LUMICKS

High-throughput dynamic single-molecule measurements of DNA-binding proteins with DNA repeat assembly

Application note

Introduction

Biological mechanisms are not static or homogeneous, which is why dynamic single-molecule analysis has become a powerful tool to gain a more complete understanding of said mechanisms. However, this lack of homogeneity leads to the need to collect a statistically relevant number of data points to gain meaningful insights. The ability to generate high numbers of results was often a shortfall of the first dynamic single-molecule techniques. This is further compounded by traditional DNA-protein interaction studies which often only incorporate single binding sites into the tethered DNA construct. Therefore, observing enough binding events to obtain sufficient statistics to unambiguously prove a molecular mechanism can be time-consuming.

As a leader in dynamic single-molecule analysis technology, LUMICKS has already launched the C-Trap® Optical Tweezers – fluorescence microscopy system, which combines user-friendly sample handling and automated data analysis, greatly accelerating the collection and analysis of data. Further developments in biochemistry by LUMICKS has led to the development of a new DNA repeat assembly kit. This new kit will deliver the next increase in throughput, leading to the generation of statistically relevant results, in a fraction of the previous time required!

How can dynamic single-molecule analysis accelerate your DNA repair/ replication/editing/transcription research?

- Offers indisputable proof of protein-DNA interactions
- Direct measurements of thousands of site-specific protein-DNA interactions within hours
- Enables direct elucidation of molecular mechanisms in a high-throughput manner
- Get impactful results faster, using fewer methods

DNA repeat assembly, from plasmid to statistically relevant data in half a day

This application note introduces you to a new high-throughput method, called DNA repeat assembly, that enables researchers to quickly build long DNA constructs (up to 100 kb) made of many repeats of a DNA sequence of interest. High-throughput measurements in the C-Trap of fluorescent proteins binding to the target DNA sequence contained in the repeats finally integrates the insights of dynamic single-molecule analysis with high-throughput acquisition of thousands of binding events per experiment. By enabling high-throughput DNA-binding data acquisition at the single-molecule level, the C-Trap becomes a powerful tool to obtain quick answers to questions ranging from the function of the fluorescent proteins to the mechanism of enzymatic activity such as binding kinetics, characterization of binding modes (scanning/diffusion/processive/static), and binding location.

DNA repeat assembly

DNA repeat assembly is a simple two-step reaction that uses a linear DNA sequence of interest with palindromic overhangs, obtained by digestion with common restriction enzymes. During the first step of DNA repeat assembly, fast intermolecular ligation of the sequence of interest leads to the assembly of linear DNA constructs made up of multiple DNA repeats (**Figure 1**). After a brief incubation, a proprietary DNA adaptor containing 5 biotin moieties is added to the mix and incubated to allow ligation of the biotinylated adaptor to the ends of the linear DNA constructs. The result is a mix of biotinylated linear DNA with a distribution of different lengths and numbers of DNA repeats. Finally, this mix is flowed into the C-Trap, where two streptavidin coated beads trapped in the optical tweezers are maneuvered to selectively tether the longest biotinylated DNA constructs with the highest number of DNA repeats. The optimized adaptors enable fast tethering and ensure high stability of the formed tethers, enabling long data acquisition sessions.

