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## A survey on mathematical modelling on potential energy surface of proteins

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**Abstract**<sup>1</sup>. The native conformation of a protein in a solvent is viewed to correspond to the global minimum of the proteins potential energy surface. Finding the native conformation of a protein is considered as a multiple minima problem. In this paper we provide a survey on the latest investigations on the use of molecular force fields to describe the potential energy surface of a protein. We discuss several heuristic methods to search for the global minimum of potential energy surface of a protein. We also discuss fold recognition which provides an alternative method to find the native confirmation of a protein.

### 1. INTRODUCTION

Proteins play crucial roles in almost every biological process. They are responsible in one form or another for a variety of physiological functions. They function as catalysts, they transport and store other molecules such as oxygen, they provide mechanical support and immune protection, they generate movement, they transmit nerve impulses, and they control growth and differentiation. Proteins are linear polymers built of monomer units called amino acids. The construction of a vast array of macromolecules from a limited number of monomer building blocks is a recurring theme in biochemistry. Does protein function depend on the linear sequence of amino acids? The function of a protein is directly dependent on its three dimensional structure. Remarkably, proteins spontaneously fold up into three-dimensional structures that are determined by the sequence of amino acids in the protein polymer. Thus, proteins are the embodiment of the transition from the one-dimensional world of sequences to the three-dimensional world of molecules capable of diverse activities. Protein structures are commonly grouped into four levels of structure: Primary structure - the amino acid sequence of the peptide chains. The primary structure is held together by covalent or peptide bonds, which are made during the process of protein biosynthesis or translation. These peptide

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bonds provide rigidity to the protein. The two ends of the amino acid chain are referred to as the *C*-terminal end or carboxyl terminus (*C*-terminus) and the *N*-terminal end or amino terminus (*N*-terminus) based on the nature of the free group on each extremity.

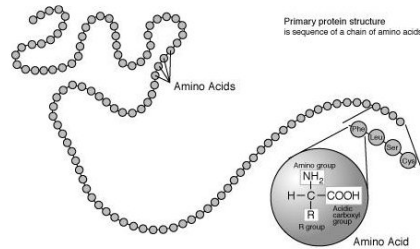


FIGURE 1. Primary structure of a protein  
(<http://www.accessexcellence.org/AB/GG/aminoAcid.html>)

**Secondary structure** – highly regular sub-structures (*alpha helix and strands of beta sheet*) which are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule.

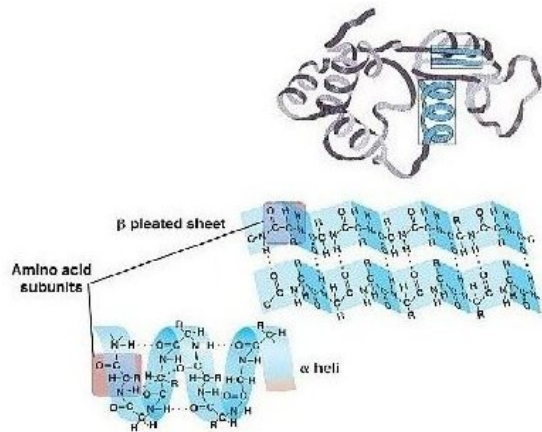


FIGURE 2. Secondary structure of protein  
(<http://kvhs.nbed.nb.ca/gallant/biology/biology.html>)

**Tertiary structure** - three-dimensional structure of a single protein molecule; a spatial arrangement of the secondary structures. It also describes the completely folded and compacted polypeptide chain.

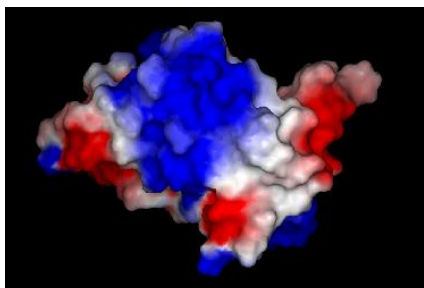


FIGURE 3. Tertiary structure of protein  
(<http://accelrys.com/discovery-studio/biopolymer/>)

**Quaternary structure** - complex of several protein molecules or polypeptide chains, usually called protein subunits in this context, which function as part of the larger assembly or protein complex.

