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Historical Perspective

A short recollection on the paper entitled "A common sense approach to peak picking in two-, three-, and four-dimensional spectra using automatic computer analysis of contour diagrams" by D.S. Garrett, R. Powers, A.M. Gronenborn, and G.M. Clore [J. Magn. Reson. 95 (1991) 214–220]

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ABSTRACT

The Contour Approach to Peak Picking was developed to aid in the analysis and interpretation and of multidimensional NMR spectra of large biomolecules. In essence, it comprises an interactive graphics software tool to computationally select resonance positions in heteronuclear, 3- and 4D spectra.

Interview with the author(s).

A video interview with the author(s) associated with this Historical Perspective and the original article can be found in the online version, at doi:10.1016/j.jmr.2011.08.009.

1. Introduction

When protein and nucleic acid NMR was confined to 2D spectroscopy, it was a relatively simple matter to analyze spectra on large paper plots (about 1 m × 1 m) using architects' illuminated drafting tables on which we would superimpose correlation and NOE spectra. The large size of the plots permitted one to easily discern small differences in chemical shifts, and the analysis of 2D NMR spectra by hand Fig. 1 was better than anything that could be done on a computer screen at the time. Then 3D NMR came along, and we recorded the first 3D spectrum of a protein in 1987 [1]. The spectrum was a homonuclear 3D NOE-HOHAHA, and it was clear that interpretation on paper plots was next to impossible. In addition, it turned out that this type of ^{1}H homonuclear 3D experiment was not only very difficult to analyze but also not particularly useful. The first 3D heteronuclear spectra of proteins from the NIH groups [2,3] were of the ^{15}N-separated variety, and analysis using stacks of paper plots for the limited number of planes (usually 64) in the ^{15}N dimension was still feasible. Indeed, when the data was converted to $^{1}H_N/^{15}N$ strips it was possible to condense all the information in a protein 3D NMR spectrum into three or four paper plots (2). However, analysis of 3D ^{13}C-separated spectra was far more complex, and strips had outlived their usefulness (since there are far more strips in a ^{13}C-separated 3D spectrum than in a ^{15}N-separated one). In addition, locating symmetry-related cross-peaks on paper plots was extremely time-consuming [4]. Finally, when 4D ^{15}N/^{13}C-separated [5] and ^{13}C/^{13}C-separated [6] NOE spectra became the norm, the spectral information was distributed over so many planes that analysis on paper plots was simply no longer an option.

In 1990, when Dan Garrett joined the Clore/Gronenborn group at NIH it had become evident that the development of interactive graphics software and peak-picking tools was absolutely essential for the efficient analysis of multidimensional NMR spectra of proteins. Fortunately, Dan had already been thinking along those lines and was very keen to get started.

The idea for Contour Approach to Peak Picking (CAPP) initially came to Dan when he was interviewing at Abbott for a position in the NMR support group that collected and analyzed 2D homonuclear spectra for the chemists. Since Dan had extensive programming experience, he discussed approaches to automate the analysis of the chemists' spectra. Abbott had automatic sample changers, automatic processing and plotting, but lacked an effective automatic peak-picking program. The spectra that were shown to Dan were almost all of high quality, with well-resolved peaks, easily above the noise level. Interestingly the Abbott NMR spectroscopists could not get an automatic peak-picking program to

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Fig. 1. AMG engrossed in NMR puzzle-solving in the late 1980s, Bld.2, NIDDK, NIH.

work well, and the algorithms that they described to Dan looked directly at the data matrix or derivatives of the data, but ignored the contours. Dan's initial idea, conceived at that time, was to base the automatic peak picking on the contour data by fitting the contours to circles.

Luckily for NIH, Dan decided to take a post-doc position there, instead of elsewhere. Since the triple resonance NMR spectra of $^{15}N/^{13}C$-labeled proteins also yielded excellent data, he wrote the program CAPP in which peaks are identified and discriminated from noise by fitting concentric contours to ellipses. The difference in linewidths in the hetero-atom and amide proton dimensions, required CAPP to use ellipses instead of circles which provided an excellent filter for distinguishing true signals from noise, since contours around noise fit better to a circle. This is the essence of the work described in the paper entitled "A common sense approach to peak picking in two-, three-, and four-dimensional spectra using automatic computer analysis of contour diagrams" by Garrett, Powers, Gronenborn and Clore, J. Magn. Reson. 95, 214–220 (1991). Moreover, this work also laid the foundation for the interactive graphics software package PIPP (**P**rimitive **I**nteractive **P**eak **P**icker) that was used exclusively for many years at the NIH and elsewhere for analyzing multidimensional NMR spectra. Indeed, PIPP allowed us to solve the structure of the 18 kDa protein interleukin-1β in 1991 [7,8], at that time a protein significantly larger than other systems tackled by NMR. Indeed, the reason that the original CAPP paper is so highly cited reflects the wide-spread use of the program PIPP in the protein NMR community as a whole.

A misunderstanding about what CAPP did led to a functional improvement in CAPP and PIPP in 1995. Ad Bax was under the impression that CAPP used the contours to determine peak-position. Dan explained to Ad that CAPP only used contours to distinguish real peaks from noise and artifacts and that, after a set of contours were considered real, CAPP fitted the top three points to a parabola to interpolate the peak position. Dan worked together with Andy Wang (now Andy LiWang) to incorporate Ad's ideas of using contours for precise peak positioning into CAPP which allowed Andy and Ad to accurately measure $^{3}J_{HN\alpha}$ and $^{3}J_{HNC'}$ couplings in $^{15}N/^{13}C$-labeled proteins for the purpose of re-parametrizing the Karplus equations for these ^{3}J couplings [9].

Recently, CAPP has been incorporated into the NMR analysis program Xipp that Dan has been working on as a replacement for PIPP. A beta version of Xipp is available at http://www.spin.niddk.nih.gov/clore/xipp.

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