Saturday, March 22, Session VI

L23

CRYO-EM ANALYSIS OF *E. coli* RIBOSOME RECOVERY MECHANISM IN THE ABSENCE OF THE 30S MATURATION FACTOR RimM

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Ribosome biogenesis is a complex, multistep process that involves the folding and modification of ribosomal RNA (rRNA), followed by the sequential assembly and integration of ribosomal proteins [1]. This intricate process is tightly regulated by numerous biogenesis factors that ensure the proper formation of functional ribosomal subunits [1]. Disruptions in these factors can lead to severe growth defects and the accumulation of immature ribosomal subunits, highlighting their critical role in ribosome maturation [1].

RimM, a key ribosome maturation factor, facilitates the correct assembly of the 30S small ribosomal subunit [2]. In *E. coli*, deletion of the *rimM* gene results in impaired growth, accumulation of immature 30S particles, and reduced translational efficiency [3-4]. Interestingly, the gradual recovery of bacterial growth suggests the presence of compensatory mechanisms that restore translation capacity over time. One such mechanism may involve the ribosomal silencing factor RsfS, which regulates protein synthesis by binding to the 50S ribosomal subunit [5]. This interaction prevents premature 70S ribosome formation, potentially shielding mature 50S subunits from associating with immature 30S particles.

In this study, we employed single particle cryo-electron microscopy (cryo-EM) to investigate the interplay between RsfS and translation initiation factors in the absence of RimM. Our structural analysis reveals that translation initiation factors bind to immature 30S subunits, preventing their association with 50S subunits until ribosomal proteins are fully assembled on the 30S subunit. Concurrently, RsfS binds to the 50S subunit, effectively inhibiting the formation of 70S ribosomes. These findings provide valuable structural and mechanistic insights into the final stages of ribosome assembly and underscore the critical roles of ribosome-associated factors in maintaining translational fidelity and cellular adaptation.

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L24

SNAPSHOTS OF THE GLUCOSE METABOLISM STUDIED BY ELECTRON CRYO-MICROSCOPY *IN VITRO* AND *IN SITU*

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Insulin is a key hormone responsible for maintaining glucose homeostasis. It is stored in pancreatic cells in a form of dense granules. We have used FIB/SEM microscopy and correlative light-electron microscopy (CLEM) to quantify the granule presence in different cell lines and studied their structure. Insulin receptor (IR) is a receptor tyrosine kinase which upon insulin binding on the extracellular receptor domain induces autophoshorylation reaction on its cytoplasmic domains. Misregulation in the insulin signalling is a cause of Diabetes melitus I and II. We have studied the mechanism of IR inhibition with insulin non-related peptidomimetics capable to fully antagonise insulin action to prevent aberrant IR signalling.

L25

CLINCELIN: A REDESIGNED LINCOSAMIDE COMBATS RIBOSOME RESISTANCE MODIFICATION THROUGH ENHANCED BINDING AND STRUCTURAL FLEXIBILITY

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Lincosamides, an important class of antibiotics in human medicine, inhibit translation by binding to the catalytic centre of the ribosome. However, their efficacy is impaired if the ribosome bears widespread A2058 methylation conferred by the *erm* resistance gene, rendering all clinical lincosamides ineffective. In this study, we present clincelin, a novel chimeric compound structurally derived from the natural lincosamides celesticetin and lincomycin [1]. Rigorous *in vitro* tests show that clincelin has significantly higher antibacterial activity compared to the two parent compounds and the clinically used lincosamide clindamycin. Remarkably, clincelin retains its efficacy also against *erm*-mediated resistant strains. Cryo-EM analysis reveals the unique mechanism underlying clincelin's evasion of resistance: Not only does it exhibit improved binding contacts with the ribosome, but it also has remarkable structural flexibility that allows different binding modes depending on the presence of Erm methylation. To the best of our knowledge, clincelin is the first antibiotic to exhibit such anti-resistance adaptation to overcome the resistance. Our detailed characterization paves the way for the development of next-generation lincosamides for clinical use and establishes a paradigm for overcoming antibiotic resistance through molecular design.

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L26

STRUCTURE OF BOTULINUM-LIKE TOXINS

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Botulinum neurotoxins (BoNTs), produced by *Clostridium botulinum*, are the most potent toxins known to man and are used to treat an increasing number of medical conditions [1]. They target neuromuscular junctions and inhibit synaptic vesicle exocytosis in motor neurons, thereby causing flaccid paralysis. Recently, a new BoNT serotype (BoNT/X) was identified in a botulism patient and *bont* genes were discovered in bacteria outside the *Clostridium* genus [2], such as *Weissela oryzae* (BoNT/Wo) or *Enterococcus faecium* (BoNT/En). These botulinum-like toxins share the molecular architecture of BoNTs and are accompanied by the non-toxic non-hemagglutinin protein (NTNH), which forms the minimal progenitor toxin complex with the toxin to protect it in the host digestive tract.

We used single-particle cryo-EM to determine the structures of the minimal progenitor toxin complexes of

botulinum-like toxins BoNT/X [3] (Figure 1) and BoNT/Wo [4]. The structures demonstrate how the toxins interact with their non-toxic protective partners and help us understand the evolutionary relationships between BoNT-like proteins. Moreover, they provide a platform for the engineering of new scientific tools and potentially also novel therapeutic toxins.

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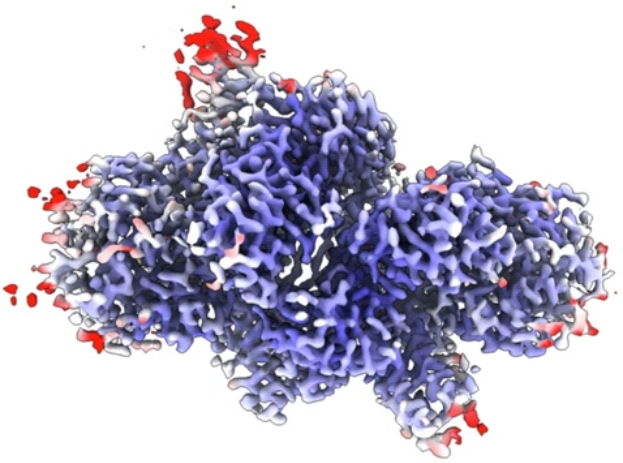

Figure 1. Cryo-EM reconstruction of the minimal progenitor toxin complex of BoNT/X.