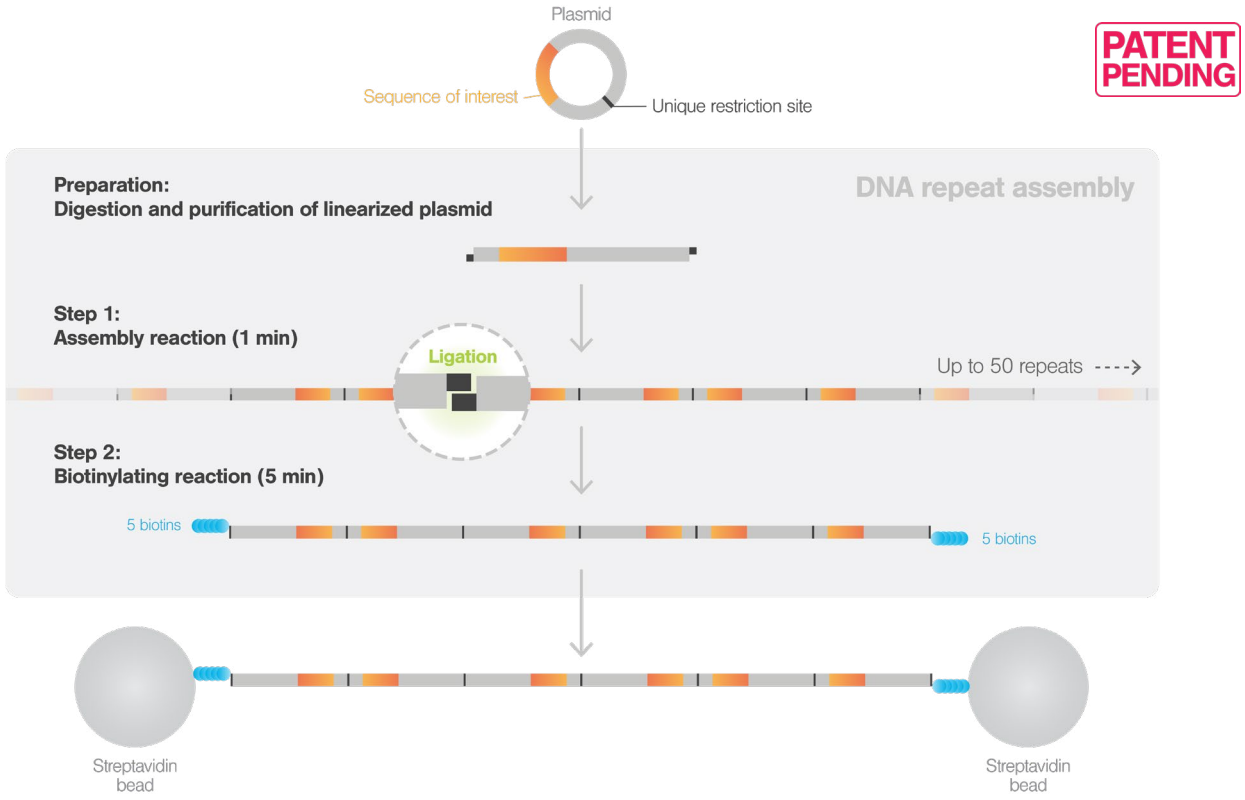


Figure 1 DNA repeat assembly schematic

As proof-of-principle, we show a highly likely scenario for any DNA sequence of interest, which is: 1) the sequence of interest is in a plasmid and 2) the plasmid contains a multi-cloning sequence with unique sites for restriction endonucleases. Here, we chose the Lac Operon (LacO) sequence as the DNA sequence of interest to measure the kinetics of binding of LacI dimer [1] labeled with ATTO 565 in the C-Trap.

> The lactose repressor protein LacI plays a crucial role in regulating the expression of genes involved in lactose metabolism in bacteria. When lactose is absent in the cell, LacI binds to the Lac Operon sequence (LacO) near the promoter and prevents RNA polymerase from transcription of genes involved in the metabolism of lactose.

When long DNA tethers made of more than 50 repeats of the plasmid with the LacO sequence were incubated with ATTO 565-LacI in the C-Trap, the results were unprecedented for a DNA-protein interaction analysis in a dynamic single-molecule set up: thousands of individual binding events of ATTO 565-LacI to the LacO sequence were acquired within a timeframe as short as 2 hours, demonstrating true high-throughput measurements of single-molecule protein binding events to DNA. By using LUMICKS new Lakeview data analysis software, the binding kinetics of 3905 individual events could be quickly analyzed.

DNA tethering and manipulation

Experiments were performed using the C-Trap, equipped with a combination of optical tweezers, microfluidics and confocal microscopy. Data analysis was performed using the Lakeview data analysis software, which allows users to go from raw data to quantitative insights in minutes, greatly reducing the time required for data analysis. To study the interaction between LacI dimer and DNA in real time at the single-molecule level, the LacO-DNA repeat array was flow-stretched and tethered between 4.3 µm streptavidin-coated polystyrene beads using the u-Flux microfluidic system (**Figure 2**). Individual dsDNA molecules containing between 40-60 LacO sequence repeats were tethered between two optically trapped beads and held at various constant forces between 1.5 and 15 pN, while monitoring LacI dimer binding using kymographs as shown in **Figure 3**. Within a little more than two hours of measurement time, a total of 3905 Lakeview-trackable, LacI binding events were obtained, with an average DNA tether length of ~120 kb (50 LacO sequence repeats). Binding events were recorded and tracked using Lakeview, showing how the dissociation rate constant varies with the applied force on the DNA (**Figure 4**). This showcases how the DNA repeat assembly kit enables rapid testing of protein functionality under different conditions, allowing for quick optimization of experimental settings and parameters.

