



ISAS - INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

A novel iterative strategy for protein design

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Chapter 1

Introduction

Proteins are the machinery of life. There are thousands and thousands of proteins in living organisms. They are responsible for catalyzing and regulating biological reactions, they are responsible for the transport of ions, they form the basis of structures as skin, hair and tendons. Despite the huge number of different proteins present in living organisms and the variety of their biological functionality, they are obtained assembling only 20 different bricks (amino acids) in a linear chain. This chain can adopt, in principle, many different conformations. However, under physiological conditions, it adopts a unique specific conformation, called structure or native state. It has been shown [2], experimentally, that the structure of a protein is encoded uniquely by its sequence, that is by the position of amino acids along the chain. This is true for globular proteins, for which the structure is the conformation with the lowest energy.

Discovering the relationship occurring between sequence and structure is a problem of primary importance in biology, at least for two reasons. Firstly, to understand biological mechanisms in living organisms. Protein sequences can easily be determined unlike structures. Recombinant DNA techniques have provided tools for the rapid determination of DNA sequences and, by inference, the amino acids sequence of proteins. The number of such sequences is now increasing almost exponentially, but by themselves these sequences tell nothing about the biology of the system. To under-

stand the biological function of proteins we would therefore like to be able to deduce or predict the structure.

Secondly, to be able to modify existing proteins or drugs (protein engineering) or to create ex-novo new proteins (protein design). The biological functionality of a protein is related to its structure. So, a full understanding of the relationship between structure and sequence is an essential intermediate step for protein design.

Protein design is the subject of this thesis. My work is based on recent theoretical advancements which showed the importance to treat carefully the conformational free energy of designed sequences (Chapter 2). In Chapter 3 I show how computational cost can be kept to a minimum by encompassing negative design features, i.e. isolating a small number of structures that compete significantly with the target one for being occupied at low temperature. Then, the method is successfully tested on minimalist protein models and using a variety of amino acids interaction potentials (Chapter 4). Finally, in the last chapter, there are the conclusions to my work.

Chapter 2

Rigorous formulation of protein design

Since the late 50's it has been established that the native state of a protein is entirely and uniquely encoded by its amino acids sequence [1, 2]. One of the fundamental issues in molecular biology is understanding the relation between protein sequence and native structure. Remarkably, this relation is not symmetric: while a given sequence folds into a single structure, a given structure can be encoded by several homologous sequences. The problem of predicting the native state of a sequence is commonly known as "protein folding". Its solution amounts to minimize the energy of the given peptidic chain over all possible conformations. The design problem, i.e. finding the sequence(s) that fold into a desired structure, has also been given a simple and rigorous formulation. At the "physiological" temperature β_p^{-1} , the sequences that correctly design a given target structure, Γ_t (their native state), maximize the Boltzmann probability [3],

$$P(s, \Gamma_t, \beta_p) = \exp \left\{ -\beta_p [H_s(\Gamma_t) - F_s(\beta_p)] \right\}, \quad (2.1)$$

where $s = (s_1, s_2, \dots, s_L)$ represents the amino acid sequence (amino acid s_1 at the first position in Γ , \dots , s_L at the last position in Γ) and H_s is the energy of s housed

on Γ . F_s in eq. (2.1) is the conformational free energy of the sequence s ,

$$F_s(\beta) = -\beta^{-1} \ln \left\{ \sum_{\Gamma} \exp[-\beta H_s(\Gamma)] \right\}, \quad (2.2)$$

where the summation is taken over all possible conformations the sequence s can assume without violating steric constraints. Maximizing (2.1) poses some serious technical difficulties since, in principle, it entails an exploration of both sequence and structure space [3, 4]. Some simplifications have been used in the past in order to limit the space of sequences; this is conveniently done by subdividing amino acids into a limited number, $q \leq 20$, of classes [5, 6]. Some approximation schemes have also been used to reduce the difficulty of calculating F_s [7, 3, 8]. Reasonable success has been obtained, for example, by postulating a suitable functional dependence of F_s on s [9, 10]. Recently, it has also been argued that, despite the huge number of conformations, Γ , the most significant contribution to (2.2) comes from the closest competitors of Γ_t [11]. These are limited in number, since they are among the ones sharing a subset of native contacts with Γ_t .

