Coarse-graining protein energetics in sequence variables

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Abstract

We show that cluster expansion (CE), previously used to model solid-state materials with binary or ternary configurational disorder, can be extended to the protein design problem. We present a generalized CE framework suitable for protein studies, in which properties such as the energy can be unambiguously expanded in the amino-acid sequence space. The CE coarse-grains over non-sequence degrees of freedom (e.g., sidechain conformations) and thereby simplifies the problem of designing proteins, or predicting the compatibility of a sequence with a given structure, by many orders of magnitude. The CE is physically transparent, and can be evaluated through linear regression on the energy of training sequences. The approach is demonstrated on two distinct backbone folds. We show that good prediction accuracy is obtained with up to pairwise interactions for a coiled-coil backbone, and that triplet interactions are important in the energetics of a more globular zinc-finger backbone.

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Protein folding and protein design stand among the most formidable challenges in contemporary computational biology. The 3-D structure of a protein is uniquely encoded in its 1-D sequence of amino acids (AA), and enormous theoretical and computational research effort has been devoted to understanding how [1, 2, 3]. The problem can be posed two ways: protein folding deals with predicting the final 3-D structure of a protein given its AA sequence, whereas protein design is concerned with finding an optimal sequence to fold to a pre-defined structure. Protein design is useful both because it allows for the engineering of macromolecules with desired properties [4, 5, 6], and because the development of computational design methods deepens our general understanding of protein folding and stability. Scoring functions that indicate the ability of sequences to fold to any given structure are central to both the folding and design problems. These range from statistical knowledge-based functions derived from databases of known protein structures [7] to empirical functions mainly based on experimental measurements [8], to more physics-based functions that attempt to model protein free energy [8, 9].

Physics-based energy functions have the potential of being the most accurate and interpretable. These express the energy of a protein sequence adopting a specified structure in terms of atomic coordinates, and account for energies arising from van der Waals (vdW) forces, electrostatics, and solvation. All atoms in a protein can be classified as either “backbone” or “side-chain”. The backbone atoms are the same for each AA and represent the overall structure or “fold” of a protein, as shown for two examples in Fig. 1. The side-chain atoms are different for different AAs, and give rise to additional degrees of freedom termed “side-chain conformations” or “rotamers” (see Fig. 1B-C). Even for a relatively small protein fold of 100 AAs there are roughly $10^{130}$ possible sequences. Accounting for side-chain conformations expands the search space to $\sim 10^{230}$ structures. The computational complexity of high-quality physics-based scoring functions makes a search for optimal sequences intractable. Because sequence determines the structure of a protein, however, a function should exist that maps sequence directly to energy. A sufficiently accurate and computationally tractable approximation of this function would find widespread use in computational studies of protein structure.

Mapping sequence to energy is similar to the configurational problem in alloy theory [10, 11, 12] where distributions of A and B atoms on a fixed topology of lattice sites specifies the energy [13, 14, 15, 16]. The technique of cluster expansion (CE) [10, 11] has proven
extremely useful for rapidly evaluating the energies of alloys and searching for low-energy
configurations. In this Letter, we apply CE to the protein design problem, deriving two
structure-specific functions that can determine the energies of a sequence adopting either
a coiled-coil or a zinc-finger geometry. Searches using these functions can be used in the
future to identify low-energy sequences that adopt these folds. Further, CE can potentially
be applied directly to the more challenging protein folding problem by deriving a function
specific to each of the \( \sim 1,000 \) known protein folds. Rapid evaluation of a sequence with the
full panel of functions could identify the best structure. This approach, termed “threading”
or “fold recognition”, is widely used for structure prediction in combination with statistically
derived energy functions.

While in alloys one typically treats binary distributions (two possible species per site) or
on rare occasions ternaries \[17, 18\], the general protein design problem requires extension
to all twenty possible AAs. For a protein of \( L \) residues let the variable \( \sigma^i = 1 \ldots m \) indicate
which of the \( m \) AAs is present at site \( i \). A sequence is then expressed by \( \vec{\sigma} = \{ \sigma^1, \ldots, \sigma^L \} \).
The energy of a protein \( E[\vec{\sigma}, \vec{\tau}] \) depends on this sequence and on the other microscopic infor-
mation \( \vec{\tau} \) (e.g. positions of all atoms on the protein and solvent molecules). The important
energy function in protein design, \( E_{\text{min}}[\vec{\sigma}] \), can be obtained by optimizing over \( \vec{\tau} \):

\[
E_{\text{min}}[\vec{\sigma}] = \min_{\vec{\tau}} E[\vec{\sigma}, \vec{\tau}] .
\]

