**FUS RNA binding assay from Gitler’s FUS paper**

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Figure 5



The solubility of various proteins, including TDP-43 and polyglutamine, can be enhanced by the addition of a glutathione-S-transferase (GST) tag [15],[41],[71]. Even so, FUS bearing a C-terminal GST-tag was also insoluble in various bacterial strains. Fortunately, an N-terminal GST-tag allowed FUS to be purified as a soluble protein under native conditions. GST-FUS remained soluble for extended periods and was competent to bind RNA in mobility shift assays (Figure 5A).

FUS-RNA binding Assay

RNA-binding assays were performed as described [105]. Briefly, FUS RNA probe was transcribed by T7 polymerase from DNA template (5′-GTAATACGACTCACTATAGGGGAAAATTAATGTGTGT​GTGTGGAAAATT-3′) with 32P-labeled UTP. Probes were gel-purified and adjusted to 104 c.p.m./µl specific activity. Standard binding reactions were carried out in 10 µl, with a final concentration of 4 mM MgCl2, 25 mM phosphocreatine, 1.25 mM ATP, 1.3% polyvinyl alcohol, 25 ng of yeast tRNA, 0.8 mg of BSA, 1 mM DTT, 0.1 µl Rnasin (Promega, 40 U/ml), 75 mM KCl, 10 mM Tris, pH 7.5, 0.1 mM EDTA, 10% glycerol, and 0.15 µM to 5 µM GST-FUS or GST. Binding reactions were incubated for 20 min at 30°C with 32P-labeled probe. After binding, heparin was added to a final concentration of 0.5 µg/ml; reactions were analyzed on a 4.5% native gel (Acrylamide/Bis 29:1, BioRad).

FUS Purification

FUS and FUS deletion mutants were expressed and purified from Escherichia coli as GST-tagged proteins. FUS constructs were generated in GV13 to yield a TEV protease cleavable GST-FUS protein, GST-TEV-FUS, and overexpressed in E. coli BL21 DE3 cells (Agilent). Protein was purified over a glutathione-sepharose column (GE) according to the manufacturer's instructions. Proteins were eluted from the glutathione sepharose with 50 mM Tris-HCl pH 8, 200 mM trehalose, and 20 mM glutathione. After purification, proteins were concentrated to 10 µM or greater using Amicon Ultra-4 centrifugal filter units (10 kDa molecular weight cut-off; Millipore). Protein was then centrifuged for 30 min at 16,100 g to remove any aggregated material. After centrifugation, the protein concentration was determined by Bradford assay (Bio-Rad) and the proteins were used immediately for aggregation reactions.

In our AROSA-FUS interaction we will modify the technique by ordering a biotin-AROSA RNA, making the interaction then testing for presence of AROSA using the biotin detection kit we used for our EMSAs in the ETS1 paper.