



Supplementary Figure S2

Binding of the ZC3H11 zinc finger to AUU repeats

- A. Recombinant trx-ZC3H11 (trx-Z) and His-tagged N-terminal fragments (indicated above by their sizes) were separated by denaturing SDS-PAGE and stained with Commassie blue. Arrows indicate the recombinant proteins. M: markers.
- B. Recombinant trx-ZC3H11 and His-tagged N-terminal fragments were separated by native PAGE and stained with Commassie blue. Bovine serum albumin (BSA) served as a control.
- C. The His-tagged N-terminal 119mer of ZC3H11 (100 pmol total protein, Z-104) was incubated with 1 pmol radioactively labelled probe, with 5µg/ml heparin, then separated by native PAGE. The phosphorimager output is shown. Radioactive RNA probes were: UAU: U(UAU)₇U; UAUU: (UAUU)₅UAU; UCU: U(UCU)₇U; U: (U)₂₃; C: (C)₂₃; A: (A)₂₃. Arrows indicate unbound probe (u), a UAU or UAUU-specific complex (s2) and a different UCU or poly(U)-specific complex (n).
- D. The His-tagged N-terminal ZC3H11 119mer (100 pmol total protein) was incubated with 1 pmol radioactively labelled U(UAU)₇U in the presence of 5µg/ml heparin and competing oligonucleotides in 5 or 20-fold excess.
- E. Competition assay for both labelled probes in the presence of 10mg/ml heparin. The non-specific band persisted.
- F. Titration of increasing amounts of recombinant trx-ZC3H11 against limiting amounts of U(UAU)₇U RNA probe. The protein was incubated with radioactively-labelled probe then separated by native PAGE. The phosphorimager output is shown. Approximate maximum and minimum protein levels (in Molar units) are shown at the top. A quantitation of radioactivity in the well relative to unbound probe (u) shown on the right. 50% binding of 10 pMol probe was seen with a concentration of about 10 nMol recombinant AC3H11-119. Since, however, we do not know how much of the recombinant protein is properly folded, and several proteins may bind to a single probe, this value cannot be taken to be a dissociation constant.