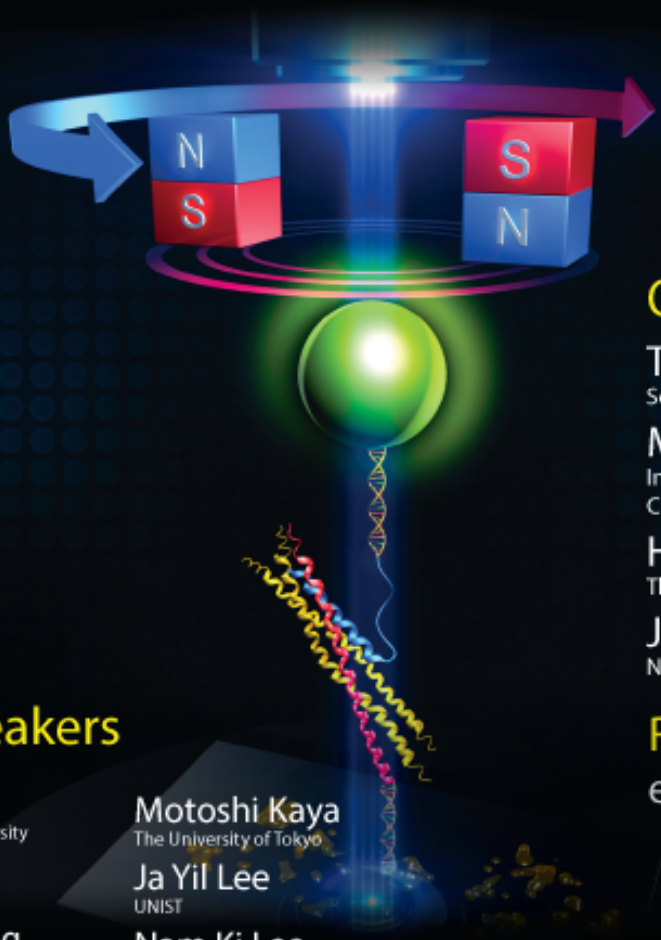
2nd East Asian Symposium on Single-Molecule Biological Sciences

July 25-27, 2019

Seoul National University

Institute of Molecular Biology and Genetics



Organizers

Tae-Young Yoon
Seoul National University

Ming Li
Institute of Physics,
Chinese Academy of Sciences

Hiroyuki Noji
The University of Tokyo

Jie Yan
National University of Singapore

Registration:

eas.snu.ac.kr

Invited Speakers

Gang Chen
Nanyang Technological University

Wei Chen
Zhejiang University

Seok-Cheol Hong
Korea University

Changbong Hyeon
KIAS

Ryota Iino
Institute for Molecular Science

Mitsuhiro Iwaki
RIKEN

Wei Ji
Institute of Biophysics,
Chinese Academy of Sciences

Pakorn Kanchanawong
National University of Singapore

Motoshi Kaya
The University of Tokyo

Ja Yil Lee
UNIST

Nam Ki Lee
Seoul National University

Ying Lu
Institute of Physics,
Chinese Academy of Sciences

Hye Yoon Park
Seoul National University

Fazhan Shi
University of Science and Technology
China

Tsuyoshi Terakawa
Kyoto University

July 25, 2019 (Thursday)

09:00-09:20 am **Opening Remarks**

Session I: New Single-Molecule Techniques

Session Chair: Sungchul Hohng (Seoul National University)

09:20 - 09:50 am Ming Li (Institute of Physics, Chinese Academy of Sciences)
"Nanotensioner-Enhanced FRET for Single-Molecule Structural Dynamics"

09:50 - 10:20 am Mitsuhiro Iwaki (RIKEN)
"DNA origami-based thick filaments for directly visualizing single-molecule dynamics of myosin force generation"

10:20 - 10:50 am Ying Lu (Institute of Physics, Chinese Academy of Sciences)
"High Precision Single Molecular Spectroscopic Rulers at Biointerfaces"

10:50 - 11:00 pm **Coffee break**

11:00 - 11:30 am Tae-Young Yoon (Seoul National University)
"Watching single membrane proteins fold"

11:30 am - 12:00 pm Wei Ji (Institute of Biophysics, Chinese Academy of Sciences)
"Pushing the resolution limit by cryogenic imaging and Repetitive Optical Selective Exposure (ROSE)"

12:00 - 12:30 pm Fazhan Shi (University of Science and Technology China)
"Electron Spin Resonance Spectroscopy of A Single Molecule"

12:30 - 16:00 pm **Lunch and afternoon break**

Short programs (for who are interested):

1. Hiking to the Gwanak Mountain (SNU is located in the Gwanak Mountain); 2. Campus tour

Session II: Observing single-molecules in live cells

Session Chair: Changbong Hyeon (KIAS)

16:00 - 16:30 am Pakorn Kanchanawong (National University of Singapore)
"Visualizing and Controlling Cell Adhesion Complexes at the Molecular Scale"

16:30 - 17:00 am Nam Ki Lee (Seoul National University)
"Single-protein tracking for direct observation of cellular process in a living cell"

17:00 - 17:30 am Hye Yoon Park (Seoul National University)
"Activity-dependent RNA dynamics in live neurons studied at single molecule resolution"

17:30 - 19:00 pm **Poster session**

19:10 – 22:00 pm **Symposium Banquet and beer happy hour**

(RockGu-Jeong, 樂口亭: Walking 10 min from the symposium hall)

July 26, 2018 (Friday)

Session III: **Molecular motors**

Session Chair: Hajin Kim (UNIST)

09:00 - 09:30 am Hiroyuki Noji (The University of Tokyo)
"Science and technology of artificial cell reactor"

09:30 - 10:00 am Ryota Iino (Institute for Molecular Science)
"Watching motor protein dynamics with plasmonic nanoprobes"

10:00 – 10:30 am Changbong Hyeon (KIAS)
"Cost-precision trade-off and transport efficiency of molecular motors"

10:30 – 11:00 am Motoshi Kaya (The University of Tokyo)
"Function of cardiac myosin essential for heart contraction"

11:00 - 11:10 pm **Coffee break**

Session IV: **Mechano biology**

Session Chair: Seok-Cheol Hong (Korea University)

11:10 - 11:40 am Jie Yan (National University of Singapore)
"The stability of mechanosensing force-transmission supramolecular linkages"

11:40 am - 12:10 pm Wei Chen (Zhejiang University)
"Mechano-chemical coupling regulates TCR antigen recognition"

12:10 - 12:40 pm Chenlu Yu(Lumicks)
"Step into the Unresolved: Versatile Tools Towards Real-time Single-molecule Biology"

12:40 pm - **Excursion**

July 27, 2018 (Saturday)

Session V: **Genome maintenance at single-molecule resolution**

Session Chair: Hye Yoon Park (Seoul National University)

09:30 – 10:00 am Tsuyoshi Terakawa (Kyoto University)
"A new type of DNA molecular motor: Condensin"

10:00 – 10:30 am Seok-Cheol Hong (Korea University)
"Z-DNA: Cornucopian topic for single-molecule studies"

10:30 – 11:00 am Gang Chen (Nanyang Technological University)
"Combining single-molecule manipulation and peptide nucleic acid binding studies for unraveling how RNA structures regulate ribosomal frameshifting and alternative splicing"

11:00 – 11:30 am Ja Yil Lee (UNIST)
"Lesion search mechanism of human NER protein"

11:30 – 11:50 am **Poster awards and Concluding remarks**

12:00 pm- **Lunch** (Lunch box)

Speakers:

Nanotensioner-Enhanced FRET for Single-Molecule Structural Dynamics

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Fluorescent energy transfer has become an indispensable technique for studying dynamics of molecular motors and membrane proteins. The lack of angstrom resolution made it difficult to correlate molecular dynamics with structural biology. Here, we report on our recent efforts in taking advantage of bending elasticity of double-stranded DNA to exert tension on the molecules of interest to improve the resolution of FRET to a few angstroms. The method is both simple and efficient, and is expected to find wide applications in smFRET studies of helicases and other molecular motors that interact with nucleic acids. We validated the method by applying it to study DNA unwinding kinetics of helicases. The resolution is high enough to uncover the differences in DNA unwinding by yeast Pif1 and E. coli RecQ whose unwinding behaviors cannot be differentiated by currently practiced methods. The method also enabled us to understand the function of RQC domain that is specific to RecQ-family helicases. When applied to a ring-shaped T7 replicative helicase (gp4), our data revealed that gp4 translocates along DNA in random step sizes different from that deduced from the static structure of gp4-DNA complex. Gp4 steps backward and forward repeatedly when it encounters an abasic lesion, suggesting the existence of a hypothetic intermediate state absent in the currently available gp4-DNA complex structures.

