ELSEVIER
www.ComputerScienceWeb.com
POWERED BY SCIENCE @ DIRECT®
Computational Geometry 25 (2003) 139–159
Computational Geometry
Theory and Applications
www.elsevier.com/locate/comgeo

Long proteins with unique optimal foldings in the H-P model [☆]

Oswin Aichholzer [a,1], David Bremner [b,2], Erik D. Demaine [c,*], Henk Meijer [d],
Vera Sacristán [e,3], Michael Soss [f]

[a] *Institut fur Grundlagen der Informationsverarbeitung, Technische Universität Graz, Inffeldgasse 16b, A-8010 Graz, Austria*
[b] *Faculty of Computer Science, University of New Brunswick, P.O. Box 4400, Fredericton, N.B. E3B 5A3, Canada*
[c] *MIT Laboratory for Computer Science, 200 Technology Square, Cambridge, MA 02139, USA*
[d] *Department of Computing and Information Science, Queen's University, Kingston, Ontario K7L 3N6, Canada*
[e] *Departament de Matemàtica Aplicada II, Universitat Politècnica de Catalunya, Pau Gargallo 5, 08028 Barcelona, Spain*
[f] *Chemical Computing Group, 1010 Sherbrooke St. West, Suite 910, Montreal, Quebec H3A 2R7, Canada*

Received 13 July 2001; accepted 21 January 2002

Communicated by H. Alt

Abstract

It is widely accepted that (1) the natural or folded state of proteins is a global energy minimum, and (2) in most cases proteins fold to a unique state determined by their amino acid sequence. The H-P (hydrophobic-hydrophilic) model is a simple combinatorial model designed to answer qualitative questions about the protein folding process. In this paper we consider a problem suggested by Brian Hayes in 1998: what proteins in the two-dimensional H-P model have *unique* optimal (minimum energy) foldings? In particular, we prove that there are closed chains of monomers (amino acids) with this property for all (even) lengths; and that there are open monomer chains with this property for all lengths divisible by four.

Keywords: Protein folding; Hydrophobic-hydrophilic model; Folding stability; Energy minimization; Combinatorial geometry

[☆] A preliminary version of this paper appeared at the 17th European Conference on Computational Geometry [2].

[*] Corresponding author.

E-mail addresses: oaich@igi.tu-graz.ac.at (O. Aichholzer), bremner@unb.ca (D. Bremner), edemaine@mit.edu (E.D. Demaine), henk@cs.queensu.ca (H. Meijer), vera@ma2.upc.es (V. Sacristán), soss@chemcomp.com (M. Soss).

[1] Supported by the Austrian Programme for Advanced Research and Technology (APART).

[2] Partially supported by NSERC Canada.

[3] Supported by DURSI Gen. Cat. 1999SGR00356 and Proyecto DGES-MEC PB98-0933.

0925-7721/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0925-7721(02)00134-7

1. Introduction

Protein folding [14,22,30] is a central problem in molecular and computational biology with the potential to reveal an understanding of the function and behavior of proteins, the building blocks of life. Such an understanding would greatly influence many areas in biology and medicine such as drug design. In broad terms, the protein-folding problem is to determine how proteins so consistently fold into a stable state. The most ambitious goal is to understand the entire *folding pathway* (see e.g. [33]), i.e., the complete dynamics and/or chemical changes involved in going from an unfolded linear state into a compact folded state. Although naturally posed as a numerical simulation, there are several problems of scale, including the small energy differences between folded and unfolded states, and the extremely short interval (approximately 10^{-15} seconds) for which the dynamics equations remain valid, compared to the milliseconds to seconds over which the folding takes place [15]. The *thermodynamic hypothesis*, first developed by Anfinsen [4], proposes that proteins fold to a *minimum energy* state. This motivates the attempt to predict protein folding by solving certain optimization problems. There are two main difficulties with this approach: there is as yet no scientific consensus on what the precise energy function to be minimized might be, and the functions commonly used lead to extremely difficult optimization problems [20,31].

One of the most popular models of protein folding is the hydrophobic-hydrophilic (H-P) model [14,17, 22]. In the H-P model, proteins are modelled as chains whose vertices are marked either H (hydrophobic) or P (hydrophilic); the resulting chain is embedded in some lattice. H nodes are considered to attract each other while P nodes are neutral. An *optimal* embedding is one that maximizes the number of H-H contacts. This combinatorial model is attractive in its simplicity, and already seems to capture several essential features of protein folding such as the tendency for the hydrophobic components to fold to the center of a globular (compactly folded) protein [14]. Unlike more sophisticated models of protein folding, the main goal of the H-P model is to explore broad qualitative questions about protein folding such as whether the dominant interactions are local or global with respect to the chain. For a nice survey of the kinds of questions asked and conclusions drawn, see [18].

While the H-P model is most intuitively defined in 3D to match the physical world, it is arguably more realistic as a 2D model for currently computationally feasible sizes. The basic reason for this is that the perimeter-to-area ratio of a short 2D chain is a close approximation to the surface-to-volume ratio of a long 3D chain [14,22].

Much work has been done on the H-P model [1,5–9,11–13,16,19,21,26–28,34–36]. Without theoretical guarantees, there are many heuristic approaches (e.g., [11,19]) and exhaustive approaches (e.g., [5,6]). In theoretical computer science, Berger and Leighton [9] proved NP-completeness of finding the optimal folding in 3D, and Crescenzi et al. [16] proved NP-completeness in 2D. Hart and Istrail [21] have developed a $3/8$-approximation in 3D and a $1/4$-approximation in 2D of the number of H-H contacts in the H-P model. Newman [32] just developed a $1/3$-approximation in 2D. Agarwala et al. [1] have developed constant-factor approximation algorithms for a generalized H-P model allowing multiple levels of hydrophobicity in the 2D triangular lattice and the 3D face-centered cube (FCC) lattice.

In this paper we are concerned with the question of whether or not H-P chains have *unique* optimal embeddings. This is a natural interpretation of the thermodynamic hypothesis in this model, and a natural model of folding stability. There are other factors to consider, e.g., Šali et al. [37,38] consider a folding stable if there is a large score gap between it and the next best folding, but uniqueness seems like a good candidate for making H-P strings more “protein-like” for the following reasons (among others):

- Insisting on uniqueness of optimal embeddings defeats the known proofs of NP-hardness [9,16];
- The H-P chains that produce protein-like 3D structures have a small number of optimal foldings [18];
- Algorithmically it is easy to design an H-P chain that folds to particular shape [25] as *one* of its optimal states;
- Experiments have shown that synthetically designed polymers tend to have many optimal embeddings, and also not fold stably [18].

In particular we explore a problem suggested by Brian Hayes [22] about the existence of stable protein foldings of all lengths. We solve this problem in a positive sense for circular protein strands. We also nearly solve the problem for open strands by exhibiting an infinite class of proteins having unique optimal foldings. More precisely, we prove the following main results, in a sense establishing the existence of stable protein foldings in the H-P model:

(1) We exhibit a simple family of closed chains of monomers, one for every possible (even) length, and prove that each chain has a unique optimal folding according to the H-P model.
(2) We exhibit a related family of open chains of monomers, one for every length divisible by 4, with the same uniquely-foldable property. Note that a result as strong as (1) cannot be obtained for open chains, because there are some lengths for which no uniquely foldable open chains exist.

In addition, we observe a complementary result about the ambiguity of folding:

(3) We exhibit a family of (open or closed) chains of monomers, one for every length divisible by 12, and prove that each chain has $2^{\Omega(n)}$ different optimal foldings, each with $\Omega(n)$ contacts. In biological terminology, these proteins have a highly degenerate ground state [22].

2. H-P model

In this section we review the H-P model and introduce some terminology common to the rest of the paper.

