

Single-molecule science: an emerging discipline

Katharina Gaus and
Sue Min Liu

(University of New South
Wales, Australia)

All forms of life have a molecular basis. A cell—the smallest unit of life—is essentially a bag full of molecules. But simply putting molecules together in a bag will not create a life. So where is the magic? What brings a bag full of molecules to life?

To get closer to the point at which a bunch of molecules take on a coordinated process, become an integrated network and, ultimately, a self-regulating biological system, we use a hybrid discipline coined single-molecule science. This is an emerging field in which we aim to combine broad perspectives from studying whole systems and networks with investigations at the molecular scale (Figure 1).

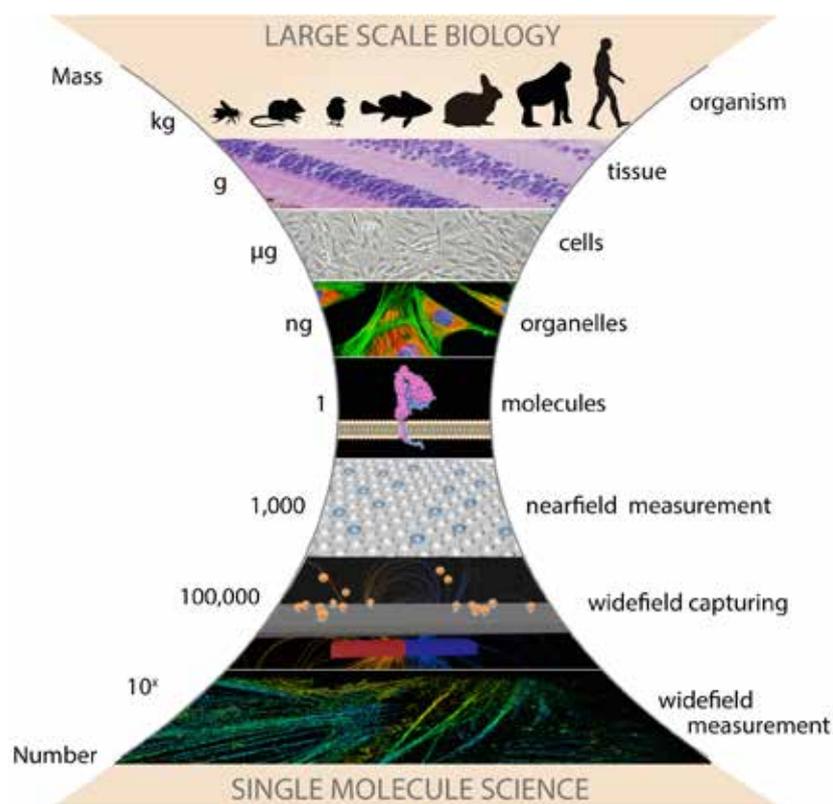


Figure 1. The scale of biological investigations. Analytical techniques used to measure whole organisms down the scale to single molecules; the complexity of the data collected increases as the scale of the subject increases. Figure from *Angew. Chem. Int. Ed.* (2016) **55**, 11354–11366; reproduced with permission. Copyright 2016 Wiley.

The secret life of ants

The challenge of single-molecule science is to retain the molecular details—for example, the protein motif that confers specificity to binding events—and capture the many functional possibilities of individual molecules in the complex and crowded milieu of a cell. How can we combine nanoscale information, on a sub-second timescale, with a system that occurs on the micron scale and operates in hours or even days?

Imagine an ant colony in the forest; how do we go about finding out what makes the colony work? A ‘top-down’ approach would be to analyse the whole ecosystem: the number and types of plants in the vicinity of the ant colony, the soil composition, the climate and so on, and then correlate the number of ants in relation to these aspects of the forest. Alternatively, a ‘bottom-up’ approach would see us studying the anatomy and behaviour of individual ants. Both approaches will yield valuable information, but we doubt that either of them, in isolation, would reveal the underlying functional dynamics of a colony.

Magic at the crossroads

An intersectional discipline would create a new language and a powerful way of investigating biological science that merges traditional analysis methods and draws upon their disparate strengths to provide new perspectives (Figure 2). To us, single-molecule science represents a new platform on which to work together to build on the rigour and culture established in the traditional disciplines over generations, to forge a new language for understanding biology. We see a convergence between the more traditional ‘top-down’ disciplines of physiology and cell biology with the ‘bottom-up’ disciplines of structural biology and biophysics as the key to understanding the dynamics of life.

