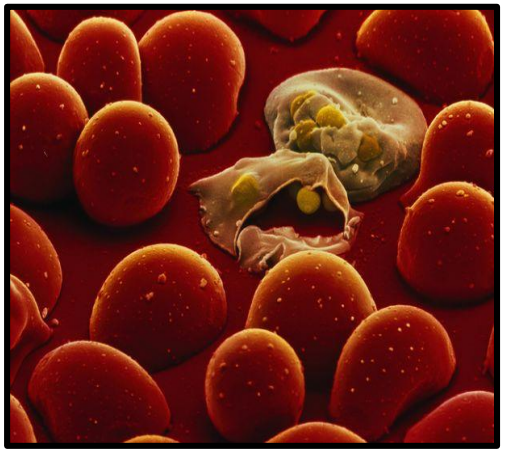
Small angle scattering studies of Chromatin Proteins in the Human Malarial Parasite

Ashley Jordan

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

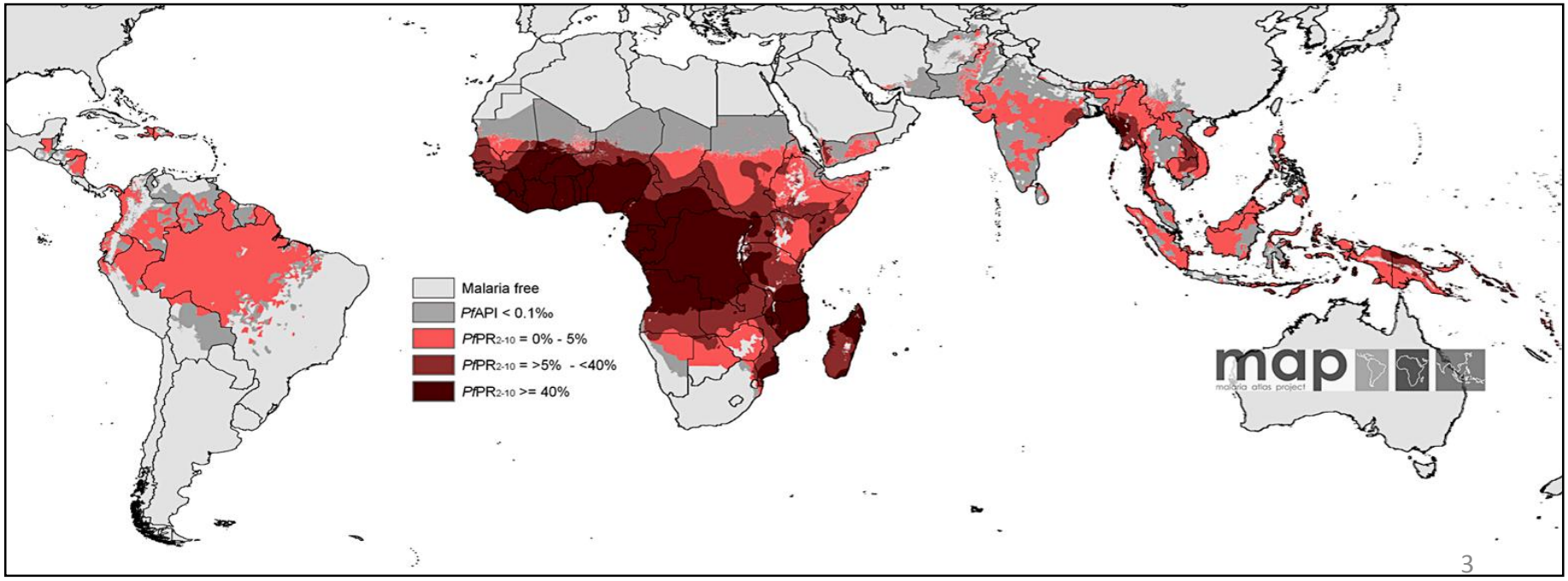


Overview

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

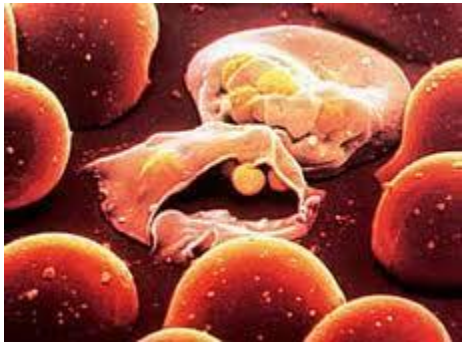
Introduction to Malaria

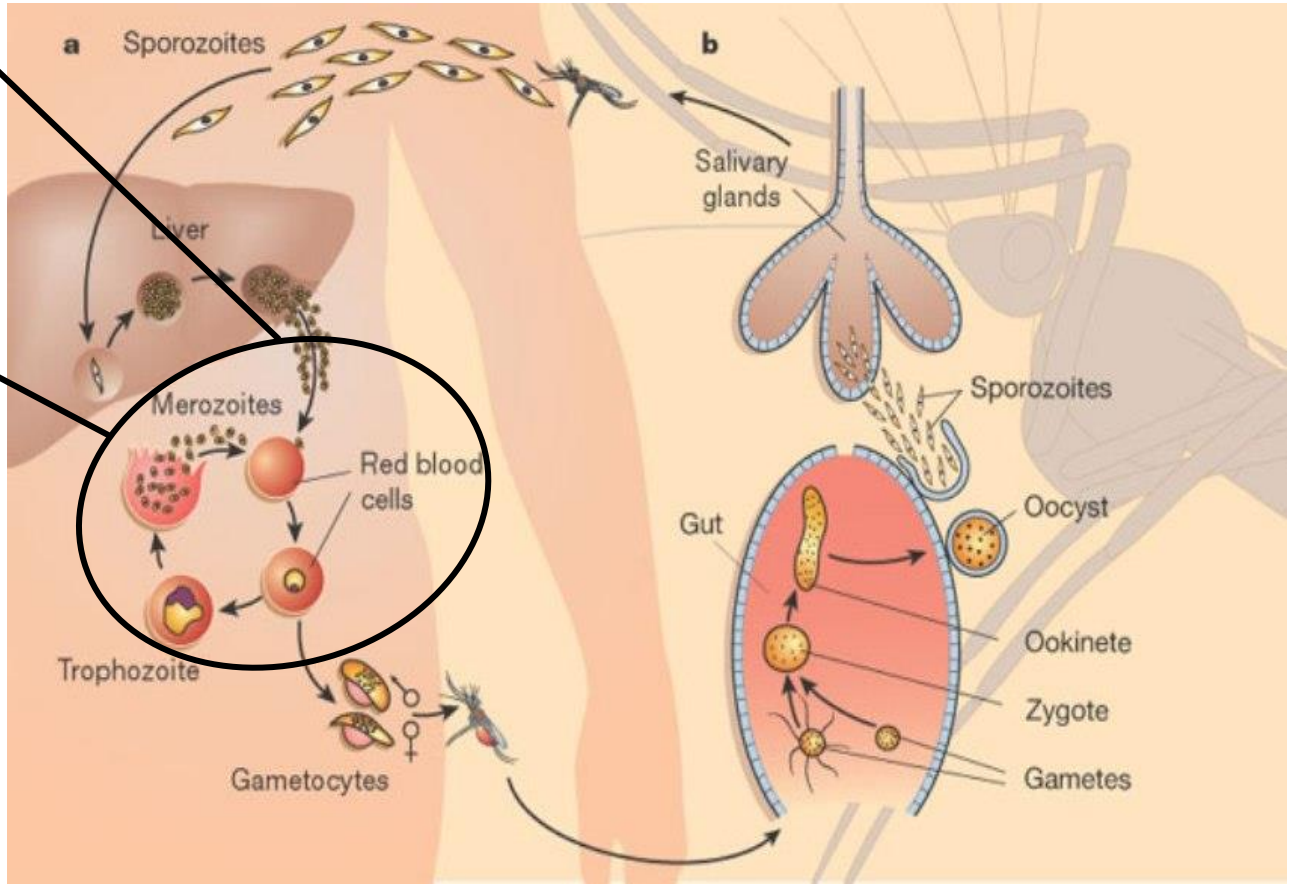
- Infection caused by parasitic organism – *Plasmodium falciparum*
- Approximately 214 million cases of infection with an estimated 438,000 deaths each year (WHO Malaria Report 2014).
- Mortality predominantly occurs in the sub-Saharan Africa region amongst young children and the elderly.



Plasmodium falciparum **lifecycle**

- 2 hosts: mosquitoes and humans.



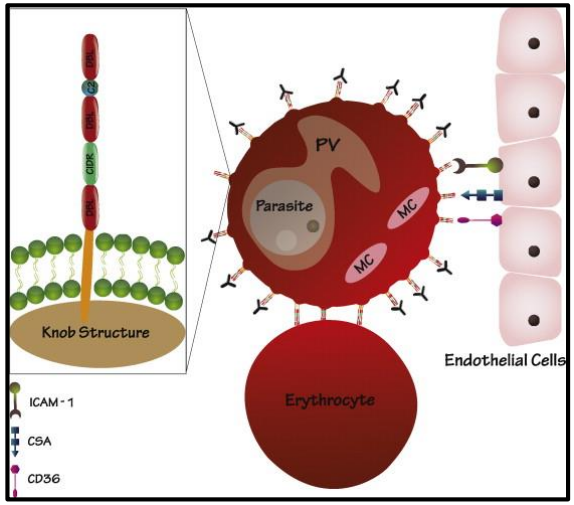


Nature.com 433, 113-114 (2005)

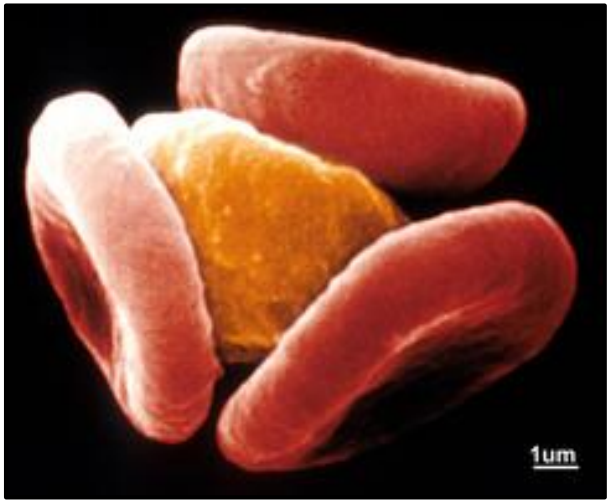
- Growth in red blood cells causes all the symptoms of malaria.

Introduction to Malaria

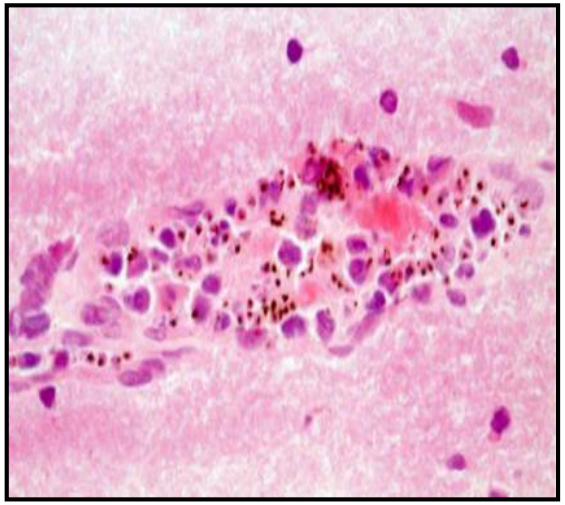
PfEMP-1



Infected RBC's

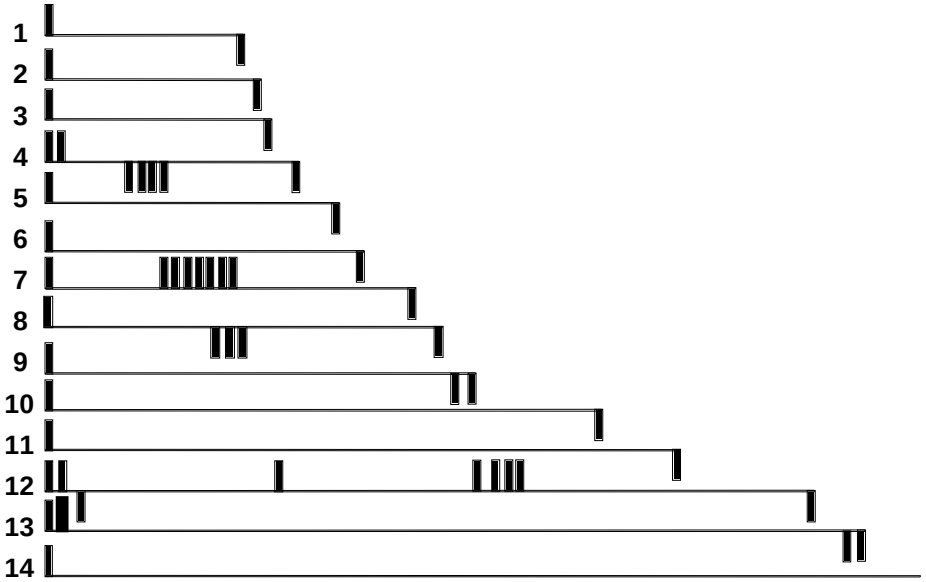


Sequestration

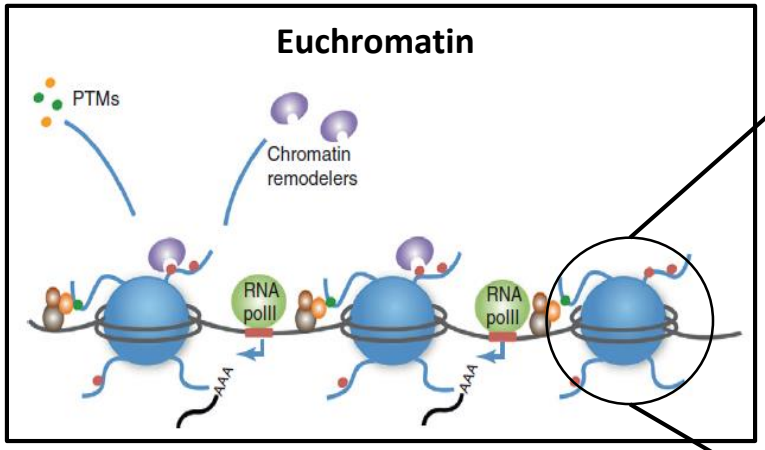


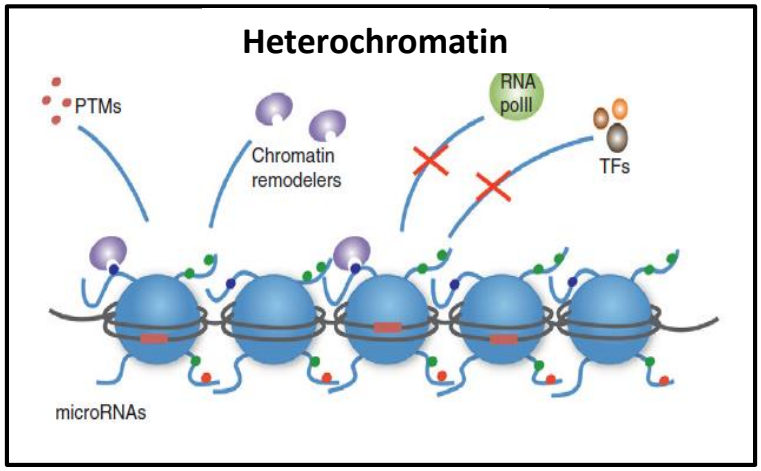
Virulence Genes & Antigenic Variation

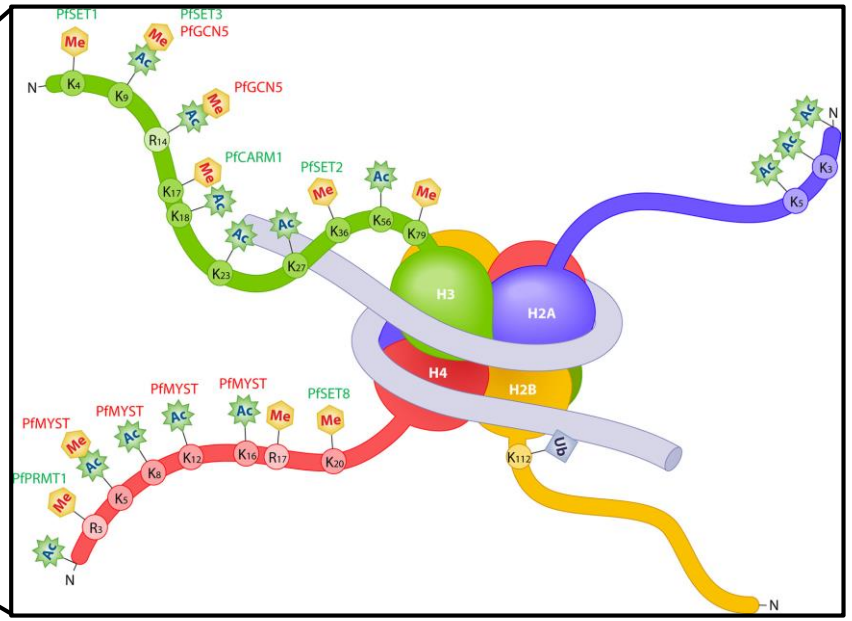
- PfEMP-1 variants encoded by a large multi-gene family – '*var*' genes.
- Over 60 variants present within *P. falciparum* genome.
- Able to switch between different variant forms of PfEMP-1 to avoid host immune response.
- Only 1 active *var* gene at any given time.
- **How is it regulated?** **- Epigenetics**



