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

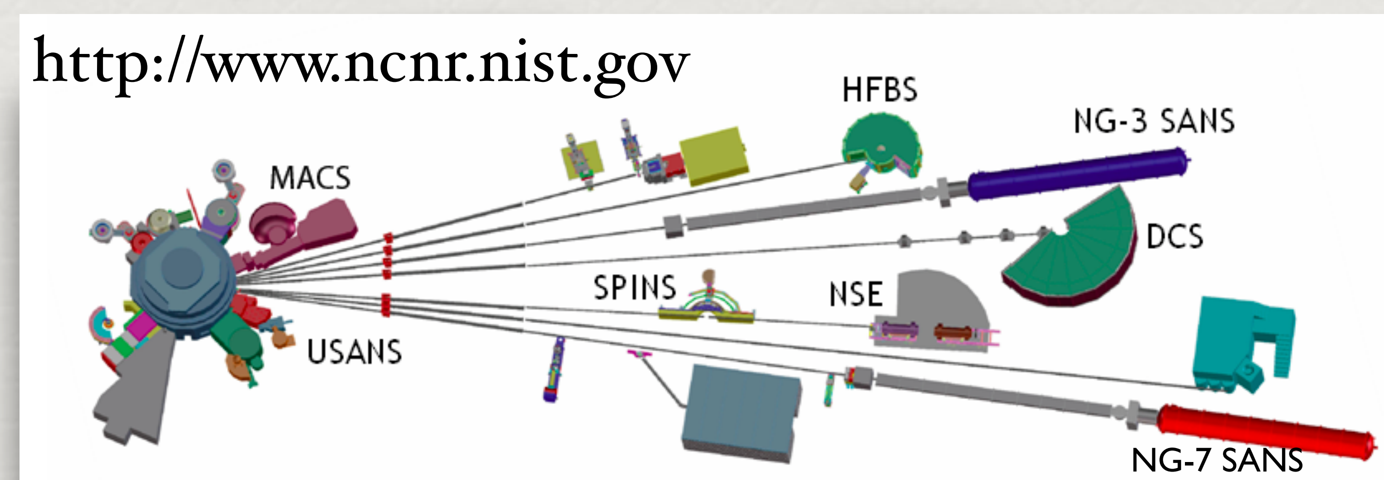
Nicholas Clark
NIST-Amgen post-doc fellow
Low-q Seminar
September 10th 2014

My background

- BSc in Biochemistry and Molecular Biology from University of New Mexico HSC
  - Performed researched 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.

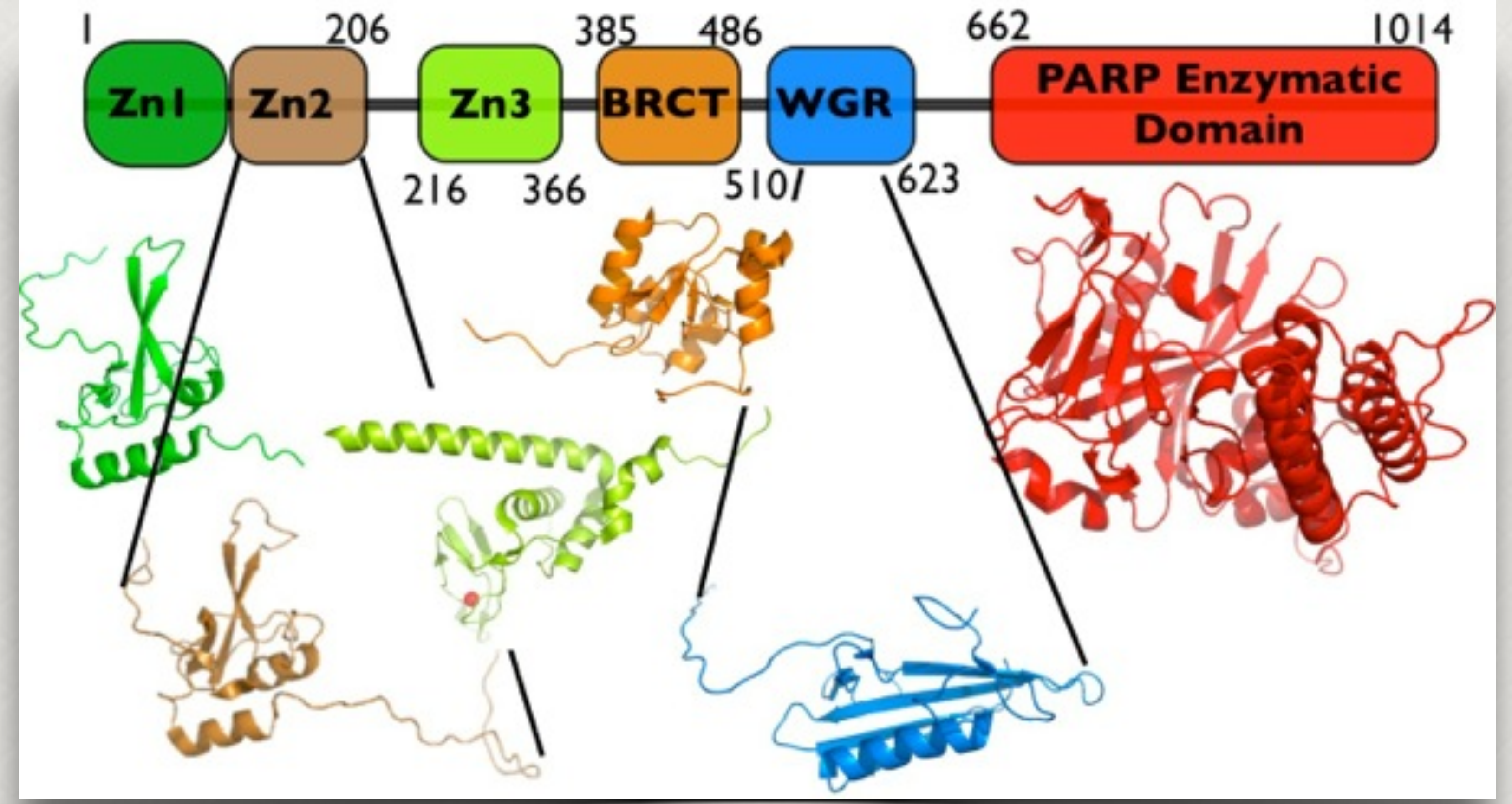
National Institute of Standards and Technology Center for Neutron Research (NCNR)

- Began going to NCNR as a user (March 2009)
- Attended the MD Summer School (July 2011)
- Hired into my post-doc (January 2012)



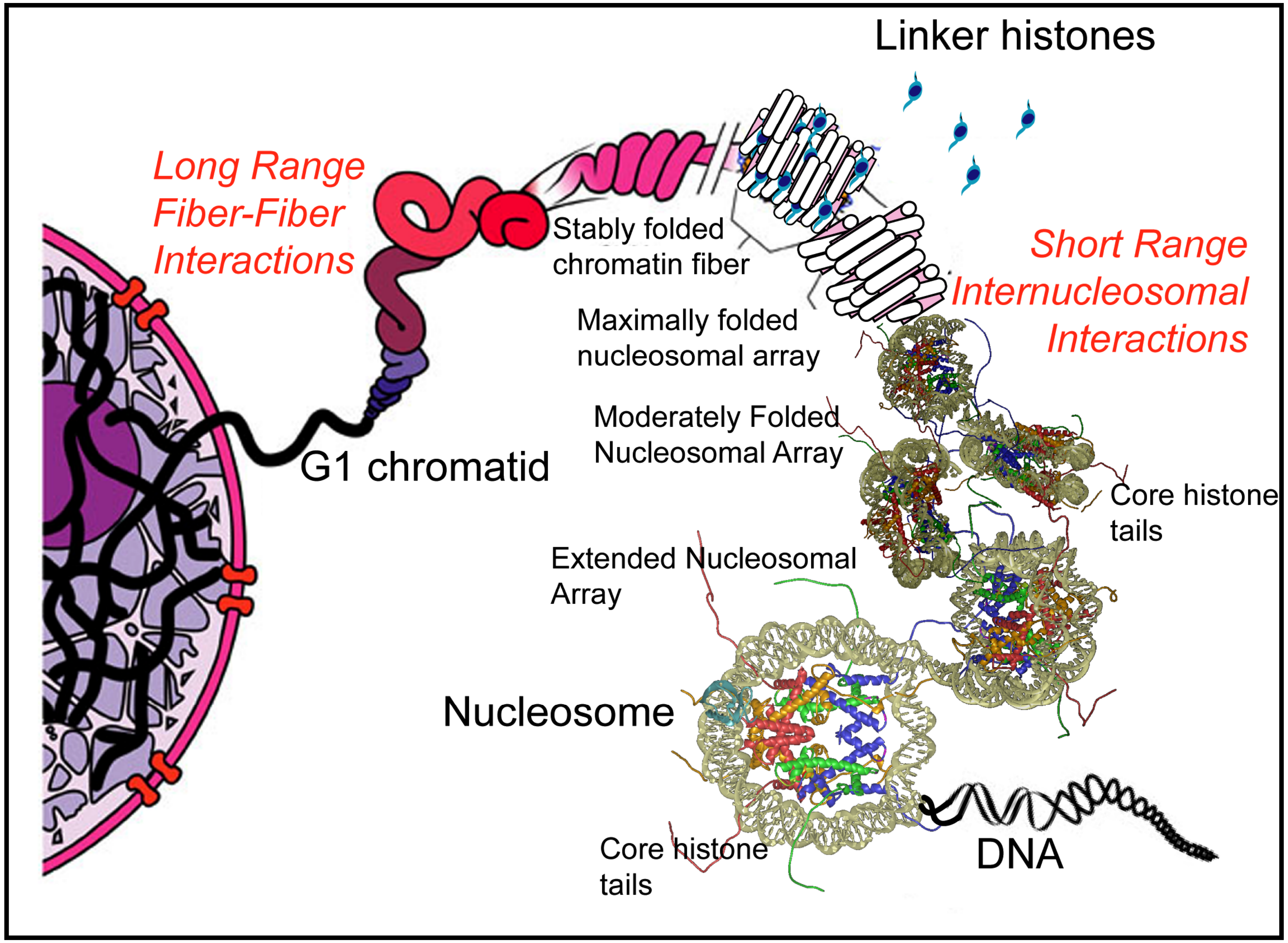
Poly(ADP-ribose) Polymerase-1 (PARP-1)

- Transcription
- Chromosomal stability and telomere length
- Long-term memory formation
- **Chromatin architecture**
- **DNA-damage response and repair**



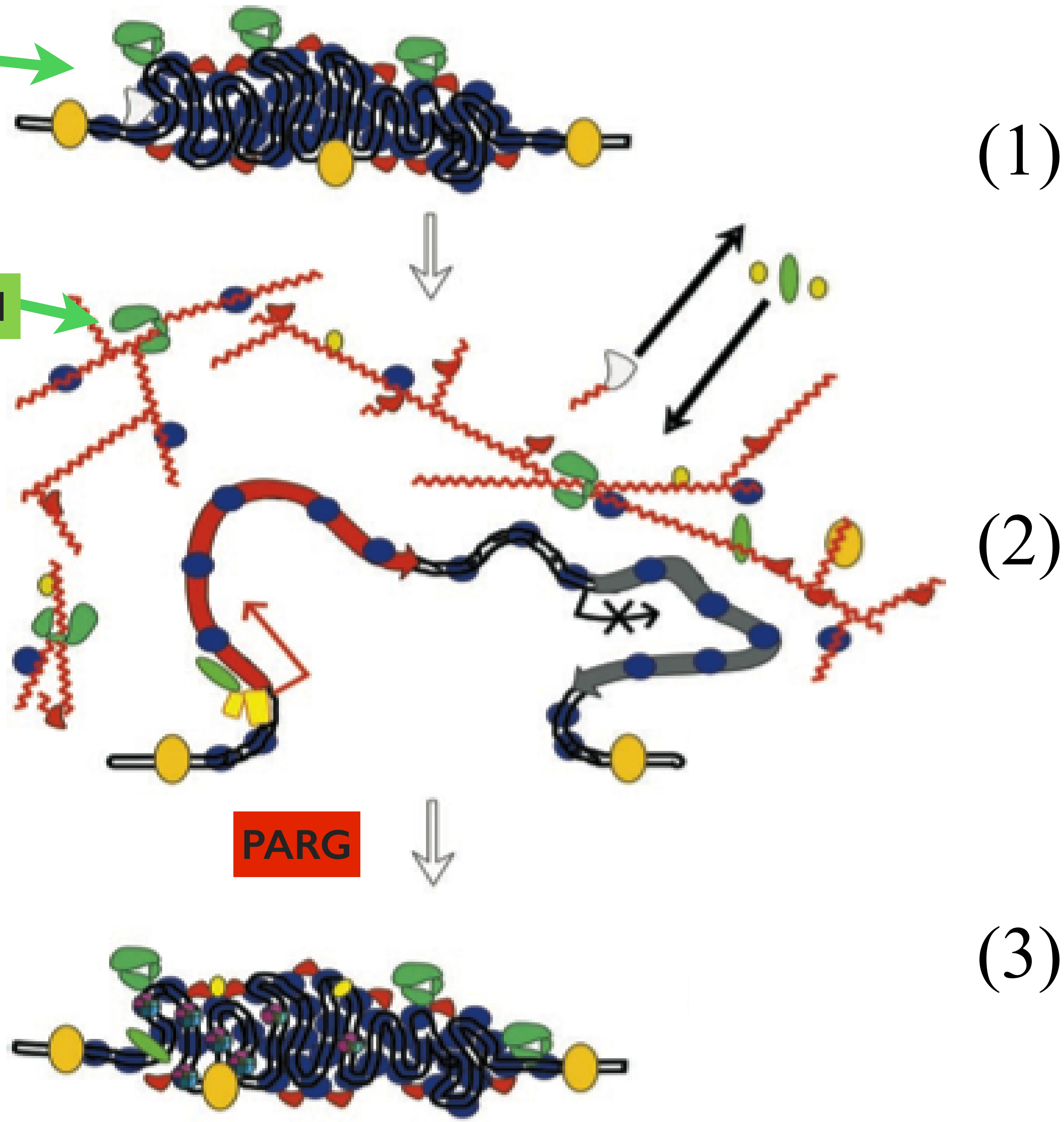
How is PARP-1 involved in regulating opposing chromatin states?

- Euchromatin
- Heterochromatin



-Jeff Hansen Lab
Colorado State Univ.

PARP-1 mediated chromatin loosening



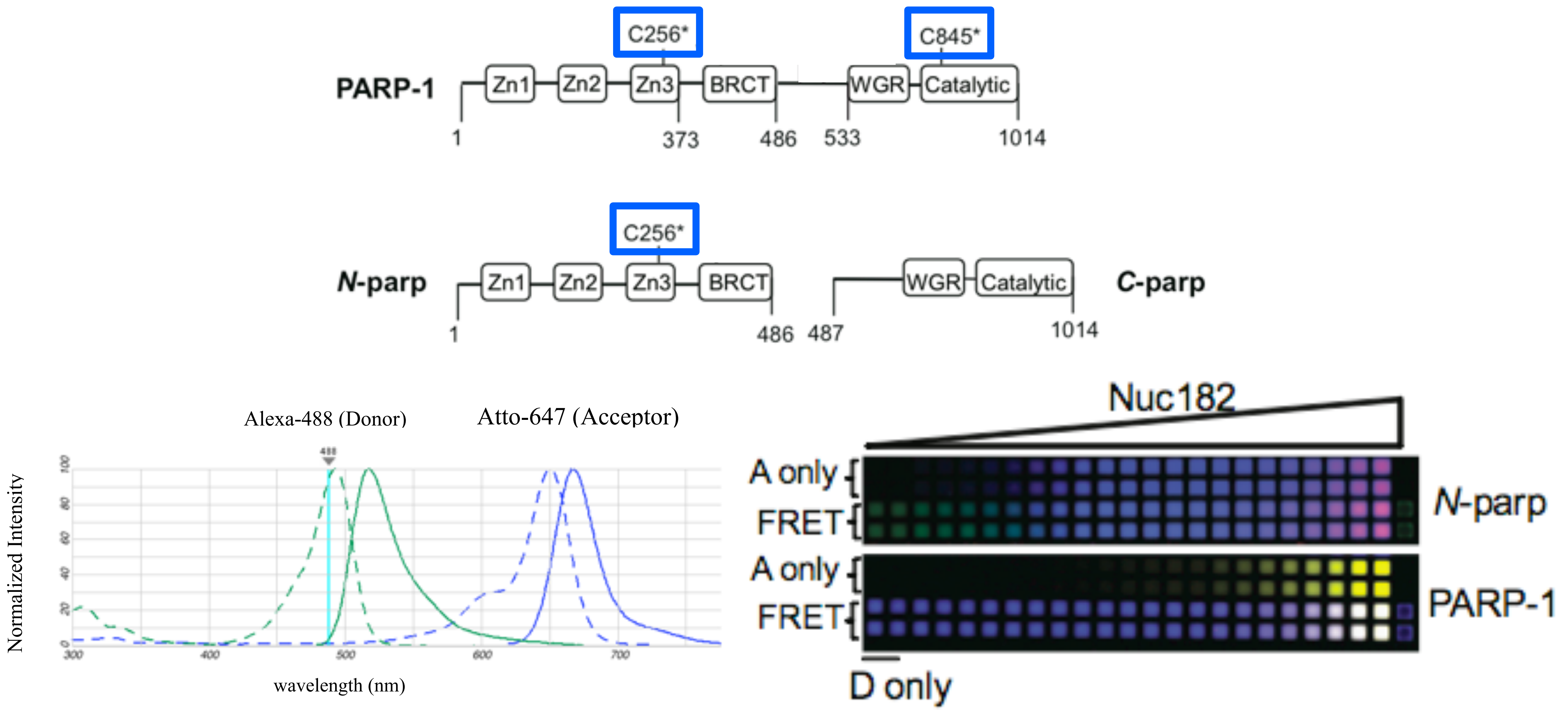
(1) PARP1 is Associated With Silent Chromatin

(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



J. Biol. Chem. 2012 Sept;287(39):32430-9

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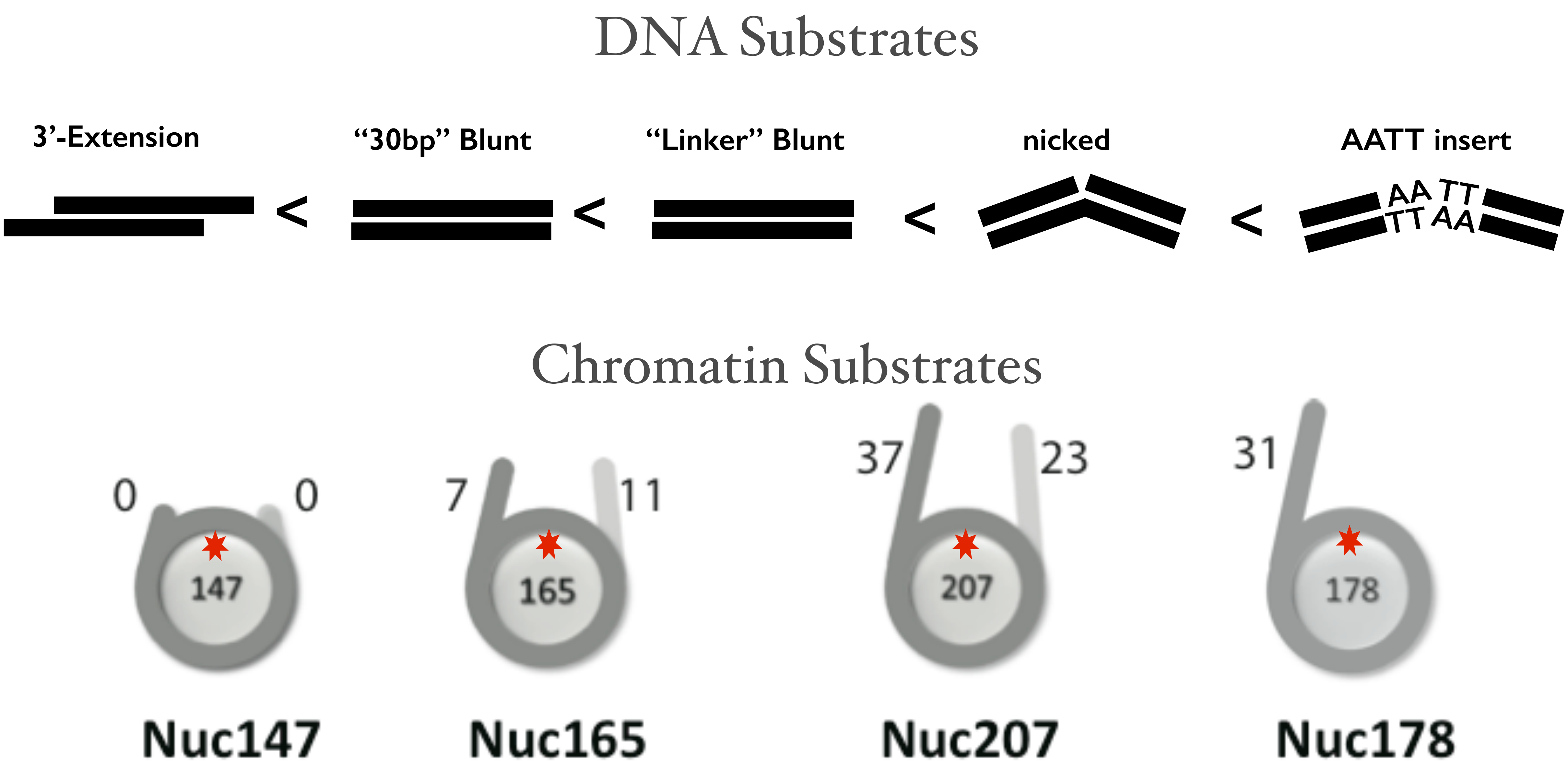
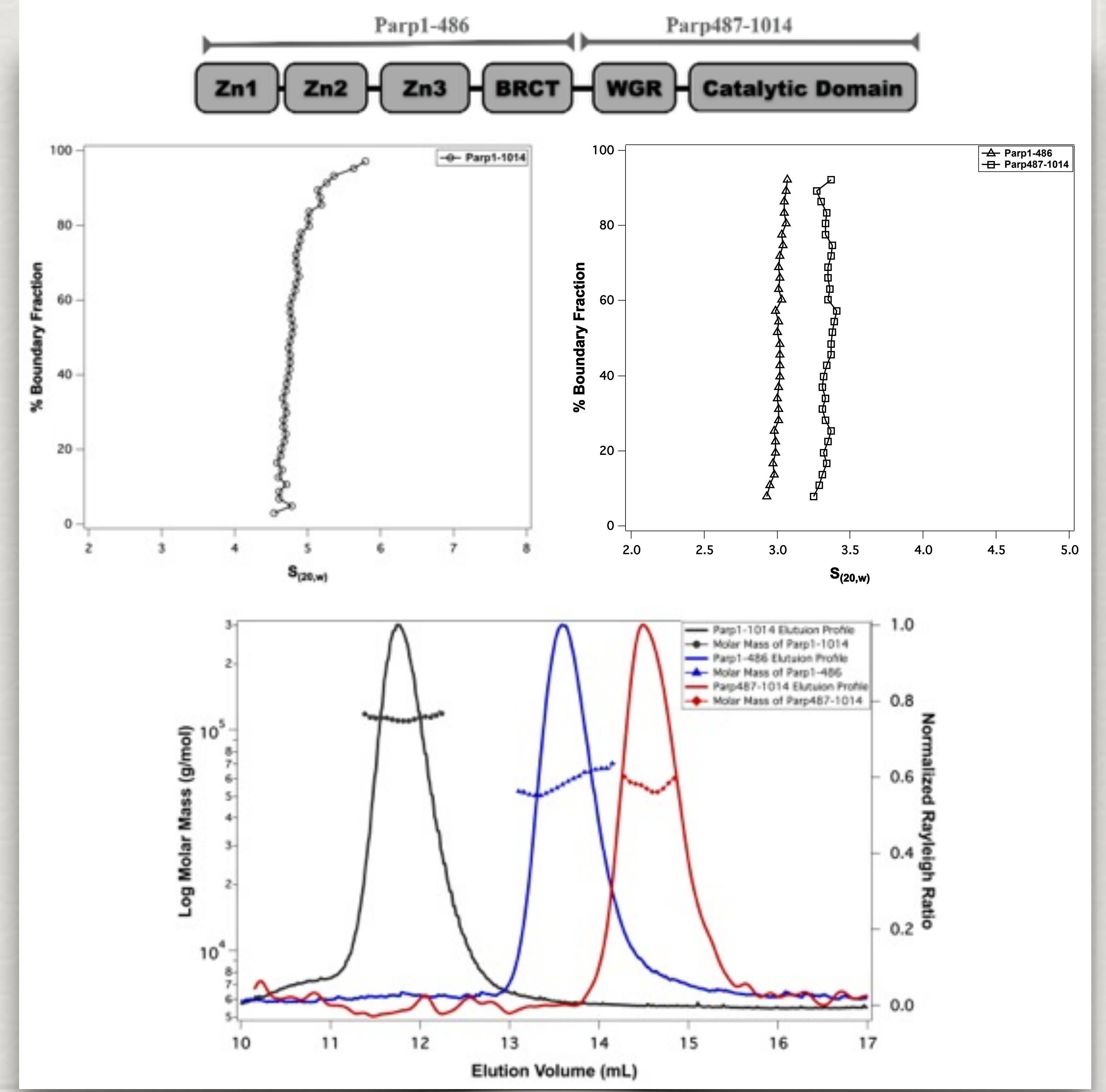


J. Biol. Chem. 2012 Sept;287(39):32430-9

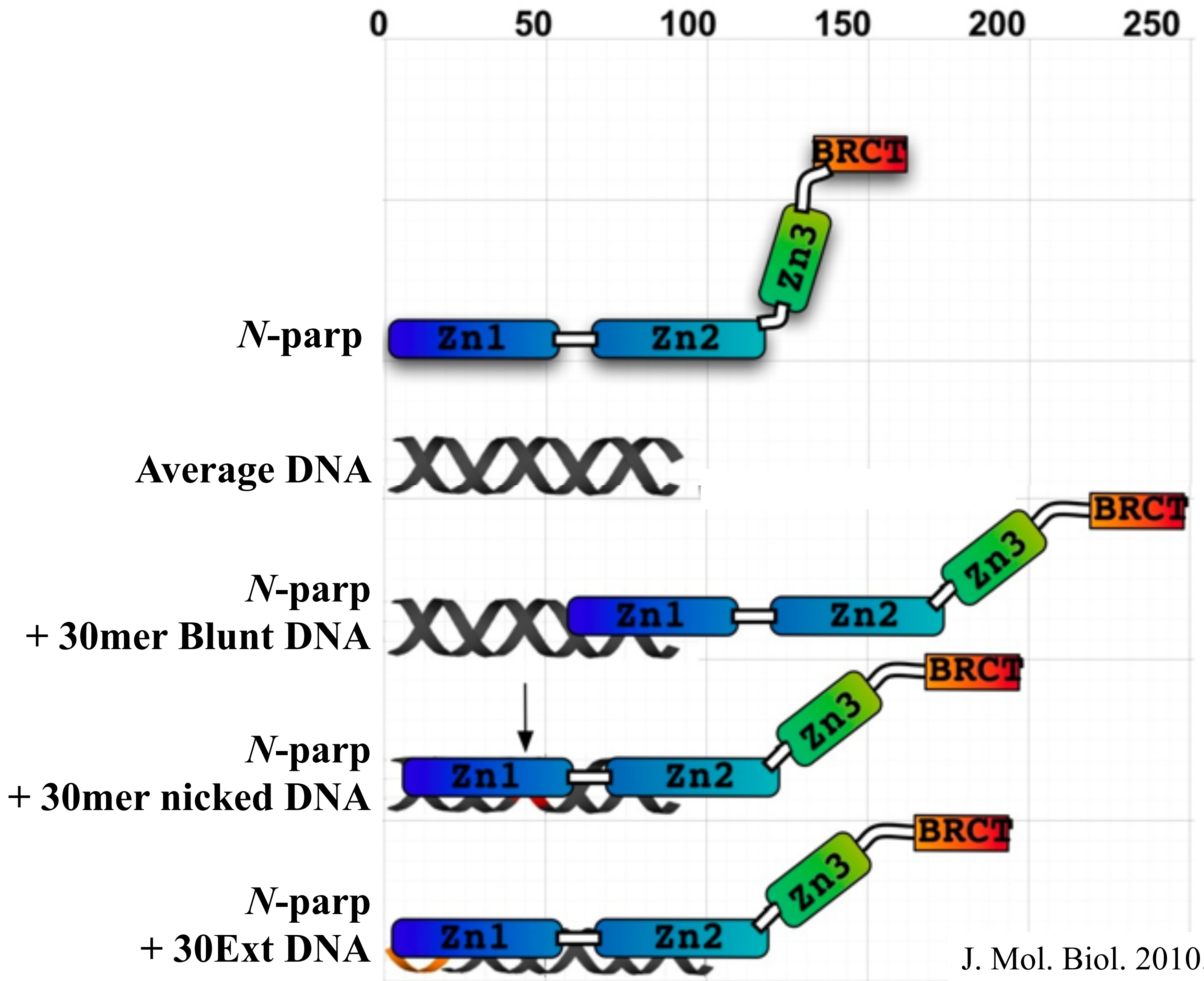
Biophysical characterization of PARP-1

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

Results:
-Monomeric in solution
-PARP-1 relatively elongated.
-Elongates further upon binding to DNA-damage models.

