Supplementary Information

Molecular envelope and atomic model of an anti-terminated *glyQS* T-box regulator in complex with $tRNA^{Gly}$

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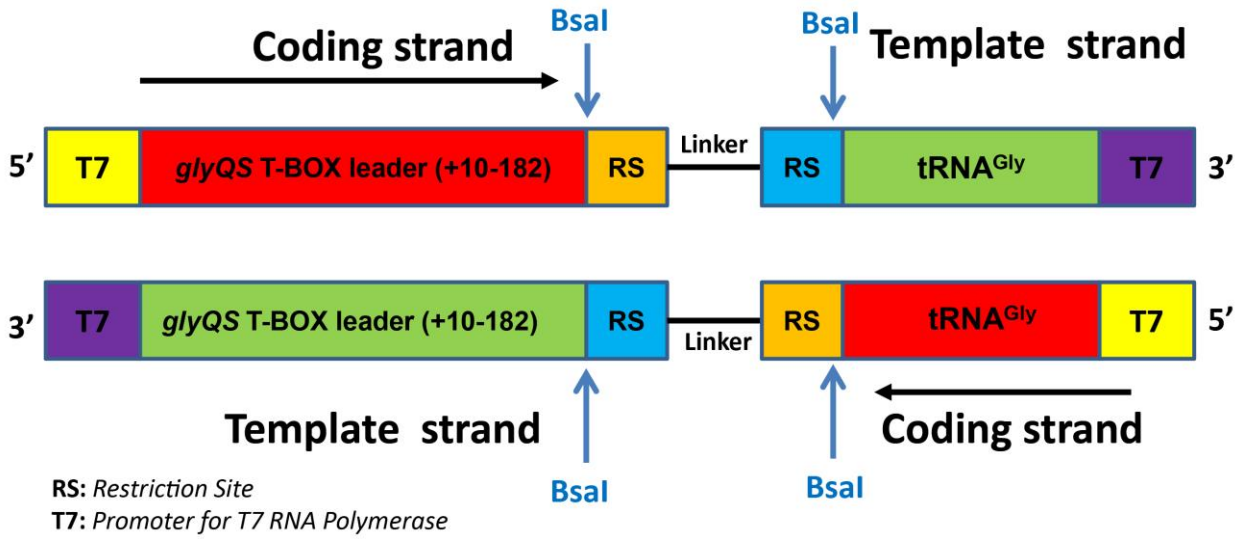
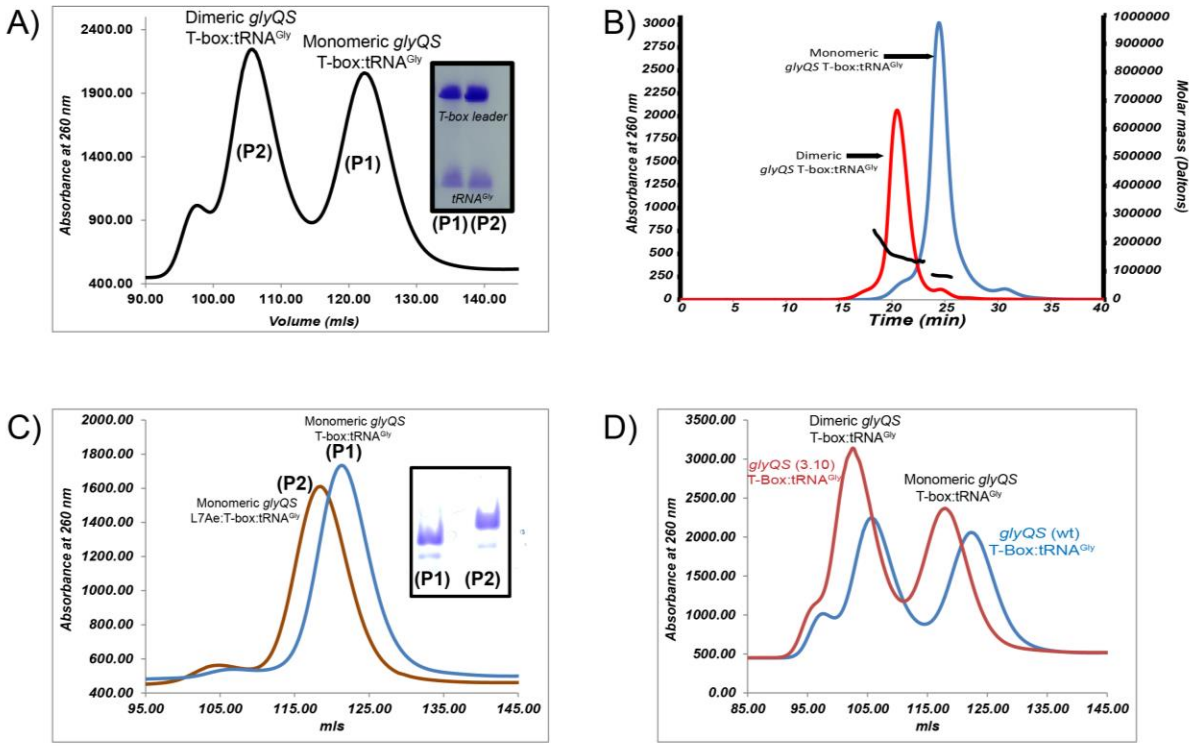
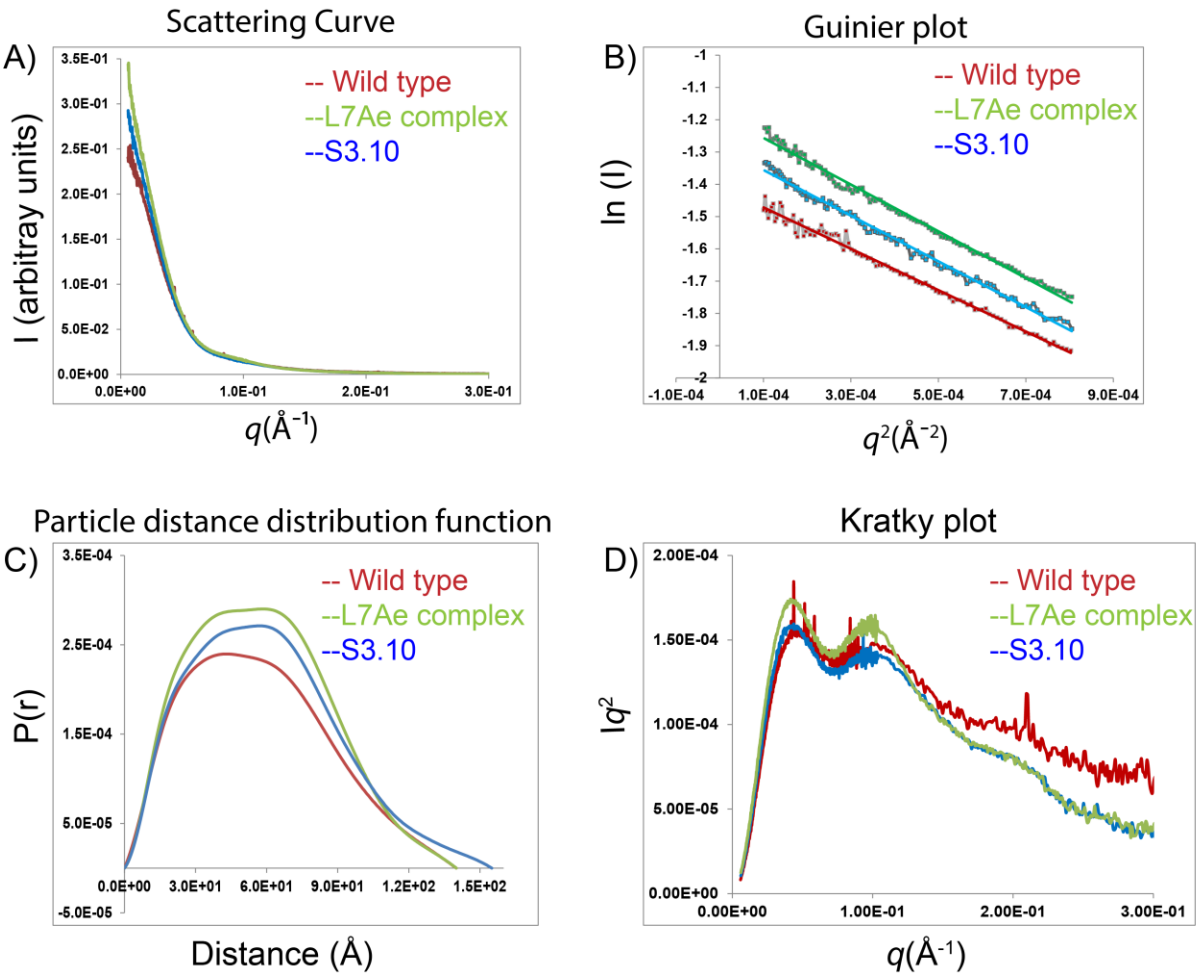


