20th Chinese Biophysics Congress

Abstract

July 19-23, 2023
Changsha,China

S01-1-033

Structural Basis of nucleosome deacetylation by Sin3 HDAC complex

JUN HE

Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences 510530
No. 190, Kaiyuan Avenue, Huangpu District, Guangzhou
he_jun@gibh.ac.cn

Abstract

In Saccharomyces cerevisiae, cryptic transcription is prevented by the activity of Sin3 histone deacetylase (HDAC) complex Rpd3S in coding regions. Rpd3S is carried by the transcribing RNA polymerase II (RNAPII) to deacetylate and stabilize chromatin. Despite its fundamental importance, the mechanisms of Rpd3S deacetylating nucleosomes and regulating chromatin dynamics remain elusive. Here, we determined several cryo-EM structures of Rpd3S in complex with nucleosome core particles (NCP). These states demonstrate that Rpd3S utilizes a conserved Sin3 basic surface to progress through the nucleosomal DNA in a left-handed superhelical manner.

S01-1-035

Structural insight into the human putative nucleic acid channel SIDT2 reveals its lipid hydrolytic activity

Dandan Qian[1#], Ye Cong[2#], Runhao Wang[1], Quan Chen[1*], Chuangye Yan[2*], Deshun Gong[1*]

[1]*State Key Laboratory of Medicinal Chemical Biology and College of Life Sciences, Nankai University, Tianjin 300350, China.*

[2]*State Key Laboratory of Membrane Biology, Beijing Frontier Research Center for Biological Structure, Beijing Advanced Innovation Center for Structural Biology, Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, 100084, China.*

Deshun Gong: gongds@nankai.edu.cn; 15222653627.

Abstract

The systemic RNAi-defective (SID) transmembrane family member 2 (SIDT2) is a putative nucleic acid channel that plays essential roles in nucleic acid transport. Here, we report the cryo-EM structures of human SIDT2, which forms a tightly packed dimer with extensive interactions mediated by two previously uncharacterized extracellular/luminal β-strand-rich domains and the unique transmembrane domain (TMD). The TMD of each SIDT2 protomer contains eleven transmembrane helices (TMs), and no discernible nucleic acid conduction pathway has been identified within the TMD or the dimerized TMDs, suggesting that it may act as a transporter. Intriguingly, TM3-6 and TM9-11 form a large cavity with a putative catalytic zinc atom coordinated by three conserved histidine residues and one aspartate residue lying approximately 6 Å from the extracellular/luminal surface of the membrane. Notably, SIDT2 can hydrolyze C18 ceramide into sphingosine and fatty acid, with a comparable rate to those of another two kinds of ceramidases, adiponectin receptor and alkaline ceramidase, suggesting that the SID1 family is a novel class of lipid hydrolases. The information presented advances the understanding of the novel structure-function relationships and marks an important step toward elucidation of the functional mechanisms of SID1 family proteins.

S01-1-042

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

Quan Meng[1§], Lin Jian[1§], Zhulou Wang[1], Qian Xiao[1], Shaohui Huang[1,2*]

[1]*Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

[2]*University of Chinese Academy of Sciences, Beijing 100049, China*

[§]*These two authors contributed equally to the research project*

[*]*Corresponding Author: shaohuih@ibp.ac.cn*

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.

To probe the potential structural conformations of biomolecules, a pair of FRET fluorophores was attached to both ends of the DNA hairpin and G4 molecules. The different structural conformations of biomolecules were identified with unique fluorescence lifetimes. Without prior knowledge of the sample compositions, we used MEM to fit the time-correlated single photon counting (TCSPC) histogram of micro-time of photons, which allowed us to obtain the lifetime distribution curves. The sample components were identified and resolved from the obtained lifetime distribution curve. The filter function for each sample component was further determined based on its unique lifetime. In addition, the conformational transition rates between different components were calculated by fitting the ratio of the cross-correlation function and auto-correlation function separated with filter functions. Our results showed that MEMFLCS can disclose the biomolecular equilibrium dynamics in the timescale of microseconds to milliseconds in a homogenous environment.

S01-1-080

Structural Basis for the Inhibition of Coronaviral Main Proteases by S-217622

Cheng Lin,[1,#] Haihai Jiang,[2,#] Wenwen Li,[3,4] Pei Zeng,[4] Xuelan Zhou,[4] Jin Zhang,[2,*] Jian Li[1,6,*]

[1]*College of Pharmaceutical Sciences, Gannan Medical University, Ganzhou 341000, China;*

[2]*School of Basic Medical Sciences, Nanchang University, Nanchang 330031, China;*

[3]*Shenzhen Crystalo Biopharmaceutical Co., Ltd., Shenzhen 518118, China;*

[4]*Jiangxi Jmerry Biopharmaceutical Co., Ltd., Ganzhou 341000, China;*

[#]*These authors contributed equally to this work.*

[*]*Correspondence: zhangxiaokong@hotmail.com (J.Z.), rmsl_2040@163.com (J.L.)*

Multiple coronaviruses, such as SARS-CoV, MERS-CoV, and SARS-CoV-2, as well as some SARS-Cov-2 variants have emerged and posed a serious threat to global health, which strongly supports the need to develop antiviral drugs with broadly spectrum properties. Main protease (Mpro) is a highly conserved cysteine protease that plays a vital role in the replication of coronaviruses, making it an attractive pan-coronaviral therapeutic target. Ensitrelvir (S-217622), developed by Shionogi, is the first orally active non-covalent, non-peptidic SARS-CoV-2 Mpro inhibitor, which also displays antiviral efficacy against other human coronaviruses as well as SARS-CoV-2 variants of concern (VOC) and variants of interest (VOI). However, the molecular basis for the broadly inhibition of coronaviral Mpros by S-217622 remains to be fully understood. In this study, we solved the structures of the main proteases from SARS-CoV-2, SARS-CoV-2 VOC/VOIs, SARS-CoV, MERS-CoV, and HCoV-NL63 bound to the inhibitor S-217622 by using X-ray crystallography method. Subsequent structural analysis illuminates key structural determinants essential for S-217622 inhibition and elucidates the binding modes of the main proteases from different coronaviruses with S-217622. Given the importance of the main protease for the treatment of coronaviral infection, structural insights obtained from this study could accelerate the design of novel antivirals with broad-spectrum efficacy against different human coronaviruses.

S01-1-099

Structure basis for dislocation of tail-anchored membrane proteins by the AAA+ ATPase Wsm1

Simin Wang[1*]

[1]*School of life science, University of Science and Technology of Chain, Hefei, China*

[*]*E-mail address:2870153470@qq.com*

Abstract:

Wsm1 is an meiotic ATPase of the AAA+ (ATPases Associated with various cellular Activities),that is a kind of motor in cell.There is great amount research on AAA+protein.However,meiotic AAA+-ATPase is a kind of unstable protein in this superfamily,determination of a high-resolution structure of an assembled meiotic clade AAA ATPase has been a high priority for many years, although no such structures have been reported to date.

Wsm1 resides in the outer mitochondrial membrane (OMM) and functions in protein quality control. Although Wsm1's importance for proteostasis at the mitochondrial membrane is well established, the mechanisms used by this motor to extract its substrates remain elusive.

In vivo studies have shown that Wsm1-mediated protein extraction from mitochondria is dependent on ATP hydrolysis, and several putative substrates have been identified, including the essential peroxisomal TA protein clip that is extracted by Wsm1 when mislocalized to the OMM. And another substrates is cliy,a small TA protein targeted to the ER membrane.

In this study, we generated soluble ΔTMDWsm1 multimer by fusing the cytosolic AAA+ ATPase domain to a oligomerizing scaffolds and thereby facilitating ring formation in vitro. Furthermore,we consider Wsm1 has flexible conformations from the cryoEM.

we explore AAA+ ATPase Wsm1 how to dislocate tail-anchored membrane proteins via clip and cliy .we show that soluble multimer Wsm1 is a robust protein translocase capable of unfolding diverse substrates via identifying substrate secondary structure.In addition, the substrate has no feedback on Wsm1 bioassembly and bioactivity.

Keywords:

Wsm; AAA+-ATPase; tail-anchored membrane proteins

S01-1-105

Structural and functional studies on the transcriptional activation mechanism of the transcription factor DarR

Weiwei Wang[1], Qin Xu[1, 2], Huan Zhou[1, 2], Minjun Li[1, 2], Huating Kong[1], Zhimin Zhu[2], Liqing Huang[2], Qisheng Wang[1, 2*], Feng Yu[1, 2*]

[1]*Shanghai Institute of Advanced Study, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 201203, China*

[2]*Shanghai Institute of Applied Physics, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 201800, China*

[*]*Corresponding author Qisheng Wang: wangqs@sari.ac.cn, Feng Yu:yufeng@sari.ac.cn*

LTTR family transcription factors are widely distributed and functionally diverse in bacteria, and participate in global cellular processes by regulating the transcription of various genes. Although some crystal structures of LTTR family members have been reported, the structural composition of LTTR family member is complex and variable, and the existing crystal structures of only full-length LTTR-DNA complexes are not applicable to all LTTR family members. Consequently, we conducted structural and functional studies on DarR, an LTTR family transcription factor of Vibrio fischeri. Firstly, we screened the DNA sequence of the DarR-binding promoter through biochemical experiments, and then we successfully determined the crystal structures of DarR in the inactive and activated states, respectively, with the DNA complex. We demonstrated the novel back-to-back arrangement of the DNA-binding structural domain dimer in the quaternary structure of DarR, and observed that the angle between the two DBD dimers becomes smaller in the activated state of DarR, which would drive the position of the binding ABS to shift, exposing the RNA polymerase binding site and allowing transcription initiation. In summary, we take DarR as the object of our study, reveal the structural features of DarR in complex with promoter DNA by investigating its structure with promoter DNA, elaborate the transcriptional activation mechanism of DarR, and propose a novel and complete transcriptional model of LTTR family.

S01-1-121

Unraveling the Formation and Stabilitzation of Cell Penetration Pores: Insights from Molecular Dynamics Simulations

Zhi Zheng, Mingkun Zhang, Mian Long*, Shouqin Lü*

[1]*nstitute of Mechanics, Chinese Academy of Sciences, andSchool of Engineering Science, University of Chinese Academy of Sciences, Beijing, China.*

* *Correspondence: Drs. Mian Long (mlong@imech.ac.cn) and Shouqin Lü (lsq@imech.ac.cn)*

Cell penetration pores serve as channel-like structures enclosed by cell membranes and crossing entire cytoplasm and are presented in many cell types under physiological and pathological conditions, such as liver sinusoidal endothelial cell fenestrae and trans-endothelial cell macroaperture. These pores have key biological functions, however, it is not fully understood how this unique penetration pore structure forms and stabilizes. Here, in combination of coarse-grained molecular dynamics and steered molecular dynamics simulations, we developed a new simulation approach to elaborate the formation and stabilization of penetration pores, based on simplified spherical vesicle system containing only ions and water environment, and investigated the regulation of membrane lipid composition on pore formation and stabilization. Results showed that penetration pore could be successfully formed referring to the strategy of membrane fusion. Free energy evolutions at stalk formation stage in the formation of intra-vesicle fusion penetration pore were closely correlated with curvature change of different lipid components during fusion process, which was different from that of planar membrane fusion. In addition, the tension distribution of vesicle perforation structures was highly uneven. The feature that the inner membrane tension around pore was much larger than other regions governed the size and stability of the penetration pore. This work provided a basis for understanding perforation formation and stabilization mechanisms and proposed the clue for regulating membrane pore formation.

This work was supported by National Natural Science Foundation of China grants (32130061 and 12172366).

S01-1-139

Allosteric transcription stimulation by RNA polymerase II super elongation complex

Ying Chen,[1] Seychelle M. Vos,[1,4] Christian Dienemann,[1] Momchil Ninov,[2,3] Henning Urlaub,[2,3] and Patrick Cramer [1,5*]

[1] *Max Planck Institute for Biophysical Chemistry, Department of Molecular Biology, Am Fassberg 11, 37077 Go¨ ttingen, Germany*

[2] *Max Planck Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry, Am Fassberg 11, 37077 Go¨ ttingen, Germany*

[3] *University Medical Center Göttingen, Institute of Clinical Chemistry, Bioanalytics Group, Robert-Koch-Straße 40, 37075 Göttingen, Germany*

[4] *Present address: Massachusetts Institute of Technology, Department of Biology, Cambridge, MA 02139, USA*

[5] *Lead contact*

[*] *Correspondence: patrick.cramer@mpibpc.mpg.de*

https://doi.org/10.1016/j.molcel.2021.06.019

Abstract

The super elongation complex (SEC) contains the positive transcription elongation factor b (P-TEFb) and the subcomplex ELL2-EAF1, which stimulates RNA polymerase II (RNA Pol II) elongation. Here, we report the cryoelectron microscopy (cryo-EM) structure of ELL2-EAF1 bound to a RNA Pol II elongation complex at 2.8 Å resolution. The ELL2-EAF1 dimerization module directly binds the RNA Pol II lobe domain, explaining how SEC delivers P-TEFb to RNA Pol II. The same site on the lobe also binds the initiation factor TFIIF, consistent with SEC binding only after the transition from transcription initiation to elongation. Structure-guided functional analysis shows that the stimulation of RNA elongation requires the dimerization module and the ELL2 linker that tethers the module to the RNA Pol II protrusion. Our results show that SEC stimulates elongation allosterically and indicate that this stimulation involves stabilization of a closed conformation of the RNA Pol II active center cleft.

S01-1-155

Remodeling of rRNA in the late-stage intermediates of pre50S

Ting Yu, Mengtan Du, Qianxi Yu, Fuxing Zeng

School of Life Sciences, Southern University of Science and Technology

Postcode: 518055

zengfx@sustech.edu.cn

The biogenesis of ribosomes is a complex and highly regulated process in cells, involving intricate steps of assembly and maturation. One critical aspect of this process is the remodeling of ribosomal RNA (rRNA) within the late-stage intermediates of pre50S particles. These intermediates play a pivotal role in the formation of the large subunit of the ribosome, and understanding the remodeling events occurring within them is essential for unraveling the intricacies of ribosome biogenesis. In this study, we investigated the remodeling of ribosomal RNA (rRNA) in late-stage intermediates of pre50S particles, specifically focusing on the role of DbpA, YjgA, and BipA proteins. Through a combination of biochemical and structural analyses, we identified key mechanisms and significant structural rearrangements associated with rRNA remodeling. In a DbpA mutant strain, we observed a mis-paired H73/H74 before the maturation of the peptidyl transferase center (PTC), highlighting the critical function of DbpA in unwinding this mis-pairing. Furthermore, in a ΔyjgA strain, we observed a slowed maturation rate of PTC, as well as H89 and uL16 protein. Our findings suggest that YjgA not only prevents the location of H68, creating space for PTC maturation, but also plays a role in recruiting uL16 protein, thereby stabilizing the H89 rRNA helix. In ΔbipA cells, we observed additional intermediates, including those with immature L1-stalk, CP, H68/H69, and PTC. These late-stage intermediates of pre50S particles are crucial for the maturation of the large subunit of the ribosome. Based on our results, we propose a model pathway where the L1-stalk of pre50S forms first, followed by the CP including the 50S. After the automatic placement of H68/H69, the long helix near the PTC, YjgA binds to the base of L1-stalk and CP, pushing out H68 and preventing its folding back, creating space for PTC folding. DbpA and related assembly factors then bind to initiate the unwinding of the mismatched H73/H74, leading to the refolding of PTC. Once PTC is correctly positioned, the N-terminal of YjgA recruits and stabilizes uL16 and H89, promoting PTC maturation. Finally, YjgA dissociates, and H68/H69 move back to their correct positions. Our findings shed light on the dynamic nature of rRNA maturation and provide insights into the intricate molecular mechanisms underlying rRNA remodeling and the coordinated actions of DbpA, YjgA, and BipA during late-stage pre50S maturation. Understanding the remodeling of rRNA in late-stage intermediates of pre50S not only enhances our knowledge of ribosome biogenesis but also contributes to a deeper understanding of cellular protein synthesis machinery.

S01-1-194

Structure-based evidence for the enhanced transmissibility of the dominant SARS-CoV-2 B.1.1.7 variant (Alpha)

冠状病毒病 (COVID–19) 严重威胁全球公共卫生，而 SARS–CoV–2 病毒突变体相比与野生型病毒具有更高的传播能力，在突变体中 B.1.1.7 是首个引起大家关注的 SARS–CoV–2 突变体，于 2020 年 9 月 20 日在英国首次发现，并迅速成为当地主要的流行突变体。目前它已经蔓延到 90 多个国家，导致大约 1000 万人感染。B.1.1.7 突变体的感染效率相比于野生型显著增强，然而关于 B.1.1.7 突变体传染性增强的机制的基础研究仍然缺乏。

我们通过冷冻电镜技术解析了高分辨率的 B.1.1.7 突变体 Spike 蛋白和 ACE2 受体的复合物结构。经过结构分析，发现 B.1.1.7 突变体 Spike 蛋白上的 10 个突变位点（69，70，144 位氨基酸缺失突变 , N501Y, A570D, D614G, P681H, T716I, S982A，D1118H 氨基酸替换突变）以多种方式增强病毒的感染效率，包括增加 Spike 蛋白与 ACE2 的亲和力，增加 Spike 蛋白 prefusion 状态的稳定性，增加 Spike 蛋白 RBD 结构域“抬起“的几率，提高 furin 酶切效率等。增加了人们对新冠病毒突变体感染性增强的认识。

S01-1-206

NK1R Activation by the Natural Ligand Substance P Studied with 19F-NMR

Haoyi Ge[1, 2*], Yuxin Xu[1, 2], Lingyun Yang[1], Dongsheng Liu[1], Kurt Wüthrich[1, 2]

[1]*iHuman Institute, ShanghaiTech University, Shanghai 201210, China.*

[2]*School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China.*

[*]*Email address: gehy@shanghaitech.edu.cn*

The neurokinin 1 receptor (NK1R) is a G protein-coupled receptor (GPCR) of class A that is distributed in the central and peripheral nervous systems of mammals. NK1R has been targeted for drug design in areas such as inflammation, pain and chemotherapy-induced nausea and vomiting. Activation of NK1R is mediated predominantly through substance P (SP), an endogenous peptide consisting of 11 amino acids residues with the sequence RPKPQQFFGLM and an amidated C-terminus. To investigate SP binding kinetics and the conformation of SP bound to NK1R, 3'-trifluoromethyl-L-phenylalanine (mtfF) was synthetically introduced for 19F-labeling of SP.19F-NMR revealed that the C-terminus of SP is fixed inside the binding pocket, while the N-terminus extends outside of the pocket and is flexibly disordered. The reversible binding of SP to NK1R has an exchange rate on the milliseconds to seconds time scale. Overall, 19F-NMR thus provides a visualization of the conformation of SP bound to NK1R in solution at ambient temperature, and offers novel insights into ligand-GPCR interactions.

