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Analysis of riboswitch structure and ligand binding using small-angle X-ray scattering (SAXS)

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Summary

Small-angle X-ray scattering (SAXS) is a powerful tool for examining the global conformation of riboswitches in solution, and how this is modulated by binding of divalent cations and small molecule ligands. SAXS experiments, which typically require only minutes per sample, directly yield two quantities describing the size and shape of the RNA: the radius of gyration (R_g) and the maximum linear dimension (D_{max}). Examination of these quantities can reveal if a riboswitch undergoes cation-induced compaction. Comparison of the R_g and D_{max} values between samples containing different concentrations of ligand reveals the overall structural response of the riboswitch to ligand. The Kratky plot (a graphical representation that emphasizes the higher-resolution SAXS data) and the $P(r)$ plot or pair-probability distribution (an indirect Fourier transform, or power spectrum of the data) can provide additional evidence of riboswitch conformational changes. Simulation methods have been developed for generating three-dimensional reconstructions consistent with the one-dimensional SAXS data. These low-resolution molecular envelopes can aid in deciphering the relative helical arrangement within the RNA.

Keywords

Riboswitch; Small-angle X-ray scattering; RNA folding

1. Introduction

SAXS can yield data describing the global conformation of biological macromolecules in dilute aqueous solutions (1, 2). SAXS is a powerful tool for investigating RNAs under various conditions, for example, to examine the overall structural response of riboswitches to metal ions or cognate or non-cognate small molecules, changes in pH, temperature, etc. SAXS reports directly on the global structure of RNA and requires no labeling. This contrasts with biophysical or biochemical techniques that require labeling. For instance, fluorescence resonance energy transfer (FRET) employs fluorophores, and these report on

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³A useful forum for queries regarding general SAXS information, data analysis, and ATSAS software programs can be found at <http://www.saxier.org/forum/>.

their local environment, rather than on the global structure of the RNA. SAXS experiments do not suffer from the size and labeling limitations of NMR, or the requirement for well-ordered crystals of X-ray crystallography. The primary limitations of SAXS are that it provides only low-resolution structural information and that it does so on the ensemble of conformations present in solution.

SAXS is a rapid method for monitoring RNAs in solution. It has been employed to characterize the global collapse of catalytic RNAs as they fold in response to divalent cations, to characterize the folding intermediates of large RNAs (using time-resolved SAXS, for instance) and to characterize the ionic atmosphere surrounding RNAs (using anomalous SAXS) (3–6). Several studies have employed SAXS to characterize the response of riboswitch aptamer domains to divalent cations and small molecule ligands. These studies revealed that the folding behavior of these RNA domains is highly idiosyncratic (7–10). Specifically, while some aptamer domains such as those of the flavin mononucleotide (FMN) and class-I S-adenosylmethionine (SAM-I) riboswitches are largely preorganized in physiologic or higher concentrations of Mg^{2+} and display no additional overall compaction upon ligand binding, other aptamer domains, such as those of the class-I cyclic diguanylate (c-di-GMP-I) and thiamine pyrophosphate (TPP) riboswitches exhibit a large conformational change upon binding to their cognate ligands. SAXS studies on the glycine riboswitch have addressed the complex relationship between compaction of that RNA upon cation and ligand binding (11, 12). In addition to global size parameters, for RNAs with well-defined conformations, it is possible to obtain low-resolution shape information from three-dimensional reconstructions based on the SAXS data (13). These low-resolution reconstructions can in turn guide further studies that employ local probes (14).

SAXS can be carried out with X-rays generated in the home laboratory or using synchrotron X-radiation. Although home sources have the advantage of ready access to extensive biochemical laboratory facilities, the brightness and very high collimation of X-ray sources from synchrotron sources allow the use of considerably more dilute samples, both facilitating experiments with scarce material and reducing the likelihood of intermolecular interactions that complicate the analysis and interpretation of SAXS data. In addition, the use of synchrotron sources allows access to modalities such as time-resolved and anomalous SAXS, and makes it practical to collect radiation scattered at a higher Bragg angle (wide-angle X-ray scattering, or WAXS). In this chapter, we limit the discussion to techniques employed in basic (static, non-anomalous) SAXS characterization of riboswitch RNAs using synchrotron sources.