Proteins are chains of monomers, each monomer one of the 20 naturally occurring amino acids. In the H-P model, only two types of monomers are distinguished: *hydrophobic* (H), which tend to bundle together to avoid surrounding water, and *polar* or *hydrophilic* (P), which are attracted to water and are frequently found on the surface of a folding [14]. In our figures we use small gray disks to denote H monomers and black disks to denote P monomers. These monomers are strung together in some combination to form an *H-P chain*, either an open chain (path or arc) or a closed chain (cycle or polygon).

Proteins are folded onto the regular square lattice. More formally, a *lattice embedding* of a graph is a placement of vertices on distinct points of the (regular square) lattice such that each edge of the graph maps to two adjacent (unit-distance) points on the lattice. In the H-P model, proteins must fold according to lattice embeddings, so we also call such embeddings *foldings*.

The quality of a folding in the H-P model is simply given by the number of hydrophobic monomers (light-gray H nodes) that are not adjacent in the protein but adjacent in the folding. More formally, the *contact graph* of a folding has the same vertex set as the chain, and there is an edge between every two

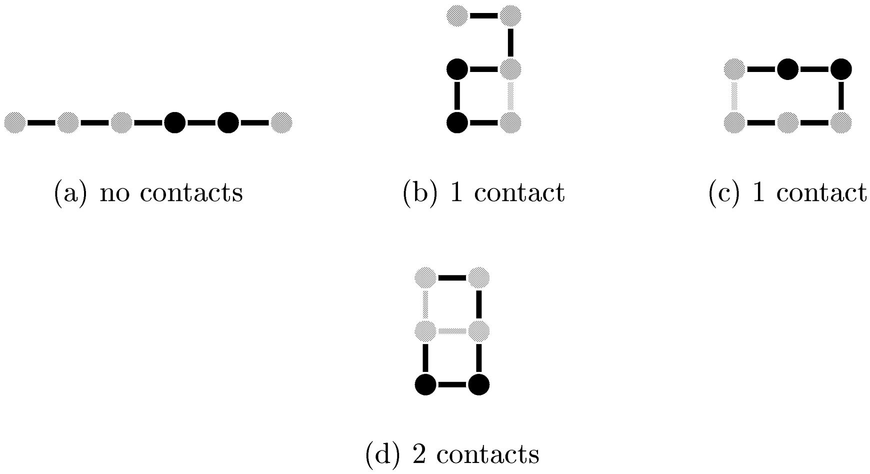


Fig. 1. An optimal folding is one that maximizes the number of contacts.

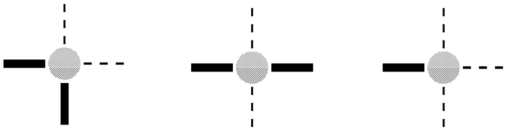


Fig. 2. Missing contacts.

H vertices that are adjacent in the folding onto the lattice, but not adjacent along the chain. The edges of the contact graph are called *contacts*; in our figures, contacts are drawn as light-gray edges.

An *optimal* folding maximizes the number of contacts over all foldings (see Fig. 1). Intuitively, if a protein is folded to bring together many hydrophobic monomers (H nodes), then those monomers are hidden from the surrounding water as much as possible.

There is a natural bijection between strings in $\{\mathsf{H}, \mathsf{P}\}^*$ and protein chains. We consider the nodes in a chain as labeled by their order in the string. We sometimes use a limited form of regular expressions to describe chains where e.g. H^k indicates k H nodes in sequence. Similarly, if we walk along an embedded chain in the order given and read off the direction of each edge, we can encode foldings as strings in $\{\mathsf{E}, \mathsf{W}, \mathsf{N}, \mathsf{S}\}^*$.

For any H node v in a lattice embedded chain, consider its 4 neighbouring lattice points. For each of the neighbouring lattice points that is occupied by neither an adjacent node on the chain, nor by an H node, we call the corresponding lattice edge a *missing contact*. We will also define the number of contacts adjacent to v as its *contact degree*. Therefore for an endpoint H node, a vertex's contact degree plus its missing contacts totals three; for a nonendpoint H node, its contact degree plus its number of missing contacts is 2.

We will further classify missing contacts into two groups. Consider the axis-parallel bounding box of the chain. If the missing contact corresponds to an edge outside the bounding box, we refer to it as an *external missing contact*. We can further classify an external missing contact by the one of four walls of the bounding box from which it emanates. (At a corner of the bounding box, we consider a missing contact to emanate from the wall to which the contact is perpendicular.) A missing contact which corresponds to an edge inside the (closed) bounding box we refer to as an *internal missing contact*.

3. General observations and ambiguous foldings

In this section we prove some basic structural and combinatorial results about contacts in the H-P model, in particular establishing that some (nontrivial) chains have exponentially many optimal foldings.

See also [7,8] for upper bounds on the number of contacts based on the patterns of lattice points occupied by H nodes.

Fact 1. *A folding of an open chain with h* H *nodes has at most $h + 1$ contacts, and a folding of a closed chain with h* H *nodes has at most h contacts.*

Proof. The sum of the number of contact and chain edges of any vertex is at most four, and every node except possibly the ends has at least two incident chain edges. □

Fact 2. *Any lattice-embeddable graph is bipartite.*

Proof. Any subgraph of a bipartite graph is bipartite, and the lattice points can be 2-colored in checkerboard fashion (see Fig. 3). □

Corollary 3. *If a folding of a closed chain* (*or an open chain with* P *endpoints*) *with h* H *nodes has h contacts, then its contact graph is a union of vertex-disjoint even cycles.*

Proof. In order to achieve h contacts, every H vertex must have contact degree 2. □

Corollary 4. *There can be a contact between two* H *nodes only if they have opposite parity* (*i.e., there is an even number of nodes between them*) *in the chain.*

Proof. The path between two nodes on the chain, along with the contact, form a cycle in the folding. Thus the result follows from Fact 2.

Fact 5. *Any optimal folding of the* (*open or closed*) *chain* $(\mathsf{PHP})^{4k}$ *has a contact graph consisting of k* 4-*cycles.*

Proof. $4k$ contacts are achievable, e.g., by a folding analogous to the one shown in Fig. 4, and no higher number of contacts is achievable by Fact 1. By Corollary 3 the contact graph is therefore a set of cycles. Now consider some cycle in the contact graph of length greater than 4, and consider the leftmost "⌜"

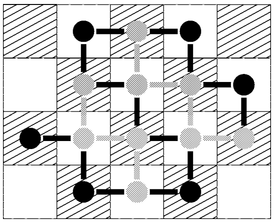


Fig. 3. Proving that all lattice-embeddable graphs are bipartite.

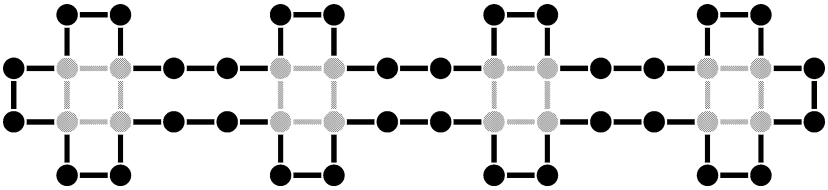


Fig. 4. Example of an optimal folding of $(\mathsf{PHP})^{4k}$.

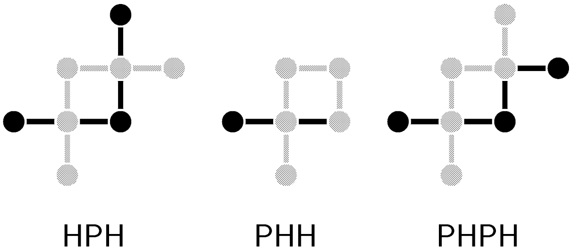


Fig. 5. Cases for Fact 5, with the forbidden subsequences they imply.