Experiments in physiology and cell biology often start by isolating tissues and cells from an animal, and analysing the constituent parts. The ‘omics’ revolution—including

genomics and proteomics—has given us a wealth of information about the molecular building blocks of life, and advanced bioinformatics has unearthed more and more of the characteristics of cell types, developmental stages and pathological conditions, almost on a daily basis. Excitingly, many of the ‘omics’ technologies have now been adapted for the analysis of single cells, shedding new light on cell-to-cell variability. Such comparisons between cells may be pivotal to helping us figure out which key elements are required to give life to a bag of molecules.

In recent years, the ‘bottom-up’ disciplines of structural biology and biophysics have accelerated enormously, making it possible to visualize the structure of ever larger multi-molecular complexes, and map fast, and often reversible, dynamics of molecular assemblies. New imaging technologies have been instrumental in what is termed the ‘resolution revolution’. Cryo-electron microscopes now achieve atomic resolution (see ‘The beginner’s guide to cryogenic electron microscopy’ elsewhere in this issue), and fluorescence microscopes, including single-molecule localization, and lattice light-sheet microscopes, allow us to see single molecules in intact cells and record videos of subcellular dynamics with breathtaking clarity (Figure 3). In our opinion, there is no better time than right now to initiate crosstalk between these disciplines; this will undoubtedly provide a completely different view of biological systems.

Rules for individuals shape the entire system

For a system with many individual units, such as an ant colony, it is useful to have a conceptual framework that is based on simple rules. For example, if we want to know how ants operate without a leader, or a blueprint, to build highways through the forest that allows them to ferry food back to the central anthill (Figure 4), we can postulate three simple rules:

- **Rule 1:** ants forage for food and return to the anthill randomly.
- **Rule 2:** ants that find food will carry the loot back to the anthill via the shortest possible route.
- **Rule 3:** ants deposit scent markers, or pheromones, along their tracks at regular intervals for others to follow either to the food source, or back to the colony.

Since it is nearly impossible to follow random tracks of many individuals, the third rule ensures that only the paths of ants carrying food back to the colony are reinforced, resulting in a highway that connects the anthill to a food source. Once all the food at one source is depleted, neither rule 3 nor rule 2 apply anymore, so ants forage



Figure 2. View at the crossroads. Research at the points where traditional disciplines intersect provides a whole new perspective to biological problems. Photo by Mike Enerio on Unsplash.

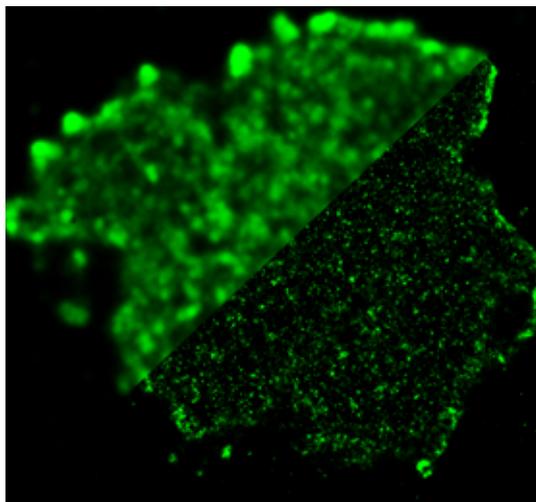


Figure 3. Molecular vision with super resolution. High-resolution microscopes like photoactivated localization microscopy (PALM) allow us to see single molecules in intact cells (bottom half of cell) compared with conventional imaging in which the details are a blur (top half of cell).



Figure 4. Rules for individuals can shape entire systems. Ants in a colony are analogous to single molecules in a cell, where their concerted behaviour governs cellular responses. Photo by Vlad Tchompalov on Unsplash.

randomly once more, and the previous highway markers evaporate. This example illustrates how a few simple rules of behaviour for individuals result in grand-scale structure and coordinated movement, the hallmarks of a society. The rule-based concept also confers flexibility and resilience to the system as a whole, as no single decision by an individual ant would threaten the entire colony.

