Structures of proteins in solution

Annette Eva Langkilde

bio saxs

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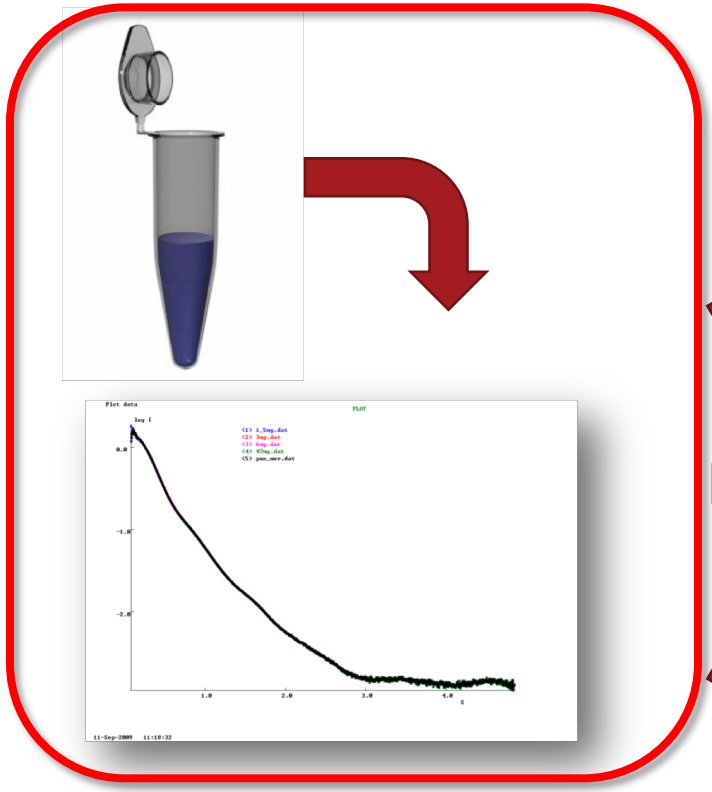
Faculty of Health and Medical Sciences

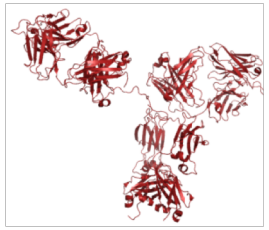
UNIVERSITY OF COPENHAGEN

Outline



Size, shape, Mw, Rg
(primary analysis)

Known atomic structure



- Homolog?
- Biological unit?
- …

Partial structure

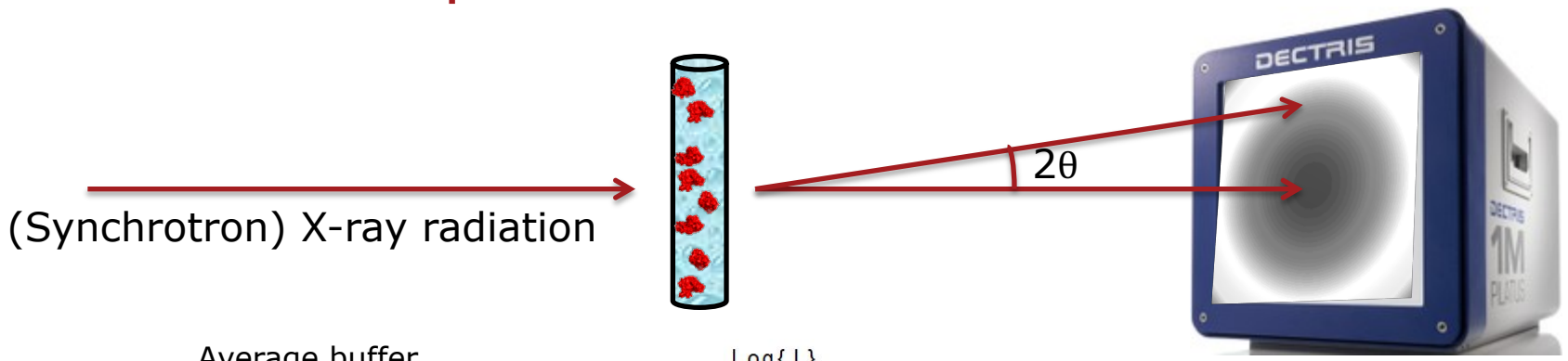
?

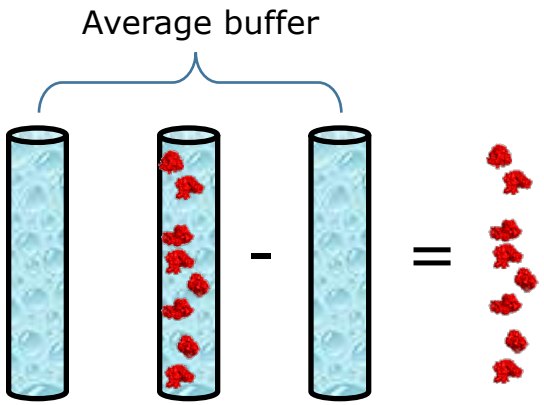
- Homolog?
- Multi domain?
- Complex?
- …

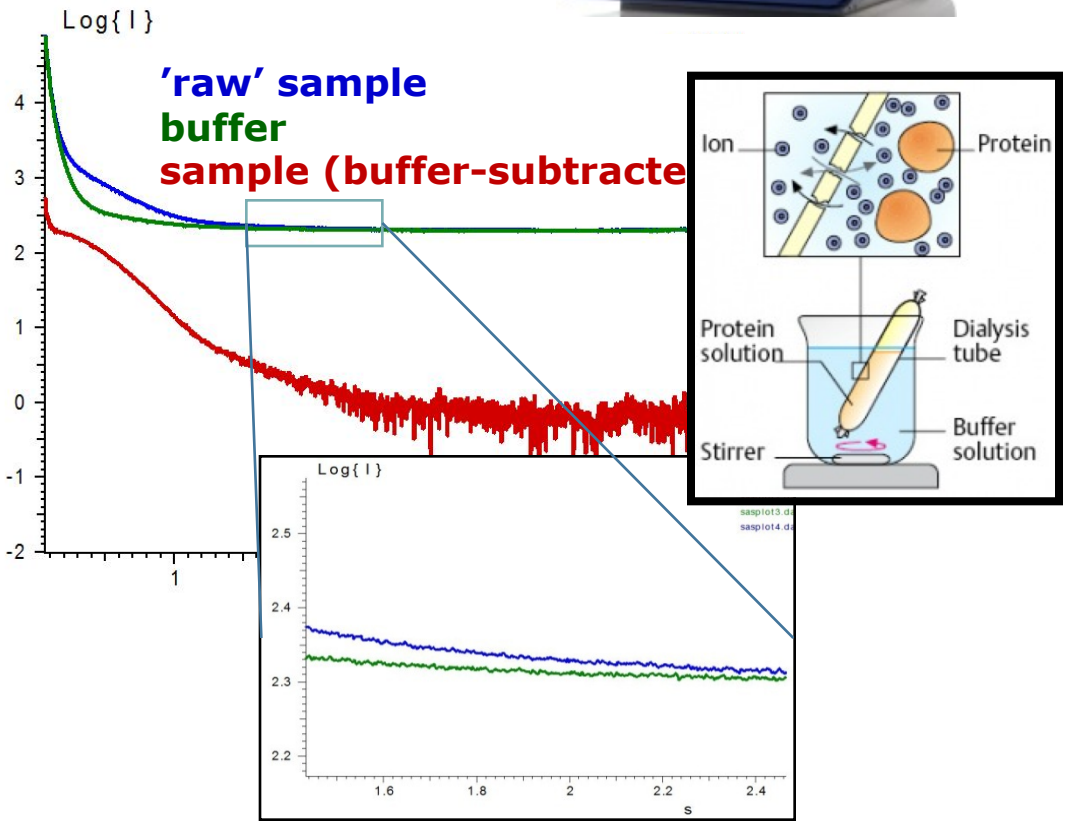
No prior knowledge

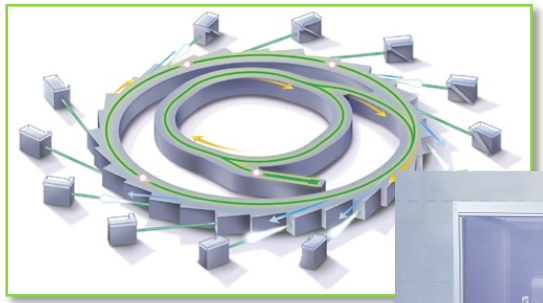
…and there's more

The experimental setup









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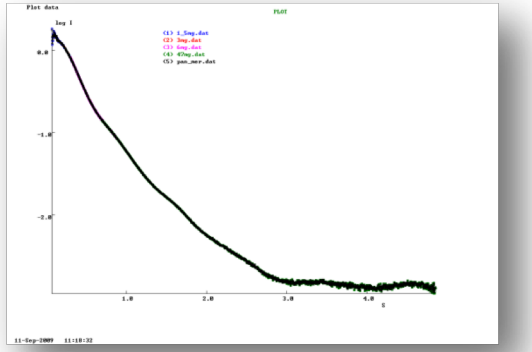

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(primary analysis)

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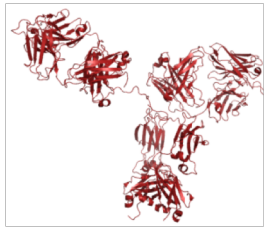

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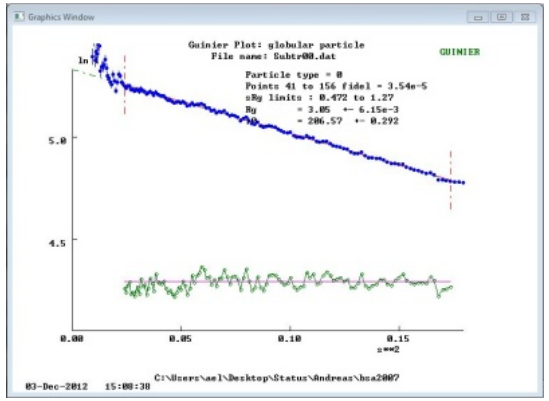
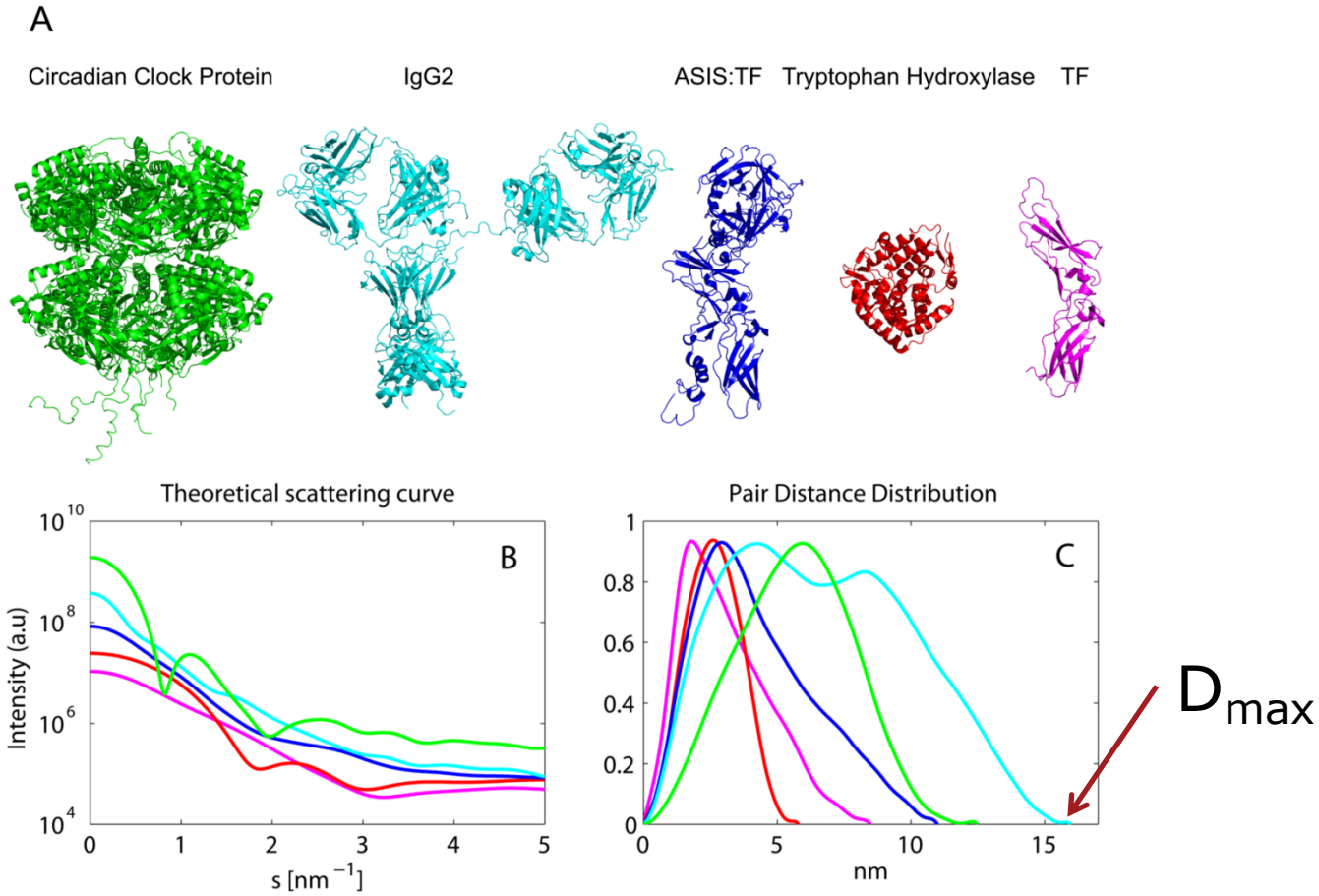
Size and shape matters!



