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Assortative mixing in Protein Contact Networks and protein folding kinetics

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ABSTRACT

Motivation: Starting from linear chains of amino acids, the spontaneous folding of proteins into their elaborate 3D structures is one of the remarkable examples of biological self-organization. We investigated native state structures of 30 single-domain, two-state proteins, from complex networks perspective, to understand the role of topological parameters in proteins' folding kinetics, at two length scales—as 'Protein Contact Networks (PCNs)' and their corresponding 'Long-range Interaction Networks (LINs)' constructed by ignoring the short-range interactions.

Results: Our results show that, both PCNs and LINs exhibit the exceptional topological property of 'assortative mixing' that is absent in all other biological and technological networks studied so far. We show that the degree distribution of these contact networks is partly responsible for the observed assortativity. The coefficient of assortativity also shows a positive correlation with the rate of protein folding at both short- and long-contact scale, whereas, the clustering coefficients of only the LINs exhibit a negative correlation. The results indicate that the general topological parameters of these naturally evolved protein networks can effectively represent the structural and functional properties required for fast information transfer among the residues facilitating biochemical/kinetic functions, such as, allostery, stability and the rate of folding.

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1 INTRODUCTION

Inside the cell, proteins are synthesized as linear chains of amino acids, which fold into unique 3D structures ('native states'). The wide range of biochemical functions performed by the proteins are specified by their detailed structures. Despite the large degrees of freedom, surprisingly, proteins fold into their native states in a very short time, which is known as Levinthal's Paradox (Levinthal, 1969). Although, given suitable conditions, some small proteins can reach their native state in a single concerted step, many others fold in stages with initial conformational events long before the final ('native') structure appears (Anfinsen, 1973). Structural changes and chemical interactions occur throughout the entire folding process, and strongly cooperative mechanisms are necessary to bring the protein in its native conformation within a very short time period (Maity *et al.*, 2005). The fast folding is a result of the catalytic effect of the formation of clusters of residues in contact with each other, which have high preferences for the early formation of secondary structures (helices, sheets and loops) in the presence of significant amounts of long-range tertiary structure interactions (Nölting and Andert, 2000).

The folding mechanism, kinetics, structure and function of proteins are intimately related to each other. Misfolding of proteins into non-native structures can lead to several disorders (Taubes, 1996). Correlating sequence with structure as well as understanding of folding kinetics has been an area of intense activity for experimentalists and theoreticians (Branden and Tooze, 1999; Fersht, 2002). Among the different theoretical approaches used for studying protein structure, function and folding kinetics, the graph theoretical approach, based on perspectives from complex networks, has been used recently to study protein structures (Amitai *et al.*, 2004; Aszódi and Taylor, 1993; Atilgan *et al.*, 2004; Bagler and Sinha, 2005; Brinda and Vishveshwara, 2005; Dokholyan *et al.*, 2002; Greene and Higman, 2003; Jung *et al.*, 2005; Rao and Caflisch, 2004; Vendruscolo *et al.*, 2002).

It is known that folding mechanisms are largely determined by a protein's topology rather than its inter-atomic interactions (Alm and Baker, 1999). With that understanding, we build graph-theoretical models of protein structures to investigate various topological properties at two different length scales, and study their possible role in the kinetics of the protein folding. We use a coarse-grained complex network model of a protein structure, namely the Protein Contact Network (PCN), by ignoring the fine-grained atomic level details, and model the 3D structure as a system constituted of amino acid units, put in place by non-covalent interactions. Long-range interactions are known to play a distinct role in determining the tertiary structure of the proteins (Epand and Scheraga, 1968), as opposed to the short-range interactions, which could largely contribute to the secondary structure formations. We consider the long-range interaction network (LIN) of each protein, which are subsets of the corresponding PCNs, constructed by ignoring

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the short-range interactions. The idea behind studying LINs is to understand the contribution of the long-range interactions to the topological properties, and their correlation to a biophysically relevant property, namely rate of protein folding.

This study aims to address the question—can general network parameters, derived from native-state structures of proteins, uncover features about the relationship of the structural properties to the folding kinetics of the proteins? To study this, we choose single domain, two-state folding proteins that belong to different structural classes (Murzin *et al*., 1995) for which the kinetic parameter of rate of folding, (k_F) is available. Our analysis of the coarse-grained network representations of protein structures uncover the exceptional topological property of a high degree of assortative mixing at both length scales (PCN and LIN) in these naturally occurring, evolutionarily selected, biological networks. Assortative mixing in LINs indicates that this feature in PCNs is independent of short-range interactions. The coefficient of assortativity (Newman, 2002), a measure of assortative mixing, are also found to be considerably high for both PCNs and LINs. By constructing appropriate control networks, we further demonstrate that the degree (connectivity) distribution of the PCNs alone can partially account for the presence of assortativity in these networks.

