PREFACE

Proteins are an important class of biological macromolecules present in every biological organism. Proteins are synthesized on ribosomes as a linear chain of amino acid residues in a specific order from information encoded within the DNA. In order to function, each newly synthesized chain must fold into the unique 3-D structure that is characteristic of the individual protein. Protein folding is a highly specific process and each protein has a specific fold to perform its specific function. Uncovering the mechanisms through which such processes take place is one of the greatest challenges in the post genomic era. This book presents current research from around the globe in the study of protein folding, including heterologous protein folding in yeast; how homodimeric proteins fold and assemble; ribosome assisted protein folding; protein disulphide isomerases; and self-assembling peptids for biomedical applications.

Chapter 1 – The 'foldase' enzyme protein disulphide isomerase (PDI) interacts with nascent polypeptides in the lumen of the endoplasmic reticulum to catalyze the formation of new disulphide bonds, as well as breakage and alternative isomerization of incorrectly formed bonds, during protein folding and maturation processes in eukaryotic cells. PDI belongs to the thioredoxin (TRX) superfamily that catalyzes cellular redox reactions and has an active site with the conserved motif WCXXC. PDI is a member of the larger PDI-like (PDIL) protein family, its subclasses varying in numbers, positions and sequences of functional domains and active sites and in subcellular locations and functions, many including PDI also playing chaperone roles. The human PDIL family has been studied in great detail, its members exhibiting activities ranging from the archetypical disulphide formation/isomerisation to a multitude of chaperone roles of relevance to cell biology and medicine, for example, in neurodegenerative diseases, apoptosis and cancer. The plant PDIL families are larger and more diverse and have been associated with some unique roles such as storage protein folding; however, information on plant PDILs is much more limited. This work overviews the key properties and functions of human PDILs, summarises the diversity and known functions of plant PDILs, and contemplates the directions for future research on PDILs, particularly in plants.

Chapter 2 – Self-assembling peptides are a category of peptides which undergo spontaneous assembling into ordered nanostructures. These designed peptides have attracted huge interest in the field of nanotechnology for its potential application in areas such as biomedical nanotechnology, cell culturing, molecular electronics, and more.

In the emerging field of tissue engineering, the development of synthetic materials promoting cell growth has led to the study of regularly alternating polar/non-polar amphiphilic oligopeptides, such as EAK16 $(AEAEAKAK)_2$, also called LEGO peptides, which have been considered particularly promising.

Self-assembling LEGO peptides have a preferential β--sheet structure, are resistant to proteolytic cleavage, and are able to form an insoluble macroscopic membrane under physiological conditions. Their ability to create such stable structures derives from the hydrophobic interactions between the aliphatic groups of non-ionic residues and the complementary ionic bonds between acidic and basic amino acids. This stability can be enhanced by the pH regulation and the presence of monovalent ions.

This chapter will be focused on some approaches useful for elucidating the influence of the sequence modifications and the interactions with a surface on the self-assembly capability of differently synthesised peptides. Eight different oligopeptides (from 16 to 19 residues), derived from EAK16, have been analysed by IR and Raman spectroscopies that are particularly useful for obtaining qualitative and quantitative information on the secondary structure of these peptides. Several modifications in the primary structure of peptides have been considered, namely acidic and/or basic substitutions (Glu → Asp; Lys → Orn), changes in the length of the aliphatic side chain of the spacer residues (Ala → Abu or Ala → Tyr), the addition of RGD (known to promote osteoblast adhesion), or scrambling of the sequence.

As these peptides are widely used as biocompatible coatings of metallic implants, the peptide folding after adsorption on different surfaces, in particular on titanium oxide, will be also discussed.

Finally, it will be shown that not all the oligopeptides examined can self-assemble into a homogeneous multilayer on metallic surfaces; however, most of the peptides take a prevailing β--sheet structure which guarantees the best peptide-surface interaction. In particular, the interactions of polar, ionic and aromatic residues with surfaces will be discussed more in detail.

Chapter 3 – Despite much progress in molecular biology, biophysics, and biochemistry, the mechanisms by which unfolded polypeptide chains gain their native conformation are not yet fully understood. Likewise, the network of forces that stabilize folded proteins and prevent them from unfolding elude a thorough accounting so far. Such an accounting is, however, necessary to engineer protein stability, which is often required for enzymes in technical applications.

