large enough volume, positive ions can screen the oxygens and then even Cl^- can permeate. Thus, by varying the size of the stewpot, this scheme of floating oxygens can be used to make many kinds of ion channels. In addition to pointing out the model's flexibility, the paper also carefully points out deficiencies, one of which is a failure to reproduce the low affinity of the Ca^{2+} channel for Mg^{2+}. This probably is related to the ability of Mg^{2+} to bind water unusually tightly, a property not mimicked by the mean spherical approximation.

This paper arrives at an exciting moment in the history of ion channels. In the past, theories attempted to reproduce functional data with the simplest possible model in the hope that some insight would be gained. But now the goals are grander than mere generic insight. With atomic structure of the K^+ channel known, theoreticians are creating various approaches to accurately describe ion trajectories within the pore and the forces that control them. In contrast to the continuum approach of PNP, Brownian dynamics models the behavior of a small group of ions in the near vicinity of a channel's mouth, and it successfully explains both functional data and the location of K^+ ions within the crystal structure of the K^+ channel (Chung et al., 1999). Molecular dynamics simulation of 4 ns in the life of the K^+ channel has now been published (Berneche and Roux, 2000). This daunting calculation of individual vibrations of each atom in the protein, three K^+ ions, and over 1000 water molecules captured a single coupled movement of the K^+ ions and waters within the selectivity filter. This appears to demonstrate that K^+ ions move in single file through K^+ channels, as first deduced in the elegant work of Hodgkin and Keynes (1955). One might expect to need about 1 μs of time in order to see an ion fully traverse the pore; this huge calculation might give an enormous payoff in our understanding of what forces control the ion at various stages of permeation. It would be valuable to see how the PNP and the mean spherical approximations do with the known K^+ channel structure and how the result compares to these other methods.

It also seems important for theoreticians to consider whether the apparent dichotomy between selectivity mechanisms for K^+ and Ca^{2+} channels is dead wrong. After all, dead wrong were the theories before crystallization of the K^+ channel about how the pore's amino acid side chains created K^+ selectivity. Perhaps backbone carbonyls rather than side chain carboxyls create a Ca^{2+} binding pocket in Ca^{2+} channels. Also, K^+ channels have powerful, but underappreciated, functional similarities to Ca^{2+} channels. At least one binds K^+ with micromolar affinity (Vergara et al., 1999) and, in precise analogy to the behavior of Ca^{2+} channels without Ca^{2+}, K^+ channels lose selectivity in the absence of K^+ (Kiss et al., 1998). Perhaps the apparently rigid K^+ channel pore was made rigid by the binding of the ions within it, i.e., that it adjusts to fit its ions and, like the Ca^{2+} channel of Nonner et al., is fluid until bound. The power emerging from new theoretical methods relating ion permeation to protein structure should allow the airing of heretical ideas, some of which are bound to be true.

REFERENCES

Berneche, S., and B. Roux. 2000. Molecular dynamics of the KcsA K(+) channel in a bilayer membrane. *Biophys. J.* 78:2900–2917.

Chung, S. H., T. W. Allen, M. Hoyles, and S. Kuyucak. 1999. Permeation of ions across the potassium channel: Brownian dynamics studies. *Biophys. J.* 77:2517–2533.

Doyle, D. A., J. Morais Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science.* 280: 69–77.

Hodgkin, A., and R. Keynes. 1955. The potassium permeability of a giant nerve fibre. *J. Physiol.* 128:61–88.

Kiss, L., D. Immke, J. LoTurco, and S. J. Korn. 1998. The interaction of Na+ and K+ in voltage-gated potassium channels: evidence for cation binding sites of different affinity. *J. Gen. Physiol.* 111:195–206.

McCleskey, E. W. 1999. Calcium channel permeation: a field in flux. *J. Gen. Physiol.* 113:765–772.

Nonner, W., L. Catacuzzeno, and B. Eisenberg. 2000. Binding and selectivity in L-type Ca channels: a mean spherical approximation. *Biophys. J.* 79:XXX-xxx.

Nonner, W., and B. Eisenberg. 1998. Ion permeation and glutamate residues linked by Poisson-Nernst-Planck theory in L-type calcium channels. *Biophys. J.* 75:1287–1305.