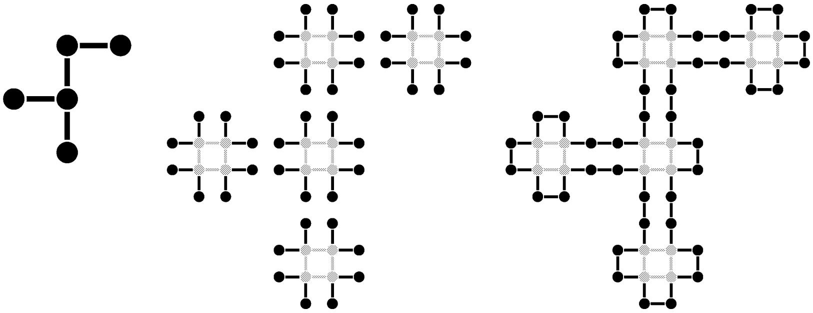


Fig. 6. Converting a lattice tree into an optimal embedding of $(\mathsf{PHP})^{4k}$.

corner. Up to symmetry, there are three cases, illustrated in Fig. 5. In each case there is either a singleton P or a double H on the chain. □

Fact 6. *For any $n = 12k$, there exists an n-node (open or closed) chain with at least $2^{\Omega(n)}$ optimal foldings, all with isomorphic contact graphs of size $\Omega(n)$.*

Proof. We argue that any lattice-embeddable tree on k nodes corresponds to an optimal folding of $(\mathsf{PHP})^{4k}$. To see this correspondence, take the embedded tree and scale by 4. Replace each node in the tree with a "gadget" consisting of a 4-cycle from the contact graph, and the associated forced chain edges (see Fig. 6). Finally replace the edges of the tree with pairs of edges between adjacent "gadgets", and close off any remaining PP pairs with chain edges (in the open-chain case, all but one pair is closed off).

Next observe that there are many lattice-embeddable trees on k nodes. A simple exponential lower bound can be obtained by considering the north/east staircase paths; because there are 2 choices at each step, this gives a lower bound of 2^k. Each tree (folding) is counted at most a constant number of times. □

The preceeding bound on the number of lattice trees can almost certainly be improved. The number of lattice trees has been studied by the Statistical Physics community, primarily from the point of view of deviation from exponential growth [23,24,29]. Based on a combination of theoretical and experimental results it is believed [24] that the number of lattice trees t_n with n nodes obeys the following bound $t_n \in \Omega(3.79^n/n)$.

4. Uniquely foldable closed chains

In this section we are concerned with closed H-P chains whose optimal foldings are unique (modulo isometries). For each $k \geqslant 1$, we define a closed chain S_k as follows. Let A_m denote the sequence $(\mathsf{HP})^m$. Define $u = \lceil k/2 \rceil$ and $d = \lfloor k/2 \rfloor$. Then define S_k as $\mathsf{P}A_u\mathsf{P}A_d$. Note that S_k has exactly two P-P edges, i.e. edges between two P nodes. We also define a folding F_k of S_k as follows (see Fig. 7). Let D_m (a "down staircase") denote the alternating path $(\mathsf{ES})^m$. Let U_m (an "up staircase") denote the alternating path $(\mathsf{WN})^m$. If k is even, define F_k as $\mathsf{E}D_d\mathsf{W}U_u$. If k is odd, define F_k as $\mathsf{E}D_d\mathsf{S}U_u$.

The main result of this section is the following theorem.

Theorem 7. *For each $k \geqslant 1$, F_k is the unique optimal folding of S_k.*

As well as providing evidence that the H-P model captures some approximation of the mechanism of protein stability for closed chains, Theorem 7 has several less direct consequences. In the next section we will use this theorem to prove a similar result about open chains. Furthermore, Theorem 7 tells us something nonobvious about the shape of optimal foldings in the H-P model, namely that there exist (closed) proteins all of whose optimal foldings are extremely "nonglobular" (noncompact). Along similar lines, in a preliminary version of this paper [2], we conjectured that every closed H-P chain had an optimal folding with the minimum possible area (enclosing no grid points). This conjecture turns out to be false [3].

The *conformation graph* of an embedding consists of the union of the chain edges and the contact graph. The idea of the proof of Theorem 7 is to show via parity arguments that the conformation graph of any optimal embedding of S_j is fixed. Once this is established, the embedding follows from the special form of the conformation graph (all but one face is a 4-cycle).

Fact 8. *There exists a folding of S_k with $k - 1$ contacts, namely F_k.*

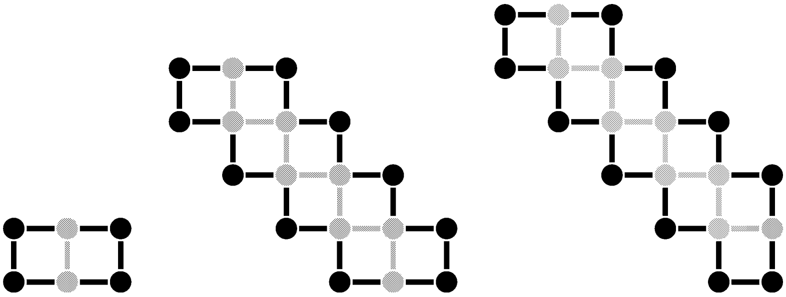


Fig. 7. Examples of S_k folded according to F_k for $k \in \{2, 8, 9\}$.

Fact 9. *The* H *nodes of S_k fall into two parity classes, separated into odd and even chains by the two* P-P *edges.*

In the case of an embedded closed chain Q, we distinguish between *chordal* contacts, i.e. those in the interior of Q, and *pocket* contacts, i.e. those exterior to Q.

Lemma 10. *There are no pocket contacts in an optimal folding of S_k.*

Proof. Each H node on the bounding box causes at least one missing contact. From Fact 8, we know that an optimal folding can therefore have at most two H nodes on the bounding box. Because every edge of the chain but two has at least one H node, and there must be at least one edge on each wall of the bounding box, there must be at least two H nodes on the bounding box, and this minimum is achievable only if both P-P edges are on distinct edges of the bounding box. For each P-P edge construct a slab by extending rays perpendicular to the edge from the endpoints (see Fig. 8). In order for a pocket contact to form, one of the odd or even chains must touch or cross one of the slabs. But if any vertex lies on a slab, the corresponding P-P edge cannot be on the bounding box. □

Lemma 11. *The contact graph is acyclic in any optimal folding of S_k.*

Proof. Consider some optimal folding of S_k. By Lemma 10, we know that all contacts must be chordal. Further observe that the conformation graph must be a planar graph. Consider an arbitrary planar embedding of the chain S_k. Note that each edge of a contact cycle must go from the odd chain to the even chain or vice-versa. After two steps along a cycle, there is no way to join the first node of the cycle to the (current) last node of the cycle without creating a crossing. □

Corollary 12. *The contact graph in an optimal folding of S_k is a path with k nodes and $k - 1$ edges.*

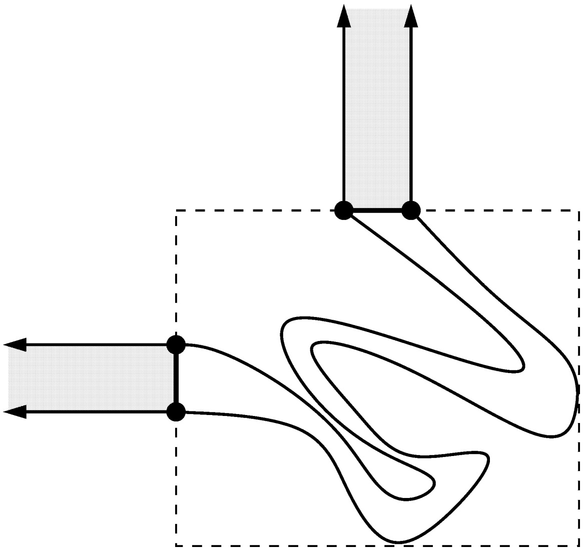


Fig. 8. Illustrating the proof of Lemma 10.