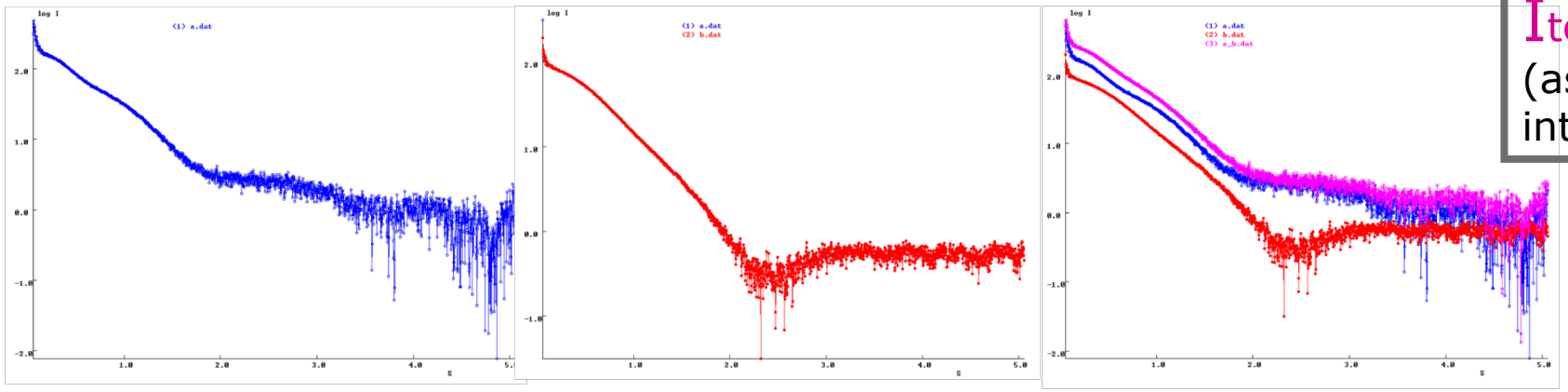
Guinier:
R_g and
I(0) –> M_w



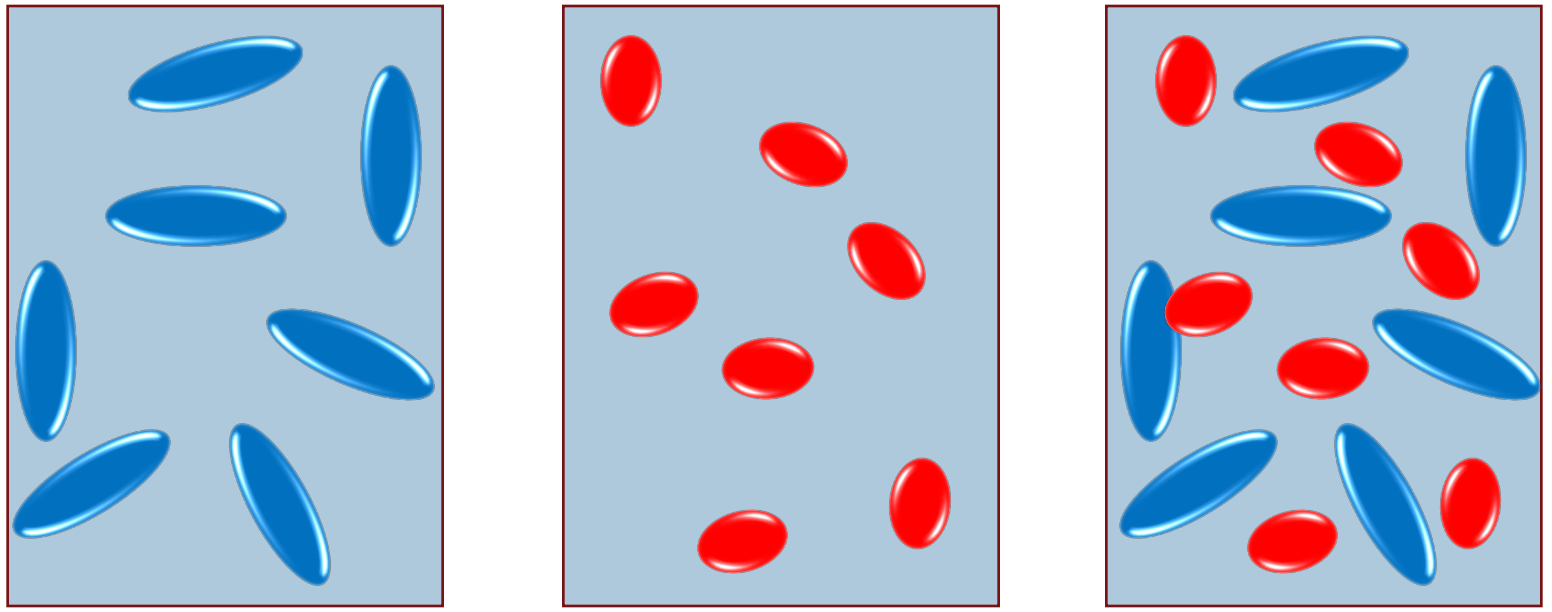
D_{max}

$$I(s)=4\pi\int_0^{Dmax} p(r)\,\sin(sr)/sr\,dr \;\leftrightarrow\; p(r)=r^2/2\pi^2\int_0^{\infty} sI(s)\,\sin(sr)/sr\,ds$$

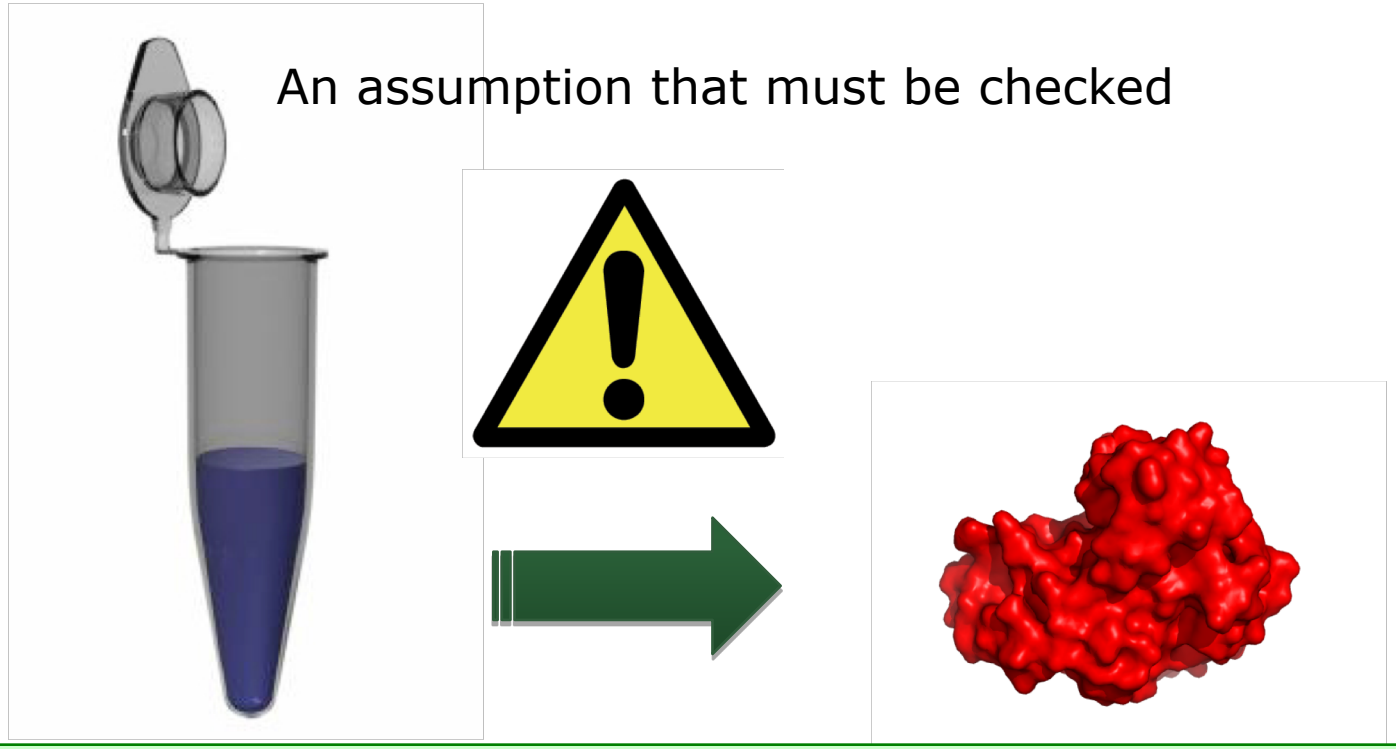
SAXS data from polydisperse samples – additive



$I_{tot} = xI_a + yI_b$
(assuming no interactions)