2. Materials

We assume that experiments will be carried out at a synchrotron beamline equipped for performing SAXS experiments with biological macromolecules. In addition to the materials listed below, the beamline should provide all necessary hardware (X-ray optics, data collection and control electronics) and software, software for initial data reduction and analysis, and software and hardware for sample manipulation. SAXS data can be analyzed using the ATSAS suite of programs (<http://www.embl-hamburg.de/biosaxs/software.html>). In reducing and analyzing data obtained at beamlines ID-12 and ID-18 at the Advanced

Photon Source (APS) at Argonne National Laboratory (Lemont, Illinois, USA), we have also used the Igor Pro (WaveMetrics) software package.

1. *In vitro* transcribed or synthetic riboswitch RNA. Methods for preparation of homogeneous RNA samples have been described elsewhere (15, 16).
2. Buffer, preferably Tris or HEPES which serve as free-radical scavengers. Data presented in this study were prepared in 20 mM Tris-HCl, pH 8 (SAM-I riboswitch, Table 1) or 20 mM HEPES-KOH, pH 7.5 (glycine riboswitch, Table 2).
3. MgCl₂, KCl and/or NaCl solutions to fold the RNA. Final solution conditions contained 40 mM NaCl and 1.5 mM MgCl₂ (SAM-I riboswitch, Table 1) or 40 mM KCl and 10 mM MgCl₂ (glycine riboswitch, Table 2).
4. Riboswitch ligands. In this study, we collected data in the absence and presence of saturating ligand concentrations (2 mM S-adenosylmethionine (SAM) or 10 mM glycine).
5. Size-exclusion chromatography (SEC) column, liquid chromatography system (optional).
6. Syringe filters for dust and aggregate removal (*e.g.* 0.02 µm Anotop filters, GE Healthcare).
7. Centrifugal concentrators of suitable molecular weight cut-off (*e.g.* Amicon concentrators, EMD Millipore).

3. Methods

3.1 General Considerations

The raw data obtained from a SAXS experiment is the scattered X-ray intensity as a function of momentum transfer, q (measured in reciprocal angstroms), where $q = 4\pi \sin \theta / \lambda$, 2θ is the scattering angle, and λ is the wavelength of X-rays used. The q -range, nominally the range of angles, is determined by the experimental geometry (distance from the sample to detector, beam-stop size and position, detector active area and angular resolution, etc.) and the energy of X-rays used. These parameters are generally set by the beamline scientist prior to data collection. The forward scattering intensity at zero angle, $I(0)$, is directly proportional to the molecular mass times the concentration (g/L) of the sample. Because of this relationship, scattering from dust particles and large aggregates, if present, will dominate the scattering signal from the macromolecules of interest. Thus, it is important to exclude aggregates from SAXS samples prior to data collection. Contributions from larger particles will dominate the observed scattering intensity, particularly at low q values where the radius of gyration (R_g) will be determined (see below), leading to errors. Finally, it is important to remember that riboswitch RNAs stability is a function of cation and ligand concentrations. We recommend monitoring the global conformational changes as a function of both of these.

3.2 Sample and Buffer Preparation

1. Each SAXS measurement will require ~100 μ L of sample at concentrations between 0.3 and 1.0 g/L (see Note 1).
2. Fold riboswitch RNA by addition of MgCl_2 and incubation of the RNA at a suitable temperature (e.g. 37°C – 50°C). After folding, equilibrate the reaction by incubation at 37°C for 15 – 30 min.
3. Samples must be homogenous. It is imperative to eliminate higher order oligomers which may result from misfolded or aggregated RNA. Demonstration of homogeneity can be accomplished at minimum by native gel electrophoresis or more explicitly by dynamic light scattering or SEC. The size-exclusion chromatogram of the SAM-I riboswitch (Fig. 1) demonstrates the presence of higher order complexes in a sample that was refolded following purification of the RNA by denaturing gel electrophoresis and desalting. The scattering data from the non-SEC-treated sample (Fig. 2b, *top*) do not demonstrate signs of aggregation and the Guinier plot (Fig. 2c) indicates only slight aggregation (see Initial SAXS data analysis below). More importantly, the chromatogram exhibits a significant peak (Fig. 1, peak A) that elutes earlier than the monomeric RNA. This peak comprises ~30% of the total absorbance, indicating that the original sample contains a significant fraction of a higher order oligomer, possibly a dimer. Analysis of SAXS data for the non-SEC treated inhomogeneous sample, containing both monomer and dimer, results in larger radii of gyration compared to the R_g 's obtained for the homogeneous monomeric samples, isolated from SEC peak B (Table 1). Furthermore, the smaller R_g of the monomeric sample bound to SAM is in good agreement with known crystal structures (17, 18) of the ligand-bound riboswitch. The discrepancy in R_g between the homogeneous and heterogeneous samples underscores the need for careful sample preparation (Table 1).
4. Samples must have matching blank buffer solutions. Close matching of blank and sample can be achieved either by SEC of the sample RNA in a column equilibrated in the blank buffer, or by performing multiple washes (sequential concentrations and dilutions) with the blank buffer using centrifugal concentrators (e.g. Amicon concentrators). Extensive dialysis will assure precise thermodynamic equilibrium, but is time-consuming and may not be compatible with integrity of the RNA.
5. If samples have not been purified by SEC, they must be passed through 0.02 μ m (i.e., 200 Å) Anotop filters to remove aggregates and dust (see §3.1, above).
6. Samples should be shipped to the beamline utilizing dry ice or cold packs or at room temperature, depending on sensitivity of samples to degradation or aggregation as a function of freezing or cooling.

