The development of Fourier transform NMR in the mid 1960’s, did parallel processing of the collection of NMR data, increased the signal/noise ratio by two orders of magnitude and made it possible to record the proton NMR spectra of small proteins which contain hundreds of resonances. The assignment of these resonances then became a challenge, which was solved with the development of two-dimensional Fourier transform NMR, also called 2D NMR. This article describes the initial start and development of the various methods of 2D NMR leading to structure determination of proteins in solution and two Nobel Prizes.

The discovery of NMR, followed by the discovery of the chemical shifts and spin–spin couplings, had already created a revolution in its applications to the practice of chemistry. However, after the discovery and till the end of the 1960’s, NMR spectra were recorded by keeping the magnetic field fixed and sweeping the frequency or vice versa. In either case, it was a slow method (known as continuous wave (CW) method) and basically did ‘serial processing’. One-dimensional Fourier transfer NMR (it was not called ‘one-dimensional’, till much later) had created a revolution in the mid-1960’s by the publication of a paper by Ernst and Anderson¹, demonstrating an improvement in S/N of detection of NMR spectra by two orders of magnitude [1]. This was achieved by simultaneous excitation and simultaneous detection of all resonances as a function of time followed by a Fourier transform (parallel processing). The process is very nicely described by a cartoon by Ray Freeman where all the keys of a piano are excited simultaneously (Figure 1).

Another advantage of Fourier transform NMR is that it transformed the practice of NMR from direct-frequency domain to

¹ This paper, became a citation classic.
timedomain, allowing the application of multiple pulses and giving rise to the subject of ‘spin-gymnastics’. By the end of the 1960’s, NMR instrument manufacturers stopped manufacturing CW NMR spectrometers and switched to FT NMR spectrometers.

The above development threw up another challenge. It allowed recording of proton NMR spectra of small proteins (having up to 50–60 amino acids) yielding nearly 500 proton resonances (Figure 2).

It then became a challenge to assign each resonance to individual protons of the protein. Up to the mid 1970’s (for small peptides up to 5–6 residues having 50–60 proton resonances), NMR spectroscopists were practicing the art of selective perturbation (decoupling or saturation) and non-selective detection using FT NMR. The selected resonance would be irradiated by a second RF field which would then either decouple it from its J-coupled

![Figure 1. Cartoon from A Handbook of Nuclear Magnetic Resonance by Ray Freeman illustrating parallel processing.](image)

![Figure 2. Proton NMR spectrum of basic pancreatic trypsin inhibitor (BPTI) [2].](image)
partners, which could be identified in the changed spectrum or in case of saturation, change the intensity of ‘nearest-neighbours-in-space’ by nuclear–nuclear Overhauser effect (NOE). The changes were detected either by direct observation or by a difference between normal and perturbed spectrum, ‘difference spectroscopy’. One would then confirm the above changes by irradiating/saturating the perturbed resonance(s) and eventually one-by-one all the resonances. This then called for selective perturbation (serial processing) and non-selective observation (parallel processing). It now looks obvious what should have been done to do ‘two-dimensional Fourier transform NMR’ – non-selective perturbation and non-selective observation. Alas! The idea did not originate from the above thinking.

The idea of two-dimensional (2D) NMR originated from a completely non-utilitarian exercise given to students in a summer school in 1971 in Polje (former Yugoslavia) by a Belgian physicist, Jean Jeener [3]. He gave an exercise to students that “if you have a system of two weakly-coupled homonuclear spins (say an AX spin system) and applied two 90° pulses with a time interval $t_1$ and detected the signal after the second pulse as a function of a second time interval $t_2$, obtaining two-dimensional time domain data $s(t_1,t_2)$, subject it to two-dimensional Fourier transform and obtain a data set as a function of two frequency variables $F_1,F_2 \rightarrow S(F_1,F_2)$, what kind of spectrum will it be?” He basically wanted them to understand ‘coherence transform’ by the second pulse in a coupled spin system. Most of the students did not understand the exercise, except one Thomas Baumann – a PhD student of Ernst.

The tradition in Ernst’s laboratory in Zürich was that after attending a conference/school you had to give a group seminar on what you had learnt there. Thomas Baumann, a perfect Swiss student, had taken detailed notes, which he described in the group seminar. Most of the group people nodded their heads but did nothing else. However, this idea kept bugging Ernst. He was fascinated by the idea and wanted to understand and exploit this two-pulse experiment and two-dimensional Fourier transform and start
working in the field. However, he did not want to step-toe on Jeener, if he was continuing on this idea.

