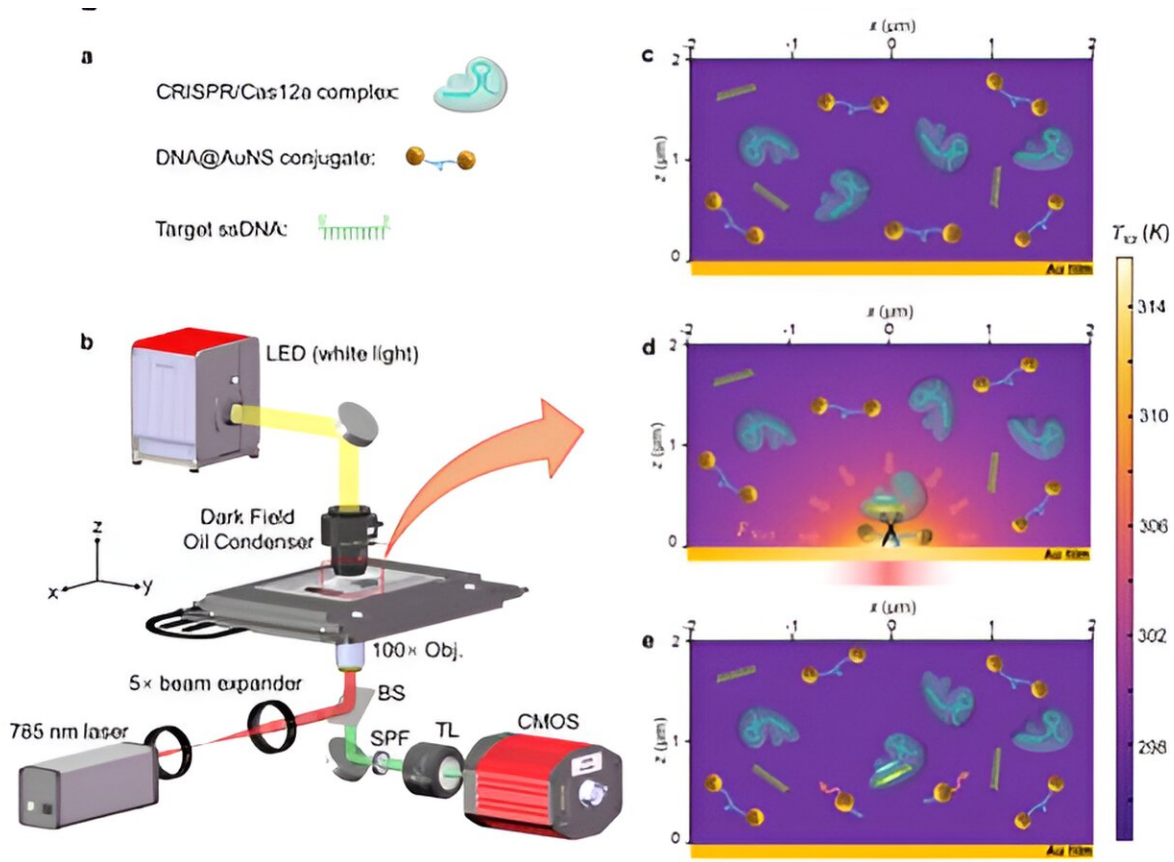
CRONT: Empowering optical tweezers with 'biometric eyes'

November 22 2023



a, The diagrammatic sketch of the three components in the solution: DNA@AuNS conjugate, CRISPR/Cas12a complex, and target ssDNA. b, Optical setup, the BS, SPF, and TL are beam splitter, short pass filter, and tube lens (f=200 mm), respectively. Additional details of the setup are provided in the Materials and Methods section. c, Dispersion of the three components in the solution without optical heating. d, Optothermal net force (F_{Net}) induced migration and DNA@AuNS conjugate cleavage upon optical heating, the heating

laser power is 0.5 mW. e, Observation of the cleavage after the optical heating is switched off. Credit: Jiajie Chen, Zhi Chen, Changle Meng, Jianxing Zhou, Yuhang Peng, Xiaoqi Dai, Jingfeng Li, Yili Zhong, Xiaolin Chen, Wu Yuan, Ho-Pui Ho, Bruce Zhi Gao, Junle Qu, Xueji Zhang, Han Zhang & Yonghong Shao

Optothermal nanotweezers, an innovative optical manipulation technique over the past decade, have revolutionized classical optical manipulation by efficiently capturing a broader spectrum of nanoparticles. While this technique has been primarily used for in-situ manipulation of nanoparticles, its potential for identifying bio-nanoparticles remains largely unexplored.