To enumerate the contribution of these global parameters obtained from the coarse-grained network model of protein structures to their biophysical properties, we show that the coefficient of assortativity of PCNs and LINs tend to have positive correlation with the experimentally determined rate of folding of these proteins. This implies that assortative mixing, that tends to connect highly connected residues to other residues with many contacts, may assist in speeding up of the folding process. In contrast, the average clustering coefficients of LINs show a good negative correlation with the rate of folding, indicating that clustering of amino acids, that participate in long-range interactions, into cliques, slows down the folding process. Interestingly, the average clustering coefficients of PCNs show negligible correlation, thereby implying that the short range interactions can reduce the negative effect on their folding kinetics.

Three parameters—CO (contact order) (Plaxco *et al*., 1998), LRO (long range order) (Gromiha and Selvaraj, 2001) and TCD (total contact distance) (Zhou and Zhou, 2002)—based on sequence distance per contact and/or total number of contacts per residue of the proteins, have also been shown to have negative correlation to their rate of folding (Gromiha and Selvaraj, 2001; Plaxco *et al*., 1998; Zhou and Zhou, 2002). The accuracy of prediction of the rate of folding, with parameters LRO and TCD, remain unchanged if short-range interactions are not included in the calculation. Here, along with delineating the role of long-range interactions, we have attempted to show that general network parameters, such as, clustering coefficient and assortativity, that are widely used in networks of diverse origins (technological, biological and social), can not only give an insight into their structural properties, but can also be used as indicators of specific biophysical processes, such as, of protein folding.

2 METHODS

2.1 Construction of PCN, LIN, and their random controls

The PCN was modeled from the native-state protein structures as available in PDB (Berman *et al*., 2000). The C_α atom of each amino acid was considered a 'node', and any two amino acids were said to be in spatial contact ('link') if there existed a threshold distance ($R_c \leq 8$Å) between their C_α atoms.

The LIN of a PCN was obtained by considering, other than the backbone links, only those 'contacts' which occur between amino acids that are 'distant' (i.e. separated by 12 or more amino acids) from each other along the backbone. Thus formed, a LIN is a subset of its PCN with same number of nodes (n_r) but fewer number of links due to removal of the short-range contacts.

Two types of random controls were created for the PCNs of the proteins. The polypeptide backbone connectivity was kept intact in both the random controls, while randomizing the non-covalent contacts. For every protein, 100 instances of each type of random control were generated from its PCN. Average of all the instances were used as a representative of the parameters and properties, and compared with that of the PCNs and their LINs.

Type I: this random control network has the same number of residues (n_r) and number of links/contacts (n_c) as those of the PCN, except that the contacts were created randomly by avoiding duplicate and self contacts.

Type II: apart from maintaining the number of nodes (n_r) and contacts (n_c), the connectivity distribution of PCNs was also conserved in this control network. To ensure adequate randomization, the pattern of pair-connectivity was randomized 2000 times.

The details of methods of construction with illustration is given in Supplementary Material.

2.2 Data

Except for Figure 1, all studies have been done on 30 single-domain, two-state folding, globular proteins, whose experimental rate of folding ($\ln(k_F)$) are available. The data include 5 all-α, 13 all-β and 12 $\alpha\beta$ class

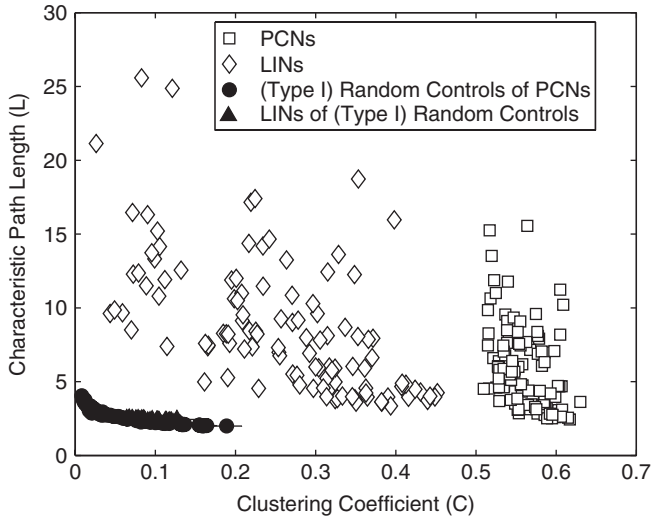

Fig. 1. L-C plot for 110 proteins from different structural classes: PCNs (open square), LINs (open diamond), Type I Random Controls of PCNs (filled circle) and LINs (filled triangle). Error—bars in the random controls data indicate SDs in L and C for each protein computed over 100 instances.

of proteins. The natural logarithms of rate of folding ($ln(k_F)$) of these proteins vary between −1.48 and 9.8 and have a range for the time of folding ($1/k_F$) of the order of 10^5 s. Sizes (n_r) of these proteins range from 43 to 126 amino acids. The structural data for these studies were obtained from the Protein Data Bank (Berman *et al.*, 2000). The preliminary network analysis (shown in Fig. 1) was done on 110 proteins ($43 < n_r < 2359$) from the major structural classes, which include the 30 single domain proteins mentioned above.

2.3 Network parameters

The following parameters were studied for the PCN, LIN and their random controls.

