The protein folding network

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Proteins are complex macromolecules with many degrees of freedom. To fulfill their function they have to fold to a unique three-dimensional structure (native state). Protein folding is a complex process governed by noncovalent interactions involving the entire molecule. Spontaneous folding in a time range of microseconds to seconds can be reconciled with the large amount of conformers by using energy landscape analysis. The main difficulty of this analysis is that the free-energy has to be projected on arbitrarily chosen reaction coordinates (or order parameters). In many cases a simplified representation of the free-energy landscape is obtained where important informations on the non-native conformation ensemble and the folding transition state ensemble are hidden. Moreover, the possible transitions between free-energy minima cannot be displayed in such projections which hinder the study of pathways and folding intermediates. The characterization of the free-energy minima and the connectivity among them, i.e., possible transitions between minima, for peptides and proteins is still an unresolved problem.

In the last five years many complex systems, like the World-Wide Web, metabolic pathways, and protein structures have been modeled as networks. Intriguingly, common topological properties have emerged from their organization. A description of the potential energy landscape without the use of any projection has been given in terms of networks for a Lennard-Jones cluster of atoms.

Here, we introduce complex network analysis to study the conformation space and folding of beta3s, a designed 20-residue sequence whose solution conformation has been investigated by NMR spectroscopy. The NMR data indicate that beta3s in aqueous solution forms a monomeric (up to more than 1mM concentration) triple-stranded antiparallel beta-sheet (Fig. 1, bottom), in equilibrium with the denaturated state. We have previously shown that in implicit solvent molecular dynamics simulations beta3s folds reversibly to the NMR solution conformation, irrespective of the starting conformation. We consider conformations sampled by molecular dynamics simulations and the transitions between them as the network nodes and links, respectively. The network analysis allows to identify the topological properties that are common to both beta3s, which folds to a unique three-dimensional structure, and a random heteropolymer which lacks a single preferential conformation like the native state despite it has the same residue composition as beta3s. These properties include the presence of several free-energy minima and highly connected conformations (hubs). On the other hand, a hierarchical modularity in the proximity of the native state is peculiar of a folding sequence.

I. MODEL AND METHODS

Molecular dynamics simulations The simulations and part of the analysis of the trajectories were performed with the program CHARMM. Beta3s was modeled by explicitly considering all heavy atoms and the hydrogen atoms bound to nitrogen or oxygen atoms (PARAM19 force field). A mean field approximation based on the solvent accessible surface was used to describe the main effects of the aqueous solvent on the solute. The two parameters of the solvation model were optimized without using beta3s. The same force field and implicit solvent model have been used recently in molecular dynamics simulations of the early steps of ordered aggregation and folding of structured peptides (alpha-helices and beta-sheets) ranging in size from 15 to 31 residues, as well as small proteins of about 60 residues. Despite the absence of collisions with water molecules, in the simulations with implicit solvent the separation of time scales is comparable with that observed experimentally. Helices fold in about 1 ns, beta-
and red nodes have \( k \) (www.visone.de) and visone. C ≲ 0 reflects structural variability within snapshots in a conformation. The yellow diamonds are folding TS conformations for coil, red for pipe colored according to secondary structure: white stands for extended, turn, and unstructured, respectively. There are 8 possible "letters" in the secondary structure "alphabet". Since the N- and C-terminal residues are always assigned an "-" a 20-residue peptide can assume \( 8^{18} \sim 10^{16} \) conformations. Conformations are nodes of the network and the transitions between them are links. A weight \( \bar{w} \) is assigned to each node to take into account the free-energy of each conformation and is equal to the number of snapshots with a given secondary structure string. The statistical weight \( w \) of a node is equal to the weight normalized by the total number of snapshots in the simulations \( (5 \times 10^5 \) and \( 10^5 \) for beta3s and the random heteropolymer, respectively). Considering all the conformations visited during a \( \mu \)s – scale simulation can yield to a computationally intractable network size. For this reason we used for the network analysis the 1287 conformations of beta3s with significant weight \((\bar{w} \geq 20 \text{ per conformation})\). Two nodes are connected by an undirected link (and called neighbors) if they either include a pair of snapshots that are visited within 20 ps or they are separated by one or more conformations with less than 20 snapshots each. For the 2 \( \mu \)s of the random heteropolymer a threshold of \( \bar{w} \geq 4 \) was used, so that \( \bar{w} \geq 4 \times 10^{-5} \) as in the beta3s network. The choice of a threshold value is somewhat arbitrary but the network properties are robust for a large range of threshold values (see Supplementary material).

