第四届全国现代生物物理方法与技术暨单分子生物学学术研讨会

摘 要 集

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目录

Elucidation of molecular mechanism of the inhibitor protein of mitochondrial ATP synthase by single-molecule and engineering approach 6

Watching single helical membrane proteins fold 7

Single molecule imaging for understanding brain function, neurological disorders, and drug action mechanism 8

Bridging-induced phase separation of SMCs for genome organization 9

Single-molecule mechanical study of rationally designed DNA molecular motors 10

Condensin's Journey on DNA with Obstacles 11

Rewiring immunity for the development of novel therapeutic platforms 12

Allosteric control of rotary molecular motor V1-ATPase by redesigning pseudo-active sites 13

Mechanistic insights into PG cleavage regulation in Bacterial Cell Division 14

Single-molecule imaging analysis of G protein-coupled receptor signalosome 15

Comprehensive computational studies of membrane proteins for the elucidation of mechanism of action and structure-based drug discovery 16

Mesoscopic architecture and molecular dynamics in focal adhesions as revealed by ultrafast single-molecule imaging 17

多种单分子技术研究细胞膜结构 18

Capturing transcriptional condensates and its relation with subnuclear structures using super-resolution microscopy 19

When Force Met Fluorescence: Single-Molecule Manipulation and Visualization of Protein-DNA Interactions 20

Number of kinesins engaged in axonal cargo transport: A novel biomarker for neurological disorders21
利用生命体编程活材料 22
A general strategy to improve the photostability of thiol-conjugated dyes and target search of Cas9 and Cas12a 23
Functional role in anti-virus infection and regulatory mechanism of nociceptive TRPV2 channels 24
Kinetic basis of G-quadruplexes selective stabilizing ligands revealed by single-molecule force spectroscopy 25
黄病毒 xrRNAs 构象动态与机械稳定性各向异性的研究 26
秀丽线虫胞间连接蛋白 HMP1-HMP2 的力学调控机制的单分子研究 27
Single-molecule conformation tracking reveals GTPase cycling dynamics in living cells 28
太赫兹生物光谱成像检测技术 29
ssDNA accessibility of Rad51 is regulated by orchestrating multiple RPA dynamics 30
冠状病毒刺突蛋白的力学适应性进化 31
单分子酶动力学分子模拟 32
面向阿尔茨海默病脑生物力学特性变化的微观测量与建模分析 33
锁核酸修饰对 CRISPR/Cas12a 的 trans 酶活的精确控制作用研究 34
胞嘧啶甲基化稳定 DNA 但阻碍 DNA 退火 35
基于 CRISPR/Cas12a 自催化效应的核酸传感应用研究 36
单分子纳米孔测序技术-原理、实现与应用 37
细胞内吞中的颗粒协同性研究 38
MOLECULAR MECHANISMS OF NUCLEASE: A SINGLE-MOLECULE PERSPECTIVE 39
Multimodal chemical imaging for tracking subcellular metabolic dynamics 40
细胞膜界面过程中的单分子动力学 41
一种原位测量胚胎组织硬度及牵引力的方法 42
In vitro reconstitution of mitochondria cristae mimics 43
Single-molecule FRET study of the conformational dynamics of Hsp70 during the functional cycle 44
流场下 VWF-A 域构象功能活化的力-化学调控机制 45

Mechanistic insight into the mechanical unfolding of an integral membrane protein 46

基于目标锁定机制的实时三维单分子动态示踪成像技术 47

1-8eV(155-1200nm)连续可调 DUV 激光系统及其 AMO 科研应用 48

Rotational Dynamics of Cellular Processes - A new paradigm powered by multi-dimensional single particle tracking 49

组蛋白泛素化修饰调控核小体动态结构的分子机制 50

病理性蛋白相变聚集与帕金森病 51

细胞(类器官)力学成像技术与应用 52

DNA 酶内在构象动力学的单分子研究 53

并行拉伸串联核酸探针进行核酸突变检测和基因分型 54

基于结构光照明的超分辨定量 FRET 显微术（SI-FRETM） 55

Molecular mechanism of synaptotagmin-1 in regulating neurotransmitter release in physiological and pathological conditions 56

“点亮”细胞的机械力信号：从亚细胞结构至单分子 57

Transition Time Determination of Single-Molecule FRET Trajectories via Wasserstein Distance Analysis in Steady-state Variations in smFRET (WAVE) 58

非共价作用调控固相分子电荷传递 59

Micro- to Millisecond Conformational Dynamics of DNA Molecules Investigated by Maximum Entropy Method-based Fluorescence Lifetime Correlation Spectroscopy 60

生物高分子材料力学：从微观到宏观 61

磁显微成像技术发展及其生物医学应用 62

单价阳离子反常降低核酸双螺旋稳定性及其机制 63

细胞和颗粒的三维动态表征 64

Single-molecule dynamics of transcription factors interrogating chromatin 65

DNA 聚合酶高选择活性与外切校对的分子机制 66

微流技术在定量生物学中的应用 67

The Force Response and Free Energy Landscape of the 10th Type III Fibronectin Domain Revealed

by Magnetic Tweezers 68
高时空分辨与双模态细胞成像新探针 69
Switch of cell migration modes orchestrated by changes of three-dimensional lamellipodium structure and intracellular diffusion 70
细胞高磷信号激活 VTC 复合物进行多聚磷酸盐合成与跨膜转运的多功能耦合与动态过程 71
基于单条染色质应变定量技术探究不同模态力学信号激活基因表达的机理研究 72
光遗传学二聚系统在细胞黏附的力感知中的应用 73
环境诱导 DNA 和 RNA 扭转的统一模型 74
A ribozyme selects mechanically stable conformations for induced catalysis 75
RNA imaging in living cells with fluorogenic RNA aptamer 76
Towards Single Molecule Biophysics using Optical Tweezers and 77
Fluorescence Microscopy 77
非天然核酸的性质与其在信息存储与读取中的应用 78
细胞与细胞外基质的生物力学模型仿真 79
DNA 探针图案化技术用于测量单细胞机械力学行为 80
Stochastically multimerized ParB orchestrates DNA assembly as unveiled by single-molecule analysis 81
Bloom Syndrome Helicase Compresses Single-Stranded DNA into Phase-Separated Condensates82
基于二聚体荧光蛋白的 DNA 纳米力镊 83
Pathogenic Mutations shift the Conformational Equilibria of α-Synuclein 84
基于冠状病毒刺突蛋白力学适应性进化的抗病毒策略 85
FNIII10 G79C 对生理拉伸作用力的响应 86
基于高速原子力显微镜(HS-AFM)的 Perforin-2 蛋白打孔机理和动力学研究 87
Exploring Mechanical Responses of Cells to Geometric Information using Micropatterned DNA-based Molecular Tension Probes 88

Elucidation of molecular mechanism of the inhibitor protein of mitochondrial ATP synthase by single-molecule and engineering approach

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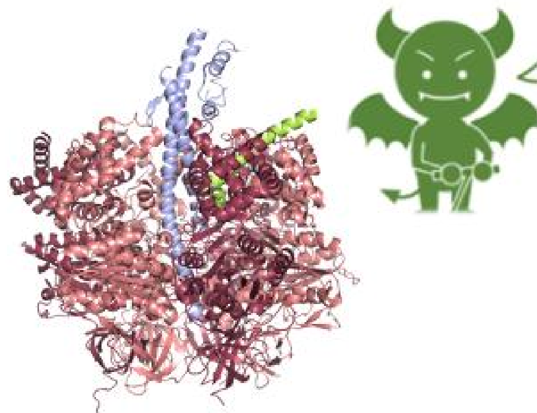
ABSTRACT.

IF_1 is a natural inhibitor protein for mitochondrial F_oF_1 ATP synthase that blocks catalysis and rotation of the F_1 by deeply inserting the N-terminal helices into F_1. A unique feature of IF_1 is the reaction-direction-dependent inhibition; although IF_1 inhibits the ATP hydrolysis of F_1, IF_1 inhibition is relieved under ATP synthesis conditions. To elucidate the reaction-direction-dependent inhibition mechanism, we performed single-molecule manipulation experiments on IF_1-inhibited *bovine* mitochondrial F_1 (*b*MF_1). The results showed that IF_1-inhibited F_1 was efficiently activated only when F_1 was rotated in the clockwise (ATP synthesis) direction, but not in the counter-clockwise direction. The observed rotational-direction-dependent activation explains the reaction-direction-dependent mechanism of IF_1 inhibition. Investigation of mutant IF_1 with the N-terminal truncation showed that the interaction with the γ subunit at the N-terminal regions is crucial for rotational-direction-dependent ejection, and the middle long helix is responsible for the inhibition of F_1. We will also share the latest works on the engineering of bacterial F1 with susceptivity with IF1 to understanding of species-specific molecular recognition mechanism of IF1



Watching single helical membrane proteins fold

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Despite advances in resolving structures of multi-pass membrane proteins, little is known about the native folding pathways of these complex structures. Using single-molecule magnetic tweezers, we report a complete folding pathway of purified human glucose transporter 3 (GLUT3) reconstituted within lipid bilayers composed of synthetic lipids and detergents. The N-terminal major facilitator superfamily (MFS) fold strictly forms first, serving as structural templates for its C-terminal counterpart that defines most of the glucose binding site. Our data further reveal folding pathways for individual MFS folds, where polar residues comprising the membrane-embedded conduit for glucose molecules present major folding challenges. The ER membrane protein complex facilitates insertion of more hydrophilic TMHs, thrusting GLUT3's microstate sampling toward folded structures. Final assembly between the N- and C-terminal MFS folds depends on specific lipids that ease desolvation of lipid shells surrounding the domain interfaces. Sequence analysis suggests that this asymmetric folding propensity across the N- and C-terminal MFS folds may prevail for metazoan sugar porters, revealing evolutionary conflicts between foldability and functionality faced by many multi-pass membrane proteins.

Single molecule imaging for understanding brain function, neurological disorders, and drug action mechanism

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Our memories are retained for decades, but lipids and proteins, the building blocks of the brain, are constantly replaced. What mechanisms allow the brain to maintain its structure and perform higher functions such as memory, learning, and thinking? Here, I will introduce our research on the mechanism that accumulates neurotransmitter receptors on postsynaptic membranes using the quantum dot single molecule tracking (QD-SPT) technique, which allows us to see the lateral diffusional motions of lipids and proteins with single-molecule resolution [1][2]. By directly visualizing the behavior of molecules at single-molecule resolution, the first events during the synaptic plasticity that is the basis of memory and learning involve the changes in the movement of neurotransmitter receptors [3][4]. QD-SPT has also shown that abnormalities in the dynamics of multiple membrane molecules occur in neuronal models of neurological diseases such as Alzheimer's disease and epilepsy [3][5]. Using neurons from Alzheimer's disease model mice, we recently found that these abnormalities in the molecular dynamics appeared in various species of membrane molecules, earlier than the initial pathological findings in vivo. This suggests that abnormalities in membrane molecular dynamics are a cellular-level pathology that appears earlier than previously considered, and therefore could be one of the important factors in elucidating the mechanism of disease development. Furthermore, in this talk, I will also introduce the application of QD-SPT for understanding the action mechanism of volatile anesthetics. Isoflurane (ISO), a halogenated ether, is one of the typical volatile anesthetics that have been widely used as general anesthetics in clinical use. The diffusion coefficient and localization of neurotransmitter receptors were modified after the application of 1 mM isoflurane, suggesting that isoflurane could impact neural activity through the diffusion dynamics and the localization of neurotransmitter receptors.

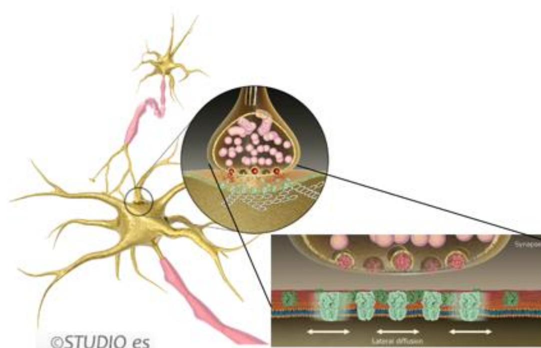

Figure: Dynamics of synaptic neurotransmitter receptors

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Bridging-induced phase separation of SMCs for genome organization

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Abstract

Structural maintenance of chromosome (SMC) protein complexes are key proteins for genome organization by extruding DNA loops. However, it is not unclear whether only DNA loop extrusion can build the genome structures at a physiologically relevant condition. Here, we show that yeast cohesin, human cohesin and yeast condensin SMC complexes exhibit pronounced clustering on DNA, with all the hallmarks of biomolecular condensation (*1)*. DNA-SMC clusters exhibit liquid-like behavior, showing fusion of clusters, rapid fluorescence recovery after photobleaching and exchange of SMC complexes with the environment. Strikingly, the *in vitro* clustering is DNA length dependent, as both yeast cohesin and human cohesin form clusters only on DNA exceeding 3 kilo–base pairs. We discuss how bridging-induced phase separation (BIPS), a previously unobserved type of biological condensation, can explain the DNA-protein clustering through DNA-protein-DNA bridges. We confirm that, in yeast cells *in vivo*, a fraction of cohesin associates with chromatin in a manner consistent with BIPS. BIPS likely is a universal phenomenon among SMC proteins. Biomolecular condensation by SMC proteins constitutes a new basic principle by which SMC complexes direct genome organization.

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Single-molecule mechanical study of rationally designed DNA molecular motors

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Abstract

A key capability of molecular motors is sustainable force generation by a single motor copy. Direct force characterization at single-motor level, though a common practice for biological motor proteins, is still challenging for artificial molecular motors. In this talk, I will discuss a recent single-molecule mechanical study of a rationally designed autonomous DNA bipedal motor using magnetic tweezers. This study(*1*) is a collaboration between our molecular motor lab and Prof. Yan Jie's single-molecule biophysics lab. This autonomous motor(*2*) is a track-walking DNA motor beyond the early bridge-burning designs, and is capable of chemically fuelled and self-directed hand-over-hand walking along a double-stranded DNA track. Real-time detection of the motor's operation requires simultaneous control of both the motor and the soft double-stranded track. This is achieved in our magnetic tweezers study using a single bead: A ~ 120nm long track plus a single motor are first assembled under the bead, which is then switched to the motor for its motility detection under a constant pulling force. The motor shows processive walking by ~ 16 nm steps up to a distance of 120 nm (covering the entire track), yielding a stall force of ~ 2 – 3 pN. These results suggest a reasonably efficient chemomechanical conversion of the motor compared to biological counterparts, and also reveal fast subsecond steps that imply a big room to improve speed of DNA motors. This talk also will cover a recently developed method(*3*) that allows extraction of a motor's stall force from single-molecule trajectories obtained at low pre-stall forces or even zero force. This method is formulated from stochastic thermodynamics and verified using trajectories of this DNA motor from the magnetic tweezers experiments. This theoretical development, which is from a collaboration with Prof. Ruizheng Hou from Xi'an University of Technology, helps simplify future single-molecule mechanical study for artificial and biological molecular motors in general.

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Condensin's Journey on DNA with Obstacles

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Condensin, a molecular motor, utilizes ATP hydrolysis energy to translocate along DNA. This motor activity is responsible for condensing chromatin during mitosis. Eukaryotic chromatin contains densely arranged protein-DNA complexes, including nucleosomes. Additionally, DNA replicative helicase Mcm2-7 remains associated with chromatin even during mitosis. Furthermore, each chromatin domain is under the influence of various tensions. Until now, the condensin translocation along naked DNA has been studied enthusiastically. However, it is highly demanded to explore condensin translocation in the presence of various obstacles, such as tension and DNA-binding proteins. In this study, we prepared DNA curtains using 1) naked DNA with various tensions and 2) DNA reconstituted with Mcm2-7 or nucleosomes and measured the condensin translocation dynamics by single-molecule fluorescence imaging. As a result, it is revealed that condensin translocates along DNA with low tension in both fast and slow modes and along DNA with high tension in only the slow mode. In our experiment, many condensins stopped translocating when they collided with a nucleosome or Mcm2-7. In contrast, a few condensins bypass these proteins. Significantly, condensins that bypassed a nucleosome consistently translocated in the fast mode. Contrary to expectations, condensins sometimes pushed Mcm2-7 along DNA. This study suggests that the mode of condensin translocation and the formation of nucleosome and pre-replicative helicase on DNA influence the chromatin condensation catalyzed by condensins.

Rewiring immunity for the development of novel therapeutic platforms

Chan Hyuk Kim

The innate and adaptive immune systems in our bodies are believed to have evolved over millions of years. Throughout this evolution, the immune system has primarily been built to protect the body against infections caused by pathogens outside the body, as well as to maintain tissue integrity and homeostasis within. Therefore, dysregulation of the immune system has the potential to not only increase susceptibility to infection but also contribute to a variety of diseases such as cancer, autoimmune disorders, and degenerative conditions. In synthetic immunology, researchers utilize advanced genetic and protein engineering tools to enhance or reprogram the effector function of immune cells, with the aim of discovering innovative therapeutic strategies for these refractory diseases. As part of this effort, I will discuss our recently published and ongoing research on engineering chimeric antigen receptor (CAR) T cells with downregulated expression of immune checkpoint receptors to treat cancer, and developing a novel anti-inflammatory phagocytosis inducer to treat Alzheimer's disease.