Shortest path length and characteristic path length Shortest path length (L_{ij}) between any pair of nodes i and j is the number of links that must be traversed between them by the shortest route. The average of all shortest path lengths, known as 'characteristic path length' (L), is an indicator of compactness of the network, and is defined as (Watts and Strogatz, 1999),

$$L = \frac{2\sum_{i=1}^{n_r-1}\sum_{j=i+1}^{n_r} L_{ij}}{n_r(n_r-1)},$$

where n_r is the number of residues in the network.

Clustering coefficient Clustering coefficient is the measure of *cliquishness* of the network. Clustering coefficient of a node i, C_i, is defined (Watts and Strogatz, 1999) as the $C_i = 2 * n/k_i(k_i - 1)$, where n denotes the number of contacts amongst the k_i neighbors of node i. Average clustering coefficient of the network (C) is the average of C_is of all the nodes in the network and is referred to as 'clustering coefficient' unless specified otherwise.

Degree and remaining degree Degree (k) is defined as the total number of neighbors a node is connected to. Degree is one of the measures of 'centrality' of a node in the network—the larger the degree more important it is. *Remaining degree* is one less than the total degree of a node (Newman, 2002). Other measures, based on degree, are maximum degree, k_{max}, average degree, $\langle k \rangle$, and the average degree of nearest neighbors, $\langle k_{nn}(k) \rangle$.

Assortative mixing and coefficient of assortativity A network is said to show assortative mixing, if the high-degree nodes in the network tend to be connected with other high-degree nodes, and 'disassortative' when the high-degree nodes tend to connect to low-degree nodes. The coefficient of assortativity (r) measures the tendency of degree correlation. It is the Pearson correlation coefficient of the degrees at either end of a link and is defined (Newman, 2002) as,

$$r = \frac{1}{\sigma_q^2}\sum_{jk} jk(e_{jk} - q_j q_k),$$

where r is the coefficient of assortativity, j and k are the degrees of nodes, q_j and q_k are the *remaining degree* distributions, e_{jk} is the joint distribution of the remaining degrees of the two nodes at either end of a randomly chosen link and σ_q is the variance of the distribution q_k.

3. RESULTS

3.1 Clustering coefficients of PCNs and LINs

PCNs from a large set of proteins have earlier been shown (Atilgan *et al.*, 2004; Bagler and Sinha, 2005; Greene and Higman, 2003; Vendruscolo *et al.*, 2002) to have high degree of clustering, which contributes to their ''small-world'' (Watts and Strogatz, 1999) nature. To study if the PCNs and their corresponding LINs of proteins have similar topological properties, such as, characteristic path length (L) and clustering coefficient (C), we plotted the L versus C graph in Figure 1 for 110 proteins from the four major structural classes (i.e. α, β, $\alpha + \beta$ and α/β). The figure also shows their corresponding Type I random controls. The Type II random controls were found to be indistinguishable from the Type I controls and not shown in Figure 1.

The results indicate two major differences between the topological properties of the PCNs and their corresponding LINs. The PCNs of these proteins have high clustering coefficients ($C_{PCN} = 0.562 \pm 0.029$) compared to their random controls, whereas the LINs show distribution in C over a range ($C_{LIN} = 0.259 \pm 0.109$), even though their random controls were almost indistinguishable from those of PCNs. L and C of random controls of PCNs were 2.621 ± 0.411 and 0.0557 ± 0.0476 and that of their LINs were 3.256 ± 0.056 and 0.075 ± 0.012. The LINs also have a little higher characteristic path lengths ($L_{LIN} = 8.72 \pm 4.564$) than PCNs ($L_{PCN} = 5.818 \pm 2.826$) owing to their reduced number of contacts as compared to those in PCNs. This indicates that the differences in C_{LIN}s may assign specificity to the protein networks at this length scale, which is otherwise lost with the short range contacts in PCNs, rendering the generic property of high clustering and compactness. The role, if any, the differential extent of clustering in the PCN at the two length scales may play in their kinetics of folding process is shown later.

3.2 Degree distributions of PCNs and LINs

The distribution of degrees in a network is an important feature, which reflects the topology of the network, and is also a possible indicator of the processes by which the network has evolved to attain the present topology. The networks in which the links between any two nodes are assigned randomly have a Poisson degree distribution (Bollobás, 1981) with most of the nodes having similar degree.

Figure 2 shows the normalized degree distributions of PCNs and LINs of the 30 proteins studied. The frequencies of nodes were scaled with the largest degree (k_{max}) in the network (PCN or LIN) to obtain the $P(k)$ of a given protein, so that proteins of different sizes can be compared. As seen in Figure 2a, the PCNs have Gaussian degree distribution that best fits the equation

$$y(x) = \frac{A}{w\sqrt{\pi/2}}\exp\frac{-2(x-x_c)^2}{w^2}$$

with $A = 5.538$, $w = 6.265$ and $x_c = 9.373$.