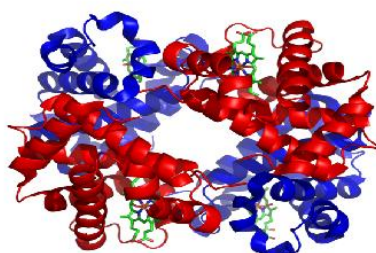


FIGURE 4. Quaternary structure of protein  
(<http://biochemistryquestions.wordpress.com>)

In addition to these levels of structure, a protein may shift between several similar structures in performing its biological function. This process is also reversible. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as chemical conformation, and transitions between them are called conformational changes.

Protein Folding is the ability of protein molecules to fold into their highly structured functional states defined by their amino acid sequence. The strings of amino acids that emerge from the protein synthesizing machinery, bend, loop, twist, coil and collapse on itself to produce the finished design as enzymes and other life-sustaining cellular components. The spontaneous self-assembly of protein molecules with huge numbers of degrees of freedom into a unique three dimensional structure that carries out a biological function is perhaps the simplest case of biological self-organization and one of the most remarkable achievements in biology.

Discovering the tertiary structure of a protein, or the quaternary structure of its complexes, can provide important clues about how the protein performs its function. Common experimental methods of structure determination include *X*-ray

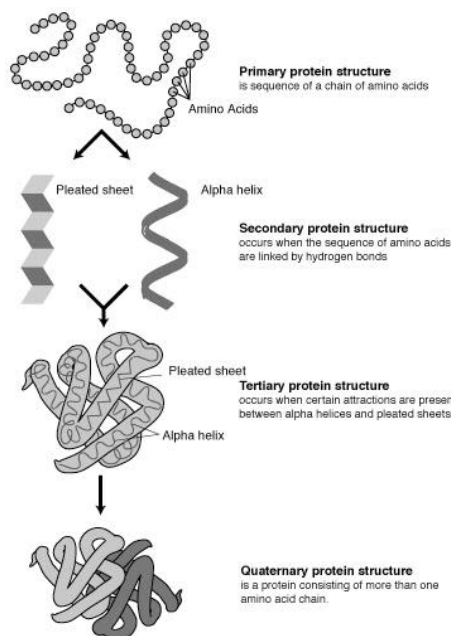


FIGURE 5. Protein structures (<http://en.wikibooks.org/>)

crystallography and *NMR* spectroscopy, both of which can produce information at atomic resolution.

## 2. PROTEIN STRUCTURE AND MINIMUM ENERGY CONFORMATION

According to proteins tertiary structure, proteins can be divided into globular and fibrous proteins. Globular proteins have nearly spherical shape. All enzymes are globular. Proteins are predominantly globular. The fibrous proteins contain a variety of structure proteins and normally exhibit regularities in their primary structures. These regularities are generally so strong that the native conformations of structural proteins are much easier to characterize than those of globular proteins. The conformational search of the global minimum energy conformation of a protein *ab initio* from the amino acid sequence is one of the greatest challenges in computational biology.

A challenge in the area of computational biology has been to develop a method to theoretically predict the correct three-dimensional structure of a protein *ab initio* from the primary structure. The two most common approaches to the problem of predicting protein structure from sequence would be either to search the native structure of the protein among the entire conformational space available to the polypeptide, or to simulate the folding process in detail.

The former appears to be beyond our reach. Even the structures of small organic molecules cannot be generated using algorithmic implementations of the “laws of

physics” for atomic interactions. Full atom protein folding simulations are completely beyond current computational resources. Short simulations from the folded state, known as molecular dynamics simulations, are possible but do not accurately recreate the behavior of folded proteins in solution.

Exhaustive conformational search is also out of reach; the number of possible conformations is immense and would take too long to explore either computationally or in vivo during folding [Levinthal, 1968]. In an attempt to reduce the search space, a common approach is to use a simplified polypeptide representation and restrain atom or residue positions to a lattice [Dill et al., 1995]. Folding or conformational search experiments are rarely successful, even for small proteins.

### 3. MATHEMATICAL OPTIMIZATION

In mathematics programming, an optimization problem is the problem of finding the best solution from all feasible solutions. More formally, an optimization problem has the general form

$$(1) \quad \min_{x \in S} f(x) \quad \text{or} \quad \max_{x \in S} f(x) ,$$

where

- $f(x)$  is a real-valued function defined on the space  $R^n$  called *objective function*;
- $S$  is a subset of the space  $R^n$  called *feasible set*;
- The points  $x^*$  in  $S$  are called *feasible*.