DNA origami-based thick filaments for directly visualizing single-molecule dynamics of myosin force generation

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To elucidate how muscle works, extensive experimental and theoretical works have proposed the swinging lever-arm model [1]. However, the dynamic features of how the myosin head swings the lever-arm, how myosin initially interacts with actin, and how the swings coordinate with each other are not well understood even though they are essential for the force generation, contraction speed, heat production, and response to mechanical perturbations of muscle [2]. This is because myosin heads during force generation have not been directly visualized. Here, we engineered thick filaments comprising DNA origami and human muscle myosin and are optimized for nanometer-precision single-molecule imaging to directly visualize the heads during force generation. We found that when a head diffuses, it weakly interacts with actin and then strongly binds preferentially to the forward region as a Brownian ratchet. Upon strong binding, the head cooperatively swings its lever-arm in a two-step manner and occasionally reverses direction. These results can explain the mechanical characteristics of muscle contraction and suggest that our DNA origami-based assay system can be used to dissect the mechanistic details of molecular motor assembly.

Reference

[1] Spudich, J. A. The myosin swinging cross-bridge model. *Nature Reviews Molecular Cell Biology* (2001) 2, 387-392.
[2] Huxley, A. F. Muscular contraction. *Journal of Physiology* (1974) 243, 1-43.

High Precision Single Molecular Spectroscopic Rulers at Biointerfaces

Ying Lu, Chun-Hua Xu, Shu-Xin Hu, and Ming Li

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The dynamic behaviors of a single biomacromolecule at the biointerface is of great importance for understanding the complexly condensed and cooperative phenomena in the life processes. Accurate measurement of the conformational changes and motions of single biomacromolecules, as well as the resulted summarization of laws and revelation of mechanisms, are the common key scientific questions taking into account both the frontiers of biological foundation and the expansion of physics connotation. By developing and exploiting various precise measurement methods based on single-molecule fluorescence resonance energy transfer technology and actively exploring typical application modes in important life research systems, the applicant has conducted the cross and integration of condensed matter physics, optics and biophysics: (1) we developed the point-to-point DNA nanotensioner method, which has measured the dynamics of molecular motors with a spatial resolution ~2 Å and effectively analyzed the step-by-step kinetics and molecular mechanism of protein-DNA interaction at the nucleic acid interface; (2) we invented the point-to-surface and point-to-liposome single-molecule fluorescence decay methods, named as SIFA and LipoFRET, respectively, which can be used in the real-time detection of membrane protein's position and movement at the membrane interface with sub-nanometer accuracy. We preliminarily build a set of high-precision measurement methods for studying the complex spatiotemporal dynamics of biological macromolecules at biointerfaces.

Watching single membrane proteins fold

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Membrane proteins are designed to fold and function in a lipid membrane, yet folding experiments within a native membrane environment are challenging to design. Here we show that single-molecule forced unfolding experiments can be adapted to study helical membrane protein folding under native-like bicelle conditions [1,2]. After inducing mechanical unraveling of target membrane protein with high mechanical tension, we relaxed the membrane protein to restore its secondary structures as well as to ensure its membrane insertion. Under a low level of mechanical tension, we observed the single membrane protein completed its folding process while exhibiting defined intermediate states. Application of this experimental method revealed the folding pathways in unprecedented details for diverse membrane proteins that ranged from GlpG, an *e. Coli* membrane protease, to human β-adrenergic receptor, indicating general applicability of the developed method. The identified folding pathways share two important features: (1) N-to-C unidirectional folding and (2) high one dimensionality in the folding energy landscape [2]. These features suggest that the folding pathways of integral membrane proteins are evolutionarily tailored to increase the fitness with co-translational folding.

Reference

[1] D. Min, R. E. Jefferson, J. U. Bowie, T.-Y. Yoon. Mapping the energy landscape for second-stage folding of a single membrane protein. *Nature Chemical Biology* (2015) 11, 981.
[2] H.-K. Choi, D. Min, H. Kang, M. J. Shon, S.-H. Rah, H. Jeong, H.-J. Choi, J. U. Bowie, T.-Y. Yoon. Watching single membrane proteins fold. *Manuscript under revision*.

Pushing the resolution limit by cryogenic imaging and Repetitive Optical Selective Exposure (ROSE)

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Remarkable progress in Single molecule localization microscopy (SMLM) has been made in the past decade. The localization precision of SMLM is mainly dependent on the number of detected photons, therefore tremendous efforts have been invested to increase photon budget by specially designed fluorophores and anti-bleaching agents. Here we developed cryogenic imaging and Repetitive Optical Selective Exposure (ROSE) which exhibit excellent localization precision performances compared to conventional SMLMs. We built an ultra-stable super-resolution cryo-FM that exhibits excellent thermal and mechanically stability. The temperature fluctuations in 10 hours are less than 0.06 K, and the mechanical drift over 5 hours is less than 200 nm in three dimensions. We have demonstrated the super-resolution imaging capability of this system. The results suggest that our system is particularly suitable for long-term observations, such as single molecule localization microscopy (SMLM) and cryogenic super-resolution correlative light and electron microscopy (csCLEM) [1,2]. We also introduced an interferometric SMLM. A fluorescence molecule is located by the intensities of multiple excitation patterns of an interference fringe, providing around two-fold improvement in the localization precision compared to the conventional centroid fitting method at the same photon budget. We demonstrate this technique by resolving a nanostructure down to 5 nm.

Reference

[1] Liu, B., Xue, Y., Zhao, W., Chen, Y., Fan, C., Gu, L., Zhang, Y., Zhang, X., Sun, L., Huang, X., Ding, W., Sun, F., Ji, W., and Xu, T. Three-dimensional super-resolution protein localization correlated with vitrified cellular context. *Sci Rep* 513017, 13017 (2015).

[2] Xu, X., Xue, Y., Tian, B., Gu, L., Li, W., Ji, W., Xu, T.. Ultra-stable super-resolution fluorescence cryo-microscopy for correlative light and electron cryo-microscopy. *Science China Life Sciences*. doi: 10.1007/s11427-018-9380-3 (2018).

[3] Gu, L., Li, Y., Zhang, S., Xue, Y., Li, W., Li, D., Xu, T., Ji, W. Molecular resolution imaging by repetitive optical selective exposure (ROSE). *Manuscript under revision.*

Electron Spin Resonance Spectroscopy of A Single Molecule

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Magnetic resonance (MR) is one of the most important techniques for characterizing compositions, structure and dynamics of molecules. Over the past several years, quantum sensing with Nitrogen-Vacancy (NV) center has opened a new door for magnetic resonance spectroscopy of a single molecule. In my talk, I will mainly introduce several new experimental results on both of methods and biology applications. (I) Zero-field electron spin resonance (ESR) spectroscopy on nanoscale. We successfully measured the zero-field ESR spectrum of a few electron spins, by precisely tune the energy levels of NV centers to be resonant with the target spins, and directly resolved the hyperfine coupling constant. This work break the sensitivity limitation and open the door of practical applications of the zero-field ESR. (II) ESR spectroscopy of single molecules under physiological conditions. The work represents a step forward towards magnetic resonance investigation of biomolecules in their native environments at the single-molecule level.

Reference

[1] Fazhan Shi, Fei Kong, Pengju Zhao, Xiaojun Zhang, Ming Chen, Sanyou Chen, Qi Zhang, Mengqi Wang, Xiangyu Ye, Zhecheng Wang, Zhuoyang Qin, Xing Rong, Jihu Su, Pengfei Wang, Peter Z. Qin, and Jiangfeng Du. Single-DNA electron spin resonance spectroscopy in aqueous solutions, Nature Methods (2018) 15, 697

[2] Fei Kong, Pengju Zhao, Xiangyu Ye, Zhecheng Wang, Zhuoyang Qin, Pei Yu, Jihu Su, Fazhan Shi, and Jiangfeng Du. Zero-field electron spin resonance spectroscopy on na-noscale, *Nature Communications* (2018) 9, 1563

[3] Fazhan Shi, Qi Zhang, Pengfei Wang, Hongbin Sun, Jiarong Wang, Xing Rong, Ming Chen, Chenyong Ju, Friedemann Reinhard, Hongwei Chen, Joerg Wrachtrup, Junfeng Wang, and Jiangfeng Du. Single-protein spin resonance spectroscopy under ambient conditions, *Science* (2015) 347, 1135

Visualizing and Controlling Cell Adhesion Complexes at the Molecular Scale

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Many complex biological functions are performed by supramolecular assemblies self-organized from a diverse ensemble of proteins. Cell adhesion structures such as the integrin-based focal adhesions and cadherin-based cell-cell junctions are multi-protein complexes known to transmit, sustain, sense, and respond to mechanical force. The knowledge of their physical organization is therefore essential for insights into their mechanobiological functions. Due to the nanometer size scale of the adhesion protein building blocks, the nanoscale is the functionally salient length scale for the spatial organization of these molecular complexes. In this talk, I will discuss our recent studies that sought to 1) decipher the nanoscale architecture of integrin-mediated and cadherin-mediated cell adhesion complexes, using interference-based super-resolution microscopy techniques[1, 2]; 2) control force transmission through specific cell adhesion molecules by chemical biology and optogenetic approaches.

References

[1] Liu, J., et al., *Talin determines the nanoscale architecture of focal adhesions.* Proceedings of the National Academy of Sciences, 2015. **112**(35): p. E4864-E4873.
[2] Bertocchi, C., et al., *Nanoscale architecture of cadherin-based cell adhesions.* Nat Cell Biol, 2017. **19**(1): p. 28-37.

