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Validating protein structure using kernel density estimates

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Abstract

Measuring the quality of determined protein structures is a very important problem in bioinformatics. Kernel density estimation is a well-known nonparametric method which is often used for exploratory data analysis. Recent advances, which have extended previous linear methods to multi-dimensional circular data, give a sound basis for the analysis of conformational angles of protein backbones, which lie on the torus. By using an energy test, which is based on interpoint distances, we initially investigate the dependence of the angles on the amino acid type. Then by computing tail probabilities which are based on amino-acid conditional density estimates, a method is proposed which permits inference on a test set of data. This can be used, for example, to validate protein structures, choose between possible protein predictions and highlight unusual residue angles.

Keywords: Circular kernel; Conformational angle; Probability contour; Variable bandwidth; von Mises density.

1 Introduction

Determination of protein structures is often carried out using X-ray crystallography, which leads to a set of co-ordinates of all the atoms — measured within some resolution. Such structures are typically made available in the Protein Data Bank, but they can be of variable quality. Currently, there is a validation suite [Laskowski et al., 1993] of software which provides a set of tools to validate and check structure data. A more recent approach, based on conformational angles, has also been proposed by Lovell et al. [2003], and this paper builds on their approach.

A *circular* observation can be seen as a point on the unit circle, and represented by an angle $\theta \in [-\pi, \pi)$. It is periodic, *i.e.* $\theta = \theta + 2m\pi$ for $m \in \mathbb{Z}$, which sets apart circular statistical analysis from standard real-line methods. Recent accounts are given by Jammalamadaka and SenGupta [2001] and Mardia and Jupp [1999]. Concerning nonparametric density estimation, there exist a few contributions focused on data lying on the circle or on the sphere (Bai et al. [1988], Beran [1979], Klemelä [2000], Taylor [2008]). Recently, Di Marzio et al. [2010] obtained general results for kernel estimation of densities (and their partial derivatives) defined on the d-dimensional torus $\mathbb{T} := [-\pi, \pi]^d$.

Data on the (two-dimensional) torus are commonly found in descriptions of protein structure. Here, the protein backbone is given by a set of atom co-ordinates in $\mathbb{R}^3$ which can then be converted (without any loss of information) to a sequence of *conformation angles*. The sequence of angles can be used to assign [Kabsch and Sander, 1983] the structure of that part of the backbone (for example α-helix, β-sheet) which can then give insights into the functionality of the protein. A potential higher-dimensional example is provided by NMR data which will give replicate measurements, revealing a dynamic structure of the protein. For shorter peptides the modes of variability could be studied by an analysis of the replicates, requiring density estimation on a high-dimensional torus. In Section 2.1 we introduce toriodal kernels for kernel density estimation and review a simple way to select the smoothing parameter. Our application, of conformational angles in a protein backbone, is introduced in Section 3, and in Section 4 we investigate whether the bivariate distributions of angles are dependent on the amino acid type. Various *validation* scores, which can be used for “new” proteins, are introduced in Section 5. We conclude with a discussion.

2 Density estimation on the torus

2.1 Toroidal kernels

A *kernel density estimate* on the circle is easily constructed by adopting a circular density (with mean zero, and concentration parameter λ) for the kernel function. In this case, given angles $\theta_1, \ldots, \theta_n$, the kernel density estimate is simply

$$\hat{f}_\lambda(\theta) = \frac{1}{n} \sum_{i=1}^{n} K_\lambda(\theta - \theta_i)$$

where $\lambda > 0$ is the (inverse of the) smoothing parameter, and $K_\lambda(\cdot)$ is a circular (symmetric) probability density function.

On the torus, we can use a d-fold product $K_{\boldsymbol{C}} := \prod_{s=1}^{d} K_{\lambda_s}$, where $\boldsymbol{C} := (\lambda_s \in \mathbb{R}_+, s = 1, \ldots, d)$ is a set of smoothing parameters. Most kernels are continuous and symmetric about the origin, so the d-fold products of von Mises, wrapped normal and wrapped Cauchy distributions are all valid. However, we note that the cardioid density (which was used by Lovell et al. [2003]): $(2\pi)^{-1}\{1 + 2\lambda \cos(\cdot)\}$ with $|\lambda| < 1/2, \theta \in \mathbb{T}$ gives a very inefficient kernel (see [Di Marzio et al., 2009]) relative to the von Mises and wrapped normal kernels. This can be seen by considering the Fourier series representations of the probability density function and the cardioid kernel. Indeed, this kernel function was excluded in the definition of [Di Marzio et al., 2009] because it failed to satisfy a limiting “concentration” criterion.

