Methyl-TROSY spectroscopy is a powerful technique that extends the solution-state NMR regime to probe the structures and conformational dynamics of large biomolecular systems (1, 2). However, the main drawback of the application of this technique is the difficulty of assigning the resonances to methyl groups in a given protein. Several different approaches have been proposed to tackle this problem. Traditional through-bond approaches, such as a combination of triple resonance and methyl out-and-back experiments (3), rely on back-bond assignments and suffer from low sensitivity. The brutal force method, using mutagenesis, is time-consuming, and the changes in chemical shifts due to mutagenesis can cause ambiguity when assigning the resonances (4). Recently, inspired by a method by Matthews and co-workers (5), we develop a new algorithm FLAMEnGO to assign methyl resonances by combining the crystal structure and sparse and ambiguous NMR restraints (6), which illuminates a new approach to assign methyl resonances.

A new version of FLAMEnGO is incorporated with a newer version of fuzzy logic and is able to make use of the TOCSY experiment. The new algorithm can tolerate missing experimental data, converge more quickly during sampling, and use spin systems obtained from TOCSY data to reduce sampling space. It is used to assign methyl groups in 40kDa protein kinase A (PKA). Using only 26% NOE, 78% PRE, and 98% TOCSY data, nearly complete methyl assignments (98%) are acquired without the need of amide assignments. After the assignments are subsequently confirmed by through-bond experiments, ~20% of methyl groups are mis-assigned, but above half of mis-assignments are simply due to the swap of assignments with nearby methyl groups. Other types of data, such as through-bond experiments and assignments from mutagenesis, can be used to further increase the accuracy of the resulting assignments. Moreover, the methyl assignments can also be used to accelerate the assignment processes of amide resonances, if through-bond experiments are available. This new algorithm, FLAMEnGO 2.0, can significantly resolve the difficulties generated by limited availability of resonance assignments, and make methyl-TROSY spectroscopy more practical.

References