S01-1-209

Effects of Salt Concentration on the Conformational Dynamics of an Ultrafast Folding Protein, Engrailed Homeodomain (EnHD)

Chengzhen XU and Xiakun CHU*

Advanced Materials Thrust, Function Hub, The Hong Kong University of Science and Technology (Guangzhou), Nansha, Guangzhou, Guangdong 511453, China

Email: cxu807@connect.hkust-gz.edu.cn; xiakunchu@ust.hk

Abstract:

The Engrailed Homeodomain (EnHD) protein is a widely expressed transcription factor composed of 54 amino acid residues, 19 of which are charged. This unique sequential feature allows EnHD to form stable protein-DNA complexes through electrostatic interactions. However, the effects of these interactions on the conformational dynamics of EnHD are not well understood. Recent experimental studies have revealed that ionic interactions can modulate the conformational ensemble of EnHD. To investigate the microscopic-level mechanisms of ionic effects on EnHD structure and dynamics, we performed all-atom simulations and coarse-grained structure-based model simulations for EnHD at different salt concentrations. Our results show that increasing salt concentration enhances protein stability and folding cooperativity. We also analyzed the global folding dynamics and local native dynamics of EnHD at different salt concentrations and found significant differences between these two aspects. Using a reweighting method, we compared the effects of salt concentration on the conformational dynamics of EnHD with varying degrees of structural disorder. Our findings, which are in good agreements with experimental results, improve our understanding of the effects of salt concentration on the conformational dynamics of EnHD and provide insight into the relationship between structure, dynamics, and function of EnHD.

S01-1-223

Study on the mechanism of eggcase silk fiber formation

Qin Ruiqi Fan Tiantian Yuan Wensu Fan Jingsong Yang Daiwen Linzhi

Tianjin University, School of Life Science, 300072, qinruiqi12@163.com

National University of Singapore, Department of Biological Sciences, 119077

Spider silks have excellent properties such as high stress、extensibility and biocompatibility. Revealing the relationship between structure and function is essential to fully understand the mechanism of silk fiber formation and form the solid fibers. We mainly focused on the minor component of eggcase silk TuSp2, the results of NMR revealed that the repetitive domain of TuSp2(TuSp2-RP) displayed a dimeric structure. TuSp2-RP interacted with the different domains of the TuSp1 the main component of the eggcase silk was due to the charge interface. Fiber spun from the complex of the TuSp1 and TuSp2-RP exhibited higher strength and Young's modulus compared with the natural spider silk fiber. Above results indicated the important of the minor component of the eggcase silk and provide a new insight into the biomaterials.

S01-1-233

Enhancing Thermostability and Structural Flexibility of IsPETase through Mutations: A Molecular Dynamics Simulation Perspective for Rational Enzymatic Design

Shiqinrui Xu, Xiakun Chu

Advanced Materials Thrust, The Hong Kong University of Science and Technology (Guangzhou)
Nansha, Guangzhou, Guangdong 511400, China
sxu128@connect.hkust-gz.edu.cn, xiakunchu@ust.hk

Poly(ethylene terephthalate) (PET) constitutes around 10% of global plastic production and is commonly utilized in the manufacturing of water and soda bottles. However, less than 30% of PET products are currently recycled, posing significant environmental challenges. In contrast to other thermophilic hydrolases, IsPETase exhibits the ability to catalyze the hydrolysis of PET at a mild temperature of approximately 30℃. Regrettably, the enzymatic activity of IsPETase declines considerably within 24 hours at higher temperature (40℃) in vitro, which severely hampers its industrial applicability. Numerous studies have been undertaken to enhance the thermostability of IsPETase, resulting in an interesting observation: the degradation efficiency of PET also increases along with the improvements in thermostability. In this study, we employ all-atom molecular dynamics (MD) simulations to investigate the effects of mutations on both thermostability and structural flexibility. Three variants of IsPETase, namely Thermo PETase, FAST PETase, and Dura PETase, are selected. These three mutant PETase were previously found to have profound improvements in PET degradation efficiency with respect to the wide-type one. Our findings reveal that mutations lead to a more funneled energy landscape with elevating local native dynamics. This suggests a positive role of structural stability and flexibility in the catalytic function of PETase. We develop a methodology that combines the shortest pathway map (SPM) and frustration analysis to predict potential mutation sites. SPM aims to identify distal residues that assist IsPETase in attaining the catalytically competent conformation, whereas frustration analysis identifies residues that contribute to the overall system stabilization. Based on our prediction method, we propose several mutants, which may be potential mutation sites for future investigations. Notably, this prediction method holds promise for application in the rational design of other systems.

S01-1-292

Mechanism of abnormal activation of MEK1 induced by dehydroalanine modification and impact of FDA-proved inhibitors

Li Zhao*, Yue Zhao

School of Life Science, Jilin University, China

* *Tel: 18186861339; Email: zhaoli2011@jlu.edu.cn*

Mitogen-activated protein kinase kinase 1 (MAPKK, MEK1), the key kinase in the mitogen-activated protein kinase (MAPK) signaling pathway, is closely related to the malignant growth and spread of various tumors, such as melanoma, because of its mutations. Trametinib, Cobimetinib, Binimetinib, and Selumetinib have been approved by FDA as MEK1 inhibitors. Recent studies showed that dehydroalanine (Dha) modification can also lead to abnormal activation of MEK1(Cell, 2021, 184(10): 2680–2695), promoting tumor development potentially. In this work, molecular dynamics simulations were used to study effects of Dha modification on MEK1 structure, explore the mechanism of MEK1 abnormal activation, and predict the inhibitory effects of existing FDA-approved inhibitors on the Dha-modified MEK1. The results showed that Dha modification induces the movement of the activation loop, makes the catalytic site to expose and therefore keeps MEK1 in persistent abnormal activation state. Among the four inhibitors, Selumetinib blocks the catalytic activity of Dha-modified MEK1 significantly. After binding Selumetinib, the secondary structure of the activation loop changes from α-helix to disordered, blocking the active site obviously. Our study will be helpful to reveal the mechanism of abnormal activation of MEK1 caused by Dha modification and provide clues for the application of corresponding inhibitors.

S01-1-331

Structural basis of Spns2 facilitated sphingosine-1-phosphate transport and sphingosine-1-phosphate receptor activation

Leiye Yu (玉蕾叶)[1,2], Bin Pang[1,2], Bing Gan[1,2], Tong Li[1], Licong He[3], Sheng Wang[3], Wei Dai[1], Li Chen[1], Ruobing Ren[1,2*]

[1] Institute of Metabolism and Integrative Biology, Fudan University, Shanghai, 200438. [2] Kobilka Institute of Innovative Drug Discovery, the Chinese University of Hong Kong. [3] Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, [*]Email: renruobing@fudan.edu.cn

As one of the critical sphingolipid metabolites in eukaryotes, sphingosine-1-phosphate (S1P) plays a vital role in multiple tissues' physiology and pathology. Extracellular S1P binds with different S1P receptors (S1PR1-S1PR5) on cell membranes, activating different downstream signaling pathways to regulate cellular proliferation, differentiation, survival, and migration. Extracellular S1P is exported by transporters. Spinster homolog 2 (Spns2), belonging to the major facilitator superfamily (MFS), is the first identified and most extensively studied S1P transporter. However, the molecular basis of S1P export across the membrane remains unclear. Here, we present two cryo-EM structures of human Spns2 in inward-open conformations bound to S1P or inhibitor 16d. Combining the structural information and extensive mutagenesis studies using a cell-based S1P efflux assay and an in vivo assay by evaluating the heart development in zebrafish, we decipher critical residues involved in S1P recognition, translocation, and conformational changes of Spns2 through S1P transport. Besides, we also determined four high resolution cryo-EM structures of Gi-coupled human S1PR1 complexes: bound to endogenous agonist d18:1 S1P, benchmark lipid-like S1P mimic phosphorylated Fingolimod ((S)-FTY720-P), or non-lipid-like therapeutic molecule CBP-307 in two binding modes, and decipher the common feature of the S1PR1 agonist recognition and activation mechanism.

S02-141

Dissecting the mechanism of homotypic membrane fusion mediated by atlastin

Lijun Shi, Chenguang Yang, Mingyuan Zhang, Keying Wang, Kangning Li, Yunyun Wang, Ming Li, Yong Wang*, Lu Ma*, Shuxin Hu*, Xin Bian*

Nankai University, Tianjin 300071, China;

**xin.bian@nankai.edu.cn*

In eukaryotes, homotypic membrane fusion of the endoplasmic reticulum (ER) is mediated by dynamin-like GTPase atlastin (ATL). Mutations in human ATL1 are implicated in hereditary spastic paraplegia (HSP) and hereditary sensory neuropathy type I (HSN1). The ER membrane fusion process relies on the rearrangements of GTPase domain and three-helix bundle (3HB) in N-terminal ATL. However, the conformatonal dynamics of ATL during GTPase cycle remains elusive. Here, we combine single-molecule Förster resonance energy transfer (smFRET) imaging and molecular dynamics (MD) simulations to address this conundrum. Our data provide evidence that upon GTP binding, ATL can form a Form 2-like loose crossover dimer, which is a surprising observation given that the current models in the literature only predict a Form 1 or Form 3 dimer conformation for GTP-bound ATL. After GTP hydrolysis, the loose association of 3HBs is completely tightened for membrane fusion. Furthermore, the membrane-embedded and self-associated α-helical motif between the 3HB and transmembrane domain, is proposed to be structurally linked to the energy supply for membrane fusion. To recycle the proteins, the frequent relative movements between the GTPase domain and 3HB is activated by Pi release for disassembling the ATL dimer, and the subsequent GDP dissociation alters the conformational preference of the ATL monomer towards the Form 2 conformation for entering the next round of GTPase cycle. Finally, we also found that HSP-causing mutations S398Y and N440T in ATL1 destabilize the GTP-bound loose crossover dimer formation and the helix in the linker region, respectively. These results provide new insights into the mechanisms of ATL-mediated fusion and the pathological mechanisms in HSP.

S03-018

Cryo-EM structure of the cytosolic Hsp90-p23-AhR-XAP2 complex

Zuoling Wen, Fei Sun, Yujia Zhai, Yun Zhu

National Key Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences,
100101, Beijing, China
zhuyun@ibp.ac.cn

Aryl hydrocarbon receptor (AhR) is an important ligand-activated transcription factor that belongs to the basic helix-loop-helix (bHLH) Per-Arnt-Sim (PAS) family. AhR is also known as the dioxin receptor since 2,3,7,8-tetrachlorodi-benzo-p-dioxin (TCDD) was the first AhR ligand identified. Along with the identification of an increasing number of endogenous ligands, AhR seems to be important and irreplaceable in normal physiological processes. In the absence of ligand, AhR forms a stable multiprotein complex in the cytosol, including chaperone Hsp90, cochaperone p23, and AhR interacting protein XAP2 (AIP). The ligand binding event triggers conformational changes in AhR to expose the N-terminal nuclear localization signal (NLS), leading to the translocation of the AhR complex into the nucleus.

Here, we report the cryo-EM structures of the Hsp90-AhR-p23 complex with or without bound XAP2, where the structure of the mouse AhR PAS-B domain is resolved. A highly conserved bridge motif of AhR is responsible for the interaction with the Hsp90 dimeric lumen. The ligand-free AhR PAS-B domain is attached to the Hsp90 dimer and is stabilized in the complex with bound XAP2. In addition, the DE-loop and a group of conserved pocket inner residues in the AhR PAS-B domain are found to be important for ligand binding. These results reveal the structural basis of the biological functions of AhR. Moreover, the protein purification method presented here allows the isolation of stable mouse AhR protein, which could be used to develop high-sensitivity biosensors for environmental pollutant detection.

S03-061

CD146 Associates with Gp130 to Control a Macrophage Pro-inflammatory Program that Regulates the Metabolic Response to Obesity

Hongxia Duan*, Lin Jing, Jianquan Xiang, Chenhui Ju, Zhenzhen Wu, Jingyu Liu, Xinran Ma, Xuehui Chen, Zheng Liu, Jing Feng, Xiyun Yan*

Laboratory of Protein and Peptide Pharmaceutical, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

E-mail: cherryshoen@ibp.ac.cn; yanxy@ibp.ac.cn

Abstract

The mechanism of obesity-related metabolic dysfunction involves the development of systemic inflammation, largely mediated by macrophages. Switching of M1-like adipose tissue macrophages (ATMs) to M2-like ATMs, a population of macrophages associated with weight loss and insulin sensitivity, is considered a viable therapeutic strategy for obesity-related metabolic syndrome. However, mechanisms for reestablishing the polarization of ATMs remain elusive. This study demonstrates that CD146+ ATMs accumulate in adipose tissue during diet-induced obesity and are associated with increased body weight, systemic inflammation, and obesity-induced insulin resistance. Inactivating the macrophage CD146 gene or antibody targeting of CD146 alleviates obesity-related chronic inflammation and metabolic dysfunction. Macrophage CD146 interacts with Glycoprotein 130 (Gp130), the common subunit of the receptor signaling complex for the interleukin-6 family of cytokines. CD146/Gp130 interaction promotes pro-inflammatory polarization of ATMs by activating JNK signaling and inhibiting the activation of STAT3, a transcription factor for M2-like polarization. Disruption of their interaction by anti-CD146 antibody or interleukin-6 steers ATMs toward anti-inflammatory polarization, thus attenuating obesity-induced chronic inflammation and metabolic dysfunction in mice. The results suggest that macrophage CD146 is an important determinant of pro-inflammatory polarization and plays a pivotal role in obesity-induced metabolic dysfunction. CD146 could constitute a novel therapeutic target for obesity complications.

S03-070

Regulation of Na+/bicarbonate cotransporter NBCe1

Ying Liu, Li-Ming Chen

Key Laboratory of Molecular Biophysics of Ministry of Education, School of Life Science & Technology, Huazhong University of Science & Technology, Wuhan, Hubei 430074, China

Email: liming.chen@hust.edu.cn

Abstract

Solute Carriers (SLC) superfamily are a large group of secondary active transporters encompassing at least 65 families with up to 440 members. The SLC4 family includes five Na+/HCO3− cotransporters (NBCs) that play central roles in maintaining the systemic electrolyte and fluid in body. Dysfunctions of the NBCs are associated with a series of human diseases, including but not limited to severe metabolic acidosis, hypertension, epilepsy, migraine, cancers.

NBCe1 (SLC4A4) was the first discovered of the NBCs. The 3-dimension structure of NBCe1 has been resolved. The transmembrane domain of NBCe1 consists of a scaffold domain and a carrier domain. NBCe1 employs an elevator-like mechanism for ion transport. NBCe1 activity depends on the cyclic movement of the carrier domain relative to the scaffold. By molecular docking and mutational studies, our group showed that NBCe1 (essentially the carrier domain) can incoorporate two anions (HCO3− or CO3=) and one Na+. The binding of HCO3− procceeds that of Na+ in NBCe1 (Wu H et al, 2022). Our kinetic model provided a framework for understanding the functional regulation of NBCe1.

NBCe1 contains a large amino-terminal domain (NTD) topologically located in the cytosol. The NTD plays dual roles in NBCe1. Firstly, the NTD represents an essential component of the ion translocation machinery of NBCe1. Secondly, the NTD also contains structural elements that represent important mechanisms for NBCe1 regulation. Some NBCe1 variants contain an auto-inhibitory domain (AID) at the Nt end. NBCe1 can be inactivated by the action of AID, but be activated by protein interaction with IRBIT. Our group has disclosed the structural mechanism for the auto-inhibition of NBCe1 and its activation by IRBIT (Su P et al, 2021). The NTD of NBCe1 also contains several regions that can be heavily phosphorylated. I will talk about our latest progress in understanding the structural and kinetic mechanism for the regulation of NBCe1 by phosphorylation (Wu H, unpublished data).

S03-073

IL18 uses IL18r and NCC to support β-cell development and insulin secretion, and adipocyte thermogenesis and insulin sensitivity

Xian Zhang[1], Liu Jian[1*]

[1] *School of Food and Biological Engineering, Hefei University of Technology, 193 Tunxi Road, Hefei, Anhui 230009, China*

Xian Zhang, PhD, Email: zhangxian@hfut.edu.cn;

[*]*Corresponding author: Jian Liu, PhD, Email: liujian509@hfut.edu.cn*

With the development of obesity, hyperglycemia and insulin resistance causes islet dysfunction and type-2 diabetes. Plasma IL18 levels are elevated in obese and diabetic patients. IL18 has two receptors, IL18 receptor (IL18r) and Na-Cl co-transporter (NCC). No evidence supports that IL18 can display different actions by using different receptors in obesity and diabetes.