¹RNA purification can be performed under either denaturing or native conditions. Regardless, the sensitivity of SAXS data to higher order oligomeric contaminants still requires demonstration that the sample is homogeneous.

3.3 SAXS Data Collection

The following instructions are based on our experience collecting SAXS data at two beamlines at the APS, ID-12 BESSRC and ID-18 BioCAT. Both beamlines utilize a similar experimental set-up and data analysis software. Data are collected under continuous flow to limit the exposure of the sample to X-ray radiation. For simplicity, we refer to any data collection event as a 'sample' regardless of whether the sample contains buffer or macromolecule. Although the specific unit operations will be somewhat different at different synchrotron beamlines, the overall procedures will still resemble those described below.

1. To remove any residual macromolecule from previous runs purge the sample flow cell using the Hamilton titrator syringe at the beamline with 2 mL of each of the following solutions in the given order: water, 20% bleach, water, isopropanol, and water.
2. Wash the flow cell extensively with sample buffer, making sure to leave no air in the sample line.
3. Initiate the beamline-programmed titration program, which is used to fill the flow cell with sample and subsequently to control the sample flow during data collection. Continuous flow of sample reduces radiation damage.
4. First, aspirate 20 μ L of air to generate a gap between the buffer-filled line and the sample being loaded. This air gap is more critical during the loading of the RNA sample in order to avoid mixing and dilution.
5. Aspirate 70 – 100 μ L of sample for data collection.
6. Close and interlock the experimental hutch. Open the beamline shutter.
7. Using the beamline-specific control script, enter the desired exposure time, number of exposures, and time between exposures. Collect twenty individual 1-second exposures without any time delay between exposures. Provide succinct sample names. It might be helpful to indicate in the filename whether the sample is a blank or contains RNA.
8. Initiate the data collection script while simultaneously initiating the titrator program for continuous sample flow.
9. After data collection has been completed, enter the experimental hutch and recover the sample.
10. Repeat steps 1 – 9 for each buffer/RNA combination.
11. If SAXS data will be measured on multiple RNA samples in the same buffer (*e.g.*, a dilution series), collection of buffer data can be carried out less often but must be obtained at least prior to the first RNA sample and following the last RNA sample utilizing that buffer. Agreement between the initial and final buffer samples is an indication that there have been no aberrant changes in the delivered beam intensity or focus during data collection and no deposition of macromolecule on the capillary during exposure to X-rays.

12. Samples can be re-used for data collection several times. However, after 3 or 4 collections, samples will begin to show signs of radiation damage (*see below*).