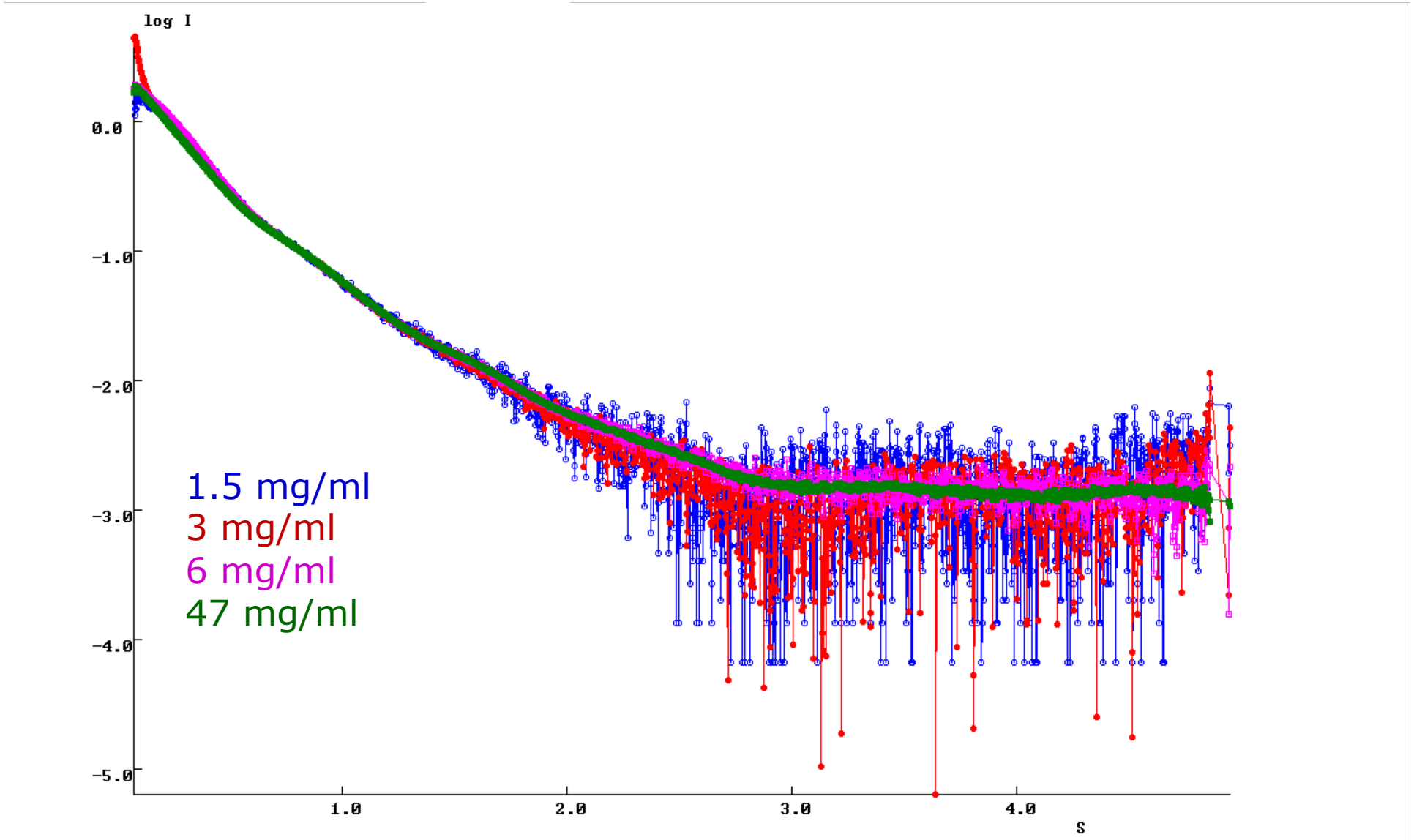
Ideality and monodispersity – an assumption



Ideality: No intermolecular interactions
Non-ideality: Attraction or repulsion between molecules

Monodispersity: Identical Particles
Polydispersity: Size/shape/state/… differs among particles

Concentration series



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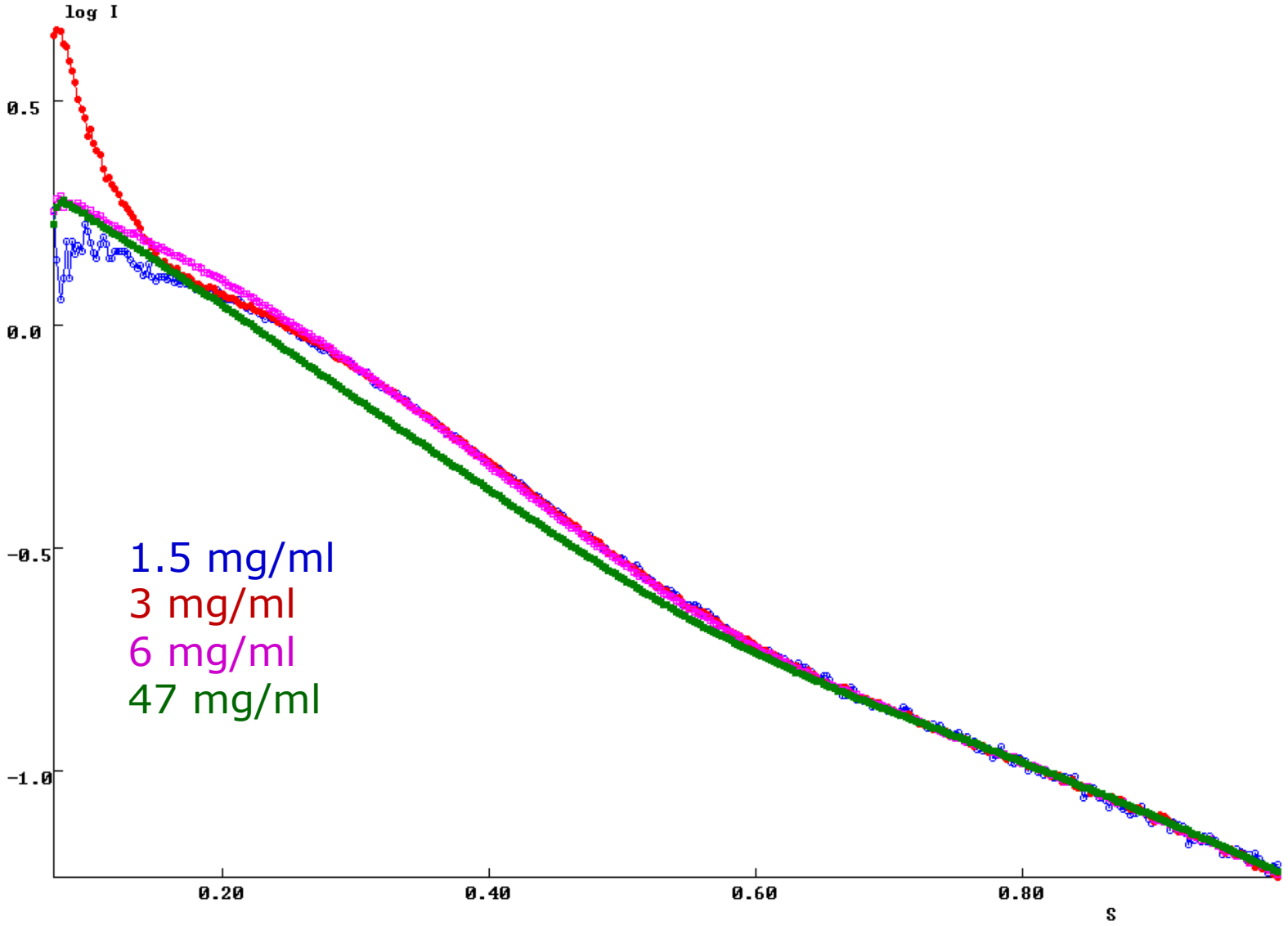

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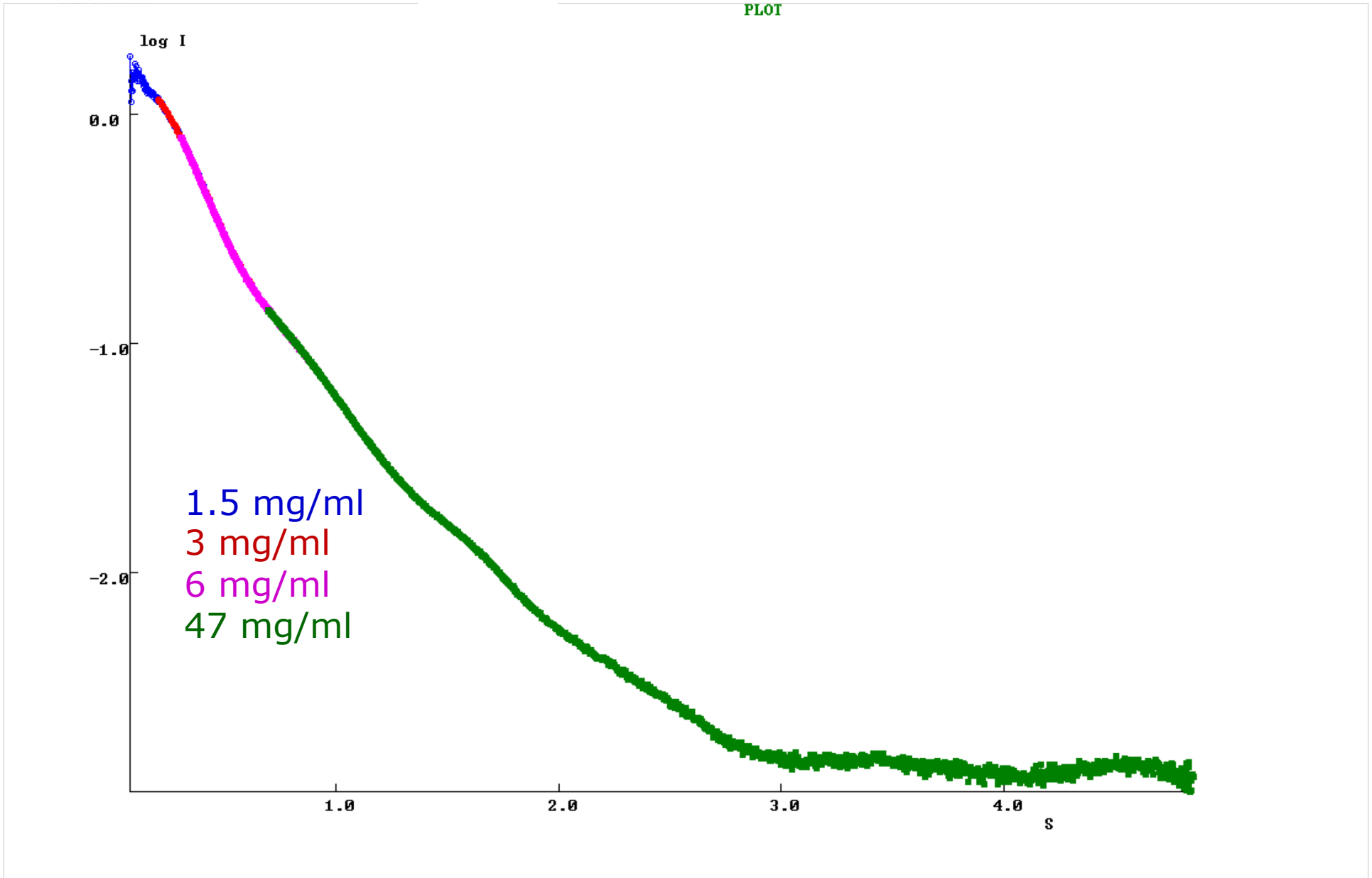
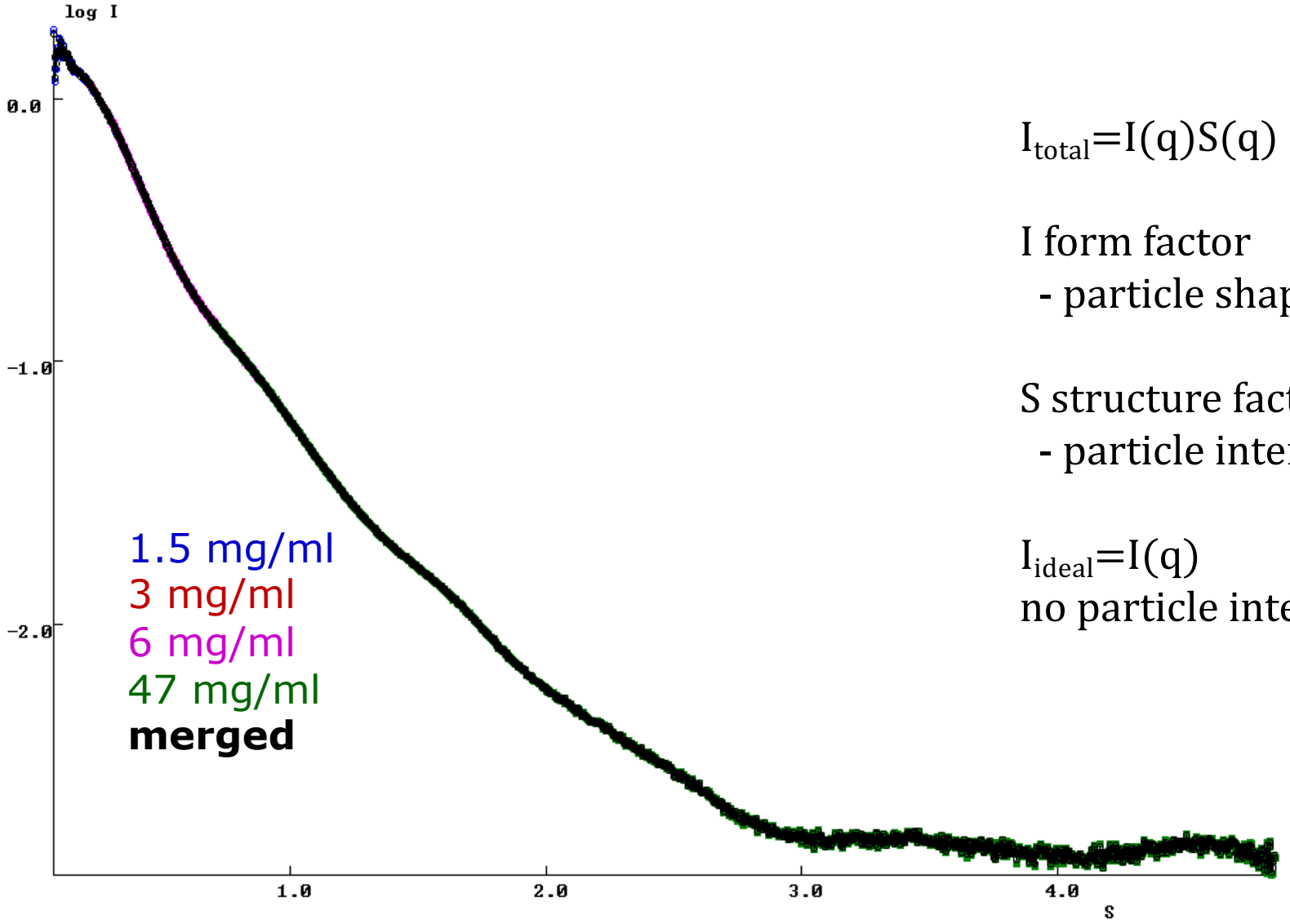


