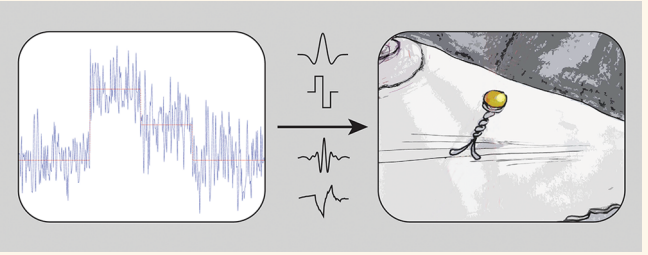
Revealing Hidden Dynamics within Living Soft Matter

Dino Ott, Poul M. Bendix, and Lene B. Oddershede*

Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark

ABSTRACT In the study of living soft matter, we often seek to understand the mechanisms underlying the motion of a single molecule, an organelle, or some other tracer. The experimentally observed signature of the tracer is masked by its thermal fluctuations, inherent drift of the system, and instrument noise. In addition, the timing or length scales of the events of interest are often unknown. In the current issue of *ACS Nano*, Chen *et al.* present a general method for extracting the underlying dynamics from time series. Here, we provide an easily accessible introduction to the method, put it into perspective with the field, and exemplify how it can be used to answer important out-standing questions within soft matter and living systems.



Motility at the nanoscale in living soft matter can be highly complex as the environment is often compartmentalized and hosts both passive and active processes.[1−3] This causes the tracers of interest, *e.g.*, single molecules, to undergo heterogeneous dynamics, and their experimentally observed trajectories are further complicated by the action of stochastic forces, originating both from the tracer's thermal fluctuations and from the inherent noise of the equipment and detection systems. To identify the locations of certain types of behaviors in time and space correctly, a scientist has to be equipped with experimental and analytical tools allowing the extraction of signals from noisy backgrounds. The standard output from many experiments consists of lengthy time series recordings of molecular dynamics or tracking of either endogenous objects or externally added tracer particles. The events of interest contained in such time series may occur randomly in time and furthermore be embedded within random noise. This renders extraction and the following correct interpretation of the data challenging. Existing analytical methods to discriminate active or anomalous behavior from random noise include the application of physical models in which thresholds are assigned to separate Brownian noise from heterogeneous events.[4] Also, transformation of time recordings into the frequency domain is frequently used to extract spectral information about biophysical processes and has been applied in the analysis of, *e.g.*, acto-myosin networks to identify nonequilibrium activity fueled by ATP hydrolysis.[1] However, spectral analysis based on Fourier transformations, even short time Fourier transformations, are best suited for analyzing stationary processes in which the localization of certain events in the time domain is not necessary. The abundance of processes in nature exhibiting nonstationary behavior renders analysis purely in frequency domain inadequate since all information regarding the localization of events in the time domain is lost.

The localization of events in time is utterly important, *e.g.*, for understanding signaling processes, cellular trafficking, or the highly nonrandom chromosomal organization within the nucleus and how this relates to the cellular clock. Therefore, we are in need of general methods that both provide information regarding the scale of heterogeneous events and are able to localize biophysical events in time. This would allow robust extraction of important information embedded within time series originating from living cells and soft matter systems. Inspired by previous work[5,6] and by the challenges met during the analysis of nanoscale soft matter systems, Chen *et al.* present in the current issue of *ACS*

* Address correspondence to oddershede@nbi.dk.

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Nano[7] a general wavelet-based method that can discriminate heterogeneous behavior from random noise in time series. They elegantly do this by applying a multiscale wavelet transform, thus preserving both the information contained in the time domain and the scale of the heterogeneous events. The success rate of the method in detecting heterogeneous events is tested both on simulated data and on real data obtained from three distinct areas of science. The methodology is independent of predefined physical models, thus making it powerful for analyzing time series originating from many different research fields. As outlined below, we expect this methodology to advance the field of nanoscale motility significantly, thereby answering important questions regarding fundamental transport mechanisms within living soft matter systems. In particular, new information is needed and will arise on the topic of nuclear organization and transportation in relation to the cell cycle, and light will be shed on the complicated aging mechanisms that give rise to weak ergodicity breaking in living organisms and how this alters the inherent cellular dynamics.

