Jungtiniai cheminės fizikos seminarai

Seminaro pavadinimas: From monitoring DNA polymerases in vitro to target search of CRISPR-Cas in vivo.

Pranešėjas: Johannes Hohlbein [Laboratory of Biophysics, Wageningen University & Research].

Johannes Hohlbein holds a Ph.D. in Physics (2008, University of Halle-Wittenberg, Germany) and worked between 2008 and 2012 as a postdoctoral research assistant in the 'Gene Machines' group of Achilles Kapanidis at the University of Oxford, UK. In 2012 he became an assistant professor in the Laboratory of Biophysics at Wageningen University & Research (NL) and his group studies the amazing world of DNA-protein interactions utilising methods of single-molecule fluorescence spectroscopy.

Anotacija

Single-molecule detection schemes offer powerful means to overcome static and dynamic heterogeneity inherent to complex samples. Probing interactions and reactions with high throughput and high spatiotemporal resolution, however, remains challenging.

In the first part, I will introduce DNA based FRET sensors that allow binding of DNA polymerases and even DNA synthesis to be monitored at the single-molecule level and in real time [1]. The sensors are further characterized in novel glass-made nanofluidic devices that confine the diffusional movement of the probes thereby enabling high throughput measurements and, in a mixing geometry, even the continuous observation of chemical reactions [2].

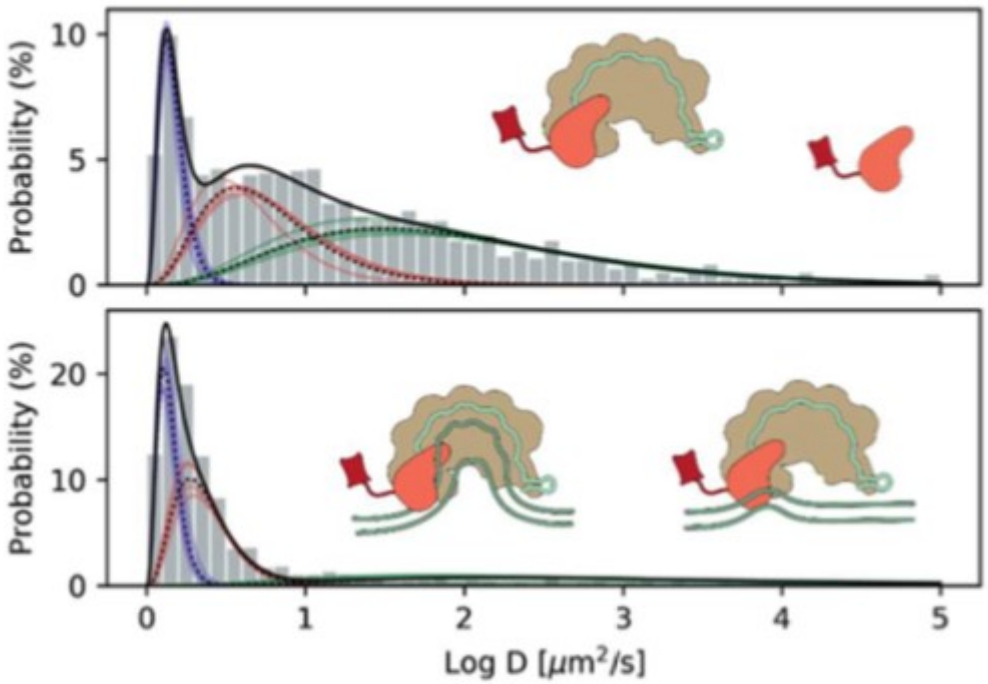

Fig. 1 Histogram of diffusion coefficients of the fluorescently tagged Cas8e subunit and its associated Cascade complexes in Eschericia coli. In presence of suitable DNA targets, the mobility significantly decreases.

In the second part, I will demonstrate how single-particle tracking can help to unravel details of a CRISPR-Cas system in its native bacterial host. CRISPR-Cas systems encode versatile machineries that have evolved to store, recognize and cleave specific DNA sequences in prokaryotic cells. Using fluorescent proteins as tags, we monitored a CRISPR-Cas system to understand how foreign DNA targets are found in a crowded, DNA-packed cellular environment. By tracking individual surveillance complexes in live cells, we show that these complexes interrogate potential binding sites much faster than reported before, enabling around 100 effector complexes to find a single invader DNA sequence within 20 minutes. We further observed mechanisms that prevent self-targeting of genomic DNA which otherwise would have a disastrous effect on the survival of the cell. Taken together, our results reveal new links between

target search kinetics, host self-avoidance and CRISPR-Cas interference in its natural environment.

[1] C. Fijen, A. Montón Silva, A. Hochkoeppler, and J. Hohlbein; Physical Chemistry Chemical Physics 19 (2017), pp. 4222-4230
[2] C. Fijen, M. Fontana, S.G. Lemay, K. Mathwig, and J. Hohlbein; bioRxiv, (2017), doi:10.1101/201079
[3] K. J. A. Martens, S. van Beljouw, S. van der Els, S. Baas, J. N.A. Vink, S. J.J. Brouns, P. van Baarlen, M. Kleerebezem, J. Hohlbein. bioRxiv, (2018), doi:10.1101/437137

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