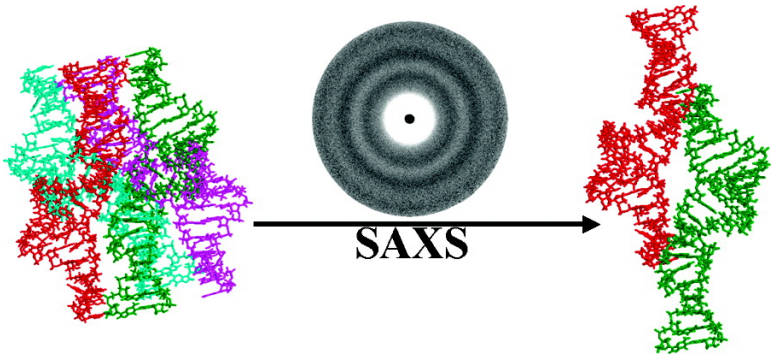
Communication

Global Molecular Structure and Interfaces: Refining an RNA:RNA Complex Structure Using Solution X-ray Scattering Data

Xiaobing Zuo, Jingbu Wang, Trenton R. Foster, Charles D. Schwieters, David M. Tiede, Samuel E. Butcher, and Yun-Xing Wang

J. Am. Chem. Soc., **2008**, 130 (11), 3292-3293 • DOI: 10.1021/ja7114508



J|A|C|S
COMMUNICATIONS

Published on Web 02/27/2008

Global Molecular Structure and Interfaces: Refining an RNA:RNA Complex Structure Using Solution X-ray Scattering Data

Xiaobing Zuo,[†] Jingbu Wang,[†] Trenton R. Foster,[‡] Charles D. Schwieters,[§] David M. Tiede,[||] Samuel E. Butcher,*[,‡] and Yun-Xing Wang*[,†]

Protein Nucleic Acid Interaction Section, Structural Biophysics Laboratory, NCI-Frederick, National Istitutes of Health, Frederick, Maryland 21702, Department of Biochemistry, University of Wisconsin at Madison, Madison, Wisconsin 53706, Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, Maryland 20892, and The Chemical Sciences and Engineering Division, Argonne National Laboratory, Argonne, Illinois 60439

Received December 28, 2007; E-mail: butcher@biochem.wisc.edu; wangyu@ncifcrf.gov

We demonstrate in this report that solution X-ray scattering data can be utilized to precisely define the global structure and, therefore, implicitly the interfaces of an RNA:RNA complex. Defining the interfaces among components and the global structure of multicomponent systems is one of the essential problems in understanding biological interactions on a molecular level. However, identifying molecular interfaces is not an easy task for solution NMR spectroscopists. To resolve this problem, a widely used protocol is to prepare various sophisticated but labor-intensive and sometimes costly isotope-labeled samples and apply NMR isotope-filter experiments.[1−4] Moreover, global structures are often underdetermined, due to a general lack of experimentally measured NMR-derived restraints that define the overall dimensionalities and shapes of biomacromolecules and complexes in solution, even if residual dipolar coupling has been utilized to provide global orientation restraints.[5] This lack is particularly severe in the structural determination of RNA molecules or complexes, where the proton spin density is much lower than that in protein counterparts, the structures generally tend to be elongated, and there are few options in selective-labeling sample preparation schemes. Furthermore, isotope filter/edited nuclear Overhauser effect (NOE) experiments are in general rather insensitive. Even when there are observable NOEs, assigning them is often challenging and time-consuming.

Small-angle X-ray or neutron scattering (SAXS and SANS) data contain information about the overall shape and dimensionality of biomacromolecules in solution[6−8] and have recently been utilized directly to refine protein solution structures in combination with NMR restraints in order to achieve accurate global orders of single-chain multidomain proteins.[9,10] The utilization of SAXS data to define the global structure and consequently identify the interfaces of complexes of an RNA complex has not been reported. We report here a method that utilizes SAXS data to define the global structure and consequently to identify the interfaces of an RNA complex without intermolecular NOE distance restraints and to refine the global shape of the RNA complex. We demonstrate the utility of the method using a 30 kDa homodimeric tetraloop−receptor RNA complex, which is a commonly occurring RNA tertiary structural motif involved in helical packing.[11] The structure of the complex has been determined using heteronuclear solution NMR spectroscopy.[12,13] In the previous determination, the relative position and the orientation between the two subunits were restrained using 36 × 2 intermolecular NOE distance and hydrogen bond restraints together with 9 × 2 imino residual dipolar couplings (RDCs).