Allosteric control of rotary molecular motor V1-ATPase by redesigning pseudo-active sites

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Concerted functions of protein complexes in biomolecular systems are exerted by orchestrating the cooperative works between the constituent subunits. A variety of orchestrations is controlled by allosteric mechanism. Recently, we have reported an approach to designing allosteric sites (where by binding an effector molecule, activity at the distal active site is regulated) to provide novel orchestration into protein complexes (T. Kosugi, et al., *Nat. Chem.*, 2023). By the approach, we have succeeded in designing artificial allosteric sites into a rotary molecular motor, *Enterococcus hirae* V_1-ATPase. The allosteric sites were created by restoring lost functions of pseudo-active sites in a pseudo enzyme, of which function is predicted to have been lost during the evolution. Single-molecule experiments together with X-ray crystallography analyses revealed that binding of ATP to the designed allosteric site boosts this V_1's activity compared with the wild-type, and the rotation rate is accelerated. This is the first time achievement of rotation accelerated by engineering a rotary molecular motor. Our strategy have potential for creating allosteric sites into various kinds of protein complexes and artificially controlling concerted functions of the protein complexes. I will show about the future potentials and recent applications of the approach, too.

Mechanistic insights into PG cleavage regulation in Bacterial Cell Division

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Abstract

Bacterial division necessitates the processing of a shared peptidoglycan (PG) septum, a mechanism largely mediated by enzymes known as amidases. In Gram-negative bacteria, amidases, such as AmiB, are autoinhibited by a regulatory helix to prevent unintended cell wall cleavage. Their activation at the division site is orchestrated by the EnvC activator, which itself is regulated by the ATP-binding FtsEX complex. Through detailed structural studies in *Pseudomonas aeruginosa*, we unveil how ATP binding likely activates the FtsEX-EnvC assembly, promoting its interaction with AmiB. This activation involves a regulatory helix swap between EnvC and AmiB, facilitating PG cleavage. Notably, such regulatory helices are ubiquitous in Gram-negative bacteria, indicating a conserved activation mechanism. Conversely, in mycobacteria and Gram-positive bacteria, the FtsEX system directly governs PG-hydrolases, with our studies in *Mycobacterium Tuberculosis* illuminating the unique "Match and Fit" mechanism of RipC recognition and activation. This comprehensive understanding of the FtsEX complex, spanning diverse bacterial genera, not only elucidates the conserved and distinctive regulatory mechanisms but also underscores potential therapeutic avenues for the design of antibiotics targeting PG processing.

Single-molecule imaging analysis of G protein-coupled receptor signalosome

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Abstract

G-protein-coupled receptors (GPCRs) are major drug targets that act as a signaling hub via interaction with G proteins and arrestins on the plasma membrane. Biased ligands with pathway-selective activity have attracted much attention as drugs with lower side effects. However, it is yet to be clear how the multiple signaling pathways of GPCR are spatial-temporally regulated in living cell membrane.

Here we show that a co-clustering of GPCRs and signaling molecules in a membrane domain is closely related to their function including G protein binding, GRK-dependent phosphorylation and arrestin/clathrin-dependent endocytosis. First, the single-molecule imaging analysis of various GPCRs demonstrates a general feature that activated GPCRs are entrapped into an immobile membrane domain. Then, we show a novel signalosome that regulates the signal-bias of the angiotensin signaling by AT1R, a class AGPCR. The NanoBiT assays and BRET imaging revealed the AT1R/G protein/GRK preassembly complex in living cell membrane. The dual color single- molecule imaging analysis suggested that the preassembly complex regulates the signal bias in a confined region of the plasma membrane. Finally, we discuss future applications of the next-generation high-content analyzer, which automates the single- molecule imaging analysis, for future pharmacology and membrane biology.

Comprehensive computational studies of membrane proteins for the elucidation of mechanism of action and structure-based drug discovery

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Transmembrane 4 L six family member 5 (TM4SF5) is a transmembrane protein known to interact with other TM4SFs, growth factor receptors, signaling proteins, and integrins, leading to uncontrollable cell growth and proliferation which eventually results in fibrosis and cancer. TM4SF5 contains 4 transmembrane (TM) helices, two extracellular loops, an intracellular loop, and N- and C-terminal cytosolic tails. The LEL region of TM4SF5 has previously been noted as integral to its function and interactions with molecular partners. Recently, we have identified TM4SF5 as an arginine sensor for mTORC1 activation and subsequent phosphorylation of its downstream effectors. Mutations of conserved residues in the LEL region were found to significantly affect mTORC1 function supporting the involvement of TM4SF5 in this pathway. To investigate important structural features of TM4SF5, we generated a homology model, and based on sequence conservation and mutational studies in the LEL region, we applied molecular dynamics (MD) simulations for the apo wild-type and mutant structures. Protein-protein docking with mTORC1 and MD simulation of ensuing complexes were also performed. Trajectory and network analysis allowed us to distinguish the importance of each conserved residue to TM4SF5 structural integrity and function in the mTORC1 pathway. We have also analyzed the binding mode of L-arginine and compounds we have found. In addition, it’s interaction with c-Src is also studied with the extensive computational studies along with the biological studies. These results could be utilized for the rational structure-based drug discovery.

Mesoscopic architecture and molecular dynamics in focal adhesions as revealed by ultrafast single-molecule imaging

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Regulation of molecular dynamics in the plasma membrane (PM) is fundamentally important for organizing PM functions. We have developed an ultrafast camera system to perform single fluorescent-molecule imaging at a time resolution greater than 100 μs and revealed the actin-induced compartmentalization of the PM. Virtually all lipids and transmembrane proteins in the PM undergo confined diffusion within ~100-nm-compartments and hop movements between the compartments, which we call hop diffusion.

Focal adhesion, or FA, is a micron-sized structure in the plasma membrane (PM), responsible for the movement of the cells involved in cell differentiation or cancer cell invasion. How FA structures fit into the view of the compartmentalized PM was addressed by simultaneous two-color PALM-dSTORM and PALM-ultrafast single fluorescent-molecule imaging. They revealed the dynamic nano-meso organization of the FA, leading to the compartmentalized archipelago FA model, consisting of FA-protein islands with broad diversities in size (13~100 nm; mean island diameter ≈30 nm), protein copy numbers, compositions, and stoichiometries, which dot the partitioned fluid membrane (74-nm compartments in the FA vs. 109-nm compartments outside the FA). The FA-protein islands were found to form loose ≈300 nm clusters, and function as units for recruiting FA proteins. Integrins are recruited to these islands by hop diffusion through the channels between the FA-protein island clusters, which would facilitate rapid FA formation and disintegration.

多种单分子技术研究细胞膜结构

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细胞膜是生命体基本结构单元“细胞”的天然屏障，它把细胞与外界环境分离开来。细胞膜有许多重要的生物功能，如物质隔离、物质交换和细胞通讯等。在分子水平研究细胞膜的结构对解释细胞膜的功能和治疗细胞膜相关疾病有重要的指导意义。细胞膜结构研究已经有近百年的历史；然而由于结构复杂、研究条件有限，细胞膜这一超分子结构尚处在模型假说阶段。我们利用多种单分子技术（原子力显微镜、超分辨荧光显微镜、冷冻电镜和单分子力谱等技术）在接近生理条件下对多种细胞膜结构（包括多种血红细胞膜、多种哺乳动物有核体细胞膜）进行了深入系统的研究[1-3]，从整体角度揭示了细胞膜的非对称性和种类差异性，提出了红细胞的“半镶嵌”模型（Semi-mosaic Model）和哺乳动物组织细胞的“蛋白层-磷脂-蛋白岛”模型(Protein Layer-Lipid-Protein Island model)，并通过力学测量、冷冻电镜等技术验证了新模型结构；同时，基于活细胞荧光动态成像技术和细胞膜结构新认知，我们又提出新的“膜非对称性决定的有序囊泡转运”模型，它可以从膜整体结构角度解释细胞内物质有序运输的过程和现象。

关键词：细胞膜；原子力显微镜；超分辨光学显微镜；冷冻电镜；单分子

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Capturing transcriptional condensates and its relation with subnuclear structures using super-resolution microscopy

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Mammalian genome is organized into a hierarchical structure at multiple scales in a cell nucleus. Recent intensive studies using live-cell imaging techniques have characterized behaviors of subnuclear compartments, exemplified with transcriptional condensates and nuclear speckles. They are functionally specialized non-membrane bound organelles, mostly follow liquid properties. However, how the genome structure and subnuclear organelles are spatially organized in the nucleus has not been elucidated. We found that chromatin looping by CTCF, a chromatin architectural protein, acts as an architectural prerequisite for the assembly of phase-separated transcriptional condensates. Moreover, we observed a layered structure of transcriptional and splicing condensates near the nuclear matrix discovered by super-resolution imaging of RNA polymerase II, nuclear speckles, and Scaffold attachment factor A (SAF-A, a nuclear matrix associated proteins).

When Force Met Fluorescence: Single-Molecule Manipulation and Visualization of Protein-DNA Interactions

Liu shixin

DNA is both a fundamental building block of life and a versatile natural polymer. A myriad of DNA-binding proteins carry out their biological functions by recognizing specific sequences, shapes, and structures of DNA, or by actively altering the DNA configuration through force generation and energy consumption. Single-molecule techniques are ideally suited for studying these interactions thanks to their ability to follow dynamic processes in real time, while precisely exerting and measuring forces on the system of interest. In this talk, I will describe recent work in my laboratory that uses correlative single-molecule force and fluorescence microscopy to simultaneously control the mechanical state of DNA and observe the behavior of its associated proteins. This powerful approach enables us to discern intricate dynamics and discover unexpected phenomena in the protein-DNA interplay. Our findings underscore the physical characteristics of DNA as an integral dimension of biological regulation and open new avenues for interrogating these regulatory mechanisms for genome transactions.

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Number of kinesins engaged in axonal cargo transport: A novel biomarker for neurological disorders

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Abstract The motor protein kinesin plays a crucial role in anterograde transport of cargo vesicles in neurons, moving them from the cell body towards the synaptic region. Not only the transport force and velocity, but also the number of kinesin molecules involved in transporting a single cargo vesicle, is pivotal for synapse formation. This collective transport by multiple kinesins ensures stable cargo transport in neurons. Abnormal increases or decreases in the number of engaged kinesin molecules could potentially act as biomarkers for neurodegenerative diseases such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ALS), spastic paraplegia, polydactyly syndrome, and virus transport disorders. Our aim, by employing novel physical measurements to quantify the number of kinesin molecules, is to shed light on the molecular mechanisms of neurodegenerative diseases related to axonal transport.

Key Words Axonal transport, Neurodegenerative diseases, Kinesin, Dynein, Biophysical Model, Fluorescence observation

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利用生命体编程活材料

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摘要：自然界中很多生物材料体系—例如骨骼组织和细菌生物被膜—能够生长、自修复并适应环境，具有人工合成材料所不具有的独特“活体”属性。如何效仿自然创建可编程、功能可调的“活”材料一直是材料合成生物学领域中的重要挑战。在本次报告中，报告人首先将就课题组利用合成生物技术创建基于细菌生物被膜（大肠杆菌或枯草芽孢杆菌生物被膜）活体功能材料方面的工作做一简要介绍。具体例子包括：（1）可编程、可 3D 打印的枯草芽孢杆菌生物被膜活体材料；（2）具有环境响应和自修复功能的细菌活体胶水；（3）基于光诱导生物被膜和生物仿生矿化创建的活体梯度复合材料和半导体-微生物人工光合作用系统等。此外，报告人还将讨论材料合成生物学交叉领域新的研究机遇和挑战。课题组的前期研究为创建环境耐受性、环境响应性和有机-无机复合活体材料提供了范式研究，并为未来从头理性设计智能活材料打下坚实基础。

关键词：活材料，生物被膜，自修复材料，淀粉样蛋白材料

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A general strategy to improve the photostability of thiol-conjugated dyes and target search of Cas9 and Cas12a

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Abstract

Single-molecule fluorescence technique utilizes highly-sensitive fluorescence microscopes to accurately measure the dynamic properties of biological macromolecules at the molecular level, to elucidate their functions and regulatory mechanisms, thereby to guide the functional modification and optimization of biomolecules. Our lab has developed several unique novel technologies at various levels including a general strategy to improve the photostability of dyes for protein labeling, new protein and RNA labeling techniques for single-molecule fluorescence measurements, single-molecule photoactivation FRET measurement for high concentrations of labeled species, scanning fluorescence resonance energy transfer-fluorescence correlation spectroscopy (FRET-FCS) technique with high temporal and spatial resolution, and dual-color fluorescence cross-correlation spectroscopy (dcFCCS) technique for precise measurements of biomolecular condensates at the nanoscale, etc. With these tools, we have mainly characterized important metastable states and dynamic processes of the molecular machinery that cannot be resolved by structural biological tools to explain their molecular mechanisms, including the molecular mechanism of Cas protein's target search and cleavage mechanisms and etc. These discoveries have guided us to optimize and remodel functions of Cas9, Cas12a, and cGAS proteins.

In this talk, I will mainly describe a general strategy to improve the photostability of thiol-conjugated dyes for single-molecule measurements and our interesting findings when using single-molecule assays to examine one-dimensional diffusion of Cas9 and Cas12a on dsDNA during target search.

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Functional role in anti-virus infection and regulatory mechanism of nociceptive TRPV2 channels

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The transient receptor potential vanilloid 2 (TRPV2) channel is a calcium-permeable cation channel belonging to the TRP superfamily. As a multimodal ion channel, TRPV2 channel is widely distributed in nervous and non-nervous system, and has been implicated in diverse biological functions. Here, we show that TRPV2 facilitates virus penetration through the Ca^{2+}-LRMDA axis in myeloid cells by regulating the tension and mobility of cell membrane. We found that knockout of TRPV2 in myeloid cells significantly inhibited the penetration but not the attachment of multiple viruses including HSV-1, VSV, IAV, and SV40 and that the Lyz2-Cre;Trpv2$^{fl/fl}$ mice were more re- sistant to lethal HSV-1 and VSV infection than the Trpv2$^{fl/fl}$ mice. In addition, we have determined that reconstitution of wild-type TRPV2 or TRPV1 but not TRPV2^{E572Q} and TRPV1$^{D646N/E648/651Q}$ (which lost the Ca^{2+} permeability) or TRPV2$^{E594/604Q}$ (which lost the channel activity) into TRPV2- deficent BMDCs restored the penetration of viruses, indicating that TRPV channels-mediated Ca^{2+} permeability controls viral entry in myeloid cells. In addition, knockout of TRPV2 leads to downregulation of Lrmda in BMDCs and BMDMs, and knockdown of Lrmda significantly downregulates the mobility and tension of cell membrane and inhibits viral infections in Trpv2$^{fl/fl}$ but not LyZ2-Cre;Trpv2$^{fl/fl}$ BMDCs. Consistently, complement of LRMDA into LyZ2-Cre;Trpv2$^{fl/fl}$ BMDCs partially restores the tension and mobility of cell membrane and promotes viral penetration and infection. Furthermore, we identify that the protein tyrosine kinase JAK1 mediates TRPV2 phosphorylation at the molecular sites Tyr(335), Tyr(471), and Tyr(525). JAK1 phosphorylation is required for maintaining TRPV2 activity and the phagocytic ability of macrophages. We further show that TRPV2 phosphorylation is dynamically balanced by protein tyrosine phosphatase non- receptor type 1 (PTPN1). PTPN1 inhibition increases TRPV2 phosphorylation, further reducing the activation temperature threshold. Our data thus characterize a previously unknown function of myeloid TRPV2 in facilitating viral infection though the Ca^{2+}-LRMDA axis, and also unveil an intrinsic mechanism where the phosphorylation/dephosphorylation dynamic balance sets the basal chemical and thermal sensitivity of TRPV2.

Kinetic basis of G-quadruplexes selective stabilizing ligands revealed by single-molecule force spectroscopy

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Abstract G-quadruplexes are non-canonical nucleic acid structures and have attracted increasing attention as novel anti-cancer targets. ~99.5% of G4-targeting ligands contain aromatic groups and thus can also intercalate into double-stranded DNA. Here， we systematically characterized the binding properties of eight most commonly used G4 ligands to a long dsDNA using single-molecule stretching assay. Our results showed that most of G4 ligands except NMM can intercalate into dsDNA, resulting in significant dsDNA elongation. We further analyzed the folding/unfolding kinetics of oncogene promoter and telomeric G-quadruplexes at physiologically relevant solutions. Our results showed that small ligands can selectively stabilize G4s thus explain the G4-dependent transcription regulaiton function. The G4s selective stabilizatoin is maingly through two main mechanisms: G4 stabilizer that directly bind to the folded G4 structures, leading to a reduction in the unfolding rates k_u of G4s; G4 inducers that accelerate the folding rates k_u of G4s. Current study shed light on the potential of manipulating G4 folding/unfolding kinetics for precise regulation of G4-associated biological activities.

Key Words Single-molecule manipulation,Folding/unfolding kinetics,G-quadruplexes, Magnetic Tweezers.