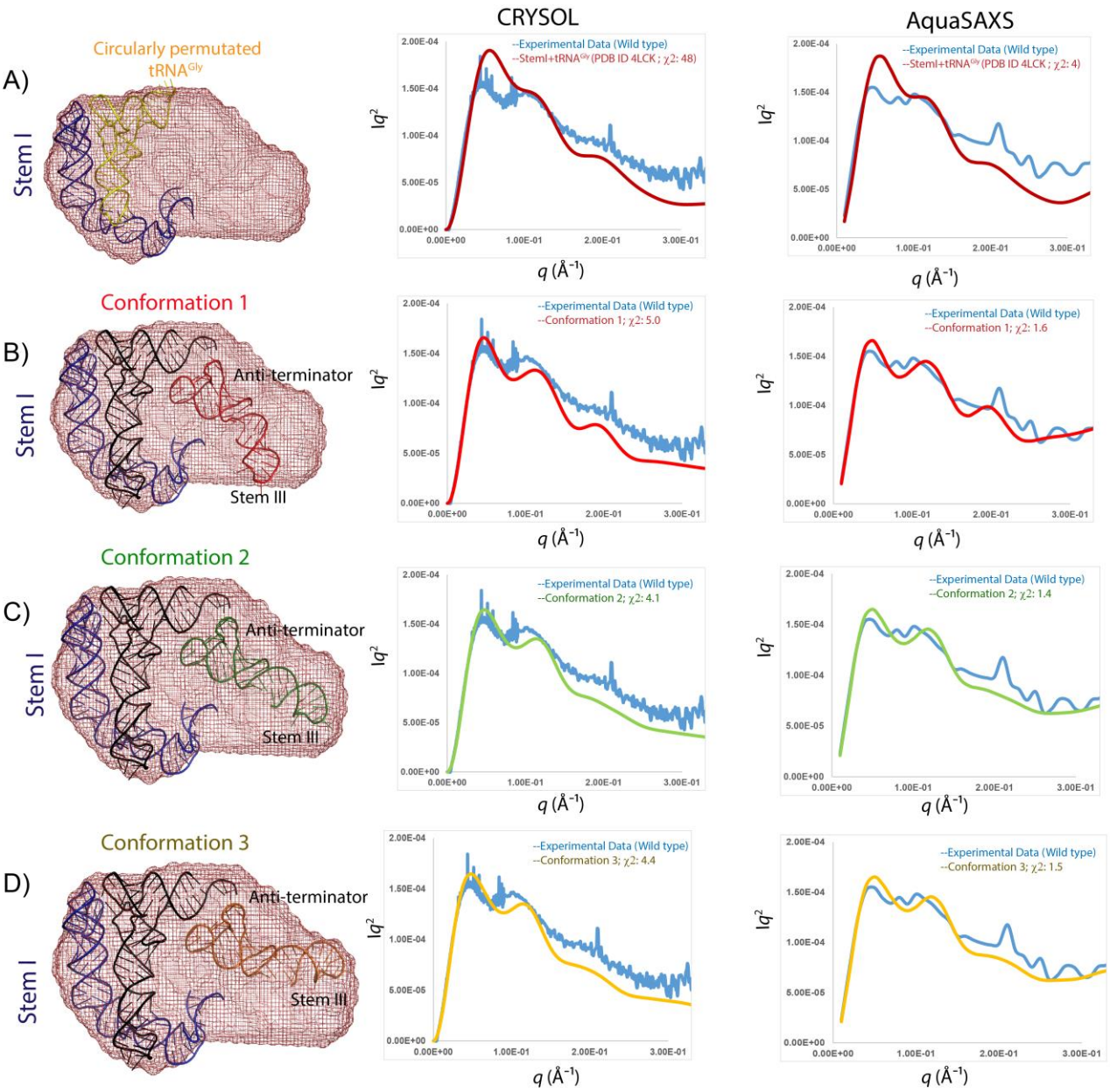
Supplementary Figure 1. Schematic diagram showing the design of the bicistronic DNA construct for *in vitro* transcription of the *B. subtilis glyQS* T-box leader and its cognate tRNA^{Gly}. RS corresponds to enzyme restriction sites used to linearize the circular plasmid, T7 to the T7 RNA polymerase promoter sequence. Sequences for the two RNAs are shown in Figure 1.