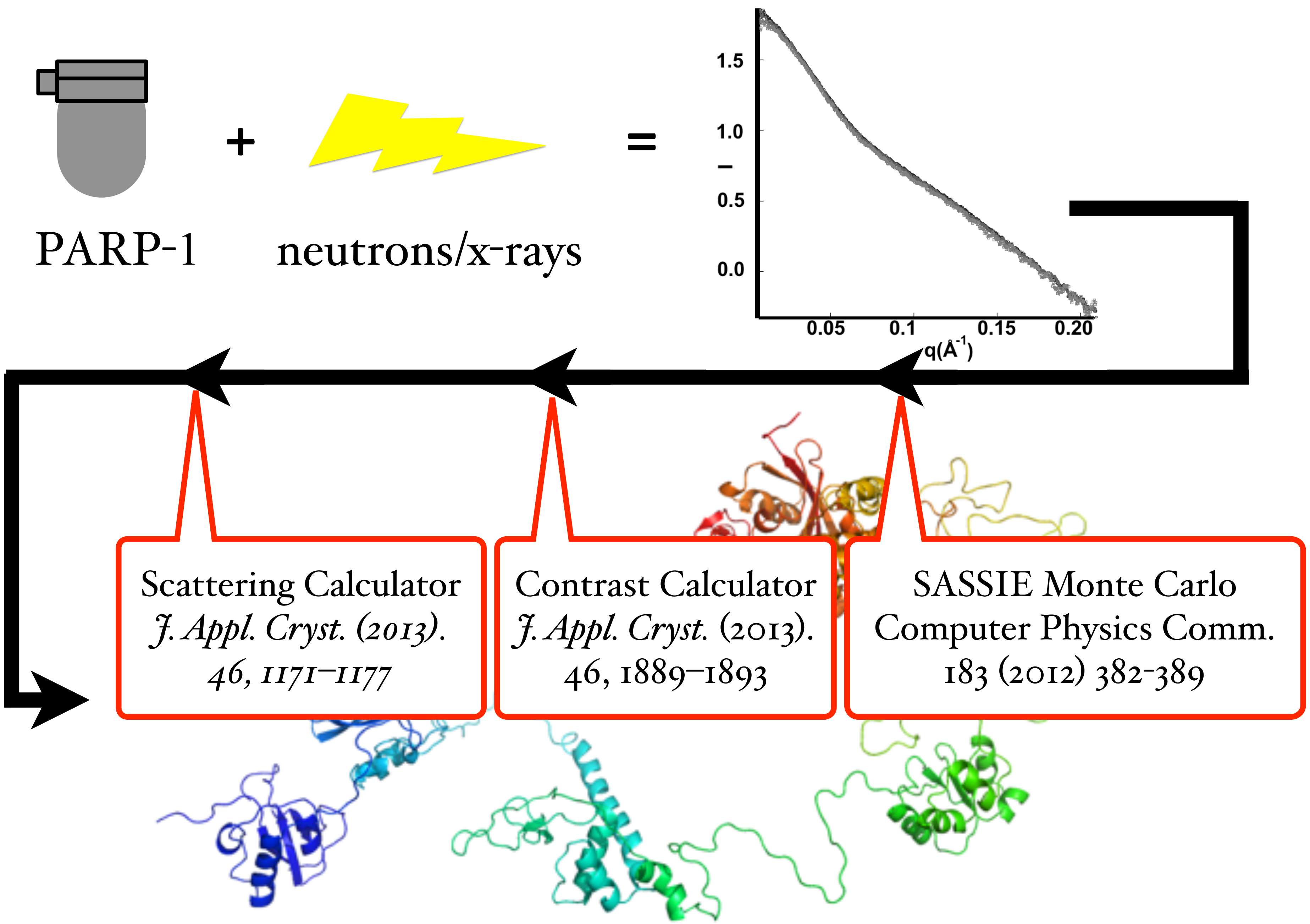


Small angle scattering (SAS) confirms an increase in particle dimension upon DNA binding



J. Mol. Biol. 2010. 395(5):983-994

How is small angle scattering used to help determine the structure-function relationship of PARP-1 in **solution**?



A solution structure of PARP-1

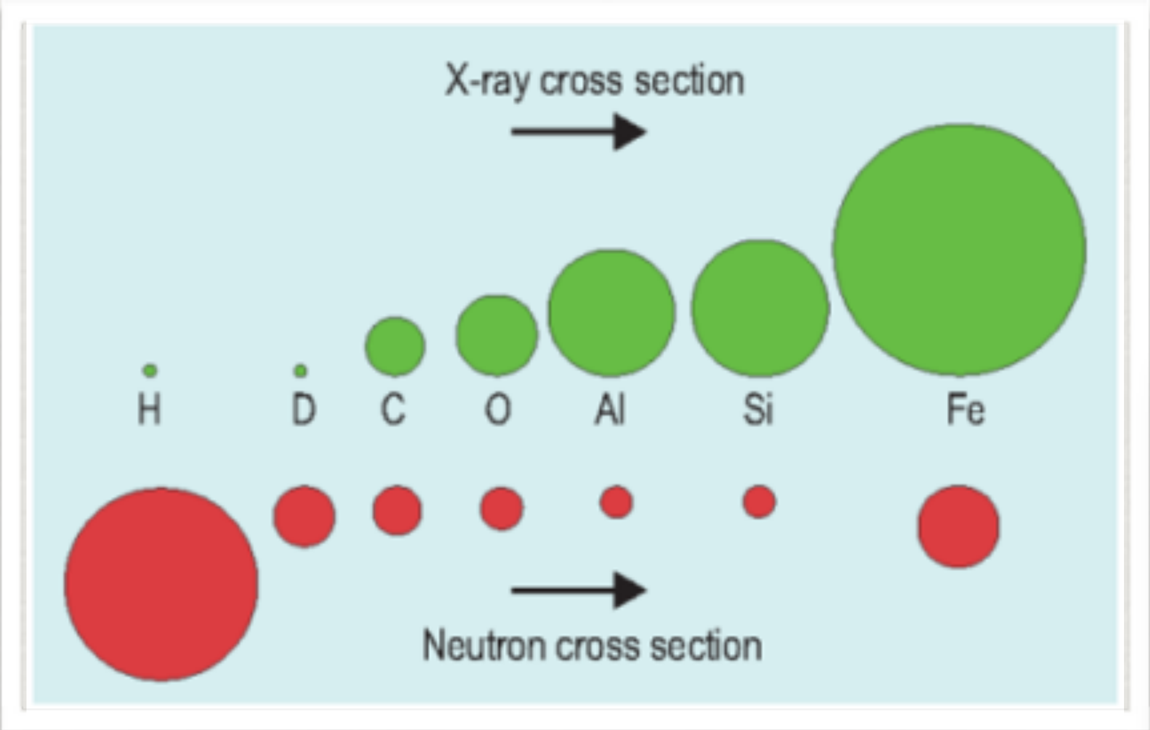
Small angle scattering (SAS) is a set of low-resolution solution characterization techniques that have been developed over the last half century.

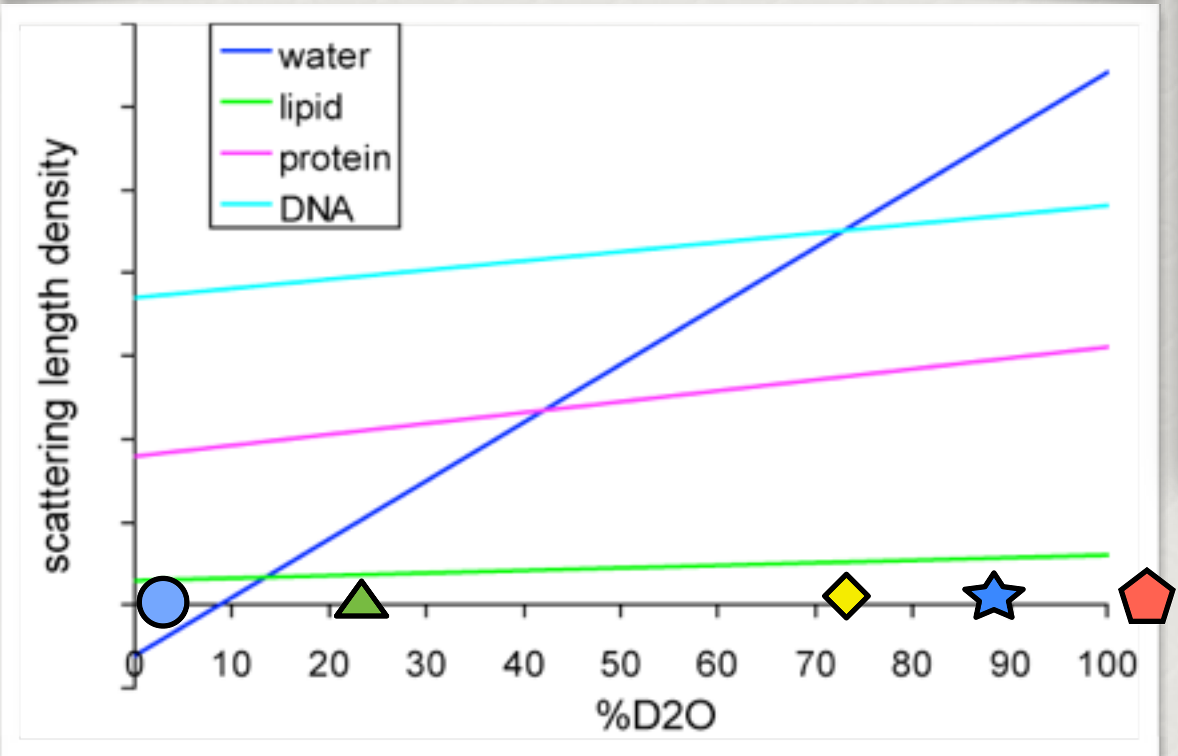
SAS sources we have used for our studies

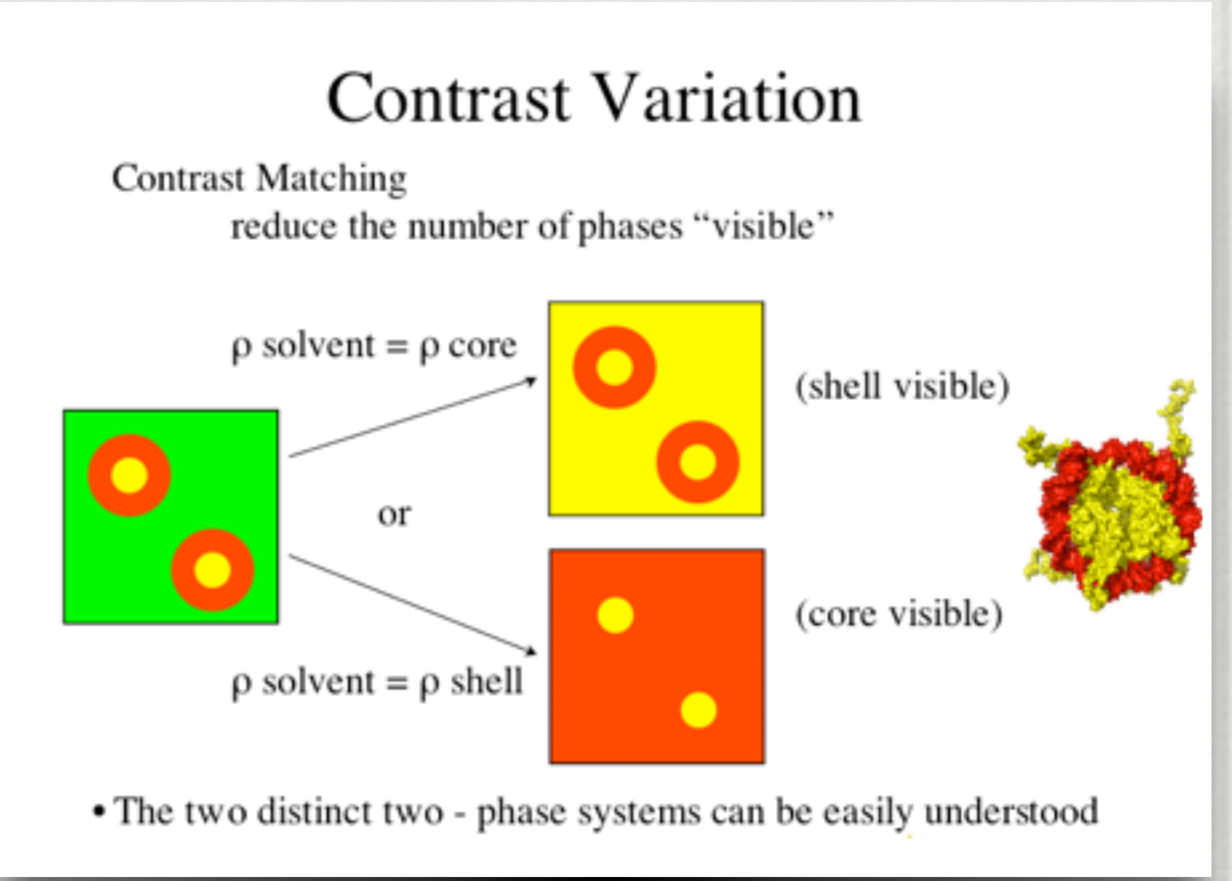
and ILL...

How do particle physics and particle accelerators relate to biochemistry and structural biology?

X-rays vs. Neutrons

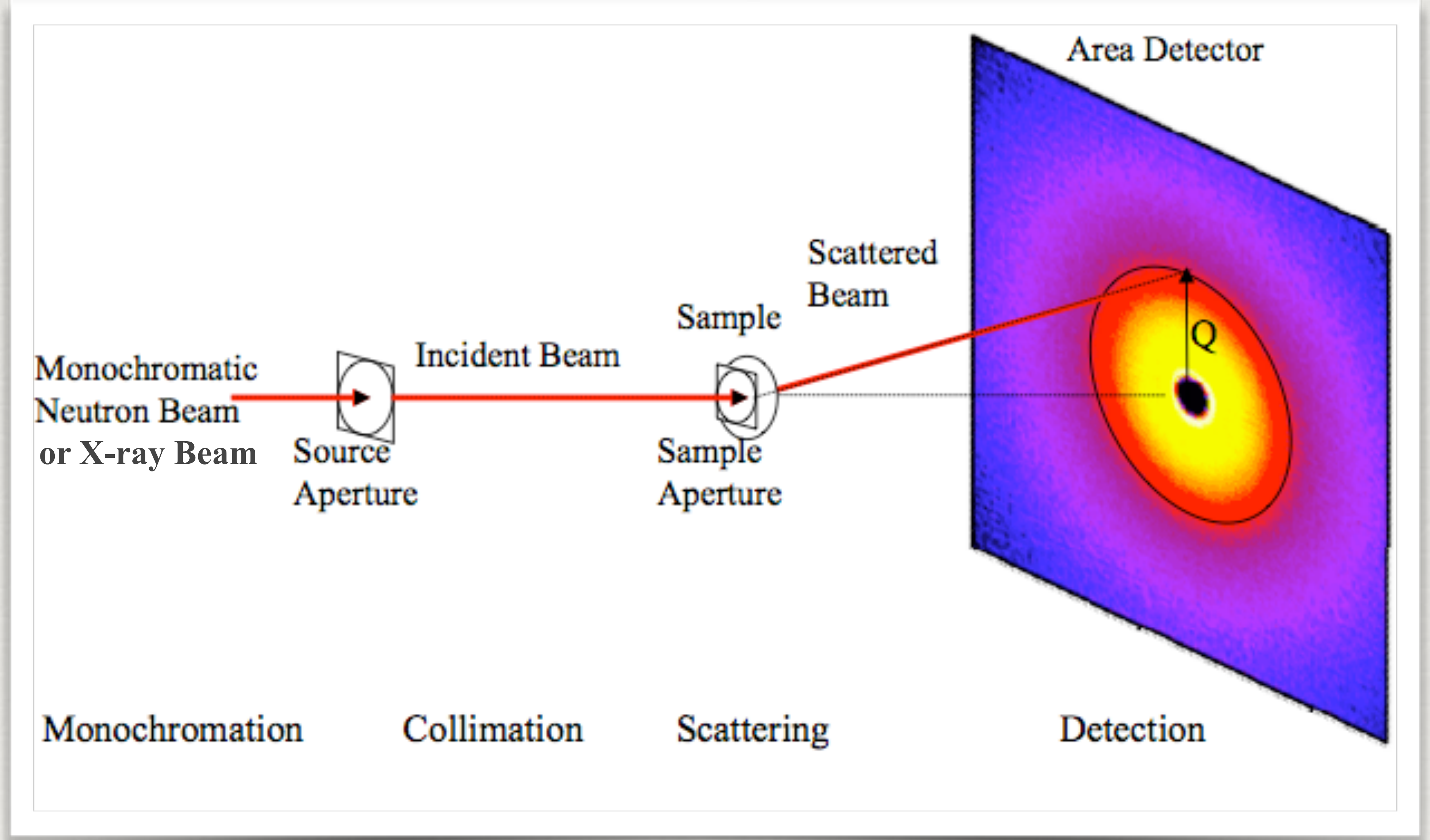




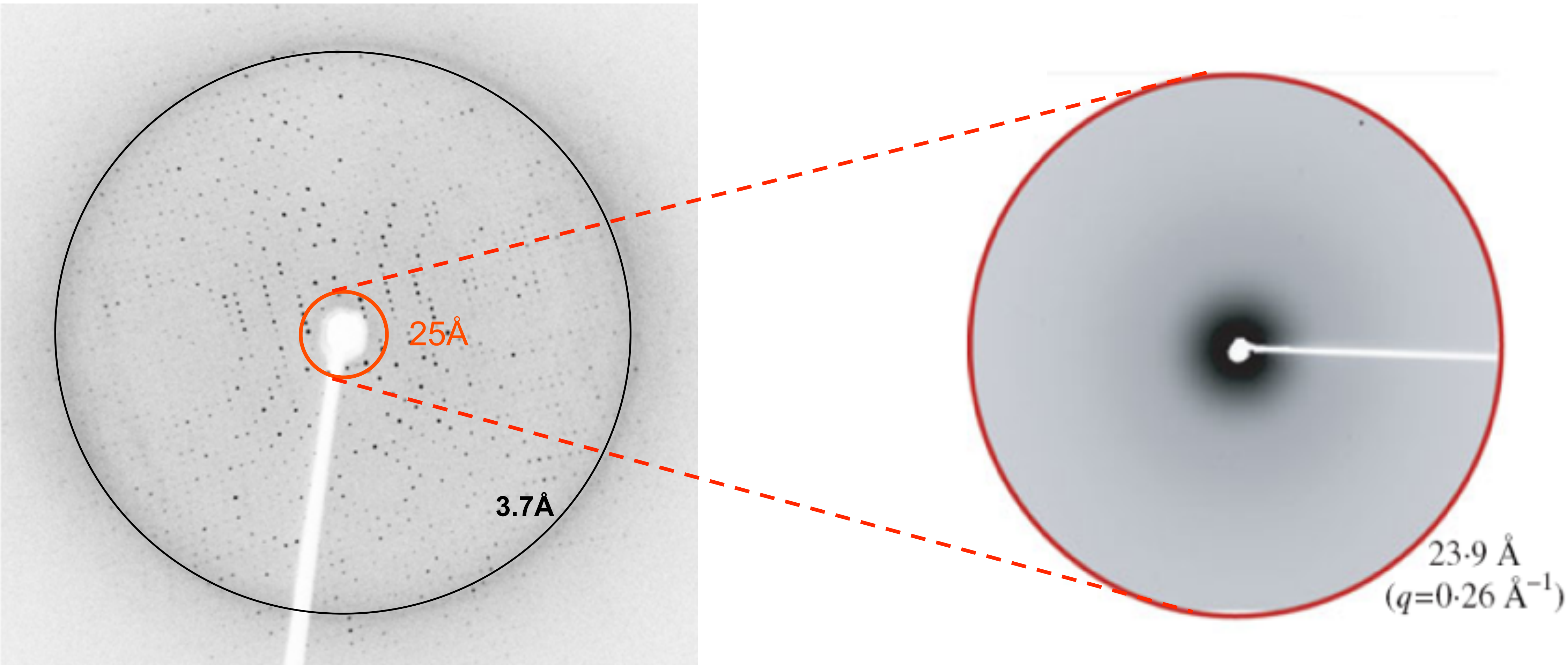


Small angle scattering (SAS)

(similar to crystallography)