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黄病毒 xrRNAs 构象动态与机械稳定性各向异性的研究

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抗核酸外切酶RNA（xrRNA）广泛存在于黄病毒基因组3’非编码区，可以折叠为独特的环状结构，抵抗宿主5’→3’方向核酸外切酶Xrn1的降解，使病毒亚基因组RNA（sfRNA）在宿主体内积累，与病毒的致病性和免疫逃逸相关。黄病毒xrRNAs独特的结构与功能使得其近年来得到广泛的关注，然而我们对其构象动态特性、方向依赖的机械稳定性与功能之间的相互关系还知之甚少。

单分子荧光共振能量转移技术（smFRET）是研究RNA构象动态的有力工具，但是需要对RNA进行荧光标记。为了克服现有标记技术的局限性，我们首先发展了基于非天然碱基的RNA位点特异性标记技术。进一步，我们将此标记方法与smFRET、小角X射线散射（SAXS）和酶切实验相结合探究含有不同假结（PK2）长度的xrRNAs的折叠、构象动态及抗酶切活性，结果表明其折叠、构象动态及抵抗酶切的活性是高度相关且依赖Mg^{2+}的，并且受到PK2长度的调控。PK2较长的xrRNAs需要较少的Mg^{2+}折叠成天然的环状结构及抵抗酶切，构象动态特性较低，同时在高Mg^{2+}浓度下可以耐受由于其他三级相互作用突变对xrRNAs结构和功能的影响。这些结果揭示了黄病毒xrRNAs折叠和构象动态调控的新机制。

与smFRET技术不同，单分子纳米孔技术则在研究RNA方向依赖的机械稳定性中有独特的优势。本研究另一部分借助单分子纳米孔技术并结合抵抗酶切实验、分子动力学模拟揭示了寨卡病毒xrRNA1具有明显的机械稳定性各向异性。这种性质是高度依赖于Mg^{2+}和三级相互作用的，低Mg^{2+}或三级相互作用的破坏严重影响其环状结构的完整性并削弱其机械稳定性各向异性。

综上所述，本研究阐释了黄病毒xrRNAs折叠、构象动态及功能之间的关系，揭示了其机械稳定性各向异性的分子机制，为我们进一步理解xrRNAs抵抗酶切的机制、设计基于环状结构的生物材料及拓展其在生物医学上的应用奠定了基础。

关键词：黄病毒抗核酸外切酶RNA，构象动态，机械稳定性各向异性，单分子荧光共振能量转移技术，单分子纳米孔技术

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秀丽线虫胞间连接蛋白 HMP1-HMP2 的力学调控机制的单分子研究

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细胞黏附、迁移与分化，组织生长、修复与维持，胚胎发育等一系列重要的生理、病理过程都受到力信号的调控。细胞感知力信号的分子基础是一系列富集于细胞连接上的、对力敏感的蛋白。对这些力敏感蛋白力学调控机制的深入探究是理解细胞力学生物学调控的关键。在本研究中，我们以经典模式生物秀丽线虫（Caenorhabditis elegans）的胞间连接的关键力敏感蛋白HMP1与HMP2为研究对象，利用基于磁镊的单分子力学操控技术，探究其生理力范围的调控机制。我们直接刻画了HMP1所有结构域，特别是其受力的调控结构域（modulation domains, M1-M3）在生理机械力范围的折叠去折叠动态。我们发现，与其哺乳动物同源蛋白相比，调控结构域间的互作极大地增强了M1结构域的力学稳定性，从而提高了依赖于M1结构域去折叠的vinculin互作发生的阈值力。进一步，我们直接定量了HMP1-HMP2互作的力调结合寿命，并发现HMP2上关键位点的拟磷酸化突变显著降低了HMP1-HMP2互作的力调结合寿命。综上，这项研究为秀丽线虫胞间连接关键力敏感蛋白HMP1与HMP2的力调机制提供了单分子层次的理解。

关键词：胞间连接，HMP1，HMP2，单分子力学操控

Single-molecule conformation tracking reveals GTPase cycling dynamics in living cells

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Abstract: Revealing the activity or conformation while following the diffusion of individual proteins inside living cells is challenging, demanding biosensors that are sensitive, easy to deploy, and versatile. GTPases are switch-like enzymes that interact with downstream effectors only when they are in their GTP-bound conformation. We have developed biosensors for GTPase activity based on environment-sensing fluorescent dyes, purposefully using dyes that undergo spectral changes suitable for ratiometric imaging in living cells. For simple construction of biosensors within cells, we used membrane-permeable dyes that attach to the SNAP-tag incorporated in the biosensors. The SNAP/dye was positioned in a linker between the GTPase and a peptide that binds specifically to the active conformation of the GTPase. Through optimization, both Cdc42 and Rac1 biosensors showed a 25 nm shift in emission upon activation, along with a significant fluorescence lifetime increase.

Use of a single dye enabled ratiometric imaging without the need for bleach correction, greatly facilitating quantitative imaging. The biosensors were sensitive because they were directly excited (in contrast to the indirect excitation of FRET). Single-molecule spectrum imaging and ratiometric imaging allow us to follow the spectra shift of individual molecules. Finally, we quantitatively mapped single Cdc42 molecule recruitment and activity across the cell, particularly around cell edge. We also studied the mechanism of nanoscale Cdc42 clusters in regulating its activity and determined the activation kinetics of Cdc42 at single molecule levels. Interestingly, we revealed the ultra-fast activity twinkling phenomenon of membrane-associated Cdc42, suggesting new insights into GTPases cycling.

Keywords: Biosensor, Single molecule tracking, GTPase, environment-sensing, dyes

太赫兹生物光谱成像检测技术

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摘要：太赫兹光谱成像技术在生物医学领域表现出了良好的应用前景。近年来，本研究团队在毫米、微米和纳米级空间分辨水平对不同生物样品（组织、细胞和生物大分子）开展了太赫兹光谱成像检测研究。首先采用透射式的远场太赫兹时域光谱检测系统对离体的BALB/c 小鼠黑色素瘤组织进行了太赫兹成像检测，结果显示：太赫兹检测技术能很好地辨别组织中黑色素瘤区域和正常区域。其次，利用自主研制的基于光电导微天线的近场太赫兹时域光谱成像检测系统对脑组织切片和单个细胞分别进行了微米级空间分辨检测，结果显示：脑组织的不同功能区域能够从太赫兹图像中清楚地区分开来；细胞在脱水过程中发生的变化可以从太赫兹图像中清晰地观察到。最后，基于金属纳米探针的散射式近场太赫兹时域光谱成像检测系统，利用单分子制备技术和近场信号增强技术，实现了单个蛋白分子的纳米级空间分辨太赫兹成像检测。上述研究结果表明：利用远场和近场太赫兹光谱成像技术可以在组织、单细胞和单个生物大分子三种不同层次开展检测研究，这对推进太赫兹技术在生物医学领域的应用具有重要意义。

关键词：太赫兹，生物，光谱成像，近场

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ssDNA accessibility of Rad51 is regulated by orchestrating multiple RPA dynamics

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Abstract:

The eukaryotic single-stranded DNA (ssDNA)-binding protein Replication Protein A (RPA) plays a crucial role in various DNA metabolic pathways, including DNA replication and repair, by dynamically associating with ssDNA. While the binding of a single RPA molecule to ssDNA has been thoroughly studied, the accessibility of ssDNA is largely governed by the bimolecular behavior of RPA, the biophysical nature of which remains unclear. In this study, we develop a three-step low-complexity ssDNA Curtains method, which, when combined with biochemical assays and a Markov chain model in nonequilibrium physics, allow us to decipher the dynamics of multiple RPA binding to long ssDNA. Interestingly, our results suggest that Rad52, the mediator protein, can modulate the ssDNA accessibility of Rad51, which is nucleated on RPA coated ssDNA through dynamic ssDNA exposure between neighboring RPA molecules. We find that this process is controlled by the shifting between the protection mode and actionmode of RPA ssDNA binding, where tighter RPA spacing and lower ssDNA accessibility are favored under RPA protection mode, which can be facilitated by the Rfa2 WH domain and inhibited by Rad52 RPA interaction.

Key Words:

DNA metabolism, DNA repair, Single-molecule biophysics, DNA Curtains

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冠状病毒刺突蛋白的力学适应性进化

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摘要：

力可以调控生物体所有过程， 包括病毒入侵等，并且贯穿生物体一生。适应力学微环境对每个生物体的生存而言至关重要。然而，力学调控是否驱动病毒突变演化及其潜在的调控机制仍然不清楚。在此，我们利用一系列单分子力谱动态操控与表征技术，结合结构生物学、生物信息学和病毒学等技术，发现了冠状病毒刺突蛋白进化遵循了力学适应性的演化轨迹。我们发现：力学调控诱导了一系列中间的冠状病毒刺突蛋白-血管紧张素转换酶2（ACE2）结合状态，从而增强了结合的力学稳定性[1,2]；此外，力学调控重塑了冠状病毒刺突蛋白的构象网络，降低了刺突自身的力学稳定性。随着刺突的突变演化，刺突与 ACE2 结合的力学稳定性逐渐增强，而自身的力学稳定性逐渐降低，以更好的促进病毒入侵。我们建立动力学模型，预测新出现的 XBB.1.5 变异株刺突蛋白的力学调控行为，证明 XBB.1.5 具有更高的力学适应性。综上，我们揭示了力能塑造冠状病毒刺突蛋白的突变演化，让病毒更好地适应细胞力学微环境，更全面地阐明了新冠病毒刺突突变演化进化的规律，也为其他病毒包膜蛋白的突变演化机理研究提供了启示。

关键词：冠状病毒，刺突蛋白，力学调控，病毒进化

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单分子酶动力学分子模拟

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摘要：随着近年来蛋白质静态结构预测问题的重大突破，蛋白分子的动力学研究越来越受到人们的关注。作为蛋白分子机器的典型代表，酶分子被广泛用于研究蛋白分子的功能动力学机制。自然界的酶由于进化通常具备了惊人的工作效率。酶分子采取怎样的物理策略来实现催化循环中多个物理化学步骤的精巧耦合与协同运作，从而克服催化循环中的限速步骤、实现高效率催化是一个尚未完全理解的基本生物物理问题。单分子实验技术的发展使得人们可以观测单个酶分子的催化动力学，这为酶催化理论模型提出新要求。本报告中，我们以腺苷酸激酶AdK为例，尝试建立描述单分子酶催化动力学的理论模型，并基于分子模拟、能量统计以及序列进化分析等方法，探讨酶分子实现高效率催化所采取的物理策略，揭示出酶分子多尺度能量阻挫特征、别构性质、以及酶催化动力学之间的关联。在此基础上，进一步探讨突变、机械力学、拥挤环境等因素调控酶催化动力学的分子机制以及提高酶催化速率的可能方案。

关键词：酶催化动力学，能量阻挫，别构耦合, 力学调控，单分子

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面向阿尔茨海默病脑生物力学特性变化的微观测量与建模分析

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摘要：神经退行性疾病是影响老龄化人口的主要疾病之一，给社会带来沉重负担，而阿尔茨海默病（AD）占其中主要部分。前期临床研究发现，AD早期的轻度认知障碍（MCI）患者，其脑组织剪切模量比正常老年人显著下降。为进一步揭示AD早期MCI患者脑组织力学参量变化机制，对神经元模型并对包含多神经元的培养基、神经元和细胞骨架开展原子力显微镜模量测量分析。本研究使用人神经母细胞瘤细胞系SH-SY5Y进行体外培养，并采用20μmol/L浓度的$A\beta_{1-42}$诱导SH-SY5Y细胞建立AD细胞模型，诱导时间为48h[1]。完成诱导后使用搭载PFQNM-LC-A-CAL探针的原子力显微镜峰值力液下测量模式分别测量正常细胞与AD细胞的杨氏模量，重复测量3组NC细胞与3组AD细胞，每组选取6个样本进行测量。测量完成后使用双样本t检验及多重比较校正（n_{NC}=18, n_{AD}=18）对比两种细胞杨氏模量差异。结果进一步与细胞计算力学模型开展对比，有助于更好地理解AD的发病机制及早期变化，为基于生物力学原理的AD早期筛查及治疗提供新思路。

关键词：阿尔茨海默病, 脑生物力学，细胞力学，原子力显微镜

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锁核酸修饰对CRISPR/Cas12a的trans酶活的精确控制作用研究

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摘要：CRISPR/Cas12a蛋白是一种等效的核酸内切酶，可催化靶链dsDNA进行位点特异性切割（*cis*活性）和非靶链ssDNA进行非特异性的随机切割（*trans*活性）。CRISPR/Cas12a系统的这种功能特性使其成为一种极具潜力的可编程核酸回路而广泛应用于多种信号放大反应和逻辑电路中。因此，精确控制其酶活作用的研究有助于进一步开发更为丰富的核酸回路。已报道的酶活控制化学修饰包括2'-O-甲基核苷酸（2'-OMeN）、锁定核酸（LNA）、硫代磷酸酯修饰核苷酸（PS），均表现出对某些限制性核酸内切酶和核酸外切酶的抵抗效应。尽管一些研究报道了修饰的crRNA可以增强Cas9蛋白在基因编辑中的特异性，而部分寡核苷酸修饰可以显著增强Cas12a蛋白参与的核酸检测方法的单核苷酸多态性检测能力。但是目前很少有研究系统深入地探讨不同DNA修饰对CRISPR/Cas12a的*cis*及*trans*酶活的影响，以及这种修饰对Cas12a蛋白等效内切酶活性的精确控制作用。本研究报告了一种利用锁核酸（LNA）修饰实现对Cas12a的*cis*及*trans*酶活进行精确控制的方法。通过特异性的引入和改变靶DNA中的LNA修饰位点，可以将Cas12a的切割活性限制在某个特定位点。本研究利用超高分辨的凝胶成像方法，成功验证了这种基于LNA修饰对酶活的精确控制效应，并基于实验结果构建了一个完整的Cas12a核酸酶活控制模型，用于解释和指导LNA的修饰方式以及精确控制酶活的效果。本研究中构建的LNA修饰控制Cas12a核酸酶活的模型为基于CRISPR/Cas12a系统的可编程核酸回路的设计提供了更为高效的方法，也为基于该系统的逻辑电路开发提供了更为多样化的选择。

关键词：CRISPR/Cas12a系统，锁核酸，*cis*酶活，*trans*酶活，定点切割，随机切割

胞嘧啶甲基化稳定 DNA 但阻碍 DNA 退火

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摘要：

在真核生物中，5mC 甲基化是基因组上最常见的 DNA 修饰，具有多种生物学功能。单分子技术已经被广泛应用于研究包括 5mC 甲基化 DNA 在内的多种核酸的结构和功能 [1]。在细胞生命活动过程中，DNA 解链过程至关重要，尽管对于 5mC 甲基化能够稳定 dsDNA 已有广泛研究，5mC 甲基化影响 DNA 解链和退火动力学过程的作用机制仍处于未知状态。

本研究使用单分子磁镊技术测量了 5mC 甲基化和非甲基化 DNA 发夹结构打开和重新闭合的动力学过程，发现 5mC 甲基化增大了 DNA 发夹打开的拉力，但出人意料地显著减小了 DNA 发夹重新闭合的拉力；测量恒力下 DNA 发夹打开和闭合的过程发现，非甲基化 DNA 发夹在恒力下打开和重新闭合的过程中都会在不同的平衡态间跳跃，表明能垒较低，而 5mC DNA 在完全打开后无法重新闭合，除非施加更小的拉力，才会以一步式的方式随机闭合，表明 5mC DNA 退火中存在高能垒，5mC 甲基化在动力学上阻碍了 DNA 退火。我们同时使用全原子分子动力学模拟揭示了 5mC 甲基化在动力学上阻碍 DNA 退火是由于额外的甲基造成的空间位阻效应，而不是影响了所带电荷导致的 [2]。

在细胞中，DNA 解链和退火在高速的 DNA 复制和转录过程中至关重要，5mC 甲基化造成的 DNA 退火过程减慢可能会对 DNA 复制和转录有重要影响，本研究的发现为 5mC 的生物学功能提供了新的见解。

关键词：DNA甲基化，DNA解链，DNA退火，单分子磁镊，全原子分子动力学模拟

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基于 CRISPR/Cas12a 自催化效应的核酸传感应用研究

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摘要：CRISPR-Cas系统来源于细菌体内的获得性免疫系统，具有精准靶向识别目标核酸序列和核酸高效催化切割的双重功能，因此在生物传感和临床诊断领域具备广阔的应用前景。然而，现有的基于CRISPR-Cas系统的检测方法存在检测系统冗余、信号放大效率有限，以及需要复杂的核酸结构设计等缺陷。为了更高效地发挥Cas12a蛋白核酸识别和切割的双功能特征，本研究通过引入两段式单链DNA（ssDNA）可控激活Cas12a和锁核酸修饰调控Cas12a外切酶活（*trans*）实现位点特异性切割的两种创新机制，构建了一种全新的基于Cas12a自催化正反馈级联信号放大的可编程核酸回路（CRISPR-Cas Autocatalysis Amplification driven by LNA-modified Split Activators, CALSA）。利用CALSA方法，本研究以合成的基因组DNA为靶标物系统性的验证了方法的信号放大效率，检测灵敏性以及核酸识别的特异性，发现与CRISPR/Cas12a直接检测方法相比，信号放大效率提升接近3个数量级，在短于1小时的检测时间内达到飞摩尔的灵敏度，并且具备单碱基突变识别的超高特异性。基于上述结果，本研究进一步利用CALSA方法通过对不同肿瘤细胞系分泌的无细胞DNA（cell free DNA, cfDNA）的实时检测，实现了对乳腺癌细胞系（MCF-7）的准确定量识别。