2.2 A plug-in rule for the von Mises kernel

The performance of a kernel density estimate is usually measured by the integrated mean squared error

$$\text{IMSE} = \int \mathsf{E}\left(\hat{f}_\lambda(\theta) - f(\theta)\right)^2 \mathrm{d}\,\theta$$

which seeks a trade-off between the bias-squared and variance. In the case that $d = 2$ and a multiplicative von Mises kernel function is adopted, we have a kernel density estimate of $f(\phi, \psi)$ given by

$$\hat{f}_\lambda(\phi, \psi) = \left\{n(2\pi)^2 I_0(\lambda)^2\right\}^{-1} \sum_{i=1}^{n} \exp\{\lambda \cos(\phi - \phi_i) + \lambda \cos(\psi - \psi_i)\}$$

where

- the bivariate data is given by $(\phi_i, \psi_i), i = 1, \ldots, n$
- $I_r(\lambda)$ is the modified Bessel function of order r
- $\lambda \geq 0$ is the concentration parameter of the von Mises density. In this case $\lambda = 0$ corresponds to a uniform density, and as $\lambda \to \infty$ the density concentrates around the mean (ϕ_i, ψ_i). Hence, when used in the kernel function λ is (inverse of the) smoothing parameter (assumed — for simplicity — to be equal for both variables).

Note that distance between two angles is measured by taking the cosine of the difference, which is important when the data may be distributed around the torus.

When f is assumed to be a bivariate von Mises distribution, with independent components, and common concentration κ, then we can approximate the asymptotic integrated variance of the kernel density estimate (see [Di Marzio et al., 2010]) as

$$\lambda/(4n\pi)$$

with asymptotic integrated bias-squared as

$$\kappa\left[3\kappa I_0(2\kappa)^2 - I_0(2\kappa)I_1(2\kappa) + \kappa I_1(2\kappa)^2\right]/(32\pi^2 I_0(\kappa)^4\lambda^2)\ .$$

As usual, we see a trade-off between bias-squared and variance: as λ increases (corresponding to less smoothing) the bias decreases whilst the variance increases, but when λ decreases the bias increases whilst the variance decreases. In this setting (assuming von Mises data) we can obtain an *asymptotic* choice for λ to minimize the asymptotic IMSE (integral of bias-squared plus variance). We obtain a plug-in rule

$$\lambda^* = \left[n\hat{\kappa}\left\{3\hat{\kappa} I_0(2\hat{\kappa})^2 - I_0(2\hat{\kappa})I_1(2\hat{\kappa}) + \hat{\kappa} I_1(2\hat{\kappa})^2\right\}/(4\pi I_0(\hat{\kappa})^4)\right]^{(1/3)} \tag{1}$$

where $\hat{\kappa}$ is an estimate of the concentration of the data.

As will be seen in the next section, angles associated with protein structure do not follow a von Mises distribution so we will not adopt (1). In some cases they can be modelled by a mixture of von Mises densities with an EM algorithm being used to fit the components [Mardia et al., 2007]. In the case of data which are not von Mises, Taylor [2008] investigates robust ways to obtain useful estimates of κ which can be used in (1), though cross-validation provides a more objective approach to the choice of λ. In this case, we choose

$$\lambda_{\mathrm{CV}} = \arg\min_{\lambda} \prod_{i=1}^{n} \hat{f}_\lambda^{(i)}(\phi_i, \psi_i) \tag{2}$$

where

$$\hat{f}_\lambda^{(i)}(\phi_i, \psi_i) = \left\{n(2\pi)^2 I_0(\lambda)^2\right\}^{-1} \sum_{j \neq i}^{n} \exp\{\lambda \cos(\phi_j - \phi_i) + \lambda \cos(\psi_j - \psi_i)\}.$$

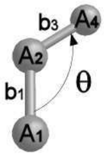
3 Conformational Angles

The *backbone* of a protein comprises a sequence of atoms

$$\mathrm{N}_1{-}\mathrm{C}_1^\alpha{-}\mathrm{C}_1{-}\mathrm{N}_2{-}\mathrm{C}_2^\alpha{-}\mathrm{C}_2{-}\ldots{-}\mathrm{N}_p{-}\mathrm{C}_p^\alpha{-}\mathrm{C}_p,$$

By choosing 4 atoms $A_1, \ldots, A_4$ with A_3 directly behind A_2, A_1 directly below A_2 and A_4 as shown in the figure below, we can specify 3 *dihedral angles*: ϕ, ψ, ω.