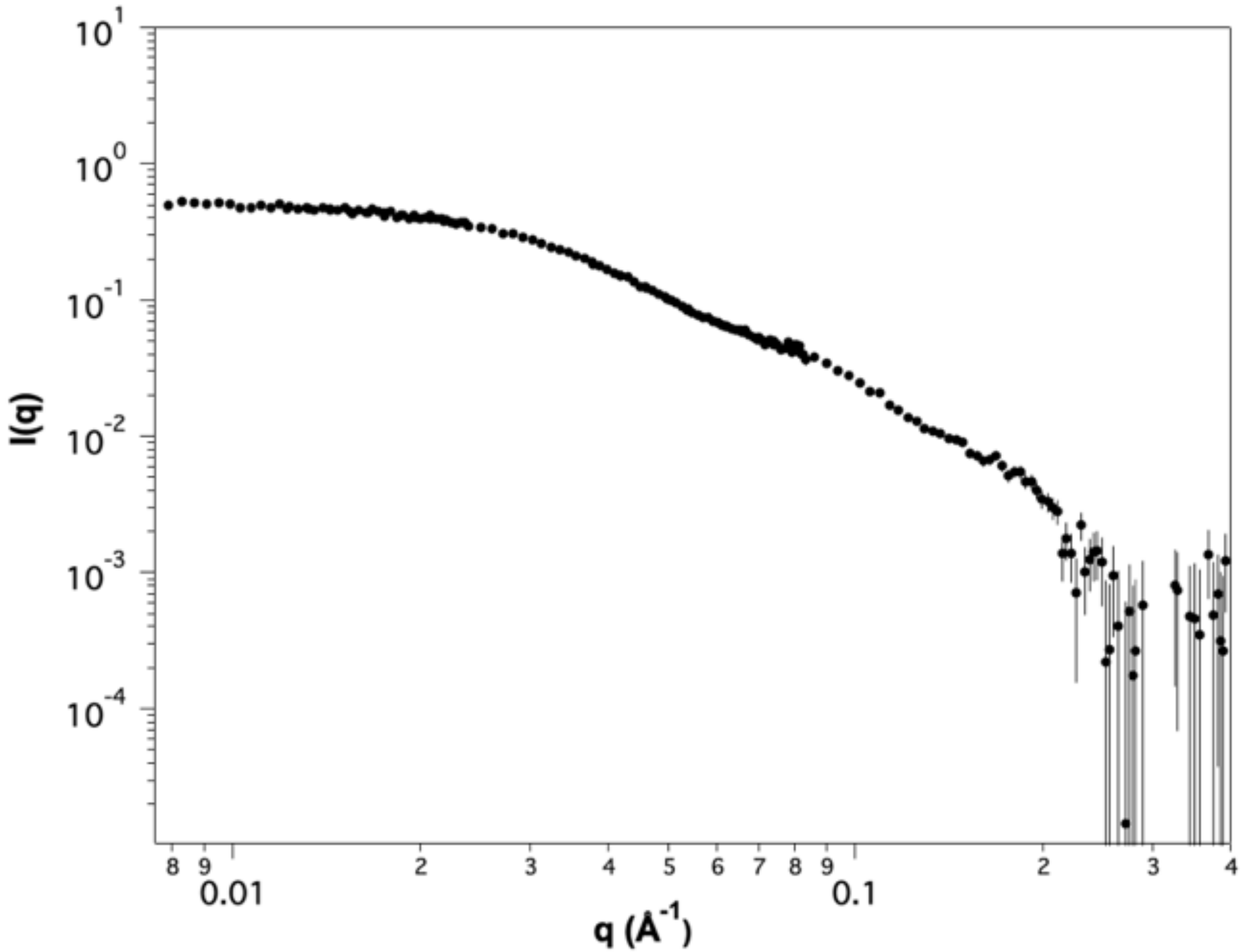
SAS provides low-resolution (~15-250Å) information



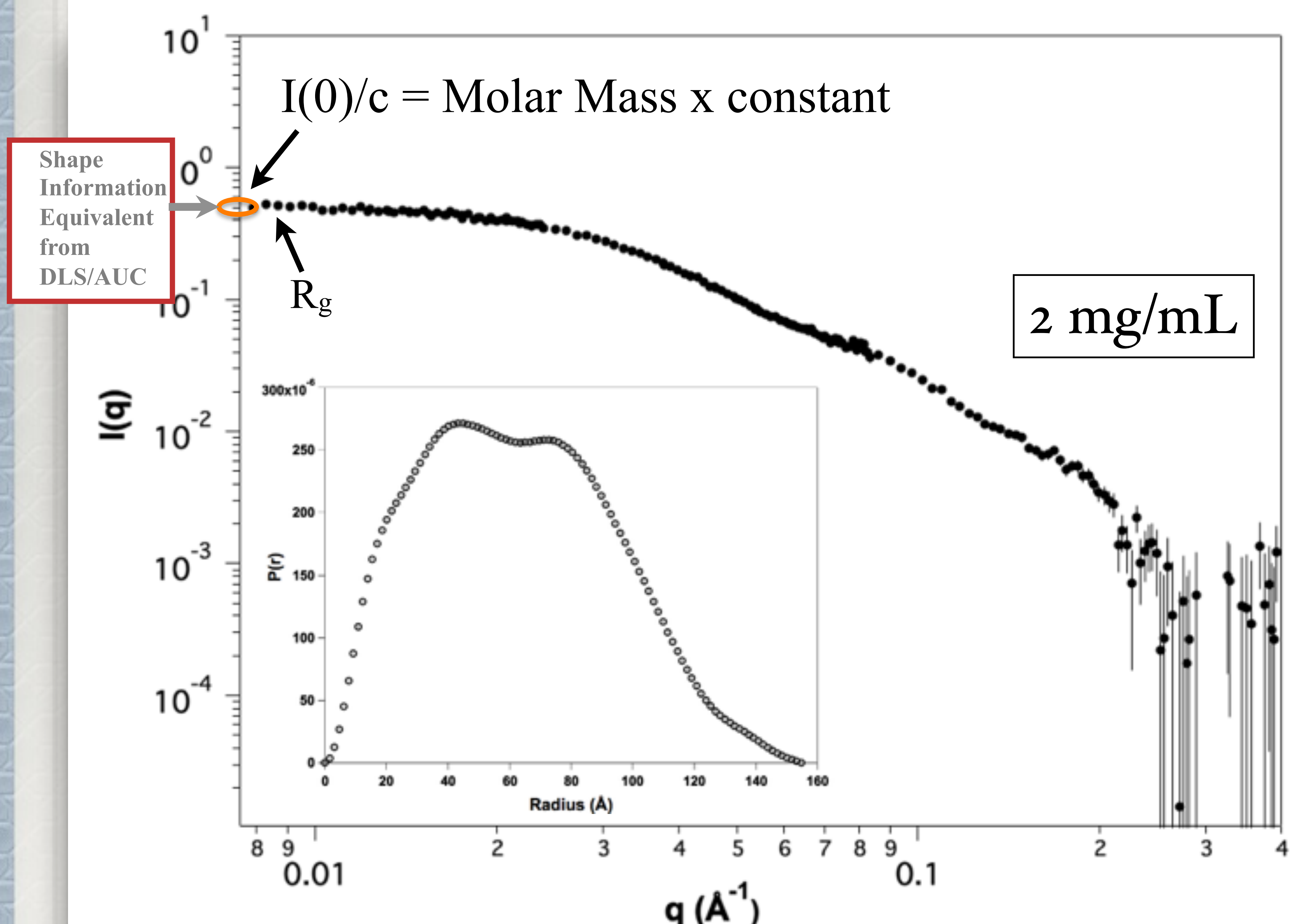
X-ray Diffraction

Small Angle X-ray Scattering

Small-angle scattering profiles are composed of intra- and intermolecular components



Small angle neutron scattering (SANS) profile of dilute anti-streptavidin immunoglobulin-2 (ASA-IgG2)



SANS of an IgG1

Yearly *et al.*
Biophys J. 2013; 105(3): 720–731

SAXS/SANS of an IgA

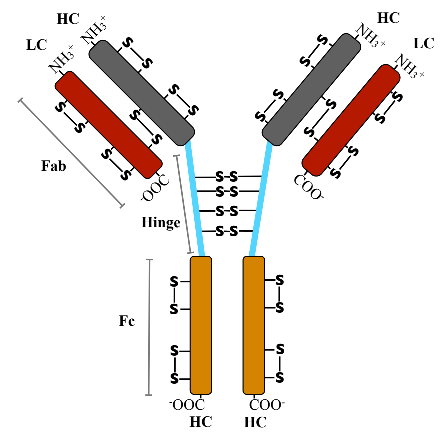
Perkins *et al.*
J. Phys. Chem. B 2012, 116, 9611-9618

SAXS of an IgG1

Lilyestrom et. al
J. Phys. Chem. B 2012, 116, 9611-9618

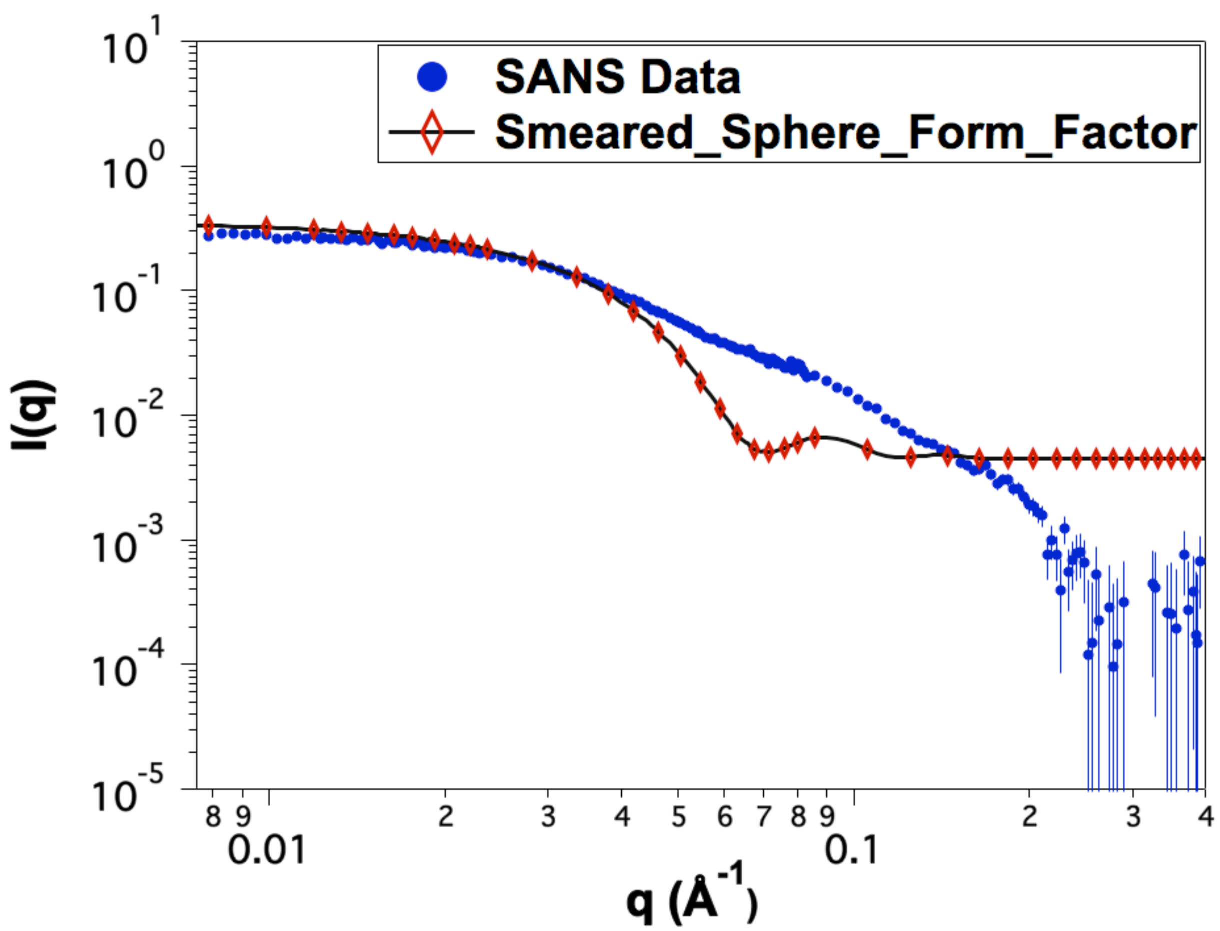
SAXS of an IgG2

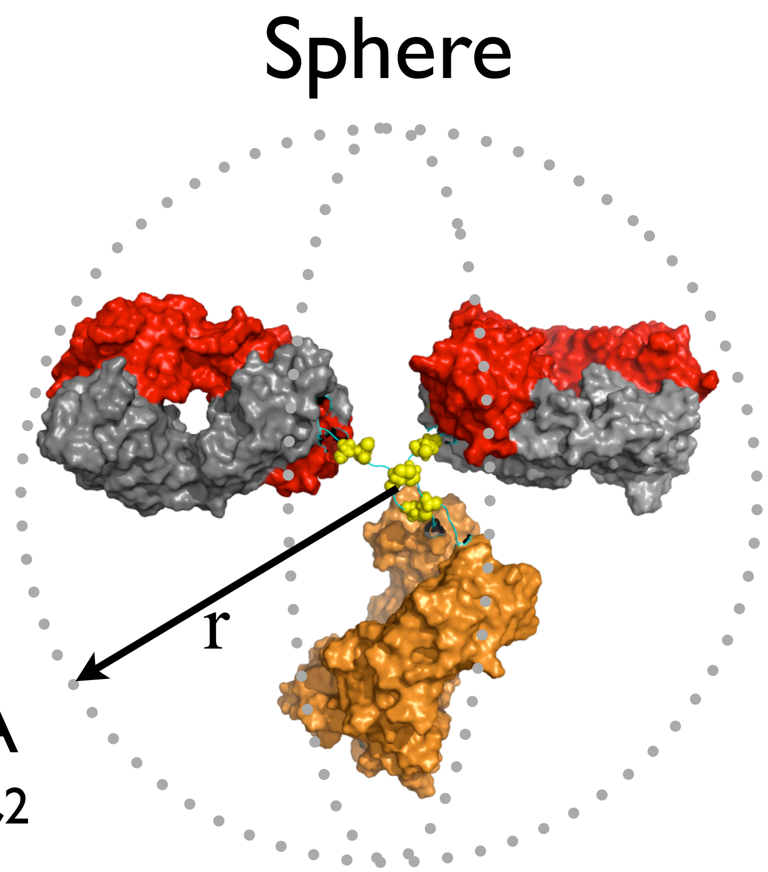
Mosbæk et al.
Pharm Res (2012) 29:2225–2235



ASA-IgG2

Geometric forms fit a portion of the low-q but atomistic models provide a better fit to SAS data



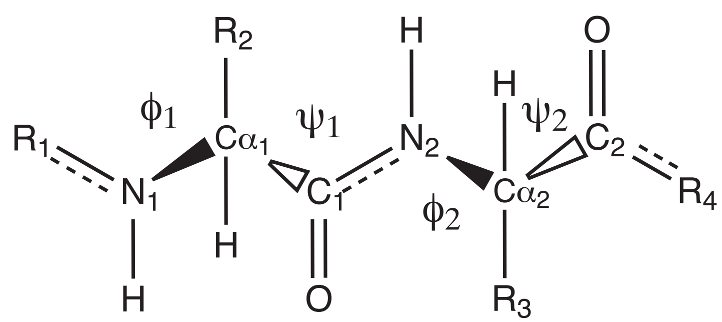


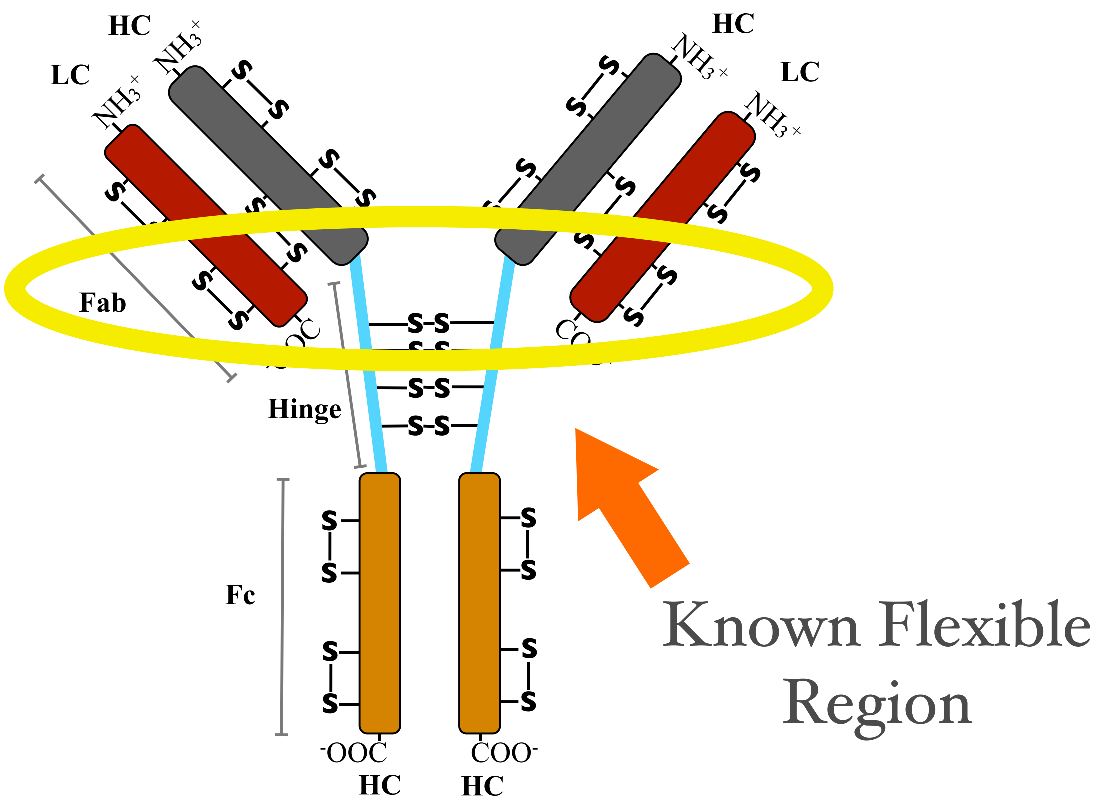
r = 65Å

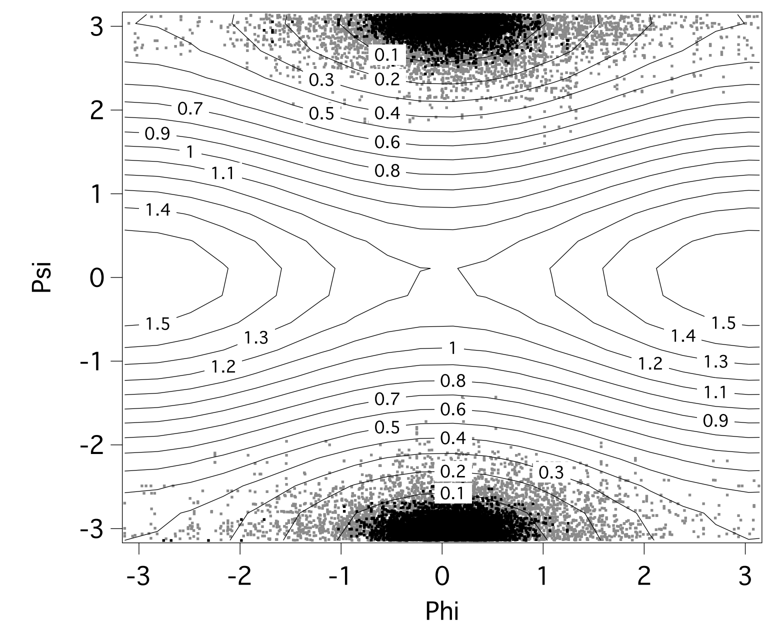
$Rg^2 = {}^3/_5\ r^2$

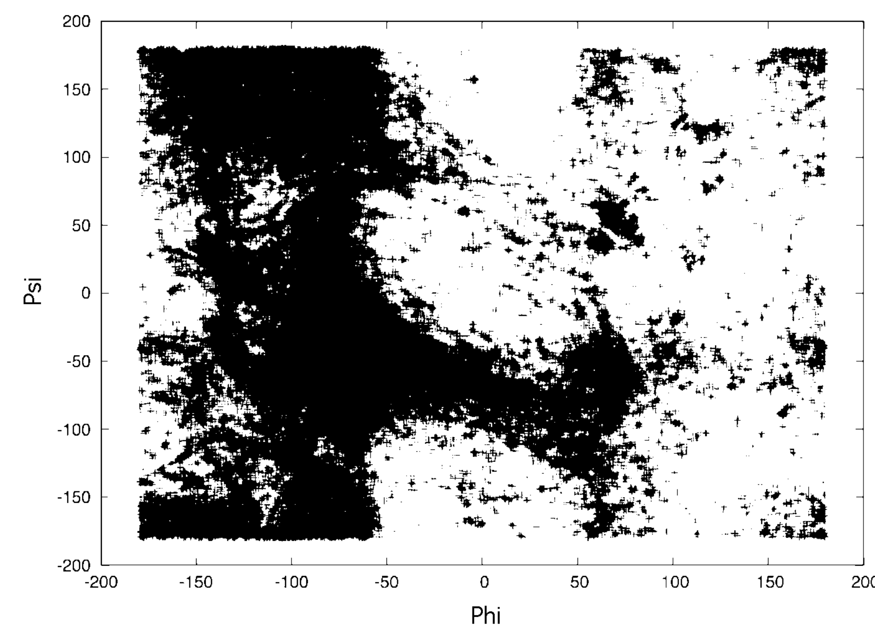
Molecular Monte Carlo (MMC) allows for rapid sampling of the mAb conformations that are possible in solution

