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Supplemental Information

Elucidating the Role of Microprocessor Protein DGCR8 in Bending RNA Structures

Suzette A. Pabit, Yen-Lin Chen, Emery T. Usher, Erik C. Cook, Lois Pollack, and Scott A. Showalter

Supporting Materials for "Elucidating the role of microprocessor protein DGCR8 in bending RNA structures"

by Suzette. A. Pabit, Yen-Lin Chen, Emery T. Usher, Erik C. Cook, Lois Pollack, and Scott A. Showalter

Consistent with the "2017 Publication Guidelines for Structure Modeling of Small Angle Scattering Data from Biomolecules in Solution: An Update" paper [Trewhella, J. *et al. Acta Crystallogr. Sect. D Struct. Biol.* **73**, 710–728 (2017)], we report the following information about our SAXS samples, experimental conditions and analysis, summarized as Table S1 and in the text below:

Table S1: Summary of SAXS results and analysis

(a) Sample details		
	Pri-miR16-1 RNA	DGCR8-core protein
Organism	Human	Human
Source	T7 transcription, collected single-peak though FPLC size-exclusion column (Superdex 200 Increase 10x300 GL), showed a single transition in a UV melting experiment	*E. coli* expressed, was passed through a Ni-NTA column, further purified by FPLC on a Sephacryl S100 gel filtration column and fractions were chosen using results from an SDS-PAGE gel
Extinction coefficient $(M \cdot cm)^{-1}$	1,125,400 at 260 nm	544,800 at 278 nm
Molecular weight (g/mol)	36170	26000
Sample concentration (μM)	5, 10, 20	10, 20
Solvent	50 mM KCl, 50 mM HEPES, pH 7.5, 5 mM DTT, 1% glycerol, 0-50% w/v sucrose	

(b) SAXS data-collection parameters	
Instrument/data processing	Cornell High Energy Synchrotron Source BioSAXS, Pilatus 100k detector
Wavelength (Å)	1.25
Beamsize (μM)	250 × 250 (beam defining slits)
Sample to detector distance (m)	1.517
q measurement range ($Å^{-1}$)	0.0080 - 0.2792
Absolute scaling method	Comparison with scattering of pure H_2O
Normalization	Beamstop pin diode
Monitoring for radiation damage	Sample oscillation, frame-by-frame comparison
Exposure time	20 1-second exposures
Sample configuration	2-mm quartz capillary flow cell
Sample temperature (^oC)	23

(c) Software employed for SAXS data reduction, analysis and interpretation	
SAXS data reduction	BioXTAS RAW and MATLAB in-house scripts
Extinction coefficient estimate	Oligocalc Analyzer for RNA, ProtParam for protein
Determination of sucrose match point	Determined empirically by experimentation as described
Guinier analysis	BioXTAS RAW and MATLAB in-house scripts
P(R) analysis	GNOM
Shape/bead modeling	DAMMIF, DAMAVER, SUPCOMB through ATSAS
Atomic structure modeling	AMBER for RNA, CRYSOL for protein

3D graphic model representation	PyMOL	
(d) Structural parameters (representative curves)		
	Pri-miR16-1 RNA	*DGCR8-core protein*
Guinier analysis		
$I(0)$ (cm^{-1})	0.0294 ± 0.0002	0.00631 ± 0.00003
R_g (Å)	43.8 ± 0.3	24.4 ± 0.2
q_{min} ($Å^{-1}$)	0.0177	0.0177
qR_g max	1.3	1.3
Coefficient of correlation, R^2	0.994	0.983
M from $I(0)$ (ratio to predicted)	33300 g/mol (0.92)	21,400 g/mol (0.80)
P(R) analysis		
$I(0)$ (cm^{-1})	0.0317	0.0065
R_g (Å)	48.3	24.6
D_{MAX} (Å)	175	80
q range	0.0143 to 0.190	0.0166 to 0.279
Total quality estimate	0.72	0.86
M from $I(0)$ (ratio to predicted)	35970 g/mol (0.99)	21,400 g/mol (0.82)
(e) Shape model-fitting results		
	Pri-miR16-1 RNA [by itself]	*RNA complexed to DGCR8-core [protein blanked with sucrose]*
DAMMIF (default parameters, 15 calculations, slow mode)		
q range for fitting ($Å^{-1}$)	0.0183 to 0.2792	0.0183 to 0.2792
Symmetry, anisotropy	P1, unknown	P1, unknown
NSD (standard deviation)	0.755 (0.024)	1.108 (0.065)
(f) Atomistic Modeling		
	Pri-miR16-1 RNA (by itself)	*RNA complexed to DGCR8-core [protein blanked with sucrose]*
All-atom models	Molecular Dynamics model generated from AMBER as described in the text	Generated from the AMBER model and the SAXS reconstruction using the FCC algorithm as described in the text

