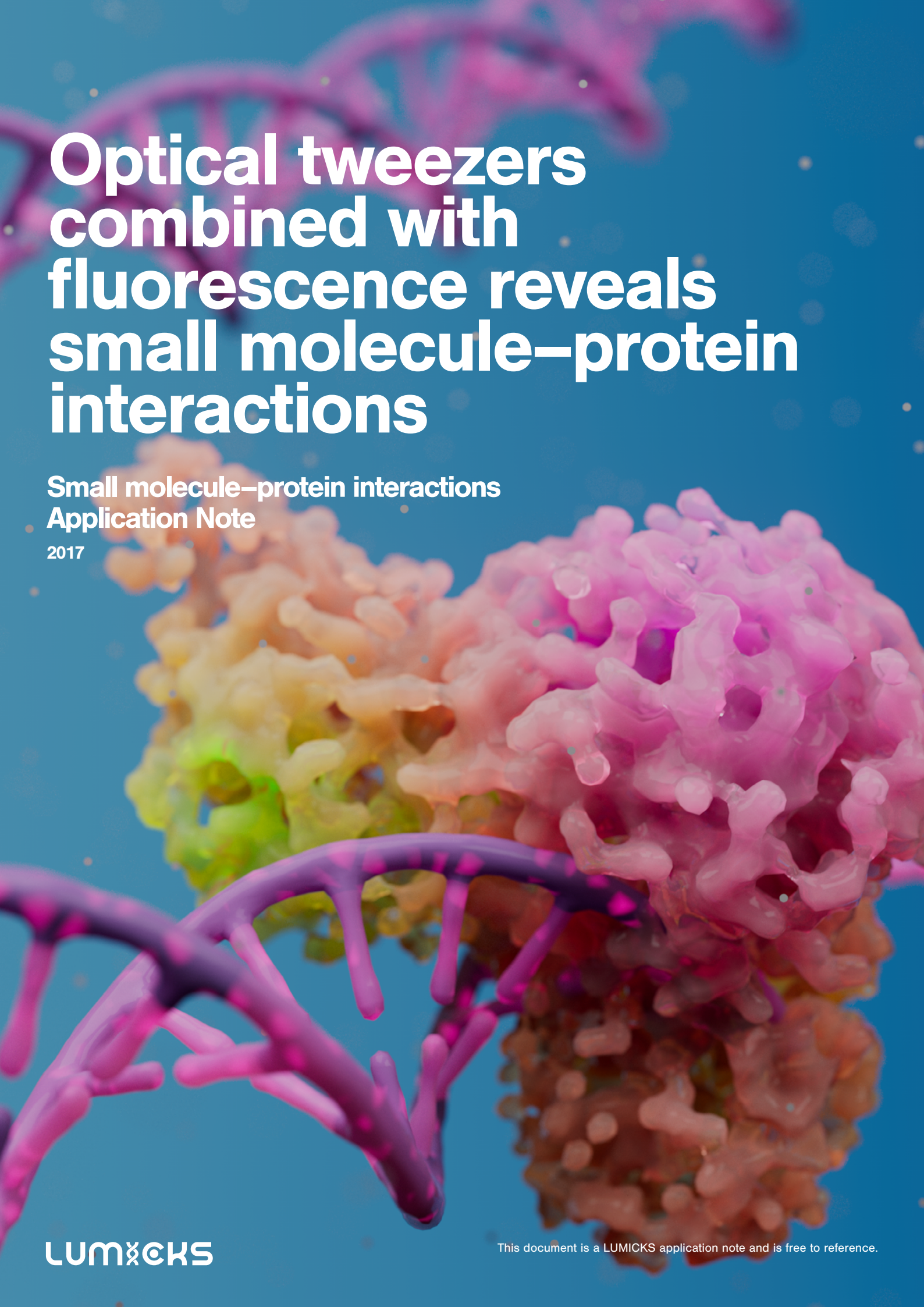
Optical tweezers combined with fluorescence reveals small molecule–protein interactions

