

Small angle scattering studies of Chromatin Proteins in the Human Malarial Parasite



Ashley Jordan



Juliette Devos, Catherine Merrick, Michael Haertlein, Trevor Forsyth, Edward Mitchell

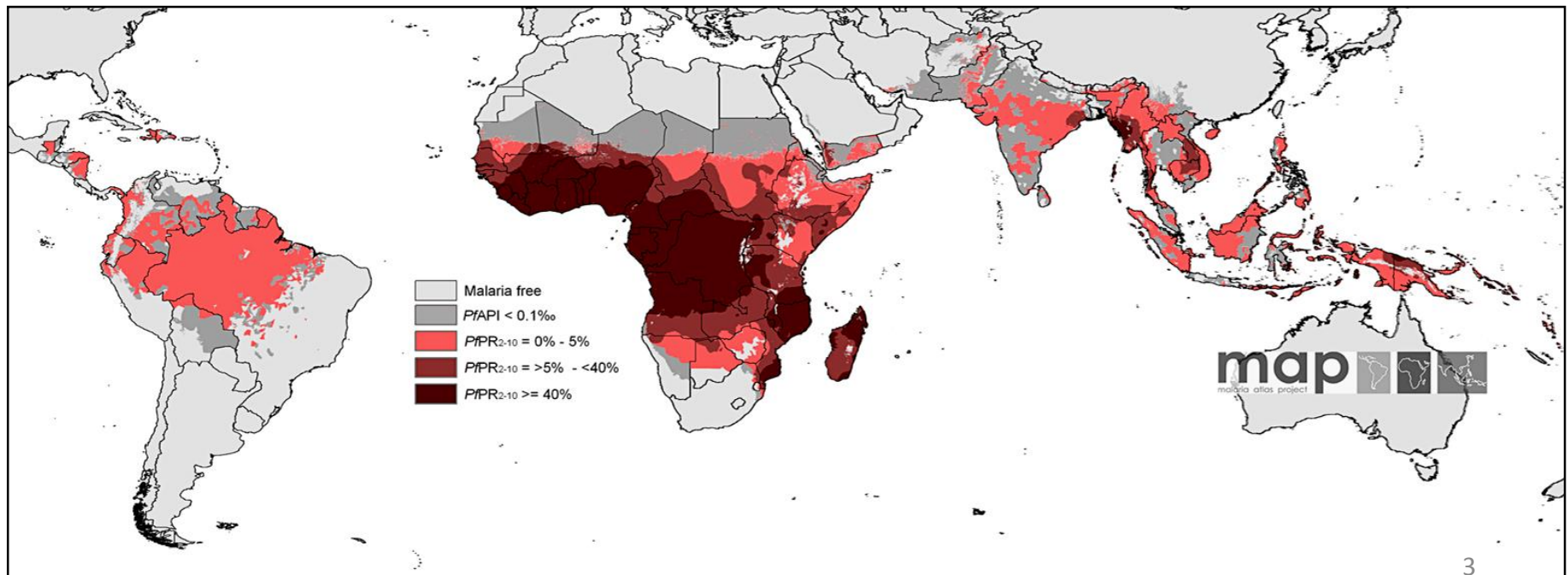


Overview

- Introduction to malaria
- Proteins of Interest
- Characterisation & Preparation
- Small angle scattering studies
- Summary

Introduction to Malaria

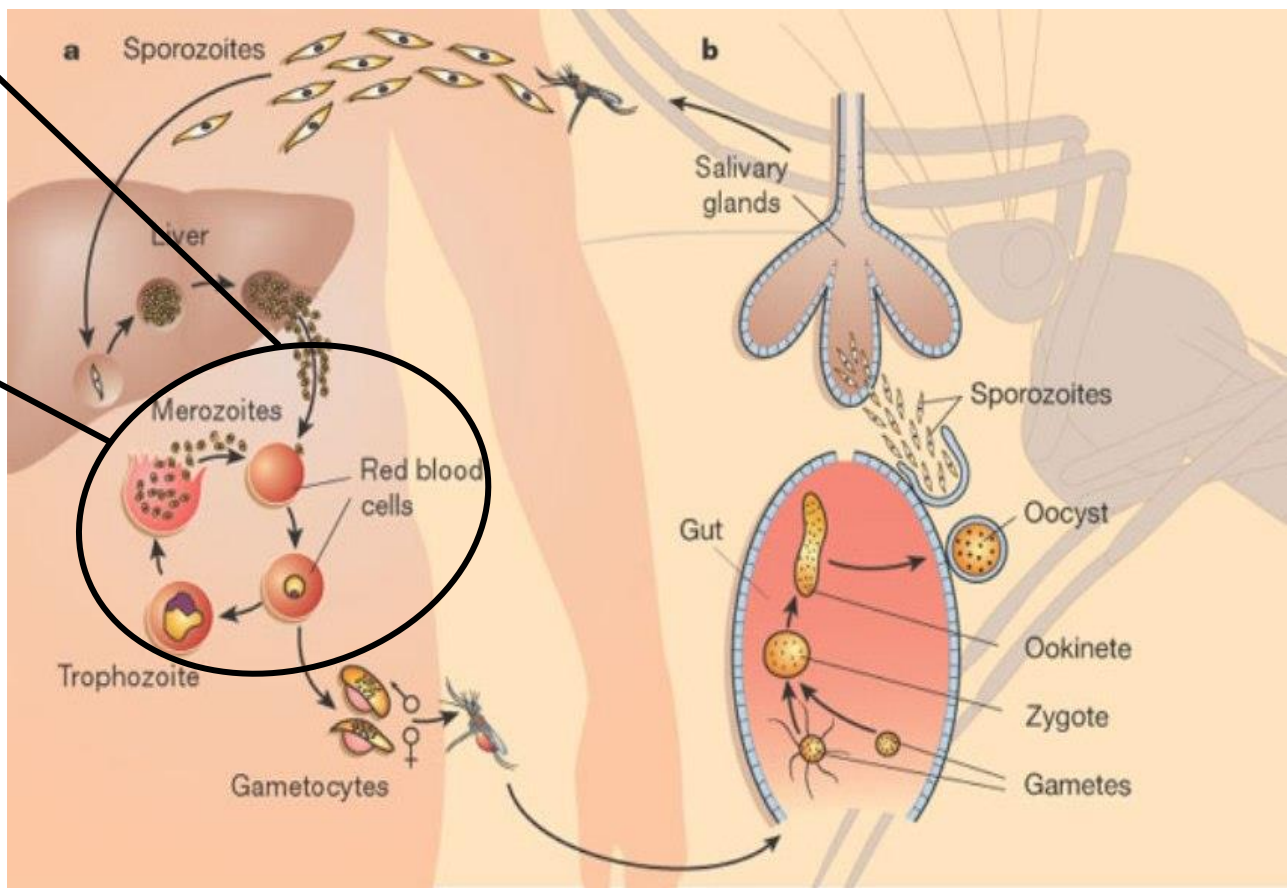
- Infection caused by parasitic organism – *Plasmodium falciparum*
- Approximately 214 million cases of infection with an estimated 438,000 deaths each year (WHO Malaria Report 2014).
- Mortality predominantly occurs in the sub-Saharan Africa region amongst young children and the elderly.



Introduction to Malaria

Plasmodium falciparum lifecycle

- 2 hosts: mosquitoes and humans.

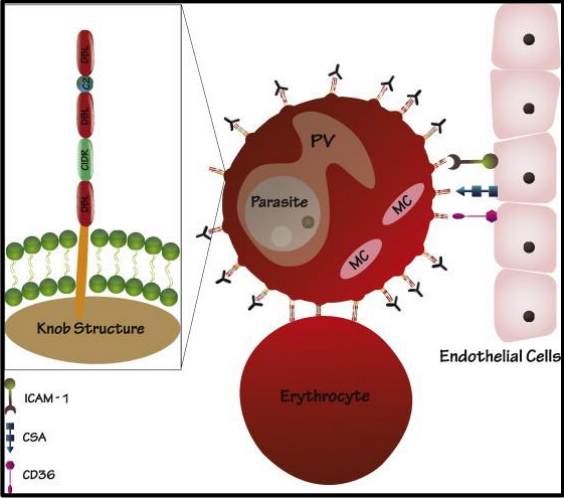


Nature.com 433, 113-114 (2005)

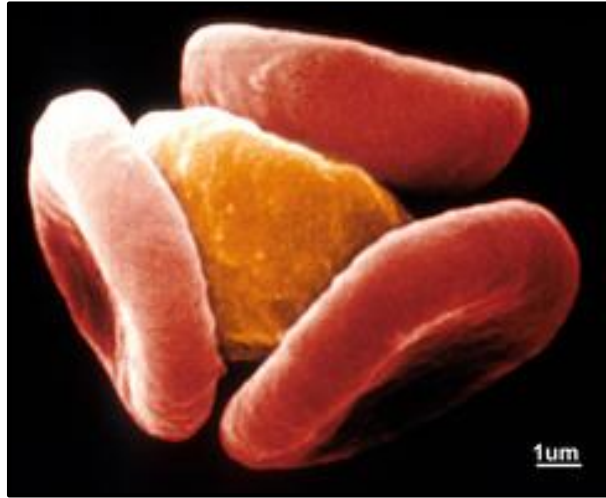
- Growth in red blood cells causes all the symptoms of malaria.

Introduction to Malaria

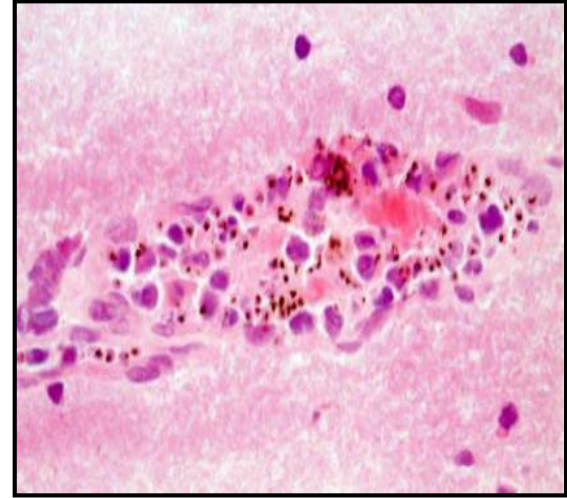
PfEMP-1



Infected RBC's

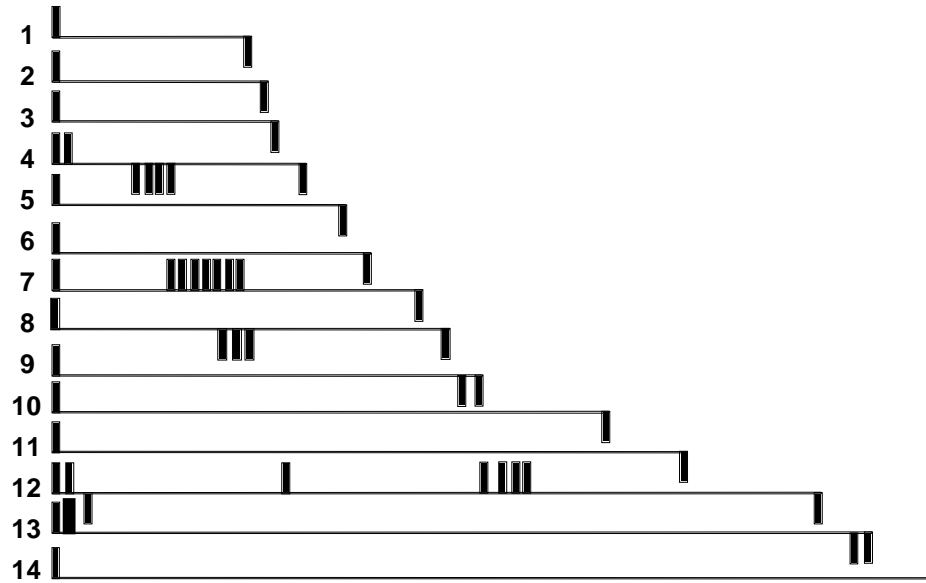


Sequestration

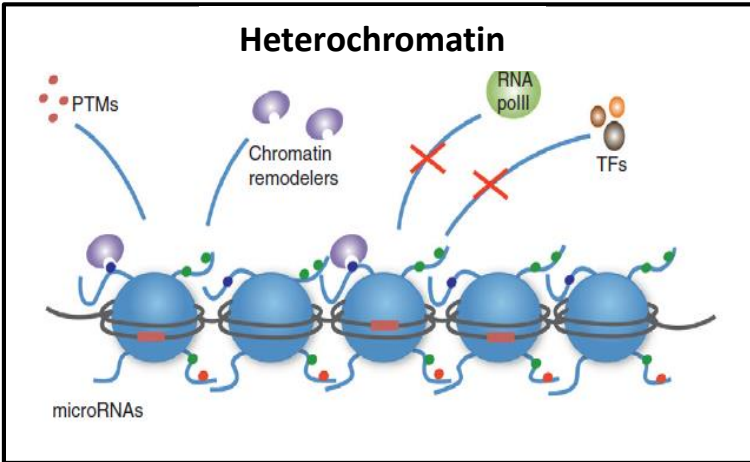
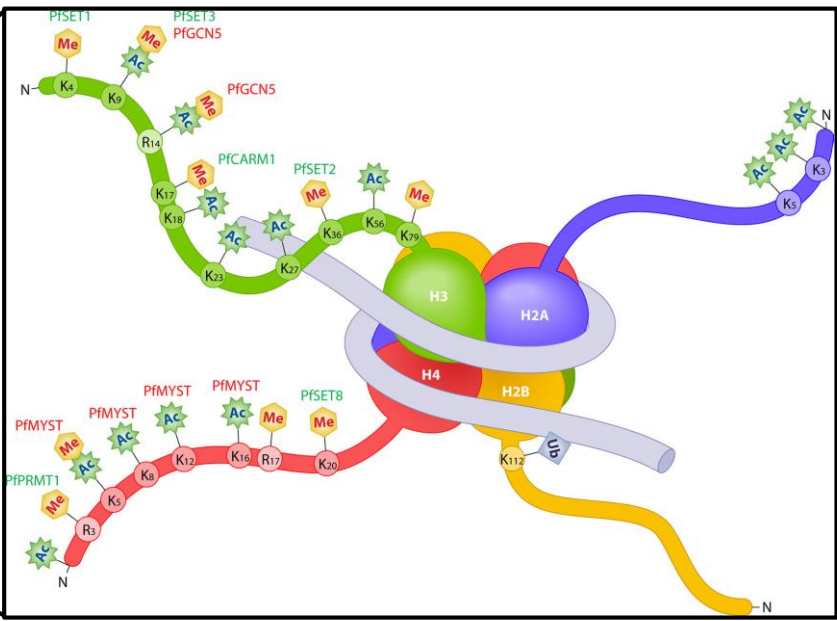
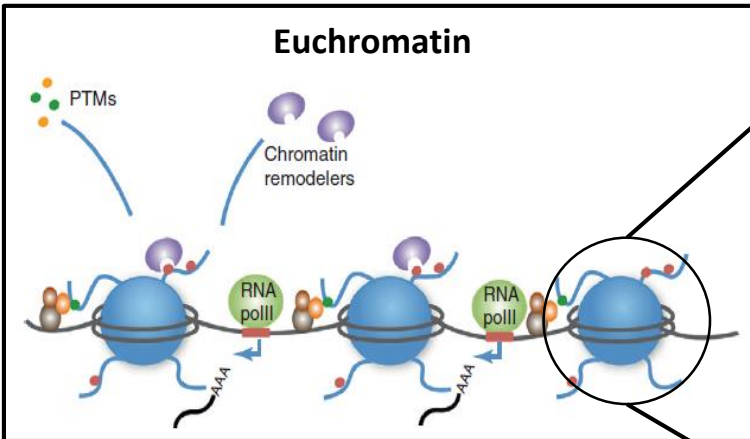


Virulence Genes & Antigenic Variation

- PfEMP-1 variants encoded by a large multi-gene family – ‘var’ genes.
- Over 60 variants present within *P. falciparum* genome.
- Able to switch between different variant forms of PfEMP-1 to avoid host immune response.
- Only 1 active *var* gene at any given time.
- **How is it regulated? - Epigenetics**