A point  $x^*$  in  $S$  is said to be a local minimum of the  $f(x)$  if

$$(2) \quad f(x^*) \leq f(x) \quad , \quad \forall x \in S \cap \{x, \|x - x^*\| < \delta, \delta > 0\} ,$$

A point  $x^*$  in  $S$  is said to be a global minimum of the function  $f(x)$  if

$$(3) \quad f(x^*) \leq f(x) \quad , \quad \forall x \in S .$$

Local and global maximum points can be similarly defined. Maximization and minimization are related by the following relation

$$(4) \quad \max\{f(x) , \forall x \in S\} = - \min\{-f(x) , \forall x \in S\} .$$

Therefore any maximization problem can be converted into an equivalent minimization problem, and vice versa.

### 4. POTENTIAL ENERGY SURFACE DEFINED BY FORCE FIELDS

Let us consider a molecule with  $N$  atoms. The position of the  $i$ -th atom is denoted by the vector  $x_i$ . We describe the potential energy surface of a protein by molecular mechanics. Molecular mechanics states that the potential energy of a protein can be approximated by the potential energy of the nuclei. Therefore, the energy contribution of the electrons is neglected. This approximation allows one to write the potential energy of a protein as a function of the nuclear coordinates. A typical molecular modelling force field contains five types of potentials. These potentials correspond to deformation of covalent bond length and bond angles, torsional motion associated to rotation about bonds, electrostatic interaction, and van der Waals interaction.

$$(5) \quad V(x) = V_{\text{length}} + V_{\text{angle}} + V_{\text{torsion}} + V_{\text{electrostatic}} + V_{\text{weak}} .$$

The potential energy  $V = V(x)$  is a function of the atomic coordinate  $x$  of the molecule. The distance is measured in Ångstrom ( $\text{\AA}$ ), energy in *kcal/mol*, and mass in atomic mass unit (Dalton). The bond length potential is given by

$$(5a) \quad V_{\text{length}} = \sum_{i,j \text{ (bonds)}} k_0(r_{ij} - r_0)^2 .$$

Where  $r_{ij} = \|x_i - x_j\|$  is the bond length,  $r_0$  is the reference bond length, and  $k_0$  is a force constant. Reference bond lengths and force constants depend on the bond type. The bond potential corresponds to covalent bond deformation. The bond length deformations are sufficiently small at ordinary temperatures and in the absence of chemical reactions. The bond deformation energy between the  $i$ th and  $j$ th atom is given by a harmonic potential

$$k_0(r_{ij} - r_0)^2 .$$

The bond angle potential is given by

$$(5b) \quad V_{\text{angle}} = \sum_{\theta \text{ (angle)}} k_0(\theta - \theta_0)^2 ,$$

where  $\theta_0$  is the reference bond angle and  $k_0$  is a force constant. Reference bond angle and force constant depend on the type of the atom involved. The angle  $\theta$  between the bonds  $\mathbf{p} = x_j - x_i$  and  $\mathbf{r} = x_k - x_j$  is given by

$$\cos(\theta) = \frac{\mathbf{p} \cdot \mathbf{r}}{\|\mathbf{p}\| \|\mathbf{r}\|} , \quad \theta \in [0, \pi] .$$

The bond angle potential corresponds to angle deformation. Bond angle deformations are sufficiently small at ordinary temperatures and in the absence of chemical reactions.

The potentials for bond length and bond angle deformation are considered as the hard degrees of freedom in a molecular system in the sense that considerable energy is necessary to cause significant deformation from their reference values. The most variation in structure and relative energy comes from the remaining potential energy terms.