Epigenetics & Chromatin Remodelling

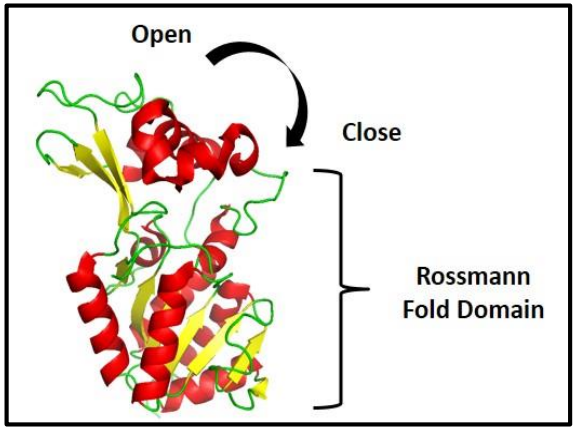






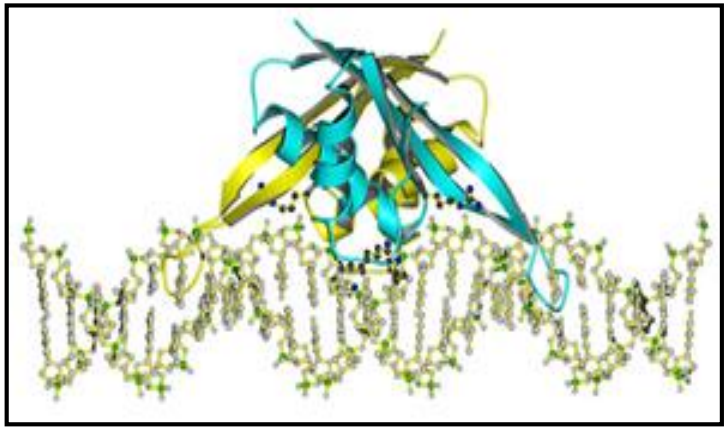
Proteins of Interest

PfSir2a
33.8kDa

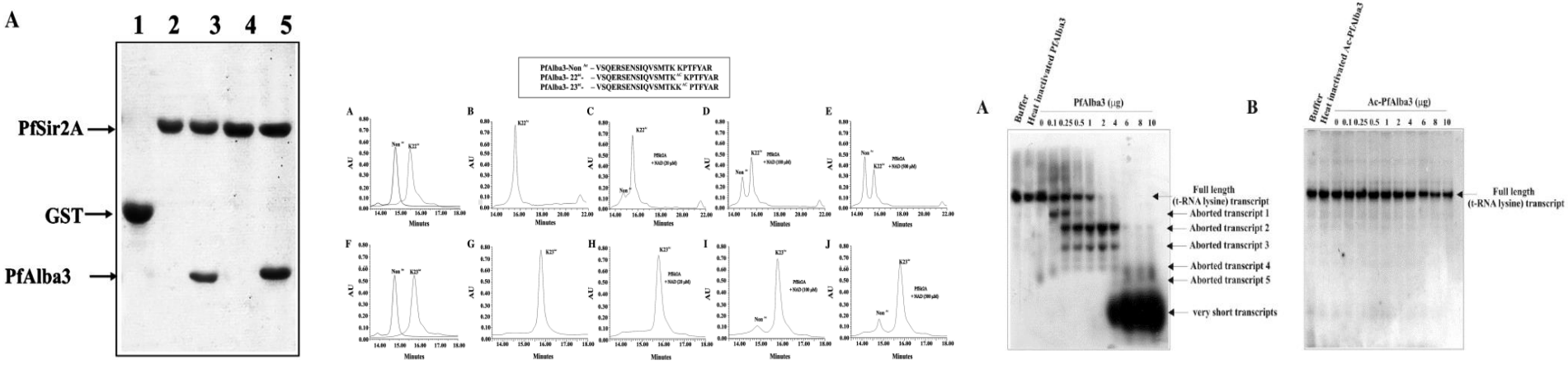


PDB ID: 3JWP

Alba3
13.04kDa

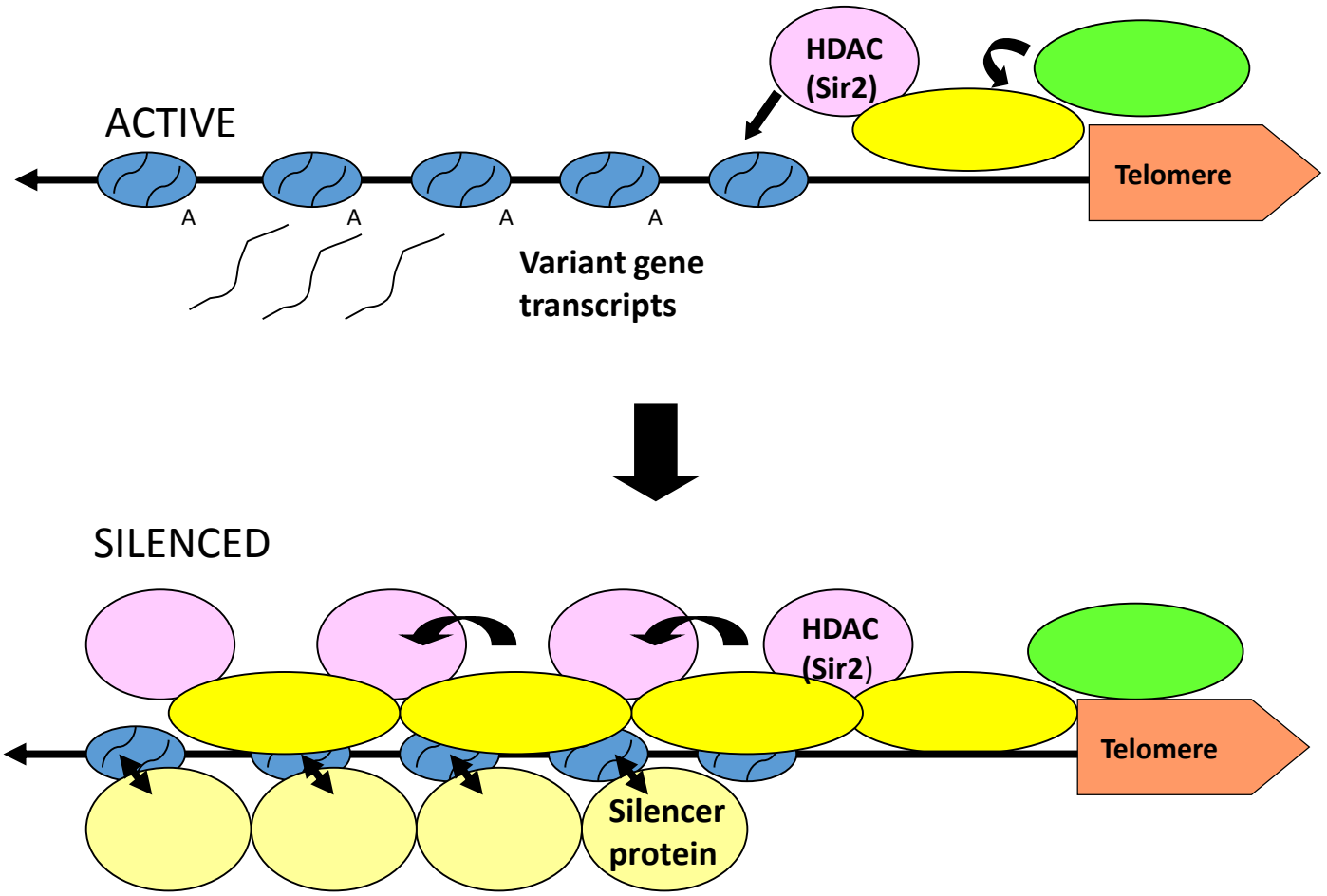


Wardleworth *et al* 2002 EMBO Journal

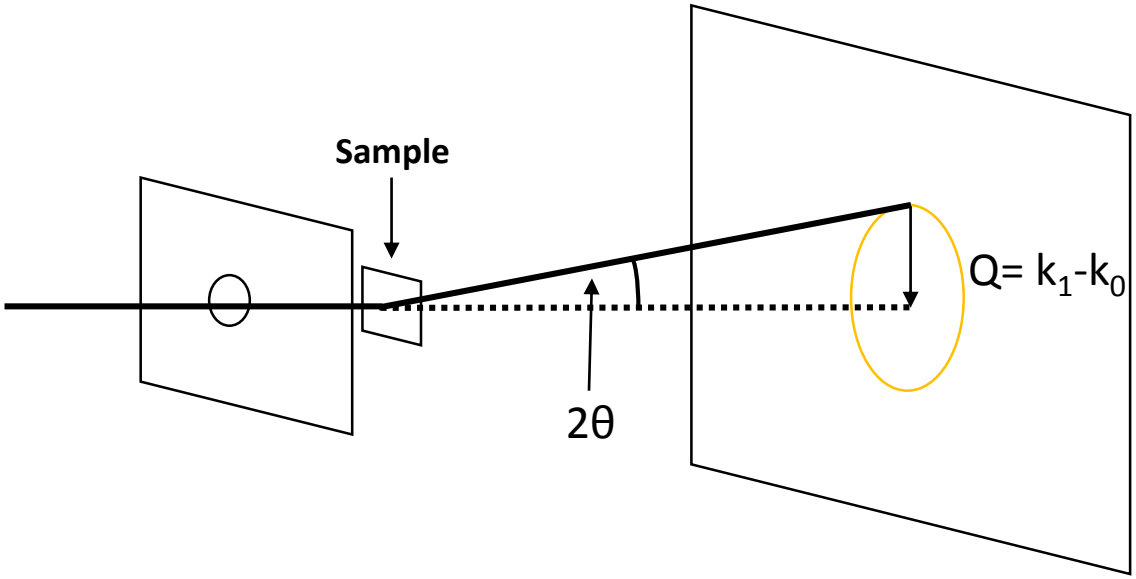


Goyal *et al* 2011 Nucleic Acids Research.

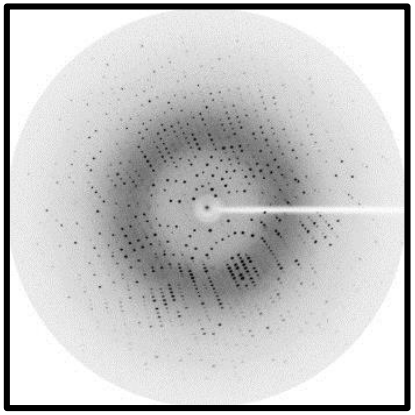
Proposed Silencing Mechanism



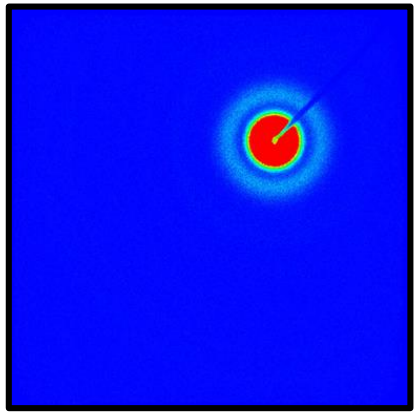
Small Angle Scattering - Overview



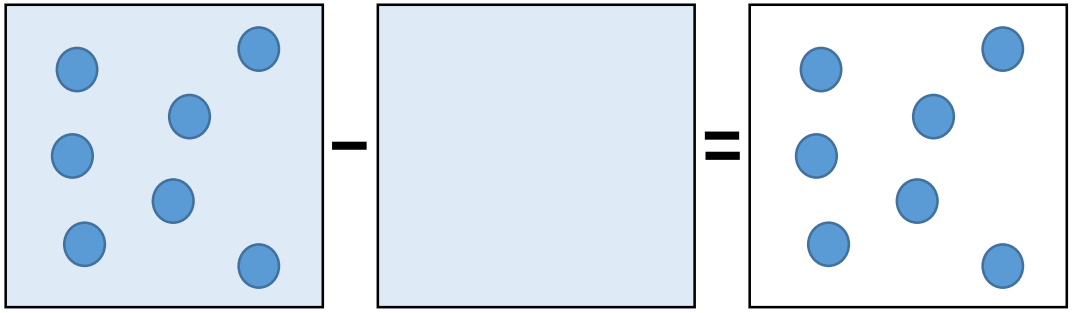
Crystallography



SAXS/SANS



Solution = Solvent + Particle



Don't Forget!

Empty beam
Blocked Beam
Empty Cell

S(Q)

P(Q)

I(Q)

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

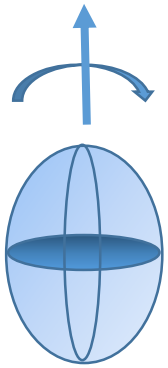
Small angle scattering:

Radius of gyration: Rg

Guinier's Law:

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

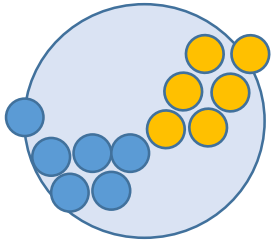
$$0 < q < 1/R_g$$



Globular Protein

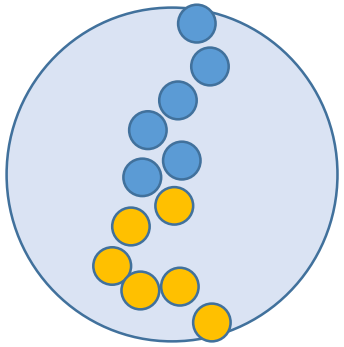
3nm

Oligomeric state



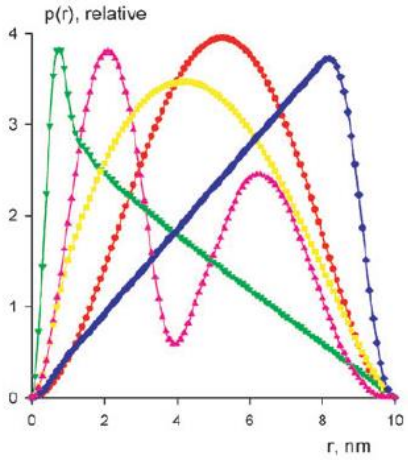
5nm

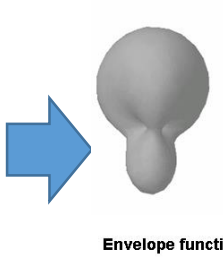
Extended/IDP



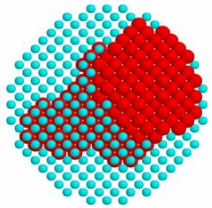
10nm

Pair-Distance Distribution Function: P(r)