(g) SASBDB IDs for data and models	***ID numbers***
Pri-miR16-1 RNA (by itself)	SASDJV7
Pri-miR16-1 RNA complexed to DGCR8-core (in sucrose)	SASDJW7

Notes on Contrast-Variations SAXS experiments for RNA-protein interactions:

Demonstration of sample quality

First, we established the quality (e.g. homogeneity) of the RNA and protein samples prior to complex formation. As described in the main text, we showed that the RNA and protein SAXS samples are from highly purified and dilute solution of monodisperse particles. Below, we show representative Guinier fits to scattering profiles from the RNA alone, protein alone, and the RNA-protein complex. We use Guinier analysis to find the radius of gyration, R_g, in regular buffer (no sucrose). In the main text, we report the average R_g taken from several Guinier fits on different SAXS samples.

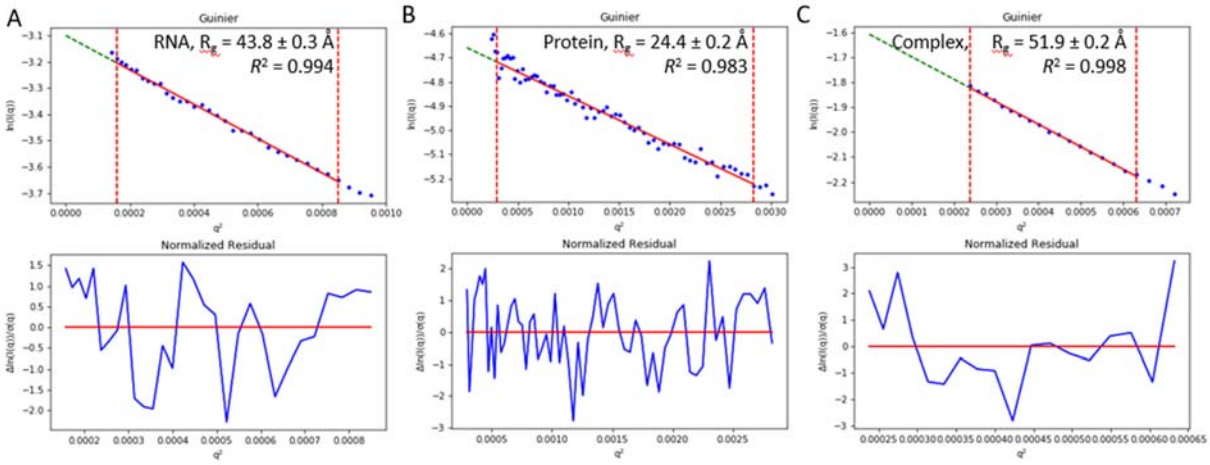

Figure S1: Guinier approximation is used to find radius of gyration of (A) RNA alone, (B) protein alone, and the (C) RNA-protein complex in regular buffer. We show the Guinier fits, the normalized residuals and display the R_g and the coefficient of correlation, R^2.

In the main text (Figure 2D), we show the corresponding SAXS scattering profiles in linear scale to demonstrate the differences in signal strength between the protein, RNA and protein-RNA complex, as demonstrated in eq. (8) and eq. (9).

Demonstration of contrast variation using sucrose

Prior to performing a contrast variation experiment using sucrose to match or blank out the protein SAXS signal, we established that sucrose does not affect the RNA structure.

