

SEMINAR

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Hosted by Assist. Prof Lin Zhewang

Tracking RNA binding protein dynamics during bacterial growth

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About the Speaker

Mie completed her bachelor's degree in Natural Sciences at the University of Cambridge before joining the Kathryn Lilley group (Cambridge Center for Proteomics, CCP) for MSc in Systems Biology. During her time at the CCP she co-developed Orthogonal Organic Phase Separation (OOPS [1,2]), an assay for the concurrent systematic recovery of RNA-binding proteins and protein-bound RNA. Following this work, she went on to join the Anne Willis Lab (MRC Toxicology Unit, University of Cambridge) for her graduate studies focusing on the exploration of tRNA responses to different environmental stimuli.

While RNA-protein interactions are fundamental for bacterial homeostasis, we lack a systems-wide understanding of how they are rewired upon environmental changes. In this study, we have characterised the dynamics of 91% of the *E. coli* proteome and the RNA-interaction profile of 271 bacterial RNA-binding proteins (RBPs) at different growth phases. This includes 170 proteins with previously uncharacterised RNA binding properties. We find that 68% of RBPs differentially bind RNA across growing conditions, indicating a profound RBP network remodelling during cell growth. We reveal novel RBP functions for proteins such as the molecular chaperone HtpG, a novel stationary-phase RBP we find to bind tRNA sequences. Moreover, we discover that 17 unannotated proteins are indeed bacterial RBPs, including YfiF, a predicted methyltransferase protein. CLIP-seq. analyses reveal this protein to indeed bind ribosomal and transfer RNA. While these new RBPs are mainly present in proteobacteria, 3 of them possess human orthologs in the form of mitochondrial proteins required for organelle function. Altogether, we provide the first dynamic RBPome of a bacterium, showcasing how this approach can reveal the function of uncharacterised proteins, and identify critical RNA-protein interactions for cell growth which could inform new antimicrobial therapies.

[1] Queiroz, R. M. L. et al. Comprehensive identification of RNA-protein interactions in any organism using orthogonal organic phase separation (OOPS). *Nat. Biotechnol.* 37, 169–178 (2019).

[2] Villanueva, E. et al. Efficient recovery of the RNA-bound proteome and protein-bound transcriptome using phase separation (OOPS). *Nat. Protoc.* 15, 2568–2588 (2020)