Concentration series –mimicking ideality



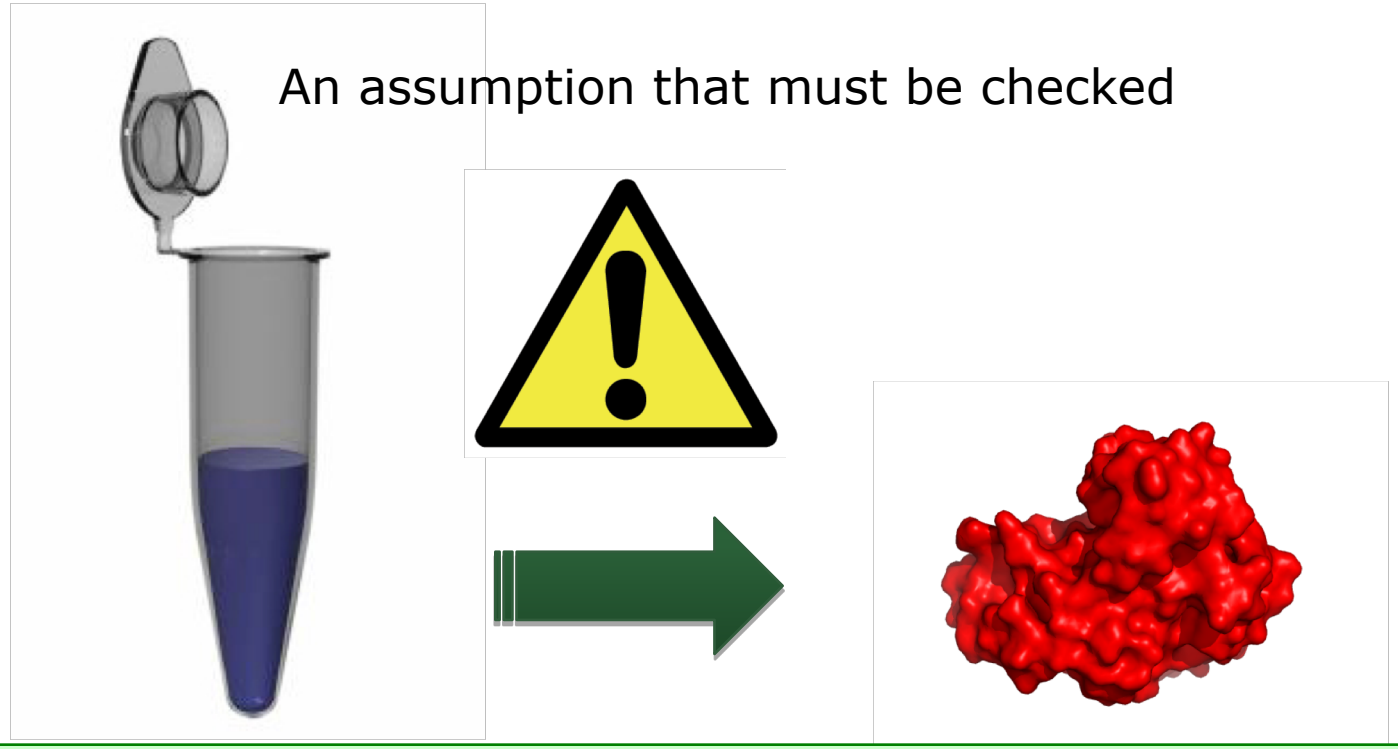
$I_{total}=I(q)S(q)$

I form factor
- particle shape & size

S structure factor
- particle interactions

$I_{ideal}=I(q)$
no particle interactions, $S(q)=1$

Ideality and monodispersity – an assumption



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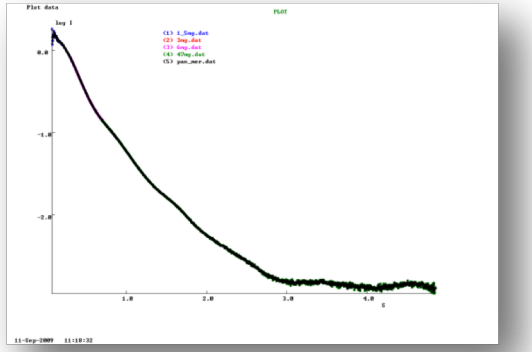
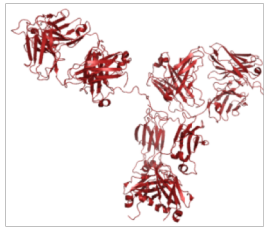


Size, shape, Mw, Rg
(primary analysis)

Known atomic structure



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Partial structure

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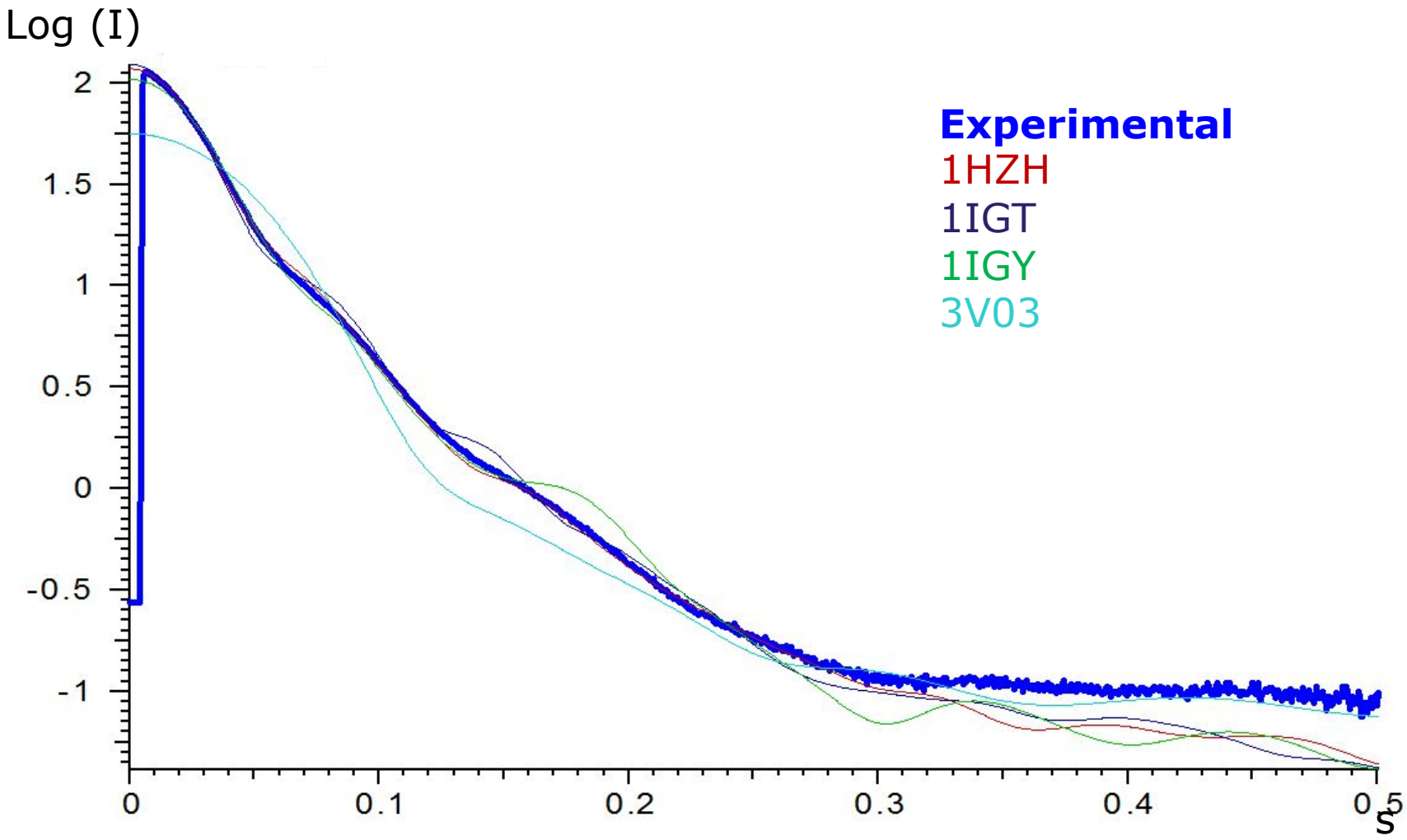
No prior knowledge

…and there is more

Fitting known structures (CRYSOL, FoXS, …)



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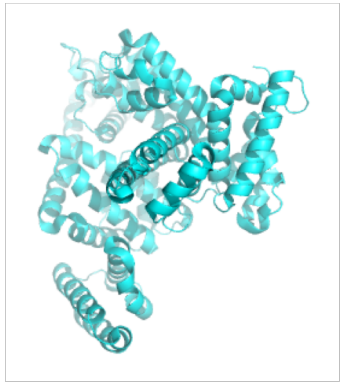
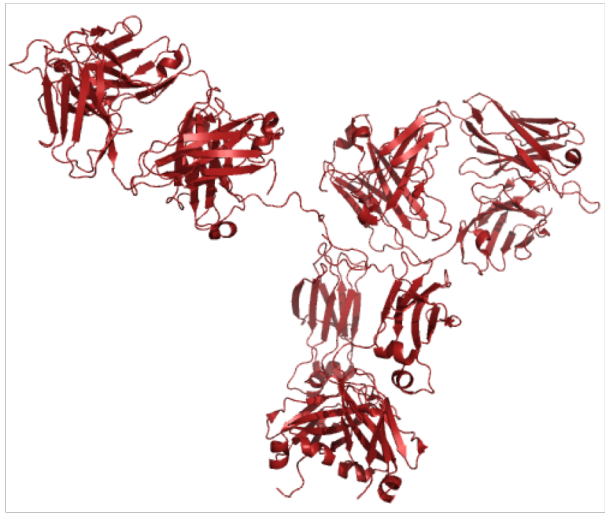