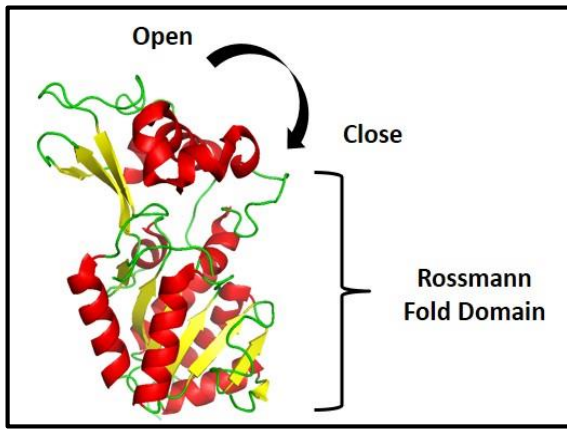


Epigenetics & Chromatin Remodelling



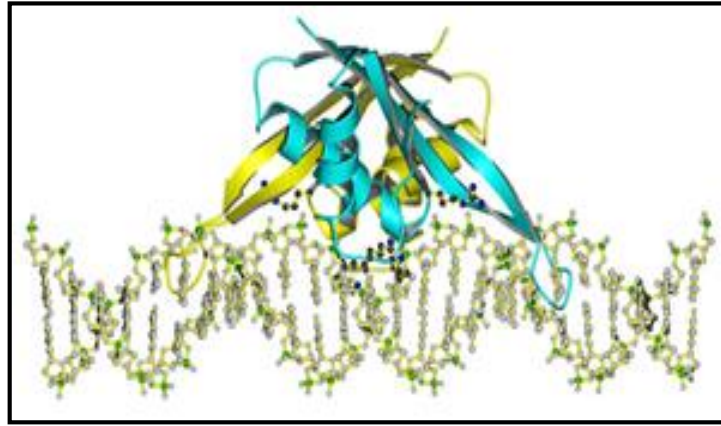
Proteins of Interest

PfSir2a
33.8kDa

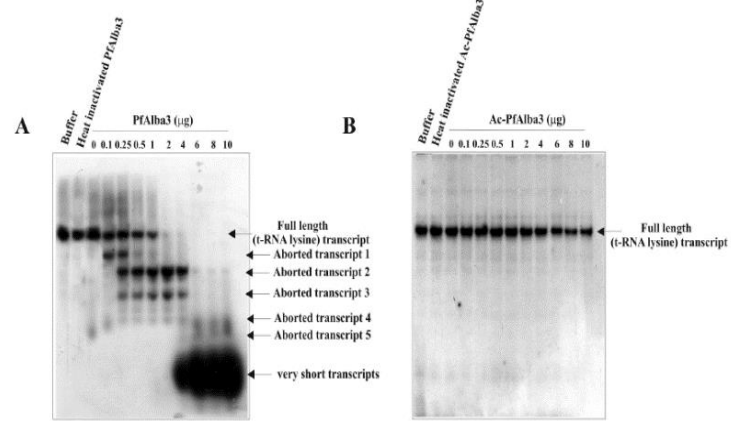
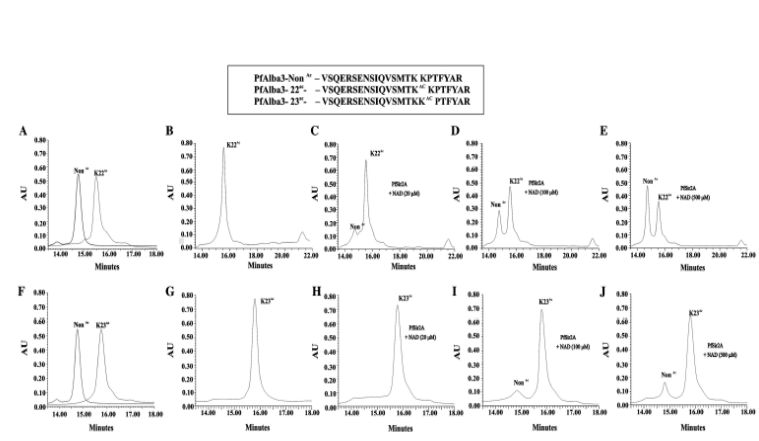
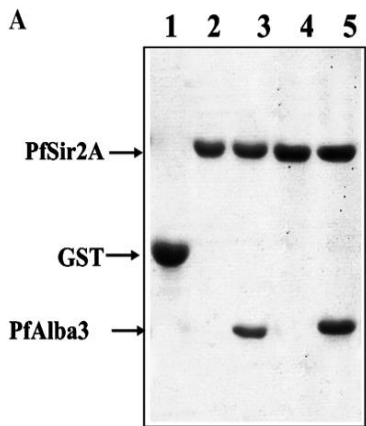


PDB ID: 3JWP

Alba3
13.04kDa

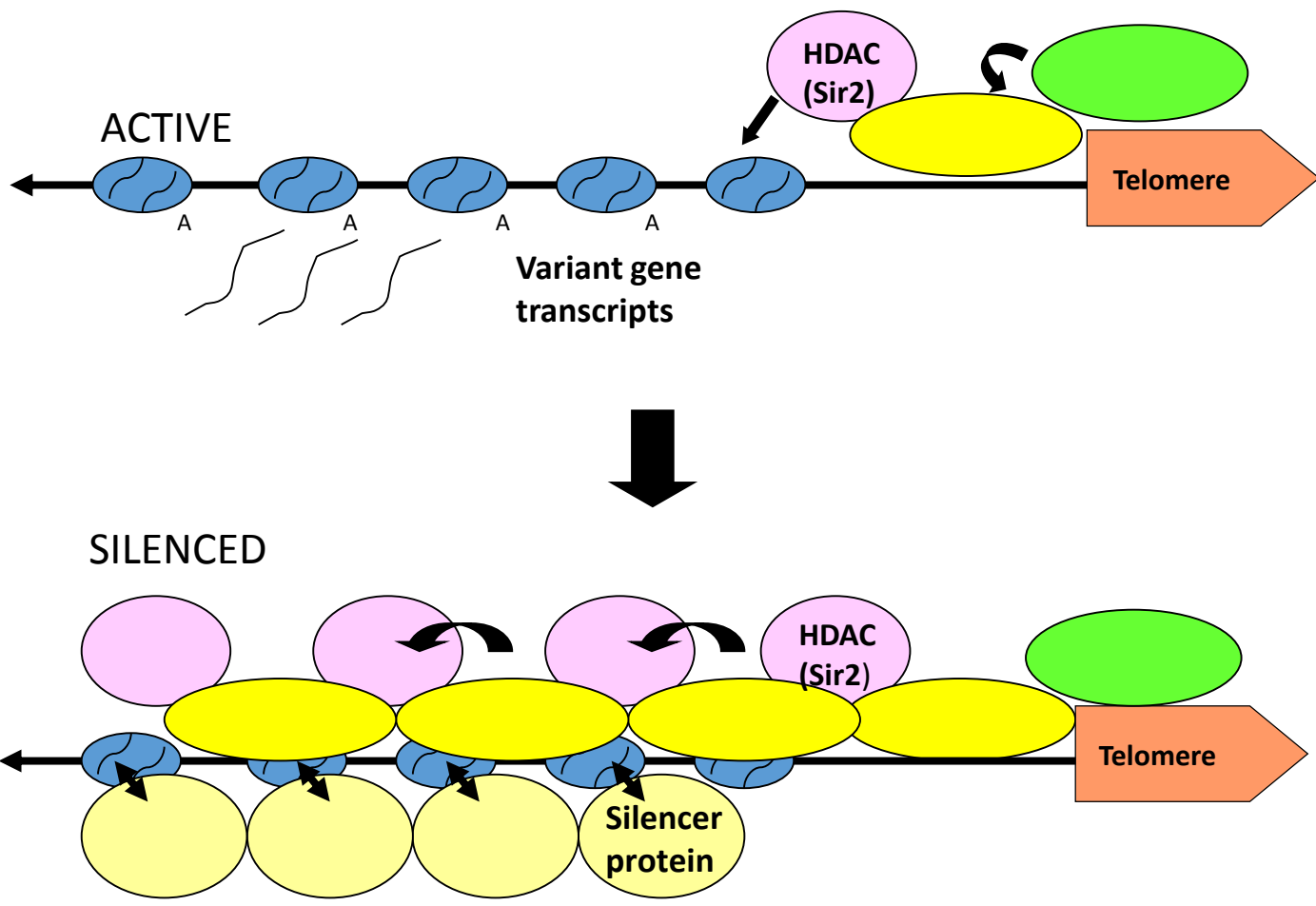


Wardleworth *et al* 2002 EMBO Journal

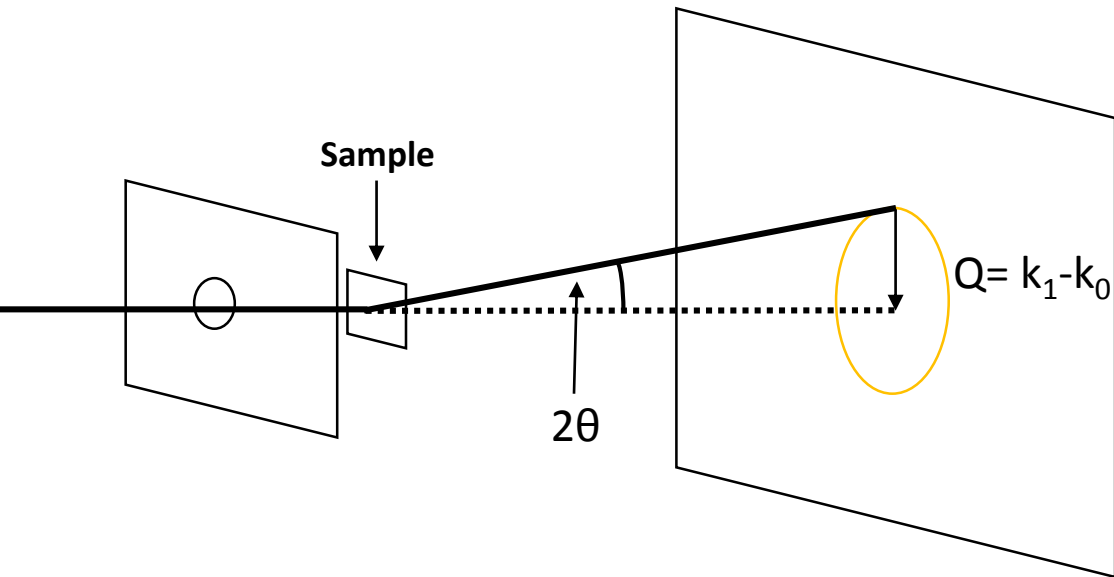


Goyal *et al* 2011 Nucleic Acids Research.

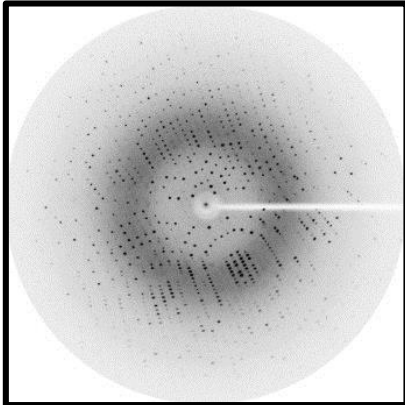
Proposed Silencing Mechanism



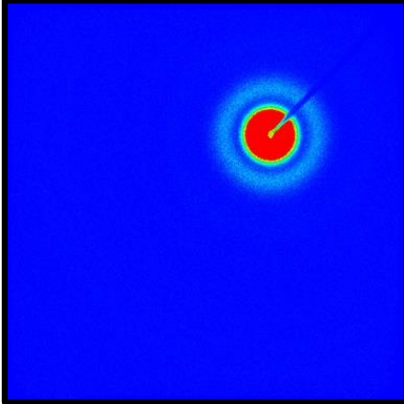
Small Angle Scattering - Overview



Crystallography



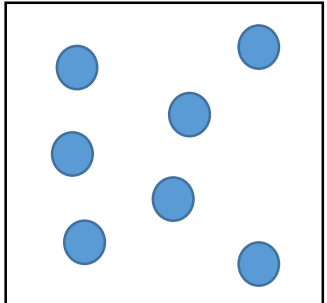
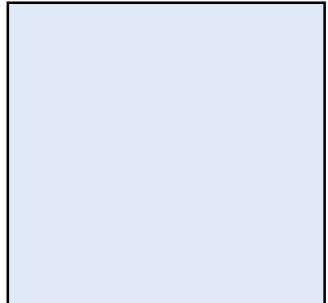
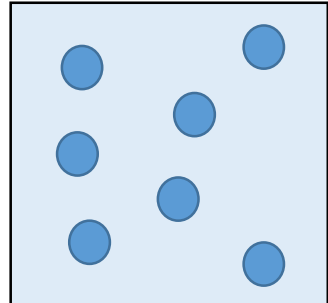
SAXS/SANS



Solution

Solvent

Particle



Don't Forget!

$S(Q)$

$P(Q)$

$I(Q)$

$$I(Q)_{\text{Total}} = P(Q) \times S(Q)$$

- Empty beam
- Blocked Beam
- Empty Cell

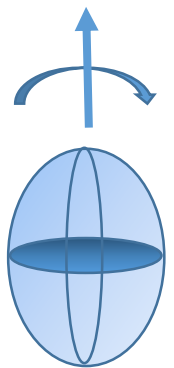
Small angle scattering:

Radius of gyration: R_g

Guinier's Law:

$$I(q) \approx I(0) e^{\frac{-q^2 R_g^2}{3}}$$

$$0 < q < 1/R_g$$

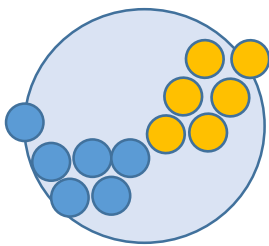


Globular Protein



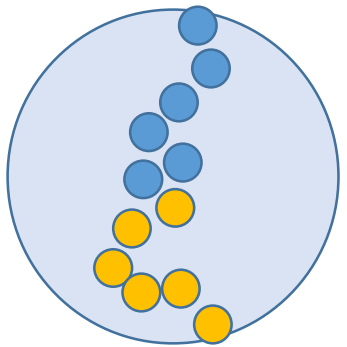
3nm

Oligomeric state



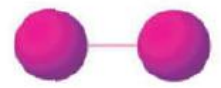
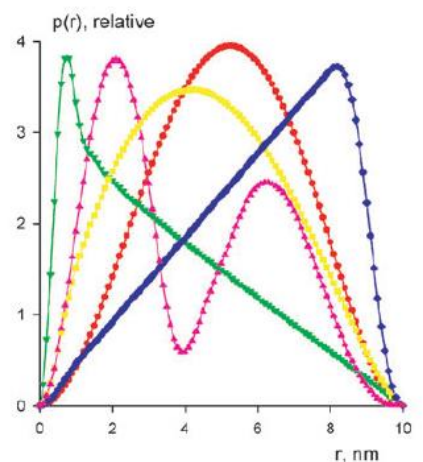
5nm

Extended/IDP

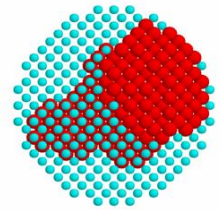


10nm

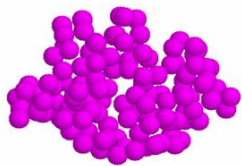
Pair-Distance Distribution Function: $P(r)$



Envelope function



Bead models

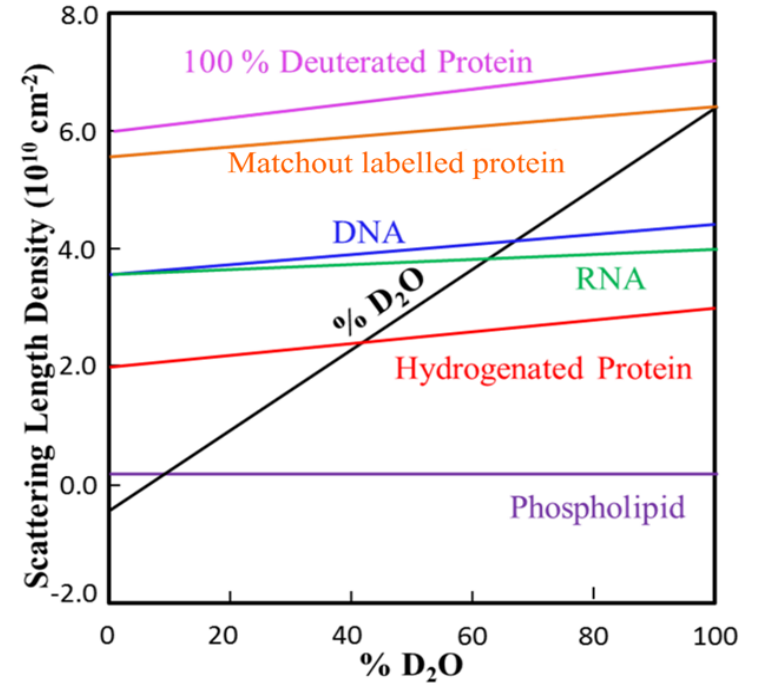


Dummy residues model

Small Angle Scattering Studies

Project Goals

- Produce recombinant proteins for structural characterisation
- Produce deuterium labelled variants
- Perform contrast 'match-out' experiments to investigate protein-protein interactions.