In this article we propose a new technique apt for designing a given structure, Γ_t , using a minimal set of structure for calculating (2.2). The technique is simple to implement and does not require to constrain sequence composition and/or to search solutions with lowest possible energy [12].

Chapter 3

Theory: the iterative design scheme

In order to discuss the design method we introduce a Hamiltonian function, $H_s(\Gamma)$ depending only on coarse grained degrees of freedom. A commonly used form is the one in terms of the contact matrix $\Delta_2(\vec{r}_i, \vec{r}_j)$ which is 1 when $|\vec{r}_i - \vec{r}_j| < a$ with $a \approx 6-8\text{\AA}$, and 0 otherwise. Other forms which smoothly interpolate between 0 and 1 are also used in practice. Two amino acids, s_i and s_j , which are in contact contribute to the energy by an amount $\epsilon_2(s_i, s_j)$, a phenomenological symmetric matrix (see e.g. refs. [13, 14, 15, 16, 17]). Many body interactions can also be easily included in terms of a generalized contact maps $\Delta_k(\vec{r}_{i_1}, \dots, \vec{r}_{i_k})$ depending only on relative distances and on extra energy parameters $\epsilon_k(s_{i_1}, \dots, s_{i_k})$. Thus the energy can be written as

$$H_s(\Gamma) = \sum_{k \geq 2} \sum_{i_1 < i_2 < \dots < i_k} \epsilon_k(s_{i_1}, \dots, s_{i_k}) \Delta_k(\vec{r}_{i_1}, \dots, \vec{r}_{i_k}). \quad (3.1)$$

Two structures which have the same values for all the Δ_k 's, i.e. the same generalised contact map, will be regarded as identical. This useful coarse-graining procedure neglects the fine structure fluctuations (e.g. due to thermal excitations) and, for a sequence s with native state Γ , allows to define its folding temperature, β_F^{-1} , such that

$$P(s, \Gamma, \beta) > 1/2 , \quad (3.2)$$

for all $\beta > \beta_F$. Conversely, all s 's satisfying inequality (3.2) have their unique ground state in Γ and folding temperature greater than β^{-1} .

Based on this observation the novel strategy for protein design can be formulated in terms of a scheme similar in spirit the one described in ref [[16]]. The essence of the procedure relies on the fact that the sum in (2.2) is carried out only on a limited set of structures D . Initially, D contains only the target structure itself and another structure with a different contact map and similar degree of compactness (chosen at random or with other criteria). Upon iterating the procedure several design solutions will be identified and stored in set S . This set is, of course, initially empty. The steps to be iterated are as follows,

1. An optimization procedure, like simulated annealing, is used to explore sequence space and isolate the sequence \bar{s} (not already included in S), such that

$$\beta[H_{\bar{s}}(\Gamma_t) - \tilde{\mathcal{F}}_{\bar{s}}(\beta)] < \ln 2 . \quad (3.3)$$

$\tilde{\mathcal{F}}$ is calculated approximately by restricting the sum in (2.2) over the competitive structures held in D :

$$\tilde{\mathcal{F}}_s(\beta) = -\beta^{-1} \ln \left\{ \sum_{\Gamma \in D} \exp[-\beta H_s(\Gamma)] \right\} . \quad (3.4)$$

2. Then the lowest energy state(s), $\bar{\Gamma}$ of \bar{s} are identified and the corresponding energy compared with that obtained by \bar{s} on Γ_t . By definition, if $\bar{\Gamma} \neq \Gamma_t$ and $H(\bar{s}, \bar{\Gamma}) \leq H(\bar{s}, \Gamma_t)$, then \bar{s} is not a solution to the design problem and $\bar{\Gamma}$ is added to D . Otherwise, \bar{s} , is added to the set of known solutions, S .

The iterative procedure is repeated from step 1. The scheme stops when it is impossible to find sequences, satisfying (3.3) not already included in S , or when a

sufficient number of solutions has been retrieved. It is easy to see, using (3.3) and (3.4) that, in step 2, it can never happen that a newly chosen $\bar{\Gamma} \neq \Gamma_t$ is already contained in D . Thus, at each iteration, new informations are collected, either in the form of a putative solution (added to S) or as a new decoy (added to D).

Notice that, if the exact form of F_s were used instead of (3.4), then the sequences in S would have a folding temperature greater than β^{-1} . However, since approximation (3.4) leads to systematically overestimating $F_s(\beta)$, it is not guaranteed that the selected sequences have $\beta_F^{-1} < \beta^{-1}$. The inequality should however be satisfied to a better extent for larger decoys sets.

