Supplementary information for: Power of protein/tRNA functional assembly against aberrant aggregation

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Supplementary figures

Figure S1. Size exclusion chromatography carried on a Superdex 200 increase 10/300 GL for A) TrmFo Y346A mutant in 50 mM sodium phosphate pH 8 and 100 mM NaCl; B) TrmFo Y346A mutant after heating up to 70 °C in 50 mM sodium phosphate pH 8. Their respective molecular weights and hydrodynamic radius (Rh) as determined by SEC-MALLS are also shown.

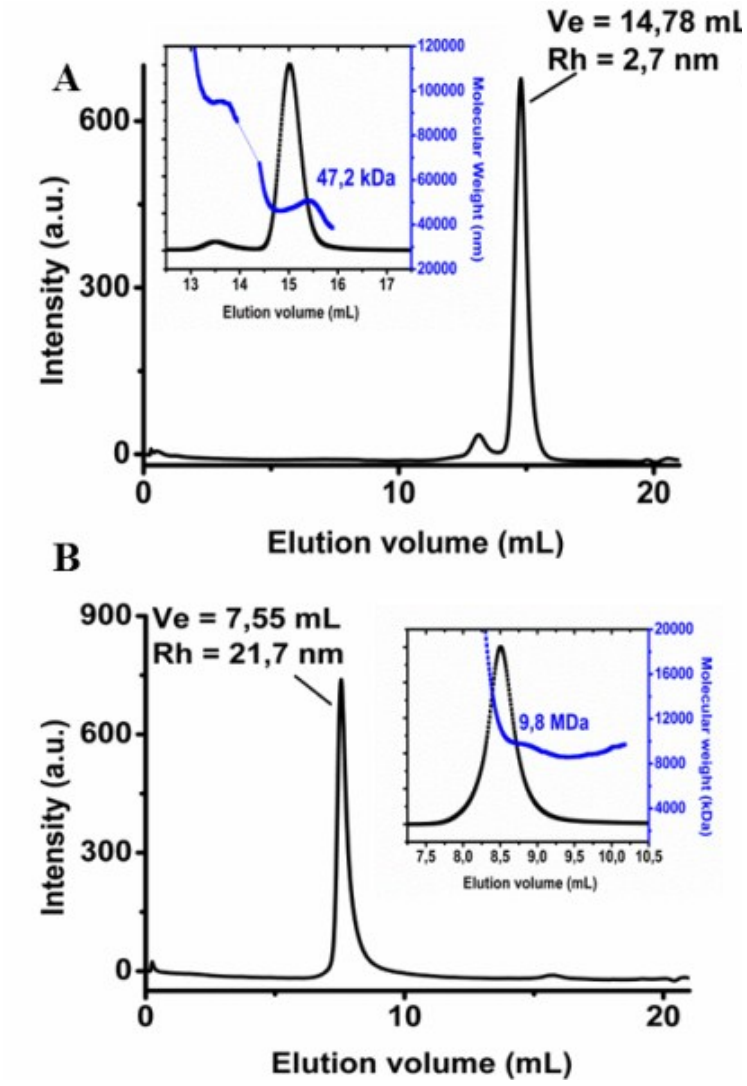

Figure S2. Gel shift assay. Gel shift assays with 1 µM of bulk tRNA in the presence of increasing amount of A) TrmFo Y346A mutant and B) TrmFo Y346A mutant after heating at 70 °C for 20 minutes. Final protein concentration reached was 120 µM.