Wavelet Analysis (WLA). The so-called 'wavelet transform' mathematical technique was established in the 1980s and is now widely used for digital signal processing, such as in image compression, denoising, and pattern recognition.[8] Wavelet transform analysis allows for local, time-resolved, multiscale detection of dynamic processes in time series, revealing important features that various other established signal analysis techniques, like the Fourier transform, fail to do. For instance, the WLA method has been used for characterizing the rotational and translational Brownian motion of a nanostructure that changes size over time;[6] however, it has not yet found widespread use for the analysis of soft matter living systems. The paper of Chen *et al.* shows that WLA is a most useful and advantageous technique for nanoscale soft matter systems as well, and the following section will take the reader through a 'crash-course' on WLA.

The WLA method comprises the three following steps: (i) To analyze a given time series (as shown in Figure 1A), one chooses a wavelet. Wavelets can take different forms and their capabilities to detect certain signal features are well-known.[5,8] A wavelet is a function that is square integrable and that integrates to zero. Typically, a wavelet is limited in time and could be a single oscillation, *e.g.*, the commonly used Haar wavelet shown in Figure 1B. (ii) During the wavelet transform, the time-dependent data, *e.g.*, the trajectory of a single marker, is transformed into a time- and scale-dependent representation of the original data (shown in Figure 1C). Mathematically, this involves convolution of the time series with scaled and translated versions of the wavelet. Translation simply means moving the wavelet along the time axis and forms the basis for locating the dynamics of interest in time, whereas scaling is achieved by stretching/compression of the wavelet in time and provides information on the scale of the dynamics. This scaling of the wavelet allows one to conduct the time series analysis on multiple scales without being bound to a specific scale. In other words, the transformation returns information regarding at which points in time and on which scale the features of the wavelet match the time series. This information is given by the color scale in Figure 1C. In practice, the transformation is easy to conduct due to the availability of easily accessible toolboxes in, *e.g.*, MATLAB (MathWorks) and Mathematica (Wolfram Research). (iii) In the final step, the researchers impose a physically motivated restriction to a range of scales on which the heterogeneous dynamics are expected to occur, and an adaptive universal threshold is set. This threshold (a certain color between blue and pink in Figure 1C) serves as the decision criterion for classifying the type of dynamics and identifying dynamical heterogeneity. In the current issue of *ACS Nano*, Chen *et al.* present a general wavelet-based method that can discriminate heterogeneous behavior from random noise in time series.

By applying this methodology to analyzing the trajectories of fluorescently labeled endosomes in living cells, Chen *et al.* demonstrate the potential for detecting the switching between changing dynamics, in this case between active transport and passive diffusion (as sketched in Figure 2).

Furthermore, the authors conduct a performance evaluation using simulated data and show that the method is rather insensitive to multiple noise sources. The generality of the WLA method makes it valuable for analyzing time series of different origin. Moreover, it not only provides the means for more detailed insights, but also, importantly, precludes false

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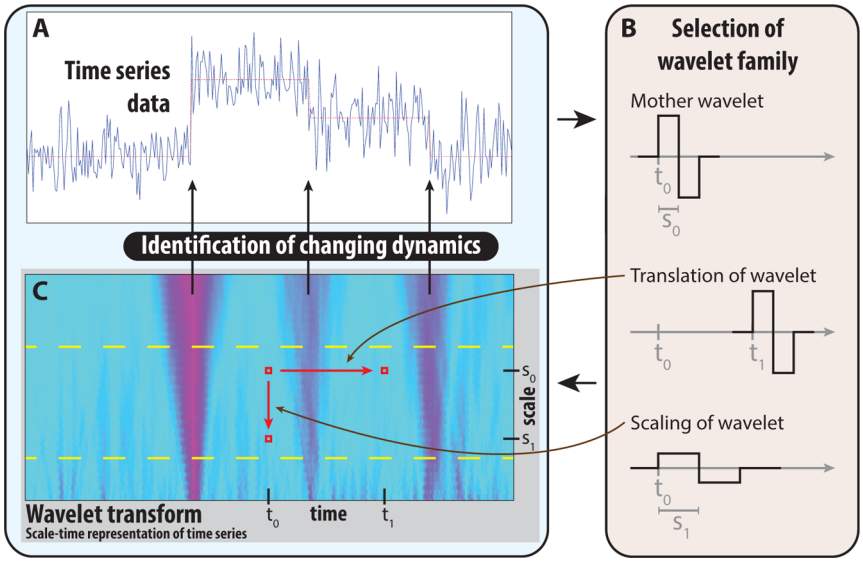

