Requirements for the presenting of biological macromolecule small-angle scattering data in publications

It is not the intention of this document to define a quality requirement for SAS experiments that would be acceptable for publication. Rather, the purpose is to outline the way in which SAS experiments should be presented in order to enable the reader to independently assess the quality of any interpretations made by the authors.

Sample Quality

High sample quality is required for SAS experiments. As such the authors must demonstrate as clearly as possible that any sample is of sufficient quality to yield meaningful results in a SAS experiment.

Essential

- A complete description of the sample must be made available; including sequences for proteins and polynucleotides (including purification tags), modifications and cofactors.
- Sample purification procedure must be reported, along with an estimate of the final purity and how this was estimated.
- Solvent blank composition (including pH for aqueous systems) must be reported.
- For SANS contrast variation experiments, a statement describing the level of deuteration in biomolecules and their solvents and *how it* was determined is required.
- Sample concentration(s) and method(s) of determination must be reported, including extinction coefficients when A280 nm absorbance measurements are used.

Preferable

- A SAS-independent assessment of monodispersity (DLS and/or aggregate-free gel filtration and/or MALLS) gives the reader confidence in the results and should be reported for globular proteins where available.
- A statement describing how the solvent blank was obtained (eg dialysis, column flow through) should be made.

Data Acquisition

Details on the execution of the SAS experiment must be provided.

Essential

- Instrument type (eg model or beamline) and configuration (point or line source, collimation details, detector details) must be reported. In the case of SANS there may be several configurations (eg detector positions, no. guides, apertures, etc) for a single experiment.
- Wavelength (including $\Delta\lambda/\lambda$ for neutrons) and measured q-range must be reported.
- Sample environment (including cell pathlengths, temperature) and exposure times.
- Standards measured and controls (secondary scattering standards for assessing $I(0)$ data (*e.g.* lysozyme, water).

- For synchrotron X-ray studies, radiation damage must be monitored and the ways of ensuring the absence of the damage must be reported (addition of scavengers, sample flow, analysis of time frames)

Preferable

- Data reduction protocol and software should be reported.
- Where a line source is used, beam geometry must be provided (either in terms of dimensions of a defined shape [e.g. parameters of a trapezoidal profile], or as a plot of the beam profile file).

Experimental Validation

In order for a reader to be able to assess the quality of a SAS experiment, it is necessary that the data be presented in a clear, well-described manner.

Essential

- Where possible, scattering profiles ($I(q)$ vs q) and P(r) profiles ($P(r)$ vs r) should be reported in the bulk manuscript. If space is restricted, plots may be reported in supplementary materials. $I(q)$ plots should be presented either as linearX-logY or logX-logY axes. the linearX-LinearY representation should be avoided. Where possible, data should be plotted on an absolute scale; however, multiple curves may be offset on the same plot for clarity, provided that this is explained in the figure caption.
- For structural characterisation of monodisperse samples, Guinier plots must be shown. The Guinier range should be explicitly given and the linear fitting should be displayed in the range not exceeding $q.R_g = 1.3$. Gunier plots may be included as stand-alone figures, insets to $I(q)$ vs q plots, or reported in supplementary material.
- The plots containing experimental data must show propagated errors (usually based on counting statistics).
- If the presented data were desmeared (to correct for beam geometry or polychromaticity), this fact must be reported as well as the method for desmearing.
- Molecular weight estimates using either $I(0)$ or the molecular volume determined by Porod invariant must be reported, including uncertainties in the parameters used in the determination.
- The data must be recorded at multiple solute concentrations to eliminate the possibility of concentration-dependent oligomerisation or interparticle interference.
- For contrast variation experiments (both SAXS and SANS) the nature and number of contrast points should be reported. The plot of normalized $\pm\sqrt{I(0)}$ vs solvent density particle matching point must be presented.

Preferable

- Theoretical contrasts and molecular weights for globular samples should be reported along with the method of calculation.
- Extrapolation to infinite dilution is desirable to avoid interference effects. Where no change in R_g or $I(0)/C$ is observed with increasing concentration (C), this fact should be reported.

- For the contrast variation experiments , Stuhrmann plots of R_g^2 versus the reciprocal of contrast are desirable. Extracted component scattering functions (including cross-term) are desirable.

Modeling

Where the experimenter is looking to support a three dimensional model, any modeling must be justified and thoroughly described.

Essential

- All software used for modeling (including generating $P(r)$ profiles) must be reported.
- χ^2 values and a plot of the model fit to the experimental $I(q)$ vs q must be shown for at least the best model.
- Analysis of the ambiguity of the reconstruction (averaging or clustering) must be done.
- For rigid body modeling, a description of how the starting models were obtained (eg crystal structure of a domain, homology model, etc) as well as any connectivity or distance constraints and how they were chosen.
- Any modeling assumptions (eg symmetry) must be stated.

Preferable

- If multiple modeling protocols were utilized, all models and fits to the data should be shown (in supplementary materials if necessary).
- Any additional experimental evidence supporting modeling assumptions and therefore enabling modeling restraints should be reported.