Supplementary materials:

When clusters of conformations were used as states, the final result might depend on the clustering method, or threshold used.[1] To estimate how much the result depended on the clustering method, we did clustering by all-atom root-mean-square-deviation (RMSD), main chain hydrogen bonds pattern and combinations of them with different threshold values. Follow Krivov and Karplus,[1] each subsequent structures were compared with all clusters so far; it would form a new cluster if the RMSD between this structure and/or the first structure of all clusters exceeded the RMSD threshold or the difference of the hydrogen bonds pattern of this structure and the first structure of all clusters exceeded the hydrogen bonds pattern dissimilarity threshold. This method was called algorithm one. The algorithm employed in the manuscript was called algorithm two.[2]

We employed clustering algorithm one first and did clustering by RMSD only with threshold 4.0 Å. The corresponding minimal cut tree (or disconnectivity graph, TRDG) was shown in Fig S1. The vertical axis was the free energies and the unit was kcal/mol. Only the lowest 300 minima were kept and the minima were numbered from the lowest in the free energy to the highest and this theme was through to Fig S4. There were one anti-parallel amyloid aggregation minimum (9) and two parallel amyloid aggregations minima (1 and 2), which were identical to each other if the two chains are indistinguishable. Parallel aggregation with displacement zero or one between the two chains could not be discriminated. This implied that the threshold was a little large. Then we reduced the threshold to 3 Å. The corresponding minimal cut tree was shown in Fig S2. Minima 1, 2, 4 and 5 were parallel aggregations with displacement equal to one.

Minimum 8 was anti-parallel aggregation. Minima 1 and 4, 2 and 5 were identical by the exchange of the two chains. Minima 1 and 2 (4 and 5) had the same pattern of alignment but different hydrogen bond number and side chain packing. Most conformations in minimum 16 and minimum 19 were in register parallel amyloid aggregation. However, some conformations belong to these minima had only two main chain hydrogen bonds and they took an out of register arrangement. This implied that RMSD was not sensitive to the displacement of the two chains.

Hydrogen bond pattern itself might also work as clustering criteria. However, the shortcoming was that it could not discriminate conformations with and without bending, since the bending of the two chains did not affect the hydrogen bonds pattern. The minimal cut tree by combined criteria from RMSD and hydrogen bond pattern was shown in Fig S3, which was generated with RMSD 4.0 Å and the threshold of hydrogen bond pattern off by one. Algorithm one was used here. Minima 14 and 20 were in-registry parallel aggregation. Minima 1, 2, 3 and 4 were parallel aggregations with displacement 1. Minima 41 and 58 were parallel aggregations with displacement 3 between the two chains. Minimum 18 was anti-parallel aggregations. Minima 1 and 2, 3 and 4, 14 and 20, as well as 41 and 58 were identical to each other, respectively, if the two chains were indistinguishable. Minima 1 and 2 had the same pattern of alignment but different number of hydrogen bonds and side-chain packing. This minimal cut tree was very like the one shown in Fig. 1 of the manuscript, which was from clustering algorithm two using the same threshold values, except that the orders of the minima of these two figures were different. Reduce of the RMSD and/or hydrogen bond pattern threshold

would not modify the main property of the minimal cut tree. We also employed algorithm two with RMSD 3 Å and threshold of hydrogen bond pattern off by one. The minimal cut tree was shown in Fig. S4. Minimal 1,2,3,4 were parallel aggregations with displacement 1 and minimum 9 was in-registry anti-parallel aggregation. Minimal 13 and 14 were in-registry parallel aggregations. Minimal 31 and 36 were parallel aggregations with displacement 3. Minimum 50 was bent anti-parallel aggregation (Minimum 77 in Fig. 1). Minima 1 and 3, 2 and 4, 13 and 14, as well as 31 and 36 were identical to each other, respectively, if the two chains were indistinguishable. Minima 1 and 2 had the same pattern of alignment but different number of hydrogen bonds and side-chain packing.