Herein, based on the synergistic effects of optothermal manipulation and CRIPSR-based bio-detection, authors developed CRISPR-powered optothermal nanotweezers (CRONT). Specifically, by harnessing diffusiophoresis and thermo-osmotic flows near the substrate upon optothermal excitation, authors successfully trapped and enriched bio-nanoparticles, including gold nanoparticles, CRISPR-associated proteins, as well as DNA molecules.

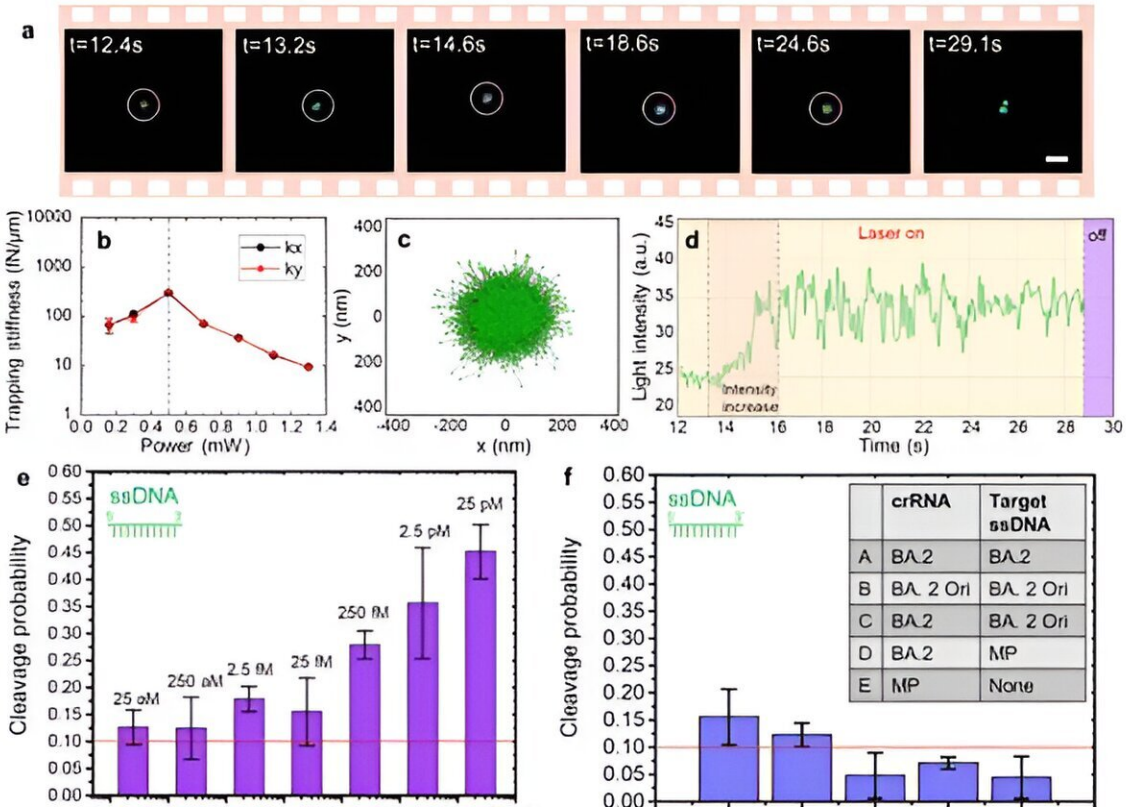
In a recent publication published in *Light: Science & Applications*, a team of scientists led by Professor Jiajie Chen, Zhi Chen, Zhang Han, Yonghong Shao from Shenzhen University, along with their collaborators, Professor Ho-Pui Ho from The Chinese University of Hong Kong have devised an optothermal approach for enhancing CRISPR-based single-nucleotide polymorphism (SNP) detection to achieve single molecule level.

