PROFILE

Profile of Marius Clore

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In a hangar-style space draped in dim blue light stand seven tall, cylindrical machines. The machines, NMR spectrometers, resemble wingless man-made satellites. Most of them rest at floor level, with a sample loading port reached by a small staircase, but a few protrude above the floor.

NMR spectrometers operate at the atomic level, and the size of a spectrometer reflects the strength of its superconducting magnets. By exploiting the magnetic fields of atomic nuclei and using high-pulse radio waves, NMR spectrometers help researchers like Marius Clore, a molecular biophysicist and structural biologist at the National Institute of Diabetes and Digestive and Kidney Diseases at the NIH in Bethesda, Maryland, determine the structure and dynamics of molecules such as proteins and nucleic acids.

For nearly four decades, Clore, elected to the National Academy of Sciences (NAS) in 2014, has studied the molecular structure and dynamics of biological macromolecules, and spearheaded the development and refinement of biomolecular NMR. Through work carried out in three countries on two continents, he has pushed the limits of NMR technology, laying the foundation for 3D and 4D NMR techniques and ushering in discoveries such as the high-resolution, 3D structure of the immune modulator interleukin-1β. However, Clore's path to NMR was not straightforward.

Marius Clore. Image courtesy of Marius Clore.

Science at Home

Clore grew up in the Chelsea area of central London during the 1960s. Along with his mother and father, a film producer who worked on the 1981 film *The French Lieutenant's Woman*, which starred actress Meryl Streep, Clore lived in a two-story apartment near the home field of Chelsea Football Club. Around the age of five years, Clore, who started attending the French Lycée school in London, already knew what he wanted to do when he grew up.

"I was a little bit of a hypochondriac," Clore says. "I decided very early on that I wanted to do medicine. I knew that by the time I was age five."

Clore stayed at the French Lycée school throughout grade school and, along with many of his school friends, continued to explore science. At the time, Clore says, pharmacists carried chemicals for purchase such as sulfuric acid, nitric acid, and potassium permanganate, leading Clore to do "a lot of chemistry at home, including nearly blowing the place up."

It also happened that Clore's mother was distantly acquainted with Peter Pauling, the son of Linus Pauling, who won the Nobel Prize in Chemistry in 1954 and Nobel Peace Prize in 1962. When Clore was around 12 years old, Peter Pauling, by then a chemistry professor at University College London (UCL), invited Clore and a friend to his laboratory, where Clore says Pauling "allowed us to take every piece of glassware, every chemical we could lay our hands on, and carry them home." In addition to finding another supply of science equipment, the UCL trip foreshadowed Clore's career at UCL.

Gaining Structure

Clore entered UCL and University College Hospital (UCH) Medical School in 1973. After two years of preclinical study, he obtained a biochemistry degree and graduated with first-class honors in 1976. Three years later, he earned a medical degree from UCH Medical School. In 1980, after completing a year of medical residency, Clore took up an independent principal investigator position at the Medical Research Council's National Institute of Medical Research (NIMR) in Mill Hill, London.

During Clore's last year of medical school, the NIMR's director, Sir Arnold Burgen, an early proponent of using NMR in molecular pharmacology, recruited Clore to the NIMR. Following a handwritten letter sent by Clore, Burgen took an interest in Clore's kinetics work and asked Clore to interview at the NIMR. At the time, Clore was primarily interested in studying the flow of electrons in the respiratory pathway using spectroscopic and kinetic techniques and had no plans to make a career out of NMR (1). The NIMR did not have the

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necessary equipment for Clore's kinetics studies, however, and Burgen encouraged him to work on NMR, a still-burgeoning field.

Clore accepted the job and settled into a small office that he shared with structural biologist Angela Gronenborn, now at the University of Pittsburgh School of Medicine, and set to work on NMR. Although new to Clore, NMR was not too difficult because his mathematics background gave him a leg up. "I could figure out a whole bunch of things very quickly, especially since many of the processes boil down to the same sort of differential equations used in kinetics," Clore says.

Today, the most powerful NMR spectrometer in the world is a 1-GHz spectrometer at the CNRS in Lyons, France. However, in 1980, the NMR machine Clore used was the NIMR's 270-MHz spectrometer, which could only run experiments in one dimension; the development of 3D and 4D NMR, to which Clore contributed, was nearly a decade away.

Clore used NMR to investigate the cAMP receptor protein (CRP), which is involved in cellular signaling. Using NMR, he examined ligands bound to CRP, and gained insight on ligand–protein binding states during the transferred nuclear Overhauser effect (TRNOE), an effect related to the nuclear spin of protons (2). In subsequent experiments, Clore used the TRNOE to study how small molecules such as peptides, nucleotides, and single-stranded DNA bind to proteins.