Small molecule–protein interactions Application Note

2017



LUMICKS

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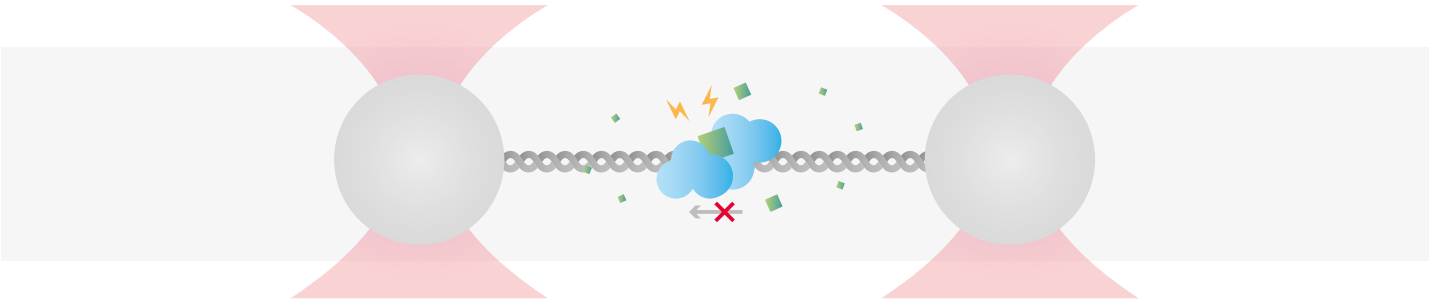


1 A schematic representation of an experiment involving small molecule-protein interactions. A double-stranded DNA molecule is attached to optically trapped microspheres, while a motor protein is interacting with the DNA. At the same time, multiple small molecules interact with the motor protein, thereby reducing or altering its activity.

Study how small molecules interact with proteins or DNA to modulate enzymatic activity

Small molecule inhibitors are widely used in the pharmaceutical industry. They are often used in cancer therapy and they still remain one of the most effective agents in clinical use. Intercalation of small molecules within the DNA template, or binding to the active binding sites of various enzymes, are broadly used as drug treatments to compromise DNA-associated processes that progress in an abnormal fashion.

By using correlated high-resolution optical tweezers and fluorescence microscopy, not only can the binding properties of small molecules be studied (e.g., kinetics or diffusive features) but also their effect in the inhibition of the activity of DNA-processing motors.

These processes can be grouped into two main categories:

- those in which small molecules interact with the DNA template, resulting in roadblocks that hamper the motion of the proteins, and
- those in which small molecules interact with the active binding site of the enzymes, thereby reducing or altering their performance.

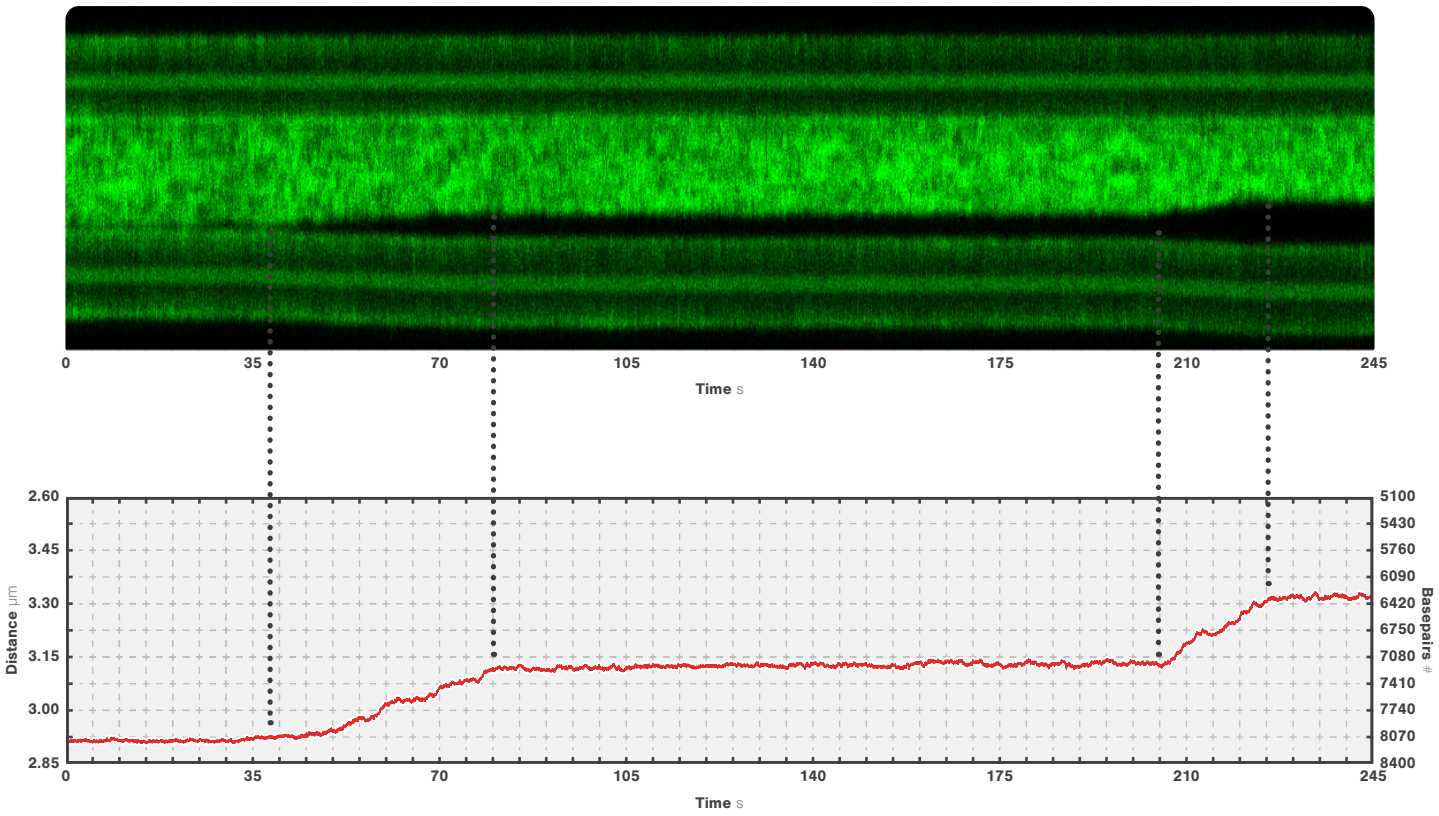
The C-Trap™ Optical Tweezers – Fluorescence Microscopy system provides the ability to apply and measure force and extension of the tethered DNA while simultaneously visualizing proteins as they interact with the DNA. In this way, it is possible to film proteins and directly characterize their effect.