The folding process of proteins starts with a hydrophobic collapse or local events at so-called 'nuclei' or 'initiation sites' and proceeds stepwise to the natively folded protein. The occurrence of rate-limiting steps often leads to the population of folding intermediates. In contrast to the successive protein folding process, protein *un*folding is highly cooperative. Due to the principle of microscopic reversibility, unfolding must be a progressive process as well, which is postulated to start at a confined region—'unfolding region', 'critical region' or similar—of the tertiary structure of the native protein molecule.

The rate constants of the folding (k_f) and unfolding reaction (k_U), both of which determine the respective free energy of activation $\Delta G_f^{\ddagger}$ and $\Delta G_U^{\ddagger}$, provide information on the contribution of these reactions to the thermodynamic stability (Gibbs free energy $\Delta G = \Delta G_U^{\ddagger} - \Delta G_f^{\ddagger}$). The comparison of proteins with their chemically modified or, preferably,

genetically engineered variants yields information on the involvement of specific residues in the folding or unfolding reaction.

Ribonuclease A (RNase A) has been a model protein for half a century and is one of the most thoroughly studied examples concerning the protein folding problem. Whereas the folding reaction is complex due to proline isomerization reactions, the unfolding reaction can be described by a single-exponential reaction. Modifications by, *e.g.*, genetic engineering have revealed the importance of confined regions for the unfolding and refolding reaction, respectively.

Starting from basic principles of thermodynamics and kinetics, the effect of protein modifications on the stability and tools for protein stabilization are presented with special focus on work on RNase A.

Chapter 4 – Yeast is an important platform for the production of therapeutic proteins. It has principally been used for the production of relatively simple proteins that lack *N*-linked glycosylation. However, with recent developments in technologies focused on glycoengineering yeast to produce humanized *N*-glycans, its application to the field of human therapeutics becomes greatly potentiated. A constant challenge in the biotechnology industry is the production of recombinant proteins at high levels. Like other protein expression platforms, productivities in yeast depend on the host strain, its process of cultivation and the type of protein being expressed. In addition, it has been demonstrated that yeast lack some molecular chaperones found in higher eukaryotic cells. Protein folding in the endoplasmic reticulum is a key process that can influence the fate of a protein, directing it either to secretion or ER-associated degradation. Some proteins require specific molecular chaperones for their protein maturation and others require general protein folding chaperones. In this review, yeast folding machinery as well as attempts made to improve protein folding will be discussed.

Chapter 5 – The problem of protein self-organization is one of the most important problems of molecular biology nowadays. Despite the recent success in the understanding of general principles of protein folding, details of this process are yet to be elucidated. Moreover, the prediction of protein folding rates has its own practical value due to the fact that aggregation directly depends on the rate of protein folding. The time of folding and transition state ensembles for 67 proteins with known experimental data at the point of thermodynamic equilibrium between unfolded and native state have been calculated using a Monte Carlo method and Dynamic programming one where each residue is considered to be either folded as in the native state or completely disordered. The times of folding for 67 proteins which reach the native state within a limit of 10 Monte Carlo steps are in a good correlation with experimentally measured folding time at mid-transition point (the correlation coefficient is -0.82). A lower correlation was obtained if to use Dynamic programming approach (the correlation coefficient is -0.72). The capillarity model allows us to predict the folding rate at the same level of correlation as by Monte-Carlo simulations. The calculated model entropy capacity (conformational entropy per residue divided by the average contact energy per residue) for 67 proteins correlates by about 78% with the experimentally measured folding rate at the mid-transition point. Theoretical consideration of a capillarity model for the process of protein folding demonstrates that the difference in the folding rate for proteins sharing more ball-like and less ball-like folds is the result of differences in the conformational entropy due to a larger surface of the boundary between folded and unfolded phases in the transition state for proteins with more ball-like fold.