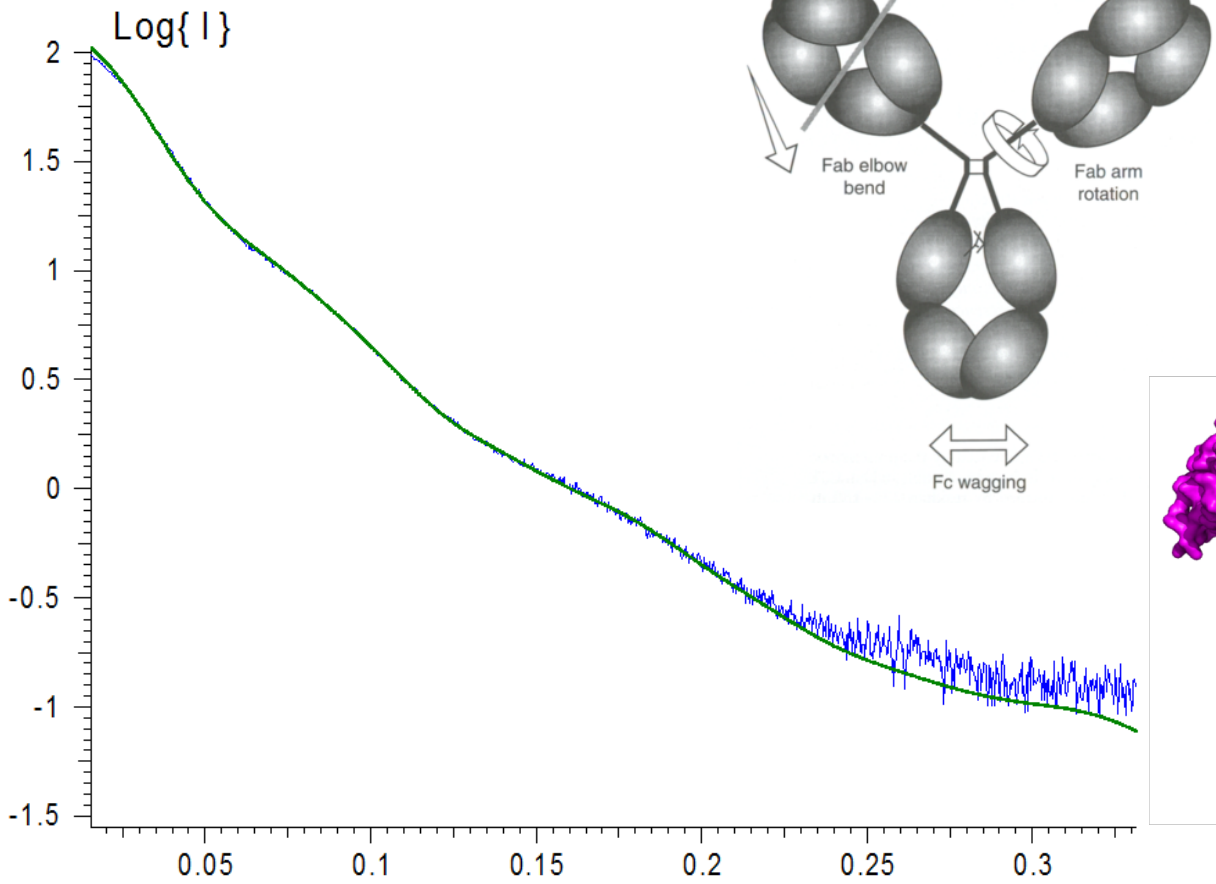
Fitting known structures (CRYSOL , FoXS, …)

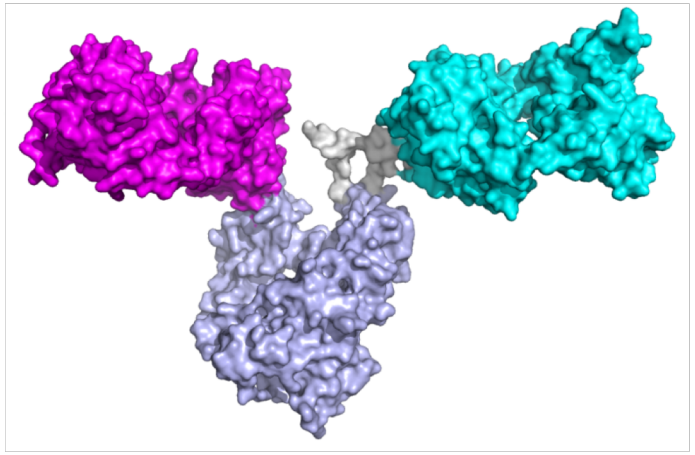
Experimental
1HZH
1IGT
1IGY

Fitting known structures (CRYSOL , FoXS, …)

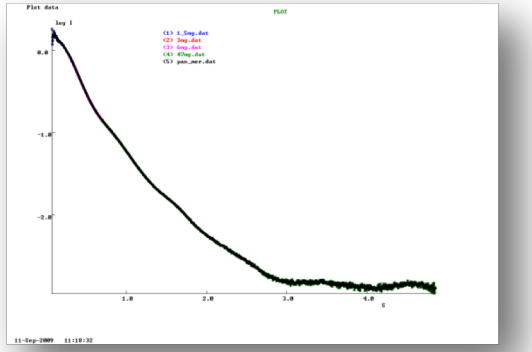


Rigid body refinement (e.g. SASREF)





Outline



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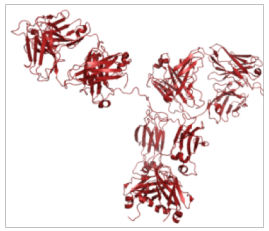

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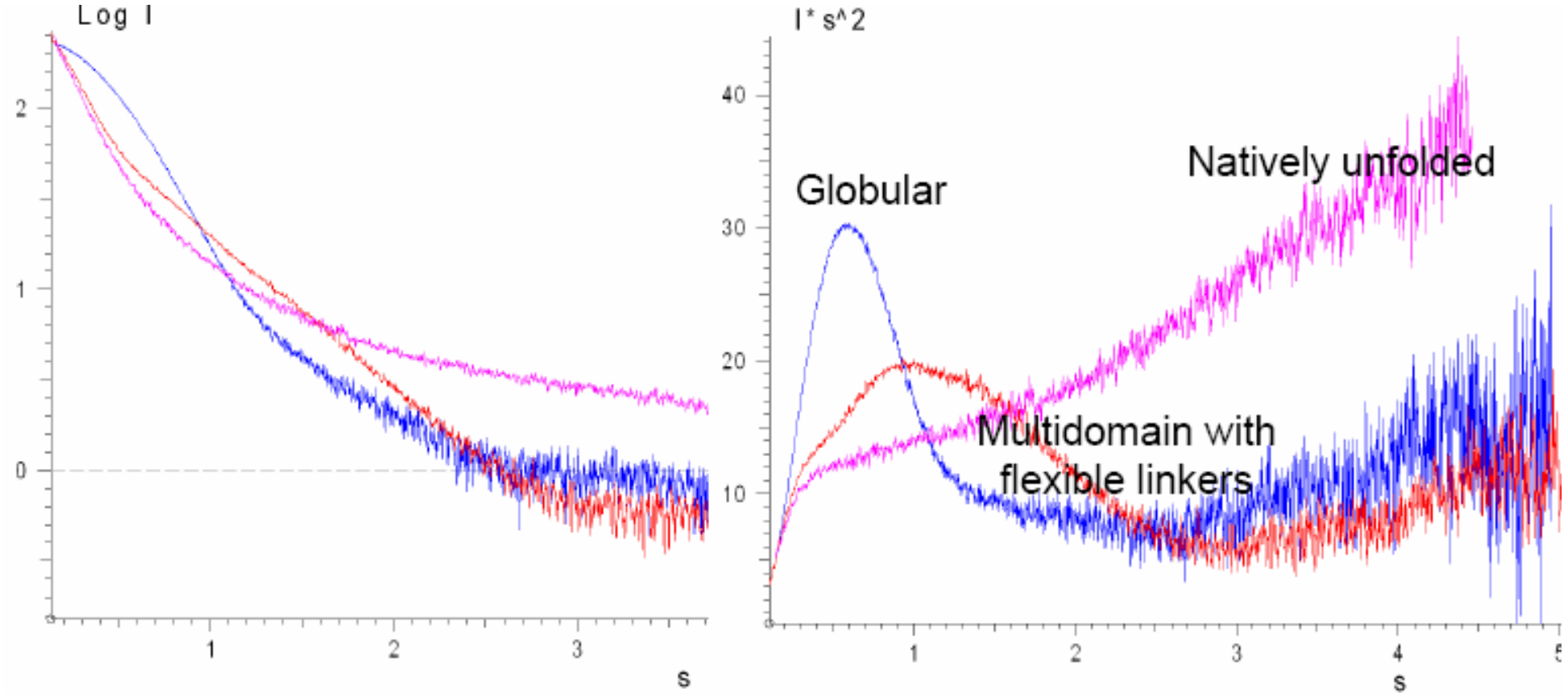
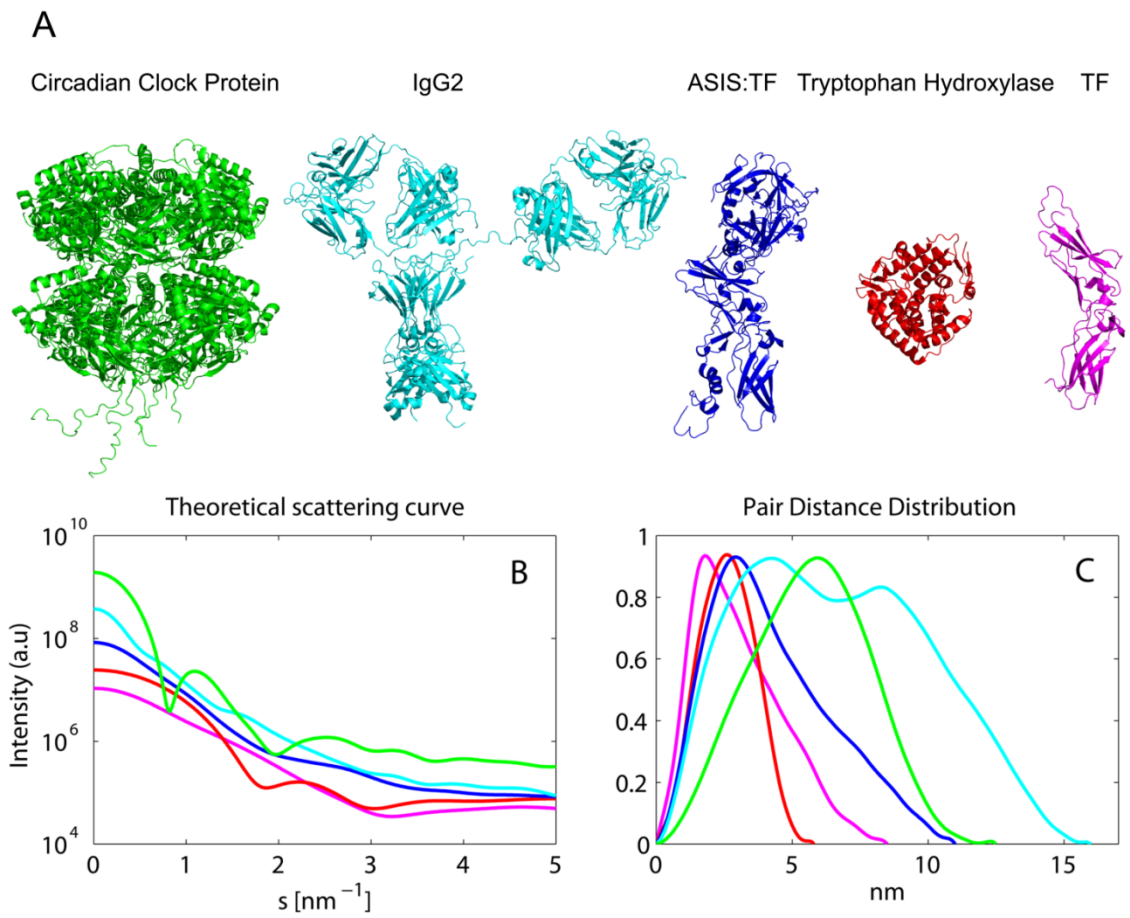
No prior knowledge