Here we report that IL18 is a brown adipokine, and uses NCC in brown adipose tissue (BAT) and IL18r in white adipose tissue (WAT). Deficiency of both IL18r and NCC increases bodyweight gain and insulin resistance, impairs thermogenesis in BAT, and confounds lipid and glucose metabolism and inflammation in WAT from high-fat diet (HFD)-fed mice. WAT insulin resistance is increased in HFD-fed Il18r-/- and Il18r-/-Ncc-/- mice, whereas thermogenesis is decreased in BAT from HFD-fed or β3-adrenoceptor agonist-treated Ncc-/- and Il18r-/-Ncc-/- mice. In addition to exacerbated bodyweight gain, glucose intolerance, and insulin resistance, BAT-selective depletion of NCC or IL18 reduces thermogenesis, whereas IL18r specific depletion in WAT impairs insulin signaling. In the meanwhile, IL18 is also expressed in islet α cells, NCC in β cells, and IL18r in acinar cells in human and mouse pancreas. Deficiency of both IL18r and NCC reduces islet β-cell proliferation and insulin secretion, but increases β-cell apoptosis and exocrine macrophage accumulation in diabetic mice. β-cell-selective depletion of NCC or acinar cell-selective IL18r depletion reduces glucose tolerance and insulin sensitivity with impaired β-cell proliferation, enhanced β-cell apoptosis and macrophage expansion and inflammation in mouse hyperglycemic pancreas.

Together, IL18 uses NCC to enhance thermogenesis in BAT but uses IL18r to control glucose uptake and insulin signaling in WAT (Nature Communications,2022). IL18 uses NCC in islets to support β-cell development and insulin secretion, and uses IL18r in acinar cells to block hyperglycemic pancreas macrophage expansion and inflammation to assist β-cell function (Developmental Cell,2022).

Keywords: IL18; IL18 receptor; Na-Cl co-transporter; adipocyte; β-cell function; pancreas inflammation

S03-076

Serum levels of polychlorinated biphenyls and polybrominated diphenyl ethers in early pregnancy and their associations with gestational diabetes mellitus

Shijie Geng, Xiaodie Yao, Juan Wen

Author affiliation: Nanjing Maternity and Child Health Care Institute, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210004, Jiangsu, China

E-mail addresses: g928748376@163.com (Shijie Geng)

xiaodieyao@163.com (Xiaodie Yao)

wenj2010@139.com (Juan Wen)

ABSTRACT

Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) are extensively present in humans and may disturb glucose metabolism during pregnancy. However, previous reports on the associations between PCBs/PBDEs levels and gestational diabetes mellitus (GDM) have been inconsistent. We performed a nested case-control study to measure the serum levels of 6 PCB and 7 PBDE congeners in early pregnancy, and to assess their associations with GDM risk and blood glucose levels. Totally, 208 serum samples (104 GDM cases and 104 controls) were included based on a prospective cohort which was carried out in Jiangsu province, China, from 2020 to 2022. The results showed that PCB-153 was the major PCB congeners, whereas PBDE-47 was the predominant PBDE congeners. The continuous concentrations of PCB-153, PBDE-28, and total PCB were significantly related to an increased risk of GDM, with adjusted ORs (95%CI) of 1.25 (1.04-1.50), 1.19 (1.02-1.39), and 1.47 (1.12-1.93), respectively. Potential dose-response relationships were also observed between serum levels of PCB-153 (P = 0.020), PBDE-28 (P = 0.032), total PCB (P = 0.006), and total POP (persistent organic pollutants, P = 0.029) and GDM risk. Interestingly, a stronger effect of total POP on GDM risk was observed among women with gestational weight gain < 11.5 kg compared with that in women with gestational weight gain ≥ 11.5 kg (Pmultiplicative interaction = 0.044). Moreover, PCB-153 and PBDE-28 levels were positively related to 1-h OGTT blood glucose (adjusted βPCB-153: 0.14, 95%CI: 0.00-0.28; adjusted βPBDE-28: 0.20, 95%CI: 0.08-0.32), whereas total PCB was positively related to both 1-h and 2-h OGTT blood glucose (adjusted β1-h: 0.34, 95%CI: 0.13-0.54; adjusted β2-h: 0.20, 95%CI: 0.00-0.40). Further meta-analysis also supported the associations of PCBs/PBDEs exposure with GDM risk. Our study provides affirmative evidence that PCBs/PBDEs exposure may increase GDM risk during pregnancy.

S03-084

CD146 is a Novel ANGPTL2 Receptor that Promotes Obesity by Manipulating Lipid Metabolism and Energy Expenditure

Zhenzhen Wu[1], Jingyu Liu[1], Xiyun Yan[1], Jing Feng[1] and Pengcheng Bu[1]

[1] *Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101, China*

E-mail: sdwhwuzhenzhen@163.com; bupc@ibp.ac.cn

Obesity and its related complications pose an increasing threat to human health; however, targetable obesity-related membrane receptors are not yet elucidated. Here, the membrane receptor CD146 is demonstrated to play an essential role in obesity. In particular, CD146 acts as a new adipose receptor for angiopoietin-like protein 2 (ANGPTL2), which is thought to act on endothelial cells to activate adipose inflammation. ANGPTL2 binds to CD146 to activate cAMP response element-binding protein (CREB), which then upregulates CD146 during adipogenesis and adipose inflammation. CD146 is present in preadipocytes and mature adipocytes, where it is mediated by its ligands ANGPTL2 and galectin-1. In preadipocytes, CD146 ablation suppresses adipogenesis, whereas the loss of CD146 in mature adipocytes suppresses lipid accumulation and enhances energy expenditure. Moreover, anti-CD146 antibodies inhibit obesity by disrupting the interactions between CD146 and its ligands. Together, these findings demonstrate that ANGPTL2 directly affects adipocytes via CD146 to promote obesity, suggesting that CD146 can be a potential target for treating obesity.

S03-211

The role of selenoprotein K in neuronal function and AD pathology

Selenoprotein K (SELENOK), an endoplasmic reticulum (ER) resident protein, is regulated by dietary selenium and expressed at a relatively high level in neurons. Our previous research found that SELENOK gene knockout markedly enhanced intracellular Ca^{2+} levels and oxidative stress, accompanied by obvious endoplasmic reticulum stress phenomenon, and induced neuronal apoptosis in vivo and in vitro through m-calpain/caspase-12-level response (Antioxidants, Endoplasmic reticulum stress and calpain inhibitors can reverse this phenomenon)
. In addition, SELENOK knockout significantly reduced cognitive ability and increased anxiety in 7-month-old mice. The study found that the expression level of SELENOK was significantly reduced in Alzheimer's disease patients and 3×Tg-AD model mice. Through gene knockout and hybridization technology, SELENOK-/- AD mice were screened out to detect their learning and cognitive ability and anxiety level, and study the regulatory effect of $SELENOK^{-/-}$ on antioxidant enzymes in AD mice. The results found that SELENOK gene knockout significantly increased the levels of oxidative stress (MDA), decreased the learning and memory ability of AD mice, and increased the level of anxiety in AD mice. To further explore the effect of SELENOK on AD pathology, we used shRNA to knock down SELENOK in 3×Tg-AD mice. The study found that SELENOK knockdown further reduced the learning and memory ability of 3×Tg-AD mice and increased anxiety levels. Further studies found that SELENOK knock down impaired mitochondrial function, decreased ATP Levels, increased Aβ aggregation and Tau protein phosphorylation levels. Therefore, we speculate that SELENOK plays an important role in regulating AD pathological processes and exerts neuroprotective effects in vivo. Further studies on the function of SELENOK will better reveal the important biological roles of selenium in vivo and its underlying mechanisms.

S03-256

SELENOM knockout induces synaptic deficit and cognitive dysfunction by influencing brain glucose metabolism

Shujing Lina, Pei Ouyanga, Zhiyu Caia, Jiaying Penga, Zhonghao Zhanga, GuoLi Song[a,b,c,*]

Current affiliation: Shenzhen Key Laboratory of Marine Bioresources and Ecology, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China;

Postal code: 518060

Email address: lssj1162@163.com

Abstract

Selenium, a trace element associated with memory impairment and glucose metabolism, mainly exerts its function through selenoproteins. SELENOM is a selenoprotein located in the endoplasmic reticulum (ER) lumen. Our study demonstrates for the first time that SELENOM knockout decreases synaptic plasticity and causes memory impairment in 10-month-old mice. In addition, SELENOM knockout causes hyperglycemia and disturbs glucose metabolism, which is essential for synapse formation and transmission in the brain. Further research reveals that SELENOM knockout leads to inhibition of the brain insulin signaling pathway (phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR/p70 S6 kinase pathway), which may impair synaptic plasticity in mice. High-fat diet feeding suppresses the brain insulin signaling pathway in SELENOM knockout mice and leads to earlier onset of cognitive impairment in 5-month-old mice. In general, our study demonstrates that SELENOM knockout induces synaptic deficits via the brain insulin signaling pathway, thus leading to cognitive dysfunction in mice. These data strongly suggest that SELENOM plays a vital role in brain glucose metabolism and contributes substantially to synaptic plasticity.

Keywords: SELENOM; Cognitive dysfunction; Glucose metabolism;

S03-306

Loss of glucocorticoid receptor in oxytocin neurons of the paraventricular hypothalamus leads to P2ry12-dependent suppression of the melanocortin signaling and obesity.

Yu Liu[1], Ilya A. Vinnikov[1*]

[1] Laboratory of Molecular neurobiology, Sheng Yushou Center of Cell Biology and Immunology, Department of Genetics and Developmental Biology, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dongchun Rd., 200240, Shanghai, China. [*]Emails address: i.vinnikov@sjtu.edu.cn

Modern societies are currently facing one of the biggest global health care crises with obesity and obesity-related pathologies. In this project, we aimed to identify the cell-autonomous role of the main hypothalamic-pituitary-adrenal (HPA) axis effector, glucocorticoid receptor (GR), within the oxytocin (OT) neurons of the paraventricular nucleus of the hypothalamus. These neurons are critical regulators of appetite and energy expenditure and constitute the second order neurons of the melanocortin system. Strikingly, OT neuron-specific in situ CRISPR-Cas9-mediated knock-out of GR caused food intake-independent obesity and insulin resistance. This phenotype was associated with "ectopic" emergence and drastic up-regulation of microglia-specific marker, P2ry12, a GPCR receptor of ADP which Gi-dependently inhibits cAMP/PKA/pERK/pCREB/c-Fos pathway. We validated both in vitro and in vivo that this "ectopic" expression of P2ry12 prevents the melanocortin 4 receptor (MCR4)/cAMP/PKA/pCREB/c-Fos axis activity to counteract obesity. Strikingly we detected such "ectopic" expression of P2ry12 also in neurons of HFD-treated mice, while application of P2ry12 inhibitors to obese mice normalized the phenotype suggesting that it can be used as a novel therapeutic to treat metabolic disorders.

S04-034

Graphitic-Like Hexagonal Phase of Alkali Halides in Quasi-Two-Dimensional Confined Space under Ambient Conditions

Yimin Zhao[1*], Quan Zhang[1*], Yunzhang Li[2], Liang Chen[3,4], Ruobing Yi[1,4], Bingquan Peng[1], Dexi Nie[1], Lihao Zhang[1], Guosheng Shi[2], Shengli Zhang[1†] & Lei Zhang[1†]

Abstract

The discovery of specific matter phases with abnormal physical properties in low-dimensional systems and/or on particular substrates, such as the hexagonal phase of ice and two-dimensional (2D) CaCl with abnormal valence state, continuously reveals more fundamental mechanisms of the nature. Alkali halides, represented by NaCl, are one of the most common compounds and usually thought to be well-understood. In the past decades, many theoretical studies suggested the existence of one particular phase, i.e., graphitic-like hexagonal phase of alkali halides at high-pressure or in low-dimension states, with the expectation of improved properties of this matter phase but lack of experimental evidence due to severe technical challenges. Here, by optimized cryo-electron microscopy (cryo-EM), we report the direct atomic-resolution observation and in situ characterization of the prevalent and stable graphitic-like alkali halide hexagonal phases, which were spontaneously formed by unsaturated NaCl and LiCl solution respectively, in the quasi-2D confined space between reduced graphene oxide (rGO) layers under ambient conditions. Combined with control experiment, density functional theory (DFT) calculation and previous theoretical studies, we believe that a delicate balance among the cation-π interaction of the solute and substrate, electrostatic interactions of anions and cations, solute-solvent interactions and thermodynamics under confinement synergistically results in the formation of such hexagonal crystalline phases. These findings highlight the effects of the substrate and the confined space on the formation of specific matter phases and provide a universal scheme for the preparation of special graphitic-like hexagonal phases of alkali halides.

S04-134

Development and application of a novel cryo-EM strategy on in-situ high-resolution structural analysis

Xing Zhang, Xin You, Sen-Fang Sui, Hong-Wei Wang

Tsinghua University, School of Life Sciences, Beijing, China

Email: zhxix@126.com

In the field of cryo-electron microscopy (cryo-EM), in-situ structural analysis directly from cells presents significant challenges. The cryo-focused ion beam technique can produce cell lamellas with a suitable thickness of 100-200 nm, enabling cryo-EM imaging. Cryo-electron tomography and sub-tomogram averaging (STA) methods are commonly employed to resolve protein structures from such information crowded lamellas at a sub-nanometer resolution. To overcome the resolution limitation of STA, we have developed a novel strategy that combines STA with single-particle analysis (SPA), leveraging the advantages of both approaches. This hybrid strategy was applied to investigate photosynthesis-related protein structures in red algae using FIB lamella sample. Through optimizing the structure-refinement protocol, we accomplished a significant breakthrough by determining the native structure of the intact phycobilisome-photosystem II-photosystem I-LHCs megacomplex at 3.3 Å resolution (with local resolution reaching 2.6 Å). This result enabled us to identify novel complex components, uncover both intra- and inter-complex interactions, and gain a comprehensive understanding of the entire energy transfer pathway involved in photosynthesis. Furthermore, our strategy has also been validated in other applications, demonstrating its effectiveness in resolving structures from complex in-situ sample.

S04-390

Cryo-EM structure of Z-genome phage and ZTCG DNA

Jing Lin[1,2]; Suwen Zhao[1,2]

[1] *iHuman Institute, ShanghaiTech University, Shanghai, 201210*

[2] *School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210*

Email: linjing@shanghaitech.edu.cn

2-aminoadenine (Z) fully replaces adenine in the genomes of hundreds of bacteriophages. It is known that Z genome DNA (ZTCG DNA) has different physical, chemical and mechanical properties since Z pairs with T through three hydrogen bonds. However, the structural details behind the special properties of Z-genome phage and ZTCG DNA remain elusive. Here, we present cryo-EM structures of Z-genome phage SH-Ab 15497, including its capsid, portal, major tail protein and the ZTCG DNA located within the portal. The structural protein of SH-Ab 15497 exhibits a simple architecture without additional modified proteins. Notably, the ZTCG portal-DNA exhibits an A-like form, distinguished from the known conformation of bacteriophage DNA. From a structural perspective, our research sheds light on the unique features of Z-genome phages. These insights contribute to a better understanding of the structural properties and potential functional implications of ZTCG DNA and Z-genome phages.

S05-036

Eukaryotic transcriptional dynamics at single-molecule level

Ying Xiong, Shuang Wang

Institute of Physics, Chinese Academy of Sciences, Beijing, 100190, China

Abstract

Transcription is a complex process that transfers genetic information from DNA to RNA for protein translation. It is accomplished by an RNA polymerase with or without the regulations of a variety of transcription factors whose dynamics are highly required for cell functions. Here, we use single-molecule magnetic trap and fluorescence assays to explore the mechanisms of eukaryotic RNA polymerase II (Pol II) during its extrinsic transcription termination. We developed a DNA scaffold which allows us to efficiently initiate and elongate yeast Pol II using either in bulk assays or single-molecule magnetic trap assay. We used this DNA scaffold together with single-molecule magnetic trap to study the termination mechanics of Sen1 helicase, a homolog to human Senataxin (SETX) functional for transcription termination of short non-coding RNAs in human. We found that Sen1 follows the classical Michaelis-Menten model to diffuse, recognize and form an intermediate termination complex with Pol II elongation complex (Sen1-Pol II TEC). This Sen1-Pol II TEC intermediate represents a partially collapsed transcription bubble prior to termination. To explore the fate of such intermediate, we implemented single-molecule fluorescence assays to measure the formation and dissociation kinetics of each component (RNA polymerase II, Sen1, RNA and DNA) when extrinsic termination occurs. Interestingly, we recognized another fine Michaelis-Menten process involved in the formation of the Sen1-Pol II TEC intermediate which is possibly the Sen1 translocation process on RNA transcript. These results may deeper our interpretation of the mechanisms of eukaryotic transcription termination.

S05-122

Genomic DNA is a crowded track where motor proteins frequently collide. It remains underexplored whether

these collisions carry physiological function. In this work, we develop a single-molecule assay to visualize the trafficking of individual E. coli RNA polymerases (RNAPs) on DNA. Based on transcriptomic data, we hypothesize that RNAP collisions drive bidirectional transcription termination of convergent gene pairs. Single-molecule results show that the head-on collision between two converging RNAPs is necessary to prevent transcriptional readthrough but insufficient to release the RNAPs from the DNA. Remarkably, co-directional collision of a trailing RNAP into the head-on collided complex dramatically increases the termination efficiency.