When DNA-protein interactions are measured at the single-molecule level the exact mechanisms of DNA organization, replication, translation, and repair can be studied in high detail, including conformational changes.

Why use single-molecule techniques for drug discovery?

Identification of the most potent drug candidates with single-molecule methods has enormous potential in the future of drug-discovery, offering major benefits over conventional assays used for screening. The ability of single-molecule tools to characterize new properties and specific transient steps in complex biochemical pathways, which are otherwise obscured in ensemble-average systems, allows you to look for inhibition of specific steps. Given that most biochemical pathways usually consist of multiple steps, the total number of potential drug targets is considerably increased. Because of the C-Trap's ability to directly visualize multi-step processes (even the most transient ones), it is extremely straightforward to observe at which step inhibition occurs and how it works. This, in turn, allows the development of more precise drugs while requiring significantly fewer secondary targets, and thus reducing the probability of side effects.

Common concerns such as long experimental time and high costs completely diminish when using the C-Trap. Bulk assays are often indirect methods of screening and require many experiments to reveal the molecular mechanism in question. Moreover, since only single molecules are required for the C-Trap experiments, the material consumption is several orders of magnitude lower. The intuitive software, the integrated microfluidics, and the automation capabilities guarantee an unprecedently quick workflow. The C-Trap gives access to novel information and ensures the precise screening of small molecules, quickly and effectively.



2 (Top) Kymograph showing SYTOX Orange bound to double-stranded DNA, thus indirectly visualizing the position and activity of DNA polymerase.

(Bottom) Simultaneous measurement of the end-to-end DNA distance shows activity steps of DNA polymerase with high-resolution as single-stranded DNA is generated.

Visualization and characterization of small molecule–protein interactions

The C-Trap's optical tweezers can be used to catch a DNA molecule tethered between two beads, while a motor protein and differently labeled small molecules can be visualized by correlative fluorescence microscopy.

Figure 1 shows how small molecules interact with the motor protein, thereby reducing or altering its activity. Alternatively, the small molecules can interact with the DNA template, resulting in barriers that restrict the movement of the proteins.

Figure 2 shows the results of an experiment in which initially, the DNA template (8.3 kbp) was fully coated by SYTOX Orange, a small molecule that fluoresces when intercalated between the base pairs of double-stranded DNA. A single bound DNA polymerase proofreading the nucleotides by removing them from the template can be monitored via fluorescence microscopy, by the appearance of an increasing dark region over time.

Simultaneously, by maintaining a constant tension, a clear increase of the end-to-end DNA distance is observed, due to the generation of single-stranded DNA. The presence of small molecules, in this case, compromises the motility of the motor protein, by drastically reducing its velocity by increasing the frequency and duration of pauses during its performance.

