The complete VS ribozyme in solution studied by small-angle X-ray scattering

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SUPPLEMENTARY MATERIAL

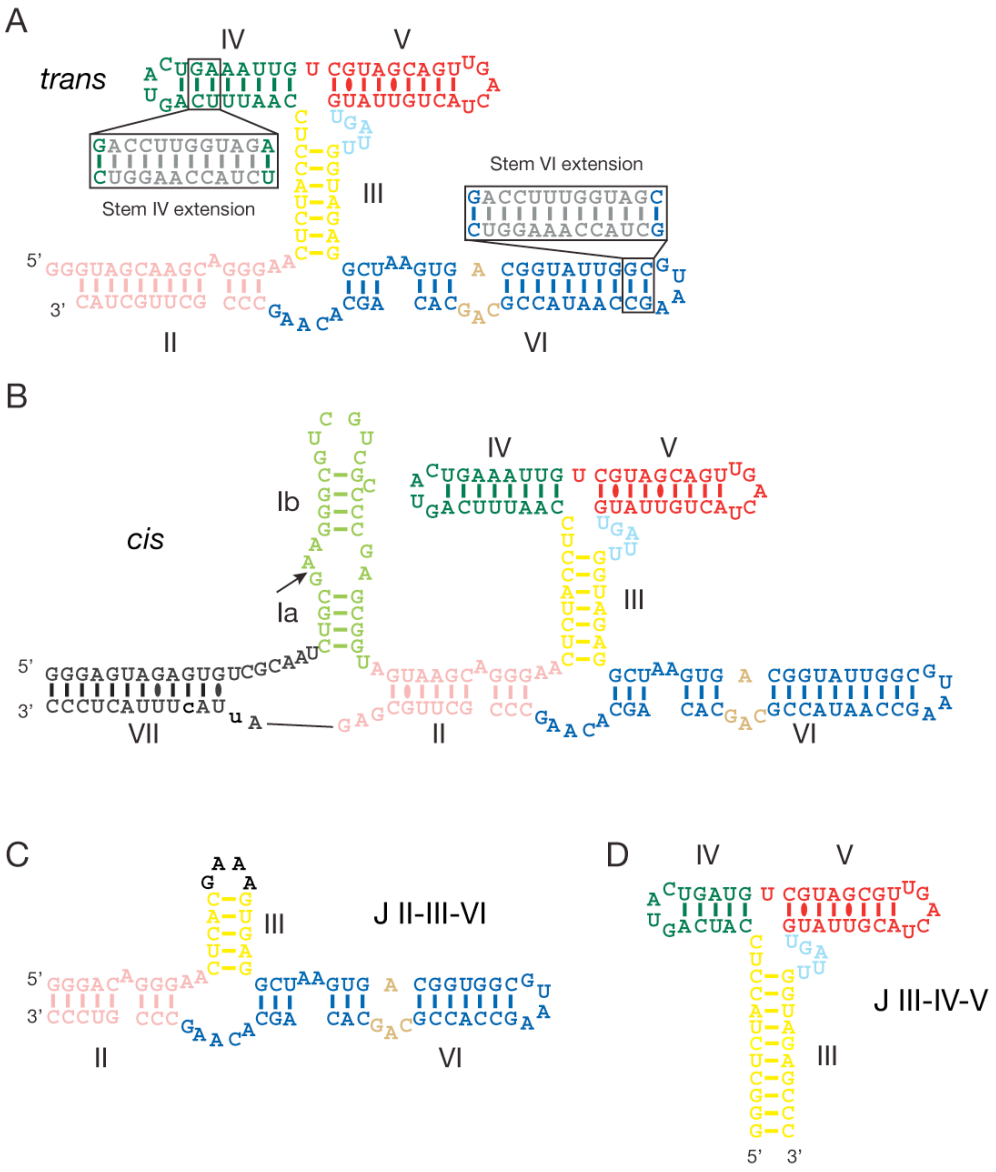

Figure S1. The sequences of all the constructs used in these studies.

A. The *trans*-acting ribozyme. Extra sections added to the constructs in order to lengthen helices IV and VI in separate species are shown boxed, in grey.