θ	A_1	A_2	A_3	A_4
ϕ_i	C_{i-1}	N_i	C_i^α	C_i
ψ_i	N_i	C_i^α	C_i	N_{i+1}
ω_i	C_{i-1}^α	C_{i-1}	N_i	C_i^α



The angle ω is usually restricted to be about zero. The remaining angles (ϕ, ψ) are measured between $-\pi$ and π. Scatter plots of the (ϕ, ψ) angles for a given protein are known as *Ramachandran plots*; for further details, see Lesk [2010]. For any protein, it would be possible to compute a kernel density estimate with λ being chosen by cross-validation. The kernel density estimates can be used: (i) to indicate sub-groups in the data; (ii) for classification purposes [Kabsch and Sander, 1983]; (iii) for estimation of quantiles; and (iv) for clustering. However, it should be noted that — in general — the observations $(\phi_i, \psi_i), i = 1, \ldots, n$ will not be independent, and so the usual considerations of IMSE, and general principles underlying cross-validation, may not hold. This lack of independence has been investigated by Berkholz et al. [2009] and has also been modelled by Boomsma et al. [2008] using a Markov model — with bivariate von Mises mixture components; a similar approach might be possible here.

4 Amino acid dependence and inference

Note that each pair (ϕ_i, ψ_i) is associated with an amino acid. There are twenty amino acids, each coded by a single letter — for example Alanine (A) — and we use the letter Z to denote a pre-proline amino acid. For a large database of proteins we can collect all bivariate angles associated with each amino acid. Then we can estimate the probability density for each, say

$$\hat{f}_\mathrm{A}(\phi, \psi), \hat{f}_\mathrm{C}(\phi, \psi), \hat{f}_\mathrm{E}(\phi, \psi), \hat{f}_\mathrm{F}(\phi, \psi), \ldots$$

It could be of interest to visually compare the distributions and this can be shown graphically using contour representations for the densities.

To make formal comparisons between the densities of angles for two amino acids is feasible using booststrap methods, or circular analogues of the Kolmogorov-Smirnov test [Mardia and Jupp, 1999]. Such tests reveal that it is possible to detect (statistically significant) differences in the distribution between most amino acids. That is, for most pairs of amino acids we can formally reject the hypothesis $H_0 : f_k(\phi, \psi) = f_j(\phi, \psi)$, $k \neq j$. In an all-against-all comparison we can obtain a test statistic based on "energy" [Rizzo, 2007], and associated p-value for each pair of amino acids. (Note that in such a multiple comparison situation, the threshold for an interesting p-value would be much less than the usual 0.05.) These matrices have the potential for use as additional information within a substitution matrix. Here we use the test statistics as a distance matrix to which multi-dimensional scaling [Mardia et al., 1979] can be applied. This allows a graphical representation of which amino acids are more similar; the results are shown in Figure 1. It is interesting to note that the energy test cannot detect a difference between the distributions of Alanine (A) and Glutamic Acid (E) even though these have different side-chain polarities and charges. We have tried, with some difficulty, to intepret the two axes in Figure 1. Although it is quite straightforward to describe the difference (or similarity) between any pair, we have found it almost impossible to characterize the meaning of either dimension. We have observed that the amino acids towards the left of the plot tend to have more complex contours in the region corresponding to the β sheet. However, given that very few pairings are found to have similar distributions, it seems important *not* to pool together angles from different amino acids.

The kernel density estimate can be converted to *probability* contours, or *level sets* as follows. Given α, with $(0 \leq \alpha \leq 1)$ define the set $B_\alpha = \{(\phi, \psi) \mid \hat{f}(\phi, \psi) \geq t(\alpha)\}$ where $t(\alpha)$ is a threshold determined so that

$$\iint_{(\phi,\psi)\in B_\alpha} \hat{f}(\phi, \psi) \mathrm{d}\phi \mathrm{d}\psi = 1 - \alpha.$$

A $\alpha-$probability contour can then be drawn at the boundary of B_α. Some examples are shown in Figure 2 (for similarities refer to Figure 1). Conversely, given a specific point (ϕ_0, ψ_0) a contour passing through this point will have a specific value of $\alpha = \alpha_0$, say which can be interpreted as a measure of likelihood of occurrence at that location. It should be noted that these numbers are not probabilities but correspond to the usual p-value in a hypothesis test in the sense that, if the null hypothesis is true, then the values will be uniformly distributed in $[0, 1]$. These numbers can be used for validation as described in the next section.

5 Validation for new proteins

Given existing density estimates for each amino acid: $\hat{f}_{\mathrm{A}}(\phi, \psi), \hat{f}_{\mathrm{C}}(\phi, \psi), \ldots$ these can be converted (as above) to "probability functions", say $\hat{P}_{\mathrm{A}}(\phi, \psi), \hat{P}_{\mathrm{C}}(\phi, \psi), \ldots$. Then for a "new" n-residue protein (which did not contribute training data to the density estimates) with $\{(\mathcal{A}_i, \phi_i, \psi_i), i = 1, \ldots, n\}$ we can compute a measure for the ith residue conditional on the amino acid type $\mathcal{A}_i$, $i = 1, \ldots, n$. We can then create an overall measure of quality using the arithmetic or geometric

