Inverse design of proteins with hydrophobic and polar amino acids.

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A two amino acid (hydrophobic and polar) scheme is used to perform the design on target conformations corresponding to the native states of twenty single chain proteins. Strikingly, the percentage of successful identification of the nature of the residues benchmarked against naturally occurring proteins and their homologues is around 75% independent of the complexity of the design procedure. Typically, the lowest success rate occurs for residues such as alanine that have a high secondary structure functionality. Using a simple lattice model, we argue that one possible shortcoming of the model studied may involve the coarse-graining of the twenty kinds of amino acids into just two effective types.

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I. INTRODUCTION

The gigantic efforts spent by the scientific community over the past decades have unravelled many of the mysteries lying behind the chemistry and biological functionality of proteins ², ⁴, ⁶. However, two fundamental questions remain unanswered:

1. what are the mechanisms that guide the folding of a string of amino acids into a complicated three-dimensional structure of helices, loops and β-sheets,
2. how does one identify the sequence of amino acids that folds into a pre-assigned native structure.

This study, building on the pioneering work of Shakhnovich and Gutin ¹³ and Sun et al. ²⁰, deals with the second issue which is commonly referred to as the inverse folding problem ¹, ³, ⁵, ⁸, ¹⁰, ¹³, ¹⁴, ¹⁵, ¹⁶, ¹⁷, ¹⁸, ²¹. This problem is central in many areas of biology and medicine; indeed the possibility of designing artificial proteins would open far-reaching pharmaceutical applications.

The complexity of the problem is enormous because, in principle, it entails an exhaustive comparison of the native states of all sequences in search for the one(s) matching the desired target structure. This procedure has been recently formulated into a general mathematical form appropriate for numerical implementation ¹³ which shows that solving the design problem for a structure, Γ, amounts to the identification of the amino acid sequence, S, that maximizes the occupation probability, \( P_Γ(S) \),

\[
P_Γ(S) = \frac{e^{-\beta E_Γ(S)}}{\sum_{Γ'} e^{-\beta E_{Γ'}(S)}} = e^{-\beta(E_Γ(S) - F(S))} = e^{-\beta K(Γ, S)},
\]

where \( \beta \) is the Boltzmann weight, \( E_Γ(S) \) is the energy of the sequence \( S \) over the structure \( Γ \) and the sum in the denominator is taken over all possible structures, \( \{Γ'\} \) having the same length of \( Γ \). The winning sequence will maximize \( P_Γ(S) \) at all temperatures below the folding transition temperature (where the occupation probability of the native state is macroscopic).

The previous studies of ¹³ and Sun et al. ²⁰ consisted of finding a sequence that has the lowest possible energy in the putative native state. This is equivalent to the assumption that \( F(S) \) in the above equation is independent of \( S \). The latter study used a modified Hamiltonian that included a chemical potential term that controlled the number of hydrophobic residues and may be interpreted as a first approximation for the inclusion of an \( F(S) \) term (see later).

Two main obstacles need to be overcome to implement (1). The first is that one needs to know how to calculate \( E_Γ(S) \) and \( F(S) \); second, it is necessary to explore the whole space of sequences to find the one maximizing (1).

A commonly used simplification of the latter problem is effected on coarse graining the 20 types of naturally occurring amino acids into just two dominant classes: hydrophobic (H) and polar, (P). The evidence in favour of this subdivision is considerable ³, ⁶.
In fact, it has been shown that, to a large extent, the folding of proteins is driven by the collapse of H residues into a compact hydrophobic core surrounded by polar amino acids or solvent molecules [3, 7, 9]. On top of that it appears possible to take a protein and exchange some of its amino acids within the H (or P) class without changing its native structure notably [7].

In this paper we will adopt this point of view and perform a design on “real structures” (i.e., structures extracted from the Protein Data Bank) within the HP framework. We will address the difficulty of maximizing (1) by considering a series of approximate forms for $E_r(S)$ and $F(S)$ of increasing complexity and refinement. For each design attempt, we present a detailed summary of the failure rates of identifying the correct class of each amino acid. We find that, though the failure rates on individual amino acids varies significantly over the various design attempts, the overall design success remains nearly constant. This is possibly suggestive of a limitation of the HP coarse-graining; in support of this we will present evidence showing that the the HP coarse-graining hinders the success of design strategies in a solvable protein model.

