ENCORE, which has parallel code, on the Digital Research Alliance of Canada Cedar computing cluster (https://alliancecan.ca).

Figure 3D shows the wall clock time required to calculate the pairwise ensemble dissimilarity for the 5 ubiquitin partially folded ensembles, with varying number of conformations in each ensemble using PROTHON and ENCORE, respectively. That is, we performed $\binom{5}{2}$ ensemble dissimilarity calculations to calculate 10 values of D in eq 9 for PROTHON and 10 values of the Jensen−Shannon divergence for ENCORE. While the time required to run ENCORE increases exponentially with the number of conformations (the y-axis of the plot is on a log scale), the time required to run PROTHON essentially remains the same at ≈300 s = 5 min. We anticipate that for large ensemble sizes the scaling should be linear in the number of conformations (times the chain length), because filling in

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J. Chem. Inf. Model. XXXX, XXX, XXX−XXX

the matrix $\mathbb{X}$ in eq 1 is sufficient to determine the distribution X_p in eq 3. When $i = 6$ and each ensemble had $\frac{1}{6} \times 5000 = 834$ conformations, it takes ENCORE 596 s = 9 min, 56 s, which is twice that of PROTHON (which takes 298 s = 4 min, 58 s), to run. When $i = 1$ and each ensemble had $\frac{1}{1} \times 5000 = 5000$ conformations, the time it takes ENCORE to run increases dramatically to 28,406 s = 7 h, 53 min, 26 s, which is more than 88 times that of PROTHON (which takes 322 s = 5 min, 22 s) despite ENCORE using 48× the cores of PROTHON. If ENCORE runs on a single core, the corresponding calculation time for 5000 conformations is 58 h (2 days and 10 h), which would be $2^{17.7}$ in Figure 3D. We also note that this is not linear scaling for ENCORE; the 48 core calculations show a speedup of only 7.4×.

Although it may be possible to achieve speedup in practice by substituting the RMSD matrix used in ENCORE with a matrix using an alignment-free structural comparison, such as dRMSD,[57−59] fraction of common contacts (Q),[60] local distance test,[61] global distance test (GDT),[62] or minimal Euclidean distance,[18] the computational complexity would still remain at $O(M^2)$, where M is the number of structures to be compared. This is in contrast to PROTHON, which achieves a computational complexity of $O(M)$. We reserve for future work a more extensive, systematic comparison between the metric we propose here and other metrics proposed in the literature.

SOFTWARE AND DATA AVAILABILITY

PROTHON is freely available. Users can run PROTHON by downloading the Python file Prothon.py from github under the GPLv3 license at https://github.com/PlotkinLab/Prothon. The snippet below shows how to use PROTHON to calculate ensemble dissimilarity in only 12 lines of Python code. The code below was used to generate the data for the plot in Figure 3A; a description follows.

```
1  from Prothon import Prothon
2  import numpy as np
3
4  data = ['Q99.dcd','Q75.dcd','Q80.dcd','Q85.dcd','Q90.dcd','Q95.dcd']
5  topology = 'topology.pdb'
6
7  prothon = Prothon(data = data, topology = topology)
8  ensembles = prothon.ensemble_representation(measure = 'CBCN')
9  x_min, x_max = (np.min(ensembles), np.max(ensembles))
10
11 dissimilarity = []
12
13 for ensemble in ensembles[1:]:
14     d = prothon.dissimilarity(ensemble, ensembles[0], x_min=x_min, x_max=x_max)
15     dissimilarity.append(d[0])
16
17 print(dissimilarity)
```

The Prothon class is first imported from the Prothon package (line 1), followed by the creation of a Prothon object (line 7) initialized with the topology in pdb format (line 5) of the conformations contained in these ensembles and a list of the ensembles (line 4) to be compared. The dcd format has been used here for the ensemble data, but any format supported by the MDTraj package[33] is allowed. The Prothon *ensemble_representation* function is then used to represent the ensembles as described in the present work using the C_β contact number (CBCN) local structural property (line 8). The minimum and maximum values of CBCN in all ensembles are obtained in line 9 using numpy, which is imported in line 2.

To calculate the dissimilarity between two ensembles, the Prothon *dissimilarity* function is used (line 14), which returns a list d with 3 objects: the global dissimilarity, local dissimilarity values, and the p-values for the local dissimilarity (i.e., statistical significance, with dissimilarity being significant if the p-value <0.05). There are 70 elements for the local dissimilarity and for the p-values, corresponding to the number of C_β atoms. The global dissimilarity between each ensemble and the first ensemble is saved (line 15) to the dissimilarity list (created in line 11), which is finally displayed in line 17.

The ubiquitin molecular dynamics-generated ensemble data set (229 MB zip file) is available at 10.5281/zenodo.7792288.

CONCLUSION

We have developed a new generalized method for the efficient representation and comparison of protein ensembles. Our newly developed method was implemented in the Python programming language and made freely available as a Python package called PROTHON to the computational structural biology community. The method involves the representation of a protein ensemble as a vector of probability distribution functions of a local quantity involving each amino acid. Each probability distribution function is estimated, using Gaussian kernel density estimation, from the distribution of a local structural property of the polypeptide chain. The Jensen−Shannon distance between corresponding probability distribution functions then quantifies the dissimilarity between protein ensembles. Here, the C_β contact number (CBCN) was used as a local structural property, but in principle, any local structural property can be used in PROTHON. Examples include solvent accessible surface area (SASA) per residue,[32,33] virtual $C_\alpha-C_\alpha$ bond angle (CABA) and torsion angle (CATA),[34] and C_α contact number (CACN). PROTHON was shown to be effective in correctly distinguishing computationally generated ensembles of ubiquitin and experimentally derived ensembles of a 130 amino acid fragment of tau protein. The computational efficiency of PROTHON, when compared to the publicly available software ENCORE,[28] can simultaneously yield up to an 88-fold gain in wall-clock time while benefiting from a 48-fold reduction in the number of computing cores required.

ASSOCIATED CONTENT

Data Availability Statement

The ubiquitin molecular dynamics-generated ensemble data set (229 MB zip file) is available at 10.5281/zenodo.7792288.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jcim.3c00145.

Representation, dimensionality reduction, and comparison of ubiquitin ensembles (PDF)

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Notes

The authors declare no competing financial interest.

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J. Chem. Inf. Model. XXXX, XXX, XXX−XXX