Single-protein tracking for direct observation of cellular process in a living cell

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Recent advances in single-molecule fluorescence microscope techniques have allowed single-molecule sensitivity to probe various protein-DNA interactions, their structural changes, and fundamental cellular processes in a living cell [1-3]. Transcription, a process of mRNA generation by RNA polymerase (RNAP), is highly coupled with translation by ribosome in bacteria. The effect of the transcription-translation coupling on the transcriptional dynamics and the localization of genes in a living cell is poorly understood [3]. Here, we directly observe the dynamics of transcription and the movement of the subcellular localization of genes actively transcribed by RNAP in living cells at the sub-diffraction limit resolution. The subcellular localizations of the non-membrane protein' genes, actively transcribed by RNAPs, move toward outside nucleoid or to plasma membrane by the effect of translation by ribosome. The movement of genes by transcription-translation coupling is general for both E. coli RNAP and T7 RNAP [4]. Our observation demonstrates how two spatially separated processes of transcription and translation are coupled in bacteria and the movement of genes by the cooperation between transcription and translation plays a crucial role in the effective expression of genes in E. coli.

Reference

[1] D.-H. Kim, S. Park, D.-K. Kim, M. G. Jeong, J. Noh, Y. Kwon, K. Zhou, N. K. Lee, S. H. Ryu,PLoS Biol. 16, 12 (2018)
[2] Kim, D.-H.; Kim, D.-K.; Zhou, K.; Lee, N. K.; Ryu, S. H., Chemical Sciences, 8, 4823, 2017
[3] Yang, S; Kim, S. H.; Lim, Y. R.; Sung, J.; Lee, N. K., Nature communications 5, 4761, 2014
[4] Yang et al., Under revision (2019).

Activity-dependent RNA dynamics in live neurons studied at single molecule resolution

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The dynamics of RNA - the synthesis, transport, and degradation - plays significant roles in a variety of neuronal processes. Abnormal mRNA processing and transport are implicated in neurological disorders such as autism and Alzheimer's disease. However, understanding the mechanistic roles of RNA dynamics has been hampered by the lack of techniques to observe the endogenous molecules in the native tissue environment. Here I will describe a systems approach, combining single-particle tracking, genetic engineering, and intravital microscopy. Recently we have developed a new mouse model to fluorescently label endogenous *Arc* mRNA [1]. The immediate early gene *Arc* (also known as *Arg3.1*) is highly involved in the formation of long-term memory. Expression of *Arc* is tightly coupled to the activity of the neuron; *Arc* mRNA is rapidly produced in response to neural activity and transported to distant dendrites. Based on our previous work to visualize single endogenous β-actin mRNA [2], we generated Arc-PBS mouse by knocking in 24 tandem arrays of PP7 binding site (PBS) in the 3' untranslated region (3' UTR) of the *Arc* gene. Using this mouse model, we are studying activity-dependent transcription of *Arc* in live hippocampal neurons. By simultaneously imaging relative Ca^{2+} concentrations and *Arc* mRNA transcription, we are investigating the relationship between the activity of neurons and gene expression in a single cell level with single RNA resolution. The next step of our study is to image *Arc* mRNA in a more physiological condition such as in brain slices, or even in the brain of live mice using a two-photon microscope. Ultimately, our research will allow us to link behavior and gene expression in live animals in real time.

Reference

[1] S. Das, H. C. Moon, R. H. Singer, and H. Y. Park. A transgenic mouse for imaging activity-dependent dynamics of endogenous Arc mRNA in live neurons. *Science Advances* (2018) 4, eaar3448.
[2] H. Y. Park, H. Lim, Y. J. Yoon, A. Follenzi, C. Nwokafor, M. Lopez-Jones, X. Meng, and R. H. Singer. Visualization of dynamics of single endogenous mRNA labeled in live mouse. *Science* (2014) 343, 422.

Science and technology of artificial cell reactor

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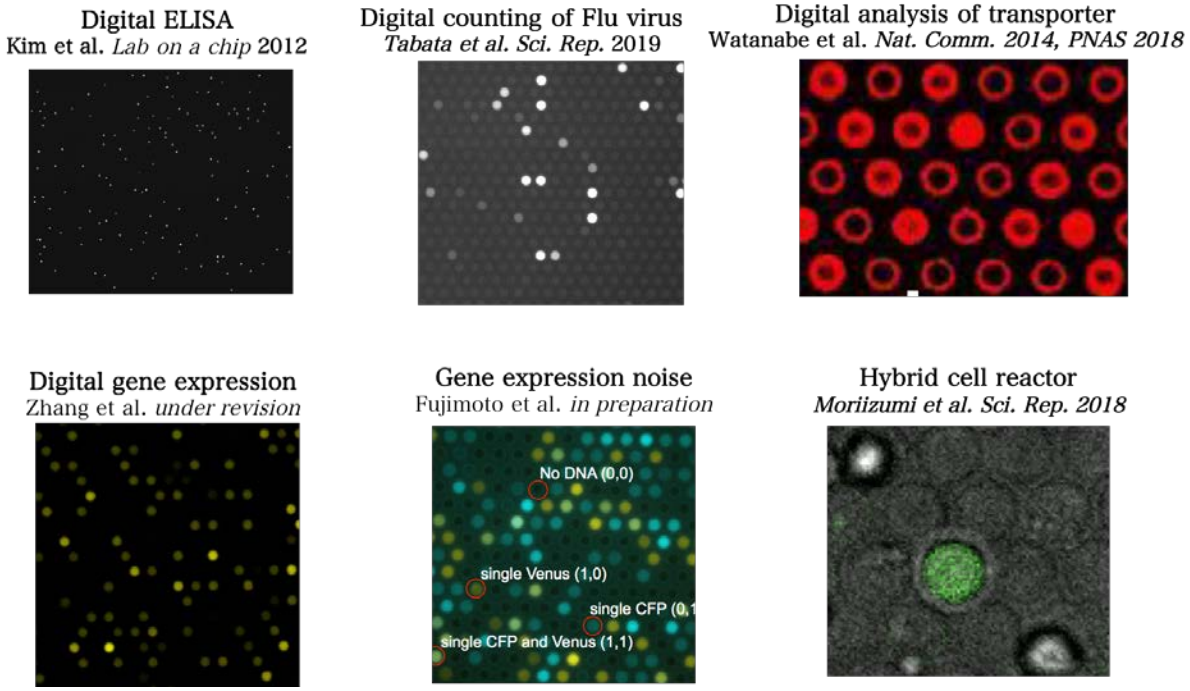
We developed femto-liter reactor array device (FRAD) that display over million water-in-oil droplets with size of femto-liter range. One can readily encapsulate bio- or non-biological molecules into the FRAD reactors, by spreading assay mixture on the device and sealing the reactors with oil. The extremely small volume features of the reactors on FRAD allows very sensitive bioassay at single-molelule referred as to 'digital bioassay'. The massive number of the reactor enables to select highly active but very rare functional molecules among a large numbers of library. The high biocompatibility of FRAD reactors also allows the reconstitution of molecular systems such as cell-free gene expression and replication. In this presentation, I will firstly introduce the concept of FRAD in the context of synthetic biology, and show some applications including enzyme screening for the engineering of alkaline phosphatase to enhance kcat. The last part of the talk includes the implementation of large DNA amplification system and/or cell-free gene expression system in FRAD with the attempt for cell-free DNA assembly and/or cell-free gene cloning systems.



Watching motor protein dynamics with plasmonic nanoprobes

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To understand operation and design principles of protein molecular motors [1], we have been developing high-precison and high-speed single-molecule imaging methods visualizing fast motions of protein molecular motors with angstrom localization precision and microsecond temporal resolution [2]. Our methods are based on dark-field scattering imaging of gold nanoparticles [3] and nanorods [4]. As probes of single-molecule imaging, the gold nanoparticles and nanorods have advantages over the commonly used ones such as fluorescent dyes and quantum dots, because much stronger signals can be obtained without suffering from photobleaching and blinking. In my talk, I will discuss chemo-mechanical coupling mechanisms and coordination mechanisms of linear molecular motors kinesin-1 [5], dynein, and processive chitinase [6], and a rotary molecular motor V_1-ATPase [7-9], revealed by our single-molecule methods. I will also introduce our recent approaches to achieve multi-color single-molecule imaging with plasmonic nanoparticles.

Reference

[1] R. Iino et al., *Biochim Biophys Acta* (2018) 1862, 241-252
[2] J. Ando et al., *Biophys. J.* (2018) 115, 2413-2427
[3] H. Ueno et al., *Biophys. J.* (2010) 98, 2014-2023
[4] S. Enoki et al., *Anal. Chem.* (2015) 87, 2079-2086
[5] H. Isojima and R. Iino et al., *Nat. Chem. Biol.* (2016) 12, 290-297
[6] A. Nakamura et al., *Nat. Commun.* (2018) 9, 3814
[7] Y. Minagawa and H. Ueno et al., *J. Biol. Chem.* (2013) 288, 32700-32707
[8] H. Ueno and Y. Minagawa et al., *J. Biol. Chem.* (2014) 289, 31212-31223
[9] T. Iida et al., *J. Biol. Chem.* (2019) Submitted.

