

Supplementary material

Cap-related modifications of RNA regulate binding to IFIT proteins

Jingping Geng^{1†}, Magdalena Chrabąszczewska^{2†}, Karol Kurpiejewski³, Anna Stankiewicz-Drogon², Marzena Jankowska-Anyszka³, Edward Darzynkiewicz^{1,2}, Renata Grzela^{2*}

1 - Interdisciplinary Laboratory of Molecular Biology and Biophysics, Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland

2 - Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland

3 - Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

Correspondence to Renata Grzela: Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland

rgrzela@fuw.edu.pl

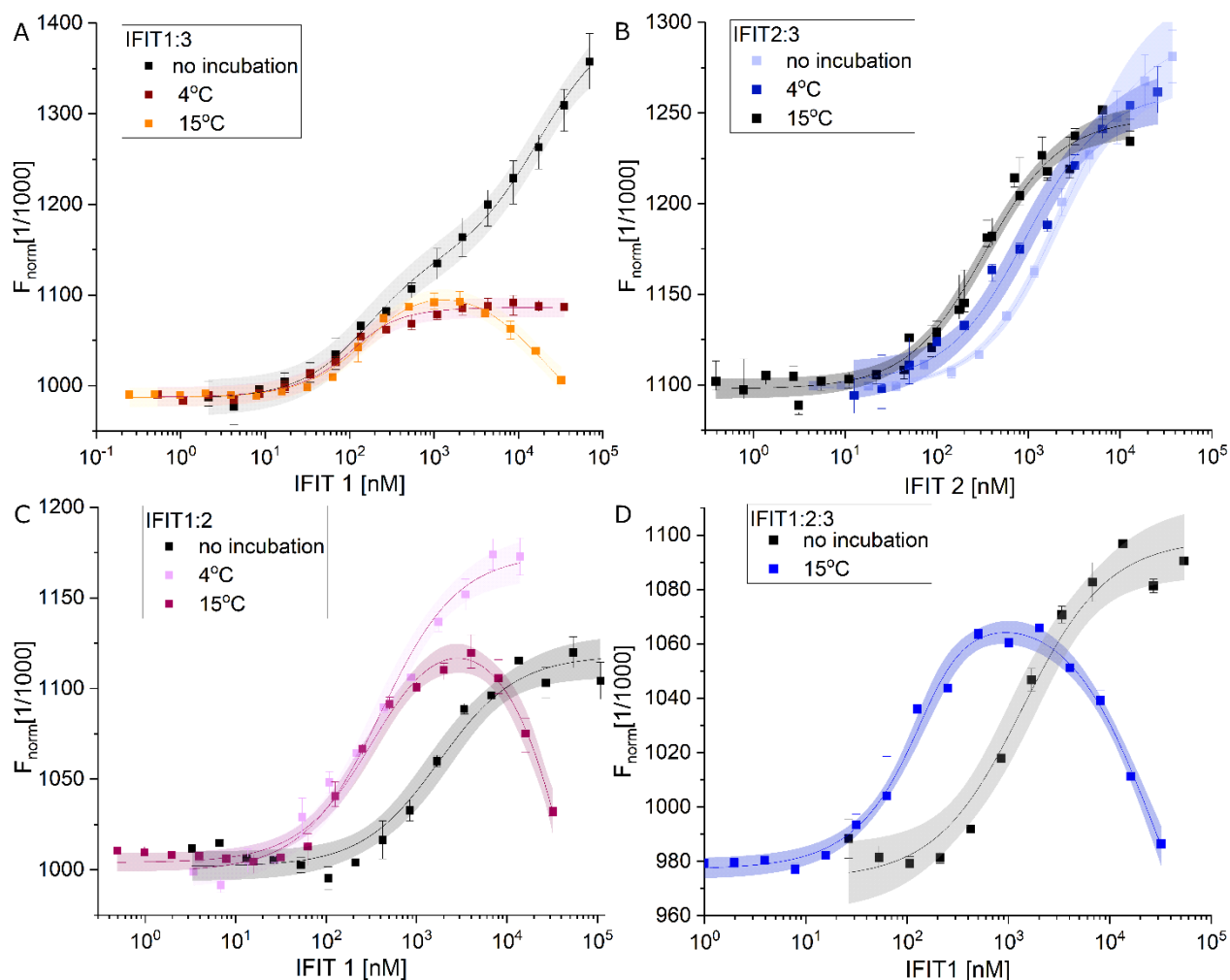


Figure S1

Representative MST pseudo-titration data for the formation of IFIT complexes. The measurements were conducted at 22 °C, after 30 minutes pre-incubation at 4 °C and 15 °C or without the pre-incubation step. Squares represent experimental points, solid lines represent results of fitting and color area represents 95% confidence bands for the used model. IFIT1 added in the increasing concentration range served as a ligand protein in experiments (A), (C) and (D). Formation of IFIT1/IFIT2/IFIT3 trimer was examined with the preformed IFIT2/IFIT3 complex (D). For IFIT2/IFIT3 complex formation, IFIT2 was used as a ligand protein (B).