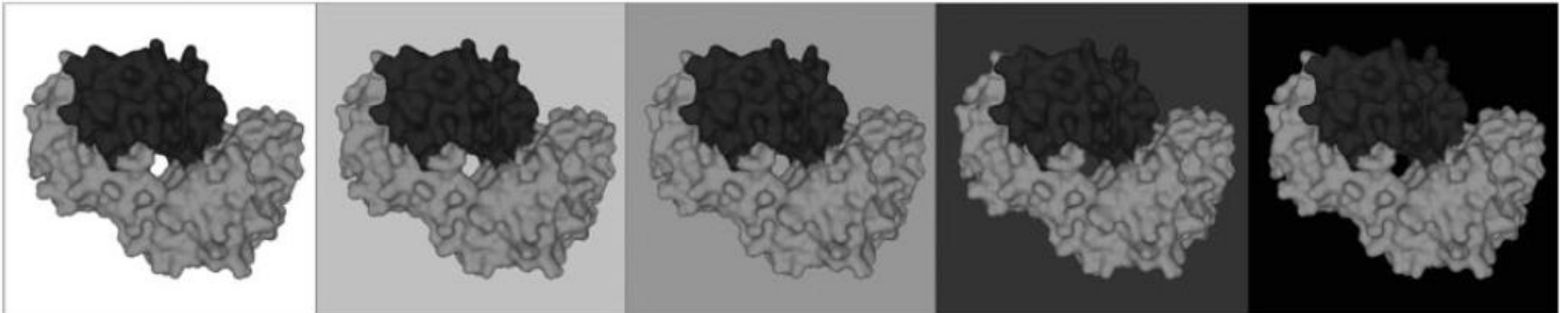
0 % D_2O

20 %

40 %

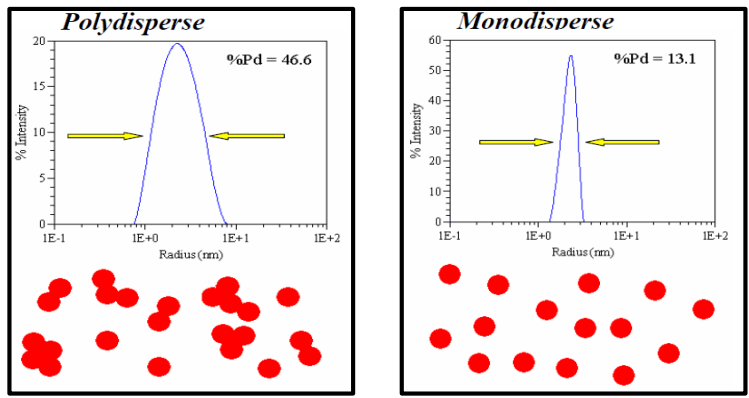
90 %

100 %

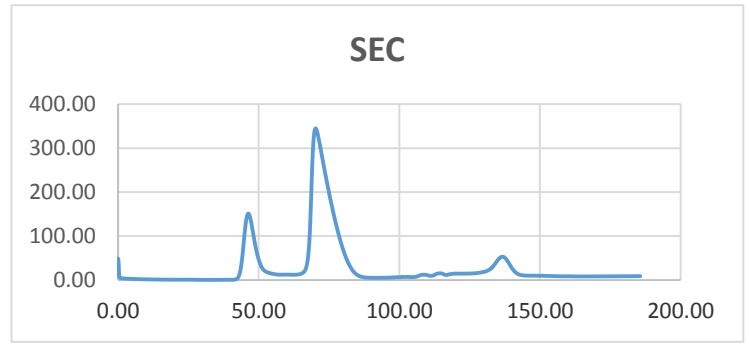


Small angle scattering theory requires:

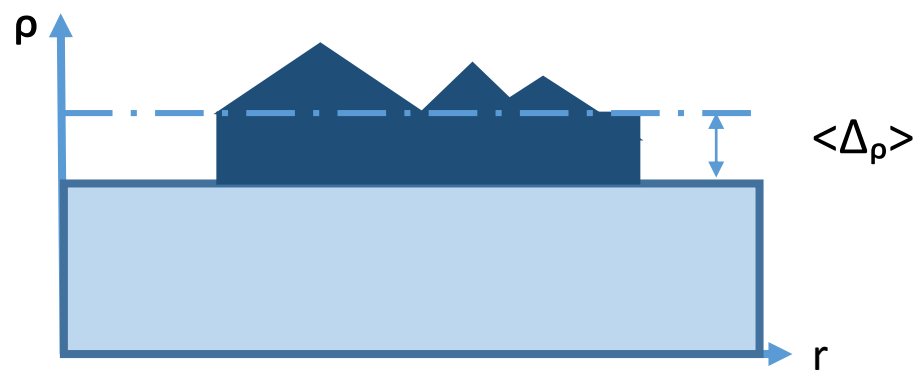
- Monodisperse sample



- Free from aggregates




- Particle to have sufficient contrast from solvent
Concentrated solutions 1-10mg/ml



- Free from concentration effects – oligomerisation, interparticle interactions, aggregation.


A PRELIMINARY CHARACTERISATION

PURITY



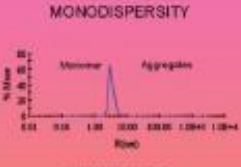
SDS-PAGE and GEL FILTRATION
High molecular weight species must be removed

CONCENTRATION




MEASURE ABSORBANCE
Also use A280/A290 to detect nucleic acids

MONODISPERSITY

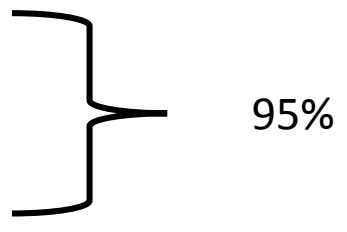


DYNAMIC LIGHT SCATTERING
Sample must be aggregate-free

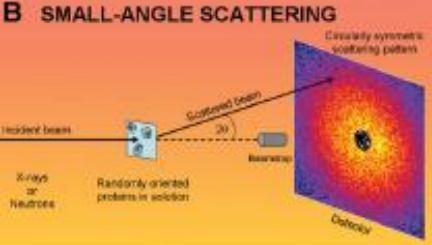
MATCHED SOLVENT



DIALYZE SAMPLE
Matched solvent required for accurate solvent subtraction



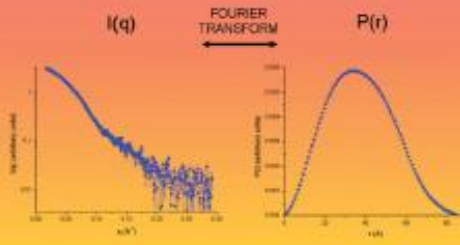
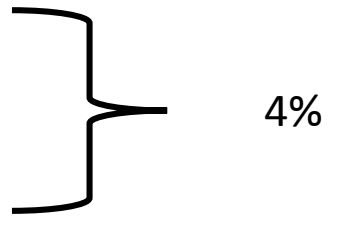
B SMALL-ANGLE SCATTERING



Coaxially symmetric scattering pattern


DATA REDUCTION → $I(q)$ → FOURIER TRANSFORM → $P(r)$

SOLVENT SUBTRACTION →


C DATA VALIDATION

INITIAL INSPECTION




GUINIER PLOT
Linearity is necessary but not sufficient for further analysis

RADIATION DAMAGE




MEASURE TIMECOURSE
 $I(q)$ and R_g should show no time-dependence

CONCENTRATION EFFECTS

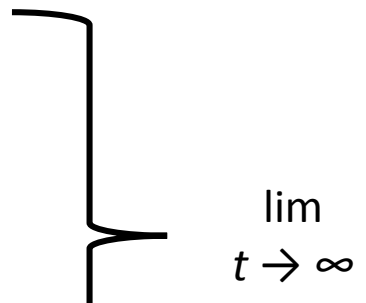


MEASURE CONCENTRATION SERIES
 $I(q)/C$ and R_g should remain constant. Increase with C indicates aggregation, decrease indicates interparticle interference

STANDARDS




MEASURE WATER AND/OR SECONDARY PROTEIN STANDARD
Normalise scattering to standard. Calculated molecular weights will be accurate for high quality samples




D MODELING

HIGH-RESOLUTION VALIDATION



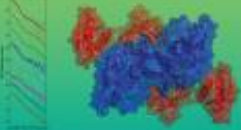
CRYSTALLOGRAPHY
Compare atomic models to solution scattering

MULTI-DOMAIN MODELLING




DAMMIN/DAMMIF/SASREF/BLINDCH
Ab-initio and/or rigid-body refinement against scattering data

SUBUNIT MODELING



MULTICOMBSASREFIT
Use neutron contrast variation data to extract component scattering functions, and/or model subunits

FLEXIBILITY



EDM
Model an ensemble of rigid structures against the scattering data


E ADDITIONAL INFORMATION

ATOMIC MODELS




CRYSTALLOGRAPHY/EM/BIOPHYSICS/BIOPHYSICS MODEL
Useful for all modeling

DISTANCE RESTRAINTS



NMR/EM/BIOPHYSICS/BIOPHYSICS
Powerful restraints for all rigid-body modeling

SYMMETRY



CRYSTALLOGRAPHY/NMR/EM/BIOPHYSICS
Powerful restraint for rigid-body and elastic modeling

DISORDER



EM/BIOPHYSICS/BIOPHYSICS
Essential for meaningful ensemble modeling



Recombinant Protein Production & Characterisation

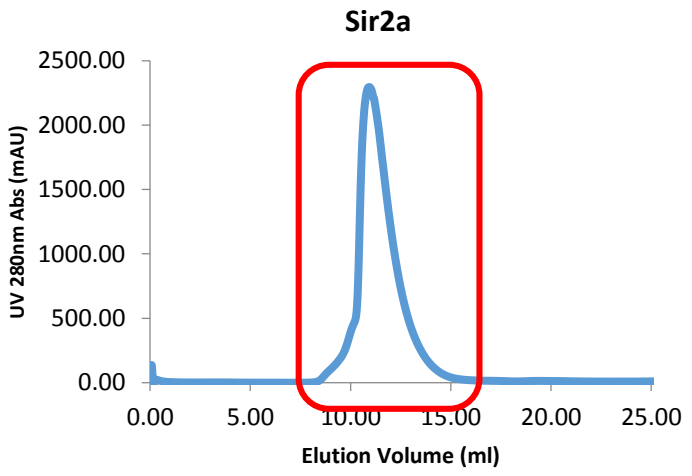
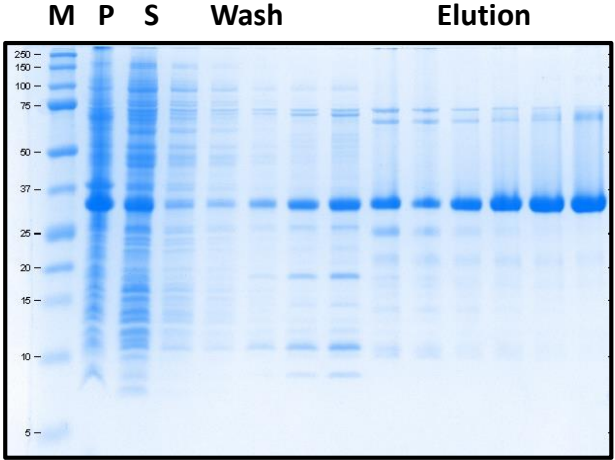
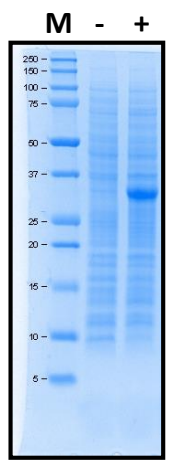
Expression
E. coli

Purification
IMAC/IEX

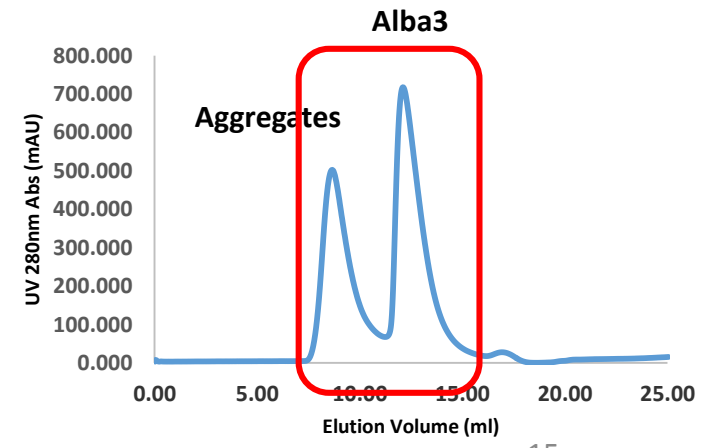
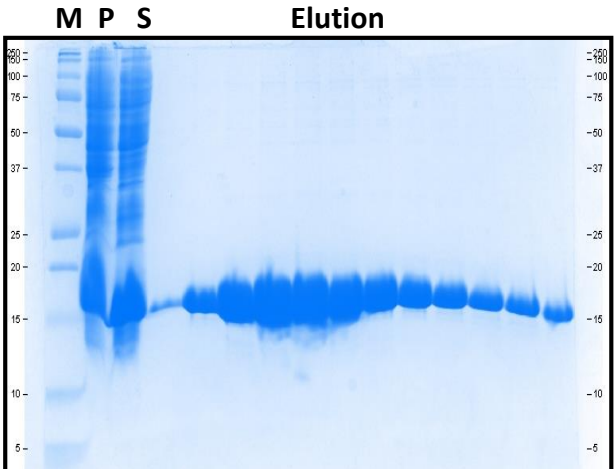
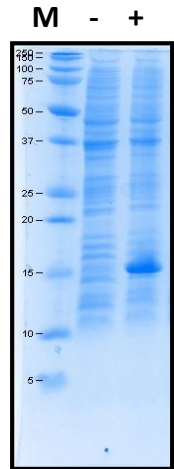
Final Steps
Size exclusion chromatography

Ni-NTA/Cation exchange chromatography

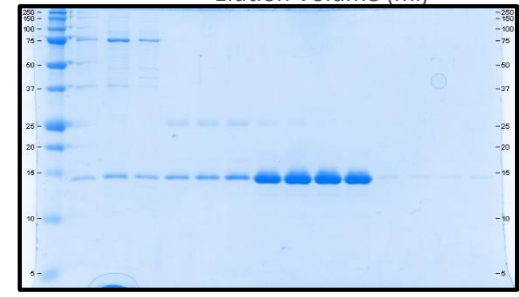
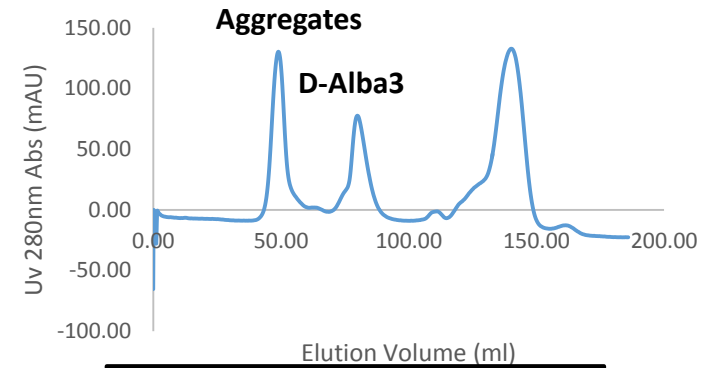
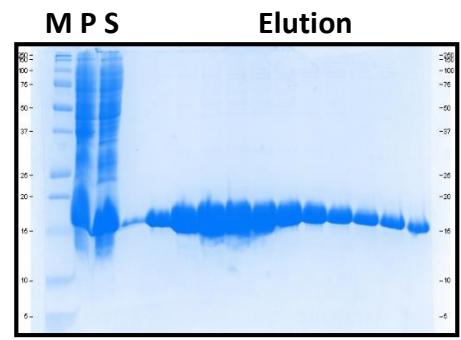
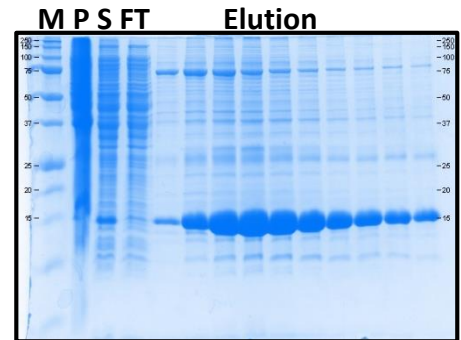
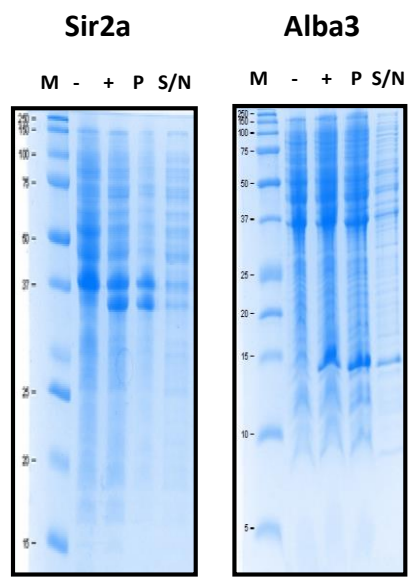
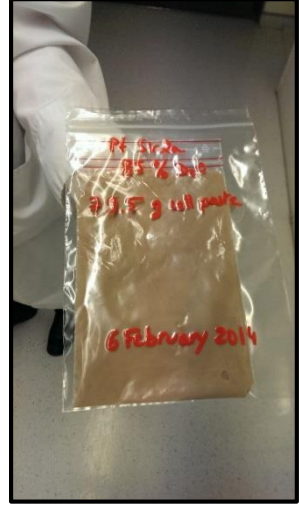
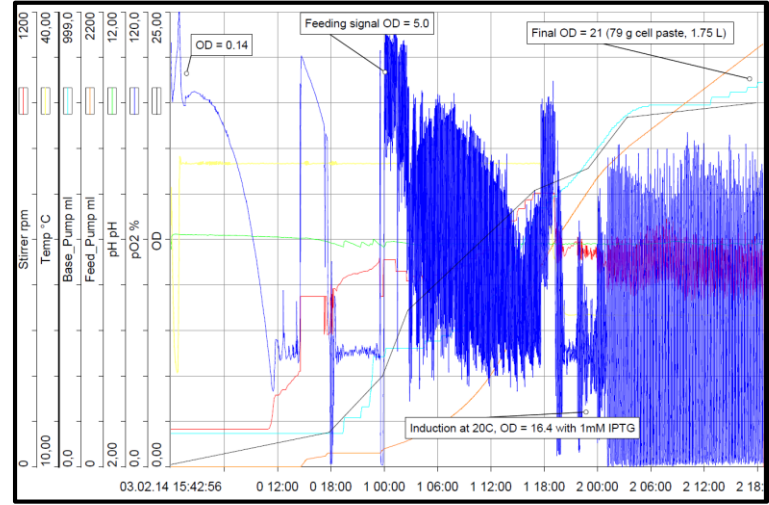
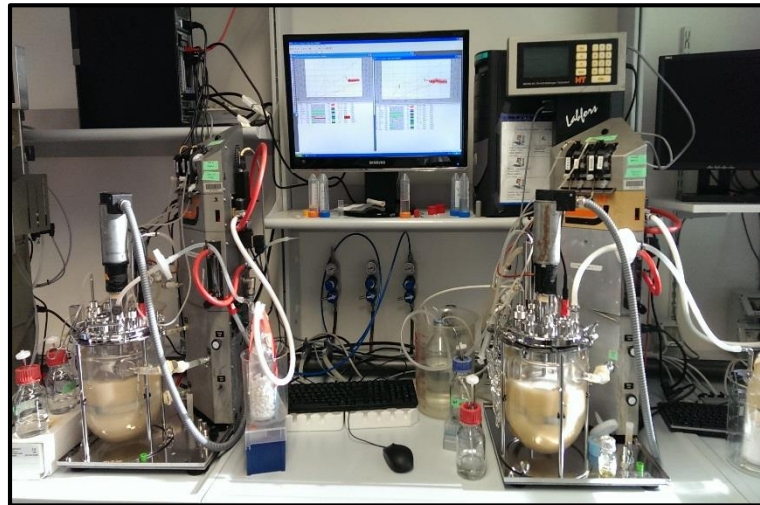
Sir2a: 33.8 kDa



