Nanopores as versatile single-molecule tools

From DNA turbines to protein sequencing

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the challenges in preclinical drug discovery, and present a few real-life drug discovery projects demonstrating the various ways these computational models were able to impact the discovery process.

1585-Wkshp
Computation of conformational transitions and activation of Src tyrosine kinase
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[1]Purdue University, West Lafayette, IN, USA, [2]Van Andel Institute, Grand Rapids, MI, USA.
A protein molecule can adapt different structural forms depending on solution conditions or ligation state. The stable forms can be defined experimentally, but the transition pathways and metastable states between them cannot. A computational method to explore equilibrium conformational transition pathways is the adaptive bias path optimization (ABPO) algorithm. ABPO, implemented in the CHARMM program, differs from other path methods by using enhanced sampling along a path between two known states without restraining the system to the reduced coordinates of the evolving path. ABPO converges to a physically reasonable path even in the case of a highly rugged energy landscape and is amenable to assessing the power of the reduced variables chosen to explore the transition. ABPO was applied to three cases where a conformational transition is localized to one part of the polypeptide chain. With proper selection of the reduced coordinate set, sampling without restraints converged to a physically reasonable path. We also applied ABPO to a more complex transition of Src tyrosine kinase catalytic domain that undergoes a more complex transition between enzymatically down-regulated and activated conformations. The results using coarse-grained and all-atom models identified a switched electrostatic network (SEN) that could confer an entropic advantage to reduce energetic barriers by limiting the search space of the activation loop. The richness in atomic detail provided by the ABPO path reveals how the SEN couples the dynamics of the key αC-helix and the activation loop, which provided a new rationale for the highly conserved HRD sequence of kinases. Further, the results related to energetics of the rotation of the αC-helix provide a rationale for the interactions observed in a number of kinase regulatory complexes, some for which the basis for regulation from inspection of the structure along is puzzling.

Workshop: Developments in Nanopore Biosensors

1586-Wkshp
Probing protein structural dynamics at the single-molecule level by nanopore tweezers
Min Chen.
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Proteins are dynamic entities that sample multiple conformations over a range of time scales. Understanding how proteins work requires a full description of their conformational dynamics over time. Nuclear magnetic resonance (NMR) spectroscopy can measure protein dynamics at multiple time scales, however, application is laborious and limited to relatively small proteins that can be stably prepared at high concentrations. Conformational heterogeneity, such as multiple weakly populated conformational states, is challenging to resolve from ensemble methods such as NMR due to averaging effects. Integrative approaches and single-molecule techniques can be advantageous for revealing transient protein dynamics and functional heterogeneities. Nanopore tweezers have emerged as a powerful, single-molecule analytical tool to monitor the protein's conformational dynamics. Nanopore tweezers confine a single protein within the pore lumen and measure ionic current changes induced by the protein analyte's motion. Here we will present several studies of ClyA nanopore tweezers to showcase their utility in resolving ligand interaction modes, screening allosteric drugs and investigating intrinsically disordered protein interactions.

1587-Wkshp
Ultra-precise single-molecule nanopore tool to watch enzymes at work
Jens H. Gundlach.
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We developed a nanopore tool to measure how enzymes move along nucleic acid chains. In this technique, which evolved from our effort in developing nanopore sequencing, the ion current through an engineered protein nanopore is used to measure the motion of the DNA or RNA. With its unprecedented spatiotemporal resolution this new single-molecule tool resolves sub-millisecond and sub-nucleotide-long enzyme steps while providing the exact nucleotide sequence context within the enzyme. In addition, the tool has high throughput and is relatively simple to set up. We find surprisingly strong sequence-dependence in the kinetics of many types of helicases such as in Hel308, PcrA, RecQ, or the SARS-CoV-2 helicase nsp13. Furthermore, we have recorded data with DNA- and RNA-polymerases and reverse transcriptases. With each enzyme we discover behavior that had not been observable with any other single-molecule technique.

1588-Wkshp
Nanopores as versatile single-molecule tools: From DNA turbines to protein sequencing
Cees Dekker.
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Nanopores offer ample opportunities for studying single biomolecules. I will present some recent examples of nanopore research from my lab: (1) DNA origami turbines powered by nanoscale flow. We demonstrated driven rotary motion of a nanoscale DNA origami turbine which harnesses energy from a water/ion flow generated by a static chemical or electrical potential gradient in a solid-state nanopore. Sustained unidirectional rotary motion of up to 20 revolutions/s were observed. These artificial nano-engines operate autonomously in physiological conditions, converting energy into useful mechanical work. (2) Nanopore-based sequential reading of peptides. We demonstrated a nanopore-based single-molecule peptide reader capable of reliably detecting single amino-acid substitutions within individual peptides. A peptide is linked to a DNA molecule and sequentially pulled through a biological nanopore by a DNA helicase in single amino-acid steps. Stepping ion-current signals enable discrimination of single-amino-acid substitutions in single reads. Notably, we demonstrated the capability to 'rewind' peptide reads, obtaining indefinitely many independent reads of the same molecule, yielding an undetectably low read errors. Recently, we expanded this concept to discriminating single post-translational modifications within peptides of mixed charge. These proof-of-concept experiments constitute a promising basis for the development of a single-molecule protein sequencer.

1589-Wkshp
New pores, new tricks
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Over the past three decades, biological nanopores have been developed for the rapid, low-cost, and portable sequencing of nucleic acids. A wide range of new applications are now been explored, including the sequencing of proteins, and the real-time identification of molecules in complex biological samples. All these applications, however, require developing nanopores with bespoke size, shape, and biophysical properties that can tackle the specific challenges associated with molecular recognition and transport for such a wide variety of analytes. Here I will describe the efforts in our laboratory to control the capture and transport of molecules across nanopores, to identify tiny differences in proteins and to engineer nanopores to recognize molecules in a complex biological sample.

Workshop: Vibrational Tools for Biomolecular and Cellular Studies

1590-Wkshp
Stimulated Raman scattering microscopy: The next frontier of optical imaging
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All molecules consist of chemical bonds, and much can be learned from mapping the spatiotemporal dynamics of these bonds inside cells, tissue and animals. Since its invention in 2008, stimulated Raman scattering (SRS) microscopy has become a powerful modality for imaging chemical bonds with high sensitivity, resolution, speed and specificity. The past dozen years have witnessed the blossoming of SRS microscopy. Herein I will highlight exciting development along single-molecule vibrational imaging, metabolic imaging, and super-multiplexed imaging.