The torsion potential corresponds to the barriers of bond rotation which involves the dihedral angles of the rotatable bonds. The barriers of torsion can be expressed as a series of cosine functions. The mathematical expression of the torsion potential is given by

$$(5c) \quad V_{\text{torsion}} = \sum_{\theta::(\text{dihedral})} |k_0| - k_0 \cos^2(n_0\theta) ,$$

where  $n_0$  is the multiplicity of the angle and  $k_0$  is a force constant. Both multiplicity and force constants depend on the type of the atoms involved. The dihedral angle  $\theta$  can be obtained from

$$\cos(\theta) = \frac{|(\mathbf{p} \times \mathbf{r}) \cdot (\mathbf{r} \times \mathbf{q})|}{\|\mathbf{p} \times \mathbf{r}\| \|\mathbf{r} \times \mathbf{q}\|} , \quad \theta \in [-\pi, \pi] ,$$

where

$$\mathbf{p} = x_j - x_i \quad , \quad \mathbf{r} = x_k - x_j \quad , \quad \mathbf{q} = x_\ell - x_k ,$$

and the sign of the angle  $\theta$  is given by the sign of the inner product  $(\mathbf{p} \times \mathbf{q}) \cdot \mathbf{r}$ . The complementary angle  $\pi - \theta$  is the torsion angle of the bond  $x_j - x_k$ .

The electrostatic potential corresponds to the non-bounded interaction between the charged atoms in a molecule. The interaction is attractive when the charges have opposite sign and repulsive when the charges have the same sign. The electrostatic potential of a molecule is given by

$$(5d) \quad V_{\text{electrostatic}} = \sum_{i < j \text{ (atoms)}} \frac{q_i q_j}{4\pi\delta_0 r_{ij}},$$

where  $q_i$  is the point charge of the  $i$ -th atom and  $\delta_0$  is the dielectric constant of vacuum, and  $r_{ij}$  is the distance between  $i$ -th and  $j$ -th atoms.

The van der Waals potential corresponds to the interaction between nonbounded atoms in a molecule. This interaction comes from attractive and repulsive forces. The van der Waals potential is given by

$$(5e) \quad V_{\text{electrostatic}} = \sum_{i < j \text{ (atoms)}} \left[ \left( \frac{A_{ij}}{r_{ij}^{12}} \right) - \left( \frac{B_{ij}}{r_{ij}^6} \right) \right],$$

where  $A_{ij}$  and  $B_{ij}$  are given by

$$A_{ij} = \frac{1}{2} B_{ij} (R_i + R_j)^6$$

$$B_{ij} = \frac{3}{2} \frac{1}{\sqrt{4\pi\delta_0}} \frac{1}{m_e} \frac{e\hbar\alpha_i\alpha_j}{\sqrt{\alpha_i/N_i} + \sqrt{\alpha_j/N_j}},$$

where  $e$  is the electron charge,  $\hbar$  is the reduced Planck constant,  $m_e$  is the electron mass,  $\alpha_i$  is the polarizability of the  $i$ -th atom,  $N_i$  is the effective number of outer shell electrons in the  $i$ -th atom.  $R_i$  is the van der Waals radius of the  $i$ -th atom.

## 5. CONFORMATIONAL SEARCH METHODS

The conformational search of the global minimum energy surface of a protein from the amino acid sequence is one of the challenging problems in bioinformatics. In recent years, several optimization approaches to solve this problem have appeared in the literature. The most common approach is to model the protein surface by using a force field. Among the most commonly used force fields are CHARMM developed by Brooks et al. [Brooks et al, 1983]. Conformational search based on force fields can be approached by global optimization techniques by Horst and Pardalos [Horst and Pardalos, 1994]. These methods are currently better suited for lower dimensional problems. For higher dimensional problems, one of the most successful optimization techniques for conformational search is Conformational Space Annealing (CSA) introduced by Lee, et al [Lee, et al, 1997]. CSA has been designed to search a large portion of the potential energy surface. It is an iterative algorithm maintaining in each iteration a population of local minimum energy conformations. It has been successfully applied to proteins with 100 to 150 residues [Scheraga, 1996]. It is currently one of the leading conformational search algorithms.

The smoothing method also known as diffusion equation method invented by Kostrowicki, et al [Kostrowicki, et al, 1991 and 1992] is another useful techniques for conformational search. This method can be used to approximate the potential energy surface such that the number of local minima largely decreases while the deepest local minimum is retained. When a force field is smoothed in a way that the potentials for bond lengths and bond angles are smoothed as well, the whole molecular structure will become a single point. A comprehensive coverage

