EDITORIAL

The thermodynamic signature of a single molecule or a single particle in dilute liquids and live cells: Single-Molecule Biophysics & Biochemistry based on the stochastic nature of diffusion

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°In memoriam R.F.-P. (1918-2008).

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INTRODUCTORY REMARKS

In this article, I would like to briefly introduce my work in single molecule analysis in dilute liquids and live cells to a cross-border readership. Motion is fundamental to life sciences. Jittering molecules or particles move in every direction by random collisions amongst themselves or other molecules or particles driven by an effect, studied by Einstein, among others, called, for example, Brownian motion or translational diffusion. In order to describe the stochastic nature of translational diffusion at the molecular scale, we need probability equations. For this reason, I considered the thermodynamic jitter in a small volume of the bulk phase, which I called detection or observation volume. Stochastic thermodynamics rules the physical formulation (Földes-Papp 2006, 2007; Földes-Papp et al. 2005; Baumann and Földes-Papp 2022) Why are the novel formulas so attractive to life scientists?

i) There is the straightforward relationship to the diffusion times of molecules/particles (diffusion coefficients of molecules/particles).
ii) There is the concentration dependency for molecules/particles of the same kind in the bulk phase.
iii) There is the dependency on the size of the detection or observation volume.
iv) The relationships are mainly based on the existence of a continuity equation and the existence of a probability current (Földes-Papp et al., 2005).

The physical phenomenon of translational diffusion and the special case of the Brownian motion were derived from stochastic thermodynamics (Földes-Papp 2007, 2013) so that we can better understand the phenomenon. These mathematical operations enable many new formulas/ expressions for one-, two- and three- dimensional diffusive problems in dilute liquids, live cells as wells as artificial and biological membranes without immobilization/ attachment or significant hydrodynamic flow.

What is new about these approaches based on stochastic thermodynamics and the exact formulas derived from them in single molecule analysis? The key question was how far apart do two molecules / two particles have to be in the time

domain so that the required degree of separation between the two individual molecules / the two individual particles can be quantified at the molecular scale in order to distinguish them as separate entities without immobilization or significant hydrodynamic flow. The novel formulas of single molecule analysis answer exactly this question and enable the new theory of single molecule detection / single particle detection as discussed in the next section.

DISCUSSION OF OUR STUDIES/ PUBLICATIONS AND OTHER REFERENCES

The language of stochastic thermodynamics is the main advantage of the theory of single-molecule detection / single particle detection at the level of the individual molecule / individual particle (Baumann and Földes-Papp 2022; Földes-Papp and Baumann 2011). The deductive reasoning does not require data, but the mathematical derivations are based on experimental facts. This is the effectiveness of the formulas used to precisely describe the real physical and experimental conditions. Exact answers to the key question posed in the above section “INTRODUCTORY REMARKS” of this original article are the Földes-Papp's limits in measurement time, which should NOT be exceeded if you want to measure the selfsame molecule / selfsame particle with high probability in one, two or three dimensions (Földes-Papp 2021).

Some of the resulting consequences are far beyond what can be observed with current technologies and biotechnologies. In other words, the theory and its natural laws have practical uses and consequences because it is the physical theory about the thermodynamic jitter of single molecules/single particles in dilute liquids and live cells without immobilization or significant hydrodynamic flow. It is groundbreaking and fundamental research. Since mathematics provides a way to answer questions about the thermodynamic jitter in a clear, rational manner, with evidence to support it, mathematics is the reliable method necessary to get the best information on the motion of a single molecule / a single particle at the molecular scale in dilute liquids and live cells as well as artificial and biological membranes without immobilization/attachment or significant hydrodynamic flow.

The thermodynamic jitter in liquids and live cells is quantified by the Földes-Papp's limits. Thus, the thermodynamic Földes-Papp's limits are reference measures (so called 'golden standard') for any temporal resolution calculations / algorithms / analyzes based on parameter sets of specific measurement techniques. The previous shortcomings of missing any profound and valid theoretical basis for the thermodynamic jitter of molecules / particles currently characterize all photon counting statistics, the Nyquist limit, single-molecule FRET, fluorescence auto-correlation and two-color cross-correlation spectroscopy, single-molecule localization microscopy / nanoscopy (super-resolution microscopy) / spectroscopy, single-molecule laser scanning microscopy, single-molecule image analysis, triple-color coincidence analysis, single-molecule cluster analysis, etc., which are widely used in single molecule analysis in order to justify whether or not a single molecule / a single particle (individual molecule / individual particle, selfsame molecule / selfsame particle) was measured during the detection / observation time time in dilute liquids or live cells without immobilization on a solid phase or membrane as well as without significant hydrodynamic flow (Baumann and Földes-Papp 2022). The thermodynamic jitter is simply ignored in the present single molecule literature (Földes-Papp 2021). Földes-Papp's limits are formally the counterpart of Abbe’s spatial resolution limit.

