DIVISION OF BIOCHEMICAL TECHNOLOGY
OF THE AMERICAN CHEMICAL SOCIETY

BIOT

The Best of BIOT Awards: October 18, 2017

Date	Area	Time	Presenter	Institution
Wednesday, October 18th	Emerging Technologies	12:00-12:30 PM	**Jesse Zalatan**	University of Washington
		Rewiring genome structure with programmable CRISPR-Cas tools to physically reposition genes		
	Bio-based Products	12.30 -1:00 PM	**Miguel Suastegui**	Iowa State University
		Multilevel engineering of the upstream aromatic module in Saccharomyces cerevisiae for high production of polymer and drug		

Webinar registration can be made at ACS BIOT WebEx Webinars

"Rewiring genome structure with programmable CRISPR-Cas tools to physically reposition genes"

Jesse Zalatan, University of Washington, Seattle, WA

The physical organization of the genome plays a central role in biological processes ranging from cell division to gene regulation. Understanding the functional significance of genome spatial organization is currently hampered, however, by the lack of tools to systematically perturb genome structure in space and time. To address this challenge, we have developed a new method to physically reposition genes within the nucleus of eukaryotic cells. Using CRISPR-Cas DNA binding complexes, we can tether genomic sites to targeting protein domains that localize to specific subnuclear sites. We demonstrate this approach by recruiting genes to the nuclear periphery in yeast and human cells. Notably, only a single dCas9:gRNA complex targeting a single genomic site is necessary for peripheral recruitment. A key advantage of this approach is that it directly targets endogenous sites, unlike prior methods that require introducing heterologous binding sites into the genomic site of interest. By perturbing genome structure and assessing the functional consequences at many different sites, this new tool will enable us to systematically probe how the physical structure of the genome contributes to gene regulation.

“Multilevel engineering of the upstream aromatic module in Saccharomyces cerevisiae for high production of polymer and drug precursors”

Miguel Suastegui, Iowa State University, Ames, IA/Pennsylvania State College, State College, PA

The aromatic amino acid pathway is exemplary for hosting a great diversity of chemicals relevant to the polymer, pharmaceutical, and nutraceutical industries. The precursor module, composed mainly of the shikimic acid pathway, houses the important metabolites shikimic acid (SA), used as a drug precursor, and dehydroshikimic acid (DHS), the branching point for production of the polymer precursor muconic acid (MA). A multilevel approach to engineer the precursor module of this pathway in Saccharomyces cerevisiae was implemented. It consisted of (i) relieving the pathway from strong transcriptional repression, (ii) removal of competing pathways to ensure high carbon capturing, and (iii) rewiring of precursor pathways to increase the carbon funneling to the desired target. By combining computational tools for the prediction of novel transcriptional repression targets, optimization-based OptForce procedure for the design of pathway interventions, and implementation of the CRISPR/Cas9 platform for fast and efficient gene deletion, we constructed S. cerevisiae strains with titers as high as 2.48 g L-1 SA, 13-fold higher than the initial strain. Further expansion of the platform led to the production of MA and intermediates together close to 1.6 g L-1. The novel approach presented here that combines identification of both the transcriptional regulators and novel metabolic targets can be easily extrapolated for engineering the downstream modules in the aromatic pathway leading towards high-titer production of plant-sourced secondary metabolites.