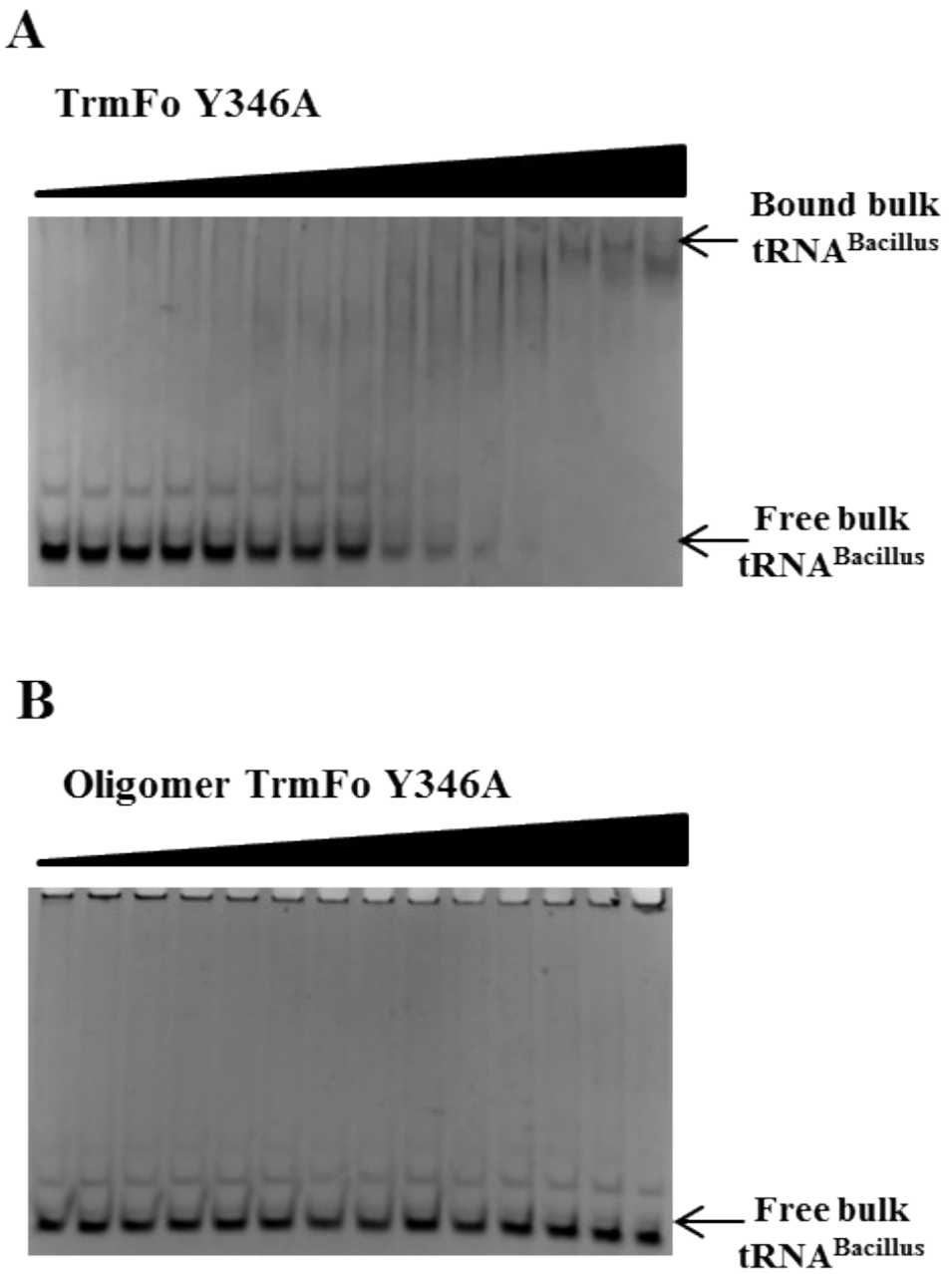

Figure S3. (A) Thermal-induced structural denaturation of Y346A TrmFO followed by circular dichroism at 222 nm in the presence of 0, 0.1, 0.2, 0.4, 0.8 or 1.6 M NaCl. The temperature ramping rate was set to 1.5 K/min. (B) Far-UV spectra of Y346A TrmFO as function of temperature 5from 20 to 80°C) in the presence of 0.2 M NaCl.