...and there is more

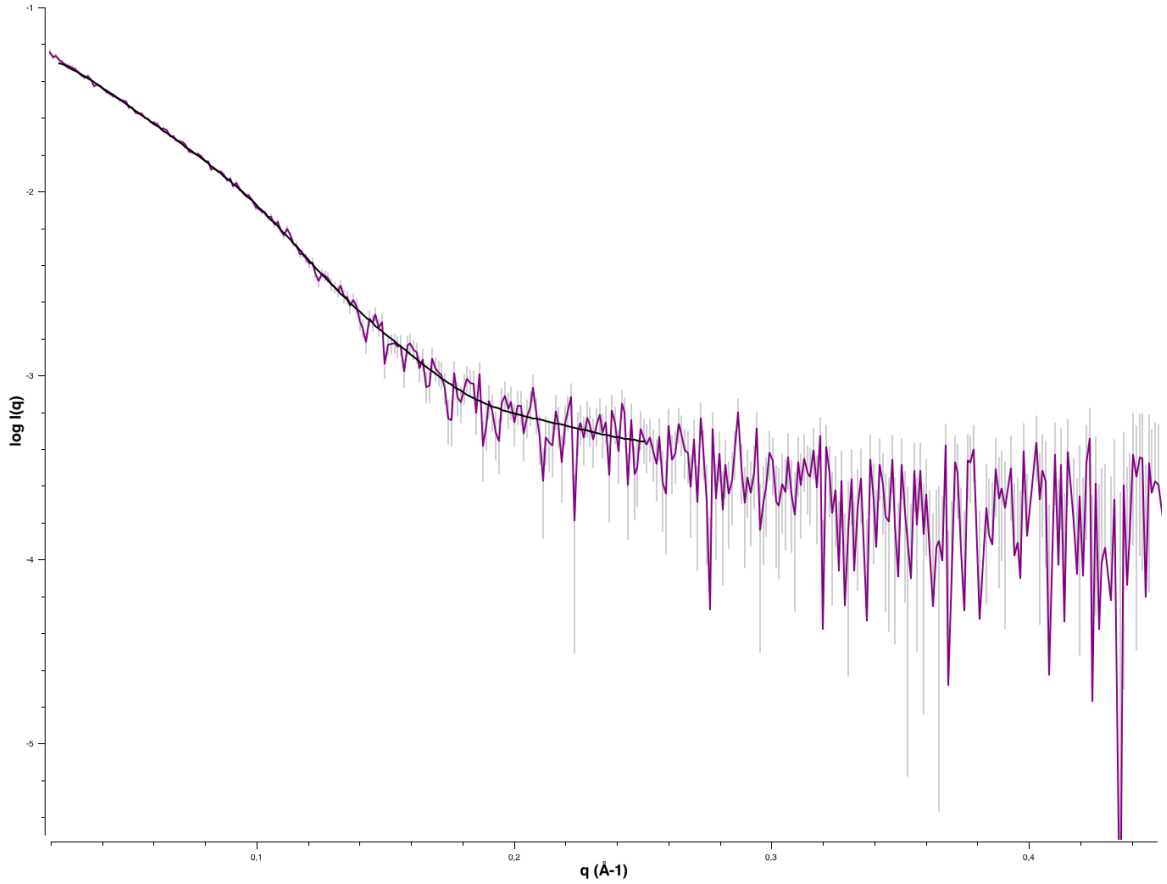
Globular or flexible proteins?

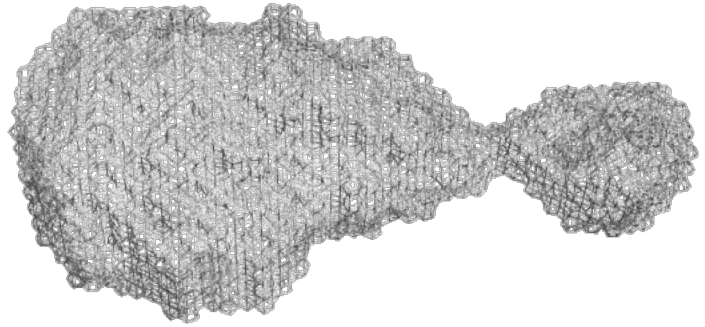


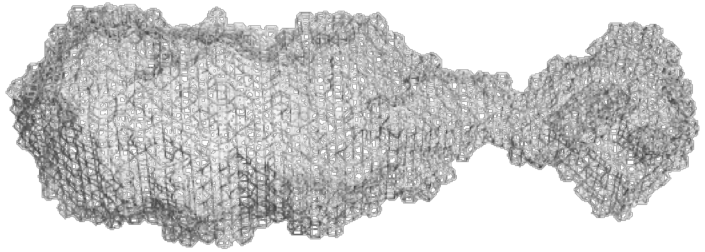
Remember: Size and shape matters!

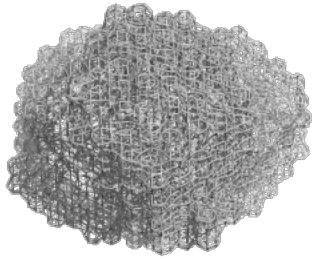


Ab-initio shape reconstruction (DAMMIF/N)









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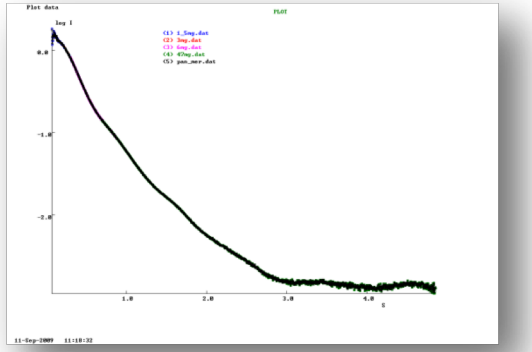

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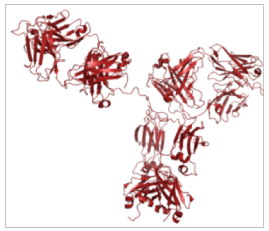

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Partial structure

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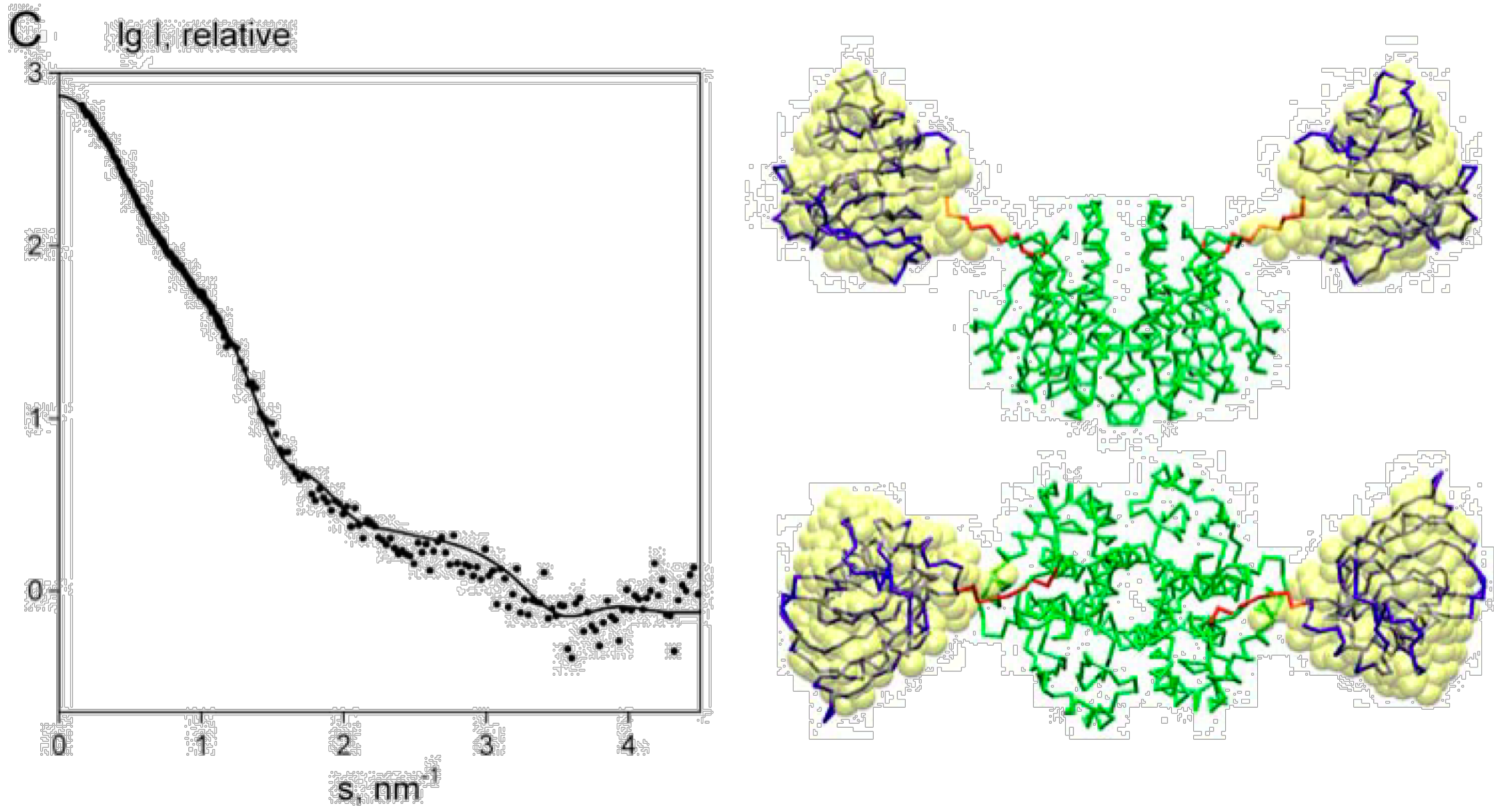
Combined methods

- Rigid body + ab-initio (dummy residues)



Petoukhov & Svergun 2005 Biophys J

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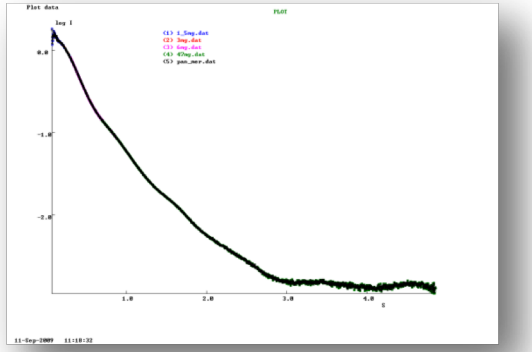

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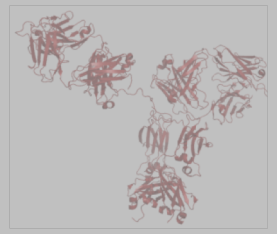

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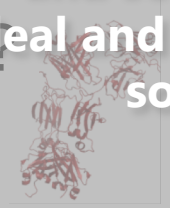
!!!