The properties of the network are robust also with respect to the length of the simulation time and the definition of the nodes. The topological properties are independent from simulation lengths if one considers more than 2 \( \mu \)s. The correlation between statistical weight and connectivity, as well as power-law behavior of the connectivity distribution and 1/k behavior of the clustering coefficient distribution (see below) are essentially identical after 2, 4, and 10 \( \mu \)s. As an example, the exponent of the power-law is 2.0 for the beta3s networks based on 2, 4 and 10 \( \mu \)s of simulation time. Defining nodes by grouping snapshots according to root mean square deviations (rmsd) in coordinates of C\(_\alpha\)-C\(_\beta\) atoms yields the same overall properties i.e., power-law distribution of the links (with similar \( \gamma \) value) and 1/k tail of the clustering distribution. Grouping snapshots according to secondary structure motifs does not require the use of an arbitrarily

**FIG. 1:** Beta3s conformation space network. The size and color coding of the nodes reflect the statistical weight \( w \) and average neighbor connectivity \( k_{nn} \), respectively. White, cyan, and red nodes have \( k_{nn} < 30, 30 \leq k_{nn} \leq 70, \) and \( k_{nn} > 70 \), respectively. Representative conformations are shown by a pipe colored according to secondary structure: white stands for coil, red for a-helix, orange for bend, cyan for strand and the N-terminus is in blue. The variable radius of the pipe reflects structural variability within snapshots in a conformation. The yellow diamonds are folding TS conformations (TSE1, TSE2, see text for details) characterized by a connectivity/weight ratio \( k/2\bar{w} \) > 0.3, a clustering coefficient \( C < 0.3 \), and \( 60 < k_{nn} < 80 \). This figure was made using visone (www.visone.de) and MOLMOL visualization tools.

hairpins in about 10 ns\(^{22}\) and triple-stranded \( \beta \)-sheets in about 100 ns\(^{33}\), while the experimental values are \( \sim 0.1 \mu s\(^{23}\), \( \sim 1 \mu s\(^{23}\) and \( \sim 10 \mu s\(^{3}\), respectively. Recently, four molecular dynamics simulations of beta3s were performed at 330 K for a total simulation time of 12.6 \( \mu s\(^{14}\). There are 72 folding events and 73 unfolding events and the average time required to go from the denatured state to the folded conformation is 83 ns. The 12.6 \( \mu s \) of simulation length is about two orders of magnitude longer than the average folding or unfolding time, which are similar because at 330 K the native and denatured states are almost equally populated\(^{14}\). For the network analysis the first 0.65 \( \mu s \) of each of the four simulations were neglected so that along the 10 \( \mu s \) of simulations there are a total of \( 5 \times 10^5 \) snapshots because coordinates were saved every 20 ps. The sequence of the random heteropolymer is a randomly scrambled version of the beta3s sequence with the same residue composition. It was simulated for 2 \( \mu s \) and \( 10^5 \) snapshots were saved. The conditions for the molecular dynamics simulations, i.e., force field, solvation model, temperature, and time interval between saved snapshots were the same for both peptides.
chosen RMSD cutoff, and is able to capture the fluctuations of partially structured conformations.

Evaluation of $P_{\text{fold}}$ The TS ensemble can be defined as the set of structures which have the same probability of folding ($P_{\text{fold}}$) or unfolding in trajectories started with varying initial conditions. For each putative TS conformation, the probability to fold before unfolding was calculated by 100 very short trajectories at 330 K started from ten snapshots within a node. The only difference between the ten runs was the seed for the random number generator used for the initial assignment of the atomic velocities. A trajectory was considered to lead to folding (unfolding) if it visits first structures with a fraction of native contacts $Q > 22/26$ ($Q < 4/26$).

II. RESULTS AND DISCUSSION

To study the conformation space network of polypeptides we concentrate on the analysis of topology, i.e., on the study of the connectivity between different conformations, leaving for a later study the analysis of transition rates. We have investigated the network topologies of several peptides but on this paper we focus on beta3s and the random scrambled version of it. Additional details can be find in the Supplemental material where the network properties of another structured peptide and a glycine homopolymer are presented.

