Protein-nucleic acids interactions: new ways of connecting structure, dynamics and function

Maria G. Spies[1] & Brian O. Smith[2]

[1]Carver College of Medicine, University of Iowa, 51 Newton Rd., 4-532 BSB, Iowa City, IA 52242, USA

[2]Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary & Life Sciences, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK.

Molecular machines that act on nucleic acids, DNA and RNA, are at the heart of the cellular information processing. A coherent description of the interactions involved in their assembly, activities and regulation affords a quantitative understanding of how transcription factors and DNA repair proteins find their unique targets among millions of nonspecific sequences and undamaged DNA bases, how the intricate choreography of DNA replication, recombination and repair, and gene expression is regulated, how viral particles self-assemble and how chromosomes are organized inside living cells. These important questions are not easy to answer. This is because transactions between proteins and nucleic acids commonly involve extended surfaces with multiple interaction epitopes, the resulting macromolecular assemblies are non-homogenous and dynamic; and the structures of multicomponent protein-DNA and protein-RNA complexes are often refractory to analysis by traditional X-ray crystallography and nuclear magnetic resonance (NMR). New techniques and clever adaptations and combinations of the state-of-the-art approaches are therefore needed.

Our selection of speakers cover exciting new developments, both technological and conceptual, in determining the structures of protein-DNA and protein-RNA complexes, as well as in connecting the structure, dynamics and activity of these complexes using methods of single-molecule biophysics. While understanding of nucleoprotein architecture at atomic resolution and how it changes in response to regulatory events can be achieved using structural biology techniques, single-molecule techniques allow us to visualize and interrogate biologically important molecules individually, in real time. For both structural biology and single molecule techniques, the ability to validate observations made *in vitro* under physiological conditions is both more important and more feasible (Sakakibara, Sasaki et al. 2009, Ito, Mikawa et al. 2012, Hamatsu, O'Donovan et al. 2013). The ability to observe and directly manipulate individual protein and nucleic acid molecules provides further unprecedented insight into the kinetics of the molecular recognition steps, which are often obscured in bulk

biochemical and biophysical studies (Cornish and Ha 2007, Boehm, Subramanyam et al. 2016). During the last decade or so, technological advances in structural biology and single-molecule biophysics have brought new vibrancy and excitement to the field of nucleoprotein interactions, which we hope we capture in the selection of this session's topics.

The three invited talks and the three oral presentations selected for this session exemplify the breadth of excellence at the frontiers of structural and single-molecule research:

The ParB protein of *Bacillus subtilis* is the subject of Fernando Moreno-Herrero's talk. Fernando Moreno-Herrero (Centro Nacional de Biotecnologia, Spain) has been leading the field in atomic force microscopy (AFM) studies of protein-nucleic acid interactions (Fuentes-Perez, Dillingham et al. 2013). ParB condenses and bridges DNA molecules in the context of bacterial chromosome organisation and dynamics which the Moreno-Herrero lab investigates in collaboration with the Dillingham (Univ Bristol), Crump (Univ. Bristol), Sobbot (Univ. Atwerp) and Murray (Univ. Newcastle) groups. In his talk he shows that the C-terminal domain of B. subtillis ParB is critical for dynamic DNA binding and bridging using magnetic tweezers (MT) and a combined MT - total internal reflection microscopy (TIRF) setup (Taylor, Pastrana et al. 2015).

The CRISPR-Cas system is one of the hottest topics in biology currently due to its rapid adoption as the genome editing tool of choice, but there is still plenty to discover about its mechanism and this knowledge will inform the development of more faithful and less promiscuous tools (Spies 2014). Ralf Seidel (Leipzig University, Germany) describes work using MT (Daldrop, Brutzer et al. 2015) to interrogate the mechanism of target recognition by CRISPR-Cas system. By applying force and torque to the ends of the DNA molecule his group were able to probe the binding affinity of CASCADE for DNA targets with differently positioned imperfections (Szczelkun, Tikhomirova et al. 2014, Rutkauskas, Krivoy et al. 2017).

Metabolic disorders that originate from mitochondrial defects have been the subject of much public interest in recent years and Hanna Yuan (Academia Sinica, Taiwan) presents her group's latest work on Endonuclease G (EndoG), which digests paternal mitochondrial DNA during embryogenesis. EndoG also has a role in mature mitochondria integrating DNA replication and repair, apoptosis and response to oxidative stress. Using X-ray crystallography and studies in C. elegans, the Yuan group reveal how EndoG degrades DNA without sequence specificity and shown how EndoG acts as a ROS

sensor presenting new avenues for the prevention and treatment of the diseases that result from oxidative stresses (Lin, Wu et al. 2016, Zhou, Li et al. 2016).

Electron Paramagnetic Resonance (EPR) has recently had a renaissance in biomolecular structural biology and Christoph Gmeiner (ETH Zurich, Switzerland) describes how the long-range distance restraints produced by combining site-directed spin labelling with pulsed EPR can be used in conjunction with short-range NMR restrains to determine the 3D structure of the PTBP1/EMCV protein-RNA complex (Duss, Yulikov et al. 2014, Duss, Yulikov et al. 2015).

NMR spectroscopy remains the only technique that can simultaneously provide detailed structural and dynamic information on biomolecules in solution and Bruno Kieffer (IGBMC, France) presents work that reveals the complex allosteric mechanism by which post-translational phosphorylation regulates the retinoic acid receptor's recognition of target DNA allosterically to control gene expression (Martinez-Zapien, Delsuc et al. 2014, Belorusova, Osz et al. 2016).

The assembly of virus particles is of fundamental interest and potentially a process that can be targeted to disrupt the spread of disease causing viral pathogens. Margherita Marchetti (Vrije Universiteit Amsterdam, the Netherlands) presents an elegant single-molecule study where a combination of optical trapping and confocal microscopy (Hashemi Shabestari, Meijering et al. 2017) was used to visualize the self-assembly of artificial virus-like particles.

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Duss, O., M. Yulikov, F. H. Allain and G. Jeschke (2015). "Combining NMR and EPR to Determine Structures of Large RNAs and Protein-RNA Complexes in Solution." Methods Enzymol **558**: 279-331.

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Rutkauskas, M., A. Krivoy, M. D. Szczelkun, C. Rouillon and R. Seidel (2017). "Single-Molecule Insight Into Target Recognition by CRISPR-Cas Complexes." Methods Enzymol **582**: 239-273.

Sakakibara, D., A. Sasaki, T. Ikeya, J. Hamatsu, T. Hanashima, M. Mishima, M. Yoshimasu, N. Hayashi, T. Mikawa, M. Walchli, B. O. Smith, M. Shirakawa, P. Guntert and Y. Ito (2009). "Protein structure determination in living cells by in-cell NMR spectroscopy." Nature **458**(7234): 102-105.

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