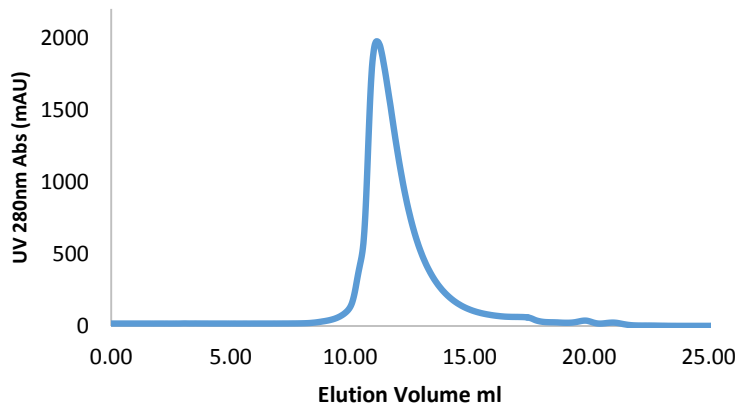
Alba3: 13.04 kDa



Deuterated Protein Production

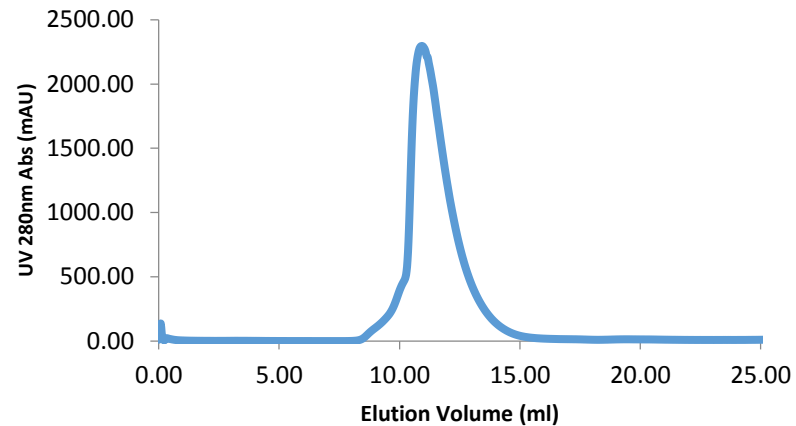


Sir2a



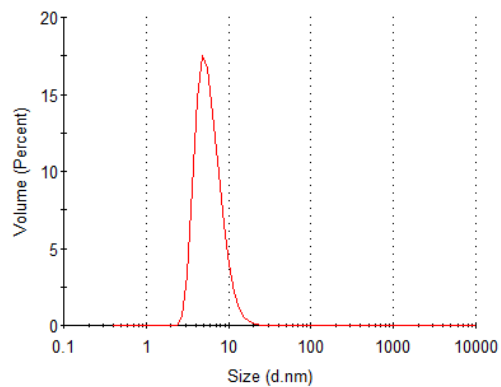
50mM HEPES pH 7.5
50mM NaCl

Sir2a



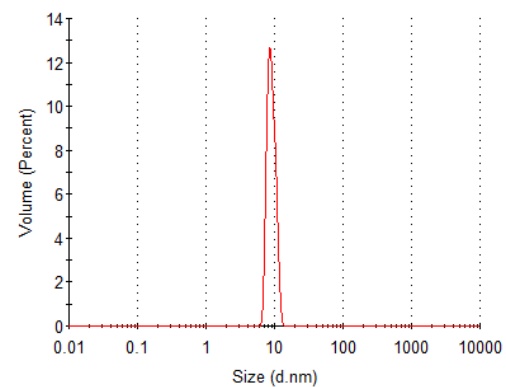
50mM Naphos pH 7.0
500mM NaCl

Size Distribution by Mass

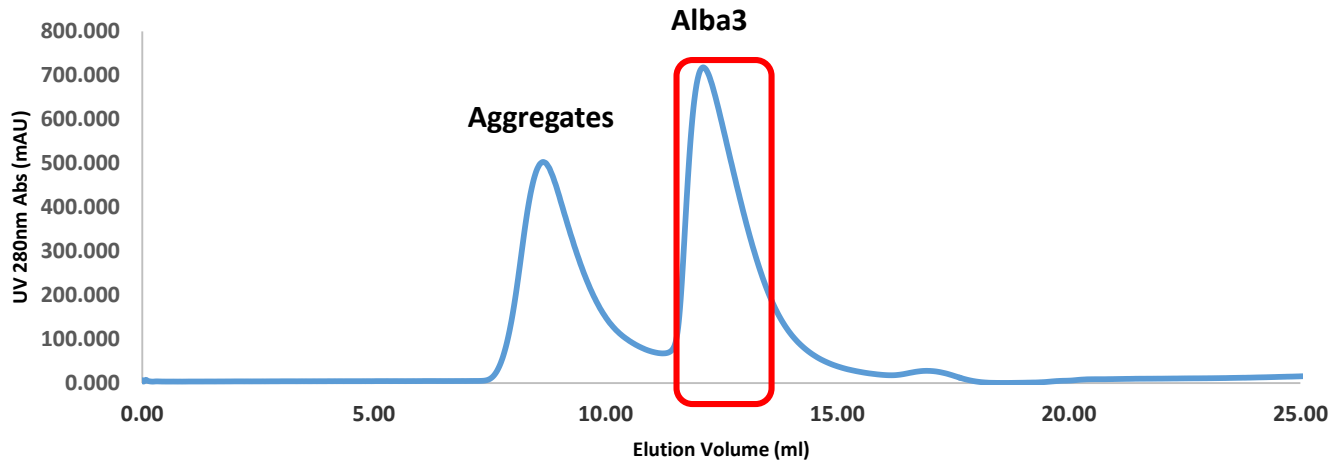


PD = 44%

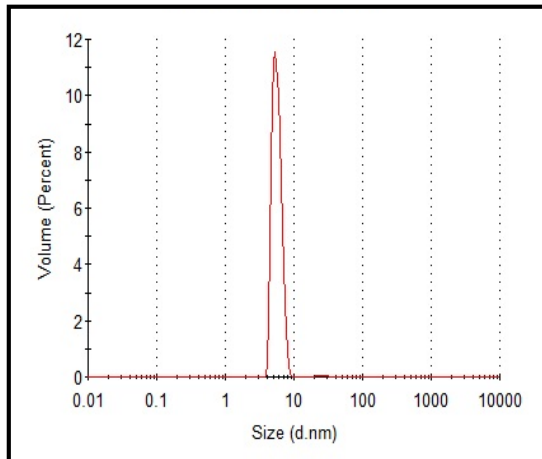
Size Distribution by Mass



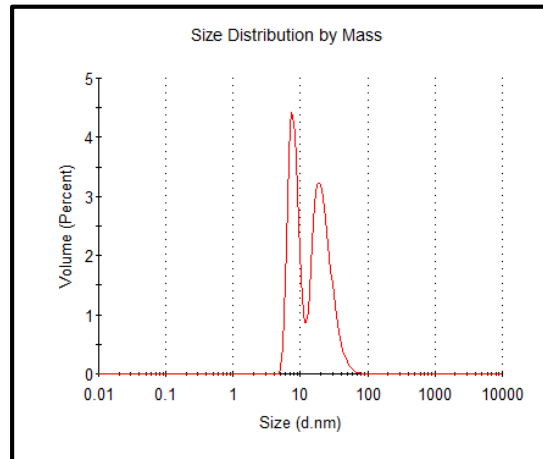
PD = 13.4%



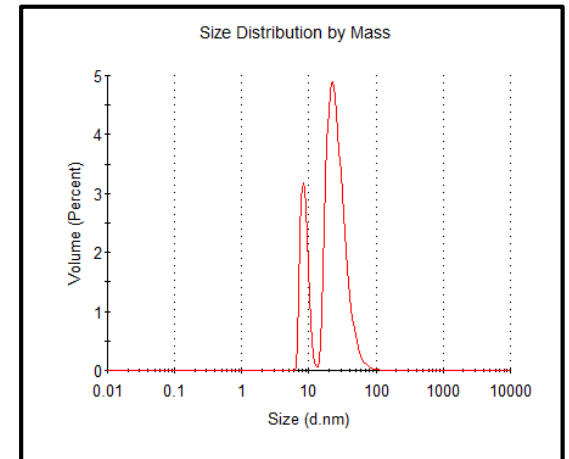
5min



15min

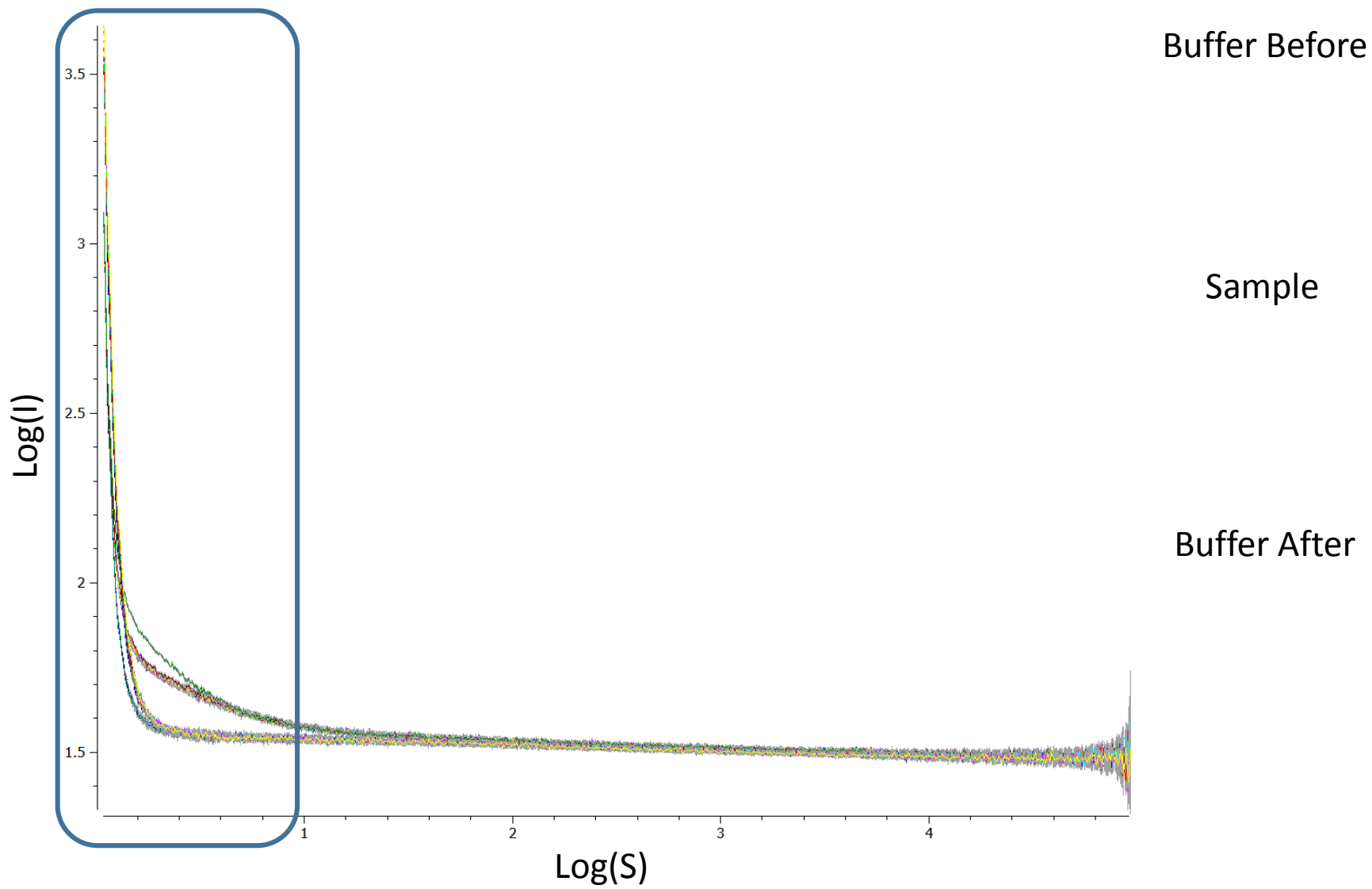


30min

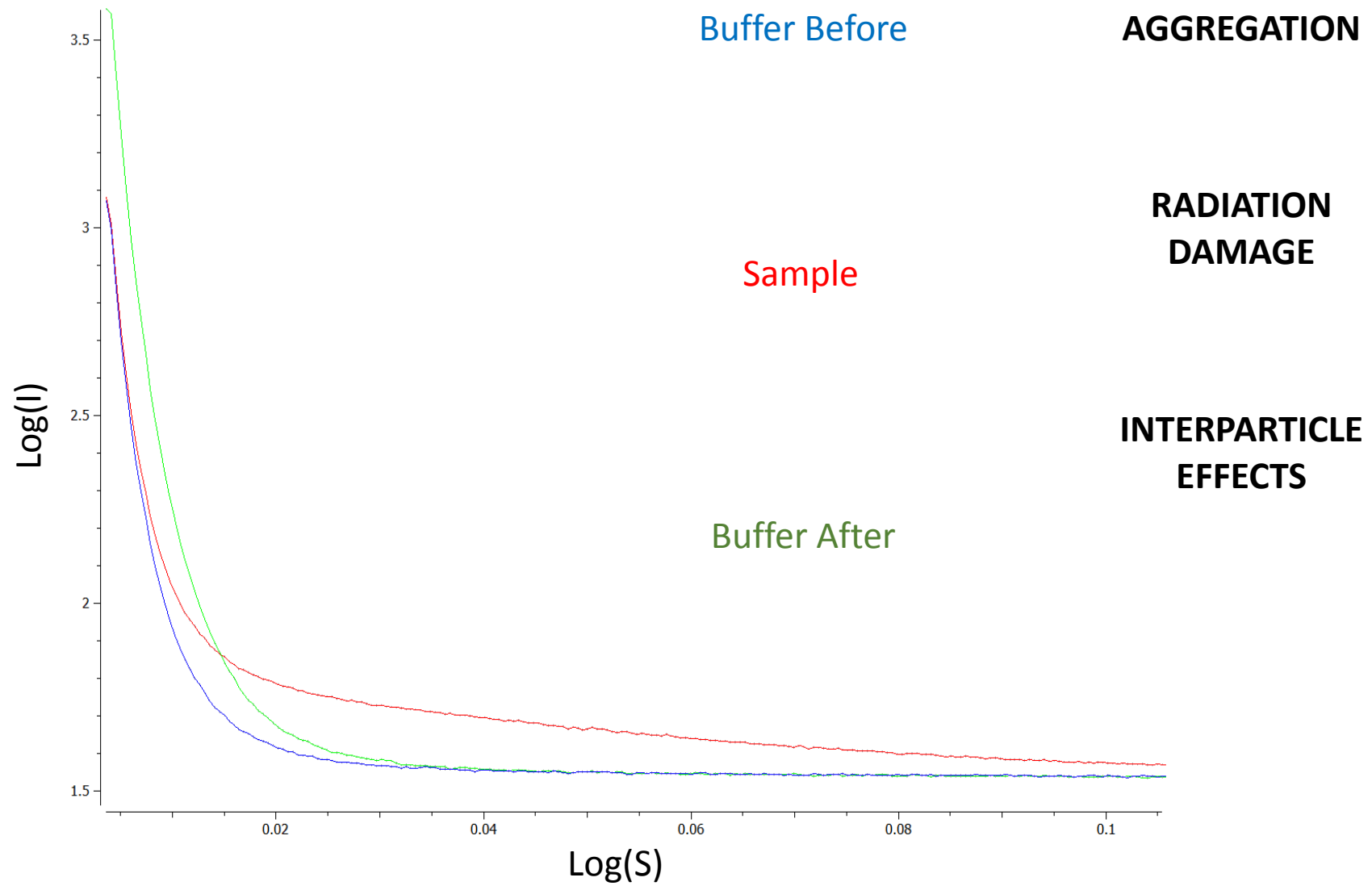


%PD

SAXS Studies

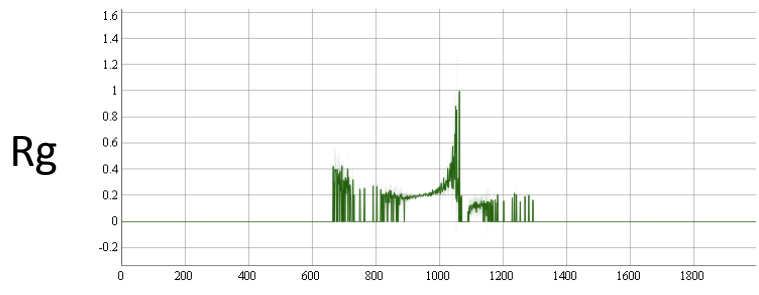
