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Relevance of Single Cell and Single Molecule Studies at Different Biological and Physical Length Scales

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Abstract

Scale transcends multiple levels of biological and physical organization and is the critical factor that determines success of any scientific investigation. Specifically, the scale at which a question is posed plays a crucial role in influencing the type of experiments and apparatuses needed. Single cell and single molecule experiments came to the fore of experiment science due to its capability at addressing a fundamental problem in biology and physical science: does the same behaviour in cells and molecules transcend different length and population scales? Thus far, single cell experiments could be achieved with trapping of single cell using optical tweezer traps and microfluidic channels. The same, however, is not true for single molecule studies, which remains in the realm of theoretical and simulation studies. Specifically, single molecule experiment remains at the hundreds to thousands of molecules level, where possible skew in the population of molecules sampled could provide a false depiction of molecular reality of a larger population. But what do scientists learn from single cell and single molecule studies? Is it the uncovering of mysteries of the probabilistic behaviour at the single entity level, guided by perhaps quantum mechanics? The answer is no for single cell studies, given that cellular decision making require the input of tens to hundreds of molecular sensors and effectors within a cell. Hence, single cell behaviour is not random, but directed at a nutrient or concentration gradient or signalling source. On the other hand, enzymatic catalysis of a single molecule substrate with the active site involves a quantum mechanical crosstalk. Thus, reaction between the substrate molecule and the active site of an enzyme proceeds if suitable energy levels (i.e., quantum mechanical states) are found for both parties. Given that distribution of quantum mechanical states is probabilistic, stochasticity rules single molecule interaction such as a covalent bond formation reaction between reactant A and B. Thus, single cell and single molecule studies do hold relevance in biological and physical sciences research if the correct experiment tool is used for a pertinent question at an appropriate length and population scale. For example, while tremendous amount of basic understanding could be derived from single cell experiments, single cell perspective is not relevant to questions examining the interactions between two large subpopulations of cells. Single molecule experiments, on the other hand, remains in the theoretical and simulation realm for highlighting the effect of quantum mechanics in guiding the behaviour of molecules at the nanoscale.

Keywords: Single cell; Single molecule; Length scale; Population scale; Subpopulation; Quantum mechanics; Stochastic behavior; Aggregate behavior; Single cell trapping; Skew in sampling

Improved sensitivity has driven the articulation of experimental design to the single cell and single molecule level [1,2]. Specifically, there is a preponderance of single molecule and single cell studies in top journals since the trend first started about a decade ago. Motivated by the curiosity to understand molecular level processes at the single molecule level, where statistical distribution meant that not all molecules behave in the same way or participate in each reaction, single molecule studies are aided by the advent of high sensitivity mass spectrometry tools and spectroscopy techniques that allow the investigation of molecular processes at the hundreds to thousands of molecules level [3-5]. However, instruments remain as accessories of research without careful thinking and experiment design aimed at addressing the problem at hand in research. Specifically, single molecule studies, if not properly

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designed would easily lose relevance for the experiment system under investigation; for example, delivering observations based on small population size of molecules when large system scale analysis is needed to solve a macroscopic riddle in science. At its heart, single molecule studies provide scientists with a lens to compare macroscopic observations with molecular data at the hundreds to thousands of molecules level to elucidate new understanding as single molecule interact with each other. More importantly, given the probabilistic nature of molecular behaviour at small population size, single molecule studies provide a unique perspective on how stochastic effects influence chemical reactions or molecular binding that, in aggregate, manifest as a macroscopic event such as a high yield of a chemical from a reaction, or the high adsorption of copper on seaweed surface. Doing so allow the scientist to develop conceptual and mathematical models on which computer simulations could be performed to understand the behaviour of the system under different environmental conditions. Thus, what is single cell and single molecule studies? Can we achieve single molecule resolution in investigating an experiment system? The answer depends on the type of analytical tool used. With cellular studies, modern optical tweezer approaches together with microfluidics technique afford the ability of trapping single cell for subsequent analysis such as single cell proteomics, transcriptomics, or genomics [6-8]. On the other hand, achieving single molecule sensitivity in analytical sciences is significantly more difficult. Specifically, no technique at present is able to perform single molecule studies. What manifest as single molecule studies are typically experiments interrogating hundreds to thousands of molecules, where a statistical distribution remains in the non-concordant behaviour of the molecules in aggregate. However, given the significant reduction in sample size compared to the macroscopic, there could be substantial skew in the behaviour of the molecules under analysis, which do not provide a real snapshot of molecular behaviour at the small sample size level. Given that the goal of single molecule studies is in understanding how individual molecules behave at the microscopic level, the presence of skewness in the subset of molecules analyzed by contemporary high sensitivity techniques meant that molecular data derived from single molecule studies would need to be interpreted with caution. Specifically, data obtained should be from multiple small samples of the same population of pooled molecules, which provides a statistical average snapshot of how small subset of molecules behave in relation to an analyzed parameter such as electrical charge. But what is the purpose of single cell or single molecule studies with respect to the macroscopic phenomenon? Or, from another perspective, do single cell and single molecule studies hold relevance for understanding biological or physical phenomena, which occurs in the aggregate, and whose effects manifest at the macroscopic scale? The answer offered by researchers to the above question is to understand the individuality of molecules and cells distinct from that of the aggregate population. However, biological processes occur over a range of length scales, ranging from the single molecule as substrate to an enzyme, to the population level average of an enzyme kinetic rate of reaction. Similarly, while single cell studies offer a rare glimpse at the behaviour of a cell in its environment, more interesting questions lies in understanding how cell-cell interaction aggregates to more easily observable macroscopic phenomenon. Thus, in thinking whether single molecule or single cell studies offer relevance to biological problems, the question of importance is the scale at which an angle of framing the problem is posed. For example, if the problem is posed at understanding cellular behaviour of subpopulations of cells within a larger clonal population, the relevant and interesting spatial and population scale for framing the problem could be between 1000 to 10000 cells. On the other hand, if the purpose is to determine the effect of a metabolite in influencing the cellular differentiation of a subpopulation of cells known to exist within a larger consortium of cells from different species, the relevant population scale for examining the problem may be between 10 to 100 cells. Hence, the chosen population scale for understanding a biological phenomenon depends critically on the minimum number of cells necessary for a particular contextual based action to occur at the cellular level, with or without interactions with other cells.