In our analogy of the ant colony, an effective experimental approach could be to observe the colony at work, that is, map the location and movement of ants over time. Simply tracking a single ant, if that ant is in its natural environment, may not reveal the rules that underpin the formation of highways, nor is it helpful to watch a few ants in a sterile and controlled environment. Instead, we obtain better insights observing the behaviour of many individual ants, in relation to each other, in their natural ecosystem. The equivalent is true for single-molecule science. Despite the naming of this approach, we observe as many molecules in a living cell as we can, not just one molecule, or the average of those molecules in a system. It is not surprising that fluorescence microscopy has become the tool of choice for many of us since it is compatible with live cell imaging, and the fluorescent markers allow us to selectively observe the molecular species under investigation. However, we have now reached a point where the images contain more information than we can see by eye, or interpret manually. We thus need computer vision and data scientists to extract the wealth of biological information imbedded in these data.

Making sense of the molecular chaos

Identifying the underlying rules of a cell—or an ant colony—is not easy. Part of the challenge of single-molecule science is to develop the concept and collect the experimental evidence in parallel. As our technologies get closer to the molecular level, and our data captures more and more of the biological complexities, we will undoubtedly need new concepts and frameworks. We therefore welcome theoreticians, computational modellers and data scientists to join the conversation. For example, molecules inside a cell can encounter different spaces and types of spaces: a cytosolic protein that reversibly binds to actin filaments toggles between exploring 3D and 1D spaces; and a transcription factor in the nucleus diffuses through a cave-like space that may encode information of

DNA binding sites. In other words, living systems in which molecules operate, like inside cells, are vastly different to the controlled and homogeneous environment of test tubes. This distinction has important implications. Like the ant highways, the actin cytoskeleton is also constantly rebuilding while it continues to confer mechanical stability to the cell. On the macroscopic scale however, it is hard to imagine how the walls of a house could continue to hold up the roof while the bricks are constantly shuffling around. The relationship between the individual parts and whole system must therefore be very different in the single-molecule world.

An often-overlooked aspect of living systems is that molecular reactions take place far from equilibrium. Most of us are familiar with reaction kinetics that achieve a state of stability at equilibrium. In thermodynamics, far-from-equilibrium is a type of dynamic equilibrium in which the state of a system is constantly changing over time. The term is also used in social science, or to describe phenomena like swirling storms and swarming schools of fish. Can far-from-equilibrium concepts explain the diversity in signalling reactions? In cell signalling, there is convincing evidence that one type of receptor can activate multiple different signalling pathways leading to different, and often opposing, cellular outcomes. Advanced proteomics analysis can identify the multiple interaction partners across various pathways for this receptor. Is it possible that the fluctuating concentrations of binding partners in a living cell gives rise to a far-from-equilibrium system that ultimately determines cellular function and cell fate? We would only be able to answer this, and related questions, when experimental cell and molecular biologist collaborate with experts in condensed matter and statistical mechanics.

So, what exactly is single-molecule science? For us, it is a growing conversation at the crossroads of many disciplines with a common goal to understand the relationship between the building blocks of life and the living system as a whole.

We thank the University of New South Wales, Sydney, for establishing one of the first research centres dedicated to single-molecule science in 2015, and realizing that a cell is, of course, more than a bag full of molecules. We deliberately chose an unusual name to start a conversation on the direction and vision of molecular biosciences in the 21st century. ■



Scientia Professor Katharina Gaus is an NHMRC Senior Research Fellow at the University of New South Wales (UNSW, Sydney) and is the founding Head of the EMBL Australia Node in Single Molecule Science. She is also the Deputy Director of the ARC Centre of Excellence in Advanced Molecular Imaging (2014–2020). Katharina received her PhD from the University of Cambridge in 1999 and has led an independent research group since 2005. She was awarded the Gottschalk Medal from the Australian Academy of Science (2012), the New South Wales Science and Engineering Award for Excellence in Biological Sciences (2013), and the Khwarizmi International Award (2018). Email: k.gaus@unsw.edu.au



Dr Sue Min Liu is a former researcher turned science communicator. She was awarded her PhD in Immunology (2005) from the University of New South Wales (UNSW, Sydney). Funded by a C.J. Martin Overseas Biomedical Fellowship from the NHMRC, Sue completed postdoctoral training at Harvard Medical School and Brigham and Women's Hospital in Boston, MA, USA (2009), and then at the Garvan Institute of Medical Research in Sydney, Australia (2014). Sue now holds dual roles as Communications Officer at the EMBL Australia Node in Single Molecule Science at UNSW, Sydney, and Associate Coordinating Editor for ACS Sensors. Email: sue.liu@unsw.edu.au

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