Single-Molecule Methods in Polymer Science

STEVE GRANICK, SUNG CHUL BAE, BO WANG, SUBHALAKSHMI KUMAR, JUAN GUAN, CHANGQIAN YU, KEJIA CHEN, JAMES KUO

Department of Materials Science and Engineering, University of Illinois, Urbana, Illinois 61801

Received 7 July 2010; accepted 31 July 2010
DOI: 10.1002/polb.22133
Published online 13 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

KEYWORDS: diffusion; ensemble average; fluorescence; imaging; nanotechnology; optics; single molecule; transport

No one can forget the exhilaration of seeing an individual molecule or atom for the first time, or of manipulating one. Like children we use them as building blocks—think of writing with ink made of single atoms by using scanning tunneling microscopy[1] and of constructing complex origami by using DNA.[2] Or, like a voyeur, just watching single molecules go about their business.[3] To those who studied quantum mechanics with a reflective turn of mind, this may seem magical and seem to risk violating the laws of quantum mechanics, but it does not. This viewpoint will focus on single-molecule fluorescence methods[4] and leave for another day the equally interesting problem of single-molecule force measurements, for example, the tour de force of measuring the stress–strain properties of individual chains.[5] We will focus on how the problems of polymers differ from those of biophysics, where single-molecule fluorescence methods have had so much impact. We emphasize unsolved problems and areas for useful future research.

One wonders what Boltzmann would have said—after all, to see each molecule in a gas wouldn't teach us anything that we don't know already. Boltzmann might have argued that to visualize single molecules is a giant step backward. The point of statistical mechanics is to put discussion on a higher level than this; concepts such as entropy allow us to avoid clumsy accounting of each molecule. In a gas, we know the underlying distribution of interesting observables, and anyway we are more interested in aggregate properties such as pressure and temperature than in the trajectories of individual molecules. Ensemble averaging allows one to see forests without getting lost in their trees.

It becomes more interesting when ensemble averaging goes wrong. Consider a room full of young children. About half of them have learned to walk, and about half of them are still crawling on all fours: on the average, children walk on three limbs! This of course is too simple minded; the naïve average masks a meaningful bimodal distribution.

One reason that single-molecule fluorescence experiments have had so much impact in biophysics is that the problems of biophysics are also so heterogeneous. In taking images of where molecules are located, most commonly this is done on spatial scales limited by diffraction,[4] but emerging subdiffraction fluorescence methods do even better than this. Unlike electron microscopy, fluorescence measurements can be performed *in situ*, though it remains true that the spatial resolution of electron microscopy is better. The field of polymers has seen few spatially resolved microscopy investigations at the level of single or just a few molecules, probably because problems requiring this resolution have not been identified. Unlike Biophysics, where it is interesting and relevant to know (for example) the static distribution of molecules between different organelles in a cell and how this changes *in situ* with time, present-day polymer problems rarely carry, outside the subdiscipline of block copolymer morphology, this aspect of compartmentalization over scales largely relative to the wavelength of light.

Regarding dynamics it becomes even more interesting. Since now what matters is relative position, time-dependent changes can be quantified with far better discrimination. Using FRET, Förster resonance energy transfer, it may be a distance of a few nanometers. Using single-molecule tracking to follow the Gaussian center of a blurry diffraction-limited image, it may be a few nanometers to a few tens of nanometers. Conformational mobility and transport can then be studied. It is interesting to consider why FRET techniques, discovered long ago, became popular only with the study of enzyme and other protein conformational changes; the reason is probably that these biomolecules present distinct conformational states, the steps between which can be discriminated from background noise. When polymer science identifies distinct conformational states in our synthetic polymers, FRET methods are likely to find wider application. Similarly, tracking the transport of individual polymer molecules will likely find wider application when the moving molecules become larger, large enough that the molecule exceeds the spatial resolution, as is really only so now for biopolymers such as DNA and actin. This may happen with supramolecular polymer constructs.

