A physically meaningful method for the comparison of potential energy functions

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Abstract

In the study of the conformational behavior of complex systems, such as proteins, several related statistical measures are commonly used to compare two different potential energy functions. Among them, the Pearson's correlation coefficient r has no units and allows only semi-quantitative statements to be made. Those that do have units of energy and whose value may be compared to a physically relevant scale, such as the root mean square deviation (RMSD), the mean error of the energies (ER), the standard deviation of the error (SDER) or the mean absolute error (AER), overestimate the distance between potentials. Moreover, their precise statistical meaning is far from clear. In this article, a new measure of the distance between potential energy functions is defined which overcomes the aforementioned difficulties. In addition, its precise physical meaning is discussed, the important issue of its additivity is investigated and some possible applications are proposed. Finally, two of these applications are illustrated with practical examples: the study of the van der Waals energy, as implemented in CHARMM, in the Trp-Cage protein (PDB code 1L2Y) and the comparison of different levels of the theory in the ab initio study of the Ramachandran map of the model peptide HCO-L-Ala-NH$_2$.

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1 Introduction

The most fundamental way to account for the behavior of a physical system is through its energy function $H(\vec{q}, \vec{p})$, which depends on the coordinates $\vec{q}$ and

the momenta $\vec{p}$ of all the particles. In normal situations, this function can be expressed as the sum of the kinetic energy $K(\vec{q},\vec{p})$[1] and the potential energy $V(\vec{q})$. Because the former is of general form for any type of system and, normally, it does not affect the equilibrium properties, the latter is enough for a complete characterization of the problem.

In the study and simulation of complex systems, such as proteins, researchers often face the dilemma of choosing among many different ways of calculating a conceptually unique potential energy V [1–7]. Others tackle the problem of designing new algorithms to perform this calculation looking for the improvement of the relation between accuracy and numerical complexity [8–19].

The energy V studied may be the total potential energy of the system or any of the terms in which it is traditionally factorized[2] and the different ways of calculating it may stem from distinct origins, namely, that different algorithms or approximations A are used, that the potential energy function depends on a number of free parameters $\vec{P}$ or that it is computed on different but somehow related systems S (for a proper definition of this, see Sec. 6).

Changes in these inputs produce different *instances* of the same physical potential energy, which we denote by subscripting V. For example, if it is calculated on the same system S, the algorithm and approximations A are held constant but two different set of parameters $\vec{P_1}$ and $\vec{P_2}$ are used, our notation made explicit would read as in the following equations[3]:

$$V_1(\vec{q}) := V(A, \vec{P_1}, \vec{q}, S) \qquad \text{and} \qquad V_2(\vec{q}) := V(A, \vec{P_2}, \vec{q}, S) \ . \qquad (1)$$

For each practical application of these two potential energy functions, there is a limit on how different V_1 could be from V_2 to preserve the relevant features of the system under scrutiny. Clearly, if V_1 is *too distant* from V_2, the key characteristics of the system behavior will be lost when going from one function to the other.

In the literature, a number of different methods are used to quantify this distance. Among them, the Pearson's correlation coefficient r does not have units and its meaning is only semi-quantitative. Some others, such as the root mean square deviation (RMSD), the mean error of the energies (ER), the standard deviation of the error (SDER) or the mean absolute error (AER), do have units of energy and their values can be compared to the physically relevant scale in each problem. However, they tend to overestimate the sought distance even in the interesting situations in which the potential energy functions under study are physically proximate. The aim of this article is to define, justify and describe a new meaningful measure $d(V_1, V_2)$ of the distance between two instances of the same potential energy that overcomes the aforementioned difficulties, and

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[1] The kinetic energy K depends, in general, on the positions and the momenta. However, if Cartesian coordinates are used, the dependence on positions vanishes.

[2] For example, in the case of proteins [20, 21], some of the terms in which the total potential energy is typically factorized are the hydrogen-bonds energy, the van der Waals interaction, the excluded volume repulsion, the Coulomb energy and the solvation energy.

[3] Analogous definitions may be made if different algorithms or approximations, A_1 and A_2, are used or if V is computed on two related systems, S_1 and S_2.

that allows to make precise statistical statements about the way in which the energy differences change when going from V_1 to V_2[4].

In Sec. 2, the hypothesis made on V_1 and V_2 to accomplish this are outlined and, in Sec. 3, the central object, $d(V_1, V_2)$, is defined. The statistical meaning of the distance herein introduced is discussed in Sec. 4 and 5 and some of its possible applications to practical situations are proposed in Sec. 6. A comparison to other commonly used criteria is made and illustrated with a numerical example in Sec. 7. The important issue of the additivity of $d(V_1, V_2)$, when the potentials studied are only a part of the total energy, is investigated in Sec. 8 and, in Appendix A, the metric properties of our distance are discussed. The robustness of the van der Waals potential energy (as implemented in CHARMM [23, 24]) under a change in the free parameters and the ab initio Ramachandran plots of HCO-L-Ala-NH$_2$ at different levels of the theory are studied in Sec. 9 as examples of applications of the distance. Finally, Sec. 10 is devoted to the conclusions and to a useful summary of the steps that must be followed to use the distance in a practical case.

2 Hypothesis

In some cases traditionally studied in physics, the dependence of the potential energy V on the parameters is simple enough to allow a closed functional dependence $V_2(V_1)$ to be found[5]. However, in the study of complex systems, such as proteins, this dependence is often much more complicated, due to the high dimensionality of the conformational space and to the fact that the energy landscape lacks any evident symmetry. The set $C(V_1)$ of the conformations with a particular value of the potential energy V_1 typically spans large regions of the phase space containing structurally different conformations (see Fig. 1). When the system is slightly modified, from S_1 to S_2, or an approximation is performed (or the algorithm is changed), from A_1 to A_2, or the free parameters are shifted, from $\vec{P}_1$ to $\vec{P}_2$, each conformation $\vec{q}$ in $C(V_1)$ is affected in a different way and its potential energy is modified, from $V_1(\vec{q})$ to $V_2(\vec{q})$, in a manner that does not depend trivially on the particular region of the phase space which the conformation $\vec{q}$ belongs to. In such a case, a simple functional relation $V_2(V_1)$ is no longer possible to be found: for each value of V_1, there corresponds now a whole distribution of values of V_2 associated with conformations which share the same value of V_1 but which are far apart in the conformational space. Moreover, the projection of this high-dimensional $\vec{q}$-space into the 1-dimensional V_1-space makes it possible to treat V_2 as a random variable parametrically dependent on V_1 (see Fig. 2), in the already suggested sense that, if one chooses at random

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[4]The convenience of this approach has been remarked in [22]. Note, however, that in this article a different distance is defined.

[5]For example, if the recovering force constant of a harmonic oscillator is changed from k_1 to k_2, the potential energy functions satisfy the linear relation $V_2(\vec{q}) = (k_1/k_2)V_1(\vec{q})$ for all the conformations of the system; if the the atomic charges are rescaled by a factor α (being actually αQ_i) and α is changed from α_1 to α_2, the free energies of solvation calculated via the Poisson equation satisfy the linear relation $V_2(\vec{q}) = (\alpha_1/\alpha_2)^2 V_1(\vec{q})$, etc.

a particular conformation $\vec{q}_i \in C(V_1)$, the *outcome* of the quantity $V_2(\vec{q}_i)$ is basically unpredictable[6].
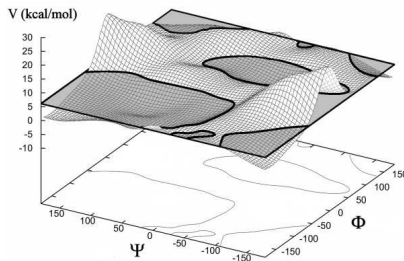

Figure 1: Space of constant potential energy V in a simple system with only two degrees of freedom: an alanine dipeptide in vacuo. Potential energy surface (PES) calculated ab initio at the B3LYP/6-311++G(d,p) level of the theory in (Perczel, A. et al. 2003, *J. Comp. Chem.* 24:1026–1042).
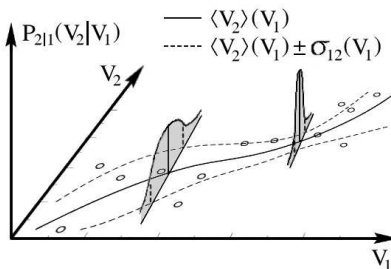

Figure 2: Illustration of the conditional-probability density function $P_{2|1}(V_2|V_1)$ at different V_1-positions. The points represent single conformations of the system. The V_1-conditioned mean of V_2, $\langle V_2 \rangle(V_1)$, is depicted as a solid line and, although in the hypothesis it is assumed to depend linearly on V_1, here it is shown as a more general function to better illustrate the concepts involved. Analogously, the V_1-conditioned standard deviation of V_2, $\sigma_{12}(V_1)$ (which is assumed to be constant in the hypothesis) is added to $\langle V_2 \rangle(V_1)$ (and subtracted from it) and the result is depicted as broken lines.

