which of several energetically similar crystal structures will form. Moreover, Wuest's materials do not have any built-in component that could perform catalytic chemistry. Others have made progress towards this objective. For example, Richard Robson of the University of Melbourne has constructed porous metal coordination networks based on a porphyrin building block known to have catalytic activity[5].

Even though there is a long way to go before organic zeolite analogues come to fruition, Wuest's discovery demonstrates an essential step. The essence of chemical reactivity is the ability to bring together a substrate and a reagent through molecular diffusion. We now know that this diffusion can occur on a reasonable timescale through a nanoporous organic crystal. Although organic zeolites will never be as robust as their inorganic counterparts, they can still be useful so long as molecules can enter and leave the shape-selective portholes without causing the lattice to crumble. □

Jeffrey S. Moore is in the Departments of Chemistry, and Materials Science and Engineering, University of Illinois-Urbana, Illinois 61801, USA.

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MOLECULAR BIOPHYSICS

Taking a shine to myosin

Robert M. Simmons

SOME 20 years ago Hirschfeld[1] detected the fluorescence from an antibody labelled with about 80 fluorophores. As reported by Funatsu *et al.* on page 555 of this issue[2], techniques have now been improved to the point where it is possible to detect single fluorophores using a video-camera with a light microscope, with good resolution of intensity and time. Similar achievements in focused beam experiments have already been reported[3,4].

Detecting single-molecule fluorescence in solution has presented a challenge because of scattering from the solution. But the rewards for success are considerable in three main areas.

First, there could be some interesting physics of the excitation–emission process itself, which might be averaged out in an ensemble of fluorophores. Second, and this is the major impetus, it might be possible to sequence single molecules of DNA: the notional experiment is to make complementary DNA with bases having different fluorophores, tether the molecule in a capillary tube and digest it with an exonuclease while flowing solution along the tube; then, with a sensitive detector downstream, to distinguish between the fluorophores by spectral or lifetime differences. The research on spectroscopy and sequencing has concentrated on reducing the volume illuminated by the laser beam used for excitation, so as to minimize the background scatter, and on improving the efficiency of detection. The detection of single-molecule fluorescence was inferred from correlation analysis ten years ago[5], but it is only recently that bursts of photons have been shown convincingly to arise from single fluorophores traversing the laser beam. It has also been possible to measure lifetimes, using a pulsed laser and measuring the delay before a photon is emitted[4].

The third reason comes from motility assays in which the action of motor proteins is studied *in vitro*. One example is the imaging of actin filaments sliding over a glass surface coated with the motor protein myosin. Experiments in which single motor-protein interactions are detected[6,7] would be greatly enhanced by positional information about the myosin molecules and the biochemical kinetics of the interactions. One goal is to identify unambiguously the position of single motor-proteins, from which mechanical measurements can then be made. This would resolve lingering uncertainties about how many molecules are needed for an effective interaction. A video-microscopy technique is needed for the purpose, one that can tell the difference between one fluorophore and two, and which has a video-rate temporal resolution.

Funatsu *et al.*[2] have now achieved this end using conventional instrumentation, but taking extreme care to reduce background scattering and fluorescence. Measured over the area of resolution of a single fluorophore, these precautions reduced the background scattering from more than 6,000 photons per second to 140 photons per second, to be compared with 500 photons per second from the fluorophore itself. Background counts were then finally nearly abolished using an evanescent-wave technique to illuminate the surface on which the fluorophores (attached to myosin molecules) were located. Lastly, electron micrographs of the myosin molecules on a surface superposed well on the fluorescence image.

Game and set, if not match, in single motor-protein studies would be won by simultaneous measurements of mechanics and biochemical kinetics. This could settle once and for all the issue of which biochemical transition accompanies force generation (popularly thought for myosin to be phosphate release). It would also lay to rest (or resurrect) the spectre of multiple interactions of myosin with actin for each ATP hydrolysed[8]. The first experiments towards this end were reported two years ago by Sowerby and colleagues[9], who measured single turnovers of ATP in myosin filaments containing about 10^4 molecules.

Funatsu *et al.* have brought these measurements to the single-molecule level, using a fluorescent analogue of ATP at low concentration (10 nM), and watching molecules of the analogue bind to myosin molecules and then disappear again as the ATP analogue is hydrolysed and the products diffuse away. One limitation is the need to use very low concentrations to avoid high background counts, although, as Sowerby *et al.*[9] point out, it may be possible to use a photoactivatable fluorophore and photolyse it locally. Another limitation is the comparatively poor time resolution, arising from the low photon detection rate; in all these single-fluorophore measurements, it is tantalizing that the detection rate appears to be 1 per cent at best[3], and also that the number of photons is limited to about 10^5–10^6 by photobleaching[1]. So it seems that as few as 10^3–10^4 photons are available for measurement, necessitating a compromise between temporal resolution and duration.

Game, set *and* match in single motor-protein experiments would be to measure the mechanics, biochemistry and structural changes accompanying force generation in the same experiment. If this were to involve both fluorescence and a scanning-probe technique, one might be tempted to say that there was — at last — light at the end of the tunnel. □

Robert M. Simmons is in the MRC Muscle and Cell Motility Unit, Randall Institute, King's College London, 26–29 Drury Lane, London WC2B 5RL, UK.

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