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In order to investigate and understand the dynamics of protein fluctuations, specifically the interaction between dynamical structure and function, we have started a program to use resonantly enhanced light scattering to probe the fluctuation spectrum. Here we report on a new technique, the use of a quartz microbalance to confirm the existance of motion within myoglobin at 5 MHz. We report on modelling the temperature rise for an absorbing sample with incident laser light. Finally, we give some preliminary results of quasi-elastic light scattering on samples of membrane fragments containing the bacteria Bacteriorhodopsin.

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Measurement of Protein Hydration Shells
Using a Quartz Micro-Balance

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Abstract

The protein myoglobin is dried on the surface electrodes of a 5 MHz quartz crystal oscillator. As the humidity above the film increases, the protein absorbs water and develops a hydration shell. The oscillator frequency shift measures the mass of absorbed water. Dramatic incremental decreases in the amplitude of vibration are also observed, corresponding to a *softening* of the protein structure and subsequent energy dissipation (damping) of the oscillations. The transition of a protein from a dry more rigid structure to a *semifluid* hydrated state is observed to occur in discrete steps.

This first study is presented to demonstrate the viability of the technique, and the important information one can obtain with the quartz microbalance. The experiment can be repeated as a function of crystal frequency, using a number of crystals from 10 kHz to 100 MHz. The damping of the crystal should, of course, decrease when the frequency of the crystal differs from the frequencies of the protein fluctuations. In addition, the temperature can be varied. The temperature of protein will change the spectrum of protein fluctuations. New information concerning the temperature dependence of the protein internal viscosity could be obtained from this technique. The experiment can also be repeated using different proteins. We anticipate a number of interesting and informative studies to follow.

This work has been submitted to Physical Review Letters (1989).

**Laser Induced Heating and Thermal Propagation,
A Model of Tissue Interaction with Light**

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Abstract

The temperature distribution in space and time is modeled for an absorbing tissue with incident laser light. We use *Beer's Law* with absorption coefficient α to describe the light absorbed within the tissue. Heat is diffused away in all directions by the thermal conductivity, κ , of the tissue, and the rate of heat flow is proportional to the temperature gradient. The specific heat, c_p , and the density, ρ , are the remaining parameters in the model. We give the resulting heat flow equation with a source term as the inhomogeneous partial differential equation:

$$\kappa \nabla^2 T - (c_p \rho) \frac{\partial T}{\partial t} = -I_0 \alpha \quad (1)$$

where I_0 is the incident laser intensity. Equation (1) is solved with a *Green's function*. The resulting function:

$$T(\mathbf{r}, t) = \frac{I_0 \alpha \sqrt{c_p \rho}}{16(\pi \kappa)^{3/2}} \int_0^t dt' \int d^3 x' \frac{\Psi(\mathbf{r}', t')}{(t - t')^{3/2}} \exp\left[\frac{-|\mathbf{r} - \mathbf{r}'|^2 (c_p \rho)}{4\kappa(t - t')}\right] \quad (2)$$

of the change in temperature for the tissue is numerically integrated. An arbitrary intensity distribution for the incident laser intensity can be chosen using a density distribution $\Psi(\mathbf{r}', t')$. In one example calculation, we use a gaussian intensity distribution with an f/5.7 focussed beam to obtain the temperature distribution in cell membranes during a micro-beam experiment. In the micro-beam experiment, a laser is focussed with a microscope and "aimed" at a cell membrane. A micron size hole is subsequently "burned" through the membrane. We assume that the hole is created whenever the absorbed laser light raises the temperature of the water to its boiling point. The values from water are used for density, thermal conductivity and specific heat in our calculation. The absorption coefficient of the cells is measured with a spectrophotometer. Correlations between the volumes where the calculated temperature exceeds the boiling point of water and the measured hole size from micro-beam experiments are compared.

This work is used to characterize the temperature changes in the resonantly absorbing samples during the light scattering experiments. The resonant light can induce thermal effects (*i.e.*, an increase in diffusion rates from a higher temperature and a decrease in solvent viscosity), as well as stimulating the molecular fluctuations. It is, therefore, important to know the temperature in the sample under the exact experimental conditions. Since our calculations agree well with the "calibration" of the microbeam technique—a hole is created when the water reaches boiling—we expect it to correctly describe the temperature profile of a scattering solution.

This was reported at the Conference on Lasers and Electro-Optics in Baltimore, April 1989. A written manuscript is in preparation.

**Photo-Induced Membrane Fluctuations from Purple Membrane
Detected with Homodyne Quasi-elastic Light Scattering**

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Abstract

Membrane fragments containing the protein Bacteriorhodospin are known to pump protons after absorbing light. The photocycle progresses through several intermediate states, as characterized by the absorption spectra, after the absorption of a photon. During this cycle the protein is also known to undergo several conformational transitions in addition to pumping a proton. With low intensity light, the homodyne autocorrelation function shows the membrane fragments to diffuse with rates that correlate well with the size distribution of the membrane fragments. However, when the light is increased, the autocorrelation function shows a dramatic increase the frequency spectrum. Initial measurements have shown a gaussian shaped band of fluctuation frequencies, centered near 1 kHz to be stimulated by the increased light. Thermal effects are currently being checked to explain these additional fluctuations. In addition, the fluctuations are the greatest on the large membrane fragments—those with a diameter greater than 2 μm .

The idea to use quasi-elastic light scattering to measure fluctuations within biomolecules has received little attention. The changes in the autocorrelation spectrum are generally believed to be very small. Using a combination of carefully controlled experimental techniques, computer assisted data analysis and sample preparation, we believe that we have been successful in measuring changes in the scattered light autocorrelation function. We currently interpret the additional motion as a ruffling of the membrane fragment during the photocycle. Work is continuing to identify this motion.

The light scattering experiments using an autocorrelator were started on the purple membrane fragments instead of heme proteins because the larger membrane fragments scatter light more effectively and the small protein molecules in solution. The purple membrane can also resonantly scatter light and one would expect them to exhibit biomolecular fluctuations. Thus the purple membrane is an excellent molecule to study, in addition to a "training molecule." A manuscript is in preparation and the experiments continue.

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