Furthermore, they have introduced a novel CRISPR methodology for observing nucleotide cleavage. Moreover, this innovative approach has endowed optical tweezers with DNA identification ability in aqueous

solution, which was unattainable before. Given its remarkable specificity and feasibility for in-situ manipulation and identification of bio-nanoparticles, it is poised to become a universal tool in point-of-care diagnosis, biophotonics, and bio-nanotechnology.

The CRONT can be exquisitely tuned to manipulate bio-nanoparticles and meet the working conditions of CRISPR-based target bio-nanoparticle identification. Specifically, by incorporating optothermal-induced diffusiophoretic force, authors have successfully manipulated bio-nanoparticles, including ssDNA, dsDNA, BSA, Cas12a protein, and DNA functionalized gold nanoparticles.

By incorporating a CRISPR-based DNA biosensing approach, in which the cleavage of a single trapped DNA@Gold-nanoparticle conjugate is interrogated, authors turned this optothermal tweezer into a molecular probe for the in-situ DNA molecules (SARS-CoV-2 or Monkeypox) identification without nucleic acid amplification and achieved detection limits of 25 aM for ssDNAand 250 aM for dsDNA.



a, A single DNA@AuNS is captured by the CRONT at the laser heating region. The heating laser is turned off at 28.8s, and cleavage is observed afterward. b, Trapping stiffness measurements at varying laser powers in x/y direction, with the dashed line denoting the maximum stiffness at 0.5 mW. c, Position distribution of the trapped single DNA@AuNS at 0.5 mW. d, Light intensity variation of a trapped DNA@AuNS during the laser activation. The target ssDNA is from part of the Monkeypox (MP) virus sequence. Frames were recorded using dark-field microscopy, and the scale bar is 2 μm. e, Cleavage probability of the DNA@AuNS at different target ssDNA (MP) concentrations. f, Cleavage probability at different crRNA and target ssDNA combination groups (A-E) for specificity test, the target ssDNA concentrations is 250 fM. g, Cleavage probability of the DNA@AuNS at different target dsDNA (MP) concentrations. The optical power set as 0.5 mW in a, c-g. h, Cleavage probability of the DNA@AuNS under dsDNA at a lower optical power of 0.16 mW, the inset indicates the temperature distribution. Each capturing event was conducted for 2 minutes, and each data point comprised 10-17 capturing events over a 40-minute period. Each concentration was tested three times. The PEG

mass fraction is 10%. The concentration of AuNS and Cas12a is 0.5 μM and 0.125 nM respectively. Credit: Jiajie Chen, Zhi Chen, Changle Meng, Jianxing Zhou, Yuhang Peng, Xiaoqi Dai, Jingfeng Li, Yili Zhong, Xiaolin Chen, Wu Yuan, Ho-Pui Ho, Bruce Zhi Gao, Junle Qu, Xueji Zhang, Han Zhang & Yonghong Shao

Remarkably, they have demonstrated that these nanotweezers offer single nucleotide polymorphisms (SNPs) identification at ultra-lower detection volumes (10 μL), which play a crucial role in genetic diversity and are associated with various phenotypic traits, including disease susceptibility and drug response. Therefore, this innovation in SNP detection techniques is essential to meet the diverse demands of genomic research and medical applications in the future.

These authors summarized the Work and Outlook of the CRONT as follows:

"CRONT has enabled the immediate implementation of CRISPR-based biosensing within ultra-low detection volume. Optical tweezers are now endowed with DNA identification ability through the CRISPR-based biosensing system. The localized heating properties of CRONT have provided not only an avenue for biomolecule enrichment but also a necessary thermal environment for the cleavage of the CRISPR complex."

"Further development of this optothermal-based CRISPR bio-detection scheme may involve the utilization of an array of laser heating spots for parallel high-throughput detection, which makes the technique more suitable for quantitative detection and significantly reducing detection time. CRONT may also be employed to guide the CRIPSR/Cas complex to the target DNA and initiate the gene editing process. It also allows the

researchers to monitor the gene editing process in real-time at the single-molecule level," they added.

"We anticipate that such non-contact nanoprobes will contribute to a deeper understanding of various complex biological processes, high lighting optical, thermal, biological similarities at the single-particle level."

More information: Jiajie Chen et al, CRISPR-powered optothermal nanotweezers: Diverse bio-nanoparticle manipulation and single nucleotide identification, *Light: Science & Applications* (2023). DOI: 10.1038/s41377-023-01326-9

Provided by Chinese Academy of Sciences