Furthermore, stem-loop structures formed in the nascent RNA are required for collisions to occur at well-defined positions between convergent genes. These findings suggest that physical collisions between RNAPs furnish a mechanism for transcription termination and that programmed genomic conflicts can be exploited to co-regulate the expression of multiple genes.

S05-157

3D structural determination of large RNAs by integrative methods

Zhonghe Xu[1, #], Jie Zhang[1, #], Burkhard Endeward[2], Akiva Feintuch[3], Jaideep Singh[4], Peter Z. Qin[4], Daniella Goldfarb[3], Thomas F. Prisner[2], Xianyang Fang[5*]

[1]School of Life Sciences, Tsinghua University, Beijing 100084, China. [2]Institute of Physical and Theoretical Chemistry and Center of Biomolecular Magnetic Resonance, Goethe University Frankfurt am Main, Germany. [3]Department of Chemical Physics, Weizmann Institute of Science, Rehovot 76100, Israel. [4]Department of Chemistry, University of Southern California, Los Angeles, California 90089, United States. [5]Key Laboratory of RNA Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101, China

[*]Correspondence: fangxy@ibp.ac.cn; #These authors contributed equally.

Abstract

RNAs are important biomolecules that play diverse roles in many cellular processes and have been linked to human health and diseases. Studies of RNA 3D structure, dynamics and interactions are crucial to understand the mechanisms of their functions and development of new therapeutics, which however remains challenging using conventional techniques including X-ray crystallography, NMR and cryo-EM, due to RNA's inherent flexibility. There is a growing trend in the field to comprehensively analyze RNA structure and dynamics by combined use of multiple complementary experimental and computational techniques, in other words, integrative methods. Recently, we have developed a novel method for posttranscriptional site-specific labeling of large RNA using the TPT3-NaM unnatural base pair system, opening new possibility for application of molecular ruler techniques, such as X-ray scattering interferometry (XSI), Pulsed electron-electron double resonance spectroscopy (PELDOR) and single-molecule fluorescence resonance energy transfer (smFRET) in investigating the structure and dynamics of large RNAs. Using the novel RNA labeling method and molecular rulers, we develop an integrative method for 3D structure determination of large RNA by combined use of PELDOR, SAXS and computational modeling. We have benchmarked and applied the methods to a variety of large RNAs from flavivirus and coronavirus.

S05-250

Intermolecular Interactions Modulate Mechanical Stability and Trimer Assembly of the Transmembrane Protein Diacylglycerol Kinase

Huiying Yang[1,3], Mojie Duan[2,3,*], Daihong Zhou1, Hao Yu[1,*]

[1]*School of Physics, Huazhong University of Science and Technology, Wuhan 430074, China.*

[2]*Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences, Wuhan 430071, China*

[3]*These authors contributed equally.*

[*]*Email: 1711875627@qq.com.*

Understanding the molecular mechanisms of membrane protein folding is of broad interest. Atomic force microscopy-based single molecule force spectroscopy (SMFS) has proven to be an important technology to study membrane proteins. However, the interpretation, molecular mechanism and significance of unfolding intermediates remained poorly explored and underappreciated. In this work, we used SMFS and molecular dynamics simulations to study the unfolding pathway of an integral membrane protein, diacylglycerol kinase (DAGK). We were able to localize intermediate states to the precision of single amino acid by simulations and carefully designed experiments. We also identified intra- and inter-molecular interactions that affects the mechanical and thermal stability of DAGK, and verified the results by making point mutations. This work provides new understanding on the significance of mechanical unfolding of membrane proteins.

S05-304

Transport dynamics in senescent cells

Bo Gao[1], Hui Li1[2*]

[1]School of Systems Science, Beijing Normal University, 100875, Beijing, China; [2]Institute of Nonequilibrium Systems, Beijing Normal University, 100875, Beijing

E-mail: huili@bnu.edu.cn; 202131250026@mail.bnu.edu.cn

Cell is a non-equilibrium living system, in which biomolecules and organelles constitute a highly complex and dynamic environment. As such, the intracellular transport is tightly regulated by the biophysical properties inside the cells, and is associated to the cellular physiological states. Moreover, the dynamics of intracellular transports also provides a way to explore the intracellular structural information, as well as the physical characteristics of the intracellular world have been poorly studied. In this study, we utilize live-cell single-molecule fluorescence tracking techniques to investigate the macromolecule diffusion and directed motion in senescent cells. We build two kinds of senescent cell systems by treating with Doxorubin. And we quantify the cell spread area and volume using immunofluorescence technology and discover that the senescent cells spread about 6 times area of young cells and volume is about 7 times. Then we determine the intracellular diffusion dynamics of quantum dots and find that is faster in senescent cells. However, the intracellular diffusion dynamics of fluorescent beads is slower than that in young cells. The diffusion results with different particles sizes are opposite. We also measure the directed movement of fluorescent beads after endocytosis and find to be faster than that in young cells. Our results reveal the complex dynamics within cells, providing some new insights into understanding the physical characteristics of senescent cells.

S08-110

Characterization of a novel heterozygous frameshift variant in NDP gene that causes familial exudative vitreoretinopathy in female patients

Abstract

Familial exudative vitreoretinopathy (FEVR) is a severe inherited disease characterized by defective retinal vascular development. With genetic and clinical heterogeneities, FEVR can be inherited in different patterns and characterized by phenotypes ranging from moderate visual defects to complete vision loss. This study was conducted to unravel the genetic and functional etiology of a 4-month-old female FEVR patient. Targeted gene panel and Sanger sequencing were utilized for Genetic evaluation. Luciferase assays, western blots, quantitive real-time PCR (RT-qPCR), and immunocytochemistry were performed to verify the functional defects in the identified candidate variant. Here, we report a 4-month-old girl with bilateral retinal folds and peripheral avascularization and identified a novel frameshift heterozygous variant c.37dup (p.Leu13ProfsTer13) in NDP. In vitro experiments revealed that the Leu13ProfsTer13 variant causes post-translationally degradation of the encoded protein and results in compromised Norrin/β-catenin signaling activity. This study reports the identification and characterization of a novel variant c.37dup (p.Leu13ProfsTer13) in the NDP gene and demonstrates that this variant produces a premature translation-termination codon, leading to post-translationally degradation. Furthermore, we propose, preliminarily, that the severity of FEVR in heterozygous female NDP variant carriers might be positively correlated with the extent of functional defects. Thus, the pathogenic mechanism by which heterozygous frameshift or nonsense variants in female carriers cause FEVR might largely result from a loss-of-function variant in one X chromosome allele and skewed X-inactivation. Further recruitment of more FEVR-affected females carrying NDP variants and genotype-phenotype correlation analysis can ultimately offer valuable information for the prognosis prediction of FEVR.

S09-001

Stress granules protect ER-exit sites from TDP43 aggregation

Hongyi Wu[1], Loo Chien Wang[2], Belle M. Sow[1], Damien Leow[3], Jin Zhu[1], Kathryn M. Gallo[4, 5], Kathleen Wilsbach[4, 5], Roshni Gupta[1], Lyle W. Ostrow[4, 5, *], Crystal J. J. Yeo[6 - 10, *], Radoslaw M. Sobota[2, *], Rong Li[1, 11 - 13, *]

[1] *Mechanobiology Institute, National University of Singapore (NUS), Singapore*

[2] *Functional Proteomics Laboratory, SingMass National Laboratory, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore*

[3] *Department of Anatomy, Yong Loo-Lin School of Medicine, National University of Singapore, Singapore*

[4] *Department of Neurology, School of Medicine, Johns Hopkins University, USA*

[5] *Department of Neurology, Lewis Katz School of Medicine, Temple University, USA*

[6] *National Neuroscience Institute and A*STAR, Singapore*

[7] *Duke-NUS Medical School, Singapore*

[8] *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*

[9] *Department of Neurology, Feinberg School of Medicine, Northwestern University, USA*

[10] *School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, UK*

[11] *Department of Biological Sciences, National University of Singapore, Singapore*

[12] *Center for Cell Dynamics and Department of Cell Biology, School of Medicine, Johns Hopkins University, USA*

[13] *Lead contact: mbihead@nus.edu.sg*

[*] *Correspondence*

Abstract

Aggregation of proteins under cellular stress plays key roles in age-related degenerative diseases, but how different proteins coalesce to form inclusions that vary in composition, morphology, molecular dynamics and physiological consequences is poorly understood. We employed a general reporter to identify proteins forming aggregates under proteotoxic stress in human cells. Over 300 proteins were identified, forming different inclusions containing subsets of aggregating proteins. In particular, TDP43, implicated in Amyotrophic Lateral Sclerosis (ALS), partitions dynamically between two distinct types of aggregates: stress granule (SG) and a previously unknown solid inclusion containing components of the ER exit sites (ERES), such as SEC16A. TDP43 accumulation at ERES is antagonized by SG assembly, but enhanced by certain ALS-associated mutations. TDP43-ERES aggregation biases toward nascent TDP43 and, unlike SG, does not contain RNA. Such aggregation causes defects in ER-to-Golgi protein transport, providing a link between TDP43 aggregation and compromised cellular function in ALS patient neurons.

S09-025

PDE4D single nucleotide polymorphism rs918592 is associated with ischemic stroke risk in Chinese: A meta-analysis

Xinrui Yu[1†], Guiying Zhang[1†], Xuelei Tang[1], Rong Lin[1,2*]

[1]*Department of Biology, Hainan Medical University, Haikou571199, China*

[2]*Center of Forensic Medicine of Hainan Medical University, Hainan Provincial Academician Workstation (tropical forensic medicine), Hainan Provincial Tropical Forensic Engineering Research Center, Haikou571199, China*

[*]*Corresponding author*

E-mail: xianronglin@gmail.com, Tel: +86-18689506639

[†]*Xinrui Yu and Guiying Zhang contributed equally.*

Abstract: Several studies have investigated the correlation between phosphodiesterase 4D (PDE4D) single nucleotide polymorphism (SNP) rs918592 and the risk of ischemic stroke (IS) in Chinese populations. But the results were inconsistent and inconclusive. Therefore, to resolve this conflict, we conducted a meta-analysis to further elucidate their relationship in Chinese populations. Studies focused on SNP rs918592 and IS risk were electronic searched in the databases of PubMed, Embase, ISI Web of Science, Weipu, China National Knowledge Infrastructure (CNKI), Chinese Biomedical (CBM) and Wanfang. The meta-analysis was performed with STATA 11.0 statistical software. We also adopted two online prediction websites (HaploReg and RegulomeDB) to explore the functions of SNP rs918592. The meta-analysis ultimately included 10 studies involving 2,348 cases and 2,289 controls. The results showed that there was a significant correlation between SNP rs918592 and IS risk in Chinese. The G allele had reduced risk of developing IS compared to the A allele (odds ratio (OR) 0.83, 95% confidence interval (CI) 0.74-0.95, P=0.005). HaploReg and RegulomeDB analyses suggested that SNP rs918592 and its strongly linked SNPs (e.g. rs34168777) might have regulatory functions. This study shows that SNP rs918592 in PDE4D may be a contributor of IS risk in Chinese. It offers a good answer for the association of PDE4D SNP rs918592 with IS risk in Chinese for the first time.

Keywords: PDE4D, rs918592, single nucleotide polymorphism, ischemic stroke, meta-analysis

S10-003

Nitrogen Doped Carbon Dots Based on Maltose with Antimicrobial Activity against Escherichia coli

Yuanyuan Hu [1*], Yuheng Wang [2], Liquan Tian [3], Yili Wang [4], Xingyue Liu [5], Nuonuo Zhang [6]

[1*,2,3,4,5] *Medical College, China Three Gorges University, No. 8, Daxue Road, Yichang, 443002, huyuanyuan@ctgu.edu.cn*

[6] *College of Material and Chemical Engineering, China Three Gorges University, No. 8, Daxue Road, Yichang, 443002*

Pathogenic bacteria and their drug-resistant strains pose a serious threat to human health worldwide. A number of nanomaterials have been synthesized to directly inhibit pathogenic bacteria. In our study, nitrogen-doped carbon dots (NCD-p) are prepared by one-pot dry heat treatment of maltose and p-phenylenediamine (PPD). NCD-p exhibit excellent photoluminescence and stable chemical properties. More importantly, NCD-p exhibit obvious inhibitory effect on E. coli including drug-resistant strains and O157:H7 with low in vivo and in vitro toxicity. The inhibition rate of NCD-p on all E. coli strains in this test was more than 80 %. NCD-p are able to result in deformation and rupture of E. coli cytoderm, which leads to death of E. coli. Therefore, our study suggests that NCD-p can function as an effective antibiotic against E. coli.

Key words: nano materials, nitrogen doped carbon dots (NCD), antimicrobial

S10-100

Molecular and supramolecular engineering on lipopeptide-based hole-punching nanodrugs to trigger multimodal death of drug-resistant tumors: apoptosis, necrosis and autophagy

Xiao Zhang[1,*], Xianghui Xu[2]

[1]*Department of Pharmacy, Second Clinical Medical College, Shanxi Medical University, Taiyuan, Shanxi, 032000*

[2]*Department of Pharmacy, College of Biology, Hunan University, Changsha, Hunan, 410082*

Email: xiao.zhang1@hotmail.com; zhangxiao24@a.sxmu.edu.cn

Discovering bioactive and efficient new agents for broad-spectrum multidrug resistance reversal is a worldwide challenge in the biomedical field. Herein, bioactive nanomaterials assembled from specially-designed lipopeptides are developed as a novel type of hole-punching nanodrugs to trigger multimodal tumor death for multidrug resistance reversal in vitro and in vivo. The dendritic arginine-rich peptides as hydrophilic parts could incorporate with multivalent scaffolds and multiple-interaction residues to boost membrane-binding intensity, and cholesterol as hydrophobic blocks are hopeful to improve and ameliorate membrane-targeting/inserting ability against drug-resistant tumor membranes. The amphiphilic lipopeptides are designed to drive supramolecular self-assembly into nanostructures for improving hole-punching potential and stability. As envisaged, these lipopeptide-based nanodrugs could maintain high stability, obtain strong hole-punching activity and trigger multimodal tumor death with 80.3% TUNEL-positive apoptotic cells, 97.8% PARP-positive necrotic cells and 71.0% LC3-positive autophagic cells for multidrug resistance reversal in vivo. This work opens a new opportunity to develop new-style toxins for antitumor treatment, and the lipopeptide-based nanodrugs are expected to be a candidate agent for dealing with multidrug resistance.

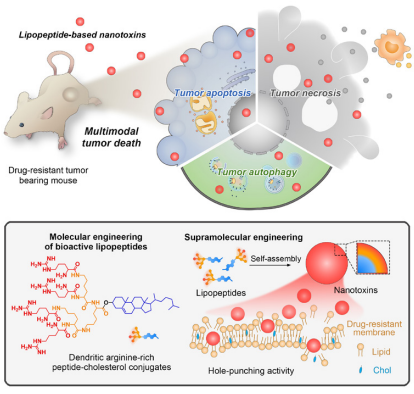


Fig. 1 Schematic illustration of hole-punching NTs for efficient induction of multimodal tumor death and multidrug resistance reversal. Bioactive lipopeptides self-assemble into nanodrugs to generate hole-punching ability against drug-resistant tumor membrane for induced apoptosis, necrosis and autophagy.

References

[1] Zhang X., Li Y., Yang Y., Deng K., Liu R., Hou R., Xu X. Chem. Eng. J. 2023, 465, 142698.

S10-106

Conformational and allosteric structural signatures of proteins resolved by nanopore sensing

Mingkun Zhang [1,2], Zhi Zheng [2], Shenbao Chen [2], Shouqin Lü [2,*], Mian Long [2,*]

[1] *Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, 400714, China*

[2] *Institute of Mechanics, Chinese Academy of Sciences, Beijing, 100190, China*

*zhangmk@cigit.ac.cn, * lsq@imech.ac.cn, * mlong@imech.ac.cn*

Abstract: The conformational changes of proteins play a vital role in implementing their functions and revealing underlying mechanisms in various biological processes. Accurate detection and identification of protein-conformational evolutions are essential to describe the micro-structural mechanisms of protein allostery. Nanopore technique is promising for protein detection, especially when recent advances in the regulation of molecular translocations have made it possible to detect the allosteric processes of single proteins. However, it is challenging in monitoring intermediate- state conformations by those implicit characteristics of the attenuated amplitudes and temporal widths of current-resistance pulses during protein allostery, since the corresponding relationship between structural information of evolutive conformations and characteristic current recordings is still unclear. Here a molecular dynamics-based approach is first developed to theoretically predict the volume and shape identifiers of protein, and the current modulations of protein conformation and translocation orientation are decoupled [1]. Further, a new approach based on electrically- and mechanically-coupled sensing is established to probe conformational dynamics of protein in a nanopore, and the structural signatures of intermediate conformers are resolved in real time via a novel SMD-spheroid approximation. Results indicate that nanopore confinement increases the energy barrier of conformational extension of a typical protein of αXβ2 integrin. Competitive effects of intramolecular domains in compensating for the stretched height and determining alternative allosteric patterns are further clarified. This work proposes a new method to real-time visualize the conformational dynamics during protein allostery, favoring to depict structural characteristics for the conformations of those transient states and evaluate the impacts of the nanopore confinement on protein allostery.

References:

[1] Zhang MK, Chen SB, Hu JR, Ding QH, Li LD, Lü SQ*, Long M*, Nanoscale 2021, 13 (12), 6053.

