Analytical techniques – from proteome analysis to mechanical phenotypes of multiprotein assemblies

Editorial overview

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The development of physical methods, as well as progress in producing samples that are adapted to these physical methods, is the motor in analytical techniques for life sciences. These methods enable the investigation of basic phenomena and allow one to address a series of important questions. Which proteins are in a given cell and in what quantity? What are the structures of the proteins and with what are they interacting? How do they adopt their structures by folding? How do these structures change when the proteins exert their function and what forces can they produce in doing so? The answer to these questions is essential for the quantitative analysis of the proteome, for structural genomics and for the elucidation of enzyme mechanisms and the function of molecular machines. These questions are addressed in the following six reviews.

Among the most basic analytical information concerning a cell is the quantities of compounds in a given compartment and the constitution of these compounds. The task is formidable for cells where the compounds are proteins that are expressed over several magnitudes of dynamical range and that have very different molecular masses and properties. Steven P Gygi and Ruedi Abersold (pp 489–494) review the most recent techniques for identifying proteins by mass spectrometric analysis. A special thrust of the review lies in methodology for examining large collections of proteins and multiprotein complexes without the need for separating them on two-dimensional gels. Progress in analyzing proteins with extremely low solubility or extreme isoelectric points is also described. Quantitative proteome analysis is a special focus of the review and is obtained by isotope-labeling techniques and by chemically modifying the proteins based on the presence of cysteine. Progress is described for identifying the components of multiprotein assemblies and detecting protein–protein interactions.

Proteome analysis and structural genomics are tools that help us to understand the function of cells and develop new drugs. For structural genomics, fast methods are required to solve new structures. In the review by Steven E Ealick (pp 495–499), the rise of multiple wavelength anomalous diffraction (MAD) crystallography from an esoteric technique to the method of choice for the structure elucidation of macromolecules is presented. In 1999, 100 new protein structures were solved with MAD. This has been facilitated by new beamlines, better X-ray detectors, improved programs for data handling and, last but not least, improved sample preparation techniques. For proteins, selenomethionine is the most frequently used amino acid to introduce anomalously diffracting atoms. This can be done in bacteria but also in higher cells using a baculovirus. Other ways of incorporating heavy atoms for MAD are also reviewed.

There are whole classes of protein structures that are very difficult to solve by X-ray crystallography. Some of these proteins can be attacked by solid-state NMR. Amyloid-forming peptides and proteins that are associated with ‘misfolding’ diseases such as Alzheimer’s or Creutzfeldt–Jakob’s are of special interest in Robert Tycko’s review (pp 500–506), which summarizes the available information about the β-amyloid peptide from NMR and other methods. Selective labeling of the peptides with stable isotopes enables the measurement of interatomic distances as well as torsional angles to define the conformation of the peptide in the amorphous fibrils. In addition, multiple quantum experiments provide information about the topology of the peptide strands. For the β-amyloid peptide, a parallel assembly of the peptide strands was found that has disproved quite a number of three-dimensional models of the fibres.

Misfolding diseases leading to amyloidal fibrils clearly imply that one protein can exist in at least two conformations, namely one that is soluble and another that is not. David MJ Lilley and Timothy J Wilson (pp 507–517) review fluorescence resonance energy transfer (FRET), which allows the study of biomacromolecular conformations and their changes with time resolution and on single molecules. The review focusses on oligonucleotides, for which FRET has been instrumental in the elucidation of the global structure of four-way and three-way junctions as well as ribozymes (most prominently the hammerhead ribozyme), conformational transitions from B- to Z-DNA and protein-induced winding and unwinding as well as bending of DNA. Measurements ranging to distances of over 60 Å between chromophores are possible. These long distance restraints are rather uniquely obtained from this method. The precision and accuracy of these measurements is discussed. FRET is a single-molecule method, a feature that it shares with the methods

reviewed in the final two articles. Single-molecule FRET has been used for time-resolved folding of RNA and interactions of proteins with DNA.

Another most successful method that can be applied in a time resolved way and to minute quantities of molecules is reviewed by Reiner Vogel and Friedrich Siebert (pp 518–523). Of the different types of vibrational spectroscopy, they focuses mainly on Raman, resonance Raman and Fourier transform infrared (FTIR), which allow the elucidation of enzyme mechanisms with special impact when the structure of the protein is known and when strongly absorbing chromophors are present in the protein. Technical improvements have increased the sensitivity of the measurement to allow, for example, the study of bacterial and plant photosystems I and II. With progress in techniques for isotopic labelling and point mutations, a better interpretation of the data is possible. The time resolution can be driven down to a couple of picoseconds. One striking development is the possibility of obtaining structural information much along the lines of NMR because of dipolar coupling of vibrational modes through space. Important developments for the interpretation of vibrational spectroscopy come also from quantum chemical calculations when combined with classical molecular dynamics. Caged compounds make it possible to trigger reactions if the reaction cannot be triggered by light directly. This has been used for signal transduction in membrane proteins.

The final review dwells on a method that also enables the investigation of energy profiles. The review by Hermann E Gaub and co-workers (pp 524–530) discusses force spectroscopy of single biomolecules, which allows mechanical profiles to be obtained over the range from a few piconewtons to several nanonewtons (using devices that have been developed by physicists over the past decade). Force spectroscopy allows the determination of the mechanical phenotype of biomolecules. This is the force/path diagram that bears immediate significance for biomolecules involved in motors, such as titin or myosin. This important review covers cell–cell adhesion, unfolding of proteins, strain-induced transitions of DNA from the familiar B form to the strain-induced form.

All the reviews describe important developments in analytical fields. Their successful application to the life sciences requires an interdisciplinary approach from cell biologists, biochemists, chemists and physicists. The papers review progress in several fast-developing fields that promise to assist us in our understanding of cellular function at the atomic level.