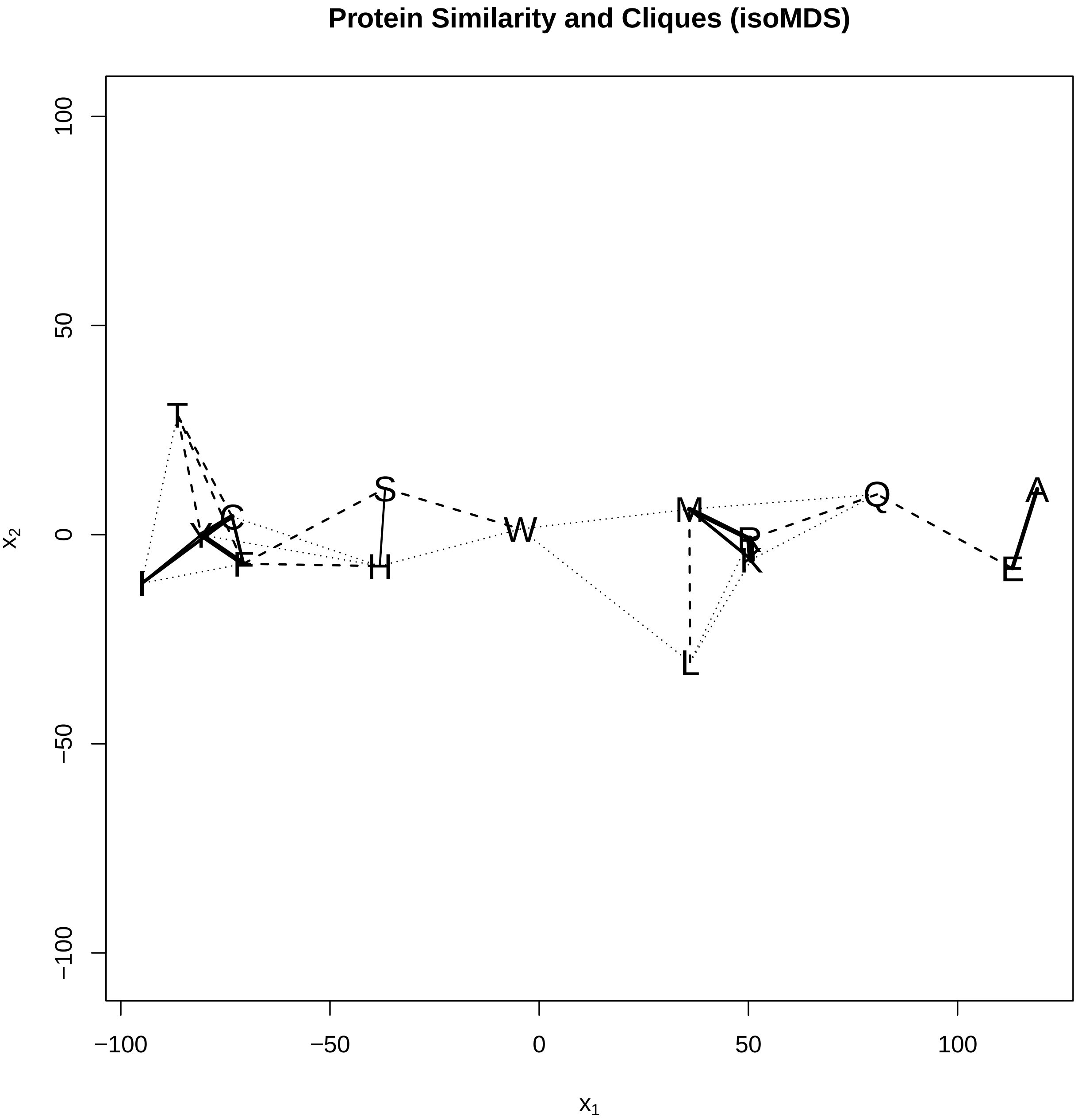


Figure 1: Kruskal's multidimensional scaling for the bootstrap test statistic to measure similarity between the distributions of each pair of amino acids. Those connected by a continuous line have distributions which are not significantly different at 5%; dashed lines are similar at 1%, and dotted lines at 0.1%. Those amino acids which are not connected — and those which are not shown (D, G, N, P, V and pre-proline) — have no similarities with any other (at 0.1% level).