Vergara, C., O. Alvarez, and R. Latorre. 1999. Localization of the K+ lock-in and the Ba2+ binding sites in a voltage-gated calcium-modulated channel: implications for survival of K+ permeability. *J. Gen. Physiol.* 114: 365–376.

Yang, J., P. T. Ellinor, W. A. Sather, J. F. Zhang, and R. W. Tsien. 1993. Molecular determinants of Ca2+ selectivity and ion permeation in L-type Ca2+ channels. *Nature.* 366: 158–161.

Molecular Motions in Fourier Transform Space

J. Michael Schurr

Department of Chemistry, University of Washington, Seattle, Washington 98195 USA

Molecular Brownian motions in dense macromolecular suspensions and glassy liquids may involve extensively correlated particle movements and substantial temporal and spatial heterogeneity (Cicerone et al., 1996; Ediger et al., 1996; Marcus et al., 1999; Weeks et al., 2000). Living systems may exhibit even more complex Brownian dynamics due to the dispersion in their molecular sizes and shapes. In addition they may also exhibit complex non-thermal motions that are driven ultimately by ATP hydrolysis. Although the translations of individual molecular motors along their conjugate filaments have been extensively studied, little is known about other non-thermal motions, such as

Received for publication 11 August 2000 and in final form 21 August 2000.

Address reprint requests to J. Michael Schurr, Department of Chemistry, P.O. Box 351700, University of Washington, Seattle, WA 98195. Tel.: 206-543-6681; Fax: 206-685-8665; E-mail: schurr@chem.washington.edu.

0006-3495/00/10/1692/03 $2.00

how cytoskeletal motions within the cell affect the movements of other molecules, complexes, and organelles. The use of fluorescent labels to track the motions of particular species is now common, and in principle digital video fluorescence microscopy (DVFM) is the tool of choice for simultaneously tracking the positions of all labeled particles in the field of view in order to study their complex motions. However, DVFM suffers from certain limitations: 1) photobleaching of the fluorescent labels restricts the time over which any given particle can be tracked; 2) the temporal resolution (time between images) may be inadequate to examine certain rapid motions; and 3) extensive computation is generally required to reduce the data for many particle trajectories down to the relevant statistical quantities that characterize their motions. The new technique of Fourier imaging correlation spectroscopy (FICS) introduced by Margineantu et al. in this issue of *Biophysical Journal* provides an alternative approach that substantially alleviates these problems. FICS can be regarded as a fluorescence-based analogue of dynamic light scattering (DLS) that takes full advantage of both the greater sensitivity and labeling specificity that is afforded by fluorophores. A traveling fringe excitation method (Hattori et al., 1996) is extended in order to vary the wavelength of the fringe (or wave vector $\mathbf{k}$) and to provide potentially much greater time resolution, and is applied to analyze microscopically imaged samples. The sample, which contains fluorophores at positions $\mathbf{r}_j$, $j = 1,\ldots N$, is excited by an optical grating of constant wavelength that translates continuously across the sample at a constant velocity, so the resulting fluorescence intensity oscillates in time with a period that is much shorter than the time scale of any molecular motion of interest. The fluorescence signal varies as a consequence of the oscillatory excitation and the motion of the fluorophores, which alters the amplitudes, $A(\mathbf{k}, t) = \Sigma_j \exp[i\mathbf{k} \cdot \mathbf{r}_j]$, of the different Fourier components of the fluorophore spatial distribution. The fluorescence is detected by a lock-in amplifier, whose average output over several cycles of oscillation is proportional to $A(\mathbf{k}, t)$ for the particular optically excited Fourier component. Subsequent autocorrelation of $A(\mathbf{k}, t)^2$ yields the relevant statistical information regarding the fluorophore dynamics in a manner directly analogous to the intensity autocorrelation function of dynamic light scattering. Provided the real and imaginary parts of $A(\mathbf{k}, t)$ are nearly gaussian random variables, which is the case whenever the fluorescence comes from numerous independently moving or fluctuating domains, the autocorrelation function of $A(\mathbf{k}, t)^2$ provides the square of the dynamic structure factor, $S(\mathbf{k}, t) = (1/N) <\Sigma_j \Sigma_m \exp[i\mathbf{k}\cdot(\mathbf{r}_j(0) - \mathbf{r}_m(t))]>$. This two-time correlation function has been theoretically investigated for many different models of particle motions and fluctuations, and has provided numerous insights into the dynamics of complex systems studied by DLS. By varying the grating spacing, the dynamics of different Fourier components can be examined one at a time in serial fashion. This is crucial for understanding the diffusive dynamics of strongly interacting or confined species, as well as certain non-thermal motions.