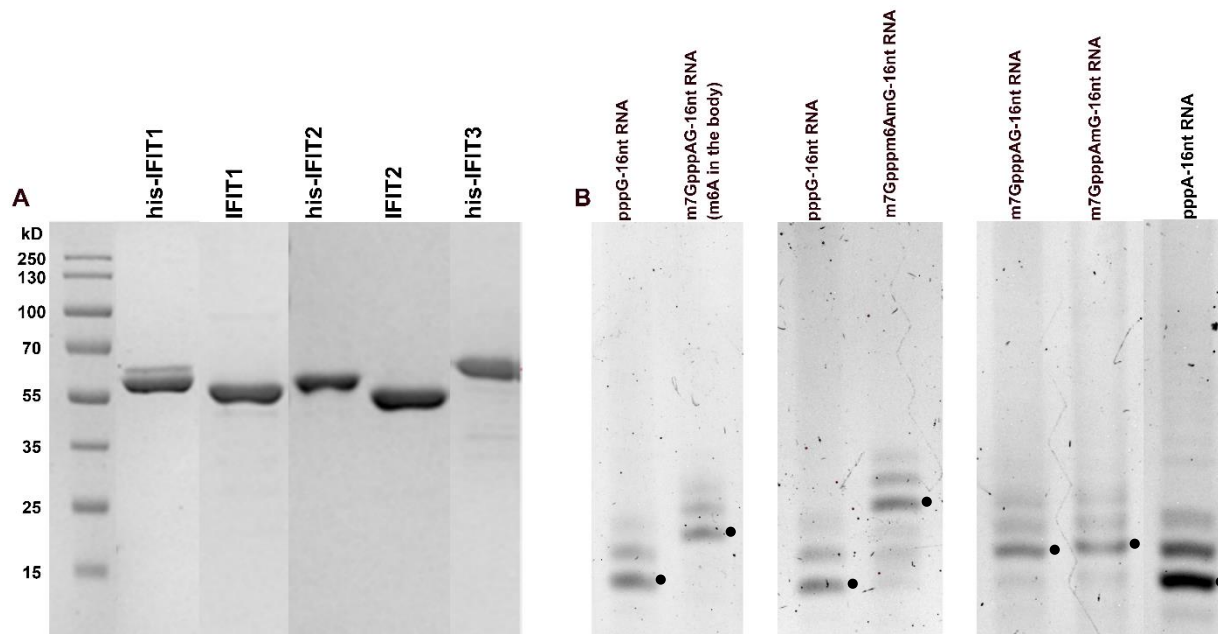
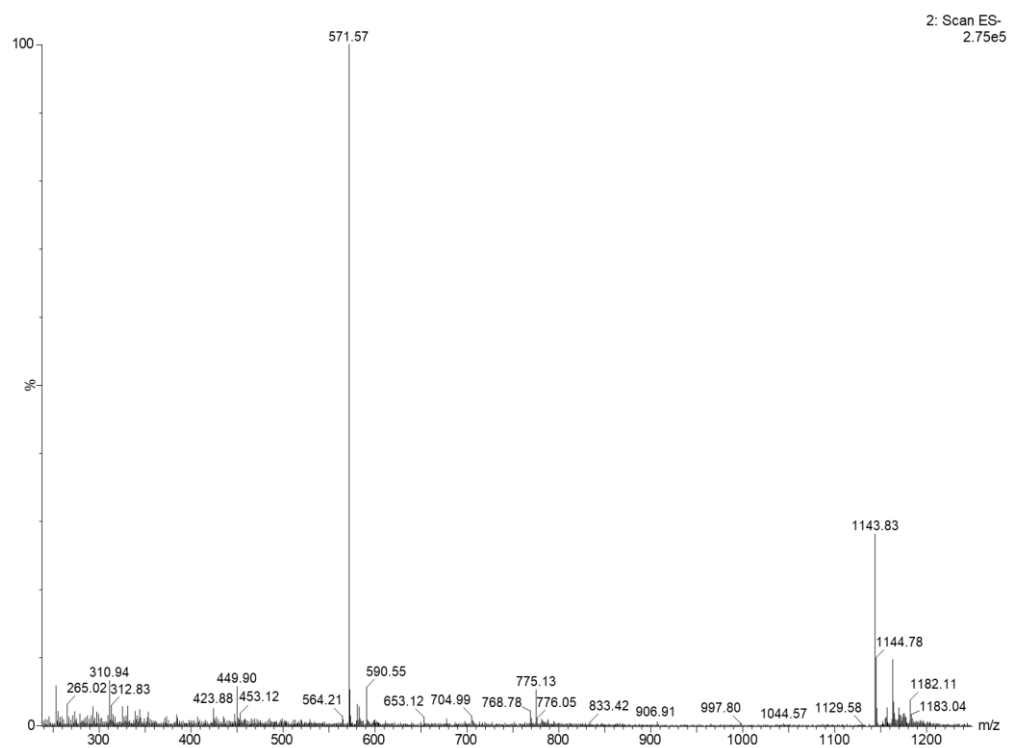


Figure S2.

IFIT proteins and short RNA bearing various modifications of the cap structure at the 5' end used in this work. (A). SDS-page of IFIT proteins after purification by Ni-NTA and size exclusion chromatography. From left to right: IFIT1 with his-tag, IFIT1 without his-tag, IFIT2 with his-tag, IFIT2 without his-tag and IFIT3 with his-tag. (B). Denaturing polyacrylamide gel of short 16-nt RNAs with different cap analogues (marked with dots). Additional $n + 1$ and $n + 2$ bands originated from nontemplated addition of nucleotides during in vitro transcription.

m⁷Gppp(A_m)pG

MS [M-H]⁻ ESI



m⁷Gppp(m⁶A_m)pG

MS [M-H]⁻ ESI