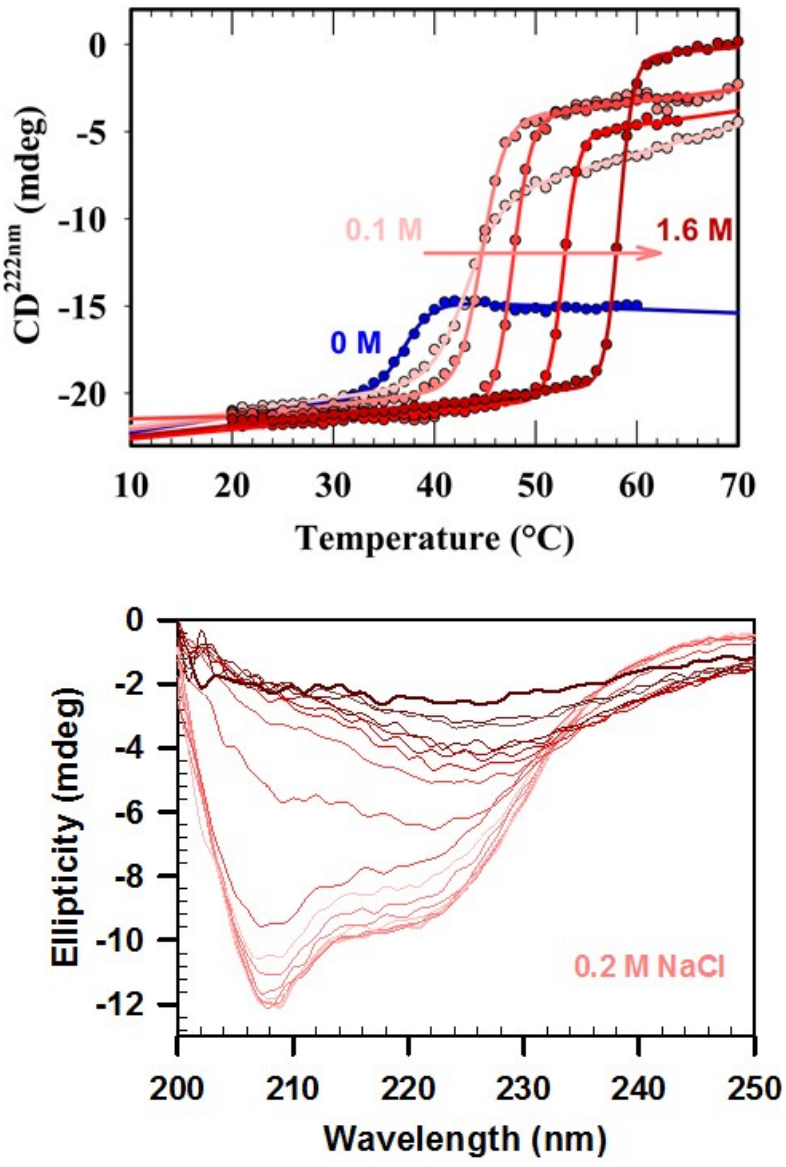

Figure S4. (A) Normalized scattered intensity I/I0 measure by DLS for wild type and Y346A TrmFO (0.2 mg.ml^{-1}) in the presence of 200 mM NaCl. (B) Intensity distribution derived from the NNLS analysis of the sample at 20 (black), 36 (blue) and 48°C (pink) in the presence of 200 mM NaCl.