Chapter 6 – The interest in intrinsically unordered proteins (IUPs) has greatly increased, as it has become clear that they are very widespread, especially in eukaryotic organisms. The presence of unordered regions in functional proteins is implicated in important biological roles, such as translation and transcriptional regulation, cell signaling and molecular recognition. A number of studies report that for mammals about 75% of their signalling proteins are predicted to contain long unordered regions (>30 residues), about half of their total proteins are predicted to contain such long unordered regions, and about 25% of their proteins are predicted to be fully unordered. Other studies report examples of unordered proteins implicated in important cellular processes, undergoing transitions to more structured states upon binding to their target ligands, DNA, or other proteins. The accumulation of such evidence led to the suggestion that the classical protein structure-function paradigm needs to be reassessed. In fact, the assumption that a folded three-dimensional structure is necessary for function has been modified. Although the functions of many proteins are directly related to their three-dimensional structures, numerous proteins that lack intrinsic globular structure under physiological conditions are now recognized. As the amino acid sequence contains the information for protein folding, it was reasoned that, also for proteins that do not fold into three-dimensional structures, the residue sequence should specify protein "nonfolding". Therefore, to test this hypothesis, a large number of methods were developed to predict regions of protein sequences that fail to fold. Their prediction accuracy has been better than expected and this suggests that the information for failure to fold into a regular structure is likely to be inherent within the residue sequence. Because there is an enormous interest in predicting unordered regions of proteins, the analysis of the possibility of predicting protein "unordered" regions was addressed also in the last four editions of CASP experiment.

The main aim of this chapter is to present the state of the art of prediction methods of protein unordered regions developed in the last years and their application to specific protein families. In particular, the authors will focus their discussion on human Sirt-1 and cytokine membrane receptors because the author data suggest that these proteins, involved in chronic inflammatory diseases, have unordered structural segments very important for their function.

Chapter 7 – Protein folding is an increasingly important field in biomedical research. Tools of physical, chemistry, protein engineering, computer simulation and structural biology have been combined to investigate the folding mechanism of many monomeric proteins.

However, a large percentage of proteins, from all organisms, are oligomeric and most commonly homodimers. In recent years, numerous folding studies have been carried out to understand the folding and assembly pathways of multisubunit proteins and homodimers in particular. This review chapter describes the current knowledge on the thermodynamic and kinetic stability, the folding process and subunit association of homodimeric proteins. The experimental design and interpretation of data from equilibrium and kinetic techniques are revised, which provide practical information for determining the folding pathways. In addition, several folding models are discussed ranging from the simplest two-state model to those that involve one or more intermediate conformations. This information is of interest for understanding the folding reaction as well as protein–protein interactions of natural multimeric proteins as well as abnormal complexes implicated in disease.

Chapter 8 – Methionine adenosyltransferases (MATs) are a family of highly conserved oligomers that catalyze the only known reaction for the synthesis of S-adenosylmethionine (AdoMet), the main cellular methyl donor. Their catalytic subunits exhibit a characteristic structure, organized in three domains formed by nonconsecutive stretches of the sequence.

The active sites locate at the interface between subunits in the dimer with amino acids of each monomer contributing to catalysis. Changes in activity, oligomerization level and expression have been detected in several hepatic diseases; the knockout mouse for *MAT1A* spontaneously developing hepatocellular carcinoma (HCC). However, none of the patients with persistent hypermethioninemia caused by mutations in this gene exhibits hepatic problems, instead a few cases showing demyelination have been described. This chapter discusses aspects related to the structural features of these enzymes and the impact that the mutations found in the human *MAT1A* gene may have in the final protein structure. The influence of the redox environment in MAT folding and association is also analyzed, in light of the effects that drugs and metals that alter the GSH/GSSG ratio produce in the activity and association level. The recent report of the nuclear localization of the MAT I/III isoenzymes, along with their presence in tissues other than liver opened the option to MAT moonlighting. The possibility exists that disease development is related not only to a decrease in AdoMet production, but also to the role of these particular isoenzymes in different subcellular compartments. Therefore, the influence of *MAT1A* mutations, especially those leading to protein truncations, on folding and subcellular localization is discussed, paying special attention to the Hazelwood's hetero-oligomerization hypothesis to explain the demyelination process in patients with persistent hypermethioninemia.

Chapter 9 – The most abundant molecules in nature other than water are proteins, which are involved to stimulate or control virtually every chemical process. Majority of proteins must be converted into tightly folded compact structures in order to function and their ability to fold to a unique structure generate enormous selectivity and diversity in their functions. Conformational changes in proteins result in misfolding, aggregation, and intra- or extraneuronal accumulation of amyloid fibrils in many neurodegenerative disorders. Proteins appear to fold by diverse pathways, the rates of folding are largely determined by the topology of the native structure. In general, secondary structure is inherently unstable and its stability is enhanced by tertiary interactions. The most conspicuous feature of many neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease, is the occurrence of protein aggregates in ordered fibrillar structures known as amyloid found inside and outside of brain cells. The appearance of aggregates in diseased brains implies an underlying incapacity in the cellular machinery of molecular chaperones that normally functions to prevent the accumulation of misfolded proteins. Molecular chaperones provide a first line of defense against misfolded, aggregation-prone proteins, and are among the most potent suppressors of neurodegeneration known for animal models of human disease. A detailed understanding of the molecular basis of protection by chaperones against neurodegeneration might lead to the development of therapies for neurodegenerative disorders that are associated with protein misfolding and aggregation.

