THE LIGAND-FREE STATE OF THE TPP RIBOSWITCH, A PARTIALLY FOLDED RNA STRUCTURE

Supplementary Information

Mona Ali[1], Jan Lipfert[2], Soenke Seifert[3], Daniel Herschlag[4], Sebastian Doniach[1]

[1]Deparment of Applied Physics, Stanford University, Stanford, CA 94305, USA

[2]Kavli Institute of Nanoscience, Delft Institute of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

[3]Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439 USA

[4]Deparment of Biochemistry, Stanford University, Stanford, CA 94305, USA

Discrepancy in experimental data for TPP bound state

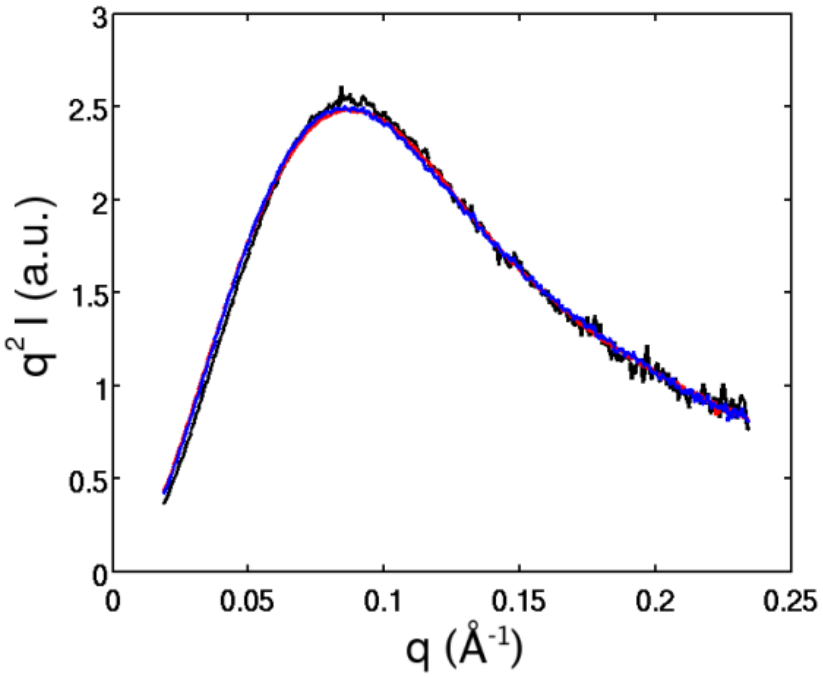

Figure S1: Experimental SAXS profiles obtained for the TPP bound state of the riboswitch for three independent sample preparations and measurements. Each independent preparation and measurement is shown in a different color: red, blue or black. Guinier analysis of the profiles yielded an R_g of 24 Å for the measurements represented by the red and blue profiles. The black profile gave an R_g of 22 Å, which is closer to the R_g of 20 Å calculated from the crystal structure. The preparation and purification of the RNA was identical for each of the measurements shown. The black profile has been used in the data analysis presented in our results, due to better agreement with the theoretical profile calculated from the crystal structure of the bound state of the riboswitch.