It was obviously that both the free energy of the minima and the free energy barriers between them depended on the clustering criteria weakly, though the minimal cut trees by different clustering method and thresholds showed similar result. This is not hard to understand. Larger threshold would enlarge the region associated with the minima in the phase space and thus lowed the calculated free energy. We might define a group of clusters, which has lower free energy barrier between them as one basin. If the conformation space was divided into well-separated basins and the RMSD distance between different basins were much larger than those within each basin, the total visited time of each basin would not be modified by the threshold, though individual minimum would be. And if this was the case, the calculated free energy barrier between different basins should also be independent on the threshold. However, the free energy surface in present work (and all proteins) was complex and the above statement was obviously not satisfied. Larger threshold would merge minima separated by short RMSD distance,

while the free energy barrier between them was not negligible. Thus the calculated free energy barrier along each route was underestimated. This effect was illustrated in Fig S5. Suppose that the RMSD between node 2 and node 3 was small, while the free energy barrier between them was relatively high. The calculation of max flow from node 1 to node 4 was 5. Once a larger threshold was used in the clustering process, which cause node 2 and node 3 were merged into one node, the calculated max flow along this route would be 20 and this was actually an overestimation. On the other hand, the threshold must not be too small. Otherwise the system was very easy to visit several clusters within the sampling time interval. Thus, the number of re-crossings would be very large and the transition possibility would be underestimated largely. Suppose an extreme condition that the RMSD threshold was zero and each cluster had only one conformation, the calculated max flow between any two clusters would be only one and this was obviously under-estimation. Too small threshold would also cause each cluster to be visited too less times and the statistic error might be high. Unfortunately, the choice of clustering threshold should depend on the detailed energy surface. The strict requirement that free energy barrier within each cluster was always smaller than those between different clusters could not be granted from merely the clustering of the MD trajectory. This difficult actually existed in all clustering algorithm of MD data.[3-6] However, the fact that minimal cut tree (or connected graph, folding network presented in the above references) obtained by different threshold and clustering algorithm showed similar qualitative result indicated that the analysis employed in this manuscript was robust and was enough for the qualitative analysis of the energy surface.

Reference:

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Figures captions:

Fig. S1. Minimal cut tree obtained with RMSD threshold of 4.0 Å. Only the lowest 300 minima were shown. The vertical axis was the free energy and the unit was kcal/mol. The minima were numbered from the lowest to the highest in free energy. This theme was through to Fig. S4. Minimal 1 and 2 were parallel aggregations and minimum 9 was anti-parallel aggregation.

Fig. S2. Minimal cut tree obtained from algorithm one with RMSD threshold 3.0 Å. The typical conformations of two-strand β-sheet are: 1), parallel out-of-registry displaced by one residue (minima 1, 2, 4, and 5); 2), parallel in-registry (minima 16 and 19); and 3), anti-parallel in-registry (minimum 8).

Fig. S3. Minimal cut tree obtained from algorithm one with RMSD threshold of 4.0 Å and hydrogen bond pattern off by 1. Minima 14 and 20 were in-registry parallel aggregation. Minima 1, 2, 3 and 4 were parallel aggregations with displacement 1. Minima 41 and 58 were parallel aggregations with displacement 3 between the two chains. Minimum 18 was anti-parallel aggregations.).

Fig. S4. Minimal cut tree obtained from algorithm two with RMSD threshold 3.0 Å and hydrogen bond pattern off by 1. Minimal 1,2,3,4 were parallel aggregations with displacement 1 and minimum 9 was in-registry anti-parallel aggregation. Minimal 13 and 14 were in-registry parallel aggregations. Minimal 31 and 36 were parallel aggregations with displacement 3. Minimum 50 was bent anti-parallel aggregation

Fig. S5. An illustration of two clusters with a short RMSD distance but separated by high free energy barrier. Too large clustering threshold might cause merging of two clusters and over-estimation of the max flow between node 1 and node 4.