In this review, the advances on the protein folding in neurodegenrative disorder and unfolding the role of molecular chaperones in neuroprotection are discussed.

Chapter 10 – In this chapter the authors intend to describe in detail the most usual DSC equilibrium and kinetic models that explain the folding behavior of small proteins, domains and peptidic fragments, paying special attention to the concrete experimental and computational aspects that should be taken into account to obtain as much thermodynamic information as possible. The authors will also explain the best strategies to properly discern between the different folding models and to improve the accuracy of the respective thermodynamic parameters.

Chapter 11 – The folding/unfolding kinetics of a three-dimensional lattice protein was studied using a simple statistical mechanical model for protein folding that the authors had developed earlier. The model considers the specificity of an amino acid sequence and the native structure of a given protein. The authors calculated the characteristic relaxation rate on the free energy surface starting from a completely unfolded structure (or native structure) that is assumed to associate with a folding rate (or an unfolding rate). The chevron plot of these rates as a function of the inverse temperature was obtained for four lattice proteins, *a1*, *a2*, *b1*, and *b2*, in order to investigate the dependency of the folding and unfolding rates on their native structures and amino acid sequences. Proteins *a1* and *a2* fold to the same native structure, but their amino acid sequences differ. The same is true for proteins *b1* and *b2*, but their native structure is different from that of *a1* and *a2*. To elucidate the roles of individual amino acid residues in protein folding/unfolding kinetics, the authors calculated the kinetic properties for all possible single amino acid substitutions of these proteins and examined their responses. The results are discussed with respect to the roles of short- and long-range interactions and formation of a folding nucleus in the kinetics of protein folding/unfolding.

Chapter 12 – One of the central characteristics of a living system is the ability to self-assemble its component molecular structures with precision and fidelity. The folding of proteins into their compact three-dimensional structures is the most fundamental and universal example of biological self-assembly. Understanding this complex process will therefore make available a unique insight into the way in which evolutionary selection has influenced the properties of a molecular system for functional advantage. The final goal of folding studies is to predict structure from sequence, allowing the design of new functional proteins and prevention of abnormal disease-associated protein conformations.

Chapter 13 – Peptidyl-prolyl cis/trans isomerases (PPIases; EC 5.2.1.8) are folding helper enzymes which accelerate the formation of the cis/trans equilibrium of peptidyl-prolyl bonds, a rate-limiting process during protein folding events. PPIases are ubiquitous proteins present in almost all living organisms. They can be classified into three structurally and biochemically divergent subfamilies: cyclophilins, FKBP (FK506 binding proteins), and parvulins. No sequence homology exists between the three families, and they have characteristic substrate specificities. In this chapter, the authors review the current knowledge about bacterial cyclophilins: biochemical characteristics, diversity, and evolutive relationships.

Chapter 14 – The effort to develop better description of protein three-dimensional folding structures has dominated biochemistry and drug discovery research for more than 70 years since Pauling first defined the helical configurations as secondary structure for protein in 1940. The challenge is how to acquire a complete description of protein folding shapes from N-terminal to C-terminal, including regular secondary structure as well as irregular tertiary structure. Here, a novel description method is introduced, which a set of 27 vectors is rigorously derived mathematically from an enclosed space. Each vector represents a three-dimensional folding shape of five successive $C_{\square}$ atoms, and the protein conformation can be completely described along protein backbone. These vectors are expressed by 27 alphabetic symbols, which are called as protein folding shape code (PFSC). Consequently, with PFSC, the folding conformation of any protein with given three-dimensional structure is able to be converted into a simple one-dimensional alphabetic string without gap. Furthermore, to take the advantage of one-dimensional description of folding shapes, the protein conformational structures are able to be compared with Needleman-Wunsch alignment algorithm. The global

similarity of protein 3D structures is able to be assessed by a value of protein folding structure alignment score (PFSA-S) as a quantitative measurement, and the similarity and dissimilarity of local structures is able to be examined by alignment table. The results show that this approach has the capability not only to distinguish protein conformers with relatively high similarity, but also to compare proteins with diverse degrees in structural homology. Therefore, this approach provides a consistent procedure, and it produces a unique score for assessment of similarity in protein structure comparison. The significant is that the complete description of protein folding shapes provides a simple and effective means to screen protein database, compare protein structures, search protein fragment and probe drug binding site, study protein mutation and protein misfolding and so on.