This work was supported by National Natural Science Foundation of China grants (32130061 and 12172366).

S10-190

基于 SpyStapler 的固相共轭产物纯化

孙晨博，寇松姿，孙飞 *

深圳湾实验室粤港澳生物医学创新中心，广东深圳

生物偶联是连接生物分子的有效技术之一。然而，灵活的连接片段对于这些共轭生物分子的远程变构，以实现较高自由度的构象变化来进行各种生化活动是必不可少的。在此，我们使用 SpyStapler，一种本质上无序的连接酶来催化 Spytag 和 BDtag 之间的异肽键形成，这两个肽标签来自化脓性链球菌的蛋白质结构域。并且引入了 SpyStapler 的固相固定方法，它可以通过去除未反应的前体以及未固定的 SpyStapler 来产生更纯的偶联产物，它还可以提供灵活无结构的连接片段。固定的 SpyStapler 可以重复使用多次，从而降低成本。这种固相结合分离方法展示了生物偶联和扩展连接酶效用的实用方法。

S10-216

Antibacterial effect of rod-like ZnO on *S.mutans*

Ruihua Liu[1,2], Qin Du[1,2,*]

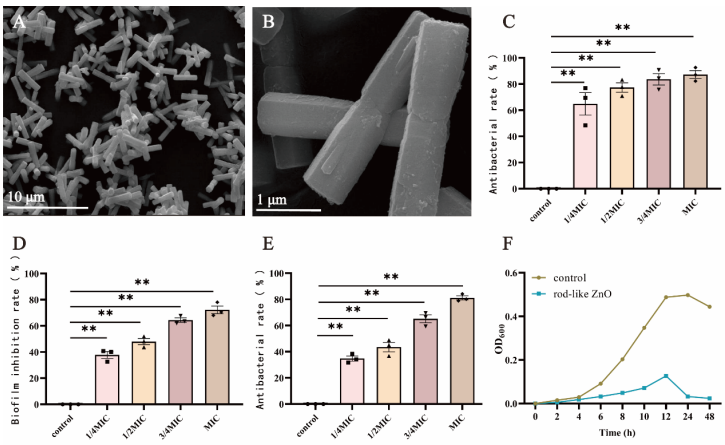
[1] *School of Medicine, University of Electronic Science and Technology, Chengdu ,Sichuan, China, 610054;*

[2] *Department of Stomatology, Sichuan Academy of Medical Science & Sichuan People's Hospital, Chengdu, Sichuan, China, 610072*

*Email: Norahliuu@163.com; * Correspondence, dududicn@163.com*

Abstract

Dental caries is the most common oral disease that poses a threat to human health. In this study, we developed a composite resin material with good antibacterial activity and biosafety by utilizing the inherent features of ZnO as an inorganic antibacterial material, to address the consequences of filling failures caused by secondary caries. Rod-like ZnO was synthesized by the atmospheric pressure hydrothermal method and characterized using Scanning Electron Microscope(SEM) to observe its morphology. The antibacterial effect of rod-like ZnO was studied by assessing the minimum inhibitory concentration (MIC), antibacterial rate, biofilm formation and destruction of *S.mutans*, and its impact on the growth curve. The rod-like ZnO crystal had a length of approximately 2.5 μm, the diameter of roughly 0.8 μm (Fig A, B), with the minimum inhibitory concentration against *S.mutans* being 3.0 mg/mL. At MIC and sub-MIC concentrations, it exhibited an apparent bacteriostatic effect (Fig C, $P < 0.01$) and significantly inhibited biofilm formation (Fig D, $P < 0.01$), as well as destroyed the formed biofilm (Fig E, $P < 0.01$). The growth curve of *S.mutans* was notably inhibited only at 1/2 MIC concentration (Fig F). Based on the principle of atmospheric pressure hydrothermal method, rod-like ZnO material could be prepared by modifying the reaction conditions, which demonstrated significant inhibitory effects on the growth, adhesion, and aggregation of the primary oral cariogenic bacteria *S.mutans*. These findings suggest excellent antibacterial properties, providing an adequate foundation for its application in preventing secondary caries in the oral cavity.



S10-265

Ultraviolet-B persistent phosphors luminescence smart window for Inhibiting bacteria and Food preservation

Xiaohui Lin[1], Chonghui Li[1,*], Shicai Xu,[1*]

[1]*Shandong Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou, 253023, P. R. China*

E-mail: XiaohuiLinSCI@163.com

The potential to commercialize UV afterglow phosphors light technologies as new methods for preserving food products has caught the attention of a food industry that wishes to fulfill consumers' demands for fresh products. In this work, photoluminescence (PL) persistent luminescence (PersL) and optical stimulated luminescence (OSL) with 300 nm emission wavelength have been realized on SrAl12O19: Ce3+, Sc3+ phosphor, which fills the gap of PersL in UVB range. Wherein□UVB persistent has good bactericidal effects on pathogenic bacteria and yeast in the process of food spoilage. Thermoluminescence (TL) spectra show that the shallow trap which is induced by the Sc3+ co-doping plays an important role in contribution to PersL, while the deep trap dominates the generation of OSL. Owing the appearance of deep trap, the OSL under x light excitation (x=700-900 nm) are also observed. Due to the features of PersL in UVB range,thus, a UVB persistent phosphors smart window for inactivating bacteria is fabricated, and offers new insights into developing UV light for sterilization applications and food preservation.

S10-329

Regulation of neurobiofilm homeostasis and diagnosis and treatment of brain diseases based on Nanotechnology

Xue Xue*, Tianqi Li, Heping Wang

State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, Nankai University, Tianjin, China, 300350

**Email: xuexue@nankai.edu.cn*

Recently, the morbidity and mortality of brain diseases are increasing year by year, which has become a serious public health problem endangering human health and causing a heavy social burden. The effective diagnosis and intervention of major brain diseases is a very important research area in the field of brain science and technology in the future, which is in line with a Chinese policy known as the 14th Five-Year Plan. Due to the complex pathogenesis of brain diseases, there is a lack of effective treatment and clinical drugs for them. Our research has shown that modulating the homeostatic imbalance of neurobiological membranes (including the blood-brain barrier and nerve cell membranes) is one of the important ways to intervene in brain diseases. Under pathological conditions, the stability and integrity of neural biofilms are easily disturbed and disrupted, leading to disturbances in the internal and external environment of the nervous system and the development of diseases. The application of new nanomaterials in the diagnosis and treatment of brain diseases can not only achieve high bioavailability and low toxicity of drugs by utilizing the superior physical and chemical properties of the materials themselves, but also directly interact with neurobiofilms layer by layer. These new nanomaterials maintain the homeostasis of nerve cell membrane by regulating the content, structure and localization of key components in the cell membrane, and finally realize efficient and specific repair of brain diseases. This approach helps to reveal the nature of the disease, establish diagnostic criteria, and develop targeted interventions. At the same time, it provides a new Angle for the functional development of nanobiomaterials and helps the further development of the field of neuromedicine.

S11-1-177

A diet high in sugar and fat affects social memory in adult female rats

Xing Cai, Haiyang Zhou, Yifan Ye and Li Lu

Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, 650201, China.
Email: caixing@mail.kiz.ac.cn

Background and aim: In light of rapid societal development, substantial changes have occurred in the daily dietary patterns of individuals, leading to a prevalent increase in the consumption of high-sugar and high-fat (HSHF) foods. Emerging evidence has highlighted the significant role of HSHF diets in adversely affecting social recognition and cognitive function during adolescence. This study aims to examine the impact of a HSHF diet on social memory in adult rats.

Methods: We administered a HSHF diet to adult male and female Long-Evans (LE) rats for 3 or 8 weeks. Subsequently, we conducted behavioral experiments to evaluate anxiety level, locomotor activity, spatial and social memory. Additionally, immunofluorescence staining was performed on the corticolimbic system to assess the number of parvalbumin interneurons (PVIs), which play a crucial role in social memory.

Results: Our findings demonstrate that an 8-week HSHF diet reduced anxiety and improved social memory in adult female LE rats. However, this diet did not affect the social memory of adult male LE rats, nor did it affect spatial learning and memory in either male or female LE rats. Furthermore, an 8-week HSHF diet significantly increased the number of PVIs in the dorsal hippocampal CA2 region, while it did not affect PVI numbers in the remaining regions of the hippocampus, medial prefrontal cortex, and basolateral amygdala.

Conclusion: Our study suggests that the HSHF diet enhances social memory by promoting the expression of parvalbumin in the dorsal hippocampal CA2 region in adult female LE rats, which may provide valuable insights into the complex relationship between diet and the encoding of social memory.

S11-2-316

Circulatory pro-CTSD stimulates cerebral microvascular transcytosis in diabetes

Dan Zhao, Qing-Hua Gao, Xue Sun, Jun-Xiu Shi, Ming-Xin An, Wei-Dong Zhao*

Department of Developmental Cell Biology, China Medical University, Shenyang 110122

**Email: wdzhao@cmu.edu.cn.*

Microvascular complications are the major outcome of type 2 diabetes mellitus progression and the underlying mechanism remains to be determined. To explore the contribution of circulating monocytes to the microvascular lesions, high-throughput RNA sequencing was conducted and we found high-level expression of cathepsin D (CTSD) in the monocytes of type 2 diabetes mellitus patients. The transgenic mice expressing human CTSD in the monocytes showed increased brain microvascular permeability resembling the diabetic microvascular phenotype, accompanied with cognitive deficit. Mechanistically, we found the monocytes release non-enzymatic pro-CTSD to upregulate caveolin expression in brain endothelium triggering caveolae-mediated transcytosis, without affecting the paracellular route of brain microvasculature. The circulating pro-CTSD activated the caveolae-mediated transcytosis in brain endothelial cells via its binding with low density lipoprotein receptor-related protein 1 (LRP1). Importantly, genetic ablation of CTSD in the monocytes exhibited protective effect against the diabetes-enhanced brain microvascular transcytosis and the diabetes-induced cognitive impairment. Our findings thus uncover the novel role of circulatory pro-CTSD from monocytes in the pathogenesis of cerebral microvascular lesions in diabetes. The circulatory pro-CTSD is thus a potential target for the intervention of microvascular complications in diabetes.

S15-005

Title: Autologous cord blood mononuclear cells for preventing preterm complications in very preterm monozygotic twins: the first-in-human randomized, placebo-controlled, double-blinded phase I trial and long term follow-up outcomes

Zhuxiao. Ren[1†], Fang. Xu[1†], Jingjun. Pei[2†], Wei. Wei[3†], Shumei. Yang[1†], Jiangxue. Han[1], Liling. Yang[1], Qi. Zhang[4], Gengying.Liu[1], Liu. Yang[1], Zhipeng. Liu[3], Qiuping. Li[5], Feng Zhichun[6], Jie. Yang[2*]

Zhuxiao Ren: renzhx1990@163.com; Fang Xu,MD:15975463496@163.com; Jingjun. Pei,MD: youknowjun@163.com; Wei. Wei,PHD: wwei@chinacord.org; Shumei. Yang,MD: 234815987@qq.com; Jiangxue. Han,MD:376440818@qq.com

Liling. Yang,MD: 947534220@qq.com; Qi. Zhang,MD: gotai@163.com; Gengying Liu,MD,liugengy2018@163.com; Liu. Yang,MD, Baylee_young@163.com; Zhipeng. Liu,MD,zpliu@chinacord.org; Qiuping. Li,MD,PHD: zhjhospital@163.com; Feng Zhichun,MD:byfengzc@163.com; Jie. Yang: jieyang0830@126.com;

[1]Department of Neonatology, Guangdong Women and Children Hospital, Guangzhou, China; [2]Department of Neonatology, Nanfang Hospital, Southern Medical University, China. [3]Guang Dong Cord Blood Bank, Guangzhou, China. [4]Department of Clinic genetic center, Guangdong Women and Children Hospital, Guangzhou Medical University, Guangzhou, China. [5]National Engineering Laboratory for Birth defects prevention and control of key technology, Beijing, China. [6]Department of Neonatology, the [7]th Medical Center of PLA General Hospital, Beijing, China.

[†] These authors contributed equally to this work.

[*]To whom correspondence should be addressed:

Dr. Jie Yang

Department of Neonatology, Nanfang Hospital, Southern Medical University, China.

Email: Jieyang0830@126.com; E-Tel: 8620-39151600

Dr. Feng Zhichun, MD. PhD

Department of Neonatology, the 7th Medical Center of PLA General Hospital, Beijing, China

National Engineering Laboratory for Birth defects prevention and control of key technology, Beijing, China.

email: byfengzc@163.com; Tel: 8620- 66721786

One-sentence summary

This first-in-human randomized, placebo-controlled, double-blinded phase I clinical trial showed autologous cord blood mononuclear cells (ACBMNCs) infusion early after birth was feasible, safe and may alleviate preterm complications via a potent immunomodulatory function.

Abstract

Persistent inflammation is the common main contributor to the pathogenesis of preterm complications. Immunomodulation effect was an important mechanism underlying the beneficial results of stem cell therapy. We conducted a randomized, placebo-controlled, double-blinded phase I trial to establish whether autologous cord blood mononuclear cells (ACBMNCs) infusion was feasible, safe and may improve outcomes in very preterm monozygotic twins (VPMTs), and we assessed the immunoregulation effects via multi-omics approaches. Eight pairs of VPMTs received intravenous ACBMNCs or placebo within 24 hours after birth. ACBMNCs treatment was safely conducted, well tolerated, and led to a reduction trend in preterm complications. We found significant increase in Treg proportion, decreased inflammatory cytokines level, induced transcriptional up-regulation of processes facilitating Treg differentiation in immune cells, improved diversity and favorable components of lung microbiota after ACBMNCs infusion. Our findings nominated intravenous ACBMNCs intervention as a potent immunomodulatory and protective therapy for alleviating preterm complications.

Keywords: very preterm monozygotic twins, autologous cord blood mononuclear cells, safety, feasibility, immunomodulation, outcomes

Trial registration: The trial is registered at Clinicaltrials.gov (NCT05087498).

Graphic abstract

S15-120

Spatial metabolomics to discover hypertrophic scar relevant metabolic alterations and therapeutic strategies

Enyuan Zhang, Siqi Zeng, Jingbin Jiang, Yiliang Yin, Qian Li, Ling Chen, Jun Li, Jingyun Li*

*Women's Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital), 123rd Tianfei Street, Mochou Road, Nanjing 210004, China. Mobile phone:18951679968. Email: drlijingyun@163.com

Abstract

Mapping hypertrophic scar and surrounding normal skin tissues' metabolic remodeling spatially can fundamentally improve our understanding of scar formation, facilitates the development of advanced therapeutic strategies. Here, we performed matrix-assisted laser desorption/ionization (MALDI), a mass spectrometry imaging-based spatial metabolomics, to hierarchically visualize the metabolic heterogeneity in hypertrophic scar and surrounding normal skin tissues. A total of 1631 metabolites were identified. Top 4 identified classes were benzene and substituted derivatives, heterocyclic compounds, amino acid and its metabolites, and glycerophospholipids. 22 metabolites were upregulated and 66 metabolites were downregulated in hypertrophic scar tissues. Top 4 altered metabolites were glycerophospholipids, glycerolipids, benzene and substituted derivatives, and heterocyclic compounds between hypertrophic scar and surrounding normal skin tissues. We then selected 7 available metabolites, analyzed their spatial characteristics and added them into the cell culture medium of primary hypertrophic scar fibroblasts respectively to check their actions. The results revealed that 1-pyrrolidinecarboxamide, glycerol trioleate and Lyso-PAF C-16 inhibited expressions of COL1A1, COL1A2 and ACTA2 at specific concentrations. We also analyzed the potential bound proteins of these 88 altered metabolites based on bioinformatic websites. Being mild and non-toxic, bioactive metabolites show good pharmacokinetics and pharmacodynamics, and their role in drug development has attracted much attention. Thus, this study is expected to provide new therapeutic clues for hypertrophic scar.

S15-262

Hard tissue research of a family with dentinogenesis imperfecta type II

Ning Yue[1,2], Qin Du[1,2,*]

[1] *School of Medicine, University of Electronic Science and Technology, Chengdu ,Sichuan, China, 610054;*

[2] *Department of Stomatology, Sichuan Academy of Medical Science & Sichuan People's Hospital, Chengdu, Sichuan, China, 610072*

*Email: 1092664717@qq.com; * Correspondence, dududicn@163.com*

Abstract

Objective: Dentinogenesis imperfecta type II is a kind of hereditary dentin dysplasia. It is an autosomal dominant disease, characterized by abnormal dentin structure, which will cause dentin tissue defects and affect the function and appearance of patients' teeth. In this research, one family line of dentinogenesis imperfecta type II was collected, and microhardness, scanning electron microscopy, and energy spectrum analysis were performed on it to find the differences between its phenotype and normal people.

Methods: The clinical data of the family were collected, and microhardness, scanning electron microscopy, and energy spectrum analysis were performed. Statistical analysis of its data.

Conclusion: In this study, a family with hereditary dentin hypoplasia type II was collected, and the microhardness of the patient group was significantly different from that of the normal group, which was significantly lower than that of the normal group. Scanning electron microscopy revealed that the dentinal tubules of the patient's tooth were highly irregular in size and shape and decreased in number (Panel E), whereas normal dentinal tubules were highly regular in shape and size (Panel F). EDS results showed that there were significant differences in the elements of the DGI teeth and the control group.