On the other hand, Figure 2b shows that the degree distribution of LINs is significantly different than those of PCNs. In LINs, most nodes were populated in the low-degree region and very few of them have high degrees. The best-fit for the LINs represent a single-scale exponential function (Greene and Higman, 2003), $P(k) \sim k^{-\gamma} \exp(-k/k_c)$, with $\gamma = 0.24$ and $k_c = 4.4$. The nodes of degree 1 in LINs' degree distributions, are the N- and C-terminal amino acids that are at the either end of the protein backbone. As expected (Bollobás, 1981), the

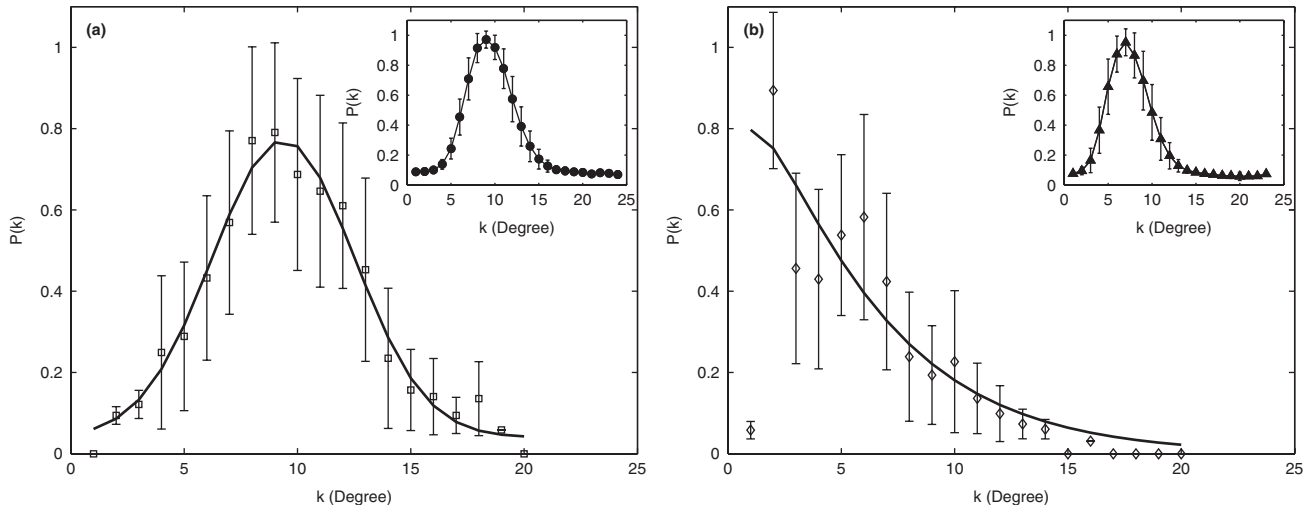

Fig. 2. Normalized degree distributions $P(k)$ of (**a**) PCNs and (**b**) LINs. Shown in the insets are (a) Type I Random Controls of PCNs and (b) their LINs. Thick lines are the best-fit curves for the means of the data. Error—bars indicate SD of the data for $P(k)$ of nodes with degree k across the 30 proteins analyzed.

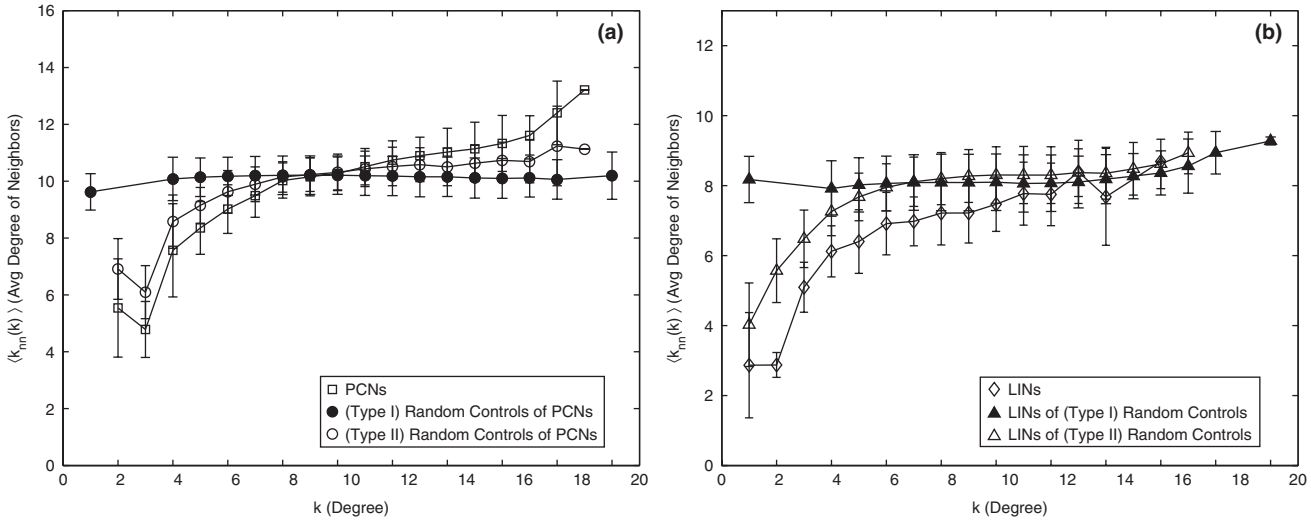

Fig. 3. Degree correlation pattern for (**a**) PCNs and (**b**) LINs. Assortative mixing of PCNs (open square) and LINs (open diamond) as compared to Type I Random Controls of PCNs (filled circle) and their LINs (filled triangle), and Type II Random Controls of PCN (open circle) and their LINs (open triangle). Error—bars indicate SD of the data for $\langle k_{nn}(k)\rangle$ of nodes with degree k across the 30 proteins and their controls.