Chapter 15 – Many organisms grow in extreme environments on Earth. Microorganisms whose optimal growth temperature is above 80°C are called hyperthermophiles. Proteins from hyperthermophiles usually exhibit higher stability than those from organisms that grow at lower temperatures. In this paper, the authors describe their studies of the stability and folding of ribonuclease HII from the hyperthermophile *Thermococcus kodakaraensis* and introduce other studies of hyperthermophilic proteins. These studies focus on hyperthermophilic proteins with reversible unfolding and examine their equilibrium and kinetic aspects. The results indicate that kinetic stability (slow unfolding) is a general strategy by which hyperthermophilic proteins adapt to higher temperatures. Mutational analysis indicates that hydrophobic interaction is a factor in the molecular mechanism of the slow unfolding of hyperthermophilic proteins. Next, the authors report on studies of subtilisin-like serine protease from *Thermococcus kodakaraensis*. The results demonstrate that this protein is extremely stable and that the maturation (folding) process of this protein differs from those of mesophilic homologues in terms of the role of Ca^{2+} and propeptide. This protein is thought to be stabilized due to a high kinetic barrier between the unfolded and folded states. To reduce this barrier, the folding may be fine-tuned by utilizing Ca^{2+} and propeptide. The authors discuss protein folding from the viewpoint of adaptation to hot environments.

Chapter 16 – One of the ultimate goals of bioinformatics is to discover the principles governing protein folding. However, information on folding mechanisms of proteins cannot be easily decoded from amino acid sequences using standard bioinformatics techniques such as multiple sequence alignment and so on. We still do not sufficiently understand how the determinants of protein folding are encoded in amino acid sequences. In this review, the authors summarize how information about protein folding mechanisms can be extracted from amino acid sequences using inter-residue average distance statistics. The authors' kind of predicted contact map (Average Distance Map, ADM) pinpoints regions of possible folding nuclei for proteins. The amino acid sequence of a given protein suffices for construction of the ADM of a protein. An inter-residue effective potential is defined from the average distance statistics and is utilized to predict folding nucleation sites of a protein sequence. The authors review examples of the applications of these methods to the analyses of the protein folding of members of several protein families. The general possibility of predicting protein folding mechanisms from only sequences is also discussed.

Chapter 17 – Protein disulfide isomerase (PDI) is a protein-folding assistant and a molecular chaperone found in the endoplasmic reticulum. This enzyme catalyzes the formation and the isomerization of disulfide bonds. The binding and release of substrate from molecular chaperones are usually triggered by cycles of ATP hydrolysis. It has been proposed that the substrate binding / release cycle of PDI could be correlated with the redox states of its

active sites. The idea that PDI might act as redox-dependent molecular chaperone opened a new path in the field of chaperoning.

However, this redox dependence has not yet been fully established and remains subjected to controversy. The authors wish to address this issue here on the basis of the current knowledge on PDI including their recent findings on conformational change of thermophilic fungal PDI. Toward this end, based on inspection of the characteristics of the modular structure of PDI, the author present in this chapter the differences between the domains that compose PDI in regards to their functions and the consequences of the conformational alteration they experience upon redox condition changes. Finally, the mechanistic model the authors propose for the action of PDI supports the hypothesis of redox-dependent chaperoning.