The translational dynamics of the same mitochondrial reticulum under different physiological conditions are investigated by both DVFM and FICS. $S(\mathbf{k}, t)$ is computed directly from the DVFM images for comparison with FICS, and the two methods are found to agree quantitatively. The observed dynamics are shown to arise from a superposition of thermally excited motions and presumably driven motions that require ATP synthesis, and both kinds of motions are characterized in considerable detail. These results shed significant new light on the complex motions of the mitochondrial reticulum and their dependence upon metabolic state. However, the full potential of the FICS method is not yet fully realized and the best may be yet to come.

In subsequent work, the FICS method is extended to obtain the trajectories of the separate real and imaginary parts of $A(\mathbf{k}, t)$ (A. Marcus, personal communication), which allows a determination of the complex $S(\mathbf{k}, t)$, instead of just its absolute magnitude. Although $S(\mathbf{k}, t)$ is purely real for ergodic thermal motions, it becomes complex in the presence of directional driven motions, including uniform translation. Knowledge of the complex $S(\mathbf{k}, t)$ should allow one to ascertain the presence of directional driven motions and to characterize them in regard to direction and velocity. Thus, FICS should become a powerful tool for investigating protoplasmic streaming and the intracellular trafficking of particular labeled particles. It should also be possible to examine the in- and out-of-phase responses of particle positions to sinusoidal perturbations, such as alternating electric or shear fields. In addition, any long-lived spatial heterogeneity of the molecular dynamics should be manifested by a difference in the decays of the autocorrelation functions of the real and imaginary parts of $A(\mathbf{k}, t)$, and can be detected in that way. The spatial scale of those heterogeneities could assessed by comparing such differences for a range of $\mathbf{k}$ values. Another promising new application of FICS is the study of ligand binding kinetics (A. Marcus, personal communication). Whenever binding events are accompanied by a change in quantum yield of a fluorophore, they contribute along with particle translation to the decay of $S(\mathbf{k}, t)$. By combining measurements at two or more different values of $\mathbf{k}$, the relevant chemical relaxation time can be unambiguously assessed. An important difference between the FICS method and conventional fluorescence correlation spectroscopy (FCS) with spot illumination, is that the signal-to-noise ratio increases with increasing N in FICS, whereas it decreases with increasing N (for zero fluorescence background) in FCS. Thus, in FICS it should be possible to work with much

greater signals, and thereby access much more rapid chemical reactions. The FICS method can also be extended to study the rotational dynamics of fluorophores in a manner analogous to depolarized dynamic light scattering.

FICS is one of the most promising new techniques for studying molecular motions in complex systems, including living systems, that has appeared in recent years. Moreover, the method can be implemented using Raman scattering, two-photon fluorescence, and four-wave mixing in addition to normal fluorescence (A. Marcus, personal communication).

REFERENCES

Cicerone, M. T., and M. D. Ediger. 1996. Enhanced translation of probe molecules in supercooled *o*-terphenyl: signature of spatially heterogeneous dynamics? *J. Chem. Phys.* 104: 7210–7218.

Ediger, M. D., C. A. Angell, and S. R. Nagel. 1996. Supercooled liquids and glasses. *J. Phys. Chem.* 100:13200–13212.

Marcus, A. H., J. Schofield, and S. A. Rice. 1996. Experimental observation of non-Gaussian behavior and stringlike cooperative dynamics in concentrated quasi-two-dimensional colloidal liquids. *Phys. Rev. E.* 60: 5725–5736.

Weeks, E. R., J. C. Crocker, A. C. Levitt, A. Schofield, and D. A. Weitz. 2000. Three-dimensional direct imaging of structural relaxation near the colloidal glass transition. *Science.* 287:627–631.

Hattori, M., H. Shimizu, and H. Yokoyama. 1996. Fluorescence correlation spectroscopy with traveling interference fringe excitation. *Rev. Sci. Instrum.* 67:4064–4071.