

Temporal resolution of NAIL-MS of tRNA, rRNA and Poly-A RNA is overcome by actinomycin D

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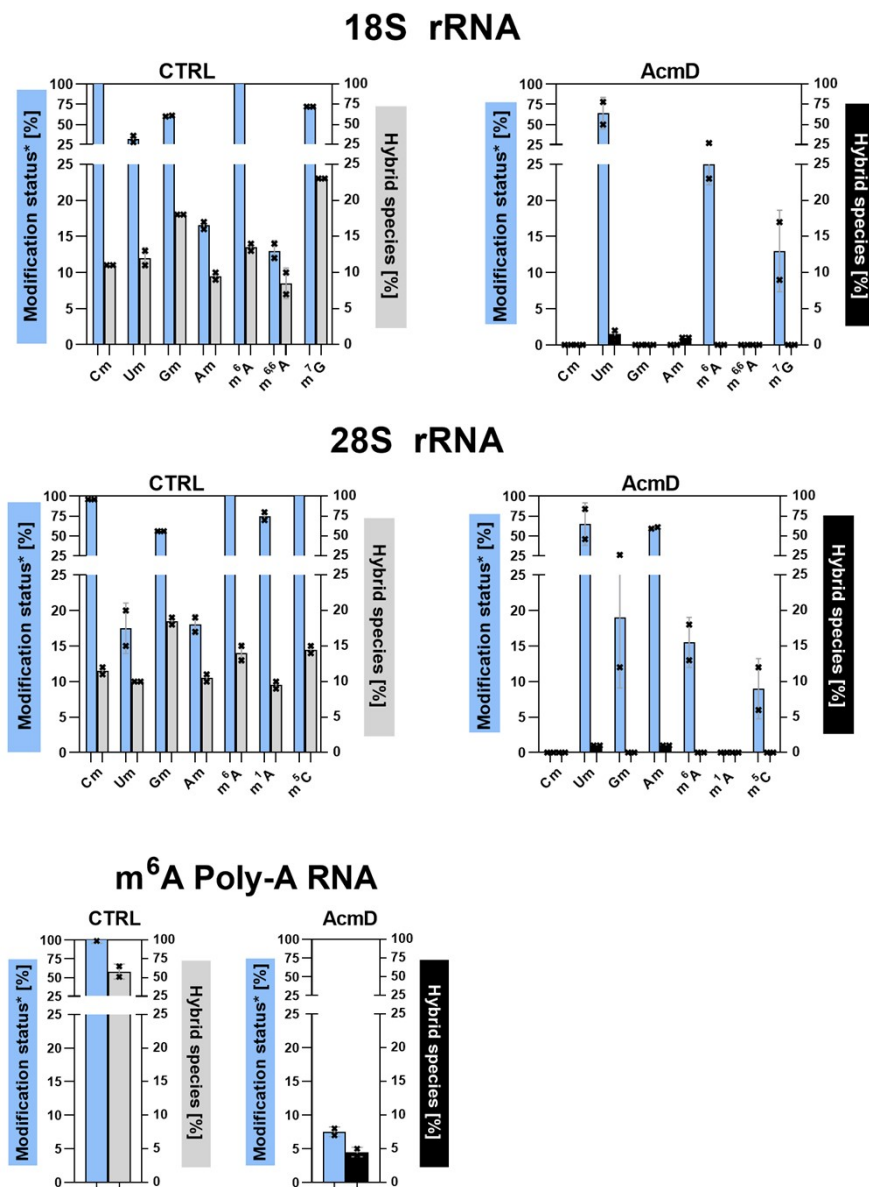


Fig S1: Occurrence of hybrid and new modifications in rRNA and Poly-A RNA 8 hours after AcmD treatment. Modification status of new transcripts is calculated by dividing the absolute abundance of new modifications (per new adenosine) at time point 8 hours by the absolute abundance of original modifications (per original adenosine) at time point 0 hours (equation 2). Hybrid species [%] is calculated according to equation 1 given in the result section.

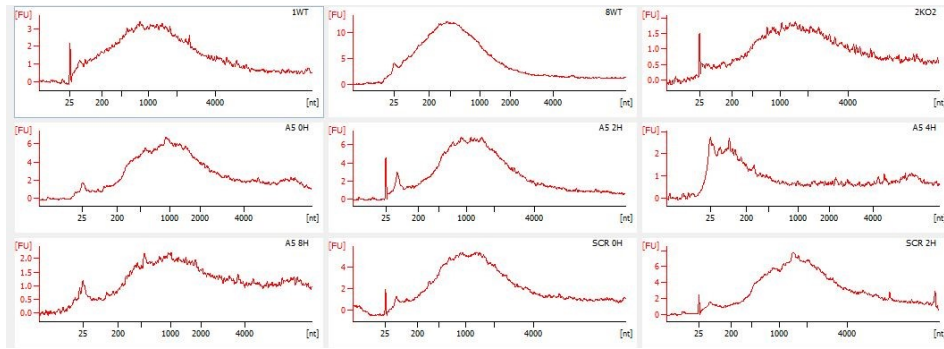
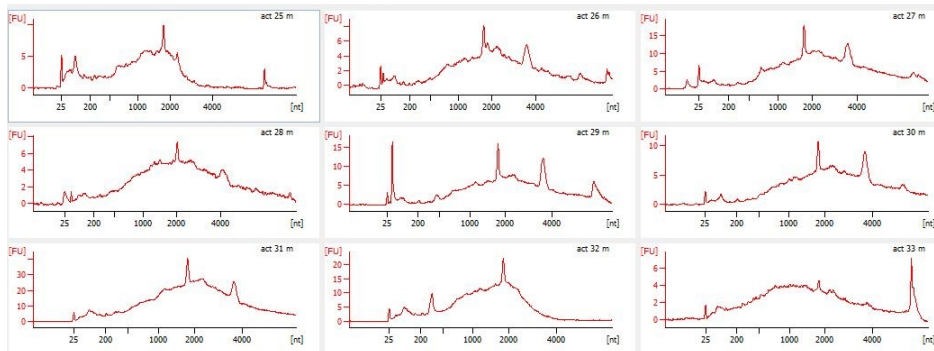
A**B**