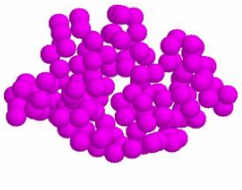




Envelope function



Bead models

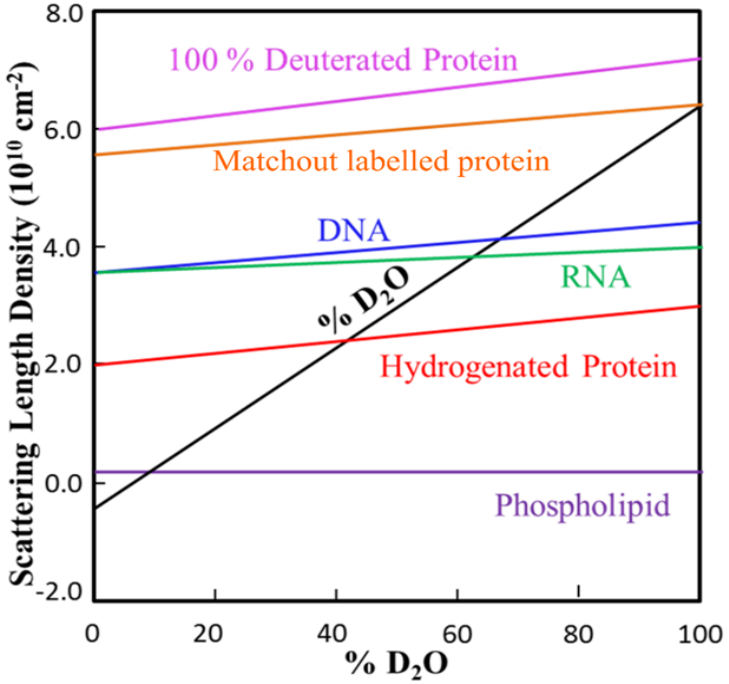


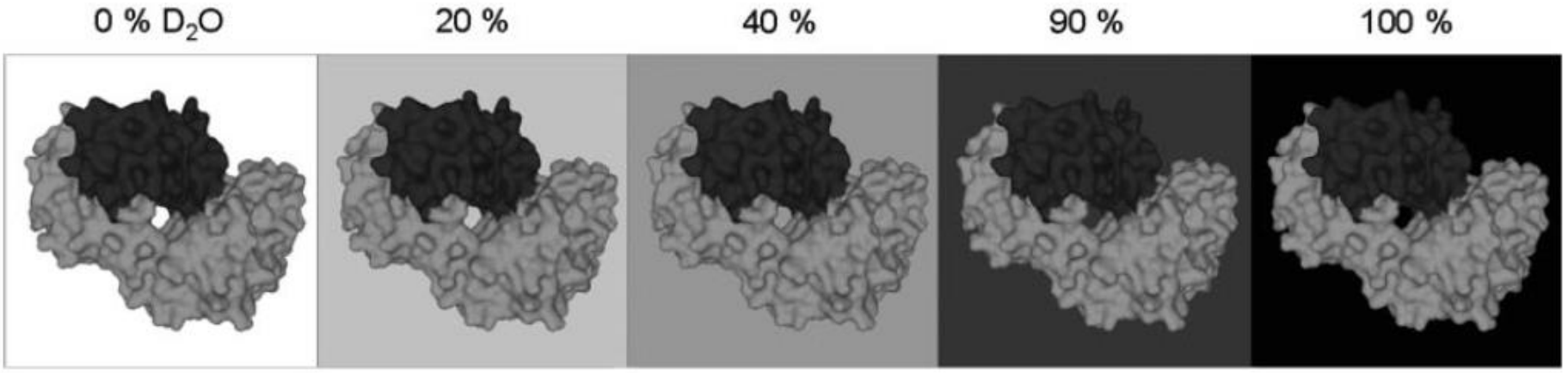
Dummy residues model

Small Angle Scattering Studies

Project Goals

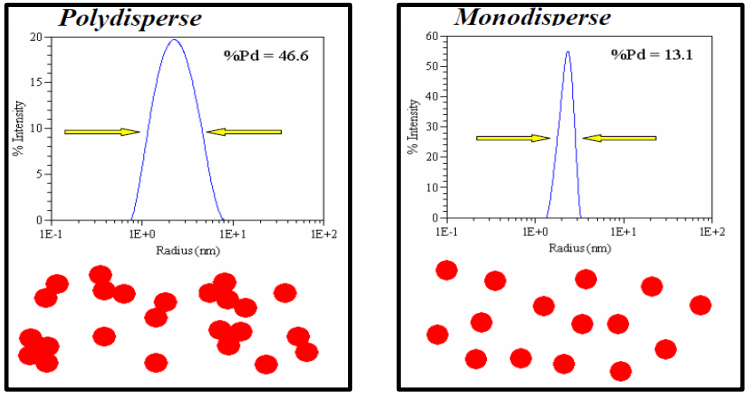
- Produce recombinant proteins for structural characterisation
- Produce deuterium labelled variants
- Perform contrast ‘match-out’ experiments to investigate protein-protein interactions.



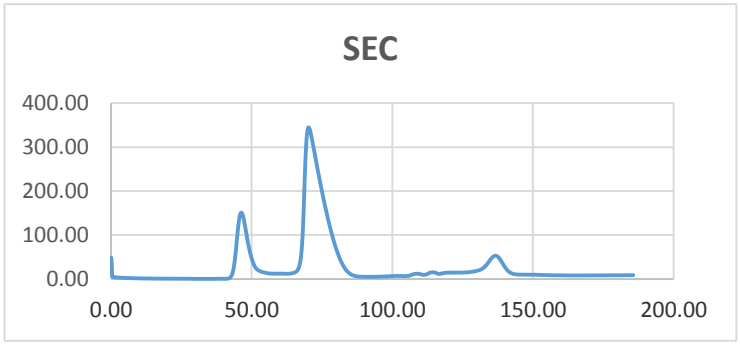


Small angle scattering theory requires:

- Monodisperse sample

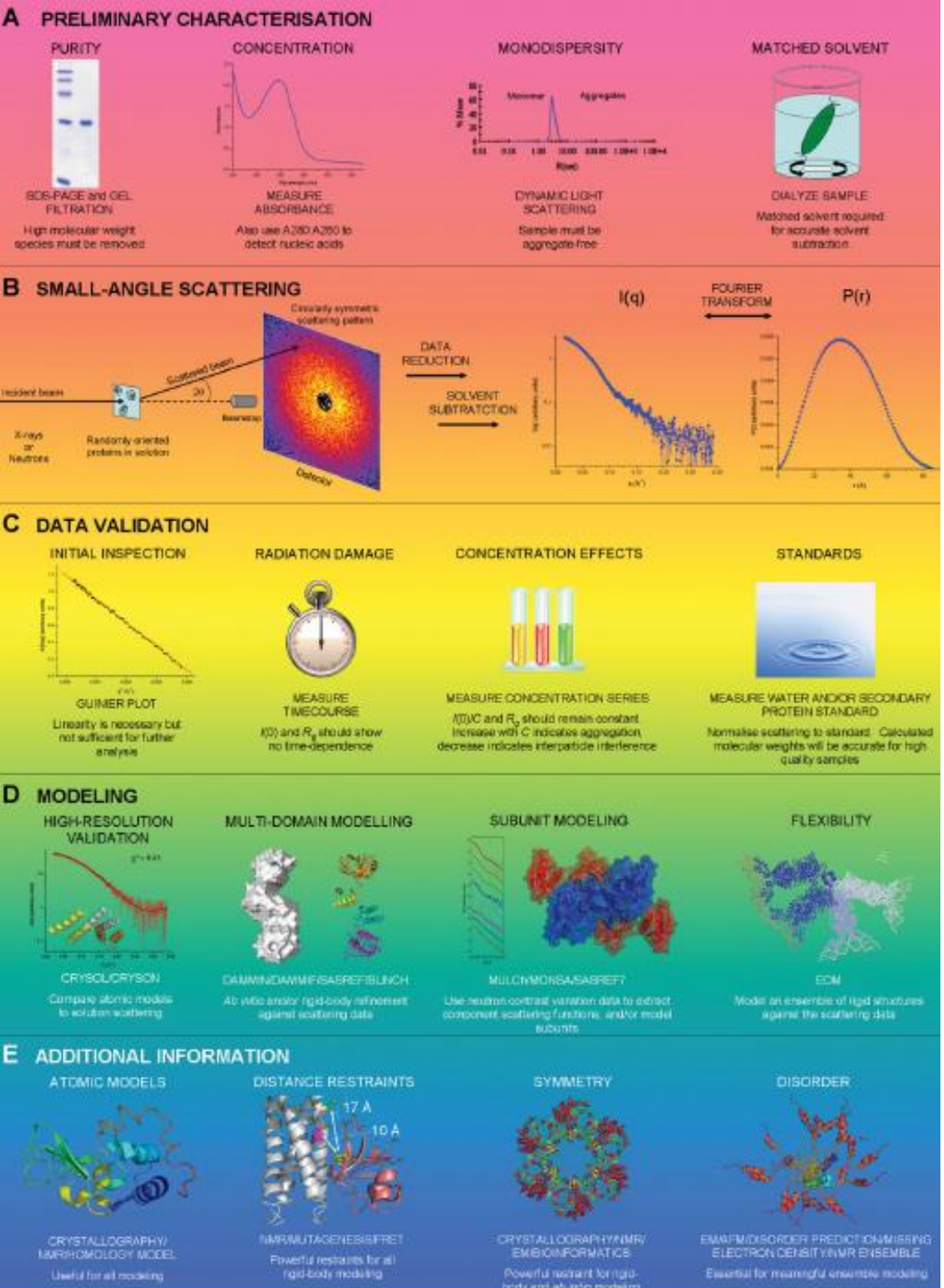


- Free from aggregates



- Particle to have sufficient contrast from solvent
  Concentrated solutions 1-10mgml

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



95%

4%

lim
$t \to \infty$

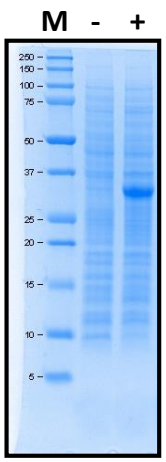
Recombinant Protein Production & Characterisation

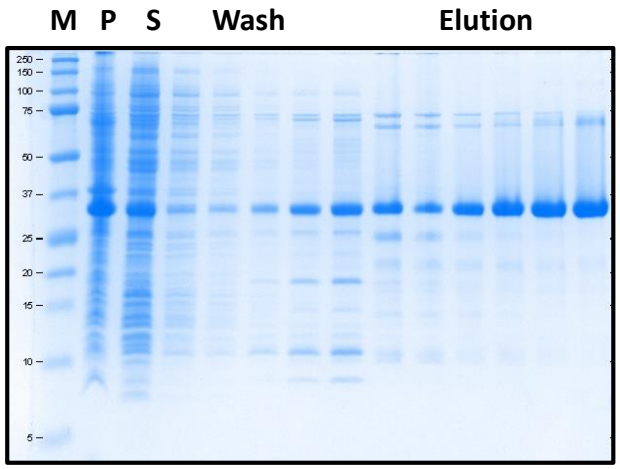
Expression
E. coli

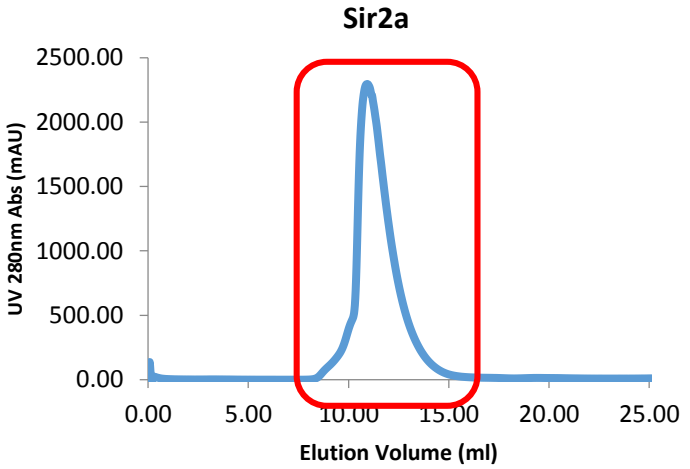
Purification
IMAC/IEX
Ni-NTA/Cation exchange chromatography