Type I random controls of the PCNs (Fig. 2a, inset) have a Poisson degree distribution. LINs of Type I random controls (Fig. 2b, inset) too have a Poisson degree distribution. The figure clearly shows that these properties are the same for proteins irrespective of their functions and structural classifications (Bagler and Sinha, 2005; Greene and Higman, 2003).

3.3 Assortative nature of PCNs and LINs

The pattern of connectivity among the nodes of varying degrees can affect the interaction dynamics in the network, and their degree correlation is used as a measure to compute the strength and pattern of connectivity in a network. Average degree of the nearest neighbors, $k_{nn}(k)$, of nodes of degree k, is a parameter by which one can measure and visualize the degree correlation pattern on a network. In the presence of correlations, $k_{nn}(k)$ increases with increasing k for an 'assortative network', and decreases with k for a 'disassortative network' (Pastor-Satorras *et al.*, 2001).

Figure 3 shows $\langle k_{nn}(k)\rangle$ versus k plots for the PCNs (a) and LINs (b) and the two types of random controls. The nature of the curves for the PCNs (open square in Fig. 3(a)) and their LINs (open diamond in Fig. 3(b)) shows that both networks are characterized with 'assortative mixing', as the average degree of the neighboring nodes increased with k. The curve shows a tendency to saturate at larger k—a feature that may be due to

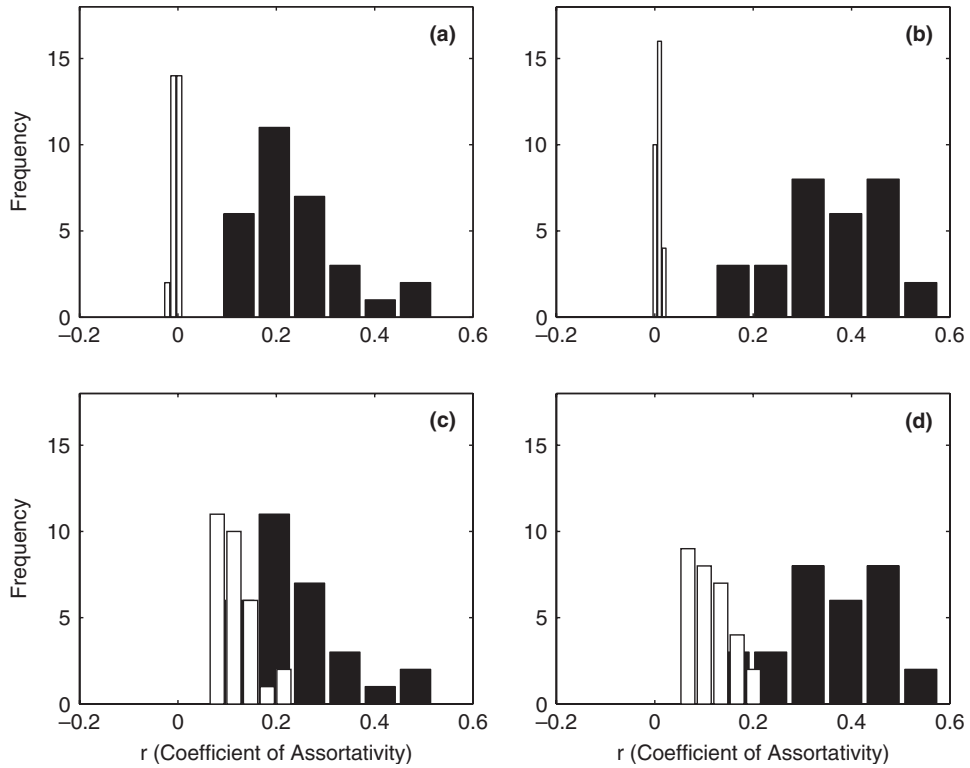

Fig. 4. Histograms of 'Coefficient of Assortativity (r)' of PCNs, Type I and Type II Random Controls of PCNs and their LINs. (**a**) and (**b**) PCNs and LINs (filled square) and their (Type I) Random Controls (open square). (**c**) and (**d**) PCNs and LINs (filled square) and their (Type II) Random Controls (open square).

the steric hindrance experienced by the connecting amino acids in the 3D structural organization of the protein. This steric hindrance restricts the position of an amino acid in the three dimensional conformational space, and results in a maximum values of degree ($k_{\max}$) of a node. In comparison, the $\langle k_{nn}(k)\rangle$ remained almost constant for the Type I random control for both PCNs (filled circle)) and LINs (filled triangle), indicating lack of correlations among the nodes' connectivity in these controls.