3.4 Initial SAXS Data Analysis

1. Radially integrate the data for each of the twenty 2-D scattering images, to produce twenty 1-D scattering profiles.
2. Import integrated data into data analysis software as scattering profiles of intensity vs. q ($\text{\AA}^{-1}$). Confirm that the 20 replicate exposures from each sample overlay. A few aberrant curves are likely the result of an air bubble in the flow cell line. These may be safely deleted. If there exists a general spread of non-overlapping curves, it may indicate radiation damage to the sample and the sample must be repeated and/or discarded.
3. Separately average and save the well-overlaid curves for each buffer or RNA sample (Fig. 2a).
4. Perform background subtraction of the averaged buffer scattering curve from the averaged RNA scattering curve. The buffer corrected intensity should exhibit a plateau at low q for globular macromolecules (Fig. 2b). Aggregation is often observed as a monotonically increasing intensity while moving toward the low q region.
5. Evaluation of the Guinier plot, $\ln [I(q)]$ vs. q^2 , at low q is the next step in analyzing the data quality. This plot should be linear; upward curvature in the plot is indicative of aggregation. Note that Fig. 2c (*top*) demonstrates a slight curvature in the Guinier plot, indicating a small degree of aggregation, while the background subtracted data in Fig. 2b (*top*) did not immediately indicate aggregation, as discussed above. The data in the two panels of Fig. 2c are fit in a similar low q region. In the top panel (non-SEC purified sample), the data deviate from the linear fit at low values of q^2 . This upturn is indicative of aggregation of the sample. Though only slight upturn is observed in this plot (*top panel*), samples suffering from severe aggregation will exhibit more considerable deviation from linearity. The data in the bottom panel are linear over the whole range of the fit. Aggregation may be removed by passing the sample through a $0.02 \mu\text{m}$ filter. Then the experiment can be repeated.
6. The Guinier analysis is valid in the low q region (as $q \rightarrow 0$), where the scattering data can be approximated as $I(q) \approx I(0)e(-q^2 R_g^2/3)$. A fit to Guinier plot in the low q region ($qR_g < 1.3$) results in a slope equal to $-R_g^2/3$ and a y-intercept equal to $\ln I(0)$. This extrapolated $I(0)$ scattering intensity can be compared with RNA standards which have been well-characterized, such as tRNA, to demonstrate that the sample is monomeric (or oligomeric, see next step below). Data at the lowest (and highest) q have increased noise, owing to the similarity in scattering intensity of the buffer and sample (Fig. 2a) and are much more sensitive to buffer mismatches. Hence, slightly mismatched buffer subtraction can also give rise to non-linearity at low q .

7. If a crystal structure of the riboswitch is available, the R_g can be calculated from the coordinates using CRY SOL, a program within the ATSAS suite (19). In such a case, agreement of the experimental R_g with the crystal structure R_g will suffice to demonstrate that the solution sample is monomeric and homogeneous. If no structural information is available for the riboswitch, it is important to compare forward scattering intensities, $I(0)$, with a standard sample to demonstrate that the riboswitch is monomeric (or a specific oligomer) and homogeneous. Data should be collected for the standard sample during each trip to the synchrotron. Because the forward scattering is proportional to the product of the molecular weight (MW) and the concentration (C, in g/L), comparison with a standard allows for estimation of the RNA sample molecular weight, $MW_r \propto MW_s C_s I(0)_r / C_r I(0)_s$, where the standard and RNA are indicated by the subscripts s and r, respectively. Table 2 provides an example. The isolated second aptamer of the dual-aptamer glycine riboswitch from *Fusobacterium nucleatum* exhibits an increase in R_g upon binding glycine. This was initially unexpected as the generally expected outcome is global compaction (decrease in R_g) in the presence of ligand. A closer inspection revealed a concomitant increase in $I(0)$, suggesting the presence of an oligomer. Increasing the $[Mg^{2+}]$ to 20 mM results in an additional increase in R_g and $I(0)$. The $I(0)$ nearly doubles under these conditions, indicating dimerization of this aptamer. In contrast, the first aptamer from this glycine riboswitch does not exhibit a significant increase either in R_g or $I(0)$ upon introduction of ligand, indicating that it does not dimerize. A closer look at the data from the first aptamer is useful as well. Not only does the first aptamer remain monomeric under all experimental conditions (no change in $I(0)$), but there is no change in R_g for this aptamer upon introduction of its cognate ligand, glycine. This could be the result of non-saturating ligand concentrations at the given $[Mg^{2+}]$. Alternatively, the riboswitch may not undergo a global conformational change upon ligand binding. A few additional examples have been reported in which ligand binding does not induce a global compaction. In the presence of saturating ligand concentrations neither the lysine riboswitch nor the FMN riboswitch undergo global conformation changes. The R_g 's of these riboswitch aptamers are unchanged in the absence and presence of ligand (8, 20).

3.5 Further SAXS Data Analysis

Observing conformational changes by SAXS goes beyond measurements of R_g . The Kratky and pair-distance probability distribution function, or $P(r)$, plots are helpful in comparing data obtained under various conditions (e.g. riboswitch +/- ligand, or low vs. high concentrations of Mg^{2+}).