In this context, the hypothesis to be done about the two instances of V are, first, that the pair of values $(V_1(\vec{q}_i), V_2(\vec{q}_i))$ is independent of $(V_1(\vec{q}_j), V_2(\vec{q}_j))$ if $i \neq j$ and, second, that the probability distribution of V_2 conditioned by V_1 is normal with mean $b_{12}V_1 + a_{12}$ and standard deviation σ_{12} and that, conversely,

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[6]The same may be said in the case that the conformations belong to $C(V_2)$ and the random variable is, in turn, V_1. The role of the two instances of V is interchangeable in the whole following reasoning, however, for the sake of clarity, this fact will be made explicit in some cases and will be tacitly assumed in others.

the probability distribution of V_1 conditioned by V_2 (i.e., the distribution of the random variable V_1 in the space $C(V_2)$, analogous to $C(V_1)$) is normal with mean $b_{21}V_2 + a_{21}$ and standard deviation σ_{21}. Where a_{12}, b_{12}, σ_{12}, a_{21}, b_{21} and σ_{21} are constants not dependent on V_1 or V_2. This can be summarized in the following expressions for the conditional-probability density functions:

$$P_{2|1}(V_2|V_1) = \frac{1}{\sqrt{2\pi}\sigma_{12}} \exp\left[-\frac{(V_2 - (b_{12}V_1 + a_{12}))^2}{2\sigma_{12}^2}\right] , \qquad (2a)$$

$$P_{1|2}(V_1|V_2) = \frac{1}{\sqrt{2\pi}\sigma_{21}} \exp\left[-\frac{(V_1 - (b_{21}V_2 + a_{21}))^2}{2\sigma_{21}^2}\right] . \qquad (2b)$$

In general, one may reason about the whole conformational space of the system C and regard each randomly selected conformation $\vec{q}_i$ as a single *numerical experiment* to which the value of two random variables, $V_1(\vec{q}_i)$ and $V_2(\vec{q}_i)$, can be assigned. However, no hypothesis need to be made about the joint probability density function $P_{12}(V_1, V_2)$[7]. For the distance herein introduced to be meaningful, it suffices to assume Eq. 2.

Regarding the question of whether in a typical case these hypothesis are fulfilled or not, some remarks should be made. First, the satisfaction of the independence hypothesis depends mainly on the process through which the working set of conformations $\{\vec{q}_i\}_{i=1}^N$ is generated. For example, if the conformations are extracted from a single molecular dynamics trajectory letting only a short simulation time pass between any pair of them, their energies will be obviously correlated and the independence will be broken. If, on the contrary, each conformation $\vec{q}_i$ is taken from a different trajectory (see the first example in Sec. 9), one may reasonably expect this assumption to be fulfilled, i.e., the independence hypothesis is normally under researcher's control.

The normality hypothesis, however, is of a different nature. That the distribution of V_2 be normal for a particular value of V_1 may be thought as a consequence of the large number of degrees of freedom the system possesses, of the usual pairwise additivity of the forces involved and of the Central Limit Theorem (this, in fact, can be proved in some simple cases). Nevertheless, that the V_1-conditioned mean of V_2, $\langle V_2 \rangle(V_1)$, is linear in V_1 and that the V_1-conditioned standard deviation of V_2, $\sigma_{12}(V_1)$, is a constant must be regarded as a zeroth order approximation that should be checked in each particular case (see Fig. 2). It is worth pointing out, however, that, for the commonly used statistical quantities r, RMSD, etc. to be useful, this assumption must also be made (see Sec. 7) and also that it has been found to be approximately fulfilled in several cases studied (see, for example, [22] and Fig. 3b).

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[7]The hypothesis that $P_{12}(V_1, V_2)$ is bivariate normal, for example, is stronger than the assumptions in Eq. 2. The latter can be derived from the former.

3 Definition

For the aforementioned cases in which the dependence of the potential energy on the parameters is simple enough (see footnote 5), one can describe $V_2(V_1)$ by a closed analytical formula and exactly compute a_{12}, b_{12} and σ_{12} (this last quantity being equal to zero in such a situation). However, in a general situation, the parameters entering Eq. 2 can not be calculated analytically. In such a case, one may at most have a finite collection of N conformations $\{\vec{q}_i\}_{i=1}^N$ and the respective values $V_1(\vec{q}_i)$ and $V_2(\vec{q}_i)$ for each one of them.

From this finite knowledge about the system, one may statistically estimate the values of a_{12}, b_{12} and σ_{12}. Under the hypothesis assumed in the previous section, the least-squares estimators [25,26] of these quantities are optimal in the precise statistical sense that they are maximum-likelihood and have minimum variance in the class of linear and unbiased estimators[8] [27].

If we denote $V_1^i := V_1(\vec{q}_i)$ and $V_2^i := V_2(\vec{q}_i)$, and N is the number of conformations in the working set, the mean-squares maximum-likelihood estimators[9] of a_{12}, b_{12} and σ_{12} are given by the following expressions [25, 26]:

$$b_{12} = \frac{\mathrm{Cov}(V_1, V_2)}{\sigma_1^2} \ , \tag{3a}$$

$$a_{12} = \mu_2 - b_{12}\mu_1 \ , \tag{3b}$$

$$\sigma_{12} = \left[\frac{\sum_{i=1}^N (V_2^i - (b_{12}V_1^i + a_{12}))^2}{N}\right]^{1/2} \ , \tag{3c}$$

where:

$$\mu_1 := \frac{1}{N}\sum_{i=1}^N V_1^i \ , \tag{4a}$$

$$\mu_2 := \frac{1}{N}\sum_{i=1}^N V_2^i \ , \tag{4b}$$

$$\sigma_1 := \left[\frac{1}{N}\sum_{i=1}^N (V_1^i - \mu_1)^2\right]^{1/2} \ , \tag{4c}$$

$$\mathrm{Cov}(V_1, V_2) := \frac{1}{N}\sum_{i=1}^N (V_1^i - \mu_1)(V_2^i - \mu_2) \ . \tag{4d}$$

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[8]The same letters are used for the ideal parameters a_{12}, b_{12}, and σ_{12} and for their least-squares best estimators, because the only knowledge that one may have about the former comes from the calculation of the latter.

[9]In this article, the maximum-likelihood estimator for σ_{12} (with N in the denominator) is preferred to the unbiased one (with $N-2$ in the denominator) for consistency. Anyway, for the values of N typically used, the difference between them is negligible.

The quantities with 21 subscripts are found by changing $1 \leftrightarrow 2$ in the preceding expressions and the central object of this article, the distance $d(V_1, V_2)$ between two different instances of the same potential energy V is defined as follows:

$$d(V_1, V_2) := \left(\sigma_{12}^2 + \sigma_{21}^2\right)^{1/2} . \tag{5}$$

It must be stressed here that the measured distance depends on the working set, $\{\vec{q}_i\}_{i=1}^N$, of conformations chosen. More precisely, it depends on the occurrence probability of an arbitrary conformation $\vec{q}$ in the set. This probability must be decided a priori from considerations regarding which regions of the phase space are more relevant to answer the questions posed and up to what extent. For example, if one believes the system under study to be in thermodynamical equilibrium, then, it would be reasonable to generate a working set in which the probability that $\vec{q}$ occurs is proportional to its Boltzmann weight. If, on the contrary, one doubts whether the system is ergodic or not (as in the case of proteins) or one simply wants to study in detail the dynamical trajectories out of equilibrium, then, all the conformations in the phase space should be weighted equally and the probability should be flat.

4 Meaning

Under the hypothesis made in Sec. 2 (independence and Eq. 2), a simple expression may be written for the probability density function of the V_2-energy differences ΔV_2 conditioned by the knowledge of the V_1-energy differences ΔV_1:

$$P_{\Delta 2|\Delta 1}(\Delta V_2 | \Delta V_1) = \frac{1}{\sqrt{2\pi}d_{12}} \exp\left[-\frac{(\Delta V_2 - b_{12}\Delta V_1)^2}{2d_{12}^2}\right] . \tag{6}$$

The quantity d_{12} in this equation is defined as follows:

$$d_{12} := \sqrt{2}\sigma_{12} . \tag{7}$$

It is related to the distance defined in Eq. 5 via the following expression:

$$d(V_1, V_2) = \left(\frac{d_{12}^2 + d_{21}^2}{2}\right)^{1/2} . \tag{8}$$

And it encodes the *loss of information* involved in the transit from V_1 to V_2 through the following important properties:

1. The addition of an energy reference shift a_{12} between V_1 and V_2 has neither an implication in the physical behavior of the system nor in the numerical value of d_{12}.

2. One of the novel features of the distance herein defined is that no loss of information is considered to occur (i.e., $d_{12} = 0$) if there is only a constant rescaling b_{12} between the two potential energy functions studied.
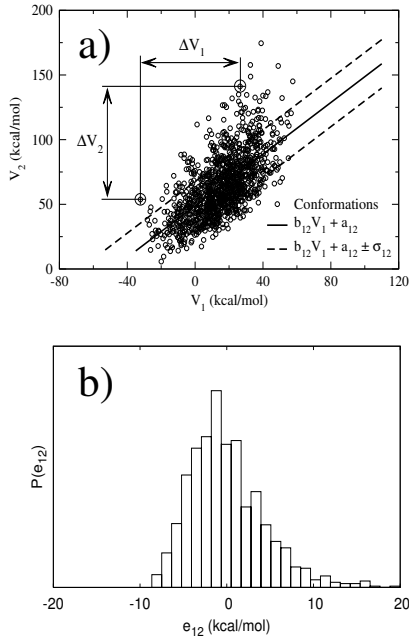
Figure 3: **(a)** V_1- and V_2-energies of the set of 1100 conformations of the Trp-Cage protein used in the first example of Sec. 9. Both potentials are the van der Waals energy as implemented in CHARMM; V_1 corresponds to $R_C = 1.275$ Å and V_2 to $R_C = 3.275$ Å, ε_C is fixed to -0.020 kcal/mol. The values of ΔV_1 and ΔV_2 for a selected pair of conformations are also depicted. The solid line represents the least-squares fit and the region where the probability of finding a conformation is largest is enclosed by broken lines. **(b)** Histogram of the residues $e_{12}(\vec{q}_i) := V_2(\vec{q}_i) - [b_{12}V_1(\vec{q}_i) + a_{12}]$ associated to the mean-squares fit in Fig. *a*.

