Structural characterization of proteins and complexes using small-angle X-ray solution scattering

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Presentation outline

Background

- Small angle x-ray scattering (SAXS)
- Study Objectives

Analysis techniques

- Guinier plots
- *Ab inito*
- Rigid body modelling

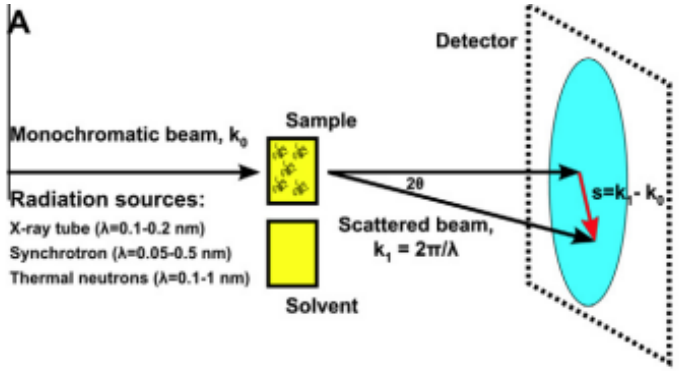
Application

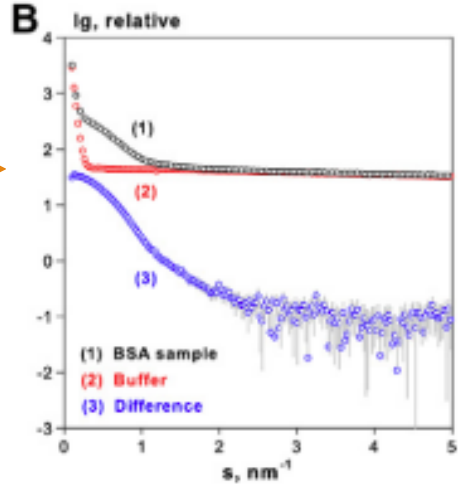
- Flexible systems
- Mixtures

Future developments

Summary

Background info





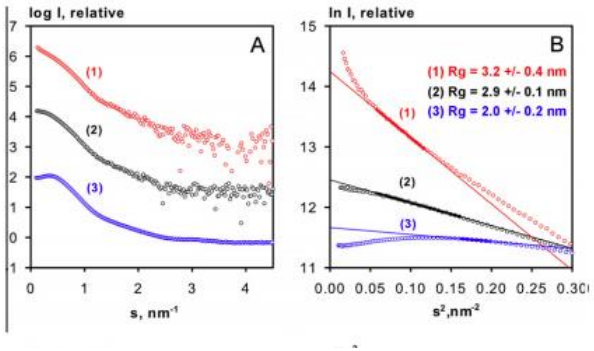
Size, oligomeric state, overall shape of molecule and 3-d structures

Study Objective

Evaluate main approaches to the characterization of proteins and protein complexes using SAXS

Analysis methods: Guinier plots

First step in sample characterization by SAXS



Forward scattering intensity
radius of gyration
non-specific aggregation
presence of inter-particle repulsion

Limitations:

- If we do not get a linear relationship, we can't analyze anything
- Linear plot ≠ monodisperse sample

Ab inito models

Automated bead modelling lead to detailed ab initio reconstructions

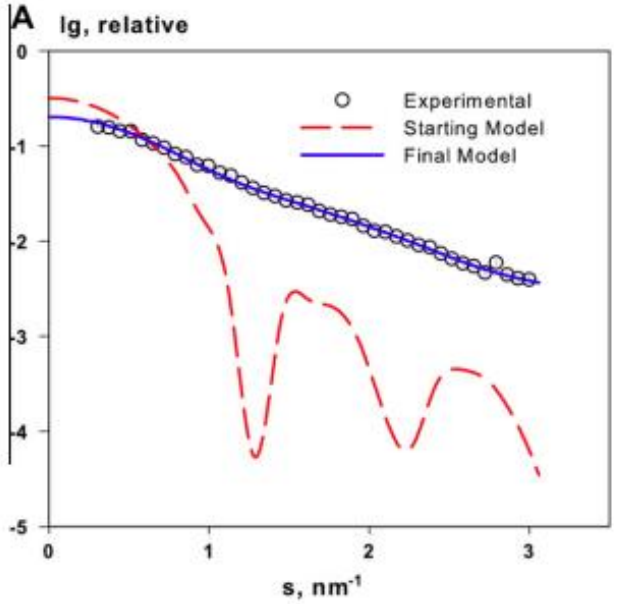
Most popular program: DAMMIN

- Collection of M densely packed beads
- Define Dmax
- Assign each bead randomly to the solvent/solute
- Shape reconstruction occurs using random initial approximation by simulated annealing (SA)