S16-191

基于水溶性叶绿素结合蛋白产生单线态氧的酶法有机合成方法学研究

徐英杰 寇松姿 孙飞 *

深圳湾实验室粤港澳生物医学创新中心，广东深圳

水溶性叶绿素结合蛋白可开发作为一种在生物兼容条件下的新型光敏剂。水溶性叶绿素结合蛋白是一种稳定、水溶性、无毒的四聚体复合物，含有 4 个叶绿素。可作为稳定的光敏剂产生活性氧（ROS 或单线态氧）用于多种用途，例如有机小分子的生物合成。研究表明，重组水溶性叶绿素结合蛋白不仅克服了传统光敏剂在水溶性差、生物相容性差等方面的缺点，叶绿素结合蛋白可保护包埋其中的叶绿素使其免受光敏产生单线态氧的氧化，而且其在红光下表现出优异的稳定性和强吸收。本研究展示了利用重组水溶性叶绿素结合蛋白进行单线态氧双烯 [4+2] 反应的例子。为合成生物学在生物小分子的生物合成方面提供一定的参考价值。

S16-333

Effects of different altitudes and different exercise intensities on cardiac function in rats

ZHU minxia*;LI xiaofeng;LIU bing;LEI yaqi;GUO jing;Key Laboratory of High Altitude Hypoxia Environment and Life Health, School of Medicine, Xizang Minzu University□Xianyang 712082, China.
E-mail: mxzhu@xzmu.edu.cn

[Abstract] Purpose: The purpose of this study was to investigate the effects of different exercise intensity on cardiac function in rats at different altitudes. Methods: In this study, SD rats were fed at four altitudes of plain (600m), 3600m, 4600m and 5600m respectively. Three training modes of non-exercise, low-intensity exercise and high-intensity exercise were set up at each altitude, a total of 12 groups. Exercise training Rats received 4 weeks of treadmill exercise. The weight gain of rats was monitored. The SV, LVEDV, EF and HR of rats were measured by echocardiography. The contents of CK, MB and cTnI in myocardium were detected by biochemistry. Results: The weight gain of rats slowed down with the increase of altitude and exercise intensity. Under the same exercise intensity, cardiac function such as SV and LVEDV increased at 3600 m, and decreased with altitude after 4600 m. When non-exercise or low-intensity exercise was performed below 4600 m altitude, although there was no obvious pathological change in myocardial tissue, a small amount of damage factors such as CK and MB were secreted by myocardium. Conclusion: In the altitude range of 3600m-4600m, there may be a functional turning point of the heart. When it is higher than this ' turning altitude ', even in a quiet state, slight inflammation of the myocardium may be induced.For early monitoring of myocardial injury, it's more meaningful to observe the molecular indicator such as CK and MB than the structure.

S17-081

Tumor Microenvironment Responsive Nanocomposites for Synergistic Therapy of Tumors

Chunxia Li*

Institute of Frontier and Interdisciplinary Science, Shandong University, Qingdao, 266237
E-mail: cxli@sdu.edu.cn

At present, some progress has been made in cancer treatment based on nano catalysts, but it is still a huge challenge to achieve precise treatment for specific tumor microenvironment. Additionally, it is difficult to achieve a satisfactory therapeutic effect by a single treatment mode. We mainly focus on rare earth upconversion nanoparticles (UCNPs) and photothermal conversion nanomaterials. A variety of nanocomposites have been designed based on the characteristics of tumor microenvironment (TME) to overcome the above problems, so as to achieve tumor synergistic therapy.

(a) A UCNPs-based TME responsive smart nanosystem UCNPs@Cu-Cys-GOx (UCCG) for starvation therapy/chemodynamic therapy/immunotherapy. It remained inert in normal tissues and was activated specifically only in TME. A series of enzyme cascades occured to enhance the production of ROS in tumor site in situ. Furthermore, it effectively inhibited the growth and tumor metastasis of primary tumor in combination with PD-L1 antibody. In addition, UCCG exhibited upconversion fluorescence enhancement in TME.

(b) A new strategy for ferroptosis-boosted low-temperature photothermal therapy based on artificial single atom Pd nanozymes was proposed for the first time. Pd nanozymes have double enzyme activities of peroxidase and glutathione oxidase in tumor microenvironment, and good photothermal conversion performance, resulting in ferroptosis featuring the up-regulation of lipid peroxides and reactive oxygen species.

(c) Degradable nanomaterials as pyroptosis inducers for cancer immunotherapy. Two new pyroptosis inducers ZnO2 and Ca2+, which can be biodegraded in the tumor microenvironment, were developed. The pyroptosis caused by mitochondrial calcium overload was confirmed for the first time, and the mechanism of inducing immune response was clarified. It was found that the nanomaterials can significantly inhibit primary tumor and lung metastasis.

References:

[1] M. Wang, M. Y. Chang, C. X. Li, J. Lin, et al. Adv. Mater. 2022, 34: 2106010.
[2] M. Y. Chang, Z. Y. Hou, C. X. Li, J. Lin, et al. Angew. Chem. Int. Ed. 2021, 60: 12971.
[3] P. Zheng, B. B. Ding, C. X. Li, J. Lin, et al. Angew. Chem. Int. Ed. 2022, 61: e202204904.
[4] P. Zheng, B. B. Ding, C. X. Li, J. Lin, et al. Chem. Eng. J. 2022, 450: 137967.
[5] P. Zheng, B. B. Ding, G. Q. Zhu, J. Lin, C. X. Li, Small 2023, 19: 2300370.

S17-114

Designed of Antioxidative Nanocatalysts for Cardioprotection

Minfeng Huo[1], Jianlin Shi[1,2*]

[1] *Shanghai Frontiers Science Center of Nanocatalytic Medicine, School of Medicine, Tongji University, 200072, Shanghai, P. R. China. E-mail: mfhuo@tongji.edu.cn*

[2] *Shanghai Institute of Ceramics Chinese Academy of Sciences; Research Unit of Nanocatalytic Medicine in Specific Therapy for Serious Disease, Chinese Academy of Medical Sciences (2021RU012), 200050, Shanghai, P. R. China. E-mail: jlshi@mail.sic.ac.cn*

Distressing and lethal cardiotoxicity is one of the major severe side effects of using anthracycline drugs such as doxorubicin for cancer chemotherapy. The currently available strategy to counteract these side effects relies on the administration of cardioprotective agents such as Dexrazoxane, which unfortunately has unsatisfactory efficacy and produces secondary myelosuppression. In the present work, aiming to target the characteristic ferrous iron overload in the doxorubicin-contaminated cardiac microenvironment, a biocompatible nanomedicine prepared by the polyvinylpyrrolidone-directed assembly of magnesium hexacyanoferrate nanocatalysts is designed and constructed for highly efficient intracellular ferrous ion capture and antioxidation. The synthesized magnesium hexacyanoferrate nanocatalysts display prominent superoxide radical dismutation and catalytic H2O2 decomposition activities to eliminate cytotoxic radical species. Excellent in vitro and in vivo cardioprotection from these magnesium hexacyanoferrate nanocatalysts are demonstrated, and the underlying intracellular ferrous ion traffic regulation mechanism has been explored in detail. The marked cardioprotective effect and biocompatibility render these magnesium hexacyanoferrate nanocatalysts to be highly promising and clinically transformable cardioprotective agents that can be employed during cancer treatment.

Keywords: Nanocatalytic antioxidation; cardioprotection; prussian blue analogue; chemoprotection.

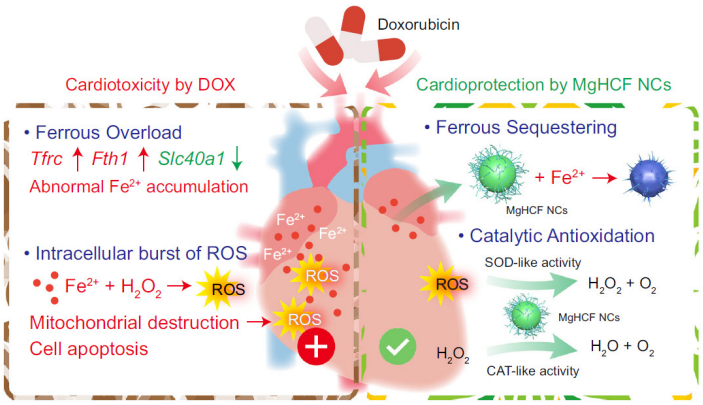


Figure 1. Schematic cardiotoxicity generation during chemotherapy using doxorubicin as well as herein proposed cardioprotective strategy by the synthetic MgHCF NCs.

Reference:

1.Huo M, et al. Nat Commun. 2022;13: 7778.

S17-115

Title: Bioinspired hierarchical self-assembled nanozyme for efficient antibacterial treatment

Author list: Ningning Song[1], Yue Yu[1], Yinuo Zhang[1], Zhengdi Wang[1], Zhanjun Guo[1], Jianlin Zhang[1], Changbin Zhang[2*], Minmin Liang[1*]

[1]*Experimental Center of Advanced Materials, School of Materials Science & Engineering, Beijing Institute of Technology, Beijing, China.*

[2]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China.*

[*]*Correspondence: mmliang@bit.edu.cn and cbzhang@rcees.ac.cn*

Along with the rapid development and ever-deepening understanding of nanoscience and nanotechnology, nanomaterials hold promise to mimic the highly evolved biological exquisite nanostructures and sophisticated functions. Here, inspired by the ubiquitous antibacterial nanostructures on the wing surfaces of some insects, we develop a NiCo2O4 nanozyme with self-adaptive hierarchical nanostructure that can capture bacteria of various morphotypes via the physico-mechanical interaction between the nanostructure and bacteria. Moreover, the developed biomimetic nanostructure further exhibits superior peroxidase-like catalytic activity, which can catalytically generate highly toxic reactive oxygen species that disrupt bacterial membranes and induce bacterial apoptosis. Therefore, the mechano-catalytic coupling property of this NiCo2O4 nanozyme allows for an extensive and efficient antibacterial application, with no concerns of antimicrobial resistance. This work suggests a promising strategy for the rational design of advanced antibacterial materials by mimicking biological antibiosis.

S17-133

Title: A series of functional hydrogenase-like nanozymes for efficient and robust H2 evolution in aqueous environment

Author list: Zhanjun Guo[1], Ningning Song[1], Kang Li[1], Shuo Wang[2], YunPeng Liu[3], Meishuai Zou[2], Minmin Liang[1*]

[1]*Experimental Center of Advanced Materials, School of Materials Science & Engineering, Beijing Institute of Technology, 100081, Beijing, China.*

[2]*School of Materials Science & Engineering, Beijing Institute of Technology, Beijing, 100081, China.*

[3]*Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.*

[*]*Correspondence to: mmliang@bit.edu.cn*

Abstract:

Hydrogen, as one of the clean and high energy-density carriers, is attracting much attention now. However, its sustainable and green production methods are still under continuous developments. [NiFe]-Hydrogenase, with turnover rate around 103 s-1, offered a great template for clean hydrogen production catalyst, but its implementation is currently limited by its low resistance to environmental changes (such as temperature, pH, etc.). Here, inspired by the active center structure of the hydrogenase, we designed and synthesized a variety of transition metal based hydrogenase-like nanozymes and applied those nanozymes to different green hydrogen production methods. We were able to achieve a high hydrogen production (HER) rate of 915 L h-1 per grams of nanozyme using aluminum as electron donors and achieved a fully consumption of Al. Also, we assembled a perovskite oxide/[NiCo]-based nanozyme composite and realized a 60-fold increase in photocatalytic HER rate compared to perovskite oxide alone. In conclusion, we envision that the transition mental based hydrogenase-like nanozymes can potentially offer an upgrade for current hydrogen production.

S18-291

In silico study on internal structure of condensate formed by full-length FUS proteins

Si-cheng Tong[1], You-liang Zhu[1], Li Zhao[2,*], Zhong-yuan Lu[1]

[1] *College of Chemistry, Jilin University, China*

[2] *School of Life Science, Jilin University, China*

[*] *Tel: 18186861339; Email: zhaoli2011@jlu.edu.cn*

Specially focus is on the condensate formed by full-length fused in sarcoma (FUS), an RNA-binding protein, which is involved in transcription initiation and regulating many aspects of RNA processing, also linked to several neurodegenerative diseases (Nature Structure & Molecular Biology. 2018 25(4):341-346.). To explore the spatiotemporal characteristics of FUS protein condensate, we built FUS protein simulation model and performed ~tens of microsecond MD simulations based on a novel coarse-grained force field developed for describing LLPS of intrinsically disordered proteins (PNAS. 2021 118(44): e2111696118-10). Based on as-formed condensate with size over 70 nm, we calculated its radial density profile with respect to the center of mass, which indicates that there is a core region with almost uniform density and the density decreases gradually in the outer layer. The diffusion coefficient of FUS protein indicates that both “core” of the condensate and its outer layer possess liquid characteristics, and the protein in outer layer has better mobility. For the first time, the internal structure of full-length FUS condensate was illustrated here, which further showed domain and charge distribution. Especially, RRM domain in FUS protein distributes with a large ratio in outer layer, implying the Nature-optimized RNA binding function of the FUS condensate. Comparing size and dynamic behavior of FUS protein in dilute solution and in condensate, we found that the FUS chain in dilute solution is more collapsed, which implies structural origin for different functions of FUS protein in LLPS and non-LLPS states.

S20-166

Tumor Microenvironment Heterogeneity Management and Therapeutic Response Enhancement with Bioresponsive Prodrugs

Yachao Li, Xianghui Xu

Department of Pharmacy, College of Biology, Hunan University, Changsha, Hunan 410082, China.
Email: yachao_li@hun.edu.cn

The development of bioresponsive nano-systems has gained significant attention in the field of oncology. which hold great promise for precise regulation of the tumor microenvironment (TME) and effective antitumor therapy. By harnessing the unique characteristics of the TME, such as acidic pH, hypoxia, and overexpressed enzymes, bioresponsive nano-systems can be designed to respond to these physicochemical signals and deliver therapeutic payloads selectively to cancer cells. Addressing the challenge of poor clinical molecular therapy response caused by TME heterogeneity remains an enormous hurdle in the treatment of serious diseases. Our research presents a practical solution through the development of a bioresponsive nano-system that combines the advantages of macromolecular and small-molecular drugs for effective antitumor treatment. The nano-system utilizes a dynamic dual-drug conjugation strategy to achieve programmable multidrug delivery, employing nanoparticulate prodrugs comprising small-molecular and macromolecular drug conjugates. By leveraging the unique characteristics of the TME, the bioresponsive nano-system selectively modulates the TME, including the tumor stroma matrix, interstitial fluid pressure, vasculature network, blood perfusion, and oxygen distribution. Furthermore, the nano-system enables rapid intracellular release of small-molecular drugs, enhancing therapeutic efficacy by improving pharmacokinetics and pharmacodynamics. This work validates the potential of bioresponsive nano-systems to effectively regulate tumor microenvironment heterogeneity, leading to improved therapeutic responses, elucidating mechanisms for drug resistance reversal, and inhibiting metastasis. The nanoparticulate prodrugs composed of small-molecular and macromolecular drug conjugates serve as a compelling example of nanomaterial-based drug design, showcasing the promising role of bioresponsive nano-systems in advancing antitumor therapy for the future.

Reference:

[1]. Yachao Li, et. al. Small 2023, DOI: 10.1002/smll.202301656.
[2]. Yachao Li, et. al. Journal of Controlled Release 2021, 329, 1129-1138.
[3]. Yachao Li, et. al. Journal of Controlled Release 2020, 323, 483-501.
[4]. Yachao Li, et. al. Materials Horizons 2018, 5, 1047-1057.
[5]. Yachao Li, et. al. ACS Nano 2017, 11, 416-429.

S22-027

Characteristics of gut microbiota, changes in the HPA axis and immune response in mice with comorbidity of type 2 diabetes and depression

Yang Yao, Wenjing Song, Heling Zhang, Peng Liu

Department of Pharmacology, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang Liaoning, 110016, P.R. China. Email: pengliu@syphu.edu.cn

Increasing evidence shows that type 2 diabetes mellitus (T2DM) is associated with depression. However, the pathogenesis of T2DM with comorbid depression (T2DD) is not fully understand. In this study, we hope to explain the pathogenesis of T2DD from the perspective of gut microbiota. First, we used db/db mice to study the difference in the gut microbiota of T2DM and T2DD and then discussed the effect of gut microbiota on the hypothalamic–pituitary–adrenal (HPA) axis and immune response in T2DD. We found that db/db mice (about 20% db/db mice showed depressive behavior, which were described as db/dp mice) exhibited depressive behavior at 20-week-old in forced swimming and tail suspension tests. Next, we used 16S rRNA gene sequencing to test the difference in intestinal flora between db/db mice and db/dp mice. After that, we found that the db/dp mice exhibited sparse microvilli, vacuolation of mitochondria, reduction of goblet cell numbers and tight junction protein expression in the duodenum and colon; decreased levels of the hippocampal neurotransmitters norepinephrine, 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid, dopamine and its metabolites homovanillic acid, and dihydroxyphenylacetic acid; increased serum corticosterone and adrenocorticotropic hormone; and decreased the expression of glucocorticoid receptor (GR). The db/dp mice also showed severe inflammatory responses and increased levels of inflammatory factors. Moreover, we performed fecal microbiota transplantation (FMT) from db/db mice to db/dp mice and found that the FMT-db/dp mice exhibited reduced depressive behaviors and decreased inflammatory factors, increased dopamine levels, and attenuated HPA axis to GR dysfunction in the hippocampus. In this study, we found difference of the gut microbiota between db/db mice and db/dp mice, and through the difference of the gut microbiota, db/dp mice exhibited HPA axis hyperactivity and severe immune response.

