2018

CDLC Symposium

Single Molecule Biophysics and Chemical Dynamics in Living Cells

Date: June 04th (Mon) 14:00~18:40, 2018

Place: Room B119 (B1F), BLDG 310, Chung-Ang University

CDLC Creative Research Initiative
Center for Chemical Dynamics in Living Cells
National Research Foundation of Korea

PROGRAMME

04th June (Monday)

13: 50 Opening Remarks: Jaeyoung Sung (CDLC)	
Session 1 (Chair, Hye Ran Koh)	
14:00 - 14:50	**Peng Chen (Cornell University)** Single-molecule chemistry: from transcription regulation to living polymerization
14:50 - 15:40	**Kang Taek Lee (GIST)** Unconversion nanoparticles (UCNP): from fundamental physical chemistry to biological applications
15:40 - 15:50	**Break Time**
Session 2 (Chair, Kang Taek Lee)	
16:00 - 16:50	**Hye Yoon Park (Seoul National University)** Activity-dependent RNA dynamics in live neurons studied at single molecule resolution
16:50 - 17:40	**Hye Ran Koh (CDLC, Chung-Ang University)** Correlating transcription initiation and conformational changes by a single subunit RNA polymerase with near base pair resolution
17:40 - 17:50	**Break Time**
Session 3 (Chair, Jaeyoung Sung)	
17:50 - 18:40	**Ji-Hyun Kim (CDLC, Chung-Ang University)** Novel stochastic kinetics for quantitative biology
18:40 - 21:00	**Discussions and Dinner**

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Abstracts

Single-molecule chemistry: from transcription regulation to living polymerization

Peng Chen

Department of Chemistry and Chemical Biology, Cornell University, USA

I will present two stories that use single-molecule approaches to interrogate the mechanism and dynamics of biological and chemical processes. In the first story, I will present our work in using super-resolution single-molecule tracking to study transcription regulation in living cells. Binding and unbinding of transcription regulators on chromosome constitute a primary mechanism for gene regulation. While many cellular factors are known to regulate their binding, little is known on how cells can modulate their unbinding for regulation. I will describe that two metal-sensing transcription regulators show unusual concentration and chromosome-conformation dependent unbinding kinetics in bacterial cells, which provide novel mechanisms for facile switching between transcription activation and deactivation *in vivo* and in coordinating transcription regulation of resistance genes with the cell cycle. In the second story, I will present our work in using magnetic tweezers to track single polymer growth in real time under living polymerization catalysis conditions. I will describe how the real-time growth dynamics of single polymers reveal the formation and unraveling of conformational entanglements that play key roles in the polymerization kinetics and kinetic dispersion among individual polymers.

References:

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3. C. Liu, K. Kubo, E. Wang, K.-S. Han, F. Yang, G. Chen, F. A. Escobedo,* G. W. Coates,* P. Chen* “Single polymer growth dynamics” *Science* **2017**, *358*, 352-355.

Upconversion nanoparticles (UCNPs): From fundamental physical chemistry to biological applications

Kang Taek Lee
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Lanthanide-doped upconverting nanoparticles (UCNPs, $NaYF_4$:Yb^{3+},Er^{3+}) are well known for their emission of visible a photon upon absorption of two or more near-infrared (NIR) photons through energy transfer from the sensitizer (Yb^{3+}) to the activator (Er^{3+}). However, there still remain numerous mysteries regarding the complicated photophysical pathway in the dopants. For example, they never photobleach and photoblink, the luminescence depends on the spectral bands. They also attracted huge research interests in bioimaging because of the biocompatible NIR: low photodamage, free of autofluorescence. In this talk, I would like to discuss the origin of photoblinking (SPEM) in UCNPs, the band-dependent photophysical pathways revealed by stimulated emission depletion (STED). The biological application of UCNPs is demonstrated by the endocytosis through live cells (cancer and neuronal), and transport dynamics thereof in 2 and 3 dimensions. Finally, a simple statistical model was applied to find the rate determining step in the upconversion process.

References:

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Activity-Dependent RNA Dynamics in Live Neurons Studied at Single Molecule Resolution

Hye Yoon Park

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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. 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 (Park et al., Science 343 (2014): 422-424), 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.

Correlating transcription initiation and conformational changes by a single subunit RNA polymerase with near base pair resolution

Hye Ran Koh

Department of Chemistry, Chung-Ang University

We provide a comprehensive analysis of transcription in real-time by T7 RNA Polymerase (RNAP) employing single-molecule fluorescence resonance energy transfer (FRET). We could monitor the entire life history of transcription initiation by a single RNAP including stepwise initial RNA synthesis with near base pair resolution, abortive cycling, and transition into elongation by single RNAP molecules. We observed that abortive initiation takes place via RNAP exchange or RNAP recycling, and that there are multiple pathways of transition into elongation. By monitoring abortive cycling in real-time using three probes analysis, we could sort out productive and failed transcription events and show that abortive products are primarily generated from failed initiation and that a majority of the productive events transit to elongation without generating abortive products.

Novel Stochastic Kinetics for Quantitative Biology

Ji-Hyun Kim

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Gene expression is a complex stochastic process composed of numerous enzymatic reactions with rates coupled to hidden cell-state variables. Despite advances in single-cell technologies, the lack of a theory accurately describing the gene expression process has restricted a robust, quantitative understanding of gene expression variability among cells. Here we present a novel stochastic kinetics for living cells, providing an accurate relationship between the environment-coupled chemical dynamics of gene expression and gene expression variability. Combined with a general, accurate model of environment-coupled transcription and translation processes, we provide a unified, quantitative explanation of various gene expression statistics observed across a number of experimental systems. This work suggests promising new directions for quantitative investigation into not only cellular control over biological functions but also heterogeneous activity of single clonal enzymes and universal transport dynamics of complex fluids.