Although such a transformation does have physical implications and would change the transition rates in a molecular dynamics simulation and alter the effective temperature in any typical Monte Carlo algorithm, V_1 can be easily recovered from V_2, if pertinent, upon division of V_2 by b_{12}. If the two potential energy functions are on equal footing (e.g., they correspond to different values of the free parameters (see Sec. 6)), there is no *correct* energy scale defined. However, in the case that the distance is used to compare an approximated potential to a more ab initio one or even to experimental data, the *correct* energy scale must be considered to be that of the more reliable potential and the rescaling b_{12} may be safely removed as indicated above. Note that the quantity d_{12} changes, when this removal is performed, from d_{12} to $d_{12}/|b_{12}|$ (to see this, take the analogous for σ_2 of Eq. 4c and change V_2^i by V_2^i/b_{12}, finally, take the result to Eq. 12a) and it is the second value which must be considered as the relevant one.

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3. Directly from its very definition in Eq. 7, one has that $d_{12} = 0$ is equivalent to V_2 being exactly a linear transformation of V_1, i.e., to $V_2(\vec{q}_i) = b_{12}V_1(\vec{q}_i) + a_{12}, \forall \vec{q}_i \in \{\vec{q}_i\}_{i=1}^{N}$.

4. As stated above, $b_{12} \neq 1$ and/or $a_{12} \neq 0$ must be regarded as two different types of systematic error easily removable and not involving any loss of information when one changes V_1 by V_2. In the general case, however, the energy differences associated to each potential energy function (which are the relevant physical quantities that govern the system behavior) present an additional random error which is intrinsic to the discrepancies between the potentials and can not be removed. As can be seen in Eq. 6, in this situation, d_{12} is the standard deviation of the random variable ΔV_2 and, as its value decreases, the distribution becomes sharper around the average $b_{12}\Delta V_1$. Moreover, because the distribution is normal, the probability of ΔV_2 being in the interval $(b_{12}\Delta V_1 - Kd_{12}, b_{12}\Delta V_1 + Kd_{12})$ is $\sim 38\%$ for $K = 1/2$, $\sim 68\%$ for $K = 1$, $\sim 95\%$ for $K = 2$, etc. Hence, d_{12} quantifies the random error between the trivially transformed potential $b_{12}V_1 + a_{12}$ and V_2, i.e., the unavoidable and fundamentally statistical part of the difference between V_1 and V_2 which stems from the complex character of the system.

5. To gain some insight about the meaning of d_{12}, the following *gedanken experiment* may be performed: if a Gaussian *noise* with zero mean and variance equal to s^2 were independently added to the linearly transformed V_1-energy, $b_{12}V_1(\vec{q}) + a_{12}$, of each conformation and the resulting potential were denoted by V_2, then one would have that the hypothesis in Eq. 2a is fulfilled and that $d_{12} = \sqrt{2}s$. Therefore, d_{12} may be regarded (except for a harmless factor $\sqrt{2}$) as the size of the Gaussian noise arising in the whole energy landscape when one changes $b_{12}V_1 + a_{12}$ by V_2.

6. Closely related to the properties in the two preceding points, an illuminating statistical statement about the energetic ordering of the conformations can be derived from Eq. 6. The probability that the energetic order of two randomly selected conformations is maintained when going from V_1 to V_2 (more precisely, that $sign(\Delta V_2) = sign(b_{12}\Delta V_1)$) conditioned by the knowledge of ΔV_1, can be easily shown to be:

$$P_{\text{ord}}\left(\frac{|b_{12}\Delta V_1|}{d_{12}}\right) = \frac{1}{2} + \frac{1}{\sqrt{2\pi}} \int_0^{|b_{12}\Delta V_1|/d_{12}} \exp\left[-\frac{x^2}{2}\right] \mathrm{d}x \ . \qquad (9)$$

The intuitive meaning of this expression is that d_{12} is the V_1-energy difference at which two randomly selected conformations can be typically *resolved* using V_2 after the removal of the harmless rescaling b_{12} (see Pt. 4). Certainly, if one has that $|b_{12}\Delta V_1| \ll d_{12}$, then $P_{\text{ord}} = 1/2$, reflecting a total lack of knowledge about the sign of ΔV_2 and, consequently, V_2 can not be used to resolve V_1-energy differences. If, on the contrary, $|b_{12}\Delta V_1| \gg d_{12}$, then $P_{\text{ord}} = 1$ and the conformations ordering is exactly

conserved. In any intermediate point, P_{ord} is a rapidly increasing function of $|b_{12}\Delta V_1|/d_{12}$ that reaches a *reasonable* value ($\sim 84\%$) when its argument equals 1, i.e., when $|b_{12}\Delta V_1| = d_{12}$. Some other interesting points are $P_{\mathrm{ord}}(1/2) \simeq 69\%$ or $P_{\mathrm{ord}}(2) \simeq 98\%$.

7. Finally, some clarifying properties of the distance associated to its relation to the Pearson's correlation coefficient will be investigated in Sec. 7 (see specially Eq. 12a).

The same considerations may be done about d_{21} regarding the transit from V_2 to V_1 and, as can be seen in Eq. 8, the square of $d(V_1, V_2)$ is the mean of the squares of d_{12} and d_{21}. Therefore, this measure of the difference between potential energy functions quantifies the average size of the uncertainty in the energy differences of the system that arises from changing one of the potentials studied by the other. If the comparison is performed between potential energy functions that stand on the same footing (see, for example, the second possible application in Sec. 6), the symmetric quantity $d(V_1, V_2)$ should be used as a summarizing measure of the loss of information involved in the transit from V_1 to V_2 and vice versa. However, if one of the potentials is a priori considered to be more ab initio or more accurate and it is compared to a less reliable instance, V_1 may denote the former, V_2 the latter and one may use only d_{12} as the measure of the discrepancies between them[10].

Hence, although both the discussion regarding the relevant values of the distance in the following section and the investigation of its mathematical properties in Sec. 8 and Appendix A are referred to $d(V_1, V_2)$ for generality, they may be equally applied to d_{12}. Conversely, the comparison between d_{12} and the quantities commonly used in the literature done in Sec. 7 may be extended to $d(V_1, V_2)$ upon symmetrization of ER, which is the only asymmetrical one.

5 Relevant values of the distance

Regarding the value of $d(V_1, V_2)$ in a practical case, some remarks must be made. One may expect two special values of the distance to exist: $d_{\min}$ and $d_{\max}$. In such a way that, if $d(V_1, V_2) < d_{\min}$, one potential energy function may be substituted by the other without altering the key characteristics of the system behavior, and that, if $d(V_1, V_2) > d_{\max}$, then, the substitution is not acceptable. This limiting values must be set depending on the particularities of the system studied and on the questions sought to be answered, and it may even be the case that some special features of the energy landscape are the main responsible of the behavior under scrutiny. For example, we are not going to establish any strict limit on the accuracy required for a potential energy function to successfully predict the folding of proteins [20, 21]. We consider this question a difficult theoretical issue, whose solution probably requires a much deeper knowledge of the protein folding problem itself than the one that exists at present, and

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[10]Note, from Eq. 8, that, if $d_{12} = d_{21}$, then $d(V_1, V_2) = d_{12} = d_{21}$.

we believe that it may be possible a priori that some special features of the energy landscapes of proteins (such as a funnel-like shape [28,29]) are the main responsible of the high efficiency and cooperativity of the folding process [20,21]. If this were the case, a different procedure for measuring the distance between potential energy functions could be devised for this situation [30–32], as any change of V_1 by V_2 which did not significantly alter these special features would be valid even if the value of $d(V_1, V_2)$ were very large. Our definition of $d(V_1, V_2)$, being based in characteristics shared by many complex systems and statistically referred to the whole energy landscape, is of more general application but cannot detect such particular features as the ones mentioned.

However, due to the laws of Statistical Mechanics, a rather stringent but general value for $d_{\min}$ can be used to a priori assess the interchangeability of V_1 and V_2. As can be seen in the thermodynamical equilibrium Boltzmann distribution, in which the probability p_i of a conformation $\vec{q}_i$ is proportional to $\exp(-V(\vec{q}_i)/RT)$, the order of the physical uncertainty in the potential energies of a system in contact with a thermal reservoir at temperature T is given by the quantity RT[11]. This typical energy sets the scale of the thermal fluctuations and it also determines the transition probability, $\min[1, \exp(-(V(\vec{q}_{i+1}) - V(\vec{q}_i))/RT)]$, in the Metropolis Monte Carlo scheme and the spread of the stochastic term in the Langevin equation [33,34]. Consequently, in this article, RT (which equals ~ 0.6 kcal/mol at room temperature) will be used as a general lower bound for $d_{\min}$. The results will be presented in units of RT and any two instances V_1 and V_2 of the same potential energy function whose distance $d(V_1, V_2)$ be smaller than RT will be regarded as physically equivalent[12].

Regarding d_{max}, no estimations of its value can a priori be made without referring to the particular potential energy functions compared and the relevant behavior studied. The fact that Eq. 12b has an absolute maximum when $r_{12} = 0$ sets only the worst possible upper bound and is only of mathematical interest.

6   Possible applications

There are at least three basic situations in which the distance defined in this article may be used to quantify the discrepancies between two different instances, V_1 and V_2, of the same potential energy:

- If the difference between V_1 and V_2 arise from the use of two distinct algorithms or approximations, $d(V_1, V_2)$ (or d_{12}, see the final lines of Sec. 4) may help us to decide whether the less numerically complex instance could be used or not. For example, one may compare the electrostatic part of the solvation energy calculated via solving the Poisson equation [36–39]

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[11] RT is preferred to $k_B T$ because per mole energy units are used in this article.