Chapter 18 – The authors' goal in this work was the evaluation of the structural stability of horse heart myoglobin in organic solvents and cosolvents. The selection of an organic solvent or solvent mixture able to maintain the structural stability of myoglobin in organic phase can make viable the use of such media to replace the aqueous one. Changes in the structure of myoglobin were monitored in spectroscopic tests, UV-visible and circular dichroism, CD. The former was used to infer the changes in heme group as a result of the addition of organic solvent whereas the latter was performed to assess the secondary structure of the protein. The increase in the solubility of myoglobin in nonpolar solvents was investigated by means of hydrophobic ion pairing. Sodium dodecyl sulfate, SDS, was used to replace small counter ions on the protein surface and change the miscibility in nonpolar solvents. The results of myoglobin in alcohols indicated that the increase in hydrocarbon content and the decrease in alcohol branching increased their denaturing ability. The most interesting result was the dissolution of myoglobin in pure methanol, which corroborates the molecular dynamic simulation of proteins in this organic solvent. In general, the UV-visible spectra revealed that polar organic solvents have better performance in the dissolution of myoglobin than the polar ones. Protic solvents, like alcohols and glycols, exhibited low denaturing ability compared to the aprotic ones. This behavior can be reasoned in terms of the interaction between the solvent and the protein. Protic solvents probably establish hydrogen bonds with the protein polar groups, similar to water. However, solvents with high polarity can damage the native folding of proteins, because of the removal of water shell. CD spectroscopy was used as a complementary test for the systems with unaltered UV-visible absorbance profile to infer the secondary structure of the protein. The CD results showed the potential of glycols, namely glycerol and ethylene glycol, as cosolvents of myoglobin, up to 50% in volume. Regarding the change in myoglobin superficial moiety, the absorbance profile was maintained for the molar ratio SDS/myoglobin of 5, showing that the protein was effectively extracted to the organic phase (hexane) as the result of SDS pairing on its surface. Higher content of SDS led to turbidity. This result showed the potential of the technique to increase the solubility of myoglobin in nonpolar solvents.

Chapter 19 – In spite of well established fact that gene regulation by the transcription factors (TFs) involves the regulated assembly of multi-protein complexes on enhancers and promoters, scientific community is still actively searching for answers to questions pertaining to the mechanisms that govern this complex process. In this context the structural determinant of activation domains (ADs) of TFs, which provide a basic platform for the assembly of other coregulatory proteins (critical in gene regulation), is of immense importance. It has become quite clear that many TFs possess AD(s), which exist in an intrinsically disordered (ID)

conformation. In recent years, it has been shown that significant biological functions are associated with ID states of proteins. Among others, the flexibility of intrinsic disorder in ADs facilitates post-translational modifications, adoption of different conformations with various binding partner proteins, which in turn may lead to its diverse role in gene regulation by the TFs. Thus, proper understanding of ID AD regions in the action of signaling molecules may lead to a new approach for drug discovery, targeted at the assembly of multi-protein complexes involving TFs. This article reviews the available understanding of disorder-order transition in the gene regulation by the TFs, and provides future perspectives for the utilization of this knowledge for novel approaches to drug discovery.

Chapter 20 – Coarse-grained models are experiencing a renewed interest as they grant access to biologically relevant length and time-scales, they allow to single out molecular driving forces from a plethora of biochemical details. Topological models, discussed in the first part of the chapter, are based on the minimal frustration principle that justifies the stability and fast accessibility of the native state. Despite the several underlying approximations, this rich class of models also including Ising-like and Elastic Network Models, has been successfully applied in several contexts, from biomedical problems to the study of mechanical unfolding and translocation. Conversely, the necessity to elucidate the role of the amino-acid sequence on protein folding, in agreement with Anfinsen's postulates, inspires sequence-based models that are reviewed in the second part of the survey. As discussed, each model presents advantages and limitations and the choice of a model is dependent on the problem that must be addressed. PACS 87.15.H-, 87.15.hm, 87.10.-e

Chapter 21 – In this chapter, the authors introduce an approach to the protein folding problem from the point of view of statistical physics. Protein folding is a stochastic process by which a polypeptide folds into its characteristic and functional 3D structure from random coil. The process involves an intricate interplay between global geometry and local structure, and each protein seems to present special problems. The first part of this chapter contains a concise discussion on kinetics versus thermodynamics in protein folding, and introduces the statistical physics basis of protein folding. In the second part, the authors introduce CSAW (conditioned self-avoiding walk), a model of protein folding that combines the features of self-avoiding walk (SAW) and the Monte Carlo method. In this model, the unfolded protein chain is treated as a random coil described by SAW. Folding is induced by hydrophobic forces and other interactions, such as hydrogen bonding, which can be taken into account by imposing conditions on SAW. Conceptually, the mathematical basis is a generalized Langev in equation. Despite the simplicity, the model provides clues to study the universal aspects while the authors overlook details and concentrate only on a few general properties. To illustrate the flexibility and capabilities of the model, they consider several examples, including helix formation, elastic properties, and the transition in the folding of myoglobin. From the CSAW simulation and physical arguments, the authors find a universal elastic energy for proteins, which depends only on the radius of gyration Rg and the residue number N. The elastic energy gives rise to scaling laws $Rg \sim N^{\nu}$ in different regions with exponents ν= 3/5,3/7,2/5, PACS 87.10.Mn, 87.14.et, 87.15.Cc, 87.15.ad.