Supplementary Figure 2. Purification of the *B. subtilis glyQS* T-box:tRNA^{Gly} complexes for structural studies by SAXS. A) Elution profile of the *in vitro* transcription reaction for the wild-type *B. subtilis glyQS* T-box:tRNA^{Gly} complex. The horizontal and vertical axes correspond to elution volume (ml) and absorbance at 260 nm (arbitrary units), respectively. The RNA content of the two main peaks (P1 and P2) was tested on an 8M urea gel (inset). **B)** The molecular weight and homogeneity of the T-box:tRNA complex in P1 and P2 was determined by SEC-MALS, which shows that P1 and P2 corresponds to monomeric (1:1) and dimeric (2:2) complexes, respectively. The horizontal and vertical axes correspond to the elution time (min), absorbance at 260nm (arbitrary units), and molar mass (daltons), respectively. **C)** SEC analysis showing that the monomeric *B. subtilis glyQS* T-box:tRNA^{Gly} complex forms a stable ternary complex with the K-turn binding L7Ae protein. The inset shows a gel shift assay stained for RNA showing complex formation. The horizontal and vertical axes correspond to elution volume (ml) and absorbance at 260 nm (arbitrary units). **D)** Elution profiles of the *in vitro* transcription reaction for the *B. subtilis glyQS* (S3.10) T-box:tRNA^{Gly} and the wild-type *B. subtilis glyQS* T-box:tRNA^{Gly} complexes. The horizontal and vertical axes correspond to elution volume (ml) and absorbance at 260 nm (arbitrary units) respectively.