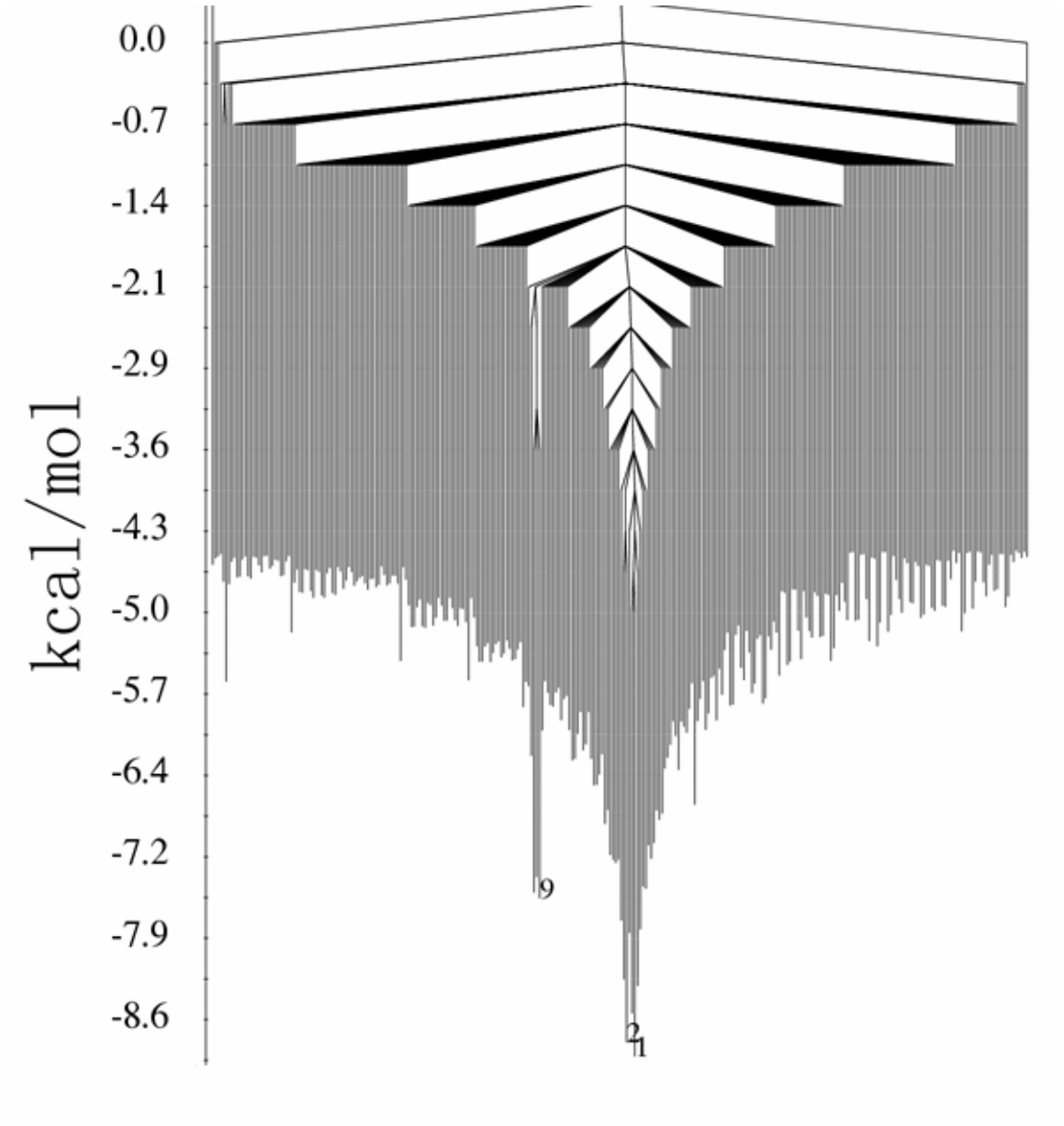

Fig. S1