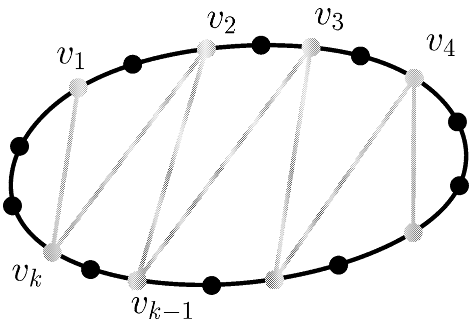


Fig. 9. Up to reversal of the labeling, the labeled contact graph of S_k is fixed.

Proof. This follows from Fact 8 and Lemma 11. □

Lemma 13. *If k is odd, every optimal folding of S_k has the same labeled contact graph. If k is even, there are two possible labeled contact graphs and the mapping from one to the other is given by the relabeling $j \mapsto k+1-j$.*

Proof. Note that the endpoints of the contact path must be adjacent to one of the P-P edges on the chain because otherwise some H node would be stranded. Further note that once the starting point of the path is chosen, the rest of the path is determined by an argument similar to the proof of Lemma 11. There is no choice of starting point for the case of k odd, because both endpoints of the contact path must be in the larger parity class of H nodes. □

We can deduce the following from the proof of Lemma 13.

Corollary 14. *In the conformation graph of an optimal folding of S_k,*

(a) *there are two* 4*-cycles of type* PPHH*,*
(b) *there are* $(k-2)$ 4*-cycles of type* PHHH*, and*
(c) *each contact edge is contained in exactly two* 4*-cycles.*

Fact 15. *Every* 4*-cycle has a unique folding, namely a square.*

Proof of Theorem 7. Consider the folded chain as a polygon, decomposed into quadrilaterals by contact edges. From Corollary 14 we can see that the dual graph of the decomposition is itself a path. We construct the folding by following this dual path. We start by choosing an orientation for one of the PPHH 4-cycles and embedding it. If $k=2$, then we have no choice for the final square. Otherwise, we choose an orientation for the second square and embed it. After the second square, by looking at degrees in the contact graph, it follows that we have no choice in embedding the next 4-cycle on the dual path. Thus our total choice in embedding was one translation, one rotation and one reflection. □

Corollary 16. *For every positive even n there is an n-node closed H-P chain with a unique optimal folding.*

Table 1
Percentage of H-P chains of length n with unique optimal embeddings

n	Unique	Total	Percentage
11	65	2,048	3.174
12	88	4,096	2.148
13	179	8,192	2.185
14	387	16,384	2.362
15	864	32,768	2.637
16	1,547	65,536	2.361
17	3,420	131,072	2.609
18	6,363	262,144	2.427
19	13,486	524,288	2.572
20	24,925	1,048,576	2.377

5. Uniquely foldable open chains

Finally we turn to open H-P chains. Dill et al. [18] computed that for chains of length up to 18, about 2% of chains have unique optimal foldings. In a similar vein, Hayes [22] found that for each $1 \leqslant n \leqslant 14$ except 3 and 5 there is an open chain with a unique optimal folding. We have duplicated the experiments of Dill et al. and extended them to chains of length 20, with (partial) results given in Table 1. We have further found experimentally that there are H-P chains with unique optimal foldings for lengths 15 through 25. Fig. 10 illustrates chains with unique optimal foldings for lengths up to 25, excluding lengths 2 (trivial) and $4k$ for $k > 3$ (covered by a theorem below). The bias towards H nodes is an artifact of our search program, which enumerates colourings in "binary-counter" order with $\mathsf{H} = 0$ and $\mathsf{P} = 1$. It has been reported elsewhere that real proteins have H to P ratio of about $2:3$ [25]. We will also see below that in the H-P model, unique optimality is achievable with a ratio very close to $1:1$.

A natural question is for what values of n there is an n-node open chain with a unique optimal folding. Based on our results about closed chains, one approach is to consider the open version of S_k with the first and last nodes removed. That is, define $Z_k = (\mathsf{HP})^u(\mathsf{PH})^d$ where $u = \lceil k/2 \rceil$ and $d = \lfloor k/2 \rfloor$. It turns out that this chain has multiple optimal folding for odd k, but only one optimal folding for even k (see Figs. 11 and 12).

In what follows, we will establish that up to isometries, the only optimal embedding of Z_{2k} is what we call the *standard* embedding, namely the P-P edge horizontal, the two adjacent edges down, the remaining edges on the right alternating right and down, and the remaining edges on the left alternating down and right (the standard embedding of Z_8 is illustrated in Fig. 11). This will establish the following theorem.

Theorem 17. *The open chain $Z_{2j} = (\mathsf{HP})^j(\mathsf{PH})^j$ has a unique optimal embedding for each positive j.*

Combining this theorem with examples illustrated in Fig. 10, it turns out that there are open chains with unique optimal foldings for $n = 2$, $n = 4$, and $6 \leqslant n \leqslant 25$.

Despite the seeming simplicity of the claim, and the similarity to Theorem 7, the proof of Theorem 17 requires a number of technical lemmas. We will argue that every embedding of Z_{2k} has at least four missing external contacts, and further prove that the standard embedding of Z_{2k} is the only embedding with exactly four missing contacts. We accomplish this by reducing the open-chain case to the closed-

