Small-Angle Neutron and X-ray Scattering and Atomistic Modeling of Intrinsically Flexible Proteins: Reflections From an AMGEN/NIST Post-doc

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Low-q Seminar
September 10th 2014
My background

- BSc in Biochemistry and Molecular Biology from University of New Mexico HSC
  - Performed research in a yeast genetics and chromatin and DNA-repair lab.

- Technician in a Molecular Epidemiology Lab.
  - Assembled, processed and archived tissue and DNA samples from cancer patients.
  - Performed functional enzymatic and biochemical assays on extracts from samples.

- PhD in Biochemistry and Molecular Biology from Colorado State University.
  - Structure function relationship of chromatin and DNA-repair associated proteins.

- NIST-Amgen post doctoral fellow.
  - Small angle scattering (SAS) to address industry questions regarding structure and stability of biologics.

- Husband and father of one.
Began going to NCNR as a user (March 2009)

Attended the MD Summer School (July 2011)

Hired into my post-doc (January 2012)
Poly(ADP-ribose) Polymerase-1 (PARP-1)

- Transcription
- Chromosomal stability and telomere length
- Long-term memory formation
- Chromatin architecture
- DNA-damage response and repair
How is PARP-1 involved in regulating opposing chromatin states?

- Euchromatin
- Heterochromatin
(1) PARP1 is Associated With Silent Chromatin

(2) PARP1 Activation Leads to Chromatin Loosening

(3) Following DNA Repair or Gene Transcription, Chromatin is Silenced Again

Science. 2003 Jan 24;299(5606):528-9
FRET was used to determine relative affinities binding stoichiometry of PARP-1 to its substrates
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Biophysical characterization of PARP-1

Characterization Methods:
- AUC-SV
- SEC-MALS
- SAXS
- SANS

Results:
- Monomeric in solution
- PARP-1 relatively elongated.
- Elongates further upon binding to DNA-damage models.
Small angle scattering (SAS) confirms an increase in particle dimension upon DNA binding.
How is small angle scattering used to help determine the structure-function relationship of PARP-1 in solution?

PARP-1 + neutrons/x-rays = A solution structure of PARP-1

- **Supplementary Figure 1.** parp486 SAXS raw data represented by Guinier, Porod and Kratky analysis.
  - **A)** Superposition of raw parp486 SAXS data at 3, 6 and 9 mg/ml concentrations depicts minimal concentration dependent scattering effects.
  - **B)** Guinier analysis of the low scattering angles depicts minimal aggregation. The residuals are illustrated in the lower portion of the graph.
  - **C)** Kratky analysis of the data illustrates a folded, yet flexible particle.
  - **D)** Porod graph of parp486 SAXS data determined that the sample follows Porod’s law and has a volume of ~100,000Å³.

**References:**
Small angle scattering (SAS) is a set of low-resolution solution characterization techniques that have been developed over the last half century.
SAS sources we have used for our studies

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How do particle physics and particle accelerators relate to biochemistry and structural biology?
X-rays vs. Neutrons

Contrast Variation

Contrast Matching reduce the number of phases “visible”

- $\rho_{\text{solvent}} = \rho_{\text{core}}$
  - (shell visible)
- or
- $\rho_{\text{solvent}} = \rho_{\text{shell}}$
  - (core visible)

- The two distinct two-phase systems can be easily understood

Scattering length density vs. %D2O
Small angle scattering (SAS)
(similar to crystallography)
SAS provides low-resolution (~15-250 Å) information.
Small-angle scattering profiles are composed of intra- and intermolecular components.

\[
q = \frac{4\pi}{\lambda} \sin(\theta)
\]

where \(2\theta\) is the angle from the incident beam.
Small angle neutron scattering (SANS) profile of dilute anti-streptavidin immunoglobulin-2 (ASA-IgG2)

\[ I(0)/c = \text{Molar Mass} \times \text{constant} \]

\[ R_g \]

2 mg/mL

SANS of an IgG1
Yearly et al.
Biophys J. 2013; 105(3): 720–731

SAXS/SANS of an IgA
Perkins et al.

SAXS of an IgG1
Lilyestrom et. al

SAXS of an IgG2
Mosbæk et al.

Shape Information Equivalent from DLS/AUC
Geometric forms fit a portion of the low-q but atomistic models provide a better fit to SAS data

\[ r = 65\,\text{Å} \]
\[ R_g^2 = \frac{3}{5} r^2 \]
Molecular Monte Carlo (MMC) allows for rapid sampling of the mAb conformations that are possible in solution.
Monte Carlo ensemble fit to data

Best-Fit Structures

We can use the average fit as a form factor

Structure-density plots for the ASA-IgG2 ensemble illustrates the large conformational space possible.

