

## SAXS and SANS Data Collection Parameters, Sample Details, Data Analysis, Model fitting Results, and Software Employed for 73%<sup>d</sup>CaM-MA

SANS data collection parameters		
Source, instrument and reference	OPAL Reactor at ANSTO, Lucas Heights, QUOKKA instrument described in Wood, K et al, J. Appl. Crystallogr. 51(2), 294-314 (2018) and <a href="https://www.ansto.gov.au/research/user-office/instruments/neutron-scattering-instruments/quokka/technical-information">https://www.ansto.gov.au/research/user-office/instruments/neutron-scattering-instruments/quokka/technical-information</a>	Bruker NanoStar with rotating anode (Cu K $\alpha$ 0.3 mm filament) pinhole collimation and VÅNTEC 2D detector at ANSTO Lucas Heights  <a href="https://www.ansto.gov.au/user-access/instruments/other-instruments-and-services/small-angle-x-ray-scattering-instruments">https://www.ansto.gov.au/user-access/instruments/other-instruments-and-services/small-angle-x-ray-scattering-instruments</a>
Wavelength	4.94 Å	1.54 Å
Camera/Beam set up	$\Delta\lambda/\lambda$ 6.65% Sample to detector settings 2m and 7m for 44% and 100% D <sub>2</sub> O samples, 2m and 10m for 19% and 88% D <sub>2</sub> O samples	Copper rotating anode (0.3 mm filament) 3 pin hole collimation, 0.7 m camera length
$q$ -measurement range (Å <sup>-1</sup> or nm <sup>-1</sup> )	0.007–0.42 Å <sup>-1</sup> for 2m and 7m data 0.007–0.3 Å <sup>-1</sup> for 2m and 10m data	0.012–0.315 Å <sup>-1</sup>
Data treatment	Detector sensitivity corrections applied using H <sub>2</sub> O or 19% D <sub>2</sub> O solvent scattering followed by radial averaging and subtraction of empty cell and blocked beam with corrections for sample and solvent transmission. Data were put on an absolute scaling using measured beam flux.  Scattering from the protein was obtained by subtraction of the (solvent + cell) scattering from the (solvent + cell + protein) scattering.	SAXS: Small Angle X-ray Scattering System V4.1.29 was used to circularly average the raw data, correct for detector sensitivity and non-linearity. All data were placed on an absolute scale using H <sub>2</sub> O as a standard.  Scattering from the protein was obtained by subtraction of the (solvent + cell) scattering from the (solvent + cell + protein scattering).
Exposure time	30 min, 60 min and 133 min for 2m, 7, and 10, respectively. Samples and solvent were acquired for equal times.	30, 60, and 120 min exposures were measured and compared for radiation effects. Samples and solvent were acquired for equal times.
Sample configuration	300 $\mu$ L solution in a 1-mm path-length, banjo-shaped cell (product No. 120-1 mm; HELIMA USA Plainview, NY)	~30 $\mu$ L of sample was loaded into a 2 mm quartz capillary in a stainless-steel holder
Sample temperature (°C)	10-20 °C	10 °C
Sample details		
Sample description: 1:1 complex of calcium saturated (4 Ca <sup>2+</sup> sites) 73% deuterated calmodulin (4Ca <sup>2+</sup> -CaM) with the HIV-1 MA protein	<p>CaM sequence (amino acids 1-148): ADQLTEEQIAEFKEAFSLFDKDGDTITTKELGTVMRSLGQNPTEAELQDMI NEVDADGNGTIDFPFLTMMARKMKDSTDSEEEIREAFRVFDKDGNGYISAA ELRHVMTNLGEKLTDEEVDEMIREANIDGDGQVNYEEFVQMMTAK</p> <p>MA sequence (amino acids 1 - 133, N-terminal His after Factor Xa cleavage then amino acids 1-133 of MA): HMGARASVLSGGELDKWEKIRLRPGGKKQYKLKHIVWASRELERFAVNPG</p>	