Cost-precision trade-off and transport efficiency of molecular motors

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An efficient molecular motor would deliver cargo to the target site at a high speed and in a punctual manner while consuming a minimal amount of energy. However, according to a recently formulated thermodynamic principle, known as the thermodynamic uncertainty relation, the travel distance of a motor and its variance are constrained by the free energy being consumed. Here we use the principle underlying the uncertainty relation to quantify the transport efficiency of molecular motors for varying ATP concentration ([ATP]) and applied load (*f*). Our analyses of experimental data find that transport efficiencies of the motors studied here are semi-optimized under the cellular condition. The efficiency is significantly deteriorated for a kinesin-1 mutant that has a longer neck-linker, which underscores the importance of molecular structure. It is remarkable to recognize that, among many possible directions for optimization, biological motors have evolved to optimize the transport efficiency in particular.

Reference

[1] "Energetic Costs, Precision, and Transport Efficiency of Molecular Motors" W. Hwang, C. Hyeon, *J. Phys. Chem. Lett.* (2018) 9, 513-520

[2] "Physical insight into the thermodynamic uncertainty relation using Brownian motion in tilted periodic potentials" C. Hyeon, W. Hwang, *Phys. Rev. E.* (2017) 96, 012156

[3] "Quantifying the Heat Dissipation from a Molecular Motor's Transport Properties in Nonequilibrium Steady States" W. Hwang, C. Hyeon *J. Phys. Chem. Lett.* (2017) 8, 250-256

Function of cardiac myosin essential for heart contraction

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In order to elucidate the molecular mechanism of how dynamics of cardiac myosins contribute to heart function, we measured forces of synthetic β-cardiac myosin filaments using optical tweezers and revealed stepwise displacements of actin filaments driven by myosins under a wide range of loads. The stepping ratio, which is the ratio of the numbers of forward steps relative to backward steps, under unloaded conditions decreased with increasing ATP concentrations. Compared with skeletal myosin [1], the stepping ratio of cardiac myosin is much lower than that of skeletal myosin, indicating cardiac myosin shows frequent backward steps. Meanwhile, the peak forces generated by cardiac myofilaments with ~15 interacting molecules were 1.5-2 times higher than those observed in skeletal myofilaments with nearly the same number of interacting molecules. Based on these findings, we developed a simulation model to understand which molecular properties critically affect on stepping behaviors and force outputs in cardiac myofilaments. The simulation suggested that reverse stroke in ADP states is a key feature to cause frequent backward steps at higher ATP concentrations, resulting lower stepping ratio. Moreover, switching between two ADP states associated with the alternate execution of power and reverse strokes keeps many myosins populated in force-generating states, enhancing the duty ratio and force outputs. Therefore, we further investigated whether single cardiac myosin can execute the power and reverse strokes in ADP state under a variety of loading conditions. When single cardiac myosins interacting with single actin filaments were stretched by optical tweezers, beads' positions were occasionally switched between two discrete levels for high loads, implying the load-dependent execution of power and reverse strokes. To know physiological meaning of reverse stroke, we simulated dynamics of myosins in sarcomere and found that the reverse stroke plays a crucial role in reducing the rate of ATP consumption during isometric contraction. Also, we implemented such molecular properties into a whole heart simulator and found that the reverse stroke is a unique future of cardiac myosin and essential for maintaining high systolic blood pressure and a rapid relaxation of diastolic blood pressure..

Reference

[1] M. Kaya, Y. Tani, T. Hisada, T. Wahio, H. Higuchi. Coordinated force generation of skeletal myosins in myofilaments through motor coupling. *Nature Communications* (2017) Jul 6;8:16036.

The stability of mechanosensing force-transmission supramolecular linkages

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The task of mechanosensing of cells involves dynamic assembly of various supramolecular force-transmission linkages, which allow the cells to properly sense and respond to the level of mechanical force in the linkages. While sufficient mechanical stability is a necessity for the mechanosensing function of the force-transmission linkages, the mechanical stability for most crucial force-transmission linkages remains poorly understood. As a force-transmission linkage typically consists of a few non-covalently linked proteins, we reason that the stability of the force-bearing interfaces between neighboring proteins in a force-transmission linkage is the most critical determinant of the linkage mechanical stability. In this talk, I will introduce our unpublished works on using a novel single-molecule detector to determine the mechanical stability of force-bearing inter-molecular interfaces, and its applications to the investigation of several inter-molecular interfaces that play crucial mechanosensing functions at cell-matrix and cell-cell adhesion sites [1,2,3,4]. In addition, I will also introduce a new theory to understand how the structural-elastic properties of biomolecular complexes determine their mechanical stability over a physiological force range [5,6].

Reference

[1] S. Le, M. Yu, J. Yan. Direct single-molecule quantification reveals surprisingly high mechanical stability of vinculin-talin/α-catenin linkages. *Manuscript under revision.*

[2] S. Le, M. Yu, J. Yan. Single-residue phosphorylation greatly impairs the highly mechanical-stable β-catenin/α-catenin complex revealed by direct single-molecule quantification. *Manuscript under revision*.

[3] Y. Wang, S. Le, Z. Guo, P. Kanchanawong, J. Yan. Label-free single-molecule quantification of rapamycin induced FKBP-FRB dimerization. *Manuscript under revision*.

[4] M. Yu, S. Le, S. Barnett, Z. Guo, X., P. Kanchanawong, J. Yan. Implementing Optogenetic Modulation in Mechanotransduction. *Manuscript submitted*.

[5] S. Guo, A.K. Efremov, J. Yan. Understanding the catch-bond kinetics of biomolecules on a one-dimensional energy landscape. Communications Chemistry, 2019, 2: 30.

[6] S. Guo, Q. Tang, M. Yao, H. You, S. Le, H. Chen, J. Yan. Structural-elastic determination of the force-dependent transition rate of biomolecules. Chemical Science, 2018, 9: 5871-5882.

Mechano-chemical coupling regulates TCR antigen recognition

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TCRs recognize cognate pMHCs to initiate T cell signaling and adaptive immunity. Mechanical force strengthens TCR-pMHC interactions to elicit agonist specific catch bonds to trigger TCR signaling, but the underlying dynamic structural mechanism is unclear. We combined steered molecular dynamics (SMD) simulation, single-molecule biophysical approaches, and *in vitro* and *in vivo* functional assays to collectively demonstrate that mechanical force induces conformational changes in pMHC-Is to enhance pre-existing contacts and activates new interactions at the TCR-pMHC binding interface to resist bond dissociation under force, resulting in TCR-pMHC catch bonds and T cell activation. Intriguingly, cancer-associated somatic mutations in HLA-A2 that may restrict these conformational changes suppressed TCR-pMHC catch bonds. Our findings not only reveal critical roles of force-induced conformational changes in pMHC-Is for activating TCR-pMHC catch bonds but also have implications for T cell-based immunotherapy.

Step into the Unresolved: Versatile Tools Towards Real-time Single-molecule Biology

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Biological processes performed by proteins interacting with and processing DNA and RNA are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry.

The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models.

Single-molecule technologies offer an exciting opportunity to meet these challenges and to study protein function and activity in real-time and at the single-molecule level.

Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using both the combination of optical tweezers with single-molecule fluorescence microscopy (C-Trap).

We show the latest applications of these technologies that can enhance our understanding not only in the field of DNA/RNA-protein interactions but also in the fields of molecular motors, protein folding/unfolding, cell membranes and genome structure and organization.

These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in many research areas

A new type of DNA molecular motor: Condensin

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Condensin plays crucial roles in chromosome organization and compaction [1], but the mechanistic basis for its functions remains obscure. Here, we used single-molecule imaging to demonstrate that *Saccaromyces cerevisiae* condensin is a molecular motor capable of ATP hydrolysis-dependent translocation along double-stranded DNA [2]. Condensin's translocation activity is rapid and highly processive, with individual complexes traveling an average distance of > 10 kilobases at a velocity of ~60 base pairs per second. Our results suggest that condensin takes steps comparable in length to its ~50-nanometer coiled-coil subunits. The finding that condensin is a mechanochemical molecular motor has important implications for understanding the mechanisms of chromosome organization and condensation.

Reference

[1] T. Hirano. Condensins: universal organizers of chromosomes with diverse functions. *Genes & Development* (2012) 26, 1659.
[2] T. Terakawa*, S. Bisht*, J. M. Eeftens*, C. Dekker, C. H. Hearing, E. C. Greene. *Science* (2017) 353, 672. (* These authors contributed equally to this work)

Z-DNA: Cornucopian topic for single-molecule studies

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DNA is the central molecule of life and heredity. Since the structure of DNA was first determined in 1953, the field of biology has been revolutionized and biology became a molecular science. The very structure discovered by Watson and Crick is not the only structure possible for nucleic acids. Many non-canonical structures are found to exist. One striking example is left-handed Z-DNA. Although it's *in vivo* existence and role are not fully established yet, Z-DNA attracts much attention from structural biologists, chemists, and biophysicists for its exotic structure, unusual properties, and potential applications. Here, I would like to talk about our research on Z-DNA. We have not only revealed the critical role of torque in the B-Z transition [1-3], but also unveiled the reaction pathway in a protein-driven B-Z transition [4]. Recently, we successfully demonstrated that the B-Z transition can be used to detect the degree of methylation of DNA [5], suggesting that Z-DNA like other non-B-DNAs can be used as a biosensor. Taken together, non-B structures are interesting subjects to study from a biophysical point of view and promise great potential as building blocks for nano devices and sensors.