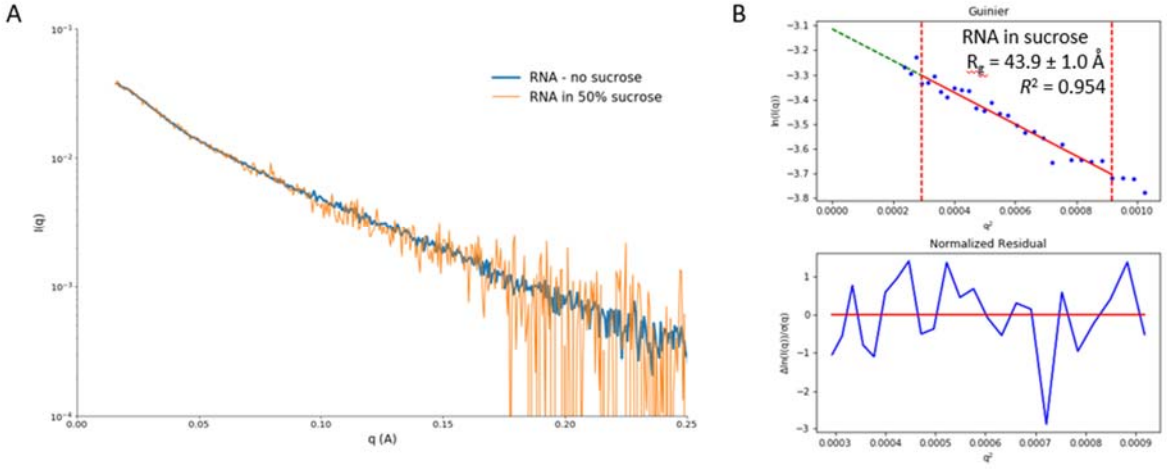

Figure S2: (A) SAXS profiles of the RNA in regular buffer (0% sucrose) and buffer with 50% sucrose showing that the curves are indistinguishable. (B) Guinier fit of the RNA in 50% sucrose reports the same R_g as RNA in regular no-sucrose buffer.

To find a sucrose match point, we looked for the sucrose concentration (in w/v) that cancels or blanks out the protein signal. Below, we show buffer-subtracted SAXS profiles of the protein at various sucrose w/v concentrations. The 50% sucrose condition shows no (or very little) protein scattering, matching with buffer.

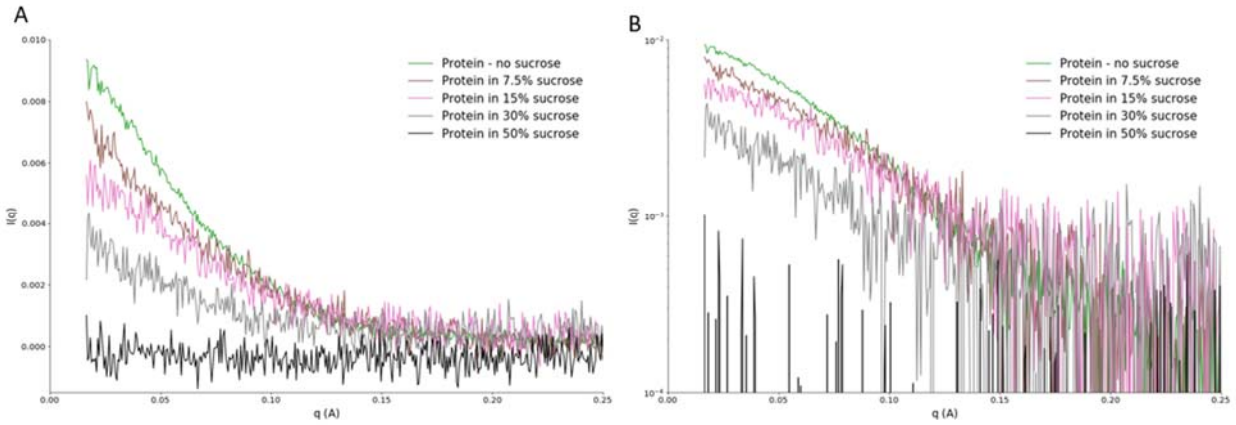

Figure S3: Buffer-subtracted protein SAXS signals at different sucrose buffer concentrations from 0% to 50% w/v sucrose displayed in (A) linear scale and (B) log scale. From this analysis, we determined that 50% sucrose is our contrast-matched point. The protein concentration was kept the same in all the curves, 20 μM.