Only valid if
data collection is done properly
and so far only for
ideal and monodisperse
solutions

Partial structure



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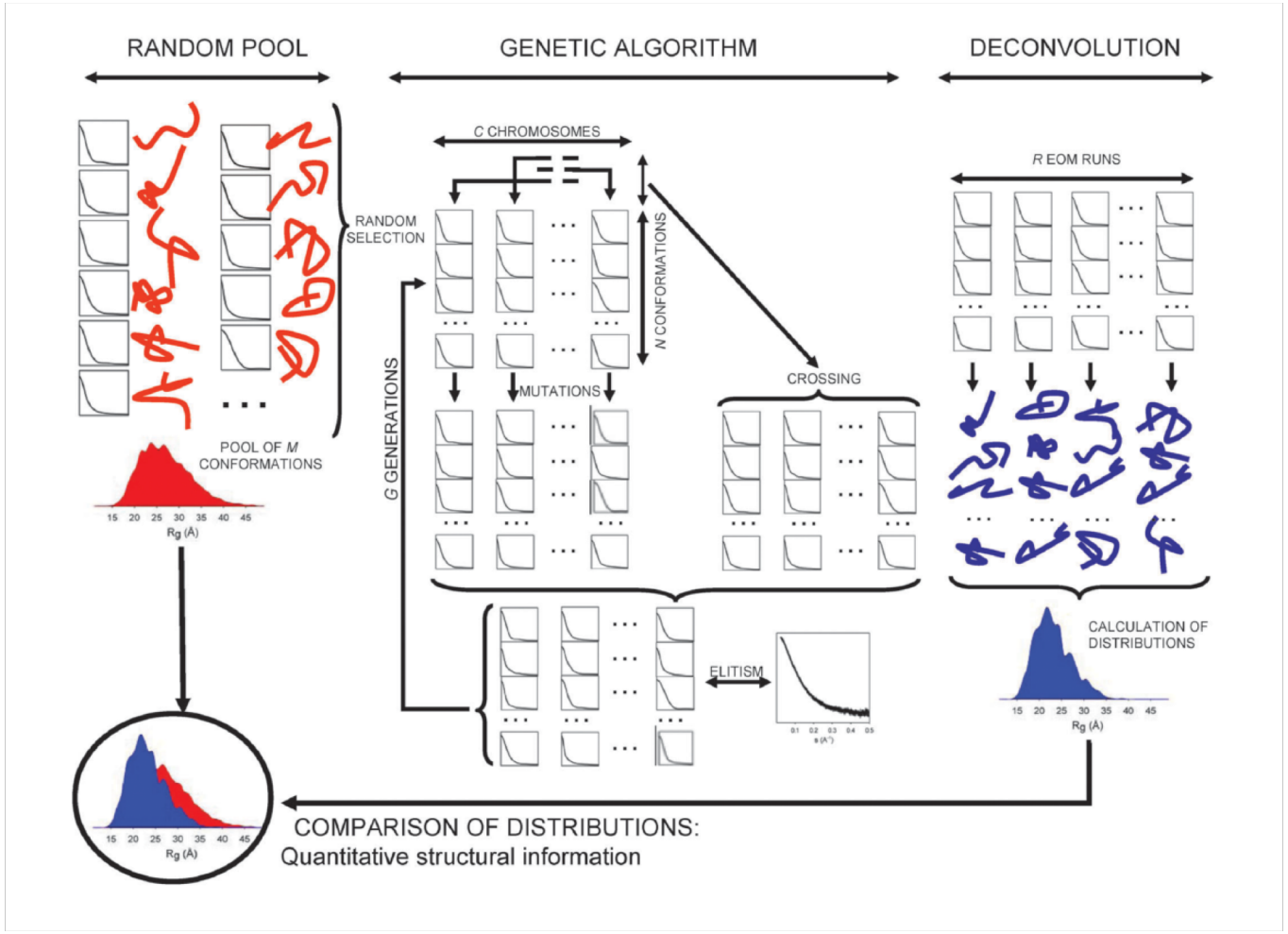
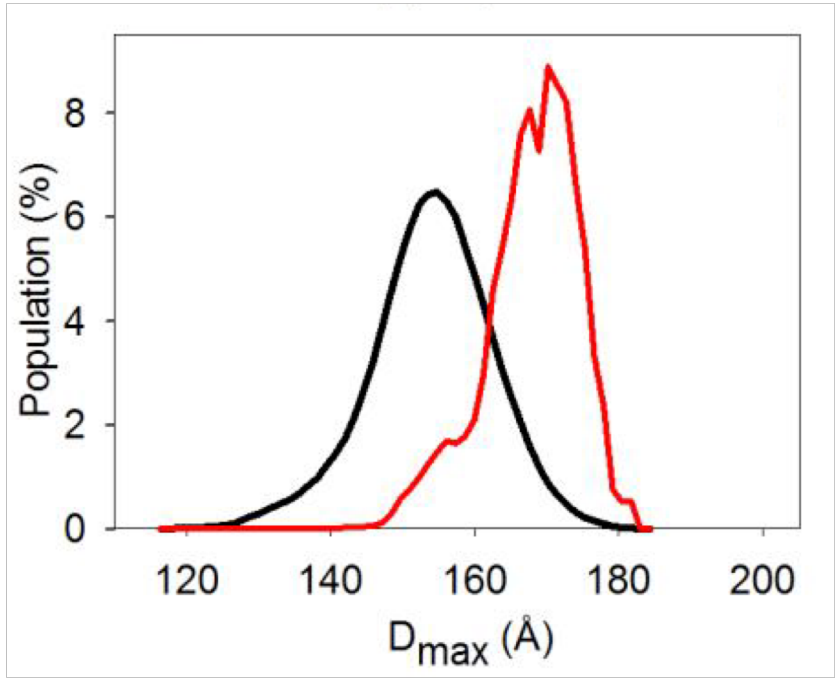
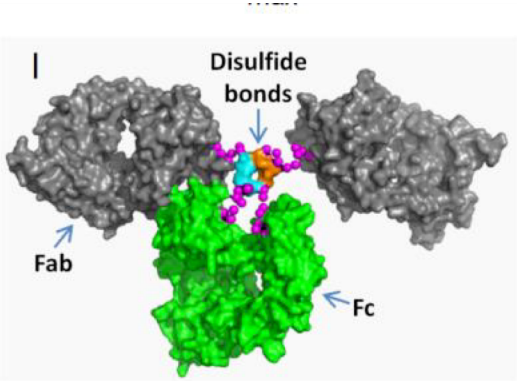
Ensemble optimization method

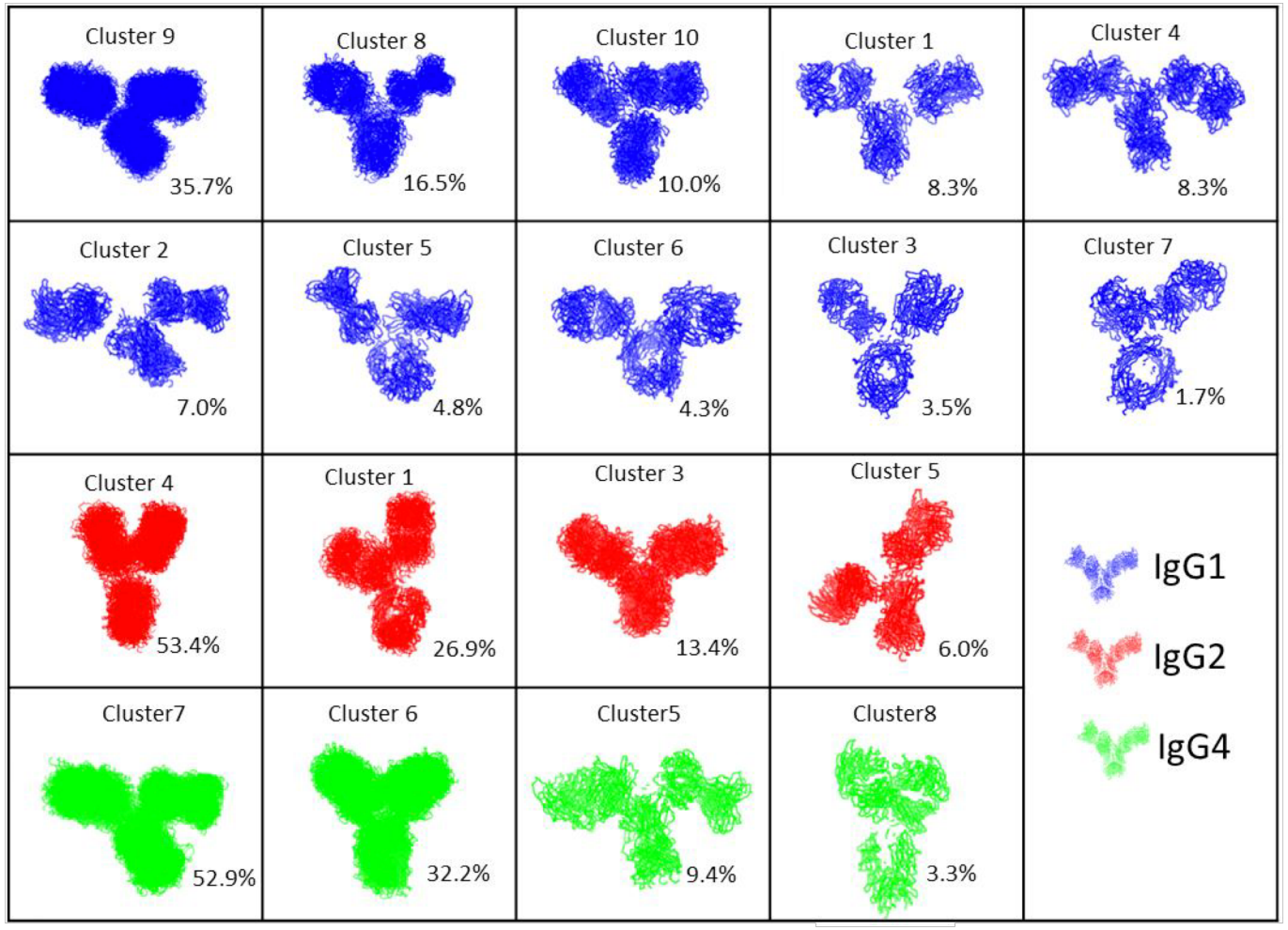




Pau Bernadó and Dmitri I. Svergun. Structural analysis of intrinsically disordered proteins by small-angle X-ray scattering. Mol. Biosyst., 8(1):151–67, jan 2012.

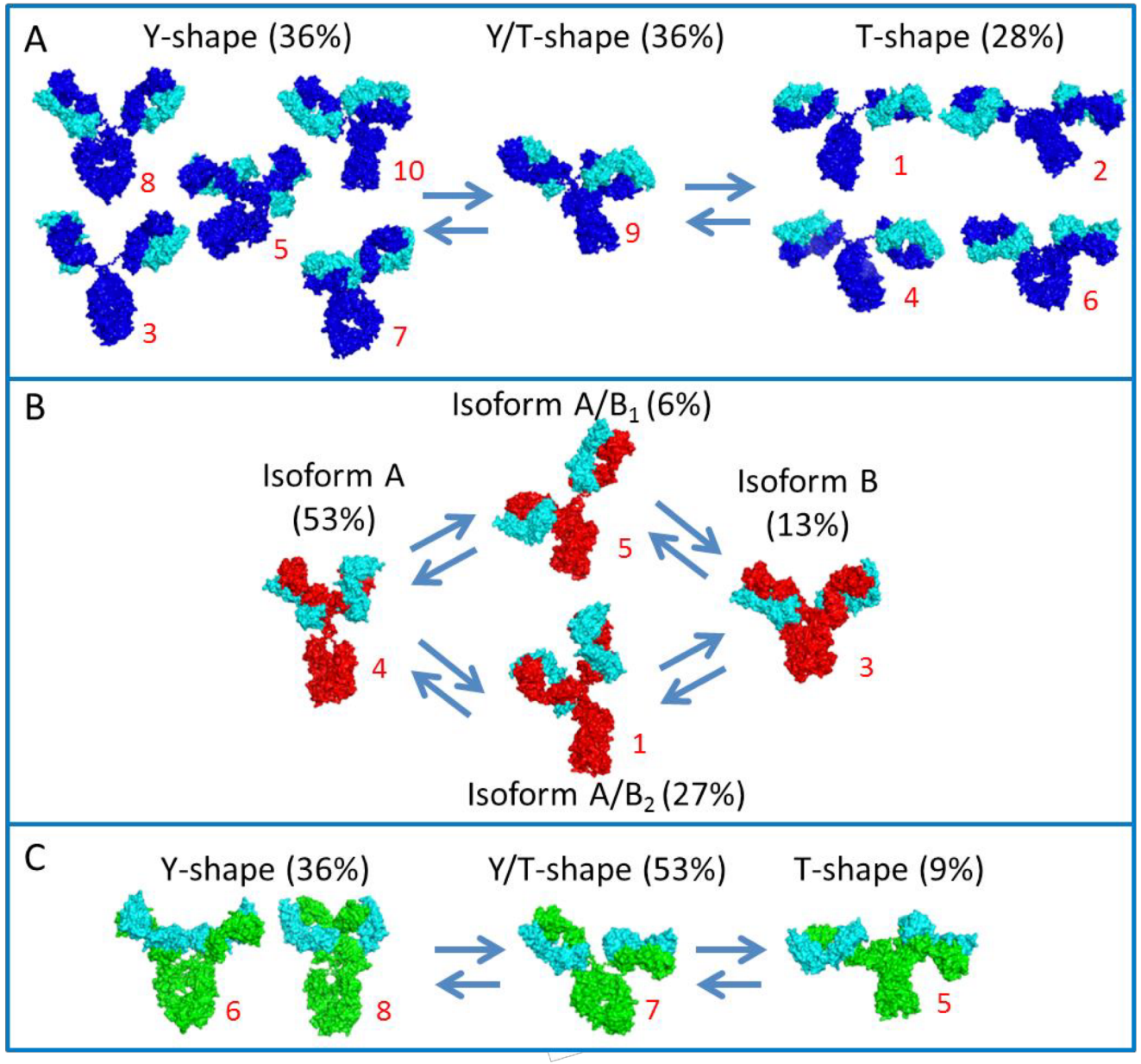
Ensembles with prior information





Tian, Vestergaard, Thorolfsson, Yang, Rasmussen & Langkilde (2014) IUCrJ , 2, 9-18.

