Part One
Introduction

NMR of Biomolecules: Towards Mechanistic Systems Biology, First Edition. Edited by Ivano Bertini, Kathleen S. McGreevy, and Giacomo Parigi.

1
NMR and its Place in Mechanistic Systems Biology

Ivano Bertini, Kathleen S. McGreevy, and Giacomo Parigi

NMR can be used to solve the structure of biomolecules and to show how they interact. Interactions among biomolecules and between biomolecules and small ligands can be studied from the thermodynamic, structural, and kinetic points of view. Through metabolomic studies, NMR can monitor the whole metabolic process. This provides a way of understanding the mechanisms of life at the molecular level and of modeling them. We can refer to these achievements as the mechanistic contribution to Systems Biology or "Mechanistic Systems Biology".

As we begin our exploration of the possibilities afforded by the application of NMR to the study of biomolecules and the resulting contribution to Mechanistic Systems Biology, we should first try to define the term "Systems Biology" itself. This is an old designation that has traditionally referred to the classical biology of a system, but which acquires a new meaning as more and more biological pathways (i.e., those series of molecular interactions in a cell that lead to certain products or changes) are discovered [1–3]. These pathways cross and intersect and, as a result, the over-expression, under-expression, or inhibition of an individual gene product leads to consequences in all of the pathways in which that product is involved. A full understanding of the biology of a system therefore requires knowledge of all of the pathways that compose that system.

We should then try to understand what the latter phrase really means. What constitutes knowledge of a pathway? In many bioengineering departments throughout the Anglo-Saxon world, it is a knowledge of kinetic parameters – how fast the reactions in a given pathway will proceed – that allows processes and alterations to be mathematically simulated [4,5]. In fact, one understanding of Systems Biology is the computational simulation of biological processes. However, while living organisms are not at the thermodynamic equilibrium, a knowledge of the thermodynamic parameters – the relative stabilities of the reactants and products – involved is still of primary importance. NMR can provide both kinetic and thermodynamic parameters, or at least their upper and lower limits, but most importantly, at present, NMR can provide structural information on all of the biomolecules in an interacting group in addition to a structural characterization of the interaction itself. The progression of biochemical pathways in terms of the mechanisms of the interactions along paths can therefore be understood at the atomic level; it is from this that the name "Mechanistic Systems Biology" originates.

As it happened, we became aware of this term in 2008 when Antonio Rosato and Ivano Bertini were explaining their contribution to Systems Biology from a structural point of view to Shankar Subramaniam, who was the Director of the Biomedical Engineering Department at the University of San Diego; he informed them that this is, in fact, called "Mechanistic Systems Biology."

NMR of Biomolecules: Towards Mechanistic Systems Biology, First Edition. Edited by Ivano Bertini, Kathleen S. McGreevy, and Giacomo Parigi.

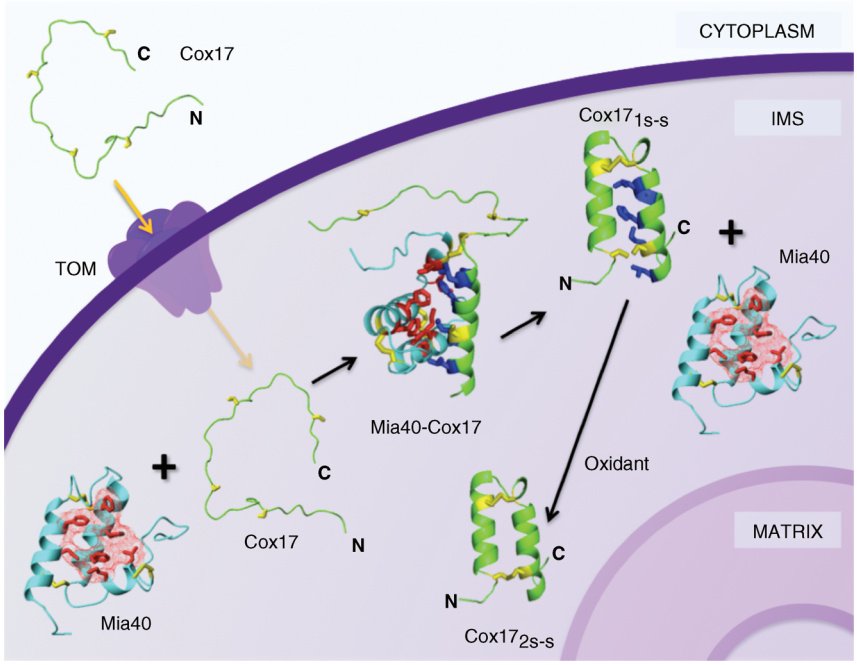

Fig. 1.1 Detection of the conformations experienced by proteins in different conditions and of their adducts allows the reconstruction of the mechanisms occurring in cells. As an example, we show here the folding process of the mitochondrial protein Cox17 induced by Mia40 [7]. Cox17 is a fully unfolded protein in cytoplasm, so that it has the conformational plasticity to enter the intermembrane space of mitochondria through a translocase of the outer membrane channel. A transient complex is formed here with the Mia40 protein, thanks to the interaction of the hydrophobic residues of Mia40 (in red) and Cox17 (in blue). Mia40 induces the formation of an α-helix on a specific region of Cox17 and of an intermolecular disulfide bond (Cys residues are shown in yellow). The next step consists of switching of the intermolecular disulfide bond to the first intramolecular disulfide bond within Cox17, so that Mia40 is released. Finally, the formation of the second α-helix is induced by the interactions of its hydrophobic residues with the first helix and the second intramolecular disulfide bond is formed. Once the two disulfide bonds are formed, the protein, now folded, is trapped in the intermembrane space.