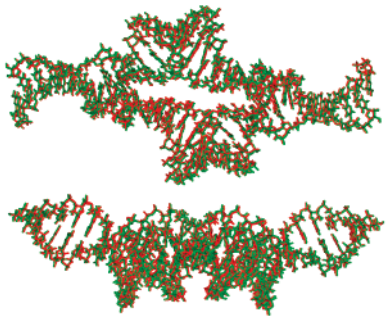


Figure 1. Side and top views of the tetraloop receptor homodimeric structures, refined with 36 × 2 distance and hydrogen bond restraints (red) (accession code: 2jyj) and defined by SAXS data using a rigid-body calculation (green) (accession code: 2jyh). The rmsd between the two structures is 0.4 Å. The global structure-defined interfaces have almost identical interface interactions, although implicit, such as base stacking and hydrogen bond interactions without explicit restraints.

For a given set of RDCs that are measured in one alignment medium, there are four satisfying discrete orientations for a subunit. In the case of a homodimer with a C_2-axial symmetry, the orientations of the two subunits are related to each other within four possible choices.[14−16] The correct orientation for the second subunit is among the three possible choices and can be determined unambiguously by applying a second alignment tensor,[14−16] or in favorable cases by applying a grid search restrained with the SAXS data (Supporting Information, SI). Therefore, in principle, the problem of defining the interfaces virtually reduces to a problem of defining the relative translational position between the two subunits, with only three degrees of freedom with respect to a fixed relative orientation between the two subunits. The approximate relative translational positions of the two subunits were also determined in the grid search (SI) and further refined using the SAXS data to optimize the relative positions of the two subunits in the complex, assuming that each subunit structure was a rigid body in a rigid-body refining protocol.[15,17] The backbone root-mean-square deviation (rmsd) between the SAXS-defined dimer and the dimer refined by the intermolecular NMR distance restraints is ∼0.4 Å (Figure 1), indicating the closeness of the two structures. The global structure-defined interfaces have almost identical interface interactions to those reported previously,[12,13] including hydrogen bonds and base stacking. The backbone rmsd of the ensemble of the top 10% of the SAXS-defined dimer structures is much less than 0.1 Å, which is due to the fact that each subunit was treated as a rigid body in the calculation, but also suggests that there is little ambiguity in defining the relative position of the two subunits using the SAXS data in the complete absence of inter-subunit distance restraints.

[†] NCI-Frederick, National Institutes of Health.
[‡] University of Wisconsin at Madison.
[§] Center for Information Technology, National Institutes of Health.
[||] Argonne National Laboratory.

10.1021/ja7114508 CCC: $40.75

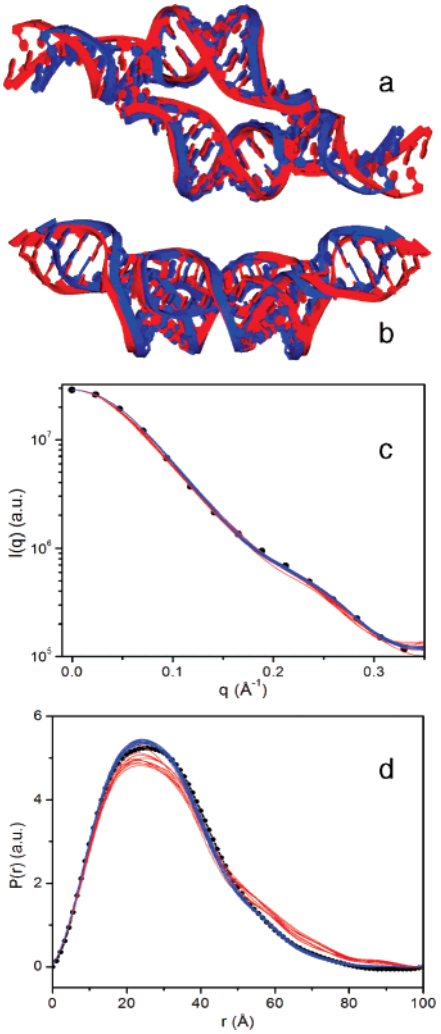


Figure 2. (a and b) Front and top views of the average structures of the top 10% refined structures without (red) and with (blue, accession code: 2jyf) experimental SAXS data. Both groups of the structures were restrained with NMR-derived distances, including intermolecular and torsion angle restraints. (c and d) Comparison of back-calculated scattering curves (c) and pair distance distribution functions (PDDFs) (d) based on the non-SAXS refined (red) and SAXS-refined structures (blue) and experimental data (●). The filled circles in (c) are the trace of the interpolated experimental SAXS data points that were used in the refinement. All PDDFs were calculated using GNOM[18] with q range of $0 \leq q \leq 0.33$ Å^{-1}.

In addition to defining the interfaces of the dimer using a rigid-body refinement protocol, we also applied the SAXS data to optimize the global shape of the dimer (Figure 2). The difference between the experimental and back-calculated SAXS curves indicates the deviation of the overall structures that were refined without the SAXS data from that in solution. This deviation likely originates from the subunit structure that was determined without the benefit of global shape restraints. Overall, the SAXS-refined structures have a slightly improved backbone rmsd to the average of the ensemble, ~1.3 Å, as compared with ~1.8 Å for the non-SAXS-refined structures. More importantly, the SAXS data complement the NMR restraints in defining the global shape of the dimer, and the impact on the global structure is noticeable with an rmsd between the two structures of about 3.2 Å (Figure 2). The pair distance distribution functions (PDDFs) of the SAXS-refined structures are more narrowly dispersed and better matched to the experimental values in real space after the refinement, and the R_g value of the structure changes from 25.1 Å (before) to 23.0 Å after the SAXS refinement, agreeing well with 23.2 Å of the experimental value.