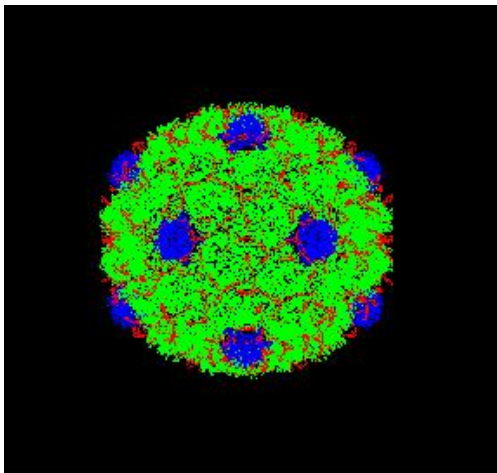


FIGURE 6. Protein conformation generation  
([www.bbmb.iastate.edu/jerniganresearch.shtml](http://www.bbmb.iastate.edu/jerniganresearch.shtml))

of smoothing various potentials can be found in [Zimmermann, 2003]. The general scheme is to define a smooth operator that is linear and each term of the potentials can be separately smoothed. For example, the exponential operator is given by

$$\Psi_t = \exp \left\{ t \frac{d^2}{dx^2} \right\} .$$

This smooth operator is linear and transforms polynomial functions into polynomial functions of the same degree. For instance

$$\Psi_t(x^4) = x^4 + 12x^2t + 12t^2 .$$

This operator is well connected with the definition of two-variable Hermite polynomial.

$$H_4^{(2)}(x, y) = H_4(x, y) = \exp \left\{ t \frac{d^2}{dx^2} \right\} (x^4) .$$

For the general definition of this operator, acting on analytic functions, see e.g. [Ricci and Tavkhelidze, 2009].

Here we describe the process of smoothing the torsion potential of a protein. Let us recall, the torsion potential of a protein is expressed as a linear combination of cosine terms of dihedral angles (5c). In order to smooth this potential, we express the dihedral angles by distances. We assume that bond lengths and bond angles are fixed to their reference values. Then cosine of a dihedral angle  $\theta$  can be expressed by the distance  $r = \|x_\ell - x_i\|$  of the first and last of the involved atoms:

$$\cos(\theta) = \alpha + \beta r^2 ,$$

where  $\alpha$  and  $\beta$  are constants depending on the reference bond lengths and reference bond angles. In general  $\cos(n\theta)$  of a multiple dihedral angle can be represented as a Chebyshev polynomial in  $\cos(\theta)$ , which is a polynomial in  $r^2$ .

Let  $x = \cos(\theta)$ , then the Chebyshev polynomials can be written as

$$T_n(x) = \cos(n\theta) = \cos(n \arccos(x)) .$$

Furthermore, we have

$$T_n(x) = \cos(n\theta) = T_n(\alpha + \beta r^2) .$$

Consequently, the torsion potential can be expressed as a linear combination of Chebyshev polynomials

$$(6) \quad V_{\text{torsion}} = \sum_{\theta::(\text{dihedral})} |k_0| - k_0 T_n^2(\alpha + \beta r^2) .$$

Each term is a polynomial in  $r^2$  and so the torsion potential  $V_{\text{torsion}}(x)$  can be smoothed by the linear operator  $\Psi_t$ ,

$$\tilde{V}_{\text{torsion}}(x, t) = \Psi_t V_{\text{torsion}}(x) .$$

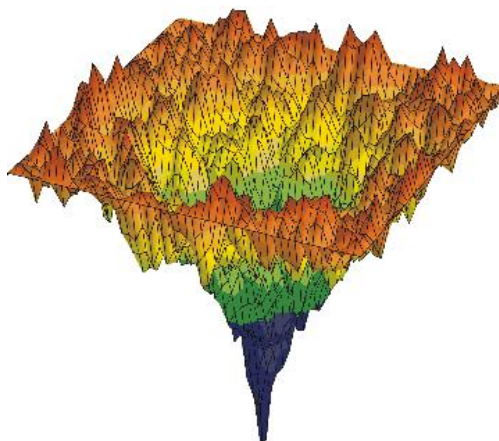


FIGURE 7. Potential energy surface of protein  
([www.lsbu.ac.uk/water/protein2.html](http://www.lsbu.ac.uk/water/protein2.html))

## 6. PROTEIN FOLD RECOGNITION

The objective of conformational search is to find all preferred conformations of a molecule. An alternative approach to conformational search is fold recognition. Proteins may have similar tertiary structures even if their primary structures are not sufficiently similar or different. This observation has led to the hypothesis that there are only a limited number of significantly distinct tertiary structures. The main goal of fold recognition is to predict the tertiary structure of a protein from its amino acid sequence by finding the best match between the amino acid sequence and some tertiary structure in a protein database.