Conformation space network of a structured peptide The conformation space network and relevant structures of beta3s are shown in Fig. 1. The group of nodes at the bottom of Fig. 1 (red nodes) represents the native state basin (FS). The native basin is connected to a wide region of nodes with significant native content (cyan circles in the middle of Fig. 1). Although many heterogeneous routes can be taken to reach the folded state (in agreement with lattice simulations), most of the folding events have common structural features that define two average folding pathways. The less frequently average pathway (see Ref. but also the density of transitions in Fig 1 bottom right) consists of conformations that have the N-terminal hairpin formed while the C-terminal strand is mostly unstructured with non-native hydrogen bonds at the turn (TSE1 in Fig. 1). The second and most frequent average pathway includes conformations with a well formed C-terminal hairpin while the N-terminal strand is disordered (TSE2 in Fig. 1), namely it can be out-of-register or mostly unstructured. It is interesting to note that the same two folding pathways were observed experimentally for a 24-residue peptide with the same folded state as beta3s. Furthermore multiple folding pathways have recently been detected by kinetic analysis of a β-sandwich protein.

The denatured state ensemble is very heterogeneous and includes high enthalpy, high entropy conformations (e.g., the partially helical conformations, denoted HH in Fig. 1) but also low enthalpy, low entropy conformations (e.g., the curl-like trap, TR). The former are loosely linked clusters of conformations with similar secondary structure (see Tab. 1) which are characterized by an unfavorable effective energy (sum of peptide potential energy and solvation energy) and fluctuating unstructured residues (e.g., the terminal of the helix shown on top left of Fig. 1). On the contrary, low enthalpy, low entropy traps form tightly linked clusters with almost identical secondary and tertiary structure, favorable effective energy (similar to the one of the native structure, see Tab. 1) and no fluctuating residues (e.g., Fig. 1, top right). Taken together, these results indicate that FS is entropically favored over low enthalpy conformations like TR, i.e., FS has more flexibility than TR. A possible explanation is that the C-terminal carboxy is involved in four hydrogen bonds in TR (with the backbone NH’s of residues 4-7), whereas both termini undergo rather large fluctuations in FS. In addition, a more favorable van der Waals energy in TR is consistent with a denser packing in TR than in FS.

| Conformation | $\langle E \rangle$ | $\Delta F$ |
|--------------|-----------------|----------|
| Helical conformations (HH) | | |
| ---HHHHHHHHH----- | 0.9 | 3.1 |
| ---HHHHHHHHH----- | -1.9 | 3.3 |
| ---HHHHHHHHH--- | 0.9 | 3.5 |
| ---HHHHHHHHH--- | 0.5 | 3.7 |
| ---HHHHHHHHH--- | -0.8 | 3.7 |
| ---TT---HHHHHHH--- | -0.8 | 3.8 |
| Curl-like trap (TR) | | |
| ---SSGGG---EEE---STTTE--- | -7.8 | 3.4 |
| ---SSGGG---EEE---STTTE--- | -7.0 | 3.5 |
| ---SSGGG---EEE---STTTE--- | -9.3 | 3.7 |
| ---SSGGG---EEE---STTTE--- | -9.6 | 3.7 |
| ---SSGGG---EEE---STTTE--- | -8.4 | 3.7 |

$^a$Average effective energy  
$^b$Free-energy relative to the most populated conformation. All values are in kcal/mol. The conformational entropy of the peptide is equal to $(\langle E \rangle - F)/T$. Note that the curl-like traps are entropically penalized with respect to the native state.

Note that the network description of non-native conformations is more detailed than the one obtained by projecting the free energy surface on progress variables (e.g., based on fraction of native contacts). In such projections, for low values of the fraction of native contacts structures as diverse as helices and the curl-like conformations mentioned above are not distinguished. Even
the ensemble with half of the native contacts is heterogeneous and hard to classify. Using as reaction coordinate the RMSD (with respect to a given structure) or the radius of gyration is even less selective. Only when a clever combination of variables is used it is possible to have a more detailed description of the free-energy landscape. The network description of the conformation space gives a synthetic and systematic view of all the possible conformations accessed by the system and their transitions. By considering the statistical weight of the nodes a thermodynamical description of the system is obtained.

![Graph](image)

**FIG. 2:** Correlation between the statistical weight $w$ and the connectivity $k$ for beta3s. The connectivity is proportional to $\log^2(w)$ with a correlation coefficient of 0.88 (solid line). The correlation and the fit are calculated over all nodes of the network but in the figure logarithmic binning is applied to reduce noise.

