JOURNAL OF SYNCHROTRON RADIATION

Volume 22 (2015)

Supporting information for article:

Limiting radiation damage for high brilliance biological solution scattering: practical experience at the EMBL P12 beam line, PETRAIII

Cy M. Jeffries, Melissa A. Graewert, Dmitri I. Svergun and Clément E. Blanchet

Table S1 Beam dimensions.

Beam Parameters	Dimensions (FWHM)	Dimensions (max)
horizontal, cm	0.020	0.050
vertical, cm	0.011	0.025
Area, cm^2		0.00125

Table S2 Capillary Dimensions.

Capillary	Dimensions
External Diameter, cm	0.180
Internal diameter, cm	0.17*
Capillary wall thickness, cm	0.005

*Used as the sample pathlength, *L*, for Gy calculations.

Table S3 Beam flux and energy parameters.

Beam Parameters	Beam flux, ph.s^{-1}	Capillary wall transmission *	Sample Flux, ph.s^{-1}**	λ (m) and energy per photon (J.ph^{-1})***	Energy delivered to sample per second, J.s^{-1}	Energy delivered to sample per second per unit beam area, J.s^{-1}.cm^{-2}
No Attenuation	5.1E+12	0.7817	3.9868E+12	1.2398E-10 m 1.6022E-15 J.ph^{-1}	6.3875E-03	5.1100
Medium Attenuation	7.3E+11	0.7817	5.7065E+11		9.1429E-04	0.7314
High Attenuation	1.8E+11	0.7817	1.4071E+11		2.2544E-04	0.1804

*The capillary wall transmission is calculated from a SiO_2 thickness of 50 μm with a mass density, ρ_m, 2.648 g.cm^{-3}.

**The flux experienced by the sample takes into account the attenuation of the first 50 μm SiO_2 wall of the capillary.

***The wavelength, λ (m) and energy per photon (J.ph^{-1}) is consistent for each level of attenuation: λ = 1.2398 Å, 10 keV. Energy per photon: E = *hc*/λ, λ in m, where *h* is Planck's constant, *c* is the speed of light.

Table S4 Sample parameters and absorbed dose.

Sample*	Mass Density, ρ_m, g.cm^{-3}**	Mass attenuation coefficient, μ/ρ, cm^2.g^{-1}***	Time to critical dose, s	Gy, J.kg^{-1}
Glucose Isomerase				
10 mg.ml^{-1}	1.029	5.5090	0.390	7046
5 mg.ml^{-1}	1.029	5.5170	0.360	6510
2.5 mg.ml^{-1}	1.029	5.5210	0.390	7056
10 mg.ml^{-1} + ascorbate	1.029	5.509	0.360	6504
10 mg.ml^{-1} + DTT	1.029	5.5110	0.330	5964
BSA				
11 mg.ml^{-1}	1.023	5.140	0.315	5470
5.5 mg.ml^{-1}	1.023	5.146	0.315	5473
2.75 mg.ml^{-1}	1.023	5.149	0.270	4693
11 mg.ml^{-1} + ascorbate	1.023	5.139	0.360	6250
11 mg.ml^{-1} + DTT	1.023	5.142	0.315	5471
11 mg.ml^{-1} + glycerol	1.037	5.062	0.450	7700
Cytochrome C				
10 mg.ml^{-1}	1.027	5.408	0.060	1073
5 mg.ml^{-1}	1.027	5.412	0.060	1073
2.5 mg.ml^{-1}	1.027	5.414	0.060	1074
10 mg.ml^{-1} + ascorbate	1.027	5.4070	0.150	2682
10 mg.ml^{-1} + DTT	1.027	5.410	0.420	7513
Lysozyme				
8.8 mg.ml^{-1}	1.028	5.406	0.020	365
4.4 mg.ml^{-1}	1.028	5.410	0.018	325
4.4 mg.ml^{-1} (medium attenuation)	1.028	5.410	0.135	346
4.4 mg.ml^{-1} (high attenuation)	1.028	5.410	0.450	284
2.2 mg.ml^{-1}	1.028	5.412	0.016	293
8.8 mg.ml^{-1} + ascorbate	1.028	5.405	0.060	1072
8.8 mg.ml^{-1} + DTT	1.028	5.408	0.060	1073
8.8 mg.ml^{-1} + glycerol	1.042	5.328	0.150	2643
RNAse				
10 mg.ml^{-1}	1.027	5.409	0.018	319
5 mg.ml^{-1}	1.027	5.413	0.016	280
2.5 mg.ml^{-1}	1.027	5.414	0.014	252
10 mg.ml^{-1} + ascorbate	1.027	5.409	0.060	1073
10 mg.ml^{-1} + DTT	1.027	5.412	0.090	1610
10 mg.ml^{-1} + glycerol	1.041	5.332	0.300	5291