Reference

[1] M. Lee, S. H. Kim, S.-C. H., PNAS, 107, 2985 (2010).
[2] A.-Y. Kwon, G.-M. Nam, A. Johner, S. Kim, S.-C. Hong, N.-K. Lee, PRE, 93, 022411 (2016).
[3] S. H. Kim, …, S.-C. Hong, in preparation.
[4] S. H. Kim, S.-H. Lim, A.-R. Lee, D. H. Kwon, H. K. Song, J.-H. Lee, M. Cho, A. Johner, N.-K. Lee, S.-C. Hong, NAR, 46, 4129 (2018).
[5] S. H. Kim, …, S.-C. Hong, in preparation.

Combining single-molecule manipulation and peptide nucleic acid binding studies for unraveling how RNA structures regulate ribosomal frameshifting and alternative splicing

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RNA structures are involved in regulating many biological activities such as programmed ribosomal frameshifting and pre-mRNA alternative splicing. mRNA structures stimulate minus-one ribosomal frameshifting resulting in the production of multiple proteins from one mRNA. Pre-mRNA structures at the splice sites are involved in the regulation of alternative splicing resulting in the production of protein isoforms through the inclusion or exclusion of exons. Studying the RNA mechanical stabilities, unfolding and folding dynamics, as well as interactions with ligands such as peptide nuclei acids can help facilitate a deep understanding of RNA functions. We combined single-molecule manipulation using optical tweezers and peptide nucleic acids binding studies to unravel the biophysical properties of model RNA hairpin and pseudoknot structures, and their correlations with minus-one ribosomal frameshifting [1-4] and pre-mRNA alternative splicing [5, and unpublished data] activities, respectively.

Reference

[1] Z. Zhong, L. Yang, H. Zhang, J. Shi, J. Vandana, D.T.U.H. Lam, R.C.L. Olsthoorn, L. Lu, and G. Chen, Mechanical unfolding kinetics of the SRV-1 *gag-pro* mRNA pseudoknot: possible implications for −1 ribosomal frameshifting stimulation, *Sci. Rep.* (2016) 6, 39549.

[2] L. Yang, Z. Zhong, C. Tong, H. Jia, Y. Liu, and G. Chen, Single-molecule mechanical folding and unfolding of RNA hairpins: Effects of single A-U to A·C pair substitutions and single proton binding and implications for mRNA structure-induced −1 ribosomal frameshifting. *J. Am. Chem. Soc.* (2018) 140, 8172-8184.

[3] R.Y. Puah, H. Jia, M. Maraswami, D.F.K. Toh, R. Ero, L. Yang, K.M. Patil, A.A.L. Ong, M.S. Krishna, R. Sun, C. Tong, M. Huang, X. Chen, T.P. Loh, Y.G. Gao, D.X. Liu, and G. Chen, Selective binding to mRNA duplex regions by chemically modified peptide nucleic acids stimulates ribosomal frameshift. *Biochemistry* (2018) 57, 149-159.

[4] L. Yang, M.S. Krishna, D.F.K. Toh, Z. Zhong, and G. Chen, Base triple formation between stem 1 and loop 2 in SRV-1 pseudoknot inhibits antisense PNA invasion of stem 1 and stem 2. In preparation

[5] J. Tan, L. Yang, A.A.L. Ong, J. Shi, Z. Zhong, M.L. Lye, S. Liu, J. Lisowiec-Wachnicka, R. Kierzek, X. Roca, G. Chen, A disease-causing intronic point mutation C19G alters tau exon 10 splicing via RNA secondary structure rearrangement. *Biochemistry*, (2019) 58, 1565-1578.

Lesion search mechanism of human NER protein

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DNA damage repair is critical for the genomic stability and integrity. In particular, searching for DNA lesions is important because it initiates the entire repair process. In nucleotide excision repair (NER) that is a conserved and versatile repair mechanism, Xeroderma pigmentosum complementation group C protein (XPC) finds DNA lesions and recruits downstream factors. structural studies revealed the molecular feature of damage identification by XPC, and single-molecule approach reported the diffusion of XPC on DNA. However, how XPC can recognize the defects on DNA while it diffuses along DNA and what factors influence the diffusive motions of XPC still remain elusive. To reveal the detailed mechanism behind damage search of XPC, we visualized the motion of human XPC-Rad23B (hXPC-Rad23B) on undamaged or lesion-containing DNA using a high-throughput single-molecule imaging technique, DNA curtain. We observed the heterogeneity in motions of hXPC-Rad23B, exhibiting diffusive, constrained, and immobile species. We found that the heterogeneity results from the interaction between hXPC-Rad23B and DNA breathing on consecutive AT-tracks. In diffusive motion, the diffusion coefficient dramatically increases according to ionic strength, suggesting that hXPC-Rad23B diffuse along DNA via hopping, which was further supported by our finding that hXPC-Rad23B can bypass protein obstacles upon collision. Furthermore, we found that hXPC-Rad23B recognized cyclobutane pyrimidine dimers (CPDs) with low efficiency, proposing that another factor is necessitated. Taken together, our results give an insight into how hXPC-Rad23B can rapidly finds DNA lesions in billions of base pairs of human genome.

Poster session

P-1 **Dongwook Kim (Seoul National University)**
"Single mRNA imaging with CRISPR-Cas13"

P-2 **Chanwoo Kim (UNIST)**
"Single Molecular Study of Looping and Flexibility of Bacterial Genes"

P-3 **Changwon Kim (Seoul National University)**
"Subunit Study on N-ethylmaleimide sensitive factor (NSF) using Single-Molecule Technics"

P-4 **Haesoo Kim (Seoul National University)**
"Focused clamping of a single neuronal SNARE complex by complexin under high mechanical tension"

P-5 **Sook Ho Kim (Korea University)**
"Nuclease-free detection of DNA methylation via the B-Z transition."

P-6 **Hyeon-Min Moon (Korea University)**
"Cisplatin fastens nucleosomal DNA under physiological salt conditions"

P-7 **Hyungseok Moon (Seoul National University)**
"Formation of Arc mRNA granules in P-bodies"

P-8 **Soojin Park (Seoul National University)**
"Mechanism of Tightly-bound SSB Displacement by RecO Without Consuming ATPs Revealed by Single-molecule Studies"

P-9 **Keewon Sung (Seoul National University)**
"A regulatory mechanism of CRISPR-Cas9 nuclease specificity revealed from single-molecule structural dynamics"

P-10 **Jae Youn Shim (Seoul National University)**
"The role of beta-actin mRNA localization in single dendritic spines studied by two-photon uncaging"

P-11 **Sanghun Yeou (POSTECH)**
"Unexpected bending property of short dsDNA appeared in D-shaped DNA nanostructure"

P-12 **Gee Sung Eun (Seoul National University)**
"PD-1 and PD-L1 interaction observation at the single-molecule scale"

P-13 **Byung Hun Lee (Seoul National University)**
"Imaging activity-dependent transcription of endogenous Arc mRNA in live mouse brain"

P-14 **Il-Buem Lee (Korea University)**
"Z sectioning of intracellular organelles with remote-focusing-enabled iSCAT microscopy"

P-15 **MinKwon Cha (KAIST)**
"Venetoclax companion diagnostic based on single molecule imaging of protein interaction"

P-16 **Byoungsan Choi (Seoul National University)**
"Single-molecule functional anatomy of endogenous dimers of human receptor tyrosine kinases"

P-17 **Hyunkyu Choi (Seoul National University)**
"Watching single membrane proteins fold"

P-18 **Hong-Young Choi (Seoul National University)**
"Modeling Transcription Dynamics of Arc mRNA in Neurons"

Single mRNA imaging with CRISPR-Cas13

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Single-molecule imaging has been a useful technique to investigate the regulation of mRNA inside a cell. However, most of the current techniques for single mRNA imaging require either fixation of the cell or insertion of the RNA tagging system. In order to visualize endogenous mRNAs without introducing any tagging sequences, we are developing a new RNA imaging method based on clustered regularly interspaced short palindromic repeats (CRISPR)-Cas13 system. CRISPR-Cas13 systems have recently emerged as a platform for RNA knockdown and editing. Using catalytically inactive variants of Cas13 (dCas13) fused to msfGFP, we are pursuing single-molecule imaging of endogenous mRNAs in living mammalian cells. As the first demonstration, we transfected dCas13-msfGFP and the guide RNA targeting the MS2 binding site (MBS) into the mouse embryonic fibroblasts (MEFs) bearing 24 repeats of MBS in β-actin mRNA. Also, we tried to develop multiple gRNA expression methods to image U2OS β-actin mRNA. By using these methods, we expect to visualize the location and dynamics of endogenous mRNA inside a living cell with minimal perturbation.