(1)
The CE is a general approach to obtain \( E_{\text{min}} \) by expanding in a suitable set of independent
basis functions. Let \( i, j, k = 1 \ldots L \) denote AA sites and \( \alpha, \beta, \gamma = 1 \ldots m - 1 \) index basis
functions \( \{1, \phi^i_\alpha, \phi^j_\alpha, \phi^j_\beta, \phi^j_\gamma, \ldots \} \). The energy can be expanded as:

\[
E_{\text{min}}[\vec{\sigma}] = J_0 + \sum_{i,\alpha} J^i_\alpha \phi^i_\alpha(\sigma^i) + \sum_{ij,\alpha\beta} J^{ij}_{\alpha\beta} \phi^i_\alpha(\sigma^i)\phi^j_\beta(\sigma^j) \\
+ \sum_{ijk,\alpha\beta\gamma} J^{ijk}_{\alpha\beta\gamma} \phi^i_\alpha(\sigma^i)\phi^j_\beta(\sigma^j)\phi^k_\gamma(\sigma^k) + \ldots
\]

(2)
where the \( J_s \) are expansion coefficients. We leave it to a future paper to describe the
mathematical properties of this basis set and to show its completeness in the space of all
possible \( L \)-site AA sequences. Eqn.\( \boxed{2} \) is in principle exact, though in practice the expansion
has to be truncated. While the \( J \) coefficients depend on the choice of basis functions, the
sum over terms spanning an cluster of AA sites \( \{i, \ldots, j\} \) has a physical interpretation, and
can be defined as effective interaction (EI) between the AA’s on these sites:

\[
EI(\sigma^i \ldots \sigma^j) = \sum_{\alpha \ldots \beta} J_{\alpha \ldots \beta}^i \phi_{\alpha}^i(\sigma^i) \ldots \phi_{\beta}^j(\sigma^j).
\] (3)

The choice of point basis functions \(\phi_{\alpha}\) is in principle arbitrary though we have found that previously proposed basis functions [10] have poor numerical stability for the high dimensional configuration spaces of proteins and make the expansion converge slowly. In this Letter we use \(\phi_{\alpha}(\sigma) = \delta(\sigma - \alpha)\). Hence \(\phi_{\alpha}(m) \equiv 0\) and the hypothetical sequence \(\{m, \ldots, m\}\) has energy \(J_0\). If we assign \(m\) to Alanine (Ala) any point EI(\(\sigma^i\)) equals the energetic contribution of \(\sigma^i\) relative to Ala. Therefore, point EIs exactly correspond to the change in energy upon mutating a residue to alanine, a quantity that is frequently measured experimentally to assess the importance of a residue to stability. Pair EI(\(\sigma^i, \sigma^j\)) is the interaction of an AA pair. This is also a measure well known to biochemists [19, 20]. This concept can be taken beyond pairs – contributions purely from triplets can be measured similarly. Although this is difficult to do experimentally, the CE allows one to systematically analyze the importance of higher order interactions.

Given \(E_{\text{min}}\) for enough sequences, \(J_s\)s can be extracted by standard fitting procedures. Determining which \(J_s\)s to keep in the fit is not always obvious. While one may be guided by the idea that point terms are larger than pairs, which in turn are larger than triplets, this is not always true. We use a more systematic way for evaluating important \(J_s\)s based on the cross-validation (CV) score [21]. Essentially, the CV score is the average error with which each sequence is predicted when left out of the fitting, and as such is a good measure of the prediction power. Our procedure consists of fitting a selected set of candidate clusters and order them by the average \(|J|\). Clusters for which the \(J\) value largely arises from numeric noise increase the CV score, and are excluded. When a cluster is included, so are all of its sub-clusters.