Read more:

Manosas et al. Nature Communications (2017)
Almaqwashi et al.

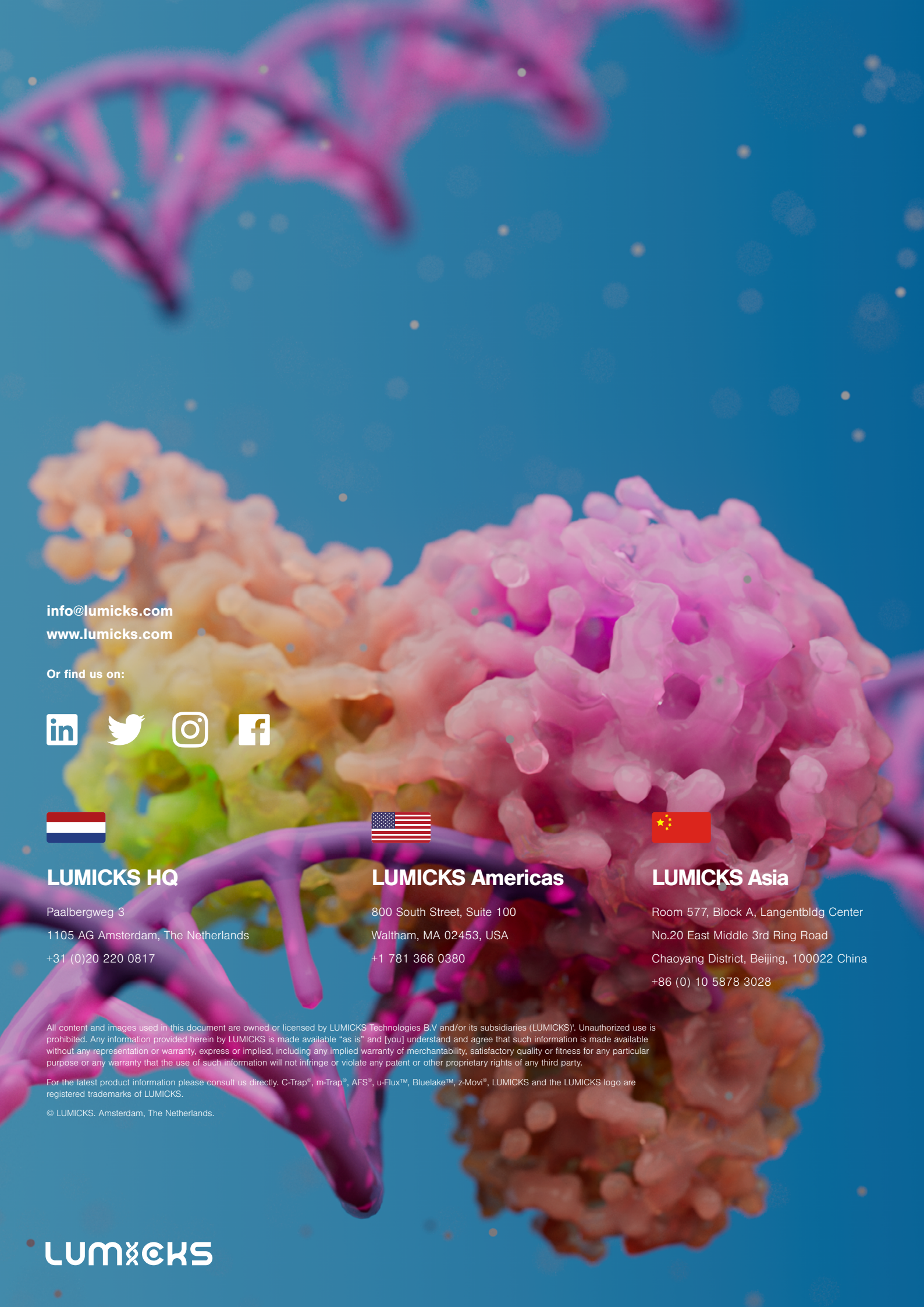
Nucleic Acid Research (2016)

User insights

Prof. Sarah Köster

Georg August University

"Using our C-Trap we manipulate cellular components and study their dynamic behavior while visualizing them in real-time. Moreover, we are new users to optical tweezers, which makes it even more impressive that with this instrument we have been able to really start from scratch and go to 100% and perform experiments within a day."



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