The 'coefficient of assortativity' (Newman, 2002), r, is a global quantitative measure of degree correlations in a network, and takes values as $-1 \leq r \leq 1$. r is zero for no correlations among nodes' connectivity, and takes positive or negative values for assortative or disassortative mixing, respectively. The r for both PCNs and LINs of the 30 proteins were found to be positive, indicating that the networks are assortative. Figure 4 shows the histograms of r of (a) PCNs, (b) LINs, both in (filled square), and their Type I random controls (open square). The r values of both PCNs as well as LINs of all the proteins show significantly high positive values (range: $0.09 < r < 0.52$ for PCNs and $0.12 < r < 0.58$ for LINs) when compared to other networks of diverse origins (Newman, 2002). Thus, the networks modeling the native protein structures are clearly characterized by high degree of assortative mixing at both short and long contact scales. The Type I random controls in Figure 4 a and b, for both PCNs and their LINs, are distributed around zero, confirming the observation of lack of degree correlations of the controls, made in Figure 3.

These properties of positive r and assortative degree correlations were also observed (data not shown) for a large number of protein structures performing various cellular functions and belonging to diverse structural categories (used in Bagler and Sinha, 2005). This conclusively proves that the assortative mixing in PCNs and LINs is a generic feature of protein structures. The role, if any, the assortative nature of the PCN at both length scales may play in their kinetics of folding process is shown later.

3.4 Degree distribution partially accounts for assortativity

To investigate whether the patterns of connectivity in the PCNs and LINs of the 3D structures of the proteins contribute towards the observed assortativity, we studied the assortative mixing and the 'coefficient of assortativity' of Type II random controls, in which the degree distribution of the PCNs were preserved while randomizing the pair connectivities. Figure 3c and d show the degree correlation plots of the Type II random controls of PCN (open circle) and their LINs (open triangle). It is clear that, unlike Type I random controls, the average degree of the neighboring nodes increased with k in Type II random controls, as seen for the PCNs and LINs.

The histograms of the 'coefficient of assortativity' (r) of Type II random controls (open square) are shown in Figsure 4 c and d. Here also, it can be seen that the assortativity is partially recovered in the Type II random controls for both PCNs and

their LINs. Thus degree distribution partially explains the observed assortative mixing. It implies that preserving the degree distribution of PCN, even while randomizing the pair-connectivities, is important to partially restore the assortative mixing in the random controls of PCNs as well as their LINs. The recovery of assortative mixing in the LINs by Type II random controls of PCNs is even more surprising, as the degree distribution of LINs (Fig. 2b) is very different compared to the PCNs (Fig. 2a). This is especially significant in the light of the observation (Xulvi-Brunet and Sokolov, 2004) that one can rewire the links in a (scale-free) network to obtain assortativity or disassortativity, to any degree, without any change in the degree distribution.

3.5 Correlation of protein network parameters to protein folding rates

The general network parameters (e.g. L, C and r) have been used to shed light on the topology, growth and dynamics of widely different networks—physical, social and biological. Here, we show the relationship of these general topological parameters (specifically, C and r) obtained from our coarse-grained model of protein structures (the PCNs and LINs), to a biophysical property underlying the organization of the 3D structure of the protein chains, i.e. with the kinetics of protein folding. Below, we have correlated the available experimental data on the rate of folding of the 30 proteins with the two network parameters, C and r of the PCNs and their LINs.

3.5.1 Average clustering coefficient and rate of folding Figure 1 shows that the PCNs and their LINs differ in their clustering coefficients (C), with PCNs having similar but high C, and their LINs having C distributed over a range from low to medium values. We did not find any significant relationship between the clustering coefficient of the PCNs (C_{PCN}) and the $\ln(k_F)$ for all the 30 proteins (correlation coefficient $= -0.2437$; $p < 0.2$). On the other hand, $\ln(k_F)$ showed a high negative correlation with the average clustering coefficient of the corresponding LINs (C_{LIN}). Since the clustering coefficient depends on the degree of the node, we plot, in Figure 5, the $C_{\mathrm{LIN}} * k_{\max}$ with $\ln(k_F)$ of all the proteins. The plot shows significantly high negative correlation (correlation coefficient $= -0.7712$; $p < 0.0001$) between the C_{LIN}s and the rate of folding for these single-domain, two-state folding proteins. Figure 5 also shows that neither Type I nor Type II random controls show any correlation with the rate of folding of the corresponding LINs.

C_{LIN} enumerates number of loops of length three in the LIN. Thus C_{LIN} essentially correlates to the number of 'distant' amino acids (nodes), those separated by a minimum of 12 or more other amino acids along the backbone, brought in mutual 'contact' with each other in the native state structure of the protein. Understandably, more the number of such long-range mutual contacts are required to be made in order to achieve the native state, more is the time taken to fold, and hence slower is the rate of folding. Interestingly, our result shows that this feature is completely neutralized through the short-range contacts in the PCNs. It may be mentioned that a comparable correlation (-0.7574; $p < 0.0001$) is observed between the (CO) of these 30 proteins with their $\ln(k_F)$. It is interesting to note that despite dissimilar quantities that CO and C_{LIN} measure, the similar correlation coefficients essentially indicate the important role of long-range contact formation in the rate of folding.