II. THE DESIGN ALGORITHMS

To test the design algorithm we first chose a set of 20 single chain proteins from the protein data bank (PDB); here and below the proteins will be identified with their index number as in Table I.

In order to perform the HP design, we begin by substituting each amino acid unit with a fictitious residue placed at a distance of 3 Å from the protein backbone along the $C_\alpha - C_\beta$ direction of the true residue, following Sun et al. [20]. For GLY, which does not have a $\beta$-Carbon, the fictitious residue coincided with the $\alpha$-Carbon [11]. Following this procedure, one obtains the bare backbone of the original protein stripped from any information that could distinguish different types of amino acids.

The goal of the design procedure was to identify the polarity or hydrophobicity of fictitious residues exactly as in the true sequence, where the 20 aminoacids were coarse-grained as in Table II.

From table II it can be calculated that the fraction of H residues is about 41%. If one were to use a totally random method for guessing the correct class of residues, while respecting the H/P ratio $r = 0.41/0.59$ one would obtain a relative success of

$$\frac{1}{2} + \frac{1}{2} \left( \frac{r - 1}{r + 1} \right)^2 \approx 52\%$$ (2)

only slightly higher than without constraining $r$. This result is to be borne in mind when assessing the performance of the design procedures presented in the remainder of this section.

A. Method 1

The coarsest design attempt that can be tried is to establish the polarity of a residue according to the number of neighbours in contact with it. Customarily two non-consecutive residues, $i$ and $j$, are said to be in contact if their distance, $r_{ij}$, falls within a range of around 7 Å and no other residue is between them. To each residue, $i$, in the target structure we assigned a contact score according to the rule

$$n_c(i) = \sum_j \frac{1}{1 + e^{r_{ij} - 6.5}},$$ (3)

which weights the strength of contact interactions with a smooth sigmoidal function [20, 11]. The superscript tilde in equation (3) indicates that the sum is not taken over $j \in \{i - 1, i, i + 1\}$, nor over residues that are spaced more than 10 Å apart. The latter constraint is used to avoid the possibility that an intervening residue is between sites $i$ and $j$. The residues which have a contact score greater than 5 were chosen to belong to the H class while the others were considered polar (P). Finally, the obtained string was compared with the true (HP-coarse-grained) protein sequence. It turned out that, on average, the design procedure identified the correct class of amino acid residues 73% of the time. This very simple design strategy matches the performance of previous design attempts which were based on more complex algorithms where, for example, the polarity of residues was assigned according to the exposed area of their Van der Waals spheres (Sun et al. [20]).

The detailed success rate on each protein in our chosen set is given in Fig. 1. Fig. 2, on the other hand, shows the frequency with which a given amino acid was assigned to the wrong HP class. These results, which to our knowledge
were not considered in previous studies, provide useful insight for the design failure. For example, from Fig. 3, it appears that the class identification failure is highest for alanine, for which it is slightly higher than 50%. This can probably be ascribed to the fact that the sites occupied by alanine are determined more on the basis of steric interactions than hydrophobic ones. The failure of identifying alanine as hydrophobic seriously affects the overall design success rate; in fact, nearly 10% of proteins residues are of this type (see Table II).

B. Method 2

It is perhaps surprising that the previous design method can yield a success rate of 73% especially in view of the fact that it only uses local geometrical information about the target structure, \( \Gamma \). In this subsection, we go beyond this approximation and take as the solution to the design problem, the sequence, \( S \) minimizing the following form (see also Sun et al. [20] for \( K(\Gamma, S) \) appearing in [1]).