Figure 1. Wavelet analysis in a nutshell: (A) time series displaying discrete steps that are masked by stochastic noise. (B) the commonly used Haar wavelet, its translation, and scaling. (C) result of convoluting the time series shown in (A) with scaled and translated Haar wavelets. The color map shows the goodness of the match of the wavelet with the time series as the wavelet is moved in time (horizontal red arrow) and scaled (vertical red arrow). The area between the yellow lines denote the scales of interest and in the colormap, pink colors signify strong correlations and hence identify the masked steps in the time series shown in (A)).

conclusions from being drawn when blindly assuming homogeneous dynamics.

Inside Cells. The dynamics of single molecules or organelles inside living cells are known to be rich, complex, and of diverse character and origin.[9] Tracing individual molecules provides insight into the fundamental processes responsible for trafficking, signaling, and gene regulation inside living cells. There are several technical challenges connected to retrieving reliable trajectories of individual molecules inside the living cell. First, the molecules of interest need to carry a label (*e.g.*, a fluorescent marker) such that they can be followed in two or three dimensions. Some organelles (*e.g.*, lipid granules)[10] are relatively large and carry enough contrast themselves that they are visible in the microscope and need no further labeling. The technique of single particle tracking is extremely useful for tracing individual molecules or particles inside living cells, but optical traps operated at low laser powers can also provide traces with very high temporal and spatial resolution. A typical time trace is subject to a significant amount of noise, not the least of which are the thermal fluctuations of the tracer, which are substantial at physiological temperatures and at the single-molecule level.

Inside the cell, most molecules and organelles exhibit subdiffusion, *i.e.*, slower diffusion than in normal Brownian motion. This is the case for single mRNA molecules inside bacteria[11] and also for lipid granules inside yeast.[10] Interestingly, although normal Brownian motion would be a faster way to transport molecules in a passive manner, subdiffusion is much more abundant in living cells than is Brownian motion. This could be due to two factors: one is that the cytoplasm needs to be tightly packed and dense in order to accommodate all the necessary cellular machinery, and the second is that it might be advantageous for a process involving a protein binding to DNA or RNA, for example, that the proteins or nucleic acids do not diffuse away quickly but rather stay in the vicinity of their correct targets longer, thus increasing the chance of successful attachment.

In addition to these passive types of motility, there is also active, biochemical energy-consuming transport inside living cells, for instance of myosin along actin inside muscle cells or kinesin-mediated transportation along microtubules,[12] as sketched in Figure 2. Further complicating the picture, a given tracer, for example an endosome, may at certain times experience passive thermal diffusion and at other times active transport. The challenge of the scientist is then to deduce backward, from the observed heterogeneous time trace to the basic mechanisms governing the motion.

Another environment displaying highly heterogeneous dynamics that presently is gaining a great deal of attention is inside the nucleus. Chromosomes are spatially organized in the nucleus, where diffusion is extremely slow; it has been shown that the telomeres certainly do not explore the entire nucleus.[13] In addition, it was recently shown that certain genetic sequences mediate transportation of genetic information to a specific location.[14] Hence, nuclear transportation is highly regulated and far from random; however, still very little is known about the dynamics of the nucleus that are highly important for correct genetic regulation and expression.