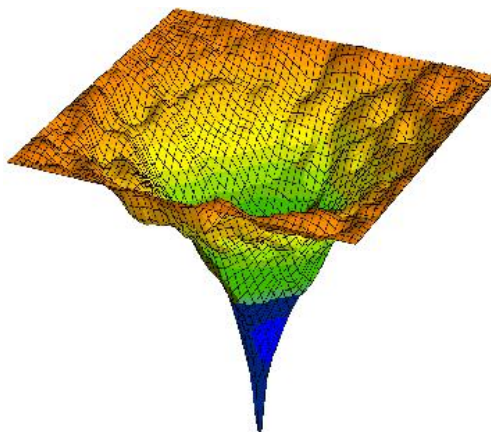


FIGURE 8. Smoothed potential energy surface of protein ([www.lsbu.ac.uk/water/protein2.html](http://www.lsbu.ac.uk/water/protein2.html))

A basic approach to fold recognition is comparative modelling. Let  $\mathbf{A}$  be the amino acid sequence of a protein with unknown tertiary structure, align the sequence  $\mathbf{A}$  to the primary structures of all proteins in the database of tertiary protein structures. Suppose the sequence  $\mathbf{A}$  best aligns to the primary structure of  $\mathbf{B}$ . This sequence alignment can be used to infer the structural alignment. For example, if the residue  $a_i$  of  $\mathbf{A}$  aligns with the residue  $b_j$  of  $\mathbf{B}$ , then the position of the residue  $a_i$  in the unknown tertiary structure is defined as the position of the residue  $b_j$  in the tertiary structure in the database. Subsequences of the sequence of  $\mathbf{A}$  aligned with a series of blanks of the sequence of  $\mathbf{B}$  are modelled as coil region.

A more sophisticated approach to fold recognition makes use of method of 3D profile-sequence alignment. For this, we make use of both sequence database and protein database. Let  $\mathbf{A}$  be a sequence of amino acid and  $\mathbf{P}$  be the 3D profile of a protein. We align  $\mathbf{A}$  to  $\mathbf{P}$ . Let  $\sigma(\mathbf{P}, \mathbf{A})$  be the corresponding alignment score. To estimate the significance of these alignment scores, we align the protein with 3D profile  $\mathbf{P}$  against all amino acid sequences of a sequence database. The  $Z$  score for aligning the amino acid sequence  $\mathbf{A}$  to the protein with 3D profile  $\mathbf{P}$  is given by

$$(7) \quad Z(\mathbf{P}, \mathbf{A}) = \frac{\sigma(\mathbf{P}, \mathbf{A}) - \mu(\mathbf{P}, \mathbf{A})}{\sigma(\mathbf{P})},$$

where  $\mu(\mathbf{P})$  is the mean score of alignment scores given by

$$\mu(\mathbf{P}) = \frac{1}{M} \sum_{\mathbf{A}} \sigma(\mathbf{P}, \mathbf{A}),$$

with  $M$  as the number of sequences in the sequence database, and  $\sigma(\mathbf{P})$  is the standard deviation of the scores given by

$$\sigma(\mathbf{P}) = \sqrt{\frac{1}{M} \sum_{\mathbf{A}} [\sigma(\mathbf{P}, \mathbf{A}) - \mu(\mathbf{P})]^2}.$$

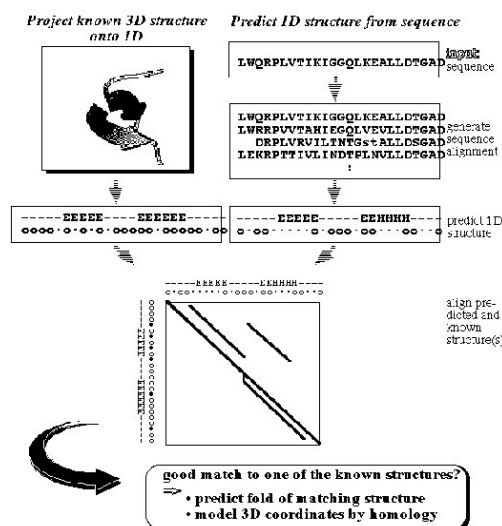


FIGURE 9. Threading predicted 1D structure profiles into known 3D structures ([www.rostlab.org/papers/1997\\_topits/paper.html](http://www.rostlab.org/papers/1997_topits/paper.html))

A high  $Z$  score  $Z(\mathbf{P}, \mathbf{A})$  may indicate that amino acid sequence  $\mathbf{A}$  has similar tertiary structure as the protein with the 3D profile  $\mathbf{P}$ .