	LLETSEGCRQILGQLQPSLQTGSEELRSLYNTIAVLVCVHQRIDVKDTKEALDKIEEEQNKSKKKAQQAADTGNNSQVSQNY				
Source	Bacterially expression (see James et al. (2012) Biophys J. <b>103</b> :541–549)				
Organism, UniProt sequence ID (residues in construct)	Xenopus laevis, CaM uniprot ID <b>P0DP34</b> HIV-1, gag-poly MA protein uniprot ID <b>P12497</b>				
Extinction coefficient (A280, 0.1% w/v solution)	CaM: 0.178 MA: 1.141 1:1 CaM:MA complex: 0.634				
Partial specific volume from sequence ( $\vartheta$ cm <sup>3</sup> g <sup>-1</sup> )	CaM-MA complex: 0.724				
Particle contrast from sequence and solvent constituents ( $\Delta\rho$ , 10 <sup>10</sup> cm <sup>-2</sup> )	SANS    19% D <sub>2</sub> O    3.09 44% D <sub>2</sub> O    1.70 88% D <sub>2</sub> O    -0.74 100% D <sub>2</sub> O   -1.41		SAXS    3.081		
Molecular mass from sequence (Da)	32,612				
Solvent blank	50 mM MOPS, pH 7.0, 5 mM CaCl <sub>2</sub> , 2 mM TCEP, solvent blank was the dialysate taken after final dialysis.				
<b>SAXS Data analysis</b>					
	SANS 19% D <sub>2</sub> O	SANS 44% D <sub>2</sub> O	SANS 88% D <sub>2</sub> O	SANS 100% D <sub>2</sub> O	SAXS 100% D <sub>2</sub> O
Sample concentrations measured (mg/mL)	7.7	7.0	7.4	7.5	7.2
<b>Guinier analysis*</b>					
$I(0)$ (cm <sup>-1</sup> )	0.27 ± 0.009	0.081 ± 0.004	0.013 ± 0.001	0.059 ± 0.008	0.20 ± 0.002
$R_g$ (Å)	29.4 ± 1.4	20.8 ± 1.5	10 ± 2	38 ± 8	29.68 ± 0.039
$qR_g$ max	1.29	1.28	1.2	1.29	1.27
$q_{\min}$ (Å <sup>-1</sup> )	0.014	0.019	0.012	0.013	0.014
Quality of fit (fidelity)	0.65	1.00	1.00	0.91	0.94
Molecular Weight from $I(0)$	42160	45831	36880	45718	33618
<b><math>P(r)</math> analysis*</b>					
$I(0)$ (cm <sup>-1</sup> ) (normalised to unit $C$ in mg/mL)	0.270 ± 0.006	0.079	0.014 ± 0.009	0.057 ± 0.005	0.201 ± 0.002
$R_g$ (Å)	30.0 ± 0.8	20.7 ± 1.0	13.3 ± 0.9	41 ± 2	31.5 ± 0.4
$d_{\max}$ (Å)	105	64	43	110	110
$q$ range (Å <sup>-1</sup> )	0.014-0.304	0.013-0.304	0.014-0.304	0.013-0.219	0.014-0.270
Total estimate from GNOM	0.81	0.86	0.88	0.65	0.78