Reference

[1] S. Konermann *et al*., Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors, *Cell* **173**, 665–676, (2018).
[2] DA. Nelles *et al*., Programmable RNA Tracking in Live Cells with CRISPR/Cas9, *Cell* **165**, 488–496, (2016).
[3] HY. Park *et al*., Visualization of dynamics of single endogenous mRNA labeled in live mouse, *Science* **343**, 422-424, (2014).
[4] Yan *et al*., Cas13d Is a Compact RNA-Targeting Type VI CRISPR Effector Positively Modulated by a WYL-Domain-Containing Accessory Protein, *Molecular Cell* **70**, 327–339, (2018).

Single Molecular Study of Looping and Flexibility of Bacterial Genes

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Recent studies on the fundamental physical properties of DNA suggest that local nucleotide sequence and chemical modifications critically influence the physical properties, possibly controlling looping and compaction behavior of DNA. Such control of DNA geometry and dynamics at the fundamental level may relate to the mechanism of spatio-temporal organization of DNAs and chromatin at larger scale. Here, we employ single molecule fluorescence resonance energy transfer (smFRET) technique, atomic force microscopy (AFM), and molecular dynamics (MD) simulations to discover the factors determining the conformational dynamics of DNA molecules, taking Escherichia coli genes as examples. It is known that DNA wraps around histone octamer without forming kinds, which implies that the intrinsic bending property of DNA plays a crucial role in controlling nucleosome formation and hence chromatin compaction. Moreover, nucleosome-forming DNA sequence was found to show a pattern of sequence motifs, implying that the bending property strongly depends on the sequence.

Subunit Study on N-ethylmaleimide sensitive factor (NSF) using Single-Molecule Technics

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N-ethylmaleimide sensitive factor (NSF) disassembles the SNARE complex for recycling after synaptic vesicle fusion. NSF is a member of the AAA+ ATPase family and evolutionarily highly conserved among eukaryotes due to its indispensable role in cell. Six NSF monomers form an asymmetric split-ring structure, making a highly efficient disassembly machinery. The hexameric NSF requires only a single round of ATP hydrolysis to disrupt the SNARE complex. How NSF harnesses energy from ATP hydrolysis and converts it to the mechanical work for disassembly remain largely unexplored. Here, we develop a combined method of single-molecule measurement and mutagenesis to study the molecular mechanism of NSF-mediated SNARE disassembly at the subunit level. Our assay is capable of identifying the number of functionally deficient subunits within a single NSF hexamer assembled around a SNARE complex. By monitoring the enzymatic activities of the partially deficient individual NSF hexamers, we find the relationship between the subunits and their roles in the functioning NSF.

Reference

[1] Ryu *et al*., Spring-loaded unraveling of a single SNARE complex by NSF in one round of ATP turnover. *Science* **2015,** *347* (6229), 1485-9.

[2] Zhao et al., Mechanistic insights into the recycling machine of the SNARE complex. *Nature* **2015,** *518* (7537), 61-7.

Focused clamping of a single neuronal SNARE complex by complexin under high mechanical tension

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Neuronal SNAREs (soluble N-ethylmaleimide–sensitive factor attachment protein receptors) catalyze the fusion of synaptic vesicles with presynaptic membranes through the formation of SNARE complexes[1)]. Complexin (Cpx) is the only presynaptic protein that tightly binds to the neuronal SNARE complex and therefore regulates synaptic vesicle fusion[2)]. However, it remains unclear how Cpx modulates the energy landscape involved in the SNARE complex assembly, especially when mechanical tension is loaded on the SNARE complex. Using magnetic tweezers, we studied how Cpx interacts with a single neuronal SNARE complex, and found that the molecular effects of Cpx manifested only under high mechanical tensions above 13 pN. We found that Cpx mechanically stabilized the central four-helix bundle composed of the SNARE motifs. At the same time, Cpx prevented the zippering of SNARE complexes from reaching completion by inhibiting assembly of the linker domains. These results suggest that Cpx generates a focused clamp for the neuronal SNARE complex in a linker-open conformation.

The last step of neurotransmitter release is joining of Ca^{2+} sensor Synaptotagmin-1(Syt1). Syt1 is expected to cue Ca^{2+} triggering release of neurotransmitters by interacting with intact lipid membranes[3) 4)]. To reconstitute the synaptic terminal environment on magnetic tweezers, we casted synaptotagmin and artificial lipid membranes with SNARE complex.

Reference

[1] Weber, T. et al. SNAREpins: minimal machinery for membrane fusion. Cell 92, 759–772 (1998).

[2] McMahon, H. T., Missler, M., Li, C. & Südhof, T. C. Complexins: cytosolic proteins that regulate SNAP receptor function. Cell 83, 111–119 (1995).

[3] Yoshihara, M., & Littleton, J. T. Synaptotagmin functions as a calcium sensor to synchronize neurotransmitter release. Neuron, 36(5), 897–908, (2002).

[4] Elledge, S. J., Jackson, S. P., Weinberg, R. A., Kleiman, F. E., Manley, J. L., Ouchi, T., … Hohfeld, J. How Synaptotagmin Promotes Membrane Fusion, (May), 1205–1209. (2007).

Nuclease-free detection of DNA methylation via the B-Z transition.

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DNA methylation is one of the most frequent and critical epigenetic modifications. Abnormal DNA methylation has been implicated in various health issues such as metabolic syndromes and a variety of cancers(*1*). It is therefore not only of fundamental interest but also of practical significance to probe the degree of DNA methylation by DNA methyltransferases (DNMT) and to characterize DNA methylation profile(*2*). To this end, several methods have been developed(*3*).

Here, we propose a new single-molecule-based approach to measure the degree of DNA methylation. It is known that the B-to-Z-DNA transition is sensitive to the extent of DNA methylation: GC repeat sequences with methylated cytosines are easier to convert to Z-DNA than unmethylated, otherwise, identical sequences(*4*). Thus, we monitored the B-Z transition occurring to individual DNA molecules in various methylation states by single-molecule FRET and could quantitate DNA methylation in a facile manner and establish the relationship between Z-DNA forming capability and the degree of DNA methylation. Besides, we also tested the effects of various DNMT inhibitors, some of which are clinically tested as anti-cancer drugs, discovering that our technique provides a convenient way to evaluate the efficacy of such drugs. Our work clearly demonstrates that the new method is a sensitive, selective, versatile, and viable sensor platform to detect DNMT activity and screen DNMT inhibitors.

Reference

[1] El-Osta, A. & Wolffe, A. P. DNA methylation and histone deacetylation in the control of gene expression: basic biochemistry to human development and disease. *Gene Expr.* **9,** 63–75 (2000).

[2] Varinder Singh, P. S. & Capalash, N. DNA Methyltransferase-1 Inhibitors as Epigenetic Therapy for Cancer. *Current Cancer Drug Targets* **13,** 379–399 (2013).

[3] Nazmul Islam, M. *et al.* Optical biosensing strategies for DNA methylation analysis. *Biosens Bioelectron* **92,** 668–678 (2017).

[4] Lee, M. *et al.* Minute negative superhelicity is sufficient to induce the B-Z transition in the presence of low tension. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 4985–4990 (2010).

Cisplatin fastens nucleosomal DNA under physiological salt conditions

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Cisplatin is one of the most potent anti-cancer drugs, displaying clinical activity against various tumors. It is known that cisplatin binds to DNA and induces local kinks[1], which can lead to cell death[2]. The working mechanism of cisplatin has, however, not been fully understood yet. It is accepted that cisplatin delivery into a cell and its activity on DNA depends on local ionic states of cisplatin which is regulated by the ambient concentration of Cl^-. In reality, other cellular anionic species also suppress cisplatin activity[3,4]. Moreover, recent studies highlighted several intriguing roles of histones in cisplatin's anti-cancer effect[5-7]. Here we investigated these issues via magnetic tweezers. The reduced activity of cisplatin under physiological salt conditions is still sufficient to impair the integrity of a nucleosome by retaining its condensed structure firmly, even against severe mechanical and chemical disturbances. The cisplatin induced fastening of nucleosomal DNA can hamper DNA repair and inhibit nucleosome remodeling required for normal biological functions. Our direct physical measurements on cisplatin-nucleosome adducts suggest that formation of such adducts can be the key to the anti-cancer effect by cisplatin under physiological salt conditions and provide a new insight into understanding the mechanism of platinum-based anti-cancer drugs.

Reference

[1] Rice et al., The major adduct of the antitumor drug cis-diamminedichloroplatinum(II) with DNA bends the duplex by ap-proximately equal to 40 degrees toward the major groove. Proc. Natl. Acad. Sci. U. S. A. **1988**, 85, 4158-4161.

[2] Siddik et al., Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene **2003**, 22:7265-7279

[3] Binter et al., Formation of monofunctional cisplatin-DNA adducts in carbonate buffer. Inorg. Biochem. **2006**, 100, 1219-1224.

[4] Todd et al., Understanding the effect of carbonate ion on cisplatin binding to DNA. J. Am. Chem. Soc. **2007**, 129, 6370-6371.

[5] Mymryk et al., Cisplatin inhibits chromatin remodeling, transcription factor binding, and transcription from the mouse mammary tumor virus promoter in vivo. Proc. Natl. Acad. Sci. U. S. A. **1995**, 92, 2076-2080

[6] Wang et al., Nucleotide excision repair from site-specifically platinum-modified nucleosomes. Biochemistry **2003**, 42, 6747-6753.