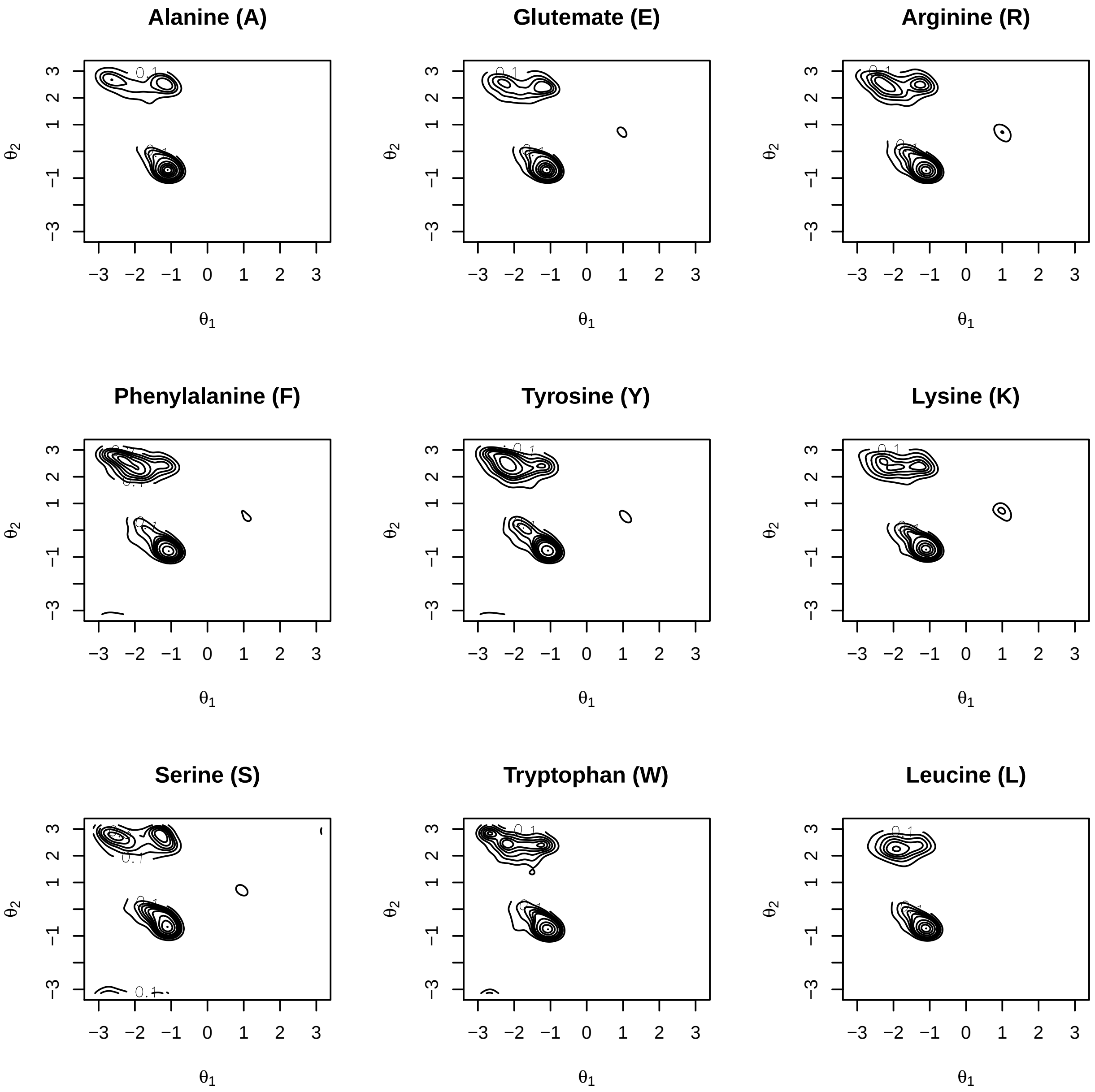


Figure 2: Contour (probability) plots of kernel density estimates for some example amino acids. Comparison with Figure 1 shows that the distributions of pairs (A,E), (R,K), (F,Y) are indistinguishable ($p > 0.05$), the distributions of (S,W), (F,S) are very similar ($0.05 > p > 0.01$) , and the distributions of (K,L), (R,L), (L,W) are probably distinct ($0.01 > p > 0.001$).

mean. In practice, if there is a very small quantity, then the geometric mean, which is given by:

$$\left\{ \prod_{j\text{th amino acid type}} \prod_{\{i:\mathcal{A}_i=j\}} \hat{P}_j(\phi_i,\psi_i) \right\}^{1/n} \tag{3}$$

is more likely to reveal this through a formal test. As is well-known, if the $\hat{P}$ satisfy the null hypothesis, then they will be uniformly distributed. Alternatively (or in addition), we can consider $\min_{i,j} \hat{P}_j(\phi_i,\psi_i)$, or the list $p = (p_1, \ldots, p_{21})$ where

$$p_j = \min_{\{i:\mathcal{A}_i=j\}} \hat{P}_j(\phi_i,\psi_i).$$

When a previously validated training dataset is used, a leave-one-out approach can be adopted to provide a benchmark for the above quantities, by which future data can be compared. The proposed validation is formally intended to check both the labels (amino acids) and the angles. Since the angles are derived from a set of 3-D co-ordinates, this would imply that the (relative) co-ordinates have been accurately determined. So, in the event that a protein "fails" the validation test, this could be due to an incorrect amino acid label or inaccurate co-ordinates obtained from the X-ray technology. To distinguish between these two situations could be possible in some cases. However, in practice, the situation is a little more complicated as the sequence of labels is assumed to be known, and often used (with prior knowledge) to suggest the co-ordinate structure. Note that this that this is a somewhat circular argument!

