polarization, and because of the difficulty that the high conductivity of biological samples causes with dielectric measurements.

A dielectric polarization process with a large dielectric constant associated with the aqueous environment of membranes may exert a dominant effect upon the mobilities of ions and charged functional groups near the membrane surfaces. Consequently, the dielectric process that we observed may exert a major effect upon both the structure and function of biological membranes. We can now interpret these physical measurements in terms of biochemical properties of membranes. We have shown that high-frequency dielectric measurements are physiologically relevant to active membrane systems that constantly regenerate, especially if they are closely spaced in vivo, e.g., photoreceptor membranes in rod outer segments of the retina.

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SUBNANOSECOND FLUORESCENCE LIFETIMES BY TIME-CORRELATED SINGLE PHOTON COUNTING USING SYNCHRONOUSLY PUMPED DYE LASER EXCITATION

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The measurement of fluorescence parameters, particularly fluorescence lifetimes, represents one of the principal techniques for studying the photophysical properties of organic molecules and for elucidating the dynamic chemical and physical processes seminally important in molecular biology. Particularly informative are changes in lifetimes with different environmental factors that mimic physiological states. Time-correlated single-photon counting has been applied to nanosecond fluorescence measurements since the mid-1960s. Because of the inherent characteristics of the traditional excitation source, the air gap-discharge arc, there are major problems associated with lifetime measurements when fluorescence intensities are low. Recently a syn-

chronously pumped tunable dye laser has been developed (1) that is ideally suited as an excitation source for measurements of relaxation phenomena on the nanosecond and subnanosecond time scales (2). We have used a modified version of such a mode-locked laser with a time-correlated single-photon counting system to extend markedly the possibilities of determining nanosecond and subnanosecond lifetimes under experimental conditions where such measurements have thus far not been achieved (3). The particular advantages of this excitation source are: (*a*) very short (<35 ps full width at half maximum [FWHM]) excitation pulses are produced, (*b*) the peak pulse power is high (140 W at 580 nm), (*c*) high repetition rates of 10 kHz to 10 MHz are possible, (*d*) the pulse profiles are uniform over short and long times, (*e*) the light is monochromatic, wavelength-adjustable, and polarized, (*f*) frequency doubling provides tunable UV wavelengths, and (*g*) there is no radio frequency noise. Very narrow excitation pulse widths are necessary to accurately determine subnanosecond lifetimes as well as multiple exponential decay parameters. The mode-locked pump laser output, a 76.802 MHz pulse train with 13.020-ns period, was used to pump rhodamine 6G. A Bragg cell in the dye laser cavity was used to "dump" a preselected fraction of the mode-locked pulses. Single-pass and double-pass beams arise from the same dye laser pump and provide two optical output channels. The cavity-dumped output consisted of a main pulse with preceding and trailing pulses of 98% smaller amplitude, spaced by 13.020 ns, and with spatial separation permitting spatial filtering. Optimum timing resolution of the photon counting system was achieved by using start channel pulses of −2.5 V obtained from a fast photodiode that monitors the single-pass cavity dumped beam and by connecting the photomultiplier (PM) tube (RCA 8850, RCA Solid State, Somerville, N.J.) directly to the stop channel input with no preamplifier. The overall timing resolution of the photon counting system was 25.1 ± 2.5 ps when fast photodiodes were used to monitor the single- and double-pass beams from the Bragg cell, with one output going to the start channel and the other output going to the stop channel. Thus the start channel timing resolution was <25 ps. The laser pulse profile obtained by scattering from a Ludox solution (E. I. DuPont, Wilmington, Del.) had a pulse width of 0.34 ± 0.01 ns FWHM. The principal reason for the measured light pulse FWHM appearing greater than the actual <35 ps value is the inherent transit time dispersion in the PM tube, 0.32 ns FWHM minimum (4). With excitation at 570 nm, the fluorescence emission of 10^{-6} M rhodamine B in the presence of various amounts of KI quencher was monitored through a sharp cut-off red filter. Data acquisition times never exceeded 5 min with 40,000 counts in the peak channel. Fluorescence lifetimes were calculated by deconvolution of the decay profile by the method of moments (5). Using fabricated data, we obtained a precision of ±20% for for lifetimes that correspond to $\frac{1}{6}$ of the excitation pulse FWHM. For this study we analyzed for a single exponential decay component and obtained the following lifetimes (ns): 1.48 (0.00 M KI), 0.921 (0.075 M KI), 0.671 (0.15 M KI), 0.352 (0.30 M KI), 0.162 (0.60 M KI), and 0.068 (1.20 M KI). These results are presented to illustrate fluorescence relaxation behavior on the subnanosecond time scale. It should be pointed out that the observed fluorescence relaxation actually displays multiple expo-

nential behavior principally due to static and dynamic KI quenching effects and time-dependent depolarization effects arising from molecular rotation due to Brownian motion. Also, systematic errors were introduced because of the wavelength dependence of the PM tube temporal response (excitation and fluorescence profiles were determined at different wavelengths) and convolution by the Ludox scattering solution compared to the clear sample solution of the excitation profile. In conclusion, we obtained fluorescence excitation pulse widths of 225 ps FWHM using a static crossed-field PM tube.[1] Because the transit time dispersion of a static crossed-field PM tube is <30 ps[2] it should be possible to display excitation pulse widths limited by the timing resolution of the photon-counting electronics, and with refined experimental and deconvolution methods, to determine relaxation times of the order of 10 ps with better than 20% accuracy.

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ALLOSTERY IN AN IMMUNOGLOBULIN LIGHT-CHAIN DIMER

A CHEMICAL RELAXATION STUDY

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The light chain dimer of the murine immunoglobulin MOPC 315 (L_2 315) has been previously shown to bind the same nitroaromatic haptens as the parent molecules (HL), with similar fine specificity (1, 2). 2 mol of hapten were found to bind per 1 mol of the dimer (1). This finding may be explained in terms of a local twofold rotation axis, with both "original" light chain residues and "new" heavy-chain-homologous residues forming two distinct symmetry-related binding sites for the hapten (2). Recently we reported (3) that hapten binding to L_2 315 involves positive cooperativity, most probably mediated by an allosteric transition of the protein. Two haptens, ϵ-*N*-2-4-dinitrophenyl-L-lysine (DNPL) and 4-(α-*N*-L-alanine)-7-nitro-benz-2-oxa-1,3-