B. The *cis* ribozyme. The position of ribozyme cleavage is indicated by the arrow,

C. The II-III-VI junction.

D. The III-IV-V junction.

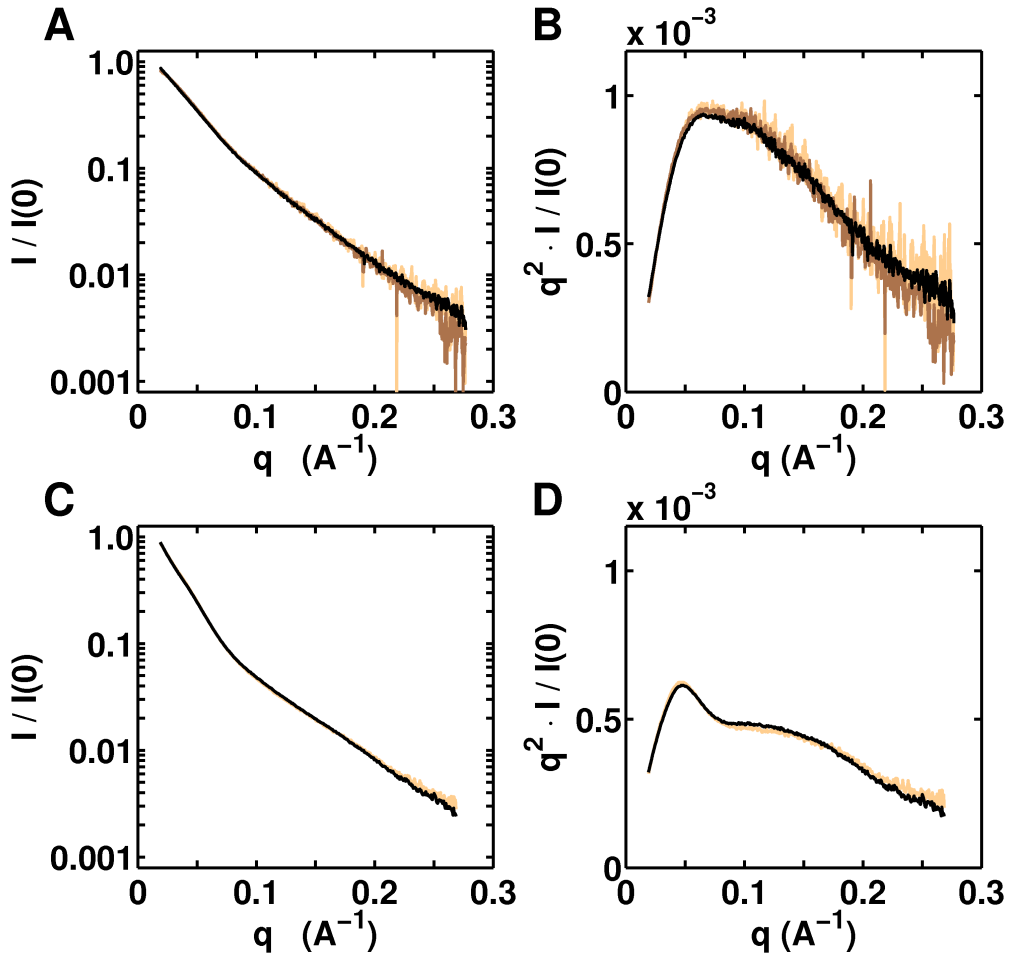

Figure S2. The shape of the scattering profiles is independent of RNA concentration.

Scattering profiles of the *trans* VS construct measured at RNA concentration of 0.5 (light brown), 1 (dark brown), and 2 (black) mg/ml in the presence of 10 mM Mg^{2+} (A, B). Scattering profiles of the *cis* VS construct measured at RNA concentration of 1 (light brown) and 2 (black) mg/ml in the presence of 100 mM Mg^{2+} (**C, D**). SAXS profiles are normalized by forward scattering intensity and are shown as log(I) as a function of q (**A, C**), and in Kratky representation [q^2I as a function of q] (**B, D**).