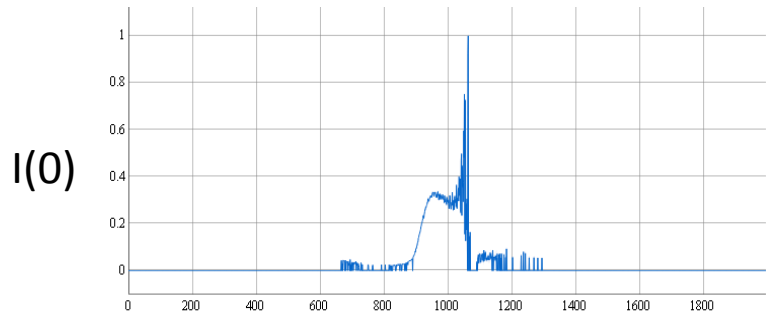
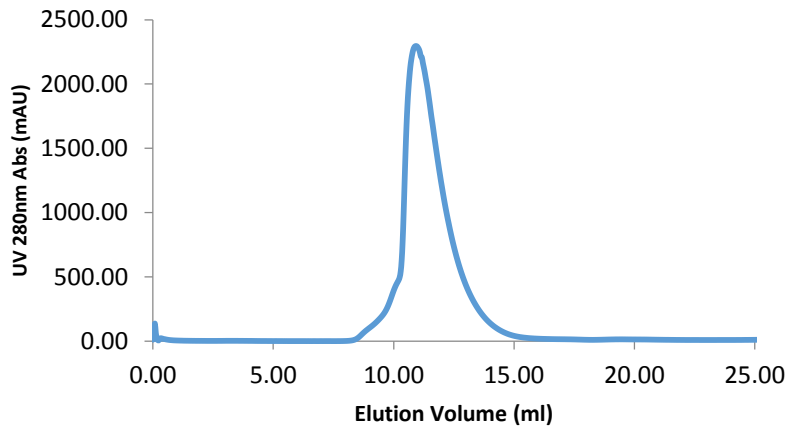


SAXS Studies

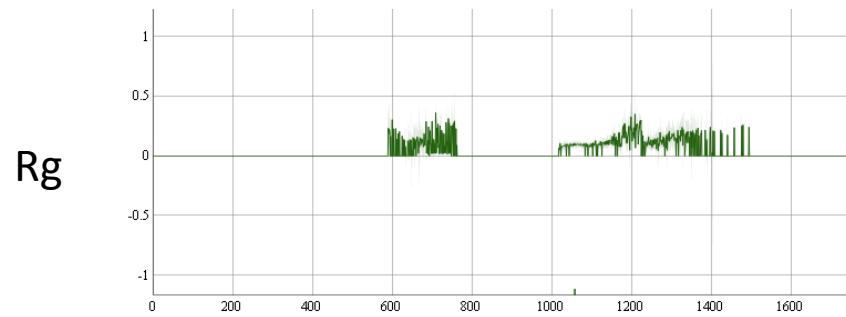
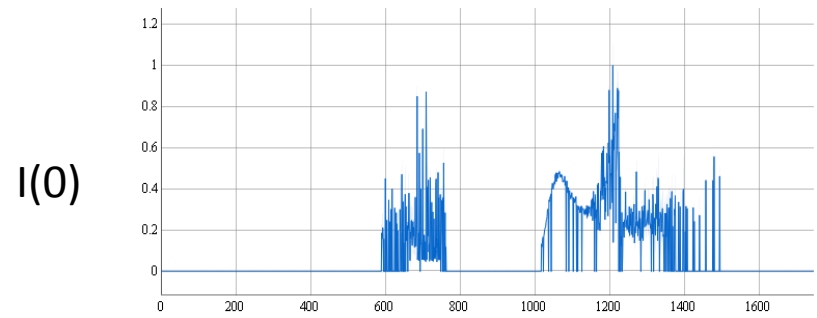
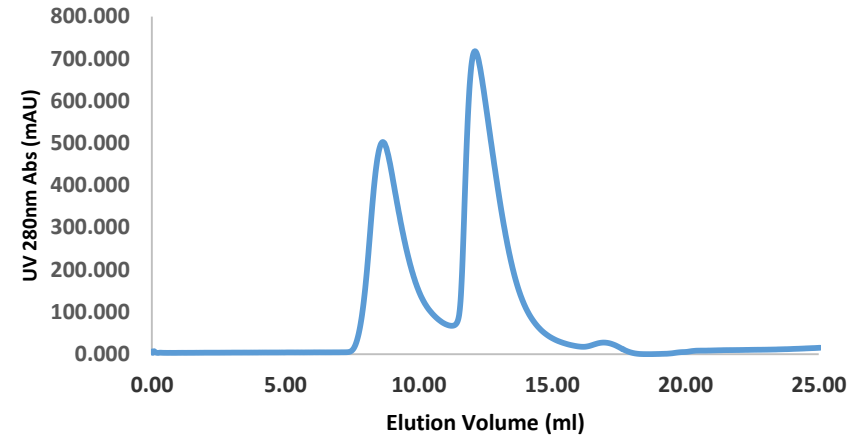


SAXS Studies – Online HPLC

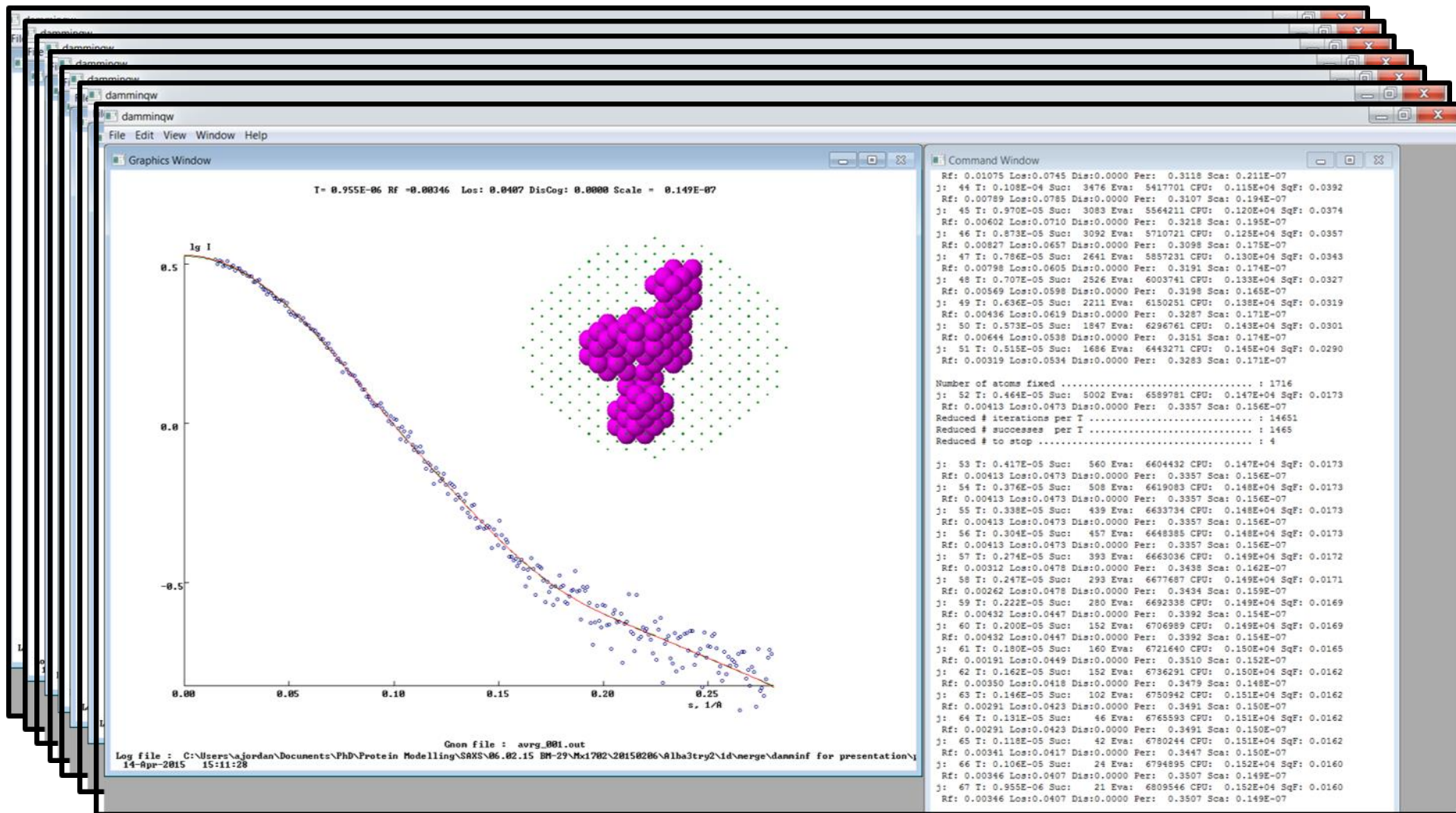
Sir2a



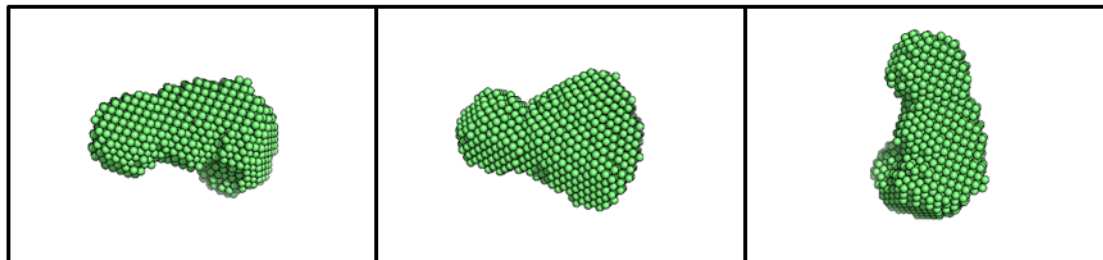
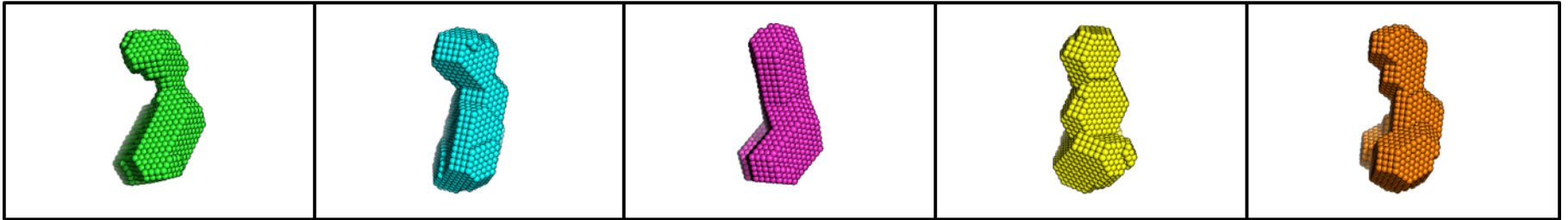
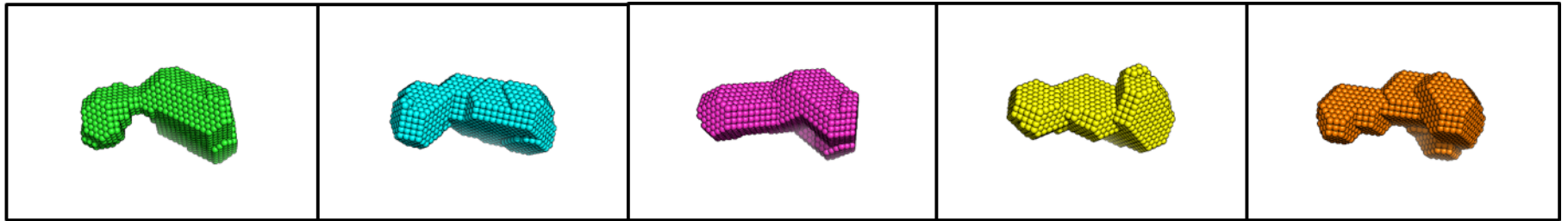
Alba3



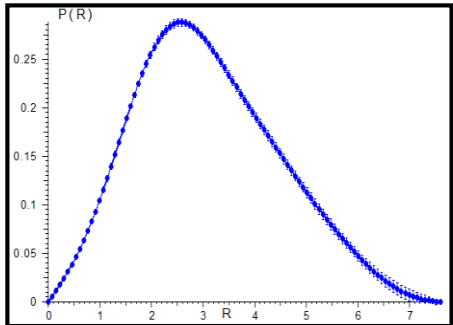
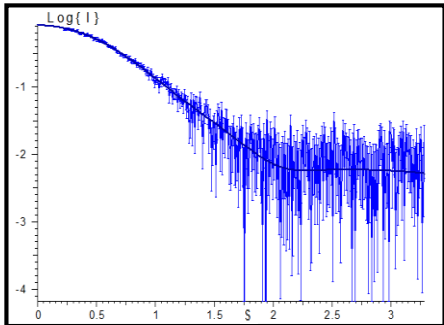
Ab initio modelling



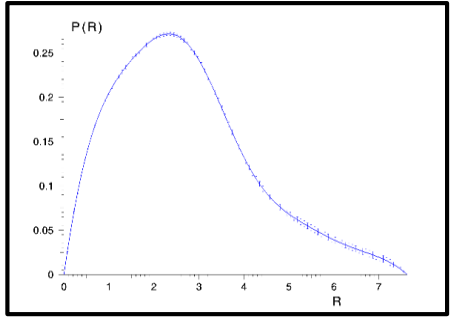
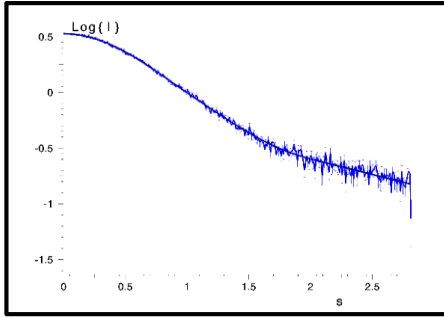
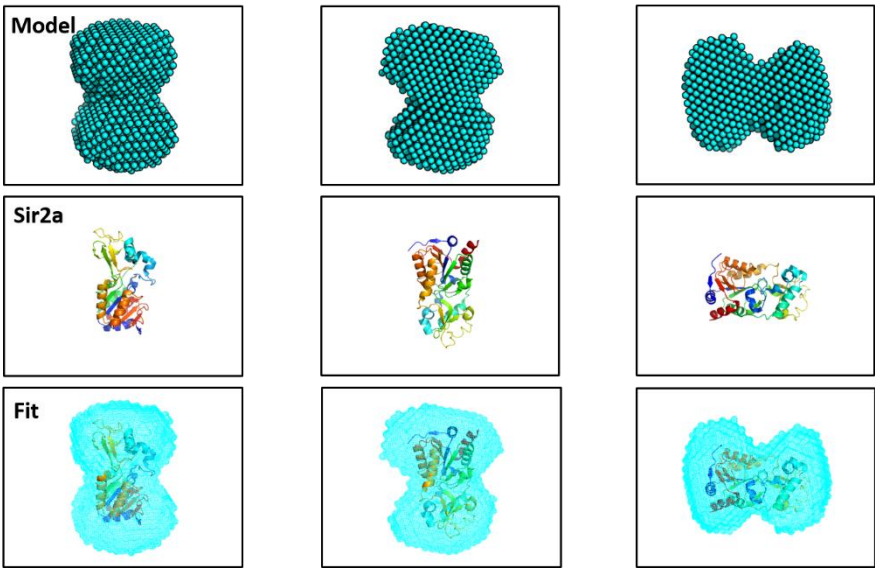
Ab initio modelling