di-alanine peptide





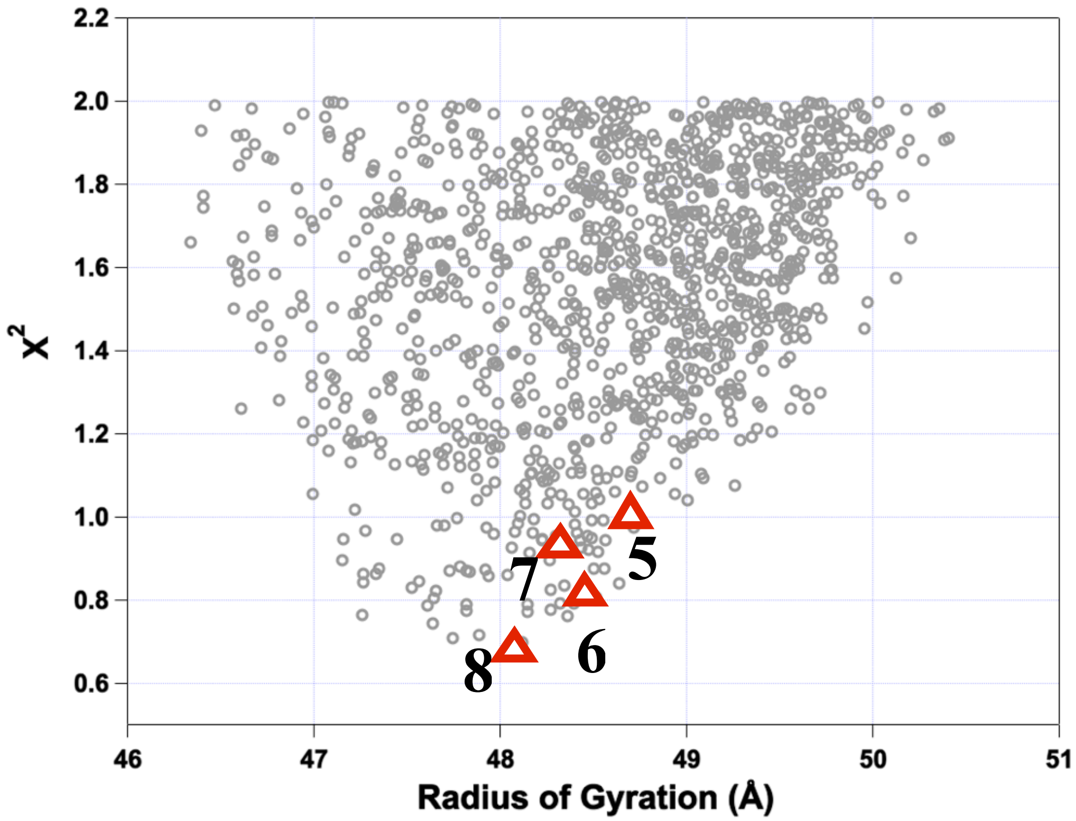


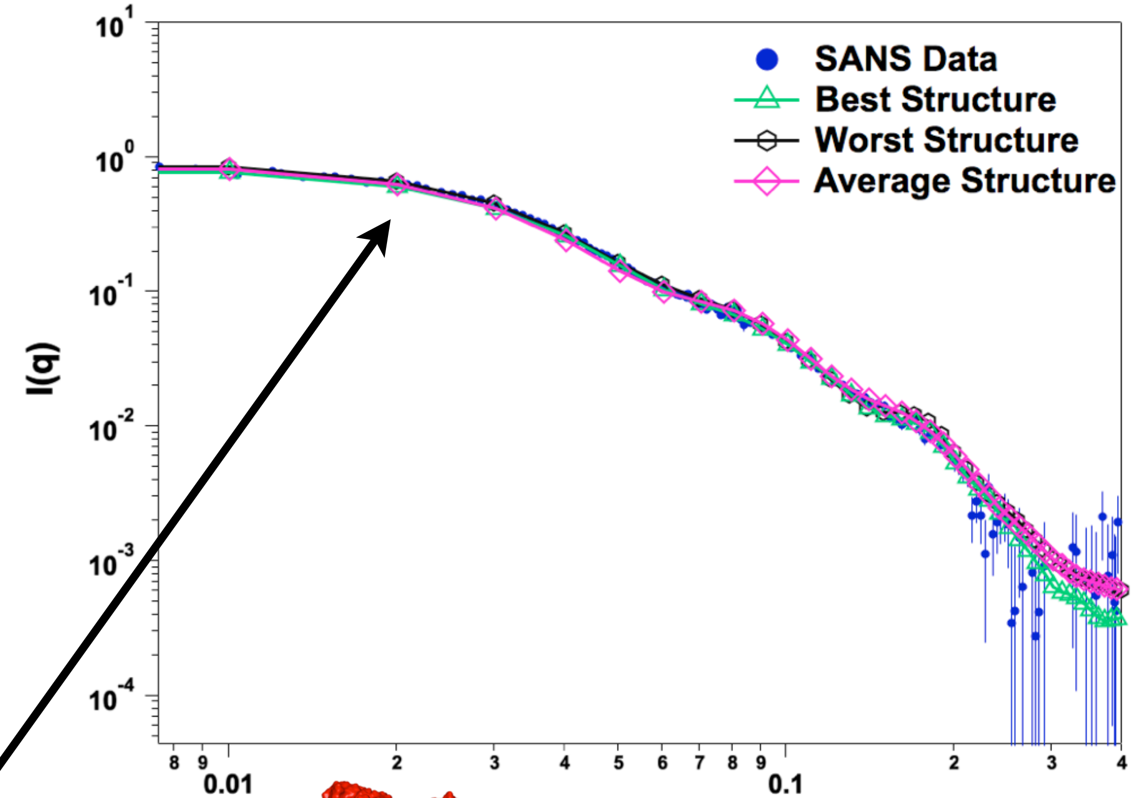


Computer Physics Communications 183 (2012) 382–389

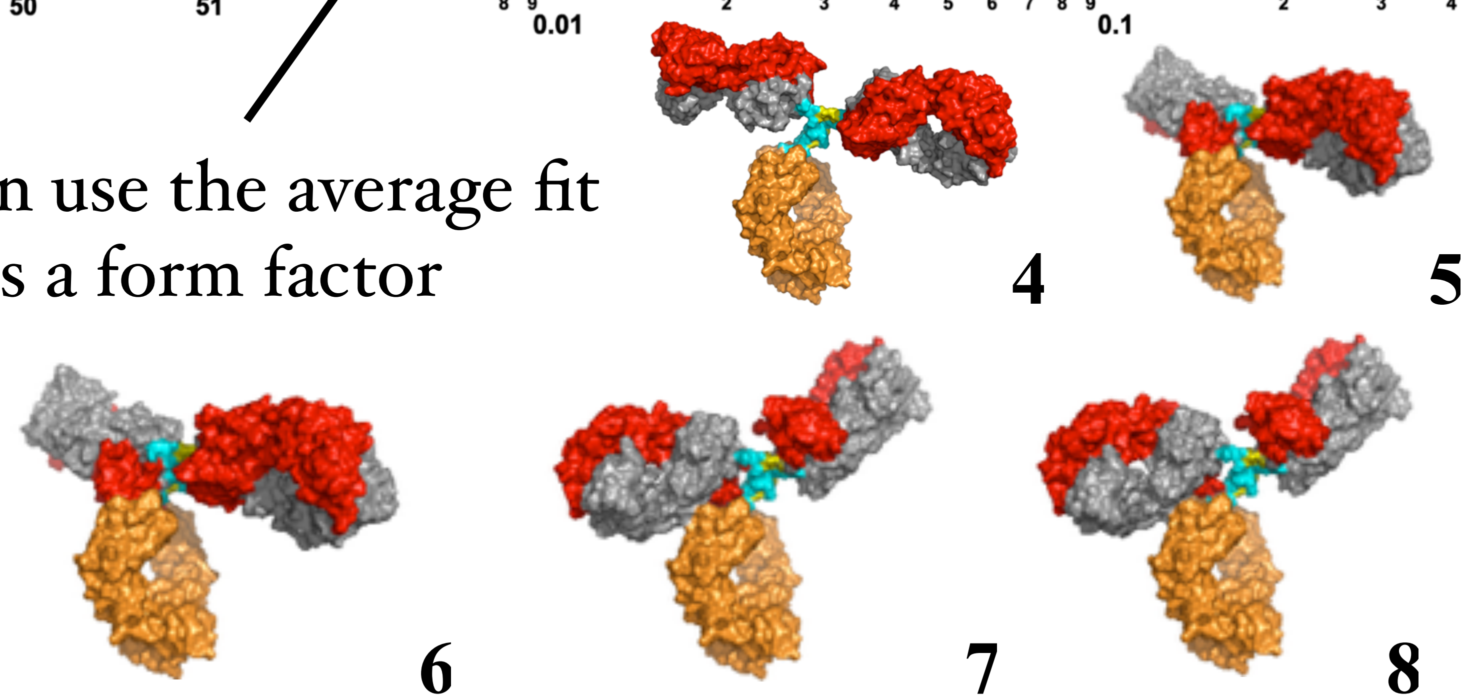
Monte Carlo ensemble fit to data

Best-Fit Structures





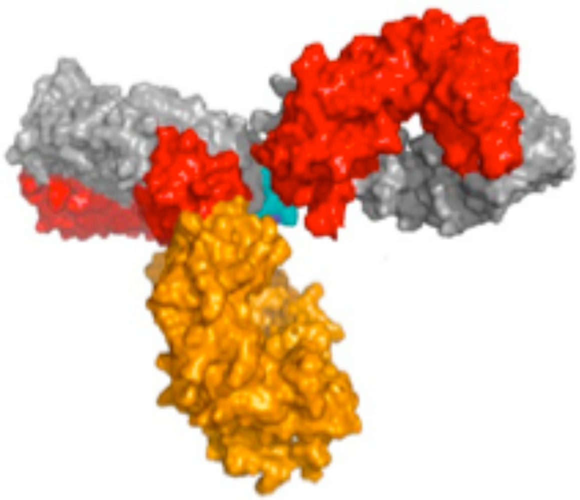
We can use the average fit as a form factor



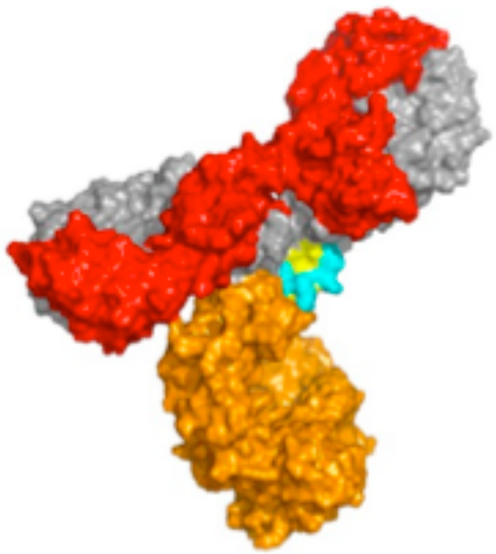
J. Phys. Chem B., 2013, 17 (45):14029-38

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

Free-energy analysis



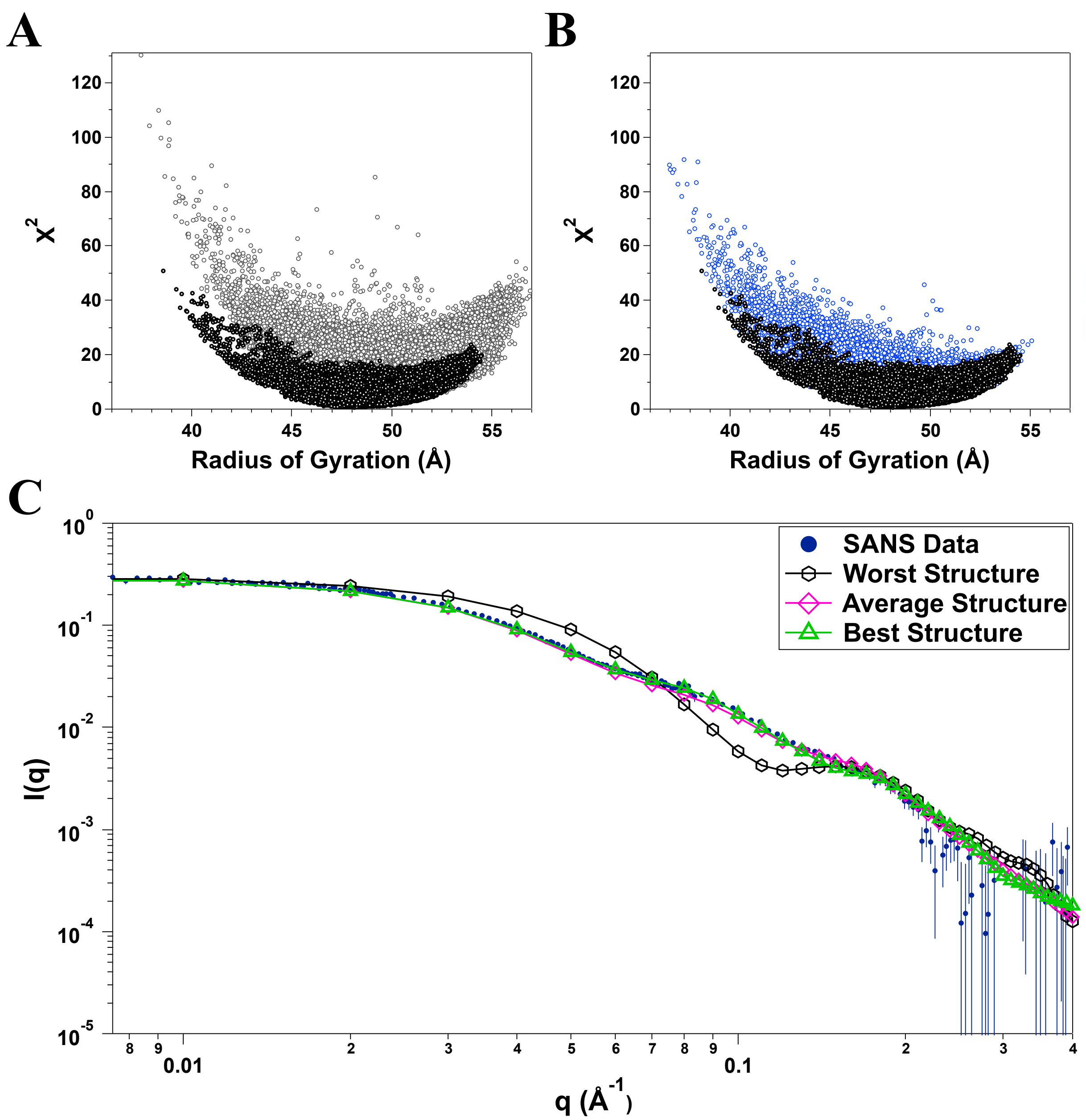
-16400 kcal/mol

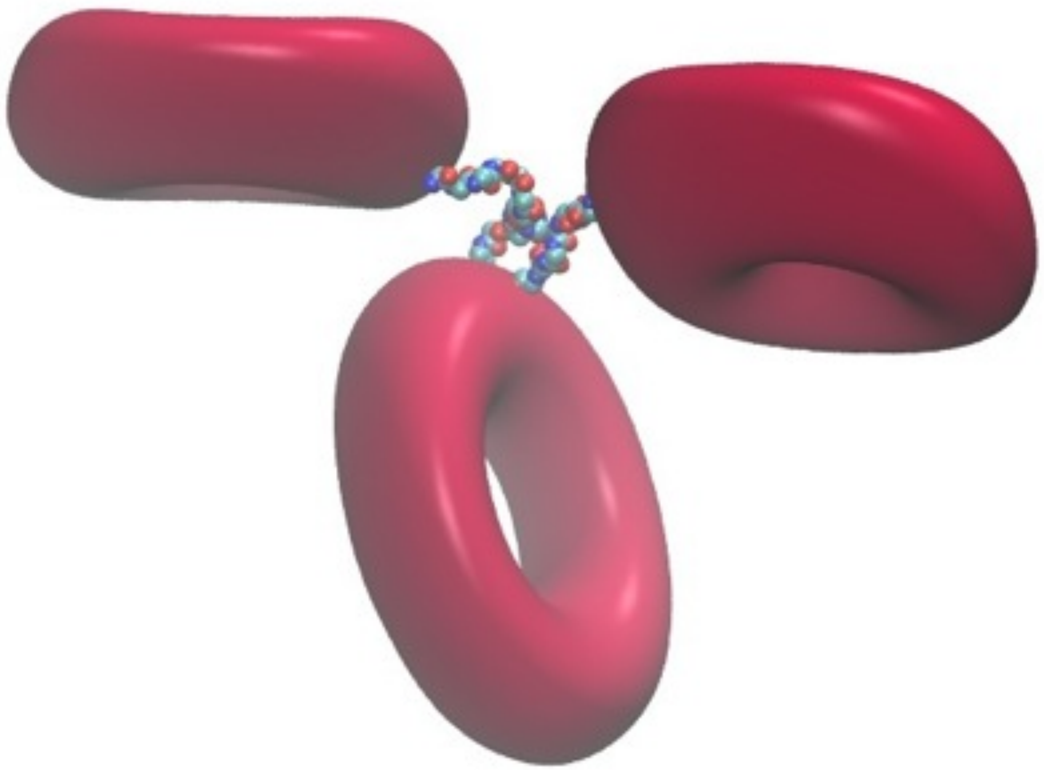


-16800 kcal/mol

J. Phys. Chem B., 2013, 17 (45):14029-38

Hybrid MMC toroid approach (proprietary and simulation advantages)



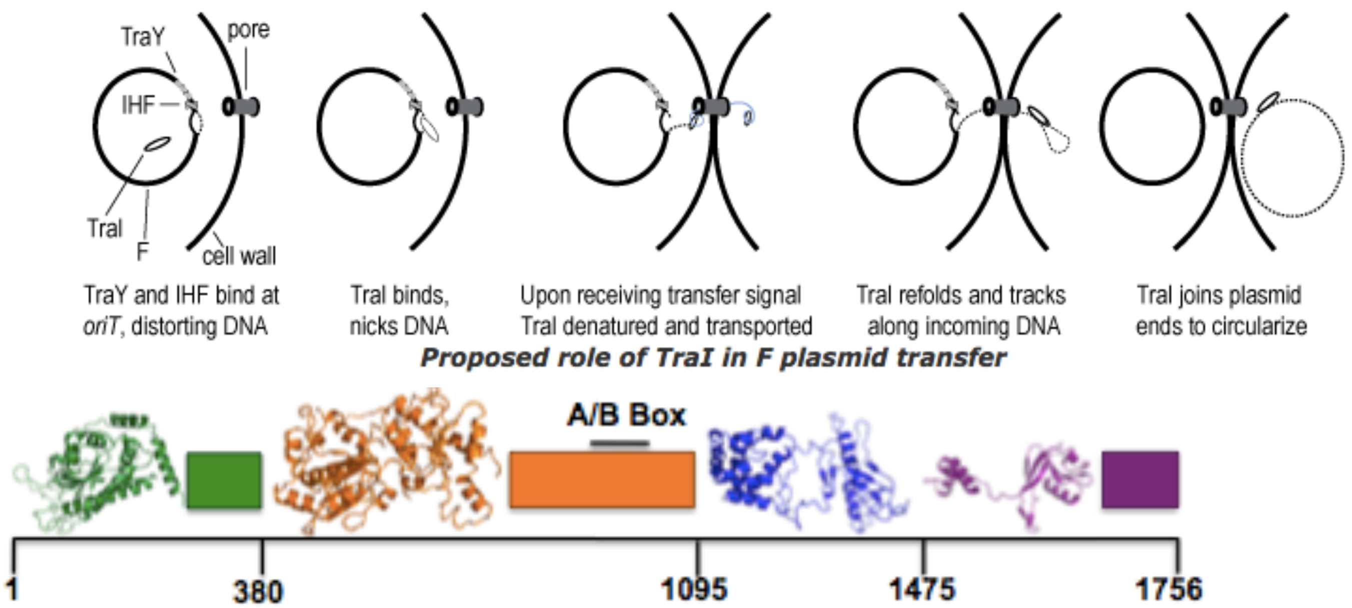


Manuscript in preparation... (Sarachan, K. et al...)

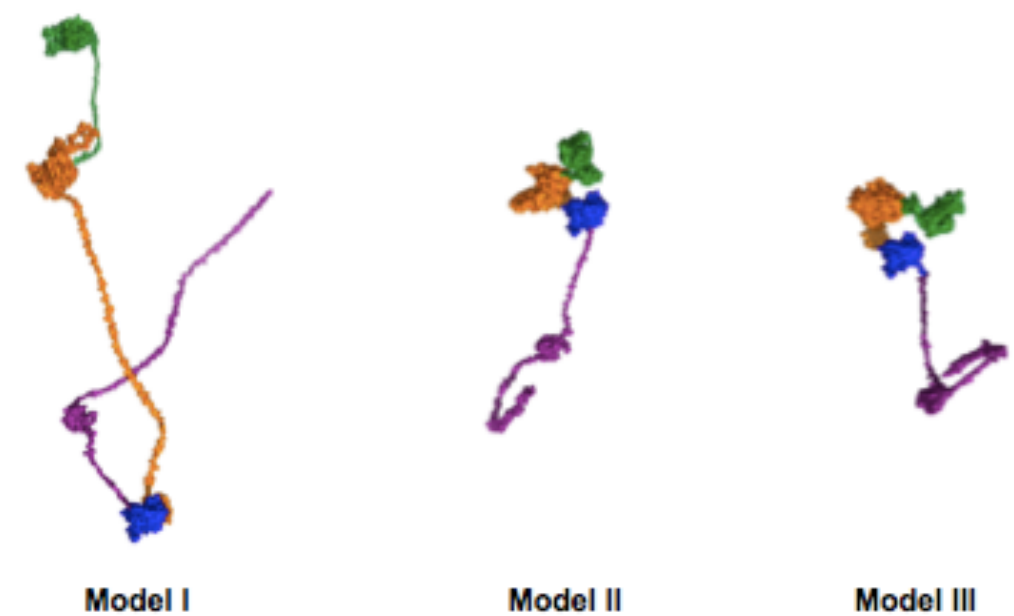
Other Structural Biology Questions

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



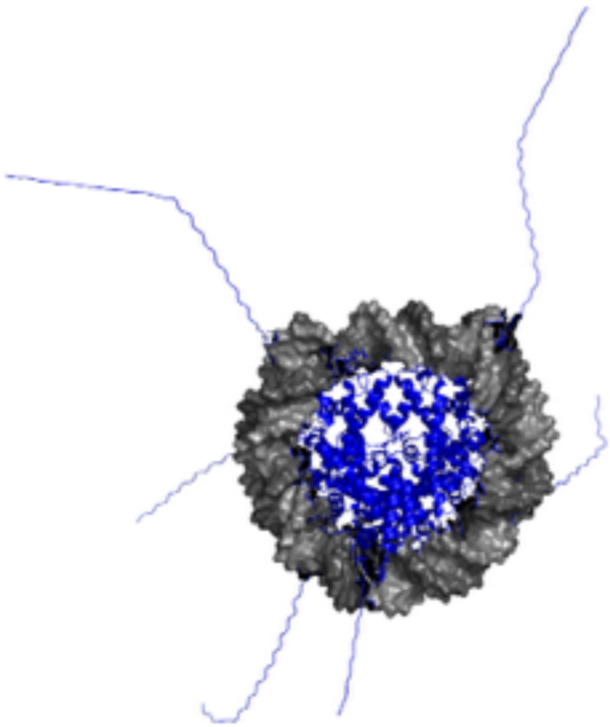
Beth Buenger's graduate thesis work underway...

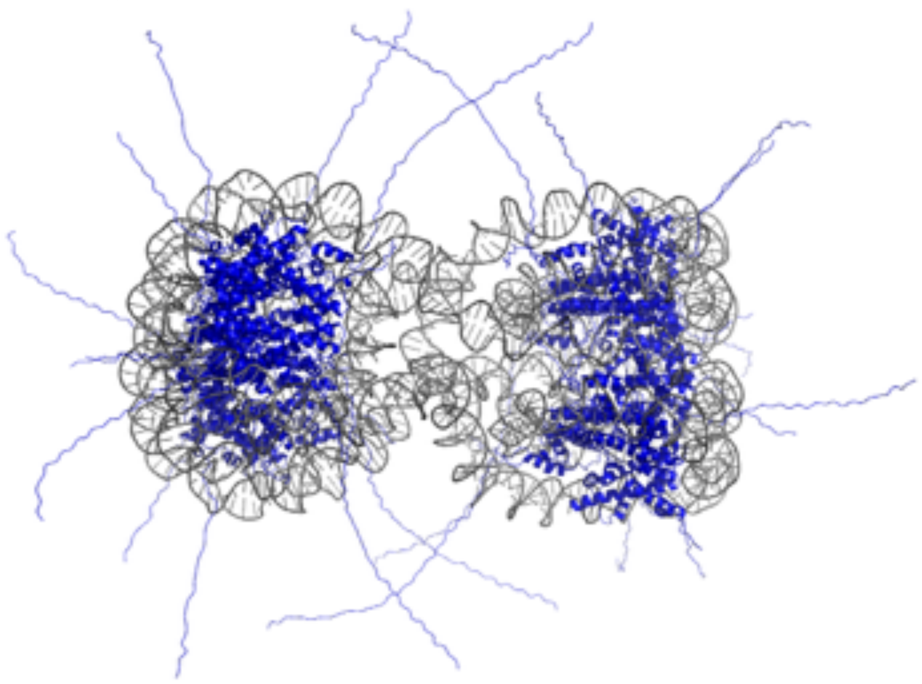
Clark *et. al.*, 2014, *J Mol Model* 20:2308

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?



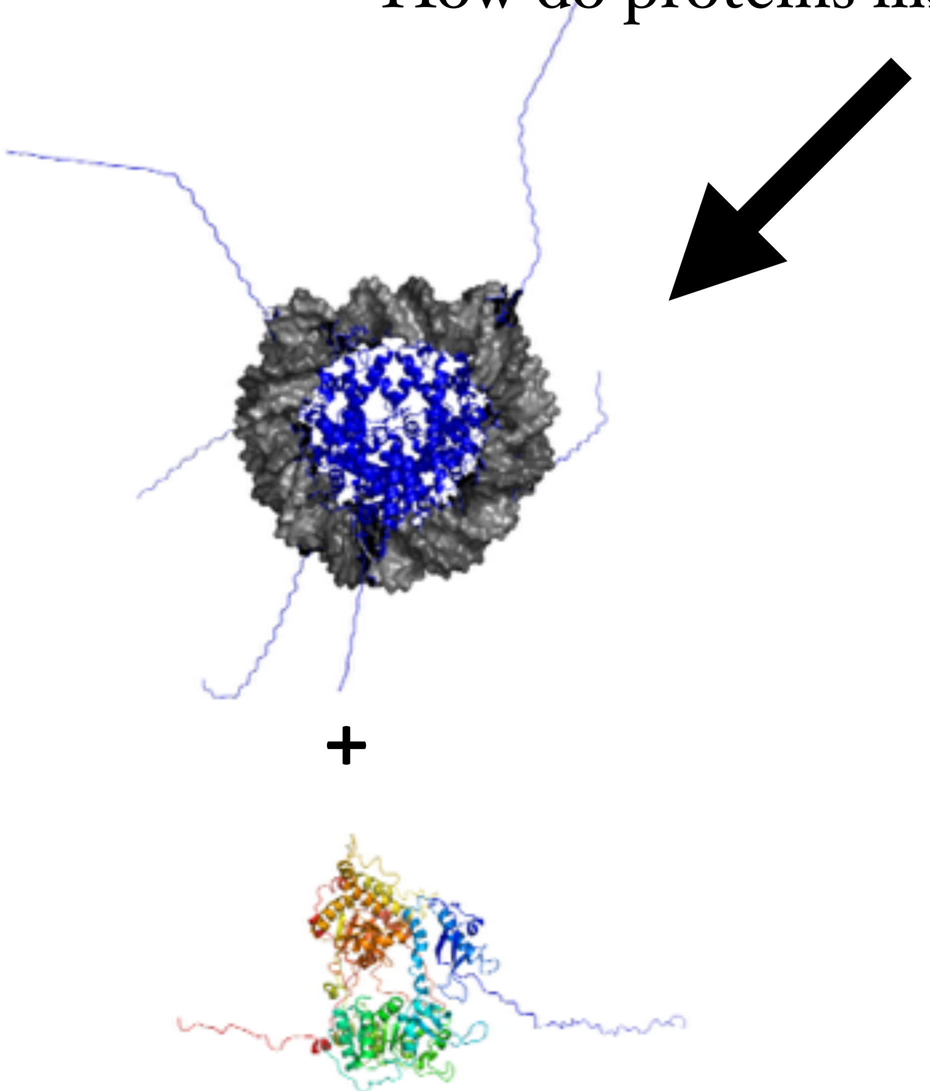


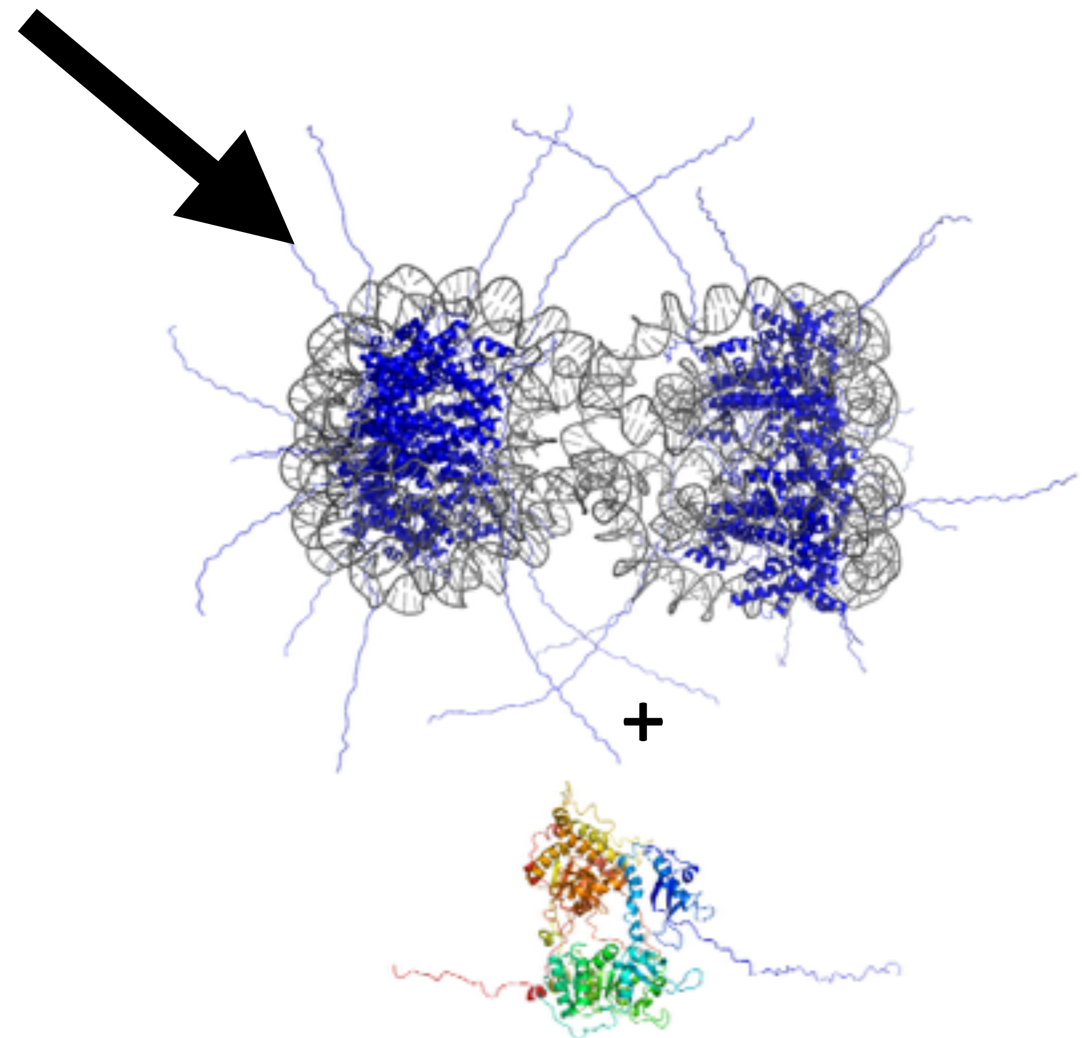
Modeling work underway...thanks to new double-stranded DNA MMC

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?