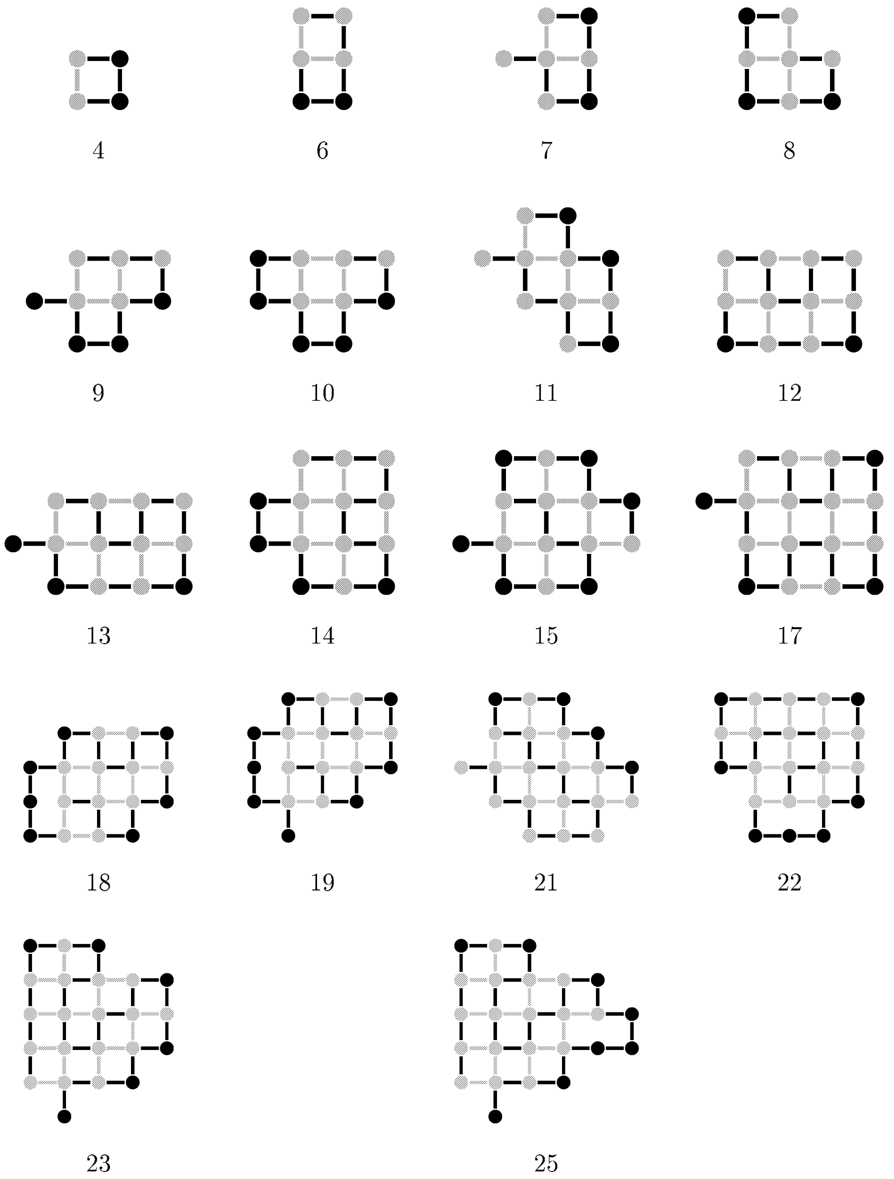


Fig. 10. Examples of H-P chains with unique optimal foldings, shown in their optimal embedding.

chain case discussed in the previous section. In particular, we will show that in an optimal embedding of Z_{2k}, the H endpoints are on the bounding box and in contact. This will allow us to extend any optimal embedding of Z_{2k} to an optimal embedding of S_{2k}. The proof can be summarized as follows:

(1) An optimal embedding has at most 4 missing contacts (Fact 18, Corollary 19).
(2) An optimal embedding has at least 3 external missing contacts and at most one internal missing contact (Fact 20, Corollaries 21 and 22).

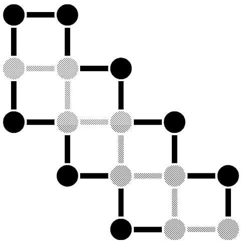


Fig. 11. Unique optimal folding of Z_8.

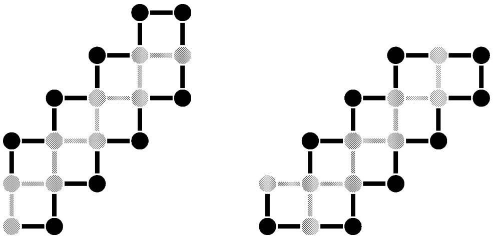


Fig. 12. Two optimal foldings for Z_9.

(3) There are no H corners (i.e., turns in the chain) on the bounding box (Lemmas 24 and 25).
(4) If an endpoint is on the bounding box, either it is in contact with the other endpoint, or creates an internal missing contact (Lemma 26).
(5) In an optimal embedding of Z_{2k}, there is at most one H node on the bounding box that is neither a corner, nor an endpoint (Facts 27 and 28, Lemmas 29 and 30).

In the rest of this section, we present the details of the proof of Theorem 17. As in the previous section, we start by observing that any optimal embedding must be at least as good as our example embedding.

Fact 18. *The standard embedding of Z_{2k} has only four missing contacts, all external.*

Corollary 19. *Any optimal embedding of Z_{2k} has at most four missing contacts.*

We begin with the following observation.

Fact 20. *In any embedding of Z_{2k}, either*

(a) *three bounding-box walls contain* H *nodes, and one contains only the* P-P *edge of Z_{2k}, or*
(b) *four bounding-box walls contain* H *nodes.*

Proof. Every bounding-box wall must contain either an edge or an endpoint of Z_{2k}. Only one edge of Z_{2k} does not contain an H node, and each endpoint is a H node. □

Corollary 21. *In an optimal embedding of Z_{2k}, there are missing external contacts emanating from at least three walls of the bounding box. The fourth wall either contains the* P-P *edge, or has a fourth missing external contact.*

Corollary 22. *Any embedding of Z_{2k} has at least three external missing contacts, and an optimal embedding has at most one internal missing contact.*

Fact 23. *For $k > 1$, the bounding box of any optimal embedding of Z_{2k} has both height and width at least two.*

Proof. If either dimension of the bounding box is less than 2, then all of the H nodes are on the bounding box. □

Fact 20 implies that there is at least one nonendpoint H node v on the bounding box. We break this down into two cases. Two edges adjacent to v could be on the bounding box, in which case we call v a *straight* H *node*. Alternatively, only one edge adjacent to v could be on the bounding box, in which case we call v an H *corner*. We first argue that in an optimal embedding of Z_{2k} there are no H corners at the corner of the bounding box.

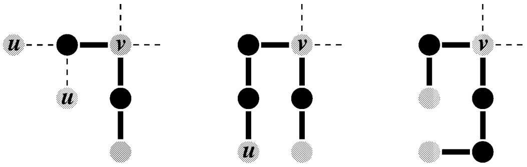
Lemma 24. *In an optimal embedding of Z_{2k}, there are no* H *corners at the corner of the bounding box.*

Proof. Let v be an H corner which is also on the northeast corner of the bounding box. We distinguish three cases, as illustrated in Fig. 13. In the first case, v is not adjacent to the P-P edge. In the second, v is adjacent to the P-P edge but not on the same wall of the bounding box. In the third, v is adjacent to the P-P edge and on the same wall of the bounding box.

We first consider the case where v is not adjacent to the P-P edge. Because the two H nodes nearest v (two edges away) cannot both occupy the lattice point southwest from v, one of these two nodes must also be on the bounding box. Suppose there exists an H node on the bounding box two edges south from v, and consider the position of the other node u (refer to Fig. 13(a)). If u is on the bounding box then there exists a fourth external missing contact along only two walls (north and east) of the bounding box. This implies the presence of a fifth external missing contact. Thus the embedding is not optimal by Corollary 19. The other possibility is that the path from v to u is west-south, but this creates a missing internal contact between u and a P node. Because the presence of a fourth external contact is necessary, this embedding cannot be optimal.

We next consider the case where v is adjacent to the P-P edge, but where the P-P edge is not on the same bounding box wall as v (refer to Fig. 13(b)). This implies that the P-P edge occupies the lattice point southwest from v, and therefore there exists an H node two edges south from v. Because the east wall has two external missing contacts and the P-P edge cannot be on the south wall, by Corollary 21 the P-P edge must be on the west wall of the bounding box (otherwise, there would be external missing contacts on each wall, for a total of five, and the embedding would not be optimal). By Fact 23, this is a contradiction.

Finally, we consider the case where v is adjacent to the P-P edge and on the same wall (see Fig. 13(c)). We assume that the P-P edge lies directly south of v. Because the north and east walls have two external missing contacts and the P-P edge, any additional external missing contact on these walls would imply suboptimality by Corollary 21. Therefore the two H nodes nearest v cannot lie on either of these walls, and must lie as indicated in the figure. This causes an internal missing contact between an H node and a P node. Thus there are at least five missing contacts, and the embedding is not optimal. □



(a) (b) (c)

Fig. 13. Illustrating the proof of Lemma 24.