The high correlation between the statistical weight of a node and its number of links (Fig. 2) shows that the most connected nodes are also low lying minima on the free-energy landscape. This indicates that the conformation space network describes the significant free energy minima and their dynamic connectivity, without projection, where highly populated nodes are minima of free-energy and the set of nodes densely connected to them make up the basins of such minima.

**Folding and network topology** The average neighbor connectivity $k_{nn}$ of beta3s (Fig. 3a), i.e., the average number of links of the neighbors of a given node, is rather heterogeneous, highlighting the presence of different connection rules in different regions of the network. This is not the case for the random heteropolymer (Fig. 3b) whose basins have organization and statistical weight similar among each others as previously found for most homopolymers. Note that for beta3s the native state is well discriminated by $k_{nn}$ (red nodes in Fig. 1 and top band in Fig. 3a).

The connectivity distribution of conformation space networks shows a well pronounced power-law tail $P(k) = k^{-\gamma}$ with $\gamma = 2.0$ for both beta3s and the random heteropolymer (Fig. 4a) as well as another structured peptide and homoglycine, i.e. (Gly)$_{20}$ (see Supplementary material). The power-law is due to the presence of few largely connected "hubs" while the majority of the nodes have a relatively small number of links. This behavior has been previously observed for several biological, social and technological networks, which in the literature take the name of scale-free networks. In terms of free-energy this means that only a few low lying minima are present but they act as "hubs" with a large number of routes to access them.

The average clustering coefficient $C$ is a measure of the probability that any two neighbors of a node are connected. Beta3s and the heteropolymer have $C$ values of 0.49 and 0.28, respectively. These values are one order of magnitude larger than random realizations of the two networks with the same amount of nodes and links. The native basin of beta3s includes the nodes with the largest number of links of the network. These nodes give rise to the $1/k$ tail of the clustering distribution (Fig. 4b), i.e., an inherently hierarchical organization of the conformations in the native basin of beta3s. Such organization is not observed for the non-native region of beta3s and the random heteropolymer. Note that the power-law scaling of the connectivity distribution can be considered as a general property of free-energy landscapes of polypeptides, whereas a hierarchical organization of the nodes reflects a single pronounced free-energy basin of attraction (like the native state).

![Graph](image)

**FIG. 3:** Average neighbor connectivity $k_{nn}$ plotted as a function of the statistical weight for the 1287 nodes of beta3s (A) and for the 2658 nodes of the random heteropolymer (B). $k_{nn}$ of node $i$ is the average number of links of the neighbors of node $i$. The yellow diamonds are folding transition state conformations (see also Fig. 1 and text) characterized by a connectivity/weight ratio $k/2w > 0.3$, a clustering coefficient $C < 0.3$, and $60 < k_{nn} < 80$.

**Transition state ensemble** As mentioned above folding is a complex process with many degrees of freedom involved and it is difficult (or even not possible) to define a single reaction coordinate to monitor folding events. Hence, it is very difficult to isolate transition state (TS) conformations from equilibrium sampling. The TS conformations are saddle points, i.e., local maxima with respect to the reaction coordinate for folding and local min-

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**Note:** The text contains diagrams and mathematical expressions that are not included in the plain text representation. For a complete understanding, visual aids and detailed mathematical expressions are necessary. The diagrams and mathematical expressions are crucial for the clarity of the text.
Formations). The straight line corresponds to a power-law fit
of neighbors of node \( i \) within a certain range. For
node \( i \), it is defined as
\[ C_i = \frac{2k_i}{n_i(n_i - 1)} \]
where \( k_i \) is the number of neighbors of node \( i \) and \( n_i \) is the total number of connections between them. Values of \( C \) are averaged over the nodes with \( k \) links. The straight line corresponds to a power-law fit
\[ y = x^{-\gamma} \] on the tail of the distribution with \( \gamma = 2.0 \). (B) The clustering coefficient \( C \) describes the cliques of a node. For node \( i \), it is defined as
\[ C_i = \frac{n_i(n_i - 1)}{2k_i} \]
where \( k_i \) is the number of neighbors of node \( i \) and \( n_i \) is the total number of connections between them. Values of \( C \) are averaged over the nodes with \( k \) links. The straight line corresponds to a power-law fit
\[ y = x^{-1} \] on the tail of the distribution of beta3s.