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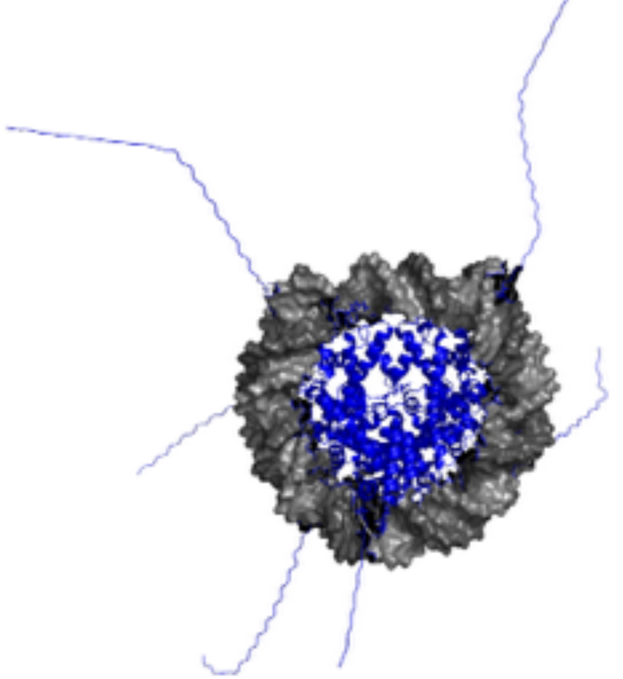
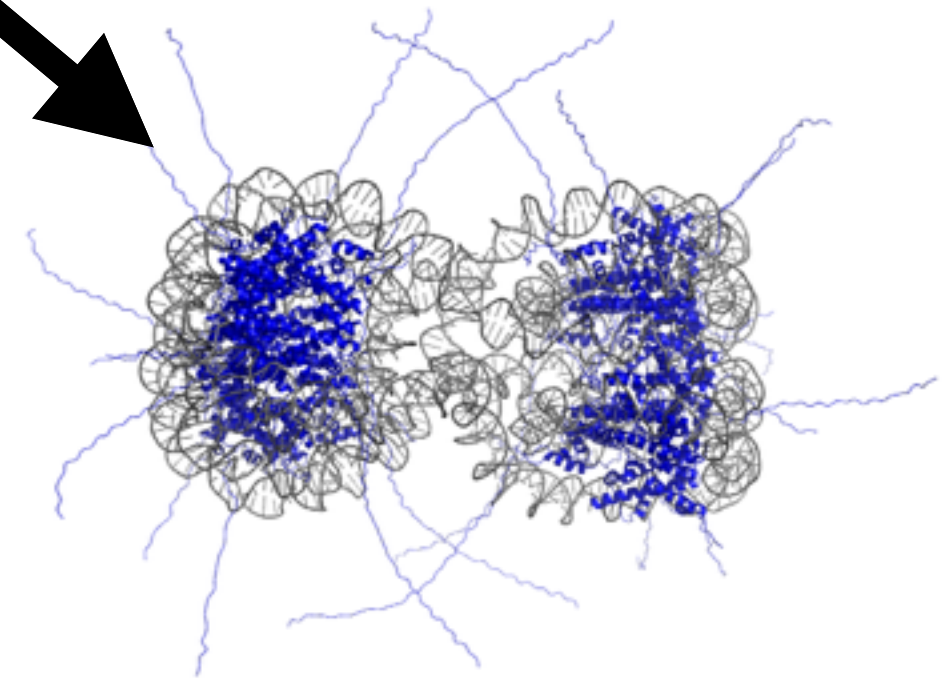
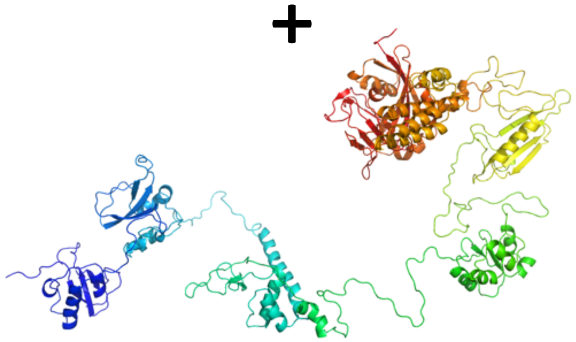
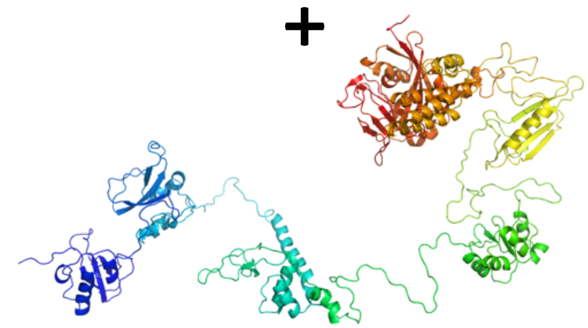
How do proteins like PARP-1 interact with chromatin?









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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.

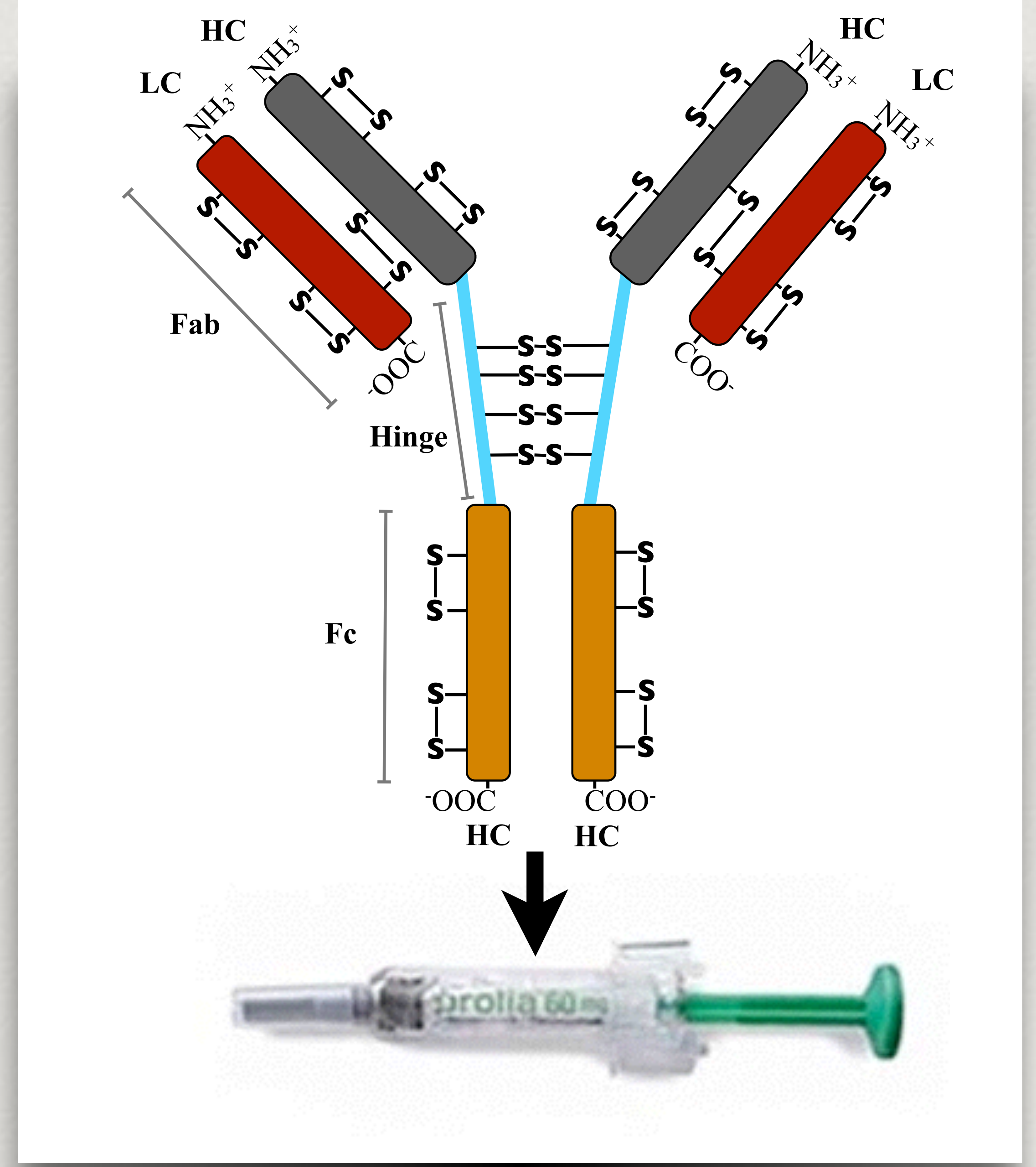
Biopharmaceuticals or Biologics
Specifically; Monoclonal Antibodies (mAbs)

Pros

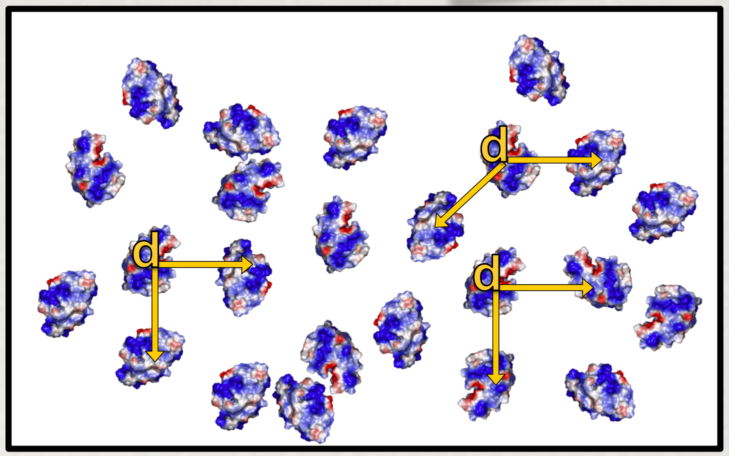
- Lower toxicity with higher specificity
- Biologics can be custom designed to have specific activity

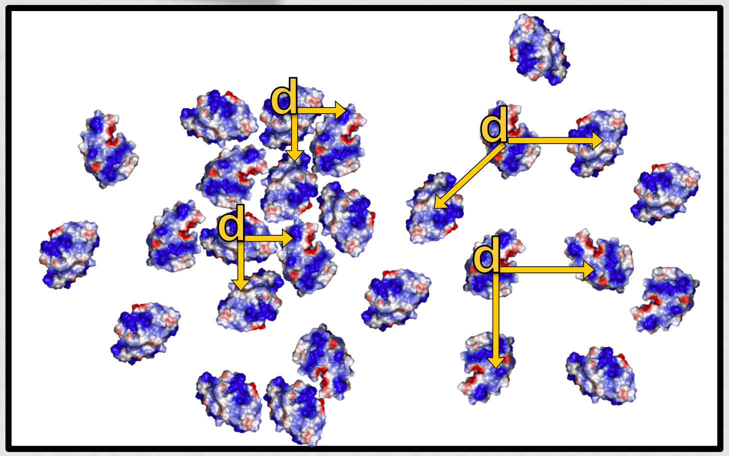
Cons

- Low potency requires that they be administered in high concentrations (≥1.0 g / kg)
- Highly concentrated solutions have unwanted side effects
  - high viscosity
  - tendency to crystallize, phase separate or aggregate

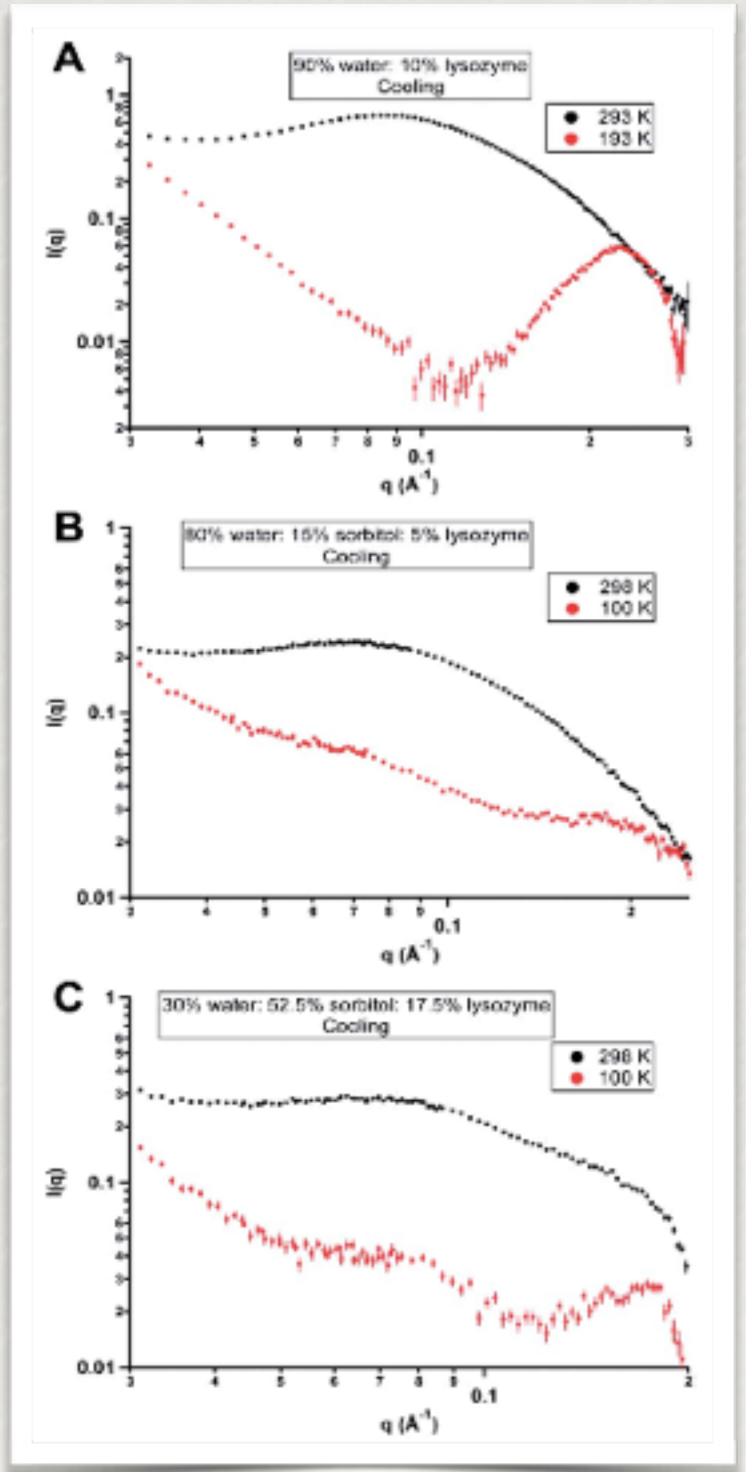


Neutrons be useful for studying proteins in different phases
(liquid, frozen and powdered formulations)



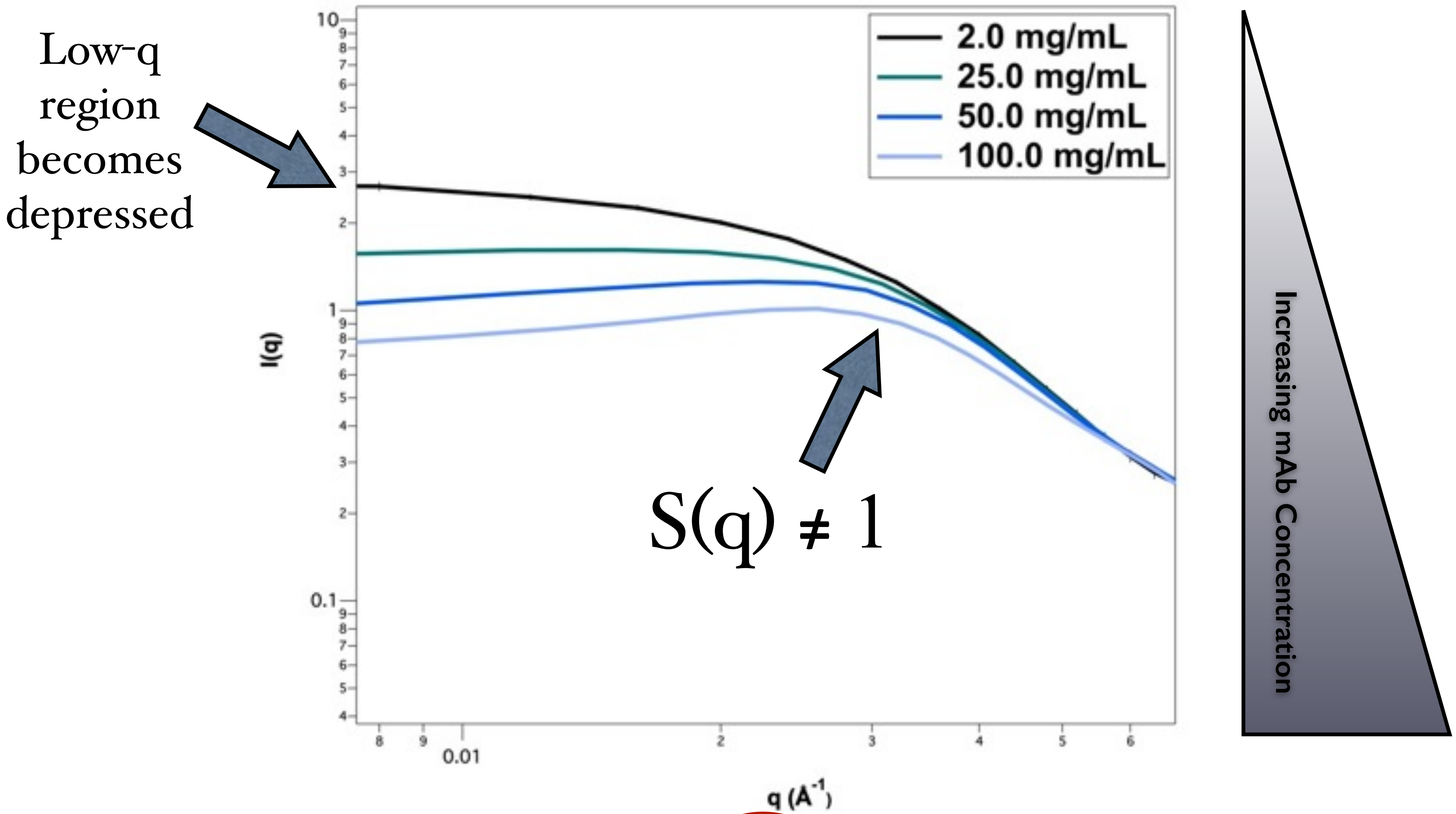


Soft Matter (2014), 10, 4056 DOI:10.1039
and *J Phys Chem B.* 2012 Apr 19;116(15):4439-47
and *Faraday Discuss.* 2012;158:285-99
lyophilized powder work in preparation…



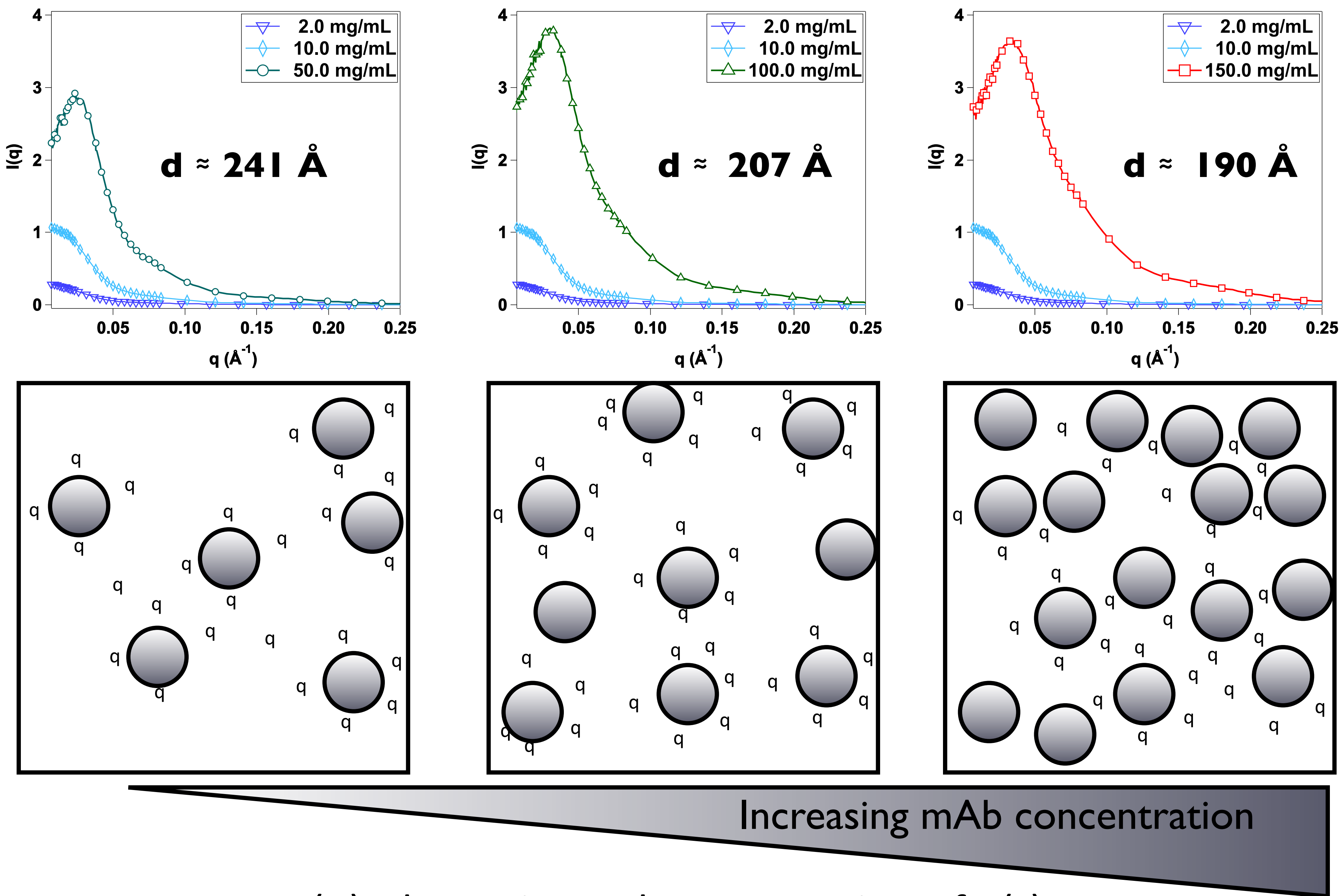
What happens at high concentrations?