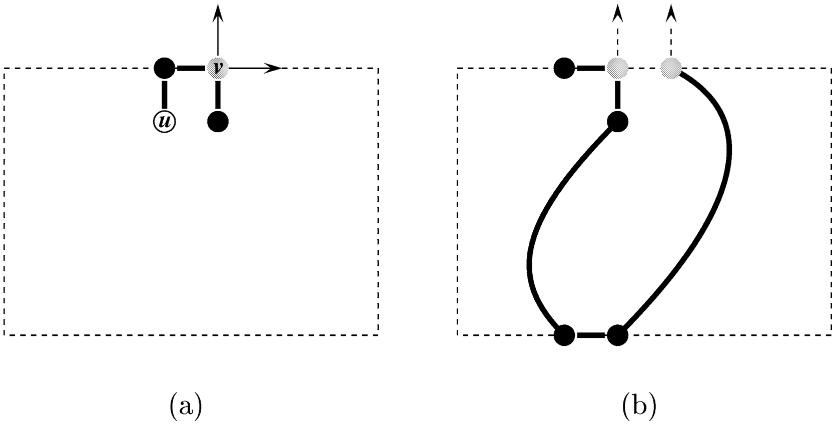


Fig. 14. Illustrating the proof of Lemma 25.

We now expand the restriction on H corners to include the entire bounding-box wall.

Lemma 25. *There are no* H *corners on the bounding box in an optimal embedding of Z_{2k}.*

Proof. By Lemma 24, we need only consider the case of an H corner in the relative interior of a bounding box edge. Let v be such an H corner. We assume that the edges of the chain adjacent to v are to the west and the south. We distinguish two cases, as illustrated in Fig. 14. In the first case, v has no contacts and thus has a missing external contact and a missing internal contact (along the wall of the bounding box). In the second case, v has an internal contact, which must be with a second H corner or an endpoint.

We first consider the case where v has no contacts. Because there is a missing internal contact, there can only be three external missing contacts if the embedding is to be optimal. Therefore the P-P edge must be on the bounding box, and cannot be on the north wall. Furthermore, no other H node can be on the north wall. Consider the edge adjacent to v on the north wall, and the node u which follows it. Because u cannot be on the north wall, its only possible position is south west off. This creates a second internal missing contact between u and a P node (if u is an H node) or an extra external missing contact (if u is part of the P-P edge), causing suboptimality.

We finally consider the case where v has an internal contact, which must be with a second H corner or endpoint w. Because w has a second external missing contact on the north wall, the P-P edge must lie on the bounding box by Corollary 21. Furthermore, the P-P edge must lie on the path from v to w because v and w have different parity. Thus the path from v to the P-P edge creates a barrier between all points west of this path and all points after the P-P edge. The endpoint of the same parity as v must lie west of the path, as the chain cannot go outside the bounding box. This endpoint has three missing contacts, for a total of five for the chain. Therefore the embedding is not optimal. □

We have now established that if an H node is on the bounding box, it must either be straight or an endpoint. With respect to endpoints, we observe the following:

Lemma 26. *In an optimal embedding of Z_{2k}, if an endpoint is on the bounding box, then either there is an internal missing contact, or the two endpoints are in contact.*

Proof. An endpoint has three potential contacts; at least one of which lies along the wall of the bounding box. Either this is an internal missing contact, or the endpoint is adjacent to an H node. Because there are no H corners in an optimal embedding, this H node must be the second endpoint. □

Because there are only two endpoints and one P-P edge, we know there must be at least one straight H node on the bounding box. We define two kinds of straight H nodes. We say a straight H node v is *coupled* if the preceding or following H node is also on the same wall of the bounding box as v; otherwise it is *solitary*. These cases are illustrated in Fig. 15.

Fact 27. *In an optimal embedding of Z_{2k}, a solitary straight* H *node must either be adjacent to the* P-P *edge or contact with an endpoint.*

Proof. Let v be a solitary straight H node on the north wall of the bounding box which is not adjacent to the P-P edge. Then there must be two H nodes immediately southwest and southeast of v, as illustrated in Fig. 16. The lattice point south of v cannot be the adjacent P node of any of the H nodes, nor can it be vacant, because there would be at least two missing internal contacts, violating optimality. □

Fact 28. *In an optimal embedding of Z_{2k}, there is at most one pair of coupled straight* H *nodes.*

Proof. Each m-tuple of coupled H nodes causes m external missing contacts on the same bounding box wall. There can be at most one wall with two external missing contacts, and none with three or more. □

The following two lemmas establish that there is at most one solitary straight H node.

Lemma 29. *In an optimal embedding of Z_{2k}, there is at most one solitary straight* H *node in contact with an endpoint.*

Proof. Assume there are two such straight H nodes, n and s, which contact with two endpoints, n' and s', respectively. We assume n is on the north wall and s on the south; the argument can be slightly modified

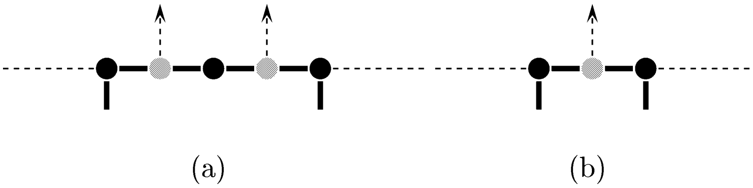


Fig. 15. A pair of coupled straight H nodes, and a solitary straight H node.

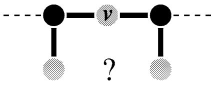


Fig. 16. Illustrating the proof of Fact 27.

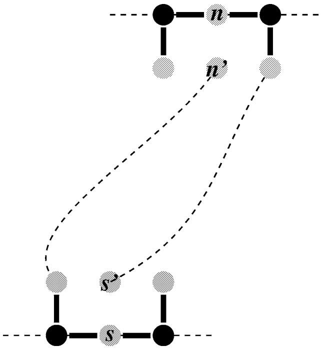


Fig. 17. Illustrating the proof of Lemma 29.

for any other case. Because n' and s' are of different parity, so too must be n and s. Therefore there is a path on the chain from n to s' which does not pass through s, and a path from s to n' which does not pass through n. Assume without loss of generality that the path from n to s' leaves n to the east, as in Fig. 17. Then the path from s to n' must leave s to the west to avoid intersection. Because the chain is connected, there must be a path from n to s. The only possibility left is that the path leaves n to the west, and enters s from the east. However, this requires the path to either leave the bounding box, or intersect the rest of the chain, neither of which is possible. □

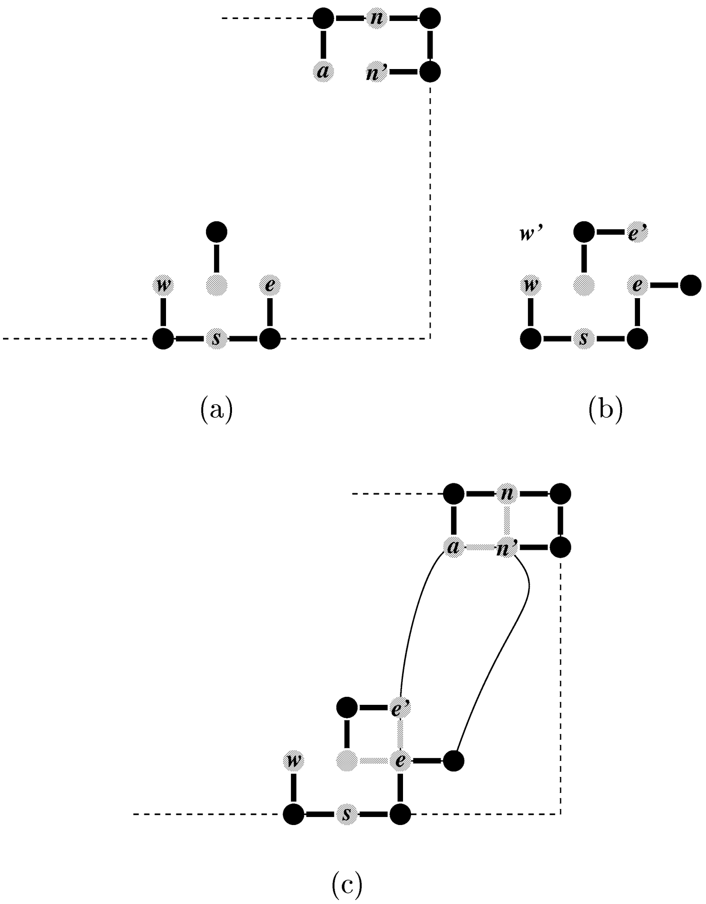
Lemma 30. *In an optimal embedding of Z_{2k}, there is at most one solitary straight* H *node on the bounding box.*

Proof. Suppose there is more than one straight H node on the bounding box. By Fact 27 and Lemma 29 there must be exactly two: one must be adjacent to the P-P edge; the other must contact with an endpoint. We observe that the P-P edge must be on the bounding box, because each wall not containing a solitary H node must otherwise contain either a pair of coupled straight H nodes or an endpoint; any combination of these two possibilities leads to a total of at least 5 missing contacts (apply Lemma 26 in the case of an endpoint).