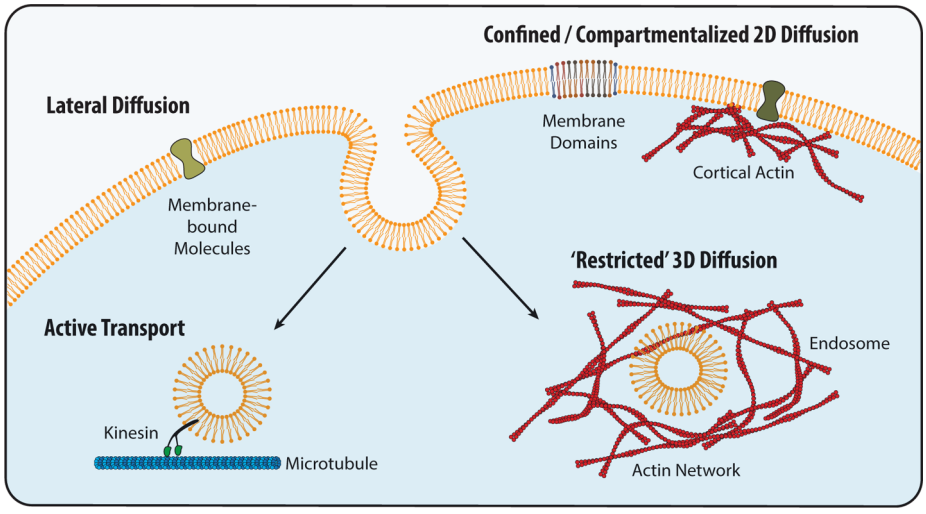

Figure 2. Sketch of the variety of transport mechanisms inside living cells. Proteins restricted to the membrane perform lateral diffusion, this possibly being complicated by the presence of membrane domains containing other types of lipids or by tethering or confinement of the protein by the underlying cortical actin mesh. Inside the cell, a tracer can perform passive thermal diffusion, but this process is hindered by the presence of the cytoskeleton and other cellular components. Furthermore, molecular motors, for instance kinesin, perform active transportation of cargos inside the living cell.

From the single-molecule or particle trajectories, it can be rather difficult to distinguish one type of motion from another and different measures exist for this purpose. One frequently applied measure is the mean square displacement, $\mathrm{MSD} = \langle (x(t) - x(t_0))^2 \rangle \sim t^{\alpha}$, where the exponent, α, provides information regarding whether the motion is confined ($\alpha = 0$), subdiffusive ($0 < \alpha < 1$), normal ($\alpha = 1$), or superdiffusive ($\alpha > 1$). Another useful method is the mean maximal excursion method,[15] which can be used to determine the physical nature underlying the observed stochastic motion. However, two major problems faced during *in vivo* detection are the noise and drift inherently present in experimental data taken at the single-molecule level over extensive periods of time. Also, it is most often not known *a priori* at which time and spatial scales the phenomenon of interest is taking place. It is likely that the multiscale wavelet method presented in this issue of *ACS Nano*[7] can overcome some of these challenges as the method extracts features without limiting the search to a certain time or spatial interval, while also being quite robust with respect to noise.

It is likely that widespread use of the multiscale wavelet method will significantly advance the field and expand our knowledge on the origin of stochastic motion inside living systems.

Ergodic *versus* Nonergodic Behavior. Recent reports have shown that time averages obtained over *in vivo* single-particle traces often are not reproducible. In other words, ensemble averages do not yield the same results as temporal averages, which means that one of the hallmarks of ergodicity is violated.

This phenomenon has been observed both for lipid granules in living yeast cells[16] and, at certain locations, in living endothelial cells,[17] as well as for membrane proteins.[18] As living organisms undergo life cycles and age over time, it is reasonable that their physical observables also change properties with time and that such systems therefore violate strict ergodicity.

In the above-mentioned experiments, not all hallmarks of ergodicity are broken, only some of them, and therefore, this effect has been named 'weak ergodicity breaking'. The concept of weak ergodicity breaking is currently highly debated; it is certainly not yet well-defined and poses serious challenges to statistical physics. One of the problems is the difficulty in pointing out the signatures that may provide clues as to the underlying processes from time traces. Moreover, it is likely that tracers at certain time scales exhibit ergodic behavior, possibly well described by the fractional Brownian motion, whereas at other time scales, the tracer's dynamics may be better defined by a continuous time random walk, which has nonergodic properties. The scientific community interested in anomalous diffusion has long been in need of tools to distinguish between different stochastic models, and it is likely that widespread use of the multiscale wavelet method will significantly advance the field and expand our knowledge on the origin of stochastic motion inside living systems.