The fact that scattering profiles measured at different RNA concentrations are superimposable after rescaling by forward scattering intensity indicates that there are no significant aggregation or interparticle interference effects. Additionally we found that the forward scattering intensity increases linear with RNA concentration (data not shown), and we have determined the molecular mass of the samples from the forward scattering intensity by comparison to a nucleic acid molecular mass standard, using the formula

$$MM = \frac{I(0)}{I(0)_S}\frac{c_S}{c} MM_S$$

where *MM* is the molecular mass estimate, *I(0)* and $I(0)_S$ are the measured forward scattering intensities of the sample and of the molecular mass standard, respectively, c and c_S are the concentrations of the sample and molecular mass standard in mg/ml, and MM_S is the known molecular mass of the standard (Glatter and Kratky, 1982; Lipfert et al., 2008). We used a 24 bp DNA duplex prepared as described (Bai et al., 2005) at a concentration of 2 mg/ml in 50 mM Na MOPS, 10 mM $MgCl_2$ as molecular mass standard. The experimentally determined molecular mass values for all constructs analyzed in this work are shown in Table S1. The experimental values agree, within experimental errors, with the molecular mass predicted from the RNA sequences – see Table S1.

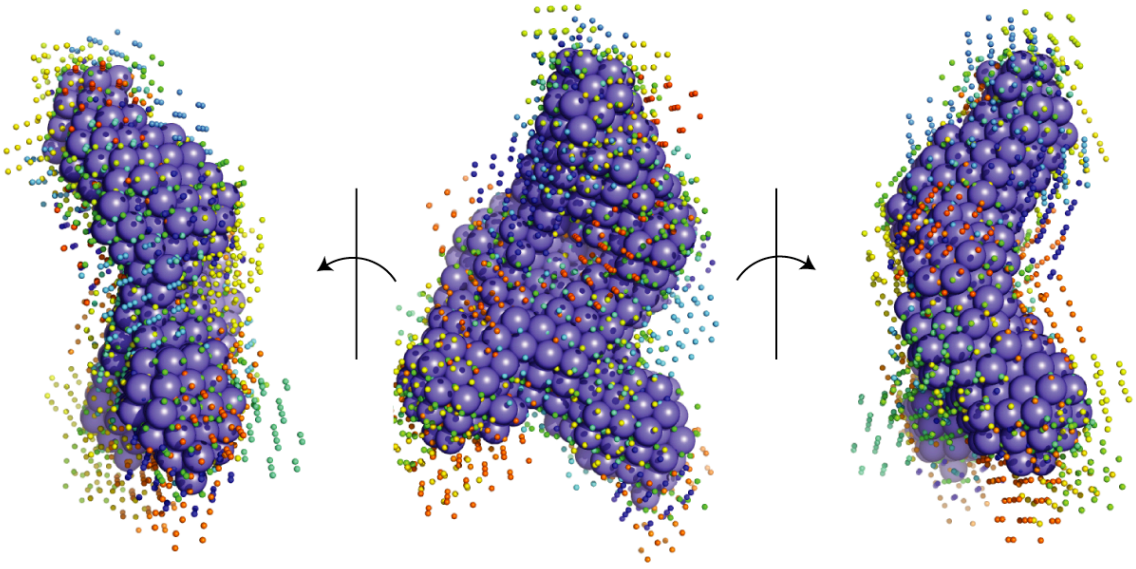

Figure S3. Superposition of individual bead reconstructions of the complete cis VS ribozyme with the average. Individual reconstructions are shown using the smaller bead radius, each in a separate color. The average structure is shown using the larger bead radius. Three views of the structure are shown, each rotated 90° about the vertical axis.