[7] Kim et al., Inhibition of histone deacetylase increases cytotoxicity to anti-cancer drugs targeting DNA. Cancer Res. **2003**, 63, 7291-7300.

Formation of Arc mRNA granules in P-bodies

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Structural plasticity is essential for the maintenance of memory. Arc mRNA travels long distances along dendrites of neurons for localized translation at their destinations to modify strength of synapses nearby. However, it is not well known whether these mRNAs are moving in the state of a single Arc mRNA or in the granular state of multiple Arc mRNAs. Here, we investigated the density of Arc mRNA in neuronal mRNP particles and found that multiple Arc mRNAs tend to form a granule and move together as their density increases. Liquid-liquid phase separation involving the interaction between proteins with intrinsically disordered region (IDR) is known to play a key role in the formation of membrane-less organelles. By simultaneous detection of Arc mRNAs and diverse IDR containing proteins, we found that Dcp1a, which is a marker of the Processing body (P-body), co-localizes with Arc mRNA granules. We are currently investigating the mechanism of Arc mRNA granule formation and its role in structural plasticity.

Mechanism of Tightly-bound SSB Displacement by RecO Without Consuming ATPs Revealed by Single-molecule Studies

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RecO is a key protein in the RecFOR pathway which binds to single-stranded DNA(ssDNA) and displaces SSB from ssDNA for the loading of other homologous recombination proteins in the DNA repair process. However, the molecular mechanism of how RecO works is largely unknown. In this work, we directly observed the binding of Deinococcus radiodurans RecO to SSB-coated ssDNA using single-molecule FRET techniques. Even though SSB binds to ssDNA approximately 300 times more strongly than RecO, we found that RecO efficiently displaces SSB from ssDNA without consuming ATPs. We revealed that the sequential binding of the two major DNA-binding sites of RecO located at both ends overcomes the strong binding of SSB to ssDNA and facilitates the SSB displacement.

Reference

[1] Kowalczykowski, S. C., An Overview of the Molecular Mechanisms of Recombinational DNA Repair. Cold Spring Harbor Perspect Biol. 2015, 7 (11).

[2] Inoue, J.; Nagae, T.; Mishima, M.; Ito, Y.; Shibata, T.; Mikawa, T., A Mechanism for Single-stranded DNA-binding Protein (SSB) Displacement from Single-stranded DNA upon SSB-RecO Interaction. J. Biol. Chem. 2011, 286 (8), 6720-6732.

[3] Leiros, I.; Timmins, J.; Hall, D. R.; McSweeney, S., Crystal structure and DNA-binding analysis of RecO from Deinococcus radiodurans. Embo J. 2005, 24 (5), 906-918.

A regulatory mechanism of CRISPR-Cas9 nuclease specificity revealed from single-molecule structural dynamics

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The CRISPR-Cas9 nuclease has been widely used for genome engineering, for which understanding the molecular principles of its target specificity is required to overcome off-target cleavage effects. The RNA-guided CRISPR-associated nuclease Cas9 searches for target DNA using base-complementarity between the DNA and RNA sequences, but the regulatory mechanism of its target-specific nuclease activation remains largely unknown. Using single-molecule FRET spectroscopy, we investigate real-time structural dynamics of the non-target strand (NTS) of DNA upon Cas9 binding to on- and off-targets. Together with Cas9-mutational analysis, we find that a non-nucleolytic domain of Cas9 called REC2 regulates NTS rearrangement for the cleavage reaction with the help of positively charged surface residues, by inducing a cleavage-incompetent intermediate conformation for off-target DNAs [1]. Our result uncovers the dynamical and structural mechanism of the NTS regulation for the nuclease specificity and thereby would provide molecular insights into the development of specificity-improved Cas9 variants.

Reference

[1] K. Sung *et al.*, Target Specificity of Cas9 Nuclease via DNA Rearrangement Regulated by the REC2 Domain. *J. Am. Chem. Soc.* **140**, 7778-7781 (2018).

The role of beta-actin mRNA localization in single dendritic spines studied by two-photon uncaging

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The purpose of this research is to investigate the physiological role of beta-actin mRNA localization in stimulated dendritic spines on long-term potentiation (LTP). LTP is an important form of synaptic plasticity underlying learning and memory. In order to understand the role of beta-actin mRNA localization in neurons, we hypothesized that local translation of beta-actin mRNA plays a key role in structural remodeling of dendritic spines during LTP. We employed two-photon glutamate uncaging to stimulate single dendrite spines with or without beta-actin mRNA localization. Hippocampal neuron culture from a knock-in mouse in which all endogenous beta-actin mRNAs are fluorescently labeled was used for live-cell imaging of beta-actin mRNA. The activity of all neurons was blocked by applying tetrodotoxin (TTX), which is an inhibitor of sodium channels. Two-photon uncaging of glutamate was carried out to stimulate only a confined area near a single dendritic spine. The structural LTP was assessed by measuring the changes in the volume of dendritic spines. Our results suggest that localization of beta-actin mRNA has a strong correlation with the structural LTP process. This study sheds a light on the implication of beta-actin mRNA localization and local translation for LTP in neurons and their role in long-term memory formation.

Unexpected bending property of short dsDNA appeared in D-shaped DNA nanostructure

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DNA bending is known as one of the major factors of DNA-protein binding, like nucleosome [1]. In order to analyze bending properties of DNA, models like freely jointed chain (FJC) or worm-like chain (WLC) have been suggested [2]. These models have been appropriate well with empirical data of long dsDNA (~kbp) for several decades. However, recent studies of extremely short dsDNA (<100bp) showed that dsDNA is much more flexible than the expectation of these models [1][3]. To explain the inconsistency in detail, we used alternating-laser excitation confocal microscopy (ALEX-FRET) and D-shaped DNA nanostructure which is predicted to form bent dsDNA generated by the extension force of the ssDNA portion [4]. Using ALEX-FRET, we could determine accurate distance of dye pair in nano-scale which allowed to calculate curvature of dsDNA. As a result of the experiments, we found that the curvature about length and sequence of ssDNA length were not appropriate with previous models. This result suggested that the curvature of dsDNA be much larger than expected. Therefore, the modification of model should be required to explain these unexpected bending tendency of DNA oligo.

Reference

[1] Hernan G. Garcia et al., Biological Consequences of Tightly Bent DNA: The Other Life Biological of a Macromolecular Celebrity. Biopolymers 85, 115-130 (2007)
[2] Carlos Bustamante et al., Ten years of tension: single-molecule DNA mechanics, Nature 421, 423-427 (2003)
[3] Yih-Fan Chen et al., Entropic boundary effects on the elasticity of short DNA molecules, Phys. Rev. E 80, 020903 (2009)
[4] Cheolhee Kim et al., Dynamic Release of Bending Stress in Short dsDNA by Formation of a Kink and Forks. Angew. Chem. Int. Ed. 54, 8943 (2015)

PD-1 and PD-L1 interaction observation at the single-molecule scale

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Using single-molecule imaging using TIRFM (Total internal reflection fluorescent microscope, our team has focused on measuring protein-protein interaction of cancer cells in vitro, not only for the cytosolic proteins but also for the membrane proteins. Programmed cell death protein-1 (PD-1), an immune checkpoint protein interacts with its ligand PD-L1 to propagate inhibitory signal into T-cells. As membrane proteins, the extracellular domains of PD-1 and PD-L1 molecules are well studied, but the structure or structural dynamics of the proteins including the cytosolic part are yet to be discovered [1]. Here, we utilize the full-length form of PD-1 and PD-L1, to observe the PD-1:PD-L1 protein-protein interaction at the single-molecule level. With the help of single-molecule fluorescence spectroscopy, we discovered the nanocluster of PD-1, which are pre-formed on the membrane acting as a seed unit of PD-1:PD-L1 interactions. Experiments done by truncation of PD-1 c-terminal tail demonstrate that the cytosolic domain of PD-1 is responsible for the cluster formation. Since very little is known about PD-1 full-length protein, our investigation reveals which part of PD-1 is a key initiator of the PD-1:PD-L1 interactions, in T-cell receptor (TCR) independent manner. Our results suggest that the unstructured region of membrane proteins can be utilized to dynamically regulate their distances (or signaling units), thus affecting cell-to-cell interaction and cell signaling.

Reference

[1] C. Xiaoxiao, *et al.,* Structure and interactions of the human programmed cell death 1 receptor. *Journal of Biological Chemistry* **17**, 288 (2013).

Imaging activity-dependent transcription of endogenous Arc mRNA in live mouse brain

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During learning and memory formation, the immediate-early genes (IEGs) such as Arc, c-fos, and egr-1 are rapidly induced in the subset of neurons. Recent findings revealed that these IEG expressing neurons store the information that are needed for the memory recall. Since memories are theoretically thought to be distributed in specific neurons or 'engrams', IEG expression has been used as a marker for engram cells. However, little is known how the engram representing a specific memory changes over time. To investigate the dynamics of engrams in a live animal, we used a transgenic mouse in which endogenous Arc mRNAs are fluorescently labeled by the PP7-GFP system. By using in vivo two-photon imaging through hippocampal windows, we were able to find neurons transcribing Arc mRNA in a few minutes after behavioral experiments. We compared the engram patterns in the CA1 region of the hippocampus after contextual fear conditioning and fear memory recall. This longitudinal imaging of engrams will shed a light on the dynamic processes of encoding, consolidation, and retrieval of memory in vivo.

