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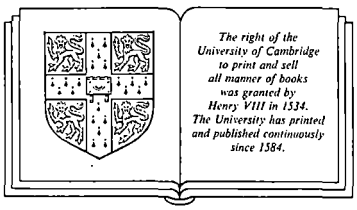

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DIELECTRIC SPECTROSCOPY OF THE ROTATIONAL AND TRANSLATIONAL MOTIONS OF MEMBRANE PROTEINS: THEORY AND EXPERIMENT

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Within the framework of so-called localised theories [1] of energy coupling in electron transport phosphorylation, a knowledge of the dynamic, topological disposition of membrane protein complexes assumes importance [2,3].

In principle [4], dielectric spectroscopy (see e.g. [5]) should provide a powerful tool for assessing the electrical organisation and ion conducting pathways of energy coupling membrane vesicle suspensions. We have therefore developed [6] a computerised, frequency-domain dielectric spectrometer, covering the range 5Hz-13MHz, and have observed, for the first time, dielectric dispersions apparently corresponding to the rotational and translational motions of the protein complexes in Rhodopseudomonas capsulata chromatophores [7,8].

Calculations of the (dielectric) relaxation time for rotational motion of charged membrane proteins of typical size indicate that, for membrane (micro-)viscosities in the range 1-10 poise (0.1-1 Pa s), a dielectric dispersion should indeed be observable with a characteristic frequency in the range 1-10 kHz.

If translational (2-dimensional) diffusion is unrestricted, as in the "fluid mosaic" model (see [9]), the characteristic frequency for relaxation due to translational motion (which scales as the inverse square of the vesicle radius) should lie in the range 0.5-5 Hz for a 'spherical' microbial cell of radius 0.5 μm. In contrast, if lateral diffusion is restricted, say by "long-range" protein-protein interactions, f_c values may be as great as 1-10 MHz, depending on the distance diffused before, on average, a 'barrier' is encountered.

The effects of cross-linking reagents (dimethyl suberimidate and glutaraldehyde) on chromatophores [8] and on cells and protoplasts of P. denitrificans (in preparation) indicate that whilst rotation is limited essentially by frictional (hydrodynamic) forces alone, translational

diffusion in these systems is somewhat more restricted. This conclusion contrasts with certain measurements of "lateral electrophoresis" carried out at higher field strengths than those used herein ($\leq$0.3V/cm) [10,11].

Dielectric spectroscopy constitutes a wholly non-invasive approach to such studies. Such measurements might also be used to optimise, and further to understand the mechanisms underlying, electric field-mediated cell fusion [12].

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