The method outlined here is rigorous and its iterative application allows, in principle, to extract all sequences designing a given structure. Its practical implementation may encounter difficulties at step 2, where it is required to find the low energy conformation(s) of a sequence. Sequences selected at step 2 with a low β will correspondingly have a high folding temperature and are expected to be good folders. Hence, it is plausible that identifying the corresponding low energy states is much simpler than solving the general folding problem. In fact, we have gathered numerical evidence showing that strategy can be stopped as soon as a one finds a structure where is attained an energy lower than on Γ_t (even if true native state has still lower energy). Notice that it is still necessary to have folding technique to allow to test if the design procedure is successful. Our iterative scheme is able to use informations of failed attempts in order to improve design at subsequent iterations.

Chapter 4

Testing on lattice the iterative scheme

4.1 Methods: implementation and test of the procedure

To carry out a rigorous and exhaustive test of the proposed strategy we have restricted the space of structures by discretizing the positions of amino acids, \vec{r}_i . We choose to follow the common practice of restricting the \vec{r}_i 's to occupy the nodes of a cubic lattice [6, 18, 19, 4, 20]. This simplification allows for an exhaustive search of the whole conformation space for chain of a few dozen residues, albeit at the expenses of an accurate representation of protein structures, as discussed in ref. [[21]]. To mimic the high degree of compactness found in naturally occurring proteins, we first considered all the maximally compact self-avoiding walks of length $L = 27$ embedded in a $3 \times 3 \times 3$ cube. There are 103346 distinct oriented walks modulo rotations and reflections. This restriction is a good approximation if the interaction energies between amino acids are sufficiently negative, so that compact conformations are favoured over loose ones. Without loss of generality we adopt a Hamiltonian where only pairwise interactions are considered (corresponding to $k = 2$ in (3.1)). If the

	relative structures	Enc.
Γ_1	URDDLLFFRBRFULLBBUFFRRBDLU	25
Γ_2	UURFLFDBRBDLFRRBBUUFFLDRB	337
Γ_3	UURRFDLULDDFUURRDBBULDDFFU	1224
Γ_4	UURRDLFFRBULLDDRBRFFLLUURR	1303

Table 4.1: The four structures used for benchmarking the design strategy. The conformations are encoded in bond directions: U, up; D, down; L, left; R, right; F, forward; B, backward. The encodability in the rightmost column is defined as the number of sequences admitting the corresponding structure as their unique native state (HP interactions are assumed).

interaction energies are sufficiently attractive it is guaranteed that the native state is compact. Step 2 of the iterative procedure was carried out in two distinct ways. In a first attempt we found the true lowest energy state of s by exhaustive search. In a second attempt we tried to mimic the difficulty of finding the ground state in a realistic context and hence carried out a random partial exploration of the structure space. Although the first method was expected to be more efficient than the second, their performance turned out to be almost identical, as we discuss below.

The four target conformations used to test the procedure are given in Table 4.1 and shown in Fig 4.1a-d.

We used three possible choices for the ϵ 's. First, we adopted the standard 2-class HP model with $\epsilon_{HH} = -1 - \alpha$ and $\epsilon_{HP} = \epsilon_{PP} = -\alpha$. α is a suitable constant ensuring that native conformations are compact. Since all conformations considered here have the same number of contacts the value of α is irrelevant and will be omitted from now on. The second case is a 6-class model and the ϵ 's are shown in Table 4.1.

For the last case we considered the full repertoire of 20 amino acids used the Miyazawa and Jernigan energy parameters given in Table 3 of ref. [[13]]. With the

