The Small-Angle X-ray Scattering Core Facility of Center for Cancer Research

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Abstracts

Small-angle X-ray scattering (SAXS) is a complementary technique to Crystallography and NMR techniques and is becoming more widely used in structural biology. Crystallography requires good crystals and NMR limits to biomolecules with low molecular mass. SAXS allows studying the structure of macromolecules and their complexes in near physiological environments and studying structural changes with external conditions. The SAXS core facility of Center for Cancer Research (CCR) has an in-house state-of-art SAXS instrument and access to beamtime in the Advanced Photon Source at Argonne National Lab. The research field includes but not limited to structural studies of nucleic acids, proteins, protein assemblies, virus particles, lipid membranes and membrane-protein/DNA complexes. This poster gives introduction to the SAXS core facility of CCR and highlights recent scientific achievements by SAXS core facility users.

Introduction to SAXS Core Facility

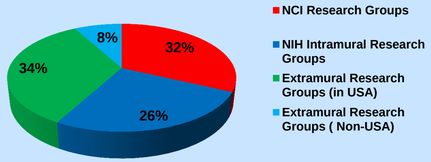
The SAXS core facility was established in 2013, with a strong support from Dr. Jeffrey Strathern of the Center for Cancer Research (CCR) of National Cancer Institute. It is open to all intramural and extramural research communities. The SAXS core facility offers routine access to the synchrotron beamline source at Advanced Photon Source (APS) of Argonne National Laboratory through the existing Partnership User Program (PUP) agreement. The SAXS core also has an in-house SAXS instrument since 2015, which serves the SAXS core user communities with a routine daily access to the SAXS techniques for multiple purposes.

The Mission of SAXS Core Facility

The mission of SAXS core facility is to provide support to research projects from CCR principle investigators (PIs), NIH intramural PIs and extramural academic research groups/laboratories. The support includes providing routine access to the APS PUP SAXS/WAXS beamline and in-house SAXS/WAXS instrument and expertise in experimental design, data collection, processing, analysis and interpretation.

Productivity of SAXS Core Facility

About 70 laboratories and research groups from US and other countries have used the CCR SAXS resources.



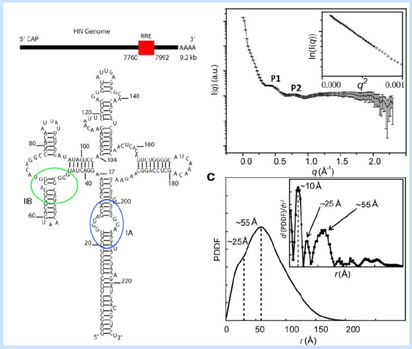
16 publications in peer reviewed journals in 2013-2014

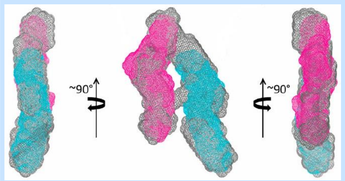
SAXS Core Facilities

APS PUP Beamline Source: SAXS/WAXS Setup at Beamline 12ID-B

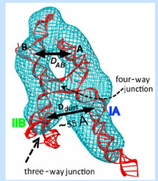
In-house SAXS Instrument -BIOSAXS 2000

Rev Response Element In HIV-1 RNA





RRE molecular envelope derived from SAXS data



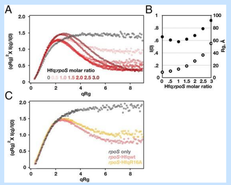
Atomic model(red) based on SAXS envelope

The Rev-protein response element (RRE) in the HIV-1 RNA genome is crucial for HIV virus life cycle because RRE helps some RNA genome escape the host nucleus to pass the gene to child virus before it is spliced and loses necessary genes. This RNA genome translocation from nucleus to cytoplasm is enabled through forming a complex between RRE domain and Rev-Protein remained elusive for many years because of the difficulties in the structural determination for the RRE RNA. Recently, the structure of the RRE RNA was solved using solution SAXS technique. This 3D topology resolves the two-decade-long mystery of how HIV virus specifically selects it own mRNA to export for packaging.

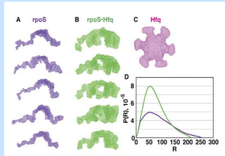
This work is done by a team led by Dr. Yun-Xing Wang (NIH/NCI). Fang et al, Cell, 155(3), 594-605 (2013)

Complex of rpoS RNA and Protein Hfq

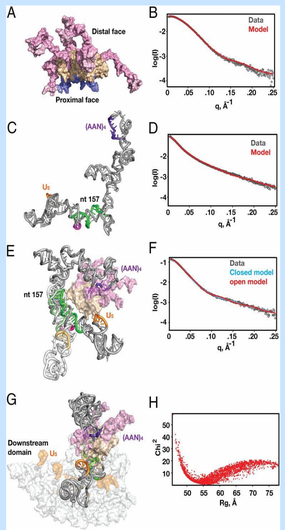
Small noncoding RNAs optimize bacterial gene expression under stress and increase the virulence of many bacterial pathogens. The RNA-binding protein Hfq (host factor Q-beta phage) promotes base pairing between small RNAs and target mRNAs, but it is not known how Hfq brings the two RNAs together in the proper orientation. SAXS along with footprinting, and molecular dynamics simulations reveal that the mRNA wraps entirely around the Hfq protein, specifically contacting both surfaces. This destabilizes the mRNA structure around the small RNA target site, poising it to base pair with a complementary small RNA also bound to Hfq.