1. The Kratky plot, $q^2 I(q)$ vs. q , is useful for comparing the compaction of the molecule under varying conditions. In such a plot, folded macromolecules exhibit a steep rise and peak followed by a pronounced decline (21, 22). Unfolded molecules do not exhibit a steep decline, and may continue rising throughout the q range. Intermediate conformations may exhibit a decline in the Kratky plot after the peak but the shape of the curve will be distinct from the most folded conformation, exhibiting a broader peak. Fig. 3a depicts differences in a Kratky plot between apo-

and ligand-bound SAM-I riboswitch samples. In the absence of SAM, the riboswitch is partially folded, as represented by a well-defined peak and decline. The riboswitch undergoes further structural organization to a final compact conformation in the presence of SAM, where the shape of the curve becomes more pronounced both in its peak and its decline.

2. The $P(r)$ plot (Fig. 3b) is a discrete Fourier transformation of the data which provides a measure of the likelihood of two electrons within the molecule being present at a distance r from each other. It is analogous to the power spectrum in physics or the Patterson function in crystallography. The curve provides information, in real-space, on the shape of the molecule and approaches zero at its maximum dimension, $D_{\max}$. This plot is useful for comparison of different samples to demonstrate changes in shape and $D_{\max}$.
3. Generation of the $P(r)$ plot is commonly performed using the program GNOM from the ATSAS suite (23). GNOM requires the background subtracted scattering profile as its input. An initial $D_{\max}$ estimate must be provided by the user.
4. The $P(r)$ plot must be iteratively tested, with the user varying the initial $D_{\max}$, to determine the most robust transformation of the raw data (*see* Note 2). A reasonable plot has three diagnostic features. First, the curve must approach zero at distances greater than or equal to $D_{\max}$. This requirement is imposed by the software. Second, the curve must be positive at all points. Finally, the curve should be generally smooth, particularly near the $D_{\max}$.
5. Because the scattering intensity is proportional to the concentration and molecular weight, Kratky and $P(r)$ plots generated from samples containing the same RNA (e.g. apo-riboswitch RNA and ligand-bound riboswitch RNA) can be normalized to the forward scattering intensity of each sample. This normalization avoids the pitfall of over-interpreting differences in peak heights between the samples that arise from differences in concentration.

3.6 Three-Dimensional Shape Reconstructions Based on SAXS Data

Beyond one-dimensional scattering plots and numerical descriptions of RNA conformations (R_g , $D_{\max}$), *ab initio* reconstructions based on the scattering data can provide low-resolution models of the riboswitch conformation in solution. Dummy atom models can be created using the program DAMMIF from the ATSAS suite (24). This program uses simulated annealing procedures to generate models whose scattering profiles are consistent with the experimental data. Several previous SAXS studies of riboswitches have employed dummy atom models to interpret conformational changes induced by ligand binding. For instance, in the TPP and cyclic-diguanylate riboswitches large-scale reorientations of specific helical elements have been observed by examination of the reconstructed models in the absence and presence of ligand (8, 10, 25). In these cases and others, general agreement has also been reported between solution SAXS reconstructions and available crystal structures.

²Jacques and Trewhella have recently published a helpful review regarding SAXS experimentation and data treatment. Their review provides additional descriptions that complement the protocol described here, particularly regarding data evaluation (27).

1. Within the ATSAS suite of programs, the DAMMIN/F programs are used to reconstruct a dummy atom model containing an array of beads. The two programs differ in their algorithms (and required processing time) but yield comparable results.
2. The output from GNOM is the input required for DAMMIF. We recommend utilizing the output from three individual GNOM runs, which themselves differ by the user-input $D_{\max}$, near the actual $D_{\max}$ and larger. Perform 10 – 15 DAMMIF reconstructions for each GNOM output file used. Use of the default parameters and 'slow' mode is sufficient (13). Running 10 DAMMIF reconstructions simultaneously requires 50 min on an iMac equipped with a 2.66 GHz Intel Core 2 Duo processor and 4 GB of RAM.
3. Use DAMAVER (26) from the ATSAS suite to align, average and filter the ten 3-D reconstructions. The DAMAVER log file will list the normalized spatial discrepancy (NSD) for each model after comparison with all other models. $NSD < 1$ indicates similarity of each model to those against which it was compared. Visually inspect each of the three filtered dummy atom models (generated from the three GNOM output files used) to demonstrate convergence of the data to a consistent dummy atom model structure. This comparison also provides a visual evaluation of the general variance between the dummy atom models. Understanding the variance between the reconstructed models of the same data will help avoid over-interpretation of the structural changes observed between models from *different* solution conditions. For reference, Fig. 4 demonstrates the agreement between the filtered DAMMIF reconstruction of the SEC-treated SAM-I SAXS data in the presence of 2 mM SAM with an available crystal structure of the SAM-I riboswitch (17).