[12] This discussion is closely related to the common use of the concept of *chemical accuracy*, typically defined in the field of ab initio quantum chemistry as predicting bond-breaking energies to 1 kcal/mol [35].

with the instance of the same energy calculated using one of the many implementations of the Generalized Born model [9, 11–13, 16, 19, 40, 41], which are much less computationally demanding and more suitable for simulating macromolecules. If the distance between them is small enough for the behavior under study not to be much modified (see Sec. 5), the latter could be used. The second example in Sec. 9 is devoted to illustrate this type of comparison, which, of the three possible applications proposed in this section, is the one most commonly found in the literature.

- If the algorithm and the approximations are fixed and only one system S is studied, any reasonable functional form used to account for V will be a simplified model of physical reality and it will contain a number of free parameters $\vec{P}$. These parameters, which, in most of the cases, are not physically observable, must be fit against experimental or more ab initio results before using the function for practical purposes. For any fit to yield statistically significant values of the parameters, the particular region of the parameter space in which the final result lies must have the property of *robustness*, i.e., it must occur that, if the found set of parameters values is slightly changed, then, the relevant characteristics of the potential energy function which depends on them are also approximately kept unmodified. If this were not the case, a new fit, performed using a different set of experimental (or more ab initio) points, could produce a very distant potential. If V_1 and V_2 come from the same family of potential energy functions and they correspond to different values of the free parameters $\vec{P}$, the distance $d(V_1, V_2)$ between them may help us to assess the robustness of the potentials under changes on the parameters. In the first example of Sec. 9, the robustness of the van der Waals potential energy implemented in the well-known molecular dynamics program CHARMM [23, 24] is quantified as an example of this.

- The last application of the distance is fundamentally different of the ones in the previous points but, although the reasoning throughout the article is intentionally biased, for the sake of clarity, toward the study of potential energies of the same system, one may appeal to the same underlying assumptions to compare two different systems, S_1 and S_2, provided that a meaningful mapping can be established between both conformational spaces[13]. For example, if the conformations of a particular protein are described only by their backbone angles, one can define an unambiguous mapping between the conformations of, say, the wild-type chain and any mutated form, in such a way that $V_1(\vec{q})$ would represent the energies of the former and $V_2(\vec{q})$ those of the latter. The distance $d(V_1, V_2)$, in this case, quantifies how different the energy landscapes of the two systems are and, depending on the features under study, how sensitive the behavior of

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[13]In short, for the distance criterion to be applied, one needs to be able to assign two energies, $V_1(\vec{q})$ and $V_2(\vec{q})$, to each conformation $\vec{q}$. This is done trivially in the first two points but it requires a mapping between the conformational spaces of S_1 and S_2 in the third case.

the protein is to mutations. The comparison of a potential, V_1, to another one, V_2, which comes from the first via *integrating-out* certain degrees of freedom and which is commonly termed *effective potential energy* [42], may be considered to be another example of this type of application.

7 Relation to other statistical quantities

In the literature, some comparisons between potentials[14] are performed a posteriori, i.e., not directly studying the energies but computing some derived quantities, such as the pK_a of titratable groups [9, 12], investigating molecular dynamics trajectories [3, 6, 8, 13], comparing the ability of the different instances of V to select the correct native state of a protein from a set of decoys [8], etc.

For the a priori comparison of two ways of calculating the same potential energy, one may investigate the whole energy landscape visually if the system has no more than two degrees of freedom [14, 17], but, if the object of study is a protein or another complex system, the vastness of the conformational space and its lack of symmetries require the utilization of statistical quantities calculated from the energies of a finite set of conformations. Among the most common such measures, one may find the root mean square deviation (RMSD) [1, 6, 13, 14, 16], the mean error of the energies (ER) [1, 7, 10], the standard deviation of the error (SDER) [7], the mean of the absolute error (AER) [11], all of which have units of energy, and the Pearson's correlation coefficient r [2, 4, 6, 7, 10, 15, 19], which does not have units. Finally, in [16], a root mean square of the difference in the relative energies (REL) (see Eq. 10e for a clarification) which is proximate to d_{12} is defined, however, it has not been found to be used in any other work.

If we use the same notation as in Eq. 3 and we define $\Delta V_{12}^i := V_2^i - V_1^i$, the statistical quantities mentioned in the preceding paragraph (except r, which will be discussed later) are given by the expressions:

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[14]It must be pointed out that we have only found in the literature examples of the comparison between two potentials corresponding to the first case described in Sec. 6, which is associated to different algorithms or approximations. No examples of robustness studies have been found and, regarding the third case, in which the differences arise from a slight change in the system, such as a mutation in a protein, only articles investigating the total free energy of folding have been found [43, 44].

$$\mathrm{RMSD}(V_1, V_2) := \left[\frac{1}{N} \sum_{i=1}^{N} (\Delta V_{12}^i)^2 \right]^{1/2} , \tag{10a}$$

$$\mathrm{ER}(V_1, V_2) := \frac{1}{N} \sum_{i=1}^{N} \Delta V_{12}^i , \tag{10b}$$

$$\mathrm{SDER}(V_1, V_2) := \left[\frac{1}{N} \sum_{i=1}^{N} (\Delta V_{12}^i - \mathrm{ER}(V_1, V_2))^2 \right]^{1/2} , \tag{10c}$$

$$\mathrm{AER}(V_1, V_2) := \frac{1}{N} \sum_{i=1}^{N} |\Delta V_{12}^i| , \tag{10d}$$

$$\mathrm{REL}(V_1, V_2) := \left[\frac{2}{N(N-1)} \sum_{i=1}^{N} \sum_{j=i+1}^{N} (\Delta V_{12}^j - \Delta V_{12}^i)^2 \right]^{1/2} . \tag{10e}$$

In the following points, this measures of the difference between potential energy functions are individually compared to the distance defined in Sec. 3 and their limitations with respect to d_{12} are pointed out[15]:

- The first one, the RMSD, which is one of the most commonly used, presents the major flaw of overestimating the importance of an energy reference shift between V_1 and V_2. This transformation, which has no physical implications in the conformational behavior of the system, must not influence the assessment of the difference between potentials. This fact is, for example, detected in some of the comparisons performed in [6] and recognized to be conceptually erroneous in [1], where the shift is removed by minimizing the RMSD with respect to it. In addition, the RMSD also overestimates the effect of a slope $b_{12} \neq 1$ between the two potentials, a fact that, as has been remarked in Sec. 4, is not desirable (for a practical case in which the loss of information is small but $b_{12} \neq 1$ see [10]; for a numerical example see Fig. 4 and the discussion at the end of this section). It can be proved that, if $b_{12} = 1$ and $a_{12} = 0$, then $\mathrm{RMSD}(V_1, V_2) = d_{12}/\sqrt{2}$.

- ER, in turn, only accounts for a systematic error between the two potentials (an offset). The relation $\mathrm{ER}(V_1, V_2) = \mu_2 - \mu_1$ holds and ER equals the energy-reference shift a_{12} if $b_{12} = 1$ (see Eq. 3b). Thus, the changes in the conformational behavior of the system are not reflected by this quantity.

- In SDER, the standard deviation associated to ER, the reference shift is removed by subtracting ER from each difference ΔV_{12}^i. However, this

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[15]The quantity ER is not symmetrical. This is why all the measures in Eq. 10 are compared to d_{12} and not to its symmetrized version $d(V_1, V_2)$ (see Appendix A and the final part of Sec. 4).

quantity still overestimates the effect of a slope $b_{12} \neq 1$, in fact, only if $b_{12} = 1$, one has that $\mathrm{SDER}(V_1, V_2) = d_{12}/\sqrt{2}$.

- To establish precise relations between AER and d_{12} is difficult because of the modulus function that enters this quantity. Nevertheless, it is clear from its definition that AER, like ER, overestimates both the effect of an energy reference shift a_{12} and of a slope $b_{12} \neq 1$. For a numerical check of this fact, see Tab. 1.

- Finally, the measure REL, introduced in [16], has much of the spirit of the distance defined in this work. On one hand, it focuses on the energy differences, which are indeed the relevant physical quantities to study the conformational behavior of the system, on the other hand, it correctly removes the effect of an energy reference shift a_{12}. However, it still overestimates the importance of a slope $b_{12} \neq 1$ and one only has that $\mathrm{REL}(V_1, V_2) = d_{12}$ if $b_{12} = 1$.

There is yet another quantity commonly used for measuring the differences between two potentials: the Pearson's correlation coefficient (denoted by r_{12} in the following):

$$r_{12} := \frac{\mathrm{Cov}(V_1, V_2)}{\sigma_1 \sigma_2} \ . \tag{11}$$

This statistical measure differs from the ones discussed above in several points. On one hand, it has no units; a fact that renders difficult to extract from its value relevant statements about the energies studied. Some statistical statements can be made about the real value of r_{12} (the value in an infinite sample, denoted by ρ_{12}), however, to do this, the sampling distribution of r_{12} must be known. Without making stringent assumptions about the joint distribution $P_{12}(V_1, V_2)$ (see Sec. 2) only the null hypothesis of ρ_{12} being equal to 0 can be rejected from the knowledge of r_{12} in a finite sample [45]. This is clearly insufficient, because, in the vast majority of the cases, the researcher *knows* that the two potentials are correlated, i.e., the null hypothesis can be easily rejected from a priori considerations. If, in turn, one assumes $P_{12}(V_1, V_2)$ to be bivariate Gaussian, the Fisher transformation can be used to make inferences about ρ_{12} which are more general than $\rho_{12} \neq 0$ [45]. In any case, unfortunately, these type of statements are not directly translated into statements regarding the energies; a fact that undermines much of the physical meaning in r_{12}.