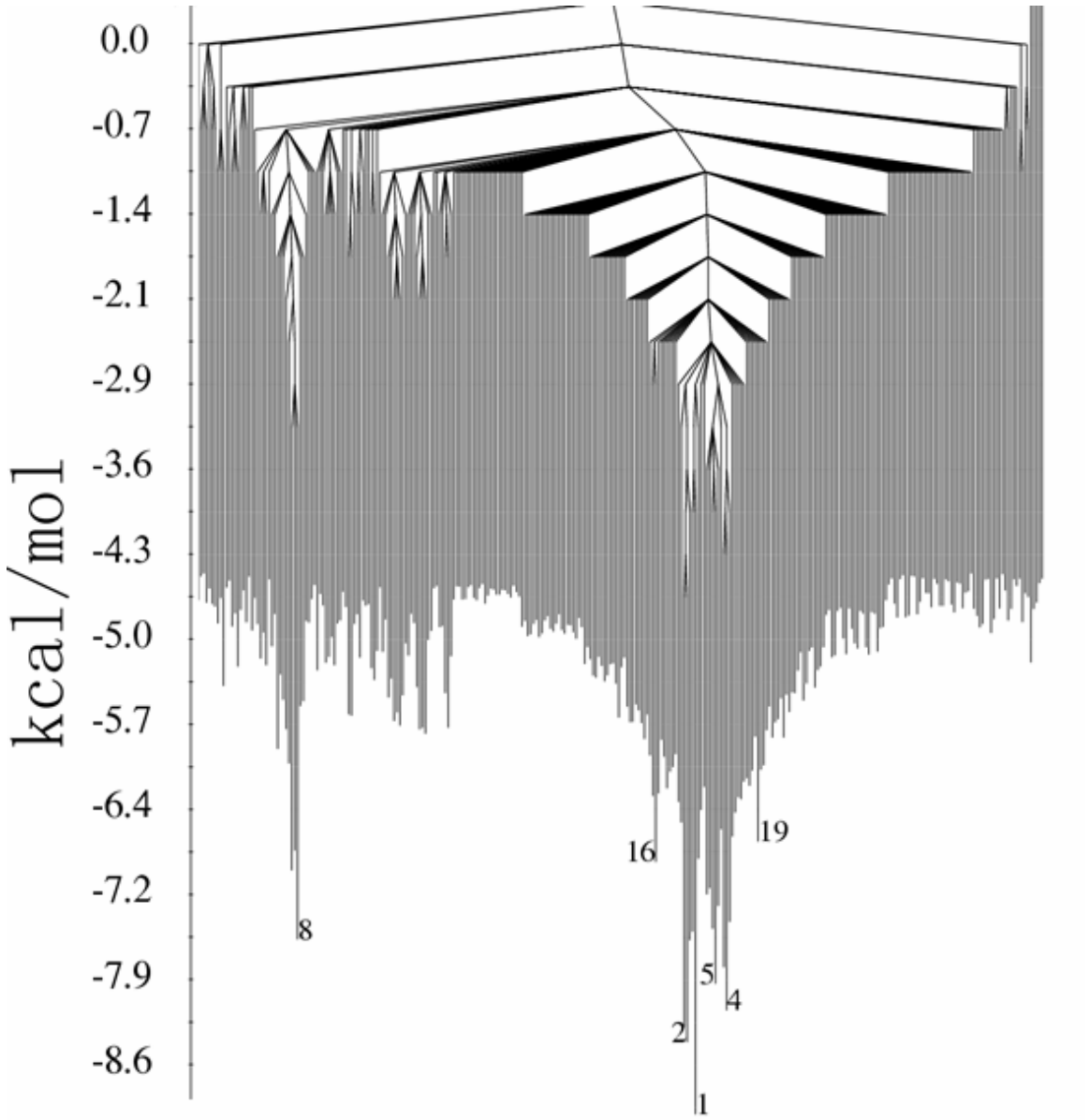

Fig. S2

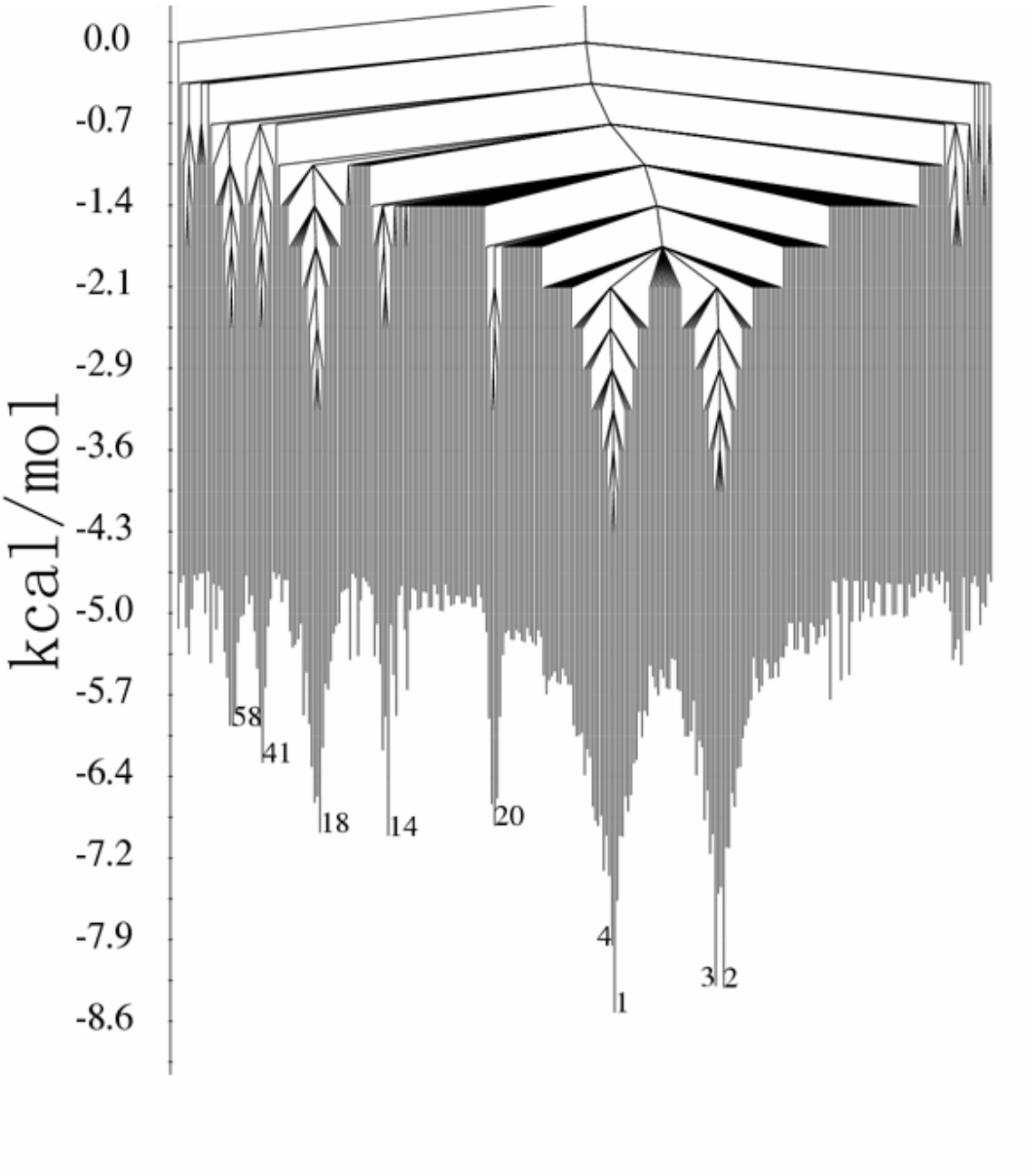