Cutoff rank number for isolated junctions

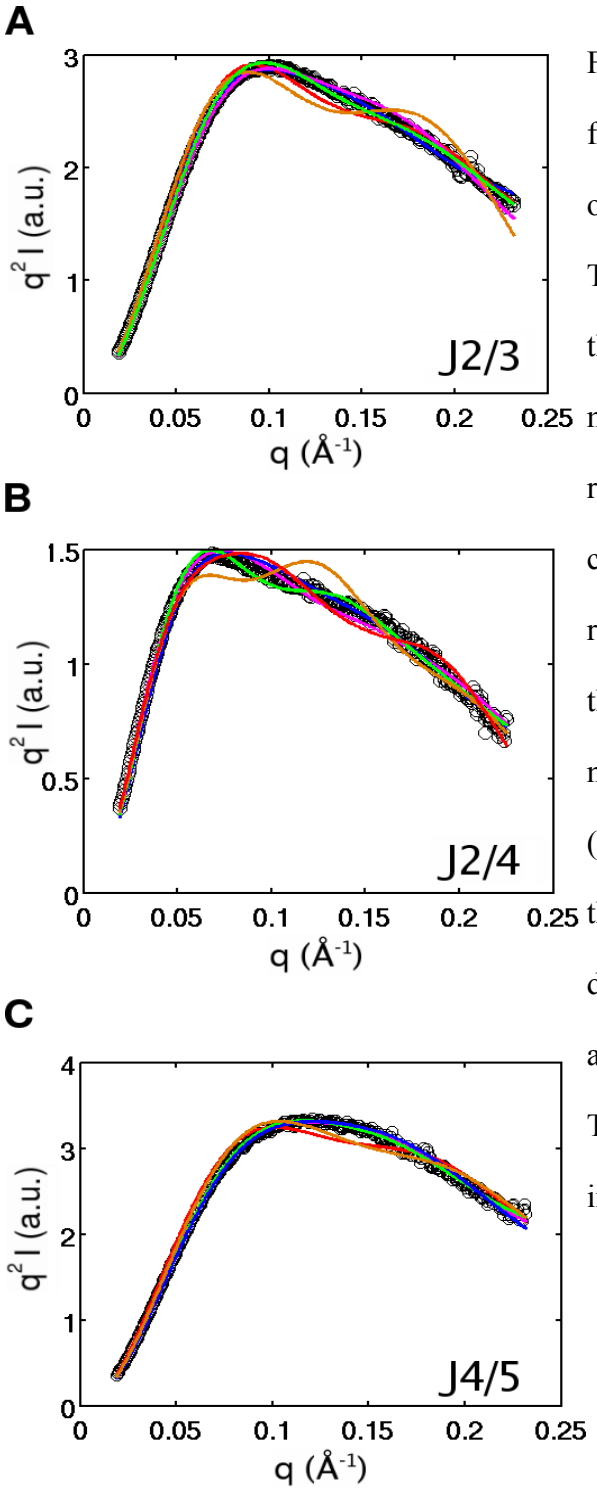

Figure S2: Theoretical SAXS profiles for the first five ranked models of the junctions overlaid on experimental SAXS data for that junction. The black circles show experimental data, and the solid lines show the profiles from the models. The models for each junction were ranked according to the goodness of fit using a chi-squared criterion (Methods). The first five ranked models for each junction are shown, and the lines are colored according to the rank number: 1st (magenta), 2nd (blue), 3rd (green), 4th (red), 5th (brown). For each junction, the first three models give good fits to the experimental data (p-value > 0.95), whereas the fourth model and beyond give progressively worse fits. Therefore, models ranked from 1 to 3 are included in the divide-and-conquer approach.

Highest ranking models of ligand-free state from global approach

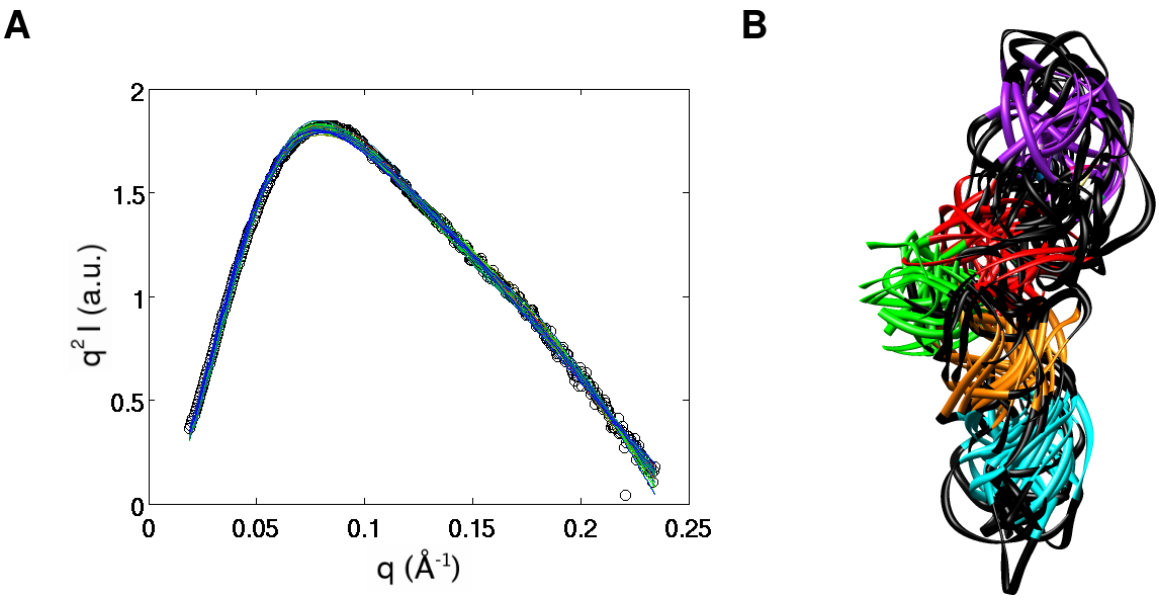

Figure S3: Models of the ligand-free state from global approach. (A) Theoretical SAXS profiles of models of ligand-free state superimposed on experimental data for the riboswitch in excess Mg^{2+}. Black circles show experimental data, and the solid lines indicate profiles from the different models. (B) Models of the ligand-free state that give good fits to experimental data in (A), superimposed on one another. The helices are colored as defined in Figure 1: P1 (green), P2 (red), P3 (purple), P4 (orange), P5 (cyan). The non-base paired regions are colored black. The models all adopt an extended conformation, where L3 and L5 are far apart from one another.