Final Steps
Size exclusion chromatography

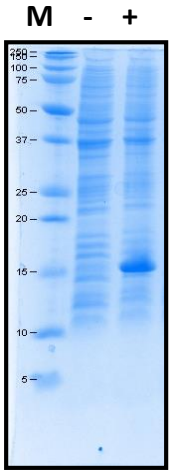
Sir2a: 33.8 kDa

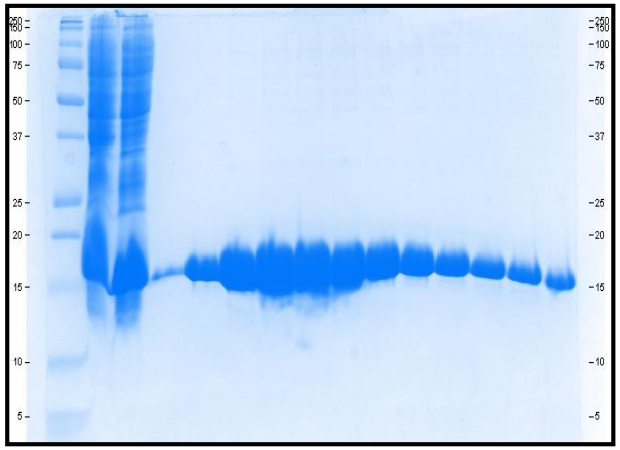


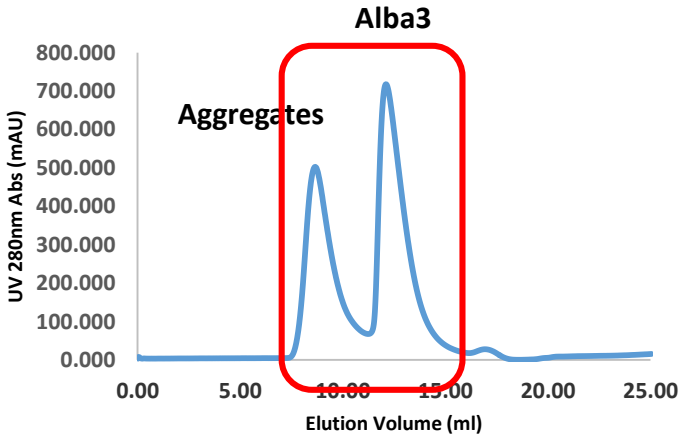




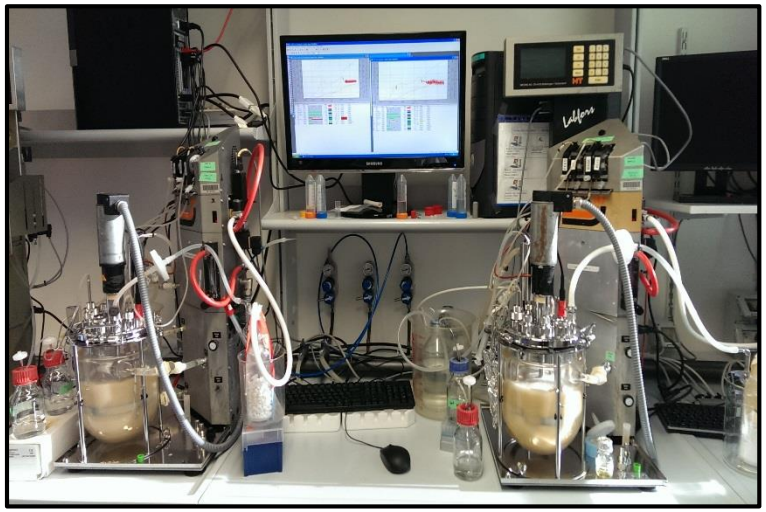
Alba3: 13.04 kDa

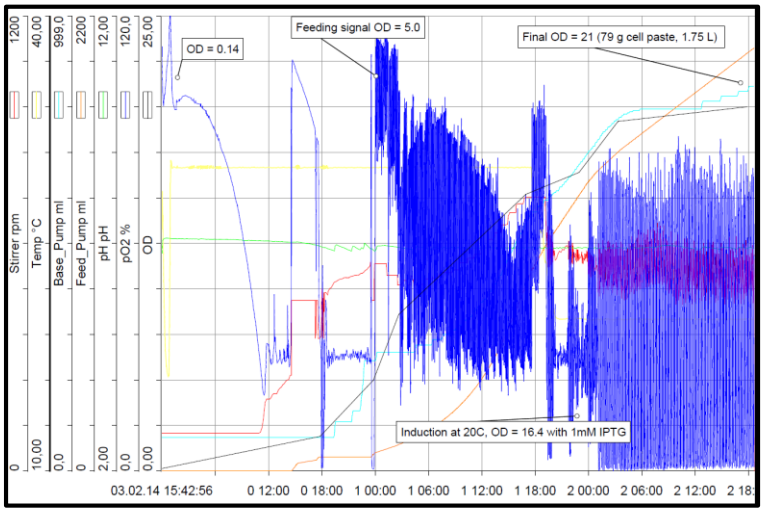


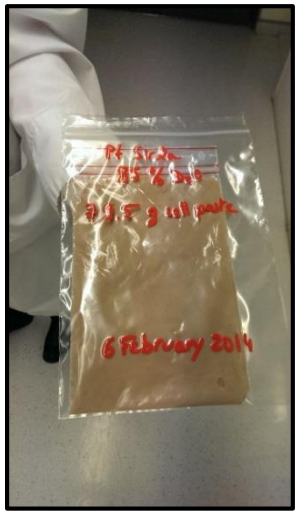




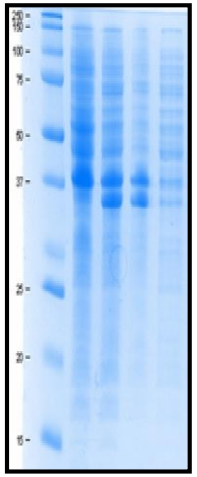
Deuterated Protein Production



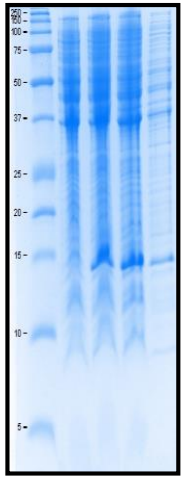




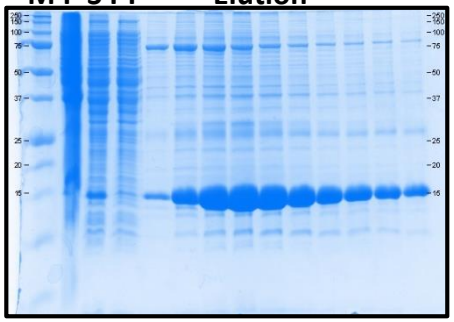
Sir2a



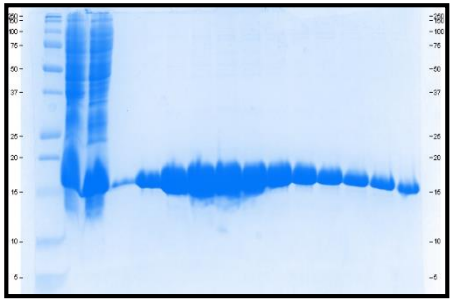
Alba3



M P S FT Elution

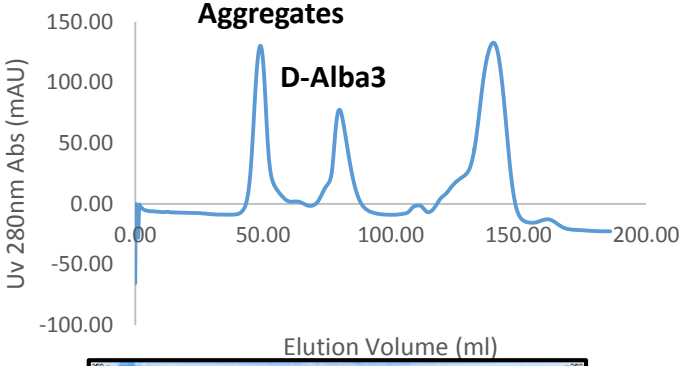


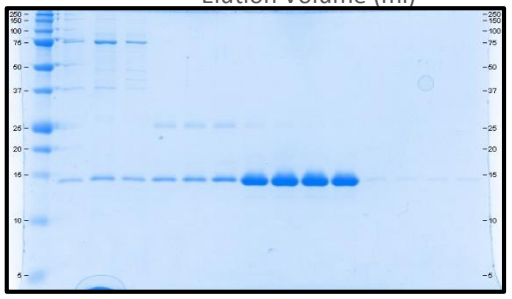
M P S Elution

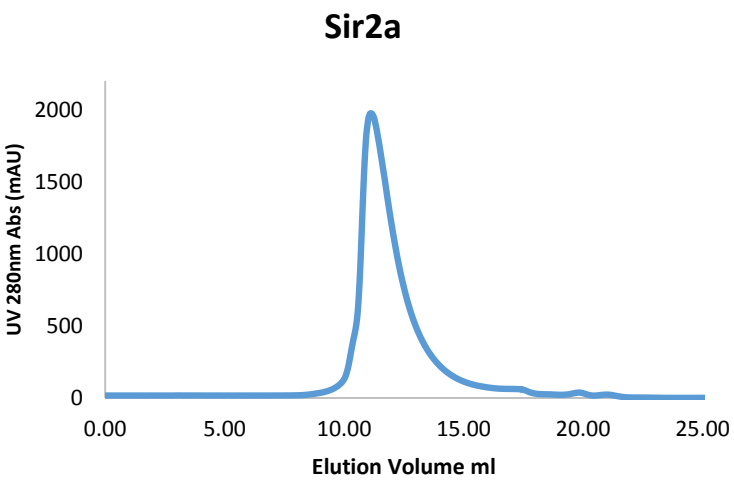


Aggregates

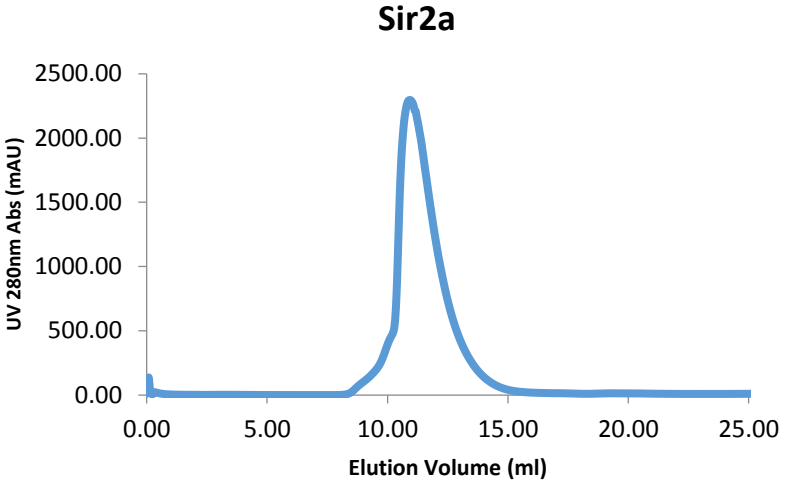
D-Alba3



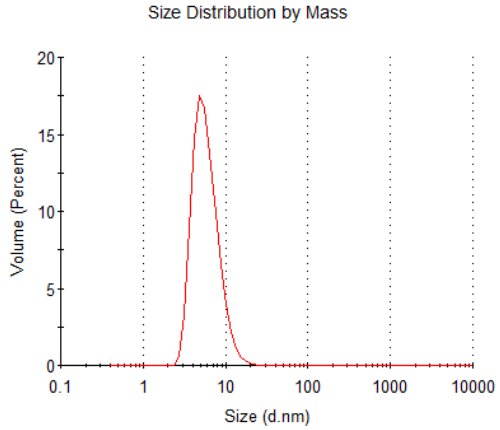




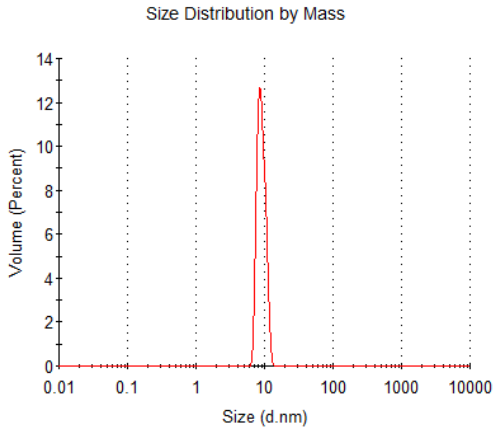
50mM HEPES pH 7.5
50mM NaCl