Molecular Weight from $I(0)$	42160	44699	39717	44168	33786
Porod Volume ( $\text{\AA}^{-3}$ )	-	-	-	-	54507
Guinier parameters for all data and the SAXS $P(r)$ analysis are the autoRg and autoGNOM solutions, respectively, from the latest version of PRIMUS Qt version 5.12.6. $P(r)$ parameters for the SANS data are reported from GNOM as implemented in Version 4.5a revised 09/02/02 that was used for the original publication and included the correction for instrument resolution (filename smear.res). Errors are propagated counting statistics only.					
Model fitting results					
Ab initio Modelling – MONSA bead modelling against the combined SAXS and SANS data					
$q$ range for fitting ( $\text{\AA}^{-1}$ )	0.012-0.304 $\text{\AA}^{-1}$				
Symmetry, anisotropy assumptions	P1, none				
$\chi^2$ values for fits of presented model	SAXS 0.98 SANS, D <sub>2</sub> O content 0.19 0.62 0.44 0.72 0.88 0.80 1.0 0.85				
Atomistic model – SASREF7 rigid body modelling against the combined SAXS and SANS data					
CaM component	CaM coordinates from the 1 <sup>st</sup> structure on the NMR ensemble 2KDU				
MA component	Coordinates from 2HMX were divided into 8 sections (numbering here starts at -1 for the N-terminal His) consisting of 7 helical sections are 1 - 10, 11 - 25, 26 - 46, 47 - 72, 73 - 90, 91 - 96, 97 - 113, plus a flexible C-terminal tail 114 - 133 that was not included in the rigid body modelling.				
$q$ range for fitting ( $\text{\AA}^{-1}$ )	0.012-0.304 $\text{\AA}^{-1}$				
Symmetry, anisotropy assumptions	P1, none				
$\chi^2$ values for fits of presented model	SAXS 1.19 SANS, D <sub>2</sub> O content 0.19 0.44 0.44 0.72 0.88 0.80 1.0 0.85				
Software employed for SAXS data reduction, analysis and interpretation					
Data reduction to sample minus solvent	SANS: IGOR Pro 6.1 (Wavemetriss, Portland OR) with QOKKA data reduction installed  SAXS: Small Angle X-ray Scattering System V4.1.29 (Bruker Software)				
Extinction coefficient estimate	ProtParam (Gasteiger et al., 2005)				
Calculation of $\Delta\rho$ and $\vartheta$ values from sequence	MULCh 1.1 (06/10/16) (Whitten et al., 2008) for the MW calculations deposited in SASBDB				
Basic analyses: Guinier, $P(r)$ , Porod volume	Guinier parameters and Porod volume are from the PRIMUSQT from ATSAS program suite (Franke et al., 2017; Petoukhov et al., 2012)				

	$P(r)$ parameters are reported from GNOM (Svergun et al., 1988) as implemented in Version 4.5a revised 09/02/02 that was used in the original publication. For the SANS data the instrument resolution correction is included (filename smear.res)
Ab initio modelling	MONSA (Svergun, 1999), locally run version MONSA144 from the ATSAS suite
Atomistic modelling	SAREF (Petoukhov and Svergun, 2005), locally run version SASREF7 from the ATSAS suite
Model profiles	CRYSON (Svergun et al., 1998) and CRYSOL (Svergun et al., 1995) from the ATSAS suite
3D graphic model representations	The PyMOL Molecular Graphics System, Schrödinger, LLC.

Svergun, D.I., Semenyuk, A. V., Feigen, L. A. (1988). Small-angle-scattering-data treatment by the regularization method. *Acta Crystallogr A* **44**, 244-250.

Franke, D., Petoukhov, M.V., Konarev, P.V., Panjkovich, A., Tuukkanen, A., Mertens, H.D.T., Kikhney, A.G., Hajizadeh, N.R., Franklin, J.M., Jeffries, C.M., *et al.* (2017). ATSAS 2.8: a comprehensive data analysis suite for small-angle scattering from macromolecular solutions. *Journal of applied crystallography* **50**, 1212-1225.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M.R., Appel, R.D., and Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In *The Proteomics Protocols Handbook*, J.M. Walker, ed. (New York: Humana Press), pp. 571-607.

Petoukhov, M.V., Franke, D., Shkumatov, A.V., Tria, G., Kikhney, A.G., Gajda, M., Gorba, C., Mertens, H.D., Konarev, P.V., and Svergun, D.I. (2012). New developments in the ATSAS program package for small-angle scattering data analysis. *Journal of applied crystallography* **45**, 342-350.

Petoukhov, M.V., and Svergun, D.I. (2005). Global rigid body modeling of macromolecular complexes against small-angle scattering data. *Biophysical journal* **89**, 1237-1250.

Svergun, D., Barberato, C., and Koch, M.H.J. (1995). CRY SOL - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates. *Journal of applied crystallography* **28**, 768-773.

Svergun, D.I. (1999). Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing. *Biophysical journal* **76**, 2879-2886.

Svergun, D.I., Richard, S., Koch, M.H., Sayers, Z., Kuprin, S., and Zaccai, G. (1998). Protein hydration in solution: experimental observation by x-ray and neutron scattering. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 2267-2272.

Whitten, A.E., Cai, S.Z., and Trewthella, J. (2008). MULCh: modules for the analysis of small-angle neutron contrast variation data from biomolecular assemblies. *J Appl Crystallogr* **41**, 222-226.