The most accurate approach to fold recognition is based on a knowledge-based potential. Knowledge-based potentials [Sippl, 1995] have been successfully applied to detecting errors in experimentally determined structures [Sippl, 1993]. In addition, stochastic sampling methods can be used to explore the potential energy surface. There is a vast literature on statistical mechanics [Amit and Verbin, 1999; Gallavotti, 1999; Phillips, 1994]. Comprehensive accounts on stochastic sampling methods for conformational search are given in [Allen and Tildesley, 1987; Leach, 1996].

#### REFERENCES

- [1] M.P. Allen & D.J. Tildesley, *Computer simulation of liquids*, Clarendon Press, Oxford, 1987.
- [2] D.J. Amit, Y. Verbin, *Statistical physics: an introductory course*, World Scientific, Singapore, 1999.
- [3] B. Brooks, R. Bruccoleri, B. Olafson, D. States, S. Swaminathan & M. Karplus, *CHARMM: a program for macromolecular energy minimization and dynamics calculations*, J. Comp. Chem., 4(1983), 187–217.
- [4] K.A. Dill, S. Bromberg, K.Z. Yue, K.M. Fiebig, D.P. Yee, P.D. Thomas, & H.S. Chan, *Principles of protein-folding – a perspective from simple exact models* Prot. Sci., 4(1995), 561–602.
- [5] G. Gallavotti, *Statistical mechanics*, Springer, New York, 1999.
- [6] C. Guerra, S. Istrail, *Mathematical methods for protein structure analysis and design*, Lecture Notes in Bioinformatics, Springer, 2000.
- [7] D. Halperin, M.H. Overmars, *Spheres, molecules and hidden surface removal*, Comput. Geom., 11(2)(1998), 83–102.

- [8] R. Horst, P.M. Pardalos, *Handbook of global optimization*, Kluwer, Dordrecht, 1994.
- [9] J. Kostrowicki, L. Piela, J. Cherayil & H.A. Scheraga, *Performance of the diffusion equation method in searches for optimum structures of clusters of Lennard-Jones atoms*, J. Phys. Chem., 95(1991), 4113–4119.
- [10] J. Kostrowicki, H.A. Scheraga, *Application of the diffusion equation method for global optimization of oligopeptides*, J. Phys. Chem., 96(1992), 7442–7449.
- [11] A.R. Leach, *Molecular modeling: principles and applications*, Addison Wesley, 1996.
- [12] J. Lee, H.A. Scheraga, S. Rackovsky, *New optimization method for conformational energy calculations on polypeptides: conformational space annealing*, J. Comp. Chem., 18(1997), 1222–1232.
- [13] A.M. Lesk, *Introduction to protein architecture: the structural biology of proteins*, Oxford Univ. Press, 2001.
- [14] C. Levinthal, *Are there pathways for protein folding?* J. Chem. Phys., 65(1968), 44–45.
- [15] G.D.J. Phillies, *Elementary lectures in statistical mechanics*, Springer, New York, 1994.
- [16] P.E. Ricci, I. Tavkhelidze, *An introduction to operational techniques and special polynomials*, J. Math. Sci., 157(1)(2009), 161–189.
- [17] H.A. Scheraga, *Recent developments in the theory of protein folding: searching for the global energy minimum*, Biophys. Chem., 59(1996), 329–339.
- [18] M.J. Sipple, *Knowledge-based potentials for proteins*, Curr. Biol., 5(1995), 229–235.
- [19] M.J. Sipple, *Recognition of errors in three-dimensional structures of proteins*, Proteins: Struct. Funct. Genet., 12(1993), 355–362.
- [20] D. Voet, J.G. Voet, *Biochemistry*, Vol. 1, 3rd ed. Wiley, 2004. See esp. pp 227–231.
- [21] K.H. Zimmermann, *An introduction to protein informatics*, Kluwer Academic Publishers, 2003.