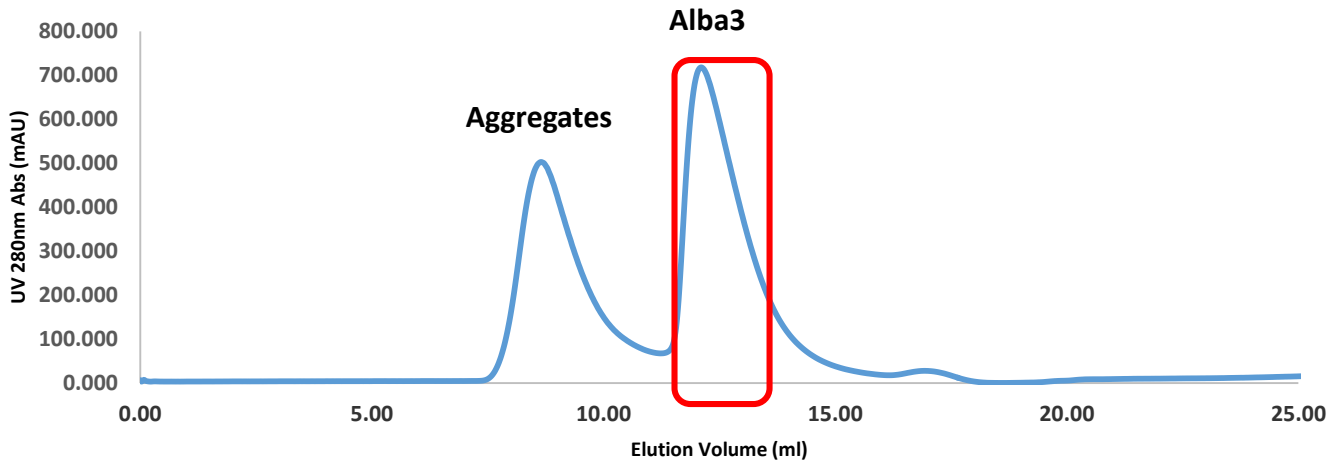
50mM Naphos pH 7.0
500mM NaCl



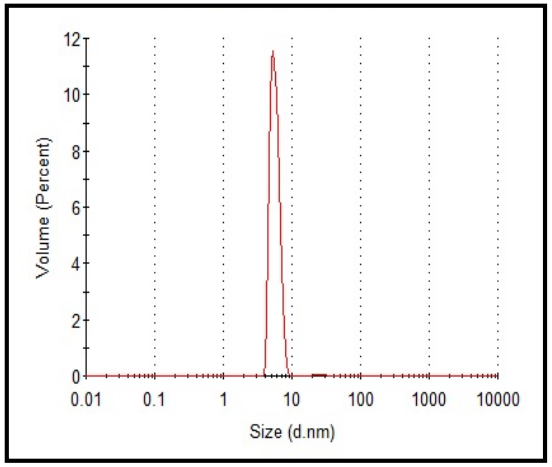
PD = 44%



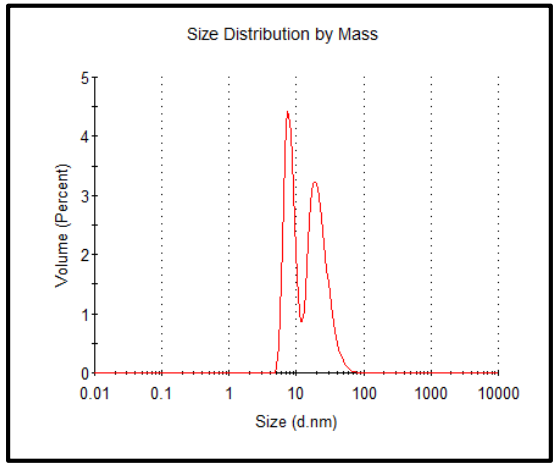
PD = 13.4%



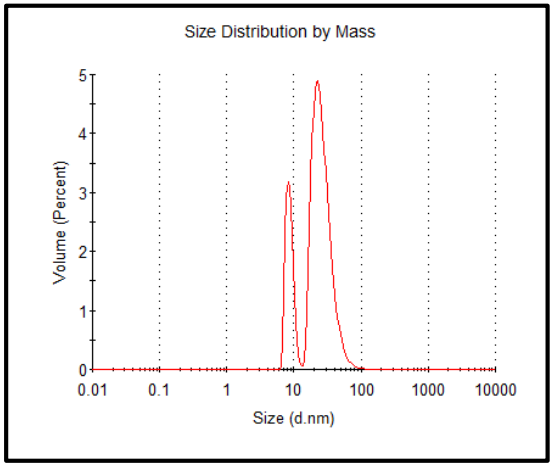
5min



15min

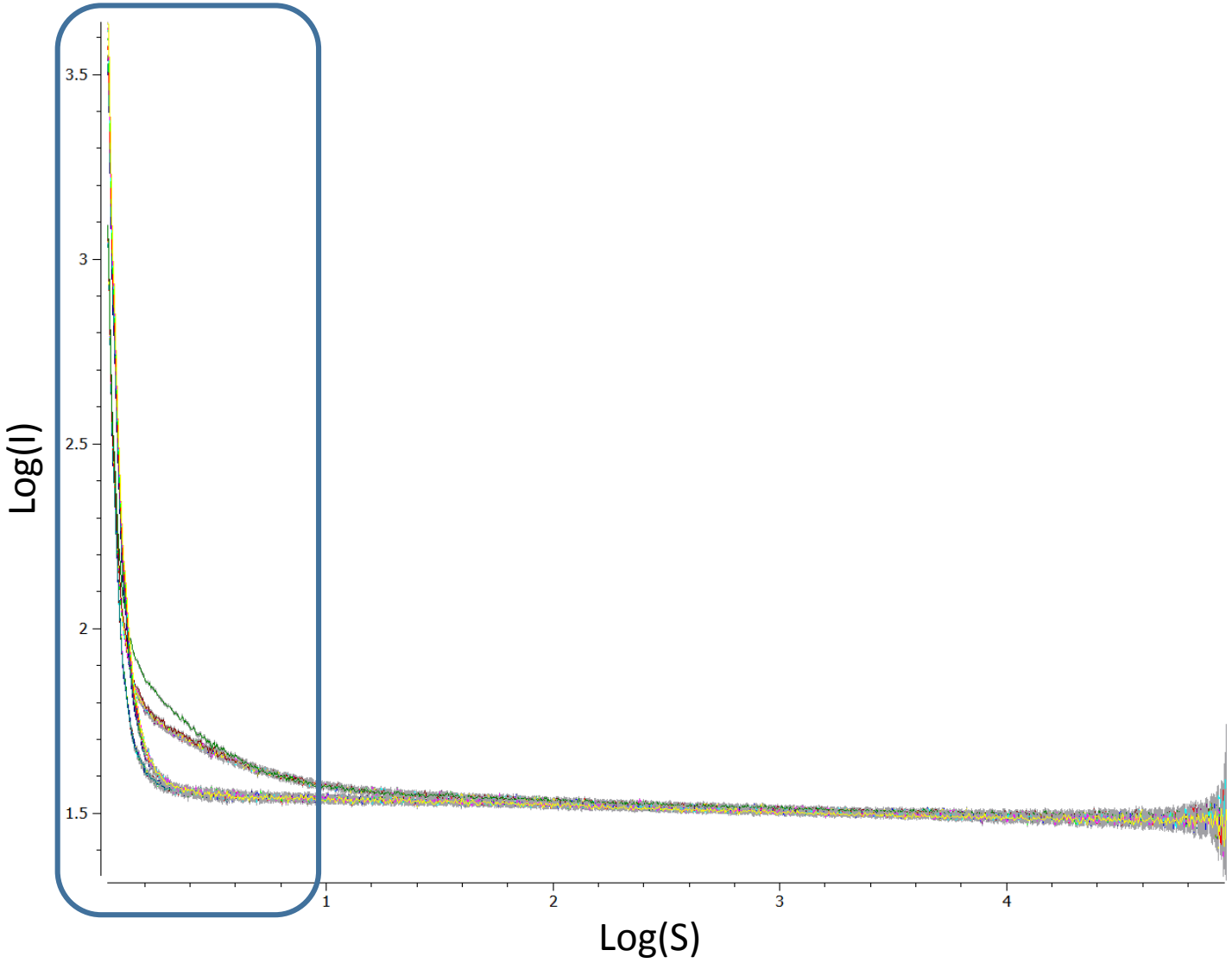


30min



%PD

SAXS Studies

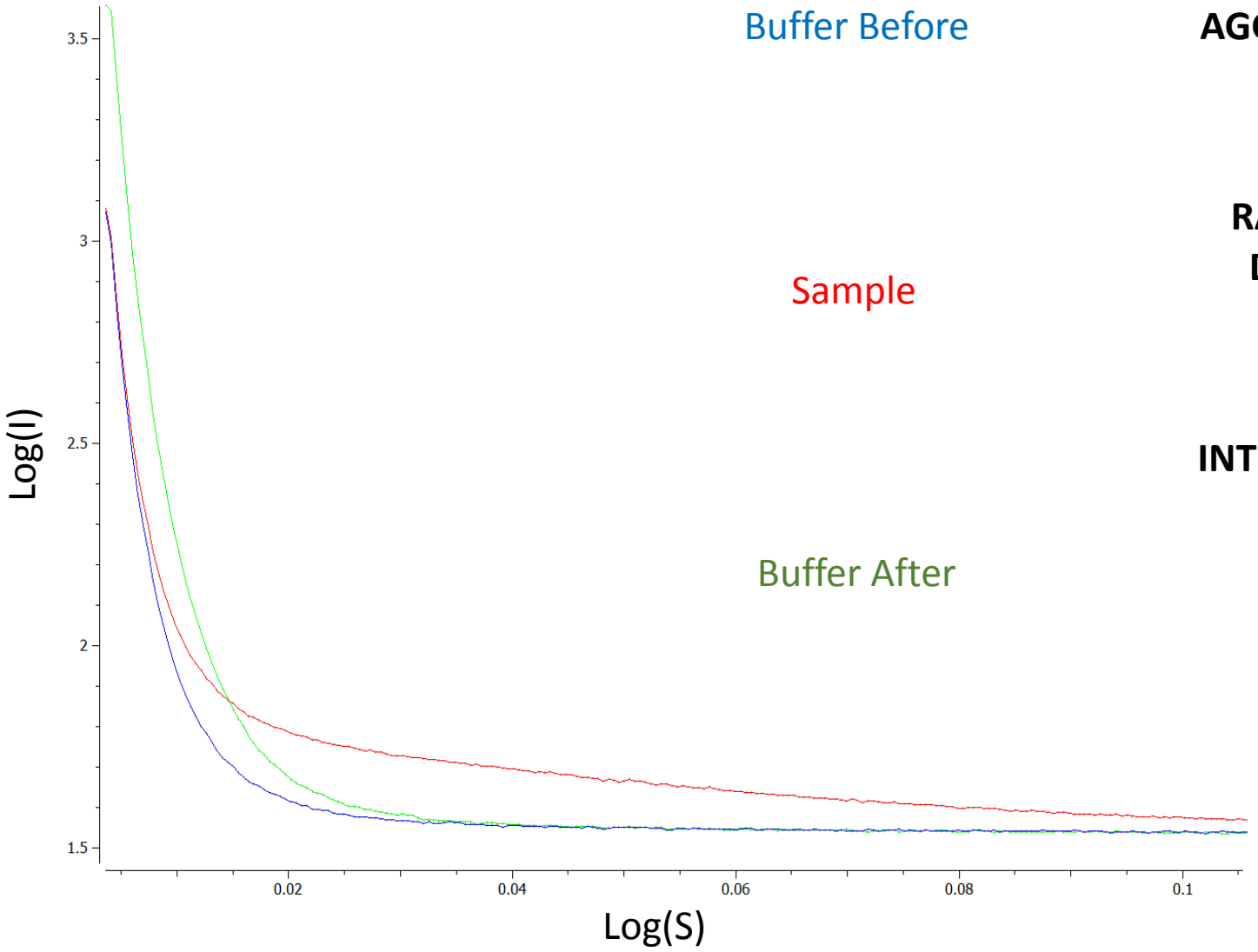


Buffer Before

Sample

Buffer After

SAXS Studies



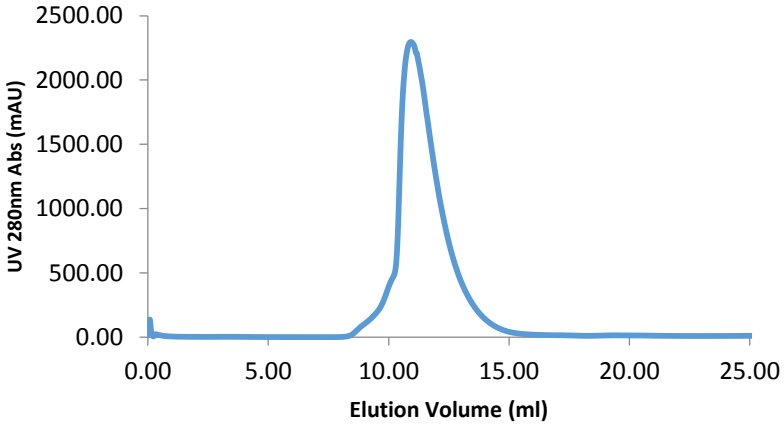
AGGREGATION

RADIATION DAMAGE

INTERPARTICLE EFFECTS

SAXS Studies – Online HPLC

Sir2a



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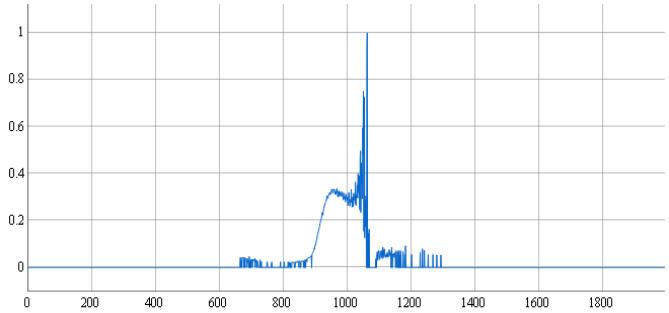
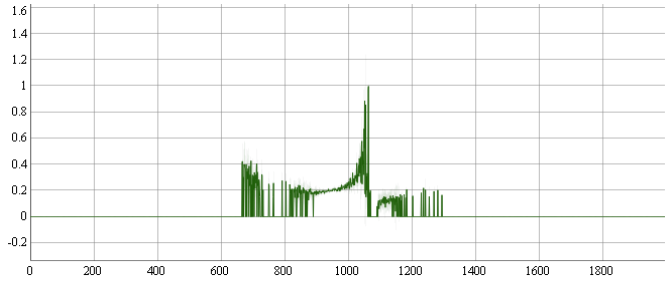
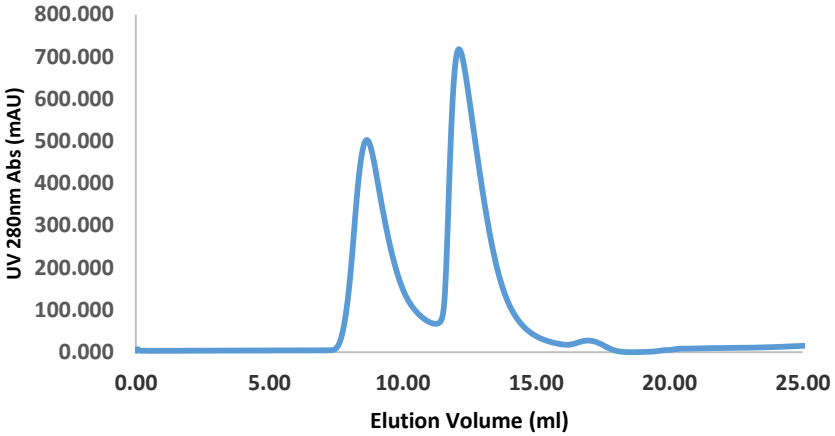


Rg



Alba3



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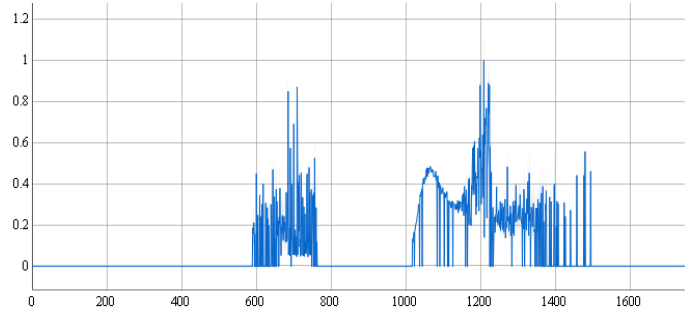
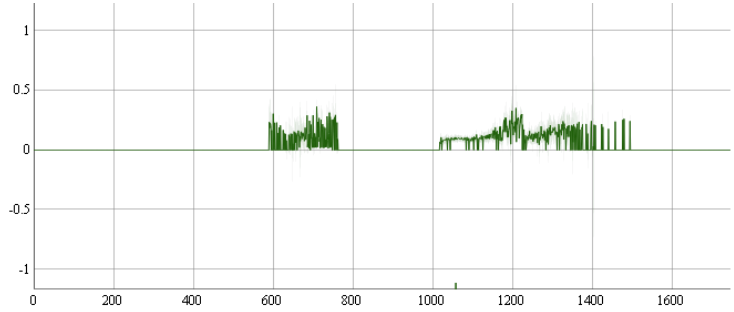
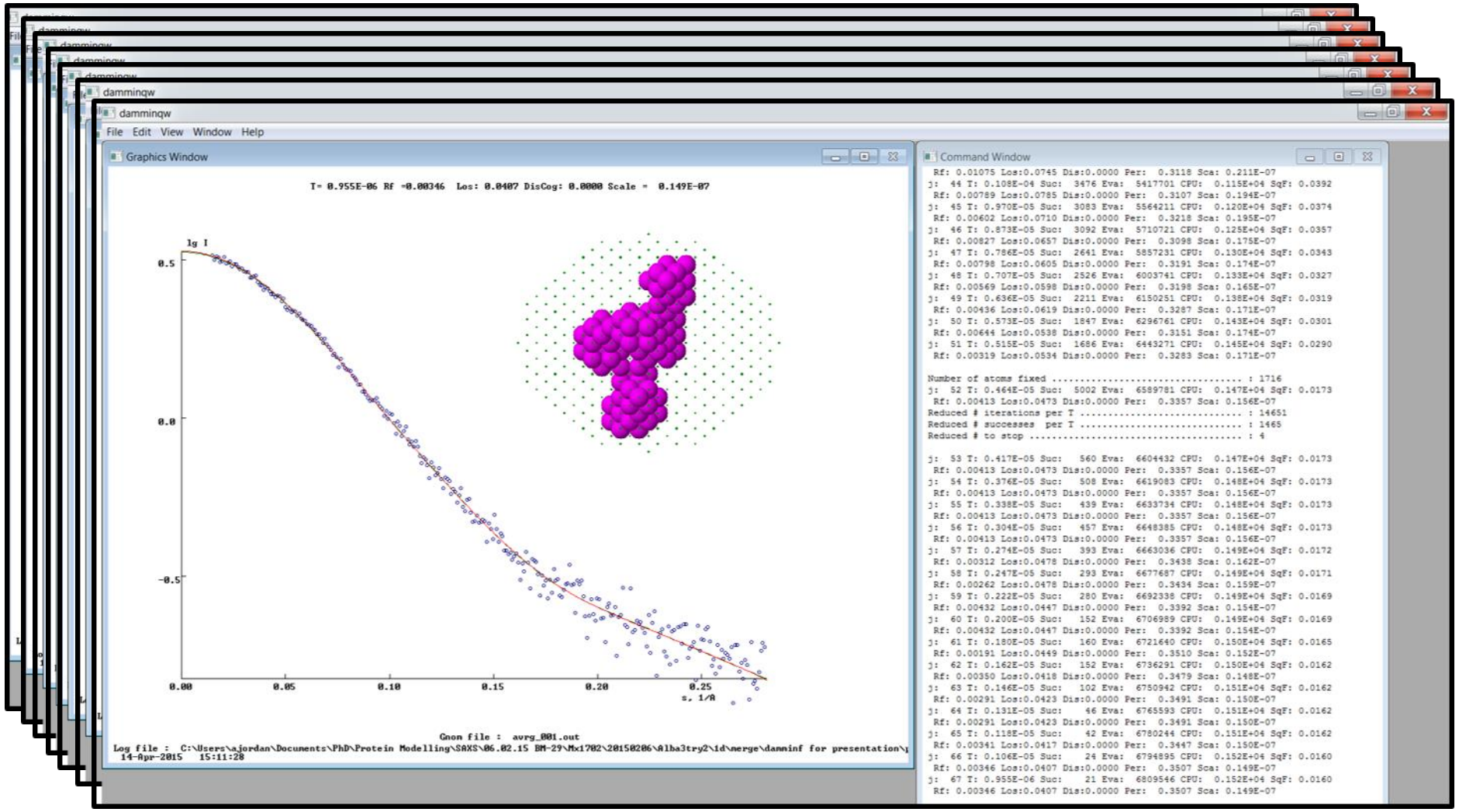