*Unless stated, Gy data are derived for full beam measurements with no beam attenuation or sample flow.
The sample path length, *L*, used for the Gy calculation corresponds to the internal capillary diameter (0.17 cm).

**Calculated using MULCh, Whitten *et al*. (2008) J. App. Cryst. 41: 222-226.

***Calculated from atomic composition weight fractions using XCOM: http://www.nist.gov/pml/data/xcom/index.cfm

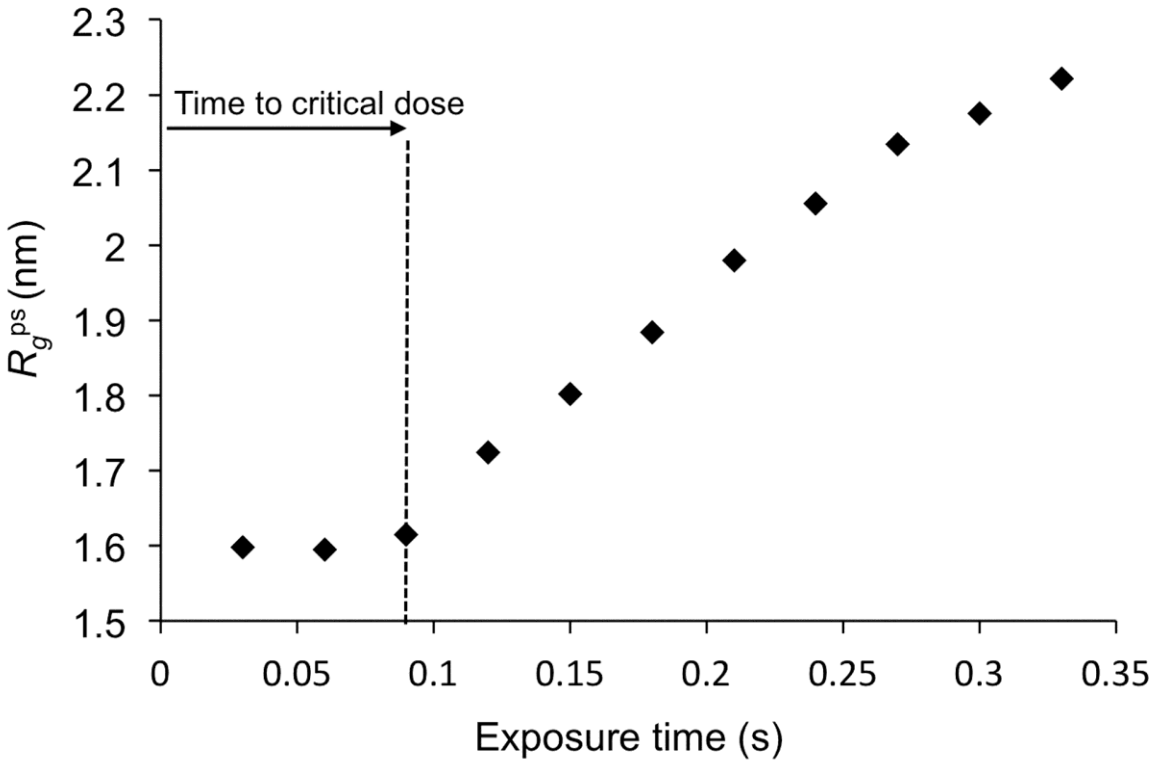

Figure S1 Calculation of critical dose time (an example). A plot of R_g^{ps} vs exposure time (s) for RNAse (10 mg.ml^{-1}) in the presence of 1 mM DTT showing the evaluation of the critical dose time whereby $\Delta R_g^{ps} \leq 0.1$ nm.