上述结果不仅证明了本研究中所开发的基于CRISPR/Cas12a的核酸回路CALSA方法在即时诊断领域的显著应用潜力，同时由于CALSA方法具有显著的可编程性，因此也进一步展示了CALSA在逻辑电路和其他生物传感技术开发领域的重要价值。

关键词：CRISPR/Cas12a系统，锁核酸，级联信号放大，无细胞DNA（cfDNA），即时诊断

单分子纳米孔测序技术–原理、实现与应用

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摘要

单分子纳米孔基因测序在测序读长、测序仪便携性、测序流程简便性、潜在的测序低成本、RNA直接测序以及表观遗传信息检测等方面都优于其它测序技术，但之前其测序准确度偏低，尚未能成为最主流的测序应用技术。近期纳米孔测序技术的有了突破性进展，在准确度上已经接近或达到二代测序技术的水平。纳米孔测序技术是一个典型的多学科交叉的技术，需要融合不同领域的先进前沿技术才能提升测序性能和指标。虽然多个技术环节都影响着纳米孔测序的准确度，但最关键的因素来自于测序化学复合体系，特别是纳米孔蛋白和控速蛋白。我们通过分子动力学模拟、人工蛋白质设计和蛋白质工程化改造对测序复合体系进行优化，获得具有更好碱基分辨能力的测序核心蛋白，为高通量测序设备的完成奠定基础。

细胞内吞中的颗粒协同性研究

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摘要：纳米颗粒在生物医药领域有着广阔的应用前景。内吞是细胞摄取纳米颗粒的一个主要途径。但目前的研究大都集中于细胞对同一类纳米颗粒的摄取行为，而对异种颗粒的细胞摄取却知之甚少。我们结合实验和模拟发现实际上细胞对功能化纳米颗粒（Functional NP，FNP）的吞噬能够有效促进非功能化纳米颗粒（或旁观者纳米颗粒，Bystander NP，BNP）的细胞摄取率。分子模拟和PMF计算表明，这一协同效应主要是由于FNP/BNP 与膜的相互作用以及由此所产生的膜曲率介导的纳米颗粒之间的有效吸引作用所导致的。并且，我们的理论分析表明，细胞对于一个特定的BNP的摄取率存在一个最佳FNP/BNP的数量比；而BNP 形状的各向异性和尺寸在一定范围内的增加也有利于旁观者摄取效应发生。我们的结果对如何利用FNP 或BNP 的性质来调控旁观者摄取效应给出了定性或定量的建议，为此效应的进一步应用奠定了基础。

关键词：细胞内吞,细胞膜,纳米颗粒,伴吞效应,计算机模拟

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MOLECULAR MECHANISMS OF NUCLEASE: A SINGLE-MOLECULE PERSPECTIVE

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Key Words: EXD2, RNase, Nuclease, Single molecule, Optical tweezers.

Ensemble studies have contributed tremendously to comprehending biological reactions. However, these studies characterize the average molecular population and have limited ability to detect intermediate states or distinguishing heterogeneities. Single-molecule techniques, such as optical tweezers (OT) and fluorescence resonance energy transfer (FRET), have proved to be exceedingly powerful in addressing this knowledge gap. By studying one molecule at a time, these approaches have enabled significant advances in the understanding of a wild variety of biomolecular systems. Employing these single-molecule techniques, we aim to understand the mechanisms and functions of indispensable enzymes in DNA replication, repair, and recombination. In this talk, I will share our recent findings regarding the nucleic acid degradation mediated by two types of nucleases – RNase H1 and EXD2.

Multimodal chemical imaging for tracking subcellular metabolic dynamics

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Abstract: Understanding the spatio-temporal dynamics of metabolites in living systems is fundamental for addressing important biomedical questions. Vibrational spectroscopic imaging techniques are increasingly recognized as unique tools for investigating biomolecules in their natural microenvironment. By employing a pump-probe detection scheme, both Raman scattering and infrared spectroscopy have greatly improved sensitivity and spatial resolution, enabling high-speed chemical imaging to monitor metabolites at the subcellular level and quantitatively track the physiochemical properties of biomolecules in real time.

Our previous work has focused on developing stimulated Raman scattering (SRS) and mid-infrared photothermal microscopy (MIP) imaging approaches to track compositional changes in micron-sized lipid droplet organelles. We have recently developed a novel multimodality spectroscopic imaging method, enabling comprehensive analysis of chemicals in cells and tissues at submicron resolution. Furthermore, through a photothermal relaxation localization (PEARL) mechanism, we have developed a novel super-resolution imaging technique for non-fluorescent molecules. Significant improvements in spatial and spectral resolutions have allowed resolving interactions between protein and lipid droplets by directly locating chemical bonds. Overall, these technological advancements have facilitated quantitative mapping of chemical dynamics in single cells, enabling us to decode the physiological status of cells and providing new insights into the subcellular dynamics of biomolecules for studying mechanisms regulating the physiological status of cells.

Keywords: chemical imaging, stimulated Raman scattering, super-resolution, metabolism, lipid droplet

细胞膜界面过程中的单分子动力学

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细胞膜隔绝细胞内部与外部环境，实现高效率和选择性的物质传递、能量传输、信号传导。许多基本的生理学过程皆发生于细胞膜界面。细胞膜的厚度仅约5纳米，并且成分复杂，具有异质性、流动性等特征，这为细胞膜界面过程的相关研究带来很大难度。单分子追踪和分析技术的发展为针对细胞膜时间、空间异质性特点的生物学过程研究带来了一种有效策略。此次报告将以多肽分子与细胞膜的界面相互作用过程为例，介绍本团队在细胞质膜单分子追踪与运动行为分析方面的研究进展。主要包括两个部分：单个脂质及多肽分子在细胞膜面内的扩散行为非高斯特性分析，以及单个分子在垂直细胞膜方向的动力学透化过程。这两类单分子追踪分析技术的发展，为实时研究细胞膜界面动力学过程、揭示活性分子与细胞膜的相互作用机制、进而设计靶向特定细胞膜的抗菌及化疗药物，提供了备选方案。

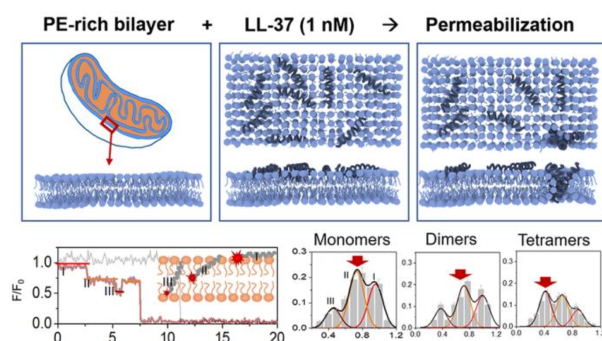

Fig. 1 Peptide-induced permeabilization of a membrane.

关键词：细胞膜；多肽；界面相互作用；单分子运动

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一种原位测量胚胎组织硬度及牵引力的方法

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摘要：

胚胎组织的硬度及其产生的牵引力对胚胎发育和分化具有重要影响。在活体胚胎中测量硬度和牵引力是一个具有挑战性的任务。本实验室前期发明了弹性微球，可在斑马鱼胚胎中定量测量胚层组织产生的三维牵引力[1]。目前已有的方法，只能对牵引力或硬度进行测量，这限制了对胚胎发育过程中力学性能变化的研究。本课题组制备了一种铂钴十字合金与聚乙二醇交联的探针，该探针在紫外光的照射下硬度会发生改变，可以用于原位测量胚胎组织的硬度与牵引力[2]。

通过显微注射将探针注射到斑马鱼胚胎内，利用细胞磁力扭曲仪对其进行磁化，随后施加15高斯的交变磁场，此时探针处于高硬度状态，会发生刚性旋转，通过测量旋转的角度，可以测出胚胎组织的硬度。随后用紫外光对探针进行短时间的照射，使探针整体变软。在周围组织的挤压下，探针发生形变，通过探针的形变程度即可计算出胚胎组织产生的牵引力。通过对斑马鱼胚胎发育的长时间观测，发现斑马鱼胚胎躯体部位产生的牵引力显著大于卵黄部位。

关键词：胚胎硬度, 牵引力，细胞磁力扭曲仪

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In vitro reconstitution of mitochondria cristae mimics

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Mitochondria inner membrane forms invaginated cristae architecture, providing a hub for highly dynamic biochemical reactions inside mitochondria. The biophysical properties of cristae structure and how it affects mitochondrial functions are not well understood. To study biochemistry on cristae-based environment, we developed an in-vitro cristae mimicking membrane system, named DNA-tethered lipid bilayer (DNA-TLB). DNA-TLB is a curvature-controllable, artificial membrane system, formed by the fusion of small unilamellar vesicles onto DNA-array-anchored glass surface. The double stranded DNA functions as robust pillars that lift up the lipid bilayer with a predictable height. Single molecular imaging showed DNA-TLB's good integrity and lipid diffusivity. Importantly, membrane curvature can be induced by mixing DNAs with different lengths to mimic cristae organization. With this system, we discovered that cardiolipin, a mitochondrial signature lipid, spontaneously accumulated at curved regions. This 3-dimensional structural stability overcomes the intrinsic repelling electrostatic force from the 2 negative charges carried on cardiolipin's head, elegantly organizes the densely charged phosphates with a certain orientation at the edge. Furthermore, mitochondrial transcription factor A (TFAM) was found strongly colocalized with cardiolipin on patterned DNA-TLB, regulating mitochondria DNA organizations. Therefore, the in-vitro reconstitution system provides new insights of how cristae structure regulates mitochondria functions.

Single-molecule FRET study of the conformational dynamics of Hsp70 during the functional cycle

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Abstract

Hsp70 is a conserved molecular chaperone which plays an indispensable role in regulating protein folding, translocation and degradation. The conformational dynamics of Hsp70 and its regulation by cochaperones is vital to its function. Using bulk and single molecule fluorescence resonance energy transfer techniques, we studied the interdomain conformational distribution of human stress-inducible Hsp70 and constitutively-expressed Hsc70 and the kinetics of conformational changes induced by the cochaperone Hdj1. We found that the conformations between and within the nucleotide- and substrate-binding domains show heterogeneity. The conformational distribution in the ATP-bound state can be induced by Hdj1 to form an NBD-SBD undocked conformation, which is an ATPase-stimulated state. Kinetic measurements indicate that Hdj1 binds to monomeric Hsp70 as the first step, then induces undocking of the two domains, which subsequently facilitates dimerization of Hsp70 and formation of a heterotetrameric Hsp70-Hsp40 complex. Our results provide a kinetic view of the conformational cycle of Hsp70 and reveals the importance of the dynamic nature of Hsp70 for its function. This work also reveals the difference between the highly homologous Hsp70 and Hsc70 in interdomain conformational dynamics and their regulation mechanism by Hdj1, which will provide clues to design drugs that specifically target stress-inducible Hsp70.

Key words： single-molecule FRET, conformational change， molecular chaperone, Hsp70, protein interactions

流场下 VWF-A 域构象功能活化的力-化学调控机制

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摘要：血管受损时内皮细胞会分泌血管性血友病因子VWF，与暴露的内皮下胶原结合，介导循环血小板的粘附和聚集。突变或病理性高流体剪切应力可诱导VWF封闭构象变为伸展构象，促进VWF与GPIbα结合。然而，在流体下力和突变诱导VWF-A域激活的关键事件仍不清楚。为此，我们利用 AFM和平行平板流动腔技术，研究了不同流体剪切应力下VWF-A的构象和功能变化。我们的AFM扫描成像数据显示，随着预加载剪切应力的增加，VWF-A分子先缩短后伸长，剪切应力的阈值约为100 dyn/cm2，表明增加预加载剪切应力会使处理后的VWF-A构象从松散的球形结构逐渐转变为致密结构，然后变为开放的伸展结构。此外，GPIbα在VWF-A基底上的粘附频率随预加载壁剪应力的增大先减小后增大，且具有相同的阈值。这些结果表明，VWF-A的力诱导激活仅发生在高剪切应力(> 100 dyn/cm2)。A1A2A3封闭构象的机械稳定性会被A1的GOF突变体R1308L削弱，被LOF突变体G1324S增强。为了进一步揭示力诱导VWF-A激活增强或减弱的分子机制，我们通过AFM在单分子水平上测定VWF-A1与A2或A3的互作。A1与A2的粘附频率大于A1与A3的粘附频率，说明A1更倾向于结合A2而不是A3。A1与A2或A3结合时表现出"逆锁-滑移键"转换，表明在低剪切应力作用下，力通过逆锁键机制抑制VWF活性，增强了封闭A1A2A3构象的稳定性；而在高剪切应力作用下，力通过滑移键机制增强VWF活性，降低封闭A1A2A3结构的稳定性，促进VWF-A由封闭构象向伸展构象转变。GOF突变体R1308L可缩短A1/A2复合物的键寿命，而LOF突变体G1324S可延长其键寿命。上述结果表明，GOF突变体R1308L会下调A1与A2的结合亲和力，导致封闭的VWF-A结构打开时屏障较低。相比之下，LOF突变体G1324S通过上调A1与A2的结合亲和力来增强封闭VWF- A构象的稳定性，从而抑制VWF活性。

关键词：VWF，剪切应力，突变，构象

Mechanistic insight into the mechanical unfolding of an integral membrane protein

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Single-molecule atomic force spectroscopy allows individual membrane proteins to be mechanically unfolded and has been widely used to study the dynamics and energetics of membrane proteins. Yet, the interpretation, molecular mechanism and significance of unfolding intermediates remained poorly explored and underappreciated. Here, we performed force spectroscopy experiments and molecular dynamics simulations to study the unfolding pathway of an integral membrane protein, diacylglycerol kinase (DAGK). The remarkable agreement between experiments and simulations allowed precise structural assignment and interaction analysis of unfolding intermediates, bypassing existing limitations on structural mapping, and thus provided mechanistic explanations to the formation of these states. DAGK unfolding was found to proceed helix by helix. We identified intermolecular van der Waals packing formed by hydrophobic residues as one of major contributions to the stability of unfolding intermediates. Mutagenesis creating packing defects induced dramatic decrease to the mechano-stability of corresponding intermediates and also to the thermo-stability of DAGK trimer, in good agreement with predictions from simulations. The more accurate structural assignment established and microscopic mechanism revealed here may significantly expand the scope of single molecule studies of membrane proteins.

基于目标锁定机制的实时三维单分子动态示踪成像技术

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摘要

细胞内生物分子之间的动态相互作用构成了生命活动的基础，研究其相互作用动态过程有望揭示最本质的生命活动的分子机制。受限于传统光学显微镜的时空分辨率，在纳米尺度上研究生物分子相互作用动态过程仍然存在挑战。实时三维单颗粒示踪技术已成为研究动态生物相互作用的强大工具，而单分子示踪由于其高空间和时间分辨率以及高灵敏度，有可能革新生物学动态过程研究方式。当前单分子动态观测方法的成像视野大多被限制在二维上，其使用的面探测器的曝光时间限制了单分子成像的时空分辨率。此外，单分子在溶液内的快速扩散运动阻碍了对单分子的高时空精度测量。对此，我们开发了一种基于主动反馈机制的实时三维单分子动态示踪成像技术（3D single-molecule active real-time tracking method, 3D-SMART）。3D-SMART利用电光偏转器和可调声梯度透镜实现激光焦点的快速三维扫描，通过关联荧光光子的探测时间信息和激光焦点的位置信息实现实时三维单分子定位，并通过主动反馈控制纳米位移台，使得目标荧光分子被锁定在激发区域之内。这种基于目标锁定机制的单分子示踪方法有效的解决了时间精度、空间定位精度和观测时长之间难以平衡问题。3D-SMART具有高时空分辨率、高成像深度、高灵敏度长观测时长等优点，为在单分子水平上研究生物分子之间的三维相互作用动态提供了一个有力的工具。

关键词：单分子成像，单分子示踪，荧光成像，单分子动态，三维示踪

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1-8eV(155-1200nm)连续可调DUV激光系统及其AMO科研应用

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AMO是研究原子、分子尺度量级上微观物质之间或物质与光之间相互作用的微观科学。这些相互作用的能量量级通常在几个电子伏（eV）。因此，光子能量为几个电子伏（eV）的窄线宽、连续可调的激光光源在AMO科研应用中起着至关重要的作用。在过去10几年，课题组长期从事深紫外AMO激光的研究，成功研制一系列1-8eV无间断连续可调的深紫外AMO激光系统，并成功应用在多个AMO科研领域之中，如激光核谱技术、激光离子源技术、共振拉曼技术、光镊技术等等。

Rotational Dynamics of Cellular Processes - A new paradigm powered by multi-dimensional single particle tracking

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Abstract： Viruses, drug delivery vectors, and other external particles exhibit a variety of complex behaviors on the cell membrane and inside the cell. Understanding the characteristic translational and rotational motion of nanoparticles is crucial to understand the underlying mechanisms. However, the knowledge of rotational dynamics in and on live cells remains highly limited due to technical limitations. A multi-dimensional single particle tracking technique has been developed to fully disclose the 3D translational and rotational motions of anisotropic imaging probes, as well as relevant biomolecular information. Using plasmonic gold nanorods as probe in our recent studies, we have visualized the live endocytic and intracellular transport events of nanoparticle cargos with unprecedented details in live cells. These experiments have led to new insights on the working mechanisms of molecular motors (dynamin in clathrin mediated endocytosis and kinesin/dynein in microtubule-based intracellular transport). This research initiates a shift in the current research paradigm on cellular structure and function by demonstrating the importance of rotational dynamics at the single molecule and nanoparticle level.