S24-144

SARS-CoV-2 RdRp uses NDPs as a substrate

Maofeng Wang, Kundi Zhang & Lichuan Gu*
Shandong University, 72 Binhai Road, Qingdao 266237, lcgu@sdu.edu.cn

RNA synthesis is one of the most fundamental processes in life. It is generally believed to follow a reaction process similar to DNA replication, where RNA polymerase sequentially recognizes nucleoside triphosphates (NTPs) based on the template strand's sequence. It forms new phosphodiester bonds at the 3' end of the nascent RNA chain, releasing one pyrophosphate for each added nucleotide. This process is a crucial aspect of the central dogma and forms the theoretical basis for our understanding of biological phenomena.

This fundamental theory has also profoundly influenced the principle of nucleoside analog antiviral drugs design. A common consensus is that nucleoside analog drugs must undergo a series of phosphorylation processes inside cells to ultimately become triphosphate forms, which can be utilized by viral RNA polymerase (or reverse transcriptase) to exert antiviral activity. Therefore, the design of nucleoside analog antiviral drugs has always followed the principle that prodrugs must possess a structure that allows easy triphosphorylation upon cellular entry.

SARS-CoV-2 RdRp is an appealing target for antiviral drug development. Here, our research reveals that RdRp can recognize and utilize nucleoside diphosphates as a substrate to synthesize RNA with an efficiency of about two thirds of using nucleoside triphosphates as a substrate. Nucleoside diphosphates incorporation is also template-specific and has high fidelity. Moreover, RdRp can incorporate β-d-N4-hydroxycytidine into RNA while using diphosphate form molnupiravir as a substrate. This incorporation results in genome mutation and virus death. It is also observed that diphosphate form molnupiravir is a better substrate for RdRp than the triphosphate form molnupiravir, presenting a new strategy for drug design.

S24-229

Mechanisms of miRNA-126 Regulating DNMT1 in Allergic Rhinitis

Honglin Jia a, Xiaofang Jiang b*

a The Fourth Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830054, China, E-mail: jiahonglin0621@163.com; b HaploX Future Medical Laboratory, Shenzhen, Guangdong 518000, China, E-mail: jiangxf@haplox. com, Adress: No. D333 Yinxing science and technology building, No. 1301, sightseeing Road, Longhua District, Shenzhen, China.*

Abstract

Methods: RT-qPCR was used to detect the expression of DNMT1 gene in AR patients and healthy controls. miR-126 was transfected into Jurkat cells, and the expression of DNMT1 was detected by RT-qPCR and western blot. The rat model of AR was established by OVA and Al (OH) 3 methods. HE staining was used to observe the pathological damage of nasal mucosa. ELISA was used to detect the expression level of IgE in serum to determine whether the model was successfully established. RT-qPCR was used to detect the expression levels of miR-126 and DNMT1 in rats. miR-126 was transfected into AR rats, and the expression of DNMT1 was detected by RT-qPCR and western blot; the ultrastructural damage of nasal mucosa was scanned by transmission electron microscopy. Results: DNMT1 gene expression was increased in the AR patient group; effective transfection of miR-126 into Jurkat revealed that DNMT1 expression increased with miR-126. The pathological damage of nasal mucosa was aggravated and the expression of IgE in serum was increased in AR model rats, indicating that the model was successfully established, and the expression of miR-126 and DNMT1 was increased in AR rats. Efficient transfection of miR-126 into rats also showed that elevated miR-126 resulted in increased DNMT1 expression, and elevated miR-126 expression was observed to aggravate local pathological damage of the nasal mucosa under transmission electron microscopy. Conclusion: During the development of AR, miR-126 expression is increased, DNMT1 expression is increased, and the increase of miR-126 expression level will cause the increase of DNMT1 expression and promote the development of AR. miR-126 can participate in AR by regulating DNMT1 expression.

Keywords: Allergic rhinitis; miR-RNA; miR-126; DNA methylation; DNMT1

S26-067

Fluorogenic and cell-permeable rhodamines for live-cell protein labeling in superresolution imaging

Lu Wang[1]

1Fudan University, School of Pharmacy, Shanghai, 201203, China

Email: lwangfd@fudan.edu.cn

Proteins play crucial roles in nearly every cellular activity, and advances in fluorescence-based imaging techniques like superresolution microscopy (SRM) provide powerful tools to investigate protein functions at the molecular level in real-time within living cells. However, the limited availability of appropriate fluorophores, which often exhibit poor cell permeability and produce nonspecific background signals, has hampered the widespread use of this technique. Rhodamines are one of the most essential classes of fluorophores for applications in live-cell fluorescence microscopy. In this study, we have presented a novel strategy to transform regular fluorophores into fluorogenic probes with an excellent cell permeability and a low unspecific background signal. Conversion of a carboxyl group found in rhodamines and related fluorophores into an electron-deficient amide does not affect the spectroscopic properties of the fluorophore, but allows us to rationally tune the dynamic equilibrium between two different forms: a fluorescent zwitterion and a non-fluorescent, cell-permeable spirolactam. Furthermore, the equilibrium generally shifts towards the fluorescent form when the probe binds to its cellular targets. The resulting increase in fluorescence can be up to 1,000-fold. The simplicity of this design principle allowed us to create a range of fluorogenic probes in various colors for different cellular targets, enabling wash-free, multicolor, live-cell stimulated emission depletion (STED) microscopy. Importantly, by systematically tuning the spirocyclization equilibrium, we were able to convert conventional rhodamines into spontaneously blinking dyes for single-molecule localization microscopy (SMLM). These new probes not only provide exceptional tools for bioimaging and biosensing, but also offer a promising strategy for the continued improvement of fluorescent probes for future use in cellular biology research.

S26-338

基于光遗传学的机械力刺激器的开发及其在线粒体和内质网力学生物学的研究

段丽婷，香港中文大学生物医学工程系

细胞感知和响应机械力信号的能力对于许多生物活动至关重要。大量研究揭示了细胞膜、细胞骨架和许多细胞内信号通路在细胞力学传感和转导中的作用。最近，细胞核也被确定为重要的细胞机械力传感器。然而，由于缺乏对活细胞内细胞器精确施加力刺激的实验手段，线粒体和内质网是否以及如何感知、响应力刺激尚不清楚。因此，我们开发了基于光遗传学设计的光控机械力刺激器，能够远程、精准地对活细胞内的内质网或线粒体施加机械拉力、并不干扰其他的细胞结构，并且在时间、空间和力强度方面具有可控性。我们揭示了机械拉力可以驱动线粒体的“断尾”式分裂：首先线粒体生成细长的管状结构，随后在此发生分裂。这种分裂涉及 DRP1/Mff 和内质网小管的包裹，并产生了不含线粒体内膜的线粒体衍生囊泡来募集 Parkin 和 LC3B。另外一方面，使用针对内质网的光控机械力刺激器，我们发现力刺激可以引发内质网快速释放钙离子，抑制内质网到高尔基体的运输，并诱导内质网应激。我们的结果证明了内质网和线粒体的机械敏感性，为力学刺激调节细胞器功能提供了直接证据。

S27-263

Comparative genomic analysis of five freshwater cyanophages and reference-guided metagenomic data mining

Kang Du[12], Feng Yang[2], Jun-Tao Zhang[2], Rong-Cheng Yu[2], Wei-Fang Li[2], Yuxing Chen[12], Qiong Li[2*] and Cong-Zhao Zhou[12*]

[1] *Hefei National Laboratory for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei, Anhui 230027, China.*

[2] *School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, China.*

[*] *Correspondence: zcz@ustc.edu.cn, liqiong@ustc.edu.cn*

Abstract

As important producers using photosynthesis on Earth, cyanobacteria contribute to the oxygenation of atmosphere and the primary production of biosphere. However, due to the eutrophication of urban waterbodies and global warming, uncontrollable growth of cyanobacteria usually leads to the seasonal outbreak of cyanobacterial blooms. Cyanophages, a group of viruses that specifically infect and lyse cyanobacteria, are considered as potential environment-friendly agents to control the harmful blooms. Compared to the marine counterparts, only a few freshwater cyanophages have been isolated and genome-sequenced to date, largely limiting their characterizations and applications.

Here we isolated five freshwater cyanophages varying in tail morphology, termed Pam1~Pam5, all of which infect the cyanobacterium Pseudanabaena mucicola Chao 1806 that was isolated from the bloom-suffering Lake Chaohu in Anhui, China. The whole-genome sequencing showed that cyanophages Pam1~Pam5 all contain a dsDNA genome, varying in size from 36 to 142 Kb. Phylogenetic analyses suggested that Pam1~Pam5 possess different DNA packaging mechanisms, and are evolutionarily distinct from each other. Moreover, comparative analyses of the reference genomes of Pam1~Pam5 and previously reported cyanophages enabled us to identify three circular and seven linear contigs of virtual freshwater cyanophages from the metagenomic data of the Lake Chaohu.

We propose a high-throughput strategy to systematically identify cyanophages based on the currently available metagenomic data and the very limited reference genomes of experimentally isolated cyanophages. This strategy could be applied to mine the complete or partial genomes of unculturable bacteriophages and viruses. Transformation of the synthesized whole genomes of these virtual phages/viruses to proper hosts will enable the rescue of bona fide viral particles.

S29-182

Mitochondrial oxidative stress promotes sinus node dysfunction in RyR2-R2474S mice

Rui Xu, Jing-Jing Li, Yi Zhang, Wenjun Xie

School of Life Science and Technology, Xi'an Jiaotong University, No. 28 West Xianning Road, Xi'an, Shaanxi 710049, China, 993924335@qq.com

Abstract

Aims: Catecholaminergic polymorphic ventricular tachycardia (CPVT) patients are usually associated with sinus node dysfunction (SND), however, the underlying mechanism remains largely unknown. In this study, we sought to determine the link between mitochondrial oxidative stress and aberrant intracellular Ca2+ release via the type 2 ryanodine receptor (RyR2) that promotes SND in a CPVT mice model.

Methods and Results: Mice harboring human CPVT-mutated RyR2 (RyR2-R2474S) developed slowed heart rate and prolonged sinus node recovery time (SNRT) without structural and functional alterations in heart. Acute injection with isoproterenol (ISO) induced sinus rhythm variation followed by ventricle arrhythmias. Sinoatrial node (SAN) myocytes isolated from RyR2-R2474S mice displayed increased intracellular and mitochondrial oxidative stress as determined with DCF or MitoSox Red fluorescence, while no such alteration occurred in atrial and ventricular myocytes. Inhibition of mitochondrial reactive oxygen species production by overexpressing the human catalase gene targeted to mitochondria or treating with mito-TEMPO for 2 weeks improved sinus function and reduced ISO-stimulated ventricular tachycardia in RyR2-R2474S mice.

Conclusion: Our findings reveal, for the first time to our knowledge, that mitochondrial oxidative stress plays a pivotal role in the development of SND in CPVT mice. Targeting this previously unrecognized mechanism could be useful in developing effective interventions to prevent and treat SND as well as CPVT.

S29-314

Screening and identification of regulators of STIM1 calcium affinity

Yangchun Du Youjun Wang*

Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing, P. R. China

**Email: wyoujun@bnu.edu.cn*

Stromal interaction molecule 1 (STIM1) is an endoplasmic reticulum (ER) Ca^{2+} sensor. The Store-operated Ca^{2+} entry(SOCE)mediated by it is crucial for Ca^{2+} homeostasis and regulating gene expression in most cell types. Its abnormal function is closely related to human autoimmune diseases, atherosclerosis and various cancers. Although some progress has been made in the identification of STIM1 regulators and the analysis of its regulatory mechanism in recent years, there is still a lack of research on the regulators of STIM1 activation in the ER lumen. Our previous research results suggested that there may be some factors that regulate the calcium affinity of STIM1 in the ER lumen. It has also been reported that the glycosylation and disulfide bond formation of STIM1 can change its affinity or activity, but the corresponding enzyme has not been reported yet. Therefore, we used miniTurbo mediated in situ biotin labeling of live cells combined with mass spectrometry identification to obtain STIM1 adjacent proteomes in the ER lumen. Afterwards, confocal imaging and bioinformatics analysis obtained several protein modifying enzymes that dynamically co-localized with STIM1. Through molecular biology operations, combined with calcium imaging, FRET and super-resolution imaging, it was found that a key regulatory factor can post-translationally modify STIM1 to inhibit SOCE. Further mechanistic exploration results showed that this enzyme can regulate the calcium affinity of STIM1 through post-translational modification of STIM1. In summary, we have identified a key enzymes that can post-translationally modify STIM1, and revealed their mechanism of action, laying the foundation for further understanding of the regulatory mechanism of STIM1-mediated calcium signaling in animal cells.

S29-325

Genome-Wide Identification of miRNA Associated with Endoplasmic Reticulum (ER) Calcium Homeostasis and Their Potential mechanism

Yiping Wang, Shuai Zhang, Youjun Wang*

Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing, China. 100875 wyoujun@bnu.edu.cn.

Abstract: Calcium ion (Ca^{2+}) is an ubiquitous second messenger within the cell., and proper maintenance of Endoplasmic reticulum (ER) Ca^{2+} homeostasis is essential to ensure normal physiological state and function of the cell. To gain a comprehensive understanding of the molecular mechanisms underlying miRNA regulation of ER Ca^{2+} homeostasis, we performed genomic-scale screening of regulators involved in the regulation of ER Ca^{2+} homeostasis, and tested ER basal Ca^{2+} level by Ca^{2+} imaging. We found 10 miRNA that can regulate ER Ca^{2+} homeostasis. Then we utilized transcriptome sequencing technology and bioinformatics analysis to identify target genes and patterns. Our analysis revealed that the up-regulated genes were related to ion transport and transmembrane transport pathways. Moreover, we found that the down-regulated genes were associated with ubiquitination and growth factor binding membrane surface receptors. The results suggest that miRNAs may indirectly promote the expression of ion channels or transmembrane transport by downregulating the expression of ubiquitin-related protein encoding genes. Additionally, miRNAs may directly modulate the basal ER Ca^{2+} level through the growth factor binding membrane surface receptor pathways. Furthermore, the up-regulated genes were enriched in neuroactive ligand-receptor interaction pathways. In contrast, the down-regulated differential genes were enriched in pathways related to aging-related diseases such as Alzheimer's disease, prion disease, Parkinson's disease, neurodegeneration-multiple diseases, arrhythmogenic right ventricular cardiomyopathy, and hepatocellular carcinoma. These findings suggest that the function of both upregulated and downregulated genes are closely associated with the nervous system, and downregulated differential genes are strongly associated with aging-related diseases including neurodegenerative and cardiovascular diseases. Furthermore, the enrichment analysis of all miRNA down-regulated differential genes revealed the involvement of protein processing in the ER, indicating the vital role played by these genes in the ER. Our study comprehensively demonstrated the regulatory effect of miRNAs at the mRNA level. We systematically screened miRNAs that altered the Ca^{2+} homeostasis of the ER, which represents a novel finding. we achieved a panoramic display of information related to miRNAs, Ca^{2+} homeostasis, and disease.

Keywords: Endoplasmic Reticulum Ca^{2+} Homeostasis; miRNA; Bioinformation

S30-109

Quantifying stiffness and forces in zebrafish embryo through same probe

Fuxiang Wei, Junyu Luo, Chenyang Ji

Laboratory for Cellular Biomechanics and Regenerative Medicine, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China

Email: weifx@hust.edu.cn

Abstract

Forces and stiffness are two fundamental mechanical properties of the living cells and tissues. It has long been acknowledged that one can calculate stiffness or forces within cells or tissues separately by different probes. However, in the living embryos, the forces and stiffness varied dynamically with the significant change of the cells position, so it is imperative to acquire the forces and stiffness at the same position. Here we show that a probe makeup of the ferromagnetic cobalt-platinum microcross which was trapped inside an arginine–glycine–apartic acid–conjugated stiff poly(ethylene glycol) (PEG) round microgel, can be used to measure the stiffness and force in same location. This stiff probe can be injected into zebrafish embryos and magnetized by the 3D magnetic twisting cytometry (3D-MTC). Through analyzing the rotation angles of the probe under oscillatory magnetic field, we can calculate the stiffness of the embryo. Then, brief episodes of ultraviolet light exposure were applied to dynamically photodegrade and soften the fluorescent nanoparticle–embedded PEG microgel, whose deformation and traction forces can be quantified inside the zebrafish embryos. Thus, stiffness and 3D traction forces can be measured in same location of the embryo. In addition, both normal and shear traction force oscillations were observed in zebrafish embryos from blastula to gastrula. Our findings provide a method which could quantify the stiffness and forces in the living embryos through one probe at same position.

S30-130

Global architectures and hemodynamics of in situ hepatic lobular vascular microcirculation

Jin Zhoua, Lüwen Zhouc, Shouqin Lüa,b, Mian Longa,b*

aInstitute of Mechanics, Chinese Academy of Sciences, Beijing 100190, China; bSchool of Engineering Science, University of Chinese Academy of Sciences, Beijing 100049, China; cSmart Materials and Advanced Structure Laboratory, School of Mechanical Engineering and Mechanics, Ningbo University, Ningbo Zhejiang 315211, China.

Objective: Three-dimensional structures and corresponding hemodynamics in hepatic lobular vascular microcirculation play a key role for liver function. While those profiles for localized lobule or individual sinusoids are extensively investigated, the global structural features and hemodynamic distributions in the in situ lobular vascular microcirculation remains unclear.

Method: We characterized global architectures of in situ murine lobule using tissue cleaning technique, confocal scanning and Imaris analyses during the development of liver fibrosis, and quantified global distributions of flow velocity and pressure by microscopic particle tracking velocimetry (μ-PTV) in combination with graphic model.