So, after a two-year wait, he used the idea in an experiment, far removed from Jeener’s two-pulse experiment, to do two-dimensional Fourier NMR imaging experiment. The story is as follows:

A year earlier, Paul Lauterbur had suggested imaging of small objects (two capillaries of $\text{H}_2\text{O}$ in a 5 mm NMR sample tube filled with $\text{D}_2\text{O}$) using linear gradients [5]. He had demonstrated that if you apply a linear gradient in some direction, one gets a spectrum giving projection of the distribution of two tubes perpendicular to the gradient. Several projections in different directions were obtained and projection reconstruction algorithm [6,7] (same as that used in a CT scan) would then yield the image of two tubes (Figure 3). Ernst came up with the idea that using only two orthogonal gradients, collection of data as a function of two time variables and a double Fourier transform would also yield the complete image and in much shorter time (Figure 4). This is like replacing serial processing by parallel processing. Collection of two-dimensional time domain data sets and processing by two-dimensional Fourier transform required writing the software ab-initio. The intensities were digitized into blanks, dots, star and a few alphabets. The teletype spitted a crude image of the two tubes of water (Figure 4) and 2D NMR found its first application into MRI. Ernst and his group, laughed at the experiment thinking that nothing will come out of it, and published it without patenting [3, 8]. Today, the two-dimensional Fourier transform NMR method is central to all medical MR imaging.

After this, Ernst launched a full-fledged study of applying the idea of two-dimensional FT to NMR spectroscopy. The first application was to the carbon-13 spectrum, of n-hexane. In one-dimension, it was coupled and in the other, decoupled (no second 90° pulse). The three-carbon site had only chemical shifts in one-dimension and had both chemical shift and J-coupling in the other [9]. He then launched a systematic study of hetero-nuclear and homo-nuclear...
2D spectroscopy. Simultaneous work was started in Ray Freeman’s laboratory at Oxford (UK) with PhD students, Ad Bax and Geoffrey Bodenhausen. Many experiments were developed and a quiet revolution was underway. Ernst wrote a detailed paper in the Journal of Chemical Physics [10]. This paper contains detailed information on 2D NMR including 2D line shapes, phase cycling to retain various coherence orders as well as indirect detection of forbidden multiple quantum coherences; the 2-pulse experiment of Jeener had been fully exploited.

Development of various 2D NMR techniques and their applications continued at feverish pace during the 1970’s, but applications to biomolecules were highly limited. Ernst and Wüthrich therefore, jointly obtained funding to “develop and apply 2D NMR to proteins”. The first person hired in this joint project was Kuniaki Nagayama. Nagayama wrote a massive computer program to apply 2D NMR to proteins. With this software, he made two developments. He developed ‘2D resolved spectroscopy’ of protons in a protein and with 45° rotation followed by a projection, demonstrated resolving the chemical shifts of all the 19 methyl protons of a protein. Limited computer memory also prompted him to develop ‘spin-echo correlated spectroscopy’ (SECSY), an experiment which contains coupling information but retains the chemical shifts only in one dimension [11]. He recorded the SECSY spectrum of a protein and demonstrated the applica-

3 This paper also became a citation classic.
tion of 2D NMR to proteins. However, SECSY was not very successful for assignments, since it had mixed-phase line-shapes and one had to calculate absolute intensities at the cost of resolution.

At this juncture (August 1979), Nagayama was leaving for Japan and I reached Zürich to work in the ‘joint’ project for one year. I was given a sheet of paper in which several projects were mentioned, including possible 2D experiment for NOE. I remember Ernst telling me that “2D-NMR was ‘cute’ but of not much use”. However, I found a preprint of Meier and Ernst’s JACS paper on the use of three 90° pulses with a mixing time for the study of chemical exchange [12]. I immediately recognized that since both chemical exchange and NOE take place with ‘non-equilibrium Z magnetization’, this was also the experiment for studying NOE. I launched a full-blooded effort to do this experiment in a protein, using and modifying Nagayama’s computer programme to put out three 90° pulses instead of only two. The details of the “troubles and triumphs” of this experiment are contained in an article in Magnetic Resonance in Chemistry titled ‘First NOESY’ [13]. The NOESY spectrum of BPTI, (Figure 5) has literally hundreds of cross-peaks, identifying proton-pairs near (less than 5 Å) each other, in the protein, thus containing distance information [15]. This information when fed to computer programmes
such as distance geometry algorithm, yielded the three-dimension structures of the proteins (Figure 6).

The 2 two-pulse (original Jeener sequence), now named ‘COSY’, yielded detailed information on the assignment of resonances. It was only a matter of time before the 2D-COSY and 2D-NOESY were used by everyone for obtaining 3-dimensional structures of their proteins in solution by NMR (Figure 6). With this development, 2D NMR had arrived and contour plots dominated the posters and talks in many Experimental NMR Conferences (ENC)’s during the next two decades.

Later many more developments took place. Three and four-dimensional FT NMR experiments were developed using N-15 and C-13 isotope labelling of the proteins. Another useful experiment was TROSY [15] which made it possible to study larger bio-molecules, which needed higher magnetic fields, leading to development of 800–1000 MHz NMR spectrometers.

Ernst received the Chemistry Nobel in 1991 for ‘development of one- and two-dimensional Fourier transform NMR’ and Wüthrich also the Chemistry Nobel in 2002 for ‘structure determination of biomolecules (proteins) in solution by NMR’.

Suggested Reading


