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EDITORIAL

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Proteins: from chemical to physiological mechanism

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A special one-day meeting of the Molecular Enzymology group of the Biochemical Society was held in October 2001 at the Royal Society, London, to celebrate the 80th birthday of Professor H. (Freddie) Gutfreund. The aim of the meeting was to stimulate discussion on kinetic approaches applied to the life sciences. The meeting brought together work on the dynamic aspects of protein function, from studies of detailed chemical mechanism to molecular structures and how such studies inform thinking about physiology. The underlying theme was therefore the time scale on which proteins sense and respond to their environment, how such behaviour can be studied and how the time scales of protein activity relate to those of physiological responses.

Professor Gutfreund has made many contributions to the study of both biochemistry and biophysics. His early prominence came from developing the methodology to study fast events on enzymes and this led him on to detailed mechanistic studies that now are part of classical enzymology. His career began in physical chemistry and moved on from studies of the physical properties of proteins to mechanisms of enzyme catalysis. In particular, he identified the importance of ligand-induced changes in protein conformation for defining the biological function of proteins. Seen in hindsight, this appears like a natural progression from physicochemical mechanisms to ligand-induced conformational changes in proteins to their role in mediating the response of an organism to its environment. His recent interests have centred on signal and energy transduction, particularly the mechanism of mammalian rhodopsin and its associated heterotrimeric G-protein signalling system, monomeric G-proteins and ATP-dependent myosin motors.

Professor Gutfreund's contributions to methodology in transient and relaxation approaches for the study of enzymes are legendary. These include stopped-flow calorimetry and fluorimeters, the use of rapid pressure perturbations for probing protein-protein and protein-ligand interactions and, recently, a return to continuous flow methods. Relaxation methods are ideally suited to the study of complex systems since rapid changes of temperature or pressure can be readily applied to cellular systems. Such studies in the 1980s were used by him to monitor protein isomerizations of bovine rhodopsin in vesicles or whole rod outer segments and this was followed by the application of pressure and temperature relaxation on isolated actomyosin and contracting muscle fibres.

Professor Gutfreund has always striven to encourage young scientists to attempt challenging projects and has fought for long-term support that such research requires. An example of the complex technical background required for molecular approaches to physiological problems is illustrated by the records shown in Fig. 1 from a research programme that sees as its roots the inspiration afforded by his leadership of the Molecular Enzymology Laboratory in the Biochemistry Department at Bristol University for 25 years. The experiment done in a project investigating muscle energetics used laser flash photolysis to liberate ATP from ''caged'' ATP in single fibres. The force generated by the fibre was monitored simultaneously with the amount of P_i liberated from ATP hydrolysis through use of a P_i sensor. The sensor is a fluorescently labelled phosphate-binding protein that sequesters phosphate rapidly with micromolar affinity and with a concomitant fluorescence change. Such a study demonstrates elegantly how the rate of ATP hydrolysis (as measured by release of P_i into the medium) is accelerated as soon as the fibre is allowed to shorten. The experiment illustrates the multidisciplinary

Dedicated to Professor H. Gutfreund on the occasion of his 80th birthday

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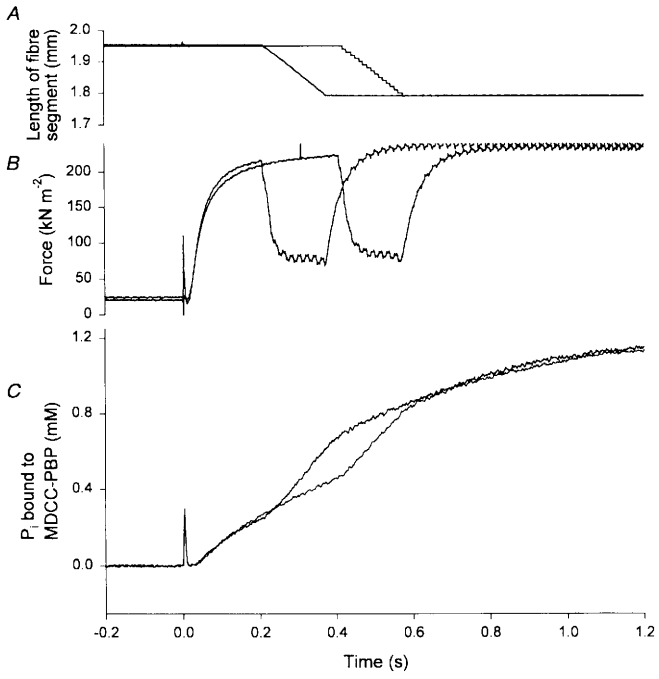

Fig. 1. Simultaneous measurement of length (**A**), force (**B**) and phosphate release (**C**) in a single skinned muscle fibre, illustrating the acceleration of P_i release rate during shortening. A permeabalized muscle fibre was mounted between two hooks, one attached to a length-adjusting motor and the other to a force transducer. The figure shows two consecutive measurements on a single fibre. The fibre was initially at rest length in a rigor solution (no ATP). At zero time a contraction was initiated by the release into a muscle fibre of ~1.5 mM ATP by laser photolysis of caged ATP. **A** The length of the fibre controlled by the motor. At either 0.2 or 0.4 s the fibre was allowed to shorten by 8% of its length. **B** Force measurements: at approx. 0.2 s the fibre, which was prevented from shortening, reaches is maximum level of force development. **C** The amount of phosphate bound to the P_i-sensor incubated with the muscle fibre. The trace shows clearly that the rate of P_i liberation was increased during shortening [from fig. 5 of He et al. (1999) J Physiol 517:839–854 with permission of the publishers]

approach required for this type of study: synthesis of the caged ATP and the fluorescent label, the molecular biology to design, express and label the phosphate-binding protein, and the photochemistry and optics required to release caged ATP and monitor the fluorescence signal in a single muscle fibre on the millisecond time scale. All of these technologies are allied to the classical physiological techniques of controlling the length of a 3 mm long muscle fibre and measuring its tension.

The culmination of a lifetime thinking about proteins and working with proteins was brought together in Professor Gutfreund's most recent book "Kinetics for the Life Sciences" (his fourth influential textbook on the enzymology and thermodynamics of proteins). This seminal work illustrates common conceptual approaches to the study of dynamic phenomena from the level of purified individual proteins to whole cells, organisms and populations. As such it forms a fitting climax to a career which began on the firm foundation of the physicochemical studies of proteins and gradually embraced more and more of the biological processes which proteins direct.

In the following pages are four reports of some of the work presented at the meeting and which illustrate the diversity of ways to probe biological mechanisms so inspired by Professor Gutfreund.

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