NMR Spectral Fingerprinting of Biologic Therapeutics Using Interferograms: Exploiting Non-Uniform Sampling Without the Need for Spectral Reconstruction

F Delaglio, LW Arbogast, RG Brinson, Y Aubin, and JP Marino

1 Institute for Bioscience and Biotechnology Research, National Institute of Standards and Technology and the University of Maryland, 9600 Gudelsky Drive, Rockville, Maryland 20850, United States

2 Centre for Biologics Evaluation, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, K1A 0K9, Canada

Systematic collections of HSQC spectra are often analyzed in terms of the changes in corresponding peak positions, and possibly also changes in peak height or linewidth. A complementary approach is to apply Principal Component Analysis (PCA) directly to the matrix of spectral data, correlating spectra according to similarities and differences in their overall shapes, rather than according to parameters of individually identified peaks. This is particularly well-suited for spectra of mAbs, where individual peaks might not be resolved.

Characterization of biologic therapeutics for development, evaluation of equivalence, and manufacture requires monitoring their high order structure (HOS), since misfolding or aggregation can lead to loss of efficacy or cause unintended and potentially harmful immune responses. Two-dimensional (2D) heteronuclear NMR is a logical tool to probe HOS, since even small changes in chemical environment and structure give rise to readily observable changes in corresponding HSQC spectra. Furthermore, it has been demonstrated that combinations of fast measurement techniques combined with Non-Uniform Sampling (NUS) strategies can generate useful ¹³C HSQC spectra at natural abundance for molecules as large as intact monoclonal antibodies (mAbs, ~150kDa). This makes it possible to apply 2D spectral fingerprinting approaches directly to drug products in order to systematically characterize structure and excipient effects.

NUS schemes generally require a non-linear alternative to the Fourier transform in order to generate a spectrum, and several effective approaches for reconstructing NUS data have been demonstrated, including Multidimensional Matrix Decomposition, Maximum Entropy reconstruction, artifact subtraction methods, and Compressed Sensing methods. The Fourier transform is linear and reversible, so a Fourier transform NMR spectrum contains exactly the same information contained in the measured time-domain data, but reorganized in a way that is more convenient for visual evaluation and many types of analysis, in particular for identifying peaks. By contrast, while non-linear spectral methods will not add information, except perhaps in the form of assumptions about the underlying signals, they can reduce information or distort it. In many biomolecular applications, simply identifying the existence and the location of peaks is sufficient, and many applications are forgiving of small changes to lineshape or peak height. Also, while non-linear methods might reduce information content from a rigorous point of view, they can still produce a spectrum where peaks can be identified more effectively by eye or by a particular peak detection method, so in practice non-linear spectral processing methods are effective and important tools.

In the case of spectral fingerprinting by PCA, it is not a requirement to identify peaks, or even to have a spectrum with visually identifiable features, so long as the properties of interest are sufficiently captured by the measured data. This makes it possible to apply PCA spectral fingerprinting to 2D interferograms instead of spectra, since the interferograms encode more or less the same information. This also obviates the need for any special non-linear reconstruction methods if NUS or Random Phase Detection schemes are used. We demonstrate this PCA interferogram fingerprinting concept on HSQC spectral series, including ¹⁵N HSQC data for the protein therapeutic Filgrastim.