Variation in the SAXS shape reconstructions

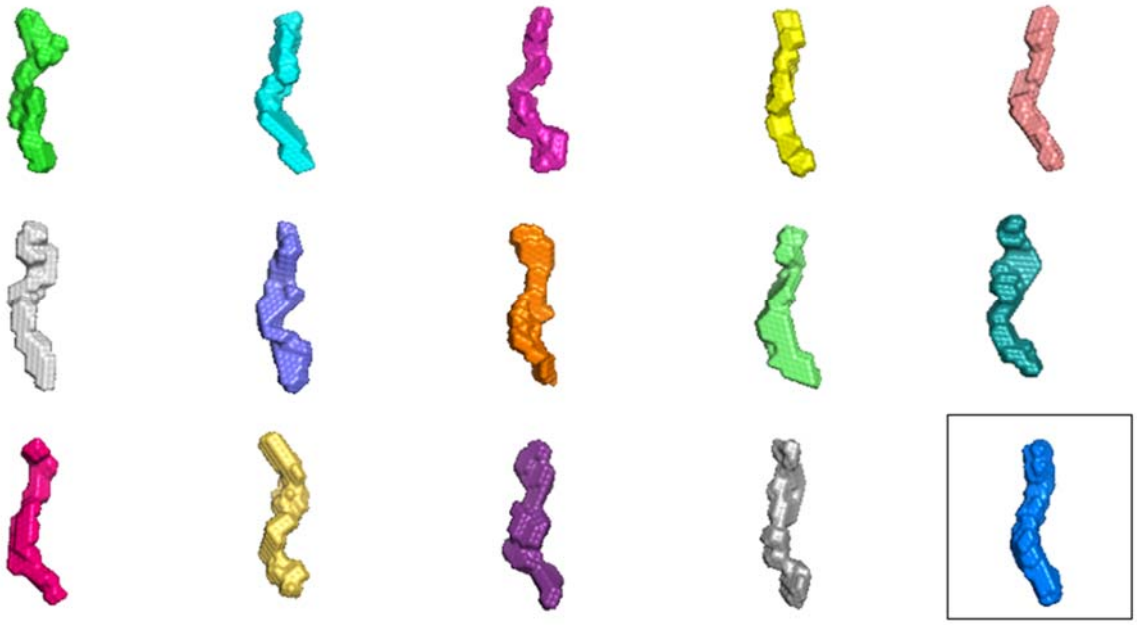

Figure S4: DAMMIF shape reconstructions of the pri-miR16-1 RNA (in regular buffer, by itself) with the DAMAVER-DAMFILT-averaged dummy atom model boxed.

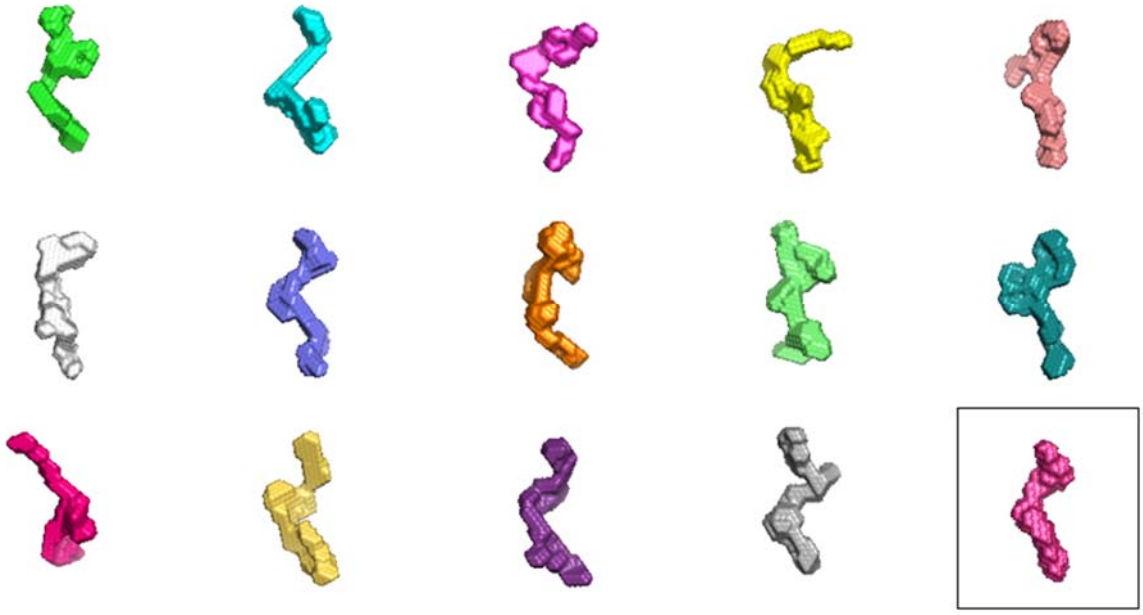

Figure S5: DAMMIF shape reconstructions of the pri-miR16-1 RNA in complex with the DGCR8-core protein (blanked at 50% sucrose) with the DAMAVER-DAMFILT-averaged dummy atom model boxed.

Shape reconstructions allowed us to compare the RNA alone to the RNA in complex with the protein, with the protein scattering blanked by sucrose. The averaged dummy atom model resulting from the reconstructions is shown in the main part of the manuscript and compared with PDB models. In Figures S4 and S5 above, we show the different dummy atom models derived from DAMMIF, prior to averaging and filtering using DAMAVER and DAMFILT. Note that the NSD for the reconstructions are 0.755 ± 0.024 for the RNA alone and 1.108 ± 0.065 for the RNA in complex. An NSD greater than 1 implies that there are systematic differences in the individual models, which could indicate some heterogeneity in the sample. However, the bend in the RNA persists as an overall feature in the reconstructed profiles for the RNA in complex. The SAXS measurements capture a bending or conformation change in the RNA due to the protein binding. In the main text, we use FRET as a complementary tool to confirm binding and bending the RNA due to the DGCR8-core protein.