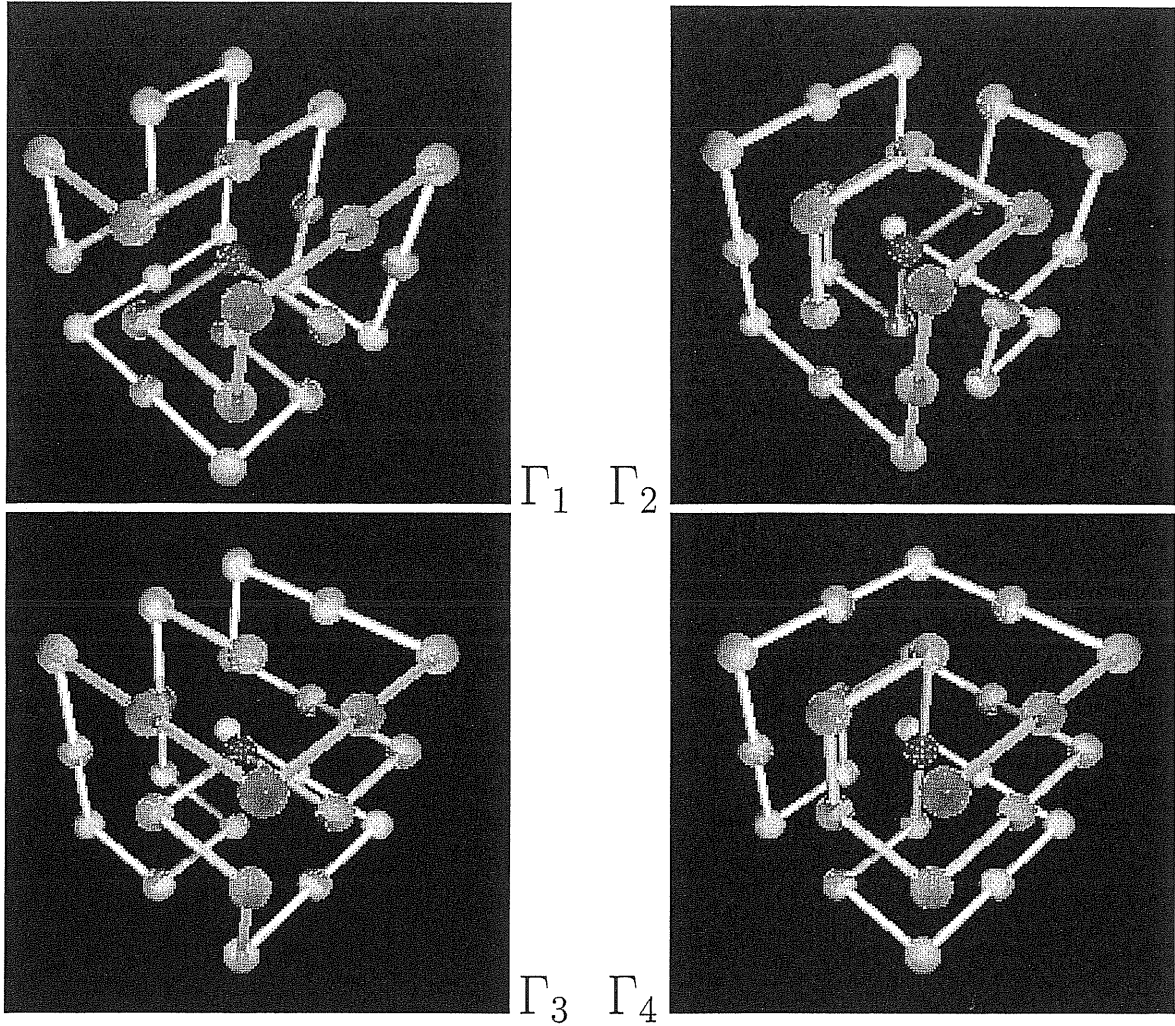


Figure 4.1: The target structures $\Gamma_1, \Gamma_2, \Gamma_3, \Gamma_4$.

	1	2	3	4	5	6
1	-50.00	-20.49	-38.20	-6.65	-43.65	-10.63
2	-20.49	-14.91	-18.13	-4.00	-15.56	-3.81
3	-38.20	-18.13	-35.75	-5.07	-23.96	-26.02
4	-6.65	-4.00	-5.07	-1.65	-5.17	-9.47
5	-43.65	-15.56	-23.96	-5.17	-43.71	-18.63
6	-10.63	-3.81	-26.02	-9.47	-18.63	-26.70

Table 4.2: Energy parameters for the 6-class model. Parameters obey the segregation principle [25].

standard HP parameters, structures $\Gamma_1 - \Gamma_4$ have various degree of designability. The latter is defined as the number of sequences admitting them as unique ground states [22]. Hence, the encodability of Γ_1 and Γ_2 is poor and average respectively, while Γ_3 and Γ_4 have very large encodability. It was shown that the degree of encodability is mainly a geometrical property of the structure and not too sensitive to the number of amino acid classes or the values of interaction parameters [22, 20, 16]. For this reason we expect that the relative encodability of $\Gamma_1 - \Gamma_4$ remain different when using all the three sets of parameters.

