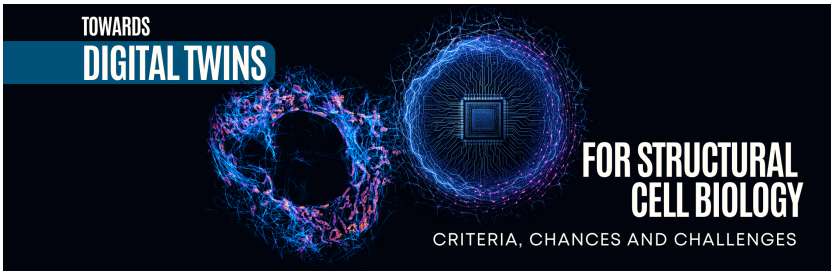

Contribution ID: **58** Type: **not specified**

Molecular basis of specificity in alternative splicing regulation through splicing regulator SRSF6

The human serine-arginine rich splicing factor 6 (SRSF6) is part of the SR-protein family consisting of 12 members and as such is involved in (alternative-) splicing regulation. It is composed of an N-terminal RRM domain, followed by a pseudo RRM and a C-terminal serine-arginine rich disordered domain. With SRSF6 being an integral part of the splicing machinery, all three domains have been implicated in interacting with RNA and/or proteins, but individual interactions mediating SRSF6 specificity remain poorly understood. Therefore, our goal was to structurally and biochemically analyse single domains and their combinations to decipher their RNA interaction sites as well as their sequence requirements. To this end, we used RNA Bind-n-Seq to obtain RNA consensus motifs for the single and tandem RRMs. We then used nuclear magnetic resonance (NMR) spectroscopy combined with electrophoretic mobility shift assay, fluorescent polarization, spectral shift assay and X-ray crystallography, applied to recombinant SRSF6 variants and the derived RNA motifs. We found the two single RRMs to have significantly different binding affinities and sequence requirements towards RNA: RRM1 binds to cytosine- and adenine-rich RNAs in a canonical way, whereas RRM2 prefers purine-rich sequences in a non-canonical mode of interaction. To understand the latter on an atomistic level, we solved the NMR-structure of RRM1 alone to verify the canonical binding mode. For RRM2, we solved the crystal structure both in the apo- and RNA-bound forms and confirm the NMR derived non-canonical RNA-binding mode mediated by RRM2's α-helix 1. Additionally, we found the linker between RRMs to play an important part in increasing affinity towards RNA in concert with RRM2. Furthermore, by combining NMR relaxation data with SAXS-based modelling, we were able to shed light on the arrangement of the two RRMs with respect to each other and the role of the linker. Finally, we confirmed our data from E. coli produced protein by a comparison with protein produced in the human cell line HEK293, which allows examining the role of the SR region, not accessible with bacterial protein. Altogether, our data provide a strong structural basis for understanding the functions and target specificity of SRSF6 as opposed to the other 11 members of the SR protein family on a molecular level.

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Session Classification: POSTER SESSION