Mechanistic Systems Biology has the potential to become an important field within biology. With the term "mechanistic," we can imagine a play whose plot is represented by a biochemical pathway. From our seats in the audience, we can see the three-dimensional structures of the biomolecular actors as they interact with one another in various scenes (Figure 1.1). In real terms, we can achieve an atomic-level, three-dimensional view of proteins interacting with one another or with DNA/RNA to achieve a biochemical result. Observing three-dimensional structures allows one to better understand the biomolecule, and provides a further means to intervene and monitor its function. The goals and challenges of Systems Biology remain the same. Mechanistic Systems Biology will provide the basis for molecular pharmacology, where drugs can be described as inhibitors or activators of a pathway and the side-effects due to the consequences on other pathways can be predicted.

NMR has a primary role within this framework. It can provide a biomolecule's structure and can monitor its interactions in a mechanistic frame. Both thermodynamic and kinetic information on the interactions between biomolecules can be obtained, thus providing a powerful tool for the modeling of the system. NMR is especially important when the interactions are weak and the interacting species are in fast exchange. The interacting molecules can be observed and it is often possible to intervene in the interaction. Furthermore, NMR is not yet a mature science – it must still develop in the field of membrane proteins and immobilized (though not necessarily crystalline) forms, and must extend its investigative power to larger biomolecules.

NMR is a technique capable of monitoring single nuclei. The initial information comes from the chemical shift – the resonance frequency when the substance and the nucleus are in a magnetic field. The chemical shift tells us about the surroundings of the atom whose nucleus is observed. Typically, in a protein, the chemical shift of a ^{13}C backbone nucleus provides information on its secondary structure (see Chapter 2.1). A closer look at the spectra tells us which other atoms/nuclei are bound through chemical bonds. Another type of experiment provides information on the distances of nearby nuclei. All these pieces of information may provide a structural model through dedicated computer software. Another nuclear parameter that should be mentioned is the rate of return to equilibrium of an ensemble of nuclear spins after a perturbation with a radiofrequency pulse. These rates provide precious information on the mobility of atoms.

Magnetic nuclei are needed to perform NMR experiments; if an atom has multiple isotopes, the one that provides the sharpest signal is preferred. The naturally most abundant ^{12}C has no magnetic moment and is silent in NMR experiments. ^{14}N is magnetic, but not good, while ^{15}N is. Nowadays procedures are well-developed to produce biomolecules that are 100% labeled with ^{13}C and ^{15}N. ^{1}H is a good nucleus, as is ^{31}P.

Structural and dynamic information represents the strength of NMR: given a biomolecule, the average structure in solution can be obtained, dynamics of nuclei can be monitored, and conformational equilibria can be followed. Unfortunately, there is a limit – the size. The larger the protein, the more crowded and less informative the spectrum. The NMR lines are also broader. More intense magnetic fields provide better resolution, which is why there is a drive to build higher and higher magnetic fields, from the 2.2 T of 1970 up to 22 T in 2010 with the prospect of 25 T in 2015. In parallel, probe technology has also been developed up to the modern cryoprobes able to increase the signal-to-noise by a factor of 4 with respect to the traditional, noncryogenically cooled probes. Proteins or domains up to 35 kDa can currently be determined routinely, with 70 kDa as an upper limit [6]. Partial information can be obtained up to 1 MDa.

It is not only the developments in NMR instrumentation that are advancing the frontiers of NMR investigations in the Life Sciences. Simultaneous advances in labeling strategies make increasingly complex experiments feasible, thus improving the possibilities of understanding the structural and dynamic features of biosystems. The development of new methodologies such as in-cell NMR, which is attracting a significant amount of attention due to its unique role in providing information directly in cells, cell-free expression, and advances in solid-state NMR applied to biomolecules, allowing the study of immobilized proteins such as membrane proteins and fibrils, provide plenty of new opportunities. NMR is now tackling the Systems Biology approach with a new technology called metabolomics that provides information on metabolites in biological fluids, tissues, and bacterial cultures, thus providing an independent source of data for Systems Biology itself. Furthermore, the information technology approach, in our view, is closely bound to the development of NMR; software programs for the analysis of the NMR data are continuously upgraded and interconnected to keep pace with the methodological advances and for satisfying the demand for fast (and accurate) analysis of increasing amounts of data. The contribution of NMR to Mechanistic Systems Biology is thus foreseen as being of increasing importance.