4.2 Results and discussion

The “dynamical” performance of the algorithm can be seen in Figs. 4.2a-c.

	HP		6 classes		20 classes	
	N_{it}	N_{sol}	N_{it}	N_{sol}	N_{it}	N_{sol}
Γ_1	62	8	1000	895	500	388
Γ_2	722	337	1000	891	500	419
Γ_3	1898	1219	1000	906	500	423
Γ_4	1719	1297	1000	911	500	457

Table 4.3: Number of extracted solutions, N_{sol} , after N_{it} iterations of the design procedure. For the HP model N_{it} is the number of iterations at which the iterative scheme stopped. It was verified that the 1297 extracted solutions for structure Γ_4 have a folding temperature between 0.15 and 0.6.

The plots show the number of solutions retrieved as a function of the number of iterations at a “physiological” temperature equal to 0.1, 10.0 and 0.7 for 2, 6 and 20 classes of amino acids, respectively. The different values of the physiological temperature are related to the different energy scales of the interactions.

It can be seen that, after an initial transient, the performance of the method (given by the slope of the curves) is very high. In particular, for a large number of classes, it is nearly equal to 1 for all structures. Table 4.3 provides a quantitative summary of the performance of the method.

For the HP model, first column of Table 4.3, the method was iterated until it could not find further solutions with (estimated) folding temperature greater than 0.1. For the cases of 6 and 20 classes, a very large number of solutions exist. Hence, we stopped the procedure after 1000 or 500 iterations, depending on the number of classes.

An appealing feature is that the extracted solutions show no bias for sequence composition (see Fig. 4.2d) or ground-state energy. This can be seen in Fig. 4.3, where we have plotted the energies of 1000 designed solutions of fixed composition