IgG conformations



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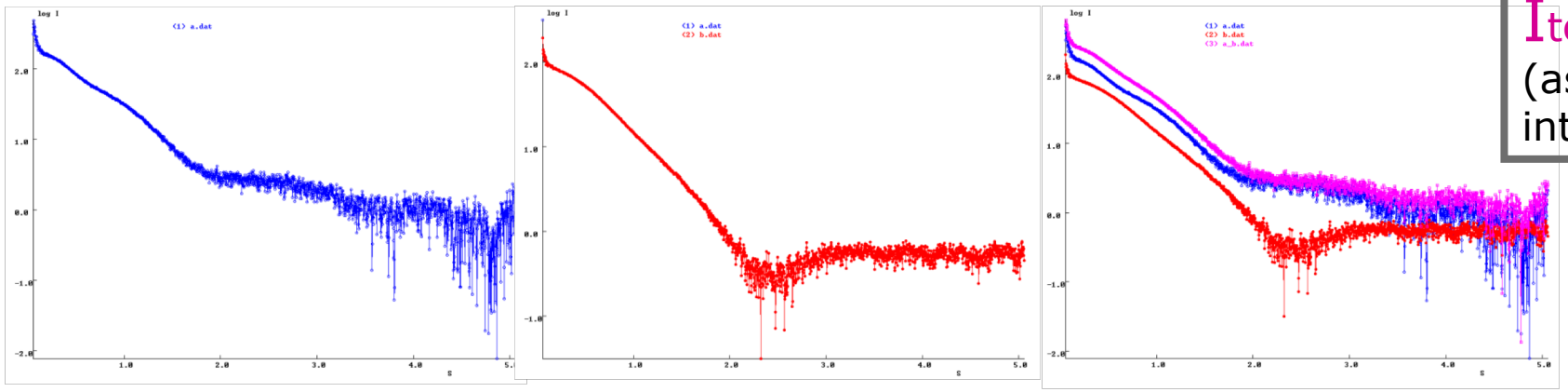

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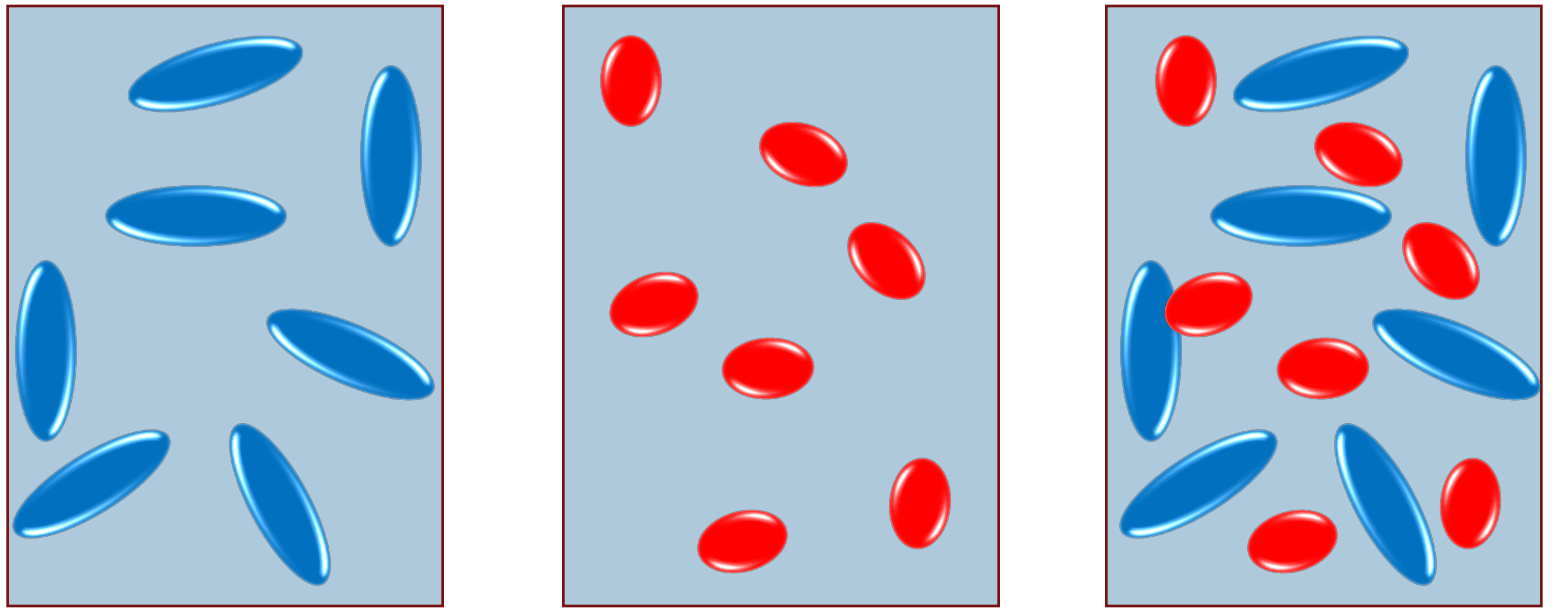
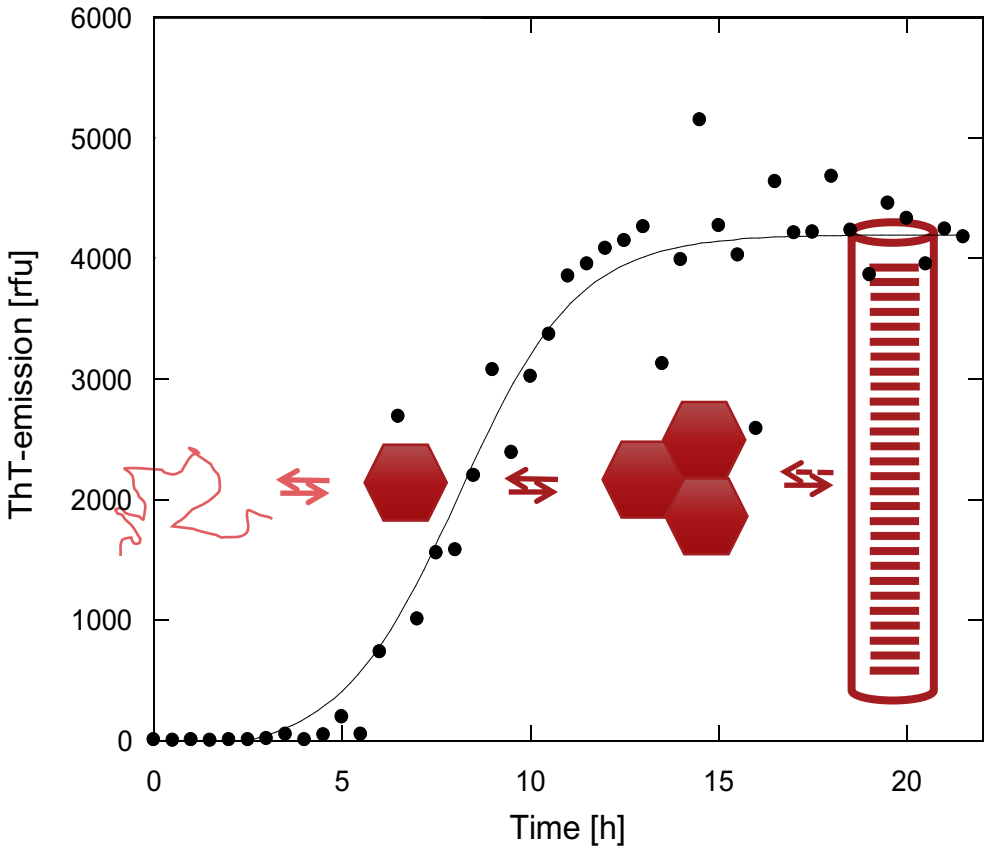
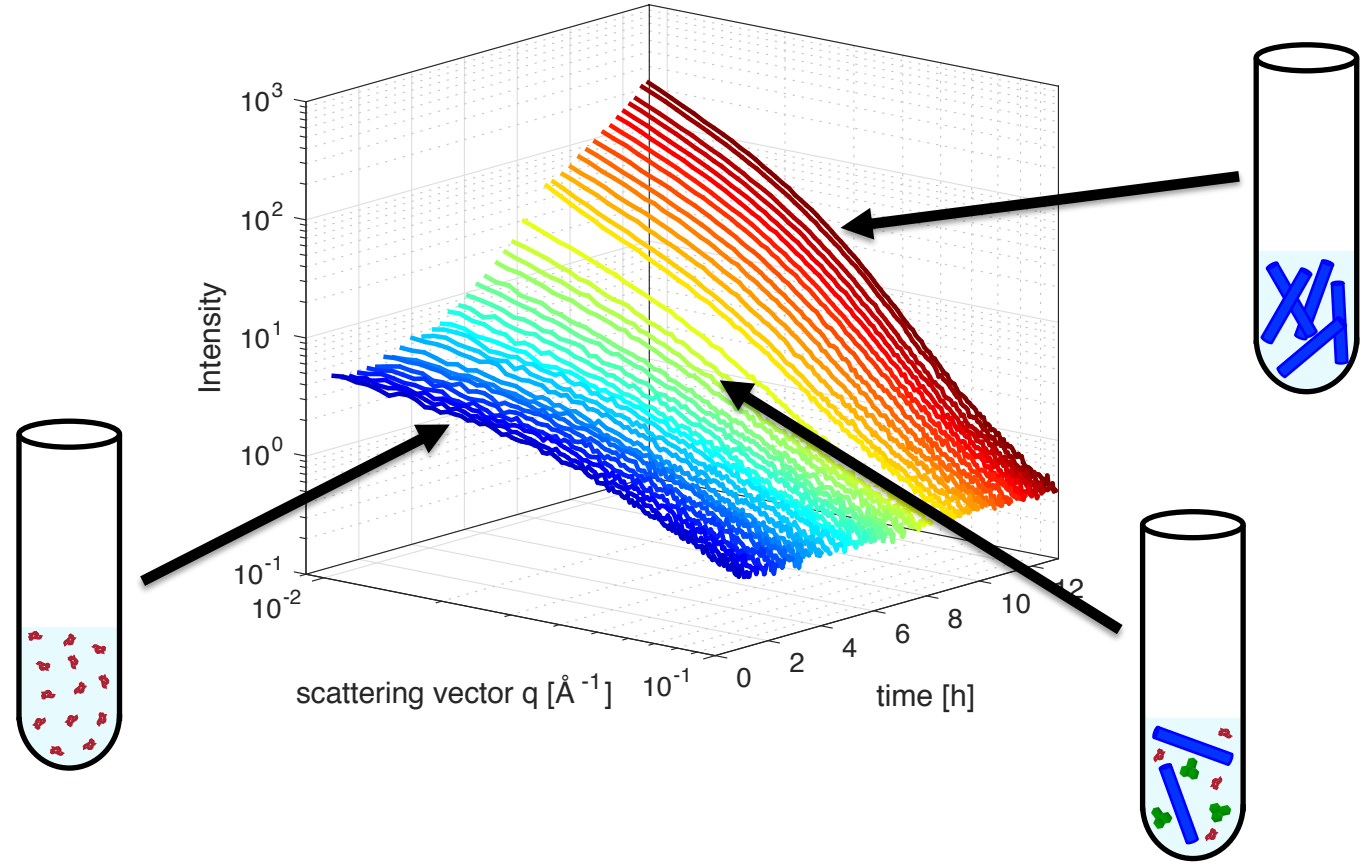


Decomposition and structural characterization using SAXS





$$I_{\text{tot}} = a \cdot I_{\text{monomer}} + b \cdot I_{\text{oligomer}} + c \cdot I_{\text{fibril}}$$