\[
K_F(\Gamma, S) = \sum_{i,j} \epsilon(S_i, S_j) f(r_{ij}) + \mu \sum_i S_i
\]

where \( S_i = 0 \) \([S_i = 1]\) if residue \( i \) is of type \( P \) \([H]\), \( \epsilon \) is the contact energy matrix, \( f(r_{ij}) \) is the sigmoidal contact-strength function,

\[
f(r_{ij}) = \begin{cases} 
(1 + e^{r_{ij} - 6.5})^{-1} & \text{if } j \notin \{i - 1, i, i + 1\} \text{ and } r_{ij} < 10 \text{Å}, \\
0 & \text{otherwise,}
\end{cases}
\]

and \( \mu \) is a positive quantity. The contact matrix, \( \epsilon \) is chosen to be symmetric (i.e., \( \epsilon(0, 1) = \epsilon(1, 0) \)); moreover the energy scale is set so that \( \epsilon(1, 1) = -1 \). The second term in (4) does not depend on the target structure, \( \Gamma \), and is to be regarded as an approximate expression for the free-energy, \( F(S) \), whose effect is to control the ratio of \( H \) to \( P \) residues in designed sequences. This particular approximation for \( F \) was inspired by the fact that, on HP lattice models, sequences with the same number of \( H \) and \( P \) residues have nearly the same free-energy [22]. The introduction of the "chemical potential", \( \mu \), appears the simplest way of sifting through the sequence space to retain only sequences with the designed H-P ratio. Among this subset, the putative solution to the design problem will be the sequence minimizing the contact energy term. On the basis of the success of an analogous HP design strategy for three-dimensional lattice structures [23], it can be expected that the sequence minimizing (4) will be close to the true protein sequence.

The quantities \( \epsilon(1, 0), \epsilon(0, 0) \) and \( \mu \) appearing in (4) are regarded as parameters to be optimized in order to maximize the overall design success rate. This step can be viewed as a way of extracting the HP contact energies from proteins of known sequence and conformation.

The design procedure engine consists of the following two steps:

1. for a given set of parameters the sequence \( S \) minimizing (4) is identified with a simulated annealing procedure (the elementary move being the mutation of a fraction of residues from one class to the other),

2. the parameters are varied, and step 1 is repeated, in the attempt to identify the set of values giving the highest average design success rate.

The highest success rate was found for

\[
\epsilon(0, 1) = 0.006025 \, , \, \epsilon(0, 0) = 1.481606 \, , \, \mu = 6.1909
\]

and was equal to 73.4% as shown in Fig. 3.

Figure 4 represents the ribbon plot of protein 3rn3, where the design success was 73.3%. Helices and \( \beta \) – sheets are coloured in purple and yellow respectively, while the black portions mark the residues whose hydrophobicity/polarity was not recognized correctly by the design method.

Remarkably, despite the increased computational effort, the overall success rate has not improved appreciably over the previous design attempt. In particular it can be noted that, while the failure rate over the individual files is roughly the same as in Fig. 3, Figs. 2 and 3 differ significantly. The average failure in identifying P-type residues has decreased significantly while the failure rate for alanine has grown from 50% to 60%. As mentioned before this can be ascribed to the small volume of alanine which favours its location in protein structures on the basis of excluded volume reasons. A high failure rate also affects methionine, which is a common helix-former. Hence, it appears that the highest failure rate is found among residues whose location in the protein is dictated by specific functionality rather than mere energetic considerations.
C. Method 3

In a third attempt we tried to improve the approximation for the free energy term, \( F(S) \), used in the previous method. A possible way of constructing approximate forms for \( F(S) \) is to include in it all possible constraints that are satisfied by real protein sequences. One of these constraints is that, using the coarse-graining scheme of Table I, the ratio of \( H \) to \( P \) residues must be,

\[
r \approx 0.71.
\]  

(7)

The previous method tried to tune in to the right value of \( r \) by optimizing the “chemical potential”, \( \mu \).

By studying the statistical properties of protein chains we have been able to identify another constraint which, to our knowledge, had not been previously identified. In fact, we have established that the number of \( H \) segments, \( \Sigma_H \), in a variety of proteins grows linearly with the length, \( L \), of the chain. As can be seen in Fig. 6 the linear behaviour is marked and, in fact, the linear correlation coefficient over our set of 20 proteins was equal to 0.964. A linear regression of the points in Fig. 6 gives the following equation for the interpolating line:

\[
\Sigma_H(L) = 0.547889 + 0.252676 \cdot L
\]  

(8)

For chains of length \( L \approx 130 \) the difference between the true value of \( \Sigma_H \) (\( \approx 35 \)) and the one estimated with (8) was of the order of 3 units. Given the high degree of reliability of constraint (8) we decided to incorporate it in our expression for \( F(S) \). In fact, it turned out that the sequences designed with the previous method had rather low values of \( \Sigma_H \); in other words the \( H \) residues tended to cluster together in relatively long segments.