Z sectioning of intracellular organelles with remote-focusing-enabled iSCAT microscopy

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Interferometric scattering (iSCAT) microscopy can localize nanoscopic objects such as nanoparticles[1,4], single protein molecules,[3] and biomolecular components in the cells[5] with high spatial precision. It also enables us to observe the spatial organization of subcellular organelles, dynamical behaviors and co-localization of vesicles, promising great potential in biological research. Three-dimensional image of living cells and organisms can be achieved by optical sectioning at various depths along the optical axis by changing the distance between the specimens and the objective lens. In iSCAT microscopy, however, such a displacement of focus position gives rise to undesirable modulation in iSCAT contrast due to variation of the phase difference between scattering and reference fields[1], and thus the relative phase between scattering and reflection fields must remain constant in time and irrespective of the depth of focus. Such an intensity modulation may provide a way to determine the vertical position of a particle[2], but it is of limited utility, in particular, for iSCAT microscopy and the range of trackable height of particles is very limited, within the depth of focus.

Here, we adopted the remote-focusing technique[6] to the iSCAT microscopy in order to overcome this limitation. The remote-focusing technique is an ideal approach here because the relative phase between the fields remains the same and the plane of focus can be swiftly swept for fast 3D imaging because a light mirror, not heavy and bulky objective, is translated for focus change. As a proof-of-principle experiment, we imaged nuclei and vesicles within them. First we visualized the nuclear boundary and found that the shape of z-sectioned nuclear envelope or nuclear cross-section was transformed as the plane of focus was ascended or descended, suggesting the 3D structure of the whole nucleus. Next, we visualized and tracked nuclear vesicles lying at different heights in the cell while keeping the distance between the specimens and the objective lens.

In summary, we successfully demonstrated that our remote-focusing-equipped iSCAT microscope can track three dimensional objects such as cells along the z-direction over a much larger range of 10 um, which reforms iSCAT microscopy into a powerful 3D imaging technique and provides great opportunities in biological sciences.

Reference

[1] K. Lindfors, T. Kalkbrenner, P. Stoller, and V. Sandoghdar. “Detection and Spectroscopy of Gold Nanoparticles Using Supercontinuum White Light Confocal Microscopy.” Phys. Rev. Lett. **93,** 297–4 (2004).

[2] Madhavi Krishnan, Nassiredin Mojarad, Phillip Kukura, and Vahid Sandoghdar. Geometry-induced electrostatic trapping of nanometric objects in a fluid. Nature **467,** 692–695 (2010).

[3] Marek Piliarik and Vahid Sandoghdar. “Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites.” Nat Commun **5,** 4495–8 (2014).
[4] Il-Buem Lee, Hyeon-Min Moon, Jong-Hyeon Joo, Kyoung-Hoon Kim, Seok-Cheol Hong, and Minhang Cho. “Interferometric Scattering Microscopy with Polarization-Selective Dual Detection Scheme: Capturing the Orientational Information of Anisotropic Nanometric Objects.” ACS Photonics **5,** 797–804 (2017).
[5] Jin-Sung Park, Il-Buem Lee, Hyeon-Min Moon, Jong-Hyeon Joo, Kyoung-Hoon Kim, Seok-Cheol Hong, and Minhaeng Cho. “Label-free and live cell imaging by interferometric scattering microscopy.” Chem. Sci. **9,** 2690–2697 (2018).
[6] Edward J. Botcherby, Rimas Juskaitis, Martin J. Booth, and Tony Wilson. “Aberration-free optical refocusing in high numerical aperture microscopy.” Opt. Lett., OL **32,** 2007–2009 (2007).

Venetoclax companion diagnostic based on single molecule imaging of protein interaction

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Venetoclax(Venclexta) is the BCL2 specific inhibitor that works by preventing the protein interaction with apoptotic proteins. Even though, Venetoclax is proven to be effective by inducing the objective responses among 80% of CLL patients, there are no established diagnostic methods to prescribe the venetoclax to the various type of blood cancer.

Efficacy toward the venetoclax depends on how much apoptotic proteins of such cancer cell are sequestered by BCL2. But, since apoptotic protein can interact with multiple anti-apoptotic proteins from BCL2 family, it is critical to quantitatively measure and compare the effect of each anti-apoptotic protein.

But, due to post-translational modification(PTM) which can alter the molecular behavior of protein, it is hard to measure the effect of each anti-apoptotic proteins with conventional methods such as mRNA expression or protein expression level. [1],[2]

Here, we introduced single molecule imaging technique that directly image the protein interaction that reflects every effects of molecular changes thereby, Enabling the quantitative measurement of anti-apoptotic signal by each BCL2 family proteins.

By analyzing the protein interaction signal from various anti-apoptotic proteins, we've found that venetoclax sensitive cell-line shows strong protein interaction signal specifically related to BCL2, suggesting that protein-protein interaction(PPI) imaging can be used for companion diagnostic methods for prescribing venetoclax.

Reference

[1] Kale, Justin, Elizabeth J. Osterlund, and David W. Andrews. "BCL-2 family proteins: changing partners in the dance towards death." *Cell death and differentiation* 25.1 (2018): 65.

[2] Boidol, Bernd, et al. "First-in-human response of BCL-2 inhibitor venetoclax in T-cell prolymphocytic leukemia." *Blood* 130.23 (2017): 2499-2503.

Single-molecule functional anatomy of endogenous dimers of human receptor tyrosine kinases

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Human epidermal growth factor receptors (HERs) are the primary targets of many directed cancer therapies. However, the reason a specific dimer of HERs generates a stronger proliferative signal than other permutations remains unclear. Here, we used single-molecule immunoprecipitation to develop a biochemical assay for endogenously formed, entire HER2 dimers. We observed unexpected, large conformational fluctuations in juxta-membrane and kinase domains. Nevertheless, the HER2-HER3 heterodimer catalyzes tyrosine phosphorylation at an unusually high rate, while simultaneously interacting with multiple copies of downstream signaling proteins. This high catalytic rate, coupled to the multi-tasking capacity, constitutes the key biochemical contributor to the strong proliferative signals of HER2-HER3 heterodimers. By scaling up our assay to a high-throughput screening system, we identified tyrosine kinase inhibitors that potently inhibit the HER2-HER3 pathway in breast cancer cells and tumor xenografts. Our results provide a general roadmap toward the construction of biochemical assays for large cellular complexes, as well as a high-throughput screening system for drugs targeting the corresponding complexes.

Reference

[1] Lee, H.W. et al. Profiling of protein-protein interactions via single-molecule techniques predicts the dependence of cancers on growth-factor receptors. Nature Biomedical Engineering **2**, 239-253 (2018).
[2] Wilson, T.R. et al. Widespread potential for growth-factor-781 driven resistance to anticancer kinase inhibitors. Nature **487**, 505-U1652 (2012).
[3] Novotny, C.J. et al. Overcoming resistance to HER2 inhibitors through state-specific kinase binding. Nat Chem Biol **12**, 923-+ (2016).

Watching single membrane proteins fold

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Comprehending the folding pathways of integral membrane proteins is one of the key steps to understanding biogenesis[1]. So far, due to a paucity of the proper experimental techniques, previous studies have exclusively focused on the unfolding pathways and their intermediate states[2,3]. In this work, we here demonstrate an experimental method that permits observation of the folding pathways of integral membrane proteins (*Escherichia coli* (*E. coli*) membrane protease GlpG and human β2-adrenergic receptor (β2-AR)). We initiated the folding process from a loosely stretched state where the TM helices were aligned in a zigzag manner with minimal constraints. Despite their remarkable distances along the evolutionary path, the identified folding pathways share striking coincidences, including N-to-C-terminus unidirectional folding, usage of helical hairpins as the basic folding unit and a high level of one-dimensionality in the folding energy landscapes.

Reference

[1] J. U. Bowie, Solving the membrane protein folding problem. *Nature* 438, 581 (2005).
[2] D. Min, R. E. Jefferson, J. U. Bowie, T.-Y. Yoon, Mapping the energy landscape for second-stage folding of a single membrane protein. *Nat. Chem. Biol.* 11, 981 (2015)
[3] H. Yu, M. G. Siewny, D. T. Edwards, A. W. Sanders, T. T. Perkins, Hidden dynamics in the unfolding of individual bacteriorhodopsin proteins. *Science* **355**, 945-950 (2017).

Modeling Transcription Dynamics of Arc mRNA in Neurons

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When trying to understand cell activity, the process of mRNA transcription is very important given that it is the beginning of protein synthesis. Larson et al. [1] determined the initiation and elongation rates of the transcription of mRNA in the yeast gene at the transcription site (TS) of living cells using correlation spectroscopy. After fitting the autocorrelation curve of the fluorescence intensity fluctuation from the TS, the initiation and elongation rates were measured simultaneously. In our case, we simulated the Arc mRNA transcription model using simple computational methods to determine the contributions of the two stages of transcription, i.e., initiation and elongation and the corresponding rates. We then compared the results of the simulation and actual observations through experiments involving mammalian cells.