SAXS of rpoS•Hfq complexes reveals a compact structure



Ab initio models of rpoS RNA, Hfq protein, and the rpoS•Hfq complex. Five ab initio structures for (A) free rpoS RNA and (B) the rpoS•Hfq complex (1:1 molar ratio). The averaged ab initio model of Hfq (C).



Model of the *rpoS* RNA•Hfq regulatory complex. All-atom models of full-length Hfq, (*A* and *B*), *rpoS* RNA (*C* and *D*), and *rpoS*•Hfq complex (*E* and *F*). Open structures from Monte Carlo simulations (G). The best-fitting 917 models from the trajectory (*H*)

This work is done by a team led by Prof. Sarah A. Woodson (Johns Hopkins University)
Peng et al, PNAS, 111, 17134-17139 (2014)

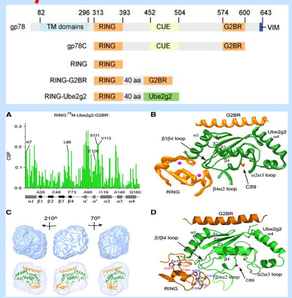
The RING:Ube2g2:G2BR Complex

RING finger proteins constitute the large majority of ubiquitin ligases (E3s) and function by interacting with ubiquitin-conjugating enzymes (E2s) charged with ubiquitin. How low-affinity RING–E2 interactions result in highly processive substrate ubiquitination is largely unknown. The RING E3, gp78, represents an excellent model to study this process. Combining NMR and SAXS structural analysis of the RING:Ube2g2:G2BR complex reveals that gp78 is a ubiquitination machine where multiple E2-binding sites coordinately facilitate processive ubiquitination.

SAXS indicates a well-behaved 1:1:1 complex. SAXS envelope model encompasses 100% of the atoms in the solution NMR structure. SAXS data confirm that RING:Ube2g2:G2BR is a monomeric complex in solution.

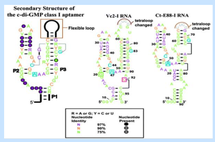
This work is done by a team led by Dr. Andy Byrd (NIH/NCI)

Das et. al, The EMBO Journal, 32, 2504-2516 (2013)

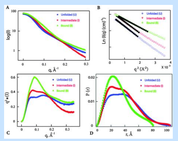


(A) CSPs in ^{15}N-Ube2g2 backbone
(B) The 20 lowest energy NMR structures of RING:Ube2g2:G2BR
(C) SAXS envelopes of RING:Ube2g2:G2BR
(D) The combined RING–G2BR:Ube2g2 crystal structure

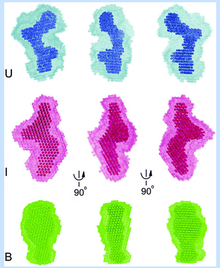
E88 Riboswitch Aptamer



Predicted secondary structures of the selected c-di-GMP riboswitch aptamers.



SAXS analysis of Ct-E88 RNA under three different conditions.



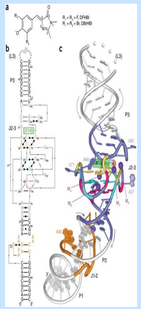
Low-resolution ab initio models of the Ct-E88 aptamer under three different folding states

Cyclic-di-GMP has emerged as an ubiquitous second messenger, which regulates the transition between sessile and motile lifestyles and virulence factor expression in many pathogenic bacteria using both RNA riboswitches and protein effectors. SAXS and 1D NMR titration studies shows that E88, a new c-di-GMP class I riboswitch aptamer from Clostridium tetani, has a similar fold to the prototypical class I riboswitch, Vc2, but differentially binds to c-di-GMP analogs.

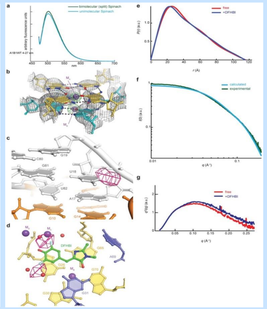
This work is done by a team led by Prof. Dayie and Sintim (University of Maryland)
Luo et al, Mol Biosyst., 10(3), 384-90 (2014)

Spinach RNA Mimics of Green Fluorescent Protein

Green fluorescent protein (GFP) has transformed the study of proteins at the level of single molecules to whole organisms. Spinach is an *in vitro* evolved RNA mimic of GFP. Combining SAXS, NMR and Crystallography structural analysis of Spinach–DFHBI complex reveals the structural basis for fluorescence activation. It provides a foundation for structure-driven design and tuning of fluorescent RNAs.



Structure of the Spinach–DFHBI



SAXS studies of free and DFHBI-bound Spinach RNA are shown in e, f, g.

This work is done by a team led by Dr. Adrian R. Ferré-D'Amaré (NIH/NHLB)
Warner et. al, Nature structural & molecular biology, 21, 658-663 (2014)

Acknowledgement

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