Our expectation was that the simultaneous requirement that design sequences should obey both (8) and (9) would be an efficient sieve for isolating good sequences. Hence we adopted the following form for \( K(\Gamma, S) \),

\[
K_1(\Gamma, S) = \sum_{i \neq j} \epsilon(S_i, S_j) f(r_{ij}) + 
\begin{align*}
V_1(\Sigma_H(S) - \tilde{\Sigma}_H(L))^2 + \\
V_2(n_H(S) - 0.415 \cdot L)^2
\end{align*}
\]  

(9)

where \( L \), \( N_H(S) \) and \( \Sigma_H(S) \) are, respectively, the length of the chain, the number of \( H \) residues in \( S \) and the number of \( H \) segments in \( S \). The term in square bracket in equation (9) can be regarded as an expansion of the free energy, \( F(S) \) (see eqn. (8)), to fourth order in the spin variables, \( S_i \). In fact, it can be equivalently recast into,

\[
\frac{V_1}{2} \sum_{i=1}^{L-1} (S_i - S_{i+1})^2 + S_0 + S_L - \tilde{\Sigma}_H(L) + V_2 \sum_{i=1}^{L} \left( S_i - \frac{r}{r+1} \right)^2.
\]  

(10)

In equation (9), the amplitudes of the two potential wells, \( V_1 \) and \( V_2 \), were chosen to be of the order of 100 (the energy unit is \(|\epsilon(1,1)| = 1\) ). By using the strategy described in subsection B we obtained the best results for \( \epsilon(1,0) = 0.285509 \) and \( \epsilon(0,0) = 1.520444 \), for which the design success rate was 73.1%, slightly lower than for the previous attempt. The detailed results are summarized in Figs. 7 and 8. Once more, the failure rates on individual amino acids differ appreciably from previous attempts (the amino acid failure rates are, however, robust against small changes in the optimized parameters).

An artificial way of increasing the design success would be to include alanine in the class of polar residues. This would make the success rate of method 1 grow to 73.7% . For the second method the success increase is, \textit{a priori} not as easy to estimate. In fact, it can be expected that changing the class of alanine could trigger a cascade effect modifying the overall success score.

Surprisingly this was not the case: by changing alanine from \( H \) to \( P \) the best overall success grew to 75.1%. This is almost exactly the value that one would have obtained by taking, for alanine, the complementary of the failure rate in Fig. 6, suggesting an apparently weak correlation between the position of alanine and other residues.

D. Structural homology

We complete the discussion of our design attempts by considering the case of protein homology. It is well-known that there is no strict one-to-one correspondence between structures and sequences; indeed, it is often the case...
that native states of proteins whose mutual identity is around 80% fold into nearly the same structure (the RMSD being typically less than 0.8 Å per residue). In this case the two proteins are said to be homologous. A thorough check of a design procedure should then allow for the possibility that the designed sequence, $S'$, is homologous to the target one, $S$. This can conveniently be done by comparing $S'$ with all the known sequences homologous to $S$.

Table IV lists, for each of our 20 target structures, the names of proteins with the same length and high structural identity (this includes, of course, the true target sequence). It can be noticed that, in a number of cases, it was not possible to identify any homologous sequence.

We carried out the same design procedure as in method 3, but with the following proviso: in case a target structure is associated with more than one sequence in Table IV, we measure the design success against each sequence in this set and then take the highest value as the design score.

It turned out that, for nearly all cases, the highest sequence identity was attained with the true target sequence, not the homologous ones. For this reason the overall success was 73.4%, very close to the previous result.

The inability to improve significantly the success score over the four attempts discussed above is, possibly, suggestive that important features of real proteins have been mistreated.

Another possible explanation for our inability to correctly recognize as many as 25% of the residues may be the coarse graining of the 20 types of amino acids into just two classes, H and P. In the following section we will try to explore this possibility by studying a lattice model for proteins. Indeed it will appear that the HP coarse graining seriously limits the maximum achievable success rate.