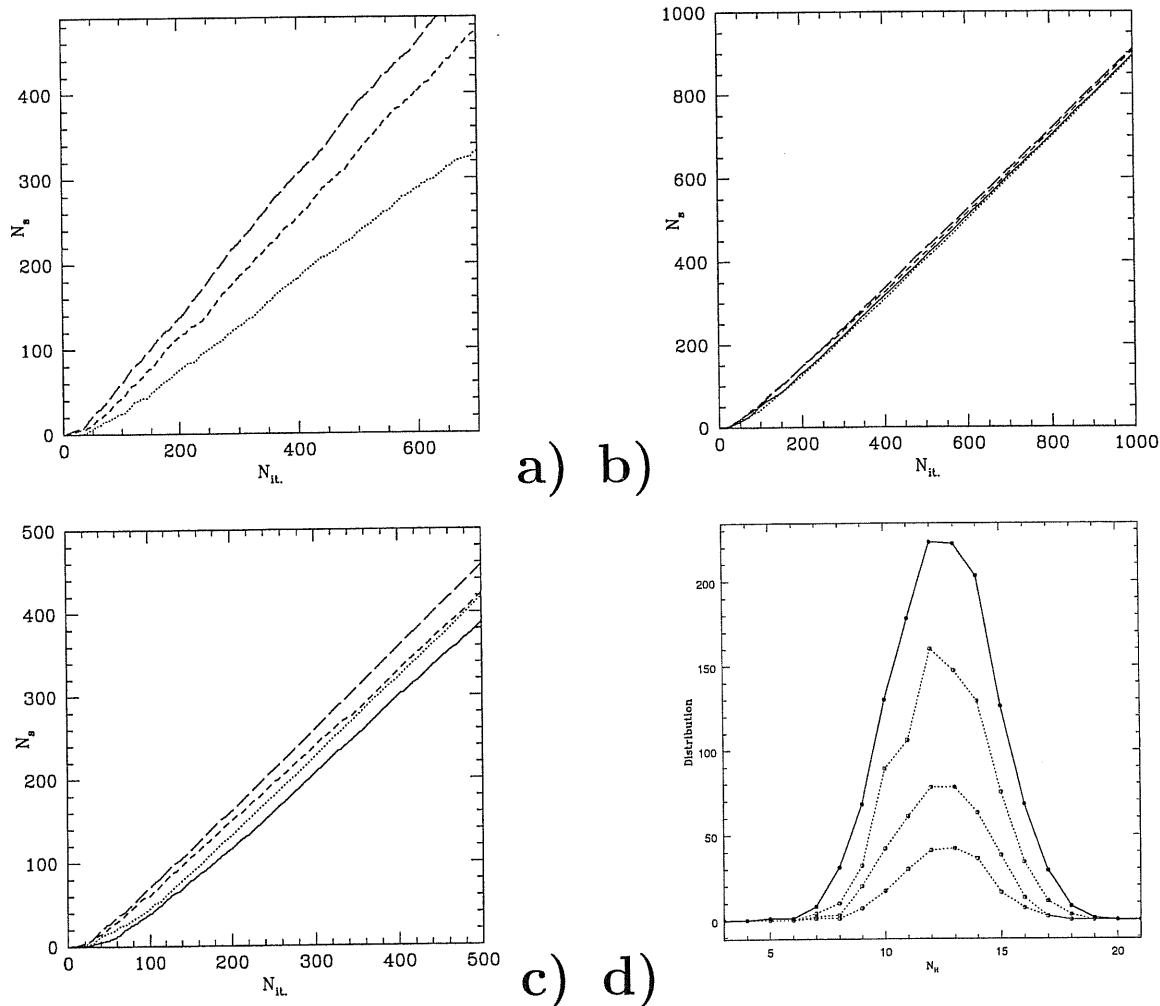


Figure 4.2: Number of extracted solutions versus the number of iterations for (a) HP interactions, (b) 6 amino acid classes and (c) 20 classes. The ideal curve, corresponding to efficiency 1, should have slope 1. Plots referring to structures Γ_1 , Γ_2 , Γ_3 , Γ_4 are denoted with continuous, dotted, dashed and long-dashed lines respectively. Panel (d) represents the histogram of the number of extracted solutions as a function of sequence composition (HP model). Curves pertain to an HP-design attempt on structure Γ_4 at different values of N_{it} : 200, 400, 800, 1719. It can be seen that the efficiency of the design technique is independent of the sequence composition.

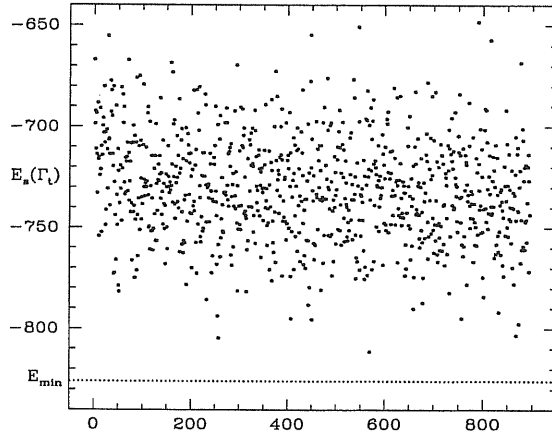


Figure 4.3: Energy of the solutions found for structure Γ_4 (6-class model) at fixed composition (4, 4, 4, 5, 5, 5)

for the 6-classes case.

Solutions do not exhibit packing around the minimum energy (≈ -830) and their energy spread is fairly wide (the estimated maximum energy is ≈ -170). Furthermore, for each extracted sequence we also calculated its folding temperature, to compare it with $1/\beta$. As we remarked, if all the significant competitors of Γ_t were included in D , then sequences satisfying (3.3) should have folding temperatures greater than $1/\beta$. As shown in the typical plot of Fig. 4.4 this is almost always the case, ensuring that solutions can be extracted with a desired thermal stability.

Finally, we analysed the degree of mutual similarity between extracted solutions. For the 6-classes case, the sequence similarity between solutions was rather low, being around 20%, as can be seen in Fig. 4.5.

This rules out the possibility that solutions correspond to few point mutations of a single prototype sequence.

