Understanding the enzymatic activity of RNA helicases DDX3X and DDX3Y using multi-parameter confocal fluorescence spectroscopy

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DDX3X and DDX3Y are sex-chromosome encoded RNA helicases that unwind double-stranded RNA for translation initiation. Even though the protein sequences of DDX3X and DDX3Y are 92 % identical, it has been previously found that these proteins have different RNA targets and different propensities to phase separate. Despite the fact that DDX3X has been previously studied, DDX3Y has not been studied in great depth, and this investigation will lay the foundation to further examine the enzymatic activities of these proteins in order to scrutinize the differences in catalytic activities that lead to their varying activities. We used multi-parameter confocal fluorescence spectroscopy (PicoQuant) and employed single molecule fluorescence resonance energy transfer (smFRET) assays to understand DDX3X and DDX3Y's RNA binding affinities and the dynamics of their ATP-dependent interactions with RNA. Using multi-parameter confocal technique we get access to various signals such as FRET and fluorescence fluctuations when the double-stranded RNA (dsRNA) labeled with Cy3 on the long strand and Alexa647 on short strand diffuses in and out of the detection volume. The obtained FRET signals were further analyzed using FRETBursts software to understand the enzymatic activity of DDX3X and DDX3Y, whereas the fluorescence fluctuations were auto-correlated to shed light on the distinct propensities of DDX3X and DDX3Y to phase separate by comparing the differences between the slowed diffusion rate of DDX3X and DDX3Y upon binding to RNA.