Concentration effects on scattering profiles

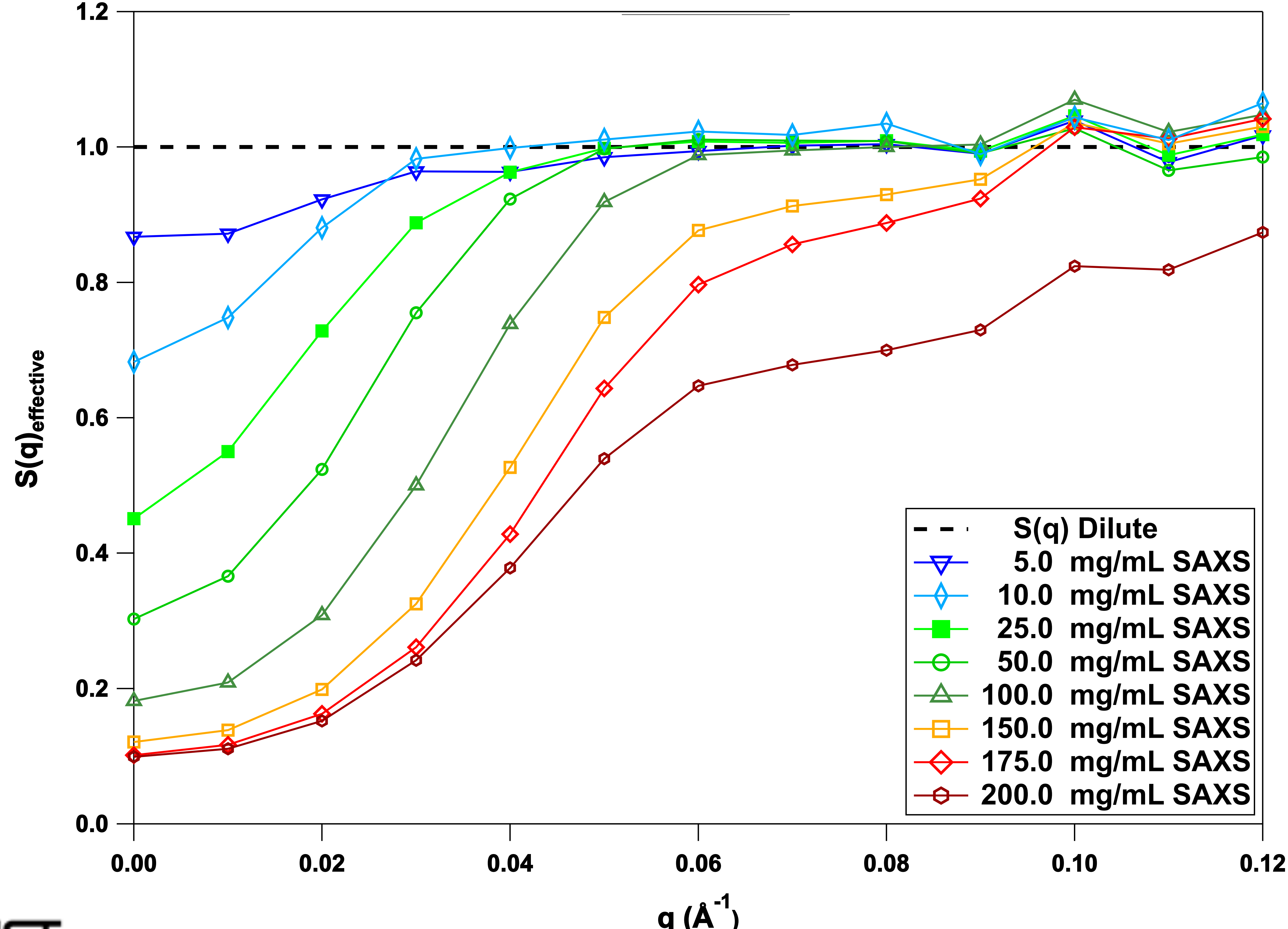


$$I(q) = \frac{N_p}{V} P(q) S(q) \quad \nRightarrow \quad P(q) = |F(q)|^2$$

Small angle scattering profiles reveal intermolecular interactions



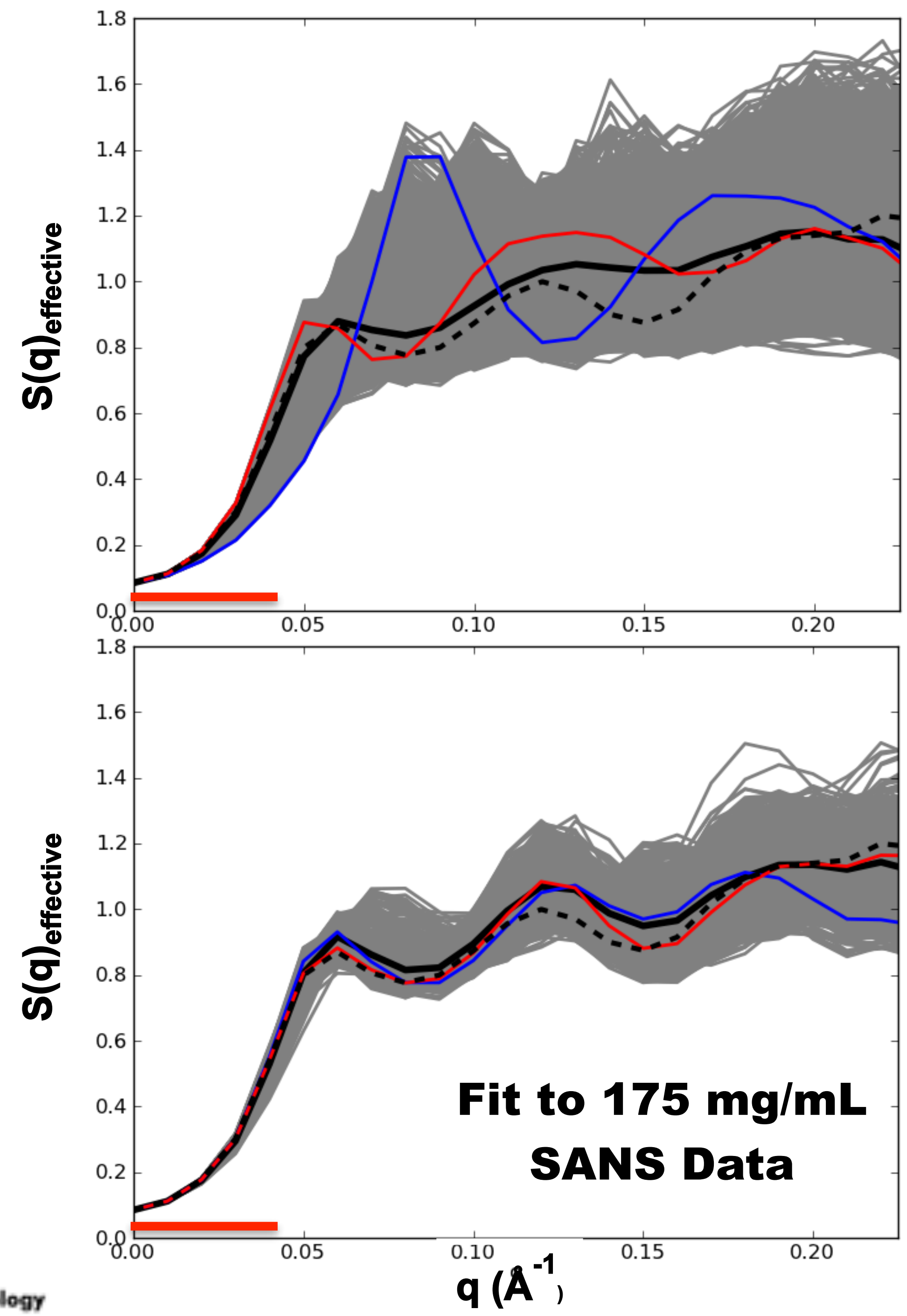
S(q)≈the reciprocal space version of g(r)

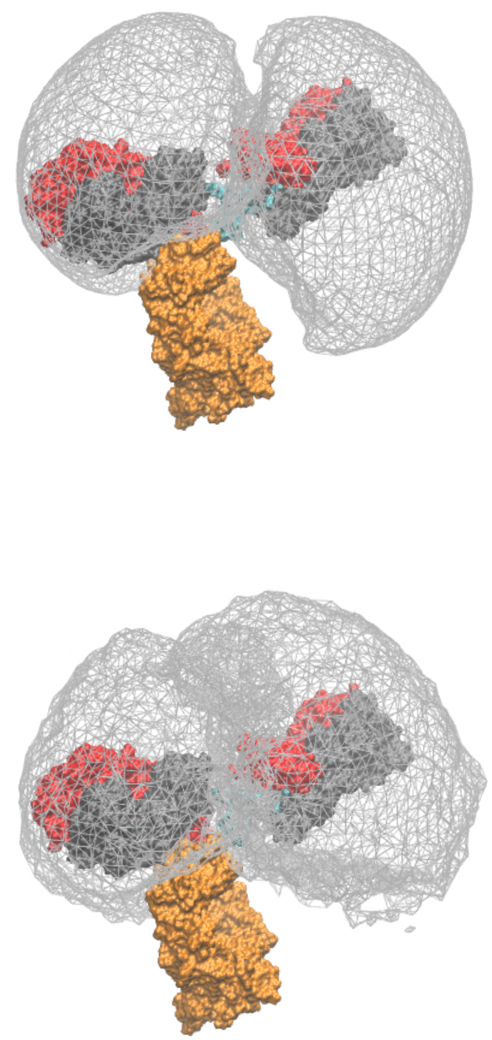


NIST National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

$$S(q)_{effective} = I(q)_{concentrated} / I(q)_{dilute}$$

Conformational effects on $S(q)_{effective}$-plots derived from atomistic ensemble of ASA-IgG2

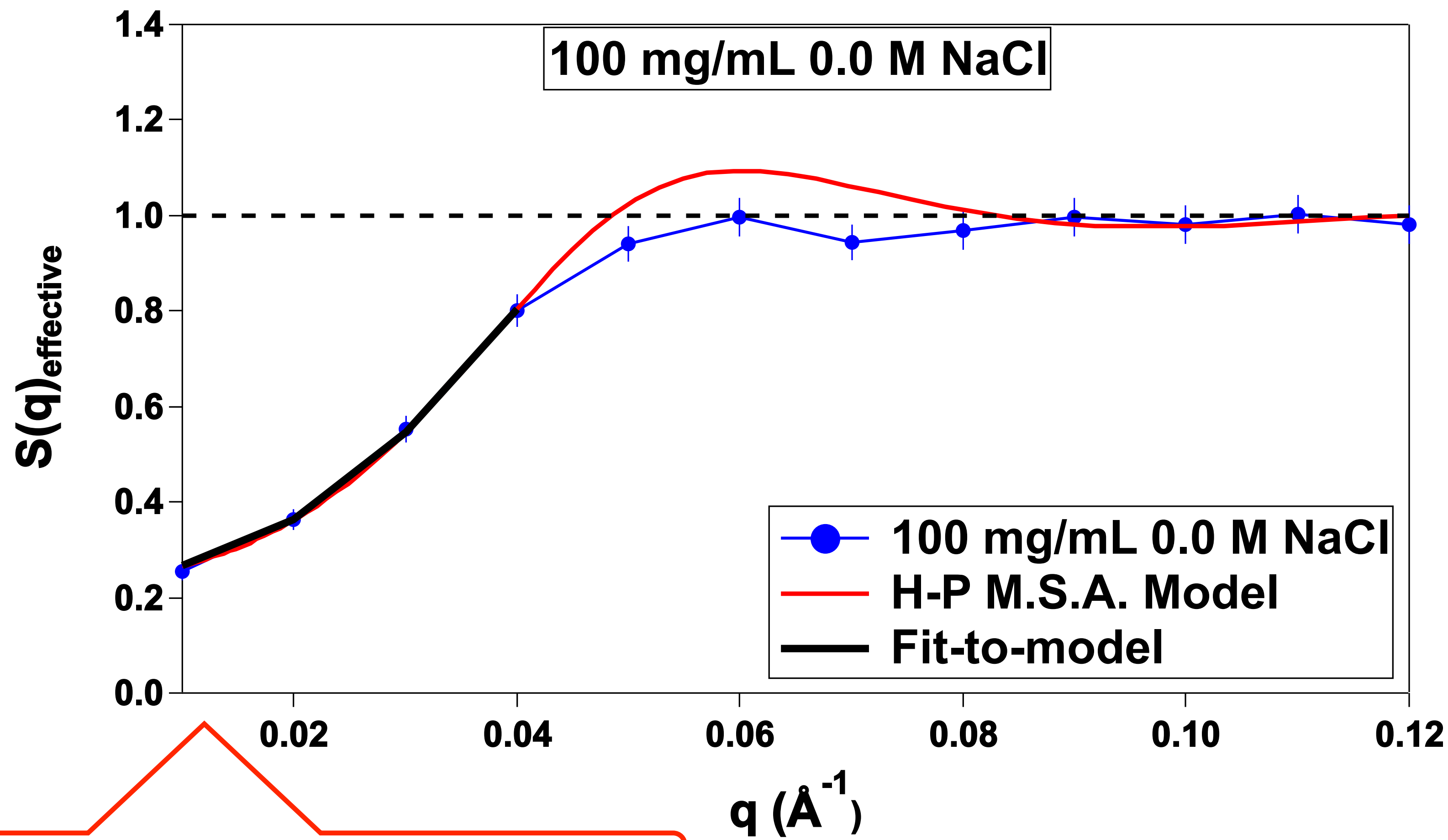




Clark et. al., in progress

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

$S(q)_{effective}$ plots can be fit to simple colloidal interaction models



Under these conditions ASA-IgG2 is repulsive

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

Variables used;

-Volume fraction
-Dielectric constant
-Monovalent salt Conc.

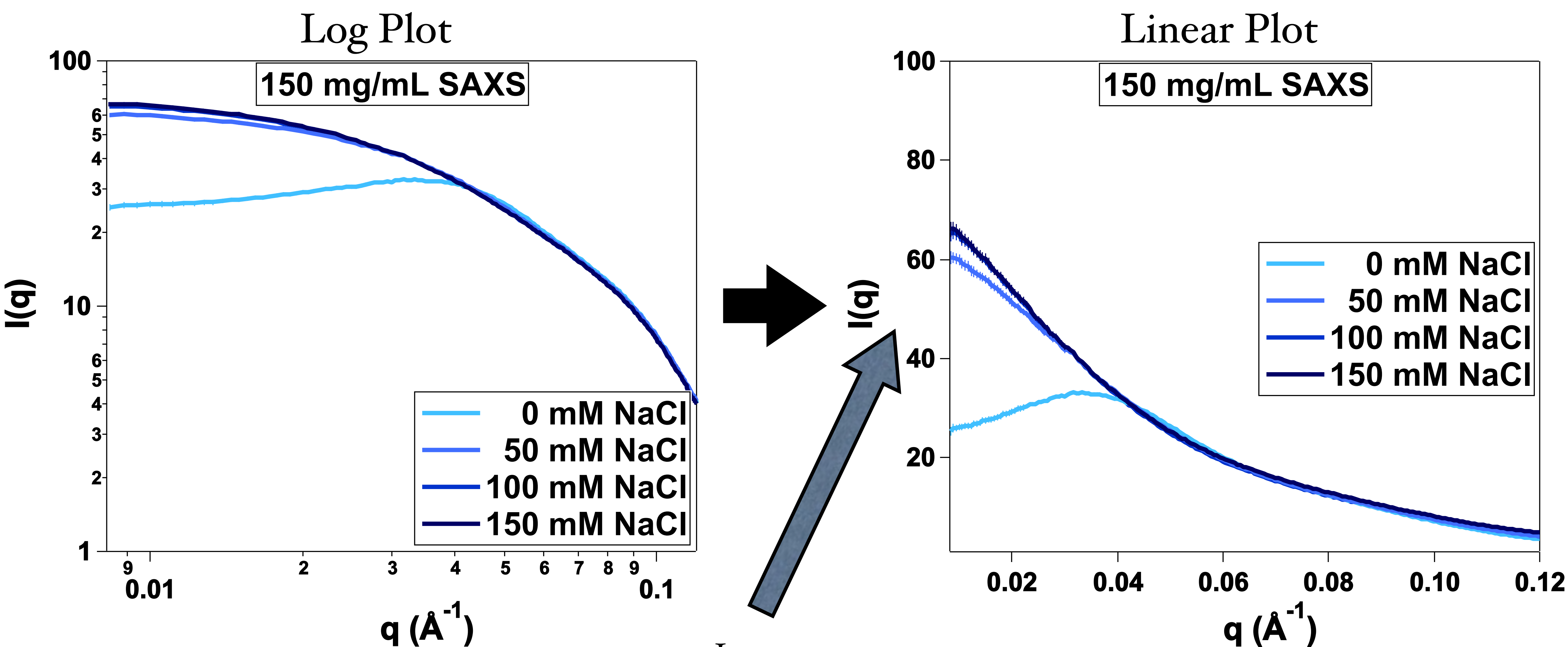
Variables obtained;

-Charge of particle
-Effective Diameter (Å)

1. JP Hansen and JB Hayter *Molecular Physics* 46, 651-656 (1982).
2. JB Hayter and J Penfold *Molecular Physics* 42, 109-118 (1981).

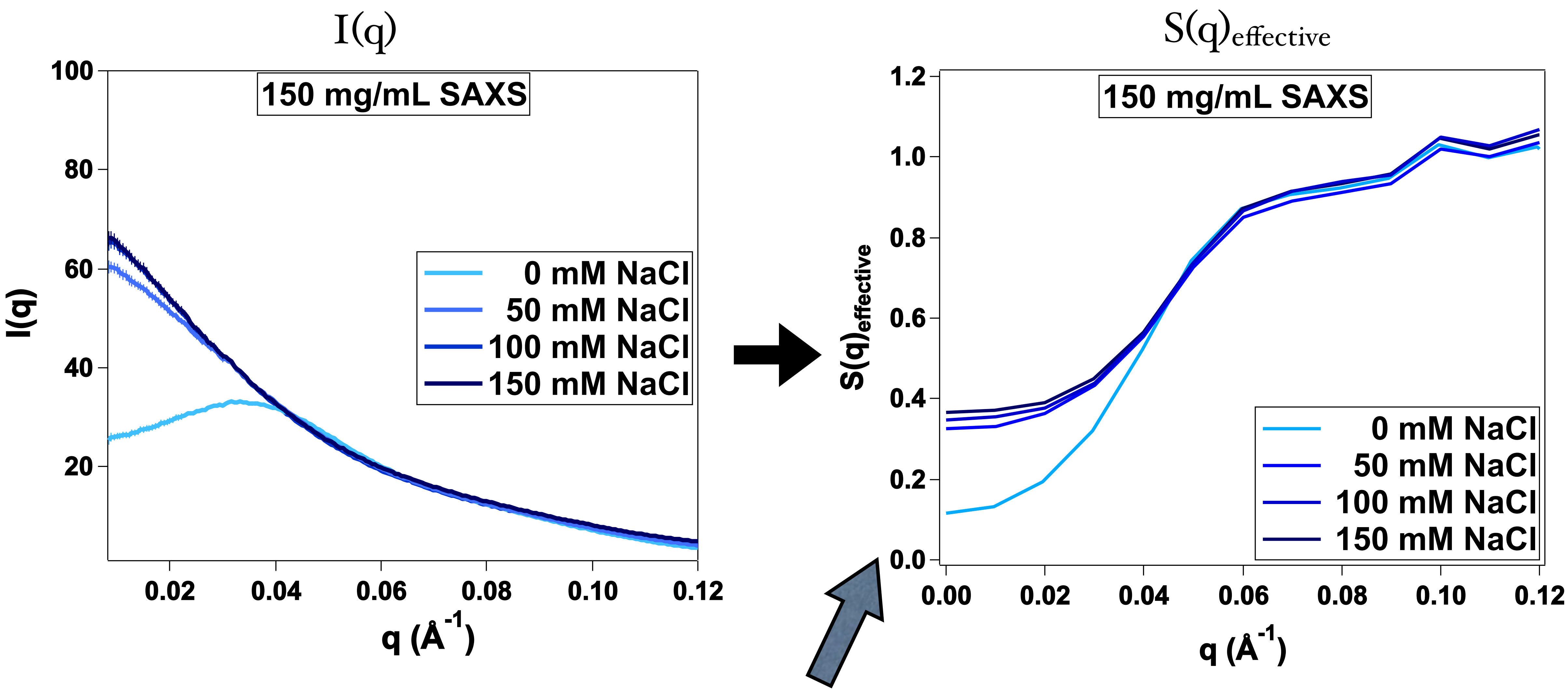
What happens when you add salt?

Salt effects on scattering profiles
(150 mg/mL sample)



Low-q
region becomes less depressed
with increasing NaCl

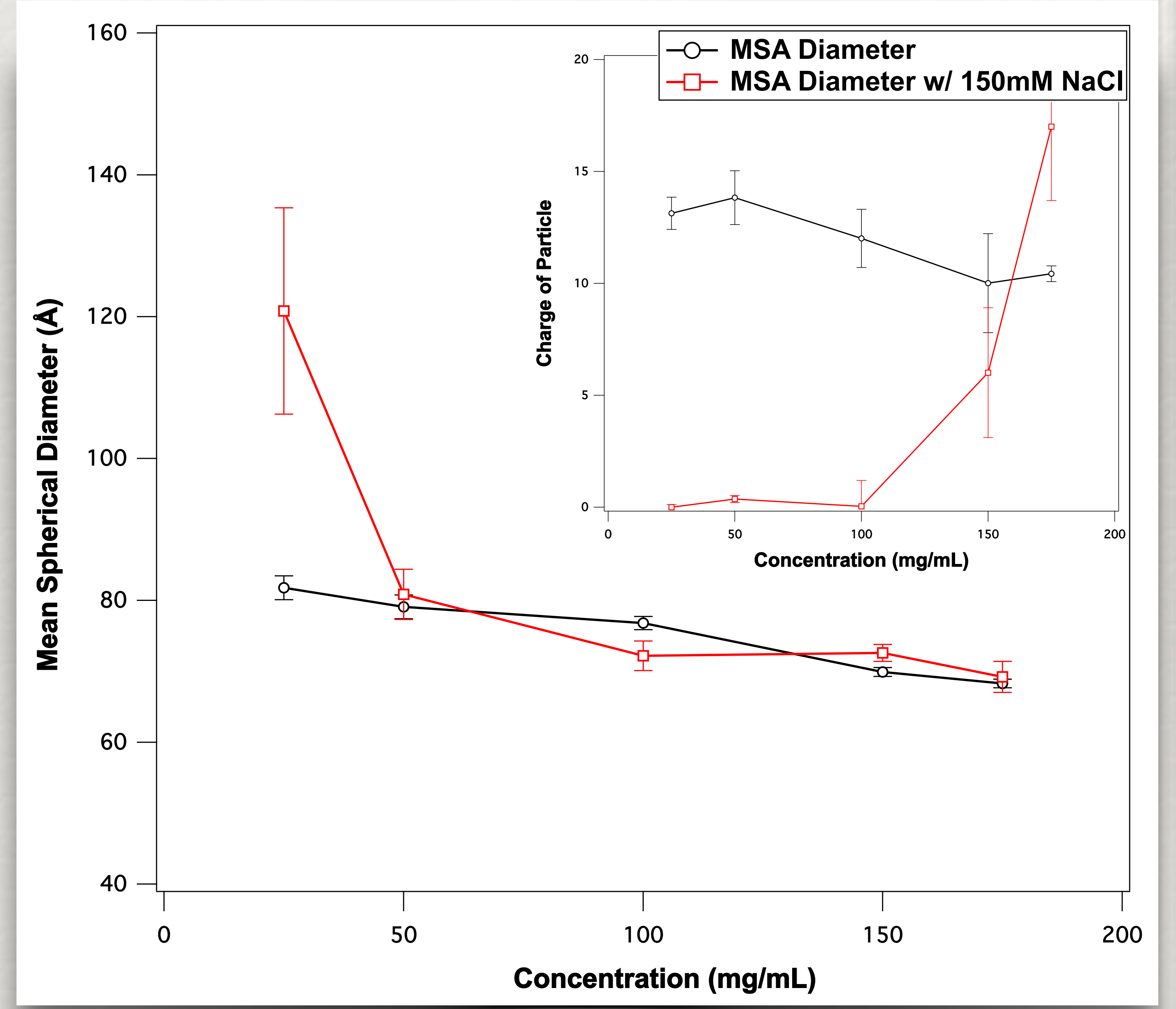
Salt effects on scattering profiles
(150 mg/mL sample)