Clore's stay at the NIMR lasted just over four years. Although the NIMR acquired a 500-MHz spectrometer shortly before Clore's departure, the Max Planck Institute of Biochemistry in Martinsried, Germany, had made Clore an offer that he says was "impossible to refuse."

Making Moves

Clore, along with Gronenborn, was chosen to head the Max Planck Institute of Biochemistry's new NMR department, which came equipped with a 500-MHz spectrometer. Over a few days, Clore and Gronenborn moved their belongings to Germany in just one car. "We didn't have much to move then," Clore remarks.

At the Max Planck Institute of Biochemistry, Clore laid the foundations for the NMR technology that he would later use to determine the structure of large proteins and protein–DNA complexes. At first, Clore picked up where he left off at the NIMR and continued work on DNA, collaborating with structural biologist Axel Brünger in determining the 3D structure of a DNA duplex using NMR. The research resulted in the development of restrained molecular dynamics protocols for NMR-based protein structure determination, as well as simulated annealing methods for the determination of 3D structures of nucleic acids and small peptides from short interproton distances.

Clore then applied the concepts in subsequent NMR experiments aimed at solving the structure of α1-purothionin. The α1-purothion protein consists of just 45 residues, or amino acids, making it a small protein. After 3 weeks, Clore et al. (3) determined α1-purothionin's structure.

The structural determination of α1-purothion was only the second example of the uncovering of a protein's structure by NMR without incorporating a preexisting model for the protein, and the work led to the determination of structures of other small proteins. For proteins larger than 100 residues, however, Clore recognized that the spectral resolution provided by 2D NMR would not be enough, and set out to develop 3D NMR further, research that enabled the recording of a 3D NMR spectrum of a protein and the demonstration of 3D NMR's ability to increase spectral resolution (4). "At the Max Plank, we started developing protein structure determination properly," Clore says.

Clore's experience at the Max Plank Institute of Biochemistry would serve him well in his next role at the NIH, to which NIH structural biologist William Eaton had recently recruited him.

Pushing the Technology

When Clore arrived at the NIH in 1988, he was principally interested in developing and refining NMR technology for studying proteins. Over the next few years, Clore, along with structural biologist Ad Bax, turned his attention to the development of 3D and 4D NMR.

Higher dimensional NMR affords researchers the ability to study the increasingly complicated structures of larger molecules such as those in the 150- to 300-residue range because the higher dimensions eliminate much of the spectral signal overlap that would otherwise obscure the NMR results. In a review article on the development of 3D and 4D NMR that Clore coauthored in *Science*, he used the analogy of an encyclopedia to represent the spectral dimensionality of NMR (5). In one dimension, words and sentences form a single, superimposed line that becomes a single page in two dimensions, spreading out the information and making it increasingly comprehensible. Three dimensions further separate the text onto different pages, but it is only in four dimensions, comparable to a multivolume book, that the information becomes entirely clear. According to the researchers, dimensionality in NMR presents itself in a similar way, with higher dimensions allowing for clearer spectral readings for the study of complex structures.

Clore played a major role in developing 3D and 4D NMR, and his early work included the 3D structural determination of interleukin-1β, an immune modulator implicated in the human immune and inflammatory responses (6). Following interleukin-1β, Clore went on to solve the structures of other immune modulators as well as calmodulin–peptide and protein–DNA complexes (7, 8).

The Next Pulse

Today, Clore, an NIH Distinguished Investigator and Chief of the National Institute of Diabetes and Digestive and Kidney Diseases' Protein NMR section, shares building space with eight other NAS members. Clore's inaugural article describes the use of NMR to investigate how the chaperone molecule, GroEL, influences protein folding activity, which Clore found

was mediated by various hydrophobic interactions of GroEL (9).

Recently, Clore has also focused on interactions between proteins as well as on sparsely populated protein states, which are highly transient protein states involved in various biological processes. For instance, the bacterial phosphotransferase system, which uses biomolecular protein–protein interactions to control the transport of sugars across the bacterial membrane, has provided Clore a platform with which to study how signal transduction proteins recognize structurally dissimilar proteins (10). In addition, his laboratory's role in developing NMR technology for detecting sparsely populated protein states has provided new insights into the biochemistry and interactions of biological macromolecules, and earned him the 2013 Biochemical Society Centenary Award (11).

Although Clore has been involved in biomolecular NMR since its infancy and contributed to its evolution, the versatility of NMR and advancements in the technology keep him intrigued. Like his longtime hobby of cave diving, the unknown drives him forward.

"One of the things about NMR that never ceases to amaze or excite one is that just as one thinks the field has basically reached the end of what it's capable of, something new comes along that produces a quantum jump in the capabilities of the method and what it can do," Clore says. "As I often tell people, if I knew what I was going to be working on in, say, three to five years' time, I'd already be working on it now."

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