Assume the north, east and south walls are covered by the two solitary straight H nodes and the P-P edge. We assume the configuration in Fig. 18(a) without loss of generality (the argument here will not depend on whether the two solitary nodes are on opposite walls of the bounding box). Call the H node on the south wall s, and the H nodes preceding and following (northeast and northwest of s) e and w. One endpoint of the chain is in contact with e, w and s.

Consider the possibilities for covering the west wall of the bounding box: there is either a pair of coupled straight nodes, or an endpoint. By Lemma 26, either case results in two missing contacts, both either contained in or emanating from the west wall. It follows that we need only find one more missing contact to establish suboptimality.

Consider the H node z east of s; e cannot be on the bounding box as this would create an external missing contact. Placing an H node east of e just creates another internal missing contact, which in turn cannot be blocked without creating a missing contact with the P node between s and e on the chain. It follows that the chain must turn east at e; in order to avoid an internal missing contact, there must be an H node north of e (at e' in Fig. 18(b)), and a chain edge west from this H node. Note that lattice point north of w (i.e. w') cannot contain an H node, as this would create an internal missing contact.



(a) (b)

(c)

Fig. 18. Illustrating the proof of Lemma 30.

By planarity, the subchains containing e and e' must be connected to the P-P edge as shown in Fig. 18(c). Consider the polygon Q formed these connecting chains, along with the contacts ee' and an'. By an argument similar to Lemma 11 (with the extension that no contacts can be formed with w), all of the remaining contacts for H nodes on this polygon must be internal to Q. Because there is an imbalance in the parity of potential contacts for nodes on Q, this forces an internal missing contact. □

We are finally ready to characterize the intersection of an optimal embedding of Z_{2k} with its bounding box.

Lemma 31. *In an optimal embedding of Z_{2k}, the two endpoints are on the bounding box and in contact, and the* P-P *edge is adjacent to a solitary* H *node, both of which are also on the bounding box. Furthermore, there are no internal missing contacts.*

Proof. By Lemma 30 there is at most one solitary straight H node on the bounding box, and there is of course only one P-P edge. On the other two walls of the bounding box, there are either two endpoints, or one endpoint and one pair of coupled straight H nodes. In the last case, the endpoint causes one

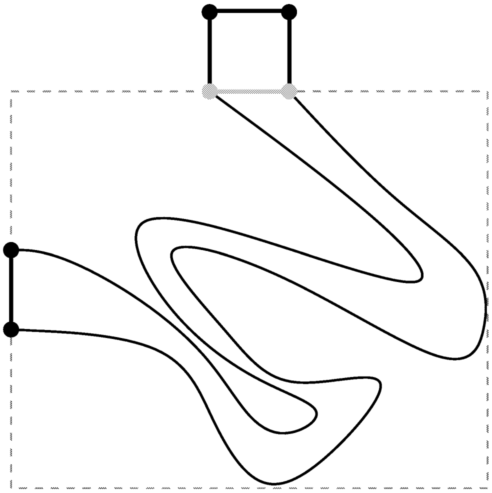


Fig. 19. Converting an optimal embedding of Z_{2k} into an optimal embedding into an optimal embedding of S_{2k}.

internal missing contact by Lemma 26, and the coupled straight H nodes cause two external missing contacts. Therefore there are no coupled straight H nodes. Furthermore, because both endpoints are on the bounding box, they must be in contact by Lemma 26 to avoid having two internal missing contacts. Because the two endpoints contact, and are both on the bounding box, they have two external missing contacts on the same wall. Therefore there are four external missing contacts in an optimal embedding, and thus no internal missing contacts. □

The previous lemma claims in essence that the open chain Z_{2k} behaves just like a closed chain in any optimal embedding. We formalize this intuition as follows:

Theorem 32. *There are as many optimal embeddings of S_{2k} as there are of Z_{2k}.*

Proof. By the preceding lemma, in an optimal embedding the endpoints Z_{2k} are in contact and on the bounding box; thus we can convert an optimal embedding of Z_k into an optimal embedding of S_{2k} by adding a second P-P edge, outside the bounding box (see Fig. 19). □

Theorem 17 is a straightforward consequence of the preceding theorem and Theorem 7.

We expect that by similar methods we can prove the following:

Conjecture 33. *For odd $k \geqslant 5$, the open chain Z_k has exactly two optimal embeddings.*

We have computationally verified this conjecture for chains of length up to 26, that is, for odd $5 \leqslant k \leqslant 13$. For $k = 1$ and $k = 3$, Z_k in fact has a unique optimal folding.

6. Conclusions and directions for future work

In this paper we considered a natural characterization of stable protein folding in the 2D H-P model, namely uniqueness of optimal folding. We established that

(1) there exist closed H-P chains with unique optimal folds for all (even) lengths, and
(2) there exist open H-P chains with unique optimal folds for all lengths divisible by 4.

We further observed that

(3) there exist arbitrarily long H-P chains with linear sized contact graphs and an exponential number of optimal foldings.

There are several natural directions for future work, involving more general lattices, asymptotic bounds, and algorithmic questions, as summarized below.

There is a great deal of natural skepticism about the biological relevance of results stemming from the 2D square lattice. While certain qualitative properties are independant of the lattice used [18], in the case of the present work it seems clear that the bipartiteness of the lattice plays an important (and difficult-to-motivate) role. It is thus important to consider nonsquare lattices in 2D, and preferably nonbipartite lattices in 3D. Examples include the triangular lattice in 2D, and the face-centered cubic (FCC) lattice suggested by Neumaier [31] as a minimal approximation of chemical bond distances and angles. For example, Agarwala et al. [1] consider both of these lattices.

The existence of H-P chains with unique embeddings in a given lattice is only a first step to understanding the behaviour of these models with respect to uniqueness. Experimental results [18] have shown that about 2% of open chains up to length 18 have unique optimal foldings. It would be very nice to have asymptotic bounds for the fraction of H-P chains with unique optimal foldings. Rather than considering arbitrary H-P chains, it would also be useful to see what fraction of the proteins in the Protein Data Bank [10] fold uniquely in the H-P model. It is likely that the best that can be hoped for is that real proteins have a small number of optimal foldings.

From a sequence-design point of view, the more interesting question is not whether there exists an H-P sequence with a small number of optimal foldings, but how to design sequences with this property. From a combinatorial point of view, this asks for a characterization of what sequences have unique (or a small number of) optimal foldings.

From an algorithmic point of view, there are two natural questions. The first problem is whether there is an efficient algorithm to recognize sequences with unique optimal foldings. The second problem is whether the problem of finding a minimum energy folding of an H-P chain is still NP-hard when restricted to chains with unique optimal foldings.

