

MS12-O2 New Pipelines for Automated High Throughput Ligand ScreeningIrina Cornaciu¹, Mari Ytre-Arne², Guillaume Hoffmann¹, Vincent Mariaule¹, Peter Murphy¹, Matthew Bowler², Didier Nurizzo², Gordon Leonard³, Bjørn Dalhus², José A. Márquez¹

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While both crystallization and data collection can be performed in a highly automated, high-throughput fashion, the numbers of ligands that can be investigated with macromolecular crystallography is strongly limited by manual crystal soaking and mounting. Here we describe a novel generations of pipelines for automated, high-throughput ligand screening with X-ray crystallography. They are based on the combination of the EMBL CrystalDirect technology, enabling completely automated crystal harvesting and cryo-cooling and the MASSIF beamline at ESRF, a fully automated hands-off data collection facility. In addition to automated crystal cryo-cooling and harvesting, the CrystalDirect Technology provides an opportunity to automate soaking experiments through controlled diffusion¹. This approach supports a gentler delivery (lower osmotic stress) and enables the achievement of higher final ligand concentrations. These new ligand screening pipelines were applied to screen small-molecule libraries targeting proteins of high biomedical relevance leading to the identification and structural characterization of novel ligand protein complexes. These high-throughput, automated, ligand screening pipelines can help streamline the process of structure-based drug design by removing critical bottlenecks and are available to European scientists through the E.C funded H2020 *i*NEXT project.

¹ Zander U, Hoffmann G, Cornaciu I, Marquette J-P, Papp G, Landret C, Seroul G, Sinoir J, Röwer M, Felisaz F, Rodriguez-Puente S, Mariaule V, Murphy P, Mathieu M, Cipriani F, Márquez J. *Automated harvesting and processing of protein crystals through laser photoablation*. Acta Cryst. 2016. D72, 454-466

Keywords: ligand screening, high-throughput

MS12-O3 DNA Structure and Dynamics – A Combinational ApproachJames P. Hall^{1,2}, Sarah P. Gurung^{1,2}, Paraic M. Keane^{1,2,3,4}, Fergus E. Poynton⁵, David J. Cardin¹, Thomas Sorensen², Graeme Winter², Thorfinnur Gunnlaugsson³, Igor V. Sazanovich⁴, Mike Towrie⁴, John M. Kelly³, Susan J. Quinn⁶, John A. Brazier⁷, Christine J. Cardin¹

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DNA is an incredibly flexible and versatile molecule. It can adopt a number of different structures ranging from the iconic Watson-Crick double helix to the 4-stranded I-motif. The structure adopted by different DNA sequences strongly affects the dynamics observed in the solution environment. DNA can be damaged by UV light or the introduction of ligands designed to induce DNA damage. As such, linking data from a crystal structure, which gives a static picture of the biomolecule, with spectroscopic measurements is key in establishing how DNA behaves and why.

Here we present our research into the structure and dynamics of DNA and DNA-ligand complexes, using a variety of techniques including circular dichroism¹, X-ray crystallography² and ultrafast transient infra-red spectroscopy³. By combining these techniques together, we can determine how DNA behaves, and why, under many different conditions and in multiple sample environments.

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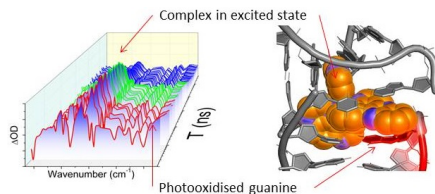


Figure 1. (Left) Transient infra-red spectroscopic measurements recorded from crystal fragments. (Right) This is combined with the knowledge of the crystal structure to assign a specific damage

site.

Keywords: DNA, photooxidation, time-resolved spectroscopy

MS12-04 Racemic DNA Crystallography: advantages and applications

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The use of racemic mixtures of naturally chiral macromolecules such as proteins and nucleic acids can significantly increase the chances of crystallization. Racemates allow molecular contacts to be formed in a greater number of ways and give access to centrosymmetric space groups. The benefits of applying racemic crystallographic methods to proteins are considerable (1). Curiously, however, racemic DNA crystallography had not been established despite the commercial availability of L- deoxyribo-oligonucleotides. We present a study into racemic DNA crystallography over a diverse range of sequences and folded conformations including duplexes, four-way Junctions and G-quadruplexes. This study revealed a strong propensity of racemic DNA mixtures to form racemic crystals (2). The crystals obtained from racemic mixtures were invariably achiral, showing a preference for the space group P1(bar) and the structures determined were nearly identical to those determined from classical D-enantiopure solutions (2). Following this proof-of-concept, we evaluated the racemic crystallographic approach for the crystallization of an intractable, biologically relevant DNA sequence – that of the Pribnow box consensus sequence 5'-TATAAT-3' in a duplex with its complementary sequence. Four high-quality crystal structures were determined with resolutions in the range of 1.65 – 2.3 Å, providing insights into the racemic crystallization process as well as structural details of this biologically relevant sequence (3). The racemic crystallization technique provides an attractive alternate to conventional crystallization using D-enantiopure solutions alone. It shows potential for the study of native DNA sequences beyond the classical model sequences, and thus may be of interest to those investigating structural aspects of fundamental biological processes and structure-based DNA-directed drug development programs. This work was supported by the European Union's Seventh Framework Programme through the European Research Council (Grant Agreement No. ERC-2012-AdG-320892, post-doctoral fellowship to PKM).

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