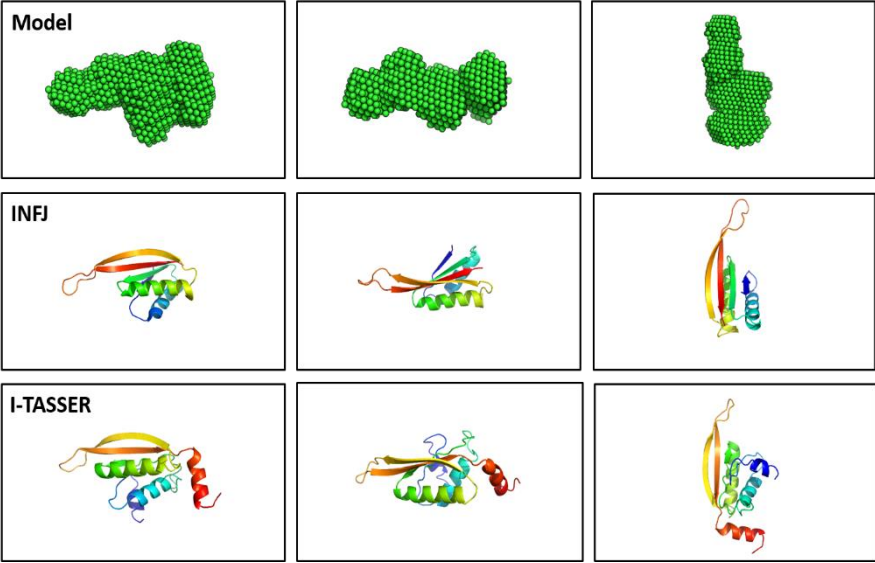
SAXS Studies – Online HPLC



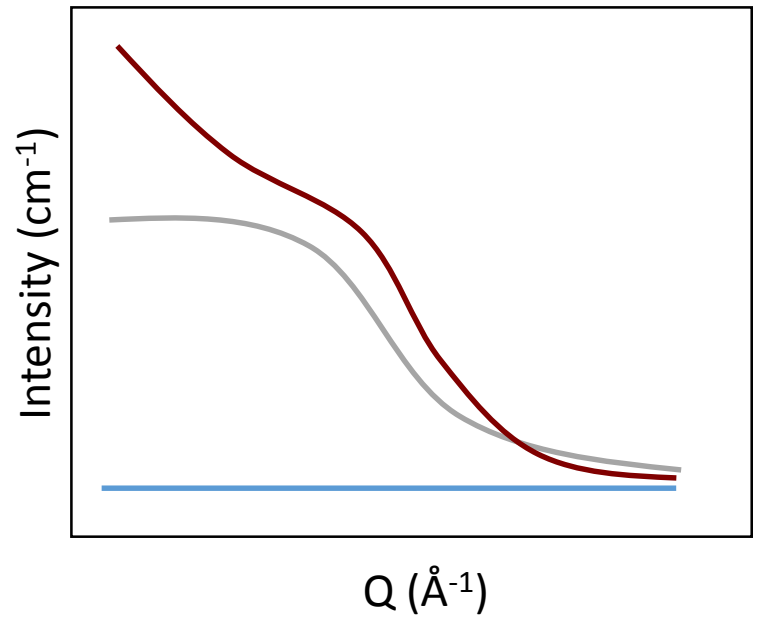
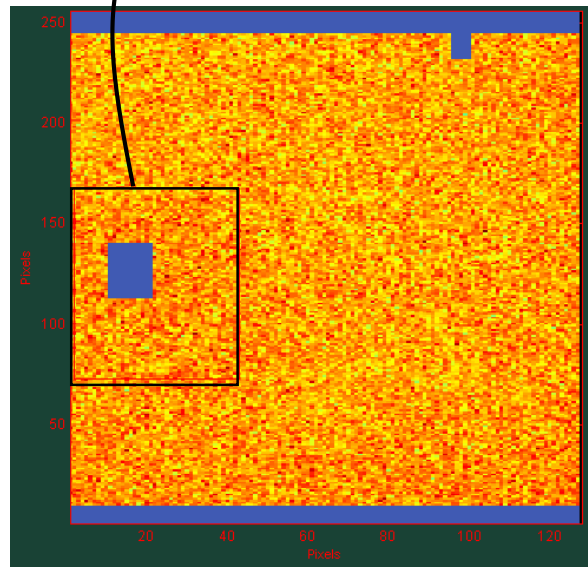
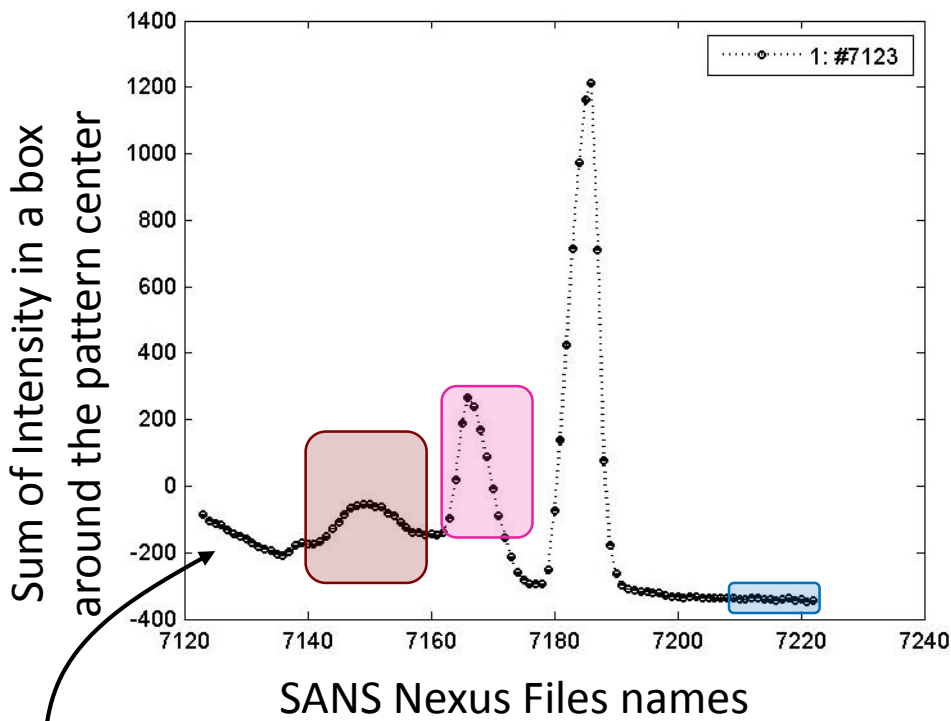
Exp Rg = 2.40 +/- 0.027nm
 Calc Rg = 2.1nm



Exp Rg 2.19nm +/- 0.018nm
 Calc Rg = 1.8nm



SANS Online FPLC



Resulting scattering curve of the protein of interest and comparison with what we obtained without *in situ* chromatography.



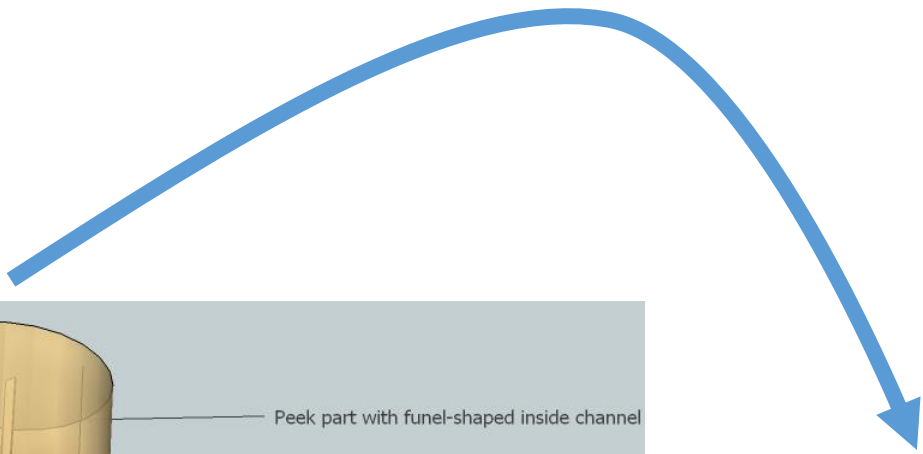
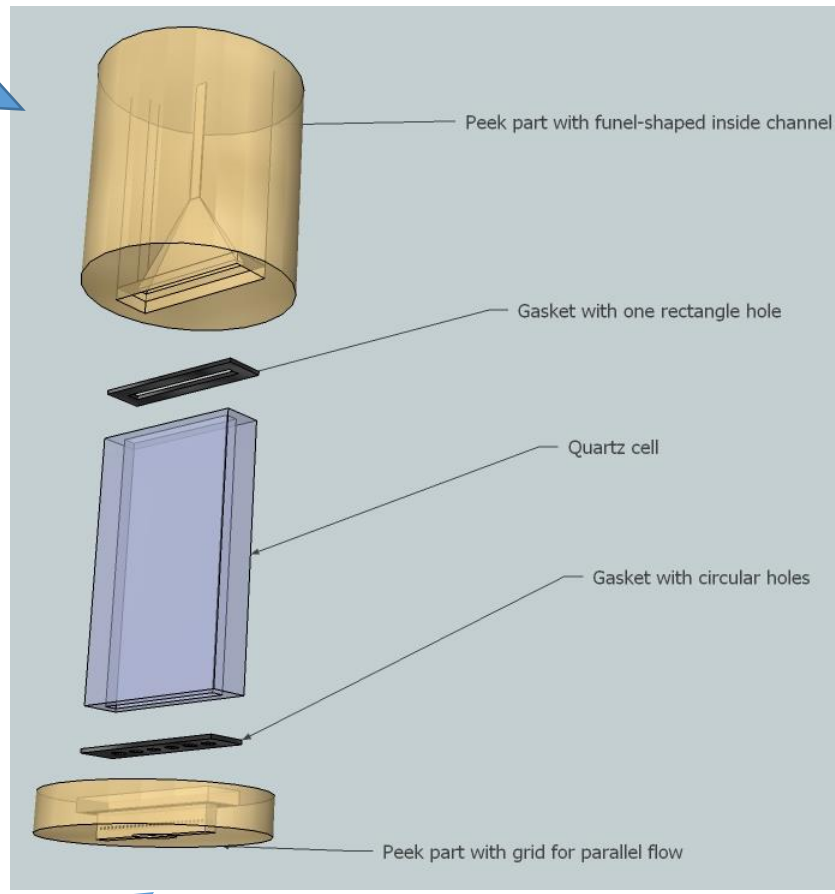
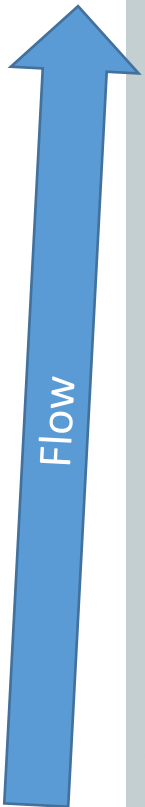
Guinier analysis and Rapid *Ab initio* modelling

SANS Online FPLC

30s – 1 min exposure time

S-75 or S-200
Analytical
column

Flow rate
0.2 – 0.4ml
min

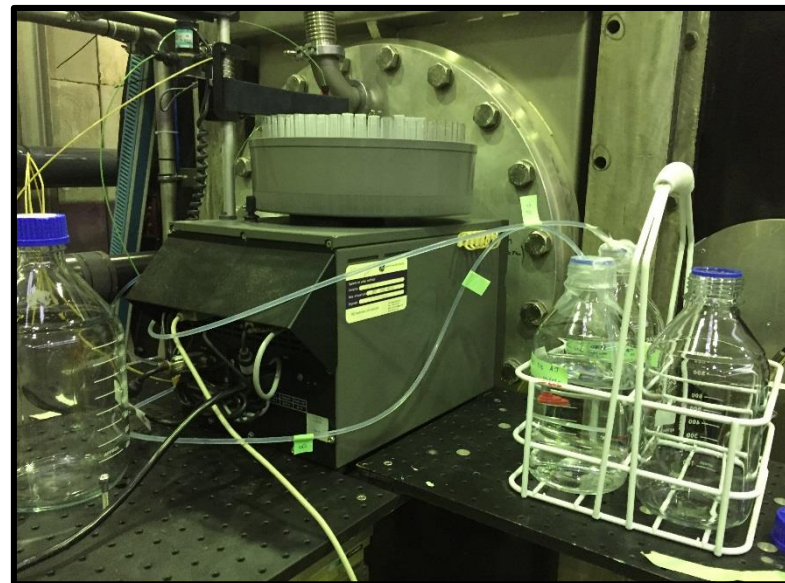
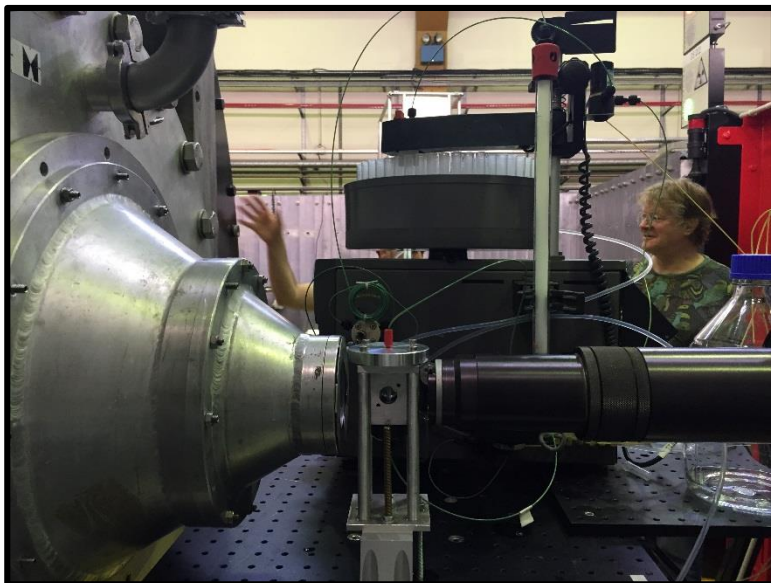
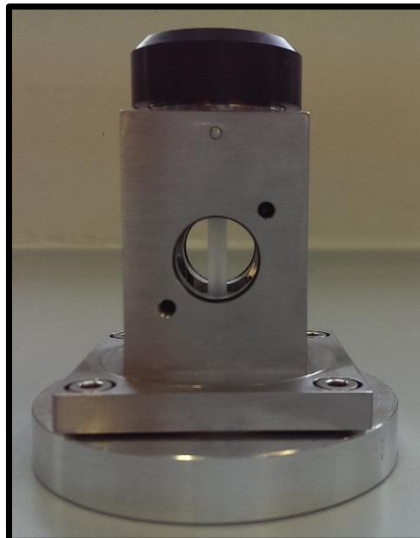
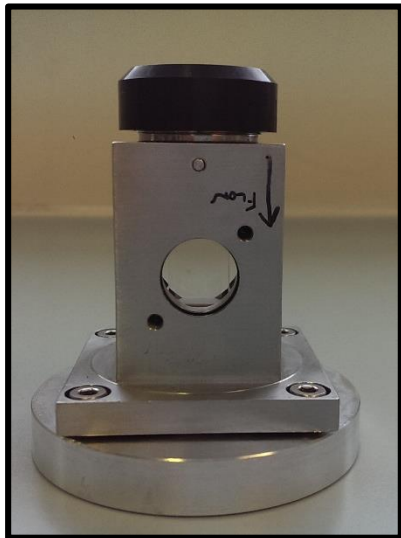