Key Words： Live Cell Imaging, Single Particle Tracking, Endocytosis

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组蛋白泛素化修饰调控核小体动态结构的分子机制

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摘要：真核细胞中，基因组DNA被组蛋白包装形成染色质，为所有DNA相关生物过程如基因转录与复制等，提供了结构平台。组蛋白分子伴侣FACT（Facilitates Chromatin Transition）复合物在真核生物中高度保守，是细胞存活的必要条件。FACT复合物最早由HHMI的Danny Reinberg实验室发现，其在促进RNA Pol II染色质水平上的基因转录延伸中发挥重要作用。随后的研究发现FACT几乎参与所有染色质相关过程，如基因转录、复制、修复等，并在维持染色质全基因组完整性中发挥作用。但是，对于FACT如何与染色质直接互作，进而在不同染色质状态中发挥不同生物学功能的机制仍然不清楚。我们团队首次揭示了FACT复合物对核小体具有降低稳定性和维持完整性的双功能调控机制；并揭示组蛋白H2A泛素化修饰不影响FACT分子伴侣功能，但抑制FACT的结合从而阻止其对染色质的调控，最终维持泛素化H2A染色质的稳定状态，在基因转录抑制中发挥作用。在此工作基础上，进一步阐明了组蛋白H2B泛素化修饰与FACT协同作用的分子机制。研究通过单分子磁镊技术，揭示了组蛋白H2B的K120位单泛素化修饰（ubH2B）可促进FACT将H2A-ubH2B二聚体组装进入染色质，形成ubH2B完整核小体的分子伴侣功能。ubH2B组装进入核小体后，破坏核小体的稳定性，并招募FACT结合到染色质上。有趣的是，结合FACT后的ubH2B核小体因为协同作用，会形成一个稳定可逆的核小体变构状态，在基因转录激活过程中发挥重要作用。全基因组ChIP-seq和RNA-seq数据分析也证实了这一点，富含ubH2B的基因区域同时有更多的FACT复合物富集，并且具有更高的转录水平。进一步的特定基因动态转录水平分析发现，在基因诱导表达的过程中H2B的泛素化水平逐渐上升，FACT复合物随之被逐渐招募到该基因区域激活基因转录。

关键词：组蛋白H2AK119，组蛋白H2BK120，单泛素修饰，FACT，核小体

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病理性蛋白相变聚集与帕金森病

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摘要：蛋白的液-固相变导致的病理性聚集与多种重要的人类神经退行性疾病密切相关。帕金森病（Parkinson’s disease，PD）是仅次于阿尔茨海默病的第二大神经退行性疾病。α-Synuclein (α-Syn)蛋白通过液-固相变形成病理性蛋白聚集为主要成分的路易小体是PD的核心病理学标志, α-Syn聚集的形成及其在神经元间的传播与PD的发生与发展密切相关。在本次报告中，我将以α-syn与PD作为一个具体研究案例，介绍蛋白病理性相变在神经退行性疾病中的关键作用，以及针对蛋白病理相变的疾病早期诊断及药物干预的相关研究。主要包括以下两个方面：（1）α-syn病理性相变聚集的结构多态性（amyloid polymorphism）以及其在决定疾病临床病理高度异质性的关键作用；（2）基于α-syn病理性聚集的抑制剂以及分子示踪剂的设计与筛选。相关研究对于深入理解蛋白相变与疾病提供新的视角和研究方向。为疾病的诊断与药物研发提供了新的思路。

细胞(类器官)力学成像技术与应用

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摘要：研究表明，生命体中的细胞既会对外界力学刺激产生各种生物学响应，也会由于生理、病理原因产生的生物学改变引起力学信号的变化。将细胞力学表型作为一种“生物标志物”来反应细胞的生物学状态，实现高通量、非标记、低成本的细胞分类识别和药敏测试，已逐渐成为细胞力学转化应用的一个前沿课题。要实现这一目的，就需要力学、生物学、图像处理、光电工程、人工智能和高通量生物芯片等深度交叉融合。本次报告将介绍近期我们课题组的一些研究进展，包括：发展基于高内涵成像的高通量细胞力显微镜技术，开展淋巴细胞活化、心肌细胞药物毒性测试等应用研究，并拓展到3D细胞类器官等研究；研制基于介电泳力的细胞粘弹形变检测芯片，实现细胞的非标记力学分型，并探索在疾病诊断、药物评价等应用；开发力-电耦合芯片，实现了3D细胞侵袭迁移的实时、免标记、高通量定量表征，并验证其在抗肿瘤药物评价筛选、临床肿瘤样本转移性检测等应用潜力。

关键词：细胞力学，显微成像，类器官，器官芯片

DNA 酶内在构象动力学的单分子研究

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摘要：DNA酶（DNAzyme）是一类具有催化功能的DNA分子，对特定金属离子有极高的选择性。由于其在生物传感器和医疗方面的潜在应用，DNA酶已被广泛研究并表征生化特性和功能之间的关系。与蛋白质酶和核酶类似，DNA酶具有以金属离子依赖性方式发生构象变化以进行催化。尽管构象在催化过程中发挥着重要作用，但传统方法可能无法揭示其变化动力学信息。使用单分子荧光共振能量转移（smFRET）技术，我们研究了对铀酰离子（UO^{2+}）具有识别特异性的DNA酶-39E的详细构象变化动力学。我们观察到在Mg^{2+}离子存在的情况下,39E的构象切换至折叠态，而在加入UO^{2+}后39E的构象处于一种比天然构型更延伸状态，以行使自酶切活性。此外，我们还发现39E即使在没有二价金属离子存在的情况下亦可在天然态，折叠态和延伸态之间切换，表明其构象变化是一种自发的过程。这种自发的构象变化可能是其对铀酰离子具有特异选择性的机制之一。

关键词：smFRET, DNA酶，构象动力学，金属离子

并行拉伸串联核酸探针进行核酸突变检测和基因分型

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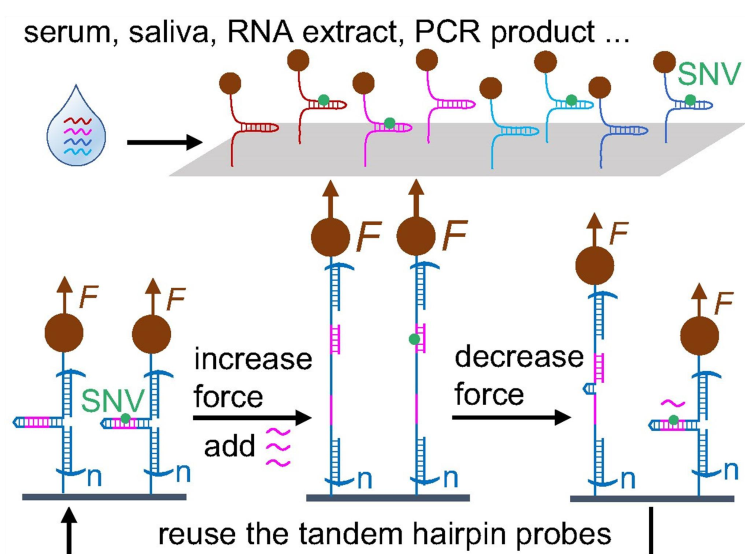
核酸序列的检测以及对 SNV（Single Nucleotide Variation）的鉴别，在科学研究和临床应用中都具有至关重要的意义。然而，存在一些限制因素，使得这一过程在技术上及具挑战性：SNV 引起的杂交能量损失使得其引起的检测信号变化微弱，难以与野生型区分；非特异性核酸片段的扩增容易引发假阳性结果；光谱的重叠限制了在并行检测中可选用的荧光染料类型等。为了克服这些限制，我们基于多通道单分子磁镊技术，发展了一种名为 THREF（Hybridization-Induced Tandem DNA Hairpin Refolding Failure）的新型单分子核酸检测方法。该方法无需扩增待测核酸，可在 30 分钟内实现飞摩尔浓度的检测灵敏度。同时，该方法以探针长度变化作为检测信号，解决了对荧光信号的依赖问题，极大增加了并行检测通量，这使得我们既可以量化临床样本中 microRNA 的表达水平，还可以识别核酸序列中多种类型的 SNV，包括难以检测的 G-U 或 T-G 摆动突变。我们在模拟临床样本上的示范性测试中，成功分析了血清中序列高度相似的 let-7 家族成员，以及唾液中 SARS-CoV-2 病毒株的基因型。此外，通过重复使用探针，该方法还可以进一步降低检测成本并缩短检测时间。综合而言，该方法具备高灵敏度、高特异性、高通量并行检测、可重复使用、样本无需处理以及探针设计简单等诸多优点。

该工作已在《Nucleic Acids Research》上公开发表：https://doi.org/10.1093/nar/gkad601.

关键词：核酸检测，单分子磁镊，单核苷酸变异，串联核酸探针。



基于结构光照明的超分辨定量 FRET 显微术（SI-FRETM）

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结构光照明显微术（SIM）已经是活细胞超分辨结构研究的重要技术手段[1]，荧光共振能量转移（FRET）显微术则是研究活细胞分子间结合分离以及构象变化不可获取的技术[2]。在超分辨显微术诞生后，发展活细胞超分辨 FRET 成像分析技术就内生物学家们基于厚望[3]。但是，尽管十几年中很多课题组做了很多努力，但是至今却没有实现活细胞中的定量 FRET 成像分析。SR-SIM 图像重建产生的“伪像”和定量 FRET 成像的“定量”间的矛盾是发展定量 SIM-FRET 成像的技术瓶颈。本文提出了利用 FRET 原理约束 SIM 图像重构发展活细胞超分辨 SI-FRETM 成像术的思想，并介绍了 SI-FRETM 概念的提出、发展、和研究进展。SI-FRETM 主要包括两种超分辨 FRET 成像理论：SIM-FRET 和 SI-FRETM。SIM-FRET 理论包括两步[4]：第一步基于 FRET 空间滤波的结构光照明图像重构（SIM）FRET 三通道的超分辨 SIM 图像，第二步基于该三通道图像进行定量 FRET 成像；SI-FRETM 理论则将 FRET 成像系统看成一个由 FRET 约束的光学成像系统，由供体结构光和受体结构光分别激发分别获得供体通道、受体敏化通道和受体通道的结构光照明原始图像，然后直接在空域进行 FRET 三通道的进行整体 SIM 图像重构直接获得 FRET 图像。根据 SI-FRETM 理论，搭建了双通道 SIM 成像系统，并实现了活细胞中线粒体膜蛋白结构 120 nm 分辨的定量 FRET 成像[4]。基于海森 SIM（His-SIM）技术和显微成像系统，全面开展了 His-SIM-FRET 成像技术研究以及成像系统的研发。研究了基于高保真 SIM 算法（HIFI-SIM）的定量 FRET 成像，探索了 HIFI-SIM-FRET 成像的技术问题和可行性。

关键词： FRET，超分辨成像，结构光照明显微术(SIM)

Keyword: FRET, Super-Resolution Imaging, Structure Illumination Microscopy

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Molecular mechanism of synaptotagmin-1 in regulating neurotransmitter release in physiological and pathological conditions

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In pre-synapse, membrane fusion is an essential process for neurotransmitter release in the nervous system. The synaptic fusion machinery includes the neuronal SNAREs, synaptotagmin-1(Syt1), and other fusogenic proteins. In physiological condition, prior to Ca^{2+}-triggered neurotransmitter release, synaptic vesicles are locked at the priming state by Syt1, a process that enables the synaptic vesicles to undergo fast-triggered fusion. In pathological condition, the imbalance of intracellular ion during diseases may result in uncontrolled neurotransmitter release prior to Ca^{2+} influx. Syt1, as the Ca^{2+} sensor on synaptic vesicle, regulates both the spontaneous release and Ca^{2+}-evoked neurotransmitter release. However, the mechanism of Syt1 together with SNAREs, orchestrate to regulate neurotransmitter release is still not fully understood. In our work, combining the single-channel membrane fusion electrophysiology, cell-based electrophysiology and single molecule fluorescence experiment, we found that interactions among Syt1, SNAREs and anionic membrane synergically orchestrate the dynamics of fusion nanopore opening in synaptic vesicle exocytosis, and Syt1 may employ an alternative mode to regulate neurotransmitter release in pathological condition.

Key words: Neurotransmitter release; SNARE; synaptotagmin-1; membrane fusion; fusion pore

“点亮”细胞的机械力信号：从亚细胞结构至单分子

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细胞在组织中是高度动态的，它们的功能不断被其他细胞和细胞外基质（ECM）产生的各种形式的推、拉、挤的机械力所调节。在这些过程中，在受体分子上产生的机械力非常微小，在几 pN 到几十 pN 之间，但这些力可以在时间和空间上精确调节信号转导过程，从而直接或间接控制一些生物反应，如细胞分化、基因表达和细胞凋亡。因此，在分子水平上表征机械和生化信号之间的相互作用是理解细胞机械信号传递机制的一个重要部分。在这里，我们提出了一种基于 DNA 的纳米技术，能够对活细胞中通过膜受体传输的 pN 级分子力进行实时成像。利用这种技术，我们揭示了以前未曾见过的承力超分子结构（即 "机械热点"），这些结构作为 "机械支点 "稳定了焦点粘附结构并促进其成熟。此外，为了了解细胞如何在分子水平上感知和响应 ECM 硬度，我们开发了一种基于水凝胶的分子张力荧光显微镜（mTFM）方法，以可控和可靠的方式将这些 DNA 张力探针功能化在软水凝胶表面。此外，荧光纳米球被连接在同一水凝胶表面，使我们能够将分子张力荧光显微镜（mTFM）与传统的 TFM 结合起来，并收集更全面的数据，包括分子力图、净细胞牵引力和力取向。利用基于水凝胶的 mTFM，我们研究了 ECM 刚度如何调节整合素介导的力传递过程。我们发现，细胞主要通过招募更多的受力整合素和调节整合素对 ECM 采样的频率来感知 ECM 刚度的增加，而不是增加 FAs 的整合素力的平均幅度。我们的结果表明，基于水凝胶的 mTFM 可以用来研究在软水凝胶上扩散的细胞的机械传导过程，这在力学上与组织更相似，并可能提供关于细胞刚度感应和机械传导的分子机制的新见解。

Transition Time Determination of Single-Molecule FRET Trajectories via Wasserstein Distance Analysis in Steady-state Variations in smFRET (WAVE)

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Abstract: Many biological molecules shift their conformational states from one steady state to another due to external stimuli, such as light, a sudden change in ionic concentration, temperature, hormones, electric potential, or even magnetic fields. Single molecule FRET (Fluorescence Resonance Energy Transfer) is of particular interest to not only define the steady state conformational ensemble usually averaged out in the ensemble of molecules, but to characterize the dynamics of biomolecules. To study steady-state transitions, i.e., non-equilibrium transitions, a data analysis methodology is necessary to analyze single-molecule FRET intensity-time trajectories, which contain mixtures of contributions from two steady-state statuses and include non-equilibrium transitions. In this study, we introduce a novel methodology called WAVE (Wasserstein distance Analysis in steady-state Variations in smFRET) to test whether a single molecule has transitioned from one steady state to another, and if yes, also to determine the transition time, which is the time lapse between an external stimuli and when the system set into another steady-state status, through detecting and locating non-equilibrium transition positions in FRET trajectories. Our method first utilizes a combined STaSI-HMM (Stepwise Transitions with State Inference Hidden Markov Model) algorithm to determine the number of (sub-)states and exchange rates before and after the external excitation that changes the molecular condition and convert the original FRET trajectories into discretized trajectories. We then apply Maximum Wasserstein Distance (MWD) analysis to differentiate the FRET state compositions of the fitting trajectories before and after the non-equilibrium transition, and figure out the point of each FRET trajectory where MWD is attained as a preliminary estimate of the non-equilibrium transition position. In the last step, the forward and backward algorithms, based on the Minimum Description Length (MDL) principle, are used to find the refined positions of the non-equilibrium transitions. We showcase the feasibility of our method by applying it on simulated smFRET intensity-time trajectories based on realistic experimental parameters, and the results reveal excellent performance in various scenarios with high detection sensitivity and accurate identification of non-equilibrium transition positions. Our method's applicability to real experimental data has been verified through its successful application in studying TAR-DNA hairpin folding, in which a proof-of-principle experiment is carried out to study the transition patterns of the denaturing of a TAR−DNA hairpin. WAVE provides a reliable decoupling tool for FRET data obtained from real-time in-situ observations under variable conditions. It opens a new avenue for dynamical studies of biological molecules, and we believe it would benefit studies focusing on complex and dynamic biological systems.