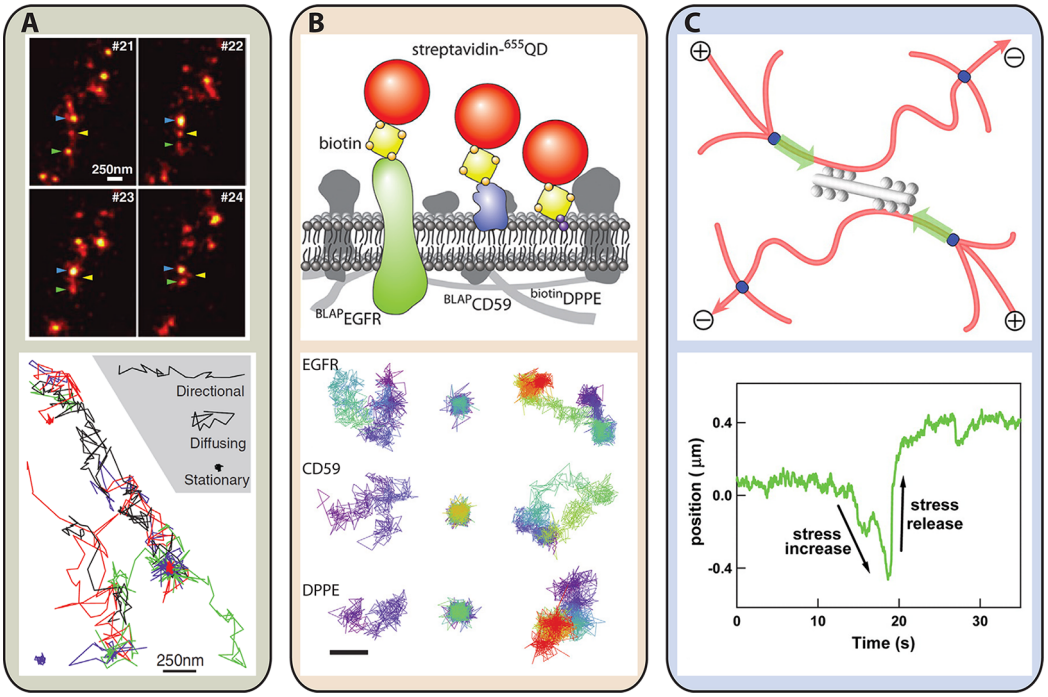

Figure 3. Heterogeneous dynamics in membranes and reconstituted systems. (A) Upper images show stimulated emission depletion images of fluorescently labeled synaptic vesicles. The lower plot shows the two-dimensional vesicle motility, which exhibits signs of stationarity, directional motion, and normal diffusion. Reprinted with permission from ref 2. Copyright 2008 American Association for the Advancement of Science. (B) Upper sketch shows a schematic drawing of the epidermal growth factor receptor (EGFR) transmembrane protein, the CD59 lipid anchored protein, and the DPPE glycerophospholipid (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine), all specifically attached to quantum dots. The lower figure shows the traces of these proteins and lipids inside the membrane of mouse fibroblast cells, the scale bar is 400 nm. Reprinted from ref 20. Copyright 2013 American Chemical Society. (C) Upper drawing shows how the sliding of myosin filaments along actin cables generates tension in the actin network. Lower graph shows that the contractile event is detected by probe particles embedded in the actin network. Reprinted with permission from ref 1. Copyright 2007 American Association for the Advancement of Science.

Membrane Dynamics. Membranes are essential components of living systems, and the lipids composing the membranes as well as the proteins embedded therein display highly interesting dynamics at different levels. Within the two-dimensional membrane sheet, proteins undergo different types of lateral diffusion dictated by the phase state of membrane domains as well as by interactions with cytoskeletal elements (as sketched in Figure 2). Inside the cytoplasm, trafficking of endosomal membranes takes place by vesicle diffusion and active transport through the cytoplasm, a process which is important to understand in the context of nanomedicine delivery.[19] Super-resolution microscopy techniques enable localization of multiple fluorescent markers with spatial resolution down to 20 nm, and are thus powerful tools to extract time series displaying heterogeneous dynamics from living and soft-matter systems. Recently, experiments performed using stimulated emission depletion (STED) microscopy revealed that synaptic vesicle mobility in the boutons of axons performs stick-and-diffuse motion due to the transient association of vesicles with cellular elements[2] (Figure 3A). The data analysis used in ref 2 relied on both temporal averaging and some user-defined thresholds to identify 'hot spots' where vesicles became transiently trapped. The WLA method presented in this issue of *ACS Nano*[7] would enable a more objective treatment of the raw data obtained from super-resolution microscopy and could likely retrieve new hidden dynamics embedded within the time traces acquired.