Free-energy analysis

-16400 kcal/mol

-16800 kcal/mol

Hybrid MMC toroid approach (proprietary and simulation advantages)

Manuscript in preparation... (Sarachan, K. et al...)

[Graph A: Radius of Gyration (Å) vs. X²]

[Graph B: Radius of Gyration (Å) vs. X²]

[Graph C: Intensity (I(q)) vs. q (Å⁻¹)]
Other Structural Biology Questions
Small angle scattering can be used to help determine the structure-function relationship of highly disordered proteins in solution?

How do important proteins like TraI function in solution?

Combination of NMR, crystallographic, homology modeling and circular dichroism (CD)-guided simulated annealing

Beth Buenger’s graduate thesis work underway...

Clark et. al., 2014, *J Mol Model* 20:2308

Additional work in progress with Schildbach Lab (JHU)
How is small angle scattering used to help determine the structure-function relationship of Chromatin! in solution?

How does chromatin behave structurally in solution?

Modeling work underway...thanks to new double-stranded DNA MMC

In progress with Luger Lab (CSU/HHMI), van Duyne Lab (Penn), Bowman Lab (JHU), Irving Group (APS/IIT) and Qiu Lab (GWU)
How is small angle scattering used to help determine the structure–function relationship of Chromatin Associated Proteins (ChAPs) in solution?

How do proteins like CHD-1 interact with chromatin?

Modeling work underway...thanks to new double-stranded DNA MMC

In progress with Bowman Lab (JHU) and Irving Group (APS/IIT)
How is small angle scattering used to help determine the structure-function relationship of Chromatin Associated Proteins (ChAPs) in solution?

How do proteins like PARP-1 interact with chromatin?

Modeling work underway...thanks to new double-stranded DNA MMC

In progress with Luger Lab (CSU/HHMI), van Duyne Lab (Penn), Bowman Lab (JHU), Irving Group (APS/IIT) and Qiu Lab (GWU)
How can we use small angle scattering to help address mAb-related biotech problems?

Problems may include but are not limited to: concentration dependent aggregation, high viscosity, phase separation, spontaneous crystallization, investment withdrawal and sudden job loss.
Biopharmaceuticals or Biologics
Specifically; Monoclonal Antibodies (mAbs)

**Pros**
- Lower toxicity with higher specificity
- Biologics can be custom designed to have specific activity

**Cons**
- Low potency requires that they be administered in high concentrations ($\geq 1.0 \, \text{g/kg}$)
- Highly concentrated solutions have unwanted side effects
  - high viscosity
  - tendency to crystallize, phase separate or aggregate
Neutrons be useful for studying proteins in different phases (liquid, frozen and powdered formulations)

lyophilized powder work in preparation...
What happens at high concentrations?
Increasing mAb Concentration

Low-q region becomes depressed

Concentration effects on scattering profiles

\[ I(q) = \frac{N_p}{V} P(q) S(q) \]

\[ P(q) = |F(q)|^2 \]
Small angle scattering profiles reveal intermolecular interactions.

$S(q) \approx$ the reciprocal space version of $g(r)$

Increasing mAb concentration
\[ S(q)_{\text{effective}} = \frac{I(q)_{\text{concentrated}}}{I(q)_{\text{dilute}}} \]
Conformational effects on $S(q)_{\text{effective}}$-plots derived from atomistic ensemble of ASA-IgG2

Fit to 175 mg/mL SANS Data

Clark et. al., in progress
S(q)_{effective} plots can be fit to simple colloidal interaction models

Hayter/Penfold
Mean Spherical Approximation (M.S.A.)

Variables used;
- Volume fraction
- Dielectric constant
- Monovalent salt Conc.

Variables obtained;
- Charge of particle
- Effective Diameter (Å)

Under these conditions ASA-IgG2 is repulsive

What happens when you add salt?
Salt effects on scattering profiles
(150 mg/mL sample)

Low-q region becomes less depressed with increasing NaCl
Salt effects on scattering profiles
(150 mg/mL sample)

Low-\(q\) region becomes less depressed with increasing NaCl
Results of the fit to MSA model

- Effective diameter decreases with increasing protein concentration
- Particles become less repulsive with NaCl
- NaCl does not alter the diameter of the particles
- NaCl decreases the charge of the particles up to ~100 mg/mL (will confirm with simulations)
What happens when you add salt above physiological concentrations?
Increasing the salt concentration above physiological levels results in attractive interactions.

\[ S(q) \text{ effective} \]

\[ r/2R \]

from SHS can obtain interaction energies (units of kT)

Phase diagrams help to characterize mAb behavior over a wide range of protein and salt concentrations.

Similar phase diagrams have been used to predict protein crystallizability!

\[ \gamma-\text{Crystallin} \]
Thousand-mAb simulations (in progress...)