We demonstrate the power of the CE by testing it on two different protein folds, mimicking the protein design problem. The folding energy is defined as the energy difference between the folded and the unfolded states: \(E_{\text{folding}} = E_{\text{folded}} - E_{\text{unfolded}}\). Although the CE can in principle be used with any energy model, we test it here with a physically meaningful but relatively simple expression similar to Hamiltonians commonly used in the design field [22]:

\[
E[\vec{\sigma}, \vec{\tau}] = E^{\text{vdW}} + E^{\text{elec, wat}} + E^{\text{solv, sc}} + E^{\text{torsion}},
\] (4)
FIG. 1: A) Two protein folds - the coiled coil (top - side view; left - helix axis view) and the zinc-finger (right). Orange spheres are backbone atoms and the ribbons are a cartoon representation of the backbone geometry. The coiled-coil unit cells are highlighted. B) The optimal rotamers for two AA’s in an all-atom representation. C) A set of common rotamers for one AA shown superimposed.

where $E^{\text{vdW}}$ is the vdW interaction modeled as a 6-12 Lennard-Jones potential, $E^{\text{elec, wat}}$ is the total electrostatic energy (excluding intra-sidechain interactions), $E^{\text{solv, sc}}$ is the solvation energy of all backbone and sidechain atoms [23], and $E^{\text{torsion}}$ is the sidechain torsional energy. All energy terms are calculated using the CHARMM package [24] with the param19 parameters. The unfolded state is modeled by retaining only sidechain self energies and local interactions between sidechains and their surrounding penta-peptide backbone. Because $E[\vec{\sigma}, \vec{\tau}]$ in Eq. 4 is pairwise-decomposable, we are able to apply the dead-end-elimination (DEE) algorithm [25, 26] followed by a branch-and-bound search to arrive at the optimal sidechain conformations corresponding to $E_{\text{min}}$. Thus in a CE derived from these $E_{\text{min}}$, the $J$s, and hence EIs, parameterize optimized energies whereby all the sidechain degrees of freedom are coarse-grained out. The EI, defined at the sequence level, may include higher order terms even though the initial energy expressions at the conformational level are pairwise decomposable. The advantage of this procedure is an enormous reduction in the search space, from $(20m)^L$ to $m^L$, where 20 is the average number of rotamers considered per AA.

In order to more accurately fit the important low energies, our fitting is weighted by $\max(e^{-(E-E_0)/K}, w_0)$, where $E_0$ is the lowest energy in the data set, $K$ is approximately the range of interest above $E_0$ and $w_0$ is the minimal weight at large $E$ to avoid numeric instability.
Our first case study involves the coiled coil, a common and well-characterized protein interaction interface. (Fig 1). An ideal coiled-coil backbone possesses a screw axis with a repeating unit every 7 residues (a heptad) as well as C2 symmetry about the coil axis [27]. We use a unit cell (highlighted on Fig. 1) consisting of 4 heptads. With the assumption that significant interactions are short range, the unit cell incorporates all clusters important to describe coiled-coil stability. Only 4 sites in each heptad are each modeled as one of 16 selected AA species (the 3 remaining sites are set to Ala). These 4 sites have been shown, in many cases, to be sufficient to determine coiled-coil dimerization preferences and other properties [28, 29]. The backbone is extended by an identical unit cell sequence at both ends to avoid edge effects. The optimized sidechain configurations correspond to $E_{\text{min}}$ of the entire protein. The energy of the central unit cell plus half of its interaction with the rest of the system is presented.

Our training set consists of 21,066 randomly chosen sequences weighted by $\max(e^{-(E+26)/120}, 0.01)$. Truncating the CE at the pair level is sufficient to accurately reproduce the energetics of the system. The structural symmetry reduces all 137 clusters up to pairs to 1 constant, 4 point and 36 pair-level independent cluster (7741 independent $J$s).
We are therefore able to include all of them as candidate clusters in the fitting. Fig. 2 shows the weighted RMS and CV scores of the least square fitting versus the number of included clusters (ordered by \(\langle|J|\rangle\)). Although the RMS decreases monotonically as expected, the CV score reaches a minimum at 22 clusters, and fluctuates (mostly increases) slightly afterwards. We thus come to an “optimal” set of 22 clusters (3676 Js) for energy prediction, with weighted RMS = 1.0 kcal/mol and CV = 1.1 kcal/mol. The most significant EIs are found to correspond to residues that mediate contacts between different helices, in agreement with biologists’ intuition about the system.