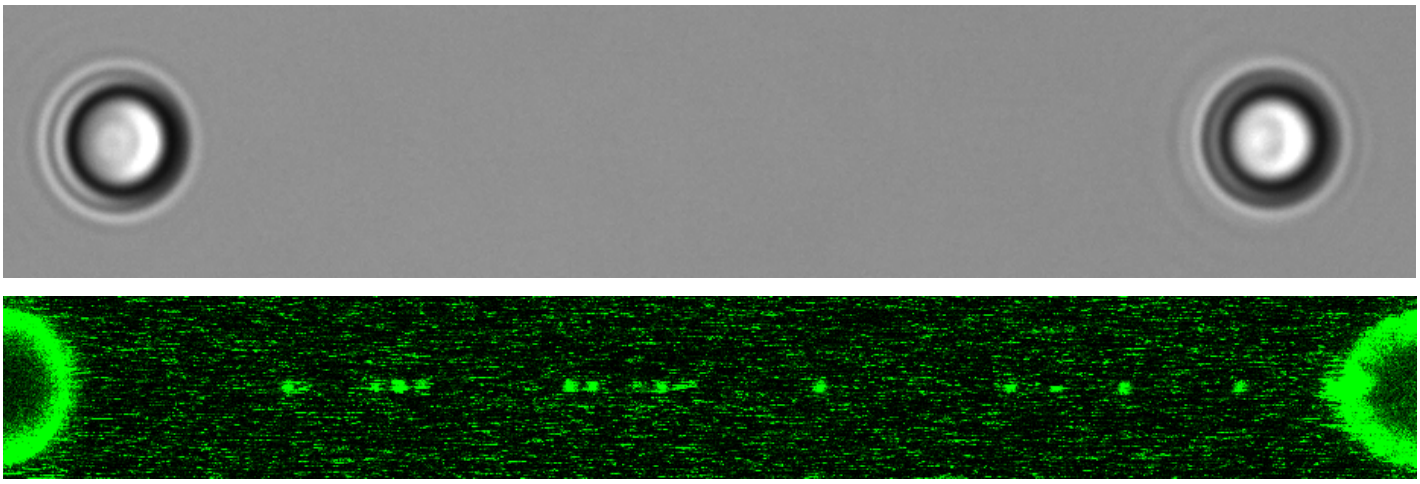


Figure 2 Brightfield image and corresponding confocal 2D image of a 40 µm long tandem DNA repeats construct containing ~60 LacO sequence repeats, showing individual ATTO 565-LacI dimer binding events.