On the other hand and despite the disadvantages remarked in the preceding lines, r_{12} behaves satisfactorily when an energy reference a_{12} is added or when a rescaling $b_{12} \neq 1$ is introduced between V_1 and V_2; like d_{12}, and in contrast to RMSD, ER, SDER, AER and REL, the Pearson's correlation coefficient does not overestimate such transformations, in fact, r_{12} is completely insensitive to them. Therefore, it is not surprising that a simple general relation can be written between both r_{12} and d_{12}:

$$d_{12} = \sqrt{2}\sigma_2(1 - r_{12}^2)^{1/2} \; , \tag{12a}$$

$$d(V_1, V_2) = [(\sigma_1^2 + \sigma_2^2)(1 - r_{12}^2)]^{1/2} \; . \tag{12b}$$

In these expressions, it can be observed that the distance herein introduced depends on two factors: on one side, the width of the probability distributions associated to the potentials σ_1 and σ_2, which set the physical scale and give energy units to $d(V_1, V_2)$, on the other, the quantity $1 - r_{12}^2$, which measures the degree of correlation between V_1 and V_2. The second factor is completely insensitive to a change in the energy reference shift or in the slope (due to the properties of r_{12}); the part that depends on the width of the distributions, in turn, makes the distance sensitive to a change in the slope (remaining insensitive to a change in the reference), through σ_2 if the rescaling is performed on V_2 ($\sigma_2 \rightarrow \sigma_2/|b_{12}|$). However, contrarily to the case of the quantities in Eq. 10, the implications of such a transformation are not overestimated. In the case of our distance, the sensitivity to a rescaling arises only from the dilatation of the random errors, whereas the other quantities take erroneously into account the fact that the best fit line is not necessarily parallel to the line $V_2 = V_1$ (see the following numerical example).

In short, *the distance defined in this article consists in a physically meaningful way of giving energy units to the Pearson's correlation coefficient.*
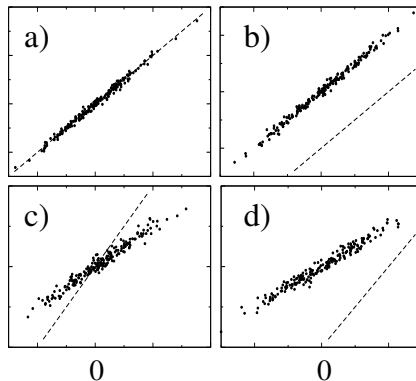

Figure 4: Numerical examples of the possible situations found in practical problems. 200 conformations are depicted for each case with the values of V_1 in the x-axis and the ones of V_2 in the y-axis (both in arbitrary energy units). The broken line corresponds to the line $V_2 = V_1$. **(a)** $b_{12} \simeq 1$ and $a_{12} \simeq 0$. **(b)** $b_{12} \simeq 1$ and $a_{12} \simeq 200$. **(c)** $b_{12} \simeq 1/2$ and $a_{12} \simeq 0$. **(d)** $b_{12} \simeq 1/2$ and $a_{12} \simeq 200$.

To close this section, a numerical example is presented that summarizes the situations that may be found in practical examples (see [10] for a real case of the issues raised) and that makes explicit the aforementioned disadvantages

		RMSD	ER	SDER	AER	REL	r_{12}	d_{12}
$b_{12} \simeq 1$	$a_{12} \simeq 0$	9.6	-0.7	9.6	7.7	13.6	0.995	13.5
$b_{12} \simeq 1$	$a_{12} \simeq 200$	199.8	199.6	9.4	199.6	13.3	0.996	13.2
$b_{12} \simeq 1/2$	$a_{12} \simeq 0$	52.7	-8.4	52.0	41.9	73.8	0.980	14.4
$b_{12} \simeq 1/2$	$a_{12} \simeq 200$	205.0	198.0	52.9	198.0	74.9	0.985	13.2
Overestimates $a_{12} \neq 0$		Yes	Yes	No	Yes	No	No	No
Overestimates $b_{12} \neq 1$		Yes	Yes	Yes	Yes	Yes	No	No
Has units of energy		Yes	Yes	Yes	Yes	Yes	No	Yes

Table 1: Values of the statistical quantities RMSD, ER, SDER, AER, REL, r_{12} and d_{12} computed in the situations depicted in Fig. 4. All the values are in arbitrary energy units except the ones of r_{12} which have no units. A summary of the properties of each quantity is presented in the bottom part of the table.

of the commonly used statistical quantities. In Fig. 4, four samples of 200 conformations are depicted with the values of V_1 in the x-axis and the ones of V_2 in the y-axis (both in arbitrary energy units). The different situations correspond to all generic cases in which $a_{12} = 0$ or $a_{12} \neq 0$ and in which $b_{12} = 1$ or $b_{12} \neq 1$. All the quantities discussed in this section, including d_{12}, have been computed in each case and their values are presented in Tab. 1.

From these data and the preceding discussion, some conclusions may be extracted. First, among the quantities with energy units, SDER and REL are the most proximate to the distance d_{12}, although they will overestimate the difference between potentials in situations in which there is a constant rescaling $b_{12} \neq 1$ between them. In Fig. 4c, for example, the contribution of the points that lie further apart from the origin of coordinates is overestimated by all the quantities in Eq. 10 for the sole fact that the best fit-line and the line $V_2 = V_1$ are not parallel (note that $a_{12} = 0$ and that the random noise associated to these points is not particularly large compared to the one that corresponds to the points in the central region of the figure). This is due to the fact that all quantities in Eq. 10 are based on ΔV_{12}^i, which measures the distance of each point to the line $V_2 = V_1$. A disadvantage that is not shared by d_{12}, which measures the differences with respect to the best-fit line.

Second, the Pearson's correlation coefficient r_{12} has good properties, although no physically relevant statements can be extracted from its value due, among other reasons, to the fact that it does not have units. In Tab. 1, for example, the value of r_{12} is close enough to 1 to be considered as a sound sign of correlation, however, the value of d_{12} (if we pretend it to be in kcal/mol, which could be the case) tells us that the typical indetermination in the energy differences, when substituting V_1 by V_2, is around 13 kcal/mol, a value an order of magnitude larger than RT. As explained in Sec. 5, this suggests that the relevant behavior of the system may be essentially modified.

Finally, it is worth stressing that all the considerations made in this section

17

and throughout the article are valid when the physical quantities compared are potential energy functions of the same system or closely related systems (see Sec. 6). When other quantities, such as the pK_a, charges, dipoles, Born radii, etc. or energies of distinct systems are the object of the comparison, the assessment of the discrepancies rests on different theoretical basis and, frequently, only semi-quantitative statements can be made. Acknowledging this limitation, the use of any of the quantities studied in this section, *including* d_{12}, may be fully justified. Note, in addition, that the numerical effort needed for the calculation of d_{12} is both low and very similar to the one required to compute any of the other quantities (see Sec. 10).

8 Additivity

Frequently, the potentials compared are instances of only a part of the total potential energy of the system. If the conclusions extracted, via $d(V_1, V_2)$, in such a case are pretended to be meaningfully transferred to the total energy, this measure of the difference between potentials must obey some reasonable additivity rules. Here, we will see that, for some relevant cases in which certain independence hypothesis are fulfilled, our distance is approximately additive, although, in other relevant situations, it is not.

For the sake of brevity, the notation will be much relaxed in this section and we will assume that we are working with six different potentials, x, y, p, r, q and s, that satisfy the following relations:

$$x = p + q \qquad \text{and} \qquad y = r + s . \tag{13}$$

Conceptually, x and y must be regarded as instances of the same potential energy and the same can be said about the pair p and r and the pair q and s. Hence, the study of the additivity of our distance rests on finding a way of expressing $d(x, y)$ as a function of $d(p, r)$ and $d(q, s)$. If one assumes that p is independent from q and that r is independent from s (see the discussion at the end of this section for the implications of such an hypothesis), one has that $r_{pq} = 0$, $r_{rs} = 0$, $\sigma_x^2 = \sigma_p^2 + \sigma_q^2$ and $\sigma_y^2 = \sigma_r^2 + \sigma_s^2$. In such a case, the following additivity relation can be written:

$$d^2(x, y) = d^2(p, r) + d^2(q, s) + \Delta d , \tag{14}$$

where:

$$\Delta d := (\sigma_p^2 + \sigma_r^2)(r_{pr}^2 - r_{xy}^2) + (\sigma_q^2 + \sigma_s^2)(r_{qs}^2 - r_{xy}^2) . \tag{15}$$

And the correlation coefficient r_{xy} can be expressed in terms of quantities associated to p, r, q and s in the following way (note that r_{xy} is indeed not additive):

$$r_{xy} = \frac{\sigma_p \sigma_r r_{pr} + \sigma_q \sigma_s r_{qs}}{(\sigma_p^2 + \sigma_q^2)^{1/2}(\sigma_r^2 + \sigma_s^2)^{1/2}} . \tag{16}$$

18

Now, one can see in Eq. 14 that, if Δd were zero, the square of the distance would be exactly additive in the aforementioned sense, making it possible to assert, for example, that, if p is proximate to r and q is proximate to s, then $x = p + q$ is proximate to $y = r + s$. Unfortunately, this is not the case. It can be shown that $\Delta d \geq 0$ (the distance is *over-additive*) and, without imposing any restriction on the potentials studied, nothing satisfactory can be said. For example, a particularly undesirable, albeit also uncommon, situation is that for which $\mathrm{Cov}(p, r) = -\mathrm{Cov}(q, s)$. Such a relation, makes zero the numerator in Eq. 16 and, consequently, r_{xy}. Substituting $r_{xy} = 0$ in Eq. 15 and taking Δd to Eq. 14, one has that, for every allowed value of r_{pr} and r_{qs}:

$$\mathrm{Cov}(p, r) = -\mathrm{Cov}(q, s) \Rightarrow d^2(x, y) = \sigma_p^2 + \sigma_q^2 + \sigma_r^2 + \sigma_s^2 = \sigma_x^2 + \sigma_y^2 \ , \qquad (17)$$

which is the worst possible value of $d(x, y)$.