Key Words: smFRET; non-equilibrium transition; maximum Wasserstein distance

非共价作用调控固相分子电荷传递

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摘要：分子电子器件的电信号对外界环境响应灵敏，在分析传感中有重要的潜在应用。许多分子电子器件电信号的响应与分子非共价作用在化学环境中的变化密切相关。精准调控分子非共价作用引起的电荷传递变化，设计和区分电学特征信号，对研制功能型分子电子器件，实现目标物灵敏检测至关重要。通过分子结构设计和固相有序自组装单层膜，构建结构较为明确的分子结。通过准确调控分子内、分子间非共价作用，我们探讨了主客体作用、氢键、静电力和范德华力等非共价作用调控分子电学性质的规律和可能的理论模型，为发展基于固相分子结的分析检测新方法提供了重要信息。

关键词：分子电子学，电荷传递，非共价作用

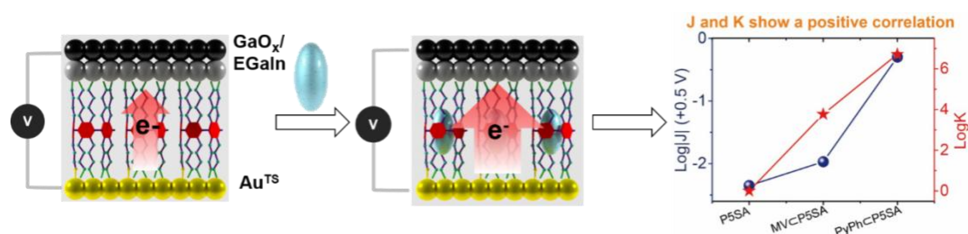

Fig. 1 The host-guest interactions enhance the charge transport via Pillar[5]arene-based self-assembled monolayers as an example

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Micro- to Millisecond Conformational Dynamics of DNA Molecules Investigated by Maximum Entropy Method-based Fluorescence Lifetime Correlation Spectroscopy

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To decipher a biomolecule's dynamic structure-function mechanism, it is critical to analyze its conformations and conformational dynamics occurring at the functionally relevant micro- to millisecond timescales. Fluorescence correlation spectroscopy (FCS) is an effective technique for investigating fast structural changes using biomolecules labeled with Förster resonance energy transfer (FRET) probes. However, traditional FCS method is typically limited to studies of transitional dynamics between two priorly known conformations. In this study, we developed a novel technique of maximum entropy method-based fluorescence lifetime correlation spectroscopy (MEM-FLCS), which can resolve molecular confirmations without prior knowledge, and simultaneously determine transitional rates for at least three conformations. We first validated this new technique using simulated data, and then used it to investigate conformational dynamics of FRET-labeled DNA hairpin and MYC G-quadruplex (G4) molecules. At physiological relevant NaCl concentrations, DNA hairpin exhibits three conformational states: folded (F), intermediate (I) and unfolded (U). MEM-FLCS was able to resolve these three confirmations, and measured the relaxation constants for the F↔I, F↔U transitions to be 143±12μs and 156±15μs respectively, while that for the I↔U transition is less than 10μs. At physiological relevant KCl concentrations, MYC G4 prominently showed two conforms: Folded (F) and Unfolded (U), with a little understood Intermediate (I) conformation detectable at low KCl concentrations. Interestingly, the relaxation constant for the F↔U transition decreases steadily with increasing salt concentrations, which we interpret as faster conformational transition bypassing the Intermediate state. Overall, we demonstrated a valuable tool for resolving multiple biomolecular conformations and quantitatively evaluating the functionally relevant conformational dynamics.

生物高分子材料力学：从微观到宏观

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摘要：在自然界中，生物材料呈现出多样的力学特性，例如血管具有高度的延展性，肌肉展现出优异的弹性，蜘蛛丝则表现出卓越的韧性等。这些材料的基本构成是弹性蛋白，作为主要的结构单元，它们承担着承受外部力的重要角色，其力学属性直接塑造了整体材料的力学特性。与此同时，弹性蛋白的构象变化还在细胞内以及细胞与细胞外基质之间传递着力信号。

随着单分子操作技术的进步，特别是基于原子力显微镜的单分子力谱技术的应用，我们得以在单个分子层面上研究自然界和人工合成高分子材料的力学特性。这一发展使我们能够揭示许多弹性蛋白的力学性能与其分子结构之间的紧密联系，进一步加深了对高分子链的力学化学响应机制的理解。借助自下而上的方法，我们日益能够理性设计蛋白质的宏观力学特性。

受贻贝足丝蛋白中富含组氨酸的序列的自组装启发，我们设计了带有“花边”的自组装多肽纤维。利用这些纤维作为构筑元件，我们制备了具有高强度、高韧性、抗疲劳性和快速机械恢复的水凝胶【1】。在这种设计中，我们巧妙地将一部分提供延展性的高分子链以“花边”的形式嵌入多肽纤维的beta片之间，而不是像通常双网络水凝胶那样作为额外的冗余网络，解决了水凝胶中常见的刚度-韧性不可兼得的设计难点。同时，由于螯合金属离子的自组装多肽纤维中多重弱相互作用的协同，自组装纤维力学稳定性高，组装速度快。这些特殊的分子尺度的设计赋予了水凝胶高强度（~4.1 MPa）、良好的韧性（25.3 kJ m^{-2}）、高疲劳阈值（~451 J m^{-2}）和快速恢复（数秒量级）的优良宏观力学性能。我们的研究强调了在分子水平上设计水凝胶力学性能的重要意义，所获得的材料有望在组织工程支架、可拉伸传感器和软机器人组件等方面得到应用。

关键词：单分子力谱, 水凝胶，多肽，蛋白质，力学性能

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磁显微成像技术发展及其生物医学应用

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摘要：磁共振技术分别在医学影像和分子结构研究中取得了巨大成功，然而，受限于检测方式，传统磁共振的空间分辨率和灵敏度不高。金刚石磁传感器（NV 色心）由于其优良性质，提供了一种非常好的微观磁共振测量手段，既保留了传统磁共振的优势，又在分辨率和灵敏度方面有很大的提升，有很好的应用前景。然而，虽然经过了系统的物理原理验证、量子调控和精密测量技术的开发，但由于测量技术与生物医学应用场景的脱节，缺乏针对性有效检测方法，导致金刚石量子传感技术还没有在生命科学领域发挥应有的作用。我们主要从事物理与生物医学的交叉研究，着重从生命科学与健康领域的重大科学问题和应用需求出发，开发基于金刚石磁传感器的微观磁共振技术，进而用于解决重要的生物医学问题，取得了多项重要研究成果。近 5 年，基于金刚石量子传感器，自主搭建了多台套微观磁共振检测装置，开展了从组织到细胞、再到单分子水平的多层级技术开发与应用研究，开发出微米分辨率的肿瘤组织免疫磁成像技术、纳米分辨率的细胞磁成像技术、单分子灵敏度的生物分子相互作用磁学检测技术，主要研究成果发表在 PNAS、Science Advances 和 Nano Letters 上。在相关研究方向，我们将进一步发展基于金刚石量子传感器的微观磁共振技术，用于生物医学基础研究和临床检测与诊断。

关键词：金刚石 NV 色心，磁显微成像，肿瘤组织，单分子，免疫检测

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单价阳离子反常降低核酸双螺旋稳定性及其机制

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关键词：磁镊、双螺旋、DNA、单价盐、Tm

摘要：核酸双螺旋的两条链带有大量负电荷，互相排斥而降低双螺旋的稳定性。双螺旋的负电荷吸附溶液中的阳离子，影响核酸双螺旋的性质。例如，我们发现在大概 65 pN 的拉力下，较低的盐浓度下 DNA 双螺旋稳定性降低而解链 [1-3]，较高的盐浓度（<1 M）稳定了双螺旋结构。另外，我们发现核酸吸附高价阳离子时，双螺旋会电荷中和甚至电荷反转，通过不同的路径影响 DNA 和 RNA 双螺旋：1）高价阳离子主要嵌入 RNA 大沟，使 RNA 变硬 [4]，同时旋紧 RNA [5]；2）高价阳离子主要结合在 DNA 的磷酸基团，通过扩增直径旋松 DNA [6-7]，而 5mC 甲基化会强化这些作用 [8-9]。

一般认为，双螺旋的一个负电荷最多吸附一个阳离子，因此单价阳离子不能使双螺旋电荷反转，单价阳离子浓度单调稳定双螺旋，但缺乏直接验证。为验证该猜测，我们测量了 DNA 和 RNA 双螺旋的力学和热稳定性，意外发现更高浓度（>1 M）单价阳离子降低双螺旋的稳定性。通过分子动力学模拟，我们发现两种机制导致了该反常现象：1）双螺旋的负电荷呈分数分布，因此一个负电荷可以吸附超过一个溶液中的单价阳离子，导致双螺旋电荷反转；2）双螺旋结合的单价阳离子过于拥挤而互相排斥，从而降低双螺旋的稳定性。我们的新发现对于理解核酸双螺旋结构以及离子与带电界面相互作用具有新的理论意义，也在核酸分型定量 [10] 和纳米孔测序等技术中对优化溶液条件具有应用价值。

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细胞和颗粒的三维动态表征

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摘要

真实环境中诸如药物纳米颗粒、细胞和细菌等微粒通常进行着难以预测、各向异性的快速运动。对这些体系进行无扰动的原位三维动态观测是了解它们行为规律的基础。然而，要实现对复杂场景下微粒动态行为的无损、高精度、原位三维观测仍存在很大的技术挑战。近期，我们建立了一种基于光学干涉的三维显微成像技术-数字全息显微镜(Digital Holographic Microscopy (DHM))并发展了相应的三维追踪算法。在DHM的测量中，光场在目标样品周围形成干涉。结合高速相机，我们可实时记录物体的全息图像序列。 结合光学衍射重构,定位及多粒子三维追踪算法，微粒的三维轨迹及形貌变化可被成功获取。该方法的观测范围可从界面至远离界面数百微米，无需标记，适用于同时追踪多个目标物，并具有小于100 nm的三维定位分辨率，并可对不定形和可形变的物体进行不间断的三维示踪。利用DHM，我们观察了药物颗粒、细菌和细胞在多种材料和环境中的三维动态行为。同时，利用DHM对海洋污损菌的粘附和细胞在三维凝胶中的运动研究等典型例子揭示了细胞和细菌对环境的主动响应是其动态行为的重要组成部分。

关键词：三维示踪；数字全息显微镜；细胞动态表征；颗粒三维表征；

Single-molecule dynamics of transcription factors interrogating chromatin

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The presence of closed chromatin often hinders transcription factors (TFs) and the transcription machinery from accessing nucleosomal DNA templates. Some TFs, known as pioneer transcription factors (PTFs) or pioneer factors, have a remarkable ability to access their target DNA even when it is nucleosome-bound in compacted chromatin and initiate cell fate changes. However, the dynamic process of PTF engaging with and targeting chromatin in cellular context remain elusive. In this study, we used a combination of live-cell single-molecule tracking (SMT), fixed-cell multi-color super-resolution imaging PALM and computational simulation and modeling to elucidate the dynamic process of PTFs searching for targets on chromatin in living cells. We discovered a notable "confined target search" mechanism that facilitates PTFs to access targets in closed chromatin. In this mechanism, the PTF alternates between fast free diffusion in the nucleus and slower confined diffusion within mesoscale compacted chromatin domains. The latter leads to repetitive binding trials to the nucleosomal targets and successful binding. Therefore, these findings suggested that densely packed closed chromatin could play a previously unrecognized positive role in facilitating PTFs binding nucleosomal targets. Our results reveal a new mechanism whereby TFs exploit chromatin architecture to access closed chromatin.

Keywords: Single-molecule tracking, Super-resolution chromatin organization, pioneer factors, closed chromatin

DNA 聚合酶高选择活性与外切校对的分子机制

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摘要：高保真度的 DNA 复制对基因遗传的稳定性和生命新陈代谢起着关键作用，而 DNA 聚合酶的高选择活性和外切校对功能是实现高保真度的关键。此前的研究表明，DNA 聚合酶的选择性主要依赖于其手指结构域不同的构象：打开、部分闭合和闭合构象。前两个构象可能会对碱基进行选择，使得结合错配碱基后难以转变为闭合构象。然而，由于打开和部分闭合构象的持续时间很短，直接观察其选择碱基的过程是困难的。当聚合酶遇到错配的碱基时，其外切校对功能会切除该碱基。如果校对失败，聚合酶继续往下游合成。外切功能还能否再对上游包埋的错配进行校对尚不清楚，其具体分子机制仍未厘清。我们通过亚毫秒时间精度的单分子荧光共振能量转移技术(smFRET)观察了 DNA 聚合酶 I 对配对与错配碱基的选择过程。结果表明，当聚合酶结合到正确的碱基时，打开构象向部分闭合构象的转变速度要快于结合到错配碱基时。在部分闭合构像中，错配的碱基有很大的概率解离，而正确的碱基会促使部分闭合构象转变为闭合构象。此外，我们还观察了 DNA 聚合酶 I 和 T7 DNA 聚合酶 gp5 对包埋错配的校对，发现其在切除碱基后有可能进行连续切除，这既提高了切除包埋错配的效率又有可能切除配对碱基，形成损耗。通过理论计算，我们发现存在一组连续切除的参数，使其在校对和损耗之间达到平衡，而这一参数也十分接近实验测量值。

关键词：DNA聚合酶，保真度，单分子荧光共振能量转移技术

微流技术在定量生物学中的应用

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近期的细胞动力学分析以及定量的生物学研究中，一些新的方法例如微流技术已经开始被广泛的应用。其能精确的控制细胞 / 细菌所处的微环境，高时空精度的记录细胞 / 细菌的表型行为，蛋白表达水平的变化，同时高通量的进行实验对照为细胞水平的动力学研究提供了最佳的实验工具。该实验方法结合生物物理模型可以实现精准的改变外部单参数，对基因调控回路的进程进行定量的分析以及物理建模；也可以测定人工基因元件的参数并对人工合成生命回路的动态性质进行精准预测，极大的扩展了物理学在细胞水平的生物学问题研究的应用场景。

在报告中报告人将展示近年来课题组开发的针对不同模式细胞的蛋白动力学定量分析的高通量微流芯片系统；利用该系列微流系统，一方面我们开展了不同糖浓度环境下酵母细胞应对渗透压变化应激响应行为的定量研究工作，分析得到低糖浓度渗透压应激响应蛋白高合成速率的生物物理原因；另一方面在系列合成生物转录翻译调控元件的定量表征及应用中，我们采用稳态与动态变量分离的数理模型并结合微流系统中测定的高通量细菌蛋白表达动态数据，实现了转录翻译调控元件的精准刻画以及各类复杂回路的动力学行为预测。

关键词:微流技术，定量生物学，合成生物学，蛋白表达动力学，应激响应，转录翻译

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The Force Response and Free Energy Landscape of the 10th Type III Fibronectin Domain Revealed by Magnetic Tweezers

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Abstract: The tenth domain of type III fibronectin (FNIII10) plays a crucial role in cell adhesion to the extracellular matrix. This protein has a simple native structure similar to immunoglobulin domains but displays different unfolding behaviors. Our study used magnetic tweezers to investigate FNIII10's unfolding and folding dynamics under stretching forces in a physiological force range (4-50 pN). Results showed that FNIII10 follows a consistent transition pathway, with an intermediate state involving detached A and G beta−strands from its native state. We determined all force-dependent transition rates of FNIII10 and found that the rates of unfolding from the native state to the intermediate state and from the intermediate state to the unfolded state deviate from Bell's model. We constructed a quantitative free energy landscape with well-defined traps and barriers that exhibits a hierarchical symmetrical pattern. Our findings provide a comprehensive understanding of FNIII10 conformation dynamics and demonstrate how the free energy landscape of multi-state biomolecules can be precisely dissected.

Key Words: Fibronectin, Free Energy Landscape, Magnetic Tweezers, Force Response

高时空分辨与双模态细胞成像新探针

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摘要：

单分子定位成像是实现细胞结构纳米尺度可视化研究的强大工具，其发展高度依赖于探针、仪器和算法的协同创新。近年来，随着领域内先进成像仪器和算法的快速发展，荧光标记探针逐渐成为首要制约因素。例如：由于探针亮度和标记密度低，单分子定位成像的空间分辨率受限，难以实现看得更清的目的。其次，由于探针的光控动力学不佳，难以捕捉活细胞中毫秒级纳米尺度的分子动态变化，无法达到看得更快的目的。其三，由于荧光探针不耐受电镜制样，高精度超分辨光电关联成像存在困难，细胞环境的全景结构信息缺失。针对以上问题，我们开展了一系列探针开发工作：1）建立普适性探针开发策略，发展单分子定位成像金标准荧光蛋白探针，提升成像空间分辨率；2）开发高时间分辨率荧光探针和由探针衍生的成像算法，将活细胞单分子定位成像的时空分辨率推进到当前技术极限（0.1 s，50 nm）；3）设计发展第一个耐常规电镜制样的光控荧光蛋白，解决同层切片高精度光电关联成像难题，实现全景超微结构信息的同步获取。

关键词：

单分子定位成像，光控荧光蛋白探针，活细胞超分辨成像，超分辨光电关联成像

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Switch of cell migration modes orchestrated by changes of three-dimensional lamellipodium structure and intracellular diffusion

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Abstract: Cell migration plays important roles in many biological processes, but how migrating cells orchestrate intracellular molecules and subcellular structures to regulate their speed and direction is still not clear. Here, by characterizing the intracellular diffusion and the three-dimensional lamellipodium structures of fish keratocyte cells, we observe a strong positive correlation between the intracellular diffusion and cell migration speed and, more importantly, discover a switching of cell migration modes with reversible intracellular diffusion variation and lamellipodium structure deformation. Distinct from the normal fast mode, cells migrating in the newly-found slow mode have a deformed lamellipodium with swollen-up front and thinned-down rear, reduced intracellular diffusion and compartmentalized macromolecule distribution in the lamellipodium. Furthermore, in turning cells, both lamellipodium structure and intracellular diffusion dynamics are also changed, with left-right symmetry breaking. We propose a mechanism involving the front-localized actin polymerization and increased molecular crowding in the lamellipodium to explain how cells spatiotemporally coordinate the intracellular diffusion dynamics and the lamellipodium structure in regulating their migrations.