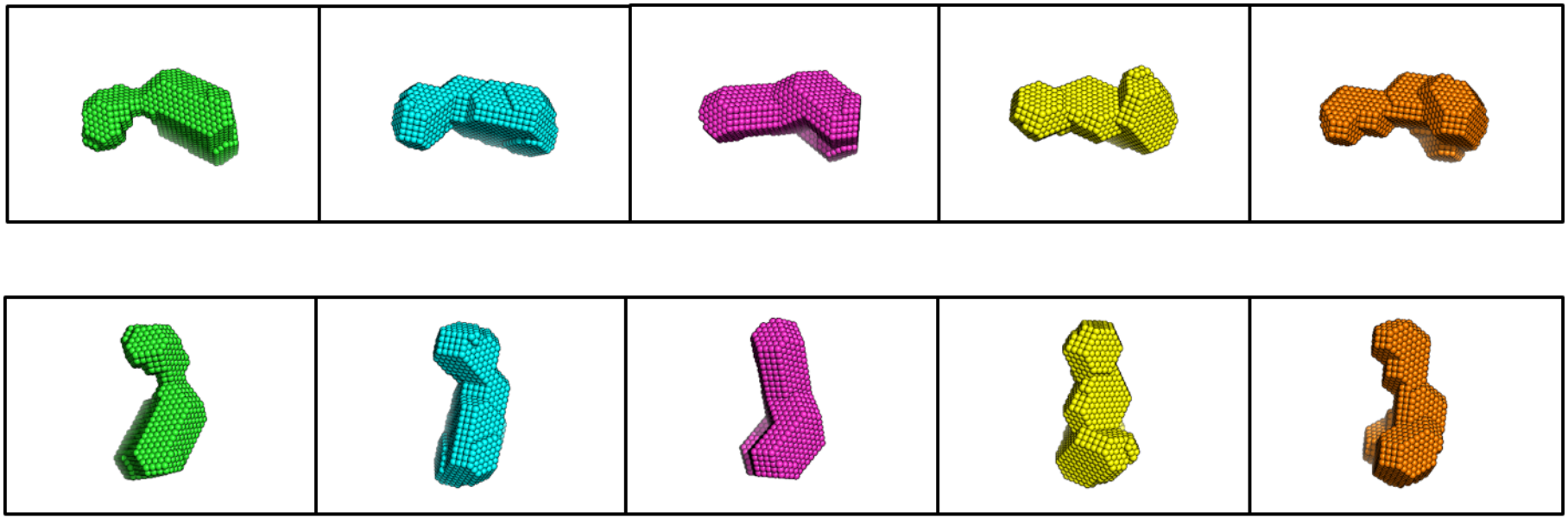
Rg



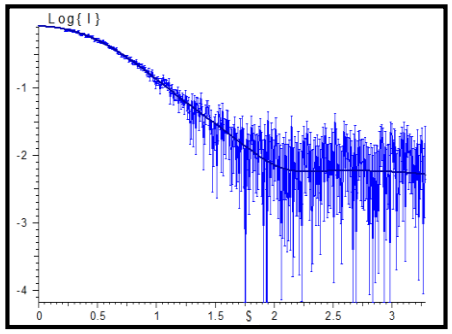
Ab initio modelling

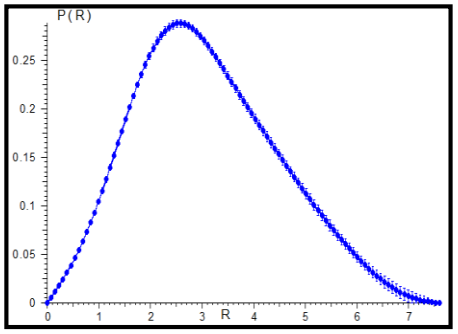


Ab initio modelling



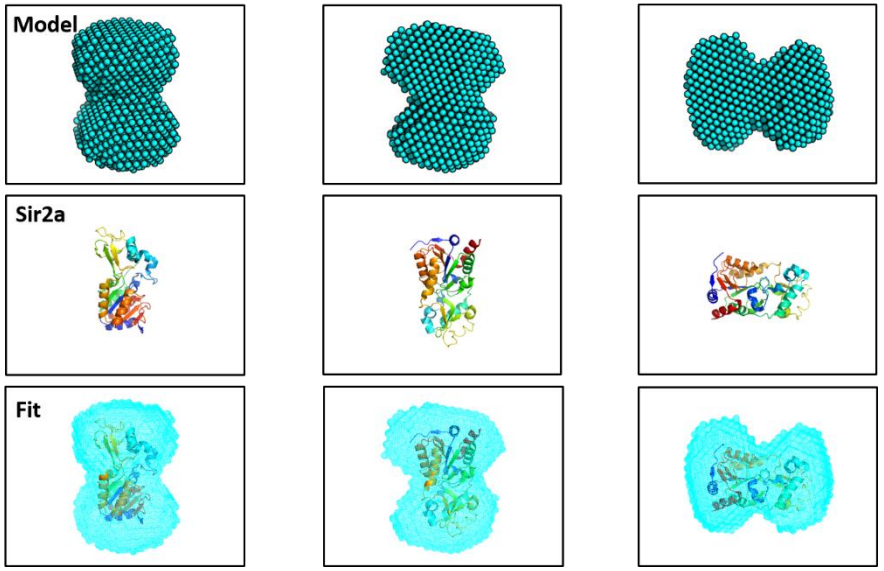
SAXS Studies – Online HPLC

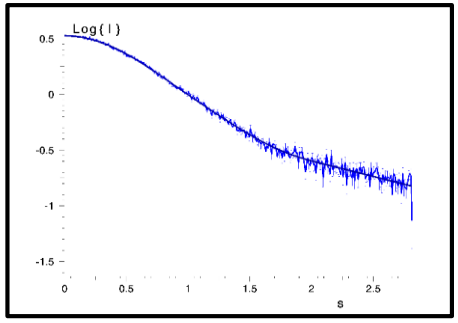


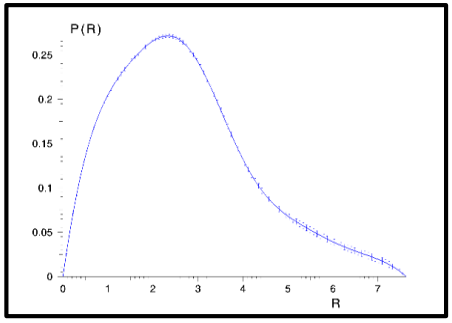


Exp Rg = 2.40 +/- 0.027nm

Calc Rg = 2.1nm

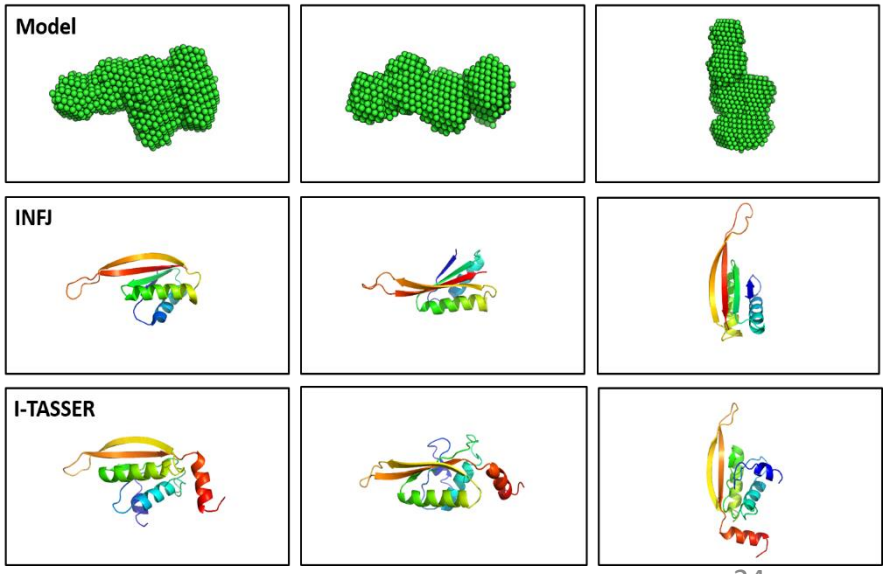




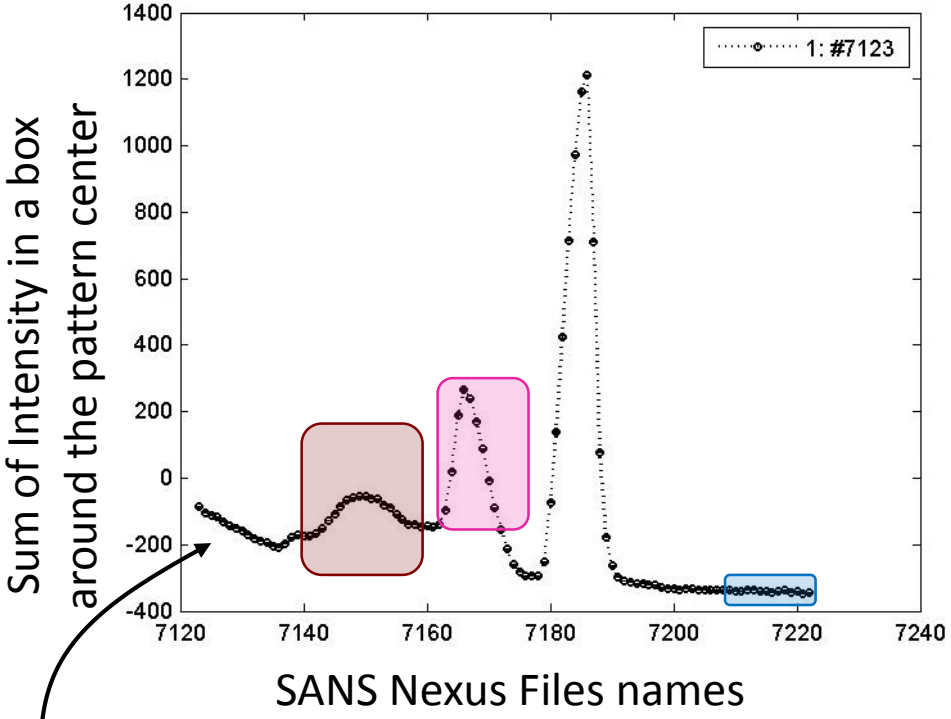


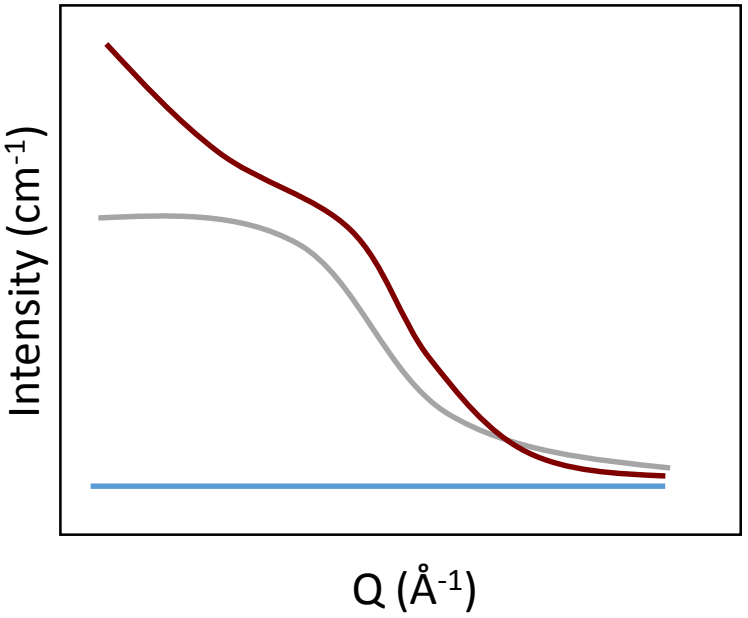
Exp Rg 2.19nm +/- 0.018nm

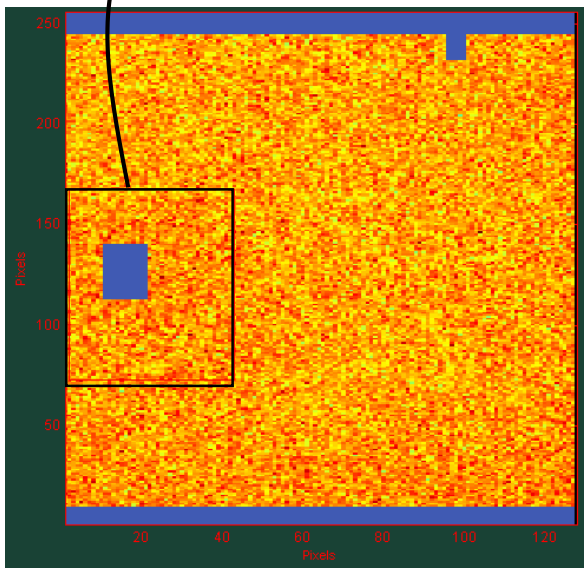
Calc Rg = 1.8nm



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

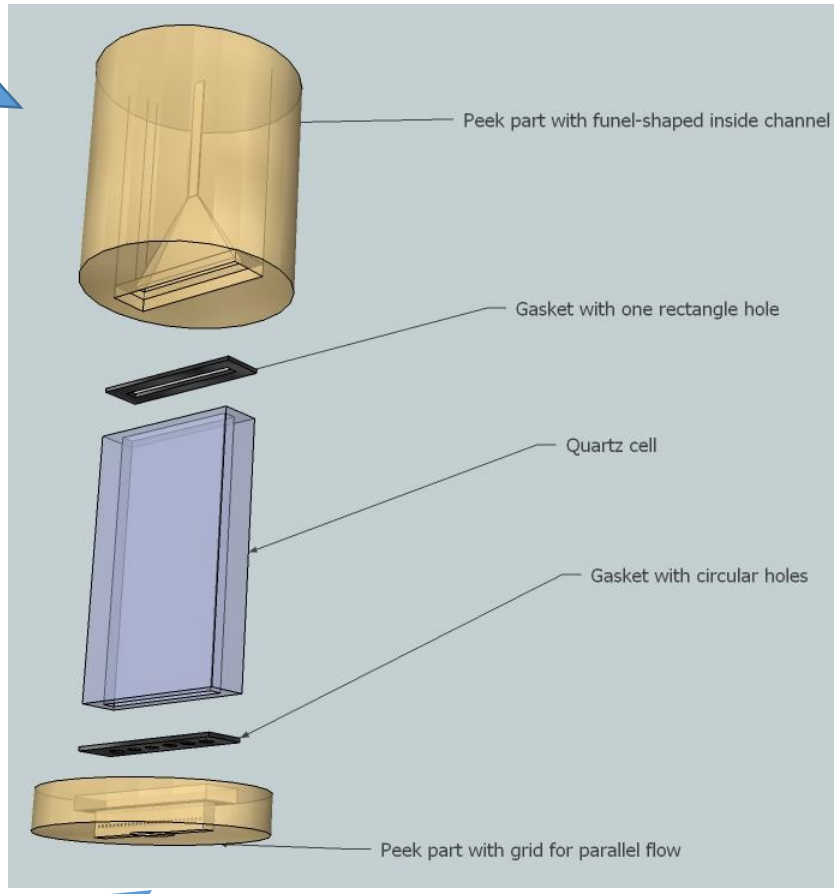
30s – 1 min exposure time

S-75 or S-200 Analytical column

Flow rate 0.2 – 0.4ml min

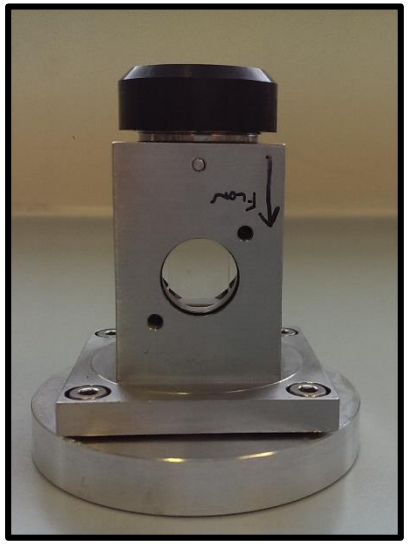
Chromatography column

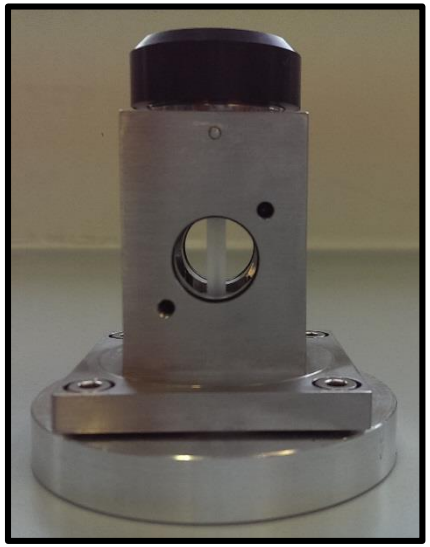