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Fig. 1.

Size-exclusion chromatogram of refolded SAM-I riboswitch. RNA was heated in 20.3 mM Tris-HCl, pH 8, 40.6 mM NaCl for 2 min at 85 °C followed by incubation at room temperature for 5 min. $MgCl_2$ was added to yield final concentrations of 20 mM Tris-HCl, 40 mM NaCl, and 1.5 mM $MgCl_2$. The sample was loaded onto a Superdex 200 HR10/30 column (GE), pre-equilibrated in a buffer matching the final sample conditions. Two distinct peaks are observed, labeled A and B, along with an early 'shoulder' which may contain higher-order oligomers. The maximum absorbance of peak B is beyond the sensitivity range of the detector. Absorbance at 290 nm was monitored for the same reason; both peaks A and B were outside the range of the detector at 260 nm and 280 nm wavelengths.

Fig. 2.

SAXS data obtained for the SAM-I riboswitch aptamer. Data in top panels were collected on samples not purified by SEC. Data in bottom panels were collected from samples purified by SEC (RNA from peak B in Fig. 1 was used for SEC purified SAXS data collection). The ordinate in each plot is scaled to maximize differences between the apo (black) and bound (grey) samples. (a) Primary data in the form of integrated, averaged one-dimensional scattering profiles for sample and buffer are plotted as intensity vs q . The buffer curves in the absence and presence of SAM are identical. (b) The background-subtracted primary data exhibit the typical plateau at low q . (c) Guinier plots of the SAM-I riboswitch from which

R_g and $I(0)$ are obtained (see text). The curves and fits in the top panel have been manually offset for clearer inspection.

Fig. 3.

Transformations of the primary SAXS curve (SEC-treated samples). (a) A Kratky plot is used to evaluate the compaction and relative degree of folding of the molecule. The SAM-I riboswitch in both the absence and presence of SAM is generally folded, exhibiting a characteristic peak and subsequent decline in the Kratky plot. The addition of SAM results in additional compaction and folding, as observed by the increased peak and steep decline (see text). (b) The $P(r)$ plot is a real-space transformation of the primary SAXS data and reports on the general shape changes of the riboswitch in the absence and presence of SAM. Additionally, the maximum distance within the molecule, $D_{\max}$, is obtained from this plot.

Fig. 4. Three-dimensional reconstructions of SAXS data. Ten independent DAMMIF reconstructions were run using GNOM output from the SEC-purified SAM-I riboswitch aptamer bound to SAM (+ 2 mM SAM). The dummy atom models were then aligned, averaged, and filtered using DAMAVER. The top panel illustrates the overlay of the dummy atom reconstruction and the SAM-I crystal structure (PDB ID 3GX5). The bottom panel shows the crystal structure in the same orientations as the top panel. The resulting filtered DAMMIF model matches the dimensions and general shape of the crystal structure well. Images were prepared using CHIMERA (28).

Table 1SAXS R_g analysis of *T. tengcongensis* SAM-I riboswitch

	R_g (Å) – SAM	R_g (Å) + SAM
Non-SEC treated RNA	29.9	26.3
SEC-treated RNA	25.3	22.8
Crystal Structure	n.a.	21.5

Experiments were performed in 20 mM Tris-HCl, pH 8, 40 mM NaCl, 1.5 mM MgCl₂.

n.a. – not applicable. The crystal structure R_g was calculated from PDB ID 3GX5 using CRY SOL from the ATSAS suite.

Table 2SAXS analysis of *F. nucleatum* glycine riboswitch single aptamers

	Aptamer #1 R_g (Å)	Aptamer #2 R_g (Å)	Aptamer #1 $I(0)$	Aptamer #2 $I(0)$
– glycine	23.2	29	43.6	52.6
+ glycine	24.1	33.6	52.2	71.2
+ glycine, high Mg^{2+}	24.2	37.6	54.6	93.9

All experiments were performed in 20 mM Tris-HCl, pH 8, 40 mM KCl, 10 mM $MgCl_2$, excepting the high Mg^{2+} condition which contained 20 mM $MgCl_2$. All samples were prepared to ~0.5 mg/mL.