From another perspective, does the oft-quoted stochastic behaviour of cells and molecules at the single cell and single molecule level manifest microscopically? Does stochasticity guide cellular and molecular behaviour at the low numbers' regime? The answer may not be true at the cellular level, given that cellular decision making at the single cell level is guided by a consortium of sensing and effector mechanisms punctuated at each stage with aggregate number of molecular sensors and effectors. Specifically, the decision to migrate to another site with better nutrient availability is not based on the state of binding of a single sensing or effector molecule. Rather, aggregates of tens to hundreds of sensing or effector molecules would likely be needed to trigger a response at the single cell level. But does current technology afford us the window to look at single molecule binding or reaction at the single cell level? No, the equipment and methodology available lack the analytical capability for detecting binding of a substrate to a single sensing molecule in a single cell. Thus, decision making at the single cell level is an aggregate decision at the molecular level, given that tens to hundreds of sensing and effector molecules coordinate their action for a specific cellular decision.

On the other hand, single molecule studies seek to understand the hypothesized stochastic behaviour of molecules. But do molecules behave in a stochastic manner based on a probability density function? The answer lies in quantum mechanics. For example, take the case of a molecule interacting with an enzyme for a catalytic reaction. Is this a single molecule experiment with respect to both the molecule (substrate) and the enzyme? The answer is no, for the enzyme is an aggregate of atoms that manifest a function due to the coordinated movement of all the constituent atoms. From another perspective, however, is an enzymatic reaction always successful at the single molecule level, i.e., all substrate molecules are converted to product molecules at the active site of the enzyme? The answer is no, because at the quantum state level, conditions may not align for a reaction to occur between a single substrate molecule and the active site of

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an enzyme. As another example, consider the reaction between two molecules, A and B, forming a product. At the quantum mechanical level, the two molecules may collide at a speed and energy level higher than that able to form a covalent bond stably; thus, reaction, when considered at the single molecule level, can be depicted via a probability density function, which introduces the concept of stochasticity.

Hence, what is single cell and single molecule studies? Is it really single cell and single molecule or is it a smaller subset of cells and molecules from a population being studied? The second question needs to be answered first as one digest a research paper. While single cell studies are possible through technologies such as single cell trapping via an optical tweezer or microfluidic channel, the same is not true for single molecule studies, except at the theoretical and simulation level. But what is the relevance of single cell and single molecule studies at the macroscopic level? This depends on the degree in which behaviour observed at the subpopulation level could manifest at the aggregate level. From another perspective, relevance of single cell and single molecule studies depends critically on the type of research question posed and the scale (both spatial and population) at which a problem is tackled. Thus, is there stochasticity at the single cell and single molecule level? The answer is no for the case of a single cell given that aggregate sensing and effector molecules hold the key to cellular decision making at the single cell level. However, the issue is more nebulous at the single molecule level. Depending on the interaction partner of the single molecule, stochasticity or probability could rule the success of the interaction or reaction. For example, an interaction between a single substrate molecule with an enzyme is not stochastic with respect to the enzyme, as the larger molecular complex behaviour is an aggregate of all the atoms that constituted it. On the other hand, interaction between the single molecule with the active site may be ruled by quantum mechanics and is thus stochastic in nature.

Conflicts of interest

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