Low-q region becomes less depressed with increasing NaCl

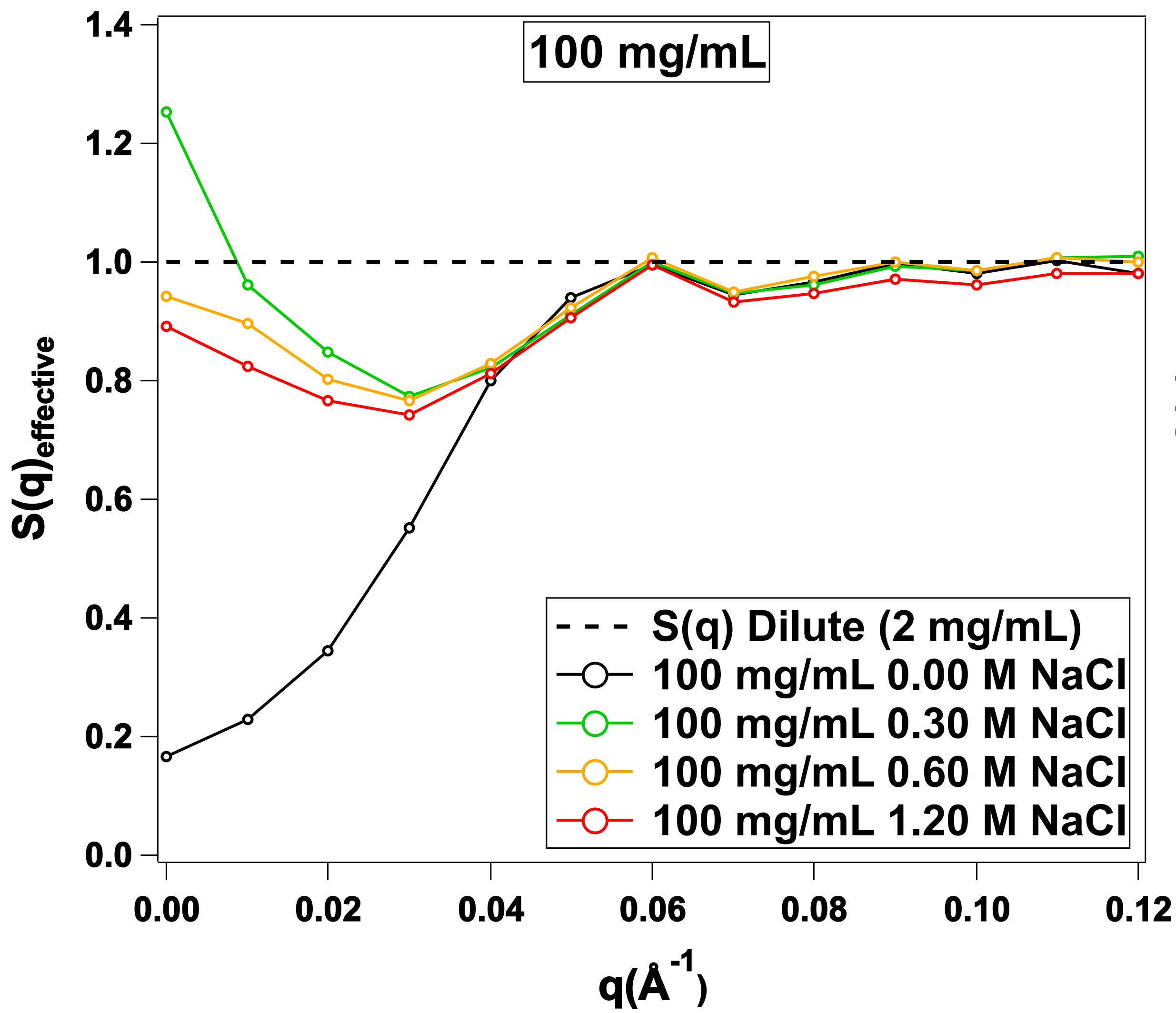
Results of the fit to MSA model

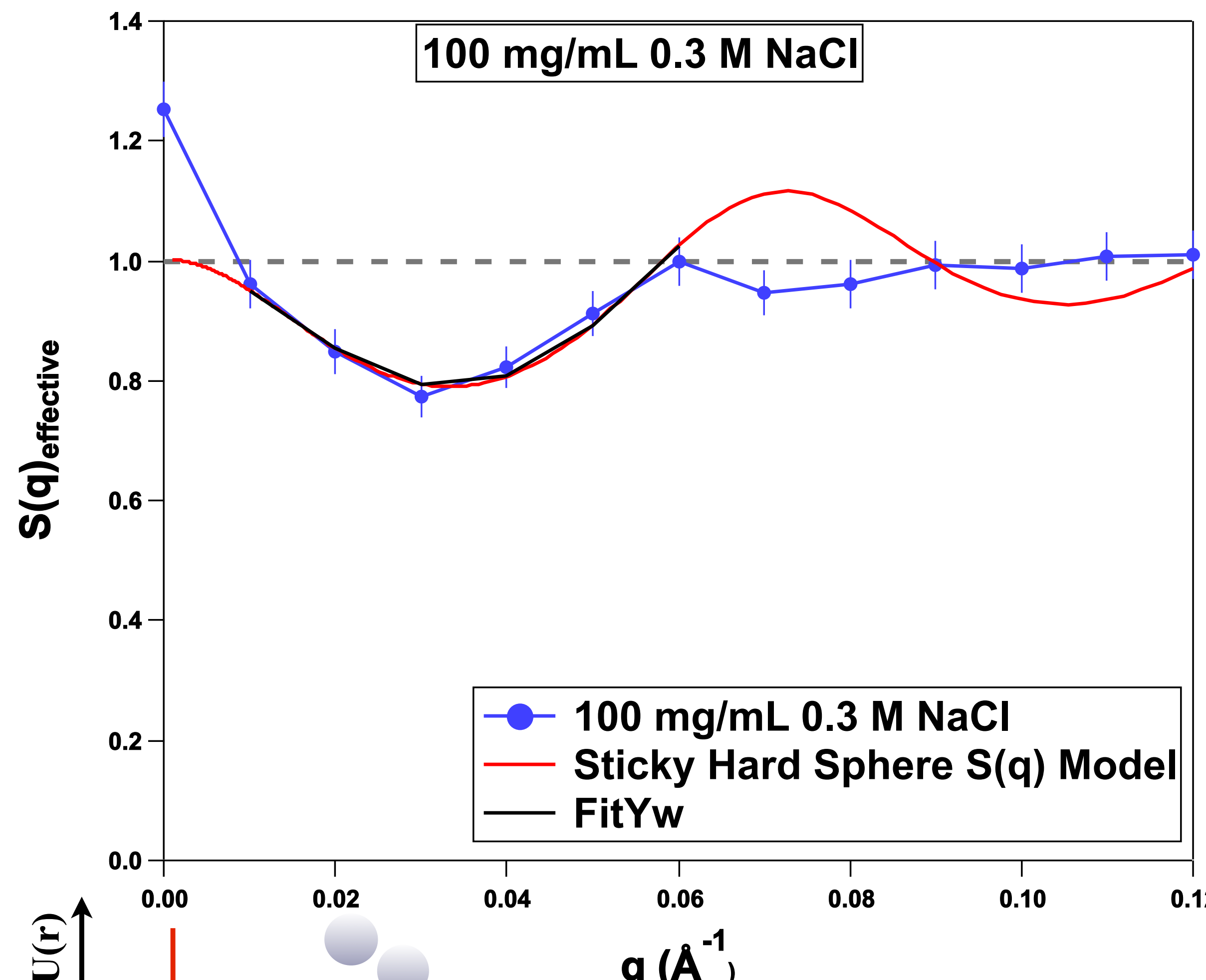
- Effective diameter decreases with increasing protein concentration
- Particles become less repulsive with NaCl
- NaCl does not alter the diameter of the particles
- NaCl decreases the charge of the particles up to ~100 mg/mL (will confirm with simulations)

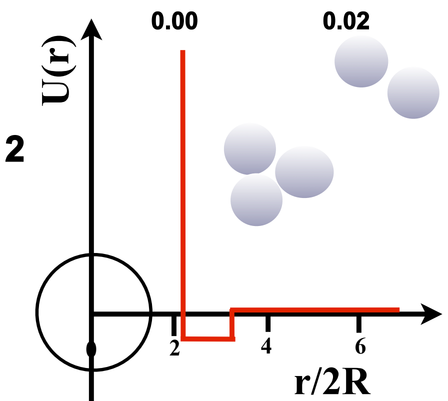


What happens when you add salt above physiological concentrations?

Increasing the salt concentration above physiological levels results in attractive interactions



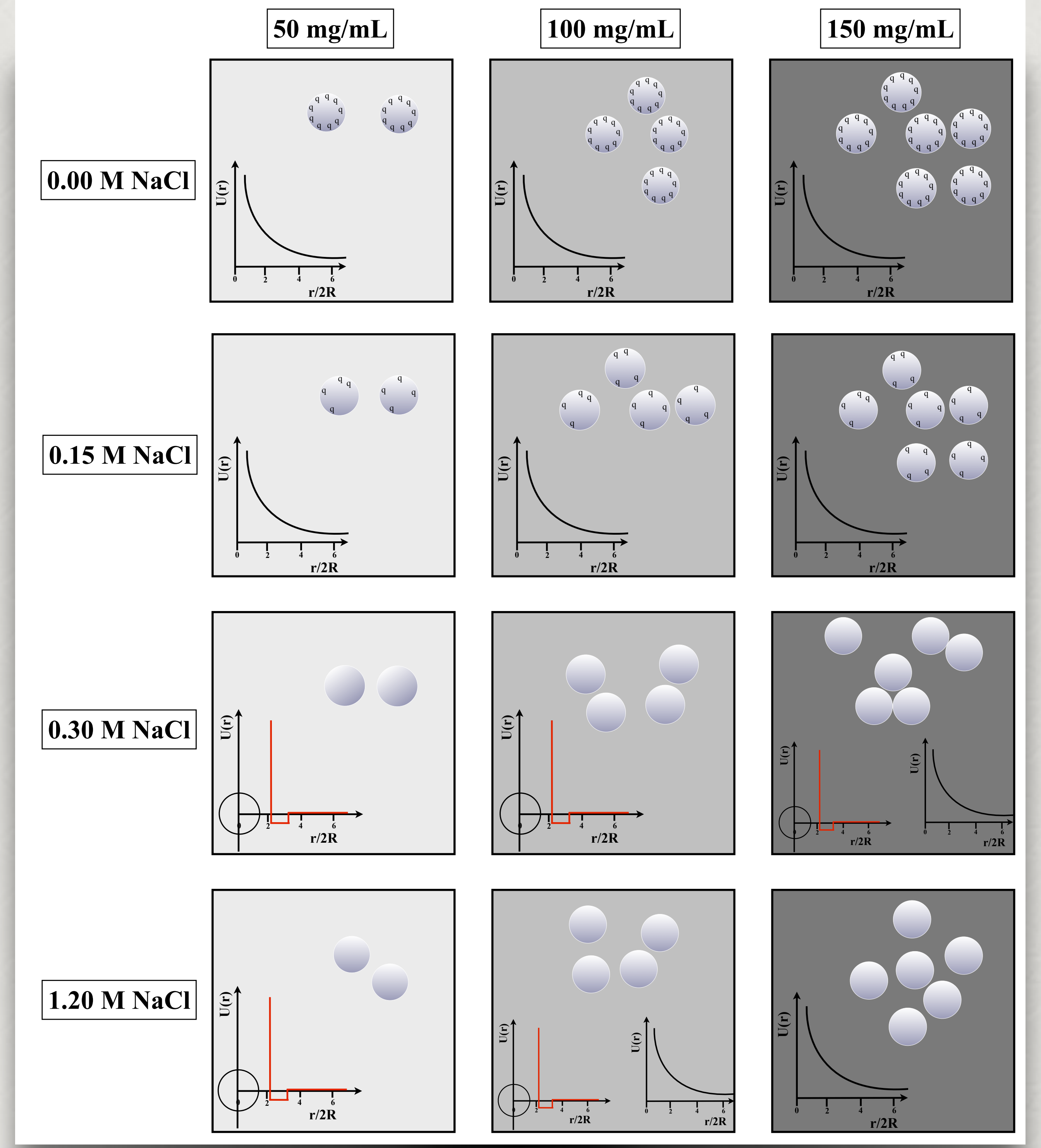




from SHS can obtain interaction energies (units of kT)

Menon, S. V., et al, *J. Chem. Phys.*, 1991, 95(12), 9186-9190

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!

i.e.

γ-Crystallin

Bonnete, F., et al., *Acta Cryst.* (1997) D53,438-47

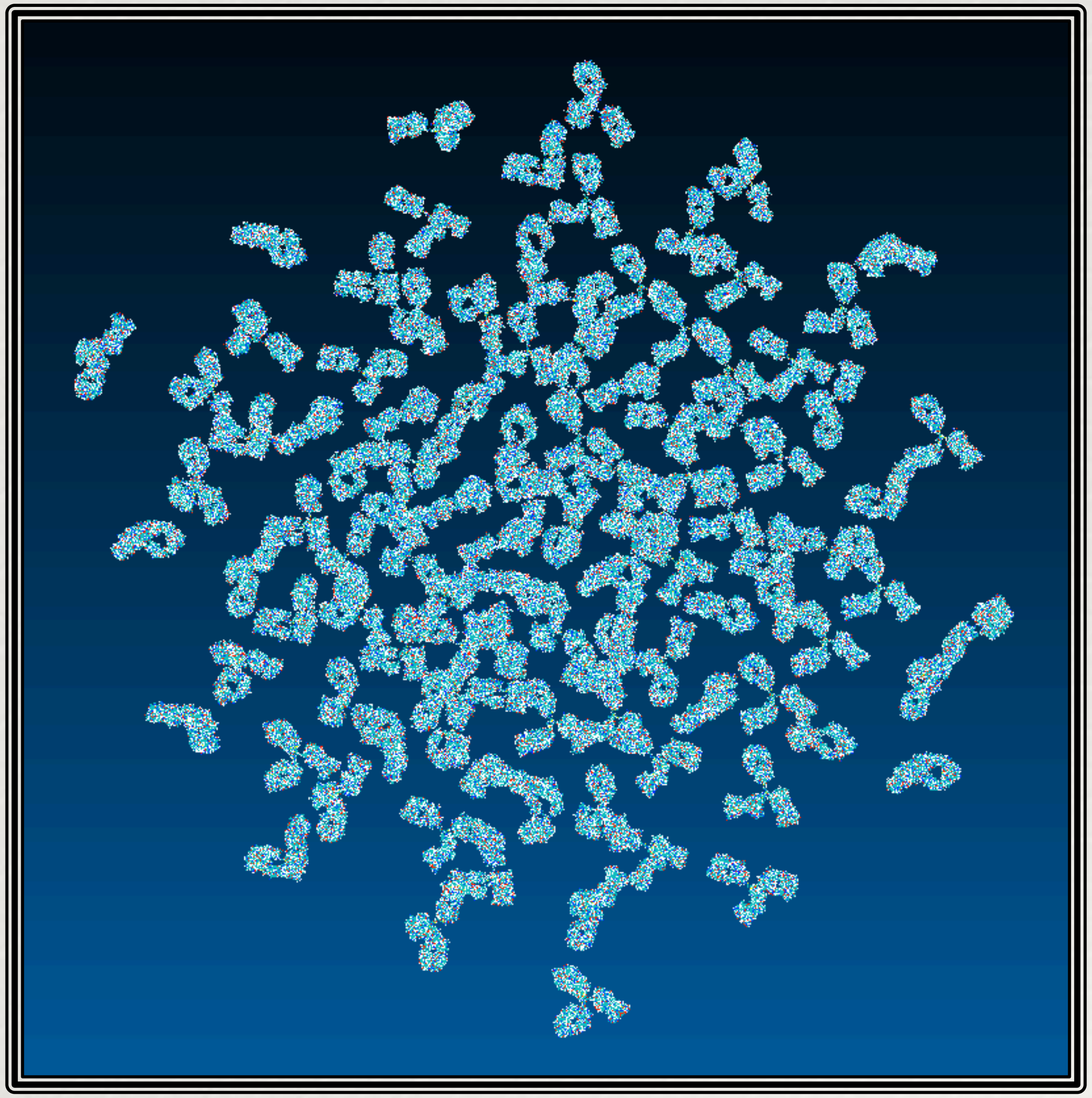
Thousand-mAb simulations
(in progress...)

- 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.SA.) 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)

HT compatible
<u>BioSAXS beamlines</u>

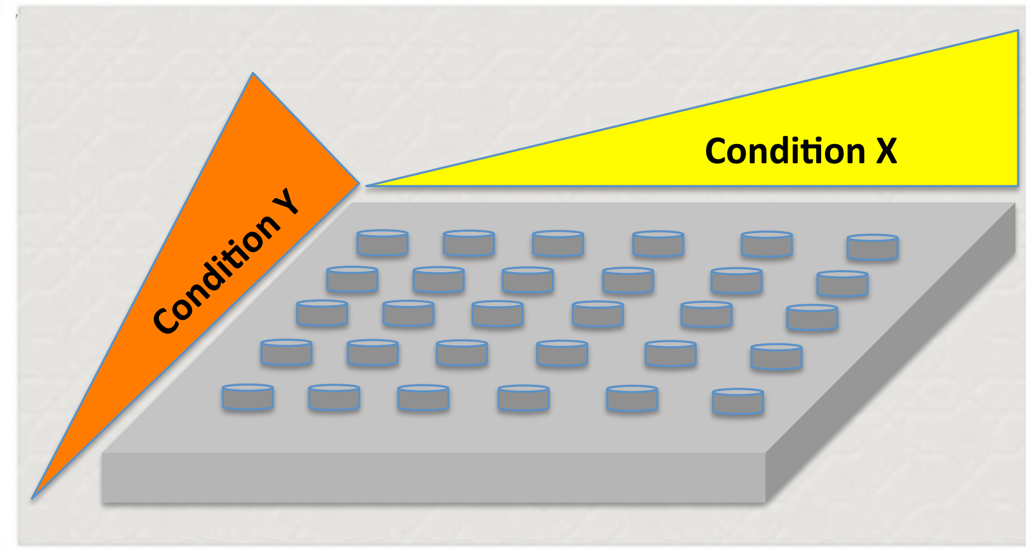
- CHESS-G1
- ALS-12.3.1
- SSRL-4.2

<u>HT SANS</u>

- Spallation Source ?

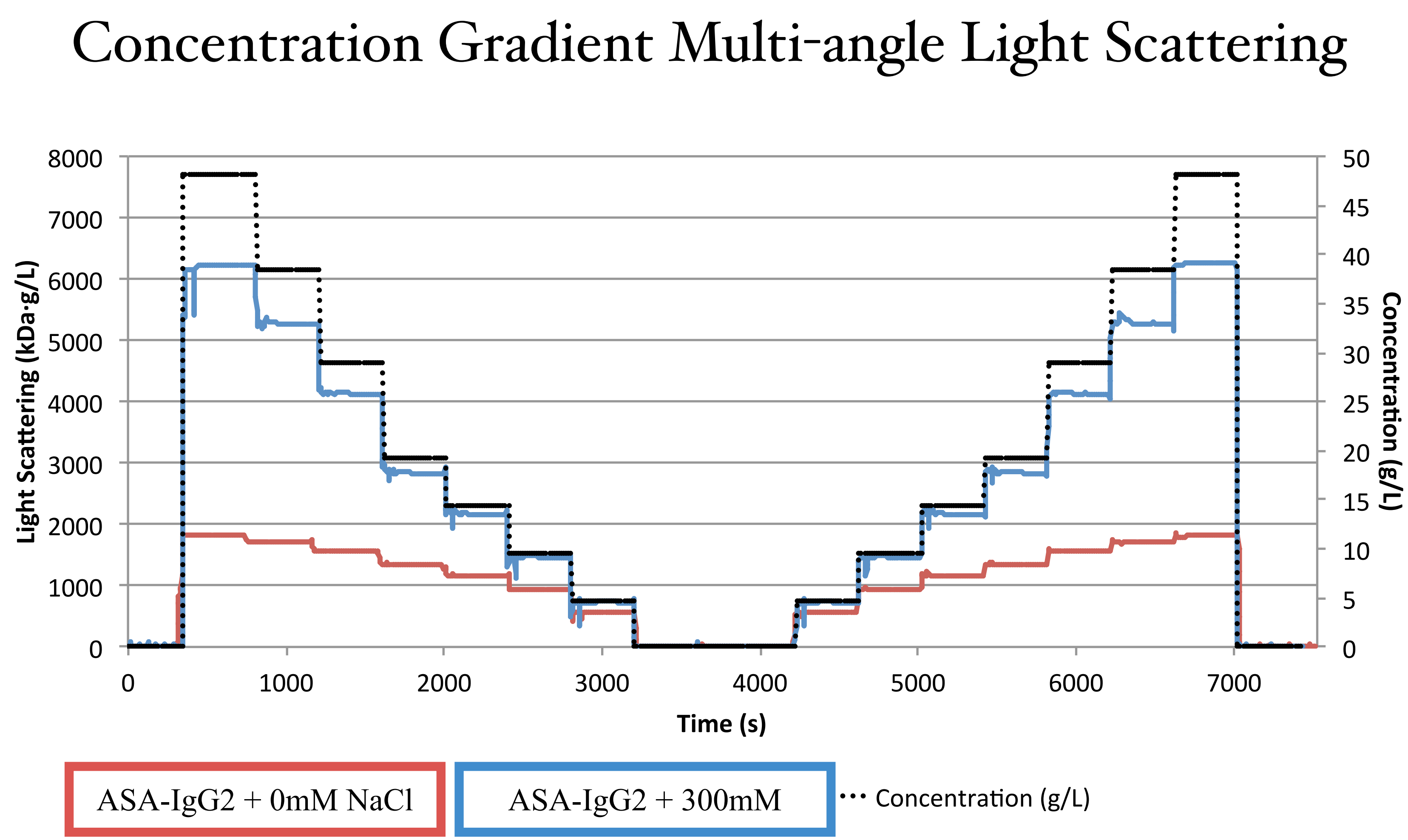
Future directions:
Dynamic Light Scattering (DLS)
(plate reader?)

- 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 bench 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!

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(NCNR)

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Rob Dimeo
Max Watson
Katie Sarachan

Cornell High-Energy Synchrotron Source
(CHESS)

Richard Gillilan
Kathy Dedrick

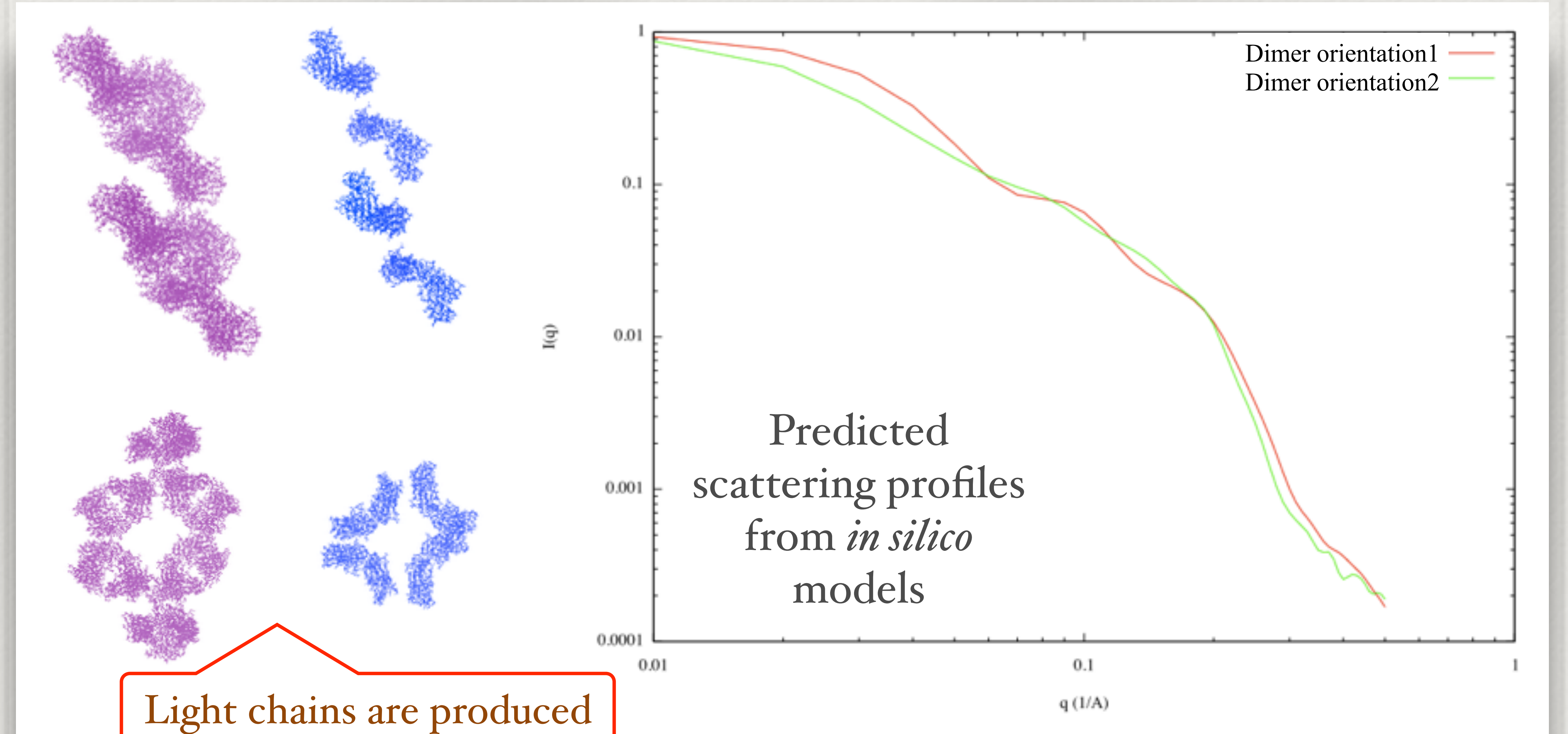
Local Celebrities

AMGEN

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

Questions?

