SIMS-24
24th International Conference on Secondary Ion Mass Spectrometry
8-13 September 2024
La Rochelle, France
www.sims-24.com

Cryo-OrbiSIMS Enables Integrative Modelling of RNA Structures at Atomic Resolution

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RNAs are one of the most challenging systems to study using conventional structural biology techniques. To address this challenge, we recently pioneered the use of OrbiSIMS mass spectroscopy in RNA folding and structure refinement algorithms to model native structures with atomic precision (Ward et al. 2024 Nat Comms, final revision). First developed in 2017 for label-free metabolic imaging, OrbiSIMS is a breakthrough solid-state mass spectroscopy technology with unprecedented chemical specificity, mass resolution and picomolar sensitivity. This has enabled us to overcome the sample limitations of conventional structural techniques and characterise native RNA complexes at nanoscale.

Our results indicate that the OrbiSIMS spectrum contains more chemical information under cryogenic conditions and the mass spectrum range is sufficient to probe neutral losses of RNA fragments up to 6 nucleotides in length. We further ascertained that this mass range is sufficient to characterise more than 80% of the biologically relevant RNA complexes deposited in the protein databank. Next, we show that even though the ballistic fragmentation of the samples by the argon beam is stochastic in nature, the Cryo-OrbiSIMS experiments can reproducibly generate unique mass fingerprints for all bimolecular complexes studied under different physiological conditions and / or biological conditions. Further, peak assignments of the mass spectrum revealed that the mass data also encoded information about the native structures and plasticity of the complexes studied. Furthermore, we identified that the frequency of the presence of an RNA residue in the OrbiSIMS spectrum is correlated to its RNA-RNA contacts in the native structure. Using this information as a base-pairing probability (equivalent to SHAPE restraints) in 2D and 3D structure prediction algorithms, we accurately modelled atomic-resolution native RNA structures that align with the experimental data. Going beyond method validation, we also unravelled RNA structural plasticity in free, protein-bound and disease-remodelled states during HIV infection.

In conclusion, by benchmarking OrbiSIMS against existing methodologies, we have provided a critical validation of the technique. This positions OrbiSIMS as a transformative tool for uncovering the intricate details of native biomolecular complexes and propelling a step-change in structural characterisation by native Mass Spectroscopy techniques.