However, there exists a particular class of situations than can be argued to be proximate to the situations found in typical cases and for which the additivity is approximately accomplished. These special situations are characterized for the satisfaction of the following relation:

$$\sigma_p/\sigma_r = \sigma_q/\sigma_s := k \ . \qquad (18)$$

When this equality is satisfied, it can be proved that the following quotient:

$$\Delta d_{\mathrm{rel}} := \Delta d/(d^2(p, r) + d^2(q, s)) \ , \qquad (19)$$

which measures the relative deviation from the exact additivity, does not depend on k and can be expressed as a function of only σ_r, σ_s, r_{pr} and r_{qs}. If, in addition, we define c through $\sigma_s = c\sigma_r$, without loss of generality, we can write Δd_{rel} as a function of only r_{pr}, r_{qs}, and c as follows:

$$\Delta d_{\mathrm{rel}} = \frac{c^2(r_{pr} - r_{qs})^2}{(1 + c^2)(1 - r_{pr}^2 + c^2(1 - r_{qs}^2))} \ . \qquad (20)$$

Representing this equation as a three-dimensional surface (see Fig. 5a), one has a *valley* whose lowest region lies in the line $r_{pr} = r_{qs}$ and has zero height, i.e., $\Delta d_{\mathrm{rel}}(r_{pr} = r_{qs}) = 0$. The slopes of the valley are curved and ascend as one moves away from the minimum line, eventually reaching arbitrarily large values of Δd_{rel} when $(r_{pr}, r_{qs}) \to (1, -1)$ or $(r_{pr}, r_{qs}) \to (-1, 1)$.

Numerically, the region for which the value of Δd_{rel} is acceptable is rather large. In Fig. 5b, the contour lines corresponding to $\Delta d_{\mathrm{rel}} = 10\%$ are depicted for some values of c that may be found in practical cases. It can be seen that, as one departs from $c = 1$, the region for which $\Delta d_{\mathrm{rel}} < 10\%$ gets larger, occupying, in any case, the majority of the (r_{pr}, r_{qs})-space. Therefore, one can conclude that, for the cases in which Eq. 18 is satisfied, the square of the distance introduced in this article is approximately additive in the relevant situations in which the correlations between p and r and between q and s are similar. Moreover, for continuity arguments, one has that, in the case that
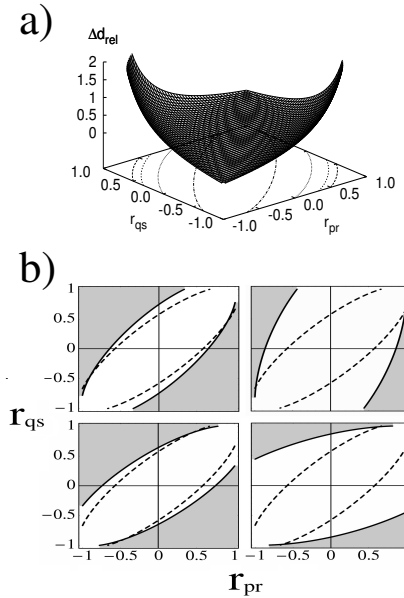
Figure 5: Graphical study of the additivity of the distance. **(a)** $\Delta d_{\rm rel}$ as a function of r_{pr} and r_{qs} for $c = 1$ (see Eq. 20). Contour lines are plotted at the levels $\Delta d_{\rm rel} = 10\%, 50\%, 100\%, 150\%$. **(b)** In white, the regions in (r_{pr}, r_{qs})-space with $\Delta d_{\rm rel} < 10\%$ for different values of c. From left to right and from top to bottom, each figure corresponds to $c = 1/2, 1/5, 2, 5$. In each case, the borders of the $\Delta d_{\rm rel} < 10\%$ region for $c = 1$ are shown with broken lines for comparison.

Eq. 18 were only approximately satisfied, the situation would be proximate to the one described in the previous lines.

Finally, some remarks must be made about the assumption of independence between p and q and between r and s. At first sight, one would say that this hypothesis, as the independence hypothesis in Sec. 2, is under researcher's control. In the case of a generic complex system (a spin glass, a random heteropolymer, etc.), this is indeed the case, however, if the object of study is a protein, one must be cautious. It is widely believed that the sequences of proteins are the result of a million-years-long selection process whose driving force is the search for the ability to fold rapidly and robustly [20,21,28,29]. Regarding the interactions responsible of the folding process, this means that they have been optimized in the sequence space to be *minimally frustrated* [46], i.e., maximally cooperative. In such a case, the correlations between different parts of the total potential energy may be large and the study of the additivity done in this section should be regarded as a privileged reference situation.
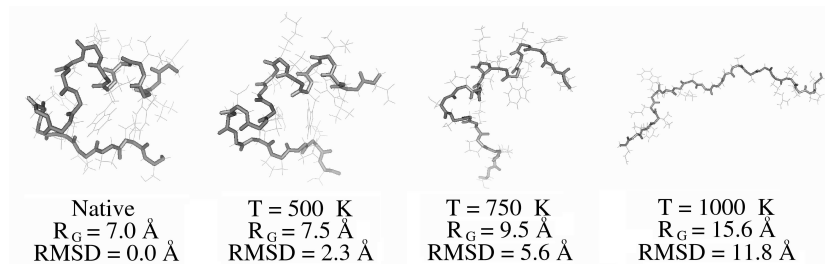
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9 Application

Figure 6: Native conformation of the Trp-Cage protein together with arbitrarily chosen structures from three particular subsets of the working set. The average radius of gyration $\langle R_G \rangle$ and the average RMSD with respect to the native structure is presented for each set. Both quantities have been computed taking into account only the α-carbons. Pictures generated with `PyMOL` (DeLano, W. L., 2002, `http://www.pymol.org`).

To illustrate one of the possible practical applications of the distance, we first study the robustness of the van der Waals energy, as implemented in the CHARMM molecular dynamics program [23, 24], in a particular system: the de novo designed protein known as Trp-Cage [47] (PDB code 1L2Y).

The program CHARMM itself was used as a conformation generator. From the native conformation stored in the Protein Data Bank [48], a 10 ps heating dynamics[16] was performed on the system, from an initial temperature $T_i = 0$ K to eleven different final temperatures (from $T_f = 500$ K to $T_f = 1000$ K in steps of 50 K). This was repeated 100 times for each final temperature with a different seed for the random numbers generator each time. The overall result of the process was the production of a working set of 1100 different conformations of the protein, whose structures range from *close to native* (the $T_f = 500$ K set) to *completely unfolded* (the $T_f = 1000$ K set) (see Fig. 6). It is worth remarking that the short time in which the system was heated (10 ps) and the fact that there was no equilibration after this process cause the final temperatures to be only *labels* for the eleven aforementioned sets of conformations. They are, by no means, the thermodynamical temperatures of any equilibrium state from which the structures are taken. This sets of conformations are only meant to sample the representative regions of the phase space. In Fig. 6, arbitrarily chosen structures from three particular sets are shown together with the native conformation. The average radius of gyration $\langle R_G \rangle$ of each set, depicted in the

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[16]The `c27b4` version of the CHARMM program was used. The molecular dynamics were performed using the *Leap Frog* algorithm therein implemented and the `param22` parameter set, which is optimized for proteins and nucleic acids. The water was taken into account implicitly with the [13] version of the Generalized Born Model built into the program.

same figure, must be compared to the radius of gyration of the native state[17]. The average RMSD of the structures in each set with respect to the native structure, calculated via the quaternion-based method described in [49], is also presented[18].

The van der Waals energy implemented in CHARMM may be expressed as follows:

$$V := \sum_{i<j} \left[(\varepsilon_i \varepsilon_j)^{1/2} \left(\left(\frac{R_i + R_j}{r_{ij}} \right)^{12} - 2 \left(\frac{R_i + R_j}{r_{ij}} \right)^{6} \right) \right] , \qquad (21)$$

where the sum is extended to all the pairs of atoms and the free parameters ε_i and R_i only depend on the type of atom (i.e., two atoms i and j of the same type have the same parameters assigned).

Using the working set of conformations described above of the Trp-Cage protein, the robustness of this potential energy function to changes in the free parameters ε_C and R_C associated to the aliphatic sp3 carbon CH (denoted by CT1 in CHARMM) is investigated. To do this, a finite grid-like set of points (ε_C^k, R_C^k) is chosen in the bi-dimensional parameter space, with ε_C^k ranging from -0.10 kcal/mol to -0.02 kcal/mol and R_C^k ranging from 2 Å to 4 Å. Then, for each point in this set, different values $\delta \varepsilon_C$ are added to and subtracted from ε_C^k, or different values δR_C are added to and subtracted from R_C^k independently. The potential that corresponds to $\varepsilon_C = \varepsilon_C^k - \delta \varepsilon_C$ is denoted by V_1, the one that corresponds to $\varepsilon_C = \varepsilon_C^k + \delta \varepsilon_C$ is denoted by V_2 (analogously with R_C) and the distance $d(V_1, V_2)$ between the two instances is computed in each case (i.e., for each *central point* (ε_C^k, R_C^k) and for each $\delta \varepsilon_C$ (or δR_C))[19].