Key Words: Cell migration, single-particle tracking, three-dimensional, lamellipodium

细胞高磷信号激活 VTC 复合物进行多聚磷酸盐合成与跨膜转运的多功能耦合与动态过程

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摘要：多聚磷酸盐（ployP）存在于所有生命体中，是重要的能量代谢物质。PolyP 的合成是以 ATP 为原料，通过形成高能磷酸酐键，将正磷酸盐聚合形成线性多聚物，大量储存能量和磷酸盐，维持 ATP 和磷酸盐稳态。PolyP 广泛分布于线粒体、细胞核、液泡等细胞器，它通过能量和 Pi 的可逆储存与释放，维持 ATP 和 Pi 稳态，与癌症、神经退行性、细菌感染等疾病相关。真核生物中，目前唯一已知的 polyP 合酶是 VTC 膜蛋白复合物。酵母 VTC 复合物包含多个亚基（Vtc1, Vtc2, Vtc3, Vtc4），主要定位于液泡膜，它感知细胞高磷信号（PP-InsP），以 ATP 为原料在胞质侧合成 polyP，并将其跨膜转运至液泡内储存。因此，VTC 作为一种特殊的膜蛋白质机器，它同时具备信号受体、酶和转运体的功能，耦合信号感知、产物合成与跨膜转运。我们联用冷冻电镜、活细胞胞内核磁共振、单分子荧光等多种生物物理化学手段和技术，基于生化与功能分析，阐明了 VTC 复合物中细胞高磷信号的真实受体亚基，揭示了 VTC 复合物耦合信号感知、产物合成与跨膜转运的动态过程与调控机制，为理解真核生物中 polyP 的生物发生提供了新的见解，也为其它高等生物中 polyP 合酶的发现提供了线索和指导。

基于单条染色质应变定量技术探究不同模态力学信号激活基因表达的机理研究

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力学信号及胞外基质的力学特性可调控细胞增殖及分化、蛋白质合成及基因表达，在组织发育，疾病发生及生理调节中起到非常重要的作用[1-3]。我们之前的研究表明，力学信号可直接拉伸染色质激活基因表达[4]，然而其机制尚不清楚。除此之外，细胞在生理条件下感受的力有不同频率、大小和模态（如压缩力或拉伸力），但是不同模态的力学信号是否调控差异性基因表达尚且未知。

本研究基于前期构建的染色质可视化细胞系，结合三维细胞磁力扭曲仪以及细胞拉伸仪两种不同的细胞加力系统，实现不同模态、大小和频率的力对染色质拉伸的定量，并结合 RNA 原位杂交（RNA FISH）技术定量力激活基因表达的程度。研究发现低频率（0.3-20Hz）的力学信号会快速上调内源基因 *egr1* 以及外源基因 *DHFR* 的表达，而高频率（100Hz）力学信号虽能提高细胞硬度但不能上调基因表达，同时发现拉伸染色质可激活基因表达，而压缩染色质则不能激活基因表达，说明不同模态力学信号激活基因表达具有差异性。

本研究发现，力只能激活位于常染色质的基因，该部分染色质 H3K9me3 低表达，处于疏松状态，可被力拉伸，而上调 H3K9me3 可固缩染色质从而阻止力激活基因表达，说明力激活基因表达需要 H3K9 去甲基化。同时发现，基于拉伸力实现的染色质拉伸可暴露基因启动子位点，从而募集转录机器 RNA Polymerase II 结合基因启动子，激活基因表达，而压缩力实现的染色质压缩由于阻止 RNA Polymerase II 结合基因启动子，无法激活基因表达。揭示了不同模态力学信号激活基因表达的机理。

综上所述，我们的研究表明，力激活基因表达依赖力对染色质的拉伸而非压缩，同时力激活基因表达需要 H3K9me3 低甲基化。

关键词：染色质可视化，染色质应变定量，单细胞力学加载，基因表达，表观遗传

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光遗传学二聚系统在细胞黏附的力感知中的应用

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细胞通过动态富集于细胞连接上的力敏感蛋白来感知与转导一系列来自细胞内外微环境的物理信号。这些力敏感蛋白，例如在细胞黏附上的integrin, talin, vinculin等，在生理时间尺度上动态地、非共价地结合形成受力的信号通路。若能够在时间和空间上实时地调控这些力敏感的信号通路将有助于理解细胞力感知与转导的分子机制。广泛应用的光遗传学二聚系统是一种潜在的强有力的实现力敏感通路实时调控的工具。由于力敏感信号通路承受着生理范围机械力的调控，光遗传学二聚系统应用于力敏感通路的一个必要前提是其能够承受相应生理范围的机械力。为此，我们利用单分子力学操控技术，探究了包括iLID，LOVTrap等经典光遗传学二聚系统在生理机械力范围的力学稳定性，并将其应用于talin介导的细胞黏附过程的力感知调控。我们的研究为细胞力学生物学机制探究提供了一种光遗传学二聚系统调控的方法。

关键词：力敏感蛋白，光遗传学二聚系统，力学生物学，单分子力学操控

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环境诱导 DNA 和 RNA 扭转的统一模型

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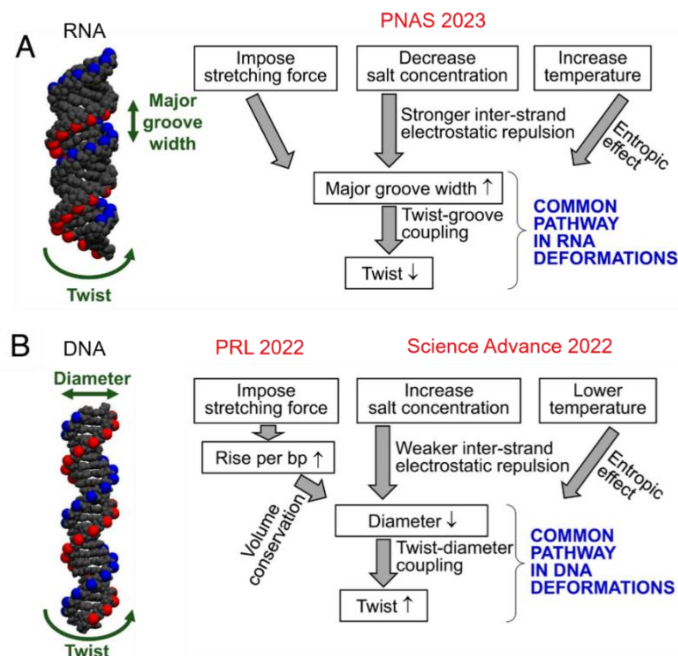
环境因素，如拉力、盐离子浓度和温度变化，会引起核酸结构和性质的变化，这在众多生物过程和材料应用中是至关重要的。微小的扭转角变化在超长的 DNA 分子中积累，最终导致显著的结构变化。引人关注的是，拉力对 RNA 和 DNA 的扭转产生的效果是相反的，这一现象一直未得到解释。我们使用单分子磁镊实验准确测量了各种环境因素对 DNA 和 RNA 扭转的影响，并利用分子动力学模拟以及理论建模来建立了环境诱导的 DNA 和 RNA 扭转的统一模型。

我们的研究发现，DNA 和 RNA 分子在特定环境刺激下，均可以展现出正和负的扭转-拉伸耦合。其主要取决于在拉伸过程中哪种形变路径占主导：1）通过改变直径形变，产生负的耦合参数；2）当存在高价离子使直径不可变时，通过改变沟槽的宽度形变，产生正的耦合参数。而哪种形变路径占主导地位，取决于 DNA 和 RNA 在拉伸时的结构状态及其受到的外部约束 (Physical Review Letters, 2022)

我们还发现，在盐浓度变化、温度和拉力三种不同刺激下引起的 RNA 和 DNA 变形具有一定的普遍性。对 DNA 来说，这些刺激首先改变了 DNA 的直径，并通过扭曲-直径耦合转化为 DNA 的扭曲变化（Science Advances, 2022）。对于 RNA，这些刺激首先改变大沟槽宽度，通过扭转-沟槽耦合转导成扭转变化（PNAS 2023）。扭转-沟槽耦合和扭转-直径耦合可以降低蛋白质结合时 DNA 和 RNA 时的变形能量成本。



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A ribozyme selects mechanically stable conformations for induced catalysis

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Abstract： Ribozymes come from the RNA world and are widely found in prokaryotes and eukaryotes. These catalytic RNA molecules target nucleic acids and can be engineered as biotechnical tools or for gene regulation. Among them, hammerhead is the smallest and best characterized ribozyme, capable of cleaving a specific phosphodiester of an RNA strand in a cis or trans fashion. However, the structure and biochemical data of hammerhead ribozymes have long been disagreed, making the understanding of its catalysis mechanism a longstanding issue. Particularly, the role of conformational dynamics in ribozyme catalysis remains elusive. Here we show a concerted catalysis mechanism of mechanical conformational selection and induced-fit for a trans hammerhead ribozyme revealed at the single-molecule level. We identified a conformational set containing five mechanical conformers of the ribozyme, including the active conformation that is independent of magnesium ions and substrate RNA. Moreover, we observed that magnesium ions select the active conformation with well-folded catalytic core domains. Our results also revealed that the ribozyme can be induced to fit substrate recognition. Our demonstration strongly confirms the theory of conformational selection for biomolecular recognition. The single-molecule mechanical method for understanding the RNA catalytic mechanism presented here will be beneficial for future ribozyme engineering.

Key Words： Ribozyme, Conformational selection, Magnetic Tweezers

RNA imaging in living cells with fluorogenic RNA aptamer

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摘要：

荧光响应RNA适体是一类可以特异性结合并“点亮”小分子探针的成像工具，与荧光蛋白不同，荧光RNA适体与小分子探针复合物尺寸小，并且具有荧光响应性，因此在用于细胞成像时具有低干扰、高灵敏度、高信噪比的优势，为研究RNA的时空分布与功能提供了新的方法。

为了成像信使RNA(mRNA)，我们开发了小分子探针BI用于结合Broccoli适体，Broccoli/BI复合物能够在细胞内产生持续且高度稳定的荧光信号。我们将Broccoli适体插入到mRNA的3’非翻译区，在细胞内表达这类RNA，并加入BI荧光小分子，促进Broccoli在细胞内的正确折叠，增强荧光信号，实现了对单分子mRNA的标记。

此外，我们还设计了基于荧光响应RNA适体的高灵敏传感器，用于检测细胞内各类RNA。这类传感器在未结合到靶标时不发光，具有更低的背景信号，此外，传感器只有不到1 kDa的大小，具有更小的分子体积，降低了对靶标RNA功能和动力学的影响，使其成为理想的RNA成像工具。

综上，利用荧光响应RNA适体和小分子探针，我们在高等哺乳动物活细胞内实现了对RNA的高灵敏度和高精度的标记和追踪，为揭示RNA的分子机理和生命过程提供了新工具。这一技术不仅可广泛应用于基础生物学研究，也为疾病诊断与治疗开拓了新思路。

关键词：RNA标记；荧光响应RNA适体；小分子荧光探针；活细胞RNA成像

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Towards Single Molecule Biophysics using Optical Tweezers and Fluorescence Microscopy

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ABSTRACT: Biological processes occur across various time and length scales, connecting molecular details with collective, emerging phenomena. Detailed insights into the most fundamental building blocks of life such as individual proteins and nucleic acids provide essential information for understanding the fundamentals of intra- and extracellular organization. Direct, real-time observations of biomolecular interactions are required to validate and complete the current biological and biophysical models.

Single-molecule technologies offer an exciting opportunity to meet these challenges and enable researchers to study molecular function and activity in real-time. Here, we present our efforts towards further enabling discoveries in the field of biomolecular interactions by correlating optical tweezers and fluorescence microscopy. We present several examples in which our technologies enhanced the understanding of DNA protein interactions, membrane-less compartments, genome organization and biomolecular folding. Furthermore, we show how advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new avenues for breakthrough discoveries in biology and biophysics.

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KEY WORDS: Biomolecular interactions, Single-molecule methods, Optical tweezers, Fluorescence microscopy.

非天然核酸的性质与其在信息存储与读取中的应用

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天然核酸如DNA与RNA是生命体最基础的遗传物质载体，是天然的适合用于信息存储的物质。目前，利用核酸进行冷数据的信息存储是科研界与工业界正在探索的前沿方向[1]。然而，由于天然核酸仅能够提供有限的排列，对于更大量的信息难以有效存储。更重要的是，由于天然核酸经常能够被体内与环境中的酶识别并降解，天然核酸的保存成为了亟待解决的问题。目前，非天然核酸与人工修饰核酸被研究者广泛关注。由于引入非天然核酸和人工修饰核酸能够增加信息存储密度，并且能够抵抗核酸酶的降解达到持久保存的目的，利用非天然核酸与人工修饰核酸能够为基于核酸的信息存储领域提供新的方法与思路。然而，不同的非天然核酸或人工修饰核酸链内相互作用的方式尚不明确，其自身链内、链间以及与DNA之间复杂的相互作用会导致在信息存储与读取过程中存在错误[2]（如TNA）。此外，对于长链非天然核酸的合成而言，其单体合成方法复杂、价格昂贵；传统 DNA 聚合酶、逆转录酶等对非天然核酸的容忍度极低，难以直接用于非天然核酸序列的延伸，需要定向进化适配非天然核酸的聚合酶[3]。因此，探究非天然核酸之间相互作用的性质并解决长链非天然核酸合成问题，是推动非天然核酸信息存储的关键。在此，我们从非天然核酸与DNA之间相互作用的生物物理学性质出发，针对FANA、TNA与DNA三者之间相互作用的热、动力学等基础性质进行了单分子力谱分析，为后续对非天然核酸酶的研究提供基础的生物物理学数据。此外，我们还针对FANA的延伸进行了聚合酶的定向改造。将DNA聚合酶Tgo突变成为Tgo-D4K。突变后的Tgo-D4K能够将FANA延伸至4000 nt以上的长度。利用Tgo-D4K进行延伸的FANA-cssDNA链能够有效的进行信息存储与读取，并具有较强的抵抗酶解能力。进一步增加聚合酶的延伸速度，通过人工智能方法继续对Tgo-D4K聚合酶进行单点突变，获得了Tgo-D4K-I693W，其延伸速度较Tgo-D4K聚合酶提高约三倍。综上所述，我们的工作为利用非天然核酸进行信息存储与读取提供了基础的理论支持与技术支持，能够进一步促进基于非天然核酸的信息存储与读取技术。

关键词：非天然核酸、单分子磁镊、核酸信息存储与读取

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细胞与细胞外基质的生物力学模型仿真

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关键字：细胞力学，细胞外基质，原子力显微镜，有限元仿真

研究细胞的生物力学特性对于了解和认识细胞具有重要意义，现有研究多聚焦于细胞本身的力学特性，对于细胞与细胞外基质连接的力学性质研究少有涉及，该结构是在体细胞存在的普遍形式，对于理解组织的力学特性具有重要作用。本研究针对细胞与胞外基质整体结构的生物力学特性，通过建立计算力学模型分析该力学特性。

首先利用荧光染色获取细胞骨架、细胞膜以及细胞外基质的 3D 结构，基于原子力显微镜采用尖端探针测量细胞骨架、细胞膜以及细胞外基质的力学特性。同时，采用粘附纳米小球的悬臂梁探针测量细胞和细胞外基质整体的力学性质。基于测量的结构和组件力学特性在 COMSOL 中构建力学模型，将细胞构建为细胞膜、细胞质和细胞骨架三个部分，细胞膜和细胞质使用粘弹性材料模拟，细胞骨架和细胞外基质使用线弹性模型模拟，仿真获得整体的力学特性，并与整体测量的结果进行比较。

结果表明构建生物力学仿真模型和实测的细胞与细胞外基质生物力学特性吻合度较好，并且表明细胞外基质对于解释组织的力学特性具有重要作用。

DNA 探针图案化技术用于测量单细胞机械力学行为

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摘要：细胞形状受细胞骨架调节，而细胞骨架又受粘着斑（FA）和细胞外基质 (ECM) 之间的机械相互作用影响。在经典的细胞培养条件下，细胞呈现不同的形状、大小和方向，使得研究细胞力学和获得可重现的结果变得困难。现有微图案力学测量技术在蛋白微接触印刷的基础上发展了集成微图案的细胞牵引力测量方法，这些技术虽然证明了细胞-ECM牵引力与ECM几何信息以及细胞形状尺寸之间的相关性，但是受限于光学分辨率以及微柱几何尺寸，仅可实现纳牛/亚纳牛级灵敏度及微米级空间分辨率，难以提供细胞-ECM间单个整合素介导的分子间作用力大小及其空间分布随时间及微图案特征的精细变化信息。这里，我们提出一种结合分子张力荧光显微镜 (mTFM) 的单细胞微图案化技术，可以控制细胞形状的同时在分子水平上表征细胞-ECM的机械力特征。结果表明，细胞能感知几何形状和面积从而调控机械力的大小和分布，随着微图案面积越大，56pN信号（力学热点）密度在下降且在不同形状的图案有显著分布差异。此外，我们还发现曲率是细胞用以膜张力依赖性方式来区分形状的关键因素，不仅会影响到FA大小，还会影响力学热点的密度和空间分布。最后，我们探究了整合素两种亚型（αv和β1）在机械力识别几何形状中的作用，发现β1重点参与细胞机械响应图案形状的重要调节。以上，我们相信mTFM和单细胞微图案化技术的结合为研究机械信号与细胞几何响应的关系提供了强大的工具，进一步推动机械生物学领域的发展。

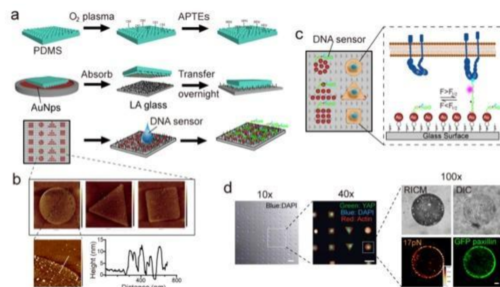

Fig. 1 Micropatterned DNA-based Molecular Tension Probes

关键词：整合素机械力；分子张力探针；微图案技术；

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Stochastically multimerized ParB orchestrates DNA assembly as unveiled by single-molecule analysis

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Abstract The tripartite ParAB*S* system mediates chromosome segregation in a wide range of bacteria. Dimeric ParB was proposed to nucleate on *parS* sites and spread to neighboring DNA. However, how properly distributed ParB dimers further compact chromosomal DNA into a higher-order nucleoprotein complex for partitioning remains poorly understood. Here, using a single-molecule approach, we show that tens of Bacillus subtilis ParB (Spo0J) proteins can stochastically multimerize on and stably bind to nonspecific DNA. The introduction of CTP promotes the formation and diffusion of the multimeric ParB along DNA, offering an opportunity for ParB proteins to further forgather and cluster. Intriguingly, ParB multimers can recognize *parS* motifs and are more inclined to remain immobile on them. Importantly, the ParB multimer features distinct capabilities of not only bridging two independent DNA molecules but also mediating their transportation, both of which are enhanced by the presence of either CTP or *parS* in the DNA. These findings shed new light on ParB dynamics in self-multimerization and DNA organization and help to better comprehend the assembly of the ParB-DNA partition complex.