Flow

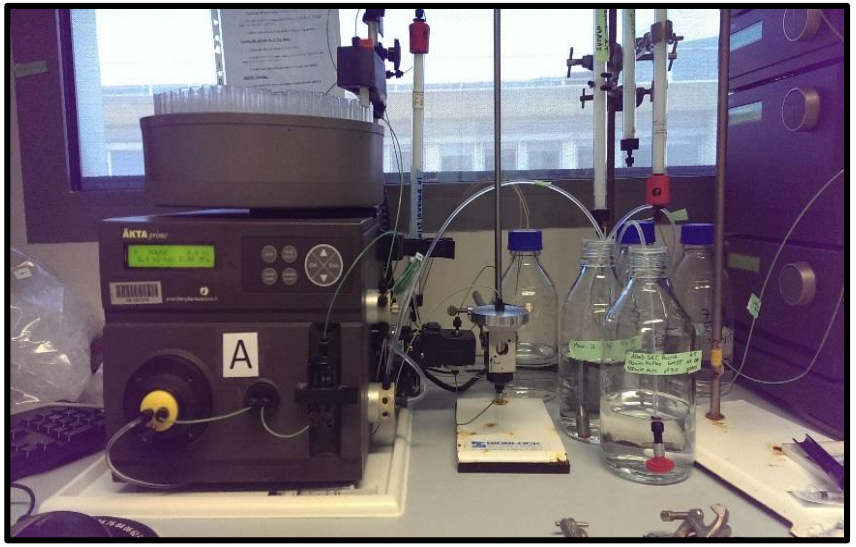


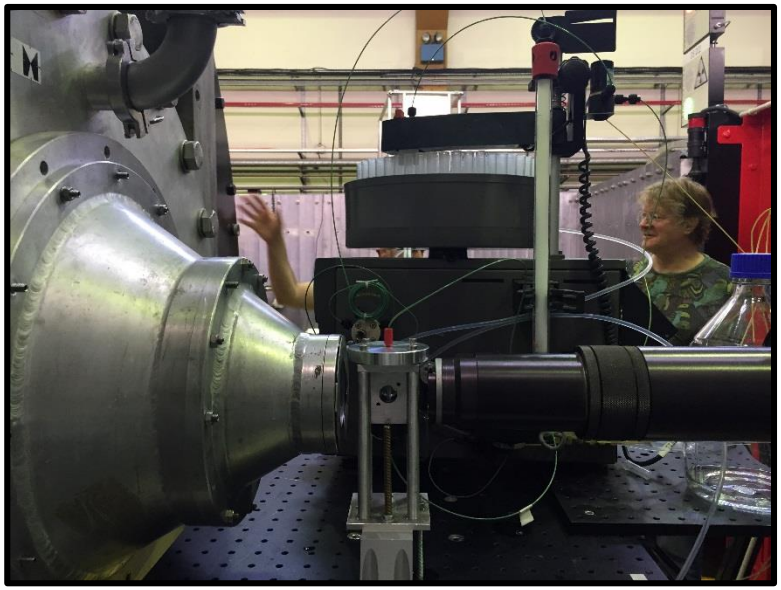
Fraction collector

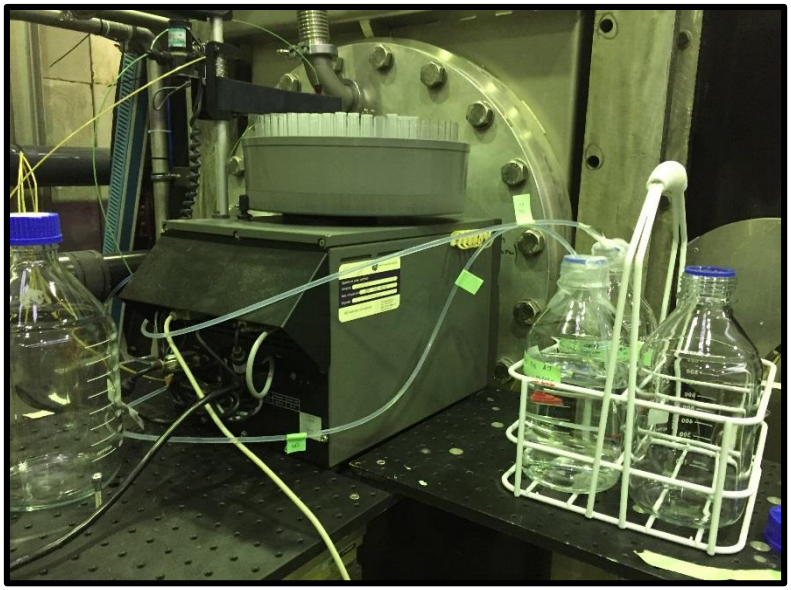
SANS Online FPLC – D22



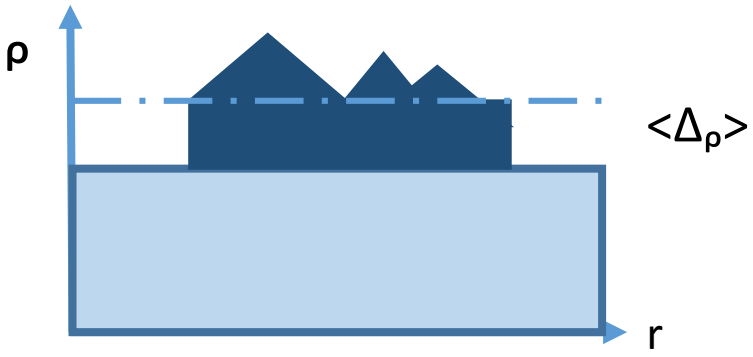








SANS Online FPLC



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

8m Data

Intensity reduced by 4 due to increased distance but see lower Q information – S/N poor

2m Data

Higher Intensity but less low Q information – S/N improved

I(Q)

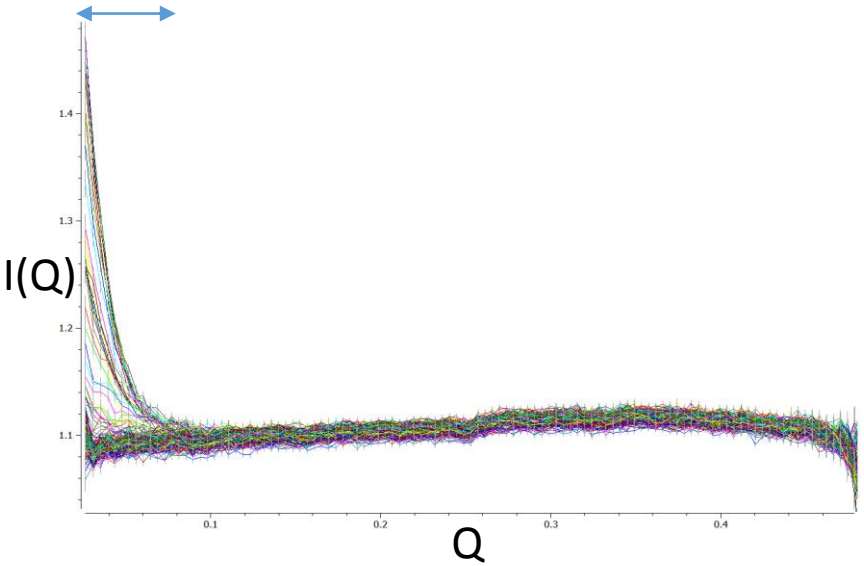
Q

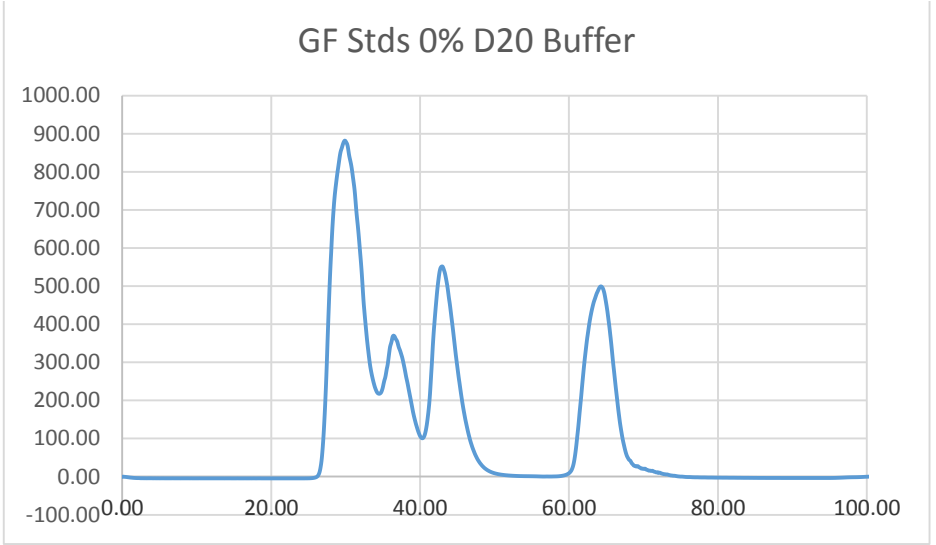
2m Data Total

Table 1. Gel Filtration Standard Components

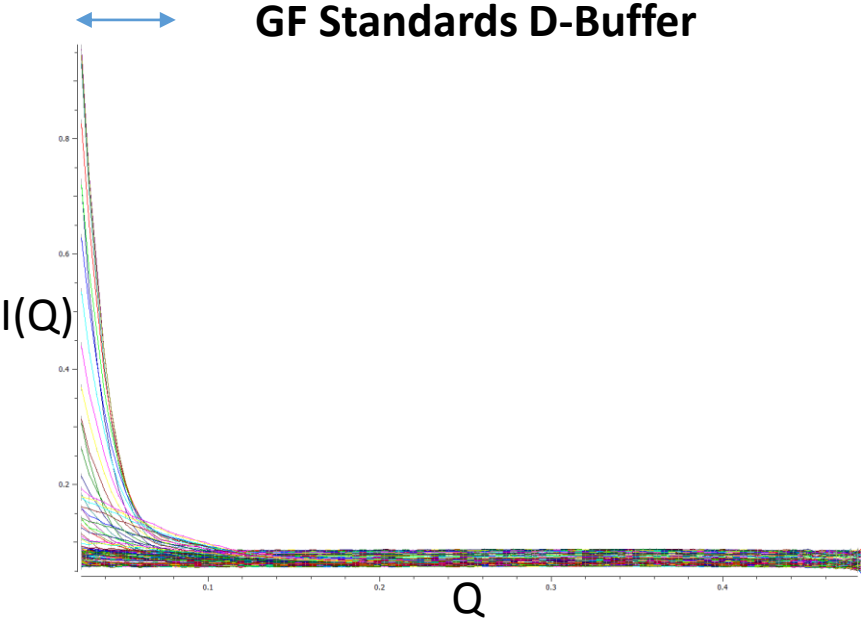
Component	Molecular Weight*	Amount per Vial (mg)
Thyroglobulin (bovine)	670,000	5.0
γ-globulin (bovine)	158,000	5.0
Ovalbumin (chicken)	44,000	5.0
Myoglobin (horse)	17,000	2.5
Vitamin B_{12}	1,350	0.5
		18