Utilizing SAXS data together with the NMR-derived restraints one can easily determine interfaces of proteins or heterodimeric complexes, if discrete choices of possible orientations are known or if interfaces between two highly asymmetrical subunits is roughly known by other means. In the latter case, the grid search program can be tailored to search for the accurate interfaces that best fit the SAXS data. In addition, we would expect that refinement using SAXS data would have a relatively greater impact on the global structural determination of RNA molecules compared to those for proteins, because RNA structures are typically elongated and underdetermined. Furthermore, it is noteworthy to point out that, compared to multidimensional NMR experiments, typical solution SAXS experiments require an order of magnitude smaller amount of non-isotope labeled samples; the data collections are relatively straightforward, and interpretations are streamlined with the protocols and software that we described in this communication. In conclusion, applying SAXS data for optimizing the global structures of RNA molecules and for defining the interfaces of complexes represents a significant step forward in developing more rapid and robust strategies for solution structure determination.

Acknowledgment. This work was supported in part by NSF Grant MCB-0445613 to S.E.B, DOE Contract DE-AC02-06CH11357 to D.M.T., and by the NCI intramural research grant to Y.-X.W. Work at Argonne National Laboratory (DMT) and the Advanced Photon Source was supported by the Office of Basic Energy Sciences, DOE under contract. We thank Drs. L. Guo and S. Seifert and staffs of Sectors 18-ID and 12-ID of APS at Argonne National Laboratory for their support for synchrotron experiments and Dr. R. Andrew Byrd for helpful discussions.

Supporting Information Available: Procedures used to record and to analyze the SAXS data are presented, and calculation protocols are deposited in the PDB databank together with the coordinates and can also be downloaded from the authors' websites. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Clore, G. M.; Appella, E.; Yamada, M.; Matsushima, K.; Gronenborn, A. M. *Biochemistry* **1990**, *29*, 1689−96.
(2) Griffey, R. H.; Redfield, A. G. *Q. Rev. Biophys.* **1987**, *19*, 51−82.
(3) Matsuo, H.; Shirakawa, M.; Kyogoku, Y. *J. Mol. Biol.* **1995**, *254*, 668−80.
(4) Venters, R. A.; Huang, C. C.; Farmer, B. T., II; Trolard, R.; Spicer, L. D.; Fierke, C. A. *J. Biomol. NMR* **1995**, *5*, 339−44.
(5) Al-Hashimi, H. M.; Valafar, H.; Terrell, M.; Zartler, E. R.; Eidsness, M. K.; Prestegard, J. H. *J. Magn. Reson.* **2000**, *143*, 402−406.
(6) Feigin, L. A.; Svergun, D. I. *Structure Analysis by Small Angle X-Ray and Neutron Scattering*; Plenum Press: New York, 1987.
(7) Glatter, O.; Kratky, O. *Small Angle X-ray Scattering*; Academic Press: New York, 1982.
(8) Lipfert, J.; Doniach, S. *Annu. Rev. Biophys. Biomol. Struct.* **2007**, *36*, 307−27.
(9) Grishaev, A.; Wu, J.; Trewhella, J.; Bax, A. *J. Am. Chem. Soc.* **2005**, *127*, 16621−8.
(10) Lee, D.; Walsh, J. D.; Yu, P.; Markus, M. A.; Choli-Papadopoulou, T.; Schwieters, C. D.; Krueger, S.; Draper, D. E.; Wang, Y. X. *J. Mol. Biol.* **2007**, *367*, 1007−22.
(11) Costa, M.; Michel, F. *EMBO J.* **1995**, *14*, 1276−85.
(12) Davis, J. H.; Tonelli, M.; Scott, L. G.; Jaeger, L.; Williamson, J. R.; Butcher, S. E. *J. Mol. Biol.* **2005**, *351*, 371−82.
(13) Davis, J. H.; Foster, T. R.; Tonelli, M.; Butcher, S. E. *RNA* **2007**, *13*, 76−86.
(14) Saupe, A. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 97−112.
(15) Bewley, C. A.; Clore, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 6009−6016.
(16) Al-Hashimi, H. M.; Majumdar, A.; Gorin, A.; Kettani, A.; Skripkin, E.; Patel, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 633−40.
(17) Schwieters, C. D.; Clore, G. M. *Biochemistry* **2007**, *46*, 1152−1166.
(18) Svergun, D. I. *J. Appl. Crystallogr.* **1992**, *25*, 495−503

JA7114508