Fig. S3

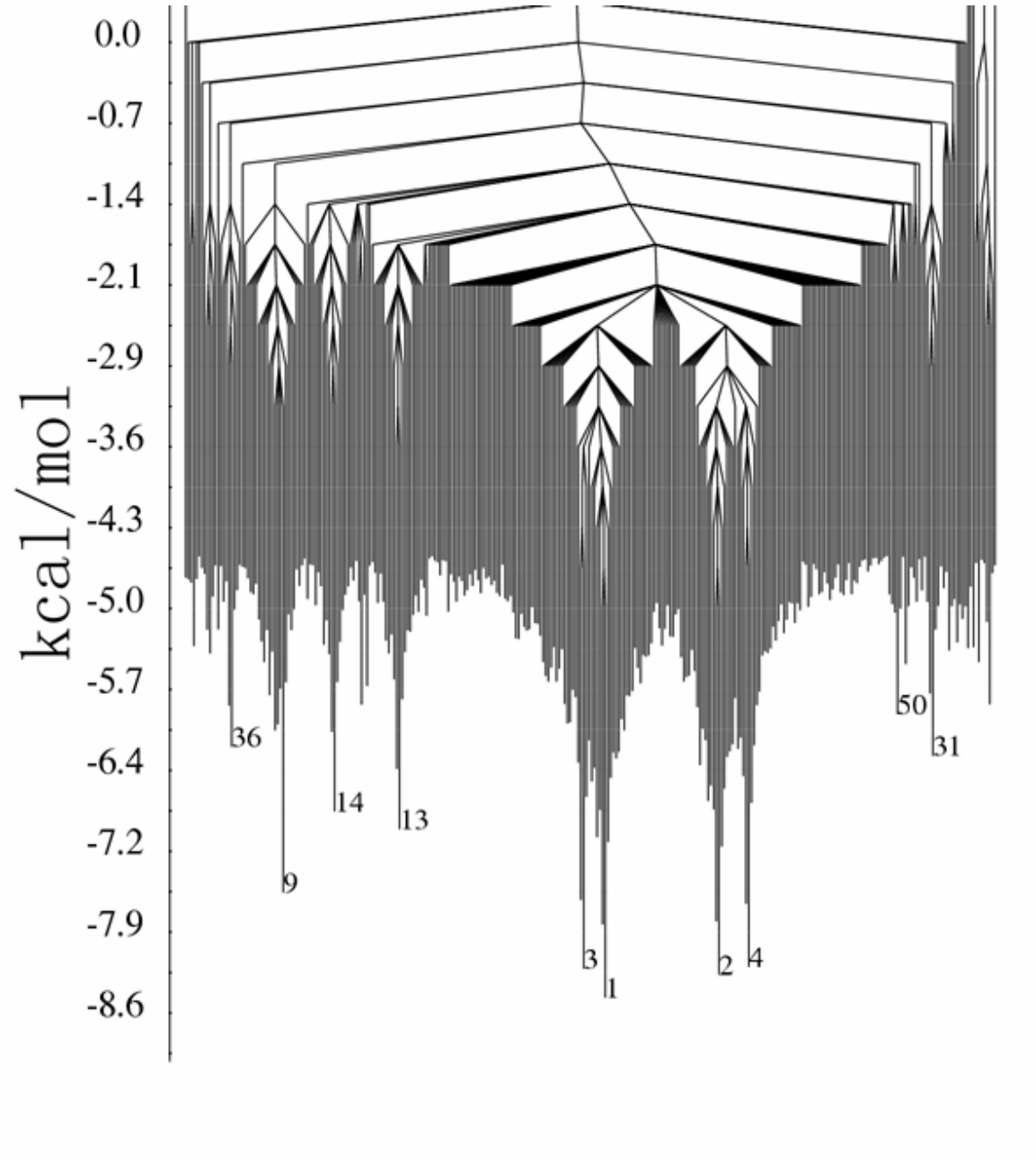