The physical theory on individual single molecules was drawn upon experimental experiences and certainities by forming the concept of "THE MEANINGFUL TIME *Tm*" acting AS FUNDAMENTAL NATURAL LAWS OF SINGLE-MOLECULE TIME RESOLUTION of freely diffusing molecules (i.e., with negligible external forces like a significant hydrodynamic flow or without firm binding or attachment (immobilization) on a solid phase like artificial surfaces and biological membranes) in dilute liquids and live cells (3D-diffusional normal and anomalous processes as well as in or on artificial or biological membranes as 2D-diffusional normal and anomalous processes). The physical laws correspond to the mathematical equations, which we present to describe every single molecule / single particle. It must be made crystal clear that the underlying physics and molecular biochemistry can be modified easily but things hardly get worse when there is any trouble with the

mathematics which rules the physical and biological formulations. The advantages of this new theory of single-molecule biophysics and biochemistry, based on individually diffusing molecules in dilute Liquids and live cells, are evident (see also the section "INTRODUCTORY REMARKS" above).

The Brownian motion (normal diffusive systems) and generally speaking the thermodynamic jitter (anomalous diffusive systems) are ultimately the direct or indirect cause of every measurement signal at the molecular scale in diffraction limited and unlimited optical systems performed in dilute liquids and live cells without immobilization or hydrodynamic flow. For example, emitted photons are epiphenomena of the underlying process of thermodynamic jitter of single molecules / single particles at the molecular scale. The 'noise' of the measurement signal in the signal-to-noise ratio does not come from the molecular motion of single molecules / single particles. It comes from the background (for example, autofluorescence) generated by the dilute liquids or the live cells under laser excitation, the laser sources, the detectors and the optical diffraction limited and unlimited setups. So far, studies have limited themselves to examining slow events at the molecular level. Not because slow events are particularly interesting to biology in general but because of how our detectors, laser sources, optical diffraction limited and unlimited setups, i.e. our detection techniques, work.

We unravel common misunderstandings on the role of thermodynamic jitter and provide theoretical insights with significant practical implications, especially on the design and use of time-resolved instrumentation, even if our research and the new theory are not about how detection techniques work (Földes-Papp 2007, 2013, 2015, 2021; Földes-Papp and Baumann 2011).

The so-called 'single molecule' data from the 1990s, 2000s and 2010s form an impressive collection of measurements performed in dilute liquids and live cells without immobilization or without significant hydrodynamic flow (Digman and Gratton 2011; Xia et al. 2013; Yu 2016). These data are of the same type as today's single-molecule data in dilute liquids and live cells without immobilization or without hydrodynamic flow. However, the current state of knowledge suggests a different interpretation, namely that an averaging takes place over many molecules / many particles and for fundamental reasons no statements can be made about the single molecule / single particle that is the individual molecule / individual particle (Baumann and Földes-Papp 2022; Földes-Papp 2021). Thermodynamic jitter is provided as a correction parameter to go from many molecules / many particles to a single molecule / a single particle in the experiments.

HIGHLIGHT

The paper presents properties of the thermodynamic signature of a single molecule or a single particle in dilute liquids and live cells based on the stochastic nature of translational diffusion. Along with these properties, we illustrate the esoteric and abstruse ideas and approaches currently used in single molecule spectroscopy and imaging seemingly devoid of any practical and theoretical values for measuring just one single molecule / particle, which is the selfsame molecule / particle. We hope that our humble scientific work will be well received by the communities of single molecule imaging and spectroscopy and by all users of these technologies as well as biotechnologies in the various and different disciplines. The theoretical basis of the novel formulas is given in the original research articles. The esoteric publications of ignorance presenting abstruse ideas in 'single molecule' studies are exempflified in Földes-Papp 2021 as well as in Baumann and Földes-Papp 2022, they are being replaced with that bright of future.

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