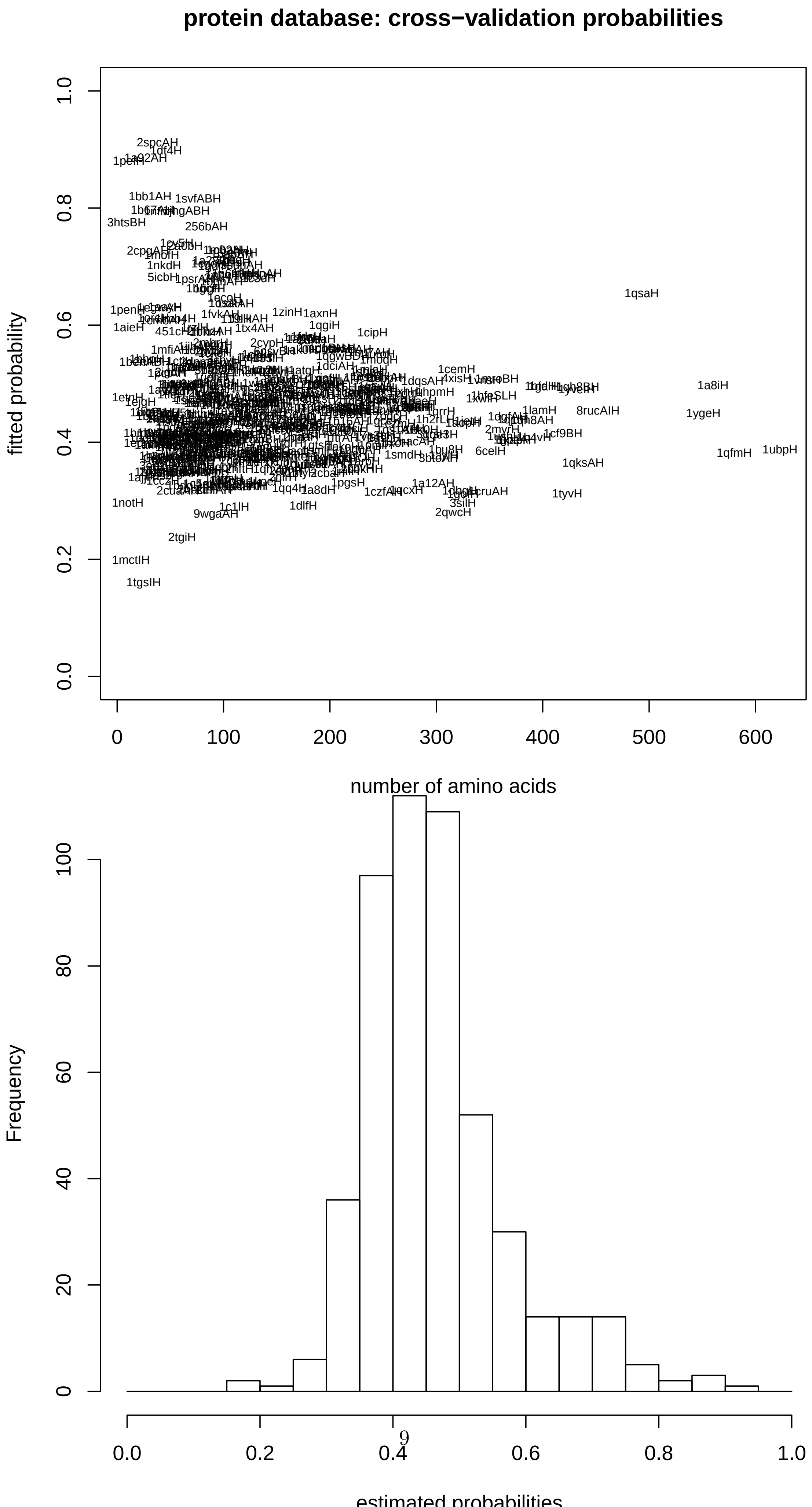
Using a cleaned up subset [Lovell et al., 2003] of the top 500 proteins from the Kinemage[1] database, we can obtain a validation probability (using leave-one-protein-out cross-validation) for each protein. This dataset has 74,414 bivariate angles, with frequencies for each amino acid given in Table 1.

