**Automated NMR assignment of the N-terminal SH3 domain of Nck and Y111N mutant of Sticholysin II.**

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There is much interest in improving the efficiency of NMR structure determination methods. Within the general scheme for protein structure determination from NMR spectra, resonance assignment is the 'bottle-neck'. For this reason, it is necessary to accelerate the spectral assignment step of a NMR project. With such aim we have been using the program, GARANT[1](General Algorithm for Resonance AssignmeNT). In this program, sequence-specific assignments are the result of the optim match between a set of expected peak lists obtained from the amino acid sequence, and the equivalent set of experimental peak lists. The program relies on evolutionary algorithms to carry up the optimization process. The aim of this work is the automatic chemical shift assignment of Y111N mutant of Sticholysin II and N-terminal SH3 domain of Nck by means of GARANT.

Sticholysin II is a 175 amino acids protein belonging to the actinoporin family. Proteins from this family bind to the plasma membrane and oligomerize to produce functional pores which result in cell lysis[2]. Nck is a protein with multidomain architecture consisting of three consecutive SH3 domains and a C-terminal SH2 domain; biological function of Nck is to link cell surface receptors to the actin cytoskeleton[3]. This work is focus on N-terminal SH3 domain, that recognizes a proline-rich sequence contained in the cytoplasmic tail of CD3ε3, a subunit of the T cell receptor complex, that is crucial for T cell activation.

A set of 3D spectra was obtained for $^{13}C$ and $^{15}N$ doubly labeled proteins. Peak list were manually extracted from these spectra and then used to perform the automatic assignment. Partial chemical shifts assignments were obtained for both proteins. In the case of the mutant of Sticholysin II, the use of suplementary information from the wild-type protein structure clearly improves the number of final assignments obtained by GARANT. In sumary, we have been able to obtain around 70 % of the backbone assignment by using fully automated methods. The rest of the assignments must be done 'manually'. In conclusion, automatic methods, such as GARANT, do not resolve but facilitate the assigment of protein NMR spectra.

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