Key Words Single-molecule, chromosome segregation, ParAB*S* system

Reference Lijuan Guo, Yilin Zhao *et al*, Stochastically multimerized ParB orchestrates DNA assembly as unveiled by single-molecule analysis, Nucleic Acid Research，*2022，50（16），9294-9305.*

Bloom Syndrome Helicase Compresses Single-Stranded DNA into Phase-Separated Condensates

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Abstract: Bloom syndrome protein (BLM) is a conserved RecQ family helicase involved in the maintenance of genome stability. BLM has been widely recognized as a genome 'caretaker' that processes structured DNA. In contrast, our knowledge of how BLM behaves on single-stranded (ss) DNA is still limited. Here, we demonstrate that BLM possesses the intrinsic ability for phase separation and can co-phase separate with ssDNA to form dynamically arrested protein/ssDNA co-condensates. The introduction of ATP potentiates the capability of BLM to condense on ssDNA, which further promotes the compression of ssDNA against a resistive force of up to 60 piconewtons. Moreover, BLM is also capable of condensing replication protein A (RPA)- or RAD51-coated ssDNA, before which it generates naked ssDNA by dismantling these SSB proteins. Overall, our findings identify an unexpected characteristic of a DNA helicase and provide a new angle of protein/ssDNA co-condensation for understanding the genomic instability caused by BLM overexpression under diseased conditions.

Key Words: BLM, Helicase, Phase separation, Condensation, Single-molecule

Reference: Wang, T., Hu, J., Li, Y., Bi, L., Guo, L., Jia, X., Zhang, X., Li, D., Hou, X.M., Modesti, M. *et al.* (2022) Bloom Syndrome Helicase Compresses Single-Stranded DNA into Phase-Separated Condensates. *Angew Chem Int Ed Engl*, **61**, e202209463.

基于二聚体荧光蛋白的DNA纳米力镊

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基于DNA碱基互补配对的DNA纳米机器由于其良好的可编程性和生物相容性，已经被广泛用于各种生物技术的开发[1]。但是相比于利用DNA纳米结构实现对小分子的检测和控制，利用DNA纳米机器精确调控蛋白质的结构和功能的研究相对较少。同时，目前研究DNA结构的生物物理性质的手段仍然较为单一，例如探究DNA及DNA与相互作用蛋白的力学性质通常使用光镊、磁镊等单分子工具[2, 3]，虽然近年开发的DNA帘幕等技术可以高通量地检测DNA链在溶液中的行为来探究DNA的力学性质[4]，但上述技术都难以研究DNA两端都处于外力约束状态下的扭曲结构——如cohesin-CTCF约束下的DNA环[5]、RNA聚合酶聚合过程中产生的双超螺旋结构[6]的力学性质。研究上述问题的常用方法是单分子荧光成像[5-7]，单分子荧光成像虽然可以直观地反应DNA结构的动态变化，但是很难对DNA施加特定的约束力。

因此，我们通过共价连接DNA单链和水溶性二聚体荧光蛋白，利用不同长度的单链DNA与上述DNA-蛋白偶联物杂交，就可以获得两端被二聚体蛋白约束的DNA环状结构，且二聚体荧光蛋白的结合常数可以反映DNA两端所受到的约束力。我们通过生化分析和荧光偏振实验，证明了DNA双链杂交、聚合和结合特定蛋白质可以精确调控二聚体蛋白的行为和构象分布，并且此过程中的能量变化和能量传递效率受到二聚体蛋白结合常数的影响。我们希望DNA-二聚体蛋白模型可以成为某些特殊条件下DNA生物物理性质研究的新工具。

关键词：DNA纳米机器，二聚体荧光蛋白，蛋白力镊，蛋白调控

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Pathogenic Mutations shift the Conformational Equilibria of α-Synuclein

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2023年度青年学者论坛

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Pathogenic Mutations shift the Conformational Equilibria of α-Synuclein

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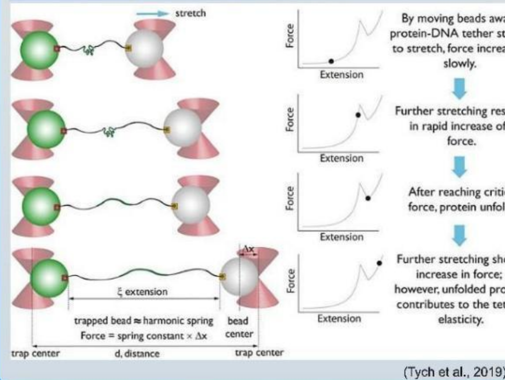
Abstract

α-Synuclein (α-Syn) is a natively unfolded protein whose misfolding and aggregation into amyloid fibrils is involved in the pathology of Parkinson's disease. A full comprehension of the misfolded conformation and aggregated dynamics is an unsolved problem that is vital for deciphering the molecular mechanisms of the formation of fibrils. We applied the optical tweezer-based single–molecule mechanical unfolding methodology to map the conformational space of the WT α-Syn and α-Syn mutants (A30P and A53T). We monitored marked differences in the conformation heterogeneity of the mutants with respect to the WT sequence. Our comprehensive analysis of the conformational heterogeneity reveals potential conformations involving aggregation rates. We further confirmed those aggregated conformations by the presence of metal ion (Fe^{3+}) and drug (Fasudil). Besides, we characterized the structural details of dimers formed by WT α-Syn and α-Syn mutants to find the folded pathways of potential conformations.

Method



(Tych et al., 2019)

Results

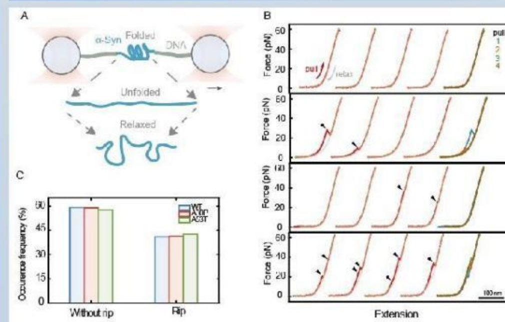


Figure 1. Heterogeneous unfolding of WT and mutants . (A) Schematic of force spectroscopy experiment: A single α-Syn molecule is attached to beads held in optical traps by DNA. (B) Four sets of successive pulling (grey) and relaxing (red) curves of α-Syn show various behavior. (Far Right) Unfolding curves for the four successive pulls. The rip (arrow) indicates a structural behavior. (C) Distributions of the type of unfolding event about WT and mutants (A30P and A53T).

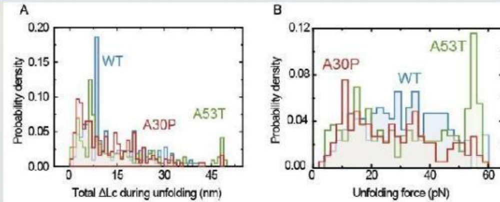


Figure 2. Characteristics of Force-extension curves exhibits different conformations between WT and mutants. (A) The total contour-length (ΔLc) change for the three different constructs. All show a different distribution. (B) The unfolding force distribution from the three different molecules, which show significant variability.

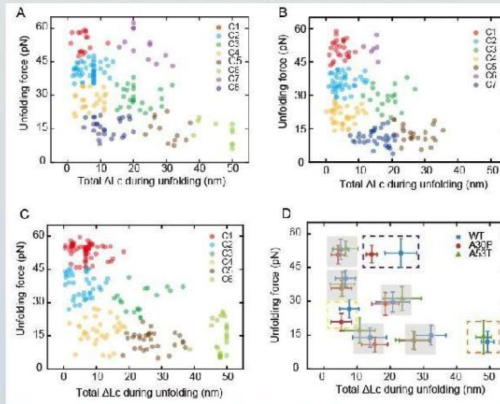


Figure 3. Pathogenic mutations shift the conformational equilibria of α-Syn. Cluster analysis permits to group and classify the complex conformations in term of the total contour-length change and force. (A) the conformations of WT could be optimally classified into eight different clusters (C1-C8). (B) the data of A30P could be optimally classified into seven different clusters (C1-C7). (C) the conformations of A53T could be optimally classified into six different clusters (C1-C6). (D) The conformational equilibria from the three different molecules shows difference. The total ΔLc, force and s.d. for the unfolded conformation is estimated by calculating the average of distribution of clusters.

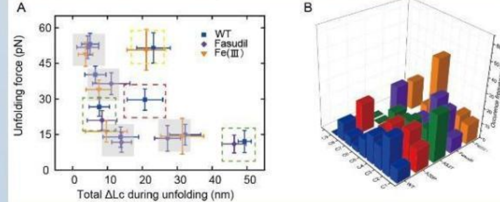


Figure 4. Effect of Fasudil and Fe^{3+} (delaying α-Syn aggregation) with α-Syn. (A) The population shift of α-Syn in two different conditions (Fasudil and Fe^{3+}). (B) The conformation equilibria of A30P and Fasudil are similar, and comparing with Fe^{3+} indictes C8 is potential conformations involving delaying aggregation rates.

Conclusion

- Pathogenic Mutations shift the conformational equilibria of α-Syn.
- There are several conformations related to aggregation rates, such as C8.
- Fasudil might play a role in stabilizing conformation equilibria.

分子与发育生物学国家重点实验室

基于冠状病毒刺突蛋白力学适应性进化的抗病毒策略

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摘要：力学调控在分子、细胞和组织水平上对生物体的组织起着至关重要的作用，适应其力学环境对每个生物体的生存而言至关重要。然而，力学调控是否驱动病毒的进化及其潜在的分子机制仍然未知。在此，我们发现了冠状病毒刺突蛋白进化遵循了力学相关的进化轨迹。我们的发现揭示了力学调控诱导了一系列中间的冠状病毒刺突蛋白-血管紧张素转换酶 2（ACE2）结合状态，从而增强了结合的力学稳定性[1,2]。此外，力学调控重塑了冠状病毒刺突蛋白的构象网络，降低了其自身的力学稳定性。有趣的是，随着 SARS-CoV-2 关注变异株（VOCs; Alpha、Beta、Gamma、Delta、BA.1、BA.2、BA.4 和 BA.5）的进化，这两个与力学调控相关的特征都受到影响，并且与这些 VOCs 的适应性之间存在强烈的相关性。基于我们的研究结果，我们提出了一种全新的病毒抗体阻断策略。该策略可以规避高频率突变的受体结合域，并通过抑制冠状病毒刺突蛋白 S1/S2 的解离来提高其机械稳定性，从而阻止病毒与宿主细胞进行膜融合，从而阻碍病毒感染宿主的过程。这种抗体的设计被针对性地定位在高度保守的区域，因此可以展现更广泛的抗体病毒效果，并有效对抗病毒不断变异演化的挑战。综上所述，探索力学因素介导病毒入侵和演化的机制有助于发展基于力学调控规律的抗病毒新策略。

关键词：冠状病毒，刺突蛋白，力学调控，病毒进化，抗病毒策略

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FNIII10 G79C 对生理拉伸作用力的响应

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摘要：III 型纤维连接蛋白 (Fibronectin, FN) 的第 10 个结构域 ($FNIII_{10}$) 中的 RGD 活性位点通过粘附于细胞表面的跨膜蛋白整合素从而感受细胞内部的作用力。我们利用单分子磁镊技术研究了 $FNIII_{10}$ 在生理受力模式下对机械作用力 (4-50 pN) 的响应。结果发现不受力的 G 链给蛋白质提供了额外的稳定作用，从而使得 $FNIII_{10}$ 的去折叠作用力相对较大。根据作用力与步长关系的理论曲线，我们发现在大多数情况下 A 链会在蛋白质完全去折叠之前自发地去折叠，这是造成 $FNIII_{10}$ 去折叠步长相对较小的原因。我们还通过力跳变实验测量得到 $FNIII_{10}$ 力依赖性的折叠和去折叠速率，比较有趣的是，实验结果表明 $FNIII_{10}$ 去折叠速率在对数坐标下呈现出一种连续变化的非线性关系。另外，在 8-9 pN 的恒定作用力下我们观察到 $FNIII_{10}$ 长达数天的平衡态转变信号。考虑到体内错综复杂的机械作用力干扰，$FNIII_{10}$ 的这一特性使其可以长时间稳定的行使其生物功能，从而保证细胞内外的有效沟通。

关键词：单分子磁镊技术，$FNIII_{10}$，三态蛋白质

基于高速原子力显微镜(HS-AFM)的 Perfor in-2 蛋白打孔机理和动力学研究

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摘要：近年来随着原子力显微镜技术的发展和完善，特别是以同时具有亚纳米级空间分辨率[1]和毫秒级时间分辨率[2]为代表的新型高速原子力显微镜（HS-AFM）技术的成熟，使得利用原子力显微镜在准生理环境中对生物分子实时成像，观察和追踪单个生物分子的结构动态、行为机理和动力学成为可能。高速原子力显微镜开始被越来越多应用于生物大分子（蛋白质、DNA 等）的结构动态机理、生物纳米机器组装及生物纳米功能材料开发等方面研究，成为一种新兴生物分子研究技术。基于高速原子力显微镜系统，我们对新型穿孔素蛋白（Perforin-2）在细胞膜表面打孔机理和动力学进行研究，发现 Perforin-2 会先在磷脂膜表面组装形成紧密排列的圆环形孔前体复合物；受溶液 pH 降低激发，孔前体复合物沿顺时针方向发生快速结构转变进行打孔，其中单个蛋白会经历两步结构转变[3, 4]。我们还定量分析了各个步骤的动力学。

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Exploring Mechanical Responses of Cells to Geometric Information using Micropatterned DNA-based Molecular Tension Probes

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Abstract ： The geometric shape of a cell is strongly influenced by the cytoskeleton, which in turn is influenced by mechanical interactions between focal adhesions (FAs) and the extracellular matrix (ECM). Under conventional cell culture conditions, cells exhibit diverse shapes, sizes, and orientations, making it challenging to study cell mechanics and obtain reproducible results. Existing micro-patterned mechanical measurement techniques have developed integrated micro-patterned cell traction force microscopy (mTFM) methods based on protein micro-contact printing. While these techniques have demonstrated correlations between cell-ECM traction forces and ECM geometric information as well as cell shape dimensions, they are limited by optical resolution and micro-pillar geometry, achieving only nano- to subnano-newton sensitivity and micrometer-level spatial resolution. They struggle to provide detailed information about the magnitude and spatial distribution of molecular-level interactions mediated by individual integrins between cells and the ECM over time and in response to fine pattern changes. Here, we propose a single-cell micro-patterning technique combined with molecular tension fluorescence microscopy (MTFM), thus allowing us to characterize the mechanical features of cells with prescribed geometry. Our results indicate that cells can sense geometric shape and area to regulate the magnitude and distribution of mechanical forces. As micro-pattern area increases, the density of 56pN signals (mechanical hotspots) decreases, and there are significant distribution differences in patterns of different shapes. Additionally, we found that curvature is a key factor by which cells distinguish shapes in a membrane tension-dependent manner. It not only affects FA size but also influences the density and spatial distribution of mechanical hotspots. Finally, we investigated the roles of two subtypes of integrins (αv and β1) in mechanical force recognition of geometric shapes. We found that β1 plays a crucial role in regulating cell mechanical response to pattern shapes. In summary, we believe that the combination of mTFM and single-cell micro-patterning techniques provides a powerful tool for studying the relationship between mechanical signals and cell geometric responses, further advancing the field of mechanobiology.

Keywords: Integrin force; DNA tension sensor; Micropattern technique;