One of the most significant features of the novel design procedure is that the number of structures, D , used to calculate the approximate free energy, (3.4), can be kept to a negligible fraction of the total structures and yet allow a very efficient

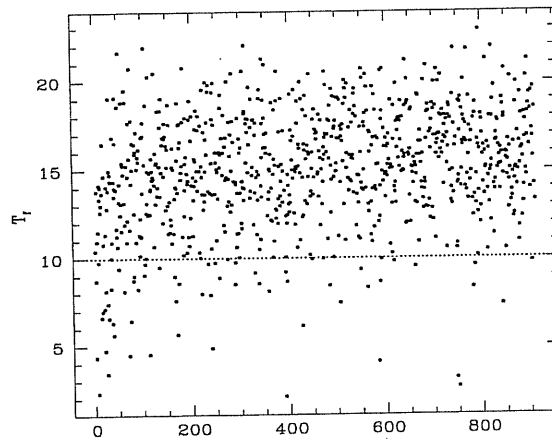


Figure 4.4: Folding temperatures of solutions designing structure Γ_4 (6-class model) as a function of the order of extraction. Very few solutions turn out to have a folding temperature below the simulation temperature $T = 10$ (shown with a dotted line).

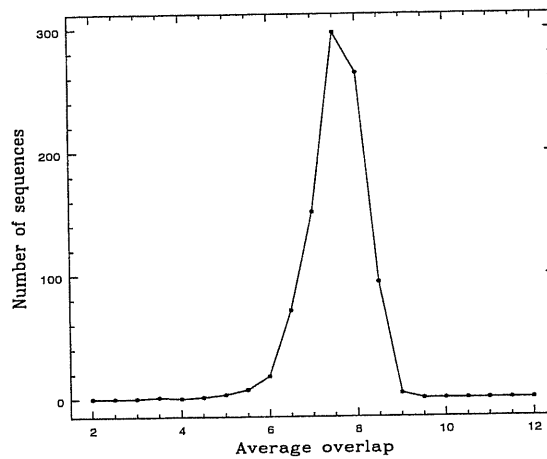


Figure 4.5: Histogram of the average overlap (sequence identity) of solutions for Γ_4 (6-class model). For a given sequence the average overlap is calculated over all other extracted solutions.

design. This is proved even more strikingly by a further test of our design strategy in the whole space of both compact and non-compact conformations. We carried out a design of structure Γ_2 by using the HP parameters with the constant α set to 0. This amounts to allow for non-compact conformations to be native states. Since it is unfeasible to explore this enlarged structure space, step 2 was carried out with a stochastic Monte Carlo process, as described in refs. [3, 4], which generated dynamically growing low-energy conformations at a suitable fictitious Monte Carlo temperature. The correctness of the putative solutions was carried out by using an algorithm known as Constrained Hydrophobic Core Construction (CHCC)[23, 24]. The algorithm relies on an efficient pruning of the complete search tree in finding possible low energy conformations for a sequence. At the heart of the algorithm is the observation that the most energetically convenient conformations for the hydrophobic monomers is to form a compact, cubic-like, core. This ideal situation may not be reachable for arbitrary sequences, due to frustration effects; these are taken systematically into account to build a compact core with a number of cavities sufficient to expose P singlets (i.e. a P flanked by two H monomers in the sequence) on the surface, which is energetically more effective than burying them in the core. Then, exhaustive search algorithms are used to check the compatibility of a sequence with cores of increasing surface area (i.e. decreasing energy). A detailed description of the method can be found in Refs. [23, 24]. The time required by CHCC to find the ground state energy of a sequence increases significantly, on average, with the increase of the number of H residues. For this reason we limited the search for design solutions to sequences with $n_H = 13$. The solutions, obtained in about one hundred iterations, appears in table (4.4).

All the 23 extracted solutions had Γ_2 as the unique ground state among the compact structures, and 17 of them retained Γ_2 as ground state even when non-compact structures are considered. Given the vastity of the enlarged structure space this represent a remarkable result.

Correct solutions
010111001110110001010100001
000011011100111101000100101
000010011100111101000101101
000010000111100101110101101
000010010100101111000110111
000010000110100111010110111
000010110100100111000101111
000010000110100111010110111
000010110100100111000101111
000010010100101111000101111
000010000110100111010101111
000010110100100101000111111
000010010100101101000111111
000010100100100111000111111
000010010100100111000111111
000010000110100101010111111
000010000100100111010111111
Incorrect solutions
110010001110110001010101001
010011001110110100010100101
110010001100110101000101101
100011001100110101000101101
000010100100100111010101111

Table 4.4: Extracted solutions for structure Γ_2 . The design attempt was carried out in the whole space of conformations with arbitrary degree of compactness.