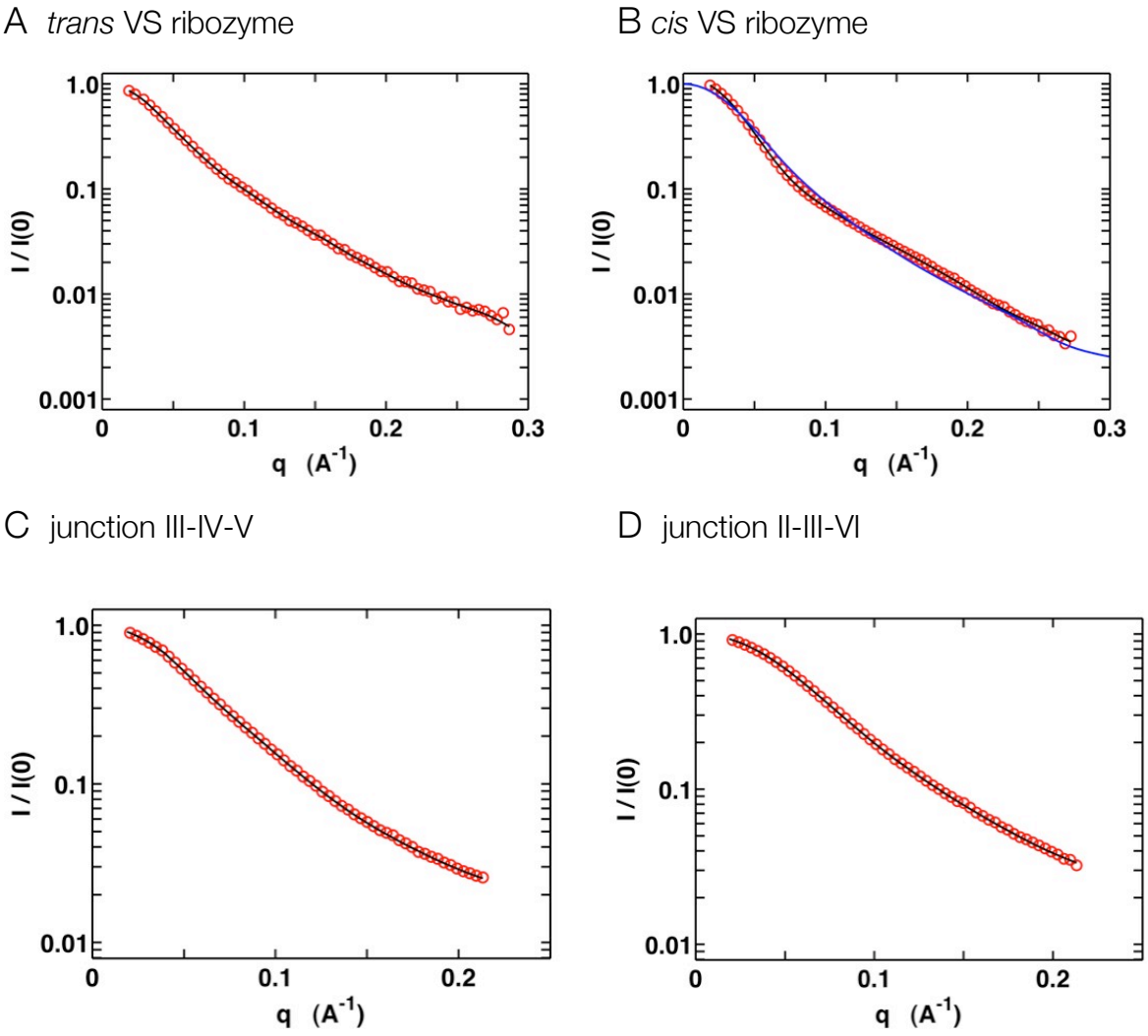

Figure S4. Experimental and calculated scattering profiles for the *trans* (A) and *cis* (B) VS ribozyme and the individual three-way junctions III-IV-V (C) and II-III-VI (D).

Experimental scattering profiles are shown as red circles, and the profiles calculated from *ab initio* constructed bead models are shown in black. The scattering profile computed from the atomic model of the *cis* ribozyme is shown in part **B** in blue. The number of points in the experimental profiles has been reduced for clarity.

	Mg^{2+} conc. / mM	measured MM / kDa	calc. MM / kDa	size / nt
trans	0	47 ± 4	45.5	141
trans	10	46 ± 5	45.5	141
trans; IV + 10 bp	10	53 ± 5	51.9	161
trans; VI + 11 bp	10	58 ± 6	52.5	163
cis	0	58 ± 6	64.2	199
cis	10	60 ± 6	64.2	199
III-IV-V junction	10	24 ± 3	20.2	63
II-III-VI junction	10	25 ± 3	24.1	74

Table S1. Molecular masses of the VS ribozyme constructs.
Experimental values were determined from the forward scattering intensity (see the legend of Figure S2). These can be compared with the molecular mass (MM) calculated from the sequence.

References

Bai, Y., Das, R., Millett, I.S., Herschlag, D. and Doniach, S. (2005) Probing counterion modulated repulsion and attraction between nucleic acid duplexes in solution. *Proc. Natl. Acad. Sci. USA*, **102**, 1035-1040.

Glatter, O. and Kratky, O. (1982) *Small-angle X-Ray scattering*. Academic Press, London.

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