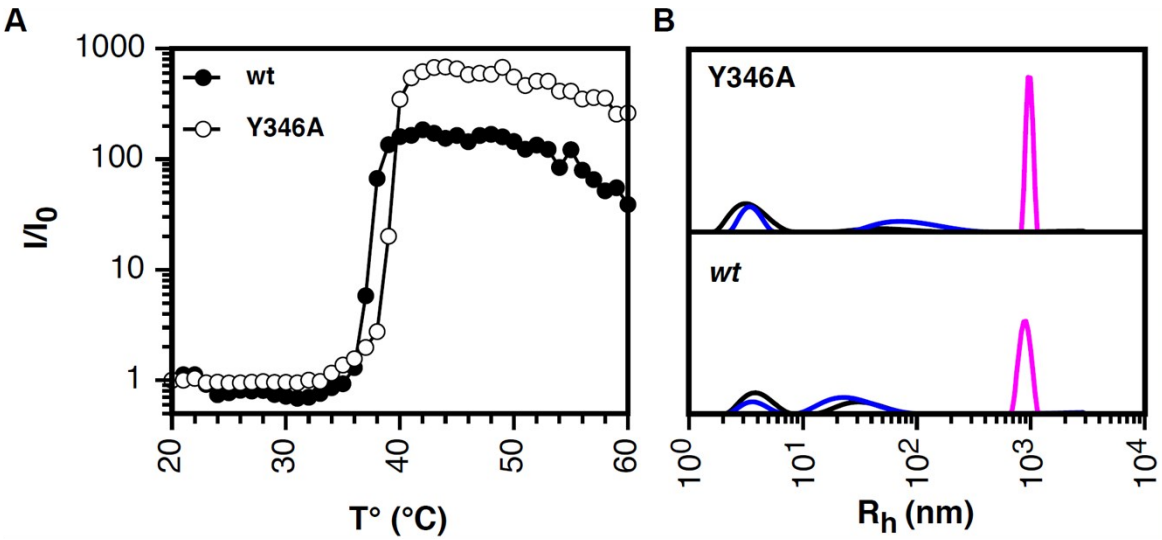

Figure S5. Limited proteolysis profiles obtained at different incubation time with 1/400 trypsin ratio for A) TrmFo Y346A mutant in 50 mM sodium phosphate pH8 and B) TrmFo Y346A mutant after heating at 70 °C in 50 mM sodium phosphate pH 8. In C) are shown the intensity profiles of the respective bands observed in B) upon time.

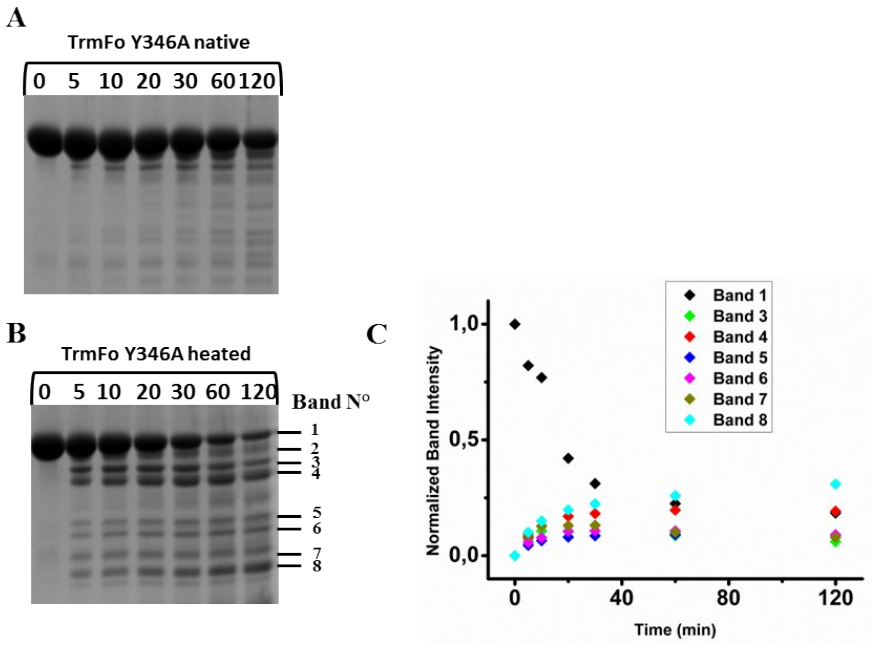

Figure S6. Structural characterization of TrmFO oligomers by limited proteolysis mass spectrometry

(1-8) Zooms of MALDI MS spectra of full-length (upper panel) and truncated forms of Y346A TrmFO after trypsin (left panels) and Glu-C (right panels) proteolysis. Protein coverage (not shown) was identical for full-length and fragments but terminal peptides peaks (annotated on spectra) were detected with significant lower relative intensities in truncated products (only significantly different spectra are represented). Trypsin-generated C-terminal peptides 415-423 and 417-423 detected equally in all bands apart from fragment 07, indicates mild proteolysis occurred mainly within N-terminal regions. N-terminal peptide 110-122 detected predominantly in band 04 after Glu-C proteolysis precises one of limited trypsinolysis sites at R109. Peaks marked with asterisk correspond to peptides with cysteine modified by propionamide.