New developments with ultrasensitive high-speed cameras have allowed tracking of membrane proteins diffusing in the two-dimensional plasma membrane with impressive time resolution[20,21] (Figure 3B). Data from such experiments has fundamentally contributed to our understanding of how the underlying actin matrix interacts with the membrane and the cytoplasmic domain of transmembrane proteins. The cytoskeleton splits the membrane into compartments and causes the membrane proteins to perform hop-diffusion between the individual membrane compartments;[21] examples of such hop-diffusion motility within membranes are shown in Figure 3 for a transmembrane protein (epidermal growth factor receptor, EGFR), a lipid-anchored protein (CD59), and a glycerophospholipid (1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, DPPE).[20] In this context, an unresolved question remains regarding the effect on the diffusional motion from disputed membrane rafts. It is known that diffusion in liquid disordered domains

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is only approximately twice that in liquid ordered domains (resembling these rafts) and it is rather challenging to detect the dynamic partitioning of lipids or proteins between different domains. Here, wavelet analysis could be an appropriate analytical tool to improve the analyses of such data and to contribute to the understanding of the nanoscale membrane structure and motility within membranes.

***In Vitro* Reconstituted Biological Systems.** To elucidate the cellular dynamics and cytoskeletal architecture in a more detailed manner, it is often beneficial to design and to study an artificial system composed of only a few essential components selected from the complex *in vivo* system. A simplified *in vitro* reconstituted and minimal system allows for investigation of the influence of one well-controlled parameter at a time. For instance, the influence of the network structure on tracer diffusion has been studied successfully in an actin network where the actin concentration was varied in a controlled manner.[22] In this and other *in vitro* studies of crowded environments, subdiffusion appears to be the predominant passive motility mode. To elucidate how myosin motors collectively remodel an actin network, the beforementioned experiment was further developed by adding molecular motors to the actin network.[1,23] Myosin motors from bundles would walk along the actin filaments and create substantial tension in the actin network, as depicted in Figure 3C. In the analysis of Stuhrman *et al.*,[23] they used thresholds to segment the time series locally to discriminate between oriented and random motion. In general, even reconstituted biological systems display rich heterogeneity and could benefit greatly from WLA to extract quantitative information of the hidden dynamics.

OUTLOOK AND FUTURE CHALLENGES

Wavelet analysis has by no means yet reached its full potential in the life sciences. A combination of WLA and new experimental techniques, like super-resolution microscopy, provides powerful tools to unravel detailed kinetics embedded within time series recordings of processes in living systems. The examples presented above point to the fact that there already exists a huge amount of data on anomalous diffusion of tracers displaying heterogeneous dynamics. Although much valid analysis has been done, existing data cries out for more sophisticated methods of analysis that are capable of extracting the underlying interesting heterogeneous dynamics, which are the footprints of the biological processes. It is straightforward to use the multiscale wavelet method, presented in the current issue of *ACS Nano*, to this overwhelming amount of existing data and thereby uncover fundamental new insights hidden in the original analyses.

Conflict of Interest: The authors declare no competing financial interest.