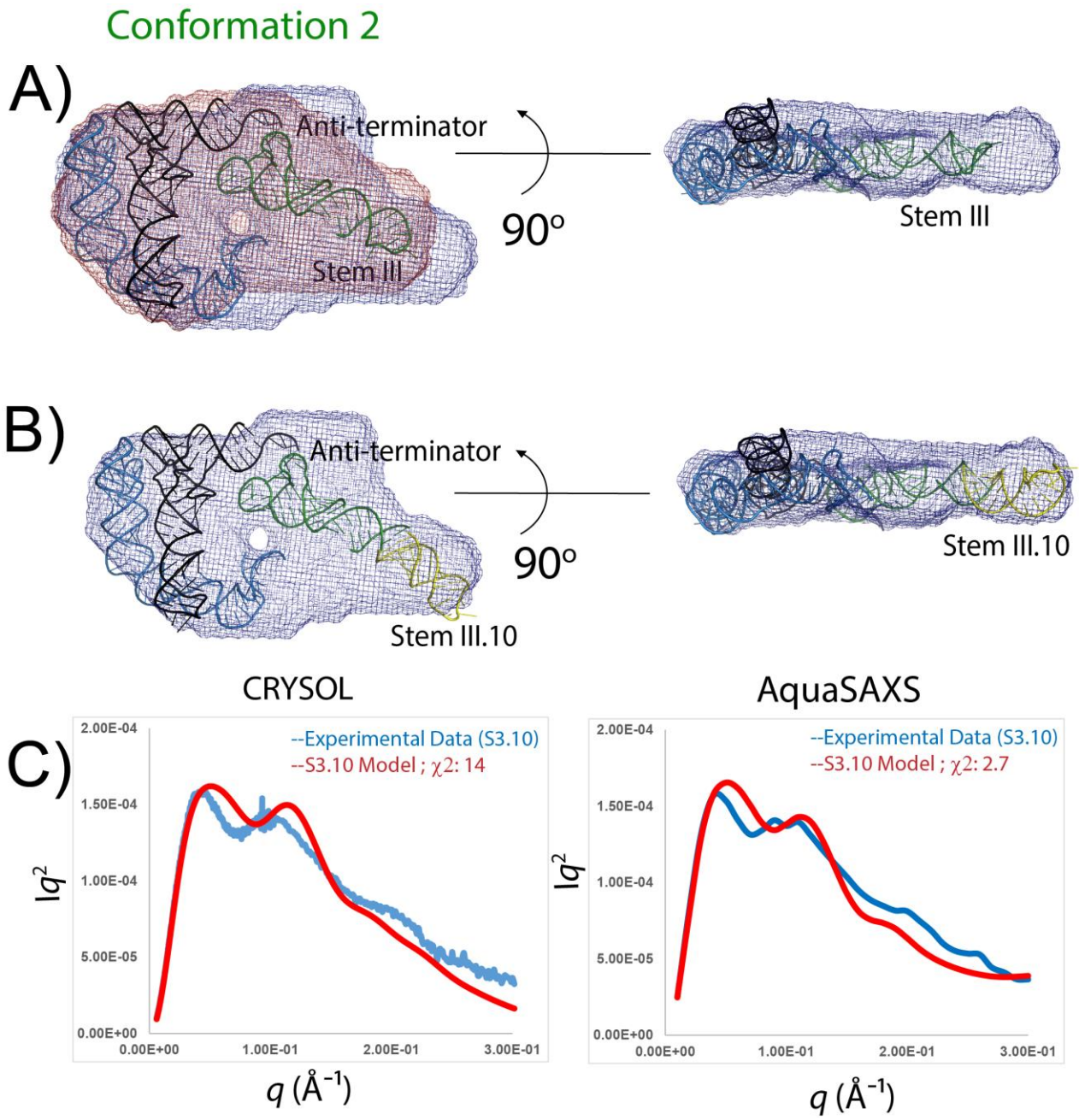


Supplementary Figure 3. Small-angle X-ray scattering data of the different monomeric *B. subtilis glyQS* T-box:tRNA^{Gly} complexes analyzed. A) Experimental SAXS curves. The horizontal and vertical axes correspond to q (Å^{-1}), the momentum transfer, and the scattering intensity (I) (arbitrary units). **B)** Guinier plots. The horizontal and vertical axis correspond to q^2 (Å^{-1})^2 and the logarithm of the scattering intensity, respectively. **C)** Pairwise distribution functions P(r) calculated from the final data sets. The horizontal and vertical axes correspond to the Distance (Å) and the calculated P(r). **D)** Kratky plot calculated from the final data sets. The horizontal and vertical axis correspond to q (Å^{-1}) and Iq^2 respectively.



Supplementary Figure 4. Comparison of theoretical scattering profile computed from different structural models with the experimental scattering profile for wild type *glyQS* T-box:tRNA^{Gly} complex. The left panels in **A)** to **D)** show the location of various structural models in the SAXS derived molecular envelope of the wild type *glyQS* T-box:tRNA^{Gly} complex. The middle and right panels depict Kratky plots computed from the theoretical and experimental scattering curves in which the horizontal and vertical axis correspond to q (Å^{-1}) and Iq^2 respectively. The middle panels in **A**) to **D**) show the fit of experimental and theoretical scattering profile computed using CRYSOL (1). The right panels show the fitting of the same experimental and theoretical scattering profile computed using AquaSAXS (2). Note that the two programs use different algorithms for modelling the primary hydration shells and, overall, AquaSAXS produces a better fit of the theoretical data to the experimental data. **A)** shows the fit of the

crystal structure of Stem I in complex with a circularly permuted $tRNA^{Gly}$ **(PDB ID: 4LCK)** (3)**. B)-D)** shows the fit of three representative conformers with different relative orientation (Conformation 1 to 3) of Stem III with respect to the anti-terminaor helix.



Supplementary Figure 5. Location of the anti-terminator helix and the elongated Stem III in the molecular envelope of the *glyQS* (S3.10): tRNA^{Gly} complex. The left panel in **A)** depicts the superposition of the molecular envelopes of wild type *glyQS*:tRNA^{Gly} complex (red) onto the *glyQS* (S3.10): tRNA^{Gly} complex (blue) and the structural model which fits best to the wild type experiemental data as a cartoon. **B)**. The conformation of Stem III that fits best to the wild type experimental data was extended by adding 10 additional base pairs (yellow). In **B)**, orthogonal views of the molecular envelope are shown, which support the position and orientation of the elongated Stem III helix. **C)** Fit of the theoretical scattering profile computed from the model *glyQS* (S3.10): tRNA^{Gly} complex to the experimental profile. The left panel in **C)** was computed using CRYSOL (1) and right panel was computed using AquaSAXS (2). Note that the two programs use different algorithms for modelling the primary hydration shells and, overall, AquaSAXS

produces a better fit of the theoretical data to the experimental data. The theoretical and experimental scattering profile in **C)** are depicted as Kratky plots in which the horizontal and vertical axis correspond to q ($Å^{-1}$) and Iq^2 respectively.