Correspondence to: S. Granick (E-mail: sgranick@uiuc.edu)
Journal of Polymer Science: Part B: Polymer Physics, Vol. 48, 2542–2543 (2010)

One mustn't blindly insist on watching the same molecule. The technique of fluorescence correlation spectroscopy averages over the course of time over many molecules, each of them measured with single-molecule sensitivity for a short time, allowing single-polymer diffusion to be measured at surfaces and in ultra-dilute solutions.[6] Of great conceptual importance is that this allows one to accumulate large amounts of statistics, more than one could accumulate for any single molecule. There seems to be much potential to further develop, in polymer science, the "few molecule" approach.[7]

"Caveat emptor"—let the potential user beware of the technical difficulties. This experiment usually senses not the (nonfluorescent) polymer itself but instead a fluorescent dye attached to it as a fiducial marker. Experimentalists struggle with "photobleaching," which is the fact of life that the lifetime of any given fluorescent dye under light illumination is limited, and also with "blinking"; however, these difficulties are softened when using few molecule methods. Photobleaching becomes even less of a problem with polymers so large that many fluorescent dyes can be attached to them, so many that the loss of a few to photobleaching is not serious. Another benefit to labeling a polymer with numerous dyes is that fluorescent background impurities become proportionately less prominent, which facilitates discrimination between signal and noise. Adding to this, it may be worthwhile for polymer scientists to notice that our molecules are, in favorable situations, so large that the presence of a fluorescent dye is of less concern regarding potential perturbative effects than when these methods are used to study small-molecule systems. When a macromolecule is labeled with many dyes along its backbone, internal conformations can be followed.[8] This approach, adopted frequently from the perspective of considering DNA as a semiflexible polymer, surely in the future will be extended to more long-chain synthetic polymers.

Many credos about polymer science are found to have limitations. When chains stretch under elongational flow, is the mean-field prediction correct? Not so.[9] When particles diffuse by Brownian motion in a polymer environment, is the motion necessarily Gaussian? and is mean-square distance proportional to time elapsed? Not for every particle. Only on the average, and anyway the statistics of having a finite number of random walk steps muddies these waters.[10] In the same spirit, we anticipate surprises when single-molecule studies are extended to study additional problems of polymers out of equilibrium, such as electrophoresis and nonlinear rheology. Between the following single molecules and the ensemble average obtained using the more traditional methods of polymer science lies the fertile land where the experimenter patiently watches single molecules, one-by-one, and then dissects the distribution of multitudinous single-particle trajectories.[11]

REFERENCES AND NOTES

1 Eigler, D. M.; Schweizer, E. K. Nature 1990, 344, 524–526.

2 Chen, J.; Seeman, N. C. Nature 1991, 350, 631–633.

3 Moerner, W. E.; Orrit, M. Science 1999, 283, 1670–1676.

4 Principles of Fluorescence Spectroscopy, 2nd ed.; Lakowicz, J. R., Ed.; Springer: New York, 2006.

5 Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. Science 1997, 275, 1295–1297.

6 Sukhishvili, S. A.; Chen, Y.; Muller, J. D.; Gratton, E.; Schweizer, K. S.; Granick, S. Nature 2000, 406, 146.

7 Senning, E. N.; Marcus, A. H. Annu Rev Phys Chem 2010, 61, 111–128.

8 Maier, B.; Rädler, J. O. Macromolecules 2000, 33, 7185–7194.

9 Perkins, T. T.; Smith, D. E.; Chu, S. Science 1997, 276, 2016–2021.

10 Wang, B.; Anthony, S. M.; Bae, S. C.; Granick, S. Proc Natl Acad Sci USA 2009, 106, 15160–15164.

11 Wang, B.; Guan, J.; Anthony, S. M.; Bae, S. C.; Schweizer, K. S.; Granick, S. Phys Rev Lett 2010, 104, 118301(1/4).