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REFERENCES AND NOTES

1. Mizuno, D.; Tardin, C.; Schmidt, C. F.; Mackintosh, F. C. Nonequilibrium Mechanics of Active Cytoskeletal Networks. *Science* **2007**, *315*, 370–373.
2. Westphal, V.; Rizzoli, S. O.; Lauterbach, M. A.; Kamin, D.; Jahn, R.; Hell, S. W. Video-Rate Far-Field Optical Nanoscopy Dissects Synaptic Vesicle Movement. *Science* **2008**, *320*, 246–249.
3. Pierce, S. K.; Liu, W. The Tipping Points in the Initiation of B Cell Signalling: How Small Changes Make Big Differences. *Nat. Rev. Immunol.* **2010**, *10*, 767–777.
4. Arcizet, D.; Meier, B.; Sackmann, E.; Rädler, J.; Heinrich, D. Temporal Analysis of Active and Passive Transport in Living Cells. *Phys. Rev. Lett.* **2008**, *101*, 248103.
5. Daubechies, I. *Ten Lectures on Wavelets*; SIAM: Philadelphia, PA, 1992.
6. Yang, H. Detection and Characterization of Dynamical Heterogeneity in an Event Series using Wavelet Correlation. *J. Chem. Phys.* **2008**, *129*, 074701.
7. Chen, K.; Wang, B.; Guan, J.; Granick, S. Diagnosing Heterogeneous Dynamics in Single Molecule/Particle Trajectories with Multiscale Wavelets. *ACS Nano* **2013**, DOI: 10.1021/nn402787a.
8. Percival, D. B.; Walden, A. T. *Wavelet Methods for Time Series Analysis*; Cambridge University Press: Cambridge, U.K., 2006.
9. Barkai, E.; Garini, Y.; Metzler, R. Strange Kinetics of Single Molecules in Living Cells. *Phys. Today* **2012**, *65*, 29.
10. Tolić-Nørrelykke, I.; Munteanu, E.-L.; Thon, G.; Oddershede, L.; Berg-Sørensen, K. Anomalous Diffusion in Living Yeast Cells. *Phys. Rev. Lett.* **2004**, *93*, 078102.
11. Golding, I.; Cox, E. Physical Nature of Bacterial Cytoplasm. *Phys. Rev. Lett.* **2006**, *96*, 098102.
12. Schnitzer, M. J.; Block, S. M. Kinesin Hydrolyses One ATP per 8-nm Step. *Nature* **1997**, *388*, 386–390.
13. Bronstein, I.; Israel, Y.; Kepten, E.; Mai, S.; Shav-Tal, Y.; Barkai, E.; Garini, Y. Transient Anomalous Diffusion of Telomeres in the Nucleus of Mammalian Cells. *Phys. Rev. Lett.* **2009**, *103*, 018102.
14. Jakociunasa, T.; Jordo, M. D.; Mebareka, M. A.; Bunnera, C. M.; Verhein-Hansen, J.; Oddershede, L. B.; Thon, G. Subnuclear Relocalization and Silencing of a Chromosomal Region by an Ectopic rDNA Repeat. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, in press.
15. Tejedor, V.; Bénichou, O.; Voituriez, R.; Jungmann, R.; Simmel, F.; Selhuber-Unkel, C.; Oddershede, L. B.; Metzler, R. Quantitative Analysis of Single Particle Trajectories: Mean Maximal Excursion Method. *Biophys. J.* **2010**, *98*, 1364–1372.
16. Jeon, J.-H.; Tejedor, V.; Burov, S.; Barkai, E.; Selhuber-Unkel, C.; Berg-Sørensen, K.; Oddershede, L.; Metzler, R. *In Vivo* Anomalous Diffusion and Weak Ergodicity Breaking of Lipid Granules. *Phys. Rev. Lett.* **2011**, *106*, 048103.
17. Leijnse, N.; Jeon, J. H.; Loft, S.; Metzler, R.; Oddershede, L. B. Diffusion Inside Living Human Cells. *Eur. Phys. J.: Spec. Top.* **2012**, *204*, 75–84.
18. Weigel, A. V.; Simon, B.; Tamkun, M. M.; Krapf, D. Ergodic and Nonergodic Processes Coexist in the Plasma Membrane as Observed by

Single-Molecule Tracking. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6438–6443.
19. Zhao, F.; Zhao, Y.; Liu, Y.; Chang, X.; Chen, C.; Zhao, Y. Cellular Uptake, Intracellular Trafficking, and Cytotoxicity of Nanomaterials. *Small* **2011**, *7*, 1322–1337.
20. Clausen, M.; Lagerholm, B. Visualization of Plasma Membrane Compartmentalization by High-Speed Quantum Dot Tracking. *Nano Lett.* **2013**, *13*, 2332–2337.
21. Kusumi, A.; Shirai, Y. M.; Koyama-Honda, I.; Suzuki, K. G. N.; Fujiwara, T. K. Hierarchical Organization of the Plasma Membrane: Investigations by Single-Molecule Tracking *vs.* Fluorescence Correlation Spectroscopy. *FEBS Lett.* **2010**, *584*, 1814–1823.
22. Wong, I.; Gardel, M.; Reichman, D.; Weeks, E.; Valentine, M.; Bausch, A.; Weitz, D. Anomalous Diffusion Probes Microstructure Dynamics of Entangled F-Actin Networks. *Phys. Rev. Lett.* **2004**, *92*, 178101.
23. Stuhrmann, B.; Soares e Silva, M.; Depken, M.; MacKintosh, F. C.; Koenderink, G. H. Nonequilibrium Fluctuations of a Remodeling *In Vitro* Cytoskeleton. *Phys. Rev. E* **2012**, *86*, 020901.