III. CONCLUSIONS

Complex network theory was used to analyze the conformation space of a structured peptide and the one of a random heteropolymer of same residue composition. Four main results have emerged. First, as it was already observed for a variety of networks as diverse as the World Wide Web and the protein interactions in a cell, the con-

FIG. 4: Topological properties of conformation space networks. Red and blue data points are plotted for beta3s and a random heteropolymer, respectively. For a direct comparison, the connectivity \( k \) is normalized by the average connectivity \( \langle k \rangle \) of each network. Logarithmic binning is applied to reduce noise. (A) The connectivity distribution \( P(k) \) is the probability that a node (conformation) has \( k \) links (neighbor conformations). The straight line corresponds to a power-law fit
\[ y = x^{-\gamma} \] on the tail of the distribution with \( \gamma = 2.0 \). (B) The clustering coefficient \( C \) describes the cliques of a node. For node \( i \), it is defined as
\[ C_i = \frac{2k_i}{n_i(n_i - 1)} \]
where \( k_i \) is the number of neighbors of node \( i \) and \( n_i \) is the total number of connections between them. Values of \( C \) are averaged over the nodes with \( k \) links. The straight line corresponds to a power-law fit
\[ y = x^{-1} \] on the tail of the distribution of beta3s.
formation space network of polypeptide chains is a scale-free network (power-law behavior of the degree distribution). Second, the native basin of the structured peptide shows a hierarchical organization of conformations. This organization is not observed for the random heteropolymer which lacks a native state. Third, free energy minima and their connectivity emerge from the network analysis without requiring projections into arbitrarily chosen reaction coordinates. As a consequence it is found that the denatured state ensemble is very heterogeneous and includes high entropy, high enthalpy conformations as well as low entropy, low enthalpy traps. Fourth, the network properties were used to identify transition state conformations and two main average folding pathways. It was found that the average neighbor connectivity $k_{nn}$ correlates with $P_{fold}$, the probability of folding. $P_{fold}$ is computationally very expensive to evaluate. Hence, it will be important to generalize this result by analyzing other structured peptides which is work in progress in our research group. In conclusion, the network analysis seems particularly useful to study the conformation space and folding of structured peptides including the otherwise elusive transition state ensemble.

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FIG. S1: Dependence of the beta3s network properties on the node-weight threshold. The threshold value used in the present work ($\tilde{w} = 20$) is shown as an empty circle while filled circles correspond to threshold values of, from left to right, 500, 200, 100, 50, 10, 5, 2 and 1. (A) Relation between the threshold value and the number of nodes. (B) Number of links as a function of the number of nodes. When the threshold is very large (i.e., small number of nodes) the network approaches a topology where all possible connections are present (solid line, $N_{\text{links}} = N_{\text{nodes}}(N_{\text{nodes}} - 1)/2$). When the threshold is small (i.e., large number of nodes) the network approaches a topology with only one link per node (dashed line, $N_{\text{links}} = N_{\text{nodes}}$). (C) Average number of links per node ($\langle k \rangle$) as a function of the number of nodes. (D) Average clustering coefficient $C$ as a function of the number of nodes.
FIG. S2: Dependence of the beta3s connectivity distribution (A) and clustering coefficient distribution (B) on the node-weight threshold. This plot shows that the scale-free behavior and the $1/k$ tail of the clustering coefficient distribution are robust with respect to the choice of threshold values.

FIG. S3: Connectivity distribution (left) and clustering coefficient distribution (right) for beta3s (filled circles), another structured peptide, i.e., residues 101-111 of α-lactalbumin (empty diamonds, Demarest et al., (1999) Biochemistry, 38, 7380), and a 20-residue homo-glycine which is unstructured (filled diamonds).
## Table S1: Supplementary material. Nodes used for $P_{fold}$ evaluation.

