Main research areas

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Research interests

- Biophysical chemistry
- Biological membranes and membrane proteins
- Fluorescence spectroscopy
- Rapid reaction kinetics
- Regulation and mechanism of ion transport across biological membranes
- Voltage-sensitive fluorescent dyes
- Na+,K+-ATPase
- Membrane electrical properties

Ion-transporting Membrane Proteins: Ion-transporting membrane proteins play a decisive role in the metabolism of all cells and in numerous physiological processes, e.g., ATP production, nerve impulse propagation, and muscle contraction. A deeper understanding of their mechanisms and regulation can be obtained by the determination of the kinetics of their individual reaction steps. One approach to this research goal is to make use of the electrical current they generate across the cell membrane and to apply electrophysiological methods, such as the patch-clamp technique. Another is to convert the electrical voltage that the proteins produce into an optical signal by incorporating a voltage-sensitive dye into the membrane. Voltage-sensitive styrylpyridinium dyes allow a rapid detection (within nanoseconds) of local changes in electrical field strength within membranes. The great advantage of the dyes is that they allow one to investigate electrogenic reaction steps of membrane proteins in open membrane fragments, i.e. conditions under which electrophysiological methods are not applicable.

Na^+,K^+-ATPase: The Na^+,K^+-ATPase is the enzyme responsible for maintaining the physiological essential Na^+ and K^+ concentration gradients across the plasma membrane of all animal cells. Jens-Christian Skou (University of Aarhus, Denmark) was awarded the Nobel Prize for Chemistry for its discovery in 1997. Using the voltage-sensitive probe RH421 in conjunction with the fluorescence stopped-flow method we have investigated in detail the enzyme's kinetics and mechanism. This has allowed us to determine rate constants for most of the steps of the enzyme's reaction cycle and to locate the rate-determining steps. The reaction cycle of the Na^+,K^+-ATPase is universally described in biology and chemistry textbooks by the Albers-Post model, which represents the catalytic unit of the enzyme as a monomer undergoing a sequence of ion-binding, conformational changes and ATP phosphorylation/dephosphorylation steps. Recently we have discovered that this description is inadequate and, based on our experimental results, have proposed a new model, in which the enzyme exists in dimeric form with two gears of ion pumping depending on the number of ATP molecules bound. We are currently investigating further the mechanism by which the two gears of pumping come about.

Membrane Dipole Potential and Orientational Polarisability: Ion-transporting membrane proteins, such as the Na^+,K^+-ATPase, are very sensitive to the composition

of their lipid surroundings. The mechanisms by which lipids and membrane proteins interact are still unclear. One possibility which we are investigating is via an electrostatic interaction between charged or dipolar groups on both the lipid and the protein. Within the head-group region of phospholipid membranes there exists an electrical potential (the dipole potential) of ca. 200-400 mV, positive in the membrane interior. This produces a very large electric field strength of around 10_9 V m-1 within the membrane, which could potentially influence the energy of intermediate states in the pumping cycle of ion-transporting membrane proteins, thus changing the activation energies of steps of the cycle and hence their rate constants. However, another important consideration is the degree to which charged groups of the lipid are free to reorientate around the intermediate states of ion pumps, which is determined by the order or the fluidity of the membrane. The effects of the dipole potential and membrane fluidity are combined in the concept of the membrane orientational polarisability, for which we recently developed a fluorescence-based method of determination using the probe di-8-ANEPPS. Future work will involve analysing for a correlation between orientational polarisability and ion pump activity.

Fluorescent Styrylpyridinium Voltage-Sensitive Membrane Probes: Voltage-sensitive membrane probes based on the styrylpyridinium fluorophore have proven to be very useful in the neurosciences for the optical imaging of voltage transients of neurons and in biophysical research for the investigation of the kinetics of ion-transporting membrane proteins. However, in spite of the successes which have been achieved through their use, they suffer from the disadvantage that they are often photochemically unstable and phototoxic. In order to develop improved probes it is necessary to obtain fundamental knowledge on the origin of the dyes' photochemistry. We have been investigating in particular the probe RH421, which displays the unusual property of undergoing an increase in its fluorescence on excitation when bound to lipid membranes, and the more recently developed probe ANNINE 5.