3.5.2 Coefficient of assortativity and rate of folding Unlike the clustering coefficients, the protein networks show high coefficient of assortativity (r) at both length scales (i.e. for the PCNs and their LINs). In Figure 6, the rate of folding of the proteins are plotted as a function of the coefficient of assortativity of their LINs. There is an increasing trend of $\ln(k_F)$ with increase in r. The five α proteins, all having high rate of folding, do not follow the trend very well. The correlation coefficient between the rate of folding ($\ln(k_F)$) and r of their LINs, excluding the

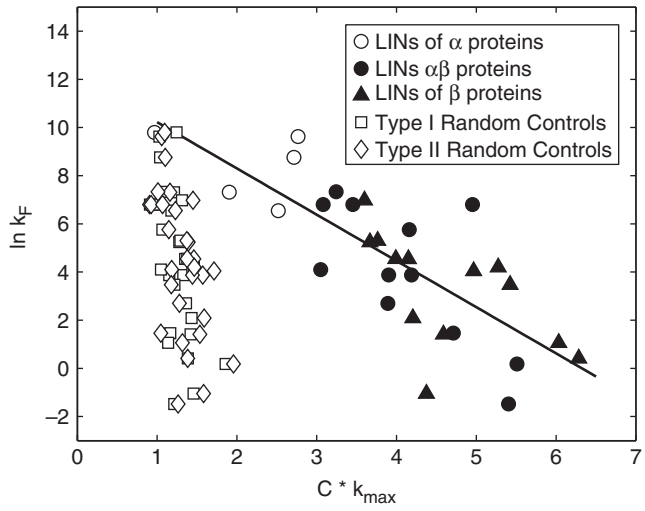

Fig. 5. Rate of folding, $\ln(k_F)$, has a negative correlation, as indicated by the trendline, with clustering coefficient (C) LINs.

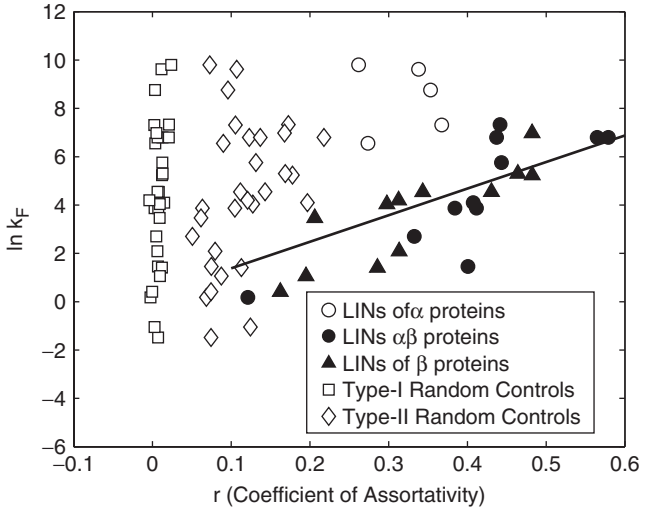

Fig. 6. Positive correlation between the rate of folding, $\ln(k_F)$, and the coefficient of assortativity (r) of LINs. The trendline is also shown.

five α proteins, is 0.6981 ($p < 0.0005$). The same for the PCNs is calculated to be 0.5943 ($p < 0.005$). The result implies that, along with showing assortative mixing, the PCNs and their LINs both show significant positive correlations with the rate of folding. Thus, the generic property of assortative mixing in proteins tends to contribute positively towards their kinetics of folding, and is fairly independent of the short- and long-range of interactions. Here also the Type I random controls, due to their coefficient of assortativity being clustered around zero (Fig. 4b), do not show any correlation with the rate of folding. As is expected from Figures 3 and 4, the Type II random controls, on the other hand, are scattered owing to the partial gain in assortativity, though they do not show any definite trend with the rate of folding.

4 DISCUSSION

In recent years, much interest is seen in the study of structure and dynamics of networks, with application to systems of diverse origins such as, society, technology and biology, etc. (Albert and Barabási, 2002; Dorogovtsev and Mendes, 2002). The aim of these studies has been to identify the common organizational principles within these wide variety of systems, and identify general network parameters that can correlate to the structure, function and evolution of each of the specific processes. Of these, biological networks are of special interest as they are products of long evolutionary history. The PCN is exclusive among other intra-cellular networks for their unique method of synthesis as a linear chain of amino acids, and then folding into a stable 3D structure through short- and long-range contacts among the residues. In this study, our aim is to understand if the general network parameters can offer any clue to the biophysical properties of the existing 3D structure of a protein, thereby reflecting the commonalities in network organization in general.