### III. DISADVANTAGES OF THE HP COARSE-GRAINING

In this section we want to highlight the fact that an H-P coarse-graining can partially mislead the design procedure. To substantiate this claim we perform a lattice test in which proteins are schematically represented by self-avoiding walks on a square lattice. The amino acids are located at the sites touched by the chains and they are divided in four types, according to their different degree of hydrophobicity or polarity: $H_1$, $H_2$, $P_1$, $P_2$. Two non-consecutive amino acids will be said to be in contact if they are spaced only one lattice unit apart. The contact potentials, $u(\sigma_i, \sigma_j)$ (where $\sigma \in \{H_1, H_2, P_1, P_2\}$) between two interacting sites were chosen to be

$$ u = \begin{pmatrix} -2 & -4 & -2 & -4 \\ -4 & -1 & -4 & -1 \\ -2 & -4 & -2 & -4 \\ -4 & -1 & -4 & -1 \end{pmatrix}. $$

The entries of the matrix $u$ were chosen so that the residues $H_1$ and $H_2$ are energetically favoured to stay in the interior of the protein, unlike $P_1$ and $P_2$. We consider chains made of 12 residues, a case where an exhaustive enumeration of all possible conformations can be carried out with modest numerical effort. Thus, for a given sequence of amino acids $S = \{\sigma_1, \sigma_2, \ldots, \sigma_{12}\}$, we can verify if it has a unique ground state (i.e. if it is a stable protein) or not. We then generated and stored a set of 1000 sequences $S_1, S_2, \ldots, S_{1000}$ with unique ground state conformations, $\Gamma(S_i)$. Our aim was to verify whether a coarse graining of the original 4 types of amino acids into just two HP classes still allows a correct design over the 1000 conformations. As a first attempt, we gathered the amino acids $H_1$ and $H_2$ in class H and $P_1, P_2$ in class P and introduced a new set of interaction potentials, $\tilde{u}(\sigma_i, \sigma_j)$ between these two new classes by averaging out the corresponding 2x2 H-P blocks in the matrix (11).

$$ \tilde{u} = \begin{pmatrix} -\frac{3}{2} & -\frac{1}{2} \\ -\frac{1}{2} & -\frac{3}{8} \end{pmatrix}. $$

Then we transformed each sequence $S_i$ in the corresponding coarse grained form, $\tilde{S}_i$ and by means of exact enumeration we tried to verify if $\tilde{S}_i$ still had a unique state of lowest energy (adopting the coarse grained interactions) on conformation $\Gamma(S_i)$. Disappointingly, only in 718 cases was the answer affirmative. A case where the procedure fails is shown in Fig. 9.

To make our test more stringent, we repeated the procedure by setting

$$ \tilde{u}(H, H) = -1, \quad \tilde{u}(H, P) = \tilde{u}(H, P) = z, \quad \tilde{u}(H, H) = y. $$

5
and optimally varied y and z in order to maximize the number of ground states correctly predicted. We have found an optimal solution for \( z = y = 0.86 \) for which 830 of the original 1000 conformations were identified as native states of the coarse-grained sequences.

Although one cannot extend the same quantitative study of the effects of the HP coarse graining on real protein design, the results given above suggest that not only they can be non negligible, but they can significantly reduce the maximum design success that can be achieved.

### IV. SUMMARY

To summarize we have presented several protein design methods for identifying the correct hydrophobic or polar (H/P) class of residues on a set of 20 proteins. The simplest of these techniques, which is readily implemented and takes nearly no CPU time, gave a success rate of 73%. More sophisticated methods, encompassing negative design features, failed to improve appreciably upon the previous attempt.

We have presented evidence showing that the highest failure rate was found on residues with high secondary-structure functionality. On the basis of numerical results on exactly solvable models, it was also suggested that the HP coarse-graining may significantly impair the design algorithms, thus possibly accounting for the failure to increase the design success score by using algorithms of growing complexity.