Finally, there may be better definitions of folding stability in the H-P model. We have mentioned the notion of a large gap in the number of contacts between the optimal folding and the next best folding [37, 38]. Given that the H-P model is only approximate, it may also be inappropriate to distinguish between chains having e.g. 1 and 2 optimal embeddings.

Acknowledgements

The authors would like to thank Godfried Toussaint for pointing us to this topic and for organizing the workshop at which the research was initiated. The authors would also like to thank Vida Dujmović, Jeff Erickson, Ferran Hurtado and Suneeta Ramaswami for stimulating conversations on this and other topics, and to thank Tom Shermer and Joe Horton for helpful discussions on lattice trees.

References

[1] R. Agarwala, S. Batzoglou, V. Dancik, S.E. Decatur, M. Farach, S. Hannenhalli, S. Muthukrishnan, S. Skiena, Local rules for protein folding on a triangular lattice and generalized hydrophobicity in the HP model, J. Comput. Biology 4 (2) (1997) 275–296.

[2] O. Aichholzer, D. Bremner, E.D. Demaine, H. Meijer, V. Sacristán, M. Soss, Long proteins with unique optimal foldings in the H-P model, in: Proceedings of the 17th European Workshop on Computational Geometry, 2001.

[3] G. Aloupis, E.D. Demaine, P. Morin, M. Soss, On the area of minimum energy foldings of H-P chains, Manuscript, 2001.

[4] C. Anfinsen, Studies on the principles that govern the folding of protein chains, in: Les Prix Nobel en 1972, Nobel Foundation, 1972, pp. 103–119.

[5] R. Backofen, Constraint techniques for solving the protein structure prediction problem, in: Proceedings of the 4th International Conference on Principles and Practice of Constraint Programming, in: Lecture Notes in Computer Science, Vol. 1520, 1998, pp. 72–86.

[6] R. Backofen, Using constraint programming for lattice protein folding, in: Proceedings of the 3rd Pacific Symposium on Biocomputing, 1998, pp. 387–398.

[7] R. Backofen, An upper bound for number of contacts in the HP-model on the face-centered-cubic lattice (FCC), in: Proceedings of the 11th Annual Symposium on Combinatorial Pattern Matching, Montreal, in: Lecture Notes in Computer Science, Vol. 1848, 2000, pp. 277–292.

[8] R. Backofen, S. Will, Optimally compact finite sphere packings – hydrophobic cores in the FCC, in: Proceedings of the 12th Annual Symposium on Combinatorial Pattern Matching, Jerusalem, in: Lecture Notes in Computer Science, Vol. 2089, 2001, pp. 257–271.

[9] B. Berger, T. Leighton, Protein folding in the hydrophobic-hydrophilic (*HP*) model is NP-complete, J. Comput. Biology 5 (1) (1998) 27–40.

[10] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids Res. 28 (2000) 235–242.

[11] E. Bornberg-Bauer, Chain growth algorithms for HP-type lattice proteins, in: Proceedings of the 1st Annual International Conference on Computational Molecular Biology, 1997, pp. 47–55.

[12] H.S. Chan, K.A. Dill, Origins of structure in globular proteins, Proc. Nat. Acad. Sci. USA 87 (1990) 6388–6392.

[13] H.S. Chan, K.A. Dill, Polymer principles in protein structure and stability, Annual Reviews of Biophysics and Biophysical Chemistry 20 (1991) 447–490.

[14] H.S. Chan, K.A. Dill, The protein folding problem, Physics Today (February 1993) 24–32.

[15] P. Clote, R. Backofen, Computational Molecular Biology: An Introduction, Chapter 6, in: Wiley Series in Mathematical and Computational Biology, Wiley, 2000.

[16] P. Crescenzi, D. Goldman, C. Papadimitriou, A. Piccolboni, M. Yannakakis, On the complexity of protein folding, J. Comput. Biol. 5 (3) (1998).

[17] K.A. Dill, Dominant forces in protein folding, Biochemistry 29 (31) (1990) 7133–7155.

[18] K.A. Dill, S. Bromberg, K. Yue, K.N. Fiebig, D.P. Yee, P.D. Thomas, H.S. Chan, Principles of protein folding – A perspective from simple exact models, Protein Sci. 4 (1995) 561–602.

[19] K.A. Dill, K.M. Fiebig, H.S. Chan, Cooperativity in protein-folding kinetics, Proc. Nat. Acad. Sci. USA 90 (5) (1993) 1942–1946.

[20] C. Floudas, P. Pardalos (Eds.), Optimization in Computational Chemistry and Molecular Biology, Nonconvex Optimization and its Applications, Vol. 40, Kluwer Scientific, 2000.

[21] W.E. Hart, S. Istrail, Fast protein folding in the hydrophobic-hydrophilic model within three-eights of optimal, in: Proceedings of the 27th Annual ACM Symposium on the Theory of Computing, Las Vegas, NV, 1995, pp. 157–168.

[22] B. Hayes, Prototeins, American Scientist 86 (1998) 216–221.

[23] E.J. Janse Van Rensburg, On the number of trees in $\mathcal{Z}^d$, J. Phys. A 25 (1992) 3523–3528.

[24] I. Jensen, Enumerations of lattice animals and trees, J. Statist. Phys. 102 (3–4) (2001) 865–881.

[25] J.M. Kleinberg, Efficient algorithms for protein sequence design and the analysis of certain evolutionary fitness landscapes, in: Proceedings of the 3rd International Conference on Computational Molecular Biology, Lyon France, ACM, 1999, pp. 226–236.

[26] K.F. Lau, K.A. Dill, A lattice statistical mechanics model of the conformation and sequence spaces of proteins, Macromolecules 22 (1989) 3986–3997.

[27] K.F. Lau, K.A. Dill, Theory for protein mutability and biogenesis, Proc. Nat. Acad. Sci. USA 87 (1990) 638–642.

[28] D.J. Lipman, W.J. Wilber, Modelling neutral and selective evolution of protein folding, Proc. Royal Soc. London, Ser. B 245 (1312) (1991) 7–11.

[29] N. Madras, G. Slade, The Self-Avoiding Walk, Birkhäuser, 1996.

[30] K.M. Merz, S.M. Le Grand (Eds.), The Protein Folding Problem and Tertiary Structure Prediction, Birkhäuser, Boston, 1994.

[31] A. Neumaier, Molecular modeling of proteins and mathematical prediction of protein structure, SIAM Rev. 39 (3) (1997) 407–460.

[32] A. Newman, A new algorithm for protein folding in the HP model, in: Proceedings of the 13th Annual ACM-SIAM Symposium on Discrete Algorithms, San Francisco, 2002, pp. 876–884.

[33] G. Song, N. Amato, Using motion planning to study protein folding pathways, in: Proceedings of the 5th International Conference on Computational Molecular Biology, ACM, 2001, pp. 287–296.

[34] R. Unger, J. Moult, Finding the lowest free energy conformation of a protein is a NP-hard problem: Proof and implications, Bull. Math. Biology 55 (6) (1993) 1183–1198.

[35] R. Unger, J. Moult, A genetic algorithm for 3D protein folding simulations, in: Proceedings of the 5th International Conference on Genetic Algorithms, San Mateo, CA, 1993, pp. 581–588.

[36] R. Unger, J. Moult, Genetic algorithms for protein folding simulations, J. Molecular Biology 231 (1) (1993) 75–81.

[37] A. Šali, E. Shaknovich, M. Karplus, How does a protein fold, Nature 369 (1994) 248–251.

[38] A. Šali, E. Shaknovich, M. Karplus, Kinetics of protein folding: A lattice model study of the requirements for folding to the native state, J. Molecular Biology 235 (1994) 1614–1636.