This procedure allows us to study the dependence of the distance between V_1 and V_2 on the corresponding difference, $\delta \varepsilon_C$ or δR_C, between this two potentials in the parameter space for each central point (ε_C^k, R_C^k). This relation may be regarded as one between indetermination in the values of the free parameters and its influence on the conformational behavior of the system. From this point of view, the difference $\delta \varepsilon_C$ (or δR_C) in the parameter space for which the distance associated equals RT (see Sec. 5) must be considered an amount of indetermination in the parameters that does not involve relevant physical changes in the system. Therefore, if the parameters are known to a precision equal or greater than the one associated to these particular values of $\delta \varepsilon_C$ or δR_C, the statistical indetermination of the parameters may be regarded as harmless. The values of this differences (as a function of the central point (ε_C^k, R_C^k)) computed for the system studied in this section are depicted in Fig. 7.

Although this study only pretends to be an illustration of the concepts introduced in the previous sections and more features of the van der Waals energy

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[17]Both R_G and the RMSD have been computed taking into account only the α-carbons.

[18]The notation for this quantity, which is the root mean square deviation of the atomic coordinates of two structures after optimal superposition [49], is the same as the one used for the RMSD of the energies in Sec. 7. This choice has been made for consistency with the literature, in which this ambiguity is very common.

[19] It can be proved that, in this particular case, the normality hypothesis in eq. 2 is approximately fulfilled.
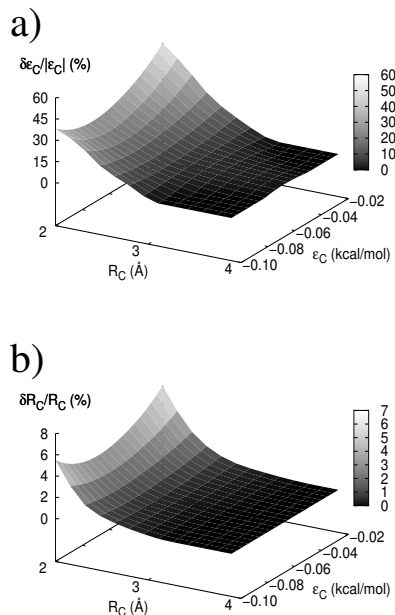
a)

b)

Figure 7: Robustness of the van der Waals energy to changes in some free parameters. Relative indetermination in ε_C **(a)** and in R_C **(b)** associated to $d(V_1, V_2) = RT$ as a function of the central point in the parameter space. Larger values of the relative indetermination correspond to greater robustness.

should be investigated elsewhere, some interesting remarks may be made about the results herein presented. One one hand, directly from Fig. 7, one can see that the precision needed in R_C is much greater than the one needed in ε_C, i.e., the van der Waals energy is more sensitive to changes in R_C than in ε_C. This is reasonable because V depends on R_C raised to the 12th and 6th power whereas ε_C only enters the expression raised to $1/2$ (see Eq. 21). On the other hand, the allowed indetermination in the parameters grows, in both cases, as R_C diminishes (the dependence on ε_C is much weaker). The reason for this being probably that, when the van der Waals radius R_C is large enough, the atoms begin to clash, i.e., the 12th power in Eq. 21, associated to the steric repulsion, begins to dominate over the 6th power term, associated to the attractive dispersion forces.

Finally, we would like to mention that, for the values $\varepsilon_C = -0.02$ kcal/mol and $R_C = 2.275$ Å, which are the ones used in the CHARMM `param22` parameter file, the allowed indeterminations in the parameters are $\delta\varepsilon_C/|\varepsilon_C| = 35$ % and $\delta R_C/R_C = 3$ %, in the region of relatively lower required precision (i.e., the relatively more favorable region). Note, however, that the indetermination for

23

R_C corresponds to ~ 0.07 Å, which is a rather demanding accuracy and suggests that, if the van der Waals radii set is changed, the behavior of the system may be significantly modified.

Comparison cell (row RHF 6–31+G(d), column RHF 3–21G):
d_{12} 3.06 RT
N_{res} 0.11
a_{12} 1459.5 kcal/mol
b_{12} 1.06

Comparison cell (row RHF 6–311++G(d,p)):
	RHF 3–21G	RHF 6–31+G(d)
d_{12}	3.13 RT	0.29 RT
N_{res}	0.10	12.13
a_{12}	1528.6 kcal/mol	69.1 kcal/mol
b_{12}	1.08	1.03

Comparison cell (row B3LYP 3–21G):
	RHF 3–21G	RHF 6–31+G(d)	RHF 6–311++G(d,p)
d_{12}	2.72 RT	4.65 RT	4.64 RT
N_{res}	0.13	0.05	0.05
a_{12}	1537.0 kcal/mol	78.7 kcal/mol	9.7 kcal/mol
b_{12}	1.02	0.85	0.82

Comparison cell (row B3LYP 6–31+G(d)):
	RHF 3–21G	RHF 6–31+G(d)	RHF 6–311++G(d,p)	B3LYP 3–21G
d_{12}	2.26 RT	1.75 RT	1.87 RT	3.60 RT
N_{res}	0.19	0.32	0.29	0.08
a_{12}	2991.1 kcal/mol	1531.9 kcal/mol	1462.9 kcal/mol	1454.6 kcal/mol
b_{12}	1.18	1.08	1.05	1.09

Comparison cell (row B3LYP 6–311++G(d,p)):
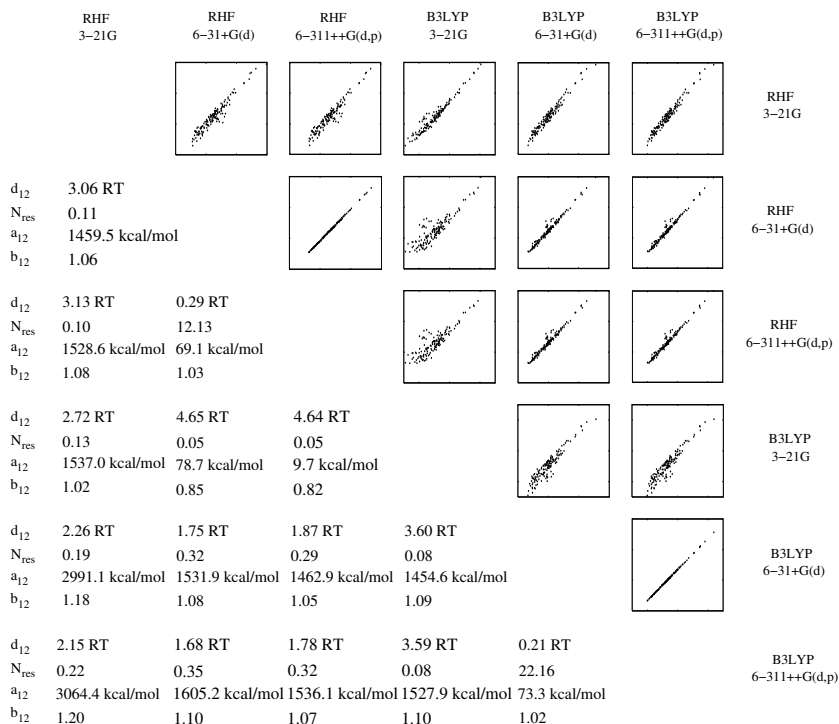|	RHF 3–21G	RHF 6–31+G(d)	RHF 6–311++G(d,p)	B3LYP 3–21G	B3LYP 6–31+G(d)
d_{12}	2.15 RT	1.68 RT	1.78 RT	3.59 RT	0.21 RT
N_{res}	0.22	0.35	0.32	0.08	22.16
a_{12}	3064.4 kcal/mol	1605.2 kcal/mol	1536.1 kcal/mol	1527.9 kcal/mol	73.3 kcal/mol
b_{12}	1.20	1.10	1.07	1.10	1.02

Figure 8: Comparison between different levels of the theory in the quantum mechanical ab initio study of the Potential Energy Surface (PES) associated with the Ramachandran angles of the model dipeptide HCO-L-Ala-NH$_2$. The figure must be read as follows: **1)** Any numerical set of measures is associated to the comparison between the level of the theory in the corresponding *row* (denoted by V_1) and *column* (denoted by V_2). **2)** The conformations scatter plot that belongs to a particular set of measures is the one that lies in the position which is obtained via reflection (of the set) with respect to the blank diagonal.

As a second brief example of the possible applications of the method, we present an exploratory comparison of different levels of theory in the quantum mechanical ab initio study of the Potential Energy Surface (PES) associated with the Ramachandran angles of the model dipeptide HCO-L-Ala-NH$_2$. This comparison is an example of the first point discussed in Sec. 6.

In [4], the PES of HCO-L-Ala-NH$_2$ is calculated with two methods, RHF and B3LYP, using, for each one, three different basis sets, 3-21G, 6-31+G(d) and 6-311++G(d,p). To do this, the Ramachandran space is divided in a 12x12 grid and, fixing the values of the Φ and Ψ torsional angles, a geometrical optimization

24

of the structure is performed at each point. This process produces the values of six different instances of the same potential energy on a working set of 144 conformations of the system.

In Fig. 8, each one of the six levels of the theory is compared to the other five (using the data provided by A. Perczel) and some relevant numerical measures are presented. The distance d_{12} is given in units of RT (at 300 K), the fitted energy reference shift a_{12} and slope b_{12} are also shown and the only quantity that requires further explanation is N_{res} (see Eq. 23 below).

One of the interests in studying PESs of peptide models lies on the possibility of using the results for modeling short oligo-peptides or even proteins [20]. If we imagine that we use the PES of HCO-L-Ala-NH$_2$ for constructing a potential that describes the behavior of a peptide formed by N alanine residues, the first *naive* attempt would be to simply add N times the potential energy surface of the individual HCO-L-Ala-NH$_2$ (making each term suitably depend on different pairs of Ramachandran angles). We may now ask whether the distance between two different instances of the N-residue peptide potential can be related to the distance between the corresponding mono-residue ones. It can be proved, appropriately choosing the working set of conformations of the larger system and using the relations presented in Sec. 8, that the following relation holds:

$$d_{12}(N) = \sqrt{N} d_{12}(1) \; , \tag{22}$$

where we have denoted by $d_{12}(N)$ the distance between the V_1 and V_2 potentials of the N-residue peptide.

