# Saturday, March 21, Session VIII

**L30**

## MAKING LIGHT WITH DNA

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Once thought to play a passive role in the storage of genetic information, nucleic acids are now known to be capable of a wide range of functions. Notable examples include allosterically activated ribozyme and deoxyribozyme sensors, RNA aptamers that enhance the fluorescence of small molecule fluorophores, and polymerase ribozymes that can generate transcripts of more than 100 nucleotides in a sequence-directed manner. Discovery of these motifs has been greatly facilitated by a method of artificial evolution called *in vitro* selection, in which DNA or RNA molecules with useful or interesting functions are isolated from large random sequence libraries using multiple rounds of selection and amplification. Inspired by these examples, in this study we used *in vitro* selection to identify a novel deoxyribozyme which catalyzes a chemiluminescent reaction. Under optimized conditions this deoxyribozyme generates light more than 10,000-fold more efficiently than the nonenzymatic background reaction. Comparative analysis of more than 100,000 variants obtained using *in vitro* selection and high-throughput sequencing revealed the secondary structure and sequence requirements of the deoxyribozyme. It also made it possible to design a light-producing sensor in which this deoxyribozyme was used as the signaling component.

**L31**

## EFFECT OF HELICAL KINK IN ANTIMICROBIAL PEPTIDES ON MEMBRANE PORE FORMATION

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Every cell is protected by a semipermeable membrane. Peptides with the right properties, e.g. Antimicrobial peptides (AMPs), can disrupt this protective barrier by formation of leaky pores. Unfortunately, matching peptide properties with their ability to selectively form pores was reported to both increase and decrease antimicrobial activity. We used computer simulations and fluorescence experiments to show that a kink in helices affects the formation of membrane pores by stabilizing toroidal pores but disrupting barrel-stave pores. The position of the proline/glycine kink in the sequence further controls the specific structure of toroidal pore. Moreover, we demonstrate that two helical peptides can form a kink-like connection with similar behavior as one long helical peptide with a kink. The provided molecular-level insight can be utilized for design and modification of pore forming antibacterial peptides or toxins.

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L32

# UREA-DERIVED INHIBITORS OF CYTOKININ OXIDASE/DEHYDROGENASE FOR AGRICULTURAL APPLICATIONS

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Numerous improvements in agriculturally important parameters of plants have been achieved by manipulating cytokinin content in plants. Cytokinins are plant hormones and their levels are regulated by a flavoenzyme family of FAD-dependent cytokinin oxidases/dehydrogenases (CKO/CKX). Thidiazuron (TDZ) and N-(2-chloro-pyridin-4-yl)-N´-phenylurea (CPPU) are well-known synthetic cytokinins and CKX inhibitors. These urea compounds compete with cytokinin metabolites, bases and ribosides, for the binding site of CKX. Development of novel potent CKX inhibitors reflects the ban on genetically engineered food and crops in EU. These inhibitors might increase the lifetime of endogenous cytokinins and affect different cytokinin functions, thereby having positive effects on seed filling, delayed senescence and stress tolerance toward biotic and abiotic stresses and thus improving crop yield.

Here, we report a development and biological activity of novel CKX inhibitors, which are derived mainly from diphenylurea. Several CKX isoforms from maize (*Zea mays*) were used to study the inhibitory strength of new inhibitors by analyzing enzyme kinetics as well as their binding mode by X-ray crystallography. We identified several compounds with $IC_{50}$ values in nanomolar range and solved crystal structure complexes up to 1.6Å resolution. These compounds can alleviate stress responses to drought and salt as well as increase seed/grain yield in field trials on barley, wheat and barley. Hopefully, they can find place on the market of plant growth substances.

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L33

# COMPARATIVE ULTRAFAST SPECTROSCOPY AND STRUCTURAL ANALYSIS OF OCP1 AND OCP2 PROTEIN FROM TOLYPOTHRIX

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The orange carotenoid protein (OCP) is a structurally and functionally modular photoactive protein involved in cyanobacterial photoprotection. Recently, based on bioinformatic analysis and phylogenetic relationships, new families of OCP have been described, OCP2 and OCPx. The first characterization of the OCP2 showed both faster photoconversion and back-conversion, and lower fluorescence quenching of phycobilisomes relative to the well characterized OCP1. Moreover, OCP2 is not regulated by the fluorescence recovery protein (FRP). In this work, we present a comprehensive study combining ultrafast spectroscopy and structural analysis to compare the photoactivation mechanisms of OCP1 and OCP2 from Tolypothrix PCC 7601. We show that despite significant differences in their functional characteristics, the spectroscopic properties of OCP1 and OCP2 are comparable. This indicates that the OCP functionality is not directly related to the spectroscopic properties of the bound carotenoid. In addition, the structural analysis by X-ray footprinting reveals that, overall, OCP1 and OCP2 have grossly the same photoactivation mechanism. However, the OCP2 is less reactive to radiolytic labeling, suggesting that the protein is less flexible than OCP1. This observation could explain fast photoconversion of OCP2.

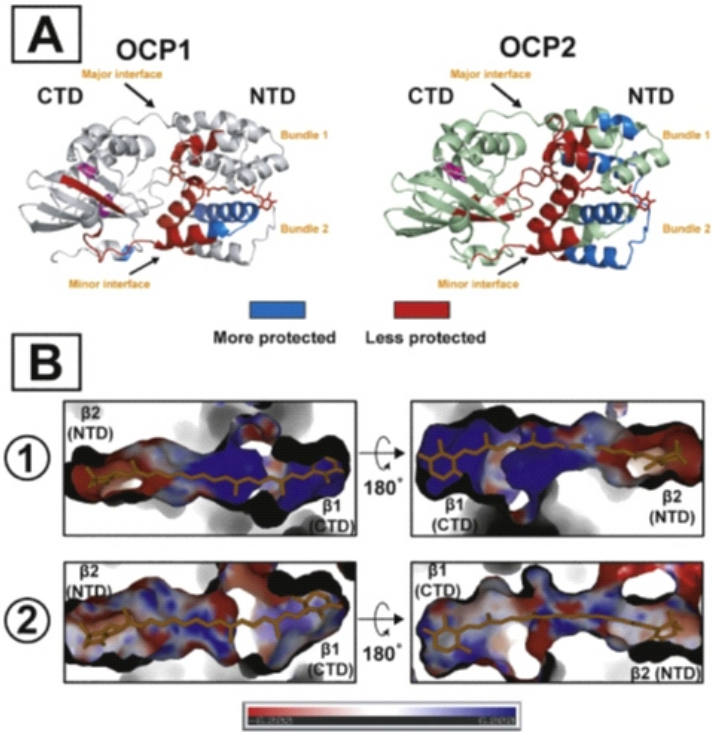


**Figure 1.** A. Local structural changes in the Tolypothrix OCP1 and OCP2 upon light activation as measured by XFMS. The changes in solvent accessibility (> 1.5-fold changed) upon light activation are plotted on the OCP1 crystal structure (PDB 6PQ1) and on the OCP2 homology model showing regions becoming less protected (red) and more protected (blue). The carotenoid in OCPR form is shown in red sticks. The domain separation is not shown. The conserved Y201 and W288 are highlighted in magenta. B. Charge distribution of the carotenoid tunnel in OCP1 (1) and OCP2 (2). 1 and 2 regions are highlighted, colored by electrostatic potential from –6kT/e (red) to 6kT/e (blue).