To test the predictive character of the CE we compare its energy for 3995 random sequences not included in training to the directly calculated energy (Fig. 2 inset). The unweighted RMS error is 2.4 kcal/mol for all energies and 1.7 kcal/mol for \(-26 < E_{\text{min}} < 10\) kcal/mol. The error is sufficiently small for such applications as sequence optimization, and is comparable with the accuracy of the underlying energy model. We trade such a small error for being able to predict the optimal energy of any sequence by summation of EIs for 22 clusters, as opposed to performing global optimization in side-chain conformation space of \(5.9 \times 10^{55}\) on average. Even compared to the highly efficient DEE method for sidechain positioning, the time to calculate \(E_{\text{min}}\) of a sequence is reduced from \(~200\) sec to \(~1\) \(\mu s\) with our coarse-grained Hamiltonian, a \(2 \times 10^8\)-fold acceleration.

As a second application we consider the zinc-finger, a conserved DNA-binding fold (Fig II A). The backbone of Zif268 (PDB ID 1ZAA) residues 33-60 is used as a model Zn-finger structure. Following Mayo et al. [30], we consider a sequence space in which 2 sites are fixed, 1 site has 7 candidate species, 18 sites have 10 and the other 7 sites have 16. No symmetry condition is applied. The training set consists of 29,864 random sequences weighted by max(e\(^{-\frac{(E+35)}{100}}\), 0.01). Since there are too many pairs (325 pairs, or \(4 \times 10^4\) Js) to easily include in one single fitting, we start with constant and point terms and add pairs one by one to the existing clusters, retaining a pair if it decreases CV. We iterate until no new pair can be selected. However, truncation at pairs leads to an unsatisfactory fitting with CV > 6 kcal/mol. Instead of trying all 2,600 triplets, we use the characteristics of the poorly fitted sequences \(\mathcal{A}:|E| > 10\) kcal/mol to locate important triplets. We calculate the information content \(I^i = \ln m^i - S(p(\sigma^i|\mathcal{A}))\), \(I^{ij} = \ln m^i m^j - S(p(\sigma^i \sigma^j|\mathcal{A})) - I^i - I^j\) for each site \(i\) and each pair \(\{i, j\}\) out of the AA distribution in \(\mathcal{A}\) (\(S(p) = -\sum_{(p)} p\ln p\) denotes entropy). Four sites have large \(I^i\) that are almost exclusively occupied by aromatic sidechains W, H,
Y and F. Five out of the 6 pairs formed by these sites have significant $I^{ij}$. Located in proximity to each other, these sites constitute 2 triplet clusters (see fig. 1A). Thus we use one constant, 26 point, 24 pair, and 2 triplet clusters (5692 $J$s in total) for fitting. RMS and CV scores versus the number of clusters included are shown in Fig. 3. The two triplets are found to be indispensable in correctly reproducing the energies. This demonstrates the existence of complex correlation in a globular protein, and the CE provides a systematic, quantitative way of identifying such correlated sites. Prediction of 4000 random Zn-finger sequences is shown in Fig. 3 inset. Again a reasonably good accuracy of 2.7 kcal/mol for $-35 < E_{\text{min}} < 10$ kcal/mol is obtained. Although a larger prediction error 15.4 kcal/mol is obtained with all energies, high energy sequences are correctly detected. Such error is traded for a remarkable reduction in search space: from $1.4 \times 10^{60}$ to $1.9 \times 10^{27}$ states.

In summary, we have demonstrated how the energetics of a protein with pre-defined backbone can be coarse-grained to a function of sequence only. The expansion’s accuracy can be systematically improved. We have successfully applied the method to two distinct families of proteins, and found two different types of interactions determining stability. The accuracy of the CE predictions implies that this much simpler expression can be used in

FIG. 3: RMS and CV scores for Zn-finger fitting. Inset: CE predicted vs. atomistic $E_{\text{min}}$ for 4000 random sequences (only $E_{\text{min}} \leq 10$ kcal/mol shown).
place of traditional Hamiltonians, dramatically improving computational efficiency.

The CE methodology can be coupled with any energy model, e.g. more accurate Hamiltonians or experimentally determined energies, and properties other than energy are potentially expandable. Thus, it can be extended to treat any multi-species search problem for which an appropriate scoring scheme can be generated. In structural biology, this includes modeling not only protein stability, but protein interaction specificity, DNA and RNA structure, protein-DNA interactions, and potentially the interactions of small-molecule pharmaceuticals. We are optimistic that the method will find a wide range of practical applications in biology research.

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