Chromatography column

Flow

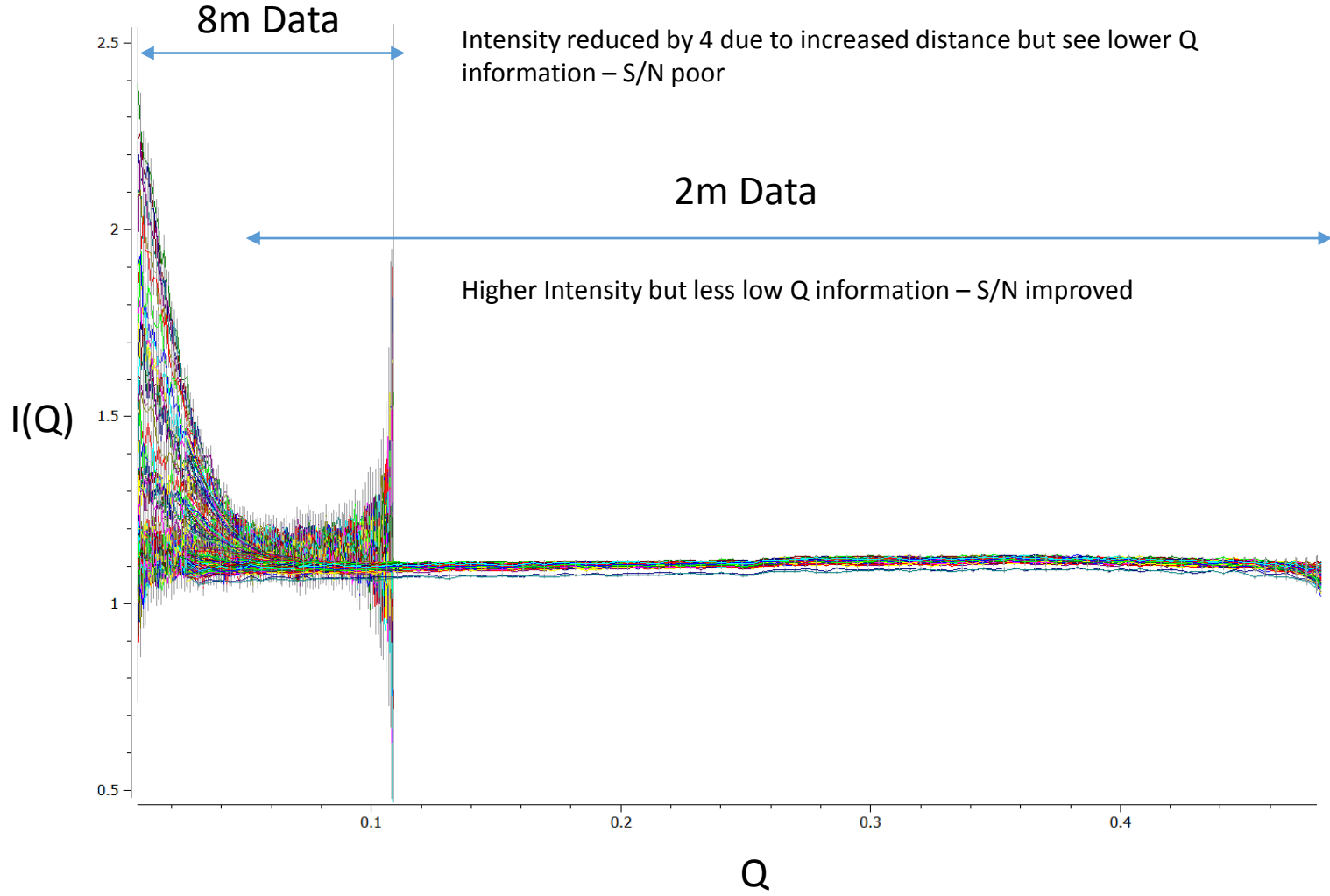
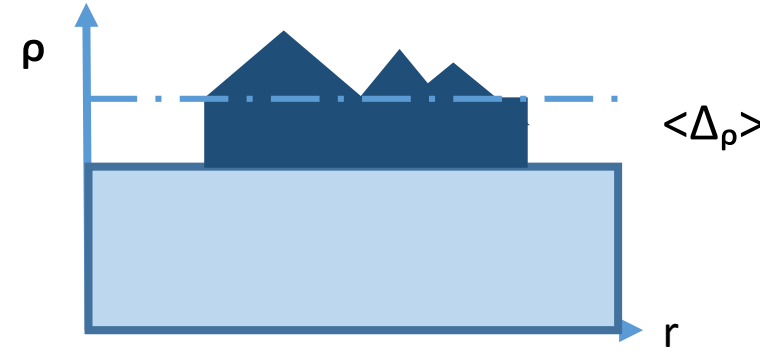
Fraction collector

SANS Online FPLC – D22



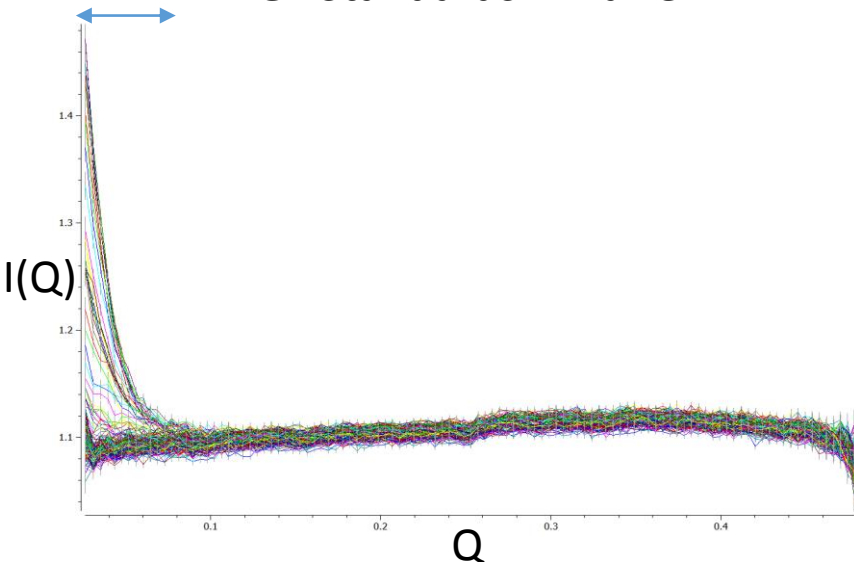
SANS Online FPLC

Concentration – Signal & interparticle effects
Contrast – S/N buffer



2m Data Total

GF Standards H-Buffer



GF Standards D-Buffer

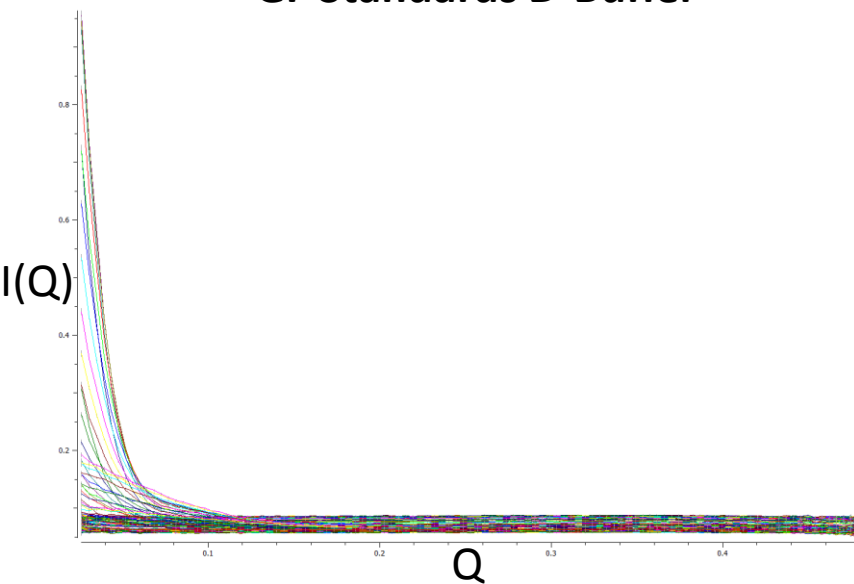
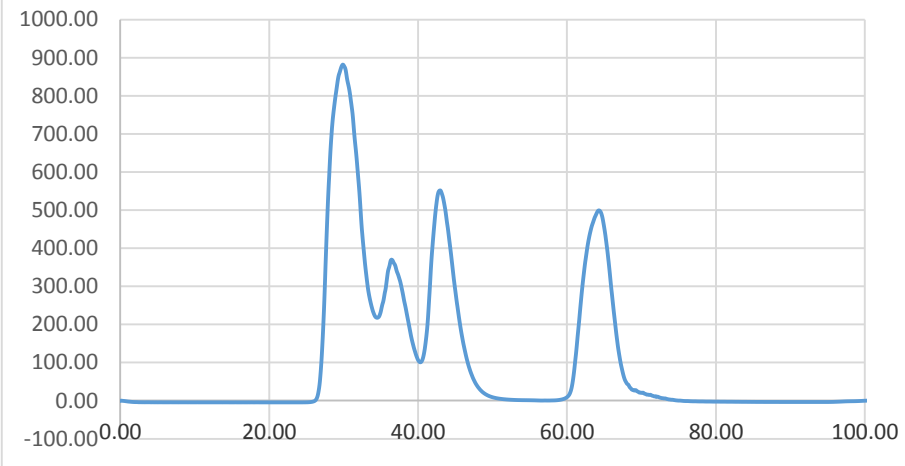


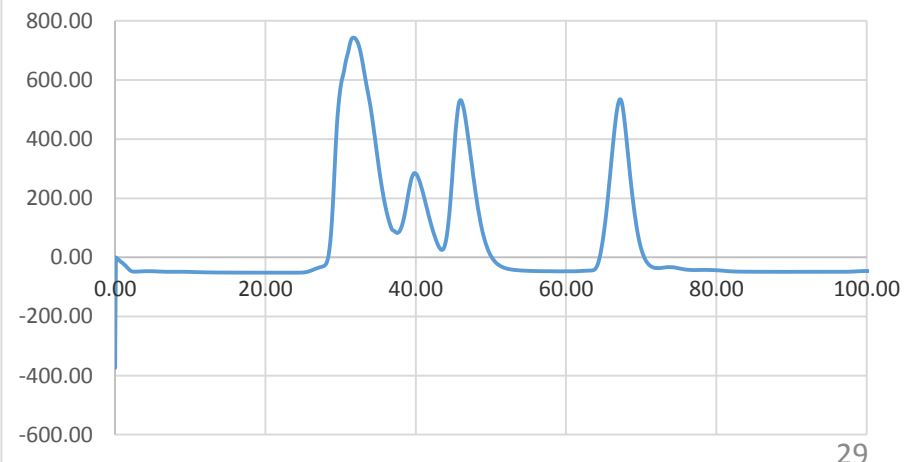
Table 1. Gel Filtration Standard Components

Component	Molecular Weight*	Amount per Vial (mg)
Thyroglobulin (bovine)	670,000	5.0
γ -globulin (bovine)	158,000	5.0
Ovalbumin (chicken)	44,000	5.0
Myoglobin (horse)	17,000	2.5
Vitamin B ₁₂	1,350	0.5
		<u>18</u>