Highest ranking models of ligand-bound state from global approach

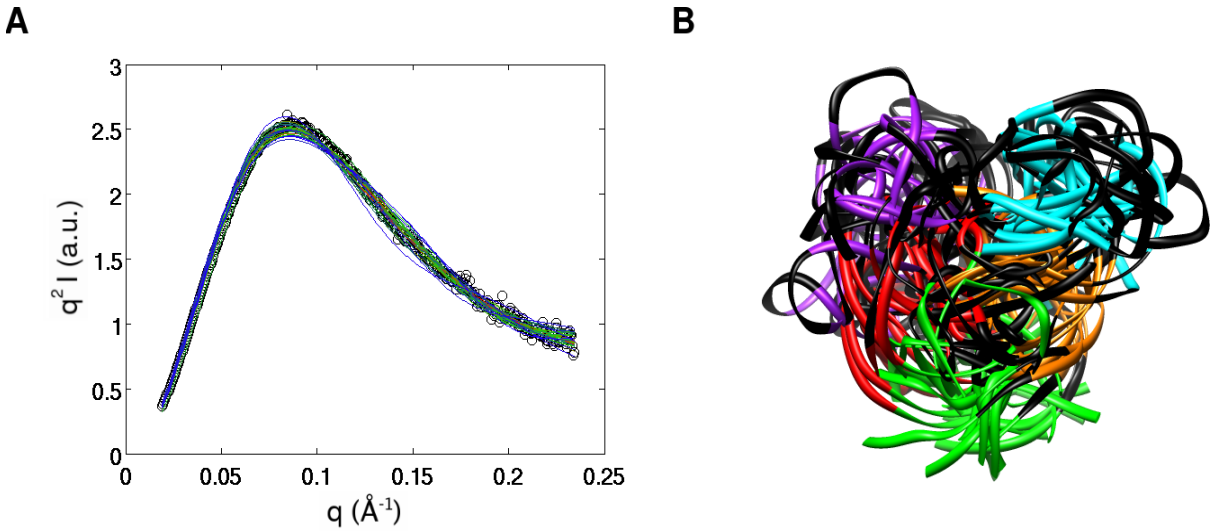

Figure S4: Models of ligand-bound state from global approach. (A) Theoretical profiles (solid lines) calculated from models, which give good fits to experimental data (black circles). The lines are colored from red to blue, showing the profiles from different models of the ligand-bound state. (B) Models of ligand-bound state, which give good fits to experimental data in (A), superimposed on one another. The helices are colored as in Figure 1: P1 (green), P2 (red), P3 (purple), P4 (orange), P5 (cyan). The non-base paired regions are shown in black. Although variations exist between the models, they all consistently show a conformation where L3 and L5 are close together, in a compact form, similar to that observed in the crystal structure.

Models of bound-state isolated using experimental profile with higher R_g value

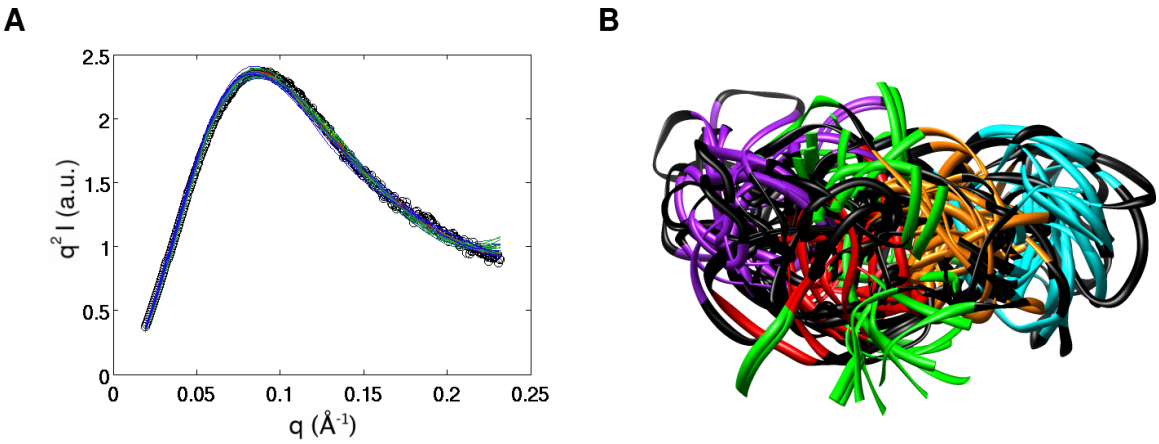

Figure S5: Highest ranked models of the ligand-bound state compared to experimental SAXS profiles giving R_g value of 24 Å, as opposed to 22 Å (which is used to obtain the structures shown in Figure S4, and is described in the results). (A) Fits of theoretical profiles of models to experimental data, shown in Kratky representation. Experimental data is shown in black circles, and the theoretical profiles are shown in solid lines. Different color solid lines indicate different models. (B) Highest ranked models superimposed on one another. The models are colored as described in Figure 1. The density of P1 helix (green) is divided into two main places, indicating that P1 is bent towards the P2/P3 stalk, or the P4/P5 stalk. The models show variability despite good agreement of all the SAXS profiles in (A). The overall conformation of the models is

more compact than that observed for the ligand-free state. However, there is no one clear conformation that is populated by most of the models, and these models do not emulate the crystal structure of the bound-state adequately. Hence, the use of experimental data with a higher R_g value does not give a good representation of the bound-state as compared to the crystal structure.