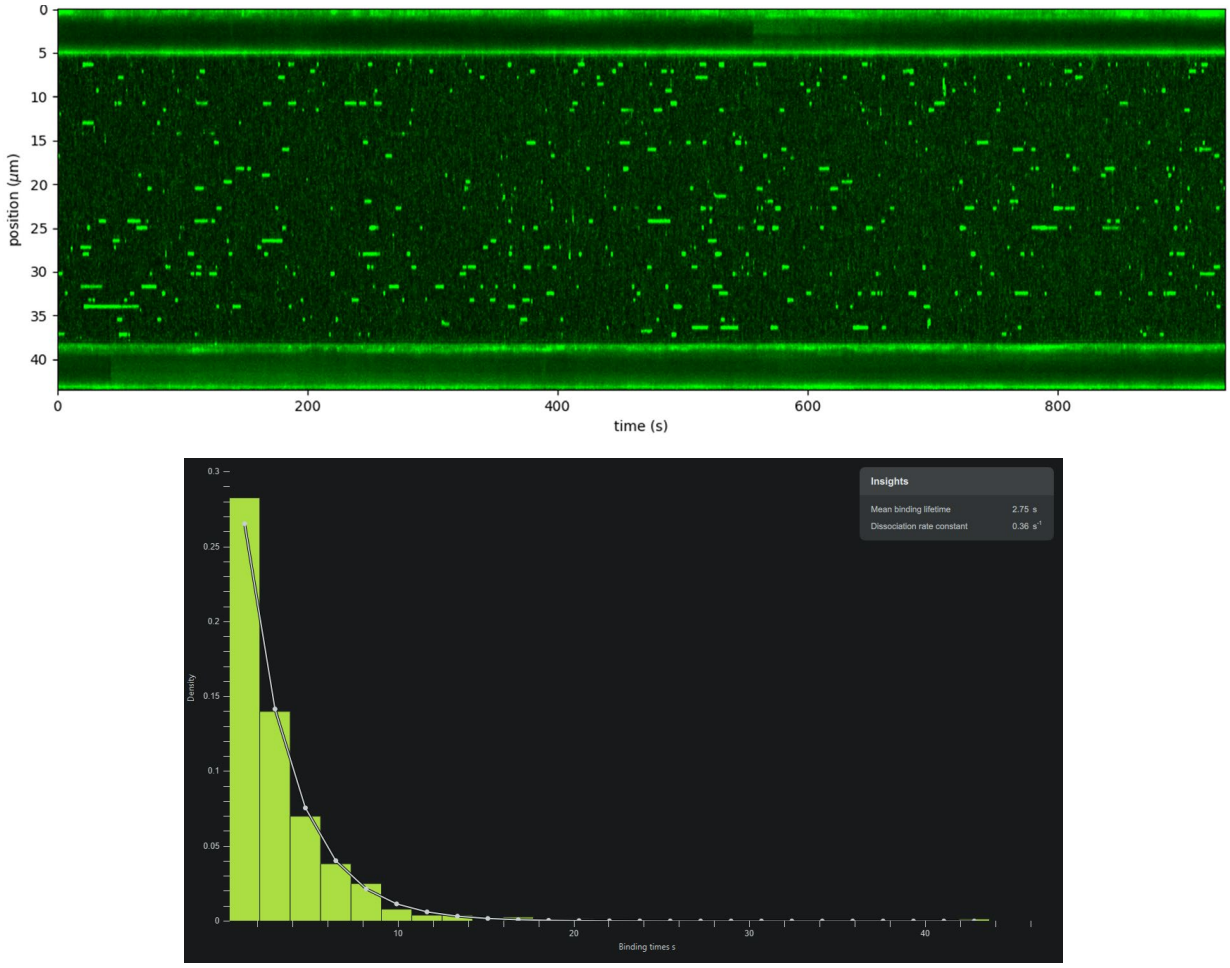


Figure 3 Kymograph of ATTO 565-LacI dimer binding to 100 kb long DNA, held at a constant force of 10 pN for 15 minutes. **Top:** Raw image data. **Bottom:** Lakeview-analyzed binding time distribution with the calculated force-dependent mean binding lifetime and corresponding dissociation rate constant.

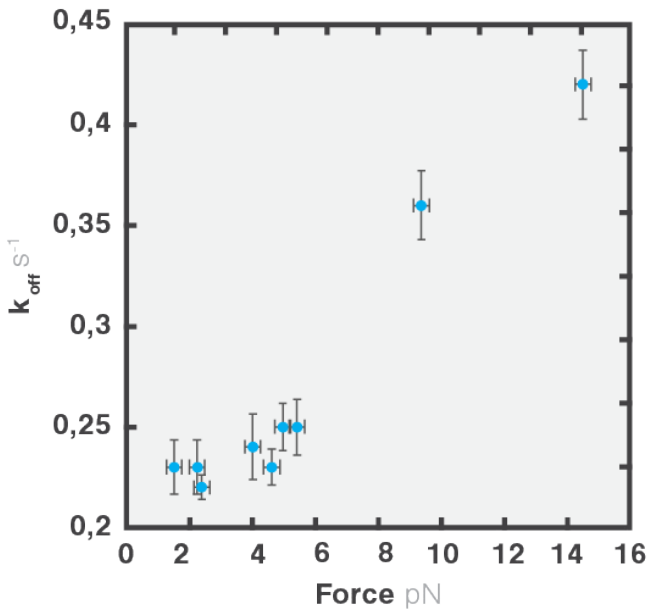


Figure 4 Lakeview-calculated dissociation rate constants showing the force-dependence of LacI-dimer binding to DNA. The dissociation rate constant increases with increased tension on the DNA, revealing how greater forces applied on the LacO sequence destabilizes LacI binding.

Conclusion

Here we demonstrate how utilizing the new LUMICKS DNA repeat assembly kit, in combination with the C-Trap and Lakeview analysis software can lead to a 50-fold increase in experimental throughput. Using Lakeview, it is possible to automatically output all the mechanistically-relevant parameters, such as the protein binding position on the DNA, binding kinetics with binding rates and dissociation rate constants, as well as the measured diffusion coefficient, allowing for fast characterization of mechanism models under different experimental conditions. This approach allows for direct visualizing and tracking of each individual binding event, leading to precise, statistically significant measurements of individual molecular mechanisms, in real time.

The DNA repeat assembly kit, combined with the C-Trap optical tweezer and Lakeview software, enables researchers to generate statistically viable, direct evidence of molecular mechanisms in as little as half a day. It reduces the need to prepare customized biotinylated DNA constructs and the time required to analyze the subsequent results. This approach greatly reduces the challenges of time-consuming biochemistry, challenging operation and complex analysis often associated with earlier dynamic single-molecule experiments, now making these unique and powerful insights accessible to all scientists.