| Node number | Probability of folding $P_{fold}$ | Standard deviation $\sigma_{P_{fold}}$ | Neighbor connectivity $k_{nn}$ | Weight $\tilde{w}$ | Number of links $k$ | $k/2\tilde{w}$ | Clustering coefficient $C$ | Native contacts $Q$ | Standard deviation $\sigma_Q$ | Secondary structure string |
|-------------|----------------------------------|--------------------------------------|--------------------------------|------------------|------------------|----------------|--------------------------|----------------|---------------------------|--------------------------|
| 432         | 0.22                             | 0.17                                 | 55.1                          | 54               | 40               | 0.37           | 0.31                     | 0.38           | 0.09                      | -----SS--EEEESSEEED------ |
| 218         | 0.26                             | 0.20                                 | 45.5                          | 105              | 73               | 0.35           | 0.23                     | 0.42           | 0.09                      | -----SSSS--EEEESSEEED------ |
| 313         | 0.26                             | 0.16                                 | 55.6                          | 70               | 60               | 0.43           | 0.28                     | 0.46           | 0.09                      | -EEE--STTEE--SSS------ |
| 446         | 0.32                             | 0.25                                 | 56.2                          | 52               | 50               | 0.48           | 0.28                     | 0.40           | 0.10                      | -EEEESSEE--SS------ |
| 308         | 0.33                             | 0.28                                 | 65.9                          | 72               | 69               | 0.48           | 0.23                     | 0.44           | 0.08                      | -----SSSS--EEEESSEEED------ |
| 315         | 0.37                             | 0.26                                 | 52.2                          | 70               | 60               | 0.43           | 0.24                     | 0.47           | 0.08                      | -EEE--STTEE--SS------ |
| 306         | 0.43                             | 0.27                                 | 57.3                          | 73               | 60               | 0.41           | 0.31                     | 0.42           | 0.08                      | -----SS--EEE--STTEE------ |
| 208         | 0.51                             | 0.26                                 | 58.1                          | 115              | 87               | 0.38           | 0.23                     | 0.43           | 0.08                      | -----SSSS--EEEESSEEED------ |
| 589         | 0.53                             | 0.31                                 | 60.1                          | 40               | 52               | 0.65           | 0.17                     | 0.45           | 0.10                      | -----SSSSST--EEEESSEEED------ |
| 580         | 0.56                             | 0.34                                 | 65.0                          | 40               | 47               | 0.59           | 0.26                     | 0.48           | 0.08                      | -EEEESSEE--SS------ |
| 197         | 0.57                             | 0.39                                 | 80.5                          | 121              | 105              | 0.43           | 0.28                     | 0.52           | 0.09                      | -----SSST--EEEESSEEED------ |
| 540         | 0.60                             | 0.16                                 | 70.3                          | 44               | 49               | 0.56           | 0.28                     | 0.46           | 0.07                      | -----SSSS--EEEESSEEED------ |
| 285         | 0.62                             | 0.30                                 | 75.7                          | 80               | 68               | 0.42           | 0.30                     | 0.47           | 0.07                      | -----SSSS--EEEESSEEED------ |
| 630         | 0.65                             | 0.22                                 | 71.3                          | 38               | 56               | 0.74           | 0.29                     | 0.44           | 0.11                      | -----STTT--EEEESSEEED------ |
| 426         | 0.76                             | 0.20                                 | 97.7                          | 55               | 76               | 0.69           | 0.43                     | 0.55           | 0.12                      | -----BTTTB--EEEESSEEED------ |
| 280         | 0.88                             | 0.18                                 | 98.2                          | 82               | 81               | 0.49           | 0.43                     | 0.56           | 0.09                      | -----EEE--STTEE------ |

**Control simulations**

| Node number | Probability of folding $P_{fold}$ | Standard deviation $\sigma_{P_{fold}}$ | Neighbor connectivity $k_{nn}$ | Weight $\tilde{w}$ | Number of links $k$ | $k/2\tilde{w}$ | Clustering coefficient $C$ | Native contacts $Q$ | Standard deviation $\sigma_Q$ | Secondary structure string |
|-------------|----------------------------------|--------------------------------------|--------------------------------|------------------|------------------|----------------|--------------------------|----------------|---------------------------|--------------------------|
| 174         | 0.09                             | 0.10                                 | 60.0                          | 139              | 51               | 0.18           | 0.61                     | 0.37           | 0.07                      | -----EE--STTEEESTTEE------ |
| 179         | 0.09                             | 0.10                                 | 25.0                          | 135              | 19               | 0.07           | 0.33                     | 0.44           | 0.07                      | -----EEE--EEEESSEEED------ |
| 15          | 0.10                             | 0.13                                 | 35.8                          | 3243             | 73               | 0.01           | 0.28                     | 0.47           | 0.08                      | -----EEE--EEEESSEEED------ |
| 200         | 0.10                             | 0.27                                 | 62.9                          | 119              | 51               | 0.21           | 0.31                     | 0.34           | 0.07                      | -----SSSB--EEEESSEEED------ |
| 475         | 0.15                             | 0.17                                 | 61.7                          | 48               | 34               | 0.35           | 0.68                     | 0.43           | 0.06                      | -----EEE--STTEERTT--EEE------ |