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| Label | PDB File | Len |
|-------|----------|-----|
| 1     | 1bba     | 36  |
| 2     | 1bbl     | 37  |
| 3     | 3ebx     | 62  |
| 4     | 1aba     | 87  |
| 5     | 2hpr     | 87  |
| 6     | 1aps     | 98  |
| 7     | 1aaq     | 105 |
| 8     | erv      | 105 |
| 9     | ycc      | 108 |
| 10    | 5cpv     | 108 |
| 11    | 3rn3     | 124 |
| 12    | h1el     | 129 |
| 13    | 1lfb     | 131 |
| 14    | 1ecd     | 136 |
| 15    | 1osa     | 148 |
| 16    | 1mbd     | 153 |
| 17    | 1ra8     | 159 |
| 18    | 192      | 162 |
| 19    | 2lzm     | 164 |
| 20    | 9pap     | 212 |

**TABLE I.** The names of the 20 proteins taken from the Protein Data Bank and used in our design studies are given in column two. The number of amino acids in each protein is given in the last column.

| Amino Acid | Type | Freq. (%) |
|------------|------|-----------|
| ALA        | H    | 8.85      |
| VAL        | H    | 6.59      |
| LEU        | H    | 6.85      |
| ILE        | H    | 5.36      |
| CYS        | H    | 2.04      |
| MET        | H    | 2.42      |
| PHE        | H    | 4.38      |
| TYR        | H    | 3.32      |
| TRP        | H    | 1.40      |
| GLY        | P    | 7.91      |
| PRO        | P    | 3.23      |
| HIS        | P    | 2.17      |
| SER        | P    | 5.70      |
| THR        | P    | 5.87      |
| LYS        | P    | 8.08      |
| ARG        | P    | 4.64      |
| ASP        | P    | 5.96      |
| ASN        | P    | 5.15      |
| GLU        | P    | 6.76      |
| GLN        | P    | 3.32      |

**TABLE II.** Following Sun et al. we distributed the 20 amino acids in H/P classes as in the second column. The third column gives the relative frequency of a given amino acid in the 20 proteins of Table I.
| Target structure | Homologous Sequences |
|------------------|----------------------|
| 1bba             | 1bba                 |
| 1bbl             | 1bbl                 |
| 3ebx             | 3ebx, 5ebx, 1nxb     |
| 1aba             | 1aba                 |
| 2hpr             | 2hpr                 |
| 1aps             | 1aps                 |
| 1aaj             | 1aaj                 |
| 1erv             | 1erv, 1eru, 3trx, 1trw |
| 1ycc             | 1ycc, 1csu, 1raq, 1crj, 1cig |
| 5cpv             | 5cpv                 |
| 3rn3             | 3rn3                 |
| 1hel             | 1hel                 |
| 1ifb             | 1ifb                 |
| 1ecd             | 1ecd                 |
| 1osa             | 1osa, 3cln, 4cln, 1cll |
| 1mbd             | 1mbd                 |
| 1ra8             | 1ra8, 1jom           |
| 1l92             | 1l92, 7lzm, 1l74, 1l72, 1l64 |
| 2lzm             | 2lzm                 |
| 9pap             | 9pap                 |

TABLE III. For each protein in the original set (first column) we identified, when possible, a set of proteins with high structural identity (second column). The outcome of our design procedure was then checked against the set of structurally homologous sequences.
FIG. 1. Histogram of the design success rates on each of the 20 proteins of Table I obtained using method 1.

FIG. 2. Histogram of the failure rates in identifying the correct H/P class of the 20 amino acids obtained with method 1.
FIG. 3. Histogram of the design success rates on each of the 20 proteins of Table I obtained using method 2.

FIG. 4. Ribbon plot of protein 3rn3. The black sections highlight residues which were wrongly assigned by the second design procedure.
FIG. 5. Histogram of the failure rates in identifying the correct H/P class of the 20 amino acids obtained with method 2.

FIG. 6. Plot of the number of H segments, $\Sigma_H$, for our set of 20 proteins as a function of the protein length, $L$. The solid curve is the interpolating line (see equation 8).
FIG. 7. Histogram of the design success rates on each of the 20 proteins of Table I obtained using method 3.

FIG. 8. Histogram of the failure rates in identifying the correct H/P class of the 20 amino acids obtained with method 3.
FIG. 9. a) The unique ground state conformation for sequence $S = \{P_1, H_1, P_1, H_2, H_1, P_2, H_1, H_1, P_2, H_2, P_2\}$. The ground state energy of $S$ is -8. The HP coarse graining of $S$ yields a sequence, $\tilde{S} = \{P, H, P, H, P, H, H, H, P, H, P\}$ which has energy -6.5 on conformation (a). The true ground state of $\tilde{S}$ has energy -6.875 and is represented in (b).