A	7506	C	1299	D	4711
E	4132	F	3313	G	6865
H	1579	I	3772	K	3482
L	5968	M	1399	N	3083
P	1898	Q	2177	R	2621
S	4767	T	4639	V	5607
W	1153	Y	2838	Z	1605

Table 1: Frequencies of amino acids in the database of Lovell et al. [2003], with "Z" denoting pre-proline

The probabilities can be plotted (vs number of residues to improve clarity) and this plot, together with a histogram, is shown in Figure 3. Given the nature of the data, it is not surprising that the smallest probability is about 0.158 (for protein 1tgsIH), which gives no cause for concern. It is also reassuring that these probabilities do not seem to depend on size.

[1] http://kinemage.biochem.duke.edu/databases/top500.php


protein database: cross−validation probabilities
fitted probability
number of amino acids
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
2spcAH
1df4H
1a92AH
1pefH
1bb1AH
1svfABH
1mhgABH
3htsBH
256bAH
2cpgAH
1nofH
1nkdH
5icbH
1penH
1aieH
451cH
1zinH
1axnH
1qgiH
1cipH
2cypH
1cemH
4xisH
1dqsAH
1hpmH
1hfeSLH
1kwfH
1lamH
8rucAIH
1qsaH
1a8iH
1ygeH
1cf9BH
1qfmH
1ubpH
1qksAH
6celH
1smdH
3btaAH
1a12AH
1czfAH
1qcxH
1pgsH
1q4H
1a8dH
1dlfH
1tyvH
1cruAH
3silH
2qwcH
1c1lH
9wgaAH
1notH
2cuaAH
1ajjH
2tgiH
1mctIH
1tgsIH
1etnH
1ejgH
1fvkAH
1tx4AH
1ecoH
1atgH
1dciAH
1rroBH
2myrH
Frequency
estimated probabilities
0
20
40
60
80
100
0.0
0.2
0.4
0.6
0.8
1.0

We now consider a hypothetical "new" protein `1xb1` — which is not in the training database — using the proposed validation procedure, and compare the outcomes with Procheck [Laskowski et al., 1993], which is a commonly used tool for the validation of protein structures. (Procheck, as well as MolProbity [Lovell et al., 2003] are used as part of a bigger validation tool provided by the Protein Data Bank.) The geometric mean for the angles is 0.28, which is well within a "normal" range of overall validation probabilities (Figure 3). The minimum $\hat{P}$ is 1.7×10^{-11}, which — at first sight — does look significant, and it is a particular residue (E) that Procheck associates with a "disallowed region". However, if we consider a similar calculation for each of the 500 Kinemage proteins, then this minimum ranks 14 — see Figure 4. Boxplots for each $\log p_j$ over the Kinemage database, with a comparison of the corresponding values for protein `1xb1` provides another visual check (Figure 4). More formal tests which compare the distribution of the minimum amino acid tail probabilities of the Kinemage proteins with `1xb1` give p-values ranging around 0.1.

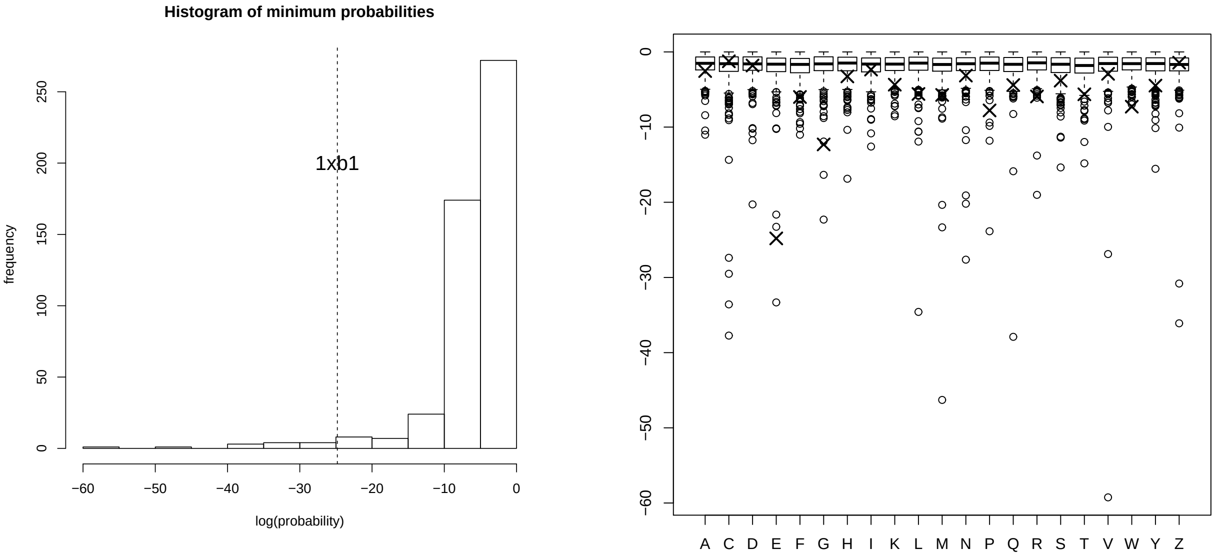


Figure 4: Left: Histogram of log of the minimum tail probabilities for each of the Kinemage proteins, with minimum of the "test" protein `1xb1`. Right: Boxplots of the log of the minimum tail probabilities by amino acid for the database, with extra points (×) corresponding to `1xb1`.

