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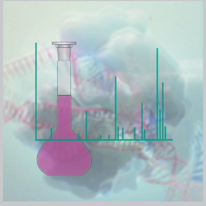

Highlights of Analytical Sciences in Switzerland

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Beyond 3D Structures: New Ways to Study Biomolecular Gymnastics

Sonja Schmid*

**Correspondence:* Prof. Dr. S. Schmid, E-mail: sonja.schmid@unibas.ch
Biomolecular Nano-Dynamics Lab, Department of Chemistry, University of Basel, Mattenstrasse 22, CH-4058 Basel; Swiss Nanoscience Institute, University of Basel, Klingelbergstrasse 82, CH-4056 Basel.

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Traditionally, analytical chemistry has relied on measuring billions of molecules at once, to obtain one characteristic ensemble average. While this approach has underpinned the success of chemistry for centuries, it is naturally limited in its ability to resolve differences between individual molecules. Such differences exist in mixtures as well as in pure samples, *e.g.* if molecules undergo conformational changes or transient interactions (like those essential for biomolecular function). Just as the average posture of all players in the Euro 2025 football tournament fails to capture their individual movements, an ensemble average is similarly insufficient to fully characterize intrinsic molecular heterogeneity.

Therefore, in biophysical chemistry, a range of techniques were established that now enable the investigation of single molecules. For example, mixtures can be studied with the ultimate resolution of one single molecule, as in our work using label-free electrical nanopore recordings.[1] And fluorescence-based experiments can observe single proteins at work: how they undergo intra- and inter-molecular rearrangements to perform their biomolecular function.[2] The resulting single-molecule trajectories uniquely reveal the timing and sequence of distinct functional events (*e.g.* a conformational change), which is usually 'averaged out' and lost in ensemble experiments. This is because the latter rely on observing averaged equilibrium relaxation kinetics, while single-molecule trajectories can provide direct observations under all thermodynamic conditions, including the out-of-equilibrium steady-state of the biological cell, and also thermal equilibrium. Ultimately, these studies offer combined kinetic and thermodynamic descriptions of biomolecular mechanisms, including quantitative kinetic rate constants and free-energy landscapes (Fig. 1).

Recently, our group described a new Förster Resonance Energy Transfer (FRET) approach offering up to 100-fold more information per single molecule.[3,4] We achieved this advance with a trick we term *DyeCycling*, where we continuously replace the fluorescent markers to prevent them from bleaching, which would end the experiment. In this way, we can record the gymnastics of a single biomolecule for over an hour, offering unprecedented kinetic insight and data credibility.

While 3D structural models of biomolecules are readily available today, they lack temporal information to link protein structure and function. **Time-resolved single-molecule techniques, such as DyeCycling, bridge this gap by providing a better mechanistic understanding of biomolecular function, to stimulate progress in biomedicine and biotechnology.**

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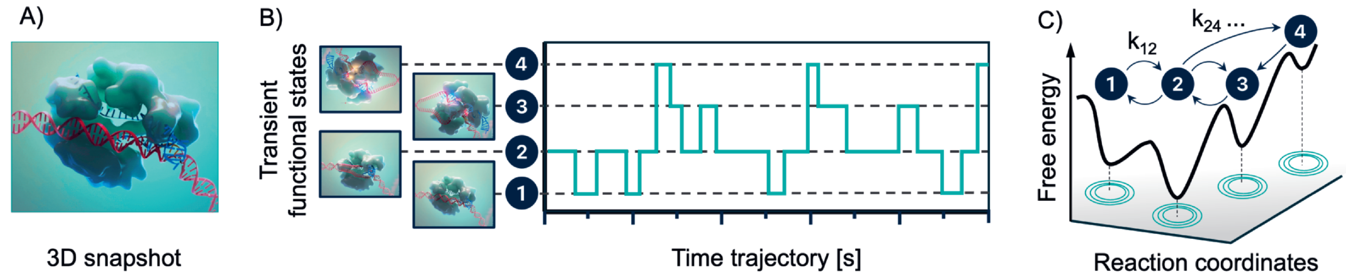

Fig. 1. Bio-macromolecules, such as proteins, DNA, RNA, are dynamic systems. (A) A three-dimensional (3D) structural snapshot represents one out of many functional states, *e.g.* conformational or interaction states adopted by the molecule in solution. (B) Time-resolved single-molecule techniques zoom in on one individual molecule and track these excursions into transient functional states in real time (here schematic states 1 to 4), revealing the timing and order of events. These data offer direct access to kinetics and thermodynamics that would be lost by ensemble averaging: (C) Schematic kinetic model (here 4 states, 6 rate constants k_{ij}) and the resulting free energy landscape. Structures in A and B) adapted from *BioInteractive*, HHMI, 4000 Jones Bridge Road, Chevy Chase, MD 20815.

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Please contact: Dr. Bodo Hattendorf, ETH Zürich, HCI G105, Vladimir-Prelog-Weg 1, CH-8093 Zürich, Tel.: +41 44 632 44 72
E-mail: analytical_highlights@chimia.ch