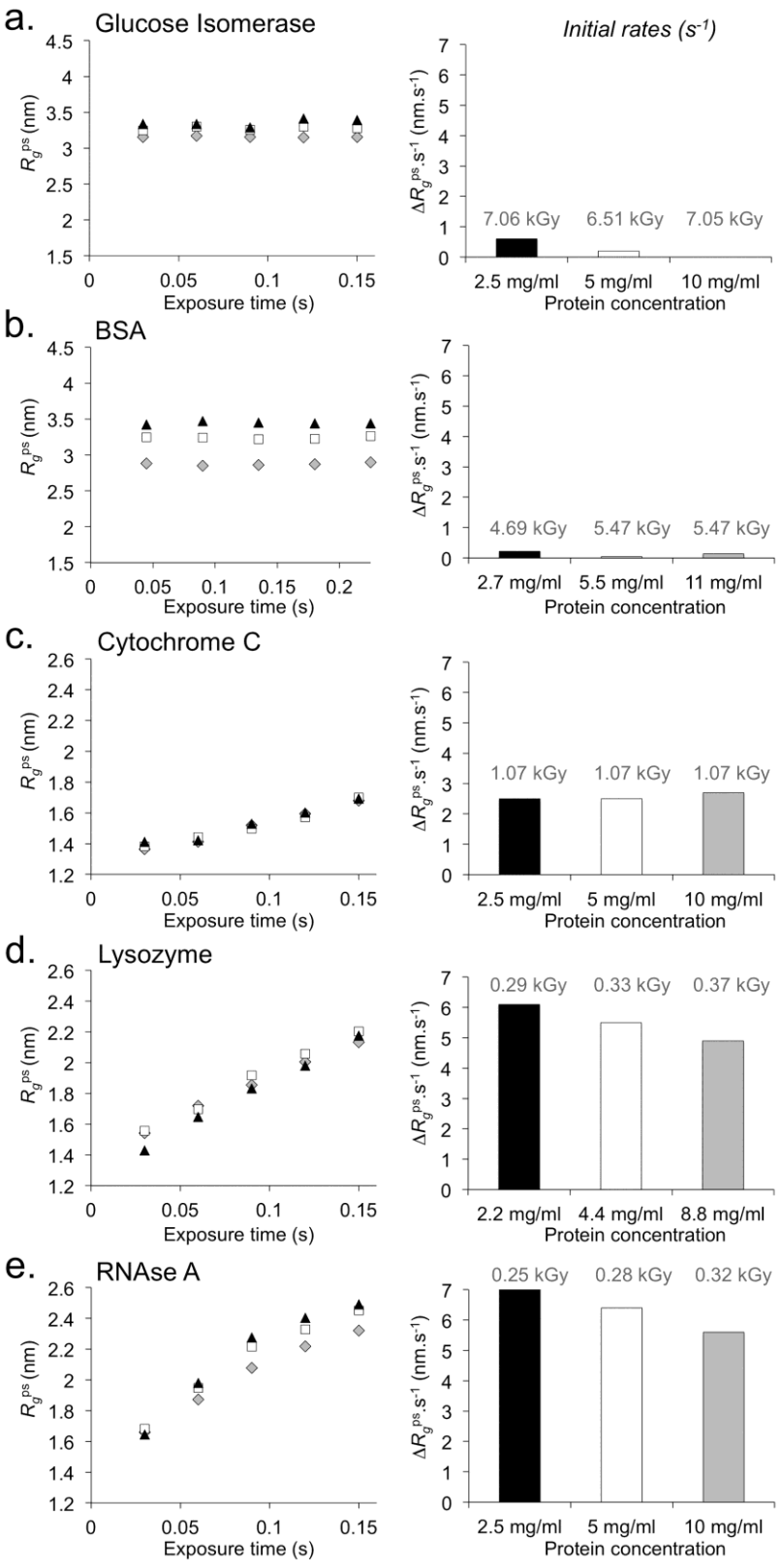

Figure S2 Protein concentration screening. Plot of R_g^{ps} vs exposure time (s) of different protein samples at various sample concentrations and estimates of the initial rates of aggregation, $\Delta R_g^{ps}.s^{-1}$ (unattenuated beam, no sample flow) and absorbed dose (kGy).