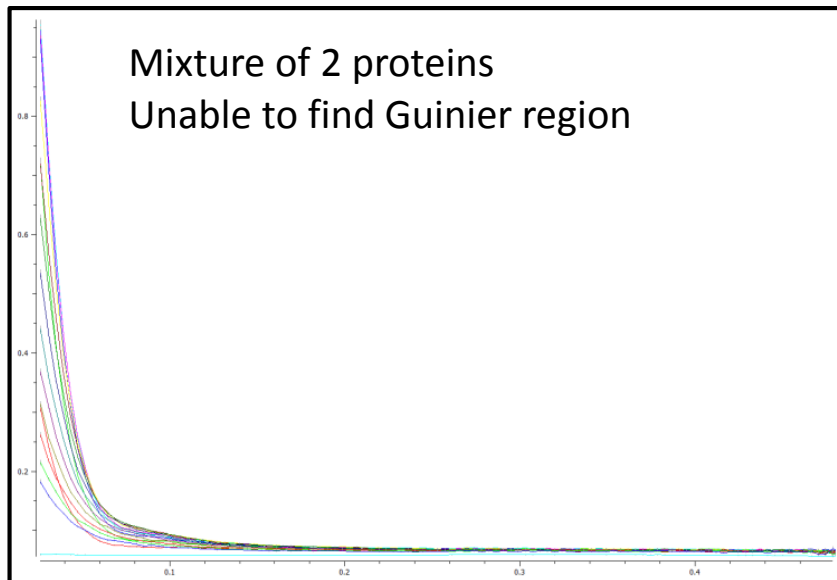
GF Stds 0% D2O Buffer



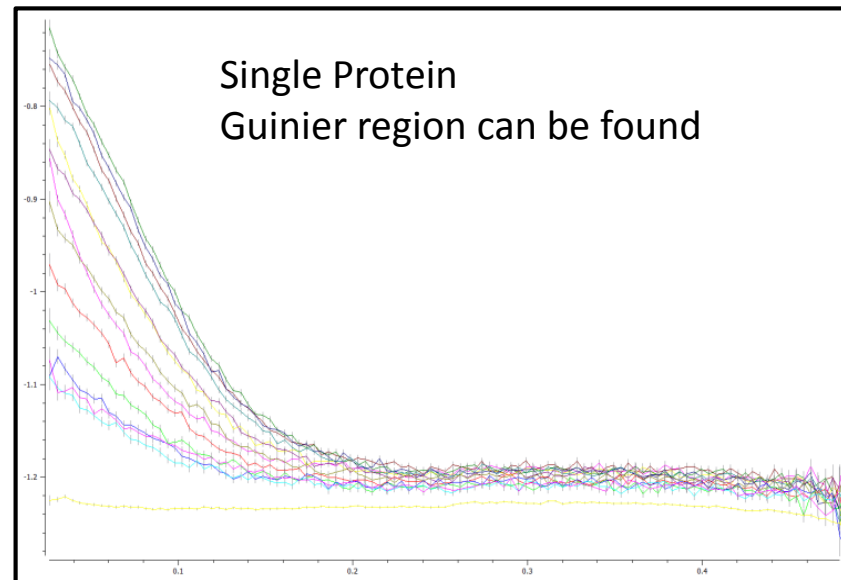
GF Stds 100% D-Buffer



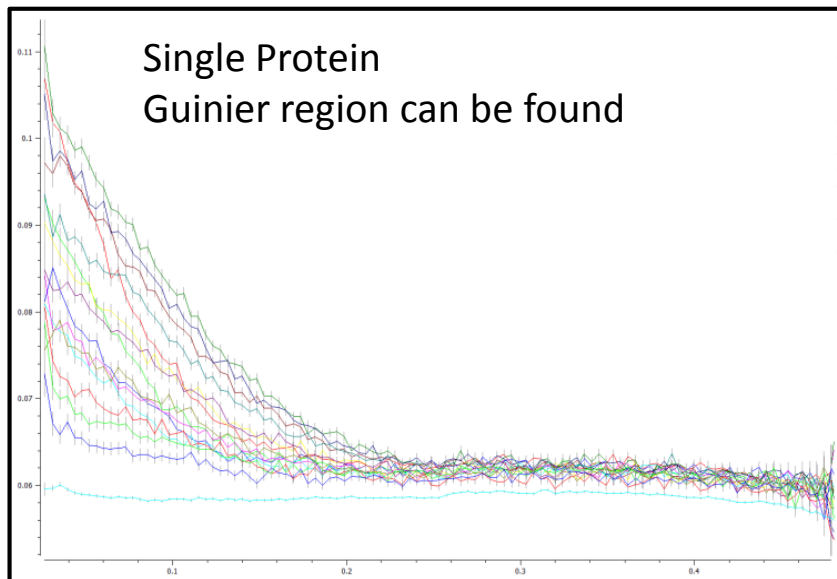
Peak 1



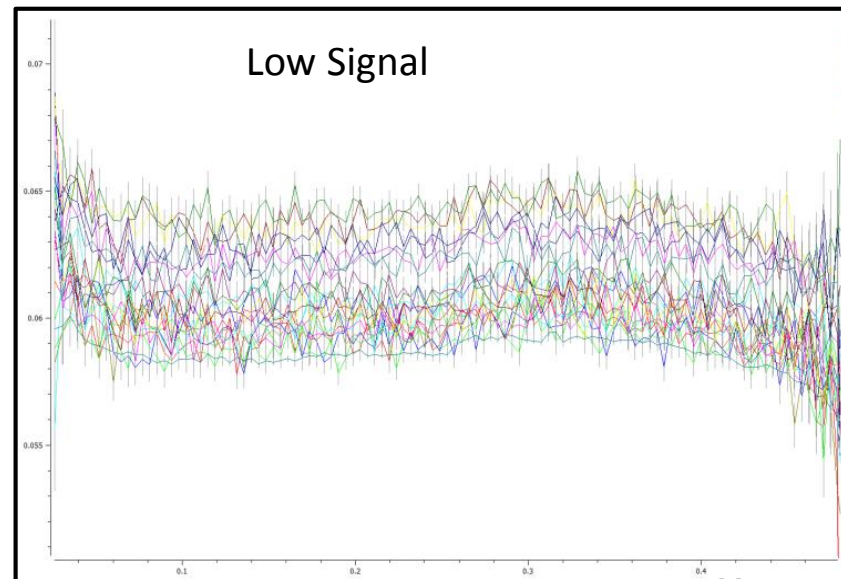
Peak 2

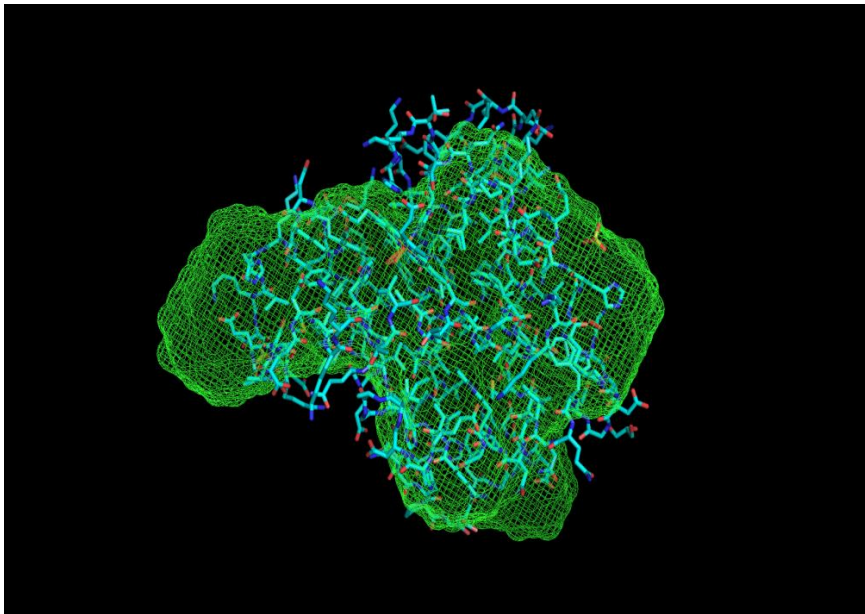
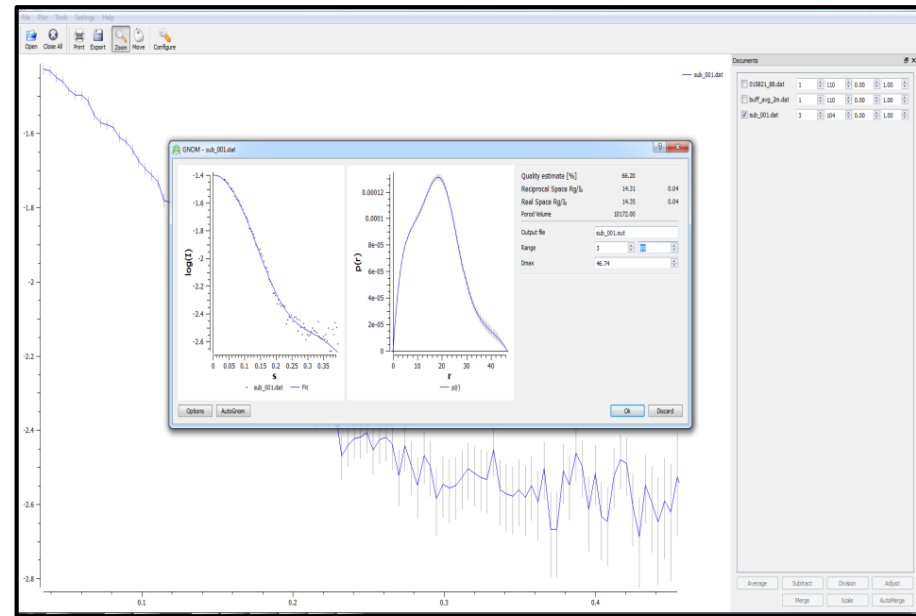
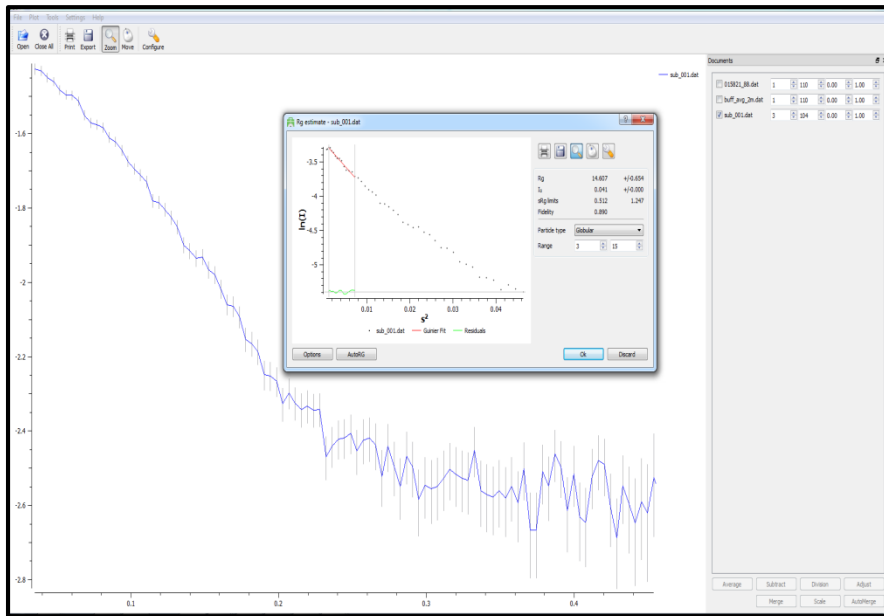


Peak 3



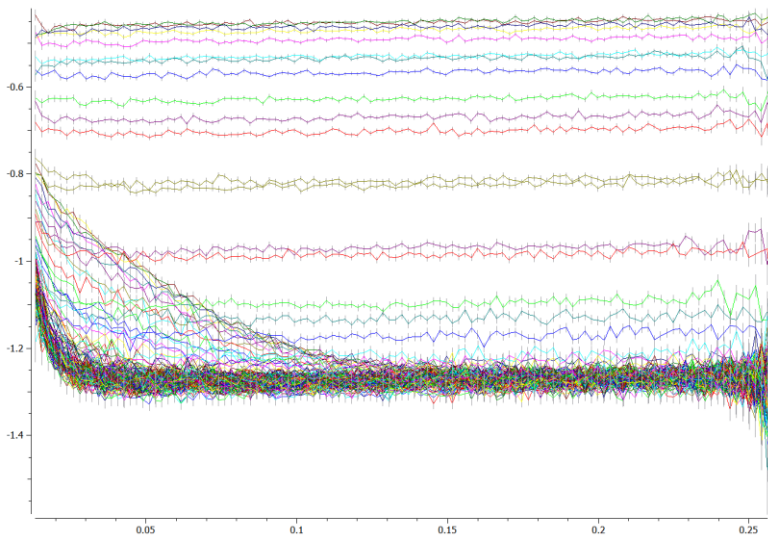
Peak 4



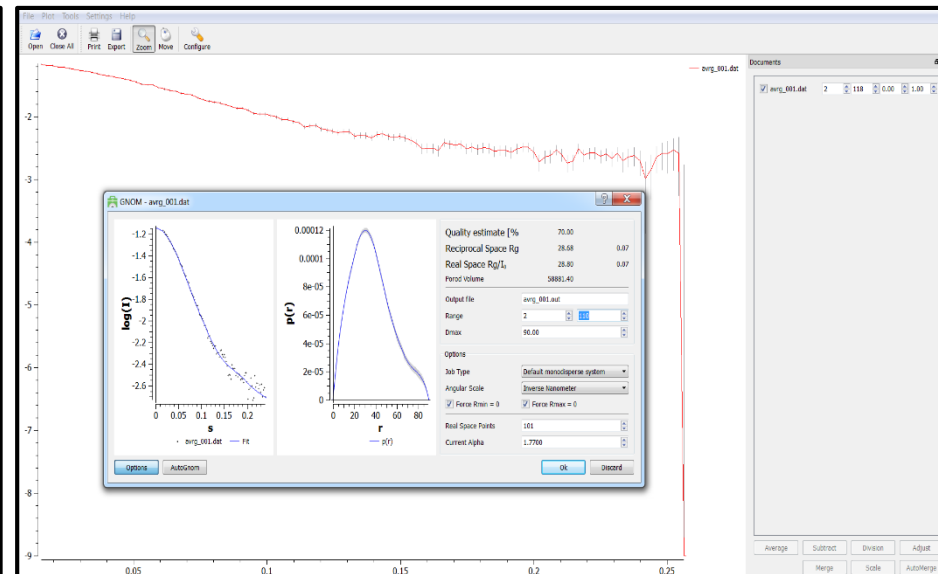
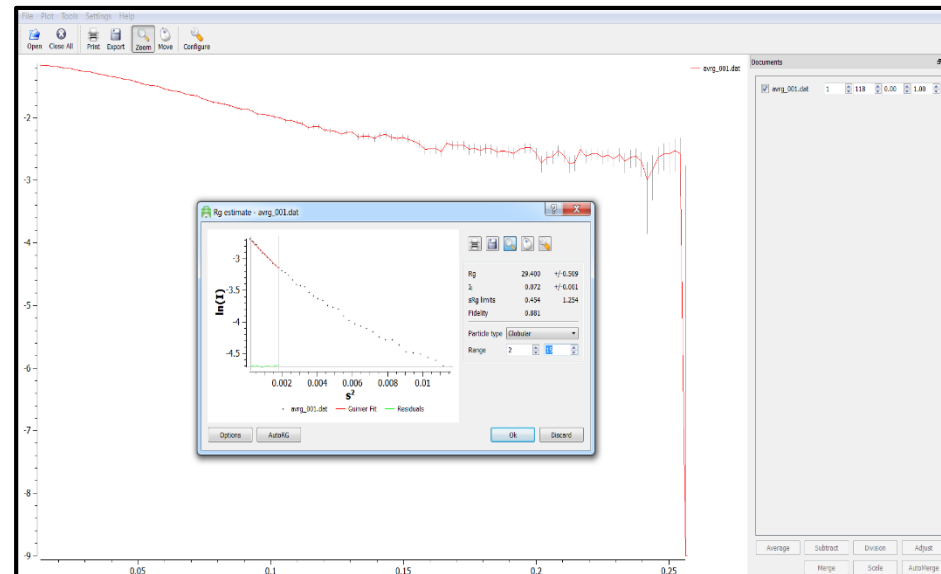
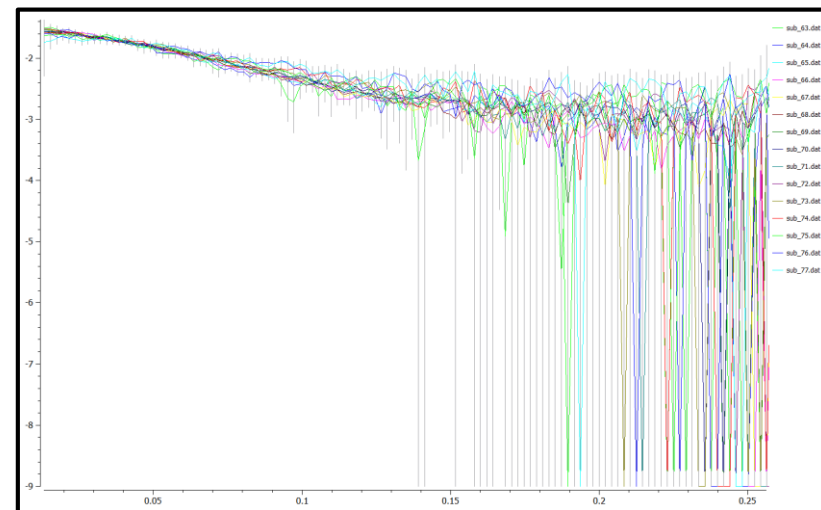


- Can distinguish between protein species
- BUT!
- Carefully optimized – sample concentration, buffer composition, det distance, flow rate etc.
- Recovery of sample for corresponding SAXS measurements for envelope checking.

Sir2a 100 %D Buffer

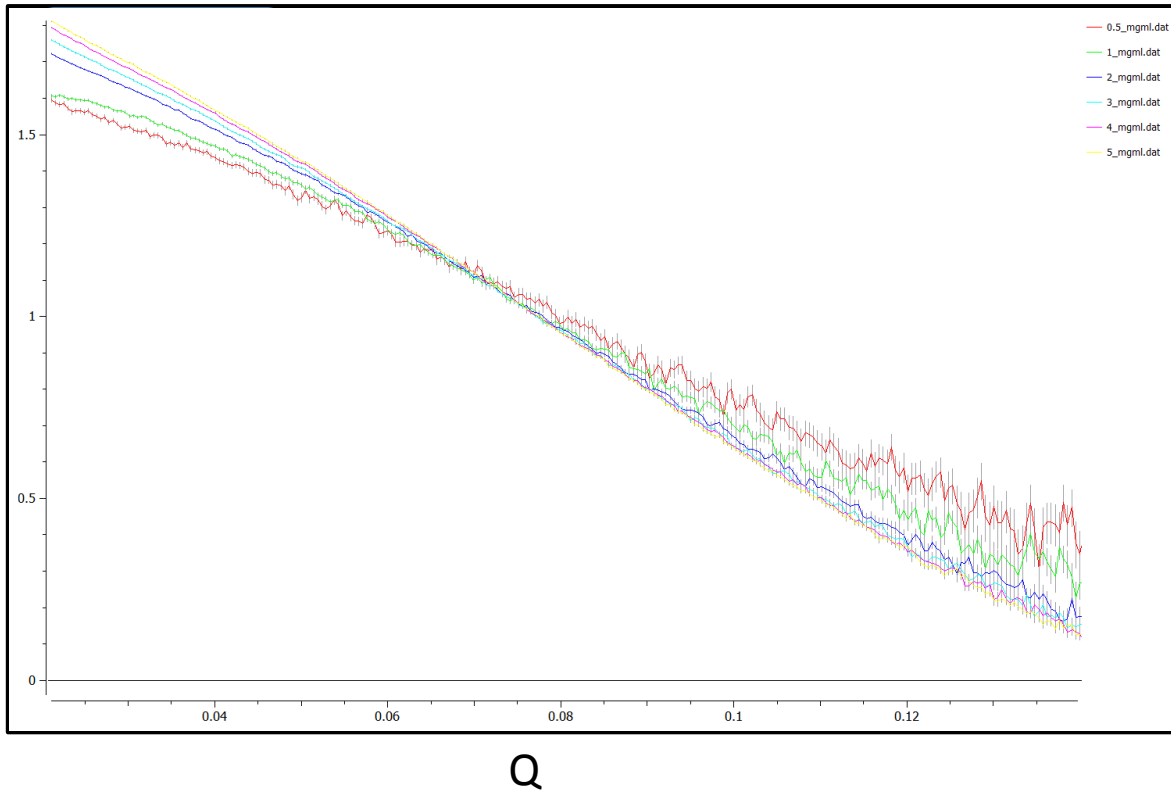
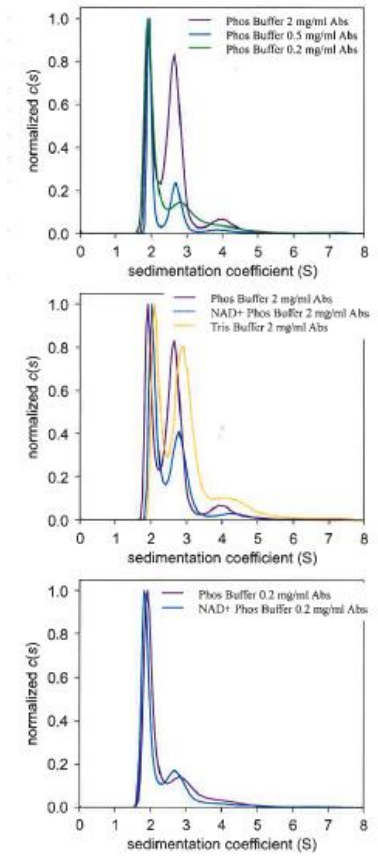


Subtracted



SANS Exp $R_g = 2.9\text{nm} \pm 0.569\text{nm}$

AUC



Concentration effects observed

- Monomer/Dimer populations
- Increase in observed R_g

Summary & Outlook:

IN SAXS/SANS – SAMPLE PREPARATION & CHARACTERISATION IS KEY!!!

- Particularly true for proteins – several techniques needed to characterise properly
- Good samples must be pure, monodisperse, free from concentration effects – Garbage in = Garbage out
- SAXS can be used to study fast aggregating systems and lower concentration systems but is susceptible to radiation damage
- Online SEC systems can help to moderate these effects
- SANS FPLC can be a viable way to study difficult systems which suffer from radiation damage and stability issues
- Still some constraints – suffers from lower flux, requires adequate concentrations to detect protein signal, sufficient contrast between buffer and protein (H₂O/D₂O buffers)

Acknowledgements



Supervisors:

Catherine Merrick
Michael Haertlein
Edward Mitchell
Trevor Forsyth

Special Thanks:

Adam Round
Anne Martel
Barbara Calisto
Martha Brennich



Lab Members:

Florent Bernaudat
Juliette Devos
Orla Dunne
Sam Lenton
Valerie Laux
Martine Moulin
Alycia Yee



Thanks for listening!