The resulting discovery of mechanisms of DNA-binding proteins will be invaluable in fields such as DNA repair, replication, organization, and transcription. These insights will help to underpin our understanding in these research areas and will support the next wave of scientific breakthroughs.

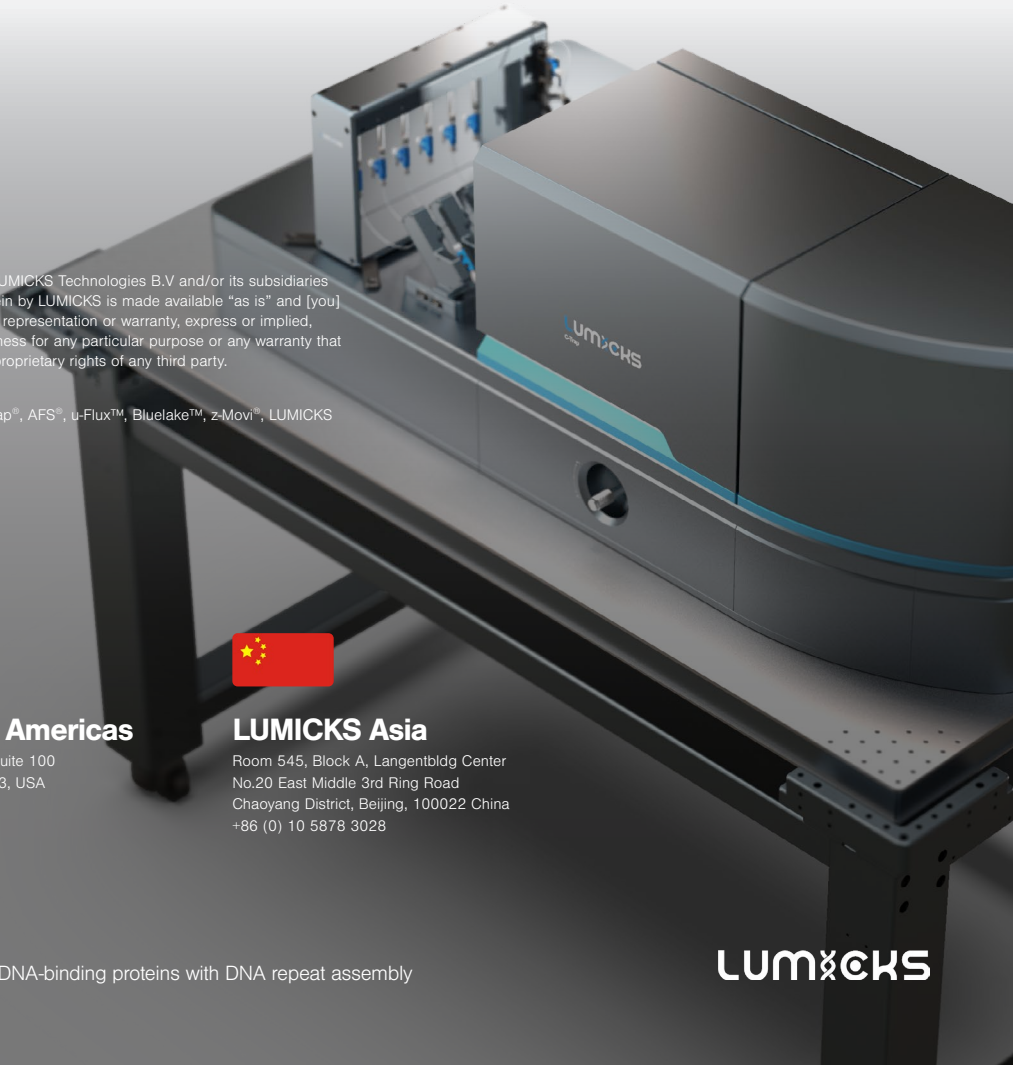
The LUMICKS C-Trap Dymo is designed for **research use only**. Not for use in diagnostic procedures. The system is intended for use by professional users trained in the operation of the C-Trap Dymo.

For more information on the repeat kit, please visit
https://store.lumicks.com/

References

[1] Marklund, E., van Oosten, B., Mao, G. et al. DNA surface exploration and operator bypassing during target search. Nature 583, 858–861 (2020). https://doi.org/10.1038/s41586-020-2413-7

Or find us on:



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