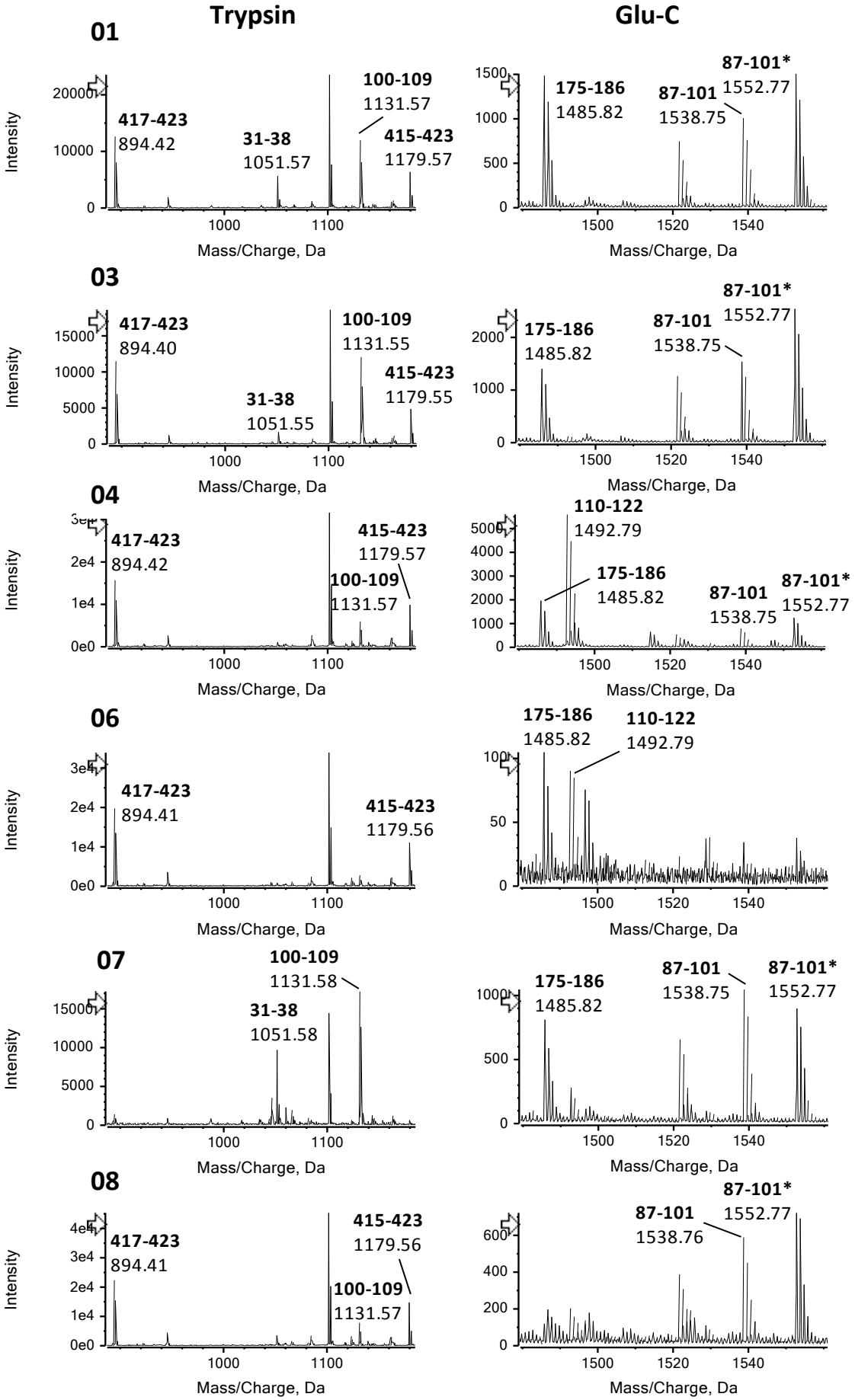
Trypsin
Glu-C
01
417-423
894.42
31-38
1051.57
100-109
1131.57
415-423
1179.57
175-186
1485.82
87-101
1538.75
87-101*
1552.77
03
417-423
894.40
31-38
1051.55
100-109
1131.55
415-423
1179.55
175-186
1485.82
87-101
1538.75
87-101*
1552.77
04
417-423
894.42
100-109
1131.57
415-423
1179.57
110-122
1492.79
175-186
1485.82
87-101
1538.75
87-101*
1552.77
06
417-423
894.41
415-423
1179.56
175-186
1485.82
110-122
1492.79
07
417-423
894.41
31-38
1051.58
100-109
1131.58
175-186
1485.82
87-101
1538.75
87-101*
1552.77
08
417-423
894.41
100-109
1131.57
415-423
1179.56
87-101
1538.76
87-101*
1552.77
Intensity
Mass/Charge, Da