Our coarse-grained complex network model of protein structures uncovers, for the first time in a naturally evolved biological system, the interesting, and exceptional topological feature of assortativity at both short- and long- length scale of contacts. The assortative nature is found to be a generic feature of protein structures. We show that the assortativity positively correlates to the folding mechanisms at both length scale. This feature corroborates the known fact that the folding mechanisms are largely independent of the finer details of the protein structure (Alm and Baker, 1999). Since strongly cooperative mechanisms are necessary to bring the protein in its native conformation within a very short time (Maity *et al*., 2005), we have shown that assortative mixing contributes positively towards speeding up the folding process at different contact-length scales. The generality of assortative mixing in PCNs assume greater importance in the light of the debate on whether protein folding kinetics is under evolutionary control (Larson *et al*., 2002; Mirny *et al*., 1998; Scalley-Kim and Baker, 2004). Given the genetic basis and mode of formation of protein chains, the signature of assortativity as an indicator to the rate of folding is clear.

We also delineate the difference in the property of clustering of the nodes in the native structure at short- and long-length scales. The PCNs have high degree of clustering, which contributes to their 'small-world' nature helping in efficient and effective dissipation of energy needed in their function (Atilgan *et al*., 2004; Bagler and Sinha, 2005). Our results show that, in contrast, the corresponding LINs have significantly lower and distributed clustering coefficients (Fig. 1), and they show a negative correlation with the rate of folding of the proteins (Fig. 5). This indicates that clustering of amino acids that participate in the long-range interactions, into 'cliques' can slow down the folding process—possibly due to the backbone connectivity and steric factors. However, the clustering coefficient of PCNs *do not* have any significant correlation to the rate of folding, clearly indicating that the short-range interactions may be playing a constructive and active role in the determination of the rate of the folding process by reducing the negative contribution of the LINs. Our results thus show that the separation of the types of contacts in the PCNs and LINs clearly delineate the length scale of contacts that play crucial role in protein folding. It was recently shown that the CO of the transition state ensemble (TSE) is highly correlated to that of their native state structure, and they both correlate equally well with their rate of folding (Paci *et al*., 2005). This has been attributed to the fact that the long-range contacts are mainly located in the structural core that are formed early in the folding process, and the formation of such contact networks leads to the inverse correlation with the folding rates. Our results with general parameters of the LIN (C_{LIN} and r_{LIN}) corresponding to the native PCNs also reflect the crucial role that long-range interactions play in their rate of folding.

After the synthesis in the cell, folding of the amino acid chain is important for attaining the structure required to reach a functional state as soon as possible. This happens through inter-residue non-covalent interactions at many length and time scales. The folded structure have to confer stability, regions for binding of ligands of specific shapes and sizes, transmit the information of binding/unbinding to other parts of the protein, scaffold for retaining the functional regions along with the shape suitable for the protein function. It is likely that many of these properties may require opposing features to operate at different time and space scales. For example, the 'small-world' nature (high clustering) in the native protein structure is useful in inter-residue signaling required for its function on binding and allostery. On the other hand, the LIN have reduced clustering, which may facilitate communication among distant residues in the native structure to some extent, but such a feature can also increase the folding time as it requires distant residues in the chain to come closer during the folding process. Thus, the evolved native structure of the proteins show differential levels of clustering at two length scales. The assortative mixing, on the other hand, helps in enhancing the folding process at both length scales.

A large number of networks of diverse origin have been found (Newman, 2002) to be of disassortative nature, and questions regarding the origin of this property and whether this is an universal property of complex networks, has been adjudged as 'one of the ten leading questions for network research' (Amaral *et al*., 2004). Our discovery of assortativity in the amino acid networks in protein structures at short- and long-contact scales questions the invoked generality of the property in natural networks. The assortative nature of the

social networks has been claimed to be originating from their unusually high clustering coefficients and community structure (Newman, 2003). In proteins, LINs have high assortativity without necessarily having high clustering coefficients. It would be interesting to study if the secondary structures provide any role in shaping the 'community structure' in these molecular networks that help in conferring assortative mixing at both contact length scales (Newman, 2003; Palla *et al*., 2005).

Disassortative mixing observed in certain biological networks (metabolic signaling pathways network, and gene regulatory network) is conjectured to be responsible for decreasing the likelihood of crosstalk between different functional modules of the cell, and increasing the overall robustness of a network by localizing effects of deleterious perturbations (Maslov and Sneppen, 2002). In contrast to these two networks, PCNs are not disassortative. For the PCN, one may put forward the possibility of the backbone chain connectivity as a means of conferring greater robustness against perturbations.

From computational studies, it has been observed (Newman, 2002; Xulvi-Brunet and Sokolov, 2004) that assortative networks percolate easily, i.e. information gets easily transferred through the network as compared to that in disassortative networks. Protein folding is a cooperative phenomenon, and hence, communication amongst nodes is essential, so that appropriate non-covalent interactions can take place to form the stable native state structure (Maity *et al*., 2005). Thus, percolation of information is very much essential and could lead to the observed cooperativity and fast folding of the proteins. Hence, assortative mixing observed in proteins could be an essential prerequisite for facilitating folding of proteins.

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