Fig S2: Exemplary electropherograms of Poly-A RNA purified in the NAIL-MS context of this manuscript. A RNA size distribution of Poly-A RNA isolated through Poly-A enrichment, subsequent rRNA depletion and 2nd Poly-A enrichment. B RNA size distribution of Poly-A RNA isolated through Poly-A enrichment and subsequent rRNA depletion omitting the 2nd Poly-A enrichment step. Here, some residual 18S and 28S rRNA remains visible in the profile.

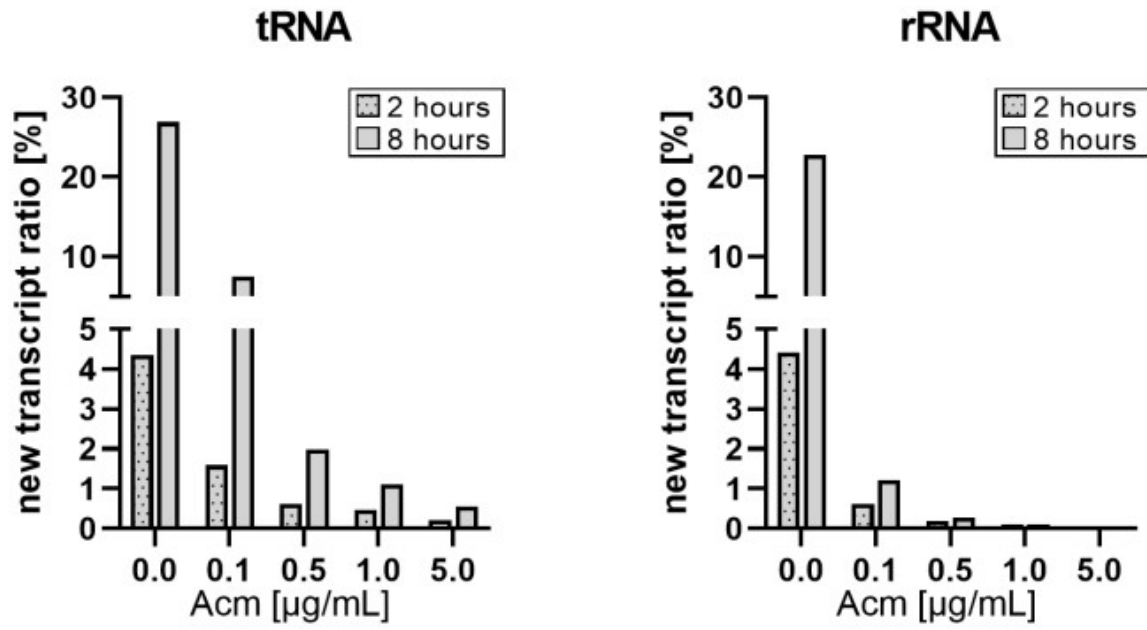


Fig. S3: New transcript ratio after 2 and 8 hours after ActinomycinD (Acm) treatment.

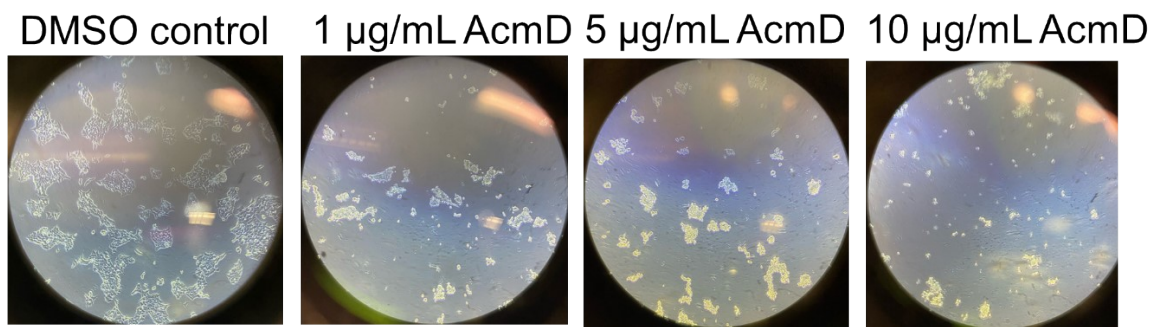


Fig. S4: Disturbed distribution of HEK cells 8 hours after DMSO and AcmD incubation.