Future directions: Contrast variation orientational information of oligomeric species or from concentrated solutions



Light chains are produced in deuterating conditions

X-ray Sources?
(Synchrotron or Home Sources)

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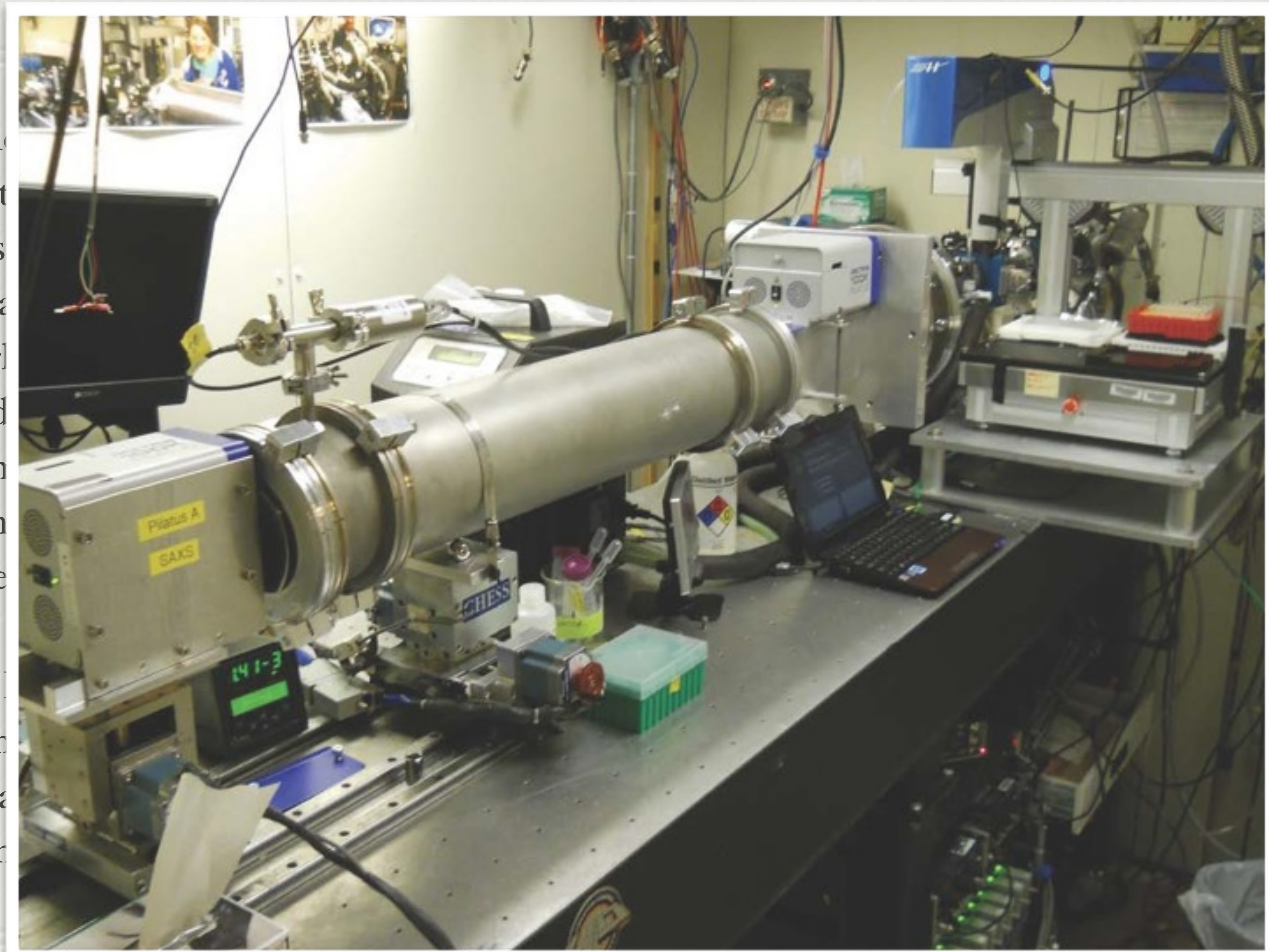
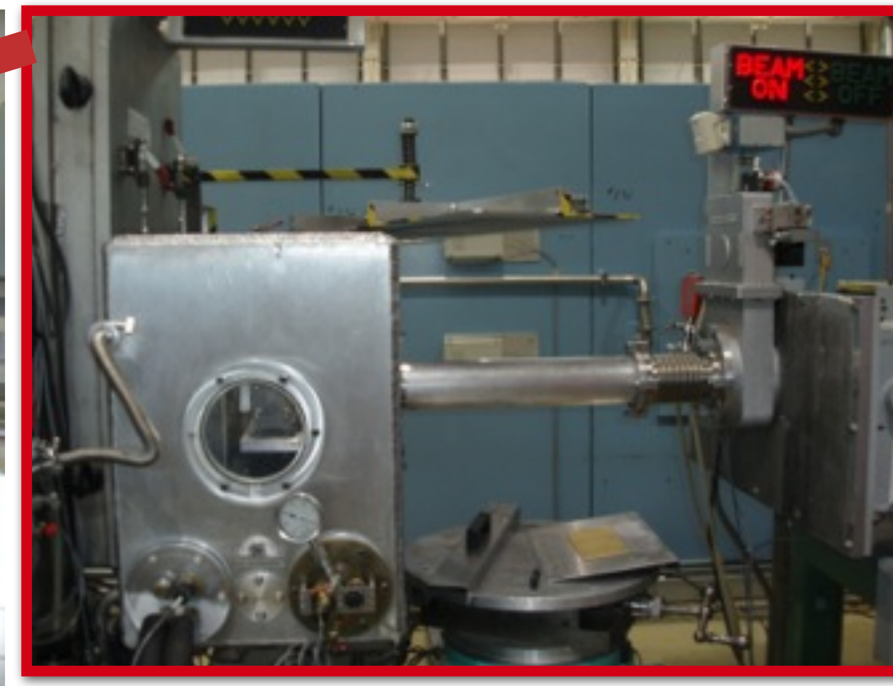
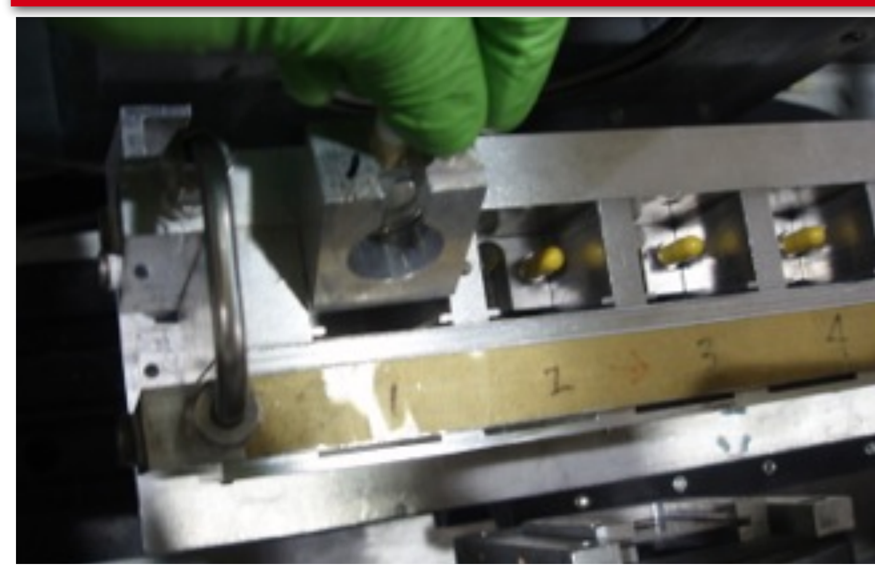


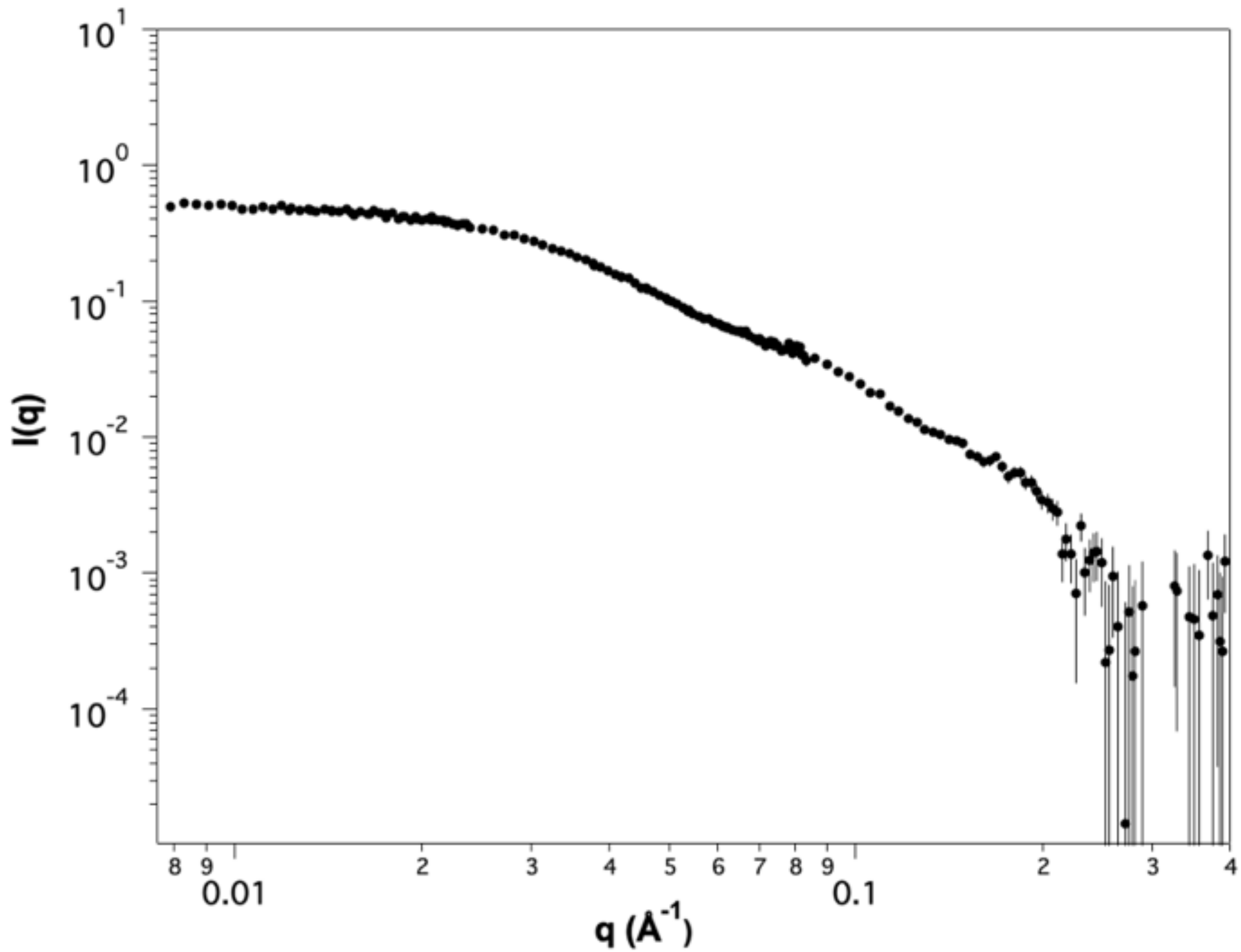
Neutron Sources?
(Nuclear Reactor or Spallation Sources)

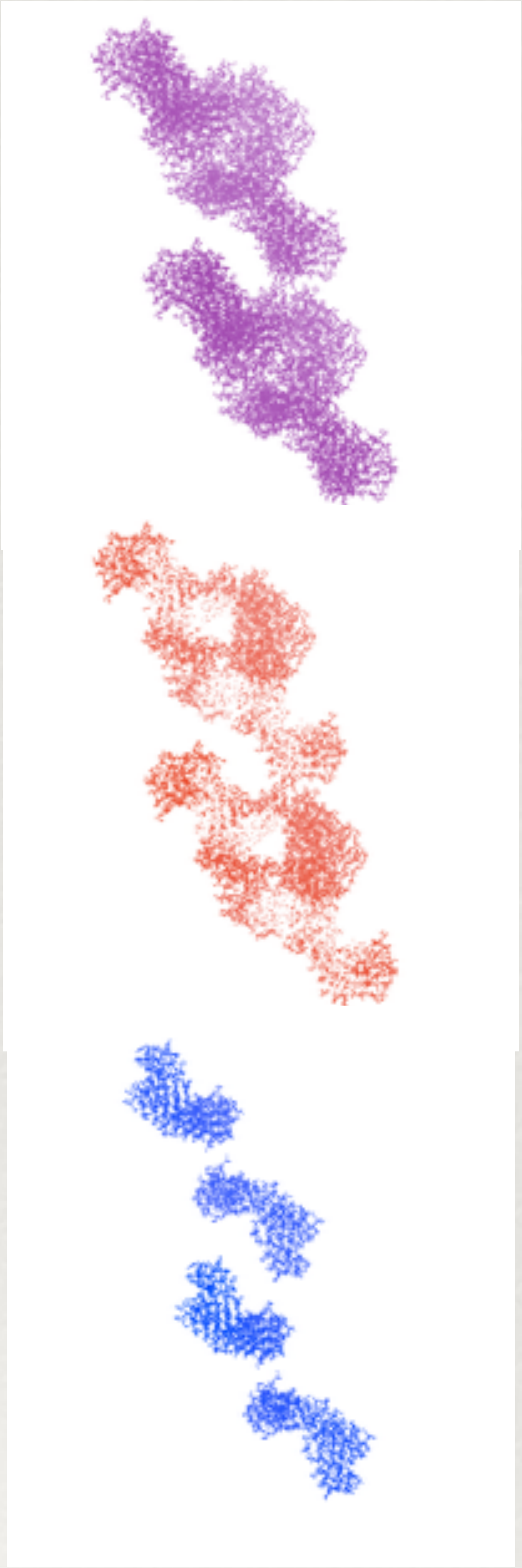


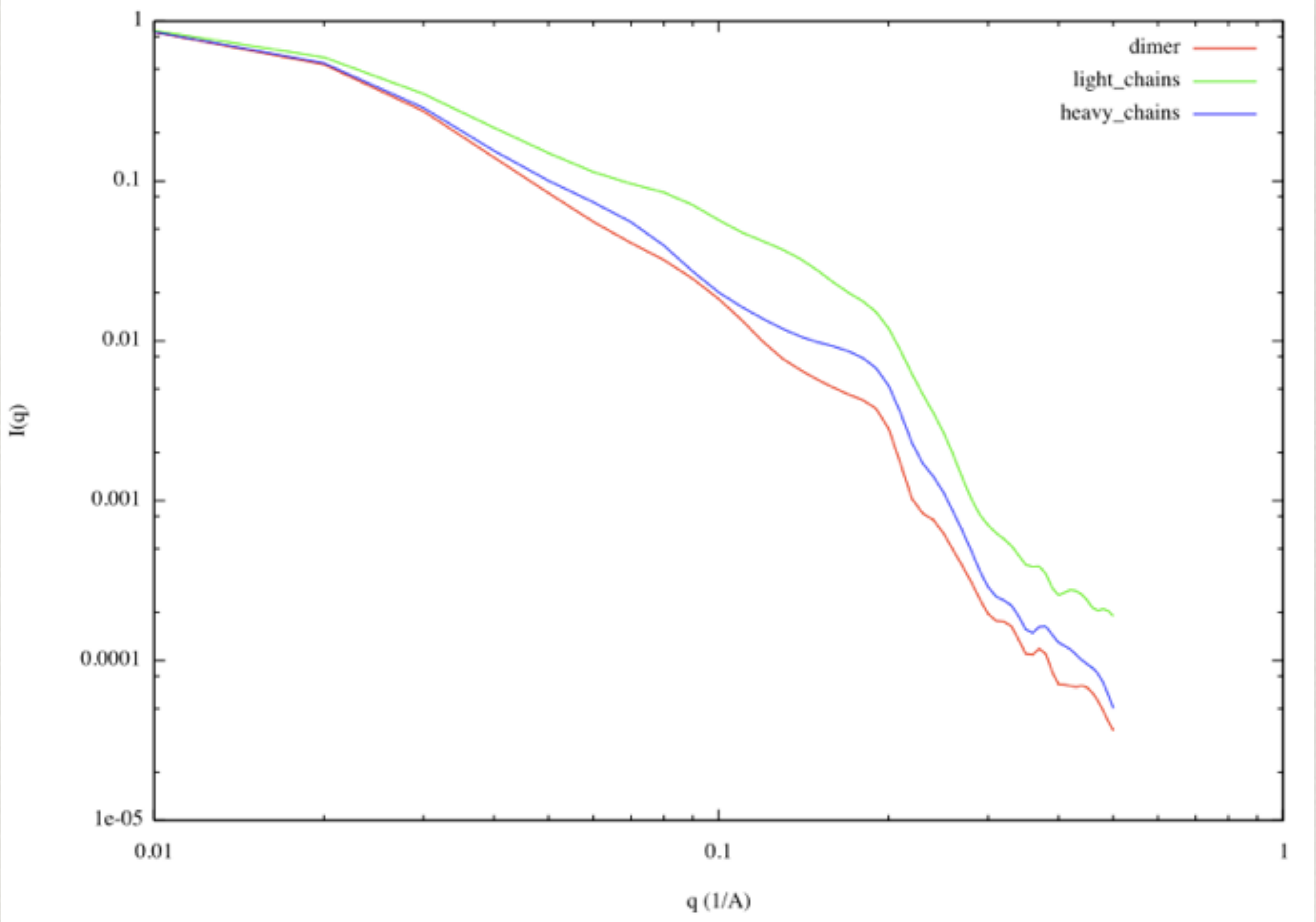


Disclaimer: The Federal Government does not support, endorse or favor any one NFL team over another!

Small angle neutron scattering (SANS) profile of dilute anti-streptavidin immunoglobulin-2 (ASA-IgG2)







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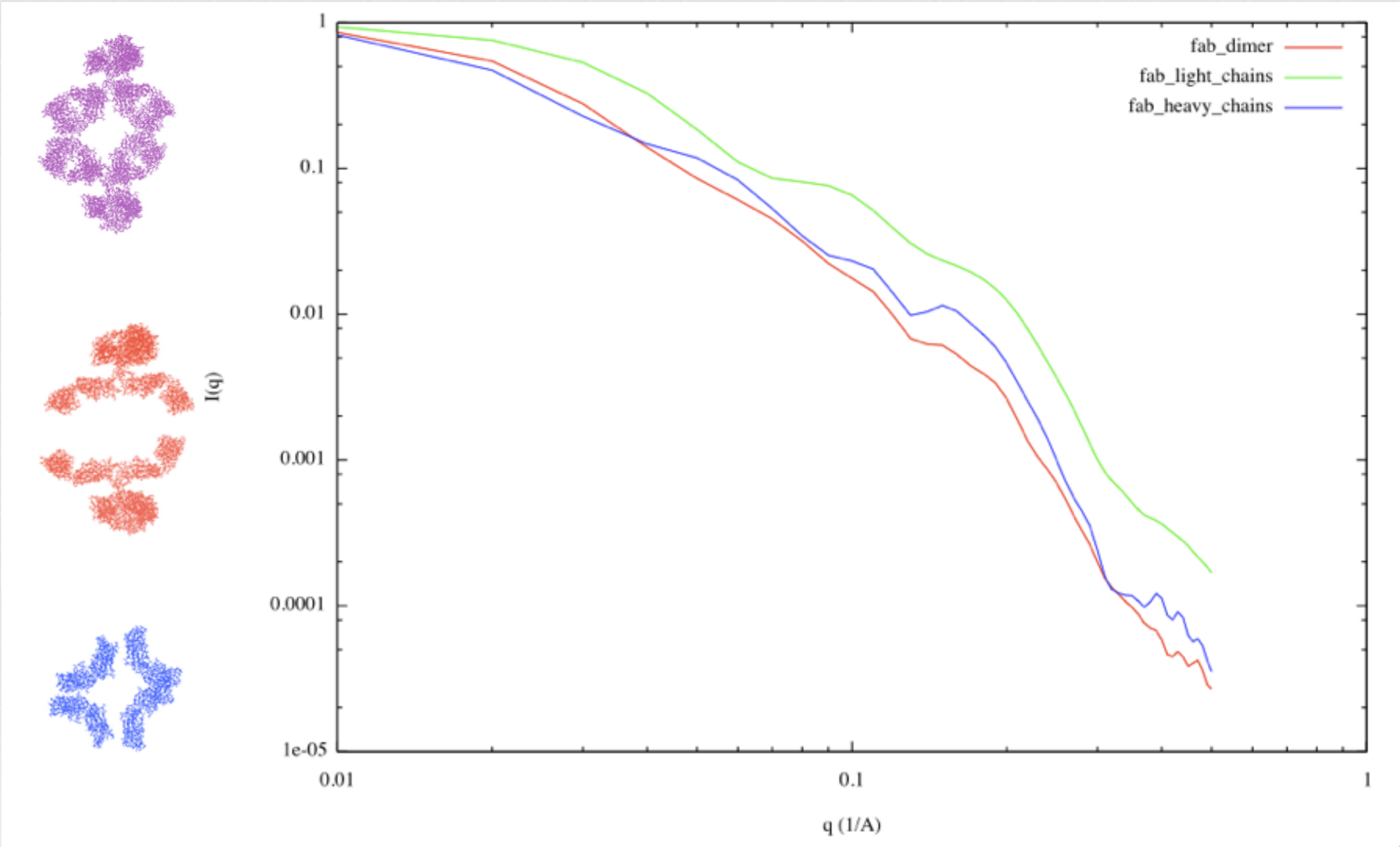
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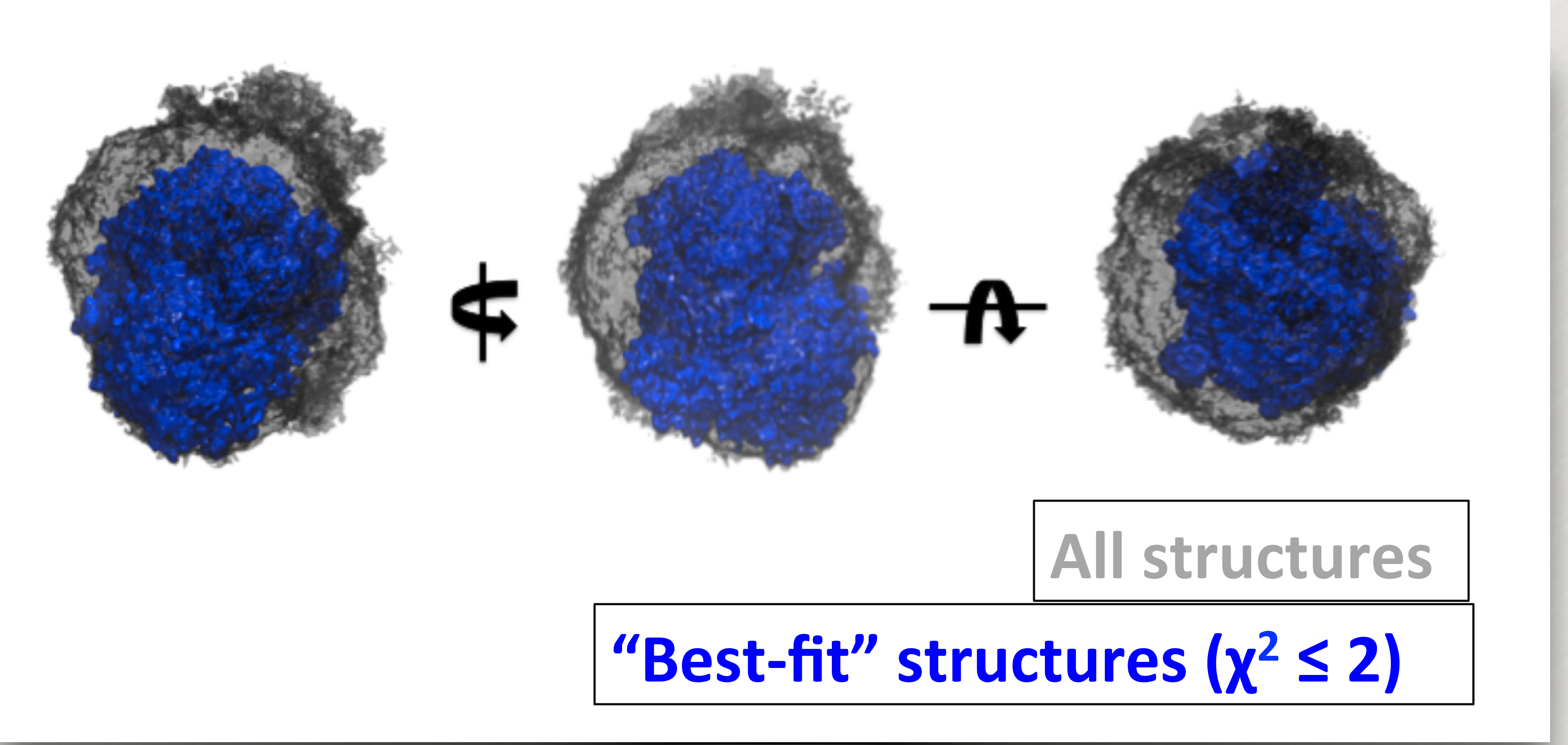
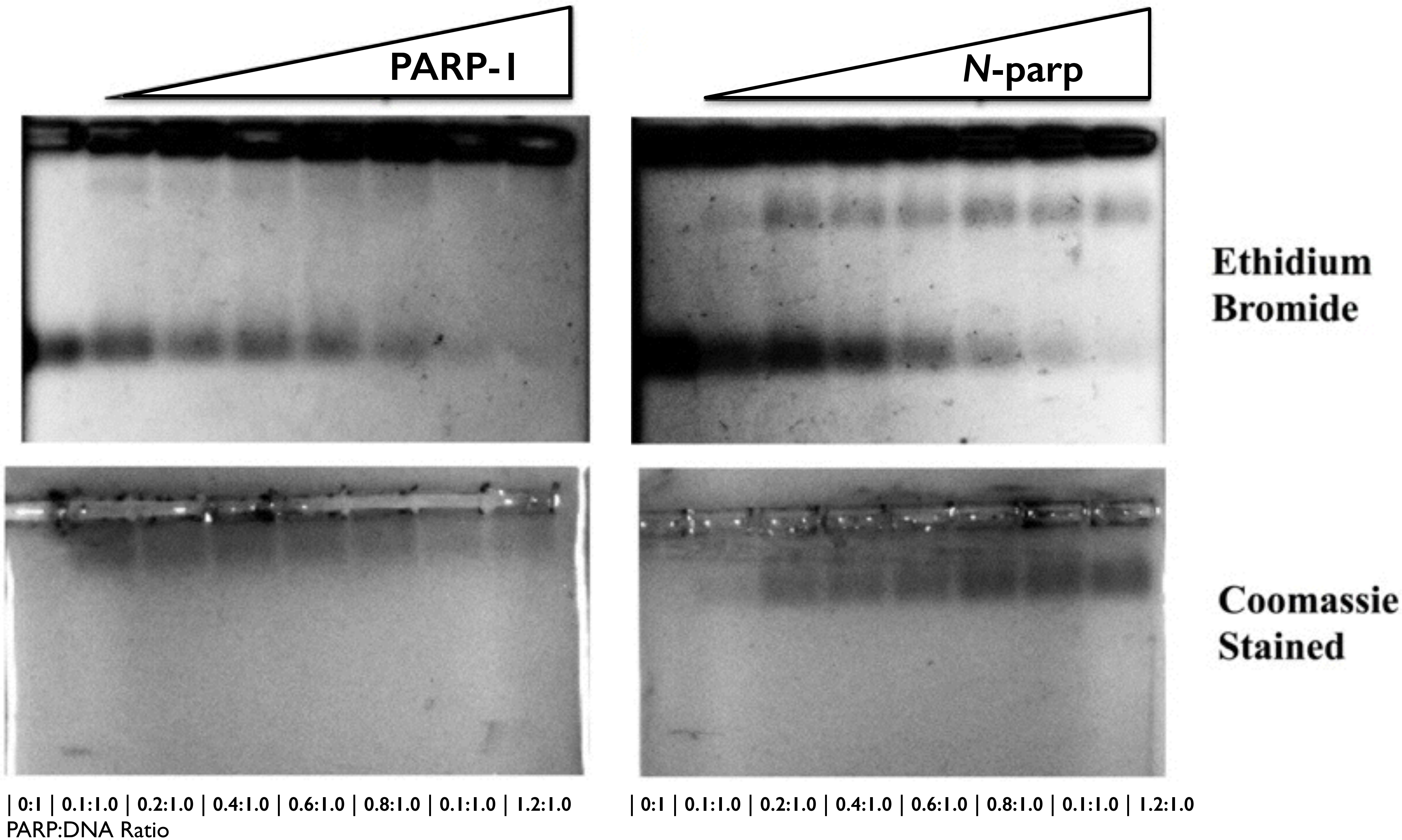
PARP-1



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	SAXS
-Sample volume (350-650 µL) -Measurement time (1≤ hours) -Concentrations Ranges (1 - ≥100 mg/mL)	-Sample volume (15-50 µL) -Measurement time (≤1-5 minutes) -Concentrations Ranges (0.5 - ≥100 mg/mL)