Fig. S4

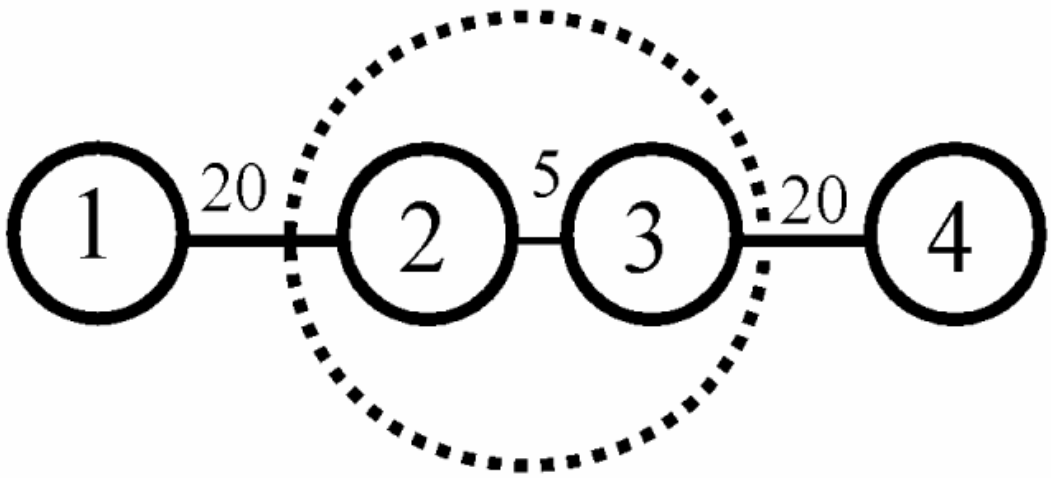

Fig. S5