Supplementary Table I. SAXS data collection and processing parameters

	T-box (wt):tRNA^{Gly}	T-box (wt):tRNA^{Gly}:L7Ae	T-box(S3.10):tRNA^{Gly}
Data-collection parameters			
Source	ID5-D at DND CAT (APS)	ID5-D at DND CAT (APS)	ID5-D at DND CAT (APS)
Instrument	Rayonix SAXS/MAXS/WAXS CCD	Rayonix SAXS/MAXS/WAXS CCD	Rayonix SAXS/MAXS/WAXS CCD
Beam geometry	0.25 mmx 0.25 mm pin-hole	0.25 mmx 0.25 mm pin-hole	0.25 mmx 0.25 mm pin-hole
Energy (KeV)	10	10	10
q range (Å^{-1})	0.0015-2.6	0.0015-2.6	0.0015-2.6
Total exposure time (sec)	50	50	50
Concentration range (mg/ml)	0.2-0.74	0.2-0.55	0.21-0.54
Temperature (°K)	293	293	293
Structural Parameters			
I(0) (cm^{-1}) [from P(r)]	0.24	0.29	0.27
Rg (Å) [from P(r)]	44.6	44.72	46.35
I(0) (cm^{-1}) [from Guinier]	0.24±0.00041	0.30±0.00069	0.27±0.00058
Rg (Å) [from Guinier]	43.17±1.29	44.98±1.19	45.52±1.34
D_{max} (Å)	140	140	155
Porod volume estimate (Å^3)	194341	217116	227480
Dry volume (Å^3) calculated from model	92990	--	--
Molecular Mass determination			
Partial specific volume (cm^3/mg)	5.4x10^{-4}	5.4x10^{-4}	5.4x10^{-4}
Molecular Mass Mr [from I(0), *q*=0.3] (KDa)	77.4 ± 8.3	103±5.5	100.7±7.1
Calculated monomeric Mr from sequence (kDa)	82	96	88
Software employed			
Primary data reduction	In-house package at DND CAT	In-house package at DND CAT	In-house package at DND CAT
Data processing	PRIMUS (ATSAS)	PRIMUS (ATSAS)	PRIMUS (ATSAS)
Ab initio analysis	DAMMIN	DAMMIN	DAMMIN
Validation and averaging	DAMVER	DAMVER	DAMVER
Rigid body modelling	PYMOL	PYMOL	PYMOL
Computation of model intensities	CRYSOL;AquaSAXS	CRYSOL;AquaSAXS	CRYSOL;AquaSAXS
3D graphics representations	PYMOL	PYMOL	PYMOL

Supplementary Table II. Sequence features of Stem III helix from *glyQS* T-box riboswitch in closely related gram positive bacteria.

Organism	Stem III Sequence	Length	Stability (ΔG)[#]
Bacillus subtilis	AGGCTGGGATTTTGTTCTCAGC	22	-9.3 kcal/mole
Bacillus amyloliquefaciens	AGGCTGAGTGATTCACATCCTCAGC	25	-9.6 kcal/mole
Bacillus atrophaeus	AGGCTGGGGGTTAAATCCCGGC	22	-12.7kcal/mole
Oceanobacillus iheyensis	TGGCTCATGTATCTTGAGC	19	-5.2 kcal/mole
Geobacillus kaustophilus	TGGGTGCGCGTTTGGCGCATC	21	-10.2kcal/mole

[#] Values obtained from Mfold server (4) by using Stem III sequence as an input.

References.

1. Svergun, D., Barberato, C. and Koch, M.H.J. (1995) CRYSOL - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates. *J. Appl. Crystallogr.*, **28**, 768-773.
2. Poitevin, F., Orland, H., Doniach, S., Koehl, P. and Delarue, M. (2011) AquaSAXS: a web server for computation and fitting of SAXS profiles with non-uniformally hydrated atomic models. *Nucleic Acids Res.*, **39**, W184-189.
3. Zhang, J. and Ferre-D'Amare, A.R. (2013) Co-crystal structure of a T-box riboswitch stem I domain in complex with its cognate tRNA. *Nature*, **500**, 363-366.
4. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*, **31**, 3406-3415.