$$\Phi = \chi^2 + \alpha P(p)$$

Limitations

- Focus is solely on shape (Low resolution)
- Can't provide a unique solution



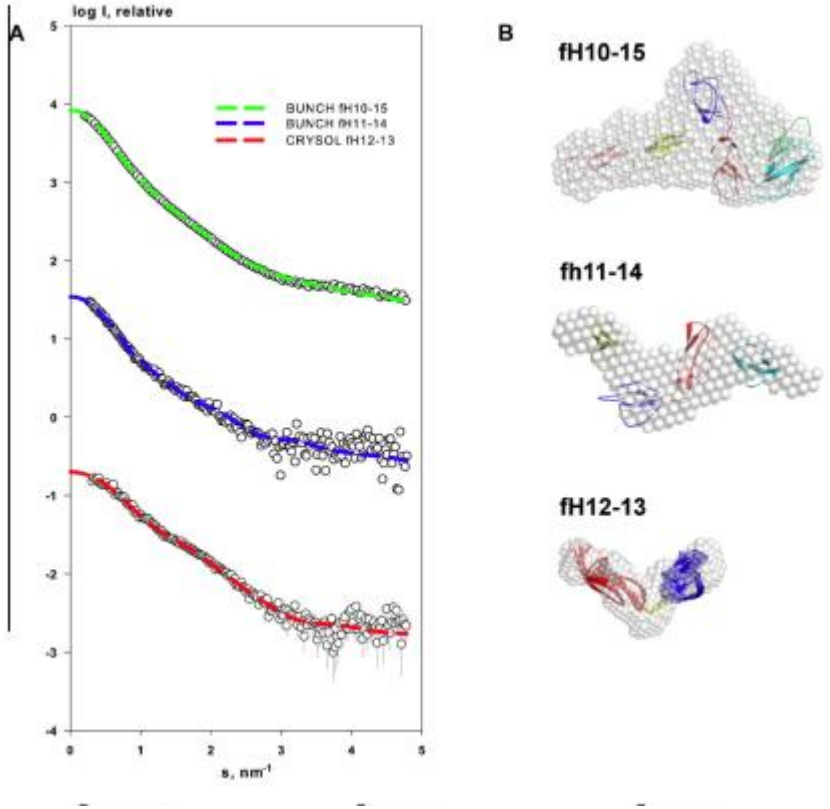
Rigid body modelling

SASREF is comprehensive automated rigid bead modelling program

- Starting from arbitrary position of subunits
- Use SA to search for best fit of computed complex to scattering profiles
- Models generated always are interconnected and no main-chain/backbone steric hindrances

$$\Phi = \chi^2 + \alpha P(\mathbf{p})$$

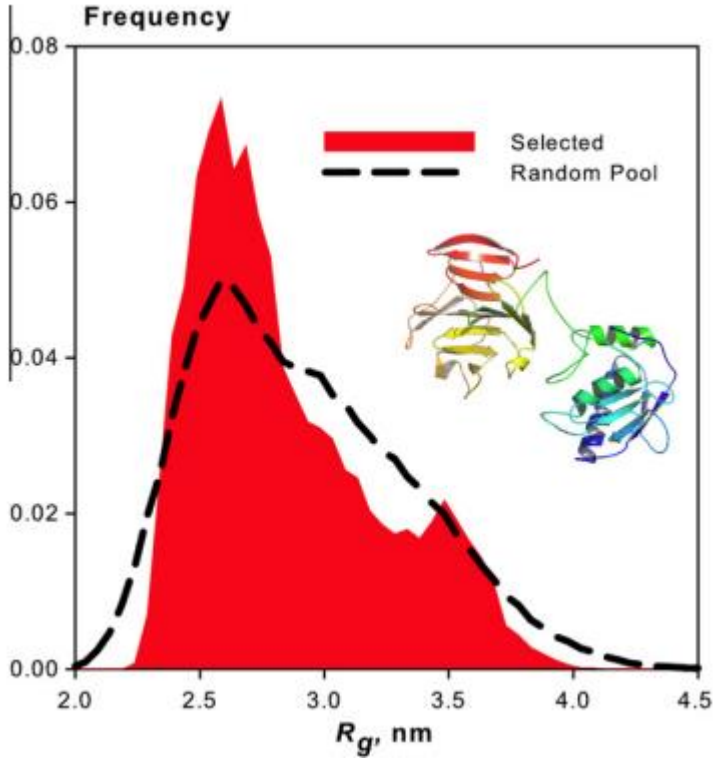
BUNCH: ab initio + rigid body modelling



Flexible systems

EOM (ensemble optimization method)

- High resolution structures of domains is required
- Proteins=ensemble of structures
- Random configurations is generated of the ensemble
- If both selected and random are as broad = flexible
  - Narrow peaks = rigid



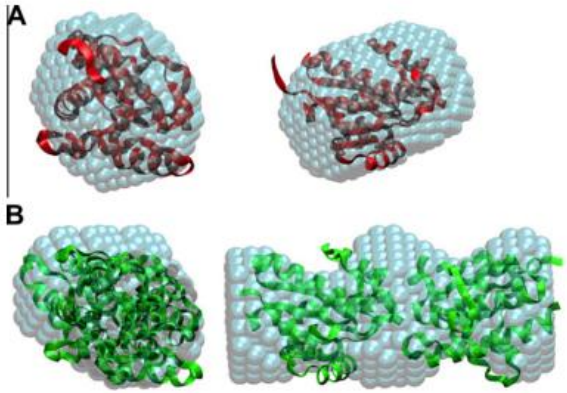
Analysis of Mixtures

$$I(s) = \sum_{k=1}^{K} v_k I_k(s),$$

If scattering patterns of components is known → OLIGOMER can compute volume fractions

Deconvolution of SAXS → *ab initio* models of the minority species

Protein/RNA folding → TR-SAXS



Future outlooks

Automation of data collection, data reduction and analysis

Availability of beamlines has improved

Summary

SAS allows for rapid sample characterization

- Size, shape, structure and volume of macromolecules

Ab initio and Rigid body modelling

- Ab initio provide low resolution 3D models from scattering data lone
- Rigid body determines structure of the macromolecular complexes
- Works best when we employ them in conjunction with other methods