Figure S7. Size exclusion chromatography carried on a Superdex 200 increase 10/300 GL of Y346A TrmFO in the presence of CH_2THF (5 molar excess) after heating to 70°C for 20 minutes in 25 mM sodium phosphate pH 8. The wavelength used for the chromatogram is 280 nm.

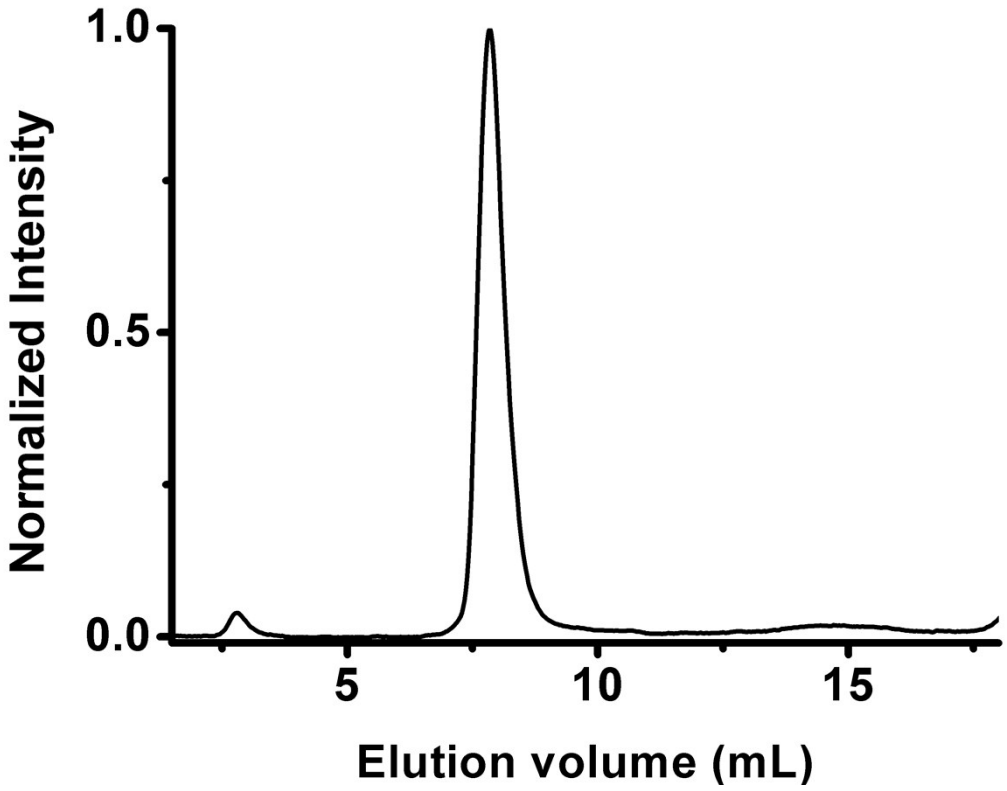

Figure S8. Size exclusion chromatography carried on a Superdex 200 increase 10/300 GL of Y346A TrmFO (red curve), tRNA alone (black curve) and Y346A TrmFO/tRNA complex before heating to 70°C for in 25 mM sodium phosphate pH 8 (blue curve); after heating up to 70 °C for 20 minutes in 50 mM sodium phosphate pH 8 (green curve). The units are reported in a.u x 10^{-3}. The wavelength used to follow the elution of the molecules is 280 nm.