6 Discussion

The above probabilities all critically depend on the choice of smoothing parameter — λ for the von Mises kernel. In general, the smaller is λ (which corresponds to more smoothing), the larger is the validation probability, and the less sensitive is the test.

In principle, the above method could be used on any dataset. Ideally, one would like *training data* which consists of a large database of independent bivariate observations which are known to be "correct". (Note that our use of the Kinemage database does not have the sought-after independence.) Probability estimates $\hat{P}_{\mathrm{A}}(\phi, \psi), \hat{P}_{\mathrm{C}}(\phi, \psi), \ldots$ for each amino acid require selection of the λ's which could be obtained by cross-validation (or a plug-in rule). Having stored the $\hat{P}$'s (on a reasonably fine grid on the torus) then formula (3) could be used to validate any new protein structure.

An alternative to cross-validation for selection of the λ's is to consider an *adaptive* kernel bandwidth. Theoretical results — see, for example Terrell and Scott [1992] — suggest that using a separate bandwidth for each observation, with a bandwidth that depends on density, will have better theoretical properties. This approach has been adopted by Lovell et al. [2003], although they have used an inefficient Cardioid kernel, and an adaptation that is not consistent with theory which suggests that, for a von Mises kernel with concentration λ, the adaptive bandwith for observation i should satisfy $\lambda_i \propto f(\phi_i, \psi_i)$. However, limited simulation experiments suggest that $\lambda_i \propto f(\phi_i, \psi_i)^\gamma$ with $\gamma \approx 0.7$ may work better for large datasets. Further work will investigate this more closely, as well as examining which of the above proposed scores are more useful.

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References

Z. D. Bai, R. C. Rao, and L. C. Zhao. Kernel estimators of density function of directional data. *Journal of Multivariate Analysis*, 27 (1988), 24–39.

R. Beran. Exponential models for directional data. *The Annals of Statistics*, 7 (1979), 1162–1178.

D. S. Berkholz, M. V. Shapovalov, R. L. Dunbrack, Jr. and P. A. Karplus. Conformation dependence of backbone geometry in proteins. *Structure*, 17 (2009), 1316–1325.

W. Boomsma, K. V. Mardia, C. C. Taylor, J. Ferkinghoff-Borg, A. Krogh, and T. Hamelryck. A generative, probabilistic model of local protein structure. *PNAS*, 105 (2008), 8932–8937.

M. Di Marzio, A. Panzera and C.C. Taylor. Local polynomial regression for circular predictors. *Statistics and Probability Letters*, 79 (2009), 2066–2075.

M. Di Marzio, A. Panzera and C.C. Taylor. Kernel density estimation on the torus. *Journal of Statistcal Planning and Inference*, 141 (2011), 2156–2173.

S. R. Jammalamadaka and A. SenGupta. *Topics in Circular Statistics.* World Scientific, Singapore, 2001.

W. Kabsch and C. Sander. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, 22 (1983), 2577–2637.

J. Klemelä. Estimation of densities and derivatives of densities with directional data. *Journal of Multivariate Analysis*, 73 (2000), 18–40.

R. A. Laskowski, M. W. MacArthur, D. S. Moss, J. M. Thornton, PROCHECK - a program to check the stereochemical quality of protein structures. *J. App. Cryst*, 26 (1993), 283–291.

A. M. Lesk, *ntroduction to protein science : architecture, function, and genomics. 2nd edition* Oxford: Oxford University Press, 2010.

S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson, and D.C. Richardson. Structure Validation by $C\alpha$ Geometry: ϕ, ψ and $C\beta$ Deviation. *Proteins: Structure, Function and Genetics*, 50 (2003), 437–450.

K. V. Mardia and P. E. Jupp. *Directional Statistics.* John Wiley, New York, NY, 1999.

K. V. Mardia, J. T. Kent and J. M. Bibby. *Multivariate Analysis.* London: Academic Press, 1979.

K.V. Mardia, C.C. Taylor, and G.K. Subramaniam. Protein bioinformatics and mixtures of bivariate von mises distributions for angular data. *Biometrics*, 63 (2007), 505–512.

M. L. Rizzo *Statistical Computing with* R. Boca Raton: Chapman & Hall, 2007.

C. C. Taylor. Automatic bandwidth selection for circular density estimation. *Computational Statistics & Data Analysis*, 52 (2008), 3493–3500.

G. R. Terrell, and D. W. Scott. Variable kernel density estimation. *Annals of Statistics*, 20 (1992), 1236–1265.