Chapter 5

Conclusions

We have presented a novel approach to protein design that encompasses negative design features. Taking the latter into proper account has been shown to be crucial for a successful protein design. From a practical point of view this amounts to calculating the conformational free energy of all sequences which are candidate solutions. This computational intensive task is kept to a minimum in our scheme thanks to the identification of a limited number of structures which are close competitors of the target conformations. The strategy, is easy to implement and has been tested on minimalist models. The method appears to be very efficient and reliable for a variety of different sets of amino acid interactions. Contrary to other design techniques, the extracted solutions show no bias in sequence composition or native state energy and can be chosen to have a desired thermal stability.

Chapter 6

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Bibliography

- [1] C. Branden, & J. Tooze, (1991) in *Introduction to protein structure*, Garland Publishing, New York; T.E. Creighton, (1992) in *Proteins: structures and molecular properties*, W. H. Freeman ed., New York
- [2] C. Anfinsen, *Science* **181**, 223(1973).
- [3] F. Seno, M. Vendruscolo, A. Maritan, and J.R. Banavar, *Phys. Rev. Lett.* **77**, 1901 (1996).
- [4] C. Micheletti, F. Seno, A. Maritan and J. R. Banavar, *Phys. Rev. Lett.*, **80**, 2237 (1998).
- [5] S. Kamtekar, J.M. Schiffer, H. Xiong, J.M. Babik, and M.H. Hecht, *Science* **262**, 1680 (1993).
- [6] K.F. Lau and K.A. Dill, *Macromolecules* **22**, 3986 (1989).
- [7] J.M. Deutsch, and T. Kurosky, *Phys. Rev. Lett* **76**, 323 (1996).
- [8] M.P. Morrissey and E.I. Shakhnovich, *Folding and Design* **1**, 391 (1996).
- [9] C. Micheletti, F. Seno, A. Maritan and J. R. Banavar, *Proteins: Structure Function and Genetics*, **32**, 80 (1998).
- [10] F. Seno, C. Micheletti, A. Maritan and J.R. Banavar, *Phys. Rev. Lett.* **81**, 2172 (1998).

- [11] J. R. Banavar, M. Cieplak, A. Maritan, G. Nadig, F. Seno and S. Vishveshwara, *Proteins: structure function and genetics*, **31**, 10 (1998).
- [12] E.I. Shakhnovich, A.M. Gutin, *Proc. Natl. Acad. Sci. USA* **90**, 7195 (1993).
- [13] S. Miyazawa and R.L. Jernigan, *J. Mol. Biol.* **256**, 623 (1996).
- [14] M.J. Sippl, *J. Mol. Biol.* **213**, 859 (1990); M. Hendlick, P. Lackner, S. Weitckus, H. Floeckner, R. Froschauerer, K. Gottsbacher, G. Casari and M.J. Sipp, *J. Mol. Biol.* **216**, 167 (1990); D.A. Hinds and M. Levitt, *Proc. Natl. Acad. Sci. USA* **89**, 2536 (1992); D.T. Jones, W.R. Taylor and J.M. Thornton, *Nature* **358**, 86 (1992); A. Godzik and J. Skolnick, *Proc. Natl. Acad. Sci. USA* **89**, 12098 (1992);
- [15] L.A. Mirny and E.I. Shakhnovich, *J. Mol. Biol.* **264**, 1164 (1996).
- [16] C. Micheletti and A. Maritan, "A study of energy minimization techniques applied to protein design", SISSA preprint, cond-mat/9808154 (1998).
- [17] A. Sali, E.I. Shakhnovich and M. Karplus, *Nature* **369**, 248 (1994).
- [18] H.S. Chan and K.A. Dill, *J. Chem Phys.* **95**, 3775 (1991).
- [19] E.I. Shakhnovich, *Phys. Rev. Lett.* **72**, 3907 (1994).
- [20] C. Micheletti, J. R. Banavar, A. Maritan and F. Seno, *Phys. Rev. Lett.* **80**, 5683 (1998).
- [21] B. H. Park and M. Levitt, *Journal of Molecular Biology*, **249**, 493-507 (1995).
- [22] H. Li, R. Helling, C. Tang, N. Wingreen, *Science*, **273**, 666 (1996).
- [23] K. Yue and K.A. Dill, *Proc. Natl. Acad. Sci. USA* **92**, 146 (1995).
- [24] K. Yue and K.A. Dill, *Phys. Rev. E* **48**, 2267 (1993).
- [25] H. Li, C. Tang, N. Wingreen, *Phys. Rev. Lett.* **79**, 765 (1997).