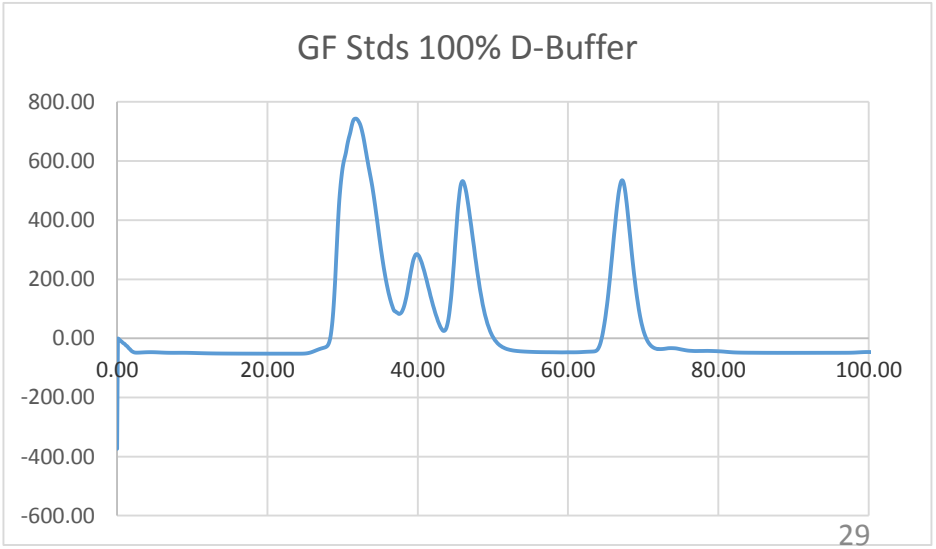
GF Standards H-Buffer



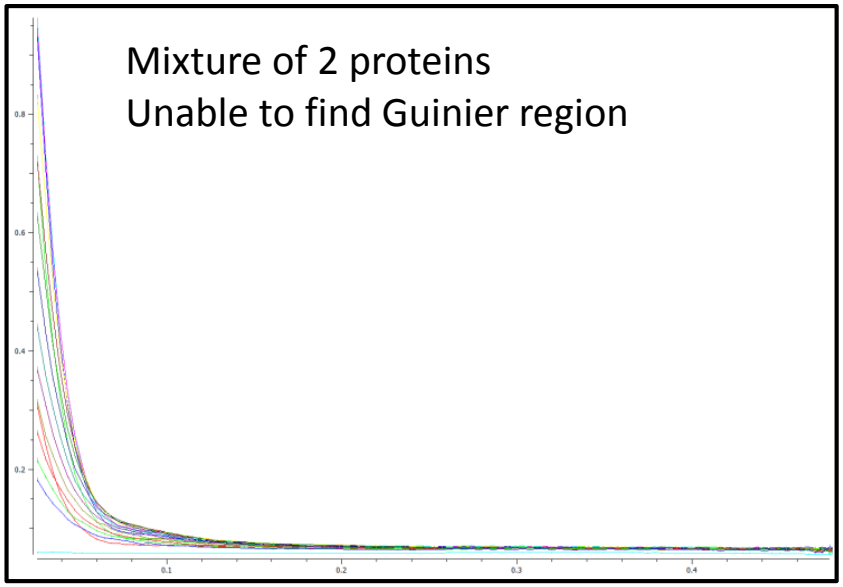


GF Standards D-Buffer

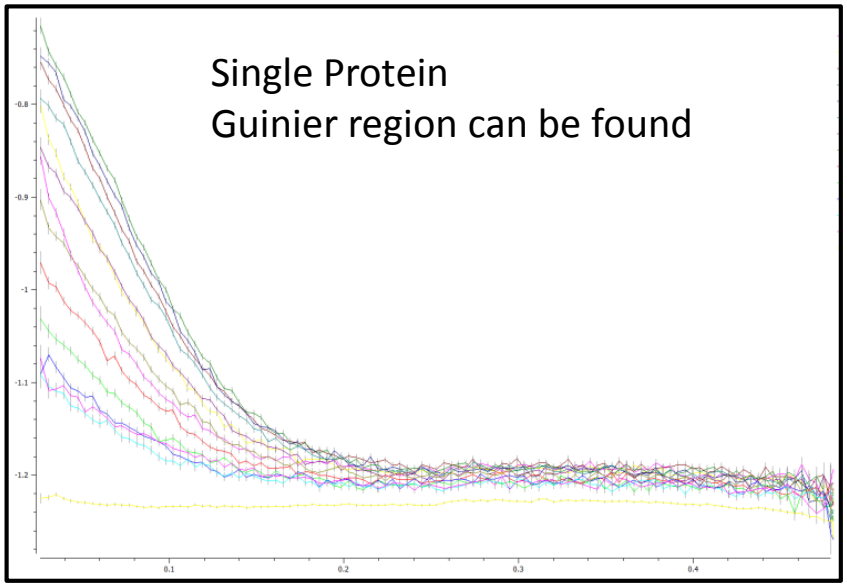




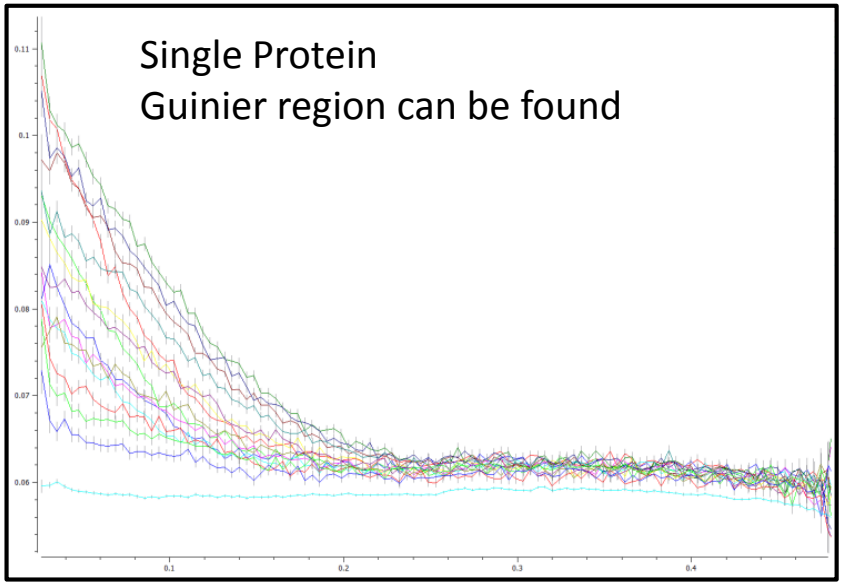
Peak 1



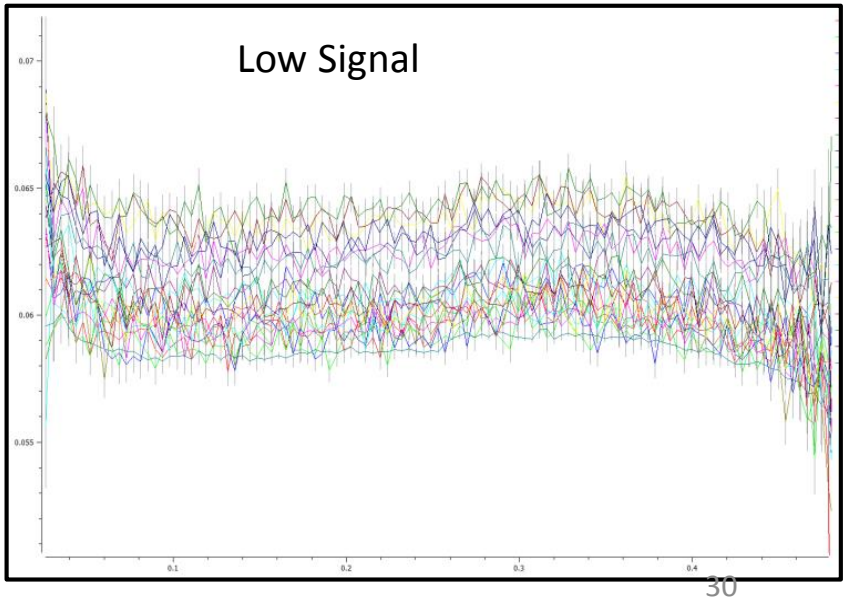
Peak 2

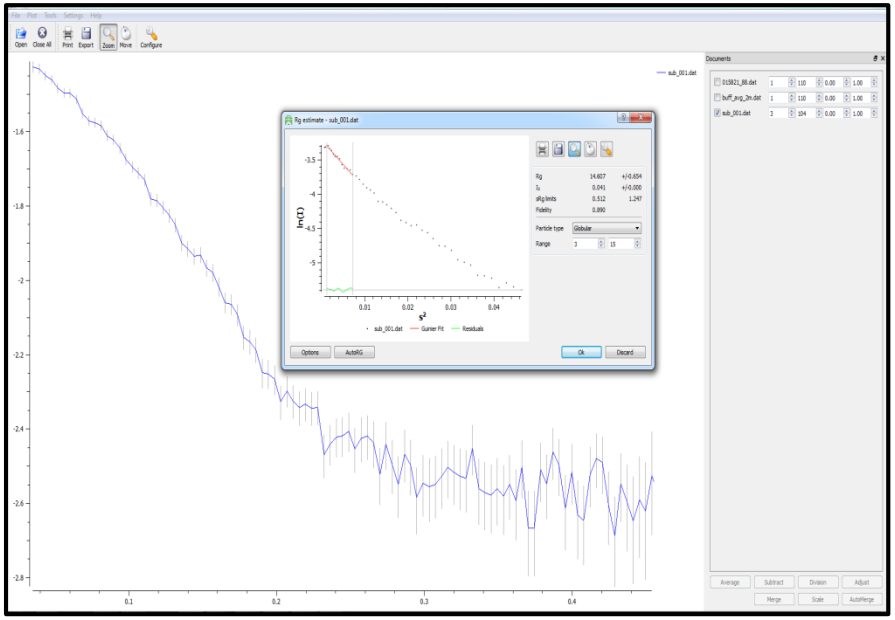


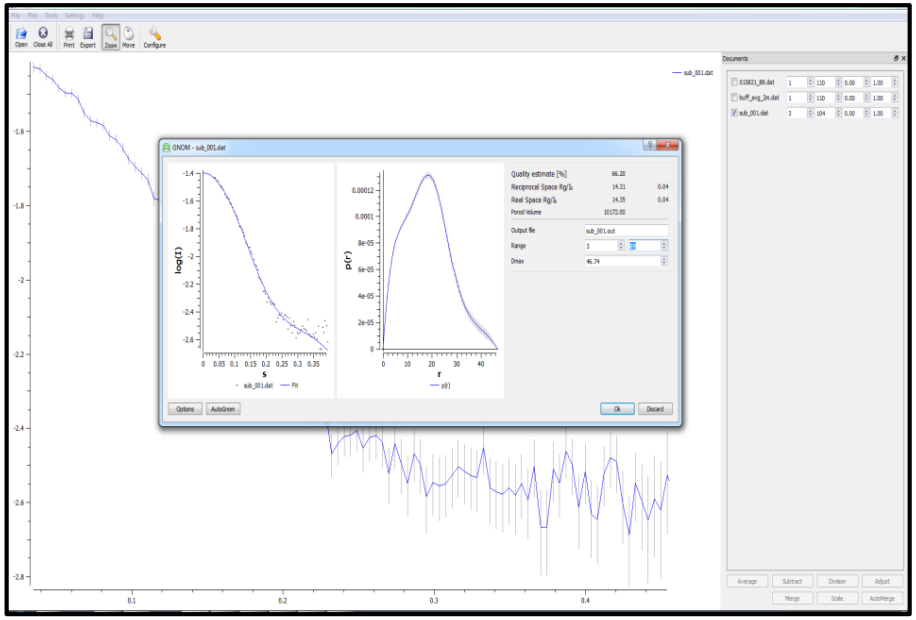
Peak 3

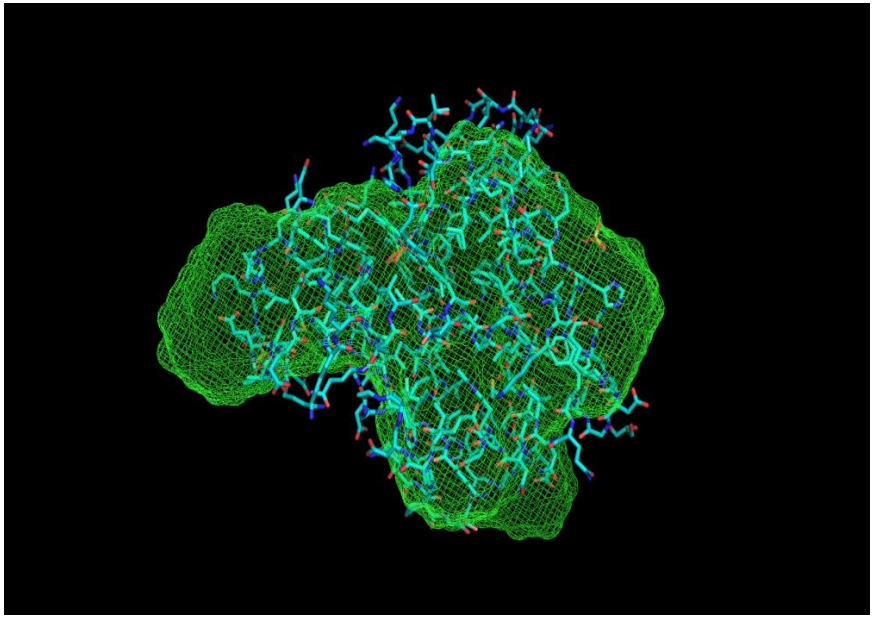


Peak 4



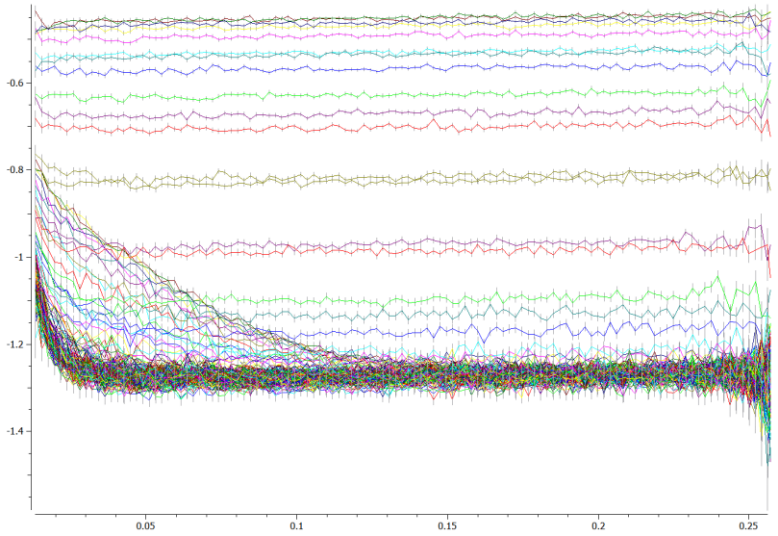




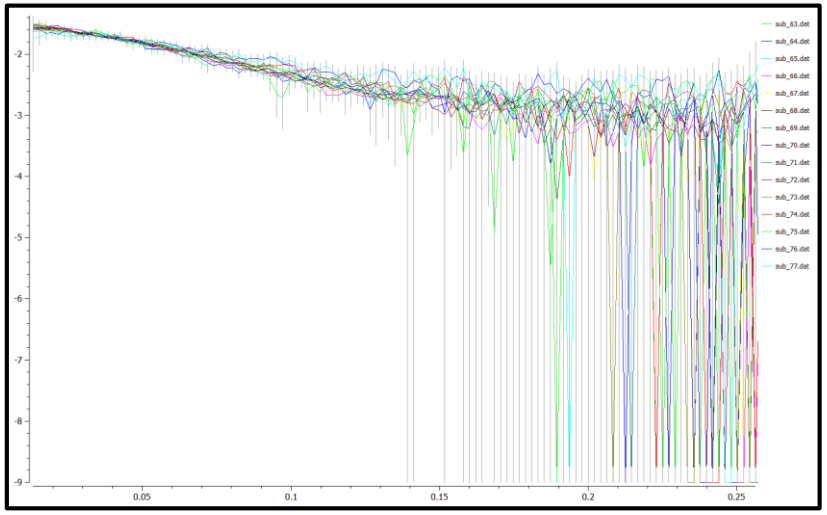


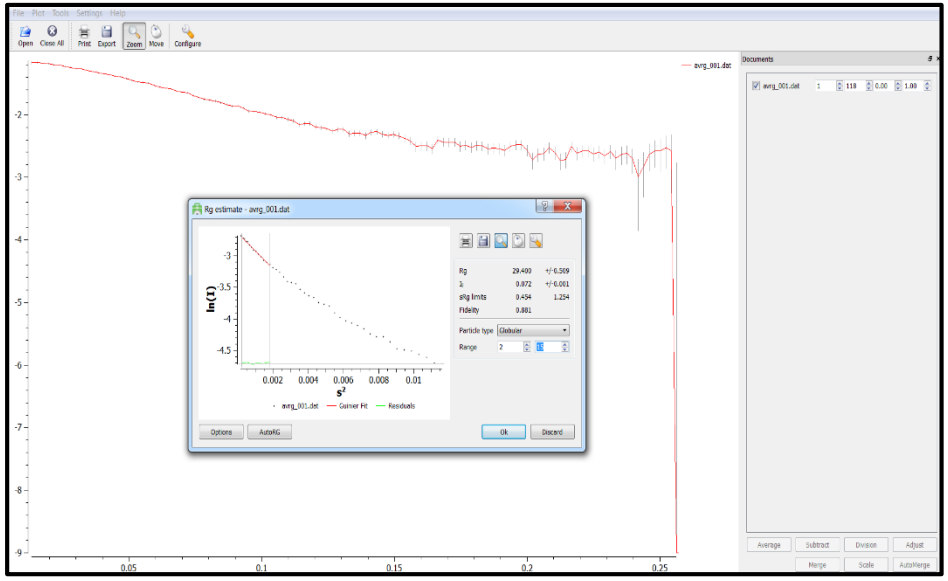
- Can distinguish between protein species
- BUT!
- Carefully optimized – sample concentration, buffer composition, det distance, flow rate etc.
- Recovery of sample for corresponding SAXS measurements for envelope checking.

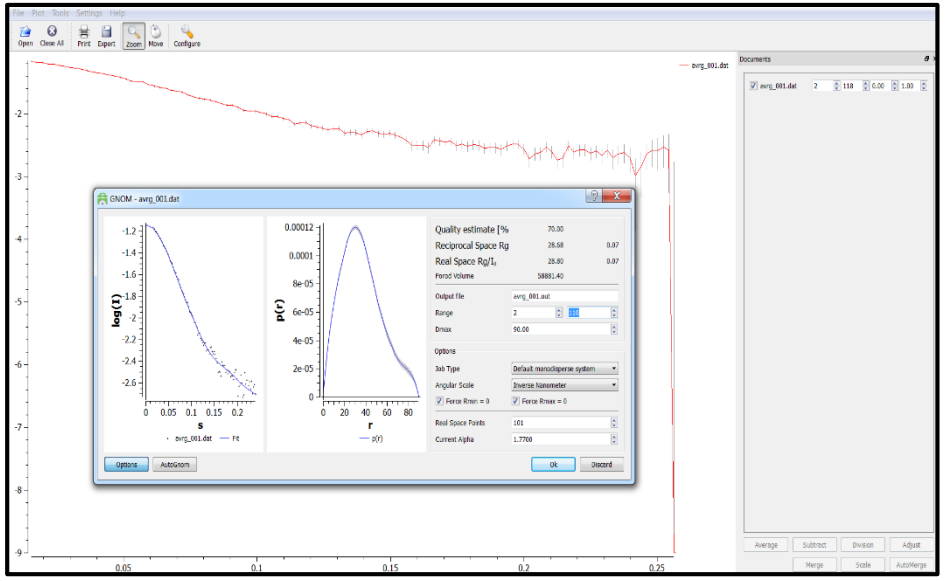
Sir2a 100 %D Buffer



Subtracted

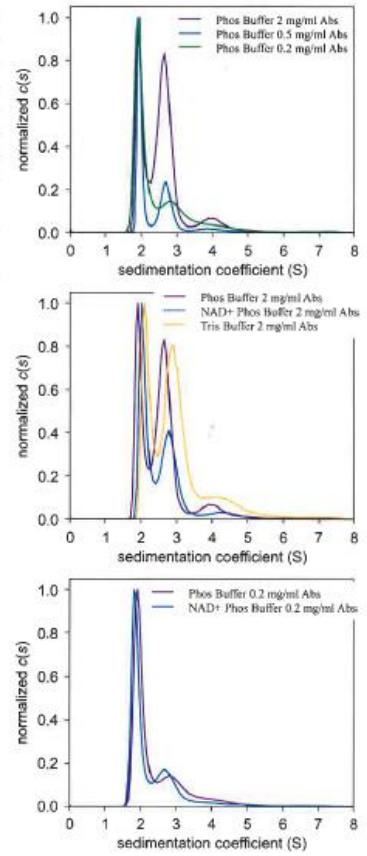


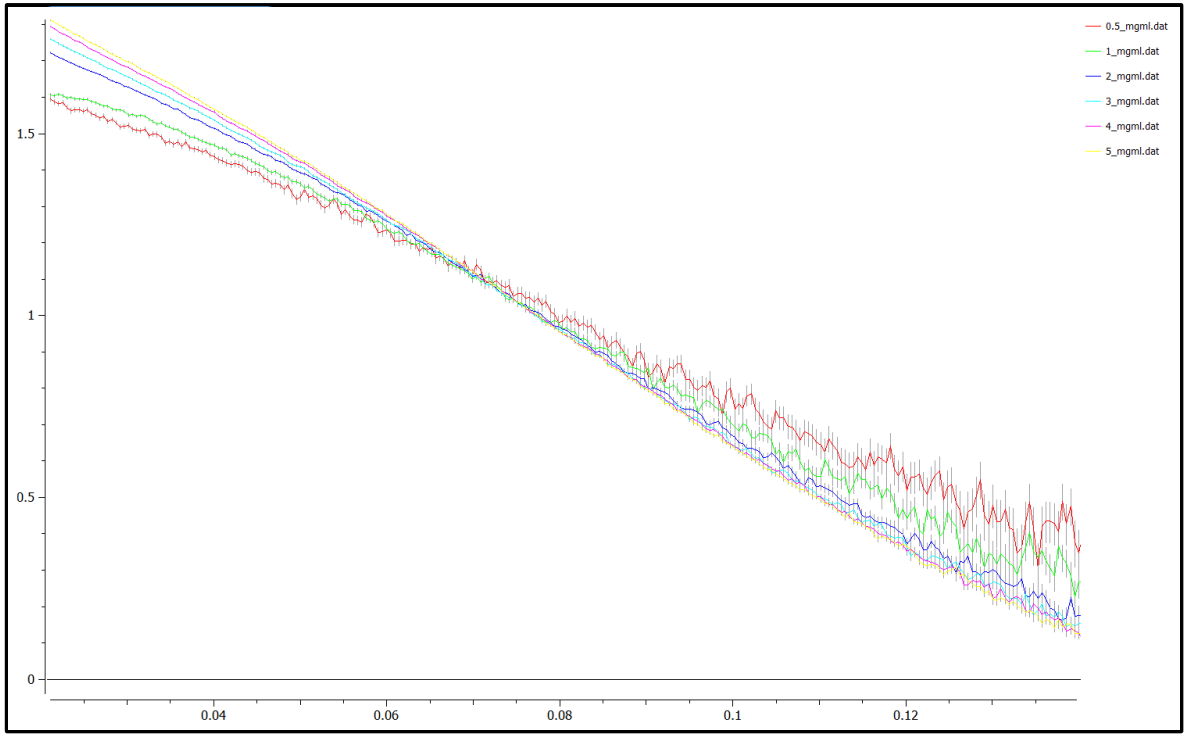




SANS Exp Rg = 2.9nm +/- 0.569nm

AUC





Concentration effects observed

- Monomer/Dimer populations
- Increase in observed Rg

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 (H_2O/D_2O 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!