Result: Spatial atlases of multiple zonation features of hepatic lobular vascular network were reconstructed by combining the vessel branching level and depth, presenting the progressively increasing of vessel diameter, straightness, angle, velocity and flow along the blood flow direction of the central venous in physiological condition. During the development of liver fibrosis, the vessel diameter and straightness were increased, the velocity and flow decreased in the early fibrosis and gradually increased following the fibrosis degree upon the same pressure difference setting between central vein and portal vein, particularly in the central vein and pericentral sinusoids, but the vessel angle presented opposite trend.

Conclusion: This work not only provides insights into elaborating hepatic lobular hemodynamics to maintain functional homeostasis under physio-pathological cases, but also offers the related models and methods for elucidating the biomechanical issues related to the pathogenesis of liver fibrosis, liver tissue repair and reconstruction.

This work was supported by National Key Research and Development Program of China grant 2021YFA0719300, and National Natural Science Foundation of China grants (32130061 and 32101056).

S30-147

Implementing Optogenetic Modulation in Mechanotransduction of Cell

Miao Yu [1], Shimin Le[2], Samuel Barnett [3], Pakorn Kanchanawong[4] and Jie Yan [4]

[1]*School of Basic Medical Sciences, Zhejiang University, Hangzhou, China*

[2]*Department of Physics, Xiamen University, Xiamen, China*

[3]*Max Planck Institute for Medical Research, Heidelberg, Germany*

[4]*Mechanobiology Institute, National University of Singapore, Singapore*

Email: *yu.miao@zju.edu.cn*

Abstract: Mechanotransduction is a critical physiological process in which cells sense physical forces and translate them into biochemical responses. Mechanotransduction processes begin with mechanosensing, which involves dynamic assembly of various supramolecular force-transmission linkages each consisting of multiple physically non-covalently linked mechanosensing proteins, such as integrin, talin, vinculin, paxillin, etc. at cell-matrix adhesions. To decode the molecular mechanisms of mechanotransduction mediated by these mechanosensing proteins, it is critical to be able to control the activity of the proteins or the connectivity of the supramolecular linkage, to achieve the modulation of the mechanotransduction in a temporally and spatially controlled manner. Optogenetic dimerization systems provide a potential means for such control. Here, by utilizing a single-molecule stretching assay, we directly quantified the mechanical stability of a widely-used optogenetic dimerization system, iLID, and showed that the mechanical stability of the iLID meets the physiological requirement for the modulation of machanotransductions of cells. This is demonstrated by applying the iLID to modulate the talin-mediated mechanotransduction at cell-matrix adhesions through controlling the linkage connectivity in living cells.

Keywords: Mechanotransduction, Optogenetic Modulation, iLID, Talin, Single-molecule manipulation

S30-255

Impact of cell-substrate adhesion on embryonic stem cell differentiation

Lu Zheng[1,2], Dongyuan Lü[1,2], Fan Zhang[1,2], Yi Wu[1,2], Mian Long[1,2]

[1] *Institute of Mechanics, Chinese Academy of Sciences. Beijing. 100190.*

[2] *School of Engineering Science, University of the Chinese Academy of Sciences. Beijing. 100049.*

[*] *mlong@imech.ac.cn*

Substrate stiffness can regulate the morphology, proliferation, and differentiation of stem cells. It also maintains the stemness of human embryonic stem cells (hESCs) and facilitates the formation of definitive endoderm (DE). This study explored the distribution and expression of mechanosensitive proteins during the differentiation from cloned hESCs into DE cells, and quantified the role of cell-substrate adhesion in the process of differentiation. Key mechanosensitive proteins involved in the mechanotransductive signals within the cells were specified.

Three kinds of polyacrylamide hydrogel with varied stiffnesses (0.14, 6.1 and 46.7 kPa) were prepared, and collagen was pre-coated on the substrate. hESCs (cell line H1) were seeded on different stiffness substrate, and induced to DE cells after three days of routine culture. At the time points prior to (DE 0 day) and the beginning of hESCs differentiation (DE 1 day), the traction force distribution of clones/DE cells, the expression of β1-integrin, adhesion plaque associated proteins, F-actin and YAP were detected.

Results indicated that the expression of DE markers were positively correlated with substrate stiffness, yielding high expression for the cells locating at the clone periphery than those at the clone interior. The traction force of clone increased with the stiffness, also presenting high forces at the clone periphery compared to those at the clone interior. This biomechanical signaling pattern was consistent with those biochemical expression levels of β1-integrin, adhesion plaque associated proteins, and YAP. Meanwhile, the DE markers expression decreased and their difference among three stiffness substrates disappeared after inhibiting β1-integrin. Inhibiting the entry of YAP into the nucleus tended to reduce the DE differentiation. These data suggested that substrate stiffness could promote DE differentiation of hESCs through β1-integrin, adhesion plaque protein, YAP pathway, which furthered the understanding of biomechanical regulation of substrate stiffness in stem cell differentiation.

Acknowledgments: This work was supported by the National Key Research and Development Program of China grant 2021YFA0719302 and the National Natural Science Foundation of China (Grants 32130061, 31661143044 and 31627804).

S31-342

Modulation of TNFR juxtacrine signaling and cellular functions

Min Ma, Zhongwen Chen*

Interdisciplinary Research Center on Biology and Chemistry, Shanghai institute of Organic Chemistry, Chinese Academy of Sciences, 100 Hai-Ke Road, Shanghai 201210

**E-mail: chenzw@sioc.ac.cn*

Neuroinflammation leads to surrounding neuronal loss, which is related with the cognitive impairment of Alzheimer's diseases. Tumor necrosis factor (TNF) is the main mediator of neuroinflammation, and its receptors TNFR1 and TNFR2 play antagonistic roles in neurodegenerative diseases. TNFR1 mainly promotes inflammation and cell death, while TNFR2 has anti-inflammatory and cytoprotective effects. It is very important to understand the differences in the activation mechanisms of TNFR1 and TNFR2, however, previous studies mainly focused on the paracrine functions of cells by using soluble TNF (sTNF). In this project, we applied a micropatterned supported lipid bilayer (SLB) platform, where TNF was reconstituted on the fluid membranes to mimic cellular mTNF. In the meanwhile, RGD peptide was applied to allow cell spreading by integrin engagement. Using this technique, we found that mTNF can interact with TNFR1 and TNFR2 to form clusters. In the future, we will continue to explore the dynamic processes of TNFR signaling, and analyze the underlying physical chemical mechanisms. This will help us understand neuronal fate regulation mechanisms in neurodegenerative diseases, which is of great significance for future treatment.

S31-343

The physiochemical regulation of the interaction between Sialyl molecules and their Siglecs receptor

Xiyi Chen, Min Ma, Li Shu, Zhongwen Chen*

Interdisciplinary Research Center of Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, 201210, China. chenxiyi@sioc.ac.cn

The immune system is regulated by both activating and inhibitory signals. The latter is a vital reason why malignant cells can escape immune system, which is also the main difficulty faced by immunotherapy. In fact, the lipids and proteins on malignant cells are often highly glycosylated, with sialic acid (SA) frequently present at the tail of these oligosaccharide chains. These molecules containing SA often bind a class of proteins called Siglecs which located on the surface of NK cells and other immune cells, thereby producing immunosuppressive signals. Despite the downstream signaling pathway has been confirmed, the physical model and the regulation mechanism of this inhibitory signaling pathway are unclear. Using supported lipid bilayer(SLB) system, we can mimic the interface of malignant cell and immune cell to analyze the interaction between sialyl molecules and Siglecs. Our preliminary results showed that GD3, a disialoside-containing ganglioside, can bind to Siglec-7 and Siglec-9, both of which are inhibitory receptors on NK cells. The more complete understanding of the physiochemical regulation mechanisms of sialyl molecules and Siglecs may lay a foundation for the development of immunotherapy targeting Siglecs.

S32-174

NLRP3 upregulation related to sleep deprivation-induced memory and emotional behavior changes in TRPV1-/- mice

Ahsawle Ozathaley, Yunqing Ma, Danwei Luo, Junli Chen, Chang Liu, Zhaowei Liu*

Medical School, Nankai University, Tianjin, China, 300071

zhaoweiliu@nankai.edu.cn

ABSTRACT

Sleep deprivation, which is a common problem in modern society, impairs memory function and emotional behavior. TRPV1, a subfamily of transient receptor potential cation channels, is abundantly expressed in the central nervous system and is associated with animal behavior. In this article, we report that TRPV1 deficiency in mice alleviates sleep deprivation-induced abnormal behaviors. We found that in the sleep-deprived mice, TRPV1 knockout increased the duration and visits in the central area in the open field task and increased visits to the open arms in the elevated plus maze. The TRPV1-/- mice performed better during the test stage in the Morris water maze phase after sleep deprivation. In the mPFC and hippocampus regions, western blotting results showed that TRPV1-/- attenuated sleep deprivation-induced increases in GFAP, NLRP3, and ASC and increased the expression of the mitochondrial marker Tom20. Immunofluorescence results showed that the action of TRPV1 knockout on NLRP3 was negatively correlated with Tom20 after sleep deprivation. Our results confirm that TRPV1 knockout attenuates sleep deprivation-induced behavioral disorders. The effect of TRPV1 on the behavior of sleep-deprived mice may be related to the neuroinflammation associated with mitochondria in the mPFC and hippocampus.

KEYWORDS: Sleep deprivation, Memory, Emotion, TRPV1, NLRP3

S32-222

Selenoprotein K-mediated palmitoylation of CD36 regulates microglial functions to affect Aβ phagocytosis

Pei Ou-Yang, Zhi-Yu Cai, Jia-Ying Peng, Guo-Li Song*, Zhong-Hao Zhang*

College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China, 518060

**Email: zzh@szu.edu.cn, lilys@szu.edu.cn*

Alzheimer's disease (AD) is the most common type of dementia. β-amyloid (Aβ) is considered the main cause and driver of AD. Selenium (Se) and its compounds have been reported to reduce Aβ deposition, ameliorate synaptic damage, and improve cognitive ability in AD mice. However, the functional mechanism of Se in these processes, especially with regard to Aβ-related pathological processes, is not clear. Se exerts its biological functions mainly through selenoproteins. Our previous data suggest that selenoprotein K (SELENOK), which has an immune regulation effect in vivo, is related to pathological processes of AD and may be involved in the effects of Se in AD. Here, we show that changes in the expression of SELENOK significantly affect the level of inflammatory factors and the ability of migration and phagocytosis in microglia. Meanwhile, SELENOK knockdown inhibits the phagocytosis and degradation of exogenous Aβ oligomers by microglia. More importantly, the level of Aβ in the brain of 5×FAD mice with SELENOK knockout are significantly increased, which is directly related to the impaired ability of microglia to phagocytosis of Aβ induced by SELENOK knockout. Interestingly, it is found that the effects of SELENOK-mediated CD36 palmitoylation on its localization and expression in the plasma membrane is a potential mechanism of SELENOK's regulation of microglia function and Aβ phagocytosis. Thus, this study clarifies a series of regulatory mechanisms from selenoprotein-dependent Se to Aβ-related pathological processes, which may provide the significant experimental basis for the clinical application of Se compounds in therapy for AD.

S34-212

Near-infrared-responded high sensitivity nanoprobe for steady and visualized detection of albumin in hepatic organoids and mouse liver

Guofeng Liu, Chonghui Li, Congcong Shen, Jihua Wang*, Shicai Xu*,

Shandong Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou, Shandong 253023, China

Tel: 0534-8982081 :Email: jhwang@dzu.edu.cn（王吉华）*; Email:shicaixu@dzu.edu.cn (*许士才*)*

Exploring the advanced techniques for protein detection facilitates cell fate investigation. However, it remains challenging to quantify and visualize the protein with one single probe. Here we reported a luminescent approach to detect hepatic cell fate marker albumin in vitro and living cell labeling with upconversion nanoparticles (UCNPs), which were conjugated with antibody (Ab) and rose bengal hexanoic acid (RBHA). To guarantee the detection quality and accuracy, we adopted an "OFF-ON" strategy: in the presence of albumin, the luminescence of nanoparticles remains suppressed owing to energy transfer to the quencher. Upon albumin binding to the antibody, the luminescence was recovered under near-infrared light. In various bio-samples, the UCNPs-Ab-RBHA (UCAR) nanoprobe could sense albumin with a broad detection range (5-315 ng/mL). When applied to liver ductal organoid culture medium, the UCAR could monitor hepatocyte differentiation in real time by sensing the secreted albumin. Further, UCAR enabled live imaging of cellular albumin in cells, organoids and tissues. In a CCl4-induced liver injury model, UCAR detected reduced albumin in liver tissue and serum. Thus, we provide a biocompatible nanoprobe for both quantification and imaging of protein in complex biological environment with superior stability and high sensitivity.

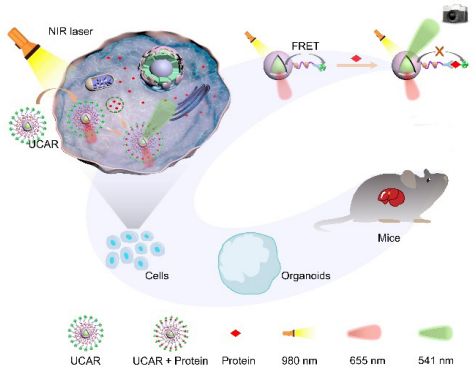


Figure 1. Förster Resonance Energy Transfer (FRET) based UCNPs-Ab-RBHA (UCAR) nanoprobe adopts an "OFF-ON" strategy to detect target protein.

S34-228

Capillary–SERS Label Coupling Fabry–Pérot Cavity with Local Surface Plasmon Resonance for in situ Detection of Molecular Aqueous Solutions

Chonghui Li (李崇辉), Xiaohui Lin (林晓卉), Jihua Wang (王吉华), Guofeng Liu (刘国锋) and Shicai Xu (许士才)

Shandong Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou 253023, China
E-mail: chlisdnu@126.com, XiaohuiLinSCI@163.com, shicaixu@dzu.edu.cn

In situ surface-enhanced Raman scattering (SERS) detection of trace analytes with high sensitivity, especially in complex aqueous solution environments such as polluted water and biological fluids, remains an urgent task. Herein, a capillary–SERS label was developed by folding a plasmonic structure composing Au@AgNPs and CuO nanospikes (NSs). In the the-oretical simulation using COMSOL software, we demonstrated that this structure with a parallel facing state has excellent qualities of Fabry–Pérot (FP) cavity and local surface plasmon resonance (LSPR) coupling. Moreover, it detected the mo-lecular aqueous solution via capillary action in only 5 min. Benefitting from the dense volumetric hotspots and the sim-ple trace extraction solution method, we utilized the proposed capillary–SERS label for ultrasensitive in situ SERS detec-tions of microcystin–LR toxins in drinking water, adenosine biological fluid (10–10 M), and carcinogenic malachite green in seafood using a portable Raman instrument. The findings of this study provide promising insights for achieving in situ SERS detection of trace analytes in complex aqueous environments.

S34-232

Robust emission in near-infrared II of lanthanide nanoprobes conjugated with Au (LNPs-Au) for temperature sensing and controlled photothermal therapy

Guofeng Liu, Zhenxing Wang, Wan Sun, Xiaohui Lin, Rui Wang, Chonghui Li, Li Zong, Zuoling Fu*, Hanping Liu*, Shicai Xu*

Shandong Key Laboratory of Biophysics, Institute of Biophysics, College of Physics and Electronic Information, College of Medicine and Nursing, College of Chemistry and Chemical Engineering, Dezhou University, 253023.

gfliu26@163.com, shicaixu@dzu.edu.cn, 253023

Abstract

To address the poor activity and inaccurate in situ temperature measurements of composite photothermal probes, herein we report lanthanide nanoparticles-gold (LNPs-Au) nanoprobes for fluorescence temperature sensing and controlled photothermal therapy (PTT). The conjugation between LNPs and Au is done via electrostatic interactions. LNPs doped with rare earth cations (Yb^{3+}, Ho^{3+}, Er^{3+}, and Ce^{3+}) fluoresce strongly in the NIR-II region and their emission spectrum does not overlap with the absorption spectrum of Au. The intensities of LNPs-Au emission peaks at 1185 and 1560 nm highly depend on temperature, which is utilized in this study for the non-contact temperature measurements in the biologically relevant range. Deep tissue penetration of NIR-II radiation is observed, which is favorable for real-time temperature sensing. In addition, this radiation does not damage the normal tissue. Moreover, LNPs-Au nanoparticles reduce HeLa cell viability below 40% and adequately inhibited tumor tissue in mice upon 808-nm irradiation. The results of this study will motivate the development of multifunctional photothermal probes for safer, controllable PTT of cancers with simultaneous temperature monitoring.

S34-236

Near-infrared molecular biosensors for detection of molecular events in live cells and in vivo

Minghai Chen*, Xian-En Zhang*

Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

E-mail: mh.chen1@siat.ac.cn, zhangxe@siat.ac.cn

Molecular events such as protein-protein and protein-RNA interactions in living cells play pivotal roles during the process of life. Identifying and visualizing these key biomolecular interactions in live cells and in vivo is of great significance for understanding the underlying mechanisms of the biological processes including the occurrence and development of the diseases. As for the strong green background fluorescence produced in living cells, which will interfere the signal of molecular biosensors based on the green fluorescent proteins. To break the obstacles, we successfully created a series of molecular biosensors with long wavelength based on the near-infrared fluorescent proteins. These near-infrared molecular biosensors were then applied to detect the protein-protein or protein-RNA interactions during the virus infection process or the development of tumor in live cells or in vivo, which provide powerful tools for detection of molecular events with low spontaneous background fluorescence both in living cells and in vivo.