- Atomistic modeling of high concentration mAbs offers insight into the types of intermolecular interactions.
- Incorporating flexibility into simulations is challenging but it will serve to better represent the solution.
- New scattering engine needed to be developed (Watson and Curtis)
- Simulations in conjunction with SAS data will help to better design mAb-candidates even in the early stage of development (low sample volumes)
Conclusions and Future Directions

- Atomistic modeling combined with dilute SAS data can result in low resolution structural information (used to obtain a Form Factor).

- SAS can be combined with models like the “mean spherical approximation” (Hayter/Penfold M.S.A.) to better understand concentrated mAb solutions.

- Interactions can be moderated with the addition of NaCl.

- Can interactions be moderated with the addition other excipients?

- Atomistic simulations of concentrated solutions are in progress and can replace the Mean Spherical Approximation (Joseph Curtis and Hailiang Zhang).
High-throughput (HT) SAS to rapidly characterize proteins in multiple formulation conditions

- Multi-well platform is ready in use for other measurements.
- Can readily screen multiple protein concentrations in a given set of conditions.
- SAS offers a Q-dependent look not available for the more commonly accepted/used DLS measurements.
- W/ SAS we can observe structural affects on mAbs during stability studies.
- SAS/WAS offers a unique look at solvent- and co-solute-protein interactions. (WAS = wide angle scattering)
Future directions: Dynamic Light Scattering (DLS) (plate reader?)

- Multi-well platform is ready in use for other measurements.

- Can readily screen multiple protein concentrations in a given set of conditions.

- Combined DLS and DSC can offer insights into structural stability as a function of temperature.

- Combining SAS, DSC, MALS and DLS on specific “behavior classes” of mAbs can offer benchmark marks for new mAbs or mAb-formulations.
Incorporate concentration gradient multi-angle light scattering (CG-MALS) to better characterize self-associations present in mAb solutions.

- CG-MALS offers insight into self-associations.
- Models derived from CG-MALS will help to build better in silico models.
- Combining SAS and GC-MALS will help to build better simulations and possibly enhance mAb design.

Joey Pollastrini, Amgen
Thank you!

Acknowledgments

NIST Center for Neutron Research (NCNR)

Joseph Curtis
Susan Krueger
Hailiang Zhang
Dan Neumann
Rob Dimeo
Max Watson
Katie Sarachan

Cornell High-Energy Synchrotron Source (CHESS)

Richard Gillilan
Kathy Dedrick

AMGEN

Arnold McAuley
Sekhar Kanapurem
Mike Treuheit
Bruce Kerwin
Joon Huh
Tom Dillon
Bruce Mason
Maribel Espinoza
Meghan Moore

Local Celebrities
Questions?
Future directions:
Contrast variation orientational information of oligomeric species or from concentrated solutions

Light chains are produced in deuterating conditions

Predicted scattering profiles from *in silico* models
X-ray Sources?
(Synchrotron or Home Sources)

- Synchrotrons are storage rings for accelerated particle beams.
- First synchrotrons came on line in the middle of last century.
- Early on, x-rays were unwanted byproducts.
- Today, synchrotrons are user facilities that cater to materials science and biology researchers.
- Benchtop SAXS instruments are now available (use vacuum tube and copper anode sources).

**SAXS Experiment**
- Sample volume: 15-50 μL
- Measurement time: ≤1-5 minutes
- Concentration ranges: 0.5-≥100 mg/mL
Small "research" reactors became popular in the middle of the last century (even w/ universities and companies like Kodak)!

NCNR has a 20 MW fission reactor.

First came on line in the late 60's.

The NCNR is now a user facility that caters to academic and industry researchers.

Neutron Sources? (Nuclear Reactor or Spallation Sources)

SANS Experiment

Sample volume (350 - 650 μL)

Measurement time (1 - 2 hours)

Concentrations Ranges (1 - ≥ 100 mg/mL)

Neutron Sources? (Nuclear Reactor or Spallation Sources)

Borrowed from NIST Center for Neutron Research (NCNR)

Disclaimer: The Federal Government does not support, endorse or favor any one NFL team over another!
Small angle neutron scattering (SANS) profile of dilute anti-streptavidin immunoglobulin-2 (ASA-IgG2)
PARP-1

"Best-fit" structures ($\chi^2 \leq 2$)

All structures
Electrophoretic mobility shifts suggest PARP-1 can bind DNA as a monomer
Small angle scattering (SAS) experimental set-up
(neutrons or x-rays)

SANS
Banjo Cell Cuvet

**SANS**
- Sample volume (350-650 μL)
- Measurement time (≤ hours)
- Concentrations Ranges (1 - ≥100 mg/mL)

**SAXS**
- Sample volume (15-50 μL)
- Measurement time (≤1-5 minutes)
- Concentrations Ranges (0.5 - ≥100 mg/mL)