Hence, we define N_{res} as the N for which $d_{12}(N) = RT$, representing up to which number of residues the criterium given in Sec. 5 will be satisfied:

$$d_{12}(N_{\text{res}}) := RT \quad \Longrightarrow \quad N_{\text{res}} = \left(\frac{RT}{d_{12}(1)} \right)^2 \; . \tag{23}$$

Although a much more exhaustive study will be carried out in future works, let us extract some meaningful conclusions from the data in Fig. 8. Note, first, that the only two cases for which $d_{12} < RT$ are RHF/6-31+G(d) vs. RHF/6-311++G(d,p) and B3LYP/6-31+G(d) vs. B3LYP/6-311++G(d,p). This means that the convergence in basis is achieved for both methods somewhere between 6-31+G(d) and 6-311++G(d,p) and suggests (for HCO-L-Ala-NH$_2$) that there is no need in going above 6-31+G(d). Of course, the fact that $N_{\text{res}} \simeq 22$, in the B3LYP case, and $N_{\text{res}} \simeq 12$, in the RHF case, places a limit on the size of the system for which the similarity of the two levels should be considered as sufficient. Finally, note that the distance between RHF/6-311++G(d,p) and B3LYP/6-311++G(d,p) is 1.78 RT, which means that the convergence in methods has not been achieved and some more accurate method should be studied.

10   Conclusions

In this work, a measure $d(V_1, V_2)$ of the differences between two instances of the same potential energy has been defined and the following points about it have

been discussed:

- It rests on hypothesis whose validity stems from general characteristics shared by many complex systems and from the statistical laws of large numbers. We believe that, without knowing specific details of the system, the statistical approach is unavoidable and, among the many criteria, our distance is the most meaningful way of quantifying the differences between potentials.

- It allows to make physically meaningful statements about the way in which the energy differences between conformations change (or how the energetic ordering of the conformations is altered) upon substitution of one potential by the other.

- It may be applied to at least three practical situations characterized by the origin of the differences between the potentials.

    - Different algorithms or approximations are used (potential design).
    - The potential energy function depends on free parameters and the two instances correspond to different values of them (robustness).
    - Slightly different systems are compared (mutational studies, effective potentials).

- It presents advantages over the commonly used quantities RMSD, ER, SDER and AER that consist mainly of not overestimating irrelevant transformations on the potentials, such as adding an energy reference or rescaling one of them. Regarding the Pearson's correlation coefficient r, our distance may be considered as a physically meaningful way of giving him energy units. Finally, the numerical complexity involved in the calculation of $d(V_1, V_2)$ (see below) is similar to the one associated to any of the other quantities.

- It is approximately additive for most of the interesting situations encountered in practical cases.

In addition, a first practical example, which consists in the study of the robustness to changes in the free parameters of the van der Waals energy in CHARMM, and a second one, in which the ab initio potential energy surfaces of the HCO-L-Ala-NH$_2$ molecule calculated at different levels of the theory are compared, have been presented to illustrate the concepts discussed.

Finally, we would like to summarize the steps that must be followed to compute the distance in a practical case. Although all that follows has been said, we believe that a brief *recipe* could be useful for quick reference:

1. Generate a working set of independent conformations $\{\vec{q}_i\}_{i=1}^N$ (see Sec. 2 and the last paragraph of Sec. 3).

2. Denote $V_1^i := V_1(\vec{q}_i)$, $V_2^i := V_2(\vec{q}_i)$ and compute the statistical quantities μ_1, μ_2, σ_1 and $\mathrm{Cov}(V_1, V_2)$ in Eq. 4.

3. With them, calculate the mean-square estimators through Eq. 3. First b_{12}, then a_{12} and, finally σ_{12}.

4. If comparing a potential to a more accurate instance, use $d_{12} = \sqrt{2}\sigma_{12}$ to find the asymmetrical version of the distance between them, and rescale V_2 dividing it by b_{12} if desired. Otherwise, repeat the steps 2 and 3 changing $1 \leftrightarrow 2$ in all the expressions to compute σ_{21} and use Eq. 5 to finally arrive to $d(V_1, V_2)$.

5. If $d(V_1, V_2) < RT$ (or $d_{12} < RT$, depending on the case), the two potentials may be considered physically equivalent.

Appendix A: Metric properties

For completeness, and because, in the case of our distance, it is illustrative to do so, we will investigate, in the following, in which situations (which will turn out to be rather common) the behavior of $d(V_1, V_2)$ approaches that of a traditional mathematical *distance*. However, it must be stressed that the measure introduced in this article was never intended to be such an object. Its meaning is encoded in the statistical statements derived from its value (see Sec. 4) and the name *distance* must be used in a more relaxed manner than the one traditionally found in mathematics.

The object $\mathcal{D}(x, y)$ is said to be a *distance* (also a *metric*) in mathematics if it satisfies the following properties:

1. $\mathcal{D}(x, y) = 0 \Leftrightarrow x = y$

2. Positivity: $\mathcal{D}(x, y) \geq 0$

3. Symmetry: $\mathcal{D}(x, y) = \mathcal{D}(y, x)$

4. Triangle inequality: $\mathcal{D}(x, z) \leq \mathcal{D}(x, y) + \mathcal{D}(y, z)$

Whereas, in the case of $d(V_1, V_2)$:

1. The first property is not fulfilled. One certainly has the implication to the left, but the direct implication is false in general. As has been stated in the Pt. 3, in Sec. 4, the analogous property that $d(V_1, V_2)$ satisfies is that $d(V_1, V_2) = 0$ is equivalent to V_2 being a linear transformation of V_1 and vice versa, i.e., to $V_2(\vec{q_i}) = b_{12}V_1(\vec{q_i}) + a_{12}$ and $V_1(\vec{q_i}) = b_{21}V_2(\vec{q_i}) + a_{21}$, $\forall \vec{q_i} \in \{\vec{q_i}\}_{i=1}^{N}$. Where, additionally, one has that $b_{21} = 1/b_{12}$ and $a_{21} = -a_{12}/b_{12}$. The fact that this property of a mathematical distance is not satisfied by $d(V_1, V_2)$ must be considered an advantage, because, as has been remarked in previous sections, it is reasonable to regard as equivalent two potentials if there is only a linear transformation between them.

2. $d(V_1, V_2) \geq 0$ for every V_1 and V_2.

3. Directly from its definition in Eq. 5 or from Eq. 8, it is evident that $d(V_1, V_2)$ is symmetrical under change of V_1 by V_2. This property is not fulfilled by the quantity d_{12}. However, the situation in which it is reasonable to use it (the comparison of a particular instance of a potential energy V to a less accurate one) is also intrinsically asymmetrical (see the final part of Sec. 4).

4. The triangle inequality, in this context, is a relation that must be expressed as a function of the statistical quantities related to three different potentials, V_1, V_2 and V_3, as follows:

$$\sqrt{\sigma_1^2 + \sigma_3^2}\sqrt{1 - r_{13}^2} \leq \sqrt{\sigma_1^2 + \sigma_2^2}\sqrt{1 - r_{12}^2} + \sqrt{\sigma_2^2 + \sigma_3^2}\sqrt{1 - r_{23}^2} . \quad (24)$$

This relation is not fulfilled for every triplet (V_1, V_2, V_3), i.e., the distance introduced in this article does not satisfy, in general, the triangle inequality. A simple counterexample is found if one makes σ_3 grow, keeping the rest of the quantities in Eq. 24 constant. For σ_3 large enough, the relation above may be approximated by:

$$\sigma_3 \left[\sqrt{1 - r_{13}^2} - \sqrt{1 - r_{23}^2} \right] \leq \sqrt{\sigma_1^2 + \sigma_2^2}\sqrt{1 - r_{12}^2} . \quad (25)$$

Then, if $r_{13}^2 < r_{23}^2$, one may make σ_3 even larger and eventually break the inequality (in the case that it were not already broken for the value of σ_3 for which Eq. 25 is a good approximation). As a final remark, it is worth pointing out that, despite the general mathematical facts stated above, there is a particular situation, which is also expected to be similar to the situations relevant to be studied, in which the distance has been found to satisfy the triangle inequality. If one has that $\sigma_1 = \sigma_2 = \sigma_3$ (something that is expected to be approximately true in the case that the three potentials are proximate), Eq. 24 turns into a relation involving only the correlation coefficients:

$$\sqrt{1 - r_{13}^2} \leq \sqrt{1 - r_{12}^2} + \sqrt{1 - r_{23}^2} . \quad (26)$$

In addition, assuming the hypothesis discussed in Sec. 2, the following inequalities can be proved [45] without any further assumptions about the potentials:

$$r_{13} \geq r_{12}r_{23} - \sqrt{1 - r_{12}^2}\sqrt{1 - r_{23}^2} , \quad (27a)$$

$$r_{13} \leq r_{12}r_{23} + \sqrt{1 - r_{12}^2}\sqrt{1 - r_{23}^2} . \quad (27b)$$

We have numerically found that, if the relations in Eq. 27 are satisfied, so is the one in Eq. 26. Hence, if $\sigma_1 = \sigma_2 = \sigma_3$, then, for all values of r_{12}, r_{23} and r_{13}, the distance satisfies the triangle inequality. Clearly, for continuity, if $\sigma_1 = \sigma_2 = \sigma_3$ is not exactly but approximately satisfied,

then, although the triangle inequality may be broken, it will broken by a small relative amount. In short, if one has $\sigma_1 = \sigma_2 = \sigma_3$ approximately, one has the triangle inequality also approximately.

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